SYDNEY FUNGAL STUDIES GROUP INC.
COLLECTING MACROFUNGI

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CONTENTS

General

There is a growing interest in mapping the macrofungal flora of many areas to obtain distribution records similar to those already existing for flowering plants. However, unlike plants the identification of fungi relies on the collection of fruiting bodies, which in turn is largely dependent upon the availability of moisture, that is, in most cases, seasonal rains.

In the Eastern States of Australia the main collecting period occurs generally in autumn, around April – June, (actually from the first heavy storms in March), with a lesser fruiting sometimes in spring; around September – October. Also a range of species will fruit from January (and sometimes December) after some good summer rains (these include many Boletes and *Amanita* species). In areas where there is abundant moisture year long, such as along permanent watercourses, the collection period may be considerably extended. Nevertheless, there may be sporadic and occasional fruiting when suitable environmental conditions occur throughout the year. In some drought years fruiting may not occur, while in damp places such as fern gullies and areas of rainforest, fruiting bodies may be collected at most times of the year.

In view of the restricted availability of fungal fruiting bodies, the fungal mapping of an area may require that the area be surveyed over an extended time, perhaps a number of years. To obtain a good idea of the fungi present in a particular area, it should be visited every week over the period from March to June. Also, since they often do not fruit every year, it may take 5-7 years to have seen the full range of species

Collecting Macrofungi

Before any collection of specimens occurs it is necessary that approval to survey and collect specimens be obtained from the owner, manager or relevant statutory authority of the proposed collecting area. Not unusually, conditions are often attached to this consent, such as, disturbance to vegetation should be minimised, the taking of material for identification purposes should be minimised and only when necessary, a copy of the final survey results and report may be required by the consenting authority, the number and destination of voucher specimens may be nominated, etc.

Since the aim of collecting specimens from an area is to be able to identify those fungi so that they are included in the mycological flora for that area, it is important to appreciate that the greater the amount of data obtained relative to the specimen the easier will it be to determine an identity.

The actual collecting process should begin with a photograph of the specimen in situ, or as soon as possible after collection, to illustrate colour, size, habit and other characters. An identifying number or accession number or legend and scale should be included in the photograph. The scale, ideally in centimetres, is best placed alongside the specimen, preferably along the bottom edge of the photograph, and it is most useful if a neutral background is used. Although a natural background provides some habitat information, and may make a pretty picture, a neutral background allows the fungus, the base of one stem and a section to be more clearly shown, i.e. to achieve the primary aim of illustrating the fungus. Where a photograph is not possible, an accurate scale colour sketch with identifying or accession number should be drawn, with the major dimensions of the specimen included. Large specimens may be drawn at a reduced scale while for small ones the scale should be multiplied two to ten. Try to draw one or two complete specimens, of different ages. As much of the field identification sheet should be completed as possible on the spot and at the time of collecting the specimen. Proper records will avoid the frustrating experience of having insufficient data to complete identification.

It is important that entire specimens are collected, particularly the base of the stem of those that grow in the ground and those that have rhizoids or other similar delicate features at near soil level, such as a volva. Consequently it is advisable to dig up the fungus with a trowel rather than attempt to otherwise remove the specimen. The more care taken when collecting specimens the more readily will the specimen be identified.

While many fungi grow on the ground, fungi growing on buried wood, animal dung or mycorrhizal with plants may give the appearance of growing in soil unless the whole specimen is carefully dug up.

Where the specimen is growing on wood some of the substrate should be removed together with the specimen, usually using a sharp knife or similar.

While those fungal specimens which grow above ground (epigeal fungi) are relatively straightforward to collect, subterranean fungi (hypogeal fungi)

requires the disturbance of the soil and combing of the humus layer with a knife or a small fine-tined gardener's 'hand' or rake (a bent table fork is a reasonable substitute), even sometimes lightly hoeing the ground.

