ANNEX 4: LABORATORY MANUAL FOR COLLECTING AND PROCESSING PLANT SPECIES

LABORATORY MANUAL

FOR

COLLECTING AND PROCESSING OF PLANT SAMPLES

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PAPUA NEW GUINEA FOREST AUTHORITY

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Preface

The documentation and botanical exploration in Papua New Guinea as it is known today is a result of efforts made by many botanists, foresters, researchers, agriculturalists, lecturers and their students. Many institutions have supported this efforts by way of providing resources, logistics and technical assistants to many long term and short term researchers and scientists both residents and non residents.

Botanical explorations in PNG remain the most challenging both physically and culturally. Much of the forest areas in Papua New Guinea remain to be explored botanically due to inaccessibility and isolation. The plant collections made under this project will come from sites that had not been explored in the past and these will increase the number of herbarium collection significantly.

The methodology for documenting, collecting and preserving plant specimens remain the same over the years except for recent improvement in preservation techniques for samples for DNA studies, which provides the basis for phylogenetic studies and taxonomic classification.

This manual was prepared to assist forestry officers and scientists collecting and documenting the flora of Paua New Guinea in the Multipurpose National Forest Inventory project. It focuses on field collection techniques and laboratory processes to ensure that plant specimens are sampled correctly in the field and that they are processed correctly after it is received in the laboratory.

Acknowledgement

This manual was put together base on materials prepared by the Forest Biology Program team for the Dendrology Refresher Training for PNGFA officers in preparation for the Multipurpose National Forest Inventory project. The team also provided comments on the draft of this document. We thank Penniel Lamei, Oliver Paul, Peter Homot and Tiberius Jimbo for their input.

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1.0 Introduction

This manual is provided as an easy reference for the implementation of the Multipurpose National Forest Inventory (MNFI) in Papua New Guinea. It focuses on sampling methods designed for sampling plant materials under the project for higher plants, lower plants, lianas, and leaf traits. The standard botanical collection and processing procedures are described when sampling materials in the field and processing in the laboratory.

The manual describes the equipment to be use when collecting plant samples in the field, how to collect samples, how to describe a plant in the field and write the notes into the field note book, how to collect floral and fruiting materials, how to preserved samples in alcohol and stored in the field and how to packed specimens and send them to the herbarium at the Forest Research Institute (FRI) for processing.

The manual also described methods for collecting samples of different plant groups. Some plant groups have larger leaves and are difficult to collect specimens in the field. The different methods for collecting these plant groups are detailed in the manual.

2.0 Botanical collecting tools and equipment

Sufficient tools and equipment are required for collecting and preparing plant samples in the field. A good plant specimen will enable the botanist make an accurate identification on a plant species sampled and therefore maintain the quality of the data. The following tools and equipment are required for field collection and must be available when working in the field.

Figure 1. Equipment for collecting and preserving plant specimens

1. Prunner
2. Bush knife
3. Secateures
4. Safety helmet
5. Silica gel
6. Sticky tapes
7. Old news papers
8. Polythene tubes (plastic)
9. Plant presses (wooden) & straps
10. Card boards
11. Ethanol (95%)

Figure 2. Equipment used for recording data on plant specimens

1. Binoculars
2. Camera
3. GPS
4. Tape measure
5. Dissecting kit
6. Hand lens (X 10 / 12)
7. Field book
8. Pencils

Figure 3. Sling shot (catapult) use for collecting samples from canopy trees.

1. Sling shot (Catapult – Locally made)
2. Hand gloves
3. Fishing line rheels (L)
4. Fishing line rheels (M)
5. Nylon twine (4mm thick x 150m long)
6. Fishing line (21 lbs x 150 m long)
7. Sinker (0.1kg lead)

3.0 Plant samples collected from the NFI clusters

Plant specimens collected from the plots/clusters under the NFI project will be processed and stored for further study and confirmation of species names. It is anticipated that most of the samples will be sterile and therefore will take time to confirm their species names. In the lab, the specimens will be dried and stored before they are identified.

Incoming specimens will be processed and identified immediately after they are received from the field. The lab will cater for general specimens as well as samples collected for Leaf Area and DNA studies.

4.0 Sample numbering and labelling

The number of specimens in the field is very important because it is the only way to track specimens and data back to their correct plot and cluster. The Tag will be used on both sides and the following procedure will apply to: Upper Plants; Super Plots; Lianas; Regeneration; and Vegetation Cover, Leaf Area and DNA.

Using Cluster 108924 and Centre Plot at Radius 3m as an example, the following applies;

1. Upper Plants

Side 1:	**108924 - C**	Side 2:	**R3 – NE - Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, R3 is Radius 3, NE is North-East Direction, Spec # is Spp No

2. Super Plots

Side 1:	**108924 - C**	Side 2:	**SUP – SEG 1 – Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, SUP is Super Plot, SEG 1 is Segment No, Spec # is Spp No

3. Lianas

Side 1:	**108924 - C**	Side 2:	**L – R3 - Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, L is Liana, R3 is Radius 3, Spec # is Spp No

4. Regeneration

Side 1:	**108924 – C**	Side 2:	**REG – N - Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, REG is Regeneration, N is North Direction, Spec # is Spp No

5. Vegetation Cover

Side 1:	**108924 - C**	Side 2:	**VC – R10 – Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, VC is Vegetation Cover, R 10 is Radius 10 or 15, Spec # is Spp No.

