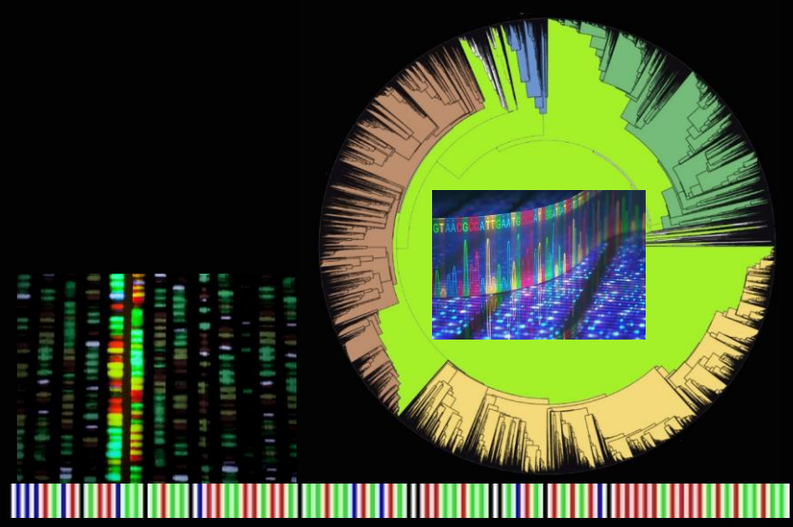
ADVANCE EXPERIMENTAL TAXONOMY

(BOT 621)

Capparis rotundifolia Rottler



Professor (Dr.) M. Ajmal Ali, PhD

Department of Botany and Microbiology
College of Science, King Saud University
P Box 2455,Riyadh-11451, Saudi Arabia
Email: alimohammad@ksu.edu.sa

هيئة تقويم التعليم والتدريب
Education & Training Evaluation Commission

اعتماد
NCAA

2024
TPG-153

Course Specification

(Postgraduate Programs)

Course Title:	*Advanced Experimental Taxonomy*
Course Code:	*BOT621*
Program:	*Ph.D. in Botany*
Department:	*Botany and Microbiology*
College:	*Science*
Institution:	*King Saud University*
Version:	4th
Last Revision Date:	2/2/2026

هيئة تقويم التعليم والتدريب
Education & Training Evaluation Commission

Table of Contents

A. General information about the course:

1. Course Identification:

1. Credit hours: 2 (2+0)

2. Course type

A.	☐University	☐College	☒ Department	☐Track
B.	☐ Required		☒ Elective	

3. Level/year at which this course is offered: (1st level/ 1st year)

4. Course General Description:

The course will detail the theory and practices of classic and modern plant systematics.

5. Pre-requirements for this course (if any)**:**

None

6. Pre-requirements for this course (if any)**:**

None

7. Course Main Objective(s):

To introduce the students to the explanation of advances in tools and techniques, and relevance of molecular plant taxonomy to understand the principles of Plant taxonomy

2. Teaching Mode: (mark all that apply)

No	Mode of Instruction	Contact Hours	Percentage
1	Traditional classroom	30	100%
2	E-learning		
3	Hybrid • Traditional classroom • E-learning	0	0
4	Distance learning	0	0

3. Contact Hours: (based on the academic semester)

No	Activity	Contact Hours
1.	**Lectures**	30
2.	**Laboratory/Studio**	
3.	**Field**	
4.	**Tutorial**	
5.	**Others (specify).....**	
	Total	30

هيئة تقويم التعليم والتدريب
Education & Training Evaluation Commission

Code	Course Learning Outcomes	Code of CLOs aligned with program	Teaching Strategies	Assessment Methods
1.0	Knowledge and understanding. At the end of the program, the graduate will be able to			
1.1	Analyze taxonomic evidence derived from morphological, anatomical, chemical, and molecular data to resolve complex taxonomic problems.	K2, K3	Advanced lectures, guided reading, case studies, and group discussions	Written reports, assignments, and a final exam
1.2	Evaluate contemporary theories, methodologies, and technologies in experimental and molecular plant taxonomy.	K3, K4	Seminars, literature review sessions, student-led discussions	Literature review report, oral presentation
2.0	Skills			
2.1	Synthesize scientific literature in plant taxonomy and molecular systematics to develop research-based arguments.	S3, S4	Journal club, guided reading, group discussions, research writing workshops	Literature review paper, research proposal mini-project, written assignment rubric
2.2	Communicate scientific findings effectively through oral presentations, written reports, and scholarly discussions.	S4, S6	Student-led presentations, peer-review sessions, academic writing workshops, and seminar discussions	Oral presentation rubric, written report rubric, participation and discussion assessment
3.0	Values, autonomy, and responsibility			
3.1	Apply ethical principles and research integrity standards in taxonomic and molecular research.	V1	Ethics case studies, research integrity workshops, guided discussions, policy brief reviews	Ethics reflection report, plagiarism check, rubric-based assessment, participation evaluation
3.2	Collaborate effectively to plan and execute advanced research tasks,	V2	Group research projects, collaborative learning	Peer evaluation rubric, group project report, participation

	demonstrating accountability and professional conduct.		activities, mentoring sessions, peer-learning workshops	assessment, teamwork performance rubric

B. Course Learning Outcomes (CLOs), Teaching Strategies and Assessment Methods:

C. Course Content:

No	List of Topics	Contact Hours
1.	Principles and practices of Plant taxonomy	2
2.	Taxonomic circumscription and the need for taxonomic evidences	4
3.	Structural information as a source of taxonomic evidences	4
4.	Non-structural information as a source of taxonomic evidences	6
5.	Molecular Systematics (tools and techniques of sampling to sequencing), Choosing a molecular marker for phylogenetic analyses	6
6.	Phylogenetic analyses: DNA sequences as taxonomic evidence, Interpretation of molecular phylogenetic trees	4
7.	Assessment of genetic diversity using molecular data, DNA barcoding	4
	Total	30

D. Students Assessment Activities:

No	Assessment Activities *	Assessment timing (in week no)	Percentage of Total Assessment Score
1.	1st presentation	4th	20%
2.	2ND presentation	8th	20%
3.	3ed presentation	12th	20%
4.	Final exam	14th -15th	40%

*Assessment Activities (i.e., Written test, oral test, oral presentation, group project, essay, etc.)

E. Learning Resources and Facilities:

1. References and Learning Resources:

Essential References	Michael G. Simpson (2010) Plant Systematics. Elsevier Science Publishing Co Inc, San Diego, United States (ISBN10 012374380X).
Supportive References	
Electronic Materials	▪ American Journal of Botany (http://www.amjbot.org)

	▪ Botanical Journal of the Linnaean Society (http://www.blackwellpublishing.com/jnl_default.asp) ▪ Molecular Biology & Evolution (http://mbe.oupjournals.org) ▪ Molecular Phylogenetics & Evolution (http://www.elsevier.com) ▪ Systematic Botany (http://www.sysbot.org/) ▪ Taxon (http://www.botanik.univie.ac.at/iapt/s_taxon.php) ▪ http://www.plantsystematics.org/index.html ▪ The plant list (http://www.theplantlist.org/) ▪ NCBI (https://www.ncbi.nlm.nih.gov/) ▪ Phylogeny programs (http://evolution.genetics.washington.edu/phylip/software.html) ▪ Flora of Saudi Arabia (http://plantdiversityofsaudiarabia.info/Biodiversity-Saudi-Arabia/Flora/Flora.htm) ▪ https://www.tropicos.org/home ▪ Online Resources for Taxonomic Research (https://stories.rbge.org.uk/archives/1002) ▪ Eflora (http://www.efloras.org/flora_page.aspx?flora_id=2) ▪ Royal Botanic Gardens, Kew (https://www.kew.org/)
Other Learning Materials	Molecular phylogenetic analysis software (BioEdit, ClustalX, MEGA) is available with the instructor.

2. Educational and Research Facilities and Equipment Required:

Items	Resources
facilities (Classrooms, laboratories, exhibition rooms, simulation rooms, etc.)	Classrooms, laboratories
Technology equipment (Projector, smart board, software)	Projector & smart board
Other equipment (Depending on the nature of the specialty)	• A computer with a digital camera and suitable programs -New laboratory equipment for plant surface

F. Assessment of Course Quality:

Assessment Areas/Issues	Assessor	Assessment Methods
Effectiveness of teaching	The students	Indirect
Effectiveness of students assessment	Faculty	Direct
Quality of learning resources	Faculty & students	Indirect
The extent to which CLOs have been achieved	Program Leaders	Direct
Other		

Assessor (Students, Faculty, Program Leaders, Peer Reviewer, Others (specify)
Assessment Methods (Direct, Indirect)

G. Specification Approval Data:

COUNCIL/ COMMITTEE	ACADEMIC ACCREDITATION COMMITTEE OF THE DEPARTMENT OF BOTANY AND MICROBIOLOGY
REFERENCE NO.	
DATE	2 FEBRUARY 2026

Course Assignment Details – BOT 621

Semester: 1447 AH – 2nd Semester

Assignment Components

Assignment Title	Mode of Submission	Marks	Notes
Research project on a topic related to **Experimental Taxonomy**	Written / Oral presentation	**30**	—
• Report writing	Written	**15**	—
• Oral presentation	Oral	**10**	—
• Academic integrity	< %15	**5**	—
Submission of **15 identified herbarium specimens**	Practical	**30**	Group cooperation in the collection and identification; each student submits individually
Final Exam	Written	**40**	—
Total		**100**	

Research Topics and Student Names

Research Topic	Student Name
Taxonomic Keys	
Scientific Nomenclature	
Numerical Taxonomy	
Anatomical Taxonomy	
Chemical Taxonomy	

Mona S. Alwahibi

1. INTRODUCTION

- Taxonomy is the science concerned with identification, nomenclature, and classification of organisms.
- Plant taxonomy is essential for understanding biodiversity and evolutionary relationships.
- Traditional taxonomy mainly relied on morphological and anatomical characters.
- Advances in molecular biology have transformed taxonomy into an interdisciplinary science.
- The integration of classical and molecular approaches is called advanced experimental taxonomy.
- Experimental taxonomy uses controlled experiments and analytical techniques for classification.
- Cytological, biochemical, and molecular data help resolve complex taxonomic problems.
- This approach is important in groups showing hybridization and phenotypic plasticity.
- DNA sequencing and molecular phylogenetics have revolutionized modern taxonomy.
- Experimental taxonomy has become predictive, analytical, and integrative.

2. Principles and Practices of Plant Taxonomy

- ❑Plant taxonomy deals with identification, naming, and classification of plants.
- ❑It organizes plant diversity into natural hierarchical groups.
- ❑Taxonomy follows the International Code of Nomenclature rules.
- ❑Morphological characters are traditionally used for identification.
- ❑Herbarium specimens are important reference materials.
- ❑Identification keys help distinguish taxa using diagnostic characters.
- ❑Taxonomy integrates field observations with laboratory studies.
- ❑Classification systems aim to reflect evolutionary relationships.
- ❑Modern taxonomy combines classical and molecular approaches.
- ❑Accurate taxonomy supports biodiversity research and conservation.

3. Classical Plant Taxonomy
3.1 Concept and Scope

- Classical taxonomy is based mainly on observable morphological characters.
- Characters include leaves, flowers, fruits, seeds, and anatomy.
- Early taxonomists such as Linnaeus established foundational systems.
- Field observations and herbarium studies are essential methods.
- Dichotomous keys are widely used for plant identification.
- Classical taxonomy is simple and cost-effective.
- It is widely applied in floristic and biodiversity studies.
- Morphological characters are accessible under field conditions.
- It provides the base for modern systematics.
- Classical taxonomy remains essential for practical identification.

3.2 Limitations of Classical Taxonomy

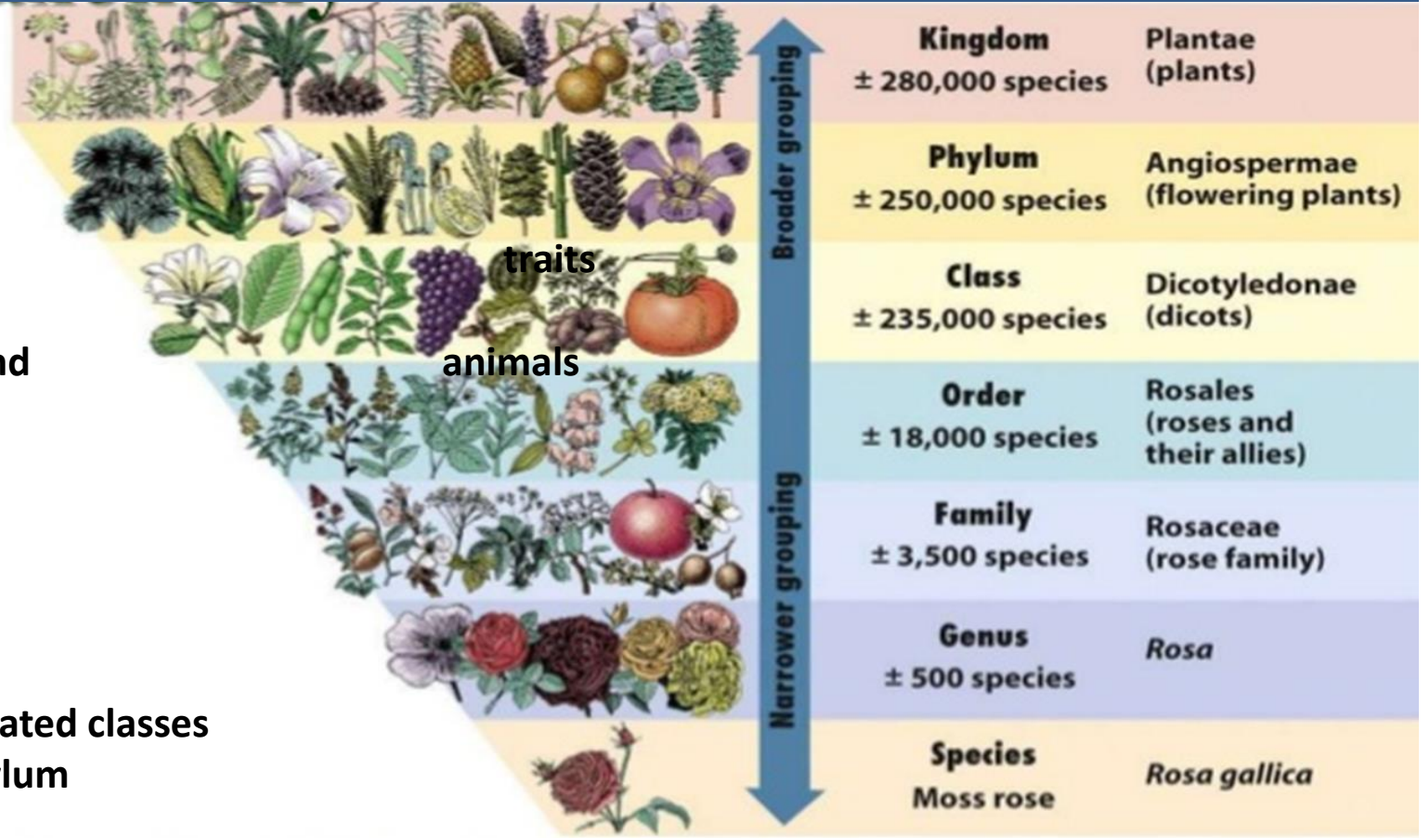
- ❑Morphological characters can be influenced by environmental conditions.
- ❑Phenotypic plasticity may lead to misidentification.
- ❑Convergent evolution may create misleading similarities.
- ❑Closely related species may appear morphologically similar.
- ❑Hybridization complicates species delimitation.
- ❑Polyploidy causes classification challenges.
- ❑Cryptic species remain hidden under morphological approaches.
- ❑Asexual reproduction reduces diagnostic variability.
- ❑Artificial groupings may arise from limited data.
- ❑Molecular approaches are required to overcome these limitations.

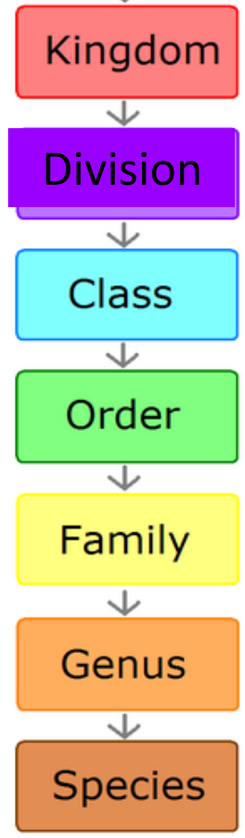
4. Taxonomic Circumscription and Need of Taxonomic Evidences

- Taxonomic circumscription defines boundaries of taxa.
- Species delimitation requires careful evaluation of variation.
- Single-character classification often causes errors.
- Multiple evidences improve taxonomic accuracy.
- Morphological similarity does not always indicate relatedness.
- Genetic data clarify taxonomic boundaries.
- Circumscription depends on species concepts.
- Taxonomic evidences provide objective classification.
- Integrative taxonomy combines multiple data sources.
- Proper circumscription ensures classification stability.

TAXONOMIC HIERARCHY

- Carrolus Linnaeus first adopted the hierarchic system of taxonomy classification in 1753.
- The succession groups are as follow:
- **Species**: Organisms sharing a set of biological traits and reproducing only their exact kind.
- The lowest major group, representing plants and animals referred to as Species.
- **Species is the fundamental unit in taxonomy**
- **Genus**: Genus are the closely related species
- **Family** : Family is the closely related genera
- **Order** : Order is the closely related families
- **Class** : Class are the closely related order
- **Division / Phylum**: Division or Phylum is the related classes
- **Kingdom**: Kingdom is the related Division / Phylum





Objective / Goals / Aims of Plant Taxonomy

- ❑ To provide an inventory of plant taxa for local, regional or continental needs.
- ❑ To establish suitable method for identification, nomenclature and description of plant taxa.
- ❑ Classification of organism into classes, Order, Families, Genera, and species
- ❑ To provide significantly valuable information concerning wild and medicinal species, endangered species, unique plants, genetic and ecological diversity

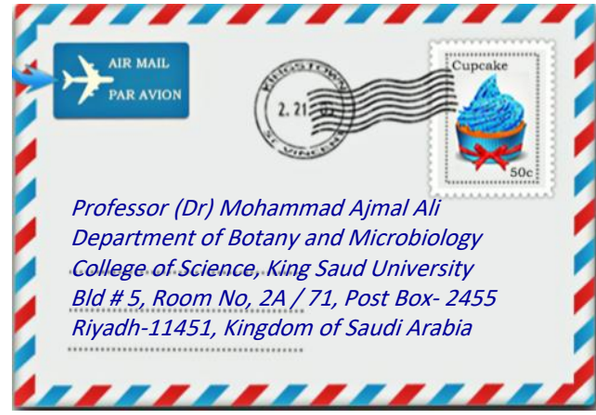
Scope of Taxonomy

- ❖ Taxonomy is one of the oldest sciences.
- ❖ It provides thorough knowledge of living species and their various forms.
- ❖ All the branches of biology are dependent on taxonomy for proper identification the species.
- ❖ It has been proceeded further incorporating data from phytochemistry, cyto-genetics supported by proper computation.

Basic components (Principles) of Plant Taxonomy / Plant Systematics

- Plant collection, Preservation and Documentation
- Plant Structure (Taxonomic Terminology, Taxonomic description of external and internal morphology)
- Taxonomic Identification
- Scientific Nomenclature / Botanical nomenclate : Nomenclature deals with the application of a correct name to a plant or a taxonomic group. Scientific names are necessary because the same common name is used for different plants in different areas of the world.
- Taxonomic Classification (History and Systems of Plant Classification)
- Taxonomic evidences / Source of data (Morphology, Anatomy, Embryology, palynology, Micromorphology, Chemistry, DNA etc.) in plant taxonomy

Kingdom: Plantae
Class: Angiosperms
Order: Arecales
Family: Arecaceae
Genus: *Phoenix*
Species: *P. dactylifera*



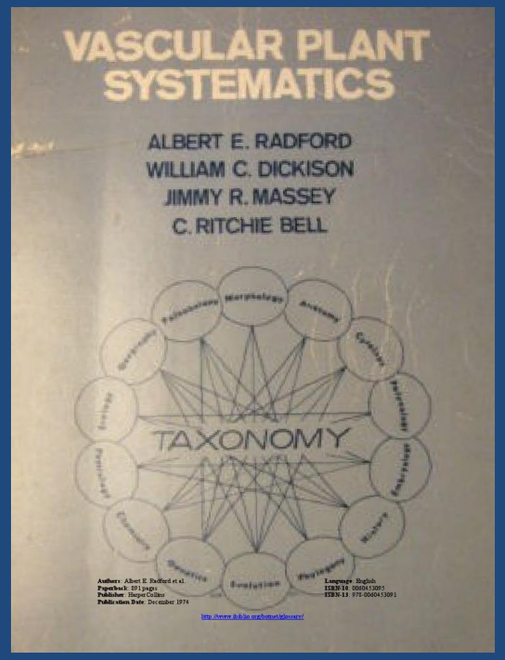
Types of Taxonomy / Taxonomic Studies / Plant Taxonomic Classification

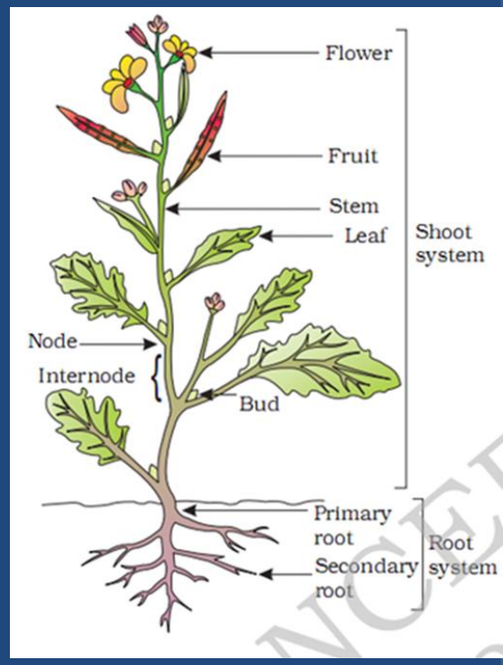
Alpha (α) Taxonomy / classical taxonomy:- It involves description and naming of organisms. It is the parent of other types of taxonomy.

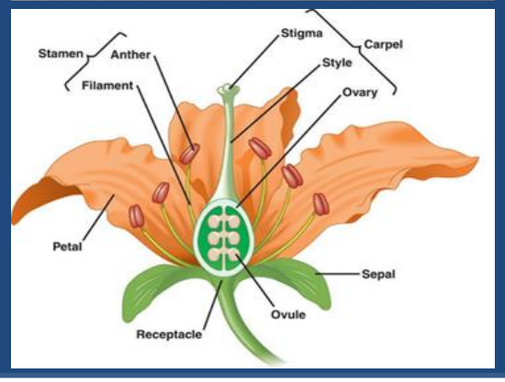
Omega (Ω) Taxonomy: - It is the modern experimental taxonomy in which the taxonomic activities have been enriched with data from ecology, phyto-chemistry, phyto-geography, cyto-genetics and physiology coupled with adequate computation.

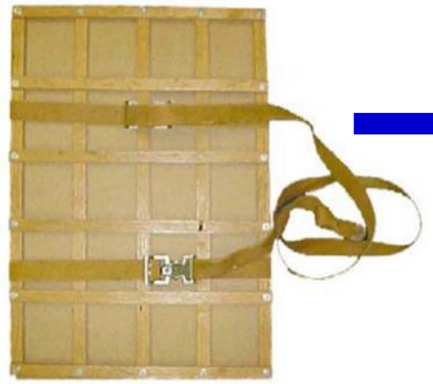
Herbarium: Plant collecting, Preservation and Documentation

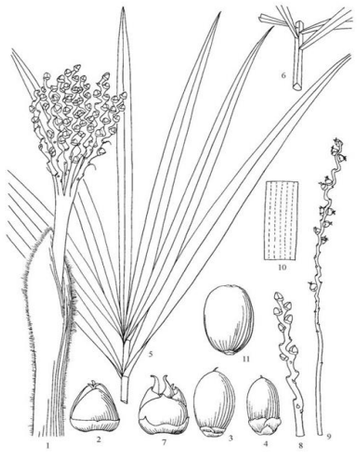
- **To make a herbarium specimen, the plant is collected, and notes are made about it. The plant is then pressed until dry between blotters that absorb moisture and mounted onto a herbarium sheet with a suitable label, and stored in steel cabinet arranged into some system of classification.**
- **Herbarium techniques involve : (i) Collection, (ii) Drying, (iii) Poisoning, (iv) Mounting, (v) Stitching, (vi) Labelling, and (vii) Deposition.**
- **Flora = it is the documentation of plants occurring in a particular region.**
- The FLORA is the main Resources of Taxonomic Information
- **A HERBARIUM is a collection of dried plants systematically named and arranged for ready reference and study.**



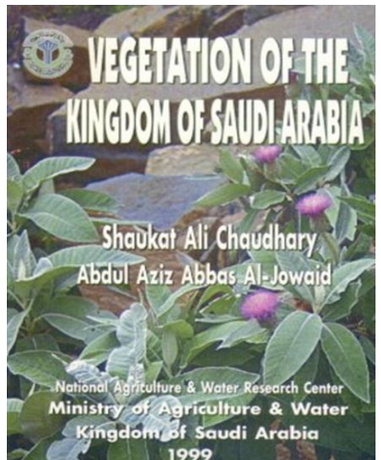


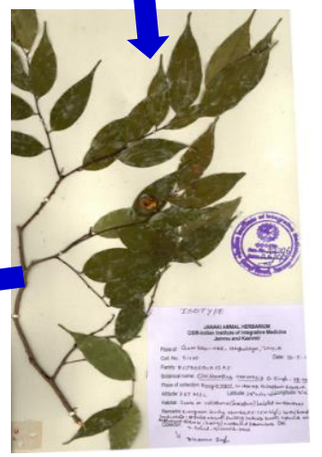




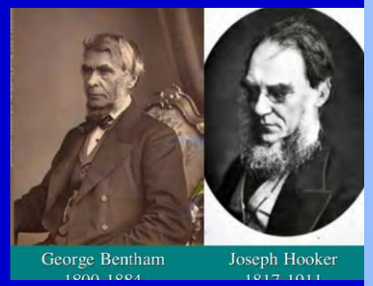


Phoenix dactylifera Linnaeus, Sp. Pl. 2: 1188. 1753. Stems solitary or clustered and then with few shoots, to 30 m tall, to 50 cm in diam., rough with persistent, diamond-shaped leaf bases. Leaves 3-5 m; sheath and petiole to 1 m; rachis 1-2 m; acanthophylls many per side of rachis; pinnae to 200 per side of rachis, linear, irregularly arranged and spreading in different planes; middle pinnae to 40 × 2 cm. Male inflorescences erect, to 1 m, with many rachillae, these ca. 30 cm; female inflorescences erect, becoming pendulous, to 2 m, with to 150 rachillae, these to 40 cm. Fruits variable in shape, usually oblong, to 7 × 3 cm, brown or black; endosperm homogeneous.





Bentham and Hooker System of Plant Classification



- Bentham and Hooker, two English botanists, represented the most well developed natural system of plant classification. The classification was published in a three-volume work Genera plantarum (1862-83).
- Hooker supervised the publication of Index Kewensis (2 volumes, 1893), listing the names of all known species and their synonyms.
- Many important herbaria of the world have specimens arranged according to Bentham and Hooker system of plant classification.
- Bentham and Hooker recognized three class:

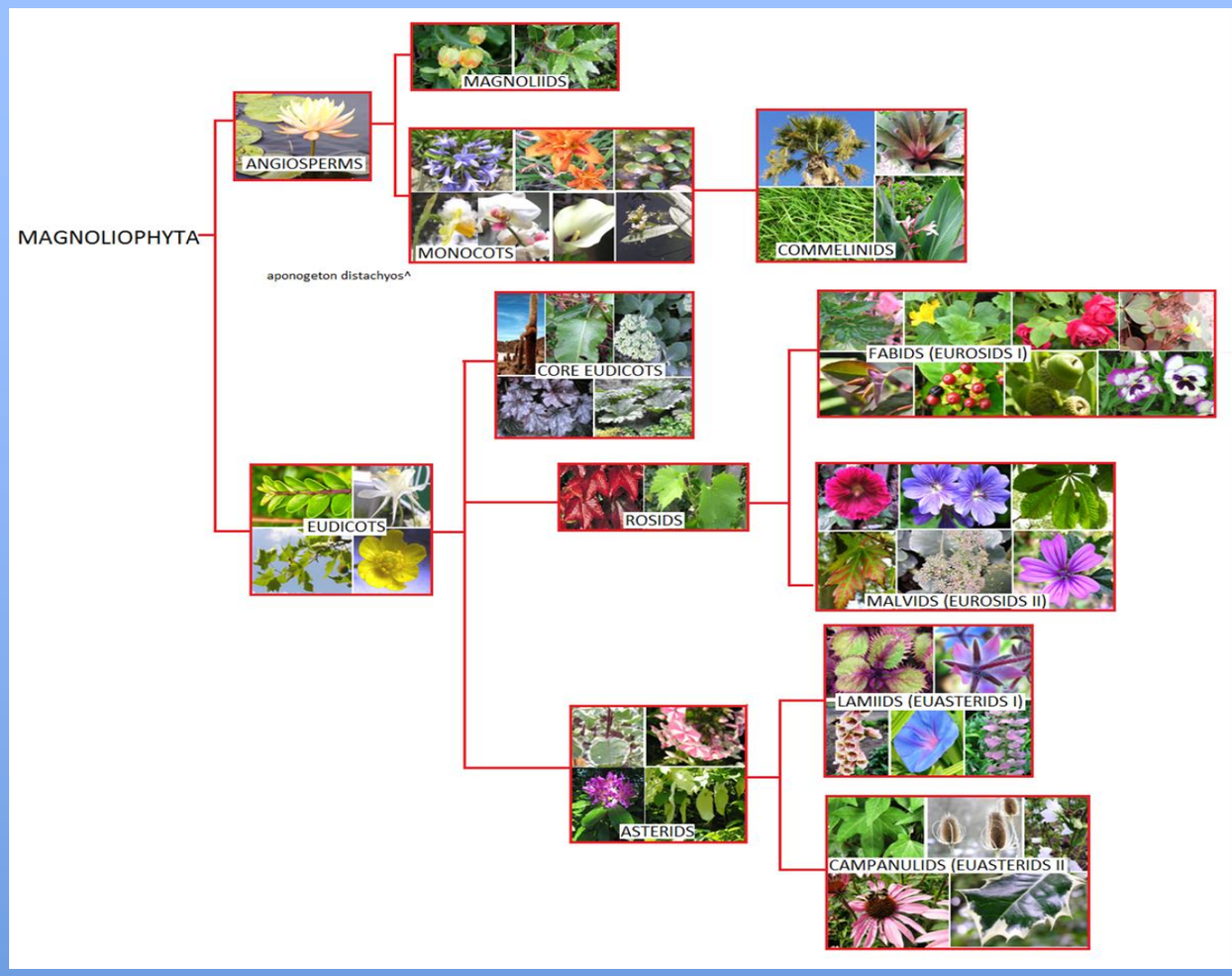
Class Dicotyledones:

Subclass POLYPETALE with three series Series 1. THALAMIFLORÆ, Series 2. DISCIFLORÆ, Series 3. CALYCIFLORÆ;
Subclass DICOTYLEDONES (GAMOPETALÆ) with three series that is Series 1. INFERÆ, Series 2. HETEROMERÆ, Series 3. BICARPELLATÆ, and
Subclass DICOTYLEDONES MONOCHLAMIDEÆ.

Class Gymnospermeæ (Gymnosperms are placed between Dicotyledons and Monocotyledons)

Class Monocotyledones

❖ Angiosperm Phylogeny Group (APG)



- The APG system of flowering plant classification is the modern, mostly molecular-based, system of plant taxonomy for flowering plants (angiosperms) being developed by the Angiosperm Phylogeny Group (APG).
- The APG was first published in 2008.
- Currently the APG IV system recognizes a total of 64 angiosperm orders and 416 families.
- The families in APG classification have been grouped into 40 putative monophyletic orders under a small number of informal monophyletic higher groups: monocots, commelinoids, eudicots, core eudicots, rosids, eurosids I, eurosids II, asterids, euasterids I and euasterids II

SCIENTIFIC NOMENCLATURE / BOTANICAL NOMENCLATE :

Nomenclature deals with the application of a correct name to a plant or a taxonomic group.

- ❖ **We have millions of species distributed in different geographical regions of the world.**
- ❖ **The Scientific names (Botanical name and Zoological name)of the living organism (Plants and Animals) are necessary because the same common name is used for different plants / Animals in different areas of the world.**
- **Swedish Botanist Carolus Linnaeus introduced Binomial Nomenclature.**
- **The Binomial nomenclature uses two Latin words to indicate the genus and the species. The first word is the genus and the second word is the species. Example- the botanical name of Dates is *Phoenix dactylifera***

Species Concept

- **Species is the basic unit of classification**
- **Plants in the same species consistently produce plants of the same types**
- **The name of the plants must should be written in italics. For example Phoenix dactylifera**

TAXONOMIC RANKS OF LAND PLANTS	ENDING	EXAMPLE TAXON
Kingdom	(various)	**Plantae**
Phylum [Division]	**-phyta**	**Magnoliophyta**
Subphylum [Subdivision]	-phytina	Magnoliophytina
Class	**-opsida**	**Asteropsida**
Subclass	-idae	Asteridae
Order	**-ales**	**Asterales**
Suborder	-ineae	Asterineae
Family	**-aceae**	**Asteraceae**
Subfamily	-oideae	Asteroideae
Tribe	-eae	Heliantheae
Subtribe	-inae	Helianthinae
Genus	(various)	***Helianthus***
Subgenus	(various)	*Helianthus*
Section	(various)	*Helianthus*
Series	(various)	*Helianthus*
Species [abbr. sp. (sing.), spp. (pl.)]	(various)	***Helianthus annuus***
Subspecies [abbr. subsp. or ssp. (sing.), subspp. or sspp. (pl.)]	(various)	*Helianthus annuus* ssp. *annuus*
Variety [abbr. var. (sing.), vars. (pl.)]	(various)	*Helianthus annuus* var. *annuus*
Form [abbr. f.]	(various)	*Helianthus annuus* f. *annuus*

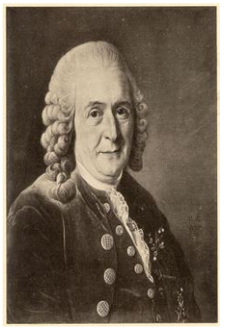
- ❖ **Binomial Nomenclature and Carolus Linneaus System of Plant Classification**
- ❖ **Taxonomic Systems of Classification: Ideally our systems of classification should allow us to place similar species of plants together in the same category.**
- ❖ **There are two types of Classification Schemes:**
- ❑ **Artificial taxonomy was a system of grouping unrelated plant species by a common criteria (i.e. a flowers sexual organs)**
- ❑ **Natural classification reflects relationships among taxon**
- ➢ **Carolus Linneaus was a Swedish botanist.**
- ➢ **Carolus Linneaus traveled to Lapland (Blue Lake, CA) and collected large number of plants.**
- ➢ **Carolus Linneaus introduced Binomial Nomenclature.**

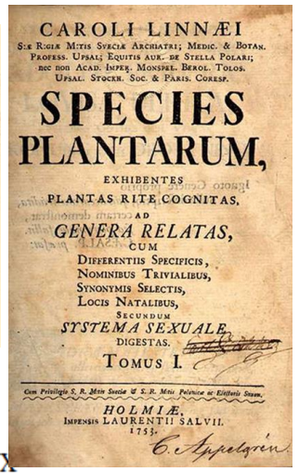
Binomial nomenclature = Uses two Latin words to indicate the genus and the species. The first word is the genus and the second word is the species. Example- the botanical name of dates is *Phoenix dactylifera*

- ➢ **Carolus Linneaus published the book 'Species Plantarum' in 1753.**
- ➢ **Carolus Linneaus classified the plants based on the plant's method of reproduction and structure of reproductive parts.**
- ➢ **Produced his sexual system of classification (Artificial classification)**
- ➢ **Carolus Linneaus divided plants into 24 classes. The Classes in the Linneaus is based largely on the amount, union and length of stamens**

Classes

1. Monandria- stamen one
2. Diandria- stamens two
3. Triandria- stamens three
4. Tetrandria- stamens four
5. Pentandria- stamens five
6. Hexandria- stamens six
7. Heptandria- stamens seven
8. Octandria- stamens eight
9. Ennandria- stamens nine
10. Decandria- stamens ten
11. Dodecandria- stamens 11-19
12. Icosandria- stamens 20 or more, on the calyx
13. Polyandria- stamens 20 or more, on the receptacle
14. Didynamia- stamens didynamous; 2 short, 2 long
15. Tetradynamia- stamens tetradynamous; 4 long, 2 short
16. Monadelphia- stamens monadelphous; united in 1 group
17. Diadephia- stamens diadelphous; united in 2 groups
18. Polyadelphia- stamens polyadelphous; united in 3 or more groups
19. Syngenesia- stamens syngenesious; united by anthers only
20. Gynandria- stamens united with the gynoecium
21. Monoecia- plants monoecious
22. Dioecia- plants dioecious
23. Polygamia- plants polygamous
24. Cryptogamia- flowerless plants





International Code of Botanical Nomenclature (ICBN)

The current activity of botanical nomenclature is governed by the International Code of Botanical Nomenclature (ICBN) published by the International Association of Plant Taxonomy (IAPT).

The Code is divided into 3 divisions:

I. Principles

II. Rules and recommendations

III. Provisions for the governance of the Code

Principles of ICBN

- Botanical Nomenclature is independent of Zoological Nomenclature. The Code applies equally to the names of taxonomic groups treated as plants whether or not these groups were originally so treated.
- The application of names of taxonomic groups is determined by means of nomenclatural types / **TYPIFICATION**.
- Nomenclature of a taxonomic group is based upon **Priority Of Publication**.
- Each taxonomic group with a particular circumscription, position and rank can bear **Only One Correct Name**, the earliest that is in accordance with the rules.
- Scientific names of taxonomic groups are treated as **LATIN**, regardless of derivation.
- The rules of nomenclature are **Retroactive (Date)**, unless expressly limited.

- **Generic Name:** The Generic name is usually a noun and singular, which is spelled or written with a capital letter.
- **Specific Epithet:** The specific epithet is often an adjective and it is written with a small initial letter.
- **In the hand written manner, both the generic names and specific epithet should be underlined, while if printed it should be in italics.**

Synonyms and related terminology

- **Synonyms:** A name rejected due to misuse or difference in taxonomic judgement.
- **Basionym:**
  - The basionym is the first name ever given to a taxon. Further studies and revisions may reject the basionym as the most correct one, but it still is useful as a nomenclatural reference for that species.
  - Also, according to the priority rules of the ICBN, after a taxonomic revision that results in a species being reclassified in another genus, the specific epithet must remain the same as the one in the Basionym.
  - A short example: Linnaeus classified the Tea Plant as *Thea sinensis*. Some decades later, Sweet noticed that the genus *Thea* was not really different from the genus *Camellia*, and renamed all the *Theas* as *Camellias*. *Thea sinensis* became *Camellia sinensis*, because he had to keep the specific epithet the same as the original name (Basionym) for that species, given by Linnaeus.
- **Homonym:** A case in which two or more identical names are baed on different type , of which only one can be a legitimate name , is called as homonym.
- **Tautonym:** A case in which name of genus and the name of the species is the same.

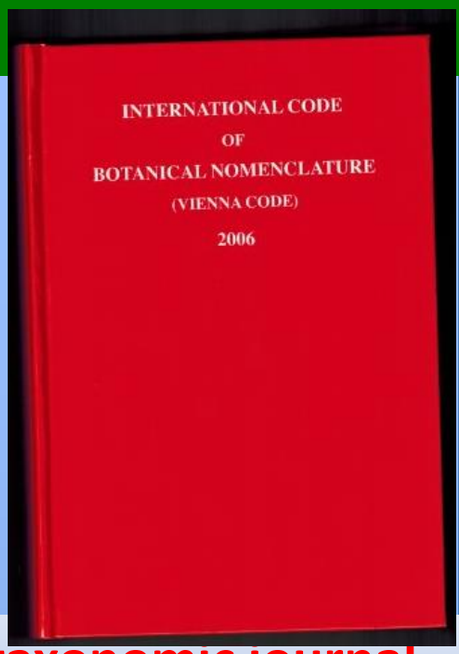
Names of Taxa

Rank	*Ending*	*Example*
Kingdom	-bionta	Chlorobionta
Division	-phyta	Magnoliophyta
	-mycota (Fungi)	Eumycota
Subdivision	-phytina	Pterophytina
	-mycotina (Fungi)	Eumycotina
Class	-opsida	Magnoliopsida
	-phyceae (Algae)	Chlorophyceae
	-mycetes (Fungi)	Basidiomycetes
Subclass	-opsidae	Pteropsidae
	-idae (Seed plants)	Rosidae
	-physidae (Algae)	Cyanophysidae
	-mycetidae (Fungi)	Basidiomycetidae
Order	-ales	Rosales
Suborder	-ineae	Rosineae
Family	-aceae	Rosaceae
Subfamily	-oideae	Rosoideae
Tribe	-eae	Roseae
Subtribe	-inae	Rosinae
Genus	*-us, -um, -is, -a, -on*	*Pyrus, Allium, Arabis, Rosa, Polypogon*
Subgenus		*Cuscuta* subgenus *Eucuscuta*
Section		*Scrophularia* section *Anastomosanthes*
Subsection		*Scrophularia* subsection *Vernales*
Series		*Scrophularia* series *Lateriflorae*
Species		*Rosa canina*
Subspecies		*Crepis sancta* subsp. *bifida*
Varietas		*Lantana camara* var. *varia*
Forma		*Tectona grandis* f. *punctata*

Typification: Type Specimen is the one representative of the taxon.

- **Holotype:** A specimen designated by the author in the original publication (nomenclatural type).
- **Isotype:** A duplicate specimen of the holotype collected at the same time and place (may be in other herbarium).
- **Lectotype:** A specimen chosen from the author's original material when no holotype has been designated.
- **Neotype:** A specimen selected when all original specimens have been destroyed

Author Citation, Effective Publication and Principle of Priority



Author Citation

- For a name to be complete, it should be accompanied by the name of the author or authors who first published the name validly. The names of the authors are commonly abbreviated, Example L. for Carolus Linnaeus
- *Aizoon canariense* L.
- *Tribulus macropterus* var. *arabicus* (Hosni) Al-Hemaid & J. Thomas

Basic structure of a taxonomic Research papers / Recent publication of a new species in taxonomic journal

Ann. Bot. Fennici 53: 37–39 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 4 January 2016

Silene langshanensis (Caryophyllaceae), a new species from Inner Mongolia, China

Li-Qing Zhao[1,*], Zhi-Ming Xin[2] & Yi-Zhi Zhao[1]

[1)] *College of Life Science, Inner Mongolia University, Hohhot 010021, China (*corresponding author's e-mail: zhaotieniu@126.com)*
[2)] *Experimental Center for Desert Forestry, Chinese Academy of Forestry, Dengkou, Inner Mongolia 015200, China*

Received 22 Apr. 2015, final version received 9 Oct. 2015, accepted 9 Oct. 2015

Zhao L.Q., Xin Z.M. & Zhao Y.Z. 2016: *Silene langshanensis* (Caryophyllaceae), a new species from Inner Mongolia, China. — *Ann. Bot. Fennici* 53: 37–39.

Silene langshanensis L.Q. Zhao, Y.Z. Zhao & Z.M. Xin *sp. nova* (Caryophyllaceae), is described and illustrated from Inner Mongolia, China. It appears to be most closely related to *S. scabrifolia* of *Silene* sect. *Holopetalae*. *Silene langshanensis* can be distinguished by the basally pubescent carpophore, petals with obtuse auricles, stems and leaves with dense, short hairs, and by the glabrous calyx.

In total, there are about 600 species of *Silene s. lato* (Caryophyllaceae) (Zhou *et al.* 2001). They are distributed mainly in the northern temperate regions, but occur also in Africa and South America (Zhou *et al.* 2001). Among these species, 110 are known from China, of which 67 are endemic. Twenty of the endemics (nine species of *Silene s. stricto*, nine of *Melandrium*, one of *Cucubalus* and one of *Lychnis*) are found in Inner Mongolia.

In September 2008 and later, in 2014, the authors Zhao and Xin collected specimens of *Silene* from Langshan in Bayannaoer (Inner Mongolia) from desert steppe communities on mountain slopes at 1150–1400 m a.s.l. After careful study, we concluded that the specimens represented an undescribed species of *Silene*.

Silene langshanensis L.Q. Zhao, Y.Z. Zhao & Z.M. Xin, *sp. nova* (Fig. 1)

Holotype: China. Inner Mongolia, Bayannaoer, Dengkou, Mt. Langshan, 40°43′58.4″N, 106°22′28.5″E, on stony mountain slopes, 1371 m a.s.l., 9 June 2014 *Li-Qing Zhao, Zhi-Ming Xin, Shuai Qin & Long Chen N14-001* (HIMC). — Paratypes (all HIMC!): Same location as holotype, 9 June 2014 *Li-Qing Zhao, Zhi-Ming Xin, Shuai Qin & Long Chen N14-002, N14-003, N14-004*; Mt. Langshan, 40°39′27.7″N, 106°23′14.1″E, on stony mountain slopes, alt. 1185 m a.s.l., 10 September 2008 *Li-Qing Zhao & Zhi-Ming Xin N08-001*.

Herbs perennial, 20–50 cm tall. Roots robust, lignified. Plants cespitose with erect stems, multibranched or sparsely branched, pubescent, upper parts glabrescent. Basal leaves oblanceolate, 20–60 × 2–6 mm, both surfaces pubescent, margins ciliate, base attenuate into a long petiole, apex acute; cauline leaves 3–7 pairs, linear-oblanceolate or linear-lanceolate, with short, axillary sterile branches or sometimes elongated flowering axillary branches. Flowers in a racemiform-like thyrse; cymes alternating (resulting from suppression of opposite cyme at same node) or opposite, 1-flowered (rarely 2), peduncles nearly equal or shorter than pedicels. Pedicel 2–6 cm, glabrescent; bracts ovate-lanceolate, ciliate, base connate, apex acuminate. Calyx tubular, green, sometimes violet, narrowly campanulate, slightly inflated in fruit, 10–13 × 4–5 mm, glabrous; calyx teeth broadly triangular-ovate, apex obtuse or acute, margin membranous, ciliate. Carpophore 2–3 mm, basally pubescent. Petals ca. 15 mm, claws cuneate, glabrous, auricles obtuse; limbs yellowish green, narrowly obovate, margin entire or emarginate, coronal scales absent. Stamens and styles prominently exserted; filaments glabrous. Styles 3. Ovary ovate-elliptic, ca. 5 mm long. Capsule 6-valved. Flowering and fruiting in June–September.

Silene langshanensis is placed in the section *Holopetalae*, of which five species are now known from China (Zhou *et al.* 2001). It resembles *S. scabrifolia* (*see* Shishkin 1936: 677) but can be distinguished by several characters (Table 1). The following key should be useful when identifying species of sect. *Holopetalae* in China.