For identification purposes it is helpful to collect a range of specimens of differing ages, where these are available. Additionally, it is more useful to have a collection of a few specimens in good condition rather than a large number of poor and usually deteriorating specimens. A collection of a single small fruit body is usually useless. However, sometimes with large specimens (e.g. *Boletus*, *Amanita*) a single fruit body is often the only one available and this can be adequate, if it is in good condition. The ideal is to collect a range of fruiting bodies, in good condition, of different ages. For a useful collection, usually a minimum of three fruit bodies is needed. It is usual to include with the specimen a small amount of the substrate, to indicate the substrate. Of course, only sufficient specimens should be collected for identification and when required for storing as herbarium material and whenever possible specimens should be identified in situ.

Small fungal specimens such as those growing on moss, on animal droppings or the smaller hypogeal fungi are better handled with a small pair of tweezers rather than attempting to do so with the hands.

Generally fungal specimens are relatively fragile, accordingly they should be handled carefully to avoid bruising and in some cases discolouration. Transportation may require special packing precautions be taken to minimise the effects of vibration.

The fruit bodies in the collection should be kept separate from other collections. Where care needs to be taken to avoid squashing the specimens later the specimens should be placed in greaseproof paper, or for a short period of time, in one of a range of small plastic boxes. The greaseproof paper should enclose the specimen so that the specimen is at the centre of a loosely rolled cylinder the ends of which are twisted closed. Each specimen roll should have an identifying number or legend. This method of packaging isolates and reduces desiccation of specimens and to some extent provides cushioning between specimens for transportation. Obviously the more robust or heavier specimens should be placed in the bottom of the collecting container, although smaller or the more fragile specimens may be kept separately in another container.

If boxes are used for short-term storage and transport of specimens, they should be as small as possible. A plastic bag or wrap is not suitable for the packaging of specimens; since they sweat, become slimy and rapidly deteriorate, making them unsuitable for identification purposes.

Some collections may be made with a view to immediately identifying the specimens after collection, in which case, it is probable most specimens will be discarded soon thereafter, only those requiring more extensive examination being packaged. Under these circumstances, and as the specimens will not be held for any extended period, they may be conveniently

carried and transported over a short distance, in a multi-compartmented case, such as used for storing fishing tackle, which provides the necessary mechanical protection and separation. Discarded specimens should be replaced in an upright position for subsequent spore dispersal.

Where possible, when transporting packaged specimens it is helpful to keep them cool. An inexpensive polystyrene 'cooler' serves this purpose satisfactorily. Similarly after the return from the field, the greaseproof wrapped specimens may be held in a domestic refrigerator, for a short period. It is important that the colour, in natural light, be recorded prior to refrigeration since colours fade in some species. However, it is important to note that the specimens not be held in a closed container as they will rapidly spoil.

Collecting Equipment

To reduce possible damage to specimens when collecting, a small amount of simple equipment is utilised. The amount and type of equipment used to collect and keep records of fungal specimens includes: -

1. A single lens reflex camera with colour film (suitable for colour slides) or a modern digital camera that is competitive in image quality, to record the appearance of the fungi when fresh and in situ. Camera films vary slightly and significantly with respect to their colour accuracy and stability over time. One that is still widely used is Kodachrome 64.
2. A small trowel to remove fungi from their substrate. An old tablespoon makes a convenient substitute while a small short-handled rake, or bent fork as a reasonable substitute, is useful when looking for underground fruiting bodies (hypogeal fungi).
3. A robust stick is useful to remove leaf litter or dead twigs that may obstruct the view of emerging fruiting bodies.
4. A sharp knife; any good strong sharp penknife is satisfactory for most purposes, while a safety razor blade is probably more useful when cutting sections from specimens. For larger tougher species and bracket fungi a large broad-blade knife is useful and in some cases a tomahawk and a small bush saw is useful to collect a small piece of wood together with the specimen.
5. A hand lens, 10x or 20x power
6. A small hand mirror (a dentist's mirror is satisfactory) to view in situ the underneath of a specimen.
7. A small painter's brush is useful to gently remove debris such as soil, humus etc., prior to photography.
8. Greaseproof paper to wrap specimens to prevent desiccation. Note that plastic bags or wrapping causes specimens to 'sweat' and deteriorate. Absorbent paper may be used for viscous or moist specimens.
9. Field data sheets, equipment to measure specimen dimensions, notebook, pens, and pencil for drawing; to record details of date, location, specimen identification number or legend, habit, smell, taste, colour and other characteristics at the time of collection. Note that a small cassette tape recorder may be used to advantage to record some of these field details.

