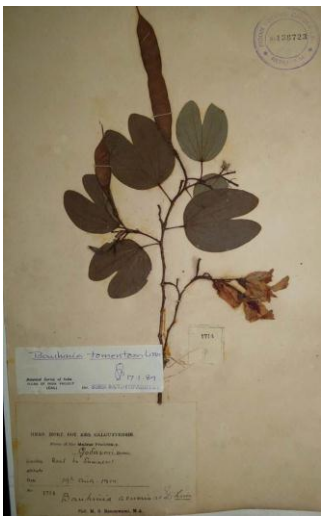
TRAINING COURSE ON HERBARIUM TECHNIQUES AND METHODOLOGY



भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

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Environmental Information System
INDIA

1. INTRODUCTION TO HERBARIUM

A herbarium (plural herbaria) is a storehouse of dried plant specimens collected from far and wide, mounted on appropriate sheets, arranged according to some known system of classification and kept in pigeonholes of steel or wooden cup-boards and are generally associated with botanic gardens and educational or research organizations. The word 'Herbarium' was derived from *Herbar* means plant specimens and *arium* means an artificial place.

Tournefort (1700) used the term *Herbarium* as an equivalent to *Hortus siccus* and Linnaeus also used this term. It was mainly through Linnaean's influence the word *Herbarium* superseded the former term *Hortus siccus,* in Latin literally meaning dry garden.

Lawrence (1951) defines it, as "the arrangement of specimens in the sequence of an accepted classification and the specimens are available for reference or other scientific studies. " Herbarium" used in its original sense referred not to a collection of plants, but to a book about medicinal plants. According to Fosberg and Sachet (1965), a modern herbarium is "a great filing system for information about plants, both primarily in the form of actual specimens and secondary in the form of published information, pictures and recorded notes".

HISTORY OF HERBARIUM

- Luca Ghini (1556) is the sole initiator of the art of herbarium making, who started collecting, drying and pasting them over paper.
- Gherardas Cibo, student of Ghini began collecting plants and preserving them from 1582 and continued till his death.
- In those days, the herbarium sheets were bound into volumes and just like books these herbaria volumes were also arranged vertically as in Libraries.
- However, in 18^{th} Century, Linneaus started a new method in which he mounted his specimens on single sheets and started storing them flat horizontally, which is followed by almost all the museums and herbaria in the world.

OBJECTIVES OF HERBARIUM

- To provide facilities for determination of any material including new taxa.
- To enable preparation of new monographs and floras.
- To preserve specimens of historic importance.
- To assemble data for working out ranges and ecological distribution.

- To bring together in a relatively permanent form of specimens for comparative morphological or phylogenetic studies and
- To provide material for specific research as in plant anatomy, palynology and ethnobotany and also for molecular research.

HERBARIUM SHEET

The general size of the mounting board or herbarium sheet is 42 x 28 cm. The label with the size of 12 x 8 cm is generally pasted on the bottom right hand corner of the mounting board. The specimen is pasted as far as possible in the middle, field number at middle of left margin and accession number and/or barcode at right top of the herbarium sheet.

ROLE OF HERBARIUM IN TEACHING AND RESEARCH

Teaching: The herbarium serves as an aid in teaching botany to degree and post-graduate students. It is difficult for any student to identify local plants without the help of a proper herbarium. Many specimens, which the teacher would like to show to his students, may not be available fresh at the time of giving the course. In such situations, available specimens in the herbarium serve the purpose.

Research: Herbarium, which was considered to be the concern and tool of an "orthodox taxonomist" is totally unanimously believed to be an essential requirement for biosystematic research. For biosystematic studies (including population studies) the worker needs material of his taxon from far and wide. As it is not conveniently possible always to visit different areas of occurrence of the taxon, one has to largely fall back on resources of the herbaria.

For ethnobotanical researches, the herbaria have proved to be very valuable source of information. Many native uses of plants recorded on the herbarium sheets have never gone into published literature and therefore have never been subjected to scientific scrutiny. Herbaria also provide a meeting place for discussions and exchange of ideas among scientists from far and wide.

TYPES OF HERBARIA

Herbarium ranges from small personal collections, mostly of a few hundred specimens to large collections of colleges, universities, private foundations and government agencies.

There are different types of herbaria and they are being used for various activities and generally the following types of herbaria are categorised.

- International herbaria (e.g. Royal Botanic Gardens, Kew)
- National herbaria (e.g. Central National Herbarium, Howrah)

- Regional herbaria (e.g. Andaman and Nicobar Regional Centre, BSI, PortBlair)
- University herbaria (e.g. Calcutta University Herbarium, Kolkata)
- Medicinal plant herbaria (e.g. Central Institute of Medicinal and Aromatic Plants, Lucknow)
- Economically important plant herbaria (e.g. Industrial section Indian Museum, Kolkata)
- Local herbaria (e.g. Malabar Botanical Herbarium, Calicut)
- Agricultural herbaria (e.g. Tamil Nadu Agricultural University Herbarium, Coimbatore)

The contents or holdings in a herbarium vary according to the interest of the organization or institution. The labels and notes on the sheets also slightly vary accordingly a) the herbaria of organizations like Botanical Survey of India contain all collections from any explored area. b) The herbaria of the institution interested in drugs and medicines include plant specimens of known medicinal value or plants that are reported or supposed to have medicinal properties. c) The Herbaria of the universities and colleges generally contain specimens only for teaching and research. d) Weeds of cultivated fields form the contents of the herbaria of Agricultural colleges and universities.

ACRONYM

An acronym (from Greek *acro-* in the sense of *extreme* or *tip* and *onyma* or *name*) in a strict sense is an abbreviation of several words in such a way that the abbreviation itself forms a pronounceable word. But for herbarium, the acronym is an abbreviated form to denote a particular herbarium and it is assigned by the Index Herbariorum (IH), in which each institution is assigned a permanent unique identifier in the form of a one to eight letter code, a practice that dates from the founding of IH in 1935. For example, Central National Herbarium, (Calcutta) Howrah – **CAL**, Madras Herbarium, Coimbatore – **MH**, Royal Botanic Gardens, Kew – **K**, Natural History Museum, London – **BM**.

FUNCTIONS OF HERBARIUM

Herbarium is a conservatory of material and data. The specimens in the herbarium carry valuable data on their labels. Large herbaria have collections from far and wide, and thus, provide at one place, basic material for study of flora and vegetation of different places or regions. The material in the herbarium remains a permanent record of flora of these regions and in certain cases, where catastrophes or other factors have totally destroyed the vegetation

the collections in the herbarium provide evidence of what once existed there. Thus herbaria serve as invaluable conservatory of flora of different parts of this earth. Properly collected plants bear labels with abundant data on habit, habitat, local names, native uses of the plant, abundance or frequency of the species, associated plants etc. Such notes on labels once incorporated on sheets in the herbaria continue to provide data for botanical, ethnobotanical and phytogeographical studies for all times to come. Thus herbaria also serve the valuable function of data banks on plants.

An active herbarium continues to receive fresh material either through the collections of its own staff or through gifts, exchanges, etc. The collections and the data on collection are never static or closed resource; they continue to increase and expand in contents and value. Therefore, the herbarium basically a conservatory of material and data is a "living organism" which continues to grow.

IMPORTANT HERBARIA OF THE WORLD

There are approximately 3,000 herbaria in the world today, as per the data published by the *Index Herbariorum*. Collectively the world's herbaria contain an estimated 350,000,000 specimens that document the earth's vegetation for the past 400 years. A list of the largest herbaria of the world with approximate number of specimens in each is given below.

LOCATION	**ACRONYM(S)**	**NO. OF SPECIMENS**
Museum National d'Historie Naturaelle, Paris	P	8,000,000
Newyork Botanical Garden, New York, USA	NY	7,800,000
V.L. Komarov Botanical Institute Leningrade, Russia	LE	7,200,000
Royal Botanical Gardens, Kew	K	7,000,000
The National Herbarium Nederland (NHN), The Netherlands	L, U, WAG	7,000,000
Missouri Botanical Garden, St. Louis, USA	MO	6,600,000
Conservatorie et Jardin Botaniques de la Ville de Geneve, Geneva, Switzerland	G	6,000,000
Harvard University Herbaria, Cambridge, USA	A, AMES, ECON, FH, GH	6,000,000
Natural History Museum, London	BM	5,200,000

MAJOR HERBARIA IN INDIA

1. The Central National Herbarium (CAL) located at Howrah, established in 1795 and comprises about 2,000,000 (2 million) specimens. This is the first herbarium in the country and one of the most important Asian Herbaria.
2. Forest Research Institute, Dehra Dun contains 350,000 specimens (DD)
3. The National Botanic Gardens, Lucknow contains 260,000 specimens (LWG)
4. Blatter Herbarium, St. Xavier's college, Fort Bombay contains 200,000 specimens. (BLAT)
5. Botanical Survey of India has herbaria attached to their regional centres and units in different parts of India.