6. Leaf Area

Side 1:	**108924 - C**	Side 2:	**LA – R3 – Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, LA is sample material, R3 is Radius 3, Spec # is Spp No.

7. DNA Samples

Side 1:	**108924 – C**	Side 2:	**DNA – R3 – NE - Spec #**

Where 108924 is Cluster No, C is Centre Plot Id, DNA is sample material, R3 is Radius 3, NE is North-East Direction, Spec # is Spp No.

Table 1. Equipment for collecting and sampling plant samples per team

	Item	Quantity	Purpose
1	Secateurs	2	Use to cut and trim a specimen in the field
2	Field note book	2/person	Record field characters of specimens
3	Specimen tags	1 box	Tag all specimens with field numbers
4	Pencil	1	Use pencil to write on tags and field books
5	Old newspapers	1 bundle	To cover and separate specimens
6	Plant press & straps	2	To press specimens before preserving in alcohol
7	Plastic bags (polythene)	20	To store and preserved specimens
8	Pruner	1	To collect samples from higher branches
9	Bush knife	1	To slash the bark of trees to view exudate and texture
10	Safety helmet	1	Wear helmet when using the sling shot
11	Binoculars	1	Identify trees in the canopy
12	Camera	1	Take image of specimens
13	GPS	1	To record Lat/Long and elevation of sites
14	Batteries	1 Box	Spare batteries
15	Clip Lock Bags	1 Packet	Storing samples
16	Tape measure	1	Take measurement of trees
17	Dissecting kit	1	confirming anatomy of flowers and fruits
18	Hand lens (X 10 / 12)	1	Closer view of character states of flowers, hairs, and spores
19	Sling shot (Catapult)	1	Collect samples from canopy trees
20	Hand gloves	1	Use for collecting thorny plants
21	Fishing line reels	1	To tie the sinker and shoot over a branch
22	Nylon twine (4mm thick x 150m long)	1	Pull down branches for sample
23	Sinker (0.1kg lead)	10	Use to shoot a line over a branch
24	Ribbons	10	Use to tag/ demarcate sections in plot

5.0 Materials to be used for preserving plant samples in the field

Use a plant press and a strap to prepare materials for preservation with 75% ethanol. For flowers and fruits, these can be preserved in small jars filled halfway with ethanol, the jars must be airtight and lids further secured with heavy duty masking tape. For DNA materials, these can be stored in filter papers with labels and stored in small jars with dried silica gels and properly labelled on the outside.

1. Plant presses (wooden) & straps
2. Card boards
3. Ethanol (75%)
4. Collecting vases for spirit material
5. Silica gel
6. Masking tape

6.0 General collection of plant specimens

Collecting plant samples for preservation in the herbarium is a skill that gets better with more field collections. It is important that all samples collected as herbarium specimens or for identifications be fertile so the botanist can use all the features to identify the plant and have its correct species name placed on the specimen.

Fertile specimens are samples with fruit and flowers whilst a sterile specimen is one without a fruit or flower. The fruit or flower may be separate from the specimen however; they must have the same tag number. In many cases plants may not be in flowering or fruiting season, then all effort should be made to collect a good plant material with complete leaves and habit.

A botanical or plant specimen is not just a single leaf, a half leaf or fragments. A good plant specimen should have at least some leaves attached to the twigs or even better if the flowers and fruits are present.

It is important to note that different plant groups (angiosperms, gymnosperms, monocotyledons, pteridophytes, bryophytes etc.) will have different collection methods. These methods ensure that samples collected have the correct features that will be use to differentiate and identify one species from another.

The following are basic procedures to follow when collecting plant samples in the field.

1. Look for a branch with flowers and or fruits on it and collect a twig of a branch that has good leaves, flowers and or fruits.
2. Make at least 3-5 specimens per tree.
3. Tag the specimen with a tag and write the collection number on the tag.
4. Wrap the specimens in a newspaper and place in a collecting back

