The taxonomy and ecology of wood decay fungi in *Eucalyptus obliqua* trees and logs in the wet sclerophyll forests of southern Tasmania

by

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Declarations

This thesis contains no material which has been accepted for a degree or diploma in any university or other institution. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due acknowledgment is made in the text.

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Abstract

The wet sclerophyll forests in southern Tasmania are dominated by *Eucalyptus obliqua* and are managed on a notional silvicultural rotation length of 80 to 100 years. Over time, this will lead to a simplified stand structure with a truncated forest age and thus reduce the proportion of coarse woody debris (CWD), such as old living trees and large diameter logs, within the production forest landscape. Course woody debris is regarded as a critical habitat for biodiversity management in forest ecosystems. Fungi, as one of the most important wood decay agents, are key to understanding and managing biodiversity associated with decaying wood. In Australia, wood-inhabiting fungi are poorly known and the biodiversity associated with CWD has not been well studied.

This thesis describes two studies that were undertaken to examine the importance of CWD as habitat for wood-inhabiting fungi in the wet sclerophyll forests of Tasmania.

Study one examined the effect of changing tree age on the fungal species richness, fungal community composition and rotten wood associations within the tree. Six living *E. obliqua* trees in each of three age-classes (69, 105 and >150 years old) were felled. Each tree was cross-cut at nine standard sampling points and the decay profile was mapped. Fungi were isolated from rotten wood at each sampling point, and from control samples of clear heartwood and sapwood. Samples of each rotten wood type were collected and classified, based on their colour and texture.

Wood-inhabiting fungi in Australia are not well known, making the identification of fungal cultures problematic. In study one, cultures of wood-inhabiting fungi were grouped into putative species groups and identified using both morphological techniques and polymerase chain reaction (PCR) and sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA.

Ninety-one species of wood-inhabiting fungi were isolated from the 18 trees examined in study one. Eight different rotten wood types were identified and studied. The community composition of wood-inhabiting fungi in trees greater than 150 years old (the oldest age-class examined) was very different compared with those found in the younger two age-classes; more than half of all species were only

found in these older trees. In addition, trees in the oldest age-class contained greater volumes and proportions of rotten wood habitat.

The second study examined the wood-inhabiting fungi that had been isolated in a previous study by Z-Q Yuan (University of Tasmania) from large (>85 cm) and small (30-60 cm) diameter *E. obliqua* logs in mature, unlogged forests and 20-30 year-old logged forests that were regenerating after clearfelling. The previously described morphospecies were tested using PCR-sequencing of the ITS region of ribosomal DNA and a consensus final species groupings was obtained.

This study determined that a total of 60 species of wood-decay fungi had been commonly isolated from the 36 logs examined. Significant differences in fungal community structure were found between mature forests and regenerating forests. Some differences in fungal species richness and community composition were also found between logs of different sizes.

These studies are among the first to examine wood-inhabiting fungi in mature *E. obliqua* trees and logs in Tasmania. The ecological information obtained from this research will assist in the development and deployment of strategies for the management of mature living trees and logs in wet eucalypt forests in Tasmania. This research suggests there is a need for forest managers to consider instigating measures that allow for some trees in the production forest landscape to live long enough to develop decayed wood habitat. This will provide important habitat for fungi as both trees and large diameter logs, sustaining an important component of forest biodiversity.

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CHAPTER 1: FUNGI IN EUCALYPT FORESTS

1.1 Introduction

This thesis examines the taxonomy and ecology of the wood decay fungi present in living trees and fallen logs in the wet eucalypt forests in Tasmania, Australia and how they are influenced by substrate and forest type. In order to provide a context for the significance of research into wood decay fungi, this chapter begins with an exploration of the global significance of Australian eucalypt forests and argues that the high biological diversity (or biodiversity) associated with eucalypt forests has important implications for forest management. The review then discusses potential threats to biodiversity and the ecological significance of disturbance in Australian eucalypt forests, and then focuses specifically on the implications of these disturbance factors for fungi and fungal diversity.

1.2 Fungi in Australian eucalypt forests

Fungi play numerous key functional roles in forest ecosystems ranging from saprotrophs and pathogens of plants and animals through to symbionts of phototrophic organisms such as those in lichens and mycorrhiza (Dix and Webster 1995). Fungi are also an extremely taxonomically diverse group of organisms; within Australia there are thought to be approximately 250 000 species of fungi compared with only 22 000 species of vascular plants (Hawksworth 1991; May and Pascoe 1996). Despite their importance within ecosystems, fungi are often overlooked. As a result, the taxonomy and ecology of fungi are very poorly known compared with the majority of other organisms present in forest ecosystems (May and Simpson 1997). In Australia for example, only 5% of the estimated total number of species of fungi have been formally described, and those that are named are often represented by only a few collections (May and Pascoe 1996). Given their importance for ecosystem processes, several studies have suggested the use of fungi as focal species, or indicators of ecological continuity, in forest ecosystems world-wide (e.g. Butler *et al.* 2002; Heilmann-Clausen 2003; Høiland and Bendiksen 1996; Sverdrup-Thygeson and Lindenmayer 2003).

1.3 Australian eucalypt forests have high levels of biological diversity

Native eucalypt forests and woodlands are a quintessential part of the Australian landscape; they dominate the less arid regions of Australia (i.e. areas with over 250 mm annual rainfall) and covered almost 20% of Australia's landmass at the time of European settlement (NLWRA 2001; Williams and Brooker 1997). These eucalypt forests and woodlands cover a diverse range of environments, from the tropics and sub-tropics of northern Australia, to the cool temperate, sub-alpine and alpine regions of southern Australia (Ladiges 1997; NLWRA 2001). Plants in the genus *Eucalyptus* L'Hér. are key components of these systems[1] (Brooker 2000; Ladiges 1997), and are collectively termed eucalypts. They are long-lived, and tend to dominate the ecosystems they inhabit by virtue of their height and size (Kirkpatrick 1997). The genera include more than 700 species, the vast majority of which are tall forest and woodland species (Brooker 2000; Williams and Brooker 1997). Almost all species of *Eucalyptus* are endemic to Australia, with only 14 species found elsewhere, in south-east Asia (Ladiges 1997; Williams and Brooker 1997). Forests *per se*, with trees ≥ 10 m tall and with ≥ 30% projected foliage cover, are more restricted in their geographical extent occupying 1.2% of the Australian landmass at the time of European settlement, being limited to areas generally exceeding 500 mm annual rainfall (NLWRA 2001). The following discussion will focus on these taller vegetation types.

Australian temperate eucalypt forests are biologically and evolutionarily very different from other temperate forests world-wide (Norton 1996b; Potts and Wiltshire 1997; Recher *et al.* 1996; Wardell-Johnson *et al.* 1997). In contrast with most temperate forests, they have an evolutionary history that features a comparative lack of recent glaciations, so much of their evolutionary development has been *in situ* (Nix 1982; Norton 1996b). As a result of this long period of *in situ* evolution, of Australia's relative isolation, and of the major climatic pulses of the past circa 2.5 million years (Hopper *et al.* 1996), Australian eucalypt forests are

[1] The classification of eucalypts is controversial (e.g. Brooker 2000; Ladiges and Udovicic 2000). This thesis uses the genus *Eucalyptus* to describe species in all associated subgenera including *Angorphora, Corymbia, Blakella, Eudesmia, Symphomyrtus, Minutifructa* and *Eucalyptus* as described by Brooker (2000).

extremely biologically diverse, not only in terms of the number of different species of eucalypt but also in relation to the vast number of other species eucalypt forests support (e.g. Kirkpatrick 1997; Recher *et al.* 1996; Wardell-Johnson and Horwitz 1996). Australian temperate eucalypt forests are among the most species rich, if not the richest temperate terrestrial communities in the world (Recher *et al.* 1996) and include some internationally recognised areas of high biodiversity and regional endemism, such as the south-west biodiversity hotspot (Western Australia), the south-east forest region (New South Wales and Victoria) and the Tasmanian eucalypt forests (Norton and May 1994; Recher *et al.* 1996). In addition to these specific areas of recognised high biodiversity, eucalypt forest ecosystems generally support a high number of species at the site level and the change in species and genotypes between sites can be very high (Recher *et al.* 1996; Wardell-Johnson and Horwitz 1996).

Given that the conservation and maintenance of biological diversity is commonly a major goal for contemporary forest management (Brown 1996; Brown *et al.* 2001; Grove *et al.* 2002), the high biological diversity associated with eucalypt forests and its high regional variation have important consequences.

Fungi in Australian eucalypt systems also have high levels of biological diversity

While the taxonomy and biology of fungi in eucalypt systems is less studied than that of most other types of organisms, the high biological diversity of eucalypt systems is also thought to relate to high fungal diversity (May and Simpson 1997). Estimates of fungal biodiversity in eucalypt forests *per se* are not available; however the expected ratio of number of species of fungi to their vascular plant host is thought to be about 10:1 (Pascoe 1990). If there are more than 700 species of eucalypts in Australia, there are at least 7000 species of fungi hosted by them (May and Simpson 1997). Clearly, the actual diversity of fungi in eucalypt systems is much higher as this estimate does not include any fungi associated with other organisms found in eucalypt systems such as understorey plants, invertebrates or mammals. Not only is fungal diversity high in eucalypt communities in Australia, but diversity on any one site is also very high. Sampling at individual sites in Victoria by May and others (see May and Simpson 1997) yielded more than 50

ectomycorrhizal fungi and more than 130 species of saprotrophic and parasitic macrofungi.

Although our understanding is hampered by a lack of taxonomic knowledge, a large number of the fungi in eucalypt systems are also thought to be endemic to Australia, particularly for parasitic and symbiotic fungi (May and Simpson 1997). Ectomycorrhizal fungi, for example, are mostly endemic; the eucalypt systems in southern Australia are considered to be a global biodiversity hotspot for hypogeous fungi with more than 35% of genera and 95% of species endemic to Australia (Bougher and Lebel 2001). In contrast, wood decay polypores and corticioids demonstrate low endemism at a species level (May and Simpson 1997). Compared with vascular plants such as eucalypts, fungi display fairly limited local endemism and are generally considered to be endemic at a continental level (May and Simpson 1997).

1.4. Australian eucalypt forests have high structural complexity

The structural complexity present in natural Australian eucalypt forests is thought to be one of the key features leading to their capacity to support high biological diversity (Butler *et al.* 2002; Cork and Catling 1996; Franklin *et al.* 2002; Grove 2002; McComb and Lindenmayer 1999; Scotts 1994). Australian eucalypt forests are characteristically heterogeneous systems, both spatially and temporally, often with a wide variety of trees of different sizes and ages, in different states of growth and decay (e.g. Duncan 1999; e.g. Jackson and Brown 1999; Lindenmayer *et al.* 1999b; Lindenmayer *et al.* 1991). These structures can range from different diameter living trees to standing dead trees (stags) and different diameter logs and branches on the forest floor in different phases of decay (Lindenmayer *et al.* 2000; Lindenmayer *et al.* 1999a; Woodgate *et al.* 1996). This is particularly the case for wet forests such as those in southern Tasmania, which are often multi-aged forests, resulting from a mosaic of fire histories (Wells and Hickey 1999).

1.5 Anthropogenic disturbance in Australian eucalypt forests

Most forest systems are highly adapted to natural disturbances such as fire, drought, wind storms, pathogens and climate change (see Burgman 1996). In many Australian eucalypt forests for example, fire is one of the most important factors determining their ecological dynamics (Gill 1997). Natural disturbances rarely

remove entire stands; generally they leave numerous biological legacies such as large dead trees, logs and dense understorey vegetation (Hansen *et al.* 1991; Lindenmayer and McCarthy 2002) and can play an important role in ecosystem function such as regeneration and habitat creation (Lindenmayer and Franklin 1997). This contrasts with human or anthropogenic disturbance which often occurs at scales, patterns and intensities which do not mimic natural disturbance (Lindenmayer and McCarthy 2002).

Throughout Australia, eucalypt forests are managed for a wide range of uses including conservation, recreation, water supply and timber harvesting (National Forest Inventory 2005). While almost 15% of Australia's forest estate is formally protected in nature conservation reserves within IUCN reserve categories I-VI (including formal nature conservation reserves as well as reservation within leasehold land, multiple-use forests and private land National Forest Inventory 2005), the majority of Australian native eucalypt forests (including some of those in reserves) are subject to varying levels of anthropogenic-induced disturbance (Norton and May 1994). Since European settlement of Australia (starting in 1788) approximately half of Australia's forests have been cleared or severely modified (Resource Assessment Commission 1992). Between 1972 and 1980, for example, more than 17 000 hectares of native vegetation, primarily eucalypt forest, were cleared or harvested as part of forestry activities in Tasmania (Kirkpatrick 1991). This clearing and modification has not been uniform; it has been concentrated in areas which are relatively accessible and have high productivity (Norton 1996b; Pressey *et al.* 1996). This means that some forest types have been subjected to heavy anthropogenic disturbance while, in contrast, others are largely unaffected (Gill and Williams 1996; Resource Assessment Commission 1992).

Anthropogenic disturbance can take many different forms. Hobbs and Hopkins (1990) describe four categories of anthropogenic disturbance in Australia: complete removal of vegetation (and complete disruption of ecosystem processes); replacement of vegetation (partial disruption of ecosystem processes); exploitation or utilisation of vegetation; and little deliberate modification or disturbance. Complete removal of vegetation can be the result of a range of factors including clearing for urbanisation, mining and roads (Gill and Williams 1996; Norton 1996b; Wardell-Johnson and Horwitz 1996). Replacement of vegetation generally refers to

clearing for agriculture, horticulture or plantation forestry (Hobbs and Hopkins 1990) and exploitation can refer to the use of forests for grazing and wood extraction and removal of other natural materials. Land which has had no deliberate modification is usually either unused land (e.g. desert) or land set aside for nature conservation, protection and tourism. This final category usually has had only minor disturbance (Norton 1996b).

1.6 The ecological significance of anthropogenic disturbance

There are many changes that are likely to occur in eucalypt forests as a result of disturbance regimes (Gill and Williams 1996; Hickey 1993; Laurance and Laurance 1996; Lindenmayer and McCarthy 2002; Mackey *et al.* 2002; Norton 1996a). It can be difficult to measure how disturbance will affect biodiversity however (Ehrlich 1996), particularly as in Australia, where large components of the biodiversity remain unknown. With reference to forest harvesting, Norton and May (1994) suggest that the ecological significance of anthropogenic disturbance on biodiversity in eucalypt forests will depend on several factors. These include: the proportion of the total forest system which is undergoing modified disturbance regimes, the pattern of disturbance in the landscape, the frequency of disturbance in relation to historical levels of ecosystem turnover, the frequency and duration of other pressures on the system (such as climate change) and the overall resilience of the system. For example, forest clearing over a small area of a total forest ecosystem may have little lasting impact on the forest ecosystem, however clearing extensive areas in space and time may diminish the capacity of the forest to regenerate in the short to medium term (Norton and May 1994). Similarly, a forest is likely to become severely modified or degraded if it is subjected to a significantly more, or less, frequent fire regime than would be naturally present in that forest.

The following section focuses on two specific factors which can be influenced by anthropogenic disturbance in forests and investigates how changes to these factors might affect biodiversity. These two factors are loss in structural complexity and fragmentation of forest landscapes. The focus of the former will be primarily at a stand-level, while the latter will be dealt with at a landscape-scale. These factors have been chosen from the broader suite of physical and biological changes in disturbed forests as they have received significant attention recently (e.g. As 1993;

Bengtsson *et al.* 2000; Bunnell 1995; Gibbons and Lindenmayer 2002; Gill 1997; Gill and Williams 1996; Grove *et al.* 2002; Kirkpatrick *et al.* 2002; Lindenmayer and Franklin 1997; Lindenmayer *et al.* 1999a; Lindenmayer *et al.* 1999b; Mackey *et al.* 2002; Mazurek and Zielinski 2004; Niemelä 1999; Pharo *et al.* 2004; Shifley *et al.* 2006; Vesk and MacNally 2006; Wardell-Johnson and Horwitz 1996). In addition, both loss of habitat, through reduction in structural complexity, and fragmentation have been identified as the major causes of high species extinction rates in the past 50 years (Burgman and Lindenmayer 1998).

The structural diversity of forest ecosystems is clearly an important factor in maintaining forest biodiversity (Franklin *et al.* 2002; Lindenmayer *et al.* 2006). Some of the anthropogenic disturbances which can lead to a change in forest structural diversity include clearfell forest harvesting (creating more even-aged stands and the removal of large trees and logs) (Acker *et al.* 1998; Bobiec 2002; Lindenmayer and McCarthy 2002; Lindenmayer *et al.* 1991; Sippola and Renvall 1999); the removal of debris for energy generation at time of forest harvest (Grove *et al.* 2002; Mac Nally 2006; Rudolphi and Gustafsson 2005); and coppicing and burning (Mac Nally 2006).

The fragmentation of eucalypt forests is another factor which may contribute to significant losses in biodiversity (e.g. Gill and Williams 1996; Henle *et al.* 2004a; Henle *et al.* 2004b; Hobbs 2005; Melbourne *et al.* 2004; Pharo *et al.* 2004; Saunders *et al.* 1991). Gill and Williams (1996) suggest that the three primary causes of fragmentation in the forest systems of south-eastern Australia are: agriculture, urbanisation and plantation forestry. Two other factors, forest harvesting and mining, could also be included as timber removal and clearing for mining can significantly alter the landscape and act as barriers between undisturbed forest remnants (Norton and May 1994; Wardell-Johnson and Horwitz 1996). Fragmentation of the forest landscape can result in a wide range of specific modifications such as the introduction of weed species, changes in nutrient flows due to increased run-off from adjacent areas, removal of natural materials and rubbish dumping (Gill and Williams 1996).

The adverse impacts of fragmentation on forest ecosystems arise principally from the fragmentation of the remaining habitat into smaller, more isolated patches, and the impacts of changes in the surrounding matrix on the remaining forest fragments

(Hobbs 2005). For example, widespread clearing in some regions of Australia has resulted in a very fragmented landscape with only 2-3% of the original native vegetation remaining, often in small patches of only a few hectares (Saunders *et al.* 1993). These small patches are physically isolated from each other by the surrounding matrix of disturbance and can also be subject to disturbance from the surrounding matrix (Saunders *et al.* 1993). For simplicity, in this review, the only aspect of fragmentation that will be thoroughly considered is the isolation of remnant native forest patches.

1.7 Disturbance effects on fungi

This section focuses specifically on one aspect of biodiversity, fungi, and examines how the diversity of fungi is affected by the two disturbance factors discussed in the previous section.

1.7.1 Changes in structural complexity

Structural complexity in forest ecosystems has received widespread attention for its importance for fungi (Bader *et al.* 1995; Berg *et al.* 1995; Heilmann-Clausen and Christensen 2004; Lindhe *et al.* 2004; Renvall 1995; Sippola *et al.* 2004). In well studied forests such as those in northern Europe, different elements of structural diversity appear to support different numbers of species, different species or different assemblages of fungi (Heilmann-Clausen and Christensen 2004; Lindhe *et al.* 2004; Nordén and Paltto 2001; Renvall 1995; Sippola *et al.* 2004; Tedersoo *et al.* 2003). Some of these elements include: diversity of substrate types; diversity of substrate ages and/or sizes; diversity of features within a substrate; diversity of decay stages within a substrate; and microclimatic changes. In the following discussion, each of these factors will be treated separately; however in a forest system they are all interrelated.

Diversity of Substrate Types

A number of studies have demonstrated that different types of substrates such as living trees, fallen logs, stags and stumps support very different species of fungi (e.g. Berg *et al.* 1994; Jonsell and Weslien 2003; Lindhe *et al.* 2004). In a study of wood decay fungi in *Picea abies, Quercus robur, Populus tremula* and *Betula pendula* in Sweden, Lindhe *et al.* (2004) found that over a nine year period, logs

and stumps contained different species assemblages, with logs hosting significantly more species than stumps. This was thought to be primarily due to differences in decay rate as logs decay faster and therefore the turnover of species is higher. Similarly, Berg *et al.* (1994) found that wood decay fungi in northern Sweden were dependent on specific substrates such as old trees, logs and stags. This may have been the result of different species adaptations to the defence mechanisms present in the living tree, and not in logs and stags, as well as differences in the microclimate within each substrate (Boddy 2001). Factors such as the concentration of carbon dioxide, moisture and temperature within the wood have been found to influence the fungi able to colonise it and can differ between substrate types (Boddy 1992). Different host species also generally support different fungal assemblages (Berg *et al.* 1994; Rayner and Boddy 1988; Renvall 1995).

Diversity of Substrate Ages and/or Sizes

Maintaining a diversity of substrate ages and sizes appears to be particularly important for fungal diversity. In many cases however, it is difficult to differentiate between the effect of substrate size and age on the associated fungal diversity and thus no distinction will be made here. Living trees and logs of different sizes are known to support different elements of fungal biodiversity, with large old trees and large diameter logs being particularly important, due to their reduced abundance in managed forests in Australia (Simpson and Eldridge 1986) and overseas (e.g. Andersen and Ryvarden 2001; Bader *et al.* 1995; Basham 1991; Berry and Lombard 1978; Edmonds and Lebo 1998; Heilmann-Clausen and Christensen 2005; Høiland and Bendiksen 1996; Johannesson and Stenlid 1999; Lumley *et al.* 2000; McAfee and Taylor 2001; Nakasone 1993; Nordén and Paltto 2001; Renvall 1995; Tedersoo *et al.* 2003; Vasiliauskas and Stenlid 1998). The importance of the latter may be due to a range of factors including the larger wood volume available for colonisation, the larger surface area for fruitbody production (Høiland and Bendiksen 1996) and the relative temporal and microclimatic stability of large diameter substrates (Grove *et al.* 2002; Kruys and Jonsson 1999). The temporal element is also important: large trees tend to be significantly older than small diameter trees and thus have had more time for colonisation by fungi and more chance for exposure to events such as fire which may predispose them to fungal attack (Aho 1977; Basham 1958). Old, large diameter trees, in particular, tend to contain a very high variety of features and this

may also contribute to their species richness (see paragraph below and Franklin *et al.* 2002; Grove 2002; Groven *et al.* 2002; Lindenmayer and Franklin 1997). The larger diameter of branches found on old, large diameter trees may also act as a more effective infection court for fungi than small diameter branches on smaller diameter trees (Wardlaw 2003). Although large diameter substrates are generally acknowledged as supporting large numbers of fungi, the species associated with fine woody debris and small diameter substrates should not be overlooked. Several studies have found fungi specifically associated with small diameter substrates (Heilmann-Clausen and Christensen 2004; Kruys and Jonsson 1999; Nordén *et al.* 2004), particularly Ascomycete species (Nordén *et al.* 2004).

Diversity of Features and Microclimates

Features of substrates such as hollows, dead or decaying tree tops and fire scars are known to support specialist organisms which are restricted to these features (in Tasmania, (e.g. Grove 2001a; Kantvilas and Jarman 2004) and overseas (e.g. Ranius and Wilander 2000)). This may also be the case for fungi. If this is so, then greater species richness may be achieved by increasing the number of these features in a substrate or forest stand. Similarly in Scandinavia, changes in forest microclimate such as sun exposure can affect the fungi present within substrates, particularly those with smaller diameter which have limited buffering capacity (Lindhe *et al.* 2004).

Diversity of Decay Stages

One aspect of structural complexity which appears critical for fungal diversity is maintaining dead wood substrates in a variety of decomposition stages. Renvall (1995) identified five decay stages in logs in Scandinavia, ranging from freshly fallen (stage 1) through to almost completely decomposed (stage 5). He found that the species assemblage of fungi present in the logs was highly dependent on the decay stage of the log, with decay stage 3 supporting the greatest number of species. Many other studies in Europe have found the species of fungi present on decomposing logs to be significantly related to the amount of decomposition within the log which has already taken place (Bader *et al.* 1995; Heilmann-Clausen and Christensen 2005; Høiland and Bendiksen 1996; Niemelä *et al.* 1995; Pyle and Brown 1999; Sippola and Renvall 1999). These studies, and others, indicate that

there is often a succession of fungi which will inhabit a log or other substrate as it decomposes (Chapela *et al.* 1988; Juutinen *et al.* 2006; Lindhe *et al.* 2004; Lumley *et al.* 2000; Niemelä *et al.* 1995; Willig and Schlechte 1995). A good example is *Phellinus nigrolimitatus*, a wood decay fungus which has only ever been found in logs in decay classes 3-5 in Europe, despite extensive surveys (Stokland and Kauserud 2004). This element of succession has important ramifications for the maintenance of fungal diversity within forests since there must be a continuous supply of logs within the dispersal range of the species to maintain the fungi present at each stage of the logs' decomposition (Edman and Jonsson 2001).

As can be seen from the previous discussion, studies of the ecology of fungi in Australia lag well behind those in many other countries (May 2001; May and Simpson 1997) and so there is very little literature investigating the importance of forest structural complexity for fungi in Australia. Broader Australian literature highlighting the biological importance of structural complexity in forest ecosystems is plentiful, however, and has been briefly included here to demonstrate similarities between Australian systems and those overseas which have been more thoroughly investigated. Large old trees and logs are clearly recognised as critical habitat for more than 400 species of hollow-dwelling mammals and birds (e.g. Abbott 1998; Gibbons and Lindenmayer 2002; Gibbons *et al.* 2002; Lindenmayer *et al.* 1993; Mackowski 1987; Whitford and Williams 2001). The importance of large trees and logs for more cryptic organisms such as bryophytes (Jarman and Kantvilas 2001) and saproxylic beetles (Grove 2002; Grove and Bashford 2003; Meggs 1996; Michaels and Bornemissza 1999; Yee 2005) has also been demonstrated in a limited number of systems.

1.7.2 Fragmentation

How fungi cope with forest fragmentation in Australia is unclear. As with other types of organisms, however, there is likely to be a wide variation in adaptability and resilience to fragmentation. Studies in the northern hemisphere have demonstrated that most fungi have very good dispersal abilities, although their dispersal is often imprecise and can be limited by the ability of spores to withstand UV radiation (Gustafsson 2002; Heilmann-Clausen 2003; Komonen 2005; Nordén 2000; Rayner and Boddy 1988; Stenlid and Gustafsson 2001). The majority of fungi

disperse over long distances using vast numbers of tiny (5-30 μm) airborne spores (Rayner and Boddy 1988). For example, each fruitbody of the polypore *Fomitopsis pinicola* typically produces 10^{11} spores (Nordén 1997). While the majority of these spores appear to settle within a short distance of the fruitbody (e.g. Nordén and Larsson 2000; Stenlid and Gustafsson 2001), long distance dispersal of airborne spores has been recorded: more than 300 m for some wood decay fungi (Nordén 2000) and, on rare occasions, thousands of kilometres for some rusts (e.g. on wind currents from Africa to Australia: Knox *et al.* 1994). Although effective in terms of spreading spores long distances, this method of dispersal is somewhat haphazard, as airborne spores have no method for locating and settling on suitable substrates (Heilmann-Clausen 2003), aside from small variations in size, shape and ornamentation which may help in adapting to specific environments (Nordén 2000; Nordén *et al.* 2000).

Although spore dispersal could be successful throughout fragmented landscapes, fragmentation may reduce genetic differentiation and spore viability in fungi. The haphazard dispersal of spores is even more important when sexual spores are the dominant mode of long-distance dispersal. The spores most commonly used for long-range dispersal by Basidiomycetes fungi are haploid sexual spores (Nordén 2000). To successfully establish a population, the mycelium produced by two compatible haploid spores must join within a substrate to complete the sexual cycle and produce a fruitbody with more spores for dispersal (Stenlid and Gustafsson 2001). Thus, where many types of sexual spores are concerned, the successful colonisation of a new substrate or isolated forest fragment requires not one, but two compatible spores to arrive in the same area. Studies of wood decay fungi in Sweden by Edman and Gustafsson (Edman *et al.* 2004a; Edman *et al.* 2004b) have indicated that geographically isolated fungal populations in southern Sweden suffered from negative genetic effects including reduced spore viability and low spore deposition. This was particularly apparent for rare species and species on the margin of their geographic range (Edman *et al.* 2004a; Högberg and Stenlid 1999) and may be the result of inbreeding caused by isolation of populations. Edman *et al.* (2004a) suggest that even though most wood decay fungi have the capacity to disperse between forest fragments, the quantity of dispersed spores may not be enough to prevent inbreeding.

For certain of types of fungi, particularly truffle-like species and wood decay fungi, vectors may be important modes of local dispersal (Fogel and Trappe 1978; Lawrence 1989; Rayner and Boddy 1988). One well studied example is Dutch elm disease, where the bark beetles *Scolytus multistriatus* and *Hylurgopinus rufipes* act as vectors for fungi in the genus *Ophiostoma* (e.g. Brasier 1991). Clearly, the dispersal of fungi that is reliant on vectors is dependent on the ability of the vectors to disperse effectively through a fragmented landscape and on their resilience to fragmentation. In her study of dead-wood dependent beetles, Yee (2005) found that 25% of beetles appeared to disperse by crawling (rather than flying) and these putative vectors would be expected to be strongly affected by fragmentation.

Hyphal cords, rhizomorphs and other vegetative propagules are also important for fungal dispersal although they are often effective only at a local scale. Some species of root rot fungi in the genus *Armillaria*, for instance, disperse poorly by spores and rely on spread by rhizomorphs as their primary dispersal mechanism (Redfern and Filip 1991). Related to this, newly arrived spores compete poorly for substrates and resources with already established mycelia (Nordén 2000; Stenlid and Gustafsson 2001). In a fragmented landscape with a reduced availability of substrates this is likely to be significant, as it would mean that fungi attempting to disperse between fragments using spores would compete poorly with fungi already established in these fragments and this situation may lead to a decrease in diversity of fungi within individual fragments.

While studies of the effects of forest fragmentation on fungi are still in their early stages internationally, there is some strong evidence to suggest that fungi will respond negatively, in a similar manner to many other groups of organisms. In Australia, there have been no studies dealing with forest fragmentation and fungi. As forests continue to be subjected to anthropogenic disturbance, there is an increasing need to examine the ways in which fungi respond to disturbance. As fungi are often considered indicators of ecosystem health (e.g. Tommerup and Bougher 1999), understanding the ways in which they respond to disturbance may be of particular importance.

1.8 Outline of the thesis

This thesis takes one small but crucial element of the above discussion of fungi and disturbance in Australian eucalypt forests and examines it in detail. It provides critical baseline data on the taxonomy and ecology of wood decay fungi associated with elements of structural diversity in the wet eucalypt forests in southern Tasmania, Australia. Wood decay fungi are defined in this thesis as fungi found on or inhabiting woody substrates (Nordén 2000).

Two primary studies were conducted: the first examined the wood decay fungi found inhabiting living *Eucalyptus obliqua* trees of different ages while the second examined the fungi present within *E. obliqua* logs on the forest floor.
The specific objectives of this thesis are:

- To provide a morphological and molecular analysis of the taxa of wood decay fungi inhabiting *Eucalyptus obliqua* trees in the wet forests of southern Tasmania (Chapter 3).
- To compare the wood decay fungi found within living *E. obliqua* of different ages, examining patterns of succession and the importance of large, mature trees for fungal diversity (Chapter 4).
- To examine the relationship between wood decay fungi, rotten wood type and tree age in living *E. obliqua* (Chapter 5).
- To identify the wood decay fungi associated with decomposing *E. obliqua* logs on the forest floor and investigate their relationship with rotten wood type (Chapter 6).
- To investigate the associations between assemblages of wood decay fungi in large and small diameter *E. obliqua* logs in unlogged and logging regenerated forests (Chapter 7).
- To discuss the ecological importance of structural complexity for fungal diversity within a production forest matrix, examining the possible impacts of forest management and making recommendations for future research directions and forest management (Chapter 8).

Each chapter has been written in the format of a journal article or journal article in development, meaning some repetition between chapters is unavoidable. Relationships between chapters are highlighted where appropriate. Study site history, descriptions and locations, as well as general sampling methods are presented in Chapter 2.

Authorities of fungal species are listed only in reference tables, not throughout the text.

CHAPTER 2: GENERAL MATERIALS AND METHODS

2.1 The Study System

Wet eucalypt forest is a widespread forest type in Tasmania, making up approximately 883 000 hectares of the 3.17 million hectares of native forest in the state (National Forest Inventory 2005). Wet eucalypt forests are tall open forests, characterised by a canopy of one or more eucalypt species and an understorey of either broadleaved shrubs and ferns or rainforest species (Kirkpatrick 1988). Dominant eucalypts in this system include *Eucalyptus obliqua* L'Hérit., *E. regnans* F.Muell. and *E. delegatensis* R.T.Baker. Of these *E. obliqua* is the most widespread and can live for up to 400 years, reaching sizes of 75 m tall and over 2 m girth (Alcorn *et al.* 2001).

Over 48% of wet forests are reserved, while the remainder are managed for a range of values including timber harvesting, water supply, conservation, recreation and environmental protection (National Forest Inventory 2005). Since the 1960s, wet eucalypt forests managed for timber production have been subjected to clearfell, burn and sow silviculture (Gilbert and Cunningham 1972; Hickey and Wilkinson 1999) as this attempts to mimic the natural regeneration after severe wildfire. The current planned rotation time is 80-100 years (Whiteley 1999). Natural fire frequency has been estimated to occur once every 20-100 years, however, with fires varying in intensity to create multi-aged stands (Hickey *et al.* 1998). Thus, after successive rotations under this silvicultural regime, a simplified stand structure is projected, combined with a truncated forest age (Grove *et al.* 2002). In particular, the number and volume of mature trees and large diameter logs in the landscape will be drastically reduced in the long-term (Grove *et al.* 2002) and this could have a profound effect on wood-inhabiting organisms such as fungi.

This thesis describes two studies which aim to collect base-line data on the effect of current CBS silvicultural systems on wood-inhabiting fungi. The first study investigates the decay and fungal communities present within living *E. obliqua* trees of varying ages while the second study looks at fungi within different sized *E. obliqua* logs on the forest floor.

2.2 Study Sites and Sample Selection

2.2.1 Study 1: Decay and wood decay fungi in living Eucalyptus obliqua.

This study took place at the Warra Long Term Ecological Research (LTER) site in southern Tasmania, 60 km southwest of Hobart. Warra LTER is part of a world-wide network of long-term monitoring sites and it is particularly focussed on assessing the biological, social and economic impacts of a range of silvicultural alternatives to clearfell, burn and sow silviculture (Hickey *et al.* 2001).

Trees were selected from the two 'aggregated retention' coupes WR008I and WR001E, located less than 1km apart (Figure 2.2.1, Hickey *et al.* 2001). Three age classes of *Eucalyptus obliqua* trees are present at these sites. Two of these (69 yrs and 105 yrs) comprise trees that regenerated after the two most recent severe wildfire events in the area; in 1934 and 1898 respectively (Alcorn *et al.* 2001; Hickey *et al.* 1998). The third age-class (mature trees, >150 yrs) comprises trees that were already well established at the time of the 1898 fire and survived both fire events, although their exact age is uncertain (Alcorn *et al.* 2001). Both sites had similar understorey species composition, slope and aspect, described in detail in Alcorn *et al.* (2001).

Selection of study trees

Six *E. obliqua* trees in each age class (4 each from WR008I and 2 each from WR001E) were selected along a 100 m by 10 m transect within each coupe (Figure 2.2.2). The first 2-4 trees along the transect within each age class were selected, provided they fitted specific selection criteria. These criteria were as follows:

1) Trees must be *E. obliqua*;
2) Trees must be in dominant/co-dominant strata;
3) Trees must be within a similar size class for each age class (based on DBHOB); and
4) Variables such as aspect and slope should be kept as constant as possible.

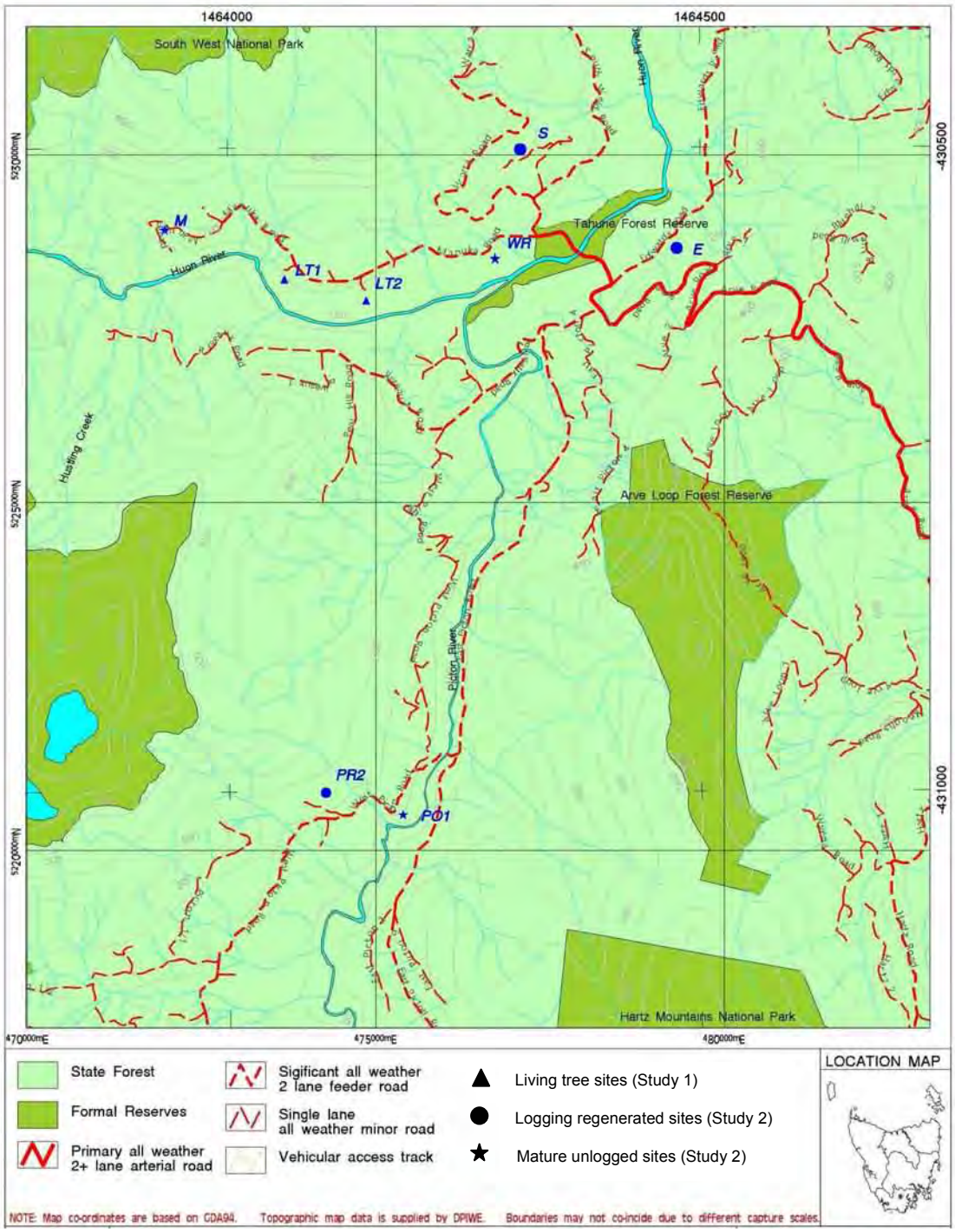
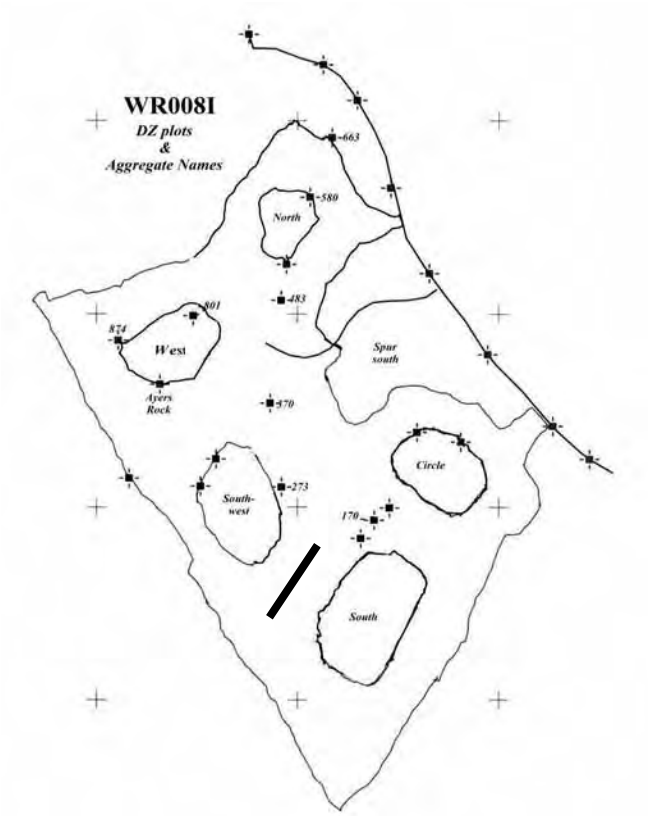


Figure 2.2.1. Location of the study area in southern Tasmania, showing the two study sites for Study 1 and six study sites for Study 2, as well as the State forest and Forest reserve boundaries. LT1 is WR008I and LT2 is WR001E. Refer to Table 2.3.1 for descriptions of the alphanumeric site codes for Study 2. Map prepared by Forestry Tasmania.



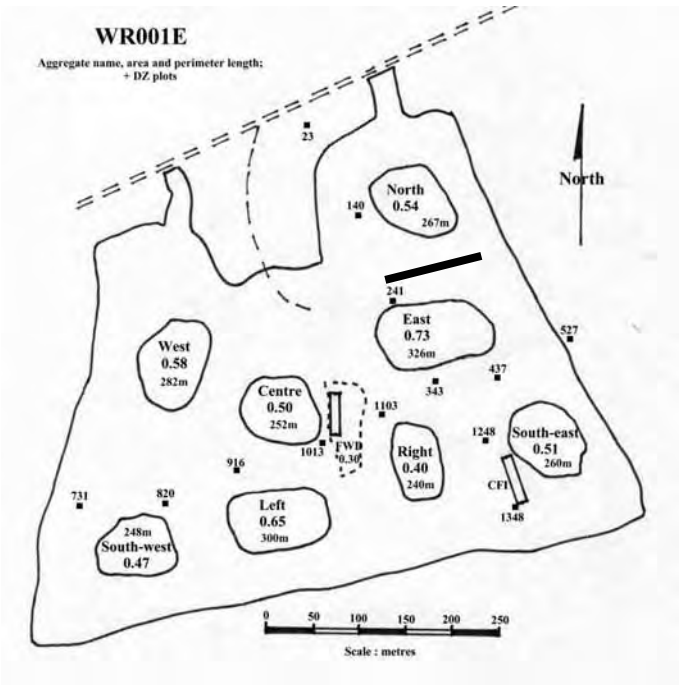


Figure 2.2.2. Location of 100 m by 10 m transects (▬) in Aggregated Retention Coupes (A) WR008I (grid reference 473785E, 5228171N) and (B) WR001E (grid reference 474935E, 5228057N). Maps provided by Forestry Tasmania.

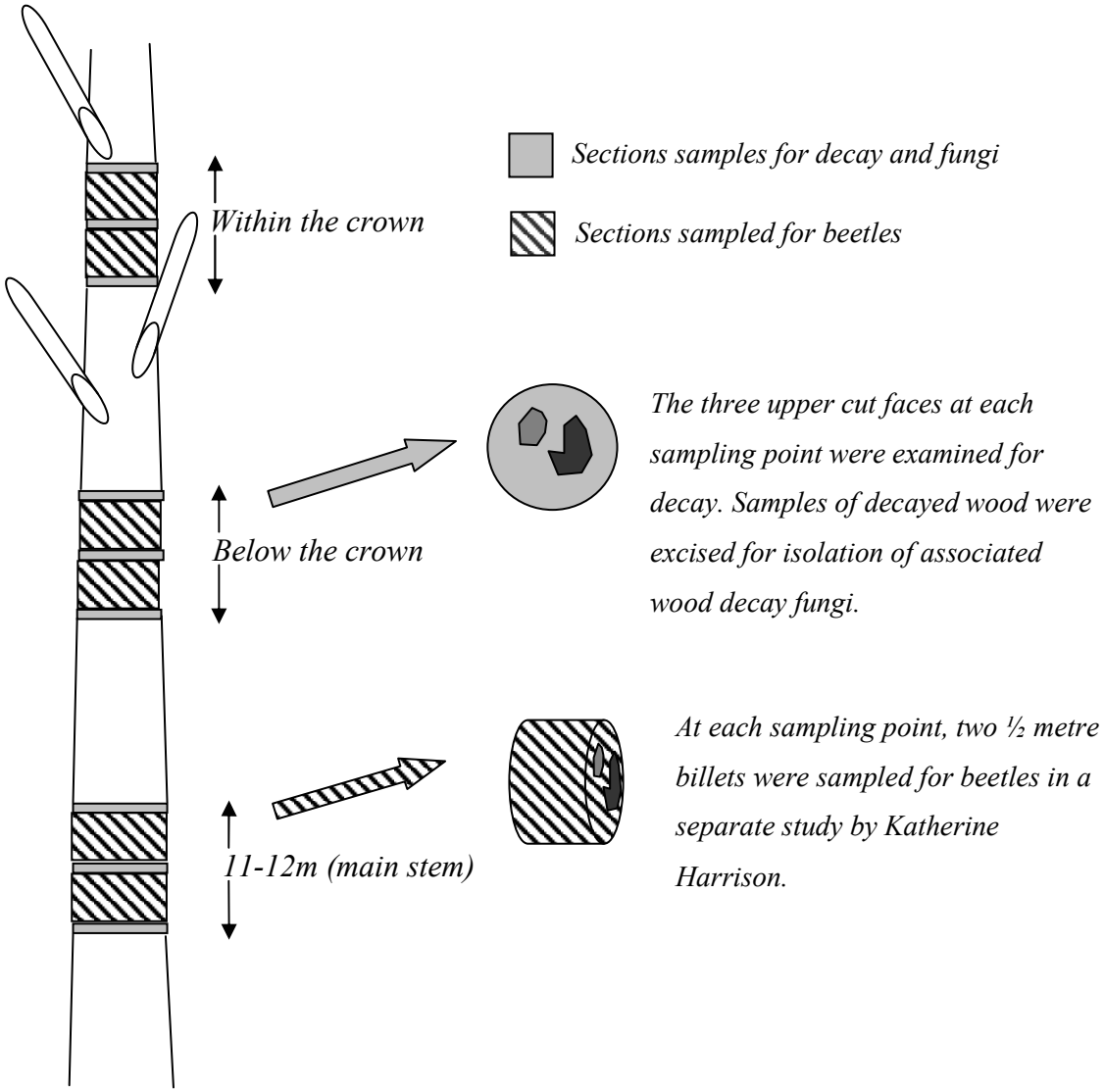


Figure 2.2.3. Live tree sampling method used in this study, showing three standard sampling heights, each cut into two ½ metre billets to expose three different cut faces.

Trees were felled to coincide with current logging regimes. Each was assessed for structural characteristics and examined for fungal colonisation and for decay. Prior to felling, trees were marked to distinguish the north side and the diameter at breast height over bark (DBHOB) was marked and measured. Once felled, other key features of each tree were recorded such as tree height, crown height and the height, cardinal point and size of all branches, branch stubs, knots, hollows and fire scars. Other features such as the presence of a dead top were also recorded. Tree age class was confirmed by ring counting all sample trees in the field (Leigh Edwards, pers. comm. 2003). Where the butt of the tree was too decayed to enable ring counting, an estimate of the age of the tree was obtained by counting the number of rings at 11 m

height where decay was not as apparent. The characteristics of each tree are shown in Table 2.2.1.

Table 2.2.1. Selected architectural features of all trees measured in each age class. Tree age class was verified by counting growth rings of each individual.

Tree Code	Age Class	DBHOB (cm)	Tree Height (m)	Number of Stem Hollows	Fire Scar Area (m^2)	Number of Large Dead Branches[#]	Dead Top Present
T41	69	24	19	0	0	0	No
T9		29	24	0	0	0	No
T25		29	27	0	0	0	No
T43		30	25	0	0	0	No
T24		31	26	0	0	0	No
T7		41	23	0	0	0	No
Mean		30.6±5.61	24±2.82	0	0	0	
T10	105	43	31	0	0	4	No
T30		43	28	0	0	5	No
T2		64	30	0	0	0	No
T40		64	33	0	0	0	No
T42		64	32	0	0	0	No
T3		76	32	0	0	0	No
Mean		59±13.24	31±1.78	0	0	1.5±2.35	
T6	>150[*]	73	23	0	0.40	5	Yes
T4		95	21	0	1.81	3	Yes
T44		96	22	0	0.47	2	Yes
T5		99	30	1	0.26	10	Yes
T45		99	39	2	1.16	6	Yes
T21		111	28	0	2.54	2	Yes
Mean		95.5±12.42	27.2±6.79	0.5±0.84	1.11±0.91	4.7±3.08	

[*] Trees in this age class could be any age ranging from 150 years to more than 350 years old.
[#] Large dead branches are those >5cm in diameter.

Selection of sampling points

Once the structural attributes were recorded, the stem of each tree was cut at three standard sampling points (Figure 2.2.3). These were within the main stem at 11-12m height, immediately below the crown, and within the live crown. These points were chosen as they have been found to be concentration points for decay in other studies (Wardlaw 1996; Wardlaw 2003). Sampling point 1 (11-12m) was representative of decay within the main stem of each tree and to encompass the area most prone to decay identified by Wardlaw (2003, 6-12m height in the main stem). Sampling point 2, immediately below the crown, was chosen to incorporate the majority of senescent branch stubs which are thought to be good colonisation points for fungi (Wardlaw 1996). Finally, sampling at point 3, within the crown, attempted to find

decay and fungi which were colonising through large living or dying branches. At each standard sampling point, the stem was cut into two 50cm billets, creating three upper cut faces.

2.2.2 Study 2: Wood decay fungi in large and small diameter **E. obliqua** *logs*

This study was based on the results of field work and fungal isolate morphology carried out by Zi-Qing Yuan from the University of Tasmania. The background/ field data collected by Yuan are described below. I continued this study, and undertook all molecular studies, wood decay enzyme tests, final fungal species groupings and statistical analyses. This study, by Yuan, was carried out concurrently with a study of saproxylic beetles in large and small diameter logs presented in Yee (2005).

Location of study sites

Sampling took place in five mature (unlogged) sites and five logging regenerated sites in the southern forests of Tasmania, in the vicinity of the Warra LTER site (Figure 2.2.1, Table 2.3.1). The mature forests were representative of unharvested forest with a multi-aged stand of two or more cohorts of eucalypts which had regenerated from successive wildfires. The logged forests were forests regenerating from a single rotation of clearfell, burn and sow silviculture (Alcorn *et al.* 2001). The oldest logged site was less than 33 years old, representing the first CBS silviculture in these forests in the 1960s. All sites were lowland wet eucalypt forests dominated by *E. obliqua,* with similar understorey species composition, slope, elevation and aspect. For more information on site selection see Yee (2005).

Within each site a 50 x 50 m plot was established. This was located at least 50 m from the road to minimise edge effects. All sampling was conducted within this quadrat.

Table 2.3.1. Name, location and recent disturbance history of study sites in study 2 (log study).

	Site name and Access Road	Latitude x Longitude	Year of clearfelling	Year of last wildfire
Logging regenerated forest	S South weld Spur 1	43.0826 S x 146.7223 E	1975	-
	E Edwards Rd	43.0918 S x 146.7473 E	1969	-
	PR2 West Picton Rd	43.1672 S x 146.6869 E	1976	-
Mature-unlogged forest	M Manuka Rd	43.0933 S x 146.6442 E	-	1906
	WR Manuka Rd	43.0935 S x 146.713 E	selectively logged 1983	1914
	P01 West Picton Rd	43.1667 S x 146.6869 E	-	1934

Selection of study logs

At each site (Figure 2.2.1, Table 2.3.1), three large and three small diameter logs were sampled for wood decay fungi. These logs were all at an intermediate decay stage (Lindenmayer *et al.* 1999a; Meggs 1996), characterised by a lack of bark, soft sapwood, heartwood still intact but rotting in places and the logs retaining their original shape. In the mature forests these logs would have been recruited naturally, while in the logging sites, they were the result of logging harvest debris. The large logs all had a diameter greater than 85 cm (Table 2.3.2), representative of mature trees (>150 years old), while the small logs were a similar size to a tree approaching commercial maturity (80-100 years old) ranging from 30-60 cm diameter. Shape and curvature of the small diameter logs was examined closely to ensure the logs were not simply large branches from mature trees.

Selection of sampling points

Each log was sampled at six points across its length by removing a thin cross sectional disc and examining the face for decay (Figure 2.2.4). The six sampling discs were located in pairs one metre apart, with at least four metres separating each pair. Where decay was found in a disc, a sample of decay was removed from the leading edge of the decay column and returned to the lab for isolation of associated wood decay fungi.

Table 2.3.2. Names and diameter of study logs used for destructive sampling.

	Site	Large log	diameter (cm)	Small log	diameter (cm)
Logging regenerated forest	E	EDL1	87.5	EDS1	42
		EDL2	100	EDS2	30
		EDL3	99	EDS3	35
	S	SDL1	92	SDS1	39
		SDL2	95	SDS2	55
		SDL3	85	SDS3	49
	PR2	PR2DL1	94	PR2DS1	43
		PR2DL2	100	PR2DS2	52
		PR2DL3	95	PR2DS3	49
Mature unlogged forest	M	MDL1	97.5	MDS1	32
		MDL2	125	MDS2	35
		MDL3	95	MDS3	46
	PO1	PO1DL1	100	PO1DS1	49
		PO1DL2	90	PO1DS2	53
		PO1DL3	100	PO1DS3	50
	WR	WRDL1	90	WRDS1	43.5
		WRDL2	110	WRDS2	44
		WRDL3	105	WRDS3	33

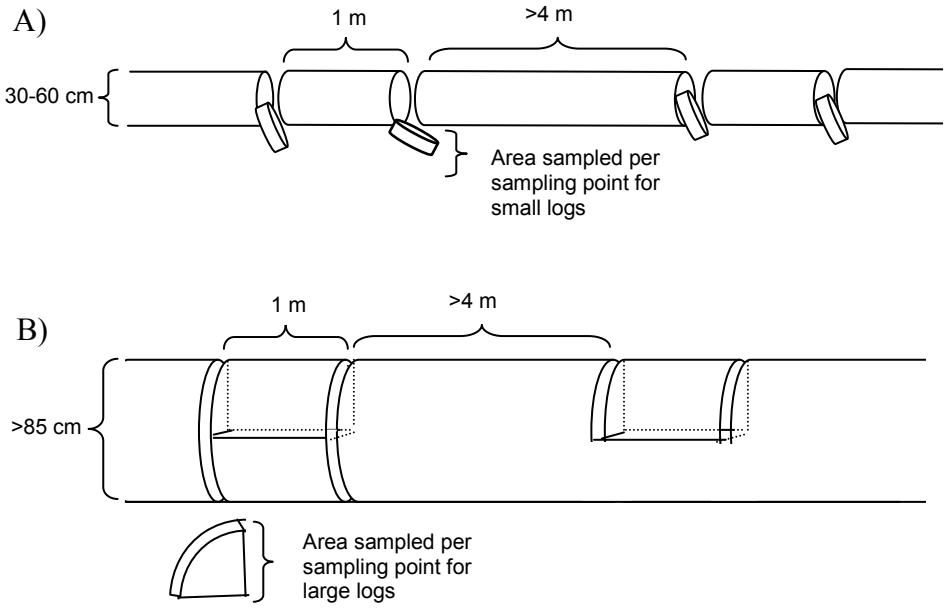


Figure 2.2.4. Schematic representation of the location of sampling discs on A) small; and B) large diameter logs. Note that only four of the six sampling points are shown in each diagram. For the large logs, only one quarter of the cross-sectional area of each disc was examined to attempt to account for differences in sample size.

2.3. Laboratory Sampling Methods

2.3.1 Classification of rot types

For the living trees in study one, the cut face of each billet was photographed, the number of patches of decay (i.e. decay columns) counted and the area of decay measured (Figure 2.3.1). A sample of rotten wood from each decay column was taken back to the laboratory for further examination. Control samples of clear heartwood and sapwood were also collected from each cut face.

Rotten wood samples from both living trees and logs (study one and two) were grouped into preliminary rot types based on similarities in colour, texture and wetness of the wood and the presence of hyphae or other fungal markings such as zone lines. In this preliminary grouping, colour and texture were used as the main indicators of rotten wood type (white or brown rot). The texture of the rotten wood was described as blocky, stringy, pocketed or crumbly.

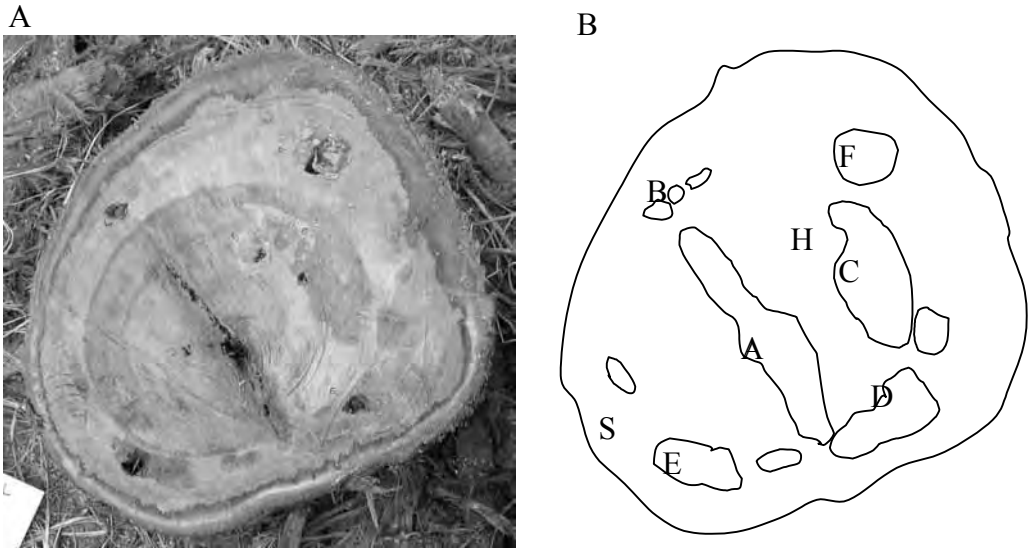


Figure 2.3.1. An example of a) a photograph taken for each cut face at each sampling point and b) a corresponding diagram showing the patterns of decay and the code assigned to each. Letters A-F represent the location of sampling of decay patches, letters H and S represent the location of sampling for clear heartwood and sapwood respectively.

The preliminary rot types were then visually compared with those described by Yee (2005) from decaying logs in the southern forests of Tasmania and a final consensus of rot types was obtained using Yee's (2005) original classification (see Chapter 5).

2.3.2 Fungal isolation from wood samples

The isolation of fungi from decayed wood was considered a useful method for studying wood decay fungi on eucalypts as fruitbody surveys do not necessarily accurately reflect the community composition of fungi within a substrate (Rayner and Boddy 1988). Fruitbodies are usually formed in response to a range of environmental variables and so fruitbody surveys require repeated sampling to obtain accurate results (May and Simpson 1997). In addition, macrofungal fruitbodies are only occasionally encountered on living eucalypts in wet forest systems either in regrowth forest (T. Wardlaw, pers. comm. 2003) or mature stands containing old growth trees (Y. Bar-Ness, pers. comm. 2004). Different isolation conditions will favour different groups of fungi, however, and this is an important consideration when interpreting the results of these studies (May and Simpson 1997).

Fungi were isolated from wood samples by excising subsamples ($1cm^3$ pieces) of wood from at least three different places around the edge of each decay column found in living tree and log sampling faces. Control wood samples were also collected from both clear heartwood and clear sapwood. All wood samples were then surface sterilised for 2 minutes in domestic White King bleach (approximately 2.5% available chlorine) and incubated at 20°C for four-six weeks on specialised fungal media (Malt extract agar (MEA) and MAT). Malt Extract Agar is a standard fungal medium regularly used for the culture of wood decay fungi, while MAT is a more specialised medium for Basidiomycetes which is based on MEA but contains antibacterials and fungicides for inhibition of lower fungi and yeasts (Hopkins *et al.* 2005). The specialised medium (MAT) was used as this was known to effectively isolate slow growing wood decay Basidiomycetes from wood (C. Mohammed, pers. comm., 2002; Barry *et al.* 2002) and it is similar to other common media selective for Basidiomycetes in wood and soil (Hale and Savory 1976). Basidiomycetes were chosen as the focus of this study, since they are one of the dominant phyla of wood decay fungi (Dix and Webster 1995; Simpson 1996).

