Abstracts from the Scientific meeting of the Australasian Mycological Society

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Plenary Talk I

The impact of molecular data on the delimitation of species and genera in the Agaricomycetes

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Abstract. Phylogenetic analysis of molecular data has revolutionised the classification of the Fungi. However, in the species-rich and functionally diverse Agaricomycetes, the focus has been mostly on higher level classification. The ectomycorrhizal genera *Cortinarius* and *Laccaria* will be used as examples to demonstrate the utility of multi-gene phylogenies for elucidating species boundaries. About twice as many species are established from molecular data in comparison to species detected by multivariate analysis of comprehensive suites of macro- and microscopic characters, and, for *Cortinarius*, pigment composition. However, morphological data assists in 'calibrating' the degree of molecular divergence expected within phylogenetic species. A barcode gap exists for several DNA regions, including the universal fungal barcode region (ITS); but this is not always the best barcode region. The performance of the ITS in identification of existing species and detection of novel species needs to be balanced against the lack of universal primers for other regions. Herbarium specimens have proved a rich source of novel taxa, and formal taxonomic designation of environmental sequences is unlikely to be necessary. Implications of molecular phylogenies for delimitation of supraspecific taxa (such as genera) will also be discussed, including in relation to sequestrate taxa, paraphyly, rank, singleton taxa and groups that lack molecular data.

Keywords. systematics, species delimitation, phylogenetic species.

Plenary Talk II

The devil is in the detail – some stories about small fungi

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Abstract. This talk will explore the idea that all groups of fungi, if they are studied intensively for extended periods of time, will inevitably reveal valuable insights into fungal evolution, ecology, pathogenicity, and biogeography. I will use the inoperculate discomycetes (Leotiomycetes), a physically insignificant part of the ecosystems in which they are found, as examples. These often barely visible fungi are usually plant-associated, often host-specialised, and have a diverse range of lifestyles – pathogens of fruits, leaves and wood, mycorrhizas, leaf endophytes, saprobes, fungal parasites, and aquatic hyphomycetes. The species found in Australia, New Zealand and South America will be used to speculate on the origins of New Zealand's fungi, the evolution of fungi, and fungal ecology, pathogenicity, diversity and distribution.

Symposium 1: Fungal Interactions

Chair: John Dearnaley

Fungi Increase Stores of Organic Carbon in Soil

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Abstract. Organic carbon (OC) in soil is critically important for many ecosystem functions including plant productivity, water storage and maintenance of biodiversity. Half of the OC in Australian soils has been lost in the last 30 years. Loss of OC has very serious global implications for the sustainable use of soil. Conservation agriculture (no-till, green

manure crops, composting) has an unpredictable impact on stocks of soil OC. Organic matter (OM) may be degraded by hydrolysis (food for microbes) and oxidation (lost as CO_2). We know OC is protected in aggregates. Unpredictable impacts are probably because aromatic carbon is not necessarily placed by plants in protected locations. Thus we sought a mechanistic explanation of how OC is sequestered in soil. Arbuscular mycorrhizal fungi (AMF) and OM were critically important for developing and stabilising soil structure. Hyphal remains of AMF become an important supply of OM in soil. Melanised endophytic fungi transformed the energy from their host and OM in aggregates and deposited aromatic compounds that remain protected from oxidation in aggregates. These finding provide a practical means to predictably increase stores of OC in soil.

Keywords. Melanin, endophyte, arbuscular mycorrhiza, agriculture, mine restoration.

Insect-Fungal symbioses – How important are they?

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Abstract. The importance of symbioses between fungi and plants as expressed in endophytic and mycorrhizal relationships is well recognised and studied. However the ubiquity and importance of symbiotic relationships between fungi and insects is less well appreciated, except for a limited number of well known examples. Most of these were revealed through studies conducted before the middle of last century, including extensive research by Buchner that led him to suggest that endosymbiotic yeasts were extremely important to insect development. Few studies were conducted during the intervening years until a recent revival of interest with molecular studies revealing the vast diversity of yeasts carried in the gut of many insects. Investigations into the ecology of the small hive beetle (*Aethina tumida*), a recently established exotic apairy pest in eastern Australia, suggest that the yeast *Kodamaea ohmeri* is symbiotic with this beetle. The symbiotic relationship between this yeast and the small hive beetle appears to be responsible for the total destruction of honey bee hives helping *A. tumida* produce very large populations leading to the rapid establishment of this pest. These studies have led to speculation as to whether we underestimate the importance of fungal-insect symbiosis in the ecology of many insects.

Keywords. Yeasts, Fungal symbiosis, Insects.

Investigation of the mycorrhizal fungi of the vulnerable *Sarcochilus hartmannii*

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Abstract. Mycorrhizal fungi are essential for seed germination and inorganic nutrient uptake for all orchids. To improve the conservation status of threatened orchids, it is essential that the mycorrhizal fungal partner of species is isolated and identified. This can enhance ex situ horticultural growth, as well, be used to guide suitable reintroduction sites. The *Sarcochilus* genus of orchids contains a number of vulnerable and endangered species which are largely restricted to rainforest habitats in eastern Australia. Previous research on the vulnerable *S. weinthalii* showed that the species associated with a single taxon of *Ceratobasidium* fungus at a number of sites in southern Queensland. *Sarcochilus hartmannii* is a vulnerable lithophytic/epiphytic orchid species confined to northern NSW and south-eastern Queensland. Threats to the species include overcollecting, fire and weed invasion. This project is aimed at identifying the mycorrhizal fungus of *S. hartmannii*. Fungal coils will be isolated from roots of plants at multiple field sites. DNA will be extracted, PCR amplified and sequenced from fungal pure cultures. DNA will also be extracted from whole colonized orchid roots, PCR amplified, cloned and sequenced. This latter procedure will determine if non-culturable mycorrhizal fungi are present. Preliminary results of the study will be outlined.

