Training Manual on Plant Taxonomy (Dicots) in Southeast Asia

ASEAN CENTRE FOR BIODIVERSITY

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LIPI

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FOREWORD*

It is a distinct pleasure for me to join you in the opening ceremony of the Training-Workshop on Taxonomy of Terrestrial Plants. This is a great event for the future of young taxonomists in East and Southeast Asia, providing a venue to exchange knowledge, views and technical expertise in studying, evaluating and maintaining plant resources of Southeast Asia. It is common knowledge that floristically, our region is one of the richest areas of the world, containing many native species of plants that are still untapped.

On behalf of the Indonesian Institute of Sciences (LIPI), allow me to welcome you all to the Herbarium Bogoriense – the Research Center for Biology in Cibinong Science Center-LIPI. A special warm welcome is extended to the participants, observers and lecturers. Indeed, it is a great pleasure for me to be here with you to witness this very important course.

I really appreciate that this training is receiving enormous support from many parties as reflected by the presence of distinguished delegates from the region and international institutions.

We realize that the lack of trained human resources and inadequate capacities in taxonomy has been stressed as one of the obstacles in the implementation of Convention on Biological Diversity commitments, especially in the ASEAN region. It is the mandate of LIPI as a National Focal Point of the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA) and National Focal Point of the Global Taxonomy Initiative (GTI) and Global Strategy for Plant Conservation (GSPC) to promote basic and strategic research on biodiversity especially in plant taxonomy.

I am very confident that this training-workshop will be an important milestone to enhance our expertise in the region, as well as strengthen our networking for the fruitful cooperation among ASEAN taxonomists and East Asian experts.

At this juncture, I wish to stress that the training of young experts will give them opportunities to hone their skills and gain additional knowledge in their field of expertise. As for research,the participants will be given the chance to perform research work in taxonomy.

Finally, on behalf of the Indonesian Institute of Sciences, I would like to acknowledge the generous support from the Ministry of Environment of Japan, ASEAN Center for Biodiversity and Japan Wildlife Research Center. I would like also to express my gratitude to Herbarium Bogoriense-Research Center for Biology-LIPI who organized and hosted this course.

My heartiest gratitude is extended to all lecturers from the Philippines, Japan and Herbarium Bogoriense, RC Biology-LIPI. I hope that the participants, lecturers, organizers and sponsors will continue to communicate and strengthen your collaboration to increase our understanding of our biodiversity.

**Welcome Remarks delivered by Prof. Dr. Ir. Bambang Prasetya, Deputy of Life Sciences, LIPI at the opening session of the Training Workshop on Taxonomy of Terrestrial Plants, 16 February 2011*

BACKGROUND

The lack of trained human resources and inadequate capacities in taxonomy has been stressed as one of the obstacles in the implementation of Convention on Biological Diversity (CBD)requirements, especially in the Association of Southeast Asian Nations (ASEAN) region. The dynamic growth of the ASEAN region and its neighboring countries Japan, China and Korea (also referred to as ASEAN + 3) in recent years has increased the pressure on their natural resources. Human activities, the driving force behind the regional growth, threaten the biological resources. Lack of scientific information on biodiversity in this region is a crucial issue in the assessment and prediction of biodiversity changes, caused mainly by the lack of taxonomic capacity in data collection and analysis.

During the Ninth Meeting of the Conference of the Parties to the CBD (CBD-COP9), the Programme of Work on the Global Taxonomy Initiative (GTI)with deliverable outcomes was adopted (Decision IX/22). The Programme of Work, as revised, was adopted in CBD COP10 through Decision X/39.Parties to the Convention were urged to promote/carry-out the Programme of Work for the GTI through coordination of its implementation with existing national, regional, sub-regional, and global initiatives, partnerships and institutions; designation of national GTI focal points; provision of updated information about legal requirements for exchange of genetic/biological specimens; about current legislation and rules for access and benefit-sharing in terms of the needs for the GTI; and initiatives of setting-up of national and regional networks to aid the Parties in their taxonomic needs in implementing the CBD.

Adequate taxonomy is one of the necessary fundamental tools required for the global community to be able to implement the Millennium Development Goals and the development targets from the World Summit for Sustainable Development. Without adequate long-term investment in the human, infrastructural (including, important biological collections) and information resources necessary to underpin the science of taxonomy, the now well-recognized taxonomic impediment will continue to prevent adequate implementation of sound, scientifically-based sustainable, environmental management and development policies.

It is in this context that the project *"Taxonomic Capacity Building and Governance for Conservation and Sustainable Use of Biodiversity"* was approved by the Japan-ASEAN Integration Fund in July 2010. An Inception Meeting/Workshop was conducted in Manila, Philippines on 31 August – 01 September 2010 to provide direction and work out the details of the project.The workshop identified three training topics to be conducted, one of which is the taxonomy of plants.

The main objective of this manual is to provide a clear, simple and concise technical handbook for young researchers in plant taxonomy. The handbook aims to increase scientific expertise, and improve skills in management of specimens, writing, and in preparing taxonomic publications. This manual is an attempt to bring together the current knowledge on the theory and practice on plant taxonomy, from morphology, anatomy, geography, ecology, cytology and molecular biology.

Many people have been involved in the production of this manual, the content of whichwas based on the training-workshop conducted at the Herbarium Bogoriense, RC Biology-LIPI, and the experiences of the Herbarium Bogoriense staff. It is hoped that the manual will become a widely used and accepted handbook to help in-services training in many institutions of the different ASEAN member States.

The organization of this manual is in two parts. Part I provides a full report of the Training Course conducted at the Herbarium Bogoriense, RC Biology – LIPI in Bogor, Indonesia, from 16 to 23 February 2011. Part II is a Training Manual largely based on the training course.

We are indebted to the Japan ASEAN Integration Fund for funding this manual and to the ASEAN Centre for Biodiversity (ACB) and the Research Center for Biology-LIPI for organizing the course. Our gratitude goes to the following staff for their contributions: Dr. Teguh Triono, Dr. Rugayah, Dr. Nanda Utami, Dr. Hary Wiriadinata and Dr.Marlina of Herbarium Bogoriense, RC Biology-LIPI; Dr. Eiji Suzuki from the Graduate School of Science and Engineering, Kagoshima Universityand Dr. Shiji Fujii fromtheUniversity of Human Environments inJapan; and Dr. Edwino Fernando of the College of Forestry and Natural Resources at the University of the Philippines Los Banos for their lecture materials during the Training-Workshop.

PART I – THE TRAINING COURSE

Objectives of the Training Workshop

Generally, the training workshop aimed tocapacitate participants in the rigors of taxonomy especially on the dicotyledonous plants group. Specifically, the training workshop:

1. Introduced the participants to the taxonomy of terrestrial plants particularly on selected families of dicots
2. Familiarized the participants with the general biology of these plants
3. Upgraded the taxonomic skills of the participants on the following:
   a. Methods of morphological observation
   b. Sample collection, processing and managing
   c. Databasing
4. Introduced the participants to advanced taxonomic methodologies such as molecular techniques, bar coding and databasing
5. Provided hands-on experience through a field exercise in collection, DNA coding and molecular analysis

The outputs of the training workshop were the following:

1. Skills of the participants in terrestrial plants taxonomy upgraded
2. Advanced taxonomic methodologies introduced
3. Hands-on experience in collections management, cataloguing and storage provided

Organization and Participation of the Training Course

The training course was organized by the ASEAN Centre for Biodiversity (ACB) and the Ministry of the Environment, Japan (MoE-J), and hosted by Herbarium Bogoriense, R.C.Biology, CSC-LIPI in Bogor, Indonesia.

The course requiredparticipants from each ASEAN Member State who had background in botany, plant ecology and related fields, not older than 35 years old, and with academic or government position working on terrestrial botany or plant ecology.

There were 15 participants representing eight ASEAN Member States, plus three additional participants from Indonesia andan observer from China.

There was a balance in the number of male and female participants. The age distribution was also very much within the range of the age requirement, except for two participants who were above the 35 years old age limit. Professionally, nine participants were working with academe or research institutions, and tenwith the government. The directory of participants can be found in Appendix 1.

The Training Course Programme

The training course on plant taxonomy had three methodologies: lectures, specimen management, and hands-on field collection. A copy of the course programme can be found in Appendix 2.

The lectures were on the following:

1. General lectures on:
   a. Taxonomy
   b. Morphology
   c. Biogeography
   d. Cytology
   e. Bar coding
   f. Molecular analysis
   g. Specimen collection methodologies
2. Special lectures on the following dicot families:
   a. Fabaceae / Legumes
   b. Rubiaceae
   c. Rosaceae
   d. Ericaceae
   e. Sapotaceae
   f. Euphorbiaceae
   g. Cucurbitaceae
   h. Myristicaceae

The participants were divided into groups to work on cytology and on DNA extraction as part of the specimen management. For the field collection exercise, a field trip to the Cibodas Botanical Garden was conducted.

As a culminating activity, the participants chose specific dicot families for their group presentations and reports. Putting together their learning from the lectures, they collected samples from the botanical garden, identified the specimen, made the taxonomical keys based on morphology, and provided the plant description.

A CD was distributed to the participants containing all the presentations of the groups, the lecturers, as well as the pictures taken throughout the training course. In addition, the Darwin Core, a biodiversity information database standard to facilitate exchange about geographic occurrence of species, and integrating this with other information about the organisms was presented and given to the participants.

Abstracts of Presentations

Plant taxonomy is a basic and fundamental research in understanding plant diversity. A specific technical course is usually needed for each group of plants, such as courses on Monocotyledoneae, Dicotyledoneae, Ferns, Bryophyte and Fungi. The broad scope of the course, from morphology to molecular analyses, requires sufficient period of time for its conduct. For a two-week course, a special topic can be selected as a main course, while the other topics can read to develop general understanding. For anyone interested in molecular analyses, he/she can directly follow the technical manual for DNA analyses; however, additional subjects are required to understand the relationship among the other subjects.

Genetic information through cytology and molecular analyses are interesting and exciting subjects for young scientists. However, in any taxonomic work, one needs to understand other subjects such as morphology, anatomy, paleontology, ecology and geography etc. During the training course, all subjects were discussed.

The training course was divided into lectures (basic taxonomy, morphology, dicot families), laboratory work (herbarium work, cytology, molecular analyses), database, and field collection. The complete lectures during the training course are provided as appendices (Appendix 1).

Plant Taxonomy (Introduction)

Dr. TeguhTriono, Head of Plant Taxonomy Group, RCBiology-LIPI, Indonesia

The foundation principles of taxonomy stem from the natural need for classification, recognizing that a 'name is a key to information'.

"The urge to classify is a fundamental human instinct; like the predisposition sin, it accompanies us into the world at birth and stays with us to the end." (A.Tindell Hopwood 1959 in Davis & Heywood 1963).

The aims of taxonomy are for identification and communication; to provide natural classification; and to reconstruct evolutionary pathway. The phases in taxonomy are exploratory phase (identification); consolidation phase (adding more materials); biosystematic phase (taxonomic evidence); and encyclopaedic phase (drawing evolutionary relationship).

The common terminologies being used in taxonomy are identification, description, classification and key, and phylogeny. In plant classification, relationship is being assessed, delimiting taxa and assigning the rank of a species. Criteria for classifying plants would include genetically fixed variation; based on correlation and discontinuity of characters; consideration of all materials used; covers an entire geographical range; and having consistent treatment.

Characters as used in taxonomy may be analytic and synthetic, qualitative and quantitative, good and bad. Taxonomic evidences would also need to be produced, since classification will often be based on total evidence, such as morphology, cytology and anatomy, metabolites and DNA, and field, herbarium and library specimens. Taxonomic products contain information, and these could be in the form of a checklist, notes, flora, monograph or a phylogenetic paper.

Plant morphology of Dicotyledonae

Dr. TeguhTriono, Head of Plant Taxonomy Group, RCBiology-LIPI, Indonesia

Plant morphology simply refers to "the way in which plants are constructed". This is necessary for quick and easy identification, in writing descriptions, in using literature (keys, plant descriptions), laying-out, collecting, and for field notes. Careful observation is always the key. A set of standard works is usually adopted, such as that of Hickey (1979) to

follow leaf morphology, and the Systematics Association Committee (1962) for shapes. Key characters of plants, in morphological units, include habit, sap, leaves, stipules, hairs and glands, flowers, and fruits.

Biogeography

Dr. Edwino S. Fernando, Professor, University of the Philippines - Los Banos, Department of Forest Biological Sciences, UPLB, Philippines

Plant geography or phytogeography is often regarded as one of the oldest of plant studies. It deals with the distribution of plants over the Earth's surface, past and present, and is the science that attempts to describe and interpret the geographic distributions of organisms. A common concern in plant biogeography is the distribution and dynamics of plant biodiversity, often simplified in terms of numbers, or proportions of endemic species.

