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Accumulating and managing DNA sequence data for New Zealand's fungi

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Identifications based on a DNA sequence comparison is becoming a standard method of choice, especially in applied fungal ecology and for many taxa of high biosecurity significance. In ecology, this relates to the need to track fungal hyphae, rather than the macroscopic fruiting bodies, through space and time. It is the hyphae through which fungi interact with the ecosystems to which they belong, through which they secrete metabolites, and through which they capture and exchange nutrients. The genetic diversity of hyphae can be assessed only by using molecular identification tools. In biosecurity the need for molecular identification tools relates in part to a loss of traditional mycological taxonomic skills, as well as the fact that many groups of fungi with high biosecurity significance are difficult to identify morphologically to the species level. Identification using DNA sequences relies on a library of reliable, existing sequences against which to compare unknowns. Although the accumulation of DNA sequences in databases such as Genbank is increasing exponentially, this data is provided from many different sources, and is haphazard in terms of both taxon selection and reliability of the original identification. In many cases the data is not linked back to a voucher specimen, making verification impossible. This talk will discuss the value of being able to provide a New Zealand-focussed set of DNA sequences that can be linked back to reliably identified and publicly available voucher specimens. Some of the data needed for such a resource is being accumulated through the existing NZFungi database, linking specimens vouchered in the PDD herbarium and ICMP culture collection to Genbank records generated from them. Also being accumulated is a set of DNA sequences deposited in Genbank, based on collections from New Zealand, but with vouchers deposited in other herbaria. A crude, BLAST-based search interface will be demonstrated that allows unknowns to be queried against reliable data sets. A major limitation to the present effectiveness of such a resource will be its incompleteness, and the likelihood that many searches will provide no close match. This can be overcome through research groups (ecological and plant pathogenic, as well as taxonomic) around the country having a collaborative approach to data accumulation and management, and through targeted sequencing of specific groups of particular ecological and biosecurity interest.

Evolution of Australasian Inocybaceae: support for multiple lineages and diversification during the Paleogene

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A molecular phylogeny of the ectomycorrhizal agaric family Inocybaceae is used to evaluate competing historical biogeographical hypotheses and to examine patterns of diversification in the family. This research incorporates a sample of taxa from the tropics and southern hemisphere, which have yet to be integrated in any worldwide monograph of the Inocybaceae. Ancestral state reconstruction analyses, combined with a relaxed molecular clock analysis, indicate support for immigration of early lineages out of the palaeotropics by the late Cretaceous and into temperate regions of both hemispheres by the Paleogene where the family underwent considerable diversification. The Australasian component of the Inocybaceae originated at least ten times at various stages primarily during the Paleogene between 65 and 24 million years ago.

Comparative biogeography of ectomycorrhizal and saprotrophic mushrooms

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Although fungi are ubiquitous and play an important role in terrestrial ecosystems, fungal biogeography (or myco-geography) has not been extensively studied within a phylogenetic framework. The cryptic nature of many fungi makes them difficult to sample, and thus hindering global scale biogeographic studies. In this talk, I will examine my preliminary results from multigene analyses of Hysterangiales and Geastrales, two of the four major groups within Phallomycetidae (Basidiomycota), to understand the comparative biogeography of closely related, but ecologically distinct groups. For example, unlike Hysterangiales, which is characterized by ectomycorrhizal habit and hypogeous fruiting bodies with spore dispersal by animal (rodents and small marsupials) mycophagy, Geastrales is characterized by saprobic habit and above- or below-ground fruiting bodies with spore dispersal by wind. Comparative biogeography of four closely related groups within Phallomycetidae will provide exciting insight into the fungal biogeography, which is still in a developing stage as compared to plant and animal studies. No such comprehensive biogeographic studies for macrofungi are yet available.

Evaluation of stream baiting for *Phytophthora* spp. as a surveillance tool in NZ

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The genus *Phytophthora*, once classified with the fungi but now considered more closely allied to the algae is an oomycete. Oomycetes share many of the characteristics of true fungi but are different in their genetics and reproductive mechanisms. Phytophthoras are responsible for some of the most destructive plant diseases known to man. Examples include the European potato famine of the 19th century, which was caused by *P. infestans* and *P. cinnamomi*, which has caused devastating plant losses in some ecosystems in Australia. Commonly known as water moulds *Phytophthora* spp. produce motile zoospores and

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require water for dispersal. Many species can be found in waterways.

