

UC Flora e Vegetação

Flora and vegetation: from field to molecular Analysis

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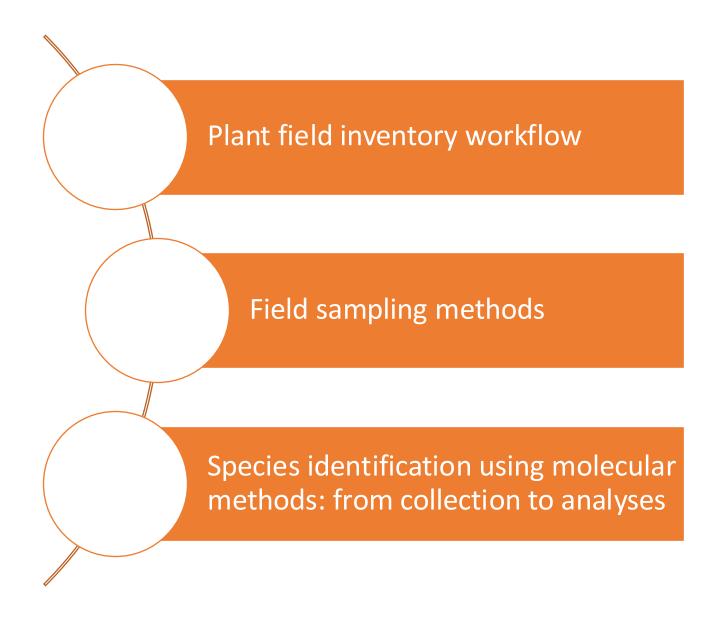


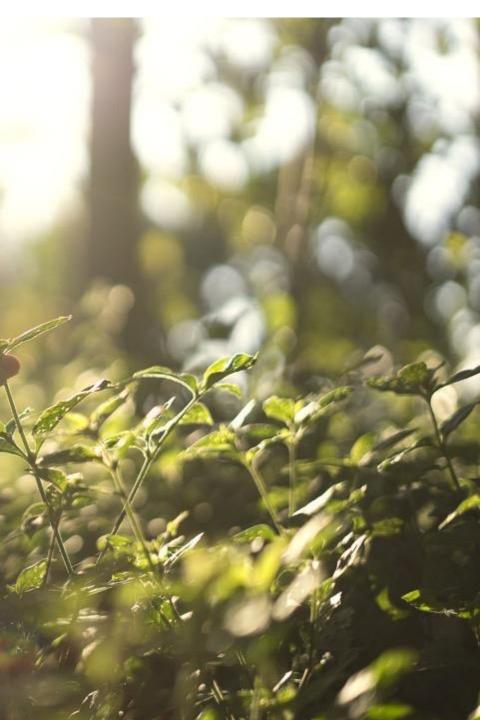






Outline





A **Field Plant Inventory** typically focuses on how to identify, catalog, and manage data about plant species in a specific natural or agricultural area. Here's an overview of what such a course would generally include:



Learn to identify native and invasive plant species.



Conduct accurate field surveys and inventories.



Use tools like GPS, plant ID guides, and data sheets or digital platforms.



Understand ecological relationships and vegetation types.



Apply data for land management, conservation, or research.

1. Introduction to Plant Taxonomy

Scientific names & classification

Binomial System of Nomenclature

- · Binomial (two names)
- Nomenclature (naming)
- All organisms have a scientific name consisting of a genus and a species
- · Names derived from Latin
- Guidelines set by the International Code of Botanical Nomenclature



Ash Ordinary

Scientific Names

A full "species" is comprised of the genus, species epithet and author name. For example, for our native salmonberry shrub:

Rubus spectabilis Pursh





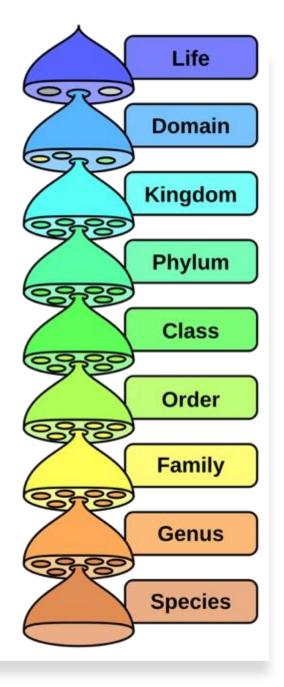
species epithet



A few common species epithets (typically derived from Latin or Greek):

- macrophylia (-um) = big-leaved
- heterophylla (-um) = different types of leaves
- sativa = cultivated
- · arvensis = from the fields
- pratensis = from the meadows
- oreganum = from Oregon
- · californicum = from California
- columbianum = from Columbia River area
- lewisii = named for explorer Meriweather Lewis
- menziesii = named for naturalist Archibald Menzies
- nootkaensis=named for Nookta Sound or the Nuu-chahnulth peoples

Note: Species epithet endings differ (-a, -um, -es, -is, -ium, etc.) depending on the gender of the genus name, as per Latin grammar rules.



Overall phylogeny – living land plants **Bryophytes** Mosses, liverworts, hornworts Lycophytes Clubmosses, etc. Ferns and Fern Allies Ferns, horsetails, moonworts, etc. **Gymnosperms** Conifers, pines, cycads and cedars, etc. Magnoliids Monocots Fabids Ranunculales Rosids Malvids Eudicots Caryophyllales **Ericales** Lamiids The treatment for flowering plants follows Campanulids the APG IV (2016) classification. Not all branches are shown.

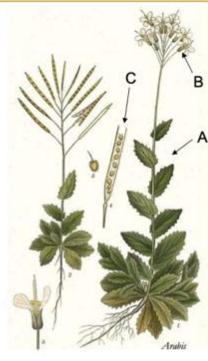
Plant family characteristics

Understanding plant family characteristics can be helpful for identifying plants, understanding their evolutionary relationships, and even for predicting their growth habits and potential pest problems.

Example

Brassicaceae s. str. MUSTARD FAMILY

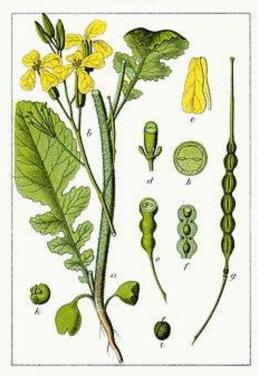
- Herbaceous
- With mustard oils
- Leaves simple, alternate (A), often lobed, with pinnate venation
- Leaf edge often dentate (A) or lobed
- Inflorescence a raceme
- Petals 4, not fused, forming a cross + from above (B), white, yellow, or pink
- Stamens 6 (4 longer, 2 shorter)
- Fruit a dry capsule with inner wall (silique; C)



Note: This family circumscription refers to Brassicaceae s. str. and does not include Capparaceae (capers) and Cleomaceae.