No.	CENTRES/ UNITS	LOCATION	DATE OF INITIATION	ACRONYM	NO. OF SPECIMENS
1.	Central National Herbarium	Howrah	1795	CAL	2,000,000
2.	Industrial Section Indian Museum	Kolkata	1887	BSIS	20,000
3.	Southern circle	Coimbatore	1955	MH	2,80,000
4.	Western circle	Pune	1955	BSI	1,75,000
5.	Eastern circle	Shillong	1956	ASSAM	2,60,000
6.	Northern circle	Dehra Dun	1956	BSD	1,10,000
7.	Central circle	Allahabad	1962	BSA	65,000
8.	Andaman and Nicobar circle	Port Blair	1972	PBL	25,000
9.	Arid zone circle	Jodhpur	1972	BSJO	25,000
10	Arunachal Pradesh circle	Itanagar	1977	ARUN	15,000
11.	Sikkim Himalayan circle	Gangtok	1979	BSHC	35,000
12.	Deccan circle	Hyderabad	2005	BSID	10,000

2. COLLECTION

FIELD EQUIPMENTS

Following items are very essential during plant collection trips:

Altimeter,
Axe,
Binoculars,
Camera,
Candles,
Clothes,
Contingency vouchers,
Cutter,
Drying / Blotting sheets,
Field book,
Field shoes,
First Aid Box,
Global Positioning System (GPS),
Ice axe,
Identity cards,
Ink,
Kerosene,
Khurpi,
Knife,
Letter-heads,
Matchbox,
Medicines,
Old newspapers,
Pencils,
Petromax,
Pocket lens,
Polythene bags,
Presses,
Raincoat,
Ropes,
Rubber,
Scale,
Seal and Rubber Stamp,
Service stamps,
Shoulder bags,
Soap,
Specimen tubes,
Stove,
Straps,
Tents,
Torch with batteries,
Tree Pruner,
Utensils,
Vasculum

Fig. 1: Global Positioning System (GPS) instrument

KINDS OF FIELD WORK

Depending upon the purpose of plant collections fieldwork can be broadly classified into three types. 1. *Collection trip*: It is of short duration usually of only one day to nearby place. 2. *Exploration*: It is done for preparing detailed floristic accounts and for study of economic plants of some region and. 3. *Expedition*: It is undertaken to remote and difficult areas and are usually of several months duration.

COLLECTION

There are three ways of collecting the plant specimens in the field.

1. If the trip is for one day, one can carry the plant press and newspapers or blotters. The specimens are pressed then and there in the field.
2. The second method is to keep the collected specimens in metal can called *Vasculum.* Wet newspapers must be placed inside the Vasculum to keep the specimens cool. The plants kept in the Vasculum must be transferred to newspaper that day itself or the Vasculum along with the plants may be kept in a cool place overnight.
3. Now-a-days polythene bags are available and they are of varying sizes. The collected plants are placed inside and the mouth is tied tightly. This is easy to carry and there will be no serious loss of the plant material kept inside. The plants can be pressed after reaching the headquarters.

WHAT TO COLLECT?

What plants shall be collected depends on the purpose of study. If the object of study is the preparation of flora of a region, collection should be exhaustive and samples of all plants of that area should be collected. Collections should contain at least flowers or fruits or preferably both.

In case of grasses and other herbs the whole plant including the underground part should be collected. The size of the herbarium sheet on which the specimens will finally be mounted is approximately 28 x 42 cm and this limits the size of the collected plants or twigs.

Due to various considerations, at least four specimens of each plant are collected; this is done particularly to facilitate distribution and exchange. Of the four specimens, two will be mounted and kept in the herbarium and the rest will be kept as duplicates.

The numbers given to collections are very important record. All the four specimens of the same plant are given the same field number. The number must be attached to the specimen in the field itself and this number will always go with the specimens even if there is a possibility

of change of name at a later date. Collection number refers to even the most valuable specimens like type specimens. A type is an element on which the description associated with the original publication of a name was based.

A very important part of the plant collection work is the recording of field notes. Detailed notes should be entered in the field note-book at the time of collection in the field itself. Generally, the following details should be recorded in the field notebook.

a) Date b) Vernacular name c) Locality d) Habitat e) Description f) Collector's name, etc.

This is the most important part of the plant collection trip and one must remember that the specimen we are going to collect in the field will be used by some persons on a later date. So the following points are to be borne in mind.

1. Do not collect scraps of plants.
2. If the specimen is herbaceous whole plant including the underground parts must be collected or if it is woody, a twig that can easily fit into the herbarium sheet can be collected. It must by 35-40 cm long.
3. Very rare specimens like orchids, insectivorous plants and endangered specimens must be collected sparingly.
4. Collection should be made of the material in all stages of development. If necessary, 2 or 3 trips to the same spot must be made to collect different stages of the specimen.
5. Sometimes bulbous specimens must be collected for planting in the experimental garden.
6. All the areas of the locality must be visited and then only the collection will be complete.

Collection of certain groups like succulents, aquatic plants, aroids large bamboos, and very tall trees require special methods and precautions. Succulent plants like Cacti, *Euphorbia* and members of Crassulaceae present unusual difficulties in making herbarium specimens. Their thick succulent tissues take very long time to dry and so they require special attention. Hence, either the tissues should be killed by dipping in boiling water or excess of tissues removed by hollowing out the thick organs. Treating with alcohol or strong Formalin can also kill the tissue.

While recording the characters of succulents, especially the spiny succulents, details of shape, size and the arrangement of spines and joints should be noted.

Some plants like *Lemna* and *Wolfia* are microscopic and cannot be processed for the herbarium in the usual way. These plants should be collected in mass with the collection number, notes etc., sun dried and put in a packet and the whole packet pasted on the

mounting board. These can also be preserved in any of the liquid preservatives used for embryological or anatomical studies. The common liquid preservative is:

Ethyl alcohol 95%	50 cc
Glacial acetic acid	5 cc
Formaldehyde 40%	5 cc
Water	40 cc

The collection number and place, date etc. should be written on a slip in lead pencil or Indian ink and put inside the jar or pasted on it.

Ferns should be collected with their basal portion, because the shape of rhizome and hairs and scales on rhizome are important taxonomic characters. Fronds without sori are of little value.

Slender aquatic plants, after collection is placed in a tray containing water and is spread out. Then a wire press or sieve plate with which paper or muslin cloth is inserted below the specimen and taken out; the paper or cloth is lifted slowly with both hands and placed between the dryers. Some aquatic lithophytes like the members of Podostemaceae cannot be detached from the rocky substratum and a portion of the rock needs to be broken apart for collecting them. The rock with the plant has to be either dried or preserved in liquid medium.

FIELD NOTE BOOK (FIELD DIARY)

While preparing herbarium specimens close attention should be given to recording all necessary data concerned with the plants, which may not be present or detected after drying

The following points to be noted during plant collection in field notebook:

1. Colouration of foliage and floral parts.
2. Corolla venation.
3. Anther colouration before and after dehiscence.
4. Viscidity of parts.
5. Pollinating agencies.
6. Texture of foliage and perianth parts.
7. Colour and nature of fleshy matured fruit.
8. Habitat.
9. Exact location; use proximate object near the site.
10. Waxed pattern of shoot and root system.
11. Insecticides and repellents.
12. Branching pattern of shoot and root system.
13. Type of soil, moisture content, slope and light conditions.

The notes in the field note must be taken in pencil, if ink is used, it may get smudged when drenched. As far as possible all the points to be noted at the spot itself as postponing or planning to write these notes in the base camp in the evening will lead to confusion or some points might be forgotten.

BOTANICAL SURVEY OF INDIA

Flora of …… State……

Serial …… Date……

Name……

Family……

Local Name……

Habit……Height……..Fl…… Fr……

Locality…….. Alt……

Soil……

Vegetation type……

Associated plants……

Distribution…… Abundance……

Uses……

Significant notes……

……

……

……

Photograph…….. Collector……

Identified by……

Fig. 2: Sample of a page of field-book, used in Botanical Survey of India

3. PROCESSING OF SPECIMENS

The processing of specimens includes a. Poisoning, b. Pressing, c. Drying, d. Mounting, e. Stitching, f. Labelling, g. Identification / Determination of plants, h. Incorporation and i. Arrangement. These are discussed in detail below.

a. Poisoning

The specimens are poisoned either immediately in the camp or after reaching the headquarters. If possible, such as in the case of one-day trips, it is advisable to poison the plants immediately after collection; poisoning kills the plant and thereby the formation of abscission layer is prevented. The poisoning is generally done by dipping the whole plant in a saturated solution of mercuric chloride in ethyl alcohol. The solution is poured in a tray and the specimen is dipped in it with the help of forceps. **Dipping fingers in the solution should be avoided and rubber gloves should be used while poisoning.** All parts of the plant are dipped in the solution and left there for 15-20 seconds, depending up on the thickness of the plants. Mercuric chloride is a deadly poisonous chemical and its effect on human beings is cumulative. Lauryl pentachlorphenate (LPCP) is used in some herbaria as substitute for mercuric chloride and it is reported to be very effective and comparatively safer in handling.

Fig. 3: Poisoning chemicals

Fig. 4: Poisoning of specimens

Recently another method has often been adopted for collecting and poisoning plants during explorations and expeditions involving longer durations. This is called as Formalin method. This method is highly suitable for tropical countries. The collections are spread out in ordinary old newspapers and bundled up. Each bundle is then placed in a large polythene bag. 70% ethyl alcohol with 5 cc of 10% Formalin mixtures is poured over the bundles, so that the bundles just get soaked thoroughly without however having excess of Formalin in the bags. The bags are then tied airtight. No further change of folders is necessary till reaching the headquarters. On

reaching the headquarters, the specimens are spread out for pressing and drying as usual. This method is advantageous in many ways.