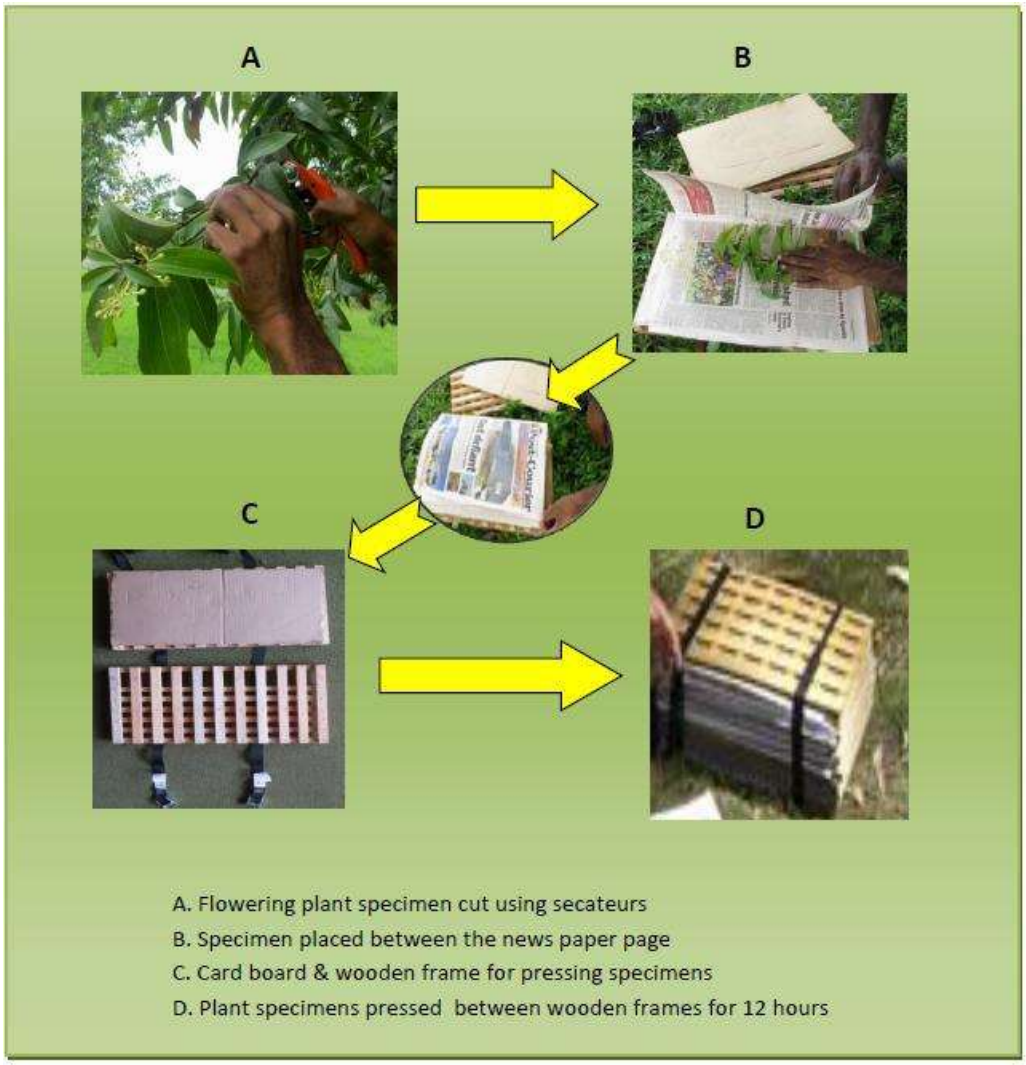
5. Measure the dbh and height of the tree and take notes of its habit, buttress, bole, bark characters, exudate, branching and canopy.

6. Describe the general environment in which the plant was collected from, soils, topography, forest type, and elevation and GPS location.

7. If live materials are collected, such as seeds, rhizomes, cuttings or wildings for planting, these should be tagged and given the same tag number as that of the specimen voucher.

8. If plant samples are collected for DNA studies, these materials should be place into the sampling jar with silica gel and given the same voucher number.

Figure 4. Collecting and preserving plant samples.



7.0 Sampling trees using a catapult

In the past specimens from very tall trees were collected using rifles and shot guns by gun men, they aim at the branches with flower and fruits and shoot bring down the branches. The method is no longer used in PNG due to risks involved. Long pruners were used for taller trees but can only reach certain height depending on the number of extensions to the pole. Climbers were often used but only for smaller trees.

The alternative method introduced to PNGFRI in the mid 1990s by a former director Dr. Geoff Stocker was a sling shot or catapult method (Damas, 2014).

This method of collecting samples uses a lead (sinker) tied to one end of a fishing line (approx. 21lbs) and is shot up to a branch of a tree. The branch must have flowers and or fruits. The sinker goes over the desired branch and it is lowered down to the ground, then the sinker is removed from the string and the string is tied to a nylon twine and pulled up over the branch until that end of the nylon twine reaches the ground. The string is removed and both ends of the nylon twine held together and pull until the branch breaks.

The catapult or a sling shot method will have the following components (see Figure 3).

1. 21 lbs fishing line approximately 150 m long.
2. Fishing line reel (large size)
3. Fishing line reel (medium size)
4. Sinkers (lead)
5. Sling shot (Catapult) locally made
6. Nylon twine (4mm thick and 150 m long)
7. Hand gloves

8.0 Preserving plant samples

At the end of each day, all collections made must be put into press and ready for preservation the following day. Plant samples (voucher) are to be trimmed to fit into a one page of a newspaper, tagged and place into a plant press, interspacing each collection with a cardboard to ensure specimens are pressed flat and ready for preservation. Tie the plant press with a strap or cord and press down equally on all sides ensuring that the force is evenly distributed (Womersley, 1976).

After 24 hours, remove the specimens from the press, remove the cardboards and tie the specimens as a bundle. Place the bundle of specimens into a polythene tube and add 3-4 cups of 75% ethanol, ensuring that all specimens are treated. Shake the bundle to allow the ethanol to spread evenly and seal the tube with a masking tape. Ensure that the bundle is air tied and the ethanol is not leaking.