Examples: white mustard (Sinapis), garlic mustard (Alliaria), horseradish (Armoracia), cabbage, broccoli, brussels sprouts, kale, collards, rutabaga, canola, black mustard, turnip (Brassica), arugula (Diplotaxis, 'rustica' type), mouse-ear and thale cress (Arabidopsis), yellow rocket (Barbarea), radish (Raphanus), woad (Isatis), water cress (Nasturtium).

Tafel 40.



Gemeiner Hederich, Crucifera raphanistrum.

Raphanus raphanistrum



Sinapis alba

Use of dichotomous keys

• Dichotomous keys are valuable tools used to identify plants by guiding users through a series of paired choices or characteristics

AS TREE DICHOTOMOUS KEY Franching (2) N2 branching (4) impound leaves (3) imple leaves: Maple species (see a-c below) a. Leaf margins smooth, 5 lobes Sugar Maple (Acer saccharum) b. Leaf margins double-toothed, 3 to 5 lobes Red Maple (Acer rubrum) c. Leaf margins single-toothed, 3 to 5 lobes, lobes separated by deep, angular openings......Silver Maple (Acer saccharinum) 3. Three (rarely 5) leaflets...... Box Elder (Acer negundo) 3. Five to 11 leaflets: Ash species (see a-c below) a. 9 to 11 leaflets, leaflets do not have petiole...... Black Ash (Fraxinus nigra) b. 5 to 9 leaflets, leaflets have petiole, smile-shaped leaf scar extending up sides of new bud.......White Ash (Fraxinus americana) c. 7 to 9 leaflets, leaflets have petiole, leaf scar ends at base of new bud..... Green Ash (Fraxinus pennsylvanica) 4. Compound leaves (5) 4. Simple leaves (8) 5. 7 or fewer (usually 5) leaflets, egg-shaped nut...... Shagbark Hickory (Carya ovata) 5. 7 or more leaflets (6) 6. Leaflets rounded Black Locust (Robinia pseudonacacia) 6. Leaflets pointed (7) 7. Leaf 6 to 8 inches long Mountain Ash (Sorbus americana 7. Leaf 8 to 24 inches long Butternut (Juglans cinerea). 8. Leaves not lobed (9) or Black Walnut (Juglans nigra) 8. Leaves lobed: Oak species (see a-f below) a. Rounded lobes, 5 to 9 deep even lobes and sinuses, leaves hairless...... White Oak (Quercus alba) b. Rounded lobes, pair of deep sinuses near middle of leaf, hairy underside of leaves Bur Oak (Quercus macrocarpa) c. Rounded lobes, leaf narrow at base and broad near middle, hairy underside of leaves.... Swamp White Oak (Quercus bicolor)

Benefits of Dichotomous Keys

Dichotomous keys offer numerous advantages across scientific and educational domains, simplifying the process of identification and classification.

Fruits Vegetables Leafy Green Boot Vegetable Tube Bulb Yan Publip Green Garig

Accurate Identification

Ease of Use

Consistency: These keys adhere to a standardized format, employing specific terminology and criteria, ensuring consistent identification outcomes across different users.

Research Utility: cataloging and documenting biodiversity, contributing to the comprehension of species relationships and ecological dynamics.

Fieldwork Facilitation: Portable and adaptable for field use, dichotomous keys are invaluable for on-site surveys, ecological studies, and biodiversity assessments.

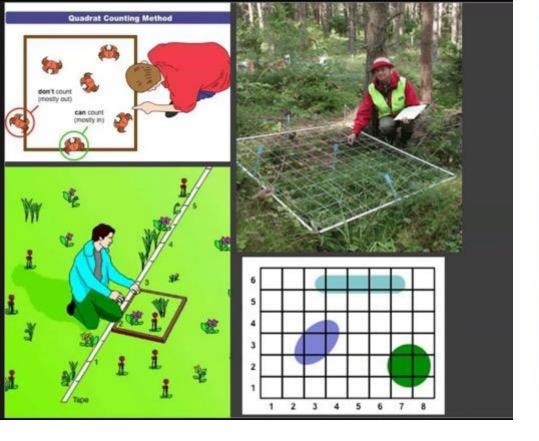
Simplicity and Accessibility: By breaking down complex identification tasks into binary choices, dichotomous keys make the process accessible to both experts and novices, streamlining identification efforts.





Obstacles of Dichotomous Keys

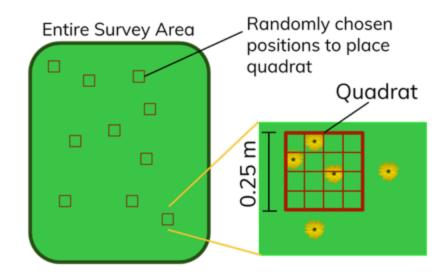
Limited distinguishing forms
Incomplete coverage of the characteristics
Differing observation conditions
Language as a Barrier
Obsolescence





2. Methods of Sampling Plant Communities

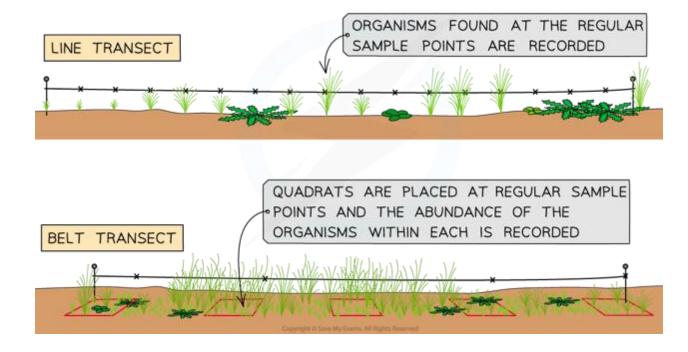
Quadrats



Transects



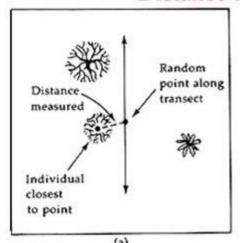
• Transects involve sampling along a straight line or a series of lines across a study area. They are particularly useful for studying gradients or changes in vegetation over space.

