Fungal Network of New Zealand
27th New Zealand Fungal Foray and 12th Mycology Colloquium
Matawai, Wednesday 15th May, 2013

Program

Time	Speaker*	Title
9:00–9:20	Sapphire McMullan-Fisher	Fungi all sorts: macrofungi, ecology, fire and Australia
9:20–9:40	Suliana Teasdale	Fungal diversity from the canopy and terrestrial root-systems of a southern *Nothofagus menziesii* forest in New Zealand
9:40–10:00	Xinyue (Cecilia) Wang	Are truffles animal dispersed?
10:00–10:20	Takamichi Orihara	Phylogeny and systematics of the sequestrate basidiomycete genus, *Rossbeevera* and allies (Boletaceae, Boletales)
10:20–11:00		**Morning Tea**
11:00–11:20	Teresa Lebel	The truffle-like genus *Stephanospora* (Stephanosporaceae) in Australasia
11:20–11:40	Mahajabeen Padamsee	Molecules and morphology: an exploration of morphological characters in the Psathyrellaceae using molecular phylogenetic analyses.
11:40–12:00	Vincent Hustad	Geoglossomycetes of Australasia and the Northern Hemisphere
12:00–1:00		**Lunch**
1:00–1:20	Jerry Cooper	Sequencing NZ mushrooms—a medley of interesting results
1:20–1:40	David Orlovich	Editing DNA sequences with polymorphic indels to improve sequence quality and phylogenetic analyses
1:40–2:00	Jerry Cooper	Naturewatch NZ—Citizen Science portal mark 2
2:00–2:20	Inga Meadows	Chestnut Blight and Ink Disease: A few lessons learned
2:20–3:00		**Afternoon Tea**
3:00–3:20	Bevan Weir	A species concept for *Phytophthora* “taxon Agathis” (PTA) — causal agent of root and collar rot of *Agathis australis* in New Zealand
3:20–3:40	Peter Johnston	Genetic diversity of *Botrytis* in New Zealand vineyards

*Contact details for speakers are listed at the end of this document.

Fungi all sorts: macrofungi, ecology, fire and Australia

Sapphire McMullan-Fisher

Many people are focused on what species are. I like names but am interested in what species do—so focus on the ecology of species. Over the last seventeen years I have been involved in a number of macrofungal projects across Australia. I will take people on a short tour of these projects highlighting the ecology and conservation issues. This includes the collaborative work I have done on the understanding of fire on Australian fungi. I will include as many pretty images as I can too.

Fungal diversity from the canopy and terrestrial soil systems of a southern *Nothofagus menziesii* forest in New Zealand

Suliana Teasdale, Xinyue Wang, David Orlovich

Department of Botany, University of Otago, PO Box 56, Dunedin 9054, New Zealand. Email teasdale703@hotmail.com

The formation of canopy soil in old growth temperate rainforests has been globally documented. Canopy soil is a term used to describe the accumulation of humus rich soil along canopy branches of woody tree species. Canopy soils can promote growth of traditionally non-epiphytic plants as well as ‘canopy roots’ from the host. As with terrestrial roots of some species, canopy roots have the potential to host ectomycorrhizal fungi, however this relationship is largely understudied. Old-growth *Nothofagus* (Southern beech) forests are known to accumulate canopy soils, and have also been shown to grow canopy roots that were colonised with ectomycorrhizal fungi. The knowledge of *Nothofagus* canopy fungi is, however, limited to descriptions of ectomycorrhizal genera present. While the presence/absence and abundance of ectomycorrhizal colonisation has been compared between the canopy and terrestrial systems of some host species, to date there are comparisons between the diversity of these fungi. This project aimed to identify and describe differences and similarities of ectomycorrhizal fungal communities in *Nothofagus menziesii* canopy and terrestrial soil systems. There are a number of techniques to explore the diversity of fungal communities; the most successful involve environmental DNA investigated by molecular methods. Hyphal ingrowth bags were incubated for twelve months in canopy and terrestrial environments of five *Nothofagus menziesii* trees. Fungal ergosterol was extracted from hyphal ingrowth bag samples to calculate biomass. DNA was extracted and amplified, then analysed using terminal restriction fragment length polymorphism (TRFLP). TRFLP profiles represent communities of fungi; these communities were combined to describe richness, evenness, and diversity of the canopy and terrestrial environments. Ergosterol concentrations were not significantly different between the canopy and terrestrial environments, showing that the hyphal growth rates of fungi into the ingrowth bags were not affected by environment. Analysis of community TRFLP profiles from the canopy and terrestrial hyphal ingrowth bag samples show that the TRF richness, evenness and total