Keywords. Orchid mycorrhizas, *Ceratobasidium*, *Sarcochilus*.

Biodiversity of fungal endophytes in Semi-evergreen vine thickets

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Abstract. Endophytes form a large but relatively understudied group of fungi. Rainforests, with their high moisture environments and host plant richness are thought to harbour the greatest diversity of endophytic fungi. Semi evergreen vine thickets (SEVT), a type of dry rainforest, are a nationally endangered ecosystem occurring sporadically along the east coast of Australia. Very little is known about the fungal diversity within this ecosystem. In this study, six leaves were sampled from each of 21 plants in SEVT in the south-east Queensland region and were identified using molecular and morphological methods. A total of 239 different fungal species were isolated, averaging 10 endophytes per plant (std=5.4). Common species were *Nigrospora* spp, *Preussia* spp, *Cladosporium* spp, *Xylaria* spp, *Epicoccum* spp, *Pestalotiopsis* spp, and *Phomopsis* spp. Many of these are cosmopolitan endophytes, with the exception of the *Preussia* spp. which are more commonly known as dung dwelling species. When a number of *Preussia* isolates were tested for bioactivity, 87% of isolates showed some level of activity against gram positive bacteria. These findings highlight the importance of preserving endangered vegetation types such as Australian SEVT.
Keywords. fungal distribution; diversity.

Symposium II: Molecular Mycology

Chair: Dee Carter

The unusual genetic structure of *Cryptococcus gattii*

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The yeast species *Cryptococcus neoformans* and *C. gattii* cause cryptococosis in humans and a range of animal. Both species are composed of a number of distinct molecular genotypes that vary in their ecology, their geographic distribution, and various virulence-associated phenotypes. In particular, *C. gattii* molecular type VGII is responsible for outbreaks that have expanded the fungus beyond its normal geographic range. Our interests lie in understanding the ecology and evolution of *C. gattii* in the environment, and how these relate to its ability to cause disease. We have found the level of sexual recombination varies by molecular type, and that while in general *C. gattii* population structure is sexual, this is punctuated by periodic clonal lineages that may be associated with disease outbreaks. Here we refine our analysis using MLST data, haplotype networks and coalesence theory. We find the level of diversity within genotypes to be highly constrained and comparable to some recently evolved plant pathogens, and evidence for purifying selection at the master regulator of mating type.
Keywords. *Cryptococcus,* population genetics, evolution

The Crz1/Sp1 transcription factor of *Cryptococcus neoformans* is regulated by calcineurin-dependent and independent mechanisms in response to different environmental stresses

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Abstract. *Cryptococcus neoformans* survives host temperature and regulates cell wall integrity via a calcium-dependent phosphatase, calcineurin. In *S. cerevisiae,* calcineurin dephosphorylates the zinc finger transcription factor Crz1, which translocates to nuclei and regulates expression of target genes. We now show that the *C. neoformans* Crz1 ortholog (Crz1/Sp1) physically interacts with subunit A of calcineurin (Cna1) and is a *bona fide* target of calcineurin. Both *Δcna1* and *Δcrz1* mutants are susceptible to cell wall

perturbing agents. Furthermore, expression of *CHS6,* which encodes chitin synthase, is reduced in both mutants under control and inducing conditions. These findings suggest that in *C. neoformans*, Crz1/Sp1-directed maintenance of cell wall integrity is calcineurin-dependent. We tracked the subcellular localization of Crz1-Gfp in WT *C. neoformans* and *Δcna1* in response to different stimuli, in the presence and absence of the calcineurin inhibitor, FK506. Exposure to elevated temperature and extracellular calcium caused calcineurin dependent nuclear accumulation of Crz1-Gfp. Unexpectedly, 1M salt and heat shock triggered calcineurin-independent Crz1-GFP sequestration within cytosolic and nuclear puncta. To our knowledge, punctate cytosolic distribution, as opposed to nuclear targeting, is a unique feature of *C. neoformans* Crz1. We conclude that Crz1 is selectively activated by calcium/calcineurin-dependent and independent signals depending on the environmental conditions.

Keywords. *Cryptococcus neoformans*, calcineurin, Crz1.

Investigating transcriptional regulation of growth and development

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Abstract. In response to developmental and environmental cues, fungi can produce a diverse array of cell-types that are morphologically distinct from the predominant growth form. This requires highly regulated changes in key aspects of growth including cell cycle, polarity establishment and cell wall biosynthesis. *Penicillium marneffei* is a dimorphic pathogen displaying multicellular hyphal growth at room temperature (25°C), while the single-celled fission yeast growth form is observed at 37°C both *in vitro* and *in vivo.* Similar to the model ascomycete *Aspergillus nidulans, P. marneffei* can undergo conidiation (asexual development) to elaborate multicellular conidiophore structures that subsequently bud uninucleate conidia. This work demonstrates the C_2H_2 zinc finger transcription factor HgrA/MsnA as a strong inducer of hyphal growth in *P. marneffei* and *A. nidulans*. The activity of HgrA/MsnA must be appropriately regulated to allow cellular differentiation to occur in both species, suggesting that the hyphal growth state is actively specified and is not merely the default growth mode until a developmentally competent state is reached and appropriate inductive signals can be perceived. Further to investigating how HgrA/MsnA ontributes to cellular identity, we are undertaking varied approaches to identify novel transcription factors governing the onset and establishment of development in these model fungi.