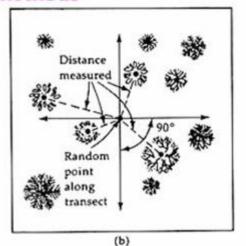


Plotless Sampling Methods

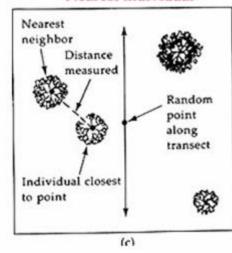
Plotless methods are used when setting up quadrats or transects is impractical. These methods rely on measuring distances between plants or random points.

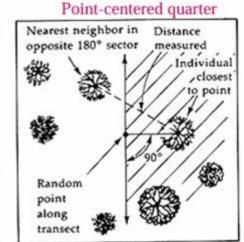
Distance methods





Nearest individual





Nearest neighbor

Random pairs

• Different measures on flora and vegetation

From field data collection to diversity analyses

How is biodiversity measured?

01 Species Richness



This method involves counting the number of different species within a specific area or ecosystem.

02 Simpson's Diversity Index



Simpson's Diversity Index is a calculation that measures the number of species and their relative abundance.

03 Genetic Diversity



Thie method measures the variations in DNA sequences among individuals of the same species.

04 Ecosystem Diversity



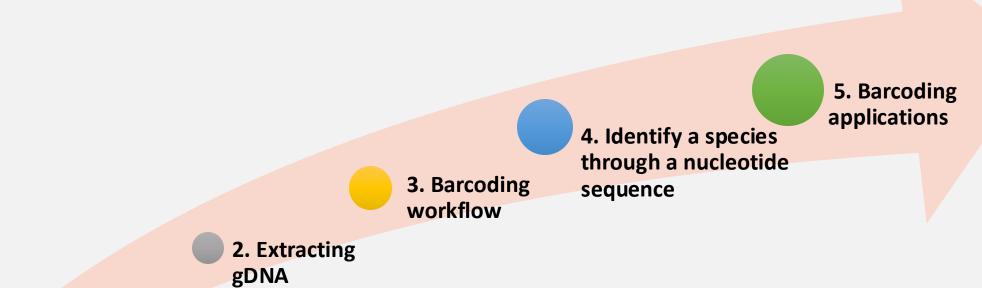
This method measures how many different types of ecosystems exist within a region.



www.biodiversityfacts.com



3. Species identification using molecular methods: from collection to analyses



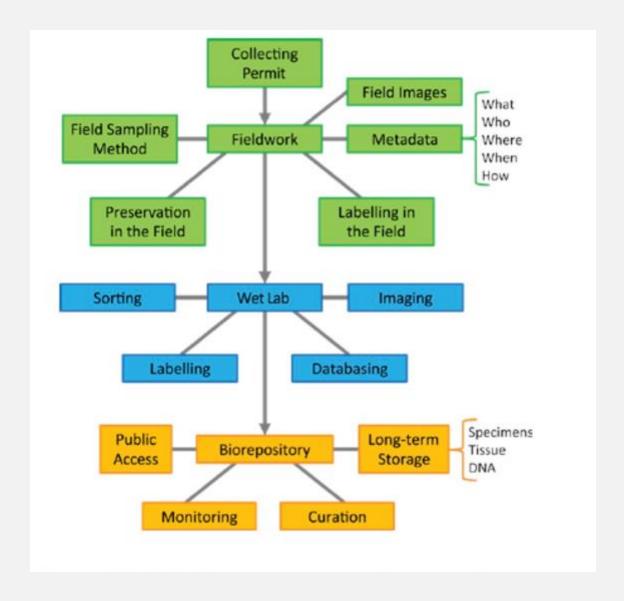
1. Collecting plant samples for molecular analysis



1. Collecting plant samples for molecular analysis

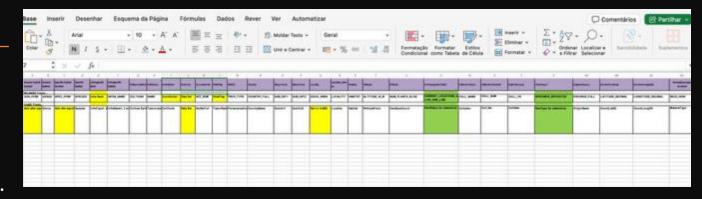
Planning the collection

Field notebook, hand lens, GPS device or smartphone, measuring tape, plant press



Registering plant collection information

- **ID**: Unique identifier for each entry.
- **Scientific Name**: Latin name of the species.
- Common Name: Commonly used name.
- Family: Botanical family (e.g., Fabaceae, Rosaceae).
- Category: Type of plant (Tree, Shrub, Herb, Vine, Grass, etc.).
- Location: Where the plant was found (GPS coordinate, plot number, or habitat type).
- **Abundance**: Rare, Occasional, Common, Abundant.
- Health Status: Healthy, Fair, Poor, Dead.
- Date Observed: Date the observation was recorded.
- **Notes**: Additional observations (e.g., flowering, pests, invasive species).







1. Collecting plant samples for molecular analysis

A- Preservation in the field



B- Voucher specimen

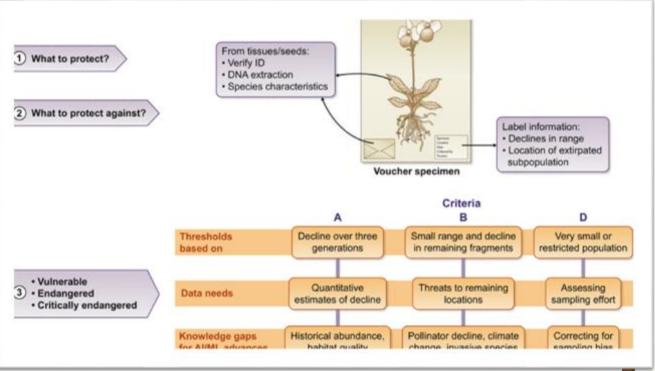








• Importance of voucher specimen







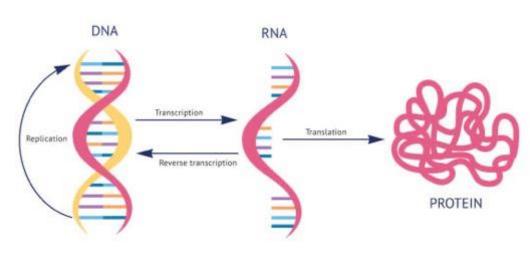






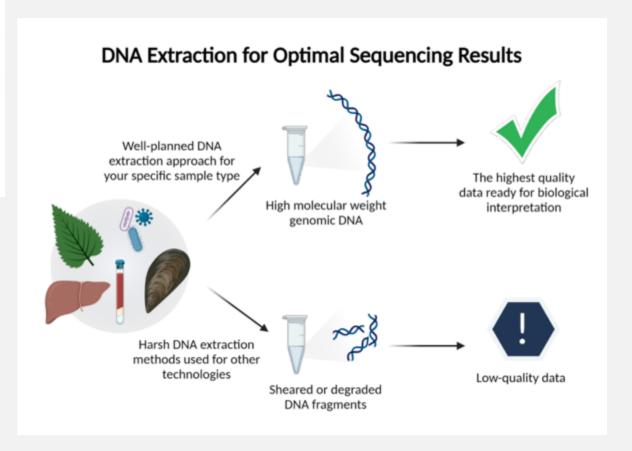


Genomic DNA as the key molecule



DNA e RNA são ácidos nucleicos que possuem diferentes estruturas e funções.