The Earth's surface is often divided into geographical units based on similarities and differences in the occurrences of species and ecosystems such as Biogeographical realm, Biogeographical provinces, Biome, Ecoregion, Vegetation, Flora, Floristic areas, and Endemism, among others. A general question in historical biogeography is why not a uniform Flora worldwide? To which the obvious reply would be the differences in biotic and abiotic environmental factors in various parts of the world.

Disjunctions – which are natural gaps in the geographic ranges of taxa – account for the many reasons why in seemingly identical conditions of different areas there are still different floras, as well as for the discontinuous distributions of species. Causes of disjunction may be chance dispersal and vicariance. Phylogenetic analyses may reveal the evolutionary relationships among the resultant lineages and allow for reconstruction of the events that resulted in their original separation from one another.

Phylogeography is the study of the geographical distribution of genealogogical lineages as determined by DNA sequence variation from individuals across a species range. Conservation biogeography is the application of biogeographical principles, theories and analyses, being those concerned with the distributional dynamics of taxa individually and collectively, to problems concerning the conservation of biodiversity.

Plant age

Dr. Eiji Suzuki, Professor, Earth and Environmental Sciences, Graduate School of Science and Engineering, Kagoshima University, Kagoshima, Japan

Plants live longer than animals. The oldest recorded plant, the Pinus aristata in the USA, was recorded to be 4,862 years old, while the longest living animal, the turtle, was recorded at 152 years old. In a cold climate zone, many trees have annual rings, and these are counted to estimate the age. The ages of trees without annual rings are estimated by C methods. Age of animals is mostly from breeding records in zoos. For whales, their earwax accumulates, making annual scars, which are then estimated as records of their age. Shelled species, on the other hand, make annual scars in their shells.

Some animal physiologists, however, contest that plants do not live longer than animals. Plants, they say, live for only a few decades because, for instance in tree cells, the vessels or tracheid are dead cells from the beginning, and that the living cells, such as those in leaves and phloem, live for only 1-30 years. In animals, however, most of their cells are alive, with the same cells living throughout their lifespan. The brain cells of a 150 year-old turtle is actually alive throughout its life span. The difference of life between plants and animals are in (1) structure of cells; and in (2) reproduction. In determining the difference between angiosperms and gymnosperms, the age, physiology and evolution should be considered.

Cytology

Dr. DedyDarnaedi, Plant Taxonomist, Senior Staff, Herbarium Bogoriense, RCBiology-LIPI, Indonesia

Plant systematics serve as a major unifying force in biology because it uses data from a multitude of discipline, such as molecular biology, biochemistry, comparative phytochemistry, electron microscopy as well as morphology, ecology, biography to develop a framework upon which classification can be developed. Indeed without the system of nomenclature provided by plant taxonomy, there could be no comparative reference in naming of plants. Cytology is more commonly known as cell biology, and is also an important discipline relevant to taxonomy. Specifically, the genetic or chromosome information in the cell, such as the number of chromosomes in each cell, the size of chromosome, centromere position, and the behavior of chromosomes, are being studied.

Molecular systematics

Dr. Marlina, RCBiology-LIPI, Indonesia

There are several ways to collect material for DNA extraction. The ultimate thing to remember is how we prevent the cells from undergoing destruction. Collecting methods may be through Silica gel, FTA PlantSaver Card (Whatman), Liquid Nitrogen and CTAB Solution. The polymerase chain reaction (PCR) is a technique widely used in molecular biology. As PCR progresses, the DNA generated is used as a template for replication. This sets in motion a chain reaction in which the DNA template is exponentially amplified.

Database IBIS

Mr. Bambang Hartoko and Mr. Basuki Bundi Mulyono

The Indonesian Biodiversity Information System (IBIS) is an application developed by the Center for Research Biology-LIPI. Developed by using open source software, the IBIS is used to manage data of specimens of flora, fauna and microorganisms (specimen data are derived from flora specimens, fauna specimens, and living micro-organisms specimens). Its development began in 1991.The migration of data and records took place from 1994 to 2003, and since then the IBIS has been utilized and implemented. The last version of IBIS was developed in 2011, consisting of five tables – Collection, Specimen, Locality, Determination, and Component.

THE DICOT FAMILY

Ericaceae

Dr. Edwino Fernando

Ericaceae are mostly trees and shrubby plants, rarely lianas or herbs, sometimes epiphytic, mycorrhizal, some achlorophyllous. Leaves are simple, often spiral (including pseudoverticillate), decussate or whorled, and always exstipulate, with glands below, venation pinnate (sometimes with three or more veins from the base). Flowers in bracteate racemes, sometimes solitary, often pendulous, usually actinomorphic, usually bisexual; petals connate and typically campanulate, funnel-shaped, cylindrical, tubular or urceolate; stamens are five to ten, sometimes free or adnate to the corolla, the anthers often dehiscing by apical pores and sometimes have appendages; ovary five-locular. Fruit is capsule (loculicidal or septicidal), berry, or drupe; seeds are numerous, usually small, sometimes winged. Many species are edible, as well as used as ornamental plants. It usually grow in elevations of 1,500 meters to very high elevation such as alpine. They usually grow as shrub, or small trees, but in the lower elevation they can grow as epiphytes. They have very attractive, colorful flowers,have potential as an ornamental plant, and have medicinal properties.

Spot character:

- Always: Leaves spiral (incl. *pseudoverticillate*), exstipulate; flowers hermaphroditic; corolla tubular or urceolate, anthers opening by apical pores, style 1; seeds numerous.
- Usually/often: Woody; leaves glandular below, venation pinnate, sometimes with 3 or more secondary veins from base; flowers actinomorphic; ovary 5-locular; stamens 10, anthers with appendages.
- Striking features: Saprophytes *(Andresia, Monotropastrum);* leaves lepidote (most *Rhododendron*); glands on leaf margin at base
- Different from: *Epacridaceae:* venation parallel.

One group who choses ***Ericaceae*** studied some species of *Rhododendron*. In Java, there are 13 species. Near Cibodas Botanic Gardens the wild *Rhododendron* usually grows as epiphyte on the branches of big trees, several species can be found in the gardens as terrestrial shrubs.

Sapotaceae

Dr. Teguh Triono

Identifying the plant morphology of Sapotaceae includes determining the presence or absence of cuts or wounds and color of the sap or exudate. Leaves could be simple, opposite/alternate, crowded/dispersed, and with margins of the entire plant. Sapotaceae may be hermaphrodite or unisexual, with the ovary of its flowers either superior (hypogynous) or semi-inferior (epigynous).

Begoniaceae

Dr. HaryWiriadinata

Begoniaceae is a big family containing more than 1,500 species found wild throughout tropical and subtropical Asia, Africa and America. It is characterized by its herbaceous habit, rarely woody stem, asymmetric leaf, unisex flower and many tiny seeds. Many species have beautiful leaves so they become ornamental plants which have commercial importance in the horticultural trade. Over 10,000 kinds of hybrids and cultivars are available.

- **Herbaceous** plants, sometimes tuberous, monoecious.
- **Leaves** simple alternate.
- **Stipules** always present on the base of the petiole, sometimes caducous.
- **Inflorescence** basically cymose or racemose.
- **Flowers** always uni-sexual; the sepals, petals sometimes similar. In **male** flowers usually have 2 outer sepals and 2 smaller inside sepals, free; stamens numerous, filament free, anthers are spherical or hemispherical cluster, dehiscing longitudinally. The **female** flowers usually have 4-5 sepals; **ovary** inferior which has 3 locules (chambers); ovules many per locule. Fruits a capsule rarely berry.

Spot character

- **Always:** Herbs, stem succulent, sometimes woody at base; leaves simple, alternate, asymmetrical, stipulate; flowers unisexual (plants monoecious); stamen many; ovary inferior, 3 locular, ovules numerous.
- **Usually/often:** Sepals and petals free, fruit 3-winged.
- **Different from:** The family is allied to the Datiscaceae (huge trees); sterile *Begoniaceae* might be mistaken for Balsaminaceae (exstipulate, symmetric leaves) - *Gesneriaceae (Cyrtandra):* leaves opposite – *Urticaceae: Cystoliths*.

One group chose *Begoniaceae* for their study due to its potential as ornamental plant. There are several species that occur in the wild, few are in botanic garden collections. Is there a natural hybrid between *Begonia robusta* and *B. multiangula* since they grow in the same ecological niche? The dense red hairs on the young leaf of *Begonia robusta* sometimes loses an old leaf. *Begonia isopteran* is characterized by its equal 3-wing fruit.

Fabaceae

Dr. Hary Wiriadinata

Majority of the members of the Fabaceae family occurs from sea level to mountainous flora.

Rosaceae

Dr. Hary Wiriadinata

The majority members of Malesian Rosaceae belong to the mountain flora and occur only above 1000 meters above sea level. It is a large family with worldwide distribution.

- **Tree** or **herbaceous** plants.
- **Leaves** always spirally arranged, sometimes distichous, simple or compound.
- **Stipules** on the twig or on the base of the petiole, free or adnate to petiole, rarely absent. Inflorescence various.
- **Flowers** always bisexual and actinomorphic; hypanthium usually very distinct, from saucer-shaped to tubular or campanulate; the sepals petals and stamens inserted on its rim, its inside usually lined by a nectariferous disk.
- **Sepals** usually 5, free, sometimes an epicalyx present.
- **Petals** usually 5, free, from large and showy to small and not or hardly distinct from sepals or absent.
- **Stamens** usually numerous, filament free, anthers bilocular, dehiscing longitudinally
- **Pistil (s)** one to numerous, free.
- **Ovary (ies)** superior to inferior; **style** present; **ovule** (s) one to several per locule.

Spot characters:

- Always: Leaves spirally arranged, stipulate (except *Spiraea*); flowers actinomorph and style present.
- Usually: Leaves serrate, flowers hermaphrodite, stamens numerous.
- Striking features: leaves drying reddish brown, black glands below, fruit often weakly bilobes *(Prunus);* hooked bristles on hypanthium *(Acaena, Agrimonia);* spiny plants *(Rosa* p.p., *Rubus.)*
- Different from: Chrysobalanaceae: always trees, flowers more or less zygomorphic, style more or less exentric. Symplocaceae: exstipulate, ovary inferior; Saxifragaceae: usually exstipulate.

During the field trip, one teamfound and studied some species of the genus *Rubus.* This groupis characterized by spiny stem, compound leaflets, persistent calyx, 5 free white petals, many stamen, and many pistils. Fruit are berries.

Rubiaceae

Dr. Hary Wiriadinata

The family Rubiaceae is trees, shrub, vines or herbaceous plants that are sometimes ant-inhabited. Members of this family can be found from sea level to mountainous areas.

- **Trees, Shrub, Vines** or **herbaceous** plants, sometimes ant-inhabited (as *Hydnophytum, Myrmecodia, Myrmeconauclea*).
- **Leaves** simple or compound, sometimes opposite arranged, sometimes distichous. **Stipules** on the twig or on the base of the petiole, free or adnate to petiole, rarely absent. **Inflorescence** various.
- **Flowers** always bisexual and actinomorphic; hypanthium usually very distinct, from saucer-shaped to tubular or campanulate; the sepals, petals and stamens inserted on its rim, its inside usually lined by a nectariferous disk.
- **Sepals** usually 5, free, sometimes an epicalyx present.
- **Petals** usually 5, free, from large and showy to small and not or hardly distinct from sepals or absent.
- **Stamens** usually numerous, filament free, anthers bilocular, dehiscing longitudinally.
- **Pistil (s)** one to numerous, free.
- **Ovary (ies)** superior to inferior
- **Style** present.
- **Ovule (s)** one to several per locule.

Spot characters:

- **Always:** Leaves decussate, including verticillate (sometimes one leaf of a pair reduced), simple, entire, interpetiolar (rarely intrapetiolar) fused stipules; corolla tubular, stamens isomerous, alternate with lobes.
- **Usually/often:** Leaves with raphides; flowers hermaphrodite, actinomorphic, corolla 4 or 5 lobed; ovary inferior, 2-locular, placentation axile.

- **Striking features:** Leaves of a pair unequal, flowers like those of *Solanum (Argostemma);* stipule ochreate, ovary superior *(Gaertnera);* climber with hooks *(Uncaria);* one sepal enlarged and showy *(Mussaenda);* epiphytes with swollen stems inhabited by ants *(Myrmecodia, Hydnophytum)*
- **Different from:** *Caprifoliaceae:* leaves exstipulate; *Loganiaceae:* no raphides, ovary superior; *Rhizophoraceae:* petals free.

There are several genera and species along the trail to Cibeureum waterfall such as *Borreria, Lasianthus, Mussaenda, Mycetia, Pavetta, Psychotria, Urophyllum* and *Wendlandia.* The genus *Lasianthus* and *Urophyllum* have similar morphological characters and they have also similar habitat. *Lasianthus* sometimes misidentified as *Urophyllum*. In this training some species of *Lasianthus* were investigated in more detail.