10. A map of the collecting area, of a suitable scale, sufficient to provide the latitude and longitude of the collection locations.
11. A flat-bottomed collecting tray, basket or rigid bag. (Where specimens will be identified immediately, a multi-compartmented fishing tackle box is suitable,)
12. A pair of small tweezers, while not essential, is helpful for handling tiny specimens.
13. A field key to the fungi. Texts that are useful in identifying larger fungi are detailed in Appendix A.
14. Chemical reagents used for the identification of some species of fungi, which may be included in the field equipment, are best carried in small bottles securely packed in a box or tin rather than carried in a pocket.

Clearly clothing worn should be as practical as possible, comfortable footwear and a brightly coloured hat or high visibility clothing contributes to safety and comfort. As the collection of many fungi, particularly those that grow in the ground, necessitates kneeling, wearing a good pair of kneepads can save some considerable discomfort at the end of a long day of collecting. The use of a strong comfortable haversack for the collecting equipment, some light refreshments, water and a first-aid kit provides a convenient way to carry the necessary supplies for a day's collecting.

Identification of Macrofungi

It is usual to begin the identification process by describing the gross morphology of the specimen. Much of this information will be recorded on the field data sheet for the specimen. The photograph of the specimen in situ supplements the data from the field data sheet and provides an excellent real life view of the specimen particularly where the specimen is dried for storage. Nevertheless, where only one photograph is taken, to ensure that the maximum detail relevant to the fungus is shown, a neutral background should be used to photograph the specimen(s) and sectioned specimen(s).

Some fungal characteristics are relatively ephemeral; as such the time of collection is when it is best to carry out several identification checks.The smell of the fresh specimen and the taste of a small sample should be noted. Only a small portion should be tasted briefly and then spat out. Most fungi are quite harmless, but where the specimen is suspected of being toxic, the taste test should be omitted. With some species, their pigments are water soluble and wash out in heavy rain. Nevertheless, it is important to record specimen colour, and if possible of specimens at several stages of development. Other species exhibit characteristic colour changes when their flesh is cut or broken and these changes are helpful for identification. Tests using chemical reagents particularly on some fungi difficult to identify otherwise, should be carried out on fresh specimens. Similarly, fresh specimens are necessary for those fungi that exude a milky fluid or

latex, when the cap is cut or broken, the change or not of the colour of the latex when exposed to air is a useful diagnostic feature.

Another useful aid in identifying macrofungi is to determine the colour of a mass of its spores, usually obtained from a spore deposit or spore print. Spore prints should be taken from fresh specimens or at least as soon as reasonably possible after collection. The most fragile specimens should be those prepared for a spore print first. Where specimens have a cap, the cap should be cut from the stem leaving a small portion of the stem. The cap is placed on a piece of paper, labelled with the specimen identification number and which is alternatively coloured black and white, positioning the cap so that it is held by the remaining section of stem slightly clear of the paper and half of the underneath of the cap is over black and half over white. To prevent the cap from drying out and air currents disturbing spores, a clear glass jar, basin or other similar utensil is placed so as to completely cover the cap. A small drop of water may be placed on the top of the cap to help maintain a high humidity.

For spores of other fungi that because of their morphology or the number of spores deposited are insufficient to form a reasonable mass or for some other reason are not suitable for the method described above, they may be placed on a glass microscope slide covered to maintain high humidity, and a microscope used to search for the spores, allowing observation of their colour, shape, size and ornamentation.

