The Diversity and Distribution of Microfungi in Leaf Litter of an Australian Wet Tropics Rainforest

Thesis submitted

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STATEMENT ON THE CONTRIBUTION OF OTHERS

In this section, a number of individuals and institutions are thanked for their direct contribution to this thesis. Many more have provided assistance in some other way and are gratefully acknowledged in the next section.

Dr Paul Gadek, James Cook University, and Dr Kevin Hyde, The University of Hong Kong, supervised this project and provided academic guidance and helpful editorial comment.

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ABSTRACT

This thesis examines aspects of the diversity, distribution and taxonomy of microfungi in leaf litter of several tree species in an upland tropical rainforest of Far North Queensland, Australia.

The first study assessed the advantages and limitations of the particle filtration method as a potential complementary approach for estimating microfungal diversity. The observed microfungal diversity was comparable to that reported for neotropical leaf litter fungi, with a total of 253 morphotypes observed among 1365 isolates from eight samples of *Neolitsea dealbata* leaf litter. The isolation rate was negatively correlated with the time that leaves had been stored in a dried state while the number of observed morphotypes was similar to the control after three weeks of storage. Surface treatment with sodium hypochlorite did not affect the isolation of internal colonisers while it reduced the number of propagules on the leaf surface.

The diversity of microfungi could in part be explained by the dynamic nature of tropical leaf litter where decay processes advance rapidly. In a second study that examined decaying leaves of *Ficus pleurocarpa*, a total of 105 taxa were recorded using a direct observational method. Applying a particle filtration method, 53 taxa were detected among 562 isolates. Distinct differences in microfungal assemblages were observed at different stages of decay, which were characterised by a rapid replacement of microfungal species at early decay and increasing similarity of collections with advancing decay.

Microfungal diversity was characterised in leaf litter of six tree species belonging to four plant families common to the region, namely the Elaeocarpaceae, the Lauraceae, the Moraceae and the Proteaceae using two isolation protocols. A total of 185 taxa were observed using the direct method and 419 morphotypes were recorded in the wet season and 276 morphotypes in the dry season using a particle filtration protocol. The observed diversity of microfungi differed between some tree species and also between isolation protocols. However, both isolation methods provided congruent results in terms of microfungal distributions. Microfungal leaf litter communities were strongly shaped by host phylogeny and seasonal factors. These results indicate that microfungi in tropical leaf litter are not random assemblages but rather communities with ‘recognisable and measurable differences among repeating assemblages of fungi that occur simultaneously

in similar habitats'. Species richness on leaves of different tree species was correlated with the level of total phenolics, leaf thickness and manganese. The role of chemical and physical leaf attributes in shaping overall distributional patterns as well as those of individual microfungal species requires further detailed studies. A high percentage of observed fungi were anamorphs and approximately 50 % of taxa could not be integrated into a phylogenetic scheme below the level of class. Nevertheless, families and orders previously reported from tropical habitats were also dominant among those fungi that could be integrated.

While an assessment of interspecific interactions among fungi was beyond the scope of this study, interactions between a discomycete and a scolytine beetle were demonstrated and it was hypothesised that insect-fungi interactions may increase the efficiency of decomposition processes.

For future studies of microfungal diversity, a centrifugal-phylogenetic approach may provide a useful strategy to extend the baseline information established in the present study. With this approach, closely related hosts are studied first and then more and more distantly related plants are included. Due to the high diversity of tree species at all taxonomic levels, the rainforests of the wet tropics of Australia would provide an ideal study site for ongoing research into the host recurrence of microfungal species.

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INTRODUCTORY OVERVIEW

Background

> *"One of the most striking and perhaps characteristic features of life on Earth is its rich variety."*
>
> *E. O. Wilson (1992)*

Fungi are among the most diverse organisms on Earth (Hammond, 1995) but the magnitude of their diversity is still unknown. They are vital contributors to ecosystems, for example through their roles in nutrient cycling (Jordan, 1985; Lodge, 1992), their mycorrhizal and endophytic associations with plants (Allen, 1991; Rodrigues and Peterini, 1997; Kumaresan and Suryanarayanan, 2002), and their interactions with insects (Wilding et al., 1989; Cafaro, 2002). Fungi also hold a vast unknown genetic potential for human endeavours, including pharmaceutical research (e.g. Bills, 1995; Wildman, 2003) and other biotechnological applications (e.g. Hyde, 1995; Vandamme, 2003). Despite the important services fungi provide to ecosystems and humans alike, fungi are an understudied element particularly of tropical regions and are rarely considered in conservation plans (Hyde, 2003). This is especially true for those fungi that cannot be observed by the unaided human eye, commonly referred to as 'microfungi'. Among this taxonomically and functionally diverse group, those microfungi involved in the decay of leaf litter in an Australian tropical rainforest will be the focus of this project.

Research strategy

General approach

This study provides a rare opportunity to assess aspects of microfungal taxonomy, diversity and ecology in a tropical ecosystem. Since information about these aspects is limited both on a regional and global scale, this project intends to be an explorative baseline survey rather than a solely experimentally based study. Understanding the

diversity and distributions of microfungi is an important first step towards understanding fungal ecology in general and any information will assist in the design of future studies to more fully elucidate the role of fungi in ecosystem processes (Cooke and Rayner, 1984).

This study therefore had the following aims:

- To assess and make recommendations with respect to sampling and isolation methods for microfungi
- To characterise the diversity and structure of microfungal assemblages from the rainforests of north Queensland
- To assess the distribution of microfungi in leaf litter and to generate hypotheses regarding their ecology
- To assess the taxonomy of selected microfungal taxa and to provide a reference collection of observed microfungi for future studies.

Geographical context

The wet tropics of Australia (15° to 19° South, 145° to 146° East; Tracey, 1982) contain the most extensive continuous area of rainforest in Australia (Winter et al., 1991) and were declared a world heritage area in 1988. This region is characterised by an extraordinary diversity and a high degree of endemism among plants and animals (Wet Tropics Management Authority, 2004). This project was undertaken in upland rainforest on the Atherton Tablelands, north Queensland. The two study sites are part of an area of continuous forest, which also includes Bellenden Ker National Park (79,500 ha). Both sites were selected on the basis of the high diversity among tree species and were approximately matched for rainfall and rainforest type.

Choice of host species

Four common plant families of this region provide a framework for this study. These include the Lauraceae, the Proteaceae, the Moraceae and the Elaeocarpaceae (Chapter 1). Microfungi were assessed on leaf litter of one or two representative species of each family, namely *Cryptocarya mackinnoniana* F. Muell. (Lauraceae), *Elaeocarpus*

angustifolius Blume (Elaeocarpaceae), *Ficus pleurocarpa* F. Muell. (Moraceae), *Ficus destruens* F. Muell. ex C.T. White (Moraceae), *Neolitsea dealbata* (R. Br.) Merr. (Lauraceae), *Opisthiolepis heterophylla* L.S. Smith (Proteaceae), and *Darlingia ferruginea* J.F. Bailey (Proteaceae). Plant families are discussed in Chapter 1 and photos of leaves and a description of each species are provided in Appendix A.

Choice of collection methods

All methods of studying microfungi impose some filter on the observed diversity. To overcome this filtering effect to some extent, I elected to use a combination of two methods. These included direct observation of fungal fruiting bodies following humid chamber incubation and the particle filtration method (Chapter 1 and 2).

Time allocation

A maximum of two years could be allocated for field and laboratory work as part of this PhD project. To examine an adequate number of sampling units within each study year, I needed to weigh up whether to replicate the study over two years using the same method or whether to cross-check results with a second method in two separate years. My rationale for selecting the latter option was that if different isolation methods provided congruent results over two years with respect to the central questions, the conclusions of this study would be strengthened.

Limitations

A number of limitations were encountered during this project. The amount of work that can be achieved by a single researcher using a replicated sampling strategy is a prime limitation in working with microfungi due to the labour-intensive nature of isolating and identifying these organisms. As a result, the replication within studies was low compared to some ecological studies of macro-organisms. Athough it was adequate to detect meaningful patterns in multivariate analyses, it is necessary to exercise caution when attempting to generalise these results to other forest types, ecosystems and time frames. In addition, a limitation outside my control was that one of the study years

(2002) was the driest year on record and it is not clear whether and how this has influenced microfungal diversity estimates.

Another limitation was that few taxonomic resources are available for microfungi of north Queensland and testing species relationships and delimitations for more than some selected taxa was beyond the scope of this study. To circumvent this limitation to some extent, I contacted mycologists experienced in the taxonomy of tropical microfungi to assist in identifications or to confirm my preliminary identifications in some instances. These mycologists are gratefully acknowledged earlier in this thesis. Nevertheless, this limitation resulted in a conservative approach in identifying specimens to species levels.

Relevance of research

Advances in the study of fungal diversity and ecology occur in small increments. In the short-term, this project adds to this incremental advance by confirming and extending the results of previous studies and by providing new information on isolating methods, sampling protocols and estimation procedures. This project also adds to the knowledge base of microfungal diversity and distributions in tropical rainforests, and generated hypotheses, which can form the basis for further synecological and autecological studies.

In the medium term, the development of appropriate sampling and estimation strategies depends on an understanding of the factors, which shape fungal distributions (Lodge and Cantrell, 1995). More efficient and reliable sampling strategies for estimating microfungal diversity will benefit diverse areas of scientific research, such as conservation biology and biotechnology (Rossman, 1994; Cannon, 1997b; Hyde et al., 1997b; Hawksworth, 1998b). Despite the vital roles that microfungi play in ecosystems, a major gap exists in our understanding of the relationship between fungal diversity and ecosystem function (die Castri and Younes, 1990). Reliable methods for estimating fungal diversity are required to even begin unravelling this question. Together with advances in the taxonomic knowledge of tropical microfungi, it can also progress the utilisation of fungal genetic resources and novel compounds for biotechnology (Bills, 1995).

Thesis outline

This thesis is divided into eight chapters, each dealing with a separate aspect of this project.

The current state of knowledge with respect to microfungal taxonomy, diversity and distributions is reviewed in **Chapter One**. This is also where the reader will find definitions of terms and descriptions of relevant concepts.

In **Chapter Two**, I will explore aspects of one isolation method for microfungi, i.e. particle filtration, and its usefulness for estimating microfungal diversity. The results of this preliminary study will be compared to those of previous studies.

Successional patterns of microfungi in leaf litter of one tree species are reported in **Chapter Three**.

In **Chapter Four**, I will discuss microfungal diversity and the patterns observed within microfungal assemblages. Aspects that may influence diversity estimates are also considered.

An examination of the distribution of microfungi in leaf litter of six tree species is provided in **Chapter Five**. The distribution of fungi is discussed in relation to a number of factors such as host phylogeny, season, and site and I propose a number of hypotheses about the ecology of microfungi.

In **Chapter Six**, I explore an association between a fungus and a beetle in decaying fig leaves. The spatial and temporal distribution of the fungus is also described and this information is integrated to generate a number of hypotheses about the nutritional modes of both organisms and their effect on decomposition processes.

In **Chapter Seven,** I describe selected taxa, which are new to science, and provide a summary of the observed taxonomic diversity.

Finally, I integrate the information gained from these separate studies and make recommendations with respect to future studies of microfungal diversity in **Chapter Eight.**

CHAPTER ONE

A REVIEW OF CURRENT KNOWLEDGE

1.1 Introduction

The object of this chapter is to review current knowledge with respect to three different areas of research, namely the taxonomy, the diversity and the ecology of microfungi. This division into three sections serves as a frame work for discussion but is somewhat arbitrary as all three areas of research are interlinked and inform each other. In **Section One**, I provide a definition of fungi and their basic divisions. I discuss aspects of ascomycete taxonomy and describe available taxonomic resources in general and of the wet tropics in specific. In **Section Two**, I define the term diversity and summarise what is known about microfungal diversity, particularly in tropical regions. Factors that may affect diversity estimates are also reviewed; these include isolation methods for microfungi and statistical tools for estimating diversity. The significance of fungal biodiversity is considered and finally, a brief discussion is devoted to estimates of global fungal species numbers. **Section Three** is dedicated to a review of fungal ecology. In particular, I discuss some basic concepts and challenges of myco-ecology and review distributional patterns among microfungi. Potential factors underlying microfungal diversity and distributions, and the role of saprotrophic microfungi are considered. Finally, this studyis put into its geographic context of the region and the environmental context of leaf litter.

While this chapter is focussed on microfungi, some examples are drawn from other fungal groups; this was necessary due to the paucity of data that exists for some areas of microfungal research. Where I considered it appropriate, I have also borrowed concepts from microbiology, plant ecology and general ecology.

1.2 Taxonomy of microfungi

1.2.1 Fungi and microfungi defined

The term ‘fungi’ refers to a diverse assemblage of eukaryotic organisms. The majority of these organisms are ‘true fungi’ and are placed in the kingdom Eumycota, but some

belong in the kingdoms Chromista and Protoctista (Walker, 1996; Kirk et al., 2001). There is now increasing consensus based on chemical, ultrastructural and molecular data that Eumycota encompass four phyla: Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota (Bruns et al., 1991; Walker, 1996; Kirk et al., 2001). These organisms are united by a lack of plastids (chloroplasts and amyloblasts). They are heterotrophs with an absorptive mode of nutrition Kendrick, 1992; Kirk et al., 2001). Their growth form is usually filamentous and consists of multicellular, coenocytic haploid hyphae although some fungi, such as yeasts, may be unicellular. Their diploid phase, if present, is usually short-lived. In contrast to plants, their cell walls contain chitin and ß-glucans (Kirk et al., 2001). Unlike many organisms grouped in Protoctista and Chromista, they are non-flagellate, or if flagella are present as in chytrids, they are lacking mastigonemes (Kendrick, 1992; Kirk et al., 2001). Fungi also have in common a number of other characters at the intracellular level (Kendrick, 1992; Kirk et al., 2001).

Fungi can reproduce either sexually or asexually and are referred to as pleomorphic fungi, if more than one form or spore state is present. The whole fungus with both sexual and asexual states is referred to as 'holomorph' with the 'teleomorph' being the sexual morph and the 'anamorph' the asexual morph (Kendrick, 1979a; Kendrick and DiCosmo, 1979; Seifert and Samuels, 2000). The term 'microfungi' describes those fungi that cannot be detected with the naked human eye. It is a term of convenience and thus it may include a varied assemblage of organisms from all of the above phyla and kingdoms. In the context of this study, the majority of 'microfungi' detected were ascomycetes and their anamorphs, and these are discussed in more detail. The reader is referred to other texts for information on chytrids, zygomycetes and basidiomycetes (e.g. Kendrick, 1992; Alexopoulos et al., 1996).

1.2.2 Ascomycete taxonomy

1.2.2.1 Teleomorphic ascomycetes

The largest phylum of the Eumycota is the Ascomycota. The teleomorphs of these fungi are characterised by carrying spores in sac-like structures called asci (singular: ascus). Ascospores are formed following the fusion of nuclei (karyogamy) and meiosis. Each ascus usually contains eight spores, although variable numbers have been observed in many taxa (Kendrick, 1992). A further possible uniting character among ascomycetes is

their lamellate hyphal wall, by which anamorphic ascomycetes can also be recognised using ultrastructural studies (Kirk et al., 2001). Asci and spores are contained within protective structures called ascomata, which can have several basic forms (apothecial, perithecial, pseudothecial and cleistothecial; Kendrick 1992). While the early taxonomy of teleomorphic ascomycetes relied mostly on fruit body shape and ascus arrangement (e.g. Engler and Prantl, 1897; Saccardo, 1882-1928; von Höhnel, 1907), in later decades increasing importance was placed on the development of ascomata (Nannfeldt, 1932) as well as the structure of asci (e.g. Luttrell, 1951; Eriksson, 1981) and their mode of discharge (Minter, 1988; Kirk et al., 2001). A comprehensive review of ascomycete taxonomy has been published in Volume 1A of the Fungi of Australia series (Walker, 1996) and an excellent review of taxonomic characters for teleomorphic ascomycetes is provided in Fröhlich & Hyde (2000); therefore these aspects will not be further discussed here.

In recent years, the classification of ascomycetes has been shaped by the use of molecular sequence data and is updated continuously (Kirk et al., 2001). The most recent edition of the Dictionary of Fungi recognises 6 classes, 56 orders, 226 families, 3409 genera and 32,739 species (Kirk et al., 2001). It must be stressed that ascomycete taxonomy, in particular at higher taxonomic ranks, is still in a state of flux (Walker, 1996). For example, 41 accepted families could not be placed in orders and over 680 genera could not be assigned to families (Kirk et al., 2001). An outline of the current classification and the most recent changes are available on-line in Outlines of Ascomycetes (Eriksson, 2004).

1.2.2.2 Anamorphic fungi

Anamorphic fungi have been variously called ‘Deuteromycotina’, ‘deuteromycetes’, ‘mitotic fungi’, ‘Fungi Imperfecti’, ‘conidial fungi’ and ‘asexual fungi’ (Carmichael et al., 1980; Kirk et al., 2001). These fungi produce propagules from cells, which do not undergo meiosis (Kendrick, 1992). Most propagules are conidia but some may be derived from unspecialised mycelium (Kirk et al., 2001). Sterile mycelia or those fungi that form sclerotia, bulbils, or chlamydospores are also included in this group (Carmichael et al., 1980, Subramanian, 1983). Although many of these fungi have been linked to a sexual state classified among ascomycetes or basidiomycetes (Anateleo,

2004), a considerable number of anamorphs are still unconnected or, in some instances, appear to have lost their sexuality (Kirk et al., 2001).

Reported anamorph-teleomorph connections have been established with varying degrees of rigour. These range from the least reliable, a single report of the physical proximity of an anamorphic and teleomorphic fungus on a natural substratum, to a connection proven by repeated cultural experiments (Kendrick, 1979a). The only way to unequivocally ascertain an anamorph-teleomorph relationship was to establish an axenic culture and observe the transition from one state to the other (Kendrick, 1979a). In recent years, molecular techniques have revolutionised the integration of anamorphs into a phylogenetic system. However, establishing fungal holomorphs using molecular methods remains a challenge due to inconsistent intraspecific sequence variation among genera (Egger and Sigler, 1993).

In present-day taxonomy, the integration of anamorphic fungi in the teleomorph based system is considered an absolute necessity by many mycologists (Kendrick, 1979b; Seifert and Samuels, 2000; Gams et al., 2003). In fact, the latest edition of the 'Dictionary of Fungi' already assigns anamorphic taxa to the appropriate known level of the teleomorphic hierarchy (Kirk et al., 2001). Ultimately, the complete integration of anamorphs into a phylogenetic system is likely to be a complex task and may require numerous name changes (Hennebert and Gams, 2002). Concerns have been raised that name changes have the potential to disrupt the work of mycological practitioners and even destabilise taxonomy. These fears may be the reason why a vote taken at the Seventh International Mycological Congress 2001 indicated support for no or slow changes in fungal nomenclature (Gams et al., 2003).

Currently, a dual naming system remains in use (Reynolds and Taylor, 1993; Sutton, 1993; Walker, 1996; Greuter et al., 2000; Kirk et al., 2001). In this system, the use of the suffix 'form' for genera and species of asexual fungi is a recognition of the artificial nature of their classification (Sutton, 1993). Formal taxonomic ranks above genera have been rejected by many (e.g. Sutton, 1993) but not all authors (e.g. Walker, 1996). In many instances, artificial classes continue to be used in an informal sense and these include

- hyphomycetes, which are mycelial in form and bear conidia on separate hyphae or aggregations of hyphae

- coelomycetes which produce conidia in pycnidial, pycnothyrial, acervular, cupulate or stromatic conidiomata and
- aganomycetes which are sterile mycelial forms but which may produce vegetative structures such as chlamydospores or sclerotia (Kirk et al., 2001).

1.2.2.3 Taxonomic tools

The rapid advance in ascomycete systematics has not been matched by the availability of up to date identification tools. This may be in part due to the fact that the sheer size of the group makes it difficult to determine, which morphological features should be stressed in their delimitation (Kirk et al. 2001). This problem is compounded in those situations where molecular and morphological data do not agree (Kirk et al. 2001). "The Fungi. Volume IV A." (Ainsworth et al. 1973) is a valuable resource and includes keys to orders, families and genera of ascomycetes; unfortunately, it is lacking recent changes and additions. A more recent key to orders of Ascomycota is that by Walker (1996). In addition to identification tools that target specific ecological groups such as palm ascomycetes (e.g. Fröhlich and Hyde, 2000) or geographical regions (Dennis, 1978), other important sources of information are treatments of orders of Ascomycota listed in Walker (1996), treatments of families, for example Rhytismataceae (Johnston, 1986, Johnston, 1989, Johnston, 1990, Johnston, 1991, Johnston, 1992), Gnomoniaceae (Monod, 1983), families of Hypocreales (Rossman et al. 1999), and other more broadly based texts (e.g.Sivanesan, 1984; Hanlin, 1998).

Although an entirely artificial group, numerous identification schemes exist for anamorphic fungi; this may be due their perceived economic importance (Subramanian, 1983). For example, taxonomic treatments are available for coelomycetes (Sutton, 1980), for appendaged coelomycetes (Nag Raj, 1993), and for hyphomycetes (von Arx 1970; Ellis 1971; Matsushima 1971, 1980, 1981, 1983, 1985, 1987, 1989, 1993, 1995, 1996; Ainsworth et al. 1973, 1975; Ellis 1976; Carmichael et al. 1980; Domsch et al. 1980; Castañeda Ruiz 1985a, 1985b, 1987; Castañeda Ruiz and Arnold 1985; Castañeda Ruiz and Kendrick 1990a, 1990b, 1991; Barnett and Hunter 1998; Kiffer and Morelelt 2000; Kirk et al. 2001).

Improved accessibility of mycological taxonomic resources and improved efficiency in documenting new taxa are vitally important for the progress of mycology (May, 1999).

The development of databases based on coded taxonomic characters (e.g. B. Kendrick pers. comm., W. Ho pers. comm.) is one step in this direction. In addition, taxonomic resources are increasingly becoming available on-line and these include databases (Index Nominum Genericorum, 2003; The CABI Bioscience and CBS Database of Fungal Names, 2003; The NZFungi databases, 2004), keys (e.g. Rossman et al., 2003; The NZFungi databases, 2004) and digital exsiccate (e.g. Langer, 1997).

1.2.3 Microfungi of the wet tropics

Hawksworth (1993b) pointed out that our knowledge of the tropical mycobiota remains in the 'pioneer phase' or the first portion of *alpha* taxonomy (Davis and Heywood, 1963). This is certainly true for the wet tropics of Australia. While plant pathogens have received more attention than saprotrophic leaf litter fungi (e.g. Fröhlich, 1992; Hyde and Alcorn, 1993; Shivas and Alcorn, 1996; Fröhlich et al., 1997; Pearce, 1999), it is estimated that even among those only a fraction has been discovered to date (Shivas and Hyde, 1997). In the early days of European settlement, some microfungal collections, mainly of those causing leaf spots, were made by a network of collectors and these were sent to overseas mycologists for identification and description (May and Pascoe, 1996). More recently, visiting mycologists have further contributed to the knowledge of microfungi of Queensland, including leaf litter fungi (Matsushima, 1971; Taylor, 1997; Fröhlich, 1997; Hyde and Goh, 1998; Whitton, 1999; Fröhlich and Hyde, 2000; Guo et al., 2000; Johnston, 2000; Yanna et al., 2001a, 2001b; Parungao et al., 2002). A significant contribution to the taxonomy of saprotrophic microfungi was also made by K.D. Hyde during his period with the Northern Australian Quarantine Strategy at Mareeba (May and Pascoe, 1996), and W. Shipton of James Cook University published on species delimitation in *Cunninghamella* (Lunn and Shipton, 1983). Despite these efforts, the taxonomic diversity of leaf litter microfungi has been relatively underexplored in this region.

1.3 Diversity of Microfungi

1.3.1 Diversity defined

The term 'biodiversity' was coined by Wilson (1988) as a contraction of biological diversity and describes the diversity of life at all levels including genetic, species and

ecosystem diversity (Hawksworth, 1991). In recent years, this term has been increasingly used in arenas outside of science and has become charged with political and social meaning (e.g. Commonwealth of Australia, 1996; Takacs, 1996; ten Kate and Laird, 1999). For this thesis, it is preferable to apply the term diversity in the sense of the richness and abundance of species (Magurran, 1988, Hubbell, 2001). Diversity has been described at different levels. For example, Whittaker (1960, 1977) defined seven levels of diversity in two main categories, namely inventory and differentiation diversities (Table 1). The former describes the diversity in one habitat at different scales, while the latter is a measure of how different (or similar) a range of habitats or samples are in terms of species richness and in some instances species abundances (Magurran, 1988). In this thesis, I assess the alpha diversity of microfungi in a particular habitat, i.e. leaf litter (Chapter 4). I also consider the pattern diversity between microfungi on different leaf species (Chapter 5) and on the same leaf species at different stages of decay (Chapter 3).

1.3.2 Current knowledge of microfungal diversity

"The knowledge gap between the known and unknown fungi is enormous."

(Hawksworth, 1998b)

This statement describes the difficulty of reviewing current knowledge of microfungal diversity beyond a mere listing of studies. The diversity of soil microfungi in temperate regions has been considered 'indeterminable' (Christensen, 1989), tropical endophytic assemblages have been described as 'hyperdiverse' (Arnold et al., 2001) and a very high species richness was observed among saprotrophic leaf litter fungi in a neotropical rainforest where, for example, between 134 and 228 species were reported per five grams of leaf litter (Polishook et al., 1996). In all these instances, there was an indication that greater sampling effort would have yielded a greater number of species than was actually observed. Although there is a general agreement that microfungi are very diverse and that only a fraction of their diversity is known (e.g. Hyde, 1997a), drawing any conclusions from the body of diversity literature is difficult. This is because diversity studies are almost impossible to compare across space and time as standardised sampling and estimation protocols are lacking (Hyde, 1995; Cannon, 1997b; Cannon, 1999). Sampling approaches and hypotheses about fungal diversity

could be tested if a 'known universe' of fungal species was available. For this reason, a number of mycologists have proposed an all taxa biodiversity for fungi (e.g. Rossman, 1994, 1997). A complete inventory for fungi will be costly in terms of human and material resources (Rossman, 1994) and to date this has not eventuated.

Table 1.1 Levels and types of species diversity (adapted from Whittaker, 1960, 1977)

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The taxonomic diversity of tropical microfungi has recently been reviewed (Hyde, 1997a; Hyde et al., 2004) with a focus either on specific microfungal taxonomic groups (e.g. Johnston, 1997), on microfungi on particular substrata, hosts or habitats (e.g. Hyde et al. 1997c; McKenzie, 1997; Hyde et al., 2004) or on modes of life (Shivas and Hyde, 1997; Rodriguez and Petrini, 1997). For many groups of organisms a latitudinal diversity gradient has been reported, with maximum species richness reached in the tropics (e.g. Ehrlich and Wilson, 1991). Whether this trend exists for fungi is still unclear. Some fungal groups such as the Phyllachoraceae (Cannon, 1997a), Hypocreales (Samuels, 1997) and the Xylariaceae (Whalley, 1997) appear to have a strong tropical representation. For other groups, for example the Rhytismatales (Johnston, 1997), such

a generalisation is not possible and some authors have noted the cosmopolitan nature of many microfungal genera (e.g. Johnston, 1997; McKenzie, 1997). There have also been reports that teleomorphs of some species appear restricted to the tropics while their anamorphic states were observed to be cosmopolitan (Samuels and Rossman, 1992). Similarly, the overlap between the majority of freshwater ascomycetes from tropical and temperate regions was noted to be very small (Hyde et al., 1997c) while many freshwater hyphomycetes are apparently cosmopolitan (Kuthubutheen, 1993; Goh, 1997). A greater number of plant pathogenic fungi are likely to occur in the tropics as many plant pathogenic fungi are host-specific and plants are known to be more diverse in the tropics (Shivas and Hyde, 1997). Similarly, pathogenic microfungi associated with invertebrates in the tropics appear to harbour many new taxa (Hywel-Jones, 1997) while non-pathogenic Laboulbeniales appear to have similar levels of diversity in both temperate and tropical regions (Weir and Hammond, 1997). Some substrata seem to harbour a particularly rich diversity of microfungi, for example palms (Fröhlich, 1997; Taylor, 1997), bamboo (Zhou and Hyde, 2002), Pandanaceae (McKenzie, 1997; Whitton, 1999), endemic plants of Mauritius (Dulymamode et al., 2001) and particular plant species in Great Britain (J. Cooper, pers. comm.). Most authors stressed that sampling remains incomplete in terms of the geographical regions, host taxa or niches examined and that any conclusions with respect to patterns of diversity need to be viewed in the light of this limitation.

1.3.3 Diversity estimation

1.3.3.1 Overview

An assessment of diversity is at the heart of many questions in theoretical and applied ecology. Although the concept of diversity can be grasped intuitively, it is "rather like an optical illusion" (Magurran, 1988). Our perception of diversity is shaped by numerous factors, among them our definition of diversity, the filters we inadvertently apply when we collect diversity data, and the choice of mathematical tools to measure and estimate diversity. These problems are compounded in species-rich groups, in groups that require special methods to detect 'individuals' and even more so in groups, such as microfungi, where 'individuals' are ill-defined (Seifert, 1981). For this reason, I will review methods of detecting microfungi and provide a broad overview of mathematical tools that are applied in diversity estimation.

1.3.3.2 Methods of detecting microfungi

The systematic study of microfungi became possible only with the advent of microscopes and its history stretches back to the end of the 19th century (e.g. Seifert, 1981). While the observation and collection of fungal fruiting bodies remains a central focus for fungal taxonomy, in the 20th century mycologists increasingly recognised the value of "small scale gardening", as a pioneer of this method, Dr Johanna Westerdijk, called the cultivation of microfungi (Commonwealth Agricultural Bureau, 1960). Numerous methods of isolating fungal cultures from various substrata have since been developed, each beset with their own limitations for the estimation of microfungal diversity (e.g. Booth, 1971). To overcome these limitations, it has been suggested that complementary protocols should be utilised if a more complete understanding of microfungal diversity is required (Frankland et al., 1990; Grigorova and Norris, 1990). Two main approaches for isolating fungi, the direct and indirect approach, provide such a complementary approach (Booth, 1971).

Direct approach

The observation and isolation of fungal fruiting bodies from a substratum is termed the 'direct method' (Booth, 1971). This can either be undertaken on dried herbarium specimens, freshly collected material or following incubation of substrata in moist chambers. If desired, stains can be applied to fungal material and slides are then prepared either from squash mounts or microtome or handsections in a suitable mounting medium (Booth, 1971; Hawksworth, 1974; Gams et al., 1998). Sections are particularly useful in the study of coelomycetes and ascomycetes (Michaelides et al., 1979). Single spore cultures can be isolated from fresh specimens by plating dilutions of a spore suspension onto a suitable antibiotic medium and by transferring single spores to a fresh plate once germination has been observed (e.g. Booth, 1971). As this approach provides specimens from natural substrata and in many instances also from cultures, it is the most useful approach for taxonomic studies. In addition, single spore cultures of known genetic make-up are required for the elucidation of anamorph/teleomorph connections (Kendrick and Nag Raj, 1979). On the other hand, microfungal fruit bodies are ephemeral structures and their appearance is easily missed

by this approach; this may lead to an underestimation of the total number of species present in a particular resource unit (Booth, 1971).

Indirect methods

Indirect methods have the potential to provide a more complete assessment of the fungal assemblage in a resource unit than direct methods (Booth, 1971). In general, a fungal 'resource', e.g. soil suspension or leaf particles, is plated onto a weak agar medium containing antibiotics and a growth inhibitor. The aim is to suppress the growth of bacteria and fast growing fungi and to facilitate the isolation of a representative sample of fungi present in the substratum (Bills, 1995). The isolation of a representative sample may be hindered by the fact that some fungi may not grow in artificial culture or on the particular media used (Bills, 1995). Nevertheless, indirect methods, in particular dilution plating, have been employed widely for the assessment of the soil microfungal assemblages (Christensen, 1968; Booth, 1971; Mahoney, 1972; Bissett and Parkinson, 1979a; Widden, 1986b; Hawksworth et al., 1995). Similar methods have been used for studying microfungi in leaf litter, for example by plating out macerated leaves (Kuter, 1986; Cornejo et al. 1994) or wash water of leaves (Booth, 1971). For the isolation of endophytes, surface sterilised leaf discs are usually placed on agar plates (Schultz et al., 1993; Hyde et al., 1997a).

The identification of fungi is based on the morphology of their fruiting bodies. It is, therefore, necessary to induce sporulation in cultures and a number of methods have been developed to achieve this (Booth, 1971; Matsushima, 1971; Guo et al., 1998). While many fungi will readily sporulate under artificial conditions (e.g. Kuter, 1986; Bills and Polishook, 1994a), a percentage of cultures may remain sterile and therefore cannot be formally identified. In this case, non-sporulating isolates are usually grouped into morphotypes on the basis of macroscopic and microscopic characters (e.g. Bills and Polishook, 1994a, 1994b; Cornejo et al., 1994; Polishook et al., 1996; Taylor, 1997; Brown et al., 1998; Fröhlich et al., 2000; Suryanarayanan et al., 2000, 2003). Two recent papers have assessed the fidelity of this approach by comparing morphotypes with genetically delineated species based on DNA sequences. Arnold et al. (2000) reported that their morphotypes concept overestimated diversity by 17.1 % ±10.3 % in endophyte and litter fungi. In contrast, Lacap et al. (2003) found a high degree of reliability in their assessment of six endophyte morphotypes. Although morphotypes

introduce a degree of uncertainty, it currently provides the most practical approach to deal with non-sporulating cultures. Identification of non-sporulating taxa using DNA sequence comparisons (Horton and Bruns, 2001, Schadt et al., 2001) was not considered for the present project because it was anticipated that species identifications would be hampered by the relatively small number of DNA reference sequences currently deposited in molecular databases relative to the fungal diversity found in leaf litter (Guo et al., 2000; Horton and Bruns, 2001). Therefore, the cost of applying this method may currently outweigh its benefits for diversity studies.

Indirect isolation methods in general have been criticised because they cannot distinguish between cultures isolated from hyphal fragments and those isolated from spores (e.g. Lussenhop, 1981). This means that the relative abundance of heavily sporulating species is likely to be overestimated (Booth, 1971). In the case of leaf litter, spores deposited on the leaf surface from the surrounding environment, may also lead to an overestimation of the number of species involved in decay (Kirby, 1987). To overcome this problem, Kirby & Webster (1990) pioneered the particle filtration method. With this method, a standard amount of leaf litter is blended in a mixer and extensively washed through a series of fine propylene mesh under sterile conditions. A subsample of the leaf particles collected in the finest mesh is then plated out onto antibiotic media. Theoretically, the extensive washing undertaken during the filtration procedure will reduce the number of contaminating spores on the leaf surface. However, this assumption has not formally been tested for terrestrial leaf litter. The particle filtration method holds some promise for diversity assessments (Bills, 1995), but only a small number of studies have so far utilised this method (Kirby and Webster, 1990; Bills and Polishook, 1994a, 1994b; Polishook et al., 1996; Miriam and Bhat, 2000). These studies have demonstrated that the diversity observed by particle filtration is several times greater than that observed by the direct method.

1.3.3.3 Rapid biodiversity assessment

This review would be incomplete without a discussion of rapid assessments of biodiversity, which circumvent the need for taxonomic identifications. Alternative options for assessing diversity have been considered by a number of authors (e.g. Hawksworth, 1993b; Hyde, 1995; Commonwealth of Australia, 1996; Cannon, 1997b; Hyde, 1997b; Cannon, 1999) for two reasons: the lack of specialist taxonomists and the

time required to formally identify organisms. In rapid biodiversity assessments, trained technicians sort organisms into recognisable taxonomic units (RTUs; Beattie et al., 1993; Cannon, 1997b; Hyde and Hawksworth, 1997). The application of RTUs has been tested for groups such as spiders, ants and polychaetes (Oliver and Beattie, 1993) and rapid biodiversity assessments have already been extensively used to assess insect diversity in the rainforests of Papua New Guinea (Basset et al., 2000). For fungi, this method has not been formally tested (Hyde, 1997b). Although criticised for providing uncertain data that cannot be compared across space and time, it has been suggested that if results of parataxonomic sorting show clear and biologically meaningful patterns, the sorting is likely to be reliable (Krell, 2002).

In recent years, another approach of rapidly estimating diversity in the absence of formal taxonomy has become feasible: the estimation of diversity using molecular tools. Most methods are based on the extraction of community DNA from the substratum and the amplification of rDNA sequences using kingdom specific primers. Subsequently, amplicons can be separated, for example by denaturing temperature gradient gel electrophoresis (Cannon, 1999), or cloned and sequenced (Martin, 2002). Although comparisons between studies that have used the latter option have been hampered to date by a lack of consensus as to what degree of sequence divergence defines a species or operational taxonomic units (OTUs), it is likely that molecular diversity estimation will prove useful in the estimation of fungal diversity (Cannon, 1999). Molecular diversity estimation has already been extensively applied to bacterial communities (e.g. Kroes et al., 1999; McGaig et al., 1999) and to a lesser extent also to fungal assemblages (Vainio and Hantula, 2000; Nikolcheva et al. 2003; Nikolcheva and Bärlocher, 2004). For the purpose of the present project, the advantages of developing fungal reference and culture collections in the course of a diversity survey using direct and indirect methods outweigh the speed of molecular methods.

1.3.3.4 Statistical tools for estimating diversity

Diversity can be described and estimated from sample based observations using statistical tools, which have often been developed for macro-organisms (e.g. James and Rathbun, 1981; Magurran, 1988; Coddington et al., 1990). These include species richness measures, species abundance models, and indices based on the proportional abundance of species (Magurran, 1988; Colwell and Coddington, 1994; Hughes et al.,

2001). The following discussion is not intended to be a complete review of tools for estimating diversity but focuses on measures that either have been applied in mycology or may be useful in the future.

Among various species richness measures, the observed number of species is a useful measure if the study area can be delimited in space and time (Magurran, 1988). Species richness can also be reported as a relative measure, for example as numerical species richness, which is defined as the number of species per specified number of individuals (Kempton and Wedderburn, 1978) or species density, which is the number of species per specified collection area (Hurlbert, 1971). To compare datasets of unequal sample sizes, the expected number of species in each sample if all samples were of a standard size can be calculated using a technique called rarefaction (Hurlbert, 1971; James and Rathbun, 1981). The disadvantages of rarefaction are a loss of information about species abundances (Magurran, 1988) and that its variance measures variation due to the reordering of samples rather than precision (Hughes et al., 2001). The latter problem can be overcome by using richness estimators, which can be grouped into three main classes, i.e. those extrapolated from accumulation curves, and parametric and non-parametric estimators (Colwell and Coddington, 1994; Hughes et al., 2001).

Most extrapolation methods use the observed species accumulation curve to model the process of observing new species with increasing sampling effort (Colwell and Coddington, 1994; Hughes et al., 2001). These include for example the Michaelis-Menten equation of enzyme kinetics (Holdridge et al., 1971; Raaijmakers, 1987) and the novel estimators, F3 and F5 (Rosenzweig et al., 2003). Species-rich assemblages, such as microfungi in leaf litter, are usually undersampled relative to their diversity and extrapolation may, therefore, multiply bias and random error. For this reason, extrapolation methods may not be useful for species-rich groups (Colwell and Coddington, 1994; Hughes et al., 2001). The next group of estimators, i.e. parametric estimators, are based on species abundance models and include for example the lognormal (Preston, 1948). For microfungal diversity estimation, these estimators have the disadvantage that they require a full species by abundance matrix (Colwell and Coddington, 1994; Hughes et al., 2001), which may not be available for data obtained by the direct method.

The non-parametric estimators include those adapted from mark-release-recapture statistics. Examples include the Chao2 estimator (Chao, 1987), the incidence-based

coverage estimator (ICE; Lee & Chao 1994) and the Burnham and Overton jackknife procedure (B&O, Burnham and Overton, 1979). These statistics have in common that they perform well in datasets skewed towards low abundance classes, as for example in the case of species datasets with an abundance of rare species (Colwell and Coddington, 1994; Hughes et al., 2001). Calculation of these statistics can be undertaken on either a matrix of presence/absence data for each sample, or of abundance data (Colwell and Coddington, 1994; Turner et al., 2000). Non-parametric estimators, which utilise abundance data, include the Chao1 (Chao, 1984) and the abundance-based coverage estimator (ACE; Chao & Lee 1992). Although the evaluation of diversity estimators for microorganisms is still in the early stages (Hughes et al. 2001), they have been increasingly employed in molecular diversity estimation of microbial communities (e.g. Kroes et al., 1999; Nubel et al., 1999; Bishop-Hurley et al., 2002; Breitbart et al., 2002; Valinsky et al., 2002, Horner-Devine et al., 2003) and to a limited extent in mycology (e.g. Lodge & Cantrell, 1995; Suryanarayanan et al., 2003).

Species abundance distributions utilise all the information gathered in an assemblage and therefore have been strongly advocated by some workers (e.g. May, 1975; Southwood, 1978). Among the many distributions that have been described, the four most frequently examined are the lognormal distribution, the geometric series, the logarithmic series and MacArthur's broken stick model (Magurran, 1988). The mathematical fit of data to a distribution can be tested but some authors favour a graphical inspection of data over tests that have a low power to discriminate between distributions (e.g. Lambshead and Platt, 1985). Although fungal diversity studies often include graphical representations of species abundances (e.g. Fröhlich et al., 2000; Yanna et al., 2002; Zhou and Hyde, 2002; Parungao et al., 2002), few have attempted to describe the distribution of the data (e.g. Thomas and Shattock, 1986; Schnittler et al., 2002). In addition, fungal species abundance models have been rarely considered in assessing biological phenomena. This may be in part due to the fact that 'abundance' is difficult to assess for fungi as 'individuals' are ill-defined (see below). It is not entirely clear how this would affect diversity estimates derived from species abundance models. However, an index called Fisher's alpha based on the logarithmic series model has been shown to be robust, even when the log series distribution is not the best descriptor of the underlying species abundance pattern (Kempton and Taylor, 1974; Taylor et al., 1976; Southwood, 1978). It also has a low dependence on sample size and good discriminant

ability (Magurran, 1988). This index may, therefore, be suitable to assess and compare microfungal diversity.

Diversity indices, which take into account both the evenness and species richness, provide an alternative approach to assessing species abundance distributions (Magurran, 1988). Two major groups of indices are available. The first group includes those indices derived from information theory, such as the Shannon-Wiener and the Brillouin index (Pielou, 1975). The Shannon-Wiener index assumes that all species present in a sample are known (e.g. May, 1975), and may, therefore, not be the best choice for microfungal assemblages. Nevertheless, this index is commonly calculated in fungal diversity studies and, therefore, may facilitate comparisons with previous studies. Although these indices take into account the evenness of species, it also possible to calculate additional evenness measures, for example Shannon's evenness (Magurran, 1988) or a number of measures provided by Legendre & Legendre (1983). The second group are dominance measures that are weighted towards the abundances of the commonest species; these include for example the Simpson's index and the Berger-Parker index (Magurran, 1988). In selecting an appropriate diversity index, it is necessary to take into account the underlying assemblage structure, the discriminant ability of diversity indices, and their sensitivity to sample size (Magurran, 1988). A review of the characteristics of a selection of indices was provided by Magurran (1988).

1.3.3.5 Differentiation diversity

Differentiation diversity can be described using either similarity or distance measures. The choice of index is in part dependent on the type of data that is available. For example, the Jaccard (Jaccard, 1901, Southwood, 1978) and Sorenson (Janson and Vegelius, 1981) indices are commonly applied when only presence/absence data are available. In contrast, for abundance data the Bray-Curtis index (Bray and Curtis, 1957), also called the Sorenson quantitative (Magurran, 1988) or Motyka index (Motyka et al., 1950), and the Morisita-Horn index are the most commonly used. Among these, the Bray-Curtis distance, has been shown to have a robust relationship with ecological distance (Faith et al., 1987) and has been recommended as an index of choice for ordination techniques (Clarke and Warwick, 1994). Simpson's Coefficient of Similarity (Simpson, 1943) expresses the overlap of species in two datasets or the number of

shared species as a proportion of the smaller dataset. This may be useful in some instances for example when comparing datasets of unequal sample sizes.

Another method of describing differentiation diversity that has been used in mycology is the calculation of complementarity of species (e.g. Polishook et al., 1996). Complementarity has been defined as 'the proportion of all species in two sites that occurs in only one or the other of them' (Colwell and Coddington, 1994). Although an intuitively simple approach to assessing the distinctness of diversity data, this method has a number of disadvantages if samples of insufficient size are used. This is likely to be the case in species rich groups and complex effects may arise. For example, complementarity may underestimate geographical range or other variables estimated from discrete points in time or space and overestimate the degree of distinctness (Colwell and Coddington, 1994).

1.3.4 Significance of assessing fungal diversity

Despite the challenges inherent in assessing the microfungal diversity, developing strategies to produce reliable estimates that can be compared across space and time is essential for a number of reasons. For example, biodiversity or the extent of biological variation on earth has become a key issue in science and politics during the final decade of the 20th century. A focal point was the Biodiversity Convention, which was signed in Rio de Janeiro in June 1992 by 156 countries (Stork, 1993). In effect, this convention requires countries to assess and conserve the biodiversity of ecosystems and this requirement was also brought to the attention of the scientific community and policy makers by a number of mycologists (Hawksworth, 1991, 1993a, 1993b, 1997, 1998a, 1998b; Hyde, 1995, 1997b, 2003; Hyde and Hawksworth, 1997; Cannon, 1997b; 1999).

The significance of fungi is based on both the ecosystem services they provide and on their importance to humans. For example, leaf litter fungi are involved in nutrient cycling (Lodge, 1992) and serve as important food source to many insects (Wilding et al., 1989; Bengtsson et al., 1996). In their roles as decomposers, leaf litter fungi also sequester carbon and move it to the recalcitrant carbon stores of the soil (Jordan, 1985). In turn, carbon storage is highly relevant to mitigating the effects of greenhouse gas emissions associated with global warming and therefore has become a new currency in the recently evolved economy of carbon credit trading (Australian Greenhouse Office,

2004). While carbon storage in standing trees is readily quantifiable, less is known about carbon stores of the soil (Australian Greenhouse Office, 2004). However, it is estimated that approximately 64 percent of all carbon in US forest ecosystems, for example, occurs below ground (Birdsey, 1992).

In addition to their vital roles in ecosystems, microfungi have a number of practical applications. For example, medicine has been revolutionised with the advent of fungal antibiotics and in more recent years other important pharmaceutical products have been developed from fungal metabolites (Rossman, 1997). These include for example cyclosporin (from *Tolypocladium inflatum*), an immunosuppressant important in organ transplantation and lovastatin (from *Apergillus terreus*), a cholesterol lowering drug (Hyde and Hawksworth, 1997; Rossman, 1997). Despite the ongoing discovery of many novel fungal compounds potentially useful for medicine, the contribution of tropical fungi to the discovery of novel compounds has been questioned (Wildman, 1997). Nevertheless, fungi present a vast source of genetic potential, which may be only realised in the future. Areas of active research include the investigation of fungal pigments for use as dyes (Hyde, personal communication) and utilisation of fungal enzymes in the detoxification of polyphenols and degradation of plastics (Rossman, 1997). Fungi also have important agrochemical applications, for example as biocontrol agents, mycopesticides and mycoherbicides (Evans and Ellison, 1990) and are a source of industrially important enzymes (Hyde and Hawksworth, 1997). The elucidation of habitats and patterns of high fungal diversity and its conservation are, therefore, of interest to a variety of industries (ten Kate and Laird, 1999).

1.3.5 Global fungal species estimates

Although estimating the numbers of fungal species has important implications for planning and resource allocation for conservation (Hallenberg, 1987; Hawksworth 1991, 1993b), the magnitude of fungal diversity is still unknown to date. Estimates range from around 100,000 extant species of fungi (Ainsworth and Bisby, 1943) to 9.9 million (Cannon, 1997). A frequently cited estimate is 1.5 million fungal species worldwide (Hawksworth, 1991). This figure was extrapolated from the ratio of six fungi for each recorded vascular plant in the British Isles and was supported by ratios derived from other Northern Hemisphere floras.

Very few studies have addressed the ratio of fungi to vascular plants in tropical regions (Hyde et al., 1997b; Fröhlich and Hyde, 1999). Evidence from other organisms such as plants, suggests that species diversity increases with decreasing latitude (Hawksworth, 1993) but it is not clear whether the same is true for fungi. In fact, it has been proposed that the ratio of fungi to hosts may be lower in the tropics than in temperate regions (May, 1991). This is because dispersal distance between widely dispersed hosts in areas of high tree species diversity may act as selective pressure on the organisms to become less specialised in their host or substrate requirements (May, 1991). The question of host or substratum specificity or preference is, therefore, central to the issue of global fungal species estimates.

Based on extrapolations from microfungi on tropical palms, the hypothesis of a lower fungi to plant ratio in the tropics has been refuted (Hyde et al., 1997a; Fröhlich and Hyde, 1999). In these studies, 10% and 25% of fungal species were estimated to be 'host specific' and a ratio of 26 and 33 unique fungi to each of the palm species studied was proposed. While the magnitude of the estimate may be applicable for palms of these regions, it is impossible to generalise these results to tropical microfungi in a variety of substrata and clearly, more work is required to assess the level of fungal host specificity or preference.

1.4 Ecology of microfungi

1.4.1 Definitions and concepts

Similar to the term 'biodiversity', the term 'ecology' describes a political and social concept as much as a scientific one. Even within the scientific field, definitions of ecology abound (McIntosh, 1985). For the purpose of this thesis, ecology is defined as the study of the diversity and distribution of organisms and their reciprocal relations with their environment (Griffin, 1972; Cooke, 1979; Cooke and Rayner, 1984). Since aspects of microfungal diversity have been addressed in the previous section, I will focus on the distribution of fungi and the relationship with their environment in this final section.

Ecology as a scientific discipline can be broadly divided into three areas of research: autecology or population ecology, synecology or community ecology, and function ecology (Christensen, 1989). Autecology strives to understand a particular organism or

a small association of organisms in relation to its environment; these studies are often undertaken in a laboratory setting. In contrast, synecology refers to the characterization of communities in terms of the totality of species in a given environment, and function ecology seeks to elucidate the functional roles of organisms within an ecosystem (Christensen, 1989). Rarely have two or more approaches been combined (e.g. Frankland, 1966, 1969; Christensen, 1989; Cornejo et al., 1994). The present study is concerned mainly with the synecology of microfungi (Chapter 4 and 5) although an autecological study has also been included (Chapter 6).

Synecological studies of fungi, in particular, have to contend with problems of definitions and methodological challenges (Cooke and Rayner, 1984). For example, the indeterminate growth form of fungi, the hidden nature of their vegetative state, their eccentric patterns of fruiting, their unusual genetic system (e.g. heterokaryosis and parasexual recombination), their phenotypic plasticity and their ability to clone themselves have made it difficult to define the concept of a fungus 'individual' (Seifert, 1981; Cooke and Rayner, 1984; Moncalvo, 1997). This has a direct effect on how fungal survey data is evaluated statistically and places limitations on the interpretations of results. Since individuals remain elusive, the term 'population' (i.e. an assemblage of individuals of the same species) also has little meaning (Seifert, 1981). With the advent of molecular techniques, it is now possible to accurately assess the boundaries of an individual mycelium (Stenlid et al., 1993). The practicalities and cost involved in employing molecular techniques have so far limited its application to ecological questions. Similarly, mating studies undertaken in wood decay fungi have demonstrated the individuality of mycelia (Jennings and Rayner, 1984) but similar delimitations are not feasible for broad scale surveys of leaf litter. In the present project, therefore, surrogate measures of 'individuals' have been applied, i.e. fungal occurrences for data obtained by the direct method and number of cultures for data obtained by particle filtration.

The concept of 'community' has also been difficult to delineate in fungal ecology. Some researchers have taken a broad view of communities and have defined a community as an assemblage of diverse species occupying the same, functionally discrete environment (e.g. Cooke and Rayner, 1984). Other researchers have based their fungal community concept on the ability to repeatedly recognise and measure differences among repeating assemblages of fungi that occur simultaneously in similar

habitats (States, 1981). Yet other authors have questioned the existence of fungal communities altogether and have proposed that fungi occurring together on a substratum are random associations of ubiquitous organisms (Baker and Meeker, 1972; States, 1981). Due to a lack of consensus of what defines a microfungal community, I have applied the term 'assemblage' instead of 'community' as has been proposed by Frankland (1992) and the definition of community will be revisited in the final chapter of this thesis. Despite these limitations, synecological studies of microfungi have provided valuable information about distributional patterns, which I will review next.

1.4.2 Current knowledge of microfungal distributions

1.4.2.1 Effects of host, substrata and tissue type

Traditionally, saprotrophic fungi have been considered to be generalists that do not show any or limited specificity (e.g. Hawksworth, 1974; Kendrick, 1985). However, this view has been challenged in recent years. For example, quantitative differences of fungi on different litter species have been reported for studies from Nevada by Cowley (1970), from Panama by Cornejo et al. (1994) and from Mauritius by Dulymamode et al. (2001). Similarly, in a study from the neotropics, Polishook et al. (1996) reported greater similarity of microfungal assemblages from the same litter species at different sites compared to those of different litter species at the same site. A study from north Queensland observed a high percentage of fungi that occurred on only one leaf type but this result was confounded as a nearly equally high percentage of species was known only from one record (Parungao et al., 2002). Differential colonisation of leaf litter by aquatic hyphomycetes has been described in studies from Belarus (Gulis, 2001), Spain (Chauvet et al., 1997) and Australia (Thomas et al., 1992). Hyde et al. (2004) have reviewed information from hosts at different levels of the taxonomic hierarchy and have concluded that a degree of host preference or host specificity exists in saprotrophic microfungi. Although indications are that differences in species richness and composition exist in leaf litter of different tree species, some of the evidence is circumstantial and few studies have examined this question systematically.

