Wood-inhabiting fungi found within living *Eucalyptus obliqua* trees in southern Tasmania

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Abstract

This paper describes the wood-inhabiting fungi found in an intensive study of living *Eucalyptus obliqua* trees in Tasmania. Three hundred and twelve isolates of wood-inhabiting fungi were obtained from 18 trees, representing 91 species or operational taxonomic units (OTUs). A number of these were new records for Australia or for the living tree substrate. The cultural characteristics of the 20 most common species are described and illustrated in detail.

Key words: Wood decay, fungal morphology, cultural taxonomy, ITS sequencing, basidiomycetes.

Introduction

Wood-inhabiting fungi play a critical role in the decay and formation of habitat in Australian eucalypts (Simpson & Eldridge 1986; Mackowski 1987). Despite this important ecological role, many wood-inhabiting fungi in Australia remain poorly known (May & Simpson 1997; Kile & Johnson 2000). Most studies of wood-decay fungi have been conducted in the context of reducing decay to improve commercial forest management in native forests (e.g., Tamblyn 1937; Refshuage 1938; Parkin 1942; Greaves *et al.* 1967; Marks *et al.* 1986; Wardlaw 2003). Wood-inhabiting fungi observed fruiting on living eucalypts, include species of *Armillaria, Fistulina, Gymnopilus, Hymenochaete, Inonotus, Phellinus, and Piptoporus* (May & Simpson 1997; Kile & Johnson 2000). A few common pathogenic species have been quite extensively studied (e.g., *Armillaria luteobubalina* (Kile 1981; Shearer & Tippett 1988)), however, for the vast majority of wood-inhabiting fungi on eucalypts little is known about their taxonomy or biology.

The poor understanding of fungal taxonomy in Australia is further highlighted when the taxonomy of cultures of wood-inhabiting fungi is considered. In countries with a longer history of fungal taxonomy, it may be possible to identify a large proportion of wood-inhabiting 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-inhabiting 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.

This paper documents the species of fungi associated with decayed wood in living *Eucalyptus obliqua* trees in southern Tasmania. It is based on isolations from wood rather than sporocarp surveys. Isolates were identified using two complementary identification methods: traditional morphological methods and sequencing of the rDNA ITS region. Due to the shortage of taxonomic information available for cultures of wood-inhabiting fungi in Australia, this paper includes descriptions of the 20 most commonly isolated taxa.

Methods

Sample collection and isolation

A total of 18 *Eucalyptus obliqua* trees, ranging in age from 69 years old to more than 150 years old, were felled and examined for wood-inhabiting fungi as described in Hopkins *et al.* (2005). The trees were taken from two adjacent sites in southern Tasmania. The wood-inhabiting fungi were examined in each tree by sampling cross-sectional discs that were taken at nine sampling points along the main stem. Subsamples (1 cm^3 pieces) of wood were excised from clear sapwood and heartwood as well as the leading edge of any decay columns observed at each sampling point. All decay columns found were within this heartwood. 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 4–6 weeks on specialised fungal media (Malt extract agar (MEA) and malt extract agar with added antibiotics (MAT; see Hopkins *et al.* 2005)). Fungi were considered to be of interest to this study if they either tested positive for wood-decay enzymes or displayed characteristics typical of basidiomycetes in culture (Stalpers 1978).

Morphological identification

Fungal isolates were sorted into broad morphological groups (referred to as morphospecies) based on their macroscopic appearance, followed by a closer examination using traditional morphological taxonomy (Stalpers 1978; Nakasone 1990). Isolates were grown on 1.5% MEA at 20ºC in the dark and examined 2 weeks and 6 weeks after subculturing. At both ages, the macroscopic and microscopic characteristics of

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the isolates were recorded. The specific terminology used to describe these features follows that of Stalpers (1978). Macroscopic characters recorded include colour and texture of aerial hyphae, characteristics of culture margin and changes in colour of the reverse or underside of the culture medium. At 2 weeks, the radius of each isolate was measured to indicate growth rate.

Three different regions of hyphae, the marginal hyphae, submerged hyphae and the aerial hyphae, were examined microscopically 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 were carried out to detect the production of the wood degrading enzymes laccase and tyrosinase by each isolate (Stalpers 1978). 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 colour change to brown following 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.