Incubating plates were examined weekly and subcultures of possible Basidiomycete fungi were taken when found and maintained on MEA. More than 80% of cultures were obtained from isolations on MAT; very few fungi of interest were found on MEA isolation plates. Fungi were considered to be potential wood decay fungi (and

therefore of interest to this study) if they either tested positive for wood decay enzymes or displayed some of a range of characteristic Basidiomycete characters in culture (Refer to section 2.4.1; Stalpers 1978).

2.3.3 Collection and Processing of Fungal Fruitbodies

Fungal fruitbodies were collected from a number of different locations to form a reference collection of identified fruitbodies, identified cultures and identified ITS sequences used for comparison with fungi isolated in both studies one and two. Macrofungal fruitbodies were collected during the fieldwork for both study one and two and a more general collection was made from fungi growing on wood throughout Tasmania. These fungal fruitbodies were usually identified to species. Where this was not possible, tag names were used, equivalent to those lodged in the Tasmanian Herbarium (TAS). Access was also granted to a collection of reference cultures collected internationally. For the specific details of the individual collections, see Section 2.4.3. These fruitbodies are being held at the CSIRO fungal collection in Hobart.

Fungal cultures of all fresh fruitbodies collected were obtained by excising 8-15 small sterile pieces of fruitbody from within the cap or stipe. These pieces were then sterilised for 1.5 minutes in domestic White King bleach (approximately 2.5% available chlorine) and incubated for four to six weeks on both MEA and MAT. An isolation was considered successful when the majority of fruitbody pieces resulted in the same mycelium. This mycelium was then subcultured and maintained on MEA 20 ºC for the duration of the study. Copies of each isolate have been lodged with the CSIRO Forest Health culture collection in Hobart.

A clean, 1 cm^3 piece of each fruitbody was removed and stored in an eppendorf tube at –80 ºC to be used for DNA extraction if required. All fruitbodies were then described and air dried according to the instructions in Brundrett *et al.* (1996).

2.3.4. Testing the reliability of isolation techniques from fruitbodies

It was critical to the reference collection that the isolation techniques used were reliable and targeted appropriate species of wood decay fungi. The reliability of the isolation techniques used was tested by examining the accuracy of mycelial isolations from fruitbodies from the reference collection. Both fruitbodies and

mycelial cultures were subjected to PCR-RFLP analysis and the results were compared. This was thought to be a quick, reliable way to determine the accuracy of isolations as PCR-RFLP of the ITS region has been shown to differentiate well between species of fungi on eucalypts (Glen *et al.* 2001a).

Twenty-five species of wood decay fungi were chosen from the fruitbodies collected by G. Gates and D. Ratkowsky (Section 2.4.3, Table 2.3.1). The 25 were mostly well known wood-decay or wood-inhabiting species and were therefore thought to be appropriate representatives for isolates obtained from the living trees or logs in studies one and two.

The aerial mycelium was collected from 14-21 day old cultures of each isolate and the hymenial surface of dried fruitbodies and DNA was extracted from all samples using the glassmilk method described in Glen *et al.* (2002). Amplification of the DNA was carried out by PCR in 50 µl volumes with similar conditions to those in Glen *et al.* (2001b). For more information see section 2.4.2. The fungal specific primer ITS1F (Gardes and Bruns 1993) for the ITS region of the nuclear rDNA was used in combination with a universal reverse primer ITS4 (White *et al.* 1990) as this pair were known to successfully amplify the ITS from nearly all fungal species tested (M. Glen, pers. comm. 2004; Gardes and Bruns 1993)

Following PCR, a 7.5-10 µl aliquot of unpurified PCR product was digested for 3-4 hours with 5 µl of reaction mix containing 1U of the restriction enzymes *Alu*I and *Taq*I (Biotech International) according to the manufacturer's instructions. These enzymes were selected since they gave unique RFLP-PCR patterns for the ITS region in up to 85% of Basidiomycete species previously tested (Glen *et al.* 2001a; Glen *et al.* 2001b). The entire digest reaction was subject to electrophoresis on a 2.5% agarose gel for 5 hours at 4V cm^{-1}. Gels were post-stained for 15 minutes in 1µg ml^{-1} ethidium bromide and photographed under UV light. Fragments were sized using Kodak Digital Science 1D Image Analysis Software by reference to five equidistant lanes of size standards on each gel (*Hind*III digested bacteriophage lambda DNA, *Hpa*II digested pUC19 DNA, Biotech International and Geneworks 20 and 100bp ladders). Only those fragments larger than 100bp were used to discriminate RFLP groups due to poor resolution and visibility of smaller bands. The range of sizes considered for a single fragment varied by 5% as suggested in

Glen *et al.* (2001b). Where RFLP matches were found between the fruitbodies and cultures, the culture was lodged in the culture reference library.

Table 2.3.1. Species of wood-inhabiting fungi for which cultures and fruitbodies were subjected to PCR-RFLP analysis to determine the accuracy of isolation techniques. Collection number is the number used in the CSIRO culture collection in Hobart, Australia. RFLP match indicates whether a successful match was obtained for RFLP patterns for both fruitbody and mycelial culture and thus a successful isolation.

Name	Collection Number	RFLP Match
Anthracophyllum archeri (Berk.) Pegler	WR079	Yes
Armillaria hinnulea Kile & Watling	WR056	Yes
Beenakia dacostae D.A. Reid	WR051	Yes
Clitocybe semiocculta Cleland	WR052	No
Conchomyces bursiformis (Berk.) E. Horak	WR092	No
Datronia brunneoleuca (Berk.) Ryvarden	WR022	No
Ganoderma applanatum (Pers.) Pat.	WR023	Yes
Heterotextus peziziformis (Berk.) G.Cunn.	WR026	No
Hymenochaete "brown resupinate″	WR087	Yes
Panellus longinquus (Berk.) Singer	WR016	Yes
Perenniporia ochroleuca (Berk.) Ryvarden	WR031	Yes
Phellinus sp.	WR036	Yes
Pleurotus purpureo-olivaceus (G. Stev.) Segedin, P.K. Buchanan & J.P. Wilkie	WR012	No
Polypore, all-white, resupinate	WR066	Yes
Polypore, yellow-buff, resupinate	WR086	Yes
Polyporus melanopus (Sw.: Fr.) Fr.	WR062	Yes
Postia cf. *caesia* (Schrad.: Fr.) P. Karst.	WR018	Yes
Pycnoporus coccineus (Fr.) Bondartsev & Singer	WR114	Yes
Rigidoporus laetus (Cooke) P.K. Buchanan & Ryvarden	WR029S	Yes
Schizophyllum commune Fr.: Fr.	WR097	Yes
Stereum vellereum Berk.	WR061	Yes
Stereum ostrea (Blume & Nees: Fr.) Fr.	WR055	Yes
Trametes ochracea (Pers.) Gilb. & Ryvarden	WR116	Yes
Xylaria castorea Berk.	WR095	Yes
Xylaria castorea Berk.	WR090	Yes

Of the twenty-five species tested, twenty showed successful matches between RFLP patterns for fruitbodies and mycelial isolations (Table 2.3.1). These included all species of polypores (except *Datronia brunneoleuca*) and both isolates of *Xylaria.* With the exception of *D. brunneoleuca,* the five species which did not have successful matches between fruitbodies and cultures were mushroom-like or jelly fungi. Due to their small surface area to volume ratio, it is more difficult to isolate from these species as there are few regions which have not been exposed to external

contamination and the fruitbodies degenerate quickly. Jelly fungi are especially difficult to culture (Fenwick 1995). In addition, the *Clitocybe semiocculta* fruitbody was not as fresh as most other material, potentially reducing chances of successful isolation and increasing the chance of contamination. Given that 80% of cultures tested showed successful mycelial isolation, the technique used was deemed suitable for the target wood decay fungi.

2.4 Identification of Fungi Isolated From Wood

2.4.1 Morphological grouping of fungal isolates

Fungal isolates were sorted into broad morphological groups based on their macroscopic appearance, then identified to morphospecies using traditional morphological taxonomy (Nakasone 1990; Stalpers 1978). Isolates were grown on 1.5% MEA at 20ºC and examined two weeks and six weeks after subculturing. At both ages, the macroscopic and microscopic characteristics of the isolates were recorded.

Macromorphology

Macroscopic characters recorded include colour and texture of aerial hyphae, characteristics of culture margin and changes in the colour of the reverse of the culture medium. The specific terminology used to describe these features followed that of Stalpers (1978).

At two weeks, the radius of each isolate was measured to indicate growth rate.

Micromorphology

The three different regions of hyphae, the marginal hyphae, submerged hyphae and the aerial hyphae, were examined for each culture as these often contain different features. In each region, the characteristics and dimensions of the generative hyphae were noted especially the presence, or absence, and characteristics of clamp connections, hyphal diameter, wall thickness, branching, pigmentation and ornamentation. The presence and characteristics of specialised hyphae (e.g. skeletal hyphae, binding hyphae, lactiferous hyphae) and other structures such as terminal swellings, cystidia, chlamydospores and conidia were also recorded (Stalpers 1978).

Enzyme Tests

Enzyme tests were carried out to detect the production of the wood degrading enzymes laccase and tyrosinase by each isolate. Enzyme testing is a useful technique as it can be both taxonomically and ecologically important (Kaarik 1965 in Stalpers 1978). Laccase (a lignin-degrading enzyme) and tyrosinase (a cellulose-degrading enzyme) were chosen for testing as these are the key enzymes for culture identification used by Stalpers (1978) and have been found to give a good indication of the decay capacity of more than 90% of isolates. Drop testing was chosen in preference to the use of specialised media (such as Gallic/Tannic Acid (GTA)) as it is a quick, reliable and repeatable way of determining enzymatic capacity (Gramss *et al.* 1998; Worrall *et al.* 1997), factors which are particularly important when large numbers of isolates are examined. One test of each of two solutions was applied to the culture margin of each 2 week-old isolate. The presence of laccase was indicated by a purple colour change to the application of 0.1M α-naphthol dissolved in ethanol. The presence of tyrosinase was indicated by a brown colour change to the application of 0.1M ρ-cresol dissolved in ethanol. Isolates were monitored for enzyme colour changes after 3 hours, 24 hours and 72 hours (Stalpers 1978). Each drop test was performed at least twice to test for reliability.

2.4.2 Sequence analysis of fungal isolates

Isolates from each morphospecies were selected for sequencing of the ribosomal DNA internal transcribed spacers (rDNA ITS) to confirm the morphological groupings and attempt to identify them to species or genus level. Fruitbodies and cultures from fruitbodies were also sequenced. The rDNA ITS is widely considered to be an appropriate region for fungal species differentiation as interspecific species variation in the ITS of many fungal species has been observed (e.g. Farmer and Sylvia 1998; Glen *et al.* 2001a; Kåren *et al.* 1997). The level of intraspecific heterogeneity does vary between species, however, and some intrageneric homogeneity has also been observed (Farmer and Sylvia 1998; Glen *et al.* 2001a). The conserved nature of the subunits surrounding the ITS (18S, 5.8S and 28S) also makes the development of universal fungal primers possible for phylogenetically diverse groups of fungi (White *et al.* 1990). As the ITS region is widely used for fungal studies, it is well suited for identification of unknown fungal isolates as it is

possible to compare their ITS sequences with those found on public databases e.g. Genbank, EMBL (Hoff *et al.* 2004; Horton and Bruns 2001).

The aerial mycelium was collected from 14-21 day old cultures of each isolate or the hymenial surface of dried fruitbodies and DNA was extracted from all samples using the glassmilk method described in Glen *et al.* (2002).

Amplification of the DNA was carried out by PCR in 50 µl volumes with similar conditions to those in Glen *et al.* (2001b). The fungal specific primer ITS1F (Gardes and Bruns 1993) for the ITS region of the nuclear rDNA was used in combination with a universal reverse primer ITS4 (White *et al.* 1990) as this pair successfully amplified the ITS from nearly all fungal species tested (Gardes and Bruns 1993, M. Glen, pers. comm.) The concentration of each primer was 1 µM, dNTPs concentration was 0.2 mM and $MgCl_2$ concentration was 2 mM. Each 50 µl PCR contained 1.1U TTH+ polymerase (Fisher Biotech) and 1x polymerisation buffer (Fisher Biotech). Amplification parameters were an initial denaturation at 95ºC for 3 minutes, followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds and extension at 72ºC for 30 seconds, with a final extension at 72ºC for 7 minutes. Following PCR, 90-100 µl of PCR product was purified, precipitated and concentrated using the MO BIO Laboratories Inc. UltraClean PCR Clean-up Kit to remove primers and dNTPs.

DNA sequencing and final ethanol precipitation were carried out according to the instructions provided with the Beckman Coulter GenomeLab Dye Terminator Cycle Sequencing with Quick Start Kit with the following modifications. Between 1 and 5 µl of DNA template were used in a 10 µl sequencing reaction with 3.2 pmol of primer and 2 µl of DTCS Quick Start Master Mix. During ethanol precipitation, 0.25 µl of 20 mg/ml of glycogen was used. Sequences were determined on a Beckman Coulter CEQ 8000. Forward sequences were obtained for all isolates with reverse sequences only when additional information was required. At least two complete (forward and reverse) sequences were obtained for each morphospecies.

DNA sequences were edited using BioEdit (Hall 1999). Sequences of isolates from the same morphospecies were aligned in ClustalW (Thompson *et al.* 1994) and manually assessed for similarity. Once a consensus sequence was obtained for each morphospecies, Blast (Altschul *et al.* 1997) searches of public databases were

carried out using BioManager (ANGIS). Phylogenetic trees of each morphospecies were created in ClustalW and viewed in TreeView (Page 2001), including similar sequences downloaded from BioManager (ANGIS 2005). Sequences from related but different genera were used as outgroups where possible. Molecular designation of isolates was determined by sequence groupings from dendrograms. Where highly similar but non-identical sequences were obtained from several isolates, chromatograms were reviewed to verify nucleotide variation.

2.4.3 Comparison of isolates with reference fungal collection

The cultures in the reference collection came from three different sources, outlined below. The first two sources were collections of fresh fruitbodies which were cultured and maintained as described in section 2.3.3. A complete list of all fruitbodies collected can be found in Appendix 2.1. The third collection was a set of identified cultures developed and maintained by CSIRO Clayton Laboratories. Sequences of the ITS region of cultures from this third source were prepared by K. Potter and T. Trang (University of Tasmania). A list of these sequenced cultures can be found in Appendix 2.2.

Collection of fungi from during Studies 1 and 2

Fruitbodies found on, or in the vicinity of the study trees in Study 1 at the time of the original field work were collected. Two years following the field work in Study 1, the study site (now logging regeneration) was revisited and any potential wood decay fungi were collected. Fungi from both surveys were cultured (section 2.3.3), described and dried (Brundrett *et al.* 1996). Identifications were carried out in consultation with Genevieve Gates and David Ratkowsky (University of Tasmania).

Similarly, any fruitbodies found on, or in the vicinity of the study logs in Study 2 were collected, cultured, identified and dried by Z-Q Yuan (University of Tasmania) following the methods outlined in Brundrett *et al.* (1996).

Collection of wood inhabiting fungi from Tasmania

This reference collection also included both fruitbodies and cultures. Reference fruitbodies were also obtained from more than 130 identified fruitbodies growing on wood collected from throughout Tasmania by D. Ratkowsky and G. Gates (University of Tasmania) (Ratkowsky and Gates 2005). These fruitbodies were then

cultured and maintained as described in section 2.3.3. The surveyed sites are listed in Ratkowsky and Gates (2005).

National and international collection of wood inhabiting fungi

This reference collection only contained mycelial cultures. Access was granted to the large culture collection of identified wood decay fungi developed and maintained by CSIRO Clayton Laboratories. Sequences of the ITS region of cultures from this third source were prepared by Karina Potter and Tran Tranh Trang (University of Tasmania). A list of these sequenced cultures can be found in Appendix 2.2.

Comparing reference collections with study isolates

The morphological features of all reference cultures were compared with the cultures isolated from wood in studies one and two. Where similarities were found between study isolates and reference cultures, sequences of the ITS region of the original reference fruitbody was obtained and compared with sequencing of the study isolate. More details of this process are given in section 3.2.

2.5 Statistical Analysis

The specific details of statistical methods used are discussed in each chapter however a general outline is given below.

2.5.1 Univariate statistics

One-way analyses of variance (ANOVA) were undertaken in SAS 9.1 (Anon. 2002), and unless otherwise stated, a follow up multiple comparison test (Ryan-Einot-Gabriel-Welsch Multiple Range Test: REGW test) was used to determine the differences between pairs of treatment means. All standardisation techniques such as Chao Estimator and Rarefaction were performed in EstimateS unless otherwise stated.

2.5.2 Multivariate statistics

Both constrained and unconstrained ordination methods were used to explore changes in assemblage structure. Non-metric Multidimensional Scaling (NMS), an unconstrained ordination technique, was used to explore fungal assemblage variation in relation to various treatments. NMS is a non-parametric ordination technique and

relates the similarity of entities (e.g. trees, logs) based on ranked distances in multidimensional space. It is generally considered to be one of the most effective ordination methods for ecological community data as it deals well with zero-inflated data and non-normal data (McCune and Grace 2002). NMS was performed using a Sorensen (Bray-Curtis) distance measure in PC-ORD (McCune and Mefford 1999) choosing the slow and thorough autopilot method.

The constrained technique Canonical Analysis of Principal coordinates (CAP) analysis (Anderson and Willis 2003) was also used to explore fungal assemblage structure relationships. CAP uses *a priori* canonical axes to examine community structure (Anderson and Willis 2003). CAP was performed in CAP12 (Anderson 2004) using 9999 unrestricted random permutations of the data.

Indicator Species Analysis was run in PC-ORD for Windows 4.25(McCune and Mefford 1999) and used to investigate whether particular species were significantly associated with parameters such as tree age class or log size. A cut-off value of IndVal $\geq$25, p<=0.05 was used.

Mantel tests were performed in PC-ORD for Windows 4.25 (McCune and Mefford 1999) to compare community distribution patterns such as the relationship between fungal and beetle community structure in living trees. Mantel tests were based on Non-metric Multi-dimensional Scaling (NMS) ordinations prepared in PC-ORD for Windows 4.25 (McCune and Mefford 1999) for each group. The Mantel tests gave p values based on Randomisation (Monte Carlo) tests using 1000 randomised runs.

Appendix 2.1

Species of wood-inhabiting fungi cultured and sequenced as part of the reference collection for Studies 1 and 2. Fruitbodies in Study 2 were collected and identified by Z-Q Yuan (University of Tasmania) and fruitbodies in the Tasmania-wide collection were collected and identified by G. Gates and D. Ratkowsky (University of Tasmania). Species which were not able to be identified are shown with tag names only. Numbers indicate the number of fruitbodies collected in each study.

Species name	Fruitbodies Collected in Study 1	Fruitbodies Collected in Study 2	Tasmania-wide Collection	Sequence Obtained
Amauroderma rude (Berk.)Torrend.			1	Yes
Anthracophyllum archeri (Berk.)Pegler			1	
Antrodia sp.		1		Yes
Antrodiella zonata (Berk.)Ryvarden			2	Yes
Armillaria hinnulea Kile & Watling		1	1	Yes
Armillaria novaezelandiae (G. Stev.) Herink		1	1	Yes
Ascocoryne sarcoides (Jacq.) J.W. Groves & D.E. Wils			1	
Australoporus tasmanicus (Berk.) P.K. Buchanan & Ryvarden			1	Yes
Austroboletus sp.		1		
Beenakia dacostae D.A. Reid			1	
Beige "apricot fungus"		1		Yes
Bisporella citrina (Batsch ex Fr.) Korf & S.E. Carp.			1	
Bisporella sp.			1	
Bisporella sulphurina (Quél.) S.E. Carp.			1	
Boletellus obscurococcineus (Höhn.) Singer	1	1		
Byssomerulius corium (Pers.: Fr.) Parmasto			2	Yes
Callistosporium sp. "maroon on wood'			1	
Chlorociboria aeruginascens (Nyl.) Kanouse ex Ramamurthi, Korf & Batra		1	1	
Chondrostereum purpureum (Pers.) Pouzar			1	
Clavicorona piperata (Kauffman) Leathers & A.H. Sm.			1	
Clitocybe semiocculta Cleland			1	
Conchomyces bursiformis (Berk.) E. Horak			1	Yes
Coprinus "small grey"			1	Yes
Coprinus disseminatus (Pers.: Fr.) Gray			1	
Corticioid "red-brown"		1		Yes
Cortinarius abnormis		1		
Cortinarius sanguineus (Wulfen) Fr.		1		
Crepidotus aff. *applanatus* (Pers.) Kumm.		1	2	
Crepidotus stromaticus (Cooke & Massee) Sacc.			1	
Crepidotus variabilis (Pers.: Fr.) P. Kumm.			2	
Datronia brunneoleuca (Berk.) Ryvarden			1	Yes
Dermocybe austroveneta (Cleland) M.M. Moser & E. Horak		1		
Entoloma viridomarginatum (Cleland) E. Horak		1		
Fistulina hepatica (Schaeff.) With.		2		Yes (all)
Flammulina velutipes (Curtis: Fr.) Singer			2	
Fomes hemitephrus (Berk.) Cooke			1	Yes
Fomitopsis lilacinogilva (Berk.) J.E. Wright & J.R. Deschamps			1	Yes
Galerina sp.		2		
Ganoderma applanatum (Pers.) Pat.			1	Yes

Gloeoporus phlebophorus (Berk.) G.Cunn.		1	Yes
grey jelly fungus		1	
Gymnopilus allantopus (Berk.)Pegler		1	
Gymnopilus junonius (Fr.:Fr.)P.D.Orton	1		
Heiorganum curtisii (Berk.) Singer, Garcia & Gomez		1	
Heterotextus peziziformis (Berk.) Lloyd		1	
Hohenbuehelia sp.	1	2	Yes
Hymenochaete "brown resupinate"		1	Yes
Hymenoscyphus pezizioideus (Cooke & W.Phillips) Gamundi		1	
Hyphodontia sp.1	1		Yes
Hyphodontia sp.2	1		Yes
Hypholoma brunneum (Massee) D.A. Reid	1	1	
Hypholoma fasciculare (Huds.: Fr.) P. Kumm.	1		Yes
Hypholoma fasciculare (Huds.: Fr.) P. Kumm. var *armeniacum* Y.S. Chang & Mills ined.		1	Yes
Hypholoma sublateritium (Cooke) Sacc.	2		Yes
Hypocrea aff. *sulphurea* (Schwein.) Sacc.		1	
Hypoxylon aff. *placentiforme* Berk. & M.A. Curtis		1	
Hypoxylon archeri Berk.		1	
Hypoxylon howeanum Peck		1	
Inonotus hispidus (Bull.) P. Karst.	1		Yes
Lachnum lachnoderma (Berk.) G.G. Hahn & Ayers		1	
Lactarius sp.	1		
Lentinellus castoreus (Fr.)Kühner & Maire		1	
Lentinellus pulvinulus (Berk.) Pegler		1	
Lentinellus tasmanica R.H.Petersen		1	
Lycoperdon pyriforme Schaeff.: Pers.		1	
Marasmiellus affixus (Berk.) Singer		2	
Marasmius sp.	2		
Mollisia sp.		1	
Mucronella pendula (Massee) R.H. Petersen		2	
Multiclavula sp.		2	
Mycena "gelatinous grey frilly"		1	
Mycena "grey rubbery"		1	
Mycena interrupta (Berk.) Sacc.		1	
Mycena nargan Grgur.		1	
Mycena sp.	3		
Panellus ligulatus E. Horak		1	
Panellus longinquus (Berk.) Singer		1	
Panellus stipticus (Bull.: Fr.) P. Karst.		2	
Peniophora "resupinate purple"		1	Yes
Peniophora incarnata (Pers.) P. Karst.		1	Yes
Perenniporia ochroleuca (Berk.) Ryvarden		1	Yes
Phellinus sp.		1	Yes
Phellinus wahlbergii (Fr.) D.A. Reid	3	3	Yes
Phellodon niger (Fr.) P. Karst.	1		
Phlebia sp.		1	Yes
Pholiota pallidocaulis Y.S. Chang & A.K. Mills		1	
Pleurotus purpureo-olivaceus olivaceus (G. Stev.) Segedin, P.K. Buchanan & J.P. Wilkie		1	
Pluteus atromarginatus (Konrad) Kühner		1	
Podoserpula pusio (Berk.) D.A. Reid		1	
Polyporaceae sp.1	1		Yes

Polyporaceae sp.2		1		Yes
Polyporaceae sp.3		1		Yes
Polypore, all-white, resupinate			1	Yes
Polypore, yellow-buff, resupinate			1	Yes
Polyporus aff. *dictyopus* Mont.		1		Yes
Polyporus cf. *gayanus* Lév.			1	Yes
Polyporus melanopus (Sw.: Fr.) Fr.			1	Yes
Polyporus sp.1		1		Yes
Poria sp.		1		Yes
Postia cf. *caesia* (Schrad.: Fr.) P. Karst.			6	Yes
Postia pelliculosa (Berk.) Rajchenb.			2	Yes
Postia sp.		1		Yes
Postia, blackish brown upper surface			1	Yes
Psathyrella echinata (Cleland) Grgur.			1	Yes
Pseudohydnum gelatinosum (Scop.: Fr.) P. Karst.			1	
Psilocybe brunneoalbescens Y.S.Chang & A.K.Mills			1	
Psilocybe sp.			1	
Pycnoporus coccineus (Fr.) Bondartsev & Singer			2	
Resupinate fungus, unknown genus.			1	Yes
Resupinatus "grey"			1	
Rigidoporus laetus (Cooke) P.K. Buchanan & Ryvarden			3	Yes
Russula persanguinea Cleland		1		
Ryvardenia campyla (Berk.)Rajchenb.		3		Yes
Ryvardenia campyla (Berk.) Rajchenb.			1	Yes
Ryvardenia cretacea (Lloyd) Rajchenb.			1	Yes
Schizophyllum commune Fr.: Fr.	1		1	Yes
Schizophyllum-like sp.1		1		
Steccherinum(?) sp.			1	Yes
Stereum ‘all brown'			1	
Stereum hirsutum (Willd.: Fr.) Pers.	1	2	3	Yes
Stereum illudens Berk.	1		1	Yes
Stereum ochraceoflavum (Schwein.) Peck			2	
Stereum ostrea (Blume & Nees: Fr.) Fr.	1	1	1	Yes
Stereum rugosum Pers.			1	
Stereum sp.		1		
Trametes ochracea (Pers.) Gilb. & Ryvarden			1	Yes
Trametes versicolor (L.: Fr.) Lloyd	1	1	2	Yes
Tremella fimbriata Pers.:Fr.			1	
Tremella fuciformis Berk.			1	
Tremella mesenterica Retz.: Fr.			1	
Tricholomataceae "Gelatinous white-on-wood”			1	
Tubaria "Bettye's brown"			1	
Tubaria rufofulva (Cleland) D.A. Reid & E. Horak			1	Yes
Tubaria sp.		2		Yes
Tyromyces merulinus (Berk.) G. Cunn.			2	Yes
Tyromyces sp.		1		Yes
Unknown corticioid species			1	Yes
Vibrissea dura G. Beaton & G. Weste			1	
Xylaria apiculata Cooke			1	
Xylaria castorea Berk.			3	

Appendix 2.2.

Species of wood-inhabiting fungi cultured and sequenced by K. Potter and T. Trang from the CSIRO Clayton Culture Collection. DFP Code is the Clayton Collection Code. Name is the original name assigned to the culture and may now be out of date. Collection location indicates the country where the isolate was collected.

DFP Isolate Code	Name	Collection Location
10679	*Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh.	USA
7384	*Flavodon flavus* (Klotzsch) Ryvarden	Japan
6413	*Fomitopsis palustris* (Berk. & M.A. Curtis) Gilb. & Ryvarden	India
7381	*Fomitopsis pinicola* (Sw.) P. Karst.	Spain
10323	*Fomitopsis spraguei* (Berk. & M.A. Curtis) Gilb. & Ryvarden	USA
11112	*Gloeocystidiellum porosum* (Berk. & M.A. Curtis) Donk	New Zealand
7872	*Gloeophyllum odoratum* (Wulfen) Imazeki	USA
6241	*Gloeoporus dichrous* (Fr.) Bres.	USA
10274	*Hymenochaete corrugata* (Fr.) Lév.	India
11114	*Hyphodontia sambuci* (Pers.) J. Erikss.	New Zealand
7380	*Laricifomes officinalis* (Vill.) Kotl. & Pouzar	Spain
10807	*Lentinus cyathiformis* (Schaeff.) Bres.	Germany
7382	*Lentinus squarrosulus* Mont.	Spain
10806	*Lentinus tigrinus* (Bull.) Fr.	Germany
5259	*Lenzites betulina* (L.) Fr.	Portugal
2543	*Lenzites elegans* (Spreng.) Pat.	New Guinea
2378	*Neolentinus lepideus* (Fr.) Redhead & Ginns	UK
2384	*Neolentinus lepideus* (Fr.) Redhead & Ginns	UK
2385	*Neolentinus lepideus* (Fr.) Redhead & Ginns	UK
2386	*Neolentinus lepideus* (Fr.) Redhead & Ginns	UK
2387	*Neolentinus lepideus* (Fr.) Redhead & Ginns	UK
7519	*Neolentinus lepideus* (Fr.) Redhead & Ginns	USA
16544	*Phanerochaete chrysosporium* Burds.	USA
6235	*Phellinus conchatus* (Pers.) Quél.	USA
6987	*Phellinus lloydii* (Cleland) G. Cunn.	New Zealand
2390	*Phellinus weirii* (Murrill) Gilb.	UK
2445	*Phellinus weirii* (Murrill) Gilb.	Canada
2447	*Phellinus weirii* (Murrill) Gilb.	Canada
2447	*Phellinus weirii* (Murrill) Gilb.	Canada
10022	*Pleurotus ostreatus* (Jacq.) P. Kumm.	UK
5250	*Polyporus brumalis* (Pers.) Fr.	Portugal
7956	*Postia sericeomollis* (Romell) Jülich	USA
7378	*Pycnoporus sanguineus* (L.) Murrill	Spain
6225	*Radulomyces confluens* (Fr.) M.P. Christ.	USA
7726	*Rigidoporus crocatus* (Pat.) Ryvarden	USA
8330	*Rigidoporus sanguinolentus* (Alb. & Schwein.) Donk	UK
2377	*Serpula lacrymans* (Wulfen) J. Schröt.	UK
14579	*Tinctoporellus epimiltinus* (Berk. & Broome) Ryvarden	Zambia
14579B	*Tinctoporellus epimiltinus* (Berk. & Broome) Ryvarden	Zambia
6242	*Trichaptum biforme* (Fr.) Ryvarden	USA

CHAPTER 3: IDENTIFICATION OF WOOD DECAY FUNGI FROM LIVING *EUCALYPTUS OBLIQUA* TREES

3.1 Introduction

Wood decay fungi are central to many ecosystem processes including the recycling of nutrients and the creation of decayed wood habitat in natural ecosystems (Grove *et al.* 2002; Heilmann-Clausen and Christensen 2003) and are also recognised as one of the major factors threatening solid wood production in eucalypts (Kile and Johnson 2000; Wardlaw 2003). Wood decay fungi are also biologically important in their own right as a diverse and speciose group of organisms (Simpson 1996). More than 1500 species of macrofungi are known to inhabit wood in Finland (Siitonen 2001) while 1250 species are estimated from Denmark (Heilmann-Clausen 2003) and 1100 species from Sweden (Gustafsson 2002). Microfungi, while less well known, are also thought to frequently grow within or on wood (e.g. Lumley *et al.* 2000).

Despite their ecological and commercial importance, the taxonomy of wood decay fungi in Australia is poorly developed (Hood 2003; May 2001; Simpson 1996). In contrast with Europe and North America, where decay species are relatively well characterised (Boddy 2001; Nordén and Paltto 2001), the identification of wood decay fungi in Australia is often problematic since many decay species are not yet named (or even discovered!) (Buchanan 1989; Buchanan 2001). Of the predicted 250 000 species of fungi in Australia, as few as 5% of these have been identified and described in detail (Hawksworth 1991; May and Pascoe 1996). This is primarily due to lack of professional mycologists and the short history of mycological research in Australia. In 1996 there was the equivalent of less than ten full time taxonomic mycologists in Australia (May and Pascoe 1996) and the situation is little different today. Much of the taxonomy of wood decay fungi in Australia is therefore based on outdated monographs of Australasian fungi (e.g. Cunningham 1963; Cunningham 1965) or applications of northern hemisphere works (see Buchanan 2001; Simpson 1996) although a few good Australian publications do exist (e.g. Fuhrer and Robinson 1992; Hood 2003; see also McCann 2003). Since the taxonomy of higher fungi is primarily based on the description of fruitbodies, the sporadic and cryptic

appearance of many fungal fruitbodies (an international phenomenon) also complicates matters.

The poor understanding of fungal taxonomy in Australia is further highlighted when the taxonomy of cultures of wood decay fungi is considered. In countries with a longer history of fungal taxonomy, it may be possible to identify a large proportion of wood decay fungi from their cultures. In Australia, however, this is rarely the case unless they can be matched to a culture from a known fruitbody (Buchanan 1989). There are few publications to assist in the description and identification of basidiomycete wood decay fungi in culture, however, they include Nobles (1948), Stalpers (1978) and Nakasone (1990). While all provide useful information about fungal taxonomy, they include few, if any, Australian species and so may not result in identification even to family level. Stalpers (1978) is the only comprehensive key to include fungal isolates from Australia and for this reason, is the basic key followed here.

Molecular techniques are increasingly being used to augment cultural morphology and attempt to identify wood decay fungi and their phylogenetic relationships directly (Adair *et al.* 2002; Glen *et al.* 2006a; Johannesson and Stenlid 1999; Oh *et al.* 2003; Vainio and Hantula 2000). PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) has consistently been used to discriminate fungi to species level and beyond (e.g. Gardes and Bruns 1996; Glen *et al.* 2001b; Johannesson and Stenlid 1999; Kåren *et al.* 1997; Kauserud and Schumacher 2002) due to its speed and accuracy. With the development of improved technology however, PCR and sequencing of fungi is increasingly proving a fast, reliable and inexpensive method of detecting fungal species relationships, providing more taxonomic information than can be achieved with PCR-RFLP (Bougoure and Cairney 2005; Hoff *et al.* 2004; Horton and Bruns 2001; Silva-Pinhati *et al.* 2004).

Although a wide variety of organisms are known to inhabit decayed wood, the majority of active decay species are Basidiomycetes together with some Ascomycetes such as the Xylariaceae (Rayner and Boddy 1988; Schwarze *et al.* 2000). Agarics, corticioid and polypore species are the most commonly observed morphological groups (Bader *et al.* 1995; Buchanan 1989; Heilmann-Clausen 2003; Nordén 2000). These three groups accounted for more than three quarters of the 277 species of macrofungi found on deciduous wood in Danish forests (Heilmann-

Clausen 2003). Examples of well known cosmopolitan pathogenic fungi include the agarics *Armillaria* spp., the polypores *Phellinus* spp., *Inonotus* spp. and *Ganoderma* spp. and the corticioids *Phlebia, Coniophora* and *Peniophora* spp. (Boddy 2001). Although relatively rare, fungi found fruiting on living eucalypts include species of *Armillaria, Fistulina, Gymnopilus, Hymenochaete, Inonotus, Phellinus,* and *Piptoporus* (Kile and Johnson 2000; May and Simpson 1997). Very little is known about their ecology however, and their taxonomy is generally not well developed (Buchanan 2001; Buchanan and May 2003; May 2001; May and Simpson 1997).

This chapter documents the species of fungi associated with decayed wood in living *Eucalyptus obliqua* trees in southern Tasmania in three age classes: 69 year old and 105 year old regrowth[1] trees, and mature trees greater than 150 years old. The fungi described in this study were obtained by isolation from decayed wood, not by the collection of fungal fruitbodies, thus making the development of effective methods for the identification of Australian fungal cultures central to this study. While this thesis is predominantly ecologically focussed, the taxonomy of cultures of wood decay fungi was quickly identified as needing more thorough attention. Two identification methods, traditional morphological methods and DNA sequencing, were used to provide complementary information to determine robust groupings at a probable species level. As well as determining groupings of isolates, sequence data were used to provide tentative identifications based on searches of public databases and similarities to reference collections.

[1] NB Trees in both these age classes (i.e. 69 and 105 years old) are regrowth from wildfire, not regrowth from logging.

3.1.1 Glossary

This study examines a number of different methods used to group isolates into putative species or species groups. The different terminology used to describe these groupings is explained below.

Morphological species: putative species determined by morphological examination of cultures.
ITS species: putative species determined by dendrograms based on molecular sequence information.
Species /Final species: inferred species determined using the combined morphological and molecular information. These are the putative species which were used for the remainder of the analyses in this study.

Main species: species which contain more than one isolate.
Singletons: Species which contain only one isolate (ie were only found once).
Ungrouped isolates: Isolates with unremarkable morphological features which were not able to be allocated to a morphological grouping.

3.2 Methods

Species delineation

A total of 18 *E. obliqua* trees, six from each of three age classes (69, 105 and 150 years old) in two adjacent sites in southern Tasmania, were felled and examined for wood decay fungi as described in Section 2.2. Isolations were carried out from clear sapwood and heartwood as well as samples of rotten wood collected at each sampling point.

Two methods were used to group and identify the fungal species present (Section 2.4). First, a traditional morphological analysis of all fungal isolates was carried out (Stalpers 1978), followed by a DNA sequencing study of the isolates to test the morphological groupings and gain a better understanding of their taxonomic affiliations. Representatives of each morphological group were sequenced. Between two and 15 isolates of each morphological group were chosen to cover the full spectrum of morphological and tree/location variation within each morphological group. Ungrouped or singleton isolates were also subjected to sequencing, both for identification and classification purposes. If sequence variation was found, further isolates were sequenced to enable the determination of two or more groups. Based

on the results of the molecular study, the morphological grouping was then revisited and the macroscopic and microscopic features re-examined. The final fungal species groupings used in this study were based on the results of both the morphological and molecular data. For more details of this process refer to Section 2.4**.** Public databases were searched for the best match to consensus sequences from the final fungal species groupings using BLAST (Altschul *et al.* 1997) to assist in providing tentative identifications.

Comparison with reference isolates

Sequences of study isolates and reference fungi were aligned and dendrograms were created using Clustalw (Thompson *et al.* 1994) and DNAml of the Phylip package (ANGIS; Felsenstein 1989) and viewed in TreeView (Page 2001). Sequences were considered likely to belong to the same species if there was less than 1-2% variation between them, however this did depend on the species involved (for more information see Section 3.4 or Glen *et al.* 2001a). A more general search of sequences in the reference collection was also carried out for matches with the sequence of each final fungal species grouping using FastA (Pearson and Lipman 1988).

3.3 Results

Isolation rates

Three hundred and twelve isolates of wood decay fungi were obtained from 18 living trees. The majority of these isolates were obtained from samples of wood from the leading edge of decay columns (70.8%, Table 3.3.1). Only a few isolates of wood decay fungi were obtained from the samples of clear heartwood and sapwood (15.1% and 14.1% respectively). The rotten wood samples resulted in the greatest isolation success rate at 65.7% while only 23.8% of the heartwood and 27.5% of the sapwood samples yielded wood decay fungi. Those wood samples that did not yield isolates of wood decay fungi yielded either common contaminant fungi (e.g. *Penicillium* spp., *Mucor* spp.) or did not grow any fungi while incubated on media for six weeks.

Table 3.3.1. Proportion of fungal isolates obtained from the different wood samples, and the isolation success rate for each wood sample type. Isolation success rate was determined by dividing the total number of isolates from each wood sample type by the total number of wood samples collected for that same wood sample type.

Wood sample type	Decayed Wood	Clear Heartwood	Clear Sapwood	All
Proportion of isolates (%)	70.8	15.1	14.1	-
Isolation success rate (%)	65.7	23.8	27.5	46.5

Morphological grouping of fungal isolates

All isolates were described morphologically and were sorted into nine main morphological species and 145 ungrouped isolates. These nine main morphological species are described briefly in Table 3.3.2. Two morphological species (M04 & M06) were thought to belong to the Hymenochaetaceae due to the presence of setae or setal hyphae (Stalpers 1978). The remaining morphological species could not be identified from morphological information alone.

Table 3.3.2. The primary distinguishing features of each of the nine main morphological groups found in the study.

Morphological group code	Distinguishing features: Macroscopic	Microscopic
M01	Culture white, becoming brown and white with age. Aerial mycelium silky to felty.	Single clamp connections present at every septum, monomitic, oil filled terminal swellings in aerial and submerged hyphae.
M02	Culture white, becoming yellow with age. Aerial mycelium woolly to felty. Can produce small brown fruitbody primordia after 6 weeks.	Single clamp connections present at every septum, monomitic, oil filled terminal swellings in aerial and marginal hyphae.
M03	Culture white to cream. Aerial mycelium woolly becoming silky with age.	Multiple clamp connections present in marginal hyphae, sometimes aerial hyphae. Chlamydospores and arthroconidia present.
M04 (Hymenochaetaceae)	Culture white quickly becoming brown to dark brown. Aerial mycelium silky, reverse dark.	Hyphae simple septate, often pigmented brown, septa ampullate. Setal hyphae and setae present in submerged hyphae.
M05	Culture hyaline, becoming orange or reddish with age. Aerial mycelium silky to absent.	Single clamp connections occasionally present, thick-walled generative hyphae, gloeocystidia and chlamydospores.

Morphological group code	Distinguishing features: Macroscopic	Microscopic
M06 (Hymenochaetaceae)	Culture white, becoming greenish-yellow with age. Aerial mycelium cottony to woolly. Reverse becomes greenish-yellow/brown with age.	Hyphae simple septate, often highly branched and pigmented light brown. Chlamydospores and setal hyphae often present.
M07	Culture white, becoming yellow in places with age. Aerial mycelium silky.	Single clamp connections present at every septum, thick-walled generative hyphae, early binding hyphae, chlamydospores present.
M08	Culture white, aerial mycelium woolly.	Single clamp connections present at every septum, arthroconidia, chlamydospores and terminal swellings present.
Xylariaceae	Cultures white, becoming black in patches with age. Some produce black, stick-like fruitbodies with age.	Simple septate hyphae, Intercalary hyphal swellings often present.

Sequence information changed the morphological species grouping

Useful sequence information was obtained from 167 isolates which were then compared using ClustalW and TreeView. Examples of these comparisons can be seen in Appendix 3.2. For the majority of isolates, the sequence information confirmed the morphological species groupings. Where this was not the case, one of two situations occurred. Either the sequence of some ungrouped isolates matched those within existing morphological species and all were combined into one ITS group, or there were found to be two or more distinct sequence types within a morphological species. In the second situation, the morphological species was divided into two or more ITS species. Some ungrouped isolates were also found to have similar sequences to other ungrouped isolates and so were combined to form a new ITS species. Based on this information, all the isolates were classified into 18 ITS species and 80 singletons.

Obtaining the final species

Based on the combined information from the morphological and ITS sequence analyses, a total of 91 final species were identified, 20 of which were common. In the few cases in which the morphological and molecular analyses gave conflicting results, both sets of data were revisited. In cases where the sequence information was similar but the morphology markedly different, isolates were considered to be

different species. The reverse was also true; if culture morphology was similar but sequence information was very different (>3%), isolates were considered different species. The determination of final species for each species is shown on Table 3.3.3. The relationships between ITS sequences of the main species are shown in Appendix 3.2.

For example, M01 was considered a discrete morphological group based on the presence of single clamps, a distinctive brown and white aerial mycelium and predominantly positive tests for both laccase and tyrosinase. An examination of DNA sequences grouped a number of ungrouped isolates with M01, resulting in two similar, yet distinct putative ITS species. Thus two ITS groups were recognised: *Postia*-like sp.1 and *Postia*-like sp.2 (Figure 3.3.1). The majority of isolates in *Postia*-like sp.2 were originally morphologically ungrouped isolates. A further examination of the morphology isolates in *Postia*-like sp.1 identified a few isolates (originally morphologically ungrouped) which had markedly different morphology from the majority of isolates in *Postia*-like sp.1. Thus these were determined to be separate species and designated *Postia*-like sp.6 and *Postia*-like sp.7 in the final species grouping. From the DNA sequence and dendrogram information, it was unclear whether *Postia*-like sp.2 was comprised of one or two species. The morphology of isolates within these putative species was revisited and two distinct morphologies were identified which coincided with slight sequence variation. These were designated *Postia*-like sp.4 and *Postia*-like sp.5 in the final species grouping. Another putative morphological species, M07 was also found to have approximately 90% sequence similarity to *Postia*-like sp.1, however this group falls into a distinct clade in the ML tree, a distinction supported by the morphological data. It was designated as *Postia*-like sp.3. Each of the final *Postia*-like species groups, except for *Postia*-like sp.1, was a monophyletic group in the ML tree, even though, for example species 1, 6 and 7 together or species 4 and 5 together also form monophyletic groups.

Table 3.3.3. A diagrammatic representation of the process of determining the final species groupings.

Morphological species	ITS species	Final Species
M01	*Postia*-like sp.1	*Postia pelliculosa**
Ungrouped isolates		*Postia*-like sp.6
		Postia-like sp.7
	Postia-like sp.2	*Postia*-like sp.4
		Postia-like sp.5
M07	*Postia*-like sp.3	*Postia*-like sp.3
M02	*Fistulina*-like sp.1	*Fistulina*-like sp.1
M03	*Coniophora*-like sp.1	*Coniophora*-like sp.1
	Coniophora-like sp.2	*Coniophora*-like sp.2
Ungrouped isolates	*Coniophora*-like sp.3	*Coniophora*-like sp.3
M04	Hymenochaetaceae sp.1	Hymenochaetaceae sp.1
M05	Basidiomycete sp.1	Basidiomycete sp.1
	Basidiomycete sp.2	Basidiomycete sp.2
M06	Hymenochaetaceae sp.2	Hymenochaetaceae sp.2
Xylariaceae	Xylariaceae sp.1	Xylariaceae sp.1
Ungrouped isolates		
Ungrouped isolates	Ascomycete sp.1	Ascomycete sp.1
	Ascomycete sp.2	Ascomycete sp.2
M08	Basidiomycete sp.3	Basidiomycete sp.3
Ungrouped isolates	*Athelia*-like sp.1	*Athelia*-like sp.1
	Basidiomycete sp.4	Basidiomycete sp.4
	Hypholoma fasciculare	*Hypholoma fasciculare*
	Phlebia-like sp.1	*Phlebia*-like sp.1
	Stereum-like sp.1	*Stereum*-like sp.1
	Trametes versicolor	*Trametes versicolor*

* In the final species analysis, the largest division of *Postia*-like sp.1 was re-named *Postia pelliculosa* due to a strong match with ITS sequences from a *Postia pelliculosa* fruitbody in the reference collection.

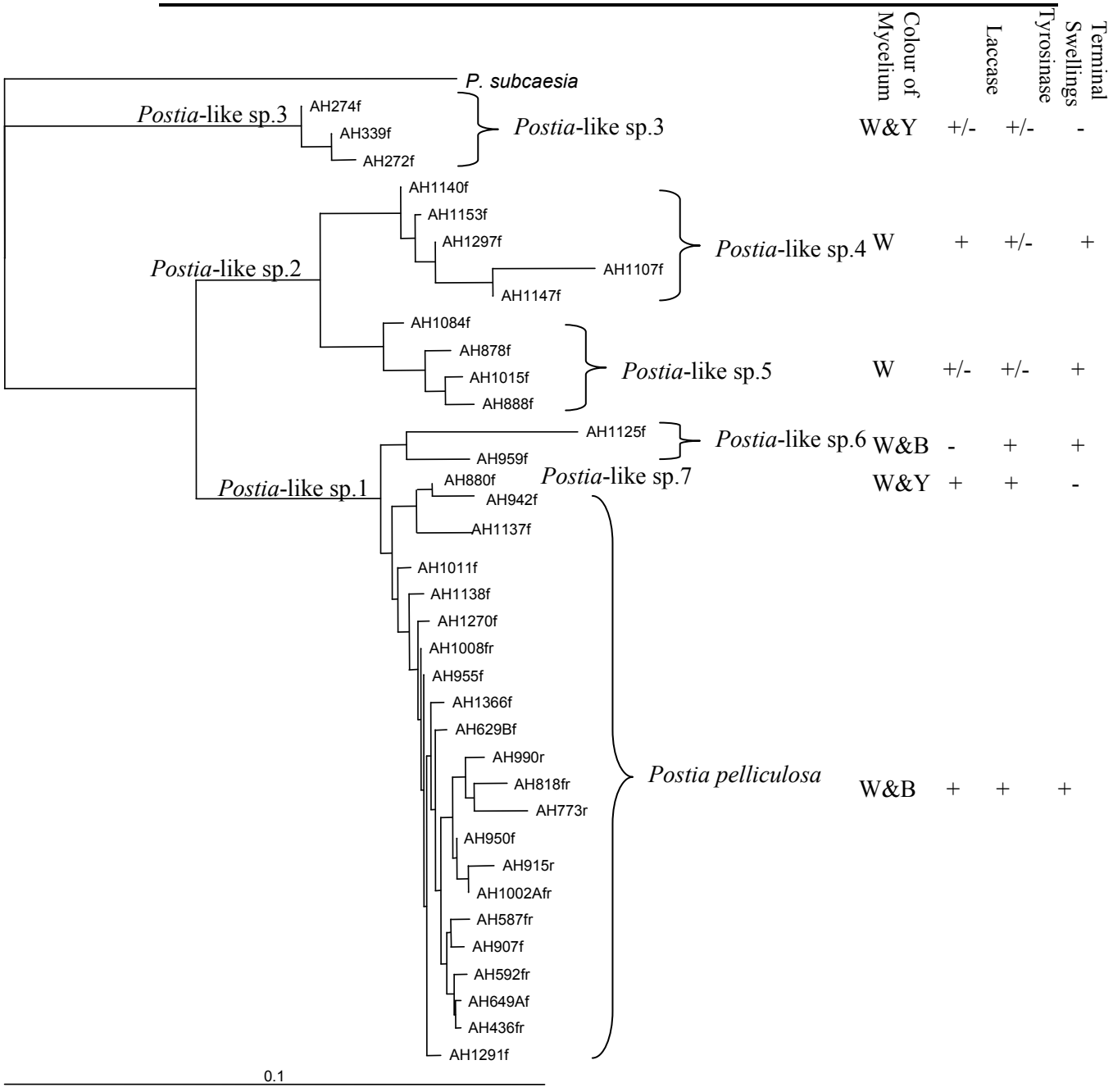


Figure 3.3.1. Maximum likelihood tree from analysis of ITS sequences of all *Postia*-like isolates. The *Postia subcaesia* outgroup is a sequence downloaded from GenBank (Accession No. AY599577). The initial molecular designation is shown on the left-hand side of the dendrogram while the final species groups are shown on the right-hand side of the dendrograms. The bar represents an expected sequence variation of 10%. The columns on the right-hand side of the graph show a few of the characters used to discriminate the isolates morphologically. Mycelial colours are W=white, B=brown, Y=yellow. The presence or absence of the wood decay enzymes laccase and tyrosinase is indicated by + or – with +/- indicating a variable result. The presence of terminal swellings in the mycelium is also indicated by + or -. In the final species analysis, *Postia pelliculosa* was named due to a strong match with ITS sequences from a *Postia pelliculosa* fruitbody in the reference collection (AH436).

Identifying the fungi: Matches with public databases

Blast searches using all 167 sequences were carried out on public databases (e.g. GB, EMBL, DDBJ). One of the 20 species showed very high similarity to a sequence from a known species (*Hypholoma fasciculare*, Table 3.3.4, Appendix 1), giving a good indication of the identity of the fungus (based on percentage similarity and length of matching region). Of the remaining 19 species, 14 had lower sequence similarity (87-97%) to sequences of fungi lodged on GenBank, giving a good indication of genus or family. Five of the species did not have successful matches to family level, however their identity as fungal sequences was confirmed to division, usually by high similarity of the 5.8S region. A number of singletons also showed good matches with sequences from GenBank (Table 3.3.5).

Table 3.3.4. Results of searches for sequences matches in GenBank with main species groups. Sequence match quality indicates the value of the taxonomic information obtained from the Blast search, based on percentage similarity and length of matching region. Sequences are shown in Appendix 1.

Final Species Group	Closest Blast Match	Number of bases matched[1]	% Match	Origin of Closest Match	Sequence Match Quality
Ascomycete sp.1	Assorted Ascomycete species	478/513	94-96%	Canada, USA	Poor
Xylariaceae sp.1	*Xylaria* sp.	493/561	98-100%	USA, Spain	Moderate
Athelia-like sp.1	*Athelia arachnoidea, A. epiphylla*	581/690	96-97%	USA	Moderate
Basidiomycete sp.1	*Phlebia brevispora,* and assorted Aphyllophorales	170/623	99%	Japan	Poor
Basidiomycete sp.2	Assorted unspecified fungi				Poor
Basidiomycete sp.3	*Antrodia, Oligoporus, Fomitopsis rosea*	240/602	91-100%	France, Germany	Poor
Basidiomycete sp.4	*Stereum sanguinolentum*	193/506	99%	Sweden	Poor
Coniophora-like sp.1	*Coniophora marmorata*	522/670	94-96%	Germany	Moderate
Fistulina-like sp.1	*Fistulina hepatica*	502/761	95%	USA	Moderate
Hymenochaetaceae sp.1	*Hymenochaete adusta, Inonotus hispida*	427/687	91-94%	South Korea	Moderate
Hymenochaetaceae sp.2	*Inonotus hispida*	396/687	86-89%	South Korea	Moderate

Final Species Group	Closest Blast Match	Number of bases matched[1]	% Match	Origin of Closest Match	Sequence Match Quality
Hypholoma fasciculare	*Hypholoma fasciculare*	579/592	97%	Sweden	Good
Phlebia-like sp.1	*Phlebia radiata*	476/550	88-89%	USA	Moderate
Postia pelliculosa	*Postia subcaesia*	309/632	88-96%	China	Moderate
Postia-like sp.3	*Oligoporus rennyi, Postia* spp.	460/681	87-89%	UK	Moderate
Postia-like sp.4	*Postia balsamea, Postia subcaesia*	355/640	90-93%	UK, USA	Moderate
Postia-like sp.5	*Oligoporus rennyi, Postia* spp.	334/623	90-91%	UK	Moderate
Postia-like sp.6	*Postia subcaesia*	299/601	91%	China	Moderate
Postia-like sp.7	*Postia balsamea, Postia caesia*	254/590	90%	China	Moderate
Stereum-like sp.1	*Stereum annosum, S. hirsutum*	395/556	97%	Tunisia	Moderate

[1]Length of matching sequence/full length of sequence

Table 3.3.5. Results of searches for sequences matches in Genbank with singleton species groups. Sequence match quality indicates the value of the taxonomic information obtained from the Blast search, based on percentage similarity and length of matching region. Only Moderate or Good matches shown. Sequences are shown in Appendix 1.

Final Species Group	Closest Blast Match	Number of bases matched	Percentage Match	Origin of Closest Match	Sequence Match Quality
Ascocoryne sp.1	*Ascocoryne sarcoides*	482/544	92%	USA	Moderate
Metarhizium flavoviride	*Metarhizium flavoviride*	502/536	97%	Australia	Good
Nectria radicicola	*Nectria radicicola*	454/476	97%	Sweden	Good
Xylaria sp.1	*Xylaria arbuscula*	463/500	93%	USA	Moderate
Athelia sp.2	*Fibulorhizoctonia/ Athelia* spp.	422/495	92%	USA	Moderate
Coniophora-like sp.2	*Coniophora marmorata*	522/670	94%	Germany	Moderate
Fomitopsis-like sp.1	*Fomitopsis rosea*	411/461	92%	Norway	Moderate
Gymnopilus allantopus	*Gymnopilus allantopus*	418/415	99%	Australia	Good
Hypholoma sp.1	*Hypholoma fasciculare*	371/430	95%	Sweden	Moderate
Hypholoma sp.2	*Hypholoma fasciculare*	289/297	96%	Germany	Moderate
Peniophora aurantiaca	*Peniophora aurantiaca*	513/525	97%	Sweden	Good
Peniophora cinerea	*Peniophora cinerea*	290/294	99%	Sweden	Good
Phanerochaete	*Phanerochaete*	569/584	97%	Korea	Good

Final Species Group	Closest Blast Match	Number of bases matched	Percentage Match	Origin of Closest Match	Sequence Match Quality
sordida	*sordida*				
Polyporus gayanus	*Polyporus gayanus*	436/442	98%	Sweden	Good
Psathyrella-like sp.1	*Psathyrella gracillis*	539/578	93%	Canada	Moderate
Steccherinum-like sp.1	*Steccherinum litschaueri*	463/527	93%	Sweden	Moderate
Trametes ochracea	*Trametes ochracea*	539/540	99%	Russia	Good
Trametes versicolor	*Trametes versicolor*	573/575	99-100%	USA	Good
Typhula-like sp.1	*Typhula* spp.	189/438	97%	USA	Moderate

Comparison to reference isolates

There were only three matches between the study isolates and the reference collection for either culture morphology or ITS sequence information. A significant ITS sequence match (>97% similarity) was found between *Postia*-like sp.1 and *Postia pelliculosa* from the reference collection of Gates and Ratkowsky (see Chapter 2). In addition, two species which were previously identified through sequence matches on Genbank, also matched the same species found within the reference collection. These were *Trametes versicolor* and *Hypholoma fasciculare.* No other useful information was obtained from the reference collection data that was not already known from searches of public databases.

Final species

A total of 91 fungal species were determined from the 18 living trees examined. Of these 91 species, 20 species were isolated more than once and are herewith referred to as the main species. Two of the main species were ascomycetes and 18 were basidiomycetes (Table 3.3.6). Of the ascomycetes, one species group was in the Xylariaceae while the other was of uncertain family. Four of the basidiomycetes were of uncertain family while the remainder showed ITS sequence matches with members of a range of families within the Polyporales, Agaricales, Hymenochaetales, Boletales and Russulales. The singleton species groups showed similar taxonomic patterns: Four were ascomycetes and 67 were basidiomycetes. Fifty-three of the basidiomycetes remained un-named even to order. This is either due to difficulty obtaining a sequence or due to there being no sequence matches on

public databases. Of the 14 singleton basidiomycetes which returned a reasonable match in sequence searches, seven were in the Polyporales, five in the Agaricales, and one in each of the Russulales and Boletales.

Table 3.3.6. Final species (follows taxonomy of Kirk *et al.*(2001)). Fifty-three of the un-named basidiomycete singletons are not included. Isolation frequency refers to the number of cut faces from which a species was isolated.

SPECIES	ISOLATION FREQUENCY
ASCOMYCOTA	
Incertae sedis	
Ascomycete sp.1	9
Helotiaceae	
Ascocoryne sp.1	1
Nectriaceae	
Metarhizum flavoviride Sorokïn.	1
Nectria radicicola Gerlach & L. Nilsson	1
Xylariaceae	
Xylariaceae sp.1	11
Xylaria sp.1	1
BASIDIOMYCOTA	
Incertae sedis	
Basidiomycete sp.1	14
Basidiomycete sp.2	3
Basidiomycete sp.3	2
Basidiomycete sp.4	2
Coprinaceae	
Psathyrella-like sp.1	1
Cortinariaceae	
Gymnopilus allantopus (Berk.) Pegler	1
Fistulinaceae	
Fistulina-like sp.1	4
Strophariaceae	
Hypholoma fasciculare (Huds.) P. Kumm.	6
Hypholoma sp.1	1
Hypholoma sp.2	1
Typhulaceae	
Typhula-like sp.1	1
Coniophoraceae	
Coniophora-like sp.1	7
Coniophora-like sp.1	1

SPECIES	ISOLATION FREQUENCY
Hymenochaetaceae	
Hymenochaetaceae sp.1	5
Hymenochaetaceae sp.2	3
Atheliaceae	
Athelia-like sp.1	4
Athelia-like sp.2	1
Fomitopsidaceae	
Fomitopsis-like sp.1	1
Postia pelliculosa (Berk.) Rajchenb.	30
Postia-like sp.3	4
Postia-like sp.4	5
Postia-like sp.5	4
Postia-like sp.6	2
Postia-like sp.7	2
Meruliaceae	
Phlebia-like sp.1	2
Phanerochaetaceae	
Phanerochaete sordida (P. Karst.) J. Erikss. & Ryvarden	1
Polyporaceae	
Polyporus gayanus Lév.	1
Trametes-like sp.1	1
Trametes versicolor (L.) Lloyd	1
Steccherinaceae	
Steccherinum-like sp.1	1
Peniophoraceae	
Peniophora aurantiaca (Bres.) Bourdot & Galzin	1
Stereaceae	
Stereum-like sp.1	2

3.4 Discussion

This study demonstrates the high fungal species richness of living eucalypts, with 91 different species of fungi isolated from the wood of just 18 *E. obliqua* trees. Of these 91 species, 20 were considered to be common. Using a wood cutting method similar to that used in this study, Hood *et al.* (2004) identified just 6 common species of fungi (along with an unspecified number of rare species) on 16 freshly felled podocarp stems in New Zealand. In Sweden, 25 fungal species were isolated from 10 recently dead Norway spruce logs (Gustafsson 2002) and in Japan, 10 species of fungi were isolated from five Japanese beech logs (Fukasawa *et al.* 2005).