In contrast, tissue specificity or preference has been demonstrated for microfungal assemblages on palms (Yanna et al., 2001) and on the mangrove palm *Nypa fruticans* (Hyde and Alias, 2000). Numerous individual examples of tissue specificity can be found in the taxonomic literature, for example for *Mycosphaerella* spp. on *Eucalyptus*

(Crous, 1998), for *Gnomonia* spp. (Monod, 1983; Paulus et al., 2003a) and for a new genus of Dermateaceae on *Ficus* (Hyde et al., 2004).

1.4.2.2 Seasonal variation

A number of synecological studies have investigated the effect of environmental variables on soil fungal assemblages (e.g. Bissett and Parkinson, 1979b; Widden, 1986b) and leaf litter fungi (e.g. Hudson, 1968; Gessner, 1977; Kuter, 1986) in cold temperate, alpine and tundra environments. In these studies, distinct summer and winter fungal assemblages were identified. For tropical regions, a relative paucity of research into the seasonality of leaf litter microfungi is apparent and contradictory results have been reported. For example, Cornejo et al. (1994) reported significant differences in fungal species numbers between wet and dry seasons with the highest numbers observed during the driest months. In contrast, variations in frequencies of fungal species during litter decomposition could not be attributed to climatic conditions in a study of microfungi in a Mexican cloud forest (Heredia, 1993) while studies of different substrata, for example submerged wood and bamboo baits, reported a higher species richness for the hot wet season (Ho et al., 2002; Zhou and Hyde, 2002). A seasonal effect was also proposed by Hutton and Rasmussen (1970) who found that 35 % of the 23 plant species studied had no epiphyllous fungi in common between the wet and dry season and 44 % had only one fungus in common.

1.4.2.3 Spatial heterogeneity

Spatial heterogeneity can be observed at different scales. For example, tropical decomposer fungi are frequently restricted to particular size classes and types of substrata and to certain positions relative to the ground (Lodge, 1997). Vertical stratification has been reported a number of times, for example in endophytes (e.g. Rodriguez and Samuels, 1990), in decaying cooksfoot culms (Webster, 1956, 1957), and in decaying bamboo leaf culms (Dalisay, 1998; Hyde et al., 2002). Stratification of microfungi was also observed in the litter layers of pine needles (e.g. Kendrick and Burges, 1962). In sites 200 metres apart, a relatively high heterogeneity (complementarity 62 to 66 percent) was observed in a study from the neotropics, for which leaf litter of the same tree species was collected (Polishook et al., 1996). At a

greater scale, Taylor (1997) found that the mycota of disjunct tropical rainforest palms differed significantly and that their mycobiota in tropical countries has a higher diversity than temperate areas. Similarly, the spatial relationship of hosts was found to have a greater influence on the distribution of saprotrophic palm fungi than host phylogeny in collections from Australia, Brunei and Hong Kong (Fröhlich, 1997).

These observations raise the question "What are potential causes of these distributions?" The next section will review general ecological theories and factors that potentially contribute to microfungal diversity and distribution in leaf litter.

1.4.3 Potential factors affecting diversity and distribution of microfungi

1.4.3.1 General ecological theory

Ecological theory abounds with views of the underlying causes of diversity and distributions of species. Most of these can be summed up in two general perspectives, the niche assembly and the dispersal assembly perspective (Hubbell, 2001). In broad terms, the niche assembly view proposes that communities are "groups of interacting species whose presence or absence and even their abundance can be deduced from assembly rules that are based on the ecological niches or functional roles of each species" (Hubbell, 2001). The dispersal assembly view, on the other hand, states that "communities are open, nonequilibrium assemblages of species largely thrown together by chance, history, and random dispersal" (Hubbell, 2001). Most dispersal assemblage theories, such as the theory of island biogeography (e.g. MacArthur and Wilson, 1967), are neutral; this means that the underlying assumption is that all species are equal in their probabilities of immigration or extinction (Hubbell, 2001). In mycology, only a small number of studies have tested these assumptions. For example, the theory of island biogeography has been tested using leaves and cellophane squares as resource islands (e.g. Andrews et al., 1987; Kinkel et al., 1987; Wildman, 1987). These studies have concluded that the theory of island biogeography is a useful starting model for describing fungal colonisation of resource islands.

By far the most frequently applied view in mycology and plant ecology alike has been the niche assembly view. Under this framework, it is generally assumed that two species cannot coexist if they use resources in an identical manner; this principle has been termed the competitive exclusion principle (Gause, 1934). Coexistence of species,

therefore, occurs when resources vary spatially, and presence of each species is determined by its superior competitive ability under particular conditions (Ashton, 1969). Both plant ecologists and mycologists have pondered how a very large number of species can co-exist in the same habitat (Christensen, 1989; Lodge and Cantrell, 1995; Lodge, 1997; Wright, 1999). Could interspecific differences in the few parameters required for colonisation and growth explain the levels of diversity observed in some habitats such as tropical rainforests?

To consider this question for leaf litter fungi, it is necessary to describe the parameters, which potentially make up the ecological niche of a fungus. At its simplest, the concept of an ecological niche describes the characteristics of the substratum on which a fungus lives and from which it obtains nutrients, and the environmental conditions to which the fungus is exposed (Cooke and Rayner, 1984). In addition, the genetic properties of a fungus also determine its niche and the occurrence of other organisms in its vicinity control its activity within that niche (Cooke and Rayner, 1984; Christensen, 1989). Furthermore, the concept of a niche also describes the functional role of a fungus within an ecosystem (Cooke and Rayner, 1984). With this in mind, the following discussion will consider what is known about some factors that may define ecological niches of leaf litter fungi.

1.4.3.2 Potential niche defining factors

Substratum dependent factors

Leaves of different rainforest tree species provide unique habitats for leaf litter fungi because of differences in their chemistry and texture (e.g. Kanowski, 1999). In turn, fungi vary in their minimum requirements for sources of carbon, nitrogen, phosphorus and other minerals and also in their ability to produce enzymes required to degrade some of the complex polymers found in plant tissues, such as cellulose, cutin, lignin, suberin and waxes (Cooke and Whipps, 1993; Jennings, 1995). While many fungi produce an array of enzymes, no species is capable of utilising all potential substrates within leaves. Therefore, a large number of species may be supported in the same habitat, each exploiting different substrates (Neville and Webster, 1995).

In addition to nutrients and complex polymers, plant tissues commonly contain specific and non-specific biologically active substances, which have the potential to inhibit and

stimulate fungal growth (Neville and Webster, 1995). Rainforest trees, in particular, have evolved a wide variety of compounds in their leaves, such as phenols, oils, alkaloids and other secondary metabolites, which act as defence against herbivores and pathogens (e.g. Wicklow, 1988; Coley and Aide, 1991; Kutchan, 2001). In addition to inhibitors, plants may produce flavonoids and other phenolics, which stimulate growth in fungal taxa (Black and Dix, 1976; Neville and Webster, 1995). Potentially, this differential interaction between inhibitors and stimulators and the response of different fungi is a factor in shaping the distribution of fungi.

Leaf texture may impact twofold on the fungal assemblage structure. Differences in texture may be reflected by differences in both the chemical composition and the physical attributes of leaves. For example, tougher leaves are more likely to have a higher cellulose and lignin content compared to soft leaves (Cooke and Rayner, 1984). Similarly, petioles and midribs contain more lignin than the leaf lamina (Raven et al., 1992). In addition, variation in texture and presence of auxillary organs such as trichomes may also impact indirectly on physical factors such as water holding capacity (Cooke and Rayner, 1984). These factors may be especially relevant for the attachment of spores and early colonisation (Andrews and Hirano, 1991). The colonisation of the substratum by later arriving fungi may also be influenced by fungi present in living and senescent leaves that persist after abscission (Cooke and Rayner, 1984). Therefore, any factors, such as leaf age, shade or light leaves and general tree health, that govern the development of fungal assemblages in and on living leaves (Andrews and Hirano, 1991), may potentially also affect fungal assemblages in decaying leaves.

Leaf litter is a dynamic environment and undergoes constant change. Changes in both leaf chemistry and leaf texture with advancing decay are mirrored by changes in fungal assemblages but the actual mechanisms responsible for the apparent species replacement are poorly understood (Frankland, 1998). The replacement of fungal species on a particular substratum over time has been termed ‘succession’ and has been the focus of numerous studies (e.g. Kendrick and Burgess, 1962; Hering, 1965; Frankland, 1966; Hogg and Hudson, 1966; Kuter, 1986; Kjøller and Struwe, 1992).

Abiotic factors

The effect of abiotic factors on fungal colonisation and growth have been mostly established in laboratory based studies (Cooke and Rayner, 1984; Cooke and Whipps, 1993; Neville and Webster, 1995). These factors include for example temperature and water availability, aeration, pH, and light. In natural environments, many of these factors do not vary independently and may also work interactively on fungi. For example, water potential and aeration of a substratum are inversely related and the effect of water potential on the growth rate of a fungus may vary according to temperature (Boddy, 1992).

Interactions with other fungi and insects

Some researchers have proposed that interspecific interactions may be one factor that overrides all others in regulating fungal species abundance (Gochenaur, 1984; Shearer and Zare-Maivan, 1988). Although interspecific interactions have often been envisaged in terms of interspecific struggle, such as combat and competition, fungi may be equally involved in neutral or positive interactions (Cooke and Rayner, 1984). In addition, interactions may be strongly influenced by abiotic factors. For example, the competitive ability of five species of *Trichoderma* was shown to vary at different temperature ranges (Widden and Hsu, 1987).

Numerous interactions exist between litter inhabiting animals and fungi that potentially affect fungal assemblages. For example, selective grazing of certain fungal taxa by fungivores such as mites and collembola has been reported to influence the colonisation of leaves by specific fungal taxa (Newell, 1980). In addition to mycophagy, fungi may also be impacted by detritivores, which consume vast amounts of decomposing organic matter containing living fungal material. This leads not only to the destruction of fungal biomass but also to the disappearance of fungal resources and habitats (Cooke and Whipps, 1993). For example, in soils where earthworms are active, leaves are rapidly fragmented and integrated into the soil, thereby reducing the time frame available for fungal assemblages to establish (Cooke and Rayner, 1984). Finally, invertebrates are involved in the dispersal of fungal propagules and may, therefore, play an important role in determining fungal distributions within a site (Bengtsson et al., 1996).

Nutritional modes

The inherent strategy that fungi apply to access resources may also shape microfungal distributions. For example, fungi can be broadly combined into three groups to describe their predominant mode of resource acquisition. Saprotrophs utilise non-living organic material as a resource but never cause the decline or death of their host. Necrotrophs kill living tissues of a host first before utilising it whereas biotrophs only exploit living host cells as a nutrient source (Cooke and Rayner, 1984). These nutritional modes are not mutually exclusive and fungi may show varying degrees of flexibility during their life cycles (Cooke and Whipps, 1993). Depending on the position of a fungus on the continuum of nutrient acquisition modes, nine principal categories have been recognised (Table 1). In leaf litter, microfungi belonging to all nutritional modes can be expected to be found, albeit in varying abundance and at different times. Even obligate biotrophs may persist for a brief period following leaf drop while facultative saprotrophs and necrotrophs may have a competitive advantage over later arrivals due to their establishment within the leaf lamina before senescence (Cooke and Whipps, 1993).

Functional role of leaf litter fungi

Leaf litter microfungi are vital contributors to decomposition in rainforest ecosystems (e.g. Lodge, 1996). Decomposition of plant litter refers to the physical and chemical processes involved in reducing litter to its elemental chemical constituents and as such is a major determinant of nutrient cycles (Aerts, 1997). Complex organic compounds containing carbon, nitrogen and phosphorus are broken down externally by an array of hydrolytic enzymes into soluble low-molecular weight subunits that fungi can assimilate (Cromack and Caldwell, 1992). Besides producing new fungal biomass, assimilated materials can be modified and excreted in a wide variety of fungal byproducts including organic and humic acids. These acids may play an important role in the weathering of the soil minerals (Cromack and Caldwell, 1992). Saprotrophic microfungi, therefore, make available nutrients to higher plants in more than one way (Lodge, 1996).

This list of potential niche defining factors is unlikely to be complete and, yet, it is apparent that a large number of factors may influence the distribution of fungi in leaf litter. These factors may also interact in numerous ways and thus a manifold increase the number of potential niches may result. Following the general review of microfungal

distributions and potential factors affecting these distributions, the next section will provide the geographical and local context for the proposed study

Table 1.2 Econutritional groups of fungi

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Adapted from (Cooke and Whipps, 1993)

1.4.4 Context of this study

1.4.4.1 The wet tropics of Australia

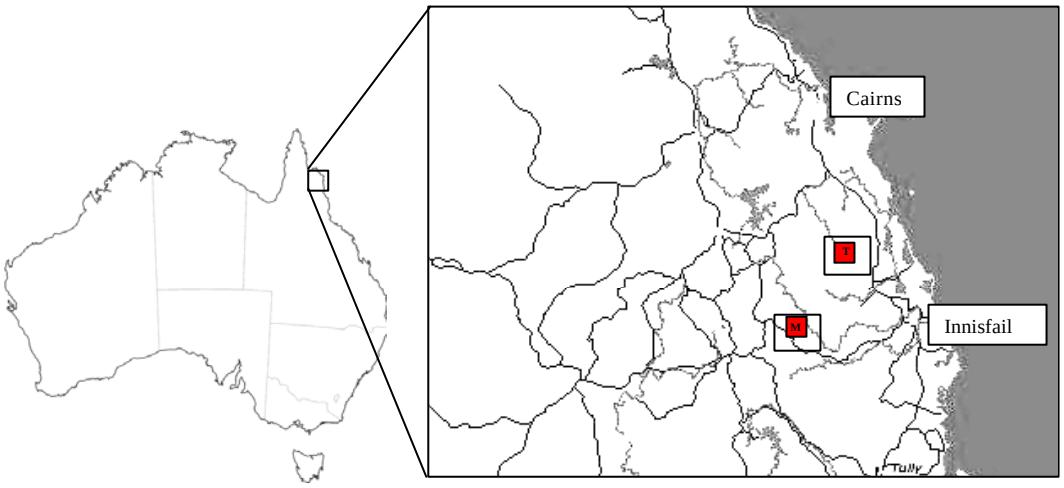
The wet tropics of Australia lie in a region between the latitudes of 15° to19° South and the longitudes of 145° to 146°30' East (Tracey, 1982). This region contains the most extensive continuous area of rainforests in Australia (Winter et al., 1991) and has been declared a world heritage area in 1988. The rainforests of this region are characterised by high plant diversity, particularly at the generic level (DASETT, 1986) and may represent ancient refugia where Gondwanan warm mesic forests have persisted continuously since the Cretaceous (Barlow and Hyland, 1988). The flora of the region includes an array of primitive angiosperms; in fact, the concentration of primitive families in the Australasian region is the greatest in the world (Webb and Tracey, 1981). Of 14 primitive angiosperm families, eight occur in north-east Queensland and these include two narrowly endemic families, Austrobayleaceae and Idiospermaceae (Webb and Tracey, 1981). There is also a tropical intrusive element in the rainforest flora, which has reached Australia from the north-west since the Miocene (Barlow and Hyland, 1988). The intricate pattern of humid forest communities observed today may have further developed as the result of climatic oscillations in the Quaternary (Barlow and Hyland, 1988). Among the extant rainforests, thirteen main structural types have been described and these have been further subdivided into 27 broad rainforest communities (Tracey and Webb, 1975; Tracey, 1982).

The major influence on the weather of this region is the seasonal movement of the intertropical convergence zone characterised by tropical lows and cyclones during the months of January to April; these depressions bring much of the rainfall to the region (Spain, 1991). The wet tropics region has a considerable range of climates, associated with altitudes from sea-level to over 1600m and rainfall gradients from 1300 to over 8000 mm per annum (Tracey, 1982; Bureau of Meteorology, 2000). Soils are variable and correlated with a variety of parent material, e.g. basalt and granite, drainage status and rainfall (Tracey, 1982).

Two study sites are proposed for the present project (Fig. 1.1). These sites are situated on the Atherton tablelands at an altitude of approximately 710 m for the Topaz site (17°24'00"S, 145°43'30"E) and 570 m for the Millaa Millaa site (17°32'30"S, 145°42'50"E). Mean annual rainfall has been reported as 3810 mm for Topaz (Bureau

of Meteorology, 2000) and 3400 mm for Millaa Millaa (N. Tucker, pers. comm.). Average temperatures are 22.6°C (mean maximum) and 9.9°C (mean minimum) in August and 29.6°C (mean maximum) and 17.3°C (mean minimum) in January at the nearest recording station in Atherton (Spain, 1984). Rainforest of both sites is a complex mesophyll vine forest on basalt soils in the very wet uplands, which has been classified as 1b rainforest (Tracey and Webb, 1975). These sites were selected on the basis of high tree species diversity and presence of mature trees. They were approximately matched for rainfall and for rainforest type, although the tree species composition differed to some extent between sites.

Figure 1.1. Map of Australia and the Cairns region with study sites. 'T' denotes the site near Topaz and 'M' the site near Millaa Millaa



1.4.4.2 Leaf litter environment

Litter on the rainforest floor forms a heterogenous habitat, which is subject to seasonal fluctuations. In rainforests of north Queensland, the maximum leaf litter fall occurs in the period December to March and varies in mass from 8 to 10 tons per hectare per year (Brassell et al., 1980; Spain, 1984). The crop of litter on the forest floor also varies seasonally depending on the balance between litter fall and breakdown rates. By the start of the wet season, a layer of dead leaves has built up, the mass of which ranges from 3.5 to 11 tons per hectare at upland sites (Brassell, 1981; Spain, 1984). Breakdown

rates are similar as those reported for other tropical rainforests with slower rates reported for cooler upland sites as compared to lowland forest (Brassell, 1981; Spain, 1984). Considerable annual variations in breakdown rates were observed but generally, breakdown of litter occurs in less than a year (Spain, 1991). The habitat of 'leaf litter' is, therefore, rather unstable compared to long lived substrata such as logs. Data on the physical and chemical attributes of leaf litter in the region are lacking but for one exception. Brassell et al. (1980) undertook a litter analysis for sodium, potassium, calcium and magnesium but this study was restricted to pooled litter samples (including small branches and fruits) from two upland sites. More comprehensive information is available for living leaves from upland sites including data on the elemental composition and attributes of leaves of selected species (Kanowski, 1999).

1.4.4.3 Selection of host species

A phylogenetic frame work formed the basis for host selection for the main part of the study. This selection criterion was based on the assumption that, although the production of secondary metabolites differs between species of the same family and even between individuals of the same species (Shelton, 2000; Theis and Lerdau, 2003), some secondary metabolites would be shared between members of the same phylogenetic lineage (Soltis et al., 1997). This was relevant because microfungal species may be affected differentially by biologically active compounds in abscised leaves (Neville and Webster, 1995). From 119 families reported in the wet tropics region (DASETT, 1986), four common plant families belonging to four major angiosperm clades were chosen for this study. The selected families were the Lauraceae (Laurales), the Proteaceae (Proteales), the Moraceae (Rosales) and the Elaeocarpaceae (Oxalidales). The Lauraceae are one of the most ancient plant families and have a pantropical distribution, extending into temperate regions in some instances. This family is characterised by the production of flavonols (Watson and Dallwitz, 2000). The Proteaceae have a largely Southern Hemisphere distribution with the main centres being Australia and South Africa; members of this family produce cyanogenic glycosides (Watson and Dallwitz, 2000). The Moraceae occur in tropical or warm temperate regions and are characterised by the production of latex (Watson and Dallwitz, 2000). Finally, the Eleaocarpaceae have a mostly tropical distribution and are predominantly found in Papuasia and Australia but also extend into temperate regions. Members of the

Elaeocarpaceae are characterised by the production of ellagic acid, alkaloids and flavonones (Watson and Dallwitz, 2000).

At the species level, mostly pragmatic selection criteria were applied. Firstly, only species were considered for which a minimum of three individual trees could be located at both study sites. Secondly, abscised and decaying leaves had to be identifiable as belonging to the selected species without any doubt and without requiring specialised identification methods such as cuticle studies. Furthermore, I endeavoured to include species for which leaf chemistry data were available. Four species were selected to form the main framework for this project (asterixed). An additional two species were added on the basis of their close phylogenetic relationship with one other species; therefore the study included two congeneric species and two species belonging to different genera within the same family. Another tree species, *Neolitsea dealbata* was utilised in a separate study that examined the particle filtration protocol. On this basis, the following tree species were selected for this project.

- *Cryptocarya mackinnoniana* F. Muell. (Lauraceae) - rusty laurel *
- *Elaeocarpus angustifolius* Blume (Elaeocarpaceae) - blue quandong *
- *Ficus pleurocarpa* F. Muell. (Moraceae)- banana fig *
- *Ficus destruens* F. Muell. ex C.T. White (Moraceae) - rusty fig
- *Neolitsea dealbata* (R. Br.) Merr. (Lauraceae) - grey bollywood
- *Opisthiolepis heterophylla* L.S. Smith (Proteaceae) - blush silky oak *
- *Darlingia ferruginea* J.F. Bailey (Proteaceae). - hairy silky oak

Photos of leaves and a description of each species are provided in Appendix A.

1.5 Conclusion

In this chapter, I have reviewed current knowledge and concepts with respect to the taxonomy, diversity and distribution of leaf litter microfungi. While a large body of literature covers various aspects of microfungal biology well, a number of gaps in our knowledge could be identified. These include the limited taxonomic information available for saprotrophic fungi in leaf litter of the wet tropics, the paucity of diversity data for microfungi in tropical rainforests in general and for the wet tropics of Australia

in particular and the difficulty in obtaining meaningful diversity estimates due to a lack of standardised protocols. In addition, it was noted that some hypotheses about fungal distributions, for example host preference, were based on circumstantial evidence or derived from studies that were limited by their sampling design.

Therefore, the following aims are proposed for the present project:

1. To assess and make recommendations with respect to sampling and isolation methods for microfungi
2. To characterise the diversity and structure of microfungal assemblages from the rainforests of north Queensland
3. To assess the distribution of microfungal assemblages and to generate hypotheses regarding their ecology
4. To assess the taxonomy of selected microfungal taxa and to provide a reference collection of observed microfungi for future studies.

CHAPTER TWO

Particle filtration: a tool for estimating microfungal diversity in leaf litter?

2.1 Introduction

> *"All methods of quantifying soil fungi are imperfect. Success depends to a great extent on soundly based sampling and cross checks between methods rather than reliance on a single procedure."*
>
> *Frankland et al. (1990)*

The truth contained in this statement also holds for litter fungi. Although much valuable work has been undertaken in the study of litter fungi, any inventory of fungi is likely to represent an incomplete list, biased in respect of some physiological feature selected for by the method (e.g. Swift 1976). In addition, developing 'soundly based sampling protocols' for studies of microfungal diversity and ecology remains challenging for two reasons. Firstly, data are still lacking with respect to factors that may affect estimates of microfungal diversity. Secondly, the replication and number of samples required to satisfy statistical validity, in particular in the face of great variability between samples, often exceeds the number that can be handled adequately (Swift, 1976). Despite these difficulties and limitations, a number of studies combining two or more methods have extracted valuable information about microfungal ecology and have set a benchmark in overcoming the specific challenges that the study of fungal life forms pose (e.g. Kendrick and Burgess, 1962; Frankland, 1966; Christensen, 1968).

A number of methods are used to detect or isolate fungi, and these can be broadly subdivided into direct or indirect approaches (Chapter 1; Booth, 1971). The direct method has been applied extensively, in particular for diversity studies with a strong taxonomic component (e.g. Fröhlich, 1997; Taylor, 1997; Whitton, 1999). This chapter will be mainly concerned with an indirect approach of estimating microfungal diversity. Indirect methods, such as soil dilution plating, have been criticised because the origin of

mycelial colony forming units (e.g. hyphae or spores) cannot be determined (Barron, 1971; Swift, 1976; Lussenhop, 1981). This means that isolates arising from inactive fungal propagules on the surface of leaf litter may provide an exaggerated and erroneous impression of the diversity of fungi associated with leaf decay (Barron, 1971). Methods such as serial washing combined with particle filtration may remove a major proportion of inactive fungal spores from the leaf surface (Kirby and Webster, 1990). This assumption was previously investigated in an aquatic plant, *Ranunculus penicillatus* var. *calcareus,* Kirby (1987) but has not been examined for substrata such as rainforest leaves.

Other factors that may affect estimates of fungal diversity obtained by particle filtration include leaf storage and isolation media. Both the conditions under which leaves are stored and the storage period have been shown to influence the survival of fungal hyphae and propagules in selected groups of fungi (Hong et al., 2001). Therefore, freshly collected substrata should be used, whenever possible, to reduce distortion of the diversity data. In some situations, however, this may be impractical. For example, distant study sites, or the need to compare substrata collected within the same time frame combined with the labour-intensive nature of isolating fungi, may prohibit the use of fresh material (e.g. Bills and Polishook 1994a). Therefore, an assessment of the effect of leaf storage was undertaken.

With indirect methods of isolation, a major filter for fungal diversity is the selectivity of isolation media (Swift, 1976). Even so-called nonselective, nutritionally enriched media such as malt or potato dextrose agar are in reality highly selective for the recovery of species with a robust and rapid radial growth (Bills, 1995). In contrast, low nutrient media or those containing sublethal doses of fungitoxic or colony restricting agents can effectively suppress rapidly growing fungi, but these media may not meet the nutritional requirements for initiating colonies of slower growing species (Bills, 1995). A systematic assessment of the effect of media type on the recovery of fungal diversity, however, has not been undertaken (Bills, 1995).

Achieving an adequate sample size is difficult in microfungal studies but increases the robustness and interpretative power, in particular in answering ecological questions. A number of methods have been devised to estimate an adequate sample size for macro-organisms, for example observing an asymptote in a species accumulation curve (Magurran, 1988) or observing a decrease in standard error with increasing sample size

(Underwood, 1997). These methods may be of limited value for species rich groups, such as microfungi, due to the distribution of their sample populations (e.g. Hughes et al., 2001; Ugland et al., 2003). For this reason, Polishook et al. (1996) suggested that sample size estimation using complementarity between samples (Colwell and Coddington, 1994) may provide a better tool. In this chapter, an initial survey of the structure of microfungal assemblages as detected by particle filtration is provided. The issue of estimating adequate sample sizes will be developed further in subsequent chapters.

Aims

The major aims of this chapter are

- to assess whether particle filtration is a viable method for diversity estimation
- to assess the effect of dry leaf storage over a period of four weeks
- to determine a method that will reliably isolate interior leaf colonisers in decaying rainforest leaves while largely excluding external colonisers and inactive propagules on the leaf surface
- to assess the effect of isolation media on the observed morphotype frequency

A general discussion of the observed diversity and structure of microfungal assemblages as well as advantages and limitations of this method is provided in the context of previous studies.

2.2 Materials and Methods

2.2.1 Collection of leaves

Intact leaves at early and moderate stages of decay were collected randomly from a 7 x 7 m quadrat under a single tree of *Neolitsea dealbata* (Lauraceae) in September and October 2000. These leaves are leathery, glabrous, and have a waxy cuticle that remains until the final stages of decay (Appendix A). The leaves were returned to the laboratory in sealed plastic bags in an airconditioned vehicle within three hours and were processed within 24 h unless otherwise stated.

2.2.2 Direct isolations

Sixteen decaying leaves (approximately 20 g) were incubated for six weeks in a moist chamber containing tissue paper moistened with sterile distilled water. Decaying leaves were observed approximately every third day under a stereomicroscope and semipermanent slides of fungal fruiting bodies were prepared in lactic acid. Fungi were identified to the lowest possible taxonomic level.

2.2.3 Particle filtration protocol

Leaves of *N. dealbata* were washed extensively to remove adhering soil particles and bryophytes and then air-dried at approximately 25 °C for eight hours. For each particle filtration, 5 g of leaves were selected to include both leaf lamina and midrib/petioles. These standardised samples were washed three times in 100 ml of sterile distilled water by agitating for two minutes each time in a 500 ml Schott bottle and a fourth time in 20 ml of sterile distilled water. Aliquots (0.1 ml) of the final wash water were plated out onto three plates for each of four isolation media (Kirby, 1987).

Each sample was processed using a particle filtration protocol (Bills and Polishook, 1994b). The leaves were homogenised in a sterile Waring blender at high speed in 100 ml of sterile distilled water for two minutes. Approximately 10 L distilled water were applied with pressure from an elevated tank via a hose to wash the particle suspension through three sterile stainless steel sieves (21 cm diameter, 0.5 mm mesh size). The two lowest sieves were lined with sterile polypropylene mesh (105 and 210 µm, Swiss Greens, Lab Supply, Australia). Leaf particles collected on the lowest mesh were suspended in 20 ml of sterile distilled water. Control plates were set up to detect spores in the laboratory atmosphere and in the distilled water.

Aliquots (0.1 ml) of leaf particle suspension were plated onto triplicate plates of four isolation media at a density of 40 and 80 particles per plate. Plates were incubated at room temperature (ca.23 °C) and checked daily over a period of three weeks. Leaf particles from which hyphae emerged were transferred to a Petri dish of potato dextrose agar (Merck). Isolates were incubated at room temperature (ca. 23 ºC) in the dark for three weeks and sorted into groups based on morphological characters such as hyphal and soluble pigments, shape of margin, colony surface textures and growth rates (Bills and Polishook, 1994a). These groups were rechecked after another four weeks.

Representative isolates of each morphotype were subcultured onto Potato Carrot Agar containing a 2 cm piece of wheat straw and a 2 cm^2 piece of *N. dealbata* leaf and incubated under black light for a 12 h photo period to induce sporulation (Booth, 1971). The majority of sporulating isolates were identified to genus and isolates of different species within the same genus, were designated species 1, species 2, etc. (Bills and Polishook, 1994a).

For the purpose of this discussion, each group of isolates defined either by a taxonomic identification or by colony morphology is termed a 'morphotype'. The terms 'common' and 'rare' morphotypes refer to the relative number of isolates among the morphotypes rather than their overall abundance in the natural environment (Bills and Polishook, 1994a).

2.2.4 Media

All media used for the isolation of fungal isolates were made up to 1 L with distilled water. Bandoni's medium contained 4 g L-sorbose, 0.5 g yeast extract, and 20 g agar (Bandoni, 1981). Malt yeast extract (MYE) agar contained 10 g malt extract, 2 g yeast extract, 20 g agar and 25 mg rose bengal (adapted from Dreyfuss 1986). Commercial cornmeal agar (CMA, Merck) was made up to manufacturer's instructions and rose bengal (25 mg L^{-1}) was added. An adapted medium based on dichloran rose bengal chloramphenicol agar ('DRBC'; Gams et al. 1998) contained 5 g yeast extract, 10 g dextrose, 1 g potassium phosphate, 0.5 g magnesium sulfate, and 25 mg rose bengal. After cooling, 50 mg L^{-1} chlortetracycline was added to the Bandoni's and MYA media; 50 mg L^{-1} streptomycin was added to CMA; and 50 mg L^{-1} streptomycin and 10 mg L^{-1}chloramphenicol were added to 'DRBC'.

2.2.5 Effect of leaf storage

Air-dried leaves of *N. dealbata* were subdivided into four 5 g samples and stored in sealed plastic bags at room temperature (ca.25 ºC) for one day (sample 1), seven days (sample 2), 21 days (sample 3) and 28 days (sample 4). To assess the effect of leaf storage on the recovery of fungal isolates, each sample was processed using the particle filtration method outlined above. Isolates were grouped into morphologically similar morphotypes.

2.2.6 Effect of surface treatments

Leaf samples were collected on two occasions in September and October 2000 as the labour intensive nature of isolations prohibited the simultaneous processing of all treatment and control leaves. Five intact air-dried leaves from the first collection were immersed in 96 % ethanol for 30 seconds, washed in distilled water for 30 seconds and then immersed in 33 % sodium hypochlorite (Sigma, final concentration 3.3 % aq. chlorine) for 5 minutes (Petrini and Dreyfuss, 1981). This treatment was designated 'EtOH/NaOCl'. For the treatment designated 'NaOCl', five leaves from the second collection were treated with 5 % sodium hypochlorite (Sigma, final concentration 0.5% aq. chlorine) for two minutes. Five leaves from each collection were immersed in sterile distilled water as a control group for the corresponding treatment. Following the treatments, all leaves were washed as outlined above and processed using the particle filtration protocol. Comparisons were made between the treated leaves and the corresponding control leaves from the same collection date.

2.2.7 Effect of isolation media

The effect of media on morphotype frequencies was assessed using data from a survey of leaf litter fungi in four tree species using particle filtration. This survey was undertaken during the wet season 2001 and details of study sites, survey design and hosts are available in Chapter 5.

2.2.8 Statistical analyses

2.2.8.1 Effect of leaf storage

The isolation rate was determined using the following equation:

$$\text{Isolation rate} = \frac{\text{Total number of particles yielding one or more isolates} \times 100}{\text{Total number of particles}}$$

Percent morphotype abundance is the number of isolates in a morphotype divided by the total number of isolates in a sample multiplied by 100. To compare fungal taxon similarity, the Jaccard Index was calculated pair-wise among samples based on the presence/absence of a species (Magurran, 1988; Polishook et al., 1996). The Kruskal-

Wallis Test tested for differences in sample populations (Mendenhall and Beaver, 1991). The cumulative number of morphotypes derived from four particle filtrations was plotted against the storage period in an accumulation curve (Magurran, 1988). Morphotypes that occur only once in the dataset are referred to as singletons in this project.

2.2.8.2 Effect of surface treatment of leaves

Fungal morphotypes arising from surface treated and control leaves were classified as belonging to one of three categories: from leaf particles only, from wash water only, or from both leaf particles and wash water. Chi square values were calculated separately for the number of morphotypes derived from leaf particles in each pair of treatment and control groups using a Goodness-of-Fit Test (Mendenhall and Beaver, 1991). A rarefaction index, $E_{(200)}$, was calculated for the complete data set (Hurlbert, 1971).

2.2.8.3 Effect of isolation media

Only fungal morphotypes with four or more isolates were included in the analyses. Morphotypes were grouped according to the isolation media from which they were isolated. A Kruskal-Wallis test was used to test for differences in morphotype frequencies arising from the four isolation media described above (SPSS, 2001).

2.3 Results

2.3.1 Effect of leaf storage

A total of 736 isolates in 112 morphotypes were isolated from four samples of *N. dealbata* leaf litter. The number of morphotypes ranged from 41 in sample 1, to 29 in sample 4 (Table 2.1). The percent abundance for the most common taxa decreased with increasing storage time from 38.7 % in sample 1, 21.2 % in sample 2, 21.0 % in sample 3 and 16.9 % in sample 4 (Fig. 2.1). The isolation rate was negatively correlated with storage time (R^2 = 0.977; Fig. 2.2) and declined from 54 % in sample 1, to 13 % in sample 4. The morphotype diversity of the four samples did not differ significantly in a Kruskal-Wallis Test (p=0.39). The rarefaction index $E_{(200)}$, i.e. the expected number of

taxa in a random sample of 200 isolates, was 54 for the combined data sets. Rarefaction indices, $E_{(65)}$ for each sample ranged from 16 in sample 1, to 29 in sample 4 (Table 2.1). Only between 3 and 5 morphotypes per sample had a percent abundance greater than 5 % of the total number of isolates. Unique isolates comprised 8.2 % of sample 1, 7.8 % of sample 2, 11.7 % of sample 3 and 21.5 % of sample 4 (Fig. 2.1). The Jaccard Index showed a low degree of similarity between samples, ranging from 0.14 between sample 1 and 2, to 0.04 between sample 1 and 4 (Table 2.2). The cumulative number of morphotypes isolated from *N. dealbata* leaves increased from 41 on day 1, to 112 after on day 28 (Fig. 2.3). No asymptote was evident after isolating 736 isolates in four particle filtrations but the accumulation curve levelled off when singletons were removed (Fig. 2.3).

Table 2.1 Numbers of isolates and actual versus expected numbers, (E65), of morphotypes derived from *Neolitsea dealbata* leaf litter after 1 to 28 days of storage

Sample	1	2	3	4
Storage time (days)	1	7	21	28
No. of isolates	292	217	162	65
No. of morphotypes	41	39	38	29
$E_{(65)}$	16	22	24	29

Table 2.2 Jaccard Index of similarity calculated pair-wise for each of four cohorts of isolates derived from *Neolitsea dealbata* leaf litter after storage from 1 to 28 days

Sample	1	2	3	4
1	-			
2	0.14	-		
3	0.10	0.08	-	
4	0.04	0.10	0.11	-

Figure 2.1 Frequency of isolates per morphotype derived from four *Neolitsea dealbata* leaf litter samples that had been dried and stored at ambient temperatures for a period of 1, 7, 21 and 28 days (samples 1 to 4)

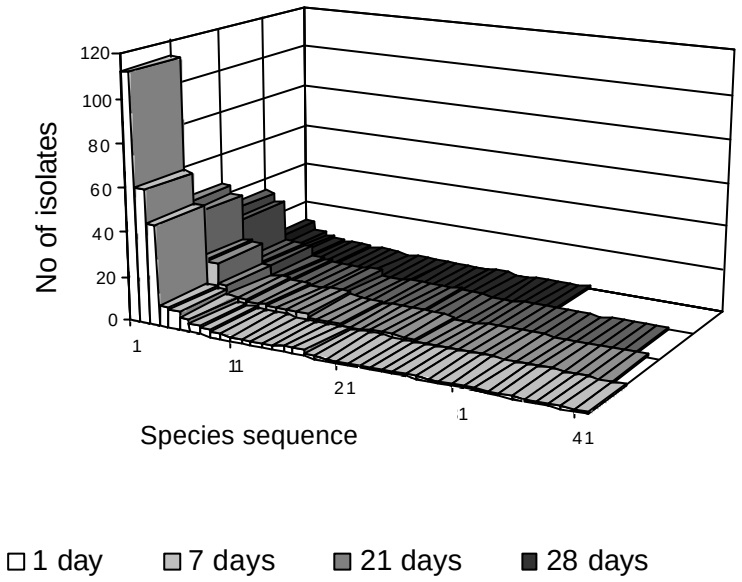


Figure 2.2 Scatterplot of isolation rate of leaf particles versus storage time (in days)

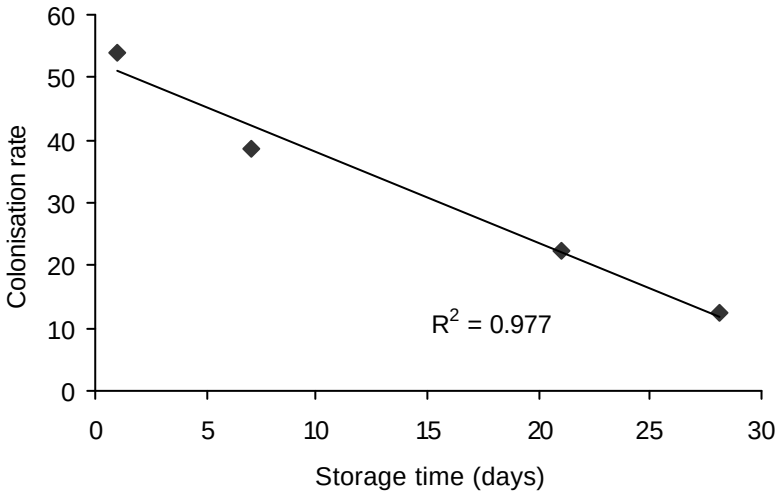
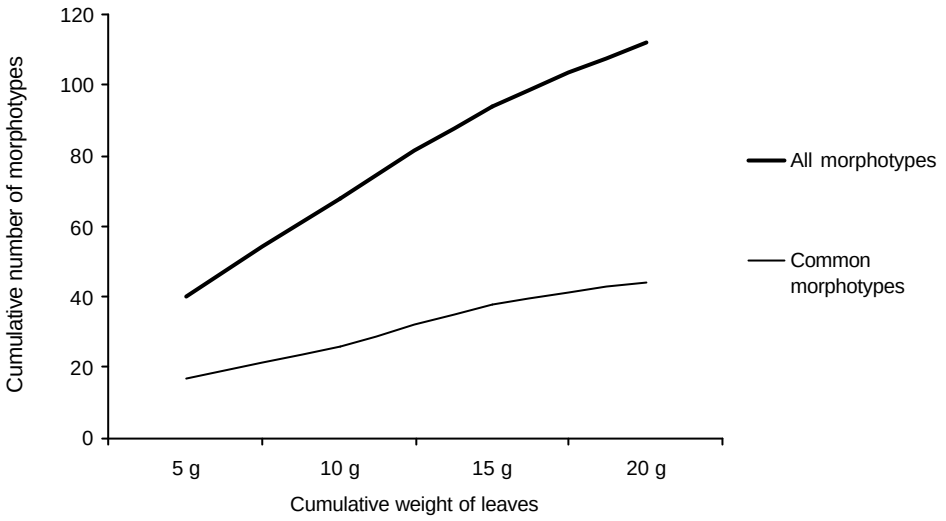


Figure 2.3 Cumulative number of all morphotypes and common morphotypes isolated from 5 g of decaying *Neolitsea dealbata* leaf litter after 1 to 28 days of dry storage



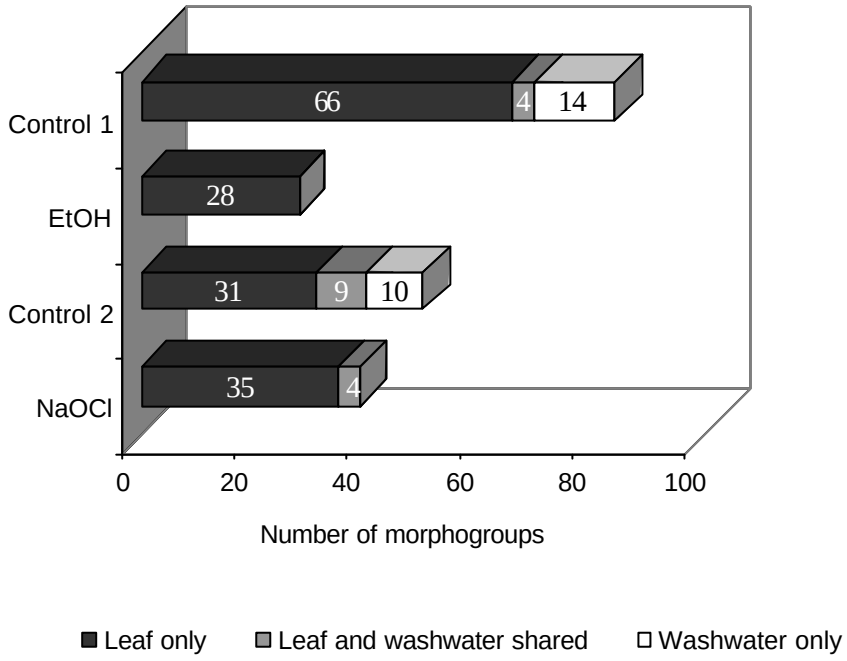
2.3.2 Effect of surface treatment

A total of 639 isolates belonging in 141 morphotypes were isolated from four leaf litter samples of *N. dealbata.* The number of isolates isolated was 154 for control leaves of the ethanol treatment and 178 for control leaves of the sodium hypochlorite treatment while the number of isolates isolated from treated leaves was 41 for the EtOH/NaOCl and 162 for the NaOCl treatment group (Table 2.3). The numbers of isolates isolated from wash water were 52 and 41 respectively for control groups. The wash water did not yield any isolates after EtOH/NaOCl treatment but eleven isolates in four morphotypes were isolated following the NaOCl treatment (Table 2.3). These taxa were also found in leaf particle isolations (Fig. 2.4).

Compared to the control group, the EtOH/NaOCl treatment significantly reduced the number of morphotypes recovered from leaf particles ($p<0.001$; Fig. 2.4). In contrast, the number of morphotypes isolated from NaOCl treated leaves was not significantly different from control leaves ($p=0.62$). A list of taxa, either identified to genus level if sporulating or placed in to morphologically similar groups if sterile, is provided in Appendix B. The rarefaction index, $E_{(200)}$, for the combined data set was 58. Of the 28 fungal taxa isolated by the direct method, 53.6 % were also isolated using the particle

filtration while 46.4 % taxa were identified only with the direct method (Appendix B). Mitosporic fungi (hyphomycetes 75.4 %, and coelomycetes 24.6 %) dominated the samples (Appendix B).

Figure 2.4 Morphotypes isolated from *N. dealbata* leaf litter, wash water and/or both from treatment and control groups. Black bar: morphotypes derived from leaf particles, grey bar: morphotypes derived from leaf particles and wash water; white bar: morphotypes derived from wash water only.



2.3.3 Effect of isolation media

A total of 89 morphotypes among 863 isolates were included in analyses; morphotypes with three or less isolates were excluded from analyses. On Bandoni's medium, 80 morphotypes were isolated, 70 on CMA, 67 on 'DRBC' and 60 on MYA. The differences in frequencies of morphotypes were not significant at the 5 % level ($p=0.09$) but significant at the 10 % level.

2.4 Discussion

2.4.1 A tool for estimating fungal diversity

A number of authors have suggested that the particle filtration protocol for the isolation of fungi from leaf litter and other substrata may prove useful in the assessment of fungal diversity in leaf litter and other substrata (Kirby and Webster, 1990; Bills and Polishook, 1994a). However, few studies have utilised this method, presumably because it is costly in media, consumables and labour. The most extensive work to date has been undertaken by Bills & Polishook (1994a, 1994b) and Polishook et al. (1996) who studied fungal diversity in a number of leaf litter types from Costa Rican and Puerto Rican rainforests.

In the present study, a total of 1365 isolates in 253 morphotypes were isolated from eight samples of *N. dealbata* leaf litter. There were few abundant taxa and a high proportion of taxa with unique or few isolates (Fig. 2.1). Similar distributions have been reported previously for both soil and leaf litter fungal assemblages studied by indirect methods (Christensen, 1989; Bills and Polishook, 1994a, 1994b; Polishook et al., 1996). Between three to five morphotypes per sample had abundances higher than 5 % and ranged from 16.9 % to 38.7 % for the most common taxon. This fell within the range of 12 % to 50 % reported by Bills & Polishook (1994a, 1994b) and Polishook et al. (1996). The most abundant species did not account for more than 7 % of total isolates in litter of *Ficus bengalensis* (Miriam and Bhat, 2000). Due to the great variability, abundance values of common taxa do not appear to be a good parameter for comparison of fungal communities. Therefore, similarity indices based on the most abundant species, such as the Morisita-Horn index (Magurran, 1988), are not appropriate for microfungal assemblages. In contrast, rarefaction indices were comparable to those reported from neotropical lowland rainforests (Bills and Polishook, 1994a, 1994b; Polishook et al., 1996). While rarefaction indices of mixed leaf litter samples were variable, i.e. 46 to 84 (Bills and Polishook, 1994a), those calculated from replicate particle filtrations of the same leaf type were more comparable, i.e. 40 to 59 in *Heliconia mariae* (Bills and Polishook, 1994b), 74 and 76 for *Guarea guidonia*, 58 and 60 for *Manilkara bidentata* (Polishook et al., 1996) and in the present study 54 and 58 for *N. dealbata*. These results indicate that particle filtration can provide consistent results and that species diversity in decaying leaves may differ between different leaf types.

The morphotype accumulation curve for samples 1 to 4 continued to increase without reaching an asymptote (Fig. 2.3). Therefore, it is likely that many more taxa remain to be discovered in leaf litter of the same tree (Ludwig and Reynolds, 1988). Similar results have been reported previously for microfungi in leaf litter and soil, where despite large sample sizes, the full extent of fungal diversity was not uncovered Christensen1989; Polishook et al., 1996). This observation led Christensen (1981) to conclude that the number of fungi in soils may be indeterminable. A variety of factors, for example the heterogeneity of substrata, the stage of leaf decay, the influence of microclimatic factors and chance may all contribute to the great diversity of microfungi and their patchy distribution (Kuter, 1986; Christensen, 1989; Lodge and Cantrell, 1995; Lodge, 1997). Nevertheless, the accumulation curve in the present study levelled off when singleton species were removed (Fig. 2.3). Depending on the question asked, rare species may be either excluded directly from analyses in ecological studies (e.g. Polishook et al., 1996) or indirectly through choice of similarity index (e.g. Christensen, 1968; Brown et al., 1998; Zhou and Hyde, 2002).

Typical soil taxa such as *Mucorales*, *Aspergillus*, *Geotrichum*, *Penicillium* and *Trichoderma*, that have been reported to be active in leaf litter decomposition in temperate regions (Kuter, 1986) were only found in isolations from wash water and were absent from leaf particles (Table 2.4). This observation agrees with that of Bills & Polishook (1994a) who only infrequently encountered taxa typically found in soil. It is unclear whether the discrepancy between temperate and tropical data sets is due to differences in isolation protocols or in mycotas.

All of the sporulating isolates were mitotic fungi (Table 2.4). Sporulating teleomorphs were not detected by particle filtration in this study (Table 2.4) but Bills & Polishook (1994a) recorded a small number of teleomorphs. In contrast, 16 of 57 (27.6 %) taxa detected on leaf litter from an Australian tropical rainforest using a direct isolation protocol were ascomycete teleomorphs (Parungao et al., 2002). Many fungi that produce a teleomorph stage in the natural environment were likely isolated in this study, but they may have preferentially produced an anamorph stage in culture or failed to sporulate entirely. Therefore, direct isolation approaches may be useful for augmenting indirect methods by providing more information about teleomorph stages.

I recorded 28 taxa using a direct approach (Table 2.4) as compared to 112 morphotaxa using particle filtration. These results from direct isolations are comparable to those of

Polishook et al. (1996) who isolated 24 species. The direct method appears to consistently underestimate fungal diversity in comparison to indirect isolations (Polishook et al., 1996; Miriam and Bhat, 2000), presumably because the latter method does not rely on the observation of fruiting structures. The effect of sampling intensity on diversity estimates for the direct method will be considered in later chapters.

Identification of sterile isolates remains a challenge. Of 141 morphotypes isolated for the assessment of surface treatments, 75 did not sporulate within the study period (Table 2.4). Identifications using DNA sequence comparisons are becoming routine (Horton and Bruns, 2001; Schadt et al., 2001). However, this approach was not applied in the present study because it was anticipated that species identifications would be hampered by the relatively small number of DNA reference sequences currently deposited in molecular databases relative to the fungal diversity found in leaf litter (Guo et al., 2000; Horton and Bruns, 2001). Instead I grouped nonsporulating isolates on the basis of morphological characters, which is a commonly used method (e.g. Bills and Polishook, 1994a; Cornejo et al., 1994; Brown et al., 1998; Fröhlich et al., 2000).

Other issues still need to be addressed in designing sampling protocols for fungal diversity assessments. It has been suggested that many biological data sets are non-replicate assemblages of samples rather than true replicates (Lambshead and Platt, 1985). This was true for microfungal community data recorded in the present study, which showed a low degree of similarity in species composition between samples with the Jaccard Index (0.04 to 0.14). In order to approximate an adequate sample size and to avoid the problem of combining data from different communities, the most effective approach may be to maximise the information obtained from tightly defined samples of greatest similarity (Cannon, 1999).

2.4.2 Effect of leaf storage

While substrata are often stored before microbial analyses are undertaken (Heredia, 1993; Bills and Polishook, 1994a; Parungao et al., 2002), few researchers have examined the effect of storage on the recovery of isolates. Differences in substrata and isolation protocols among previous studies prevent a direct comparison and evaluation of the storage regimes. However, fungal populations were essentially unaffected by storage at -7 °C and -20°C in two separate studies of soil and litter fungi in Wisconsin

(Christensen, 1968; Kuter, 1986). This finding is not unexpected because fungi of this region are adapted to freezing for extended periods. How applicable this finding is to tropical microfungi is unknown, but Croan et al. (1999) found that tropical basidiomycetes that decay wood did not generally survive freezing. In the present study, I tested dry storage of *N. dealbata* leaves for four weeks at ambient temperatures (~23 °C) because such conditions most closely resemble those of the natural habitat during the dry season. Despite a high mean annual rainfall at the study site, decaying leaves are periodically exposed to desiccation for one month or longer during the dry season at the study site (Bureau of Meteorology, 2000).

The isolation rate from leaf particles was negatively correlated with increased storage time (Fig. 2.2). The percent abundance of the most common taxa decreased from 38.7 % after one day to 16.9 % after four weeks of storage, while the overall number of morphotypes remained essentially unaffected up to the third week (Fig. 2.1). This apparently disproportionate loss of isolates in more abundant versus rarer morphotypes may indicate that rarely isolated taxa may be present in the leaf in a physiologically less active or dormant state and may be more resistant to water stress (Cooke and Whipps, 1993). However, an alternative explanation cannot be ruled out. As discussed above, an asymptote was not reached in a morphotype accumulation curve (Fig. 2.3). Therefore, any decline in the number of morphotypes during the first three weeks of the study period may have been masked by an inadequate sample size.

The Jaccard Index indicated a low degree of similarity between samples (Table 2.2), which made it impossible to assess whether any taxa had been lost preferentially during storage. However, Johnston (1998) reported a significant reduction in the isolation of a dominant endophytic species, *Guignardia* sp., from living leaves of *Leptospermum scoparium* over even a short storage period. It is not entirely clear whether similar losses can be expected in leaf litter, where microfungal species may be better adapted to cope with strong variations in water potential compared to those in living leaves.

If leaf litter samples are to be stored, the ‘optimum’ storage time in relation to the sample size, the purpose of the study and the logistical constraints may need to be considered. Researchers also need to weigh whether changes in frequencies are acceptable for the purpose of their work. This may be the case if only presence/absence data are needed, as greater variation may be introduced by collecting leaves at different times than by dry storage (Fig. 2.4). If frequency data are vital for community analysis,

only fresh material should be utilised. In addition, the decline in isolation rates with storage time must be taken into account when planning an experiment in which a minimum number of isolates need to be isolated.