Keywords. Development, Morphogenesis, Transcriptional regulation.

Molecular targets of Miltefosine and genes involved in Miltefosine resistance in yeast

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Abstract. Miltefosine (hexadecylphospho-choline; MI), an orally active analogue of the eukaryotic membrane phospholipid, phosphatidylcholine, is marketed for treatment of leishmaniasis. It is also fungicidal, *in vitro,* via a mechanism which remains to be fully elucidated. The aim of this project is to identify molecular targets of MI using the model yeast, *Saccharomyces cerevisiae.* A *S. cerevisiae* strain M2915-6A was subjected to ethylmethane sulphonate (EMS) mutagenesis, and a stable dominant MI-resistant mutant was obtained. Genetic analysis of the strain confirmed that the mutation is located in a single gene. Whole genome sequencing of this strain is presently underway to locate the mutated target gene. We also explored candidate genes responsible for MI resistance, for which the *S. cerevisiae* strain (wild–type) was used to construct a multi-copy genomic DNA library using a yeast shuttle vector. Genetic screening of the library resulted in the isolation of a list of DNA fragments coding for genes rendering MI resistance. Sequence analysis of these DNA fragments revealed the identities of various genes including efflux pumps, mitochondrial enzymes and genes involved in important cellular metabolic pathways.

Keywords. Miltefosine, *S. cerevisiae*

Symposium III: Proferred papers

Chair: Genevieve Gates

Investigating the anti-fungal properties of Australian honey

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Abstract. Research into the antimicrobial properties of honey has led to an increasing interest in its medical use. In most honey samples, antimicrobial activity is due to hydrogen peroxide (H_2O_2) generated by the bee-derived enzyme glucose oxidase. *Leptospermum* honeys, including New Zealand manuka honey, also contain high levels of methylglyoxal (MGO) which is potently antibacterial. Previous studies have shown that pathogenic *Candida* species are not affected by manuka honey but are inhibited by honey with H_2O_2-dependent activity. In this study we examined honey derived from three native Australian floral sources that had previously been associated with H_2O_2-dependent activity. Activity ranged from MIC values of 19-38.3% (w/v) against *Candida albicans,* while the MIC of manuka honey was 36%. However, when H_2O_2 levels were measured these showed no correlation with activity, and it appeared that H_2O_2 alone was not sufficient to inhibit *C. albicans*. We assessed the effect of commercial processing, including moderate heat and filtration, and found this affected activity in an inconsistent manner. Overall, it appears that the antifungal activity of honey is complex and is likely to be modulated by various interacting factors. Floral source and H_2O_2 levels are not reliable predictors of activity, which should be assessed by standardized antifungal testing.
Keywords. Honey, *Candida albicans*, hydrogen peroxide

Isolation and identification of *Scedosporium* spp. in cystic fibrosis patients

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Abstract: The epidemiology and clinical relevance of fungal colonization/infection of the airways in cystic fibrosis (CF) is incompletely defined. In a prospective study we investigated the prevalence of *Scedosporium* spp. (a genus of particular interest) in CF patients, to be related to clinical risk factors and lung function in the future. Respiratory samples (n=755) from 177 children and 81 adults with CF were cultured on Sabouraud's and DRBC (Dicloran Rosebengal Chloramphenicol) agars. Restriction fragment length polymorphism (RFLP) analysis of the ITS1/2 region was used to identify *Scedosporium* species. *Scedosporium* colonization was evident in 11.1% adults and 11.8% children by culture, 4.9% adults and 6.2% children were colonized with *S. prolificans* whilst *Pseudallescheria boydii* complex were recovered in 7.4% adults and 6.2% children. Co-colonization with *Aspergillus fumigatus* occurred in 8.6% of adults and 5.5% of children. Of 55 isolates, 31 (56%) were recovered only on DRBC agar. Based on ITS-RFLP analysis of 54 isolates, 35.1% were *S. aurantiacum*, 20.3% were *P. boydii/S. apiospermum* and 44.4%, *S. prolificans*. Estimated frequency of *Scedosporium* colonization was ≈11%. DRBC is necessary for isolation of this fungus and ITS-RFLP accurately identifies *Scedosporium* species and distinguishes *S. aurantiacum* from other species of the *P. boydii* complex.
Key words: Cystic fibrosis; *Scedosporiu*m; ITS-RFLP.

