Module 2: Classical and digital identification tools

This module focuses on the practical tools used to identify organisms, from traditional morphology-based methods to modern digital identification systems. Learners will explore how identification keys work, and how taxonomic resources are structured.

1.1 Principles of morphological identification

What is morphological identification?

Morphological identification relies on observable physical characteristics of organisms to distinguish taxa.

Common morphological characters

- **External morphology**: size, shape, color, segmentation
- **Anatomical structures**: leaves, flowers, wings, shells, appendages
- **Microscopic traits**: spores, scales, hairs, cell shape
- **Developmental stages**: larval vs adult forms

Strengths and Limitations

Strengths encompass cost-effectiveness for field use, a vast historical literature base, and applicability to many taxa without specialized equipment.

Limitations arise from **cryptic species** (genetically distinct but morphologically identical), **phenotypic plasticity** (environmentally induced variation), and **challenges** with fragmented or immature specimens lacking diagnostic traits.

1.2 Identification Keys

Dichotomous Keys

Dichotomous keys use stepwise, two-choice decisions (e.g., "leaves compound or simple?") to guide users linearly through well-described taxa, widely featured in field guides and manuals for structured identification.

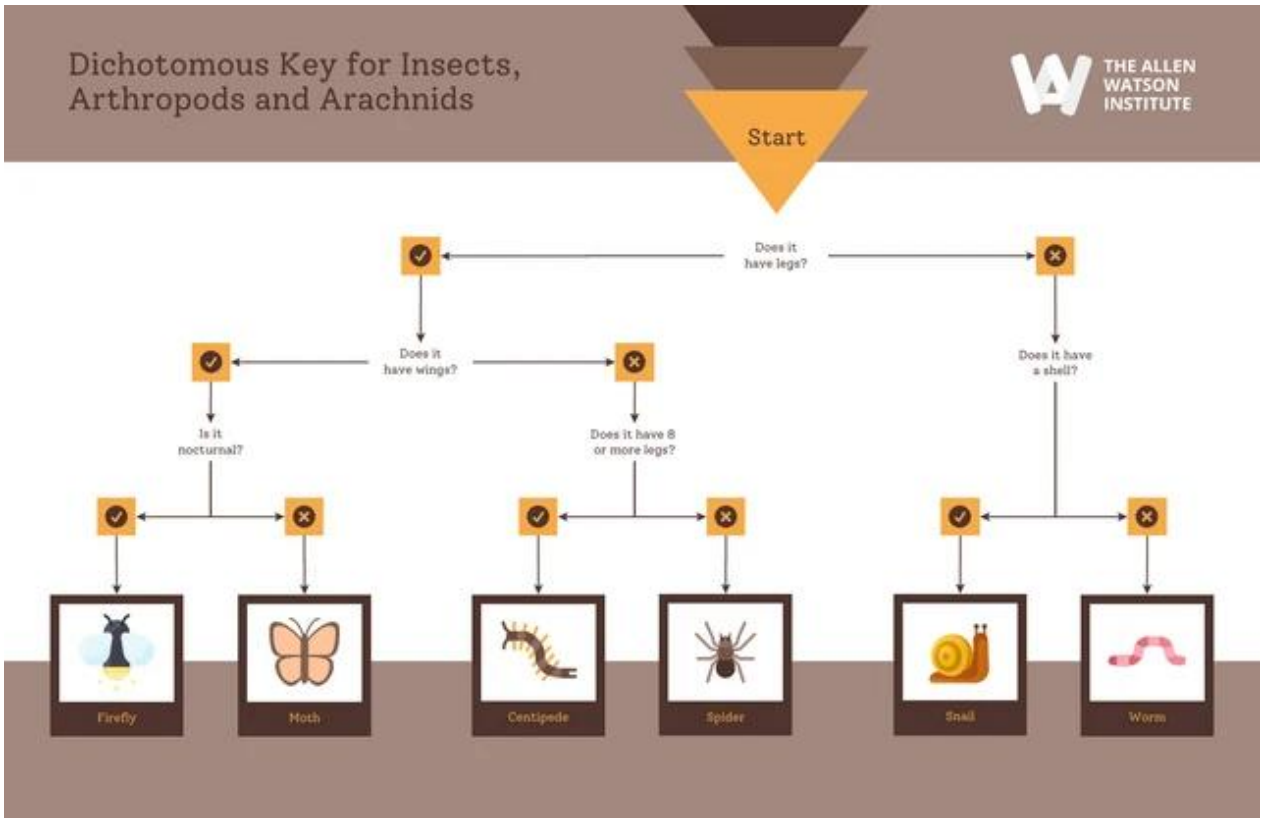

Figure 1.Dichotomous Keys (https://venngage.com/blog/dichotomous-key/)

Multi-Access (Polyclave) Keys

Multi-access (Polyclave) keys let users select characters in any order via elimination matrices, offering greater flexibility than rigid dichotomies and thriving in digital formats like LucID for partial specimens.

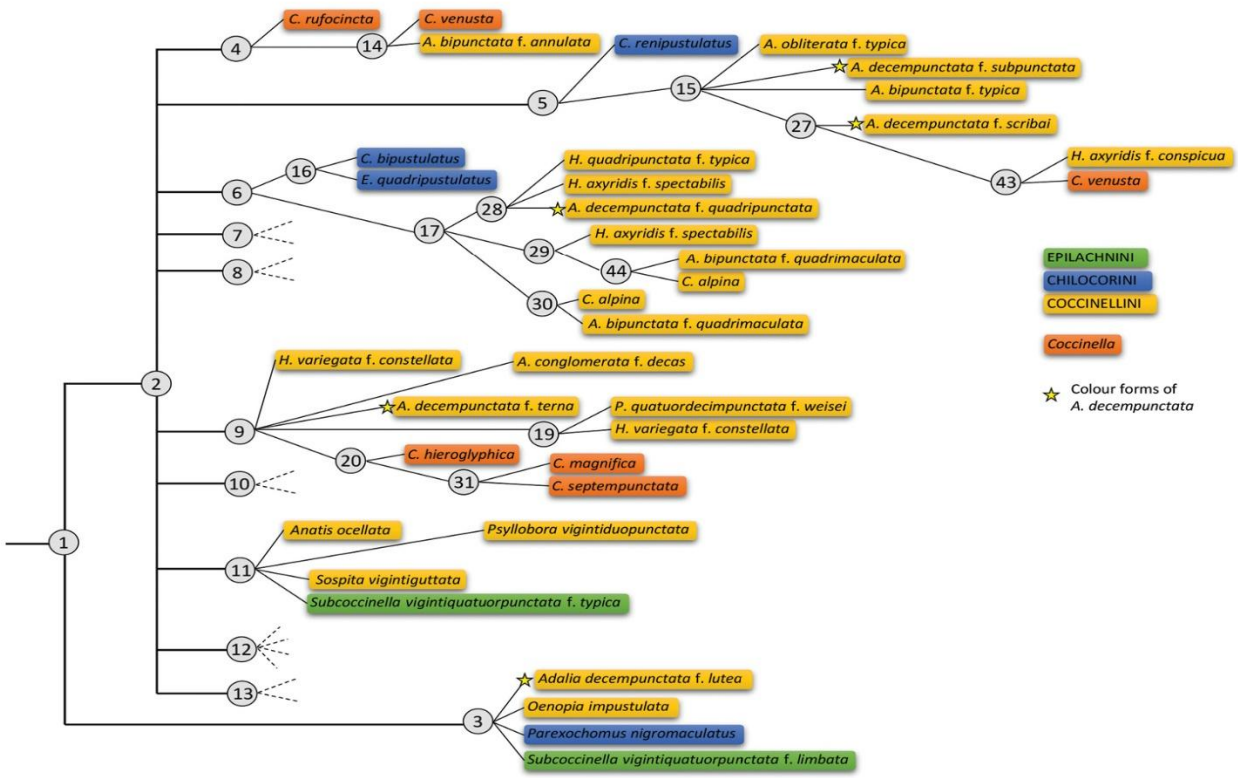

Figure 2. Representation of a part of the single-access identification key generated by IKey+ under Xper3 and the Xper score method (statistics detailed in Appendix 1). The taxonomy is highlighted (the three tribes included in this study, the genus Coccinella and the colour forms of Adalia decempunctata). Numbers in the circles represent the number of steps in the generated key. (https://zookeys.pensoft.net/article/22171/zoom/fig/12/)