Molecular identification

The aerial mycelium was collected from 14–21 day old cultures of each isolate and DNA was extracted using the glassmilk method described in Glen *et al.* (2002). Amplification of the DNA was carried out by polymerase chain reaction (PCR) in 50 µL volumes with similar conditions to those in Glen *et al.* (2001). The fungal specific primer ITS1-F (Gardes & Bruns 1993) for the ITS region of the nuclear rDNA was used in combination with a universal reverse primer ITS4 (White *et al.* 1990). 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. Sequences were determined on a Beckman Coulter CEQ 8000. Forward sequences were obtained for all isolates, while reverse sequences were only obtained when additional information was required. At least two complete (forward and reverse) sequences were obtained for each morphospecies. Sequences of isolates from the same morphospecies were aligned in ClustalW (Thompson *et al.* 1994) and visually assessed for similarity. Where sequence variation among isolates of a morphotype was less than 2%, the isolates were considered to be the same species or operational taxonomic unit (OTU). Once a consensus sequence was obtained for each OTU, BLAST (Altschul *et al.* 1997) searches of public databases were carried out using BioManager (ANGIS).

Representative isolates of all OTUs are held at University of Tasmania Forest Health Herbarium in Hobart, Australia and PPCC in Queensland, Australia. ITS sequences for all common OTUs are available on GenBank (Table 1).

Table 1 Operational taxonomic units (OTUs) of wood-inhabiting fungi isolated from within living *Eucalyptus obliqua* trees in southern Tasmania (follows taxonomy of Kirk *et al.* (2001)). Fifty-three of the unidentified basidiomycete singletons are not included. Isolation frequency refers to the number of individual cut faces from which an OTU was isolated.

Taxon	Isolation frequency	Genbank accession no.
ASCOMYCOTA		
Incertae sedis		
Ascomycete sp. 1	9	HM583804
Helotiaceae		
Ascocoryne sp. 1	1	HM583803
Nectriaceae		
Metarhizum aff. *flavoviride* Sorokïn.	1	HM583833
Neonectria aff. *radicicola* (Gerlach & L. Nilsson) Mantiri & Samuels	1	HM583834
Xylariaceae		
Nemania sp. 1	11	HM583858
Xylaria sp.1	1	HM583857
BASIDIOMYCOTA		
Incertae sedis		
Basidiomycete sp. 1	14	HM583809
Basidiomycete sp. 2	3	HM583810
Basidiomycete sp. 3	2	HM583811
Basidiomycete sp. 4	2	HM583812
Basidiomycete sp. 5	1	HM583841
Agaricales		
Agaricales sp. 1	1	HM583856
Coprinaceae		
Psathyrella-like	1	HM583851
Cortinariaceae		
Gymnopilus allantopus (Berk.) Pegler	1	HM583826
Fistulinaceae		
Fistulina sp. 1	4	HM583822

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Table 1 Continued

Taxon	Isolation frequency	Genbank accession no.
Strophariaceae		
Hypholoma fasciculare (Huds.) P. Kumm.	6	HM583831
Hypholoma sp. 1	1	HM583829
Hypholoma sp. 2	1	HM583830
Coniophoraceae		
Coniophora sp. 1	7	HM583819
Coniophora-like sp. 2	1	HM583820
Hymenochaetaceae		
Hymenochaetaceae sp. 1	5	HM583827
Hymenochaetaceae sp. 2	3	HM583828
Atheliaceae		
Athelia sp. 1	4	HM583806
Atheliaceae sp. 2	1	HM583807
Fomitopsidaceae		
Fomitopsis-like sp. 1	1	HM583823
Postia pelliculosa (Berk.) Rajchenb.	30	HM583842
Postia-like sp. 3	4	HM583843
Postia-like sp. 4	5	HM583844
Postia-like sp. 5	4	HM583845
Postia-like sp. 6	2	HM583846
Postia-like sp. 7	2	HM583847
Meruliaceae		
Phlebia-like sp. 1	2	HM583839
Phanerochaetaceae		
Phanerochaete aff. *sordida* (P. Karst.) J. Erikss. & Ryvarden	1	HM583837
Polyporaceae		
Trametes-like sp. 1	1	HM583854
Trametes versicolor (L.) Lloyd	1	HM583855
Steccherinaceae		
Steccherinum sp. 1	1	HM583852
Peniophoraceae		
Peniophora sp. 1	1	HM583835
Stereaceae		
Stereum sp. 1	2	HM583853

