Russulas

A brief introduction to my research in to the Russulaceae family

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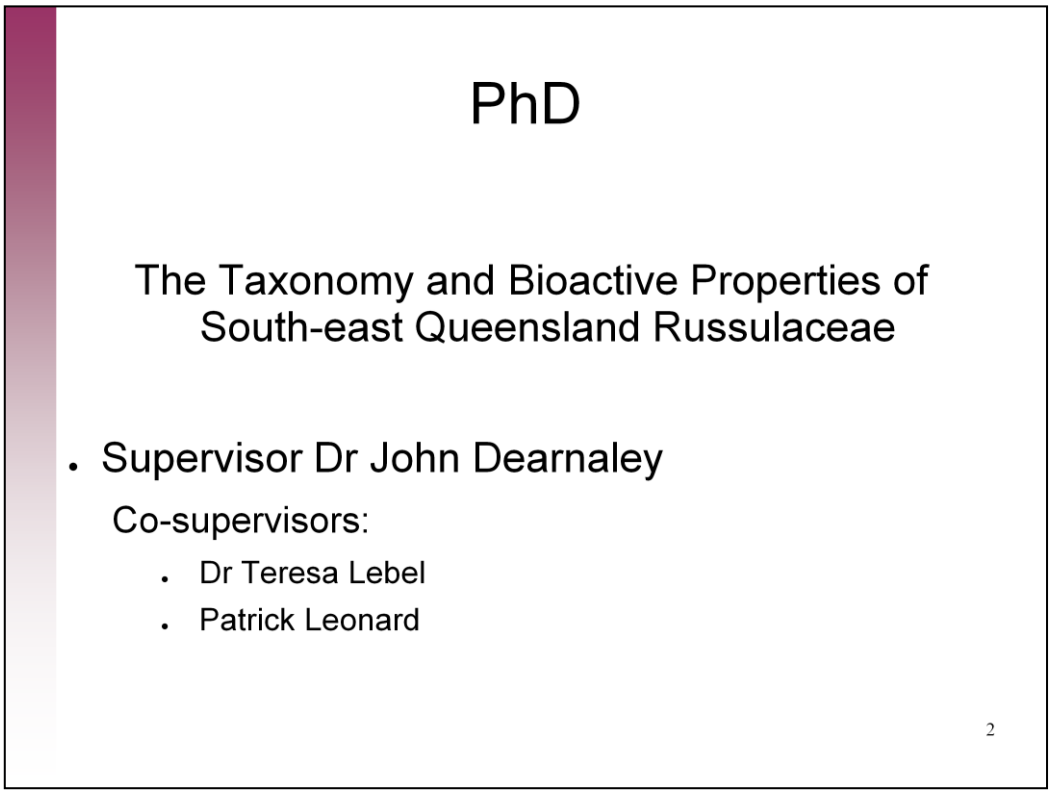
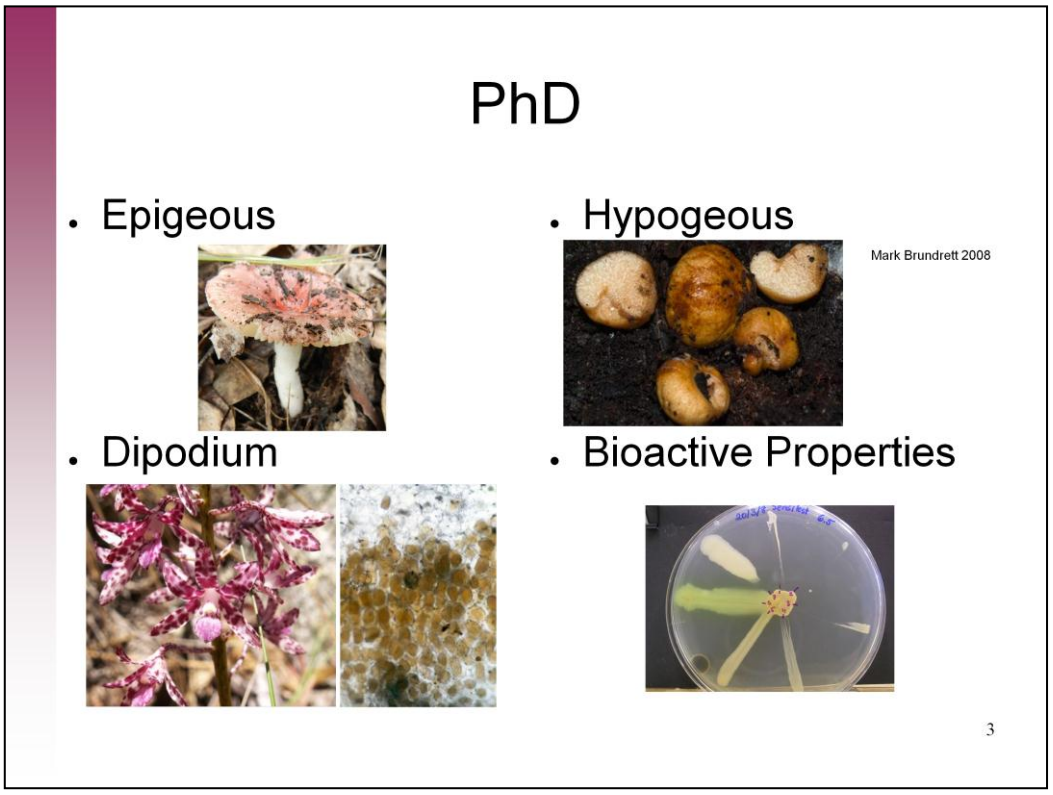