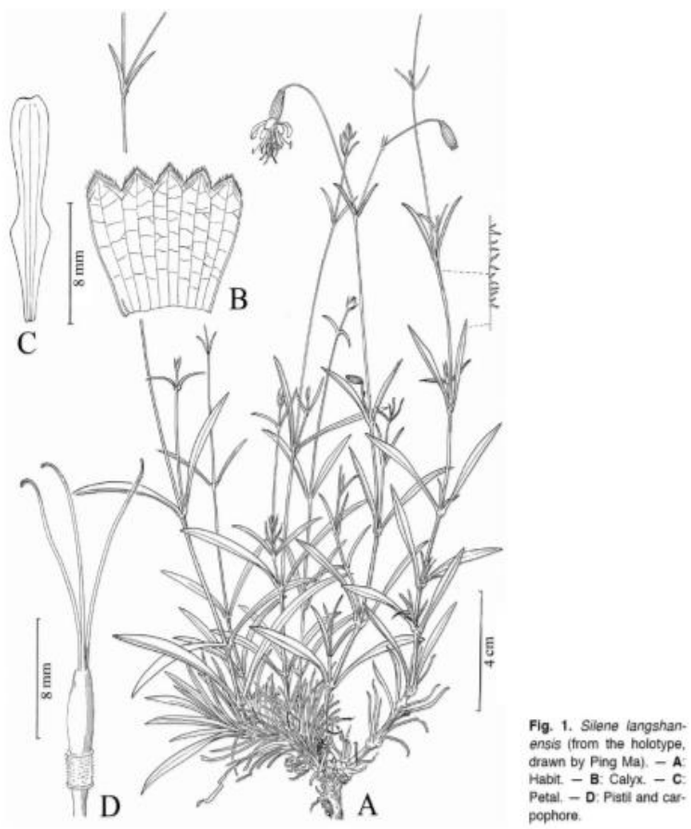


Fig. 1. *Silene langshanensis* (from the holotype, drawn by Ping Ma). — **A**: Habit. — **B**: Calyx. — **C**: Petal. — **D**: Pistil and carpophore.

Table 1. Main morphological differences between *Silene langshanensis* and *S. scabrifolia*.

Character	*S. langshanensis*	*S. scabrifolia* (= *S. komarovii*)
Stem	densely pubescent, upper part glabrescent when flowering	pubescent in lower part, glabrous and viscid above
Basal leaves	oblanceolate, 20–60 × 2–6 mm	spatulate or lanceolate, 60–80 × 5–10 mm
Cyme	1-flowered (rarely 2)	multiflowered
Pedicel	20–60 mm long, glabrescent	5–10 mm long, sparsely pubescent
Calyx	narrowly campanulate, 10–13 × 4–5 mm, glabrous	tubular-clavate, 8–12 × 2–3 mm, glabrous or sparsely villous
Carpophore	shortly pubescent	glabrous
Petal	with obtuse auricles	without distinct auricles
Limbs	yellowish green	yellowish white

1. Leaves ovate-lanceolate, 15–30 mm wide ..... *S. kungessana*
1. Leaves lanceolate or linear, 1.5–10 mm wide 2
2. Leaves linear, 10–30 × 1.5–3 mm ..... *S. holopetala*
2. Leaves oblanceolate or lanceolate, 30–80 mm long, usually more than 4 mm wide 3
3. Stems usually not branched; calyx 6–9 mm; petals pinkish abaxially ..... *S. pseudotenuis*
3. Stems branched; calyx 8–13 mm; petals yellowish green or yellowish white 4
4. Stem pubescent in lower part, glabrous and viscid above; cymes multiflowered; petals yellowish white, without obvious auricles; carpophore glabrous ..... *S. scabrifolia*
4. Stem with dense short hairs, upper part glabrescent when flowering; cymes 1-flowered (rarely 2); petals yellowish green, with obtuse auricles; carpophore basally pubescent ..... *S. langshanensis*

Acknowledgements

We are grateful to Ping Ma for the drawing. This study was financially supported by Natural Science Foundation of Inner Mongolia Autonomous Region (2014ZD02), National Key Basic Research Program of China (2014CB138802) and the Central Public-interest Scientific Institution Basal Research Fund (CAFYBB2014MA016).

References

Shishkin B.K. [Шишкин Б.К.] (ed.) 1936: [*Flora USSR*], vol. 6. — Izdatel'stvo Akademii Nauk USSR, Leningrad. [In Russian].

Zhou L.H., Wu Z.Y., Lidén M. & Oxelman B. 2001: *Silene*. — In: Wu Z.Y. & Raven P.H. (eds.), *Flora of China*, vol. 6: 66–100. Science Press, Beijing & Missouri Botanical Garden Press, Saint Louis.

This article is also available at http://www.annbot.net and http://www.bioone.org/loi/anbf

Effective publication in the journal, available to Botanist

Date of valid publication (principles of priority): If the same species will be published by some one else after this date then the publication will be not valid. (/Principles of Priority).

Ann. Bot. Fennici 53: 37–39 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 4 January 2016

Silene langshanensis (Caryophyllaceae), a new species from Inner Mongolia, China

Li-Qing Zhao[1,*], Zhi-Ming Xin[2] & Yi-Zhi Zhao[1]

[1] *College of Life Science, Inner Mongolia University, Hohhot 010021, China (*corresponding author's e-mail: zhaotieniu@126.com)*
[2] *Experimental Center for Desert Forestry, Chinese Academy of Forestry, Dengkou, Inner Mongolia 015200, China*

Received 22 Apr. 2015, final version received 9 Oct. 2015, accepted 9 Oct. 2015

Zhao L.Q., Xin Z.M. & Zhao Y.Z. 2016: *Silene langshanensis* (Caryophyllaceae), a new species from Inner Mongolia, China. — *Ann. Bot. Fennici* 53: 37–39.

Silene langshanensis L.Q. Zhao, Y.Z. Zhao & Z.M. Xin *sp. nova* (Caryophyllaceae) is described and illustrated from Inner Mongolia, China. It appears to be most closely related to *S. scabrifolia* of *Silene* sect. *Holopetalae*. *Silene langshanensis* can be distinguished by the basally pubescent carpophore, petals with obtuse auricles, stems and leaves with dense, short hairs, and by the glabrous calyx.

Abstract / Summary / Synopsis.

Previously it was required to write in Latin.

In total, there are about 600 species of *Silene s. lato* (Caryophyllaceae) (Zhou *et al*. 2001). They are distributed mainly in the northern temperate regions, but occur also in Africa and South America (Zhou *et al*. 2001). Among these species, 110 are known from China, of which 67 are endemic. Twenty of the endemics (nine species of *Silene s. stricto*, nine of *Melandrium*, one of *Cucubalus* and one of *Lychnis*) are found in Inner Mongolia.

In September 2008 and later, in 2014, the authors Zhao and Xin collected specimens of *Silene* from Langshan in Bayannaoer (Inner Mongolia) from desert steppe communities on mountain slopes at 1150–1400 m a.s.l. After careful study, we concluded that the specimens represented an undescribed species of *Silene*.

Botanical name in Latin

Rank indicated

Silene langshanensis L.Q. Zhao, Y.Z. Zhao & Z.M. Xin, *sp. nova* (Fig. 1)

Type Specimen indicated

HOLOTYPE: China. Inner Mongolia, Bayannaoer, Dengkou, Mt. Langshan, 40°43′58.4″N, 106°22′28.5″E, on stony mountain slopes, 1371 m a.s.l., 9 June 2014 *Li-Qing Zhao, Zhi-Ming Xin, Shuai Qin & Long Chen N14-001* (HIMC). — PARATYPES (all HIMC!): Same location as holotype, 9 June 2014 *Li-Qing Zhao, Zhi-Ming Xin, Shuai Qin & Long Chen N14-002, N14-003, N14-004*; Mt. Langshan, 40°39′27.7″N, 106°23′14.1″E, on stony mountain slopes, alt. 1185 m a.s.l., 10 September 2008 *Li-Qing Zhao & Zhi-Ming Xin N08-001*.

Specimens examined

Herbs perennial, 20–50 cm tall. Roots robust, lignified. Plants cespitose with erect stems, multi-branched or sparsely branched, pubescent, upper parts glabrescent. Basal leaves oblanceolate, 20–60 × 2–6 mm, both surfaces pubescent, margins ciliate, base attenuate into a long petiole, apex acute; cauline leaves 3–7 pairs, linear-oblanceolate or linear-lanceolate, with short, axillary sterile branches or sometimes elongated flowering axillary branches. Flowers in a racemiform-like thyrse; cymes alternating (resulting from suppression of opposite cyme at same node) or opposite, 1-flowered (rarely 2), peduncles nearly equal or shorter than pedicels. Pedicel 2–6 cm, glabrescent; bracts ovate-lanceolate, ciliate, base connate, apex acuminate. Calyx tubular, green,

Taxonomic Description

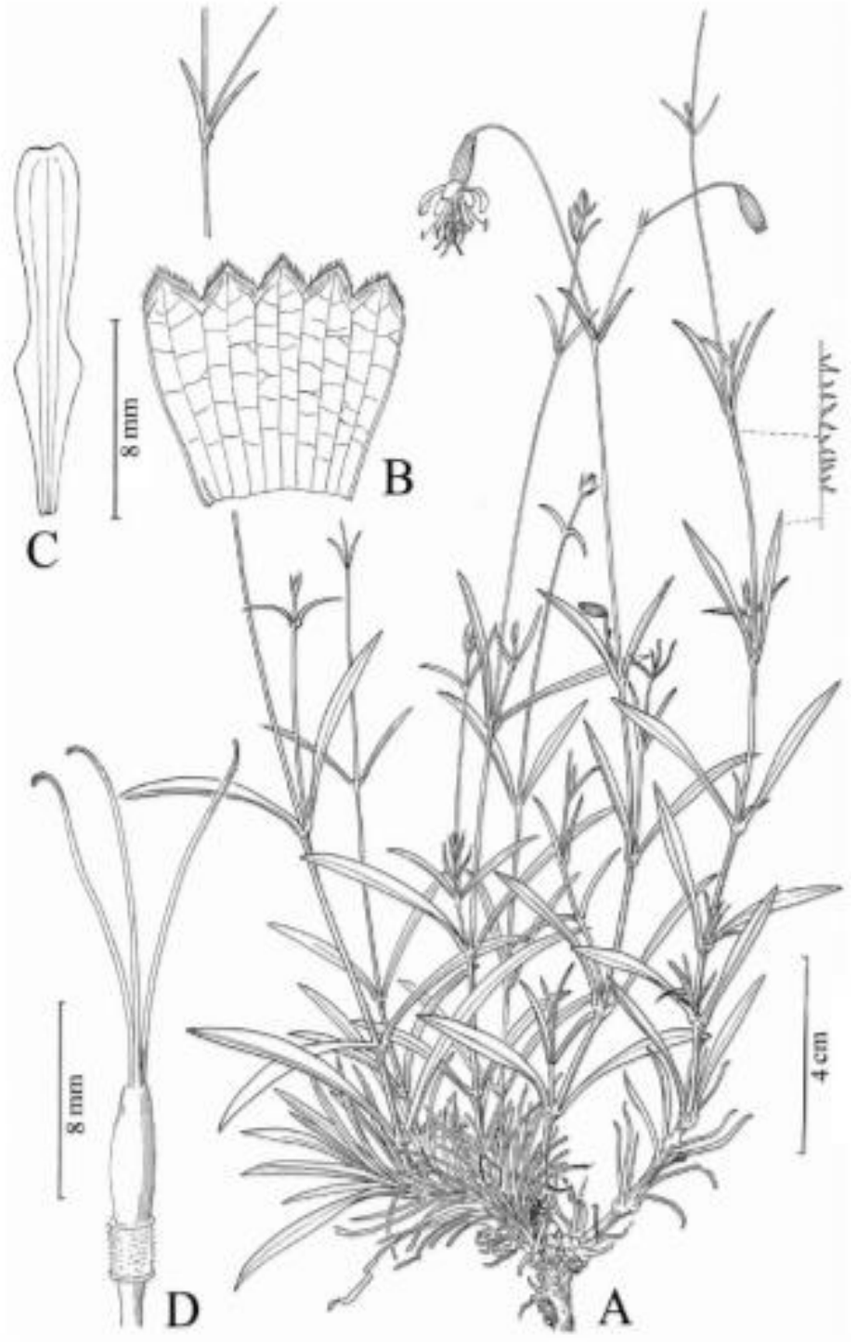


Fig. 1. *Silene langshanensis* (from the holotype, drawn by Ping Ma). — **A**: Habit. — **B**: Calyx. — **C**: Petal. — **D**: Pistil and carpophore.

sometimes violet, narrowly campanulate, slightly inflated in fruit, 10–13 × 4–5 mm, glabrous; calyx teeth broadly triangular-ovate, apex obtuse or acute, margin membranous, ciliate. Carpophore 2–3 mm, basally pubescent. Petals ca. 15 mm, claws cuneate, glabrous, auricles obtuse; limbs yellowish green, narrowly obovate, margin entire or emarginate, coronal scales absent. Stamens and styles prominently exserted; filaments glabrous. Styles 3. Ovary ovate-elliptic, ca. 5 mm long. Capsule 6-valved. Flowering and fruiting in June–September.

Silene langshanensis is placed in the section *Holopetalae*, of which five species are now known from China (Zhou *et al.* 2001). It resembles *S. scabrifolia* (*see* Shishkin 1936: 677) but can be distinguished by several characters (Table 1). The following key should be useful when identifying species of sect. *Holopetalae* in China.

Line drawing

Table 1. Main morphological differences between *Silene langshanensis* and *S. scabrifolia*.

Character	*S. langshanensis*	*S. scabrifolia* (= *S. komarovii*)
Stem	densely pubescent, upper part glabrescent when flowering	pubescent in lower part, glabrous and viscid above
Basal leaves	oblanceolate, 20–60 × 2–6 mm	spatulate or lanceolate, 60–80 × 5–10 mm
Cyme	1-flowered (rarely 2)	multiflowered
Pedicel	20–60 mm long, glabrescent	5–10 mm long, sparsely pubescent
Calyx	narrowly campanulate, 10–13 × 4–5 mm, glabrous	tubular-clavate, 8–12 × 2–3 mm, glabrous or sparsely villous
Carpophore	shortly pubescent	glabrous
Petal	with obtuse auricles	without distinct auricles
Limbs	yellowish green	yellowish white

1. Leaves ovate-lanceolate, 15–30 mm wide ..... *S. kungessana*
1. Leaves lanceolate or linear, 1.5–10 mm wide 2
2. Leaves linear, 10–30 × 1.5–3 mm ..... *S. holopetala*
2. Leaves oblanceolate or lanceolate, 30–80 mm long, usually more than 4 mm wide 3
3. Stems usually not branched; calyx 6–9 mm; petals pinkish abaxially .....
3. Stems branched; calyx
or yellow
4. Stem
cyme
obvi
4. Stem

Taxonomic Key for Identification

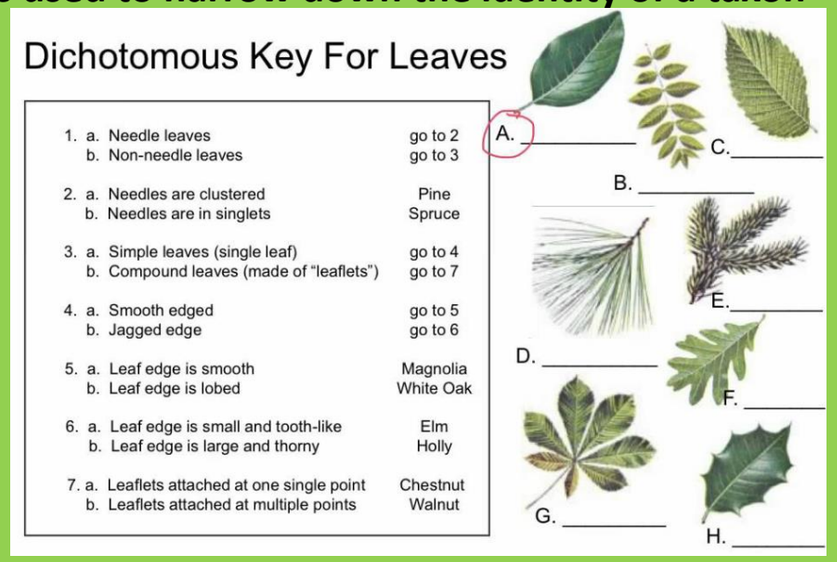
Acknowledgements

We are grateful to Ping Ma for the drawing. This study was financially supported by Natural Science Foundation of Inner Mongolia Autonomous Region (2014ZD02), National Key Basic Research Program of China (2014CB138802) and the Central Public-interest Scientific Institution Basal Research Fund (CAFYBB2014MA016).

eferences

hkin B.K [Шишкин Б.К.] (ed.) 1936: [*Flora USSR*], vol. . — Izdatel'stvo Akademii Nauk USSR, Leningrad. [In ussian].
L.H., Wu Z.Y., Lidén M. & Oxelman B. 2001: *Silene*. – In: Wu Z.Y. & Raven P.H. (eds.), *Flora of China*, vol. : 66–100. Science Press, Beijing & Missouri Botanical Garden Press, Saint Louis.

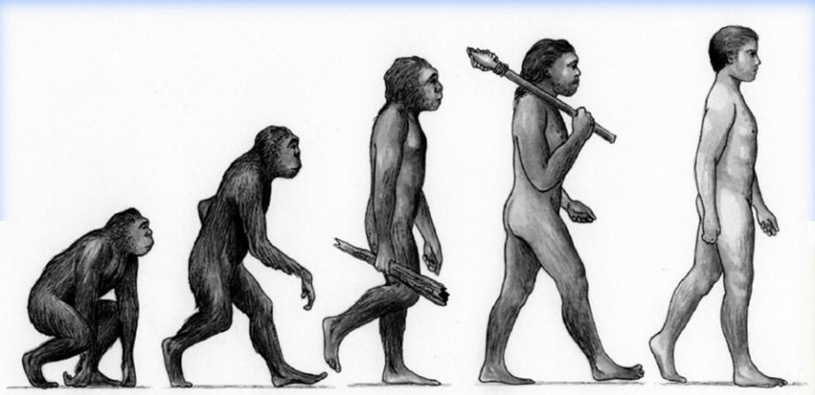
Taxonomic Key: An identification device, consisting of contrasting statements used to narrow down the identity of a taxon

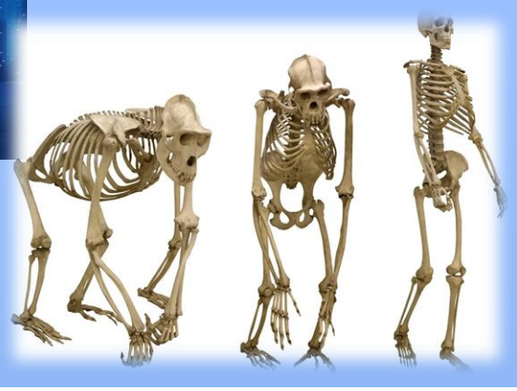


DATES

MINT

PALM





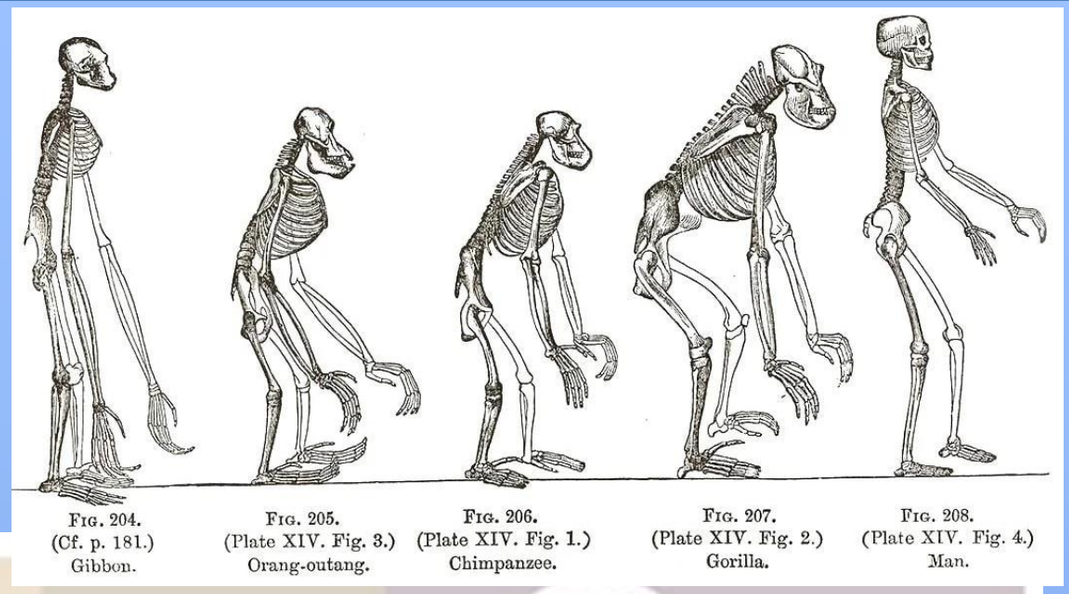


Fig. 204. (Cf. p. 181.) Gibbon.

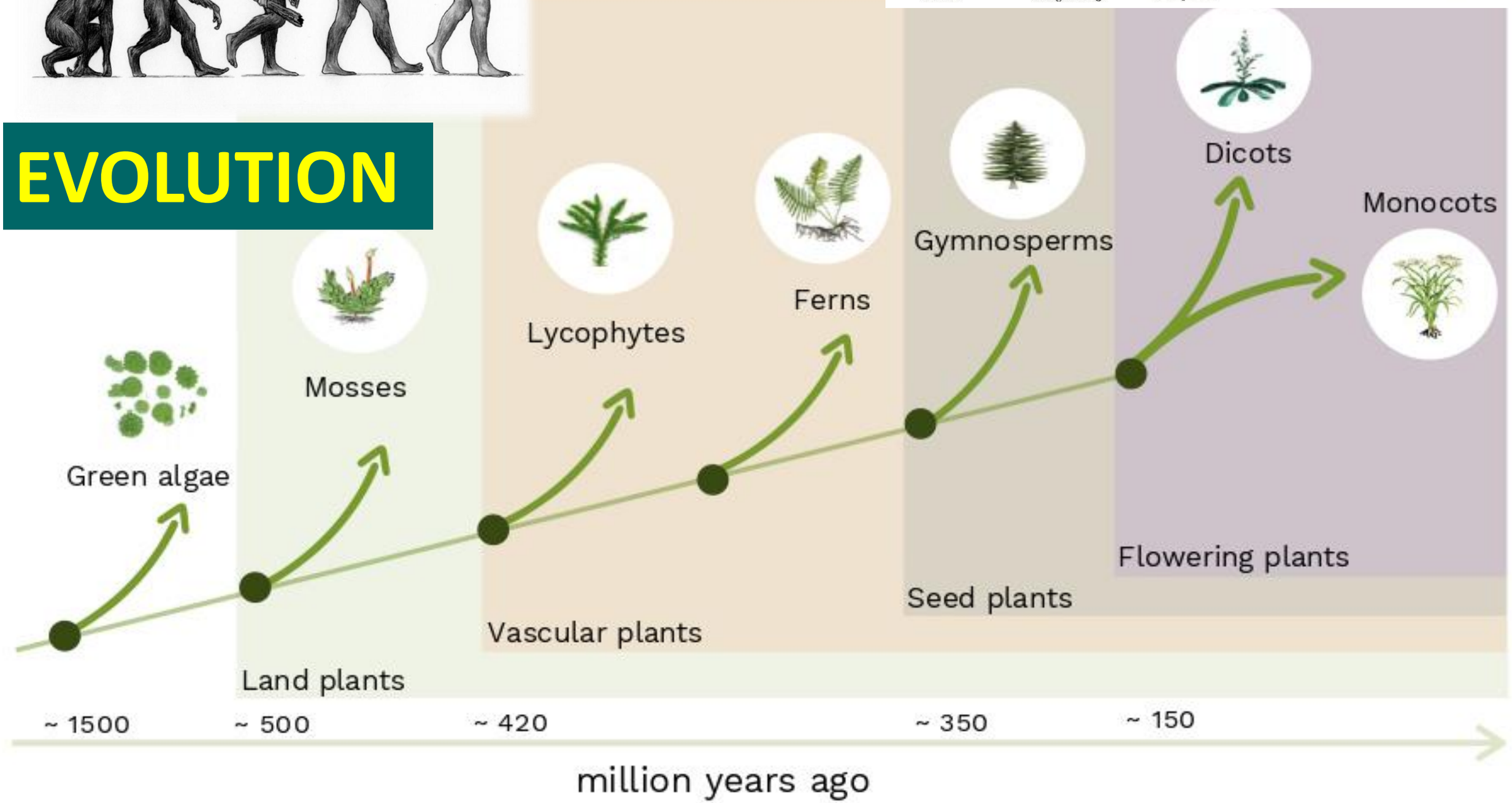
Fig. 205. (Plate XIV. Fig. 3.) Orang-outang.

Fig. 206. (Plate XIV. Fig. 1.) Chimpanzee.

Fig. 207. (Plate XIV. Fig. 2.) Gorilla.

Fig. 208. (Plate XIV. Fig. 4.) Man.

EVOLUTION



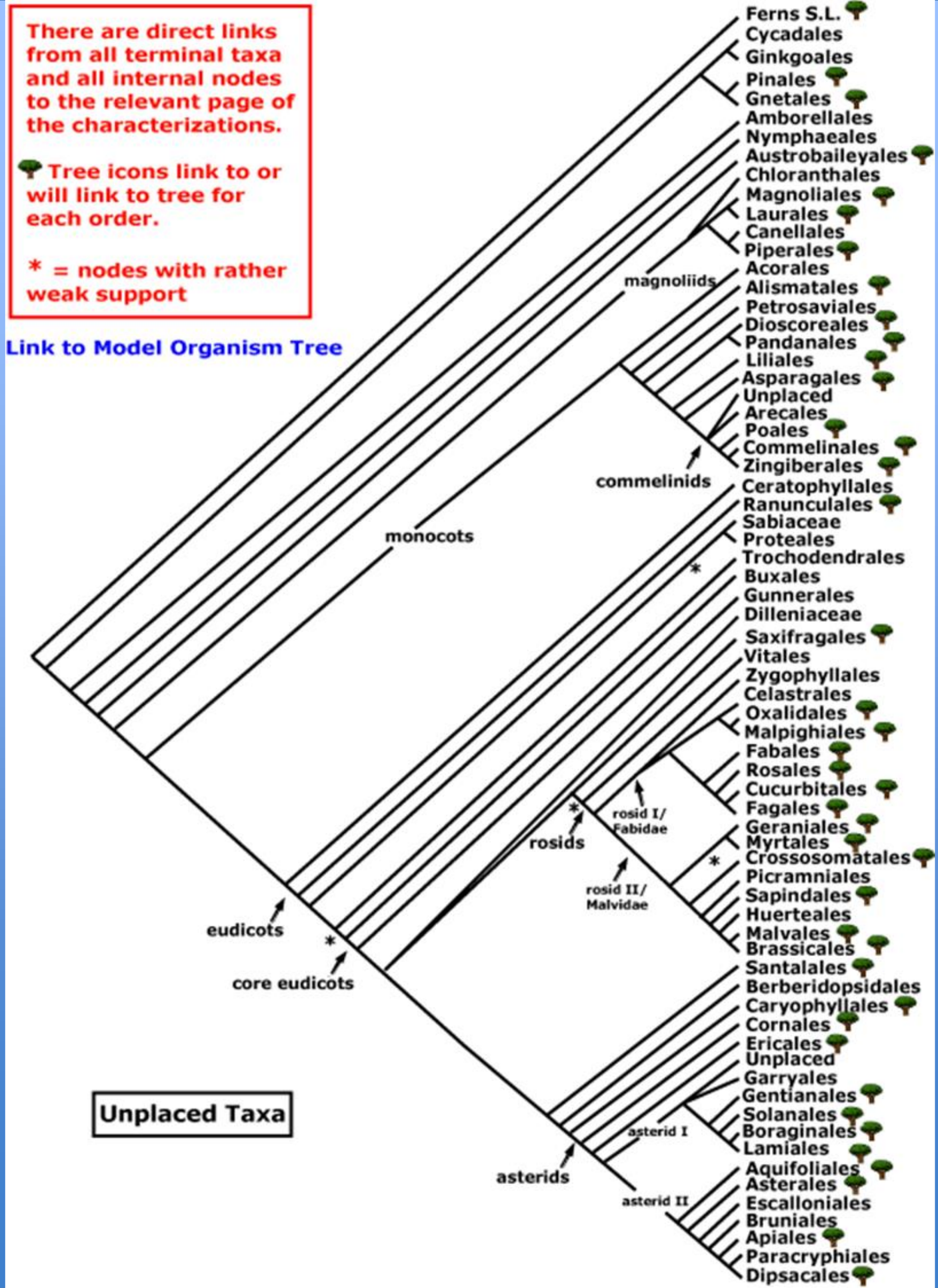
Angiosperm Phylogeny Group (APG)

There are direct links from all terminal taxa and all internal nodes to the relevant page of the characterizations.

Tree icons link to or will link to tree for each order.

* = nodes with rather weak support

Link to Model Organism Tree



Morphological variation in *Withania somnifera (Solanaceae)*

From Abha, Saudi Arabia

From Riyadh, Saudi Arabia

Morphological variation in *Panax ginseng (Araliaceae) and Nomenclature*

Page 1 of 1 100 items per page

Family	!	Scientific Name	Authority	Reference	Date
Araliaceae		Panax pseudoginseng	Wall.	Trans. Med. Soc. Calcutta 4: 117	1829
Araliaceae		Panax pseudoginseng var. angustifolius	(Burkill) H.L. Li	Sargentia 2: 118	1942
Araliaceae		Panax pseudoginseng var. bipinnatifidus	(Seem.) H.L. Li	Sargentia 2: 118	1942
Araliaceae		Panax pseudoginseng var. elegantior	(Burkill) G. Hoo & C.J. Tseng	Acta Phytotax. Sin. 11(4): 436	1973
Araliaceae		Panax pseudoginseng subsp. himalaicus	H. Hara	J. Jap. Bot. 45(7): 208–209	1970
Araliaceae		Panax pseudoginseng var. himalaicus	H. Hara	J. Jap. Bot. 45: 208	1970
Araliaceae		Panax pseudoginseng subsp. japonicus	(C.A. Mey.) H. Hara	J. Jap. Bot. 45(7): 209–210	1970
Araliaceae		Panax pseudoginseng var. japonicus	(C.A. Mey.) G. Hoo & C.J. Tseng	Acta Phytotax. Sin. 11(4): 437–438	1973
Araliaceae		Panax pseudoginseng var. major	(Burkill) H.L. Li	Sargentia 2: 119	1942
Araliaceae		Panax pseudoginseng var. notoginseng	(Burkill) G. Hoo & C.J. Tseng	Acta Phytotax. Sin. 11(4): 435	1973
Araliaceae		Panax pseudoginseng var. pseudoginseng			
Araliaceae		Panax pseudoginseng var. wangianus	(S.C. Sun) G. Hoo & C.J. Tseng	Acta Phytotax. Sin. 11(4): 436	1973

Taxonomic problems

Why is experimental taxonomy needed?

- Taxonomic problems arise due to phenotypic plasticity, where the same species shows different forms under different environmental conditions.
- Hybridization between closely related species produces intermediate forms that blur species boundaries.
- Polyploidy and chromosomal variation create multiple cytotypes within a species complex.
- Incomplete or overlapping morphological characters make it difficult to delimit species accurately.
- Wide geographical distribution leads to continuous variation and formation of ecotypes or races.
- Nomenclatural issues arise due to multiple names (synonyms) being assigned to the same taxon by different taxonomists.
- Changes in taxonomic concepts often require frequent name revisions, causing inconsistency in literature.
- Non-compliance with the International Code of Nomenclature (ICN) results in invalid or illegitimate names.
- Artificial and outdated classification systems fail to reflect true evolutionary relationships.
- Integration of new molecular data often leads to reclassification of taxa, disrupting traditional groupings.
- Disagreement among taxonomists on species limits leads to multiple competing classifications.
- Lack of universal acceptance of new classifications creates instability in plant nomenclature and taxonomy.

Types of Taxonomy / Taxonomic Studies / Plant Taxonomic Classification

From the various stages of classification, the types of taxonomy are defined: -

- **Alpha (α) Taxonomy / classical taxonomy:-**

It involves description and naming of organisms. It is the parent of other types of taxonomy.

- **Beta (β) Taxonomy: -**

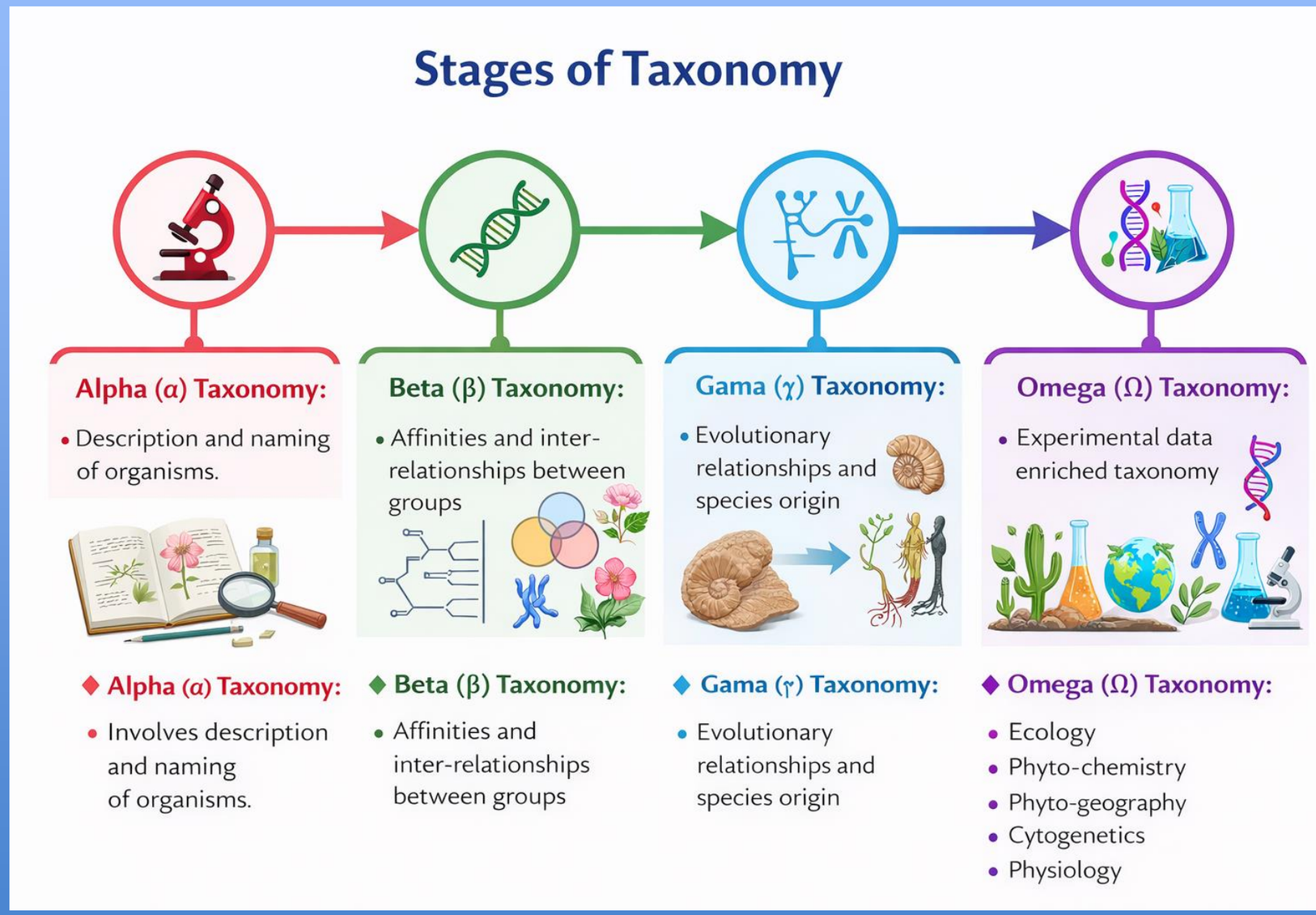
In addition to morphological description, it also involves consideration of affinities and their inter-relationship between separate group of species.

- **Gama (ɣ) Taxonomy: -**

It is concerned with description, inter-relationship and evolution of one species from the other.

- **Omega (Ω) Taxonomy: -**

It is the modern experimental taxonomy in which the taxonomic activities have been enriched with data from ecology, phyto-chemistry, phyto-geography, cyto-genetics and physiology coupled with adequate computation.



Experimental Methods in Taxonomy

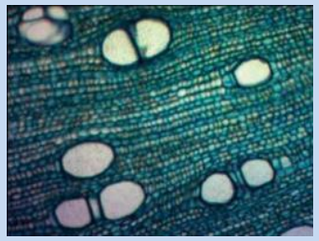
- Experimental taxonomy applies scientific experiments to study plant variation.
- Controlled hybridization experiments test species relationships.
- Cytological analysis examines chromosome number and structure.
- Growth experiments evaluate environmental influence on morphology.
- Chemical analyses reveal biochemical differences among taxa.
- Experimental methods verify natural classification systems.
- Reproductive studies help determine species boundaries.
- Cultivation experiments distinguish genetic and environmental variation.
- Physiological experiments explain adaptive traits.
- Experimental taxonomy strengthens objective plant classification.

Taxonomic Evidences

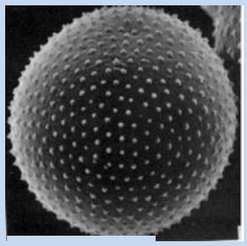
Taxonomic evidence for the establishment of classifications and phylogenies is gathered from a variety of sources

Morphology to Molecules

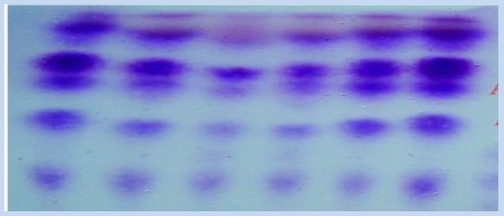
Morphology



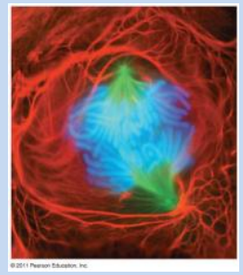
Anatomy



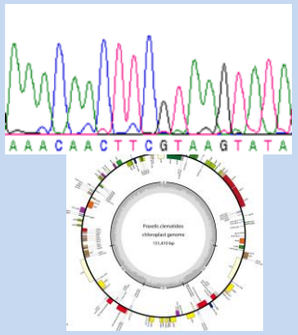
Pollen

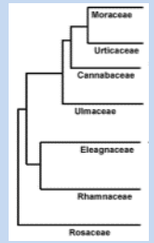


Chemistry



Chromosomes





DNA / Molecular taxonomv

- ❑ Taxonomic evidence includes all data used for plant classification.
- ❑ Morphological characters provide primary identification features.
- ❑ Anatomical traits support taxonomic differentiation.
- ❑ Cytological evidence explains evolutionary relationships.
- ❑ Palynological characters assist species recognition.
- ❑ Embryological features provide stable classification criteria.
- ❑ Chemical constituents serve as taxonomic markers.
- ❑ Molecular data provide precise evolutionary information.
- ❑ Ecological distribution patterns support classification decisions.
- ❑ Integrative taxonomy combines multiple evidences for accuracy.

5. Taxonomic Evidences in Experimental Taxonomy

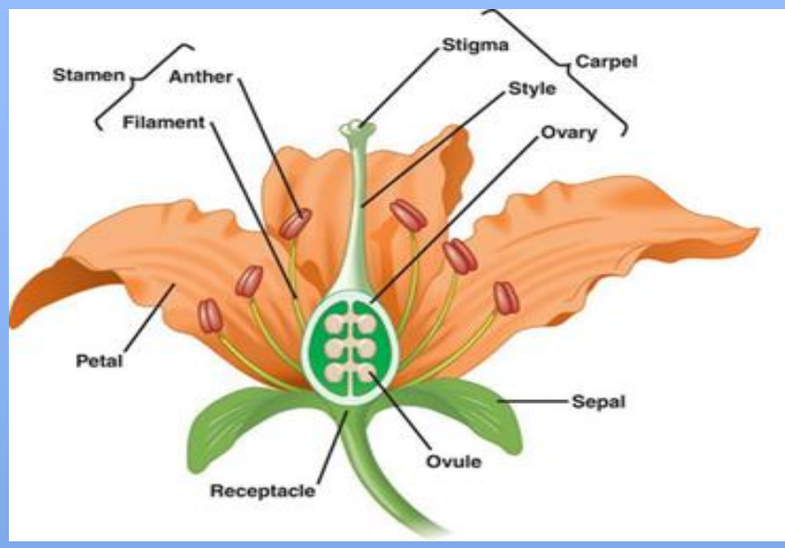
5.1 Structural Information (Morphological & Anatomical)

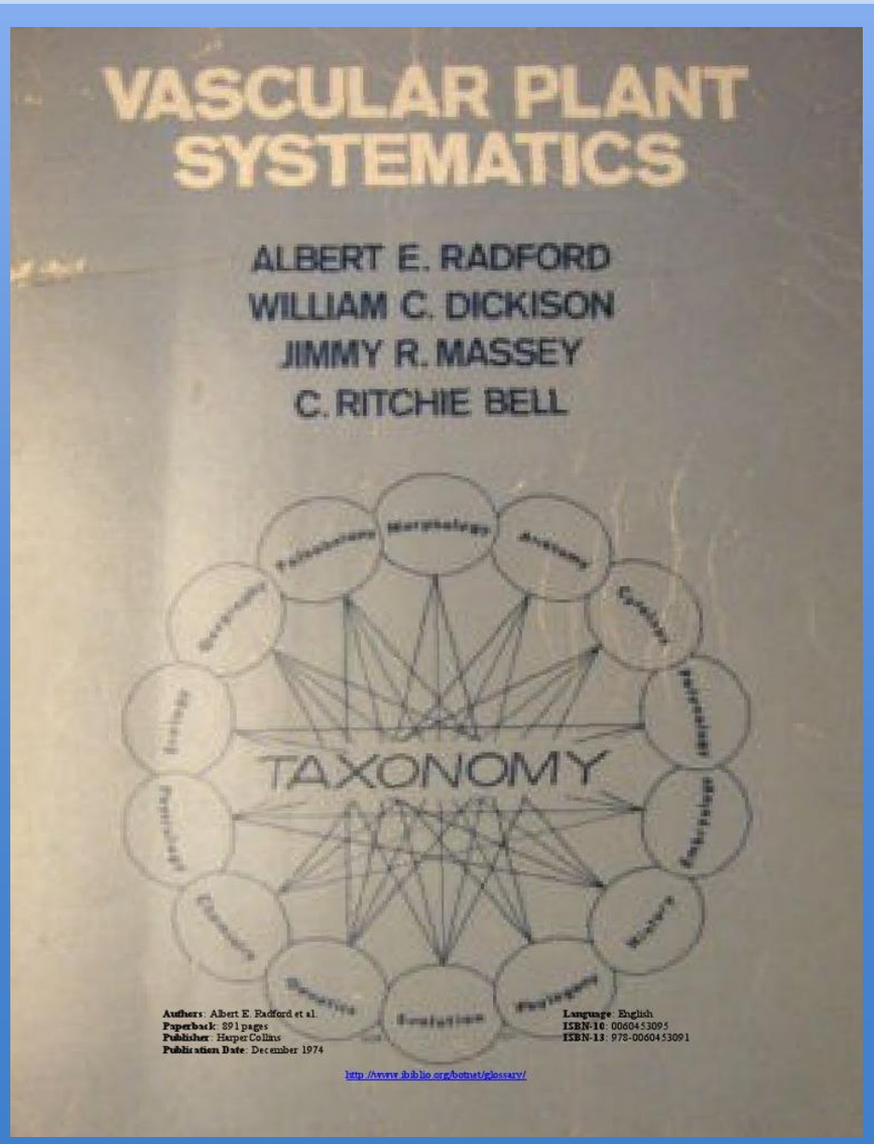
- Structural information includes morphological and anatomical characters.
- Morphological evidence remains the first step in taxonomy.
- Experimental taxonomy applies quantitative analysis of characters.
- Floral characters are important for higher-level classification.
- Leaf and seed morphology aid species identification.
- Microscopic traits improve identification precision.
- Stomatal and trichome types are valuable markers.
- Vascular and wood anatomy support family-level classification.
- Embryological characters provide taxonomic evidence.
- Structural evidence supports field-based identification.

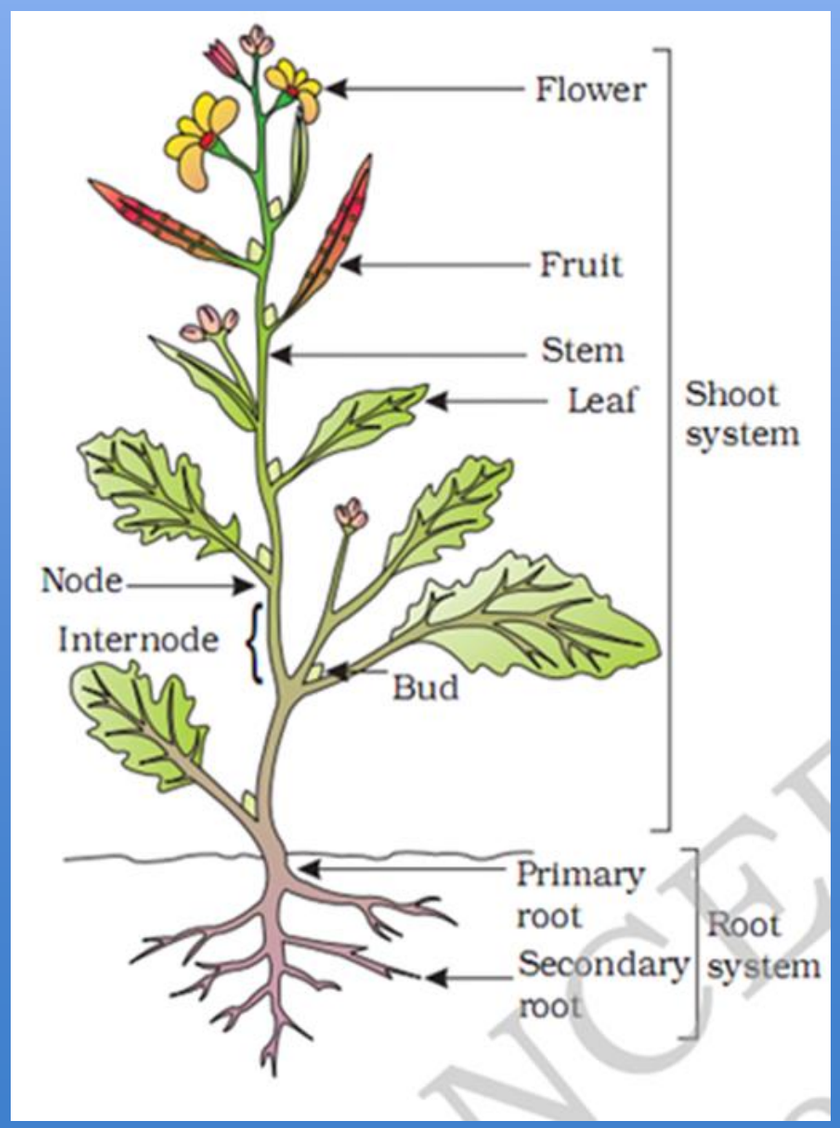
Plant Structure as Evidence of Taxonomy

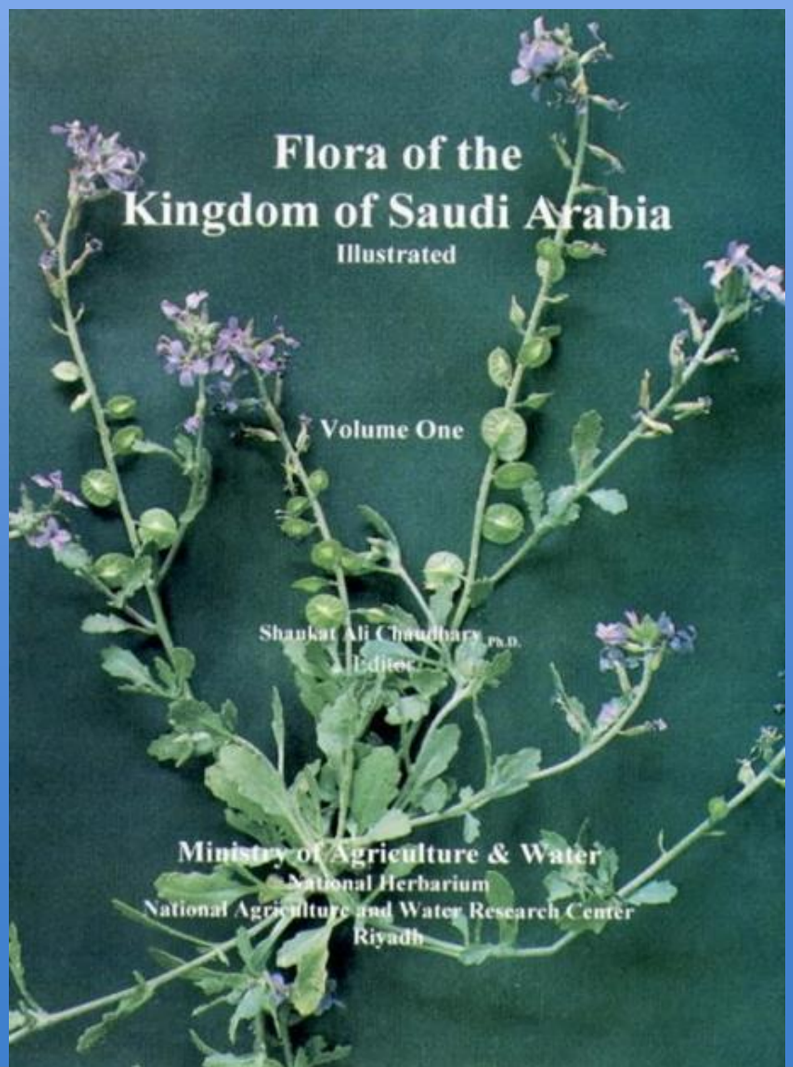
- External morphology provides fundamental taxonomic characters.
- Leaf shape and arrangement aid plant identification.
- Stem structure reflects evolutionary adaptation.
- Root systems provide ecological classification clues.
- Flower structure is highly significant in taxonomy.
- Inflorescence types assist family recognition.
- Fruit morphology supports species delimitation.
- Seed characteristics help distinguish taxa.
- Structural variations reveal evolutionary trends.
- Morphological characters remain essential in plant taxonomy.