Cucurbitaceae

Dr. Rugayah

The family Cucurbitaceaehas a species diversity of about 900 species belonging to 120 genera, with 37 genera found in Malesia. It is distributed all over the world, predominantly in tropical areas, with more than 90 percent of the species found in Africa, Madagascar, Central and South America and Southeast Asia and Malesia. Its value is usually as edible fruit and seed *(Cucurbita, Cucumis, Citrulus, Benincasa, Sechium, Luffa, Lagenaria, Momordica, Cyclanthera, Coccinea),* and for medicinal purposes *(Trichosanthes, Momordica)* as well. Cucurbitaceae is mostly found in primary or less disturbed forest, seeming to prefer growing in shady places, wet condition, or openareas along the riverside. Some may be climbing on other plants, creeping on theground or rocks. The plants are mostly unisexual, monoecious or dioecious, so both male and female flower per plant have to be collected. Collecting samples in the morning is better. Most species are nocturnal, meaning their anthesis flowers take place at night till the morning of the following day. Wet collection and taking photographs arestrongly recommended because the flowers are showy, but thin and easily damaged.The fruit is also showy, varying in shape and color, juicy (some have hardy pulp).

Balsaminaceae

Dr. Nanda Utami

The Balsaminaceae is composed of two genera – the monotypic Hydrocera and the prolific Impatiens, which are commonly referred to as the balsams. The Hydroceraare semi-aquatic plants, growing in swampy places, watersides and lakes. They canbe found in southern India, Ceylon, and parts of Southeast Asia. Impatiens is agenus of about 850-1000 species of flowering plants. The genus is concentrated in mountainous regions of Southeast Asia, south China, India and Africa. The scientificname Impatiens is Latin for impatient, in reference to their seed capsules. When thecapsules mature, they "explode" when touched, sending seeds several meters away.This mechanism is also known as "explosive dehiscence".

Fagaceae

Dr. Shiji Fujii

Distinguishing morphological characters of Fagaceae include the fruit and the cupule. The cupule is a mysterious organ. Many hypotheses had been proposed to explain its origin – intercalary growth, shoot axis, etc. One hypothesis says that it originated from sterile shoots (lateral shoot axis). In its primitive form, there are three or more flowered dichasial cyme, and with a lobed cupule (where each valve is related to a sterile shoot). There are five hypothetical patterns of distributions of species along an environmental or geographic gradient: (a) Species are distributed as discrete communities that replace each other abruptly; (b)Species are not segregated into communities, but some sets replace each other abruptly; (c) Species are distributed as discrete communities, which gradually replace each other; (d) Species behave as if they are independent of each other, neither associating in discrete communities nor replacing each other abruptly; and (e) Most species are nested within the ranges of a few dominant species, but otherwise occur independently of each other.

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Training Outputs

The participants chose specific dicot families for their group presentations and reports. Putting together their learning from the lectures, they did the collection from the botanical garden, identified the specimen, made the taxonomical keys based on morphology, and provided the plant description. The participants worked on the families Ericaceae, Begoniaceae, Rosaceae, and Rubiaceae. They produced a report on the families assigned to them and made a presentation on the last day of the training. The reports of the groups are found in Appendix 4.

EVALUATION

A pre- and post-training evaluation was conducted with the participants. A summary of their answers is found below.

Pre-training Evaluation

What skills/knowledge/behavior do you want to develop by attending this training workshop?

While majority of the participants expressed a general expectation to gain more knowledge on plant taxonomy, a few expressed their desire to develop specific skills on identification, classification, morpohology, cytology, molecular analysis, curation, collection, databasing, barcoding, herbarium work. Some mentioned that they wanted to develop specific knowledge on seed plants, freshwater (plants), vegetables and tropical trees.

What do you expect to see/hear/feel differently by developing the above skills/knowledge/behavior?

The participants expressed that they expect to be more confident and efficient, which are the skills needed tobe a professional taxonomist. Some participants said they expected their initial impressions about the difficulty of cytology work, molecular studies and other plant taxonomy work will be unfounded, and that their skills would actually improve. Other participants expected that in interacting and sharing experiences and knowledge with the other participants, their social skills will improve.

How will it benefit your job performance by developing the above skills / knowledge / behavior?

Most of the participants are working in herbariums, botanical gardens or national parks in their countries. They all believed that the training will benefit their work and job performance, as the new skills will support their tasks (research, identification and collection of plants, curation and management). Some participants expected that by improving their job performance, they will be able to speed up the completionof their projects, and may even attract potential funding agencies to support further research.

How do you want others to relate to you after attending the training workshop?

After attending the training workshop, the participants expected to have continuous contact with their fellow participants, with some hoping for strong collaboration and regional networking. One simply wanted others to relate to him as a professional taxonomist/systematist.

What do you feel you are currently not achieving due to the underdevelopment of the above skills/ knowledge/behavior?

Although most of the participants were positive in expressing that underdevelopment of skills is not necessarily a problem, they believed they can still work and improve on it.Some of them expressed that their learning and skills related to work are not enough, thus hindering them from completing projects on time. A participant said underdeveloped skills havecaused low self-esteem.

What are your personal learning goals? What do you really want to learn from this training workshop?

The participants had common personal learning goals – to learn skills related to work andto develop/establish relationships with other institutions and people.The Cambodian participant specifically mentioned "to protect and conserve forest plants in Cambodia for sustainable use".

What do yoursupervisors expect from you after attending the training workshop?

While most of the participants expressed that their supervisors expect them to improve and enhance their skills in taxonomy, as well as learn new and advance taxonomic methodologies, others said they were expected to train their colleaguesand share their learning from the training. They were also expected to network with other taxonomists and organizations.

What other expectations do you have of this training workshop?

Other expectations raised by the participants included knowing the 'science culture' of other participants; developing an international network not just for taxonomy but creating friendships as well; and other activities that would support their existing knowledge on plant taxonomy, among others.

Post-training Evaluation

Which of the training lectures/sessions need to be improved? Please elaborate which aspect needs to be given attention (time allotment, clarity of topic, instructor/trainer expertise, processes/ methodologies used).

The participants suggested that more time be allotted to lectures. They emphasized the usefulness of hands-on work (laboratory, field collection) combined with the lectures. There were mixed review on the trainers, with participants appreciating the expertise of some of the trainers, but suggesting that some of the trainers needed to improve their lecture on certain plant families.

Kindly provide your recommendations to further improve the training lectures/sessions.

The participants recommended that more detailsshould be added to each topic, such as on the principles of plant taxonomy, nomenclature, database and more in-depth lectures and hands-on training on specific or focused families, instead of including many families in a brief period of time.They also recommended inviting more experts as trainers.

Make a list of learning and results from each goal (as stated in the pre-evaluation form).

Most of the participants appreciated learning how to identify the dicot families, do keys and descriptions, as well as learn and experienceabout cytology and molecular laboratory work. Their expectations and goals to learn more about plant taxonomy were clearly met, but the participants reiterated that there was very limited time to absorb and understand everything.

What was your biggest learning experience from this training?

The answers ranged from a specific topic/lecture that the participants understood better (cytology, molecular systematic, plant identification, bar coding), the skills that they learned (field work collection, plant identification, laboratory work), and social skills they gained (networking, knowledge sharing, friends). One participant pointed out how he 'learned so many things in a short time, and still able to make a report'.

What skills/knowledge/attitude do you have now, that you didn't have before attending the training? What are your plans after this workshop?

The participants enumerated a number of specific skills and knowledge that they have acquired in attending the training, such as making a key, identifying the species in the dicot family, doing cytology work and molecular analyses, among others. Attitude-wise, they said they wereinspired to be better equipped with more plant knowledge, as the experience will be wisely applied to their work. They also acknowledged that the training allowed them to work better within a group, helping them to be better listeners in order to work within a team.

What changes will you do to apply what you have learnt from the training workshop into your day-to-day job?

There was a good mix of knowledge, skills and attitude that the participants learned from the training, which they are eager to apply to their day-to-day jobs. Participants expressed that they will try to identify the unidentified collections that they have in their botanical gardens, and expand identification to cellular/cytology level. One participant was, on the other hand, eager to prepare and submit proposals to funding agencies for taxonomic researches. Other changes that they will apply include being more critical regarding plant taxonomy problems, and to contact plant taxonomists in creating/ developing a network.

What support do you need from your supervisors and colleagues to realize the plans you mentioned above?

The participants said they needed financial support (for their researches, especially chemicals and equipment for their cytology studies), freedom (to conduct their researches, studies, as well as to attend more training and have more exposure), and support to their ideas, sharing of information, peer-to-peer review of studies, among others.

Do you have any other comments about the training workshop?

With a general sentiment that the training course was overall satisfactory, the participants emphasized the need for more time for the training course – for the lectures and field work, in order for them to practice taxonomic skills and in studying a plant species.

PART II – THE TRAINING MODULE

This Training Module is divided into four parts. The first focuses on Methodologies for Plant Herbarium Collection, the second part on Cytology, and the third section is an introduction to Molecular Systematics and Laboratory Work. The last part of the module provides brief morphological characters of selected dicotyledons.

Methodologies for Plant Herbarium Collection

The Malesia region (Malaysia, Singapore, Indonesia, Papua New Guinea, the Philippines and Bismarck Islands) is a megadiverse region. About 250 to 300 species of flowering plants, or 10 percent of higher plants, occur in the region. In the Herbarium Bogoriense, there are about three million herbarium specimens and references about plant taxonomy. Even if the herbarium specimens are stored alphabetically in new cabinets in comfortable air-conditioned rooms, it would take time to study them all within a short period. The easiest method to study higher plants within a short period is by studying the living selective group of genus with a small number of species, making herbarium specimens, and studying their morphology.

First, write all characters and character state of single individual plant, from general character to specific character, from base to top. Do the same to second individual, third individual, and so on for one species, and find out the constant characters. Compare the characters and character states of different species, and use those differences for your key to separate the species.

For the training course in Bogor, herbarium collections were made from the field at the Cibodas Botanic Gardens, and along the trail to Cibeureum waterfall within Mt. Gede Panggrango National Park at elevation 1400-1600 m high altitude. Since too many plants occur in those areas, the objective of collection was limited to several relative species of selected families such as Rosaceae, Ericaceae, Rubiaceae and Begoniaceae that grow wild in that area, with three or more species of one genus of those families. As introduction to every selected family of higher plants in this manual, for each selected family make some notes of the delimitation characters, spot the characters, and compare different characters of one family with a close relative family. Since the four large families contain many species, we will collect and analyze some sample species to find the constant characters that lead you to improve your knowledge about the groups and the families.

Collecting

A good collection is an essential aspect of herbarium practice. A good herbarium specimen must give the investigator the best possible information about the plant. Theoretically and ideally, a specimen herbarium contains all parts of the plants (flowers or fruits, leaves, wood, branches etc.) and should be well annotated so as to provide all information not visible on the dry herbarium specimen. Many plants show leaf dimorphism; plants are monoecious but the flower is unisexual (such as in Begoniaceae). To avoid misinterpretation, it is recommended to always collect more than a single specimen.

As a general rule do not collect sterile material. For scientific purposes sterile herbarium specimen are mostly useless, and for identification it is often very troublesome and time-consuming.

Small plants should be collected as a whole. Larger terrestrial herbs, shrubs, creepers and small climbers can be easily collected with secateurs or pocket knife. When collecting plant, please find a branch with leaves and flowers or fruits. Parts too large for mounting on a herbarium sheet can be reduced in size before drying. The size of specimen should be good enough to fit between folded old newspapers. Collect a fair sized sample and not a scrap.