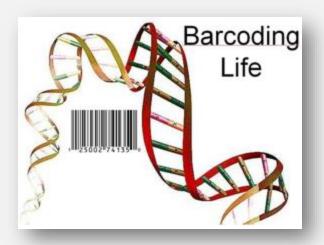
Enquanto o DNA é responsável por armazenar as informações genéticas dos seres vivos, o RNA atua na produção de proteínas







DNA barcoding





DNA barcoding is the use of short DNA sequence or sequences in a standardize locus (or loci) as a species identification tool.



- Ecological surveys

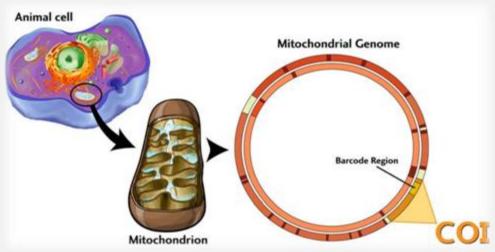
Applications:

- Identification of cryptic species (two or more distinct species classified as a single species)
- Identify and characterize species (abundant or rare, native or invasive)

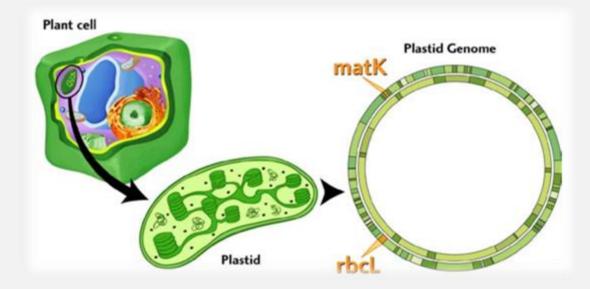


Barcode regions

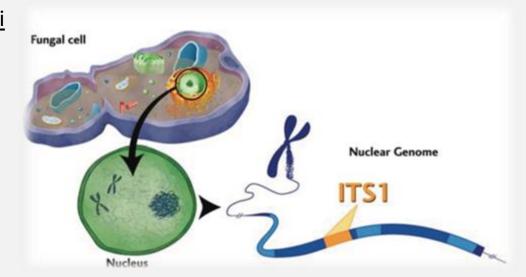
For animals



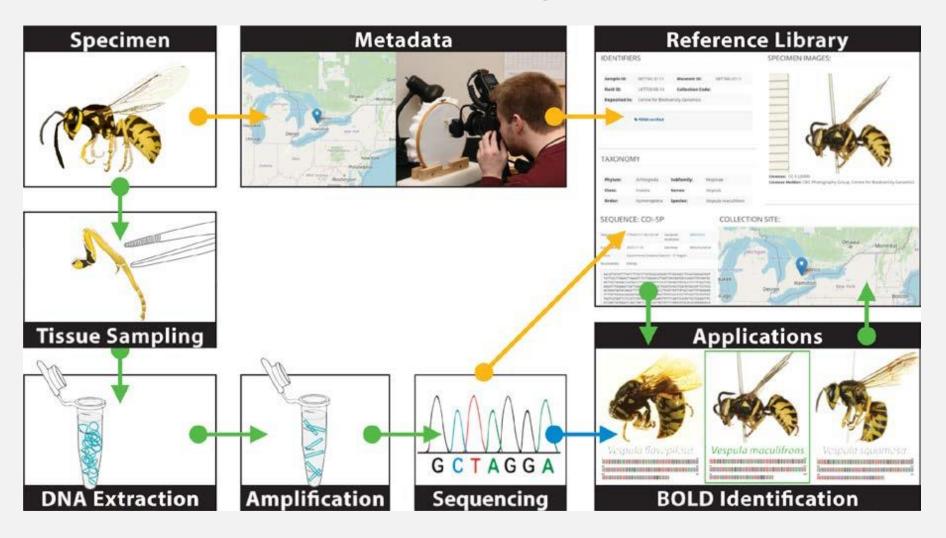
For plants



For fungi



2. Barcode Pipeline

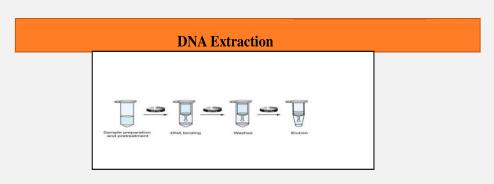


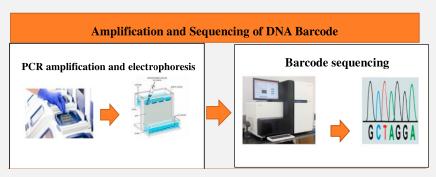


Four Barcoding components

1. Specimens: Natural history museums, herbaria, zoos, aquaria, frozen tissue collections, seed banks, type culture collections and other repositories of biological materials are treasure troves of identified specimens.

2. Laboratory Analysis: Laboratory protocols can be followed to obtain DNA barcode sequences from these specimens.







Plant DNA Barcode markers





- Polymorphic DNA region
- Conserved regions for easy amplification
- Sequence length suitable for sequencing

No unique barcode marker!

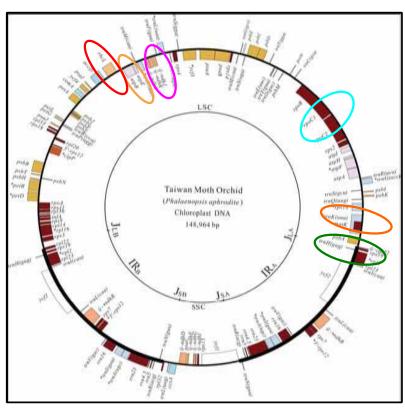
Consortion for the Barcode of Life (CBOL) Plant Working Group," proposed two "core barcode" the plastid *rbcL* and *matK* (Hollingworth et al., 2009 PNAS), the internal transcribed spacers of nuclear ribosomal DNA (nrDNA ITS, De-Zhu Li et al., 2011 PNAS).