Stream monitoring with baited traps placed in the water has recently become an important part of early detection surveillance systems for *Phytophthora ramorum*, causal organism of Sudden oak death (SOD) in western USA. The method is also used in Australia to detect the presence of *P. cinnamomi*. Previously in New Zealand surveys for the presence of *Phytophthora* spp. have relied on isolations directly from the plants or challenging the soil with a variety of baits. To date no stream monitoring has been carried out in New Zealand. Although the basic methods used and reported by practitioners of stream baiting are very similar there are differences in the type of bait used, the length of baiting period and the type of waterway sampled. To enable us to evaluate the method for conditions in New Zealand and investigate the presence of *Phytophthora* spp. a series of traps were set up in six streams on the Volcanic Plateau. The type of waterway and surrounding land use varied from site to site. Variables such as bait type, season and the length of time the baits were left in the stream before collecting and processing were trialled. During a 12-month period over 300 pythiaceous isolates were selected for further evaluation. At this time isolates have been categorised into approximately 20 groups and representatives from each group are being examined both morphologically and using molecular identification methods.

Ectomycorrhizal fungi in plantation forests of New Zealand – a PhD project and future work at Scion, Rotorua

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In Forestry, ectomycorrhizal (ECM) fungi are of great importance as they enhance the growth of plantation species and facilitate the establishment of nursery stock following outplanting. Mycorrhizal research on plantation species in New Zealand has come to a standstill after the extensive work of Chou-Chu and Chou-Chu & Grace in the mid 90's. A PhD project from 2004 – 2008 resumed the work on ECM of *Pinus radiata* in New Zealand, aiming to expand existing data and explore, especially below ground, ECM communities more rigorously by using methods such as molecular fingerprinting (Restriction Fragment Length Polymorphism, RFLP) and DNA sequencing. A nursery and four *P. radiata* stands of varying age in Kaingaroa Forest, North Island of New Zealand, were examined and sporocarp as well as soil core surveys were conducted in 2005 and 2006. Eighteen ECM species were observed fruiting above ground, 19 ECM species were identified below ground. *Inocybe sindonia, Wilcoxina mikolae, Rhizopogon pseudoroseolus*, *R. luteorubescens, Pseudotomentella* sp., *Pseudotomentella tristis* and *Tomentella* sp. were noted for the first time as ECM associates of *P. radiata* in New Zealand. The overall species richness and diversity of ECM fungi associated with the exotic host tree in New Zealand was low compared to similar forests in the Northern Hemisphere but similar to other exotic plantations in the Southern hemisphere. There was little correlation between the species fruiting and the species colonising root tips. ECM species identified from nursery seedlings did survive the first year of outplanting and were found to be dominant in the first year of outplanting. A follow up study showed that a change over from 'nursery' to 'forest' ECM occurred after two years of being in the plantation and nursery ECM were found to be completely replaced after seven years. *Rhizopogon rubescens* was found to be the most dominant species. A current project investigates the nursery ECM species and their fate in the first year of outplanting of *Pseudotsuga menziesii* seedlings and root trainer stock as well of *P. radiata* seedlings and containerised stock, each under two soil conditions. Future projects deal with the effect of fungicides and fertilisers on ECM communities as well as the aspect of pathogen protection and production of plant growth promoters by ECM fungi.