Bisphosphonates as potential synergistic agents for combination antifungal therapy

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Abstract. Synergistic combination antifungal therapy is an approach taken to reduce problems of toxicity and resistance associated with the use of antifungal drugs, such as amphotericin B (AMB) and fluconazole (FLC). AMB targets fungal ergosterol through direct

binding while FLC inhibits the ergosterol-biosynthesis enzyme 14α-demethylase. A study on protein expression during FLC treatment of the fungal pathogen *Cryptococcus gattii* identified an upregulated isoprenoid-biosynthesis related protein with homology to the farnesyl pyrophosphate synthase (FPPS) enzyme in *Saccharomyces cerevisiae*. FPPS is an ergosterol-biosynthesis enzyme active upstream from 14α-demethylase and can be inhibited by bisphosphonates, which are clinically approved for treating bone disorders. The aim of this study was to assess the ability of four different bisphosphonates to inhibit the growth of *C. gattii* and enhance the activity of AMB and FLC. This was investigated using the CLSI method for the antifungal susceptibility testing of yeasts, checkerboard assays and the program MacSynergy II. Of four bisphosphonates tested, one was able to inhibit the growth of *C. gattii*. This enhanced the activity of FLC, reducing the minimum inhibitory concentration from 8 ug/mL to 2 ug/mL. It did not, however, affect how *C. gattii* cells respond to AMB treatment.

Keywords. Antifungal drugs, synergy, bisphosphonate.

Antifungal susceptibility of the emerging pathogen *Cryptococcus gattii* molecular type VGIII

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Abstract. *Cryptococcus gattii* is a basidiomycetous yeast causing invasive fungal infection and is subdivided into four molecular types (VGI-VGIV) that seem to be genetically isolated cryptic species. Although VGIII has emerged recently in several regions worldwide including Australia, little is known about its epidemiology or response to antifungal treatment. In order to determine the susceptibility of this emerging pathogen to the commonly used antifungal drugs, 117 clinical, veterinarian and environmental *C. gattii* VGIII isolates from eight countries were tested against amphotericin B, 5-fluorocytosine and the azoles posaconazole, voriconazole, itraconazole and fluconazole, using Sensititre® Yeastone®. Minimum inhibitory concentrations (MICs) were the lowest drug concentrations that produced either 100% (amphotericin B) or ≥50% inhibition of growth compared to that of the control. The MIC90 and susceptibility ranges obtained were 0.5 (0.12-2) µg/ml for amphotericin B, 4 (0.5-8) µg/ml for 5-fluorocytosine, 0.12 (0.015-0.25) µg/ml for posaconazole, 0.12 (≤0.008-1) µg/ml for voriconazole, 0.12 (≤0.015-0.12) µg/ml for itraconazole and 16 (1-128) µg/ml for fluconazole. Flucytosine and azoles had excellent *in vitro* activity against all tested isolates. However, the high ranges to amphotericin B and fluconazole suggest that the use of these drugs may lead to tolerance or resistance of the pathogen over time.

Keywords. *Cryptococcus gattii*, molecular type VGIII, antifungal susceptibility.

Elucidating the secretome of *Cryptococcus*: analysis of the proteins secreted by *Cryptococcus* species with different virulence profiles

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Abstract. *Cryptococcus gattii* and *Cryptococcus neoformans* cause cryptococcosis, a potentially life threatening disease in humans and animals. The molecular mechanisms underlying the virulence of the pathogenic *Cryptococcus* species are yet well understood. As fungi secrete many, often potently active proteins, the composition of the secretome may play a vital role in determining virulence. In this study we analysed the secretomes of *C. gattii*, strains R272 and R265, which are hypo- and hypervirulent, respectively, along with the virulent *C. neoformans* type strain KN99α. Tandem mass spectrophotometry of total secreted proteins identified a total of seventy proteins, however only one was common to all three *Cryptococcus* strains. Approximately half of the identified proteins secreted by the hypervirulent strains R265 and KN99 were predicted to have nutrient scavenging functions, while non-classically secreted proteins, some with allergenic properties, were identified in the hypovirulent R272 secretome. These initial results suggest that there are strain specific differences in the cryptococcal secretome that may play a role in determining

virulence and host response. These proteins may be useful targets for future diagnostic or antifungal strategies.
Keywords. Pathogenesis, secreted proteins, allergenic proteins

Symposium IV: Fungal Ecology

Chair: Sandra Abell-Davis

Allocasuarina trees are important hosts of hypogeous fruiting fungal species

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Abstract. The endangered northern bettong, *Bettongia tropica*, is thought to be restricted to habitats where availability of hypogeous fungi, their principal food resource, is high. In known bettong habitat within three tropical Australian sclerophyll forests, site specific environmental variables could explain the distribution of both quantity (abundance and biomass) and richness (genus and species) of hypogeous fungal sporocarps. Significant negative relationships were found between phosphorous concentration and the quantity of sporocarps. Using a multivariate information theoretic approach, there were other more plausible models found to explain the patterns of richness. Both the mean number of fungal genera and species increased with the number of *Allocasuarina* stems, at the same time decreasing with the number of *Eucalyptus* stems. Although they are considered "invasive" native species, as they are associated with woody thickening of forests that have had a low frequency of fire, *Allocasuarina* tree species are important hosts of hypogeous fruiting fungal species, consumed by many Australian mammals.
Keywords. Hypogeous fungi, phosphorous, distribution.