Few studies have examined the species of wood decay fungi present within living eucalypt trees. Tamblyn (1937) found *Polyporus eucalyptorum* (now *Laetiporus portentosus* (Berk.) Rajchenb.) to be commonly present in mature *E. marginata* and Refshuage (1938) described eight fungi in mature *E. regnans.* In this study, a number of taxa of commonly recognised wood decay fungi were found, including closely related relatives of *Coniophora*, *Fistulina, Fomitopsis, Inonotus, Xylaria, Stereum* and *Postia* and the cosmopolitan species *Hypholoma fasciculare* and *Trametes versicolor.* Interestingly, many of the isolates were closely related to fungi with corticioid fruitbodies such as *Coniophora, Phlebia* and *Athelia.* Of the 27 final species with a tentative identification, 12 are closely related to corticioid genera. Corticioid basidiomycetes accounted for more than one-third of the 277 species of fungi observed fruiting in Danish beech forests (Heilmann-Clausen 2003).

Where fungi were able to be identified, this study has found new species records for several fungi. This study is thought to be the first record of *Peniophora aurantiaca* and *Polyporus gayanus* in Australia, both previously known only from the northern hemisphere (I. Hood, pers. comm. 2006). *Gymnopilus allantopus* has been commonly described from soil and rotten logs (Rees 2001) but this is likely to be the first record of this species isolated from within a living tree stem. It is important to remember, however, that these species have been identified primarily based on matches between their ITS sequences and those on GenBank. Without either finding a fruitbody for traditional taxonomic study or matching the sequences of further gene regions, it is not completely certain that they are exactly the same species. *Metarhizium flavoviride* is a hyphomycete commonly found on insects (Lawrence and Milner 1996) so it is unusual that it was isolated directly from wood. It is

probable that this fungus was present in the wood as a result of insect activity within adjacent wood or even grew from an insect from within the wood samples themselves.

One of the surprising outcomes of this study was the lack of matches between the study isolates and those in the reference collection. The reference collection contained a wide variety of material collected both locally and internationally and included many of the species considered as important wood decay fungi. Species from genera such as *Fomes, Hyphodontia, Phellinus* and *Poria* are thought to be common stem decay species in eucalypts (Kile and Johnson 2000; May and Simpson 1997) and, despite forming part of the reference collection, were not isolated from living trees in this study. While this is probably a reflection of the diversity of wood decay fungi in Australia and the lack of detailed studies previously published in this area, there are a number of other reasons. The reference collection contained very few corticioid species; however, sequence matches with public databases indicate that a number of the fungi found in this study were corticioid. Corticioid fungi are often overlooked in fruitbody surveys due to their cryptic habit and their difficult taxonomy (Buchanan 2001). Also, although two of the reference collections used in this study targeted living trees, the fungi from the CSIRO Clayton collection were primarily isolates from fruitbodies found on wood in service or rotten wood, not living trees and thus may reflect an entirely different suite of fungi. The low matching between the reference collection and study isolates also reinforces the findings of Johannesson and Stenlid (1999) that the fungi commonly fruiting on wood do not necessarily accurately portray the fungal taxa within.

In comparing the use of morphological and molecular techniques for grouping and identifying isolates, it is clear that neither technique is superior, each providing distinct advantages and disadvantages. The morphological analysis of fungal isolates proved a very effective method of grouping isolates into putative morphological species for isolates with sufficient distinctive features. For at least five of the morphological species, the isolates had similar sequences within morphological species and so these morphological species became accepted as final species. This was especially the case where isolates had a highly distinctive macromorphology, such as colour or texture of the aerial mycelium as in M02, M04 and M06. Where morphological species groupings did not hold as well, they were usually split further

into one or more closely related species based on their ITS sequences. Morphological group M03, for example, was a white-cream culture with several distinctive features such as multiple clamp connections in the marginal hyphae, chlamydospores and arthroconidia. Despite little apparent morphological difference, two distinct species groups with greater than 5% ITS sequence variation were identified by DNA sequence analysis. The other common disadvantage of the morphological analysis was when it failed to recognise similarities between singletons, when later ITS sequence analysis showed that they were probably the same species. This was especially the case for isolates with less distinctive morphology, such as white or hyaline aerial mycelium, a lack of clamp connections or features like chlamydospores, cystidia and specialised hyphae. In a number of cases, sequence analysis revealed relationships between otherwise morphologically indistinct isolates (e.g. *Postia*-like sp.2, *Hypholoma fasciculare, Phlebia*-like sp.1). Overall though, the morphological study was concluded to be a useful way of grouping fungal isolates into species as more than 65% of isolates were correctly grouped based on morphology alone.

The morphological study was much less useful in determining species identities. Only two morphological species (M04 & M06) were identified to a possible family and the rest remained unidentified. This highlights one of the major pitfalls of morphological identification of fungal cultures in Australia: the lack of taxonomic knowledge of cultures of Australian wood decay fungi (Simpson 1996). As previously discussed, there are few keys which can be used to describe cultures of wood decay fungi in Australia, making identification extremely difficult. In addition, identification of cultures requires highly specialised knowledge and there is a general lack of expert knowledge of cultures of Australian wood decay fungi. Of the dozen professional mycologists working in Australia, very few are expert in wood decay culture identification (Buchanan 1989; May and Pascoe 1996; Simpson 1996). At university undergraduate level, few courses provide more than a limited amount of fungal taxonomy (Simpson 1996).

PCR and DNA sequence analysis proved a very fast, efficient and relatively inexpensive way for confirming the morphological species. In comparison with the morphological study, it was very time-efficient, alleviating the need for detailed morphological identification, a process requiring a minimum of 6-8 weeks to

describe isolates completely at different growth stages (Hoff *et al.* 2004; Simpson 1996). Molecular studies also avoid the need for large amounts of temperature-controlled storage space for living culture herbaria and regular subculturing and maintenance of isolates. The molecular work alone grouped approximately 85% of the fungal isolates correctly, according to the final consensus, making it a very efficient way of determining species groupings. The sequence information was also particularly useful in providing possible species identifications by comparisons with sequences in other databases. While these comparisons may give some idea of the taxonomy of the species involved, they should be treated with caution, as the reliability of identification of species lodged in public databases is not always known (Bridge *et al.* 2003). Similar to problems encountered with the morphological keys, the low number of Australian or even southern hemisphere species on public sequence databases also makes identification difficult. Despite these difficulties, the use of public databases did help to target appropriate species for collection and sequencing in the reference collection.

Using only molecular studies would have been more time consuming and more expensive as all 312 isolates would have had to have been sequenced, rather than a subset of ~170 cultures which represented the morphological diversity of the isolates. The opportunity to examine the morphology of all the isolates provided an opportunity to examine the functional properties of the isolates (such as wood decay enzyme tests) and also helped to confirm the reliability of possible sequence matches from the database searches. For example, M04 showed 91-94% ITS sequence similarity to *Hymenochaete adusta* and *Inonotus hispida*. The morphology of this morphological species confirmed these taxonomic relationships through mycelial characters such as the presence of setae and setal hyphae (Stalpers 1978).

Based solely on the molecular data it would also be difficult to determine the intraspecific sequence variation and thus the differentiation into final species. While most authors suggest sequence variation of up to 2% within species (e.g. Johannesson and Stenlid 1999) *Russula clelandii* is known to have intraspecific variation of up to 15% in the ITS region while some groups of *Cortinarius* species are known to have extremely low interspecific variation combined with intraspecific variation (Glen *et al.* 2001a). Without a detailed knowledge of the level of intraspecific variation in a particular genus or species, it is impossible to determine

the cut-off point between species without the ability to refer to differences in morphology, particularly fruitbody morphology. For example, without being able to re-examine the morphological characteristics of the *Postia*-like isolates, the six species identified in the final species list may only have been three. A better taxonomic understanding of the species relationships in this study may be obtained by comparing the DNA sequences of more than one DNA region (Glen *et al.* 2002; Horton and Bruns 2001; Yao *et al.* 2005). For the purposes of this study however, the use of the ITS region, as the most commonly sequenced region for fungi (Hoff *et al.* 2004), makes a much wider range of sequences available for matching on public databases.

This study demonstrates the benefits of using a combination of morphological and molecular techniques to group and identify cultures of wood decay fungi. Advocation of combined morphological and molecular studies is not new (Bougoure and Cairney 2005; Hagerman *et al.* 1999; Hoff *et al.* 2004); however, it is of particular significance in Australia where it is not possible to draw on the wealth of taxonomic and ecological knowledge of wood decay fungi that is available in some other countries. While both methods can be used individually with some success, together they provide a much more robust analysis of both the species groups present and the taxonomic affiliations of these species groups. As the knowledge and taxonomy of Australia wood decay fungi becomes more developed, it may be possible to use more sophisticated molecular methods to identify wood decay fungi from cultures or even directly from wood (e.g. Adair *et al.* 2002; Johannesson and Stenlid 1999; Oh *et al.* 2003; Vainio and Hantula 2000). Until this time however, a more conservative approach is advocated which takes into account as many of the characteristics of each fungal isolate as is possible.

Appendix 3.1 Descriptions and photographs of all main species groups found in Study 1 (Table 3.3.4). Growth rate indicates colony radius. Species codes come from Stalpers (1978).

Ascomycete sp.1
Mats hyaline-white, cottony, margins appressed, dense, even at 2 weeks, by 6 weeks, cottony or absent. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Hyphae 3-5.5 μm diameter, thin-walled, hyaline, simple septate. Intercalary swellings sometimes present.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 24 hours
Growth rate: 15-25 mm after 14 days.

Closest Blast match: Assorted ascomycete species (reliability: poor)

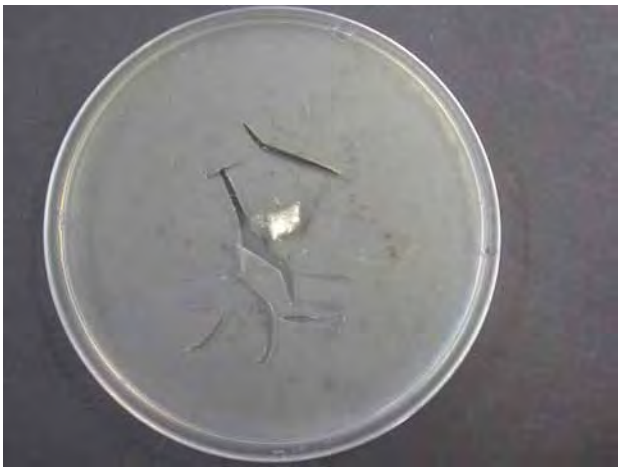


Plate 3.1.1 Ascomycete sp.1 showing mycelium of a six week old culture.

***Athelia*-like sp.1**
Mats hyaline-white, silky sometimes plumose, margins raised-appressed, dense, even at 2 weeks, by 6 weeks, white, silky. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae with frequent clamp connections in marginal hyphae, 2-5 μm diameter, thin-walled, hyaline, branching often inequivalent.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 3 hours
Growth rate: 14-20 mm after 14 days.

Closest Blast match: *Athelia arachnoidea, A. epiphylla* (reliability: moderate)

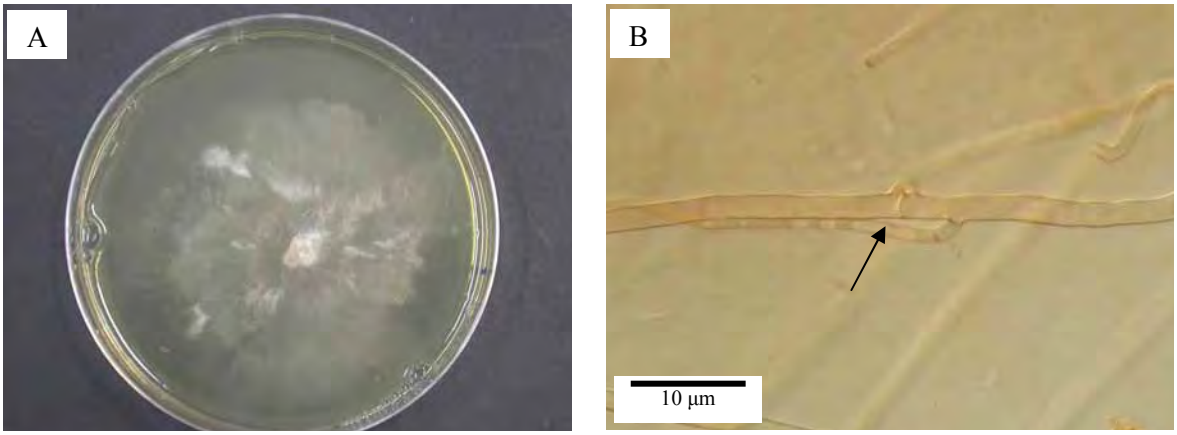


Plate 3.1.2 *Athelia*-like sp.1 showing a) mycelium of six week old culture, and b) clamp connections.

Basidiomycete sp.1

Mats hyaline, silky, margins submerged, dense, even at 2 weeks, by 6 weeks, hyaline-orange or reddish, felty to absent. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae (1.5)-2-5-(6) µm diameter, thin-walled, hyaline, single clamps rare, equivalent branching. Aerial hyphae: generative hyphae similar to that in margin, clamps frequently present, often medallion, hyphae can be encrusted with calcium oxide, rarely containing oil drops, crystals often present. Submerged hyphae: generative hyphae 1-4 µm diameter, similar to that in aerial hyphae. Thick-walled generative hyphae present in submerged zone, 5-8 µm diameter, walls 1-2-(3) µm thick. Gloeocystidia in marginal and aerial hyphae usually terminal, rarely intercalary, 6-10µm diameter, 20-25 µm long rare in aerial and submerged hyphae, clamped at base, thin-walled, darkly staining. Chlamydospores rare in submerged hyphae, thick-walled, 10-18 µm diameter.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 3 hours
Growth rate: 17-40 mm after 14 days.

Species Code: 1,8,9,13,20,25,32,39,(40),45,48,52,53,54,57,(60),73,82,85,89.

Closest Blast match: *Phlebia brevispora* (reliability: poor)

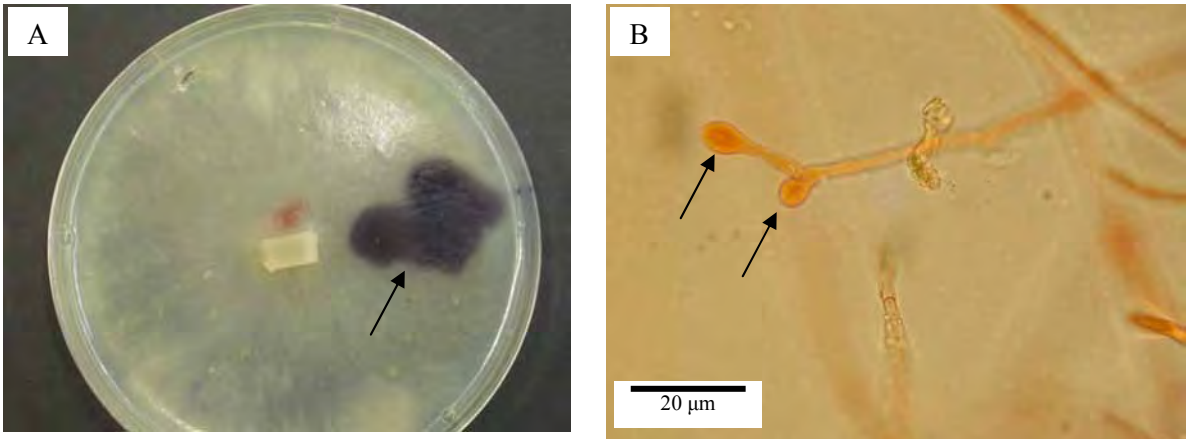


Plate 3.1.3 Basidiomycete sp.1 showing a) mycelium of two week old culture with a positive enzyme reaction to laccase, and b) gloeocystidia.

Basidiomycete sp.2

Mats hyaline-slightly orange, silky-submerged, margins appressed-submerged, dense, even at 2 weeks, by 6 weeks, hyaline-orange to brown, felty to absent. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae 2-5-(6) µm diameter, thin-walled, hyaline, single clamps rare, equivalent branching. Chlamydospores frequently present 16-20 µm diameter, thick-walled. Thick-walled generative hyphae occasionally present 6-8 µm diameter, walls 2.5-3 µm diameter. Gloeocystidia rare, 8 µm diameter, darkly stained. Crystals often present in medium.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 3 hours
Growth rate: 30-40 mm after 14 days.

Closest Blast match: Assorted unspecified basidiomycete species (reliability: poor)

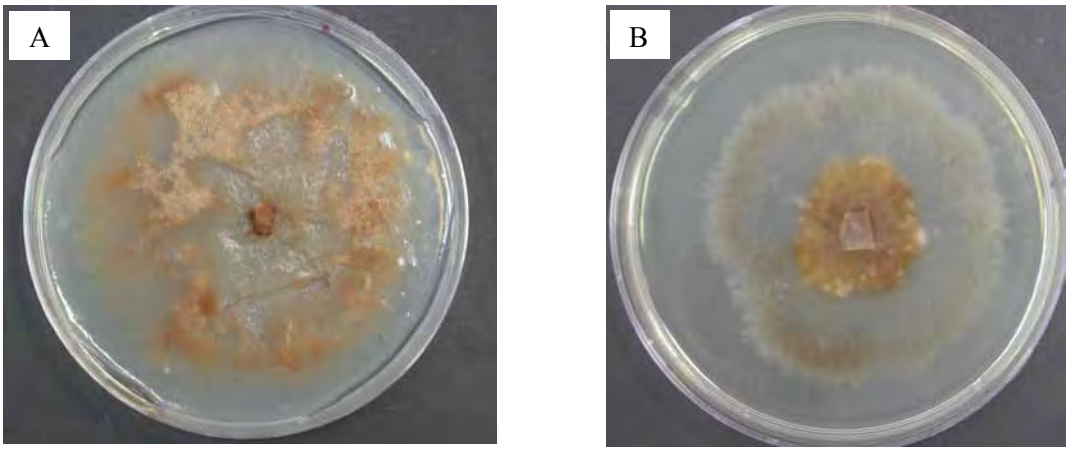


Plate 3.1.4 Basidiomycete sp.2 showing (a,b) variation of aerial mycelium of six week old cultures.

Basidiomycete sp.3
Mats white, woolly, margins appressed, dense, even at 2 weeks, by 6 weeks, white, woolly. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae 2-6 µm diameter, thin-walled, hyaline, single clamps present at every septum, equivalent branching. Aerial hyphae: generative hyphae similar to that in margin, clamps frequently present, crystals often present, hyphae occasionally monilioid. Submerged hyphae: generative hyphae similar to that in aerial hyphae. Chlamydospores in marginal and aerial hyphae, thick-walled. Arthroconidia present in aerial and submerged hyphae.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: negative after 72 hours
Growth rate: 22-28 mm after 14 days.

Species Code: 8,9,13,22,30,39,45,*53*,75,82,84,85,89.

Closest Blast match: *Antrodia, Oligoporus, Fomitopsis rosea* (reliability: poor)

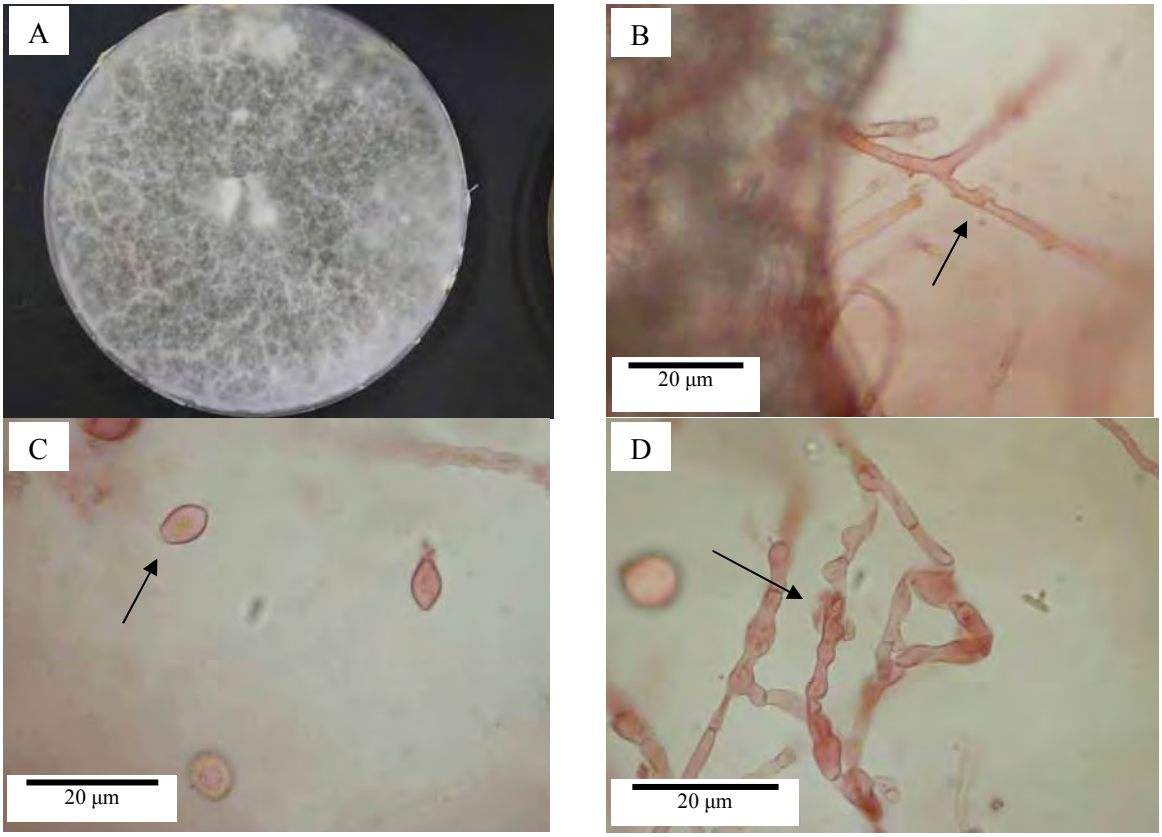


Plate 3.1.5 Basidiomycete sp.3 showing a) aerial mycelium of six week old culture, b) clamp connections, c) chlamydospores, d) monilioid hyphae.

Basidiomycete sp.4
Mats hyaline-white, almost submerged-felty, margins appressed-submerged, dense, even at 2 weeks, by 6 weeks, white, felty. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae 2-6 μm diameter, simple septate, thin-walled, hyaline, equivalent branching. Hyphal strand present.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: negative after 72 hours
Growth rate: 12-15 mm after 14 days.

Closest Blast match: *Stereum sanguinolentum* (reliability: poor)

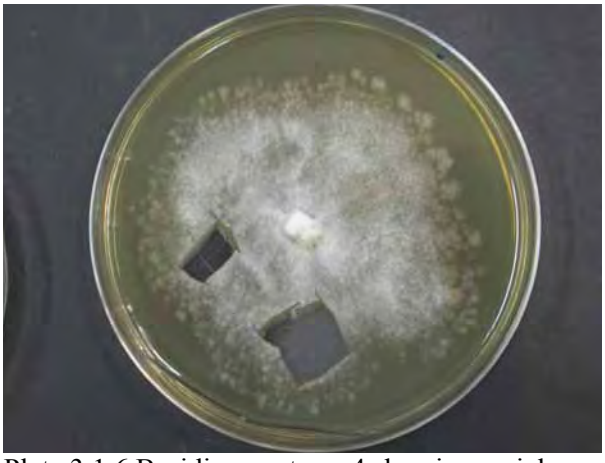


Plate 3.1.6 Basidiomycete sp.4 showing aerial mycelium of six week old culture.

***Coniophora*-like sp.1**
Mats white to cream, woolly, margins raised-appressed, distant, even at 2 weeks, by 6 weeks, cream to pale yellow, silky, margins appressed. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae 2-10 μm diameter, thin-walled, hyaline, multiple clamps frequent, often in whorls of 3-4, branching inequivalent, occasionally branching from clamps, hyphae rarely encrusted with calcium oxide crystals, septa often ampullate. Aerial hyphae: generative hyphae usually simple septate, sometimes with multiple clamps/pseudoclamps on wider hyphae, 2-10 μm diameter, hyaline, branching inequivalent, septa can be ampullate/constricted, hyphal bundles present, crystals present. Early binding hyphae rare. Submerged hyphae: generative hyphae similar to aerial hyphae, can be highly branched. Intercalary swellings (possibly early chlamydospores) often present in marginal hyphae, 10-15 μm long, thin-walled. Arthroconidia often present in marginal, rarely aerial or submerged hyphae, 2-4 μm long, square or rectangular, becoming rounded at edges.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: negative after 72 hours
Growth rate: 20-45 mm after 14 days.

Species Code:
(7),8,(9),(12),13,14,20,(22),31,39,41,(42),45,(47),50,52,53,54,55,(57),65,78,80,82,84,(85),89.

Closest Blast match: *Coniophora marmorata* (reliability: moderate)

Plate 3.1.7 *Coniophora*-like sp.1 showing mycelium of two week old culture.

***Fistulina*-like sp.1**

Mats white, woolly, margins appressed, dense, even at 2 weeks, by 6 weeks, pale yellow-yellow, felty. No odour. Reverse darkens slightly with age. Not fruiting by 6 weeks but can produce small brown mushroom primordia.

Microscopic characters: Marginal hyphae: generative hyphae 2-6 μm diameter, thin-walled, hyaline, can be oil filled, single clamps at every septum, equivalent branching, occasionally branching from clamps. Aerial hyphae: generative hyphae similar to that in margin. Submerged hyphae: generative hyphae 1-4 μm diameter, thin-walled, hyaline, single clamps at every septum, equivalent branching, often multi-branched. Some thick-walled generative hyphae also present, 4-8 μm diameter, clamps at every septum, hyaline. Allocysts/terminal swellings common in marginal and aerial hyphae, less common in submerged hyphae, 9-12 μm diameter, clamped at base, thin-walled, oil filled.

Enzyme reactions: Tyrosinase: positive after 3 hours
Laccase: negative after 72 hours
Growth rate: 10-12 mm after 14 days.

Species Code: 2,9,13,22,30,39,(42),44,45,(50),52,53,80,89.

Closest Blast match: *Fistulina hepatica* (reliability: moderate)

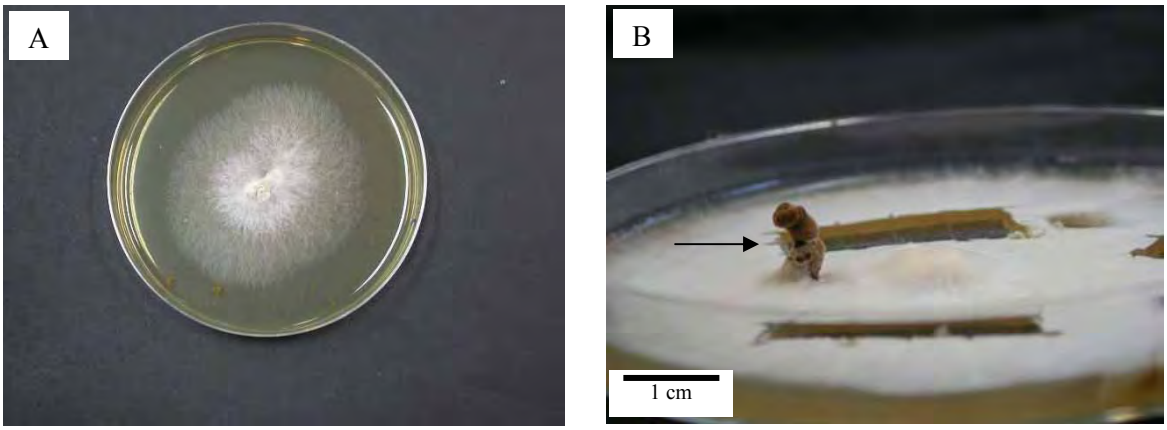


Plate 3.1.8 *Fistulina*-like sp.1 showing a) mycelium of two week old culture, b) mushroom primordia on six week old culture.

Hymenochaetaceae sp.1
Mats white, felty-cottony, margins appressed (-submerged), distant, even to fringed at 2 weeks, by 6 weeks, brown to dark brown, silky to absent. No odour. Reverse darkened, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae simple septate, 1-5 µm diameter, thin-walled, hyaline, branching equivalent, hyphae often pigmented light brown, septa can be ampullate or constricted. Early binding hyphae rare, 1 µm diameter, thickened walls. Aerial hyphae: generative hyphae similar to marginal hyphae. Submerged hyphae: generative hyphae similar to marginal hyphae. Setal hyphae and setae present in marginal and aerial hyphae, rare at 2 weeks, frequent at 6 weeks. Setae walls thickened, pigmented brown, 38-50 µm long, 4-7 µm diameter at base. Thick-walled chlamydospores present in aerial and submerged hyphae. Hyphae with many short branches and hyphal bundles can be present in aerial hyphae.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 3 hours
Growth rate: 6-9 mm after 14 days.

Species Code: 1,10,13,15,23,30,34,38,52,53,69,70,72 or 73,75 or 80,85,89.
NB. Using Stalpers (1978), this species keyed out to *Inonotus* sp. This is inconclusive, however, as it did not match the species code of any isolates described.

Closest Blast match: *Hymenochaete adusta, Inonotus hispida* (reliability: moderate)

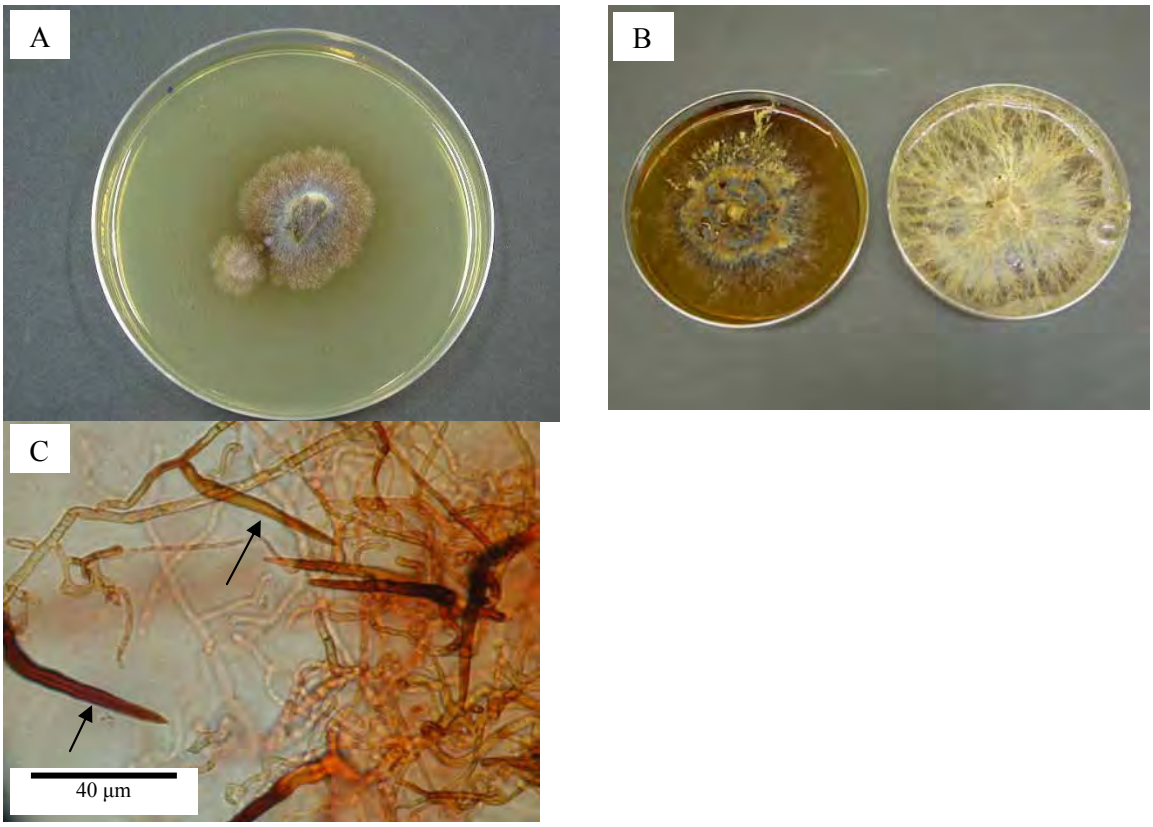


Plate 3.1.9 Hymenochaetaceae sp.1 showing a) mycelium of two week old culture, b) 6-8 week old cultures, and c) setae and simple septate hyphae.

Hymenochaetaceae sp.2
Mats white-yellow, woolly, margins appressed, dense, even at 2 weeks, by 6 weeks, yellow-brown, felty. No odour. Reverse becoming greenish-yellow, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae 2-5 µm diameter, thin-walled, hyaline, simple septate hyphae, inequivalent branching rare. Aerial hyphae: generative hyphae similar to that in margin, simple septate, hyphae often appears very curly/ highly branched and can be pigmented slightly yellow-brown. Submerged hyphae: generative hyphae similar to that in aerial hyphae, also some highly branched, thin-walled hyphae 1-2 µm diameter present. Thick-walled terminal and intercalary chlamydospores frequent in aerial and submerged hyphae 5-8 µm diameter. Conidia rarely present in submerged hyphae. Possible setal hyphae rarely present.

Enzyme reactions: Tyrosinase: negative
Laccase: positive
Growth rate: 8-10 mm after 14 days.

Species Code: 1,(2),10,13,22,30,35,38,(47),50,52,53,67,85,(86),89.

Closest Blast match: *Inonotus hispida* (reliability: moderate)

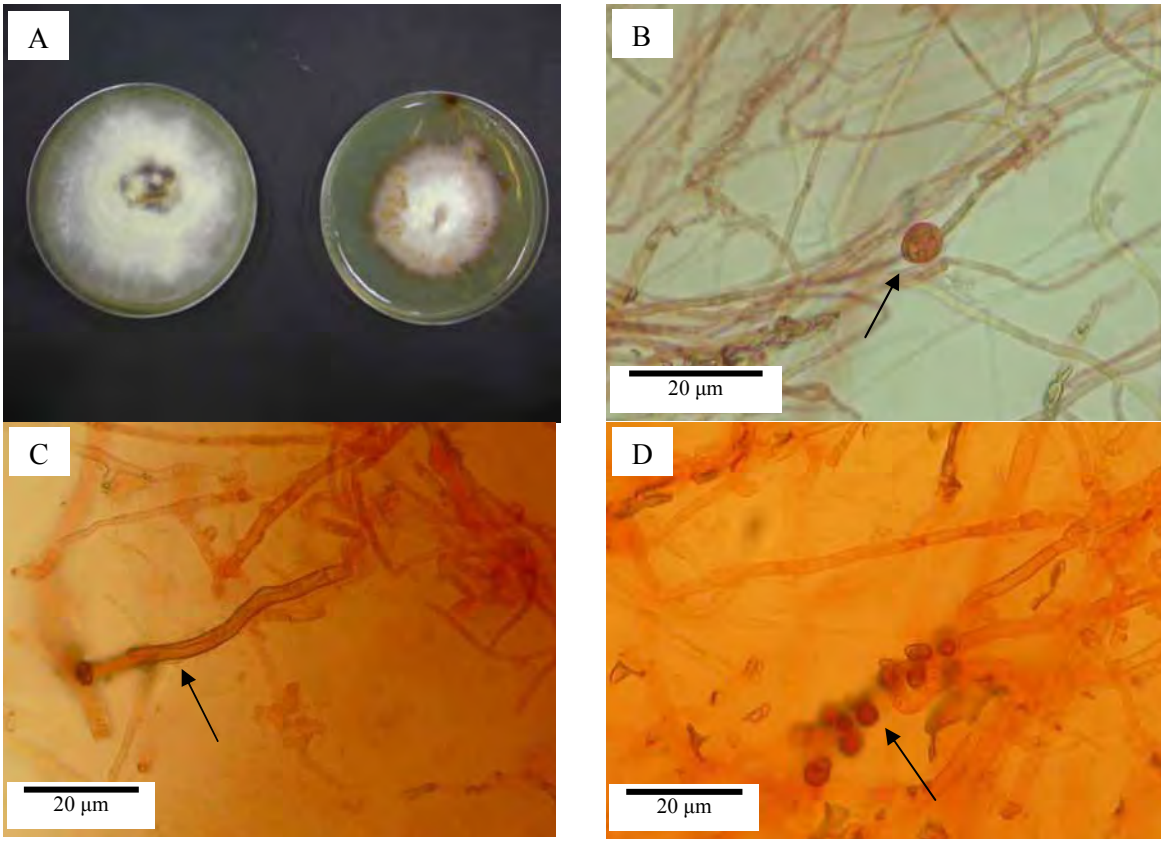


Plate 3.1.10 Hymenochaetaceae sp.2 showing a) variation in aerial mycelium of six week old cultures, b) chlamydospores present throughout mycelium, c) possible setal hyphae, d) conidia.

Hypholoma fasciculare
Mats white, woolly, at times almost farinaceous, margins appressed, very dense, even at 2 weeks, by 6 weeks, white, woolly. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae with clamp connections usually at every septum, hyaline, 1-4 µm diameter, thin-walled, inequivalent branching, can be crystal encrusted. Arthroconidia common in marginal hyphae, 2-3 µm diameter, 3-10 µm long.

Enzyme reactions: Tyrosinase: variable after 72 hours
Laccase: positive after 24 hours
Growth rate: 8-15 mm after 14 days.

Closest Blast match: *Hypholoma fasciculare* (reliability: good)

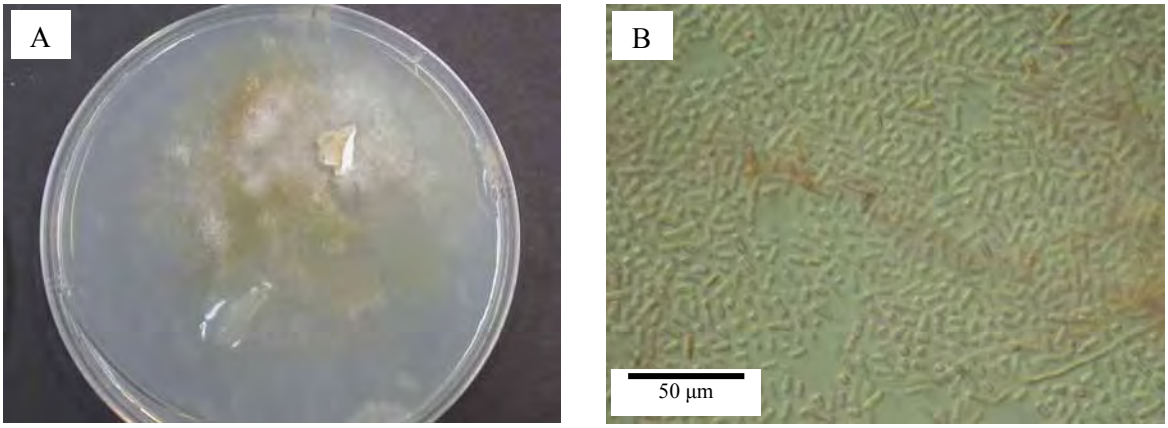


Plate 3.1.11 *Hypholoma fasciculare* showing a) aerial mycelium of six week old culture, b) arthroconidia present throughout mycelium.

***Phlebia*-like sp.1**
Mats hyaline, aerial hyphae absent or rare, margins submerged, dense, even at 2 weeks, by 6 weeks, hyaline, silky-absent. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae 1-2.5 μm and 5-7 μm diameter, thin-walled, hyaline, simple septate, equivalent branching. Thick-walled chlamydospores present in submerged hyphae, crystals in medium.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 24 hours.
Growth rate: 15-30 mm after 14 days.

Closest Blast match: *Phlebia radiata* (reliability: moderate)

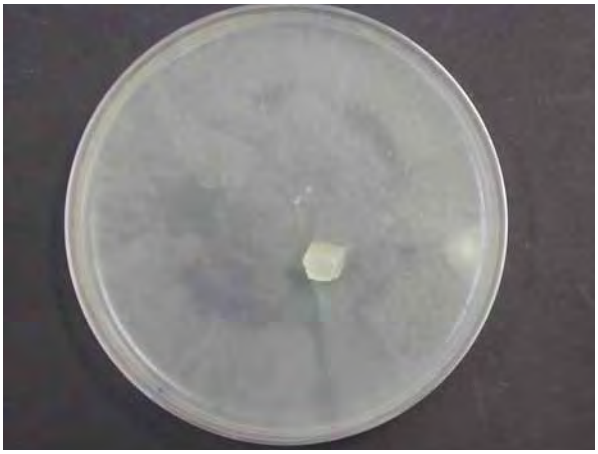


Plate 3.1.12 *Phlebia*-like sp.1 showing aerial mycelium of a six week old culture.

***Postia pelliculosa*:**
Mats white to cream, silky-cottony, then becoming raised-appressed, dense, even at margins at 2 weeks, by 6 weeks, cream and brown, silky to felty, margins appressed. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae 2-9-(10) µm diameter, thin-walled, hyaline, single clamps at every septum, equivalent branching, occasionally branching from clamps. Aerial hyphae: generative hyphae similar to that in margin rarely pigmented light brown. Submerged hyphae: generative hyphae 2-7 µm diameter, thin-walled, hyaline, single clamps at every septum, equivalent branching, often multi-branched. Allocysts/terminal swellings frequently present in aerial and submerged hyphae, 20-25 µm long, 6-8 µm diameter at head, clamped at base, thin-walled, oil filled.

Enzyme reactions: Tyrosinase: positive after 3 hours
Laccase: positive after 24 hours
Growth rate: 8-15 mm after 14 days.

Species Code: 1,2,10,13,16,20,30,38,39,45,53,54,75,89.

Closest Blast match: *Postia subcaesia* (reliability: moderate)
NB. High sequence match with *Postia pelliculosa* fruitbody from reference collection.

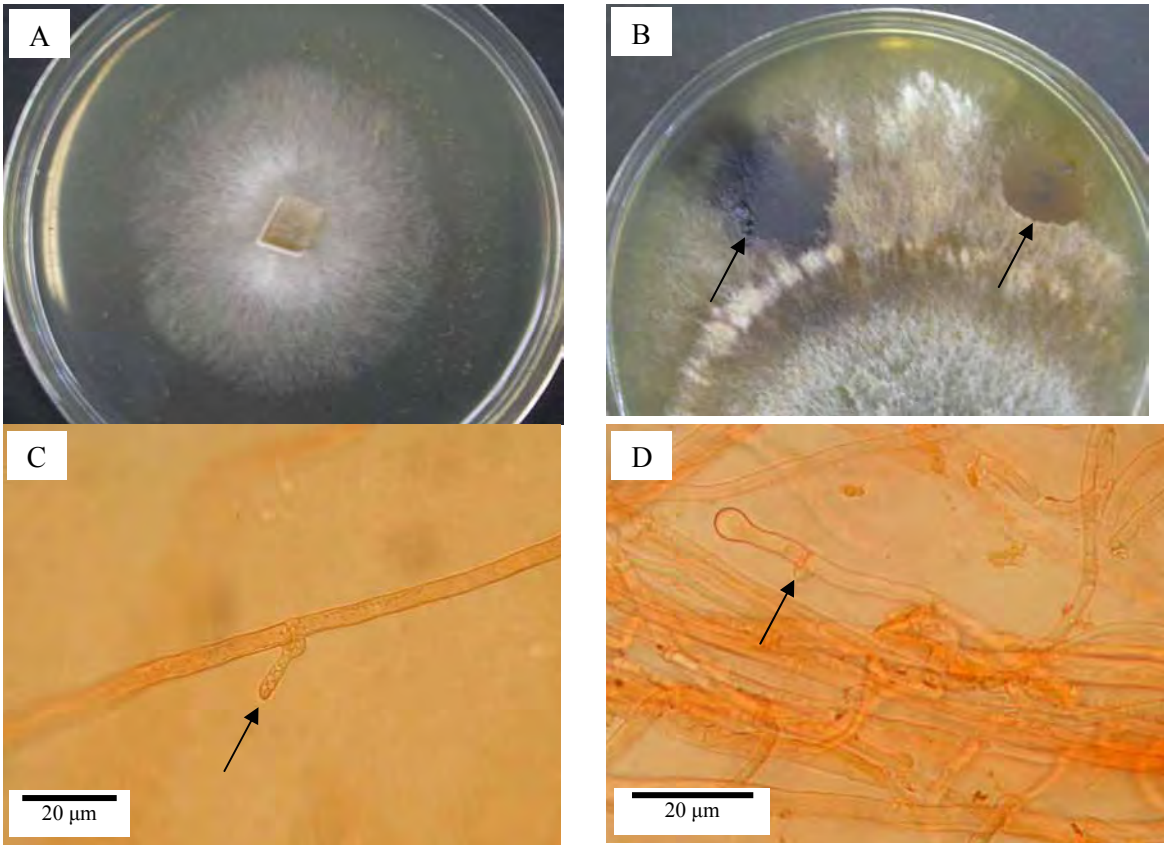


Plate 3.1.13 *Postia*-like sp.1 showing a) mycelium of three week old culture, b) positive enzyme reactions to laccase and tyrosinase, c) sprouting clamp connection, and d) terminal swelling (arrowed) with basal clamp connection.

***Postia*-like sp.3**
Mats white, silky, margins appressed, dense, even at 2 weeks, by 6 weeks, white-pale yellow, silky with yellow circles. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Marginal hyphae: generative hyphae 2-6 μm diameter, thin-walled, hyaline, equivalent branching, single clamps at every septum, clamps can be medallion and can sprout. Aerial hyphae: generative hyphae similar to that in margin, clamps always present, often medallion. Submerged hyphae: similar to that in aerial hyphae. Some thick-walled generative hyphae present in marginal zone, 6-8 μm diameter, walls 1-1.5 μm thick. Intercalary chlamydospores common in aerial and submerged hyphae, thick-walled, ovoid, 10-12 μm diameter, 12-14 μm long. Early binding hyphae rarely present in submerged zone 1-2 μm diameter,

Enzyme reactions: Tyrosinase: can show weak positive but usually negative
Laccase: weak positive after 24 hours
Growth rate: 9-15 mm after 14 days.

Species Code: (1),(2),10,13,20,30,(35),39,42,45,(47),48,52,53,54,83,85,89.

Closest Blast match: *Oligoporus rennyi, Postia* spp. (reliability: moderate)

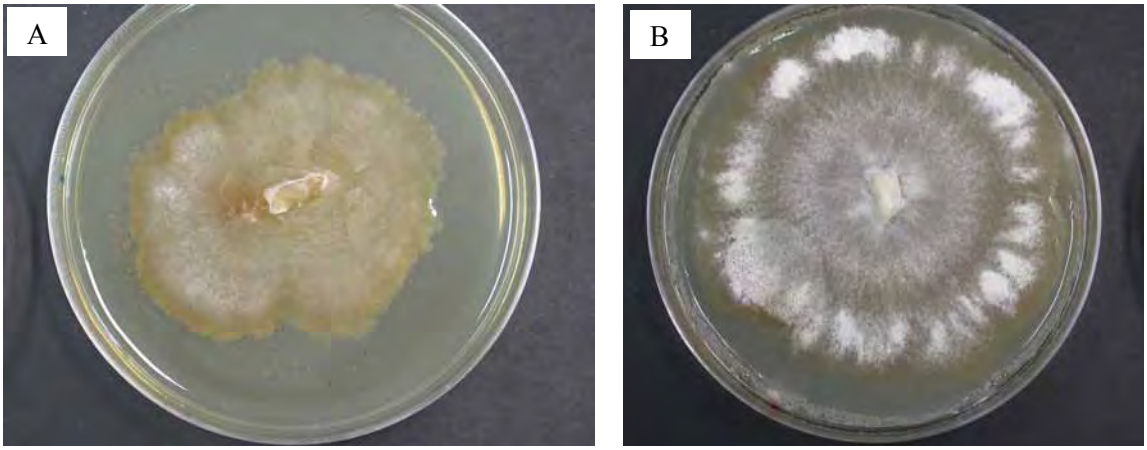


Plate 3.1.14 *Postia*-like sp.3 showing (a,b) variation of aerial mycelium of six week old cultures.

***Postia*-like sp.4**
Mats white, woolly-cottony, margins appressed, dense, even at 2 weeks, by 6 weeks, white, woolly. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae with clamps at every septum, hyphal strands rare, irregular-shaped, terminal swellings or allocysts common, crystals present in aerial hyphae.

Enzyme reactions: Tyrosinase: negative after 72 hours, rarely positive.
Laccase: positive after 3 hours
Growth rate: 16-20 mm after 14 days.

Closest Blast match: *Postia balsamea, Postia subcaesia* (reliability: moderate)

Plate 3.1.15 *Postia*-like sp.4 showing aerial mycelium of a six week old culture.

***Postia*-like sp.5**
Mats white, woolly-floccose, margins appressed, dense, even at 2 weeks, by 6 weeks, white, woolly. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae simple septate, hyaline, thin-walled, branching equivalent, 1.5-8 μm diameter. Hyphae can be ampullate/constricted at septa. Hyphal strands present in aerial hyphae. Irregular-shaped, terminal and intercalary swellings or allocysts common,7-10 μm long, 5-7 μm diameter, could be leptocystidia. Crystals present in aerial hyphae and medium.

Enzyme reactions: Tyrosinase: variable after 72 hours
Laccase: variable after 72 hours
Growth rate: 28-36 mm after 14 days.

Closest Blast match: *Oligoporus rennyi, Postia* spp. (reliability: moderate)

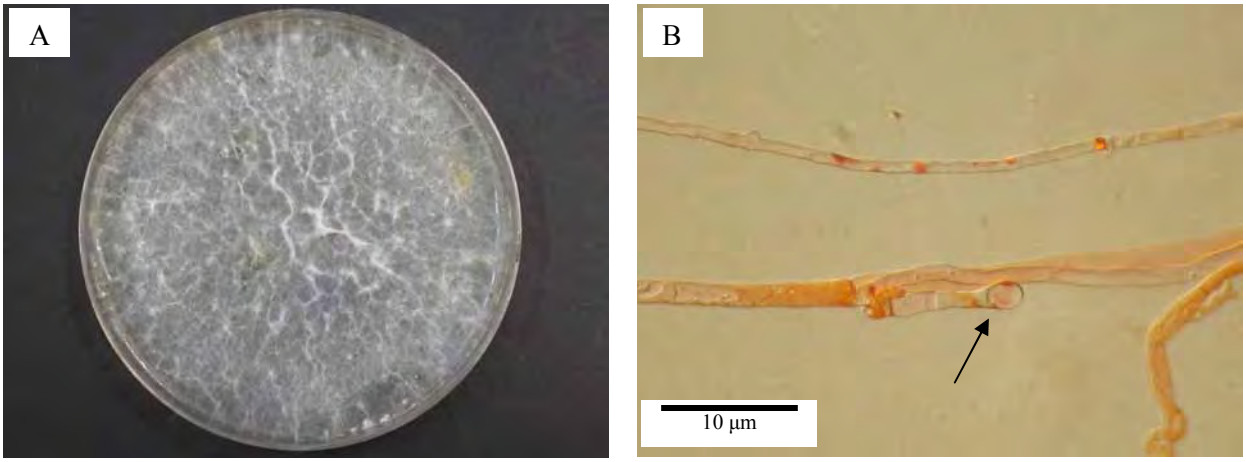


Plate 3.1.16 *Postia*-like sp.5 showing (a) aerial mycelium of six week old culture and (b) terminal swelling.

***Postia*-like sp.6**

Mats white, woolly-cottony, margins appressed, dense, even at 2 weeks, by 6 weeks, white-brown, felty, sometimes woolly or plumose. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae 3-6 µm diameter, thin-walled, hyaline, small single clamps present at every septum, equivalent branching. Terminal and intercalary allocysts present in submerged and aerial hyphae. Hyphal tips often encrusted.

Enzyme reactions: Tyrosinase: positive after 24 hours
Laccase: negative after 72 hours
Growth rate: 10-22 mm after 14 days.

Closest Blast match: *Postia subcaesia* (reliability: moderate)

Plate 3.1.17 *Postia*-like sp.6 showing aerial mycelium of a six week old culture.

***Postia*-like sp.7**
Mats white, cottony, margins appressed, dense, even at 2 weeks, by 6 weeks, white, yellow in patches, cottony. No odour. Reverse develops yellow flecks with time, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae thin-walled, hyaline, single clamps at every septum, equivalent branching. Thick-walled chlamydospores and large intercalary swellings present. Hyphal tips often encrusted, crystals present in medium.

Enzyme reactions: Tyrosinase: weak positive after 24 hours
Laccase: positive after 24 hours
Growth rate: 35-40 mm after 14 days.

Closest Blast match: *Postia balsamea, Postia caesia* (reliability: moderate)

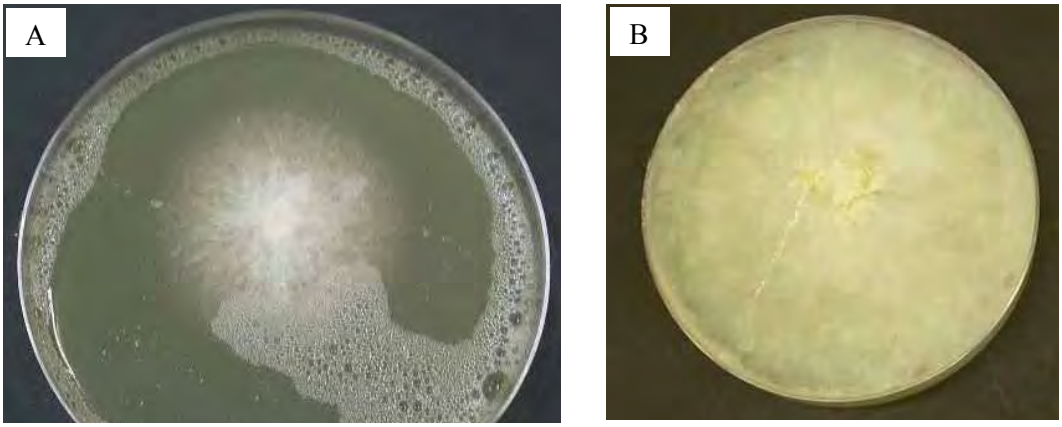


Plate 3.1.18 *Postia*-like sp.7 showing a) mycelium of two week old culture, b) mycelium of a six week old culture showing deepening colour change to yellow.

***Stereum*-like sp.1**
Mats hyaline, sparse-absent, margins submerged, dense, even at 2 weeks, by 6 weeks, hyaline-white, cottony to absent. No odour. Reverse remains unchanged, not fruiting by 6 weeks.

Microscopic characters: Generative hyphae simple septate, thin-walled, hyaline with inequivalent branching, 2-6 μm diameter. Laticiferous branched hyphae with slightly thickened walls present in marginal zone. Hyphal strands and knots can be present.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: negative after 72 hours
Growth rate:8-11 mm after 14 days.

Closest Blast match: *Stereum annosum, Stereum hirsutum* (reliability: moderate)

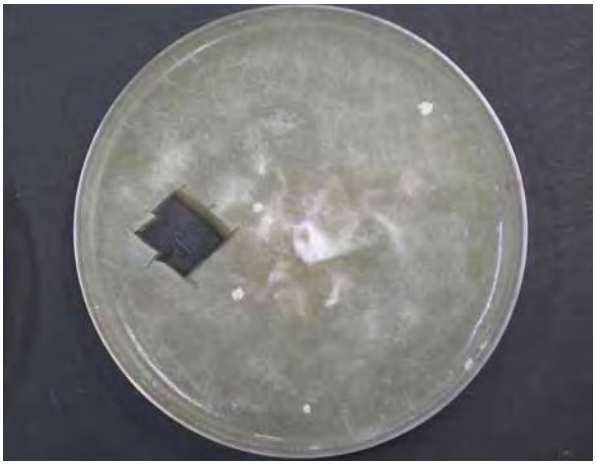


Plate 3.1.19 *Stereum*-like sp.1 showing aerial mycelium of a six week old culture.

Xylariaceae sp.1
Mats hyaline-white, silky, sometimes slightly woolly, margins appressed, dense, bayed at 2 weeks, by 6 weeks, white often with black patches. No odour. Reverse remains unchanged, often produce small black fruitbodies after 6 weeks.

Microscopic characters: Generative hyphae thin-walled, hyaline, simple septate. Septa can sometimes be ampullate. Thick-walled intercalary swellings often present in aerial hyphae, hyphal knots or coils rare.

Enzyme reactions: Tyrosinase: negative after 72 hours
Laccase: positive after 24 hours
Growth rate: 26-30 mm after 14 days.

Closest Blast match: *Xylaria* sp. (reliability: moderate)

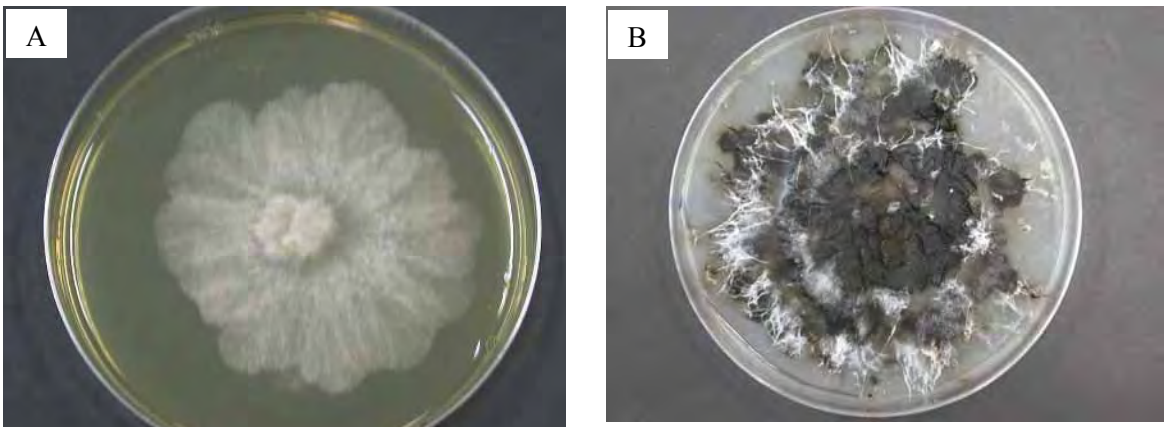


Plate 3.1.20 Xylariaceae sp.1 showing a) mycelium of two week old culture and b) mycelium of a three month old culture.

Appendix 3.2. ML dendrograms used to determine the final species grouping within similar molecular species groups.

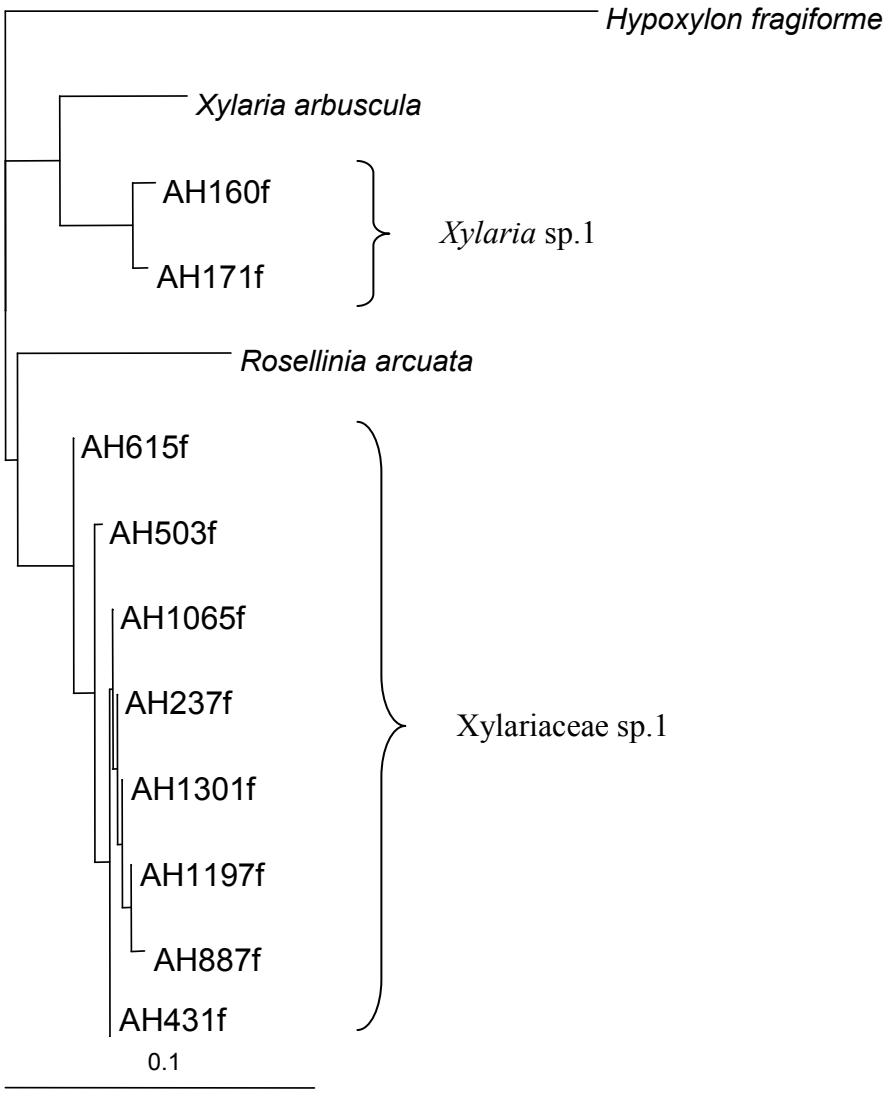


Figure A3.2.1 Maximum likelihood tree from analysis of ITS sequences of all Xylariaceae-like isolates. The outgroup *Hypoxylon fragiforme*, as well as *Xylaria arbuscula* and *Rosellinia arcuata* are sequences downloaded from GenBank (Accession Nos. AY616690, AY183369 and AB017660 respectively). AH160 is the ITS sequence from an isolate of *Xylaria* sp. from the reference collection. The final species designations are shown on the right-hand side of the dendrogram. The bar represents an expected sequence variation of 10%.