Nuclear genes usually evolve too rapidly to distinguish between the same species, but chloroplast genes evolve at a slower rate, allowing differences to occur in the DNA code between species.

Barcode markers

Plastidial markers





Nuclear markers

Internal transcribed spacers of nuclear ribosomal DNA (nrDNA ITS) yields a good resolving power



Three major problems

- 1- Fungal contamination
- 2- Paralogous gene copies
- 3- Difficult to amplify/sequence

Plant barcode markers: matk+rbcl, nrDNA ITS



Four Barcoding components

- **3. Database** construction of a public reference library of species identifiers which could be used to assign unknown specimens to known species. There are currently two main barcode databases that fill this role:
- 1. International Nucleotide Sequence Database Collaborative

GenBank, the Nucleotide Sequence Database of the EMBL in Europe, and the DNA Data Bank of Japan. - CBOL's data standards for barcode records.

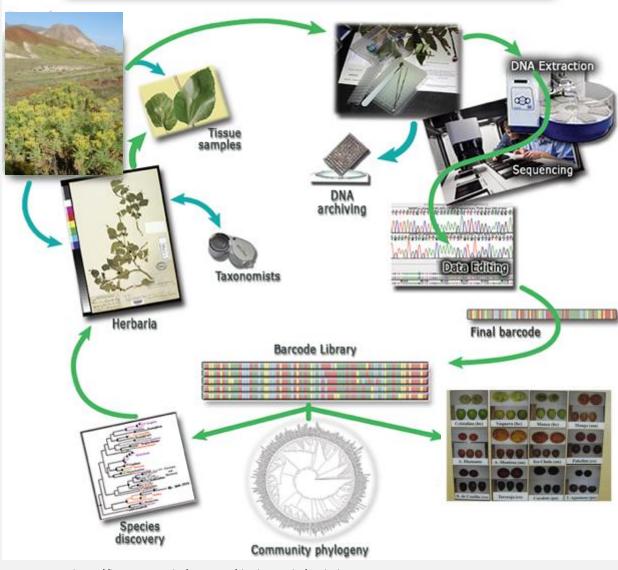
2. Barcode of Life Database (BOLD) created and is maintained by University of Guelph in Ontario.



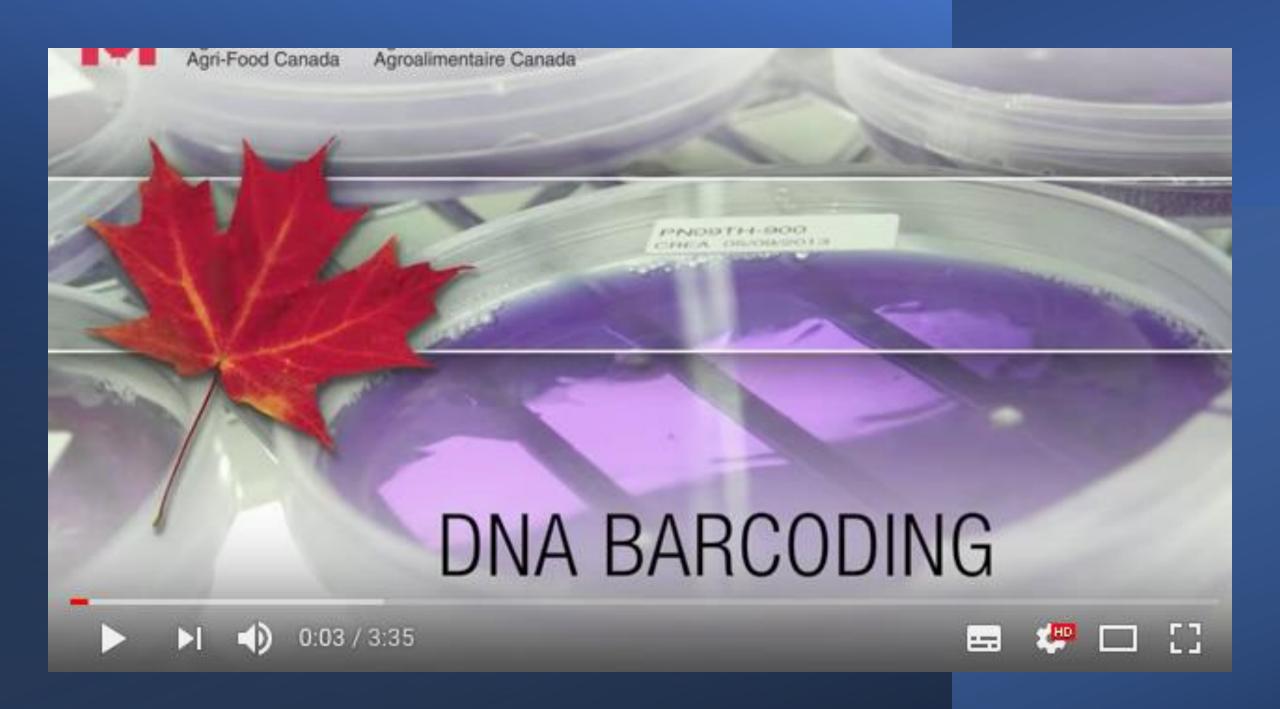




Plant DNA barcode cycle



http://botany.si.edu/projects/dnabarcode/cycle.htm

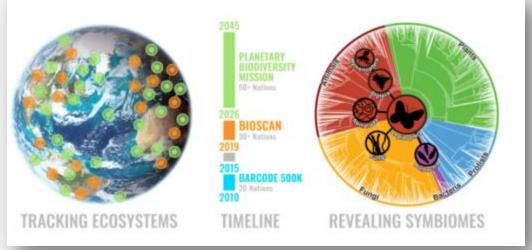




Four Barcoding components

4. Data Analysis: Specimens are identified by finding the closest matching reference record in the database. CBOL's Data Analysis Working Group





Consortium for the Barcode of Life (CBOL) is an international initiative devoted to developing DNA barcoding as a global standard for the identification of biological species.