This PhD is being undertaken on a part-time basis, so it will take six years to complete! While it sounds a long time, this is quite beneficial for this type of study – it provides me with up to six seasons in which to collect the fruiting bodies of interest, rather than three, and a bad collecting season won't be as disastrous.

It has already been a steep learning curve, but this is hopefully starting to flatten out. The following presentation provides a brief introduction to the Family Russulaceae, and a brief insight in to the techniques I am using to study it.

I hope you find it of interest...



At this stage, the study can be divided in to four chapters, which are posed as questions:

1. Are there Epigeous species of Russulaceae present in SE Queensland, and what are they? Clearly, the answer to the first part of this question is "yes", while the answer to the second part will be coming as a result of this project
2. Are there Hypogeous species of Russulaceae present in SE Queensland, and what are they? This is slightly harder to answer, as these fruiting bodies are much harder to find than the epigeous forms, but I am confident some will be found
3. What Russulaceae species are associated with the *Dipodium* species of mycoheterotrophic orchid? These plants lack chlorophyll, and obtain all of their nutritional requirements from fungi – research has shown this includes several *Russula* species.
4. Do SE Queensland Russulaceae possess any Bioactive properties? Extracts will be obtained from specimens, and these will be tested to determine if they have, for example, anti-bacterial or anti-cancer characteristics.

Study Area

- South-east Queensland

conference.auscert.org.au

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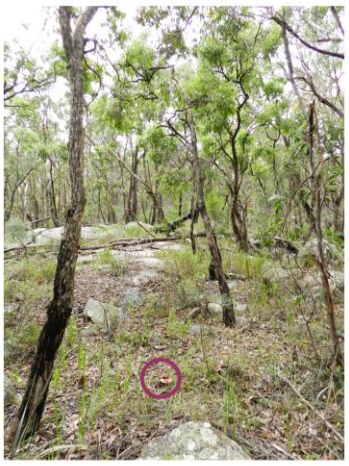
This map shows the general area for the collection of Russulaceae fruit bodies. It is a fair size, and given the short windows of opportunity for observing and collecting specimens, it will be difficult to search the entire area on my own. This is where members of associations such as the QMS can be very helpful – if you find a Russulaceae specimen, you are more than welcome to send me the dried specimens along with its characterisation and location details, for inclusion in this project.

Example sites

Crow's Nest



Stanthorpe

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Some of the sites I where I have successfully collected fruit bodies of the Russulaceae include:

Crow's Nest Falls National Park – an area frequently surveyed by QMS members

Stanthorpe – Private property. A large Russula can actually be seen in the foreground of the photograph (circled)

Example sites

Preston

ANWaR - USQ

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Preston – Private property south of Toowoomba. This is where I live, which is of benefit as it allows me to constantly search for and observe several Russula species. It isn't usually this green – this photo was taken soon after the rains earlier this year

ANWaR – The Australian Native Woodland Reserve located at Toowoomba's University of Southern Queensland campus. This area was established in 1999 to provide an educational and research resource in relation to our local native woodlands