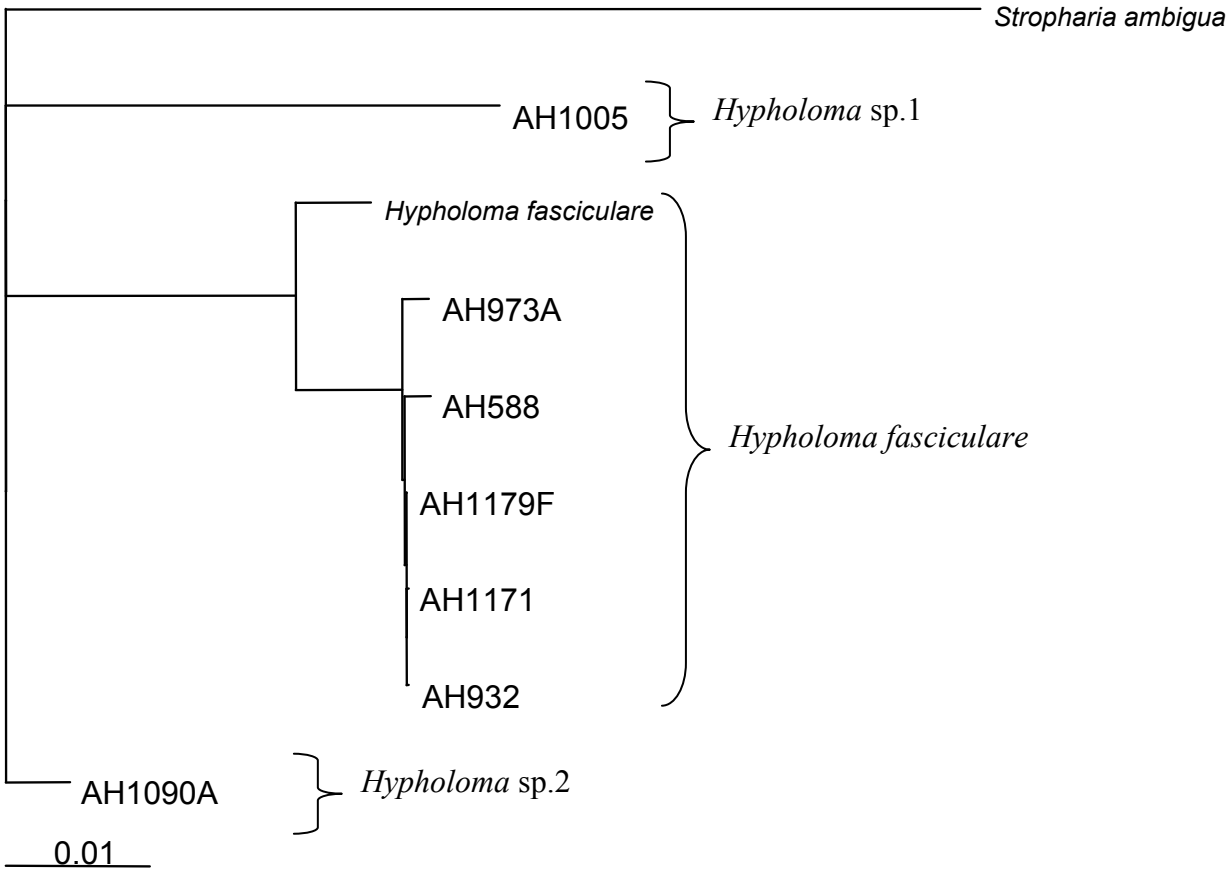


Figure A3.2.2 Maximum likelihood tree from analysis of ITS sequences of all *Hypholoma*-like isolates. The outgroup *Stropharia ambigua,* and sequence *Hypholoma fasciculare* are sequences downloaded from GenBank (Accession Nos. AY818350 and AY354216 respectively). The final species designations are shown on the right-hand side of the dendrogram. The bar represents an expected sequence variation of 1%.

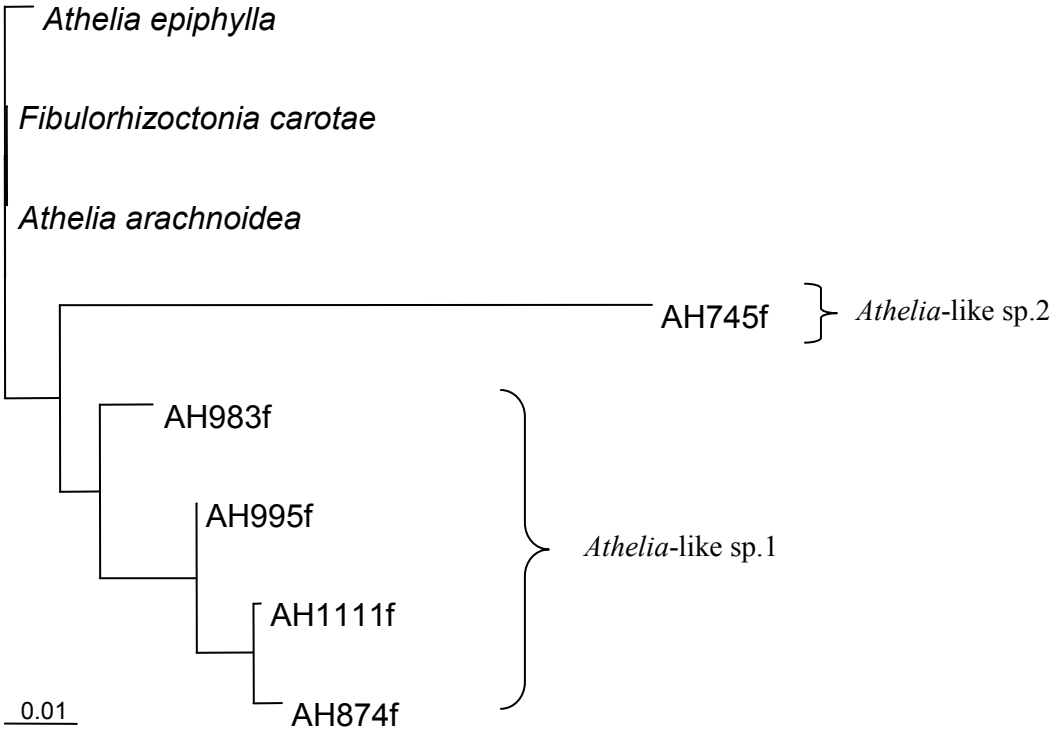


Figure A3.2.3 Maximum likelihood tree from analysis of ITS sequences of all *Athelia*-like isolates. The outgroups *Athelia epiphylla*, *Fibulorhizoctonia carotae* and *Athelia arachnoidea* are all sequences downloaded from GenBank (Accession Nos. U85793, U85791 and U85789 respectively). The final species designations are shown on the right-hand side of the dendrogram. The bar represents an expected sequence variation of 1%.

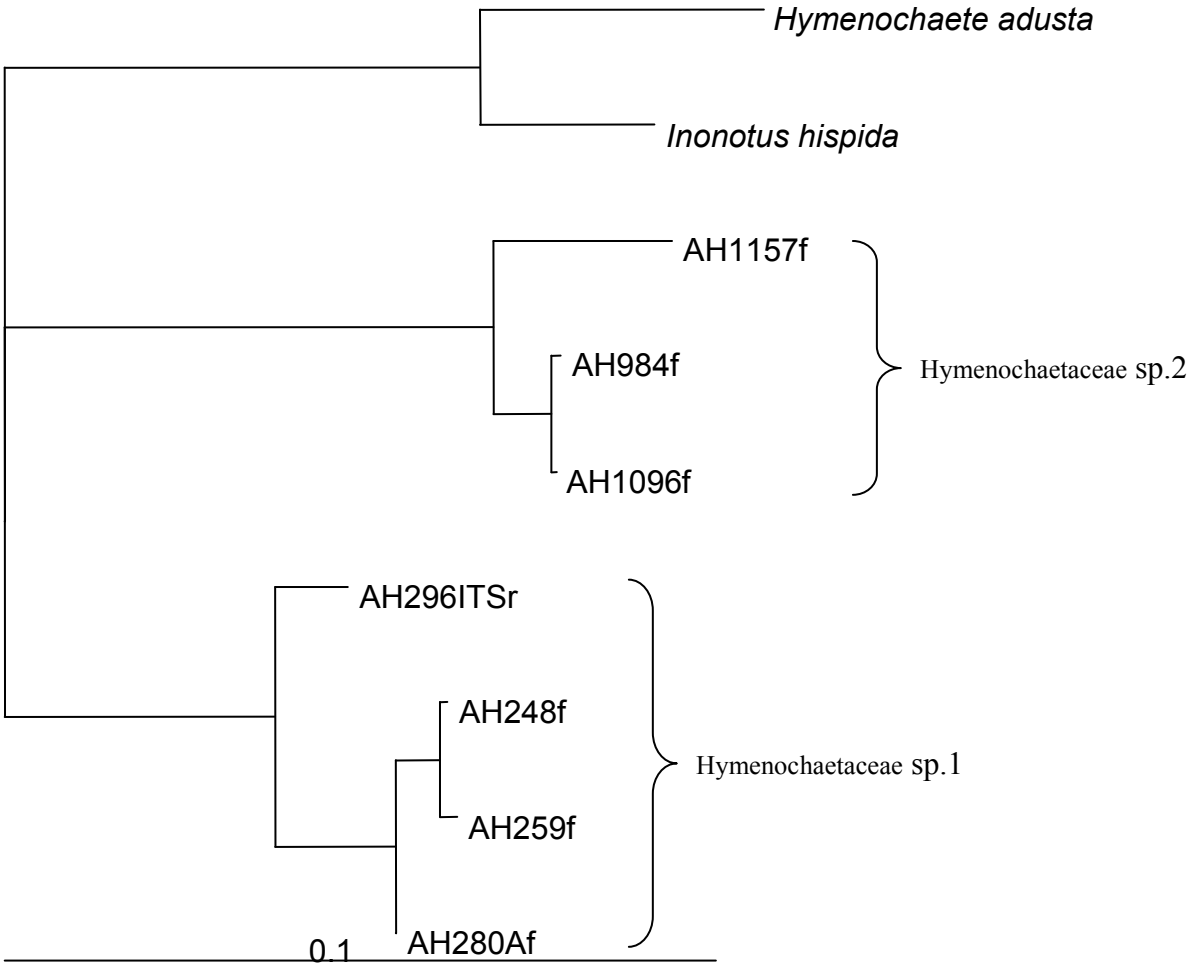


Figure A3.2.4 Maximum likelihood tree from analysis of ITS sequences of all Hymenochaetaceae isolates. The outgroups *Hymenochaete adusta* and *Inonotus hispida* are sequences downloaded from GenBank (Accession Nos. AY558594 and AY251309 respectively). The final species designations are shown on the right-hand side of the dendrogram. The bar represents an expected sequence variation of 10%.

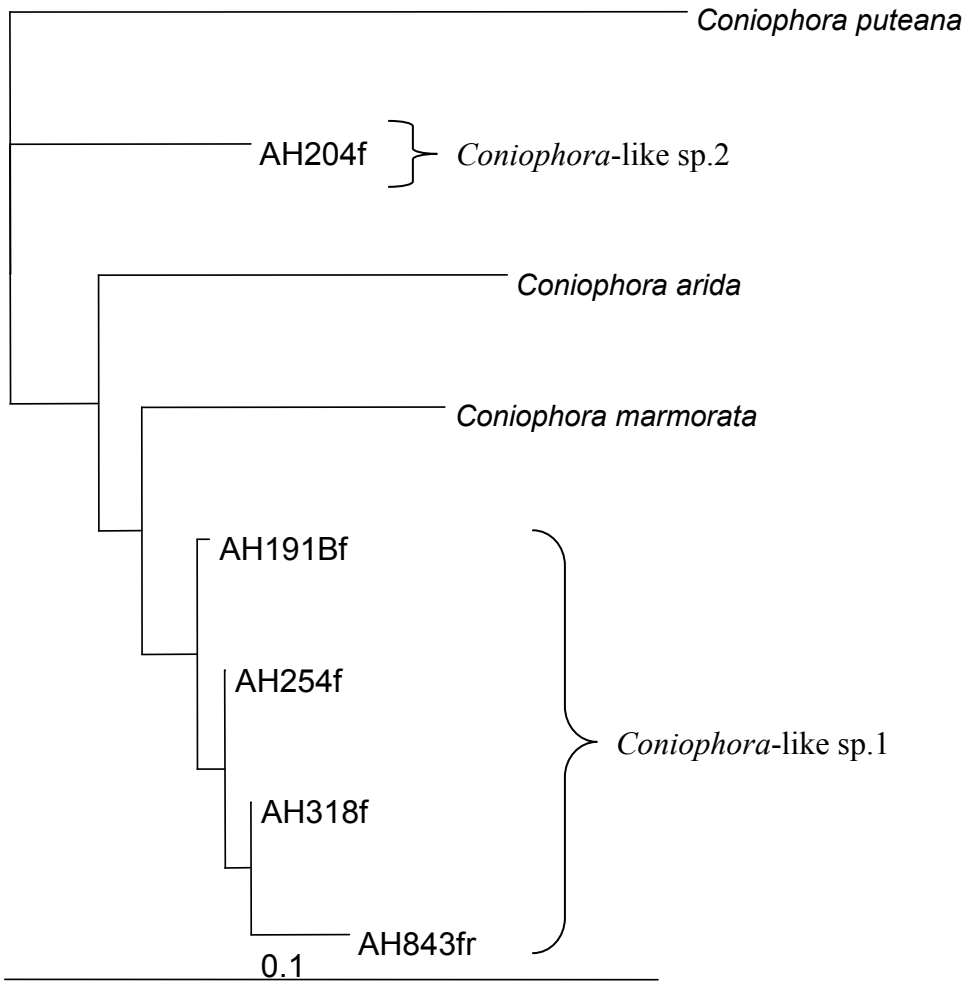


Figure A3.2.5 Maximum likelihood tree from analysis of ITS sequences of all *Coniophora*-like isolates. The outgroups *Coniophora puteana, C. arida* and *C. marmorata* are sequences downloaded from GenBank (Accession Nos. AJ419199, AJ345007 and AJ518880 respectively). The final species designations are shown on the right-hand side of the dendrogram. The bar represents an expected sequence variation of 10%.

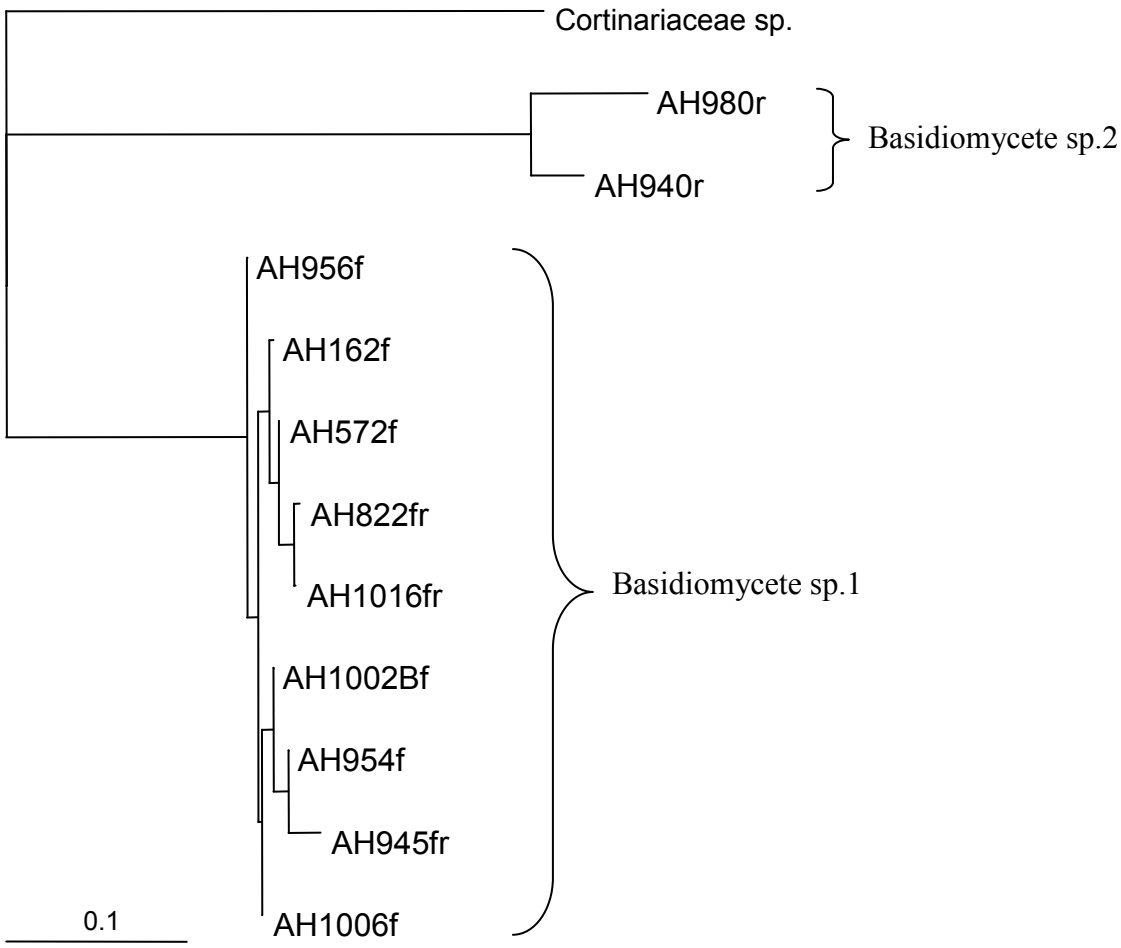


Figure A3.2.6 Maximum likelihood tree from analysis of ITS sequences of all morphospecies M05 isolates. The outgroup, Cortinariaceae sp. (*Hebeloma*), is a sequence downloaded from GenBank (Accession No. AJ549967). The final species designations are shown on the right-hand side of the dendrogram. The bar represents an expected sequence variation of 10%.

CHAPTER 4: EXAMINING THE EFFECT OF TREE AGE ON THE ASSEMBLAGE COMPOSITION OF WOOD DECAY FUNGI IN LIVING *EUCALYPTUS OBLIQUA* TREES

4.1 Introduction

Large, old, living trees, otherwise known as veteran or mature trees, are important structural and function components of the forest landscape (Franklin *et al.* 2002; Lindenmayer and Franklin 1997; Mazurek and Zielinski 2004), accounting for a large proportion of the forest biodiversity. As trees age, they develop a multitude of features including dead tops, hollows, decayed wood, crevices, sloughed bark and large diameter branches, each with important functional roles and habitat values (Franklin *et al.* 2002; Grove 2002; Groven *et al.* 2002; Lindenmayer and Franklin 1997). Many studies in the northern hemisphere have found mature trees to be important for a wide range of organisms including arboreal, hollow dwelling mammals and birds through to more cryptic fungi, lichens, bryophytes and invertebrates (e.g. Andersen and Ryvarden 2001; Berg *et al.* 1994; Hanula *et al.* 2000; Heilmann-Clausen 2003; Nilsson *et al.* 2002; Nordén and Paltto 2001; Penttilä *et al.* 2004; Ranius and Jansson 2000; Virkkala *et al.* 1994; Zack *et al.* 2002). The important biological values of mature trees are clearly recognised in the United Kingdom and northern Europe where they are well studied and important considerations in biodiversity conservation (Andersson and Östlund 2004; Kaila *et al.* 1997; Nilsson *et al.* 2002; Reid 1996). In addition, mature trees give rise to other components of coarse woody debris, such as large stags, large diameter logs and large dead branches on the forest floor, which are also known to be important for housing biodiversity (Edman and Jonsson 2001; Grove 2002; Heilmann-Clausen and Christensen 2004; Lindenmayer and Franklin 1997 ; McClelland and Frissell 1975; Samuelsson *et al.* 1994; Schiegg 2001).

Australian literature highlighting the importance of mature trees as habitat for arboreal mammals and birds is plentiful (e.g. Abbott 1998; Gibbons and Lindenmayer 2002; Gibbons *et al.* 2002; Lindenmayer *et al.* 1993; Mackowski 1987; Whitford and Williams 2001). The importance of mature trees for more cryptic organisms such as bryophytes (Jarman and Kantvilas 2001), saproxylic beetles (Grove 2002; Grove and Bashford 2003) and other arthropods (Bar-Ness

2005) has also been demonstrated in a limited number of systems. Despite this apparent focus on hollow dwelling fauna, little attention has been focussed on the importance of mature trees as habitat for wood decay fungi and the role these fungi may play as habitat creators. These fungi are of particular interest not simply in their own right as extremely diverse organisms (Hawksworth 1991; Heilmann-Clausen 2003), but because they may be crucial to the creation of decayed wood habitat for other organisms and for nutrient and carbon cycling (Edmonds and Marra 1999; Lewis and Lindgren 1999; Lindhahl 2001; Mackowski 1987; Rayner and Boddy 1988; Simpson and Eldridge 1986; Swift 1977).

Few studies have examined the ecology of fungal colonisation and decay of eucalypts in Australia. Those that have done so have generally been in the context of reducing decay to improve commercial forest management in native forests (e.g. Greaves *et al.* 1967; Marks *et al.* 1986; Parkin 1942; Refshuage 1938; Tamblyn 1937; Wardlaw 2003). Mackowski (1987) examined the ontogeny of hollows in *Eucalyptus pilularis* in dry sclerophyll forests and found that fungi, along with termites, play a role in hollow formation, although his investigations were not extensive. Other studies have found fungi to be associated with decayed wood defects in plantation eucalypts (Barry *et al.* 2002; Wardlaw *et al.* 2004; Wardlaw 1996; Wardlaw and Neilsen 1999; White and Kile 1991). Despite these early studies, we still do not have a good understanding of how assemblages of wood decay fungi develop with tree age (Wardlaw 2003), particularly from the perspective of habitat creation.

Wood decay fungi observed fruiting on living eucalypts include species of *Armillaria, Fistulina, Gymnopilus, Hymenochaete, Inonotus, Phellinus,* and *Piptoporus* (Kile and Johnson 2000; May and Simpson 1997). A few common pathogenic species have been quite extensively studied (e.g. *Armillaria luteobubalina* (Kile 1981; Shearer and Tippett 1988)) however for the vast majority of wood decay fungi on eucalypts little is known about their ecology. This may be partially due to the historically low numbers of ecological and taxonomic mycologists working in Australia (see Chapter 3.1 and May and Pascoe 1996).

This chapter explores the relationship between wood decay fungi and living *E. obliqua* trees in three different tree ages classes, including mature (greater than 150 year old) trees. Specifically, the chapter aims to address the following questions:

- What fungi are found in living *E. obliqua* trees of different tree age classes?
- Is there a change in the number of wood decay fungi with tree age?
- Does the community structure of fungi change with tree age? i.e. is there a succession of wood decay fungi with tree age?

4.2 Methods

Sample Collection

A total of 18 *E. obliqua* trees, taken from three age classes (69, 105 and 150 years old) and two adjacent sites in southern Tasmania, were felled and examined for decayed wood as described in Section 2.2. Briefly, the stem of each tree was cut at three standard points. These were within the main stem at 11-12 m height, immediately below the crown, and within the live crown. At each standard point, the stem was cut into two 50 cm billets, creating three cut faces. The cut face of each billet was photographed and the number of patches of decay (i.e. decay columns) labelled and recorded. A sample of decayed wood from each decay column was taken back to the laboratory for further examination. Control samples of clear heartwood and sapwood were also collected from each cut face.

Isolation and Determination of Wood Decay Fungi

In the laboratory, subsamples (1 cm^3 pieces) of wood from the edge of each decay column and control wood sample were surface sterilised and incubated for one month on specialised fungal media to isolate associated wood decay fungi (Hopkins *et al.* 2005). Fungal cultures were maintained at 20 ºC on MEA for the duration of the study and subcultured regularly.

The two methods were used to group and identify the fungal species present are described in detail in chapter 3. Fungal species names follow those of Kirk *et al.* (2001).

Species richness

To look for variation in species richness between tree age classes, one-way analysis of variance (ANOVA) was undertaken in SAS 9.1 (Anon. 2002), using age class as a random effect on the number of species of wood decay fungi per tree. Presence-absence data were used, pooled from the three standard sampling sections from each tree. A follow up multiple comparison test (Ryan-Einot-Gabriel-Welsch Multiple Range Test: REGW test) was used to determine the nature of the differences.

Indicator Species Analysis was run in PC-ORD for Windows 4.25 (McCune and Mefford 1999) and was used to investigate whether particular species were significantly associated with specific tree age classes. A cut-off value of IndVal ≥25, p<=0.05 was used. Untransformed species abundance data were used, where frequency data (i.e. the number of cut faces a species was isolated from) was used as a surrogate measure of abundance.

Adjustments for sampling effort

The effect of sampling effort on the species richness of biological assemblages has received much attention recently (Gotelli and Colwell 2001; Grove and Bashford 2003; Heilmann-Clausen and Christensen 2004; Schiegg 2001). In this study, two different methods were used to determine whether differences in species richness between tree age classes were confounded by sampling effort: rarefaction curves and abundance-based richness estimators.

Rarefaction computes the expected number of species for a sub-sample of the pooled total species richness based on the number of samples or individuals collected (Gotelli and Colwell 2001). This technique was used to compare species richness among tree age classes, calibrated for sampling effort. Rarefaction curves were calculated against two different variables: the frequency of individuals collected, and the cumulative cross-section area of wood faces examined. For each variable, rarefaction curves were calculated using frequency data for all species of fungi. Rarefaction curves were calculated using EstimateS (Colwell 2001) and graphs plotted in Microsoft Excel.

Total species richness was also estimated using the abundance-based coverage estimators ACE and Chao 1 in EstimateS (Colwell 2001). This was based on

frequency data for all species of fungi, pooled within each tree. Since Chao's estimated CV for abundance distribution was >0.5, it was recommended to report the larger of Chao 1 and ACE as the best estimate for abundance-based richness, using the classic rather than the bias-corrected option in the diversity settings for EstimateS (Colwell 2001).

Community composition

Both unconstrained and constrained ordinations were used to explore community composition of fungi in relation to tree age class. Log transformed frequency data from each tree were used, pooled from the three standard sampling sections. One tree greater than 150 years old (Tree 4) did not group well with the other mature trees. On closer examination, this tree was found to have very few fungal species in common with any of the other trees sampled. Instead, Tree 4 was dominated by a number of species only found in that tree. Tree 4 also had very different tree architecture to all the other trees in that it was dominated by a very large number of concave fire scars. The species of saproxylic beetles found in this tree were also very different to those in any of the other trees (K. Harrison, pers. comm., 2006). For these reasons, Tree 4 was considered to be an outlier and was removed from all analyses of community composition.

Non-metric Multidimensional Scaling (NMS), an unconstrained ordination, was used to explore fungal species assemblage variation between the three tree age classes. NMS was performed using a Sorensen (Bray-Curtis) distance measure in PC-ORD (McCune and Mefford 1999) choosing the slow and thorough autopilot method. Multi-Response Permutation Procedures (MRPP) were then applied to statistically test fungal assemblage differences among tree age classes. MRPP was carried out in PC-ORD using a Sorensen (Bray-Curtis) distance measure and using the natural group weighting of n/sum(n).

A constrained ordination method, Canonical Analysis of Principal coordinates (CAP) analysis (Anderson and Willis 2003), was then used to explore fungal assemblage structure correlated with the three tree age classes. CAP was performed in CAP12 (Anderson 2004) using 9999 unrestricted random permutations.

The distribution of individual species between age classes and their location within the tree was also examined qualitatively.

4.3 Results

Species richness

A total of 312 fungal isolates were obtained from the 18 trees examined in this study. These were sorted into 91 species groups of which 20 were found frequently and 71 were only found once (singletons). A species list can be found in Table 4.3.1. With no adjustment for sampling effort, mature trees (those greater than 150 years old) had a significantly higher number of species of fungi per tree than trees in either of the younger age classes (Figure 4.3.1, $p<0.001$). The two younger age classes (69 and 105 years old) had very similar species richness. There was a total of 56 species in the mature trees, 21 species in the 105 year old trees and 26 species in the 69 year old trees.

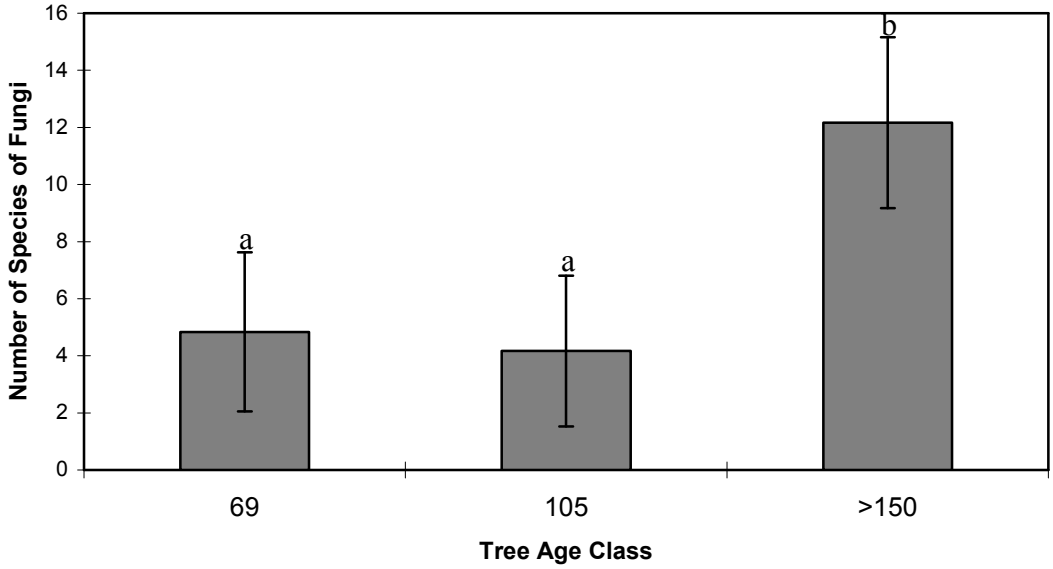


Figure 4.3.1. Average number of fungal species found in individual trees of each age class. Error bars denotes standard error, with letters a and b representing significantly different means from ANOVA.

Adjustment for sampling effort

The sample-based rarefaction curves showed that species richness for all tree age classes was very similar; although species richness was marginally greater for 69 year old trees compared with the mature trees within the comparable range (Figure 4.3.2). This pattern was further emphasised when examining the rarefaction curves for cumulative cross-sectional area (Figure 4.3.3). Species richness was markedly greater per unit area for trees in the 69 year-old age class than for trees in either of

the other age classes. In contrast, species richness was almost identical for 105 year old and mature trees within the comparable range.

Neither of the abundance-based coverage estimators, ACE or Chao 1, showed stable species richness estimates across the available range (Figure 4.3.4) and thus the results are not considered here. The richness estimators remained unstable even when the cut-off point for rare species was reduced as low as 2 (the recommended level for EstimateS is 10).

Community composition

Unconstrained ordinations (NMS and MRPP) showed that fungal assemblage differed between different tree age classes (Figure 4.3.5; p=0.004, T= -3.15). Trees greater than 150 years old seemed to have particularly distinct fungi associated with them. An exception to this was Tree 4 (a mature tree) which appeared to have a more similar fungal community to trees in the younger age classes. The patterns of fungal community composition for the two younger tree age classes were less clear, yet some of the 69 year-old trees appeared to show similar fungal community structure and some of the 105 year-old trees were also similar.

An overlay of the fungal species groups on the ordination showed that two of the *Postia*-like species (4 and 6) and *Postia pelliculosa* were strongly influencing the grouping of trees greater than 150 years old. Xylariaceae sp.1, Hymenochaetaceae sp.2 and *Athelia*-like sp.1 were partially correlated with the mature trees. *Athelia*-like sp.2, *Phlebia*-like sp.1 and *Stereum*-like sp.1 correlated with a number of the younger trees.

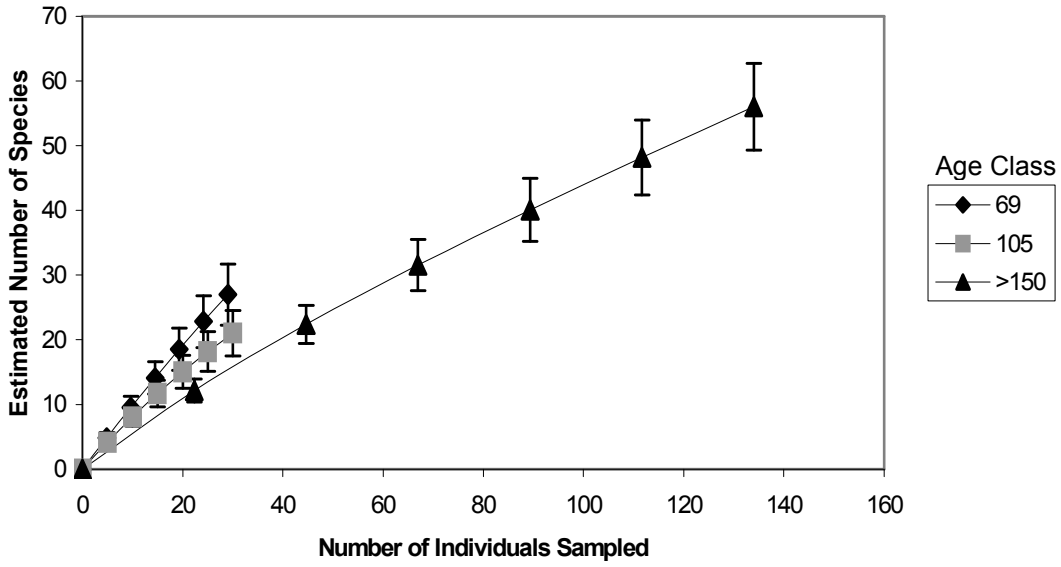


Figure 4.3.2. Rarefaction curve for all fungi in relation to tree age class. Error bars denote standard error for each age class.

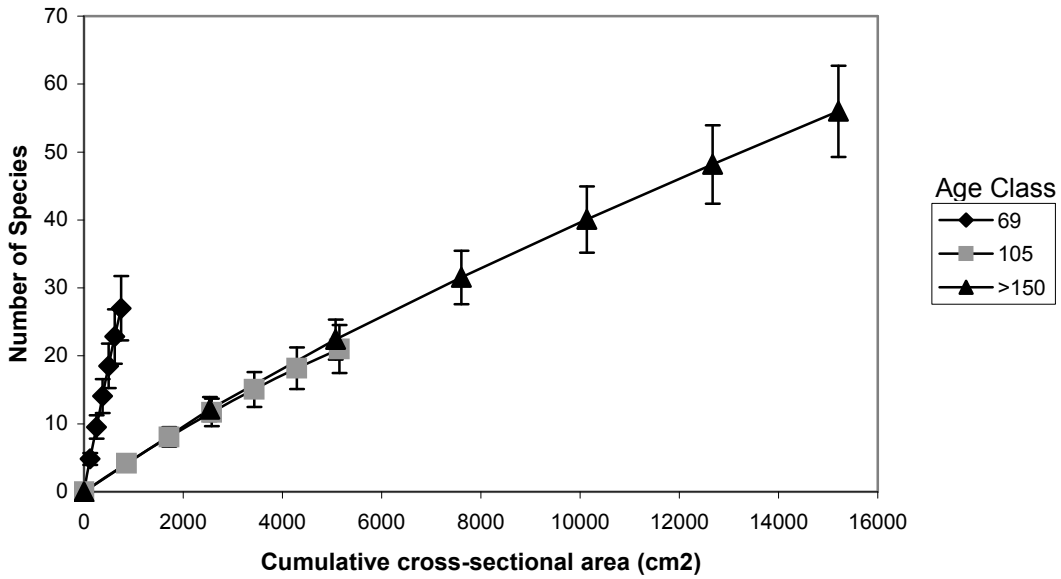


Figure 4.3.3. Rarefaction curve for all fungi based on the cumulative cross sectional area of billets examined for fungi in relation to tree age class. Error bars denote standard error.

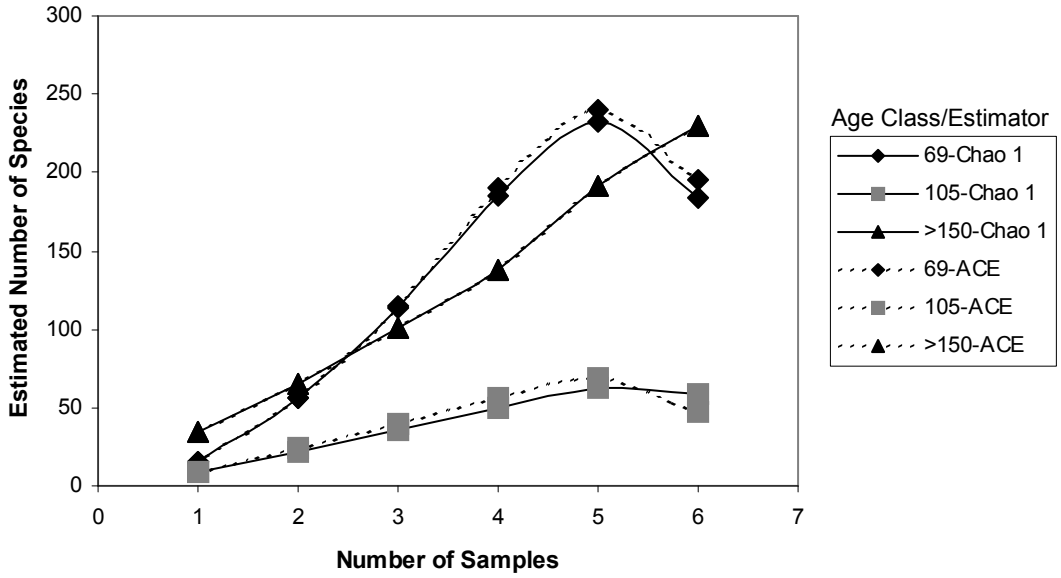


Figure 4.3.4. Graphical representation of results of the abundance-based species richness estimators Chao 1 and ACE for each tree age class. Note that none of the estimators have stabilised with the low number of trees sampled.

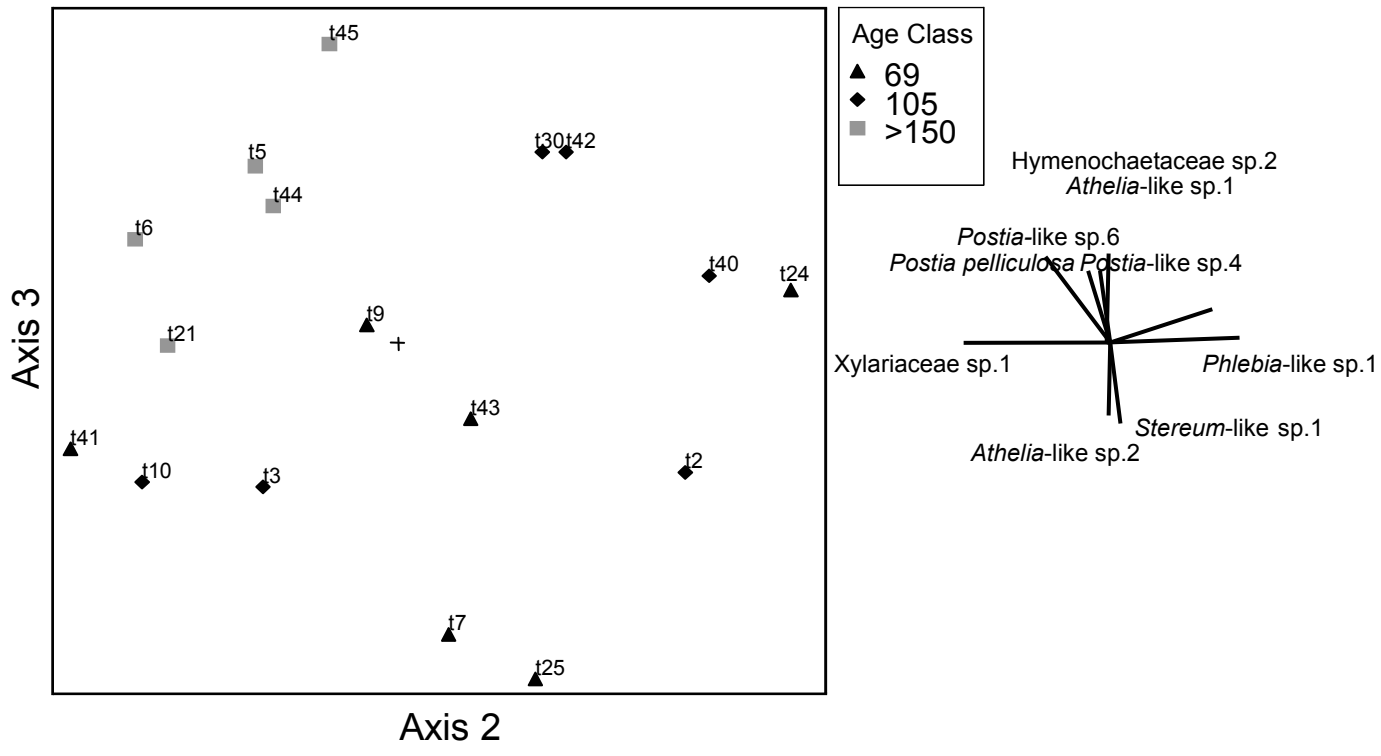


Figure 4.3.5. NMS ordination of fungal community structure with respect to tree age. The ordination is based on log transformed frequency data. Driver species are shown on the right-hand side.

The constrained ordination showed similar patterns (Figure 4.3.6) with a much stronger separation between trees greater than 150 years old and the younger age classes (along x axis). Some similarity in assemblage was also indicated within the 69 year old trees and the 105 year old trees although there was some overlap between the two age classes. Fungal species which appeared to be strongly correlated with mature trees were *Postia pelliculosa*, Ascomycete sp.1, *Postia*-like spp.6 and 4, Basidiomycete sp.1 and *Hypholoma fasciculare.* Basidiomycete sp.2 was well correlated with 105 year old trees and Basidiomycete sp.4 was correlated with 69 year old trees.

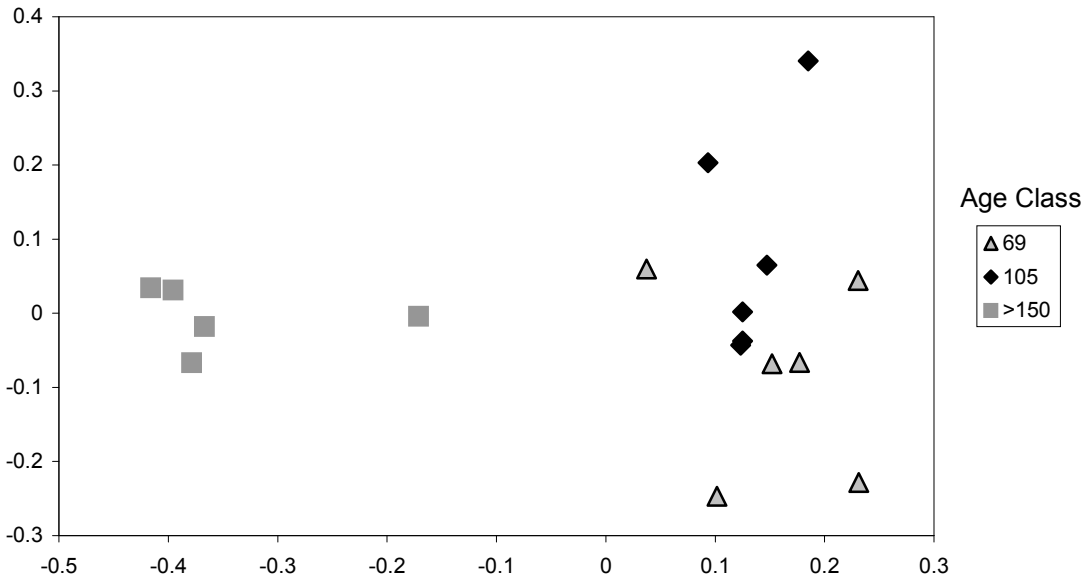


Figure 4.3.6. Constrained ordination (CAP analysis) of fungal community structure with respect to tree age class. N=91 species, and frequency data were log transformed.

Indicator species analysis found only one species, *Postia pelliculosa*, to be a significant indicator for mature trees (p=0.002). Indicator species analysis found no other fungal species to be significantly correlated with any specific tree age class. A number of main species of fungi were restricted to one tree age class, although found in very low numbers (Table 4.3.1). These include Basidiomycete sp.4 for the 69 year old trees, Basidiomycete sp.2 and Postia-like sp.7 for the 105 year old trees and Basidiomycete sp.3, *Coniophora*-like spp.1 and 2, *Fistulina*-like sp.1, Hymenochaeteaceae sp.1 and *Postia*-like spp. 3, 4, and 6 for the mature trees. Three species of fungi Basidiomycete sp.1, Xylariaceae sp.1 and *Hypholoma fasciculare*

were found in trees in all three tree age classes. A further three species *Phlebi*a-like sp.1, *Postia*-like sp.5 and *Stereum*-like sp.1 were found in both 69 year old and 105 year old trees. Only one species (Ascomycete sp.1) was found in both 69 year old trees and mature trees, while two species (*Athelia*-like sp.1 and Hymenochaeteaceae sp.2) were shared between the 105 year old trees and the mature trees.

Table 4.3.1. Species of fungi found in living *Eucalyptus obliqua* trees showing the frequency of species in each tree age class.

Species	69	105	>150
Basidiomycete sp.1	2	2	10
Hypholoma fasciculare[#]	1	1	4
Xylariaceae sp.1	1	3	7
Phlebia-like sp.1	1	1	
Postia-like sp.5	1	3	
Stereum-like sp.1[#]	1	1	
Basidiomycete sp.4	2		
Athelia-like sp.2[#]	1		
Neonectria radicicola[#]	1		
Peniophora aurantiaca	1		
Phanerochaete sordida	1		
Psathyrella-like sp.1[#]	1		
Trametes versicolor[#]	1		
Ascomycete sp.1	1		8
Athelia-like sp.1[#]		2	2
Hymenochaete-like sp.2		1	2
Basidiomycete sp.2		3	
Fomitopsis-like sp.1		1	
Postia-like sp.7		2	
Steccherinum-like sp.1		1	
Trametes-like sp.1		1	
Xylaria sp.1		1	
Ascocoryne sp.1			1
Basidiomycete sp.3			2
Coniophora-like sp.1			7
Coniophora-like sp.2			1
Fistulina-like sp.1			4
Gymnopilus allantopus			1
Hymenochaete-like sp.1			5
Hypholoma sp.1[#]			1
Hypholoma sp.2[#]			1
Metarhizium flavoviride			1
Polyporus gayanus			1
Postia pelliculosa			30
Postia-like sp.3			4

Postia-like sp.4			5
Postia-like sp.6			2
Typhula-like sp.1			1
Additional Records*	13	7	34

*Additional Records are those singleton species which were unable to be named.
#Species isolated from healthy sapwood

Sapwood species

A number of species of fungi were consistently isolated from undecayed, healthy sapwood (Table 4.3.1). Although these species demonstrated an ability to produce wood decay enzymes in culture, they were clearly not associated with decay in the living trees and were thus considered to be latently present within the tree in the sense that they were not actively growing and decaying the wood (Boddy 1994; Chapela and Boddy 1988). These included the commonly found species *Hypholoma fasciculare*, *Athelia*-like sp.1 and *Stereum*-like sp.1 as well as the singletons *Hypholoma* spp.1 and 2, *Athelia*-like sp.2, *Trametes versicolor*, *Polyporus gayanus, Neonectria radicicola, Trametes*-like sp.1 and *Psathyrella*-like sp.1 along with 13 un-named singletons.

4.4 Discussion

This study is the first to systematically examine the relationship between wood decay fungi and tree age in living *Eucalyptus obliqua* trees. While the data reported here are only preliminary (in terms of sample size), some clear patterns are present. A large number of species of wood decay fungi were isolated from living *E. obliqua* trees. Sixty-nine year old trees were the most species rich age class when equal sample sizes were compared and trees of different ages supported different assemblages of wood decay fungi. The community composition of wood decay fungi found in mature trees (those greater than 150 years old) was particularly distinct. A number of species of wood decay fungi were restricted to particular tree age classes. Given the intensive sampling methods used in this study, it is unrealistic to compare the species richness of wood decay fungi found here with previous studies on eucalypts in Australia. For instance, Tamblyn (1937) identified only three species of wood decay fungi from decay and fruitbodies associated with 12 mature *E. marginata*, while Refshuage (1938) described eight different species of fungi causing decay in *E. regnans*. These differences give an indication of the

advantages of intensive sampling (as in the present study) if the aim is to assess species richness in this habitat.

Adjustments for sampling effort indicate that within the comparable range, the 69 year old trees were in fact more species rich than the 105 year old and mature trees. This is despite the mature trees supporting a much greater number of species on an individual basis. The high species richness of the 69 year old trees could be related to their small tree diameter. Although large diameter logs and trees are generally considered to host greater numbers of species than their small diameter counterparts (e.g. Kolstrom and Lumatjarvi 2000), several studies have found the reverse to be true (e.g. Heilmann-Clausen and Christensen 2004; Schiegg 2001; Yee 2005). Schiegg (2001) suggested that the high species richness in large limbs of *Fagus sylvatica* compared with larger diameter felled logs of the same species on the forest floor may be due to the range of microclimates present across limbs in different locations. Limbs spread throughout the forest would demonstrate a high degree of variation in humidity and light intensity which would impact upon the species able to colonise them. As limbs are of smaller diameter than logs, they would be more susceptible to changes in forest microclimate. This could also be the case with young trees of small diameters. This theory is reinforced by the fact that majority of species of fungi found in the 69 year old trees in this study were only isolated from individual trees. A collection of small diameter trees also involves more separate trees than the same volume of large diameter trees. Therefore given the same volume, small diameter trees represent more colonisation events (branch stubs, dead branches etc) and a greater surface area to volume ratio than large diameter trees (Heilmann-Clausen and Christensen 2004). So despite their smaller size, the 69 year old trees in this study would represent more potential for colonisation than the same volume/size of trees in the older age classes which were of greater diameter.

There are a number of other reasons why the 69 year old trees may be the most species rich. For example, the sampling method used may have favoured faster growing, less aggressive, less specialised early colonisers found in the young trees, rather than the slower growing more aggressive decay species present in the mature trees. Attempts were made to circumvent this possibility: the media used were selected to specifically target basidiomycetes (i.e. the majority of decay species)

and the wood chips were grown on the isolation media for up to 6 weeks to allow for slow growing species. Despite this, the growth of Australian wood decay fungi in culture has not been well studied (Simpson 1996), so the influences of the isolation method are not clear. It is interesting that the 69 year old trees in the present study harboured a considerable number of species of decay fungi relative to the 105 year old trees. It is possible that in the mixed age forest sampled in the present study, the growth of these younger trees was suppressed by the surrounding older, dominant trees, making them more susceptible to colonisation by decay fungi. This idea is reinforced by relative tree height: all 69 year old trees were much shorter than 105 and >150 year old trees. In addition, their canopy was much less developed, in the sense that they had much lower numbers of branches. Perhaps these 69 year old trees actually represent a different developmental pathway compared with the 105 year old trees, and will maintain a greater species richness throughout their lifetime.

This study highlights the importance of accounting for sampling effort when examining species richness in objects of different sizes (Gotelli and Colwell 2001; Heilmann-Clausen and Christensen 2004; Schiegg 2001). Although mature trees initially appeared to be the most species rich, this was greatly affected by sampling effort. It is important to treat these results with caution however, as the current data set does not indicate the point at which species saturation would occur for the 69 year old trees. This study has only sampled a small proportion of the expected diversity of wood decay fungi in living *E. obliqua* so it is important not to extrapolate too much. That is, when the data from the rarefaction curves are extrapolated out to the point of species saturation, it is quite possible, that the young trees may have much fewer species than the mature trees. In a study of the saproxylic beetle assemblages in the same trees, Harrison found that the 69 year old trees were most species rich using rarefaction, as was found for the fungi in this study (Figures 4.3.2 and 4.3.3). However, when the data were extrapolated to consider the total volume of the trees, using four different models, the mature trees were consistently more species rich than trees in either of the two younger age classes (K. Harrison, pers. comm. 2006). Thus, simply increasing the number young trees sampled, may still not result in the large numbers of species isolated from the mature trees (Yee 2005).

It is unfortunate that this data set was unable to support the species richness indicator models attempted. This is probably due to the small size of the current dataset and the large number of singleton and doubleton species (Magurran 1988).

Many species of wood decay fungi demonstrated a preference for trees in a particular age class. In addition, the assemblages of wood decay fungi associated with particular age classes were different. These changes in fungi with tree age class may represent a succession of fungal assemblages similar to that described by Boddy (2001). She described a succession of wood decay fungi with tree decay moving from pioneer fungi, able to develop rapidly in an otherwise hostile environment, through to secondary colonisers with better combative and decay ability. Genera of commonly described pioneer fungi from the northern hemisphere include *Peniophora, Stereum, Phlebia, Nectria, Ascocoryne, Xylaria* and *Phanerochaete* (Boddy 2000; Boddy 2001; Coates and Rayner 1985; Muller and Hallaksela 2000). With the exception of *Ascocoryne*, fungi related to these genera were all isolated from 69 year old trees and were rarely found mature trees. In contrast, species of fungi isolated from the mature trees were generally related to well known decay genera and secondary colonisers such as *Coniophora, Polyporus, Fistulina, Hymenochaete* and *Postia* (Boddy 2001; Kile and Johnson 2000).

Boddy (2001) also recognised that some species of fungi, especially pioneer fungi, can colonise living sapwood and remain there as spores or mycelial fragments until there is some favourable change in condition such as the moisture content in the wood. These fungi are described as latently present in the sapwood and are able to remain dormant in the sapwood for long periods of time. Well-recognised latent fungi include a number of pioneer species such as *Stereum, Nectria, Phlebia* and *Peniophora,* as well as *Coniophora*. A number of potential decay fungi were isolated from undecayed sapwood in this study and may be considered as latent species. The majority of these species are from or related to genera of well known decay fungi such as *Trametes, Stereum, Polyporus, Nectria* and *Hypholoma* (Hood 2003; Schwarze *et al.* 2000), a number of which are recognised as latent fungi in the northern hemisphere (Boddy 2001). In this study, the vast majority of these fungi were isolated from clear wood in 69 or 105 year old trees, indicating that these fungi are acting as latent pioneer fungi. One is exception to this is *Hypholoma fasciculare*. This fungus has been intensively studied and well described in the

northern hemisphere as a highly combative decay species (Boddy 1993; Harold *et al.* 2005; Kampichler *et al.* 2004). In the present study, it was isolated from intact sapwood in all tree age classes thus, it appeared to be acting as a latent pioneer species rather than a combative secondary coloniser as would be expected.

A second explanation for the succession of fungi which appears to be associated with tree age could be related to changes in physical and chemical tree structure. These changes may be the result of tree age, tree size or natural disturbance events such as fire or extreme wind events. The mature trees in this study have been exposed to at least two wildfires (1898 and 1934) and this may have affected the fungal species able to colonise them. Fire scars are well recognised as infection points for fungi in Australian eucalypts (Perry *et al.* 1985; Simpson and Eldridge 1986; Tamblyn 1937), however it is unknown how their presence changes the assemblage of wood decay fungi present in the living tree. In this study, Tree 4 had quite different assemblages of fungi compared with all other trees and this may have been related to its extensive number of individual fire scars (pers. obs) or to other changes related to extreme exposure to fire. Other factors which may influence the assemblage of fungi present within the tree are branch stub diameter (Wardlaw 1996; Wardlaw 2003) which increases with increasing tree age (Table 2.2.1) or exposure to attack by fungi over a longer period of time (i.e. the older the tree, the longer it has been exposed to fungal attack). These points are discussed further in Chapter 5.

4.4.1 Conclusions

This study is the first to illustrate a successional relationship between wood decay fungi and tree age in living *E. obliqua* trees. Three categories of fungi appear to be present in the living trees: latent early colonisers, pioneer species and secondary colonisers. The assemblages of fungi present in the 69 and 105 year old trees are dominated by latent fungi and pioneer species while the fungi found in mature trees are mostly secondary colonisers. This demonstrates the importance of trees greater than 150 years old as habitat for secondary coloniser wood decay fungi within the sites assessed. Further studies, incorporating a wider range of sites and tree age classes should be undertaken to determine the wider applicability of these results. While this study has provided important baseline data on the species of fungi found in living eucalypts, there is a vast amount of work which remains to be done.

Ecological studies of fungi in Australia are hampered by the low numbers of identified fungi. Just as the identity of many of these species of fungi remains unclear (Chapter 3), their ecological role and habitat requirements within the tree are still largely unknown.

CHAPTER 5: THE RELATIONSHIP BETWEEN TREE AGE, ROTTEN WOOD AND WOOD DECAY FUNGI IN LIVING *EUCALYPTUS OBLIQUA* TREES

5.1 Introduction

The decomposition of wood is an important component of forest ecosystem functioning (Butler *et al.* 2002; Edmonds and Marra 1999; Franklin *et al.* 2002; Käarik 1974; Kirk and Cowling 1984; Swift 1977). The decay of living trees, stags, fallen branches and logs are critical for the recycling of nutrients, and decayed wood within these structures houses a large portion of forest biodiversity (Berg *et al.* 1994; Butler *et al.* 2002; Franklin *et al.* 1987; Grove 2002; Kruys *et al.* 1999; Samuelsson *et al.* 1994). Many fungi, bacteria, insects and other invertebrates contribute to the process of wood decomposition or use decayed or rotten wood for habitat (e.g. Berg *et al.* 1994; Boddy 2001; Edman and Jonsson 2001; Heilmann-Clausen and Christensen 2003; Jonsell and Weslien 2003; Nordén 2000; Renvall 1995; Schiegg 2001; Siitonen 2001). Numerous species of lichens and bryophytes are also known to utilise decayed wood substrates (Jarman and Kantvilas 2001; Kantvilas and Jarman 2004; Kruys *et al.* 1999; Soderstrom 1988). Decayed wood contributes to the formation of hollows, critical for the nesting and denning of many species of mammals and birds and an important habitat for many invertebrates (Crampton and Barclay 1998; Lindenmayer *et al.* 1997; Lindenmayer et al. 2000a; Ranius 2001; Virkkala *et al.* 1994). This is particularly apparent in Australia where there are no primary vertebrate excavators such as wood peckers (Lindenmayer *et al.* 1993; Mackowski 1987; Wormington and Lamb 1999). Species known to be dependent on decayed wood are known as saproxylic (Speight 1989).

Decayed wood has a dual importance in relation to wood decay fungi. Decayed wood is known to support an extremely diverse range of fungi (e.g. Andersen and Ryvarden 2001; Boddy 2001; Buchanan *et al.* 2001; Heilmann-Clausen and Christensen 2005; Vasiliauskas and Stenlid 1998). At the same time, wood decay fungi, in conjunction with other insects and microorganisms, are central to the formation of the decayed wood habitat utilised by other organisms (Käarik 1974; Kirk and Cowling 1984; Lewis 1996; Simpson and Eldridge 1986; Worrall *et al.*

1997). This chapter focuses on the second of these relationships: the contribution of wood decay fungi to the formation of decayed wood habitat.