diversity were not significantly different between the two environments. While total diversity was similar, two ordination analyses show the communities profiles form two environmentally specific groups. Dice's index (0.876) revealed that the two environments share some terminal restriction fragments (TRFs). The two environments share 30 % of TRFs, with 41 % of TRFs being unique to the terrestrial environment and 29 % unique to the canopy. These results show that both environments have similar characteristics, accumulation rates into ingrowth bags, evenness, richness and diversity of species within communities. The richness of the fungi present is similar for the number of unique and shared potential species. These results differ from other canopy fungi comparative studies where total canopy fungal biomass and colonised root-tips was much lower than terrestrial systems. This is the first report showing similarities between characteristics of ectomycorrhizal fungal communities in canopy and terrestrial systems, and showing potentially unique species associated with only canopy roots.

Phylogeny and systematics of the sequestrate basidiomycete genus, *Rossbeevera* and allies (Boletaceae, Boletales)

Takamichi Orihara [a], Teresa Lebel [b], Zai-Wei Ge [c], Matthew E. Smith [c], Nitaro Maekawa [e]

[a] Kanagawa Prefectural Museum of Natural History (KPM), Kanagawa, Japan. [b] National Herbarium of Victoria, Royal Botanic Gardens Melbourne, Australia. [c] Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, China. [d] Department of Plant Pathology, University of Florida, USA. [e] Fungus/Mushroom Resource and Research Center, Tottori University, Japan

Sequestrate fungi are highly polyphyletic within Ascomycota and Basidiomycota, and are characterized by enclosed fruitbodies that make forcible spore discharge impossible (e.g., truffle-like fungi, secotioid fungi). The sequestrate basidiomycete genus *Rossbeevera* T. Lebel & Orihara, as well as *Chamonixia* and *Octaviania*, is closely related to the epigeous bolete genera *Leccinum* and *Leccinellum*. Although they all belong to a monophyletic lineage within Boletaceae (Boletales), their genus-level relationships have not been resolved. Furthermore, additional collections from East Asia indicate that there are still a number of morphologically related but undescribed taxa.

In this study we conduct phylogenetic analyses focused on *Rossbeevera* and allied taxa based on three nuclear and two mitochondrial DNA loci (ITS, nLSU, *EF-1α*, *ATP6* and mtSSU) as well as precise morphological observations. The five-locus phylogenetic analysis provides a well-resolved phylogeny that clarifies relationships among the target genera. Moreover, ancestral state reconstruction suggests that sequestrate forms have evolved independently either two or three times in this lineage. Our analysis also detected a previously unrecognized sister clade to *Rossbeevera* as well as several new infrageneric lineages within *Rossbeevera*. Accordingly, we will propose one new *Rossbeevera* species and a new sequestrate genus, *Turmalinea* Orihara & N. Maek. nom. ined., that includes four new species and one new subspecies. The three-locus nuclear phylogeny readily resolved species-level divergence within the *Rossbeevera* - *Turmalinea* lineage, whereas the phylogenetic signal from the two mitochondrial genes followed geographic distance within each species-level lineage and suggests the possibility of gene introgression between closely related taxa. In the Australasian *Rossbeevera* species, the above-noted comparison documented significant infraspecific biogeographical diversification of the mitochondrial loci but very low differentiation in nuclear loci, suggesting that, phylogenetically, they are merely geographical variants despite the fact that they are morphologically well-differentiated. Additional collections of *Rossbeevera* species from less investigated areas in Australasia will give further insights into their taxonomy and phylogeography.