Morphological characters provide primary identification features.









Herbarium: Plant Collection, Preservation, and Documentation

- **A HERBARIUM is a collection of dried plants systematically named and arranged for ready reference and study.**
- **To make a herbarium specimen, the plant is collected, and notes are made about it. The plant is then pressed until dry between blotters that absorb moisture and mounted onto a herbarium sheet with a suitable label, and stored in steel cabinet arranged into some system of classification.**
- **Herbarium techniques involve : (i) Collection, (ii) Drying, (iii) Poisoning, (iv) Mounting, (v) Stitching, (vi) Labelling, and (vii) Deposition.**

A Typical Herbarium (KSU) in Saudi Arabia

The Herbarium at King Saud University

Ethical responsibility in handling plant materials and reporting experimental findings

- Plant specimens must be collected responsibly with proper permits and minimal environmental disturbance.
- Endangered and protected species should not be harmed during taxonomic investigations.
- Laboratory handling of plant materials must follow safety and ethical guidelines.
- Experimental data should be recorded accurately without fabrication or manipulation.
- Research findings must be reported honestly with proper citation of sources and collaborators.

MICOR-MORPHOLOGY AS A SOURCE OF TAXONOMIC EVIDENCE

Systematic Significance of Micromorphological Characters in Plant Taxonomy

Micromorphological features of the leaf surface, trichomes, and electron microscopy provide crucial taxonomic insights at the family, genus, and species levels.

1. Leaf Surface Micromorphology

Cuticular Patterns: Thickness, ornamentation, and striations help distinguish taxa.
Epicuticular Wax: Crystals, plates, or rod-like structures vary across species.
Stomatal Complex: Arrangement and type aid in family-level classification.

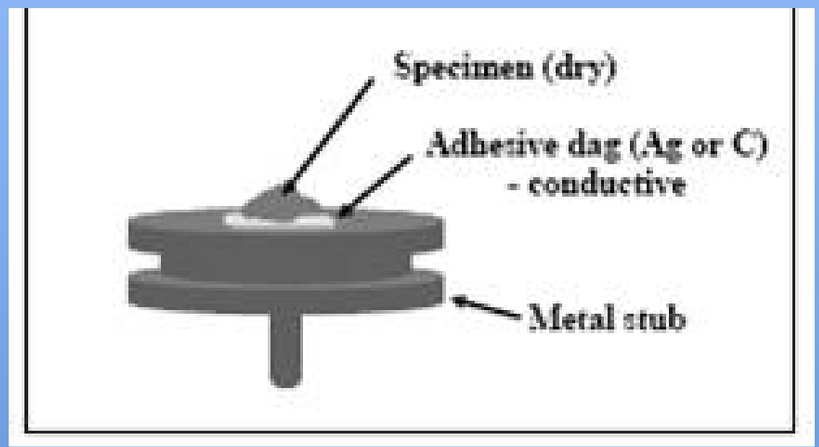
2. Trichomes (Hair-Like Structures)

Types: Glandular (secreting oils, resins) vs. non-glandular (unicellular, multicellular).
Distribution: Helps differentiate between closely related species.
Function: Adaptations to environmental stress (drought, herbivory, UV protection).

3. Electron Microscopy (Scanning & Transmission EM)

Ultrastructural Details: High-resolution imaging of cell walls, stomata, and glandular structures.
Palynology (Pollen Morphology): Shape, exine sculpturing used in classification.
Seed Coat Micromorphology: Unique textures help in species identification.

Scanning Electron Microscopy (SEM)



Systematic Significance of Seed Micromorphology & Electron Microscopy in Plant Taxonomy

Seed micromorphology, especially observed through scanning electron microscopy (SEM), provides valuable taxonomic and phylogenetic insights at different classification levels.

1. Key Micromorphological Seed Characters

Seed Coat Texture: Smooth, reticulate, striate, tuberculate (species-specific patterns).

Seed Shape & Size: Diagnostic at the genus or species level.

Hilum & Raphe Features: Position and structure help in taxonomic identification.

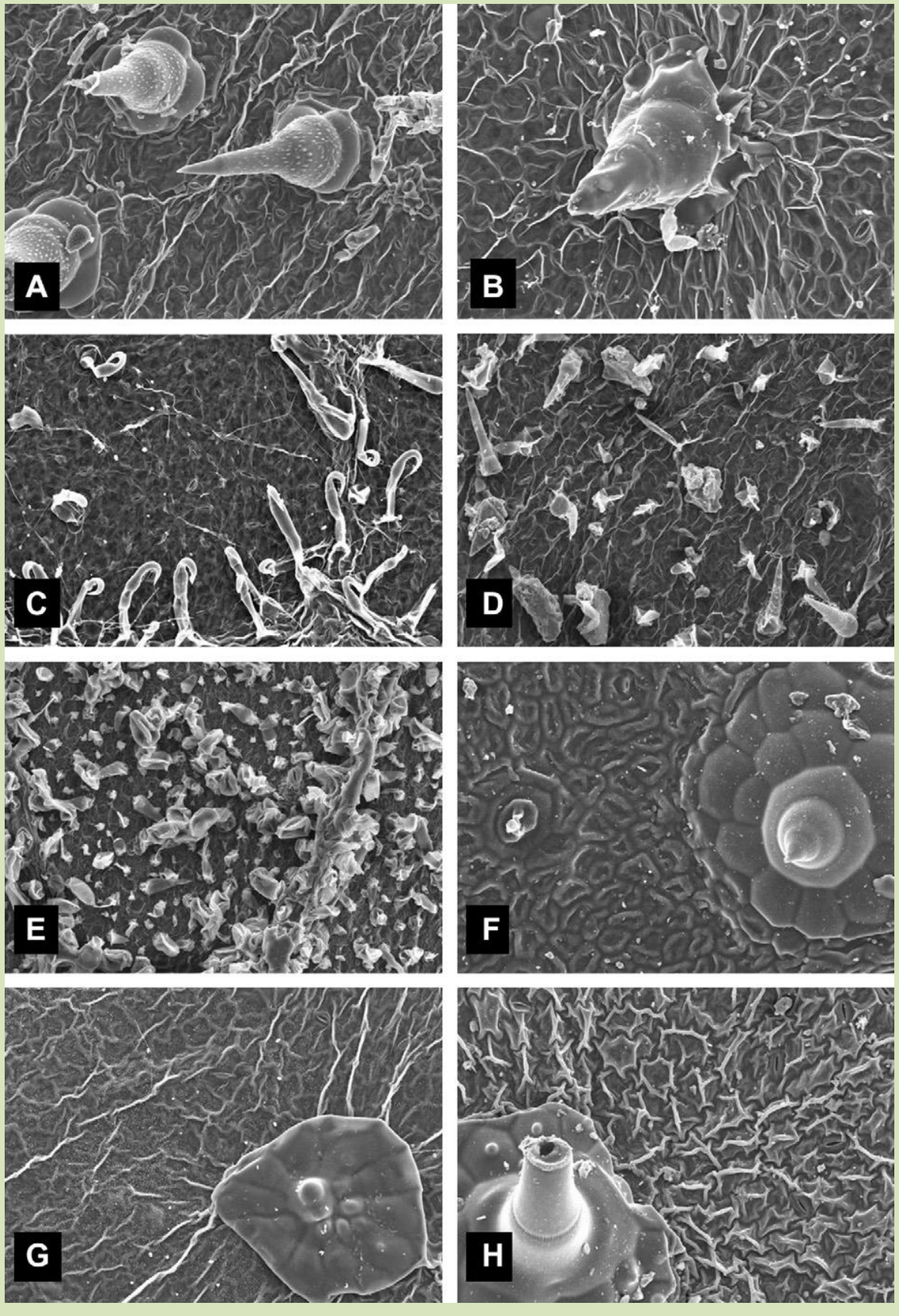
Epidermal Cell Structure: Unique cell wall ornamentation used for classification.

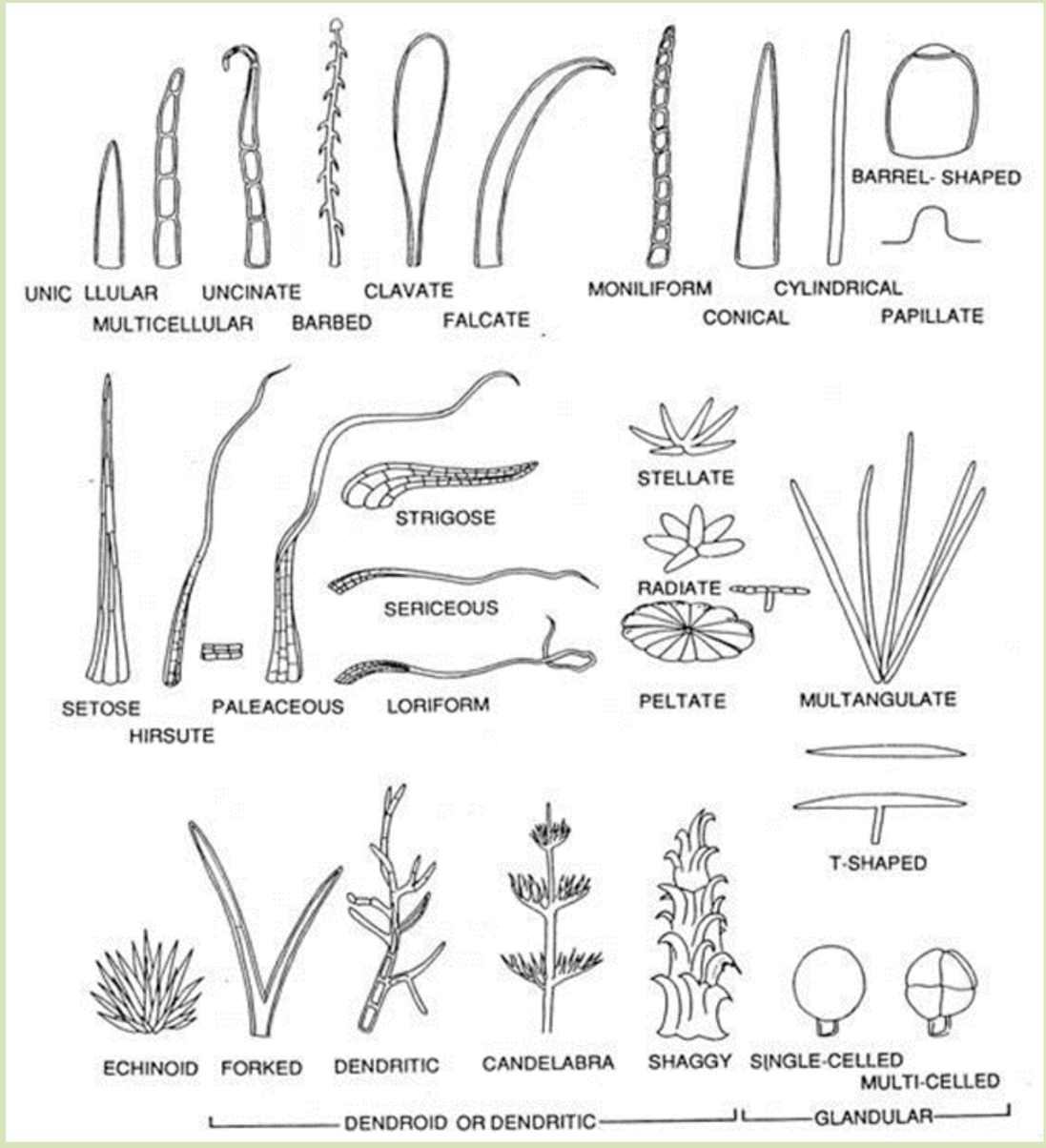
2. Electron Microscopy (SEM) in Seed Taxonomy

High-Resolution Surface Imaging: Identifies fine sculptural details not visible under light microscopy.

Pollen-Seeds Correlation: Helps in linking reproductive traits to taxonomic groups.

Comparative Studies: Differentiates closely related species or cultivars based on seed coat ultrastructure.

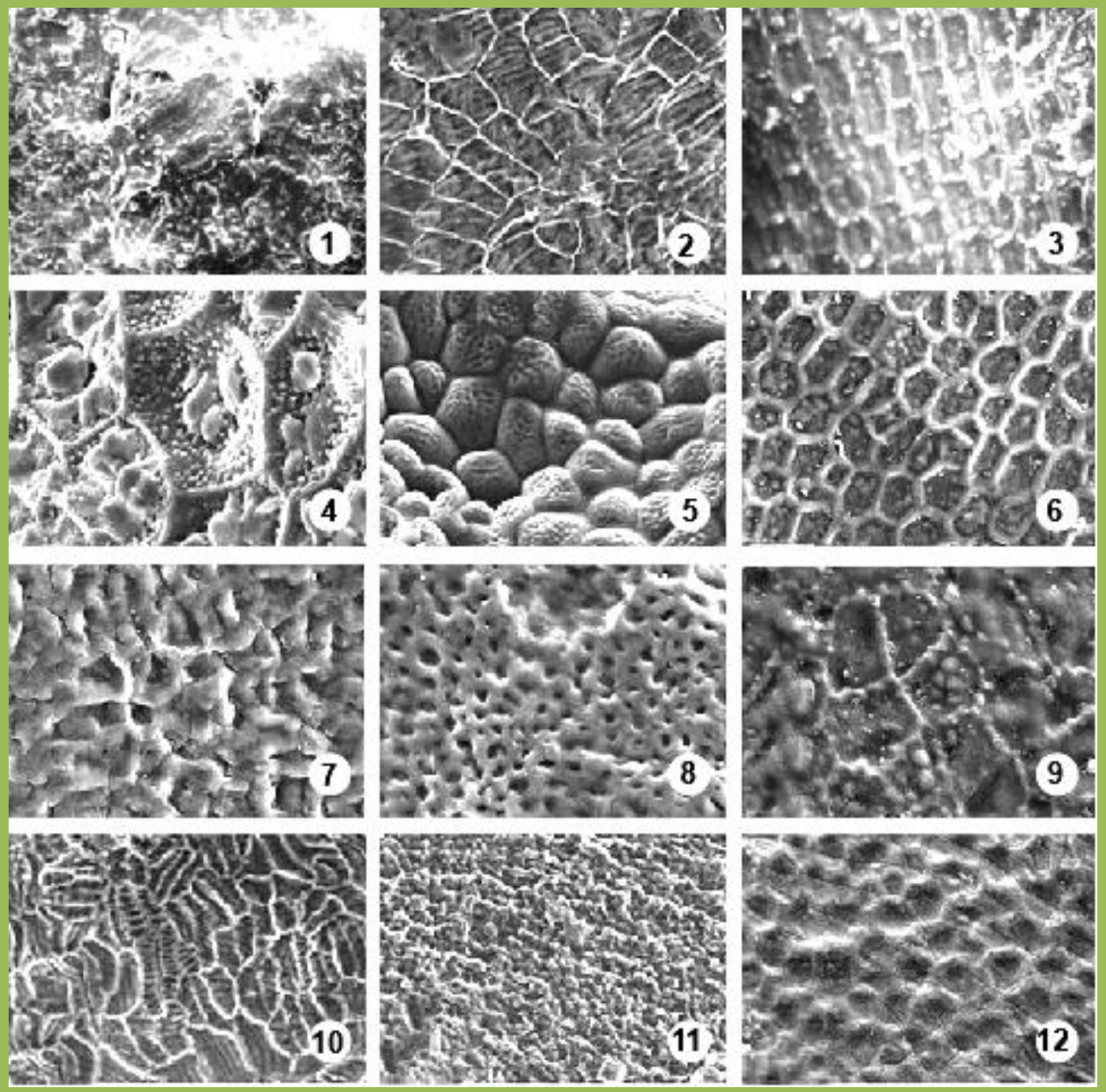




- **Trichomes meaning "hair", are fine outgrowths or appendages on plants.**
- **Ali and Al-Hemaid (2011) studies trichomes of 23 species of the member of the family Cucurbitaceae using Electron Microscope in order to find the systematic significance of micromorphological characters of trichomes**

Trichomes morphology in Cucurbitaceae: (A) *Melothria maderspatana* ·300, (B) *Sechium edulae*, (C) *Thladiantha cordifolia* ·300, (D) *Trichosanthes cucu*merina ·300, (E) *T. cucumerina* var. *anguina* ·300, (F) *T. dioica* ·300, (G) *T. lepiniana* ·300, and (H) *T. tricuspidata* ·300.

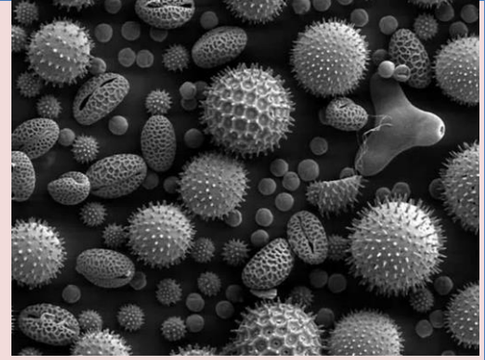
SPERMODERM



- Spermoderm refers to the pattern present on the seed coat of mature seeds.
- Seed characteristic, particularly exomorphic features as revealed by scanning electron microscopy, have been used by many workers in resolving taxonomic problems (Koul et al., 2000; Pandey and Ali, 2006) and evolutionary relationships (Kumar et al., 1999; Segarra and Mateu, 2001).
- Ali et al. (2013) studied the spermoderm pattern of the members of the family cucurbitaceae using Electron Microscope in order to find the systematic significance of micromorphological characters seed surface

Scanning electron micrograph of the seed surface in Cucurbitaceae: 1. *Benincasa hispida ×400* (rugulate); 2. *Citrullus colocynthis ×400 (reticulate); 3. Cucumis melo var. agrestis ×400 (reticulate); 4. Diplocyclos palmatus ×1000 (reticulate); 5. Gynostemma laxiflorum ×600 (colliculate); 6. Hemsleya longivillosa ×400 (reticulate); 7. Luffa echinata ×1000 (reticulate); 8. Momordica charantia ×700* (reticulate); 9. *Momordica cymbalaria ×1000 (reticulate); 10. Schizopepon bryoniifolius ×400* (reticulate); 11. *Sicyos angulatus ×300 (rugulate); 12. Trichosanthes cucumerina ×320 (reticulate).*

Systematic Significance of Palynology & Pollen Micromorphology in Plant Taxonomy



Palynology (study of pollen grains and spores) provides crucial taxonomic, phylogenetic, and evolutionary insights. **Scanning Electron Microscopy (SEM)** and **Transmission Electron Microscopy (TEM)** reveal pollen characteristics with high precision, aiding plant classification.

1. Key Pollen Micromorphological Characters

Pollen Shape & Size: Spherical, oblate, prolate (diagnostic at genus/species level).

Aperture Types:

- **Monocolpate:** Primitive type (Magnoliids, monocots).
- **Tricolpate:** Characteristic of eudicots.
- **Porate & Colporate:** Found in advanced taxa.

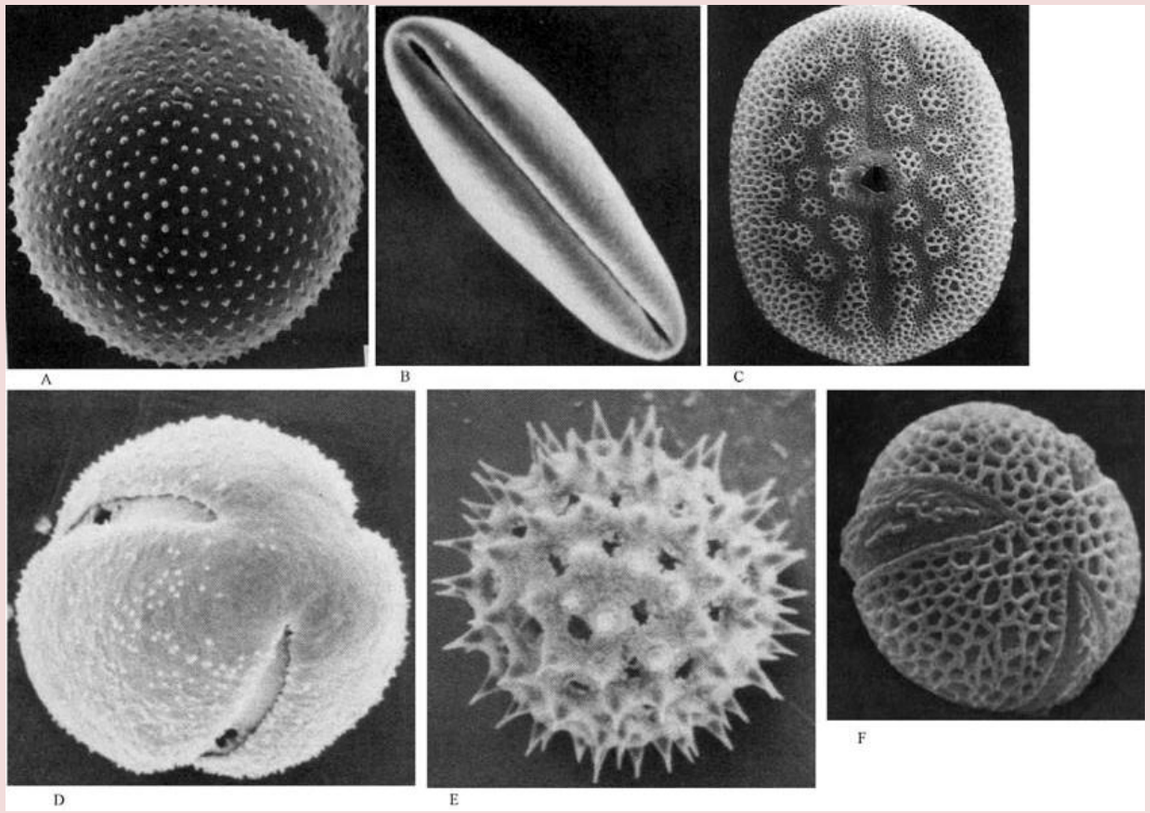
Exine Ornamentation: Reticulate, striate, spinate, psilate (family-specific patterns).

2. Electron Microscopy (SEM & TEM) in Pollen Studies

SEM: Reveals exine sculpturing, spines, and structural details.

TEM: Examines internal pollen wall layers, stratification, and ultrastructure.

Fossil Pollen Studies: Helps trace evolutionary lineages and ancient plant distributions.



SEM of pollen grains. A: Nonaperturate pollen grain of *Persea americana; B: Monosulcate* pollen grain of *Magnolia grandiflora; C: Monoporate pollen grain of Siphonoglossa; D:* Tricolporate pollen grain of *Scaevola glabra; E: Polyporate spinose pollen grain of Ipomoaea wolcottiana; F: Tricolpate pollen grain of Disanthus cercidifolius.*

- **Palynology** is the study of plant pollen and spores.
- There are two pollen types: monosulcate and tricolpate
- Monosulcate pollen are boat shaped with one long furrow and one germinal aperture (associated with primitive docots and the majority of monocots, the cycads and ferns). Triculpate pollen are found and typically have 3 apertures and is characteristic of the more advanced dicots.

Experiment Techniques:

- Today's strongest compound microscopes have magnifying powers of 1,000 to 2,000X.
- SEM (Scanning electron Microscope) or TEM (transmission electron Microscope) is required to study ultra structure.
- SEM and TEM is costly microscope (price in Million or Million plus Riyal).
- Magnification about 500,000 times.
- Material to be studies kept on aluminum stub, and then placed under vacuum condition (gold coating machine) for gold coating.
- Gold coated biological sample placed in SEM chamber.
- Specimen passed thru electron beam
- Images can be only observed at computer monitor.

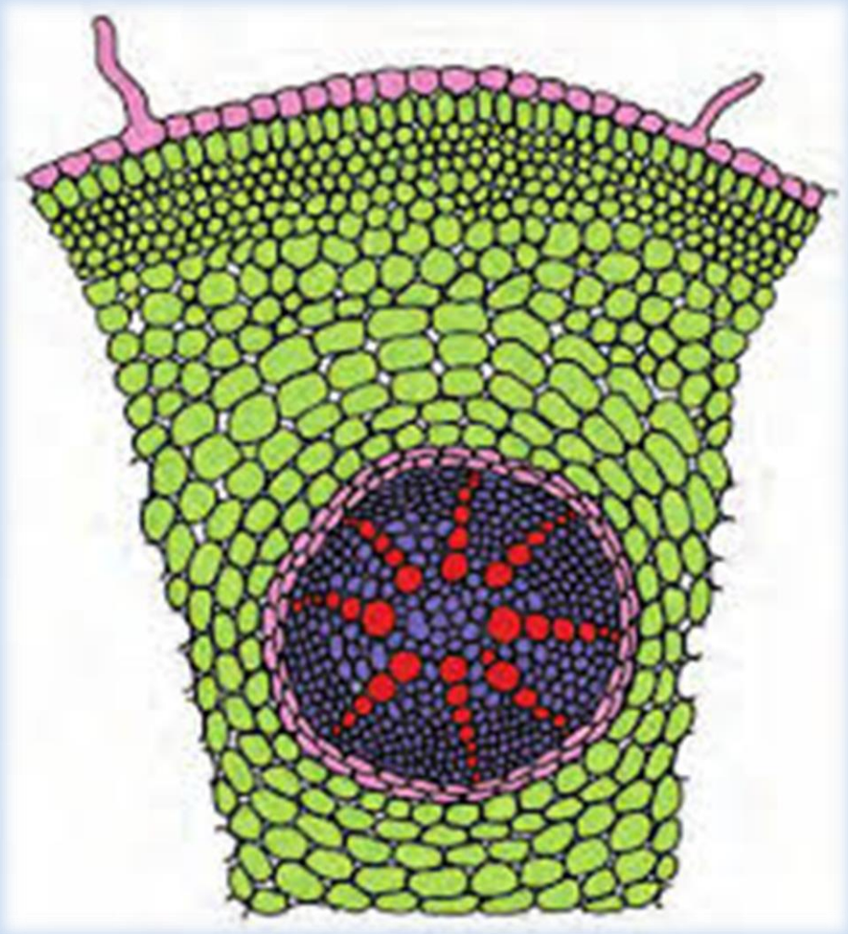
Erdtman (1963) used the pollen characters in solving the taxonomic problem of 105 family

Anatomical, cytological, chemical, and ecological evidence are used in experimental plant taxonomy

- Anatomical characters such as vascular bundle arrangement help distinguish closely related plant taxa.
- Leaf epidermal features including stomata and trichomes provide reliable taxonomic evidence.
- Cytological studies analyze chromosome number and structure to reveal evolutionary relationships.
- Polyploidy and chromosomal variation assist in species delimitation.
- Chemical constituents like alkaloids and flavonoids serve as chemotaxonomic markers.
- Secondary metabolite profiles help identify cryptic species.
- Ecological evidence evaluates plant adaptation to specific habitats.
- Geographic distribution patterns support taxonomic classification.
- Integration of anatomical, cytological, chemical, and ecological data improves natural classification systems.
- Experimental taxonomy combines multiple evidences to achieve accurate plant identification.

Plant Anatomy as Evidence in Taxonomy

- Internal plant structure provides reliable taxonomic characters.
- Vascular tissue arrangement helps differentiate families.
- Stomatal types are important anatomical markers.
- Trichome structure aids species identification.
- Wood anatomy assists classification of trees.
- Leaf anatomy reflects ecological adaptation.
- Epidermal cell patterns support taxonomic distinction.
- Anatomical traits are less affected by environment.
- Microscopic structures reveal evolutionary relationships.
- Anatomy complements morphological classification.



Laboratory techniques (microscopy, anatomical, and cytological observation) to collect taxonomic evidence responsibly

- ❑ Light microscopy is used to examine plant anatomical structures accurately.
- ❑ Proper specimen preparation ensures reliable anatomical observation.
- ❑ Sectioning techniques reveal internal tissue organization.
- ❑ Cytological staining helps visualize chromosomes during cell division.
- ❑ Microscopic observation assists identification of diagnostic characters.
- ❑ Laboratory safety protocols must be followed during specimen handling.
- ❑ Ethical collection practices ensure conservation of plant populations.
- ❑ Accurate recording of observations maintains scientific reliability.
- ❑ Preservation techniques protect specimens for future reference.
- ❑ Responsible laboratory practice ensures reproducible taxonomic research.

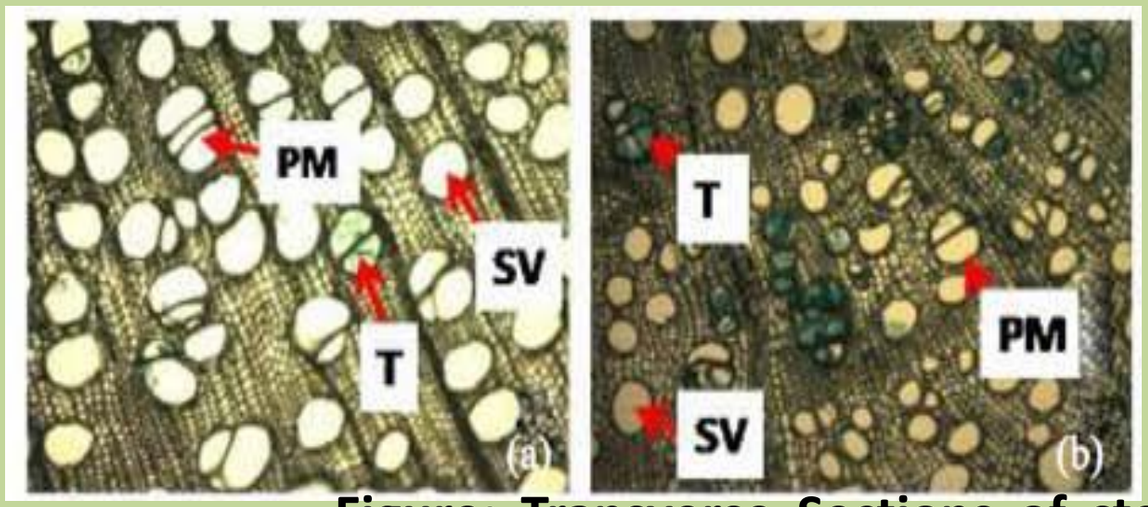
INTERNAL STRUCTURE/ INTERNAL MORPHOLOGY / ANATOMY AS A SOURCE OF TAXONOMIC EVIDENCE

Source of Taxonomic Evidence: Plant Anatomy & Physiology

1. Plant Anatomy (Internal Characteristics)

Vascular Tissues: Xylem (vessel elements vs. tracheids), phloem structure
Leaf Anatomy: Stomata type, epidermal patterns
Secretory Structures: Glandular trichomes, laticifers, resin ducts
Wood Anatomy: Growth rings, fiber arrangement

2. Plant Physiology in Taxonomy
Photosynthetic Pathways: C3, C4, CAM plants
Secondary Metabolites: Alkaloids, flavonoids, essential oils
Growth Responses: Photoperiodism, seed dormancy mechanism



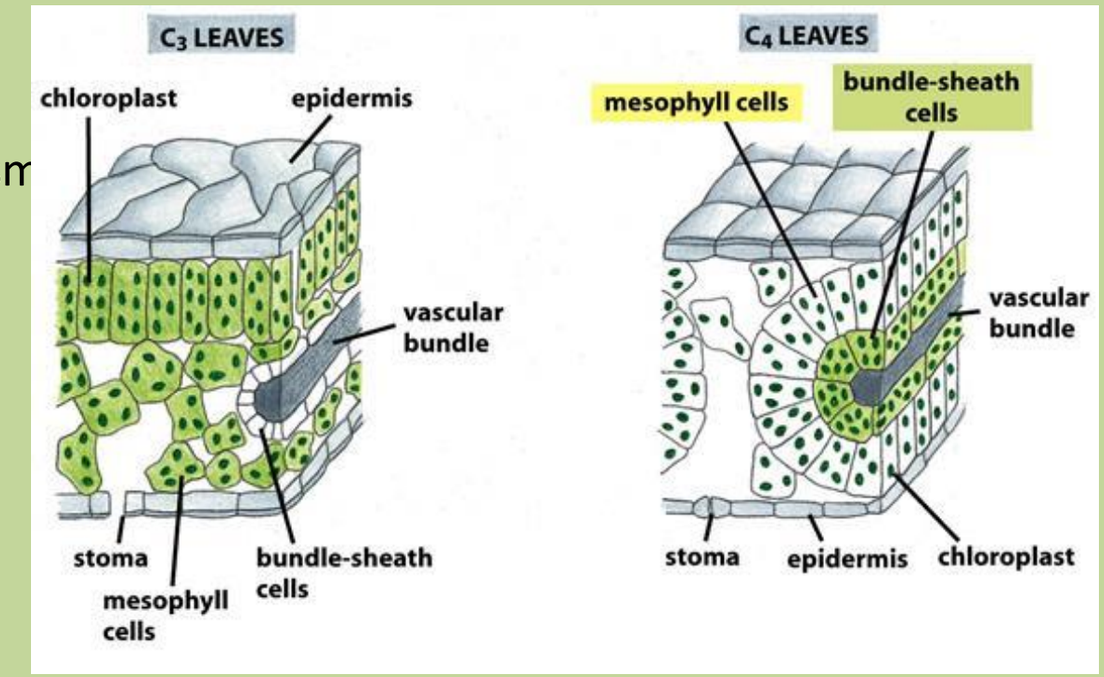
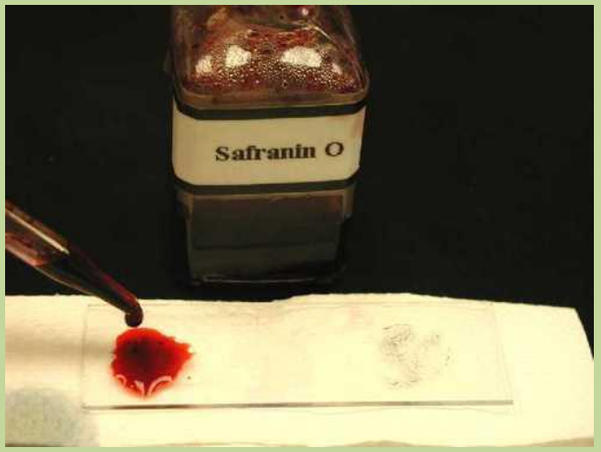
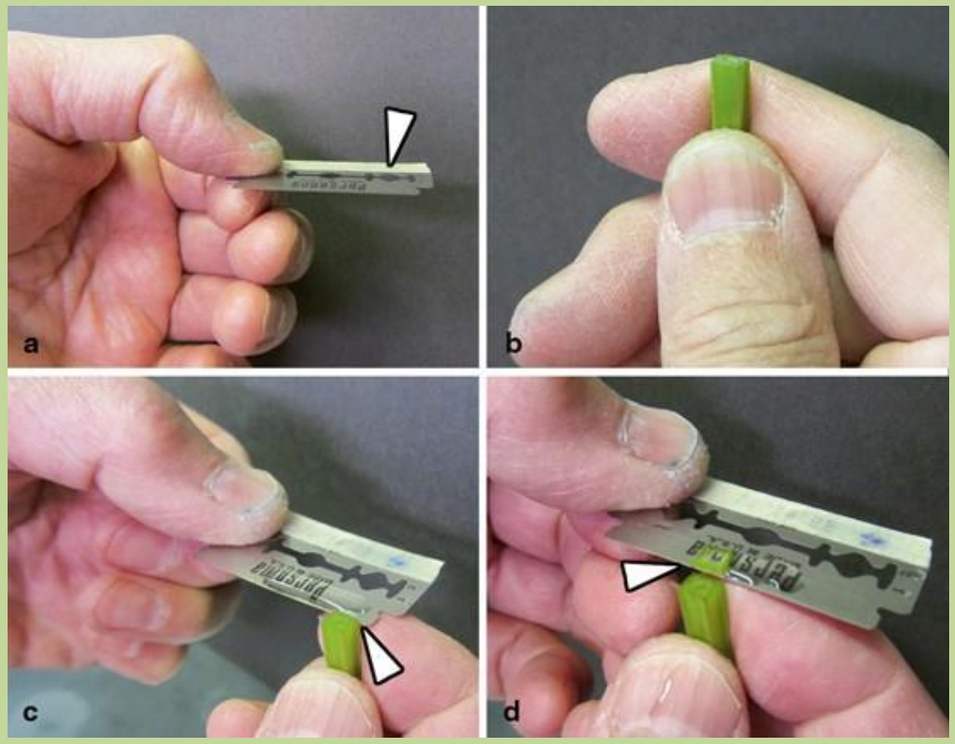


Figure: Transverse Sections of stem *Artocarpus atilis* (a) and *Artocarpus communis* (b). PM: Pore multiple, T=Tylose (Tyloses are outgrowths on parenchyma cells of xylem vessels of secondary heartwood, SV: Solitary vessel

Experimental Techniques:

- **Cutting of thin slices / section (Transverse section or Longitudinal section) of plant organs**
- **Preparation of temporary slides or permanent slides**
- **Observation under light compound microscope using tissue stain like safranin, fast green**





Systematic Significance of Stomata in Plant Taxonomy

Stomatal characteristics provide valuable taxonomic evidence for plant classification and evolutionary studies.

1. Stomatal Types (Based on Structure & Development)

Anomocytic (Ranunculaceous): No distinct subsidiary cells (e.g., Ranunculaceae).

Paracytic (Rubiaceous): Two subsidiary cells parallel to the guard cells (e.g., Rubiaceae).

Diacytic (Caryophyllaceous): Two subsidiary cells at right angles to guard cells (e.g., Caryophyllaceae).

Anisocytic (Cruciferous): Three unequal subsidiary cells (e.g., Brassicaceae).

Actinocytic: Several radially arranged subsidiary cells.

2. Stomatal Distribution Patterns

Amphistomatic: Stomata on both leaf surfaces (adapted to high light environments).

Hypostomatic: Stomata only on the lower surface (common in mesophytes).

Epistomatic: Stomata only on the upper surface (e.g., floating aquatic plants).

3. Evolutionary & Taxonomic Importance

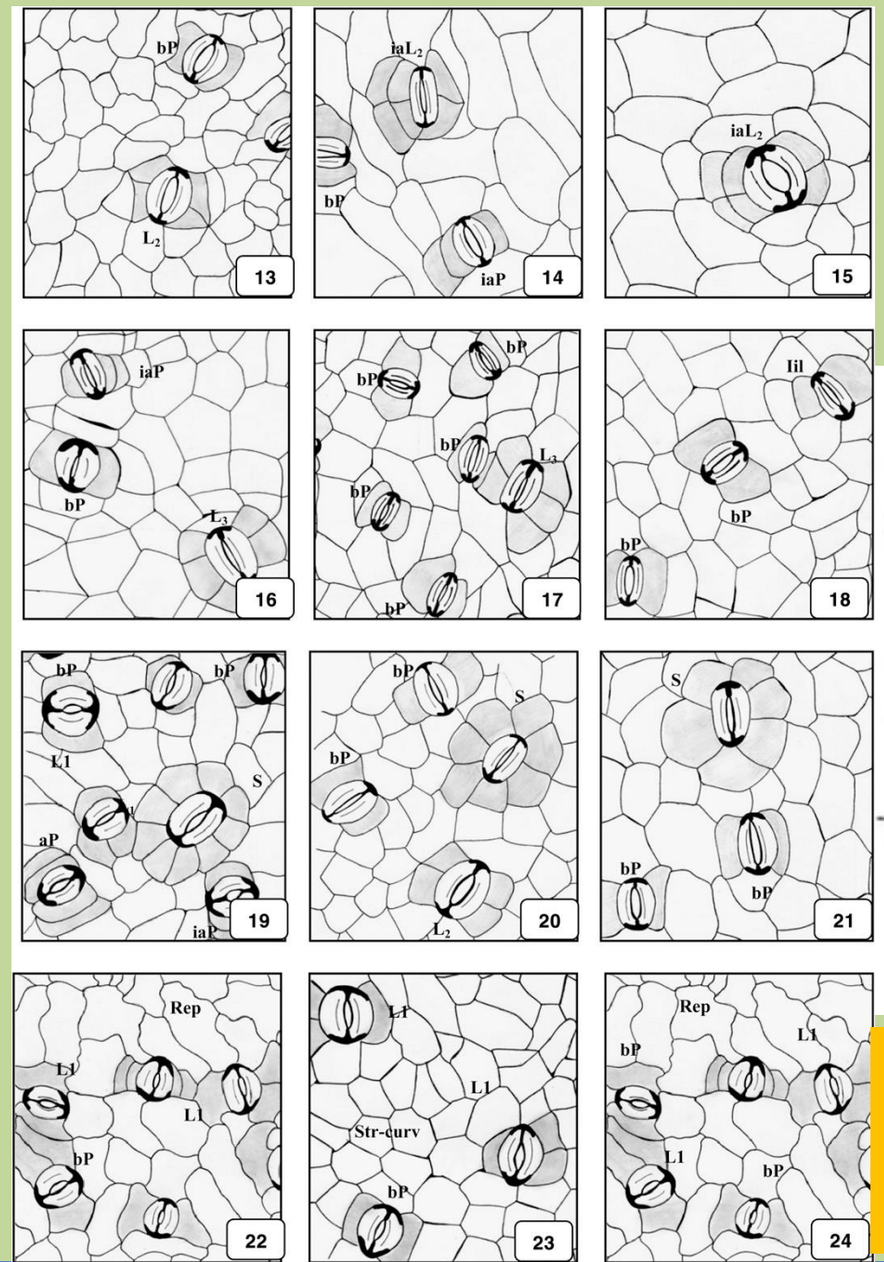
Stomatal types are stable within families and genera, aiding classification.

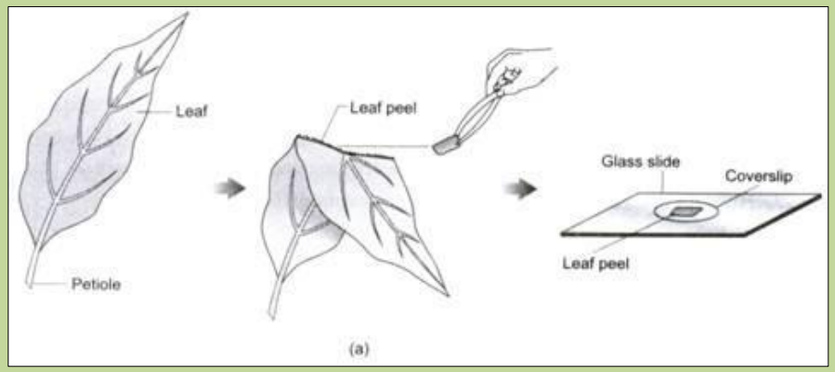
Variations reflect adaptations to ecological conditions (xerophytes, hydrophytes).

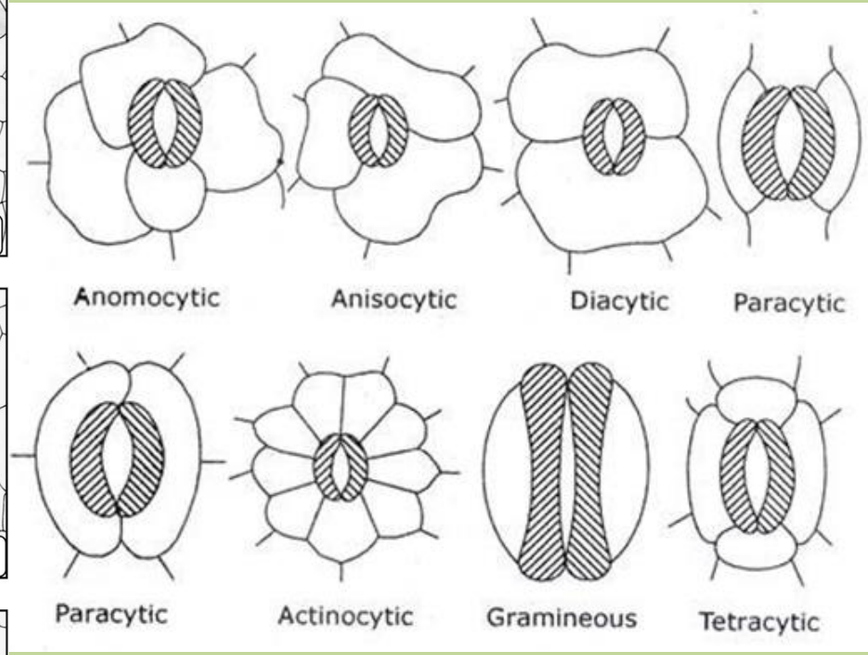
Fossil stomata help in phylogenetic and paleoenvironmental reconstructions.

Experiment Techniques:

- **Usually peeling of leaves and observation under light compound microscope (using tissue stain like safranin, fast green or without stain)**

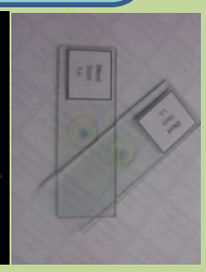






- **FARROKH et al., studies 32 *Salix speices of Salix* Species (Salicaceae) in order to find the systematic significance of trichomes in Angiosperms**

TAXONOMIC EVIDENCES: EMBRYOLOGY



Embryology (study of embryo development) provides valuable taxonomic evidence by revealing stable and conserved traits that help in plant classification and phylogenetic studies.

1. Key Embryological Characters in Taxonomy
Ovule Structure: Anatropous, orthotropous, campylotropous (helps distinguish families).
Embryo Type & Development: Variation in suspensor, cotyledon, and axis structure.
Endosperm Type: Nuclear, cellular, helobial (used to differentiate taxonomic groups).
Embryo Sac Development: Monosporic (Polygonum type), Bisporic, or Tetrasporic (variation across taxa).
Anther & Pollen Development: Tapetum type (glandular vs. amoeboid), pollen wall differentiation.

2. Systematic & Evolutionary Significance
Family-Level Differentiation:
- Onagraceae (Endosperm absent) vs. Asteraceae (Cellular endosperm).
- Solanaceae (Two-celled pollen) vs. Malvaceae (Three-celled pollen).

Phylogenetic Relationships: Conserved embryological traits indicate common ancestry.
Support for Classification Systems: Confirms or refines groupings based on external morphology.