Wood decay fungi fall into three primary categories, dependent on the type of wood decay they cause (Dix and Webster 1995; Rayner and Boddy 1988): soft rot, brown rot or white rot (Käarik 1974; Kirk and Cowling 1984; Tanesaka *et al.* 1993; Worrall *et al.* 1997). Soft rot is primarily associated with Ascomycete fungi and bacteria although some Basidiomycetes also have the ability to cause soft rot (Schwarze *et al.* 2000). Soft rot causes limited degradation of the lignified wood cell walls giving the wood a softened texture and a dull grey or brown appearance (Käarik 1974; Kirk and Cowling 1984). Brown rot fungi are Basidiomycetes that degrade cellulose and hemicellulose in the cell wall, while lignin remains only slightly modified (Rayner and Boddy 1988; Worrall *et al.* 1997). Only 6% of all known wood decay fungi are able to cause brown rot (Schwarze *et al.* 2000). Brown rot fungi cause cross-factures of the wood and a blocky, crumbly structure. White rot fungi are also predominantly Basidiomycetes but include some Ascomycetes in the Xylariales (Rayner and Boddy 1988; Schwarze *et al.* 2000). They can be separated from brown rotters by their ability to degrade lignin. White rot is characterised by bleached, stringy or fibrous wood and two types of white rot are recognised: selective delignification that only removes lignin and hemicellulose from the wood; and simultaneous white rot which removes all three of lignin, cellulose and hemicellulose (Schwarze *et al.* 2000; Worrall *et al.* 1997). Selective delignification includes white pocket rot, where the preferential lignin degradation leaves pockets of lighter, pure cellulose. These three rot types can then be broken down further depending on the morphological and chemical characteristics of the decaying wood (e.g. Refshuage 1938; Swift 1977; Yee 2005).

The amount of decay present, the type of decay and the stage of decay can influence the suitability of a decayed wood substrate for habitat. A larger amount of decay obviously means an increased volume of colonisable habitat. In living trees, studies in the northern hemisphere have generally found an increase in decay volume with tree age (Aho 1977; Basham 1958; Basham 1991). The change in the amount of decay was not generally related to a particular disturbance event, rather to an increase in the number of stem wounds and large diameter branches with tree age which provide more access to the heartwood. Aho (1977) found a strong correlation

between tree age and the amount of decay resulting from large branches, wounds and fire scars on grand fir (*Abies grandis*: Pinaceae). The distribution of rot types within decayed wood substrates, such as living trees, can also be important for habitat as some organisms prefer specific types of decayed wood over others. Brown rot may be a better habitat for invertebrates than white rot, for example, due to its lower moisture content (Edmonds and Marra 1999).

This study follows on from the work of Yee (2005; Yee *et al.* 2001) that examined the types of decayed wood found in *Eucalyptus obliqua* logs in southern Tasmania. Yee described eleven rotten wood types from large (>100 cm) and small (30-60 cm) diameter logs. One of the specific questions raised by this work was whether differences in decay patterns between large and small diameter logs resulted from different decay pathways in the stems prior to tree fall. This chapter begins to address this question by examining the types of decay found in living *E. obliqua* trees in three different age classes to determine any relationships between the rotten wood types found in living trees and those found in fallen logs of similar size. In addition, this chapter examines the relationship between decay type, amount of decay and tree age and looks at the fungi which may be associated with these rotten wood types.

5.2 Methods

Sample collection

Eighteen *E. obliqua* trees, six from each of three age classes (69, 105 and 150 years old) and from two adjacent sites in southern Tasmania, were felled and examined for decayed wood as described in Section 2.2. To examine the decay present in each tree, the cut face of each billet at each standard sampling point was photographed and the number of patches of decay (i.e. decay columns) were labelled and recorded (Chapter 2, Figure 2.3.1). Samples of the different types of rotten wood from each decay column was taken back to the laboratory for further examination. Samples of clear heartwood and sapwood were also collected from each cut face.

Classification and description of rotten wood types

Decay samples from all trees were assigned to a preliminary grouping based on similarities in colour, texture and wetness of the wood and the presence of hyphae or other fungal markings such as zone lines. In this preliminary grouping, colour and texture were used as the main indicators of wood decay type (white, brown or soft rot). The texture of the decayed wood was described as blocky, stringy, pocketed or crumbly. The preliminary rotten wood types were directly compared with samples collected and described by Yee (2005), from decaying *E. obliqua* logs in the southern forests of Tasmania. This gave a much better indication of the variability of textures and colours within and between rotten wood types and allowed a final consensus of rotten wood types to be obtained by visually comparing samples in the preliminary groupings with samples from Yee (2005).

Measuring decay area

The amount of decay in each tree was measured by digital interpretation of photographs of the three cut faces at each standard sampling point using SigmaScan Pro 4.0 (Jandel Scientific 1987-1996). The scale of each photograph was calibrated with reference to a 10 x 20 cm white board included in the photograph. A representation of the average amount of decay in each tree was calculated by determining an average of the amount area of decay for the three cut faces at each of the three standard sampling points. This was then expressed as a proportion of the surface area at each standard sampling point (%). This proportional measure was used to compare decay between trees by adjusting for differences in sampling effort between tree age classes. Differences in proportion of decay between tree age classes were compared using one-way analysis of variance (ANOVA) and a follow-up multiple comparison tests (Ryan-Einot-Gabriel-Welsch Multiple Range Test: REGW test) in SAS 9.1 (Anon. 2002). Differences among age classes were tested both at the whole tree level and at the three standard sampling heights.

Correlations between rotten wood type and tree age

For the purposes of this study, one-way analyses of variance (ANOVA) were undertaken in SAS 9.1 (Anon. 2002), using age class as a random effect on the number of rotten wood types per tree. Presence-absence data from each tree were used for all analyses, pooled from the three standard sampling sections. A follow up

multiple comparison test (REGW test) was used to determine the nature of the differences. The difference in the proportion of different rotten wood types between tree age classes was tested using Chi-square analysis.

To determine the effect of sampling effort on the number of rotten wood types in each tree age class, rarefaction curves (Gotelli and Colwell 2001) were calculated against two different variables: the number of individuals collected, and the cumulative cross-section area of wood faces examined. For each variable, rarefaction curves were calculated using incidence data for all rotten wood types including discoloured and solid wood. Rarefaction curves were calculated using EstimateS (Colwell 2001) and graphs plotted in Microsoft Excel.

Non-metric Multidimensional Scaling (NMS), an unconstrained ordination, was used to explore variation in rotten wood type assemblage between the three tree age classes. NMS was performed using a Sorensen (Bray-Curtis) distance measure in PC-ORD (McCune and Mefford 1999) choosing the slow and thorough autopilot method. Multi-Response Permutation Procedures (MRPP) were then applied to statistically test fungal assemblage differences among tree age classes. MRPP was carried out in PC-ORD using a Sorensen (Bray-Curtis) distance measure and using the natural group weighting of n/sum(n).

Isolation and determination of wood decay fungi

Fungi were isolated from the decayed wood samples as described in chapter 4 and identified as described in chapter 3. Fungal species names follow those of Kirk *et al.*(2001).

Production of wood decay enzymes by isolates

Enzyme tests were carried out on all fungal isolates to detect the production of the wood degrading enzymes laccase and tyrosinase (Stalpers 1978) and thus reinforce any associations between fungi and rot types. One drop test of each of two solutions was applied to the culture margin of each isolate. The presence of laccase (a lignin-degrading enzyme) was indicated by a purple colour change after the application of 0.1M solution of α-naphthol dissolved in ethanol. The presence of tyrosinase (a cellulose-degrading enzyme) was indicated by a brown colour change after the application of 0.1M solution of ρ-cresol dissolved in ethanol. Isolates were

monitored for enzyme colour changes after 3 hours, 24 hours and 72 hours (Stalpers 1978). Each drop test was performed at least twice on each isolate to test for reliability. The final results presented here only indicate the presence or absence of each enzyme, not the time the reaction took to appear, as this was not known to be a reliable indicator of the decay capacity of the fungal isolate (T. Wardlaw, pers. comm. 2004). Fungi were divided into four decay type, depending on the results of the enzyme tests (Table 5.2.1). Soft rot fungi are included with the white rotters as the enzyme tests used could not separate them (Worrall *et al.* 1997).

Table 5.2.1. Classification of fungal isolates into decay types, as measured by the production of laccase and tyrosinase enzymes in culture.

Decay Types	Laccase	Tyrosinase
None (No enzymes produced)	x	x
Selective White	√	x
Simultaneous White	√	√
Brown	x	√

Correlations between decay and fungi

The isolation success rate for both brown rot and white rot fungi was calculated, based on the isolation rates determined in Chapter 3.

Indicator Species Analysis was run in PC-ORD for Windows 4.25 (McCune and Mefford 1999) and was used to investigate whether particular species of fungi were significantly associated with specific rotten wood types. A cut-off value of IndVal ≥25, p<=0.05 was used. Untransformed species abundance data were used, where frequency data (i.e. the number cut faces a species was isolated from) was used as a surrogate measure of abundance.

Correlation between fungal communities, decay and beetles

Mantel tests were performed in PC-ORD for Windows 4.25 (McCune and Mefford 1999) to determine whether the patterns of fungal assemblage distribution were similar to the patterns of community distribution of rotten wood types and saproxylic beetles in the same trees. Non-metric Multi-dimensional Scaling (NMS) ordinations prepared in PC-ORD for Windows 4.25 (McCune and Mefford 1999)

were compared for each group. NMS was performed using a Sorensen (Bray-Curtis) distance measure in PC-ORD (McCune and Mefford 1999) choosing the slow and thorough autopilot method. The fungal data were based on log transformed frequency data (see Chapter 4), while for the rot type data, the NMS was based on presence-absence data (this Chapter, see previous section). The saproxylic beetle data were based on the collaborative work with Katherine Harrison (University of Tasmania) and the NMS was performed using log-transformed abundance data. The Mantel tests gave p values based on Randomisation (Monte Carlo) tests using 1000 randomised runs.

5.3 Results

Classification and description of rotten wood types

The decayed wood from 18 living *Eucalyptus obliqua* trees was classified into eight different rotten wood types (Table 5.3.1). A more detailed description of each decay type, including photographs to demonstrate colour and texture, is provided in Appendix 5.1. All decayed wood was found in the heartwood; none of the sapwood was decayed. Three of the rotten wood types were thought to be white, three brown and one a combination of brown and white. No soft rot types were identified. Two of the rotten wood types from living trees were similar to those found in logs by Yee (2005): discoloured wood, and red brown blocky fibrous decay. Two other rotten wood types, dry brown cubic decay and wet brown cubic decay, were similar to Yee's brown cubic rot, however given the striking difference between the apparent moisture content in the two rotten wood types found in living trees, they were retained as two separate rotten wood types in this study. The three white rotten wood types and the combination decay were not related to any of the rotten wood types described by Yee (2005) from logs.

Rotten wood type and tree age

With no adjustment for sampling effort, mature trees (those greater than 150 years old) had a significantly higher number of rotten wood types per tree than trees in either of the younger age classes (Figure 5.3.1, $p<0.001$).

Table 5.3.1. Types of decayed wood found in 18 living *E. obliqua* trees.

Rotten wood type	Suspected main decomposition agent*	Rot type	Brief description	Related rotten wood type in Yee (2005)
Discoloured wood	Fungi, possibly bacteria	N/A		Discoloured wood
Stringy Rot	Fungi	White	Brown and very fibrous.	-
White Spongy Rot	Fungi	White	Very bleached, soft but also quite stringy in places.	-
Small Pocket Rot	Fungi	White	Pockets very small and dispersed. Mycelium often visible in wood grain.	-
Combination Pocket and Brown Rot	Fungi	White and brown together	Looks like a pocket rot and a brown cubical rot both growing together.	-
Dry Brown Cubic Rot	Fungi	Brown	Crumbly, cubical decay, consistently very dry and light coloured even in newly fallen trees.	Brown cubic rot
Wet Brown Cubic Rot	Fungi	Brown	As above but consistently wetter even in newly fallen trees.	Brown cubic rot
Red Brown Blocky Fibrous Rot	Insects, fungi	Brown	Blocky and fibrous, often very wet with a reddish colour	Red Brown Blocky Fibrous Rot

*Adapted from Yee (2005).

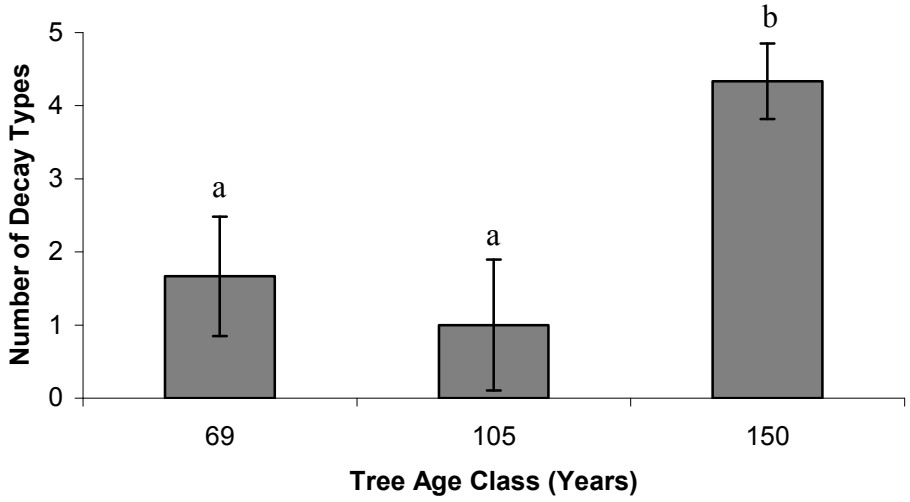


Figure 5.3.1. Average number of rotten wood types found in individual trees of each age class. Error bars denotes standard error, with letters a and b representing significantly different means from ANOVA.

The rarefaction curves based on number of individuals sampled showed that the number of rotten wood types for all tree age classes was very similar within the comparable range (Figure 5.3.2). The rarefaction curves for cumulative cross-

sectional area showed a different pattern (Figure 5.3.3). The number of rotten wood types was markedly greater per unit area for trees in the 69 year-old age class than for trees in either of the other age classes. The 105 year old trees showed fewer rotten wood types than the mature trees however this difference was minimal.

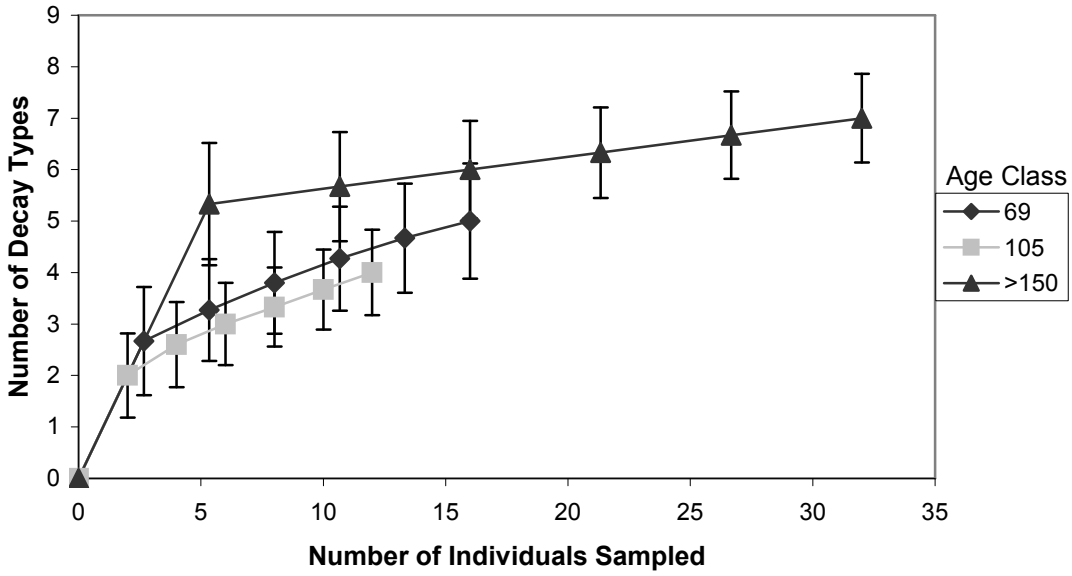


Figure 5.3.2. Rarefaction curve for all rotten wood types in relation to tree age class. Error bars denote standard error for each age class.

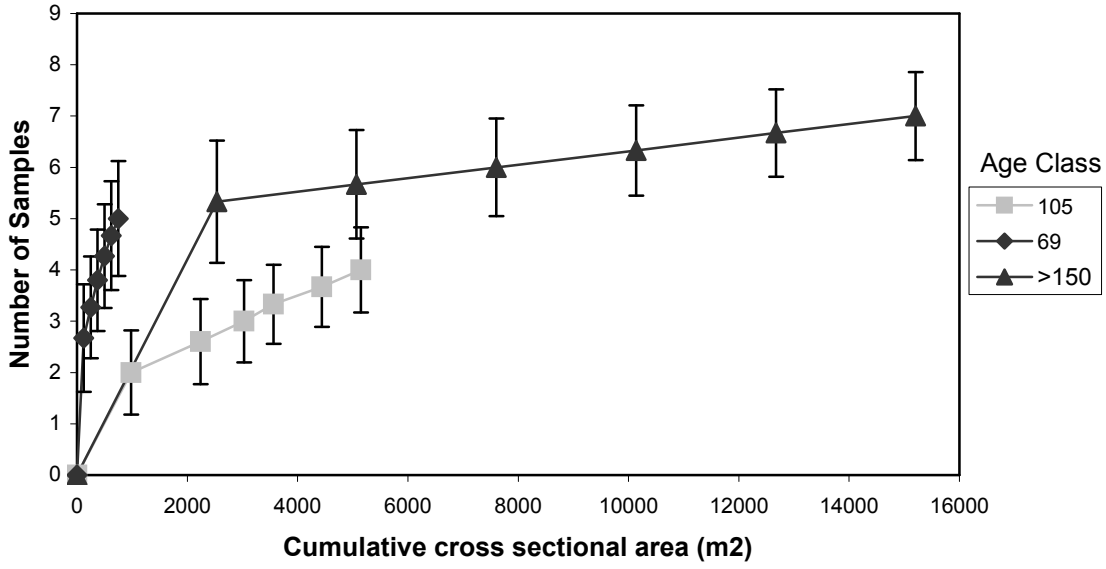


Figure 5.3.3. Rarefaction curve for all rotten wood types based on the cumulative cross sectional area of billets examined for decay in relation to tree age class. Error bars denote standard error.

Discoloured wood was found in all 69 year old and >150 year old trees, and in four of the six 105 year old trees (Figure 5.3.4). Two of the rotten wood types were confined to just one tree. Red brown blocky fibrous rot was only found in Tree 44 and combination brown and pocket rot was confined to Tree 4. Wet brown cubic rot was the only rotten wood type found in more than one tree which was confined to trees greater than 150 years old, however both dry brown cubic rot and stringy rot were both predominantly found in trees greater than 150 years old. White spongy rot was only found in two trees; one 69 year old and one 105 year old tree.

Figure 5.3.4. Frequency of occurrence of rotten wood types found in six trees in each of three age classes (69, 105 and >150 years old). Rotten wood types which differed significantly in occurrence ($p<0.05$, Chi-square analysis) between the three tree age classes are shown by *, and rotten wood types exclusive to a particular age class are denoted by **.

Decay area and tree age

The proportion of decay was highly variable both between trees and between sample sections within trees. One 69 year old tree and four 105 year old trees were completely free of decay at the standard sampling points. On average, mature trees (those greater than 150 years old) had a significantly greater proportion of decay at all three standard sampling points compared with the trees in the two younger age

classes ($p<0.001$). Similarly, 105 year old trees had significantly less decay than 69 year old trees and mature trees. The proportion of decay was relatively consistent between sample heights for each age class, except for the mature trees where the proportion of decay 2 m within the crown was significantly greater than at any other height (Figure 5.3.5, $p=0.0062$). Due to their much larger diameter and their high proportion of decay, mature trees contained a much greater amount of decay per tree than trees in either of the younger age classes.

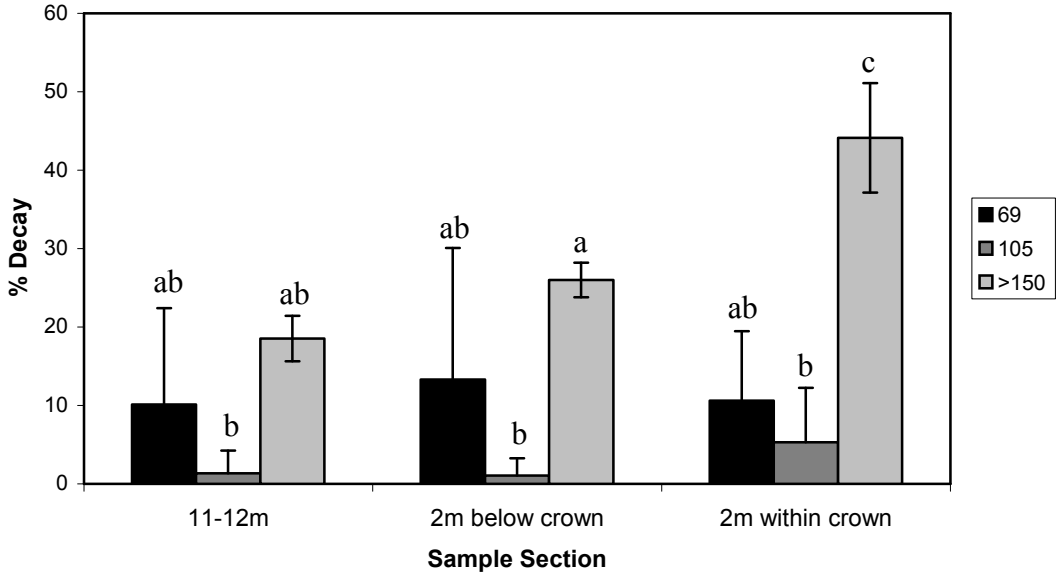


Figure 5.3.5. Proportion of decayed cross-sectional area per cut face at three different standard sampling heights. Averages are shown for each tree age class. Error bars denote standard error. Letters a, b, and c indicate means significantly different from ANOVA ($p=0.0062$).

Rot type assemblages and tree age

Mature trees had distinct communities of rotten wood compared with trees in the two younger age classes (Figure 5.3.6). The assemblage in the mature trees was particularly driven by the presence of the two types of brown cubic decay and the white stringy decay. There was little similarity in decay communities within the 69 year old tree age class and within the 105 year old age class; the majority of these trees appeared to contain quite different communities of decay from each other.

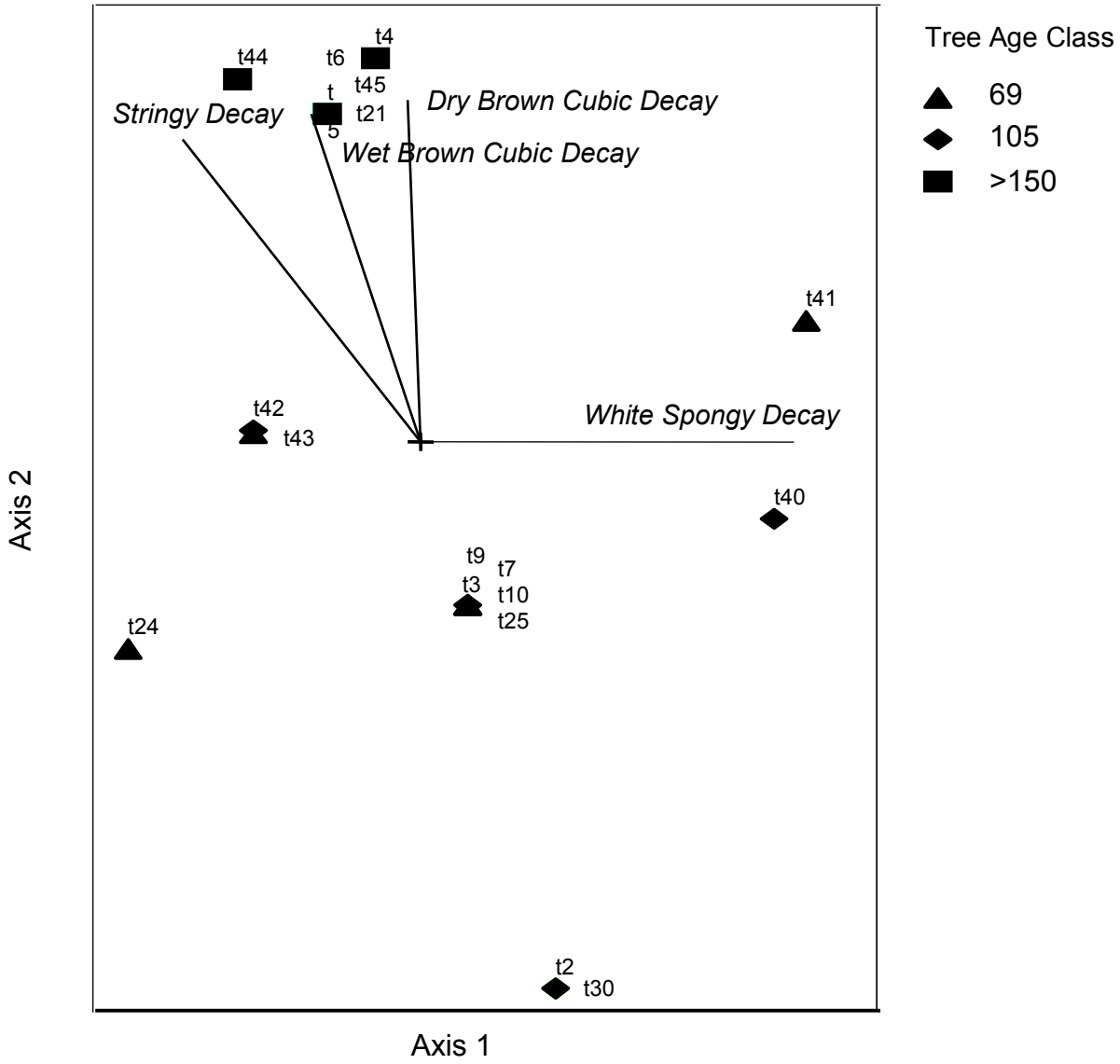


Figure 5.3.6. Non-metric Multidimensional Scaling ordination of 18 living *E. obliqua* trees based on eight rotten wood types showing axes 1 and 2 with tree age class overlaid. Rotten wood type vectors with an r^2 value >0.2 are shown. Tree numbers relate to those listed in Table 2.2.1.

Production of wood decay enzymes by fungal isolates

Of the 91 species of fungi found in this study, more than half (48) were determined as selective white rot fungi, showing a positive enzyme reaction for laccase only (Table 5.3.2). Of the remainder, 18 were simultaneous white rot fungi (positive for both laccase and tyrosinase) and just six species were brown rotters (positive for tyrosinase only). Sixteen species showed no positive enzyme reactions at all and a further three were not tested for enzyme production in culture due to contamination. All Ascomycota were selective white rot species.

Table 5.3.2. Species of fungi found in living *Eucalyptus obliqua* trees showing response of each species to wood decay enzyme tests for laccase and tyrosinase.

Species	Selective White	Simultaneous White	Brown	No Enzymes Produced
ASCOMYCOTA				
Ascocoryne sp.1	x			
Ascomycete sp.1	x			
Metarhizium flavoviride	x			
Neonectria radicicola	x			
Xylaria sp.	x			
Xylariaceae sp.1	x			
BASIDIOMYCOTA				
Athelia-like sp.1	x			
Athelia-like sp.2	x			
Basidiomycete sp.1	x			
Basidiomycete sp.2	x			
Basidiomycete sp.3				x
Basidiomycete sp.4	x			
Coniophora-like sp.1				x
Coniophora-like sp.2	x			
Fistulina-like sp.1			x	
Fomitopsis-like sp.1				x
Gymnopilus allantopus		x		
Hymenochaeteaceae sp.1	x			
Hymenochaeteaceae sp.2		x		
Hypholoma fasciculare		x		
Hypholoma sp.1		x		
Hypholoma sp.2		x		
Peniophora aurantiaca				x
Phanerochaete sordida	x			
Phlebia-like sp.1	x			
Polyporus gayanus				x
Postia pelliculosa		x		
Postia-like sp.3		x		
Postia-like sp.4		x		
Postia-like sp.5		x		
Postia-like sp.6			x	
Postia-like sp.7		x		
Psathyrella-like sp.1		x		
Steccherinum-like sp.1		x		
Stereum-like sp.1	x			
Trametes ochracea	x			
Trametes versicolor	x			

Typhula-like sp.1				x
Additional Records*	30	6	4	10
Total	48	18	6	16

*Additional Records are those singleton species which were unable to be named.

Trees in the two youngest age classes (69 and 105 year old trees) were clearly dominated by selective white rot fungi (78% and 63% respectively, Figure 5.3.7). The proportion of selective white rot species in the mature trees (trees greater than 150 years old) was much lower, accounting for only 39% of decay species. The proportion of simultaneous white rot fungi ranged from 15% in the 69 year old trees to 39% in the mature trees. The mature trees were the only age class to contain any brown rot fungi and these were relatively rare (8% of species in that age class).

Fungal isolations from wood classified as white rot had a 53% success rate, while 67% of wood chips thought to be brown rot gave rise to isolates of wood decay fungi.

Correlation between fungal communities, decay and beetles

Indicator species analysis revealed no significant correlations between specific rotten wood types and particular species of fungi.

Mantel tests indicated that there was a highly significant positive relationship between the patterns of distribution of decayed wood and fungal communities ($p=0.0025$), yet the relationship was not well correlated ($r=0.278$). Fungal assemblage structure was not significantly related to saproxylic beetle assemblage structure ($p=0.15$, $r=0.2$); however, saproxylic beetle assemblage structure was significantly related to the distribution of decayed wood in the living trees (K. Harrison, personal communication 2005).

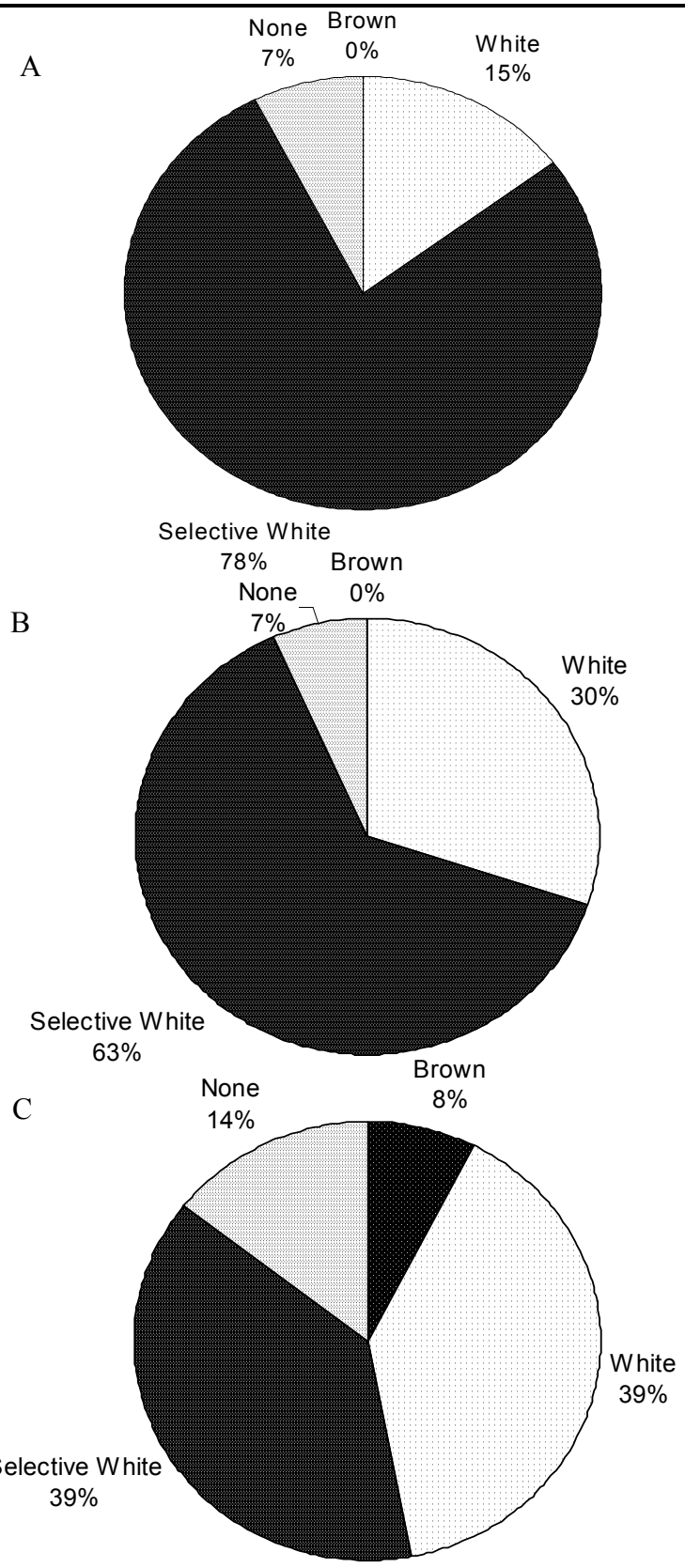


Figure 5.3.7. Proportion of fungi producing wood decay enzymes in one of four categories: brown rot, (simultaneous) white rot, selective white rot and none (no enzymes produced) for the three tree age classes a) 69 year old trees N=26, b) 105 year old trees N= 21, and c) tree greater than 150 years old N=56.

5.4 Discussion

This study is the first to examine the association between decayed wood, wood decay fungi and tree age in living *E. obliqua*. Seven rotten wood types were collected and described from 18 living trees. Table 5.4.1 compares the rotten wood types collected in this study with those collected from eucalypts in previous studies. Aside from the rotten wood types described by Yee (2005) which were described photographically and have been directly compared with decay from this study, the relationships in this table should be treated with caution. The patterns of decay and decomposition in eucalypts are not yet well understood (May and Simpson 1997; Simpson and Eldridge 1986; Wilkes 1982; Yee 2005), and many studies provide but a brief description of their rotten wood types, making comparisons difficult. Importantly, this study has built on the work of Yee (2005) to add four new, well-described rotten wood types to her study of decay in *E. obliqua* (Appendix 5.1).

Decayed wood can be found in young trees

In contrast with the findings of Basham (1991) and Aho (1977), this study showed no clear relationship between tree age and amount of decay. While mature trees had the most decay in terms of volume and percentage area, the heartwood of the 69 year old trees was proportionally more decayed than that of the 105 year old trees. This was unexpected, particularly considering the distinct lack of decay in the 105 year old trees. Adjustments for sampling effort also indicate that within the comparable range, the 69 year old trees had marginally more types of decay present than the 105 year old and mature trees. This is despite the mature trees supporting a much greater number of rotten wood types on an individual basis. It is possible that in the mixed age forest sampled in the present study, the growth of these younger trees was suppressed by the surrounding older, dominant trees, making them more susceptible to colonisation by decay fungi. This idea is reinforced by relative tree height: all 69 year old trees were much shorter than 105 and >150 year old trees (see Chapter 2). In addition, their canopy was much less developed, in the sense that they had much lower numbers of branches. Perhaps these particular 69 year old trees actually represent a different developmental

Table 5.4.1. Classification of the seven rotten wood types from this study compared with classifications from other studies. Rotten wood types in the same rows are thought to be the same. Adapted from Yee (2005). The Australian state where each study took place is shown in line two.

This study, *E. obliqua* trees	Yee (2005) *E. obliqua* logs	Meggs (1996) *Eucalyptus* spp.	Refshuage (1938) *E. regnans* trees	Tamblyn (1937) *E. marginata* trees	Parkin (1942) *E. regnans,E. viminalis* trees & logs
Tasmania	Tasmania	Tasmania	Victoria	Western Australia	Victoria
Discoloured wood	Discoloured wood				
Stringy Decay					
White Spongy Decay				Yellow straw rot	White spongy rot
Small Pocket Decay			Small white pocket rot		Small white pockets
	Fibrous surface rot	Soft yellow fibrous rot			Yellowish stringy rot
	White jelly surface rot	Other (including wet jelly rot and blue stain fungi)			
	White pocket rot		Large white pocket rot Small brown pocket rot Brown stain associated with small white pocket rot	White pocket rot	White pocket rot
	White stringy rot		White spongy rot Large white pocket rot of stringy type		White stringy rot
	Yellow dry slatey rot				
Combination Pocket and Brown Decay					
	Brown cubic friable rot				
Dry Brown Cubic Decay and Wet Brown Cubic Decay	Brown blocky crumbly rot	Orange/red/brown crumbly rot	Brown cubical rot	Brown trunk rot Xylostroma heart rot	Brown cubical rot
Red Brown Blocky Fibrous Decay	Red brown blocky fibrous rot	Red blocky rot Red blocky rot with white fungal hyphae			Yellow brown spongy rot
	Brown mudgut rot	Orange/red clayey rot			

pathway compared with the 105 year old trees, and will maintain a greater amount of decay throughout their lifetime. The unusual levels of decay in the 69 year old trees are partly confirmed by the findings of Yee (2005) who observed that the majority of small diameter *E. obliqua* logs in the Warra region had very little decay prior to tree fall. Wardlaw (2003) found that only 14.1% of 1070 20-50 year old regrowth eucalypts were free of decay between 6 and 12m bole height, while 25.3% of trees had >5% of their bole volume decayed. Despite the different measures used (volume rather than cross-sectional area), very few trees appeared to contain the high levels of decay found in the 69 year old trees in this study. This finding clearly demonstrates the importance of site variation when examining decay in living *E. obliqua* trees.

Mature trees have more decay

The greater proportion of decay in the mature trees (greater than 150 years old) could be related to a greater number of entry points in older trees for fungi. In this study, large dead branches were predominantly found in the mature trees (see Table 2.2.1, Chapter 2) while fire scars were only found in mature trees. It is probable that the presence of these features increased the opportunities for colonisation in the trees, creating more chances for fungal colonisation within the stem. Other tree features such as hollows and dead or broken tops were also only found in the mature trees and may have acted as colonisation pathways. Bar-Ness (2005) found similar patterns in *E. obliqua*, with stem hollows, fire scars and dead tops restricted to trees greater than 150 years old. Both large diameter branches and fire scars have been demonstrated as potential colonisation points for wood decay fungi and invertebrates in eucalypts (Marks *et al.* 1986; Parkin 1942; Perry *et al.* 1985; Simpson and Eldridge 1986; Tamblyn 1937; Wardlaw 1996; Wardlaw and Neilsen 1999) and other hardwoods (e.g. Basham 1958; Basham 1991; Boddy 2001). For example, Wardlaw (2003) found that almost 85% of fungal decay in young regrowth eucalypts in Tasmania was associated with dead branches and poorly occluded branch stubs, a factor which increased when branches were larger than 2.8 cm in diameter. In addition, large diameter branch stubs can be associated with colonisation by wood boring insects, providing a pathway to the central heartwood (Wardlaw 1996). Fire scars are also considered important for fungal colonisation (Parkin 1942), and may provide good colonisation points for wood boring beetles

(Simpson and Eldridge 1986). Large wound areas (as large branch stubs or fire scars) also provide opportunities for more than one species of fungus or invertebrate to coexist, thereby increasing the chance of successful entry of appropriate wood decay organisms.

The greater surface area and volume of wood of the larger diameter trees did mean there was more wood available for fungal colonisation. This might reduce the chances of competition among colonisers, resulting in a greater number of successful colonisation events. In a study in north-east Queensland, Grove (2002) considered that local tree basal area (a parameter correlated with stem diameter of component trees) was a good correlate of saproxylic beetle species richness. In addition, older trees would have had a greater time period over which colonisation could occur, again increasing the chances of a successful colonisation event. As a result of their older age, mature trees would also have been exposed to more natural disturbance events such as fires and strong winds which may make them more susceptible to infection by fungi or invertebrates (Gill 1997; Greaves *et al.* 1967; Perry *et al.* 1985). Together, these factors may begin to account for the greater amount of decay in the mature trees.

Brown rot is only found in mature trees

In addition to having a greater area and proportion of decay, mature trees also had different rotten wood types to trees in the younger age classes. Both brown rotten wood types and brown rot fungi were only found in mature trees and always at very low numbers. The low number of brown rot fungi must be related to the lower quantity of brown rot present in all trees, as the isolation success rate for brown and white rot fungi was similar.

Little is known about the relationship between tree age and rotten wood type in *Eucalyptus*, however, Wardlaw (2003) suggested that brown rot may be more common in old growth eucalypts. Similarly, surveys by Parkin (1942) found brown rot to be more common in mature eucalypt trees and logs than young trees and branches. In European systems, the brown rot fungus *Lopadostoma turgidum* is known to be a late coloniser of logs (Chapela *et al.* 1988) and brown rots are common at mid-late stages of log decay (Bader *et al.* 1995; Renvall 1995) although there is much variation between tree species (e.g. Renvall 1995). Yee (2005)

suggested that the brown rot characteristic of inner heartwood decay in large diameter *E. obliqua* logs was due to different successional processes in large and small diameter trees prior to tree fall. Given that brown rot was only found in mature (ie large diameter) trees in this study, this suggestion may well be correct. The stringy rot was also only found in mature trees in the present study and could also be a late stage successional decay type.

Associations between fungi and rot types

The majority of fungi isolated in this study showed a positive enzyme reaction for laccase, indicating they were selective or simulataneous white rotters or soft rotters. Given that over 90% of Basidiomycete wood decay fungi are thought to be white rotters (Kirk and Cowling 1984), this is not unusual. These results should be treated with caution however as many mycorrhizal and litter decay species have also been found to respond positively for laccase (Gramss *et al.* 1998). All of the Ascomycete fungi also showed positive reactions to laccase, although it was rarely very strong (personal observation). With the exception of the Xylariales, these Ascomycetes are likely to be soft rotters if they do indeed decay wood (Kirk and Cowling 1984; Schwarze *et al.* 2000; Worrall *et al.* 1997). Few browns rot fungi were identified by enzyme testing, but that is as expected since only 6% of wood decay fungi are capable of causing brown rot (Schwarze *et al.* 2000). Worrall (1997) found that only five of 98 isolates of fungi responded positively to tyrosinase.

Where neither laccase nor tyrosinase enzyme was produced, it does not necessarily indicate that the isolate had no decay capacity. Fungi can be known to lose their decay capacity over time in culture (Tanesaka *et al.* 1993) and many fungi, particularly brown rots, do not respond well to enzyme tests (Worrall *et al.* 1997). *Coniophora* species, for example, are known to cause brown rot on buildings (Dix and Webster 1995), however *Coniophora*-like sp.1 in this study showed no positive reaction to tyrosinase. This is also true for fungi from well-known decay species and genera isolated in this study such as *Fomitopsis*-like sp.1*, Peniophora aurantiaca* and *Polyporus gayanus.* This may also be because some brown rot fungi have non-enzymatic oxidative mechanism for initial break-down of carbohydrates and thus do not produce tyrosinase (Worrall *et al.* 1997).

A number of fungal species normally associated with brown rot genera showed positive reactions to laccase. This was particularly true of the majority of *Postia*-like species, generally considered to be brown rot fungi (Breitenbach and (Eds) 1986) that gave strong positive reactions to tyrosinase and laccase. There may be several reasons for this. Firstly, the isolates may be brown rot fungi either with a limited Klason lignin degrading capacity (Blanchette 1991) or they may respond to laccase for some other reason. Some brown rot fungi have a limited ability to degrade lignin and have previously been shown to respond positively to laccase (1 species Kirk and Cowling 1984; 1 species Worrall *et al.* 1997). Alternately, the placement of these isolates in *Postia* may be incorrect. The taxonomy of wood decay fungi in Australia is not well known and the placement of these isolates in *Postia* is based only on comparison of the sequences of rDNA Internal Transcribed Spacer region with sequences of overseas fungi (see Chapter 3).

Despite Mantel tests indicating a similar distribution pattern for decay and fungi, no clear relationships were found between individual species of wood decay fungi and types of decayed wood. This is consistent with the suggestion that it is difficult to show clear causal relationships between individual species of fungi and specific rot types (Wagener and Davidson 1954). One species of fungus can sometimes cause more than one rot type and one morphologically distinct rot type is often caused by the action of several different fungi (Parkin 1942; Rayner and Boddy 1988). For example, *Inonotus hispidus* is known to cause both white rot and soft rot (Schwarze *et al.* 2000). The lack of relationships between rotten wood types and specific wood decay fungi may also be due to a lack of statistical power. This is supported by the findings of Harrison in her study of the saproxylic beetles in the same living trees. Harrison's study, based on over 200 species of beetles from 18 trees, found strong relationships between specific saproxylic beetles and rotten wood types (K. Harrison, pers. comm. 2006). Assemblages of wood decay fungi are extremely complex (Rayner and Boddy 1988) and it may be that only sampling 18 trees did not capture enough information to draw specific conclusions about the fungi causing decay. Another possibility is that the primary causal fungi for a particular rot type may only be present early in the decay process. Thus, by the time isolations were carried out in this study, the causal fungi may well have already disappeared.

Mature trees are important for habitat

This study provides an example of the importance of large diameter, mature trees as habitat in forest ecosystems. Mature trees, as individual units, had a greater volume of decay, meaning that on an individual basis they would provide a greater amount of habitat for saproxylic organisms than younger trees. The type of habitat in mature trees was also different to that in the younger trees in that it contained a number of different rotten wood types. In her study of decaying *E. obliqua* logs, Yee (2005) found that certain species of beetles were specifically associated with different rot types. Brown rotten wood types in particular, appeared to be an important habitat for saproxylic beetles. For example, four beetles species, *Cossonus simsoni, Prostomis atkinsoni, Dryophthorus* TFIC sp01 and *Pycnomerus* TFIC sp02, were associated with brown decayed heartwood. This may be the result of lower water content characteristic of brown rot types compared with white rot types (Edmonds and Marra 1999). All of the mature trees surveyed in this study contained significant portions of brown rot and no brown rotten wood types or brown rot fungi were found in trees in the younger tree age classes.

Assemblages of both wood decay fungi and saproxylic beetles were strongly linked to the rotten wood types present in the living trees. This demonstrates the importance of decay as habitat for organisms other than fungi and reinforces the importance of fungi in creating this decay. Further, the patterns of assemblage distribution for fungi, decay and beetles all indicate that mature trees are an important habitat, different to that supported by trees in younger age classes (this study; K. Harrison, pers. comm. 2006).

5.4.1 Conclusions

This study has demonstrated the importance of mature trees (those greater than 150 years old) for decayed wood habitat in a *Eucalyptus obliqua* stand in southern Tasmania. Mature trees were found to contain a significantly greater area of decay than 69 and 105 year old trees and, given their larger size, had a much greater volume of decay available for use as habitat. Mature trees were the only tree age class to contain brown rot types and brown rot fungi, thought to be particularly important as habitat for invertebrates. Trees in the 69 year old age class also contained considerable proportions of decay, particularly in comparison to 105 year

old trees. These 69 year old trees may also be important for providing habitat for those species that prefer white rot habitats. Finally, this study has added to knowledge of the successional processes of wood decomposition in *E. obliqua* by describing four new rotten wood types to add to those already described by Yee (2005) and identifying the presence of a further three rotten wood types in living trees, previously only known from logs.

Appendix 5.1.

Detailed descriptions of the rotten wood types categorised from the rotten wood of living *Eucalyptus obliqua* trees in wet eucalypt forest in southern Tasmania (Study 1).

Discoloured wood

Description taken from Yee (2005), Appendix 4.6.4.

'Discoloured wood comprises any wood that has been slightly discoloured, but still has the apparent physical structure of sound wood. Discolouration can vary from light pink, to yellow, or brown. The wood can have a grainy appearance.'

This discoloured wood type was found in all three living tree age classes in this study.

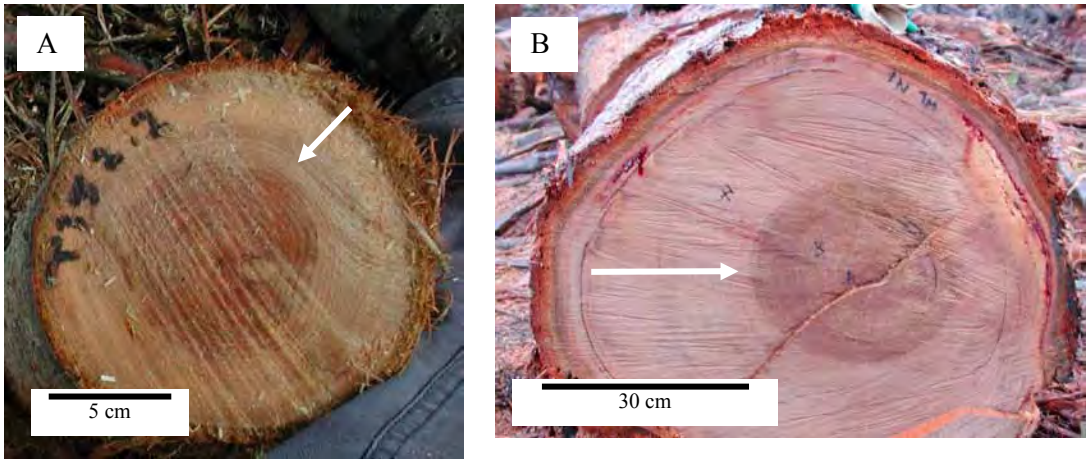


Plate 5.1.1 Discoloured wood. Arrows indicate edge of discoloured patches in (A) a 69 year old tree and, (B) and 105 year old tree.

Stringy Rot

Stringy rot has a coarse stringy texture, with the colour ranging from light brown through to dark reddish-brown. The rotten wood appears to consist of long stringy, wool-like fibres. It differs from the white stringy rot described by Yee (2005) as stringy rot lacks the bleached-white colour and softer, spongy texture of the white stringy rot. This rotten wood type was found in trees in all age classes but was most commonly associated with mature trees.

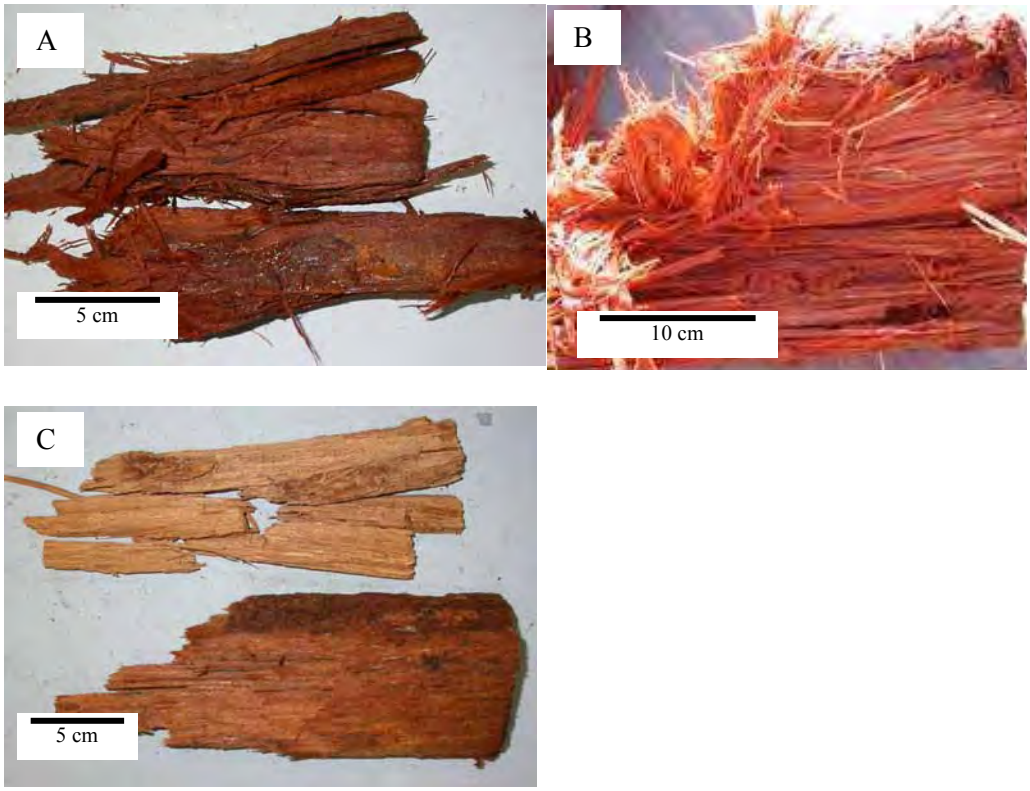


Plate 5.1.2. Stringy rot, showing colour variation from (A) dark brown, to (B) red-brown, through to (C) light brown. The fibrous texture of this rot is especially evident in (B).

White Spongy Rot

White spongy rot is characterised by continuous long, spongy, wool-like, bleached fibres. The colour can range from white through to a straw-like yellow colour. The rotten wood has a very soft texture and appears to have a very low density, making it very light. This rotten wood type was found in only two trees: one in the 69 year old age class and one in 105 year old age class.

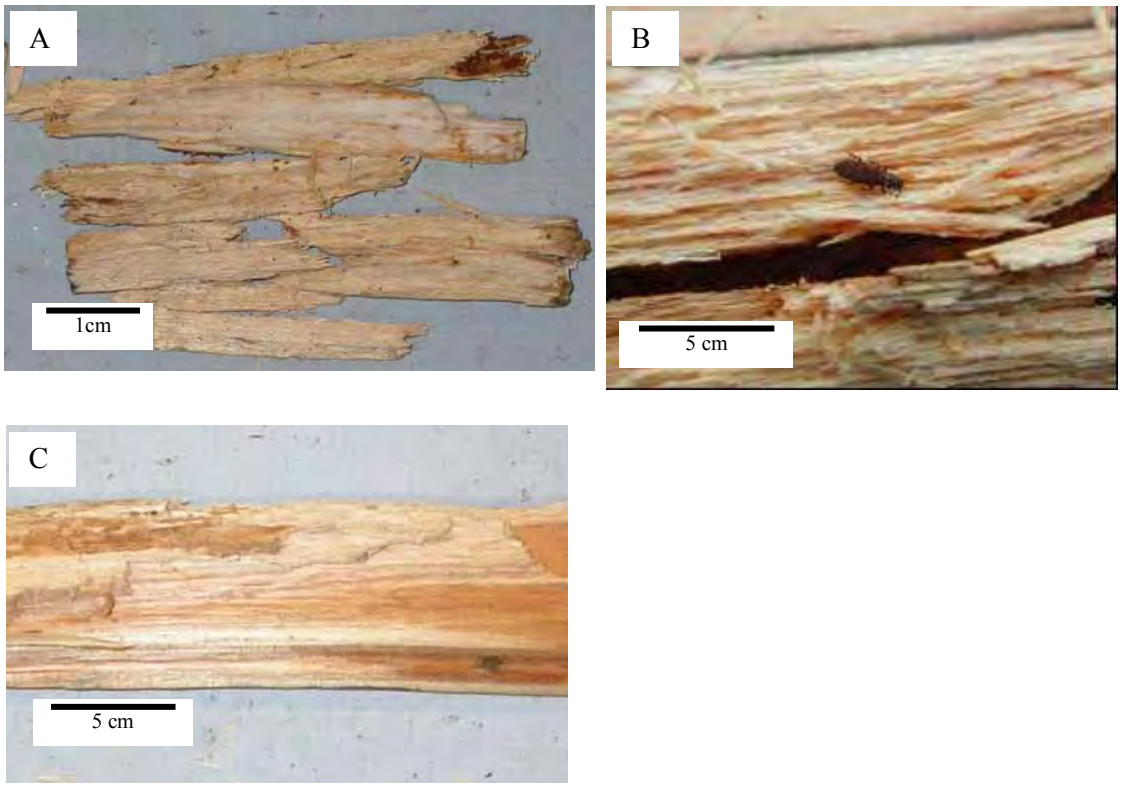


Plate 5.1.3. White spongy rot showing close-up view of the (A) bleached wood, (B) fibrous wood texture and (C) colour variation from bleached white through to straw-like yellow.

Small Pocket Rot

Small pocket rot is characterised by 2-5 mm elliptical pockets. These pockets are usually empty but are very occasionally filled with white mycelium. Pockets are separated by thin areas of seemingly intact wood. The rotten wood can range in colour from a light tan to dark reddish-brown. This rotten wood type is often associated with black 'zone' lines. It can be distinguished from the white pocket rot described by Yee (2005) as the pockets are much smaller (2-5 mm as opposed to 5-20 mm) and are often dry, rather than filled with gelatinous material or mycelium. It was only found in living trees in the 69 year old age class.

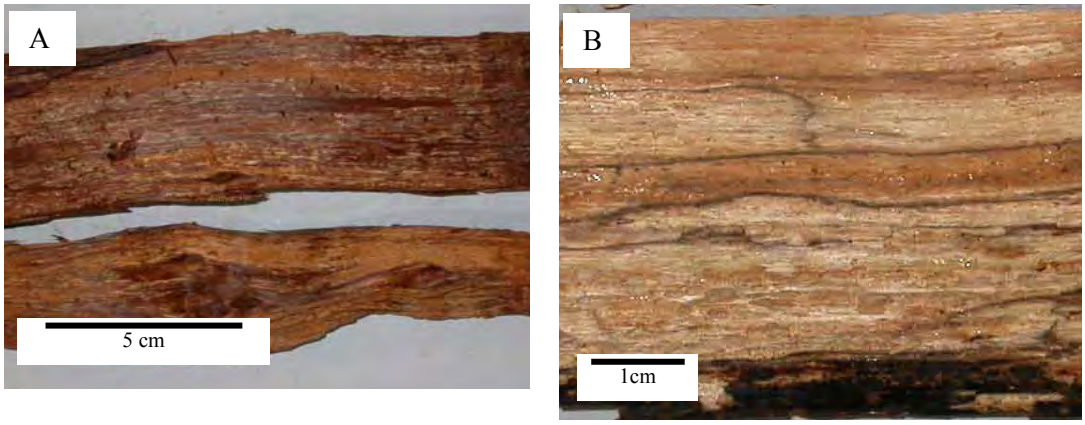


Plate 5.1.4. Small pocket rot showing small pocket size and colour variation. (A) Dark red-brown coloured rotten wood with occasional pockets filled with mycelium; and (B) light tan rot with empty pockets.

Combination Pocket and Brown Rot

Combination pocket and brown rot appears to be a rot type formed from the action of two different rots: a pocket rot and a brown rot. It is characterised by irregularly spaced 5-20 mm elliptical pockets. These pockets are generally empty but are occasionally filled with white mycelium. In contrast with other pocket rots described, the rotten wood is blocky (i.e. the wood breaks into regular blocks), rather than fibrous, and the wood in between the pockets is soft. The rotten wood is a light brown to red-brown colour. This rotten wood type was only encountered in one tree, a mature tree.

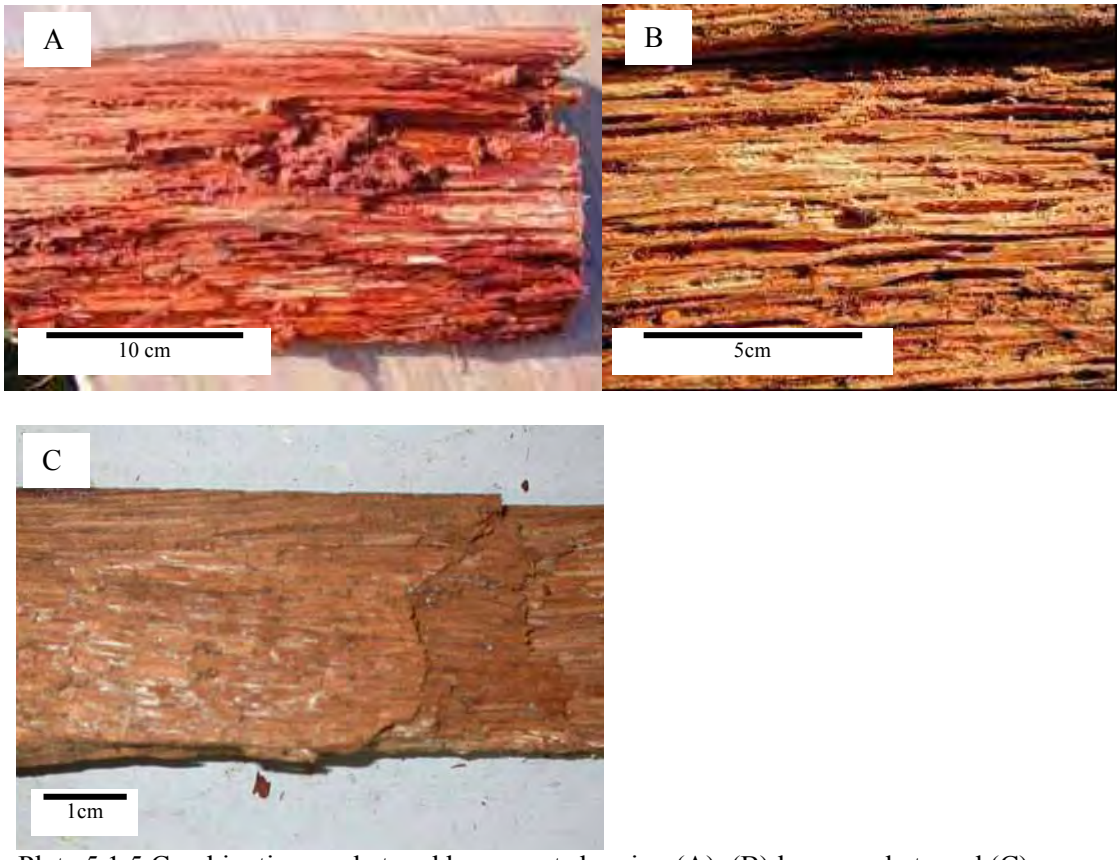


Plate 5.1.5 Combination pocket and brown rot showing (A), (B) large pockets and (C) blocky nature of the rotten wood. (C) Also shows pockets filled with white mycelium.