2.4.3 Effect of surface treatments

Surface sterilisation has been applied effectively to eliminate surface contaminants in the isolation of fungi from living leaves (Schultz et al., 1993), from bulky substrata such as seeds (Richardson, 1983), wood blocks (Coates and Rayner, 1985) and *Pinus* leaf litter (Kendrick and Burges, 1962). For spongy substrata such as lichens, those with a high surface to volume ratio such as the aquatic plant *Ranunculus penicillatus* or fine decomposed material, serial washing appears to be the most appropriate treatment (Kirby, 1987; Petrini et al., 1990). Unlike fragile substrata, many rainforest leaves of tropical Australia have a tough or leathery texture and retain a water-repellent waxy coating and a relatively intact cuticle until later stages of decay. Removing or reducing inactive propagules from the substratum surface prior to particle filtration provides a more accurate assessment of interior fungal colonizers (Kirby, 1987). Therefore, two surface treatments were tested on *N. dealbata* leaf litter. An optimal treatment for this purpose would remove or markedly reduce fungal propagules on the leaf surface (both propagules of surface colonizers and contaminating spores), while interior colonizers would remain unaffected. The effectiveness of this treatment could be confirmed as morphotypes found in wash water isolations would be reduced or eliminated, while the number of morphotypes in isolates arising from leaf particles would remain constant between treatment and control groups (Kirby, 1987).

The surface sterilisation method using ethanol and sodium hypochlorite was effective in removing detectable propagules from the wash water fraction. However, it also markedly reduced the total number of isolates (Table 2.3) and significantly reduced the number of morphotypes (Fig. 2.4) derived from treated leaves. This method is, therefore, unsuitable. In contrast, treatment with sodium hypochlorite did not significantly affect the number of morphotypes isolated from leaves while eliminating morphotypes originating from the wash water alone (Fig. 2.4). While sodium hypochlorite may be useful as a pre-treatment for many rainforest leaf species with similar texture, validation of this method is advisable for leaves that are less resistant or have a different surface texture. Morphotypes observed in both wash water and leaf

particle isolations may indicate that sodium hypochlorite treatment did not remove all viable spores on the leaf surface. Alternatively, shared morphotypes may have arisen from hyphal fragments, which were liberated during the serial washing process, since serial washing is a vigorous process in which some disintegration of the substratum may occur (Kirby, 1987).

2.4.4 Effect of isolation media

Isolation media ideally restrict colony growth to prevent the overgrowth of isolation plates by rapidly growing fungi while supporting slower growing fungi (Booth, 1971; Grigorova and Norris, 1990; Bills, 1995). For studies seeking to recover a broad range of diversity, a combination of media is usually considered appropriate (Bills, 1995). Therefore, media were selected to reflect different means of restricting colony growth (Bills and Polishook, 1994a). In Bandoni's medium, the main carbon source is L-sorbose, a monosaccharide hexose, which cannot be readily utilised by most fungi and thus retards fungal growth (Trinci and Collinge, 1973; Liu and Chen, 2002). Two media contained rose bengal, which reduces bacterial contamination and restricts the growth of rapid growing fungi by acidifying the medium (Booth, 1971). Cornmeal agar contained no inhibitors and has a carbon source that is readily utilised by fungi (Booth, 1971). Thus it served as a 'control' medium. The use of cyclosporine A, a fungitoxic compound, was considered (Dreyfuss, 1986) but rejected on the basis of its high cost for a large study such as this project.

Although differences in morphotype frequencies were observed, these were not statistically significant for the morphotypes considered in this analysis, at least at the 5 % level of significance. This would suggest that ecological studies could utilise only one isolation medium without undue loss of information. It is not possible to exclude that rarer and more fastidious species may have been affected by the type of isolation medium used on the basis of this study, and therefore, the cost of using several isolation media needs to be weighed against recovering a potentially broader diversity of microfungi. For that reason, all particle filtrations were carried out with four isolation media as outlined in the following chapters.

2.5 Summary and recommendations

During this study, particle filtration applied to leaves of *Neolitsea dealbata* has yielded consistent results that are comparable with those of previous studies. Two reasons are apparent as to why a combination of methods rather than particle filtration or direct observations alone is a more useful approach. Firstly, a high percentage of isolates did not sporulate and could therefore not be identified. While assessment of diversity using morphotypes is widely accepted for non-sporulating isolates, in particular among endophytes (e.g. Arnold et al., 2000, 2001), this approach makes comparisons difficult between seasons and between studies. Secondly, the cohort of non-sporulating isolates may hide a vast and interesting diversity of fungi that can only be accessed through expensive and time-consuming molecular methods. This is evidenced by the absence of teleomorphs among isolates in the present study compared to the high percentage isolated by the direct method. Nevertheless, the morphotype richness observed by particle filtration was much greater than that observed by the direct method. Particle filtration will also provide a useful means of cross checking results of ecological studies obtained the direct method.

The particle filtration method seems to be effective in removing contaminating propagules on the leaf surface, as 'high sporulators' abundant in soils were absent from sample populations. Propagules on the leaf surface can be further reduced but not eliminated, with a surface treatment using sodium hypochlorite. Dry storage of leaves affects the diversity and structure of microfungal assemblages. However, storage of leaves for periods up to three weeks may introduce less of a bias to diversity estimates than analysing microfungal diversity from different collections. Since the effect of isolation media on morphotype frequencies was significant at the 10 % level, the cost of using a number of isolation media is justifiable. The effect of media on rare morphotypes was not assessed in this study.

The structure of microfungal assemblages with many rare species and a few species with higher but fluctuating abundances requires consideration in planning sampling protocols and analyses. Estimation of an adequate sample size will require methods other than those generally applied in the ecology of macro-organisms. However, achieving an adequate sample size may not be impossible as the accumulation curve levelled off after singleton species were removed from datasets. The structure of microfungal assemblages will also need to be considered when analysing microfungal

datasets in ecological studies. For example, similarity indices, which are based on the most abundant species such as the Morisita-Horn index, may be inappropriate while those, which eliminate singleton species such as the Bray-Curtis distance, may be better suited. In view of the high variability of samples, adequate replication is particularly important to make results of ecological studies meaningful. The major challenge in planning sampling protocols is balancing an adequate level of replication against available time and funding. Despite the limitations that were identified, particle filtration provides a valuable tool for diversity estimates and for cross checking results of direct observations.

CHAPTER THREE

Successional Patterns of Microfungi in Fallen Leaves of *Ficus pleurocarpa* (Moraceae)

3.1 Introduction

The term 'fungal succession' describes the replacement of fungi over time at different scales, both at the level of ecosystems (macroscale) and of substrata (microscale; Suzuki, 2002). Fungal successions on plant substrata have been well-documented and a discussion of definitions and hypotheses have been provided in a number of reviews (Park, 1968; Rayner and Todd, 1979; Frankland, 1981; Dix and Webster, 1985; Neville and Webster, 1995; Frankland, 1998; Fryar, 2002). In addition, a recent volume of Fungal Diversity (vol. 10, 2002) was solely devoted to the topic of fungal succession. Numerous early succession studies were undertaken in temperate regions of the Northern Hemisphere (e.g. Webster 1956, 1957; Kendrick and Burgess 1962; Frankland 1966; Hudson 1968; Kuter 1986) and to lesser extent in tropical and subtropical regions (Hudson, 1962; Meredith, 1962; Sandhu and Sidhu, 1980). Recent years have seen a renewed interest in studying fungal succession, particularly in the subtropics and tropics (e.g. Promputtha et al., 2002; Sivichai et al., 2002; Somrithipol et al., 2002; Tokumasu and Aoiki, 2002; Yanna et al., 2002; Zhou and Hyde, 2002) and in Japan (Osono, 2002; Suzuki et al., 2002; Tokumasu and Aoiki, 2002).

The results and conclusions of succession studies depend in part on the selected isolation method. For example studies, which used a direct observational method, are limited to describing the sequence of fungal sporulation while indirect methods also provide information about the replacement of fungal mycelium within the substratum (Fryar, 2002). The majority of fungal succession studies to date have taken a synecological approach, recording species assemblages at different stages of decay. Because of their detailed nature, succession studies can potentially describe a major portion of the microfungal diversity found on a particular substratum and generate hypotheses with respect to fungal succession and other aspects of fungal ecology.

Aims

The major aims of this chapter are

- to assess both the sequence of fungal sporulation and the replacement of fungal mycelium of saprobic microfungal assemblages with advancing decay in leaf litter of one tree species, *Ficus pleurocarpa*
- to assess the ability of fungi present at early stages of decomposition to recolonise sterilised leaves

3.2 MATERIAL AND METHODS

3.2.1 Succession study

3.2.1.1 Substratum

The tropical rainforests of Australia accommodate 32 species of *Ficus*, which belong in the pantropical family *Moraceae* (Hyland and Whiffin, 1993). The strangler fig *Ficus pleurocarpa* was selected for this study because the leaves of this species provide a good substratum for microfungi. Typically, these leaves are large (160-280 × 70-110 mm) and have a thick, leathery lamina with a prominent midrib and a long, robust petiole (Appendix A). The midribs and petioles of attached and freshly fallen leaves produce a white, milky exudate (latex). Freshly fallen leaves are usually yellow/green or yellow and have a fresh abscission scar while green leaves are encountered only infrequently on the forest floor. This strangler fig is endemic to an area between Cape Tribulation and Tully, North East Queensland, and occurs in well developed rainforest at an altitudinal range from near sea level to 1000 m (Hyland and Whiffin, 1993).

3.2.1.2 Research design

As few individual *F. pleurocarpa* trees were present in the study area, it was impractical to randomise the selection process of trees and, therefore, five trees of *F. pleurocarpa* were selected on the basis of adequate leaf fall. One hundred and twenty green/yellow or yellow leaves with relatively fresh abscission scars were collected on 6 January 2002 (wet season). Twenty-four leaves were placed under each of five trees and secured to a tent peg with nylon string. Half of the leaves were placed with the abaxial and half with

the adaxial surface in contact with the soil. As a pilot study had shown that the nylon string was frequently chewed, presumably by rats, plastic mesh (15 × 15 mm grid) was laid over groups of leaves and secured with tent pegs. Ten leaves (two per tree) were removed on day 0, 7, 14, 30, 46, 62, 78 and 94, and transported in plastic bags to the laboratory within four hours. Collections were abandoned after approximately three months when 80 leaves had been examined as leaves had disintegrated to such an extent that fragments could not readily be identified as belonging to study leaves. Ten green leaves were also collected across the first six visits (n=10). To assess to what extent microfungal communities varied in the short term, ten freshly fallen leaves were collected two weeks after the initial collection and microfungal species richness and composition was evaluated as outlined below.

3.2.1.3 Direct isolations

All leaves were incubated in separate humid chambers containing tissue paper moistened with sterile distilled water. To ensure that a large proportion of fungi fruiting on the leaves was captured, leaves were initially observed after two days (Hudson, 1968) and again after a longer period of incubation (Hering, 1965), i.e. between 7 and 17 days. As my aim was to maximise detection of fungi on leaves, a 15 × 15 mm sampling grid was applied. A slide was prepared for one representative fruiting structure of each morphologically distinct fungal entity in each quadrat. Slides were rendered semipermanent by addition of 90 % lactic acid and identifications were completed once all leaves had been examined. Herbarium specimens were prepared for each taxon by removing a section of leaf with a scalpel blade and drying it at 37°C for three days. Single spore isolations were undertaken for selected taxa (Booth, 1971). Fungal specimens of taxa, which were recognised as new to science were deposited at the Queensland Plant Pathology Herbarium (BRIP), Department of Primary Industries.

3.2.1.4 Indirect isolations

Leaves collected on day 0, 46 and 94 were also analysed within 48 hours of collection using replicate particle filtrations after leaves were surface treated as outlined in Chapter 2. Isolates were sorted into morphotypes as outlined in Chapter 2. To induce sporulation, representative isolates of each morphotype were subcultured onto Potato

Carrot Agar (PCA, Johnston and Booth, 1983) containing a 0.02 m^2 piece of banana leaf and incubated for a 12 h photo period under black light (Booth, 1971). The majority of sporulating isolates were identified to genus and isolates of different species within the same genus, were designated species 1, species 2, etc. (Bills and Polishook, 1994a).

A standard isolation protocol, which had been described for the isolation of endophytic fungi, was applied to ten green leaves collected during the first six visits (Petrini and Dreyfuss, 1981). Green leaves were surface sterilised (96 % ethanol for 30 seconds, 33 % sodium hypochlorite for 5 minutes, four washes with sterile distilled water; Petrini and Dreyfuss 1981). Ten squares (5 × 5 mm) were cut from each of ten leaves with a sterile scalpel blade and placed on replicate plates of Cornmeal Agar (Merck) containing streptomycin 50 mg L^{-1}. Petri dishes were checked daily and emerging hyphae were subcultured onto MYA. Isolates were treated as described above.

3.2.1.5 Statistical analyses and definitions

For the direct method, fungal species were recorded as either present or absent for each leaf, as it was not possible to determine whether fruiting structures occurring in different quadrats on the same leaf were produced by the same mycelium. The number of leaves on which a particular fungal species was found was then designated the 'occurrence of a fungus' and was used to calculate the 'percent abundance' of a species during each time period using the following formula (Yanna et al., 2002):

$$\text{Percent abundance} = \frac{\text{occurrence of taxon A} \times 100}{\text{occurrence of all taxa}}$$

The 'percent abundance' was also assessed for morphotypes isolated by particle filtration. The term 'dominant species' refers to species with percent abundances over 5 percent and is not intended to express area coverage of a mycelium on individual leaves.

Shannon's diversity index (H') was used to express the species richness of microfungal assemblages at each time point (Magurran, 1988).

$$H' = -S\, p_i \ln p_i$$

where p_i denotes the number of isolates or the occurrence of fungi for a fungal species divided by the total number of isolates or occurrences. The relationship between Shannon's diversity indices and the number of days that leaves had been on the ground was assessed for direct data only using a Pearson's correlation analysis as not enough data points were available from the particle filtration study (SPSS 2001).

The relative similarity of microfungal assemblages from leaves at different stages of decay identified by the direct method was visualised in two-dimensional space by Non-metric Multidimensional Scaling (NMDS; SPSS 2001). This ordination was chosen because the data did not conform to the assumptions of normality, linearity and similar variance of data and independence of variables, which need to be met for most other ordination methods (Shepard, 1962; Kruskal, 1964; Clarke and Warwick, 1994). Calculations were based on a matrix of Bray-Curtis distances (Bray and Curtis, 1957; SPSS, 2001) as this measure has been shown to have a robust monotonic and linear relationship with ecological distance (Bray and Curtis, 1957; Faith et al., 1987).

$$\text{Bray-Curtis index} = \left[\frac{S \; | y_{ij} - y_{ik} |}{S \; (y_{ij} + y_{ik})} \right]$$

where y denotes the abundance of species *i* in collections *j* and *k* respectively.

3.2.2 Recolonisation of leaves

A second study was undertaken to assess colonisation patterns in freshly fallen leaves. Twenty leaves were collected on 6 August 2002 (dry season). Of these, ten leaves were sterilised with ethylene oxide (EtO) at ambient temperature by RA Dibbs & Sons Pty Ltd, Brisbane, Queensland, and ten were stored as controls at room temperature. To assess the effectiveness of sterilisation, two leaf sections (5 × 5 mm) were removed from each sterilised and control leaves and placed on corn meal agar. Plates were checked after 1 and 6 weeks for growth of mycelia. Three days after collection, all leaves were placed randomly under one *F. pleurocarpa* tree and secured as described above. The leaves were collected after 14 days exposure on the rainforest floor and

microfungi were observed directly after a period of incubation in humid chambers as outlined above.

3.3 RESULTS

3.3.1 Succession study

A total of 105 taxa were recorded among 629 occurrences over a period of 94 days using a direct observational method (Appendix C). When known anamorph-teleomorph connections were taken into account, 104 species were recorded. Other potential anamorph-teleomorph connections such as *Guignardia* and a *Leptodothierella* state and *Chaetosphaeria* and *Dictyochaeta* have not been confirmed. Four replicate particle filtrations yielded 562 isolates of which 265 (47 %) sporulated. These belonged to 53 taxa (Appendix D; Fig. 3.1). The remaining 297 isolates were sterile and were assigned to 100 morphotypes. No attempt was made to determine the equivalency of sterile isolates from different isolation periods.

Figure 3.1 Percent abundance of sporulating microfungi observed in green leaves (GL) and in leaves of *Ficus pleurocarpa* collected on day 0, day 46 and day 94 using a particle filtration protocol.

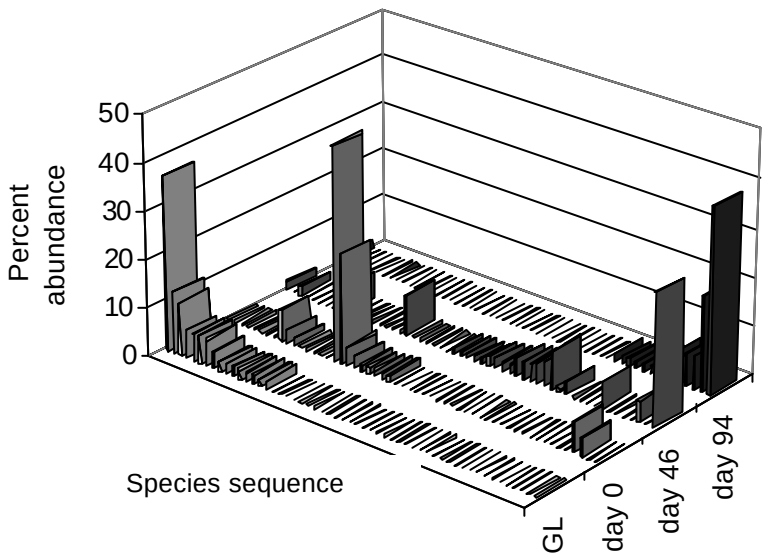


Table 3.1 Number of species, total occurrence and Shannon's diversity indices for direct isolations and number of morphotypes, number of isolates and Shannon's diversity index for indirect isolations

	Sampling time	GL [a]	d 0	d 7	d 14	d 30	d 46	d 62	d 78	d 94
Direct	No. of species	21	33	39	28	23	31	20	23	13
	Total occurrence	66	111	109	79	59	65	60	56	27
	H' [b]	2.74	3.12	3.43	3.00	2.83	3.16	2.64	2.84	2.36
	Fisher's alpha		16.6	22.3	12.7	14.8	19.0	8.0	12.7	7.9
Indirect	No. of morphotypes	38	23				54			52
	No. of isolates	109	146				169			138
	H' [b]	3.11	2.37				3.34			3.20
	Fisher's alpha									

[a] GL = green leaves; data not directly comparable due to differences in sampling and isolation protocols

[b] H' denotes the Shannon-Wiener index

Different taxa dominated in early, intermediate and late periods (Figs. 3.2, 3.3 and 3.4). Early species included *Colletotrichum* sp. 1 & 2, a *Gaeumannomyces*-like ascomycete, *Meliola* sp., *Pestalotiopsis* cf. *breviseta*, *Phomopsis* sp. 1, *Zygosporium echinosporum* and *Z. mansonii*, an unidentified ascomycete (F450) and the anamorph of a Dermateaceae F472, which represents a genus new to science (paper in prep.). Some of these early species sporulated up to day 14 and a small number up to day 30 and 46 (Fig. 3.2). Although the overall species numbers were high between day 7 and day 30 (Table 3.1), the group of dominant taxa was comparatively smaller (Fig. 3.3). These included *Volutella ramkurii, Selenodriella fertilis, Phomopsis* sp. 2, *Ophiognomonia elasticae, Lanceispora amphibia* and the teleomorph of Dermateaceae F472. Some species commenced sporulation as early as day 7 but did not become dominant until

later in the decay while others only formed fruiting bodies from day 62 onwards (Fig. 3.4). These included *Dactylaria ficusicola*, *Chaetospermum camelliae*, *Gliocladiopsis tenuis, Helicosporium griseum, Helicosporium* sp., *Speiropsis pedatospora*, an ascomycete in the Hysteriaceae and a *Xenogliocladiopsis*-like species. An *Asterina* sp. was present for much of the study period with fruiting structures deteriorating in the latter stages.

Figure 3.2 Percent abundance of microfungal species dominant in early stages of decay. Fungi were observed in green leaves (GL) of *Ficus pleurocarpa*, in freshly fallen leaves (day 0) and in leaves that had been exposed on the rainforest floor for up to 62 days

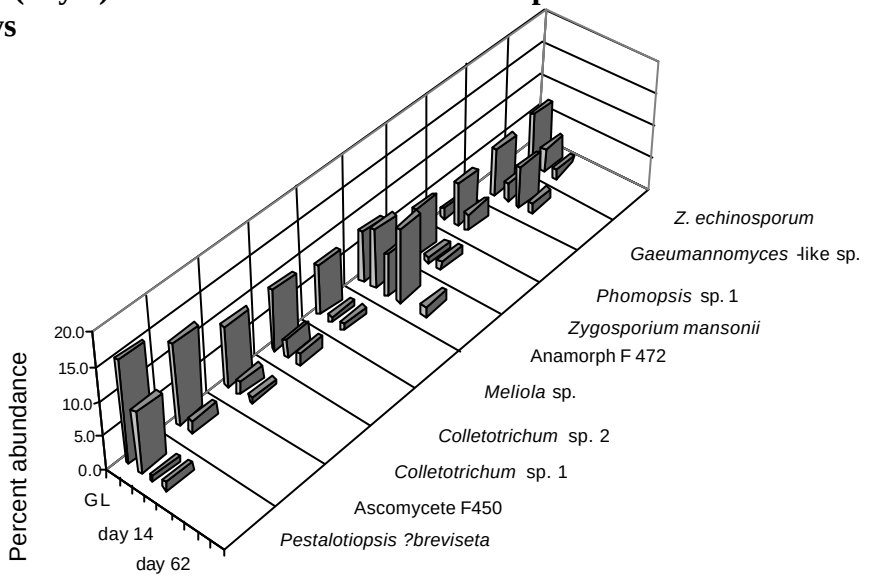


Figure 3.3 Percent abundance of microfungal species dominant in *Ficus pleurocarpa* leaves at the intermediate stages of decay. Leaves had been exposed on the rainforest floor between 7 and 94 days

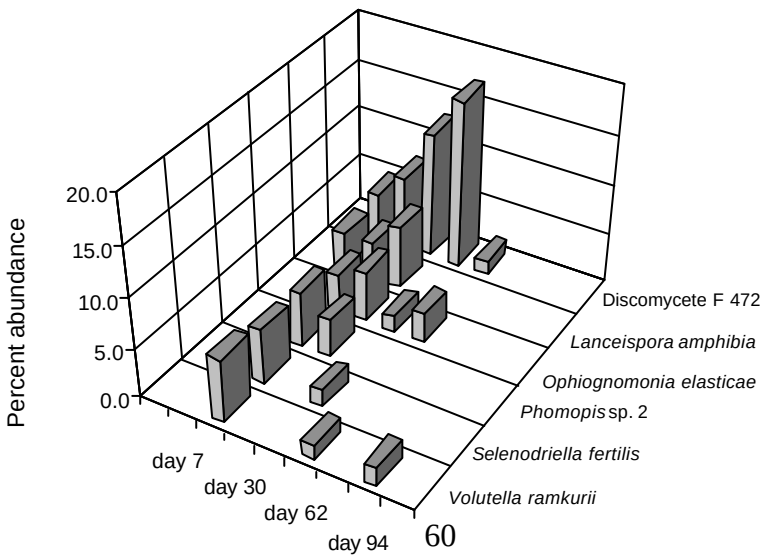
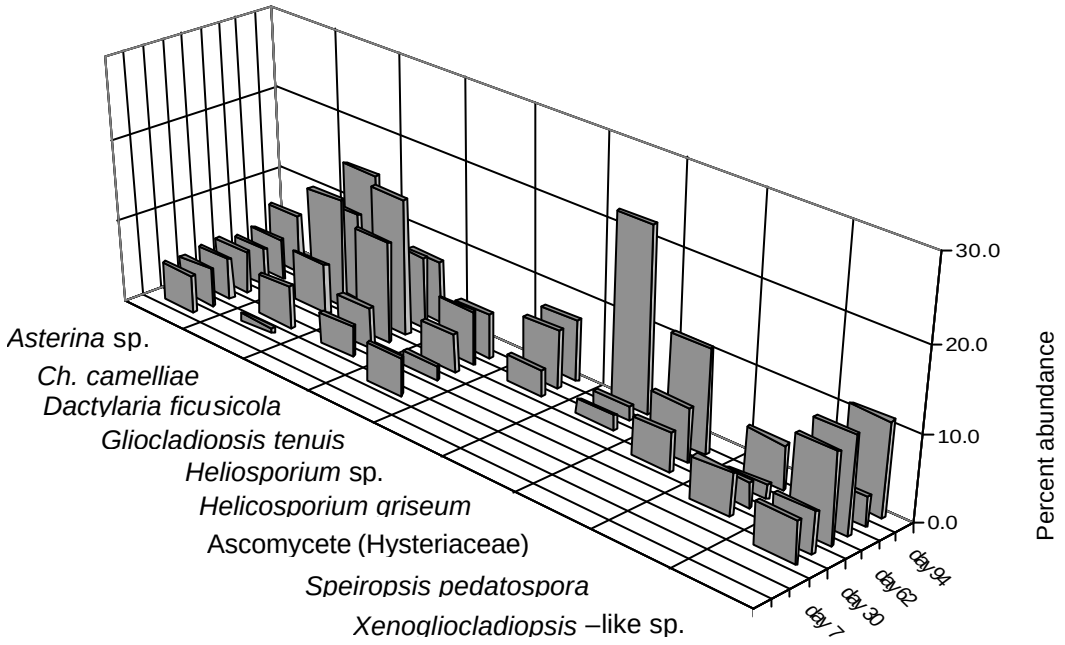
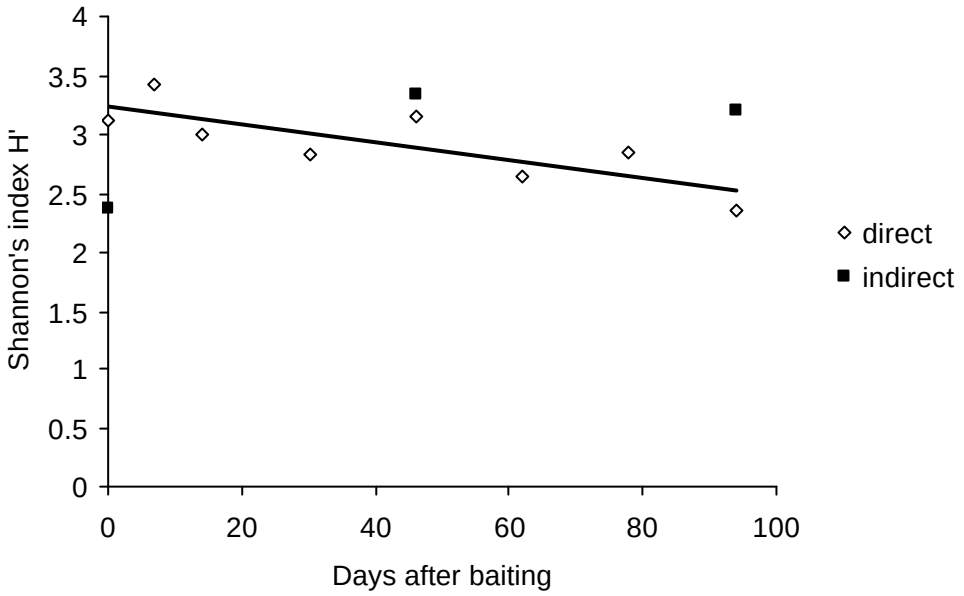


Figure 3.4 Percent abundance of microfungal species, which had become dominant in *Ficus pleurocarpa* leaves at later stages of decay. Leaves had been exposed on the rainformest floor between 7 and 94 days



Species numbers, occurrence and Shannon's diversity index (H') are presented in Table 3.1. Shannon's diversity indices and number of days on the ground showed a significant negative correlation (R^2 = 0.638, P = 0.009) for data collected by the direct method. A slight increase in Shannon's diversity index was detected with advancing decay for the particle filtration data (Fig. 3.5). Diversity measurements using Fisher's alpha mirrored these results. Due to the few available data points, it was not appropriate to undertake a correlation. In a two-dimensional NMDS ordination of data derived by direct observation, microfungal assemblages of the first 30 days were more distant from each other than those of later collections that encompass a similar time frame (Fig. 3.6). Day 46 to day 94 collections were the most similar. Microfungi in freshly fallen leaves that were collected two weeks after the initial collection showed the greatest similarity to collections from green leaves and day 0 leaves (Fig. 3.6). A low scatter in a Shepard's plot (not shown) and a very low stress value of 0.02 indicated that the ordination is a good representation of the data (Clarke and Warwick, 1994).

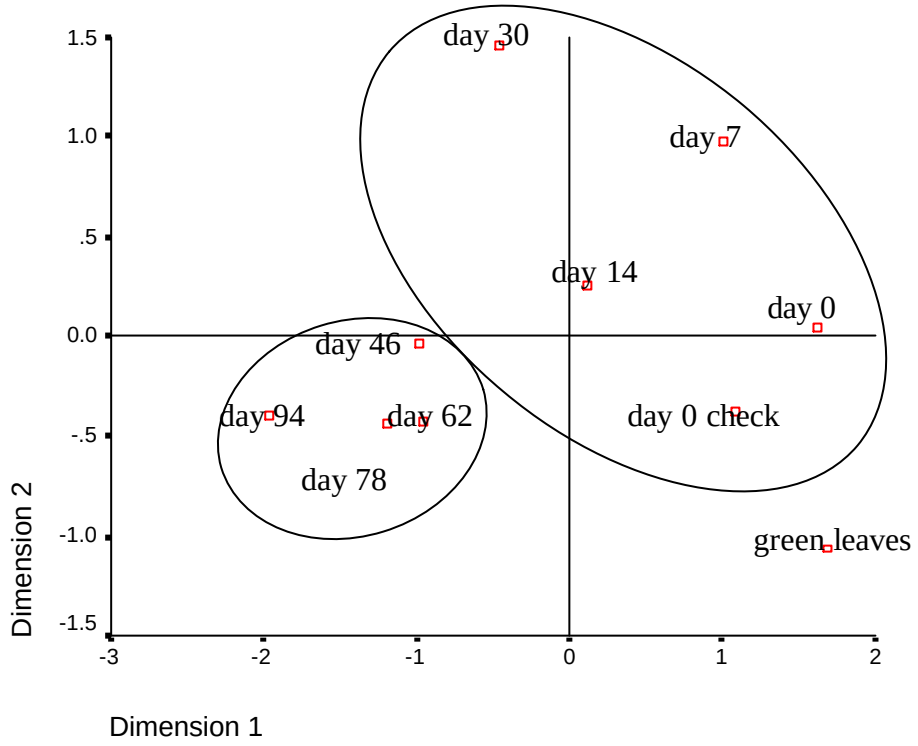
Figure 3.5 Shannon's diversity indices for microfungal assemblages on fallen leaves of *Ficus pleurocarpa* for direct and indirect isolation methods.



3.3.2 Recolonisation of leaves

Agar plates showed no fungal growth from sterilised leaves and an abundant mix of mycelia for control leaves, confirming the efficacy of EtO sterilization. From control leaves exposed on the forest floor for 14 days, twenty-four microfungal species were recorded compared to seven on sterilised leaves (Table 3.2). Two species were found on the majority of sterilised leaves, *Dictyochaeta* sp. and *Discostroma ficusicola*, and five other species were found at lower abundances. In contrast, control leaves accommodated a greater number of species with high, intermediate and low levels of relative abundance (Table 3.2). Among the dominant species, two were absent from sterilised leaves but present in control leaves; these are *Pestalotiopsis* cf *breviseta* and the anamorph and teleomorph of F472 (Table 3.2).

Figure 3.6 Ordination of Bray-Curtis distances between micro fungal assemblages on fallen leaves of *Ficus pleurocarpa* collected at different stages of decay.



3.4 DISCUSSION

A precise definition of fungal succession was provided by Rayner and Todd (1979) who termed it "the sequential occupation of the same site by thalli (normally mycelia) either of different fungi or of different associations of fungi". Many studies of microfungal substratum succession, including this study, fall short of demonstrating 'sequential occupation of the same site' as most methods are destructive (Fryar, 2002) and, unlike in large pieces of wood (Rayner and Todd, 1979), individual mycelia are not always clearly delimited in substrata such as leaves. In contrast, the definition of fungal succession as "a directional change in the composition, relative abundance and spatial pattern of species comprising communities" (Frankland, 1992) can encompass the observations of many succession studies and will therefore be applied in this chapter.

Table 3.2 Percent abundance of microfungal species on sterilised and control leaves of *Ficus pleurocarpa* after 14 days of incubation on the forest floor. The order of species corresponds with those depicted in Fig. 2.7 from most to least abundant species.

Species	Sterilised leaves (%)	Control leaves (%)
Discostroma ficusicola	90	80
Pestalotiopsis cf. *breviseta*		60
Beltraniella portoricensis	10	50
Chaetospermum camelliae	10	50
Dermateaceae F472		50
Acervular coelomycete		40
Dictyochaeta simplex	90	40
Beltrania africana		30
Beltrania rhombica		20
Gliocladium sp.		20
Minimidochium sp.		20
Ophiognomina elasticae		20
Phoma sp.		20
Sporidesmium-like sp.		20
Sphaeridium pilosum	30	20
Basidiomycete	10	10
Chalara sp.		10
Gliocladiopsis tenuis	10	10
Harpographium sp.		10
Idriella sp.		10
P*homopsis* sp.		10
Gaeumannomyces-like sp.		10
Verticillium sp.		10
Xenocladium-like sp.		10

Changes in microfungal species composition and relative abundance with advancing decay were observed with both direct and indirect methods in decaying leaves of *F. pleurocarpa* (Figs. 3.1, 3.2, 3.3 & 3.4). The species composition of the particle filtration cohorts in this study was distinct for each isolation (Appendix D; Fig. 3.1) suggesting that the observed changes in microfungal assemblages represent mycelial replacement and are true successive waves of invasion (Neville and Webster, 1995). While most dominant species isolated by the indirect methods were also detected by the direct method in this study, the relative abundances obtained by the two methods differed markedly in some instances (Appendix C and D). This discrepancy may represent sampling error (Ludwig and Reynolds, 1988; Henderson, 2003), but the work-intensive nature of isolating fungi by an indirect method prohibited more intensive sampling

within the scope of this study. Alternatively, the observed differences may reflect the fact that the sporulation patterns detected by the direct method may not match the extent of the mycelial development within the leaf lamina.

Changes in functional groups, which have been described for a number of fungal successions, include the replacement of parasitic and endophytic fungi by primary saprotrophs followed by a diverse group of secondary saprotrophs (Hudson, 1968; Frankland, 1998). Both endophytic and parasitic fungi have the advantage of colonising the leaf ahead of other species (Hudson, 1968; Neville and Webster, 1995) and some may be able to switch to a saprobic mode of nutrition once the leaf senesces (Cooke and Whipps, 1993). Primary saprotrophs include species with fast germination, rapid growth and high inoculum potential (Neville and Webster, 1995). After the initial stages of decay, fungal assemblages may be dominated by so-called secondary saprotrophs (Hudson, 1968; Frankland, 1998) and may undergo successive waves of invasion (Neville and Webster, 1995). Comparable patterns could be discerned from leaves of *F. pleurocarpa,* although decaying leaves of *F. pleurocarpa* lacked species commonly reported from the Northern Hemisphere such as *Alternaria, Aureobasidium, Cladosporium* and *Epicoccum* (Frankland, 1998). Fungi observed on freshly fallen leaves included opportunistic or obligate parasitic or endophytic genera such as *Colletotrichum* (anamorphic *Glomerella*), *Cylindrocladium, Meliola, Pestalotiopsis, Phomopsis* (Shivas and Hyde, 1997), some xylariaceous species (Rodrigues and Petrini, 1997) and a presumed endophytic fungus, Dermateaceae F472 (Chapter 6; Appendix C and D). Two species of *Zygosporium* and *Beltrania rhombica* (Appendix C and D) appear to be primary colonisers with rapid growth and high inoculum potential. Secondary saprotrophs were represented by a diverse assemblage, which reached dominance either at intermediate or later stages of decay (Appendix C and D; Fig 3.3 & 3.4).

Two models describe the changes in diversity of microfungi during succession (Dix and Webster, 1985; Neville and Webster, 1995). According to the first model, 'pioneer communities' have low species diversity while 'mature communities' are species rich. Eventually, species diversity may decline towards an 'impoverished community'(Dix and Webster, 1985). In contrast, Neville and Webster (1995) proposed that species diversity is richest and the number of 'individuals' greatest during earliest stages of colonisation and following a period of stability, diversity and total numbers begin to

decline. In the present study, I observed both patterns depending on the methodology applied. Direct isolation data showed a slow but steady decline in species numbers, occurrence, Shannon's and Fisher's alpha diversity indices with advancing decay (Table 3.1, Figs. 3.6 & 3.7) as predicted by Neville and Webster (1995). In contrast, diversity measures for the particle filtration cohort appeared to increase with advancing decay (Table 3.1, Fig. 3.6 & 3.7) thus mirroring the prediction of Dix and Webster (1985). Contradictory results have been also observed in previous studies. For example, Kuter (1986) noted a marked increase in species diversity with advancing leaf decay using an indirect isolation protocol, while Hogg and Hudson (1966) observed an increase in species abundance with a direct method and a decrease with an indirect isolation protocol. A satisfactory explanation for these observed differences is still lacking. From the observations of this current study, it can be hypothesised that with advancing decay leaves may accumulate greater numbers of propagules as earlier fungi may form dormant resting structures (Griffin, 1972; Neville and Webster, 1995); these would be detectable by an indirect method such as particle filtration. In contrast, only metabolically active mycelia producing fruiting structures would be observed by the direct method.

An ordination of microfungal assemblages by two-dimensional NMDS suggested that similarity of microfungal assemblages increased with advancing decay (Fig. 3.6). The greatest distance was observed between microfungal assemblages of collections up to day 30 while those of day 46 to day 94 leaves clustered most closely (Fig. 3.6). Increasing similarity of microfungal assemblages with advancing decay was also observed in bamboo baits (Zhou and Hyde, 2002) and in frond baits of *Phoenix hanceana* (Yanna et al., 2002). The underlying mechanisms for this trend are not known but it can be hypothesised that distinct, early microfungal assemblages may include species adapted to breaking down inhibitory compounds such as latex and alkaloids contained in plant tissues (Swenson et al., 1989; Isaac, 1992; Neville and Webster, 1995). Rapid degradation of these compounds may lead to equally rapid changes in fungal associations during early decay. However, it cannot be excluded that these rapid changes in species composition during early decay could be the result of competition in an environment of relatively high concentrations of readily accessible nutrients (Farrell, 1991). Under this model, the increasing similarity at later stages of decay could be related to those species reaching a state of equilibrium that are able to compete in

environment of reduced nutrients levels or at least of less accessible nutrients, for example those contained in lignin. The difference in species composition on freshly fallen leaves collected two weeks apart (Fig. 3.6) may indicate that other factors, for example microclimate or chance, may play an important role in the short term variability of microfungal assemblages. Further work is required to elucidate the relationship between enzymatic capabilities of early successional species and leaf chemistry and to determine the effect of factors such as competition, microclimate and chance on microfungal assemblages.

A comparison of species composition in freshly fallen sterilised and control leaves showed that two dominant species, *Pestalotiopsis* cf. *breviseta* and Dermateaceae F472, failed to recolonise leaves. Osono (2002) suggested that two early species in *Fagus crenata* were unable to recolonise sterilised leaves because they had a higher requirement for readily available, non-lignified energy sources, presumably only found in living and freshly fallen leaves. Whether this is the case or whether other life history characteristics, such as infection mode (Cooke and Whipps, 1993), play a role is not known. Irrespective of why these species have a low potential to invade dead leaves, it is possible that they undergo an endophytic or pathogenic phase during their life cycle. The species with the highest abundance in both sterilised and control leaves, *Discostroma ficusicola*, belongs to a genus, which has generally been considered to be biotrophic (Samuels and Blackwell, 2001). However, fruiting bodies of *Discostroma* have also been reported previously from decaying plant material (Müller and Loeffler, 1957; Brockmann, 1976; Barr, 1993). Further work is required to clarify whether biotrophy is a consistent characteristic of this genus or whether some species are strictly saprobic. Six species of common saprobes were also able to recolonise sterilised leaves; these include *Beltraniella portoricensis, Chaetospermum camelliae, Dictyochaeta simplex, Gliocladiopsis tenuis, Sphaeridium pilosum* and an unidentified basidiomycete. Overall species numbers were considerably lower for sterilised leaves. The reason for this observed difference is not clear but the absence of modifying effects of earlier fungi, such as *Pestalotiopsis* cf. *breviseta* and Dermateaceae F472, may inhibit colonisation by later species (Frankland, 1992, Neville and Webster, 1995). However, it cannot be excluded that some fungi were inhibited by minute amounts of residues such as glycols, chlorohydrin and bromohydrin, which may have formed following ethylene oxide sterilisation (Australia New Zealand Food Standards, 2003). Lower species

numbers in recolonised, sterilised leaves were also observed by Osono (2000) who utilised a heat sterilisation method. To reduce the possibility of the sterilisation method affecting species numbers in sterilised leaf litter, future studies might benefit from using gamma irradiation as the method of choice.

3.5 Summary

In this chapter, I have reported on the fungal diversity and distribution of microfungi on leaves of *F. pleurocarpa* during different stages of decomposition. The results of particle filtration suggest that observed changes in microfungal assemblages represent mycelial replacement. The replacement of putative functional groups detected by the direct observational method was similar to that described in previous studies from the Northern Hemisphere. At no stage was it apparent that the microfungal assemblages reached an equilibrium. An increase in diversity with advancing decay, which was observed in the particle filtration data, may be related to the isolation of fungal cultures from accumulated resting structures. In contrast, the decrease in fungal diversity with advancing decay observed by the direct method may be due to the fact that only metabolically active, sporulating taxa could be detected with this method. Greater distances between microfungal assemblages during early stages of decay may be related to the rapid degradation of inhibitory compounds, although a high species turnover related to competition in combination with changing resource availability could not be excluded. Two early, dominant species could not recolonise sterilised leaves; these may be taxa that undergo a biotrophic phase during their life cycle. Overall lower species numbers in sterilised leaves could be related to the absence of modifying effects of these species although inhibition by minute amounts of ethylene oxide by-products could not be excluded.

Investigating the ecology of microfungi, particularly those on leaf litter of tropical rainforest trees, can be daunting task due to the great number of species and the limitations of currently available methods to rapidly and reliably detect fungal diversity. The most effective approach may be to maximise the information obtained from tightly defined samples of greatest similarity using standardised methods (Cannon, 1999). The study of fungal succession meets this objective and has the potential to elucidate patterns and generate hypotheses regarding fungal ecology. In addition, the fine scale of

assessment yields data sets, which may serve as reference points for other studies (Chapter 4). Improving our understanding of the processes, which underlie fungal succession, and other aspects of fungal ecology, will require further studies that employ an autecological approach (e.g. Frankland 1998; Jones and Hyde 2002).

CHAPTER FOUR

The Diversity of Microfungi in Leaf Litter of an Australian Tropical Rainforest

4.1 Introduction

> *"Diversity is rather like an optical illusion. The more it is looked at, the less clearly defined it appears to be and viewing it from different angles can lead to different perceptions of what is involved."* (Magurran, 1988)

The high diversity of leaf litter fungi has been demonstrated in a wide range of substrata for example in monocotyledonous litter (Fröhlich, 1997; McKenzie, 1997; Taylor, 1997; Zhou and Hyde, 2002), in dicotyledonous litter (Hogg and Hudson, 1966; Kuter, 1986; Bills and Polishook, 1994a, 1994b; Polishook et al., 1996) and in litter from gymnosperms (Kendrick and Burgess, 1962; Tokumasu and Aoiki, 2002). However, its full extent is not well understood because estimating microfungal diversity is not a trivial task. Potentially a large number of species is encountered, many of which are not effectively characterised (Cannon, 1997). Therefore, exhaustive inventories are impractical and studies generally are based on samples of a finite number of observations from a finite number of habitats or microhabitats and the observed species numbers represent an estimate of the actual diversity present at a site (Rosenzweig et al., 2003). In addition, estimates of species richness differ with isolation methods and varying sampling effort (e.g. Bills and Polishook, 1994a). Although estimates of diversity may not describe the total diversity present, meaningful comparisons could be achieved across space and time if estimates were derived under tightly defined conditions (Coddington et al., 1990; Hughes et al., 2001). However, to date standardised sampling, isolation and estimation protocols are lacking (Cannon, 1997b, Coddington et al., 1990). These difficulties are exacerbated by the large range of indices and models that have been devised for measuring and estimating diversity (Chapter 1; Magurran 1988).

Aims

For the purpose of this chapter, diversity is primarily defined as species richness and abundance in one habitat. Differentiation diversity or the diversity in different substrata will be examined in Chapter 5 and taxonomic diversity in Chapter 7.

The major aims of this chapter are

- to provide estimates of microfungal diversity in leaf litter of selected tropical rainforest trees under tightly defined conditions that can serve as baseline data for leaf litter microfungi of the wet tropics and comparative datasets for future studies
- to assess patterns in microfungal diversity observed in different substrata, at different times, at different sites and with different isolation protocols
- to explore approaches to estimating microfungal diversity and their applications
- to provide recommendations with respect to findings relevant to diversity research.

To achieve these objectives, diversity information derived from two separate studies, i.e. a succession study (Chapter 3) and a substratum study (Chapter 5) will be discussed.

4.2 Methods

4.2.1 Survey design and isolation methods

Details of survey designs and isolation methods have been provided in Chapter 3 for the microfungal succession study in leaves of *Ficus pleurocarpa*. Only results of the direct method will be considered here. In short, the sampling regime for the succession study spanned over a period of three months. During this time, 80 individual leaves were examined using the direct method. Intensive subsampling at the microscopic level was undertaken using a grid system (Chapter 3).

The study assessing microfungal distributions in leaf litter of six tree species belonging in four plant families (Appendix A) will be referred to as 'substratum study'. Methodological details are discussed in Chapter 5. Four species formed the main frame work for the study (asterix) and two other species were less intensively surveyed.

- *Cryptocarya mackinnoniana* F. Muell. (Lauraceae) - rusty laurel *
- *Elaeocarpus angustifolius* Blume (Elaeocarpaceae) - blue quandong *
- *Ficus pleurocarpa* F. Muell. (Moraceae)- banana fig *
- *Ficus destruens* F. Muell. ex C.T. White (Moraceae) - rusty fig
- *Opisthiolepis heterophylla* L.S. Smith (Proteaceae) - blush silky oak *
- *Darlingia ferruginea* J.F. Bailey (Proteaceae). - hairy silky oak

The results of both direct observations and of the particle filtration will be considered for the substratum study. Thirty leaves per season (or a total of 60 leaves) of each selected tree species were examined for the direct method. Subsampling at the microscopic level was less intense than in the succession study with five 'microtransects' examined on both adaxial and abaxial leaf surfaces. For the particle filtration method, the same sampling scheme was used as for the direct method but sampling units were based on leaf mass. Methodological details of the particle filtration protocol are provided in Chapters 2 and 5.

4.2.2 Diversity estimation

In this project, 'surrogate measures' for abundance were utilised. For the direct method, fungal species were recorded as either present or absent for each leaf because it was not possible to determine whether fruiting structures occurring in different areas of the same leaf were produced by the same mycelium. The number of leaves on which a particular fungal species was found was then designated the 'occurrence of a fungus'. The numbers of isolates represented a measure of abundance for the particle filtration protocol. Rank abundance curves (Magurran, 1988) were generated for complete datasets of the succession study, the substratum study and for each individual tree species of the particle filtration study. For the succession study, a log frequency distribution of occurrences was generated to confirm the distribution by a second method. Curve estimations for species distributions were carried out in SPSS (SPSS, 2001).

Two indices were selected to reflect the observed diversity among samples. Fisher's alpha is an index that is frequently applied in plant ecology. This index was computed in the software program Ws2m (see below; Turner et al. 2000).

$$a = \frac{N(1-x)}{x}$$

where x is estimated from the iterative solution of

$$S/N = (1-x)/x[-\ln(1-x)]$$

For this index, 95 % confidence intervals were calculated from the variance provided by Taylor et al. (1976):

$$\mathrm{Var}(a) = a/-\ln(1-x)$$

The Shannon-Wiener index (Chapter 3; Pielou 1975) was included because it is among the most widely used (Magurran, 1988).

Evenness was estimated using the ratio of observed diversity to maximum diversity, calculated from the Shannon-Wiener index.

$$E = H'/H_{max} = H'/\ln S$$

Dominance indices, such as the Simpson's index, were not considered because microfungal datasets are not suited to those measures. For example, the abundances of dominant microfungal species may be strongly influenced by methodological factors, such as leaf storage (Chapter 2).

To estimate actual species numbers and sampling completeness, a variety of statistical tools are available (reviewed by Bunge and Fitzpatrick, 1993; Colwell and Coddington, 1994; Hughes et al., 2001). For presence/absence data obtained by the direct method, I selected the Chao2 estimator based on previous reports of its consistent performance in datasets, which have a high percentage of rare species (e.g. Chao, 1987; Colwell and Coddington, 1994; Hraber, 2001; Hughes et al., 2001). Two other non-parametric estimators, the incidence based coverage estimator (ICE; Lee and Chao 1994) and the jackknife procedure by Burnham and Overton (B & O; 1979), were also calculated to

assess how well species estimates agreed at maximum sample size. The related estimators, Chao1 (Chao, 1984) and the abundance based estimator (ACE; Chao & Lee 1992), were applied to particle filtration data because these estimators utilise abundance data. For the B & O jackknife procedure, the particle filtration data were transformed into presence/absence data. To facilitate comparisons with previously published data, a rarefaction index was also calculated for particle filtration data (Chapter 2; Hurlbert 1971).

Species estimates were also extrapolated from species accumulation curves using an additional estimator, a fitted Michaelis-Menten estimator (Holdridge et al., 1971; Turner et al., 2000). I have included this estimator because it has been shown to compensate well for heterogeneity of habitats at a biogeographical scale (Rosenzweig et al., 2003). While the scale differs greatly, microfungal data are often drawn from heterogenous habitats, such as leaves at different stages of decay (substratum and particle filtration study) or from different microhabitats and I was interested to compare the behaviour of this estimator with that of Chao2, ICE and B&O jackknife estimators. To assess the degree of convergence among estimators of the two groups (non-parametric estimators and those based on extrapolation), the difference between Chao2 estimates and those of other estimators was calculated as a percentage and expressed as mean ± 1 standard deviation.

All diversity estimates and species accumulation curves were generated with the computer program Ws2m (Turner et al., 2000). Individual data were randomised 100 times to remove habitat heterogeneity (shuffle individuals). The order of collections was also randomised 100 times to correct for unequal sample sizes (shuffle sample order). To estimate the number of species present in leaves rather than those in the actual dataset, I selected the setting: make samples exchangeable. As presence-absence data were used, incidences rather than abundances were randomised (shuffle incidences only). For calculations of ACE and ICE, the rare/infrequent cut-off was set at 5 occurrences. Accumulation curves for observed and estimated number of species were also generated in this program. Confidence intervals (C.I. 95 %) for Chao2 estimates were calculated manually (Chao et al., 1992).

To compare species richness of the substratum study with that observed by Parungoa et al. (2002), the observed species richness in ten leaves was assessed from randomised data generated by Ws2m. In addition, microfungal species estimates from leaves of *F*.

pleurocarpa were compared for the succession and the substratum study to assess the effect of subsampling regime on diversity estimates. Fisher's alpha and their corresponding 95 % C.I.s (Magurran, 1988) were calculated for a standard sample size of 30 leaves for both the succession and the substratum study; both datasets were randomised 100 times in the software program Ws2m (Turner et al., 2000). In addition, lognormal and log series curves were fitted in the same software program (Turner et al., 2000).

Finally, an estimate of sampling completeness was calculated for each study as follows:

$$\text{Estimated sampling completeness} = \frac{\text{observed species numbers}}{\text{estimated species numbers}} * 100$$

For the particle filtration data, an additional estimate of richness was calculated, based on Arnold et al.'s (2000) report that their morphotypes concept overestimated diversity by 17.1 % ±10.3 % in endophyte and litter fungi.

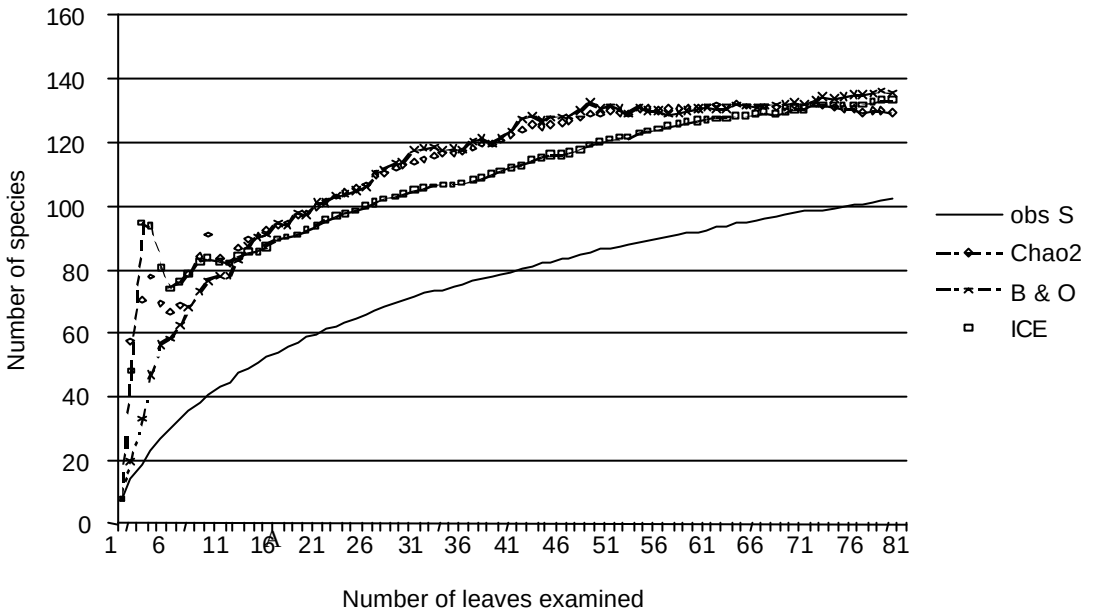
4.3 Results

4.3.1 Succession study

The succession study yielded a total of 105 taxa in 632 occurrences using a direct observational method (Table 4.1). When the single known anamorph-teleomorph connection was taken into account, 104 species were recorded. Other potential anamorph-teleomorph connections such as *Chaetosphaeria* sp. and *Dictyochaeta* sp. have not been confirmed. Fisher's alpha was 36 and Shannon's diversity index 4.02 with an evenness of 0.86 for the complete dataset. The estimated total number of species in the succession study was 130 using a Chao2 estimator, which indicates a sampling completeness of 80 % (Table 4.1). The estimates derived from the two other non-parametric estimators (ICE, B & O jackknife) differed from Chao2 estimates by an average of 3 % (± 2 %; Appendix E). The estimates derived from the Michaelis-Menten equation differed from Chao2 estimates by only one species or less than 1 % (Appendix E). The accumulation curve for observed species numbers did not level off but the species estimates generated with three non-parametric estimators converged and approximated an asymptote (Fig. 4.1). Individual collections of the succession study also reached an asymptote for Chao2 species estimates (Fig. 4.2). Both the rank

abundance graph and the log frequency distribution indicated that the species distribution approximates a logarithmic series (Figs. 4.3 and 4.4). This was confirmed by testing the fit of curves (lognormal p<001; logseries p<1.0). In a comparison of microfungal diversity on 30 leaves of the succession and the substratum study, Fisher's alpha was 32 (95 % C.I. 28-44) for the former and 21 (95 % C.I. 15-27) for the latter.

