2012

A survey of ballistosporic phylloplane yeasts in Baton Rouge, Louisiana

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A SURVEY OF BALLISTOSPORIC PHYLLOPLANE YEASTS IN BATON ROUGE, LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana Sate University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Plant Pathology

by
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December 2012

Acknowledgments

It would not have been possible to write this thesis without the guidance and support of many people. I would like to thank my major professor Dr. M. Catherine Aime for her incredible generosity and for imparting to me some of her vast knowledge and expertise of mycology and phylogenetics. Her unflagging dedication to the field has been an inspiration and continues to motivate me to do my best work. I am also very fortunate to have three other wonderful committee members-Dr. Meredith Blackwell, Dr. Ken Damann and Dr. Lowell Urbatsch- who have given me invaluable advice and have always been available when I have needed their assistance. Additionally, Dominik Begerow was extremely kind to provide several of his *Kordyana* sequences that were used in the analysis of the Exobasidiomycetes.

I have been priveledged to work with a number of people in my lab who are not only great at what they do, but have taught me valuable tools which I have used in my research. I am grateful to Alistair McTaggart for his patience in teaching me about maximum likelihood and RAxML, Merje Toome and Geg Heller for their help with various molecular techniques and Ramandeep Kaur for making the lab run smoothly. Hector Urbina and Rebecca Melanson's help with formatting and assembling this thesis is very much appreciated. My fellow students Tomas Rush, Andrew Rodriguez, Jorge Diaz, Youwen Gong, Don Nelsen, Rachel Koch and I have not only worked together, but our friendship extends outside of the lab and I know we will always feel a sense of deep camaraderie for one another. I would also like to extend many thanks to Paige Muse, Shiquita Brooks and Catherine Perino who have provided a great of help with culture work. I am also grateful to the Louisiana Board of Regents and for an NSF AFTOL grant for providing the funding for this research.

Finally, I want to acknowledge my family for years of unconditional support, love and understanding. Some of my wildest speculations have resulted from late night conversations with my brother, Alex who also appreciates a wild edible mushroom on any hiking trip. Finally, I would like to thank my parents, Valeriu and Magdalena Albu. Mere words will never express the gratitude that I feel towards them and everything they have given me.

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Abstract

A study documenting basidiomycete yeast biodiversity was conducted in Baton Rouge, Louisiana during 2010 and 2011. Using the spore-fall method, the leaf surfaces of seven ferns were sampled at biweekly intervals. Maximum likelihood phylogenetic analyses of the internal transcribed spacer (ITS) region and the D1/D2 domain of the large subunit (LSU) of nuclear ribosomal DNA were used to identify 463 isolates representing 81 species spanning 12 orders within six classes in Basidiomycota. Nearly 30 of these isolates appear to be species new to science. Data indicate fern leaf developmental stage has an effect on the number of yeasts present. A significant difference exists between the number of isolates recovered from young versus senescent leaves. On average, more yeast isolates were recovered from young leaves than from senescing ones for all classes of yeasts except Ustilaginomycetes, which were more abundant on senescing leaves. The number of yeasts recovered from lower (abaxial) versus upper (adaxial) leaf surfaces did not differ significantly, though isolates were recovered more frequently from abaxial surfaces for all classes expect Microbotryomycetes and Tremellomycetes. For all six classes there was a trend for non-fertile frond portions to yield more isolates than those with sori, but this difference is not statistically significant. Monthly records of temperature and precipitation were compiled and while they show no statistically significant correlation with yeast abundance, several patterns may be meaningful. A general downward trend was observed in the number of isolates recovered with respect to decreasing temperature and increasing precipitation levels. More isolates were recovered during the months with the highest average temperatures and lowest levels of precipitation. Additionally, two new yeast species collected during this survey are provisionally described. SA209 and SA575 represent previously undescribed yeast species in the genera *Farysia* and *Sporisorium* (Ustilaginales). The LSU and ITS regions of these isolates were compared to available sequences of *Farysizyma* and *Pseudozyma* species and related taxa in *Farysia*, *Sporisorium* and *Ustilago*. SA209 is part of a *Farysizyma/Farysia* clade in Anthracoideaceae and SA575 is sister to *Sporisorium chrysopogonis* and *S. heteropogonicola* within a clade of *Sporisorium* species that includes the type (*S. sorghi*).

Chapter 1. Literature Review

1.1 Yeasts

In the simplest terms, yeasts are single-celled ascomycete or basidiomycete fungi that reproduce asexually by budding or fission and fruiting bodies are typically absent in those species that reproduce sexually (Suh, *et al.*, 2006). In culture, yeast cells are often visible as single cells of variable sizes that produce daughter cells, but do not usually form mycelia. The frequent synonymization of the term 'yeast' with *Saccharomyces cerevisiae* is not surprising since that organism is important in food and alcohol industries and is among the most commonly used eukaryotic models of genetic research. However, there are many other lesser known yeasts which have succeeded at filling different niches. In particular, the relationships between plants and yeasts have become a focal point of mycological and botanical studies (de Jager, *et al.*, 2001, Slavikova, *et al.*, 2007).

1.2 The Phylloplane

The study of microbes on the leaf surfaces of plants has been the subject of research since the middle of the twentieth century (Fonseca & Inácio, 2006). Initial studies investigating the diversity of epiphytic microorganisms revealed that in addition to pathogens which produce signs on their hosts, a wealth of other, less conspicuous saprobes are also present. Several early studies showed that these communities are composed of filamentous fungi, bacteria, algae and yeasts (Last, 1955, di Menna, 1959, Ruinen, 1961). The hypothesis that microbe-rich nutrient and exudate runoff from the leaves contributes to the composition of the rhizosphere community (de Becze, 1956, Ruinen, 1961) initially suggested that the yeast communities of leaf surfaces and the soil were closely related. However, this was disputed by di Menna (1959), who argued that the yeast species profile of pasture plants in New Zealand did not correspond to that of plant roots and changed throughout the year. Di Menna also argued that while yeasts are ubiquitous denizens of most plant parts, leaf and soil communities are taxonomically independent of one another (di Menna, 1959). Last (1955) proposed that, just as there is an ecological niche around plant roots, which he termed the 'rhizosphere,' there is also a diverse and unique micro-environment on the leaf surface which is comprised of independent populations of microorganisms. This area was originally named the 'phyllosphere,' (Last, 1955, Ruinen, 1956), but is also referred to as the 'phylloplane' (Fonseca & Inácio, 2006). These early surveys showed that the surfaces of plant leaves are diverse ecosystems and paved the way for future work seeking to characterize species diversity in more detail.

Even though the phylloplane is teeming with microbial life, it is an extreme environment. This is not only because of the myriad different microclimates and limited availability of resources, but because of abrupt shifts in environmental conditions (Fonseca & Inácio, 2006). The survival of phylloplane microbes depends on their ability to respond and adapt to these changes as well as to exploit highly specific niches such as trichomes and stomatal depressions (Kinkel, 1997). It is now known that phylloplane ecology is affected by many factors which can influence epiphytic microbial populations (de Jager, *et al.*, 2001, Pereira, *et al.*, 2002, Nix-Stohr, *et al.*, 2008). Cuticle composition and microclimate differences may affect the distribution of the phylloplane community and numbers of epiphytic yeasts differ

among plants with different ecological distributions. Certain xerophytic plants such *Vaccinium* (blueberry) harbor fewer numbers of yeasts than other plants which have more abundant water sources available (Maksimova & Chernov, 2004). This has been attributed to a thickening of the cuticle in xerophytic plants which allows fewer nutrients to exude onto the leaf surface (Andrews & Harris, 2000). The chemical composition of nutrients that are available on the phylloplane also plays a role in determining which types of yeasts can survive and which are selected against (Inacio, *et al.*, 2002).

Epiphytic microbes must effectively cope with abrupt changes in temperature and atmospheric pressure as well as with periods of limited water and nutrient supply (Nakase, 2000). Humidity levels, solar radiation, plant exudates and physiology also affect the growth and development of phylloplane populations (Glushakova & Chernov, 2004). Microorganisms are constantly exposed to various host toxins and must adapt to an environment where carbon sources and amino acids may not be available (Phaff and Starmer, 1987). Additionally, microbes compete for resources and are often forced to devote energy to the production of secondary metabolites to counteract host and other microbial defenses (Slavikova, *et al.*, 2007). Nevertheless, the phylloplane is a rich and diverse environment, comprising one of the most extensive terrestrial niches on the planet with many species of yeasts and other fungi forming mutualistic and pathogenic relationships with plants.

Although other niches such as leaf litter and the rhizosphere contain yeast populations from both Ascomycota and Basidiomycota (Maksimova & Chernov, 2004), the dominant yeast groups of the phylloplane are basidiomycetous (Inacio, *et al.*, 2002, Fonseca & Inácio, 2006). These are often members of genera such as *Bullera*, *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* (Last, 1955, Pennycook & Newhook, 1981, Chernov, 1985, Middelhoven, 1997, Buck & Burpee, 2002, Fonseca & Inácio, 2006), and are widely distributed across many different geographic areas. Ascomycete yeasts are also phylloplane inhabitants (Inacio, *et al.*, 2004, Maksimova & Chernov, 2004), though they tend to be more common on other plant parts such as flowers (Golonka, 2002) or in substrates with high sugar content such as nectar or exudates (Phaff, 1987). However, some isolates of *Lalaria* (teleomorph = *Taphrina*) may be permanent phylloplane residents that have lost their pathogenicity (Inacio, *et al.*, 2004) and certain species of *Candida* and *Pichia* are common in forest environments (Maksimova & Chernov, 2004).

The success of fungi indicates that they are highly adaptable organisms that have developed efficient methods of reproduction. Although many phylloplane yeasts are capable of producing dikaryotic fertile mycelia by sexual reproduction with compatible mating types, many others have only ever been observed in the anamorphic stage. Like other fungi, yeasts can disperse their propagules in a variety of ways. Some rely on insects (Gilbert, 1980, Starmer, *et al.*, 1988) or larger animals (Abranches, *et al.*, 1998, Mattsson, *et al.*, 1999) and others are intentionally or unwittingly transported across great distances by humans (Lachance, *et al.*, 2012). For most yeasts, abiotic forces such as wind or rain are also efficient means of dispersal that can transport their spores over long distances introducing new species into previously uncolonized areas. However, like other basidiomycetes such as mushrooms and some rusts and smuts, certain phylloplane yeasts possess the ability to forcefully discharge their spores.

1.3 Ballistosporic Discharge

The ballistosporic mechanism was first studied in mushrooms where it was found that spores did not fall passively from the hymenium, but instead were forcefully projected from sterigmata, away from the gills (Buller, 1909). Additionally, Buller found that ballistospores had adhesive

properties which allowed them to attach tightly to different substrates. Subsequent studies of the ballistosporic process have revealed a complex mechanism that involves forced ejection of basidiospores from sterigmata through the aid of turgor pressure (Ingold, 1992), which results in a change in the center of mass of the spore (Buller, 1909, Ingold, 1992, Money, 1998, Pringle, *et al.*, 2005). For certain epiphytic fungi, ballistospore formation can occur on either the adaxial or abaxial leaf side and is believed to be stimulated by the oligotrophic nature of the phylloplane (Fonseca & Inácio, 2006). When hydrostatic pressure builds up between a fungal spore and a droplet of water, surface tension forms on the water droplet which eventually coalesces with the spore expelling it from the sterigma at extremely high velocities (Money, 1998, Pringle, *et al.*, 2005). Through this mechanism, the liberated propagules may reach new substrates where they can germinate or can then be further disseminated by other dispersal agents.

Sporobolomyces, *Bullera* and *Tilletiopsis* are common basidiomycete yeast genera, members of which release their spores through ballistosporic discharge. In particular, certain *Sporobolomyces* species can forcibly eject their spores at distances up to 2 mm (Stolze-Rybczynski et al, 2009). Because of this advantageous mechanism, the methodology employed to collect phylloplane yeasts is important and must be considered when quantifying their distribution or when performing comparative studies between different taxa. Certain ballistosporic genera such as *Sporobolomyces*, *Bullera* and *Tilletiopsis* are cosmopolitan phylloplane inhabitants frequently isolated from leaves using the spore-fall method (SFM). This technique inherently selects for ballistosporic fungi, but has limitations when applied to estimating population densities, since only the presence of actively sporulating fungi can be quantified (Pennycook & Newhook, 1978). It follows that other non-actively sporulating fungi that are either reproducing by budding or are in a dormant state may be present on the leaf. In both cases, these fungi may not be detected by the SFM.

1.4 Seasonal Studies

Surveys of yeast biodiversity have been undertaken on every continent and have explored organic, inorganic, living and dead material. Recent large-scale studies have investigated the diversity of entire biomes such as tundras, taigas, boreal, temperate and alpine forests, jungles, deserts, oceans, and even different areas within the stratosphere (Amato, *et al.*, 2007, Masuda, *et al.*, 2008, Peter, *et al.*, 2008, Sudhadham, *et al.*, 2008, James, *et al.*, 2009, Burgaud, *et al.*, 2010, Cadete, *et al.*, 2012, Kachalkin & Yurkov, 2012, Liu, *et al.*, 2012, Sterflinger, *et al.*, 2012). Other surveys have recovered yeasts from industrial wastewater treatment plants, aviation kerosene, fruits ,vegetables and processed foods, mammalian infections and insect digestive tracts (Haley, 1965, Pore & Sorenson, 1990, Suihko & Hoekstra, 1999, Fadda, *et al.*, 2001, Suh, *et al.*, 2008, Janisiewicz, *et al.*, 2010, Buddie, *et al.*, 2011). It is becoming clear that yeasts are cosmopolitan fungi that inhabit a wide variety of niches and can adapt to survive in many different environments.

Studies investigating seasonal distributions have focused mostly on gymnosperms, angiosperms or bryophytes (Last, 1955, Pennycook & Newhook, 1981, Thompson, *et al.*, 1993, de Jager, *et al.*, 2001, Glushakova & Chernov, 2004, Glushakova & Chernov, 2007, Kauserud, *et al.*, 2008, Glushakova & Chernov, 2010, Davey, *et al.*, 2012). The ideas that seasonal dynamics affect the microbial community of the phylloplane and that species density and diversity fluctuate throughout the year have been the subjects of numerous studies (Last, 1955, di Menna, 1959, Flannigan & Campbell, 1977, Pennycook & Newhook,

1981, Glushakova & Chernov, 2004). However, these parameters have not been explored for ferns and yeasts. Additionally, Louisiana has not been investigated thoroughly and little is known about the yeast flora of this area.

Studies have shown that seasonal changes in climate and precipitation affect the density and the types of fungi on the phylloplane. In a study of the seasonal influence on the microflora of apples trees in New Zealand, Pennycook and Newhook (1981) found that yeast numbers increased during spring and summer then declined in autumn. Certain common phylloplane inhabiting fungi such as *Tilletiopsis minor* and *Sporobolomyces roseus* differed in their leaf side distribution and their numbers peaked during different months. The epiphytic yeast flora varied between buds and young leaves compared to developed mature leaves. A succession of species was observed as buds became leaves and airborne spores replaced the bud flora. Non-ballistosporic genera such as *Cryptococcus* were early colonists, eventually replaced by red-pigmented *Rhodotorula* yeasts and ballistosporic species of *Bullera* and *Sporobolomyces* (Pennycook & Newhook, 1981).

A study on *Oxalis acetosella* (wood sorrel) in Russia reported that phylloplane yeast species are not specific to the plant, but are generally dominated by cosmopolitan basidiomycetous epiphytes such as members of *Rhodotorula, Sporobolomyces* and *Cryptococcus* (Glushakova & Chernov, 2004). The authors noted that *Sporidiobolus pararoseus* populations changed only minimally throughout the course of the year while *R. glutinis* was dominant in the winter and *C. laurentii* was most prevalent in the winter. Both Glushakova and Chernov (2003) and Pennycook and Newhook (1981) found that immature young leaves have a lower microbial density of phylloplane yeasts than mature and senescent leaves. This can be attributed to the fact that younger foliage exudes insufficient amounts of nutrients to sustain large populations of microbes (Glushakova & Chernov, 2004).

Maksimova and Chernov (2004) showed that species richness varies among live, senescing and dead plant parts. In a Russian forest ecosystem the greatest numbers of yeasts were found on live parts with significantly fewer colony forming units and less species richness present on senescent, lignified or dead plant matter (Maksimova & Chernov, 2004). Differences between microflora on adaxial and abaxial surfaces have also been previously observed. Seasonal sampling of *Acer platanoides* (maple) and apple leaves showed that epiphytic yeasts were consistently present in higher numbers on adaxial surfaces (Breeze & Dix, 1981, Pennycook & Newhook, 1981).

1.5 Physiological Adaptations

The remarkable potential yeasts have to colonize and thrive in different environments can be attributed, in part, to their ability to metabolize a broad range of chemicals and to withstand extreme conditions. Certain osmophilic species of *Moniliella* and *Candida* have been isolated from flower nectar and honey (Lin, *et al.*, 2010, Misra, *et al.*, 2012). Halophiles, such as *Hortaea werneckii* have been isolated from solar salterns (Turk, *et al.*, 2011) and must also find ways to survive in environments with low water activity. Many different genera of both ascomycete and basidiomycete yeasts are able to survive extreme temperatures. *Candida thermophila*, an ascomycetous soil isolate, is capable of growing at temperatures of 50° C and above (Shin, *et al.*, 2001), while several basidiomycetous genera from the Agaricomycotina and Puccinomycotina lineages have been isolated from glacial and Antarctic environments and are capable of growing at temperatures as low as 2°C (de Garcia, *et al.*, 2007, Thomas-Hall, *et al.*, 2010). These yeasts

have evolved certain adaptations in their cellular membranes that have allowed them to exploit niches that are not available for other organisms. For example, *H. werneckii* is able to survive in hypersaline environments by keeping cellular sodium concentration below a threshold level and by increasing the amount of glycerol within the plasma membrane, thereby maintaining positive turgor pressure inside the cell (Plemenitas, *et al.*, 2008). Some psychrophilic *Cryptococcus* species are thought to preserve the fluidity of their membranes by altering lipid and carotenoid synthesis within the cell (D'Amico, *et al.*, 2006).

Phylloplane yeasts have been implicated in nutrient cycling and host disease control by competition with deleterious microbes (Fonseca & Inácio, 2006). Consequently, many studies have looked at their potential as biocontrols on commercially important fruits and vegetables (Allen, *et al.*, 2004, Chanchalchaovivat, *et al.*, 2007, Janisiewicz, *et al.*, 2010, Lima, *et al.*, 2011, Robiglio, *et al.*, 2011, Sepulveda, *et al.*, 2011).

1.6 Biodiversity

There are approximately 70-100,000 species of fungi described to date, but there is much speculation and ongoing debate as to the total number of extant species (Hawksworth & Rossman, 1997). Many estimates of fungal diversity have been published and the most commonly cited figure speculates that there are 1.5 million species of fungi on the planet (Hawksworth, 1991, Hawksworth, 2001). This hypothesis is based on 270,000 vascular plant species and a fungus to plant ratio of 6:1. However, Hawksworth's estimate may be low, as he himself acknowledged after revisiting his original work (Hawksworth, 2001). Estimates of plant diversity have proposed as many as 400,000 species (http://www.bgci.org), therefore, it is possible that 1.5 million species of fungi is a very conservative figure. Hawksworth's estimate comes from a study of fungi from Great Britain, Ireland and an alpine community and assumes this fungal/ plant ratio is similar in other geographic regions. However, certain studies suggest that fungal diversity in tropical regions is higher than in temperate zones. A survey of six *Licuala* palms in Australia and Brunei found a total of 242 fungal species of saprophytes, endophytes and other morphospecies (mycelia sterilia), estimating that a ratio of 33:1 may be more accurate (Frohlich & Hyde, 1999).

Increased sampling of endophytic fungi may also boost estimates for global diversity. Endophytes are believed to be associated with every living plant (Hawksworth, 1988, Schulz, *et al.*, 1993, Arnold, *et al.*, 2000) and could represent more than a million species by themselves (Dreyfuss & Chapela, 1994). Results from a survey of tropical endophyte hyperdiversity in two species of Panamanian understory trees found nearly 350 genetically distinct species, also suggesting that the 1.5 million estimate is low (Arnold, *et al.*, 2000). The intimate associations formed by fungi and plants necessitate the continued study of their relationships. In a time where ecology, conservational biology and mycology are increasingly intertwined with rapidly-evolving DNA-based technology, it is possible to study the symbiosis of plants and their microflora in great detail. If one considers the possibility that fungi are everywhere, then it is not inconceivable to imagine that they are associated with not only plants and animals but with all other living organisms. In fact, the entire notion of what is living could be called into question since sampling of 'non-living' material such as water, soil and the air frequently recovers spores and other fungal material.

1.7 Yeast Systematics

Modern sequencing techniques and molecular phylogenetics have allowed for the revelation of previously unknown groups of fungi (Porter, *et al.*, 2008, James & Berbee, 2012). A recent study of soil samples of various origins revealed a new group of ascomycetes first identified only from DNA sequences (Porter, *et al.*, 2008). Despite their association with plant root tips throughout different soils, these cosmopolitan fungi are not mycorrhizal, but are actually saprobes (Rosling, *et al.*, 2011). The use of culture independent methods such as next generation sequencing and pyrosequencing to mine ecosystems such as the soil for data about its microflora continues to provide evidence of the vast diversity of fungal life on the planet (Jumpponen, *et al.*, 2010, Klaubauf, *et al.*, 2010, Nishizawa, *et al.*, 2010). Studies such as these exemplify just how much remains to be learned about fungal diversity and reiterates that previously accepted figures may be vast underestimates. With the knowledge of yeast biodiversity still in its infancy, intensive sampling of more ecosystems like the phylloplane will likely continue to reveal new groups of fungi while also filling in gaps for certain groups like Exobasidiomycetes, where many new species probably await discovery.

Since the sexual structures of many yeasts have never been observed, there is a paucity of taxonomically useful characters available for many species. Before the advent of molecular phylogenetics, the yeast discipline of fungal systematics relied on morphological characters, biochemical and physiological data. Although these methods are still employed in characterizing yeasts, DNA-based phylogenetics has redefined fungal taxonomy (Kurtzman, 1998, Sampaio, *et al.*, 2001, Fell & Scorzetti, 2004, Casaregola, *et al.*, 2011, Groenewald, *et al.*, 2011, Mokhtari, *et al.*, 2011). Yeast taxonomy has benefited greatly from developments in sequencing technology due to the difficulties involved in discerning between different anamorphic yeast species. Closely related, but different species may possess the same morphological traits and display similar phenotypic expression. If multiple strains are available, sexual compatibility may be tested, but in the absence of informative characters, comparison of DNA sequences can provide answers to taxonomic questions.

DNA sequence-based phylogenetic analysis of the small and large subunits (SSU, LSU) and the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) has become an integral part of fungal systematics. The SSU and LSU loci have proven to be taxonomically useful as phylogenetic markers at familial and species levels, respectively, due to their slow evolution and because they are relatively easy to amplify using PCR (Casaregola, *et al.*, 2011).

The D1/D2 region of LSU is the barcode region for ascomycete yeasts (Kurtzman & Robnett, 1998). Schoch *et al*., (2012) showed that LSU is easily amplified and is a better locus for resolving species than SSU, partly due to a larger barcode gap, meaning that interspecific variation exceeds intraspecific variation. However, the ITS region is less conserved than both SSU and LSU which makes it a valuable tool for species identification (Nilsson, *et al.*, 2008). In a comparison of potential barcoding loci which included all three rDNA loci as well as the protein-coding gene *RPB*1, it was determined that ITS and RPB1 both outperformed LSU and provided better species resolution. However, the higher PCR amplification rate for ITS resulted in it being proposed as the locus for universal fungal barcoding (Schoch, *et al.*, 2012).

1.8 Objectives

The three main objectives of this study are to:

1) Determine if there are significant differences in the number of yeasts present on the phylloplane at different times of the year, on different surfaces of the leaf and at different physiological stages of leaf development. This involves using analysis of variance and Chi Squares statistical techniques to compare the number of total yeasts recovered during periods of varying temperature and precipitation, and from young and senescing fern leaves, adaxial and abaxial sides of leaves as well as fertile and non-fertile portions of fronds.

2) Characterize the phylloplane yeast flora of seven species ferns by phylogenetic analyses in order to better understand the diversity of the yeast community and to assess species richness. An intensive biweekly sampling regime was chosen in order to generate a robust data set that could be used to analyze fluctuations of the most commonly encountered species throughout the year.

3) Use a combination of molecular, morphological and biochemical means to describe two new species found during the course of this survey.

Chapter 2. A Phylogenetic Analysis of Basidiomycete Yeasts Isolated from Seven Ferns

2.1 Introduction

Yeasts occur in two fungal phyla: Ascomycota and Basidiomycota. Ascomycetous yeasts, which include the model organism *Saccharomyces cerevisiae*, medically important *Candida* species and *Schizosaccharomyces pombe* reproduce by either by budding or fission (Kurtzman & Fell, 2006). Many are often associated with industrial processes such as brewing and baking (Suh, *et al.*, 2006), with insects (Suh, *et al.*, 2005) or are found in areas of high sugar content. On the other hand, basidiomycete yeasts do not typically ferment glucose and are more often associated with plant leaves and soils (Inacio, *et al.*, 2005, Fonseca & Inácio, 2006, Connell, *et al.*, 2008, Cecilia Mestre, *et al.*, 2011, Kurtzman, 2011, Motaung, *et al.*, 2012). They are polyphyletic across three lineages in Basidiomycota: Pucciniomycotina, Agaricomycotina and Ustilaginomycotina. Many of the ustilaginomycetous yeasts are saprobic phases of phytopathogens such as *Sporisorium* and *Exobasidium*. Certain genera within Puccinomycotina and Agaricomyctina are common inhabitants of the phylloplane and have developed spore dispersal mechanisms similar to mushrooms whereby they forcibly eject their spores outward (Boekhout, 1991, Money, 1998).

It is believed that only 1% of all yeast species are known to science (Kurtzman & Fell, 2006). This has been an impetus for mycologists to increase sampling efforts and to expand the ranges of surveys to include previously neglected environments (Butinar, *et al.*, 2005, Raspor & Zupan, 2006). This concerted push toward increased biodiscovery has been facilitated by the development of molecular tools which enable rapid and accurate identification of fungal species from their DNA. Initial methods of characterizing yeasts based on phenotypic attributes were limited by a lack of characters which in some cases led to the lumping of various taxa into species which were later identified through polyphasic taxonomy as species complexes (Fonseca, *et al.*, 2000, Sampaio, *et al.*, 2001).

The relationship between fungi and plants has been well documented in many different regions and it is generally accepted that the tropics represent an area of high fungal biodiversity, much of which still remains to be discovered (Hawksworth & Rossman, 1997, Frohlich & Hyde, 1999, Arnold, *et al.*, 2000, Hawksworth, 2001). The climate of Louisiana consists of hot humid summers and relatively mild winters- conditions which typically promote high fungal diversity. However, studies of the fungi in Louisiana have focused mostly on phytopathogens associated with cash crops such as soybeans, rice and corn (Correll, *et al.*, 2000, Cai & Schneider, 2008, Sweany, *et al.*, 2011), while the yeast flora of the region has remained vastly undocumented. This survey aims to provide the first sketch of the phylloplane yeast community of this region through intensive sampling of seven ferns in Baton Rouge, Louisiana. The following phylogenetic analyses of a large number of yeast isolates provide a detailed picture of some of the different yeasts of this region and where they fit into the larger taxonomic scaffolding of Basidiomycota.

2.2 Methods and Materials

2.2.1 Sample Collection and Yeast Isolation

Seven ferns were sampled twice a month from January to December during 2011 in Baton Rouge, Lousiana. Leaf cuttings of *Cyrtomium falcatum, Dryopteris erythrosora, Lygodium japonicum, Nephrolepis exaltata, Polypodium polypodioides, Rumohra adiantiformis* and *Thelypteris kunthii* were made twice a month during January to December of 2011 (Table 1). *Cyrtomium falcatum, D. erythrosora, N. exaltata, L. japonicum* and *R. adiantiformis* were several of many different species of plants growing in an ornamental plot on the Louisiana State University campus at the corner of Highland Road and Stadium Drive. *P. polypodioides* was collected from the trunk of a live oak near the Life Sciences Building. *Thelypteris kunthii* was collected from an apartment complex on Highland Road several miles away.

Plant material was removed, placed in small sealable plastic bags and processed immediately following collection. Three to five pinnae were randomly chosen from different fronds of the same fern at each collection date. Equal numbers of young and senescent pinnae were collected per each fern. Young pinnae did not exhibit any discoloration, but were light to dark green. Those pinnae classified as senescent exhibited some discoloration and were generally yellowish with some color variation depending on the fern. Pinnae which were brown, dry, diseased or dying were not chosen. Pinnae with sori were collected whenever possible, though certain species were not always fertile.

Pinnae were cut into approximately 1cm leaf sections. Six sections were then randomly chosen and affixed to the lid of a 5cm petri plate using a small amount of petroleum jelly. No plant material was allowed to touch the agar. The media used was either yeast malt agar (YMA): 12g yeast extract, 12g malt extract, 2g peptone, 4g glucose and 8g agar in 400ml of H_20 with 400µl of 50mg/ml chloramphenicol, or potato dextrose agar (PDA): 19.5g potato dextrose agar in 500ml H_20 with 500µl of 50mg/ml chloramphenicol. Two plates were prepared for each fern. The first plate contained 3 abaxial and 3 adaxial young leaf sections. The second plate contained 3 abaxial and 3 adaxial senescing leaf sections. Half of all sections had sori visibly present on the leaf surface for all ferns except *P. polypodioides* and *Rumohra adiantiformis* because fertile pinnae were not consistently found at each sampling date.

Plates were labeled and incubated at room temperature at natural light conditions and monitored daily for the presence of yeast colonies. Whenever a new colony was observed it was picked from the agar using a sterile toothpick and transferred to new media plates, sealed with Parafilm® and incubated at room temperature. A small mark was made on the underneath of the plate to ensure that the colony would not be picked again. All colonies were picked and counted except when ballistosporic discharge was so widespread that individual colonies quickly coalesced into one large colony and could not be distinguished from each other. Subcultures were made as necessary until pure cultures were obtained. Long term storage cultures were prepared by two methods. Cells from each axenic culture were transferred to glass slants containing PDA media and allowed to grow at room temperature for 1 week then stored at 4°C. Additionally, cells were transferred to 2ml cryovial tubes containing 40% glycerol and stored at -80°C.

2.2.2 Polymerase Chain Reaction (PCR) and Sequencing

Colony PCR was performed as described in Aime & Phillips-Mora (2005) with several modifications. Prior to polymerase chain reaction (PCR), a small amount of yeast cells were diluted in 250µl of sterile distilled water and stored at -20°C. One microliter of this suspension was further diluted 1:10 in sterile water and used as the DNA template. PCR's were performed in 25µl reaction mixtures containing Promega 2x Master Mix (Promega Corp., Madison, Wisconsin), 1.25µl of each forward and reverse primer and and 10µl of DNA template.

Amplification of 700-800 bp of the internal transcribed spacer region (ITS) was achieved using the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White, 1990). Additionally, a unique 302 bp segment within the ITS region of *Sporobolomyces pararoseus* strain CBS 484 was amplified with primers CBS484-F (5' GGCGAGCAACTTCGGTTGTGA) and CBS484-R (5' CTAGGCAAACGCCAGCAACGC) (M.C. Aime, pers. comm.). Optimal primer annealing temperature was initially determined by performing a gradient PCR with temperatures ranging from 45°C to 65°C using several isolates of *S. pararoseus* which were previously confirmed through sequencing. Subsequently, PCR using CBS484 primers was performed using an two other isolates confirmed as *S. pararoseus*, an isolate confirmed as *Bullera sinensis*, an isolate confirmed as *Aureobasidium pullulans* and an unknown pink yeast. Only the two S. pararoseus isolates and the unknown pink yeast were amplified and produced a band at approximately 300 bp. The entire ITS region of the unknown pink yeast was later sequenced and was confirmed as *S. pararoseus*. Beginning in April, 2011, all red-pigmented yeasts were screened with these strain-specific primers. Those isolates for which a band was observed at approximately 300 bp were classified as *S. pararoseus* and not sequenced. Cycle parameters for ITS PCR consisted of an initial 7 min denaturation step at 94°C followed by 36 cycles of 94°C for 30 sec, 50°C for 45 sec, 72°C for 45 sec and a final extension step of 72°C for 7 min.

Approximately 1400 bp of the ribosomal large subunit (LSU) was amplified using the primers LROR and LR7 (Vilgalys & Hester, 1990). The cycling program for LSU consisted of an initial 5 min denaturation step at 94°C followed by 35 cycles of 94°C for 30 sec, 50°C for 45 sec, 72°C for 1 min and a final extension step of 72°C for 7 min. PCR products were verified on a 1% agarose gel and sequenced at Beckman Coulter Genomics in Danvers, Massachusetts (http://www.beckmangenomics.com/genomic_services/dna_sequencing.html).

2.2.3 Phylogenetic Analysis

Sequences were manually edited and contiguous sequences were assembled in Sequencher 4.01 (Gene Codes Corp., Ann Arbor, Michigan). Putative species identifications of isolates were made by comparing consensus sequences to other isolates in the NCBI Genbank database using a Blastn search (http://www.ncbi.nlm.nih.gov/). Species identifications were based primarily on ITS sequence identity. Isolates which shared 97% or more ITS sequence identity with Genbank sequences were considered conspecific and any sequence variation of 3% or less was assumed to be intraspecific. Pairwise sequence analyses were performed in Blastn and in Sequencher 4.01 for all isolates. For those sequences which were identical, a single representative of that strain was included in the phylogenetic analyses. Any isolates which displayed sequence variation including single nucleotide polymorphisms or insertions or deletions were treated as different phylotypes and were also included in the phylogenetic analyses.

Multiple sequence alignment of concatenated ITS and LSU sequence supermatrices was constructed in MEGA5 using a MUSCLE algorithm (http://www.megasoftware.net/; Tamura et al. 2011) then edited by eye.

Preliminary phylogenetic analyses were conduced by analyzing ITS sequences of all isolates and related basidiomycete taxa based on previously shown phylogenetic relationships and outgroups were selected based on sister relationships relative to each group (Aime, *et al.*, 2006, Begerow, *et al.*, 2006, Millanes, *et al.*, 2011). Additionally, part of the data set for each tree was derived from taxa which were identified from Blastn queries in the Genbank database. Reference information pertaining to all Genbank sequences used to assemble datasets for each phylogenetic tree is shown in Tables C1-C6 (Appendix C). Gene phylogenies were inferred by maximum likelihood (ML) in RAxML (Stamatakis, 2006) using a GTR model of evolution in the CIPRES Gateway Science portal (Miller *et al*., 2010). Support for the branching topologies was evaluated by bootstrap analysis derived from 1000 replicates with 10 random additions replicated.

2.2.4 Climate Data Collection

Information pertaining to temperature and precipitation was obtained from the National Oceanic and Atmospheric Administration of the National Climatic Data Center (http://www.ncdc.noaa.gov/) and from http://weather-warehouse.com/. Individual monthly values of average temperature and total precipitation are shown in Table B-6 (Appendix B).

2.2.5 Data Analysis

Microsoft Excel was used for data preparation and to create spreadsheets. All statistical analyses were performed in SAS 9.3. Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure. The chi square goodness-of-fit test was used to check if there were any differences between the number of isolates recovered from young and senescent ferns, fertile and non-fertile ferns and from abaxial and and adxial leaf surfaces. A critical significance level of $\alpha = 0.05$ was chosen for all statistical tests.

2.3 Results

In total, 463 yeasts corresponding to the three subphyla of Basidiomycota were recovered from seven ferns during this survey (Table 1; Appendix A). These represent 6 classes, 12 orders and 81 species. Of these species, 29 (36%) cannot be assigned to any previously described yeast species. Three lineages from Pucciniomycotina were represented (Agaricostilbomycetes, Cystobasidiomycetes and Microbotryomycetes) as well as two lineages from Ustilaginomycotina (Ustilaginomycetes and Exobasidiomycetes). One lineage from Agaricomycotina was also represented (Tremellomycetes).

2.3.1 Statistics

2.3.1.1 Chi Squares Goodness of Fit Test

There is a significant difference between the number of total yeast isolates from senescent and young fern leaves ($p<0.0001$) despite the fact that nearly identical numbers of isolates were recovered from each type of leaf (Fig. 2-1). 197 isolates were recovered from senescent leaves and 199 isolates were recovered from young leaves. *Polypodium polypodioides* was excluded from this analysis because it was not possible to consistently assess the physiological condition of the individual pinnae, as they all become shriveled and appear senescent during periods without significant rainfall. The number of total yeast isolates was relatively equal between senescent and young leaves of *D. erythrosora*, *N. exaltata* and *R. adiantiformis*. Approximately twice as many isolates were recovered from senescing leaves of *C. falcatum* and *T. kunthii* while more than four times as many isolates were recovered from young leaves of *L. japonicum* (Fig. 2-1). The number of yeasts recovered from young and senescing leaves is nearly equal among the six fungal classes. However, there is still a statistically significant difference between the total number of yeasts from different classes with respect to leaf age ($p<0.001$) (Table B-1; Appendix B). This is mostly due to discrepancies between observed and expected values for Exobasidiomycetes and Ustilaginomycetes (Fig. 1; Appendix B). More exobasidiomycetous isolates were recovered from young fern leaves as from senescing ones. On the other hand, nearly five times as many ustilaginomycetous isolates were recovered from senescing leaves than from young ones.

More isolates were recovered from abaxial surfaces (54%) than from adaxial ones (46%), though this difference is not significant ($p=0.1$) (Fig. 2-2). However, the relatively low p value suggests that the difference between the number of isolates from abaxial and adaxial surfaces is not entirely negligible. Mostly, this can be attributed to *L. japonicum* where twice as many isolates were recovered from the abaxial side compared to the adaxial side (Fig. 2-2). The difference between the number of isolates recovered from different leaf surfaces among the individual classes is significant ($p<0.0073$) (Table B-2; Appendix B). In general, higher numbers of isolates were recovered from abaxial sides than adaxial ones for all classes except Agaricostilbomycetes and Microbotryomycetes, though the sample size of the former was only 4 isolates. The overall significant difference between the number of abaxial and adaxial isolates can be attributed to Microbotryomycetes and Tremellomycetes. Out of a total of 216 micobotryomycetous isolates, 54% came from adaxial sides of leaves. The observed and expected values for abaxial and adaxial isolates of this group do not coincide which results in a high Chi Square value of approximately 5.6 that represents 35% of the total Chi Square value. In contrast, more than twice as many tremellomycetous isolates were recovered from the abaxial surface than from the adaxial side. The observed and expected values of this group also do not coincide which results in a high Chi Square value of approximately 7.6, representing 48% of the total Chi Square value (Table 2; Appendix B).

The difference between the total number of isolates recovered from leaves with and without sori is significant ($p<0.0001$) (Fig. 2-3). For all ferns analyzed, (*P. polypodioides* and *R. adiantiformis* were excluded since fertile pinnae were not always present during the course of the study) more isolates were recovered from leaves without sori than from fertile leaves with sori On average, about 2.5 times as many isolates were recovered from non-fertile leaves than from those with sori. This was most pronounced for *N. exaltata* and *T. kunthii* where 86% of 63 isolates and 87% of 53 isolates, respectively, came from non-fertile pinnae. There are

discrepancies between observed and expected values for all ferns except *D. erythrosora*. The largest of these occur for *C. falcatum*. There is not a significant difference between the number of isolates from fertile and non-fertile leaves with respect to fungal class ($p<0.3914$) (Table B-3; Appendix B). This can be attributed to similar values for observed and expected values.

2.3.1.2 Analysis of Variance

There are no significant differences in the number of isolates recovered with respect to temperature ($p<0.8$) (Fig. 2-5), precipitation ($p<0.75$) (Fig. 2-6) or fern host ($p<0.81$) (Fig. 2-7). However, two trends were observed. The first shows that there was a decrease in the number of isolates that corresponds to declining average monthly temperatures. 139 total isolates were recovered during June, July and August, the months with the highest average temperature (above 80°F); 122 total isolates were recovered during April, May and September (71-76°F); 115 total isolates were recovered during October, November and March (60-66°F); 88 total isolates were recovered during December, January and February (48-52°F) (Table B-4; Appendix B). A second trend was seen corresponding to an inverse proportion of the number of isolates recovered with respect to precipitation levels. The most isolates were recovered during periods receiving the least amount of precipitation. 132 total isolates were recovered during April, May and October, the months with the lowest precipitation (<1"); 121 total isolates were recovered during December, February and August (2-3"); 106 total isolates were recovered during January, June and July (4.75-6.2"); 104 total isolates were recovered during March, September and November (7-10") (Table B-5; Appendix B). Figure 2-4 shows a graphical representation of the number of yeasts recovered during each month of the survey. On average, 39 yeasts were recovered per month. April was the most abundant month in terms of overall numbers of yeasts collected with 73 while only 17 isolates were recovered during January. Figure 2-7 shows a graphical representation of the total number of isolates recovered from each fern. On average, 66 isolates were recovered per fern. *Cyrtomium falcatum* yielded the most number of isolates while *T. kunthii* yielded the fewest.

2.3.2 Diversity of Yeasts by Class

Agaricostilbomycetes

Four agaricostilbomycetous isolates were recovered. Of these, two came from *P. polypodioides* and one each from from *D. erythrosora* and *N. exaltata*. All isolates were recovered from non-fertile leaves from early February to late April. A ratio of 3:1 was observed both for adaxial versus abaxial surfaces and for young versus senescing leaves.

Cystobasidiomycetes

Cystobasidiomycetes was the second least represented class behind Agaricostilbomycetes. 19 cystobasidiomycetous yeasts were found on all ferns except *N. exaltata*, but 79% of the total number of isolates came from *Dryopteris erythrosora*, *P. polypodioides* and *Lygodium japonicum*, while two or less were found on *C. falcatum*, *R. adiantiformis* and *T. kunthii* (Fig. 2-9). Half of the total number of cystobasidiomycetous isolates were recovered in March and the rest were found throughout the year with no other particular concentration observed.

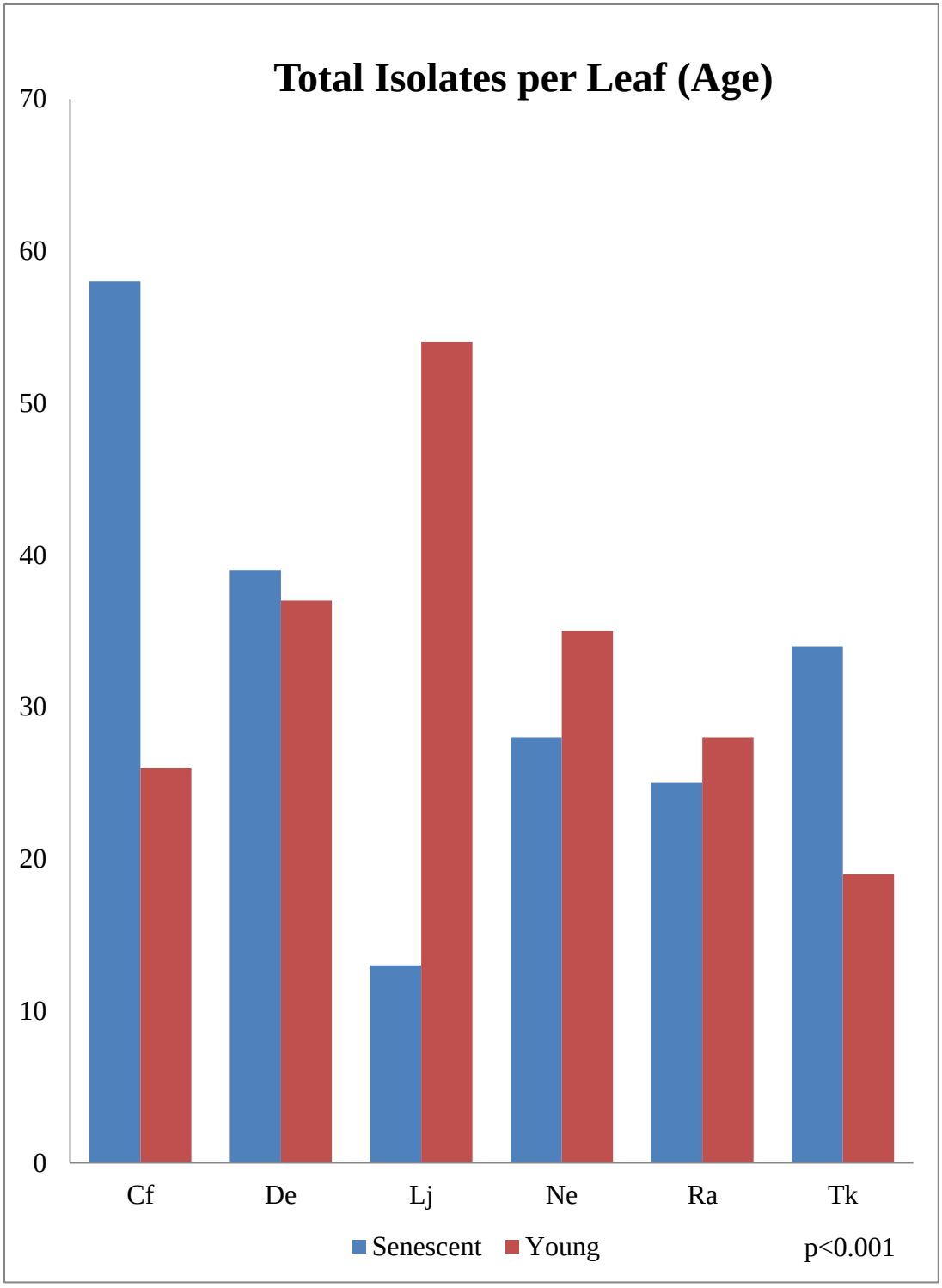


Figure 2-1. Comparison between the total number of yeast isolates recovered from senescent and young fern leaves. X axis denotes fern species; Y axis denotes number of of isolates.

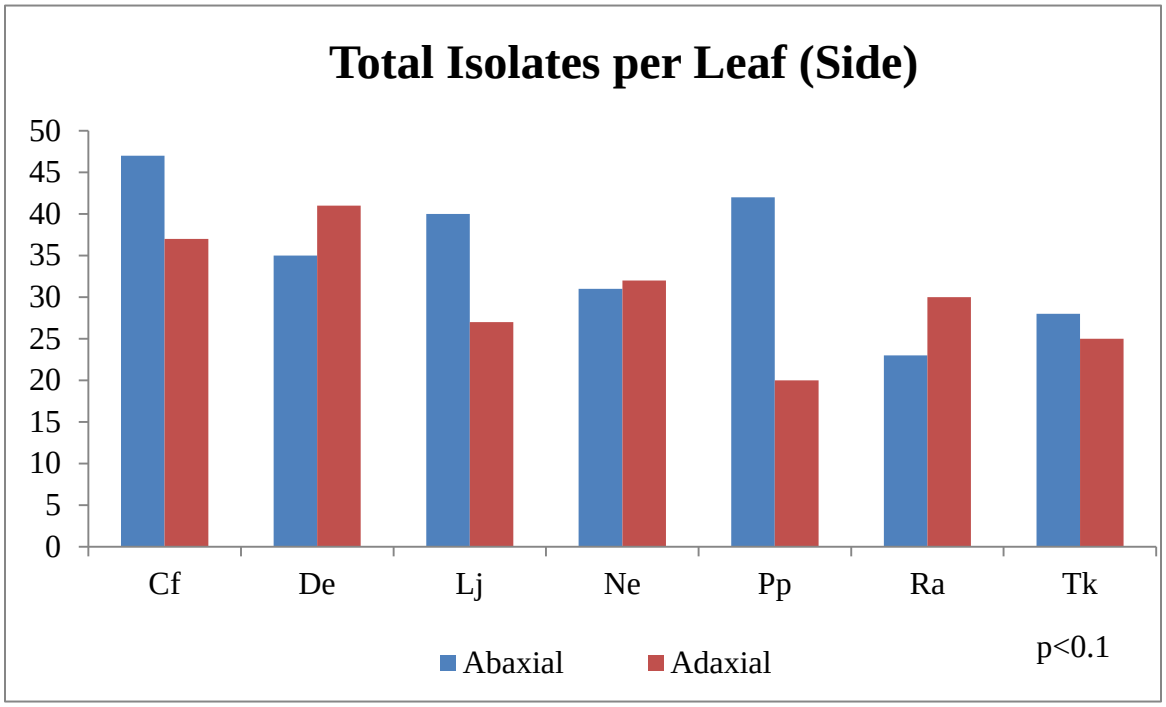


Figure 2-2. Comparison between the total number of yeast isolates recovered from abaxial and adaxial fern leaves. X axis denotes fern species; Y axis denotes number of of isolates.

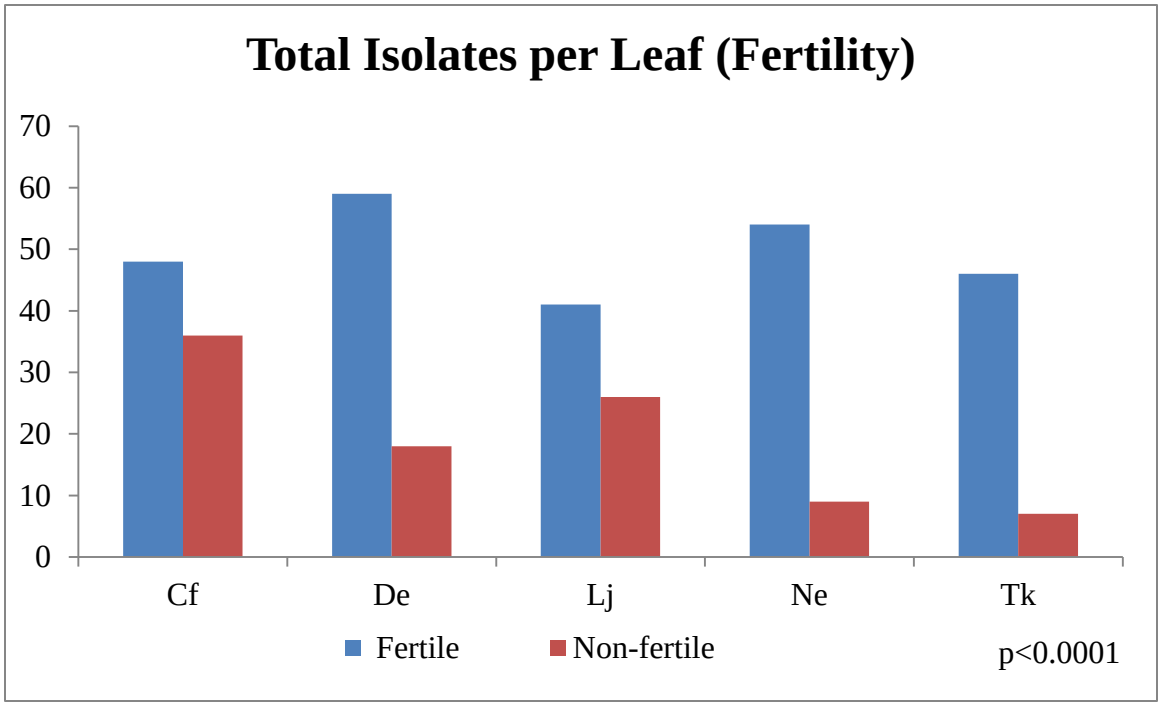


Figure 2-3. Comparison between the total number of yeast isolates recovered from fertile and non-fertile fern leaves. X axis denotes fern species; Y axis denotes number of isolates.

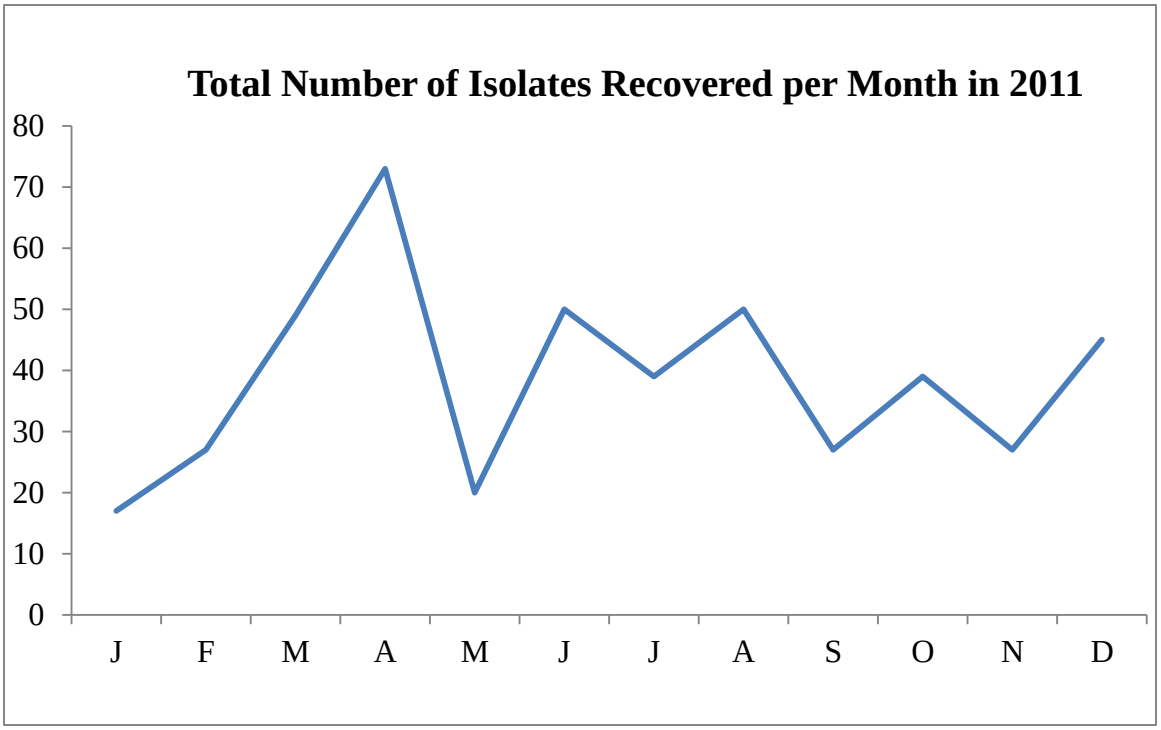


Figure 2-4. Number of isolates recovered during each month of 2011.