Station	Time (min)	Solution
1		Water
2	45	70% alcohol
3	45	80% alcohol
4	45	90% alcohol
5	45	100% alcohol
6	60	100% alcohol
7	60	100% alcohol
8	60	Clearing reagent (xylene or substitute)
9	60	Clearing reagent (xylene or substitute)
10	60	Paraffin 1
11	60	Paraffin 2
12	60	Paraffin 3

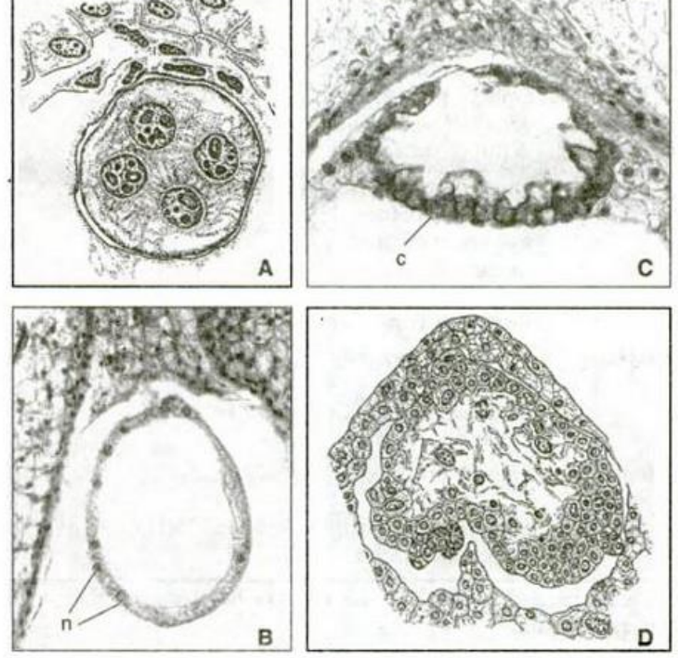


Fig. 12.14 Early stages of embryogenesis in *Paeonia* sp. **A,B.** Coenocytic embryo. **C.** Cellularization. **D.** Formation of embryos in the coenocytic-cellular stage. *n*, nuclei; *c*, cells (*from* Czapik and Izmailow, 2001)

- Embryology is the branch of biology that studies the prenatal development of gametes (sex cells), fertilization, and development of embryos and seed coats.
- The major embryological character that separates the monocots from the dicots is the number of embryonic cotyledon leaves.

- Embryological features are normally constant at the family level and below.
- The genus *Paeonia* was earlier included under the family Ranunculaceae. But *Paeonia* differs from Ranunculaceae in chromosome number, vascular anatomy, floral anatomy.
- Worsdell (1908) suggested its removal to a distinct family, Paeoniaceae.
- The separation is supported by the embryological features: (i) centrifugal stamens (not centripetal); (ii) pollen with reticulately-pitted exine with a large generative cell (not granular, papillate and smooth, small generative cell); (iii) unique embryogeny in which early divisions are free nuclear forming a coenocytic stage, later only the peripheral part becomes cellular (not onagrad or solanad type); and (iv) seed arillate.

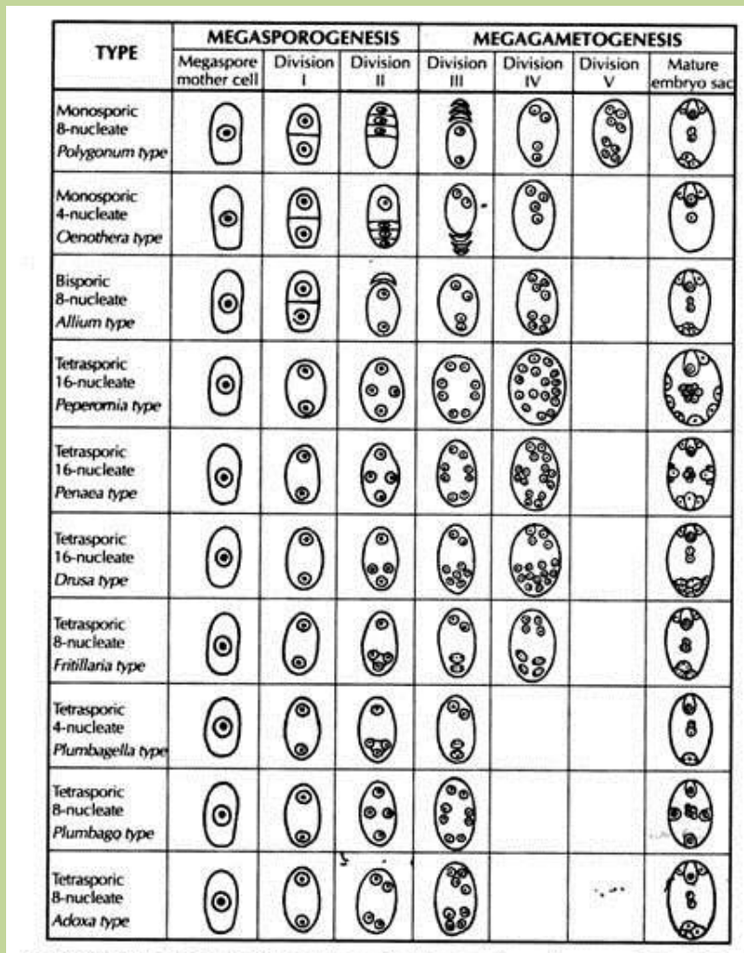


Fig. 3.8 : Development of different types of embryo sac in angiosperms (*after* Maheshwari)
[Micropyle above in all illustrations]

Cytological and Chemical Differences and Their Taxonomic Value

- Cytology examines chromosome number and structure.
- Polyploidy plays an important role in speciation.
- Chromosome behavior indicates evolutionary divergence.
- Karyotype analysis assists species delimitation.
- Chemical composition provides additional classification evidence.
- Secondary metabolites differentiate related taxa.
- Cytological variation supports phylogenetic relationships.
- Chemotaxonomic markers reveal hidden diversity.
- Combined cytological and chemical data increase accuracy.
- These differences strengthen modern taxonomic systems.

TAXONOMIC EVIDENCES: CYTO-TAXONOMY

Systematic Significance of Cytology in Plant Taxonomy

Cytology (study of chromosomes and cell structure) provides fundamental taxonomic evidence by revealing **chromosomal number, structure, and behavior**, which help in classifying and understanding plant relationships.

1. Key Cytological Characters in Taxonomy

Chromosome Number:

- Stable numbers define taxa (e.g., **Brassicaceae: 2n = 14**, **Poaceae: 2n = 14, 20**).
- Polyploidy (triploids, tetraploids) is common in evolution.

Chromosome Structure & Behavior:

- Karyotype analysis (size, shape, banding patterns) distinguishes taxa.
- Chromosomal rearrangements (inversions, translocations) provide evolutionary insights.

DNA Content & Genome Size:

- Differences in genome size help in species differentiation.
- Flow cytometry and sequencing assist in phylogenetic studies.

2. Systematic & Evolutionary Significance

Family & Genus Classification: Chromosomal variations help in plant differentiation (e.g., Asteraceae, Fabaceae).

Hybridization & Polyploidy:

- Explains speciation and adaptive evolution.
- Many crop plants (wheat, cotton) evolved via polyploidy.

Phylogenetic Insights: Chromosomal changes reflect evolutionary history and plant lineage divergence.

Cytotaxonomy (Chromosome Studies)

Definition: Study of **chromosome number, structure, and behavior** to classify plants.

Key Aspects:

Chromosome Number – Different species have specific numbers (e.g., *Triticum aestivum* has 42).

Karyotype Analysis – Examines chromosome shape and size.

Experiment Techniques:

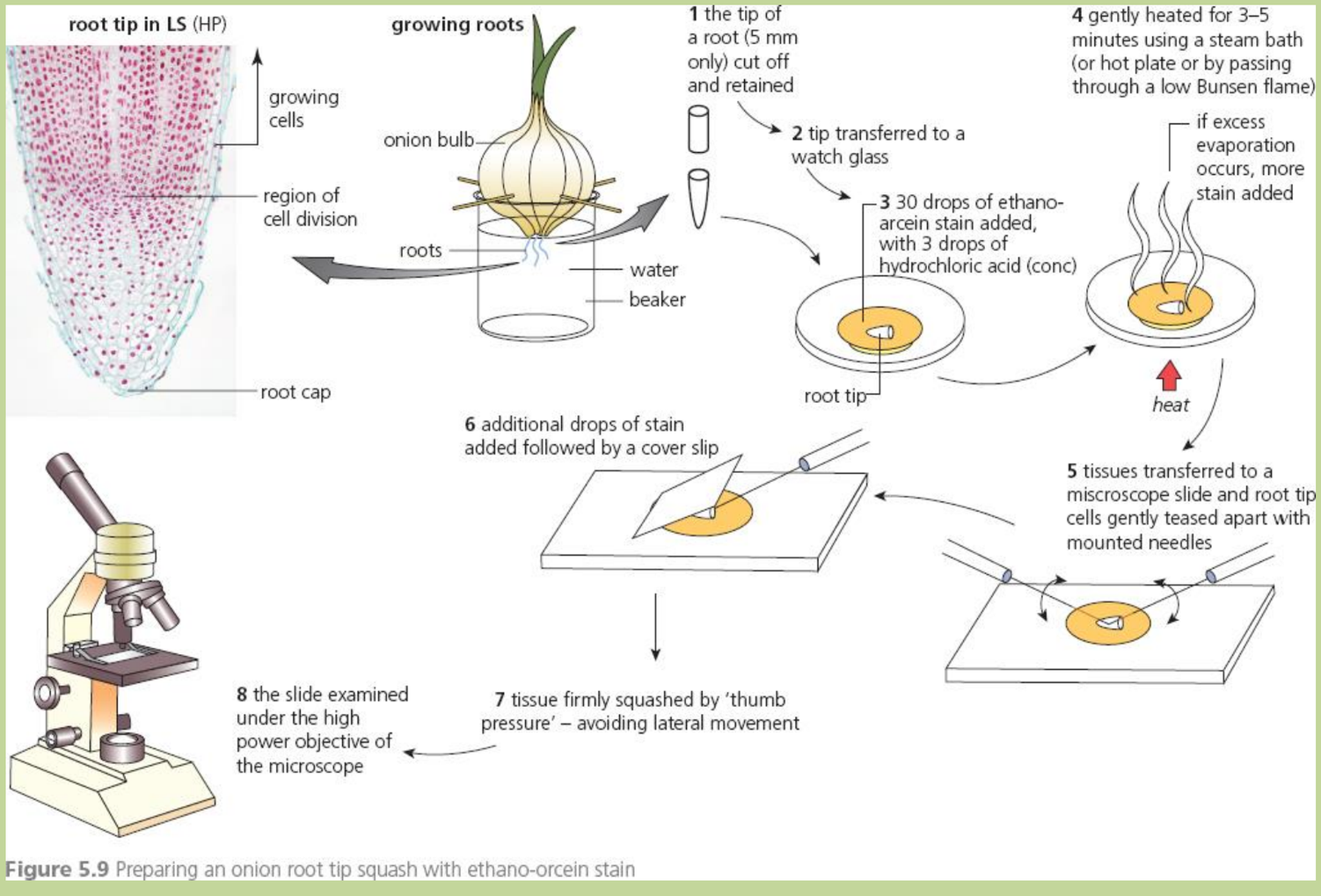
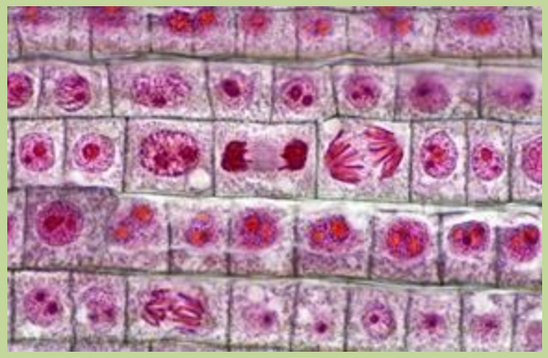
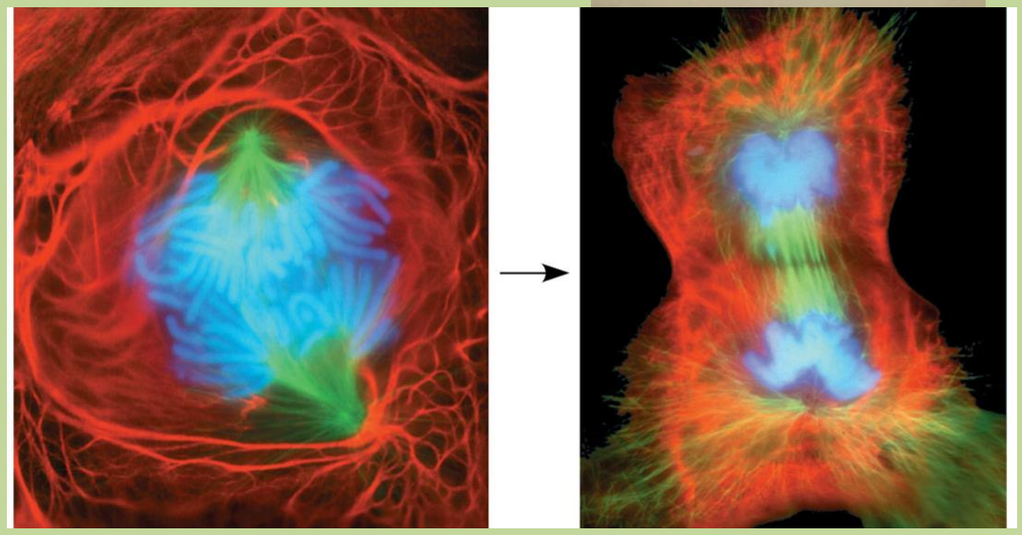


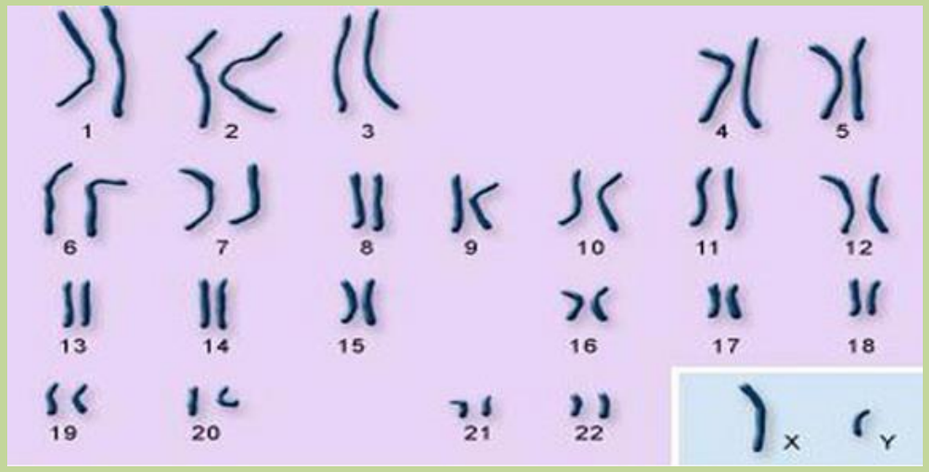
Figure 5.9 Preparing an onion root tip squash with ethano-orcein stain

- **Chromosome Set:**

- **Number of chromosome can be counted in the metaphase stage of cell division.**
- **One copy of each of the different chromosomes in the nucleus containing one copy of each different gene.**
- **Haploid Number (n): The number of chromosomes comprising one set.**
- **Diploid Number (2n): The number of chromosomes in a cell containing two sets.**
- **Human Haploid (n)= 23, Diploid (2n)=46**
- **Dates Haploid (n)= 14, Diploid (2n)=28**
- **In plants, only information about chromosome number, shape or pairing at meiosis is used for classification purposes.**
- **The term karyotype is used for the phenotypic appearance for the somatic chromosomes.**
- **The diagrammatic representation of the karyotype is termed as idiogram.**
- **The characteristic of chromosome having taxonomic values are: chromosome number, chromosome size, chromosome morphology, and chromosome behavior during meiosis.**
- **The genus *Yucca* had long been treated as a member if Liliaceae because of the superior ovary. Hutchinson shifted *Yucca* to the family Agavaceae because the genus *Yucca* possess 25 small and 5 large chromosome which is similar to the member of family Agavaceae**







Yucca carnerosana

Phytochemistry

- Phytochemistry studies chemical compounds produced by plants.
- Secondary metabolites serve as taxonomic indicators.
- Alkaloids help distinguish related species.
- Flavonoid profiles support classification.
- Terpenoids provide chemotaxonomic evidence.
- Chemical variation reflects evolutionary divergence.
- Metabolite analysis clarifies complex taxa.
- Phytochemical data support molecular taxonomy.
- Chemical markers assist medicinal plant identification.
- Chemotaxonomy enhances modern systematic studies.

5.2 Non-Structural Information

- Non-structural evidence includes cytological, biochemical, and molecular data.
- Chromosome number differentiates related species.
- Cytotaxonomy studies chromosome structure and behavior.
- Chemotaxonomy uses secondary metabolites for classification.
- Proteins and enzymes reveal genetic variation.
- Physiological and ecological traits aid taxonomy.
- Molecular data reveal genetic relationships.
- Non-structural evidence reduces reliance on morphology alone.
- Experimental data improve objectivity in taxonomy.
- Integration strengthens taxonomic conclusions.

TAXONOMIC EVIDENCES: CHEMO-TAXONOMY

Chemotaxonomy (Chemical Analysis)

Definition: Uses **chemical compounds** (secondary metabolites) for classification.

Key Chemical Markers:

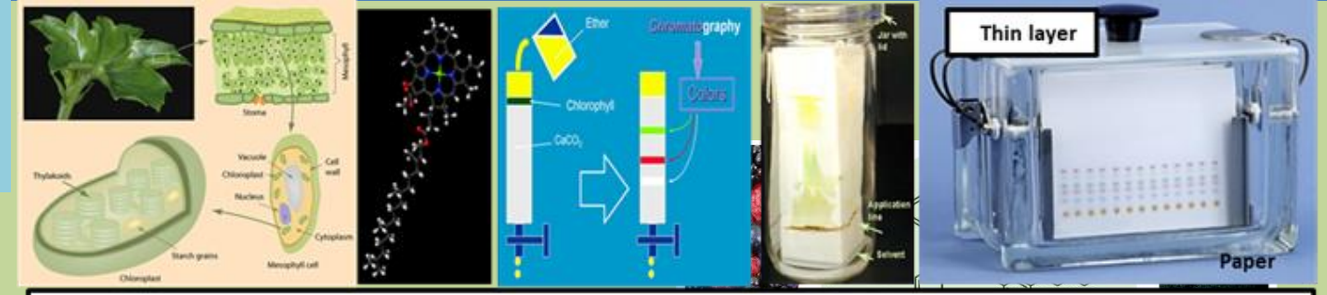
Alkaloids – Found in medicinal plants (*Papaver somniferum* - Opium poppy).

Flavonoids – Pigments help classify plant families (*Caryophyllales produce Betalains*).

Essential Oils – Common in aromatic plants (*Lamiaceae - Mint family*).

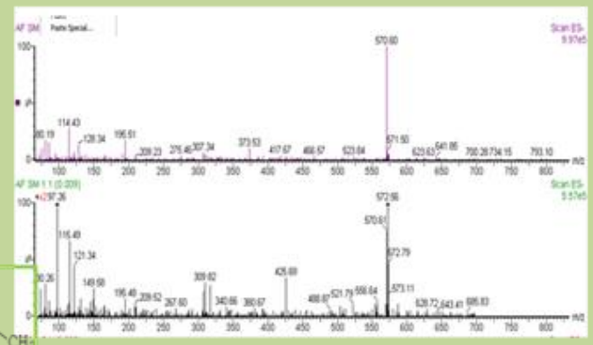
Example:
Betalains vs. Anthocyanins – Used to distinguish **Caryophyllales from Polygonales**.

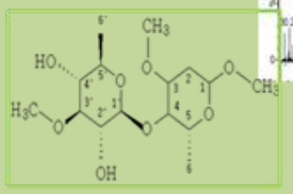
Importance:
Helps in **medicinal plant classification**.
Differentiates plants with **similar morphology**.
Useful in **pharmaceutical and agricultural research**.



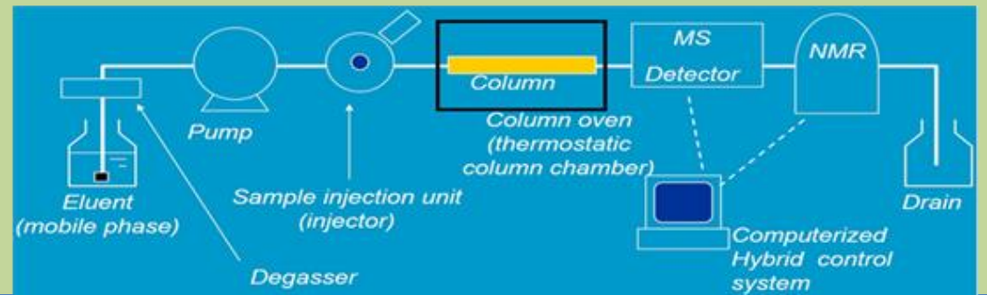
Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle. They all have a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas).

Mass spectrometry (MS) is an analytical technique that ionizes chemicals and measures the masses within a sample.





Al-Allah et al., 2018 identified as methyl β-lilacinobioside islated from *Caralluma retrospiciens*



Systematic Significance of Chemo-taxonomy in Plant Taxonomy

Chemotaxonomy uses **chemical compounds** (secondary metabolites) to differentiate plants at various taxonomic levels. These biochemical markers provide critical insights into plant relationships, classification, and evolutionary processes.

1. Key Chemical Characters in Taxonomy

Secondary Metabolites:

Alkaloids, flavonoids, terpenoids, and phenolics are commonly used for classification.
Terpenoids: Diagnostic in families like Lamiaceae and Rutaceae.
Flavonoids: Found in family-level distinctions like Rosaceae and Fabaceae.

Essential Oils:

Plant species produce distinct volatile oils (e.g., in mint, lavender) used to identify species and genera.

Amino Acids & Fatty Acids:

Unique amino acid compositions assist in distinguishing plant families.

Proteins & Enzymes:

Specific proteins (e.g., Rubisco) and enzymes can be used for genetic relationships (e.g., peroxidases).

2. Systematic & Evolutionary Significance

Family & Genus Differentiation:

Chemical profiles (e.g., alkaloid presence) help define family boundaries (e.g., Papaveraceae, Solanaceae).
Some compounds are specific to certain lineages, providing strong taxonomic markers.

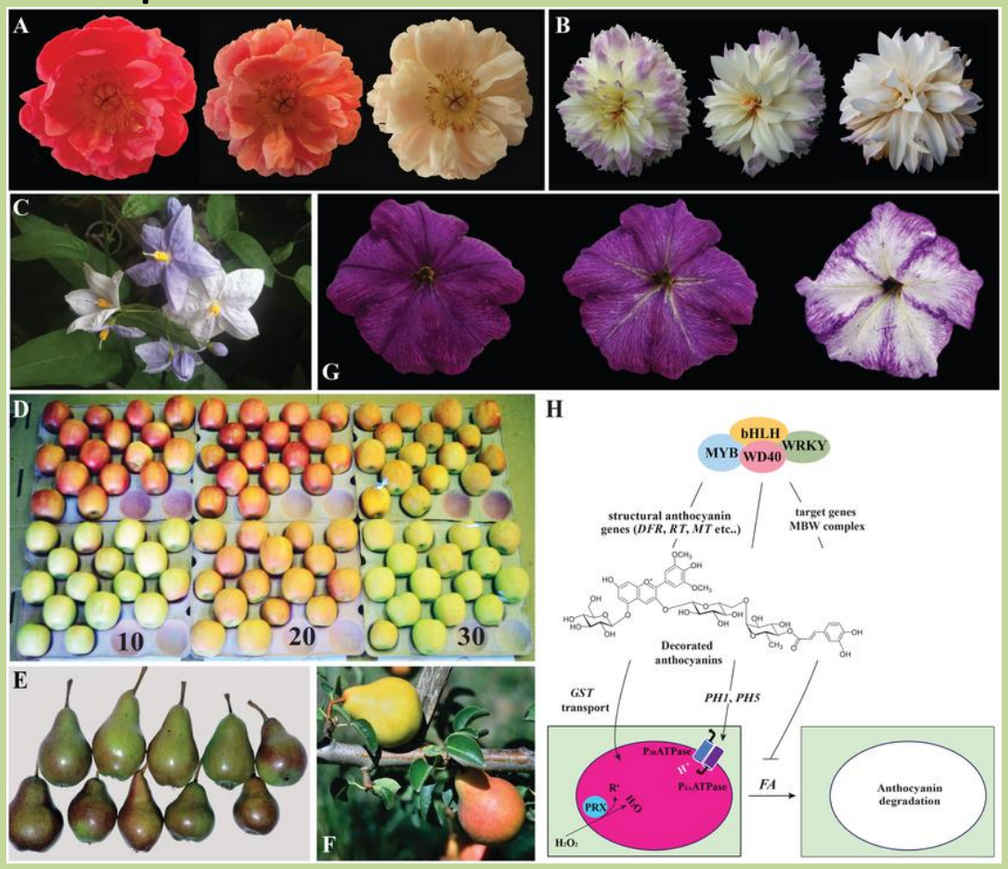
Evolutionary Insights:

Similarities in chemical composition can indicate common ancestry.
Evolutionary patterns are reflected in the presence or absence of certain metabolites.

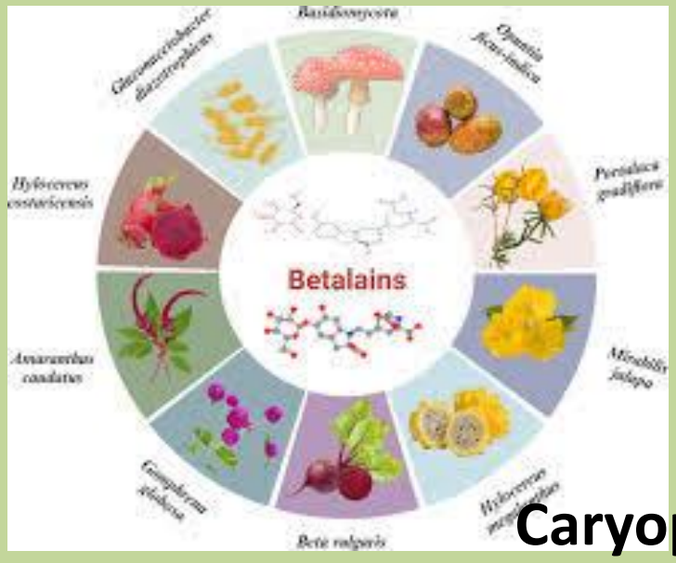
Ecological Adaptations:

Chemical compounds often relate to plant's defense mechanisms or ecological roles (e.g., phenolic compounds in drought tolerance).

- **Caryophyllales** produce **Betalins** (instead of anthocyanins).
- **Polygonales** produce **anthocyanins** (instead of Betalins).
- **Lamiaceae** are known for producing **highly aromatic compounds**.



Polygonales



Caryophyllales

Eco-Geographical Distribution and Its Taxonomic Importance

- Plant distribution patterns reflect evolutionary history.
- Geographic isolation promotes species differentiation.
- Ecological adaptation influences plant morphology.
- Habitat specialization aids taxonomic recognition.
- Climate affects species variation and classification.
- Endemism provides important taxonomic evidence.
- Biogeography helps trace plant migration routes.
- Ecological niches influence speciation processes.
- Distribution data support phylogenetic studies.
- Eco-geographical analysis strengthens taxonomic interpretation.

Systematic Significance of Ecology in Plant Taxonomy

Erect form of *Euphorbia hirta*

Prostrate form of *Euphorbia hirta*

6. DNA Sequencing and Molecular Phylogenetics

6.1 DNA Sequencing

- ❑DNA sequencing determines nucleotide order in DNA molecules.
- ❑DNA sequences act as molecular signatures.
- ❑Sequence data reflect evolutionary history.
- ❑Sanger sequencing initiated molecular taxonomy.
- ❑DNA comparison helps species differentiation.
- ❑ Sequencing provides objective taxonomic data.
- ❑Genetic evidence reduces reliance on morphology alone.
- ❑DNA data support evolutionary analysis.
- ❑Molecular signatures improve classification accuracy.
- ❑DNA sequencing is central to modern taxonomy.

6.2 Molecular Phylogenetic

- Molecular phylogenetics reconstructs evolutionary relationships using DNA.
- ITS is a common nuclear marker.
- rbcL and matK are common chloroplast markers.
- Mitochondrial genes provide additional information.
- Phylogenetic trees reveal natural relationships.
- DNA data often revise traditional classifications.
- Molecular evidence supports taxonomic revisions.
- Phylogenetics improves evolutionary interpretation.
- Different markers provide different resolution levels.
- Molecular phylogenetics supports natural classification.

7. Molecular Taxonomy and DNA Barcoding

7.1 Molecular Taxonomy

- Molecular taxonomy uses DNA for identification and classification.
- It provides objective and reproducible results.
- Molecular data detect cryptic species.
- It reduces errors caused by morphology alone.
- It is useful in groups with limited variation.
- Genetic data reveal evolutionary divergence.
- DNA analysis supports integrative taxonomy.
- Molecular approaches improve species delimitation.
- Molecular taxonomy is widely applied in systematics.
- It forms the basis of modern taxonomy.

7. Molecular Taxonomy and DNA Barcoding

7.1 Molecular Taxonomy

- Provides a solid conceptual basis for the evolutionary history of organisms
- The term 'molecular systematics' is used to refer **to macromolecular systematics**, i.e., the use of DNA and RNA sequences to infer evolutionary relationships among organisms
- Molecular techniques provide powerful tools for the study of evolution and phylogeny
- Principal advantages are the much greater number of molecular character available and greater comparability across the lineage

The genetic code

TAXONOMIC EVIDENCES: MOLECULAR TAXONOMY

Advanced Experimental Approaches in Plant Taxonomy

Introduction

Traditional taxonomy relied on **morphological features**, but modern techniques provide **greater accuracy** in plant classification.
Advanced methods include **Molecular Taxonomy, Cytotaxonomy, and Chemotaxonomy**.

Molecular Taxonomy (DNA Analysis)
Definition: Uses **DNA sequencing** to study genetic relationships between plants.

Techniques:

DNA Barcoding – Uses short DNA sequences to identify species.

RFLP (Restriction Fragment Length Polymorphism) – Identifies genetic differences.

PCR (Polymerase Chain Reaction) – Amplifies DNA for analysis.
Example:

Arabidopsis thaliana genome sequencing helped in plant evolutionary studies.

Importance:
Provides **precise plant identification**.
Helps in studying **evolutionary relationships**.
Useful for identifying **cryptic species** (visually similar but genetically different).

Molecular Systematics

Definition: Molecular systematics uses genetic data (DNA, RNA, proteins) to classify organisms and study their evolutionary relationships.

Objective Approach: Provides more accurate classifications compared to traditional morphology-based taxonomy.

Key Molecular Markers: Uses chloroplast DNA (*rbcL, matK*), nuclear DNA (*ITS*), and mitochondrial DNA.

Techniques Used: PCR amplification, DNA sequencing, and phylogenetic tree construction.

Significance: Helps in species identification, evolutionary studies, and biodiversity conservation.

Key Molecular Markers in Molecular Systematics

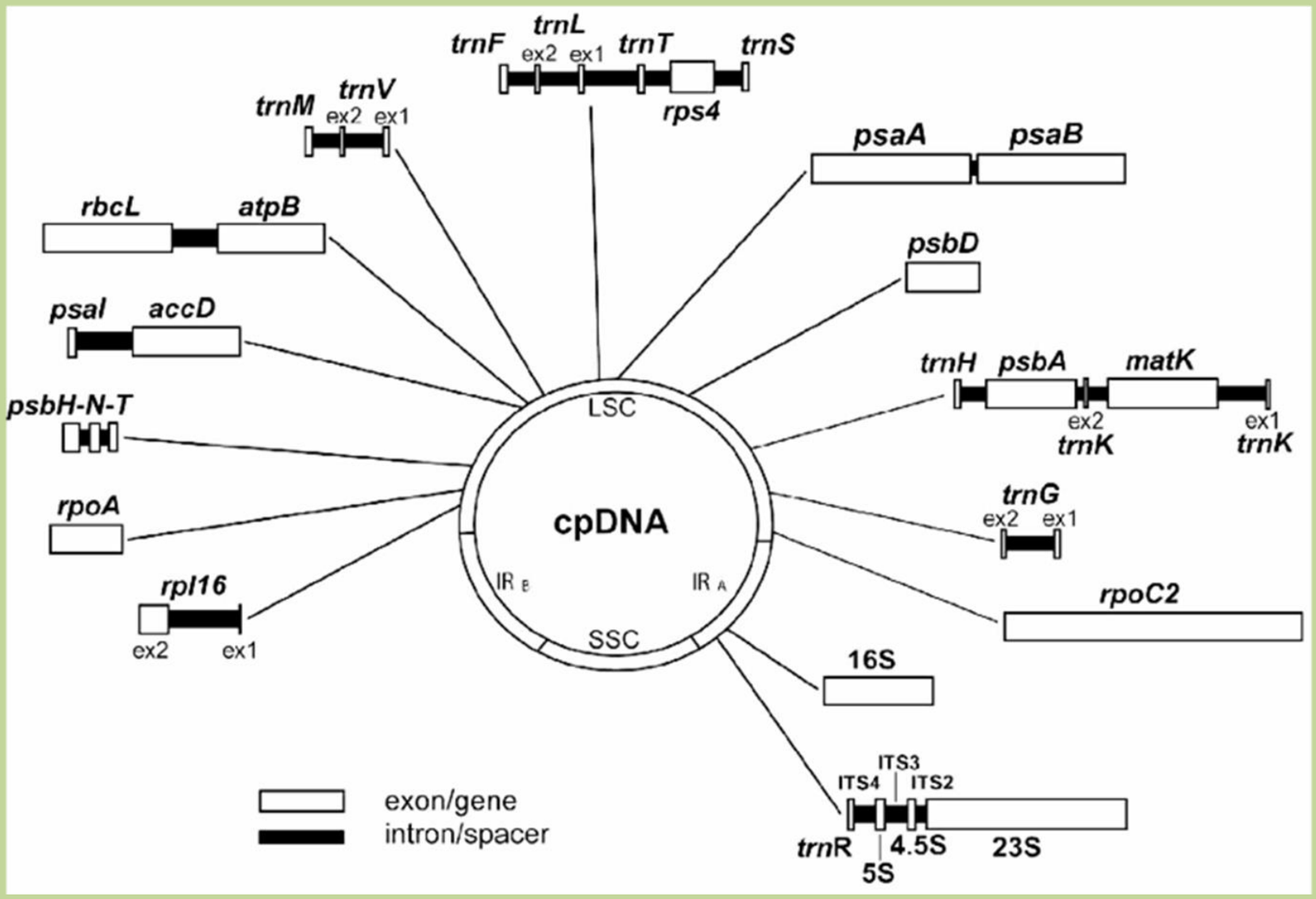
1. Chloroplast DNA (cpDNA)

Found in plant chloroplasts and maternally inherited in most angiosperms.
Highly conserved, making it useful for studying evolutionary relationships.
Commonly used **cpDNA markers:**

rbcL (*Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit*): Used in DNA barcoding and phylogenetics.

matK (*Maturase K*): Highly variable, useful for resolving species-level relationships.

trnL-F spacer, psbA-trnH, atpB-rbcL: Used in phylogenetic and population studies.



2. Nuclear DNA (nDNA)

Found in the nucleus and inherited biparentally.

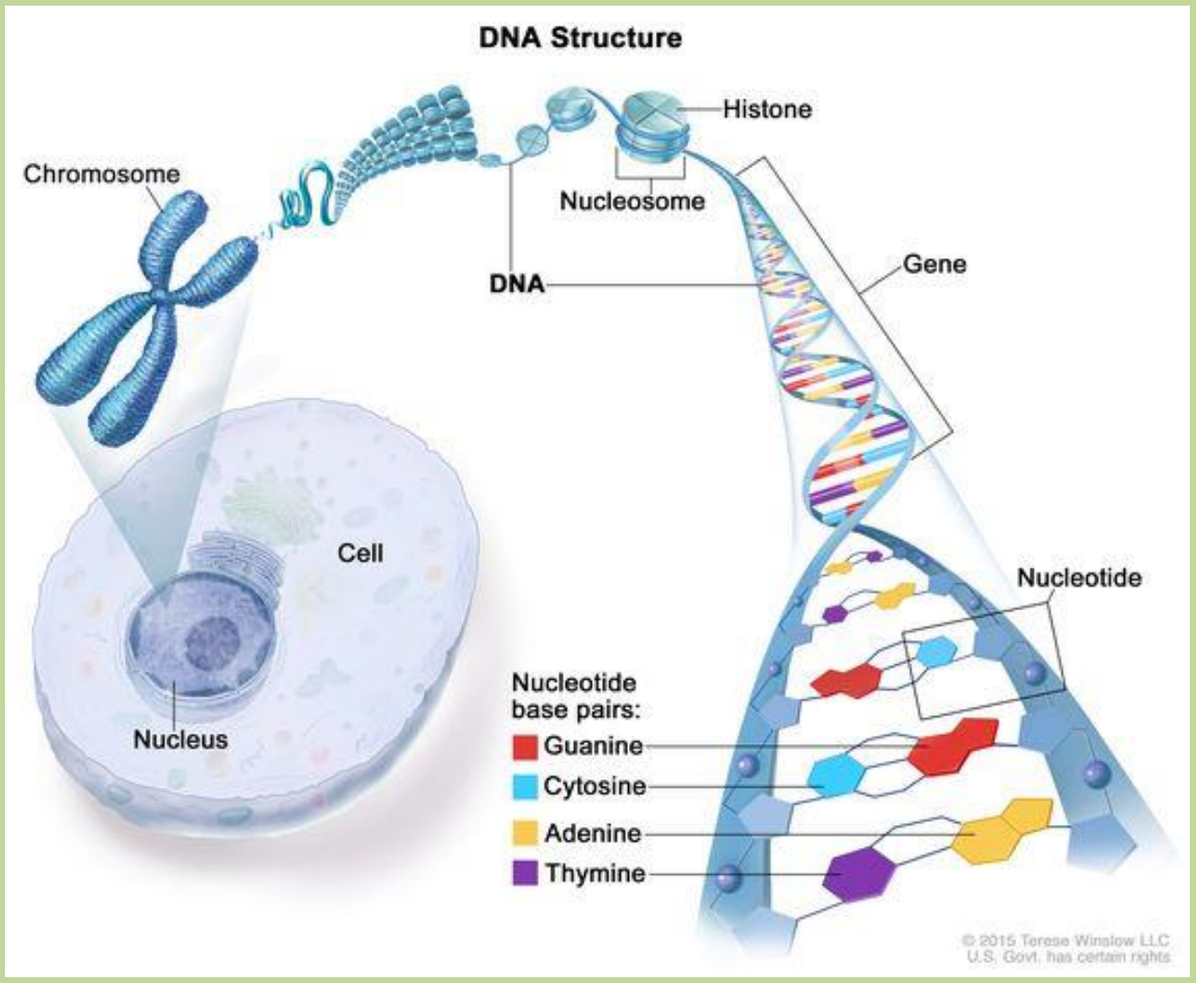
Provides high-resolution data for species differentiation and hybrid detection.

Commonly used **nuclear DNA markers:**

ITS (Internal Transcribed Spacer) regions (ITS1 & ITS2): Widely used for species-level phylogenetics.

18S rRNA, 26S rRNA: Used in deep phylogenetic studies.

Single-Copy Nuclear Genes: Provide additional resolution for evolutionary studies.



3. Mitochondrial DNA (mtDNA)

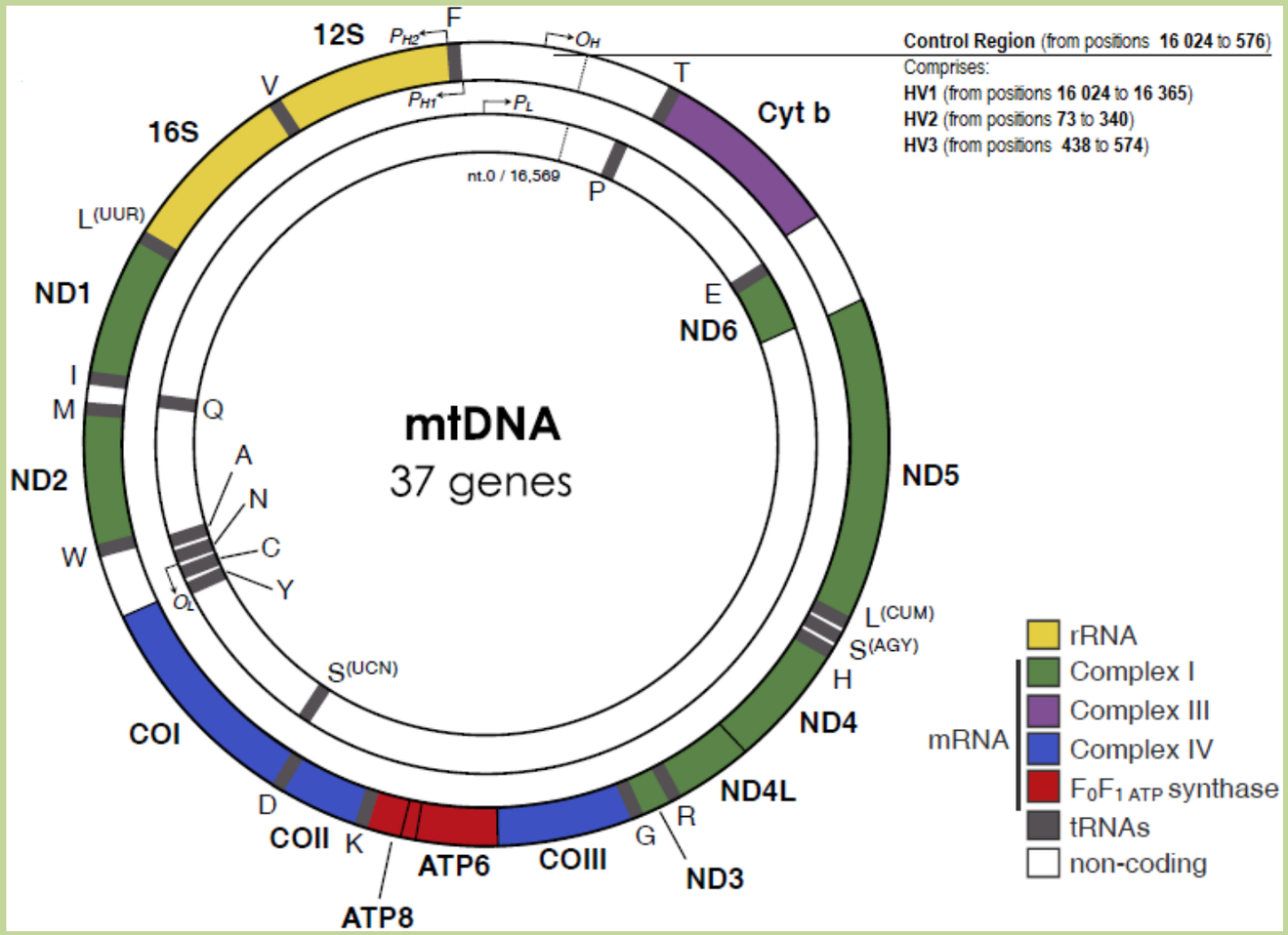
Found in plant mitochondria and typically maternally inherited.

Has a **high recombination rate** in plants, making it less useful than cpDNA for phylogenetic studies.

Commonly used **mtDNA markers:**

cox1 (Cytochrome c oxidase subunit 1): Used in some plant phylogenetic studies.

nad1, nad2: Useful in specific plant groups but less consistent.