Interactive digital keys

Interactive digital keys integrate images, character matrices, and filters in web platforms (e.g., Xper²), handling uncertain or missing data while scaling for global use.

1.3 Molecular Integration

DNA Barcoding

DNA barcoding identifies organisms using standardized genetic markers like **COI** (cytochrome c oxidase I for animals), **ITS** (internal transcribed spacer for fungi), **rbcL/matK** (for plants), and **16S rRNA** (for prokaryotes), enabling rapid species-level discrimination.

Advantages include resolving cryptic species undetectable by morphology, working with fragments or early life stages lacking traits, and providing highly reproducible results independent of observer expertise.

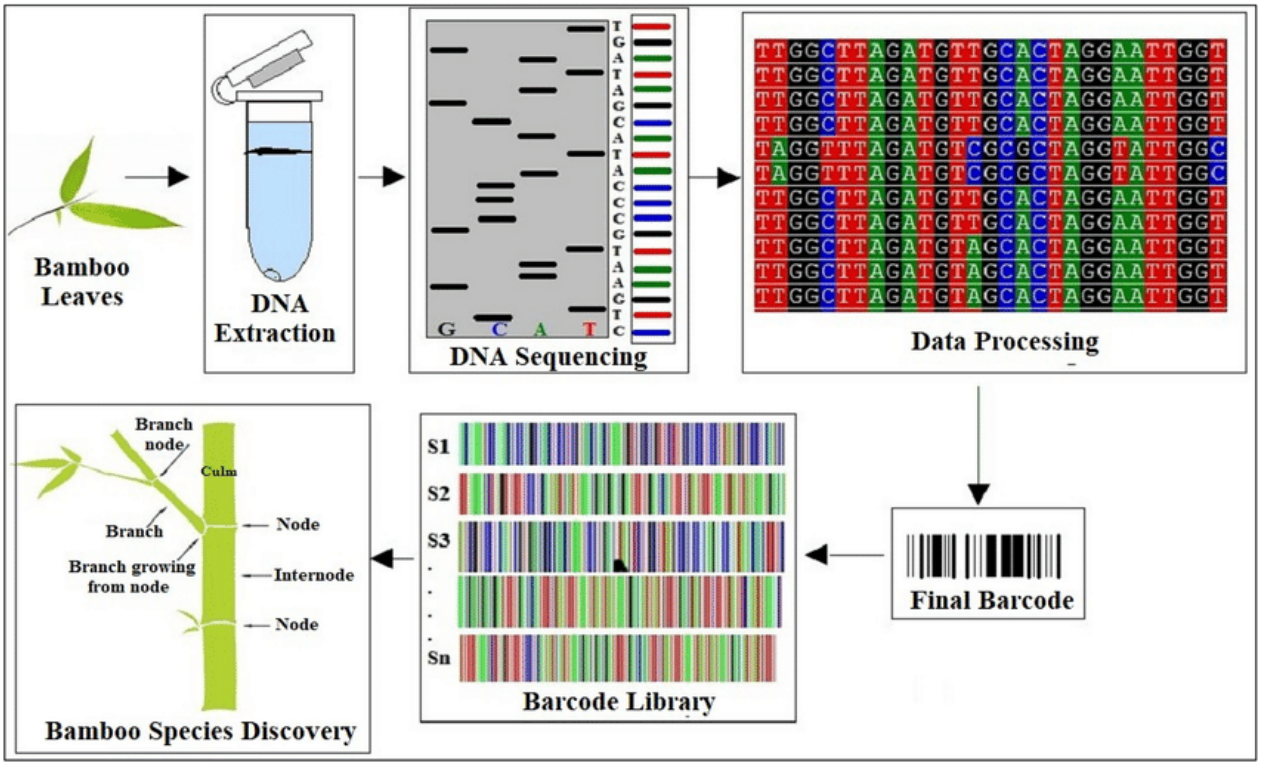

Figure 3. Schematic representation of DNA barcoding (https://www.researchgate.net/figure/Schematic-representation-of-DNA-barcoding_fig4_351600264)