The truffle-like genus *Stephanospora* (Stephanosporaceae) in Australasia

Teresa Lebel[1], Michael Castellano, R. Beever

[1]Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand and National Herbarium of Victoria, Royal Botanic Gardens Melbourne, Australia

The truffle-like genus *Stephanospora* is aligned with the resupinate polypore *Lindtneria*, based on the strong similarity in spore structure and ornamentation. Currently four species of *Stephanospora* are recognised worldwide, *S. aurantiaca* and *S. caroticolor* from Europe, *S. chilensis* from South America and Europe, and *S. flava* from Australia, New Zealand and South Africa. All four species have ochraceous, yellow or orange pigmented basidiocarps, lacking a stipe columella, with a fragile, evanescent pileus, a loculate, olivaceous-yellow to orange hymenophore, and similar spores with spines or crests and a basal appendicular corona. Recently, Castellano et al. (2007) described a new genus and species, *Mayamontana coccolobae*, from Belize. Although the basidiome morphology is similar to *Stephanospora* in pileus colour, size, texture and fruiting habit, the spores differ markedly in the lack of a basal appendicular corona and barely roughened to warty appearance.

In the hopes of gaining greater insight into species diversity and the phylogenetic relationships of *Stephanospora* and *Mayamontana*, herbarium material from Australia and New Zealand, Mexico, Panama, Guadeloupe, Puerto Rico, and New Caledonia, was examined both morphologically and molecularly. Preliminary analyses of molecular data (ITS, LSU and tef-1), suggest that four – five species can be recognised from Australia and New Zealand, *S. flava* and *S. flava forma tetraspora* which is raised to species level, and three new species, 'S. arokai sp. nov.', 'S. ellipsospora sp.nov.' and 'S. robust ornamentation sp. nov.' One new species from New Caledonia 'S. caledoneae' sp.nov.' was also distinguished. Preliminary analyses of molecular data (ITS, LSU and tef-1), suggest that *S. arokai* is likely a New Zealand endemic which has recently been introduced to Australia (only a single collection currently known from Australia). The lack of available sequences from *Lindtneria* and other allied resupinate fungi, makes placement of *Mayamontana* difficult.

Molecules and morphology: an exploration of morphological characters in the Psathyrellaceae using molecular phylogenetic analyses.

Mahajabeen Padamsee

Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand

Molecular phylogenetic analyses of mushrooms have demonstrated that various morphological characters traditionally used to delimit genera are homoplasious. However, molecular phylogenetic analyses can also provide the framework within which the evolution and taxonomic utility of morphological characters can be examined. Evidence is presented for a new genus in the mushroom family Psathyrellaceae based on a

combination of morphological and molecular evidence. This genus consists of psathyrelloid mushrooms with reddish, smooth spores that typically also possess thick-walled encrusted cystidia. Phylogenetic analyses of nuclear ribosomal large subunit DNA and elongation factor 1-alpha sequences confirmed the placement of this new genus as a unique lineage sister to the ornamented-spore genus *Lacrymaria*. The evolution of certain morphological characters is interpreted and discussed in light of the two sister lineages.