Figure 4.1 Observed (obs S) and estimated species richness of microfungi on *Ficus pleurocarpa* leaves as assessed by the direct method in the succession study. Estimated species richness is plotted for the Chao2 estimator, Burnham and Overton's jackknife estimator (B & O) and the incidence based coverage estimator (ICE).



4.3.2 Substratum study

4.3.2.1 Direct method

A total of 185 species were recorded in the wet and dry season 2002 (Appendix F); this number does not take into account unconfirmed anamorph/teleomorph connections. Of the observed species, 81 were observed on *C. mackinnoniana*, 47 on *E. angustifolius*, 63 on *F. pleurocarpa*, 61 on *O. heterophylla*, 31 on *D. ferruginea* and 41 on *F. destruens* (Table 4.1). Microfungal diversity measured by 95 % confidence intervals of Fisher's alpha differed significantly between the following four tree species which had been assessed with the same sampling intensity: *C. mackinnoniana – E. angustifolius* and *E. angustifolius – O. heterophylla.* Only a minor overlap of confidence intervals was

observed for *C. mackinnoniana – F. pleurocarpa,* and *E. angustifolius - F. pleurocarpa* while no overlap in 95 % confidence interval was noted for *C. mackinnoniana – O. heterophylla* and *F. pleurocarpa – O. heterophylla* (Table 4.1).

The number of occurrences for all observed microfungal species ranged from 81 on *Darlingia ferruginea* to 365 on *Cryptocarya mackinnoniana* (Table 4.1). The number of samples examined was significantly correlated with the number of occurrences (R^2=0.80; p=0.02) but not with species numbers (R^2=0.61; p=0.07; Appendix G) at the 5 % level.

Table 4.1 Total number of microfungal species (number of species in 10 random leaves), occurrences, number of leaves examined, Shannon's diversity index (H') and evenness, Fisher's alpha (C.I. 95 %), species numbers estimated with Chao2 estimator (C.I. 95 %) and estimated sampling completeness for direct observations in decaying leaves of *Cryptocarya mackinnoniana (Cm), Darlingia ferruginea (Df), Elaeocarpus angustifolius (Ea), Ficus destruens (Fd), F. pleurocarpa (Fp)* and *Opisthiolepis heterophylla (Oh).*

Direct	No. of species (in 10 random leaves)	Occurren-ce	No of leaves	H'	Even ness	Fisher's a (95 % C.I.)	Chao 2 estimate (95 % C.I.)	Complete-ness %
Succession								
	104	632	80	4.02	0.86	36 (29-42)	130 (114-144)	80
Substratum								
Total	185	1399	260	4.50	0.86	57 (49-65)	269 (212-326)	69
Cm	81 (33)	365	60	3.75	0.85	32 (28-37)	137 (85-189)	59
Ea	47 (21)	331	60	2.96	0.77	15 (11-19)	70 (43-97)	67
Fp	63 (23)	318	60	3.38	0.82	24 (18-29)	102 (62-142)	62
Oh	61 (24)	202	60	3.76	0.92	30 (22-37)	145 (48-242)	42
Df	31 (19)	81	20	3.04	0.89	18 (12-25)	57 (22-92)	55
Fd	41 (25)	102	20	3.40	0.92	26 (18-33)	94 (29-159)	44

Fig. 4.2 Chao2 estimates of species richness of microfungi in eight collections of ten *Ficus pleurocarpa* leaves each at similar stages of decay based on direct observations in the succession study

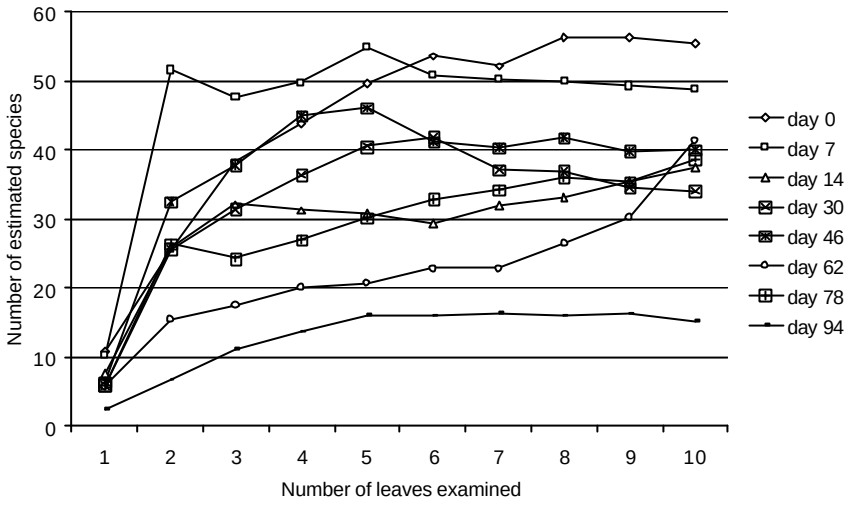


Figure 4.3 Abundance distribution for the complete dataset of microfungi in decaying leaves of *Ficus pleurocarpa* obtained in a succession study by the direct method. The solid line is the fitted logarithmic series

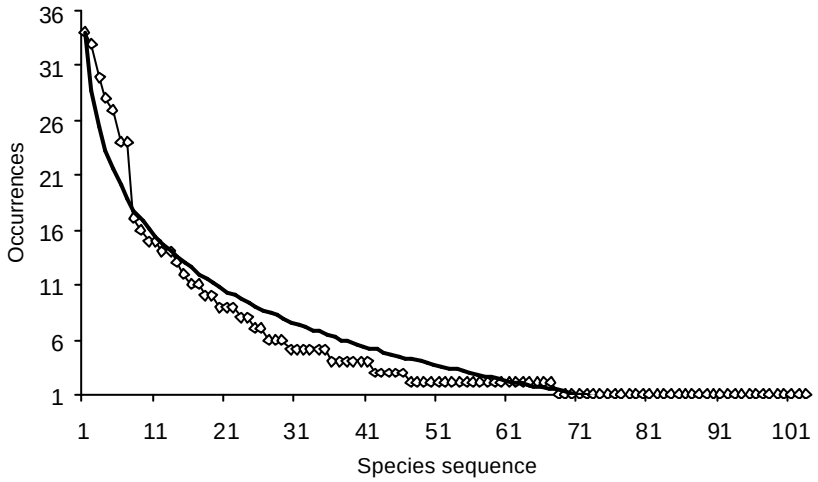
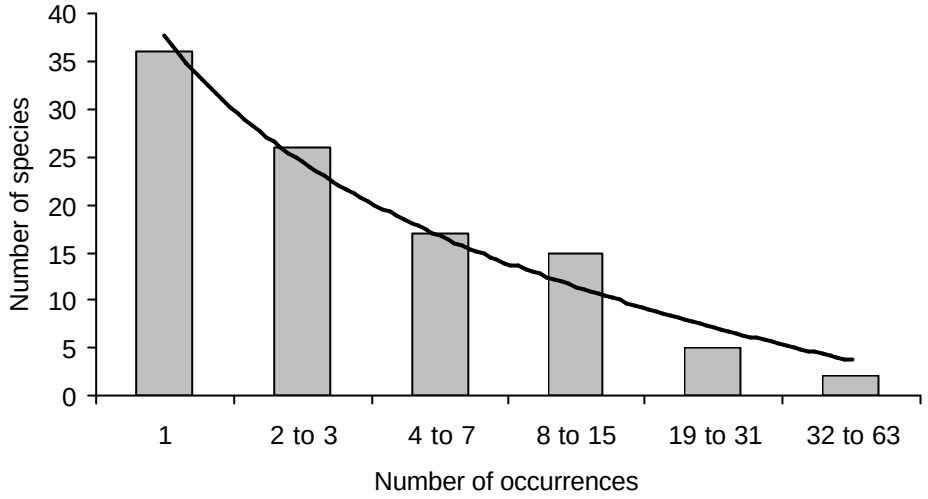


Figure 4.4 Occurrences versus number of species for the complete dataset of microfungi in decaying leaves of *Ficus pleurocarpa* obtained in a succession study by the direct method. The solid line is the fitted logarithmic series



Fisher's alpha calculated for a single season were not significantly different between tree species (Appendix H). Although Fisher's alpha was higher in the wet season, this difference was not significant at the 5 % level (Appendix H). Equally, no significant difference was observed between sites (Appendix H). The Shannon-Wiener's diversity index ranged from 2.96 to 3.76 and appeared to express a similar pattern in diversity among the six tree species as Fisher's alpha (Table 4.1). For the pooled dataset of the substratum study, the Shannon Wiener index was 4.50. Chao2 species estimates for individual tree species ranged from 57 to 145 microfungi. Significant differences between tree species could not be detected due to the wide confidence intervals of the Chao2 estimates. For the dataset pooled for all tree species, 269 species were estimated (Table 4.1). Estimates derived with two additional non-parametric estimators (ICE, B & O jackknife) differed from Chao2 estimates by an average of 10 % (± 6 %; Appendix E). In contrast, the estimates derived from the Michaelis-Menten equation differed from Chao2 estimates by 21 % (± 12 %). The estimated sampling completeness calculated from Chao2 estimates ranged from 42 to 67 % for individual species and was 69 % for the complete dataset (Table 4.1).

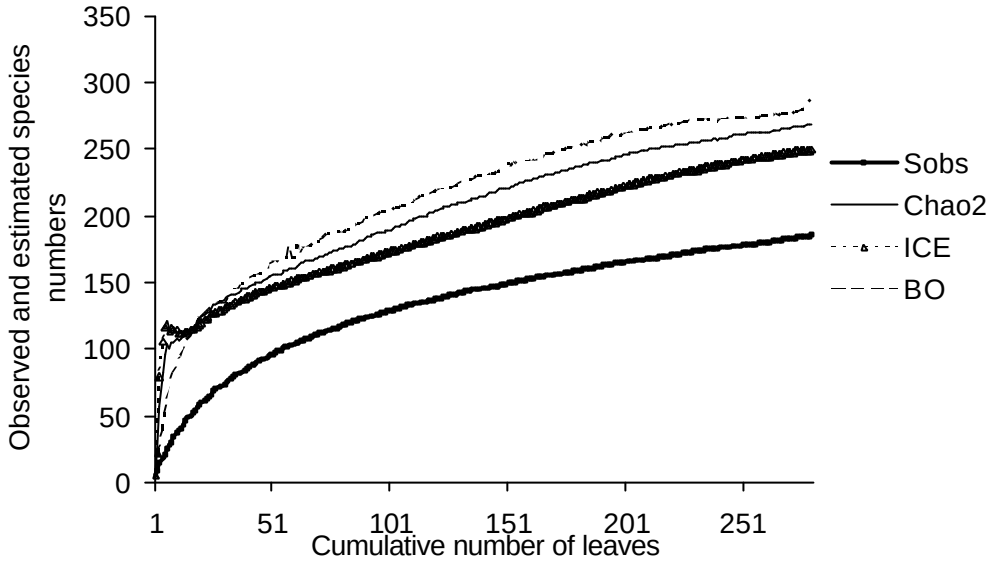
When data from individual tree species were pooled, no asymptote was achieved for observed species numbers or any of the species estimates (Fig. 4.5). For individual tree

species, accumulation curves of raw species data did not reach an asymptote, but species numbers estimated by the Chao2 estimator levelled off for most tree species, except for *Cryptocarya mackinnoniana* (Fig. 4.6). The curve for *Opisthiolepis heterophylla* appeared concave at the central part (Fig. 4.6). A visual inspection of the rank abundance curves shows that the fungal species distribution approximates a logarithmic series for both the total data set of the substratum study (Fig. 4.7) and for individual trees (Appendix I). The mathematical fit was better for the logseries compared to the lognormal distribution in all instances (all tree species excluding *O. heterophylla*: lognormal $p<0.01$; logseries $p<1.0$ and *O. heterophylla*: lognormal $p<0.01$; logseries $p<0.05$)

4.3.2.2 Particle filtration method

A total of 419 morphotypes were recorded in the wet season and 276 morphotypes in the dry season 2001 (see included compact disc for photographic record of morphotypes and species list). Morphotype numbers for individual tree species ranged from 111 to 203 in the wet season and from 112 to 162 in the dry season (Table 4.2). Microfungal diversity as assessed by Fisher's alpha differed significantly between some tree species and between seasons (Table 4.2) but not between sites (Appendix H). When 25 particle filtrations were randomly selected from the wet season data, Fisher's alpha was 177 (158-196). For both seasons, no overlap in 95 % confidence intervals was observed for the following tree species: *C. mackinnoniana – E. angustifolius, C. mackinnoniana – F. pleurocarpa.* A significant difference was detected for *F. pleurocarpa – O. heterophylla* in the wet season (Table 4.2). A comparison between seasons was not appropriate due to the increased sample size per tree species in the dry season. Shannon-Wiener's diversity indices ranged from 3.73 to 4.73 for individual tree species (Table 4.2). For wet season data pooled across all tree species, the Shannon-Wiener index was 5.35 and for the dry season it was 4.70. This index appeared to express a similar pattern in diversity among tree species as Fisher's alpha (Table 4.2).

Figure 4.5 Accumulation curves of observed (Sobs) and estimated number of microfungal species in leaf litter of six tree species, namely *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus destruens, F. pleurocarpa* and *Opisthiolepis heterophylla,* collected over wet and dry season using the direct method. Estimates were derived from Chao2, the incidence based coverage (ICE) and the Burnham & Overton jackknife (BO) estimators.

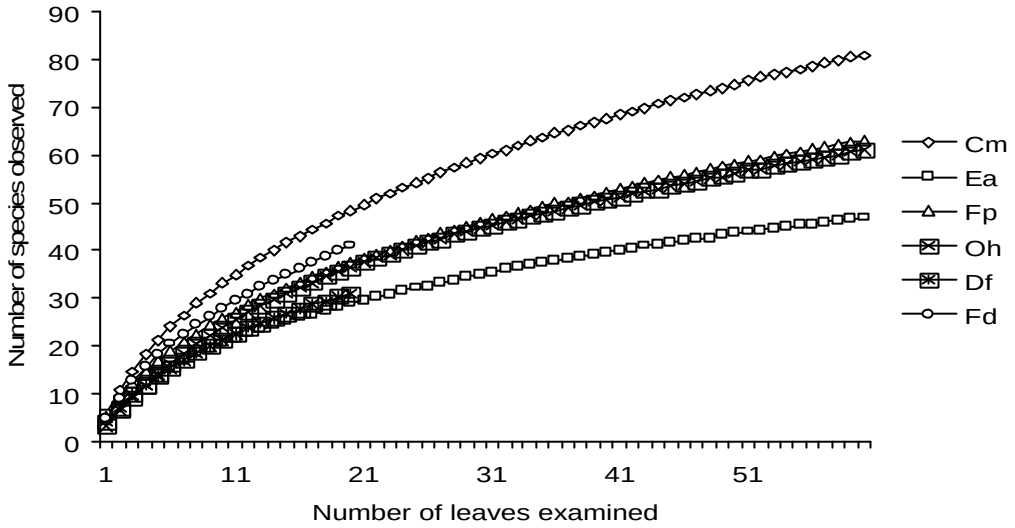


An estimated 627 morphotypes (using the Chao1 estimator) may have been present in the leaves processed during the wet season. The Chao1 estimates of morphotype numbers were lower for the dry season with 406 for the complete dataset. In the wet season, estimates ranged from 163 to 370 morphotypes and from 156 to 249 morphotypes in the dry season (Table 4.2). The estimated sampling completeness ranged from 55 % to 74 % for individual species and was 67 % and 68 % for the complete dataset of the wet and dry season respectively (Table 4.2). The estimates derived from the two other non-parametric estimators (ICE, B & O jackknife) differed from Chao1 estimates by an average of 9 % (± 6 %; Appendix E). In contrast, the estimates derived from the Michaelis-Menten equation differed from Chao2 estimates by 11 % (± 6 %).

For individual tree species, accumulation curves of raw species data did not reach an asymptote (Fig. 4.8), but species numbers estimated by the Chao1 estimator levelled off for most tree species, except for *Cryptocarya mackinnoniana* (Fig. 4.9). Species abundance distributions for both the succession and substratum study differed significantly from the lognormal but not the logseries curve (lognormal $p<0.01$;

logseries p<1.0) with the exception of the distribution for *Opisthiolepis heterophylla* (p<0.05). A visual inspection of the rank abundance curve shows that the fungal species distribution approximates a logarithmic series for both the total particle filtration data set of the substratum study (Fig. 4.10) and for individual trees (Appendix I).

Figure 4.6 Species accumulation curves for microfungi in leaf litter of six tree species, namely *Cryptocarya mackinnoniana (Cm), Darlingia ferruginea (Df), Elaeocarpus angustifolius (Ea), Ficus destruens (Fd), F. pleurocarpa (Fp)* and *Opisthiolepis heterophylla (Oh)* over wet and dry season isolated by the direct method. A. Observed number of species. B. Estimated number of species. Estimates were derived from Chao2, the incidence based coverage (ICE) and the Burnham & Overton jackknife (BO) estimators.



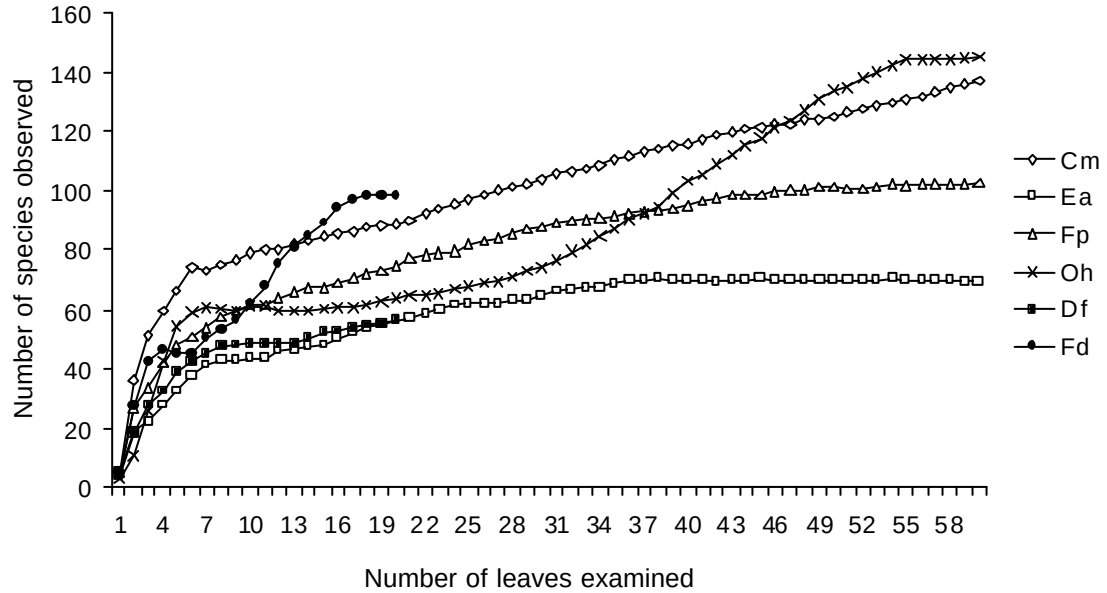


Figure 4.7 Abundance distribution for the complete dataset of microfungi in decaying leaves of six tree species obtained in the substratum study by the direct method. The solid line is the fitted logarithmic series.

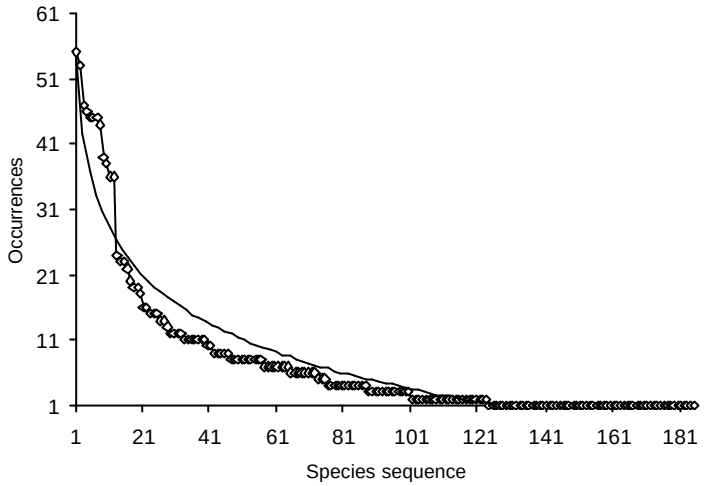


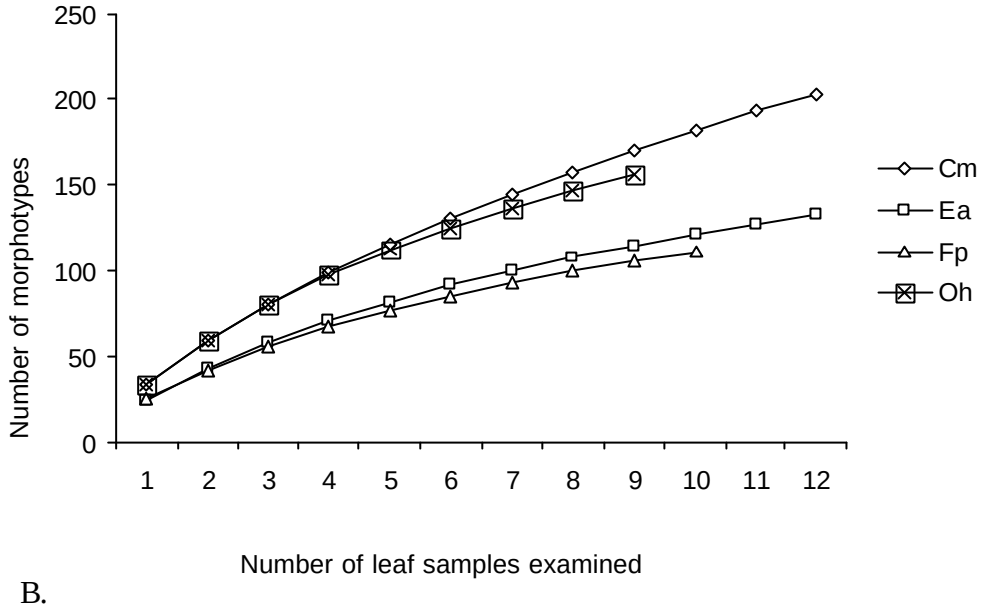
Table 4.2 Number of morphotypes, estimated species numbers (morphotype number adjusted according to Arnold et al. 2000), number of isolates, number of leaves examined, Shannon's diversity index (H') and evenness, Fisher's alpha (95 % confidence intervals), species numbers estimated with Chao2 estimator and estimated sampling completeness for the particle filtration data.

PF	No. of morpho-types	Species estimate (Arnold et al. 2000)	No of isolates	H'	Even-ness	Fisher's a (95 % C.I.)	Chao 1 estim-ate	Comple-teness %	Rare-faction index $E_{(200)}$
Wet season									
Total	419	347 (304-391)	1575	5.35	0.89	187 (169-205)	627	67	123
Cm	203	168 (147-189)	513	4.73	0.89	124 (107-141)	370	55	110
Ea	133	110 (97-124)	356	4.44	0.91	77 (64-90)	206	65	95
Fp	111	92 (81-103)	346	4.11	0.87	57 (46,-67)	163	68	86
Oh	156	129 (113-145)	360	4.69	0.93	105 (88-121)	241	65	112
Dry season									
Total	276	229 (200-257)	2059	4.70	0.84	88 (77-97)	406	68	93
Cm	162	134 (118-151)	736	4.48	0.88	64 (54-74)	249	65	88
Ea	112	93 (81-104)	614	3.73	0.79	40 (33-48)	181	62	64
Fp	115	95 (84-107)	709	3.82	0.84	39 (32-46)	156	74	62

Indices and estimates are presented for the complete dataset (Total), and for individual tree species, namely *Cryptocarya mackinnoniana (Cm), Elaeocarpus angustifolius (Ea),* and *Ficus pleurocarpa (Fp)*. For the wet season collection, an additional treespecies, *Opisthiolepis heterophylla (Oh)*, was included.

Figure 4.8 Accumulation curves of observed morphotypes for microfungi in leaf samples of *Cryptocarya mackinnoniana (Cm)*, *Elaeocarpus angustifolius (Ea)*, *F. pleurocarpa (Fp)* and *Opisthiolepis heterophylla (Oh)* isolated by particle filtration. A. wet season 2001 and B. dry season 2001.

A.



B.

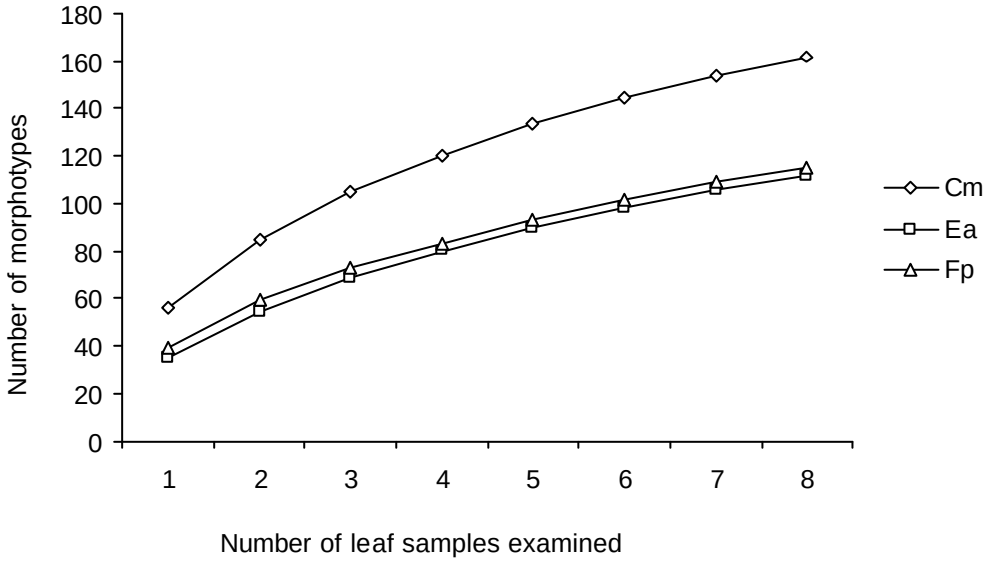
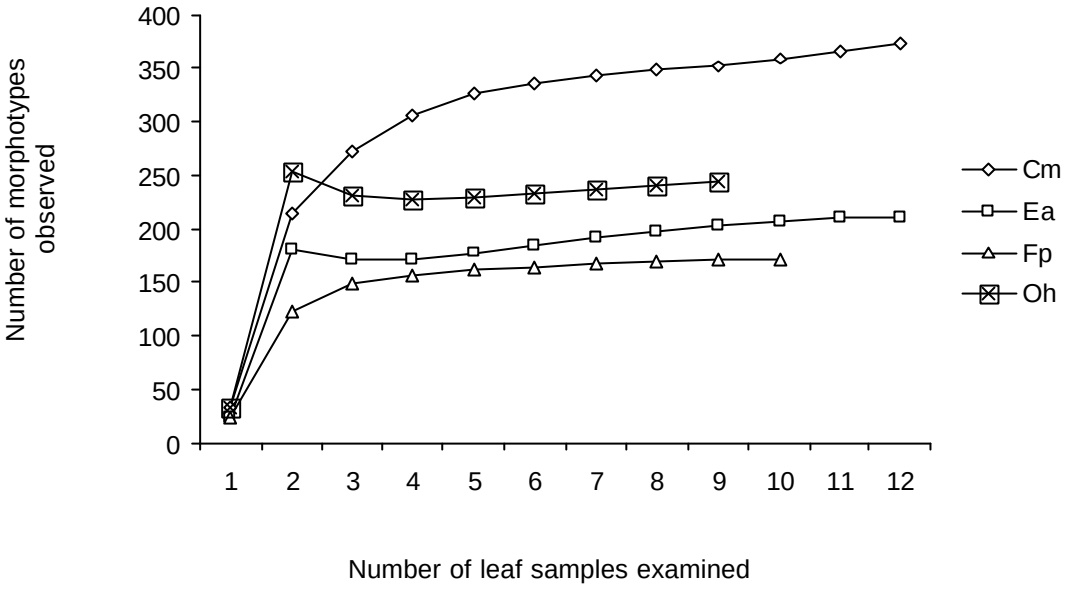


Figure 4.9 Accumulation curves of Chao1 estimates for microfungal morphotypes in leaf samples of *Cryptocarya mackinnoniana (Cm)*, *Elaeocarpus angustifolius (Ea)*, *Ficus pleurocarpa (Fp)* and *Opisthiolepis heterophylla (Oh)* isolated by particle filtration. A. wet season 2001 and B. dry season 2001

A.



B.

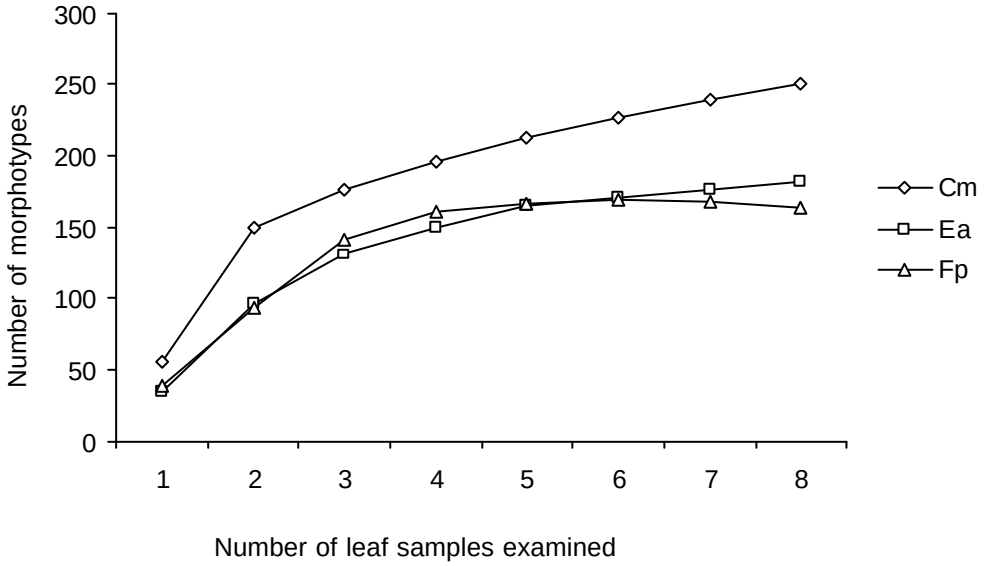
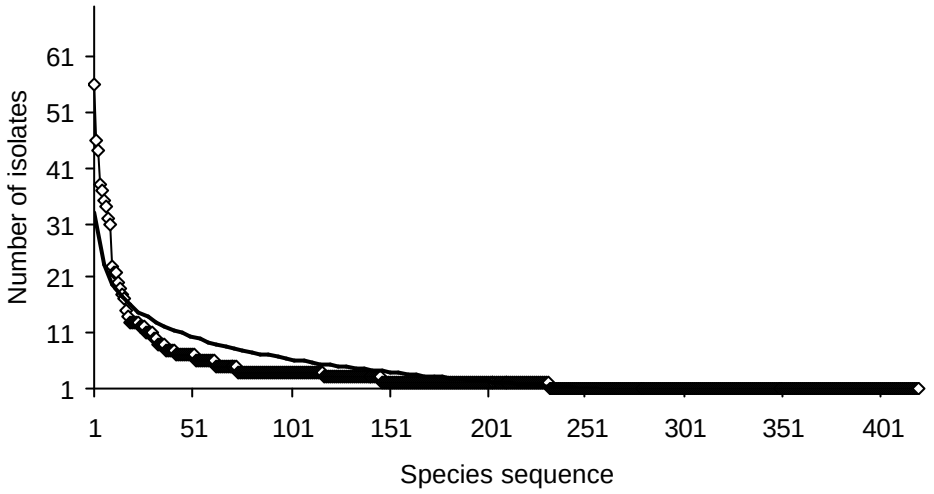
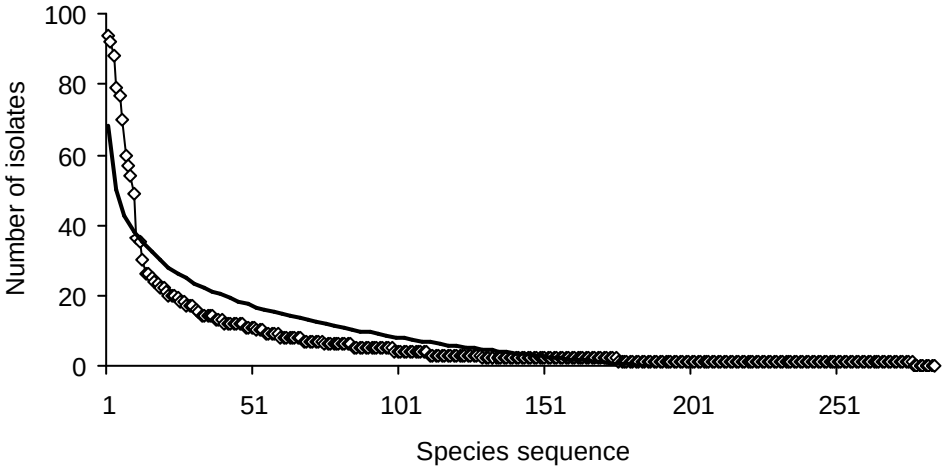


Figure 4.10 Abundance distribution for the complete dataset of microfungi in decaying leaves isolated by particle filtration in the substratum study. A. wet season and B. dry season. The solid line is the fitted logarithmic series.

A.



B.



4.4 Discussion

Microfungal diversity was assessed in leaf litter of six tree species using two different sampling approaches. A comparison of these studies indicated that differences in sampling intensity and isolation protocol have a marked effect on estimated microfungal diversity (Table 4.1 and 4.2). The greatest species richness for a single substratum type was detected by the direct method in the succession study in decaying leaves of *Ficus pleurocarpa* (Chapter 3; Table 4.1). This study used an intensive sampling regime, which is reflected in a high estimated sampling completeness (80 %; Table 4.1). A less intense sampling strategy was applied in the substratum study and this is reflected in lower estimates of species richness and sampling completeness (42 % to 67 %; Table 4.1). Similarly, marked differences in species richness were introduced by different isolation protocols with greater richness was observed and estimated for data obtained by particle filtration (Table 4.2). In addition to sampling and isolation strategies, the findings of the present project suggest that a potential bias in estimating microfungal diversity may be introduced at the microscopic level. For example, the intensive subsampling approach applied in the succession study detected a significantly higher microfungal diversity in 30 random leaves (based on 95 % C.I. of Fisher's alpha) compared to 30 leaves examined during the substratum study. Although subsampling protocols appear to influence microfungal diversity estimates, these have not often been reported in the literature, particularly for the direct method.

Irrespective of the disparity in diversity estimates introduced by varying sampling and isolation protocols, similar species abundance distributions were observed for all datasets of this project (Fig. 4.3, 4.7 & 4.10; Appendix I). All approximate a logarithmic distribution irrespective of the 'surrogate measures of abundance' applied, be it occurrences for the direct method or number of isolates for the particle filtration method. The logarithmic series model predicts a small number of abundant species and a large proportion of rare species. This distribution may be observed in situations where one or a few factors dominate the ecology of a community (see Chapter 5; Magurran 1988). It is also compatible with situations in which species arrive in unsaturated habitats at random rather than regular intervals (e.g. May, 1975). However, it has been argued that the logarithmic distribution can arise as a result of small sample sizes and that further sampling would reveal a log normal distribution (Magurran, 1988). Most microfungal datasets (e.g. Fig. 4.7 & 4.10; Appendix I) may suffer from the problem of

undersampling but the logarithmic distribution was also apparent for microfungi derived from the relatively well-sampled *F. pleurocarpa* leaves examined in the succession study. This finding is compatible with the views of a number of authors who have challenged the assertion that most communities are log normal. For example, Lambshead and Platt (1985) argued that the log normal distribution is the result of combining non-replicate samples. Similarly, Hughes (1986) suggested that the log normal distribution may arise from mistakes in grouping species and from sampling errors even when the underlying distribution is logarithmic. The significance of observing a logarithmic distribution in the present project is unknown and will need to be examined in the light of further studies. These would preferably achieve greater sampling completeness and/or include different substrata.

The properties of the logarithmic series have been thoroughly examined in a series of studies (e.g. Fisher et al., 1943; Kempton and Taylor, 1974; Taylor et al., 1976; Kempton and Wedderburn, 1978). One of the parameters that describes this distribution is an index of diversity, also called Fisher's alpha (a; Fisher et al., 1943). This index has a number of advantages, such as a relative independence from sample size and high discriminatory powers, and has been frequently used in plant and animal ecology (Magurran, 1988). Despite its apparent advantages, this index has been rarely applied in mycology (e.g. Ferrer and Gilbert, 2003). By examining its 95 % confidence intervals, it was possible to demonstrate for both isolation protocols that microfungal diversity differed significantly between some of the four main tree species that were investigated (Table 4.1 & 4.2). The reasons for these differences are not known but the lack of a significant correlation between the number of replicates and species numbers suggests that it is not a simply a sampling issue. For example, both particle filtration and direct observations agreed that leaves of *C. mackinnoniana* harbour a particularly rich mycota (Table 4.1). Leaves of this tree species are highly lignified and this may have contributed to the high diversity in this substratum. This observation is consistent with that of Wong and Hyde (2001) that grasses with more durable, strongly sclerenchymatic stems supported a higher diversity of fungi than those with more herbaceous stems. Other possible biological explanations for observed differences in diversity (and composition) of microfungal assemblages will be explored in Chapter 5.

Microfungal diversity did not differ significantly between the two sites irrespective of isolation method (Appendix H). Similarly, no significant seasonal differences in

microfungal diversity were observed with the direct method. A significantly higher diversity was noted in the wet season for data obtained by particle filtration (Table 4.2), but these results are confounded because more isolates per particle filtration were obtained during the dry season. Therefore, seasonality of microfungal assemblages will be revisited in Chapter 5 using a different approach.

Attempts to compare the results of the present study with those of other projects were frustrated for a number of reasons. Firstly, species richness is often reported for different sampling units in the literature. Some studies have examined individual leaves (this study; Parungao et al., 2002), while others utilised leaf mass (Polishook et al., 1996) or leaf discs (Heredia, 1993). Furthermore, authors use different formats for reporting data but mostly data are pooled and therefore cannot be reanalysed. Even where similar sampling units and reporting schemes were used, a number of factors confounded a comparison of studies. For example, when species richness in a standardised number of leaves was compared between the substratum study and that undertaken by Parungao et al. (2002), the diversity observed in the present study was higher. The reasons for this higher diversity are unknown and potentially include biological factors such as substratum quality, site factors, microclimatic conditions (Chapter 5), or sampling biases, for example differences in subsampling intensity (see below), or a combination of several factors. Comparisons of microfungal diversity were possible between studies, which utilised consistent subsampling protocols as the effect of uneven sample sizes could be corrected using rarefaction (Chapter 2; Bills and Polishook, 1994a, 1994b; Polishook et al., 1996; Paulus et al., 2003b). For example, comparisons of estimated morphotype richness suggested that a considerably higher diversity was present for individual tree species in the present study ($E_{(200)}$ 62-112; Table 4.2) compared to those previously reported from *Neolitsea dealbata* leaf litter at the same site that have used the same isolation technique and morphotype concept ($E_{(200)}$ 54-58; Chapter 2; Paulus et al. 2003b). Some rarefaction indices were also higher than those reported for microfungi in leaf litter of neotropical forests (E(200) 40-84; Bills and Polishook, 1994a, 1994b; Polishook et al., 1996).

Despite the high species richness that was observed in this project, it is likely that attempts to enumerate microfungi fell short of the actual number of species present in leaf litter because the reported species numbers were based on finite samples (Rosenzweig et al., 2003). A variety of statistical tools are available to estimate actual

species numbers (reviewed by Bunge and Fitzpatrick, 1993; Colwell and Coddington, 1994; Hughes et al., 2001); these have an intuitive appeal as they provide estimated species numbers rather than an abstract index. For estimating species numbers from datasets derived by the direct method, I selected the Chao2 estimator based on previous reports of its consistent performance in similar datasets (e.g. Colwell & Coddington 1994; Hraber 2001; Hughes et al. 2001). Species estimates facilitate the calculation of sampling completeness and may prove useful in assessing an adequate sample size for ecological and diversity studies.

Accumulation curves are often used to estimate an adequate sample size for diversity and ecological studies (Magurran, 1988). Although accumulation curves may approach an asymptote for datasets of species that can be enumerated exhaustively, this is rare in the case of species rich groups, such as benthic fauna (Ugland et al., 2003), insects (Erwin, 1991) or saprobic microfungi (Fig. 4.1, 4.5 & 4.6; Bills and Polishook 1994; Paulus et al 2003b). In the case of microfungi, it is conceivable that a proportion of the observed species in a particular substratum is present by chance and drawn from a vast pool of microfungal species on the forest floor. Therefore, an asymptote may not be achieved irrespective of sampling effort. In this case, valuable conclusions about microfungal diversity and distribution might still be possible in the absence of an asymptote, as long as a representative sample of 'dominant' species has been observed. To determine an adequate sample, an estimator such as Chao2 may prove to be a useful tool because it compensates for a high percentage of rare species (Colwell and Coddington, 1994; Chao, 1987; Hughes et al., 2001).

Using this approach, some intuitively logical patterns were observed among both the succession and substratum datasets. For example, Chao2 species estimates for most tree species reached an asymptote quickly if sampling units were derived from homogenous substrata (i.e. same tree species, same state of decay; Fig. 4.2). If sampling units were heterogenous in terms of their state of decay but from the same tree species, an asymptote in species estimates was only achieved after a four to five-fold increase in sampling effort (Fig. 4.1). This result is consistent with that of the succession study in which several distinct assemblages were detected with advancing decay (Chapter 3; Fig. 4.6). In contrast, when data pooled from all tree species were assessed, an asymptote was not reached for any of the species estimates (Fig. 4.5). Presumably this was due to the heterogeneity of substrata in terms of both their phylogeny and their state of decay.

This finding supports Cannon's (1997b, 1999) assertion that tightly defined samples would yield the most useful information about microfungal diversity.

Although intuitively logical patterns were observed in assessing accumulation curves of Chao2 species estimates, caution is required as confidence intervals were wide in the present study. Not unexpectedly, Chao2 species estimates appeared to behave erratically in some small samples. For example, the Chao2 estimates seemed inflated for *F. destruens* during the dry but not the wet season (Appendix H). Species estimates may also be subject to aberrations if sporulation patterns (and/or presumably subsampling) are not uniform. A concave accumulation curve and relatively low sampling completeness was observed for microfungi in *O. heterophylla* leaves by the direct method (Fig. 4.6). Leaves of this tree species curl tightly during decay (Appendix A), thereby reducing the surface area available for sporulation. This would decrease the detection rate of fungi when the direct method is applied. It is not known whether and how this affects the estimate at the maximum sample size. The accumulation curve for the particle filtration data derived from *O. heterophylla* leaf litter, did not show this aberration, presumably because this method is not dependent on observing sporulation patterns.

The validity of assessing sample size and sampling completeness using non-parametric estimators will need to be formally tested for different microfungal datasets. This can be achieved in simulations that utilise 'complete' or nearly complete microfungal datasets (Colwell and Coddington, 1994). In the absence of 'complete' reference datasets, Coddington et al. (1990) proposed that assessing convergence of estimates based on different theoretical approaches might provide evidence that each approach is measuring the same quantity. In the present study, a greater sample size and/or effort appeared to yield more precise results. This was evident as species estimates generated by different estimators were more divergent in the substratum study for both the direct method and particle filtration than in the succession study (Appendix E). In the substratum study, the Michaelis-Menten estimates, in particular, were considerably lower than those derived by ICE and B & O estimators. This finding is consistent with previous reports that Chao2, ICE and B & O estimators compensate well for a high percentage of low abundance classes (e.g. Rosenzweig et al., 2003).

An important factor in estimating the diversity of microfungi is their taxonomy (Chapter 7; Hyde et al. 2004). In this study, many species could only be named to higher

taxonomic levels and work continues where appropriate to identify and describe taxa new to science (Chapter 7). Some minor underestimation of diversity may have occurred by the direct method as only unambiguous morphological entities were counted as separate species. In a number of instances, particularly in taxonomically difficult groups such as *Pestalotiopsis, Phoma, Phomopsis* and *Acremonium,* this may have led to a 'lumping' of biological species. On the other hand, unconfirmed anamorph-teleomorph connections and synanamorph relationships were not taken into consideration (Appendix C, D & F). Nevertheless, the influence of these potential biases is probably minor relative to the total observed diversity, and thus I might expect reported species numbers to reflect relatively accurate figures. A degree of uncertainty was introduced in the particle filtration data obtained in the substratum study because morphotypes were utilised to assess richness in non-sporulating taxa. Nevertheless, even when species numbers were adjusted for a potential overestimation due to the morphotype concept (Arnold et al., 2000), the observed richness remained higher than that reported for the direct method (Table 4.1 & 4.2, Appendix H). This agrees with the results of previous studies, which also have reported the detection of a greater richness with the particle filtration protocol as compared to the direct method (Bills and Polishook, 1994a; Paulus et al., 2003b).

Diversity data are collected for a number of reasons, for example to undertake an inventory or to monitor the diversity present at one site, or to address ecological questions. Diversity assessments depend on reliable estimates of diversity that are comparable across space and time (Hyde and Hawksworth, 1997; Hawksworth, 1998b). The present study has highlighted potential methods for estimating diversity and inherent limitations using a taxon-independent approach and therefore, the next chapter will explore a different approach to comparing microfungal assemblages across space and time.

4.5 Summary and recommendations

While this study has highlighted a high diversity of leaf litter microfungi, the actual number of species that are involved in the decay of leaves of the six tree species examined remains unknown. However, diversity estimates have been derived under a set of tightly defined conditions and, therefore, could provide comparative datasets for

future studies. Within this project, a logarithmic species distribution was consistently observed for both studies, irrespective of sample size and sampling effort or isolation method. Based on this distribution, Fisher's alpha was selected to test for differences in microfungal diversity between tree species, sites, seasons and isolation protocols. It has been demonstrated that the diversity of microfungi in leaf litter differed significantly between some tree species but not between sites. Seasonal differences were observed but results were unequivocal; a significantly greater diversity was observed with the particle filtration method as compared to the direct method.

From the observations of this study, a number of recommendations can be made.

1. to facilitate comparisons of diversity between studies, matrices of species by sampling units could be made available to other researchers either on-line or via the editor of the journal. This mirrors the situation in molecular systematics where most journals require DNA datasets and corresponding analyses to be deposited in relevant databases such as GenBank or TreeBase prior to publication.
2. standardising sampling units would assist in making diversity studies comparable. For leaf litter, the natural substratum unit is a single leaf. Caution is needed when selecting and standardising subsections of leaves based on weight because certain areas of the leaf may support different microfungal assemblages (Chapter 2 & 5). Unless an appropriate approach to selecting subsections is applied, a bias may be introduced.
3. to make diversity studies comparable across space and time, standardisation of sampling strategies needs to be considered at all levels, including subsampling at the microscopic level.
4. wherever possible, other aspects of isolating microfungi, such as storage of substrata, surface treatment, and incubation times and conditions, need to be reported in publications.
5. appropriate indices and/or estimates of diversity need to be selected to provide the most reliable view of the diversity present in a substratum. Fisher's alpha has been tested extensively in other fields of biology and is recommended as a diversity index for microfungal assemblages. Non-parametric species estimators seem to be useful for estimating sampling completeness and for plotting

accumulation curves but need to be applied with caution due to wide confidence intervals.

6. there is an urgent need for 'complete' microfungal datasets to test statistical tools and sampling approaches. All-taxa biodiversity inventories may provide such datasets but on a small scale it may be more efficient to intensively sample one or a few substrata. Succession studies lend themselves to such an approach. The findings of the succession study undertaken for this project need to be verified for other substrata by studies that use a similar design.

7. although a combination of isolation methods may be desirable, this may not be achievable within the time and funding constraints under which most mycologists operate. Therefore, isolation methods need to be selected with care. A degree of uncertainty is introduced when a morphotype concept is applied. On the other hand, this method can access greater diversity and may facilitate the inclusion of parataxonomists in diversity studies. Potentially, this could speed up assessments of microfungal diversity. For single researchers, the direct method allows for greater replication and therefore may be the method of choice for synecological studies. Where the assessment of taxonomic diversity is of primary importance, the direct method will also be better suited.

CHAPTER FIVE

Distribution of Saprobic Microfungi in Tropical Rainforest Leaf Litter

5.1 Introduction

5.1.1 Potential factors affecting microfungal distributions

Fungi are vital contributors to decomposition processes (Jordan, 1985; Lodge, 1992) and their distribution patterns and interactions may affect the rates and patterns of decomposition (Swift, 1976). In tropical rainforests, the distribution patterns of saprobic microfungi show a high degree of heterogeneity (Bills and Polishook, 1994a; Lodge and Cantrell, 1995; Lodge, 1997), which may be associated with a wide range of biotic and abiotic factors (Chapter 1). These include for example the chemical and physical attributes of substrata, climatic and microclimatic conditions, and competition for resources (Swift, 1976; Cooke and Whipps, 1993; Lodge and Cantrell, 1995; Neville and Webster, 1995; Lodge, 1997). Saprobic microfungi do not need to overcome the active defences of living host cells and thus have been considered less host specific than plant pathogens or endophytes (Kendrick, 1992). However, a number of studies have indicated quantitative differences in species abundances between host substrata (Cowley, 1970; Heredia, 1993; Cornejo et al., 1994; Cantrell, 1995; Lodge and, Polishook et al., 1996; Lodge, 1997; Dulymamode et al., 2001; Parungao et al., 2002) and recent evidence suggests that some saprobic microfungi are host specific (reviewed by Zhou & Hyde 2001). Few studies to date have systematically examined the question of host specificity or preference in saprobic microfungi (e.g. Bills & Polishook 1994) and of these most were focussed on aquatic fungi (Thomas et al., 1992; Chauvet et al., 1997; Gulis, 2001). Data of host preference is especially lacking for terrestrial microfungi in tropical ecosystems (Hyde et al., 2004).

5.1.2 Definition of terms

The degree to which fungi are specific to living hosts or dead substrata may range from complete presence or absence in one host species or genus to a quantitative difference in fungal occurrences on particular substrata (Lodge, 1997). The terms host specificity and host preference have been variously applied to phenomena along this continuum and have been clarified by Zhou and Hyde (2001), who suggested that the term host specificity be restricted to the interaction between a living host and a symbiont (pathogenic, amensal or beneficial). The term 'host exclusivity' was proposed for the exclusive occurrence of a strictly saprobic fungus on a particular host or on a restricted range of related host plants. 'Host recurrence' denotes the frequent or predominant occurrence of a symbiotic, parasitic or saprobic fungus on a particular host with infrequent occurrences on other host plants in the same habitat. I have adopted these terms because they can potentially provide a more precise description of nutritive plant-fungal associations.

5.1.3 Aims of this chapter

The major aims of this chapter are

- to determine whether and to what extent the phylogeny of the host, seasonal variations and spatial heterogeneity influence microfungal distributions
- to assess species richness and its correlation with some leaf attributes
- to examine the species composition of microfungal assemblages in relation to host recurrence
- to assess whether isolation protocols provide congruent conclusions

5.2 Methods

5.2.1 Climatic factors at sites

Characteristically, both study sites have a high mean annual rainfall. At Topaz, the rainfall records for 1954 to 2000 range from 2242 mm to 5916 mm p.a., with an average of 3811 mm (Bureau of Meteorology 2003). At the Millaa Millaa site, rainfall was slightly lower with an average rainfall of approximately 3400 mm (N. Tucker, pers.

comm.). A high percentage of the annual rainfall usually occurs during the months of December to May (79 %; Bureau of Meteorology 2000). Rainfall data were accessed via the Bureau of Meteorology for the Topaz site and N. Tucker provided rainfall data for the Millaa Millaa site. Temperature and relative humidity were measured in duplicate under each study tree during the wet and dry season collections using a Therma-Hygrometer (RS). As assumptions of equal variance and normality could not be met despite data transformations, a Kruskal-Wallis test was undertaken to test for differences in temperature and relative humidity between sites, season and tree species (SPSS, 2001).