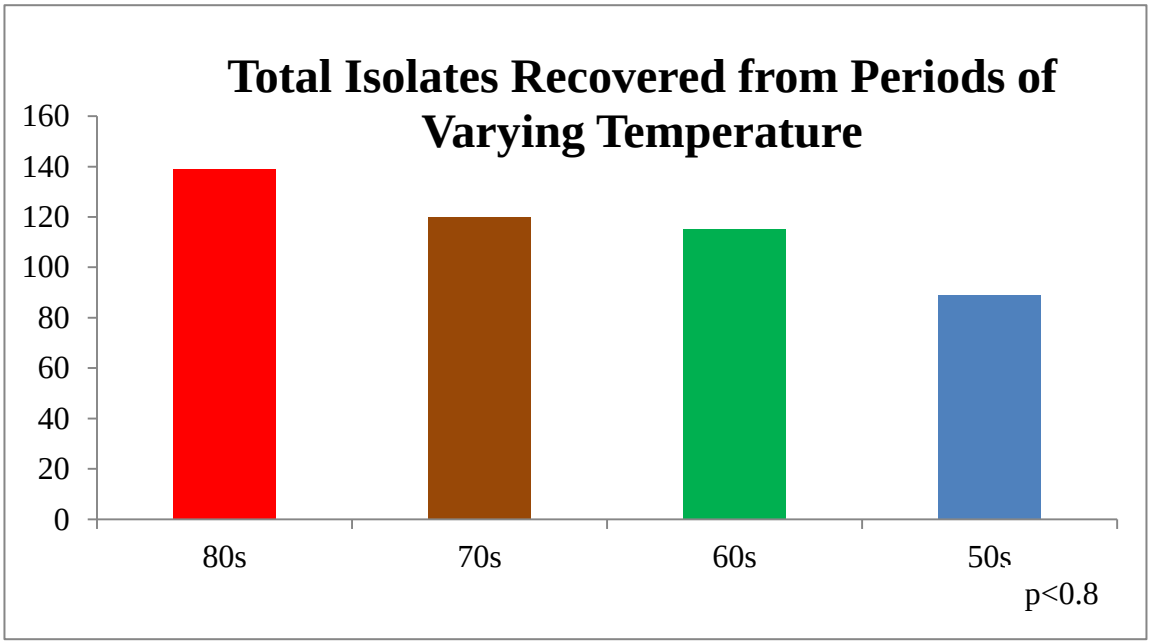


Figure 2-5. Numbers of yeast isolated in terms of temperature (Farenheit). Each column represents three months in which average temperatures were within the range displayed. X axis denote temperature values; Y axis denotes yeast numbers.

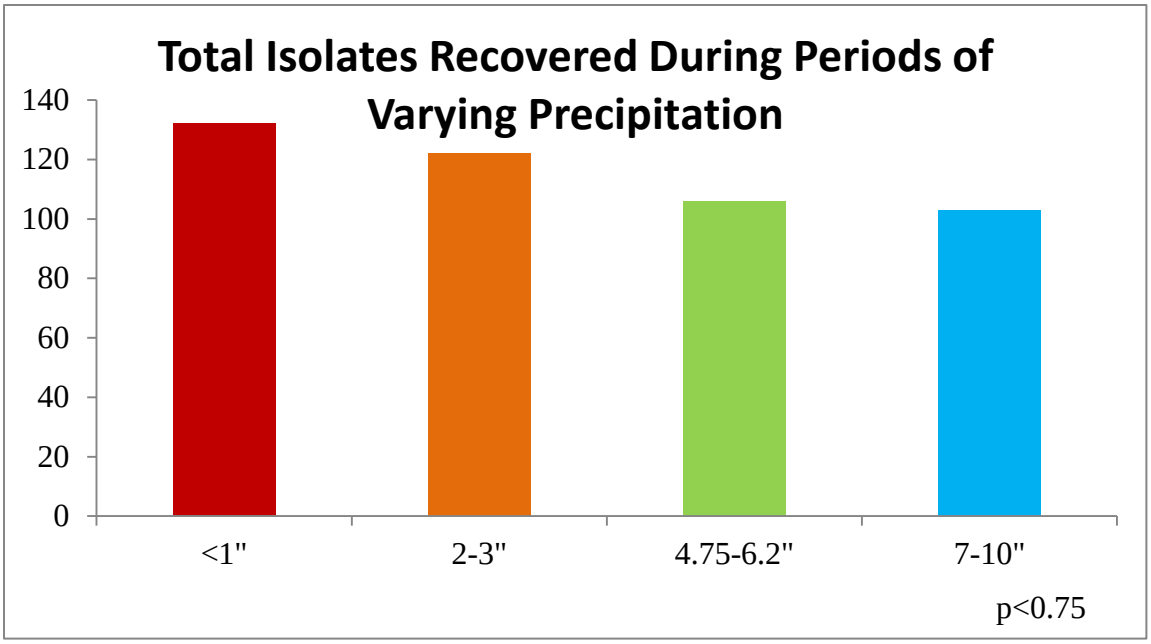


Figure 2-6. Numbers of yeast isolated in terms of precipitation in inches. Each column represents three months in which average precipitation levels were within the range displayed. X axis denote precipitation values ; Y axis denotes yeast numbers.

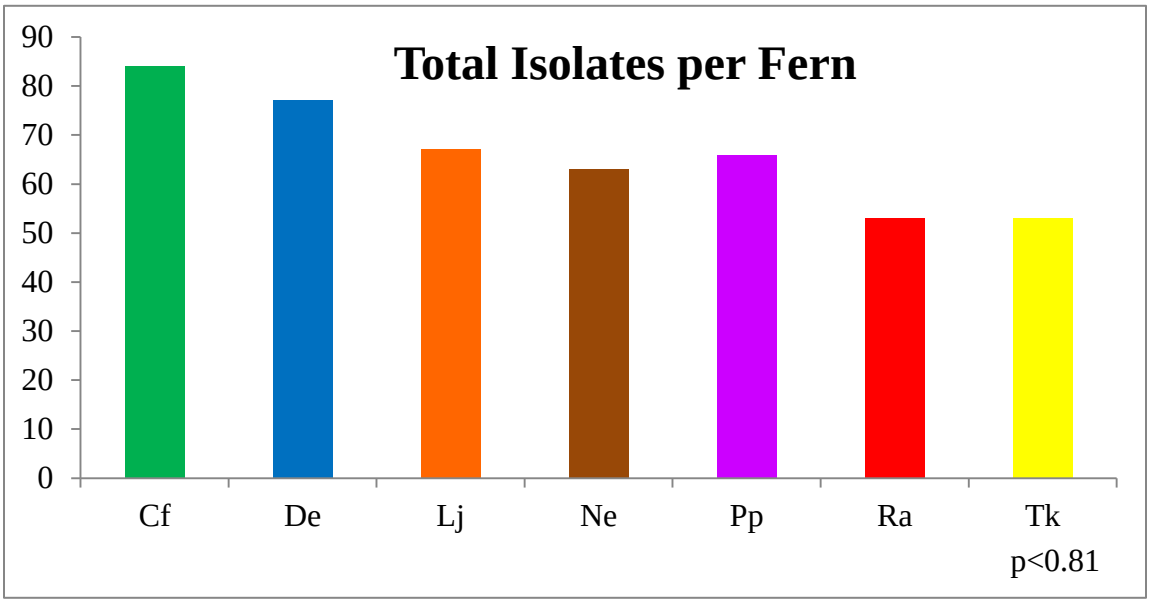


Figure 2-7. Total number of yeast isolates recovered from each fern. Cf-*Cyrtomium falcatum*; De-*Dryopteris erythrosora*; Lj-*Lygodium japonicum*; Ne-*Nephrolepis exaltata*; Pp-*Polypodium polypodioides*; Ra-*Rumohra adiantiformis*; Tk-*Thelypteris kunthii.*

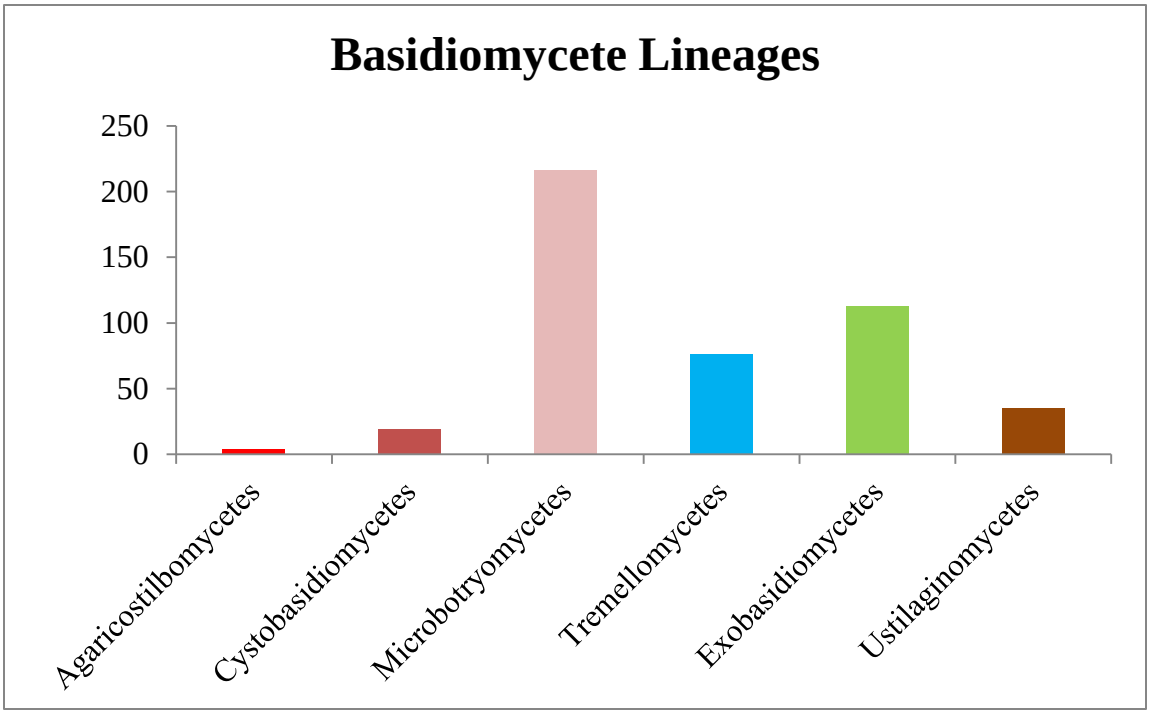


Figure 2-8. Frequency of basidiomycete yeasts by class. X axis denotes fungal lineages in Basidiomycota; Y axis indicates numbers of yeasts.

Leaf surface, fertility and age do not appear to influence yeast distribution for this group (Fig. 2-10), though this may be because of the limited amount of data available. Several phylotypes of *Rhodotorula marina* comprise 42% of all Cystobasidiomycetes isolates.Three putatively new species were recovered in Cystobasidiomycetes out of a total of eight species (Table 2-1). Two of these belong in Cystobasidiales and one in Erythrobasidiales. No isolates from Naohideales were found.

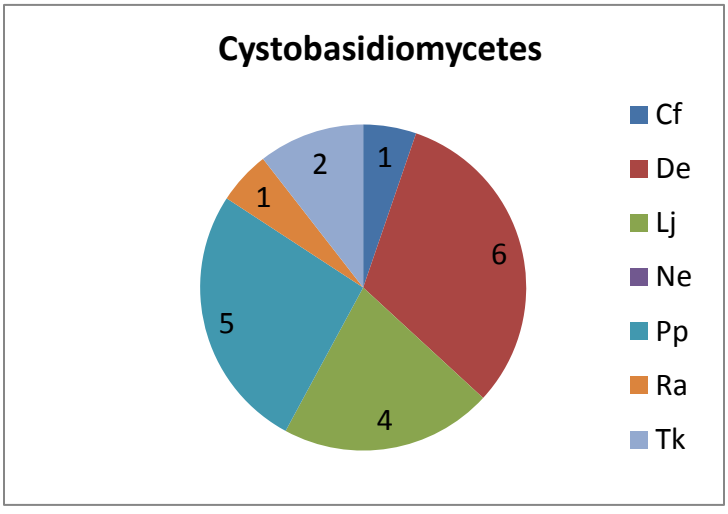


Figure 2-9. Cystobasidiomycetes distribution on 7 different fern species. Numbers indicate total yeasts. collected from each host.

Table 2-1. Cystobasidiomycetes orders and species numbers.

	Cystobasidiomycetes
Orders	2
Total Species	8
Putative sp. nov.	3
% sp. nov.	38%

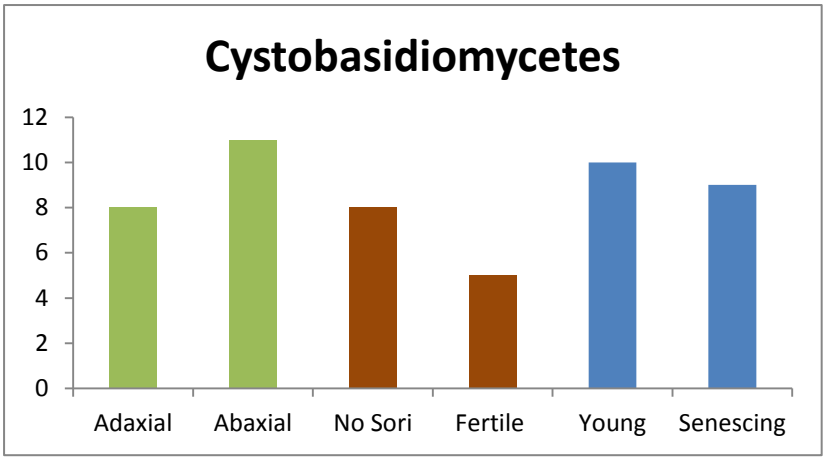


Figure 2-10. Comparison of Cystobasidiomycetes isolated from different fern leaves. X axis denotes different characteristics of host leaves; Y axis indicates numbers of yeasts collected.

Microbotryomycetes

Nearly half (49%) of the total yeasts recovered during this survey belong to Microbotryomycetes, though this mostly due to the high frequency of isolation of *Sporobolomyces pararoseus*. The same strain of *S. pararoseus* was isolated 171 separate times and comprises 79% of Microbotryomycetes and 37% of the total number of isolates. Comparatively, Agaricostilbomycetes and Cystobasidiomycetes were isolated far fewer times and represent less than 1% and 4% of all isolates, respectively. The most microbotryomycetous isolates were found on *Cyrtomium falcatum* and the least on *Polypodium polypodioides* (Fig. 2-11). More yeasts were found on adaxial surfaces than on abaxial ones and on young leaves compared to senescing ones. There is a large difference between the number of isolates recovered from fertile and non-fertile leaves. Non-fertile fronds yielded more than three times as many yeasts than fertile ones (Fig. 2-12). Eight putative new species were recovered out of a total of 14 species (Table 2-2). All isolates belong to Sporidiobolales and are exclusively of the genus *Sporobolomyces* with the exception of two isolates of *Rhodotorula nothofagi*, which are *incertae sedis*.

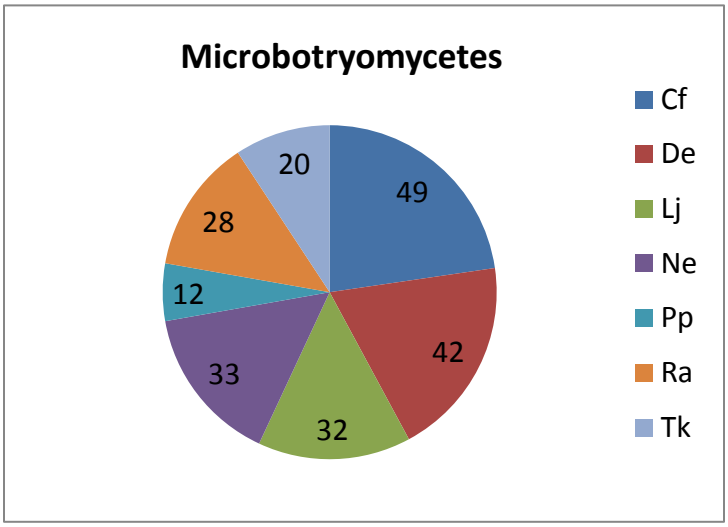


Figure 2-11. Microbotryomycetes distribution on 7 different fern species. Numbers indicate total yeasts collected from each host.

Table 2-2. Microbotryomycetes orders and species numbers.

	Microbotryomycetes
Orders	1
Total Species	14
Putative sp. nov.	8
% sp. nov.	57%

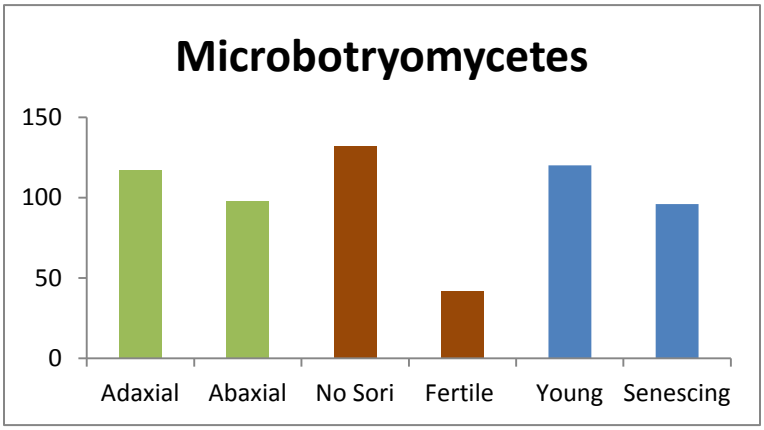


Figure 2-12. Comparison of Microbotryomycetes isolated from different fern leaves. X axis denotes different characteristics of host leaves; Y axis indicates numbers of yeasts collected.

Tremellomycetes

Tremellomycetes is the third most represented class with 76 isolates which comprise 16% of the total yeast isolates. This group is dominated by Tremellales taxa, which comprise 80% of the total Tremellomycetes isolates. Some of theTremellales isolates correspond to some commonly encountered phylloplane yeast genera such as *Bullera*, *Bulleromyces*, *Cryptococcus*, *Derxomyces*, *Dioszegia* and *Hannaella*. Several *Udeniomyces* species belonging to Cystofilobasidiales were also collected (18% of all Tremellomycetes isolates). *Bulleromyces albus* was most the commonly recovered tremellomycete, representing 17% of all tremellomycetes. *Cryptococcus terreus* was the only filobasidiaceous isolate recovered. 57% of the total amount of tremellomycetes isolates came from *P. polypodioides* and *C. falcatum* (Fig. 2-13). More tremellomycetous isolates were found on the abaxial surfaces of non-fertile, young fronds (Fig. 2-14). Four putatively new species were identified out of a total of 23 species (Table 2-3).

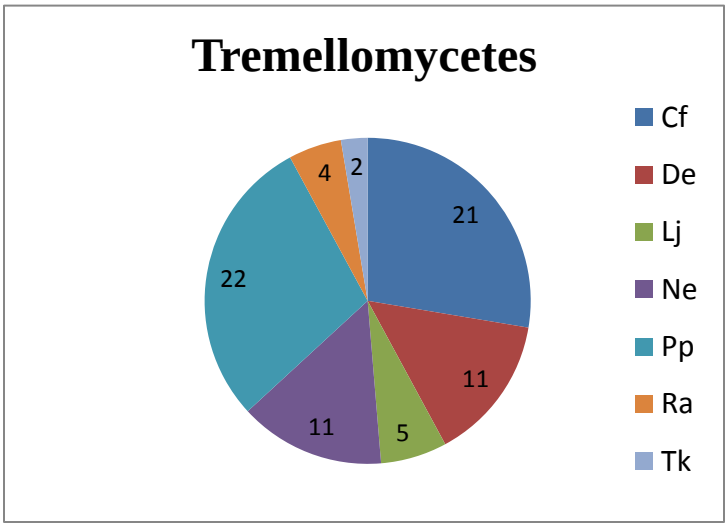


Figure 2-13. Tremellomycetes distribution on 7 different fern species. Numbers indicate total yeasts collected from each host.

Table 2-3. Tremellomycetes orders and species numbers.

	Tremellomycetes
Orders	3
Total species	23
Putative sp. nov.	4
% sp. nov.	17%

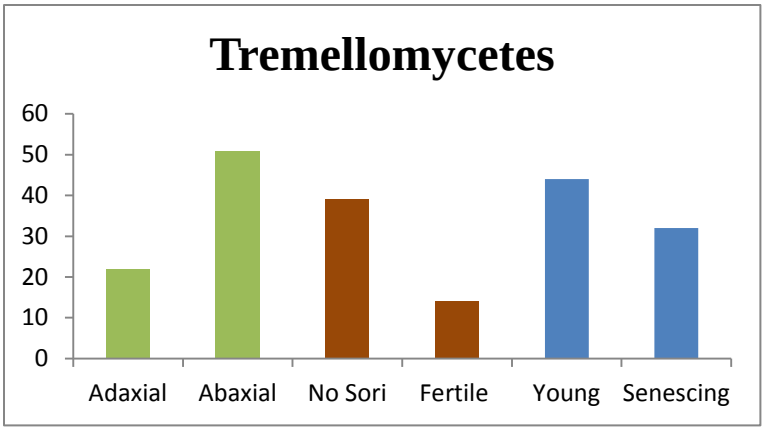


Fig. 2-14. Comparison of Tremellomycetes isolated from different fern leaves. X axis denotes different characteristics of host leaves; Y axis indicates numbers of yeasts collected.

Exobasidiomycetes

113 isolates belonging to Exobasidiomycetes were recovered, representing 24% of the total yeast isolates. Clear dominance of a single species was not observed as with *S. pararoseus*, though *Tilletiopsis lilacina* was the most common exobasidiomycete, representing 27% of all Exobasidiomycetes isolates recovered and 7% of the total yeast isolates recovered during this survey. Most isolates were recovered during the spring, especially during the month of April. The *T. lilacina* isolates were ubiquitous across their host range and were recovered from all seven ferns (Fig. 2-15). There is no indication of an Exobasidiomycete hot spot though *P. polypodioides* and *L. japonicum* yielded the most isolates and *C. falcatum*, the fewest. The abaxial sides of young, non-fertile ferns yielded the highest numbers of isolates (Fig. 2-16). Nine putatively new species were recovered in Exobasidiomycetes out of a total of 22 species spanning four orders. This is the highest number of unidentified taxa for any class in this survey and constitutes 39% of all the Exobasidiomycetes taxa recovered (Table 2-4).

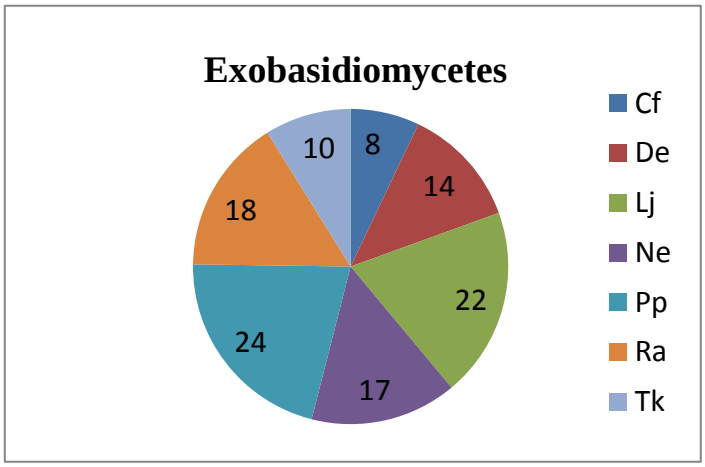


Figure 2-15. Exobasidiomycetes distribution on 7 different fern species. Numbers indicate total yeasts collected from each host.

Table 2-4. Exobasidiomycetes orders and species numbers.

	Exobasidiomycetes
Orders	4
Total Species	23
Putative sp. nov.	9
% sp. nov.	39%

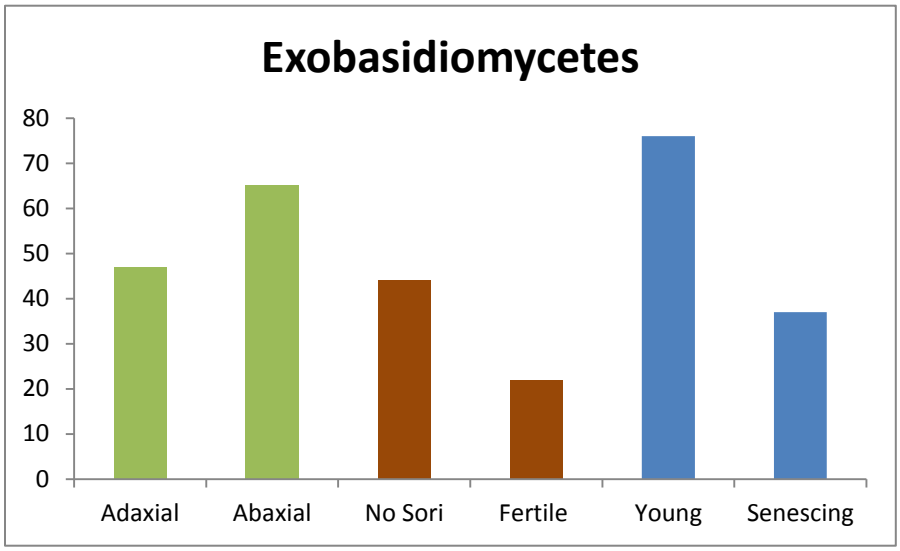


Fig. 2-16. Comparison of Exobasidiomycetes isolated from different fern leaves. X axis denotes different characteristics of host leaves; Y axis indicates numbers of yeasts collected.

Ustilaginomycetes

Eight percent of the total number of isolates belong to Ustilaginomycetes. This group is dominated by several different *Pseudozyma* species, which represent 77% of all ustilaginomycetous isolates. Of these, *Pseudozyma aphidis* was most common taxon representing 63% of the *Pseudozyma* isolates and 49% of all Ustilaginomycetes. *Farysizyma* and *Sporisorium* were the only other two genera encountered and each was isolated four times. More than half (54%) of all ustilaginomycetous isolates came from *T. kunthii* (Fig. 2-17), the majority of which were recovered from the abaxial surfaces of non-fertile, senescing fronds (Fig. 2-18). Two putatively new species were recovered out of a total of nine species (Table 2-5). All isolates belong to either Ustilaginaceae or Anthracoideaceae in the order Ustilaginales. No isolates from Urocystales were recovered.

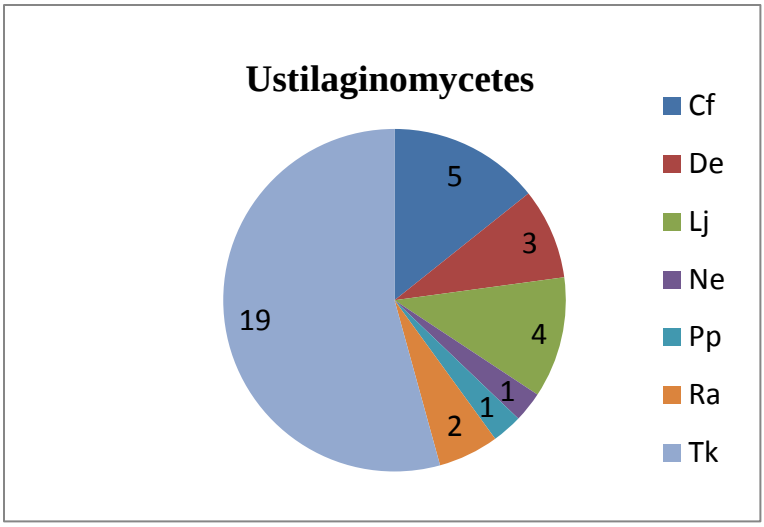


Figure 2-17. Ustilaginomycetes distribution on 7 different fern species. Numbers indicate total yeasts collected from each host.

Table 2-5. Ustilaginomycetes orders and species numbers.

	Ustilaginomycetes
Orders	1
Total Species	9
Putative sp. nov.	2
% sp. nov.	22%

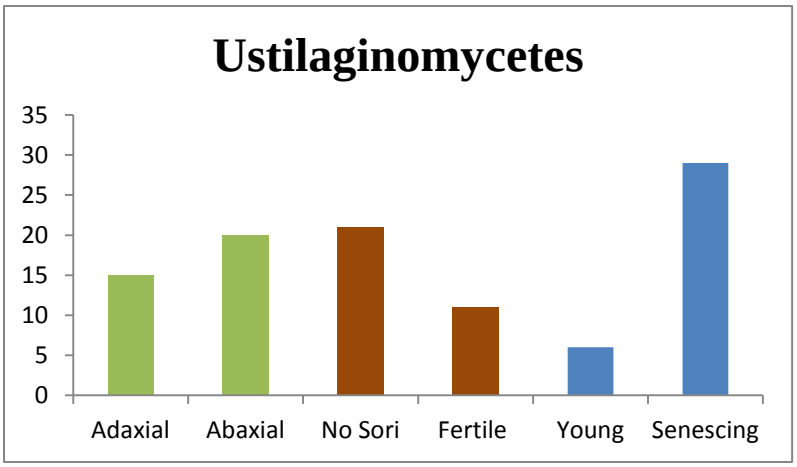


Fig. 2-18. Comparison of Ustilaginomycetes isolated from different fern leaves. X axis denotes different characteristics of host leaves; Y axis indicates numbers of yeasts collected.

2.3.3 Phylogenetic Analyses

Legend for Phylogenetic Trees

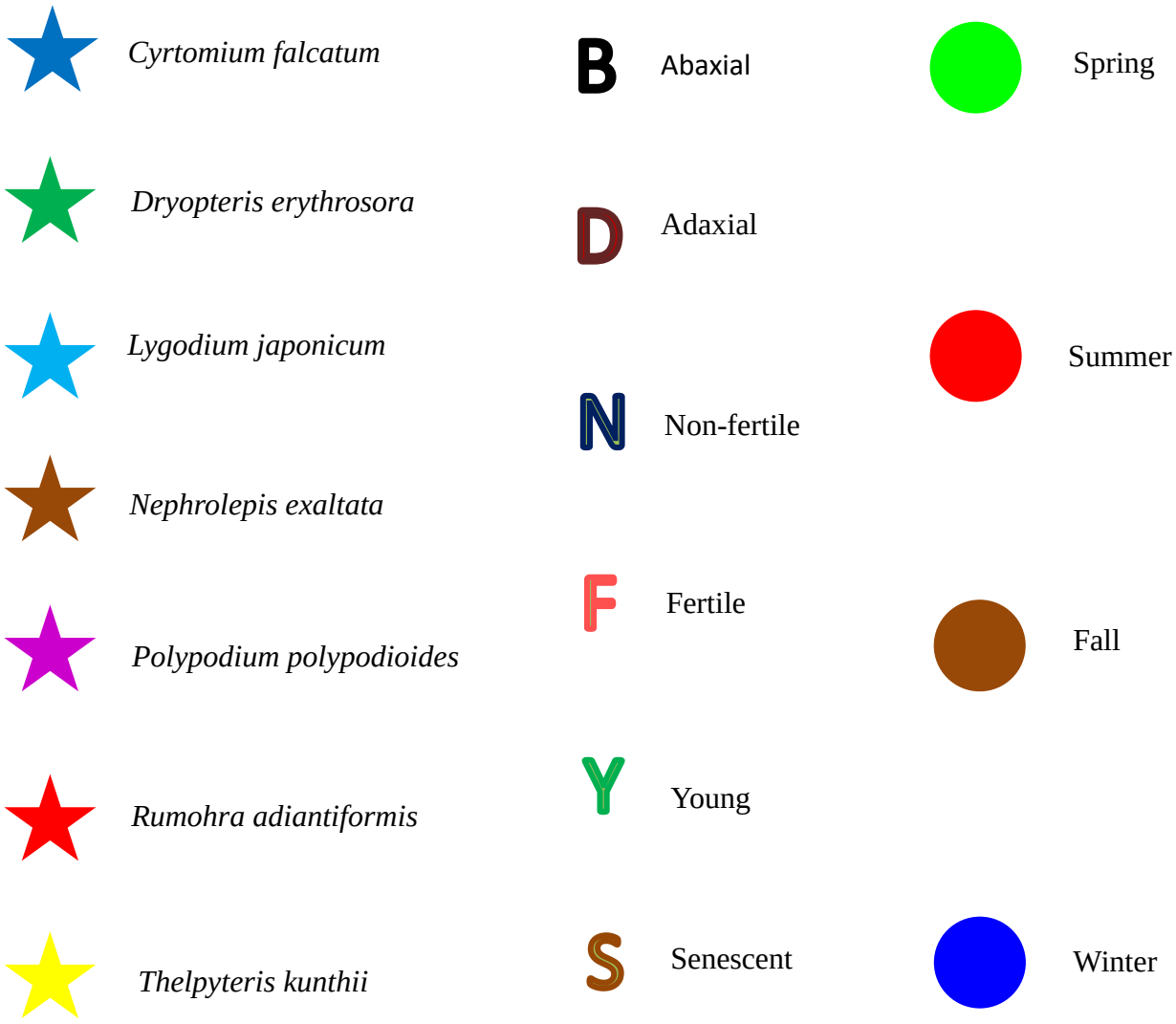


Figure 2-19. Legend depicting the labels used for phylogenetic trees. Each SA isolate represents a unique phyllotype on the following phylogenetic trees. Different colored stars refer to the different fern hosts. Different colored letters indicate the different physiological conditions and spatial orientation of the fern leaves from which isolates were recovered. Different colored circles represent the different seasons when isolates were collected. Spring- March, April and May; Summer- June , July and August; Fall-September, October and November; Winter- December, January and February. Isolates labeled with SA prefix were all recovered during this survey.

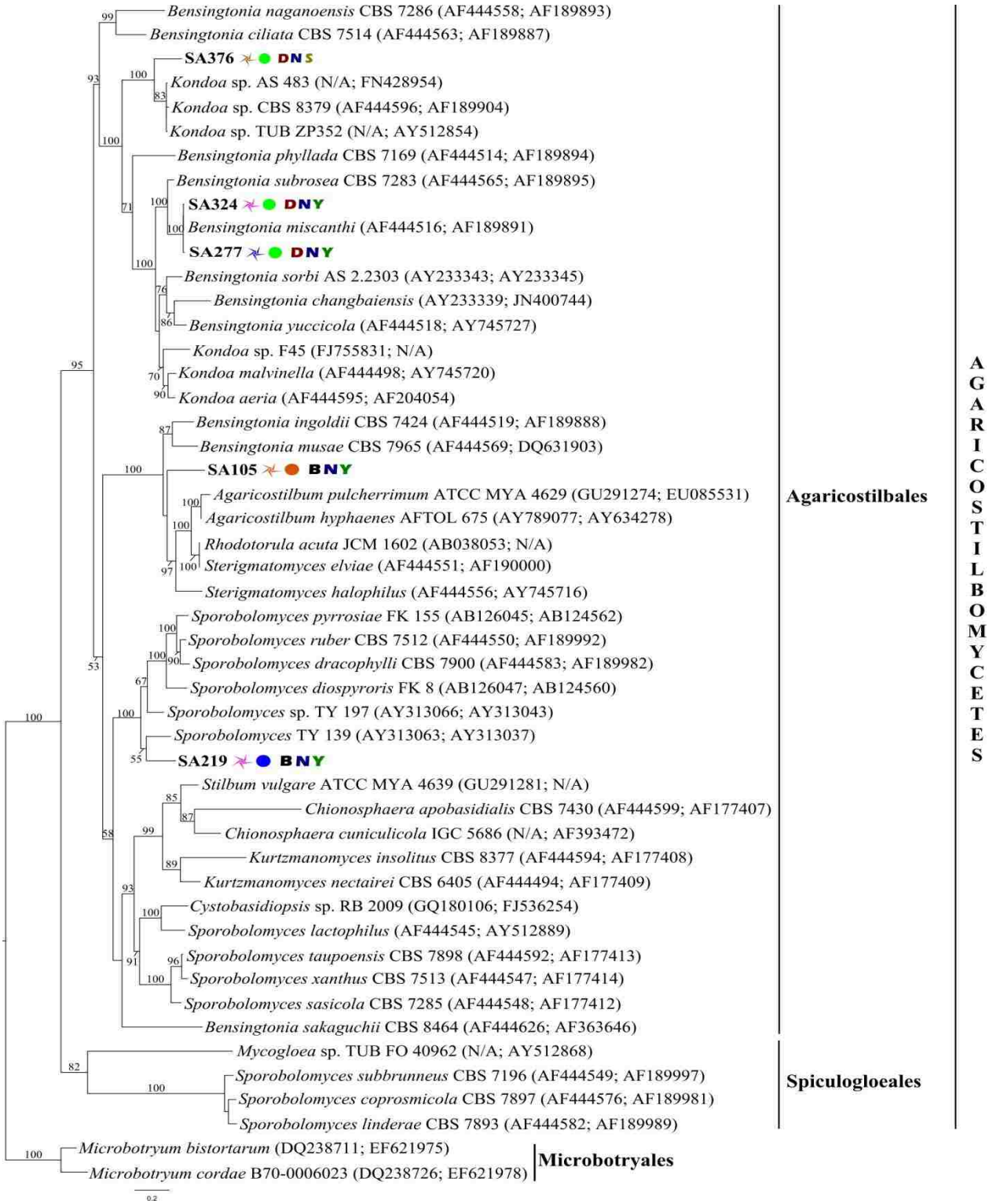


Figure 2-20. Phylogenetic relationships in Agaricostilbomycetes based on analyses of concatenated ITS and LSU sequences. ITS Genbank accession numbers are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Attractiellales was used as an outgroup.

Agaricostilbomycetes

Phylogenetic analysis of the ITS and LSU regions show that five isolates are broadly distributed across Agaricostilbales (Agaricostilbomycetes) (Fig. 2-19). Three of these belong to a clade of *Kondoa* and *Bensingtonia* species. SA376 shares 97% LSU sequence identity with three undescribed *Kondoa* isolates and appears to represent a new species in this group (Table 2-6). In this phylogeny *Kondoa* is paraphyletic and contains several species of *Bensingtonia*. SA376 is phylogenetically much more distant from *K. aeria* and the type species, *K. malvinella* than to the undescribed *Kondoa* isolates and cannot be called *Kondoa*, but will need a new generic name.

Table 2-6. Pairwise analysis of LSU sequence similarities between SA376 and related taxa. Numbers indicate percent similarity between isolates. K sp-*Kondoa* species.

	SA376	**K sp CBS 8379**	**K sp AS 483**	**K sp ZP 352**
SA376	-	97	97	97
K sp CBS 8379	97	-	99	99
K sp AS 483	97	99	-	99
K sp ZP 352	97	99	99	-

SA277 and SA324 are part of this *Kondoa*/*Bensingtonia* clade. In this phylogeny *Bensingtonia* (type species is *B. ciliata*) is also parphyletic and occurs throughout Agaricostilbales. SA277 and SA324 differ by four bases in the ITS region and also share 99% ITS sequence identity with *B. miscanthi* strain CBS 7282. In the LSU region, SA277 and *B. miscanthi* differ by one base, indicating that both SA277 and SA324 are phylotypes of *B. miscanthii*.

Table 2-7. Pairwise analysis of ITS sequence similarities between SA277 and related taxa. Numbers indicate percent similarity between isolates. Bm-*Bensingtonia miscanthi*.

	SA277	**SA324**	**Bm CBS 7282**
SA277	-	99	99
SA324	99	-	99
Bm CBS 7282	99	99	-

.

SA105 and SA219 are supported as part of Agaricostilbales, but neither can be placed in any known genus based on these results. SA105 is part of a group that contains *Agaricostilbum hyphanaes, A. pulcherrimum, Sterigmatomyces elviae, S. halophilus* and *Bensingtonia musae*, but cannot be ascribed to any species of this clade. SA105, *A. hyphanaes* and *S. elviae* share 82% ITS sequence identity (Table 2-8).

Table 2-8. Pairwise analysis of ITS sequence similarities between SA105 and related taxa. Numbers indicate percent similarity between isolates. Ah-*Agaricostilbum hyphanaes*; Ap-*Agaricostilbum pulcherrimum*; Se-*Sterigmatomyces elviae*; Sh-*Sterigmatomyces halophilus*; Bm-*Bensingtonia musae.*

	SA105	**Ah AFTOL 675**	**Ap ATCC MYA 4629**	**Se CBS 5922**	**Sh CBS 4609**	**Bm CBS 7965**
SA105	-	82	86	82	80	82
Ah AFTOL 675	82	-	99	93	84	83

Table 2-8 cont.

	SA105	Ah AFTOL 675	Ap ATCC MYA 4629	Se CBS 5922	Sh CBS 4609	Bm CBS 7965
Ap ATCC MYA 4629	86	99	-	95	86	85
Se CBS 5922	82	93	95	-	85	84
Sh CBS 4609	80	84	86	85	-	81
Bm CBS 7965	82	83	85	84	81	-

SA219 is part of a well-supported clade that includes several undescribed *Sporobolomyces* isolates, but it resides on a separate branch and is distinct from other related taxa. In the ITS and LSU regions SA219 looks like a new genus as it shares below 86% and 94% sequence identity values respectively, with its closest *Sporobolomyces* relatives (See Tables 2-9 and 2-10). The generic names of this Sporobolomyces clade will need to re-evaluated, since the type species *Sporobolomyces salmonicolor* species occurs in Sporidiobolales.

Table 2-9. Pairwise analysis of ITS sequence similarities between SA219 and related taxa. Numbers indicate percent similarity between isolates. S di-*Sporobolomyces diospyroris*; S dr-*Sporobolomyces dracophylli*; Sp *Sporobolomyces pyrrosiae*; Sr-*Sporobolomyces ruber*; S sp-*Sporobolomyces* species.

	SA 219	S di FK 8	S dr CBS 7900	Sp FK 155	Sr CBS 7512	S sp TY 139	S sp TY 197	S sp TY 223
SA219	-	82	82	84	81	83	86	83
S di FK 8	82	-	88	97	89	82	84	83
S dr CBS 7900	82	88	-	93	85	82	85	83
Sp FK 155	84	97	93	-	95	82	87	84
Sr CBS 7512	81	89	85	95	-	82	85	83
S sp TY 139	83	82	82	82	82	-	85	84
S sp TY 197	86	84	85	87	85	85	-	87
S sp TY 223	83	83	83	84	83	84	87	-

Table 2-10. Pairwise analysis of LSU sequence similarities between SA219 and related taxa. Numbers indicate percent similarity between isolates. S di-*Sporobolomyces diospyroris*; S dr-*Sporobolomyces dracophylli*; Sp *Sporobolomyces pyrrosiae*; Sr-*Sporobolomyces ruber*; S sp-*Sporobolomyces* species.

	SA 219	S di FK 8	S dr CBS 7900	Sp FK 155	Sr CBS 7512	S sp TY 139	S sp TY 197	S sp TY 223
SA219	-	90	89	89	90	94	92	85
S di FK 8	90	-	94	96	95	91	92	86
S dr CBS 7900	89	94	-	97	97	90	89	85
Sp FK 155	89	96	97	-	98	91	90	85
Sr CBS 7512	90	95	97	98	-	91	90	85

Table 2-10 cont.

	SA 219	**S di FK 8**	**S dr CBS 7900**	**Sp FK 155**	**Sr CBS 7512**	**S sp TY 139**	**S sp TY 197**	**S sp TY 223**
S sp TY 139	94	91	90	91	91	-	94	85
S sp TY 197	92	92	89	90	90	94	-	85
S sp TY 223	855	86	85	85	85	85	85	-

Cystobasidiomycetes

Twenty-one yeasts belonging to Cystobasidiomycetes were recovered. All of these isolates belong either to Cystobasidiales or Erythrobasidiales, with the majority placed in the latter. No isolates were recovered from Naohideales (Bauer, *et al.*, 2006).

SA279 is part of a small, well-supported clade that includes several one *Rhodotorula* and several *Sporobolomyces* species. Within this group SA279 shares the most ITS sequence identity with *R. armeniaca* strain CBS 8076, a phylloplane associated, orange pigment producing, non-ballistosporic species first isolated from *Callistemon viminalis* (Shivas & Rodrigues de Miranda, 1983) and *Sporobolomyces phyllomatis* strain CBS 7198. SA279 differs from *R. armeniaca* strain CBS 8076 and *Sporobolomyces phyllomatis* strain CBS 7198 by 18 and 26 bases in the ITS region, respectively.

Table 2-11. Pairwise analysis of ITS sequence similarities between SA279 and related taxa. Numbers indicate percent similarity between isolates. Ra-*Rhodotorula armeniaca*; Sp-*Sporobolomyces phyllomatis*; Ss- *Sporobolomyces salicinus*; Sk- *Sporobolomyces kluyveri-neilii*.

	SA279	**Ra CBS 8076**	**Sp CBS 7198**	**Ss CBS 6983**	**Sk CBS 7168**
SA279	-	97	96	91	91
Ra CBS 8076	97	-	95	91	91
Sp CBS 7198	96	95	-	91	92
Ss CBS 6983	91	91	91	-	91
Sk CBS 7168	91	91	92	91	-

A 219 bp portion of the ITS region of SA444was sequenced and used for phylogenetic analysis, however, this was not compared to other isolates by pairwise analysis. SA444 shares 99% LSU sequence identity with several undescribed *Rhodotorula* isolates and differs by four bases from *Rhodotorula* sp. BI218, and by five bases from *Rhodotorula* sp. 5-19 (Table 2-12), a strain isolated from a solar saltern in Korea (Shin, unpublished; Shin *et al*., unpublished). Though these three isolates share high LSU sequence identity, they are less similar to other Cystobasidiales. They clearly belong in Cystobasidiales, but based on this analysis it is not possible to assign any of them a species or generic identification.

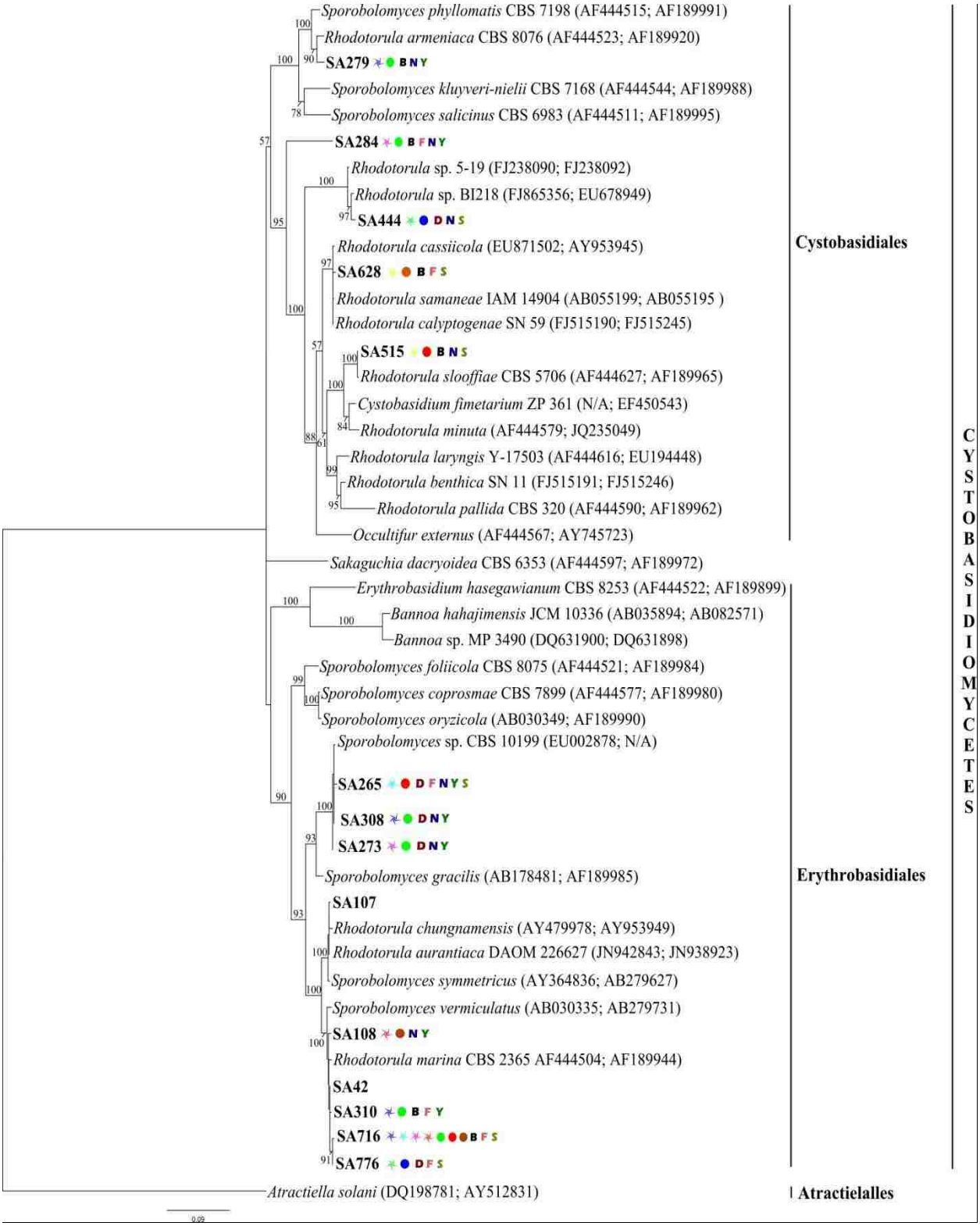


Figure 2-21. Phylogenetic relationships in Cystobasidiomycetes based on analyses of concatenated ITS and LSU sequences. ITS Genbank accession numbers are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A in parentheses. Bootstrap values above 50% are indicated. Attractiellales was used as an outgroup.

Table 2-12. Pairwise analysis of the LSU sequences of SA444 and related taxa. Numbers indicate percent similarity between isolates. R sp-*Rhodotorula* species.

	SA444	**R sp BI218**	**R sp 5-19**
SA444	-	99	99
R sp BI218	99	-	99
R sp 5-19	99	99	-

Only the the ITS region of SA628 was sequenced and the subsequent phylogenetic analyses show that it is part of a second cluster of *Rhodotorula* species (R2) including *R. cassiicola*, *R. samaneae* and *R. calyptogenae*. There is very limited inter-specific sequence variability among these taxa, especially between *R. cassiicola* and *R. calyptogenae* which differ by one base in ITS and three bases in the LSU region.
Sequence identity between SA628 and *R. minuta* isolates ranges from 93-100%. SA628 shares only 95% sequence identity with *Rhodotorula minuta* strain CBS 4408 in the ITS region, but shares 99-100% sequence identity with *R. minuta* strains SY 86, SY 87 and S 22825.
R. minuta strain CBS 4408 is part of a clade of several other *Rhodotorula* species, but it was the only isolate chosen to represent the species in this phylogenetic analysis. Judging by the nuclear rDNA sequence variation among several other different *R. minuta* isolates, it was seen that intraspecific variation within the ITS region of *R. minuta* isolates is quite high, with as much as 7% mismatches between conspecific isolates.

Table 2-13. Pairwise sequence analysis of the ITS region of SA628 and related taxa (R2 group). Numbers indicate percent similarity between isolates. R cal-*Rhodotorula calyptogenae*; R cas-*Rhodotorula cassiicola*; Rs-*Rhodotorula samanae*; Rm-*Rhodotorula minuta*.

	SA628	**R cal SN 59**	**R cas S22843**	**Rs 14904**	**Rm CBS 4408**
SA628	-	99	99	98	95
R cal SN 59	99	-	99	98	95
R cas S22843	99	99	-	98	95
Rs 14904	98	98	98	-	94
Rm CBS 4408	95	95	95	94	-

Table 2-14. Pairwise sequence analysis of the LSU regionof four related *Rhodotorula* species in Cystobasidiales (R2 group). Numbers indicate percent similarity between isolates. R cal-*Rhodotorula calyptogenae*; R cas-*Rhodotorula cassiicola*; Rs-*Rhodotorula samanae*; Rm-*Rhodotorula minuta*.

	R cal SN 59	**R cas SJ 007**	**R sam IAM 14904**	**Rm P-7**
R cal SN 59	-	99	99	96
R cas SJ 007	99	-	99	95
R sam IAM 14904	99	99	-	95
Rm P-7	96	95	95	-

Most cystobasidiomycetous isolates could be identified at least to the generic level, but SA284 could not be definitively placed in a known genus. A pairwise sequence analysis between SA284 and five Cystobasidiales species displayed low sequence identity values in both the ITS and LSU regions (Tables 2-15 and 2-16). Most of the closely related taxa are undescribed

Rhodotorula species and also *Occultifur externus* strain CBS 8732. There is strong support that SA284 belongs within Cystobasidiales, but its generic-level taxonomic position remains to be determined.

Table 2-15. Pairwise analysis of the ITS region of SA284 and four related *Rhodotorula* species in Cystobasidiales. Numbers indicate percent similarity between isolates. Rh sp-*Rhodotorula* species; Oe-*Occultifur externus*.

	SA284	**Rh sp 5-19**	**Rh sp BI128**	**Rh sp YM24636**	**Rh sp LH227**	**Oe CBS 8732**
SA284	-	91	92	91	90	89
Rh sp 5-19	91	-	100	99	92	92
Rh sp BI128	92	100	-	97	91	91
Rh sp YM24636	91	99	97	-	92	92
Rh sp LH227	90	92	91	92	-	93
Oe CBS 8732	89	92	91	92	93	-

Table 2-16. Pairwise analysis of the LSU region of SA284 and four related Rhodotorula species in Cystobasidiales. Numbers indicate percent similarity between isolates. Rh sp-*Rhodotorula* species; Oe-*Occultifur externus*.

	SA284	**Rh sp 5-19**	**Rh sp BI128**	**Rh sp YM 24636**	**Rh sp CBS 319**	**Oe AFTOL 860**
SA284	-	91	92	91	92	93
Rh sp 5-19	91	-	99	98	93	91
Rh sp BI128	92	99	-	98	92	92
Rh sp YM 24636	91	98	98	-	92	91
Rh sp CBS 319	92	93	92	92	-	93
Oe AFTOL 860	93	91	92	91	93	-

The majority of the Erythrobasidiales found in this survey belong to two groups represented by several *Sporobolomyces* and *Rhodotorula* species. *Bannoa* and *Erythrobasidium* are supported as sister to these two clades.

Five isolates are part of a well-supported group that includes *Sporobolomyces* sp. strain CBS 10199 and *S. gracilis* strain JCM 8771 (Table 2-17). The high ITS and LSU sequence identities between the SA isolates and *Sporobolomyces* sp. CBS 10199 indicate that this cluster contains several phylotypes of a new species in Erythrobasidiales that is closely related to, but phylogenetically distinct from *S. gracilis*.

Table 2-17. Pairwise analysis of the ITS region of SA265 and related taxa. Numbers indicate percent similarity between isolates. Sg-*Sporobolomyces gracilis*; Spo sp-*Sporobolomyces* species.

	SA265	**SA337**	**SA308**	**SA273**	**Sg JCM 8771**	**Spo sp CBS 10199**
SA265	-	100	99	99	96	99
SA337	100	-	99	99	96	99
SA308	99	99	-	99	96	99

Table 2-17 cont.

	SA265	SA337	SA308	SA273	Sg JCM 8771	Spo sp CBS 10199
SA273	99	99	99	-	96	99
Sg JCM 8771	96	96	96	96	-	96
Spo sp CBS 10199	99	99	99	99	96	-

There is a second cluster around *Rhodotorula marina* and *S. vermiculatus*. Of these isolates all share 98% or greater sequence identity in the ITS region (Table 2-18). *Rhodotorula marina* strain CBS 2365 and *S. vermiculatus* JCM 10224 differ by 12 bases.

Table 2-18. Pairwise analysis of the ITS regions of the *R. marina* group. Numbers indicate percent similarity between isolates. Rm-*Rhodotorula marina*; Sv-*Sporobolomyces vermiculatus*.