Family Russulaceae

- Russula

- Lactarius

- Zelleromyces
- Cystangium
- Gymnomyces
- Macowanites

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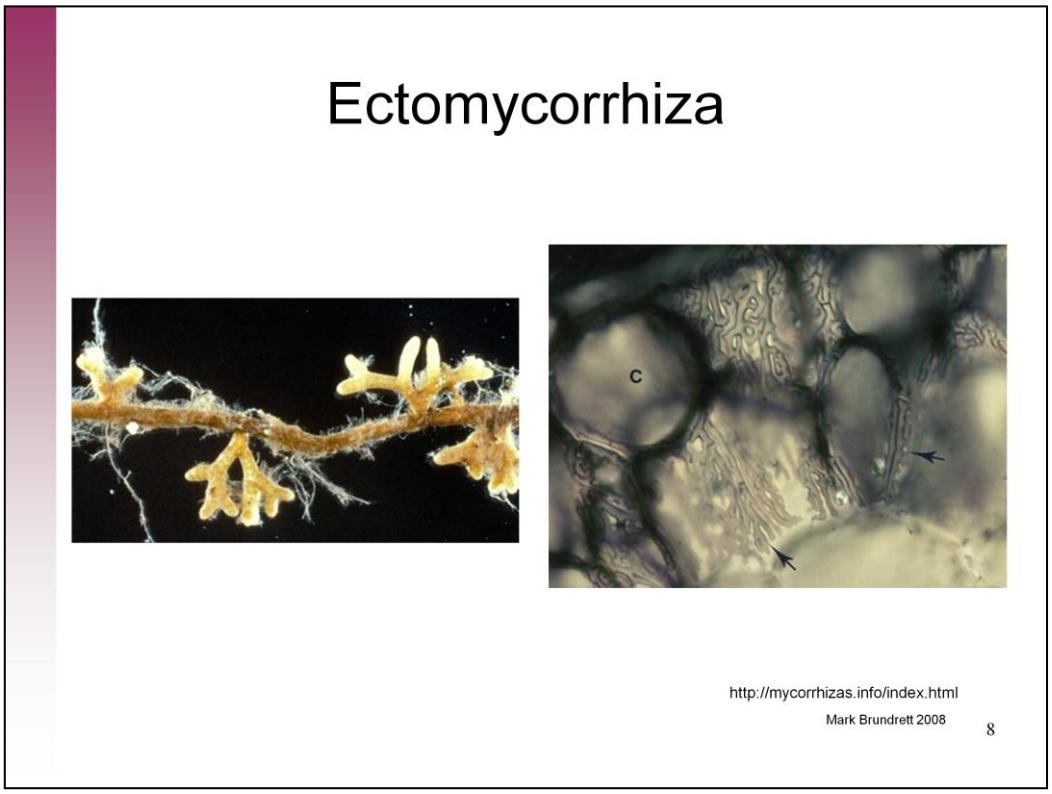
There are two major genera within the family Russulaceae – Russula and Lactarius, both of which are predominantly epigeous forms.

The hypogeous genera include Zelleromyces, Cystangium and Gymnomyces.

The genus Macowanites is described as having a secotoid form.