1. It saves the labour and time in daily changing and pressing and drying of blotters during the tour.
2. As it saves from carrying large amounts of blotters and presses, reduces the luggage. The old newspapers can be purchased as and when required in any place.
3. Since the tissues of the plants are killed instantaneously by the Formalin fumes, the formation of abscission layer is prevented, thereby preventing detachment of leaves, flowers, fruits and other plant parts.

b. Pressing

Fig. 5: Pressing of specimens

Pressing as for as possible must be carried out in the field itself. It is the process of placing specimens between the absorbents under heavy pressure. Herbaceous specimens should be washed to remove mud from roots. All plant parts such as leaves and flowers etc. are spread out neatly. This needs considerable patience. Some leaves are placed facing up and others facing down to show the characters on both surfaces. This is especially important in case of ferns with sori on the ad axial side. In case of gamopetalous flowers, if possible, one flower should be split open longitudinally and pressed with corolla spread out to show Androecium and Gynoecium. If the specimens are longer than the size of mounting sheet, they can be folded like "V" or "N" or "M" or "W". After gluing and stitching the parts easily stay in position. If there are too many leaves or branches, a few are removed so that there is a little over lapping as possible and all parts are easily visible.

While pressing the specimens should be placed in such a way that there is almost uniform thickness of the bundle in the middle and on sides. This will provide uniform pressure in the press. The main object of pressing is to flatten and dry the specimen. This is done by keeping the straps light and by changing the blotters every day for 6-10 days depending on weather. The plants gradually lose their moisture and finally become completely dry. The used and moist blotters are dried and used again.

Pressing succulents and aquatic plants is not so easy. Succulent plants may be dipped in boiling water and pressed. But this will completely alter the shape of the specimen and details may be modified. The best method is to split the succulent part and remove the fleshy inner contents. Salt may be added to cut surfaces for quick drying. For pressing aquatic plants take a big tray and pour water in it. Place a glass plate or tin sheet inside the water i.e. at the bottom of the tray and put a white paper over the plate. The size of the paper must be slightly bigger than the size of the specimen. Now transfer the aquatic plants to the tray and carefully spread the leaves, branches, etc. The lay of the specimen cannot be altered afterwards. Then carefully lift the glass plate with the paper. The specimen now will sit on the white sheet. Aquatic plants have mucilage with them and so there is no need to apply gum or paste for pasting. The plant will automatically get pasted to the white paper. Place the paper with the plant over newspaper and usually thin cloth piece is spread over the plant. Then place another newspaper over it. This is the way of pressing and pasting algae or aquatic plants. While pressing always place one specimen in each sheet. Don't always place the specimen in the center. This will make the pile uneven. In the bundle of the papers, corrugated boards must be put at regular intervals. This will enhance aeration and hasten drying. This is called *Ventilator*. The sequence of placing plants in a press is ventilator, drier, specimen in specimen paper, drier and ventilator. The press level must be even and so packing may be given wherever necessary. Then apply ropes or straps to tighten the press. Put weight over the board. The drying must be gradual.

Fig. 6A. The wrong method of pressing plants

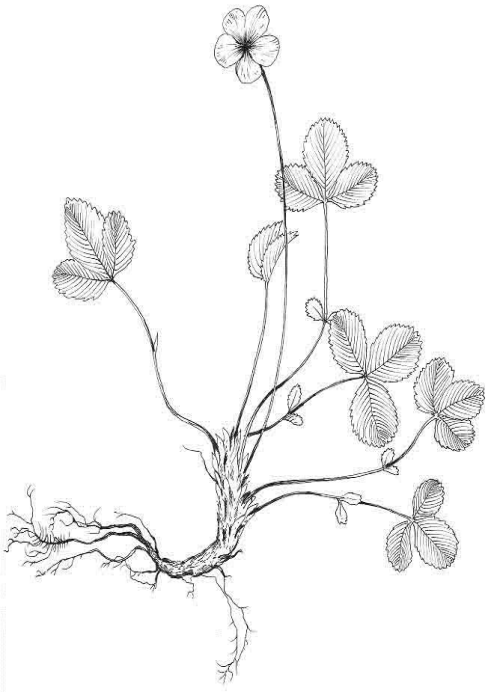


Fig. 6B. The right method of pressing plants

c. Drying

Plants should be placed in the press and be tightened. The faster the drying, greater will be the quality of the dried specimen (Fig.4). The fungal and bacterial attack will be very less or nil. If the drying is quicker, the plant parts will not lose much of their colour. Some students will immerse the plant in Copper Sulphate solution before pressing. This will make the plant appear green even after drying. Plants must be sweated in the field press for 12-24 hrs. Then open the field press and transfer the material to fresh blotters. In the course of 48 hours, the plants must be changed at least 3 times. This will hasten the drying and prevent the withering of plant parts. The wet & used blotters must be sun dried and must be made use for future drying. The sheets must be completely dry and free from mould. If sheets are found to infect, they must be discarded immediately. For one week, there must be daily changes of the sheets. The plants must be carefully transferred.

In temperate places or during rainy season, artificial drying is essential. If artificial heat is used there must be maximum airflow. Double-faced corrugated boards or aluminium ventilators are the best. The specimen should not be dried in an oven. A collapsible drying frame is used in field and camp stove or Hurricane lamp placed below it (Fig.4). In the laboratory "dry box" can be employed. The lower chamber will be having electric heater. The upper chamber will be used for placing the field press with specimens. This is extremely successful and saves labour and time. The quality of dried specimens will be great. There is a very quick method of drying. The plant is placed in between the driers and they are placed over a cloth bundle. Then apply a hot electric iron over the paper. This will hasten the drying. But this is not a proper method because the plant specimens turn brittle and break.

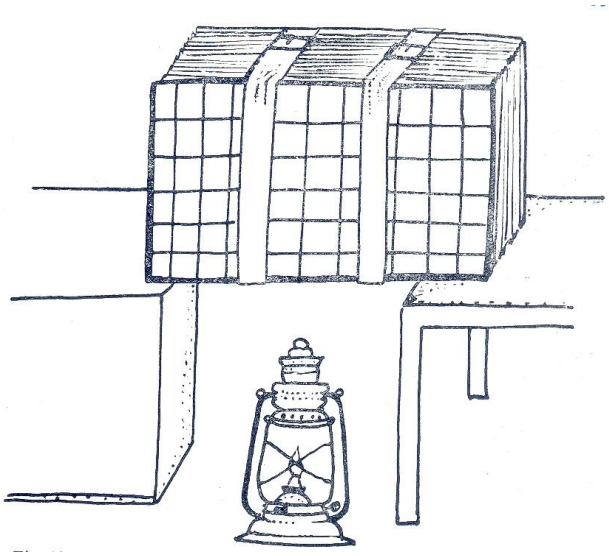


Fig. 7. Drying specimens or blotters on heat of Kerosene lamp

Disadvantages of heating & non-heating processes

No.	WITHOUT HEAT	ARTIFICIAL HEAT
1.	3 or 4 weeks are needed for complete drying.	The glaucescence or waxy bloom of the specimen will be lost.
2.	Blotters must be changed and so large quantity of newspaper is needed.	Very quickly plant becomes brittle.
3.	The wet blotters must be dried for recycling	The natural colour of the plant is lost.
4.	The labour and time expenditure is high.	The corrugated sheets used for the ventilation leave ridge markings on the plant parts.
5.	Fungal and insect attack will be there. Insect larvae present inside the flower may continue eating the floral parts.	-

d. Mounting

After the specimen is pressed, dried and poisoned, it is affixed along with a label on a mounting sheet. The mounting sheets are made from heavy long-lasting white card sheet in uniform size of 28 x 42 cm. The aim of mounting is that the specimens should be neatly and uniformly spread and fixed on the sheet and all parts of the plant should be easily visible for study.

The common technique now in use in our country is pasting specimens to sheet with glue. The common animal glue used for bookbinding are available in market as flakes or pieces is employed. The glue paste is prepared by adding flakes of glue to boiling water, gradually and in small quantities till it makes a thin syrupy paste. To give this glue some insect repellant properly, small quantity of Mercuric Chloride, Thymol crystals or Copper Sulphate (Blue vitriol) is added. The glue or paste is coated on a glass plate or tin plate. The specimen is placed over the paste and tapped gently with forceps. Then with the help of forceps carefully transfer the plant to the mounting sheet in the proper place. Now a day's fevicol also used instead of animal glue fakes. The mounting sheets with specimens glued on them are kept in press for one day for proper sticking and drying.

Fig. 8: Mounting of specimen

e. Stitching

The stitching should be small and independent and thread should not be carried from one stitch to another on the lower side of the mounting sheet. On each side of the stem/ twig a hole is made and a thread is inserted. A knot is put at the back and thread is cut after each knot.

Strapping: In this process, the specimen is not glued to the sheet, but only loosely strapped to the sheet by means of ordinary thread stitches or by some other device such as gummed cloth or paper tapes or by liquid plastic method. There are quick drying liquid plastics available in market. This is an expensive procedure.

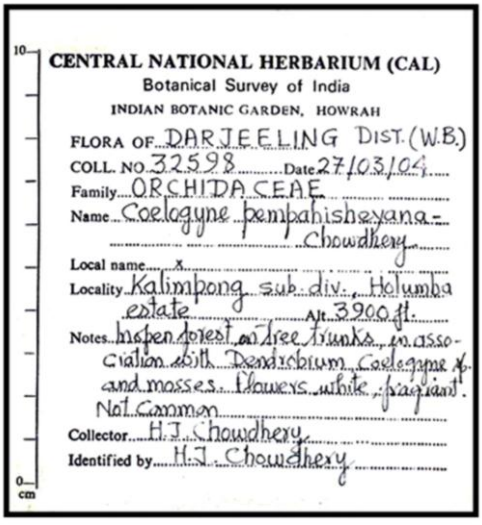
Fig. 9: stitching of specimen

f. Labelling

After the mounting of specimens on the herbarium sheets, the pasting of herbarium labels is done on the sheets. The size and design of herbarium labels slightly vary according to need. Size is about 8 x 12 cm. In general, the label should contain the following data. 1. Name of the family, 2. Name of the genus and species, 3. Locality of collection, 4. Date of collection, 5. Habitat, 6. Collector's name and field number and 7. Vernacular name and local uses.