Plant Genomes

- DNA molecule contains the genes and the total DNA content of a cell is known as its genome
- Plants have three different genomes:

Genome	Inheritance	Size (kbp)
1.Plastid	Uniparental (maternal)	120-190
2.Mitochondrial	Uniparental (maternal)	200-2500
3. Nuclear	Biparental	1.1×10^6 -1.1×10^{11}

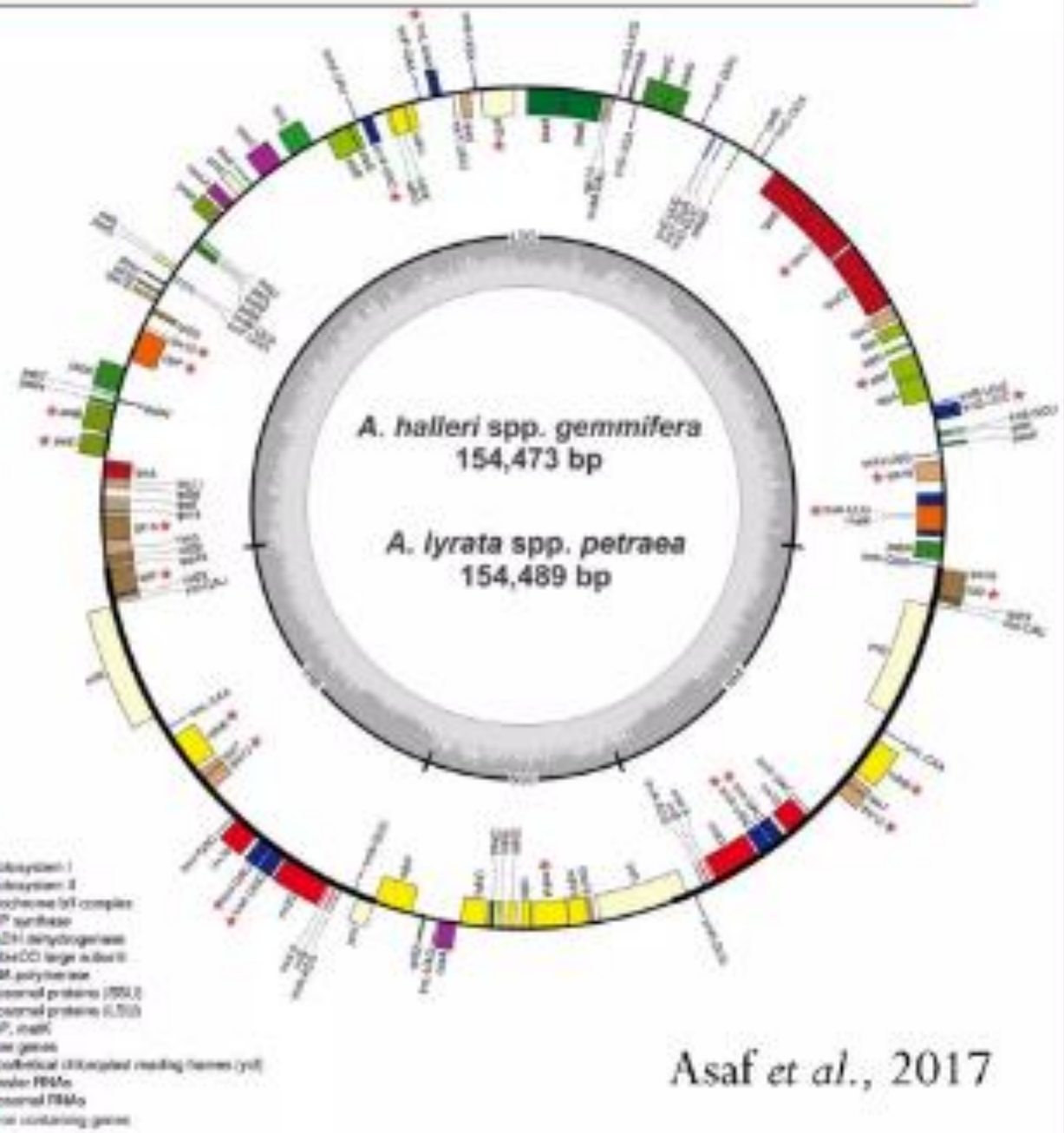
- The chloroplast and mitochondrion are generally inherited uniparentally (maternally in flowering plants)
- The nuclear genome is biparental
- Genome sizes - the number of pairs of DNA bases they contain
- The chloroplast genome is the smallest and nuclear genome is the largest among these three genomes

Molecular markers used in molecular systematic

- Chloroplast genome
- Mitochondrial Genome
- Nuclear Genome

Chloroplast genome

- Chloroplasts have circular genome
- Size of the plastid genome varies between plants, typically containing 120-190 kb
- Distinguishable into small single-copy region (SSC) and a large single copy region (LSC)
- Most genes in chloroplast genome are essentially **single copy**
- The most characteristic feature of the chloroplast genome is the presence of two regions that encode the same genes, but in opposite directions. These are known as **inverted repeats** (IRS).
- The IRS separate the SSC and LSC regions



Asaf *et al.*, 2017

Chloroplast genome

- Tend to accumulate mutations more rapidly as compared to mitochondrial genes in plants
- cpDNA used more frequently in systematic and phylogenetic studies of plants
- Plant molecular systematics has relied primarily on the chloroplast gene sequences
  - due to its small size
  - uniparental inheritance
  - conserved genome and
  - high nucleotide substitution rates
- The use of sequence data from the cpDNA has proven to be very useful in inferring both **lower and higher-level relationships**
- The entire genome chloroplast genome has been sequenced in many genera and have proven to be informative
- Some commonly used cpDNA gene and genic regions employed in plant systematics include *rbc*L, *trn*L-*trn*F, *ndh*F, matK, *trn*H-*psb*A

Molecular marker from chloroplast genome

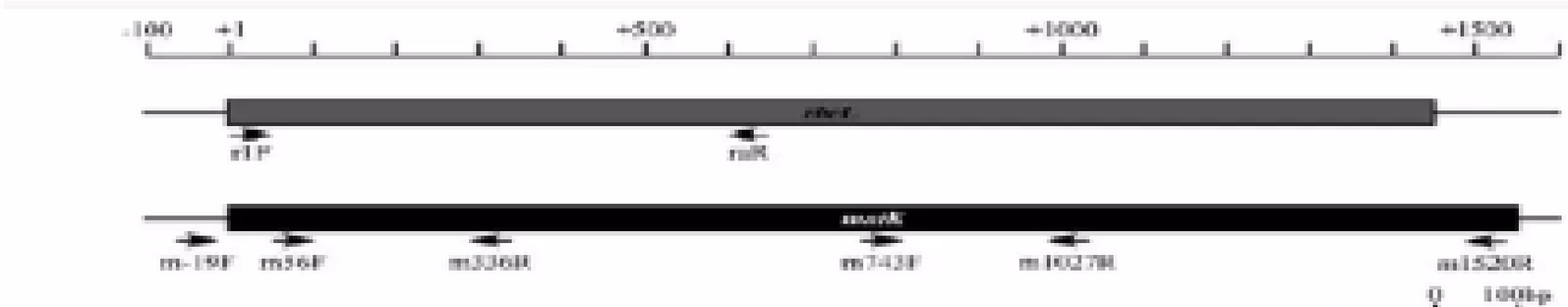
1. *rbc*L

- Among plant genes, *rbc*L (Ribulose-1,5-biphosphate carboxylase/oxygenase) was the first to be sequenced
- Encodes the large subunit of ribulose-1,5-biphosphate carboxylase (RUBISCO) and is a critical enzyme in photosynthesis
- Located in the LSC region of the chloroplast genome
- It is a slow evolving region with a length of ~ 1430 bp
- In most of the phylogenetic studies, this locus has proved to be useful for reconstructing phylogenies at the generic level and above
- The *rbc*L sequences are phylogenetically conserved and have served well across all green plants

Molecular marker from chloroplast genome

2. *mat*K

- *mat*K (Maturase K) is a group II intron present in the gene *trn*K that codes transfer RNA for lysine
- Has a length of 1550 bp and encodes for an enzyme known as maturase, which splices type II introns from RNA transcripts
- Located in the LSC region of the chloroplast genome
- It is a fast-evolving region with high substitution rates
- It is widely used in systematics due to its low transition/transversion ratio, presence of conserved regions, and the ability to discriminate between species
- *mat*K has provided useful comparative data among genera within a family and even among species within the genus



Khew and chia 2011

Molecular marker from chloroplast genome

3. *ndh*F

- The *ndh*F region codes for NADH dehydrogenase F gene
- Located in the SSC region of the chloroplast genome
- Involved in various reactions of respiration
- Consists of 2235 base pairs and its 5' region is very different from the 3' region
- It is larger than *rbc*L and has a greater number of variable sites. However, both are similar in the rate as well as the pattern of sequence change.
- Some indels of this molecular marker have also been shown to be of phylogenetic significance
- Its utility has been shown at the familial, subfamilial, tribal and generic levels due to the longer size and high sequence divergence

Molecular marker from chloroplast genome

4. *atp*B

- *atp*B, codes for beta subunit of ATP Synthetase located in the LSC region of the chloroplast genome.
- It is involved in the synthesis of ATP through proton translocation.
- Its rate of evolution and size (ca. 1497 bp) is nearly identical to *rbc*L
- Due to the absence of introns, this region is easily aligned during phylogenetic analysis
- It is conservative in nature and has also been used to analyze the phylogenetic relationships in ferns

Molecular marker from chloroplast genome

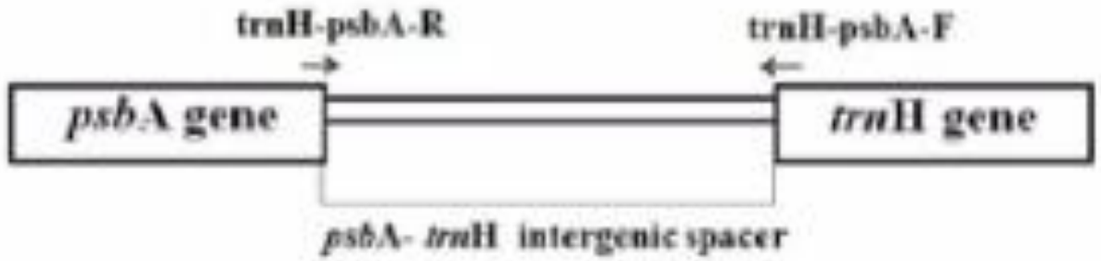
5. *rpl*16

- This **noncoding intron region** is of about 1059 bp.
- It has a lower **transition/transversion ratio** but shows higher nucleotide divergence and genetic distance
- Have more complex evolution pattern as well as frequent insertion and deletion events as compared to the coding regions

Molecular marker from chloroplast genome

6. *trn*H – *psb*A intergenic spacer

- One of the most variable **intergenic spacers** present in the chloroplast genome of angiosperms
- The tRNA- Histidine- Photosystem II protein D1 spacer varies from 296-1120 bp in length with an average of approximately 450 bp.
- Located in the **LSC region** of the chloroplast genome.
- Useful due to its high variability and the ability to discriminate between species but the small length limits its usefulness
- As the length is short, the number of variable sites provided by this locus is less.
- Also, the presence of mononucleotide repeats and inversions in this region can sometimes lead to incorrect inferences. However, the ease of amplification makes it a highly used locus

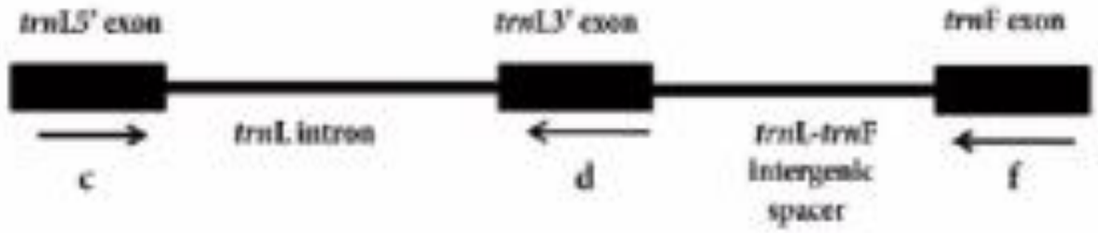


Phoolcharoen *et al.*, 2

Molecular marker from chloroplast genome

7. *trn*L- *trn*F: Intron and intergenic spacer

- The *trn*L and *trn*F code for tRNAs for **leucine and phenylalanine,** respectively
- The *trn*L- *trn*F sequence consists of **an intron** in the transfer RNA gene *trn*L (UAA) and the adjacent **intergenic spacer** between *trn*L and *trn*F (GAA)
- Located in the **LSC** region of the chloroplast genome
- Highly used in systematic studies due to its **high amplification rate, universality and good species discrimination** ability
- Useful for analyzing relationships at lower levels of the hierarchy particularly between genera and species, however, it has also been used up to tribal level



Amir *et al.*, 2010

Advantages of cpDNA for Plant Systematics

- Small (between 120-200kb) and are present in many copies per cell, making it easy to isolate in sufficient quantities from even very small amounts of plant material
- Most genes are in **single copy** and easy to examine the **entire genome**
- cpDNA is useful because of the minimal variation encountered within and among conspecific populations
- The lack of frequent structural changes (inversions, transpositions, deletions, and insertions) in the chloroplast genome makes it relatively easy to work with in comparative studies
- The relatively slow rate of nucleotide substitutions in cpDNA minimizes the problem of parallel and convergent evolution when comparing genomes of congeneric species
- Introns and spacers of cpDNA have been widely used but substitution rates are often too low to distinguish closely related species

Mitochondrial Genome

- Mitochondrion (pl. mitochondria) - important component of eukaryotic cell
- Bound by double layered membrane and is a true organelle
- Contains its own genetic information in the form of double stranded circular DNA
- mtDNA **carries several genes** and is **maternally inherited**
- In contrast to cpDNA sequences, mitochondrial DNA (mtDNA) has been employed much less frequently in plant systematics due to a high degree of intramolecular recombination and a low rate of base pair substitution
- mtDNA in plants is large, variable and is also less abundant in plant leaves as compared to cpDNA.
- mtDNA show enormous **variation in size, from about 200 kb (*Brassica*) to 2600 kb (*Cucumis melo*)**
- Some of the regions of mitochondrial genome are: **cox1 and *atp*A region**
- Presently, **very few sequences of mtDNA** are available for study in plant systematics.
- Generally, the molecule of choice in the animal kingdom

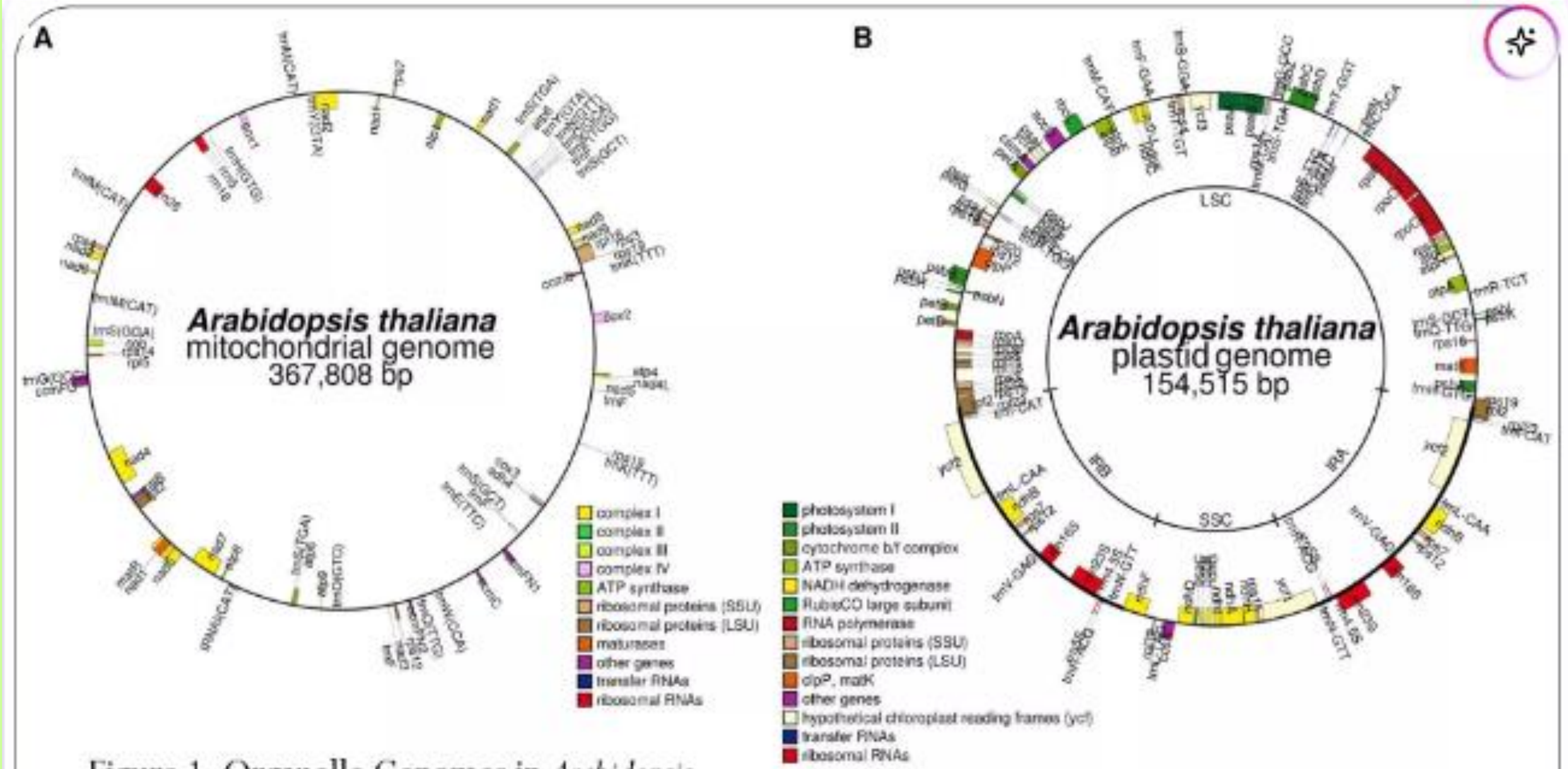


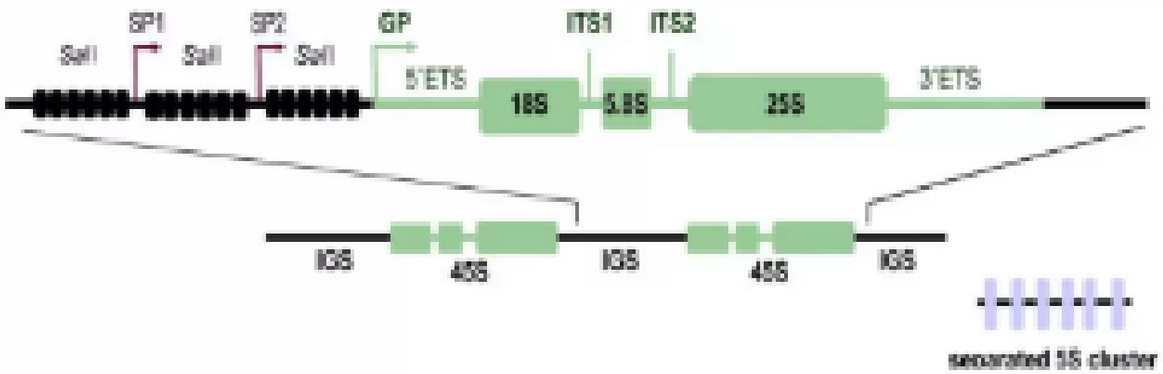
Figure 1. Organelle Genomes in *Arabidopsis*.

(A) Circular mapping of mtDNA (NCBI NC_037304), encoding some (not all) subunits of Complexes I–IV, ATP synthase, the mitochondrial ribosome, tRNAs, and rRNAs.

(B) Circular mapping of ptDNA (NCBI KX551970), encoding some (not all) subunits of photosystem complexes, electron transport chain proteins, the plastid ribosome, tRNAs, and rRNAs. The genome is divided into large and small single copy regions (LSC and SSC) separated by inverted repeat regions IRA and IRB

Nuclear Genome

- Plant nuclear genomes **vary greatly** in size
- *Arabidopsis thaliana* has one of the smallest known (1.35 x 10 bp) plant genomes and lily (*Fritillaria assyriaca*) is one of the largest (1×10^{11}bp)
- **Ribosomal genes** are arranged in tandem repeats and are subjected to concerted evolution
- Biparentally inherited
- The nuclear ribosomal genes encode the small subunit (18S) and the large subunit (26S) which are separated by a smaller (5.8S) gene
- Between the three genes, there are short **Internal transcribed spacers (ITS)**
- Each set of the three genes (18S, 26S, 5.8S and ITS regions) is separated by a larger spacer, called **intergenic spacer (IGS)**



Organization of nuclear ribosomal RNA (rRNA) genes in plants. **A schematic view of rDNA organization** in *Arabidopris thaliana* S-type. A single 45S gene unit consists of three genes (18S, 5.8S, and 26S) transcribed together as a single transcript. Genes are separated by internal transcribed spacers (ITS1 and ITS2). Borders of 45S rDNA are formed by external transcribed spacers (5'ETS and 3'ETS). Adjacent 45S rDNA units are separated from each other by intergenic spacers (IGS). 5S rDNA is located at a separate locus

Goffová *et al.*, 2021

Nuclear Genome

- Sequences of the **18S gene** (about 1800 bp) and **26S gene** (about 3300 bp) have been used for inferring relationships among large group of plants
- Both have some regions that are **highly conserved** and others that are **quite variable**, which helps in distinguishing phylogenetic groups
- ITS region, have been widely used in phylogeny reconstruction at both the generic and specific levels because of **their higher substitution rate compared to commonly used regions of cpDNA**
- ITS is a **highly suitable and powerful** region for resolving plant taxonomic and phylogenetic problems in most plant lineages
- ITS sequence data have been most valuable for inferring phylogenetic relationships at a lower level especially between closely related species as well as higher- level relationships
- The entire **ITS region** (ITS1+5.8S+ITS2) can be easily amplified using **universal primers**

Nuclear Genome

- Total length of ITS regions plus intervening 5.8S gene is fairly short and relatively uniform (600-700 bp)
- ITS provides sufficient number of parsimony informative characters, good resolution, and strong clade support
- Another region of the nuclear ribosomal DNA, i.e., **External Transcribed Spacer** (ETS) is now extensively being used to supplement the ITS data in the phylogenetic analysis
- The ETS region **evolves at the same** rate as ITS and is **longer** than ITS 1 and ITS 2 put together
- It is assumed that the restrictions posed by ITS in resolving systematic relationships might be overcome by the use of ETS
- However, the use of ETS is limited due to the **presence of highly variable non-transcribed spacer (NTS) region flanking its 5' end**
- Because of this no universal primers are available and thus sequencing becomes challenging
- To overcome this limitation, the entire IGS region (that includes NTS + ETS) is amplified using universal primers flanking 18S and 26S region

Choosing molecular marker, and application of PCR in plant molecular taxonomy / DNA taxonomy

- ❖ In DNA sequencing method based practice of plant molecular taxonomy required DNA sequences.

- ❖ To obtain DNA sequence of a taxon required extraction of whole genomic DNA first. And then amplification of gene of interest. The amplification using gene interest is achieved by the polymerase chain reaction (PCR). The PCR results into billions of copies of gene of interest which can be observed in a gel under UV light. The amplified DNA later used for the purpose of DNA sequencing. So, for the cloning of the gene of interest using PCR requires primer. The primers are also called as molecular markers. To begin plant molecular taxonomy, selection of molecular marker is very critical and important.

- ❖ The most commonly used molecular marker in molecular taxonomy are ITS, rbcL, matK, psb, ndhF, trn gene.
- ❖ The molecular marker gene could be coding gene or non coding gene.

- ❖ Properties of ideal marker genes
  - ➢ A single-copy gene may be more useful than multiple-copy gene
  - ➢ The substitution rate should be optimum so as to provide enough informative sites and alignment should be easy.
  - ➢ Primers should be available to selectively amplify the marker gene

- ❑ The nuclear ribosomal locus coding for the large subunit is represented in tandem arrays in the plant genome.

- ❑ ITS is located between the 18 and 26S rRNA genes.

- ❑ The 5.8S region on the other hand is only about 160 bp long and highly conserved within major organism groups.

- ❑ The ITS region consists of three parts: the ITS1 and ITS2 and the highly conserved 5.8S rDNA exon located in between. The total length of this region varies between 500 and 750 bp in angiosperms while in other seed plants it can be much longer, up to 1,500–3,500 bp.

- ❑ Spacer DNA is a region of non-coding DNA between genes.

- ❑ In contrast to the coding regions, spacers evolve more quickly, like the internal transcribed spacer (ITS) region, which is extensively used as a marker for phylogenetic reconstruction at different levels.

- ❑ The ITS is present in virtually all organisms. The advantages of this region are: (1) easy PCR amplification, with several universal primers available for a various kind of organisms; (2) multicopy structure; (3) moderate size allowing easy sequencing; and (4) it has a high degree of variation even between closely related species.,

- ❑ variability is due to frequently occurring nucleotide polymorphisms or to common insertions/deletions in the sequence.

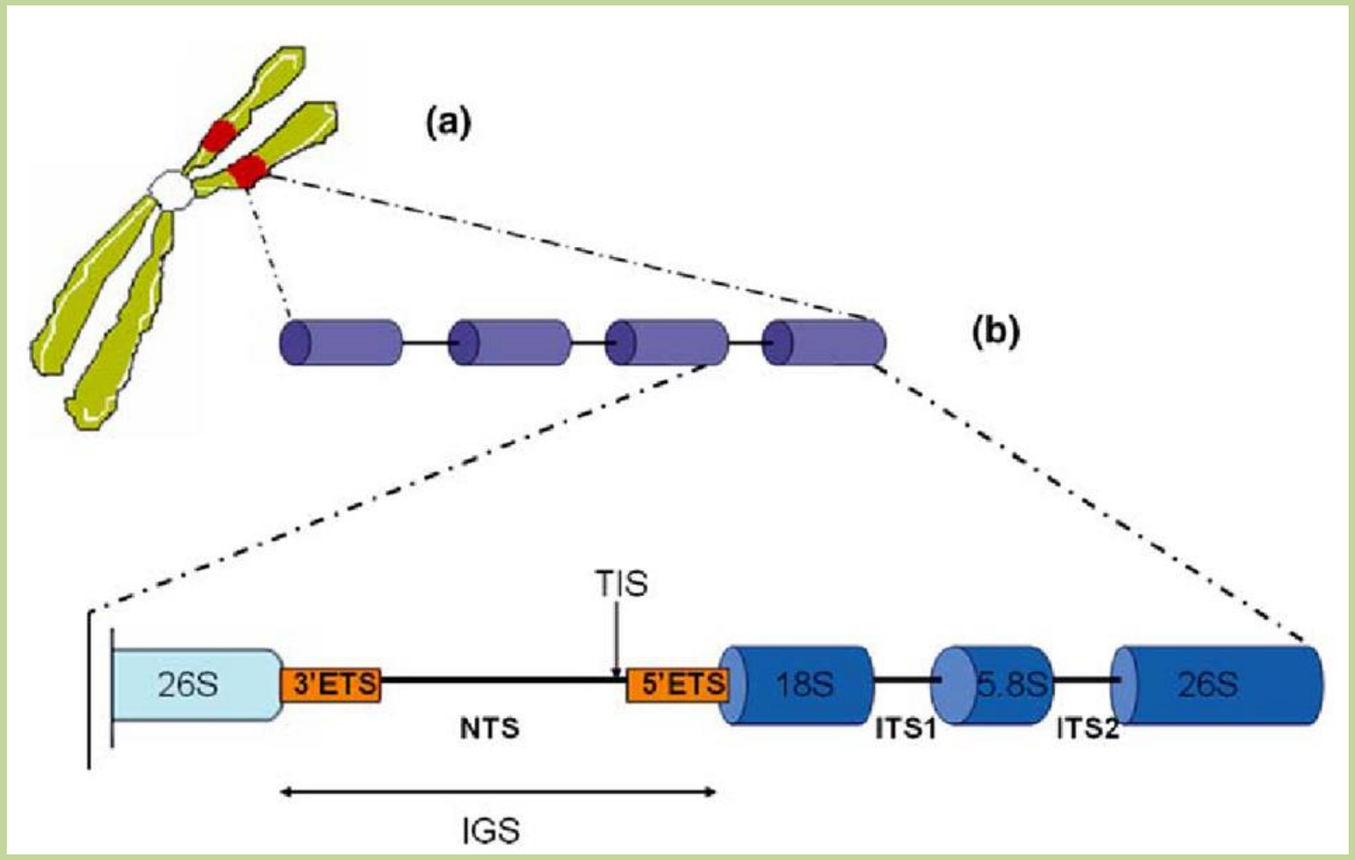
- ❑ As DNA of ITS regions is removed and it is not part of the mature RNA molecule, they are considered noncoding regions of the genome

- **A fascinating feature of biological life is the common use of the DNA genetic code and its subsequent processing into functional units of protein through the intermediate RNA molecule.**

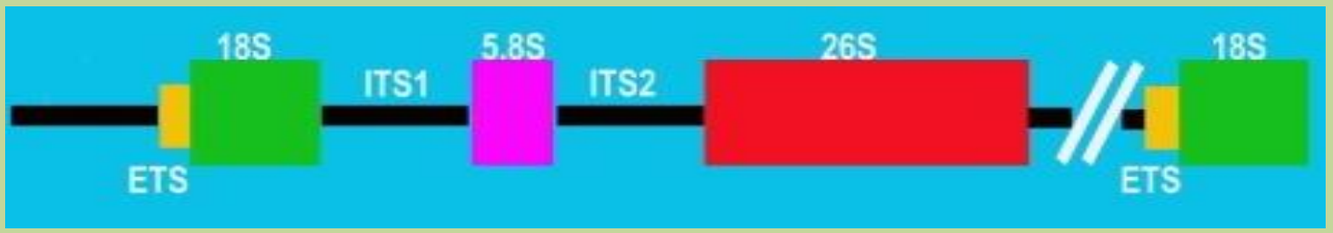
- **The transcription of DNA into RNA and translation of RNA into protein are both highly**
- **regulated and compartmentalized in all living organisms.**

- **The cellular factory responsible for the production of protein is the ribosome. As the essential functions of ribosomes are critical for survival, their physical parameters have been conserved in all forms of life.**

- **Some components within the ribosomal factories have, however, changed sometimes. These similarities, as well as the changes within genetic material can be used as tools for the identification of organisms**



MARKER	SEQUENCE	REFERENCE
ITS1 F	TCCGTAGGTGAACCTGCGG	White et al. (1990)
ITS4 R	TCCTCCGCTTATTGATATGC	White et al. (1990)
rbcLa F	ATGTCACCACAAACAGAGACTAAAGC	Levin (2003)
rbcLa R	GTAAAATCAAGTCCACCRCG	Kress and Erickson (2007)
MatK 390 F	CGATCTATTCATTCAATATTTC	Cuenoud et al. (2002)
MatK 1326 R	TCTAGCACACGAAAGTCGAAGT	Cuenoud et al. (2002)
psbA-trnH F	GTTATGCATGAACGTAATGCTC	Sang et al. (1997)
psbA-trnH R	CGCGCATGGTGGATTCACAATCC	Tate and Simpson (2003)
trn L-F R	GGTTCAAGTCCCTCTATCCC	Taberlet et al. (1991)
trn L-F F	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)



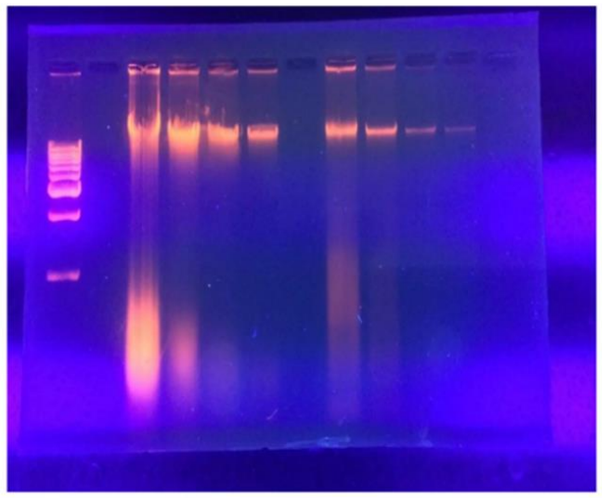
Generating DNA Sequence Data

- DNA sequence data basically refers to the sequence of nucleotide: Adenine (A), Thymine (T), Cytosine (C), Guanine (G) in a particular stretch of DNA of a given taxon
- The homologous regions of DNA (i.e., regions having similarity due to ancestry) among the taxa under study provide character and character states which are used for inferring the phylogenetic relationships
- DNA obtained from the samples are compared. DNA sequence data are generated in two ways:

1. A **gene-by gene approach** in which a gene of interest is selected, isolated from a large number of plants and sequenced
2. A **genomic approach**, in which an entire chloroplast or nuclear genome is sequenced and the sequences of many genes from the genome are analyzed

Steps in Acquiring DNA Sequence Data

- DNA is extracted from selected plant samples (preferably from live leaf material or silica dried leaf) either using CTAB method or DNA Extraction kits
- Genomic DNA is then amplified using polymerase chain reaction (PCR)
- Amplified product is cleaned and sequenced in automated sequencers



Molecular systematics

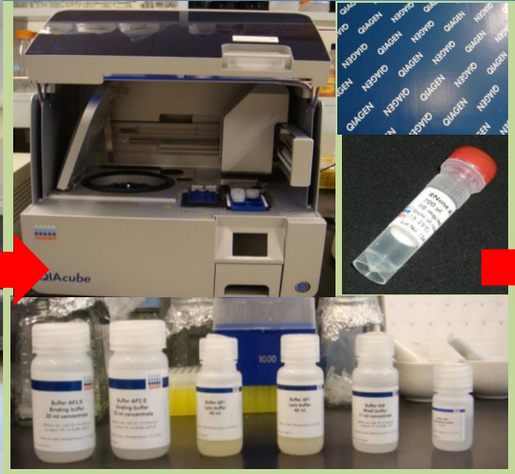
- Molecular systematics deals the utilization of nucleic acid data. As DNA sequence of a gene is constant in a species, hence advantage over morphological data for taxonomic studies.

- Taxonomist use molecular data from three different locations within a plant cell: chloroplast, mitochondrion and the nucleus.

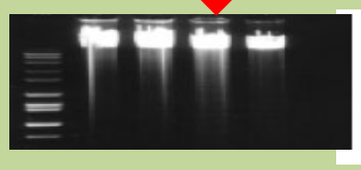
- Molecular systematics involves following steps: (1) Sample collection, (2) DNA extraction, (3) Amplification using PCR – Polymerase chain Reaction, (4) DNA / Gene Sequencing, (5) Analysis of Sequence data.
- DNA barcoding can speed up identification of species. DNA barcoding helps in Wild plant identification / Medicinal plant authentication

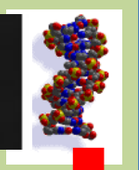
- A DNA barcode is a short gene sequence taken from standardized portions of the genome, used to identify species

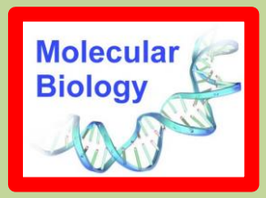
Collection of plant samples



Gel electrophoresis



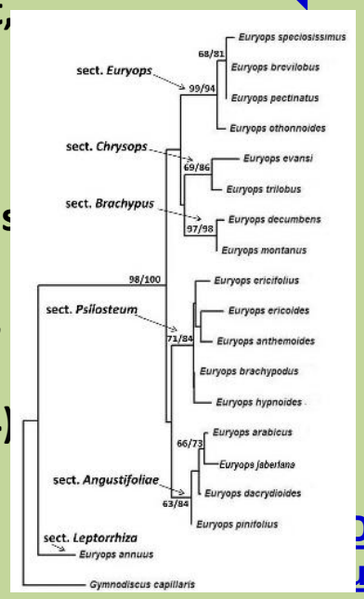


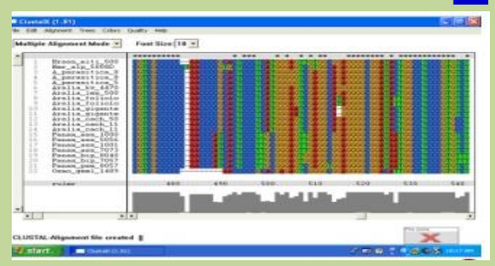


A view of molecular biology laboratory

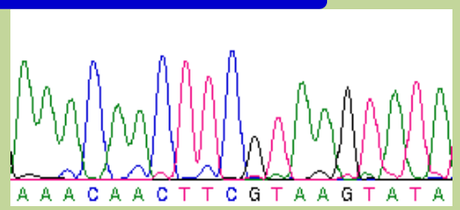
Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Building Tree

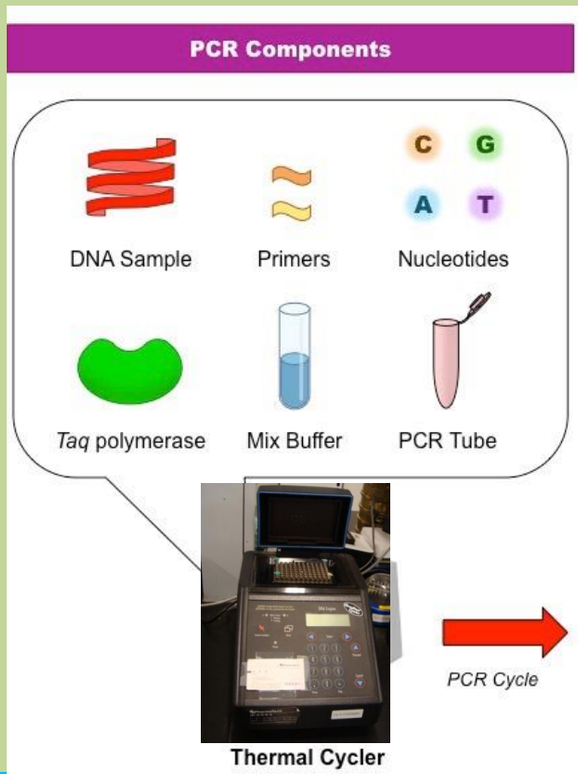


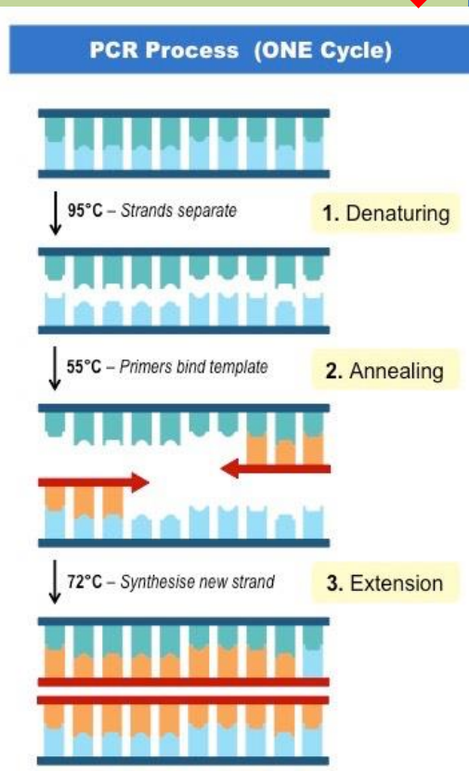


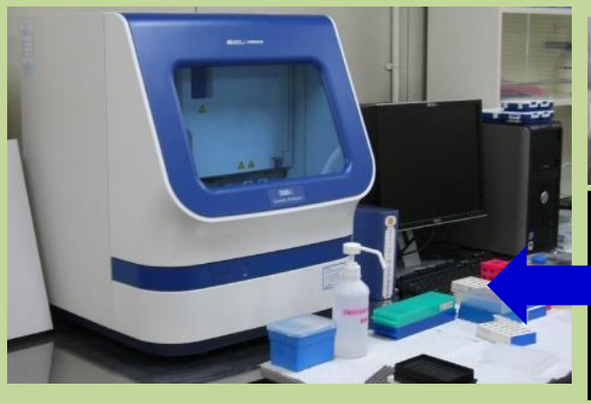
DNA sequence alignment using ClustalX

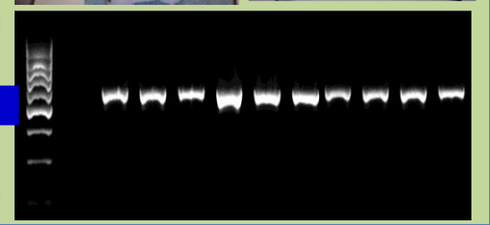


NCBI









Key Molecular Data in Taxonomy

DNA Sequencing:

rDNA (ribosomal DNA): 18S, 5.8S, 28S rDNA regions provide species- and genus-level identification.

Chloroplast DNA: matK, rbcL, and trnL regions are often used for plant family and genus classification.

Mitochondrial DNA: Markers like COX1 and ATP synthase genes reveal evolutionary relationships within certain plant groups.

Molecular Markers:

SSR (Simple Sequence Repeats): Highly polymorphic markers for species identification.

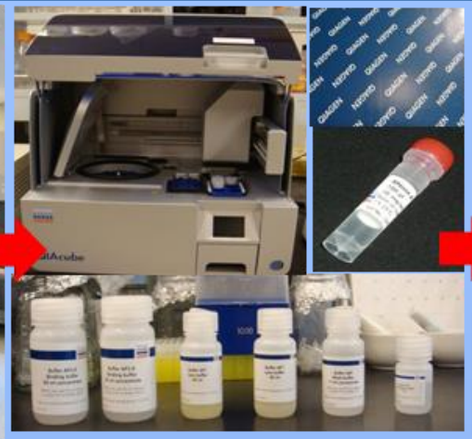
AFLP (Amplified Fragment Length Polymorphism): Used to assess genetic diversity and phylogenetic relationships.

SNPs (Single Nucleotide Polymorphisms): Help refine species boundaries and reveal population-level diversity.

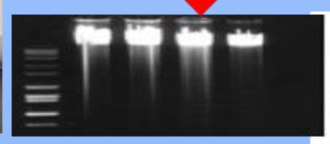
Genome Sequencing:

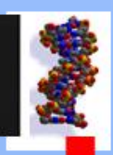
Full genome sequencing provides the most comprehensive taxonomic data, revealing gene family expansions, genome size, and synteny between species.

Collection of plant samples



Gel electrophoresis

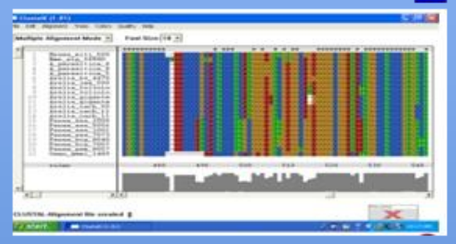




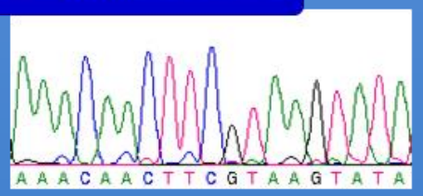
A view of molecular biology laboratory

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

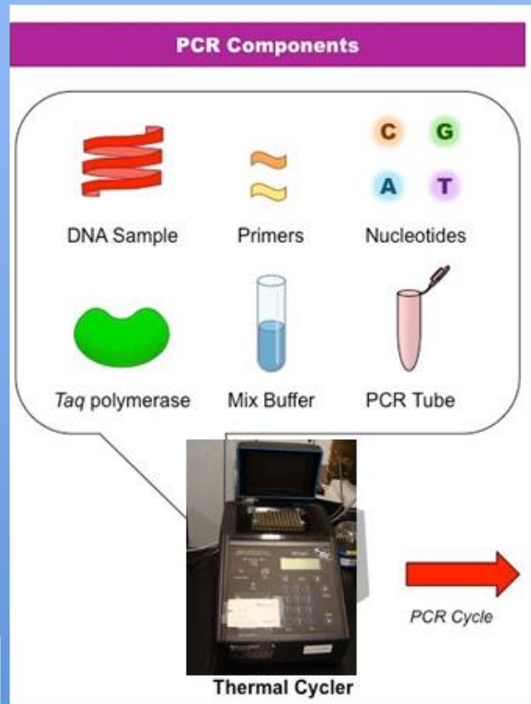
Building Tree

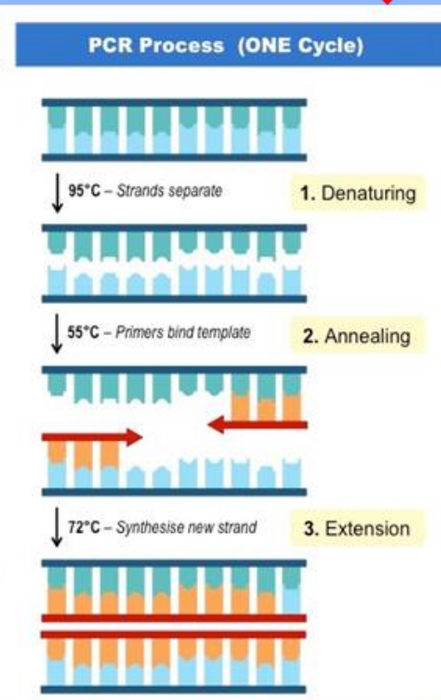


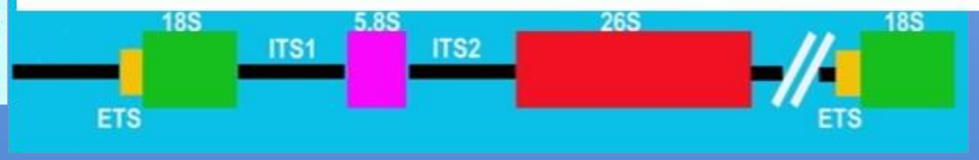
DNA sequence alignment using ClustalX

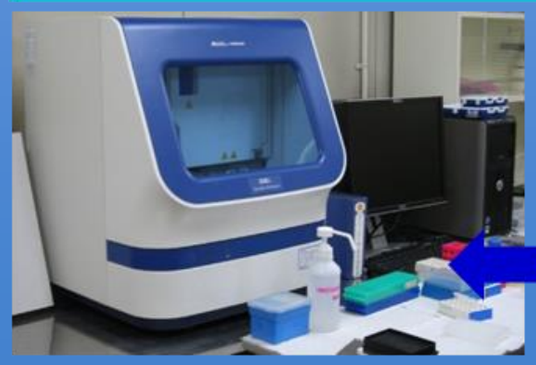


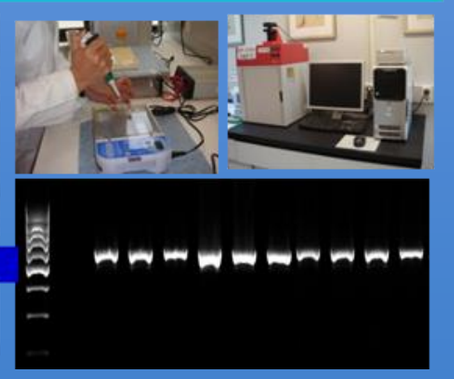
NCBI











Sampling of leaf material for the molecular taxonomic study and DNA extraction

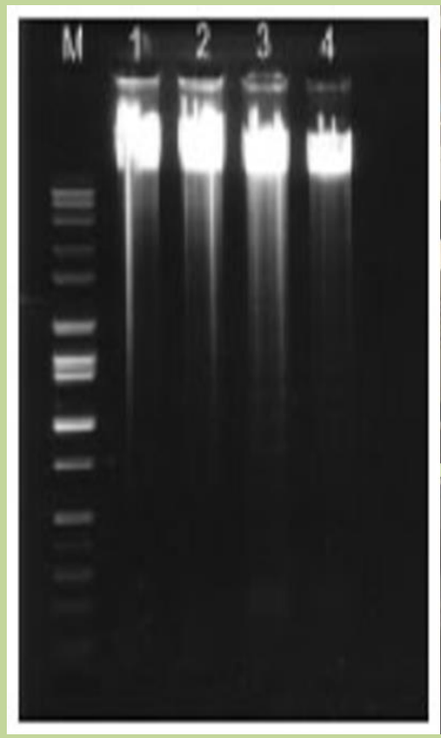
- Doyle and Doyle (1990) is widely used protocol for DNA Extraction from plant tissue. But it involves preparation of several buffer manually. It takes long times. This method atleast take more than one day preparation and about whole day in DNA extraction. It also involves several times centrifugation. This method requires large amount of fresh leaves (10 gram or even more).

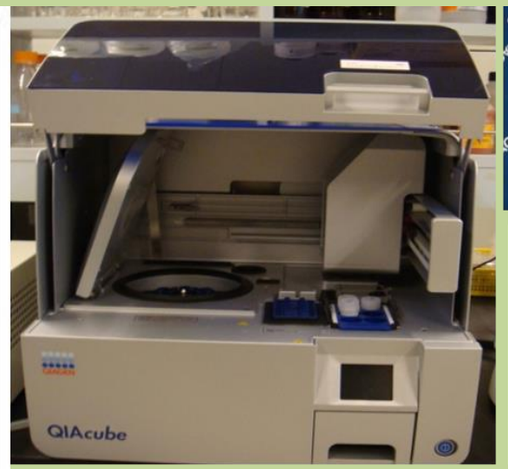
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

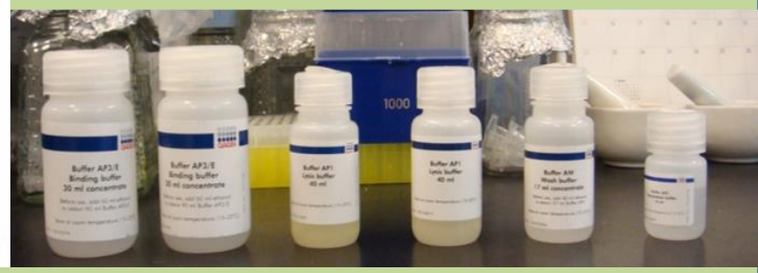
- In contrast to manual method, there are several DNA extraction kit and automated DNA extraction machine is available like Qiagen automated DNA extraction machine, and Qiagen DNA extraction Kit.

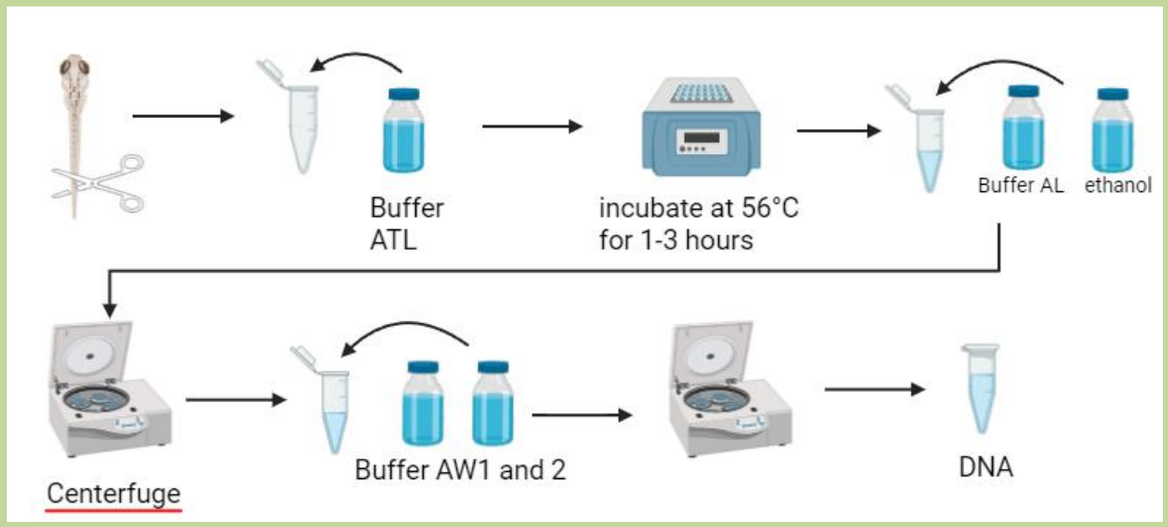
- In Qiagen DNA extraction all the buffer are provided and ready to use. DNA can be extracted from small amount of 20 mg l dried leaf tissue or from very small piece of leaf collected from even old herbarium specimens. By using Qiagen DNA can be extracted in 3 hours. It do not required centrifugation manually.