DNA Metabarcoding

Metabarcoding enables community-level identification from environmental samples like soil, water, or air by amplifying barcode genes from mixed DNA (eDNA), revealing entire assemblages without individual sorting.

Applications span biodiversity monitoring, ecology/conservation assessments, and environmental impact studies, rapidly detecting invasives or rare taxa across habitats.

Performance achieves ~95% genus-level accuracy for arthropods and ~85% species-level for vertebrates, though PCR biases and database gaps limit resolution for microbes.

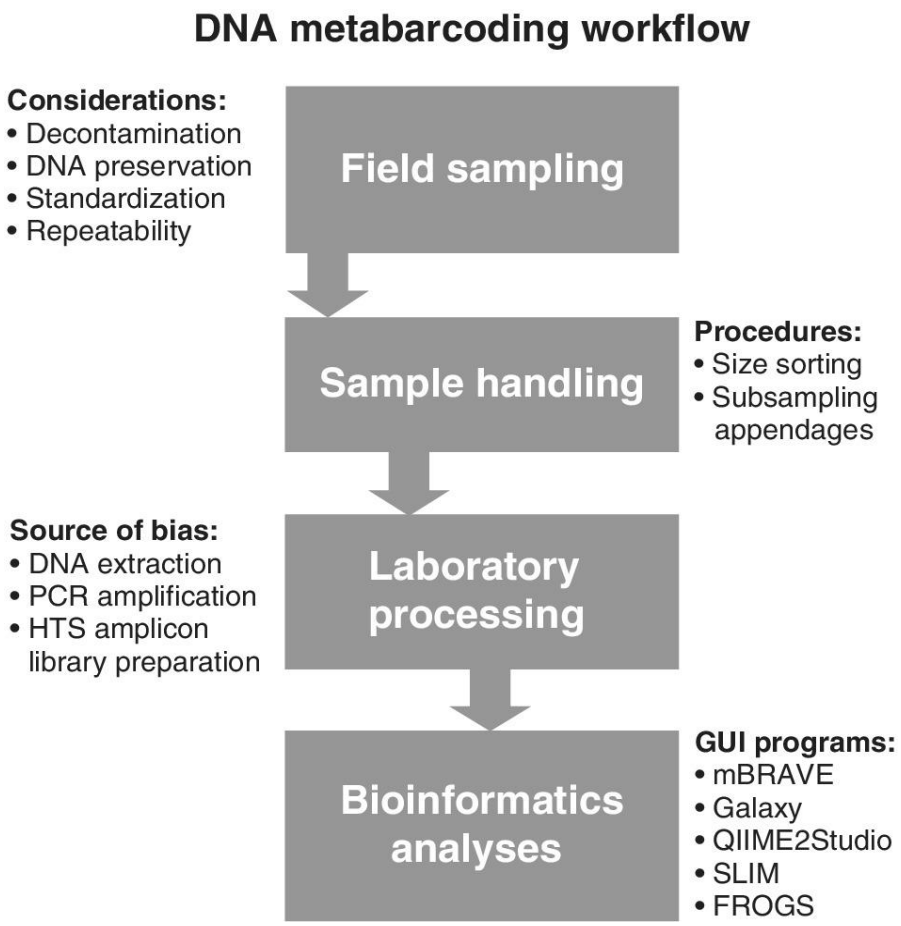

Figure 4. Metabarcoding workflow (https://resjournals.onlinelibrary.wiley.com/cms/asset/b9b1614b-74cb-4518-acd8-156e705a541f/een12831-fig-0002-m.jpg)

1.4 Protein fingerprinting and chemical signatures

MALDI-TOF Mass Spectrometry

MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) mass spectrometry identifies microorganisms through **protein fingerprinting**, primarily ribosomal protein spectral profiles in the 2–20 kDa range, achieving ~97% species-level accuracy across many bacterial and fungal taxa. This rapid, cost-effective method complements DNA-based approaches in clinical microbiology, delivering results within minutes from colonies without sequencing.