Herbarium label is fixed on the bottom right hand corner about 1 cm away from edges of the mounting sheet. It should be fixed with paste or glue.

After gluing the specimens and pasting the labels, the data on the labels should be entered clearly with some permanent ink or preferably typed before pasting. Labels are records expected to last for long and should not fade out. A specimen without any label, even if it is neatly and properly mounted, is of little value.



CENTRAL NATIONAL HERBARIUM (CAL)
Botanical Survey of India
INDIAN BOTANIC GARDEN, HOWRAH

FLORA OF DARJEELING DIST. (W.B.)
COLL. NO 32598 Date 27/03/04
Family ORCHIDACEAE
Name Coelogyne pempahisheyana - Chowdhery
Local name x
Locality Kalimpong sub div., Holumba estate Alt. 3900 ft.
Notes Mixed forest on tree trunks, in association with Dendrobium, Coelogyne sp. and mosses. Flowers white, fragrant. Not Common
Collector H.J. Chowdhery
Identified by H.J. Chowdhery

Fig. 10: Model of Label used by Botanical Survey of India

g. Identification / determination of plants

Usually, identification is considered to be the process through which a specimen whose name is not known is recognized by its characters, to be similar to some known plant and accordingly given a name. But it is now felt that since no two individuals plants are exactly identical, this process should not be called identification but determination. That is why, the annotation slips are called and marked "Determinavit" slips. However, the word identification is now so universally employed that it clearly signifies the entire process.

For the purpose of identification, the scientific method is to first study the characters of the plant, check them with the flora of the region, work through the family, genus and species keys and compare with full description and illustration. Thereafter it is to be carefully compared with earlier identified plants of the species or variety, as the case may be.

h. Incorporation

When the specimens are ready (mounted, labeled and identified), they are stamped with a distinctive mark of the herbarium or institution. The stamping is usually done on the top right hand corner of the sheet. This stamp carries the name of the institution, a serial number called the herbarium accession number and sometimes the date of accession. The sheets are listed in the accession register and now the sheets are ready for filing in the herbarium.

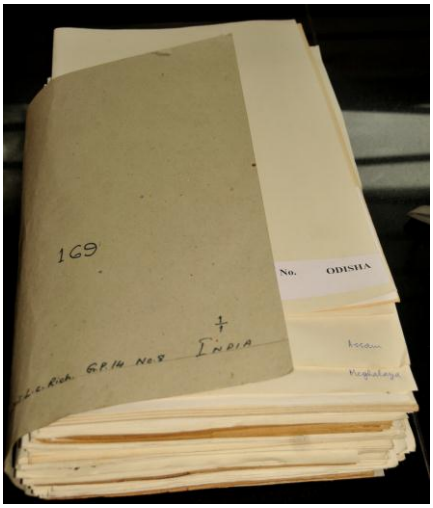


Fig. 11: Genus and Species cover

The mounted, identified and accessioned herbarium sheets are sorted out family, genus and species wise. All the sheets of the same species are filed in lighter covers called the "species cover or folders"; and all the species (with species covers or folders) belonging to one genus are placed in one more folder of heavy paper called the genus cover.

i. Arrangement of specimens

The specimens are usually arranged in the herbarium according to some recognized system of classification. In many Indian herbaria the order and numbering of families and genera is according to Bentham & Hooker's *Genera Plantarum*. In few European herbaria the families are arranged according to the recent classification, APG III (Angiosperm Phylogeny Group). The herbarium sheets are to be arranged in wooden or steel Almirahs with pigeonhole

compartments. The pigeonhole, where bundles of a new family start, is marked by a fixed label or by hinged cardboard separator. The name of the family is printed or written on this in bold letters.

The size of the species cover is usually 32 x 48 cm and that of Genus cover is 40 x 60 cm. Materials are added to the herbarium through one or more of the following means: -

a) Through actual collection by the herbarium staff.

b) By procuring collections of others through purchase, gift or exchange.

c) Sometimes it is agreed that material received for routine identification or expert opinion of specialists in the herbarium will be retained for incorporation.

Fig. 12: Sheets kept in pigeonhole

In the herbarium the most important collection is the type collection. The type specimens such as holotype and isotypes are usually kept separately in safe custody. This is to avoid unnecessary handling.

j. Lending

The specimens received on loan (for study or identification) are the property of other institutions or individuals and need great care. Soon after receipt, they should be counted and checked with the accompanying list. All the specimens received for study must be returned duly annotated by the botanist who examined them and within the period agreed upon.

4. MAINTENANCE

Collector takes lot of trouble in preparing good herbarium sheets. In some cases herbarium sheets alone act as representative of the plant, because all the plants may be missing in that locality. This is the case with the copy specimens recorded by Fyson. So to protect the sheets from fungal and insect pests is an important step. Insects like tobacco or herbarium beetle (*Lasioderma sericorne*) or drugstore beetle (*Stegolium paniceum*) and silver fish are common enemies. They complete their life cycle with the dried specimens. The life cycle period is 70 to 90 days. Unattended herbarium get lose because of these insects. The insecticide kills the insects either by contact or by being eaten. Contact insecticides used in herbaria are Cyanide gas, Paradichloro benzene (PDB), Carbon dioxide or DDT. Digestive poisons are the salts of Mercury and Arsenic. Mercuric chloride is the insecticide.

Care must be taken when new herbarium specimens and collection is brought in. One must check up whether the specimens have been poisoned properly. If a non-poisoned, infected specimen enters the herbarium it may become the source of further infection. The incoming specimens are kept in deep freezer for overnight and taken in. This is possible only with very big herbaria, where incoming specimens are of large quantity and manual poisoning is not possible.

1. FUMIGATION: This is done for killing pests in mounted as well as unmounted duplicate specimens. This process involves any one of the volatile poisonous liquids like methyl bromide, carbon disulphide or carbon tetrachloride. These are placed in small saucers or petridishes in each herbarium case and the case kept closed for about a week. Methyl bromide is used in herbarium of New York Botanical Garden. Sometimes Paradichlorobenzene is placed in all or many of the pigeonholes.
2. HEATING is another method of insect killing. Some herbaria use electric heater instead of fumigation. This requires special insulated herbarium cases with an electric heating element at the bottom. The specimens are placed inside the chamber at 44ºC and left there for few hours.
3. CHEMICAL TREATMENT is another method of insect control. There are two ways of poison action. One is permanently poisoning the specimen and the second is making the material unpalatable. The method of application is either dipping the material in the insecticide before pasting or spraying the chemical after pasting. Mercuric Chloride ($HgCl_2$) and ethyl alcohol is the best mixture, but it is deadly poisonous and should be handled carefully. Further if it is handled by barren hands, the hand may get blackened. Moreover metal forceps and trays should not be used. They get corroded.
4. HANDLING OF SPECIMENS: Sheets with specimens are intended to use by students, scholars and scientists. These specimens must be preserved as long as possible and this depends upon the handling of these sheets. Some of these points are to be borne in mind.

- Keep the sheets always flat.
- Don't shuffle or leaf through a folder like a book or pack of cards.
- Plant materials are brittle and they can break and get damaged, if handling is improper.
- Store the sheets in shelves and don't crowd the shelf.

- Use the folders namely species folders and genus folders carefully and keep the specimens inside when they are not in use.
- Don't put books or heavy articles over the herbarium sheets.
- If the parts of the specimens get detached, store them in small envelope and attach it to the sheet.
- During transport don't tie the bundle of sheets tightly. This may damage the specimen.
- Some students and scholars may try to examine the specimens or dissect the floral parts. They should not be allowed to touch the main material. The reserve materials kept in the small envelope may be used.
- For examination and dissection, dried material must be kept in boiling water and then softening agents must be added. The composition is

  1.6 m (75%) aqueous "Aerosol OT"

  73.4 ml distilled water.

  25 ml methyl alcohol.
- The materials must be placed in a watch glass and the solution is added to it, which makes dissect the specimens easier.
- The herbarium sheet must be placed below long armed dissection microscope during examination. Do not bend the sheet.
- Never write any comments or notes on the sheet. Don't make any corrections without the permission of the in charge.
- Place the sheets back in the shelf only after getting permission from the in charge.
- Dummy folders: As a result of change in nomenclature / taxonomic revision or removal of sheets for repairing or for sending on loan, a dummy folder with necessary notes will make it easy. When anybody tries to trace the sheet, the whereabouts of the sheet can be made out from the notes.

  For example in *Ionidium* write see *Hybanthus*.

Generally, the *Hybanthus* genus is known by its old name *Ionidium*. If anyone search for the specimens of *Ionidium* will not be able to locate because of nomenclatural change. So if the note in a sheet "see *Hybanthus*" in the dummy folder, leads the researcher to *Hybanthus*.