Making Notes

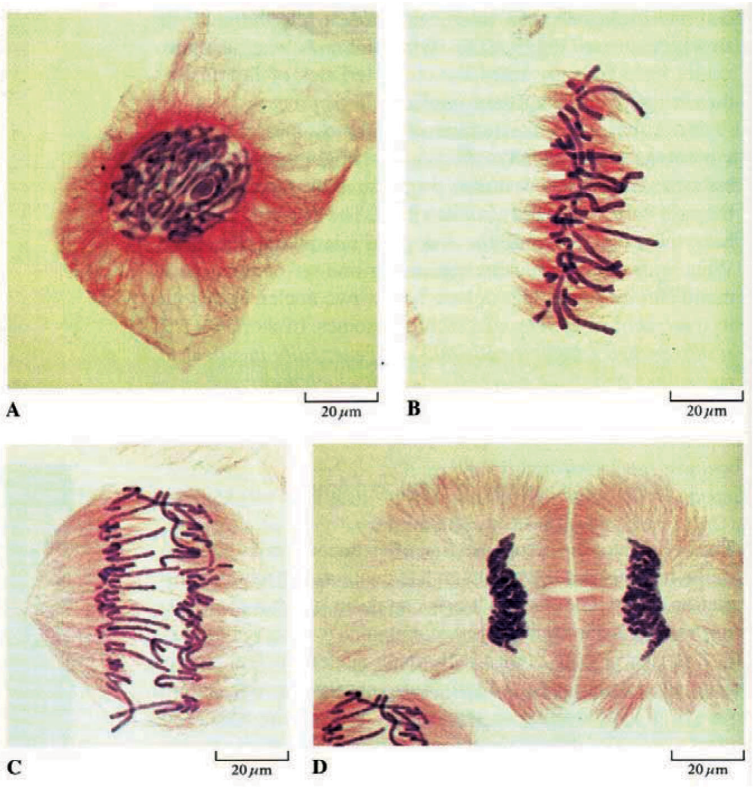
Make it a habit not to rely on your memory but to write your notes at the collecting site. Do not wait till the end of the day, or worse still, to put it off to the next day. After collecting your specimen, give it a number tag and enter notes in your collection book under the corresponding number. It is important to take note of all parts that may disappear, or are invisible in the herbarium specimen. Your field data of the living plant collections will be transferred to the herbarium label, which will then be placed on the corner of the sheet of herbarium collections, which will bear the following information:

a) Location (place of the collection, e.g., island, province, district including altitude, latitude and longitude) or a place easily located on a map.
b) Date (date of collection)
c) Collecting number (person/s) who collected the plant; the number has to be continuous; do not make new numbers for new locations)
d) Habitat (notes of vegetation type, topography, soil etc.)
e) Habit (tree, shrub, liana, herbs)
f) Exudate (latex, resin and its color, etc.)
g) Color of various parts, such as flowers, fruit or young leaf
h) Ecological and biological conservation (phenology of flowers, pollination, seed dispersal)
i) Local names (check if the local name is for a single plant or a group of plants, and consistence)
j) Local uses (as medicine, food, utensil etc., and how they are prepared)

CYTOLOGY

Chromosome information is one of the important character information in taxonomy and plant systematics. Chromosome information is also very powerful in describing the mode of reproduction (sexual, asexual, apomixis or hybrid), evolutionary biology, and relationship among species complex. Chromosome can be obtained during cell division at meiosis and/ or mitosis of any single plant. Four main stages during cell division in plant are: Prophase, Metaphase, Anaphase andTelophase. During cell division, at late metaphase stage, is usually the best time for chromosome counting, because during this stage the chromosome is fully condensed into very short form. It is important to understand the process of cell division during mitosis and meiosis.

Cell Divison in plant:
A. Prophase; B. Metaphase; C. Anaphase; D. Telophase

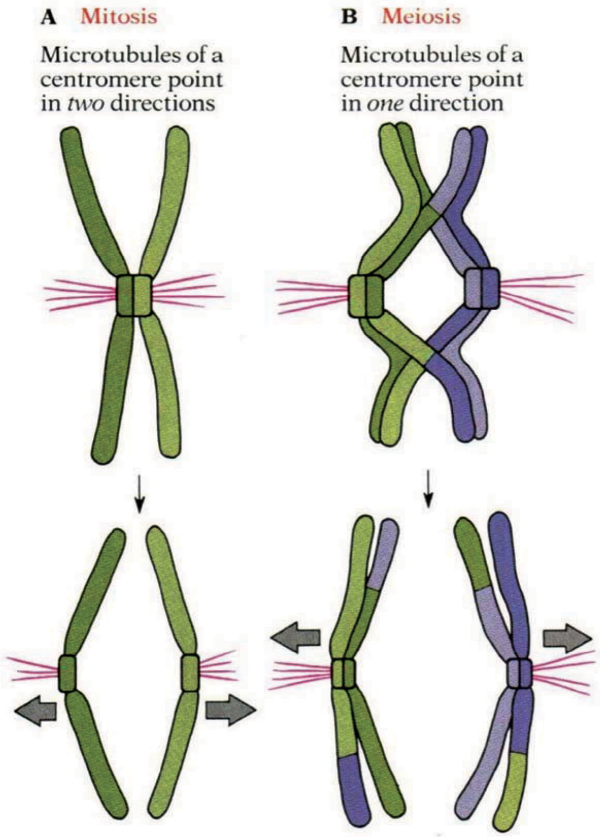


Chromosome information can be: 1)number of chromosome in each cell; 2)size and morphology of any single chromosome; 3)reaction to the staining; and 4)behavior of chromosome, especially during meiosis.

Number of chromosomes

In somatic cell division during mitosis, a mother cell produces two daughter cells with the same chromosome number. It is just a duplication of chromosome numbers. While in gametic cell division during meiosis, a mother cell produces four daughter cells with half chromosome numbers. However, in apogamous plant, gametic chromosome number during meiosis produces the same chromosome numbers as mother cell. There is no cytokinesis at the late meiosis.

Mitosis and Meiosis



9.23 Mitosis and meiosis (A) In mitosis, each centromere sends microtubules toward both poles. As a consequence, the two chromatids are pulled apart and wind up at opposite ends of the cell during anaphase. (B) In meiosis I, however, a centromere sends microtubules toward only one of the two poles, with the homologous centromere sending microtubules toward the other pole. The result is that in anaphase I the chromatids remain joined, and homologous two-chromatid chromosomes wind up in separate cells.

Size and morphology of chromosome

Size and morphology of chromosomes are important information during karyotype analyses. Some time they are quite similar like in most of Pteridophyte, but some plants have a great range in size and morphology like that of family Commelinaceae. Karyotype analyses can demonstrate their relationship of chromosome morphology among species.

Size of chromosome

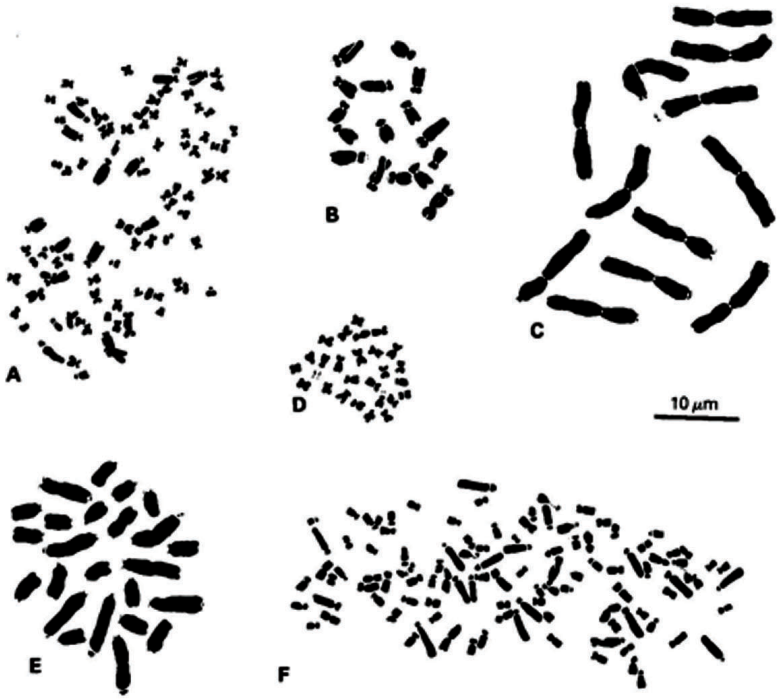


Fig. 5.5 Karyotypes of various members of the family Commelinaceae, taken from Jones and Jopling[185] and showing great range in chromosome number and morphology: **A,** *Tradescantia blossfeldiana* (2*n* = 90); **B,** *Gibasis* sp. aff. *geniculata* (2*n* = 16); **C,** *Tradescantia paludosa* (2*n* = 12); **D,** *Ballya zebrina* (2*n* = 26); **E,** *Tradescantia micrantha* (2*n* = 24); **F,** *Tradescantia fluminensis* (2*n* = 108).

Reaction to the staining and chromosome behavior

Chromosome behavior during meiosis is a good character for evaluating putative hybrids. Hybrid plant exhibits irregular meiotic divisions, producing irregular-shaped and abortive spores or pollen.

Polyploidy

Not like in animals, polyploidy in plants is very common. Duplication of basic chromosome numbers occurs often in any plant groups and ferns. The smallest chromosome number in each plant species isusually treated as basic chromosome number or (x). In gametic chromosome (n) it is half of the somatic chromosome (2n). The smallest chromosome number (x) is usually treated as sexual diploid. Refer to chart below showing the process of chromosome duplication in polyploid complex.

Polyploid complex

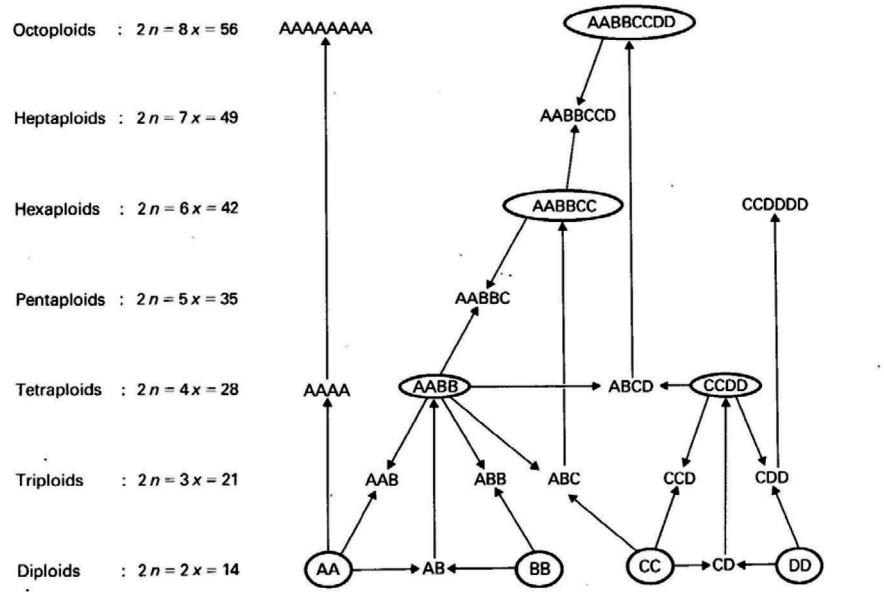


Fig. 5.1 Chart illustrating the possible origin of a polyploid complex commencing from four diploid species AA, BB, CC, DD. The ringed taxa have each genome represented twice and might be expected to exhibit full fertility, while the other taxa might be expected to exhibit varying degrees of sterility. The chromosome numbers on the left are those which would result if the whole complex had the base-number of $x = 7$.

Sources of chromosomes

Chromosome of plants can be observed in any active growing meristem, such as root tips, young leaves, young bud of flowers, or young sporogenesis of ferns. Active vegetative organs are produced by somatic cell division during mitosis,while active generative organs are produced by gametic organs during meiosis.

Root tips: Select active root tip from seedling, or young plant in the gardens. Good and healthy root tips are usually creamy in color.

Young leaves: Select good young leaves. They can be collected from plant collections in the gardens, or any young leaves in the field.

Flower buds: Select young flower buds. For meiosis observation, select new flowers in different stages of maturity. For mitosis observation, the treatment is like those of young leaves.

Sporogenesis: For meiosis observation of fern group, select one young frond with different stages of spore maturity.

Voucher specimens of any plant cytologically examined is very important.