5.2.2 Direct method

Four tree species belonging in four common plant families of the region were selected for the main part of this study, namely *Cryptocarya mackinnoniana* F. Muell. (‘*Cm*’, Lauraceae), *Elaeocarpus angustifolius* Blume (‘*Ea*’, Elaeocarpaceae), *Ficus pleurocarpa* F. Muell. (‘*Fp*’, Moraceae) and *Opisthiolepis heterophylla* L.S. Smith (‘*Oh*’, Proteaceae). Due to the high plant diversity, it was impractical to randomise the selection process of trees. Three individual trees of each species were located at each of two study sites and tagged. Most trees were spaced at least 20 m apart. Two sets of *C. mackinnoniana* and *O. heterophylla* trees at the Old Boonjie Site occurred next to each other. In addition, one *E. angustifolius* tree at the Brooke’s Road Site was situated outside the rainforest. To assess in more detail the effect of host phylogeny on microfungal assemblages, I also conducted less intensive surveys of two additional tree species. These included a second *Ficus* species, *F. destruens* F. Muell. ex C.T. White (*Fd*), and *Darlingia ferruginea* J.F. Bailey (*Df*, Proteaceae). For these species, I sampled two trees per species from one site only. This protocol allowed me to examine the leaves of these species within the same time frame as the four main species. Previous research and experience has shown that sampling during different time periods can provide markedly different estimates of diversity and species composition (Paulus et al., 2003b). For all tree species, five leaves per tree were collected on 28 May (late wet season) and 3 September (late dry season) 2002 from a 7×7 m quadrat under each study tree and returned in plastic bags to the laboratory within four hours.

Microfungi were observed following incubation as outlined in Chapter 3 but a different subsampling scheme was employed. Microfungal fruiting bodies were sampled along the leaf margins, the midrib and along two additional 'microtransects' parallel to the midrib from both adaxial and abaxial leaf surfaces (ten 'microtransects' per leaf). A slide was prepared for one representative fruiting structure of each morphologically distinct fungal entity from each microtransect.

5.2.3 Particle filtration

Particle filtration is more work intensive than the direct examination of leaves. Therefore, microfungi of four rather than six tree species were studied in the wet season and three tree species in the dry season 2001. *Opisthiolepis heterophylla* was excluded from the dry season collection to facilitate an increase in sample size, i.e. number of cultures isolated per tree. Ten leaves were collected from under each tree during the wet season (27 February and 30 March 2001) and the dry season (8 August 2001) as outlined above. Leaves were washed to remove soil and other debris and air-dried at 25º C for eight hours. As leaf sizes differed markedly between tree species, particle filtrations were based on samples standardised by mass. Small, randomly selected sections were removed from ten leaves and pooled to a total air-dry weight of 10 g. The pooled leaf samples were divided into 5 g samples and processed in two replicate particle filtrations according to the protocol described in Chapter 2. Forty eight particles with hyphae emerging (i.e. colony forming units; *cfu*s) and 96 *cfu*s per particle filtration were transferred to a fresh agar plate in the wet and dry season respectively. In an attempt to randomise the selection process of *cfu*s, the first four or eight *cfu*s touching a haphazardly drawn line on the back of each isolation plate were selected for transfer in the wet and dry season respectively. A number of *cfu*s failed to grow into a colony although hyphae had emerged initially. Rare bacterial contaminations and mixed cultures were excluded from analysis. Morphotype assessments and identifications were undertaken as outlined in Chapter 2. No attempt was made to match morphotypes across different seasons due to the number of non-sporulating isolates. Therefore, particle filtration data are presented separately for each season.

5.2.4 Definitions and statistical analyses

The overlap and complementarity of microfungi from different leaf species, sites and seasons was calculated as follows (Colwell and Coddington, 1994):

$$\text{Overlap (\%)} = \frac{\text{number of taxa shared between A and B} \times 100}{\text{total number of taxa observed in A and B}}$$

$$\text{Complementarity (\%)} = 100 - \text{overlap}$$

where A denotes the number of microfungal species in one leaf species and B the number of microfungal species in another leaf species (and season or site). The percentage of shared species was used to express the similarity of between two to six host substrata. To facilitate comparisons with previous studies, complementarity was also calculated after removal of singletons.

A similarity coefficient described by Motyka et al. (1950) was selected to compare pooled data from each tree species, site and season. This co-efficient equates to 1 – Bray-Curtis index measure frequently quoted in the literature (e.g. Faith et al. 1987).

$$\text{Motyka co-efficient of similarity} = \left(\frac{S\ 2 \min (y_{ij}, y_{ik})}{S\ (y_{ij} + y_{ik})} \right)$$

A three-dimensional NMDS ordination was undertaken based on Bray-Curtis distances and results were cross-checked using a hierarchical cluster analysis with average linkage (Clarke and Warwick, 1994; SPSS, 2001). The statistical significance of assemblage differences associated with site, season and phylogeny was examined with ANOSIM, using the program PRIMER (Clarke & Warwick 2001). Only family level assemblage differences were examined, as at the species level, *F. destruens* and *D. ferruginea* were represented by only two individuals. Nevertheless, this analysis has the potential to discriminate between the four main target trees, which all belong to different families.

Finally, possible correlations between microfungal assemblages and substrate characteristics were examined. Dr J. Kanowski provided data on leaf morphology, texture and chemistry of living leaves for five or the six study species from an extensive survey of rainforest foliage conducted in 1997 at sites on the Atherton Tablelands. Details of collection methods and analyses are given in Kanowski (1999). Data included leaf area, specific leaf area (SLA), toughness, thickness, condensed tannins, total phenolics, alkaloids, nitrogen, calcium, potassium, magnesium, phosphorus, sulphur, aluminium, boron, cobalt, chromium, copper, iron, manganese, sodium, nickel, selenium and zinc. Data from sites with different environmental attributes were pooled and mean parameters calculated. Leaf chemistry data were not available for *O. heterophylla.* Correlations between microfungal species richness and foliar attributes were examined with Pearson's Correlation Coefficient (SPSS, 2001). To control for differences in sampling effort between species (three species were represented by six individuals, and two species by two individuals), the average richness was calculated from all pairwise combinations of individuals for the former three species; these were used in correlation analyses (see Table 5.1).

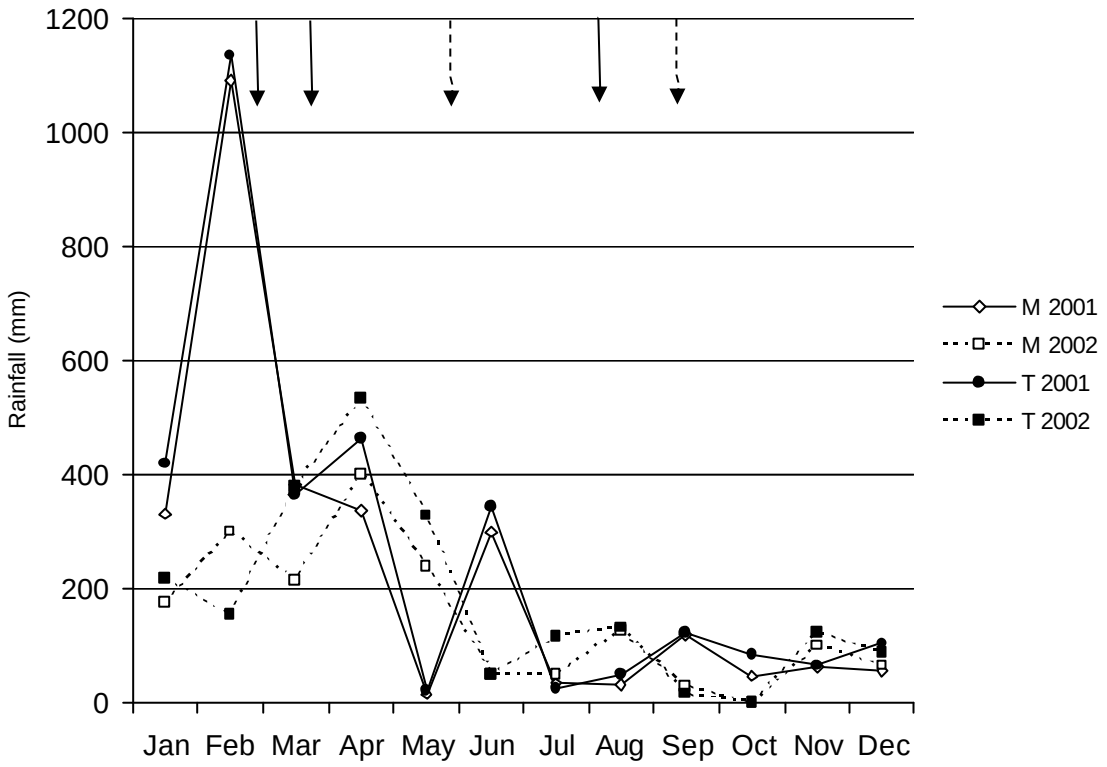
5.3 Results

5.3.1 Climatic and microclimatic conditions

Below average rainfall was recorded for both study years. In 2001, an annual total rainfall of 3207 mm was logged at Topaz and 2814 mm at Millaa Millaa. The following year, the lowest yearly rainfall since 1954 was registered with 2143 mm at Topaz and 1756 mm at Millaa Millaa. In that year, maximum rainfall was delayed until April (Fig. 5.1). In the wet season 2001, 358 mm and 1138 mm of rain were recorded in the 28 days before collections and 124 mm in the 28 days before the dry season collection (Bureau of Meteorology 2001). In the 28 days before the wet and dry season collections of 2002, the total rainfall recorded was 409 mm and 134 mm respectively (Fig. 5.1; Bureau of Meteorology 2002). The mean temperature and relative humidity on collection days for seasons, sites and tree species are provided in Appendix K. Temperatures differed significantly between the 2001 and 2002 collections; this may have been due to the relatively late wet season collection of 2002. In contrast, relative humidity did not differ significantly between the two years, despite the low rainfalls

observed during 2002 (Appendix K; Fig. 5.1). Both temperature and relative humidity differed between seasons and sites but not between quadrats of the different tree species studied. Temperature differences between sites were only significant at the 10 % level (Appendix K).

Figure 5.1 Monthly rainfall for Topaz Towalla Road (T) and Millaa Millaa (M) for the years 2001 and 2002 (Bureau of Meteorology 2003). Vertical arrows indicate collection dates (solid line 2001, stipled line 2002).



5.3.2 Direct method

The diversity of microfungi has already been discussed in Chapter 4. To recap, a total of 185 species were recorded in the wet and dry season 2002 (Appendix F). However, the actual number of species may be lower, because apart from one exception, anamorph-teleomorph connections were not confirmed. Of the observed species, 81 were observed on *C. mackinnoniana,* 47 on *E. angustifolius,* 63 on *F. pleurocarpa,* 61 on *O. heterophylla,* 31 on *D. ferruginea* and 41 on *F. destruens*. During the wet season, 135 species were detected in 753 occurrences and during the dry season 111 species in 646 occurrences. Overall, singletons comprised 34 % of microfungal species.

Pairwise comparisons of microfungal complementarity ranged from 70 % to 88 % (Table 5.1). Species, which occurred only in leaves of one host species dominated with 60 % and shared species varied from 22 % in any two host species to 2 % of microfungi detected in all six host species (Table 5.2). When singletons were removed from analyses, 39 % of fungal species occurred in leaves of one host species, 33 % of fungal species were shared between any two host species and 3 % were detected in all six host species studied (Table 5.2). Complementarity was lower in a comparison of sites than of seasons (48 % versus 65 %, Fig. 5.2). When singletons were removed complementarity changed to 22 % for sites and 48 % for seasons (Fig. 5.2).

Average Motyka similarity indices were highest in leaves of the same tree species collected from different sites but in the same season (substratum – species level; 0.53). It decreased for microfungi of the same tree species collected at the same site but in different seasons (season; 0.41) and for different seasons and different sites (site; 0.36; Table 5.3). Microfungal assemblages in leaf litter of congeneric host species were more similar (0.29) than those from host species in different genera but same family (0.14). Microfungi in leaves of host species from different families were the most dissimilar (0.09; Table 5.3). The range of Bray-Curtis values observed in each category shows high variability (Table 5.3).

Table 5.1 Percent complementarity in pairwise comparisons of microfungal assemblages in decaying leaves of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus destruens, F. pleurocarpa* and *Opisthiolepis heterophylla.* Singletons were included in analysis.

	Sp.1	Overlap	Sp.2	Complementarity
Cm-Df	68	17	16	84
Cm-Ea	58	14	28	86
Cm-Fd	62	16	22	84
Cm-Fp	51	14	35	86
Cm-Oh	50	16	34	84
Df-Ea	33	12	55	88
Df-Fd	33	18	49	82
Df-Fp	22	16	62	84
Df-Oh	23	17	61	84
Ea-Fd	45	17	37	82
Ea-Fp	30	22	48	78
Ea-Oh	33	19	48	81
Fd-Fp	21	30	49	70
Fd-Oh	29	19	52	81
Fp-Oh	37	28	35	72
Range		14-30		70-88

Table 5.2 Number of shared microfungal species detected in decaying leaves of one to six tree species. Values in brackets indicate shared microfungal species after singletons have been removed from data set (n=185 species).

No. of tree species compared (*k*)	No. of fungal species shared between *k* tree species	Percentage fungal species shared (%)
1	110 (48)	60 (39)
2	41	22 (33)
3	15	8 (12)
4	12	7 (10)
5	3	2 (2)
6	4	2 (3)

In a three-dimensional NMDS representation of Bray-Curtis dissimilarities, microfungal assemblages on decaying leaves of the four main tree species (*Cm, Ea, Fp, Oh*) were clearly separated from each other (Fig. 5.3) but collections within the same tree species clustered closely together irrespective of site or collection time. Microfungal

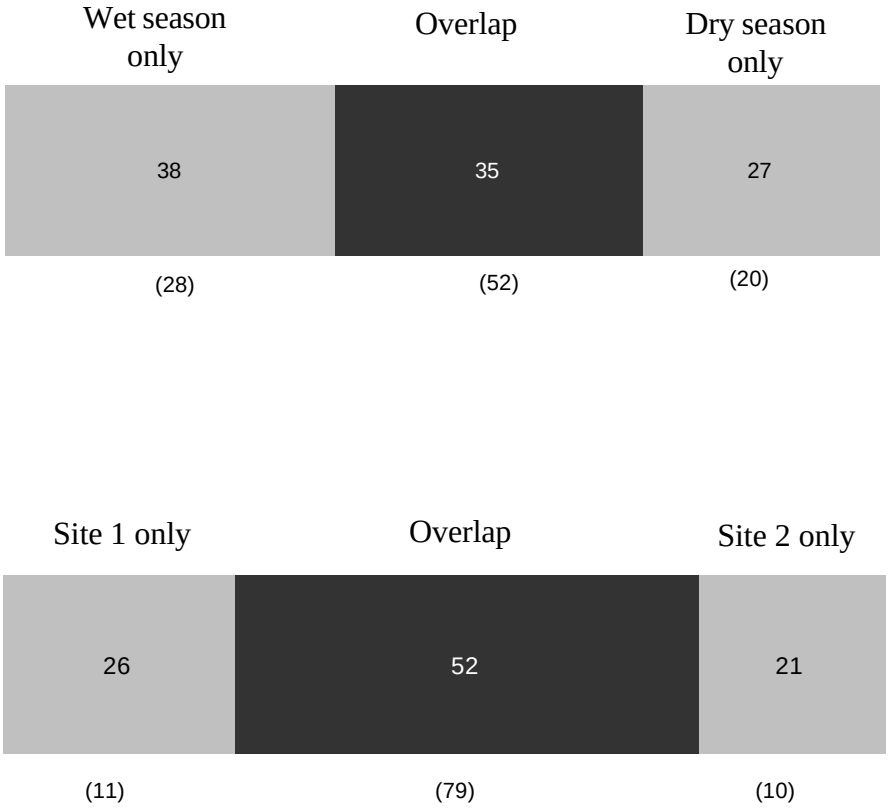
assemblages of the congenerics *F. destruens* and *F. pleurocarpa* grouped closely together. A further two species belonging to the same family but in different genera, viz. *D. ferruginea* and *O. heterophylla*, were also closely associated (Fig. 5.3). The stress level for this NMDS representation was moderate (0.15; Clarke & Warwick 1994) and hierarchical cluster analysis supported the same groupings (Fig. 5.3 & 5.4). The four main clusters of the dendrogram corresponded to the four plant families studied. Subgroups were formed by microfungal collections from *E. angustifolius, C. mackinnoniana, F. pleurocarpa,* and *F. destruens*. In contrast, collections from *D. ferruginea* and *O. heterophylla* were interspersed within two clusters, which separated at a greater distance compared to *F. destruens* and *F. pleurocarpa*. At a finer scale, collections from the same season rather than the same site were associated. The results of ANOSIM showed that variation between microfungal assemblages was strongly associated with phylogeny (host family) ($p=0.001$); all pairwise comparisons between each family were also significant ($p<0.05$). Microfungal assemblages also varied significantly between seasons ($p = 0.007$), but not between sites ($p=0.86$).

Of the leaf morphology and chemistry parameters tested, species richness was significantly and negatively correlated with total phenolics ($R^2=0.77$, $p=0.049$). Significant positive correlations were observed between species richness and thickness ($R^2=0.81$, $p=0.036$) and manganese ($R^2=0.83$, $p=0.033$; Appendix M).

Table 5.3 Summary of Motyka similarities for pairwise comparisons of microfungi in decaying leaves of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus destruens, F. pleurocarpa* and *Opisthiolepis heterophylla.*

Comparisons of categories	Mean Bray-Curtis similarities (Range)
Same host species – different site – same season	0.53 (0.41-0.67)
Same host species – same site – different season	0.41 (0.15-0.64)
Same host species – different site – different season	0.36 (0.13-0.52)
Congeneric host species (*Fd-Fp*)	0.29 (0.20-0.35)
Different host genus – same family (*Df-Oh*)	0.14 (0.04-0.26)
Different host species – different families	0.09 (0.00-0.29)

Figure 5.2 Complementarity of microfungi in decaying leaves of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus destruens, F. pleurocarpa* and *Opisthiolepis heterophylla* showing the percentage of microfungi occurring in either wet or dry season and at site 1 or site 2 and the percentage of species occurring in both seasons and both sites (overlap). Values in brackets indicate percentage after singletons were removed.



5.3.3 Particle filtration

During the wet season 2001, a total of 419 morphotypes were observed among 1575 isolates and in the dry season 276 morphotypes among 2059 isolates. These ranged from 111 to 203 morphotypes in leaves of individual tree species. A discussion of the diversity observed using particle filtration is provided in Chapter 4.

The overlap of morphotypes in pairwise comparisons of tree species ranged from 13 % to 25 % and the corresponding complementarity was 75 % to 87 % (Table 5.4). In the wet season, 69 % of all observed morphotypes were detected only in one host species; 22 % were shared between two, 6 % between three and only 3 % of morphotypes were

observed in all four host species (Table 5.5). Similarly in the dry season, 68 % of morphotypes were observed only in one host species, 22 % in two and 9 % in three (Table 5.5). The percent overlap was slightly higher when singletons were removed (Table 5.5). The average Bray-Curtis similarity indices were 0.31 and 0.36 for morphotypes isolated from the same host species at different sites for the wet and dry season respectively. In contrast, average Bray-Curtis similarities for morphotypes isolated from different tree species either at the same or at different sites ranged from 0.17 to 0.19 (Table 5.6). Due to the low number of data points, it was not appropriate to ordinate datasets obtained by particle filtration or undertake a correlation of species richness with leaf attributes. However, dendrograms based on hierarchical clustering showed that collections grouped according to host species rather than sites (Fig. 5.5).

Figure 5.3 Three-dimensional representation of the relative distance observed in microfungal assemblages from decaying leaves of *Cryptocarya mackinnoniana* (Lauraceae), *Elaeocarpus angustifolius* (Elaeocarpaceae), *F. pleurocarpa* (Moraceae) and *Opisthiolepis heterophylla* (Proteaceae) collected at two sites over two seasons. In the two instances, where more than one tree species represents a family, collections for the second tree species, i.e. *Darlingia ferruginea* (Df) and *Ficus destruens* (Fd), are indicated in the graph. Relative distances were based on a matrix of Bray-Curtis indices and were calculated using Non-metric Multidimensional Scaling. Stress level was 0.15.

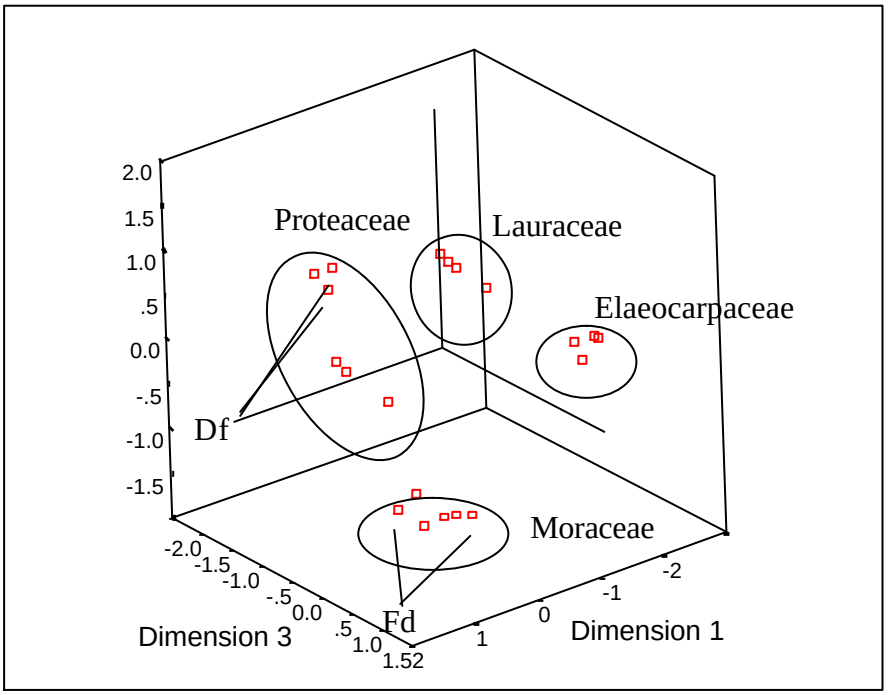


Figure 5.4 Dendrogram of microfungal assemblages in decaying leaves of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus destruens, F. pleurocarpa* and *Opisthiolepis heterophylla* collected over two seasons (wet, 'w', and dry, 'd') and at two sites (Topaz, 'T', and Millaa Millaa, 'M'). Assemblages determined from samples of five leaves from two or three trees per species per site.

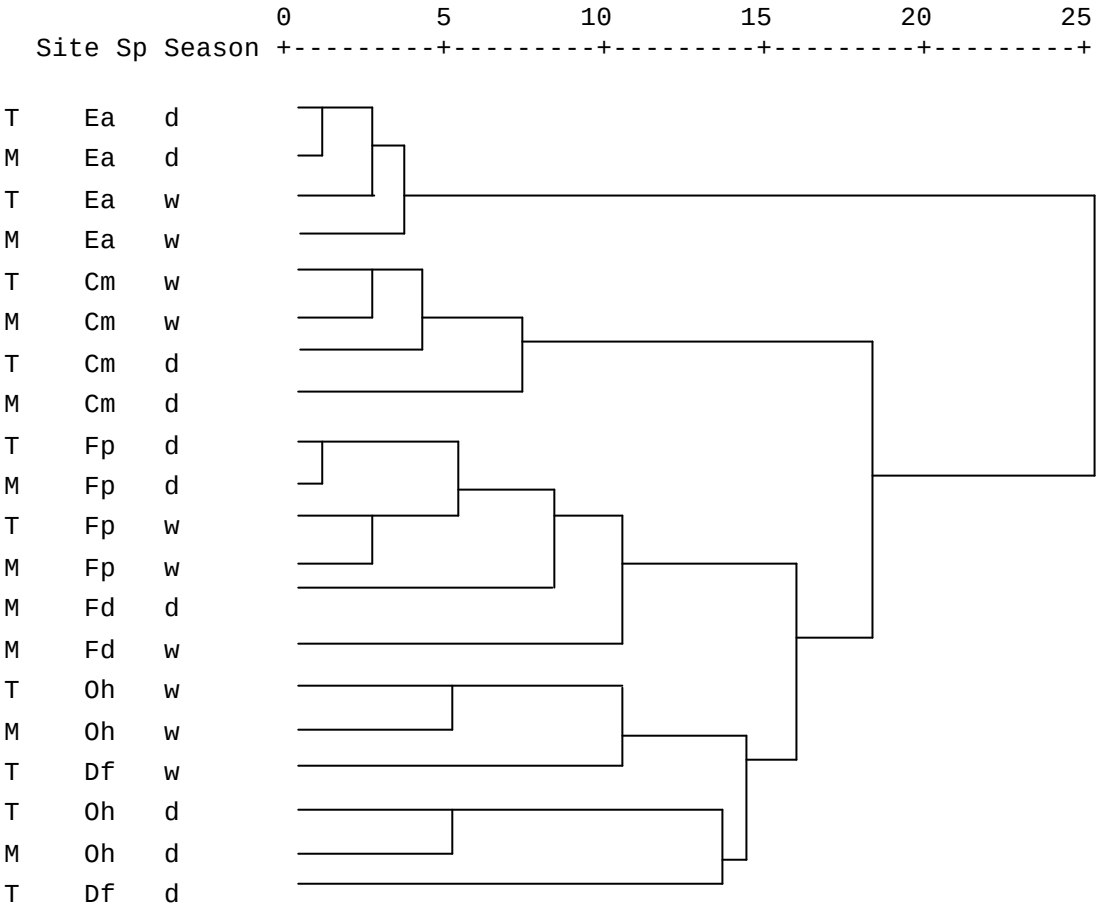


Table 5.6 Summary of Motyka similarities for pairwise comparisons of microfungi in decaying leaves of *Cryptocarya mackinnoniana, Elaeocarpus angustifolius, F. pleurocarpa* and *Opisthiolepis heterophylla* isolated by particle filtration during the wet and dry season 2001.

Comparisons of categories	Mean Bray-Curtis similarities (Range)
Wet season 2001	
Same host species – different site	0.31 (0.24-0.36)
Different host species – same site	0.17 (0.11-0.34)
Different host species – different site	0.15 (0.10-0.28)
Dry season 2001	
Same host species – different site	0.36 (0.25-0.44)
Different host species – same site	0.18 (0.11-0.24)
Different host species – different site	0.19 (0.12-0.27)

Table 5.4 Percent complementarity and overlap in pairwise comparisons of microfungal assemblages in decaying leaves of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus destruens, F. pleurocarpa* and *Opisthiolepis heterophylla,* which were isolated using particle filtration during the wet and dry Season 2001. Singletons were included in analysis.

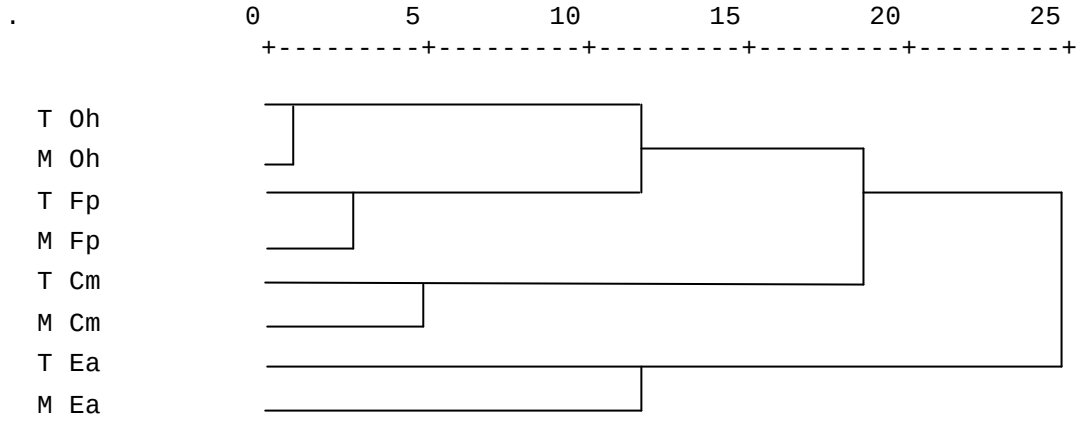
Wet 2001	Sp. 1	Overlap	Sp.2	Complementarity
Fp/Oh	28	22	50	78
Cm/Oh	48	15	37	85
Cm/Fp	56	22	23	78
Ea/Oh	41	13	46	87
Ea/Fp	50	16	34	84
Ea/Cm	35	13	51	86
Range		13-22		78-87
Dry 2001				
Cm/Ea	51	19	30	81
Cm/Fp	48	25	27	75
Ea/Fp	39	21	40	79
Range		19-25		75-81

Table 5.5 Overlap of microfungi in decaying leaves of three (dry season) and four (wet season) tree species, isolated by particle filtration. Values in brackets indicate the overlap of microfungal species after singletons have been removed from data set.

	No. of tree species compared (*k*)	No. of fungal species shared between *k* tree species	Percentage fungal species shared (%)
Wet season 2001	1	289 (102)	69 (44)
	2	90	22 (39)
	3	26	6 (11)
	4	15	3(6)
Dry season 2001	1	188 (87)	68 (50)
	2	63	23 (36)
	3	25	9 (14)

Considerable variation was observed in Motyka similarities calculated for pairwise comparisons (Table 5.6). Hierarchical cluster dendrograms for collections of both seasons formed clusters according to host species (Fig. 5.5). In a comparison of morphotypes grouped according to site, the overlap was 31 % for the wet season and 29 % for the dry season (Fig. 5.6). These percentages were considerably higher when singletons were removed from the analyses (55 % and 47 % respectively).

Figure 5.5 Dendrograms of microfungal assemblage in decaying leaves of *Cryptocarya mackinnoniana, Elaeocarpus angustifolius, F. pleurocarpa* and *Opisthiolepis heterophylla* (*Oh* in wet season only) isolated by particle filtration in A. the wet season 2001 and B. the dry season 2001. Two sites are denoted by 'T' for Topaz and 'M' for Millaa Millaa. The dendrograms were generated by hierarchical cluster analysis using average linkage.



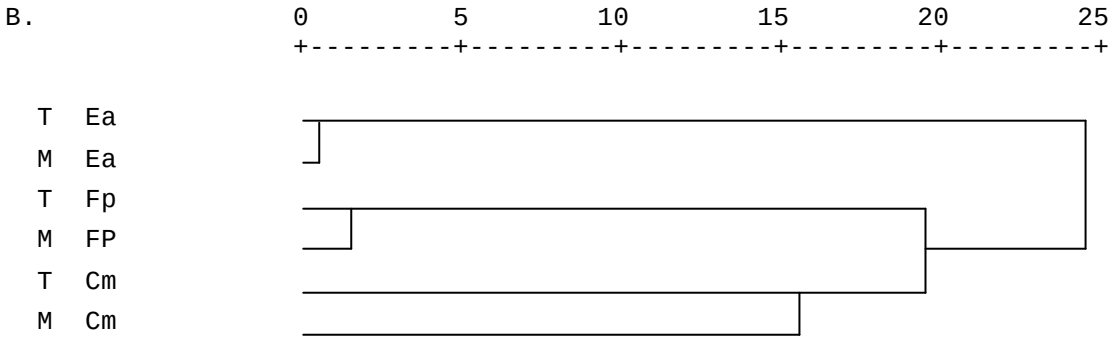
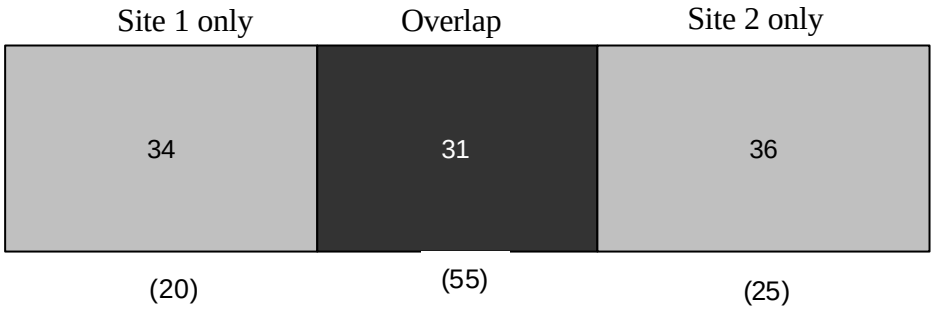
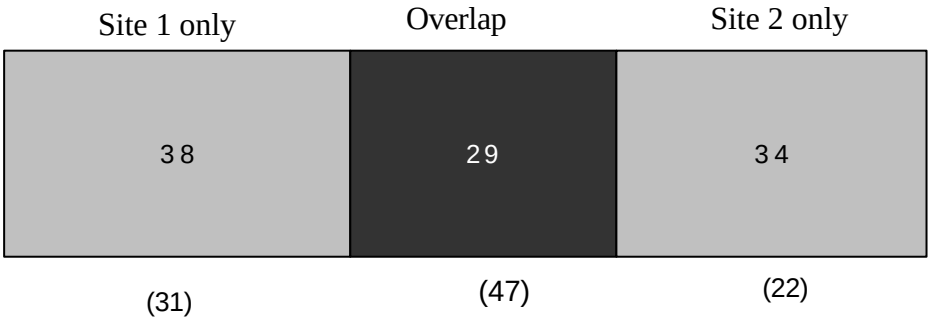


Figure 5.6 Complementarity of microfungi in decaying leaves of *Cryptocarya mackinnoniana, Elaeocarpus angustifolius, F. pleurocarpa* and *Opisthiolepis heterophylla* (*Oh* in wet season only) isolated by particle filtration. The graph shows the percentage of species occurring at either sit 1 or site 2 and the percentage of shared species. A. wet season 2001. B. dry season 2001. Values in brackets indicate percentage after singletons were removed.

A.



B.



5.4 Discussion

In this study, I assessed the relative similarity of microfungal assemblages on different tree species collected from two sites and at two time points for each of the two isolation methods applied. Due to the relatively lesser effort required for direct observations compared with particle filtration, it was possible to assess a greater number of tree species and to apply a number of analyses, such as Nonmetric multidimensional scaling (NMDS). Therefore, data from direct observations will form the main part of the discussion. The importance of applying complementary isolation techniques has been discussed previously (Dickinson 1971; Frankland 1990; Chapter 1) and therefore, I will assess whether both isolation methods will provide congruent conclusions.

In this study, 280 decaying leaves from 28 individual trees of six tree species yielded 185 microfungal species in 1399 occurrences. Microfungal species numbers differed between tree species. As microclimatic conditions did not differ significantly between the replicate quadrats below trees of the different tree species, I hypothesised that differences in species numbers may be related to various leaf characteristics such as leaf area, mass, texture, chemistry, rate of decay and susceptibility to insect attack (Cooke and Rayner, 1984; Cooke and Whipps, 1993; Neville and Webster, 1995). Of the leaf parameters that were available for testing, the levels of phenolics contained in living leaves were significantly and negatively correlated with species richness of saprobic fungi detected by the direct method (Appendix M). The role of phenolic compounds in plant defence against both herbivores and plant pathogens has been well documented (e.g. Stumpf & Conn 1981; Gutschick 1999; Kursar and Coley 2003). In addition, microbial activity during decomposition was found to be negatively correlated with concentration of the condensed tannins and total phenolics in leaf litter and wood (Swift, 1976; Neville and Webster, 1995; Zimmer, 2002). It is conceivable that phenolic compounds persist for some time during the decay process and that higher concentrations could depress overall species numbers of saprotrophic microfungi.

No significant correlation was found between leaf area and species richness but positive correlations were observed for species richness obtained by the direct method with leaf thickness and manganese levels (Appendix M). It seems intuitively correct that thicker leaves provide a more substantial substratum for mycelial growth and thus could support a greater number of species. It is not clear why higher levels of manganese are

correlated with greater species richness. Manganese is a trace element that is required for fungal growth (Jennings and Rayner, 1984) but it can be toxic at higher concentrations. It is a component of fungal enzymes, particularly of lignolytic enzymes produced by white rot fungi (e.g. Mester & Field 1998). Manganese has also been shown to stimulate sporulation in *Trichoderma* (Sierota, 1982) and to enhance biodegradation in complex ways (Aust, 1996). It is currently not known whether the correlation of manganese with species richness is associated with any of these factors. However, the effects of minerals on microfungal assemblages have been previously reported from studies of saprobic soil fungi (e.g. Christensen 1968; Bissett & Parkinson 1979; Widden 1986a). For example, Christensen (1968) postulated that calcium content of litter was a first order regulator of microfungal distributions in soil of Wisconsin conifer-hardwood forests.

Synecological studies such as these and the present study can only serve to generate hypotheses; these need to be tested in more detailed autecological studies. In addition, further data from closely or distantly related host substrata may provide evidence for other significant linear or complex, non-linear relationships between leaf attributes and species richness. For future studies, I recommend that leaf litter attributes and microfungal assemblages be analysed from leaf samples of the same study trees. This is because secondary metabolite concentrations have been shown to vary considerably within plant species, populations and even within the same plant (Shelton, 2000). In addition, matching assessments of leaf attributes and microfungal distributions could extend the analysis beyond a simple correlation between species richness and leaf attributes (Clarke and Warwick, 1994).

Relative distances between collections derived from direct observations were satisfactorily represented in three-dimensional space in a NMDS ordination. In this ordination, host phylogeny at the family level was the most important factor in influencing microfungal distributions; an Analysis of Similarity also supported this result. Microfungi from the same tree species clustered closely even though collected at different sites and in different seasons but four groups corresponding to the four plant families were well separated from each other (Fig. 5.3). Microfungal assemblages on two congeneric hosts, *F. destruens* and *F. pleurocarpa*, formed one group and microfungi from two different host genera of the same family, *D. ferruginea* and *O. heterophylla* were also associated. The wide range of Motyka similarities among

categories (Table 5.5) may point to the role of additional factors or chance in shaping these microfungal assemblages. For example, Carroll & Wicklow (1992) proposed that numerous biotic and abiotic factors may influence fungal distributions. These may include fungal competition, selective grazing by insects, the biotic history of the leaf, microclimatic conditions or chance. A hierarchical cluster dendrogram supported the results of NMDS ordination (Fig. 5.4). Phylogeny of the host appeared most important in shaping the main clusters. One exception was for microfungi from *D. ferruginea* and *O. heterophylla* leaves, which were interspersed and clustered together according to season. Within the same host species, collections of the same season rather than the same site grouped together. Due to the relatively low number of data points for particle filtration, NMDS analysis was not appropriate for these data. Hierarchical clustering applied to these data supported the finding that host species is more important than site factors in shaping microfungal assemblages (Fig. 5.5).

During 2002, the lowest total annual rainfall was recorded since records began in 1954 for Topaz Towalla Road. Nevertheless, a marked difference in total rainfall was observed in the 28 days before collections (Fig. 5.1) and microclimatic indicators differed significantly between the wet and dry season collections. Hence it is not surprising that seasonal differences were detected for microfungi observed by direct method (Fig. 5.2 & 5.5). In the present study, only 52 % of species (singletons excluded) were shared between the wet and dry season collections. To assess within and between season variability, replication of this study within the same season would have been desirable but could not be achieved due to the work intensive nature of isolating and identifying microfungi. Marked seasonal effects on microfungal assemblages have been reported from cold temperate climates of the Northern Hemisphere (e.g. Hudson 1968; Gessner 1977; Kuter 1986; Widden 1986). Fewer studies have considered seasonality in tropical forests and results are inconclusive. For example, variations in frequencies of fungal species during litter decomposition did not appear to be related to climatic conditions in a study of microfungi in a Mexican cloud forest (Heredia, 1993). In contrast, Cornejo (1994) noted significant differences in microfungal numbers in leaf litter between irrigated and control plots during the dry season in a study on Barro Colorado Island, Panama. A seasonal effect was also proposed by Hutton and Rasmussen (1970) who found that 35 % of the 23 plant species studied had no epiphyllous fungi in common between the wet and dry season and 44 % had only one

fungus in common. A major limitation of particle filtration data is apparent here as isolates obtained in different seasons could not be directly compared.

To provide an estimate of the relative importance of factors in shaping microfungal assemblages, Motyka similarities were averaged for collections from four groups of substrata, from sites and from seasons for direct data (Table 5.3) and for substrata and sites for particle filtration data (Table 5.6). The similarity of microfungal assemblages observed by the direct method were influenced by the following factors in order of importance: substratum (same species) > season > site > substratum (same genus) > substratum (same family) > substratum (different families). Thus average similarities were highest in microfungi isolated from the same tree species. Within those, differences in both site and season seemed to contribute to a decrease in similarity with season being the more important factor. The decrease in similarity in different tree species seemed to follow a phylogenetic schema with higher Motyka coefficients observed in more closely related host species than in distantly related ones (Table 5.3). Particle filtration data supported the observation of direct data that host species is the most important factor in shaping microfungal assemblages. Isolates from the same host species were more similar than those from different host species either at the same or different sites (Table 5.6).

Evidence for host recurrence has been previously presented in the literature. For example, quantitative differences of fungi on different litter species have also been reported for studies from Nevada by Cowley (1970), from Panama by Cornejo (1994) and from Mauritius by Dulymamode et al. (2001). Similarly, differential colonisation of leaf litter by aquatic hyphomycetes has been described in studies from Belarus (Gulis, 2001), Spain (Chauvet et al., 1997) and Australia (Thomas et al., 1992). Host recurrence of saprobic microfungi in leaf litter may therefore be not only a tropical phenomenon. For tropical rainforests, the results of the present studies support and extend those of previous studies. For example, a study from the neotropics reported higher Jaccard similarity indices for the same litter species at different sites compared to those of different litter species at the same site (Polishook et al., 1996). In the present study, a 33 % overlap in microfungi between any two host species was detected by direct observations and 39 % and 36 % overlap in morphotypes isolated by particle filtration for wet and dry seasons respectively (Table 5.3 & 5.8). Direct observations identified a 79 % overlap between sites and particle filtrations a 55 % and 47 % overlap (Fig. 5.2 &

5.6). The particle filtration data, in particular, are similar to those reported by Polishook et al. (1996) who observed 42 % overlap in microfungi from two host species and a 52 % overlap between microfungi from two sites. Direct data show a more pronounced difference in overlap between host species and sites. However, both the present and Polishook's et al. (1996) studies suggest that site differences were less important in shaping microfungal assemblages than host species. In fact, the spatial heterogeneity observed in the present study was not statistically significant (Fig. 5.2 & 5.6).

All of the above results suggest that host phylogeny is the most important factor in shaping microfungal assemblages; this conclusion is independent of isolation method or type of analyses applied. It is apparent from the above discussion that data derived by particle filtration offers a reduced resolution in detecting the patterns that were obvious from data derived by direct examination of leaves. Even with a doubling of relative sampling effort in the dry season, observations of potential ecological patterns among particle filtration data depended on the type of analyses applied. In contrast, all analyses applied to data derived by the direct method provided similar results. Several reasons might be evoked to explain the lower resolution of particle filtration data. Firstly, the high species richness observed by particle filtration may be derived not only from fungi actively involved in decomposition processes but also from 'contaminating' propagules on the leaf surface. Precautions were taken to remove contaminating spores before and during particle filtration (Chapter 2). As effective sterilisation of leaf surfaces in decaying leaves comes at the cost of eliminating many of the internal colonisers (Chapter 2; Paulus et al. 2003), I selected a conservative surface treatment for this study. As a result, propagules may have persisted on the leaf surface and ecologically meaningful distributions may have been blurred somewhat. Secondly, one of the advantages of particle filtration for diversity studies is that it bypasses the requirement for a fungus to sporulate before it can be discovered; it could therefore detect dormant propagules, such as chlamydospores or microsclerotia, within the leaf lamina. This could prove to be a disadvantage for ecological studies, as microfungi not actively involved in decomposition processes would also be detected. Conversely, one could argue that the results of particle filtration may be less biased than those of direct observations. With the direct method, only a narrow range of taxa may have sporulated on the leaf surface (and thus have become detectable) due to the relatively constant conditions imposed by incubation in the laboratory compared to the fluctuating

conditions in the rainforest. Whether this could have introduced a bias to the data is not known, however, a similar degree of overlap between host species was observed by both methods (Tables 5.1 & 5.4). In any case, the conclusion that host phylogeny is the most important factor in shaping microfungal assemblages on leaf litter is strengthened by the agreement of results derived from two complementary isolation methods and by different analyses.

Host exclusivity and host recurrence

Host recurrence is most evident in the abundant taxa, as less frequently observed taxa may be subject to sampling error. Between 7 and 14 species observed by the direct method per substratum type had relative abundances of more than 3 % (Table 5.1). Among the higher abundance classes, both well known, cosmopolitan species and new taxa were detected. For example, on *C. mackinnoniana* the wide spread species *Bahusutrabeeja dwaya, Beltraniella portoricensis, Dictyochaeta simplex*, *Menisporopsis theobromae, Wiesneriomyces javanicus* were among the most abundant but this also included a species new to science, *Thozetella queenslandica* (Paulus et al., 2003c). Of these abundant taxa, only two were solely found on *C. mackinnoniana* leaf litter while the others showed quantitative differences between substrata (Appendix A). In contrast, *F. pleurocarpa* leaf litter seemed to host a higher percentage of new and restricted taxa among the microfungal species with higher abundances. Of nine microfungal species in *F. pleurocarpa* leaf litter with relative abundances greater than 3 %, four were new to science. This includes a new genus of Dermateaceae, *Discostroma ficusicola* sp. nov., a new *Gaeumannomyces*-like species and a new taxon, which in molecular studies showed an affinity with *Myrothecium* but may represent a new genus (P. W. Crous, pers. comm.).

Species present on many leaf samples, which are also dominant on individual leaves, may contribute to a major extent to decomposition processes. Some observed sporulation patterns were pronounced and consistent across samples. For example, fruiting bodies of *Gnomonia elaeocarpa* were observed to cover most of the leaf lamina on 86 % of all *E. angustifolius* leaf samples. Together with *G. queenslandica,* which was restricted to petioles and midribs and present in 72 % of all leaf samples, this species must have contributed to a major extent to the decomposition of those leaves. It

is speculative whether these two species have evolved to efficiently degrade *E. angustifolius* leaves in the presence of high levels of phenolics (Appendix M). Another species showed strong tissue specificity, namely a new genus of Dermateaceae. This species was restricted to petioles and midribs of *Ficus* leaves and may be involved in the decomposition of latex (Chapter 6). Although these species may be host exclusive, information is currently lacking about their life histories and potential nutritional modes; therefore I am unable to record any of them as host exclusive under the narrow definition provided by Zhou and Hyde (2001). In contrast, Lodge and Cantrell (1995) reported that a number of *Xylaria* spp. are host specific and one of those has been confirmed as strictly saprobic (Lassoe and Lodge, 1994). Host exclusivity has also been suggested for a number of other taxa particularly from palms, for example *Oxydothis alexandrae* (Zhou and Hyde, 2001). A different distribution pattern to those on *E. angustifolius* leaves was evident on leaves of *C. mackinnoniana, F. pleurocarpa* and *O. heterophylla* on which most of the abundant species were more evenly distributed and showed a low dominance.

The various distribution patterns observed at different scales suggest that host recurrence is a complex phenomenon that may be controlled by more than one underlying factor. A number of causes for host recurrence of microfungi have been proposed, but these still await further examination. One hypothesis is that fungi occur within leaves as endophytes and when leaves senesce, these fungi switch to a saprotrophic life style. The fact that they are inside the plant gives them a competitive edge over fungi whose spores must arrive by aerial dispersal. Zhou & Hyde (2002) have suggested that the early colonizers in succession studies are originally endophytes. While it seems reasonable to hypothesise that recurrent saprobes may start out as pathogens or non-pathogenic inhabitants, Lodge (1997) found little evidence to support this hypothesis. When endophytes and saprobes were studied from the same trees, only 22 % of endophytes could be recovered from leaf litter. This amounted to only 1 % of the litter fungi detected. I found recurrent patterns in well-known, cosmopolitan saprobes, which seem to support the observation by Lodge (1997). Polishook et al. (1996) suggested that physical and chemical leaf characteristics rather than host phylogeny may influence the distributions of saprobic microfungi in leaf litter. In the present study, phylogeny and leaf attributes were confounded. Further work is required

to assess whether leaf attributes, endophytic associations or a mixture of both control fungal distributions.

Although it is impossible within the scope of this study to determine host recurrence in species of low abundance classes, they deserve consideration by the fact alone that they usually represent a high percentage of species in microfungal datasets (Chapter 4). Of all microfungal species recorded in our study, 60 % were detected in leaves of only one tree species (Table 5.2) and 34 % of all fungal species were single records (Chapter 4). A similar percentage for microfungal species found in leaves of only one tree species (63 %) was reported by Parungao et al. (2002); in that study a higher percentage of singletons (53 %) was detected. Both studies were undertaken in two rainforest sites of the same region and utilised the same method of isolation. Parungao et al. (2002) examined leaves of more tree species but a smaller number of samples. Thus, the percentage of singletons detected seems to be dependent on the sampling effort while the percentage of species observed in leaves of one tree species appears more or less consistent across the two studies. Furthermore, the percentage of morphotypes on only one leaf type detected by particle filtration, i.e. 68 and 69 % (Table 5.5), were remarkably similar to those noted by direct observations. Although an element of chance in observing these similar percentages cannot be excluded, the issue of host recurrence in singleton species warrants further exploration. For example, singletons may include a percentage of host recurrent species, which are well-adapted to leaves of a particular host species. These species could be present in low but relatively consistent numbers in leaves of a particular tree species at the time of sampling. With the onset of optimal microclimatic or substratum-specific conditions, these species could proliferate and become abundant. In a 'snap shot' study such as the present study, this changing pattern would not be detectable (Loreau et al., 2001). If host recurrence in singleton species was confirmed in future studies, estimates of fungus to host ratios in tropical rainforests would need to be scaled upwards accordingly. In an alternative scenario, singleton species may have established on a leaf by chance and persist under suboptimal conditions; these might be drawn from a common pool of litter fungi or may be adapted to different tree species. In this case, overall lower estimates of host to fungus ratios would be indicated for tropical rainforests. Further work is required to address those questions. Studies examining the nature of singleton species would need to be undertaken at a very fine temporal and spatial scale and may be hampered by the need

for destructive sampling. Despite the methodological problems facing mycologists, understanding the nature of singletons is essential in arriving at realistic quantitative estimates of host recurrent fungi in tropical rainforests.

5.5 Summary

In this chapter, I have reported on the differences in microfungal diversity, distribution and species composition in leaf litter of three to six rainforest tree species. The results of both the direct and particle filtration data suggest that microfungal species richness and patterns of distribution are strongly influenced by host phylogeny. The species richness of microfungi observed by the direct method correlates with the level of phenolics present in living leaves. Therefore, a systematic characterisation of plant secondary metabolites of the leaf species studied might provide further insights into what drives species richness. Two other factors that appear to affect microfungal species richness on various host species are leaf thickness and manganese. The influence of host phylogeny on the distribution of saprobic microfungi as observed by the direct method was significant at the family level. Although the low number of replicates at lower taxonomic ranks did not allow a test of significance, an increasing similarity in microfungal assemblages was evident for trees, which were more closely related. Microfungal distribution was also significantly affected by seasonal but not by site factors. Particle filtration data also supported the finding that host phylogeny is an important determinant of microfungal distributions but seasonal differences were not assessed for particle filtration data due to the difficulty in comparing non-sporulating morphotypes across different time frames. The detection of site differences depended on the type of analysis applied. Host recurrence appears to be a complex phenomenon that involves both wide-spread saprobes and potentially restricted taxa. It is likely that a number of factors are implicated in the development of this pattern.

CHAPTER SIX

Fungus-Insect Interactions

6.1 Introduction

> *"Although their physiology is relatively unexplored, in ubiquity and variety fungus-animal interactions have equal standing with those occurring between fungi and plants".* *Cooke and Whipps (1993)*

Ubiquitous as those interactions may be, Cooke and Whipps (1993) also pointed out that studying those interactions is inherently difficult due to great and subtle variations in associations. Opportunities for many different kinds of associations are provided in decomposing organic matter such as leaf litter, which is shared by a myriad of arthropods and fungi. For example, some arthropods are adapted to feed directly on mycelia and fruiting bodies (Kukor and Martin, 1987). In addition to mycophagy of this kind, fungi may also be impacted by detritivores, which consume vast amounts of decomposing organic matter containing living fungal material. This leads not only to the destruction of fungal biomass but also to the disappearance of fungal resources and habitats (Cooke and Whipps, 1993). In response to such attacks, some fungi have developed defence systems, such as the production of antifeedant chemicals (Wicklow, 1988).

Notwithstanding the fact, that insects can affect fungal community structure, fungi may also benefit from associations with arthropods. For example, many fungi depend on insects for the dispersal of propagules (Beaver, 1989) and for the provision of habitats and resources. The latter may range from a fungus growing on insects themselves (Wilding et al., 1989) or insect structures such as termite mounds (Batra and Batra, 1979) to fungi benefiting from 'enrichment disturbance' i.e. the nutritional input by insects through defaecation (Cooke and Whipps, 1993). In turn, fungi supply many

insects with a food source, not only directly through fruiting bodies and mycelia, but also indirectly by making plant substrata more palatable. This may be achieved for example by removing plant antifeedant chemicals and by softening tough tissues (Arsuffi and Suberkropp, 1989).

During an initial survey of leaf litter microfungi (Chapter 4, Paulus et al. 2003), I consistently observed a discomycete on petioles and midribs of the endemic strangler figs *Ficus pleurocarpa* F. Muell. and of *F. destruens* F. Muell. ex C.T. White (Moraceae). This fungus represents a new genus in the Dermateaceae (Chapter 7) and has yet to be validly published. In the majority of *F. pleurocarpa* leaves examined, this fungus co-occurred with a beetle, *Coccotrypes* aff. *vulgaris* (Eggers).