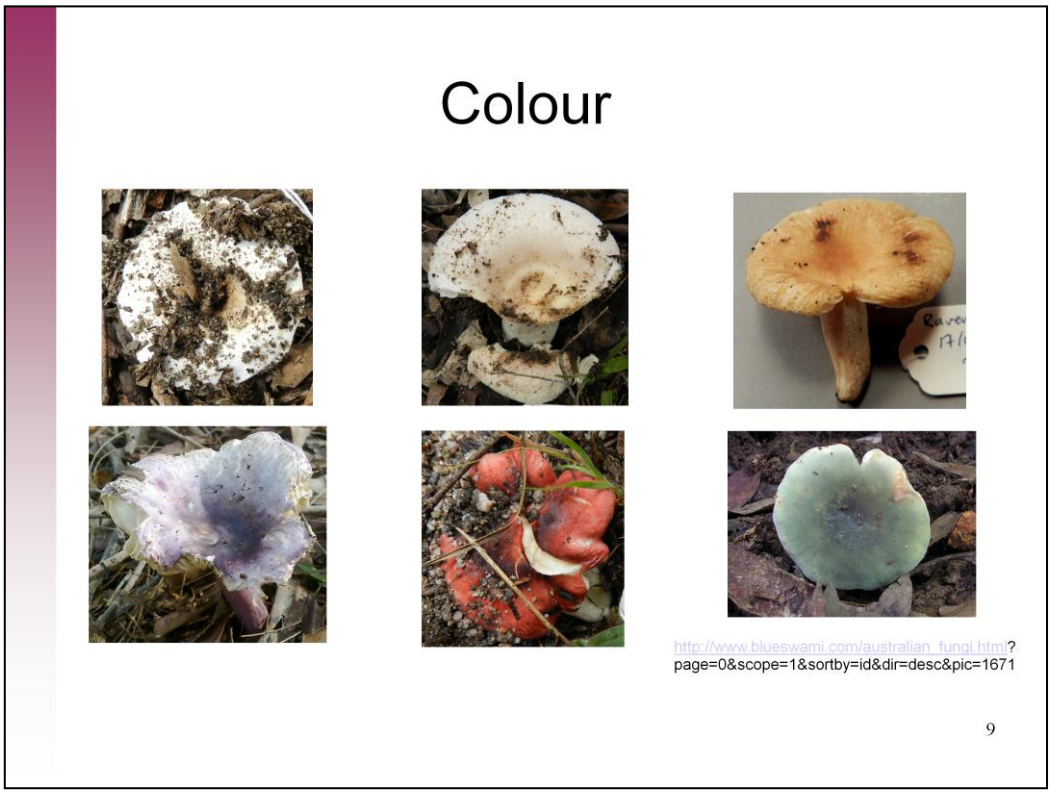


The Russulaceae are an ectomycorrhizal species – they form a symbiotic relationship with a plant host. Unlike the mycoheterotrophic orchids, this relationship is beneficial to both parties.

In Australia, eucalypts are generally the plant hosts; in Europe it tends to be pines

The left hand photo shows an ectomycorrhizal association synthesised under sterile conditions between *Pinus radiata* and *Suillus brevipes*. These dichotomously branched mycorrhizal short roots, which are a diagnostic feature of this type of association, increase in age from left to right.

The right hand photo shows the Hartig Net formed by fungal hyphae within the plant's epidermal cells



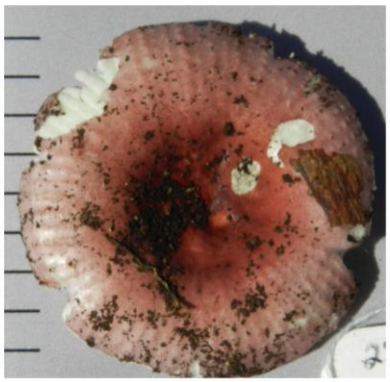
The next few slides provide a brief look at some of the features of the epigeous forms of Russulaceae

One of the first features noted is their colour, which ranges from white to gold, to pinks, to purples and greens.

However, it is a very variable and subjective characteristic, and requires the use of colour charts to provide some consistency. Unfortunately, the "standard" colour charts are difficult to obtain and are expensive, so I will be trying the Online Auction Colour Chart, which is available through Fungimap.

I currently don't have a photo of a green Russula. The green specimen above is a *Russula heterophylla* found in NSW

Cap



- Shape
  - Side view
  - Top view
- Surface Margin
- Cap Margin
- Surface Texture
- Peeling
- KOH

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Cap features predominantly found include:
A round to irregular shape when viewed from the top;
From a side view, the cap tends to be flat to convex with a central depression, or infundibuliform;
The surface margin is usually smooth or striate;
All specimens so far have a plane cap margin;
The surface is generally smooth, and will peel in varying amounts;
Potassium hydroxide dropped on to the surface can provide an olive or green colour reaction, but so far, none of the specimens tested have shown a colour change.

Stipe

- Shape
- Interior
- Attachment
  - To cap
  - To substrate
- Texture
- $FeSO_4$

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The shape of the stipe (stem) is quite variable – it can taper downwards, be cylindrical, slightly clavate, but it always tends to be very thick;
The interior is either solid, hollow, or contains some material;
Its attachment to the cap is predominantly central, while attachment to the substrate is simple;
The texture is generally smooth;
A drop of ferric sulphate produces either a green or pinky brown colour change, or no reaction.