PROCEDURE IN OBSERVING CHROMOSOMES

1. Observing somatic chromosomesin root tips
   i. Pre-treatment (at 18-20O C)
      - Prepare a small bottle with 0.002 mole 8-hydroxyquinolin aqueous solution
      - Select good root tips, cut it at 1 cm long
      - Put 4-6 good root tips into a solution bottle
      - Store this solution at 19O C (18O-20O C) for 3-5 hours
   ii. Fixation at room temperature
      - Select root tips and clean it in clean water
      - Put it into a small bottle with 45% acetic acid
      - Keep it at room temperature for 10 minutes

iii. Maceration (at 60OC)
- Prepare (1 NHCl : acetic acid = 3:1) in a small bottle
- Put the bottle at 60O C boiling water
- Keep it floating in boiling water for 2-4 minutes

iv. Staining at room temperature
- Prepare 2 clean glass slides
- Take 2 root tips from the solution and put it on glass slide, 1 root tip in each glass slide
- Cut 1-2 mm distal root tip and remove the other parts
- Drop 1.5-2 % aceto-orcein into it
- Store in a chamber containing wet filter paper with 45% acetic acid (5-10 minutes, no more than 1 hour)

v. Observe using the microscope

2. Observing somatic chromosome in young leaves

i Pre-treatment (at 18-20O C)
- Prepare a small bottle with (a): 0.02 – 0.05% aqueous solution of colchicines or (b): 0.002 mole 8-hydroxyquinolin aqueous solution or
- Select good young leaves presumably with active mitosis
- Store this solution at 19O C (18O-20O C)

ii. Fixation at ca 5OC
- Put materials into modified Carnoy's solution (absolute ethanol : glacial acetic acid : chloroform = 2 : 1 : 1) for more than 1 hr, (storing it in a refrigator can last a few months)

iii. Maceration and staining
- Put small part of young leaf on glass slide
- Cut into smaller parts using sharpened needle
- Drop 1.5-2% acetoorcein containing 1 N HCl (1/10 volume) and store in a chamber containing wet filter paper with 45% acetic acid for several minutes.

iv. Tapping and squash
- Move glass slide with the material from the chamber
- Add one drop 45% acetic acid and cover it with a cover slip (root tip in the center of cover slip)
- Warm up to 60OC for several seconds then put glass slide on a filter paper
- Fix one corner of cover slip with a finger
- Tap on cover slip gently using a sharpened stick or pencil by vertical direction to set the sample and to remove bubbles
- Put glass slide between folded filter paper, then squash gently but strongly
- Seal cover slip with nail coat or paraffin

v. Observe using the microscope

3. Gametic chromosome observation in young flower

i. Fixation at 5O C
- 45 % acetic acid for more than 15 minutes
- Modified Carnoy solution for more than 1 hour (move material into 45% acetic acid just before use)

ii. Staining
- Put anthers on glass slide
- Remove unnecessary part of anther from glass slide
- Select pollen mother cells (PMCs) on glass slide (add fixative some time to keep materials wet)
- Drop 1.5-2% acetoorcein and store in a chamber with filter paper wet with acetic acid for 2-3 minutes

iii. Squash
- Put glass slide between filter papers and squash it gently
- Seal cover slip with nail coat

iv. Observe using the microscope

4. Gametic or meiotic chromosome observation in young frond of fern during sporogenesis
   - Fix small portion of juvenile leaves with very young sporangia in glacial acetic acid: absolute alcohol (1:3) solution.
   - Keep at room temperature for 24 hours
   - Chromosome counting was made on SMCs at meiosis with aceto-carmine squash method

HOW TO PREPARE SOLUTION

1). 0.002 mole 8-Hydroxyquinolin aqueous solution
- 8-hydroxyquinolin (M.W.145.15)
- 1 liter aquadest (145.15 x 0.002) = 0.29 g
- 10 liter aquadest = 2.9 g
- Shake and store for several days after all the powder has dissolved. Then,it can be used for pretreatment(usually we need several days to dissolve the powder completely).

2). 2% acetoorcein (total volume 50 cc)
- Warm up 22.5 cc glacial acetic acid to 100O C using water bath
- Add 1 g orcein, then shake
- Cool down to room temperature
- Add 27.55 cc water, then filter
- Place stock solution in colored bottle with filter

3). Shiff's solution (total volume 220 cc)
- Boil 200 cc aquadesh (100Oc)filter and shake
- Add 1 g fuchsin basic (caution on explosive boiling)
- Or: add few grains of fuchsin basic and then add the remaining, or shake well in boiled water and then add fuchsin basic
- Cool down to ca. 500 C
- Add 20 cc 1 N HCl
- Cool down to room temperature
- Add 1 g Sodium metabisulfate (Na2S2 O5) or potassium metabisulfite (K2S2O5) and shake for several minutes
- Store at cool and dark place for 1 day
- When solution becomes clear yellowish color, store inrefrigerator

MOLECULAR SYSTEMATICS LABORATORY WORK

The discipline of systematics study involves the diversity of organisms and their evolutionary relationships among them. Systematics provides the essential framework without which we could not recognize or study biological diversity and evolution. Systematists use fossil, molecular, and genetic data to infer evolutionary relationships.

Molecular biology has revolutionized the field of Systematics. The DNA evolves by mutations and these mutations are incorporated in the DNA in which they are fixed in populations. This will lead to divergence of DNA sequences in different species. Although

diverged, we can refer to two DNA sequences as homologous (just as we would for any morphological trait).

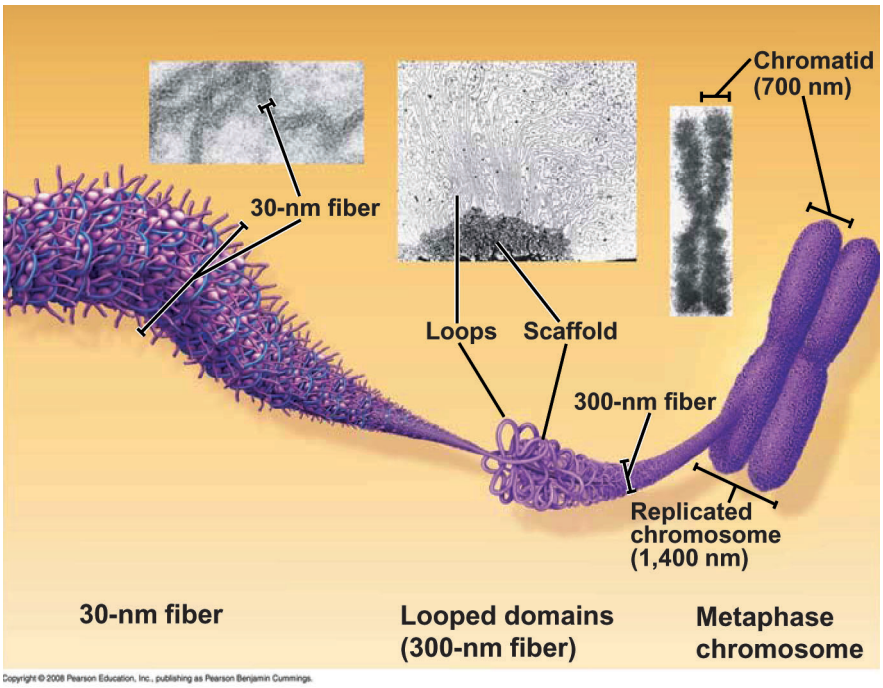
The steps in using molecular methods involve (1) DNA extraction; (2) gene amplification or PCR (Polymerase Chain Reaction) and purification; (3) Cycle sequencing and purification; and (4) the DNA sequencing. Other than sequencing method, DNA fragments are also used in molecular systematics. Upon obtaining the sequences or DNA fragment data, phylogenetic analysis are carried out to interpret the relationships among the species studied.

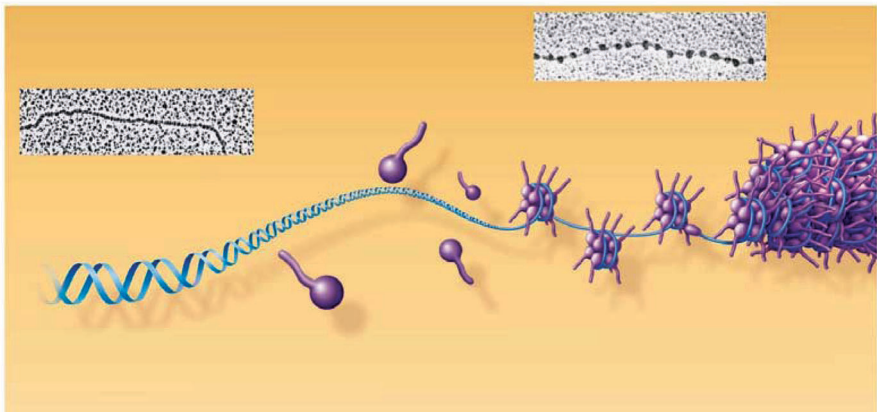
This manual is designed to introduce to beginners the theory and practice of phylogenetic inference from molecular data (DNA sequences or fragments).

What is DNA?

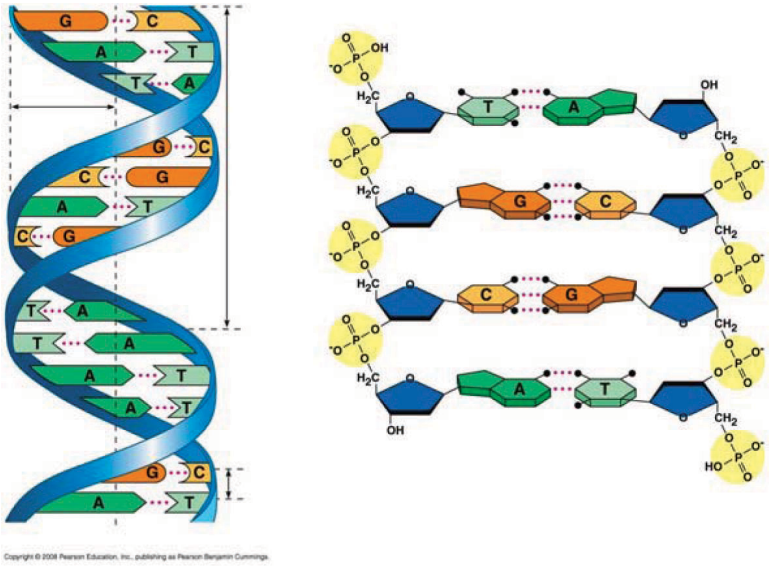
A chromosome consists of a DNA molecule packed together with proteins. The bacterial chromosome is a double-stranded, circular DNA molecule associated with a small amount of protein,while Eukaryotic chromosomes have double-stranded linear DNA molecules associated with a large amount of protein. Chromatinis a complex of DNA and protein and is found in the nucleus of eukaryotic cells. Histonesare proteins that are responsible for the first level of DNA packing in chromatin.

Fig. 16-21b





Organization of DNA within chromosome
(Source: Campbell 2008)



Structures of DNA
(Source: Campbell 2008)

Molecular Methods

Flowchart of Molecular Methods

DNA extraction

⇩ *agarose gel*

Gene amplification/PCR and purification

⇩ *agarose gel*

Cycle sequencing and purification

⇩ *agarose gel*

DNA sequencing

⇩

Phylogenetic analysis

Collecting Samples

There are some ways to collect samples for DNA extraction. The main principle to remember is to "kill" the cells as fast as possible to prevent any damage to the cells. If the living plants are near your lab, you can put the samples right away in CTAB buffer placed in an Eppendorf tube and bring to the lab for further processing. Liquid nitrogen helps in grinding the cells quickly and stopping any enzyme that will destroy the cells.

If you go to the field far away from the lab, the best method is to bring liquid nitrogen if possible or put the samples (5 x 5 cm2 leaf cut in about 1 x 1 cm2) in a tea bag in silica

gel inside a ziplock plastic bag. This would last some years, but secondary metabolites, which prevent the PCR process, are often encountered. Extraction from fresh material is still best so far for successful PCR process.

A kit is also available by scrubbing the samples on a card then it is punched and processed further according to the manual of instruction.

DNA Extraction from Plant Tissue

To extract DNA, several processes are involved, such as disrupting the cell; removal of protein, polysaccharides and other compounds; removal of CTAB; then finally precipitation of nucleic acid. There are several protocols that would suit best the samples we extract. The simple one is explained here, modified from Doyle & Doyle 1987.

Flowchart of Processes in DNA Extraction (Doyle & Doyle 1987)

Cell disruption
(mechanically by grinding, and chemically by 2x CTAB)

⇩

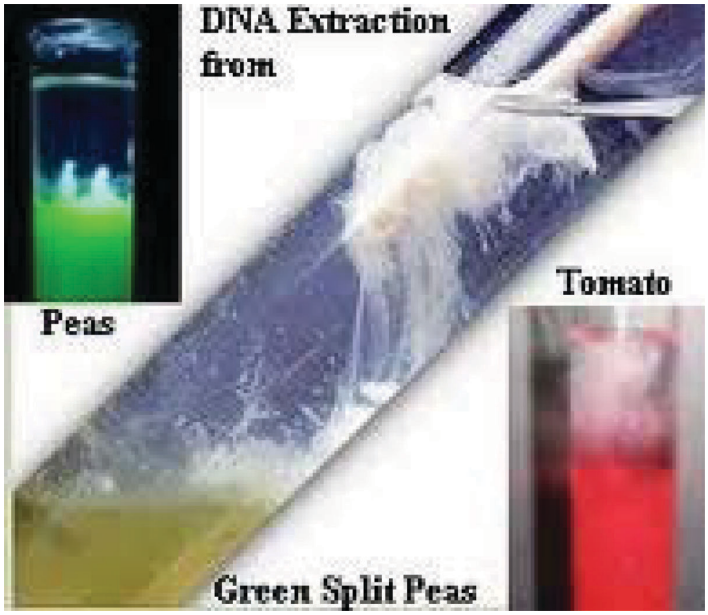
Removal of protein, polysaccharides, etc
(with chloroform-isoamyl alcohol)

⇩

Removal of CTAB
(with wash buffer)

⇩

Precipitation of nucleic acids
(with cold isopropanol)