Aims

The major aims of this chapter are

- to assess the association of 'Dermateaceae F472' and *Coccotrypes* aff. *vulgaris*, i.e. do they simply co-occur in the same habitat or does any interaction exist between these two organisms?
- to explore the distribution of 'Dermateaceae F472' over space and time

6.2 Materials and Methods

6.2.1 Spatial and temporal distribution of Dermateaceae F472

Details of study sites, host trees and survey designs for a succession study and a study of six different tree species have been reported in Chapter 3. During the succession study of microfungi in *Ficus pleurocarpa* leaf litter (Chapter 4), both the presence of 'Dermateaceae F472'and its anamorph and the presence of bore holes and frass indicative of *Coccotrypes* colonisation (Brook, 2001) was recorded for each leaf. The anamorph/teleomorph connection of 'Dermateaceae F472' and 'anamorph F455' were confirmed using standard techniques (Kendrick 1979; Chapter 7). Cultures and dried material of 'Dermateaceae F472' and its anamorph were deposited at the Queensland Plant Pathology Herbarium BRIP, Department of Primary Industries and dried specimens at RBG, Kew. The petioles of 20 leaves with frass were dissected to confirm the presence of *C.* aff. *vulgaris* and to collect beetle and larval specimens. Specimens of

Coccotrypes aff. *vulgaris* (Scolytinae) are in the private collection of Dr R. Beaver, who also identified the beetle.

The presence of 'Dermateaceae F472' was also recorded in leaf litter of six tree species in May and September 2002 (Chapter 5). The distribution of this fungus on different leaf types and during different seasons is reported. A Chi-square test of homogeneity with Yate's correction was carried out to test for differences in occurrence between the wet and dry season (SPSS, 2001).

6.2.2 Recolonisation experiment in a mesocosm

In June 2002, a total of 78 fallen leaves were collected under three *F. pleurocarpa* trees. Twenty *F. pleurocarpa* leaves were used as source of fungal spores and beetles while 58 leaves, 38 freshly fallen and 20 at later stages of decay, were used to study recolonisation by 'Dermateaceae F472' and *C.* aff. *vulgaris*. Freshly fallen leaves exuded latex when cut while those at later stages of decay were lacking visible latex exudate. Half of the leaves in each group (19 freshly fallen and 10 at later stages of decay) were sterilised with ethylene oxide (EtO) for 24 h at 21º C dry temperature, followed by a vacuum and carbon dioxide purge and six further vacuum/air changes (R.A. Dibbs, Brisbane). The other leaves were stored at 21º C as controls. A 5 × 5 mm piece was cut from each leaf using a sterile scalpel blade and placed on Malt Yeast Agar (MYA, Gams et al. 1998) to test the efficacy of EtO sterilisation. To assess whether petiole size was a determining factor in colonisation by *C.* aff. *vulgaris*, 30 unidentified, mixed leaves with petioles as large or larger than those of *F. pleurocarpa* were also collected.

A humid chamber (78–85 % R. H.) was created in an enclosed plastic box (1.5 × 0.9 × 0.2 m) by covering 30 mm of sterilised, moistened vermiculite with banana leaves, which had been surface sterilised with 70 % ethanol. All EtO sterilised and untreated *F. pleurocarpa* leaves and mixed leaf samples were labelled with a masking tape and placed in haphazard positions on the banana leaves. Leaves were incubated at ambient temperature (~25º C). After three weeks they were examined for the presence of 'Dermateaceae F472' and for bore holes and fresh frass indicative of recent *Coccotrypes* colonisation. The petioles of 20 leaves (ten control and ten EtO sterilised leaves) were dissected to confirm the presence of *C.* aff. *vulgaris* beetles and larvae. All

leaves were re-examined after six weeks for further colonisation events. Results were analysed using hierarchical loglinear analysis with leaf treatment, leaf decay, beetle colonisation and discomycete colonisation as variables (SPSS, 2001).

6.2.3 Recolonisation experiment in the field

Twenty leaves were collected in June 2003, ten of which were EtO sterilised and ten retained as control leaves. These leaves were placed in close proximity to each other under a *F. pleurocarpa* tree at the Topaz site and examined after three weeks. The presence of 'Dermateaceae F472' and of *C.* aff. *vulgaris* was recorded for all leaves as noted above. Leaves were checked again for fruiting bodies of 'Dermateaceae F472' after a further six weeks to exclude that fruiting bodies were overlooked due to slow development.

6.3 Results

6.3.1 Spatial and temporal distribution of Dermateaceae F472 and *Coccotrypes* aff. *vulgaris*

The percent occurrences of 'Dermateaceae F472', its anamorph and *C.* aff. *vulgaris* are shown in Figure 6.1. The anamorph of 'Dermateaceae F472' was observed in 10 % of green leaves and in 60 % of freshly fallen, yellow leaves. Freshly fallen leaves generally did not have any disease symptoms such as lesions or spots. The teleomorph was present in 90 % to 100 % of leaves that had been on the ground between 7 and 30 days.

No evidence was found of *Coccotrypes* aff. *vulgaris* feeding on fruiting bodies of 'Dermateaceae F472' but tunnels within petioles and midribs were observed in which both larvae and adult beetles were found. Colonisation of petioles by *C.* aff. *vulgaris* was first observed after seven days and it peaked on day 30 with 100 % of leaves affected. Petioles and midribs that were infected by both 'Dermateaceae F472' and *C.* aff. *vulgaris* decayed faster than the leaf lamina (Fig. 6.2).

The percent occurrence of 'Dermateaceae F472' during the wet and dry season 2002 is shown in Table 6.1. 'Dermateaceae F472' and/or its anamorph were observed on 80 % and 83.3 % of *F. pleurocarpa* leaves and on 50 % and 70 % of *F. destruens* leaves for dry and wet season respectively but were absent from other tree species. Differences in occurrence between the wet and dry season were not significant (p=0.701). *Coccotrypes*

aff. *vulgaris* was observed in leaves of *F. pleurocarpa* but not in leaves of *F. destruens* or in leaves of four other tree species.

Figure 6.1 Occurrence of 'Dermateaceae F472', its anamorph and *Coccotrypes* aff. *vulgaris* on *Ficus pleurocarpa* leaves collected 0, 7, 14, 30, 46, 62, 78 and 94 days after leaf fall.

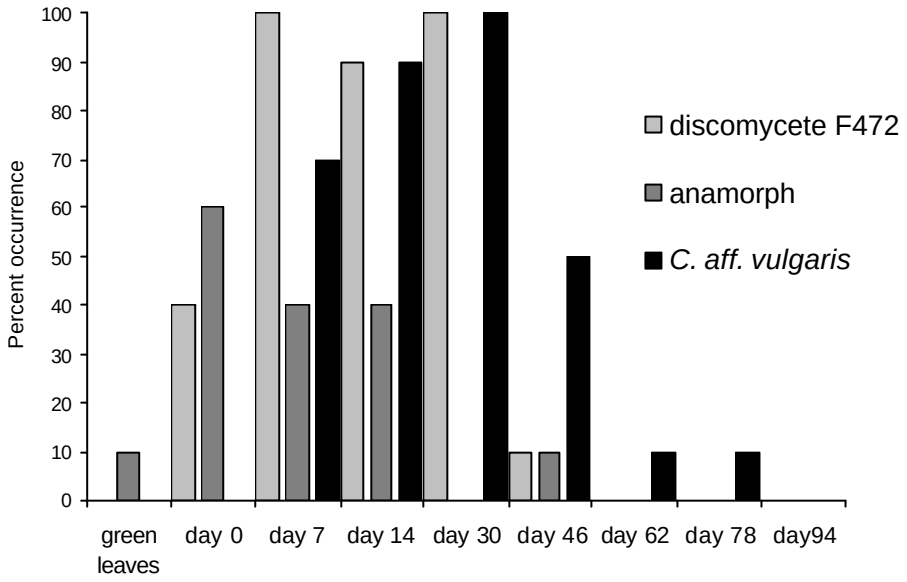


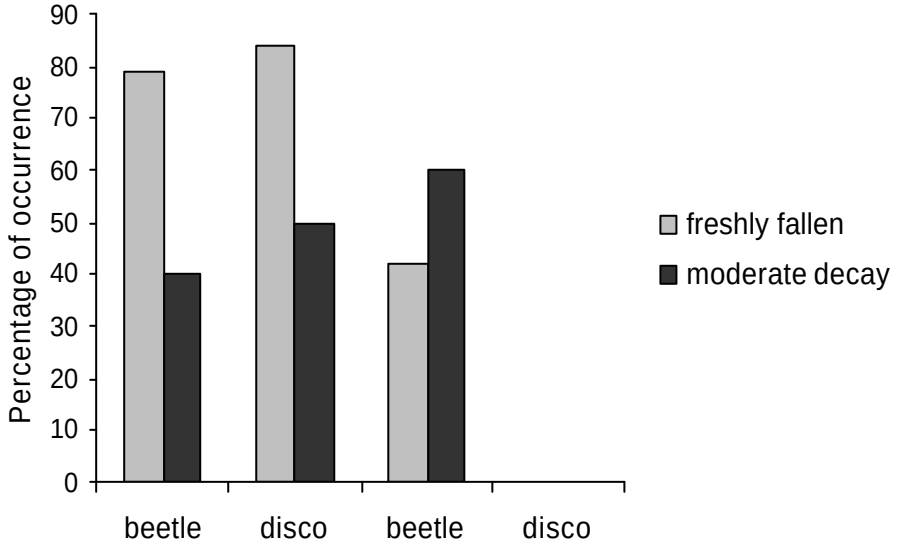
Figure 6.2 Abscissed leaves of *Ficus pleurocarpa* with decomposition occurring rapidly in midrib and lateral veins

Table 6.1. Occurrence of 'Dermateaceae F472' (anamorph and teleomorph) during the wet and dry season 2002 on decaying leaves of Ficus pleurocarpa, F. destruens and other tree species. The percentage of leaves examined is provided in brackets.

	Wet season 2002 (%)	Dry season 2002 (%)
Ficus pleurocarpa n = 30	25 (83.3)	24 (80)
F. destruens n = 10	7 (70)	5 (50)
Other leaves n = 100	0	0

a *Cryptocarya mackinnoniana*, *Elaeocarpus angustifolius*, *Opisthiolepis heterophylla* n=30; *Darlingia ferruginea* n=10.

Figure 6.3 Percent occurrence of 'Dermateaceae F472' (disco) and *Coccotrypes* aff. *vulgaris* (beetle) on sterilised and control leaves of *Ficus pleurocarpa* incubated in a humid chamber



6.3.2 Recolonisation experiment in a mesocosm

Ethylene oxide sterilisation of leaves was effective, as no fungi grew from leaf sections of sterilised leaves on MYA. Figure 6.3 shows the occurrence of 'Dermateaceae F472' and *C.* aff. *vulgaris* in sterilised and control leaves. In control leaves, both had a higher occurrence in freshly fallen leaves (78.9 and 84.2 % respectively) compared to leaves at a later stage of decay (40 and 50 % respectively). 'Dermateaceae F472' was unable to colonise sterilised leaves and the percent occurrence of the beetle was lower on freshly fallen, sterilised leaves (42.1 %) than on freshly fallen control leaves (84.2 %). A different species of fungus, *Pestalotiopsis* sp., was present in petioles and midribs of all freshly fallen, sterilised *F. pleurocarpa* leaves within three weeks. No fruiting bodies of 'Dermateaceae F472' were detected after a further six weeks.

Table 6.2 Effects of factors and interactions in beetle and 'Dermateaceae F472' colonisation of *Ficus pleurocarpa* leaves in a laboratory experiment as determined by loglinear hierarchical analysis. P-values of significant factors and interactions are given in italics.

Effect	d.f.	Likelihood ratio chi square change	p-value
Treatment	1	N/A	N/A
Decay	1	5.680	*0.017**
Beetle	1	N/A	N/A
Discomycete	1	N/A	N/A
Treatment × Decay	1	2.003	0.157
Treatment × Beetle	1	8.446	*0.004***
Treatment × Discomycete	1	48.450	*<0.001***
Decay × Beetle	1	0.123	0.726
Decay ×Discomycete	1	1.712	0.191
Beetle × Discomycete	1	23.686	*<0.001***
Treatment × Decay × Beetle	1	0.019	0.986
Treatment × Decay × Discomycete	1	0.000	0.986
Treatment × Beetle × Discomycete	1	0.552	0.458
Decay × Beetle × Discomycete	1	1.351	0.245
Treatment × Decay × Beetle × Discomycete	1	0.000	1.000

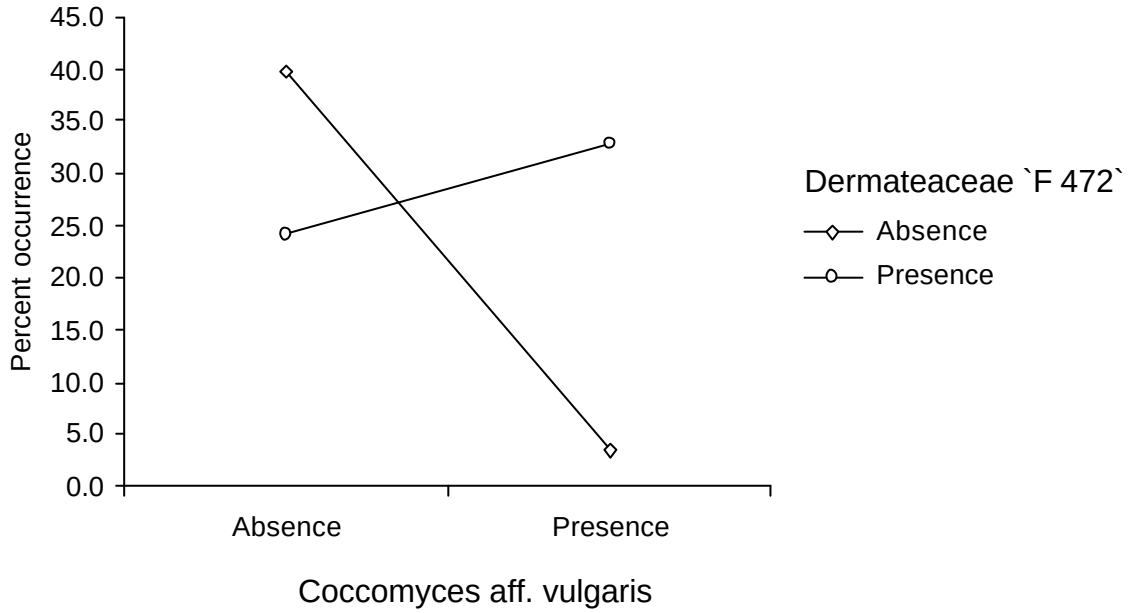
In a loglinear analysis, significant interactions were observed between leaf treatment and beetle colonisation, leaf treatment and 'Dermateaceae F472' colonisation, and beetle and 'Dermateaceae F472' colonisation (Table 6.2). Sample size was significantly

greater for freshly fallen than decaying leaves as indicated by the significant result of ‘decay’.

Figure 6.4 shows the interaction between 'Dermateaceae F472' and *C.* aff. *vulgaris*. In the presence of ‘Dermateaceae F472’, 32.8 % of leaves were infested with *C.* aff. *vulgaris* while only 3.4 % of leaves were infested with the beetle in the absence of 'Dermateaceae F472'.

Observation of petioles revealed the presence of larvae and adult beetles, suggesting that *C.* aff. *vulgaris* completes its life cycle within this habitat. None of the 30 unidentified leaves supported either ‘Dermateaceae F472’ or *C.* aff. *vulgaris*.

Figure 6.4. Interaction between 'Dermateaceae F472' and *C.* aff. *vulgaris* in *Ficus pleurocarpa* leaf baits



6.3.3 Recolonisation experiment in the field

Fruiting bodies of 'Dermateaceae F472' were observed on 50 % of control leaves while none were found on sterilised leaves. Similarly, *C.* aff. *vulgaris* was detected on 60 % of control leaves but on none of the sterilised leaves. An examination of leaves after a further six weeks did not detect any fruiting bodies of 'Dermateaceae F472' in sterilised leaves.

6.4 Discussion

This study has demonstrated that 'Dermateaceae F472' is recurrent on the petioles and midribs of at least two species of *Ficus.* Since this fungus was not detected in any of the other tree species studied, this fungus may be host exclusive. Other groups of fungi have been reported to utilise petioles and midribs of leaves as specialised habitat and resource (e.g. Barr, 1978; Monod, 1983; Barr, 1991; Yanna et al., 2001b; Paulus et al., 2003a). One general difference between leaf lamina and petioles/midribs is a greater aggregation of lignified, supportive tissues in the latter (Raven et al., 1992). In the case of *Ficus* leaves, petioles and midribs also contain latex. Latex is thought to be involved in plant defences due to its sticky texture and to inhibitory compounds such as terpenoids, proteinases and alkaloids (Doussard and Eisner, 1987; Edwards, 1987; Moore et al., 1995; Kim et al., 2003). Nevertheless, latex also contains nutrients such as proteins, carbohydrates and oils (Moore et al., 1995) and thus may provide a resource for organisms, which are able to overcome its adverse effects (Raven et al., 1992). The enzymatic capabilities of 'Dermateaceae F472' have not been investigated but its presence in latex containing petioles would suggest that it is able to tolerate if not degrade this substance. This ability to utilise latex could explain the apparent tissue and host specificity of 'Dermateaceae F472'. Furthermore, the occurrence of 'Dermateaceae F472' did not vary significantly between seasons. This may indicate that substratum dependent factors strongly shape its distribution rather than climatic factors or chance as could be postulated for some other fungi observed in this project.

'Dermateaceae F472' appears to infect senescing leaves, as its occurrence was relatively low in green leaves and much higher in freshly fallen, yellow leaves (Fig. 6.1). In addition, it was unable to recolonise sterilised leaves *in vitro* and on the forest floor. These facts and the absence of overt disease symptoms in abscised leaves point to a brief endophytic phase (Bacon and White, 2000) while its prolonged persistence in decaying leaves (Fig. 6.1) would also suggest that this fungus is able to switch to a saprobic mode of nutrition (Cooke and Whipps, 1993).

In addition to 'Dermateaceae F472', the petioles and midribs of *F. pleurocarpa* also host a scolytinoid beetle, *Coccotrypes* aff. *vulgaris* during its complete life cycle. This beetle was not observed on a second species of *Ficus*, *F. destruens,* which has smaller petioles and midribs compared to *F. pleurocarpa.* However, petiole size alone does not seem to be the determining factor for the apparent preference of *C.* aff. *vulgaris,* as leaves of other tree species with robust petioles were not colonised by this beetle. This apparent specificity of *C.* aff. *vulgaris* for petioles of *F. pleurocarpa* was observed despite the challenging environment this habitat represents to insect colonisers. The challenge of overcoming the sticky and potentially toxic latex in plant tissues has been resolved by some insect specialists by draining latex from the laticiferous system (Doussard and Eisner, 1987; Edwards, 1987) but this behaviour was not detected in the case of *C.* aff. *vulgaris.* The observations of this study suggest an interaction between 'Dermateaceae F472' and this scolytinoid beetle both in a mesocosm study and in the field (Table 6.2, Fig. 6.4). Colonisation of petioles by 'Dermateaceae F472' precedes that of the *C.* aff. *vulgaris.* In addition, more leaves were infested with *C.* aff. *vulgaris* in the presence of 'Dermateaceae F472' while lower levels of beetle infestation were observed when 'Dermateaceae F472' was absent (Fig. 6.6). In the field, where beetles had a greater choice of substrata, this effect was even more pronounced; *C.* aff. *vulgaris* was completely absent from sterilised leaves, which had not been recolonised by 'Dermateaceae F472'. It is unlikely that residues such as glycol, chlorohydrin and bromohydrin resulting from EtO sterilisation (Australian New Zealand Food Standards, 2003) would have deterred *C.* aff. *vulgaris* as EtO sterilised leaves at later stages of decay were readily colonised by this beetle in the mesocosm experiment.

The exact mechanism of the interaction between 'Dermateaceae F472' and *C.* aff. *vulgaris* is not known but this fungus may facilitate the colonisation of *F. pleurocarpa* petioles by the beetle by degrading latex and in effect neutralising the plant's

antifeedant strategy (Arsuffi and Suberkropp, 1989). Whether fungal mycelium is ingested by the beetle only incidentally, whether it increases palatability of plant tissues to the beetle in some way or whether it adds to the nutritional value of plant tissues is not known.

From the observations of this study, it appears that the association of beetle and fungus is detrimental to the fungus. This is suggested by the swift decline in fungal fruiting bodies after petioles are colonised by the beetle. In addition, the rapid destruction of its habitat (Fig. 6.2) could be expected to impact negatively on this fungus as well as the fungal species richness of this substratum (Baerlocher, 1980). Whether this association has any benefits for the fungus could not be established within the scope of this study. Mutualistic interactions between herbivorous insects and fungi have been reported previously (Batra, 1977; Wilding et al., 1989). Among the best known are the association of attine ants and their symbiotic basidiomycete fungi (Cherrett et al., 1989) and that of the ambrosia beetles and their ophiostomatoid fungal partners (Beaver, 1989). In these associations, the fungus benefits as it is dispersed to new habitats and provided with a competitive advantage under the 'care' of their insect partners. Dispersal of 'Dermateaceae F472' spores has not been examined and poses an interesting question, in particular, as different spore forms are present in the anamorph and teleomorph (Chapter 7). Spores of the teleomorph are dry and dispersal on air currents could be observed under the microscope. In contrast, the anamorph forms slimy masses of spores, which would suggest that it relies on insect vectors for dispersal.

Observations suggest that fungus-insect associations may enhance decomposition, at least in the case of *F. pleurocarpa* leaves. How common fungus-insect associations are in rainforest habitats is not known. Specialised guilds of beetles have been previously reported to utilise petioles as habitats and food source (e.g. Beaver 1979, Jordal and Kirkendall 1998) but whether interactions with fungi play a role in any of these cases has not been investigated. Among seven rainforest tree species studied in the present project, one interaction has been confirmed and circumstantial evidence exists for a second potential interaction. In that instance, an unidentified larva consistently colonised and rapidly ingested the lamina of *Elaeocarpus angustifolius* leaves. Leaves of *Elaeocarpus angustifolius* contain relatively high levels of phenolics (Kanowski, 1999) and two apparently host- and tissue-specific species of *Gnomonia* were abundant in its leaves (Chapters 5 and 7; Paulus et al. 2003b; Hyde et al. 2004). Considering that

phenolics are a group of secondary metabolites involved in plant defences (Kutchan, 2001), it is possible that *Gnomonia elaeocarpa* neutralises the plant's antifeedant chemicals. Thus, this may be a second instance in which fungus-insect interactions accelerate decomposition processes. In tropical rainforests, interactions such as these may be vital for rapid and efficient turnover of organic material and therefore ecosystem function. Further research is required to increase our understanding of the mechanisms and physiology involved and of the robustness of these interactions in times of disturbance.

6.5 Summary

In this study the spatial and temporal distribution of an apparently tissue- and host-exclusive discomycete was examined. This fungus was only detected on petioles and midribs of fallen leaves of two species of *Ficus* and showed no significant seasonal variation. Its low abundance in freshly fallen green leaves and higher abundance in freshly fallen yellow leaves as well as the absence of disease symptoms provides an indication that this fungus may infect senescing leaves while its prolonged persistence in fallen leaves suggests that it is also able to function effectively as a saprotroph. A significant interaction between this fungus and the beetle *C.* aff. *vulgaris* was detected in a mesocosm study and this was supported by observations in the field. I hypothesised that 'Dermateaceae F472' neutralises anti-feedant chemicals contained in the latex. The rapid degradation of *F. pleurocarpa* petioles, midribs and lateral veins suggest that interactions between the two organisms enhances the rate of decomposition in these tissues.

CHAPTER SEVEN

Taxonomy of Microfungi

7.1 Introduction

Taxonomy is the discipline concerned with the theory and practice of classification of organisms and is not clearly separable from systematics or the study of biological diversity and relationships among organisms. The basic unit in the hierarchy of classification is the species (Guarro et al., 1999). What defines a species has been at the centre of heated philosophical debate leading to the development of at least 22 species concepts (Claridge et al., 1997). Many of these concepts overlap and some are distinguished only by esoteric differences (Mayden, 1997). Ideally, a species concept should be as general, applicable and theoretically significant as possible (Hull, 1997). However, these are often conflicting requirements and a universal fungal species concept has not been developed. Investigating a presumed species using different concepts and methods may be most applicable for the study of selected taxa that are taxonomically difficult, economically important or of other interest, for example, for research into population structure and speciation processes. Due to time and resource constraints, it is improbable that all of fungal diversity can ever be investigated using an appropriate variety of analytical methods (Brasier, 1997). For practical and historical reasons, the morphological species concept remains currently the most frequently applied one and arguably the most operational concept for taxonomic study of a majority of fungi.

The present study has been based to a major extent on the morphological species concept, which is defined here as species being 'the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means' (Cronquist, 1978). 'Ordinary means' in the case of microfungi of the present project is understood as the examination of fungal fruiting bodies either from natural substrata and/or cultures by light microscopy. In addition to examining the morphology of fungi, additional techniques were used to clarify the placement of a small number of taxa, including molecular techniques (Appendix M).

Aims

The major aims of this chapter are

- to assess the taxonomy and systematics of selected taxa
- to provide descriptions of selected taxa observed in this study
- to summarise the taxonomic diversity detected in this study

To achieve these aims, this chapter will be divided into two separate subsections, corresponding to the aims of this study.

7.2 Description of selected taxa

This section will provide descriptions of taxa that were observed in the present study and where appropriate notes concerning the taxonomy of these taxa.

7.2.1 Methods

7.2.1.1 Direct method

All leaves were incubated in separate humid chambers containing tissue paper moistened with sterile distilled water. After a period of incubation, slides were prepared for representative fruiting structures of each morphologically distinct fungal entity. Fruiting bodies of ascomycetes and coelomycetes were cut with a razor blade and contents were mounted in sterile distilled water. Representative specimens were tested for an amyloid reaction of the apical and subapical apparatus with Melzer's reagent. Indian ink was used to assess the presence and shape of gelatinous sheaths. To assess internal structures and arrangements, hand sections were prepared. The range of measurements is derived from examining a minimum of 25 ascospores, 20 asci, 10 ascomata per specimen (except for *Anthostomella reniformis* for which three available ascomata were examined) mounted in sterile distilled water. Slides were rendered semi-permanent by addition of 90 % lactic acid and sealed with nail polish.

Hyphomycetes were mounted directly in 90 % lactic acid using a razor blade or fine needle. Some slides were stained with lactic acid cotton blue; this stain was only

necessary for taxa with small, hyaline structures. For intial placement of taxa, a total of 10 conidia, 5 conidiophores, 5 conidiogenous cells and 10 sterile elements (where present) were measured per specimen. Additional measurements (25 conidia, 10 conidiophores, 10 conidiogenous cells and 10 sterile elements) were carried out in taxa for which further characterisation was required.

Identification was undertaken from standard texts (Chapter 1) where possible but assistance with or confirmation of identifications was sought from mycologists where appropriate (refer to Acknowledgement section). Herbarium specimens were prepared for each taxon by removing a section of leaf with a scalpel blade and drying it at 37°C for three days. Isolations of fungal cultures were undertaken for selected taxa (Booth, 1971). Fungal specimens of taxa, which were recognised as new to science, were deposited at the Queensland Plant Pathology Herbarium (BRIP), Department of Primary Industries or await deposition at the School of Tropical Biology, James Cook University, Cairns.

7.2.1.2 Particle filtration

A description of the particle filtration method is provided in Chapter 2. Representative isolates of each morphotype were subcultured onto Potato Carrot Agar containing a 2 cm^2 piece of banana leaf (Matsushima, 1971) and incubated for a 12 h photo period under black light or white neon light to induce sporulation (Booth, 1971). The majority of sporulating isolates were identified to genus and isolates of different species within the same genus, were designated species 1, species 2, etc. (Bills and Polishook, 1994a).

7.2.2 Results and Notes

For photographic plates of the following species, the reader is referred to Appendix M.

Anthostomella reniformis Paulus, P. Gadek & K.D. Hyde sp.nov.

Etymology: '*reniformis*' refers to the kidney-shaped ascospores.

Ascomata solitary, infrequent, black, subglobose in vertical section, immersed and appearing as raised dome-shaped areas which are often surrounded by reddish host tissue, central and circular ostiole apparent, at maturity host epidermis may lift to form a

lid-like structure, 220–300 × 150–200 µm, peridium composed of dark brown, thick-walled, irregular cells, clypeus not seen. Paraphyses embedded in gelatinous matrix, hyaline, flexuous, apex obtuse, 2–2.5 µm wide. Asci cylindrical, unitunicate, 8-spored, uniseriate, (92.5–)105–120 µm × (7.5–)9–12 µm with a J+, subapical annulus, (3–)4–6 × 4–6 µm, apex rounded, pedicel cylindrical with blunt base, approximately 30 µm long. Ascospores reniform in lateral view, dark brown, aseptate, 10–14 × 5–9 µm with one gelatinous broad, rounded appendage on each end, 6.4–8.5 µm wide and 4.2–6 µm long, germ slit straight and nearly same length as ascospores, clearly visible in dorsal view and in that orientation, the ascospore shape is ellipsoid. Anamorph not observed.

Specimens examined: Australia, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, B. Paulus and I. Steer, 13 June 2002, BP F556 (holotype, BRIP 29312), on decaying leaves of *Ficus pleurocarpa*. Australia, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, B. Paulus and C. Pearce, 27 November 2001, BP F424 (BRIP 29325), on decaying leaves of *Ficus pleurocarpa*.

Notes

For the placement of this specimen, I considered two ascomycete genera, *Anthostomella* and *Nipicola* K.D. Hyde. *Anthostomella* is characterised by ascomata, which have a central ostiole and are typically immersed under a clypeus although the latter may be absent in some species. Its asci are mostly cylindrical, (4-)8-spored and usually short pedicillate. It is also characterised by an amyloid (sometimes inamyloid), subapical apparatus, filamentous paraphyses usually embedded in a gelatinous matrix, darkly pigmented ascospores with or without dwarf cells and germ slits. Initially *Nipicola* included only one species, *Nipicola carbospora* K.D. Hyde, which is characterised by ascomata immersed beneath a reduced clypeus, J- apical pore and black, lunate ascospores with a layered mucilaginous sheath. This generic concept of *Nipicola* was broadened by the inclusion of two further species (Hyde, 1996; Hyde and Taylor, 1996). Both *Anthostomella* and *Nipicola* now contain species with J- or J+ subapical rings, germ slits present or absent, clypeus developed or reduced, dark inequilateral ascospores and mucilaginous ascospore sheaths. However, *Nipicola* seems to differ from *Anthostomella* in the layered nature of the ascospore sheath (Whitton, 1999).

These specimens resemble *Nipicola* in the darkly pigmented, lunate or reniform ascospores and the apparent absence of a clypeus. The latter character has been reported to vary, even within different samples of the same species (Lu and Hyde, 2000). As only few ascomata were available, it will be necessary to re-examine this character when more specimens are located. Our species differs from *Nipicola* in having simple rather than layered mucilaginous appendages. Within *Anthostomella,* many species have inequilateral ascospores but no species with reniform ascospores has yet been included in this genus (Lu and Hyde, 2000). Generic placement of our species, therefore, remains problematic. In the absence of supporting molecular or ultrastructural data, I have referred it to *Anthostomella* pending further exploration of the generic delimitation between the two genera.

Cylindrosympodium cryptocaryae Paulus, Gadek & K.D. Hyde sp.nov. Fig. 7.1

Etymology. *'cryptocaryae'* referring to the occurrence of this species on decaying leaves of *Cryptocarya mackinnoniana* F. Muell.

Colonies on MYA light brown to greyish with cream margin, subfelty to farinaceous, reverse brown with cream margin, no pigment in agar, effuse, hyphae subhyaline, 1.5–2.5 µm wide, sporulation mostly on sterilised leaf on agar surface. *Conidiophores* mononematous, arising from trailing hyphae or ropes of hyphae, rarely branched, hyaline or subhyaline, cylindrical at base, geniculate or inflated and denticulate at apex, 0–1- (2-) septate, smooth, often reduced to a conidiogenous cell, on decaying host leaves 4–30 × 3–7 µm, on PCA 4–20 × 3–6 µm. *Conidiogenous cells* polyblastic, integrated, terminal, becoming intercalary, sympodially elongated, cylindrical or inflated at apex, hyaline to subhyaline, with conspicuous and cylindrical denticles, which are 1–1.5 × 1 µm. *Conidia* cylindrical, slightly attenuated and truncate at base, apex rounded, (2-)3-septate (Table 1), smooth, dry, hyaline, on decaying host leaves 22–32 × 1.5–2(–2.5) µm ($\bar{x}$ = 25.3 × 1.9 µm), on PCA (13–)25–33 × 1.5–2 µm ($\bar{x}$ = 27.2 × 1.8 µm).

Specimens examined. Australia: Queensland: Atherton Tablelands: Brooke's Road, on decaying leaves of *Cryptocarya mackinnoniana* F. Muell., *B. Paulus & I.G. Steer, BPF229,* April 2001 (BRIP 29321; holotype).

Fig. 7.1 . ***Cylindrosympodium cryptocaryae.*** **(a) conidia. (b) sympodially elongating conidiophores. Bar = 10 mm.**

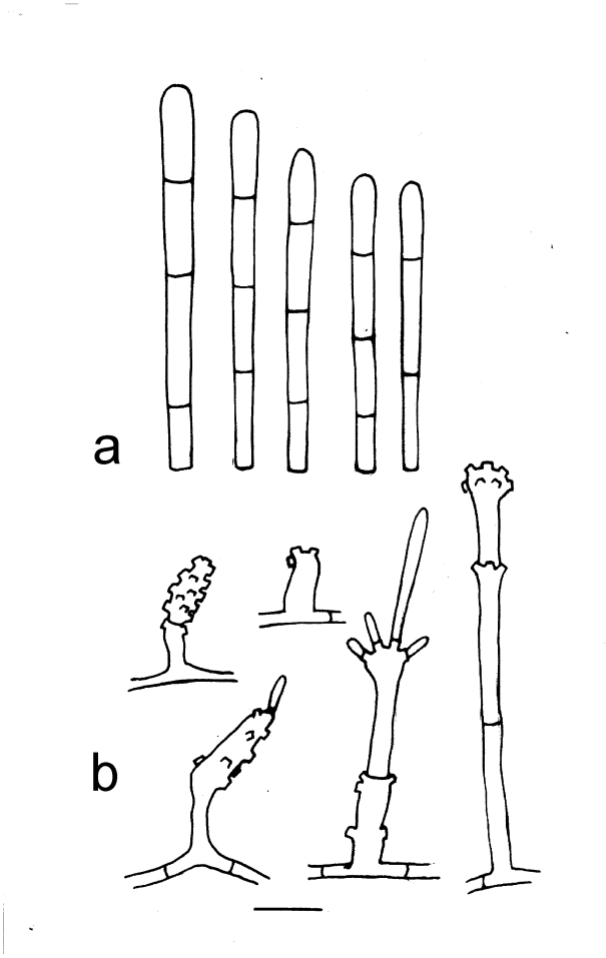


Table 7.1 Conidial septation in specimens isolated from natural substrata and from culture (PCA)

	On natural substratum (BRIP29321) n = 30				In culture (BRIP29214) n = 82			
Conidial septation	0	1	2	3	0	1	2	3
Percentage of conidia (%)	0	0	6.7	93.3	1.2	2.4	24.4	72

Key to closely related genera based on Castañeda Ruiz and Kendrick (1990a)

1.	Conidiogenous loci flat, not denticulate	*Parasympodiella*
	Conidiogenous cells denticulate	2
2.	Conidiophores pigmented	3
	Conidiophores hyaline to subhyaline	4
3.	Conidiophores solitary and robust, with broad denticles along their axis	*Subulispora*, *Solosympodiella*[1]
	Conidiophores scattered or grouped with apical, short-cylindrical denticles	*Dactylaria* section *Mirandina*
4.	Conidiophores with cylindrical denticles, conidia clavate, obclavate or filiform	*Dactylaria* section *Dactylaria*
	Conidiophore with broad, flat-topped and crowded denticles, conidia cylindrical	*Cylindrosympodium*

[1] The possible synonymy of *Subulispora* and *Solosympodiella* has been considered by de Hoog (1985) but species in *Solosympodiella* have not been transferred to *Subulispora*.

Key to species of *Cylindrosympodium*

1.	Conidia with 5 or more septa	2
	Conidia with 3 or fewer septa	3
2.	Conidia 9–15-septate, 55–110 × 3.5–4.5 µm, 2–2.5 µm at base	*C. yuhanensis*
	Conidia 5–7-septate, 50–80 × 3–3.5 µm (in PSA 6–11-septate, 50–140 × 3–3.5 µm)	*C. gracile*
3.	Conidia mostly 2-septate (1 or 3-septate conidia rarely present) 14–40 × 1–1.5 µm	*C. variabile*
	Conidia 1- or 3-septate	4
4.	Conidia 1-septate, both poles truncate, 10–26 × 1–1.5 µm	*C. eucalyptae*
	Conidia 3-septate	5
5.	Conidia 36–51 × 1–1.5 µm	*C. triseptatum*
	Conidia less than 35 µm in length	6
6.	Conidia 15–20 × 1.5–2 µm	*C. robustum*
	Conidia 25–35 × 1.5–2 µm	*C. cryptocaryae*

Notes

Cylindrosympodium is morphologically similar to several other genera, for example some species of *Dactylaria*. *Dactylaria lepida* Minter has cylindrical, hyaline conidia and broad truncate denticles arranged on a sympodially elongating axis, features which would place this species in *Cylindrosympodium*. The conidiophores are brown and thick-walled at the base and perhaps because of this Castañeda and Kendrick (1990a, 1991) did not transfer *D. lepida* to *Cylindrosympodium*. Our understanding of generic concepts is incomplete and requires additional information from other methods such as molecular analyses.

Cylindrosympodium cryptocaryae is characteristic of *Cylindrosympodium* on the basis of its hyaline, cylindrical conidia and its hyaline or subhyaline, short or reduced

conidiophores, which bear conspicuous flat-topped denticles (Castañeda Ruiz and Kendrick, 1990a). It is most similar to *C. variabile* in its morphology (Castañeda Ruiz and Kendrick, 1990a). However, it differs in having mostly 3-septate rather than the predominantly 2-septate conidia reported for *C. variabile.* This characteristic is clearest in material from natural substrata but is also evident in culture. In addition, conidia of *C. cryptocaryae* are wider but more restricted in length than reported for *C. variabile.* Two further species, *C. triseptatum* and *C. robustum,* have 3-septate conidia, but can be distinguished clearly on the basis of conidial size (Castañeda Ruiz and Kendrick, 1990a; Castañeda Ruiz and Kendrick, 1991).

Dactylaria belliana B. Paulus, P. Gadek & K.D. Hyde, sp. nov.

Etymology. 'belliana' referring to New Zealand mycologist Dr Ann Bell

Colonies on MYA light brown to grey with cream margin, subfelty, reverse light brown with cream margin, no pigment in agar, slow growing, hyphae subhyaline to light brown, 2-3.5 µm wide. *Conidiophores* macronematous, mononematous, arising from trailing hyphae or ropes of hyphae, mostly unbranched, brown, becoming light brown or hyaline towards the apex, cylindrical with apex sometimes inflated, often thick-walled at base, 0-5-septate, flexuous, smooth, sometimes reduced to conidiogenous cells, 10-60 × 2-3.5(-4) µm. *Conidiogenous cells* polyblastic, sympodial, integrated, terminal becoming intercalary, cylindrical or inflated, light brown to subhyaline, 4-10 × 2-5 µm, with conspicuous, cylindrical denticles, up to 1 µm wide. *Conidia* narrowly fusiform, base truncate and apex narrowly obtuse, 1–septate, septa visible in cotton blue, smooth, dry, hyaline, 20-26 × 1-2 µm ($\bar{x}$ = 23.3 × 1.5 µm; length:width ratio of 15.5:1).

Teleomorph: unknown.

Habitat: on decaying leaves of *Opisthiolepis heterophylla* and *Ficus pleurocarpa.*

Known Distribution: Australia.

Specimens examined. AUSTRALIA, Queensland, Atherton Tablelands, Old Boonjee Road, on decaying leaves of Opisthiolepis heterophylla, 20 April 2001, B. Paulus and I. Steer (BRIP29230, holotype); AUSTRALIA, Queensland, Old Boonjie Road, on decaying leaves of Ficus pleurocarpa (BRIP29322).

Notes

Our isolate of *D. belliana* could be included in section *Mirandina sensu* de Hoog (1985) based on its brown erect conidiophores, its short cylindrical denticles and the length to width ratio of its conidia. Within this section, *D. belliana* resembles *D. fusiformis* Shearer & Crane most closely in terms of conidial shape. Our species differs in having 1-septate conidia as compared to the 3-septate conidia of *D. fusiformis.* In addition, the pigmented conidiophores of *D. belliana* are shorter and have more pronounced denticles than those of *D. fusiformis.* Other species, which are similar to *D. belliana* in conidial shape are *D. acerosa* Matsush., *D. monticola* R.F. Castañeda & W.B. Kendr., *D. leptosphaeriicola* U. Braun & Crous and *D. xinjangensis* Z. Jiao, X.Z. Liu & Y.T. Wang. Our species differs from *D. acerosa* in having 1-septate rather than 3-septate conidia as well as pigmented, robust conidiophores and from *D. monticola* in having distinctly shorter conidia and pigmented conidiophores. It differs from *D. leptosphaeriicola* in conidial size as well as being symmetrical in both planes and from *D. xinjangensis* in the number of septa, in conidial size and in having conspicuous denticles.

Dactylaria ficusicola B. Paulus, P. Gadek & K.D. Hyde sp. nov.

Etymology. '*ficusicola*' referring to the frequent occurrence of this fungus on decaying leaves of *Ficus pleurocarpa*

Colonies on MYA brown, minimal aerial mycelium, reverse brown, slow growing. *Conidiophores* macronematous, mononematous, conspicuous, erect, straight, robust, thick-walled, tapering towards the apex, unbranched, dark brown base with light brown to hyaline apex, smooth, multiseptate, tall and short conidiophores present, 30-180 × 2-9 µm. In PCA conidiophores subhyaline to brown, mostly thin-walled, flexuous, cylindrical or inflated, variously shaped, often reduced to conidiogenous cell, 10-70 × 2.5-3.5 µm. *Conidiogenous cells* polyblastic, sympodial, integrated, terminal, 4-10 × 2-5 µm, tapering or inflated, light brown or hyaline, with cylindrical denticles less than 1 µm wide. In PCA conidiogenous cells cylindrical or inflated, subhyaline or hyaline, terminal or becoming intercalary. *Conidia* cylindrical, base conico-truncate and apex obtuse, hyaline, aseptate, smooth, dry, 12-15 × 1.5-2(-2.5) µm ($\bar{x}$ = 13.3 × 2 µm). In PCA conidia 10-16 × 2-2.5 µm (mean 14.7 × 2.1 µm).

Teleomorph: unknown.

Habitat: on decaying leaves of *Ficus pleurocarpa*.

Known Distribution: Australia

Specimens examined. AUSTRALIA, Queensland, Atherton Tablelands, Old Boonjee Road, on decaying leaves of *Ficus pleurocarpa,* 20 February 2002, B. Paulus and I. Steer (BRIP29304 [BP F535] holotype); *ibid.* (BRIP38623 [BP7148]).

Notes

The most appropriate placement of *D. ficusicola* is in section *Diplorhinotrichum* on the basis of its erect, brown conidiophores with cylindrical denticles in the apical region and its cylindrical conidia. Our specimens superficially fit the description of *D. hemibeltranioidea* R.F. Castañeda & W.B. Kendr. They differ in only producing cylindrical conidia with conico-truncate bases and despite observations made in culture and in fresh material, no fusiform or naviculate conidia as reported for *D. hemibeltranoidea* were observed. In addition, the tall conidiophores of *D. ficusicola* are thick-walled and clearly tapering towards the apex, compared with conidiophores of *D. hemibeltranoidea,* which are cylindrical. The conidiogenous cells of *D. ficusicola* are often inflated and have inconspicuous denticles rather than the conspicuous denticles reported for *D. hemibeltranioidea. Dactylaria queenslandica* Matsush. has conidia of a similar shape to *D. ficusicola.* These two species can be distinguished on the basis of conidiophore shape, size and pigmentation as well as conidial septation.

A summary of species of *Dactylaria sensu lato* described post Goh and Hyde (1997) and a discussion of species concepts in these genera is provided in Appendix N.

Discostroma ficusicola Paulus, P. Gadek & K.D. Hyde sp.nov.

Etymology: *'ficusicola'* refers to the strangler fig *Ficus pleurocarpa* from which this fungus was identified.

Ascomata numerous, mostly epiphyllous, submerged when young, with circular black clypeus visible on leaf surface, semisubmerged with central papilla emerging when mature, macroscopically appearing as pustules on leaf surface, 112.5-175 × 112.5-150 µm, peridium composed of several layers of dark brown, irregular cells. *Paraphyses*

abundant, persistent, hyaline, smooth, septate, sometimes inflated at base with long, cylindrical or tapering apical part, 3-9 µm at base, tapering to 2 µm at apex, which is obtuse or slightly inflated. *Asci* unitunicate, cylindrical or narrowly clavate when ascospores have obliquely or overlapping uniseriate arrangement or narrowly ellipsoid when ascospores are biseriate, 8-spored, apex rounded with a J+ subapical annulus, approximately 2.5 × 1.5 µm, pulvillus staining slightly blue with ink, pedicel short with inflated blunt base, (80-)90-110 µm × 10-12 µm. *Ascospores* oblong to ellipsoid, sometimes inequilateral, with one median septum, slightly constricted or not constricted at septum, hyaline, smooth-walled, usually guttulate, 17-20 × 5-6.5 µm. *Anamorph* not observed.

Specimens examined. AUSTRALIA, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, B. Paulus and I. Steer, 8 February 2002, BP F528 (holotype, BRIP 29181), on decaying leaves of Ficus pleurocarpa. AUSTRALIA, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, B. Paulus and I. Steer, 27 September 2002, BP F529, on decaying leaves of Ficus pleurocarpa. AUSTRALIA, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, B. Paulus and I. Steer, 27 September 2002, BP F833, on decaying leaves of Ficus pleurocarpa.

Notes

For the generic placement of this taxon, I considered genera developing under clypei, hyaline ascospores and asci with an amyloid apical ring. *Hyponectria* Sacc., *Physalospora* Niessl and *Rachidicola* K.D. Hyde and Fröhl. were excluded on the basis of ascospore septation, and *Charonectria* Sacc. and *Arwidssonia* B. Eriksson on ascomatal differences (Wang and Hyde, 1999). Within the Amphisphaeriaceae *sensu stricto*, *Ellurema* Nag Raj & W.B. Kendr. also has hyaline ascospores and an amyloid annulus but differs from our specimens in ascospore septation, ascal shape and relative abundance of paraphyses (Kang et al., 1999). With the exception of *Discostroma*, other genera within the Amphisphaeriaceae differ in ascospore pigmentation and/or septation or amyloidity of the ascal ring (Kang et al., 1998). A number of *Discostroma* species, namely *D. cupulum, D. empetri, D. hyperboreum, D. ledi, D. rhododendri, D. succineum* and *D. tostum* resemble our specimens in having ellipsoid, hyaline ascospores with strictly one median septum and an amyloid ascal ring. An additional two species, *D. pachystimae* and *D. caninum*, form hyaline ascospores with

predominantly one septum but these may have additional septa. *Discostroma ficusicola* differs clearly from all similar species in ascospore length.

Key to *Discostroma* and *Discostromopsis*

1. a) ascospores always muriform 2
   b) ascospores predominantly with transverse septa 4

2. a) ascospores dark brown, mostly with 3 transverse and one longitudinal septum, 18-20(-34) × 8-10(-12.5) µm
   Discostroma muricatum
   b) ascospores hyaline 3

3. a) ascospores with 3 transverse septa and one longitudinal septum in most segments, 19-22(-24) × 10-13 µm
   Discostroma propendulum
   b) ascospores with 3 to 7 (mostly 5) transverse septa, and up to 3 longitudinal septa, hyaline, in mass pinkish, 18-25 × 8.5-11 µm *Discostroma massarinum*

4. a) ascospores always with one transverse septum 5
   b) ascospore septation variable 11

5. a) ascospores 13-16 × 5-6 µm, asci 65-80 × 7.5-11.5 µm
   Discostroma succineum
   b) ascal dimensions greater, ascospore length greater or smaller 6

6. a) ascospore length ≥ 14 µm 7
   b) ascospore length predominantly ≤ 14 µm 8

7. a) ascospores 17-20 × 5-6.5 µm, asci (80)90-110 × 10-12 µm
   Discostroma ficusicola
   b) ascospores 14-17 × 7-8 µm, asci 90-100 × 11-12 µm
   Discostroma hyperboreum

8. a) ascospores hyaline, becoming yellowish to dull brown, 10.5-13(-16.5) × 5-6.5 µm, finely verruculose as longitudinal striae
   Discostroma cupulum
   b) ascospores smooth and remain hyaline at maturity 9

9. a) ascospores 12-13.5(-15) × 5.5-7 µm, asci 80-93 × 9-10.5 µm
   Discostroma empetri
   b) ascospore width ≤5.5 µm 10

10. a) on dead stems of *Epilobium*, ascospores 8-13.5 × 3-4.5 µm,

ellipsoid to slightly curved, asci 62-85 × 5-7 µm *Discostroma tostum*

b) on leaves of *Ledi groenlandici*, ascospores 10.5-12 × 4-4.5 µm, ellipsoid, asci 63-75 × 6-7 µm *Discostroma ledi*

c) on leaves of *Rhododendri californici*, ascospores 10-13 × 4.5-5.5 µm, oblong ellipsoid, asci 60-88 × 6.5-9 µm *Discostroma rhododendri*

11. a) ascospores predominantly 1-septate but additional septa are present in some spores 12

b) ascospore predominantly with more than one septum or with approximately equal proportions of 1, 2 and 3 septa 15

12. a) ascospore length mostly ≥12 µm 13

b) ascospore length mostly ≤12 µm 14

13. a) ascospores mostly 1-septate, rarely 3-septate, ellipsoid to fusoid, hyaline, 12-14 × 4.5-5 µm, ascomata erumpent *Discostroma pachystimae*

b) ascospores mostly 1-septate but also 2- or 3-septate, ellipsoid, hyaline to yellowish, 12-15(-17) × 5.3-6.5 µm, ascomata visible as round, raised dark brown shiny areas *Discostroma caninum*

14. a) asci 75-100 × 5.3-7.4 µm ascospores 10-12(-13) × 4-5(-5.3) µm, 1-septate, sometimes 2-, 3-septate *Discostroma rosae*

b) asci 55-70 × 5-7 µm, ascospores 7-12 × 3-4 µm, usually 1-septate, sometimes with further transverse septa *Discostroma strobiligenum*

15. a) ascospores mostly 2-septate, some 3-septate when mature, 16-20 × 14-22 µm, hyaline when young, brown when mature *Discostroma tricellulare*

b) ascospore mostly 3-septate or approximately equal proportions of 1, 2 or 3 septa 16

16. a) ascospores 1-, 2-, 3-septate often in same ascus, 11-14 × 4.5-5 µm *Discostroma polymorphum*

b) ascospores predominantly or always 3-septate 17

17. a) ascal ring distinct and J+ 18

b) ascal ring indistinct and J- 23

18. a) ascospores hyaline to yellowish 19

b) ascospores pigmented 21

19. a) ascospores with predominantly 3 transverse septa and rarely one longitudinal septum, 16.5-20(-25) × 7.5-9(-10) µm, asci 105-130 × 8.5-13.3 µm *Discostroma sanguineae*

b) ascospores with only transverse septa 20

20. a) ascospores 18-26 × 6-8 µm, asci 90-130 × 9-11(-15),

ascomata 200-250(-350) µm, on leaves of *Pentophylloidis fruticosae* *Discostroma fruticosum*

b) ascospores 15.5-24 × 6.5-9 µm, asci 100-160 × 9-13 µm ascomata 300-450 × 250-495 × 250-495 µm, on leaves of *Rubes* spp. *Discostroma rubicola*

21. a) ascospores 11.5-13.8 × 4-4.5 µm, brown, echinulate *Discostroma osyridis*

b) ascospores ≥15 µm with 3 transverse septa, rarely one longitudinal septum 22

22. a) ascospores olivaceous, 15-17 × 9 µm, asci 100 × 9-10 µm, ascomata 270-450 × 200-300 µm *Discostroma fuscellum*

b) ascospores light brown, 15-18 × 6.5-9 µm, asci 117-150 × 8-10 µm, ascomata 400-600 × 210-400 µm *Discostroma saccardoanum*

23. a) ascospores 14-18 × 4-5 µm, not constricted at septa, anamorph *Seimatosporium kriegerianum* *Discostromposis callistemonis*

b) ascospore length ≥ 17µm 24

24. a) anamorph *Seimatosporium leptospermi,* on dead leaves of *Leptospermum juniperini,* ascospores 17-23 × 4.5-6 µm, usually constricted at septa *Discostromopsis leptospermi*

b) ascomata on dead leaves of *Melaleuca* 25

25. a) anamorph *Seimatosporium elegans,* on leaves of *Melaleuca ericifoliae*, ascospores 17-23 × 4.5-6 µm *Discostromopsis elegans*

b) anamorph *Seimatosporium dilophosporum,* on dead leaves of *Melaleuca squarrosae*, ascospores 19-21 × 4-6 µm *Discostromopsis stoneae*

Gnomonia elaeocarpa Paulus, M.E. Barr & K.D. Hyde sp.nov.

Etymology: *'elaeocarpa'* refers to the tree species *Elaeocarpus angustifolius* from which the fungus was recorded.

