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

Thesis submitted

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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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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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A number of papers have arisen from this work (Appendix M) or are in preparation. As co-author, Dr John Kanowski critically reviewed the paper that formed the basis of Chapter Five, provided additional statistical analyses for this paper and chapter, and shared information about local rainforest sites and leaf chemistry. Co-author Dr Margaret Barr confirmed two species as new to science and provided feedback on a paper that is part of Chapter Seven. Dr Roger Beaver identified the beetle associated with one species of microfungi and provided valuable feedback on the paper that formed Chapter Six. Dr Will Edwards shared his knowledge of diversity estimation and tropical rainforest ecology. Dr Elaine Harding and Dr Shannon Bros provided an excellent introduction to multivariate analysis. Microphotographs were taken at the University of Hong Kong and the University of Chiang Mai.

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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.**