Results

Three hundred and twelve isolates of wood-inhabiting fungi were obtained from the 18 living trees examined. The majority (70.8%) of these isolates were obtained from samples of wood from the leading edge of decay columns (Table 2). Only a few isolates of wood-inhabiting fungi were obtained from the samples of clear heartwood (15.1%) and sapwood (14.1%). Isolates were obtained from 65.7% of the decayed wood samples while only 23.8% of the heartwood and 27.5% of the sapwood samples yielded wood-inhabiting fungi. Wood samples that did not yield isolates of wood-inhabiting fungi yielded either common contaminant fungi (e.g., *Penicillium* spp., *Mucor* spp.) or did not grow any fungi after 6 weeks.

Based on the combined information from morphological and ITS sequence analyses, a total of 91 OTUs were identified from the 312 isolates (Table 1). Of these, 20 OTUs were isolated more than once and are herein referred to as the common OTUs. These are described in Appendix 1 and selected features are shown in Figures 1 & 2. Two of these OTUs were ascomycetes and 18 were basidiomycetes (Table 1). One of the ascomycetes belonged to the Xylariaceae but the family of the other ascomycete OTU was unable to be determined. Similarly, four of the basidiomycetes were not able to be determined to family level, while the remainder showed ITS sequence matches with members of a range of families within the Polyporales, Agaricales, Hymenochaetales, Boletales and Russulales. OTUs isolated only once are referred to as singletons. The singleton OTU groups showed similar taxonomic patterns: four were ascomycetes and 67 were basidiomycetes. The identity of 53 of the basidiomycetes remained undetermined even to order. This is either due to difficulty obtaining a sequence or because of a lack of matching sequences available on public databases.

Discussion

This study demonstrates that a large number of wood decay fungi are found in living eucalypts, with 91 putative species isolated from just 18 *E. obliqua* trees. Of these 91 OTUs, 20 were found more than once. In a similar 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

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Table 2 Proportion of isolates of wood-inhabiting fungi 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 heart-wood	Clear sap-wood	All
Proportion of isolates (%)	70.8	15.1	14.1	100.0
Isolation success rate (%)	65.7	23.8	27.5	46.5

(1937) found *Laetiporus portentosus* (Berk.) Rajchenb. (as *Polyporus eucalyptorum*) 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 were found that are close relatives of commonly recognised wood-decay fungi such as those in the genera *Coniophora, Fistulina, Fomitopsis, Inonotus, Xylaria, Stereum* and *Postia*. The cosmopolitan wood-inhabiting species *Hypholoma fasciculare* and *Trametes versicolor* were also detected. Interestingly, many of the isolates were from species apparently closely related to fungal genera with corticioid fruitbodies, such as *Coniophora, Phlebia* and *Athelia*. Of the 27 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).

Several of the species in this study represent new habitat records. *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. *Metarhizium flavoviride* is a hyphomycete commonly found on insects (Lawrence & Milner 1996) so it is unusual that it was isolated directly from wood. It is probable that this fungus was present in the decayed wood as a result of insect activity within adjacent wood or even grew from an insect from within the wood sample itself. 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.