	SA42	SA108	SA310	SA716	SA776	Rm CBS 2365	Sv JCM 10224
SA42	-	99	99	99	99	99	99
SA108	99	-	99	99	98	98	98
SA310	99	99	-	99	99	99	98
SA716	99	99	99	-	99	98	98
SA776	99	99	99	99	-	98	98
Rm CBS 2365	99	98	99	98	98	-	98
Sv JCM 10224	99	98	98	98	98	98	-

Microbotryomycetes

Twenty-four isolates representing five strains most phylogenetically similar to *Sporobolomyces carnicolor* strain MCA 3710 were recovered. Pairwise sequence analysis of these six strains shows that all have at least one mismatch in the ITS region except for SA90 and MCA 3710, which are identical (Table 2-19). SA72 and SA791 each differ from MCA 3710 by one base and differ from SA200 and SA803 by 15 bases.

In the LSU region, only SA200 and SA803 share 100% sequence identity. Interestingly, there is a substantial degree of LSU variability between two pairs of isolates which are nearly identical in the ITS region. SA90 and SA791 differ by two bases in the ITS region and eight in LSU. Similarly, SA90 and MCA 3710 share identical ITS sequence identity, but have six mismatches in LSU. An LSU sequence was not obtained for SA72. Based on rDNA sequence data and phylogenetic analysis, SA72, SA90 and SA791 are strains of *S. carnicolor*, closely resembling strain MCA 3710. However, SA200 and SA803 appear to represent a new species, related to *S. carnicolor*.

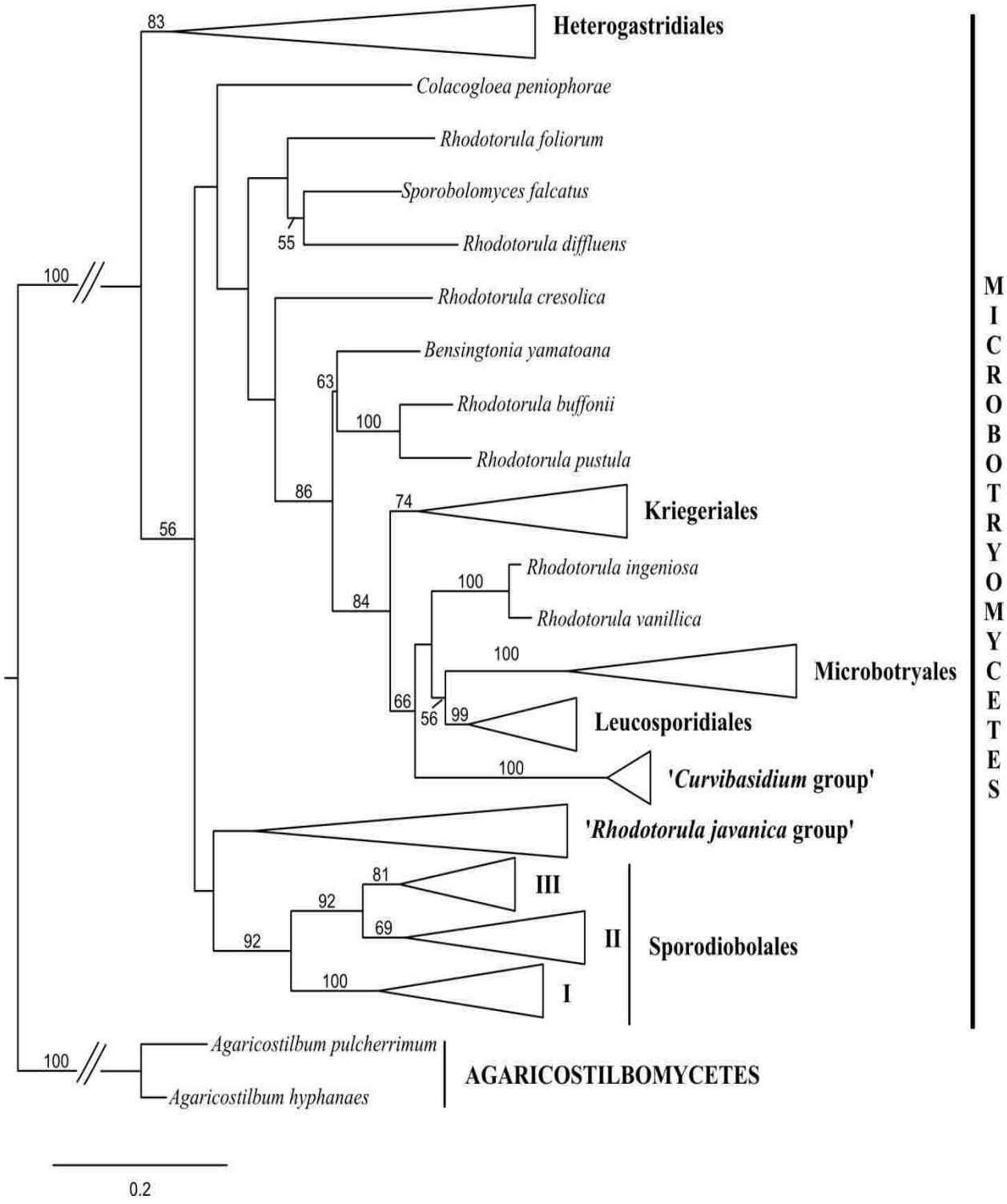


Figure 2-22. Phylogenetic relationships of the recognized orders and incertae sedis groups in Microbotryomycetes based on analyses of concatenated ITS and LSU sequences. Bootstrap values above 50% are indicated. Agaricostilbomycetes was used as an outgroup.

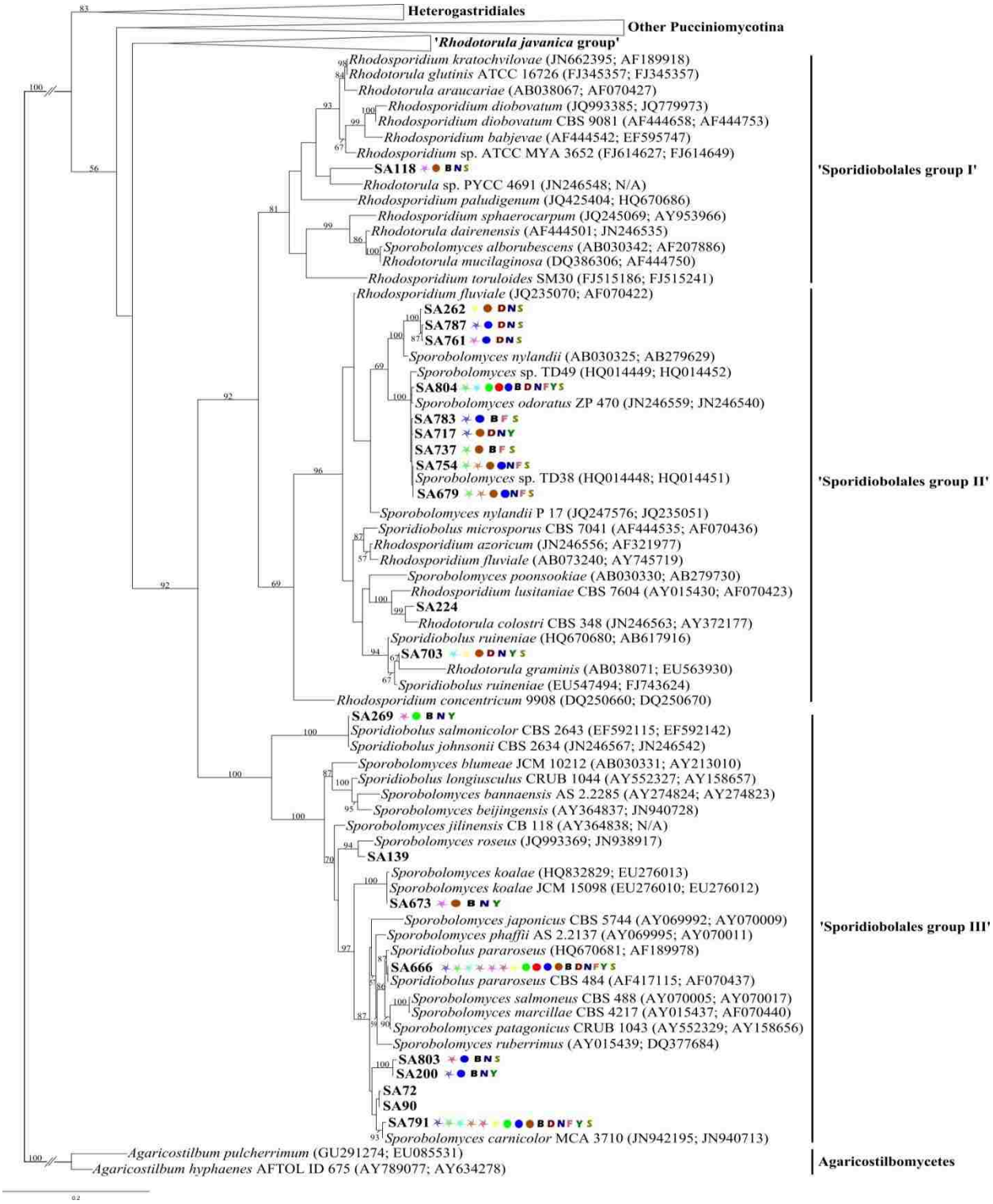


Figure 2-23. Phylogenetic relationships in Sporidiobolales (Microbotryomycetes) based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Agaricostilbomycetes was used as an outgroup.

Table 2-19. Pairwise analysis of the ITS region of members of *Sporobolomyces carnicolor* and related taxa. Numbers indicate percent similarity between isolates. Sc-*Sporobolomyces carnicolor*.

	SA72	SA90	SA200	SA791	SA803	Sc MCA 3710
SA72	-	99	98	99	97	99
SA90	99	-	97	99	97	100
SA200	98	97	-	97	99	97
SA791	99	99	97	-	97	99
SA803	97	97	99	97	-	97
Sc MCA 3710	99	100	97	99	97	-

Table 2-20. Pairwise analysis of the LSU region of members of *Sporobolomyces carnicolor* and related taxa.. Numbers indicate percent similarity between isolates. Sc-*Sporobolomyces carnicolor*.

	SA90	SA200	SA791	SA803	Sc MCA 3710
SA90	-	99	99	99	99
SA200	99	-	99	100	99
SA791	99	99	-	99	99
SA803	99	100	99	-	99
Sc MCA 3710	99	99	99	99	-

The most common isolate recovered in this survey shares 100% ITS sequence identity with *Sporidiobolus pararoseus* strain CBS 484. Out of a total of 463 isolates, 171 (49%) were positively identified as sharing 100% ITS sequence identity with strain CBS 484.

The *S. pararoseus* group is sister to the *S. carnicolor* group (Fig. 2-23). Pairwise sequence analysis shows that SA666 shares 100% ITS sequence identity with strains CBS 484 and 22261 (Table 2-21). There are at least 10 mismatches between SA666 and each of the other non-*pararoseus* isolates except between SA666 and *S. patagonicus* strain CRUB 1043, which differ by only three bases.

SA666 shares 100% LSU sequence identity with CBS 484 and differs by two bases from strain CBS 4216 (Table 2-22). There are 9-12 mismatches between SA666 and each of the non-*pararoseus* isolates except between SA666 and *S. patagonicus* strain CRUB 1043, where there are 6 mismatches. As with the *S. carnicolor* group, there are more mismatches in the LSU than in the ITS regions of certain taxa, as in the case of SA666 and strain CRUB 1043. These two isolates are slightly variable phylotypes or may represent distinct species.

Table 2-21. Pairwise analysis the ITS regions of related *Sporobolomyces* taxa. Numbers indicate percent similarity between isolates. Sp-*S. pararoseus*; Sj-*S. japonicus*; S pha-*S. phaffii*; Ss-*S. salmoneus*; Sm-*S. marcillae*; Spat-*S. patagonicus*; Sr-*S. ruberrimus*.

	SA 666	Sp 22261	Sp CBS 484	Sj CBS 5744	S pha AS 2.2137	Ss CBS 488	Sm CBS 4217	S pat CRUB 1043	Sr CBS 7500
SA 666	-	100	100	95	98	97	97	99	98
Sp 22261	100	-	100	95	98	97	97	99	98

Table 2-21 cont.

	SA 666	Sp 22261	Sp CBS 484	Sj CBS 5744	S pha AS 2.2137	Ss CBS 488	Sm CBS 4217	S pat CRUB 1043	Sr CBS 7500
Sp CBS 484	100	100	-	95	98	97	97	99	98
Sj CBS 5744	95	95	95	-	96	94	94	95	96
S pha AS 2.2137	98	98	98	96	-	96	96	98	99
Ss CBS 488	97	97	97	94	96	-	99	97	96
Sm CBS 4217	97	97	97	94	96	99	-	97	96
S pat CRUB 1043	99	99	99	95	98	97	97	-	98
Sr CBS 7500	98	98	98	96	99	96	96	98	-

Table 2-22. Pairwise analysis of the LSU regions of related *Sporobolomyces* taxa. Numbers indicate percent similarity between isolates. Sp-*S. pararoseus*; Sj-*S. japonicus*; S pha-*S. phaffii*; Ss-*S. salmoneus*; Sm-*S. marcillae*; Spat-*S. patagonicus*; Sr-*S. ruberrimus*.

	SA 666	Sp 22261	Sp CBS 484	Sj CBS 5744	S pha AS 2.2137	Ss CBS 488	Sm CBS 4217	S pat CRUB 1043	S rub VTTC 04573
SA 666	-	99	100	98	98	98	98	99	98
Sp 22261	99	-	99	98	99	99	99	99	98
Sp CBS 484	100	99	-	98	99	99	99	99	98
Sj CBS 5744	98	98	98	-	98	98	98	98	96
S pha AS 2.2137	98	99	99	98	-	98	98	98	97
Ss CBS 488	98	99	99	98	98	-	100	99	98
Sm CBS 4217	98	99	99	98	98	100	-	99	98
S pat CRUB 1043	99	99	99	98	98	99	99	-	98
S rub VTTC 04573	98	98	98	96	97	98	98	98	-

SA139 and SA673 represent *Sporobolomyces koalae* and *S. roseus*, respectively. Each isolate was only recovered once. SA139 is part of the first species cluster of Sporidiobolales and is resolved on a branch with *S. roseus* strain IWB-Y808, sister to a group of several *S. koalae* isolates. However, a pairwise comparison of all of these isolates shows that they are genetically heterogeneous in both the ITS and LSU regions (Tables 2-23 and 2-24). A Blastn search identified *S. roseus* strain IWB-Y808 as having the highest ITS sequence identity to SA139, with three mismatches separating the two isolates. In contrast, there are more than 40 mismatches between *S. roseus* strain IWBT and *S. koalae* strains LH7 and 15098. The phylogenetic distance between these species is visible (Fig. 2-22) and greater than for *S. salmoneus* and *S. marcillae*.

There is a discrepancy in sequence identities between the LSU sequence identities of SA139 and two other *S. roseus* isolates. SA139 and *S. roseus* strain AFTOL 1549 differ by four bases; SA139 and *S. roseus* strain DAOM 216360 differ by 20 bases; *S. roseus* strain DAOM 216360 and strain AFTOL 1549 differ by 22 bases. Such large differences in the LSU region make a strong case against con-specificity. A Blastn search using strain DAOM 216360 as the reference found that the closest hits were *S. pararoseus* isolates, with 98% sequence identity, while several related *S. roseus* isolates shared 97% identity and had 20 or more mismatches. In contrast, a similar search using *S. metaroseus* type strain CBS 7683 (Valerio, *et al.*, 2008) as a reference returned hits for other *S. roseus* isolates with 100% identity. A previous study reclassified several strains of *S. roseus* as *S. metaroseus* (Valerio, *et al.*, 2008) and determined that *S. roseus* represents a species complex. Despite the ambiguity regarding SA139 and its relatives, its phylogenetic position is well-defined and strongly supported as *S. roseus.*

There is less ambiguity regarding strain-specific sequence heterogeneity among the isolates pertaining to *S. koalae.* There is strong support placing SA673 in the *S. koalae* group. SA673 shares 100% ITS sequence identity with *S. koalae* strain 15098 and differs by three bases from *S. koalae* strain LH7. SA673 shares 100% sequence identity with *S. koalae* strains 15098 and 15099.

Table 2-23. Pairwise analysis of the ITS regions of *Sporobolomyces* taxa. Numbers indicate percent similarity between isolates. Sk-*S. koalae*; Sr-*S. roseus*.

	SA139	**SA673**	**Sk LH7**	**Sk 15098**	**Sr IWBT**
SA139	-	92	93	92	99
SA673	92	-	99	100	92
Sk LH7	93	99	-	99	92
Sk 15098	92	100	99	-	92
Sr IWBT	99	92	92	92	-

Table 2-24. Pairwise analysis of the LSU regions of related *Sporobolomyces* taxa. Numbers indicate percent similarity between isolates. Sk-*S. koalae*; Sr-*S. roseus*.

	SA139	**SA673**	**Sk 15098**	**Sk 15099**	**Sr DAOM**	**Sr 1549**
SA139	-	97	97	97	98	99
SA673	97	-	100	100	97	97
Sk 15098	97	100	-	100	96	97
Sk 15099	97	100	100	-	96	97
Sr DAOM	98	97	96	96	-	97
Sr 1549	99	97	97	97	97	-

SA269 was only recovered once and appears on the same branch as *Sporidiobolus johnsonii* and *S. salmonicolor* within the first Sporidiobolales cluster. SA269 shares 100% ITS and LSU sequence identity with both *S. johnsonii* strain CBS 2634 and *S. salmonicolor* strain CBS 2634.

Pairwise analysis of sequences related to SA269 revealed that there is phylogenetic diversity among different strains in the *S. johnsonii/salmonicolor* group. While SA269 is identical with *S. johnsonii* strain CBS 2643 and *S. salmonicolor* strain CBS 2643, it differs by six bases from *S. johnsonii* strain CBS 5470 and by 33 bases from *S. salmonicolor* strain ML 2241.

There is almost no variation among sequences in the LSU region. SA269 is identical to all isolates except for strain ML2241, which differ by 7 bases.

Based on the similarity of rDNA sequences, *S. johnsonii* strain CBS 2643 and *S. salmonicolor* strain CBS 2643 are synonyms. However, this data set only includes sequences of *S. johnsonii* and *S. salmonicolor* isolates that are identical, which explains the tree topology. After comparing more sequences it was observed that variation exists among different isolates.

Table 2-25. Pairwise analysis of the ITS regions of related *Sporobolomyces* taxa. Numbers indicate percent similarity between isolates. Sj-*S. johnsonii*; Ss-*S. salmonicolor*.

	SA269	**Sj CBS 2634**	**Ss CBS 2643**	**Sj CBS 5470**	**Ss ML 2241**
SA269	-	100	100	99	94
Sj CBS 2634	100	-	100	99	94
Ss CBS 2643	100	100	-	99	94
Sj CBS 5470	99	99	99	-	94
Ss ML 2241	94	94	94	94	-

Table 2-26. Pairwise analysis of the ITS regions of related *Sporobolomyces* taxa. Numbers indicate percent similarity between isolates. Sj-*S. johnsonii*; Ss-*S. salmonicolor*.

	SA269	**Sj CBS 2634**	**Ss CBS 2643**	**Sj CBS 5470**	**Ss ML 2241**
SA269	-	100	100	100	99
Sj CBS 2634	100	-	100	100	99
Ss CBS 2643	100	100	-	100	99
Sj CBS 5470	100	100	100	-	99
Ss ML 2241	99	99	99	99	-

SA703 is resolved as part of the *Sporidiobolus ruineniae* group within the second species cluster within Sporidiobolales. Two other strains of *S. ruineniae* as well as a strain of *Rhodotorula graminis* are supported as part of this group. A pairwise sequence analysis of the isolates in this group shows that there is more than 99% ITS sequence identity between SA703 and the *S. runieniae* isolates, including *R. graminis*, which has previously been shown to be a member of a phylogenetically distinct cluster that includes *R. glutinis*, *Rhodosporidium babjevae* and *R. dibovatum* (Valerio, *et al.*, 2002). Additional ITS sequences from two other *R. graminis* strains were included. SA703 shares 100% ITS sequence identity with *S. ruineniae* strain 55522 and differs only by one base from strain CO 3 and *R. graminis* strain JCM 8170. In contrast, sequence identity is lower between SA703 and the two other *R. graminis* strains, with 82 mismatches between strains CBS 2826 and SIO 108 NB PINK (Table 2-27). Strains CBS 2826 and SIO 108 NB PINK differ by four bases.

The same strains were not available for comparing the LSU region, but similar values were seen after comparing SA703 to other strains of *S. ruineniae* to *R. graminis.* SA703 differs from *S. ruineniae* strains ATT254 and LM015 by a single base, but there are 37 and 40 mismatches between *R. graminis* strains WP1 and WKZ4, respectively (Table 2-28). Strains WP1 and WKZ4 differ by four bases from each other.

Table 2-27. Pairwise analysis of the ITS regions of the *Sporobolomyces ruineniae* group. Numbers indicate percent similarity between isolates. Sr-*S. ruineniae*; Rg-*Rhodotorula graminis*.

	SA703	Sr CO 3	Sr 55522	Rg JCM 8170	Rg CBS 2826	Rg PINK
SA703	-	99	100	99	85	85
Sr CO 3	99	-	99	100	86	86
Sr 55522	100	99	-	99	86	86
Rg JCM 8170	99	100	99	-	85	85
Rg CBS2826	85	86	86	85	-	99
Rg PINK	85	86	86	85	99	-

Table 2-28. Pairwise analysis of the LSU regions of the *Sporobolomyces ruineniae* group. Numbers indicate percent similarity between isolates. Sr-*S. ruineniae*; Rg-*Rhodotorula graminis*.

	SA703	Sr ATT254	Sr LM015	Rg WP1	Rg WKZ4
SA703	-	99	99	93	93
Sr ATT254	99	-	100	94	93
Sr LM015	99	100	-	93	93
Rg WP1	93	94	93	-	99
Rg WKZ4	93	93	93	99	-

Several different strains corresponding to *Sporobolomyces odoratus* were recovered mostly during the last three months of the survey. All of these strains share similar rDNA sequences with each other and with three reference strains of *S. odoratus* (Fig. 2-22). SA679 and *S. odoratus* strain TD 38 share 100% ITS sequence identity. SA717, SA737 and strain TD 49 also share 100% ITS sequence identity (Table 2-29). Among the rest of the isolates where ITS sequences are not identical, there are four or less substitutions.

Sequence identity is higher in the LSU region. Sequences for SA679, SA737, SA754, SA783 and strain TD 38 all share 100% IS sequence identity and differ from strain ZP 470 by one base. SA804 differs by one base from strain ZP 470, by two bases from strain TD 38, by three bases from SA679, SA737, SA754, SA784 and by four bases from SA717 (Table 2-30).

The limited amount of intra-specific rDNA sequence variation between these isolates suggests that different strains of *S. odoratus* are relatively homogeneous. These results support *S. odoratus* as sister to *S. nylandi* and both are part of the second cluster within Sporidiobolales, which closely resembles the topology presented in the first description of *S. odoratus* (Valerio, *et al.*, 2002).

Table 2-29. Pairwise analysis of the ITS regions of the *Sporobolomyces odoratus* group. Numbers indicate percent similarity between isolates. So-*Sporobolomyces odoratus*.

	SA679	SA717	SA737	SA754	SA783	SA804	So ZP 470	So TD 38	So TD 49
SA679	-	99	99	99	99	99	99	100	99
SA717	99	-	100	99	99	99	99	99	100
SA737	99	100	-	99	99	99	99	99	100
SA754	99	99	99	-	99	99	99	99	99
SA783	99	99	99	99	-	99	99	99	99

Table 2-29 cont.

	SA679	SA717	SA737	SA754	SA783	SA804	So ZP 470	So TD 38	So TD 49
SA804	99	99	99	99	99	-	99	99	99
So ZP 470	99	99	99	99	99	99	-	99	99
So TD 38	100	99	99	99	99	99	99	-	99
So TD 49	99	100	100	99	99	99	99	99	-

Table 2-30. Pairwise analysis of the LSU regions of the *Sporobolomyces odoratus* group. Numbers indicate percent similarity between isolates. So-*Sporobolomyces odoratus*.

	SA679	SA717	SA737	SA754	SA783	SA804	So ZP 470	So TD 38	So TD 49
SA679	-	99	100	100	100	99	99	100	99
SA717	99	-	99	99	99	99	99	99	99
SA737	100	99	-	100	100	99	99	100	99
SA754	100	99	100	-	100	99	99	100	99
SA783	100	99	100	100	-	99	99	100	99
SA804	99	99	99	99	99	-	99	99	99
So ZP 470	99	99	99	99	99	99	-	99	99
So TD 38	100	99	100	100	100	99	99	-	99
So TD 49	99	99	99	99	99	99	99	99	-

Three strains also part of the second *Sporobolomyces* cluster (Fig. 2-23) are phylogenetically related to *Sporobolomyces nylandii* and *S. odoratus.* SA262, SA761 and SA787 are supported on a short branch with *S. nylandii*, sister to *S. odoratus.* In the ITS region SA262, SA761 and SA787 are very similar, separated by two or less mismatches. The most similar sequence found on GenBank was *S. nylandii* strain JCM 10215, which differs from SA262 and SA761 by nine bases. SA787 and strain JCM 10125 are slightly less similar with 12 substitutions. *S nylandii* strain P17 and *S odoratus* ZP 470 were also included as additional reference sequences. Based on the tree topology, these isolates were expected to differ from strain ZP 470 by 3-5% in the ITS region, which was the case. However, strain P17 is not part of the *S. nylandii* or the *S. odoratus* groups, but instead occurs on a lone branch with no support to either *S. nylandii* or *S. odoratus* groups. Strain P17 differs by more than 20 bases from SA262, SA761 and SA787 and by 19 bases from strains JCM 10215 and ZP 470 (Table 2-31) which suggests that it may not actually represent *S. nylandii*.

In the LSU region there is one mismatch between SA262 and SA761 and seven mismatches between SA262 and SA787. SA787 differs from SA262 and SA761 by seven and eight bases, respectively. SA262 and SA761 differ from strains CBS 9093, P17 and ZP 470 by 3, 16 and 13 bases, respectively. SA787differ from the same isolates by 4, 17 and 14 bases, respectively.

SA262, SA761 and SA787 appear to represent several phylotypes of one or two new species, though more work would need to be done to verify this. SA262 and SA761 are likely conspecific, but there is some evidence that SA787 is a different species as there are more mismatches in both the ITS and LSU regions. The phylogenetic relationship to *S. nylandii* is well supported by bootstrap analysis.

Table 2-31. Pairwise analysis of the ITS regions of the *Sporobolomyces nylandii* group. Numbers indicate percent similarity between isolates. Sn-*Sporobolomyces* nylandii; So-*S. odoratus*.

	SA262	SA761	SA787	Sn JCM 10215	Sn P17	So ZP 470
SA262	-	99	99	98	96	95
SA761	99	-	99	98	96	95
SA787	99	99	-	98	95	95
Sn JCM 10215	98	98	98	-	96	96
Sn P17	96	96	95	96	-	97
So ZP 470	95	95	95	96	97	-

Table 2-32. Pairwise analysis of the LSU regions of the *Sporobolomyces nylandii* group. Numbers indicate percent similarity between isolates. Sn-*Sporobolomyces* nylandii; So-*S. odoratus*.

	SA262	SA761	SA787	Sn CBS 9093	Sn P17	So ZP 470
SA262	-	99	99	99	97	98
SA761	99	-	99	99	97	98
SA787	99	99	-	99	97	97
Sn CBS 9093	99	99	99	-	98	98
Sn P17	97	97	97	98	-	97
So ZP 470	98	98	97	98	97	-

SA118 is the only isolate that was resolved in the third cluster of Sporidiobolales (Fig. 2-23), which includes of a number of *Rhodotorula* and *Rhodosporidium* species. Only the ITS region of SA118 was sequenced, but there are many nucleotide polymorphisms between it and other related taxa. There are 35 mismatches between SA118 and *Rhodotorula* sp. MAC-2011 strain PYCC 4691, which shared the most ITS sequence identity as determined by a Blastn search. Sequence similarities between SA118 and other taxa were lower and exceeded 40 substitutions for each comparison.

SA118 and strain PYCC 4691 both represent new species in the third cluster of Sporidiobolales and are sister to the *R. kratochvilovae* sub-clade, which includes *R. glutinis* strain ATCC 16726, *R. araucariae* strain JCM 3770, *R. diobovatum* strains CBS 9081 and IWBT Y840, *R. babjevae* strain CBS 7808 and *Rhodosporidium* sp. strain ATCC MYA 3652. SA118 and PYCC 4691 are not supported as sister to the *R. kratochvilovae* group, but in general, the third cluster is well supported. This analysis also shows that *R. kratochvilovae* strain LS11 and *R. glutinis* strain ATCC 16726 have identical ITS sequences and differ from *R. araucariae* by only five bases (Table 2-33). This is consistent with a previous study showing that certain strains of *R. glutinis* represent the anamorphic state of *R. kratochvilovae* (Sampaio, *et al.*, 2001). However, strictly speaking, the relationship of *R. glutinis* to *Rhodosporidium* is not entirely clear since other strains within the *R. glutinis* complex may correspond to several species of *Rhodosporidium* (Gadanho & Sampaio, 2002).

Table 2-33. Pairwise analysis of the ITS regions of the third cluster in Sporidiobolales. Numbers indicate percent similarity between isolates. Rhod sp-*Rhodotorula* species; Rk-*Rhodosporidium kratchvilovae*; Rg-*Rhodotorula glutinis*; Ra-*Rhodotorula araucariae*; Rd-*Rhodosporidium diobovatum*.

	SA 118	**Rhod sp PYCC 4691**	**Rk LS11**	**Rg ATCC 16726**	**Ra JCM 3770**	**Rd IWBT Y840**
SA118	-	94	92	92	92	92
Rhod sp PYCC 4691	94	-	94	94	93	91
Rk LS11	92	94	-	100	99	95
Rg ATCC 16726	92	94	100	-	99	95
Ra JCM 3770	92	93	99	99	-	94
Rd IWBT Y840	92	91	95	95	94	-

Several SA isolates are not resolved in Sporidiobolales, but are instead, part of a distant branch that appears to be a separate lineage distinct from Sporidiobolales (Fig. 2-24). SA780 was recovered once during this survey and displays only a distant phylogenetic relationship to any other taxa included in this analysis. A Blastn search returned sequences for *Rhodotorula javanica* and *R. crocea* as most similar to SA780. These two species are *incertae sedis*, and appear to represent a weakly-supported group sister to Sporidiobolales

A pairwise analysis of the ITS sequences of SA780, *R. javanica* strain CBS 5236 and *R. crocea* strain 2029T revealed a large number of mismatches between each isolate. SA780 differs by 66 bases from strain CBS 5236 with 77% query coverage. An accurate comparison between SA780 and strain 2029T could not be made since a Blastn search returned only 36% coverage of the two sequences.

LSU sequences also display a great deal of heterogeneity. SA780 differs from *R. javanica* strain DB 1671 by 49 bases over 69% coverage, Again, SA780 could not be thoroughly compared to *R. crocea* strain 2029 because of the low query coverage (44%). Within this clade SA780 represents a new species and possibly a new genus in Microbotryomycetidae.

SA231 and SA805 are the only two isolates which could be definitively placed with strong support outside of Sporidiobolales. In the ITS region, SA231 and SA805 differed by five bases and share high sequence identity to *Curvibasidium palleocorallinum* strain CB 6231and *Rhodotorula nothofagi* strains CBS 8166 and A45 (Table 2-34). SA231 differs from *C. palleocorallinum* by eight bases and from both strains of *R. nothofagi* by six bases. SA805 differs from *C. palleocorallinum* by three bases and from both strains of *R. nothofagi* by one base. SA231 differs from *R. fujisanensis* strain AY-31and *R. futronensis* strain JCM 9029 by 13 and 14 bases, respectively. SA805 differs from the same strains by eight and nine bases, respectively.

Pairwise analysis of the LSU region was not completed since sequences for SA231and *R. futronensis* were not obtained. However, SA805, *C palleocorallinum* and both strains of *R. nothofagi* share identical sequence identity and SA805 differs from *R fujisanensis* strain AY-31 by three bases (Table 2-35) and based on these data it appears that SA231 and SA805 represent two different strains of *R. nothofagi*.

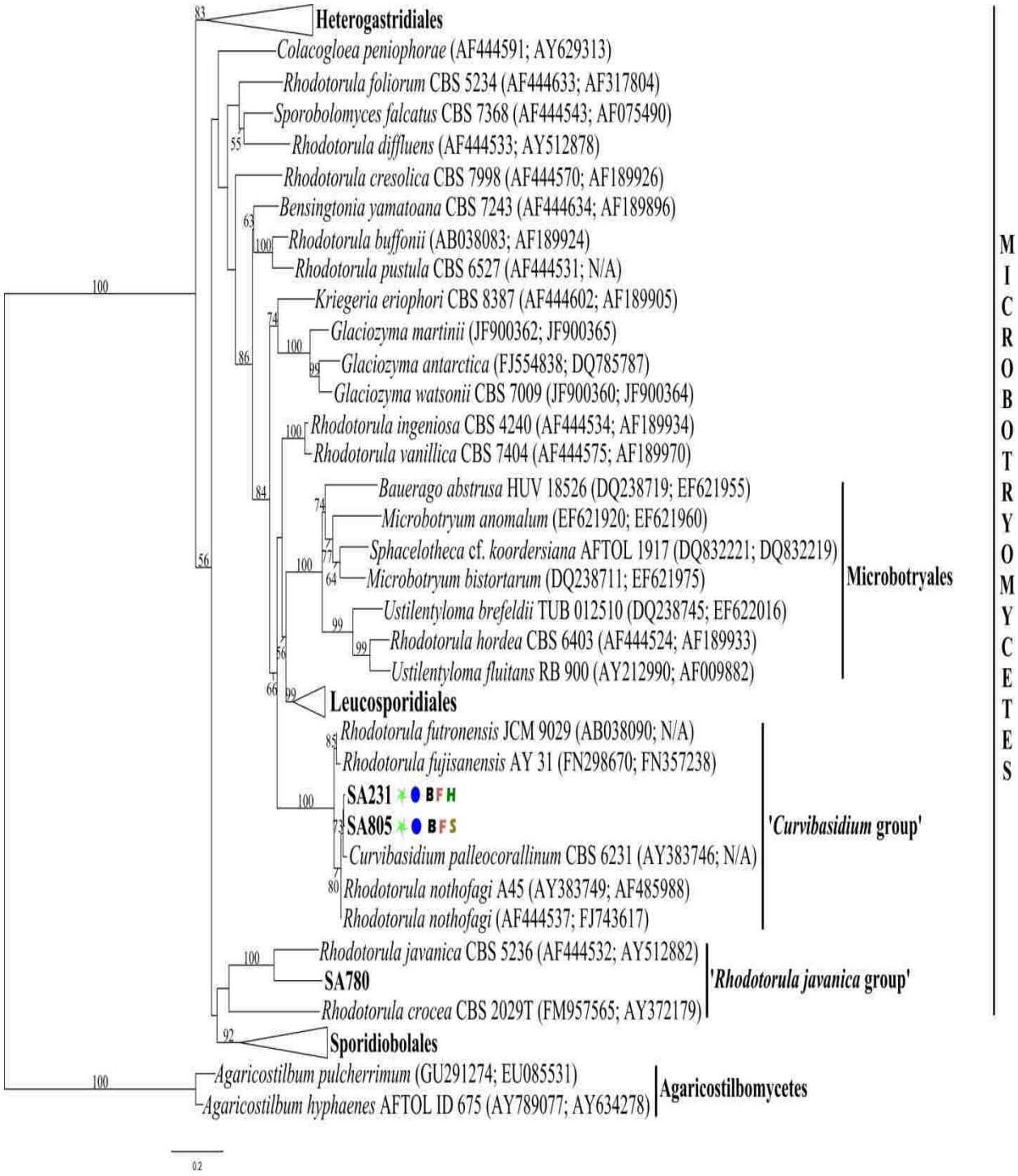


Figure 2-24. Phylogenetic relationships in the '*Curvibasidium* group' and the '*Rhodotorula javanica* group' (Microbotryomycetes) based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Agaricostilbomycetes was used as an outgroup.

Table 2-34. Pairwise analysis of the ITS regions of the *Curvibasidium* group. Numbers indicate percent similarity between isolates. Cp-*C. palleocorallinum*; Rn-*Rhodotorula nothofagi*; R fuj-*R. fujisanensis*; Rfut-*R. futronensis*.

	SA231	SA805	Cp CBS 6231	Rn CBS 8166	Rn A45	R fuj AY-31	R fut JCM 9029
SA231	-	99	99	99	99	98	97
SA805	99	-	99	99	99	98	98
Cp CBS 6231	99	99	-	99	99	99	98
Rn CBS 8166	99	99	99	-	100	99	99
Rn A45	99	99	99	100	-	99	99
R fuj AY 31	98	98	99	99	99	-	99
R fut JCM 9029	97	98	98	99	99	99	-

Table 2-35. Pairwise analysis of the LSU regions of the *Curvibasidium* group. Numbers indicate percent similarity between isolates. Cp-*C. palleocorallinum*; Rn-*Rhodotorula nothofagi*; R fuj-*R. fujisanensis*.

	SA805	Cp VKM	Rn ATT177	Rn A45	R fuj AY-31
SA805	-	100	100	100	99
Cp VKM	100	-	100	100	99
Rn ATT177	100	100	-	100	99
Rn A45	100	100	100	-	99
R fuj AY-31	99	99	99	99	-

Tremellomycetes

Cystofilobasidiales is supported as part of Tremellomycetes (Fig. 2-25) and fifteen isolates belonging to Cystofilobasidiales were recovered. These correspond to several phylotypes of *Udeniomyces* species including *U. megalosporus, U. pseudopyricola* and *U. pyricola*.

Udeniomyces belongs in Cystofilobasidiales, but the genus is not monophyletic since *U. pannonicus* is more closely related to *Itersonilia perplexans*. Pairwise analysis of both ITS and LSU regions clearly shows that *U. pannonicus* shares more sequence identity with *I. perplexans* than with other members of *Udeniomyces* (Tables 37 and 38). This has also been shown in other phylogenetic analyses (Niwata, *et al.*, 2002, Han, *et al.*, 2012).

ITS sequence variability within *Udeniomyces* is high, ranging from 2-17% (Table 2-36). SA214, SA232 and SA245 All share at least 99% sequence identity in both the ITS and LSU regions and form a cluster that represents a phylotype of *U. megalosporus*. SA213, SA340, *U. pyricola* and *U. pseudopyricola* share 98% or more sequence identity. The relatively high ITS sequence identity between *U. pseudopyricola* and *U. pyricola* does not seem to fit the profile corresponding to the other species in the genus where mismatches range from 6-15% of the entire region. SA213 and SA340 share slightly more sequence identity with *U. pyricola* than with *U. pseudopyricola* and should be designated as a strain of this species. However, future work in Cystobasidiales should re-evaluate the relationship of *U. pseudopyricola* and *U. pyricola* since they share identical LSU sequence identity and differ by only 2% in the ITS region.

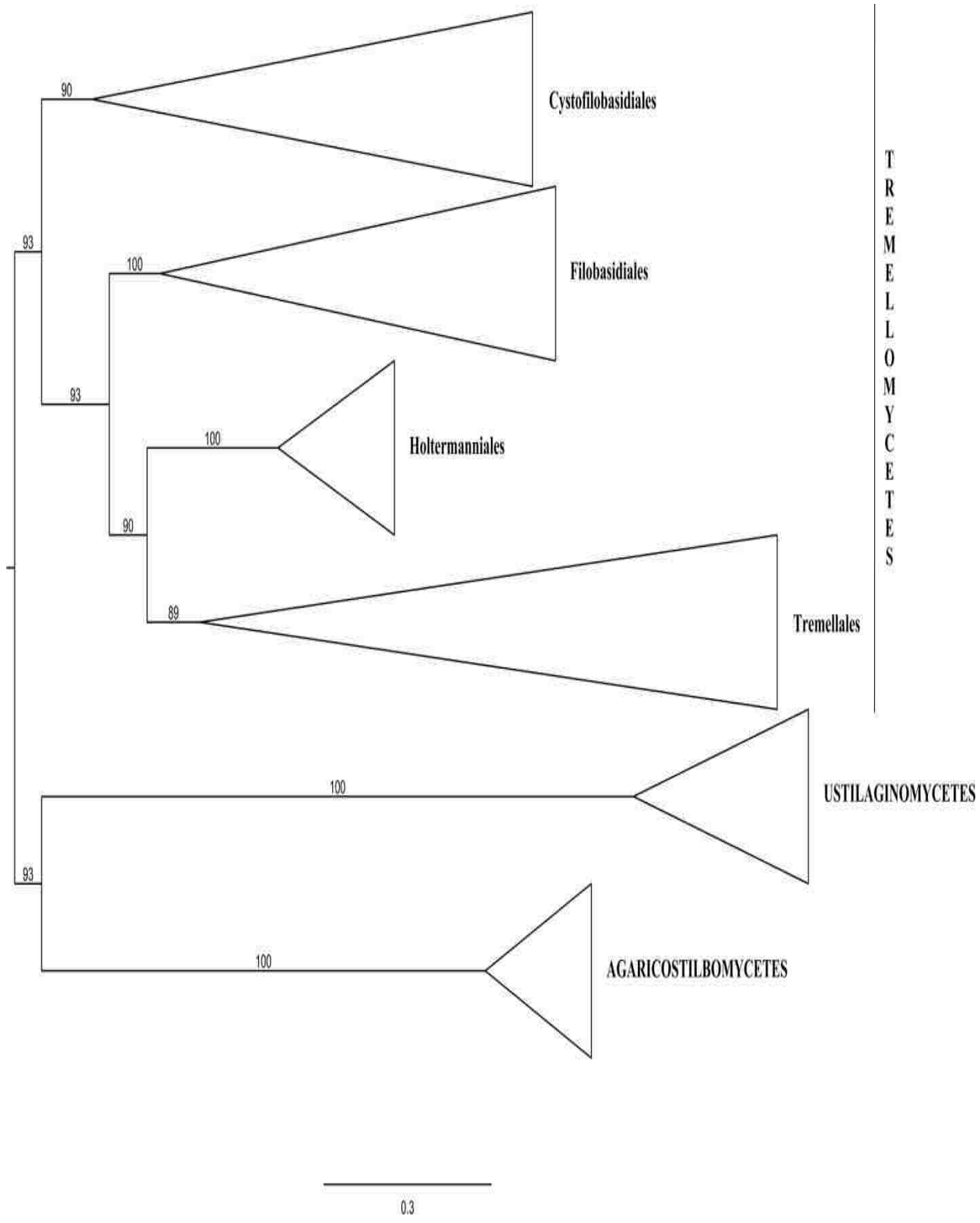


Figure 2-25. Phylogenetic relationships of four orders in Tremellomycetes based on analyses of concatenated ITS and LSU sequences. Bootstrap values above 50% are indicated. Ustilaginomycetes and Agaricostilbomycetes were used as outgroups.

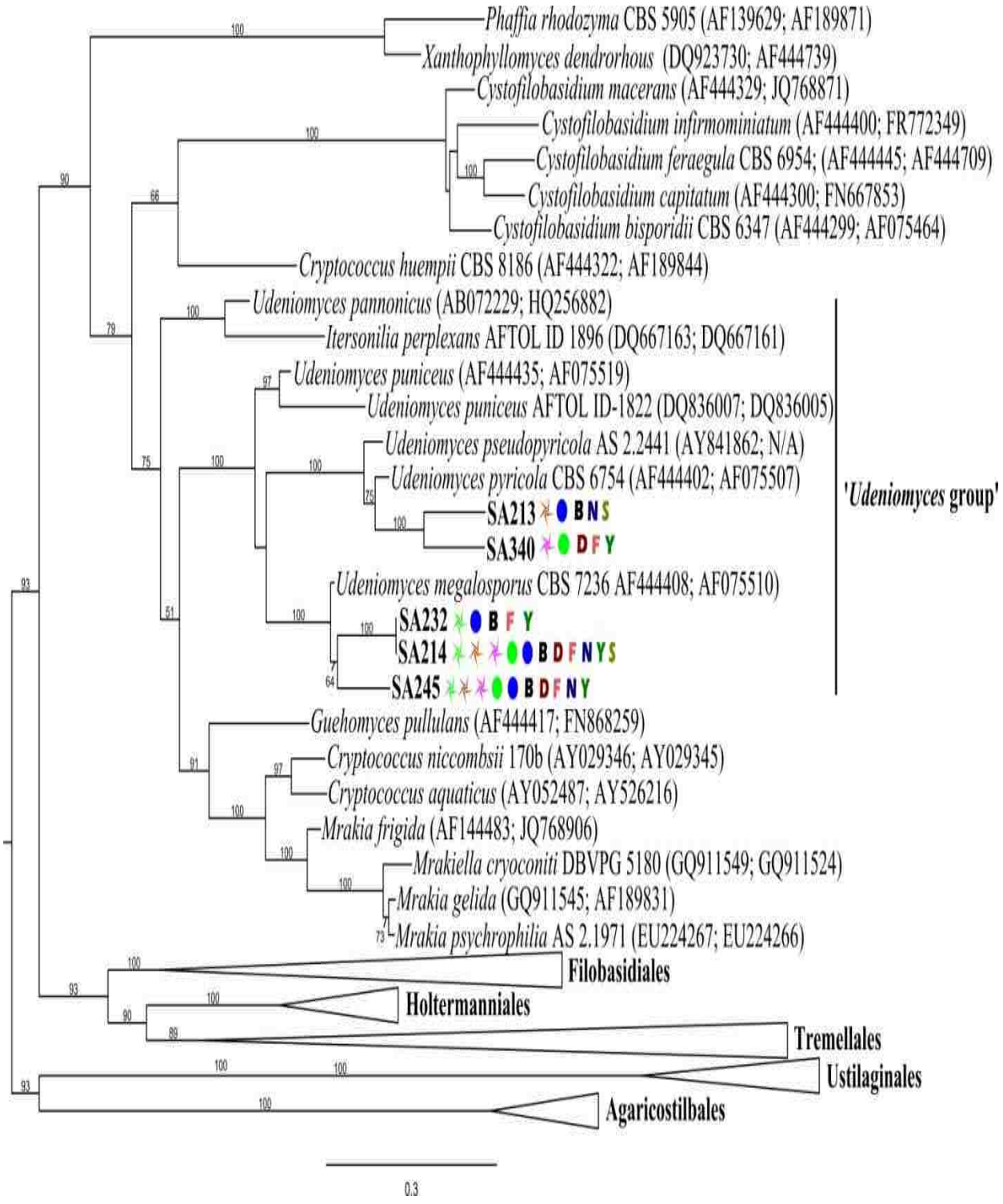


Figure 2-26. Phylogenetic relationships in the Cystofilobasidiales (Tremellomycetes) based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Agaricostilbomycetes were used as outgroups.

Table 2-36. Pairwise analysis of the ITS regions of Cystofilobasidiales. Numbers indicate percent similarity between isolates. Ip.-*Itersonilia perplexans*; Uk.-*Udeniomyces kanasensis*; Um.-*Udeniomyces megalosporus*; U. pan.-*Udeniomyces pannonicus*; U. pse.-*Udeniomyces pseudopyricola*; U. pun. *Udeniomyces puniceus*; U. pyr.-*Udeniomyces pyricola*.

	SA 213	**SA 214**	**SA 232**	**SA 245**	**SA 340**	**Ip AFTOL 1896**	**Uk XJ 10C5**	**Um CBS 7236**	**U. pan JCM 11145**	**U. pse AS 2.2441**	**U. pun AFTOL 1822**	**U. pun CBS 5689**	**U. pyr CBS 6754**
SA 213	-	91	91	91	99	84	90	91	84	98	90	90	99
SA 214	91	-	99	99	92	85	95	99	84	93	95	95	93
SA 232	91	99	-	99	92	85	95	99	84	94	95	96	94
SA 245	91	99	99	-	92	85	94	100	84	92	94	94	92
SA 340	99	92	92	92	-	86	91	92	85	98	91	91	99
Ip AFTOL 1896	84	85	85	85	86	-	85	85	97	84	85	85	84
Uk XJ 10C5	90	95	95	94	91	85	-	95	83	92	94	94	86
Um CBS 7236	91	99	99	100	92	85	95	-	88	94	95	96	94
U. pan JCM 11145	84	84	84	84	85	97	83	88	-	89	95	90	89
U. pse. AS 2.2441	98	93	94	92	98	84	92	94	89	-	85	87	98
U. pun. AFTOL 1822	90	95	95	949	91	85	94	95	95	85	-	100	85
U. pun. CBS 5689	90	95	96	94	91	85	94	96	90	87	100	-	87
U. pyr. CBS 6754	99	93	94	92	99	84	86	94	89	98	85	87	-

Table 2-37. Pairwise analysis of the LSU regions of Cystofilobasidiales. Numbers indicate percent similarity between isolates. Ip-*Itersonia perplexans*; Uk-*Udeniomyces kanasensis*; Um-*Udeniomyces megalosporus*; U. pan-*Udeniomyces pannonicus*; U. pse-*Udeniomyces pseudopyricola*; U. pun-*Udeniomyces puniceus*; U. pyr-*Udeniomyces pyricola*.

	SA 213	**SA 214**	**SA 232**	**SA 245**	**SA 340**	**Ip. AFTOL 1896**	**Uk. XJ 10C5**	**Um. CBS 7236**	**U. pan. JCM 11145**	**U. pse. AS 2.2441**	**U. pun. AFTOL 1822**	**U. pun. CBS 5689**	**U. pyr. CBS 6754**
SA 213	-	98	98	98	99	98	97	98	92	99	97	98	99
SA 214	98	-	100	100	99	99	96	100	93	99	99	99	99
SA 232	98	100	-	100	99	99	96	100	93	99	99	99	99
SA 245	98	100	100	-	99	96	96	100	93	99	99	99	99
SA 340	99	99	99	99	-	99	97	99	93	100	98	99	100
Ip AFTOL 1896	98	99	99	96	99	-	94	99	94	92	99	99	99
Uk XJ 10C5	97	96	96	96	97	94	-	94	99	94	94	97	94

Table 2-37 cont.

	SA 213	SA 214	SA 232	SA 245	SA 340	Ip. AFTOL 1896	Uk. XJ 10C5	Um. CBS 7236	U. pan. JCM 11145	U. pse. AS 2.2441	U. pun. AFTOL 1822	U. pun. CBS 5689	U. pyr. CBS 6754
Um CBS 7236	98	100	100	100	99	99	94	-	93	99	99	99	99
U. pan JCM 11145	92	93	93	93	93	94	99	93	-	93	93	94	93
U. pse AS 2.2441	99	99	99	99	100	92	94	99	93	-	98	99	100
U. pun AFTOL 1822	97	99	99	99	98	99	94	99	93	98	-	99	98
U. pun CBS 5689	98	99	99	99	99	99	97	99	94	99	99	-	99
U. pyr CBS 6754	99	99	99	99	100	99	94	99	93	100	98	99	-

The lone SA isolate from this group (SA507) shares 99% ITS sequence identity to *C. terreus* and four other *Cryptococcus* species (Table 2-38). A pairwise comparison of the sequences of these species revealed fewer substitutions in the LSU region than in ITS (Table 2-39).

Table 2-38. Pairwise analysis of the ITS regions of Filobasidiales. Numbers indicate percent similarity between isolates. Ct-*Cryptococcus aerius*; Ce-Cr*yptococcus elinovii*; Ch-*Cryptococcus himalayensis*; Cp-*Cryptococcus phenolicus*; Cf *Cryptococcus fuscescens*; Ca-*Cryptococcus aerius*.

	SA507	Ct JCM 8975	Ce PYCC 4966	Ch CBS 6293	Cp CBS 8682	Cf CBS 7189	Ca RUB 028
SA507	-	99	99	99	99	99	98
Ct JCM 8975	99	-	100	99	99	99	98
Ce PYCC 4966	99	100	-	99	99	99	98
Ch CBS 6293	99	99	99	-	99	99	98
Cp CBS 8682	99	99	99	99	-	99	98
Cf CBS 7189	99	99	99	99	99	-	98
Ca RUB 028	98	98	98	98	98	98	-

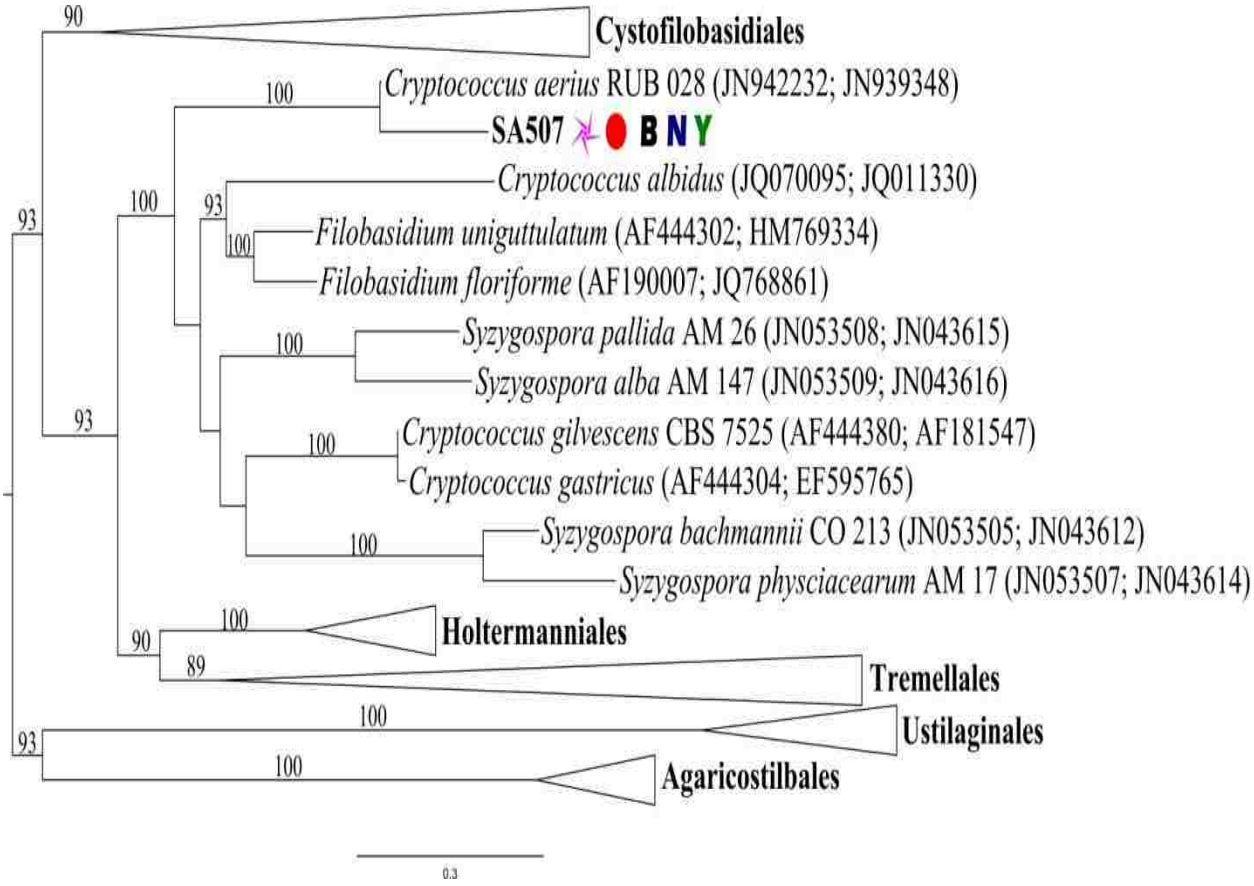


Figure 2-27. Phylogenetic relationships in the Filobasidiales (Tremellomycetes) based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Agaricostilbomycetes were used as outgroups.