Geoglossomycetes of Australasia and the Northern Hemisphere

Vincent P. Hustad

Department of Plant Biology, University of Illinois at Urbana-Champaign, Illinois Natural History Survey, 1816 South Oak Street, MC-652, Champaign, IL 61820, USA. Email vhustad@illinois.edu

The class Geoglossomycetes is among the newest classes of the fungal phylum Ascomycota and is currently comprised of one family, Geoglossaceae, and fifty species in six genera (*Geoglossum*, *Glutinoglossum*, *Nothomitra*, *Sabuloglossum*, *Sarcoleotia* and *Trichoglossum*). Commonly known as earth tongues due to their terrestrial habitat and morphology, Geoglossomycetes are characterized by large, dark, club-shaped, terrestrial fruitbodies. While most species (34/50) have been described from Eastern North America and Western Europe, seven species have been reported from Australasia. Analysis of ITS sequences available on GenBank and sequences obtained through herbarium material indicates Australasian Geoglossomycetes species are not conspecific with North American and European synonyms, despite being morphologically identical. This presentation will discuss the current progress and future directions in the assembly of a species-level taxonomic treatment of Geoglossomycetes; including cryptic speciation, species complexes and the taxonomic obstacles involved in the modern treatment of this well-studied group of fungi.

Editing DNA sequences with polymorphic indels to improve sequence quality and phylogenetic analyses

David A Orlovich

Department of Botany, University of Otago, PO Box 56, Dunedin 9054, New Zealand. Email david.orlovich@otago.ac.nz

DNA sequencing is an essential and integral part of modern systematics, but direct sequencing of amplified PCR products from mushroom collections can be problematic when the DNA sequences are polymorphic and vary in length within a collection. This can result in electropherograms that have double peaks and are difficult to interpret. Such polymorphism in the internal transcribed spacer (ITS) region can occur in 40% of sporocarp collections, reducing sequencing success considerably. I will demonstrate a workflow for dealing with these sequences that frequently permits a complete sequence read without cloning, significantly improves sequence quality and results in improved phylogenetic resolution.

Chestnut Blight and Ink Disease: A Few Lessons Learned

Inga Meadows

Plant Health & Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140

The introduction of the chestnut blight fungus (*Chryphonectria parasitica*) to New York, USA in the early 1900s resulted in the near loss of mature American chestnuts (*Castanea dentata*) in forests of the eastern United States. This iconic tree species once dominated the forest canopy, provided a major source of food for wildlife, and served as a valuable lumber species. One hundred years after the introduction of chestnut blight, The American Chestnut Foundation has succeeded in breeding blight-resistant chestnuts after three decades of work by dedicated staff and volunteers. However, blight-resistant chestnuts planted in orchards and some forested areas began dying at an alarming rate from ink disease, caused by *Phytophthora cinnamomi*. This disease was nothing new as it had already eliminated chestnuts from some parts of the southern end of their geographic range even before the blight fungus was introduced. But, as the trees disappeared from the forests, ink disease disappeared from the minds of pathologists. Not all breeding efforts have been lost—some resources that remain for the chestnut will be presented. This story re-emphasizes some lessons for plant scientists, biosecurity regulators, and forest tree growers.

A species concept for *Phytophthora* “taxon Agathis” (PTA) — causal agent of root and collar rot of *Agathis australis* in New Zealand

Bevan S. Weir, Elsa Paderes, Nitish Anand, Shaun R. Pennycook and Stanley E. Bellgard

Landcare Research Private Bag 92170, Auckland Mail Centre, Auckland, 1142, New Zealand. Email weirb@landcareresearch.co.nz

Kauri Dieback has been identified as an increasing problem affecting kauri (*Agathis australis*) across the Auckland and Northland regions. *Phytophthora* “taxon Agathis” (PTA) has been identified as a causal agent of a root and collar rot of kauri. ‘PTA’ shares a place in *Phytophthora* ITS Clade 5 with *P. heveae* and *P. katsurae*. PTA was originally misidentified as the morphologically similar *P. heveae.* It has been established that PTA has a different oogonial morphology to both *P. heveae* and *P. katsurae.* The sequencing of eight loci from both the nuclear and mitochondrial genomes has been used to resolve the species boundaries within Clade 5. Bayesian inference phylogenies reveal PTA is a discrete taxonomic entity, separate from either *P. katsurae* or *P. heveae*. Further, because of its unique colony morphology, oogonial characters, persistent sporangia and pathogenicity to *Agathis australis* we recognise PTA indet. as a new species in ITS Clade 5 of the genus *Phytophthora.*