QIAGEN automated DNA extraction method









Polymerase Chain Reaction

- **Karry Mullis (1983)-Provided the concept of PCR, Awarded Nobel Prize (1993)**
- The technique is used to amplify specific, target DNA fragments from low quantities source DNA. Different components of PCR include:

1. **DNA template**: DNA template is the sample DNA that contains the target sequence
2. **DNA polymerase:** DNA polymerase is a type of enzyme that synthesizes new strands of DNA complementary to the target sequence. PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. The DNA polymerase typically used in PCR is called Taq polymerase, after the heat-tolerant bacterium (*Thermus aquaticus)* from which it was isolated
3. **Taq buffer**: Taq buffer with MgCl, provides an optimal and stable chemical environment for the DNA polymerase to work adequately
4. **Primers:** PCR primers are short pieces of single stranded DNA (15-30 nucleotides in length) which bind to certain nucleotide sequences along the DNA strand
5. **dNTPs:** Deoxynucleotide triphosphate (dNTPs) are single units of the bases A, T, G, and C, which are essentially "building blocks" for new DNA strands

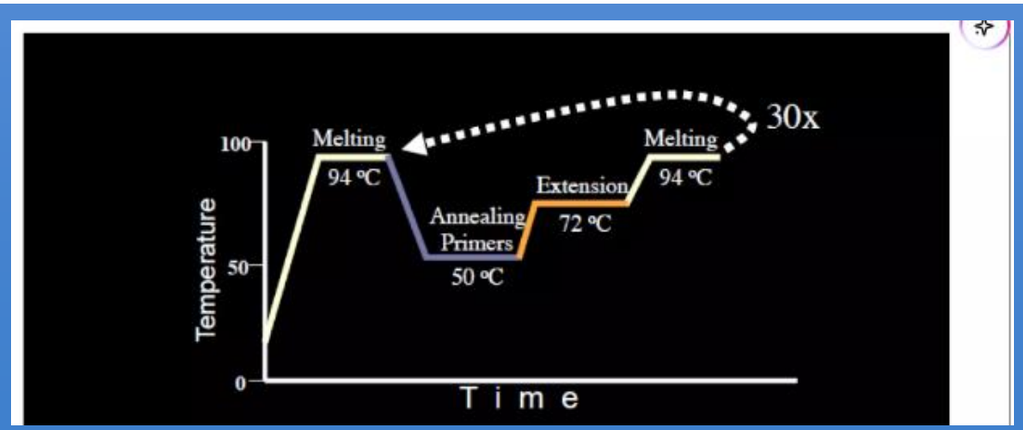
Steps in PCR

- There are three different steps involved in polymerase chain reaction: (a) Denaturation (melting), (b) Annealing and (c) Extension

- **Denaturation**: The initial denaturation step is carried out at the beginning of PCR to separate the double-stranded template DNA into single strands so that the primers can bind to the target region and initiate extension

- **Annealing:** After denaturation of the double stranded DNA, each primer hybridizes to one of the two separated strands

- **Extension**: After primer annealing, the next step in PCR is to extend the 3' end of primers, complementary to the template. In this step, 5' to 3' polymerase activity of the DNA polymerase incorporates dNTPs and synthesizes the daughter strands. PCR steps of denaturation, annealing, and extension are repeated (or "cycled") many times to amplify the target DNA



Gel Electrophoresis:

- Gel electrophoresis is the technique that is used to separate proteins as well as nucleic acids on the basis of charge in the presence of an electric field
- In molecular systematics, once the DNA molecules have been amplified by PCR, they are loaded onto a gel made of agarose and subjected to electric charge
- The negatively charged DNA molecule moves from negative pole to the positive one
- The rate of migration is dependent on the size of the DNA molecule
- The larger the size, slower is the rate of migration
- The bands on the gel are not visible with the naked eyes the gels are stained (usually with ethidium bromide) before loading the samples. A DNA ladder is loaded next to the DNA sample (amplified PCR product) to determine the size of the DNA sample
- The DNA ladder produces fragments of known size or base pairs. It can thus be used to determine the size of the corresponding bands on the gel
- The gel is visualized under UV light

Agarose Gel Electrophoresis

- **The main purpose of agarose gel electrophoresis is to determine the presence or absence of genomic DNA or PCR products and quantify the size (length of the DNA molecule).**

- **Agarose gel electrophoresis is a widely used technique for the preparation and analysis of DNA. Electrophoresis is a method of separating DNA based on the rate of movement while under the influence of an electric field.**

- **Agarose is a polysaccharide purified from seaweed.**

- **An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode.**

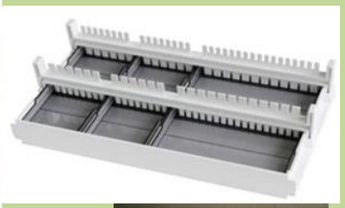
- **The DNA to be analyzed is forced through the pores of the gel by the electrical current.**
- **Under an electrical field, DNA moves to the positive electrode (red) and away from the negative electrode (black).**

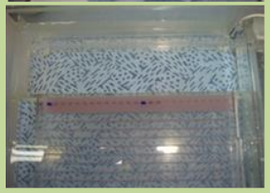
- **DNA itself is not visible within an agarose gel.**

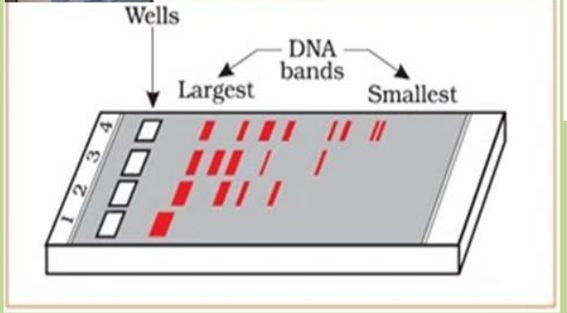
- **The DNA visualized by the use of dye that binds to DNA.**

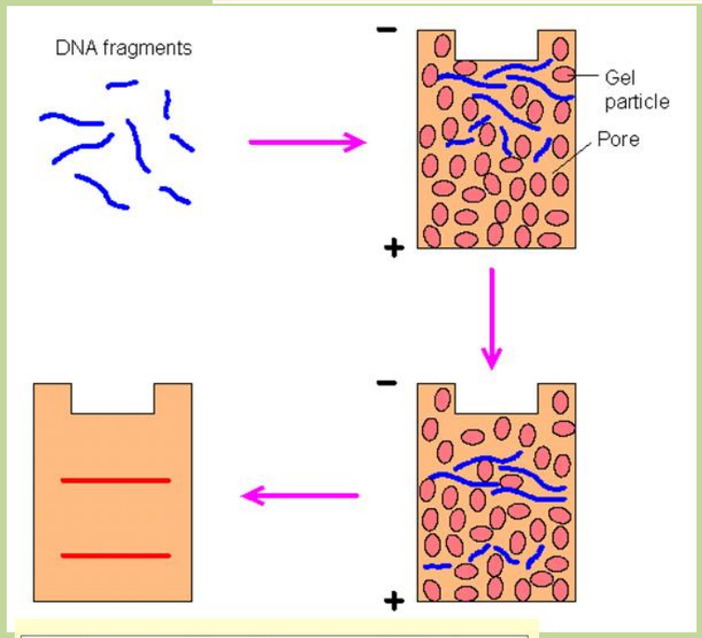
Agarose

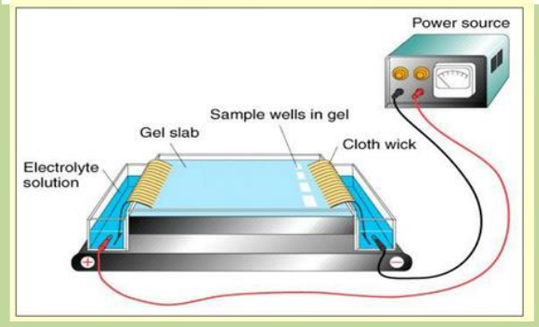

Buffer Solution



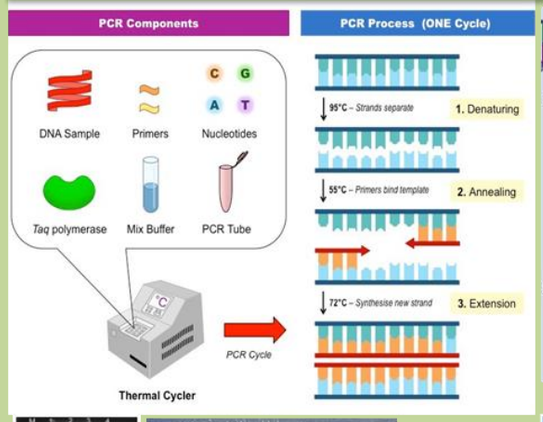


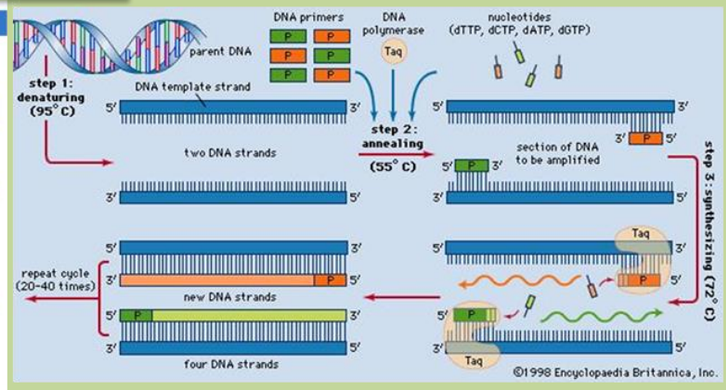


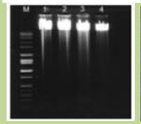


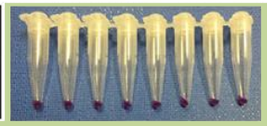


PCR (Polymerase Chain Reaction)









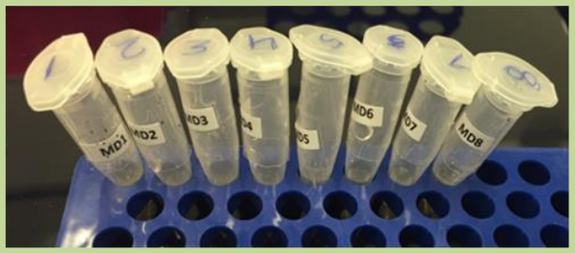
MARKER	SEQUENCE	REFERENCE
ITS1 F	TCCGTAGGTGAACCTGCGG	White et al. (1990)
ITS4 R	TCCTCCGCTTATTGATATGC	White et al. (1990)
rbcLa F	ATGTCACCACAAACAGAGACTAAAG C	Levin (2003)
rbcLa R	GTAAAATCAAGTCCACCRCG	Kress and Erickson (2007)
MatK 390 F	CGATCTATTCATTCAATATTTC	Cuenoud et al. (2002)
MatK 1326 R	TCTAGCACACGAAAGTCGAAGT	Cuenoud et al. (2002)
psbA-trnH F	GTTATGCATGAACGTAATGCTC	Sang et al. (1997)
psbA-trnH R	CGCGCATGGTGGATTCACAATCC	Tate and Simpson (2003)
trn L-F R	GGTTCAAGTCCCTCTATCCC	Taberlet et al. (1991)
trn L-F F	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)

Contents of HF PCR premix Reaction size (20 µl reaction): 1. DNA polymerase 1µl, 2. Each dNTP (dATP, dCTP, dGTP, dTTP) 250 µM, 3. 10X reaction buffer Stabilizer and tracking dye2µl

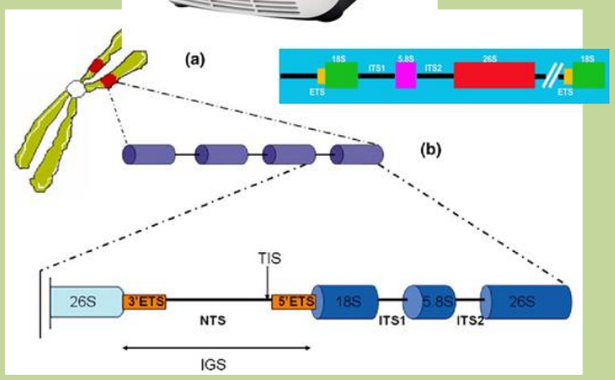
Template DNA (1µl ~ 100 ng), Primer (1µl each of F and R, 5 ~20 pmole)

1/10th genomic DNA dilution: Add 10 µl total genomic DNA in 90 µl molecular grade distilled water.

Dilution of primer for stock solution (100 pmoles/ µl): nmols X 10 Distilled water (ddH_2O) = 100 pmoles/ µl (Stock)

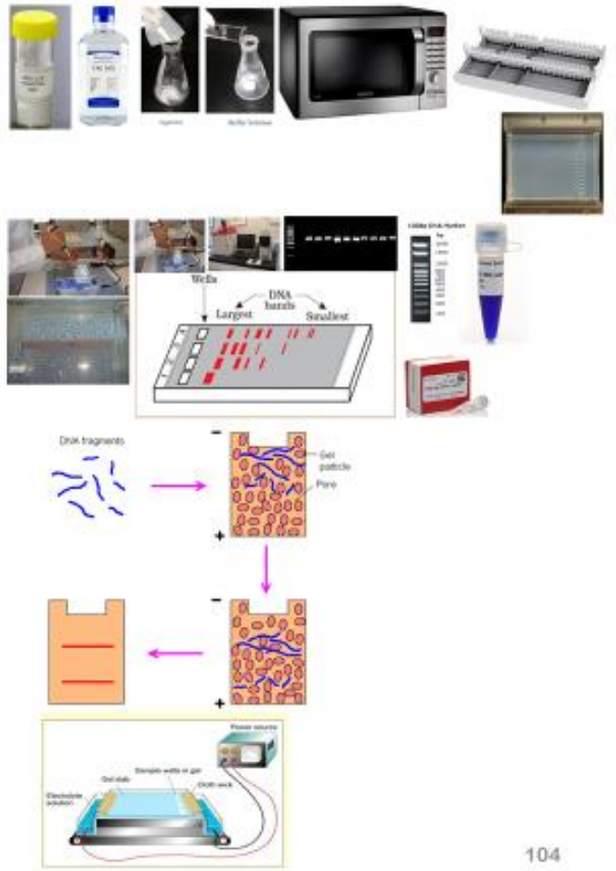


PCR Parameters			
1	Initial Denaturation	94 °C for 5 minutes	
2	Denaturation	94 °C for 1minute	Number of cycles: 40
	Annealing	49 °C for 1minute	
	Extension	72 °C for 1minute	
3	Final extension	72 °C for 5 minutes	
4	Hold	4 °C	∞



Agarose Gel Electrophoresis

- The main purpose of agarose gel electrophoresis is to determine the presence or absence of genomic DNA or PCR products and quantify the size (length of the DNA molecule).
- Agarose gel electrophoresis is a widely used technique for the preparation and analysis of DNA. Electrophoresis is a method of separating DNA based on the rate of movement while under the influence of an electric field.
- Agarose is a polysaccharide purified from seaweed.
- An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode.
- The DNA to be analyzed is forced through the pores of the gel by the electrical current.
- Under an electrical field, DNA moves to the positive electrode (red) and away from the negative electrode (black).
- DNA itself is not visible within an agarose gel.
- The DNA visualized by the use of dye that binds to DNA.



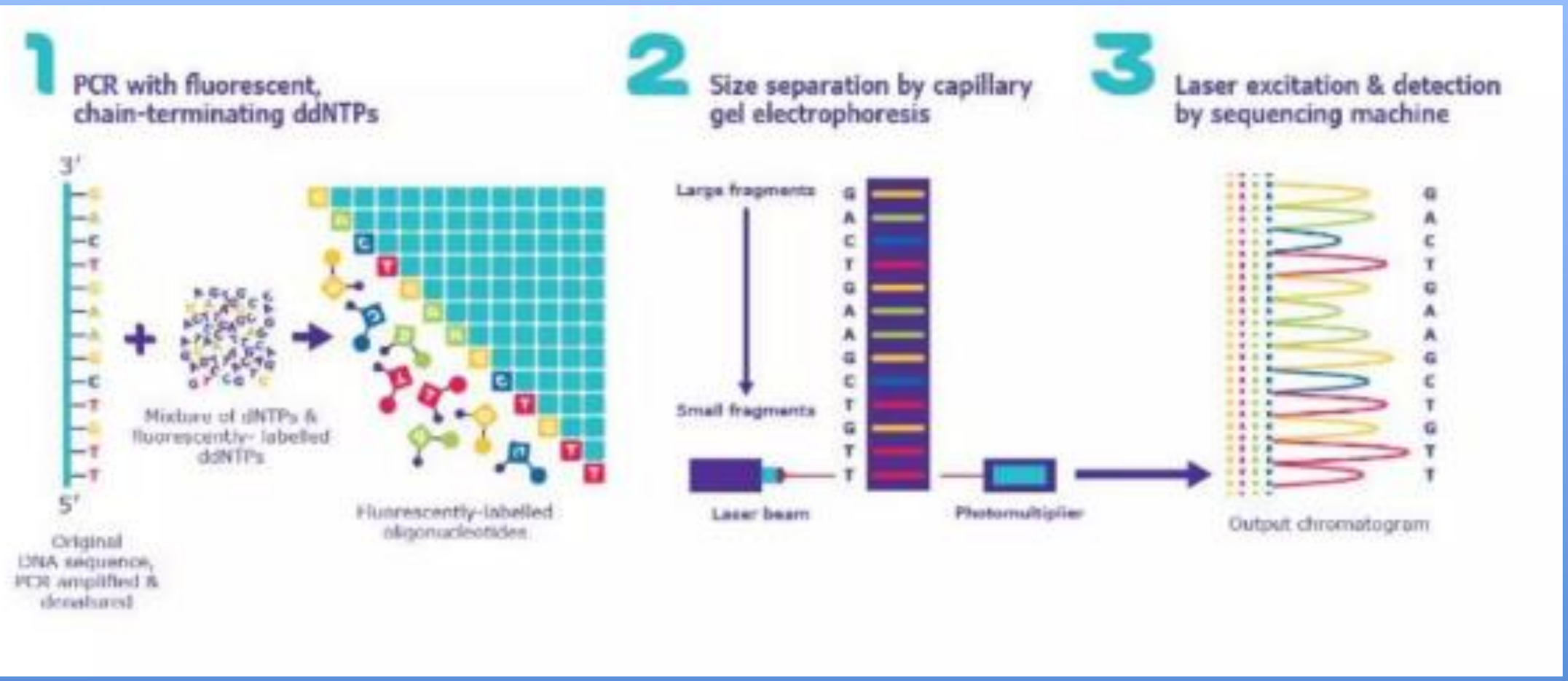
104

DNA sequencing

- DNA sequencing is the process of determining the sequence of nucleotides (A, T, C, and G) in a piece of DNA.
- In Sanger sequencing, the target DNA is copied many times, making fragments of different lengths. Fluorescent “chain terminator” nucleotides mark the ends of the fragments and allow the sequence to be determined.
- Next-generation sequencing techniques are new, large-scale approaches that increase the speed and reduce the cost of DNA sequencing.
- Sanger sequencing: The chain termination method
- Regions of DNA up to about 900 base pairs in length are routinely sequenced using a method called Sanger sequencing or the chain termination method.
- Ingredients for Sanger sequencing
- Sanger sequencing involves making many copies of a target DNA region. Its ingredients are similar to those needed for DNA replication in an organism, or for polymerase chain reaction (PCR), which copies DNA *in vitro*. They include:
- A DNA polymerase enzyme
- A primer, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a "starter" for the polymerase
- The four DNA nucleotides (dATP, dTTP, dCTP, dGTP)
- The template DNA to be sequenced
- However, a Sanger sequencing reaction also contains a unique ingredient:
- Dideoxy, or chain-terminating, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye
- Dideoxy nucleotides are similar to regular, or deoxy, nucleotides, but with one key difference: they lack a hydroxyl group on the 3’ carbon of the sugar ring. In a regular nucleotide, the 3’ hydroxyl group acts as a “hook," allowing a new nucleotide to be added to an existing chain.
- Once a dideoxy nucleotide has been added to the chain, there is no hydroxyl available and no further nucleotides can be added. The chain ends with the dideoxy nucleotide, which is marked with a particular color of dye depending on the base (A, T, C or G) that it carries.
- The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The four dye-labeled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.
- The mixture is first heated to denature the template DNA (separate the strands), then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from the primer. DNA polymerase will continue adding nucleotides to the chain until it happens to add a dideoxy nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the dideoxy nucleotide.
- This process is repeated in a number of cycles. By the time the cycling is complete, it’s virtually guaranteed that a dideoxy nucleotide will have been incorporated at every single position of the target DNA in at least one reaction. That is, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA (see figure below). The ends of the fragments will be labeled with dyes that indicate their final nucleotide.
- After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called capillary gel electrophoresis. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the “finish line” at the end of the tube, it’s illuminated by a laser, allowing the attached dye to be detected.

DNA Sequencing Reaction

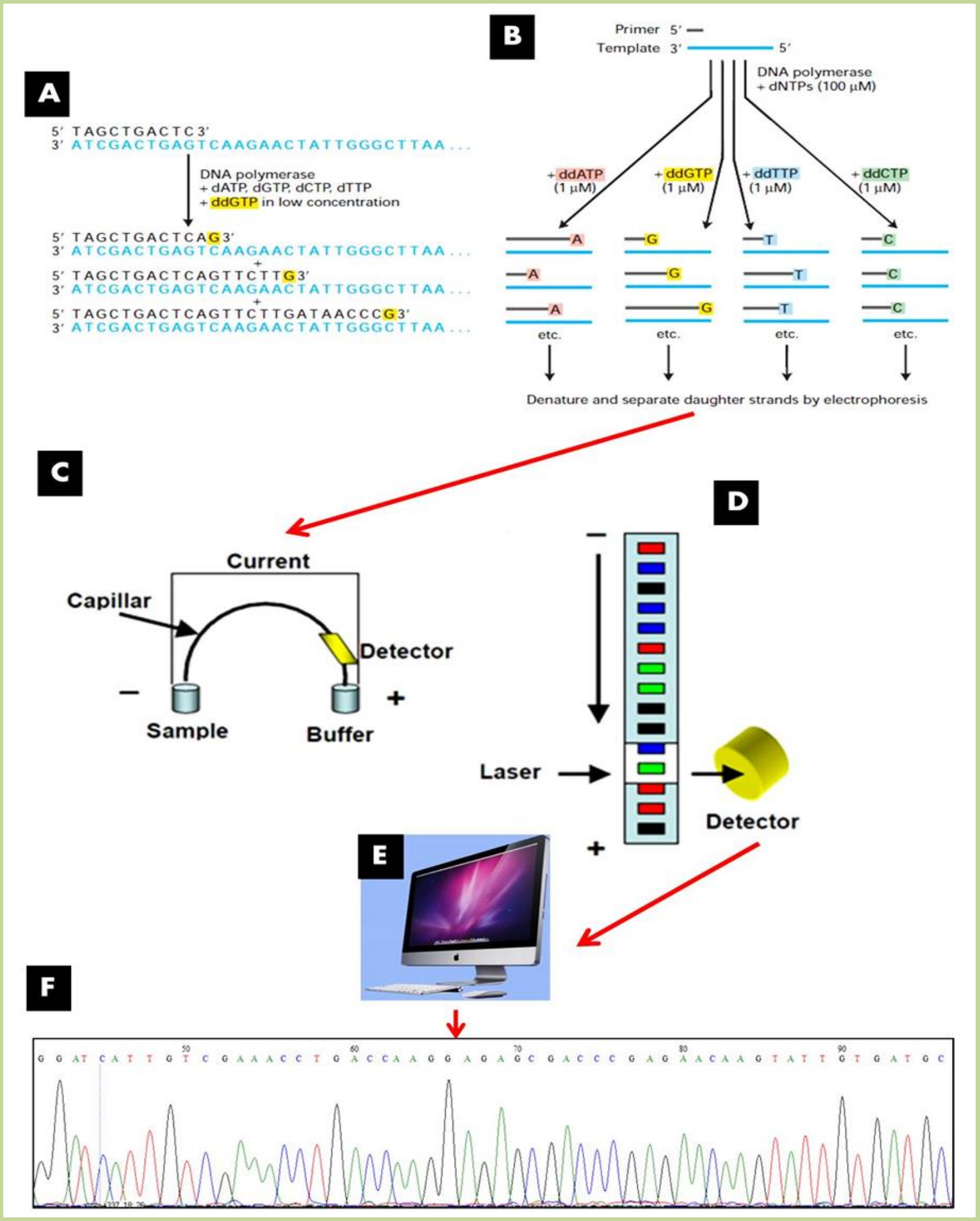
- PCR product is cleaned and processed for sequencing
- The machine which reads sequences is known as **Sequencer**
- The replicated DNA is placed in a tube with DNA polymerase, nucleotides, primers, and dNTPs
- After sequencing, electropherograms obtained from the sequencer are aligned for phylogenetic analysis
- Sequences of both individual genes and whole genomes are available in the GenBank, at the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov/). GenBank is the repository of a vast amount of publicly accessible data


1
PCR with fluorescent, chain-terminating ddNTPs
3'
5'
Original DNA sequence, PCR amplified & denatured
Mixture of dNTPs & fluorescently- labelled ddNTPs
Fluorescently-labelled oligonucleotides
2
Size separation by capillary gel electrophoresis
Large fragments
Small fragments
Laser beam
Photomultiplier
3
Laser excitation & detection by sequencing machine
Output chromatogram

- **The smallest fragment (ending just one nucleotide after the primer) crosses the finish line first, followed by the next-smallest fragment (ending two nucleotides after the primer), and so forth. Thus, from the colors of dyes registered one after another on the detector, the sequence of the original piece of DNA can be built up one nucleotide at a time. The data recorded by the detector consist of a series of peaks in fluorescence intensity, as shown in the chromatogram above. The DNA sequence is read from the peaks in the chromatogram.**

DNA sequence dataset preparation

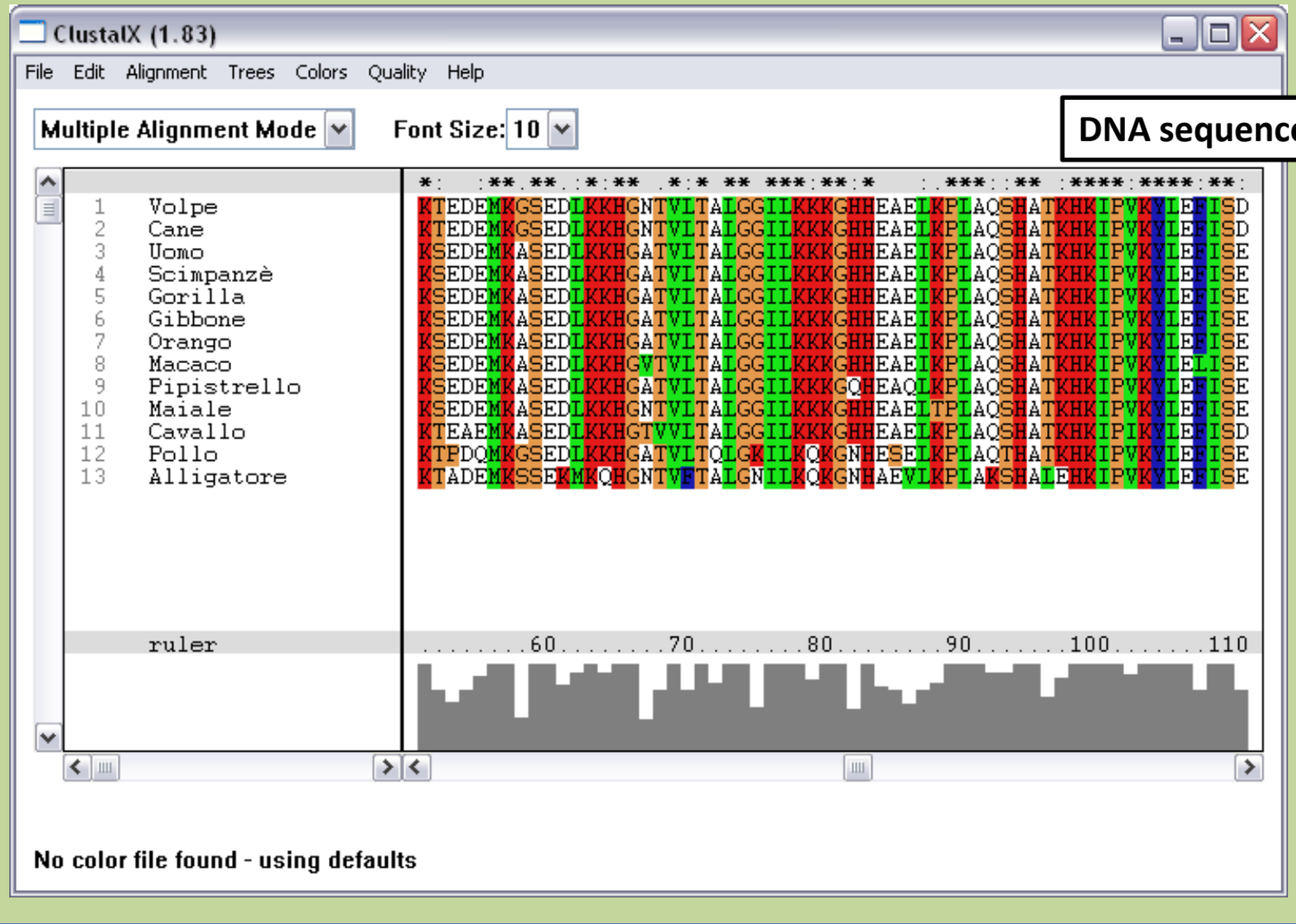
```
gene, partial sequence
GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCAGGTGAGGGACGAGGGGTGCGCAA
GCTCCCCAAGTTTCAAACCCATGGTCGGGGACCACCCTTGGGTGGCCTCGTCCGAACAACGACCCCCCGG
CGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCGTCCCCCCCGTTTGCGGGCGGCGGAAGCGTCT
TTCTAAAACACAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATG
CGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCA
TTAGGCCGAGGGCACGTCTGCCTGGGCGTCACACATCGCGTCGCCCCCCAACCCATCACTCCCTTGCGGG
AGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAATGCGAGTCCTTG
GCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATGTCGTGCGGTGACCCGTCGCCA
GCAAAATCTCTCATGACCCTGTTGCGCCGAGCCTCGACGCGCGCTCCGACCGCGACCCC
```

BIOINFORAMTICS

DNA sequence dataset preparation and sequence alignment

GTCGAAACCTGCATAGCAGAACGACCCGCGAACACGTTACACTACCAGGTGAGGGACGAGGGGTGCGCAA
GCTCCCCAAGTTTCAAACCCATGGTCGGGGACCACCCTTGGGTGGCCTCGTCCGAACAACGACCCCCCGG
CGCGGAATGCGCCAAGGAAATCAAACTGAACTGCACGCGTCCCCCCCGTTTGCGGGCGGCGGAAGCGTCT
TTCTAAAACACAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATG
CGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCA
TTAGGCCGAGGGCACGTCTGCCTGGGCGTCACACATCGCGTCGCCCCCCAACCCATCACTCCCTTGCGGG
AGTTGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAATGCGAGTCCTTG
GCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATGTCGTGCGGTGACCCGTCGCCA
GCAAAATCTCTCATGACCCTGTTGCGCCGAGCCTCGACGCGCGCTCCGACCGCGACCCC



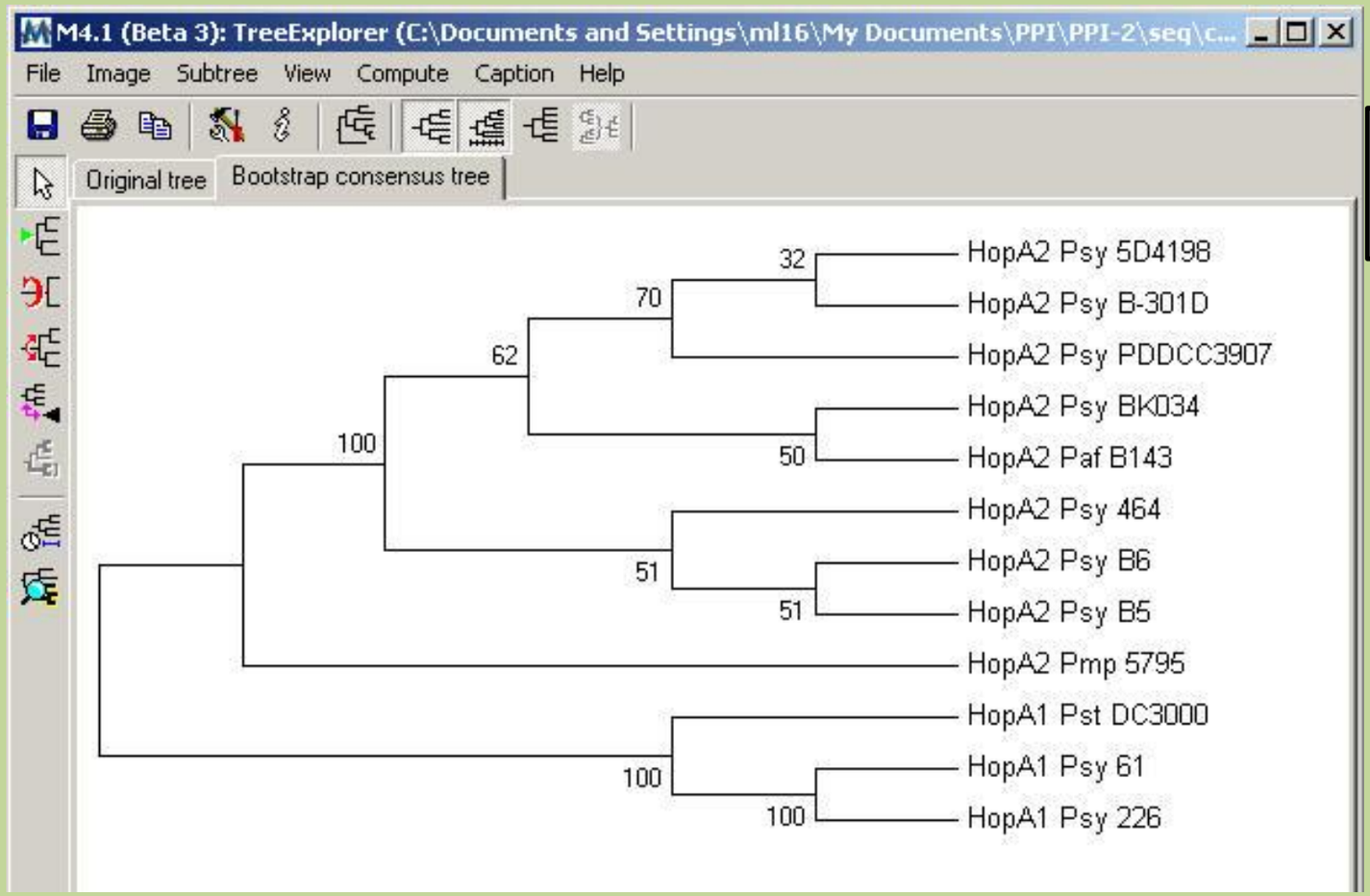
DNA sequence alignment using ClustalX

Sequence Alignment

- After the sequences have been received from the sequencer, they need to be aligned
- ***Sequence alignment: global and local***
  - Find the similarity between two (or more) DNA-sequences by finding a good **alignment** between them
- **Sequence alignment** is an arrangement of two or more sequences, highlighting their similarity.
- The sequences are padded with **gaps** (dashes) so that wherever possible, columns contain **identical characters** from the sequences involved

```
tcctctgcctctgccatcat---caaccccaaagt
||||  |||  |||||  |||||    ||||||||||||
tcctgtgcatctgcaatcatgggcaaccccaaagt
```

- **BLAST (Basic Local Alignment Search Tool)- NCBI**
- Accurate alignment is crucial to any **phylogenetic analysis**
- The alignment is necessary so that identical nucleotides can be determined
- Phylogenetic trees are generated from aligned DNA sequences
- Two DNA sequences that show high similarity are generally presumed to be homologous (evolved from a common ancestral sequence)



Molecular Phylogenetic analyses using MEGA

NCBI GenBank

Molecular phylogenetics: principles and practice

- Phylogenetic analysis is a fundamental tool used to study evolutionary relationships among species, genes, and populations.
- Advances in DNA sequencing technologies have greatly expanded the role of molecular phylogenetics in many biological disciplines.
- Phylogenetic trees represent genealogical relationships where branches indicate evolutionary lineages and nodes represent divergence events.
- Molecular phylogenetics is used not only in taxonomy and systematics but also in genomics, epidemiology, population genetics, and evolutionary biology.
- Phylogenetic trees can be reconstructed using sequence data through computational and statistical methods.
- The major phylogenetic reconstruction methods include distance-based methods, maximum parsimony, maximum likelihood, and Bayesian inference.
- Distance-based methods estimate evolutionary relationships by calculating pairwise genetic distances among sequences.
- Maximum parsimony identifies the tree that requires the smallest number of evolutionary changes.
- Maximum likelihood uses explicit evolutionary models to estimate the tree that has the highest probability of producing the observed sequence data.
- Bayesian inference incorporates prior knowledge and uses Markov chain Monte Carlo algorithms to estimate posterior probabilities of phylogenetic trees.
- Each method has strengths and limitations in terms of accuracy, computational efficiency, and robustness.
- Large genomic datasets generated by next-generation sequencing require improved computational methods and models for accurate phylogenetic analysis.
- Modern phylogenetics integrates statistical models, evolutionary theory, and computational biology to analyze genome-scale data.
- Challenges in phylogenetic analysis include missing data, systematic errors, and model selection.
- Future developments focus on improved evolutionary models, large-scale genomic analysis, and statistical phylogeography.

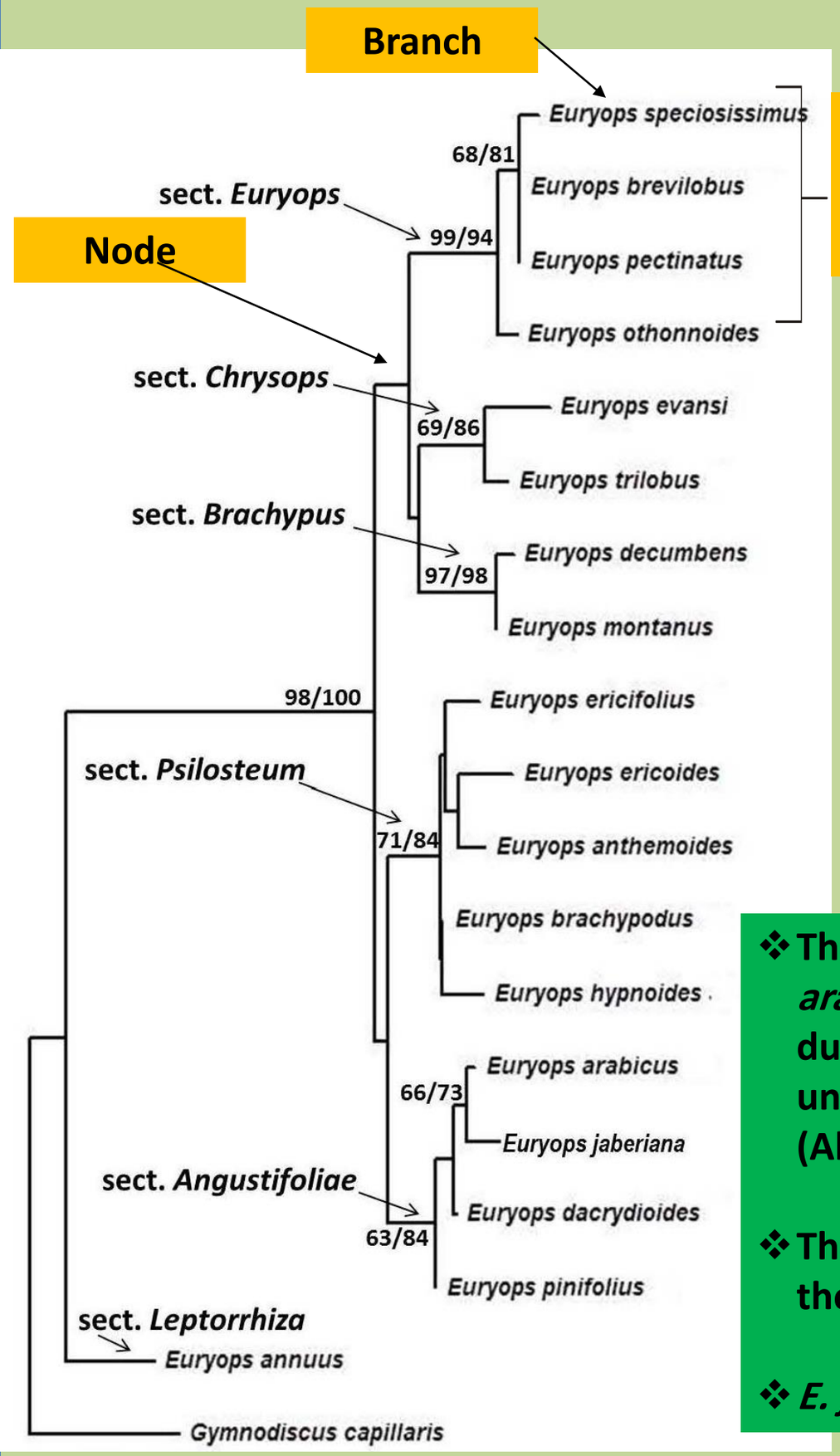
Phylogenetic Implication of Molecular Genotyping of *Euryops jaberiana* Abedin & Chaudhary (Asteraceae)

E. arabicus

- In Saudi Arabia, the genus *Euryops (*family Asteraceae) is represented by two species, *viz*. *E. arabicus* Steud. *ex* Jaub. & Spach, and *E. jaberiana* Abedin & Chaudhary.
- *E. arabicus* is is endemic to Arabian Peninsula, while *E. jaberiana* is endemic to northern Saudi Arabia.
- Morphologically *E. jaberiana* very closely resembles with *E. arabicus* / very narrow differences in m morphological characters (Abedin and Chaudhary, 2000).
- The taxonomic status of *Euryops jaberiana* Abedin & Chaudhary (tribe Senecioneae, was evaluated (Ali et al., 2016) based on molecular phylogenetic analyses of internal transcribed spacer sequence (ITS) of nuclear ribosomal DNA (nrDNA) in order to ascertain its position within the genus.

E. jaberiana

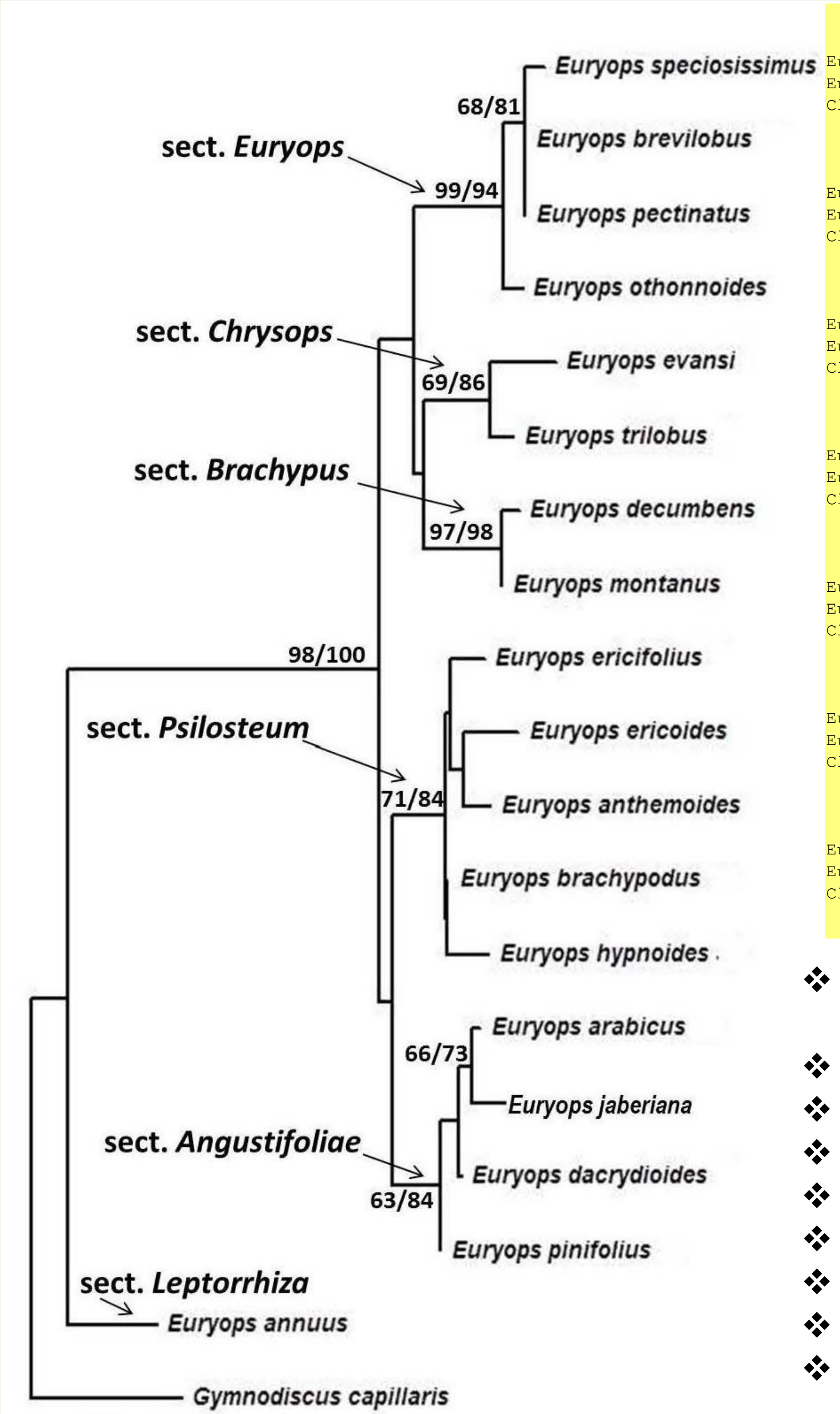
Phylogenetic Implication of Molecular Genotyping of *Euryops jaberiana* Abedin & Chaudhary (Asteraceae) Contd……



- In molecular taxonomic studies, the most convenient way of presenting taxonomic relationships among a group of organisms is the phylogenetic tree.
- Node: a branch point in a tree
- Branch: defines the relationship between the taxon
- Topology: the branching patterns of the tree
- Branch length: represents the number of changes that have occurred in the branch
- Clade: a group of two or more taxa closed together based on DNA sequences data analsysis
- Maximum parsimony is an optimality criterion under which the phylogenetic tree that minimizes the total number of character-state changes is to be preferred.
- Bootstrap: Bootstrapping is a procedure where DNA sequence data run for the phylogenetic analysis, and the reported value is the percentage of bootstrap replicates, for examples 100 means that the node is well-supported, it showed in all tress.

- The key morphological features which differentiate *E. jaberiana* from *E. arabicus* are: leaves 3-lobed at the tips, pappus hairs transparent or rarely dull white, and achenes glabrescent, while in *E. arabicus*, the leaves are unlobed, pappus hairs are dull white and achene densely lanate hairy (Abedin and Chaudhary, 2000).
- The Maximum Parsimony analyses reveals that *E. jaberiana* nested within the clade of the section *Angustifoliae.*
- *E. jaberiana* shows proximity with *E. arabicus* (66% bootstrap support.