There is:
No annulus
No volva
No veil remnants

Gills

- Attachment
- Gill Margin
- Spacing
- Lamellulae
- Arrangement

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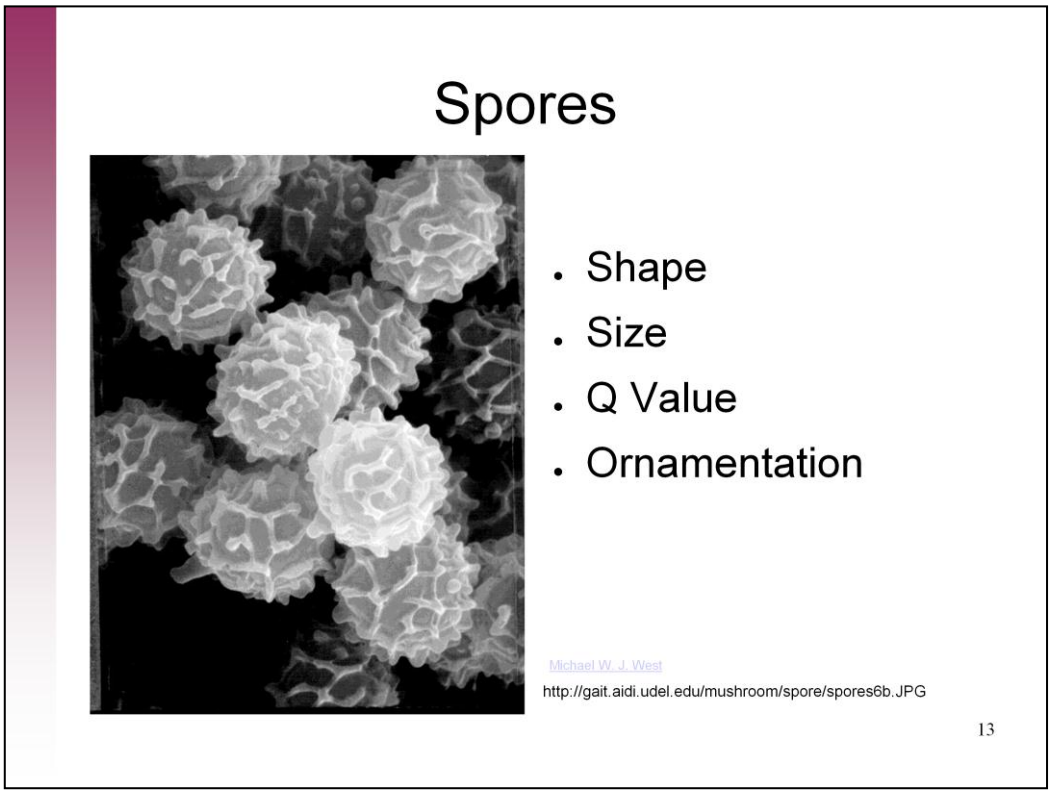
Gill attachment is mostly adnate, and occasionally subdecurrent. The gill margin tends to be smooth.
The spacing of the gills can be distant to slightly crowded, but a count of gills per cm or half-cap will be obtained for a more objective result. They always look nice and neat.
There is the occasional lamellulae, but generally each gill is full length.
The arrangement is mostly regular, but there are some specimens with bifurcate gills. A little caution is required though – gills that appear to be bifurcate can just be two gills temporarily stuck together.



The photo above shows spores from *Russula mariae* magnified 5000 times.

The spores of Russulaceae mushrooms are globose (round) to subglobose. This results in a Q value (the ratio of length/width) of around 1.
Ornamentation requires a description of the ridges and spines that occur on the spore surface. These features also produce a blue colour when Meltzer's Iodine is applied – an Amyloid reaction.
The spores are hyaline to pale coloured

Other microscopic features

- Basidia

- Cystidia

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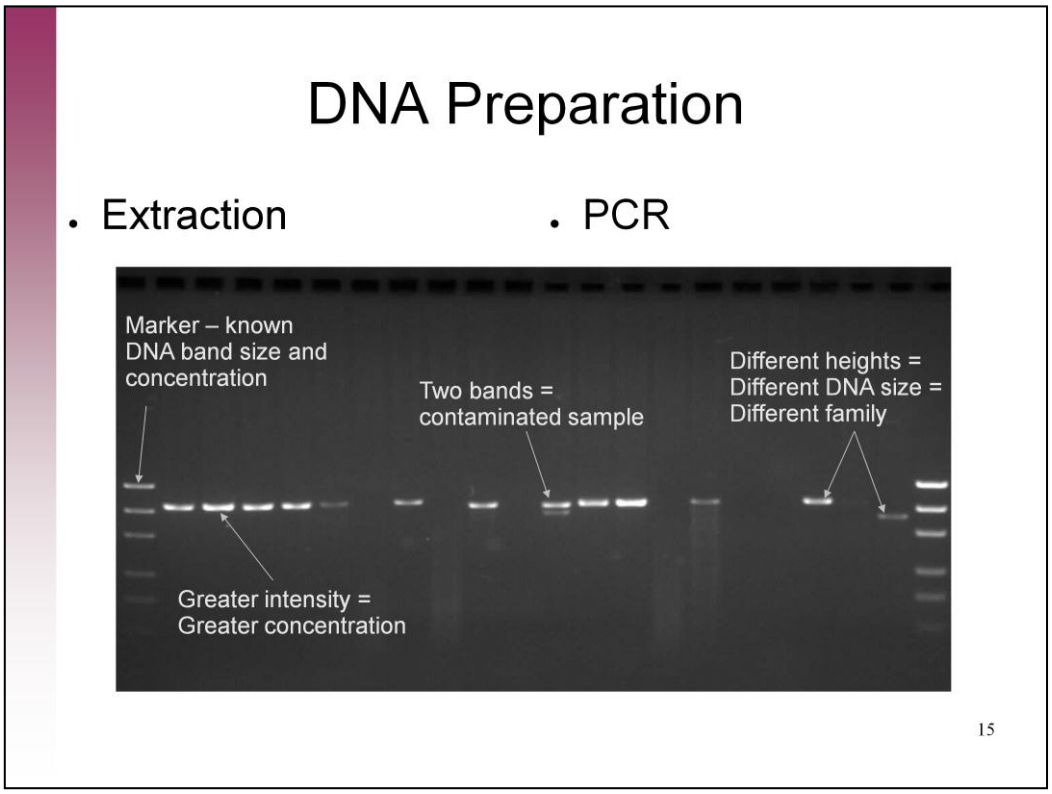
Other microscopic features to be examined and described include the basidia, which are the spore forming structures and various forms of cystidia