Table 2-39. Pairwise analysis of the LSU regions of Filobasidiales. Numbers indicate percent similarity between isolates. Ct-*Cryptococcus aerius*; Ce-Cr*yptococcus elinovii*; Ch-*Cryptococcus himalayensis*; Cp-*Cryptococcus phenolicus*; Cf-*Cryptococcus fuscescens*; Ca-*Cryptococcus aerius*.

	SA507	**Ct JCM 8975**	**Ce PYCC 4966**	**Ch CBS 6293**	**Cp CBS 8682**	**Cf CBS 7189**	**Ca RUB 028**
SA507	-	99	99	99	99	99	98
Ct JCM 8975	99	-	99	100	99	98	97
Ce PYCC 4966	99	99	-	99	99	99	97
Ch CBS 6293	99	100	99	-	99	98	97
Cp CBS 8682	99	99	99	99	-	99	97
Cf CBS 7189	99	98	99	98	99	-	98
Ca RUB 028	98	97	97	97	97	98	-

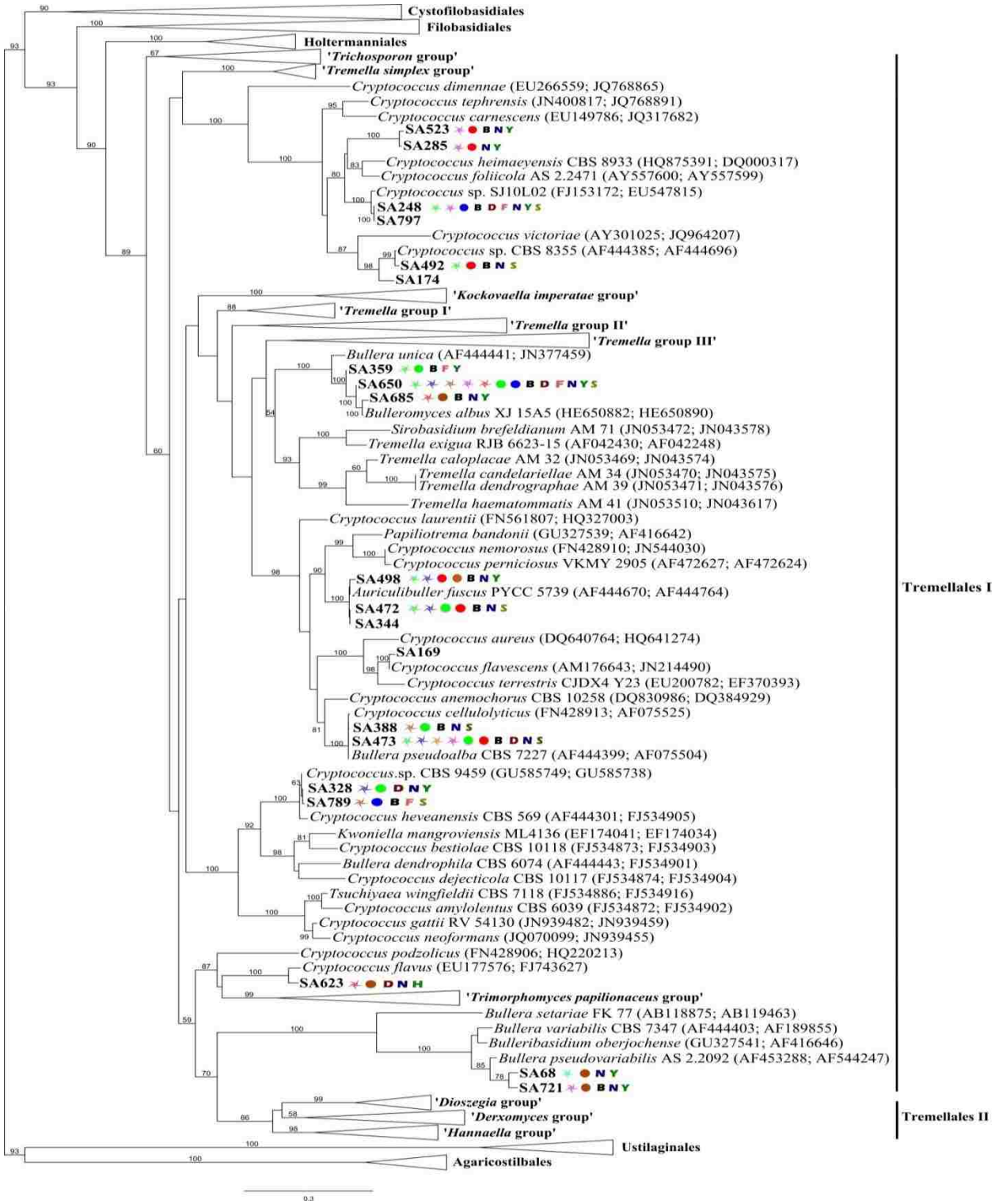


Figure 2.28. Phylogenetic relationships in the Tremellales I group (Tremellomycetes) based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Agaricostilbomycetes were used as outgroups.

Several clades are present throughout Tremellales (Fig. 2-28). SA344, SA472 and SA498 belong to a clade composed of *Auriculibuller fuscus* strain PYCC 5739 and *Papilotrema bandoni* strain PYCC 5743. All three SA isolates share more than than 99% ITS and LSU sequence identity to strain PYCC 5739. *Papilotrema bandoni* occurs on a separate branch, further away from the *A. fuscus* group. There is less sequence variation between the *A. fuscus* group and *P. bandoni* in both the ITS and LSU regions which is evidenced by the longer branch length separating them. SA169 shares 99% ITS sequence identity with *Cryptococcus flavescens* strain JCM 9909 and both isolates differ by more than 10% from their relatives in the *A. fuscus* clade (Tables 2-39 and 2-40).

A third cluster, also part of the *A. fuscus* and *C. flavescens* clade was observed. This group includes ten isolates representing two different strains corresponding to *Bullera pseudoalba* strain CBS 7227. The only sequence variation observed among the three strains is a single nucleotide substitution in the LSU region of SA388. A Blastn search of sequences related to SA388, revealed that *B. pseudoalba* strain CBS 7227 and several strains *C. cellulolyticus* share identical LSU sequences and differ by only one base in the ITS region, indicating that they are conspecific.

Table 2-40. Pairwise analysis of the ITS regions of related Tremellales taxa. Numbers indicate percent similarity between isolates. Cf-*Cryptococcus flavescens*; Af-*Auriculibuller fuscus*; Bp-*Bullera pseudoalba*; Pb-*Papiliotrema bandonii*.

	SA 169	**SA 344**	**SA 388**	**SA 472**	**SA 473**	**SA 498**	**Cf JCM 9909**	**Af PYCC 5739**	**Bp CBS 7227**	**Pb PYCC 5473**
SA 169	-	83	87	87	87	83	99	82	87	84
SA 344	83	-	87	100	87	98	83	99	88	91
SA 388	87	87	-	91	100	87	86	87	100	87
SA 472	87	100	91	-	91	99	87	99	91	93
SA 473	87	87	100	91	-	87	86	87	100	87
SA 498	83	98	87	99	87	-	86	99	88	90
Cf JCM 9909	99	83	86	87	86	86	-	82	87	84
Af PYCC 5739	82	99	87	99	87	99	82	-	88	91
Bp CBS 7227	87	88	100	91	100	88	87	88	-	87
Pb PYCC 5473	84	91	87	93	87	90	84	91	87	-

Table 2-41. Pairwise analysis of the ITS regions of related Tremellales taxa. Numbers indicate percent similarity between isolates. Cf-*Cryptococcus flavescens*; Af-*Auriculibuller fuscus*; Bp-*Bullera pseudoalba*; Pb-*Papiliotrema bandonii*.

	SA 344	**SA 388**	**SA 472**	**SA 473**	**SA 498**	**Cf 14**	**Af PYCC 5739**	**Bp CBS 7227**	**Pb IGC 5743**
SA 344	-	96	100	97	99	98	100	96	97
SA 388	96	-	96	99	96	95	96	99	96
SA 472	100	96	-	97	99	97	100	96	97
SA 473	97	99	97	-	96	95	96	100	96

Table 2-41 cont.

	SA 344	SA 388	SA 472	SA 473	SA 498	Cf 14	Af PYCC 5739	Bp CBS 7227	Pb IGC 5743
SA 498	99	96	99	96	-	97	99	96	96
Cf 14	98	95	97	95	97	-	98	96	95
Af PYCC 5739	100	96	100	96	99	98	-	97	97
Bp CBS 7227	96	99	96	100	96	96	97	-	96
Pb IGC 5743	97	96	97	96	96	95	97	96	-

A fourth group of isolates related to the *A. fuscus* clade was observed on a separate branch. This group includes 16 isolates representing two different strains. SA359 and SA685 share more than 99% sequence identity to *Bulleromyces albus* strain CBS 500 in both the ITS and LSU regions. Pairwise comparison of SA359, SA685 and *Bullera unica* strain CBS 8290 showed 3% ITS sequence variation (Table 2-42). The *B. albus* clade is sister to a group of three *Tremella* species and part of a larger branch that includes several other *Tremella* species and *Sirobasidium brefeldianum*, though this larger *Tremella* branch is unsupported (Fig. 2-28).

Table 2-42. Pairwise analysis of the ITS regions of related Tremellales taxa. Numbers indicate percent similarity between isolates. Bu-*Bullera unica*; Ba-*Bulleromyces albus*.

	SA 359	SA 685	Bu CBS 8290	Ba CBS 500
SA 359	-	99	97	99
SA 685	99	-	97	99
Bu CBS 8290	97	97	-	97
Ba CBS 500	99	99	97	-

Table 2-43. Pairwise analysis of the LSU regions of related Tremellales taxa. Numbers indicate percent similarity between isolates. Bu-*Bullera unica*; Ba-*Bulleromyces albus*.

	SA359	SA685	Bu 57474	Ba XJ 15A5
SA359	-	99	98	100
SA685	99	-	97	99
Bu 57474	98	97	-	98
Ba XJ 15A5	100	99	98	-

A fifth group of four SA isolates is related to *Cryptococcus dimenniae* strain ATCC 22024, *C. heimaeyensis* strain CBS 8933 and *C. victoriae* strain 198 Bl, and sister to another group of *Tremella* species which includes *T. mesenterica*. None of the SA isolates can be ascribed to any known species and pairwise comparison of ITS and LSU sequences suggest that these are new species in Tremellales.

SA174 and SA492 occur on a single branch and share 97% ITS sequence identity, differing by 13 bases (Table 2-44). Neither isolate cannot be assigned to any known species, however, more information is needed in order to establish of they are two phylotypes of the same new species. SA285 also occurs on a separate branch and represents another new species. ITS

sequence identity is low when compared to all other isolates, though the topolgy shows proximity to *C. heimaeyensis.* SA248 is part of the *C. heimaeyensis*/*C. victoriae* subgroup, though based on ITS and LSU sequence comparison, this isolate is likely a new species.

Table 2-44. Pairwise analysis of the ITS regions of related *Cryptococcus* spp.. Numbers indicate percent similarity between isolates. Cd-*Cryptococcus dimennae*; Ch-*Cryptococcus heimaeyensis*; Cv-*Cryptococcus vctoriae*.

	SA174	**SA248**	**SA285**	**SA492**	**Cd ATCC 22024**	**Ch CBS 8933**	**Cv 198 B1**
SA174	-	91	90	97	84	92	91
SA248	91	-	92	92	89	95	93
SA285	90	92	-	90	87	92	91
SA492	97	92	90	-	84	92	91
Cd ATCC 22024	84	89	87	84	-	97	82
Ch CBS 8933	92	95	92	92	97	-	90
Cv 198 B1	91	93	91	91	82	90	-

Table 2-45. Pairwise analysis of the LSU regions of related *Cryptococcus* spp. Numbers indicate percent similarity between isolates. Cd-*Cryptococcus dimennae*; Ch-*Cryptococcus heimaeyensis*; Cv-*Cryptococcus victoriae*.

	SA 174	**SA 248**	**SA 285**	**SA 492**	**Cd TP Snow Y90**	**Ch CBS 8933**	**Cv TP Snow Y109**
SA 174	-	97	97	99	92	97	96
SA 248	97	-	98	97	91	99	98
SA 285	97	98	-	96	92	98	97
SA 492	99	97	96	-	91	97	96
Cd TP Snow Y90	92	91	92	91	-	92	93
Ch CBS 8933	97	99	98	97	92	-	98
Cv TP Snow Y109	96	98	97	96	93	98	-

SA328 and SA789 share 97% sequence identity with *Kwoniella heveanensis,* the recently discovered teleomorph for *C. heveanensis.* (Sun, *et al.*, 2011). The clade containing the SA isolates is composed of several other branches. *Kwoniella mangroviensis* and *Bullera dendrophila* occur on the same branch, sister to the *C. heveanensis* group. *Bullera dendrophila*, (teleomorph = *Aessosporon dendrophilum*), shares 97% sequence identity with *K. mangroviensis.*

A third branch which includes the human pathogen *Cryptococcus neoformans*, *C. amylolentus* and *Tsuchiyaea wingfieldii* is also part of the larger clade. *Tsuchiyaea wingfeldii* and *C. amylolentus* share 100% ITS sequence identity (Table 2-46) and greater than 99% similarity in the LSU region (Table 2-47).

Table 2-46. Pairwise analysis of the ITS regions of the *Cryptococcus heveanensis* group. Numbers indicate percent similarity between isolates. Kh-*Kwoniella heveanensis*; Bd-*Bullera dendrophila*; Kw-*Kwoniella mangroviensis*; Cn-*Cryptococcus neoformans*; Tw-*Tsuchiyaea wingfieldii*; Ca-*Cryptococcus amylolentus*.

	SA328	**SA789**	**Kh CBS 569**	**Bd CBS 6074**	**Km ML 4136**	**Cn CBS 8336**	**Tw CBS 7118**	**Ca CBS 6039**
SA328	-	99	97	90	90	87	87	97
SA789	99	-	97	90	89	87	88	88
Ch CBS 569	97	97	-	90	89	88	97	97
Bd CBS 6074	90	90	90	-	97	90	97	97
Km ML 4136	90	98	98	97	-	90	97	97
Cn CBS 8336	87	87	88	90	90	-	96	96
Tw CBS 7118	87	88	97	97	97	96	-	100
Ca CBS 6039	97	88	97	97	97	96	100	-

Table. 2-47. Pairwise analysis of the LSU regions of members of the *Cryptococcus heveanensis* group. Numbers indicate percent similarity between isolates. Ch-*Cryptococcus heveanensis*; Bd-*Bullera dendrophila*; Kw-*Kwoniella mangroviensis*; Cn-*Cryptococcus neoformans*; Tw-*Tsuchiyaea wingfieldii*; Ca-*Cryptococcus amylolentus*.

	SA789	**Ch CBS 569**	**Bd CBS 6074**	**Km ML 4136**	**Cn CBS 8336**	**Tw CBS 7118**	**Ca CBS 6039**
SA789	-	99	94	94	92	91	91
Ch CBS 569	99	-	94	94	92	91	91
Bd CBS 6074	94	94	-	98	91	92	91
Km ML 4136	94	94	98	-	91	91	91
Cn CBS 8336	92	92	91	91	-	97	97
Tw CBS 7118	91	91	92	91	97	-	99
Ca CBS 6039	91	91	91	91	97	99	-

SA623 is a strain of *Cryptococcus flavus* and shares 98% sequence identity to *C. podzolicus*. SA623 is identical to *C. flavus* in the in the LSU region and differs by 2% in ITS (Tables 2-48 and 2-49). This clade also includes *Trimophomyces papillionaceus* and *Tremella parmellareum*, though these two taxa share less sequence identity to SA623.

Table. 2-48. Pairwise analysis of the ITS regions of members of the *Cryptococcus flavus* group. Numbers indicate percent similarity between isolates. Cf-*Cryptococcus flavus*; Cp-*Cryptococcus podzolicus*.

	SA623	**Cf WH**	**Cp YM 24343**
SA623	-	98	87
Cf WH	98	-	87
Cp YM 24343	87	87	-

Table. 2-49. Pairwise analysis of the LSU regions of members of the *Cryptococcus flavus* group. Numbers indicate percent similarity between isolates. Cf-*Cryptococcus flavus*; Cp-*Cryptococcus podzolicus*.

	SA623	**Cf ATT 259**	**Cp YM 24343**
SA623	-	100	92
Cf ATT 259	100	-	92
Cp YM 24343	92	92	-

The *C. flavus* group is sister to a large, well-supported clade which contains most of the SA Tremellales isolates (Fig. 2-29). This clade has been referred to as the '*Cryptococcus luteolus* lineage' and is comprised of three sub-groups representing the genera: *Dioszegia*, *Derxomyces* and *Hannaella* (Wang & Bai, 2008). These three genera are monophyletic in this phylogeny. Another longer branch was observed, sister to the 'C. *luteolus*' lineage that includes *Bulleribasidium oberjochense* strain CBS 9110 and *Bullera variabilis* strain CBS 7347. SA68 and SA721 share high ITS and LSU sequence identity with strain CBS 9110 and somewhat less with strain CBS 7347 (Table 2-50). ITS and LSU sequences from strains of these isolates are similar, but not identical (Table. 2-51).

Table 2-50. Pairwise analysis of the LSU regions of members of the *Bulleribasidium oberjochense* group. Numbers indicate percent similarity between isolates. Bv-*Bullera variabilis*; Bo-*Bulleribasidium oberjochense*.

	SA68	**SA721**	**Bv CBS 7347**	**Bo CBS 9110**
SA68	-	99	98	99
SA721	99	-	97	99
Bv CBS 7347	98	97	-	98
Bo CBS 9110	99	99	98	-

Table 2-51. Pairwise analysis of the LSU regions of members of the *Bulleribasidium oberjochense* group. Numbers indicate percent similarity between isolates. Bv-*Bullera variabilis*; Bo-*Bulleribasidium oberjochense*.

	SA68	**SA721**	**Bv CBS 7347**	**Bo IGC 5741**
SA68	-	100	99	99
SA721	100	-	99	99
Bv CBS 7347	99	99	-	99
Bo IGC 5741	99	99	99	-

All of the *Dioszegia* species included in this analysis form a monophyletic group in Tremellales (Fig. 2-29). SA471 represents three identical strains which share 100% ITS and LSU sequence identity to *Dioszegia zsoltii* var *zsoltii* strain CB 167 (Wang, *et al.*, 2008) and 98% and 99% compared to *D. catarinonii* strain PYCC 5859 in the ITS and LSU regions, respectively (Tables 2-52 and 2-53).

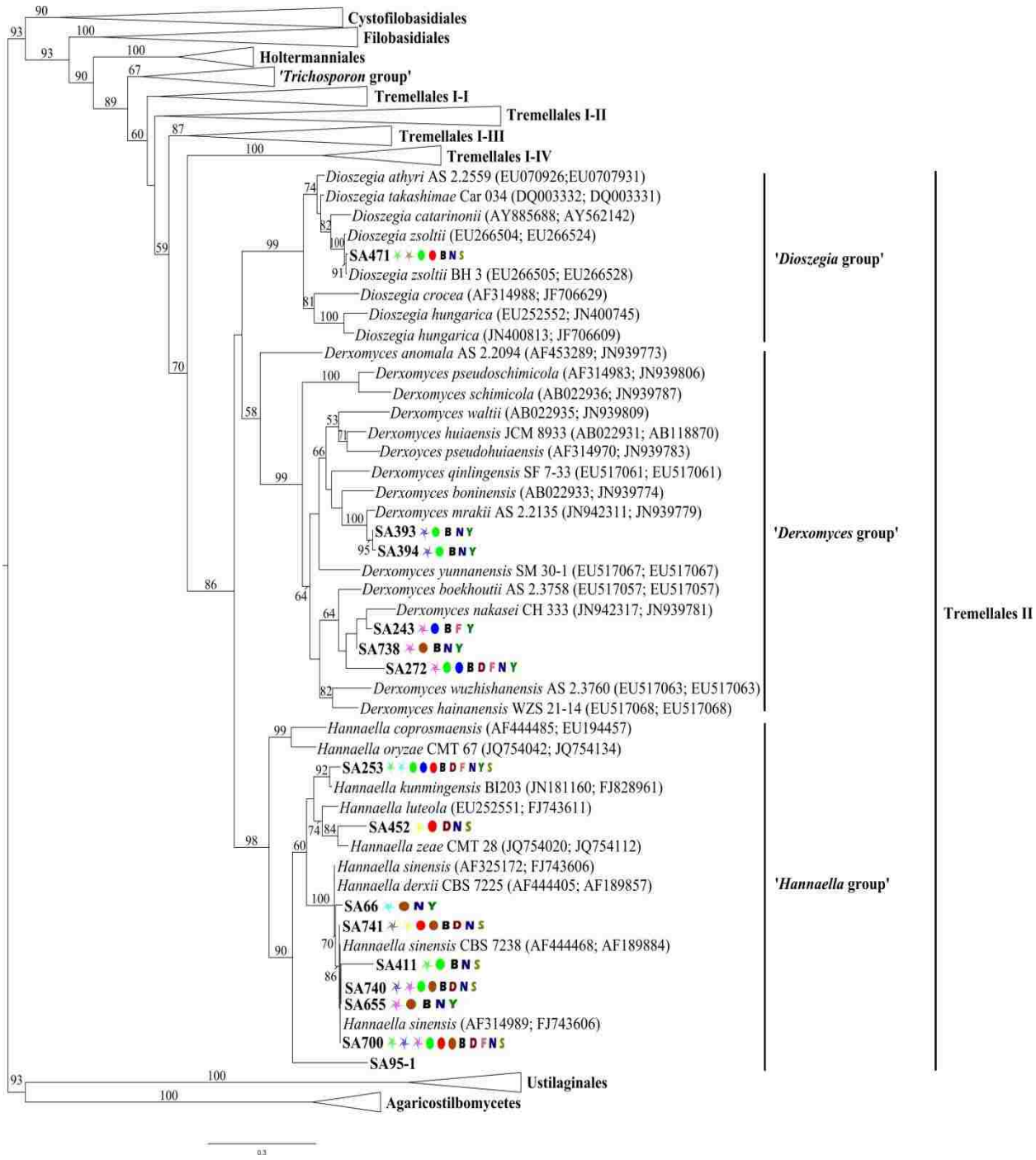


Figure 2-29. Phylogenetic relationships in the Tremellales II group (Tremellomycetes) including the genera *Dioszegia*, *Derxomyces* and *Hannaella*. Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Agaricostilbomycetes were used as outgroups.

Table 2-52. Pairwise analysis of the ITS regions of members of the *Dioszegia* group. Numbers indicate percent similarity between isolates. Dh-*Dioszegia hungarica*; Dz-*Dioszegia zsoltii*; Dc-*Dioszegia catarinonii*; Da-*Dioszegia athyri*; Dt-*Dioszegia takashimae.*

	SA 471	**Dh TP Snow Y4**	**Dz Can S 069**	**Dc A2AVS8**	**Da AS 2.2559**	**Dt Car 034**
SA 471	-	93	99	98	97	97
Dh TP Snow Y4	93	-	89	88	89	90
Dz Can S 069	99	89	-	98	97	97
Dc A2AVS8	98	88	98	-	97	97
Da AS 2.2559	97	89	97	97	-	98
Dt Car 034	97	90	97	97	98	-

Table 2-53. Pairwise analysis of the LSU regions of members of the *Dioszegia* group. Numbers indicate percent similarity between isolates. Dh-*Dioszegia hungarica*; Dz-*Dioszegia zsoltii*; Dc-*Dioszegia catarinonii*; Da-*Dioszegia athyri*; Dt-*Dioszegia takashimae.*

	SA471	**Dh PDD 40d5**	**Dz TA 204**	**Dc PYCC 5858**	**Da AS 2.2559**	**Dt Car 034**
SA471	-	98	100	99	99	99
D hungarica	98	-	97	97	97	97
D zsoltii	100	97	-	99	99	99
D catarinonii	99	97	99	-	99	99
Da AS 2.2559	99	97	99	99	-	99
Dt Car 034	99	97	99	99	99	-

Five isolates comprise two clusters around *Derxomyces mrakii* strain AS 2.2135 and *D. boekhoutii* strain AS 2.3758. Derxomyces forms a monophyletic group that is sister to *Dioszegia* (Fig. 2-29). SA243 and SA738 share 100% ITS sequence identity and differ by only one base in the LSU region (Table 2-54). These isolates both exhibit 3% incongruence in ITS sequence identity compared to *D. boekhoutii.* SA272 and *D. boekhoutii* also differ by 3% in the ITS region, but share identical LSU sequence identity (Table 2-55). This explains their closer phylogenetic position on the tree relative to SA243 and SA738. SA272 also differs by 3% and 1% in the ITS and LSU regions relative to SA243 and SA738. Therefore, while SA243 and SA738 appear to be a new species of *Derxomyces*, SA272 is a phylotype of *D. boekhoutii.*

Table 2-54. Pairwise analysis of the ITS regions of members of the *Derxomyces* group. Numbers indicate percent similarity between isolates. Db-*Derxomyces boekhoutii*; Dm-*Derxomyces mrakii*.

	SA243	**SA272**	**SA393**	**SA394**	**SA738**	**Db AS 2.3758**	**Dm AS 2.2135**
SA243	-	97	91	91	100	97	91
SA272	97	-	91	91	97	97	91
SA393	91	91	-	100	91	91	100
SA394	91	91	100	-	91	91	100

Table 2-54 cont.

	SA243	SA272	SA393	SA394	SA738	Db AS 2.3758	Dm AS 2.2135
SA738	100	97	91	91	-	98	91
Db AS 2.3758	97	97	91	91	98	-	91
Dm AS 2.2135	91	91	100	100	91	91	-

Table 2-55. Pairwise analysis of the LSU regions of members of the *Derxomyces* group. Numbers indicate percent similarity between isolates. Db-*Derxomyces boekhoutii*; Dm-*Derxomyces mrakii*.

	SA243	SA272	SA393	SA394	SA738	Db AS 2.3758	Dm AS 2.2135
SA243	-	99	98	99	99	99	99
SA272	99	-	98	98	99	100	99
SA393	98	98	-	99	98	98	99
SA394	99	98	99	-	98	98	99
SA738	99	99	98	98	-	99	99
Db AS 2.3758	99	100	98	98	99	-	99
Dm AS 2.2135	99	99	99	99	99	99	-

The *Hannaella* clade is basal in the '*C. luteolus* lineage,' sister to *Derxomyces*. Seven strains belonging to this group were recovered, which represent several new species and some different phylotypes of *H. sinensis* strain CBS 7238. SA66, SA411, SA700 and SA741 are four different strains of *H. sinensis* and all except SA411 share 100% LSU sequence identity. The LSU region was not sequenced for SA411, and it is resolved on a longer branch length than the other isolates. The ITS regions are variable to some degree among all four isolates, but all share at least 98% sequence identity with *H. sinensis* strain CBS 7238 and to each other. However, SA741 is identical to strain CBS 7238.

SA253 and *H. kunmingensis* strain BI203 share 100% ITS sequence identity and they differ by one base in their LSU sequences (Tables 2-56 and 2-57). Both isolates are part of a sub-clade which includes *H. luteola* and *H. zeae* that is sister to the *H. sinensis* group.

SA452 is unsupported on a single lone branch in between *H. luteolus* and *H. sinensis.* The LSU regions was not sequenced for this isolate, but there is 95% identity to *H. luteolus* in the ITS region. SA452 shares the most ITS sequence identity with SA253, however, a pairwise analysis of their sequences shows that they differ by at least 3% and each may be a new species of *Hannaella*.

SA95-1 is located on a branch with *H. luteolus*, though this relationship is not strongly supported (Fig. 2-29). SA95-1 is phylogenetically distinct from all other SA isolates and also from all known species of *Hannaella*.

Table 2-56. Pairwise analysis of the ITS regions of members of the *Hannaella* group. Numbers indicate percent similarity between isolates. Hk-*Hannaella kunmingensis*; Hz-*Hannaella zeae*; Hl-*Hannaella luteola*; Hs-*Hannaella sinensis*.

	SA 66	**SA 95-1**	**SA 253**	**SA 411**	**SA 452**	**SA 700**	**SA 741**	**Hk BI203**	**Hz CMT 28**	**Hl ATCC 32044**	**Hs CBS 7238**
SA 66	-	93	96	98	96	98	98	96	96	96	98
SA 95	93	-	95	93	94	93	93	95	95	94	93
SA 253	96	95	-	96	97	96	96	100	96	96	96
SA 411	98	93	96	-	95	99	99	96	95	95	99
SA 452	96	94	97	95	-	95	92	96	96	95	92
SA 700	98	93	96	99	95	-	99	96	95	95	100
SA 741	98	93	96	99	92	99	-	93	95	93	99
Hk BI203	96	95	100	96	96	96	93	-	96	96	93
Hz CMT 28	96	95	96	95	96	95	95	96	-	96	95
Hl ATCC 32044	96	94	96	95	95	95	93	96	96	-	93
Hs CBS 7238	98	93	96	99	92	100	99	93	95	93	-

Table 2-57. Pairwise analysis of the LSU regions of members of the *Hannaella* group. Numbers indicate percent similarity between isolates. Hk-*Hannaella kunmingensis*; Hz-*Hannaella zeae*; Hl-*Hannaella luteola*; Hs-*Hannaella sinensis*.

	SA 66	**SA 95-1**	**SA 253**	**SA 700**	**SA 741**	**Hk BI203**	**Hz CMT 28**	**Hl AT 122**	**Hs CBS 7238**
SA 66	-	94	97	100	100	96	96	95	100
SA 95-1	94	-	96	94	94	93	92	92	91
SA 253	97	96	-	97	97	99	97	97	96
SA 700	100	94	97	-	100	96	96	95	100
SA 741	100	94	97	100	-	96	96	95	100
Hk BI203	96	93	99	96	96	-	97	97	96
Hz CMT 28	96	92	97	96	96	97	-	98	97
Hl AT 122	95	92	97	95	95	97	98	-	96
Hs CBS 7238	100	91	96	100	100	96	97	96	-

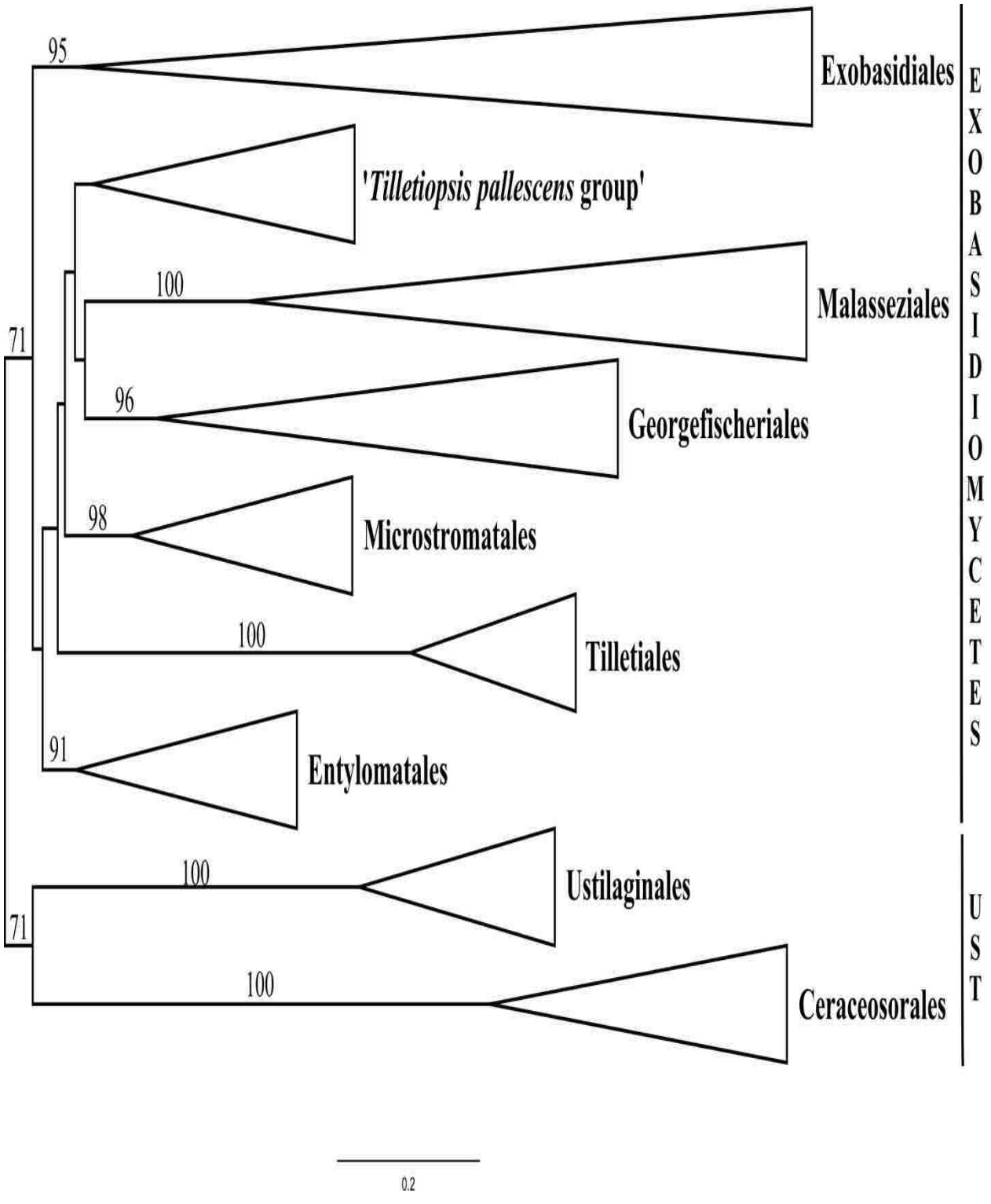


Figure 2-30. Phylogenetic relationships of seven orders in Tremellomycetes based on analyses of concatenated ITS and LSU sequences. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

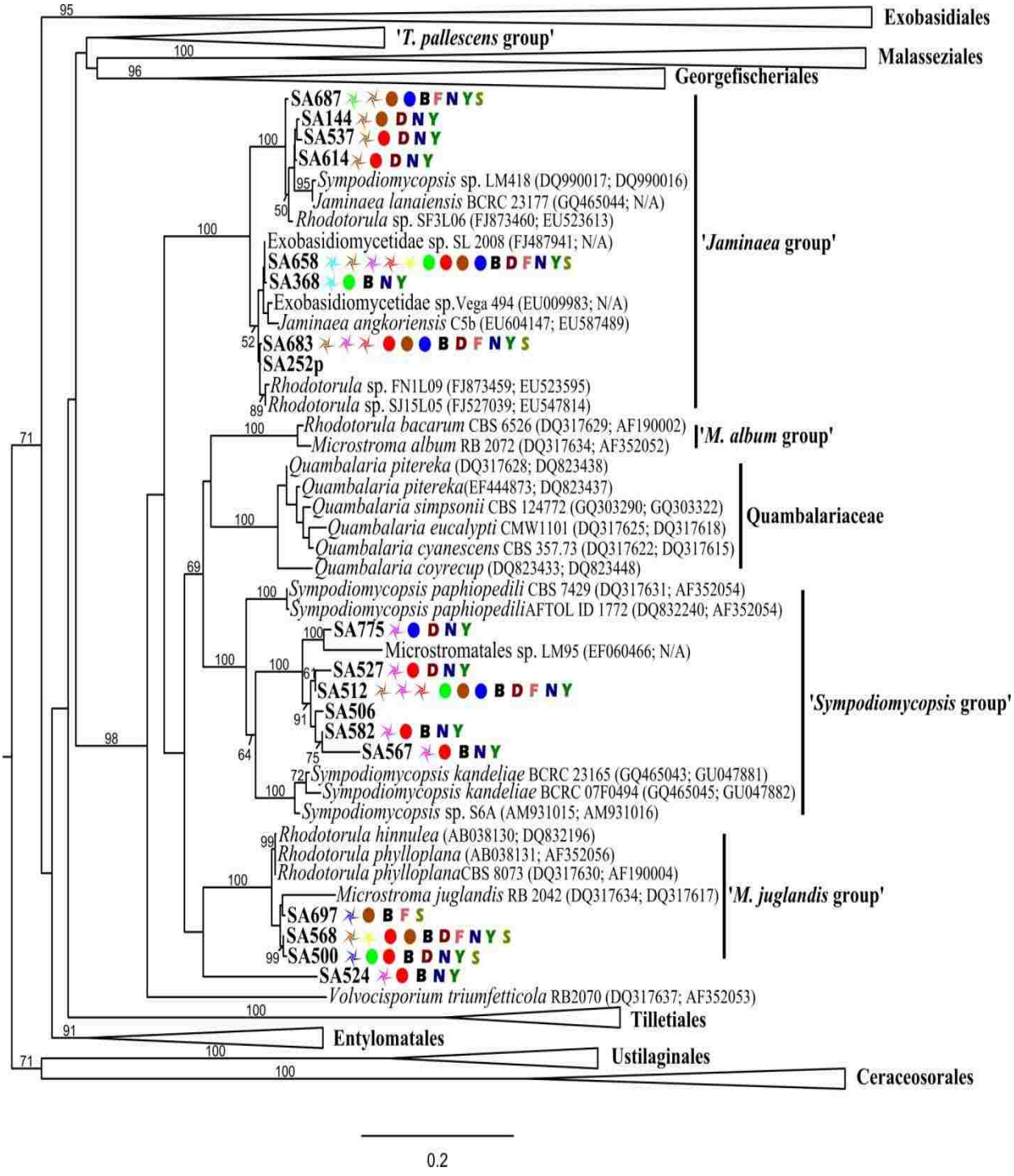


Figure 2-31. Phylogenetic relationships in the Microstromatales (Exobasidiomycetes). Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

Exobasidiomycetes

118 isolates were recovered from four of the eight recognized orders comprising Exobasidiomycetes *sensu* Begerow (2006). High bootstrap values support the placement of many isolates within various genera in the order Microstromatales. Figure 2-31 shows two dense clusters around *Jaminaea angkoriensis* strain C5b and *J. lanaiensis* strain BCRC.

Group J2 contains three small subgroups which are related to *J. angkoriensis* strain C5b in. In this group, all of the isolates for which both the ITS and LSU regions were sequenced share at least 99% sequence identity to each other in both regions. Phylogenetically, there seems to be more variation between group J2 isolates than those affiliated with *J. lanaiensis* (J1).

A second cluster of isolates was identified and can be ascribed to *Jaminaea lanaiensis* strain BCRC. The cluster around *J. lanaiensis* (group J1) shows little variability in the ITS region (Table 2-58). All isolates in this group share at least 99% sequence identity with *J. lanaiensis* strain BCRC. Additionally, the two other isolates included in this analysis (*Sympodiomycopsis* sp. strain LM418 and *Rhodotorula* sp. strain SF3L06) represent different phylotypes of *J. lanaiensis*. However, these results show that the members of groups J1 and J2 can be ascribed to *J. lanaiensis* and *J. angkoriensis*, respectively. The base pair substitutions in the ITS and LSU regions represent strain-specific single nucleotide polymorphisms, but not enough for species level variation.

Table 2-58. Pairwise analysis of the ITS regions of the J1 group. Numbers indicate percent similarity between isolates. Sym sp-*Sympodiomycopsis* species; Jl-*Jaminaea lanaiensis*; Rh sp-*Rhodotorula* species.

	SA614	**SA687**	**SA792**	**SA144**	**SA537**	**Sym LM418**	**Jl BCRC**	**Rh sp SF3L06**
SA614	-	99	99	99	99	99	99	99
SA687	99	-	100	99	99	99	99	100
SA792	99	100	-	99	99	99	99	100
SA144	99	99	99	-	100	99	99	99
SA537	99	99	99	100	-	99	99	99
Sym sp LM 418	99	99	99	99	99	-	99	99
Jl BCRC	99	99	99	99	99	99	-	99
Rh sp SF3L06	99	100	100	99	99	99	99	-

Table 2-59. Pairwise analysis of the ITS regions of the J2 group. Numbers indicate percent similarity between isolates. Ja-*Jaminaea angkoriensis*; Ex sp-Exobasidiomycetidae species; Rh sp-*Rhodotorula* species.

	SA252p	**SA368**	**SA658**	**SA683**	**Ja C5b**	**Ex sp Vega 494**	**Rh sp FN1L09**	**Rh sp SJ15L05**	**Ex sp SL2008**
SA252p	-	99	99	99	98	98	99	99	98
SA368	99	-	100	99	99	99	99	99	100

Table 2-59 cont.

	SA252p	SA368	SA658	SA683	Ja C5b	Ex sp Vega 494	Rh sp FN1L09	Rh sp SJ15L05	Ex sp SL2008
SA658	99	100	-	98	99	98	98	99	100
SA683	99	99	98	-	98	98	99	99	98
Ja C5b	98	99	99	98	-	99	98	98	99
Ex sp Vega 494	98	99	98	98	99	-	97	98	98
Rh sp FN1L09	99	99	98	99	98	97	-	99	98
Rh sp SJ15L05	99	99	99	99	98	98	99	-	99
Ex sp SL2008	98	100	100	98	99	98	98	99	-

Table 2-60. Pairwise analysis of the LSU regions of J2 group. Numbers indicate percent similarity between isolates. Ja-*Jaminaea angkoriensis*; Rh. sp-*Rhodotorula species*.

	SA252p	SA368	SA683	Ja C5b	Rh sp FN1L09	Rh sp SJ15L05
SA252p	-	99	100	99	99	100
SA368	99	-	99	99	99	99
SA683	100	99	-	99	99	100
Ja C5b	99	99	99	-	99	99
Rh sp FN1L09	99	99	99	99	-	99
Rh sp SJ15L05	100	99	100	99	99	-

In contrast to the *Jaminaea* isolates, there is considerably more sequence variability in the *Sympodiomycopsis* group (SG). *Sympodiomycopsis kandeliae* and *S. paphiopedili* share less than 90% ITS sequence identity. There is strong support for another branch sister to *S. kandeliae* that includes several SA isolates as well as Microstromatales sp. LM95 (Fig. 2-31).

SA775 occurs on the same branch as Microstromatales sp. LM95, but their ITS regions differ by 5% to each other and more than 10% when compared to *S. kandeliae* and *S. pahiopedili* (Table 2-62) and both of these isolates likely represent two different new species of *Sympodiomycopsis*.

There is high ITS sequence identity among the rest of SG. SA506, SA512, SA527 and SA583 share at least 99% ITS sequence identity to one another and also differ by more than 10% from several *S. kandeliae* and S. *paphiopedili* strains, but they occur on different branches within SG (Fig. 2-31). SA512 and SA527 are present on the same short branch, but do not appear to be identical. This may be due to the fact that the LSU region of SA527 was not sequenced. SA506 and SA583 share high ITS sequence identity, but differ by 3% from SA567, which occurs on a longer branch. Based on these results, it appears that in addition to SA775 and Microstromatales sp. LM95, there are one or two other new species in SG. SA506, SA512, SA527, SA582, and SA583 (SG2) are identical or closely related strains of a new species of *Sympodiomycopsisis* that is phylogenetically different from *S. kandeliae* strains BCRC 23165 and BCRC 07F0494 and *S. paphiopedili* strains CBS 7429 and AFTOL 1772. SA567 is also different from these two

Sympodiomycopsis species and shares only 97% identity with SG2. SA567 may be another species, or a just a different phylotype.

Table 2-61. Pairwise analysis of the ITS regions of SG. Numbers indicate percent similarity between isolates. Mic-Microstromatales species; Sk1-*Sympodiomycopsis kandeliae* strain BCRC 23165; Sk2-*S. kandeliae* strain BCRC 07F0494; Sp1-*Sympodiomycopsis paphiopedili* strain CBS 7429; Sp2-*Sympodiomycopsis paphiopedili* AFTOL 1772; Sym-*Sympodiomycopsis* species.

	SA 506	**SA 512**	**SA 527**	**SA 567**	**SA 583**	**SA 588**	**SA 775**	**Mic LM95**	**Sk1**	**Sk2**	**Sp1**	**Sp2**	**Sym S6A**
SA 506	-	100	100	97	99	89	96	93	88	88	88	89	88
SA 512	100	-	100	97	100	89	96	91	88	88	89	89	89
SA 527	100	100	-	98	100	89	96	91	88	88	89	89	89
SA 567	97	97	98	-	97	87	93	90	86	86	88	88	86
SA 583	99	100	100	97	-	89	96	92	88	88	89	89	89
SA 588	89	89	89	87	89	-	88	85	89	90	100	100	89
SA 775	96	96	96	93	96	88	-	95	87	88	87	87	88
Mic LM95	93	91	91	90	92	85	95	-	85	86	85	85	86
Sk1	88	88	88	86	88	89	87	85	-	99	89	89	98
Sk2	88	89	89	88	89	100	87	85	89	-	89	89	98
Sp1	88	89	89	88	89	100	87	85	89	89	-	100	89
Sp2	89	89	89	88	89	100	87	85	89	89	100	-	89
S6A	88	89	89	86	89	89	88	86	98	98	89	89	-

Table 2-62. Pairwise analysis of the ITS regions of SG. Numbers indicate percent similarity between isolates. Micro-Microstromatales species; Sk1-*Sympodiomycopsis kandeliae* strain BCRC 23165; Sk2-*S. kandeliae* strain BCRC 07F0494; Sp1-*Sympodiomycopsis paphiopedili* strain CBS 7429; Sp2-*Sympodiomycopsis paphiopedili* AFTOL 1772; Sym-*Sympodiomycopsis* species.

	SA506	**SA512**	**SA582**	**SA588**	**SA775**	**Sk1**	**Sk2**	**Sp1**	**Sp2**	**Sym S6A**
SA506	-	99	99	99	99	98	98	98	99	98
SA512	99	-	99	98	99	98	98	98	98	98
SA582	99	99	-	99	99	95	95	98	99	96
SA588	99	98	99	-	97	98	98	99	99	98
SA775	99	99	99	97	-	97	97	97	97	97
Sk1	98	98	95	98	97	-	99	98	98	99
Sk2	98	98	95	98	97	99	-	98	98	99

Table 2-62 cont.

	SA506	SA512	SA582	SA588	SA775	Sk1	Sk2	Sp1	Sp2	Sym S6A
Sp1	98	98	98	99	97	98	98	-	100	98
Sp2	99	98	99	99	97	98	98	100	-	98
S6A	98	98	96	98	97	99	99	98	98	-

Several putative Microstromatales isolates are part of a group (MG) comprised of *Microstroma juglandis* and several *Rhodotorula* species, though there was not strong bootstrap support for the branch with *M. juglandis*. SA500, SA568 and SA731 share 100% sequence identity in the ITS region. In the LSU region, values for SA500 and SA568 are above 99% (Table 2-63). An LSU sequence was not obtained for SA731. SA500, SA568 and SA731 differ from SA697 by 2% in the ITS region, but SA500 and SA568 shared identical LSU sequence identity with SA697 (Table 2-64). The dataset for MG also included two isolates of *R. phylloplana* and one of *R. hinnulea, R. bacarum* and *M. album.* It was noted that the *R. phylloplana* and *R. hinnulea* isolates are phylogenetically indistinguishable. The SA isolates share high sequence identity in both loci with both of these taxa and probably represent a different strain. The type species for *Microstroma* is *M. album*, but the genus is not monophyletic (Fig. 2-31). *Microstroma album* and *R. bacarum* are strongly supported as sister to *Quambalaria*, quite far from MG. Sequence analysis of these two taxa indicate that they are actually distinct species, varying significantly in their LSU sequences, but since *Rhodotorula* is polyphyletic, *R. bacarum* will eventually be synonymized.

SA254 could not be definitively placed within Microstromatales. SA524 was not strongly supported by bootstrap values, but it appears to be sister to MG. A Blastn search initially indicated that it belonged in Microstromatales, but ITS and LSU sequence data were ambiguous as to a definitive placement, though both loci place SA524 close to MG. Pairwise analysis of LSU sequences indicate that *R. hinnulea* strain AFTOL 1764 may be phylogenetically most similar (20 base pair substitutions), but ITS sequence comparisons are less telling (Table 2-63). SA524 shows only 90% ITS similarity to *M. juglandis* strain RB 2042, with query coverage just above 50%. Therefore, SA524 is certainly a new species within Microstromatales, but a new genus may need to be created to accommodate this isolate. Future phylogenetic analyses will need to include SSU sequence information and perhaps other loci to properly classify and resolve the position of this yeast.

Table 2-63. Pairwise analysis of the ITS regions of members of MG. Numbers indicate percent similarity between isolates. Mj-*Microstroma juglandis*; Rh-*Rhodotorula hinnulea*; Rp-*Rhodotorula phylloplana*; Vt- *Volvocisporium triumfetticola*; Ma-*Microstroma album*; Rb-*Rhodotorula bacarum.*

	SA 500	SA 524	SA 568	SA 697	SA 731	Mj RB 2042	Rh AFTOL 1764	Rp CBS 8073	Vt RB 2070	Ma RB 2072	Rb CBS 6526
SA 500	-	89	100	98	100	94	98	98	86	83	83
SA 524	89	-	89	89	89	90	89	89	87	88	88

Table 2-63 cont.

	SA 500	SA 524	SA 568	SA 697	SA 731	Mj RB 2042	Rh AFTOL 1764	Rp CBS 8073	Vt RB 2070	Ma RB 2072	Rb CBS 6526
SA 568	100	89	-	98	100	93	99	98	86	83	83
SA 697	98	89	98	-	98	93	98	98	86	84	83
SA 731	100	89	100	98	-	93	99	98	86	83	83
Mj RB 2042	94	90	93	93	93	-	93	93	81	83	83
Rh AFTOL 1764	98	89	99	98	99	93	-	99	86	84	84
Rp CBS 8073	98	89	98	98	98	93	99	-	85	84	84
Vt RB 2070	86	87	86	86	86	81	86	85	-	89	90
Ma RB 2072	83	88	83	84	83	83	84	84	89	-	99
Rb CBS 6526	83	88	83	83	83	83	84	84	90	99	-

Table 2-64. Pairwise analysis of the LSU regions of members of MG. Numbers indicate percent similarity between isolates. Abbreviations: Mj-*Microstroma juglandis*; Rh-*Rhodotorula hinnulea*; Rp-*Rhodotorula phylloplana*; Vt- *Volvocisporium triumfetticola*; Ma-*Microstroma album*; Rb-*Rhodotorula bacarum.*

	SA 500	SA 524	SA 568	SA 697	Mj RB 2042	Rh AFTOL 1764	Rp IGC 4246	Vt RB 2070	Ma RB 2072	Rb CBS 6526
SA500	-	98	99	100	99	99	99	94	95	94
SA524	98	-	98	97	97	99	97	94	96	95
SA568	99	98	-	100	99	99	99	94	95	94
SA697	100	97	100	-	99	99	99	94	95	94
Mj RB 2042	99	97	99	99	-	99	99	94	96	94
Rh AFTOL 1764	99	99	99	99	99	-	99	94	96	94
Rp IGC 4246	99	97	99	99	99	99	-	93	95	96
Vt RB 2070	94	94	94	94	94	94	93	-	93	91
Ma RB 2072	95	96	95	95	96	96	95	93	-	97
RbCBS 6526	94	95	94	94	94	94	96	91	97	-

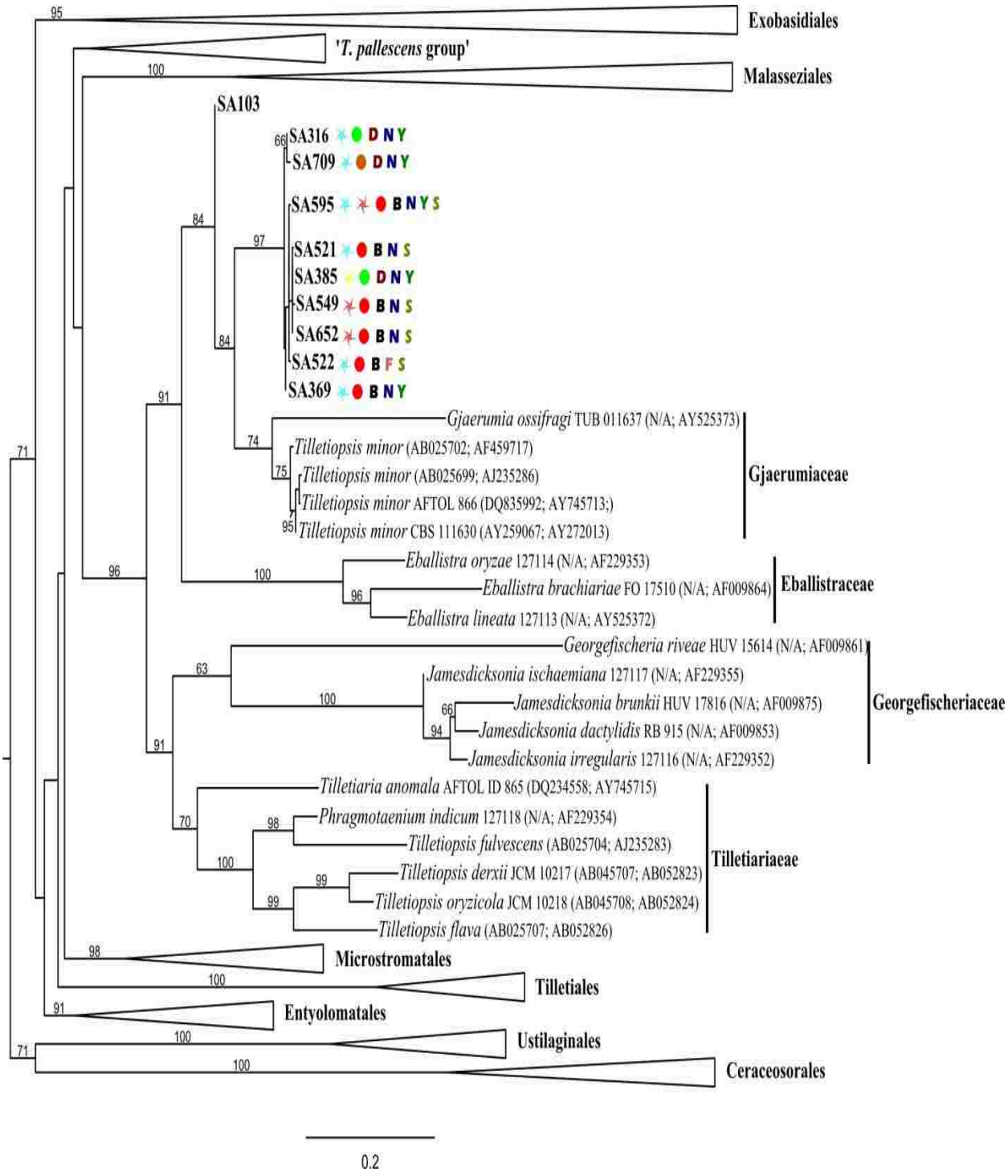


Figure 2-32. Phylogenetic relationships in the Georgefisheriales (Exobasidiomycetes). Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

The Georgefischeriales isolates from this survey are part of two well-supported clades sister to Gjaerumiaceae (Fig. 2-32). The first group (G1) contains nine isolates which are phylogenetically distinct from Gjaerumiaceae. These isolates share high sequence identity in both ITS and LSU regions with each other, but differ dramatically from *T. minor* and *G. ossifragi* with as many as 11-12% mismatches in the ITS region and 2-3% in LSU relative to *T. minor*. From a phylogenetic standpoint G1 does not belong in Gjaerumiaceae or any other family in Georgefischeriales, and may represent a new family.