Genetic diversity of *Botrytis* in New Zealand vineyards

Peter Johnston, Karyn Hoksbergen, Duckchul Park, Ross Beever

Landcare Research, Private Bag 92170, Auckland 1142, New Zealand. E-mail: johnstonp@landcareresearch.co.nz

Genetic diversity of *Botrytis* in New Zealand vineyards was surveyed over the period 2008 to 2012 from five wine growing regions. Isolates were gathered from symptomless flower buds immediately prior to flowering and, from the same vines, from diseased fruit at harvest.

Two species were found, *B. cinerea* and *B. pseudocinerea*. All of the *B. pseudocinerea* isolates detected were fenhexamid resistant. The distribution of *B. pseudocinerea* was structured geographically, common in the two Auckland vineyards sampled, infrequent elsewhere. However, even in the Auckland vineyards, it was rarely isolated from diseased fruit.

The presence of the Boty and Flipper transposons were assessed using a PCR-based method. Isolates with all four transposon states (Boty only, Flipper only, both Boty and Flipper (transposa isolates), no transposons (vacuma isolates) were found for both species. Both of the vineyards sampled in the Auckland region had relatively high numbers of Flipper-only isolates in samples taken at flowering; both of the vineyards from the Waipara region sampled had relatively high numbers of Boty-only isolates in samples taken at flowering. The large majority of isolates collected from diseased fruit at harvest contained both transposons.

The isolates collected in spring represent the total genetic diversity of *Botrytis* associated with fruit in the vineyard, whereas the isolates collected in autumn comprise only that part of the *Botrytis* population that causes disease at harvest. Our results suggest that *B. pseudocinerea*, and isolates with one or both of the Flipper and Boty transposons missing, are less capable of causing disease than *B. cinerea* or of isolates with both transposons present. Pilot scale pathogenicity testing using detached grapes in the laboratory, appear to confirm that transposa isolates are more pathogenic. *Botrytis* is known to form both latent and truly endophytic infections on some hosts and it is likely that the part of the population not associated with diseased fruit at harvest remains present in the vineyard, on fruit in symptomless infections.

Two distinct clades were resolved within *B. pseudocinerea*. Isolates in both clades share the fenhexamid-resistant phenotype, both have a similar geographic and regional distribution within New Zealand, and we accept both as *B. pseudocinerea*. Only one of these clades has been reported from Europe from grape. However, based on matching hsp60 sequences, the second New Zealand *B. pseudocinerea* clade could be the same fungus reported as an endophyte of *Centaurea* from Europe.

Phylogenetic diversity within *B. cinerea* in New Zealand was similar to that known from Europe, including the occurrence of isolates that appear to match genetically the recently reported *Botrytis* 'Group S'.

The taxonomic implications of this genetic diversity will be discussed.

Contact details of speakers

Name	**Email address**
Jerry Cooper	CooperJ@landcareresearch.co.nz
Vincent Hustad	vhustad@illinois.edu
Peter Johnston	JohnstonP@landcareresearch.co.nz
Teresa Lebel	LebelT@landcareresearch.co.nz
Sapphire McMullan-Fisher	sapphire@flyangler.com.au
Inga Meadows	Inga.Meadows@mpi.govt.nz
Takamichi Orihara	t_orihara@nh.kanagawa-museum.jp
David Orlovich	david.orlovich@otago.ac.nz
Mahajabeen Padamsee	PadamseeM@landcareresearch.co.nz
Suliana Teasdale	teasdale703@hotmail.com
Xinyue (Cecilia) Wang	wanxi542@student.otago.ac.nz
Bevan Weir	WeirB@landcareresearch.co.nz