The following tasks are to be followed when preserving the specimens;

1. Trim the samples to fit into a newspaper and tag the first voucher.

2. Make botanical notes of the specimen into a botanical field book.

3. Place the collections into the newspaper and turn a few leaves to show the under-surface, do this to all samples collected.

4. Separate each collection with a cardboard.

5. Place the collections into a plant press and tie with a strap or cord.

6. Leave for 24 hrs and then transfer the specimens from the press into a polythene tube.

7. Treat the specimens with 75% ethanol (3-4 cups) and seal the bag with a masking tape.

Figure 5. Preserving plant specimens in the field

9.0 Collecting samples of non-tree plant groups

Some groups of plants such as palms, pandanus, grasses, gingers, aroids, ferns, bananas and lianas have their own collection methods. General rule is botanical specimen must always be fertile.

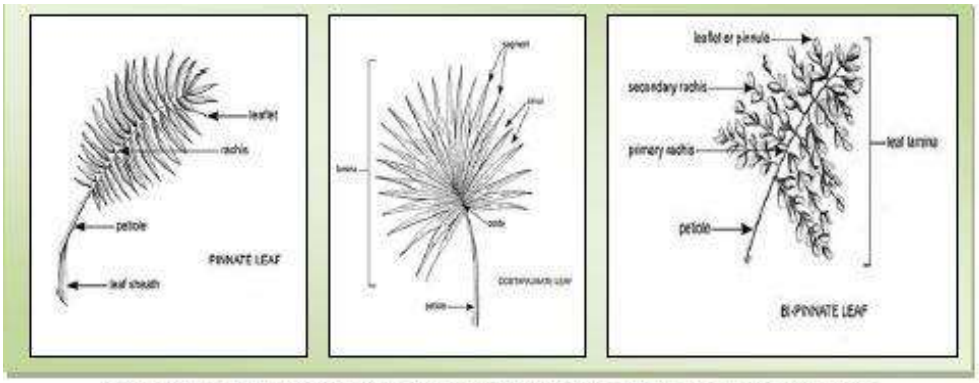
Palms, pandanus, gingers, bananas, aroids and some grasses are difficult to collect because of their sizes, only certain parts of the plants will be useful for collecting so long as their distinguishable features are present for identification. Example, the palms are too big and bulky so the fronds will be measured and several sections will be discarded and only the selected sections be maintained as samples. The inflorescence will be quiet bulky so only several sections will be selected and rest will be discarded.

10.0 Collecting palms (Arecaceae)

Palms habits and forms vary in many ways particularly the leaf structures and architectures are somewhat complex that making specimens are always tedious, time consuming and often it is bulky. It is recommended that the parts that are useful for distinguishing species must be observed and collected carefully as in all the other plant species.

Figure 6. Pictures showing different types of palms

Figure 7. Pictures showing different leaf types



Source: Anderson, P.J. 2011. Identifying Commonly Cultivated Palms. Florida department of Agricultre

Procedures for making palm specimens:

1. Take a photograph of the plant and each entire part before sectioning. Place a common object such as a pencil in the photograph to provide scale.

2. Sampling leaves:

a. Measure the petiole, blade, rachis and leaflet lengths of pinnate leaves, the petiole and blade length of palmate (segments radiating from a single point) leaves and the petiole, blade and rib lengths of costa palmate (leaf stalk extending into leaf blade - rib) leaves.

b. If leaves are small, keep and press whole leaf. For large leaves divide the petiole into mounting paper size pieces. Number the pieces on the tags to keep them in order of cutting.

c. Pinnate leaf: Take several pieces from the blade, include tip. For each piece, apart from the tip, cut the rachis into a mounting sheet size length, remove the leaflets on one side leaving the stubs near the rachis. Fold the other side back and forth to fit the mounting sheet.

d. Palmate leaf or costa palmate leaf: Keep the point of attachment to the petiole and ensure that the hastula is showing. Cut off one side of the blade, part of the other side and fold several times to fit the mounting sheet and press.

e. Inflorescence: If the flower cluster is small, fold and press all of it. If it is large, keep several portions including the base and also showing the origin of the side branches in successive order. If applicable try to keep an entire inflorescence main stem with the side branches removed. Selected side branches from noted positions should be kept and pressed.

f. All of the spathe should be kept, cutting it into sections if necessary. Some flowers may be preserved in ethanol (spirit). Fruits should be treated as for flowers. The cupule (or cup) at the base of the fruit should be kept. Large fruits may be dried quickly if cut in half. (BAKER, W. J. & DRANSFIELD, J. 2006 and BARFOD, A. S., BANKA, R. & DOWE, J. L. 2001)

11.0 Collecting Ferns

Ferns are easy to collect, especially the smaller groups of fern species. But one must keep them out of sunlight in the field as their fronts can wilt easily. Larger ferns are a bit difficult but one gets better every time new collections are made.