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COLLECTION, PRESERVATION & IDENTIFICATION OF ALGAE

Algae are the simple photosynthetic organisms that lack embryogenesis. Algae are ubiquitous, a multitude of species ranging from microscopic unicellular to gigantic kelps inhabit the widest range of habitats, from pond to ocean, soil to snow and thermal to polar habitats. They play a fundamental role in the world's ecosystems and a reliable and modern introduction to their kaleidoscopic diversity, systematic and phylogeny is indispensable. Blue green algae or Cyanophytes (Myxophyceae) are prokaryotic in nature lacking any membrane bound organelles and all other algal groups are eukaryotic. Algae are systematically classified into various groups basing on their pigment composition and reserve food material. The principal pigments in algae are chlorophyll-a, chlorophyll-b, β-carotene, xanthophylls and phycobilins. Starch is the important reserve food in all groups of algae. Other associated reserve products are Laminarin, Mannitol, fats and oils. Reproduction in algae is by vegetative, asexual and sexual modes. Algae reproduce vegetatively by fragmentation, fission and akinete or hormogonia production. Asexual reproduction is by means of formation of zoospore, aplanospore, hypnospore, autospore, auxospore, tetraspore, cyst, etc. Sexual reproduction is by union of cytoplasm and nuclear material of two gamates of two organisms of the same species.

Taxonomically, algae are classified in to 11 classes pertaining to Cyanophyceae, Xanthophyceae, Cryptophyceae, Chrysophyceae, Dinophyceae, Bacillariophyceae, Euglenophyceae, Cllorophyceae, Chloromonadineae, Phaeophyceae and Rhodophyceae (Fritsch, 1935, 1945). In India, 7309 algal taxa are recorded and described so far belonging to 741 genera and 206 families, which is about 15.29 % of the total plant wealth of the country. Unlike other plant groups, algae show a habitat specificity in their mode of occurrences, which is inferred by the restriction of certain organisms to their specific habitats. Thus the algal diversity is presented here in three broad categories, i.e. Freshwater algae, marine algae and terrestrial algae. Freshwater taxa are the dominant ones in the total algal biota of the country (approax. 67 %), followed by marine (22 %) and terrestrial (11 %) taxa.

FRESHWATER ALGAE

Freshwater composes of about 3.9 % of global water. Freshwater environments can be grouped into stagnant waters (lentic systems – including ponds, lakes, marshes, and other enclosed water bodies) and flowing waters (lotic systems – rivers, streams, canals and waterfalls). The phyco-biotic components in both ecosystems are regulated by nutrients and carbon availability and limnological parameters. Sometimes eutrophic conditions in different

lentic water bodies instigate a thick layer blue green algal biomass on the surface of the water body, called as 'bloom', which has several adverse environmental impacts. The freshwater algal populations are belonging to all groups of algae other than Phaeophyceae and Rhodophyceae, though a few freshwater red algal forms were also recorded. The freshwater algal forms are either planktonic or attached (epiphytic, epilithic, epizoic) or benthic in their occurrences.

Freshwater habitats contributed more than 60% of algal population of India. Among them the green algae (Chlorophyceae) are the most diverse, grow with different morphological forms viz., unicellular, colonial and filamentous. Many conjugating green algae, i.e. desmids, are important ecological indicators of the trophic status of the water bodies. Except a few marine forms, most of the euglenoids (Euglenophyceae) are confined to freshwater habitats. Yellow-green algae (Xanthophyceae) and silica bearing algae viz., Bacillariophyceae, Dinophyceae and Chrysophyceae, are also important components of this ecosystem.

MARINE ALGAE

India has a long coast line of about 7200 km, which enhouses several groups of marine algae including microscopic phytoplankton and macroscopic Seaweeds that live on seashores, rock pools, shallow waters of seas, etc. Seaweeds have identifying features like basal disk, called a holdfast, and a frond of varying length, shape and colour which often resembles a plant in having stem-like and leaf-like parts. They mostly belong to Brown (Phaeophyceae), Red (Rhodophyceae) and Green (Chlorophyceae) algae. Brown seaweeds are usually large, and ranges from the giant kelp to smaller species. Red seaweeds generally range from a few centimeters to about a meter in length; however, red seaweeds are not always red: they are sometimes purple, even brownish red, Green seaweeds are also small, with a similar size range to the red seaweeds. Monsoon and post monsoon seasons influence seaweeds composition and abundance.

In India, a total of 844 species of seaweeds, belonging to 217 genera have been reported so far. These seaweeds comprise of 434 species of Rhodophyceae, belonging to 136 genera, 216 species of Chlorophyceae, belonging to 43 genera, 191 species of Phaeophyceae, belonging to 37 genera and 3 species of Xanthophyceae. Seaweeds are at the base of the marine food chain. The monitoring of seaweeds is important while assessing the environmental status biodiversity of marine ecosystems, as the marine ecosystem supports to survive a large number of flora and fauna.

TERRESTRIAL ALGAE

Algal colonization is also found on different aero-terrestrial habitats like stone surfaces, soil crusts, building facades and tree barks. The algal mat on such habitats composed mostly of coccal or filamentous blue-green algae as well as filamentous green algal members of Trentepohliaceae and Vaucheriaceae, forming green, blue-green, blackish and sometimes coloured patches. They exhibit several desiccation tolerance mechanisms like secretion of exopolysaccharides, which help them in adherence and sustaining in such extreme conditions. Most important feature in such habitats is the water availability which is supplemented due to rain or atmospheric moisture. Nutrients to such habitats are transported by rainwater, aerosols and dust or soil particles.

Terrestrial algae with their microbial biocoenoses like bacteria and fungi are sometimes involved in weathering of their substratum. But in other hand the cyanobacterial components are a rich source of UV sun screen pigments like Scytonemin and MAAs (Mycosporine like Amino Acids). The members of Trentepohliaceae also develop a large amount of carotenoid pigments, which play a key role in protection from high irradiance.

FIELD & LABORATORY TECHNIQUES

Collection

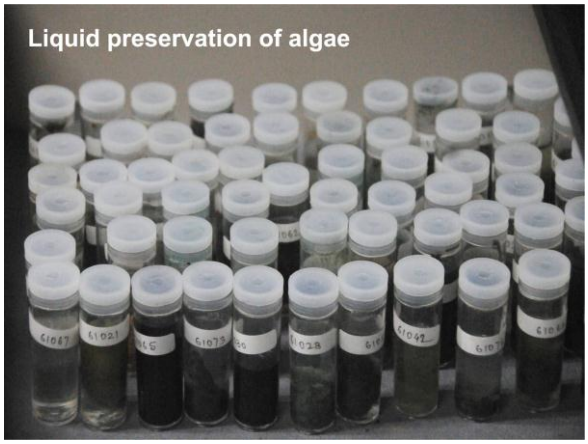
In freshwater and marine habitats, algae usually grow in consortium which may include many species and genera. But, in terrestrial habitats like moist soil, tree barks, damp walls, they are collected with a scalpel and picked up with the help of a forceps or can be scrubbed by a tooth brush. In aquatic bodies, planktonic algae are collected with the help of plankton net of various pore size ranging from 10 – 45 μm. The attached algal forms on rock (epilithic), on submerged plants (epiphytic), on sand (epipsammic), on aquatic animals (epizoic) were collected by thorough picking of them from their substratum by forceps or by scrapping with the help of tooth brush.

In specimen tubes the algae are not filled more than their quarter of their capacity. To avoid deterioration remove the cap immediately after return from the collection site, so that they may be well aerated. Apart from collecting algae, submerged twigs, stones and dead scum of aquatics are also collected to study their association with the substratum. Detail field note must be recorded for each and every sample.

Preservation

Liquid preservation -

For preservation of algae 4% of formalin is used and 2 – 3 drops of 5% glycerin should be added to minimize the evaporation. Collection preserved in liquids may become dry over a period of time. Such dried out collections cannot be restored by adding more liquids. The glycerin prevents complete drying out of the collections and enables one to add more liquid periodically and thus save the material for a long time.



Formalin – acetic acid – ethyl alcohol (FAA): {50ml of 95% ethyl alcohol, 5ml of glacial acetic acid, 10ml of 40% formalin and 35ml of water} also be used for preservation.

Dry preservation (Herbarium) –

Material required for preparing herbarium: Plastic trays, Forceps, Specimen mounting paper (herbarium sheets), Cheese cloth, Blotting paper, Herbarium wooden press, Painting brush, Pencils, knife, Poly-bags.

Procedure for preparing herbarium –

- Fresh specimen should be clean from sand particles, rocks, shells, mud and other adhering materials and epiphytes.
- A tray containing fresh water (half filled) should be taken and specimen to be mounted be placed in the water.
- A herbarium sheet, size smaller than the tray to be inserted from below the specimen and then spread the specimen on the herbarium sheet with the help of brush in such a way that overlapping of the specimen is minimized.
- After mounting the specimen on the herbarium sheet, sheet is lifted slowly and tilted to one side to allow water to drain gradually without disturbing the mounted specimen.
- Remove the sheet and properly arrange the specimen with the help of forceps or needle if required.
- To blot dry, herbarium sheets are placed on the newspaper sheets or blotting paper to remove the remaining water from the herbarium sheets.