DNA Extraction from peas, tomato and green split peas

Protocol of DNA Extraction Modified from Doyle & Doyle 1987

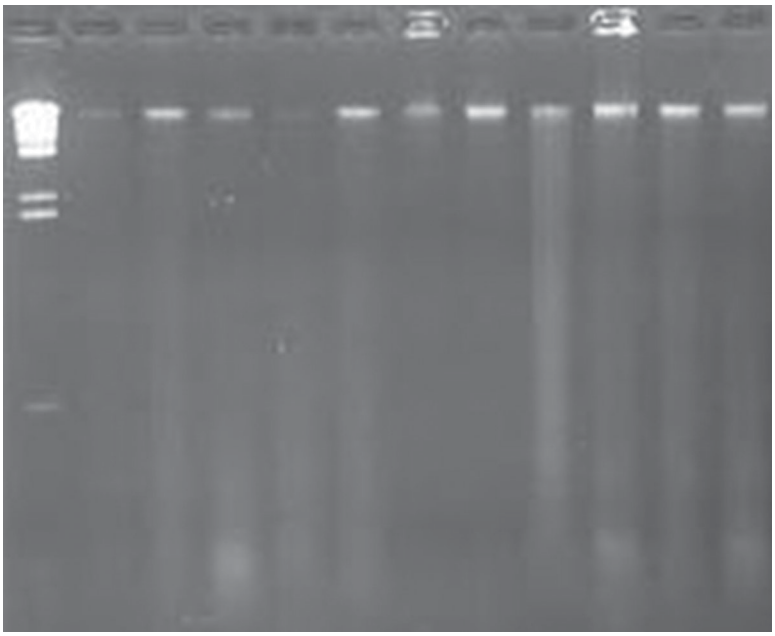
1. Label 1.5 ml Eppendorf tubes then place on a rack.
2. Prepare one circle cut out by punching fresh healthy leaves or silica gel dried material using 1.5 ml Eppendorf tube lid per sample.
3. Add a pinch of sterile sand.
4. In another 1.5 ml Eppendorf tube (one tube is needed for each DNA sample), preheat 500 µl 2x CTAB with 0.2% mercaptoethanol (1 µl) at 65oC in a water bath.
5. Using forceps, submerge the tube that contains leaf sample in liquid nitrogen (if any). If the lid is closed, openor pierce it.
6. Using a small plastic pestle, ground the tissue (sample) inside the tube to a fine dry powder. Then, dissolve the powder in 500 µl 2xCTAB and add a pinch of PVP.
7. If there is no liquid nitrogen, add half of the preheated 2X CTAB (250 µl) to the tube containing the samples, then ground the tissue using a small plastic pestle to a slurry green state. Add 250 µl more of 2xCTAB and a pinch of PVP.
8. Place the tube containing the mixture in a vortex for a few seconds, then incubate for an hour at 65oC in a water bath shaker to allow the cell to lyse for DNA liberation.
9. Take out the tubes from the water bath shaker and coolfor 10 minutes.
10. Centrifuge the samples at 13,000 rpm for 10 minutes at 20oC.
11. Gently remove the aqueous (upper) phase without removing any of the particulate matter, then place in a clean 1.5 ml Eppendorf tube.
12. Add 500 µl chloroform: isoamyl alcohol (24:1) and mix well by inversion.
13. Place the samples on a shaker for 20 minutes.
14. Centrifuge the samples at 13,000 rpm for 10 minutes at 20oC.
15. Remove the supernatant gently, being careful not to pick up any of the bottom layer, and place in a clean 1.5 ml eppendorf tube.
16. Repeat the above steps once again to re-extract the supernatant.
17. To precipitate the nucleic acids, add 2/3 volume (200-300 µl) freezer cold isopropanol (propan-2-ol) then mix well by gentle inversion.
18. Continue the mixing by inversion until the oily appearance of the mixture is gone.
19. Leave the samples at-20oC overnight.
20. Centrifuge the samples at 13,000 rpm for 10 minutes at 20oC.
21. Pour off the supernatant, being careful not to pour out the pellet.
22. Dry the pellet in a vacuum drier for 10 minutes. Do not over-dry the pellet as it will stick hard to the tube and will be difficult to dissolve.
23. Re-suspend the pellet to obtain the nucleic acid solution in 50 µl of TE buffer by flicking the tube with a finger. The genomic nucleic acids can be stored in the freezer until required.

Chemicals used and their function in DNA extraction process

- 2x CTAB buffer contains CTAB, sodium chlorides, EDTA, beta-mercaptoethanol and PVP.This extraction buffer is mostly to protect the DNA from degradation by native enzymes and secondary plant metabolites.It is also a cationic detergent that helps to lyse the cell membranes and will form complexes with nucleic acids.
- Sodium chlorides help the formation of nucleic acid-CTAB complexes.
- EDTA chelates divalent ions, especially Ca2+ and Mg2+, and prevents the activity of metal-dependant nucleases.
- Beta-mercaptoethanol is a reducing agent that protects the DNA against quinones, disulphites, peroxidases, and polyphenol oxidases.
- PVP will form complexes with secondary plant products, in particular, complex polyphenols, tannins, and quinones.

Visualizing DNA after extraction

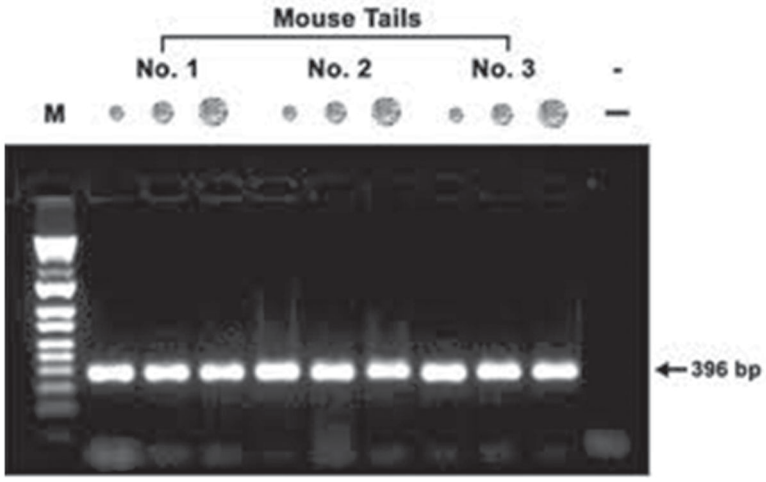
The technique called electrophoresis is used to visualize DNA to check the quality and quantity of the DNA obtained either from extraction or PCR product. Each sample is run on a 1% agarose gel (0.5 g agarosed) electrophoresis stained with Ethidium Bromide.



Agarose gel electrophoresis of DNA extraction product

Protocol of gel electrophoresis

1. Heat the agarose in TBE or TAE buffer in a microwave until all particles are dissolved. When boiling in the microwave, it is best to wait until the bubbles get bigger to avoid having small bubbles in the set gel.
2. When it is cooled, add 1 µl Ethidium Bromide then pour to a gel mould fitted with a gel comb.
3. When the gel is set, remove the comb so that wells are formed.
4. Mix 5 µl of extracted total genomic DNA with 3 µl loading solution then load in a well.
5. Load 5 µl DNA size marker in one side well of the samples to compare with the total genomic DNA.
6. Run in an electrophoretic field at 60-80V for 1-1.5 hours.
7. The negatively charged DNA will move to the positive electrode at a certain speed which depends upon the size of the molecules. Observe the bands of ethidium bromide corporated-DNA under UV light.
8. Document the results with a digital camera and print out.

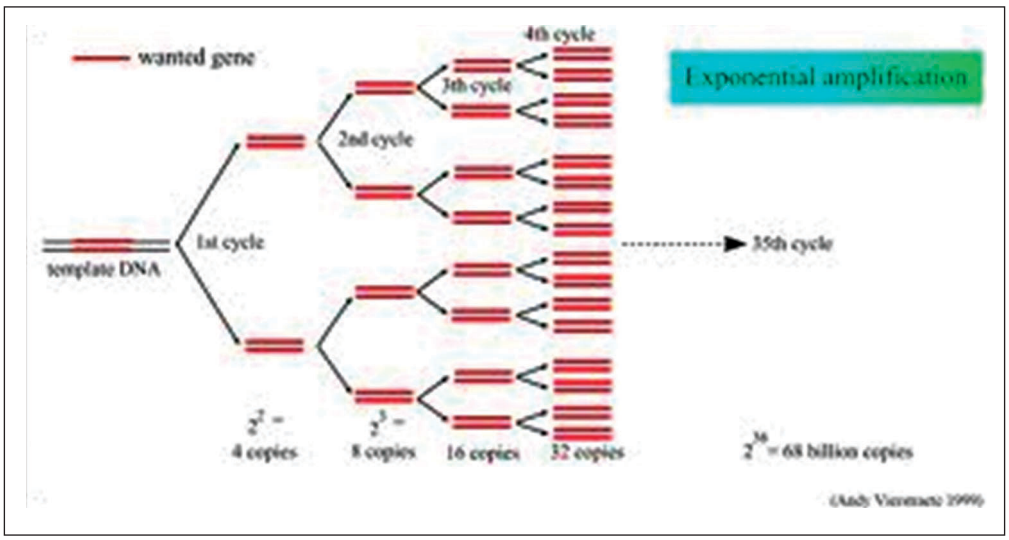


Agarose gel electrophoresis of PCR product

How to amplify DNA?

Polymerase Chain Reaction (PCR) to amplify DNA segment

Gene amplification to produce identical DNA copies is obtained via Polymerase Chain Reaction (PCR) technique. Three basic stages are involved, i.e. denaturation, annealing and synthesis or primer extension. The denaturation phase (high temperature) splits the double-stranded DNA into single-stranded DNA. The annealing phase involves lowering the temperature. In this phase, the oligonucleotide primers will bind to the single-stranded DNA. The third stage, synthesis stage, involves the binding of polymerase enzyme (Taq= Thermusaquaticuspolymerase) to deoxyribonucleotidetriphospates (dNTPs) and catalyze a reaction by attaching the nucleotides to single-stranded DNA.



PCR machine

Protocol of PCR

The PCR reaction mixtures of total volume of 50 µl in 0.2 ml PCR tube contains:

- 34.5 µl sterile distilled water
- µl of 2mM deoxyribonucleoside triphosphate (dNTP) mix
- 5.0 µl of 10x reaction buffer (160 mM (NH4)2SO4, 670 mMTrisHCl pH 8.8 at 25oC, 0.1% Tween-20)
- 2.5 µl of 50 mM MgCl2
- 1.5 µl of 10 mM Primer 1
- 1.5 µl of 10 mM Primer 2
- 0.25 µl of 5U/µl thermostable DNA polymerase
- 2 µl DNA template from aliquots of total genomic DNA

PCR cycle parameters for ITS (Internal Transcribed Spacer) amplification are as follows:

- Initial denaturation for 3 minutes at 94oC
- Denaturation of template DNA for 1 minute at 94oC
- Primer annealing for 1 minute at 55oC
- Primer extension for 1.5 minute at 72oC

After 30 cycles, a final extension step of 5 minutes at 72oC is added. This extension is meant to allow completion of unfinished strands.

Gel electrophoresis (method described previously) at 60-80 V for 1-1.5 hours using 1.5 µl of PCR products will be carried out to check successful amplification and quantity of PCR products. DNA size marker 100 bpladder is used for comparison of amplified DNA obtained.

Purification of PCR Product

Purification means to purify the DNA obtained from any unwanted artefacts from PCR such as primers, unincorporated nucleotide, polymerase and salts. There are several ways to do purification:one is by adding polyethylene glykol or using ready kit, for example QIAquick™ PCR Purification Kit. Follow the protocol provided by the kit if you use it.

Cycle sequencing and purification of sequencing reactions

Protocol of cycle sequencing

Cycle sequencing is performed prior to automated sequencing. The 20 µl sequencing PCR mixture contains:

- 13 µl of sterile distilled water
- 4 µl of Thermo Sequenase II reagent Premix
- 1 µl of 5 mM of one primer type
- 2 µl of DNA template (from purified PCR products)

The samples are then placed in a thermal cycler and run for 25 cycles with the following PCR conditions:

- Denaturation step for 10 seconds at 94oC
- Primer annealing for 5 seconds at 50oC
- Primer extension for 4 minutes at 60oC

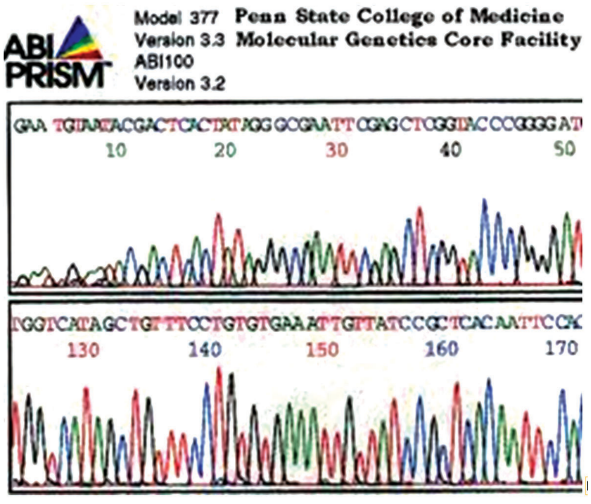
Protocol of purification of sequencing reactions

The results of cycle sequencing are purified according to the following procedure.