Figure 8. Frond of a fern

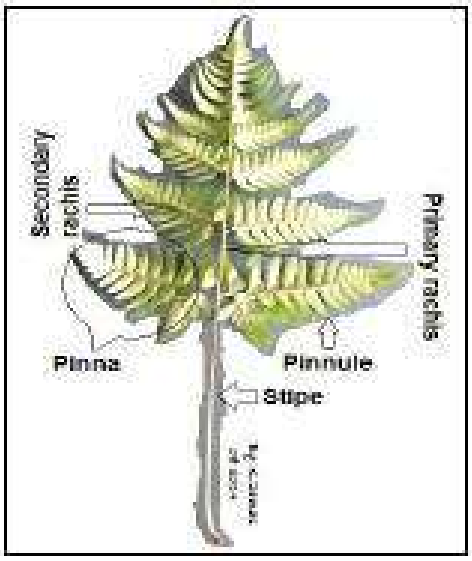
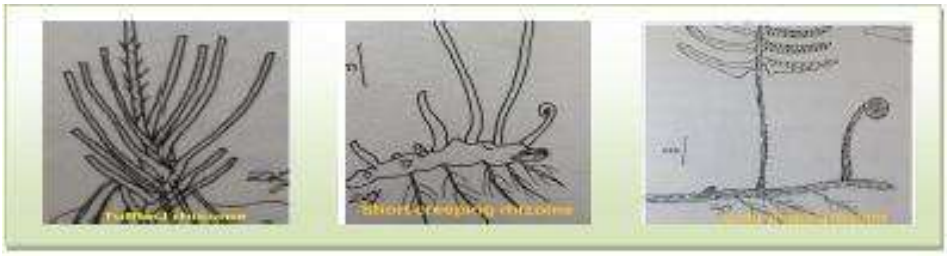


Figure 9: Rhizomes of terrestrial ferns (Source; Jones, D.L. and Clemesha, S.C. 1976)



The following steps are to be followed when collecting ferns.

1. Select a section with some new growth, good healthy fronds with spores on the under surface. Collect several plants from the same population and treat them as one collection.

2. Tag the specimen and give a voucher number

3. Wrap the sample, keep it moist and place it in a collecting bag.

4. Press the specimens as soon as you reach the camp as some ferns can lose a lot of water and wilt and will be difficult to spread out on the press.

5. For large ferns such as *Cyathea* and *Dicksonia,* collect the base of the frond (10 cm), cut the first 10-15 cm of the petiole from the base, cut a 10-15 cm from the centre of the frond and the last 15 cm from the tip.

12.0 Collecting herbs and grasses

Herbs are small plants that are rarely taller than 1 m in height and can grow as an individual within population or may be part of population that colonizes an area vegetatively. Herbaceous plants are easily collected because a whole plant can be collected by either cutting the plant at the base or uproot the plant if it is not too bulky. The following steps are to be followed when collecting herbs.

1. Select healthy individuals from the population, ones with flowers or fruits

2. Cut at least 3-4 individuals measuring 10-30 cm in length

3. Tag them and wrap all samples as one collection and place into a collecting bag

4. Describe the habitat, ecology and get the elevation of the site where the plant was collected.

5. Press the specimens as soon as you reach the camp as some herb species are succulent plants and can dry up and wilt and will be difficult to spread out on the press.

13.0 Collecting gingers

Gingers are considered as large herbs but some species are smaller in size and can be easily collected, especially the epiphytic species in high altitude forests. Some species have terminal inflorescences whilst other bears flowers and fruits at the base. In general a specimen with a flowering or fruiting materials would be sufficient to identify to the generic level. For a good species description, specimens should include a stump with a rhizome, the first 3-5 leaves, another 3-5 leaves from the centre and a terminal twig (20 cm) with flowers or fruit.

The gingers are group according to their flowering habits, one group possess terminal and axillary inflorescence while the other group bearing basal inflorescence. In Papua New Guinea the genera bearing terminal or axillary inflorescence include *Alpinia, Ridelia,Tapeinochilus Boesenbergia, Pleuranthodium* and *Curcuma.* The other genera with basal inflorescence include *Amomum, Hornstedtia* and *Etlingera*.

Figure 10. Ginger species

Figure 11. Collecting Gingers in the field.

The following steps must be followed when collecting gingers:

1. Photograph the plant and each entire part before sectioning. Place a common object such as a pencil in the photograph to provide scale.

2. Collect the best fertile (flowers and fruit) sample of a species from that population or clump,

3. For species with inflorescence attached to rhizome, dig the base carefully to avoid breaking any attached inflorescence, flowers or fruits.

4. The rhizome dug out from the ground be cleaned off the mud from the roots with water. Remove soil with care to avoid breaking the flowers.

5. Specimen collection will include rhizome (split in haves), flowers, fruits, middle leaf and top leaf attached to the pseudo stem

6. For species with terminal and axillary inflorescences carefully prepare the specimen to fit in between newspaper page

7. The leaves specimen should include top section, mid-section and the lower section of the pseudostem.

8. Described the species noting the following; life-form; i.e. terrestrial or epiphytic; habit i.e. clump or spreading, length of pseudostem; colour of flowers and fruits, position of fruit,

14.0 Collecting bananas

A good collection of a banana (*Musa* sp.) comprises herbarium sheets of pressed material, written notes, spirit material and photographs (whole plant, pseudo-stem (to show colour), whole inflorescence, bracts and male flowers). Measurements to be taken include total height of the plant, diameter of the stem, total leaf length and with, length of petiole, size of fruit and seed etc.