- A cheese cloth is placed on the top of the specimen in such a way that it covers entire specimen.
- A blotting paper is placed over the herbarium sheet for making dry.
- Once, all the specimen to be mounted are ready, herbaria are piled one above the other and then placed between the two sheets of the wooden press. The press is tied tightly with appropriate pressure by a rope.
- The press is kept at room temperature for 24 hrs. After 24 hours, blotting papers are required to be replaced. The process of replacing blotting papers is repeated till the time specimen is free of moisture.
- On drying, the specimens get attached to the paper due to the phycocolloid present in the seaweed.
- The cheese cloth is carefully removed and herbarium sheet is properly labelled containing collection number, name of the specimen, locality, date of collection and other ecological details.
- Sometimes, specimens are thick and do not stick to herbarium sheets. In such cases gum or glue may be used to stick the specimen or specimen may be tied with thread.
- Prepare three to four more sheets of each specimen. Sheets can be placed in the polyethylene bags and sealed and stored.

Algal herbarium at CNH, Howrah (CAL)

The algal herbarium at Central National Herbarium (CAL), Botanical Survey of India, Howrah has a significant collection of 986 herbarium specimens belonging to 249 genera under 115 families spread over seven classes viz., Chlorophyceae, Phaeophyceae, Rhodophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae. Most of

these were collected by European workers viz., L. Rabenhorst, J.B. Wilson, D. Prain, J. Schiller, A. Hansgirg, H.S. Kurz, G. Thuret, G.O. Allen and S. Stockmeyer from different areas of Europe, India, Australia and Hawaiian Islands. Several specimens of algae of other countries were also received through gift or on exchange basis from time to time by CAL. These also include collections of Indian botanists like K.P. Biswas and K.S. Srinivasan, made from different parts of India.

Identification

The colour, shape and size of vegetative cell, nature of cell, nature and number of chloroplast, number and length of flagella, nature of cell wall, pellicle, number of nucleus and pyrenoid, position of canal and reservoir, striae, type of branch, food reserve, ornamentation and reproduction are the taxonomic parameters which are employed in the identification of algae. Diatom frustules should be thoroughly cleaned before microscopic observation and identification.

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SURVEY, COLLECTION, IDENTIFICATION AND PRESERVATION OF WILD MUSHROOMS

Introduction: Fungi are represented by *ca* 6 phyla, 36 classes, 140 orders, 560 families, 8600 genera and nearly 10,5000 species in the world whereas, in India, there is a representation of *ca* 5 phyla, 117 orders, 378 families, 2660 genera and *ca* 14,800 species. Fungi whose fructifications are either visible through naked eye or most of the morphological features can be studied without a hand lens are called as macrofungi and commonly as mushrooms. Mainly, they are divided into two broad groups, a) Basidiomycota, the spore droppers: spores are borne on specialized reproductive cells called basidia. After reaching at maturity spores fall from the basidia and disseminated by the wind, b) Ascomycota, the spore shooters: spores are produced inside specialized reproductive cells called asci. After maturity spores are shot out through the tip of the asci. Present communication will be restricted on fleshy wild mushrooms belonging to Basidiomycota.

Season & Habitat: Fungi prefer moist areas for their growth and development. Seasonal variations amongst wild mushrooms are quite common. Though some of them are found during winter mainly, mushrooms become available naturally since the onset of spring. Summer is not the favorable season, but the damper areas may be searched for some species. At the onset of monsoon with optimum humidity, temperature and day-length brings a flush of fruiting bodies. Searching reaches its climax during July–September when most of the mushrooms appear with their fruiting bodies. Woodlands especially the mixed forests (broad-leaf and coniferous) are worth surveying for ectomycorrhizal taxa. The edge of the woods i.e. road-side are often more productive than the inner areas with profuse under-growth. Grasslands rich with organic substances are often appear to be productive for the saprophytic taxa. Tree-trunk of living trees and fallen decaying trunks are found to be infected by the wood-inhabiting or parasitic mushrooms.

Survey and Collection: To make the hunting successful, macrofungal exploration should be undertaken in systematic, methodical and careful manner. Forays undertaken throughout the year is highly appreciated to cover the mushrooms of all seasonal variants. Workout from the fresh fructifications (which is a tedious job) should be undertaken by the same day in which they are gathered, therefore, one should not attempt for collecting too many samples on a day. During collection, specimens showing all stages of development should be selected. Specimens should be carefully dug up or separated from the substrate and placed them in paper bags which are placed in airy basket. Small samples may be collected in chambered boxes. Names of associated plants (if any) should be noted. Altitude and co-ordinate for

every sample should be recorded to know their proper distribution. Good photographs of the specimens and their habitat should be taken before collecting the samples. Therefore, one should be equipped with at least the following accessories while hunting, 1) airy basket to carry macrofungi, 2) knife, 3) digger, 4) magnifying glass (hand lens × 10), 5) good camera and tripod, 6) paper packets, 7) walking stick to search, 8) note book, 9) pen & pencil, 10) altimeter, and 11) GPS system.

Fig. 1: Collection and photography in the field

Macromorphological characterization: After collecting, macrofungi should be brought at the base camp where their characterizations are undertaken systematically. Thorough characterization helps to identify a sample in due course. General stature of the fruiting body should be noted. Photographs and line drawings showing shape and relative size is helpful in this regard. Following field/ macromorphological characters should be observed.

Cap (Pileus)—Size (diameter), shape, surface, colour, texture, margin and context should be noted. Diameter of the cap of young to mature fruiting bodies should be measured in millimeters. General shape may be conic (small width compared to height), campanulate (margin flaring out and apex not sharply pointed), convex (like inverted bowl), plane (flat), uplifted (with upturned margin), umbonate (with a central blunt protrusion), papillate (with sharply delineated umbo giving breast-shaped appearance), depressed (with central depression), infundibuliform (with deep depression giving the appearance of a funnel). Cap margin is often very characteristic; may be inrolled (slightly rolled inward and directed towards gills), decurved (parallel or directed towards stipe), plane (more or less perpendicular to stipe), upturned (directed upwards), entire (giving appearance of perfect circle without any waves), crenate (wavy with regular interruptions), crisped (finely wavy), undulating (broadly wavy), eroded (with irregular interruptions), appendiculate (with irregular patches of remnant of partial veil). Margin surface may be translucent-striate (gills are visible through the top of the wet pileus), striate (with lines that are the part of the cap), sulcate (lines forming irregular grooves), tuberculately striate (with small bumps on the striae). Cap surface also bears

important features. Depending on the wetness, pileal surface (pileipellis) may be dry, glutinous (consistency like liquid glue or jelly-like), viscid (sticky when wet). Surface texture are often with different patterns: smooth (without any cracks, wrinkles or pits), scrobiculate (with shallow depressions or pits), rimose (split), areolate (with irregular splits resulting in numerous block-like areas), rivulose or corrugate (wrinkles gives lines), rugulose (with fine wrinkles). Sometimes, hyphae on the cap surface arranged in different ways to give different appearances. Surface may be glabrous (bald or naked), pruinose (covered with powder like structures), appressed fibrillose (with flattened fibrils), floccose (with fibrils giving appearance of cotton flannel), tomentose (with densely matted and wooly fibrils), velutinous (with short and fine hairs), pubescent (with short weak hairs), hirsute (with stiff hairs), squamose (with scales that are produced by the agglutination of fibrils), scabrous (rough due to presence of large scales). Features of context of pileus should be noted in terms of colour, colour changes, thickness, consistency and taste.

Fig. 2: Morphological characterization of wild mushrooms in the basecamp

Gills (lamellae)—Attachment to the stipe, spacing, colour, colour changes, etc. should be recorded for the gills. Gills may be attached to the stipe in various ways namely, adnate (attached with the full width), adnexed (narrowly attached only with the stipe apex), sinuate (curving upwards abruptly towards the juncture of the stipe as if a notch has been taken out), decurrent (running down the stem), subdecurrent (running a short distance down the stem), free (not attached to the stipe). Spacing of gills also provides valuable clue for the identification. Gills may be crowded (so close that spaces between them are not visible), close (slightly open than crowded situation), subdistant (more open than close situation), distant (spaced quite far apart). Colour of the young and mature gills should be noted. Colour changes (if any) after bruising, should also be observed. Important features are also seen at gill edge. Edge may be entire (uninterrupted), fimbriate (minutely torn), serrate (with long fringes appearing as toothed), crenate (regularly wavy), eroded (irregularly wavy with torn

waves). Number of series of lamellulae (growing along with the gills but, not reaching up to stipe) present along with gills should also be recorded.

Stem (Stipe)—Size, shape, texture, colour, context, etc. provides important features. Length and width (from the thickest portion) should be measured. Attachment of the cap is also important. Attachment may be central, lateral (attached in the margin of the pileus) or excentric (intermediate between central and lateral). Stipe are attached to the substrate by a mass of hyphae called rhizoids. The base may be rhizoidal (with large hyphae being distinct from one another), strigose (with large bristle like hyphae), rhizomorph (cord like elastic strand). The shape of the stipe should be noted. It may be equal or cylindrical (apex to base are of same diameter), tapered (becoming narrower towards apex or base), clavate (becoming club-shaped towards apex or base), subclavate (intermediate between cylindrical and clavate), bulbous (thickened like a bulb only at base). Surface may be modified in different ways. It may be glandular-dotted (with coloured spots), scabrous (roughened with erect pointed scales), reticulate (lines or fibrils forming net like pattern), longitudinal-striate (with long parallel lines), rugulose (striations interconnected forming wrinkles), rugose (coarsely wrinkled), veined (wrinkles with round edges). Colour of the stipe of young and old fruiting bodies (before and after bruising) should be observed. Consistancy of stipe is very characteristic. It may be cartilaginous, fibrous, woody, corky, leathery or chalky. Context of the stipe may be solid (closely packed with hyphae), hollow (with empty centre), stuffed (intermediate between solid and hollow). Partial veil that covers the gill-cavity at the button stage, generally left as remnant on the stipe as ring of tissue called annulus (ring). Position of annulus is to be observed. It may be superior (locating at the upper half of stipe), inferior (locating at the button half of stipe) or central (placed at middle). Another important character is the type of the volva (remnant of universal veil at stipe base). Volva may be free, adherent (attached to the stipe base and difficult to remove), saccate (as loose bag around the stipe base), membranous saccate (remains attached as membrane with the stipe base).