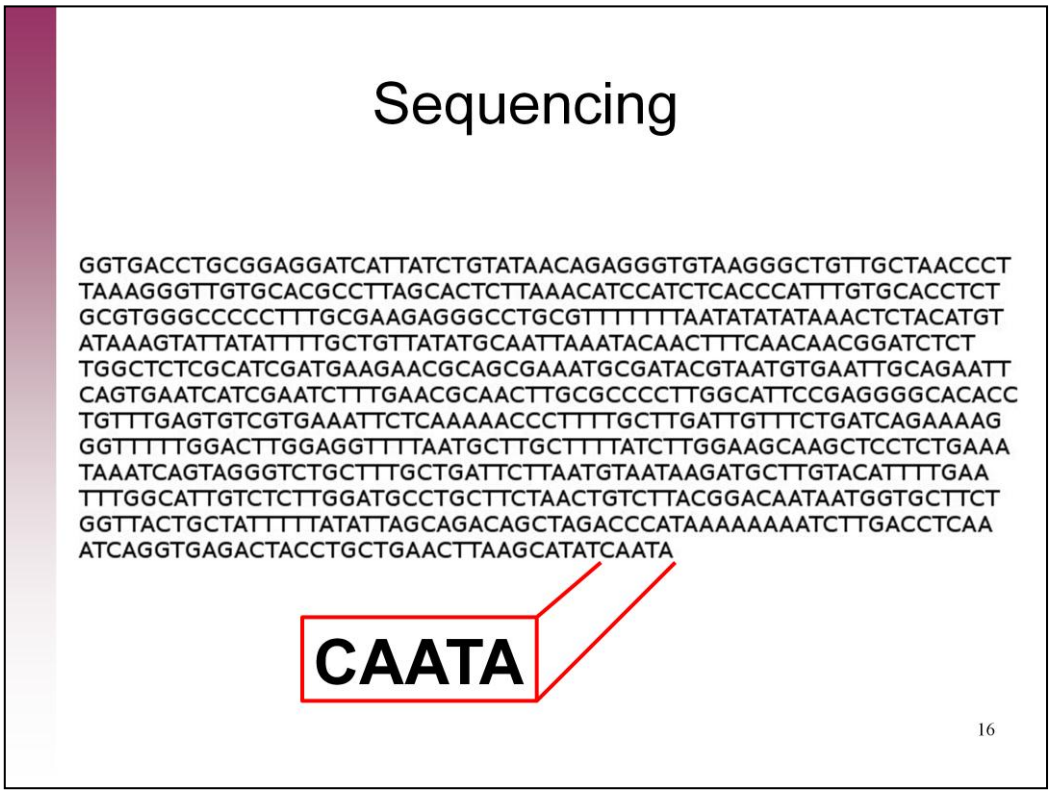


Methods for the extraction of DNA are constantly improving – what used to take several hours and required many hazardous chemicals now takes two solutions and about 10 minutes!

A maximum of 0.1g of the specimen is required. This is taken from the area where the stipe joins the cap, to avoid as much soil contamination as possible.

PCR (Polymerase Chain Reaction) is then used to increase the amount of DNA available for testing, but only one particular area of the DNA is used – this area produces very little variation within a species and a significant variation between species. It is very sensitive, and highly susceptible to contamination, so care is required.

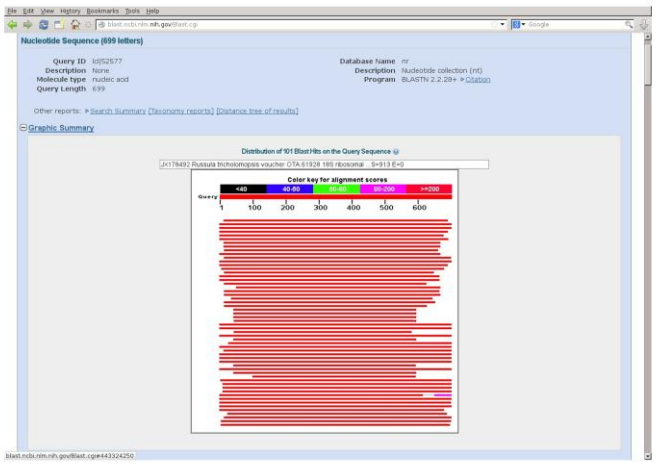
A gel is run and photographed to estimate the quality and quantity of the PCR product obtained – a good product is required to get a good sequence.