Figure 12. Banana plant and fruit with male flowers

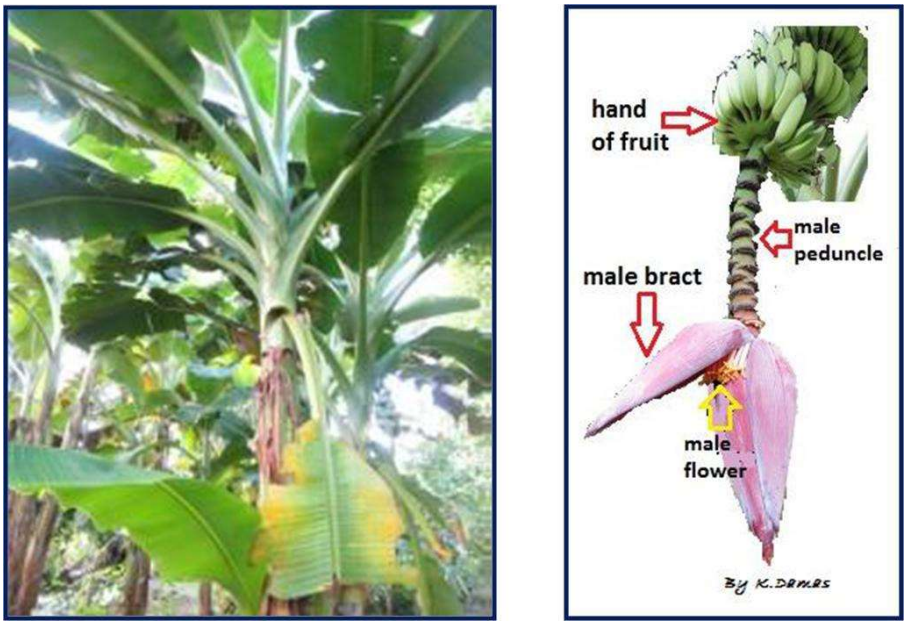
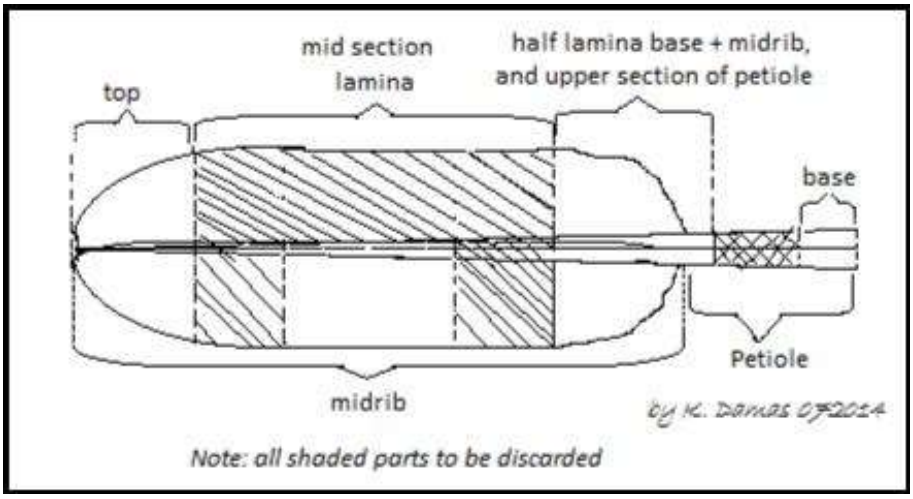


Figure 13. Banana (Musa) leaf showing sections for collection.



It is recommended that the leaf specimen must be collected from the fourth-last fully developed leaf below the inflorescence.

Procedure for collecting specimens of banana plants

1. Materials to be collected include half lamina base + midrib.

2. Upper section of petiole (junction between lamina and petiole should form the middle of the herbarium sheet), exclude the top of the leaf and bottom of the petiole

3. Split petiole in half, longitudinally and cut off one side of the lamina leaving the midrib intact so that what remains will fit onto a herbarium sheet.

4. Take one 'hand' of fruits and cut in longitudinal section of one fruit

5. Take one cluster of male flowers, attached to the subtending bract portion of the male peduncle (the hanging 'tail' between bunch and male flowers),

6. Describe the plant, taking notes of the following; plant suckering freely or hardly suckering at all; colour of sap in suckers (watery, or red to violet, or milky), colour of pseudo-stem; older bracts strongly revolute or scarcely revolute, inflorescence erect or pendulous, total length of lamina and petiole, colour of fruit, colour of male flowers.

7. For spirit materials, use a large screw-top glass jar. Include some male flowers, some female flowers, some fruits and a bract. This can be done back in the laboratory.

15.0 Collecting cycads (Cycadaceae)

Cycads are mistaken for palm or a tree fern because of their morphology especially the leaves (fronds) and their stems which almost resemble palms and tree ferns. However the inflorescence differ from palms and ferns as they are cone bearing plants. Palms bear flowers while the ferns produce spores.