- Transfer the 20 µl of PCR cycle sequencing products to a fresh 0.5 ml tube containing 2 µl of sodium acetate/EDTA buffer.
- Add 55 µl of 100% cold (-20oC) ethanol to each reaction.
- Mix briefly with a vortex mixer and place on ice for 15-20 min to precipitate the DNA.
- Centrifuge in a microcentrifuge for 15 minutes at 13,000 rpm.
- Remove the supernatant formed as much as possible.
- Add 250 µl of cold 70% ethanol to wash the pellet, then centrifuge at the same speed for 5 min.
- Remove again as much as possible the supernatant formed.
- Vacuum-dry the pellet that remains at the bottom of the tube in a vacuum centrifuge for 2-5 minutes.

Automated DNA Sequencing

Gel preparation and loading for automated DNA sequencing is performed on an ABI automatic DNA Sequencer according to the manual supplied.



Electropherogram of DNA sequences

PCR based techniques

- nucleotide sequences
- amino acids
  - synonymous (silent) vs. non-synonymous mutations
- micro-satellites (few base pair repeats)
- co-dominant markers
  - 'DNA fingerprinting'
  - RFLP, AFLP, RAPD, ISSR

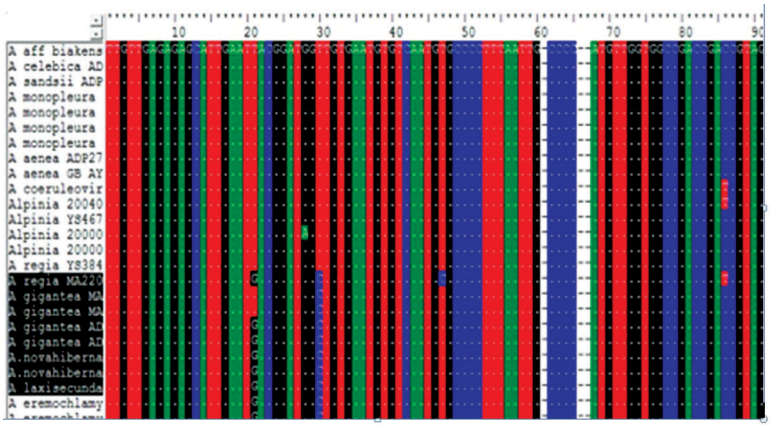
SEQUENCE ANALYSIS

Sequence Editing

The sequence boundaries of each region studied are compared with the results of existing study or compared with the sequences in the gene bank. Each region should be confirmed from forward and reverse sequences. There are several free programs to edit sequences such as 4Peaks for Macintosh or BioEdit for Windows. Licensed programs with additional function to assemble the complementary strands automatically from forward and reverse sequences are available such as Chromas, Genious, etc. These programs can build a consensus sequences. Ambigious bases are edited manually after observing the bases from forward and reverse sequences.

Sequence alignment

Sequence alignment is carried out using Clustal program. Other program called Muscle is also powerful for sequence alignment. These alignments are subsequently refined by eye.



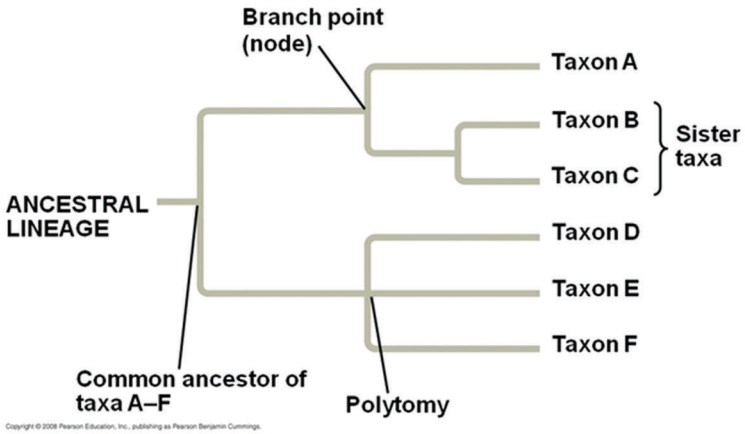
Alignment of DNA Sequences

PHYLOGENETIC ANALYSIS

Phylogeny and the Tree of life

Phylogeny is the evolutionary history of a species or group of related species. WilliHennig (1913-1976) introducedphylogenetic systematic. In 1950, he wrote GrundzügeeinerTheorie derPhylogenetischenSystematik. It was in 1966 that Phylogenetic Systematics became known.

Phylogeny is based upon evolutionary relationships, i.e., upon common ancestry. A cladogram is a tree diagram which depicts hypothesised evolutionary history, while a phylogram is a tree which indicates by branch length the degree of change believed to have occurred along each lineage.



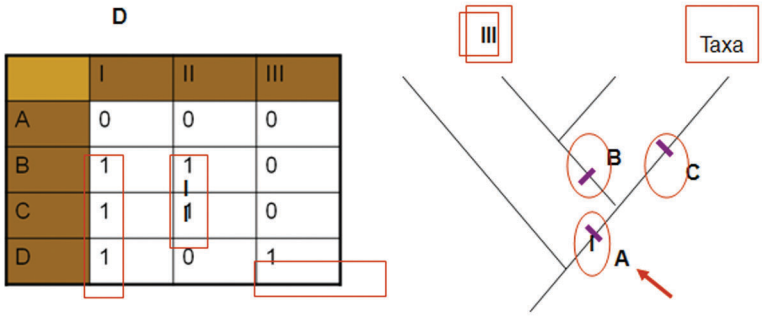
A phylogenetic tree represents a hypothesis about evolutionary relationships. Each branchpointrepresents the divergence of two species. Sister taxaare groups that share an immediate common ancestor.

A rootedtree includes a branch to represent the last common ancestor of all taxa in the tree. A polytomyis a branch from which more than two groups emerge. Phylogenies are inferred from morphological and molecular data. To infer phylogenies, systematists gather information about morphologies, genes, and biochemistry of living organisms.

Morphological and Molecular Homologies

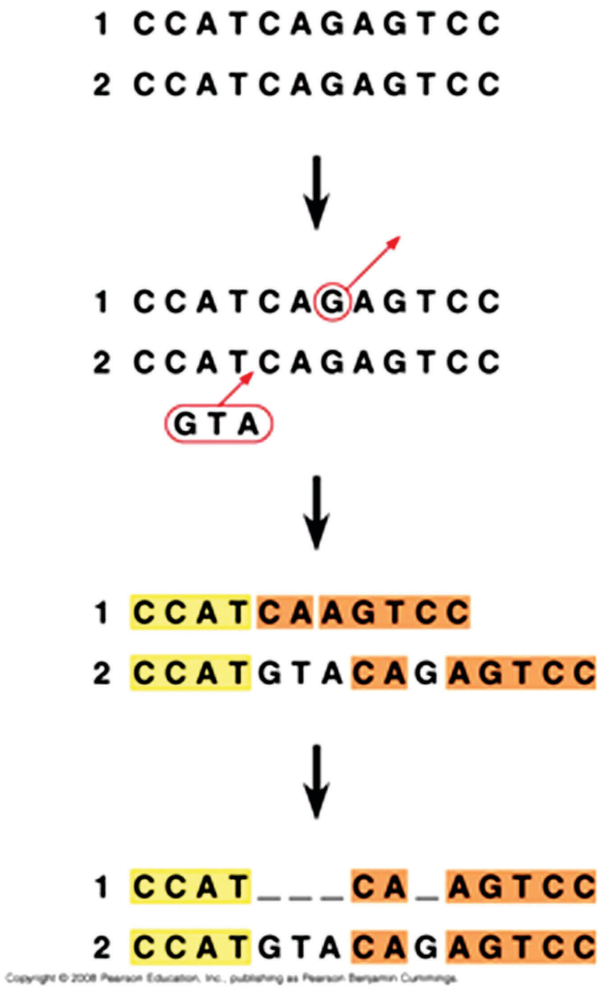
Organisms with similar morphologies or DNA sequences are likely to be more closely related than organisms with different structures or sequences. When constructing a phylogeny, systematists need to distinguish whether a similarity is the result of homology or analogy. Homology is similarity due to shared ancestry. Analogy is similarity due to convergent evolution.

Cladistics-Example



Evaluating Molecular Homologies

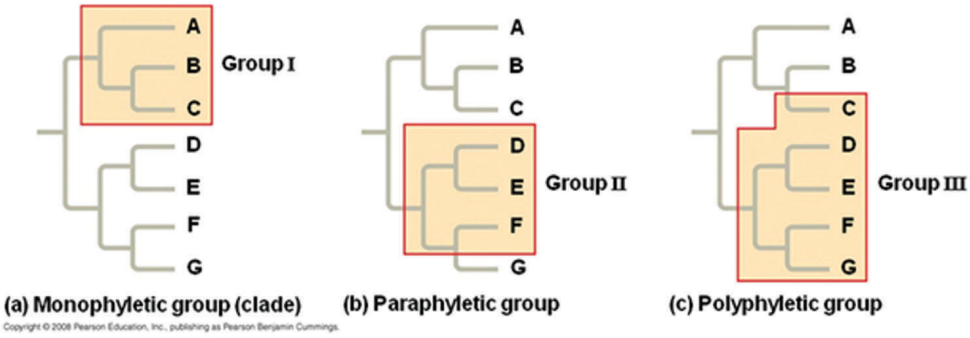
Systematists use computer programs and mathematical tools when analyzing comparable DNA segments from different organisms.



It is also important to distinguish homology from analogy in molecular similarities. Mathematical tools help to identify molecular homoplasies, or coincidences. Molecular systematics uses DNA and other molecular data to determine evolutionary relationships. Once homologous characters have been identified, they can be used to infer a phylogeny.

Cladistics

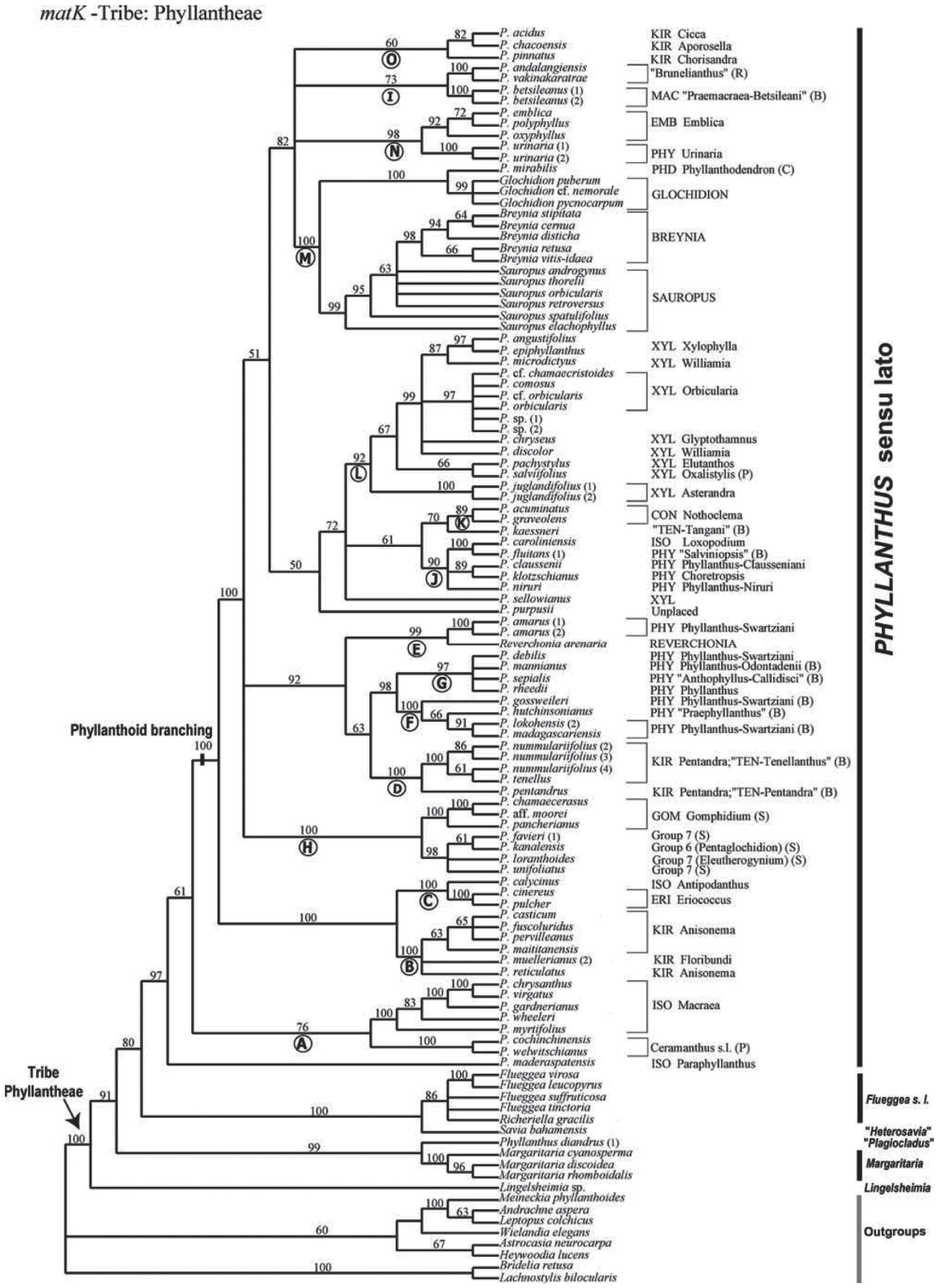
Cladistics group organisms by common descent.A clade is a group of species that includes an ancestral species and all its descendants. Clades can be nested in larger clades, but not all groupings of organisms qualify as clades. A valid clade is monophyletic, signifying that it consists of the ancestor species and all its descendants.