Universal model for the temperature dependence of biological growth rates

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Abstract. Environmental temperature is an important influence on the rate of growth and development of populations of all ectothermic organisms on this planet. We propose that there is a universal growth model applicable to all single-celled species from the three domains of life, Archaea, Bacteria and Eukarya. The model, which describes the effect of temperature on the specific rate of growth, assumes a single rate-limiting enzyme-catalysed "master reaction". Reversible thermal denaturation of the enzyme causes the growth rate to deviate from the Arrhenius-Eyring temperature dependence that usually applies to chemical reactions. Using a hierarchical Bayesian approach to simultaneously estimate model parameters, 95 data sets (Archaea: 21 strains; Bacteria: 47 strains; Eukarya: 27 yeast strains) were well fitted and yielded biologically and thermodynamically meaningful parameter estimates. The constancy over all domains and strains of important thermodynamic properties such as enthalpy and entropy changes and convergence temperatures related to protein denaturation suggests that the evolution and adaptation of microbial life on earth possesses a unifying property which is dictated by protein thermal stability. We hope to show that the same model will also apply to populations of insects, other arthropods, and many other higher life forms.
Keywords. Microbial growth, bacteria, fungi, enzyme denaturation.

Biocontrol of Weevil Borers in Cavendish Bananas by the Endophyte *Beauveria bassiana*

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Abstract. *Beauveria bassiana* is an entomopathogenic fungus capable of forming endophytic relationships with many plants of agricultural significance. *Beauveria bassiana* is considered to be a virulent pathogen against the banana weevil borer *Cosmopolites sordidus*. It was previously unknown if: (1) Australian varieties of bananas can be artificially inoculated with *B. bassiana* and (2) whether local isolates of *B. bassiana* show different levels of virulence against *C. sordidus.* To answer these questions, four locally collected isolates of *B. bassiana* were used to inoculate tissue culture Cavendish banana plants. Reisolation of *B. bassiana* from the root, corm, pseudostem and leaf was performed after 3 weeks growth. Bioassays were performed on *C. sordidus* to determine whether levels of virulence exist between these isolates. The results show that Australian isolates of *B. bassiana* are able to establish endophytic associations with Cavendish bananas and colonisation was greatest in the corm. Levels of virulence against *C. sordidus* were found to differ between isolates. Two isolates of *B. bassiana* have been identified for further research and development as a potential biological control agent of *C. sordidus* in Australia. Biological control can reduce the need for pesticides which benefits the environment while lowering costs to farmers.
Keywords. *Beauveria bassiana,* Entomopathogen, Banana.

Botryosphaeriaceae and *Ilyonectria* Fungi in Declining Young Riverina Vineyards Originate from Rootstock Source blocks and Nursery Soil

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Abstract. A survey of 20 Riverina vineyards affected by young vine decline showed that *Ilyonectria macrodidyma* or *Ilyonectria liriodendri* (producers of phytotoxin brefeldin A and cause of 'Black foot' disease of grapevines) and members of the *Botryosphaeriaceae* were isolated from rootstocks of 100% and 95% respectively of young diseased grapevines but were not isolated from healthy plants. *Togninia minima* and *Phaeomoniella chlamydospora* (cause of grapevine Petri disease) were isolated from the rootstocks of 13% and 7% respectively of diseased, but not from healthy plants, whereas *Rhizoctonia solani* was isolated from 35% of rootstocks of both diseased and healthy grapevines. A survey of a supplying nursery showed that 100% of rootstock stems of grafted plants were infected with both *Ilyonectria* spp. and *Diplodia seriata*. *I. macrodidyma* was isolated from the nursery soil and *D. seriata* was also isolated from 25% of canes sampled from the rootstock source block. This study shows that *Botryosphaeriaceae* from rootstock cuttings and *Ilyonectria* spp. from nursery soil contribute greatly to young vine decline. Although the Petri disease fungi were less common in young declining grafted grapevines in the Riverina they may contribute to the decline of surviving plants as they mature. **Keywords.** Black foot, Petri disease, Young Vine Decline.

Symposium V: Medical Mycology

Chair: Julie Djordjevic

b1,3-glucanases of *Cryptococcus neoformans* – Defining a role in pathogenesis

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Abstract: β1,3-glucans are structural components of fungal cell walls. They connect chitin with glycosylphosphatidylinositol (GPI)-anchored mannoproteins, via β1,6-glucan. b1,3-glucanases (Bgas) are involved in b1,3-glucan remodeling, yet little is known about their role in pathogenesis. Using gene deletion analysis, we describe the role of a family of

Bgas, predicted to be members of the glycosylhydrolase 16_fungal_Laminarinase 16A family, in the pathogenicity of *Cryptococcus neoformans (Cn)*. All Bga enzymes (Bga1, Bga2, Bga3, Bga5) undergo canonical secretion and Bga1, Bga2 and Bga3 are predicted to be GPI-anchored to b1,6-glucans. *BGA5* was found to be the most abundantly expressed family member. *BGA 1, 2, 3* and *5* were disrupted individually (*Δbga1, Δbga2, Δbga3, Δbga5*) and in combination (*Δbga2,5, Δbga3,5, Δbga2,3,5*) and the resulting mutants were assessed for virulence. Growth of *Cn* on laminarin (polyb1,3-glucan) required Bga5, as only mutants with disrupted *BGA5* exhibited reduced growth on this carbon source. Ability to degrade laminarin correlated with activity of secreted phospholipaseB1 (PLB1), an enzyme essential for *Cn* virulence, but not with virulence in a murine model of cryptococcosis. Only *Δbga2,3,5* displayed attenuated virulence, while *Δbga2* and *Δbga2,5* were hypervirulent. We conclude that Bgas are bona fide laminarases essential for activity of secreted PLB1, and modulators of *Cn* virulence.