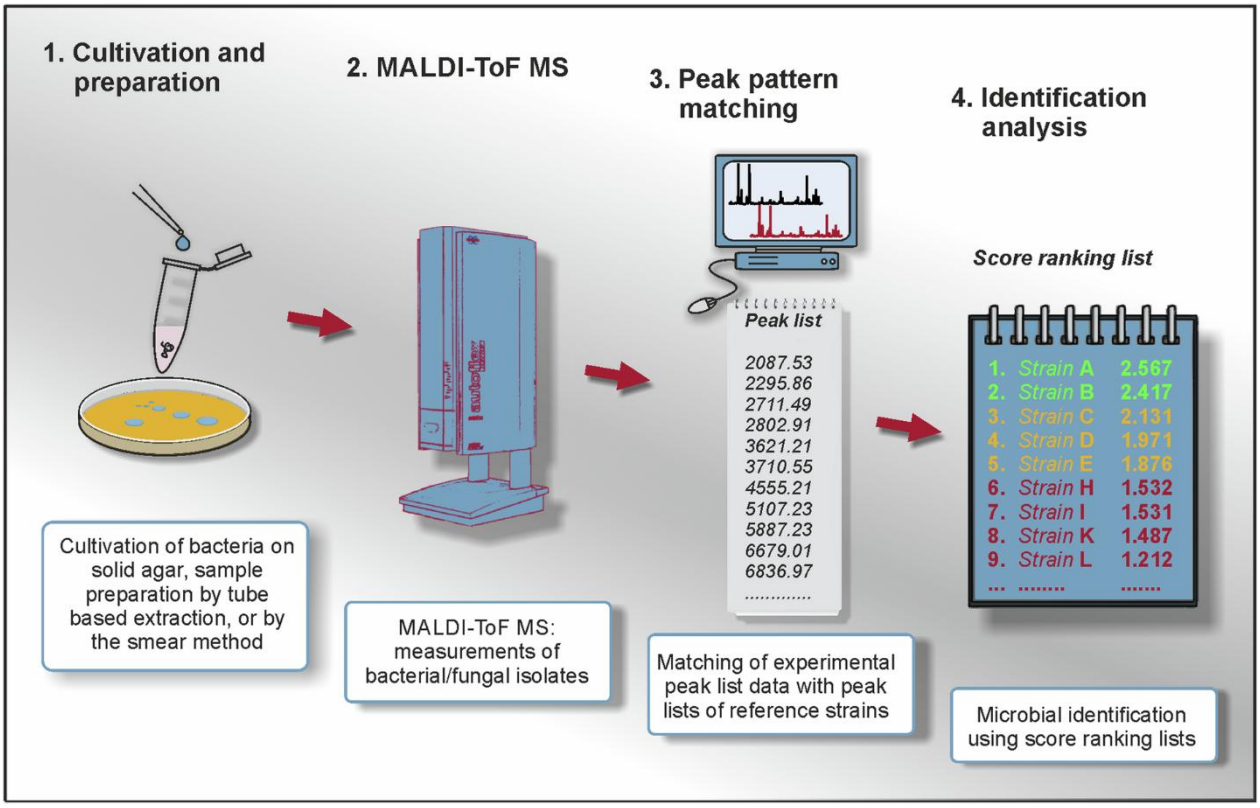

Figure 5. MALDI-TOF Mass Spectrometry (https://media.springernature.com/full/springer-static/image/art%3A10.1038%2Fs41597-025-04504-z/MediaObjects/41597_2025_4504_Fig1_HTML.png)