The second group within Georgefischeriales includes only one isolate-SA103. Like the first group, SA103 does not belong to any of the four families in the order, but it is not part of G1 either. Rather, it occurs on an entirely different branch. The ITS region could not be sequenced for SA103, but phylogenetic analysis of the LSU region indicates that it may represent a new member of Gjaerumiaceae. SA103 shares 98% sequence identity to all *T. minor* isolates and to all members of G1.

Table 2-65. Pairwise analysis of the ITS region of members of the Tm group. Numbers indicate percent similarity between isolates.Tm1-*Tilletiopsis minor* strain AFTOL 866; Tm2-*Tilletiopsis mino*r strain CBS111630; Tm3-*Tilletiopsis minor* strain JCM 8709; Tm4- Tilletiopsis minor strain JCM 8361.

	SA 191	**SA 316**	**SA 369**	**SA 520**	**SA 521**	**SA 522**	**SA 549**	**SA 595**	**SA 652**	**Tm1**	**Tm2**	**Tm3**	**Tm4**
SA 191	-	100	99	99	99	99	99	99	99	88	89	89	88
SA 316	100	-	99	99	99	99	99	99	99	88	89	89	88
SA 369	99	99	-	99	99	99	99	99	99	88	89	89	88
SA 520	99	99	99	-	99	99	98	100	99	88	89	88	89
SA 521	99	99	99	99	-	99	99	99	100	89	89	89	88
SA 522	99	99	99	99	99	-	99	99	99	88	89	88	88
SA 549	99	99	99	98	99	99	-	98	99	88	88	88	88
SA 595	99	99	99	100	99	99	98	-	99	88	89	89	89
SA 652	99	99	99	99	100	99	99	99	-	89	89	89	88
Tm1	88	88	88	88	89	88	88	88	89	-	99	99	99
Tm2	89	89	89	89	89	89	88	89	89	99	-	99	99
Tm3	89	89	89	88	89	88	88	89	89	99	99	-	99
Tm4	88	88	88	89	88	88	88	89	88	99	99	99	-

Table 2-66. Pairwise analysis of the LSU regions of members of the Tm group. Numbers indicate percent similarity between isolates.Go-*Gjaerumia ossifragi* strain TUB 011637; Tm1-*Tilletiopsis minor* strain AFTOL 866; Tm2-*Tilletiopsis minor* strain CBS111630; Tm3-*Tilletiopsis minor* strain KCTC; Tm4- *Tilletiopsis minor* strain CBS 346.33.

	SA 103	**SA 258**	**SA 316**	**SA 385**	**SA 520**	**SA 521**	**SA 522**	**SA 549**	**SA 595**	**SA 652**	**SA 709**	**Go**	**Tm1**	**Tm2**	**Tm3**	**Tm4**
SA 103	-	98	98	98	98	98	98	98	98	98	98	95	98	98	98	97
SA 258	98	-	99	100	100	99	99	100	100	100	99	94	99	99	99	98
SA 316	98	99	-	99	99	99	99	99	99	99	100	94	99	98	99	98

Table 2-66 cont.

	SA 103	SA 258	SA 316	SA 385	SA 520	SA 521	SA 522	SA 549	SA 595	SA 652	SA 709	Go	Tm1	Tm2	Tm3	Tm4
SA 385	98	100	99	-	100	99	99	100	100	100	99	98	99	99	99	98
SA 520	98	100	99	100	-	99	99	100	100	100	99	94	99	99	99	98
SA 521	98	99	99	99	99	-	99	99	99	99	99	94	98	98	99	98
SA 522	98	99	99	99	99	99	-	99	99	99	99	94	99	98	99	98
SA 549	98	100	99	100	100	99	99	-	100	100	99	94	99	99	99	98
SA 595	98	100	99	100	100	99	99	100	-	100	99	94	99	99	99	98
SA 652	98	100	99	100	100	99	99	99	99	-	99	94	99	99	99	98
SA 709	98	99	100	99	99	99	99	99	99	99	-	94	98	98	99	98
Go	95	94	94	98	94	94	94	94	94	94	94	-	94	94	94	93
Tm1	98	99	99	99	99	98	99	99	99	9	98	94	-	99	99	99
Tm2	98	99	98	99	99	98	98	99	99	99	98	94	99	-	99	100
Tm3	98	99	99	99	99	99	99	99	98	98	98	93	99	100	-	98
Tm4	97	98	98	98	98	98	98	98	98	98	98	93	99	100	98	-

SA569 represents an undescribed *Meira* species and differs by 8% from *M. geulakonigii* strain PM 2 in the ITS region. Multiple attempts at sequencing the LSU region only resulted in contigs composed of mixed products, so that locus was not included in the final analysis. However, there is strong bootstrap support that SA569 belongs to *Meira* and is distinct from *M. geulakonigii*. SA187, SA608, SA242 and SA767 share at least 99% ITS sequence identity to *M. argovae* strain AS006 and all resemble this phyloptype with very little rDNA variation. SA226 and SA227 share 100% ITS sequence identity and differ by less than 2% from *M. nashicola* strain AS 006 (Table 2-67). The eight base pair substitutions in this region are not enough to delimit a new species, so both of these isolates represent a different phylotype.

Table 2-67. Pairwise analysis of the ITS regions of members of related *Meira* species. Numbers indicate percent similarity between isolates. Ma-*Meira argovae*; Mg-*Meira geulakonigii*; Mn-*Meira nashicola*.

	SA 187	SA 242	SA 608	SA 226	SA 227	SA 569	SA 767	Ma AS 006	Mg PM 2	Mn PFS 002
SA187	-	99	100	88	88	89	99	100	91	87
SA242	99	-	99	87	87	88	99	99	91	87
SA608	100	99	-	87	87	87	100	100	89	88
SA226	88	87	87	-	100	85	87	88	88	99
SA227	88	87	87	100	-	85	87	88	87	99
SA569	89	88	87	85	85	-	87	87	92	85
SA767	99	99	100	87	87	87	-	99	89	88
Ma AS 006	100	99	100	88	88	87	99	-	89	88
Mg PM 2	91	91	89	88	87	92	89	89	-	86
Mn PFS 002	87	87	88	99	99	85	88	88	86	-

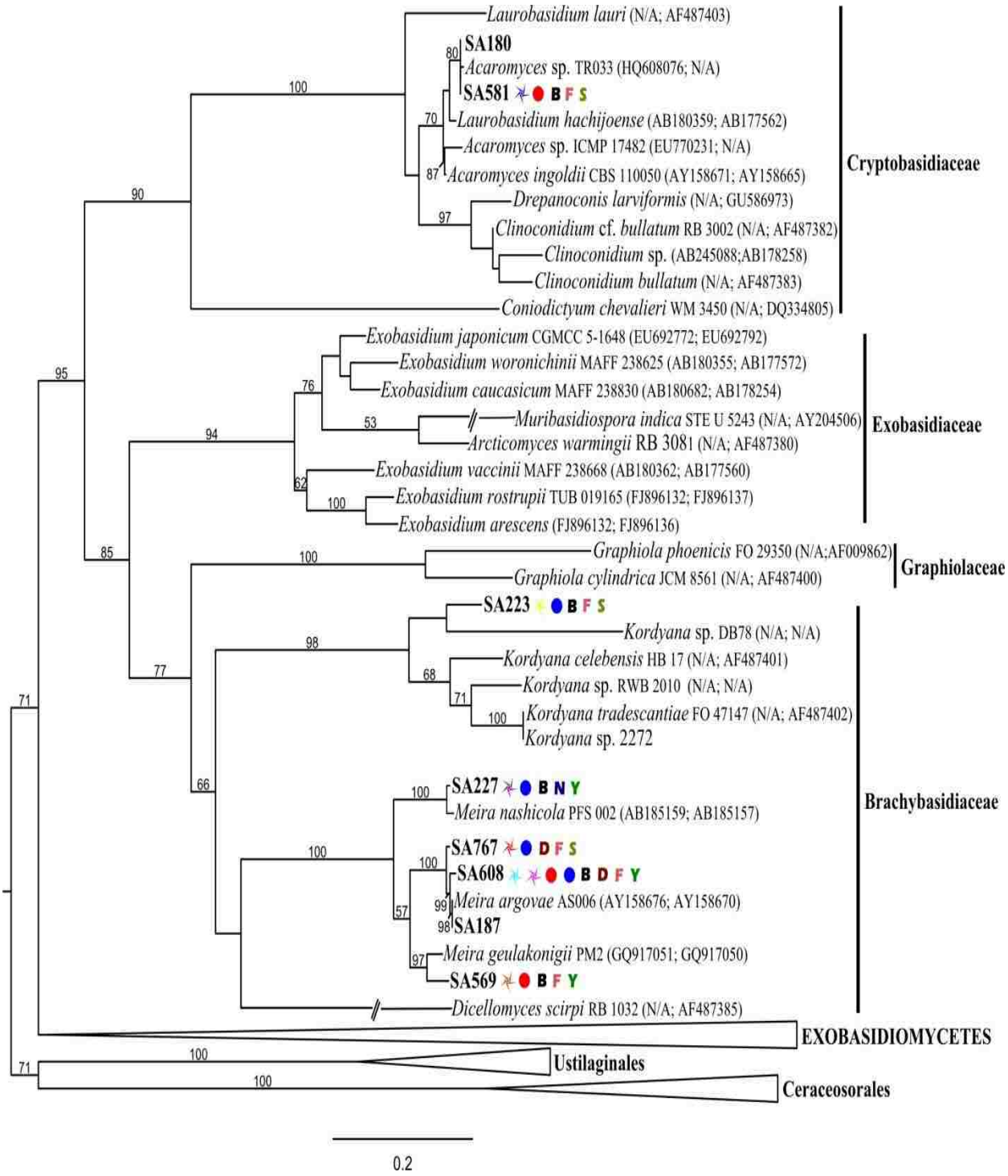


Figure 2-33. Phylogenetic relationships in the Exobasidiales (Exobasidiomycetes). Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

SA223 was isolated once from *T. kunthii* and represents a new species of *Kordyana*. This isolate was an interesting find since it is part of a group of somewhat rare tropical pathogens of *Commelina*. *Kordyana* belongs in Brachybasidiaceae and like *Dicellomyces*, *Proliferobasidium* and *Brachybasidium*, sporulates externally on the surface of the host organs instead of in host galls (Begerow, *et al.*, 2002).

The topology shows that the genus *Kordyana* is monophyletic (Fig. 2-33). According to this phylogeny, there are five phylogenetically different species within the genus. Of the five LSU sequences analyzed in addition to SA223, four of these appear to represent different species. Due to the lack of other loci, only the LSU region of SA223 was compared to the other isolates, but based on that information, there appears to be significant ITS and LSU heterogeneity between different species. *Kordyana* sp. FO 2272 (Begerow, unpublished data) was the only sequence in that could be definitely associated with one of the two described species, as it appears to correspond phylogenetically to *K. tradescantiae* strain F0 47147. SA223 shares 97% LSU sequence identity (13 base pair substitutions) with *Kordyana* sp. RWB-2010 (Table 2-68), an isolate from Brazil (Barreto, *et al*., 2010).

Table 2-68. Pairwise analysis of the LSU regions of members of the *Kordyana* group. Numbers indicate percent similarity between isolates. Kc- *Kordyana celebensis*; Kt-*Kordyana tradescatiae*; Kordy sp-*Kordyana species.*

	SA 223	**Kc HB 17**	**Kt F0 47147**	**Kordy sp RB 2010**	**Kordy sp F0 2272**	**Kordy sp DB 78**
SA223	-	96	95	97	95	95
Kc HB 17	96	-	96	97	96	93
Kt F0 47147	95	96	-	96	99	93
Kordy sp RB 2010	97	97	96	-	96	95
Kordy sp F0 2272	95	96	99	96	-	93
Kordy sp DB 78	95	93	93	95	93	-

Cryptobasidiaceae is strongly supported as part of Exobasidiales. SA180 and SA581 form part of an *Acaromyces* cluster that includes *L. hachijoense*. However, the type, *L. lauri* is not part of this group, but instead occurs on a single branch sister to this group. In a study of an outbreak of *Coniodyctium chevalieri* (Maier, *et al.*, 2006), the authors suggested the monophyly of *Laurobasidium*. However, this analysis indicates that *L. lauri* and *L. hachijoense* occur on different branches of Cryptobasidiaceae, thus making *Laurobasidium* paraphyletic. SA581 was isolated just once from *D. erythrosora*. SA180 was not found on a fern, but from the leaves of *Rhododendron indicum*. Both of these isolates were part of the *Acaromyces* clade and share high ITS sequence identity (Table 2-69).

Table 2-69. Pairwise analysis of the LSU regions of SA581 and related taxa. Numbers indicate percent similarity between isolates. Ai-*Acaromyces ingoldii*; Clino. sp.*Clinoconidium* species; Cb-*Clinoconidium bullatum*; Cc-*Conidiodyctium chevalieri*; Dl-*Drepanoconis larviformis*; Lh-*Laurobasidium hachijoense*; Ll-*Laurobasidium lauri.*

	SA 581	**Ai CBS 110050**	**Clino. sp TUK S703**	**Cb RB 3002**	**Cb 190077**	**Cc WM 3450**	**Dl MP 4520**	**Lh MAFF 238668**	**Ll MP 2371**
SA 581	-	99	96	98	96	87	96	99	96

Table 2-69 cont.

	SA 581	Ai CBS 110050	Clino. sp TUK S703	Cb RB 3002	Cb 190077	Cc WM 3450	Dl MP 4520	Lh MAFF 238668	Ll MP 2371
Ai. CBS 110050	99	-	97	98	97	85	97	100	97
Clino sp S703	96	97	-	99	97	85	97	97	95
Cb RB 3002	98	98	99	-	99	86	98	98	96
Cb 190077	96	97	97	99	-	84	98	97	95
Cc WM 3450	87	85	85	86	84	-	85	85	84
Dl MP 4520	96	97	97	98	98	85	-	97	96
Lh MAFF 238668	99	100	97	98	97	85	97	-	97
Ll MP 2371	96	97	95	96	95	84	96	97	-

Table 2-70. Pairwise analysis of the LSU regions of SA581 and related taxa. Numbers indicate percent similarity between isolates. Clino. sp-*Clinoconidium* species; Lh-*Laurobasidium hachijoense*; Ai-*Acaromyces ingoldii*; Acar. sp-*Acaromyces species.*

	SA 180	SA 581	Clino sp TUK S703	Lh MAFF 238668	Ai CBS 110050	Acar. sp TR 033	Acar. sp 1742
SA 180	-	99	84	98	98	97	99
SA 581	99	-	84	98	98	97	99
Clino sp TUK S703	84	84	-	85	84	84	85
Lh MAFF 238668	98	98	85	-	97	97	97
Ai CBS 110050	98	98	84	97	-	97	98
Acar. sp TR 033	97	97	84	97	97	-	96
Acar. sp 1742	99	99	85	97	98	96	-

No isolates from Graphiolaceae and Exobasidiaceae were recovered. In the topology, Graphiolaceae is represented by *Graphiola phoenicis* and *G. cylindrical* and is sister to Brachybasidiaceae and Exobasidiaceae includes *Arcticomyces warmingii* and *Muribasidiospora indica* (Fig. 2-33).

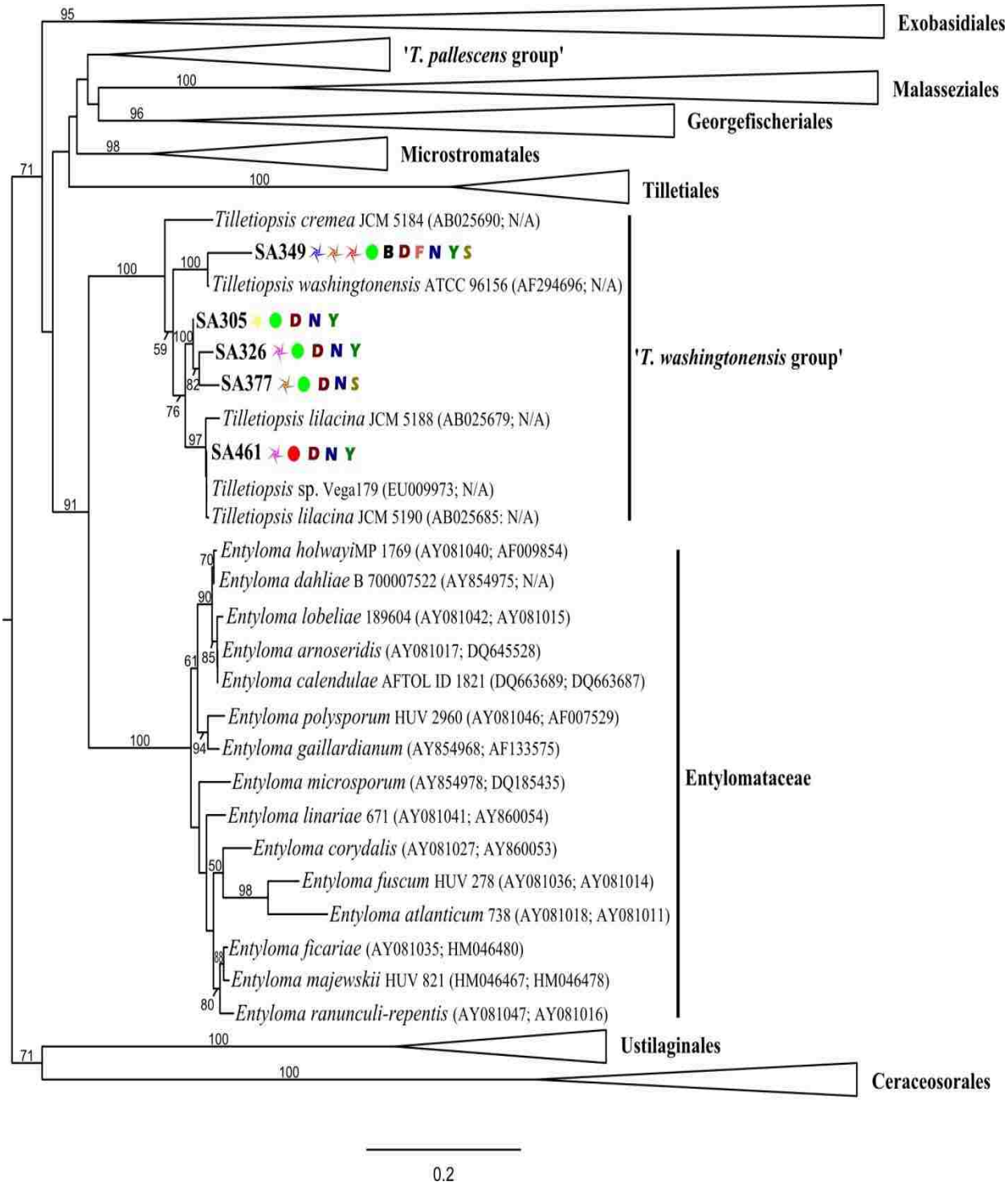


Figure 2-34. Phylogenetic relationships in the Entylomatales (Exobasidiomycetes). Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

Many isolates were recovered from Entylomatales, all of which belong to the '*Tilletiopsis washingtonensis* group' (Hamamoto, *et al.*, 2000). The overwhelming majority of these isolates were identified as *Tilletiopsis lilacina* and several are phylotypes of *T. washingtonensis*. None show close affinity to *T. cremea*. There was no heterogeneity in the ITS region of the *T. lilacina* isolates, though there are some nucleotide polymorphisms between the SA isolates and other strains of *T. lilacina* from GenBank. However, these variations are not more than 1% of the entire ITS sequence (Table 2-71).

The '*T. washingtonensis* group' is strongly supported in this analysis which agrees with previous studies which have shown that *T. washingtonensis*, *T. lilacina* and *T. cremea* are each clearly defined species (Yamazaki, *et al.*, 1985, Begerow, *et al.*, 2000, Hamamoto, *et al.*, 2000, Begerow, *et al.*, 2002). For the *T. lilacina* isolates where the LSU region was sequenced all identities were 100%. However, it was not possible to compare the SA LSU sequences of SA isolates to any others since no *T. lilacina* LSU sequences have been deposited into GenBank.

There is a 4-5% difference in ITS sequences of *T. lilacina* and *T. washingtonensis* and 6% between *T. cremea* and *T. washingtonensis*. SA304, SA326 and SA337 cannot be ascribed to any of the three described species in the '*T. washingtonensis* group.' These three isolates share greater than 99% ITS sequence identity (Table 2-71) and occur on a branch sister to *T. lilacina*. Although they appear farther from *T. lilacina* on the tree, these isolates do not seem to represent a new species in this group, but are more likely phylotypes of *T. lilacina*.

Table 2-71. Pairwise analysis of the ITS regions of the *T. washingtonensis* group. Numbers indicate percent similarity between isolates. Tl-*Tilletiopsis lilacina*; T sp-*Tilletiopsis* species; Tw-*Tilletiopsis washingtonensis*; Tc-*Tilletiopsis cremea.*

	SA 304	**SA 326**	**SA 349**	**SA 377**	**SA 451**	**SA 461**	**Tl 1 JCM 5188**	**Tl 2 JCM 5190**	**T sp Vega 179**	**Tw ATCC 96156**	**Tc JCM 5184**
SA 304	-	99	96	99	99	99	97	98	99	97	94
SA 326	99	-	96	99	98	98	98	98	98	96	93
SA 349			-								
SA 377	99	99	95	-	98	98	97	97	98	95	93
SA 451	99	98	96	98	-	100	99	99	100	96	93
SA 461	99	98	96	98	100	-	99	99	100	96	93
Tl 1 JCM 5188	97	98	95	97	99	99	-	99	99	95	92
Tl 2 JCM 5190	98	98	95	97	99	99	99	-	99	95	93
T sp Vega 179	99	98	96	98	100	100	99	99	-	96	92
Tw ATCC 96156	97	96	99	95	96	96	95	95	96	-	94
Tc JCM 5184	94	93	94	93	93	93	92	93	92	94	-

Table 2-72. Pairwise analysis of the ITS regions of members of the *Tilletiopsis* group. Numbers indicate percent similarity between isolates. Tl-*Tilletiopsis lilacina*; Til. sp-*Tilletiopsis* species; Tw-*Tilletiopsis washingtonensis*; Tc-*Tilletiopsis cremea.*

	SA 304	**SA 326**	**SA 349**	**SA 377**	**SA 451**	**SA 461**	**Tl1 JCM 5188**	**Tl2 JCM 5190**	**Til. sp Vega 179**	**Tw ATCC 96156**	**Tc JCM 5184**
SA 304	-	99	96	99	99	99	97	98	99	97	94

Table 2-72 cont.

	SA 304	SA 326	SA 349	SA 377	SA 451	SA 461	Tl1 JCM 5188	Tl2 JCM 5190	Til. sp Vega 179	Tw ATCC 96156	Tc JCM 5184
SA 326	99	-	96	99	98	98	98	98	98	96	93
SA 349	96	96	-	95	96	96	95	95	96	99	94
SA 377	99	99	95	-	98	98	97	97	98	95	93
SA 451	99	98	96	98	-	100	99	99	100	96	93
SA 461	99	98	96	98	100	-	99	99	100	96	93
Tl1 JCM 5188	97	98	95	97	99	99	-	99	99	95	92
Tl2 JCM 5190	98	98	95	97	99	99	99	-	99	95	93
Til. sp Vega 179	99	98	96	98	100	100	99	99	-	96	92
Tw ATCC 96156	97	96	99	95	96	96	95	95	96	-	94
Tc JCM 5184	94	93	94	93	93	93	92	93	92	94	-

The placement of *Tilletiopsis pallescens* is not definitively resolved within Exobasidiomycetes, though some phylogenetic analyses suggest that it may be sister to Microstromatales (Takashima & Nakase, 2001, Begerow, *et al.*, 2006, de Beer, *et al.*, 2006). There is notable rDNA sequence variability between the different GenBank isolates especially in the ITS region. The four isolates recovered during this survey are part of the '*T. pallescens* group.' In the ITS region SA448, SA591 and SA600 are all at least 98% similar to each other and to *T. pallescens* M115, *T. pallescens* JCM 10446 and to *T. pallescens* CBS 606.83 (Table 2-73). However, SA259, shares less than than 97% ITS sequence identity to every other member of the group, indicating that SA259 does not represent an isolate of *T. pallescens*.

Table 2-73. Pairwise analysis of the ITS regions of members of the *T. pallescens* group. Numbers indicate percent similarity between isolates. Tp1-*Tilletiopsis pallescens* strain F 3370; Tp2-*Tilletiopsis pallescens* strain CBS 606.83; Tp3-*Tilletiopsis pallescens* strain JCM 8711; Tp4-*Tilletiopsis pallescens* strain JCM 10446; Tp5-*Tilletiopsis pallescens* strain M 115

	SA259	SA448	SA591	SA600	Tp1	Tp2	Tp3	Tp4	Tp5
SA259	-	96	95	96	88	96	92	95	96
SA448	96	-	99	99	88	100	94	99	99
SA591	95	99	-	99	88	99	94	98	99
SA600	96	99	99	-	87	99	94	99	99
Tp1	88	88	88	87	-	88	89	88	88
Tp2	96	100	99	99	88	-	94	99	99
Tp3	92	94	94	94	89	94	-	94	94
Tp4	95	99	98	99	88	99	94	-	99
Tp5	96	99	99	99	88	99	94	99	-

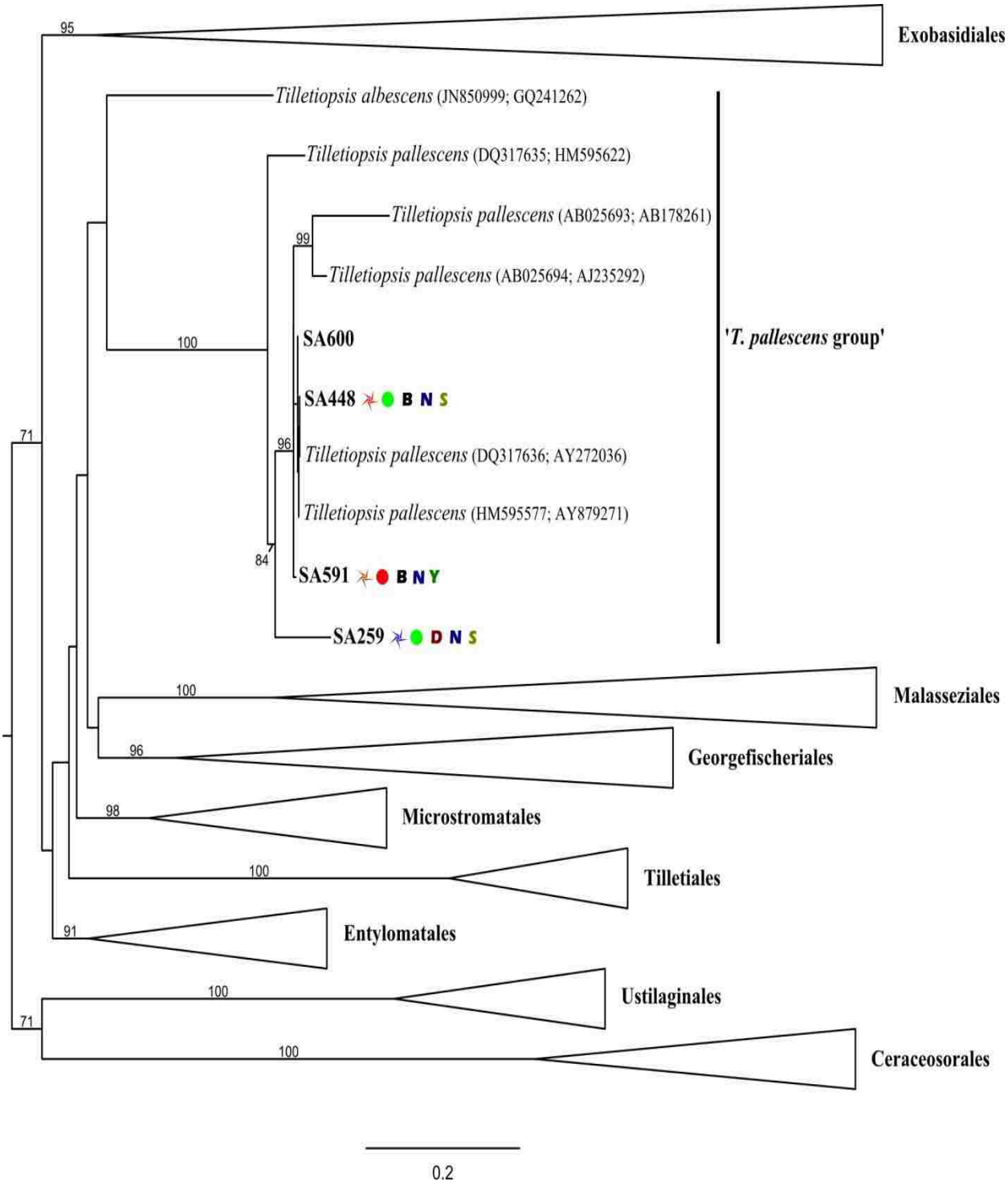


Figure 2-35. Phylogenetic relationships in the '*Tilletiopsis pallescens* group' (Exobasidiomycetes). Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

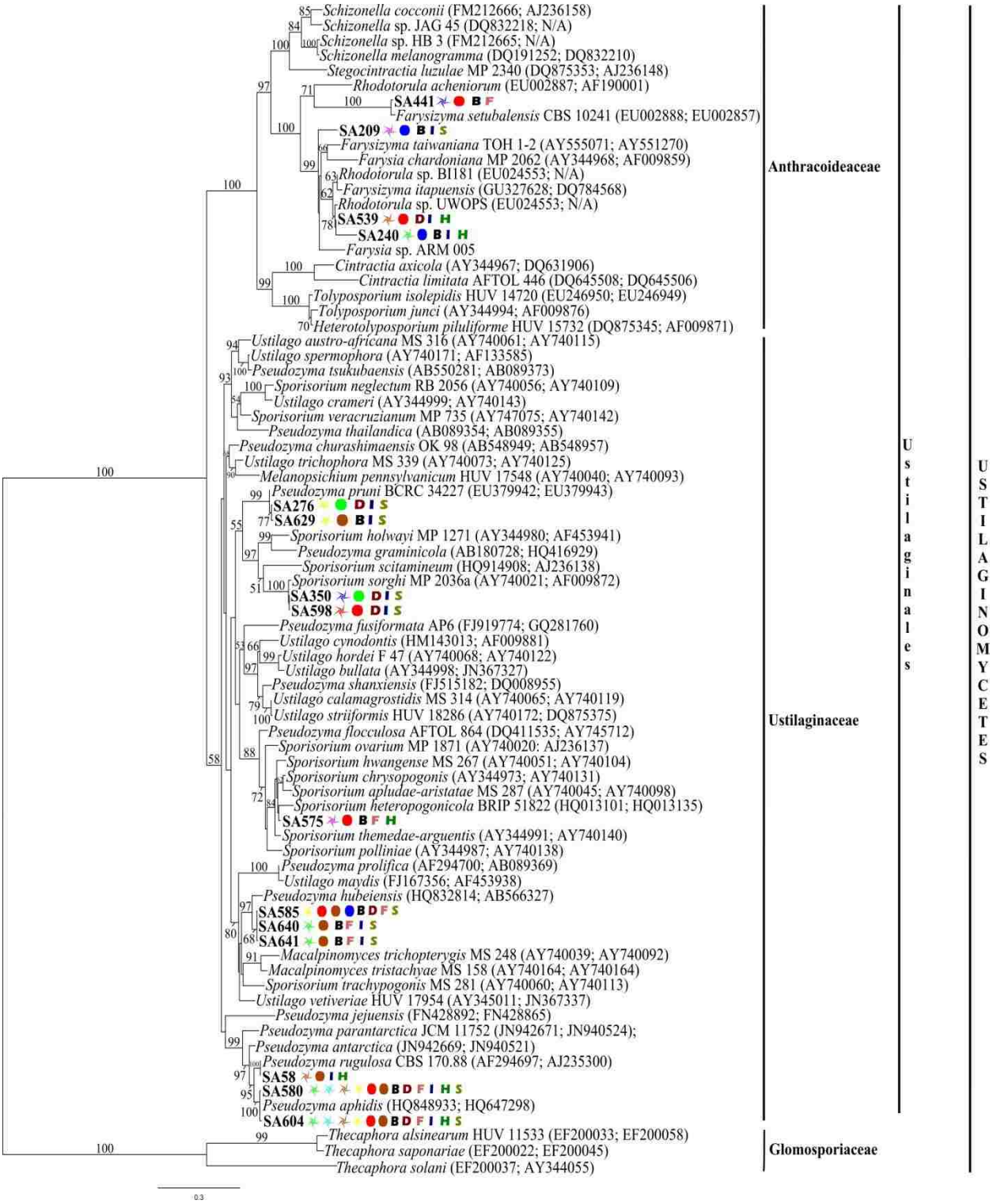


Figure 2-36. Phylogenetic relationships in the '*Tilletiopsis pallescens* group' (Exobasidiomycetes). Results are based on analyses of concatenated ITS and LSU sequences. In parentheses, ITS Genbank accession numbers of ITS sequences are listed first followed by LSU. If a sequence was not obtained this is indicated as N/A. Bootstrap values above 50% are indicated. Ustilaginales and Ceraceosorales were used as outgroups.

Ustilaginomycetes

All of the ustilaginomycetous isolates recovered during this study belong to Ustilaginales and are distributed between the families Anthracoideaceae and Ustilaginaceae. The isolates within Anthracoideaceae form a well-supported clade sister to *Schizonella melanogramma* and show high rDNA sequence identity to species within the '*Farysia* clade' which includes the anamorphic genus *Farysizyma* as well as several species of *Farysia*, a dimorphic smut pathogen on *Carex* spp. (Inacio, *et al.*, 2008).

Pairwise sequence comparison of the ITS regions of four SA isolates with all known *Farysizyma* species and all available *Farysia* sequences shows that SA209 shows differs by 38 and 41 bases with respect to *Farysizyma itapuensis* strain BI181 and SA240, respectively, and sequence identity values are even lower compared to the other taxa (Table 2-74). There are 68 mismatches between SA209 and SA539 and 66 mismatches between SA209 and SA441. SA240 and SA539 both share highest ITS sequence identity with *F. itapuensis* strains CBS 10244 and ATCC 4547, each differing by 11 and 12 bases, respectively. SA240 and SA539 differ from each other by two bases and from SA441 by 91 and 90 bases, respectively. SA441shares 100% ITS sequence identity with *Farysizyma setubalensis* strain CBS 10241, but differs starkly from all other species.

None of the other isolates are identical in the LSU region (Table 2-75). SA209 differs by more than 10 bases from all other taxa, but the fewest number of mismatches occurs between *Farysizyma taiwaniana* strain TOH 1-2 and *Farysia themenii* strain ZPU7 with 11 and 12 bases, respectively. There are eight substitutions between SA240 and SA539, but only one nucleotide polymorphism between SA539 and *F. itapuensis* strains BI120, BI181 and BI238. SA441 is identical to *Rhodotorula* cf. *taiwaniana* strain ATT 070 and differs from *F. setubalensis* strain 10241 by two bases.

Based on the phylogenetic data and results from the pairwise comparisons, SA209 has a significantly different rDNA profile than that of any known species of *Farysizyma* and represents a new species within the genus. SA240 and SA539 are two different phylotypes of *F. itapuensis* and share 98% ITS sequence identity with strains BI120, BI181 and BI238, but do not correspond exactly to any of these. SA441 is a phylotype of *F. setubalensis* similar to strain CBS 10241 and identical to *Rhodotorula* cf. *taiwaniana* strain CBS 10244, indicating that *R.* cf. *taiwaniana* actually represents *F. setubalensis*.

Table 2-74. Pairwise analysis of the ITS regions of members of the '*Farysia* clade'. Numbers indicate percent similarity between isolates. Fa-*Farysizyma acheniorum*; Fi-*F. itapuensis*; Fs-*F. setubalensis*; Ft-*F. taiwaniana*; Fc-*Farysia chardoniana.*

	SA 209	**SA 240**	**SA 441**	**SA 539**	**Fa BS 10244**	**Fi ATCC 4547**	**Fi BI181**	**Fs CBS 10241**	**Ft TOH 1-2**	**Fc MP 2062**
SA 209	-	93	86	90	87	92	93	83	91	88
SA 240	93	-	83	99	86	98	98	83	93	90
SA 441	86	83	-	83	83	84	84	100	84	84
SA 539	90	99	83	-	85	98	98	81	90	90
Fa CBS 10244	87	86	83	85	-	86	86	80	85	85
Fi ATCC 4547	92	98	84	98	86	-	99	82	91	90

Table 2-74 cont.

	SA 209	SA 240	SA 441	SA 539	Fa BS 10244	Fi ATCC 4547	Fi BI181	Fs CBS 10241	Ft TOH 1-2	Fc MP 2062
Fi BI181	93	98	84	98	86	99	-	82	90	90
Fs CBS 10241	83	83	100	81	80	82	82	-	81	82
Ft TOH 1-2	91	93	84	90	85	91	90	81	-	87
Fc MP 2062	88	90	84	90	85	90	90	82	87	-

Table 2-75. Pairwise analysis of the LSU regions of members of the '*Farysia* clade.'.Numbers indicate percent similarity between isolates. Fa-*Farysizyma acheniorum*; Fi-*F. itapuensis*; Fs-*F. setubalensis*; Ft-*F. taiwaniana*; Fc-*Farysia chardoniana*; F ch-*Farysia chardoniana.*

	SA 209	SA 240	SA 441	SA 539	Fa CBS 6386	Fi BI120	Fi BI181	Fi BI238	Fs CBS 10241	Ft TOH 2-1	F ch MP 2062	F th ZPU7
SA 209	-	99	99	99	98	98	98	98	97	98	97	98
SA 240	99	-	99	99	96	98	99	99	94	97	98	97
SA 441	99	99	-	99	98	98	98	98	99	98	96	98
SA 539	99	99	99	-	98	99	100	100	98	99	98	98
Fa CBS 6386	98	96	98	98	-	98	98	98	98	98	97	100
Fi BI120	98	98	98	99	98	-	99	99	97	99	98	98
Fi BI181	98	99	98	100	98	99	-	100	98	99	98	98
Fi BI238	98	99	98	100	98	99	100	-	98	99	98	98
Fs CBS 10241	97	94	99	98	98	97	98	98	-	98	96	98
Ft TOH 2-1	98	97	98	99	98	99	99	99	98	-	98	98
F ch MP 2062	97	98	96	98	97	98	98	98	96	98	-	97
F th ZPU7	98	97	98	98	100	98	98	98	98	98	97	

The remaining ustilaginomycetous isolates are distributed throughout Ustilaginaceae and are resolved in several subgroups comprised of *Sporisorium* and *Pseudozyma* species. Several isolates are close phylogenetic relatives of *Pseudozyma hubeiensis* strain LH 146 and form part of a larger subgroup which includes *Ustilago maydis* strain XA 0609 and *P. prolifica* strain CBS 31987. SA585 and SA641 share 100% ITS sequence identity with each other and differ from SA640 by one nucleotide polymorphism (Table 2-76). Pairwise sequence comparison of SA585 to SA641 and SA640 to *P. hubeiensis* strain LH 146 revealed 18 and 15 mismatches, respectively. Subsequent comparisons to *Sporisorium trachypogonis* strain MS 281, *Macalpinomyces trichopterygis* strain MS 158, *U. maydis* strain XA 0609, *P. prolifica* strain 31987, and *U. vetiveriae* strain HUV 17954 shows between 49-86 mismatches, suggesting much a more distant phylogenetic relationship between the SA isolates and the rest of the group.

In the LSU region there are two substitutions between SA585 and strain LH 146 (LSU sequences for SA640 and SA641 were not obtained). Sequence identity values between SA585

and all other strains are much lower, ranging from 12-39 mismatches (Table 2-77). Despite the ITS variability between SA isolates and strain LH 146, SA585, SA640 and SA641 are not new species, but phylotypes of *P. hubeiensis.*

Table 2-76. Pairwise analysis of the ITS regions of members of the *Pseudozyma hubeiensis* group. Numbers indicate percent similarity between isolates. Ph-*Pseudozyma hubeiensis*; St-*Sporisorium trachypogonis*; Mt-*Macalpinomyces trichopterygis*; Um-*Ustilago maydis*; Pp-*Pseudozyma prolifica*; Uv-*Ustilago vetiveriae.*

	SA 585	**SA 640**	**SA 641**	**Ph LH 146**	**St MS 281**	**Mt MS 248**	**Mt MS 158**	**Um XA 0609**	**Pp CBS 31987**	**Uv HUV 17954**
SA 585	-	99	100	98	90	88	89	88	87	91
SA 640	99	-	99	98	91	88	90	87	87	92
SA 641	100	99	-	98	91	88	90	88	88	92
Ph LH 146	98	98	98	-	91	89	91	88	88	91
St MS 281	90	91	91	91	-	88	90	86	86	91
Mt MS 248	88	88	88	89	88	-	94	84	84	91
Mt MS 158	89	90	90	91	90	94	-	85	86	89
Um XA 0609	88	87	88	88	86	84	85	-	99	86
Pp CBS 31987	87	87	88	88	86	84	86	99	-	86
Uv HUV 17954	91	92	92	91	91	91	89	86	86	-

Table 2-77. Pairwise analysis of the LSU regions of members of the *Pseudozyma hubeiensis* group. Numbers indicate percent similarity between isolates. Ph-*Pseudozyma hubeiensis*; St-*Sporisorium trachypogonis*; Mt-*Macalpinomyces trichopterygis*; Um-*Ustilago maydis*; Pp-*Pseudozyma prolifica*; Uv-*Ustilago vetiveriae.*

	SA 585	**Ph IFM 58554**	**St MS 281**	**Mt MS 248**	**Um MS 115**	**Pp JCM 10319**	**Uv HUV 17954**
SA 585	-	99	98	94	99	98	98
Ph IFM 58554	99	-	98	94	98	98	98
St MS 281	98	98	-	94	99	99	98
Mt MS 248	94	94	94	-	94	94	93
Um MS 115	99	98	99	94	-	100	99
Pp JCM 10319	98	98	99	94	100	-	99
Uv HUV 17954	98	98	98	93	99	99	-

SA575 was only recovered once and shares similar sequence identity with several *Sporisorium* species. In the ITS region SA575 differs by 14 and 19 bases from *S. chrysopogonis* strain 249470 and *S. heteropogonicola* strain BRIP 51822, respectively. Strains 249470 and BRIP 51822 are separated by 18 mismatches. The only *Pseudozyma* representative in this group is *P. flocculosa* strain AFTOL 864, but SA575 and this isolate differ by 57 bases implying a more distant phylogenetic relationship than with the other *Sporisorium* species. Upon comparing the ITS regions of all published *Pseudozyma* species to SA575 there was 8% or more incongruence between all isolates which implies that rDNA heterogeneity is prevalent within this genus.

In the LSU region SA575 shares more than 99% sequence identity with all other members of the group (Table 2-79). SA575 differs from *S. chrysopogonis* strain 249470 and *S. heteropogonicola* strain BRIP 51822 at one position and from *S. apludae-aristate* strain MS 287 and *S. themedae-arguentis* strain MS 95 at two positions. In contrast to the inter-specific rDNA variability which is present among this group in the ITS region, there is much less variability in the LSU region. Sequences of *S. apludae-aristate* strain MS 287 and *S. themedae-arguentis* strain MS 95 are identical and *S. chrysopogonis* strain 249470 and *S. apludae-aristate* strain MS 287 differ by only one base. Among the other *Pseudozyma* species, SA575 does not share the most LSU sequence identity with *P. flocculosa* strain AFTOL 864, as would be expected from the phylogenetic analysis. SA575 differs from strain AFTOL 864 by five bases, but differs from *P. pruni* strain BCRC 34227 by only three bases. This is interesting since the topology suggests that *P. pruni* is part of another *Sporisorium* sub-group that includes *P. graminicola*.

SA575 cannot be classified as any known species of *Pseudozyma*. The ITS region is highly variable between different *Pseudozyma* species and based on this information alone, SA575 is a new species within the genus. However, in contrast to *Pseudozyma*, the phylogenetic distances separating certain *Sporisorium* species are less well-defined, as there are different species which have identical LSU sequences and do not differ greatly in the ITS region. Since morphological characters are often inconsistent and may not support results from rDNA-based phylogenies, it is difficult to ascertain a generic profile for *Sporisorium* (Stoll, *et al.*, 2005). Thus, SA575 may represent the anamorphic state of *S. chrysopogonis* or *S. heteropogonicola*, but since teliospores or other taxonomically important morphological structures used in delimiting *Sporisorium* species (Boekhout, 1995, Shivas & Vanky, 1997, Vanky, 2005) were not observed, SA575 cannot be definitively assigned to a either of these species.

Table 2-78. Pairwise analysis of the ITS region of members of the *Sporisorium* 1 group. Numbers indicate percent similarity between isolates. Sa-*Sporisorium apludae-aristate*; Sc-*S. chrysopogonis*; S he-*Sporisorium heteropogonicola*; S hw-*Sporisorium hwangense*; So-*S. ovarium*; Sp-*Sporisorium polliniae*; St-*Sporisorium themedae-arguentis*; Pf-*Pseudozyma flocculosa.*

	SA 575	**Sa MS 287**	**Sc 249470**	**S he BRIP 51822**	**S hw MS 267**	**So MP 1871**	**Sp 24979**	**St 249483**	**Pf AFTOL 864**
SA575	-	95	98	97	96	93	91	97	91
Sa MS 287	95	-	96	92	94	95	92	95	93
Sc 249470	98	96	-	97	96	92	90	97	91
S he BRIP 51822	97	92	97	-	94	91	89	95	89
S hw MS 267	96	94	96	94	-	93	90	96	91

Table 2-78 cont.

	SA 575	Sa MS 287	Sc 249470	S he BRIP 51822	S hw MS 267	So MP 1871	Sp 24979	St 249483	Pf AFTOL 864
So MP 1871	93	95	92	91	93	-	93	93	92
Sp 24979	91	92	90	89	90	93	-	90	92
St 249483	97	95	97	95	96	93	90	-	91
Pf AFTOL 864	91	93	91	89	91	92	92	91	-

Table 2-79. Pairwise analysis of the LSU regions of members of the *Sporisorium* 1 group. Numbers indicate percent similarity between isolates. Sa-*Sporisorium apludae-aristate*; Sc-*S. chrysopogonis*; S he-*Sporisorium heteropogonicola*; S hw-*Sporisorium hwangense*; So-*S. ovarium*; Sp-*Sporisorium polliniae*; St-*Sporisorium themedae-arguentis*; Pf-*Pseudozyma flocculosa.*

	SA 575	Sa MS 287	Sc MS 135	S he BRIP 51822	S hw MS 267	So MP 1871	Sp MS 32	St Ms 95	Pf AFTOL 864
SA 575	-	99	99	99	99	99	99	99	99
Sa MS 287	99	-	99	99	99	99	99	99	99
Sc MS 135	99	99	-	100	99	99	99	99	99
S he BRIP 51822	99	99	100	-	99	99	99	99	99
S hw MS 267	99	99	99	99	-	99	98	99	99
So MP 1871	99	99	99	99	99	-	99	99	99
Sp MS 32	99	99	99	99	98	99	-	99	99
St Ms 95	99	99	99	99	99	99	99	-	99
Pf AFTOL 864	99	99	99	99	99	99	99	99	-

Four Ustilaginales isolates are part of a third cluster of *Pseudozyma/Sporisorium* species. This group is comprised of two smaller clades, each containing a different *Pseudozyma* species.Within the first group SA350 and SA598 are both located on a branch adjacent to *S. sorghi* strain MP 2036a. The ITS regions of SA350 and SA598 are identical. SA350 differs from strain MP 2036a by four bases and SA598 differs by one base. ITS Sequence identities between SA350, SA598 and strain MP 2036 are lower when compared to the other phylogenetically related isolates in this group, with more than 50 mismatches between each taxon. *Pseudozyma graminicola* strain YM 24388 is part of this group, though it differs from SA350 and SA598 by 67 and 68 bases, respectively. The second subgroup consists of three closely related isolates. SA276 and SA629 are different strains of *P. pruni*, most closely related to strain BCRC 34227. SA276 and SA629 differ from each other in the ITS region by four bases and from strain BCRC 34227 by six and five bases, respectively (Table 2-80).

SA350, SA598 and *S. sorghi* strain MP 2036a share identical LSU sequences. Interestingly, all of these *S. sorghi* isolates also show more LSU sequence identity to *P. pruni* strain BCRC 34227 than to *P. graminicola* strain YM 24388, which appears closer to *S. sorghi* in this phylogeny. SA276 and *P. pruni* strain BCRC 34227 differ by three bases and there are also 11 mismatches between *P. pruni* strain BCRC 34227 and *P. graminicola* strain YM 24388.

There is little ambiguity regarding the isolates in this group and their phylogenetic relationship to other taxa. SA350 and SA598 clearly represent a single strain closely related to *Sporisorium sorghi* strain MP 2036a. SA276 and SA629 represent two slightly variable strains of *P. pruni*, phylogenetically similar to strain BCRC 34227. This phylogeny is strongly supported at the nodes of the *P. pruni* and *S. sorghi* branches, but there is only weak support linking these two branches. The phylogeny accompanying the description of *P. pruni* resolves *P. fusiformata* as sister to *P. pruni* within a larger clade that includes *P. shanxiensis* and several *Ustilago* species (Liou, *et al.*, 2009). In their phylogenetic analysis, the authors show that *P. graminicola* and *S. holwayi* occur on the same branch which also occurs in this topology (Fig. 2-36). However, in their results there is greater phylogenetic distance between *P. graminicola* and *P. pruni*.

Table 2-80. Pairwise analysis of the ITS regions of members of the *Sporisorium* 2 group. Numbers indicate percent similarity between isolates. Pg-*Pseudozyma graminicola*; Pp-*P.pruni*; Sh-*Sporisorium holwayi*; S sc-*S. scitamineum*; S so-*S. sorghi.*

	SA 276	**SA 350**	**SA 598**	**SA 629**	**Pg LI 20**	**Pp BCRC 34227**	**Sh MP 1271**	**S sc OUCMBI 101213**	**S so MP 2036a**
SA276	-	86	85	99	85	99	85	85	85
SA350	86	-	100	86	89	86	89	87	99
SA598	85	100	-	85	88	85	89	86	99
SA629	99	86	85	-	85	99	85	85	86
Pg LI 20	85	89	88	85	-	85	91	87	87
Pp BCRC 34227	99	86	85	99	85	-	85	85	86
Sh MP 1271	85	89	89	95	91	85	-	87	87
S sc OUCMBI 101213	85	87	86	85	87	85	87	-	85
Ss MP 2036a	85	99	99	86	87	86	87	85	-

Table 2-81. Pairwise analysis of the LSU regions of members of the *Sporisorium* 2 group. Numbers indicate percent similarity between isolates. Pg-*Pseudozyma graminicola*; Pp-*P.pruni*; Sh-*Sporisorium holwayi*; S sc-*S. scitamineum*; S so-*S. sorghi.*

	SA 276	**SA 350**	**SA 598**	**Pg YM 24388**	**Pp BCRC 34227**	**Sh MP 1271**	**S sc MP 541**	**S so MP 2036a**
SA 276	-	99	99	98	99	98	97	99
SA 350	99	-	100	98	99	98	98	100
SA 598	99	100	-	98	99	98	98	100
Pg YM 24388	98	98	98	-	98	98	97	98
Pp BCRC 34227	99	99	99	98	-	98	98	99
Sh MP 1271	98	98	98	98	98	-	98	98
S sc MP 541	97	98	98	97	98	98	-	98
S so MP 2036a	99	100	100	98	99	98	98	-

The remaining isolates in Ustilaginaceae represent three strains which are part of a clade comprised of five *Pseudozyma* species In the ITS region, SA604 and *P. aphidis* strain HX 6610 are identical and differ from SA580 by one base (Table 2-82). Similarly, SA58 and *P. rugulosa*

strain CBS 17088 share 100% ITS sequence identity. Generally speaking, the ITS sequence similarities between *P. aphidis* and *P. rugulosa* are higher than between other species of *Pseudozyma.* SA58 and strain CBS 17088 differ from SA580, SA604 and strain HX 6610 by only 7, 10 and 11 bases, respectively. With the exception of *P. antarctica* strain JCM 3941, which differs from *P. rugulosa* strain CBS 17088 and *P. aphidis* strain HX 6610 by 23 and 22 bases, respectively, there are typically between 40 and 100 nucleotide substitutions among different *Pseudozyma* species.

Table 2-82. Pairwise analysis of the ITS regions of members of related *Pseudozyma taxa*. Numbers indicate percent similarity between isolates. P an-*Pseudozyma antarctica*; Pa-*aphidis*; Pj-*P. jejuensis*; Pp-*P. prolifica*; Pr-*P. rugulosa.*

	SA 58	**SA 580**	**SA 604**	**P an JCM 3941**	**P ap HX 6610**	**Pj IMUFRJ 52021**	**Pp JCM 11752**	**Pr CBS 17088**
SA58	-	98	99	97	98	86	94	100
SA580	98	-	99	97	99	86	92	98
SA604	99	99	-	97	100	86	94	99
P an JCM 3941	97	97	97	-	97	88	94	97
P ap HX6610	98	99	100	97	-	87	93	98
Pj IMUFRJ 52021	86	86	86	88	87	-	87	87
Pp JCM 11752	94	92	94	94	93	87	-	93
Pr CBS 17088	94	92	94	94	93	87	100	93

2.4 Discussion

The data recovered from this survey show that there is a wealth of diversity among phylloplane yeasts in the area surveyed. Out of a total of 463 basidiomycetous isolates, 81 correspond to either known species or previously undescribed taxa. While some ascomycete yeasts, particularly *Aureobasidium pullulans*, were commonly encountered, the majority of the phylloplane yeasts recovered were basidiomycetous. Among these isolates, certain genera like *Sporobolomyces*, *Tilletiopsis*, *Bullera* and *Pseudozyma* were cosmopolitan, while others like *Bensingtonia*, *Kondoa*, *Kordyana* and *Microstroma* were only isolated once or twice during the entire year. For most taxa, there was no evidence of any host specificity, although 85% of all 20 *Sympodiomycopsis* isolates were recovered from *P. polypodioides* and 67% of all 27 *Pseudozyma* isolates were recovered from *T. kunthii*. Even so, results such as these do no outweigh the evidence against specificity. *Sympodiomycopsis* and *Pseudozyma* isolates have been previously isolated from many different plants and other hosts in locations which are separated by large distances and differ dramatically in their climates (Sugita, *et al.*, 2003, Golubev & Golubeva, 2004, Golubev, *et al.*, 2007, Mahdi, *et al.*, 2008, Liou, *et al.*, 2009, Wei, *et al.*, 2011). It was interesting to consistently find many different exobasidiomycetous and ustilaginomycetous yeasts on non-angiosperms since it has been reported that these classes of smuts occur almost exclusively on angiosperms, most notably the Poaceae (cereals), Cyperaceae (sedges) and Ericaceae (Heath) (Begerow, *et al.*, 2006). However, it is not particulary surprising that anamorphic yeast spores are not restricted to the same hosts as their teleomorphic plant pathogenic stages. The pathogenic dikaryotic hyphal stage of many phytopathgens in

Ustilaginomycotina such as *Sporisorium*, *Farysia* and *Kordyana* has a narrow host range and is typically associated with one particular genus or family of plants (Begerow, *et al.*, 2006). On the other hand, the saprobic lifestyle is less restricted in range since it does not form an intracellular relationship with the host and those saprobes inhabiting leaf surfaces rely primarily on plant exudates for nutrients. Since phylloplane yeasts do not invade host cells they are subjected to environmental conditions such as wind and rain that transport them over great distances. This could account for the extremely wide distribution of certain yeast taxa such as *S. pararoseus*.