Dry Brown Cubic Rot

Dry brown cubic rot is characterised by brittle wood that breaks off in regular blocks and can be crumbled by hand to a powder. This rotten wood type ranges in colour from very dry tan to light brown. It is consistently very dry in texture. Distinguishing features include thin sheets of mycelium, with a chamois-like texture, progressing along the rays. This rotten wood type is very similar to the brown cubic rot described by Yee (2005)[1]. Dry brown cubic rot was found in all the mature trees examined as well as one of the 69 year old trees.

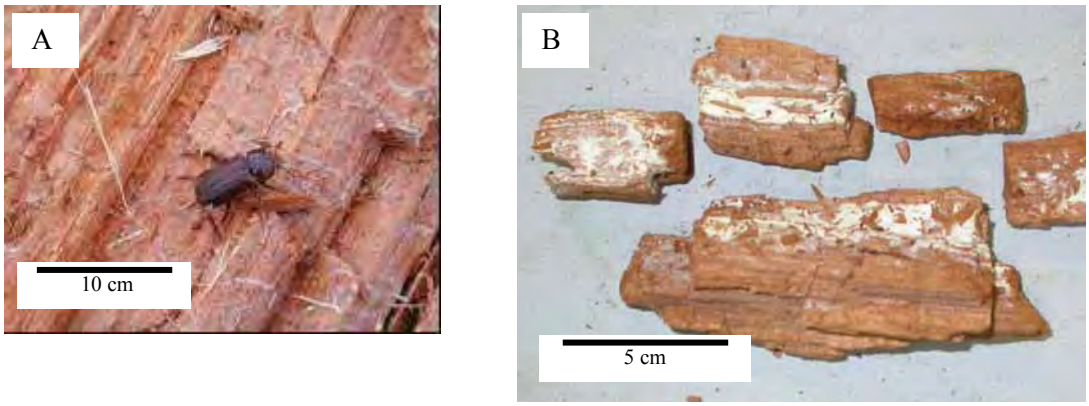


Plate 5.1.6. Dry brown cubic rot, showing (A) dry, brittle wood and (B) cubic nature of the rot with thin sheets of mycelium.

[1] Yee's brown cubic rot included both wet and dry cubic rots; however in this study, they were deemed different as they were consistently found to be either wet or dry.

Wet Brown Cubic Rot

Wet brown cubic rot is very similar to dry brown cubic rot except that it is consistently wet in texture. It is characterised by brittle wood that breaks off in regular blocks and can be crumbled by hand to a wet paste. This rotten wood type ranges in colour from red-brown to dark brown. Distinguishing features include thin sheets of mycelium, with a chamois-like texture, progressing along the rays. This rotten wood type is very similar to the brown cubic rot described by Yee (2005). Wet brown cubic rot was found in all the mature trees examined.

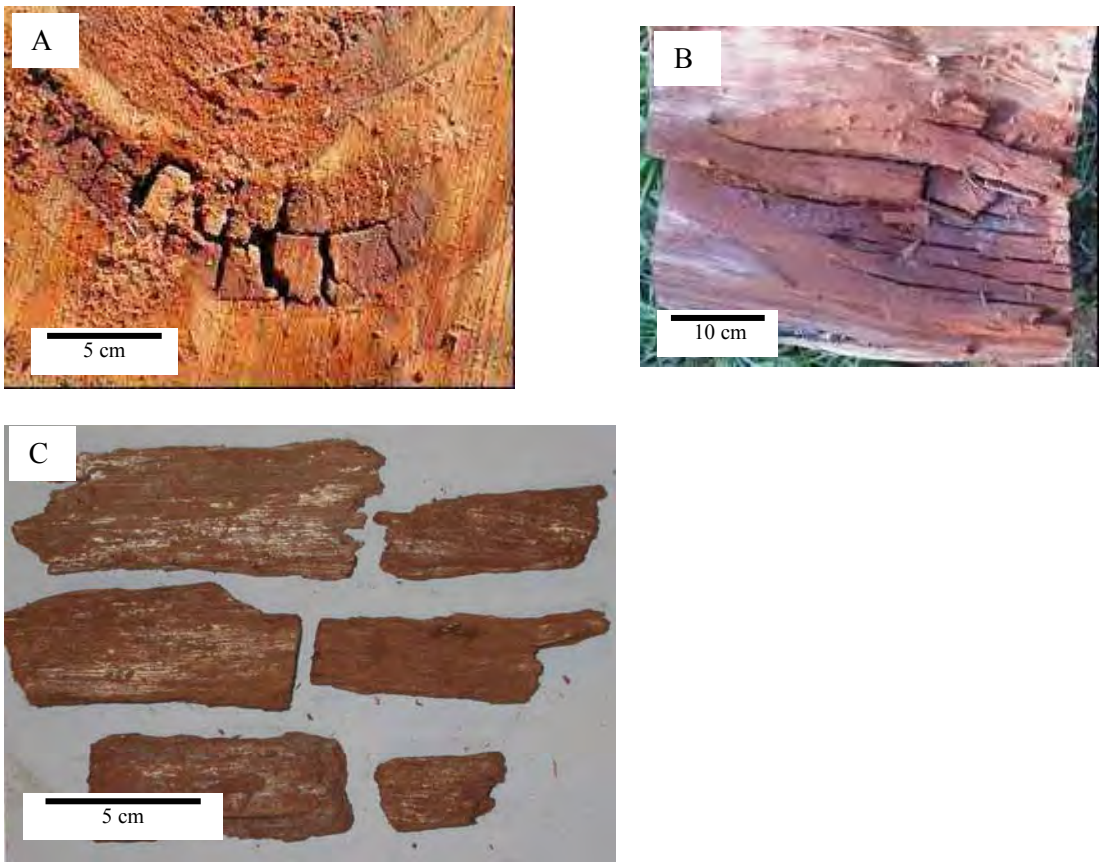


Plate 5.1.7 Wet brown cubic rot showing (A) cross-section view with blocky texture, (B) wet condition of the wood, and (C) dark, wet blocky wood sections with mycelial flecks.

Red-Brown Blocky Fibrous Rot

Description taken from Yee (2005), Appendix 4.6.4.

'Red-brown, blocky, fibrous rot has a distinctive red-brown colour. It is different from brown cubic rot in that it breaks into irregular blocks, and maintains a soft, fibrous, often relatively moist texture, rather than a crumbly, brittle one. In less decomposed wood, the wood is hard, yet the intact wood fibres can be teased apart. In more decomposed wood, the fibres are more moist and soft, giving the wood a spongy texture. This rotten wood type was mostly found in the central area of the log, but also occurred in localised patches, and occasionally in areas adjacent to the brown blocky crumbly rot.'

In living trees in this study, this rotten wood type was only found in a single mature tree (greater than 150 years old).

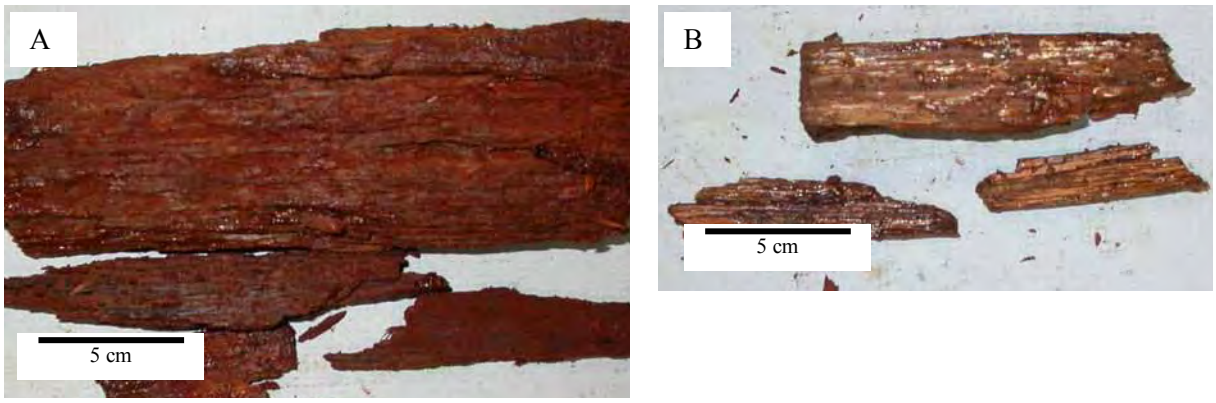


Plate 5.1.8. Red-brown blocky fibrous rot showing (A) soft, fibrous, moist wood with crumbly texture and no mycelium; and (B) spongy, more advanced rot with visible mycelium.

CHAPTER 6: WOOD DECAY FUNGI IN LOGS: IDENTIFICATION AND ASSOCIATIONS WITH ROTTEN WOOD HABITAT

6.1 Introduction

Logs on the forest floor are more than just decaying fallen trees; they are key functional resources which support a vast range of forest organisms (Franklin *et al.* 1987; Grove *et al.* 2002; Lindenmayer *et al.* 1999a; Nordén *et al.* 2004). Logs can be important nesting and denning sites for a wide range of hollow-dwelling mammals and reptiles (Butler *et al.* 2002; Gibbons and Lindenmayer 2002; Lindenmayer *et al.* 1999a; Williams and Faunt 1997), can support numerous species of bryophytes and lichens (Andersson and Hytteborn 1991; Jarman and Kantvilas 2001; Kruys *et al.* 1999), invertebrates (Ehnström 2001; Jonsell and Weslien 2003; Kaila *et al.* 1997) and fungi (Edmonds and Lebo 1998; Høiland and Bendiksen 1996; Lumley *et al.* 2000; Nakasone 1993; Nordén and Paltto 2001) and can act as a carbon, nutrient and moisture source for seedlings of vascular plants (Harmon and Franklin 1989; Swift 1977). In forests in Finland, almost a quarter of the 5000 known forest dwelling species may depend on dead-wood habitats to some extent (Siitonen 2001) and logs are one of the most important elements supporting red-listed forest species in Sweden (Berg *et al.* 1994).

The relationship between logs and wood decay fungi is complex, as wood decay fungi are thought to have two different types of relationships within logs. Fungi can be thought of as habitat creators which support or create habitat for other species, or they can be a diverse group of organisms in their own right. Studies in the northern hemisphere have shown that logs support an extremely diverse range of fungi (e.g. Andersen and Ryvarden 2001; Boddy 2001; Edmonds and Lebo 1998; Heilmann-Clausen and Christensen 2005; Høiland and Bendiksen 1996; Lumley *et al.* 2000; Nakasone 1993; Nordén and Paltto 2001). In Fennoscandia, for example, 1500 species of macrofungi are known to be associated with dead wood habitats such as logs (Siitonen 2001). At the same time, wood decay fungi are central to the formation of the decayed wood habitat utilised by other organisms such as saproxylic invertebrates and hollow-dwelling mammals (Ausmus 1977; Käarik 1974; Kirk and Cowling 1984; Lewis 1996; Simpson and Eldridge 1986; Worrall *et*

al. 1997). The type of decayed wood habitat formed, the community composition of wood decay fungi within the decayed wood, and the fruitbodies growing on the outside of logs can profoundly influence the other organisms present within the log habitat (Jonsell *et al.* 2005; Muller *et al.* 2002). Just as different types of fungi are thought to be associated with a particular rot type (see Chapter 5; Dix and Webster 1995; Käarik 1974; Kirk and Cowling 1984; Rayner and Boddy 1988; Tanesaka *et al.* 1993; Worrall *et al.* 1997), saproxylic beetles and other wood-dwelling organisms can also show preferences for particular rot types (Edmonds and Marra 1999; Yee 2005).

Recent changes in Australian forest management requirements have resulted in increased consideration of biodiversity conservation issues. This, in turn, has lead to an increased interest in research examining the broader biological diversity associated with forest ecosystems (e.g. Brown *et al.* 2001; Hickey *et al.* 2001; Lindenmayer and Franklin 1997; McCarthy and Lindenmayer 1998). Previously, the majority of studies of Australian wood inhabiting fungi were conducted from the perspective of the economic impact of decay on timber products (e.g. Barry *et al.* 2003; Shearer and Tippett 1988; Tamblyn 1937; Wardlaw 2003) and logs were largely ignored as substrates for fungi. In Tasmania, logs are clearly established as important habitat for mammals (Gibbons and Lindenmayer 2002), invertebrates (Grove *et al.* 2002; Grove and Bashford 2003; Meggs 1996; Yee 2005) and bryophytes (Jarman and Kantvilas 2001) yet their importance for fungi is less well known.

This chapter describes a study conducted in collaboration with Yee (2005), that examines some of the more cryptic biota associated with *Eucalyptus obliqua* logs in southern Tasmania. Yee (2005) described 11 rotten wood types and 360 species of saproxylic beetles from large (>85 cm) and small (30-60 cm) diameter logs in mature and regenerating wet sclerophyll forests. This chapter builds on work by Z-Q Yuan who isolated the wood decay fungi associated with these same logs (Mohammed and Yuan 2002; Yee *et al.* 2001). It takes Yuan's work a step further by attempting to identify these fungi using molecular methods. In addition, this study looks at the relationship between wood decay fungi and rotten wood type in an attempt to better understand the complex processes of habitat creation and development within log substrates.

6.2 Methods

Sample collection (prior to this study)

This study is based on fungal isolates collected by Yee and Yuan (Yee 2005; Yee *et al.* in press; Yee *et al.* 2001) from logs in the wet sclerophyll forests in southern Tasmania. For detailed site and collection descriptions refer to Section 2.2. Yee and Yuan (Yee 2005; Yee *et al.* 2001) collected these fungi from logs at six sites in two different forest types; three sites in mature, unlogged forest and three sites in forest regenerating from logging 20-30 years ago. At each site, three small diameter logs (30-60 cm) and three large diameter logs (>85 cm diameter) were examined for fungi. Thin disks were cut from each log at six points along its length and the cut face of each disc was examined for decay. To adjust for differences in disc size, only one quarter of each disc was examined for the large diameter logs, taken from a standard location on each disc. Where decay was found, samples of decayed wood were obtained and incubated on specialised media to isolate any associated Basidiomycetes and Ascomycetes (Section 2.3.2). The 758 isolates of wood decay fungi obtained were sorted into 63 major morphospecies and 77 ungrouped isolates, based on their macro-and microscopic characteristics (Mohammed and Yuan 2002). Only those 60 morphospecies which occurred on more than one log section are considered in this chapter.

Molecular determination of wood decay fungi

The morphospecies of fungi, as determined by Yuan (Mohammed and Yuan 2002; Yee *et al.* 2001), were assessed in this study by sequencing the internal transcribed spacer region of the ribosomal DNA. Between two and ten isolates from each morphological group were selected for sequencing, chosen to represent the full range of log/site variation within each group. If sequence variation was found, further isolates were sequenced to enable the determination of multiple species. For all but four of the morphological groups, sequence alignments confirmed the morphological groupings of fungi. Where sequence variation was observed within a morphological group, the morphology of the original cultures (now dried plates) was re-examined to look for morphological variation which matched the sequence variation. The origin of each isolate (forest type, log size, site and rot type) was also compared to determine whether sequence variation was related to isolate source. For

more details of this process refer to Section 2.2. The resultant 60 putative species are used for the remainder of this study. Public databases were searched for the best match to consensus sequences from the final fungal species groupings using Blast (Altschul *et al.* 1997) to assist in providing tentative identifications.

Mycelial cultures from identified fungal fruitbodies from a number of sources to form a reference collection of named cultures were also obtained. These included collections carried out concurrently with the study, collections of additional Tasmanian material and access to reference collections within Australia (Section 2.4.3). The sequence information from the identified fungi in the reference collections was compared to those of the isolates in this study. Similarly, sequence information of isolates obtained as part of study 1 (see Chapters 2 and 3) was compared with sequences of these fungi isolated from logs. Sequences were aligned using ClustalW (Thompson *et al.* 1994) and dendrograms were created with DNAml of the Phylip package (ANGIS; Felsenstein 1989) and viewed in TreeView (Page 2001). Sequences were considered likely to belong to the same species if there was less than 1-2% variation between them, however this variation did depend on the species involved (for more information see Section 3.4 or Glen *et al.* 2001a). Fungal species names follow those of Kirk *et al.*(2001).

Correlations between decay and fungi

The rot types found in the logs in this study had been previously determined and described by Yee (2005). Indicator Species Analysis was run in PC-ORD for Windows 4.25 (McCune and Mefford 1999) and was used to investigate whether particular species of fungi were significantly associated with the specific rot types. A cut-off value of IndVal $\geq$25, p<=0.05 was used. Untransformed species abundance data were used, where frequency data (i.e. the number cut faces a species was isolated from) was used as a surrogate measure of abundance.

Enzyme tests were carried out on all fungal isolates to detect the production of the wood degrading enzymes laccase and tyrosinase (Stalpers 1978) and thus help to confirm any relationships between fungi and rot types. For each fungal isolate, the presence of laccase (a lignin-degrading enzyme) and the presence of tyrosinase (a cellulose-degrading enzyme) were determined by applying one drop of each of two solutions to the culture margin. Isolates were monitored for enzyme colour changes

after 3 hours, 24 hours and 72 hours (Stalpers 1978). Each enzyme test was performed at least twice to test for reliability (Section 2.4.1). The final results presented here only indicate the presence or absence of each enzyme, not the time the reaction took to appear nor the intensity of the colour change, as this is not known to be a reliable indicator of the decay capacity of the fungal isolate (T. Wardlaw, pers. comm. 2004). Fungi were divided into four rot types, depending on the results of the enzyme tests (Table 6.2.1). Soft rot fungi are included with the white rotters as the drop tests used could not separate them (Worrall *et al.* 1997).

Table 6.2.1. Classification of fungal isolates into rot type, as measured by the production of laccase and tyrosinase enzymes in culture.

Rot Type	Laccase	Tyrosinase
None (No enzymes produced)	x	x
Selective White	√	x
Simultaneous White	√	√
Brown	x	√

Correlation between fungal communities, decay and beetles

Mantel tests were performed in PC-ORD for Windows 4.25 (McCune and Mefford 1999) to determine whether the patterns of fungal community distribution were similar to the patterns of community distribution of saproxylic beetles in the same logs described by Yee (2005). Non-metric Multi-dimensional Scaling (NMS) ordinations prepared in PC-ORD were compared for each group. NMS was performed using a Sorensen (Bray-Curtis) distance measure choosing the slow and thorough autopilot method. The fungal data were based on log transformed frequency data, while for the saproxylic beetle data were based on the collaborative work of Marie Yee (University of Tasmania) and the NMS was performed using log-transformed abundance data (Yee 2005). The Mantel tests gave p values based on randomisation (Monte Carlo) tests using 1000 randomised runs.

6.3 Results

Comparing molecular and morphological groups

More than 150 isolates were sequenced in this study, representative of 42 of the 60 common or most abundant species of wood decay fungi. The remaining 18 morphological species described by Yuan (Mohammed and Yuan 2002) were not sequenced due to contamination of stored isolates prior to the commencement of this study. Blast searches using all 150 isolate sequences were carried out on public databases (e.g. GB, EMBL, DDBJ) and a good indication of species identity was obtained for 24 of the 60 species (Table 6.3.1). Six of these species showed very high similarity to sequences from known species, giving a high degree of confidence of the identity of the fungus (e.g. *Armillaria hinnulea, Hypholoma fasciculare*). A further 12 species were able to be identified to family/genus, while the remaining six could be reliably matched only to division. Sequence identifications were not obtained for the remainder of the species either due to low sequence matches with public databases (4 species) or difficulty obtaining reliable sequences (14 species).

Five of the sequenced species produced strong matches with sequences obtained from cultures of wood decay fungi from the living trees described in Chapter 3. These were *Fomitopsis*-like sp. 1, Xylariaceae sp. 1, *Postia pelliculosa* and *Postia*-like species 4 and 5. No useful information was obtained from the reference collections beyond that achieved with searches of public databases.

Table 6.3.1. Results of searches for sequences matches in GenBank with putative species. Sequence match quality indicates the value of the taxonomic information obtained from the Blast search, based on percentage similarity and length of matching region. Sequences are shown in Appendix 1.

Final Species Group	Closest Blast Match	Number of bases matched[1]	Percentage Match	Origin of Closest Match	Sequence Match Quality
Armillaria hinnulea	*Armillaria hinnulea*	448/456	99%	Australia	Good
Ascomycete sp.2	Ascomycete from solid wood	443/463	96%	Sweden	Moderate
Athelia-like sp.3	*Athelia decipiens, Fibulorhizoctonia centrifuga, A. epiphylla*	541/571	94%	USA	Moderate
Basidiomycete sp.5	*Laetiporus sulphureus*	195+22 /478	97%	Germany	Low
Basidiomycete	*Diplomitoporus*	566/610	92%	UK	Low

Final Species Group	Closest Blast Match	Number of bases matched[1]	Percentage Match	Origin of Closest Match	Sequence Match Quality
sp.6	*lindbladii, Veluticeps fimbriata, Poria subvermispora*				
Basidiomycete sp.7	*Tyromyces chioneus*	392 + 101/553	96%	India	Moderate
Basidiomycete sp.8	*Laetiporus sulphureus*	195/402	97%	Germany	Low
Basidiomycete sp.9	*Hyphoderma setigerum, Phlebia radiata*	167/371	100%	Sweden	Low
Ceriporiopsis sp.1	*Ceriporiopsis sp., Ceriporiopsis gilvescens*	543/555	98%	Sweden	Good
Coniophora-like sp.3	*Coniophora olivacea, C. marmorata*	435/460	95%	Sweden	Moderate
Fomitopsis-like sp.1	*Fomitopsis rosea*	534/584	91%	Norway	Low
Ganoderma applanatum/ adspersum	*Ganoderma applanatum, G. adspersum*	620/622	100%	Indonesia	Good
Ganodermataceae sp.1	*Amauroderma subresinosum*	337/540	92%	Indonesia	Low
Hypocrea pachybasioides	*Hypocrea pachybasioides*	565/568	99%	Austria	Good
Hypholoma fasciculare	*Hypholoma fasciculare*	291/301	99%	Spain	Good
Phialophora-like sp.1	*Phialophora* spp.	303/360	96%	Sweden	Moderate
Phlebia-like sp.2	*Phlebia tremellosa, Ceriporiopsis* sp.	351/546	93%	Sweden	Low
Postia pelliculosa	*Postia pelliculosa, P. subcaesia, P. caesia*	530/533	100%	Tasmania	Good[2]
Postia-like sp.4	*Oligoporus rennyi*	300/497	94%	UK	Low
Postia-like sp.5	*Postia balsamea, Postia subcaesia*	348+78 +29/622	91%	UK	Low
Postia-like sp.8	*Postia balsamea, P. subcaesia*	346/476	91%	UK	Low
Postia-like sp.9	*Postia balsamea, Postia caesia*	452/528	91%	UK	Low

Final Species Group	Closest Blast Match	Number of bases matched[1]	Percentage Match	Origin of Closest Match	Sequence Match Quality
Postia-like sp.10	*Postia subcaesia, P. balsamea*	470/565	87-90%	UK	Low
Xylariaceae sp.1	Xylariaceae sp.	463/468	99%	USA	Good

[1]Length of matching sequence/full length of sequence
[2]Sequence match with *P. pelliculosa* sequence from reference database.
NB. Species names correspond to those used in Chapter 3.

Species richness

In this study, 60 species of wood decay fungi were examined, based on their isolation from 36 logs sampled as part of a previous study by Yuan (Mohammed and Yuan 2002; Yee *et al.* 2001). Of the 28 species which were able to be identified, four were Ascomycetes, one of which fell within the wood rotting Xylariaceae. The remaining 24 species, all Basidiomycetes, matched sequences from a range of genera including six *Postia*-like species, two Ganodermataceae and nine species of uncertain family (Table 6.3.2). Only 16 of the 60 species occurred in more than 10% of logs and six occurred in more than 25% of logs.

Table 6.3.2. Final species (following taxonomy of Kirk (2001)) correspond to the species names used in Chapter 3. Basidiomycete species 10-13 are Basidiomycete species for which a poor sequence match was found. The 32 un-named log decay species are not included in this table. Isolation frequency refers to the number of individual cut faces from which a species was isolated.

SPECIES	ISOLATION FREQUENCY	ENZYME TESTING
ASCOMYCOTA		
Incertae sedis		
Ascomycete sp.2	3	Selective white rot
Phialophora-like sp.1	10	No enzymes produced
Hypocreaceae		
Hypocrea pachybasioides Yoshim. Doi	33	No enzymes produced
Xylariaceae		
Xylariaceae sp.1	8	Selective white rot
BASIDIOMYCOTA		
Incertae sedis		
Basidiomycete sp.5	19	No enzymes produced
Basidiomycete sp.6	80	Selective white rot
Basidiomycete sp.7	14	Selective white rot
Basidiomycete sp.8	14	No enzymes produced
Basidiomycete sp.9	25	Selective white rot
Basidiomycete sp.10	10	No enzymes produced

Basidiomycete sp.11	23	No enzymes produced
Basidiomycete sp.12	6	No enzymes produced
Basidiomycete sp.13	16	No enzymes produced
Marasmiaceae		
Armillaria hinnulea Kile & Watling	48	Selective white rot
Strophariaceae		
Hypholoma fasciculare (Huds.) P. Kumm.	2	Selective white rot
Coniophoraceae		
Coniophora-like sp.3	2	No enzymes produced
Atheliaceae		
Athelia-like sp.3	4	Selective white rot
Fomitopsidaceae		
Fomitopsis-like sp.1	81	Brown rot
Postia pelliculosa (Berk.) Rajchenb.	9	Simultaneous white rot
Postia-like sp.4	13	Simultaneous white rot
Postia-like sp.5	28	Brown rot
Postia-like sp.8	11	Brown rot
Postia-like sp.9	7	Brown rot
Postia-like sp.10	25	Simultaneous white rot
Ganodermataceae		
Ganoderma applanatum /adspersum	20	Selective white rot
Ganodermataceae sp.1	5	Selective white rot
Hapalopilaceae		
Ceriporiopsis sp.1	25	Simultaneous white rot
Meruliaceae		
Phlebia-like sp.2	52	Simultaneous white rot

Enzyme testing

Ten of the 28 species listed in Table 6.3.2 were selective white rotters, five were simultaneous white rot species and four species showed brown rot enzyme activity. The remaining nine species showed negative responses for both tyrosinase and laccase.

Correlations between fungi and rot types

Of the sixty species of wood decay fungi isolated from logs in this study, more than half of the species (32) were found to be isolated at least once from discoloured wood. The most species rich decay type was the fibrous surface rot with 28 species,

while red-brown blocky, fibrous rot housed 21 species of fungi. Only six species were isolated from the yellow dry, slatey rot.

Table 6.3.3. The number of species of fungi associated with each rot type found in 36 *E. obliqua* logs. The rot types and rotted wood region are those described by Yee (2005). Rotten wood regions are: SF-surface, OH-outer heartwood, H-heartwood, IH-inner heartwood. The total number of species of fungi found was 60.

Rot Type	Rotted Wood Region	Number of Associated Fungal Species
Fibrous surface rot	SF	28
White jelly surface rot	SF	12
White stringy rot	OH/H	19
White pocket rot	OH	14
Yellow dry slatey rot	OH	6
Brown cubic spongy rot	OH	14
Discoloration	H	32
Brown blocky crumbly rot	IH	15
Brown mudgut rot	IH	12
Red brown blocky fibrous rot	IH	21

Indicator species analysis found five species to be significantly associated with five of the specific rot types categorised in Yee (2005, Table 6.3.4). *Armillaria hinnulea* was significantly correlated with surface white jelly rot. *Phlebia*-like sp.2 and *Ganoderma applanatum/adspersum* were associated with the outer heartwood rot types white pocket rot and white stringy rot respectively. Two inner heartwood rots, red-brown blocky, fibrous rot and brown blocky, crumbly rot were found to be indicated by the fungi Basidiomycete sp.5 and *Postia*-like sp.5 respectively. The relationship between the five species of fungi and the rot types was predominantly confirmed by the enzyme tests of the fungi. For Basidiomycete sp.5, neither tyrosinase nor laccase was produced in culture, despite the fungus being associated with a brown rot type.

Table 6.3.4. Relationship between fungi and rot types in logs. The rot types and rotted wood (RW) region are those described in Yee (2005). Rotten wood regions are: SF-surface, OH-outer heartwood, H-heartwood, IH-inner heartwood. Enzyme production related to the results of enzyme drop testing undertaken in this study. Only those species which showed significant associations with particular rot types (using indicator species analysis) are shown.

Indicator Species	Rot type	RW Region	Enzyme Production	Rot Description	P*
Armillaria hinnulea	White jelly rot	SF	Selective white rot	Surface (bark) jelly pocket rot often with black zone lines.	0.001
Phlebia-like sp.2	White pocket rot	OH	Simultaneous white rot	Hard pockets, with white cellulose (sometimes yellow mycelium) in pockets lined with hard wood	0.003
Ganoderma applanatum/adspersum	White stringy rot	OH/H	Selective white rot	Soft pockets, almost indistinguishable, very fibrous, soft and spongy, pure celluloses, looks like fine wool	0.007
Basidiomycete sp.5	Red-brown-blocky fibrous rot	IH	No enzymes produced	Red-brown hard fibrous rot	0.046
Postia-like sp.5	Brown blocky crumbly rot	IH	Brown rot	Light brown to dark brown cubic rot, dry chalky brittle often with white mycelium seams or flecks	0.043

*the p value obtained from indicator species analysis

Correlation between fungal communities, decay and beetles

A Mantel test indicated that there was a highly significant positive correlation between the patterns of distribution of fungal communities and the saproxylic beetle assemblages found in the same logs by Yee (2005; p=0.001), yet the relationship was weakly correlated (r=0.201).

6.4 Discussion

This study is the first to use molecular techniques to attempt to identify the wood decay fungi present within *E. obliqua* logs. Sixty species of wood decay fungi were isolated more than once from just 36 logs. This number of species is within the range of the values found in many other studies of wood decay fungi on logs (e.g. Allen *et al.* 2000; Andersen and Ryvarden 2001; Chapela *et al.* 1988; Gustafsson 2002; Heilmann-Clausen and Christensen 2003; Renvall 1995). Comparisons of

species richness with other studies are not meaningful, however, as there are many factors that are known to influence the species richness of fungi in logs including decay stage, log species, and log size (Heilmann-Clausen and Christensen 2003; Høiland and Bendiksen 1996; Niemelä *et al.* 1995; Pyle and Brown 1999; Renvall 1995; Vasiliauskas 1998). In addition, the results of this study are based on isolations of fungi from decayed wood rather than fruitbody surveys. The sampling method has a large influence on the species detected (as discussed later Hunt *et al.* 2004). Using a wood cutting method similar to that used in this study, Fukasawa *et al.* (2005) isolated 10 common species of fungi from five Japanese beech logs and in Sweden, 25 fungal species were isolated from 10 recently dead Norway spruce logs (Gustafsson 2002). However, these studies were not based on *Eucalyptus* logs and their isolation techniques and media were not identical to those used here.

The majority of species which were able to be identified at least to family or genus level showed strong similarities to well-known decay species, mostly polypore and corticioid species. These include the common decay species *Armillaria hinnulea, Ganoderma applanatum/adspersum, Hypholoma fasciculare, Postia pelliculosa* and putative species of *Athelia, Coniophora, Fomitopsis, Phlebia, Ceriporiopsis* and *Postia* (Kile 1981; Kile 2000; Kile and Johnson 2000; Schwarze *et al.* 2000). In a study of fungal fruitbodies in the wet forests in southern Tasmania, Packham *et al.* (2002) found *A. hinnulea, H. fasciculare, Xylaria* spp. and an unidentified resupinate species to be common in mature forests. Similarly, *A. hinnulea, H. fasciculare* and *Postia* spp. were also commonly found on wood in a fruitbody survey at Warra LTER (Gates *et al.* 2005) and throughout Tasmania (Ratkowsky and Gates 2005). Genera such as *Athelia, Coniophora, Phlebia* and *Ceriporiopsis* have also been previously recorded on *Eucalyptus* logs, although are reported less frequently from fruitbody surveys, perhaps due to their more cryptic habit (Buchanan 2001; Gates *et al.* 2005). Interestingly, no species of *Stereum, Trametes, Gymnopilus* or Hymenochaetaceae were recorded in the current study, despite being commonly recorded as fruitbodies on dead wood substrates in Tasmania (Gates *et al.* 2005; Packham *et al.* 2002; Ratkowsky and Gates 2005) and forming part of the reference collection.

One of the surprising outcomes of this study was the lack of matches between the study isolates and those in the reference collection. The reference collection

contained a wide variety of material collected both locally and within other states in Australia and included many of the species considered as important wood decay fungi. While this is probably a reflection of the diversity of wood decay fungi in Australia and the lack of detailed studies previously published in this area, there are a number of other possible reasons. The reference collection contained very few corticioid species, yet sequence matches with public databases indicate that a number of the fungi found in this study were corticioid. Corticioid fungi are often overlooked in fruitbody surveys due to their cryptic habit and their difficult taxonomy (Buchanan 2001). Also although two of the reference collections used in this study targeted logs, the fungi from the CSIRO Clayton Collection were primarily isolates from fruitbodies on wood in service, not wood in natural systems and thus may reflect an entirely different suite of fungi. The isolates from the Clayton Collection were also collected internationally not within Australia. The low matching between the reference collection and study isolates also reinforces the findings of Johannesson and Stenlid (1999) that the fungi commonly fruiting on wood do not necessarily accurately portray the fungal taxa within.

Strong relationships were found between five species of fungi and five of the rot types described by Yee (2005). The strong relationship between *Armillaria hinnulea* and white jelly surface rot is quite likely to be a causal relationship. *Armillaria hinnulea* is a commonly described wood decay fungus in Australia, known to cause white rot in the roots and butt of living *E. obliqua* (T. Wardlaw, pers. comm. in Kile 2000) in the wet sclerophyll forests of south eastern Australia (Kile *et al.* 1991). The strong relationship between *A. hinnulea* and white jelly rot found in this study is highly likely to be causal, since *Armillaria* species are known to cause white rot, with characteristic black zonation and mycelial strands (Garraway *et al.* 1991). In addition, isolates of *A. hinnulea* from this study produced strong positive reactions for the presence of laccase, a lignin-degrading enzyme. As a fungus which primarily spreads above ground using rhizomorphs (Redfern and Filip 1991), or into the tree stem via the roots (Wardlaw 2003), it is likely to colonise from the outside of the log in, once the log has fallen, as has been proposed for the white surface jelly rot (Yee 2005). In contrast with other species of *Armillaria* (such as *A. luteobubalina*) *A. hinnulea* is considered only weakly pathogenic or saprotrophic and will only affect

trees which are already stressed or damaged (Kile 2000), indicating that it is also likely to colonise logs.

Similarly, it is highly plausible that *Ganoderma applanatum/adspersum* is the primary fungus causing white stringy rot. Like *Armillaria* species, *Ganoderma* species are recognised world-wide as the cause of root rot and the cause of a soft white rot in the butt and sapwood of their hosts (Kile 2000). *Ganoderma applanatum* has been described causing white rot in plantation eucalypts in Portugal and the USA (in Kile and Johnson 2000) and in tropical *Acacia* species in Indonesia (Glen *et al.* 2006a). In this current study, *G. applanatum/adspersum* was strongly correlated with a white stringy rot which was described as having soft, almost indistinguishable pockets, being very fibrous, soft and spongy with the appearance of fine wool (Yee 2005); potentially similar to the rot previously identified as caused by *G. applanatum* above and *Ganoderma* species in general (Schwarze *et al.* 2000). Enzymatic evidence also supports this case. The stringy white rot in this current study was found primarily in the heartwood region of the log, indicating that it could either have been present in the log prior to tree fall (as a root or butt rot species) or could have colonised following tree fall from the surface of the log inwards. Either case is a possibility for *Ganoderma* species as they have been previously observed on both living trees and coarse woody debris (Kile 2000; Kile and Johnson 2000; Schwarze *et al.* 2000).

It is difficult to draw conclusions about the relationships between the three remaining fungi and their rot types, since the sequence identifications were not conclusive. In all three cases, the results of the enzyme tests provide some support for a causal relationship between the fungi and their rot types and these are further confirmed where the species were identified to putative genera. Species of *Phlebia* are known to cause white rot in living oaks (*Quercus* species, Berry and Lombard 1978), *Picea abies* logs (Bader *et al.* 1995; Renvall 1995; Rolstad *et al.* 2004), *Pinus sylvestris* logs (Sippola and Renvall 1999) and *Corylus avellana* logs (Nordén and Paltto 2001) although the type of white rot has never been thoroughly described. In the current study, enzyme testing suggests that *Phlebia*-like sp.2 is capable of causing white rot. The presence of yellow mycelium associated with this rot type supports this case, as *Phlebia*-like sp.2 has "duckling-yellow" mycelium in culture (Mohammed and Yuan 2002).

Both Basidiomycete sp.5 and *Postia*-like sp.5 were found to be strongly associated with brown rot types from the inner heartwood of logs. That Basidiomycete sp.5 did not produce any enzymes when tested in culture does not mean it does not have brown rot capacity. It is relatively common for brown rot species not to respond positively for tyrosinase (Worrall *et al.* 1997) and some isolates can lose their decay capacity once in culture (Chapter 5 Tanesaka *et al.* 1993). *Postia* species are generally considered to be brown rotters (Breitenbach and Kranzlin 1986; Yao *et al.* 2005) and are commonly found on living trees and coarse woody debris in Europe and North America (Yao *et al.* 2005). Enzyme testing of *Postia*-like sp.5 confirmed its brown rot ability by showing an unusually strong positive reaction for tyrosinase. *Postia*-like sp.5 had white mycelium which could account for the flecks of white mycelium in the brown blocky crumbly rot. The ITS sequence and morphology of *Postia*-like sp.5 were identical to those of *Postia*-like sp.5 isolated from the heartwood of the mature living trees in study 1 (Chapters 3 and 4). In study 1, this species was not found to be strongly associated with a particular rot type, however it was only found in very low numbers. Given that this species was found in the heartwood of mature living trees, it is quite plausible that it would also be associated with rot in the inner heartwood of logs.

That there is some correlation between the distribution of wood decay fungi and saproxylic beetles is not surprising given the close relationship both groups of organisms have with dead and decaying wood (Anderson 2001; Boddy 2001; Rayner and Boddy 1988; Speight 1989) and with each other (Anderson 2001; Lawrence 1989; Lawrence and Milner 1996; Muller *et al.* 2002). Yee (2005) found that a number of beetle species displayed preferences for specific rotten wood types and this preference for rotten wood types has now also been shown for several species of fungi. For the majority of fungi the details of these specific relationships are not entirely clear, as little is known about the biology and ecology of the organisms involved. It is possible that the rotten wood type is created by the fungi and that the physical conditions of the rotten wood are highly favourable to particular beetles (Yee 2005). Alternatively, the beetles and fungi may be much more closely linked; many species of beetles are known to be mycophagous and a few of these, such as the ambrosia beetle, are known to form close associations with specific fungi which break down the wood into a more palatable food source

(Gilbertson 1984; Lawrence 1989; Lawrence and Milner 1996; Speight 1989). Aside from direct associations between beetles and fungi, it is possible that both organisms are similarly affected by environmental conditions; where changes in environmental conditions occur, the community composition of fungi and beetles also changes (see Chapter 7).

6.4.1 Conclusions

This study used a destructive sampling method to examine the wood decay fungi in logs in the wet sclerophyll forests of southern Australia. It has recorded the diversity of wood decay fungi present in these habitats and, by highlighting gaps in our knowledge, has illustrated the need for more taxonomic and ecological study in this area. Strong, credible relationships were found between different species of wood decay fungi and rotten wood types within the study logs. Critically, this study has built on the work of Yee (2005) on the saproxylic beetle fauna present in the same logs and has lent considerable weight to the contention that there is a strong association between fungi, rotten wood and insects in decaying logs.

CHAPTER 7: THE EFFECT OF LOG SIZE AND FOREST TYPE ON THE WOOD DECAY FUNGI INHABITING *EUCALYPTUS OBLIQUA* LOGS

7.1 Introduction

In Australia, the relative importance of coarse woody debris for biodiversity has only recently been recognised (e.g. Grove *et al.* 2002; Lindenmayer *et al.* 1999a; Meggs 1996; Turner and Pharo 2005; Yee *et al.* 2001) and few studies are yet to thoroughly document it. Moreover, knowledge of the taxonomy and ecology of wood-inhabiting organisms in Australia is severely lacking in comparison with European and North American systems (Grove *et al.* 2002; Hopkins *et al.* 2005). This is particularly the case with fungi where less than 5% of the predicted number of species have been named (Hawksworth 1991; May and Pascoe 1996).

Coarse woody debris (CWD), such as logs and large diameter branches on the forest floor, is a key structural and biological resource in forest ecosystems (Franklin *et al.* 1987; Grove *et al.* 2002; Lindenmayer *et al.* 1999a; Nordén *et al.* 2004). Coarse woody debris provides important nesting and denning sites for a wide range of hollow-dwelling mammals and reptiles (Butler *et al.* 2002; Gibbons and Lindenmayer 2002; Lindenmayer *et al.* 1999a; Williams and Faunt 1997) and adds to the amount of physical habitat structure on the forest floor by increasing the range of microclimates and microhabitats available for exploitation by ground dwelling organisms such as mammals, fungi and invertebrates (Evans *et al.* 2003; Grove *et al.* 2002; Siitonen 2001; Sippola *et al.* 2004; Tedersoo *et al.* 2003). In forests in Finland, at least 20% of all known forest dwelling species depend on dead-wood habitats to some extent, with one of the most speciose groups being macrofungi with about 1500 dead wood dependent species (Siitonen 2001).

In the northern hemisphere, forest management practices such as logging and the removal of woody debris, have greatly reduced the volume of CWD within managed forest systems (Acker *et al.* 1998; Jonsson *et al.* 2006; Rudolphi and Gustafsson 2005) to an average of 10% the volume found within old growth stands (Fridman and Walheim 2000; Siitonen 2001). Clearfelling interrupts the continuous availability of logs in different stages of decay and can alter the initial volume and

reduce future accumulation of dead wood in regenerating stands (Fridman and Walheim 2000; Ranius *et al.* 2003). Removal of woody debris or logging residue from the forest system also reduces the amount of CWD in managed systems (Rudolphi and Gustafsson 2005). Given the large number of organisms which are known to depend on CWD for some part of their lifecycle, it is not surprising that a large number of species reliant on CWD are now red-listed in countries where industrial forestry predominates, such as Sweden (Berg *et al.* 1994). Current estimates in Sweden suggest that approximately 90% of red-listed saproxylic species are confined to CWD (Dalhberg and Stockland 2004 in Jonsson *et al.* 2006). In the northern hemisphere, increasing the volume of CWD within managed stands is now a research and management priority (e.g. Acker *et al.* 1998; Jonsson *et al.* 2006; Ranius and Kindvall 2004; Thomas 2002).

These examples from the northern hemisphere point out the effect that intensive forest management may have on wood-inhabiting organisms in Australia over time. Australia has a comparatively short history of intensive forest management, making the Australian research environment very different from that in many parts of the northern hemisphere. In Tasmania for example, clearfelling only began in the early 1960s, so most forests are in their first silvicultural rotation period of 80-100 years (Hickey and Wilkinson 1999). As a result, CWD is still well represented in these forests (Meggs 1996) and there is still time to alter forest management practices to retain CWD in managed stands, should this be required. In many parts of Australia too, large areas of unlogged (oldgrowth) forests are maintained (National Forest Inventory 2005) and so can be easily used as a real time comparison with managed forests.

This study investigates how the current planned 80-100 year silvicultural rotation may affect the diversity of wood decay fungi present in the wet sclerophyll forests in southern Tasmania. Specifically, it investigates the differences in fungal assemblage structure between large diameter logs (>85 cm, representative of mature trees) and small diameter logs (30-60 cm, representative of trees at first rotation age) to examine the potential effects of reduced CWD size on fungal biodiversity. Logs were examined in both mature forest and 20-30 year old forest regenerating from clearfelling to further elucidate the effect of changing forest conditions on fungal assemblage composition. Fungi, as key agents of wood decay and key

components of biodiversity in CWD, are central to understanding and managing coarse woody debris (Heilmann-Clausen and Christensen 2005) and so are the focus of this study.

7.2 Methods

Sample collection (prior to this study)

This study is based on fungal isolates collected by Yee and Yuan (Mohammed and Yuan 2002; Yee 2005; Yee *et al.* 2001) from logs in the wet sclerophyll forests in southern Tasmania. For detailed site and collection descriptions refer to Section 2.2.2. These fungi were collected from logs at six sites in two different forest types: three sites in mature, unlogged forest and three sites in forest regenerating from logging 20-30 years ago. At each site, three small diameter logs (30-60cm) and three large diameter logs (>85cm diameter) were examined for fungal decay. Thin discs were cut from each log at six points along its length and the cut face of each disc was examined for decay. To adjust for differences in disc size, only one quarter of each disc was examined for the large diameter logs. Where decay was found, samples of decayed wood were obtained and incubated on specialised media to isolate any associated Ascomycetes and Basidiomycetes (Section 2.3.2). From the 758 isolates obtained, 60 common species of wood decay fungi were identified, based on examination of their macro-and microscopic characteristics by Yuan (Mohammed and Yuan 2002:Yee, 2001 #37) and their ITS sequences (this study, Chapter 6). These 60 species of wood decay fungi are used for all analyses throughout the rest of this chapter unless otherwise specified.

Species richness

To look for variation in species richness between log size classes and between forest types, both one-way and two-way analyses of variance (ANOVA) were undertaken in SAS 9.1 (Anon. 2002), using log size and forest type as random effects on the number of species of wood decay fungi per log. Presence-absence data were used, pooled from the six standard sampling sections from each log. A follow up multiple comparison test (Ryan-Einot-Gabriel-Welsch Multiple Range Test: REGW test) was used to determine the nature of the differences.

Adjustments for sampling effort

In addition to the reducing sample size of the large logs, rarefaction (Gotelli and Colwell 2001) was used to compare species richness among log size classes, calibrated for sampling effort. Rarefaction curves were calculated against two different variables: the frequency of individuals collected, and the cumulative cross-sectional area of wood faces examined. For each variable, rarefaction curves were calculated using frequency data for all species of fungi. Rarefaction curves were calculated using EstimateS (Colwell 2001) and plotted in Microsoft Excel.

Assemblage composition

Both unconstrained and constrained ordinations were used to explore assemblage composition of fungi in relation to log size and forest type. Log transformed frequency data from each log were used, pooled from the six standard sampling sections. Log transformation was used to reduce the weight given to common species in the analysis (McCune and Mefford 1999). All ordination techniques were performed using the same parameters as described in Chapter 4. In summary, Non-metric Multidimensional Scaling (NMS) was used to explore fungal species assemblage variation between the log size classes and among forest types. Multi-Response Permutation Procedures (MRPP) were then applied to statistically test fungal assemblage differences between log size and among forest type.

Canonical Analysis of Principal coordinates (CAP) (Anderson and Willis 2003), was then used to explore fungal assemblage structure correlated with the two log size classes and two forest types. A similar statistical approach was used to that of Willis and Anderson (2003) where two canonical analyses were conducted. One investigated the effect of forest type, while the other was the used to investigate the effect of log size on fungal assemblages. Since both treatments were binary (i.e. mature versus regenerating forest; large versus small logs) the CAP ordination resulted in a single canonical discriminant axis for each treatment. These canonical axes scores were then plotted against each other to give a multivariate position of each log (Willis and Anderson 2003).

Indicator Species Analysis was run in PC-ORD for Windows 4.25 (McCune and Mefford 1999) and used to investigate whether particular species were significantly associated with specific log size classes or forest types. A cut-off value of IndVal

≥25, p<=0.05 was used. Untransformed species frequency data were used, where frequency was defined as the number cut faces a species was isolated from, as a surrogate for abundance.

7.3 Results

Comparing large and small diameter logs

In this study, 60 species of wood decay fungi were isolated from the 36 logs sampled. Thirty-seven species of fungi were isolated from large diameter logs and 46 from small diameter logs; 23 species were common to both. Of the 16 most abundant species (i.e. those occurring in more than 10% of logs) Basidiomycete sp.9 and *Postia*-like sp.10 were found exclusively in large logs, while *Ceriporiopsis*-like sp.1 was only found in small diameter logs (Figure 7.3.1).

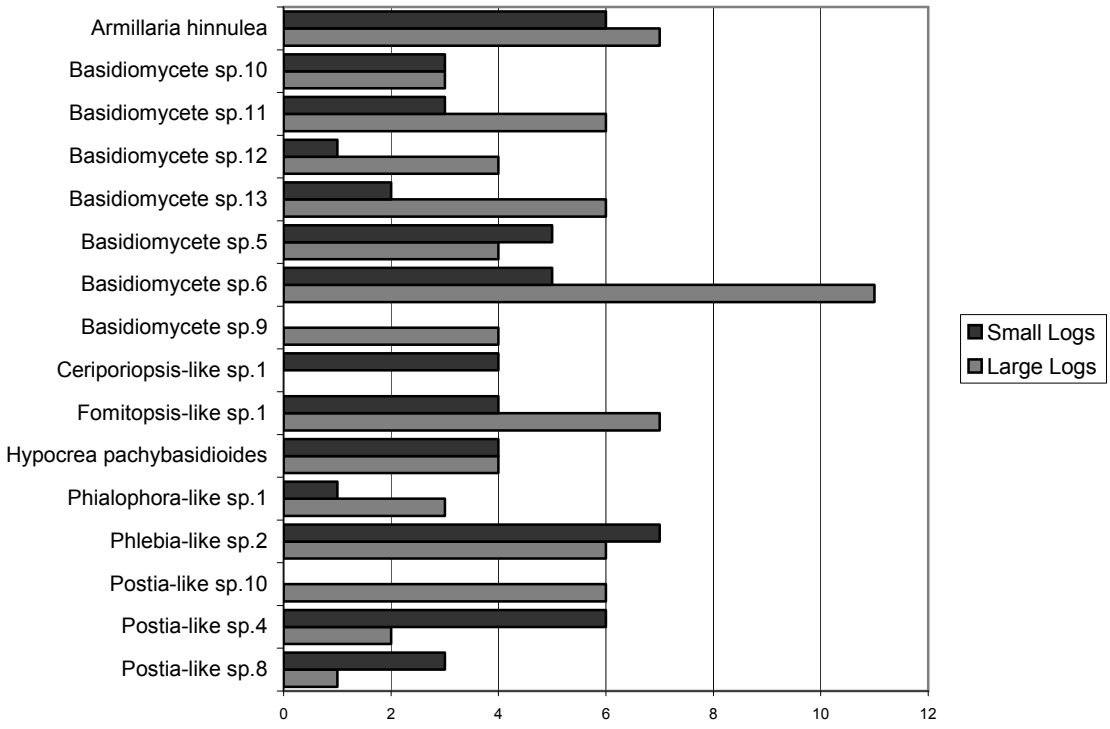


Figure 7.3.1. Frequency of common (>10% of logs) fungi found in 18 large and 18 small diameter logs at an intermediate decomposition stage.

Indicator species analysis found only one species, *Postia*-like sp.10, to be a significant indicator for large diameter logs (p=0.0110). Indicator species analysis found no fungal species to be significantly associated with small diameter logs

(Table 7.3.1). Three species were correlated with each of the canonical axis for large diameter and small diameter logs.

Table 7.3.1 Combined results of correlation analyses based on the canonical axis derived from CAP analysis for log size (Figure 7.3.9), and Indicator Species Analysis for the effect of log size. Species shown in bold also showed a preference for a particular forest type (Table 7.3.2). DSF is the code used for unidentified species.

	Species name	Correlation coefficient with canonical axis	Indicator species analysis IndVal	p value
Large diameter logs	*Postia*-like sp.10	-0.5312	33.3	0.0110
	***Phialophora*-like sp.1**	**-0.3996**		
	Basidiomycete sp.9	**-0.3503**		
Small diameter logs	DSF169	0.421		
	Ceriporiopsis-like sp.1	0.3494		
	DSF70	**0.3168**		

The sample-based rarefaction curves showed that species richness for large and small diameter logs was very similar; although species richness was marginally (although not significantly) greater for the small diameter logs compared with the large diameter logs within the comparable range (Figure 7.3.2a). Separation was enhanced when examining the rarefaction curves for cumulative cross-sectional area (Figure 7.3.2b), with small diameter logs showing significantly more species than large logs.

Unconstrained ordinations (NMS and MRPP) showed that fungal assemblage only differed slightly between log size classes (Figure 7.3.3; p=0.045, T= -1.900). Large diameter logs appeared to have particularly distinct fungi associated with them, while the patterns of fungal assemblage composition for the small diameter logs were less clear.

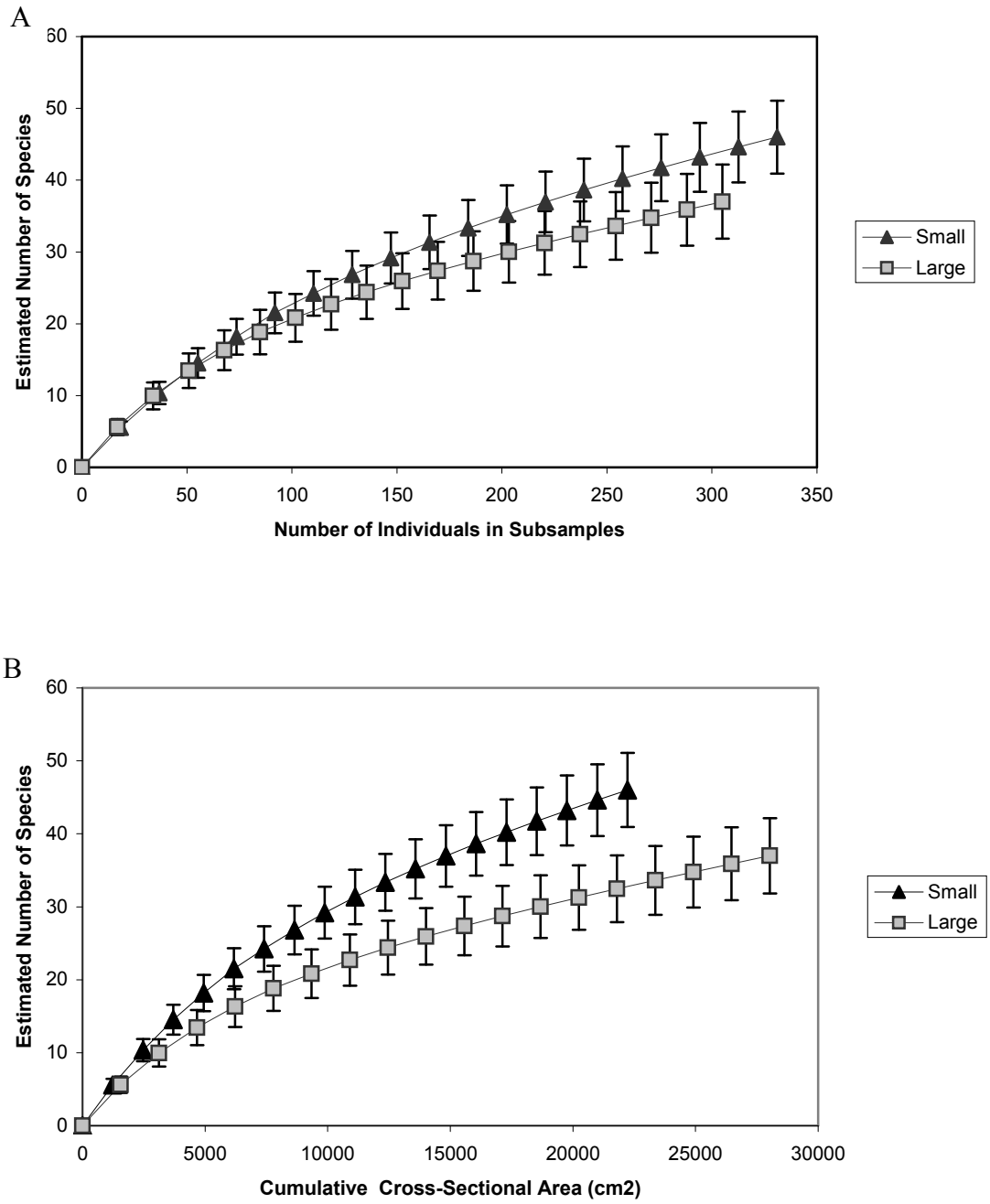


Figure 7.3.2. Rarefaction (Mao Tao) curves for species of wood decay fungi in large and small diameter logs compared by a) number of individuals and b) cumulative cross-sectional area of examined disc.

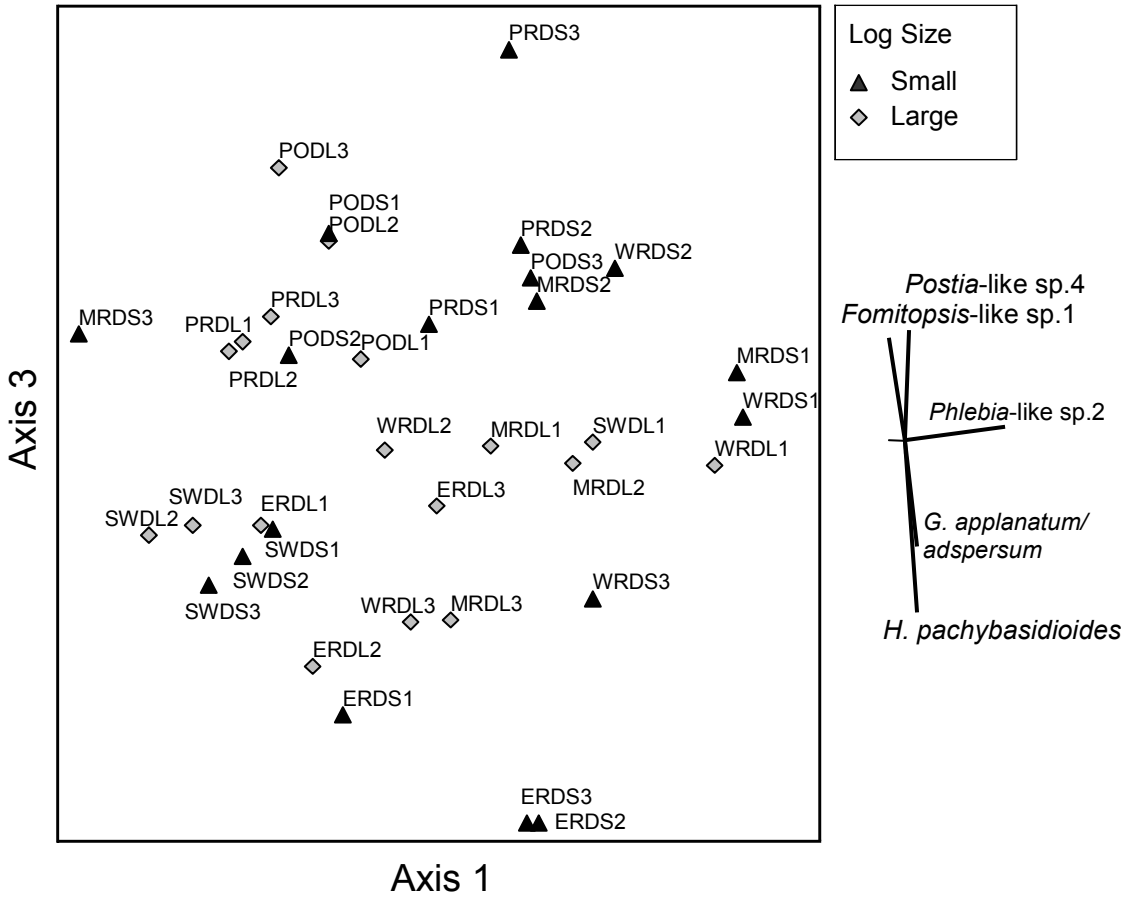


Figure 7.3.3. Non-metric multidimensional scaling (NMS) ordination plot of log transformed species abundance for the 60 most common species of wood decay fungi from 36 logs at six study sites. Only axes 1 and 3 are shown, axis 2 did not add any further information. Symbols denote log size class. Vectors are defined by fungal species abundance data; for greater clarity, these are displayed adjacent to the ordination. Stress = 0.18. Only vectors with r^2>0.2 are shown.