Keywords. *Cryptococcus neoformans*, cell wall, laminarase.

Roles of Iron and Iron Chelators in Invasive Fungal Diseases

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Abstract. Iron availability has been implicated as a major regulator of fungal growth and virulence. The antifungal roles of iron chelation was obscured by the early observation that deferoxamine (DFO) therapy was found to be a risk factor for angioinvasive mucormycosis. The discovery of novel iron chelators, deferiprone (DFP) and deferasirox (DFX), has highlighted the potential use of iron chelation for treatment of invasive fungal diseases. DFP and DFX exhibited antifungal effects against Zygomycetes, whereas DFO promoted fungal growth. In contrast, none of these iron chelators had antifungal activity against *Cryptococcus* spp. However, they showed synergistic effects when combined with amphotericin B (AMB) with fractional inhibitory concentrations (FIC) of 0.5 and 1 for DFO+AMB and DFX+AMB, respectively. Growth curve of *C. neoformans* showed a significant growth retardation when incubated yeast cells with a combination of sub-MIC concentration of AMB and DFO. *C. gattii* exhibited significantly less extent in growth retardation with DFO+AMB. Interestingly, cryptococcal growth inhibition was not observed in DFX+AMB in all serotypes. When grown in DFO, cryptococcal *CFT1*, *CFT2* and *CIR1* were up-regulated for 3.3, 2.6, and 1.8 folds, respectively. But in media with DFX, they were up-regulated for 4.1, 2.2, and 10.1 folds, respectively, suggesting that *CIR1* is a major determinant to protect *Cryptococcus* from the antifungal effect of DFX.

Keywords. iron, iron chelator, antifungal agent, mucormycosis, cryptococcosis, invasive fungal diseases

Use of *Caenorrhabditis elegans* to Study *Candida albicans* Virulence

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Candida albicans is the most common human fungal pathogen. One of the critical virulence determinants of *C. albicans* is its ability to undergo a morphological transition from a yeast to a filament form. The nematode *Caenorrhabditis elegans* has been used for biomedical science for over 30 years; however its use to study microbial pathogenesis has increased over the last decade. Recently, it has been used to study *C. albicans* virulence, more specifically, the organisms ability to form filaments and cause a lethal infection. We have developed this model to assess the role of certain genes in *C. albicans* virulence, in conjunction with mammalian infection models, and have also used the assay to assess *C. albicans* mutant libraries. This has enabled us to identify novel genes important for *C. albicans* filamentation and virulence. *C. elegans* is a powerful model system to study the virulence mechanisms of the human fungal pathogen *C. albicans*.

Host-pathogen response in *Cryptococcus* spp. with different modes of pathogenicity

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Abstract. *Cryptococcus gattii* and *Cryptococcus neoformans* are capable of causing disease in a wide range of animal hosts. These closely related species exhibit significant differences in pathogenicity and disease progression. *C. gattii* causes a more chronic infection while *C. neoformans* infection is more acute. This difference presents a unique model system with which to investigate pathogenicity during cryptococcal infection. We conducted proteomic analysis of the host lung tissue response during pulmonary infection. Rats were intratracheally infected with either *C. gattii* or *C. neoformans* and infection was allowed to progress for 2 or 6 weeks before the animals were euthanased and the lungs harvested. The host lung proteome indicates that the basic underlying response to cryptococcal infection appears to be conserved, regardless of the causative species. We identified up regulation of proteins involved directly and indirectly in fungicidal responses. Further, proteins consistent with those observed during chronic obstructive pulmonary disease were identified, including a number of proteins that suggest a loss of lung tissue structural integrity and function during cryptococcal infection. Analysis of the host inate immune response was conducted using a Bioplex Cytokine Assay kit (Bio-Rad) which detects 23 different rat cytokines. Different cytokine responses were detected between the different treatments.

Keywords. *Cryptococcus*, host-pathogen interaction, proteomics

Symoposium VI: Systematics

Chair: Teresa Lebel

Molecular phylogeny of lactarioid sequestrate fungi (Russulaceae)

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Abstract. Australia and New Zealand have an incredible diversity of sequestrate fungi, with many endemic genera, and we are still discovering new taxa. The affinities of sequestrate fungi to agaricoid or boletoid taxa have been well supported based upon morphological and molecular data. The Russulaceae is a large and diverse family, however, Australia is relatively species-poor compared to the northern hemisphere when it comes to the lactarioid lineages, *Lactarius* and *Lactifluus*. To clarify the diversity and relationships of some Australian lactarioid fungi, phylogenetic analyses were conducted using three nuclear loci (*LSU*, *ITS* and *rpb2*). We found that: 1) the sequestrate fruitbody form has arisen multiple times within at least 7 clades; 2) high, cryptic sequestrate species diversification has occurred in the *Lactarius eucalypti* lineage; and 3) *Lactarius clarkeae*/ *L. subsclarkeae*/ *L. flocktoniae*/ *L. aff. clarkeae* (NZ) form a strongly supported clade of southern hemisphere taxa.

Keywords. *Lactarius*, *Lactifluus*, truffle-like.