Phylogenetic Implication of Molecular Genotyping of *Euryops jaberiana* Abedin & Chaudhary (Asteraceae) Contd……




```
                   10        20        30        40        50        60        70        80        90       100
Euryops_jabriana   TCGAAACCTGCATAGCAGAACGACCCGTGAACATGTAATAACAATCGGGTGTCCATGGTTTCCGACTATTGTTTGATTCTTTGGATACCCTGATAATGTG
Euryops_arabicus   ............................................................................................T.......
Clustal Consensus  ******************************************************************************************** *******

                  110       120       130       140       150       160       170       180       190       200
Euryops_jabriana   CGTCTTTGGTCAGCCGCTTGGGTCCTAATGATGTCACATTAACACAATAACAAACCCCCGGCACGGCATGTGCCAAGGAAAATAAAACTTAAGAAAAGCT
Euryops_arabicus   ...............C....................................................................................
Clustal Consensus  *************** ************************************************************************************

                  210       220       230       240       250       260       270       280       290       300
Euryops_jabriana   TGTATCATGTTACGTCGTTCGCGGGGTTTGCATGATACGTGGCTTCTTTATAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGA
Euryops_arabicus   C...................................................................................................
Clustal Consensus   ***************************************************************************************************

                  310       320       330       340       350       360       370       380       390       400
Euryops_jabriana   AGAACGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCTTTTGGCCGAGGGCA
Euryops_arabicus   ....................................................................................................
Clustal Consensus  ****************************************************************************************************

                  410       420       430       440       450       460       470       480       490       500
Euryops_jabriana   CGTCTGCCTGGGCGTCACACATCGCGTCGCCCCCCACAACATCTCTTGATTGGGATGTTGTAATGGGGGCGGATATTGGTCTCCCGTTCCTAAGGTTCGG
Euryops_arabicus   ..........................................G.................G.......................................
Clustal Consensus  ****************************************** ***************** ***************************************

                  510       520       530       540       550       560       570       580       590       600
Euryops_jabriana   TTGGCTAAAATAGGAGTCCCCTTCGAAGGATGCACGATTAGTGGTGGTTGTCAAGACCTTCTTATCGACTCGCGCGTTACAAGTAGTAGGGAAGATCTCT
Euryops_arabicus   ..............................C.........................................T...........................
Clustal Consensus  ****************************** ***************************************** ***************************

                  610       620       630       640
Euryops_jabriana   TCAAAGACCCTAATGTGTTGTCTTGTGACAATGCTTCGACCGCGA
Euryops_arabicus   ..........C..................................
Clustal Consensus  ********** **********************************
```

- A total of eight specific nucleotide differences were detected between *E. jaberiana* and *E. Arabicus i.e.* at the alignment position:
- 93 (A → T)
- 116 (G → C)
- 201 (T → C)
- 443 (C → G)
- 461 (T → G)
- 531 (T → C)
- 573 (C → T)
- 611 (T → C)

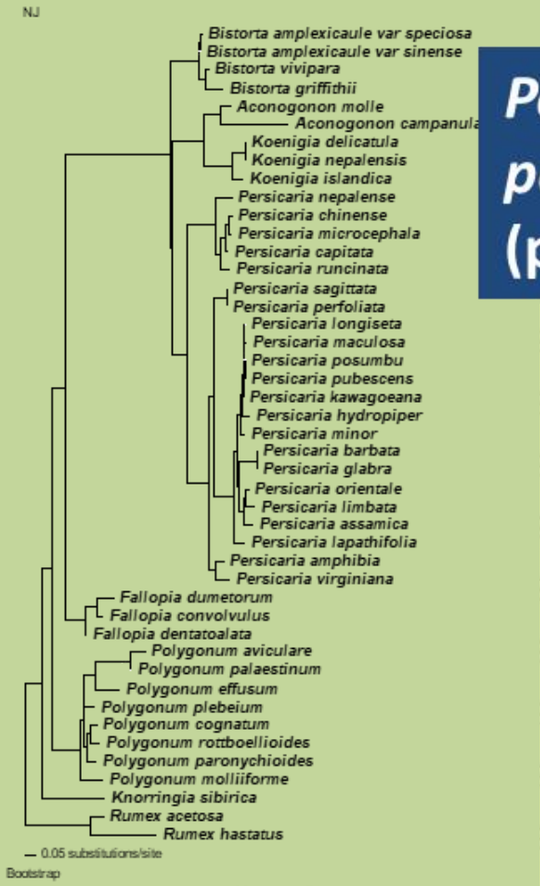
Thus on the basis of phylogenetic relationships of *E. jaberiana* within the genus and nucleotide differences, Ali et al. (2016) recognized *E. jaberiana* as a distinct species and different from *E. arabicus.*

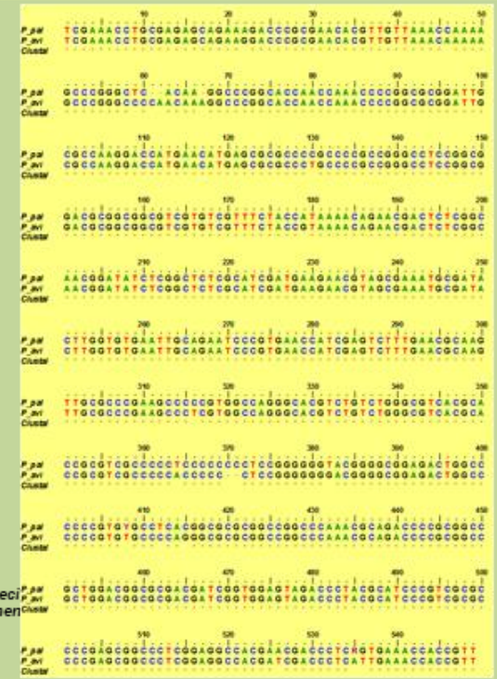
- **Molecular systematic studies on *Polygonum palaestinum* Zohary (polygonaceae) from Saudi Arabia using ITS sequences of nuclear ribosomal DNA**

- **The taxonomy of the genus Polygonum is highly controversial because of diverse variation within species among the species has resulted into lack of consensus on taxonomic circumscription. Therefore, there is disagreement among the taxonomists that to which species should be retain within the genus Polygonum and to which species should be elevated to their own genus.**
- **The genus *Polygonum* in Saudi Arabia includes P. argyrocoleum Steud. ex Kunze, P. aviculare L. and P*. palaestinum* Zohary. Two out of these Polygonums of Saudi Arabia i.e. *P. argyrocoleum* and *P. aviculare* are common weed distributed throughout. The distribution of *P. palaestinum* is restricted to Harratal Harra area of Saudi Arabia.**

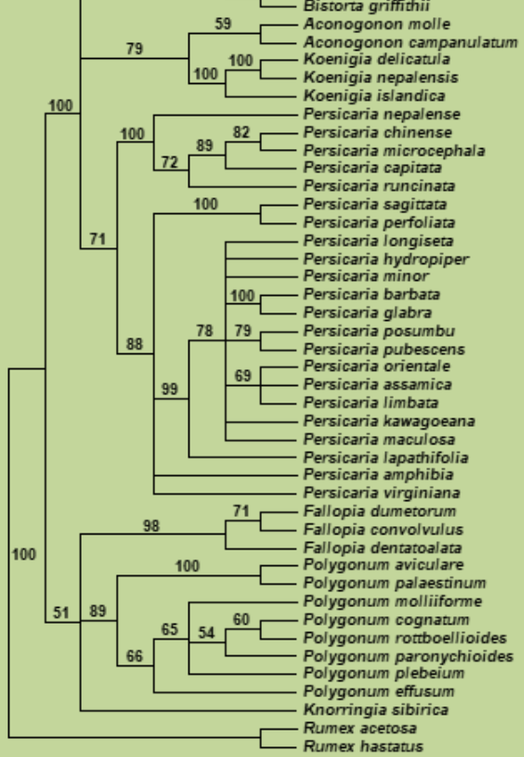
- **Decraene and Akeroyd (1988) have segregated Polygonum in the broad sense into two separate tribes, Polygoneae and Persicarieae.**

- **The systematic status of *P. palaestinum* is unresolved**





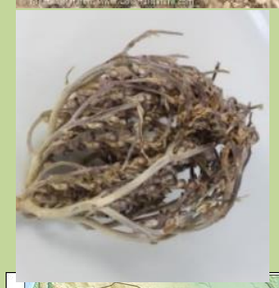
Polygonum palaestinum Zohary (polygonaceae)

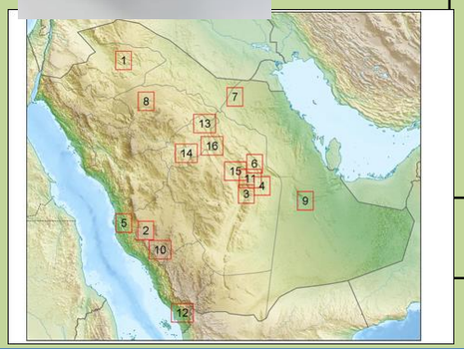


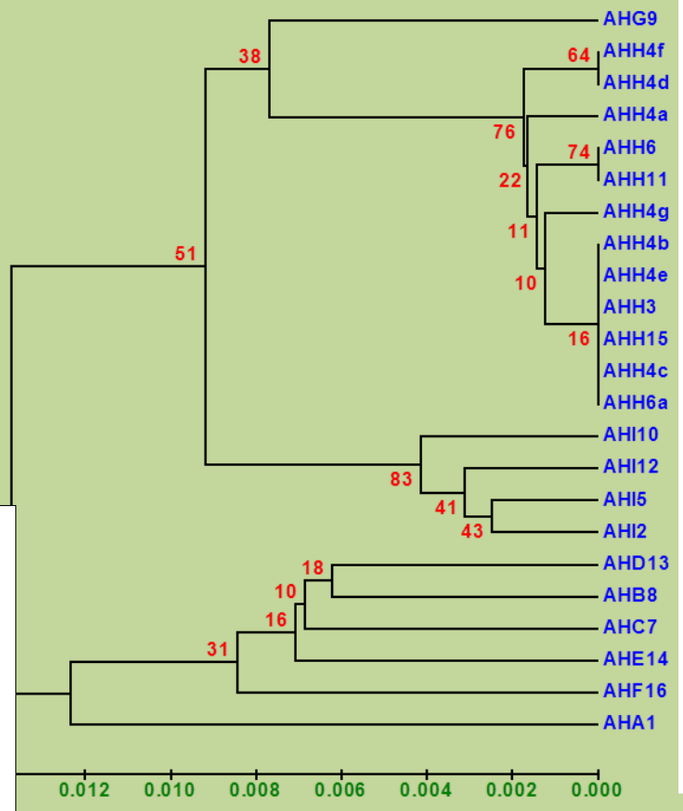
Position in sequence alignment	*Polygonum aviculare*	*Polygonum palaestinum*
22	G	A
46	A	C
59	C	T
61	C	-
62	A	-
67	A	-
130	T	C
179	G	A
316	T	C
364	A	T
370	-	C
371	-	C
382	G	T
408	T	Y C T
413	C	T
415	G	C
526	T	A
535	A	R A G
536	T	G

Assessment of genetic diversity of *Anastatica hierochuntica* (kaff maryam) from Saudi Arabia based on Internal Transcribed Spacer sequences of nuclear ribosomal DNA gene

- *Anastatica hierochuntica* (Rose of Jericho) is among the common medicinal plants widely used in Hijaz, Najd, and Al Rub'Al Khali. The plant is prescribed in folk medicine for difficult labor, uterine hemorrhage and to facilitate the expulsion of dead fetuses. A total number of 23 population of Anastatica hierochuntica from Saudi Arabia were sequenced.
- The resulted UPGMA tree reveals that the populations of different geographic location sampled in the present study grouped into three major group.
- Group I consists of population from Hanifa valley, Summan, Rumah, Hair area, Riyadh, Khurma, and Khoris;
- Group II consists of population from Al-Baha, Jeedah, Ranyah and Zazan; and
- Group III consists of population from Hail, Darb Al Hafer, Qasim Buraydah, Afif, and Marat), and the groups were according to their geographic locations;
- however it was interesting to note that population collected from the geographic location of Haradh and Buseita (Tabarjal) and were nested within the group I and II respectively, which might be due to evolution under reproductive isolation and different environmental conditions, and this may be most probably due to long distance distribution, and possibility of genetic exchange among the populations of *Anastatica hierochuntica* distributed in Saudi Arabia.





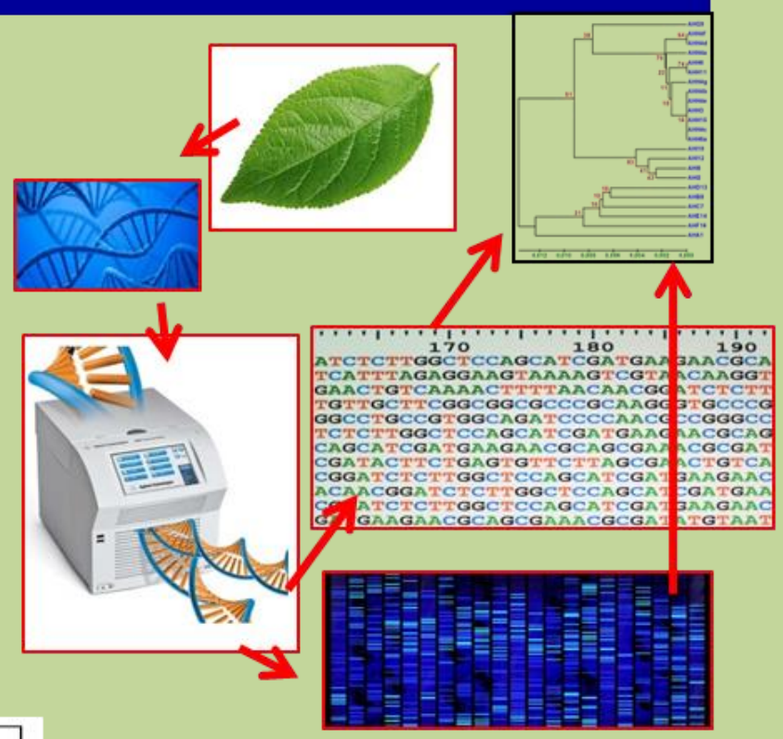


Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species.

- Molecular analyses comprise a large variety of DNA molecular markers, which can be employed for analysis of variation.

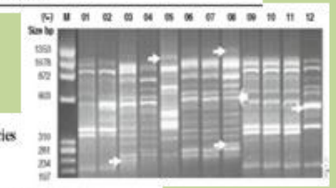
AFLP	Amplified Fragment Length Polymorphism
AP-PCR	Arbitrarily primed PCR
ARMS	Amplification Refractory Mutation System
ASAP	Arbitrary Signatures from Amplification
ASH	Allele-Specific Hybridization
ASLP	Amplified Sequence Length Polymorphism
ASO	Allele Specific Oligonucleotide
CAPS	Cleaved Amplification Polymorphic Sequence
CAS	Coupled Amplification and Sequencing
DAF	DNA Amplification Fingerprint
DGGE	Denaturing Gradient Gel Electrophoresis
GBA	Genetic Bit Analysis
IRAO	Inter-Retrotrasposon Amplified Polymorphism
ISSR	Inter-Simple Sequence Repeats
ISTR	Inverse Sequence-Tagged Repeats
MP-PCR	Microsatellite-Primed PCR
OLA	Oligonucleotide Ligation Assay
RAHM	Randomly Amplified Hybridizing Microsatellites
RAMPs	Randomly Amplified Microsatellite Polymorphisms
RAPD	Randomly Amplified Polymorphic DNA
RBIP	Retrotrasposon-Based Insertion Polymorphism
REF	Restriction Endonuclease Fingerprinting
REMAP	Retrotrasposon-Microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SAMPL	Selective Amplification of Polymorphic Loci
SCAR	Sequence Characterised Amplification Regions
SNP	Single Nucleotide Polymorphism
SPAR	Single Primer Amplification Reaction
SPLAT	Single Polymorphic Amplification Test
S-SAP	Sequence-Specific Amplification Polymorphisms
SSCP	Single Strand Conformation Polymorphism
SSLP	Single Sequence Length Polymorphism
SSR	Simple Sequence Repeats
STMS	Sequence-Tagged Microsatellite Site
STS	Sequence-Tagged-Site
TGGE	Thermal Gradient Gel Electrophoresis
VNTR	Variable Number Tandem Repeats
RAMS	Randomly Amplified Microsatellites

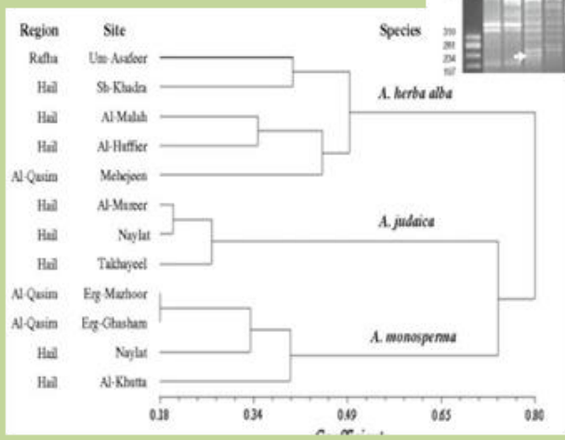
GENETIC DIVERSITY



- Genetic diversity of *Artemisia* in central and north Saudi Arabia based on RAPD

Serial	Primer	Nucleotide sequences
01	OPA-02	5'-TGCCGAGCTG-3'
02	OPA-05	5'-AGGGGTCTTG-3'
03	OPA-07	5'-GAAACGGGTG-3'
04	OPA-08	5'-GTGACGTAGG-3'
05	OPA-09	5'-GGGTAACGCC-3'
06	OPA-13	5'-CAGCACCCAC-3'
07	OPA-14	5'-TCTGTGCTGG-3'
08	OPA-18	5'-AGGTGACCGT-3'
09	OPB-10	5'-CTGCTGGGAC-3'
Total number of bands		





Badr, A., El-Shazly, H.H., Helail, N.S. et al. Genetic diversity of *Artemisia* populations in central and north Saudi Arabia based on morphological variation and RAPD polymorphism. Plant Syst Evol (2012) 298: 871)

7.2 DNA Barcoding

- DNA barcoding uses short DNA sequences for identification.
- rbcL and matK are standard plant barcodes.
- ITS and trnH-psbA improve resolution.
- Barcoding relies on interspecific variation.
- Each species has a unique barcode sequence.
- DNA barcodes act as genetic identifiers.
- Barcoding accelerates species identification.
- It reduces dependence on expert identification.
- DNA barcoding supports biodiversity assessment.
- It is widely used in modern taxonomy.

DNA barcoding

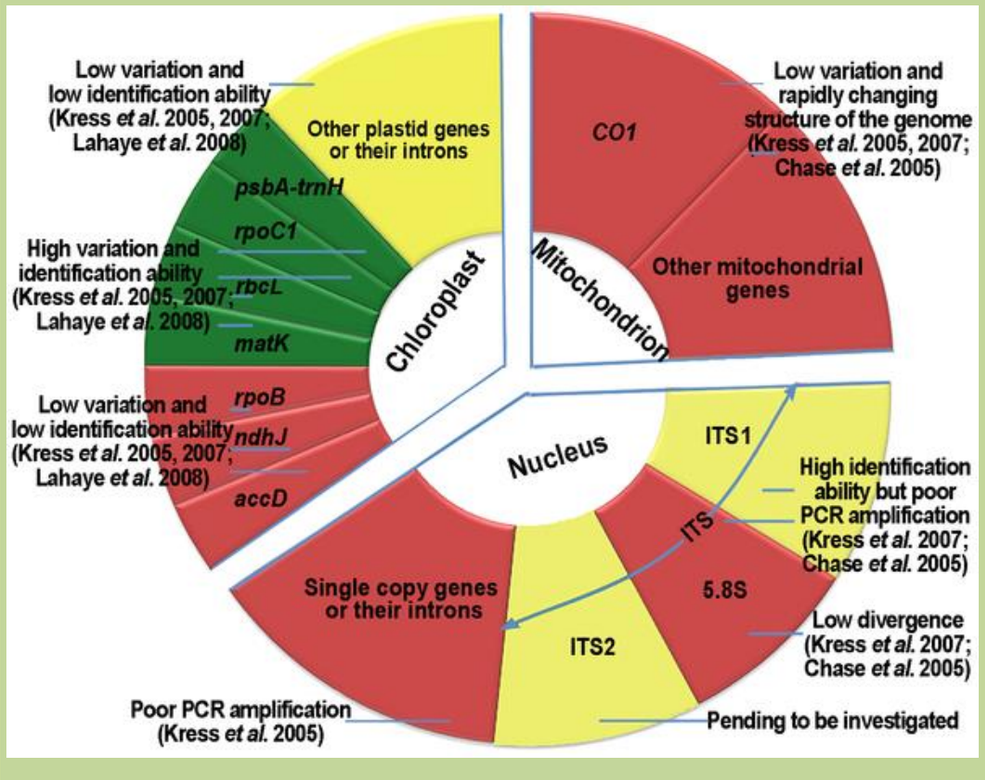
- DNA barcoding is a system for fast and accurate species identification that makes ecological system more accessible by using short DNA sequence instead of whole genome and is used for eukaryotes. The short DNA sequence is generated from standard region of genome known as marker. This marker is different for various species like CO1 cytochrome c oxidase 1 for animals, matK for plants and Internal Transcribed Spacer (ITS) for fungus. DNA barcoding has many applications in various fields like preserving natural resources, protecting endangered species, controlling agriculture pests, identifying disease vectors, monitoring water quality, authentication of natural health products and identification of medicinal plants.

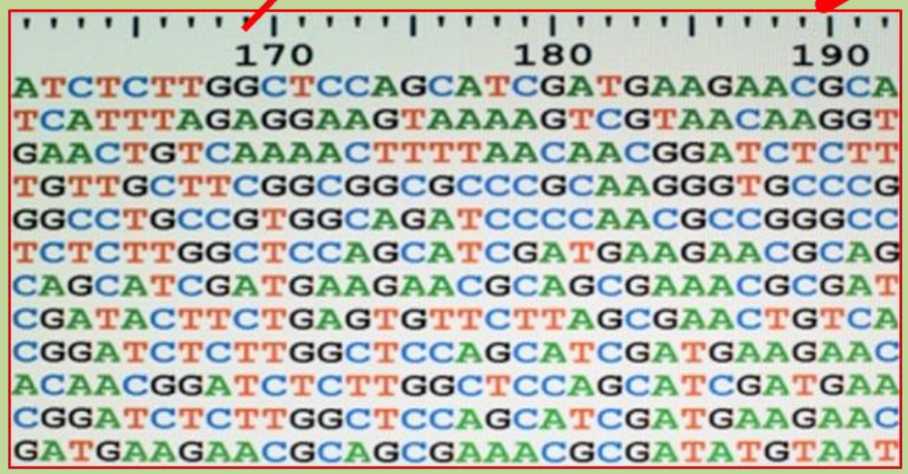
❖**DNA barcoding can speed up identification of species.**

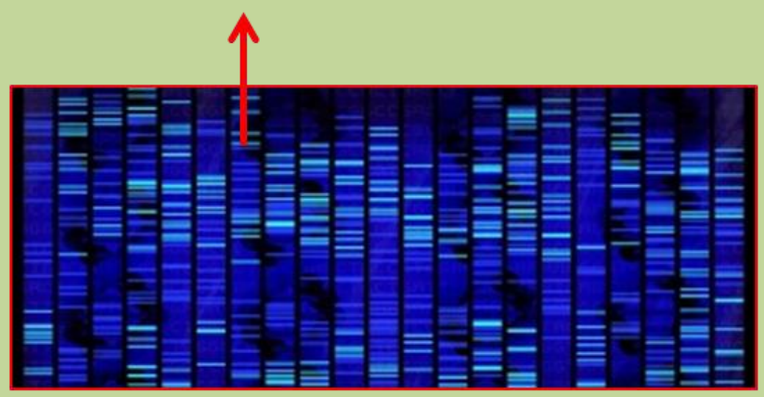
❖**DNA barcoding can provide an avenue to encourage new participants into taxonomy.**

❖**Raw drug authentication / Medicinal plant identification or authentication**

- In DNA barcoding, complete data set can be obtained from a single specimen irrespective to morphological or life stage characters.
- The core idea of DNA barcoding is based on the fact that the highly conserved stretches of DNA, either coding or
- non coding regions, vary at very minor degree during the evolution within the species.
- Sequences suggested to be useful in DNA barcoding include cytoplasmic mitochondrial DNA (e.g. cox1) and chloroplast DNA (e.g. rbcL, trnL-F, matK, ndhF, and atpB rbcL), and nuclear DNA (ITS)
- The term “DNA barcode” for global species identification was first coined by Hebert in 2003.
- The ideal DNA barcode region is reliably amplified and sequenced across large assemblages of taxa and provides a high level of species discrimination



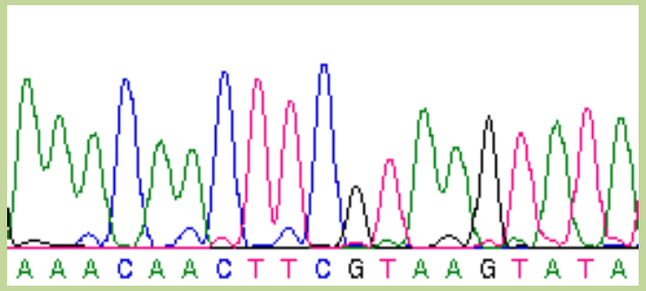




- Chen S, Yao H, Han J, Liu C, Song J, et al. (2010) Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species. PLOS ONE 5(1): e8613.
- Yao H, Song J, Liu C, Luo K, Han J, Li Y, et al. (2010) Use of ITS2 Region as the Universal DNA Barcode for Plants and Animals. PLoS ONE 5(10): e13102.
- Zuo Y, Chen Z, Kondo K, Funamoto T, Wen J, Zhou S. (2011) DNA barcoding of Panax species. Planta Med. 2011 Jan;77(2):182-7.

7.3 Applications of DNA Barcoding

- ❑Barcoding enables rapid species identification.
- ❑It detects cryptic species.
- ❑It identifies invasive species.
- ❑It authenticates medicinal plants.
- ❑ It supports conservation of endangered taxa.
- ❑Forensic studies use DNA barcoding.
- ❑Environmental DNA studies apply barcoding methods.
- ❑Barcoding improves biodiversity monitoring.
- ❑It simplifies taxonomic workflows.
- ❑It enhances classification accuracy.

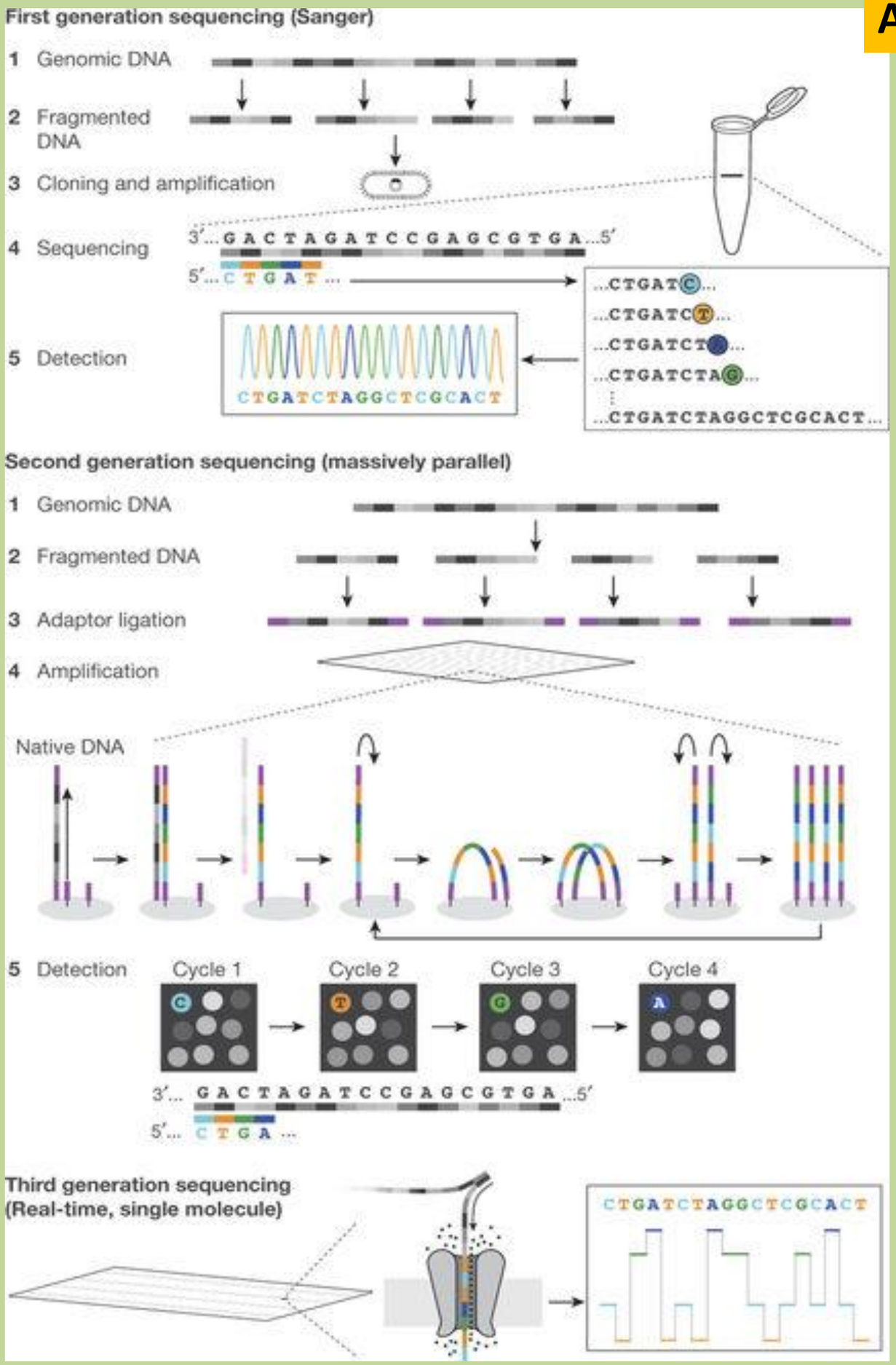


8. Next-Generation Sequencing (NGS) and Modern Taxonomy

8.1 Concept of NGS

- NGS sequences millions of DNA fragments simultaneously.
- It is faster and cheaper than Sanger sequencing.
- NGS generates large-scale genomic data.
- Illumina is widely used for short reads.
- PacBio provides long-read sequencing.
- Oxford Nanopore enables portable sequencing.
- NGS supports genome-scale taxonomy.
- High-throughput sequencing increases resolution.
- NGS integrates with bioinformatics tools.
- NGS has transformed modern taxonomy.

Advances in next generation DNA sequencing



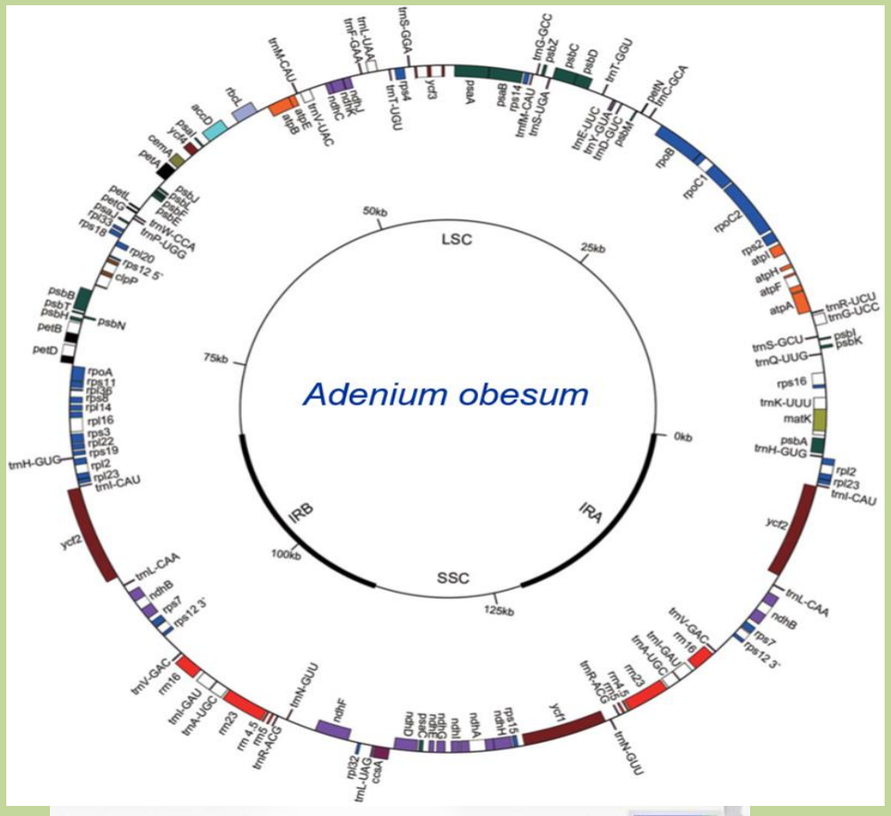
Technical milestones

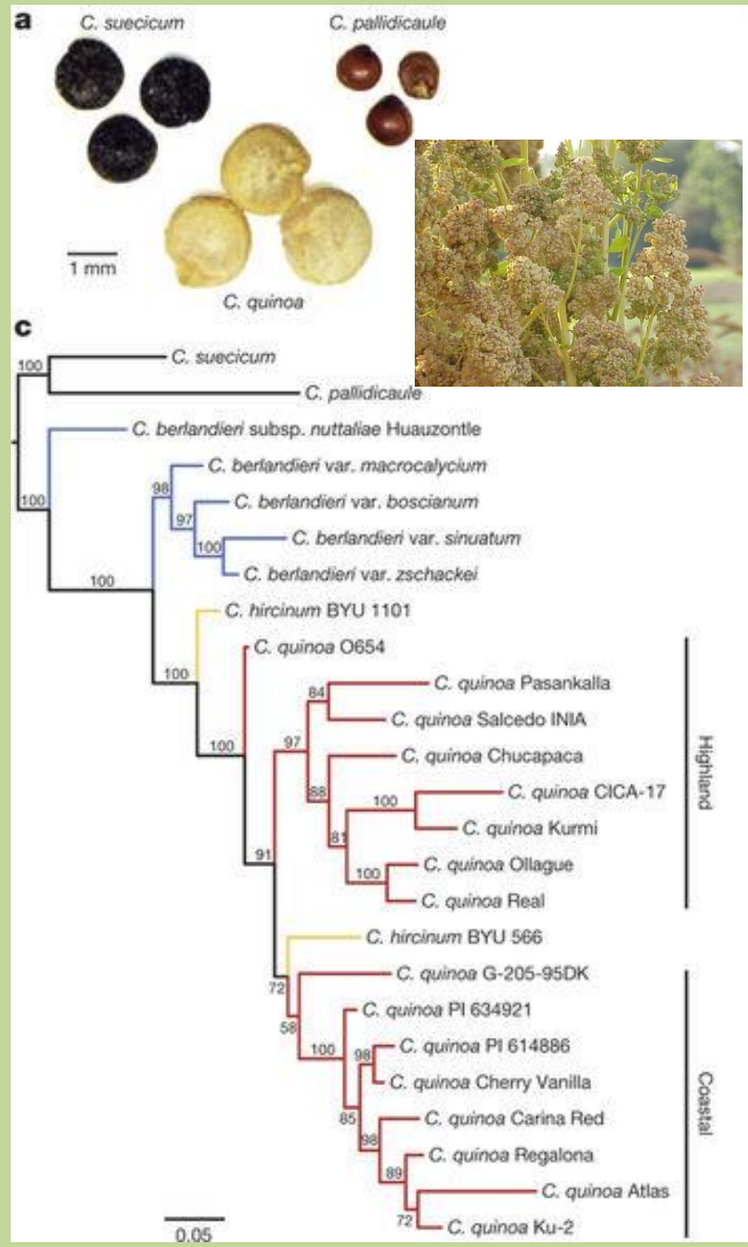
1953: Sequencing of insulin protein[2]
1965: Sequencing of alanine tRNA[4]
1968: Sequencing of cohesive ends of phage lambda DNA[6]
1977: Maxam–Gilbert sequencing[9]
1977: Sanger sequencing[8]
1981: Messing's M13 phage vector[12]
1986–1987: Fluorescent detection in electrophoretic sequencing[14,15,17]
1987: Sequenase[18]
1988: Early example of sequencing by stepwise dNTP incorporation[139]
1990: Paired-end sequencing[23]
1992: Bodipy dyes[140]
1993: *In vitro* RNA colonies[37]
1996: Pyrosequencing[44]
1999: *In vitro* DNA colonies in gels[38]
2000: Massively parallel signature sequencing by ligation[47]
2003: Emulsion PCR to generate *in vitro* DNA colonies on beads[42]
2003: Single-molecule massively parallel sequencing-by-synthesis[33,34]
2003: Zero-mode waveguides for single-molecule analysis[57]
2003: Sequencing by synthesis of *in vitro* DNA colonies in gels[49]
2005: Four-colour reversible terminators[51–53]
2005: Sequencing by ligation of *in vitro* DNA colonies on beads[41]
2007: Large-scale targeted sequence capture[93–96]
2010: Direct detection of DNA methylation during single-molecule sequencing[65]
2010: Single-base resolution electron tunnelling through a solid-state detector[141]
2011: Semiconductor sequencing by proton detection[142]
2012: Reduction to practice of nanopore sequencing[143,144]
2012: Single-stranded library preparation method for ancient DNA[145]

8.2 Applications of NGS

- ❑Whole-genome sequencing improves species delimitation.
- ❑Transcriptomics studies gene expression patterns.
- ❑Phylogenomics reconstructs deep evolutionary relationships.
- ❑Environmental DNA enables species detection.
- ❑Genome data improve evolutionary studies.
- ❑NGS uncovers cryptic diversity.
- ❑It supports large biodiversity projects.
- ❑Comparative genomics strengthens classification.
- ❑Population-level analyses become possible.
- ❑NGS expands taxonomic research scope.

Advances in next generation DNA sequencing and its application in systematics





❖ **Whole Chloroplast Genome Sequencing of *Adenium obesum***

❖ **Chloroplast (cp) is a special subcellular organelle which contains the entire enzymatic machinery for photosynthesis.**

❖ **Chloroplast contains its own small genome of 120–217 kb in size and 110-130 genes, consists of a circular double-stranded DNA.**

❖ **The cp genome can be used to investigate molecular evolution and phylogenies.**

❖ **The cp genomes are maternally inherited, which is beneficial in genetic engineering.**

Whole genome sequencing

❖ ***Chenopodium quinoa* (quinoa) is a highly nutritious grain identified as an important crop** Jarvis 2017 Nature. ; 542(7641):307-312. **The genome of Chenopodium quinoa.**

8.3 Advantages of NGS

- ❑NGS provides high taxonomic resolution.
- ❑It detects hybridization and introgression.
- ❑It reveals new species diversity.
- ❑It integrates with AI and computational tools.
- ❑Genome-level data improve accuracy.
- ❑Large datasets can be analyzed efficiently.
- ❑NGS enables predictive taxonomy.
- ❑Data generation is rapid.
- ❑It supports large-scale biodiversity studies.
- ❑NGS shifts taxonomy toward computational science.

9. Integration of Classical and Molecular Approaches

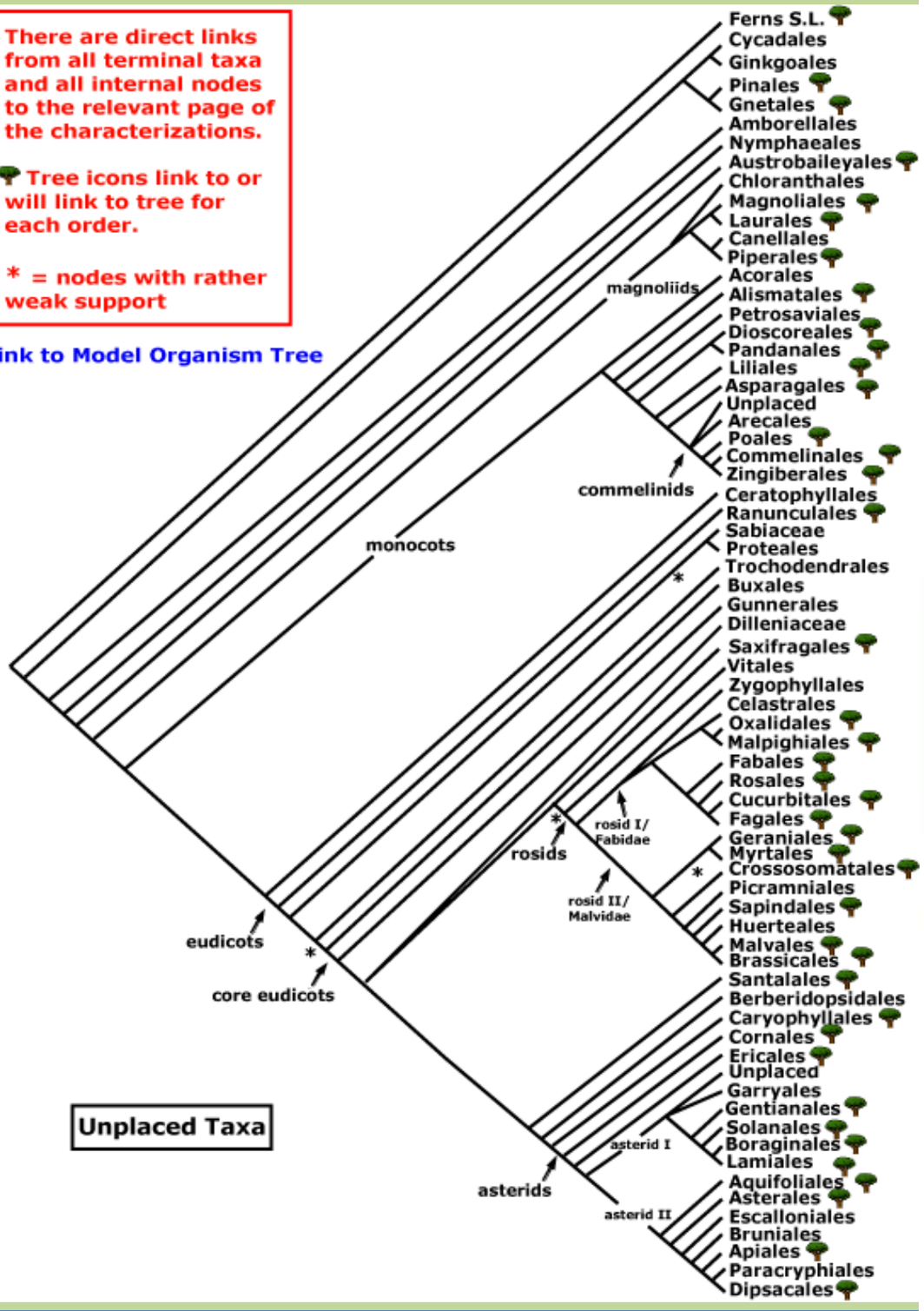
- ❑Integrative taxonomy combines multiple data sources.
- ❑No single method resolves all taxonomic problems.
- ❑Morphology provides ecological context.
- ❑Cytology reveals chromosomal evolution.
- ❑ Biochemistry supports chemical differentiation.
- ❑Molecular data reveal genetic relationships.
- ❑Combined evidence improves classification reliability.
- ❑Integrative methods improve species delimitation.
- ❑Classical and molecular methods complement each other.
- ❑Integration defines modern taxonomy.

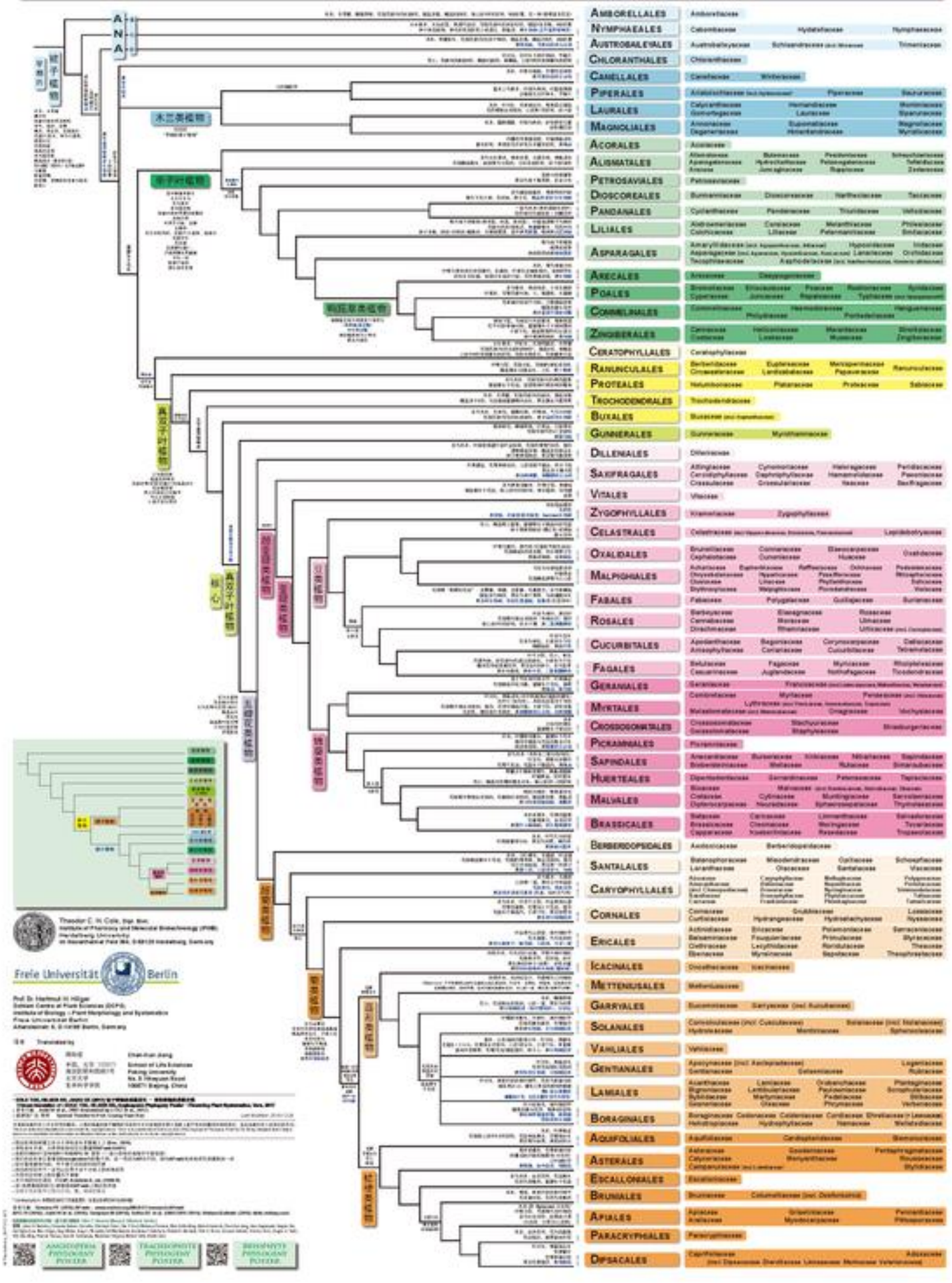
There are direct links from all terminal taxa and all internal nodes to the relevant page of the characterizations.

Tree icons link to or will link to tree for each order.

* = nodes with rather weak support

Link to Model Organism Tree





10. CONCLUSION

- Advanced experimental taxonomy integrates classical and molecular tools.
- Classical taxonomy remains the foundation of plant classification.
- Cytology and biochemistry strengthen interpretation.
- DNA sequencing improves objectivity and accuracy.
- DNA barcoding accelerates identification.
- NGS enables genome-scale analysis.
- Molecular tools reveal evolutionary history.
- Integrative taxonomy supports conservation biology.
- Modern taxonomy addresses biodiversity challenges.
- Advanced experimental taxonomy is essential for future biological research.