Though trends were observed for the influence of both temperature and precipitation, differences in the number of isolates recovered were not significant due to either condition. In general, the months with the highest total number of yeasts recovered were the ones with the highest average temperatures, but this is only an average value and not a direct correlation since four groups consisting of three months with similar temperatures were analyzed. Table B-4 (Appendix B) shows the numbers of isolates recovered per month. The average temperature during April, May and September was between 71-76°F and April was the most abundant month with 73 isolates recovered. However, May and September were among the least abundant months with only 20 and 28 siolates recovered, respectively. In contrast, December through February represents the period of time with the lowest average temperature (48-54°C), but nearly as many isolates were recovered in December (45) as in June (50), the warmest month. On the other hand, January, which had the lowest average temperature of any month, also yielded the fewest number of isolates.

There were also differences between the numbers of yeasts isolated from the different hosts during each month. In June, the average number of isolates recovered from all ferns except *C. falcatum* was 5.3. However, 18 isolates were recovered from *C. falcatum* during that month, bringing that average up to 7.1. Subsequently, during July and August, there was a sharp drop off in isolates recovered from *C. falcatum* corresponding to 4 and 0, respectively. In December and January the number of isolates recovered from *P. polypodioides* was 3 and 2, respectively. However, in February, 16 isolates were recovered from *P. polypodioides* during that month (59%). This type of random peak-and-valley distribution was characteristic of all ferns throughout the year, although *N. exaltata* displayed the least amount of spiking. These data indicate that the isolates are not normally distributed and that temperature does not appear to be a major factor affecting the amount of phylloplane yeast isolates on these hosts. The climate of Baton Rouge is subtropical with a mild winter. This may explain why there was not a more dramatic dropoff on all hosts during the coldest portion of the year. Additionally, yeast spores are produced in mass and when conditions are not suitable for germination they may remain dormant. Based on the isolation methods used in this study (i.e. spore-fall method) it is not possible to quantify exactly how many colony forming units were present on each leaf section. The spore-fall method has an inherent bias towards recovering ballistosporic isolates- especially the actively-sporulating yeast constituency of the phylloplane. It may be that taxa such as *Sporobolomyces pararoseus* were dominant because of efficient ballistospore producers and dispersion or because their cells are more tolerant of varying weather conditions or possess higher germination rates than other phylloplane yeasts. For singletons from non-ballistosporic taxa it is more difficult to assess the physiological state of the organism. For a complete listing of total yeast isolates recovered by month with respect to host, temperature and precipitation, see Table A-1 (Appendix A).

On average, the most isolates were recovered during months with the lowest levels of precipitation and the least isolates were recovered during the months with the highest levels of

precipitation. One reason for this may be that heavy rains can wash nutrients and fungal cells off of the leaf (Breeze & Dix, 1981). However, high relative humidity promotes yeast growth on the phylloplane (Inacio, *et al.*, 2002, Glushakova & Chernov, 2004) and is important for spore dispersal (Last, 1955). The constant humidity and mild climate of Baton Rouge seems to sustain phylloplane yeasts throughout the entire year, without a steep decline in the winter. Since the average monthly temperature in 2011 did not drop below 48°F it is reasonable that there was not a significant difference between seasons. It is also possible that the location of the individual pinnae on the ferns themselves may have been affected differently by the rain. Leaves were collected from the same individual ferns each time, but from different areas on the plant. Varying physical aspects involving the combination of wind and rain could have affected which parts of the ferns were subjected to the most rain during any given storm. Also, the density of leaves at locations on the ferns and surrounding plants could exhibit different levels of humidity (Last, 1955) . The location of the plants themselves could also affect the composition of their phylloplane flora and leaf height may play a role in phylloplane yeast distribution (Table A-2; Appendix A). For those ferns that were located directly beneath trees, the yeast cells which were initially washed away could have been quickly replaced by different yeasts trapped in rain drops falling from the tree above or may have been directly deposited from still other spores swirling through the air.

Most of the ferns in the ornamental plot were located directly under large trees. *Cyrtomium falcatum*, *N. exaltata* and *D. erythrosora* were part of the understory foliage beneath a live oak (*Quercus* sp.). *Rumohra adiantiformis* was located beneath a different *Quercus* species with much broader leaves and *Lygodium japonicum* was entangled within an Azalea bush (*Rhododendron* sp.). *Polypodium polypodioides* was growing on a live oak and was often collected in its shriveled, dehydrated state while *T. kunthii* was collected several miles away and was surrounded by other different plants. It seems possible that phylloplane yeast cells could be in continuous flux in certain ecosystems where rainfall and heavy strorms are common. Most cells would probably be recycled among plants that are close to one another, but others may escape and travel long distances in the atmosphere until they are eventually deposited in new environments.

More yeasts were isolated from abaxial than the adaxial sides, but the difference is not significant. Various studies of the phylloplane communities of different plants provide contrasting results. Studies of the microclimatic conditions of some plants have shown that higher humidity on the abaxial surface of leaves due to a greater number of stomatal openings provides more favorable conditions for fungi (Breeze & Dix, 1981) and abaxial surfaces may also afford some protection from ultraviolet radiation, wind and falling rain. On the other hand, other studies show that adaxial surfaces contain channels along the midrib and leaf veins where yeast cells may accumulate and obtain more nutrients due to greater amounts of exudate being present in these areas (Lindsey & Pugh, 1976). Still other studies have shown that while microbial density can be concentrated on either the abaxial or adaxial surface, segregation on one side or the other cannot be definitively predicted. Though trends are often seen, these are influenced more by abiotic factors such as humidity and ambient air temperatures (Zoberi, 1964) and leaf age than by specific physiological differences across individual sides of the phylloplane. That differences between abaxial and adaxial surfaces should be insignificant makes sense when considering the location of the survey. Differences in the number of isolates with respect to leaf side may significant in areas where microclimatic conditions play a larger role such as providing

some relief from harsh conditions. However, in this region, the mild climate doesn't seem likely to favor one leaf side over the other.

Seasonal studies have shown that *Sporobolomyces* density is greatest during summer months and closer to the base of the plant, around mature senescing leaves (Last, 1955, Pugh & Mulder, 1971). This can be attributed to the weakening of plant cell defenses, resulting in greater nutrient availability for epiphytes. The total numbers of isolates recovered from young and senescent leaves in this survey were nearly identical, but differences between those isolates pertaining to certain classes and from certain hosts were significant. The number of isolates recovered from senescent leaves of *Cyrtomium falcatum* and *T. kunthii* was approximately double those from young leaves. This especially makes sense for *C. falcatum* because the tough waxy cuticle should prevent the leaching of nutrient-rich exudate in young leaves and may actively produce antimicrobial compounds that eliminate some of the microbial population. If this is the case, phylloplane colonists are more likely to thrive on senescent leaves that are breaking down. However, more than four times as many isolates from *L. japonicum* came from young leaves compared to senescing ones. The leaves of *L. japonicum* are not particularly remarkable in terms of thickness or toughness, but its production of antimicrobial compounds has been documented (Li, *et al.*, 2006, Gou, *et al.*, 2011). It is possible that some intracellular compounds leaching out of senescing leaves may possess antibiotic properties that are not normally exuded from young leaves. This may also be possible with cupric compounds as *L. japonicum* is able to rapidly accumulate copper in its cell walls (Konno, *et al.*, 2005). If a copper-based fungicide was sprayed in the area, dying cells in senescing leaves could have leached their copper-tinged exudate back out onto the phylloplane.

More isolates were consistently recovered from non-fertile leaf sections than from fertile ones with sori. The sori themselves were never cultured, but it was observed that fern spores which occasionally dropped onto the agar did not give rise to any yeast colonies. The most obvious explanation as to why fewer isolates were recovered from fronds with sori is a matter of surface area. The average area occupied by the sori was anywhere between 25-50% of the fern section. If the sori do not harbor any yeasts, the remaining leaf area amenable for colonization is then substantially reduced. No information was found in the literature pertaining to any microbial associations with the indusium or components of fern reproductive structures such as sporangia or the spores themselves.

It was not surprising to find that ballistosporic genera such as *Sporobolomyces, Bullera, Derxomyces, Hannaella* and *Tilletiopsis* were the most common groups on the phylloplane. The production of ballistoconidia is an efficient method for spore dispersal which allows propagules to be potentially be transported over long distances. The dominance of microbotryomycetous taxa can be attributed to the cosmopolitan yeasts from the Sporodiobolales clades. Of these, *Sporidiobolus pararoseus* was the most frequently recovered isolate and represents 47% of all isolates recovered during this survey. This strain was present on all ferns and was also recovered from many other non-fern plants randomly sampled from different locations throughout the year. Red-pigmented colonies (often *Sporobolomyces pararoseus*) colonies were usually among the first yeasts seen on the plate after leaf sections were fixed to the lid of the plate. Initial deposition of cells was either by ballistosporic discharge directly from the leaf or by the expansion of single colonies which produced ballistospores on the agar, eventually coalescing with other colonies to form distinct red mucoid globs. Like many other members of Sporidiobolales, *S. pararoseus*, is commonly isolated from the phylloplane (Nakase, 2000, Libkind, *et al.*, 2005), soil (Bai, *et al.*, 2002) and is often found in aquatic and marine ecosystems (Ahearn, *et al.*, 1968).

The fact that *S. pararoseus* is such a cosmopolitan yeast suggests that it is a highly adaptable and successful organism, but it is unclear why the same strain was so ubiquitous compared to other ballistosporic yeasts in this study. It may be that *S. pararoseus* is able to utilize leaf exudates more efficiently than other yeasts or it may be more resistant to dessication, radiation or antimicrobial compounds secreted by the leaves and may also produce toxins that allow it to outcompete other phylloplane organisms. Some *S. pararoseus* strains are known to produce killer toxins which inhibit the growth of *Rhodotorula araucariae* by causing K^+ and ATP to to leak from sensitive cells (Janderova, *et al.*, 1995).

Ballistospore production probably varies between different strains and species. Although this was never quantified for any of the yeasts in this study, it was observed that *S. pararoseus* was a prolific producer of ballistoconidia. This could give it an advantage in colonizing surfaces and outcompeting other yeasts which are present in fewer numbers. During periods of low humidity water availability and nutrient availability, a large aggregate of cells would have a better chance of survival until conditions become favorable again and by that time they may have already gained a competitive advantage over newly arriving species or those fewer in number.

The diversity of fungi associated with ferns merits more attention. In comparison to fern mycorrhizal endophytes, knowledge about foliar endophytes and phylloplane epiphytes is scant. More phylloplane surveys of ferns from diferent regions would provide valuable information about fungal diversity. Future studies should examine seasonal yeast distribution on ferns in a variety of different tropical, subtropical and temperate areas. Also, the effect that sori have on phylloplane microbes is not known and a chemical characterization of these structures as well as indusia and the sporangia within would be beneficial. Additionally, future studies of phylloplane communities should employ other methods to identify the fungal flora such as pyrosequencing or simply leaf washings which will provide more resultion and fill in the gaps left by the spore-fall method. There are many more yeast species awating discovery from a plethora of different niches. Certainly, the phylloplane remains one of the most abundant yet still understudied of all these habitats.

Chapter 3. Description of Two Yeasts in Ustilaginales

3.1 Introduction

Ustilaginales G. Winter includes more than 30 genera, most of which are pathogenic on grasses in Cyperaceae and Poaceae (Begerow, *et al.*, 2006). These fungi are most conspicuous as dark masses of densely packed teliospores that destroy plant reproductive parts (Stoll, *et al.*, 2005). However, most phytopathogenic smuts have an asexual saprobic yeast stage (Begerow, *et al.*, 2006) which is ubiquitous and not host-specific. Asexual spores may use leaf surfaces as temporary resting spots during adverse conditions until they are able to fuse with a compatible mating type on a suitable host.

Typically, ballistosporic basidiomycete yeast genera such as *Sporobolomyces*, *Bullera* and *Derxomyces* are most cosmopolitan on the phylloplane (Inacio, *et al.*, 2005). However, other phylloplane surveys have shown that many other yeasts are also commonly associated with plant material (Inacio, *et al.*, 2002, Pereira, *et al.*, 2002, Golubev & Sampaio, 2009, Landell, *et al.*, 2009).

Approximately 14 species of *Pseudozyma* (Ustilaginaceae) have been described from various habitats including plant material, insect secretions, sea water (Liou, *et al.*, 2009, Statzell-Tallman, *et al.*, 2011) and the blood of immunocompromised humans (Sugita, *et al.*, 2003). Analysis of nuclear rDNA has shown that *P. tsukubaensis* and the type species *P. prolifica* are likely anamorphs of *Ustilago spermophora* and *U. maydis*, respectively (Boekhout, 1995, Wang, *et al.*, 2006), and other teleomorph connections with *Ustilago* or *Sporisorium* are likely.

On the other hand, comparatively little is known about the genus *Farysizyma* (Inacio, *et al.*, 2008). Phylogenetic analyses of ITS and LSU loci showed that *Farysizyma* is part of the '*Farysia* clade' which also includes the phytopathogens *Farysia thuemenii*, and *F. chardoniana*. This group is sister to *Schizonella* and *Stegocintractia* within Anthracoideaceae, and represents a phylogenetically related group of smut pathogens which have evolved on *Carex* species (Inacio, *et al.*, 2008). However, *Farysizyma* only contains four yeast species, all isolated from the phylloplanes and nectar of healthy plants not known to be parasitized by *Farysia* (Inacio, *et al.*, 2008).

During a survey of ferns growing in Baton Rouge, Louisiana, two new species of ustilaginomycetous yeasts were isolated from the phylloplane. Sequence data from the internal transcribed spacer region (ITS), the D1/D2 domain of the 28S large subunit (LSU), as well as physiological and morphological information show that SA209 and SA575 represent previously undescribed yeasts in Ustilaginales.

3.2 Materials and Methods

3.2.1 Sample Collection and Yeast Isolation

Both SA209 and SA575 were isolated in Baton Rouge, LA during 2011. SA209 was isolated once on January 19th from the abaxial surface of *Polypodium polypodioides* growing on the lower limbs of a live oak (*Quercus virginiana*). SA575 was also isolated only once on August 22nd from the abaxial leaf surface of a young fertile portion of *Lygodium japonicum* growing in a landscaped plot containing a wide variety of ornamental plants.

Both isolates were recovered from fern foliage by the spore-fall method (Pennycook & Newhook, 1978). Young and senescing pinnae were removed from the entire leaf blade near the rachis with sterile forceps and scissors and placed in small sterile plastic bags until processing. Pinnae were cut into 1 cm sections and affixed to the surface of 5 cm plastic petri plates containing either yeast malt agar (YMA): 12g yeast extract, 12g malt extract, 2g peptone, 4g glucose and 8g agar in 400ml of H_20 with 400µl of 50mg/ml chloramphenicol, or potato dextrose agar (PDA): 19.5g potato dextrose agar in 500ml H_20 with 500µl of 50mg/ml chloramphenicol. Each pinna was attached to the lid with a small amount of petroleum jelly using forceps and a metal spatula. For each fern, six abaxial and adaxial leaf sections were prepared per plate. Plates were incubated at room temperature with a natural light cycle and monitored daily for the presence of colonies on the agar.

Individual colonies were transferred to new plates with a sterile toothpick as soon as they were noticeable on the agar then sealed with parafilm and incubated for one week at 25°C in an opaque plastic bin. Long term storage of axenic cultures was prepared on PDA slants and stored at 4°C and cryovials containing 40% glycerol were stored at -80°C.

3.2.2 Measurements and Light Microscopy

A Carl Zeiss AxioVision Product Suite DVD 34 interference light microscope with a 40x and100x objective was used to visualize slides mounted with 50µl of liquid from actively growing cultures. Micrographs were obtained using an AxioVision digital camera and measurements were made with the Axio VS 40 v.4.8.1.0 software (Carl Zeiss Microscopy LLC, Thornwood, NY). Measurements were based on an average size of 30 spores after 7, 14, 21 and 28 days at 25°C on potato dextrose broth (PDB) and yeast malt broth (YMB).

3.2.3 Assimilation

Physiological and biochemical characteristics were determined using the methods described in Kurtzman *et al*., (2011). A basidiomycete yeast whose assimilation and fermentation properties are known was used as a positive control and double distilled water was used as a negative control. Assimilation of carbon and nitrogen sources and fermentation tests were determined at 25°C over 5 weeks.

3.2.4 PCR Amplification and Sequencing

Prior to PCR, cultures were grown on PDA for 5 days at 25°C. Colony PCR was performed following the methods of Aime & Phillips Mora (2005) with several modifications. Prior to polymerase chain reaction (PCR), a small amount of yeast cells were diluted in 250µl of sterile distilled water and stored at -20°C. One microliter of this suspension was further diluted 1:10 in sterile water and used as the DNA template. PCR's were performed in 25µl reaction mixtures containing Promega 2x Master Mix (Promega Corp., Madison, Wisconsin), 1.25µl of each forward and reverse primer and and 10µl of DNA template.

Amplification of 700-800 bp of ITS was achieved using the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White, *et al.*, 1990). PCR cycle parameters consisted of an initial 7 min

denaturation step at 94°C followed by 36 cycles of 94°C for 30 sec, 50°C for 45 sec, 72°C for 45 sec and a final extension step of 72°C for 7 min. Approximately 1400 bp of LSU was amplified using the primers LROR and LR7 (Vilgalys & Hester, 1990). The cycling program for LSU consisted of an initial 5 min denaturation step at 94°C followed by 35 cycles of 94°C for 30 sec, 50°C for 45 sec, 72°C for 1 min and a final extension step of 72°C for 7 min. PCR products were verified on a 1% agarose gel and sequenced at Beckman Coulter Genomics in Danvers, Massachusetts (http://www.beckmangenomics.com/genomic_services/dna_sequencing.html).

3.2.5 Phylogenetic Analysis

Sequences were manually edited with Sequencher 4.01 (Gene Codes Corp., Ann Arbor, MI). Consensus sequences were compared to similar taxa in the NCBI Genbank (http://www.ncbi.nlm.nih.gov/) database using a Blastn search. Multiple sequence alignment consisting of a concatenated ITS and LSU sequence supermatrix using a MUSCLE algorithm was constructed using MEGA5 (http://www.megasoftware.net/; Tamura et al. 2011) then edited by eye.

Gene phylogenies were inferred by maximum likelihood (ML) in RAxML (Stamatakis, 2006) using a GTR model of evolution. Support for the branching topologies was evaluated by bootstrap analysis derived from 1000 replicates with 10 random additions replicated.

Initial phylogenetic analyses were conducted using a dataset consisting of 208 Ustilaginales ITS and LSU sequences which consisted mostly of *Sporisorium* and *Ustilago* taxa. This matrix was eventually pared down to a final dataset of 28 taxa including the SA isolates. Glomosporiceae was selected as an outgroup based on its sister relationship to Ustilaginaceae (Vanky, *et al.*, 2008).

3.3 Results

Phylogenetic analysis of ITS and LSU show that SA209 and SA575 belong in Ustilaginales and represent two new yeast species within Anthracoideaceae and Ustilaginaceae, respectively. SA209 is part of the '*Farysia* clade,' (Inacio, *et al.*, 2008) which contains all four known species of *Farysizyma* as well as *Farysia thuemenii* and *F. chardoniana*. Anthracoideaceae and Ustilaginaceae are well supported as part of Ustilaginales.

Within Anthracoideaceae, SA209 shares most sequence identity with *Farysizyma itapuensis* strain BI181 and somewhat less with *F. taiwaniana* strain TOH 1.2. Based on rDNA sequences, there are molecular differences which separate members of the '*Farysia* clade.' In the ITS region the number of base mismatches between different species ranges from 65 to 136 out of a total of approximately 700, which corresponds to 80-92% sequence identity. *Farysizyma itapuensis* and *F. taiwaniana* share the least number of substitutions of any two species within the group and SA209 differs from both of these species by 41 and 58 mismatches, respectively. The intraspecific variation between three strains of *F. itapuensis*- the closest relative of SA209- was also examined. Strains ATCC MYA-4547 and BI238 have identical sequences and differ from BI181 by a single base.

In the LSU region, there are 10 or more mismatches between SA209 and every other sequence in this analysis. As in the ITS region, there is a clear separation of species based on sequence comparisons. Strictly speaking, all members of the '*Farysia* clade' show 98% or less similarity to each other with one exception. However, this discrepancy can be attributed to

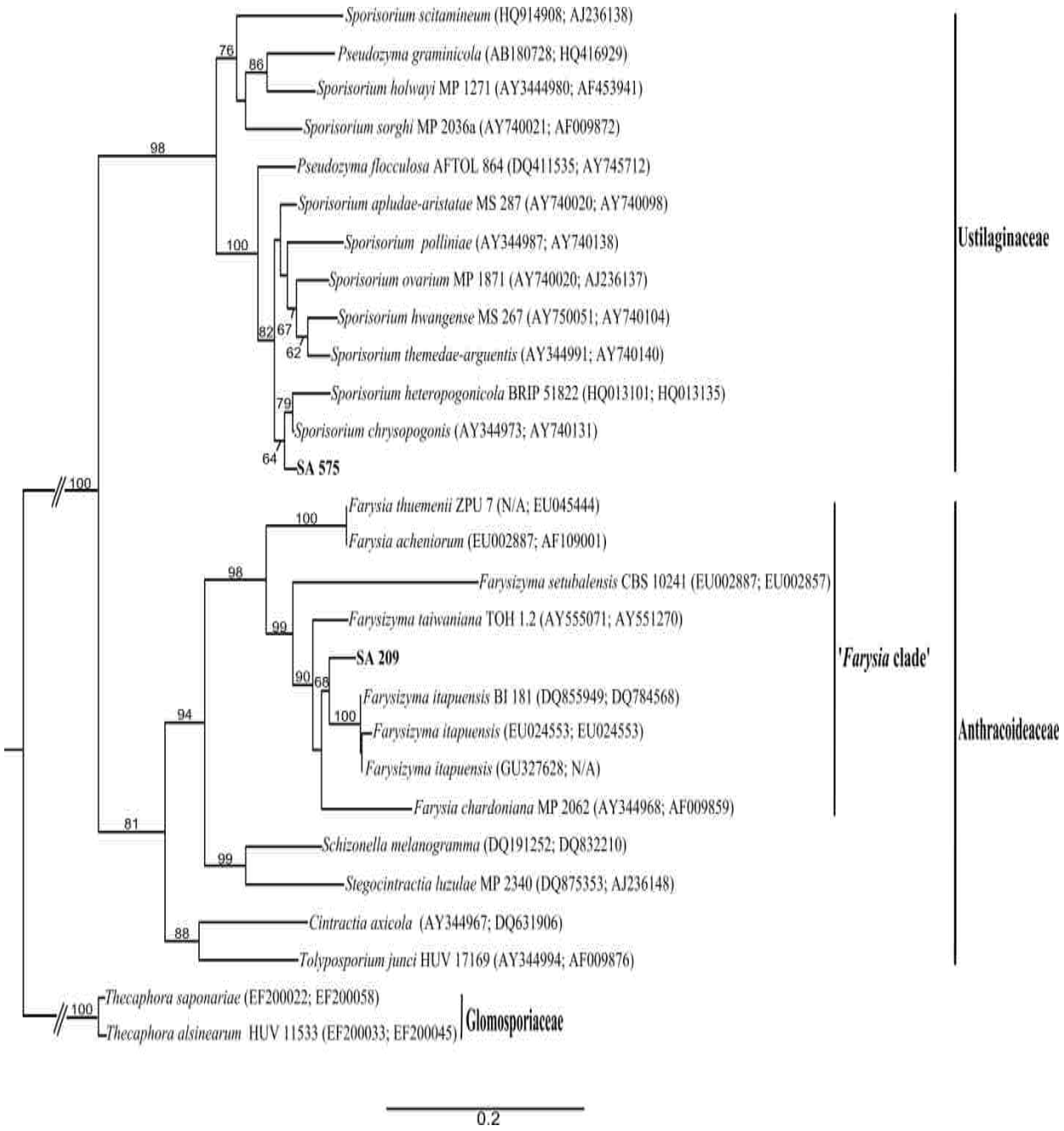


Figure 3-1. Phylogenetic tree of Ustilaginales using maximum likelihood analysis of concatenated LSU and ITS rDNA sequences as determined using a GTR model of evolution. SA209 and SA575 were compared to other taxa in Ustilaginales. Glomosporiaceae was used as outgroup based on its sister relationship. Numbers above nodes represent bootstrap values greater than 60. RAxML was used to search for the best-scoring tree using bootstrap analysis. Reference sequences were retrieved from GenBank. Strain designations were listed only when sequences of both loci were from the same strain. Accession numbers within parentheses indicate ITS and LSU sequences in GenBank, respectively.

changes in the nomenclature. In the original description of the genus, *Farysizyma*, Inacio *et al*. (2008) remarked that several of their isolates were conspecific with *Rhodotorula acheniorum*. Based on their phylogenetic position, these were removed from *Rhodotorula* and renamed *Farysizyma acheniorum*. Furthermore, the *F. acheniorum* isolates were shown to be phylogenetically very similar to *Farysia thuemenii*, indicating a possible anamorph-teleomorph connection.

Table 3-1. Physiological test responses of SA209 and SA575.

Characteristic	**SA209**	**SA575**
Carbon Source		
D-Glucose	+	+
D-Galactose	+	-
L-Sorbose	W	V
D-Glucosamine	+	+
D-Ribose	+	+
D-Xylose	+	+
L-Arabinose	+,D	+
D-Arabinose	+,D	+,W
L-Rhamnose	+,D	-,D
Sucrose	+,D	+
Maltose	+	+
Trehalose	+	+
Methyl-α-D-glucoside	+	+
Cellobiose	+,W	+
Salicin	V	V
Arbutin	V	+
Melibiose	+	+
Lactose	+	+
Raffinose	+	+
Melezitose	+	+
Inulin	-	-,D
Soluble Starch	+,W	D,W
Glycerol	+	+,W
Ribitol	+,W	+,W
Xylitol	-,W	+

Table 3-1 cont.

Characteristic	SA209	SA575
Carbon Source		
Arabinitol	-	ND
D-Glucitol	-	-
D-Mannitol	+,D	+
Galactitol	-	+,W
Myo-Inositol	-	+
D-Glucono-1,5-lactone	+	+
D-Gluconate	V	+
D-Galacturonic acid	+,W	+
DL-Lactate	+	+
Succinate	+	+
Citrate	V	+
Methanol	-	-,W
Ethanol	-	+
Quinic acid	+,W	+
Nitrogen Source:		
Thiamine	+	+
D-Glucosamine	+	+
KNO_3	+	+
$NaNO_2$	+	+
L-Lysine HCl	+	+
$(NH_4)_2S0_4$	+	+
Other Tests:		
Growth on 50% Glucose	+	+
Growth on 60% Glucose	+	+
Growth on 10% NaCl	+	+
Growth on 16% NaCl	W	-

Test results: +, positive; -, negative; W, weak; V, variable; ND, not determined.

SA575 belongs in Ustilaginaceae within a clade comprised of several *Sporisorium* species including the type, *S. sorghi* and *Pseudozyma flocculosa* (Fig. 3-1). In contrast to the *Farysizyma* and *Farysia* species analyzed above, the interspecific rDNA variation sequences among members of the SA575/*Sporisorium* clade is much lower. In the ITS region SA575 differs from *S. chrysopogonis* strain 249470 and *S. heteropogonicola* strain BRIP 51822 by 14 and 19 nucleotides, respectively. Strains 249470 and BRIP 51822 differ by 18 nucleotides. However, when compared to other *Pseudozyma* species, SA575 displays significant sequence heterogeneity. This situation is similar to what was observed between SA209 and other members

of the '*Farysia* clade.' *Pseudozyma flocculosa* strain AFTOL 864 represents the closest *Pseudozyma* relative of SA575, but the two differ by 57 bases out of 661 (91%) in the ITS region. Certain distantly related species such as *P. aphidis* and *P. graminicola* differ by more than 100 mismatches.

In the LSU region SA575 differs by only one base from *S. chrysopogonis* and *S. heteropogonicola*. SA575 and *P. flocculosa* exhibit slightly less sequence identity, represented by five nucleotide substitutions spanning a 1327 base pair region of the 28S subunit. *Pseudozyma graminicola* was also present in the *Sporisorium* clade, though it was more distantly related, sharing only 98% sequence identity (11 nucleotide substitutions) within the LSU region.

3.4 Taxonomy

3.4.1 Description of *Farysia* sp. nov. Albu, Rush and Aime

Cells are oval-shaped and proliferate by mostly unipolar budding. Pseudohyphae are observed around the growing margins of colonies after approximately two weeks. No sexual structures were seen and clamp connections and ballistoconidia are absent. The physiological profiles of the four described *Farysizyma* species are quite similar and SA209 fits this general profile, as it is able to assimilate glucose, galactose, ribose, L-arabinose, sucrose, maltose, raffinose, melezitose, D-mannitol and succinate, but not inositol and methanol. Similar to *F. itapuensis* and *F. taiwaniana,* SA209 can assimilate ethanol. All fermentation tests were negative.

Teleomorphic state is unknown. Physiological and biochemical properties are listed in Table 2. In PD broth after 3 days at 25°C, cells are ellipsoidal and elongate, 3.3—5.27 µm × 1.1—1.87 µm, mostly single, but also in pairs. Budding is mostly unipolar, rarely bipolar. After 2 weeks, sediment and ring are present, but not film or pellicle. In YM broth after 1wk at 25°C, cells are ovoid and ellipsoidal, 4.23 µm-6.28 µm × 1.62µm-3.39µm, occurring singly. Budding is unipolar. Ring and sediment are present after 1 week, but no pellicle or film. Ballistoconidia are not formed and glucose fermentation is absent. After 2 weeks at 25°C: on GYP and MYP agar, streak culture is light orange to pink, gelatinous with undulations radiating outward in ring, regular margin; on corn meal agar, streak culture is cream- colored with an irregular margin; on 1% YE agar, streak culture is cream-colored to pale green, somewhat gelatinous, highly undulate at center with regular, rounded margins; on 10% NaCl, streak culture is pale yellow and gelatinous, undulate throughout with a rounded or irregular margin; on 50% glucose and KNO_3, streak culture is whitish to cream-colored, displaying densely cerebriform or vermiform coils which taper toward a regular, slightly veined, darker butyrous margin (growth is faster on 50% glucose and margin is wider and more veined on $KNO_{3)}$; on 60% glucose, streak culture is pale yellow to flavescent composed of a tough gelatinous matrix, displaying an irregular crystalline appearance; on $NaNO_2$, streak culture is light yellow to cream, densely cerebriform to vermiform centrally, tapering into cylindrical venations, many of which are spilt open in the middle, extending into a smooth, gelatinous, flaky, mostly translucent, slightly irregular to somewhat crenelated margin; on NH_4SO_4 and thiamine, streak culture is yellowish-brown, gelatinous and yeast-like with centrally-occurring tubular structures which are split in the middle, pseudohyphal tufts extend outward becoming brown near the growing margin; on tryptophan, streak culture is icteritious with pinguid circular colonies occurring densely, no distinct margin present.

The type strain, SA209 was isolated from a frond of *Polypodium polypodioides* which was growing as an epiphyte on *Quercus virginiana* on the Louisiana State University campus in January, 2011.

3.4.2 Description of *Pseudozyma* sp. nov. Albu, Rush and Aime

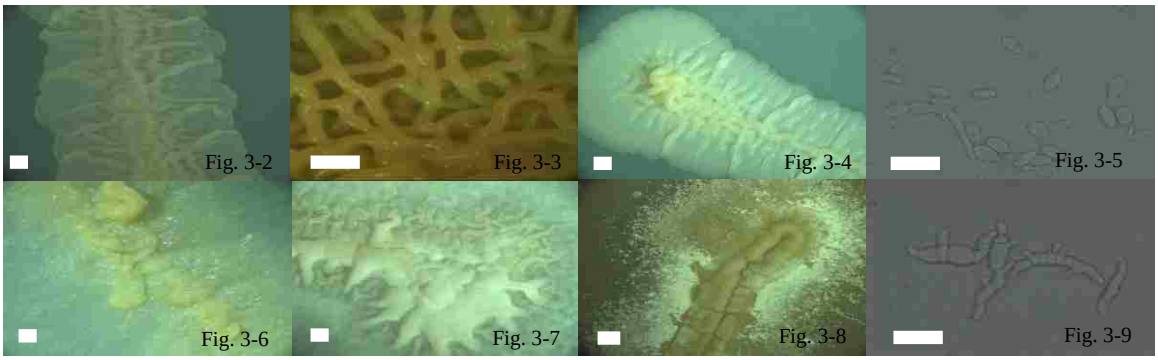
The morphology of SA575 resembles those of *Pseudozyma* yeasts as it produces pseudohyphae, proliferates through bipolar budding and lacks clamp connections, ballistoconidia and sexual structures. However, larger irregularly shaped secondarily-septate cells were formed in liquid culture after 1-2 weeks (Figs. 3-6—3-9). These types of cells have not been observed in previous descriptions of *Pseudozyma* species. The physiological characteristics of SA575 do not match those of any single species, though they do correspond to the *Pseudozyma* profile *sensu lato*. Assimilation reactions were positive for melibiose, lactose, ethanol, ribitol, mannose and inositol. SA575 does not assimilate galactose, however, which distinguishes it from all other species except *P. fusiformata*. Ballistoconidia are not formed. Fermentation of all substrates is absent. Physiological and biochemical properties are listed in Table 2. After 2 weeks: on 1% YEA, colonies are pale yellow, undulate or winkled and somewhat tough and gelatinous, small tufts of pseudohyphae emanating from margin; on 10% NaCl, streak culture is light orange and pruinose, pellicle is formed with a white layer of conidia covering culture completely, colonies are tough, dry, wrinkled and grow slowly, margin is irregular and indistinct; on 50% glucose, streak culture is yellow and vermiform, no pellicle is formed, white conidia visible on entire culture extending to lateral areas, margin rounded, smooth, pruinose, indistinct; on 60% glucose, streak culture is pale buff, densely coiled and somewhat cartilaginous growth extending radially, margin is indistinct, mostly concolorous, somewhat lighter, pruinose; on glucosamine, streak culture is yellow in the center with irregular wrinkled gelatinous growth protruding up from agar, culture is pale yellow to white flat and viscid extending out to margin, small strands of moist pseudomycelial strands protrude from center laterally, margin tip is faint white with a distinctive blurred halo beyond terminal pseudohyphae, margin edge is rounded, smooth and indistinct; on KNO_3, streak culture is yellow to pale brown, conidia are conspicuous and produced in mass, tough tooth-like protuberances are covered by conidia and rise up from agar, tapering laterally into narrow vermiform tubular structures, margin extends beyond tubular structures is round and distinctly fuzzy; on $NaNO_2$, streak culture is pale buff or tawny, conidia produced, vermiform tubular structures occur in center of culture tapering outward into thinner venous structures, white pseudomycelia formed, margin is pale and blurry, halo-shaped; on NH_4SO_4, streak culture is bright yellow, growth from initial streak is thickly gelatinous with deep convolutions, short pseudohyphae are formed at base of convolutions, moist blurry white halo begins abruptly radiating out beyond tips of pseudohyphae; on thiamine, streak culture is bright yellow, growth from initial streak is gelatinous with undulations and convolutions that taper into a viscid white mat, forming a broad circle around culture, conidia are produced in white patches distributed along mat-like structure; on tryptophan, streak culture is distinctly dark brown with raised, fragmented corrugated central axis, white conidia densely coating area next to central colonies, margin non-existent, edges of culture composed of individual colonies, flat, resembling parchment, crusty.

The type strain, SA575 was isolated from a fertile frond of *Lygodium japonicum* growing on the Louisiana State University campus.

3.5 Discussion

Maximum likelihood analysis of a concatenated alignment of ITS and LSU sequences yielded a tree with strong support for SA209 and SA575 as well as for Anthracoiceaceae and Ustilaginaceae within Ustilaginales.

The generic phylogenetic placement of SA209 and SA575 was resolved, but since sequence information for many Ustilaginales taxa is not available, it is not certain that SA209 and SA575 are not anamorphs of previously described *Farysia* or *Sporisorium* species.



Figs. 3-2—3-9: Colony and cellular morphology for SA209. 2. Two weeks on $NaNO_2$. 3. Two weeks on MYPA. 4. Two weeks on Thiamine. 5. Three days on YMB. Figs. 6-9: Colony and cellular morphology for SA575. 6. Two weeks on Glucosamine. 7. Two weeks on KNO_3. 8. Two weeks on Tryptophan. 9. One week on YMB. Bars for 2,3,4,6,7,8 = 1 mm. Bars for 5,9 = 10 µm. A Carl Zeiss AxioVision Product Suite DVD 34 interference light microscope with 40x and 100x objectives was used to visualize slides. Micrographs were obtained using an AxioVision digital camera and measurements were made with the Axio VS 40 v.4.8.1.0 software (Carl Zeiss Microscopy LLC, Thornwood, NY). Measurements were based on an average size of 30 spores after 7, 14, 21 and 28 days at 25°C on PDB and YMB.

This uncertainty is a common problem encountered when describing anamorphic yeasts, especially in groups where molecular information for many taxa is unavailable. Fungi described prior to the advent of molecular phylogenetics were initially classified and assigned to taxonomic groups using primarily morphological means and information regarding their hosts. It follows that since molecular data are not available for many Ustilaginales species, paraphyly exists among certain genera such as *Ustilago* and *Sporisorium* (Stoll, *et al.*, 2005). However, recent efforts have been able to better resolve the position of certain Ustilaginales species using a combination of molecular phylogenetics, host relationships and morphological characters (Begerow, *et al.*, 2000, Stoll, *et al.*, 2005). Species for which rDNA has not been sequenced cannot obviously be included in phylogenetic analysis. This makes the naming of new taxa within Ustilaginales somewhat problematic and will continue to leave some doubt as to their true phylogenetic placement until all sequences for all described species are available.

SA209 and SA575 appear to be anamorphs of phytopathogenic smuts which typically occur on Cyperaceae and Poaceae (Begerow, *et al.*, 2006). However, both were recovered from

ferns not displaying signs or symptoms of pathogenicity. Both SA209 and SA575 are supported in their respective clades and are described as new species of *Farysia* and *Pseudozyma*, respectively. Since assimilation data are not available for *Farysia* or *Sporisorium* species and neither teliospores or other sexual structures were observed for SA209 and SA575, the decision to place SA575 in *Pseudozyma* instead of *Sporisorium* stems from the fact that the taxonomy of *Sporisorium* and *Ustilago* remains unresolved in Ustilaginales as both genera are parphyletic across Ustilaginaceae (Stoll, *et al.*, 2005). *Pseudozyma* is comprised of approximately 15 distinctly yeast species and is something of an anamorphic repository for yeast anamorphs in Ustilaginaceae. Though direct teleomorph connections have not been shown for many of these species, *P. prolifica* and *P. tsukubaensis* have been identified by phylogenetic analysis of rDNA as anamorphs of *U. maydis* and *U. spermophora*, respectively, (Boekhout, 1995, Begerow, *et al.*, 2000). In the case of both SA209 and SA575, there is molecular, morphological and physiological evidence that distinguishes both isolates from other yeast species in *Farysizyma* and *Pseudozyma*, respectively. Likewise, although there are nearly 40 species of *Farysia* described, only *F. chardoniana* and *F. thuemenii* sequences are available for comparison.

Molecular sequencing of various phylloplane yeasts has revealed that many ustilaginomycetous anamorphs are present on a wide range of plants which are not known hosts for the phytopathogenic stage (Golubev, *et al.*, 2007, Inacio, *et al.*, 2008, Statzell-Tallman, *et al.*, 2011). Because the asexual yeast stage does not have the taxonomically informative morphological characters or restricted host range of the pathogenic teleomorph, accurate identification requires DNA sequence information. However, the lack of sequence information for many older taxa has left gaps which need to be filled in order to resolve these groups. The transition to molecular systematics has started to seal some of these taxonomic gaps but there is still a great deal of missing data. Within Ustilaginales, anamorphic genera like *Farysizyma* (Inacio, *et al.*, 2008) and *Pseudozyma* (Boekhout, 1995), share high rDNA sequence identity with *Farysia, Sporisorium, Ustilago* or other genera. However, increased phylloplane sampling and the sequencing of older herbarium specimens will likely uncover more anamorph-teleomorph connections. On the other hand, the genus Farysia is more clearly resolved and the '*Farysia* clade' is monophyletic in Ustilaginaceae and includes all anamorphic *Farysizyma* species and *Farysia chardoniana* and *F. thuemenii*. However, more molecular data from *Farysia* species would be particularly useful, as there are approximately 20 known species (Vánky & Begerow, 2007), but only rDNA sequences available for two species on Genbank.

While it is important that anamorphic yeasts continue to be identified and their biochemical and ecological properties be documented, a recent in the new International Code for Nomenclature of algae, fungi and plants has eliminated dual nomenclature for pleomorphic fungi, meaning that all fungal names now compete equally, solely based on priority (Hawksworth, 2011, Taylor, 2011, Wingfield, *et al.*, 2012). As *Pseudozyma* and *Farysizyma* are anamorphic genera, it follows that eventually they will be synonymized as teleomorph connections become clear and sequence information becomes available. However, with a dearth of *Farysia* sequences available and the taxonomy of *Sporisorium* still unresolved, both SA209 and SA575 are important new species additions to Ustilaginales.

As more fungi are discovered, it is important that a universal barcode is adopted to facilitate accurate identification of different species. The ITS region was recently proposed as the universal fungal barcode due to its ease of amplification, large barcode gap between inter-specific and intra-specific variability and high probability of correct identification of species (Schoch, *et al.*, 2012). Some critics have argued that certain ITS primers exhibit a PCR bias for

environmental samples or among different taxonomic groups (Bellemain, *et al.*, 2010, Vralstad, 2011). However, while other loci such as RNA polymerase binding protein 1 (RPB1) have shown to be good markers for correct species identification, they have been proven difficult to amplify through PCR (Schoch, *et al.*, 2012). On the other hand, the D1/D2 domain of the 28S large subunit has traditionally been the barcode for ascomycete yeasts (Kurtzman & Robnett, 1998), but is also commonly used for basidiomycetous yeasts (Bai, *et al.*, 2002, Valerio, *et al.*, 2002, Wang, *et al.*, 2011).

Today, despite the lack of an ideal locus for all fungal groups, a large database of rDNA loci already exists and has proven useful in advancing fungal taxonomy rapidly in the last decade. Given the rapid evolution of sequencing technology and molecular tools, authors submitting any new fungal species descriptions should provide not only morphological and relevant physiological information, but should also deposit molecular sequence data and accompanying chromatographs into a public database. In order to mitigate confusion, rDNA loci including SSU, LSU and ITS should serve as a standard set of phylogenetic loci that accompany all new yeast descriptions, since they are already available for many species. LSU and ITS sequence information has become integral to yeast descriptions and a concerted effort on the part of all mycologists to include all new fungi as well as those previously not sequenced could be undertaken to the benefit of the entire field.

Chapter 4. Conclusions

The remarkable amount of species and sub-specific diversity observed in this study surpassed expectations and indicates that the area sampled is a region of great fungal abundance. It is probable that subsequent studies will continue to expand on these results, providing more evidence that subtropical environments, such as the one of Baton Rouge, Louisiana are ecosystems rich in yeast biodiversity. However, another question arises: are fern phylloplanes really such rich habitats for yeasts, or is the humid subtropical climate of Louisiana responsible for the wealth of diversity? To answer this question, more data from non-ferns should be collected in Louisiana and the same survey should subsequently be undertaken in a region with a different climate, for example, one with harsher winters and less atmospheric humidity.

The vast amount of data from this project provides many different opportunities for additional research. Obviously, the remarkable amount of phylogenetic diversity among these isolates illustrates the need for increased sampling, which will lead to the description of more new species and ultimately increase our knowledge of fungal diversity. One of the tatalizing aspects to stuying fungal biodiversity is that the prospect of finding new and interesting organisms is high when sampling understudied niches. Whether it is rainforest litter, ice, sewage or the clouds, fungi do not disappoint. As more environments seemingly unfit for life are sampled, more new fungi are discovered. If we could see the world through a scanning electron microscope, much of it would probably be covered by spores. Though they are invisible to our eyes, I imagine that the air is teeming with spores, too. As is the earth and the trees and so are we.

Is everything everywhere? Baas-Becking (1934) addressed this question regarding fungi and other microbes long before thermal cyclers and DNA sequencers were commonplace in the lab. It was a difficult question to answer then and it is still not easy to answer. Becking believed that "everything is everywhere, but the environment selects." However, if I were to ask the same question regarding the fungi in this survey, the short answer would be—maybe not. At least not in terms of phylloplane yeasts. With the exception of *Sporidiobolous pararoseus*, no other yeast dominated the phylloplane to such a high degree. Perhaps a more appropriate rule of thumb when considering fungal diversity might be that s*omething* is everywhere. Nearly everything I have ever cultured has produced a fungus. This includes feathers, insects, food, the air, water, wood and other fungi. Their potential to colonize just about anything is impressive and certainly worthy of respect. While transferring yeast colonies to clean plates I have often wondered about the other phylloplane fungi which never made it onto the agar. Those non-ballistosporic yeasts that weren't dislodged from a leaf vein or remained trapped in the midrib channel were never acknowledged. Neither were the ones remaining on the plant itself. I am certain that many more slipped through the cracks as well. In retrospect, a 1 cm leaf section cut from an entire plant seems like a very small representation of the entire population. Nevertheless, the amount of diversity among the isolates I recovered far exceeded my expectations. Though it may never be possible to account for every single phylloplane fungus on one plant, this survey shows that there are very many yeasts which have no trouble moving around and finding different substrates to colonize.

The logical follow up to this survey would be to study the microflora of the leaf in more detail. Other phylloplane studies have macerated leaves and plated the dilutions to asses the actual colony forming units present on the leaf. This would provide more accurate figures as to what the actual colony numbers look like. Another method could involve simply using universal

fungal primers to sequence everything on the leaf. This kind of massive indiscriminate sequencing has led to the discovery of a new class of bizarre soil fungi and it would surely provide important information about the fungi of the phylloplane.

A third possibility would involve leaving the leaf itself and investigating the aerial microflora within a particular radius. There is already plenty of evidence that the anamorphic stages of some pathogens occur on non-host plants. Even in this study, *Sporisorium* and *Kordyana* isolates were recovered. Since the atmosphere is directly in contact with the phylloplane, it seems plausible that there is a constant ebb and flow of microbial cells coming and going. Implementing spore traps may be a useful way to monitor the different fungi which are in the vicinity and the rates at which they are moving. This idea seems easy enough and its application can be seen in entomology. However, when dealing with fungi, accounting for population numbers or diversity is not as easy as counting specimens in a trap. It it difficult to know where fungal spores coming from, where have been and where are they going. The prevalence of *Sporidiobolus pararoseus* strain CBS 484 has given me an idea about the future of mycology. Someday we will be able to trace the path of fungal spores using remote sensing. If individual cells can be engineered to emit electrical impulses or radio frequencies that could be transmitted similar to how animals are tracked with collars. We will be able to know the exact route that a spore travels and whether that cell remains in the same landfill or if it travels across the ocean and reproduces on a grape thousands of miles away. That day may not come soon, but in the meantime there is still plenty to do in other areas of mycology.