Major Sequence Repositories

GenBank or NCBI (all known nucleotide and protein sequences)

www.ncbi.nlm.nih.gov/Web/Genbank/

Ensembl (all known nucleotide and protein sequences)

www.ensembl.org/index.html

TIGR Gene Indices (non-redundant, gene oriented clusters)

www.tigr.org/tdb/tdb.html

Gene Expression

BodyMap (Human and mouse gene expression data)

bodymap.ims.u-tokyo.ac.jp

Gene Identification and Structure

EID (Protein-coding, intron-containing genes)

mcb.harvard.edu/gilbert/EID/

Exint (Exon-intron structure of eukaryotic genes)

intron.bic.nus.edu.sg/exint/extint.html

TRRD (Regulatory regions of eukaryotic genes)

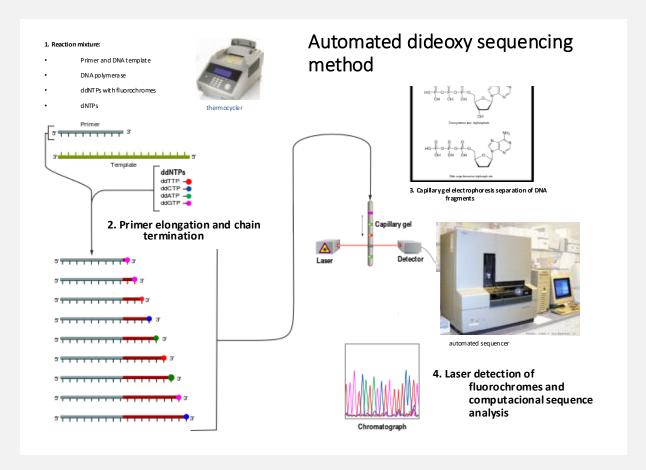
www.mgs.bionet.nsc.re/mgs/dbases/trrd4/

Sequence Databases

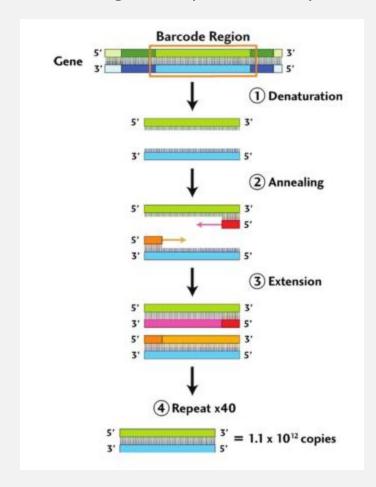


3. Acquire a DNA barcoding sequence





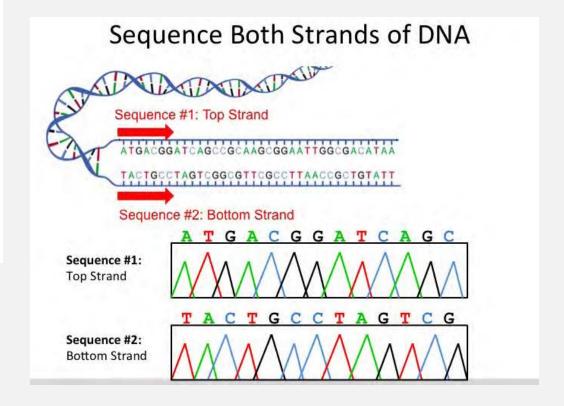
2. Barcode Region amplification by PCR



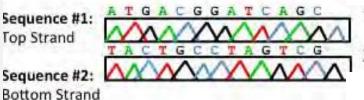


3. Acquire a Barcode sequence

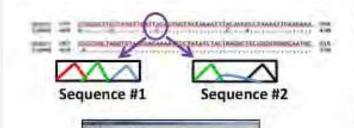
Overview of DNA Sequencing Mix with primers Perform sequencing reaction DNA Sample ...T T C A C C A A C T G G C C C A C A... **DNA Sequence** Chromatogram Adenine (A) = Green Thymine (T) = Red Cytosine (C) = Blue Guanine (G) = Black



Analyzing DNA Sequences



- Obtain two chromatograms for each sample.
- 2. Align the sequences with BLAST.



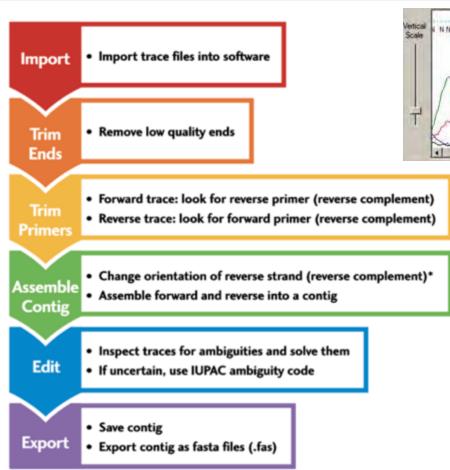
ATGCCGTAA

M P STOP

- Visualize the chromatograms using FinchTV.Compare BLAST alignments against base calls in chromatogram.
- Review any differences and determine which base is most likely correct.
- 5. Edit and trim the DNA sequence using quality data.
- 6. Translate the sequence to check for stop codons.
- 7. Use BLAST to identify origin of sequence.
- Use BOLD to confirm identity and make phylogenetic tree.



3. Viewing and analysing sequences



FASTA format

FASTA format is used to represent either nucleotide or peptide sequences. The first line is a comment line, beginning with ">" and describing the sequence. All the following lines are the sequence, in plain text.

Example DNA sequence in FASTA format:



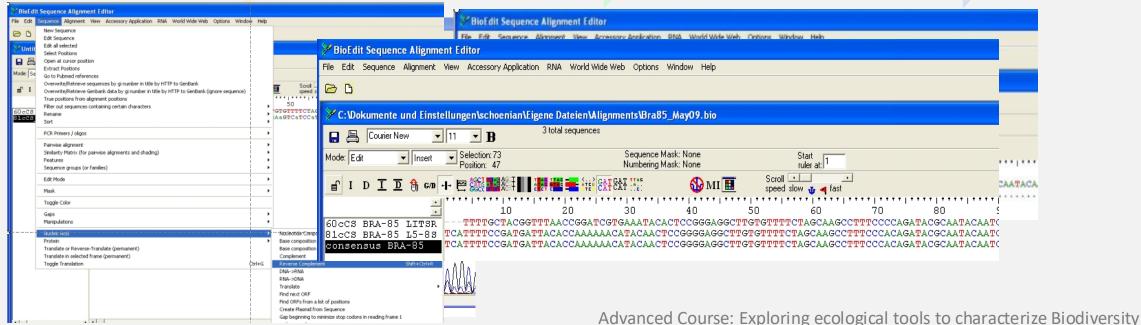
4. Building a consensus



Import forward and reverse sequences (.abi files)