Ascomata numerous, hypophyllous on leaf lamina, single, dark brown, globose to subglobose, semisubmerged, peridium composed of dark brown *textura epidermoidea,* 250–300 × 200–250 µm. Central beak protruding from substratum, 100–150 µm long and 50–90 µm wide, composed of brown *textura porrecta*, paling towards the apex, ostiolar canal lined with periphyses. At maturity an initially white mass of asci is extruded from the ostiolar pore, becoming brown on drying or with age. *Asci*

unitunicate, narrowly fusiform, 8-spored, with a J- refractive, complex subapical annulus, 2.5–4 × 2–3 µm, base deliquescing at maturity and asci freed into the centrum, 50–80 µm × 5–9 µm. *Ascospores* ellipsoid with rounded ends, hyaline, guttulate or eguttulate, uniseptate, usually constricted at the median septum, 10–15 µm × 3–4.5 µm with apparently gelatinous broad appendages at each pole when young, which unfold at maturity to become filiform, flexuous and nonbranching, 20–40 µm long; visible in water mount and disappearing in lactic acid. *Anamorph* unknown.

Specimens examined. AUSTRALIA, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Elaeocarpus angustifolius*, B. Paulus and I.G. Steer, 13 June 2002, BP F640 (holotype, BRIP 29327), Australia, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Elaeocarpus angustifolius*, B. Paulus and I.G. Steer, 28 May 2002, BP 609 (BRIP 29187).

Notes

Gnomonia elaeocarpa fits into *Gnomonia* section *Latispora* Barr, which is characterised by ellipsoid ascospores with tapering to rounded ends, a median septum and setose appendages when fully formed. Ascospores usually overlap obliquely in the ascus and the ascomata have a narrow beak (Barr, 1978). Within this section, our specimens resemble *G. ribicola* Barr in ascospore shape. However, asci, ascospores and ascospore appendages in our specimens are longer while the beak is much shorter. None of the species described by Monod (1983) resembles *Gnomonia elaeocarpa.*

Barr (1991) noted that members of *Gnomonia* are usually quite host-specific. Our observations are congruent with Barr's statement (1991) as this fungus was not identified in leaf litter of other tree species in close proximity to the *Elaeocarpus angustifolius* trees studied while being present on approximately 86 % of decaying leaves of *Elaeocarpus angustifolius* on six individual trees at two sites. Assuming a high degree of host-specificity, the type of substratum is a valuable character in species delimitation (Barr, 1978). To our knowledge, no other species of *Gnomonia* has been previously described from *Elaeocarpus* (Barr, 1978; Monod, 1983; Barr, 1991; CBS Fungi Database, Centraalbureau voor Schimmelcultures, 2003; PDD Collection Database, Landcare Research, 2003; USDA, Systematic Botany and Mycology Laboratory Databases, 2003).

Gnomonia queenslandica Paulus, M.E. Barr & K.D. Hyde sp. nov.

Etymology: *'queenslandica'* referring to the state of Queensland, Australia, where the fungus was first recorded.

Ascomata single or aggregated, predominantly found on petioles, on the adaxial surface of midribs and on domatia on the adaxial leaf surface, dark brown, base globose, semisubmerged to superficial, 300–500 µm in diameter, upper part of ascoma and long, narrow, central beak protruding from substratum; beak 320–550(–1400) µm long, 45–55 µm at base tapering to 22–27 µm at apex, yellow to orange, powdery collar at the base of beak, ostiolar canal lined with periphyses, external layer of beak composed of textura porrecta; peridium composed of dark brown to black textura epidermoidea. At maturity an initially white mass of asci is extruded from the ostiolar pore, becoming brown on drying or with age. Asci numerous, elliptical or broadly elliptical, often narrowed toward apex, unitunicate, (4-)8-spored, 20–34 × 4.5–7(–8) µm, with a distinct J- refractive, complex subapical annulus, 2.5–4.5 × 2–3 µm, base deliquescing at maturity and asci freed into the centrum. Ascospores narrowly fusiform, hyaline, guttulate or eguttulate, uniseptate, (9–)13–16 × 1–2 µm, with filiform appendage at either pole, initially gelatinous, 3–8 µm long, at maturity forming a filiform, unbranched appendage up to 20 µm long. Anamorph not observed.

Specimens examined. AUSTRALIA, Queensland, Atherton Tablelands, Millaa Millaa, Brooke's Road, on decaying leaves of *Elaeocarpus angustifolius*, B. Paulus and I.G. Steer, 13 June 2002, BP F641 (holotype, BRIP 29326). Australia, Queensland, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Elaeocarpus angustifolius*, B. Paulus and I.G. Steer, 28 May 2002, BP 608 (BRIP 29187).

Notes

Gnomonia queenslandica can be accommodated in *Gnomonia* section *Seta* Barr. This section is characterised by narrow, fusoid ascospores tapering to more or less pointed ends with a median septum and setose appendages. Members of this section also have a narrow beak (Barr, 1978). Within this section, *G. queenslandica* is similar to *G. petiolarum* (Schwein. ex Fries) Cooke in ascospore shape and size, in the presence of a

collar and in the preferred substrata, i.e. petioles and major veins (Barr, 1978). It differs in its shorter ascospores with much longer appendages, its larger ascomata, and in having a yellow or orange rather than a white collar.

Two species listed by Monod (1983) resemble *G. queenslandica* in some characters. *Gnomonia alni-viridis* Podlahova & Svrcek is similar in ascus and ascospore shape and size but differs in the morphology of the apical apparatus, in the length of ascospore appendages, in the absence of a collar and in the type of preferred leaf tissues. *Gnomonia rubi-idaei* Monod resembles our specimens in ascus and ascospore shape with long filiform appendages and as it commonly occurs on petiole and vein tissues of its host. However, it differs in ascospore length, in the morphology of the apical apparatus and in the absence of a yellow or orange collar.

Gnomonia queenslandica occurred on approximately 72 % of leaves examined and like *G. elaeocarpa* was not observed on decaying leaves of other tree species in the vicinity of the *Elaeocarpus* trees studied. Ascomata of *G. queenslandica* were invariably restricted to petioles, the abaxial surface of midribs and domatia on the adaxial leaf surface. Recurrence on certain tissue types or positions on leaves, e.g. petioles, epiphyllous versus hypophyllous, or hosts has been previously reported from other *Gnomonia* species and has been used as taxonomic character in species delimitation (Monod, 1983, Barr, 1978). As noted above, no other species of *Gnomonia* appears to have been described from *Elaeocarpus* (Barr, 1978; Monod, 1983; Barr, 1991; CBS Fungi Database, Centraalbureau voor Schimmelcultures, 2003; PDD Collection Database, Landcare Research, 2003; USDA, Systematic Botany and Mycology Laboratory Databases, 2003).

Thozetella acerosa Paulus, P.Gadek et K.D. Hyde, sp.nov.

Etymology. *'acerosa'* referring to the needle-like apices of the microawns.

Colonies cream and light brown, flat, woolly to subfelty, margin incised and indistinct. Conidiomata sporodochial or effuse, superficial, sessile, forming a convex or flat hymenium, topped by a moist spore mass, 200–1500 µm in diameter. Conidiophores macronematous, brown, irregularly cylindrical, branched, arising from a basal plate, 2.5–4 µm wide. Conidiogenous cells monophialidic, integrated, determinate, terminal, light brown, irregularly cylindrical, with no or minute collarette, periclinal wall

thickened, 12–20 × 2–4 µm. Microawns produced from conidiophores, predominantly L-shaped, basal part thin-walled and hyaline, with 0–2 septa depending on growing conditions (Appendix M), apical part acerose and slightly undulating, smooth, thick-walled with the upper portion becoming solid, refractive, 60–80 × 3.5–4.5 µm in PCA. Conidia lunate, aseptate, finely guttulate, hyaline and smooth, 14–20 × 2–3 µm in PCA, with one filiform setula at each end, 6–7 µm long.

Specimens examined. AUSTRALIA, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Cryptocarya mackinnoniana*, 8 Sep 2001, B. Paulus and C. Pearce, BP5970, HOLOTYPE: BRIP 29319.

Notes

Thozetella acerosa resembles *T. nivea* in microawn shape. Our isolate differs in having shorter and narrower conidia and longer microawns than reported for *T. nivea*. In addition, our specimens have 0–2 septa at the basal part of the microawns at 25 C while microawns of *T. nivea* are aseptate. Two other species described below, namely *T. boonjiensis* and *T. gigantea*, also have L-shaped microawns and form sporodochia. Mature specimens of *T. gigantea* are easily distinguished as they have much longer microawns. Mean values of microawn and conidial length, as well as the presence or absence of septation in microawns clearly differentiate between *T. acerosa* and *T. boonjiensis*.

Thozetella boonjiensis Paulus, P.Gadek et K.D. Hyde, sp.nov.

Etymology. *'boonjiensis'* referring to the place of collection near the Old Boonjie Road in north Queensland

Colonies cream and pale brown, subfelty, slimy, reverse pale brown. Conidiomata sessile sporodochia, 300–400 µm diam, or flat effuse development of conidiophores. Conidiophores macronematous, brown, cylindrical, branched, arising from a basal plate of cells, 1.5–3 µm wide. Conidiogenous cells monophialidic, integrated, determinate, terminal, light brown, irregularly cylindrical, lacking an apical collarette, no or slight periclinal wall thickening, 15–20 × 1.5–2.5 µm. Microawns produced from conidiophores, predominantly L-shaped, aseptate, smooth, apical part acerose, straight

or slightly undulating, thick-walled, refractive, basal part thin-walled, hyaline, aseptate, 48–75 × 3–5 µm in PCA. Conidia lunate, continuous, finely guttulate or eguttulate, hyaline, smooth, 10–15 × 2–3 µm on PCA, supplied with a single, 5–8 µm long, filiform setula at each end.

Specimens examined. AUSTRALIA, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Cryptocarya mackinnoniana*, 11 Mar 2001, B. Paulus and C. Pearce, (BP2334, BRIP 29318, holotype). AUSTRALIA, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Opisthiolepis heterophylla*, 11 Mar 2001, B. Paulus and C. Pearce (BP2383, BRIP 29316).

Notes

Thozetella boonjiensis resembles *T. tocklaiensis* in microawn shape and conidial dimensions. Our isolates differ in having longer microawns (48–75 × 3–5 µm rather than 18–38(–44) × 1.5–5 µm) and in producing sporodochia or effuse conidiomata rather than the synnemata reported for cultures of *T. tocklaiensis*. Two other species described here, namely *T. acerosa* and *T. gigantea*, also have L-shaped microawns and form sporodochia. Mature specimens of *T. gigantea* are easily distinguished because they have much longer microawns. Young specimens of *T. gigantea* may show an overlap in microawn dimensions with *T. boonjiensis*, but can be differentiated as they have longer conidia. Mean values of microawn and conidial length, as well as the presence or absence of septation in microawns clearly differentiate between *T. acerosa* and *T. boonjiensis*.

Thozetella falcata Paulus, P.Gadek et K.D. Hyde, sp.nov.

Etymology. '*falcata*' referring to the sickle-shaped microawns.

Colonies hyaline with brown, concentric rings, flat, no aerial mycelium, synnemata formed in concentric rings on agar surface and on banana leaf. Conidiomata funnel-shaped synnemata, brown to dark brown, abundant on leaf, 40–250 µm long, 12–35 µm wide at base and 75–110 µm at apex on natural substrata, in PCA much more elongated (300–1800 µm long, 12–80 µm wide at base and 120–300 µm at apex), some synnemata forming branches. Conidiophores macronematous, brown, paling towards apex,

irregularly cylindrical, densely compacted along synnematal axis, branched, 1.5–3 µm wide. Conidiogenous cells monophialidic, integrated, determinate, terminal, light brown, packed into tight palisades, irregularly cylindrical with bluntly rounded apex, lacking an apical collarette, periclinal wall thickened, 6–19 × 2–3 µm. Microawns predominantly sickle-shaped in specimens on natural substrata; L-shaped, sigmoid and straight microawns were also observed in subcultures in addition to sickle-shaped microawns, 40–95 × 2.5–5 µm on natural substrata, 50–110 × 2–4 µm on PCA. Conidia lunate, aseptate, finely guttulate or eguttulate, hyaline, smooth, 13–16 × 1.5–3 µm on natural substrata, 13–16 × 2–3 µm on PCA, provided with a single, filiform setula at either pole, 5–8 µm long.

Specimens examined. AUSTRALIA, Atherton Tablelands, Millaa Millaa, Brooke's Road, on decaying leaves of *C. mackinnoniana*, 28 May 2002, B. Paulus and I.G. Steer BP F715, BRIP29193, holotype). AUSTRALIA, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Ficus pleurocarpa*, 23 Mar 2002, B. Paulus and I.G. Steer (BP F568, BRIP 29178). AUSTRALIA, Atherton Tablelands, Millaa Millaa, Brooke's Road, on decaying leaves of *Cryptocarya mackinnoniana*, 28 May 2002, B. Paulus and I.G. Steer, (BP F634, BRIP 29192). AUSTRALIA, Atherton Tablelands, Millaa Millaa, Brooke's Road, on decaying leaves of *C. mackinnoniana*, 21 Jun 2002, B. Paulus and I.G. Steer (BP F711, BRIP 29201).

Notes

Thozetella falcata, T. cristata, T. radicata and *T. tocklaiensis* all produce synnemata. *Thozetella falcata* is also similar to *T. cristata* and *T. radicata* in microawn shape and in conidial dimensions. However, *T. falcata* differs from these two species in having considerably longer microawns on natural substrata and in culture. In addition, *T. falcata* differs from *T. radicata* in having branched and longer synnemata. Conidiomata of *T. falcata* are also different from those of *T. cristata,* in which conidiophores proliferate simultaneously to form visible ridges. In addition, *Thozetella cristata* forms consistently cylindrical synnemata, while those in *T. falcata* are funnel-shaped. The length and morphology of synnemata in *T. tocklaiensis* are similar to those of *T. falcata,* however, these two fungal taxa differ in microawn morphology and size.

Thozetella gigantea Paulus, P.Gadek et K.D. Hyde, sp.nov.

Etymology. *'gigantea'* referring to the large size of microawns.

Colonies cream with brown radial lines developing, subfelty, fast growing. Conidiomata sporodochial, superficial, sessile, forming a convex or flat hymenium, topped by a moist, white spore mass with long, straight needle-like apices of microawns protruding from it, few sporodochia produced per leaf, 100–600 × 90–500 µm. Conidiophores macronematous, brown, irregularly cylindrical, branched, arising from a basal plate, non-proliferating, 2.5–4 µm wide. Conidiogenous cells monophialidic, integrated, determinate, terminal, light brown, irregularly cylindrical, collarette missing, periclinal wall thickened, 12–20 × 2–4 µm. Microawns produced from conidiophores, predominantly L-shaped, basal part thin-walled, hyaline, continuous, long apical part acerose, smooth, straight, thick-walled with the upper portion becoming solid, narrowing down to less than 0.5 µm wide, refractive, 71–280 × 2.5–8 µm on natural substrata, 70–210 × 3–6 µm in PCA. Conidia lunate, aseptate, finely guttulate, hyaline, smooth, slightly truncate on basal pole, 14–18 × 2.5–3 µm on natural substrata and 13–17 × 2–3 µm on PCA, with a single, 6–10 µm long, filiform setula at each end.

Specimens examined. AUSTRALIA, Atherton Tablelands, Millaa Millaa, Brooke's Road, on decaying leaves of *Cryptocarya mackinnoniana*, 28 May 2002, B. Paulus and I.G. Steerm (BP F712, BRIP 29202, holotype). AUSTRALIA, Atherton Tablelands, Millaa Millaa, Brooke's Road, on decaying leaves of *C. mackinnoniana*, 28 May 2002, B. Paulus and I.G. Steer (BP F709, BRIP29200).

Notes

Thozetella gigantea resembles *T. nivea*, *T. acerosa* and *T. boonjiensis* in the production of sporodochia and in microawn shape. It differs in having considerably longer microawns on natural substrata and in culture.

Thozetella queenslandica Paulus, P.Gadek et K.D. Hyde, sp.nov.

Etymology. *'queenslandica'* referring to the state of Queensland in Australia where this fungus was first isolated

Colonies cream to grey, woolly, reverse with grey concentric rings. Conidiomata superficial, sessile sporodochia, 200–250 × 50–125 µm, forming a flat or convex hymenium on a dark brown, stromatic base, which bears a globose, ovoid or otherwise shaped white mass of conidia and microawns; some sporodochia produce dark brown transverse ridges, each representing an area of synchronous proliferation of conidiophores. Conidiophores macronematous, brown, irregularly cylindrical, branched, compact at base, more or less free towards of upper part of sporodochium. Conidiogenous cells monophialidic, integrated, determinate, terminal, light brown to subhyaline, irregularly cylindrical, without collarettes, periclinal wall thickened, 10–25 × 1.5–2.5 µm. Microawns produced from conidiophores, predominantly hamate, rarely sigmoid, refractive, with a smooth or verrucose apex, 24–33 × 2–3.5 µm on natural substrata, 21–34 × 2–4 µm on PCA. Conidia lunate, continuous, hyaline, eguttulate, smooth, 10–12 ×1.5–2.5 µm on natural substrate and 9–12 × 1.5–2.5 µm on PCA, provided with a single filiform setula at each end, 3–6 µm long.

Specimens examined. AUSTRALIA, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *Cryptocarya mackinnoniana*, 28 May 2002, B. Paulus and I.G. Steer (BP F612, BRIP 29188, holotype). AUSTRALIA, Atherton Tablelands, Topaz, Old Boonjie Road, on decaying leaves of *C. mackinnoniana*, 23 Mar 2002, B. Paulus and I.G. Steer (BP F415, BRIP 29164).

Notes

Thozetella queenslandica can be differentiated from other known species of *Thozetella* on the basis of its distinctive, short hamate microawns and the production of sporodochia, which show proliferation ridges. The microawns of *T. queenslandica* overlap in length with those of *T. effusa* and *T. havanensis*, but these species differ from *T. queenslandica* in both microawn and conidiomatal morphology.

KEY TO THE DESCRIBED SPECIES OF *THOZETELLA*

1. microawns present 2

1. microawns absent *T. ciliata*

2. microawns predominantly L-shaped 3

2. microawns not L-shaped or variously shaped [1] 6

3. microawns 0–2 septate, 60–80 × 3.5–4.5 µm (mean 72 × 3.9 µm) *T. acerosa*

3. microawns aseptate 4

4. microawns very long, 70–280 µm × 2.5–8 µm (mean 153.8 × 5 µm on natural substrata, 132.4 × 4.2 µm on PCA) *T. gigantea*

4. microawns shorter than 75 µm 5

5. conidia (17.5–) 21(–24) × 3–3.8 µm, microawns 50–70 × 3–4 µm, microawn apex undulating or geniculate *T. nivea*

5. conidia 10–15 × 2–3 µm, microawns 48–75 × 3–5 µm (mean 60.1 × 3.6 µm), microawn apex straight or slightly undulating *T. boonjiensis*

6. microawns predominantly sickle-shaped, uncinate, hamate or otherwise strongly curved 7

6. microawns predominantly straight, sigmoid or any other shape 11

7. conidiomata predominantly synnematous 8

7. conidiomata predominantly sporodochial 10

8. synnemata cylindrical, proliferating conidiophores form ridges, microawns 40–60 × 2.5–3 µm, conidia 11.5–14.5 × 2.3–2.7 µm *T. cristata*

8. synnemata funnel-shaped, conidiophores non-proliferating 9

9. microawns 40–95 × 2.5–5 µm (mean 59.9 × 3.6 µm on natural substratum, 77.2 × 3.3 µm in PCA), conidia 13–16 × 1.5–3 µm, synnemata branching

T. falcata

9. microawns 30–60 × 3–4.5 µm, conidia 11–13 × 2–2.5 µm

T. radicata

10. microawns 21–34 × 2–4 µm (mean 27.9 × 3 µm on natural substrata, 26.9 × 2.9 µm on PCA), proliferation ridges on sporodochia, conidia 9–12 × 1.5–2.5 µm

T. queenslandica

10. microawns 40–110 × 2.5–4 µm, conidia 11–17 × 2–2.5 µm, sporodochia without proliferation ridges *T. cubensis*

11. conidiomata effuse, never sporodochial or synnematous, microawns 20–30 × 3 µm, conidia 16–19 × 4–4.5 µm *T. effusa*

11. conidiomata sporodochial or synnematous 12

12. conidiomata sporodochial, microawns 32–37 × 2.5–3 µm, conidia 13–16(–18) × 2–2.5 µm *T. canadensis*

12. conidiomata synnematous 13

13. microawns variously shaped[1], bulbous base, acerose apex, straight, undulate, uncinate or bent 18–38 (–44) × 1.5–4 µm, conidia 9–13(–18) × 1.5–3 µm

T. tocklaiensis

13. microawns with +/- uniform width, sigmoid, allantoid, uncinate, verruculose, 22.4–35 × 1.5–3.2 µm, conidia 11–14 × 2.3 µm, conidiomata synnematous or effuse

T. havanensis

[1]The original descriptions of *T. tocklaiensis* shows microawns of various shapes, whereas Pirozynski and Hodges (1973) show the microawns of *T. tocklaiensis* to be L–shaped. I have accepted the original description for this key.

Notes

Further work to assess *Thozetella* species included an examination of cultures grown under different conditions and an analysis of DNA sequences of the internally transcribed spacer regions (Appendix M). In short, *Thozetella* was reported for the first time to belong in the ascomycete genus *Chaetosphaeria* Tul. & C. Tul., and the five morphological species were confirmed by five separate clades.

7.3 Taxonomic diversity of microfungi

7.3.1 Methods

A summary of data obtained by the direct method in both the succession and the substratum study is provided with respect to fungal relationships at higher taxonomic ranks. To this end, anamorphic fungi observed during this project were integrated into a phylogenetic system wherever possible (The CABI Bioscience and CBS Database of Fungal Names, 2003). Family and ordinal relationships were checked in the Outline of Ascomycetes (Eriksson, 2004).

7.3.2 Results

A total of 185 taxa were observed during the substratum study. Microfungi for which phylogenetic links were established (Table 7.3) were distributed among 17 orders, 24 families, 22 genera and 82 form genera (Table 7.2). Forty-five percent of fungal species observed could not be integrated into a phylogenetic scheme at taxonomic ranks below Ascomycota (Table 7.3). Sixteen taxa were confirmed as new to science (Appendix F and M). This is a conservative estimate and it is likely that the collections harbour further taxa new to science. In a few instances, some fungi could not be identified to generic or species level due to sparse material but the majority of these specimens may represent new taxa. In addition, some speciemens are similar to a described taxon but disagreement exists in some characters (specimens labelled either sp. or cf.). Additional characterisation, as undertaken for example for *Thozetella* (Appendix M), may establish these as taxa new to science.

In the substratum study, by far the most common orders were the Hypocreales, followed by the Xylariales, the Chaetosphaeriales, the Diaporthales and the Helotiales (Fig. 7.2).

Common families were the Nectriaceae, Chaetosphaeriaceae, Valsaceae, Bioectriaceae, Hypocreaceae and Magnaporthaceae (Fig. 7.3). The greatest number of species, however, belonged in a mixed group of hypocrealean fungi that could not be placed at the family level (Fig. 7.3, Table 2).

The results of the succession study closely mirror those of the substratum study. A total of 105 taxa were distributed among 12 orders, 22 families, 12 genera and 51 form genera (Table 3). Eleven taxa were confirmed as new to science. The greatest number of microfungal species was in the Hypocreales, followed by a mixed group of taxa that are currently included in the Dothideomycetes et Chaetothyriomycetes *incertae sedis*. The next most numerous groups were the Xylariales and the Diaporthales. Common families were the Nectriaceae, Valsaceae, Hypocreaceae, Helotiaceae, and the Chaetosphaeriaceae.

Table 7.2 Number of taxa within taxonomic hierarchy among microfungi from leaf litter of six tree species (i.e. *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus pleurocarpa, F. destruens,* and *Ophisthiolepis heterophylla*) observed during the 'substratum' study.

	Total		
No of orders	17		
No of families	28		
No of genera	22		
No of form genera	82	No of form genera integrated with teleomorph family and/or order	35
		No of form genera of uncertain positions (Ascomycota)	47
No of species	32		
No of form species	141	No of form species integrated with teleomorph family or order	69
		No of form species of uncertain positions (Ascomycota)	72
Unidentified taxa	11		
Associated organisms	1		

Table 7.3 Microfungal genera observed in leaf litter of six tree species (i.e. *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus pleurocarpa, F. destruens,* and *Ophisthiolepis heterophylla*) during the 'substratum' study. Numbers of species observed within genera are provided in brackets

Ascomycete order	Family	Teleomorphs	Anamorphs
Agaricales	Marasmiaceae	Marasmius (1)	
Agaricales	Tricholomataceae	Mycena (2)	
Calosphaeriales	Calosphaeriaceae		Bahusutrabeeja (1)
Chaetosphaeriales	Chaetosphaeriaceae		Thozetella (5), Dictyochaeta (4)
Chaetothyriales	Herpotrichiellaceae	Rhinocladiella (2)	
Diaporthales	Valsaceae	Gnomonia (4), Ophiognomonia (2)	Phomopsis (2)
Dothideomycetes et Chaetothyriomycetes incertae sedis	Botryosphaeriaceae	Guignardia (1), Botryosphaeria (1)	
Dothideomycetes et Chaetothyriomycetes incertae sedis	Incertae sedis	Brooksia (1)	
Eurotiales	Eurotiaceae		Penicillium (1)
Helotiales	Dermateaceae	gen.nov. (1), Mollisia (1)	anamorph of gen.nov.
Helotiales	Helotiaceae		Idriella (3)
Helotiales	Hyaloscyphaceae	Lachnum (1)	
Hypocreales	Incertae sedis		Stilbella (2)
Hypocreales	Niessliaceae	Niesslia (1)	
Hypocreales	Bionectriacaea	Roumeguerilla (1), Ijuhya (3)	
Hypocreales	Hypocreaceae		Trichoderma (1), Gliocephalotrichum (1), Gliocladium (2)
Hypocreales	Nectriaceae		Cylindrocladiella (1), Cylindrocladium (2), Gliocladiopsis (2), Chaetopsina (2), Cylindrocarpon (2), Fusarium (1)
Hypocreales	Incertae sedis		Acremonium (5), Cephalosporiopsis (2), Verticillium (3), Volutella (2), Myrothecium (2), Xenogliocladiopsis -like sp.nov. or gen. nov. (1)
Incertae sedis	Asterinaceae	Asterina (1)	
Incertae sedis	Magnaporthaceae	Gaeumannomyces-like sp. or gen. nov. (1)	Pyricularia (3)
Incertae sedis	Myxotrichaceae	Oidiodendron (1)	

Table 7.3 (continued)

Ascomycete order	Family	Teleomorphs	Anamorphs
Meliolales	Meliolaceae	Meliola (1)	
Mycosphaerellales	Mycosphaerellaceae		Cladosporium (2)
Onygenales	Onygenaceae	Auxarthron (1)	
Phyllachorales	Phyllachoraceae		Colletotrichum (3)
Pleosporales	Leptosphaeriaceae		Phoma (3)
Pleosporales	Tubeufiaceae		Helicosporium (2)
Rhytismatales	Rhytismataceae	Coccomyces (1), unidentified (1)	
Saccharomycetales	Incertae sedis		Geotrichum (1)
Xylariales	Amphisphaeriaceae	Discostroma (1)	Beltraniella (1), Pestalotiopsis (1)
Xylariales	Incertae sedis	Iodosphaeria (1)	
Xylariales	Hyponectriaceae	Hyponectria (2)	Microdochium (1)
Xylariales	Incertae sedis	Lanceispora (1), Linocarpon-like (1)	
Xylariales	Xylariaceae	Nodulisporium (1)	
Ascomycota incertae sedis			Scolecobasidium (3)
Ascomycota incertae sedis			Beltrania (3)
Ascomycota incertae sedis			Botryodiplodia (1)
Ascomycota incertae sedis			Catenosubulispora (1)
Ascomycota incertae sedis			Chaetospermum sp. (1)
Ascomycota incertae sedis			Chalara (2)
Ascomycota incertae sedis			Circinotrichum (3)
Ascomycota incertae sedis			coelomycete (4)
Ascomycota incertae sedis			Conioscypha (1)
Ascomycota incertae sedis			Cryptophiale (2)
Ascomycota incertae sedis			Curvularia (1)
Ascomycota incertae sedis			Cylindrosympodium (1)
Ascomycota incertae sedis			Dactylaria (5)
Ascomycota incertae sedis			Dendrosporium (1)
Ascomycota incertae sedis			Dictyochaeta-like sp. or gen. nov. (1)
Ascomycota incertae sedis			Dictyosporium (1)
Ascomycota incertae sedis			Dinemasporium (2)
Ascomycota incertae sedis			Dischloridium (1)

Table 7.3 (continued)

Ascomycete order	Family	Teleomorphs	Anamorphs
Ascomycota incertae sedis			Flabellocladia (1)
Ascomycota incertae sedis			Gliomastix (5)
Ascomycota incertae sedis			Goidanichiella (1)
Ascomycota incertae sedis			Hansfordia (1)
Ascomycota incertae sedis			Harpographium (2)
Ascomycota incertae sedis			Isthmolongispora (1)
Ascomycota incertae sedis			Kramasamuha (1)
Ascomycota incertae sedis			Kylindria (1)
Ascomycota incertae sedis			Lauriomyces (1)
Ascomycota incertae sedis			Menisporopsis (1)
Ascomycota incertae sedis			Minimidochium (1)
Ascomycota incertae sedis			Paraceratocladium (1)
Ascomycota incertae sedis			Parasympodiella (2)
Ascomycota incertae sedis			Phaeoisaria (1)
Ascomycota incertae sedis			Phialocephala (1)
Ascomycota incertae sedis			Pseudobeltrania (1)
Ascomycota incertae sedis			Pseudomicrodochium (1)
Ascomycota incertae sedis			Selenodriella (1)
Ascomycota incertae sedis			Selenosporella (1)
Ascomycota incertae sedis			Speiropsis (1)
Ascomycota incertae sedis			Sphaeridium (1)
Ascomycota incertae sedis			Spiropes (1)
Ascomycota incertae sedis			Sporidesmium (2)
Ascomycota incertae sedis			Sporodesmiella (1)
Ascomycota incertae sedis			Stachybotrys (2)
Ascomycota incertae sedis			Subulispora (1)
Ascomycota incertae sedis			Verticimonosporium (1)
Ascomycota incertae sedis			Wiesneriomyces (1)
Ascomycota incertae sedis			Zygosporium (2)

Figure 7.2 Number of microfungal species among fungal orders recorded in leaf litter of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus pleurocarpa, F. destruens,* and *Ophisthiolepis heterophylla* during the 'substratum' study

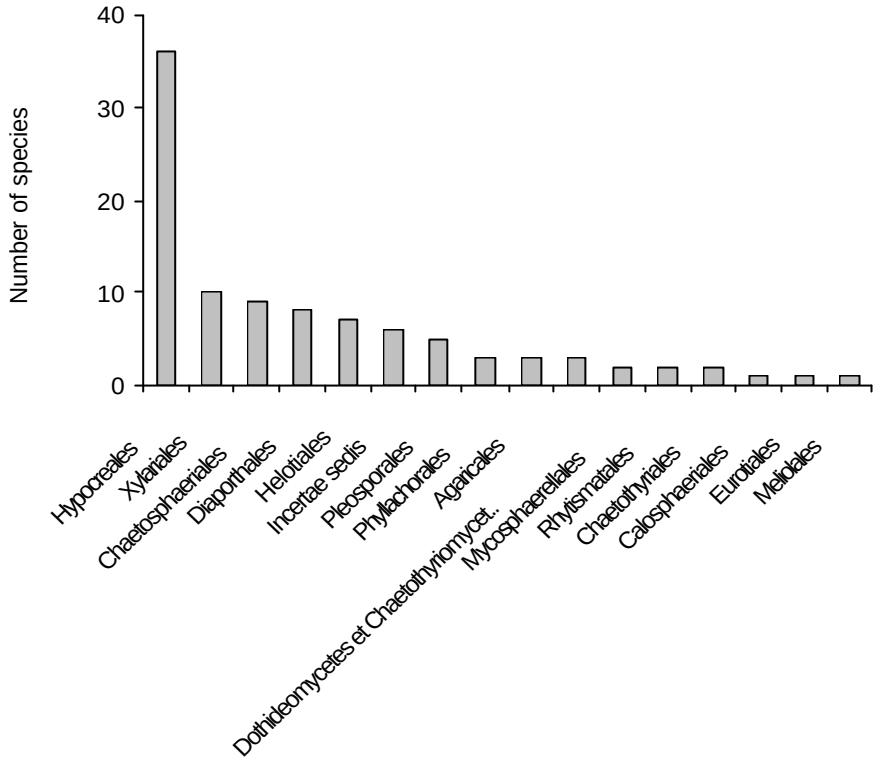


Table 7.4 Number of taxa within taxonomic hierarchy among microfungi from leaf litter of *Ficus pleurocarpa* observed during a succession study

	Totals		
No of orders	12		
No of families	22		
No of genera	12		
No of form genera	51	No of form genera integrated with teleomorph family or order	30
		No of form genera uncertain positions (Ascomycota)	21
No of species	12	No of species	12
No of form species	80	No of form species integrated with teleomorph family or order	49
		No of form species, uncertain positions (Ascomycota)	31
Unidentified taxa	10		
Associated organisms	3		

Figure 7.3 Number of microfungal species among fungal families observed in decaying leaves of *Cryptocarya mackinnoniana, Darlingia ferruginea, Elaeocarpus angustifolius, Ficus pleurocarpa, F. destruens,* and *Ophisthiolepis* heterophylla during the 'substratum' study

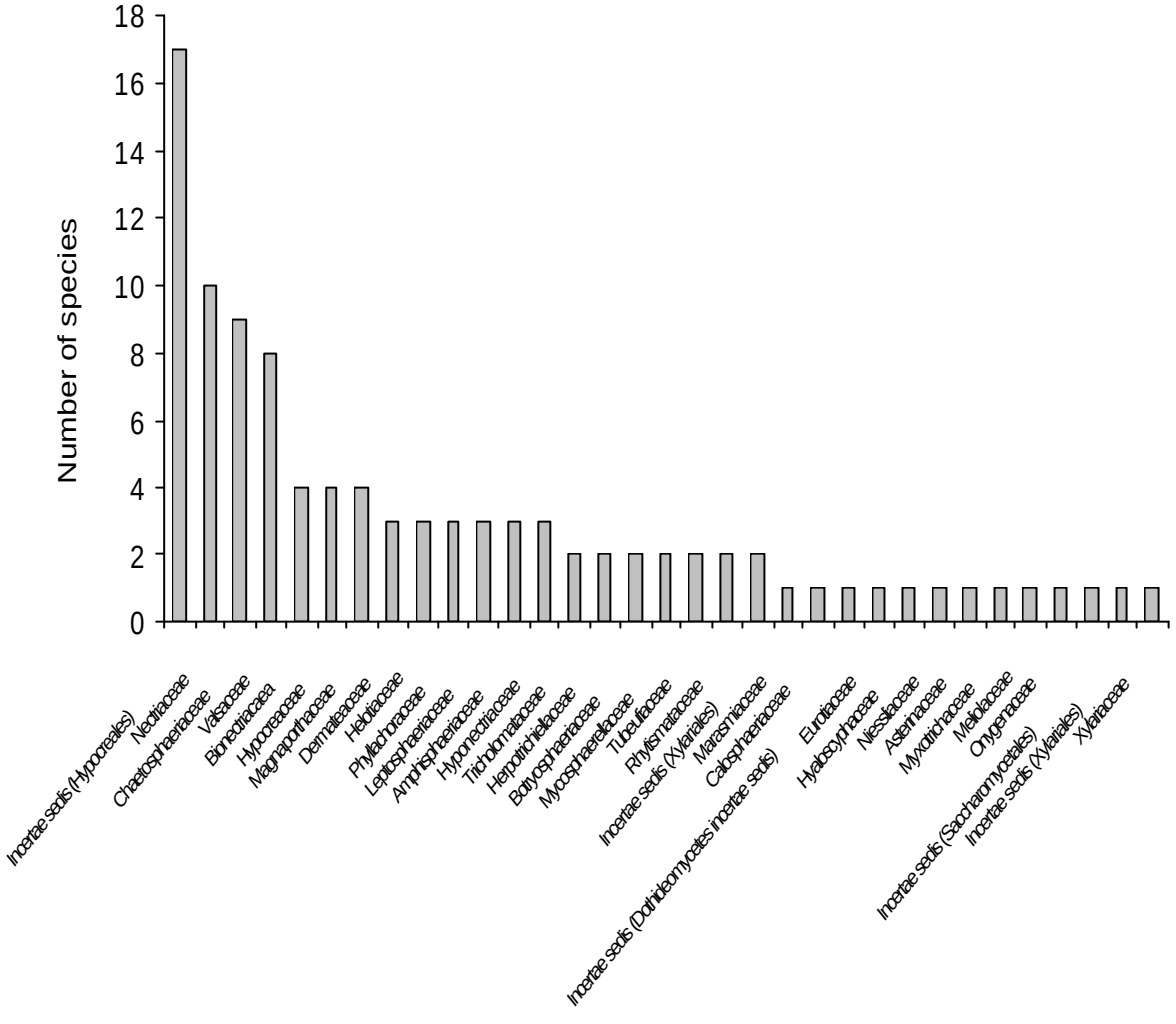


Table 7.5 Microfungal genera recorded in fungal orders and families in leaf litter of Ficus pleurocarpa during a succession study. Number of species observed within genera are provided in brackets.

Acomycete order	Ascomycete family	Teleomorphs	Anamorphs
Chaetosphaeriales	Chaetosphaeriaceae	*Chaetosphaeria* (1)	*Dictyochaeta* (2), *Thozetella* (1)
Diaporthales	Valsaceae	*Ophiognomonia* (1)	*Phomopsis* (4)
Dothideomycetes et Chaetothyriomycetes *incertae sedis*	Botryosphaeriaceae	*Guignardia* (1)	*Leptodothierella* state of *Guignardia* (1)
Dothideomycetes et Chaetothyriomycetes *incertae sedis*	Asterinaceae	*Asterina* (1)	
Dothideomycetes et Chaetothyriomycetes *incertae sedis*	*Incertae sedis*	*Brooksia* (1)	
Dothideomycetes et Chaetothyriomycetes *incertae sedis*	Mycosphaerellaceae		*Cladosporium* (2)
Dothideomycetes et Chaetothyriomycetes *incertae sedis*	Tubeufiaceae		*Helicosporium* (2)
Eurotiales	Trichocomaceae		*Penicillium* (2)
Helotiales	Dermateaceae	gen. nov. (1)	anamorph (1)
Helotiales	Helotiaceae		*Idriella* (4)
Hypocreales	Hypocreaceae		*Gliocladium* (3), *Trichoderma* (2)
Hypocreales	*Incertae sedis*		*Acremonium* (1), *Cephalosporiopsis* (2), *Myrothecium* (1), *Verticillium* (1), *Volutella* (1), *Xenogliocladiopsis*-like sp. or gen.nov.(1)
Hypocreales	Nectriaceae		*Chaetopsina* (1), *Cylindrocladiella* (1), *Cylindrocladium* (4), *Gliocladiopsis* (1)
Hysteriales	Hysteriaceae	*Hysterium* (1)	
Incertae sedis	Orbiliaceae		*Dactylella* (1)
Meliolales	Meliolaceae	*Meliola* (1)	
Phyllachorales	Phyllachoraceae		*Colletotrichum* (1)
Pleosporales	Leptosphaeriaceae		*Phoma* (2)
Pleosporales	Melanommataceae		*Pseudospiropes* (1)
Pleosporales	Pleosporaceae		*Curvularia* (1)
Sordariomycetes *incertae sedis*	Magnaporthaceae	*Gaeumannomyces* (1)	
Trichosphaeriales	*Incertae sedis*		*Nigrospora* (1)
Xylariales	Amphisphaeriaceae	*Discostroma* (1)	*Beltraniella* (1), *Pestalotiopsis* (1)
Xylariales	Hyponectriaceae		*Microdochium* (2)
Xylariales	*Incertae sedis*	*Lanceispora* (1)	
Xylariales	Xylariaceae	*Anthostomella* (1)	
Ascomycota	*Incertae sedis*		*Beltrania* (2)
Ascomycota	*Incertae sedis*		*Chaetospermum* (1)

Ascomycota	*Incertae sedis*	*Circinotrichum* (1)
Ascomycota	*Incertae sedis*	*Cylindrosympodium* (2)
Ascomycota	*Incertae sedis*	*Dactylaria* (3)
Ascomycota	*Incertae sedis*	*Dendrosporium* (1)
Ascomycota	*Incertae sedis*	*Falcocladium* (1)
Ascomycota	*Incertae sedis*	*Gliomastix* (2)
Ascomycota	*Incertae sedis*	*Hansfordia* (1)
Ascomycota	*Incertae sedis*	*Leptothyrium* (1)
Ascomycota	*Incertae sedis*	*Sphaeridium* (1)
Ascomycota	*Incertae sedis*	*Ochroconis* (1)
Ascomycota	*Incertae sedis*	*Parasympodiella* (2)
Ascomycota	*Incertae sedis*	*Selenodriella* (1)
Ascomycota	*Incertae sedis*	*Selenosporella* (1)
Ascomycota	*Incertae sedis*	*Speiropsis* (1)
Ascomycota	*Incertae sedis*	*Sporodesmium*-like (1)
Ascomycota	*Incertae sedis*	*Subulispora* (1)
Ascomycota	*Incertae sedis*	*Trichothecium* (1)
Ascomycota	*Incertae sedis*	*Wiesneriomyces* (2)
Ascomycota	*Incertae sedis*	*Zygosporium* (2)

Figure 7.4 Number of species among fungal orders recorded in leaf litter of *Ficus pleurocarpa* during a succession study

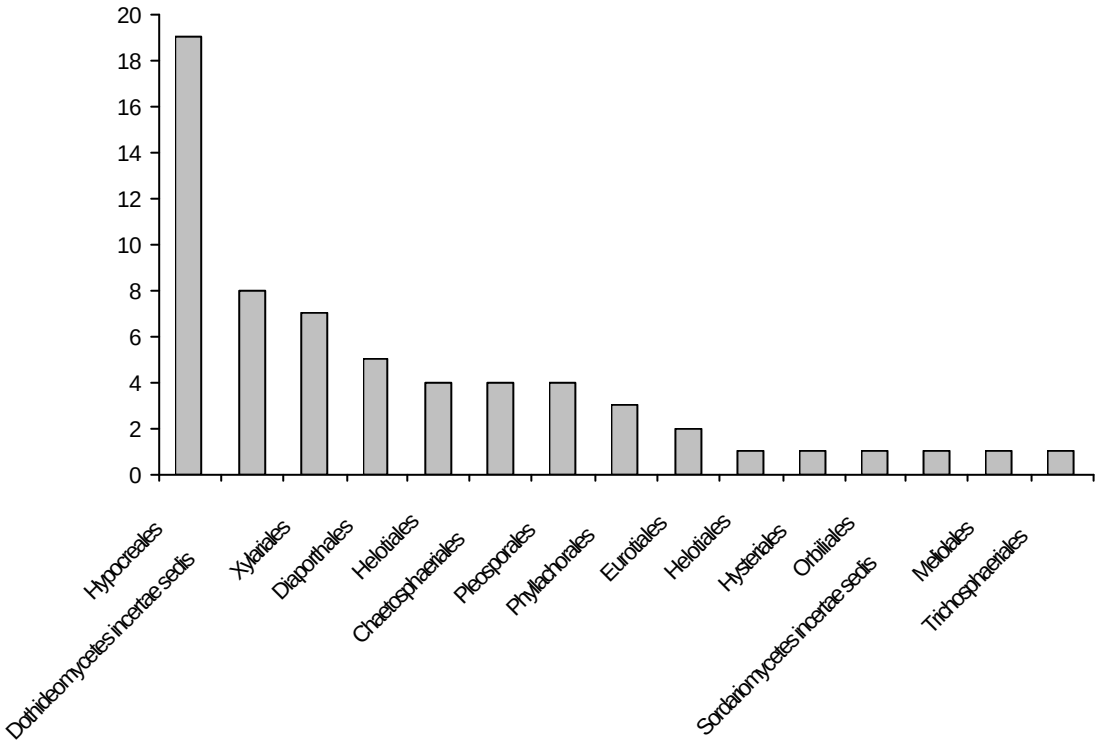
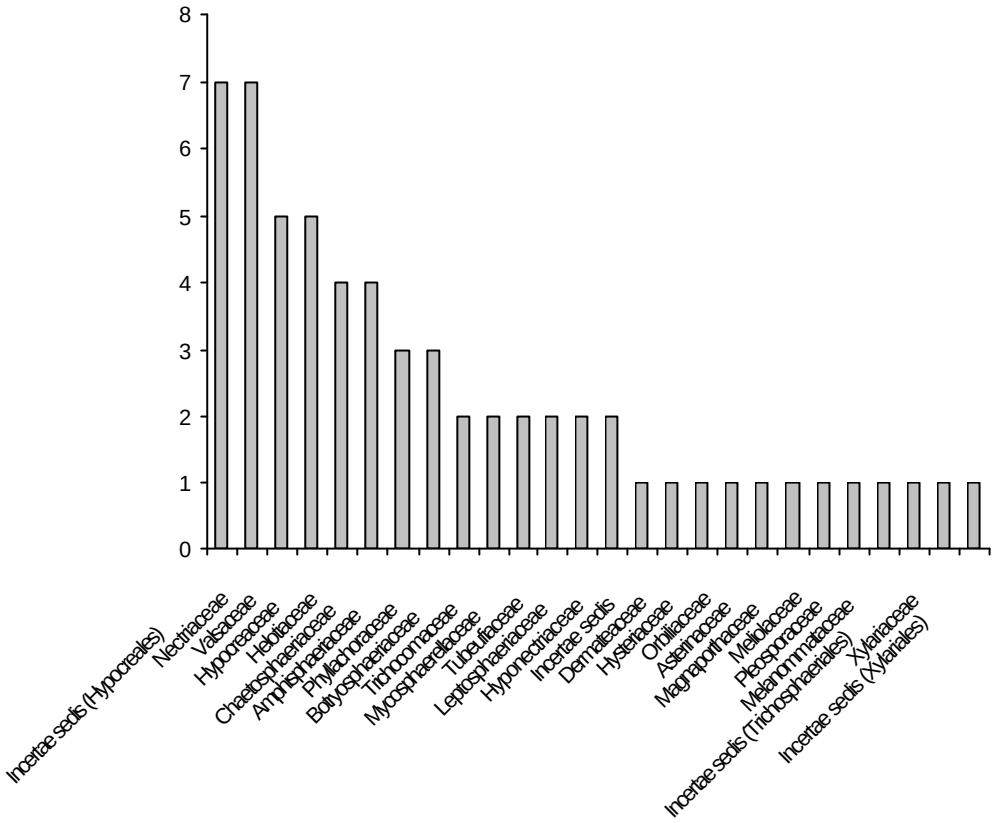


Figure 7.5 Number of microfungal species among fungal families recorded from leaf litter of *Ficus pleurocarpa* during a succession study



7.3.3 Discussion

The results of the present project indicates that the taxonomic diversity of microfungi in leaf litter is great not only at the specific level but also at higher taxonomic ranks (Tables 7.2 and 7.4). The taxonomic diversity of the succession study, which was concerned with one tree species, was only slightly lower at the generic rank and above compared to the substratum study, which included six tree species. In previous studies of the mycota in different substrata such as palms (e.g. Fröhlich, 1992; Taylor, 1997), a higher number of species and genera were observed. The replicated sampling design and sampling at only two study sites would have contributed to this result. However, differences in species numbers may also be related to differential substratum quality. The highly lignified tissues of palm petioles, for example, supported a greater number of teleomorphs (Fröhlich, 1997; Taylor, 1997) than were detected for other part of the fronds or in dicotyledenous litter of the present study.

The distribution patterns of species among orders and families were similar for both the succession and the substratum study (Figs 7.2 to 7.5). These findings are consistent with previous observations from tropical regions (Hyde, 1997a). For example, two groups of fungi, the hypocrealean and xylariaceous fungi, were reported to have a high representation in the tropics (Samuels, 1997; Whalley, 1997) and also dominated in the present study. The Xylariales (in particular Xylariaceae and Amphisphaeriaceae) and the Hypocreales (in particular the Hypocreaceae) were also commonly observed in studies of palms (Fröhlich, 1997; Taylor, 1997) but other common families of those studies, such as the Lasiosphaeriaceae and the Hyponectriaceae, were absent or represented only in low numbers in the dicotyledenous leaf litter of the present project.

In the present study, some of the taxonomic diversity at the generic level and above may have been masked due to the high percentage of anamorphic fungi encountered. Approximately 45 % of taxa could not be integrated into a phylogenetic scheme because the phylogenetic relationships of these anamorphs had not been established below the level of class (i.e. Ascomycota; Tables 7.3 and 7.5). A further limitation of undertaking an analysis based on taxonomic ranks is the fact that the Ascomycota are still in a state of flux. This is evidenced by the number of uncertain positions (*incertae sedis*) for many taxa at higher taxonomic ranks (Table 7.3 and 7.5). The high proportion of taxa observed only in their anamorphic states in this survey highlights the concerns of some practitioners that the abandonment of a dual system of nomenclature could disrupt mycological research if not considered carefully and undertaken prematurely (Gams et al., 2003). This is particularly true for surveys that do not have a primarily taxonomic focus and for which practical identification tools are essential. These surveys may be undertaken either for inventorying, monitoring, bioexploitation or as in the present project for exploring the ecology of microfungi. However, in the long-term the benefits of an integrated system for anamorphs and teleomorphs are undeniable. As is clearly evident from the taxonomic analysis, more information can be extracted from survey data if underpinned by a phylogenetic schema.

CHAPTER EIGHT

Conclusions and Recommendations

8.1 Overview

This thesis has investigated aspects of the diversity, distribution and taxonomy of leaf litter microfungi in a rainforest of the wet tropics of Australia. Chapter One provided a review of current knowledge while Chapter Two explored the effect of leaf storage and surface treatment on observed microfungal diversity as assessed by particle filtration. A study focussed on successional changes of microfungi with advancing decay was discussed in Chapter Three. Chapter Four explored the structure of microfungal assemblages and methods to describe their diversity and Chapter Five assessed the distribution of microfungi on leaf litter of different tree species, over wet and dry seasons and across two matched sites. The co-occurrence of a beetle and a discomycete is explored in Chapter Six and in Chapter Seven, selected taxonomic studies are presented. Conclusions were drawn at the end of each chapter; therefore the main aim of the present chapter is to integrate this information and to provide recommendations for future research.

8.2 Conclusions

In keeping with the aims of this project, microfungal diversity was characterised on a narrowly defined scale in leaf litter of several tree species and was compared effectively across different substrata and for the direct observational method across different time frames. To achieve this in a complex system such as a tropical rainforest, assessments were undertaken under tightly constrained conditions using standardised sampling and isolation strategies. A high variability of species presence and abundance was observed between samples of the same substratum type (Chapter 5). Despite this variability, the present study has shown consistently that microfungi in leaf litter of a tropical rainforest are not random assemblages but rather communities with 'recognisable and measurable differences among repeating assemblages of fungi that occur simultaneously in similar habitats' (States, 1981, Chapter 5). Microfungal leaf litter communities are shaped by

host phylogeny, but their boundaries are not clear-cut with both continuous and discontinuous distribution patterns observed for individual fungal species (Chapter 5). As species richness on leaves of different tree species was correlated with the level of total phenolics, leaf thickness and manganese, chemical and physical leaf attributes may also be involved in shaping overall distributional patterns as well as those of individual microfungal species. The validity of this hypothesis needs to be evaluated in more detailed studies. Achieving a realistic estimate of absolute microfungal diversity remains elusive due to methodological constraints, with isolation methods and sampling strategies effectively acting as filters on the observed microfungal diversity (Chapter 2, 3 and 5).

Microfungal communities do not only differ between substrata but also between collections undertaken at different times. In part, this can be explained by seasonal variations (Chapter 5) and the dynamic nature of leaf litter in tropical rainforests where decay processes advance rapidly (Chapter 3). After undergoing rapid changes in species composition at early stages of decay, microfungal communities appear to reach an 'equilibrium', albeit of short duration, at later stages of decay (Chase, 2003, Chapter 3). However, rapid decomposition of substrata and seasonal variation do not appear to be the only driving forces, as substrata at the same stage of decay (i.e. freshly fallen leaves) placed on the forest floor two weeks apart also differed considerably in observed microfungal species composition (Chapter 3). This observation may point to the role of other factors such as chance and microclimate in the development of a community (Neville and Webster, 1995).

While the co-occurrence of microfungal species on the same substratum may be explained by adaptation to a particular leaf environment, the question of whether beneficial and/or competitive interactions of microfungi influenced species composition and functional roles was beyond the scope of the present study. On a narrower scale, the co-occurrence of a microfungal species and a beetle was explored and an interaction between these organisms was demonstrated (Chapter 6). Observations suggested that this fungus-insect association may enhance decomposition processes. To explore the potentially more subtle interactions between microfungal species, laboratory or mesocosm studies may be better suited, in which the diversity of microfungi and variability of environmental conditions can be controlled (e.g. Fukami and Morin, 2003).

The observed community structure of microfungi with few abundant and many rare species posed a taxonomic challenge for the present study. In a number of instances, species delimitation was difficult between potential congeneric species, for which only one or two specimens were available; this required the application of more than one species concept (e.g. *Thozetella acerosa*, Chapter 7). However, extensive characterisation of taxa is impractical in broad-scale surveys and therefore an element of uncertainty remained in a small number of specimens. For assessing the distribution of microfungi, this is unlikely to introduce a serious bias because singleton species are often eliminated from analyses either directly (e.g. Polishook et al. 1996) or their importance is reduced by the choice of similarity index (e.g. Christensen 1968; Brown et al. 1998; Chapter 5). Specimens, which could not be placed at the generic or family level, were circumscribed using consistent criteria; therefore, comparisons across substrata and collections were possible. A further limitation in assessing the taxonomic diversity was that approximately 50 % of taxa could not be integrated into a phylogenetic scheme because the phylogenetic relationships of these anamorphs had not been established below the level of class (Chapter 7). Nevertheless, among those taxa that could be assessed within a phylogenetic framework, the distribution within orders was similar to those previously reported for tropical microfungi (e.g. Bills and Polishook, 1994a; Fröhlich, 1997; Taylor, 1997; Parungao et al., 2002).