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Appendix A. Yeast Strain and Host Information

Table A-1 cont. Taxonomic information, host data and date collected for all basidiomycete strains collected from ferns during 2011.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA340	Udeniomyces cf pyricola 1	Agar	Trem	Cystofilo	4/11/2011	Lj	D	1	0
SA341	Udeniomyces cf pyricola 1	Agar	Trem	Cystofilo	4/13/2011	Lj	D	1	0
SA213	Udeniomyces cf pyricola 2	Agar	Trem	Cystofilo	1/31/2011	Ne	B	0	1
SA216	Udeniomyces cf pyricola 2	Agar	Trem	Cystofilo	2/1/2011	Ne	B	0	1
SA217	Udeniomyces cf pyricola 2	Agar	Trem	Cystofilo	1/31/2011	Ne	B	0	1
SA214	Udeniomyces megalosporus 1	Agar	Trem	Cystofilo	1/31/2011	Ne	B	0	1
SA215	Udeniomyces megalosporus 1	Agar	Trem	Cystofilo	2/1/2011	Pp	B	0	0
SA235	Udeniomyces megalosporus 1	Agar	Trem	Cystofilo	2/15/2011	Cf	B	1	0
SA296	Udeniomyces megalosporus 1	Agar	Trem	Cystofilo	3/29/2011	Ne	D	0	0
SA228	Udeniomyces megalosporus 2	Agar	Trem	Cystofilo	2/15/2011	Cf	B	1	0
SA245	Udeniomyces megalosporus 2	Agar	Trem	Cystofilo	2/19/2011	Pp	B	1	0
SA293	Udeniomyces megalosporus 2	Agar	Trem	Cystofilo	3/29/2011	Ne	D	0	0
SA229	Udeniomyces megalosporus 3	Agar	Trem	Cystofilo	2/15/2011	Cf	B	1	0
SA232	Udeniomyces megalosporus 3	Agar	Trem	Cystofilo	2/15/2011	Cf	B	1	0
SA507	Cryptococcus terreus	Agar	Trem	Filo	7/12/2011	Pp	B	0	0
SA344	Auriculibuller fuscus 1	Agar	Trem	Tremel	4/25/2011	De	B	0	1
SA472	Auriculibuller fuscus 2	Agar	Trem	Tremel	6/28/2011	Cf	B	0	1
SA498	Auriculibuller fuscus 3	Agar	Trem	Tremel	7/11/2011	De	B	0	0
SA374	Bullera pseudoalba 1	Agar	Trem	Tremel	4/25/2011	Lj	D	1	0
SA405	Bullera pseudoalba 1	Agar	Trem	Tremel	6/10/2011	Cf	D	0	1
SA414	Bullera pseudoalba 1	Agar	Trem	Tremel	6/10/2011	Cf	B	0	1
SA433	Bullera pseudoalba 1	Agar	Trem	Tremel	5/11/2011	Pp	D	0	0
SA434	Bullera pseudoalba 1	Agar	Trem	Tremel	5/13/2011	Pp	B	0	0
SA435	Bullera pseudoalba 1	Agar	Trem	Tremel	5/27/2011	Pp	B	0	1
SA473	Bullera pseudoalba 1	Agar	Trem	Tremel	6/28/2011	Cf	D	0	1
SA499	Bullera pseudoalba 1	Agar	Trem	Tremel	7/11/2011	De	D	0	1
SA388	Bullera pseudoalba 2	Agar	Trem	Tremel	4/25/2011	Ne	B	0	1
SA721	Bulleribasidium oberjochense	Agar	Trem	Tremel	11/3/2011	Pp	B	0	0
SA246	Bulleromyces albus 1	Agar	Trem	Tremel	2/19/2011	Pp	B	1	0
SA255	Bulleromyces albus 1	Agar	Trem	Tremel	2/17/2011	Ra	B	0	0
SA270	Bulleromyces albus 1	Agar	Trem	Tremel	3/2/2011	Pp	B	0	0
SA303	Bulleromyces albus 1	Agar	Trem	Tremel	4/1/2011	Ne	B	1	0
SA306	Bulleromyces albus 1	Agar	Trem	Tremel	3/31/2011	De	D	0	0
SA317	Bulleromyces albus 1	Agar	Trem	Tremel	4/7/2011	De	D	0	0
SA359	Bulleromyces albus 1	Agar	Trem	Tremel	4/26/2011	Cf	B	1	0
SA362	Bulleromyces albus 1	Agar	Trem	Tremel	4/26/2011	Cf	D	1	0
SA381	Bulleromyces albus 1	Agar	Trem	Tremel	4/25/2011	Pp	n/a	0	0
SA469	Bulleromyces albus 1	Agar	Trem	Tremel	6/28/2011	Cf	B	0	1
SA470	Bulleromyces albus 1	Agar	Trem	Tremel	6/28/2011	Cf	B	0	1
SA477	Bulleromyces albus 1	Agar	Trem	Tremel	6/28/2011	De	B	0	0
SA685	Bulleromyces albus 2	Agar	Trem	Tremel	10/20/2011	Ra	B	0	0
SA315	Cryptococcus flavus	Agar	Trem	Tremel	3/18/2011	Lj	B	1	0
SA617	Cryptococcus flavus	Agar	Trem	Tremel	9/5/2011	Ra	D	0	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA623	Cryptococcus flavus	Agar	Trem	Tremel	9/4/2011	Ra	D	0	0
SA285	Cryptococcus sp cf heimaeyensis 1	Agar	Trem	Tremel	3/29/2011	Pp	n/a	0	0
SA287	Cryptococcus sp cf heimaeyensis 1	Agar	Trem	Tremel	3/28/2011	Pp	n/a	0	0
SA523	Cryptococcus sp cf heimaeyensis 1	Agar	Trem	Tremel	7/28/2011	Pp	B	0	0
SA248	Cryptococcus sp cf heimaeyensis 2	Agar	Trem	Tremel	2/17/2011	Pp	B	1	0
SA797	Cryptococcus sp cf heimaeyensis 2	Agar	Trem	Tremel	12/26/2011	Cf	D	0	1
SA328	Cryptococcus sp cf heveanensis 1	Agar	Trem	Tremel	4/13/2011	De	D	0	0
SA789	Cryptococcus sp cf heveanensis 2	Agar	Trem	Tremel	12/26/2011	Cf	B	1	1
SA193	Cryptococcus sp cf laurentii	Agar	Trem	Tremel	1/5/2011	Pp	D	0	0
SA492	Cryptococcus victoriae	Agar	Trem	Tremel	7/10/2011	Cf	B	0	1
SA494	Cryptococcus victoriae	Agar	Trem	Tremel	7/11/2011	Cf	B	0	1
SA247	Derxomyces boekhoutii 1	Agar	Trem	Tremel	2/19/2011	Pp	B	1	0
SA272	Derxomyces boekhoutii 1	Agar	Trem	Tremel	3/7/2011	Pp	D	0	0
SA393	Derxomyces mraki 1	Agar	Trem	Tremel	4/15/2011	De	B	0	0
SA394	Derxomyces mraki 1	Agar	Trem	Tremel	4/17/2011	De	B	0	0
SA738	Derxomyces sp boekhoutii 2	Agar	Trem	Tremel	11/5/2011	Pp	B	0	0
SA243	Derxomyces sp cf boekhoutii 1	Agar	Trem	Tremel	2/19/2011	Pp	B	1	0
SA387	Dioszegia zsoltii	Agar	Trem	Tremel	4/25/2011	Ne	B	0	1
SA427	Dioszegia zsoltii	Agar	Trem	Tremel	5/6/2011	Ne	B	0	1
SA471	Dioszegia zsoltii	Agar	Trem	Tremel	6/28/2011	Cf	B	0	1
SA420	Hannaella sinensis 1	Agar	Trem	Tremel	5/27/2011	Cf	B	0	1
SA436	Hannaella sinensis 1	Agar	Trem	Tremel	6/8/2011	Pp	B	0	1
SA700	Hannaella sinensis 1	Agar	Trem	Tremel	10/20/2011	De	D	1	1
SA548	Hannaella sinensis 2	Agar	Trem	Tremel	7/28/2011	Tk	D	0	1
SA741	Hannaella sinensis 2	Agar	Trem	Tremel	11/17/2011	Ne	B	0	1
SA439	Hannaella sinensis 3	Agar	Trem	Tremel	5/27/2011	Pp	D	0	1
SA740	Hannaella sinensis 3	Agar	Trem	Tremel	11/17/2011	De	B	0	1
SA411	Hannaella sinensis 4	Agar	Trem	Tremel	5/30/2011	Cf	B	0	1
SA655	Hannaella sinensis 5	Agar	Trem	Tremel	10/2/2011	Pp	B	0	0
SA253	Hannaella sp 1	Agar	Trem	Tremel	2/15/2011	Lj	B	1	0
SA490	Hannaella sp 1	Agar	Trem	Tremel	7/10/2011	Cf	B	0	1
SA491	Hannaella sp 1	Agar	Trem	Tremel	7/14/2011	Cf	B	0	1
SA452	Hannaella sp 2	Agar	Trem	Tremel	6/2/2011	Tk	D	0	1
SA219	Agaricostilbales sp	Puc	Agari	Agarico	2/6/2011	Pp	B	0	0
SA324	Bensingtonia miscantha 1	Puc	Agari	Agarico	4/14/2011	Pp	D	0	0
SA277	Bensingtonia miscantha 2	Puc	Agari	Agarico	3/22/2011	De	D	0	0
SA376	Kondoa sp 1	Puc	Agari	Agarico	4/26/2011	Ne	D	0	1
SA284	Cystobasidiales sp 1	Puc	Cyst	Cystob	3/22/2011	Pp	B	1	0
SA312	Cystobasidiales sp 1	Puc	Cyst	Cystob	3/18/2011	Pp	B	0	0
SA444	Cystobasidiales sp 2	Puc	Cyst	Cystob	5/11/2011	De	D	0	1
SA279	Cystobasidiales sp 3	Puc	Cyst	Cystob	3/14/2011	De	B	0	0
SA628	Rhodotorula calyptogenae/cassiicola	Puc	Cyst	Cystob	9/21/2011	Tk	B	1	1
SA515	Rhodotorula slooffiae	Puc	Cyst	Erythro	7/12/2011	Tk	B	0	1
SA283	Rhodotorula marina 1	Puc	Cyst	Erythro	3/18/2011	Pp	B	1	0
SA310	Rhodotorula marina 2	Puc	Cyst	Erythro	3/16/2011	De	B	1	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA311	Rhodotorula marina 3	Puc	Cyst	Erythro	3/18/2011	Pp	B	0	0
SA609	Rhodotorula marina 3	Puc	Cyst	Erythro	9/4/2011	Lj	D	0	1
SA615	Rhodotorula marina 3	Puc	Cyst	Erythro	8/30/2011	Ra	B	0	1
SA619	Rhodotorula marina 3	Puc	Cyst	Erythro	9/6/2011	De	B	0	1
SA716	Rhodotorula marina 3	Puc	Cyst	Erythro	11/1/2011	De	B	1	1
SA776	Rhodotorula marina 4	Puc	Cyst	Erythro	12/12/2011	Cf	D	1	1
SA265	Sporobolomyces sp cf gracilis 1	Puc	Cyst	Erythro	3/4/2011	Lj	D	1	1
SA333	Sporobolomyces sp cf gracilis 1	Puc	Cyst	Erythro	4/13/2011	Lj	D	0	0
SA337	Sporobolomyces sp cf gracilis 1	Puc	Cyst	Erythro	4/13/2011	Lj	D	0	0
SA273	Sporobolomyces sp cf gracilis 2	Puc	Cyst	Erythro	3/7/2011	Pp	D	0	0
SA308	Sporobolomyces sp cf gracilis 2	Puc	Cyst	Erythro	3/18/2011	De	D	0	0
SA295	Sporidiobolus pararoseus	Puc	Micro	Spor	3/29/2011	Ne	B	0	0
SA314	Sporidiobolus pararoseus	Puc	Micro	Spor	3/18/2011	Ne	D	0	0
SA421	Sporidiobolus pararoseus	Puc	Micro	Spor	5/31/2011	Ne	D	0	1
SA422	Sporidiobolus pararoseus	Puc	Micro	Spor	6/14/2011	Ne	D	0	1
SA423	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Ne	D	0	1
SA424	Sporidiobolus pararoseus	Puc	Micro	Spor	6/10/2011	Ne	D	0	1
SA425	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Ne	D	0	1
SA426	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Ne	D	0	1
SA428	Sporidiobolus pararoseus	Puc	Micro	Spor	5/13/2011	Ne	D	0	1
SA429	Sporidiobolus pararoseus	Puc	Micro	Spor	5/27/2011	Ne	B	0	1
SA430	Sporidiobolus pararoseus	Puc	Micro	Spor	6/15/2011	Ne	B	0	1
SA431	Sporidiobolus pararoseus	Puc	Micro	Spor	6/10/2011	Ne	B	0	1
SA432	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Ne	B	0	1
SA513	Sporidiobolus pararoseus	Puc	Micro	Spor	7/13/2011	Ne	D	0	0
SA532	Sporidiobolus pararoseus	Puc	Micro	Spor	7/28/2011	Ne	B	0	0
SA540	Sporidiobolus pararoseus	Puc	Micro	Spor	7/28/2011	Ne	D	0	1
SA566	Sporidiobolus pararoseus	Puc	Micro	Spor	8/11/2011	Ne	D	0	0
SA570	Sporidiobolus pararoseus	Puc	Micro	Spor	8/23/2011	Ne	D	0	0
SA610	Sporidiobolus pararoseus	Puc	Micro	Spor	9/8/2011	Ne	B	0	0
SA611	Sporidiobolus pararoseus	Puc	Micro	Spor	9/5/2011	Ne	D	0	0
SA639	Sporidiobolus pararoseus	Puc	Micro	Spor	9/19/2011	Ne	B	0	0
SA657	Sporidiobolus pararoseus	Puc	Micro	Spor	10/5/2011	Ne	B	0	1
SA694	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Ne	D	0	0
SA714	Sporidiobolus pararoseus	Puc	Micro	Spor	10/31/2011	Ne	B	1	1
SA715	Sporidiobolus pararoseus	Puc	Micro	Spor	11/2/2011	Ne	D	0	0
SA764	Sporidiobolus pararoseus	Puc	Micro	Spor	12/12/2011	Ne	D	0	1
SA274	Sporobolomyces carnicolor 1	Puc	Micro	Spor	3/2/2011	Ne	D	0	0
SA313	Sporobolomyces carnicolor 1	Puc	Micro	Spor	3/18/2011	Ne	D	0	0
SA752	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/1/2011	Ne	D	0	1
SA753	Sporobolomyces carnicolor 1	Puc	Micro	Spor	11/30/2011	Ne	D	0	0
SA763	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/12/2011	Ne	B	0	1
SA804	Sporobolomyces odoratus 2	Puc	Micro	Spor	12/30/2011	Ne	B	0	0
SA754	Sporobolomyces odoratus 4	Puc	Micro	Spor	12/1/2011	Ne	D	0	1
SA269	Sporobolomyces johnsonii	Puc	Micro	Spor	3/2/2011	Pp	B	0	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA673	Sporobolomyces koalae	Puc	Micro	Spor	10/7/2011	Lj	B	0	0
SA199	Sporidiobolus pararoseus	Puc	Micro	Spor	1/5/2011	De	B	0	0
SA202	Sporidiobolus pararoseus	Puc	Micro	Spor	1/19/2011	Lj	B	0	0
SA205	Sporidiobolus pararoseus	Puc	Micro	Spor	1/19/2011	Lj	B	0	0
SA207	Sporidiobolus pararoseus	Puc	Micro	Spor	1/19/2011	De	B	0	0
SA254	Sporidiobolus pararoseus	Puc	Micro	Spor	2/17/2011	Tk	B	1	0
SA257	Sporidiobolus pararoseus	Puc	Micro	Spor	3/4/2011	Tk	B	0	0
SA260	Sporidiobolus pararoseus	Puc	Micro	Spor	3/4/2011	Ra	D	0	0
SA261	Sporidiobolus pararoseus	Puc	Micro	Spor	3/2/2011	Ra	D	0	0
SA264	Sporidiobolus pararoseus	Puc	Micro	Spor	3/2/2011	Lj	B	1	0
SA268	Sporidiobolus pararoseus	Puc	Micro	Spor	3/4/2011	Lj	D	0	1
SA271	Sporidiobolus pararoseus	Puc	Micro	Spor	3/2/2011	Pp	B	0	0
SA278	Sporidiobolus pararoseus	Puc	Micro	Spor	3/22/2011	De	D	0	0
SA282	Sporidiobolus pararoseus	Puc	Micro	Spor	3/22/2011	Cf	D	0	0
SA297	Sporidiobolus pararoseus	Puc	Micro	Spor	3/30/2011	De	B	0	0
SA298	Sporidiobolus pararoseus	Puc	Micro	Spor	3/29/2011	De	D	0	0
SA301	Sporidiobolus pararoseus	Puc	Micro	Spor	3/29/2011	Cf	D	0	0
SA309	Sporidiobolus pararoseus	Puc	Micro	Spor	3/18/2011	De	D	0	0
SA319	Sporidiobolus pararoseus	Puc	Micro	Spor	4/4/2011	Tk	D	0	0
SA323	Sporidiobolus pararoseus	Puc	Micro	Spor	4/13/2011	Cf	D	0	0
SA330	Sporidiobolus pararoseus	Puc	Micro	Spor	4/14/2011	De	D	0	0
SA343	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	De	B	0	1
SA345	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	De	B	0	1
SA346	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	De	B	0	1
SA348	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	De	B	0	1
SA351	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	De	D	0	1
SA353	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	De	D	0	1
SA355	Sporidiobolus pararoseus	Puc	Micro	Spor	4/23/2011	De	D	0	0
SA357	Sporidiobolus pararoseus	Puc	Micro	Spor	4/26/2011	Cf	B	1	0
SA358	Sporidiobolus pararoseus	Puc	Micro	Spor	4/26/2011	Cf	B	1	0
SA361	Sporidiobolus pararoseus	Puc	Micro	Spor	4/26/2011	Cf	D	0	1
SA365	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	Ra	D	0	0
SA372	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	Lj	D	0	0
SA373	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	Lj	D	1	0
SA379	Sporidiobolus pararoseus	Puc	Micro	Spor	4/27/2011	Tk	D	0	0
SA380	Sporidiobolus pararoseus	Puc	Micro	Spor	4/27/2011	Tk	B	0	0
SA383	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	Pp	D	0	0
SA397	Sporidiobolus pararoseus	Puc	Micro	Spor	4/25/2011	Ra	D	0	0
SA399	Sporidiobolus pararoseus	Puc	Micro	Spor	4/20/2011	Cf	D	0	0
SA403	Sporidiobolus pararoseus	Puc	Micro	Spor	5/30/2011	Cf	D	0	1
SA404	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	D	0	1
SA406	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	D	0	1
SA407	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	D	0	1
SA408	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	D	0	1
SA409	Sporidiobolus pararoseus	Puc	Micro	Spor	6/15/2011	Cf	D	0	1

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA410	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	D	0	1
SA415	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	B	0	1
SA416	Sporidiobolus pararoseus	Puc	Micro	Spor	6/10/2011	Cf	B	0	1
SA417	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Cf	B	0	1
SA419	Sporidiobolus pararoseus	Puc	Micro	Spor	5/27/2011	Cf	B	0	1
SA438	Sporidiobolus pararoseus	Puc	Micro	Spor	5/30/2011	Pp	D	0	1
SA447	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	De	D	0	1
SA449	Sporidiobolus pararoseus	Puc	Micro	Spor	5/31/2011	Ra	D	0	0
SA450	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Ra	D	0	0
SA453	Sporidiobolus pararoseus	Puc	Micro	Spor	5/30/2011	Lj	B	0	0
SA454	Sporidiobolus pararoseus	Puc	Micro	Spor	5/30/2011	Lj	B	0	0
SA455	Sporidiobolus pararoseus	Puc	Micro	Spor	6/8/2011	Lj	B	0	0
SA456	Sporidiobolus pararoseus	Puc	Micro	Spor	5/30/2011	Lj	B	0	0
SA457	Sporidiobolus pararoseus	Puc	Micro	Spor	6/2/2011	Lj	B	0	0
SA458	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Lj	B	0	0
SA459	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Lj	B	0	0
SA460	Sporidiobolus pararoseus	Puc	Micro	Spor	6/13/2011	Lj	B	0	0
SA474	Sporidiobolus pararoseus	Puc	Micro	Spor	6/29/2011	Cf	D	0	1
SA475	Sporidiobolus pararoseus	Puc	Micro	Spor	6/29/2011	Cf	D	0	1
SA476	Sporidiobolus pararoseus	Puc	Micro	Spor	6/28/2011	De	B	0	0
SA478	Sporidiobolus pararoseus	Puc	Micro	Spor	6/29/2011	De	D	0	0
SA479	Sporidiobolus pararoseus	Puc	Micro	Spor	6/28/2011	Tk	B	0	1
SA483	Sporidiobolus pararoseus	Puc	Micro	Spor	6/28/2011	Pp	B	n/a	0
SA484	Sporidiobolus pararoseus	Puc	Micro	Spor	6/28/2011	Pp	D	0	0
SA486	Sporidiobolus pararoseus	Puc	Micro	Spor	6/28/2011	De	B	0	0
SA487	Sporidiobolus pararoseus	Puc	Micro	Spor	6/30/2011	Ra	B	0	1
SA488	Sporidiobolus pararoseus	Puc	Micro	Spor	6/28/2011	Ra	B	0	0
SA497	Sporidiobolus pararoseus	Puc	Micro	Spor	7/11/2011	De	B	0	1
SA501	Sporidiobolus pararoseus	Puc	Micro	Spor	7/11/2011	De	D	0	1
SA503	Sporidiobolus pararoseus	Puc	Micro	Spor	7/12/2011	Lj	D	1	0
SA505	Sporidiobolus pararoseus	Puc	Micro	Spor	7/14/2011	Ra	D	0	0
SA508	Sporidiobolus pararoseus	Puc	Micro	Spor	7/12/2011	Pp	D	0	0
SA519	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Lj	B	1	1
SA528	Sporidiobolus pararoseus	Puc	Micro	Spor	7/25/2011	De	B	0	0
SA529	Sporidiobolus pararoseus	Puc	Micro	Spor	7/28/2011	De	B	0	1
SA530	Sporidiobolus pararoseus	Puc	Micro	Spor	7/28/2011	De	D	0	1
SA543	Sporidiobolus pararoseus	Puc	Micro	Spor	8/1/2011	Ra	B	0	0
SA544	Sporidiobolus pararoseus	Puc	Micro	Spor	7/29/2011	Ra	B	0	0
SA545	Sporidiobolus pararoseus	Puc	Micro	Spor	7/28/2011	Tk	B	0	0
SA552	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Ra	D	0	0
SA553	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Ra	D	0	0
SA555	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Ra	D	0	0
SA558	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Tk	B	0	0
SA560	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Tk	B	0	0
SA563	Sporidiobolus pararoseus	Puc	Micro	Spor	8/9/2011	Tk	B	0	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA564	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Tk	D	0	0
SA642	Sporidiobolus pararoseus	Puc	Micro	Spor	9/21/2011	Cf	B	1	0
SA643	Sporidiobolus pararoseus	Puc	Micro	Spor	9/21/2011	Cf	D	0	0
SA644	Sporidiobolus pararoseus	Puc	Micro	Spor	9/21/2011	Pp	B	0	0
SA648	Sporidiobolus pararoseus	Puc	Micro	Spor	9/19/2011	Ra	B	1	1
SA651	Sporidiobolus pararoseus	Puc	Micro	Spor	8/8/2011	Ra	B	0	1
SA666	Sporidiobolus pararoseus	Puc	Micro	Spor	10/3/2011	De	D	1	1
SA667	Sporidiobolus pararoseus	Puc	Micro	Spor	10/5/2011	De	D	0	0
SA668	Sporidiobolus pararoseus	Puc	Micro	Spor	10/3/2011	Cf	B	1	0
SA669	Sporidiobolus pararoseus	Puc	Micro	Spor	10/5/2011	Cf	D	1	1
SA671	Sporidiobolus pararoseus	Puc	Micro	Spor	10/3/2011	Ra	B	0	0
SA672	Sporidiobolus pararoseus	Puc	Micro	Spor	10/3/2011	Ra	D	0	0
SA674	Sporidiobolus pararoseus	Puc	Micro	Spor	10/4/2011	Ra	D	0	1
SA677	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Cf	B	1	1
SA678	Sporidiobolus pararoseus	Puc	Micro	Spor	10/20/2011	Cf	D	1	0
SA680	Sporidiobolus pararoseus	Puc	Micro	Spor	10/20/2011	Lj	B	0	0
SA681	Sporidiobolus pararoseus	Puc	Micro	Spor	10/20/2011	Lj	B	0	0
SA682	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Lj	B	0	0
SA686	Sporidiobolus pararoseus	Puc	Micro	Spor	10/20/2011	Ra	D	0	1
SA692	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Pp	D	0	0
SA699	Sporidiobolus pararoseus	Puc	Micro	Spor	10/17/2011	De	D	1	0
SA701	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Tk	B	0	0
SA702	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Tk	D	0	1
SA704	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Tk	D	0	0
SA711	Sporidiobolus pararoseus	Puc	Micro	Spor	11/2/2011	Ra	D	0	1
SA712	Sporidiobolus pararoseus	Puc	Micro	Spor	11/1/2011	Cf	D	1	1
SA713	Sporidiobolus pararoseus	Puc	Micro	Spor	11/4/2011	Lj	B	1	0
SA720	Sporidiobolus pararoseus	Puc	Micro	Spor	11/1/2011	Pp	B	0	0
SA726	Sporidiobolus pararoseus	Puc	Micro	Spor	10/17/2011	De	D	1	0
SA728	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Tk	B	0	0
SA729	Sporidiobolus pararoseus	Puc	Micro	Spor	10/18/2011	Tk	D	0	1
SA733	Sporidiobolus pararoseus	Puc	Micro	Spor	11/18/2011	Cf	B	0	0
SA744	Sporidiobolus pararoseus	Puc	Micro	Spor	11/30/2011	Ra	D	1	1
SA746	Sporidiobolus pararoseus	Puc	Micro	Spor	12/1/2011	Lj	D	1	0
SA747	Sporidiobolus pararoseus	Puc	Micro	Spor	11/30/2011	De	D	1	1
SA748	Sporidiobolus pararoseus	Puc	Micro	Spor	11/30/2011	De	D	1	1
SA749	Sporidiobolus pararoseus	Puc	Micro	Spor	11/30/2011	De	D	0	0
SA750	Sporidiobolus pararoseus	Puc	Micro	Spor	11/30/2011	Cf	B	1	1
SA751	Sporidiobolus pararoseus	Puc	Micro	Spor	11/30/2011	Cf	D	1	1
SA758	Sporidiobolus pararoseus	Puc	Micro	Spor	12/12/2011	Cf	B	1	1
SA760	Sporidiobolus pararoseus	Puc	Micro	Spor	12/12/2011	Lj	B	1	1
SA762	Sporidiobolus pararoseus	Puc	Micro	Spor	12/13/2011	Lj	D	1	1
SA765	Sporidiobolus pararoseus	Puc	Micro	Spor	12/12/2011	Ra	B	0	0
SA766	Sporidiobolus pararoseus	Puc	Micro	Spor	12/13/2011	Ra	D	1	1
SA769	Sporidiobolus pararoseus	Puc	Micro	Spor	12/13/2011	De	D	0	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA770	Sporidiobolus pararoseus	Puc	Micro	Spor	12/13/2011	De	D	1	1
SA785	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	De	n/a	1	0
SA786	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	De	D	0	1
SA788	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Cf	B	1	1
SA794	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Cf	D	0	0
SA795	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Cf	D	0	0
SA796	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Cf	D	0	1
SA798	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Cf	D	0	1
SA799	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Cf	D	0	1
SA801	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Lj	D	0	1
SA802	Sporidiobolus pararoseus	Puc	Micro	Spor	12/26/2011	Pp	D	0	0
SA743	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/1/2011	Ra	B	0	0
SA745	Sporobolomyces carnicolor 1	Puc	Micro	Spor	11/30/2011	Lj	D	0	1
SA755	Sporobolomyces carnicolor 1	Puc	Micro	Spor	11/30/2011	Tk	D	0	1
SA756	Sporobolomyces carnicolor 1	Puc	Micro	Spor	11/30/2011	Tk	D	0	0
SA757	Sporobolomyces carnicolor 1	Puc	Micro	Spor	11/30/2011	Tk	D	0	0
SA768	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/13/2011	De	B	0	0
SA790	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/26/2011	Cf	B	1	1
SA791	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/26/2011	Cf	B	1	1
SA793	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/26/2011	Cf	D	1	1
SA800	Sporobolomyces carnicolor 1	Puc	Micro	Spor	12/26/2011	Cf	D	0	1
SA784	Sporobolomyces nylandii	Puc	Micro	Spor	12/26/2011	De	B	1	1
SA759	Sporobolomyces odoratus 1	Puc	Micro	Spor	12/12/2011	Cf	B	1	1
SA356	Sporobolomyces odoratus 2	Puc	Micro	Spor	4/26/2011	Cf	B	0	1
SA482	Sporobolomyces odoratus 2	Puc	Micro	Spor	6/29/2011	Lj	D	0	0
SA679	Sporobolomyces odoratus 3	Puc	Micro	Spor	10/17/2011	Cf	D	1	0
SA735	Sporobolomyces odoratus 3	Puc	Micro	Spor	11/16/2011	Cf	B	1	1
SA736	Sporobolomyces odoratus 3	Puc	Micro	Spor	11/18/2011	Cf	B	1	1
SA737	Sporobolomyces odoratus 3	Puc	Micro	Spor	11/18/2011	Cf	B	1	1
SA774	Sporobolomyces odoratus 3	Puc	Micro	Spor	12/13/2011	Pp	D	0	0
SA782	Sporobolomyces odoratus 3	Puc	Micro	Spor	12/26/2011	De	B	0	0
SA783	Sporobolomyces odoratus 3	Puc	Micro	Spor	12/26/2011	De	B	1	1
SA727	Sporobolomyces odoratus 4	Puc	Micro	Spor	10/20/2011	De	D	1	1
SA703	Sporobolomyces ruineniae 1	Puc	Micro	Spor	10/18/2011	Tk	D	0	1
SA200	Sporobolomyces sp cf carnicolor 1	Puc	Micro	Spor	1/7/2011	De	B	0	0
SA803	Sporobolomyces sp cf carnicolor 1	Puc	Micro	Spor	12/30/2011	Ra	B	0	1
SA787	Sporobolomyces sp cf nylandii 1	Puc	Micro	Spor	12/26/2011	De	D	0	1
SA761	Sporobolomyces sp cf nylandii 2	Puc	Micro	Spor	12/5/2011	Lj	D	0	1
SA262	Sporobolomyces sp cf nylandii 3	Puc	Micro	Spor	3/2/2011	Ra	D	0	1
SA263	Sporobolomyces sp cf nylandii 3	Puc	Micro	Spor	3/4/2011	Ra	D	0	1
SA194	Sporobolomyces sp cf ruineniae 1	Puc	Micro	Spor	1/7/2011	Lj	B	1	0
SA195	Sporobolomyces sp cf ruineniae 1	Puc	Micro	Spor	1/5/2011	Lj	B	0	0
SA196	Sporobolomyces sp cf ruineniae 1	Puc	Micro	Spor	1/6/2011	Lj	B	1	0
SA203	Sporobolomyces sp cf ruineniae 1	Puc	Micro	Spor	1/19/2011	Lj	B	0	0
SA717	Sporobolomyces sp cf ruineniae 1	Puc	Micro	Spor	11/2/2011	De	D	0	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA231	Rhodotorula nothofagi	Puc	Micro	is	2/15/2011	Cf	B	1	0
SA805	Rhodotorula nothofagi	Puc	Micro	is	12/30/2011	Cf	B	1	1
SA322	Entyloma calendulae	Ust	Exo	Enty	4/13/2011	Cf	D	0	0
SA288	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Lj	D	0	0
SA289	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Lj	D	0	0
SA290	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Lj	B	0	0
SA291	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Lj	D	0	0
SA294	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Ne	D	0	0
SA299	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Ra	D	0	0
SA300	Tilletiopsis lilacina	Ust	Exo	Enty	3/29/2011	Cf	D	0	0
SA302	Tilletiopsis lilacina	Ust	Exo	Enty	4/1/2011	Ne	D	0	0
SA307	Tilletiopsis lilacina	Ust	Exo	Enty	4/1/2011	Ra	B	0	0
SA320	Tilletiopsis lilacina	Ust	Exo	Enty	4/13/2011	Tk	D	0	0
SA326	Tilletiopsis lilacina	Ust	Exo	Enty	4/13/2011	Pp	D	0	0
SA329	Tilletiopsis lilacina	Ust	Exo	Enty	4/13/2011	De	D	0	0
SA331	Tilletiopsis lilacina	Ust	Exo	Enty	4/11/2011	De	D	0	0
SA334	Tilletiopsis lilacina	Ust	Exo	Enty	4/12/2011	Lj	D	0	0
SA335	Tilletiopsis lilacina	Ust	Exo	Enty	4/13/2011	Lj	D	0	0
SA354	Tilletiopsis lilacina	Ust	Exo	Enty	4/25/2011	De	D	0	1
SA360	Tilletiopsis lilacina	Ust	Exo	Enty	4/26/2011	Cf	D	0	1
SA364	Tilletiopsis lilacina	Ust	Exo	Enty	4/25/2011	Ra	D	0	0
SA367	Tilletiopsis lilacina	Ust	Exo	Enty	4/27/2011	Lj	B	0	0
SA370	Tilletiopsis lilacina	Ust	Exo	Enty	4/25/2011	Lj	B	0	0
SA377	Tilletiopsis lilacina	Ust	Exo	Enty	4/26/2011	Ne	D	0	1
SA378	Tilletiopsis lilacina	Ust	Exo	Enty	4/27/2011	Ne	D	0	0
SA384	Tilletiopsis lilacina	Ust	Exo	Enty	4/25/2011	Pp	D	0	0
SA386	Tilletiopsis lilacina	Ust	Exo	Enty	4/21/2011	Tk	D	0	0
SA398	Tilletiopsis lilacina	Ust	Exo	Enty	4/27/2011	Ra	B	0	0
SA461	Tilletiopsis lilacina	Ust	Exo	Enty	6/2/2011	Lj	D	0	0
SA551	Tilletiopsis lilacina	Ust	Exo	Enty	8/9/2011	Ra	D	0	0
SA561	Tilletiopsis lilacina	Ust	Exo	Enty	8/8/2011	Tk	B	0	1
SA572	Tilletiopsis lilacina	Ust	Exo	Enty	8/23/2011	Ra	D	0	1
SA620	Tilletiopsis lilacina	Ust	Exo	Enty	9/8/2011	De	B	0	1
SA621	Tilletiopsis lilacina	Ust	Exo	Enty	9/5/2011	De	B	1	1
SA451	Tilletiopsis sp cf lilacina 1	Ust	Exo	Enty	6/14/2011	Tk	B	0	1
SA304	Tilletiopsis sp cf lilacina 2	Ust	Exo	Enty	4/1/2011	Cf	B	0	0
SA382	Tilletiopsis sp cf lilacina 2	Ust	Exo	Enty	4/25/2011	Pp	n/a	0	0
SA504	Tilletiopsis sp cf lilacina 2	Ust	Exo	Enty	7/14/2011	Ra	D	0	0
SA400	Tilletiopsis sp cf lilacina 3	Ust	Exo	Enty	4/29/2011	Cf	D	1	0
SA616	Tilletiopsis sp cf lilacina 3	Ust	Exo	Enty	8/30/2011	Ra	D	0	1
SA267	Tilletiopsis sp cf lilacina 4	Ust	Exo	Enty	3/2/2011	Lj	D	1	1
SA349	Tilletiopsis sp cf washingtonensis 5	Ust	Exo	Enty	4/25/2011	De	B	0	1
SA366	Tilletiopsis sp cf washingtonensis 5	Ust	Exo	Enty	4/27/2011	Ra	D	0	1
SA375	Tilletiopsis sp cf washingtonensis 5	Ust	Exo	Enty	4/25/2011	Ne	B	1	0
SA546	Tilletiopsis washingtonensis	Ust	Exo	Enty	7/28/2011	Tk	B	0	1

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA547	Tilletiopsis washingtonensis	Ust	Exo	Enty	7/28/2011	Tk	B	0	1
SA581	Cryptobasidiaceae sp	Ust	Exo	Exob	8/25/2011	De	B	1	1
SA223	Kordyana sp	Ust	Exo	Exob	2/6/2011	Tk	B	1	1
SA608	Meira argovae 1	Ust	Exo	Exob	8/29/2011	Lj	D	1	0
SA242	Meira argovae 2	Ust	Exo	Exob	2/19/2011	Pp	B	1	0
SA767	Meira argovae 3	Ust	Exo	Exob	12/11/2011	Ra	D	1	1
SA569	Meira cf geulakonigii	Ust	Exo	Exob	8/24/2011	Ne	B	1	0
SA226	Meira cf nashicola	Ust	Exo	Exob	2/17/2011	Pp	B	0	0
SA227	Meira cf nashicola	Ust	Exo	Exob	2/17/2011	Pp	B	0	0
SA258	Tilletiopsis sp cf minor 1	Ust	Exo	George	3/4/2011	De	D	0	1
SA385	Tilletiopsis sp cf minor 1	Ust	Exo	George	4/21/2011	Tk	D	0	0
SA520	Tilletiopsis sp cf minor 1	Ust	Exo	George	8/12/2011	Lj	B	1	1
SA521	Tilletiopsis sp cf minor 1	Ust	Exo	George	8/12/2011	Lj	B	0	1
SA522	Tilletiopsis sp cf minor 1	Ust	Exo	George	8/12/2011	Lj	B	1	1
SA595	Tilletiopsis sp cf minor 1	Ust	Exo	George	8/26/2011	Lj	B	0	0
SA652	Tilletiopsis sp cf minor 1	Ust	Exo	George	8/8/2011	Ra	B	0	1
SA485	Tilletiopsis sp cf minor 2	Ust	Exo	George	7/2/2011	Ne	D	0	0
SA191	Tilletiopsis sp cf minor 3	Ust	Exo	George	1/5/2011	Cf	B	1	1
SA192	Tilletiopsis sp cf minor 4	Ust	Exo	George	1/5/2011	Cf	B	1	1
SA339	Tilletiopsis sp cf minor 5	Ust	Exo	George	4/13/2011	Lj	B	1	0
SA316	Tilletiopsis sp cf minor 6	Ust	Exo	George	3/18/2011	Lj	D	0	0
SA369	Tilletiopsis sp cf minor 7	Ust	Exo	George	4/20/2011	Lj	B	0	0
SA549	Tilletiopsis sp cf minor 8	Ust	Exo	George	8/8/2011	Ra	B	0	1
SA550	Tilletiopsis sp cf minor 8	Ust	Exo	George	8/8/2011	Ra	B	0	1
SA709	Tilletiopsis sp cf minor 9	Ust	Exo	George	10/20/2011	Lj	D	0	0
SA448	Tilletiopsis pallescens	Ust	Exo	is	5/27/2011	Ra	B	0	1
SA259	Tilletiopsis sp cf pallescens 1	Ust	Exo	is	3/4/2011	De	D	0	1
SA591	Tilletiopsis sp cf pallescens 2	Ust	Exo	is	8/28/2011	Ne	B	0	0
SA792	Jaminaea lanaiensis	Ust	Exo	Micros	12/26/2011	Cf	B	1	1
SA220	Jaminaea sp cf angkoriensis 4	Ust	Exo	Micros	2/6/2011	Pp	B	0	0
SA368	Jaminaea sp cf angkoriensis 4	Ust	Exo	Micros	4/25/2011	Lj	B	0	0
SA594	Jaminaea sp cf angkoriensis 4	Ust	Exo	Micros	8/29/2011	Lj	B	1	0
SA599	Jaminaea sp cf angkoriensis 4	Ust	Exo	Micros	8/26/2011	Tk	B	0	0
SA649	Jaminaea sp cf angkoriensis 4	Ust	Exo	Micros	9/19/2011	Ra	B	1	1
SA658	Jaminaea sp cf angkoriensis 4	Ust	Exo	Micros	10/7/2011	Ne	B	1	0
SA533	Jaminaea sp cf angkoriensis 5	Ust	Exo	Micros	7/28/2011	Ne	B	0	0
SA590	Jaminaea sp cf angkoriensis 5	Ust	Exo	Micros	8/29/2011	Ne	D	1	0
SA683	Jaminaea sp cf angkoriensis 5	Ust	Exo	Micros	10/17/2011	Ra	B	0	1
SA684	Jaminaea sp cf angkoriensis 5	Ust	Exo	Micros	10/20/2011	Ra	B	0	1
SA534	Jaminaea sp cf lanaiensis 1	Ust	Exo	Micros	7/29/2011	Ne	B	0	0
SA537	Jaminaea sp cf lanaiensis 2	Ust	Exo	Micros	7/30/2011	Ne	D	0	0
SA531	Jaminaea sp cf lanaiensis 3	Ust	Exo	Micros	7/28/2011	Ne	B	0	0
SA697	Microstroma sp cf juglandis 1	Ust	Exo	Micros	10/18/2011	De	B	1	1
SA568	Microstroma sp cf juglandis 2	Ust	Exo	Micros	8/24/2011	Ne	B	1	0
SA524	Rhodotorula hinnulea	Ust	Exo	Micros	8/1/2011	Pp	B	0	0

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA525	Rhodotorula hinnulea	Ust	Exo	Micros	7/30/2011	Pp	B	0	0
SA731	Rhodotorula hinnulea	Ust	Exo	Micros	10/18/2011	Tk	D	0	1
SA440	Rhodotorula sp cf hinnulea 1	Ust	Exo	Micros	5/11/2011	De	B	0	0
SA500	Rhodotorula sp cf hinnulea 1	Ust	Exo	Micros	7/12/2011	De	D	0	1
SA588	Sympodiomycopsis paphiopedili	Ust	Exo	Micros	8/27/2011	Ne	B	1	0
SA218	Sympodiomycopsis sp 1	Ust	Exo	Micros	2/6/2011	Pp	B	0	0
SA221	Sympodiomycopsis sp 1	Ust	Exo	Micros	2/6/2011	Pp	B	0	0
SA222	Sympodiomycopsis sp 1	Ust	Exo	Micros	2/6/2011	Pp	B	0	0
SA241	Sympodiomycopsis sp 1	Ust	Exo	Micros	2/19/2011	Pp	B	1	0
SA244	Sympodiomycopsis sp 1	Ust	Exo	Micros	2/19/2011	Pp	B	1	0
SA509	Sympodiomycopsis sp 1	Ust	Exo	Micros	7/12/2011	Pp	B	0	0
SA510	Sympodiomycopsis sp 1	Ust	Exo	Micros	7/11/2011	Pp	D	0	0
SA511	Sympodiomycopsis sp 1	Ust	Exo	Micros	7/14/2011	Pp	D	0	0
SA512	Sympodiomycopsis sp 1	Ust	Exo	Micros	7/14/2011	Pp	D	0	0
SA542	Sympodiomycopsis sp 1	Ust	Exo	Micros	8/1/2011	Ra	B	0	0
SA589	Sympodiomycopsis sp 1	Ust	Exo	Micros	8/25/2011	Ne	B	1	0
SA567	Sympodiomycopsis sp 2	Ust	Exo	Micros	8/9/2011	Pp	B	0	0
SA437	Sympodiomycopsis sp 3	Ust	Exo	Micros	6/10/2011	Pp	B	0	1
SA583	Sympodiomycopsis sp 3	Ust	Exo	Micros	8/22/2011	Pp	B	0	0
SA724	Sympodiomycopsis sp 4	Ust	Exo	Micros	10/18/2011	De	D	1	1
SA371	Sympodiomycopsis sp 5	Ust	Exo	Micros	4/15/2011	Lj	B	0	0
SA527	Sympodiomycopsis sp 6	Ust	Exo	Micros	7/29/2011	Pp	D	0	0
SA582	Sympodiomycopsis sp 7	Ust	Exo	Micros	8/22/2011	Pp	B	0	0
SA775	Sympodiomycopsis sp 8	Ust	Exo	Micros	12/12/2011	Pp	D	0	0
SA392	Tilletia buchloeana	Ust	Exo	Till	4/21/2011	De	B	0	0
SA240	Farysizyma itapuensis 1	Ust	Ustil	Ustilag	2/17/2011	Cf	B	1	1
SA539	Farysizyma itapuensis 2	Ust	Ustil	Ustilag	7/28/2011	Ne	D	0	0
SA441	Farysizyma setubalensis	Ust	Ustil	Ustilag	6/10/2011	De	B	1	n/a
SA209	Farysizyma sp nov	Ust	Ustil	Ustilag	1/19/2011	Pp	B	0	1
SA481	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	6/28/2011	Tk	B	0	1
SA557	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/8/2011	Tk	B	0	1
SA559	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/10/2011	Tk	B	0	1
SA562	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/12/2011	Tk	B	0	1
SA565	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/9/2011	Tk	D	0	1
SA573	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/24/2011	Lj	B	1	0
SA574	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/24/2011	Lj	B	1	0
SA576	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/22/2011	Lj	B	1	0
SA584	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	8/22/2011	Tk	D	1	1
SA601	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/4/2011	Tk	B	0	1
SA603	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/4/2011	Tk	B	0	1
SA604	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/4/2011	Tk	B	0	1
SA605	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/6/2011	Tk	D	0	1
SA607	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/6/2011	Tk	D	0	1
SA618	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/5/2011	Ra	D	0	1
SA626	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/21/2011	Tk	B	0	1

Table A-1 cont.

Strain	Species	SP	Class	Order	Date	Fern	LS	Fertility	Age
SA630	Pseudozyma aphidis 1	Ust	Ustil	Ustilag	9/20/2011	Tk	D	0	1
SA201	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	1/19/2011	Cf	B	0	0
SA585	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	8/22/2011	Tk	D	1	1
SA602	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	9/5/2011	Tk	B	1	1
SA606	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	9/5/2011	Tk	D	1	1
SA640	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	9/19/2011	Cf	B	0	1
SA641	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	9/21/2011	Cf	B	0	1
SA670	Pseudozyma hubeiensis	Ust	Ustil	Ustilag	10/5/2011	Cf	D	1	1
SA276	Pseudozyma pruni 1	Ust	Ustil	Ustilag	3/2/2011	Tk	D	0	1
SA629	Pseudozyma pruni 2	Ust	Ustil	Ustilag	9/20/2011	Tk	B	0	1
SA730	Pseudozyma sp cf jejuensis	Ust	Ustil	Ustilag	11/14/2011	Tk	D	0	1
SA350	Sporisorium cruentum 1	Ust	Ustil	Ustilag	4/23/2011	De	D	0	1
SA352	Sporisorium cruentum 1	Ust	Ustil	Ustilag	4/25/2011	De	D	0	1
SA598	Sporisorium cruentum 2	Ust	Ustil	Ustilag	8/26/2011	Ra	D	0	1
SA575	Sporisorium sp cf chrysopogonis	Ust	Ustil	Ustilag	8/22/2011	Lj	B	1	0

Key for Table A-1

SP (Subphylum): Agar-Agaricomycotina; Puc-Puccinomycotina; Ust-Ustilaginomycotina

Class: Trem-Tremellomycetes; Agari-Agaricostilbomycetes; Cyst-Cystobasidiomycetes; Micro-Microbotryomycetes; Exo-Exobasidiomycetes

Order: Cystofilo-Cystofilobasidiales; Filo-Filobasidiales; Agarico-Agaricostilbales; Cystob-Cystobasidiales; Erythro-Erythrobasidiales; Spor-Sporidiobolales; Enty-Entylomatales; Exob-Exobasidiales; George-Georgefischeriales; Micros-Microstromatales; Till-Tilletiales; Ustilag-Ustilaginales

Fern: Cf-*Cyrtomium falcatum*; De-*Dryopteris erythrosora*; Lj-*Lygodium japonicum*; Ne-*Nephrolepis exaltata*; Pp-*Polypodium polypodioides*; Ra-*Rumohra adiantiformis*; Tk-*Thelypteris kunthii*

LS (Leaf Side): B-Abaxial; D-Adaxial

Fertility: 0-Non-fertile frond; 1-Fertile frond

Age: 0-Young frond; 1-Senescent frond

is: *incertae sedis*

n/a: not available

Table A-2. Locations of ferns collected in this survey, names of surrounding plants and trees, indication of the density of the surrounding plant community and description of light conditions in the area.

Fern	GPS Coordinates	Other Plants in Vicinity	Plant Community	Light Conditions
Cyrtomium facatum	N 30.409093° E -91.176428°	*Asparagus densiflorus* *Canna* sp. *Dryopteris erythrosora* *Hypoestes phyllostachya* *Ipomea batatas* *Quercus virginiana* *Rhododendron indica* *Ruellia simplex* *Solenestemon scutellarioides*	Mixed (high plant diversity)	Part shade
Dryopteris erythrosora	N 30.409093° E -91.176428°	*Asparagus densiflorus* *Canna sp.* *Cyrtomium falcatum* *Hypoestes phyllostachya* *Ipomea batatas* *Quercus virginiana* *Rhododendron indica* *Ruellia simplex* *Solenestemon scutellarioides*	Mixed (high plant diversity)	Part shade
Lygodium japonicum	N 30.407817° E -91.176187°	*Lantana camara* *Cassia splendida* *Rhododendron indica* *Camellia sasanqua*	Mixed (moderate plant diversity)	Full sun
Nephrolepis exaltata	N 30.409077° E -91.176352°	*Asparagus densiflorus* *Begonia semperflorens* *Ophiopogon planiscapus* *Rubus trivialis*	Mixed (moderate plant diversity)	Part shade
Polypodium polypodioides	N 30.410784° E -91.177849°	*Quercus virginiana* *Ophiopogon planiscapus* *Tillandsia usnoides*	Epiphyte on live oak (low plant diversity)	Part shade
Rumohra adiantiformis	N 30.408786° E -91.176481°	*Celtis laevigata* *Dietes iridioides* *Indigofera kirilowii* *Quercus micheauxii* *Raphiolepis indicata* *Ruellia simplex* *Zanzibar* sp.	Mixed (high plant diversity)	Part shade
Thelypteris kunthii	N 30.396727° E -91.163255°	*Axonopus* sp. *Trachelospermum asiaticum*	Mixed (low plant diversity)	Full sun

Appendix B. Statistical Tables and Climate Information

Table B-1. Chi square goodness-of-fit test comparing the number of yeast isolates from six fungal classes recovered from young and senescing fern leaf sections.

Table of Classes by Leaf Age				
Classes				
	Senescent	**Young**	**Total**	
Agaricostilbomycetes	1	3	4	**Observed**
	1.995	2.005		**Expected**
	0.4962	0.4938		**Chi Square Value**
Cystoasidiomycetes	9	5	14	**Observed**
	6.9825	7.0175		**Expected**
	0.583	0.58		**Chi Square Value**
Exobasidiomycetes	36	53	89	**Observed**
	44.388	44.612		**Expected**
	1.5852	1.5773		**Chi Square Value**
Microbotryomycetes	96	108	204	**Observed**
	101.74	102.26		**Expected**
	0.3243	0.3227		**Chi Square Value**
Tremellomycetes	29	25	54	**Observed**
	26.932	27.068		**Expected**
	0.1587	0.1579		**Chi Square Value**
Ustilaginomycetes	28	6	34	**Observed**
	16.957	17.043		**Expected**
	7.1909	7.155		**Chi Square Value**
Total	199	200	399	
Statistic	**DF**	**Value**	**Prob**	
Chi-Square	5	20.6251	0.001	

Table B-2. Chi square goodness-of-fit test comparing the number of yeast isolates from six fungal classes recovered from abaxial and adaxial fern leaf sections.

Table of Classes by Leaf Side				
Classes				
	Abx	**Adx**	**Total**	
Agaricostilbomycetes	1	3	4	**Observed**
	2.1485	1.8515		**Expected**
	0.6139	0.7124		**Chi Square Value**
Cystobasidiomycetes	11	8	19	**Observed**
	10.205	8.7948		**Expected**
	0.0619	0.0718		**Chi Square Value**
Exobasidiomycetes	65	47	112	**Observed**
	60.157	51.843		**Expected**
	0.3899	0.4524		**Chi Square Value**
Microbotryomycetes	98	117	215	**Observed**
	115.48	99.52		**Expected**
	2.646	3.0704		**Chi Square Value**
Tremellomycetes	51	22	73	**Observed**
	39.21	33.79		**Expected**
	3.5454	4.114		**Chi Square Value**
Ustilaginomycetes	20	15	35	**Observed**
	18.799	16.201		**Expected**
	0.0767	0.089		**Chi Square Value**
Total	246	212	458	
Statistic	**DF**	**Value**	**Prob**	
Chi-Square	5	15.8437	0.0073	

Table B-3. Chi square goodness-of-fit test comparing the number of yeast isolates from six fungal classes recovered from non-fertile and fertile fern leaf sections.

Table of classes by Leaf Fertility				
Classes				
	Fertile	**Non-Fertile**	**Total**	
Agaricostilbomycetes	0	4	4	**Observed**
	1.0994	2.9006		**Expected**
	1.0994	0.4167		**Chi Square Value**
Cystobasidiomycetes	5	8	13	**Observed**
	3.5731	9.4269		**Expected**
	0.5698	0.216		**Chi Square Value**
Exobasidiomycetes	22	44	66	**Observed**
	18.14	47.86		**Expected**
	0.8212	0.3113		**Chi Square Value**
Microbotryomycetes	42	132	174	**Observed**
	47.825	126.18		**Expected**
	0.7094	0.2689		**Chi Square Value**
Tremellomycetes	14	39	53	**Observed**
	14.567	38.433		**Expected**
	0.0221	0.0084		**Chi Square Value**
Ustilaginomycetes	11	21	32	**Observed**
	8.7953	23.205		**Expected**
	0.5526	0.2095		**Chi Square Value**
Total	94	248	342	
Statistic	**DF**	**Value**	**Prob**	
Chi-Square	5	5.2052	0.3914	

Table B-4. Number of isolates recovered per fern during each month of 2011. Cf-*Cyrtomium falcatum*; De-*Dryopteris erythrosora*; Lj-*Lygodium japonicum*; Ne-*Nephrolepis exaltata*; Pp-*Polypodium polypodioides*; Ra-*Rumohra adiantiformis*; Tk-*Thelypteris kunthii*. Months are arraged in groups of three according to increasing average temperature. Average temperatures are indicated by the following colors: Blue-50°F; Green-60°F; Brown-70°F; Red-80°F

	Dec	Jan	Feb	Oct	Nov	Mar	Apr	May	Sep	June	July	Aug
Cf	17	3	6	6	7	3	12	4	4	18	4	0
De	9	3	0	8	6	11	20	2	3	6	8	1
Lj	5	6	1	5	2	10	15	3	1	7	1	11
Ne	5	3	1	4	3	7	8	4	3	8	9	8
Pp	3	2	16	2	3	11	6	5	1	4	9	4
Ra	5	0	1	7	2	5	6	2	5	3	3	14
Tk	0	0	2	7	4	2	6	0	11	4	5	12
Avg.	6.3	2.4	3.9	5.6	3.9	7.0	10.4	2.9	4	7.1	5.6	7.1
Total		**88**			**115**			**121**			**139**	

Table B-5. Number of isolates recovered per fern during each month of 2011. Cf-*Cyrtomium falcatum*; De-*Dryopteris erythrosora*; Lj-*Lygodium japonicum*; Ne-*Nephrolepis exaltata*; Pp-*Polypodium polypodioides*; Ra-*Rumohra adiantiformis*; Tk-*Thelypteris kunthii*. Months are arraged in groups of three according to increasing total precipitation. Precipitation values are indicated by the following colors: Blue-7-10”; Green-4.75-6.2”; Orange-2-3”; Red-<1”

	Mar	Sep	Nov	Jan	Jun	Jul	Dec	Feb	Aug	Apr	May	Oct
Cf	3	4	7	3	18	4	17	6	0	12	4	6
De	11	3	6	3	6	8	9	0	1	20	2	8
Lj	10	1	2	6	7	1	5	1	11	15	3	5
Ne	7	3	3	3	8	9	5	1	8	8	4	4
Pp	11	1	3	2	4	9	3	16	4	6	5	2
Ra	5	5	2	0	3	3	5	1	14	6	2	7
Tk	2	11	4	0	4	5	0	2	12	6	0	7
Avg.	7.0	4.0	3.9	2.4	7.1	5.6	6.3	3.9	7.1	10.4	2.9	5.6
Total		**104**			**106**			**121**			**132**	

Table B-6. Average temperature in degrees Farenheit and total precipitation in inches for Baton Rouge, LA during 2011.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temp (F)	48.5	54	63.8	71.4	75.1	83.9	83.7	86.3	75.9	65.6	60.4	54.2
Precip (in.)	5.3	1.9	6.9	0.99	0.59	4.8	6.2	2.2	9.9	0.49	7.35	2.8

Appendix C. Reference Information for Genbank Taxa

Table C-1. List of taxa used in phylogenetic analysis of Agaricostilbomycetes.

Species	Voucher	ITS	LSU	Reference
Agaricostilbum hypahenes	AFTOL ID-675	AY789077	AY634278	Matheny & Hibbet, UP
Agaricostilbum pulcherrimum	ATCC MYA 4629	GU291274	EU085531	Houseknecht *et al*., UP Sampaio, UP
Bensingtonia changbaiensis	AS 2.2310 TP-Snow-Y3	AY233339	JN400744	Wang *et al*., 2003 Shao, UP
Bensingtonia ciliata	CBS 7514	AF444563	AF189887	Scorzetti *et al*., 2002 Fell *et al*., 2000
Bensingtonia ingoldii	CBS 7424	AF444519	AF189888	Scorzetti *et al*., 2002 Fell *et al*., 2000
Bensingtonia miscanthi	CBS 7282	AF444516	AF189891	Scorzetti *et al*., 2002 Fell *et al*., 2000
Bensingtonia musae	CBS 7965	AF444569	DQ631903	Scorzetti *et al*., 2002 Matheny *et al*., 2006
Bensingtonia naganoensis	CBS 7286	AF444558	AF189893	Scorzetti *et al*., 2002
Bensingtonia phyllada	CBS 7169	AF444514	AF189894	Scorzetti *et al*., 2002 Fell *et al*., 2000
Bensingtonia sakaguchii	CBS 8464	AF444626	AF363646	Scorzetti *et al*., 2002
Bensingtonia sorbi	AS 2.2302	AY233343	AY233345	Wang *et al*., 2003
Bensingtonia subrosea	CBS 7283	AF444565	AF189895	Scorzetti *et al*., 2002 Fell *et al*., 2000
Bensingtonia yuccicola	CBS 7331 AFTOL ID-857	AF444518	AY745727	Scorzetti *et al*., 2002 Matheny *et al*., UP
Chionosphaera apobasidiales	CBS 7430	AF444599	AF177407	Scorzetti *et al*., 2002 Sampaio *et al*., 1999
Chionosphaera cuniculicola	IGC 5686	N/A	AF393472	Kirschner *et al*., 2001
Cystobasidiopsis sp.	RB 2009	GQ180106	FJ536254	Bauer *et al*., 2009
Kondoa aeria	CBS 8378 IGC 5565	AF444595	AF204054	Scorzetti *et al*., 2002 Sampaio *et al*., 2000
Kondoa malvinella	CBS 6082 AFTOL ID-859	AF444498	AY745720	Scorzetti *et al*., 2002 Matheny *et al*., UP
Kondoa sp.	AS 483	N/A	FN428954	Yurkov, UP
Kondoa sp.	CBS 8379	AF44459	AF189904	Scorzetti *et al*., 2002 Fell *et al*., 2000
Kondoa sp.	TUB ZP352	N/A	AY512854	Begerow *et al*., UP
Kondoa sp.	F45	FJ755831	N/A	Liu *et al*., 2010

Table C-1 cont.

Species	Voucher	ITS	LSU	Reference
Kurtzmanomyces insolitus	CBS 8377	AF444594	AF177408	Scorzetti *et al*., 2002 Sampaio *et al*., 1999
Kurtzmanomyces nectairei	CBS 6405	AF444494	AF177409	Scorzetti *et al*., 2002 Sampaio *et al*., 1999
Mycogloea sp.	TUB FO 40962	N/A	AY512868	Begerow *et al*., UP
Rhodotorula acuta	JCM 1602	AB038053	N/A	Nagahama *et al*., UP
Sporobolomyces coprosmicola	CBS 7897	AF444576	AF189981	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces diospyroris	FK 8	AB126047	AB124560	Nakase *et al*., 2005
Sporobolomyces dracophylli	CBS 7900	AF444583		Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces lactophilus	CBS 7527 DB 1580	AF444545	AY512889	Scorzetti *et al*., 2002 Begerow *et al*., UP
Sporobolomyces linderae	CBS 7893	AF444582	AF189989	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces pyrrosiae	FK 155	AB126045	AB124562	Nakase *et al*., 2005
Sporobolomyces ruber	CBS 7512	AF444550	AF189992	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces sasicola	CBS 7285	AF444548	AF177412	Scorzetti *et al*., 2002 Sampaio *et al*., 1999
Sporobolomyces sp.	TY 139	AY313063	AY313037	Fungsin *et al*., UP
Sporobolomyces sp.	TY 197	AY313066	AY313043	Fungsin *et al*., UP
Sporobolomyces subbruneus	CBS 7196	AF444549	AF189997	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces taupoensis	CBS 7898	AF444592	AF177413	Scorzetti *et al*., 2002 Sampaio *et al*., 1999
Sporobolomyces xanthus	CBS 7513	AF444547	AF177414	Scorzetti *et al*., 2002 Sampaio *et al*., 1999
Sterigmatomyces elviae	CBS 5922 CBS 7288	AF444551	AF190000	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sterigmatomyces halophilus	CBS 4609 AFTOL ID-863	AF444556	AY745716	Scorzetti *et al*., 2002 Matheny *et al*., UP
Stilbum vulgare	ATCC MYA 4639	GU291281	N/A	Houseknecht *et al*., UP

Table C-2. List of species used in phylogenetic analysis of Cystobasidiomycetes.