Comparing logs in regenerating and mature forests

Forty-two species of fungi were isolated from logs in mature forest and 35 from logs in regenerating forests; 17 species were common to both. Of the common species (i.e. those occurring in more than 10% of logs) *Phialophora*-like sp.1 was found exclusively in logs in regenerating forests and Basidiomycete sp.5 was significantly more prevalent in mature forests using a Chi^2 test (p=0.019; Figure 7.3.4).

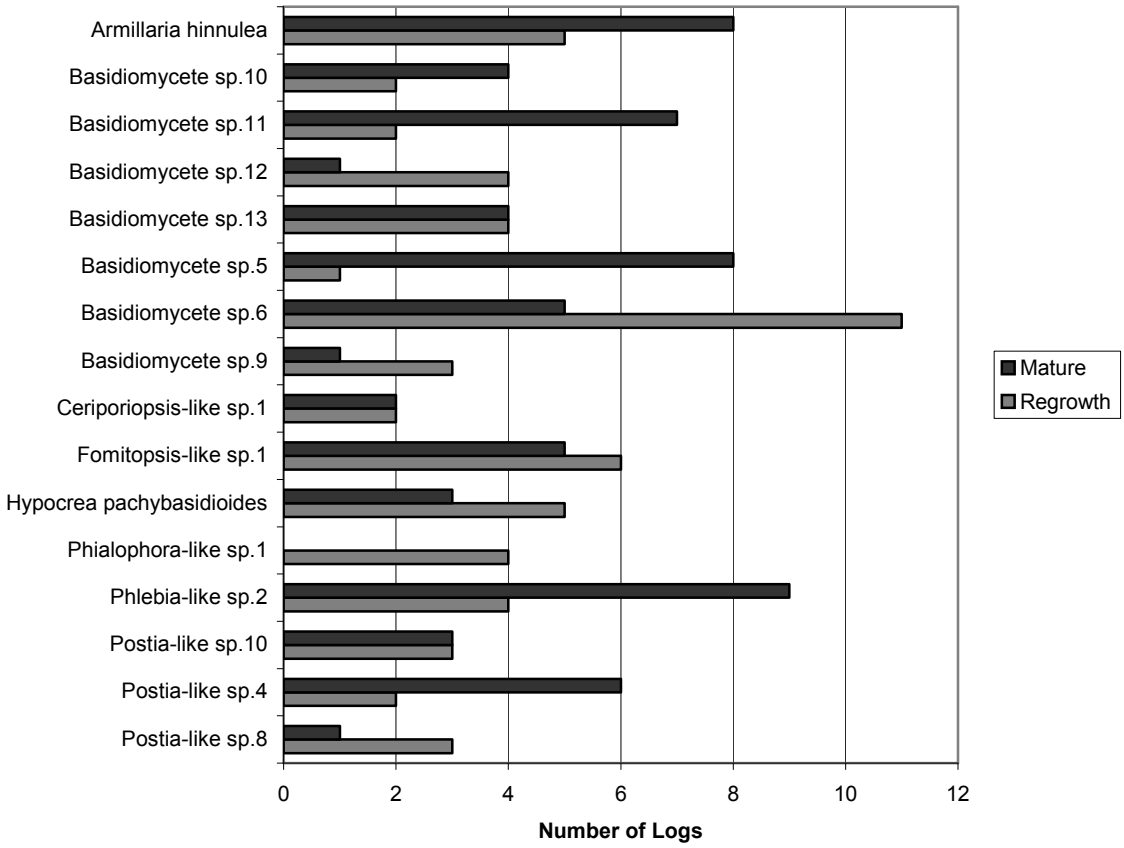


Figure 7.3.4. Frequency of common (>10% of logs) fungi found in 18 logs in mature, unlogged forest and 18 logs in regenerating forest 40 years post-logging. Logs are in an intermediate stage of decomposition.

This was confirmed by indicator species analysis which identified Basidiomycete sp.5 to be a significant indicator for logs in mature forest (p=0.0110). *Phlebia*-like sp.2 and Basidiomycete sp.11 were also found to be associated with logs in mature forest types (p=0.0670, p=0.0710 respectively; Table 7.3.2). Basidiomycete sp.6 was identified as a significant indicator species for logs in regenerating forest (p=0.0080). Three species were correlated with the canonical axis for mature forest and six species correlated with the canonical axis for regenerating forest.

Table 7.3.2 Combined results of correlation analyses based on the canonical axis derived from CAP analysis for forest type (Figure 7.3.9). Result of Indicator Species Analysis for the effect of forest type are also shown for these species. Species shown in bold also showed a preference for a particular log size (Table 7.3.1). DSF is the code used for unidentified species.

	Species name	Correlation coefficient with canonical axis	Indicator species analysis IndVal	p value
Mature forest	*Phlebia*-like sp.2	-0.4407	41.2	0.0670
	DSF70	**-0.3263**		
	DSF75	-0.3076		
Regenerating forest	Basidiomycete sp.6	0.6552	54.1	0.0080
	Basidiomycete sp.12	0.5086		
	***Phialophora*-like sp.1**	**0.4866**		
	Ganoderma-like sp.1	0.3532		
	Basidiomycete sp.9	**0.3334**		
	DSF22	0.3237		

No significant difference in fungal species richness was found between mature and regenerating forests using sample-based rarefaction curves comparing both number of individuals and the cross-sectional log area examined for fungi (Figure 7.3.5).

Unconstrained ordination (NMS) and MRPP showed that fungal assemblage differed with forest type (Figure 7.3.6; p=0.0034, T= -3.59), with logs in mature forests showing a more consistent composition. An overlay of the fungal species groups on the ordination showed that the presence of *Postia*-like sp.4 was strongly influencing the grouping of logs in mature forests. Basidiomycete spp.6 and 12 and *Hypocrea pachybasioides* were strongly influencing the grouping of many of the logs in regenerating forest.

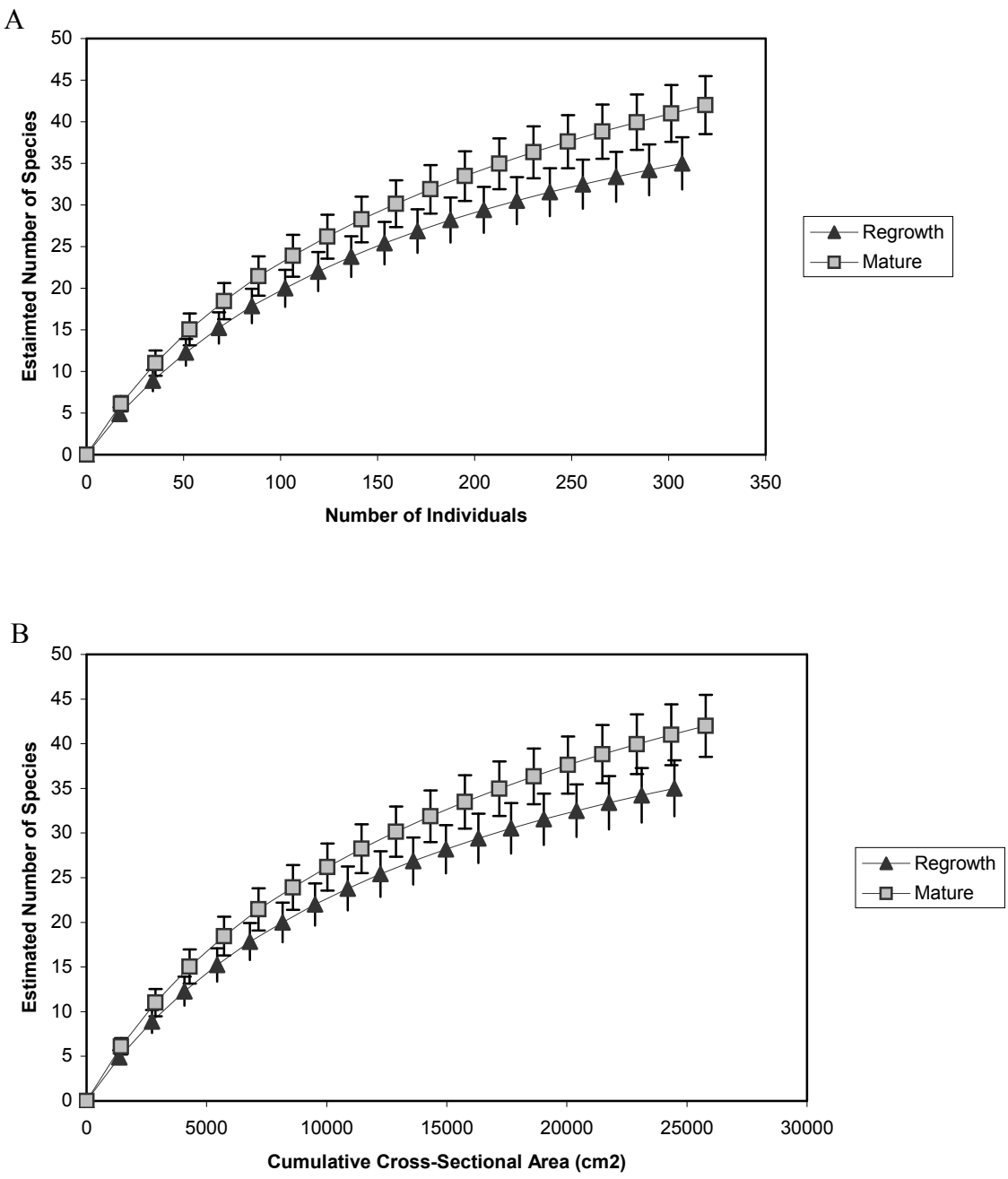


Figure 7.3.5. Rarefaction (Mao Tao) curves for species of wood decay fungi in logs in mature, unlogged forest and logging regenerated forest compared by a) number of individuals and b) cumulative cross-sectional area of examined disc.

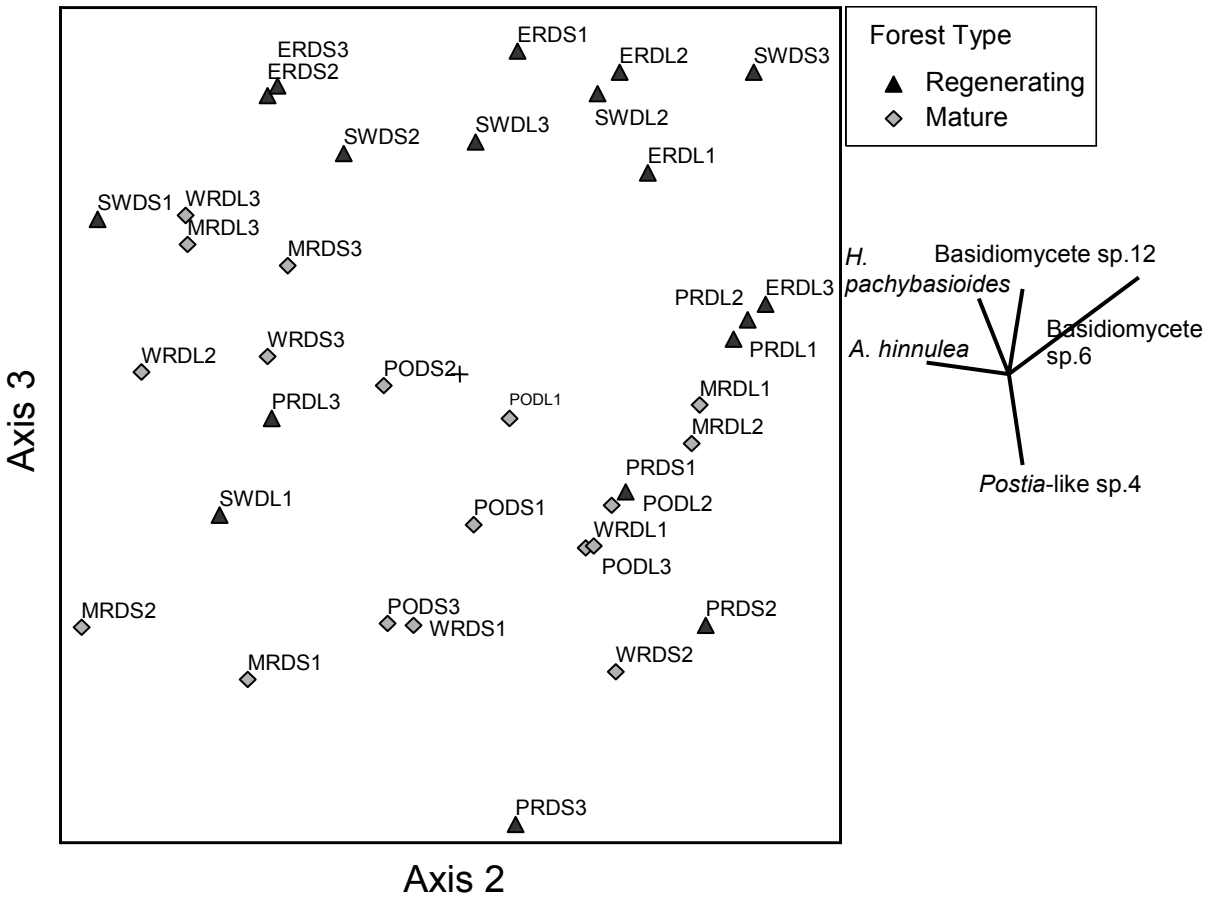


Figure 7.3.6. Non-metric multidimensional scaling (NMS) ordination plot of log transformed species abundance for the 60 most common species of wood decay fungi from 36 logs at six study sites. Only axes 2 and 3 are shown as axis 1 gave no further useful information. Symbols denote forest type. Vectors are defined by fungal species abundance data; for greater clarity, these are displayed adjacent to the ordination. Stress = 0.16. Only vectors with r2>0.2 are shown.

Comparing the effects of log size and forest type

In mature forest, 24 species of fungi were isolated from large logs and 26 species from small logs. In regenerating forests, 26 species of fungi were isolated from large diameter logs while 30 species were isolated from small diameter logs. A summary of the distribution of named species can be seen in Table 7.3.3. Basidiomycete sp.6 and 9 were found to be indicator species for large logs in regenerating forest (p=0.0490 and p=0.0460 respectively). None of the other 14 most common species (i.e. those occurring in more than 10% of logs) were significantly more common in any particular forest type/ log size treatment.

Indicator species analysis found three less common species, DSF61, DSF70 and DSF75 to be significant indicators for small logs in mature forest (p=0.0450 for all).

Table 7.3.3. Incidence of named species of wood decay fungi found in large and small diameter logs in regenerating and mature forest types.

Species	Regenerating Forest		Mature Forest	
	Large	Small	Large	Small
Armillaria hinnulea	8	11	16	12
Basidiomycete sp.5	0	3	9	6
Basidiomycete sp.6	29	25	6	1
Basidiomycete sp.9	23	0	2	0
Basidiomycete sp.10	1	1	5	3
Basidiomycete sp.11	5	0	8	10
Basidiomycete sp.12	3	2	1	0
Basidiomycete sp.13	7	0	7	2
Ceriporiopsis-like sp.1	0	7	0	12
Fomitopsis-like sp.1	15	16	29	13
Ganoderma applanatum/ adspersum	0	20	0	0
Ganoderma-like sp.1	0	5	0	0
Hypocrea pachybasioides	2	23	5	2
Phialophora-like sp.1	8	2	0	0
Phlebia-like sp.2	3	6	22	20
Postia pelliculosa	8	1	0	0
Postia-like sp.4	0	3	3	7
Postia-like sp.5	6	0	0	16
Postia-like sp.8	0	7	2	0
Postia-like sp.9	7	0	0	0
Postia-like sp.10	12	0	9	0

The sample-based rarefaction curves showed that species richness for all forest type/ log size treatments was very similar within the comparable range (Figure 7.3.7). When species richness was compared against cumulative-cross sectional area of wood examined, small logs from mature forest showed a markedly greater number of species per unit area than large diameter logs in both mature and regenerating forest. In contrast, species richness for small diameter logs in regenerating forest was very similar to that of all large diameter logs within the comparable range.

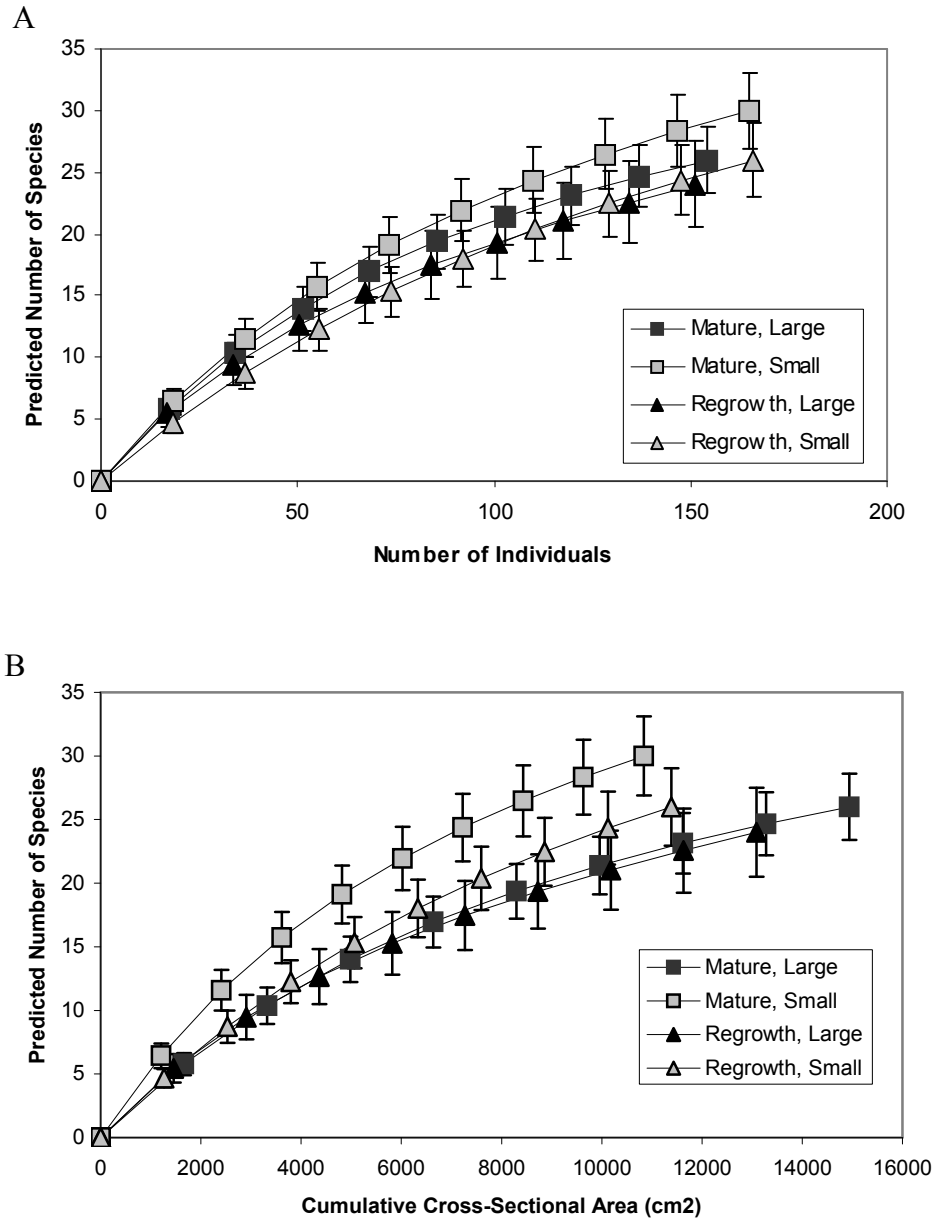


Figure 7.3.7. Rarefaction (Mao Tao) curves for species of wood decay fungi in large and small logs in mature, unlogged forest and logging regenerated forest compared by a) number of individuals and b) cumulative cross-sectional area of examined disc.

Unconstrained ordination (NMS) and MRPP showed that fungal assemblage differed between forest type/ log size treatments (Figure 7.3.8; p=0.013, T= -2.56) although the patterns were not entirely clear. Small logs in mature forest had particularly distinct fungal assemblages, while small logs in regenerating forest appeared to have highly variable fungal assemblages. Some large logs in mature forest and large logs in regenerating forest appeared to have similarities in fungal assemblage.

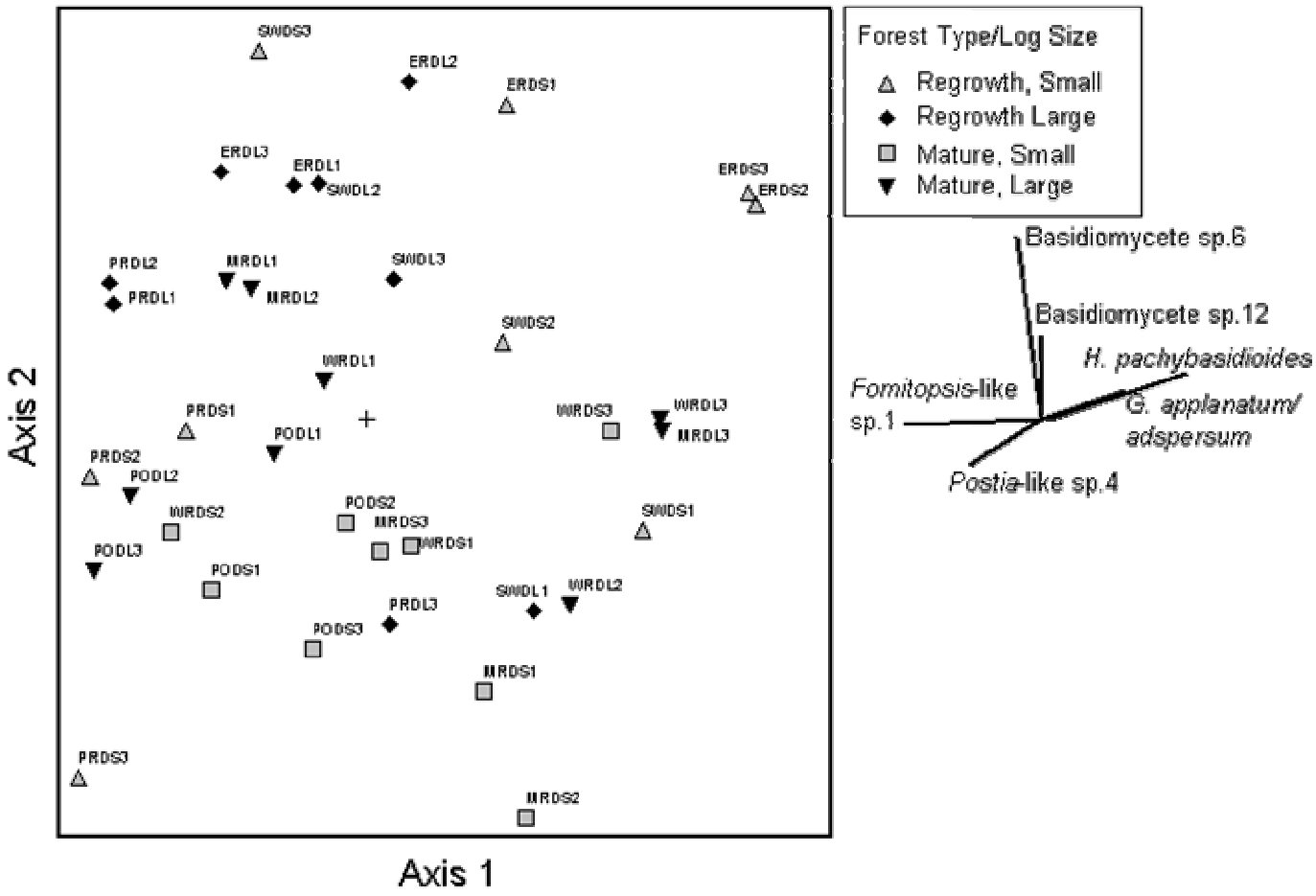


Figure 7.3.8. Non-metric multidimensional scaling (NMS) ordination plot of log transformed species abundance for the 60 most common species of wood decay fungi from 36 logs at six study sites. Only axes 1 and 2 are shown, as axis 3 provided no further useful information. Symbols denote forest type and log size. Vectors are defined by fungal species abundance data; for greater clarity, these are displayed adjacent to the ordination. Only vectors with r2>0.2 are shown.

An overlay of the fungal species groups on the ordination showed that *Postia*-like sp. 4 was strongly influencing the grouping of small logs in mature forest. Basidiomycete sp.6 was strongly correlated with regenerating logs of both large and small diameter. *Ganoderma applanatum/adspersum* was correlated with a number of the small logs in regenerating forest and *Hypocrea pachybasioides* was correlated with large logs in regenerating forest.

As shown by the constrained ordination, fungal assemblage structure was significantly related to forest type ($\delta^2 = 0.58$, $p = 0.0065$; Table 7.3.4). Fungal assemblage was also significantly influenced by log size ($\delta^2 = 0.57$, $p = 0.0160$; Table 7.3.4). A graphical representation of the logs on canonical axes corresponding

to the two main effects showed that components of the fungal assemblages were distinct among the four treatments (Figure 7.3.9). Logs grouped by mature forest type had a much higher allocation success rate than those grouped as regenerating forest. This indicates that logs in mature forest were more similar to each other than those in regenerating forest, but with a much stronger separation between small logs in mature forest, small logs in regenerating forest and all large logs.

Table 7.3.4. Results of the two canonical axes of principal coordinates (CAP), examining the effects of forest type and of log size. %Var is the percentage of the total variation explained by the first m principal coordinate axes. Allocation success is the percentage of points correctly allocated to each group. δ^2 is the squared canonical correlations.

Factor	m	% Var	----------Allocation success (%)----------			δ^2	p
			Group 1	Group 2	Total		
Forest Type	10	85.00	66.67 (regenerating)	83.33 (mature)	75.00	0.58	0.0065
Log Size	11	88.08	83.33 (small)	72.22 (large)	77.78	0.57	0.0160

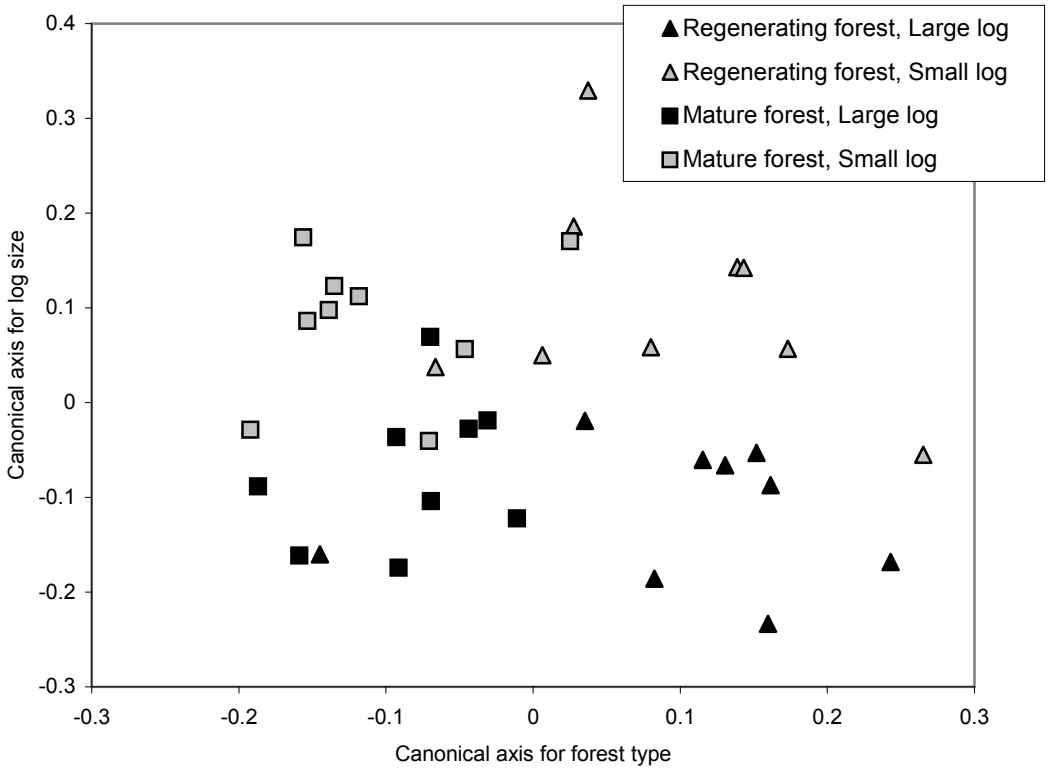


Figure 7.3.9. Constrained ordination (CAP analysis) of fungal assemblage structure with respect to forest type and log size class. N=60 species, and frequency data were log transformed.

7.4 Discussion

This study clearly demonstrates that the assemblage composition of wood decay fungi in the wet sclerophyll forests in southern Tasmania is affected by differences in log size and forest type. The broad ecological implications of these results are discussed here, and the results are revisited in the final chapter with a more specific focus on their implications for forest management. The patterns of assemblage composition and species richness identified for fungi in this study mirror those found by Yee (2005) for saproxylic beetles in the same logs. The significance of this finding is discussed in detail in the final chapter.

7.4.1 The effect of log size

One of the central foci of this study was to examine the role played by large diameter logs in the forest landscape, as they are considered to play an important ecological role in many forest systems (e.g. Edman and Jonsson 2001; Heilmann-Clausen and Christensen 2004; Lindenmayer *et al.* 1999a; Renvall 1995). In this study, small diameter logs were more species rich for wood decay fungi than large diameter logs when equal sample area were compared, however large diameter logs had distinct assemblages of wood decay fungi associated with them, irrespective of forest type. A number of species of wood decay fungi were specifically associated with large diameter logs.

Small diameter logs are more species rich

In this study, small diameter logs supported more species of wood decay fungi than large diameter logs when equal sampling areas were compared. This is consistent with the findings of Study 1 (Chapter 4), as well as several other studies (Heilmann-Clausen and Christensen 2004; Kruys and Jonsson 1999; Nordén *et al.* 2004; Schiegg 2001). It is likely to be the result of a number of factors. As discussed in relation to small diameter trees in Chapter 4, small diameter logs would be more susceptible to changes in forest microclimate than large diameter logs as a result of their smaller size. Small diameter logs spread throughout the forest floor would therefore show a higher degree of variability in light intensity and humidity than large logs, and this would impact upon the fungi able to colonise them (Boddy 1992; Renvall 1995). This is reinforced by the fact that small diameter logs in regenerating forest (the treatment with the most potential microclimatic variation)

had the greatest number of species of all treatments for a given sample area. Another possible explanation is that for a given volume of wood, small diameter logs represent more possible colonisation events and competitive exclusion (e.g. branch stubs, bark fissures), more surface area for colonisation and a greater surface area to volume ratio than large diameter logs of the same volume (Heilmann-Clausen and Christensen 2004). Thus, it is highly plausible that small diameter logs contain more species of wood decay fungi than large diameter logs at the range of volumes present in a small patch of forest.

In considering these results, it is important to bear two points in mind. Firstly, only one quarter of the volume of each large diameter log was examined for wood decay fungi to make the volumes comparable with the small diameter logs. That is, only one quarter of each cross-sectional disc removed from the large diameter logs was examined for fungi, meaning the total species richness given for the large diameter logs potentially only represents a quarter of the true species richness. Therefore, on an individual log basis, the species richness of large diameter logs is potentially much greater than that of the small diameter logs. In addition, the distribution of wood decay fungi across a disc sample is unlikely to be uniform, meaning that the quarter sampled may not represent a quarter of the fungi present on the disc. This is especially relevant for logs resting on the ground. The majority of quarter discs removed from the large diameter logs were taken from the upper half of the log. This means species of fungi which colonise large diameter logs through soil contact, such as the cord forming *Armillaria* species (Kile *et al.* 1991), may concentrate on the lower half of the log and not have been sampled from the large diameter logs. Since the entire surface of the small diameter logs was sampled, fungi colonising logs through soil contact would have been included in their species richness. Perhaps potential variation in the spatial distribution of fungi in large logs could have been better accounted for by sampling the entire disc and then comparing the number of species in large logs with small diameter logs using rarefaction curves. This is recommended for future studies of this nature.

The second point, discussed previously in Chapter 4, is that the rarefaction curves displayed in this chapter do not necessarily represent the full range of species potentially present in each log size class as the sampling was not exhaustive. It is, therefore, impossible to determine at what point species saturation will occur. Thus,

simply increasing the number of small diameter logs may not result in the accumulation of the same number of species present in large diameter logs.

Assemblage composition changes with log size

The assemblage composition of fungi in large and small logs was significantly different, with both log size classes containing unique species. This pattern appeared to be particularly driven by similarity of species within the large logs, especially those in mature forest, with greater variation among the small diameter logs. As with species richness, this greater variation in species within the small diameter logs may be due greater variation in microclimate of the logs; or the similarity in large logs may be the result of large diameter logs coming from similar origins, regardless of forest type, or from chiefly sampling their upper halves.

Differences in assemblage composition of fungi may be the result of different successional pathways between large and small diameter logs as previously suggested by Yee (2005) when examining decay and saproxylic beetle species. It is probable that the colonisation of large diameter logs by wood decay fungi would have started prior to tree fall, while the tree was still alive (Rayner and Boddy 1988; Renvall 1995). It is even possible that the weakening of the tree structure through the colonisation of fungi within the tree could have been responsible for tree fall in the mature forests (Franklin *et al.* 1987; Lewis and Lindgren 1999). Thus, wood decay fungi would have been well established within the large diameter logs /mature trees prior to tree fall. Assuming that large diameter logs in both mature and regenerating forests were mature trees in mature forest prior to tree fall, it follows that their fungal assemblage composition would have been similar.

In contrast, small diameter logs, represented by small diameter/young trees, would perhaps have few wood decay fungi present within the tree prior to tree fall, meaning the decay processes present in small diameter logs would be largely dependent on the colonisation processes taking place once the tree was on the ground. For example, in her study of large and small diameter logs, Yee (2005) found that large diameter logs were dominated by decay, especially brown rot types, within the central heartwood of the log and that these were likely to be the result of colonisation events while the sapwood (and tree) was alive. Small diameter logs had a predominance of rot types in the sapwood and outer heartwood of the log,

suggesting they were the result of fungi colonising after the death of the sapwood, from the outside inwards. The high proportion of decay, including brown rot types, and the large number of wood decay fungi reported in large old living trees in Study 1 (Chapters 4 and 5) supports this suggestion.

Due to the lack of knowledge of the taxonomy and ecology of wood decay fungi in Australia, it is difficult to draw any conclusions about the colonisation pathways of most of the species found in this study and their relationship to log size. Based on data from better studied ecosystems (Boddy 1992; Boddy 2001; Gustafsson 2002; Nordén 2000), it is probable that the fungi found in logs use one of five main strategies to arrive in or on a log. Two of these strategies relate to fungi already present in the log at the time of fall, while the other three relate to different dispersal methods employed by fungi.

Fungi present in logs at the time of tree fall would be either actively decaying wood (such as heartrot species) or latently present within the living sapwood (Boddy 1994; Boddy 2001). The former would primarily be present in large diameter trees (Chapter 4) and thus large diameter logs, while the latter could be present in logs of any size. Dispersal mechanisms employed by wood decay fungi include arrival of airborne particles such as spores, colonisation from soil by mycelial cords and rhizomorphs and transport of particles by vectors such as saproxylic invertebrates (Boddy 1992; Boddy 1993). All three of these mechanisms potentially increase with larger surface area to volume ratio and thus for a given overall volume, may favour small diameter logs. However, as these dispersal strategies are not determined by the condition of the log prior to tree fall, they are equally likely to be found in large and small diameter logs. For example, *Armillaria hinnulea* is well studied in Australia and is known to colonise substrates by means of specialised rhizomorphs (Kile *et al.* 1991). This species was found equally in both large and small diameter logs as a species which initially colonises the sapwood of the log through contact with the soil (Chapter 6).

7.4.2 The effect of forest type

Similar numbers of wood decay fungi were found in the regenerating forests and the mature, unlogged forests, demonstrating that fungi can successfully colonise *Eucalyptus obliqua* logs in 20-30 year old first rotation logging residue following

CBS treatment. The fungal assemblage composition of logs in mature and regenerating forests was, however, significantly different. These differences can most likely be attributed to two primary factors: differences in current and recent forest condition and differences in log recruitment processes.

The effect of forest condition

Both forest age and successional processes can influence the composition of wood decay fungi in natural and managed forests (Bader *et al.* 1995; Edman *et al.* 2004a; Edman *et al.* 2004b; Nordén and Paltto 2001; Rolstad *et al.* 2004; Stokland and Kauserud 2004; Vasiliauskas and Stenlid 1998). Many studies, particularly those focussing on individual species, have found species richness or number of individuals to increase with increasing forest age (e.g. Bader *et al.* 1995; Edman *et al.* 2004a; Edman *et al.* 2004b; Stokland and Kauserud 2004); however other studies have found a decrease in species richness with forest age (Nordén and Paltto 2001), perhaps as a result of increased competition between late stage combative fungal species (Boddy 2000; Boddy 2001). Whether increasing forest age leads to increased or decreased species richness, the assemblage composition of fungi is clearly different at different forest successional stages. What is not apparent is whether this is purely a result of forest age or whether it also relates to other factors such as microclimate.

The two forest types examined in this study are likely to be at very different successional stages. They have a difference in time since disturbance of more than 35 years (Alcorn *et al.* 2001; Hickey 1993; Hickey *et al.* 1998) and the type of disturbance was arguably very different. The mature, unlogged forest was subjected to a moderate fire which was not stand replacing, while the regenerating forest underwent the stand replacing process of clearfell, burn and sow. Therefore, even if the forest types were the same age, it is not certain that they would be undergoing identical successional pathways. Furthermore, the structure and composition of vascular plants in the two forest types indicate that they are at different successional stages (Hickey 1994) and that the regenerating forest has a more open canopy (Yee 2005).

Given the different successional and environmental conditions present in the two forest types it is not surprising that their fungal species composition is different.

Fungi found in logs in the regenerating forest are likely to have a much greater tolerance to fluctuating temperature and moisture extremes as a result of the more open forest structure. These may include some of the ruderal, disturbance opportunistic fungi described by Boddy (1992) but would also include some stress-tolerant species. In contrast, the mature forests would provide a much more stable habitat for some of the less stress-tolerant fungal species. This variation in microclimate would be particularly apparent when examining the combined effects of log size and forest type. Small diameter logs in regenerating forest would be much more susceptible to changing microclimates than large logs in mature forest which are protected from environmental conditions both by virtue of their size and habitat. This is clearly demonstrated by differences in fungal assemblage composition for large and small diameter logs in regenerating forest types.

Differences in log recruitment

Logs in mature and logging regenerated forest could also have different fungal species composition as a result of the different recruitment processes they have undertaken. Many of the study logs in the logging regenerating forest were probably the result of clearfelling and left on site as logging residue, felled before natural recruitment processes took place. This is especially true for those logs which contained a high proportion of rotten wood as they would not have been useful for pulp or sawlog production. Following felling, these logs would also have been subjected to a high intensity burn followed by open, sun-exposed conditions for some time. In contrast, logs in the mature forest would have been recruited by natural processes of tree fall into a relatively closed forest canopy; some may have been killed by fire but many would also have fallen as the result of rot and/or strong winds.

These different recruitment pathways are likely to favour different fungal species or species complexes. For example, wood decay fungi present in forests regenerating from logging would be more tolerant of isolation, burnt wood, desiccation and sun-exposure and these factors have been shown to strongly influence fungal assemblage composition (Lindhe *et al.* 2004; Penttilä and Kotiranta 1996; Renvall 1995). The burning of wood or the presence of charcoal on a substrate can strongly influence the fungal species able to colonise it, favouring ruderal species able to benefit from the flush of nutrients and food resources after fire (Penttilä and

Kotiranta 1996; Renvall 1995). Lindhe *et al.* (2004) described a number of species of fungi which were dependent on the amount of sun-exposure of their host logs in coniferous forests in Sweden. Four species, *Lenzites betulinus, Daedalea quercina, Fomitopsis pinicola* and *Calocera cornea* were more common in sun-exposed conditions while *Phlebiopsis gigantea* and *Peniophora incarnata* preferred shade. As wet eucalypt forests in Tasmania have evolved with fire as a major disturbance (Hickey *et al.* 1998), the effect of burnt wood on the fungal assemblage structure may not be as great as it would in less fire-prone environments; however differences in the intensity of the fires in the regenerating forest (stand replacing) and the mature forest (not stand replacing) may have had an effect. The combination of differences in fire intensity and sun-exposure would have created logs under different climatic conditions which are likely to result in different fungal communities.

As previously discussed, the fungi sampled from the logs at the present time may have been influenced by the fungal assemblage composition prior to log recruitment, and this in turn may have been affected by log recruitment processes. As logs in regenerating forests were primarily recruited as the result of logging practices, they may not have been in the same stage of fungal succession at the time of recruitment as those logs recruited through natural tree fall.

Are large logs in mature and regenerating forests similar?

One interesting result from this study was the similarity in assemblage composition between some of the large logs in mature forest and large logs in regenerating forest, as shown by NMS. Further studies would be needed to provide strong evidence that there are similarities in fungal assemblage between large logs in regenerating and mature forests, however, evidence from this study indicates it is worth exploring. This is particularly the case since large diameter logs have been suggested as legacy habitats in disturbed forests. The regenerating forests examined in this study are examples of forests in their first silvicultural rotation. In future rotations, large diameter logs may be phased out due to a lack of large diameter trees needed for their recruitment. The importance of large diameter logs in this context is discussed in Chapter 8.

There are several factors which may contribute towards similar fungi being present in large diameter logs in mature and regenerating forests. First, the composition of fungi in the large logs may have been partially driven by fungal species present in the logs prior to tree fall (and therefore prior to logging in the regenerating sites). This is reinforced in part by large diameter logs containing a number of mature tree specialist species sampled in Study 1 including *Postia pelliculosa* and *Fomitopsis*-like sp.1 (see Chapter 6). Second, large diameter logs are a more stable resource than small diameter logs with increased buffering capacity due to their size, and are therefore less susceptible to changes in forest microclimatic conditions. In comparison with small diameter logs, large diameter logs have greater moisture holding capacity (Bader *et al.* 1995), slower rates of decay (Mackensen *et al.* 2003) and are less affected by fire damage (Michaels and Bornemissza 1999). Large diameter logs also have the potential for more internal decay present at recruitment as previously discussed (and see Yee 2005). As a result, the conditions present in the large diameter logs may be similar in both mature and regenerating forests, accounting for congruence of fungal species assemblages.

If further study does show similar fungal assemblage composition in regenerating and mature forests, large diameter logs could provide an important biological link between disturbed forests and undisturbed forests. In disturbed landscapes, this type of legacy habitat may be an important way of maintaining dependent species throughout the forest matrix (Lindenmayer and Franklin 1997), especially those species with poor long-range dispersal. More than half of the large diameter logs from regenerating and mature forest had similar assemblages (as shown by the NMS; Figure 7.3.3). This could suggest that, given a big enough population of large diameter logs, the concept of legacy habitats in logging disturbed forests may indeed hold. Small diameter logs appeared to be much more susceptible to forest type, most likely as a result of the greater influence of microclimate on log conditions within the log (refer to previous discussion in Section 7.4.1). The importance of log size in this context indicates that microclimatic factors, such as changes in temperature and water content, are not the only important drivers of the fungal species composition of logs.

7.4.3 Conclusions

This study is the first to undertake detailed examination of the effect of log size and forest type on wood decay fungi in logs in southern Australia. Many of the patterns of fungal species distribution discussed in this chapter are similar to those found by Yee (2005) for saproxylic beetles and decay in the same sample logs, indicating similar or linked habitat preferences.

This study has demonstrated that wood decay fungi are capable of colonising logs/logging residue in forests regenerating from CBS logging 30-35 years previously. Despite similar species richness, the assemblage composition of wood decay fungi is different in mature forests and forests regenerating from logging. This is likely to be due to the different microclimatic conditions present in each forest type, as well as differences in forest succession and log recruitment processes.

This study also found that large and small diameter logs have different communities of wood decay fungi. This was again thought to be particularly driven by differences in log recruitment patterns as well as log microclimates. While the habitat and biology of most species of fungi are poorly understood, it is suggested that assemblage similarities in large logs in mature and regenerating forests indicates that large logs may play an important role as legacy structures in the maintenance of species of wood decay fungi in disturbed forests.

CHAPTER 8: TOWARDS THE CONSERVATION OF WOOD DECAY FUNGI IN TASMANIAN WET EUCALYPT FORESTS

8.1 Introduction

This thesis contributes new data, knowledge and understanding to the limited knowledge of wood decay fungi in the wet sclerophyll forests in southern Australia. This chapter reiterates the main findings of the thesis and discusses them in the context of the biodiversity values of wood decay fungi and their conservation within the managed forest matrix. Areas requiring further research are highlighted throughout the chapter.

8.2 Wood decay fungi in the wet eucalypt forest

This thesis has added to the taxonomic and molecular knowledge of Australian wood decay fungi, providing baseline data in a relatively undeveloped area of research. It provides descriptions of the cultural morphology and ITS sequences of 20 species of wood decay fungi (Chapter 3). Sequences of the ITS region for a further 36 species of wood decay fungi were also determined from cultures (Chapter 6). Critically, work from this thesis has initiated the creation of a reference collection of sequences and fungal cultures matched to identified fruitbodies. This reference collection currently contains more than 140 species of fungi and is specifically tailored for ecological work on wood decay fungi in southern Tasmania. It will be housed and maintained at the CSIRO/ensis laboratories in Hobart, Australia and will be able to be used for further ecological and taxonomic work with wood decay fungi in Australia.

In total, 151 species of wood decay fungi were collected over the two studies in this thesis. This result highlights the importance of using a destructive sampling or wood cutting method to study fungi in Australian eucalypts. Had fruitbody surveys been carried out alone in Study 1, for example, only three species of wood decay fungi would have been found in the 18 living trees examined, as opposed to 91 species found by culturing from wood samples.

There is a critical need to improve our understanding of the taxonomy of Australian wood decay fungi, because it improves the documentation of the biodiversity values

of these forests and, more particularly in this case, because it underpins the ability to answer ecological questions. And, as in this study, knowledge of fungal ecology may have important implications for the management of eucalypt forest systems.

This study demonstrates the benefits of using a combination of morphological and molecular techniques to group and identify cultures of wood decay fungi for use in ecological studies. Advocacy of combined morphological and molecular studies is not new (Bougoure and Cairney 2005; Hagerman *et al.* 1999; Hoff *et al.* 2004); however, it is of particular significance in Australia where it is not possible to draw on the wealth of taxonomic and ecological knowledge of wood decay fungi that is available in some other countries. While both methods can be used individually with some success, together they provide a much more robust analysis of both the species groups present and the taxonomic affiliations of these species groups.

As the knowledge and taxonomy of fruitbodies and cultures of Australian wood decay fungi becomes more developed, it may be possible to use more sophisticated molecular methods to identify wood decay fungi from cultures or even directly from wood (e.g. Adair *et al.* 2002; Allmér *et al.* 2006; Johannesson and Stenlid 1999; Oh *et al.* 2003; Vainio and Hantula 2000). Studies currently underway at the University of Tasmania, have successfully sequenced fungi directly from decayed eucalyptus wood (Glen *et al.* 2006b). Until these molecular methods are better developed and until our understanding of the taxonomy of Australian wood decay fungi is much improved, a more conservative approach is advocated which takes into account as many of the characteristics of each fungal isolate as is possible.

8.3 Maintaining habitat structural complexity for specific fungal communities

Tasmanian wet eucalypt forests are characteristically heterogeneous systems, both spatially and temporally, often with a wide variety of trees of different sizes and ages, in different states of growth and decay (e.g. Duncan 1999; Jackson and Brown 1999; Lindenmayer *et al.* 1999b; Lindenmayer *et al.* 1991). The structural characteristics of importance to fungi can range from different diameter living trees to standing dead trees (stags) and different diameter logs and branches on the forest floor in different phases of decay (Lindenmayer *et al.* 2000a; Lindenmayer *et al.* 1999a; Woodgate *et al.* 1996). The patchwork of natural fire histories characteristic

of wet eucalypt forests in particular, can lead to the formation of structurally complex multi-aged forests (Wells and Hickey 1999).

Many of the 151 species of fungi examined in this thesis demonstrated a specific preference for one of a wide range of habitat structures (Chapters 4 and 7), supporting the principle that maintaining structural diversity in wet eucalypt forests is important for species conservation (Lindenmayer and Franklin 1997; Lindenmayer *et al.* 2006). Living trees (Study 1) and decomposing logs on the forest floor (Study 2) were both found to support a rich flora of wood decay fungi within a diverse range of habitats. Some species, such as *Hypholoma fasciculare, Postia pelliculosa, Postia*-like sp.4 and 5 and Xylariaceae sp.1, were dead wood habitat generalists, found in both the heartwood of living trees and in logs; while other species preferred more specific habitats. In Study 1, only three species of fungi, Basidiomycete sp.1, *Hypholoma fasciculare* and Xylariaceae sp.1, were found in all tree age-classes. In contrast, more than 80 of the 91 species found in living trees demonstrated an apparent preference for one particular tree age, although most were found in very low numbers (Chapter 4). Three of the 60 species examined in Study 2 were apparently adapted to the long-unburnt, relatively protected logs in mature forest while six species of fungi appeared to prefer burnt, exposed logs in forest regenerating from wildfire (as demonstrated by indicator species and CAP analyses, Chapter 7). Six species of wood decay fungi also showed preferences for particular log diameters (Chapter 7) and five species were associated with specific rotten wood habitats in logs (Chapter 6). This rotten wood habitat appeared to be affected by tree age/size (Chapter 5). This is the first time that such specific associations between wood decay fungi and the structural complexity of dead wood habitat have been demonstrated in Australian eucalypt systems.

8.4 Adequacy of the current forest management practices for the conservation of wood decay fungi

This thesis has built on the work of Yee (2005) on saproxylic beetle communities in the same *Eucalyptus obliqua* logs used in Study 2. Yee's work indicated that current off-reserve management of forests in areas subject to timber harvesting was unlikely to adequately maintain saproxylic beetle diversity. In particular, timber

harvesting was likely to lead to a reduction in mature habitat structures, particularly large diameter logs; a cumulative reduction in dead wood volumes over time; and a temporal disruption to dead wood recruitment processes during forest regeneration (Yee 2005). All three of these factors were found to impact negatively on populations of saproxylic beetles.

The patterns of wood decay in *E. obliqua* logs reported in this study (Chapter 6 and 7) are very similar to those reported by Yee (2005) for the saproxylic beetles. It is reasonable to suggest therefore that the implications for forest management arising from this study will be similar too.

Loss of mature habitat structures

In Study 2, large diameter logs were found to provide an important ecological habitat for wood decay fungi (Chapter 7). The reduction or absence of these large logs from sites after successive short timber harvesting rotations will negatively impact on those fungi which show a preference for large diameter logs. Although little is known about the ecology of these species of fungi, a preference for large logs may relate to their more stable habitat, or be the result of recruitment processes which began in the large living tree prior to log recruitment (Chapter 7). In order to capture these recruitment processes, Yee (2005) also advocated the retention of large living trees in the landscape for large log production. This thesis takes Yee's work one step further by demonstrating that large living trees are important ecological habitat for biodiversity in their own right (Chapter 4). Thus, large diameter trees appear to be critical for fungal diversity and as a component of the successional processes leading to the recruitment of large diameter logs on the forest floor. Together, the work by Yee (2005) and the studies described in this thesis provide strong evidence supporting the retention of both large diameter living trees and logs in the forest landscape.

Although this discussion focuses on large diameter or mature habitat substrates, the importance of small diameter substrates for wood decay fungi should not be overlooked. In both Studies 1 and 2, small diameter substrates (20-60 cm diameter) contained a number of specialised species of fungi and had a higher species richness than large diameter substrates when equal sample volumes were compared (Chapters 4 and 7). In the production forestry landscape, small diameter substrates

are likely to remain frequent over successive short (<100 year) silvicultural rotations (Grove *et al.* 2002). As a result, the conservation of small diameter substrates is not of such critical importance for consideration by forest managers. Despite this, the high species richness of small diameter substrates does raise many potential questions which should be considered for future studies. For example, is the high species richness in small diameter trees and logs really a reflection of the diversity of microclimates present in these substrates, as suggested in Chapters 4 and 7? If so, what aspects of microclimatic change are driving changes in fungal species, as evidenced in Chapters 4 and 7? What are the colonisation strategies of the fungi inhabiting small diameter trees and logs? Are they, for example, responding to the increased opportunities for colonisation afforded by the greater surface area to volume ratio of small diameter substrates? In the managed forest landscape, changes in microclimate will inevitably occur as the result of changes in forest management patterns and forest structure (Lindenmayer and Franklin 1997). What effect these changes will have on wood decay fungi and other saproxylic organisms should form the basis of future studies.

Reduction in dead wood volumes

Modelling by Grove *et al.* (2002) shows that harvesting wet eucalypt forests at successive 100-year intervals would lead to a two-fold reduction in dead wood volumes, as compared with forests subjected to stand-replacing wildfires of the same frequency. This reduction in dead wood volumes would be exacerbated with any proposed fuel wood harvesting (Grove *et al.* 2002). These reductions in dead wood volumes will increase average distances between similar dead wood substrate types, potentially leading to fragmentation effects throughout the forest matrix. This, in turn, could lead to coupe-scale population extinctions for species dependent on specific dead wood substrates, particularly those with only short-range dispersal capabilities. European forestry provides many examples of the effect of decreasing dead wood volumes, or increasing distance between dead wood type, on reducing populations of fungi at the coupe-scale (e.g. Pentillä *et al.* 2006; Sippola *et al.* 2004) and of other saproxylic species (e.g. Jonsell and Nordlander 2002).

Future studies of Australian wood decay fungi should focus on understanding the dispersal capabilities of these fungi, giving priority to rare species and those fungi

with a high degree of habitat specialisation (e.g. Lindhe *et al.* 2004; Renvall 1995) as they are thought to be more susceptible to fragmentation (Pentillä *et al.* 2006). This will assist in determining how these fungi may respond to changes in dead wood volumes within the forest as a result of timber harvesting.

Temporal disruption to dead wood recruitment processes

Dead wood recruitment from current timber harvesting in wet eucalypt forests differs substantially from the natural recruitment patterns resulting from wildfire (Grove *et al.* 2002). In managed stands subjected to clearfelling, the majority of dead wood arises from the initial harvesting event, and it is mostly then burnt to remove this harvesting debris and to provide an ash bed for regeneration. This treatment results in a pulse of mostly severely burnt harvest debris mostly in similar early decomposition stages. Following this pulse, there is little continual dead wood recruitment (Grove *et al.* 2002). In contrast, not all wildfires in Tasmanian wet eucalypt forests are stand replacing; many wildfires are low-medium intensity and cause only partial stand replacement (Hickey *et al.* 1998). This results in multi-aged forests with a number of different cohorts of trees originating from separate wildfire events (Hickey and Wilkinson 1999), and a source of dead trees of different sizes. These dead trees, together with their limbs, may fall to the ground soon after fire or add gradually to the dead wood pool (Grove *et al.* 2002).

Many species of wood decay fungi are known to display preferences for wood at different stages of decomposition (Chapter 1; Renvall 1995). If most dead wood substrates within a forest stand are at a similar decomposition stage, as would be the case following clearfell timber harvesting (Grove *et al.* 2002), fungi reliant on specific stages of decomposed wood may ultimately have to disperse further to find new suitable habitat. For species with short-range dispersal, this is likely to be difficult. Log size is also an important factor here. Large diameter substrates are often the main components of dead wood, dominating the dead wood pool. Stands subjected to timber harvesting tend to lack large diameter trees so, over time, the proportion of large diameter substrates decreases (Grove 2001b). In addition, small diameter logs tend to decay much faster than large diameter logs (Harmon *et al.* 1986) and this can result in even lower levels of dead wood over time.

This lack of temporal continuity in dead wood substrates could have a profound effect on assemblages of wood decay fungi. This may be especially the case for fungi specifically associated with particular rotten wood types, such as those examined in Chapter 6. Given the importance of maintaining temporal continuity of dead wood substrates in various stages of decay for fungal habitat, simply extending logging rotation periods is not likely to conserve wood decay fungi. Other mechanisms, such as retaining habitat patches within stands, retaining habitat trees, retaining dead wood on the ground and the use of low and medium intensity burns should be investigated as ways to maintain temporal continuity of dead wood.

8.5 Recommendations for forest management

The outcomes of this thesis highlight the importance of maintaining habitat for wood decay fungi throughout the production forest matrix. This section focuses on the temporal aspect of dead wood continuity, rather than the spatial components, as this was the primary management focus of both Studies 1 and 2. This thesis provides supporting evidence for the recommendations of Yee (2005), in advocating the retention of living trees within stands managed for timber production to maintain diversity of wood decay fungi at scales appropriate for their long-term persistence. The retention of living trees in harvested coupes will improve stand structural complexity and continuity of dead wood supply, helping to mitigate many of the impacts mentioned in Section 8.4. Adoption of a living tree retention strategy should be immediately considered by forest managers, as the restoration of particular dead wood habitats may take some time. For example, mature living trees were found to provide important habitat for wood decay fungi (Chapter 4); some of these trees may take 150-300 years to develop habitat features suitable to support these fungi (Alcorn *et al.* 2001; Lindenmayer *et al.* 2000b). Temporal continuity of dead wood habitats is important, as any break in continuity may result in local extinctions of specific wood decay fungi. Although these fungi are important components of biodiversity in their own right, they also contribute significantly to the creation of habitats, such as rotten wood types, which are critical for the survival of many other organisms (Mackowski 1987; Simpson and Eldridge 1986). In recommending the adoption of living tree retention, there are a number of key factors which must be considered in order to successfully preserve the diversity of dead wood substrates inhabited by wood decay fungi. These include: retaining a

range of trees of different ages, sizes and states of decay; retaining trees in aggregates; and the size of aggregates and coupes. These issues are discussed further below.

The practice of retention of living trees within harvested coupes should incorporate trees in a range of age classes including old, mature and young regrowth trees. This is important not only for maintaining structural diversity within the harvested coupe (thus spatial connectivity within the production forest matrix), but also for maintaining temporal continuity of dead wood habitats. Mature trees will provide immediate habitat for those wood decay fungi that prefer this habitat (Chapter 4). Mature trees will help to provide continuity of downed wood substrates through dropping of large branches while they are still alive and eventually lead to the recruitment of large diameter logs, critical habitat for specific wood decay fungi (Chapter 7). At the same time, it is also important to retain small regrowth trees as they provide habitat for fungi which are young tree specialists. These young trees will also assist in providing temporal continuity of dead wood habitat: during the course of the silvicultural cycle, they will grow and, if left untouched, may become large diameter trees. Retaining trees in a range of age-classes and sizes may also mimic the effect of wildfire in the wet sclerophyll forests as fires leave trees in a range of age classes (such as those examined in Study 1; Chapter 2). This range of tree age classes also provides potential for the recruitment of both large and small diameter logs on the forest floor.

In addition to retaining trees of different ages in harvested coupes, it is also important to consider the state of decay of the retained trees. In some silvicultural systems, such as those in the karri forests of Western Australia, seed-trees are retained throughout harvested coupes to provide natural seed fall for the regenerating coupe (Department of Conservation and Land Management 2005; Hickey *et al.* 2001). Healthy, dominant trees are frequently selected for seed-trees as they are thought to have less genetic predisposition to decay and damage by insects and will therefore supply healthy seed (Department of Conservation and Land Management 2005). In contrast, retention of living trees for biodiversity, as recommended here, should include the retention of trees in a variety of states of health and decay, so as to increase spatial continuity in habitat types between trees and provide trees for habitat recruitment later in the silvicultural cycle.

Current silvicultural trials at the Warra LTER site in the wet sclerophyll forests in southern Tasmania are investigating the use of a range of alternatives to the current clearfell, burn and sow harvesting practices used in Tasmania. These alternatives have been developed with reference to the alternative silvicultural methods currently being investigated in North America (e.g. Franklin *et al.* 1997). The majority of these methods incorporate some level of living tree retention and include trees dispersed throughout the harvested area, aggregated or concentrated in strips (Hickey *et al.* 2001). Among the range of options investigated in Tasmania, aggregated retention (i.e. retaining 15-40% of the coupe area in aggregates) has been nominated as the most promising in combining the criteria of worker safety, timber yield, maintaining biodiversity values and social acceptability (Forestry Tasmania 2004; Hickey *et al.* 2001).