Biodiversity and host specificity of the fungal endophytes of the Wet Tropics

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Abstract. The rainforests of north-eastern Queensland house an enormous biodiversity. It is conserved as a World Heritage Area because it is a refugia of Gondwanan plant flora. The fungal component of the flora, including endophytes, is considered diverse but is not well known. The biodiversity and host specificity of fungal endophytes in one rainforest plant genus, *Elaeocarpus*, will be

investigated. The effects of agar medium, leaf piece size, leaf age, time between collection and isolation, storage temperature, and length of incubation time on the number of fungal endophytes will first be investigated. This will be the first endophyte data from north-eastern Queensland and will form the basis for further experimental protocols. The biodiversity of the endophytes will then be investigated within individual leaves and throughout the canopy of individual trees. Fungi will be isolated from leaf material and the number and abundance of known species and morphospecies will be recorded. Fungal DNA will also be extracted directly from leaf material. Host specificity will be examined using the plant host species *Elaeocarpus carolinae*. This species will be an ideal model as it has three haplotypes that are distributed across a geographic barrier, the Black Mountain Corridor. Host specificity between haplotypes and/or populations across the geographic barrier will also be evaluated using this biodiversity data.

Keywords. Endophyte, rainforest, *Elaeocarpus*.

Morphological & molecular investigation of *Russula* diversity in south-east Queensland

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Abstract. The *Russula* genus is a large, cosmopolitan group of basidiomycetous fungi. *Russula* spp. are common in temperate woodlands where they form important ectomycorrhizal associations with tree species. The fruiting bodies of many *Russula* spp. are often bright red or purple coloured and have features distinct from other gilled macrofungi including brittle stems and caps, spores with amyloid warts or ridges and unique tissue cells called sphaerocysts. Of recent times, members of the hypogeous genera *Macowanites* and *Gymnomyces,* have also been absorbed into the genus *Russula*. The catalogue of Australian fungi lists approximately 70 species of *Russula* with the majority collected in southern states. The Queensland herbarium collection of *Russula* fungi is small, and is not a reflection of the richness commonly observed in south-east Queensland woodlands. In the current project, it is aimed to increase the database of known *Russula* fungi in this region. Fungi will be sampled from a variety of habitats and will be investigated using traditional morphological approaches as well as molecular techniques. The latter will involve DNA extraction, PCR amplification of fungal ITS and LSU rDNA, sequencing and comparison to known *Russula* sequences (eg. GenBank). It is envisaged that this project will expand the distribution of known species of *Russula* within Australia as well as document new species within this genus.

Keywords. *Russula*, Ectomycorrhizas, south-east Queensland.

Entolomataceae: the family within the fringe

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Abstract. My love affair with Entolomataceae began in 1998 the moment I peered down my old monocular light microscope and saw the angled spores of my first *Entoloma* species. Although I was armed with a degree in Botany and Zoology and had a bent for taxonomy, I was thrown into the deep end of the systematics of mycology so I engaged firstly the help of Tim Baroni (*Rhodocybe* expert) and secondly that of Machiel Noordeloos (*Entoloma* expert). It has been a grand 'marriage' between me and Machiel with generosity of his part, compromise on mine and so after 14 years collecting and describing we have produced a monograph "The Entolomataceae of Tasmania". It is based on alpha taxonomy; however, molecular work done by Machiel's student, Delia Co-David, has been incorporated where appropriate. In this presentation I will outline the journey of the personal joys and discoveries, the tolerances shown by my children to, for example, opening butter containers and finding Entolomas (not butter), the strange odours of drying fungi permeating the house, and a home that became a herbarium for ca. 5000 collections of Entolomataceae.

Keywords. *Entoloma*, taxonomy,Tasmania.

Poster abstracts

Diverse routes of phospholipase C signalling in mammalian cells and the fungal pathogen *Cryptococcus neoformans*

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Abstract Cryptococcal phospholipase C1 (Plc1) produces diacylglycerol and inositol triphosphate, which in mammalian cells, activate protein kinase C (Pkc1) and calcineurin pathways, respectively. Using genetic and biochemical approaches involving deletion mutants (Δ), we investigated whether similar mechanisms operate in *C. neoformans*. Several lines of evidence suggest that Plc1 functions in parallel to the calcineurin pathway, rather than by directly activating it: firstly, growth of *Δplc1,* but not the calcineurin mutant *Δcna1*, was markedly reduced by the calcinerin inhibitor, FK506. Secondly, activation of the calcineurin-dependent transcription factor, Crz1, was unaffected by PLC inhibitors. Similarly, Plc1 was not essential for activation of the cell wall integrity pathway (Pkc1/MAP kinase cascade), as phosphorylation of the terminal MAPK Mpk1 was not repressed in *Δplc1*. Moreover, expression of the Mpk1-dependent and calcineurin-independent glucan synthase gene, *FKS1,* was elevated in *Δplc1*. Based on these findings, we hypothesize that Plc1 primarily signals via a separate pathway involving the metabolism of its IP_3 hydrolysis product, to more complex inositol polyphosphates (IPs) by the inositol polyphosphate kinases, Arg1/2, Ipk1, Asp1 and Kcs1. In conclusion, diverse IP species generated from the IP_3 precursor regulate *C. neoformans* development and virulence determinants, including melanization, capsule and growth at 37°C.