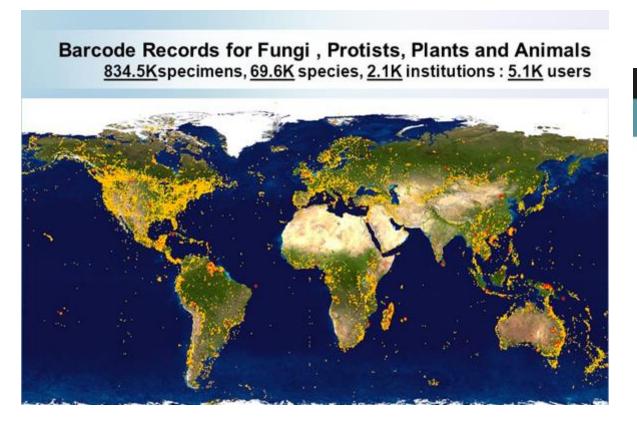
Reverse complement reverse strand and align both strands

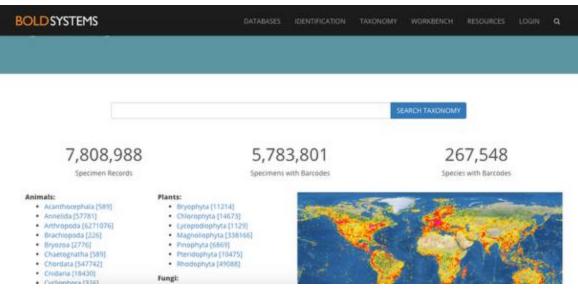
Create consensus and analyze mismatches and chromatograms (save as .fas file)



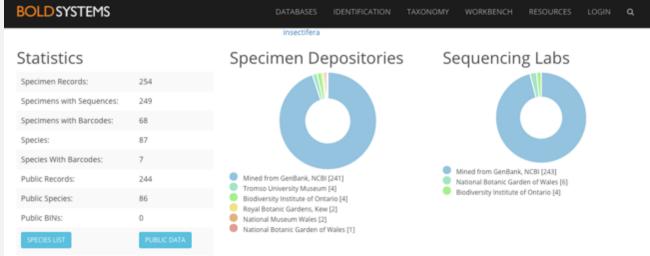
Bold database

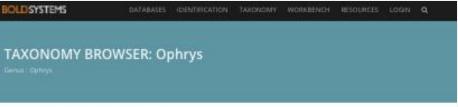
Databases





Example of BOLD information





Magnoliophyta / Liliophida / Asparagales / Orchidaceae / Ophrys



Taxon Description (Wikipedia)

The genus **Ophrys** is a large group of orchids from the alliance Orchis in the sustribe Orchidinas. They are widespread across much of Europe, North Africa, the Canary Islands, and the Middle East as far east as Turimeristan.

[http://apps.kew.org/vecsp/namedetat/do/name_id=1409.56w World Checklist of Selected Plant Families] These plants are nemarkable in that they successfully reproduce through pseudocopulation, that is, their flowers mimic female insects to such a degree that emorous males are fooled into mating with the flowers, thereby pollinating them. There are many natural hybrids. They are referred to set the "Bee erchids" due to the flowers of sects. They accesses resemblance to the flowers bodies of bees and other insects. Their scientific... full article at Wikipedia.



Organizations

Consortium for the Barcode of Life (CBOL)

(www.barcoding.si.edu)

 Barcode of Life Database (BOLD) (www.barcodinglife.org)





 FishBOL, All Birds Barcoding Initiative, MarBOL, etc. etc.

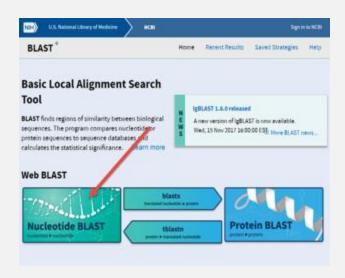
All V Leps FISH-BOL

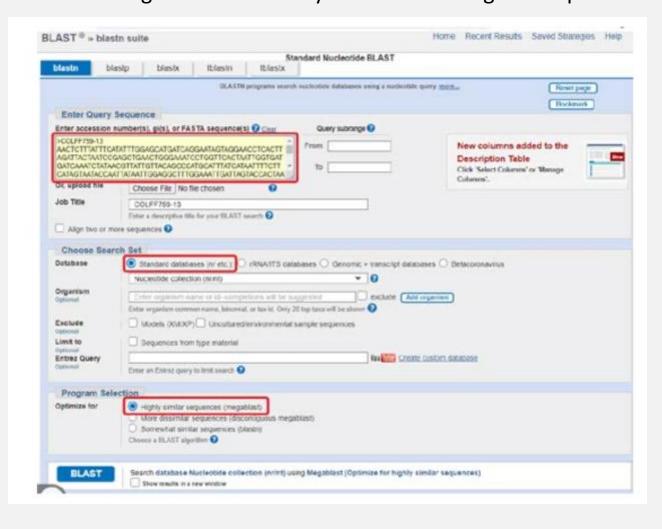


Comparing our DNA sequence with database for species identification

BLAST (Basic Local Alignment Search Tool) is an online search tool provided by NCBI (National Center for Biotechnology Information). It allows you to "find regions of similarity between biological sequences"

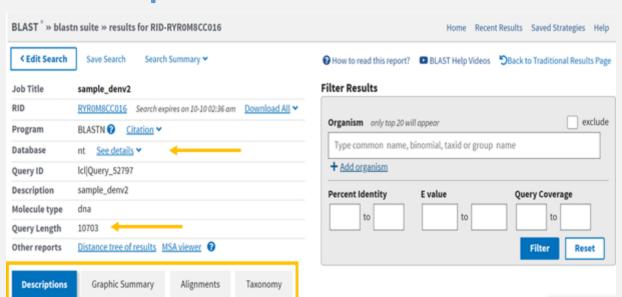
(nucleotide or protein).







ences producing significant alignments



Manage Columns Y Show Feedback

Max score: the highest bit score that is calculated from alignment matches and mismatches.

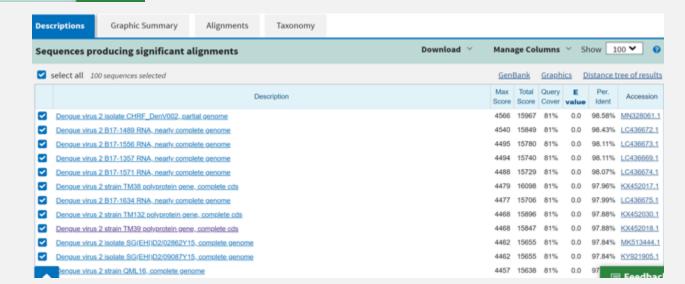
Total score: the sum of the alignment scores of all of the segments from the sequence.