Alternatively, the glass slide may be placed against the appropriate coloured background to help determine spore colour. Most spore prints require some hours, and it is often best to leave them overnight to get a good thick print.

Spore prints may be kept intact by folding paper over the print, or by sealing with hair spray, and placing the spore print in a cellophane bag together with a few flakes of naphthalene.

A good quality microscope is necessary to identify spore shape and size. It may be necessary to use x1000 magnification, although good results for relatively large spores may be obtained sometimes at only x400. A microscope with an inbuilt light source is preferable.

Prior to drying specimens, a sectional, dimensioned drawing of the specimen should be prepared, showing the general cross-sectional morphology, particularly the type of attachment of the gills or pores, as applicable, together with a detail of the spacing arrangement of the gills or pores, A detail sketch of the form of the gill margin is also sometimes a useful aid to identification.

Specimens are best preserved by drying and then stored with naphthalene, or other insecticide, to reduce insect attack. Good results may be obtained using a domestic oven on a low temperature with the

door open, or in front of a blow heater on low heat. But drying out with a commercial fruit-dryer produces best results or in a clothes-drying cabinet that works using low temperature and a good circulation of air. The aim is to use a large flow of air and a modest temperature to dry the fungus. High temperatures and low airflow will cook the specimen rather than drying it.

When dried, that is, when the specimen feels brittle, the specimen, together with a few flakes of naphthalene, and specimen identification number, should be placed in a cellophane bag, which is sealed, labelling the outside of the bag with specimen name and identification number.

Very large specimens may be cleanly cut into thinner slices to facilitate drying, while in some cases it is convenient, but not as satisfactory, to preserve the specimen as a wet preparation in a fixative (such as formalin-acetic acid-alcohol) or methylated spirits. It should be noted that most fixatives remove the colour from specimen. The dried specimen will change colour and the shape is distorted, but most of the microscopic features may be recovered when the specimen is placed in a solution of 3% or 5% potassium hydroxide (KOH), or ammonium hydroxide (NH_4OH).

All records, together with dried specimens and other relevant data should be retained in a sealed cellophane bag that includes a few flakes of naphthalene.

A number of texts useful in identifying macrofungi together with a sample field data sheet are shown in Appendix A. Most of the texts include illustrations of fungi and their features, as well; some include a glossary of terms commonly used in mycology to describe fungi.

APPENDIX A

Texts that may be used to identify macrofungi: -

Aberdeen, J.C. E. (1979). *Introduction to Mushrooms, Toadstools and LargerFungi of Queensland*. Handbook No. 1; The Queensland Naturalists Club, University of Queensland Printery.

Bougher, N. L. & Syme, K. (1998). *Fungi of Southern Australia*. University of Western Australia Press, Perth.

Courtecuisse, R. & Duhem, B. (1995). *Mushrooms and Toadstools of Britainand Europe*. Harper Collins, London.

Fuhrer, B. (1985). *A Field Companion to Australian Fungi*. Five Mile Press, Hawthorn.

Fuhrer, B. & Robinson, R. (1992). *Rainforest Fungi of Tasmania and South- East Australia*. Reid, Auckland.

Grgurinovic, C. A. (1997) *Larger Fungi of South Australia*. The Botanic Gardens of Adelaide and State Herbarium and The Flora and Fauna ofSouth Australia Handbooks Committee, Adelaide.

Hood, I. A. (1992). *An illustrated Guide to Fungi on Wood in New Zealand.* Auckland University Press, Auckland.

Horne, D. (2000). *Mushrooms and Other Fungi of New Zealand*. Reid, Auckland.

Largent, D. L. (1973). *How to Identify Mushrooms to Genus I: Macroscopic Features*. Mad River Press: Eureka.

Shepherd, C. J. & Totterdell, C. J. (1998). *Mushrooms and Toadstools of Australia*. Inkata Press, Sydney.

Young, T. (1982). *Common Australian Fungi*. New South Wales University Press, Kensington, NSW.