Latex—Some mushrooms (milkcaps) as for example *Lactarius, Lactifluus* contain latex that can be seen if gills are cut or even context are exposed. Colour and taste of latex should be recorded. Any colour change of latex in due course should be noted.

Taste and smell— Taste is also very important for several macrofungi like *Russula*. Taste of the context and the gills should be taken through the tip of the tongue and should be spit out quickly and wash out the mouth with water. Several macrofungi have their characteristic smell, for example *Russula fragrans* has characteristic smell of bitter almond. Therefore, smell for every mushroom should be recorded.

Macrochemical tests—Several chemicals are useful in establishing identification of certain macrofungi. Chemicals like $FeSO_4$ (solution or crystal), Guaiac (saturated solution), KOH (aqueous solution), diluted KOH soln. are mainly used to apply directly on the context.

Growth habit—Growth habit may be solitary (growing alone), scattered (grouped one or two feet apart), gregarious (grouped close together), caespitose (growing in extremely close association).

Spore print—Colour of the spore print is another identification-tool. To take spore print, caps are cut from the stipe apex and are placed on the pieces of white and black papers or transparent glass-slides. The cap and the paper may be covered with upturned glass. Entire set up is now kept for overnight to get the spore prints on the pieces of papers.

Apart from the gilled macrofungi (Agarics or Chanterelles), there are Boletes where spores are produced inside the tubes. Their macromorphological characters are partly similar to the gilled macrofungi. But, the spore-producing layer, the hymenium lines inside of each tube. Tubes open through pores. For characterization of these macrofungi features like tube-length, pore size, colour and colour changes of tubes and pore surface shoul be recorded. Hedgehog fungi are the other group to work with. Here the gills are replaced by the spines. Here spine characters should be noted. Few other groups are puff-balls (and allies) and stinckhorns. Puff-balls are mainly globose, pear-shaped. Stalk, spore-mass (gleba) and peridium are characterized. Similarly for stinkhorn, features of egg (immature fruiting body), receptacle and stalk should be noted.

Micromorphological characterization: Like the macromorphological characters, micromorphological features play very important role for the identification of mushrooms. Fructifications are mainly consisting of hyphae. Hyphal nature from the different parts provides numerous features that in turn help to identify respective macrofungi. Three main structures of fructifications (cap, hymenophore (tubes)/ gill and stipe) have surface layers as well as internal tissue called trama. These structures should be thoroughly observed. Similarly spores produced by the hymenophore provide important characteristics. For microscopic study following equipments are needed, 1) thin microscopic slides, 2) thin cover glass, 3) single- or double-edged razor blades, 4) dissecting needles, 5) forceps, 6) tissue paper (for cleaning), 7) dropper bottles and dropper, 8) immersion oil, 9) eyepiece and stage micrometer, 10) chemical reagents, and 11) good light microscope fitted with a drawing tube.

Surface of the cap (pileipellis)—Microscopic nature of pileipellis/ cap cuticle varies greatly in terms of the size, shape and patterns of hyphal arrangement. Shape of the hyphae varies from cylindrical (filamentous) to greatly inflated or spherical. Hyphal arrangement may be

parallel to perpendicular to the cap. Based on the shape and hyphal arrangement nature of pileipellis may be cutis (hyphae arranged more or less parallel to the cap cuticle and thin-walled), ixocutis (like cutis but, embedded in a gelatinous matrix), trichoderm (hyphae ascending, mainly oblique or perpendicular to the cap cuticle), ixotrichoderm (like trichoderm but, embedded in gelatinous matrix), lamprotrichoderm (trichoderm with thick-walled ascending elements), epithelium (pseudoparenchymatic structure, upper layer of isodiametriv cells), hyphoepithelium (with upper layer of repent septate or branched elements arising from multicellular isodiametric cells), palisade (upper layer of elongated elemented with an underlying compact layer of isodiametric cells) hymeniderm (a palisade where terminal cells are clavate), trichopalisade (a trichoderm where parts of ascending hyphae are inflated to rounded), etc. Hyphal wall is also important. Wall may be thin or thick, smooth or encrusted (with incrustations).

Trama of the cap and stipe—Trama of the cap is continuous with the trama of stipe and closely associated with gill-trama. Mainly the hyphae in trama are interwoven and branched. Hyphae may be cylindrical to inflated. They grades gradually to the cuticle. Trama sometimes bears specialized cells or hyphae, like sphaerocytes (bunch of inflated cells amongst cylindrical hyphae), oleiferous hyphae (with oil-like contents), gloeoperous hyphae (with resinous or granular contents), lactiferous hyphae (with latex). Their presence or absence is quite helpful in separating genera or groups.

Surface of the stipe (stipitipellis)—Although the nature of stipitipellis are not very helpful, studying the shape and pattern of hyphal arrangement, it provides sometimes valuable information. Often the hyphae are like that of pileipellis. Any cystidia found here are called as caulocystidia. Stipe surface of *Leccinum*, bears clusters of caulocystidia of different sizes and shapes.

Trama of the gills (hymenophore)—Hyphae of the gill trama grow downward from the cap trama to the gill edge. Hyphal arrangement in gill trama are mainly parallel to subparallel (running parallel to one another or slightly interwined), interwoven (intricately entangled), divergent (while running down toward to edge hyphae diverging out in oblique fashion), convergent (while running downward to edge hyphae converging towards the centre of trama).

Hymenium—The spore producing layer of the gills (hymenophore) composed of end cells of hyphal system of fruiting body. Three types of cells are found in the hymenium, namely, basidia, basidioles and cystidia. Basidia are the sexual spore producing cells. Mainly they are clavate to subclavate. While studying basidia, their size, shape, number of sterigmata and

colour should be noted. Basidioles (aborted basidia) are less important for identification and more or less similar in shape to basidia. Some species of macrofungi also bear cystidia along with basidia and basidioles. Cystidia are the sterile cells and usually morphologically distinct from basidia and mostly projected beyond them. Based on the position, two types of cystidia are found on gills a) pleurocystidia (produced on the faces of the gills) and b) cheilocystidia (produced on the edges of the gills). Based on the morphology, function, origin, content, etc. they may be leptocystidia (thin-walled, longer than basidia), lamprocystidia (thick-walled), metuloid (a lamprocystidia which is with round or variable shaped apex), gloeocystidia (staining readily with chemical reagents and with granular or amorphous contents), pseudocystidia and macrocystidia (originated from trama and mostly projected beyond the hymenium or the cuticle of pileus and stipe), chrysocystidia (with contents that turns yellow in aqueous alkali solution), broom cells (with the apex that have numerous pointed protuberances). Shapes of the cystidia are often characteristic. They may be capitate (with distinct swelling or protuberance at the apex), filiform (thread-like), mucronate (with short abrupt pointed tip), moniliform (beads arranged in chain), ventricose (broadest in the middle and tapering at apex and base), vesiculate (entire cell is inflated, sac-like and base is abruptly tapered), appendiculate (with appendages), etc.

Clamp connections—Special type of semicircular hyphal branch that is laterally attached to walls of two adjoining cells of hyphae and arches over the septum between them. Clamp connections are found in some groups of macrofungi. So, presence or absence of clamp connection should be checked.

Basidiospores—The features of spores are very important to characterize macrofungi. For the microscopic study, spores are taken from the spore print and mounted in water, KOH, Melzer's reagent, Cotton Blue or Cresyl Blue and seen under microscope. Most widely used mounting medium for spores is Melzer's reagent. Based on the reaction with Melzer's reagent, spores may be amyloid (spores or the ornamentations turning blue or black), inamyloid (not reacting) or dextrinoid (spores or the ornamentations turning reddish or purplish brown). Mostly spores are measured including ornamentations. But, for some groups ornamentations are measured separately and therefore spore measurement excludes the measurement of ornamentations. Spore measurements are recorded based on that of twenty basidiospores. Spores are measured in side view and sizes are given as KDa-KDc-KDb x KDx-KDz-KDy in which KDa = minimum value for the length of measured collections, KDb = maximum value for the length of measured collections, KDc = mean value for the length of measured collections and KDx = minimum value for the width of measured collections, KDy

= maximum value for the width of measured collections, KDz = mean value for the width of the measured collections. Quotient of spore indicates lenght-width ratio (Q = L/W) and is presented here as Qa-Qc-Qb where Qa = minimum quotient value amongst measured collections, Qb = maximum quotient value amongst measured collections, Qc = mean quotient value amongst measured collections. Based on the quotient value spore-shape can be defined as globose (Q = 1.00 – 1.05), subglobose (Q = 1.05 – 1.15), broadly ellipsoid (Q = 1.15 – 1.30), ellipsoid (1.30 – 1.60), elongate (1.60 – 2.00), cylindric (2.00 – 3.00). Apart from these shapes spores may be angular, allantoids (sausage-shaped), stellate (star shaped) or nodulose (with large nod-like outgrowths). Spores may be equilateral and inequilateral. In both the cases shapes are very important. Surface of many spores are smooth, however, some spore-surface are covered with ornamentations. Size, shape and pattern of spore-ornamentations are observed for spores of different macrofungi. Spore-ornamentations are often very characteristic for different macrofungi. Based on the ornamentation pattern, spore may be verrucose (with minute warts-like ornamentations), tuberculate (with knob-like ornamentations), striate (with radiating lines or furrows), reticulate (with lines or veins or ridges that are interconnected to form net-like ornamentations), winged (with wing like high ridges), punctuate (with very small dots), echinulate (with small finely pointed spines), calyptrate (with outer layer of spore wall that are loosening to form wing like folds).