MODEL QUESTIONS

THANKS

ALL THE VERY BEST FOR

YOUR FINAL EXAM

PRACTICAL

جامعة الملك سعود
King Saud University

Practical Note Book

BOT 621
Advance Experimental Taxonomy

M. AJMAL ALI

Department of Botany and Microbiology,
College of Science, King Saud University,
Riyadh -11451, Saudi Arabia
alimohammad@ksu.edu.sa

Lab Activity No 01
Title of the Activity- DNA Extraction from plant tissue

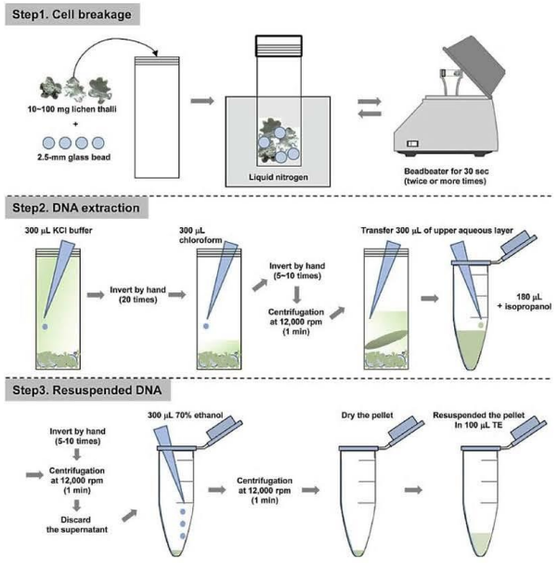
Learning Objectives:
- Understand the process of extracting DNA from plant tissue.
- Learn about the role of different reagents in DNA extraction.
- Observe the physical appearance of DNA.

Aim:
To extract and observe DNA from plant tissue using a simple extraction method.

Apparatus & Materials:
- Fresh plant tissue (e.g., banana, spinach, or strawberry)
- Mortar and pestle
- 10% dishwashing liquid or shampoo (detergent solution)
- 5% salt solution (NaCl)
- Ice-cold ethanol or isopropanol
- Distilled water
- Beaker (100 mL)
- Glass rod or toothpick
- Filter paper and funnel
- Test tube

Theory:
DNA (deoxyribonucleic acid) is a genetic material present in all living cells. It can be extracted by breaking open the cells, dissolving the cell membranes, and precipitating the DNA using alcohol. The detergent helps in breaking down the lipid membranes, while the salt stabilizes the DNA molecules. Alcohol is used to precipitate the DNA as it is insoluble in ethanol.

Diagram:



Procedure:
1. Take fresh plant tissue (about 10 g) and grind it with a small amount of saltwater using a mortar and pestle to break open the cells.
2. Transfer the crushed tissue to a beaker and add 10 mL of detergent solution. Stir gently for 5–10 minutes to break the cell membranes.
3. Filter the mixture using filter paper and a funnel to remove solid debris. Collect the filtrate in a test tube.
4. Slowly add an equal volume of ice-cold ethanol along the sides of the test tube without mixing.
5. Allow the tube to stand for a few minutes. DNA will appear as white, stringy precipitate at the interface of the alcohol and filtrate.
6. Use a glass rod or toothpick to spool out and observe the DNA.

Observations Table:

Step	Observation
Grinding plant tissue	Mixture becomes thick and greenish
Adding detergent	Solution becomes frothy and slightly clearer
Filtering	Clear liquid is obtained
Adding ethanol	White, thread-like DNA appears

Result:
DNA was successfully extracted from plant tissue and appeared as a white, stringy precipitate.

Conclusion:
The experiment demonstrates that DNA can be isolated from plant cells using simple chemical treatments. The detergent breaks cell membranes, salt stabilizes DNA, and alcohol helps in precipitation.

Precautions:
- Use fresh plant tissue for better results.
- Do not vigorously shake after adding ethanol to avoid breaking DNA strands.
- Use ice-cold ethanol for better precipitation.
- Handle chemicals carefully to avoid spillage.

Lab Activity No 02
Title of the Activity- PCR Primer and Polymerase Chain Reaction (PCR)

Learning Objectives:
- Understand the principles of Polymerase Chain Reaction (PCR).
- Learn the function and design of primers in PCR.
- Perform PCR and analyze the amplification of DNA.

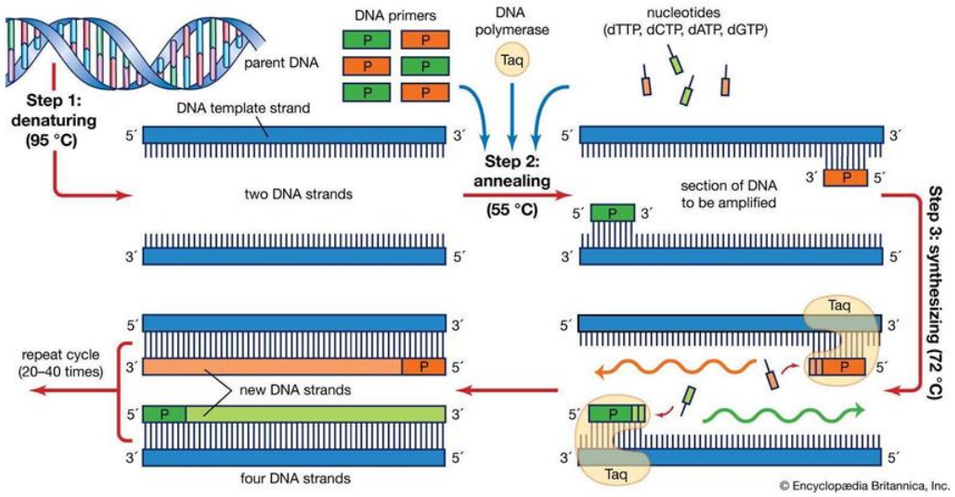
Aim:
To amplify a specific DNA sequence using Polymerase Chain Reaction (PCR).

Apparatus & Materials:
- DNA template (target DNA sample)
- Forward and reverse primers

- Taq DNA polymerase
- dNTPs (deoxynucleotide triphosphates)
- PCR buffer with Mg^{2+} ions
- PCR tubes
- Thermal cycler (PCR machine)
- Micropipettes and tips
- Agarose gel and electrophoresis apparatus (for analysis)
- UV transilluminator

Theory:
Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify a specific DNA sequence. It involves repeated cycles of denaturation, annealing, and extension. Primers are short DNA sequences that define the region to be amplified. Taq polymerase synthesizes new DNA strands using the template. The exponential amplification of DNA makes PCR an essential tool in genetics, forensic science, medical diagnostics, and research.

Diagram:



Procedure:

1. **Preparation of PCR Master Mix:**
   - In a PCR tube, mix the following components:
     - 10–50 ng of template DNA
     - 0.5–1 µM of forward and reverse primers
     - 200 µM of dNTPs
     - 1X PCR buffer (with Mg^{2+})
     - 1–2 U of Taq DNA polymerase
     - Adjust the final volume with nuclease-free water.
2. **Placing the Tube in the Thermal Cycler:**
   - Set up the PCR conditions as follows:

Step	Temperature	Time	Function
Initial Denaturation	94-98°C	2-5 min	Breaks double-stranded DNA into single strands
Denaturation	94-98°C	30 sec	Separates DNA strands
Annealing	50-65°C	30 sec	Primers bind to complementary sequences
Extension	72°C	30-60 sec	DNA polymerase extends the new strand
Final Extension	72°C	5-10 min	Ensures complete DNA synthesis
Hold	4°C	Indefinite	Stores the PCR product

- Repeat **denaturation, annealing, and extension** steps for **25-40 cycles**.

3. **Agarose Gel Electrophoresis (for visualization):**
   - Prepare a **1-2% agarose gel** and load the PCR product.
   - Run electrophoresis at **100V for 30 minutes**.
   - Visualize DNA bands under a **UV transilluminator**.

Observations Table:

Step	Observation
Sample loaded in PCR machine	No visible change
Post-PCR (before gel electrophoresis)	Clear liquid in the tube
After gel electrophoresis	DNA bands visible under UV light

Result:
A distinct DNA band corresponding to the amplified target sequence is observed on the agarose gel, confirming successful PCR amplification.

Conclusion:
The experiment successfully demonstrates the amplification of a specific DNA sequence using PCR. The results confirm the efficiency of primers in targeting the desired DNA region, and the process can be applied in various molecular biology studies.

Precautions:

- Use **sterile** micropipette tips and PCR tubes to prevent contamination.
- Keep **Taq polymerase and dNTPs on ice** to maintain enzyme stability.
- Accurately set the **thermal cycler parameters** for optimal amplification.
- Use **ice-cold ethanol or isopropanol** for DNA precipitation if required.
- Properly handle the UV transilluminator to avoid exposure.

Lab Activity No 03
Title of the Activity- Gel Electrophoresis for DNA Quality

Learning Objectives:

- Understand the principle of gel electrophoresis and its role in DNA analysis.
- Learn how to assess DNA quality based on band patterns.
- Gain hands-on experience in preparing and running an agarose gel electrophoresis experiment.

Aim:
To analyze the quality of DNA using agarose gel electrophoresis by observing the integrity, purity, and size of DNA fragments.

Apparatus & Materials:

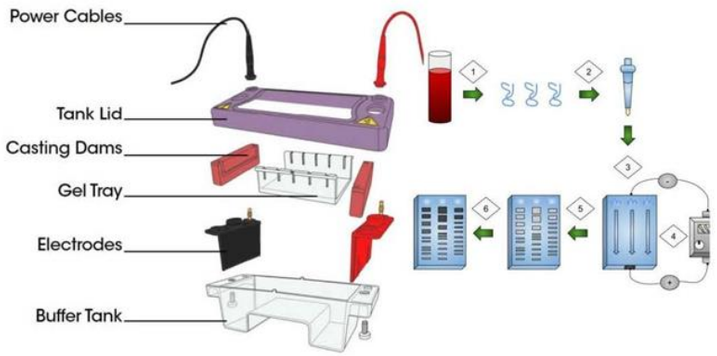
- DNA sample (extracted or PCR product)
- Agarose powder
- 1X TAE (Tris-Acetate-EDTA) or TBE (Tris-Borate-EDTA) buffer
- Ethidium bromide (EtBr) or SYBR Green (for DNA staining)

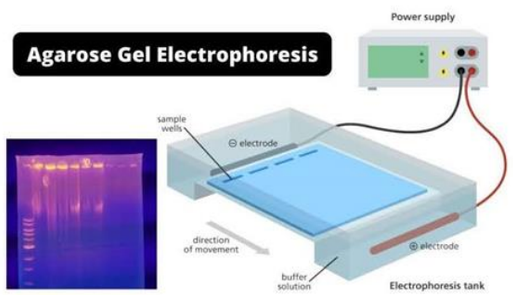
- Loading dye (e.g., bromophenol blue)
- DNA ladder (molecular weight marker)
- Gel casting tray and comb
- Electrophoresis chamber and power supply
- Micropipettes and tips
- UV transilluminator or gel documentation system

Theory:
Gel electrophoresis is a technique used to separate and analyze DNA fragments based on their size. DNA molecules are negatively charged due to their phosphate backbone and migrate towards the positive electrode when an electric field is applied.

- **Agarose Gel Concentration:** Affects resolution; lower % gels (0.8-1%) separate larger fragments, while higher % gels (2-3%) separate smaller fragments.
- **DNA Migration:** Smaller DNA fragments move faster and farther, while larger fragments move slower.
- **Staining:** DNA is visualized under UV light after binding with EtBr or SYBR Green.
- **DNA Quality Indicators:**
  - Intact DNA appears as a high-molecular-weight band.
  - Degraded DNA appears as a smear.
  - Contaminated DNA may show additional unexpected bands.

Diagram:





Procedure:

1. **Prepare the Gel:**
   - Dissolve **0.8-2% agarose** in **1X TAE/TBE buffer** by heating.
   - Cool slightly, add **EtBr/SYBR Green**, and pour into a gel tray with a comb.
   - Allow the gel to solidify (20-30 minutes).
2. **Prepare DNA Samples:**
   - Mix **5 µL of DNA sample** with **1 µL of loading dye**.
   - Prepare a **DNA ladder** in a similar way.
3. **Load the Gel:**
   - Place the solidified gel in the **electrophoresis chamber** and cover it with **1X TAE/TBE buffer**.
   - Load the **DNA samples and ladder** into separate wells using a micropipette.
4. **Run the Electrophoresis:**
   - Connect the electrodes (negative at the well side, positive at the opposite end).
   - Set voltage to **80-120V** and run for **30-45 minutes** (depending on gel size).
5. **Visualize DNA:**
   - Place the gel on a **UV transilluminator** or **gel documentation system**.
   - Capture an image to analyze the bands.

Observations Table:

Sample	Observation under UV Light	Interpretation
High-quality DNA	Single, sharp, high-molecular-weight band	Good integrity
Degraded DNA	Smear instead of distinct bands	DNA degradation
Contaminated DNA	Additional unexpected bands	RNA/protein contamination
DNA ladder	Clear bands at expected sizes	Gel running properly

Result:
Based on the gel electrophoresis results, the DNA quality was assessed. High-quality DNA appeared as intact bands, while degraded or contaminated DNA showed smearing or extra bands.

Conclusion:
Gel electrophoresis is an effective method for evaluating DNA quality. The integrity and purity of DNA can be determined by analyzing band patterns. Proper DNA extraction and storage methods help in obtaining high-quality DNA for further experiments.

Precautions:

- Use gloves and goggles when handling **EtBr** or **SYBR Green** (carcinogenic substances).
- Ensure the gel is properly set before loading samples to prevent leakage.
- Load samples carefully to **avoid cross-contamination** between wells.
- Do not run electrophoresis at excessively high voltage, as it may cause **DNA degradation**.
- Dispose of used gels and chemicals **as per lab safety protocols**.

Lab Activity No 04
Title of the Activity- Sanger Sequencing

Learning Objectives:

- Understand the principle of Sanger sequencing and its role in DNA sequencing.
- Learn how chain termination using dideoxynucleotides (ddNTPs) helps determine DNA sequences.
- Gain knowledge of the components, procedure, and interpretation of Sanger sequencing results.

Aim:

To determine the nucleotide sequence of a given DNA sample using the Sanger sequencing method.

Apparatus & Materials:

- **DNA Template:** The single-stranded DNA to be sequenced.
- **DNA Polymerase:** Taq or another thermostable enzyme for DNA synthesis.
- **Primers:** Short oligonucleotide sequences that initiate DNA synthesis.
- **dNTPs (Deoxynucleotide Triphosphates):** Standard nucleotides (A, T, G, C) for chain elongation.
- **ddNTPs (Dideoxynucleotide Triphosphates):** Modified nucleotides that terminate DNA synthesis.
- **PCR Tubes:** To set up the sequencing reactions.
- **Thermal Cycler:** For amplification and chain termination reactions.
- **Capillary Electrophoresis System or Polyacrylamide Gel:** For fragment separation.
- **Fluorescent Labeling Dye or Radioactive Labeling:** For visualization of the DNA sequence.
- **Computational Software:** For automated sequence analysis.

Theory:

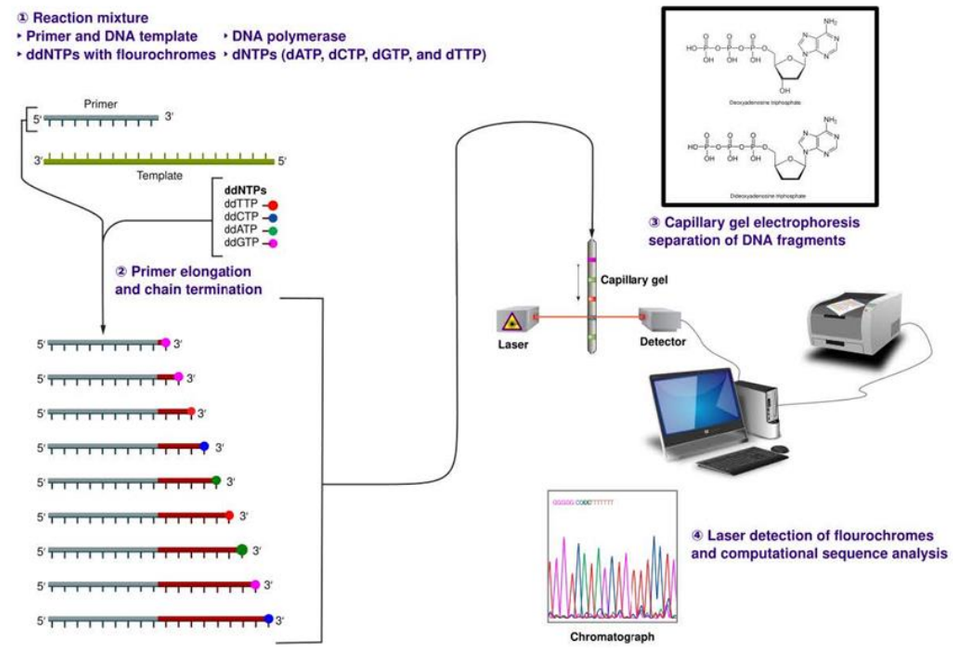
Sanger sequencing, also called the **chain termination method**, is a DNA sequencing technique developed by Frederick Sanger. The process involves DNA synthesis using a polymerase enzyme in the presence of both **normal dNTPs** (which extend the DNA strand) and **ddNTPs** (which cause termination). Since ddNTPs lack a 3'-OH group, their incorporation results in **premature termination** of strand elongation.

By running separate reactions for each nucleotide (A, T, G, C) or using **fluorescently labeled ddNTPs**, the resulting DNA fragments of different lengths can be separated via **capillary electrophoresis or gel electrophoresis** to determine the sequence.

Diagram:

Procedure:

1. **Reaction Setup:**
   - Prepare four separate reaction tubes, each containing:
     - Template DNA
     - DNA polymerase
     - Primers
     - dNTPs
     - A small amount of one of the four **ddNTPs** (ddATP, ddTTP, ddGTP, or ddCTP)



2. **DNA Amplification via PCR:**
   - Run a thermal cycling program:
     - **Denaturation (95°C, 30 sec):** DNA strands separate.
     - **Annealing (50-60°C, 30 sec):** Primers bind to the template.
     - **Extension (72°C, 1 min):** DNA polymerase incorporates nucleotides until a **ddNTP** is added, terminating the strand.
     - Repeat for 25-30 cycles.
3. **Fragment Separation:**
   - Load the amplified DNA into a **capillary electrophoresis system** or **polyacrylamide gel electrophoresis** to separate fragments by size.
4. **Detection and Analysis:**
   - If using **radioactive or fluorescently labeled ddNTPs**, detect fragment sizes using **autoradiography** (for radioactive sequencing) or **laser detection in a sequencer** (for fluorescent sequencing).
   - The banding pattern in electrophoresis corresponds to the **complementary DNA sequence**.

Observations Table:

Step	Observation	Interpretation
DNA denaturation	Single-stranded DNA formation	Ready for primer binding
Primer annealing	Primers attach to the template	DNA polymerase can initiate synthesis
DNA extension with dNTPs & ddNTPs	DNA fragments of varying lengths	Chain termination occurring
Electrophoresis result	DNA bands or peaks of	DNA sequence can be read

	different sizes	

Result:
The sequence of the given DNA template is determined based on the band pattern or **fluorescent peak readings** obtained after electrophoresis. The **shortest fragments represent the start of the sequence**, and the **longest fragments represent the end**.

Conclusion:
Sanger sequencing is a reliable method for determining DNA sequences. It uses **ddNTP-mediated termination**, and by separating the fragments based on size, the **order of nucleotides in the DNA strand can be deduced**. The method is widely used in **genetic analysis, mutation detection, and genome sequencing**.

Precautions:

- Ensure **accurate pipetting** of dNTPs and ddNTPs to prevent sequencing errors.
- Maintain **sterile conditions** to prevent contamination.
- Use **high-purity DNA** for accurate sequencing results.
- Optimize **PCR cycling conditions** to ensure proper amplification.
- Handle **fluorescent dyes and radioactive materials** with proper safety measures.

Lab Activity No 05
Title of the Activity- Retrieval of DNA sequence data from NCBI

Learning Objectives:

- Understand how to access and retrieve DNA sequence data from the **National Center for Biotechnology Information (NCBI)** database.
- Learn the use of **GenBank, FASTA format, and BLAST (Basic Local Alignment Search Tool)** for sequence analysis.
- Gain experience in navigating the **NCBI website** for DNA sequence retrieval.

Aim:
To retrieve and analyze a DNA sequence from the **NCBI GenBank database** using an accession number or a keyword search.

Apparatus & Materials:

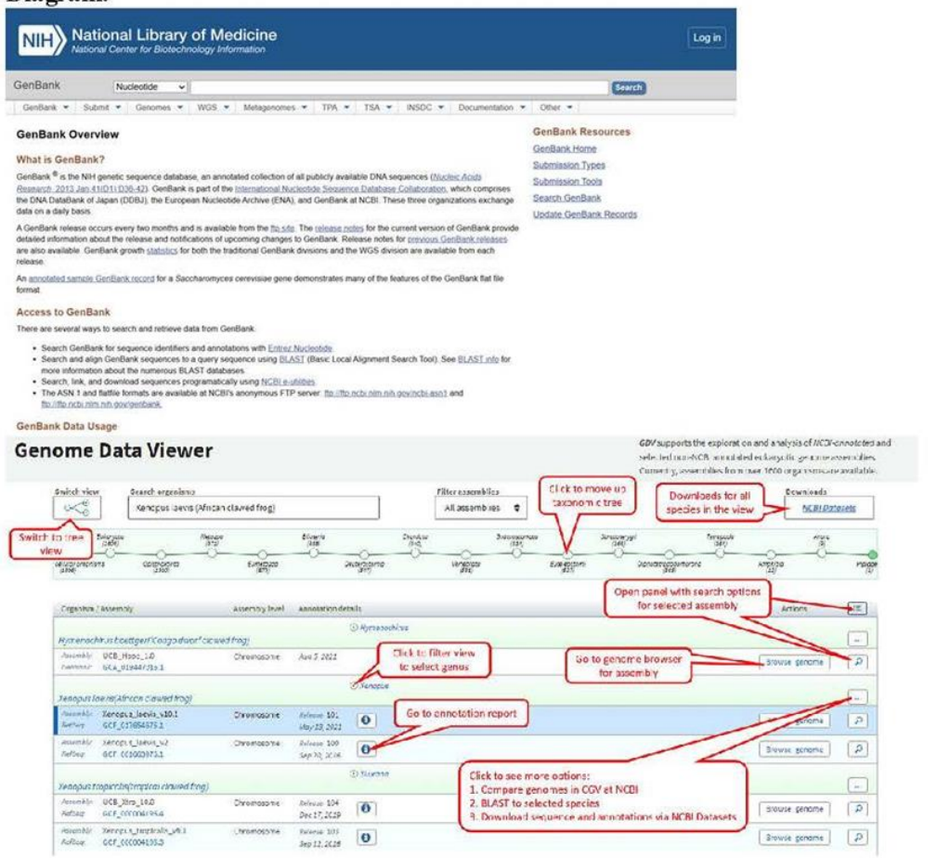
- **Computer with Internet access**
- **NCBI website (https://www.ncbi.nlm.nih.gov/)**
- **Web browser (Google Chrome, Mozilla Firefox, etc.)**
- **Accession number or search term**
- **Text editor (Notepad, MS Word, or BioEdit for FASTA files)**

Theory:
The **National Center for Biotechnology Information (NCBI)** provides a public database containing DNA, RNA, and protein sequences. The key features include:

1. **GenBank Database:** A comprehensive collection of publicly available genetic sequences.
2. **FASTA Format:** A standard format for representing DNA and protein sequences.
3. **Accession Number:** A unique identifier for each sequence entry in GenBank.
4. **BLAST (Basic Local Alignment Search Tool):** A tool to compare a query sequence with known sequences in the database.

NCBI allows users to **search, retrieve, and analyze** DNA sequences, making it a vital resource for bioinformatics research.

Diagram:



Procedure:
Step 1: Access NCBI Website

- Open a web browser and go to NCBI GenBank.

Step 2: Search for a DNA Sequence

- In the search bar, enter a **gene name, organism name, or an accession number** (e.g., **NM_001301717.2** for the human BRCA1 gene).
- Click **Search** to retrieve matching records.

Step 3: Select a Sequence

- Click on the desired entry from the search results.
- The sequence information, including **source organism, gene location, coding region, and references**, will be displayed.

Step 4: Retrieve the DNA Sequence

- Scroll down to find the **FASTA format** option.
- Click **"FASTA"** to display the sequence in a text format.
- Copy the sequence for further analysis.

Step 5: Download the Sequence File

- Click **"Send to" → "File" → Select FASTA format → Download**.

Step 6: Analyze the Sequence using BLAST (Optional)

- Go to the **BLAST tool** (NCBI BLAST).
- Paste the copied sequence into the input box.
- Click **"BLAST"** to find similar sequences in the database.

Observations Table:

Step	Observation	Interpretation
Search results	Multiple sequence entries appear	NCBI contains many related sequences
Selection of entry	Detailed sequence and metadata displayed	Correct sequence identified
FASTA sequence retrieved	A text file containing A, T, G, C bases	DNA sequence successfully extracted
BLAST result (if used)	Similar sequences with % identity appear	Sequence similarity analysis completed

Result:

A DNA sequence was successfully retrieved from **NCBI GenBank**, displayed in **FASTA format**, and stored for further analysis.

Conclusion:

The NCBI database is an essential tool for retrieving and analyzing DNA sequences. By using search queries and accession numbers, researchers can access genetic information for various applications in **bioinformatics, evolutionary studies, and genetic research**.

Precautions:

- Ensure correct spelling and scientific names while searching.
- Use **official accession numbers** for accurate sequence retrieval.
- Check the **date and version** of the sequence to get the latest update.
- Verify the source organism before using the data in further analysis.
- Avoid **editing** the retrieved sequence accidentally before saving.

Lab Activity No 06
Title of the Activity- Multiple Sequence Alignment (MSA) Using ClustalX

Learning Objectives:

- Understand the principle and importance of Multiple Sequence Alignment (MSA).
- Learn how to perform MSA using **ClustalX**, a widely used bioinformatics tool.
- Interpret sequence alignment results for evolutionary and functional analysis.

.

Aim:

To perform **Multiple Sequence Alignment (MSA)** of DNA or protein sequences using **ClustalX** and analyze sequence conservation and evolutionary relationships.

Apparatus & Materials:

- **Computer with Windows/Linux/Mac OS**
- **ClustalX software (Download from: EBI Website)**
- **FASTA format sequence files**
- **Internet access (optional, for database searches and downloads)**

Theory:

Multiple Sequence Alignment (MSA) is a method used to align three or more biological sequences (DNA, RNA, or proteins) to identify regions of similarity. These similarities may indicate **evolutionary relationships, functional regions, or conserved motifs**.

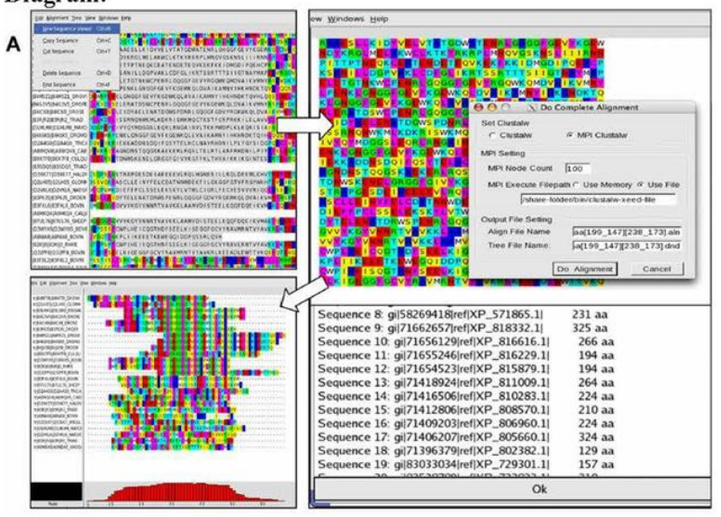
ClustalX is a graphical version of the Clustal algorithm that performs MSA using:

1. **Progressive Alignment Method** – Sequences are aligned stepwise based on similarity scores.
2. **Scoring Matrices** – Used to evaluate alignment quality (e.g., BLOSUM for proteins, PAM for DNA).
3. **Phylogenetic Tree Construction** – Helps understand evolutionary relationships.

Applications of MSA:

- Identifying **conserved domains** in genes and proteins.
- Constructing **phylogenetic trees** for evolutionary studies.
- Comparing **functional sites** in homologous sequences.

Diagram:



Procedure:

Step 1: Install ClustalX

- Download **ClustalX** from the EBI website and install it on your system.

Step 2: Prepare the Input Sequences

- Obtain DNA/protein sequences in **FASTA format**.
- Save all sequences in a **single text file** with .fasta or .txt extension.

Step 3: Load Sequences into ClustalX

- Open **ClustalX** and click **File → Load Sequences**.
- Select the prepared **FASTA file** and open it.

Step 4: Perform Multiple Sequence Alignment

- Click **Alignment → Do Complete Alignment**.
- Adjust parameters if needed (gap penalties, scoring matrix, etc.).
- Wait for the software to process the alignment.

Step 5: View and Analyze the Alignment

- The aligned sequences will be displayed with **conserved regions marked**.
- Save the results by clicking **File → Save Alignment As**.

Step 6: Construct a Phylogenetic Tree (Optional)

- Click **Trees → Draw Tree** to generate a phylogenetic tree.
- Save the tree as an image or text file for analysis.

Observations Table:

Step	Observation	Interpretation
Loading sequences	All sequences appear in ClustalX	Proper sequence input
Alignment process	Aligned sequences with gaps appear	Successful MSA
Conserved regions	Identified by asterisks (*) in the alignment	Highly conserved sequences
Phylogenetic tree	Dendrogram/tree displayed	Evolutionary relationships inferred

Result:

The given DNA/protein sequences were successfully aligned using **ClustalX**, and **conserved regions** were identified. The **phylogenetic tree** showed relationships between sequences.

Conclusion:

ClustalX is an effective tool for performing **Multiple Sequence Alignment (MSA)**, revealing conserved regions and evolutionary relationships among DNA/protein sequences. This alignment can be used for **functional analysis, comparative genomics, and phylogenetics**.

Precautions:

- Ensure **FASTA format** is correctly maintained to avoid errors.
- Choose the **appropriate scoring matrix** (BLOSUM for proteins, PAM for DNA).
- Set **gap penalties correctly** to prevent misalignment.
- Verify **sequence quality** before running MSA.
- Save results **frequently** to prevent data loss.

Lab Activity No 07
Title of the Activity- NJ Phylogenetic Tree Construction Using MEGA

Learning Objectives:

- Understand the **Neighbor-Joining (NJ) method** for constructing phylogenetic trees.

- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis)** software for tree construction.
- Analyze the evolutionary relationships between different DNA/protein sequences.

Aim:

To construct a **Neighbor-Joining (NJ) phylogenetic tree** using **MEGA** software to study the evolutionary relationships between sequences.

Apparatus & Materials:

- **Computer with Windows/Linux/Mac OS**
- **MEGA software (Download from: https://www.megasoftware.net/)**
- **FASTA format sequence file**
- **Internet access (optional, for sequence retrieval from NCBI)**

Theory:

A **phylogenetic tree** represents the evolutionary relationships among species or genes. The **Neighbor-Joining (NJ) method**, developed by Saitou and Nei (1987), is a widely used algorithm for tree construction.

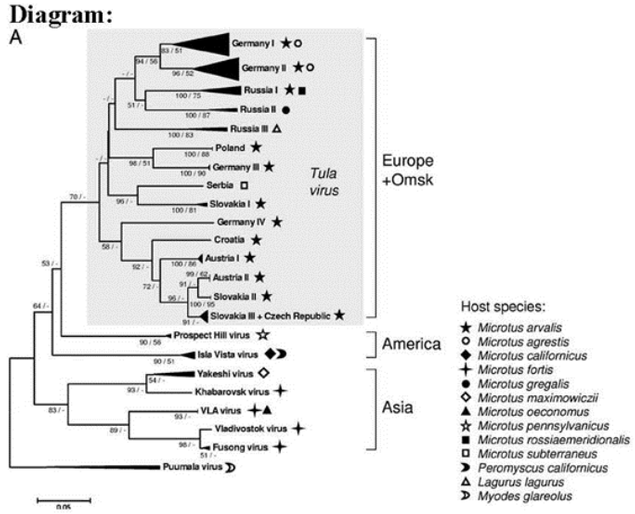
Principle of Neighbor-Joining (NJ) Method:

1. **Calculate a distance matrix** based on sequence similarity.
2. **Iteratively join the two closest taxa** (sequences) into a common ancestor.
3. **Reduce the distance matrix** and repeat until a single tree is formed.

Applications of NJ Trees:

- Understanding **evolutionary relationships** among species.
- Identifying **common ancestors** and divergence points.
- Analyzing **genetic similarities** in different populations.

Diagram:



Procedure:

Step 1: Install and Open MEGA Software

- Download and install **MEGA** from the official website.
- Open the software and select **Phylogeny → Construct/Analyze Phylogenetic Tree**.

Step 2: Prepare the Input Sequences

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Save the alignment as a **MEGA (.meg) file**.

Step 3: Create a Distance Matrix

- Click **Phylogeny → Compute Pairwise Distance**.
- Choose an appropriate substitution model (e.g., **Kimura 2-parameter** for DNA).

Step 4: Construct the NJ Phylogenetic Tree

- Go to **Phylogeny → Construct NJ Tree**.
- Select the **NJ method** and choose the substitution model.
- Click **OK** to generate the tree.

Step 5: View and Interpret the Tree

- The phylogenetic tree will be displayed with **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences displayed in MEGA	Ready for tree construction
Distance matrix calculation	Pairwise distances generated	Sequence relationships quantified
NJ tree construction	Tree structure appears with branches	Evolutionary connections established
Bootstrap values	Support values displayed on branches	Confidence level of relationships

Result:
A **Neighbor-Joining (NJ) phylogenetic tree** was successfully constructed using **MEGA software**, revealing the **evolutionary relationships** among the input sequences.

Conclusion:
The NJ method in MEGA efficiently constructs **phylogenetic trees** based on sequence similarity. It is widely used in **molecular evolution, comparative genomics, and phylogenetics** to study the relationships among species or genes.

Precautions:

- Ensure sequences are **properly aligned** before tree construction.
- Choose the **appropriate substitution model** for accurate distance calculations.
- Use **bootstrap analysis** (≥1000 replications) for statistical reliability.
- Verify sequence quality to avoid misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 08
Title of the Activity- MP Phylogenetic Tree Construction Using MEGA

Learning Objectives:

- Understand the **Maximum Parsimony (MP) method** for constructing phylogenetic trees.
- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis) software** for MP tree construction.

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Save the alignment as a **MEGA (.meg) file**.

Step 3: Create a Distance Matrix

- Click **Phylogeny → Compute Pairwise Distance**.
- Choose an appropriate substitution model (e.g., **Kimura 2-parameter** for DNA).

Step 4: Construct the NJ Phylogenetic Tree

- Go to **Phylogeny → Construct NJ Tree**.
- Select the **NJ method** and choose the substitution model.
- Click **OK** to generate the tree.

Step 5: View and Interpret the Tree

- The phylogenetic tree will be displayed with **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences displayed in MEGA	Ready for tree construction
Distance matrix calculation	Pairwise distances generated	Sequence relationships quantified
NJ tree construction	Tree structure appears with branches	Evolutionary connections established
Bootstrap values	Support values displayed on branches	Confidence level of relationships

Result:
A **Neighbor-Joining (NJ) phylogenetic tree** was successfully constructed using **MEGA software**, revealing the **evolutionary relationships** among the input sequences.

Conclusion:
The NJ method in MEGA efficiently constructs **phylogenetic trees** based on sequence similarity. It is widely used in **molecular evolution, comparative genomics, and phylogenetics** to study the relationships among species or genes.

Precautions:

- Ensure sequences are **properly aligned** before tree construction.
- Choose the **appropriate substitution model** for accurate distance calculations.
- Use **bootstrap analysis** (≥1000 replications) for statistical reliability.
- Verify sequence quality to avoid misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 08
Title of the Activity- MP Phylogenetic Tree Construction Using MEGA

Learning Objectives:

- Understand the **Maximum Parsimony (MP) method** for constructing phylogenetic trees.
- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis) software** for MP tree construction.

- Analyze **evolutionary relationships** among DNA/protein sequences using the MP approach.

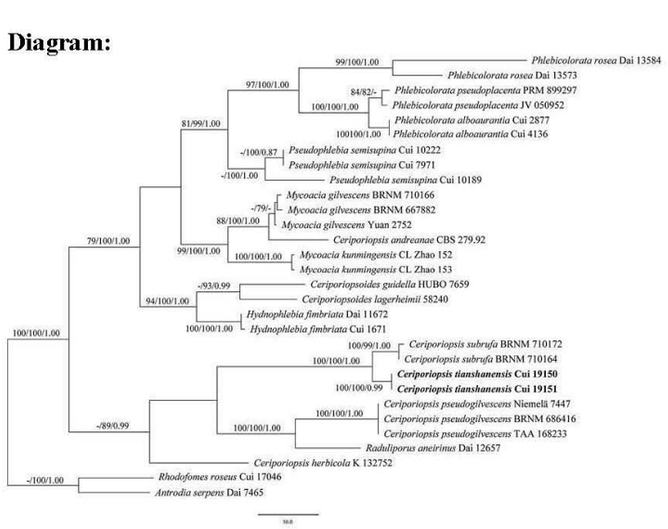
Aim:
To construct a **Maximum Parsimony (MP) phylogenetic tree** using **MEGA software** and analyze sequence relationships.

Apparatus & Materials:
- **Computer with Windows/Linux/Mac OS**
- **MEGA software (Download from: https://www.megasoftware.net/)**
- **FASTA format sequence file**
- **Internet access (optional, for sequence retrieval from NCBI)**

Theory:
A **phylogenetic tree** visually represents evolutionary relationships among species or genes. The **Maximum Parsimony (MP) method** seeks to find the simplest tree (with the fewest evolutionary changes) that explains the given sequence data.
Principles of Maximum Parsimony (MP):
1. **Minimizes total evolutionary changes** (mutations) in the tree.
2. **Uses character states (nucleotide/amino acid positions)** to determine the most likely ancestral relationships.
3. **Ideal for closely related sequences** but computationally intensive for large datasets.

Applications of MP Trees:
- Understanding **evolutionary history** of species or genes.
- Identifying **ancestral traits and lineage divergence**.
- Studying genetic variation and **conserved regions** in sequences.

Diagram:



Procedure:
Step 1: Install and Open MEGA Software
- Download and install **MEGA** from the official website.
- Open the software and select **Phylogeny → Construct/Analyze Phylogenetic Tree**.

Step 2: Load and Align Sequences

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Perform **Multiple Sequence Alignment (MSA)** and save as a **MEGA (.meg) file**.

Step 3: Select Maximum Parsimony Method
- Click **Phylogeny → Construct Maximum Parsimony Tree**.
- Choose **MP search criteria**, such as **Close-Neighbor-Interchange (CNI)** for branch swapping.
- Select the **substitution model** (e.g., Parsimony for nucleotides or proteins).

Step 4: Perform Bootstrap Analysis (Optional for Reliability)
- In the MP settings, enable **Bootstrap Test (≥1000 replicates)**.
- Higher bootstrap values indicate **stronger support** for evolutionary relationships.

Step 5: View and Interpret the Tree
- The MP tree will display **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences loaded into MEGA	Ready for tree construction
MP tree calculation	Tree structure appears with branches	Evolutionary relationships inferred
Bootstrap values	Support values displayed on branches	Confidence in evolutionary history

Result:
A **Maximum Parsimony (MP) phylogenetic tree** was successfully constructed using **MEGA software**, illustrating the **evolutionary relationships** among the input sequences.

Conclusion:
The **Maximum Parsimony (MP) method** provides a simple and effective way to infer evolutionary relationships. It is particularly useful for closely related species but may be computationally challenging for large datasets.

Precautions:
- Ensure sequences are **properly aligned** before tree construction.
- Use **bootstrap analysis** to confirm tree reliability.
- Select appropriate **parsimony criteria (CNI, SPR, TBR)** based on dataset size.
- Verify sequence quality to prevent misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 09
Title of the Activity- ML Phylogenetic Tree Construction Using MEGA

Learning Objectives:
- Understand the **Maximum Likelihood (ML) method** for phylogenetic tree construction.
- Learn how to use **MEGA (Molecular Evolutionary Genetics Analysis)** software to build ML trees.
- Analyze **evolutionary relationships** between DNA/protein sequences using the ML approach.

Aim:
To construct a **Maximum Likelihood (ML) phylogenetic tree** using **MEGA software** and interpret the evolutionary relationships among sequences.

Apparatus & Materials:
- **Computer with Windows/Linux/Mac OS**
- **MEGA software (Download from: https://www.megasoftware.net/)**
- **FASTA format sequence file**
- **Internet access (optional, for sequence retrieval from NCBI)**

Theory:
A **phylogenetic tree** is a diagram representing the evolutionary relationships among organisms or genes. The **Maximum Likelihood (ML) method** is a statistical approach that finds the **best tree** by **calculating probabilities of different evolutionary events**.
Principles of Maximum Likelihood (ML) Method:
1. Uses a **statistical model of evolution** (e.g., Jukes-Cantor, Kimura 2-parameter).
2. Evaluates multiple tree topologies to **find the most likely tree**.
3. Computes **branch lengths** based on **likelihood scores**.
4. Computationally intensive but **more accurate than NJ or MP**.

Applications of ML Trees:
- Studying **species evolution and genetic divergence**.
- Inferring **ancestral relationships** in complex datasets.
- Comparing **mutation rates and selection pressure** in genes.

Diagram:

Procedure:
Step 1: Install and Open MEGA Software
- Download and install **MEGA** from the official website.
- Open the software and select **Phylogeny → Construct/Analyze Phylogenetic Tree**.

Step 2: Load and Align Sequences

- Obtain DNA or protein sequences in **FASTA format**.
- Open **MEGA → Align → Edit/Build Alignment** to import sequences.
- Perform **Multiple Sequence Alignment (MSA)** and save as a **MEGA (.meg) file**.

Step 3: Select Maximum Likelihood Method
- Click **Phylogeny → Construct Maximum Likelihood Tree**.
- Choose an **evolutionary model** (e.g., **Kimura 2-parameter, JTT for proteins**).

Step 4: Perform Bootstrap Analysis (Optional for Reliability)
- In the ML settings, enable **Bootstrap Test (≥1000 replications)**.
- Higher bootstrap values indicate **stronger support** for evolutionary relationships.

Step 5: View and Interpret the Tree
- The ML tree will display **branch lengths and bootstrap values**.
- Save the tree by clicking **File → Export Tree as Image**.

Observations Table:

Step	Observation	Interpretation
Sequence alignment	Aligned sequences displayed in MEGA	Ready for tree construction
ML tree calculation	Tree structure appears with branches	Evolutionary relationships inferred
Bootstrap values	Support values displayed on branches	Confidence in evolutionary history

Result:
A **Maximum Likelihood (ML) phylogenetic tree** was successfully constructed using **MEGA software**, revealing **evolutionary relationships** among the input sequences.

Conclusion:
The **Maximum Likelihood (ML) method** is a powerful approach for constructing accurate phylogenetic trees. It provides **statistically robust evolutionary relationships** based on sequence data.

Precautions:
- Ensure sequences are **properly aligned** before tree construction.
- Choose an **appropriate substitution model** for accurate distance calculations.
- Use **bootstrap analysis** to confirm tree reliability.
- Verify sequence quality to avoid misinterpretation.
- Save results and **export the tree image** for documentation.

Lab Activity No 10
Title of the Activity- Construction of chloroplast genome map using GeSeq

Learning Objectives:
- Understand the process of **chloroplast genome annotation** using **GeSeq (Gene Sequence Annotation Server)**.
- Learn how to use **GeSeq** to predict and visualize **chloroplast genome features** such as protein-coding genes, rRNAs, and tRNAs.
- Construct a **chloroplast genome map** and analyze its structure.

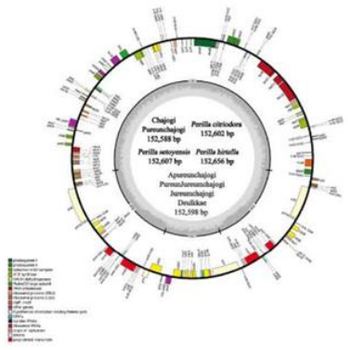
Aim:
To annotate and construct a **chloroplast genome map** using **GeSeq**, identifying genes and structural elements within the chloroplast genome.

Apparatus & Materials:
- **Computer with Internet access**
- **GeSeq (Available at: https://chlorobox.mpimp-golm.mpg.de/geseq.html)**
- **FASTA file of chloroplast genome sequence**
- **NCBI or other sequence repositories for reference genomes**

Theory:
The **chloroplast genome** is a circular, double-stranded DNA molecule found in plant cells, primarily responsible for **photosynthesis and other essential metabolic processes**. Annotation of the chloroplast genome is crucial for studying **gene functions, evolutionary relationships, and genetic engineering applications**.
What is GeSeq?
- **GeSeq (Gene Sequence Annotation Server)** is an online tool used for **annotating chloroplast genomes**.
- It identifies **protein-coding genes, rRNAs, tRNAs, and conserved regions** based on sequence comparison with reference databases.
- It also generates **graphical genome maps**, which are useful for **genome visualization and comparative genomics**.

Applications of Chloroplast Genome Annotation:
- Understanding **chloroplast gene functions**.
- Studying **plant phylogenetics and evolution**.
- Assisting in **genetic modification and synthetic biology**.
- Detecting **mutations and structural variations** in chloroplast genomes.

Diagram:



Procedure:
Step 1: Access GeSeq Online
- Open **GeSeq** by visiting: https://chlorobox.mpimp-golm.mpg.de/geseq.html.

Step 2: Upload the Chloroplast Genome Sequence
- Click **"Upload FASTA file"** and select the chloroplast genome sequence file.
- Alternatively, enter the **NCBI accession number** to fetch an existing sequence.

Step 3: Select Annotation Parameters
- Choose databases for comparison: **NCBI RefSeq, EMBL, or BLAST against existing chloroplast genomes**.
- Enable **tRNA and rRNA annotation** using **tRNAscan-SE and RNA-BLAST**.
- Set the **annotation output format** (e.g., **GFF, GenBank, or graphical genome map**).

Step 4: Run the Annotation Process
- Click **"Start Annotation"** and wait for the tool to process the genome.
- The process may take a few minutes, depending on genome size.

Step 5: View and Download Annotated Genome
- Check the **list of identified genes (protein-coding, tRNA, rRNA, and non-coding regions)**.
- Click **"Generate Genome Map"** to visualize the **circular chloroplast genome structure**.
- Download results in **GenBank format** for further analysis.

Observations Table:

Step	Observation	Interpretation
Genome upload	FASTA file successfully loaded	Ready for annotation
Annotation process	Genes, rRNAs, and tRNAs identified	Chloroplast genome elements detected
Genome map generation	Circular genome map displayed	Visualization of genome structure
Exported annotation	Gene list and sequence data available	Data ready for comparative analysis

Result:
A **chloroplast genome map** was successfully constructed using **GeSeq**, identifying key genetic elements such as **protein-coding genes, rRNAs, and tRNAs**.

Conclusion:
The **GeSeq tool** effectively annotates **chloroplast genomes** by comparing sequences with reference databases. This process provides a comprehensive view of **chloroplast gene organization**, useful for **phylogenetic studies, evolutionary biology, and genetic research**.

Precautions:
- Ensure the **input sequence is high quality** and in **FASTA format**.
- Select appropriate **reference databases** for accurate annotation.
- Verify **gene predictions** with other annotation tools if needed.
- Use **graphical visualization tools** to confirm gene positions.
- Save and backup **annotation results and genome maps** for further analysis

THANKS

ALL THE VERY BEST FOR

YOUR FINAL EXAM