The PCR samples are sent to a laboratory in Brisbane for sequencing. Once completed, you obtain several files, one of which is the sequence. DNA sequences are combinations of the letters A G C and T, which are the symbols of the nucleic acids that are the building-blocks of DNA.

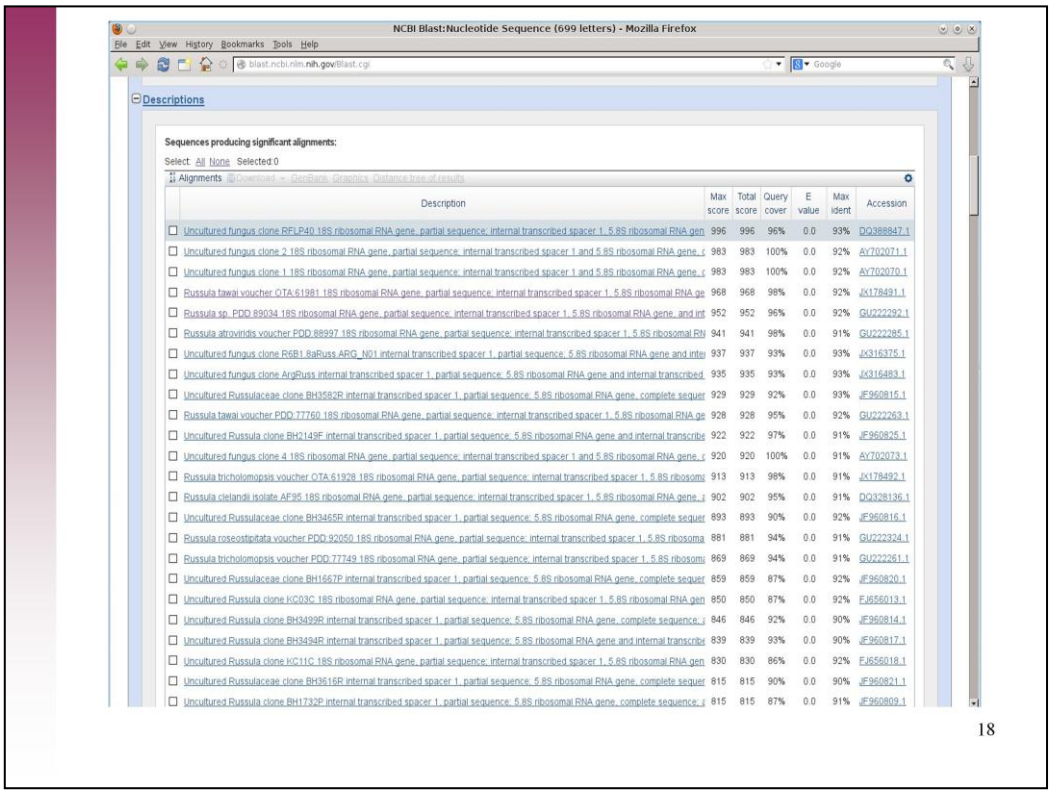
This is the sequence obtained from one of the samples shown in the previous slide.

Genbank



The sequence is then copied into the search box of a massive database of sequences, accessed via the Genbank website. Over the course of several seconds, your sequence is compared with millions of other sequences.

This is the first part of the results obtained. The chart in the middle is an overview of how the sequences in the database that are most similar to your sequence, align to your sequence – the lines produced here are predominantly red, indicating a high score



The lower part of the window lists the descriptions of the sequences that have produced the closest match to your sequence. This demonstrates two of the problems associated with this aspect of the project – firstly, you rely on people submitting the sequences that they have obtained, and more importantly, you rely on them providing a correct description for the source of their sequence. This is where people such as Patrick are invaluable, providing the initial correct identification of a specimen.

Example Results

- MB06
  - Preston
- BLAST Results:
  - *Uncultured clone*
  - 93% Identity

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The sequence and results shown in the previous slides relate to this specimen, MB06, collected at Preston. The most similar sequence currently held in the Genbank database is from an uncultured fungus clone – hardly an inspiring result! It has a 93% identity, which is a measure of how similar the two sequences are. Generally, a result above 97% is considered to be identical. Therefore, these are not the same species.