The findings of this thesis provide support for the use of aggregated retention throughout the production forest matrix as an alternative to clearfell, burn and sow which will help to maintain biodiversity. Maintaining portions of the harvested coupe as aggregates with minimal disturbance will help to protect the retained trees and will allow some of the natural forest processes, such as dead wood recruitment, to continue. Importantly, retaining aggregates of living trees will maintain logs on the forest floor within the aggregates under shaded, protected conditions, much more similar to those of unlogged, mature forest. As discussed in Chapter 7, there are a number of species of wood decay fungi which show a preference for logs in mature forest and these species should be catered for in aggregates in the harvested coupes.

If aggregated retention systems are widely adopted as an alternative to clearfelling in Tasmania (not just for old growth forests as is currently envisaged), further research into the size and spatial arrangement of aggregates is required. The current trials at Warra retain approximately 30% of coupe area as 5-6 individual aggregates of 0.5-1 hectare in area (Hickey *et al.* 2001). Very little is known about the impact of edge effects on wood decay fungi and how changes in microclimate from mature forest to aggregates (due to exposure of the edge of aggregates) may impact on the viability of fungi. Research in southern Tasmanian forests by Baker *et al.* (2006) suggests that edge effects for litter beetles can reach up to 10-25 m into the undisturbed forest habitat. On average beetle assemblages were 95% similar to

mature forest in areas greater than 22 m from the disturbed edges. Given that microclimate effects are likely to impact on wood decay fungi (Chapters 4 and 7 and Section 8.4) it is probably important to consider the size of the aggregates retained for conservation and how much they may be influenced by edge effects. In addition, the size of harvested coupes is important; smaller coupes will have a greater perimeter to area ratio, potentially facilitating more dispersal of wood decay fungi from mature forest into harvested coupes.

Although aggregated retention is suggested as a forest harvesting tool which may help to maintain wood decay fungal assemblages, this thesis advocates an adaptive environmental management approach (Holling 1978) in its adoption. Very little is known about the biology and ecology of wood decay fungi in Tasmania and without further investigation, it is difficult to predict whether a 'one-size fits all' approach to aggregated retention may have any adverse impacts on wood decay fungi or on any other poorly understood forest organisms. As a result, an approach to forest management is recommended, which chooses a range of promising silvicultural techniques across the production forest matrix, incorporating harvesting coupes and aggregates of a range of different sizes. The efficacy of these various different silvicultural techniques can then be tested *in situ*, allowing forest managers to continually examine and adapt their forest management techniques as further research is undertaken. This process of adaptive management will provide managers with the opportunity to observe, study, understand and develop forestry practices that better meet the goals of ecologically sustainable forestry.

8.6 The importance of interrelated studies

This thesis forms part of a number of interrelated studies examining the effect of timber harvesting on dead-wood dependent organisms in the wet eucalypt forests of southern Tasmania. The studies described here were both closely linked to other doctoral research: Study 1 was carried out in collaboration with K. Harrison who examined the saproxylic beetles in the same living trees (Hopkins *et al.* 2005); and Study 2 was based on work carried out in collaboration with M.Yee who examined the saproxylic beetles in the same logs and rotten wood types (Yee 2005; Yee *et al.* 2001). Both Study 1 and Study 2 tried to find links between fungi and beetles through their associations with rotten wood type. Other closely related studies have

examined the canopy arthropod fauna in mature and regenerating trees (Bar-Ness 2005), the saproxylic beetles present in newly recruited large and small diameter logs (Grove and Bashford 2003) and fungi present on logs at sites with different fire histories (G. Gates, pers. comm. 2006). The collaborative nature of these studies, and their similar focus, has allowed a clearer insight into the complex ecological processes that occur in wet eucalypt forests.

Gaining a thorough understanding of the ecology of wet eucalypt systems is important. Australian eucalypt forests are thought to differ substantially from some of the better studied northern hemisphere systems such as the Scandinavian boreal forests. In Australian wet eucalypt forests, for example, there are no woodpeckers or other vertebrate primary wood excavators. The creation of hollows and decay features in eucalypts appears to rely much more heavily on decay organisms such as fungi and saproxylic invertebrates, and on fire (Gibbons and Lindenmayer 2002, A Koch, pers. comm. 2005). In addition, Australian forests have a much shorter history of intensive forest management in comparison with many forests in the northern hemisphere. Most studies in northern Europe, for example, take place in forests which are already drastically altered by human activity (reviewed in Siitonen 2001). Intensive silviculture only began in Tasmanian eucalypt systems in the early 1960s (Gilbert and Cunningham 1972); consequently most forests are still in their first silvicultural rotation. Thus, large diameter trees and logs are still well represented in these forests (Meggs 1996), and this provides an unusual opportunity to investigate their importance as habitat before irretrievable long-term effects of forest management are evident.

8.7 Conclusions

The studies described in this thesis are the first to intensively examine the wood decay fungi present in the wet eucalypt forests in southern Australia. The morphological and molecular taxonomic work which has arisen from this thesis provides a sound basis for further investigations of wood decay fungi, either as cultures or sequences extracted directly from wood. New insights into the relationship between tree age and wood decay fungi have highlighted the importance of large, mature trees as habitat for fungi and rotten wood habitat; and in addition have demonstrated the variety of wood decay fungi found in suppressed,

small diameter trees. Logs are also identified as important habitats for wood decay fungi, with assemblage composition affected by both log size and forest type. Timber harvesting practices which lead to reductions in stand structural complexity, cumulative reductions in dead wood volumes over time and a disruption to dead wood recruitment processes have been identified as potentially threatening processes for wood decay fungi in wet eucalypt forests. An adaptive environmental management approach is advocated for Tasmanian wet eucalypt forestry, which includes the retention of large diameter trees and logs within otherwise harvested stands to maintain forest biodiversity.

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Appendix 1. Sequences of the internal transcribed spacer region for all wood decay fungi identified in Studies 1 and 2.

Armillaria hinnulea

Primer: ITS1F

TGCAATCATAGCATTGAGAACTGTTGCTGACCTGTTAAAGGGTATGTGCACGTTCGAAGTGTTGCGTT
CTATTCTTTCCACCTGTGCACCTTTGTAGACTTGGTTAACTTTCGCTCTCGAGCGGTTAGAAGGGTTG
CTTTCGAGCTCCCTTTGTCTACCAAGTCTATGTCTATATAATCTCTTGTATGTCTAGAATGTCTTGTT
TATGGGACGCAAGTCCTTTAAATCTTATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATG
AAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACG
CACCTTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTCCCCT
TCTTTTACTAGGAGTGTGGTGGATTGGACATGGGGGTTGCTGGTTTCT

Ascocoryne sp.1

Primer: ITS1F

AACCTGCGGAAGGATTCATTACAGAAGGAGGAGTCTCCTACCGTAGCAGTAGGTCCGCCTGCTGCTCG
ACGATCACGGCTCCCTCCGGGGGGTGTCCCCTCACCCTTGTGTACCCTACCTTTGTTGCTTTGGCGGG
CCGCGTTTAGCCACCGGCTCATGCTAGTGAGTGTCCGCCAGAGGCCCCAACTCTTGATTTTATAATGT
CTGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCTGTGGTATTCCGCGGGGCATGCCTGTTCGAGCGTCATTATGACCCAATCACGCCCCCGGCGTGG
TCTTGGGGCTGGCAGCTCTGCCGCCCTCAAATGCAGTGGCAGCGCCATTAGGCTCTTAGCGTAGTAAT
ACTCCTCGCTATAGGGTCCTGTGGTTGCCGCCAGCAACCCCCTATTTTCTAGGTTGACCTCGGATCAG

Ascomycete sp.1

Primers: ITS1F & ITS4

GAACCTGCGGAGGGATCATTACAGAGTTCAAGCCCTCGCGGGCAGATCTCCCACCCTTGTGTATTCAT
ACTTTTGTTGCTTTGGCAGGCCGCTGGGCTTCGGCCTGGCCACCGGCTCTAGAGCTGGTGTGCGCCTG
CCAGAGGACCCCCAAACTCTGGTTATCAGTGTCGTCTGAGTATTATACAATCGTTAAAACTTTCAACA
ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGAGAATTGCAGAA
TTCAGTGGATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTCG
AGCGTCATTTCAACCCTCAAGCTCTGCTTGGTCTTGGGCTCGGCCGTGATGGCCGGCCTTAAAATCAG
TGGCGGTGCCGTCGTAGGCTCTAAGCGTAGTACATTTCTCGCTCTGGAAGCCTGGCGGTGCCTGCCAG
ACAACCCCTAATTGTCTCTTACGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAC
AATAAGC

Ascomycete sp.2

Primer: ITS1F

CGGGCAGATCTCCCACCCTTGTGTATTCATACTTCTGTTGCTTTGACAGGCCGCTGGGCTTCGGCCTG
GCCACCGGCTCTAGAGCTGGTGTGCGCCTGCCAGAGGACCCCCAAACTCTGGTTATCAGTGTCGTCTG
AGTATTATACAATCGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGAGAATTGCAGAATTCAGTGGATCATCGAATCTTTGAACGCACATTGCGCC
CTTTGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTCTTGGG
CTCGGCCGTGATGGCCGGCCTTAAAATCAGTGGCGGTGCCGTCGCAGGCTCTAAGCGTAGTACATTTC
TCGCTCTGGAAGCCTGGCGGTGCCTGCCAGACAACCCTTAATTGTCTCTTACGGT

***Athelia*-like sp.1**

Primers: ITS1F

AACCTGCGGAAGGATCATTATTGAATTATAAGGCTTTGGCTGTGCTGGCCCCTCGGGGCATGTGCACG
TCTCTGCCCGTTAATCCCAACCACCCCTGTGAACCGACCGTGTGAGGCGCTCTGTAACGGAGCCGTCT
CATGCTTTATCATAAACACCGTATGTCTTCGAATGTAAAATCTATATCTTCGCCTTAAAACAGCGTCG
GTAAAAAAAAATTAATATAACTTTCAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGATTTTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCT
CCCTGGTATTCCGGGGAGCATGCCTGTTTGAGTATCATTAAATTCTCAACCCCGATCGGATTTGTTTT
CGCATTCGGAGGCTTGGATTGTGGAGCGTGCTGGCCCCCTCTCATGGGTCGGCTCCTCTTAAATGCAT
TCGCGGAATCGACTTTGGTCCGTTTCGGTCATCAGTGTGATAAATGTTGCGCTGTTGGCTAGGATCGA
GGTGCCGCATACAATGGTCTTCGGACAATTTTATAACCATTTGATCTCAAATCAGGTAGGA

***Athelia*-like sp.2**

Primer: ITS1F

AACCTGCGGAAGGATCATTATCGAATTATAAGGCTCTGGCTGTGCTGGCCCCTCGGGGCATGTGCACG
TCTCCGCCCGTTAATCCAACCAACCCCTGTGAACCAACCGTGGGCCGGCTCTGTGATGGAGTCGTCCC
ATGCTTTATCATAAACACTTGTATGTCTCAGAATGTAAAACGTCAATGCCTTCGCCTTAAAAACGTGA
ACGGTAATAAAATTAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATTTTCAGTGAATCATCGAATCTTTGAACGCACCTTGC
GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCCCGATCGGATTCGT
TTCTGATTCGGAGGCTTGGATCGTGGAGCGTGCTGGCCCCTGCCCGGGTCGGCTCCTCTCAAATGCAT
TCGCGGAATCGACTTTGGTCCGTTTCGGTCATCAGTGTGATAATACGTTGCG

***Athelia*-like sp.3**

Primer: ITS1F

ACCTGCGGAAGGATCATTATTGAATTATAAGGCGGTTGGTTGTGCTGGCCCCTCGGGGCATGTGCACG
CCTCCGCCGTTTAATCCCAACCCACCCCTGTGAACCAAACGTCGTGAGGCGCTCTGTGATGGAGCCGT
CTTGTGCCATTTATCATAAACACCGTATGTCTTCAGAATGTAAATCTATTGCCTTCGCCTTAAAAACG
TGTCGGTAAAAAAAATGAATACAACTTTCAACAACGGATCTCTTGGCTCTAGCATCGATGAAGAACGC

AGCGAAATGCGATAAGTAATGTGAATTGCAGATTTTCAGTGAATCATCGAATCTTTGAACGCACCTTG
CGCTCCCTGGTATTCCGGGGAGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCCCGATCGGATTTG
TTTTCGATTCGGAGGCTTGGATTGTGGAGCGTGCTGGCCCCTCACCATGGGTCGGCTCCTCTCAAATG
CATTCGCGGAATTGACTTTGGTCCGTTTCGGTCATCGGTGTGATAAATGTTACGCCGTTGGCTAGGAT
CGAGGTGCCGCATACAATGGTCTTCGG

Basidiomycete sp.1

Primers: ITS1F & ITS4

ACCTGCGGAAGGATCATTATCGAGTTTTTAAATGGGGAGCGGGTTGTATTAAGCTGGCCTTTGAAAGT
AACGAAAGTAAAAAACGATATTTTTGAAGGCATGTGCACGCCTAGCTTTTCCCATACACACTCTCTCA
CAAATAAACCCCTTTTGTGCRCCTTATAGTAGAACTCGGTGTCGAGGAAGAGACTCGATTGCCGTATG
TGGCGGTCAGTCTCGGAAAAAGAACCGTGGTCTATGTATTTTGTTTTTGTCACATACGCTTTCAGTCG
AGAATGTATTTAAATTAAATAAAAAACCCTTGAGAAAGGGGGAAAATATGTGAATACACAACTTTCAG
CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATTTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTT
TGAGTGTTATGAAATTCTCAACCTTTCGAAAGATTGTCATGATTTTTtCGTAAAGGATTGGACTTGGA
GGTTGTGTGTCGGCGAGCATAATACAGATAAATAACGATTCGTCGTTTATTATCTTTGTGTCGACTCC
TYTGAAATGCATTAGCGCGAAACGATTTAATAAACGGGATCGCCTTCGGTGTGATAAATTATTTACGC
CGCAGTCGTGAAGTTTTGAAGGAAAAAACGTAACGGACGTTTGCGCTTACGAATCGTCCTCCTCGTAC
GAGGACAAAAAAAAAATCAATTAGTAACTGATTGACAAGAAACCTAACCTCAAATCAGGTAGG

Basidiomycete sp.2

Primers: ITS1F & ITS4

ACCTGCGGAAGGATCATTATCGAGTTTTTAAATGGGGAGCGGGTTGTATTAAGCTGGCCTTTGAAAGT
AACGAAAGTAAAAAACGATATTTTTGAAGGCATGTGCACGCCTAGCTTTTCCCATACACACTCTCTCA
CAAATAAACCCCTTTTGTGCRCCTTATAGTAGAACTCGGTGTCGAGGAAGAGACTCGATTGCCGTATG
TGGCGGTCAGTCTCGGAAAAAGAACCGTGGTCTATGTATTTTGTTTTTGTCACATACGCTTTCAGTCG
AGAATGTATTTAAATTAAATAAAAAACCCTTGAGAAAGGGGGAAAATATGTGAATACACAACTTTCAG
CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATTTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTT
TGAGTGTTATGAAATTCTCAACCTTTCGAAAGATTGTCATGATTTTTtCGTAAAGGATTGGACTTGGA
GGTTGTGTGTCGGCGAGCATAATACAGATAAATAACGATTCGTCGTTTATTATCTTTGTGTCGACTCC
TYTGAAATGCATTAGCGCGAAACGATTTAATAAACGGGATCGCCTTCGGTGTGATAAATTATTTACGC
CGCAGTCAAAAAACGTGAACGGAAGTGTTGGGGCTTAACGAATTGTTCCTTCCTTCGTAACGAGGACA
CAAAAAAAAAACAATTAGTAACTGATTGAACAAGAAACCTAACCTCAAATTCAGGTAG

Basidiomycete sp.3

Primers: ITS1F & ITS4

TCATTATCGAATTCACTTTGAACGTTGGGTTGTAGCTGGCCCCTCACCGGGCATGTGCACGCCTCGTT
CAATTTTATTTTAAACCCCTGTGCACAACCTGTAGGTCGGTCTGAGGTCTCGCGGCTTCTGCCTTCCT

ATGTATATTATAAACACCAAGTAAGGTCTAAAGAATGTCTGTCGCGTTTTACACGCATATATATATAC
ATATAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATcTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAG
GAGCATGCCTGTTTGAGTGTCATGGAATTCTCAACCCCATCCACCTTTGCCGGTGAATGCGGGCTTGG
ACTTGGAGGTTTCTGTCGGACGTTCACGTTCGACTCCTCTTGAATGCATTAGCTCGAAACCTTTGTGT
GATCGGCTCATCCGGTGTGATAGTATTGTCTGCGCCGGGGGCT

Basidiomycete sp.4

Primer: ITS1F

GAACCTGCGGAAGGATCATTAGAGAAAACTCAAAGATCGAGGTTGTAGCTGGCCCTCCGGGGCATGTG
CACGCCTCTTTCGCCCATCCATCTCACACCTGTGCACCTCCGCGTGGGCTGGGCTTCACGGCTCGGCC
TGCGTCTTTTCACAAACTCTTTGTATGTCYTCAGAATGTAACTTACCGATGTAATAAAAACGCATCTA
ATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAA
GGGCACACCTGTTTGAGTGTCGTGAAATTCTCAACCCTACCGGCTTTGTTGTCGGGAGGTGTTTGGAC
TTGGAGGTTCTTGCCGGCCTAGGTCGGCTCCTCTCAAATGCATCAGTGCGTACCGTTGCGTCGCTTTG
CCTTTGGTGTGATAGATATCTACGCCGCTG

Basidiomycete sp.5

Primer: ITS1F

TTAATAGAACGAGAGACTGCGAGATTGGCTAGAGGGGGAGAGAGCTCTCTCTCGCTAGCTTGTTGAGT
GGTTCTTTACTTCATTTGCACCCTCATGTGATACCTCTGCGCGAGTTGGTACTAGGTCTCTGTGGCCT
GGAAGGCTCGCGGCTTCTATATACACCCCTTGACAAGTTGTAGAATGTACCCTTGTGCGTTTAACCGC
ATCCTAATTACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAACTTGCGCCCCTTGGTA
TTCCGAGGGGCACGCCTGTTTGAGTGTCGTGAATTCCTCCACTCGCCAATCCTTTTGTGACTGGTTGA
GCTGGGACTTGGGGGTTTTTGTGGGTCCTTTCTCTGGTCCACTCCCCTTGAATGCATGAGTGA

Basidiomycete sp.6

Primer: ITS1F

CTGCGGAAGGATCATTATCGAATCTTGAAAGGGGTTGTAGCTGGTCTTTCACGAGGCATGTGCACACC
CTGCTCATCCACTTCACACCTGTGCACACTATGTAGGATAGACGAGGATCGGGGCCCTTGGTCTCGGT
GCGAGTCTCCCCTATGTCTTTACATACTCTGTTCAGTCAATGAATGTATCTGCGATAAACGCATTTAA
TACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGG
GGCACACCTGTTTGAGTGTCGTGAAATTCTCAACTCAAATGCCTTTGTTGGTCATTTGGGCTTGGACT
TGGGGGCTTGCCGGCTTGCGTCGGCTCCCCTTGAATGTATTAGCTCGAACCCTTGCGGATCAGCTATC
GGTGTGATAATTGTCTACGCCGTGGTTGTGATGCCACCTCCGGGTGTTGAGGTGTTCGGCTTCCAATC
GTCCCTCTGGGACAATTTCTTTTTGAAATCTGACCTCAAATCAGGTGGGACTACCCGCTGAACTTA

Basidiomycete sp.7

Primer: ITS1F

AACCTGCGGAAGGATCATTATCTGAGTTTTGAAAGGGGTTGTAGACTGGCCTTTTGGGGCATGTGCAC
GCCTCGCTCATTTCCAACTCTAACACCTGTGAACTAGCTGTAGGTCGGTTTCAAAATGGGCTCTTATT
GAGTCTGTTTGTGCCTTCCTATGTCTTTAACAAACTCTTGTCGTATCAGAATGTATTCGCGCTTTATT
AAACGCATCAATATAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCATCTTGCGCCCTTTG
GTATTCCGAAGGGCATGCCCGTTTGAGTGTCATTGTATTCTCAAATCCCTTCATTTTTGTGAAGTCGA
TTTGGATTTGGAGGTTGTGCGGGTAACTCGTTGTCCGCTCCTCTTGAATGCATTAGCTGGAACCTTTT
GCTAGACCGGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTAAGGCGACTTTGTTGAGGATCCGCT
TCTAATCGTCTCTTGCAGACAAACTTATCTGAC

Basidiomycete sp.8

Primer: ITS1F

ACAATGGGGTGAGAGGTTTAGTTCATGTGACTGACCGCCGCCCCTCACCAATTTATCACTCCTGTGCA
CACTAGGCTGGCTTGTGAACGGGGGGGAAGGTTTCCCCTCGGAATCATGCCTTGCCGCATCCTTATAC
AAACCCCAGAGTATAACAGAATGTGCTTGCGTTCAACGCATCGTATCACAACTTTCAGCAACGGATCT
CTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACCTTGCGCTCCTCGGCATTCCGAGGAGCATGCCTGTTTGAGTGTCAT
GAAACCCTCAACCCTGTCATCTTTGCGGATGTAAGCAGGGCTTGGATCCTGGAGGTTTTTT

Basidiomycete sp.9

Primer: ITS1F

CATTCATAATAAGTGTTTTACTGCACTTTTTAAAAAATAGTACTCACCTTGTGTGCAATGTTGTGTTG
GRAGRAGGGTTTTTTACRAAACTTTTCCTCCCACACCTACACCAACCTAATAACCTTGAACCTCTTTG
TCTGAAAAACTATTATGAATACTTAATTCAAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCA
TCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACATTGCACTCTCTGGTATTCCGGAGAGTATGCTTGTTTGAGTATCAGTAAACACCTCAAG
TCTCTTTGTCTTTGTGACAAGGAGAGTTGGACTTGAGCAATCCCAACTCTTTTGYAAAAAAGGGGCGG
GTTGCTTGAAATGCAGGTGCAGCTGGACATTCTTCTGAGCTAAAAGCATATTTATTTAGTCCCGTCAA
ACGGATTATTACTTTTGCTCTAGCTAATATAAAGgTCAAATGGACGCGATGCTGACTGATGCAAGATT
ACCAAGTTTTT

Ceriporiopsis sp.1

Primer: ITS1F

AGGGTTGTTGCATGGGGTCCCTAACCGGGATTCAAAGTGCACGCCCTGTGTCTATTCTCAAACCCCTG
TGCACTTATTGTAGGCTCGGTGGAAAGACCGACTTCGGTTGGTCTGAAAGCCTTGCCTATGCTTTAAC
ACACGCTTCAGTCTATGAATGTAACTTTGCGGATAACGCATTAAATACAACTTTCAACAACGGATCTC
TTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA

TCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATG
GAATTCTCAACCTTCAACACTTTTGTGAAGAAGGCTTGGACTTGGAGGTCGTGTTGGCTTGCAAAAGT
CGACTCCTCTGAAATACATTAGTGCGAACCTTACGGATCGCTTCGGTGTGATAATTGTCTACGCCGTG
GTGTTGAAAGTATAGCAGTGTTCGAGCTTCTAACCGTCCGCAAGGACAACTTTCTGACAATCTGACCT
CAAATCAGGTAA

Coniophora-like sp.1

Primers: ITS1F & ITS4

GAACCTGCGGAAGGATCATTATCGATTCAAACAAATGAGAAGGGAGTTGCTTGTAGTTGTGCTGGCTT
TCTAACCAAGGCATGTGCACGCTCGACTTCTCTTTTTCTTCATTTACACACCTGTGAACCTGTTGTAG
GGCGTCTCGCAAGAGATGCTCTATGTATTTTCATATACTCCATTGATAGTCTTAGAATGTACCTTTTT
GTTCTCGTCAGAGATCAAAATAAAAGCCTTTATAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCG
ATGAAGAACGCAGCGAATTGCGATATGTAATGTGAATTGCAGATTTTCAGTGAATCATCGAATCTTTG
AACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAAATTCTCAACTCC
CTTTGATTTCTTCAAAGGTGAGCTTGGATTGTGGAGGTCTGCCGGCTGCAAAGTCGGCTCCTCTGAAA
TGCATTGGCAAAGGYGTGTGCATTAATCGGCCTTTCGGTGTGATAATGATCACCGTGGCTGGCTTGCT

Coniophora-like sp.2

Primer: ITS1F

AACCTGCGGAAGGATCATTATTGAATTTTTGAAGGAGCTGTTTGCTGGCCCTTGACGCGGGCATGTGC
ACGCTTCGTTTCAAATCTCCAACCTTCTTCATACCCCTGTGCATCTTTTGTAGGGTCGTGTCGGTCGA
AAGGCCGGTGCGCTCTATGTCATATCATAAACTCTCGTATGTGTAGAATGTTCAATGCGCACGACGCA
TCTTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATT
CCGAGGAGCATGCCTGTTTGAGTGTCATGGAATCATCAACTCTTGTTTTTTTCTATCGAAGGAATGAG
GGCTTGGACTTGGAGGCTCGTGCTGGCCCTGTTTGGGGTCAGCTCCTCTTGAATGCATTAGCTTGAAC
CTTCTGCTGTATCGGCTGCTCGGTGTGATAATTATCTACACCGTGGCTGTGAGGCTT

Coniophora-like sp.3

Primer: ITS1F

ACCTGCGGAAGGATCATTATCGATTCAACACAGAGATGAGAAGGGAGTTGTTTGTAGCTGTGCTGGCC
TCCGACAAAGGGGTATGTGCACGCTCGACTTCTCTTTTTCTTCATTGTGACACACGCTGTGAACCCGT
ATTGTAGGGTGTCTCGCAAGGGATGCTCTATGTCTATTTTCATATACCCCATTGTATGTTTTTAGCAA
TGTATCTTTTTGCTCTCGTCAGAGAACAAAAGTAAAGCCTTTATAACTTTCAGCAACGGATCTCTTGG
CTCTCGCATCGATGAAGAACGCAGCGAATTGCGATATGTAATGTGAATTGCAGATTCTTCAGTGAATC
ATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAA
GATTCTCAACTCCCTTGTGATTTCTTCAAGGGTGAGCTTGGACCGTGGAGGCT

Fistulina-like sp.1

Primers: ITS1F & ITS4

TAGGTGAACCTGACGGAAGGATCATTAACGAATAGACCCAGAGGGGTTCGTTGCTGCCGTCTCGGTGT
GCACGCCCTTCTGATTCATTCAACCATTCATCCACCTGTGAACCTTCTGCGTGATTGGGAGTCCTTCG
ACGAAGTCGCGAAGGTCGCATCTGTCTCAGCTCTGCGGCCTCGTGGCTGAGGAGCCTGACTTCCCTTC
ACGTCTCATAAACACACCTTTAAAAAAACAGTCTCAGAAAGTCAACGGTCGTAGCGGACCATAAACGT
GTCTATACAACTTTTGACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTC
CGAGGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCGCTCTCGGTTCGTCATTCGAGCTGACGA
GTTGCGTTGGATCGTGGGGGTCTCTCTTTGCCGGCTGTTCGCGGTCGGCTCCCCTCAAATGCATTAGC
GACCGTCCTCTGCTGGATCGTGCACTCGGTGTGATAGTGTTCTCATACGTCTACGCCGTCGTTGCCAT
CCCGGGGCCACATGTGGCCTCAAGGAGGTGATCACAAAAGCTTTGTCGCTCACAAATCGTCCCTATAT
GCGCGCATATATGCGCCCATTTCGTTTGGGACAGCGCTTCGGCGCTCGTTGATCATGACTTGACCTCA
AATCAGGTAGAC

Fomitopsis-like sp.1

Primer: ITS1F

AACCTGCGGAAGGATCATTAATGAATTTTGAAAGGGGTTGTAGCTGGCCTTTTCGTTTGAGAGGCATT
TTTGTGCACACCCTGATCATCATCCATCTCACACCTGTGCACATCCTGTAGGTCGGTTTGTGAGGTGA
RGCCTTCATTGGCTTCGCTTTGTGGACCTTCCTATGTTTTAATTACAAACTACTAGTTTAAAGAATGT
CTCTGCGTTTAACGCATTTAAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT
TGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAATTCTCAACTCTGTTCRCTTT
TGTGGGTGGAGTTTGGACTTGGGGGACACTGCTGAATGCATTAGYGTTTGGCTCCCCTTAAATGCATT
AGCTCGAACCTTTGTGGATCAGCTTCGGTGTGATAATTGTCTACGCCGTTCTGTTGAAGCATAATTCT
ATGGGTTTCGGCTTCCAATTGTCCTTTGCTGGACAAATRTTTCTTTGACcTTTGAcCTCAGATCARGT
AGGATTACCCGCTGAACTTAGCATAT

Ganoderma applanatum/adspersum

Primer: ITS1F

TGAACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCACGTGCACG
CCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTACGGGTCGCGAAACGGGCTCGTTTAT
TCGGGCTTGTGGAGCGCACTTGTTGCCTGCGTTTATCACAAACTCCATAAAGTATTAGAATGTGTATT
GCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC
GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTACAAGCTCTTTGC
GGGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCTTTAATGGTCGGCTCCTCTTAAATGCATTAGC
TTGATTTCCTTGCGGATCGGCTGTCGGTGTGATAATGTCTACTCCGCGACCGTGAAGCGTTTGGCAAG

CTTCTAACCGTCTCGTTACAGAGACAGCTTTATGACCTCTGACCTCAAATCARGTAGGACTACCCGCT
GAACTTAAGC

Ganodermataceae sp.1

Primer: ITS1F

GATCATTAACGAGTTTTGAAACGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCA
CTCTACCCCTGTGCACTTACTGTAGGTTTCGAGTTTGATGAGTGTCGTCCGCGGCGCTTGGATTCTCG
GGGCTTACGTTTATTACAAACTATTTAAAGTATCAGAATGTGTATTGCGATATAACGCATCTATATAC
AACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGC
ATGCCTGTTTGAGTGTCATGAAATTCTCAACCTACAAGCCTTTGCGGGTTCTGTACGTGTTGGATTTG
GAGGATTATCATTGTCGGCGCAAGTCGGCTCCTCTCAAATGCATTAGCTTGATTCCTTGCGGATCGGC
TCTCGGTGTGATAATTGTCTACGCCGCGACCGTTGAAGCGTATTGGCAAGCTTACAATCGTCTCTAGA
GACAGCTTACTTTGACATCTG

Gymnopilus allantopus

Primer: ITS1F

AACCTGCGGAAGGATCATTATTGAATAAACTTGATGTGGTTGTAGCTGACTCTCTCGAGAGTATGTGC
TCGCCCGTCATCTTTATCTTTCCACCTGTGCACTTCTTGTAGATTTGGATGTAGCTTTCCGAGGTAAC
TCGGTCGGGAGGAATGTCAACTCTTGTTGACTTTCCTTGTATGTCCAAGTCTATGTTTTTATATACTC
CAATGAATGTAACAGAATGTATCACTGGGCCTTGTGCCTATAAACTATATACAACTTTCAGCAACGGA
TCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAAGAATTCA
GTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTACCGAGGGGCATGCCTGTTCTGAG
TGTCATT

Hymenochaetaceae sp.1

Primers: ITS1F & ITS4

GATCAtTACTGAGGTTACCCGGGGTGAGTTTGTTGGGTGCTTGCTGCTGGCGTTCTCTCAGAGCGCAT
GTGCACGGCTCCTCTTCTCGCCTTCGTTCCTTTTATCAACCCCCTGTGCACTACATAGGATTAGAGAG
AAGCGTGATGCTTTAAGTTAGATTAGCATTAACCTAGTAGTCGTGGGTCCGCGACCTTCGGCGAAAGG
GGAGTTCATTTTGATCGACGTCGCGTGGTACCTCGAAGTCCTTGATTATCACAAACCACTATATATGT
CTTGTCAGAATGATCAGTCCCTTGTTGGACGCTAAATATAATACAACTTTCAACAACGGATCTCTTGG
CTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAAGGGCACGCCTGTTTGAGTGTCATGTTCA
CCTCAATCCATCAGCTTTTGCGGCTGACTTGAATTGGATTTGGGAGCCTGCTGGGCCCTTCGTGGCTC
GGCTCTCCTTGAATGCATGAGTGAGCTTCTGGCTCGCGTTGCTGGTGTGATAGTCTTTCATCATTGGC
GACGCTTGCTTAATGGGCTTGCTTTCGAACCGTCTTTTCAACGGAGACAACTATTGACATATTTGACC
TCAAATCAG

Hymenochaetaceae sp.2

Primers: ITS1F & ITS4

GAACCTGCGGAAGGATCATTACTGAGGTTACCGGGGGTGGGCTGCTCGAGTGGCTTAGTGCTGGCGCC
CTAGGCGCATGTGCACGGCTCTCCTTCTCACCTTCGTTCCTTCTATCAACCCCTGTGCACTATATTAG
GATTAGAGAGAAGCGCGTCGTTTCAGGGATTAACGTAGTAGTTGTGGGTCCGCCCGTGGCAAAAGGTT
AATCTGTGATCGGCGACGCGTGGTACCTCGAAGTCCTTAATTACAAACCACAATACATGTCTTGTCAG
AATGTTCAGTCCCTCGTTGGACGCTAAATACAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCA
TCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACCTTGCGCCCTTTGGTATTCCGAAGGGCACACCTGTTTGAGTGTCATTTTCACCTCAATC
CATCAGCTTTGCGGCTTGACTGGGTTGGATTTGGGAGGTTCTGCTGGTTCCTTAACGGGGTTCGGCTC
TCCTTGAATGCATGAGTGGGCATCTAGCTCGCACTGCCGGTGTGATAGTTTTTCATCAT

Hypholoma-like sp.1

Primer: ITS1F

CCTGCGGAAGGATCATTATTGAATAAACCTGGCTTGCGTTGCTGCTGGTCTTTTCGAAGGCATGTGCA
CACCTTGTTCATCTTTATATCTCCACCTGTGCACCTTTTGTATGACCTGGATTGCAACTTTCCGAGGC
AACTCGGTTGTGAGGAATTGCTTAATCAGTCTTTCCTTGTTATGTTTCCAGGGCTATGTTTTCATATA
CACCCTACGAATGTAACAGAATGTCATTATTAGGCTTAATTGCCTTATAAACTATATACAACTTTCAG
CAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGTAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCCAATCTTTAGAACTGCATCCTTGCGCTCCTTGGTATTCCGAGGAGCATGCC
TGTTTGAGTGTCATTAAAATTC

Hypholoma-like sp.2

Primer: ITS1F

AACCTGCGGAAGGATCATTATTGAATAAACCTGGCTTGGTTGCTGCTGGTCTTTTCGAAGGCATGTGC
ACACCTTGTCATCTTTATATCTCCACCTGTGCACCTTTTGTAGACCTGGATTCAACTTTCCGAGGCAA
CTCGGTTGTGAGGAATTGCTTAACAGCTTTCCTTGTTAGTTTCCAGGGCTATGTTTTCATATACACCC
TACGAATGTAACAGAATGTCATTATTAGGCTTAATTGCCTTATAAACTATATACAACTTTCAGCAACG
GATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGT
GTCATTAAATTCTCAACCTTTATTAGCTTTTTGGTTAGTAAATGGATTGGAAGTGGGGGTATGTTGGT
TTCTTTATTGAAATGAACTCCCCTGAAATGCATTAGCTGGTTGCCTTGTGCAAACATGTCTATTGGTG
TGATAATTATCTACGCCGTGGACTATTTGCCGTTTATAGCACTGCTTATAATCGTCTG

Hypholoma fasciculare

Primers: ITS1F & ITS4

AACCTGCGGAAGGATCATTATTGAATAAATCTGGCTTGGTTGATGCTGGTCTTTTCGAAGACATGTGC
ACACCTGGTCATCTTTATATCTCCACCTGTGCACCTTTTGTAGACCTGGATTCAACTTTCCGAGGAAA
CTCGGTTGTGAGGAGTTGCTTAATAGGCTTTCCTTGTTCGTTTCCAGGGCTATGTTTTCATATACACY

TTACGAATGTAACAGAATGTCATTATTAGGCTTAATTGCCTTATAAACTATATACAACTTTCAGCAAC
GGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAG
TGTCATTAAATTCTCAACCTTTATTAACTTTTTGGTTAGTAAGTGGATTGGAAGTGGGGGCATGTTGG
TTTCTTCATTGAAATAAACTCCCCTGAAATGCATTAGCTGGTTGCCTTGTGCAAACATGTCTATTGGT
GTGATAATTATCTACGCCGTGGGCTACTTGCCGTTTATAGCACTGCTTATAATCGTCTGTTCATTCAG
A

Hypocrea pachybasioides

Primer: ITS1F

TGTTGCCTCGGCGGGGAATTTATTCTTGCCCCGGGCGCGTCGCAGCCCCGGACCAAGGCGCCCGCCGG
AGGACCAACCAAAACTCTTTTGTATGTCCCCTCGCGGACTTTTATAATTCTGAACCATCTCGGCGCCC
CTAGCGGGCGTTTCGAAAATGAATCGAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGG
GGGTACGGCGTTGGGGATCGGCCCTT

Metarhizium flavoviride

Primer: ITS1F

GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACTTATACCTTT
ACTGTTGCTTCGGCGGGTCCGCCCCGGAACAGGTTCGCGAGAGCCGCCCCGGAACCAGGCGCCCGCCG
GGGGACCAAAACTCTTGTATTTTTTATTTGCATGTCTGAGTGGAATCATATAAAATGAATCAAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTACTCTGGCGGGCATGCC
TGTTCGAGCGTCATTTCAACCCTCAAGCCCCAGCGGTTTGGTGTTGGGGACCGGCGATGGCGCCTGCT
CCGGCAGGCGCGCGCCGCCCCCGAAATGAATTGGCGGTCTCGTCGCGGCCTCCTCTGCGTAGTAGCAC
AACCTCGCAACAGGAGCGCGGCGCGGCCACTGCCGTAAAACGCCCAACTTTTTTTTCAGA

Nectria radicicola

Primer: ITS1F

GGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCATAACTCCCTGTGAACATACCATTTAG
TATGCCTCGGCGGTGCCTGCTTCGGCAGCCCGCCAGAGGACCCAAACCCTTGTATTTTATACTAGTAT
CTTCTGAGTAAATGATTAATATAAATCAAAACTTTCAACAACGGATCTCTTGTGTTCTGGCATCGATG
AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCTGAATCTTTGAAT
CGCACTATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCTAAGTC
CCCCGGGCTTGGTGTTGGAGATCGGCGTGCCCTCCGGGGCGCGCCTGGCTCCCAATATATAGTGGCGG
TCTCGCTGTAGCTTCCTCTGCGTAGTATGCACACCTCGACACTGGAAAAACACGCGTGGCCACGCC

CG

Peniophora aurantiaca

Primer: ITS1F

GTAGGTGAACCTGCGGAAGGATCATTAGCGAAGCTCGGAATGCGTGTTCGGTCTGATGCTGCCCAGCG
ATGGGATGTGCTCGTCCGGATGCGTGTCCCTTCTCCGTTCCACCCCTTTGTGAACCAAGTGTGCGAGC
CGAAGAGAGATCGGAGGCTCGCATGCAACCCTTAACATACCCCAACGAAGTATCAGAATGTACCTTGC
GTTAACTCGCACAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCC
CTTGGCATTCCGAGGGGCACGCCTGTTTGAGTGTCGTGAACTCCTCCACCCTCCACCTTTTTCGGAAG
GCGTTGGGCTGGGATTTGGGAGCTTGCGGGTCCCTGGCCGATCCGCTCTCCTTGAATGCATTAGCGAA
GCCCTTGCGGCCTTGGTGTGATAGTCATCTACGCCTCGGTTTAGCGAAC

Peniophora cinerea

Primer: ITS1F

AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCC
TTGGCATTCCGAGGGGCACGCCTGTTTGAGTGTCGTGAACTCCTCCACCCTCCACCTTTTTCGGAAGG
CGTTGGGCTGGGATTTGGGAGCTTGCGGGTCCCTGGCCGATCCGCTCTCCTTGAATGCATTAGTGAAG
CCCTTGCGGCCTTGGTGTGATAGTCATCTACGCCTCGGTTTAGCGAACATACGGGCATCGCTTCCAAC
CGTCTCGTCAAGAGACAATCAC

Phanerochaete sordida

Primer: ITS1F

GAACCTGCGGAAGGATCATTAACGAGTAACTGAACGGGTTGTAGCTGGCCTCTCGGGGCATGTGCACA
CCTGGCTCATCCACTCTTCAACCTCTGTGCACTTGTTGTAGGTCGGCGGAAGGGCGAGTCTCTTAAAA
ACAGGCTCGCTTGGAAGCCTTCCTATGTTTTACCACAAACGCTTCAGTTTAAGAATGTAACCTGCGTA
TAACGCATTTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCC
TGGTATTCCGGGGAGCATGCCTGTTTGAGTGTCATGGTATTCTCATCCTTCATAACTTTTTGTTATCG
AAGGCATGGACTTGGAGGTCGTGCTGGTTCCTCGTTGAATCGGCTCCTCTTAAATGTATTAGCGTGAG
TGTAACGGATCGCTTCGGTGTGATAATTATCTGCGCCGTGGTCGTGAAGTAACATAAGCTTGCGCTTC
TAACCGTCCTTAAGTTGGACAAATCACTTTGACATCTGAC

Phialophora-like sp.1

Primer: ITS1F

ACAGAGTTCTAAAAGACTCCCAAAACCATTGTGAACGTACCCGTCAGCGTTGCCTCGGCGGGCGGCCC
CTCCCTGGGGCCGCTGCCTCCCTCGGGGGGTGCCCGCCGGCGTACCAAAACTATTTTGTATTTTAGTG
GCCTCTCTGAGAAAACAAGCAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCGAATTCAGTGAATCATCGAATCTTTGAACG
CCCATTGCGCCCGCCAGTACTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCGCCCTCGNAGCCCTGC
TTGGTGTTGGGGTCCTACGGGCTGGC

Phlebia-like sp.1

Primers: ITS1F & ITS4

GAACCTGCGGAAGGATCATTATCGAGTTTTGAAACGGGTTGTAGCTGGTCTTCGATCGCGGAGGCATG
TGCACGCCTGACTCATCCACTCTCAAACCCCTGTGCACTTATTGTAGGCTCGGTGGGAGAGGCTGACT
TTCATCGGTCGGTTTCGAAAGCCTCGCCTATGTTCATCACATACGCTTCAGTTTAGAATGTAACGCTT
GCGCATGACGCAAATTAAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGC
GCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCGTGAAATTCTCAACTTTCAAAACTTTTTT
GTTTCGAAAGCTTGGACTTGGAGGCCGTGTCGGTGCGTTTCGCGTCGACTCCTCTCAAATGCATTAGC
GTGAATCTTACGGATCGCCTTCGGTGTGATAATTACTGCGCCGTCGTCGTGAAGTATAACAGCGTTCG
CGCTTA

Phlebia-like sp.2

Primer: ITS1F

ACCTGCGGAAGGATCATTATCKGAGTTTTGAAACGGGTTGTCGCTGGTCCCTGCAACATGGGACATGT
GCACGCCTGGCTCATCCACTCTTCAACCCCTGTGCACTTTTTGTAGGTTCAGGCCGGACGGGTCGACT
TTGTTGTCGGCTCGAAAGCGCTTGGCCTATGTTTTCTTACAAACGCTTCAGTCACAGAATGTAAAAAC
ATTGCGGATAACGCATTTTAAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCT
TGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAATTCTCAACCTTTGAAAGCTT
CTTCTTGAGGGTTTTTAAAGGCTTGGACTTGGAGGTCGTGTCGGCTTGCAAAGGTCGACTCCTCTGAA
ATGCATCAGTGTGAACCTTTACGGATCGCCTTCGGTGTGATAAATGTCTACGCCGTAGTCGTGAAGTA
TGATTGCGTTCGTGCT

Polyporus gayanus

Primer: ITS1F

ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCT
TTGAACGCACCTTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCAGTAAATTCTCAAC
CTCTCATGCTTTGTTGGCATTGAGTGGATTGGATATGGAGGCTTGCTGGATTTTATTCAGCTCCTCTA
AAATATATTAGCAGAAACCATTGCTAAGGTTTACTGCAGCCGTGTGATAATTATCTTACGGTTGAAGT
AAATGTTTAGTTTGTCTCACATTTGGTTTGAAGAGATGCTTTTCGTAAGCTCCCTTTGCTTTTCTCTC
TTCGGAGTGATACTTATCCAAGTGTAGATTTTAATGGGTCTTATTCAGCTTCTAATCATCTGGAAACA
GATAATACTTGACCATTTGGCCTCAAATCAGGTA

Postia pelliculosa

Primer: ITS1F

GAACCTGCGGAAGGATCATTATCGAACCTTTGAAGGAGCTGTTTGCTGGCCCTTGACCCGGGGCATGT
GCACGCTCCATTCAAACTCCAACCTCTTCATACCCCTGTGCATCGTTTGTAGGGTGGCGTCGGTCGAA
AGGCCGGTGCGCTCTATGTCCATTGTAAACTCTTGTATGTATGGAATGTTCAATGCGCATGACGCATC

TTCAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTC
CGAGGAGCATGCCTGTTTGAGTGTCATGGAACCATCAACTCTTACTTCTTTTCTCAAGGAAGTGAGGG
CTTGGACTTGGAGGCTTGTGCTGGCTCTGTTTTGTGGGTCCGGCTCCTCTTGAATGCATTAGCTTGAA
CCTTCTGCTGTATCGGCTGTTCGGTGTGATAGTTATCTATGCCGTGGCTGTGAGGCT

Postia-like sp.3

Primers: ITS1F & ITS4

AACCTGCGGAAGGATCATTATTGAATCTTTGAAGGGTGAGCTGTTTGCTGGCCTCTTGCAGGCATGTG
CACGCYCCCTTCAAAATCCAACCTTCTATACACCTGTGCACTGTTTGTAGGGTCGCGGTCGAAAGGCT
CGCGCTCCTATGTTCATCATAAACCCTGTAGTACGTGAGGAATGTCATTGCGTGTAACGCATCTTTAT
ACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGA
GCATGCCTGTTTGAGTGTCGTGGAACCCTCAACTCTTGTCTTTTCTTTGCTTTGCTGAAGGGATGAGG
GCTTGGACTTGGAGGCTTTTGCTGGCTCCTCTGTGATGTCGGCTCCTCTTGAATGCATTAGCTTGAAC
CTCTGCTGTATCGGCTGTTCGGTGTGATAATTGTCTACGCCGTGGCTGTGAAGCTTCTGAATATGGGG
CTTGGCTTCCAACTGTCTCTTGGAC

Postia-like sp.4

Primers: ITS1F & ITS4

AGCTGTTTAGCTGGCCCTTGGACGGGGCATGTGCACGCTTCGTTGTCAAATCTCCAACCTCTTCATAC
CCCTGTGCATCTTTTGTAGGGTCGCATCGGTCGAAAGGCCGGTGTGCTCTATGTCATATCACAAACTC
TTGTATGTGTAGAATGTTCAATGCGCACGACGCATCTTTATACAACTTTCAGCAACGGATCTCTTGGC
TCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATC
GAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAATC
ATCAACTCTTATTCTTTCTATTGAAGGAACGAGGGCTTGGACTTGGAGGCTCATGCTGGCCCCGTTGG
GGTCGGCTCCTCTTGAATGCATTAGCTTGAACCTTCTGCTGTATCGGCTGTTCGGTGTGATAATTATC
TACGCCGTGGCTGTGAGGCTTTAAACTGGTGGGCTCAGCTTCTCAACCGTCCTTTCGAACGAGGACTA
CCCGCAAAGGGTACCATTGACCTCTGA

Postia-like sp.5

Primers: ITS1F & ITS4

ACCTGCGGAAGGATCATTATTGAATTTTTGAAGGAGCTGTTTGCTGGCCCTTGGACGGGGCATGTGCA
CGCTTCGTTTCAAATCTCCAACCTTCTTCATACCCCTGTGCATCTTTTGTAGGGTCGTGTCGGTCGAA
AGGCCGGTGCGCTCTATGTCATATCATAAACTCTCGTATGTGTAGAATGTTCAATGCGCACGACGCAT
CTTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTC
CGAGGAGCATGCCTGTTTGAGTGTCATGGAATCATCAACTCTTGTTTTTTTCTATCGAAGGAATGAGG
GCTTGGACTTGGAGGCTCGTGCTGGCCCTGTTTGGGGTCAGCTCCTCTTGAATGCATTAGCTTGAACC
TTCTGCTGTATCGGCTGCTCGGTGTGATAATTATCTACGCCGTGGCTGTGAGGCTTTGAAACTCGTGG

GCTCAGCTTCTAACTGTCCTTTTGAACGARGACTACCTGCGAAGGTACCATTGACCTCTGACCTCAAA
TCAGGTAGGA

Postia-like sp.6

Primers: ITS1F & ITS4

TAGGTGAACCTGCGGAAGGATCATTATCGAACCTTTGAAGGAGCTGTTTGCTGGCCCTTGACCCGGGG
CATGTGCACGCTCCATTCAAACTCCAACCTCTTCATACCCCTGTGCATCGTTTGTAGGGTGGCGTCGG
TCGAAAGGCCGGTGCGCTCTATGTCCATTGTAAACTCTTGTATGTATGGAATGTTCAATGCGCATGAC
GCATCTTCAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG
CGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGG
TATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAACCATCAACTCTTACTTCTTTTCTCAAGGAAGT
GAGGGCTTGGACTTGGAGGCTTGTGCTGGCTCTGTTTTGTGGGTGCTGCTGCTCTTGCATGCATGCAT
TAAACTTGAAACCTTCTGCTGTATCGGCTGTTCGGTGTGATAGTTATCTATGCCGTGGCTGTGAGGCT
T

Postia-like sp.7

Primer: ITS1F

GTAGGTGAACCTGCGGAAGGATGCATTATGCGAACCTTTGAAGGAGCTGTTTGCTGGCCCTTGACCCG
GGGCATGGTGCACGCTCCATTCAAACTCCAACCTCTTCATACCCCTGTGCATCGTTTGTAGGGTGGCA
TCGGTCGAAAGGCCGGTGCGCTCTATGTCCATTGTAAACTCTTGTATGTATGGAATGTTCAATGCGCA
TGACGCATCTTCAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCC
TTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAACCATCAACTCTTACTTCTTTTCTCAAGG
AAGTGAGGGCTTGGACTTGGAGGCTTGTGCTGGCTCTGTTTTGTGGGTCCGGCTCCTCTTGAATGCAT
TAGCTTGAACCTTCTGCTGTATCGGCTGTTCGGTGTGATAGTTATCTATGCCGTGGCTGTGAGGCTTC
AAACTTGTGGGGGCTCAGCTTCTAACCGTCC

Postia-like sp.8

Primer: ITS1F

CCTGCGGAAGGATCATTATTGAATTTTTGAAGGAGCTGTTTGCTGGCCCTTGGACAGGGCATGTGCAC
GCTTCGTTTCAAATCTCCAACCTTCTTCATACCCCTGTGCATCTTTTGTAGGGTCGTGTCGGTCGAAA
GGCCGGTGCGCTCTATGTCATATCATAAACTCTCGTATGTGTAGAATGTTCAATGCGCACGACGCATC
TTTATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAA
GTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCC
GAGGAGCATGCCTGTTTGAGTGTCATGGAATCATCAACTCTTATTTTTTTCTATCGAAGGAATGAGGG
CTTGGACTTGGAGGCTCGTGCTGGCCCTGTTTGGGGTCAGCTCCTCTTGAATGCATTAGCTTGAACCT
TCTGCTGTA

Postia-like sp.9

Primer: ITS1F

CCTGCGGAAGGATCATTATCGAATCTTTGAAGGAGCTGTTTGCTGGCCCTTGGACGGGGCATGTGCAC
GCTTCGCTCAAATCCTCCATTCATACCCCTGTGCATCGTTTGTAGGGTCGCGTCGGTCGAGAGGCCGC
ACGCGCTCTATGTCTATCATAAACTCTTGTATGTGTAGAATGTTCAATGCGTCCGACGCATCTTTATA
CAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAG
CATGCCTGTTTGAGTGTCATGGAATCATCAACTCTTTGTTTCTTTTTCGAGAGAAACCGAGAGCTTGG
ACTTGGAGGCTTGTGCTGGCTCTGTTTTTATGGGGTCGGCTCCTCTTGAATGCATTAGCTTGAACCTT
CTGCTGTATCGGCTGTTCGGTGTGATAATTATCTACGCCGTGGCTGTGAGGCTTTAAATCTGTGGGCT
CAGCTTCTAACCGTCCTTTGAACGAGGACTGCCGTGTAAAGGTATC

Postia-like sp.10

Primer: ITS1F

TCATTATTTCGAACCTTTGAAGGAGCTGTTTAGCTGGCCCTTGACCCGGGGCATGTGCACGCTCCTAT
TCAAACTCCAACCTCTTCATACCCCTGTGCATCGTTTGTAGGGTGGTGTCGGTCGAAAGGCCGGTGCG
CTCTATGTTCATTGTAAACTCTTGTATGTATGGAATGTTCAATGCGCATGACGCATCTTCAATACAAC
TTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
CCTGTTTGAGTGTCATGGAACCATCAACTCTTACTTCTTTTCTCAAGGAAAGTGAGGGCTTGGACTTG
GAGGCTTGTGCTGGCTCTGTTTTGTGGG

Psathyrella-like sp.1

Primer: ITS1F

GTAGGTGAACCTGCGGAAGGATCATTAATGAAATAACTATGGCGTTGGTTGTAGCTGGCTTCTAGGAG
CATGTGCACACCCGTCATTCTTATCTTTCCACCTGTGCACTTAATGTAGATCTGGATAACCCTCGCTC
ACACCGAGCGGATACAGAGATTGCCGTGTCGCAAGGCCGGCTCTCTTTGAATTTCCAGGTCTATGTAC
CTTTACAAACCCCAATTGTATGATAATGAATGTAGTCAATGGGCTTTCAAGCCTATAAAACAAAATAC
AACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGC
ATGCCTGTTTGAGTGTCATTAAATTCTCAACTTCATCAGTTTTGTTATGGAACTGTGTGAAGCTTGGA
TGTGGGGGTTTATGCAGAACGCGTAACAGCTGTCTGCTCCCCTGAAATGAATTAGCGAGTTCAAACTG
GGCTCCGTCTATTGGTGTGATAATTATCTACGCC

Steccherinum-like sp.1

Primer: ITS1F

AACCTGCGGAAGGATCATTAATGAATGAAATTGAGTGGGGTTGTAGCTGGCCTTTTACCGGGCATGTG
CACACCGTCATTGCAACCACCTTCTATACCTCTGTGCACTTGCTCATGAGTTGGACCGCGTCTGAAAT
ACGACAGGAGTCCGGCTCATGTGCTTTATACATAACAAATTATGTTCATATGAATGTCATTACCATGC

TTTAAAGCATATAATACAACTTTCAACAACGGATCTCTTGAGCTCTCGCATCGATGAAGAACGCAGCG
AAATGCGTATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCT
CCTTGGTATTCCGAGGAGCATGCTTGTTTGAGTGTCATGGTCTTCTCAACCCTTCTGTCTTTTTTGTT
AAAGGCAGCTGGGCTTGGACTTGGAGGTCTTGCCGGCGTCCGAATTTGTAATTCAGAAGTCGGCTCCT
CTGAAATGCATTAGCTTGAATAGAACCAAGCATGATTCAGCGTGATAATTG

Stereum-like sp.1

Primer: ITS1F

CCGTAGGTGAACCTGCGGAAGGATCATTAATGAAAATTATGACTGGAGTTGTAGACTGGCCTTTAAAA
ACGGCATGTGCACGCTCCTTTCACAATCCACACACACCTGTGCACCTTCGCGGGGGTCTCTCTGATCG
ACCTTCTGGTCTTTTGGAGAGGCTCGCGTCCCTTTACACACCCTTTGTATGTCTTAAGAATGTCTACT
CGATGTAATAAAAAACGCATCTAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
CTTGCGCCCTTTGGTATTCCGAAGGGCACACCTGTTTGAGTGTCGTGAAATTCTCAACCCTCTTCACT
TTCGTGAACGTAGGGATTGGACTTGGAGGCTTTGCCGGGCGGGCTTCACCGCTCGGCTCCTCTCAAAT
GCATTAGTGCGTCTTGTTGCGACGTGCGCCTCGGTGTGATAATTATCTACGCTGTGGTGCGCTGCTTC
TGTGGAGACGCG

Trametes ochracea

Primer: ITS1F

TGCGGAAGGATCATTAACGAGTTTTGAAACGAGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCTCTG
CTCATCCACTCTACCCCTGTGCACTTACTGTAGGTTGGCGTGGGCTCCTTAACGGGAGCATTCTGCCG
GCCTATGTATACTACAAACACTTTAAAGTATCAGAATGTAAACGCGTCTAACGCATCTATAATACAAC
TTTTAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATG
CCTGTTTGAGTGTCATGGAATTCTCAACTTATAAATCCTTGTGATCTATAAGCTTGGACTTGGAGGCT
TGCTGGCCCTCGTTGGTCGGCTCCTCTTGAATGCATTAGCTCGATTCCGTACGGATCGGCTCTCAGTG
TGATAATTGTCTACGCTGTGACCGTGAAGTGTTTTGGCGAGCTTCTAACCGTCCATTAGGACAA

Trametes versicolor

Primer: ITS1F

AACCTGCGGAAGGATCATTAACGAGTTTTGAAACGAGTTGTAGCTGGCCTTCCGAGGCATGTGCACGC
TCTGCTCATCCACTCTACCCCTGTGCACTTACTGTAGGTTGGCGTGGGCTCCTTAACGGGAGCATYCT
GCCGGCCTATGTATACTACAAACACTTTAAAGTATCAGAATGTAAACGCGTCTAACGCATCTATAATA
CAACTTTTAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAG
CATGCCTGTTTGAGTGTCATGGAATTCTCAACTTATAAATCCTTGTGATCTATAAGCTTGGACTTGGA
GGCTTGCTGGCCCTTGCGGTCGGCTCCTCTTGAATGCATTAGCTCGATTCCGTACGGATCGGCTCTCA
GTGTGATAATTGTCTACGCTGTGACCGTGAAGTGTTTTGGTGAGCTTCTAACCGTCCATTAGGACAAT
TTTTAACATCTGACCTCA

Typhula-like sp.1

Primer: ITS1F

AACCTGCGGAAGGATCATTAACGAATTCAAACCTGGGTTGTAGCTGGCCTCTCGAGGCACGTGCACAC
CCTCGTCCTAGATTCATCCCTGTGCACCCTCTGTAGGCCGGATTCGTTTCTGGCCTATGTCTTCACAC
ACCCTTTAGAAATACCGTGAACGTCTTGCGCAGCTCGACCTTAAACAAGTCGGGCTCGATATCGTACA
ACTTTTAGCAATGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATTGCGATATGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAAGGGCA
TGCCTGTTTGAGTGTCATTAAATCATCAACTCCAATCGATTCGTTTCGGGCCGTTGGAGCTTGGACTT
TGAGGGTGTGCTGGCGCGAACGTCTCGCGT

Xylaria sp.1

Primer: ITS1F

TTGGTGAACCAGCGGAGGGATCATTAAAGAGTTATTACAACTCCCAAACCCATGTGAATATACCTTCT
GTTGCCTCGGCAGGTCGCACCTACCCCGTAAGGTCTTACCCTGTAAGACACTACCCGGTAGACGCGGG
TACCCCTGCCGATGGCCCATGAAACTCTGTTTAGTATGTTATTCTGAACCTATAACTAAATATGTTAA
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCCCTGTTGCTTAGTGTTGGGAGCCTACAGCCTTTG
TAGCTCCTCAAAGTTAGTGGCGGAGTCGGTTCACACTCTAGGACGTAGTAAACTTTATTCTCGTCTGT
AGTTGCGCCGGTCCCTTGCCGTAA

Xylariaceae sp.1

Primer: ITS1F

AGTGTAATAACTCCACAAACCCATGTTGAACATACCTCATGTTGCCTCGGCAGGTCTGCGCCTACCCC
GCAGACCCCTACCCTGTAGGGCCTACCCGGAAGGCGCGGGTAACCCTGCCGGCGGCCCACGAAACTCT
GTTTAGTATTGAATTCTGAACCTATAACTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCT
TAAGCCCCTGTTGCTTAGCGTTGGGAGCCTACAGCACTGTAGCTCCCCAAAGTTAGTGGCGGAGTCGG
CTCACACTCTAGACGTAGTAAATCTTTCACCTCGTCTGTAGTTGGACCGGTCCCCTGCCG