Molecular phylogenetics and species boundaries of New Zealand's "holiest" lichens (*Menegazzia*, Parmeliaceae)

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Menegazzia is a genus of ~70 species of lichen forming fungi found mainly throughout Australasia and South America. They are characterized by the easily noticeable perforations ("holes") found throughout their upper thallus. Taxa within this group (both subgeneric and specific) have largely been described based on ascus structure, lobe morphology, and chemistry. A molecular phylogenetic dataset was constructed here to test these descriptions and help elaborate on them. Eighty recently collected specimens from throughout the South and North Island of New Zealand were used to build this dataset. We focused mainly on the internal transcribed spacer (ITS), but this data is also supplemented by preliminary results from the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and translation elongation factor- (TEF) regions. Selected morphological and chemical characters were then mapped onto the resulting phylogenetic trees to infer the evolutionary histories of these traits and assess how confidently we can use them in subgeneric delimination. Species boundaries in twelve of the New Zealand morphospecies were also examined to determine phylogenetic support, and three previously unknown species complexes are revealed.

An investigation of the genetic diversity of *Cortinarius rotundisporus* in Australia and New Zealand

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Cortinarius rotundisporus Clel. & Cheel is an ectomycorrhizal basidiomycete characterised by a green-blue pileus with a variable yellow central region, and brown, short ellipsoid to subglobose, ornamented spores. It occurs in association with *Eucalyptus* and *Casuarina* in Australia, and *Leptospermum* and *Kunzea* in New Zealand. *C. rotundisporus* ssp. *nothofagi* Soop was described from *Nothofagus* forest in NZ and this was later raised to *C. tessiae* Soop. *C. oleaginus* Clel. & J. Harris and *C. austro-evernius* Clel. & Cheel are considered by some authors to be synonyms of *C. rotundisporus* but others retain these as separate species, distinguishing them largely on the basis of basidioma size and colours. A study of genetic variation in *C. rotundisporus* (*New Phytol*. (1999) **142**, 561–568) found three phylogenetically distinct internal transcribed spacer (ITS) types (RFLP Types I, II and III), but the relationship of these ITS types to other described species was not investigated. Here we present preliminary results of an investigation of the genetic diversity of *C. rotundisporus* in Australia and New Zealand. We sequenced the ITS region for 27 putative collections of *C. rotundisporus sens. lat.* from both countries, and constructed a phylogeny by maximum parsimony analysis, with additional DNA sequences from Genbank of *C. rotundisporus* and related species. Most collections formed a monophyletic group that included RFLP Type I. Within this clade, there was some phylogenetic structure, including 2 mostly Australian clades, and 2 exclusively NZ ones. We found that RFLP Type II was related to *Cortinarius tessiae*, and that RFLP Type III is not

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closely related to the other two, with our analyses indicating a relationship to *Cortinarius* subgen. *Dermocybe*.

The time to foray

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This paper examines the concept of seasonality used by a Tasmanian indigenous population and compares it to the notion of seasonality derived from European usage. Lists of macrofungal species from three separate studies in the Warra long-term ecological research (LTER) site in southern Tasmania, where visits were made approximately fortnightly over a period of 12 months in each study, show that the majority of the fungal records and between 62.9 – 88.4% of the species occurred during the indigenous season Tunna (corresponding to the months of May – August in the European-based calendar) in at least one of the plots (or a portion of a plot) in each survey. The type of substrate supporting the fungi was also a determining factor, with wood, soil and litter appearing to have differing seasonal requirements. Information such as this may be useful in helping mycologists plan macrofungal sampling protocols.

Probing fungal diversity using DNA sequence libraries: lessons from the Duke Forest Mycological Observatory.

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Studies of ribosomal DNA sequence libraries reveal a diverse community of eukaryotic microorganisms in soils from southeastern USA Piedmont forests. These communities are dominated by fungi, and also include protistan, chlorophyte and metazoan lineages. Using phylogenetic analysis of ITS sequences from basidiome surveys and environmental sources, identification to the species level was possible for many common saprobic and mycorrhizal Agaricomycetes, including *Russula*, *Suillus*, *Mycena*, *Gymnopus* and others. We have been using this sequence database to study how fungal communities respond to environmental perturbation. Examples include study of community shifts in response to CO_2 enrichment, and community response to long-term land-use histories with different recovery histories. Our studies also identified a number of technical problems associated with molecular-based community analyses (chimeric sequences, PCR-bias, etc.), these will be discussed.