A few other results follow

Example Results

- MB16
  - Preston
- BLAST Results:
  - *Russula tawai*
  - 91% identity

Example Results

- MB24
  - Ravensbourne
- BLAST Results:
  - *Russula* species
  - 95% identity

Example Results

- MB19
  - Preston
- BLAST Results:
  - *Melanoleuca strictipes*
  - 94% Identity

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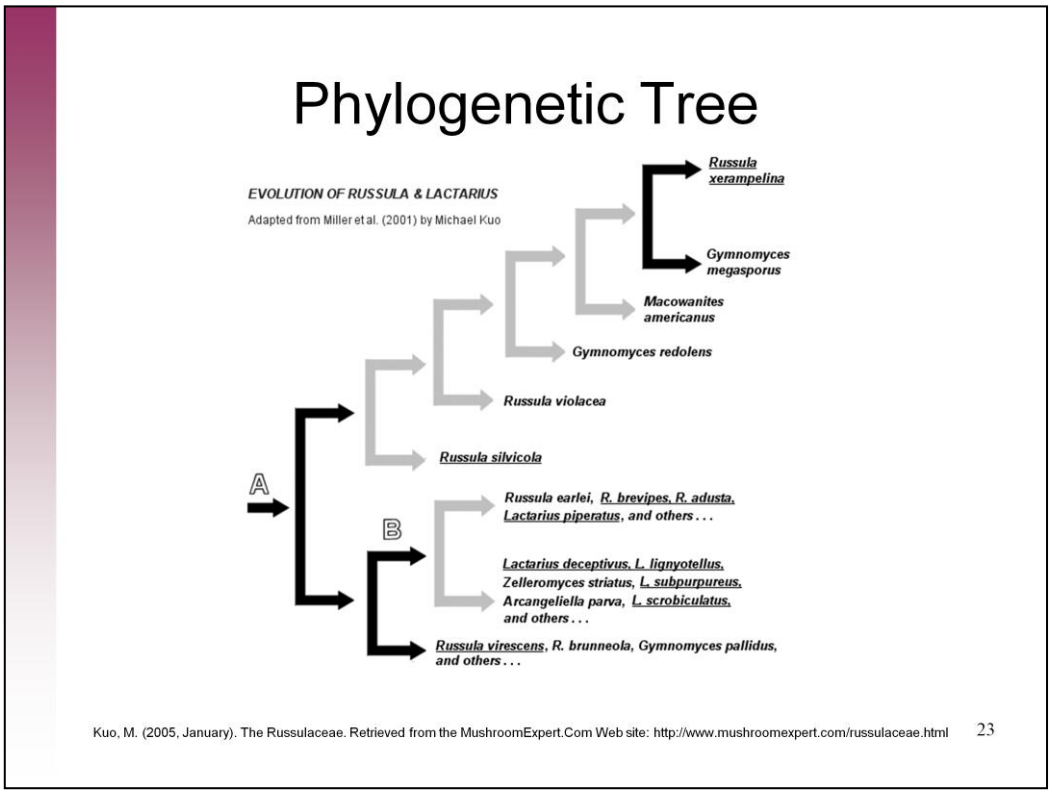
This specimen is not a member of the Russulaceae family, but has some similar characteristics – it's similar in shape and size; it's a pale pink in colour; there is no annulus, volva or veil remnant; the spores are subglobose with ornamentation that has an amyloid reaction to Meltzer's Iodine. It, and other similar specimens, will be included in order to demonstrate the robustness of the identification procedures.



The sequences obtained will then be used to create a Phylogenetic Tree. This is used to demonstrate the potential evolutionary relationship between species. This simplified example shows how species of *Lactarius* and *Russula* possibly evolved from a common ancestor. I expect the tree I create will be much more complex!

Bioactive Properties

1. *S. aureus*
2. *S. epidermidis*
3. MRSA
4. *E. coli*
5. *S. marcescens*
6. *P. aeruginosa*
7. *B. cereus*

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The final part of the project will determine whether specimens contain any chemicals which possess bioactive properties – generally anti-bacterial or anti-cancer compounds.

This photo, from my Master's research, shows a culture of *Schizophyllum commune* and radiating streaks of bacteria – the absence of growth in a streak suggests the presence of an anti-bacterial compound.

However, a lot of material is required for the extraction process, and individual specimens may not be sufficient. Laboratory cultures can be used, but members of this family are notoriously difficult to culture.

Experimentation is continuing in this area.

Acknowledgements

- Queensland Mycological Society
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I hope you have enjoyed this presentation, and I thank-you all for your interest and support...