Fig. 2: Drying mushrooms in wooden drier

Drying of specimens: Drying is the best method that helps in long-term preservation. Sun-drying is easy and economical but, not always possible in reality as mushrooms are mainly collected in the rainy season when rainfall takes place incessantly and continuously for couple of days even. So, for fleshy mushrooms artificial drying with the help of hot air blower or metallic field drier where high-power electric bulb or kerosene stove may be used for drying.

Labelling, packing and preservation: Dried materials are kept in stout, good quality paper packets (6″ × 4″) to preserve the mushrooms. Once the macro-morphological characters are recorded from the fresh samples they are allowed to dry with the help of a field drier (where specimens are placed on shelves of fine wire meshes that are arranged in tires and a mild heat source is placed at the bottom of the drier) or with the help of sun-drying. The specimens slowly become dry with the gradual removal of moisture. Dry samples are used for further study (micromorphological characterization). Dry samples are packed in a sealed bag to prevent the entry of moisture further. Once the specimens are completely dried, they are kept in the paper envelops which are placed in air tight container/ bag. To keep safe from the attack of insect or beetles, naphthalene balls are placed along with the specimens. Finally they are properly labeled and placed in the herbarium with indicator Silica gel to avoid the moisture.

Fig. 3: Preservation of identified materials in the herbarium

Free hand sections of dried samples are used to observe micromorphological features. Transverse-sections through pileus and gills are used to see hymenial characters; radial sections through pileus are used to see the nature of pileipellis (pileus cuticle); radial- and cross-sections of stipe are used to study the nature of stipitipellis (stipe cuticle).

Identification and documentation: Finally, identification of the specimens are undertaken based on the field data, macromorphological characters, photographs, micromorphological characters and thorough survey of relevant literature by placing them in proper systematic position. Once the specimens are identified they are documented in research communications or reports to the funding authority or in books with their supporting data.

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COLLECTION, PRESERVATION AND STORAGE OF BRYOPHYTES

Bryophytes grow on a variety of substrates. They may grow on soil (terricolous), on bare rock surface (saxicolous), on decaying logs or leaf litter (lignicolous), on the bark of vascular plants (corticolous), or on the surface of living leaves (foliicolous).

Collection

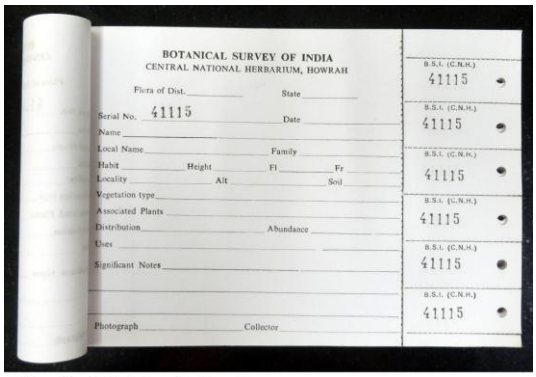
For the collection of bryophytes, some basic collecting equipment is required. These are as follows:

a) A sharp knife,
b) Small scissors (for foliicolous specimens),
c) Small hand lens (10X or 20X),
d) Paper bags,
e) Field note book,
f) Pen with water proof ink or pencil,
g) GPS and altimeter

Fig. 1. A hand lens

Collection method depends on the substratum on which the plants are growing. If they are growing in loose tufts or mats on loose soil, then they can be picked up by hand. If they grow closely appressed to the substratum, especially rock or bark, then they are collected with the help of a knife along with a small portion of the substrate. In case of foliicolous specimens, the leaves on which the bryophytes are growing are collected with the help of scissors.

In the field, a bryophyte population is studied under hand lens (Fig. 1) for careful selection, and whole plants are collected, preferably fertile ones with sporophytes. The amount of sample collected should be about the size of a palm of hand. However, in case of rare species, or very small specimens, the sample should be collected in a small quantity and always ensure that a part of the population should be left behind for natural growth.


BOTANICAL SURVEY OF INDIA
CENTRAL NATIONAL HERBARIUM, HOWRAH

Flora of Dist. State
Serial No. 41115 Date
Name
Local Name Family
Habit Height Fl Fr
Locality Alt Soil
Vegetation type
Associated Plants
Distribution Abundance
Uses
Significant Notes
Photograph Collector

B.S.I. (C.N.H.) 41115

Fig. 2. A field note book

The specimens collected are placed in a paper bag after assigning a field number. The complete field data is recorded in the field note book (Fig. 2) against each field number with a pencil or water proof ink.

Preservation

Bryophytes are usually preserved in dry condition. However, sometimes liquid preservation is also necessary, especially in case of thalloid liverworts.

1) Dry preservation

   The specimens collected in paper bags are air dried by opening the mouth of the packets and keeping them in a well ventilated place. In case of very wet samples, blotting papers are used to make packets. The specimens are then transferred to these blotting paper packets for drying. However, care should be taken to apply no pressure or very little pressure to avoid distortion and to allow the specimens retain their three dimensional form. After proper drying, the specimens are transferred to 'herbarium packets' made of brown paper having a size of 6″ × 4″.

2) Liquid preservation

   Preservation of bryophyte specimens in liquid is advisable, especially in case of thalloid liverworts and hornworts for examination of morphological and anatomical characters. For liquid preservation, the following preservatives can be used;

   a) 1 : 1: 8 : 10 – formaldehyde : glacial acetic acid : 95% ethanol : water
   b) 1 : 1 : 18 – gluteraldehyde : glacial propionic acid : 70% ethanol
   c) 10 : 1 : 1 : 8 – 96% ethanol : gluteraldehyde : glycerol : water

   Formaldehyde can also be used instead of gluteraldehyde. The presence of glycerol in the third mixture helps in keeping the tissue soft in case of accidental drying out due to evaporation of ethanol. The specimens are kept in air tight and spill proof specimen bottles (Fig. 3).

Fig. 3. A specimen bottle

   However, in Liquid preservation method, the preservatives cause degradation of chlorophyll and lipids, by which oil-bodies and the natural colour of the plants are lost. These preservatives also make the plants unusable for DNA or other molecular analysis. Hence, only a small portion of the sample should be preserved in liquid medium, and the rest should be dried properly and kept in herbarium packets. The same field number should be assigned to both the herbarium packet and the specimen bottle as they are a part of the same sample.

Storage

Bryophyte specimens are stored in herbarium packets made of brown paper having a size of 6″ × 4″. These can be made by folding a 9″ × 11″ sheet of moderately thick brown paper. The packets are then properly labeled with complete field data (Fig. 4). The herbarium label should include the following data –

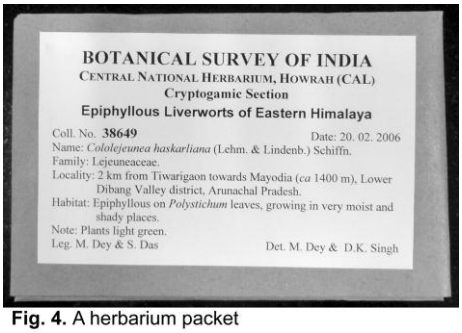

BOTANICAL SURVEY OF INDIA
CENTRAL NATIONAL HERBARIUM, HOWRAH (CAL)
Cryptogamic Section
Epiphyllous Liverworts of Eastern Himalaya
Coll. No. 38649 Date: 20. 02. 2006
Name: *Cololejeunea haskarliana* (Lehm. & Lindenb.) Schiffn.
Family: Lejeuneaceae.
Locality: 2 km from Tiwarigaon towards Mayodia (*ca* 1400 m), Lower Dibang Valley district, Arunachal Pradesh.
Habitat: Epiphyllous on *Polystichum* leaves, growing in very moist and shady places.
Note: Plants light green.
Leg. M. Dey & S. Das Det. M. Dey & D.K. Singh

Fig. 4. A herbarium packet

a) Scientific name of the species along with the author,
b) Family name,
c) Collection number,
d) Date of collection,
e) Locality with elevation and GPS coordinates (wherever possible),
f) Habitat,
g) Substrate,
h) Name of collector,
i) Name of identifier,
j) Additional notes such as vegetation type, plant colour, etc.

Fig. 5. Wooden herbarium cabinet with pull out drawers

Fig. 6. Herbarium packets kept in card board boxes

The herbarium packets are stored in air tight specialized steel or wooden cabinets having pull out drawers (Fig. 5). Alternatively, the packets can be kept in card board boxes of rectangular shape (like shoe boxes, Fig. 6), which in turn are placed on shelves or in cabinets (Fig. 7). In some herbaria, the packets are glued to herbarium sheets, covered in species covers and genus covers and stored in standard herbarium cabinets. Silica gel packets are kept in the cabinets and drawers for moisture control, while naphthalene balls, fumigants, pest strips, etc. are used for pest control.

Fig. 7. Card board boxes placed on herbarium shelves

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