The final sinking of *Endoptychum* (Agaricales)

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The sequestrate genus, *Endoptychum*, is now known to be an assemblage of species that are not closely related. The type *E. agaricoides* Czern was found to be more closely related to the agaricoid genus *Chlorophyllum* Massee, and is now listed under the name *C. agaricoides* (Czern) Vellinga. Likewise, *Endoptychum depressum* was found to be more closely related to *Agaricus*, and is now known as *A. inapertus*. Finally *Chlorophyllum* was conserved against the name *Endoptychum*, which left several European and all the Australian species of *Endoptychum* in nomenclatural limbo. Morphological and molecular characters were used to determine the final resting place of these taxa.

Examination of several hundred collections, revealed the presence of 8 Australian species, 2 matching previously published taxa. Analysis of DNA sequences suggests affinities with several different lineages within the Agariceae, including *Macrolepiota*, *Agaricus* and *Chlorophyllum*.

The major molecular genotypes of *Crytococcus gattii* vary in key virulence attributes and antifungal resistance

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Cryptococcus neoformas and *C. gattii* are environmental yeast species that are capable of causing life-threatening disease in mammals, including humans. *C. neoformans* is a cosmopolitan and predominantly opportunistic pathogen and is found in high numbers in pigeon guano. *C. gattii* usually affects otherwise healthy people, is more restricted in distribution and is associated with decaying wood. Genetic analyses have found *C. gattii* to divide into four distinct molecular types, described as VGI, VGII, VGIII and VGIV. These have been found differ in geographic range, ecology, epidemiology and fertility and may represent cryptic species. In particular, VGI is strongly associated with *Eucalyptus* wood and causes sporadic cryptococcosis, while VGII has been found on a range of tree species and has caused some apparent outbreaks, including an ongoing outbreak on Vancouver Island, Canada. VGIII has an unknown ecological niche and has been found infecting AIDS patients in California. VGIV is rare and appears confined to parts of Africa. To assess whether the molecular types might have underlying differences of clinical significance, we examined a range of virulence attributes and antifungal susceptibility in isolates belonging to VGI-III. Most VGII isolates grew at higher temperatures and produced more melanin and phospholipase than isolates of other genotypes, with VGIII having lower levels of the majority of virulence traits. Significantly, VGII was more resistant to azole-based antifungal agents and many isolates were clinically resistant to fluconazole. These data indicate that determining the isolate genotype may be important when diagnosing and treating *C. gattii* cryptococcosis.

Determining Genetic Species in *Colletotrichum*

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Filamentous fungi from the ascomycete genus *Colletotrichum* (teleomorph: *Glomerella*) are globally important phytopathogens. They cause economically significant damage to many crops including cereals, grasses, legumes, ornamentals, vegetables and fruit trees. Typical disease symptoms (anthracnose) are characterised by sunken necrotic lesions with orange conidia.

Colletotrichum species are generally recognised by morphological characteristics, such as conidial size and shape, shape of appressoria, growth rate, colour, ect. However morphological characters can change under different growth conditions, or be lost.

C. gloeosporioides is large "group-species" encompassing a set of genetically, biologically and morphologically diverse isolates. Multiple gene sequences from a global collection of vouchered specimens were used to identify genetically distinct groups within *C. gloeosporioides*. Different sequence analysis techniques will be discussed, including recently developed "species tree" methods. Such analysis techniques can be used in

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conjunction with other characteristics to resolve biologically meaningful species or subspecies.

Monitoring fungal hotspots - Stringybark Walking Trail, Deep Creek Conservation Park – a South Australian example

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A number of macrofungal hotspots in South Australia have been identified from surveys conducted in Parks in seven Regions of the State since 1997. These data, together with historic collections deposited in the State Herbarium of South Australia (AD) enable estimation of the conservation status of macrofungal taxa present at these hotspots.

One hotspot, Stringybark Walking Trail, an 18 ha site in Deep Creek Conservation Park, approximately 100 km south of Adelaide, has a particularly rich macrofungal flora. The number and diversity of fungal species recorded at this small site exceeds that at all other locations surveyed over the last twelve years in South Australia, with the exception of the much larger and ecologically more diverse Flinders Chase National Park on Kangaroo Island.