Figure 14. Cycas tree leaves, fruits and cones

Procedures in collecting cycas specimens:

1. Take pictures of whole plant, leaf (frond), flowers (cones) and fruit.

2. Measure total height of the plant, diameter of the stem, total leaf length and width, length of petiole, size of fruit and seed etc.

3. Specimens will include a cone or at least a number of the sporophylls (scales) that make up a cone, an entire leaf (i.e. the entire "frond", not just an individual pinnule (leaflet), leaf may be cut into c. 30 cm lengths for ease of handling.

4. Total number of leave present in the crown of the plant.

16.0 Collecting bamboos (Poaceae)

Bamboos comprise a section of the very large grass family Poaceae. Many species are in excess of 5 metres tall and some can reach 30 metres or more. One of the characteristics of most bamboos is that they rarely produce flowers, and so when you collect a specimen, the chances are that only sterile material will be available.

Figure 15. Picture showing bamboo culms

You will need to collect the following:

1 Photograph the plant and each entire part before sectioning. Place a common object such as a pencil in the photograph to provide scale.

2 A leaf sample from a terminal shoot.

3 Sample of the stem or culm about 30cm long (you will probably need a small saw to remove this), and one or more culm sheaths.

4 Collect flowering sprigs if the flowers are present

5 Record whether the species is tightly clumping, or ‘running’; the stem colour and any striping, overall height, and the branching pattern at the nodes. Also note from which part of the plant you collected the stem section and the culm sheaths.

17.0 Collecting DNA samples

While working on the field, selected plant families and genera will be sampled to collect material for DNA analysis: a number of leaf samples, selected from young growing buds, would be ideal. For this purpose, each team with a botanist or a staff member from the herbarium will have a copy of the list of selected taxa for the DNA sampling. To check the field identification it is a good practice to collect also a specimen for certain identification in the herbarium.

The major aim when collecting material for DNA analysis is to get the plant specimen to dry as quickly as possible, to avoid degradation or the onset of decomposition processes. For this reason, it is important to increase the surface area for water transpiration by cutting the leaves into smaller pieces. According to (Doyle 1991) it is preferable to also remove the midrib from the leaves before storing them.

To preserve the material, the leaves' fragments should be first inserted in an empty tea bag, which allows transpiration, labelled as the specimen from which the leaves are taken, and then stored in a larger container filled of silica gel, that will absorb the moisture and can be changed with ease when needed. The use of tea bags avoids the need to carry large amounts of silica gel and zip-lock plastic bags. Moreover it makes the replacement of silica gel easier (Wilkie et al. 2013).

Summary of the DNA sampling procedure

1 Identify the species to sample, take a specimen for taxonomic identification and label it.
2 Take a sample for the DNA analysis. If possible select young leaves that have no defects, like insect attacks and galls. Lichens and other dirt on the leaf surface must be removed. If the leaf is wet, wipe the leaf surface before storing it.
3 Carefully tear or break the leaves with your fingers into smaller pieces and place them in the tea bag. We recommend collecting 4–6 grams of fresh leaf material torn into pieces not exceeding 2 cm2. If possible, try to take several samples.
4 Label the tea bags with the corresponding specimen number by inserting a collector label placed against the wall of the bag so that it is easily legible (Fig. A).
5 Place the tea bags in an airtight container and completely submerged in silica gel until completely dry (Fig. B). This operation has to be done without delay.
6 The container should be shaken frequently over the first day to make sure that silica gel is always in close contact with the plant material. Once the material is completely dry, the teabag can then be removed from the silica gel and placed in an airtight container which has a fresh layer of silica gel at its base (Fig. C) for longer term storage.
7 NOTE: If the specimen has succulent leaves, it is recommended to peel and store only the leaf surface.

Materials needed for DNA sampling

1 Silica gel
2 Empty tea bags
3 Collector labels and marker
4 Small containers for drying
5 Bigger airtight container for long term storage

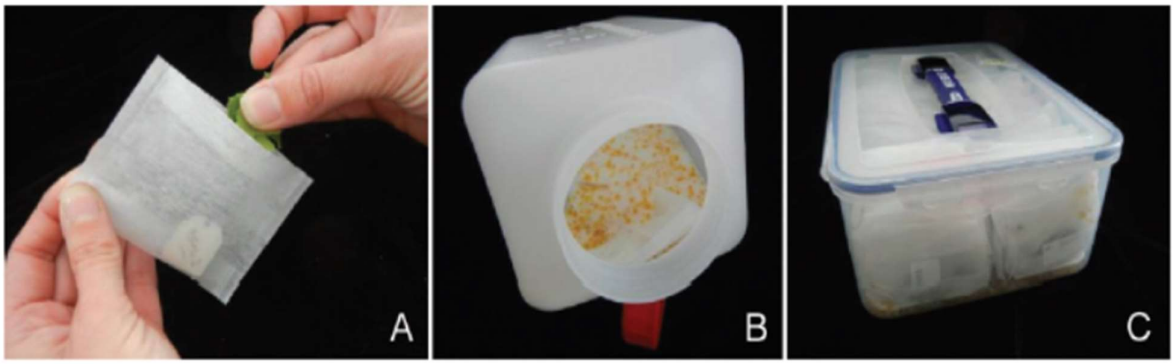


Fig. A. Ripped leaf material placed in teabag with internal paper label. **B.** Teabag submerged in silica gel (regularly shaken). **C.** Longer term storage of teabags in sealable container with layer of silica gel at base. (Photos: P. Wilkie)

18.0 Packaging and Posting Specimens to LAE Herbarium

All collections to be forwarded to the Forest Research Herbarium (LAE) must be packed and posted as follows:

1. Specimens are to be packed into polythene bags and sealed with a heavy duty masking tape. The package must be completely sealed and air tight.