Species	Voucher	ITS	LSU	Reference
Bannoa hahajimensis	JCM 10336	AB035894	AB082571	Hamamoto *et a*l., 2002
Banno sp.	MP 3490	DQ631900	N/A	Matheny *et al*, 2006
Cystobasidium fimetarium	ZP 361	N/A	EF450543	Sampaio, UP
Erythrobasidium hasegawianum	CBS 8253	AF444522	AF189899	Scorzetti *et al*., 2002 Fell *et al*., 2000
Occultifur externus	CBS 8732 AFTOL ID-860	AF444567	AY745723	Scorzetti *et al*., 2002 Matheny *et al*., UP
Rhodotorula armeniaca	CBS 8076	AF444523	AF189920	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula aurantiaca	DAOM 226627	JN942843	JN938923	Seifert, UP
Rhodotorula benthica	SN 11	FJ515191	FJ515246	Chang & Lui, UP
Rhodotorula calyptogenae	SN 59	FJ515190	FJ515245	Chang & Lui, UP
Rhodotorula cassiicola	S22843 SJ007	EU871502	AY953945	Damare *et al*., UP Fraser *et al*., 2006
Rhodotorula chungnamensis	KCTC 17150 SJ203	AY479978	AY953949	Shin, UP Fraser *et al*., 2006
Rhodotorula foliicola	CBS 8075	AF444521	AF189984	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula laryingis	Y-17503 B9-16	AF444616	EU194448	Scorzetti *et al*., 2002 Neilsen *et al*., UP
Rhodotorula marina	CBS 2365	AF444504	AF189944	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula minuta	CBS 4408 P7	AF444579	JQ235049	Scorzetti *et al*., 2002 Abdurehim, UP
Rhodotorula pallida	CBS 320	AF444590	AF189962	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula samanae	IAM 14904	AB055199	AB055195	Sjamsuridzal *et al*., UP
Rhodotorula sloofiae	CBS 5706	AF444627	AF189965	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula sp.	5-19	FJ238090	FJ238092	Shin, UP
Rhodotorula sp.	BI218	FJ865356	EU678949	Shin, UP Landell *et al*., 2008
Sakaguchia dacryoidea	CBS 6353	AF444597	AF189972	Scorzetti *et al*., 2002 Fell *et al*., 2000

Table C-2 cont.

Species	Voucher	ITS	LSU	Reference
Sporobolomyces coprosmae	CBS 7889	AF444577	AF189980	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces gracilis	JCM 8771 CBS71	AB178481	AF189985	Hagahama *et a*l., 2006 Fell *et al*., 2000
Sporobolomyces kluyveri-neilii	CBS 7168	AF444544	AF189988	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces oryzicola	JCM 5399 CBS 7228	AB030349	AF189990	Takashima & Nakase, 2000 Fell *et al*., 2000
Sporobolomyces phyllomatis	CBS 7198	AF444515	AF189991	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces salicinus	CBS 6983	AF444511	AF189995	Scorzetti *et al*., 2002 Fell *et al*., 2000
Sporobolomyces sp.	CBS 10199	EU002878	N/A	Fonseca *et al*., UP
Sporobolomyces symmetricus	CB 64 CBS 9727	AY364836	AB279627	Wang & Bai, 2004 Hamamoto, UP
Sporobolomyces vermiculatus	JCM 10224 CBS 9092	AB030335	AB279731	Takashima & Nakase, 2000 Hamamoto, 2006

Table C-3. List of species used in phylogenetic analysis of Microbotryomycetes.

Species	Voucher	ITS	LSU	Reference
Bauerago abstrusa	HUV 18526	AF189970		Fell *et al*., 2000 Kemler *et al*., 2009
Colacogloea peniophorae	PYCC 4285 AFTOL ID-709	AF444591	AY629313	Scorzetti *et al*., 2002 Matheny & Hibbet, UP
Curvibasidium palleocorallinum	CBS 6231	AY383746	N/A	Sampaio *et al*., 2004
Glaciozyma antarctica	PI12 UPM AFTOL ID-1550	FJ554838	DQ785787	Raja *et al*., UP Matheny *et al*., 2006
Glaciozyma martinii	CBS 9639 CBS 7054	JF900362	JF900365	Turchetti *et al*., 2011
Glaciozyma watsonii	CBS 7009	JF900360	JF900364	Turchetti *et al*., 2011
Heterogastridium pycnidioidum	ATCC MYA-4631	GU291276	GU291290	Housknecht *et al*., 2000
Hyalopycnis blepharistoma	CBS 591.93	AF444555	AF444555	Scorzetti *et al*., 2002 Fell et al., 2000
Kriegeria eriophori	CBS 8384	AF444602		Scorzetti *et al*., 2002 Fell *et al*., 2000

Table C-3 cont.

Species	Voucher	ITS	LSU	Reference
Leucosporidiella creatinivora	CBS 8620	AF444629	AF189925	Scorzetti *et al*., 2002 Fell *et al*., 2000
Leucosporidiella muscorum	YFB 256 CRUB 1168	FR717869	EF595759	Yurkov *et al*., 2012 Libkind *et al*., 2009
Leucosporidiella yakutica	VKM Y 2837T	AY212989	AY213001	Sampaio et al., 2003
Leucosporidium drummii	AY 220	FN908919	FN428965	Yurkov *et al*., 2012
Leucosporidium fellii	CBS 7287	AF444508	AF189907	Scorzetti *et al*., 2002 Fell *et al*., 2000
Leucosporidium golubevii	PYCC 5957T PYCC 5760	AY212987	AY212998	Sampaio *et al*., 2003
Leucosporidium scottii	CBS 5932 YM 25139-1	AF444496	JQ964224	Scorzetti *et al*., 2002 Li *et al*., UP
Mastigobasidium intermedium	CBS 11759 CBS 7281	FR717831	AF189890	Yurkov *et al*., 2012 Fell *et al*., 2000
Microbotryum anomalum	GLM 47018 GLM 59392	EF621920	EF621960	Kemler *et al*., 2009
Microbotryum bisortatum	M-0066099 TUB 015861	DQ238711	EF621975	Kemler *et al*., 2009
Rhodotorula araucariae	JCM 3770 CBS 6031	AB038067	AF070427	Nagahama *et al*., UP Fell et al., 1998
Rhodosporidium azoricum	PYCC 4648 IGC 5062	JN246556	AF321977	Coelho *et al*., 2011 Gadanho *et al*., 2001
Rhodotorula buffonii	JCM 3929 CBS 3828	AB038083	AF189924	Nagahama et al., UP Fell *et al*., 2000
Rhodotorula cresolica	CBS 7998	AF444570	AF189926	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula colostri	CBS 348	JN246563	AY372177	Coelho *et al*., 2011 Inacio & Fonseca, 2004
Rhodotorula crocea	CBS 2029T	FM957565	AY372179	Prasad & Saluja, UP Inacio & Fonseca, 2004
Rhodotorula dairenensis	CBS 446 CBS 2202	AF444501	JN246535	Scorzetti *et al*., 2002 Coelho *et al*., 2011
Rhodotorula diffluens	CBS 5233 DB 1664	AF444533	AY512878	Scorzetti *et al*., 2002 Begerow *et al*., UP
Rhodotorula foliorum	CBS 5234	AF444633		Scorzetti *et al*., 2002 Fell, UP
Rhodotorula fujisanensis	AY 31	FN298670	FN357238	Yurkov *et al*., UP
Rhodotorula futronensis	JCM 9029	AB038090	N/A	Nagahama *et al*., UP
Rhodotorula glutinis	ATCC 16726	FJ345357	N/A	Khot *et al*., 2009
Rhodotorula graminis	JCM 8170 WP 1	AB038071	EU563930	Nagahama *et al*., UP Xin *et al*., 2009

Table C-3 cont.

Species	Voucher	ITS	LSU	Reference
Rhodotorula hordea	CBS 6403	EF622016	AF189933	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula hylophila	CBS 6226	AF444622	AF363645	Scorzetti *et al*., 2002
Rhodotorula ingeniosa	CBS 4240	AF444534	AF189934	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodotorula javanica	CBS 5236 DB 1671	AF444532	AY512882	Scorzetti *et al*., 2002 Begerow *et al*., UP
Rhodotorula mucilaginosa	saar1 CBS 9078	DQ386306	AF444750	Zimmer *et al*., 2006 Scorzetti *et al*., 2002
Rhodotorula nothofagi	A45	AY383749	AF485988	Sampaio et al., 2004 Gadanho et al., 2003
Rhodotorula nothofagi	CBS 8166 ATT 177	AF444537	FJ743617	Scorzetti *et al*., 2002 Rodrigues *et al*., 2009
Rhodotorula pustula	CBS 6527	AF444531	N/A	Scorzetti *et al*., 2002
Rhodotorula sp.	PYCC 4691	JN246548	N/A	Coelho *et al*., 2011
Rhodotorula vanillica	CBS 7404	AF444575	AF189970	Fell et al., 2000
Rhodotorula yamatoana	CBS 7243	AF444634	AF189896	Scorzetti *et al*., 2002 Fell *et al*., 2000
Rhodosporidium babjevae	CBS 7808 CRUB 1142	AF444542	EF595747	Scorzetti *et al*., 2002 Libkind *et al*., 2009
Rhodosporidium concentricum	9908	DQ250660	DQ250670	Seifert *et al*., UP
Rhodosporidium diobovatum	IWBT-Y840 TEMD15	JQ993385	JQ779973	Andong *et al*., UP Tunali *et al*., UP
Rhodosporidium diobovatum	CBS 9081	AF444658	AF444753	Scorzetti *et al*., 2002
Rhodosporidium fluviale	CBS 6568 P-8	AF070422	JQ235070	Fell *et al*., 1998 Abdurehim, UP
Rhodosporidium fluviale	JCM 10311 AFTOL ID-853	AB073240	AY745719	Hamamoto *et al*., 2002 Matheny & Hibbet, UP
Rhodosporidium kratochvilovae	LS 11 IGC 4793	JN662395	AF189918	Castoria *et al*., 2011 Fell *et al*., 2000
Rhodosporidium lusitaniae	CBS 7604	AY015430	AF070423	Fell *et al*., 2002 Fell *et al*., 1998
Rhodosporidium paludigenum	AUMC 7789 8882	JQ425404	HQ670686	Soliman, UP Yang *et al*., 2011
Rhodosporidium sp.	ATCC MYA 3652	FJ614627	FJ614649	Chalkley *et al*., UP
Rhodosporidium sphaerocarpum	SDMRI SJ105	JQ245069	AY953966	Raja & Karunakaran, UP Fraser *et al*., 2006
Rhodosporidium toruloides	SM30	FJ515186	FJ515241	Chang & Liu, UP
Spacelotheca cf. *koordersiana*	AFTOL ID-1917	DQ832221	DQ832219	Matheny et al., 2006
Sporidiobolus johnsonii	CBS 2634	JN246567	JN246542	Coelho *et al*., 2011

Table C-3 cont.

Species	Voucher	ITS	LSU	Reference
Sporidiobolus longiusculus	CRUB 1044	AY552327	AY158657	Libkind *et al*., 2005 Libkind *et al*., 2003
Sporidiobolus microsporus	CBS 7041	AF444535	AF070436	Scorzetti *et al*., 2002 Fell *et al*., 1998
Sporidiobolus pararoseus	22261 CBS 4216	HQ670681	AF189978	Yang *et al*., 2011 Fell *et al*., 2000
Sporidiobolus pararoseus	CBS 484	AF417115	AF070437	Fell *et al*., 2000 Fell *et al*., 1998
Sporidiobolus ruineniae	55522 LM 015	HQ670680	AB617916	Yang *et al*., 2011 Koowadjanakul *et al*., UP
Sporidiobolus ruineniae	CO 3 ATT 254	EU547494	FJ743624	Kim, 2009 Rodrigues *et al*., 2009
Sporidiobolus salmonicolor	CBS 2643	EF592115	EF592142	Valerio *et al*., 2008
Sporobolomyces alborubescens	JCM 5352 CBS 482	AB030342	AF207886	Takashima & Nakase, UP Hong et al., 2000
Sporobolomyces bannaensis	AS 2.2285	AY274824	AY274823	Zhao *et al*., 2003
Sporobolomyces beijingensis	AS 2.2365 MCA 3740	AY364837	JN940728	Wang & Bai, 2004 Aime, UP
Sporobolomyces blumeae	JCM 10212	AB030331	AY213010	Takashima & Nakase, 2000 Sampaio *et al*., 2003
Sporobolomyces carnicolor	MCA 3710	JN942195	JN940713	Schoch *et al*., 2012
Sporobolomyces falcatus	CBS 7368	AF444543	AF075490	Scorzetti *et al*., 2002 Fell et al., 1999
Sporobolomyces jilinensis	CB 118	AY364838	N/A	Wang & Bai, 2004
Sporobolomyces japonicus	CBS 5744	AY069992	AY070009	Bai *et al*., 2002
Sporobolomyces koalae	LH 7 JCM 15099	HQ832829	EU276013	Li e*t al*., UP Satoh & Makimura, 2008
Sporobolomyces koalae	JCM 15098	EU276010	EU276012	Satoh & Makimura, 2008
Sporobolomyces marcillae	CBS 4217	AY015437	AF070440	Fell *et al*., 2002 Fell *et al*., 1998
Sporobolomyces nylandii	P 17	JQ247576	JQ235051	Abdurehim *et al*., UP
Sporobolomyces nylandii	JCM 10215 CBS 9093	AB030325	AB279629	Takashima & Nakase, 2000 Hamamoto, 2006
Sporobolomyces odoratus	ZP 470	JN246559	JN246540	Coelho *et al*., 2011
Sporobolomyces patagonicus	CRUB 1043	AY552329	AY158656	Libkind *et al*., 2005 Libkind *et al*., 2003
Sporobolomyces phaffii	AS 2.2137	AY069995	AY070011	Bai *et al*., 2002
Sporobolomyces poonsookiae	JCM 10211 CBS 9095	AB030330	AB279730	Hamamoto *et al*., 2002 Hamamoto, UP
Sporobolomyces roseus	IWBT-Y808 DAOM 216360	JQ993369	JN938917	Andong *et al*., UP Schoch *et al*., 2012

Table C-3 cont.

Species	Voucher	ITS	LSU	Reference
Sporobolomyces ruberrimus	CBS 7500 VTT C-04573	AY015439	DQ377684	Fell *et al*., 2002 Laitila *et al*., 2006
Sporobolomyces salmoneus	CBS 488	AY070005	AY070017	Bai *et al*., 2002
Sporobolomyces sp.	TD49	HQ014449	HQ014452	Carvalho *et al*., UP
Sporobolomyces sp.	TD38	HQ014448	HQ014451	Carvalho *et al*., UP
Ustilentyloma brefeldii	TUB 012510	DQ238745	EF622016	Kemler *et al*., 2009
Ustilentyloma fluitans	RB 900	AY212990	AF009882	Sampaio *et al*., 2007 Begerow *et al*., 1997

Table C-4. List of species used in phylogenetic analysis of Tremellomycetes.

Species	Voucher	ITS	LSU	Reference
Auriculibuller fuscus	PYCC 5739	AF444670	AF444764	Scorzetti *et al*., 2002
Bullera dendrophila	CBS 6074	AF444443	FJ534901	Scorzetti *et al*., 2002 Findley *et al*., 2009
Bullera formosensis	CBS 9812 FK-116	AY787859	AB090946	Okoli *et al*., 2007 Nakase *et al*., 2002
Bullera pseudoalba	CBS 7227	AF444399	AF075504	Scorzetti *et al*., 2002 Fell *et al*., 1999
Bullera pseudovariabilis	AS 2.2092	AF453288	AF544247	Bai *et al*., UP Bai & Wang, UP
Bullera setariae	FK 77	AB118875	AB119463	Takashima & Nakase, UP Nakase *et al*., UP
Bullera unica	CBS 8290 57474	AF444441	JN377459	Scorzetti *et al*., 2002 Gayevskiy & Goddard, 2012
Bullera variabilis	CBS 7347	AF444403	AF189855	Scorzetti *et al*., 2002 Fell *et al*., 2000
Bulleribasidium oberjochense	CBS 9110 IGC 5741	GU327541	AF416646	Wang *et al*., 2011 Sampaio *et al*., 2002
Bulleromyces albus	XJ 15A5	HE650882	HE650890	Han *et al*., 2012
Cryptococcus aerius	RUB 028	JN942232	JN939348	Schoch *et al*., 2012
Cryptococcus albidus	ATCC 10666 ZM-3	JQ070095	JQ011330	Gujjari et al., UP Abdurehim, UP
Cryptococcus amylolentus	CBS 6039	FJ534872	FJ534902	Findley *et al*., 2009
Cryptococcus anemochorus	CBS 10258	DQ830986	DQ384929	Pohl *et al*., 2006
Cryptococcus aquaticus	H1 VKM Y-2428	AY052487	AY526216	Birgisson *et al*., 2003 Pfeiffer *et al*., 2004
Cryptococcus aureus	G7a UL44	DQ640764	HQ641274	Sheng *et al*., 2007 Francesca *et al*., UP
Cryptococcus bestiolae	CBS 10118	FJ534873	FJ534903	Findley *et al*., 2009
Cryptococcus carnescens	CBS 10755 HAI-Y-183	EU149786	JQ317682	Connel *et al*., 2008 Gotman, UP
Cryptococcus cellulolyticus	IMUFRJ 51984 CBS 8294	FN428913	AF075525	Ribeiro, UP Fell *et al*., 1999

Table C-4 cont.

Species	Voucher	ITS	LSU	Reference
Cryptococcus dejecticola	CBS 10117	FJ534874	FJ534904	Findley *et al*., 2009
Cryptococcus dimennae	ATCC 22024 TP-Snow-Y90	EU266559	JQ768865	Gujjari & Zhou, UP Shao & Ma, UP
Cryptococcus flavescens	(59)13 14	AM176643	JN214490	Mollnar & Prillinger, UP Milanovic, UP
Cryptococcus flavus	WH ATT 259	EU177576	FJ743627	Chen *et al*., UP Rodrigues *et al*., 2009
Cryptococcus foliicola	AS 2.2471	AY557600	AY557599	Wang *et al*., 2011
Cryptococcus gastricus	CBS 1927 CRUB 1169	AF444304	EF595765	Scorzetti *et al*., 2002 Libkind *et al*., 2009
Cryptococcus gattii	RV 54130	JN939482	JN939459	Boekhout, UP Schoch *et al*., 2012
Cryptococcus gilvescens	CBS 7525	AF444380	AF181547	Scorzetti *et al*., 2002 Fonseca *et al*., 2000
Cryptococcus heimaeyensis	CBS 8933	HQ875391	DQ000317	Wang & Boekhout, UP Robert *et al*., UP
Cryptococcus heveanensis	CBS 569	AF444301	FJ534905	Scorzetti *et al*., 2002 Findley *et al*., 2009
Cryptococcus huempii	CBS 8186	AF444322	AF189844	Scorzetti *et al*., 2002
Cryptococcus humicolus	CBS 8354	AF444384	AF189851	Scorzetti *et al*., 2002 Fell *et al*., 2000
Cryptococcus laurentii	SEG-8-9 ZA-3	FN561807	HQ327003	Yurkov *et al*., UP Abdurehim, UP
Cryptococcus nemorosus	IMUFRJ 52000 228	FN428910	JN544030	Ribeiro, UP Chen, 1998
Cryptococcus neoformans	ATCC 2344 CBS 8336	JQ070099	JN939455	Gujjari *et al*., UP Schoch *et al*., 2012
Cryptococcus niccombsii	170b	AY029346	AY029345	Thomas-Hall *et al*., UP
Cryptococcus perniciosus	VKMY 2905	AF472627	AF472624	Golubev *et al*., 2003
Cryptococcus podzolicus	IMUFRJ 52006 YM 24343	FN428906	HQ220213	Ribeiro, UP Li *et al*, UP
Cryptococcus skinneri	CBS 5029	AF444305	AF189835	Scorzetti *et al*., 2002 Fell *et al*., 2000
Cryptococcus sp.	SJ10L02	FJ153172	EU547815	Lee, UP
Cryptococcus sp.	CBS 8355	AF444385	AF444696	Scorzetti *et al*., 2002
Cryptococcus sp.	CBS 9459	GU585749	GU585738	Metin *et al*., UP
Cryptococcus tephrensis	TP-Snow-Y41 TP-Snow- Y115	JN400817	JQ768891	Shao & Ma, UP
Cryptococcus terrestris	CJDX4 Y23	EU200782	EF370393	Crestani *et al*., 2009
Cryptococcus victoriae	S 762 YM 25636	AY301025	JQ964207	Woolfork & Inglis, 2004 Li *et al*., UP
Cryptotrichosporon anacardii	CBS 9549	AY549983	AY550002	Okoli *et al*., 2007
Cuniculitrema polymorpha	CBS 8088 KBP 3865	AF444320	FN554716	Scorzetti *et al*., 2002 Kachalkin *et al*., UP
Cystofilobasidium bisporidii	CBS 6347	AF444299	AF075464	Scorzetti *et al*., 2002 Fell *et al*., 1999

Table C-4 cont.

Species	Voucher	ITS	LSU	Reference
Cystofilobasidium capitatum	CBS 7420 5412	AF444300	FN667853	Scorzetti *et al*., 2002 Glushakova & Chernov, 2010
Cystofilobasidium feraegula	CBS 6954	AF444445	AF444709	Scorzetti *et al*., 2002
Cystofilobasidium infirmominiatum	CBS 323 a306	AF444400	FR772349	Scorzetti *et al*., 2002 Glushakova *et al*., UP
Cystofilobasidium macerans	CBS 2206 TP-Snow-Y95	AF444329	JQ768871	Scorzetti *et al*., 2002 Shao & Ma, UP
Derxomyces anomala	AS 2.2094	AF453289	JN939773	Bai *et al*., UP Schoch *et al*., 2012
Derxomyces boekhoutii	AS 2.3758	EU517057	EU517057	Wang & Bai, UP
Derxomyces boninensis	JCM 10570 AS2.2413	AB022933	JN939774	Sugita *et al*., 1999 Schoch *et al*., 2012
Derxomyces hainanensis	WZS 21-14	EU517068	EU517068	Wang & Bai, UP
Derxomyces huiaensis	JCM 8933	AB022931	AB118870	Sugita *et al*., 1999 Wang *et al*., 2004
Derxomyces mrakii	AS 2.2135	JN942311	JN939779	Schoch *et al*., 2012
Derxomyces nakasei	CH 333	JN942317	JN939781	Schoch *et al*., 2012
Derxomyces pseudoschimicola	NB-239 CH 155	AF314983	JN939806	Bai *et al*., 2001 Schoch *et al*., 2012
Derxomyces qinlingensis	SF 7-33	EU517061	EU517061	Wang & Bai, UP
Derxomyces schimicola	JCM 10582 AS2.2415	AB022936	JN939787	Sugita *et al*., 1999 Schoch *et al*., 2012
Derxomyces waltii	JCM 10575	AB022935	JN939809	Sugita *et al*., 1999 Schoch *et al*., 2012
Derxomyces wuzhishanensis	AS 2.3760	EU517063	EU517063	Wang & Bai, UP
Derxomyces yunnanensis	SM 30-1	EU517067	EU517067	Wang & Bai, UP
Derxoyces pseudohuiaensis	JCM 5984 AS2.2203	AF314970	JN939783	Bai *et al*., 2001 Schoch *et al*., 2012
Dioszegia athyri	AS 2.2559	EU070926	EU0707931	Wang *et al*., 2008
Dioszegia catarinonii	A2AVS8 PYCC 5857	AY885688	AY562142	Inacio *et al*., 2005
Dioszegia crocea	JCM 2961 PDD-41	AF314988	JF706629	Bai *et al*., 2001 Amato *et al*., UP
Dioszegia hungarica	ATCC 24291	EU252552	JN400745	Gujjari & Zhou, UP Shao, UP
Dioszegia hungarica	TP-Snow-Y4 PDD-40b-5	JN400813	JF706609	Shao & Ma, UP Amato *et al*., UP
Dioszegia takashimae	Car 034	DQ003332	DQ003331	Madhour *et al*., 2005
Dioszegia zsoltii	SH 12 BH 19	EU266504	EU266524	Wang *et al*., 2008
Dioszegia zsoltii	BH 3	EU266505	EU266528	Wang *et al*., 2008
Fellomyces sichuanensis	CBS 8318	AF444461	AF189879	Scorzetti *et al*., 2002 Fell *et al*., 2000
Fibulobasidium inconspicuum	AM 64	JN053498	JN043604	Millanes *et al*., 2011

Table C-4 cont.

Species	Voucher	ITS	LSU	Reference
Filobasidium floriforme	CBS 6241 TP-Snow-Y86	AF190007	JQ768861	Fell *et al*., 2000 Shao & Ma, UP
Filobasidium uniguttulatum	CBS 1730 WH 84	AF444302	HM769334	Scorzetti *et al*., 2002 Hagan *et al*., UP
Guehomyces pullulans	CBS 2532 a262	AF444417	FN868259	Scorzetti *et al*., 2002 Glushakova *et a*l., UP
Hannaella coprosmaensis	CBS 8284 P8-16	AF444485	EU194457	Scorzetti *et al*., 2002 Nielsen *et al*., UP
Hannaella derxii	CBS 7225	AF444405	AF189857	Scorzetti *et al*., 2002 Fell *et al*., 2000
Hannaella kunmingensis	BI203	JN181160	FJ828961	Landell *et al*., UP
Hannaella luteola	ATCC 32044 ATT 122	EU252551	FJ743611	Gujjari & Khou, UP Rodrigues *et al*., 2009
Hannaella oryzae	CMT 67	JQ754042	JQ754134	Costa *et al*., UP
Hannaella sinensis	JCM 6253 ATT 074	AF314989	FJ743606	Bai *et al*., 2001 Rodrigues *et al*., 2009
Hannaella sinensis	JCM 5280 ATT 074	AF325172	FJ743606	Bai *et al*., 2001 Rodrigues *et al*., 2009
Hannaella sinensis	CBS 7238	AF444468	AF189884	Scorzetti *et al*., 2002 Fell *et al*., 2000
Hannaella zeae	CMT 28	JQ754020	JQ754112	Costa *et al*., UP
Holtermaniella nyarrowii	CBS 8805	AF400697	AF400696	Thomas-Hall & Watson, 2002
Holtermannia corniformis	CBS 7675	GU937756	GU937761	Wuczowski *et al*., 2011
Itersonilia perplexans	AFTOL ID 1896	DQ667163	DQ667161	Matheny et al., 2006
Kockovaella imperatae	CBS 7554	AF444425	AF189862	Scorzetti *et al*., 2002 Fell *et al*., 2000
Kwoniella mangroviensis	ML 4136	EF174041	EF174034	Statzell-Tallman *et al*., 2008
Mrakia frigida	CBS 5270 TP-Snow-Y128	AF144483	JQ768906	Diaz & Fell, 2000 Shao & Ma, UP
Mrakia gelida	DBVPG 5218 CBS 5272	GQ911545	AF189831	Branda *et al*., 2010 Fell *et al*., 2000
Mrakia psychrophilia	AS 2.1971	EU224267	EU224266	Wang & Bai, UP
Mrakiella cryoconiti	DBVPG 5180	GQ911549	GQ911524	Branda *et al*., 2010
Papiliotrema bandonii	CBS 9107 IGC 5743	GU327539	AF416642	Wang *et al*., 2011 Sampaio *et al*., 2002
Phaffia rhodozyma	CBS 5905	AF139629	AF189871	Fell and Blatt, 1999 Fell *et al*., 2000
Sirobasidium brefeldianum	AM 71	JN053472	JN043578	Millanes *et al*., 2011
Sirobasidium magnum	AM 70	JN053497	JN043603	Millanes *et al*., 2011
Syzygospora alba	AM 147	JN053509	JN043616	Millanes *et al*., 2011
Syzygospora bachmannii	CO 213	JN053505	JN043612	Millanes *et al*., 2011
Syzygospora effibulata	AM 7	JN053500	JN043606	Millanes et al., 2011
Syzygospora pallida	AM 26	JN053508	JN043615	Millanes *et al*., 2011
Syzygospora physciacearum	AM 17	JN053507	JN043614	Millanes *et al*., 2011

Table C-4 cont.

Species	Voucher	ITS	LSU	Reference
Tremella aurantia	CBS 6965	AF444315	AF189842	Scorzetti *et al*., 2002 Fell *et al*., 2000
Tremella brasiliensis	CBS 6966	AF444429	AF189864	Scorzetti *et al*., 2002 Fell *et al*., 2000
Tremella caloplacae	AM 32	JN053469	JN043574	Millanes *et al*., 2011
Tremella candelariellae	AM 34	JN053470	JN043575	Millanes *et al*., 2011
Tremella cetrariicola	AM 111	JN053490	JN043596	Millanes *et al*., 2011
Tremella cladoniae	AM 84	JN053478	JN043584	Millanes *et al*., 2011
Tremella coppinsii	AM 38	JN053495	JN043601	Millanes *et al*., 2011
Tremella dendrographae	AM 39	JN053471	JN043576	Millanes *et al*., 2011
Tremella encephala	AM 48	JN053481	JN043587	Millanes *et al*., 2011
Tremella exigua	RJB 6623-15	AF042430	AF042248	Chen, 1998
Tremella flava	CCJ 1420	AF042420	AF042238	Chen, 1998
Tremella fuciformis	AM 68	JN053466	JN043571	Millanes *et al*., 2011
Tremella giraffa	AM 69	JN053483	JN043589	Millanes *et al*., 2011
Tremella globispora	CBS 6972	AF444432	AF189869	Scorzetti *et al*., 2002 Fell *et al*., 2000
Tremella haematommatis	AM 41	JN053510	JN043617	Millanes *et al*., 2011
Tremella leptogii	AM 81	JN053476	JN043582	Millanes *et al*., 2011
Tremella lobariacearum	AM 80	JN053473	JN043579	Millanes *et al*., 2011
Tremella mesenterica	AM 30 AM 29	JN053464	JN043568	Millanes *et al*., 2011
Tremella microspora	BPI 702328	AF042435	AF042253	Chen, 1998
Tremella moriformis	RJB 6639-16	AF042429	AF042247	Chen, 1998
Tremella mycophaga	RJB 6539-4	AF042431	AF042249	Chen, 1998
Tremella nephromatis	AM 133	JN053475	JN043581	Millanes *et al*., 2011
Tremella nivalis	CCJ 1418	AF042419	AF042237	Chen, 1998
Tremella parmeliarum	AM 120	JN053511	JN043618	Millanes *et al*., 2011
Tremella pertusariae	AM 2	JN053494	JN043600	Millanes *et al*., 2011
Tremella phaeophysciae	AM 98	JN043585	JN043585	Millanes *et al*., 2011
Tremella polyporina	AM 20	JN053501	JN043607	Millanes et al., 2011
Tremella simplex	FO 31782	AF042428	AF042246	Chen, 1998
Tremella tropica	CCJ 1355	AF042433	AF042251	Chen, 1998
Tremella tuckerae	AM 89	JN053482	JN043588	Millanes *et al*., 2011
Tremella wirthii	AM 90	JN053492	JN043598	Millanes *et al*., 2011
Trichosporon brassicae	CBS 6382	JN943750	JN939476	Schoch *et al*., 2012
Trichosporon chiarellii	FCP 540806	GQ338074	EU030272	Pagnocca *et al*., 2010
Trichosporon cutaneum	PUMCHBY 28	EU863541	EU882099	Guo *et al*., 2011
Trichosporon domesticum	CBS 8111	JN943739	JN939470	Schoch *et al*., 2012
Trichosporon gracile	CBS 7609	JN943748	JN939473	Schoch *et al*., 2012
Trichosporon inkin	PUMCHBY 23	EU863535	EU882093	Guo *et al*., 2011
Trichosporon laibachii	CBS 8900	JN943743	JN939451	Schoch et al., 2012
Trichosporon moniliiforme	CBS 8400	AF444451	AF189873	Scorzetti *et al*., 2002 Fell *et al*., 2000

Table C-4 cont.

Species	Voucher	ITS	LSU	Reference
Trichosporon ovoides	CBS 7556	AF444439	EU934805	Scorzetti *et al*., 2002 Chagas-Neto *et al*., 2009
Trichosporon porosum	CBS 5597	JN943732	JN939465	Schoch *et al*., 2012
Trimorphomyces papilionaceus	CBS 444.92	AF444483	AF416645	Scorzetti *et al*., 2002 Sampaio *et al*., 2002
Tsuchiyaea wingfieldii	CBS 7118	FJ534886	FJ534916	Findley *et al*., 2009
Udeniomyces megalosporus	CBS 7236	AF444408	AF075510	Scorzetti *et al*., 2002 Fell *et a*l., 1999
Udeniomyces pannonicus	JCM 11145 PDD-28b-11	AB072229	HQ256882	Niwata *et al*., 2002 Amato *et al*., UP
Udeniomyces pseudopyricola	AS 2.2441	AY841862	N/A	Wang *et al*., 2004
Udeniomyces puniceus	AFTOL-ID 1822	DQ836007	DQ836005	Matheny *et al*., 2006
Udeniomyces puniceus	CBS 5689	AF444435	AF075519	Scorzetti *et al*., 2002 Fell *et al*., 1999
Udeniomyces pyricola	CBS 6754	AF444402	AF075507	Scorzetti *et al*., 2002 Fell *et a*l., 1999
Xanthophyllomyces dendrorhous	ATCC 74221 CBS 6938	DQ923730	AF444739	Fell & Scorzetti, 2006 Scorzetti *et al*., 2002

Table C-5. List of species used in phylogenetic analysis of Exobasidiomycetes.

Species	Voucher	ITS	LSU	Reference
Acaromyces ingoldii	CBS 110050	AY158671	AY158665	Boekhout *et al*., 2003
Acaromyces sp.	TR033	HQ608076	N/A	Rodrigues *et al*., 2011
Acaromyces sp.	ICMP 17482	EU770231	N/A	Weir, UP
Arcticomyces warmingii	RB 3081	N/A	AF487380	Begerow *et al*., 2002
Ceraceosorus bombacis	ATCC 22867	N/A	DQ875361	Begerow *et al*., 2006
Clinoconidium bullatum	553	N/A	AF487383	Begerow *et al*., 2002
Clinoconidium cf. *bullatum*	RB 3002	N/A	AF487382	Begerow *et al*., 2002
Clinoconidium sp.	TUK-S703	AB245088	AB178258	Nagao *et al*., UP
Coniodictyum chevalieri	WM 3450		DQ334805	Maier *et al*., UP
Dicellomyces scirpi	RB 1032	N/A	AF487385	Begerow *et al*., 2002
Drepanoconis larviformis	MP 4520	N/A	GU586973	Piepenbring *et al*., 2010
Eballistra brachiariae	FO 17510	N/A	AF009864	Begerow *et al*., 1997
Eballistra lineata	127113	N/A	AY525372	Begerow *et al*., 1997
Entyloma arnoseridis	ML 1001 AFTOL-ID 1801	AY081017	DQ645528	Begerow *et al*., 2002 Matheny *et al*., 2006
Entyloma atlanticum	738	AY081018	AY081011	Begerow *et al*., 2002
Entyloma calendulae	AFTOL ID 1821	DQ663689	DQ663687	Matheny *et al*., 2006
Entyloma corydalis	670	AY081027	AY860053	Begerow *et al*., 2002 Boekhout *et al*., 2006
Entyloma dahliae	B 700007522	AY854975	N/A	Boekhout *et al*., 2006

Table C-5 cont.

Species	Voucher	ITS	LSU	Reference
Entyloma ficariae	TUB 012542 TUB 019286	AY081035	HM046480	Begerow *et al*., 2002 Vanky & Lutz., 2010
Entyloma fuscum	HUV 278	AY081036	AY081014	Begerow *et al*., 2002
Entyloma gaillardianum	TUB 012099 RB 2055	AY854968	AF133575	Boekhout *et al*., 2006 Begerow & Bauer 2000
Entyloma holwayi	MP 1769	AY081040	AF009854	Begerow *et al*., 2002 Begerow *et al*., 1997
Entyloma linariae	671	AY081041	AY860054	Begerow *et al*., 2002 Boekhout *et al*., 2006
Entyloma lobeliae	189604	AY081042	AY081015	Begerow *et al*., 2002
Entyloma majewskii	HUV 821	HM046467	HM046478	Vanky & Lutz., 2010
Entyloma microsporum	TUB 012102 TUB 012164	AY854978	DQ185435	Boekhout *et al*., 2006 Bauer *et al*., 2007
Entyloma polysporum	HUV 2960	AY081046	AF007529	Begerow *et al*., 2002 Begerow *et al*., 1997
Entyloma ranunculi-repentis	ML 1025 MP 246	AY081047	AY081016	Begerow *et al*., 2002
Exobasidium arescens	TUB 015031	FJ896135	FJ896136	Piatek *et al*., 2012
Exobasidium caucasicum	MAFF 238830	AB180682	AB178254	Nagao *et al*., UP
Exobasidium japonicum	CGMCC 5-1648	EU692772	EU692792	Li & Guo, UP
Exobasidium rostrupii	TUB 019165	FJ896132	FJ896137	Piatek *et al*., 2012
Exobasidium vaccinii	MAFF 238668	AB180362	AB177560	Nagao *et al*., UP
Exobasidium woronichinii	MAFF 238625	AB180355	AB177572	Nagao *et al*., 2004
Exobasidiomycetidae sp.	SL 2008	FJ487941	N/A	Long *et al*., UP
Exobasidiomycetidae sp.	Vega 494	EU009983	N/A	Vega *et al*., UP
Gjaerumia ossifragi	TUB 011637	N/A	AY525373	Bauer *et al*., 2005
Graphiola cylindrica	JCM 8561	N/A	AF487400	Begerow *et al*., 2002
Graphiola phoenicis	FO 29350	N/A	AF009862	Begerow *et al*., 1997
Georgefischeria riveae	HUV 15614	N/A	AF009861	Begerow *et al*., 1997
Jamesdicksonia brunkii	HUV 17816	N/A	AF009875	Begerow *et al*., 1997
Jamesdicksonia dactylidis	RB 915	N/A	AF009853	Begerow *et al*., 1997
Jamesdicksonia irregularis	127116	N/A	AF229352	Bauer *et al*., 2001
Jamesdicksonia ischaemiana	127117	N/A	AF229355	Bauer *et al*., 2001
Jaminea angkoriensis	C5b	EU604147	EU587489	Sipiczki & Kajdacsi, 2009
Jaminea lanaiensis	BCRC 23177	GQ465044	N/A	Wei *et al*., 2011
Kordyana celebensis	HB 17	N/A	AF487401	Begerow *et al*., 2002
Kordyana tradescantiae	FO 47147	N/A	AF487402	Begerow *et al*., 2002
Kordyana sp.	RWB 2010	N/A	N/A	Begerow, UP
Kordyana sp.	2272	N/A	N/A	Begerow, UP
Laurobasidium hachijoense	MAFF 238665	AB180359	AB177562	Nagao *et al*., UP
Laurobasidium lauri	MP 2371	N/A	AF487403	Begerow *et al*., 2002
Malassezia cuniculi	CBS 11721	GU733709	GU733708	Cabanes *et al*., 2011
Malassezia dermatis	29929	AB070358	AB070363	Sugita *et al*., 2002
Malassezia equi	NCYC D3201	N/A	AJ305330	Nell, UP

Table C-5 cont.

Species	Voucher	ITS	LSU	Reference
Malassezia furfur	A7A8 IFM 52635	HQ710828	AB363789	Patino-Uzcategui *et al*., 2011 Sano *et al*., 2997
Malassezia globosa	ATCC MYA 4794	JN882325	JN874505	Houseknecht *et al*., UP
Malassezia japonica	ATCC MYA 4792	JN882323	JN874504	Houseknecht *et al*., UP
Malassezia nana	CR32C	FJ998312	EU687504	De Bellis *et al*., 2009 Bond *et al*., UP
Malassezia pachydermatis	AFTOL 856	DQ411532	AY745724	Matheny *et al*., UP
Malassezia obtusa	CBS 7876	AY387137	AY743629	Gupta *et al*., 2004 Cabanes *et al*., 2005
Malassezia restricta	CBS 8747	AY387144	AY387240	Gupta *et al*., 2004
Malassezia slooffiae	MSL 29	EU915458	EU915436	Perez-Perez, *et al*., UP
Malassezia sympodialis	CBS 8740	EF140668	EF140670	Cabanes *et al*., 2007
Malassezia yamatoensis	M-9986	AB125262	AB125264	Sugita *et al*., 2004
Meira argovae	AS006	AY158676	AY158670	Boekhout *et al*., 2003
Meira geulakonigii	PM2	GQ917051	GQ917050	Jin & Peng, UP
Meira nashicola	PFS 002	AB185159	AB185157	Yasuda *et al*., 2005
Microstroma album	RB 2072	DQ317634	AF352052	de Beer *et al*., 2006 Begerow *et al*., 2001
Microstroma juglandis	RB 2042	DQ317634	DQ317617	de Beer *et al*., 2006
Microstromatales sp.	LM95	EF060466	N/A	Mahdi & Donachie , UP
Muribasidiospora indica	STE U 5243	N/A	AY204506	Crous *et al*., 2003
Phragmotaenium indicum	127118	N/A	AF229354	Bauer *et al*., 2001
Quambalaria coyrecup	WAC 12948 WAC 12951	DQ823433	DQ823448	Paap *et al*., 2008
Quambalaria cyanescens	CBS 357.73	DQ317622	DQ317615	de Beer *et al*., 2006
Quambalaria eucalypti	CMW1101	DQ317625	DQ317618	de Beer *et al*., 2006
Quambalaria pitereka	CMW 5318 DAR 19773	DQ317628	DQ823438	de Beer *et al*., 2006 Paap *et al*., 2008
Quambalaria pitereka	BRIP 48432 WAC12957	EF444873	DQ823437	Pegg *et al*., 2008
Quambalaria simpsonii	CBS 124772	GQ303290	GQ303322	Cheewangkoon *et al*., 2009
Rhodotorula bacarum	CBS 6526	DQ317629	AF190002	de Beer *et al*., 2006 Fell *et al*., 2000
Rhodotorula hinnulea	JCM 9030 AFTOL 1764	AB038130	DQ832196	Nagahama *et al*., UP Matheny *et al*., 2006
Rhodotorula phylloplana	JCM 9035 IGC 4246	AB038131	AF352056	Nagahama *et a*l., UP Begerow *et al*., 2001
Rhodotorula phylloplana	CBS 8073	DQ317630	AF190004	de Beer *et al*., 2006 Fell *et al*., 2000
Rhodotorula sp.	SF3L06	FJ873460	EU523613	Lee & Hsieh, UP Lee & Hsu, UP
Rhodotorula sp.	FN1L09	FJ873459	EU523595	Lee & Hsieh, UP Lee & Hsu, UP
Rhodotorula sp.	SJ15L05	FJ527039	EU547814	Lee, UP

Table C-5 cont.

Species	Voucher	ITS	LSU	Reference
Sympodiomycopsis kandeliae	BCRC 23165	GQ465043	GU047881	Wei *et al*., 2011
Sympodiomycopsis kandeliae	BCRC 07F0494	GQ465045	GU047882	Wei *et al*., 2011
Sympodiomycopsis paphiopedili	CBS 7429	DQ317631	AF352054	de Beer *et al*., 2006 Begerow *et al*., 2001
Sympodiomycopsis paphiopedili	AFTOL ID 1772	DQ832240	AF352054	Matheny *et al*., 2006 Begerow *et al*., 2001
Sympodiomycopsis sp.	LM418	DQ990017	DQ990016	Mahdi *et al*., 2008
Sympodiomycopsis sp.	S6A	AM931015	AM931016	Gandham & Saluja, UP
Tilletiaria anomala	AFTOL ID 865	DQ234558	AY745715	Matheny *et al*., UP
Tilletiopsis albescens	SCSGAF 0043 KUC9027	JN850999	GQ241262	Zhang & Qi, UP Kim *et al*., 2010
Tilletiopsis cremea	JCM 5184	AB025690	N/A	Hamamoto *et al*., 2000
Tilletiopsis derxii	JCM 10217	AB045707	AB052823	Takashima & Nakase, 2001
Tilletiopsis flava		AB025707	AB052826	Boekhout et al., 1995 Takashima & Nakase, 2001
Tilletiopsis fulvescens	JCM 5187 CBS 321.71	AB025704	AJ235283	Tamura *et al*., UP Boekhout *et al*., 1995
Tilletiopsis lilacina	JCM 5188	AB025679	N/A	Hamamoto *et al*., 2000
Tilletiopsis lilacina	JCM 5190	AB025685	N/A	Hamamoto *et al*., 2000
Tilletiopsis minor	JCM 8361 CBS 346.33	AB025699	AJ235286	Tamura *et al*., UP Boekhout *et al*., 1995
Tilletiopsis minor	AFTOL 866	DQ835992	AY745713	Matheny *et al*., 2006
Tilletiopsis minor	CBS 111630	AY259067	AY272013	Boekhout *et al*., 2006
Tilletiopsis oryzicola	JCM 10218	AB045708	AB052824	Takashima & Nakase, 2001
Tilletiopsis pallescens	F3370 M115	DQ317635	HM595622	de Beer *et al*., 2006 Yuan *et al*., 2011
Tilletiopsis pallescens	JCM 10446 TUK-E 57	AB025693	AB178261	Tamura *et al*., 2011 Nagao *et al*., UP
Tilletiopsis pallescens	JCM 8711 CBS 364.85	AB025694	AJ235292	Tamura *et al*., UP Boekhout *et al*., 1995

Table C-5 cont.

Species	Voucher	ITS	LSU	Reference
Tilletiopsis pallescens	CBS 606.83 CBS 111624	DQ317636	AY272036	de Beer *et al*., 2006 Boekhout *et al*., 2006
Tilletiopsis pallescens	M115 111626	HM595577	AY879271	Yuan *et al*., 2011 Boekhout *et al*., 2006
Tilletiopsis sp.	Vega 179	EU009973	N/A	Vega *et al*., UP
Tilletiopsis sp.	JCM 8709 KCTC	AB025702	AF459717	Tamura *et al*., UP Hong & Bae, UP
Tilletiopsis washingtonensis	ATCC 96156	AF294696	N/A	Avis *et al*., UP
Volvocisporium triumfetticola	RB 2070	DQ317637	AF352053	de Beer *et al*., 2006 Begerow *et al*., 2001

Table C-6. List of species used in phylogenetic analysis of Ustilaginomycetes.

Species	Voucher	ITS	LSU	Reference
Cintractia axicola	HUV 17460 MP 3490	AY344967	DQ631906	Stoll *et al*., 2003 Matheny *et al*., 2006
Cintractia limitata	AFTOL ID-446	DQ645508	DQ645506	Matheny *et al*., 2006
Farysia acheniorum	CBS 10244 CBS 6386	EU002887	AF190001	Fonseca *et al*., UP Fell *et al*.,2000
Farysia chardoniana	MP 2062	AY344968	AF009859	Stoll *et al*., 2002 Begerow *et al*., 1997
Farysia thuemenii	ZPU7	N/A	EU045444	Sampaio & Alves, 2007
Farysia sp.	ARM 005	N/A	N/A	McTaggart, UP
Farysizyma itapuensis	BI181	DQ855949	DQ784568	Landell *et al*., UP
Farysizyma itapuensis	BI238	DQ855950	DQ784569	Landell *et al*., UP
Farysizyma itapuensis	BI120	DQ767831	DQ767831	Rosa *et al*., 2006
Farysizyma setubalensis	CBS 10241	EU002888	EU002857	Fonseca *et al*., 2007
Farysizyma setubalensis	CBS 10242	EU002889	EU002858	Fonseca *et al*., 2007
Farysizyma taiwaniana	TOH 1-2	AY555071	AY551270	Inacio *et al*., 2008 Wang & Yang, 2004
Heterotolyposporium piluliforme	HUV 15732	DQ875345	AF009871	Begerow *et al*., UP Begerow *et al*., 1997
Macalpinomyces trichopterygis	MS284	AY740039	AY740092	Stoll *et al*., 2005
Macalpinomyces tristachyae	MS 158	AY740164	AY740164	Stoll *et al*., 2005
Melanopsichium pennsylvanicum	HUV 17548	AY740040	AY740093	Stoll *et al*., 2005
Pseudozyma antarctica	JCM 3941 JCM 10317	JN942669	JN940521	An, K.D., 2011
Pseudozyma aphidis	HX 6610 BCRC 34125	HQ848933	HQ647298	Xie *et al*., UP Wei *et al*., UP
Pseudozyma churashimaensis	OK 98	AB548949	AB548957	Morita *et al*., 2010

Table C-6 cont.

Species	Voucher	ITS	LSU	Reference
Pseudozyma flocculosa	AFTOL ID-864	DQ411535	AY745712	Matheny & Hibbet, UP
Pseudozyma fusiformata	AP6	FJ919774	GQ281760	Zhang *et al*., 2010
Pseudozyma graminicola	LI20 YM 24388	AB180728	HQ416929	Li *et al*., UP
Pseudozyma hubeiensis	LH 146 IFM 58554	HQ832814	AB566327	Li *et al*., 2010 Yarita *et al*., 2010
Pseudozyma jejuensis	IMUFRJ 52021	FN428892	FN428865	Ribeiro, 2009
Pseudozyma parantarctica	JCM 11752	JN942671	JN940524	An, K.D., 2011
Pseudozyma prolifica	CBS 319.87 JCM 10319	AF294700	JN367337	Avis *et al*., 2000 Sugita *et al*., 2002
Pseudozyma pruni	BCRC 34227	EU379942	EU37994	Liu *et al*., 2009
Pseudozyma rugulosa	CBS 170.88	AF294697	AJ235300	Avis *et al*., UP Boekhout *et al*., 1995
Pseudozyma shanxiensis	SN37 AS 2.2523	FJ515182	DQ008955	Chang & Liu, 2008 Wang *et al*., 2006
Pseudozyma thailandica	M9933	AB089354	AB089355	Sugita *et al*., 2003
Pseudozyma tsukubaiensis	1D9 JCM 10324	AB55028	AB089373	Morita *et al*., 2010 Sugita *et al*., 2003
Schizonella cocconii	HB111 DB 614	FM212666	AJ236158	Prillinger *et al*., 2009 Piepenbring *et al*., 1999
Schizonella melanogramma	FO 37174 AFTOL-ID 1722	DQ191252	DQ832210	Stoll & Begerow, 2005 Matheny *et al*., 2006
Schizonella sp. JAG 45	AFTOL ID-1914	DQ832218	N/A	Matheny *et al*., 2006
Schizonella sp.	HB3	FM212665	N/A	Prillinger *et al*., 2009
Sporisorium apludae-aristae	MS87	AY740045	AY740098	Stoll *et al*., 2005
Sporisorium chrysopogonis	Ust.exs.407	AY344973	AY740131	Stoll *et al*., 2003 Stoll *et al*., 2005
Sporisorium heteropogonicola	BRIP 51822	HQ013101	HQ013135	McTaggart *et al*., UP
Sporisorium hwangense	MS267	AY740051	AY740104	Stoll *et al*., 2005
Sporisorium neglectum	RB2 RB 2056	AY740056	AY740109	Stoll *et al*., 2005
Sporisorium ovarium	MP 1871	AY740020	AJ236137	Stoll *et al*., 2005 Piepenbring *et al*., 1999
Sporisorium polliniae	Ust.exs.693	AY344987		Stoll *et al*., 2003
Sporisorium scitamineum	OUCMBI 101213 MP 541	HQ914908	AJ236138	Sun *et al*., UP Piepenbring *et al*., 1999
Sporisorium sorghi	2036a	AY740021	AF009872	Stoll *et al*., 2005 Begerow *et al*., 1997
Sporisorium themedae-arguentis	Ust.exs.855	AY344991	AY740140	Stoll *et al*., 2003 Stoll *et al*., 2005
Sporisorium trachypogonis	MS281	AY740060	AY740113	Stoll *et al*., 2005
Sporisorium veracruzianum	MP 73	AY747075	AY740142	Stoll *et al*., 2005

Table C-6 cont.

Species	Voucher	ITS	LSU	Reference
Stegocintractia luzulae	MP 2340	DQ875353	AJ236148	Begerow *et al*., 2006 Piepenbring *et al*., 1999
Thecaphora alsinearum	11533	EF200033	EF200058	Vanky and Lutz, 2007
Thecaphora saponariae	TUB 012796 TUB 012772	EF200022	EF200045	Vanky and Lutz, 2007
Thecaphora solani	HUV 11180 TS15	EF200037	AY344055	Vanky and Lutz, 2007 Andrade *et al*., UP
Tolyposporium isolepidis	HUV 14720	EU246950	EU246949	Vanky & Lutz, 2008
Tolyposporium junci	HUV 17169 HUV 17168	AY344994	AF009876	Stoll *et al*., 2003 Begerow *et al*., 1997
Ustilago austro-africana	MS 316	AY740061	AY740115	Stoll *et al*., 2005
Ustilago bullata	MP 2363 DB 3758	AY344998	JN367327	Stoll *et al*., 2003 Kellner *et al*., 2011
Ustilago calamagrostidis	MS314	AY740065	AY740119	Stoll *et al*., 2005
Ustilago crameri	Ust.exs.693 MS72	AY344999	AY740143	Stoll *et al*., 2003 Stoll *et al*., 2005
Ustilago cynodontis	UE MP 1838	HM143013	AF009881	Arocha Rosete & Lucas, UP Begerow *et al*., 1997
Ustilago holwayi	MP 1271	AY344980	AF453941	Stoll *et al*., 2003 Piepenbring *et al*., 2002
Ustilago hordei	F947	AY740068	AY740122	Stoll *et al*., 2005
Ustilago maydis	XA0609 MS 115	FJ167356	AF453938	Zhang & Gao, 2008 Piepenbring *et al*., 2002
Ustilago spermophora	F 565 HUV 13634	AY740171	AF133585	Stoll *et al*., 2005 Begerow & Bauer, 2000
Ustilago striiformis	HUV 18286	AY740172	DQ875375	Stoll *et al*., 2005
Ustilago trichophora	MS 339	AY740073	Y740125	Stoll *et al*., 2005
Ustilago vetiveriae	HUV 17954	AY345011	JN367337	Stoll *et al*., 2003 Kellner *et al*., 2011

Vita

Sebastian Albu was born in Bucharest, Romania. In 2001 he earned a B.A. in Music from the University of Pittsburgh with an emphasis in jazz performance and in 2006 he received a B.S. in Biology from the Metropolitan State University of Denver. Before enrolling at Louisiana State University he worked as a research assistant in the Cardiovascular Pulmonary Division at the University of Colorado Health Sciences Center. Sebastian was a live music reviewer for the Examiner and an active member of the Denver music scene. He composed music for and led an ensemble called Golan which performed regularly throughout Colorado and won the 2008 Dazzle Jazz Clash Competition. Sebastian also enjoys playing basketball, travelling and scouring the landscape for edible plants and mushrooms.