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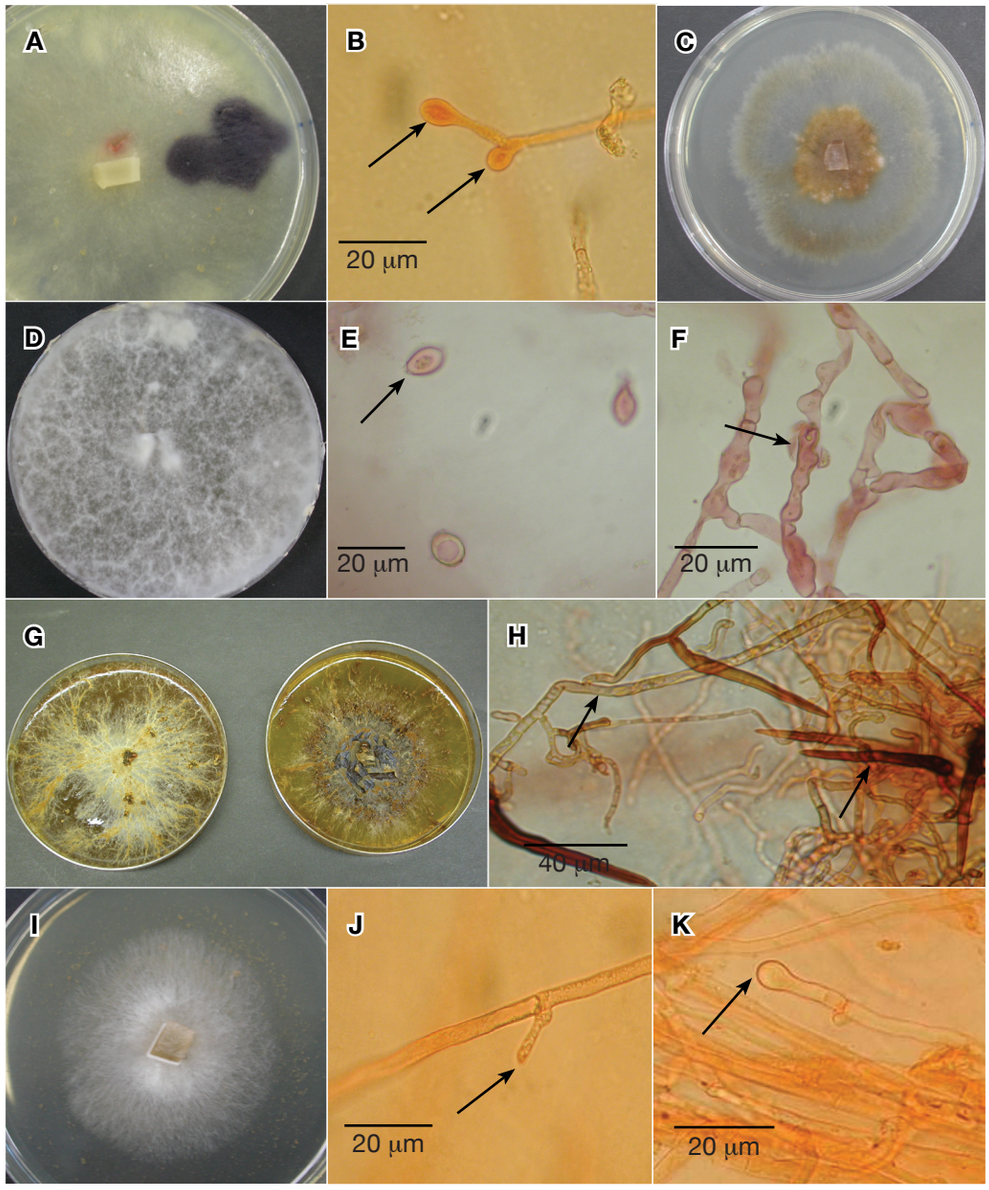

Fig 1 A Basidiomycete sp.1 showing mycelium of 2-week-old culture with a positive enzyme reaction to laccase, and **B** gloeocystidia; **C** Basidiomycete sp. 2 showing aerial mycelium of six-week-old cultures; **D** Basidiomycete sp. 3 showing aerial mycelium of six-week-old culture, **E** chlamydospores, **F** monilioid hyphae; **G** Hymenochaetaceae sp. 1 showing 6–8 week old cultures, and **H** setae and simple septate hyphae; **I** *Postia pelliculosa* showing mycelium of three-week-old culture, **J** sprouting clamp connection, and **K** terminal swelling (arrowed) with basal clamp connection.