2. The package must be packed in sequence by collection numbers and date of collection.

3. Each package must be label with; cluster number, plot, district, province and team name.

4. Place the package into a carton and sealed off using a heavy duty masking tape.

5. Label the box with a proper forwarding address.

6. Place the field books in an envelop and forward to the FRI Director, attention to the Botanists

19.0 Processing and Storing Plant Samples in the Herbarium

When specimens arrive in the herbarium, these will be registered, processed and dried in the oven dryer for 48 hours or longer if materials are bulky. The specimens will then be identified using the reference collection in the herbarium, field labels typed and place onto each specimen.

All fertile specimens will be mounted and incorporated into the herbarium and duplicates forwarded to other collaborating institutions.

The confirmed list of botanical names for each cluster will be forwarded to NFI/PNGFA HQ for correction and databasing.

Specimens received in the herbarium are to be managed in a way that can be easily tracked and checked when the need arise. The following steps will be followed;

1. Register all incoming collections in the Log Book.

2. Store the incoming specimens in the lab according to the Cluster Numbers

3. Process specimens by Cluster numbers

4. Remove specimens from their package and check for tag numbers,

5. Place single specimen into a newspaper and separate each specimen with a cardboard,

6. Use a strap to tie the specimen and place into the oven dryer.

7. Set the dryer at 60 degrees and dry the specimen for 48 hours,

8. Remove the samples from the oven dryer and remove the cardboards.

9. Identify the specimens and generate a list.

10. Confirm the identification using the reference collection in the herbarium

20.0 Field notes for NFI specimens

All specimens collected must have a field note attached to the material. The field notes are records relating to that specific sample and will include information on habitat, locality, date, plant habit etc Staff working in the herbarium will re-type the information in the field books and attached to the specimens using the tag numbers.

1. After the specimens are dried, locate and place the appropriate field book with the specimens.

2. Type the notes in the field book into a computer and save under the cluster number as a file name.

3. Change the botanical name if different from the identification made in the field.

4. Print four labels to a sheet, photocopy and cut, and place on the specimen.

5. File the specimens by clusters in the storage cabinets.

21.0 Leaf Area Measurements

The area of a leaf is the most common metric for leaf size and is defined as the one-sided or projected area of an individual leaf, expressed in mm^2. Remember that to prepare for the Leaf Area Measurements, the specimen samples have to be dried beforehand at a temperature of 60 degrees Celsius for 24 hours.

To store and catalogue the images install the free license software ImageJ and follow this procedure

1. Before the scanning of the leaves, create a folder for each cluster and store all the scanned images of leaves in the Cluster folder they belong to.

2. Scan the leaves. More leaves for each image are allowed but remove the petiole from all of them and add a ruler in every scan. Note: The background of the image must be withe or clear, the leaves have to be clearly visible.

3. Open ImageJ and follow this pathway: File > Open > Select the picture to analyse.

4. Zoom in the image so that the ruler is clearly visible.

5. Use the “line” tool (fifth button in the console) to select 1 centimetre of length along the ruler and follow this pathway: Analyse > Set Scale > Know distance: 1 > Global: check (this is needed only if all the images have the same size, true when you are scanning all the leaves in the same scanner) > Unit: cm > ok

6. Change the colours of the image for an easier selection: Image > Adjust > Colour Threshold (or Threshold if you want to work with a B/W image)

Note: if “only works with greyscale images” error appears, go to Image > Type > RGB Stacks (you will obtain a B/W image)

7. Use the two levers in the Colour Threshold panel to select only the leaves. To do so, try to keep only the green peak in the colour distribution. Look for the best fit and close the panel.

8. Open the ROI Manager to select the leaf blade: Analyse > Tools > ROI manager.

Use the “magic wand” tool (the eighth button in the console) to select a single leaf on the image, then click “Add” on the ROI manager panel. Repeat for each leaf in the image.

9. Click on “Measure” in the ROI manager panel and save the results in the correct Cluster folder using the field label as name.

Once you have finished the scanning procedure, remember to store the samples by cluster into container and keep them in a cold storage area.
Eventually update the species name after confirmation of the species.

22.0 Processing leaf samples for DNA

When materials for DNA analysis are received in the herbarium the following procedure will be followed:

1. Register all incoming materials into a Log Book

2. Record the following information base on the field label of the specimen: Species name; Specimen Number; Cluster Number; Date, Location;

3. Place sample packages from each cluster into one container and label with cluster number

4. Put containers in a refrigerator and store at 5-10 degrees celsius

5. Enter the information in the Log Book into a database and generate a checklist for the samples collected.

6. Update the checklist every month.

23.0 References

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