8.3 Recommendations

8.3.1 Methodological considerations

Microfungal diversity is studied for a variety of reasons, including taxonomic surveys, elucidation of habitats and substrata of high microfungal diversity by commercial companies, and comparisons of diversity across sites, habitats or substrata in ecological studies. Definitions of diversity and the aims of a study will vary accordingly and will guide the choice of enumeration strategies, isolation methods, and application of statistical tools. The development of a clear working definition of diversity and establishment of clear aims is, therefore, imperative in order to gain an understanding of microfungal diversity. This is particularly important in co-operative studies, as it is unlikely that one 'standardised' protocol for estimating diversity will accommodate the objectives of different disciplines. For example, synecological studies require an

adequate sample size to allow for the repeated observation of common taxa while assessments undertaken for bio-exploitation may seek the greatest complementarity between samples in order to access maximum diversity (Bills, 1995). Similarly, if the primary aim was to assess the taxonomic diversity of microfungi on a particular host or substratum, it would be reasonable to exclude specimens, which could not be identified unequivocally or for which few collections were obtained. In contrast, a different approach is required to obtain an estimate of diversity, if diversity is defined as species richness, abundance and evenness. In this case, all observed fungi need to be recorded using a defined sampling strategy even if identification or naming of a specimen cannot be achieved. This is likely in particular for tropical regions and substrata that have not been examined extensively and for which few taxonomic resources are available. To reduce the effort required for identification and to potentially facilitate a wider sampling approach, assessments at higher taxonomic ranks have been tested, in particular for insect and marine communities, and a good concordance between family and species data has been reported (e.g. Clarke and Warwick, 1994). For microfungal assemblages on leaf litter, diversity assessments at family level cannot be implemented as identification schemes for the dominant group, i.e. anamorphs, are not based on phylogenetic relationships. Nevertheless, the requirement for full species identifications can be circumvented, for example by applying a morphotype concept to endophyte assemblages (e.g. Arnold et al., 2000) and to cultures derived by particle filtration (Chapter 2, 3 and 5). Surrogate taxonomic units were also used in an assessment of macrofungi by Packham et al. (2002) who referred species of taxonomically complex genera into 'species groups'.

Biases can be reduced through the choice of appropriate sampling and isolation methods. General sampling techniques developed by plant ecologists can be adapted for sampling fungi, such as the use of plots, transects or plotless sampling (e.g. Packham et al., 2002; Henderson, 2003). In addition, diversity estimation of microfungi requires the development of unbiased subsampling strategies at the microscopic level, for example the use of 'microtransects' on leaves (Chapters 5). The present study assessed whether the same conclusions about microfungal distributions could be drawn from data obtained by different isolation methods. Although a greater diversity was observed with the particle filtration protocol, both methods concurred that host phylogeny is the most important determinant of microfungal distributions. The concordance between these

methods indicates that an adequate assessment of distribution patterns could be achieved with either method. However, the particle filtration method had a lower resolution in detecting patterns, did not facilitate comparisons across seasons and also was limited in the type of analyses due to the lower number of replicates that could be included (Chapter 5). Therefore, a direct observational method may be more practical for ecological studies. Equally, the taxonomic approach may be best served using a direct method of isolation (Chapter 2, Chapter 5).

Efficient sampling protocols for the assessment of microfungal diversity would ideally allocate sampling effort as 'thinly and widely' as is consistent with the degree of accuracy required (Colwell and Coddington, 1994). This is difficult to achieve as reliable methods of estimating an adequate sample size for species data are still lacking. For data that are independent and normally distributed, sample size may be estimated by analysing the power of a parametric statistical procedure such as an Analysis of Variance (e.g. Zar, 1999). Analysis of Variance is not suitable for comparing abundances of individual species because of potential species interactions and because species abundance patterns are likely to be 'patchy' resulting in a high percentage of zero values. Therefore, species data characteristically do not meet the assumptions of parametric analyses and non-parametric multivariate analyses may be the most appropriate tools (Clarke and Warwick, 1994). To date no appropriate procedures have been developed to estimate the power of non-parametric multivariate statistics (Clarke and Warwick, 1994). Other methods of estimating an adequate sample size for ecological studies include Pielou's method of pooled quadrats, in which the accumulated species richness or an appropriate diversity index is plotted against the number of sampling units (Magurran, 1988). Microfungal datasets are usually skewed towards rare species and characteristically new, rare species are added with each additional sampling effort; therefore, an asymptote is rarely reached (Chapter 4). However, valuable conclusions about microfungal diversity and distribution are possible in the absence of an asymptote, as long as a representative sample of 'common' species has been observed. Potential methods of assessing whether an adequate representation of 'common' species has been achieved, may include the removal of rare species from a data set prior to plotting accumulation curves (Chapter 2) or the use of an estimator, such as Chao2 (Chao, 1987), which corrects for a high percentage of low

abundance classes (Chapter 4). These methods still await validation for different datasets.

As more studies report sampling completeness based on diversity estimators, a clearer picture may emerge as to what represents an adequate level of sampling completeness to assess ecological questions. For example, an estimated sampling completeness of between 42 % and 67 % for the direct method in the present study allowed the detection of biologically meaningful patterns (Chapter 5). In the mean-time, researchers undertaking ecological or diversity studies of species-rich groups such as microfungi need to make pragmatic choices regarding the design of studies. The sampling strategy needs to take into account potential distributional patterns across hosts and seasons. In addition, an adequate sample size (e.g. observations or cultures per sampling unit) needs to be balanced against adequate replication (e.g. number of sites, trees, substratum units, isolation plates, 'microtransects') in order to undertake isolations within a given time frame (e.g. within the same season). Due to the work intensive nature of sampling microfungi, the number of replicates is usually limited. To counter this limitation, an increase in precision may be achieved by applying a stratified rather than a fully randomised sampling strategy (Ludwig and Reynolds, 1988).

Understanding microfungal diversity requires the selection of appropriate statistical tools. The common aim of these tools is to reduce the complexity of datasets in order to compare diversity across space and/or time. At one end of the spectrum, the greatest reduction in complexity (and concurrent loss of information) is achieved by univariate, taxon-independent approaches such as the calculation of diversity indices and their comparison using parametric tests such as t-test or Analysis of Variance (Magurran, 1988; Clarke and Warwick, 1994). In contrast, non-parametric multivariate techniques conserve more of the information contained in a dataset as they are usually based on similarity matrices. They have also been shown to be more sensitive than univariate methods in detecting differences in community data (Clarke and Warwick, 1994). Graphical representations of distributional patterns are intermediate between these two approaches and may be particularly useful when assessing the effect of disturbance on community structure (Magurran, 1988; Clarke and Warwick, 1994). All indices and techniques are based on underlying assumptions and these should be examined carefully. For example, the Shannon-Wiener index is frequently used to characterise fungal diversity. However, it is based on the assumption that all species in a sample

have been recorded, which is not met in most fungal studies. Similarly, the Jaccard index of similarity is based on presence/absence data and therefore is likely to overestimate the importance of rare species. Indices such as Fisher's alpha and the Bray-Curtis distance measure may provide more satisfactory approaches for the assessment of point and differentiation diversity of microfungi respectively and lead to a higher resolution in comparing microfungal communities across space and time.

Despite the challenges and limitations inherent in studying microfungal diversity, some previous studies (Tsui, 1999; Ho et al., 2001, 2002) as well as the present study have shown that a comparison of diversity across space and time is possible and that biological patterns can be detected consistently using appropriate sampling and analytical approaches.

8.3.2 Future directions

A natural extension of the present project would be to develop the framework for microfungal distributions in rainforest leaf litter on a finer scale. This would be achieved by including more tree species, both congeneric and in different genera, within the selected host families. The underlying reasons for the observed patterns could then be elucidated by matching assessments of microfungal distributions and physical and chemical leaf attributes. In addition, a more detailed characterisation of secondary metabolites might provide further leads. The potential role of manganese concentration in influencing microfungal species richness in leaf litter needs to be confirmed and the underlying mechanism in this relationship elucidated. In addition, the reference collection developed during the present study contains numerous taxa new to science that are as yet undescribed.

Although studies addressing host exclusivity and recurrence in tropical substrata will ultimately assist in refining global fungal species estimates, I refrain from making any predictions here. The observed overlap in leaf litter between six tree species was very low and this may indicate a high fungus to host ratio for tropical rainforests. However, the study was focussed at the family level of host species and as noted above further work is required to fill in the detail at the congeneric level and at the level of different host genera within the same family. Because singleton species pose a problem for estimating fungus to host ratios, understanding their nature will ultimately assist in

refining estimates of global species numbers. For example, a percentage of singleton species may be host recurrent and could become dominant under certain environmental conditions (Loreau et al., 2001). If host recurrence in singleton species was confirmed in future studies, estimates of fungus to host ratios in tropical rainforests would need to be scaled upwards accordingly. Alternatively, singleton species may establish on a leaf by chance and persist under suboptimal conditions until finally declining. If these species are drawn from a common pool of litter fungi or constitute 'dominants' on other tree species (Grime, 1998), an overall lower estimate of fungus to host ratio would be indicated. To address these questions, studies would need to be undertaken at a fine temporal and spatial scale and may be hampered by the need for destructive sampling. Despite the methodological problems facing mycologists, understanding the nature of singletons is essential in arriving at realistic quantitative estimates of host recurrent fungi in tropical rainforests.

A centrifugal-phylogenetic approach (Wapshere, 1974) may provide a useful strategy to extend the baseline information established in the present study for both singleton and more abundant species. With this approach, closely related hosts are studied first and then more and more distantly related plants are included. Due to the high diversity of tree species at all taxonomic levels, the rainforests of the wet tropics of Australia would provide an ideal study site for ongoing research into the host recurrence of microfungal species.

8.3.3 Microfungal communities as model systems

The advantages of microfungal communities as model systems outweigh any limitations and therefore, microfungi may potentially aid our understanding of fungal and general ecology. For example, microfungi can be assessed using direct and indirect methods of detection, which is not practical for most macrofungi. Using two methods of isolation can provide an independent crosscheck between results (Chapter 5). In addition, substrata can be selected randomly in the field, and stored for a period of time before incubation and examination in the laboratory (Chapter 2). This independence of observing fungi in the field simplifies the sampling strategy and reduces the need for frequent site visits. A sampling unit can be as small as a single leaf or piece of decaying wood and therefore, adequate replication is easier to achieve than for macrofungi. The aggregation of microfungal assemblages on small sampling units also makes them

amenable to field or laboratory based experiments in which conditions are manipulated (e.g. Cornejo et al., 1994; Chapter 6) and for testing theories such as MacArthur and Wilson's theory of island biogeography (e.g. Wildman, 1992).

Apart from practical considerations, the potential of microfungal communities as model systems also stems from their diversity and the key ecological roles they fill in decomposition and nutrient cycling. The relationship of diversity and ecosystem function, in particular productivity, has been at the centre of active research for some years (Loreau et al., 2001). Some ecologists have proposed that many species may be ecologically redundant and that fewer, yet functionally diverse, species can fulfil the same functions. In contrast, others view biodiversity as an 'insurance' or a buffer because different species respond differently to environmental fluctuations and this presumably leads to more predictable community or ecosystem properties (Loreau et al., 2001). These questions could be addressed for decomposition processes using microfungal communities in both observational and experimental approaches. Beyond establishing theoretical frameworks, understanding microfungal distributions and the relationship between fungal diversity and ecosystem function, in particular carbon sequestration, may also prove relevant for determining the 'value' of different forest types in mitigating greenhouse gas emissions.

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APPENDICES

Appendix A. Leaf characteristics

Scale bars = 50 mm

Elaeocarpaceae

Elaeocarpus angustifolius leaves are thin, glabrous and have a smaller surface area. They turn bright red on the tree prior to falling and decay quickly once on the forest floor.

Lauraceae

Cryptocarya mackinnoniana has large leaves with a thick, tough lamina and raised reticulate veins on the abaxial surface. Oil dots are present on the leaf surface (Hyland and Whiffin, 1993). These leaves retain their cuticle until later stages of decay and skeletonise slowly.

Neolitsea dealbata has relatively thin, glabrous leaves with a white waxy layer on the abaxial side of the leaves (Hyland and Whiffin, 1993).

Moraceae

Leaves of *F. pleurocarpa* are large, leathery with a thick and glabrous lamina and a robust petiole and midrib. They decay slowly over a period of more than 90 days.

Ficus destruens and *F. pleurocarpa* leaves are similar in morphology and both contain latex in petioles and midribs. However, leaves of *F. destruens* have a smaller surface area and thinner petioles.

Proteaceae

Leaves of *O. heterophylla* are intermediate in size and leaf margins curl tightly during the decay process. Their abaxial surface is densely covered with prostrate, brown hairs (Hyland and Whiffin, 1993).

Leaves of *D. ferruginea* are on average larger and thicker than those of *O. heterophylla* and have a tendency to repel water during early stages of decay. The leaf lamina is densely clothed in short, rusty or dark brown hairs on the underside (Hyland and Whiffin, 1993) and unlike *O. heterophylla* does not curl during decomposition.

Appendix B. List of taxa isolated from *Neolitsea dealbata* leaf litter in the assessment of surface treatments

Appendix B. List of taxa isolated by leaf washing and particle filtration protocols as well as direct isolation in the assessment of surface treatments (continued)

Species	L[1]	W/L[2]	W[3]	D[4]
Phomopsis sp. 1	62	3	0	1
Dictyochaeta sp. 1	47	1	0	4
Sterile strain 1	42	2	0	0
Phomopsis sp. 2	39	0	0	1
Gliocephalotrichum sp.	37	3	0	1
Sterile strain 2	21	2	0	0
Cylindrosympodium sp.	15	0	0	0
Phoma sp. 1	13	0	0	0
Sterile strain 3	12	1	0	0
Dactylaria sp.	11	0	0	0
Sterile strain 4	11	0	0	0
Hyphomycete sp. 1	11	1	0	0
Idriella sp. 1	10	0	0	0
Beltraniella sp.	9	0	0	1
Pestalotiopsis ?versicolor	9	3	0	1
Sterile strain 5	9	0	0	0
Cladosporium cladosporoides	8	0	0	0
Beltraniella portoricensis	7	0	0	1
Hyphomycete sp. 2	7	0	0	0
Coelomycete sp. C	6	0	0	1
Sterile strain 6	6	0	0	0
Acremonium sp. 1	5	1	0	0
Acremonium sp. 2	5	5	0	0
Fusarium sp. 1	5	2	0	0
Selenosporella curvispora	5	0	0	0
Colletotrichum sp. 1	4	1	0	0
Beltrania sp.	3	0	0	0
Speiropsis sp.	3	0	0	0
Beltrania rhombica	2	0	0	1
Myrothecium sp.	2	0	0	1
Phomopsis sp. 3	2	0	0	0
Phyllosticta sp.	2	0	0	0
Xylariaceous culture	2	0	0	0
Thozetella radicata	2	0	0	0
Torula sp.	2	0	0	0
Wiesneriomyces javanicus	2	0	0	0
cf.*Camposporium* sp.	1	0	0	0
Acremonium sp. 3	1	0	0	0
Arthrinium sp.	1	0	0	1
Beltrania-like sp.	1	0	0	0
Chalara sp.	1	0	0	1
Coelomycete sp. A	1	0	0	0
Coelomycete sp. B	1	0	0	0
Coelomycete sp. D	1	0	0	0

Species	L[1]	W/L[2]	W[3]	D[4]
Coelomycete sp. E	1	0	0	0
Coelomycete sp. F	1	0	0	0
Colletotrichum sp. 2	1	0	0	0
Dictyochaeta sp. 2	1	0	0	1
Dictyochaeta sp. 3	1	0	0	0
Dictyochaeta sp. 4	1	0	0	0
Fusarium sp. 2	1	0	0	0
Helicosporium sp.	1	0	0	0
Idriella sp. 2	1	0	0	0
Periconia sp.	1	0	0	0
Phoma sp. 2	1	0	0	0
Phoma sp. 3	1	0	0	0
Rhinocladiella sp.	1	0	0	0
Stachybotrys sp.	1	0	0	0
Thozetella sp.	1	0	0	1
Hyphomycete sp. 3	1	0	0	0
*Wardomyce*s sp.	1	0	0	0
Wiesneriomyces javanicus	1	0	0	0
Sterile strain 7	1	1	0	0
Sterile isolates 8 to 65	70	0	0	0
Penicillium sp. 1	0	0	13	0
Geotrichum sp.	0	0	11	0
Penicillium sp. 2	0	0	10	0
Trichoderma sp.	0	0	10	1
Penicillium sp. 3	0	0	7	0
Penicillium sp. 4	0	0	6	0
Aspergillus sp.	0	0	3	0
Mucor sp.	0	0	3	0
Penicillium sp. 5	0	0	3	0
Penicillium sp. 6	0	0	2	0
Sterile isolates 66-75	0	0	10	0
Anthostomella clypeata	0	0	0	1
Coelomycete sp. G	0	0	0	1
Coelomycete sp. H	0	0	0	1
Dictyoarthrinium africanum	0	0	0	1
Gliocladium sp.	0	0	0	1
Gyrothrix circinata	0	0	0	1
Gyrothrix pediculata	0	0	0	1
Iodosphaeria phyllophila	0	0	0	1
Menisporopsis ?theobromae	0	0	0	1
Pestalotiopsis sp.	0	0	0	1
Thozetella sp.nov.	0	0	0	1
Thysanophora sp.	0	0	0	1
Hyphomycete sp. 4	0	0	0	1

[1] No. of isolates obtained from leaf particles
[2] No. of isolates obtained from washwater and leaf particles
[3] No. of isolates obtained from washwater
[4] No. of fungi observed by direct method

Appendix C. Percent abundance of fungi observed on decaying leaves of *Ficus pleurocarpa* using a direct observational method in a succession study

(Chapter 3; n=10 leaves per collection)

Fungal Code	Species name	green leaves	day 0	day 7	day 14	day 30	day 46	day 62	day 78	day 94
F579	*Acanthophyses*-like structures								3.6	
F473	*Acremonium* spp.	6.2	5.5	2.8	5.1		9.2	11.7	12.5	
F556	*Anthostomella reniformis* sp.nov.				1.3					
F569	*Asterina* sp.		4.6	4.6	5.1	5.1	4.6	5.0	7.1	
F548	Basidiomycete A					1.7				
F573	Basidiomycete B						1.5		1.8	7.4
F530	*Beltrania concurvispora*							1.7		
F509	*Beltrania rhombica*			0.9						
F518	*Beltraniella portoricensis*			0.9						
F456	*Brooksia tropicalis*	3.1	0.9							
F482	*Cephalosporiopsis* sp. 1		0.9							
F491	*Cephalosporiopsis* sp. 2		0.9		1.3	1.7				
F517	*Chaetopsina fulva*				2.5	3.4	1.5			
F515	*Chaetospermum camelliae*			0.9	5.1		6.2	13.3	10.7	14.8
F495	*Chaetosphaeria* sp.		0.9	0.9	1.3		3.1	1.7		
F552	*Circinotrichum maculiforme*					3.4				
F589	*Cladosporium* sp. 1									3.7
F590	*Cladosporium* sp. 2									3.7
F455	Coelomycete A (acervular)	7.7	9.2	6.4	11.4		1.5			
F531	Coelomycete B							1.7		
F465	Coelomycete C	1.5								
F462	*Colletotrichum* sp. 1	9.2	1.8	0.9						
F463	*Colletotrichum* sp. 2	9.2	2.8	1.8						

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F520	*Colletotrichum* sp. 3			0.9						
F588	*Curvularia* sp.									3.7
F532	*Cylindrocladiella elegans*							1.7		
F489	*Cylindrocladium colhounii var. colhounii*	3.1	0.9							
F524	*Cylindrocladium floridanum*	3.1								
F493	*Cylindrocladium ilicola*			0.9						
F559	*Cylindrocladium retaudii*									
F505	*Cylindrosympodium cryptocaryae* sp.nov.			3.7						
F549	*Cylindrosympodium variabile*					1.7			1.8	
F484	*Dactylaria belliana* sp.nov.		0.9							
F535	*Dactylaria ficusicola* sp.nov.				3.8	5.1	12.3	16.7	8.9	7.4
F587	*Dactylaria* sp.nov.									3.7
F572	*Dactylella* sp.						1.5			
F586	*Dendrosporium lobatum*									3.7
F507	*Dictyochaeta* sp. 1			0.9	2.5					3.7
F533	*Dictyochaeta simplex*				1.3		1.5		1.8	
F472	Dermateaceae (gen.nov.)		4.6	7.3	12.7	16.9	1.5			
F528	*Discostroma ficusicola* sp.nov.						3.1			
F543	*Falcocladium* sp.					3.4	1.5		1.8	
F468	*Gaeumannomyces*-like sp.nov.		7.3	2.8	6.3	1.7				
F508	*Gliocladiopsis tenuis*			4.6		1.7	4.6	6.7	5.4	
F481	*Gliocladium ?solani*		2.8		1.3		1.5	1.7		
F511	*Gliocladium* sp. 2			2.8	1.3	3.4				
F544	*Gliocladium* sp. 3				1.3					
F480	*Gliomastix ?macrocerealis*		0.9							
F557	*Gliomastix elasticae*		0.9							
F558	*Guignardia* sp.	1.5								
F503	*Hansfordia pulvinata*	1.5		4.6						
F584	*Helicosporium griseum*							1.7	1.8	22.2
F574	*Helicosporium* sp.							3.3	7.1	7.4

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F570	*Hysteriaceous ascomycete*						4.6	6.7	12.5
F563	*Idriella cagnizarii*						1.5		
F475	*Idriella lunata*		1.8	4.6		11.9			
F521	*Idriella ramosa*			1.8					
F540	*Idriella* sp.					1.7			
F467	*Lanceispora amphibia*		3.7	3.7	6.3				
F525	*Leptodothierella* state of *Guignardia* sp.	1.5							
F550	*Leptothyrium* sp.					3.4			
F580	*Sphaeridium pilosum*								1.8
F560	*Sphaeridium* sp. *						1.5		
F464	*Meliola* sp.	7.7		0.9					
F561	*Microdochium phragmites*						3.1		
F582	*Microdochium* sp. *								1.8
F541	*Myrothecium* cf. *lachastrae*					1.7			
F458	*Nigrospora panici*	1.5							
F486	*Ochroconis humicola*		0.9						
F513	*Ophiognomonia elasticae*			3.7	5.1	1.7	3.1		
F564	*Parasympodiella* sp.						1.5	1.7	
F496	*Penicillium* sp. 1		1.8						
F453	*Penicillium* sp. 2			1.8					
F457	*Pestalotiopsis* cf. *breviseta*	15.4	9.2	0.9	1.3				
F502	*Phoma* sp. 1	3.1		1.8					
F527	*Phoma* sp. 2						1.5		
F461	*Phomopsis* sp. 1	1.5	6.4	2.8					
F454	*Phomopsis* sp. 2			5.5	3.8				
F497	*Phomopsis* sp. 3		0.9	0.9	2.5				
F478	*Phomopsis* sp. 4		5.5	1.8					
F501	*Parasympodiella laxa*			1.8					
F451	*Pseudospiropes pinarensis*	1.5							
F512	*Selenodriella fertilis*			5.5		1.7			
F504	*Selenosporella cristata*			0.9					
F542	*Speiropsis pedatospora*					5.1	3.1	1.7	5.4
F449	*Sporodesmium* sp.nov.	1.5	1.8						

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F466	sterile brown mycelium with distinct chlamydospores	1.5								
F506	*Subulispora procurvata*			0.9						
F566	*Thozetella falcate* sp.nov.						3.1		1.8	
F567	*Trichoderma* sp.						1.5			
F485	*Trichoderma viride*		0.9		1.3			1.7		
F483	*Trichothecium* sp.		0.9							
F578	unidentified ascomycete								1.8	
F450	unidentified ascomycete	12.3	1.8							
F547	unidentified hyphomycete					3.4				
F538	unidentified hyphomycete				1.3					
F474	*Verticillium* sp.		4.6		1.3		1.5		1.8	
F510	*Volutella ramkurii*			6.4			1.5		1.8	
F546	*Wiesneriomyces javanicus*					1.7	1.5	1.7		
F519	*Wiesneriomyces* sp. *			2.8		10.2	3.1	3.3	1.8	
F534	*Xenogliocladiopsis*-like gen. or sp.nov.				5.1	5.1	10.8	11.7	3.6	11.1
F471	*Zygosporium echinosporium*		8.3	3.7	1.3					
F460	*Zygosporium mansonii*	3.1	7.3	0.9	1.3					
Associated micro-organisms										
F487	Actinomycete 1	4.6	0.9	1.8	10.1	8.5	1.5	5.0	1.8	7.4
F492	Actinomycete 2		0.9	0.9	1.3					

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Appendix D. Percent abundance of microfungi in fallen leaves of *Ficus pleurocarpa,* observed during a succession study

Microfungi were detected by particle filtration in freshly fallen, green leaves (GL), freshly fallen yellow leaves (day 0) and leaves that were collected after 46 and 94 days.

Species	GL [a]	day 0	day 46	day 94
Acremonium sp. 1 [b]				0.7
Aureobasidium pullulans		0.7		
Beltrania rhombica [b]		18.5		3.6
Beltrania concurvispora [b]		0.7		
Beltraniella portoricensis [b]			0.6	
Cephalosporiopis sp. 1 [b]			5.9	
Cephalosporiopis sp. 2 [b]			0.6	
Cladosporium sp. [b]				0.7
Coelomycete B [b]			0.6	
Cylindrocladium retaudii [b]	7.3		1.2	0.7
Cylindrocarpon sp.		0.7		
Cylindrocladiella elegans [b]			0.6	
Cylindrosympodium cryptocaryae [b]			3.0	
Cylindrosympodium variabile [b]				1.4
Dactylaria belliana [b]				17.4
Dactylaria ficusicola [b]			1.8	
Dactylaria sp.				1.4
Dendrosporium lobatum [b]			0.6	
Dictyochaeta sp. [b]			1.8	
Geniculosporium sp.	0.9			
Geotrichum sp.			0.6	
Gliocladiopsis tenuis [b]			3.0	
Gliocladium sp. 2 [b]			0.6	
Glomerella sp. & anamorph [b]	4.6	0.7	1.2	
Guignardia sp. 1 [b]	1.8			
Guignardia sp. 2	0.9			
? Hansfordia sp.	3.7			
Idriella lunata [b]			17.8	19.6
Idriella sp. [b]			0.6	
Lanceispora amphibia [b]			1.2	0.7
Lemonniera terrestris				0.7
Microdochium sp. [b]			0.6	
Ochroconis humicola [b]		2.1	0.6	0.7
Ochroconis sp. *2*			3.0	0.7
Pestalosphaeria sp.	0.9			
Pestalotiopsis ?breviseta [b]	20.2	5.5	0.6	
Phoma sp. 1 [b]		26.0		
Phoma sp. 2 [b]			4.1	
Phomopsis sp. [b]				0.7
unidentified ascomycete A [b]		13.7		
unidentified ascomycete B [b]			0.6	
unidentified hyphomycete A	0.9			

unidentified hyphomycete B	0.9			
unidentified hyphomycete C		2.1		
unidentified hyphomycete D		1.4		
unidentified hyphomycete E		0.7		
unidentified hyphomycete F		0.7		
Verticillium sp. [b]				2.2
Xenogliocladiopsis-like *gen. nov.* [b]			2.4	1.4
Xylariaceous sp. 1	9.2	0.7		
Xylariaceous sp. 2	4.6			
Xylariaceous sp. 3	3.7			
Xylariaceous sp. 4	3.7			
Xylariaceous sp. 5	0.9			
Sterile mycelium 1	5.5			
Sterile mycelium 2	4.6			
Sterile mycelium 3	3.7			
Sterile mycelium 4	2.8			
Sterile mycelium 5 - 6	1.8			
Sterile mycelium 7-23	0.9			
Sterile mycelium 24		8.2		
Sterile mycelium 25		6.8		
Sterile mycelium 26		3.4		
Sterile mycelium 27		2.7		
Sterile mycelium 28		2.1		
Sterile mycelium 29 - 32		0.7		
Sterile mycelium 33			11.8	
Sterile mycelium 34			4.1	
Sterile mycelium 35 - 36			3.6	
Sterile mycelium 37 - 40			2.4	
Sterile mycelium 41			1.8	
Sterile mycelium 42 -63			0.6	
Sterile mycelium 64				8.7
Sterile mycelium 65 -67				2.9
Sterile mycelium 69 - 74				1.4
Sterile mycelium 75 - 100				0.7

[a] green leaf isolation differ in collection times and isolation method

[b] denotes that these species were also identified by direct isolation

Appendix E. Comparison of species estimates

Estimates based on the Incidence-based coverage estimator (ICE), Burnham and Overton jackknife estimator (B&O), and fitted Michaelis-Menton estimator (MMf) for direct observational data are compared with Chao2 estimates for direct observational data. Estimates based on the Abundance-based coverage estimator (ACE), Burnham and Overton jackknife estimator, fitted Michaelis-Menton estimator particle filtration data are compared with Chao1 estimates for the particle filtration method.

Direct	Chao2	ICE	B&O	MMf	Percent difference	ICE (%)	B & O (%)	MMf (%)
Succession	130	133	136	129		2	5	0.8
Substratum	Chao2	ICE	B&O	MMf	Percent difference	ICE (%)	B & O (%)	MMf (%)
Total	269	250	287	223		7	7	16
Cm	137	122	161	112		11	20	16
Ea	70	70	66	61		0	6	14
Fp	102	98	92	87		4	10	16
Oh	145	127	134	86		12	9	44
Fd	94	100	80	69		6	14	31
Df	57	49	47	52		14	20	11
						8 ±5	12 ±6	21±12
Particle Filtration	Chao1	ACE	B&O	MMf	Percent difference	ACE (%)	B & O (%)	MMf (%)
Wet season								
Total	627	749	693	654		19	11	4
Cm	370	370	433	415		0	17	12
Ea	206	212	219	233		3	6	13
Fp	163	174	168	190		7	3	17
Oh	241	247	264	297		2	10	23
Dry season								
Total	406	468	441	358		12	9	12
Cm	249	308	271	227		24	11	9
Ea	181	184	195	169		2	8	7
Fp	156	170	164	163		9	5	4
						9 ±4	9 ±9	11±6

Appendix F. Percent abundance of fungi observed in fallen leaves of *Cryptocarya mackinnoniana, Elaeocarpus angustifolius, Ficus pleurocarpa, Opisthiolepis heterophylla, Darlingia ferruginea* and *Ficus destruens* using a direct observational method

Code F	Name	*Cm*	*Ea*	*Fp*	*Oh*	*Df*	*Fd*
F666	*Acremonium* sp. 1				0.5		
F757	*Acremonium* sp. 2			0.3	0.5		
F895 (a)	*Acremonium* sp. 3			0.3			
F599	*Acremonium* sp. 4	0.3		1.3	1.0	1.2	
F892 (b)	*Acremonium* sp. 5		1.8	0.6	1.0		
F626	*Asterina* sp.	1.4					
F768	*Auxarthron* sp.	0.3					
F803	*Bahusutrabeeja bunyensis*	3.0					
F864	*Beltrania* cf. *concurvispora*			0.9	0.5		3.9
F815 (a)	*Beltrania rhombica*	1.6	0.6	1.9			7.8
F815 (b)	*Beltrania* sp. * (*B. rhombica*)	0.3					
F807	*Beltraniella portoricensis*	9.3	0.3	1.9		3.7	1.0
F858	*Botryodiplodia theobromae*			0.3		1.2	
F450	*Botryosphaeria*-like sp.			2.2			
F661	*Brooksia tropicalis*			0.6	0.5	1.2	
F652	*Cephalosporiopsis* sp. 1		0.6		3.5		
F892 (a)	*Cephalosporopsis* sp. 2				0.5		
F746	*Chaetopsina fulva*	0.5					
F845	*Chaetospermum* sp.	0.3	0.3	4.4	6.4	2.5	4.9
F889	*Chalara* cf. *nigricollis*	2.2			0.5		
F635	*Chalara* sp.	1.6	0.3				
F860 (a)	*Circinotrichum falcatisporum*				0.5		
F860 (b)	*Circinotrichum* sp. *				0.5		
F912	*Circinotrichum*-like sp.			1.3			
F769	*Cladosporium* sp. 1	0.3					
F812	*Cladosporium* sp. 2	0.5	1.2	0.3	0.5		
F818	*Coccomyces* cf. *limitatus*		13.3				
F645	coelomycete (acervular) A		0.6				
F455	coelomycete (acervular) B			14.2			7.8
F704	coelomycete (acervular) C		0.3				
F921	coelomycete (pycnidial) A			0.3			
F878	coelomycete (pycnidial) B					1.2	
F592	*Colletotrichum* sp. 1						1.0
F922	*Colletotrichum* sp. 2			0.3			
F835	*Colletotrichum* sp. 3			0.3			
F813 (b)	*Conioscypha* sp.	0.3					
F876	*Cryptophiale* cf. *guadalcanensis*					14.8	
F613	*Cryptophiale kakombensis*	0.8				1.2	1.0
F1006	*Curvularia* sp.			0.3			1.0
F848	*Cylindrocarpon* cf. *ianthothele*				0.5		
F859	*Cylindrocarpon* cf. *orthosporum*			0.6	2.5		
F849	*Cylindrocladiella* sp.		0.6		0.5		
F857	*Cylindrocladium coulhounii* var. *coulhounii*		0.6	0.9	0.5	1.2	1.0
F722	*Cylindrocladium floridanum*		0.3				
F839 (a)	*Cylindrosympodium cryptocaryae* sp. nov.	1.4		0.3			1.0
F1005 (b)	*Dactylaria belliana* sp.nov.				0.5		
F874	*Dactylaria ficusicola* sp.nov.						1.0

F853	*Dactylaria* section *Mirandina* sp. 1	0.3					
F710 (b)	*Dactylaria* section *Mirandina* sp. 2	0.3					
F873 (b)	*Dendrosporium lobatum*						0.3
F472	Dermataceae gen. nov.			11.9			6.9
F747	*Dictyochaeta* cf. *novae-guineensis*	0.5	0.6				
F800	*Dictyochaeta simplex*	3.3	0.9	2.2	3.0	11.1	2.0
F801	*Dictyochaeta* sp. 1	2.2	0.3	0.6			
F916	*Dictyochaeta* sp. 2	1.6	0.3				1.0
F894	*Dictyochaeta-like* gen.nov.		2.7				
F914	*Dictyosporium* cf. *australiense*				0.5		
F905	*Dinemasporium* sp. 1	0.5					
F804	*Dinemasporium* sp. 2	0.3					
F827	*Dischloridium* sp.	0.3	4.2				1.0
F833	*Discostroma ficusicola* sp. nov.			3.5			
F767	*Flabellocladia* sp.	0.3					
F726	*Fusarium* sp.			0.3			
F899	*Gaeumannomyces*-like sp.nov.			3.5			
F621	*Geotrichum* sp.	0.3					
F822	*Gliocephalotrichum simplex*		3.9				
F811	*Gliocladiopsis* sp.	2.5			4.0	1.2	1.0
F829	*Gliocladiopsis tenuis*		0.6	0.3			
F810	*Gliocladium* sp. 1	1.9	0.3	1.9	4.0		1.0
F601 (a)	*Gliocladium* sp. 2			0.3	0.5		
F843 (b)	*Gliomastix luzulae*				1.0		
F843 (a)	*Gliomastix murorum*				3.0		
F706	*Gliomastix* sp. 1				0.5		
F736 (a)	*Gliomastix* sp. 2				1.5		
F736 (b)	*Gliomastix* sp. 3 *(G. luzulae)*				2.0		
F820	*Gnomonia elaeocarpa* sp. nov.		13.9				
F819	*Gnomonia queenslandica* sp. nov.		14.2				
F901	*Gnomonia* sp. 1	1.9			0.5		
F883	*Gnomonia* sp. 2	0.3				3.7	
F863	*Goidanichiella* sp.						5.9
F821	*Guignardia* sp.		1.8				
F847	*Hansfordia pulvinata*	0.5	0.3	0.3	1.5		
F824 (a)	*Harpographium* sp. 1		0.6				
F875	*Harpographium* sp. 2					12.3	
F627	*Helicosporium griseum*	0.8			0.5		
F881	*Helicosporium* sp.	0.3			3.5	4.9	
F596	*Hyponectria* sp.						1.0
F865	*Idriella acerosa*		0.3	0.3	3.0		3.9
F873 (a)	*Idriella cagnizzari*	0.8	3.3		0.5		3.9
F861	*Idriella lunata*			0.6	0.5		3.9
F708	*Ijuhya* sp.				0.5		
F776	*Ijuhya aquifolii*				1.0	1.2	
F927	*Ijuhya leucocarpa*				2.0		
F817	*Iodosphaeria* sp.nov.	0.3	0.3				
F906	*Isthmolongispora intermedia*	0.8					
F877 (b)	*Kramasamuha* cf. *sibika*		0.9			1.2	
F884	*Lachnum* sp. nov.	5.8				3.7	
F862	*Lanceispora amphibia*						4.9
F917	*Lauriomyces helicocephala*		0.3				
F850	*Linocarpon*-like sp.	0.3					
F1012	*Marasmius* sp.	3.3		0.3	3.0	1.2	
F464	*Meliola* sp.			1.9			
F802	*Menisporopsis theobromae*	9.9					
F824 (b)	*Microdochium* sp.	0.6					0.6
F816	*Minimidochium microsporum*	0.8					
F743	*Minimidochium*-like sp.	0.3					
F907	*Mollisia* sp.	0.8					

F887	*Mycena* sp. 1			0.6		2.5	
F1001	*Mycena* sp. 2	0.3		0.3			
F724	*Myrothecium* sp. 1			0.3			
F924	*Myrothecium* sp. 2			0.3			
F772	*Niesslia* sp.			0.3			
F834	*Nodulisporium* sp.			0.6			
F915	*Oidiodendron tenuissimum*				0.5		
F513	*Ophiognomonia elasticae*			6.9			1.0
F867	*Ophiognomonia* sp.	0.3			2.5	1.2	1.0
F1004	*Paraceratocladium* sp.nov.	0.5					
F837 (b)	*Parasympodiella elongata*	0.5	0.3	0.3	2.5	4.9	1.0
F837 (a)	*Parasympodiella laxa*				0.5		1.0
F825	*Penicillium* sp.		13.3		0.5		
F828	*Pestalotiopsis* spp.	0.5	2.1	8.5	4.5	4.9	5.9
F918 (b)	*Phaeoisaria* sp			0.3			
F1010	*Phialocephala bactrospora*		0.3				
F826	*Phoma* sp. 1		2.4	0.3	2.5		
F866	*Phoma* sp. 2			0.9	2.0		1.0
F69	*Phoma* sp. 3						2.0
F789	*Phomopsis* sp. 1			0.3			1.0
F880	*Phomopsis* sp. 2					1.2	
F632	*Pseudobeltrania* sp.	1.6					4.9
F851 (b)	*Pseudomicrodochium antillarum*	0.8					
F806	*Pyricularia* sp. 1	0.3					
F877 (a)	*Pyricularia* sp. 2	0.3					
F637	*Pyricularia* sp. 3	0.3					
F873 (b)	*Rhinocladiella cristaspora*						1.0
F851 (a)	*Rhinocladiella* sp.	0.3					
F903	*Rhytismataceae*	0.5					
F909	*Roumeguerilla* sp. nov.		2.7				
F742	*Scolecobasidium* cf. *fusiforme*		1.5	0.3			
F893	*Scolecobasidium* sp. *		0.9	0.3			
F891	*Selenosporella* sp.		0.3	0.3	2.5	2.5	
F854 (a)	*Selenosporella* sp.	0.3					
F854 (b)	*Selenosporella* sp. or *Selenodriella* sp.	0.3					
F611	*Selenosporella* sp.2	1.6				2.5	
F616	*Selenosporella* sp.3	0.3					
F1008	*Speiropsis* sp.				0.5		
F898	*Sphaeridium pilosum*			0.6			
F904	*Spiropes* sp.	0.3					
F840	*Sporidesmium* cf. *ponapense*	1.1			1.5		
F449	*Sporidesmium* sp. nov.			2.2			
F813 (a)	*Sporodesmiella garciniae*	1.6					
F844 (a)	*Stachybotrys* cf. *parvispora*			0.6	6.4		
F844 (b)	*Stachybotrys* sp. *				3.0		
F823	*Stilbella* sp. 1		0.3		1.0		
F669	*Stilbella* sp. 2					1.2	
F890	*Subulispora procurvata*	0.8					
F598	synnematal hyphomycete					1.2	1.0
F856	*Thozetella boonjiensis* sp.nov.	0.8					
F919	*Thozetella falcata* sp.nov.	2.2					
F885	*Thozetella gigantea* sp.nov.	1.9				1.2	
F809	*Thozetella queenslandica* sp.nov.	4.7			0.5		
F886 (a)	*Thozetella* sp.					1.2	
F910	*Trichoderma viride*		3.0	0.3			1.0
F601 (b)	unidentified hyphomycete	0.3					
F784	unidentified hyphomycete	0.3					
F839 (b)	unidentified hyphomycete	0.3					

F846	unidentified hyphomycete				0.5		
F895 (b)	unidentified hyphomycete				0.5		
F923	unidentified hyphomycete		0.3				
F830	unidentified hyphomycete *(F784)		1.2				
F860 (c)	unidentified hyphomyete	0.3				3.7	
F732	*Verticillium* sp. 1	0.8					
F838	*Verticillium* sp. 2			0.6			1.0
F655	*Verticillium* sp. 3			1.3	2.5		2.0
F928	*Verticimonosporium ellipticum*	0.5					
F870	*Volutella* sp.						2.0
F750	*Volutella-like* sp.	0.5					
F805	*Wiesneriomyces javanicus*	7.4			3.0		2.0
F896	*Xenogliocladiopsis*-like sp. or gen. nov.**			3.5	2.5		
F900	*Zygosporium echinosporium*	1.1		1.6	3.0		
F929	*Zygosporium mansonii*			4.4	0.5		
F740	*? Catenosubulispora* sp.	0.3					
F756	*? Dactylaria* sp.		0.3				
F872	*? Hyponectria* sp.			0.3		2.5	2.9
F730	*? Kylindria* sp.	0.3					
Associated micro-organism							
F920	slime mold		0.3				

* species delimitation of taxa with congeneric taxa were based on small differences. Due to sparse material, further assessments were not undertaken. Results of statistical analyses did not vary markedly when taxa were 'lumped' with congenerics.

** DNA sequence analysis is unequivocal with respect to placement of this specimen (P.W. Crous, pers. comm.). Further systematic work of *Myrothecium* and *Xenogliocladiopsis* by a different research group is currently in progress.

Appendix G. Correlation between the number of samples, number of occurrences and number of species observed during the substratum study summed for six tree species

The six tree species included *Cryptocarya mackinnoniana, Elaeocarpus angustifolius, Ficus pleurocarpa, Opisthiolepis heterophylla, Darlingia ferruginea* and *Ficus destruens*

		No. of ccurrences	No. of samples	No. of species
No. of ccurrences	Pearson Correlation	1	.893	.783
	Sig. (2-tailed)		.02*	.07
	N	6	6	6
No. of samples	Pearson Correlation	.893	1	.778
	Sig. (2-tailed)	.02*		.07
	N	6	6	6
No. of species	Pearson Correlation	.783	.778	1
	Sig. (2-tailed)	.07	.07	
	N	6	6	6

* Correlation is significant at the 0.05 level (2-tailed).

Appendix H. Number of species, occurrences, Fisher's alpha, estimated species numbers and sampling completeness for direct observations and particle filtration data

DIRECT

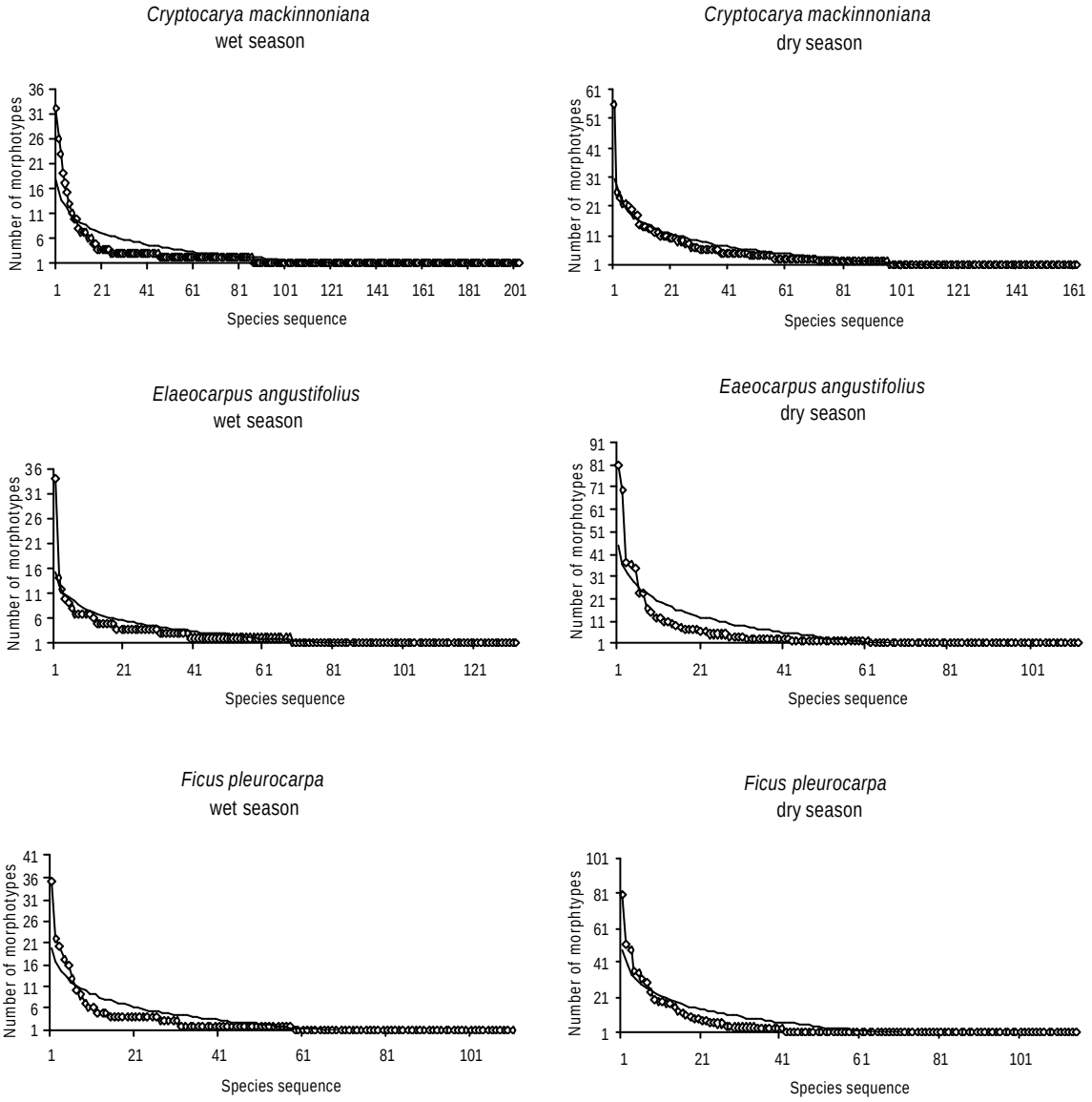
	No. of species	Occurrence	Fisher's a	Chao 2 estimate	Complete-ness %
Wet season					
Cm	49	188	22 (15-28)	153	32
Ea	34	178	13 (8-17)	54	63
Fp	46	172	21 (15-27)	68	68
Oh	35	94	20 (13-27)	76	46
Df	20	58	11 (6-16)	34	59
Fd	25	63	15 (9-21)	40	63
Total	135	753	48 (40-56)	219	62
Dry season					
Cm	50	177	23 (17-30)	70	71
Ea	28	153	10 (6-14)	38	74
Fp	32	146	13 (8-17)	45	71
Oh	36	108	19 (13-25)	56	64
Df	16	23	23 (12-35)	31	52
Fd	24	39	27 (16-37)	105	23
Total	114	646	40 (33-48)	152	75
Millaa Millaa	136	683	51 (43-59)	191	71
Topaz	146	716	56 (47-64)	207	71

PARTICLE FILTRATION

	Number of morphotypes	Number of isolates	Fisher's alpha	Chao1 estimate	Completeness
Wet season					
Millaa Millaa	279	718	148 (130,-166)	384	73
Topaz	269	880	120 (106-135)	404	67
Dry season					
Millaa Millaa	177	1029	61 (52-70)	321	55
Topaz	189	1097	62 (55-74)	248	68

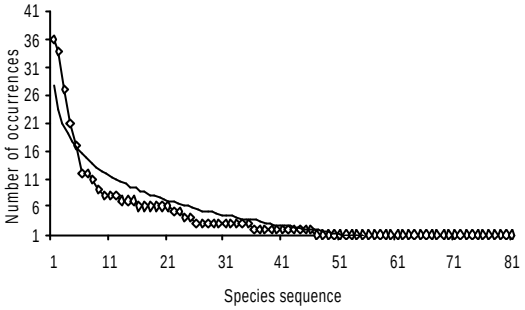
Appendix I. Abundance curves of microfungi observed by A. a particle filtration method and B. the direct method in leaf litter of six tree species

A

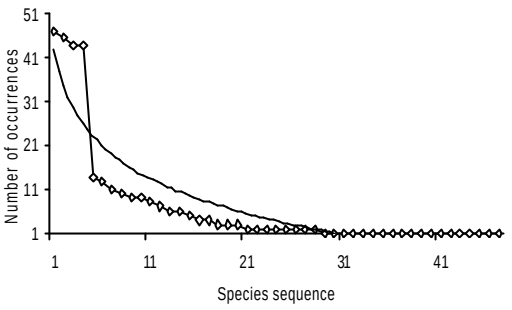


B.

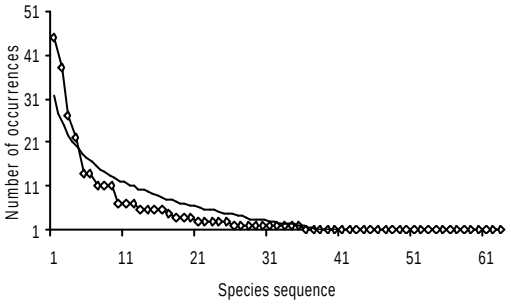
Cryptocarya mackinnoniana



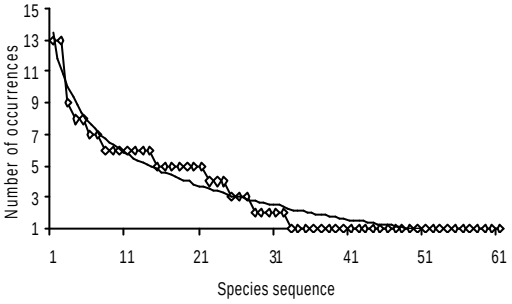
Elaeocarpus angustifolius



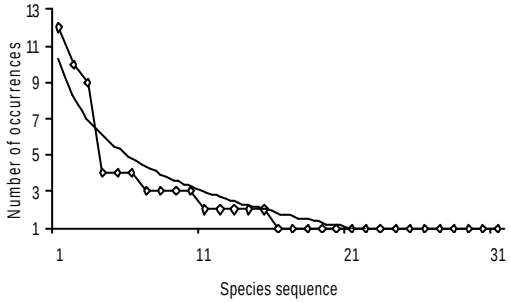
Ficus pleurocarpa



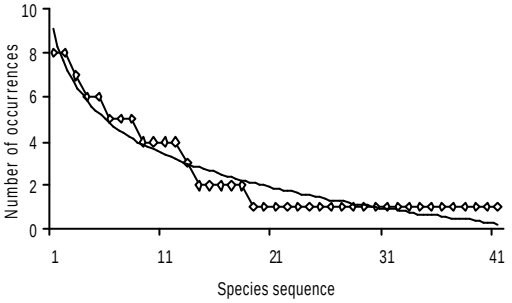
Opisthiolepis heterophylla



Darlingia ferruginea



Ficus destruens



Appendix J. Mean temperature and relative humidity measured on collection days measured over a period of two years

2001		Mean temperature	Mean relative humidity
Season	wet	26	81
	dry	21	70
Site	1	24	75
	2	23	76
Tree species	*Cm*	23	76
	Ea	24	75
	Fp	23	76
	Oh	23	76
2002			
Season	wet	23	79
	dry	21	74
Site	1	22	73
	2	21	80
Tree species	*Cm*	21	78
	Ea	23	75
	Fp	21	77
	Oh	21	77

Kruskal-Wallis Test of Significance for temperature and relative humidity

	K-W Test	Temperature	Relative humidity
Year	H	14.38	0.73
	d.f.	1	1
	p	<0.001 **	0.39
Season	H	106.94	79.48
	d.f.	1	1
	p	<0.001 **	<0.001 **
Site	H	3.25	21.13
	d.f.	1	1
	p	0.07	<0.001 **
Tree species	H	1.32	2.83
	d.f.	3	3
	p	0.73	0.42

* * significant at the 1 % level

Appendix K. Leaf attributes and chemistry for living leaves of Cryptocarya mackinnoniana, Elaeocarpus angustifolius, Ficus pleurocarpa, F. destruens and Darlingia ferruginea

Data were sourced from Kanowski (1999) with permission.

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Appendix L. Correlation between leaf attributes and chemistry of living leaves and number of species in decaying leaves of six tree species isolated by the direct method

A. To control for differences in sampling effort between species (three species were represented by six individuals, and two species by two individuals), the average richness calculated from all pairwise combinations of individuals for the former three species was used in correlation analyses. B. Only significant correlations are shown.

A.

Species richness estimate from two individuals
47 (36,54)
29 (23,37)
36 (29, 41)
36 (27, 45)
41
31

B.

Factor	Correlation
Total phenolics	r^2=0.77 p=0.05*
Manganese	r^2=0.81 p=0.04*
Leaf thickness	r^2=0.83 p=0.03*

* significant at the 5 % level

Appendix M. Publications