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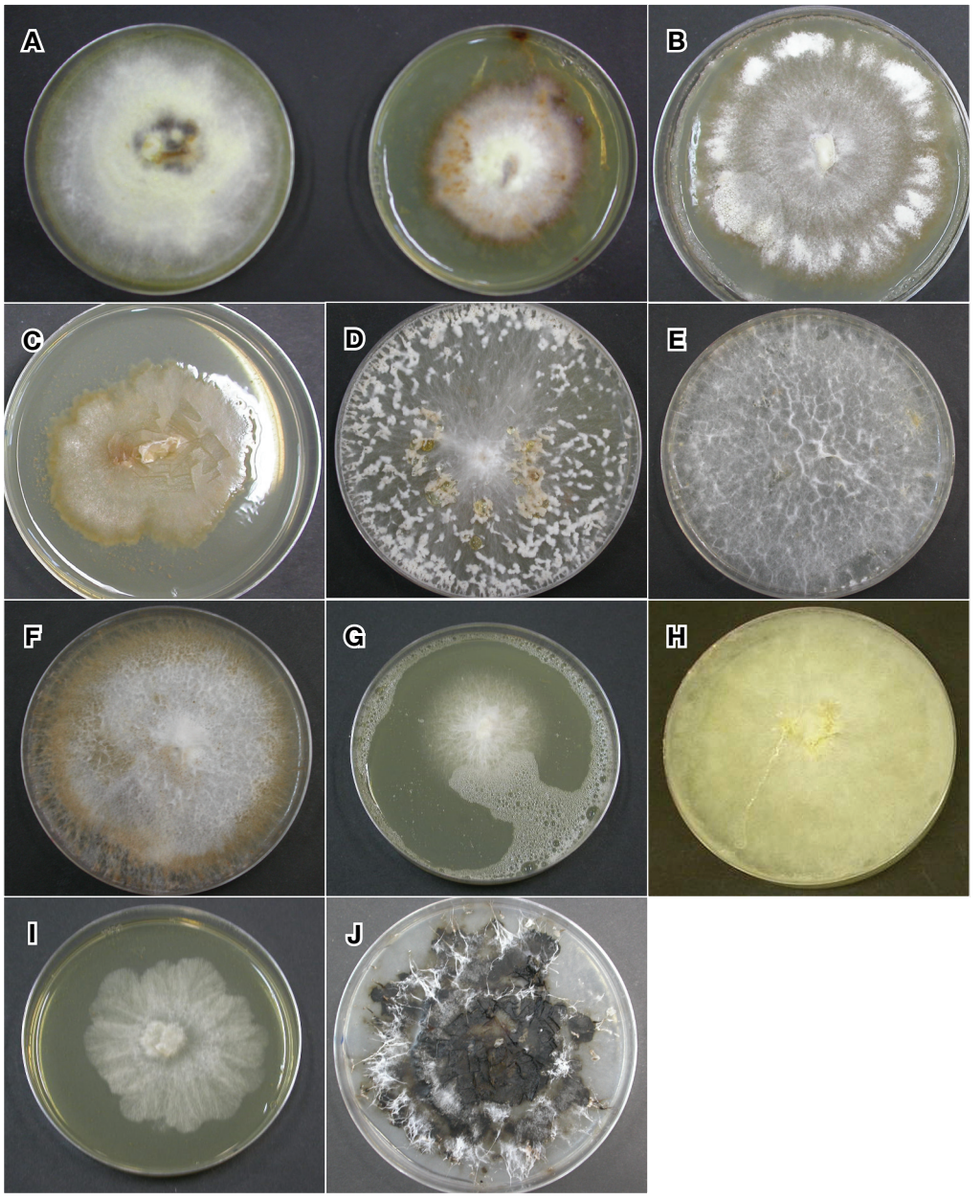

Fig 2 A Hymenochaetaceae sp. 2 showing variation in aerial mycelium of six-week-old cultures; **B**, **C** *Postia*-like sp. 3 showing variation of aerial mycelium of six-week-old cultures; **D** *Postia*-like sp. 4 showing aerial mycelium of a six-week-old culture; **E** *Postia*-like sp. 5 showing aerial mycelium of six-week-old culture; **F** *Postia*-like sp. 6 showing aerial mycelium of a six-week-old culture; **G** *Postia*-like sp. 7 showing mycelium of two-week-old culture, and **H** mycelium of a six-week-old culture showing deepening colour change to yellow; **I** *Nemania* sp. 1 showing mycelium of two-week-old culture and **J** mycelium of a three-month-old culture.

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Appendices

Appendix 1. Descriptions and photographs of cultures of all 20 common Operational Taxonomic Units (OTU) of wood decay fungi isolated from within *Eucalyptus obliqua* trees. Growth rate is indicated by 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.
BLAST results: Up to 97% sequence similarity to a range of unidentified ascomycete species, 93% similarity to GU934579.1 and several other accessions of *Scytalidium lignicola*.

Athelia 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.
BLAST results: Sequence similarity of 98% to GU187504.1 and several other accessions of *Athelia arachnoidea* and its anamorph *Rhizoctonia carotae*, 97% similarity to U85793.1, *A. epiphylla*.

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, 6–10 µm 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.
BLAST results: The 5.8S region is over 99% similar to a range of basidiomycete species, no matches were found for the ITS regions.