A Bunch of No-Good Rotters

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Sustainable forestry in Tasmania aims to retain all elements of a natural forest cycle in its management plan. In the wet *E. obliqua* forests of southern Tasmania, wildfire at different intervals has produced a mosaic of multi-aged stands with a successional climax of temperate rainforest after 400 years in the absence of fire. There are several heart rot polypores found in these forests that appear to be either host specific or confined to fruiting on large diameter eucalypts. Such trees are found in the older forests (> 250 years) or as legacies of wildfire disturbance in the younger stands. The logging of the old growth forests, the silviculture treatment of CBS (clearfell, burn and sow) and current rotation lengths of 80–100 years will lead to a loss of the large diameter *E. obliqua* and mature *Nothofagus cunninghamii* and *Atherosperma moschatum*. The fruiting bodies of the macrofungal species *Phellinus wahlbergii*, *Fomes hemitephrus* and *Australoporus tasmanicus* found in this study were exclusively in older forests and could be at risk of local disappearance. This has implications for forestry managers in Tasmania.

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Recently completed research theses in mycology

For inclusion in this section, please send thesis title, student name, name of degree, department/institution, date of completion, supervisors and abstract to the Editor, David Orlovich (david.orlovich@otago.ac.nz). Inclusion is at the discretion of the Editor and is subject to journal space constraints.

Coarse woody debris, macrofungal assemblages, and sustainable forest management in a *Eucalyptus obliqua* forest of southern Tasmania

Genevieve Maria Gates

PhD thesis, The University of Tasmania, Australia.

2009

Supervisors: Assoc. Prof.Caroline Mohammed, School of Agricultural Science UTAS; Dr Neil Davidson, School of Plant Science UTAS; Dr Tim Wardlaw, Forestry Tasmania

Abstract

This study focussed on two components of the forest ecosystem at a small spatial scale: coarse woody debris (CWD), defined as fallen dead wood ≥ 10 cm diameter and ≥ 1 m length, and the macrofungal assemblages found on wood, soil and litter in native forest at different times of regeneration since the natural disturbance of wildfire.

The CWD on the forest floor and standing dead wood (stags) in four 50 × 50m plots (= 1 ha total area) with differing wildfire histories in a *Eucalyptus obliqua* dominated native wet sclerophyll forest in southern Tasmania, Australia, were quantified and mapped. The CWD volumes obtained were amongst the highest in the world.

Analyses showed that although a plot size of 0.25 ha was too small to give an accurate measurement of volume, it was large enough to contain dead wood having attributes that reflected the stand structure resulting from wildfire disturbance. Therefore, a plot's wildfire history can be deduced from the CWD and stags of a 0.25 ha plot.

The substrates wood (dead wood and standing trees), soil and litter in each plot were surveyed for macrofungal fruit bodies at approximately fortnightly intervals for 14 months. A total of 849 macrofungal species was recorded from 1ha of native forest.

Wood supported 410 species of which 295 were on CWD but not exclusively, i.e., a few species were found on CWD and soil or on CWD and litter. The majority of the remaining species on wood was supported by 'other dead wood' (a category containing dead wood that did not fit into CWD), which contained many species not in common with those on CWD.

It was concluded that macrofungal species richness on CWD is not affected by decay class; however, length or surface area explained between 45–48% of the variation in species richness.

Of the 495 species found fruiting on soil, 330 were known to be ectomycorrhizal and 165 were considered decomposers. In addition, 146 species of macrofungi were associated with litter. It was found, using temperature and rainfall data, that the appearance of fruit bodies is seasonal but not directly attributable to rainfall events. There was a better correlation using the indigenous peoples' concept of three seasons than when using the four European-based seasons.

In essence, each plot contained a distinctive mycota, reflecting its chronosequence history, site characteristics (e.g., soil type, soil pH) or microclimate. To maintain the macrofungal diversity associated with the differing plots, a mosaic of multi-aged stands in the managed forest landscape is needed to provide inoculum for the reestablishment of macrofungal communities in forests at different times of regeneration. In addition, reserves should be as large as possible (at least 1ha) to encompass the variability (due to site characteristics, vegetation type, etc.) in the forest landscape and

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