Query coverage: the % of the contig length that aligns with the NCBI hit.

E value: the number of hits expected to be seen by chance. The closer to 0, the better.

Percent identity: the % of bases that are identical to the reference genome.

Accession [number]: a unique identifier assigned to records in the NCBI databases.





Anacardium occidentale isolate 92 maturase K (matK) gene, partial cds; Customize view chloroplast GenBank: AY594459.1 Analyze this sequence FASTA Graphics Run BLAST Go to: 🗹 Pick Primers LOCUS AY594459 1945 bp linear PLN 26-JUL-2016 Highlight Sequence Features DEFINITION Anacardium occidentale isolate 92 maturase K (matK) gene, partial cds; chloroplast. Find in this Sequence ACCESSION AY594459 VERSION AY594459.1 KEYWORDS Related information chloroplast Anacardium occidentale (cashew) SOURCE Protein ORGANISM Anacardium occidentale Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Taxonomy Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae; Pentapetalae; rosids; malvids; Sapindales; Anacardiaceae; Anacardium. 1 (bases 1 to 1945) REFERENCE LinkOut to external resources Pell,S.K. Order MATK cDNA clone/Protein/Antibody/RNAi Molecular Phylogeny of Anacardiaceae: Intrafamilial Classification TITLE [OriGene] and Evolutionary Relationships of Noted Genera JOURNAL Unpublished REFERENCE 2 (bases 1 to 1945) Pell,S.K. AUTHORS Recent activity Direct Submission TITLE Turn Off Clear Submitted (07-APR-2004) Cullman Program for Molecular Systematics JOURNAL Anacardium occidentale isolate 92 maturase Studies, New York Botanical Garden, 200th St. and Kazimiroff Blvd., K (matK) gene, partial cds; chloropla Nucleotide Bronx, NY 10458, USA FEATURES Location/Qualifiers anacardium occidentale matk (3) 1..1945 source /organism="Anacardium occidentale" /organelle="plastid:chloroplast" Anacardium occidentale voucher Zhang L /mol type="genomic DNA" sn(KUN) internal transcribed spacer Nucleotide /isolate="92" /specimen_voucher="Mori 24142" anacardium occidentale Internal (461) /db_xref="taxon:<u>171929</u>" /country="French Guiana' anacardium occidentale ITS (1591) /note="authority: Anacardium occidentale L." Nucleotide 399..>1945

Case study on Cabo Verde flora



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Research Article

SPECIAL ISSUE: Island Plant Biology—Celebrating Carlquist's Legacy

Patterns of genetic diversity in three plant lineages endemic to the Cape Verde Islands

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Species diversity and richness

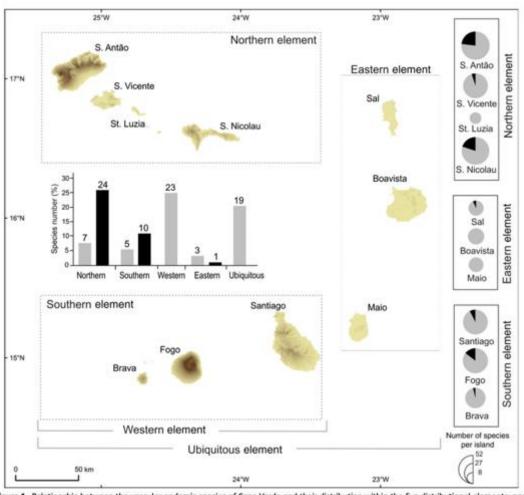
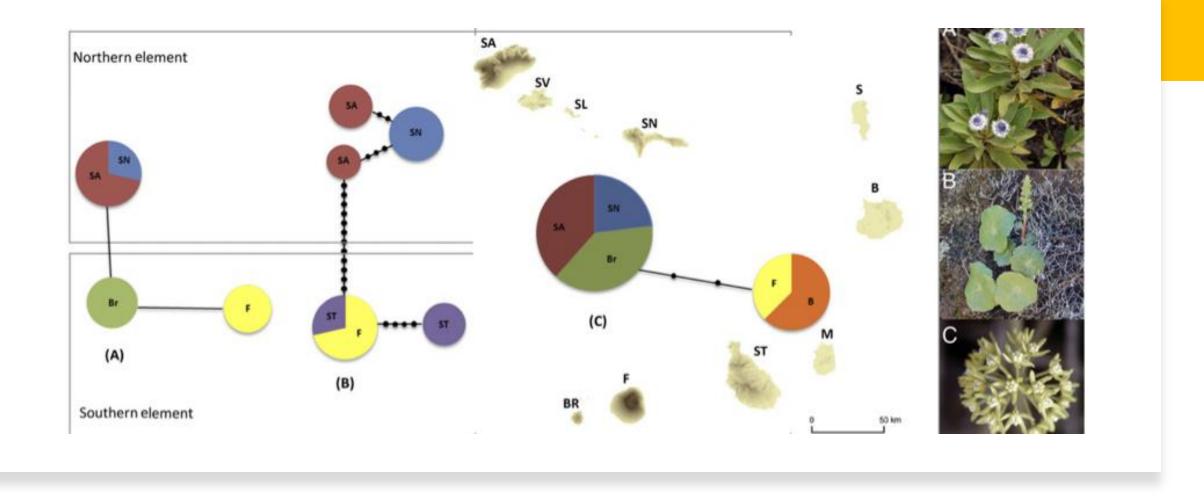


Figure 1. Relationship between the vascular endemic species of Cape Verde and their distribution within the five distributional elements: northern (i.e. Santo Antão, São Vicente, Santa Luzia and São Nicolau), southern (i.e. Santiago, Fogo and Brava), western (including species simultaneously present in northern and southern islands), eastern (i.e. Maio, Sal and Boavista) and ubiquitous (including species present in both western and eastern islands). The distribution of multi-island endemics—MIEs (grey) and single-island endemics—SIEs (black) in each island (right); and within the five distributional elements (bar graph in the centre; the number of taxa is placed above each bar).



Molecular data supporting species identification and diversity

- Potential new species disclosed by molecular data?
- Reverse taxonomy assessment and diversity analyses



Application to wild useful plants

The FAO Commission developed the <u>Genebank Standards for</u> <u>Plant Genetic Resources for Food and Agriculture</u> and recently endorsed the <u>Voluntary guidelines for national level conservation</u> <u>of crop wild relatives and wild food plants</u>.