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) µmdiameter, 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.
BLAST results: The 5.8S region is over 99% similar to a range of basidiomycete species, no matches were found for the ITS regions.

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.
BLAST results: Sequence similarity of 80–82% to a range of *Fomitopsis* and *Antrodia* species, including EU854437.1, *F. officinalis* and AY673075.1, *Antrodia vaillantii*.

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.
BLAST results: Sequence similarity up to 83% to a range of Stereaceae, including FJ799921.1, *Neoaleurodiscus fujii* and FJ810153.1, *Stereum sanguinolentum*.

Coniophora 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.

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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.
BLAST results: Sequence similarity of 95% to AM946632.1 and two other accessions of *Coniophora marmorata*, and over 90% similarity to a range of other *Coniophora* species, including *C. olivacea, C. prasinoides, C. opuntiae, C. arida, C. cerebella* and *C. puteana*.

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 mm diameter, thin-walled, hyaline, single clamps at every septum, equivalent branching, often multi-branched. Some thick-walled generative hyphae also present, 4–8 mm 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.
BLAST results: Sequence similarity of 96% to DQ486702, *Fistulina antarctica*, and 91% to AY571038, *F. hepatica*.

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.
BLAST results: Sequence similarity of 87% to AY558594.1 and FJ810171.1, *Hymenochaete adusta*, and to FJ481022, *Pseudochaete tabacina*, and 86% similarity to AY251309, *Inonotus hispida*.

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.
BLAST results: Sequence similarity of 88% to AY558594.1 and FJ810171.1, *Hymenochaete adusta*, and to FJ481022, *Pseudochaete tabacina*, and 86% similarity to AY251309, *Inonotus hispida*. This OTU has 88% sequence similarity to Hymenochaetaceae sp. 1.

Hypholoma fasciculare (Huds. : Fr.) P.Kumm
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.
BLAST results: Sequence similarity of 98.5% to AY558594.1 and another 13 accessions of *Hypholoma fasciculare*, 94% to FJ596780.1 and another 7 accessions of *H. capnoides*.

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Nemania 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 sterile 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.
BLAST results: Sequence similarity of 95–99% to DQ658238.1 and 3 other accessions of *Nemania diffusa*, and 94% to a range of *Xylaria* species, including *X. allantoidea*, *X. laevis* and *X. plebeja*.

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.
BLAST results: Sequence similarity of 91% to GQ259417.1, *Phlebia setulosa* and 90% to AB210076.1, *P. lindtneri* and several other *Phlebia species*.

Postia pelliculosa (Berk.) Rajchenb.
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.
BLAST results: Sequence similarity of 88% to AJ006666.1, *Postia balsamea*, and 87% to a range of other *Postia* species, including *P. subcaesia, P. caesia* and *P. hibernica*.
NB. High sequence match (99%) with *Postia pelliculosa* fruitbody from University of Tasmania Forest Health collection (AH436).

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 mm 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.
BLAST results: Sequence similarity of 89% to AJ006666.1, *Postia balsamea*, 88% to AJ006668.1, *Oligoporus rennyi* and 87% to a range of other *Postia* species, including *P. subcaesia, P. caesia and P. hibernica.*

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.
BLAST results: Sequence similarity of 87% to AY599576.1, *Postia subcaesia*, 86% to AJ006668.1, *Oligoporus rennyi* and to a range of other *Postia* species, including *P. sericeomollis, P. caesia* and *P. hibernica.*

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.
BLAST results: Sequence similarity of 86% to AJ006666.1, *Postia balsamea*, and 85% to a range of other *Postia* species, including *P. sericeomollis, P. caesia, P. subcaesia* and *P. hibernica.*

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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.
BLAST results: Sequence similarity of 86% to AJ006666.1, *Postia balsamea*, AY599577.1, *P. subcaesia* and AY599572.1, *P. caesia*.

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.
BLAST results: Sequence similarity of 86% to AJ006666.1, *Postia balsamea,* AY599577.1, *P. subcaesia*, AY599572.1, *P. caesia*, AJ006667.1, *P. sericeomollis*, AJ006665.1, *P. hibernica*, and AJ006668.1, *Oligoporus rennyi*.

Stereum 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.
BLAST results: Sequence similarity of 97% to FN539048.1, *Stereum gausapatum*, FN539051, *S. rugosum*, and 96–97% to AM269810.1 and 17 other accessions of *S. hirsutum*.

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