A valid clade is **monophyletic,** signifying that it consists of the ancestor species and all its descendants.

A **paraphyletic** grouping consists of an ancestral species and some, but not all, of the descendants.

A **polyphyletic** grouping consists of various species that lack a common ancestor.


matK -Tribe: Phyllantheae
P. acidus
P. chacoensis
P. pinnatus
P. andalangiensis
P. vakinakaratrae
P. betsileanus (1)
P. betsileanus (2)
P. emblica
P. polyphyllus
P. oxyphyllus
P. urinaria (1)
P. urinaria (2)
P. mirabilis
Glochidion puberum
Glochidion cf. nemorale
Glochidion pycnocarpum
Breynia stipitata
Breynia cernua
Breynia disticha
Breynia retusa
Breynia vitis-idaea
Sauropus androgynus
Sauropus thorelii
Sauropus orbicularis
Sauropus retroversus
Sauropus spatulifolius
Sauropus elachophyllus
P. angustifolius
P. epiphyllanthus
P. microdictyus
P. cf. chamaecristoides
P. comosus
P. cf. orbicularis
P. orbicularis
P. sp. (1)
P. sp. (2)
P. chryseus
P. discolor
P. pachystylus
P. salviifolius
P. juglandifolius (1)
P. juglandifolius (2)
P. acuminatus
P. graveolens
P. kaessneri
P. caroliniensis
P. fluitans (1)
P. claussenii
P. klotzschianus
P. niruri
P. sellowianus
P. purpusii
P. amarus (1)
P. amarus (2)
Reverchonia arenaria
P. debilis
P. mannianus
P. sepialis
P. rheedii
P. gossweileri
P. hutchinsonianus
P. lokohensis (2)
P. madagascariensis
P. nummulariifolius (2)
P. nummulariifolius (3)
P. nummulariifolius (4)
P. tenellus
P. pentandrus
P. chamaecerasus
P. aff. moorei
P. pancherianus
P. favieri (1)
P. kanalensis
P. loranthoides
P. unifoliatus
P. calycinus
P. cinereus
P. pulcher
P. casticum
P. fuscoluridus
P. pervilleanus
P. maititanensis
P. muellerianus (2)
P. reticulatus
P. chrysanthus
P. virgatus
P. gardnerianus
P. wheeleri
P. myrtifolius
P. cochinchinensis
P. welwitschianus
P. maderaspatensis
Flueggea virosa
Flueggea leucopyrus
Flueggea suffruticosa
Flueggea tinctoria
Richeriella gracilis
Savia bahamensis
Phyllanthus diandrus (1)
Margaritaria cyanosperma
Margaritaria discoidea
Margaritaria rhomboidalis
Lingelsheimia sp.
Meineckia phyllanthoides
Andrachne aspera
Leptopus colchicus
Wielandia elegans
Astrocasia neurocarpa
Heywoodia lucens
Bridelia retusa
Lachnostylis bilocularis
KIR Cicca
KIR Aporosella
KIR Chorisandra
"Brunelianthus" (R)
MAC "Praemacraea-Betsileani" (B)
EMB Emblica
PHY Urinaria
PHD Phyllanthodendron (C)
GLOCHIDION
BREYNIA
SAUROPUS
XYL Xylophylla
XYL Williamia
XYL Orbicularia
XYL Glyptothamnus
XYL Williamia
XYL Elutanthos
XYL Oxalistylis (P)
XYL Asterandra
CON Nothoclema
"TEN-Tangani" (B)
ISO Loxopodium
PHY "Salviniopsis" (B)
PHY Phyllanthus-Clausseniani
PHY Choretropsis
PHY Phyllanthus-Niruri
XYL
Unplaced
PHY Phyllanthus-Swartziani
REVERCHONIA
PHY Phyllanthus-Swartziani
PHY Phyllanthus-Odontadenii (B)
PHY "Anthophyllus-Callidisci" (B)
PHY Phyllanthus
PHY Phyllanthus-Swartziani (B)
PHY "Praephyllanthus" (B)
PHY Phyllanthus-Swartziani (B)
KIR Pentandra;"TEN-Tenellanthus" (B)
KIR Pentandra;"TEN-Pentandra" (B)
GOM Gomphidium (S)
Group 7 (S)
Group 6 (Pentaglochidion) (S)
Group 7 (Eleutherogynium) (S)
Group 7 (S)
ISO Antipodanthus
ERI Eriococcus
KIR Anisonema
KIR Floribundi
KIR Anisonema
ISO Macraea
Ceramanthus s.l. (P)
ISO Paraphyllanthus
PHYLLANTHUS sensu lato
Flueggea s. l.
"Heterosavia"
"Plagiocladus"
Margaritaria
Lingelsheimia
Outgroups
Phyllanthoid branching
Tribe
Phyllantheae

Computer Software Packages Available for Data Analysis

Phylogeny Programs – Joe Felsenstein's site lists 387 phylogeny reconstruction packages and 28 free servers (Department of Genome Sciences and the Department of Biology at the University of Washington)

PAUP* 4.0 – Phylogenetic Analysis Using Parsimony and other Methods

PHYLIP – package of programs for inferring phylogenies

MacClade – A useful software package for Phylogenetic Analysis

Mesquite – a modular system for evolutionary analysis, by W. P. Maddison and D. R. Maddison

Hennig86 – A PC-DOS program for phylogenetic analysis

MEGA – Molecular Evolutionary Genetics Analysis. MEGA is a DOS program for analyzing molecular data. Developed by Sudhir Kumar, Koichiro Tamura and Masatoshi Nei (1993).

MrBayes – is a program for constructing phylogenetic trees by Bayesian method (Huelsenbeck 2000)

DAMBE – Data Analysis in Molecular Biology and Evolution, developed by X. Xia at the University of Hong Kong

LVB – Reconstructing evolution with parsimony and simulated annealing

Other Programs – Maintained by Dr. Joe Felsenstein, Department of Genetics, The University of Washington

Clann– Constructing **Supertrees** from partially-overlapping datasets, designed and written by Chris Creevey at the Bioinformatics and Pharmacogenomics Laboratory at NUI Maynooth

SuperTree software by Nicolas Salamin, Biophore - 1015 Lausanne - Switzerland

SuperTree Server by Mike Sanderson, D. Gusfield, and Oliver Eulenstein, Computational Biology Laboratory, Department of Computer Science Iowa State University, Ames, IA

SuperTree software by Roderic D. M. Page, DEEB, IBLS, University of Glasgow, Glasgow G12 8QQ, United Kingdom

TreeBASE by Bill Piel, M. Donoghue and Mike Sanderson. TreeBASE is a relational database of phylogenetic information hosted by the Yale Peabody Museum

APPENDIX

1. Directory of Participants to the Training Workshop on Taxonomy of Terrestrial Plants
2. Training Course Programme
3. Lectures and Powerpoint presentations during the training course
4. Group Reports
5. Pre- and Post-Training Evaluation forms

APPENDIX 1 – Directory of Participants to the Training Workshop on Taxonomy of Terrestrial Plants

PARTICIPANTS

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APPENDIX 2 – Training Course Programme

TRAINING-WORKSHOP ON PLANT TAXONOMY
RC Biology LIPI - Indonesia, 16 - 23 February 2011

	15/02/2011		Participants arrival at CSC, Indonesia		
Day 1	16/02/2011	08.00 -09.00	Registration of participants	Committee	LMR
		09.00-09.30	Opening Ceremony Welcome remarks Opening Message	MC: Wita Wardani LIPI: Prof. Dr. Bambang Prasetya ACB/JAIF: Dr. Clarissa Arida JWRC/MoE Japan: Dr. Masaaki Yoneda MoE Indonesia: Dr. Arief Juwono	LMR
		09.30-10.00	Introduction of participants Overview of the training Workshop (Photo opportunity)	Dr. Dedy Darnaedi JAIF-ACB Project ESABII-GTI-JWRC	LMR
		10.00-10.30	Break		
		10.30-12.00	General Lecture on Plant Taxonomy	TT	LMR
		12.00-13.00	Lunch		SMR
		13.00-15.00	General Lecture on Biogeography and lecture on Ericaceae	EF	LMR LMR
		15.00-15.20	Break		
		15.20-17.00	Visit Herbarium Collection	HW	LMR
Day 2	17/02/2011	08.00-08.30	Registration of participants		LMR
		08.30-10.00	General lecture on Plant Morphology of Dicotyledoneae and lecture on Sapotaceae	TT	LMR
		10.00-10.30	Break		LMR
		10.30-12.00	Lecture on Plant Age	ES	LMR
		12.00-13.00	Lunch		SMR
		13.00-15.00	Lecture on Begoniaceae, Fabaceae, Rosaceae&Rubiaceae	HW	LMR
		15.00-15.30	Break		LMR
		15.30-16.15	Lecture on Cucurbitaceae	R	LMR
		16.15-17.00	Lecture on Balsaminaceae	NU	LMR

Day 3	18/02/2011	08.00-08.30	Registration of participants		LMR
		08.30-10.00	Lecture on Cytology	DD	LMR
		10.00-10.30	Break		LMR
		10.30-12.00	Lecture on Molecular Systematic	MA	LMR
		12.00-13.00	Lunch		SMR
		13.00-15.00	Lecture on Database IBIS and visit Database Room	BH/BBM	LMR & DBR
		15.00-15.30	Break		
		15.30-17.00	Working with herbarium specimens	HW, R, NU	BSL
Day 4	19/02/2011	08.00-08.30	Registration of participants		
		08.30-12.00	Working on Cytology (group I) Working on Molecular Analyses (group II)	DD, UH MA, WW, YS	LC LMS
		12.00-13.00	Lunch		SMR
		13.00-16.30	Working on Cytology (group II) Working on Molecular Analyses (group I)	DD, UH MA, WW, YS	LC LMS
Day 5	20/02/2011	08.00-08.30	Registration of participants		LMR
		08.30-10.00	Lecture on Ecology and Forest Dynamic	ES	LMR
		10.00-10.30	Break		LMR
		10.30-12.00	Sampling method	ES, HW	LMR
		12.00-13.00	Lunch		SMR
		13.00-15.00	Lecture on Fagaceae	SF	LMR
		15.00-15.30	Break		LMR
		15.30-17.00	Preparation for field trip	ES, HW	LMR
Day 6	21/02/2011	06.30-07.00	Registration of participants		LMR
		07.00-09.00	Move to Cibodas Bot. Gard.	Committee	CBG
		09.00-10.00	Break		CBG
		10.00-15.00	Visit Gunung Gede-Pangrango National Park and Cibodas Botanical Garden	Committee	GGNP CBG
		15.00-15.30	Break		CBG
		15.30-17.00	Data Analysis	ES	CBG
		17.00-21.00	Dinner & Discussion	Committee	CBG

Day 7	22/02/2011	07.00-08.00	Breakfast		CBG
		08.00 -12.00	Sample collections & Processing herbarium	HW	CBG
		12.00-13.00	Lunch and packing		CBG
		13.00-16.00	Back to CSC	Committee	CBG
		16.00 -.....	Preparing report and presentation (each group)		
Day 8	23/02/2011	08.00-08.30	Registration of participants		LMR
		08.30-10.00	Preparing report and presentation (each group)		LMR
		10.00.10.30	Break		LMR
		10.30-12.00	Preparing report and presentation (each group)		LMR
		12.00-13.00	Lunch		SMR
		13.00-15.00	Group presentation and discussion		LMR
		15.00-15.30	Break		LMR
		15.30-17.00	Feed back from participants		LMR
			Closing remarks: JWRC ACB LIPI : Director of RC Biology		LMR
	24/02/2011		Departure		