Keywords. *Cryptococcus neoformans*, phospholipase C, protein kinase C, calcineurin, inositol triphosphate, inositol polyphosphate, inositol polyphosphate kinase

One fungus one name/which name

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Abstract. This poster has been prepared on behalf of the International Committee for the Nomenclature of Fungi. Article 59 of the International Code of Botanical Nomenclature has long allowed an exception for fungi to the rule that a single species can have only one scientific name. This accommodated those fungi with more than one morphological state, usually a sexual and an asexual state, allowing the use of two or more scientific names for one species. Increasingly, molecular phylogeneties make it possible to unequivocally recognise that pairs of species exhibiting diverse sexual and asexual morphologies are in fact the same species, and in the same way genera applied to sexual and asexual species can also be linked. After more than 10 years of often heated discussion, the 2011 Melbourne International Botanical Congress voted to allow the use of only one scientific name for each species of fungus. This means that the Principle of Priority, i.e. the oldest name, will dictate which name to use, at both the genus and species level. Under the previous rules the names applied to the sexual state had priority. In some cases, when moving to a single name, the use of the oldest name for a species or a genus may not be practical or sensible. Perhaps 2,000–3,000 generic names and 10,000–12,000 species names will need to be assessed to ensure that familiar, well-established names are not lost under the new rules. This poster summarises a process to ensure the sensible implementation of these rules, and provides some examples of pairs of names that will need consideration.

Keywords. Code of Nomenclature for algae fungi and plants, anamorph, teleomorph, nomenclatural priority.

Effect of Copper (II) Lead (II) and Zinc (II) on four zoosporic fungi species from NSW soils

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Abstract. This study investigates the effects of soluble Copper (Cu), Lead (Pb) and Zinc (Zn) on the zoosporic true fungi (chytrid) species: *Rhizophlyctis rosea* (A13), *Terramyces sp.* (A3) and *Chytriomyces hyalinus* (A14) from soils of the Sydney Basin and Central coast regions and *Gaertneriomyces* (Mar-CC2) from a soil of north-western NSW. All species showed a similar response to Cu, with a significant increase in biomass at 10 ppm Cu and a significant decline at 30 ppm. However, there was interspecific variation in growth response to zinc and lead. All species showed least sensitivity to Pb, with only MarCC2 declining significantly at 60 ppm Pb. Cu concentrations of 10 and 60 ppm induce higher rates of zoospore discharge in some species, although zoospore production rate was adversely affected when sporangia were grown in solid PYG media with 60 ppm Cu. The higher tolerance of the three coastal species to Zn and Pb may indicate that they are Zn and Pb-tolerant ecotypes, which may be more prevalent due to anthropogenic contamination of soils in this region. Reduction in zoosporic fungal biomass in Cu, Pb and Zn contaminated soil is expected to reduce the mineralisation of soil organic matter, impacting soil ecosystem processes and soil carbon cycling.

Keywords. zoosporic, fungi, metals.

Comparing morphological species as used in ecological surveys against molecular barcoding with the ITS region: a case study of *Cortinarius* in the south-west of Western Australia

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Abstract. There is currently a disconnect between identification of fungi in ecological surveys by morphology or by molecular data. There are potential problems with both methods — morphology requires considerable experience and molecular identification relies on existence of comprehensive barcode databases. Both methods rely on delimitation of species prior to identification of field samples. Macrofungi have been included in biodiversity monitoring programs in jarrah *Eucalyptus marginata* forests in south-west Western Australia. Using material from permanent plots in these surveys, we created ITS sequences for a set of voucher collections for 118 morpho-species of *Cortinarius*, recognisable on field characters. Eighty-six morpho-species were represented by a single collection, and another 32 morpho-species by more than one collection. Analysis of ITS sequences (using a 98% similarity threshold) produced 94 molecular species. Less than half of the morpho-species were recovered exactly in the molecular analysis. Some 45% were fused with other morpho-species and 11% were split into two or three molecular species. Only 18% of molecular species matched named sequences in GenBank. Our analysis confirms the high level of diversity in the genus; allows calibration of morphological identification; highlights groups (such as *Cortinarius sublargus*) with morphologically cryptic species; and provides a databank for future molecular identification.

Keywords. *Cortinarius*, species delimitation, cryptic species, biodiversity monitoring

The role of Cryptococcal vesicles in the invasion of the central nervous system

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Abstract. *Cryptococcus neoformans (Cn)* is the primary cause of fungal meningo-encephalitis worldwide in immunocompromised patients. The ability of *Cn* to cross the blood brain barrier is of great clinical significance, hence understanding the fungal components involved in interactions with host cells is vital. This study focused on the characterisation of vesicles released by *Cn*

and their effect on brain endothelial cells and monocytes *in vitro*. Exosome-like vesicles previously described in *Cn* are associated with virulence and are taken up by macrophages. Using an alternative purification method, we focus on the larger vesicle population, which potentially has similarities to mammalian microparticles. The vesicles produced by the clinical type strain, H99, were compared to those produced by Δ*sec14*, a secretion deficient mutant, and Δ*plb1*, a phospholipase B deletion mutant, both produced from H99. The vesicles identified are <1 μm in diameter and are stained by the membrane dye, FM4-64. A subpopulation express phosphatidylserine on their surface. Vesicles are bound by a phospholipid bilayer, contain protein and are associated with laccase, urease and acid phosphatase activity. The vesicles also interact with endothelial cells and monocytes, potentially serving as 'virulence delivery bags' and playing a major role in the interaction between host and pathogen.

Keywords. *Cryptococcus*, microparticles, vesicles meningoencephalitis

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