DNA Barcoding Basics

What is DNA barcoding and why is there a need for it?

How Barcoding works

Plants are sampled

DNA is extracted

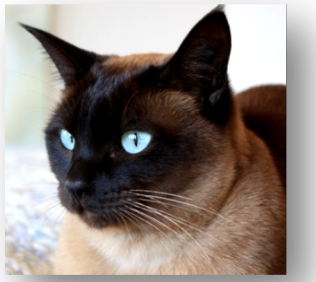
“Barcode” amplified

ACGAGTCGGTAGCTGCCCTCTGACTGCATCGAA
TTGCTCCCCTACTACGTGCTATATGCGCTTACGAT
CGTACGAAGATTTATAGAATGCTGCTACTGCTCC
CTTATTCGATAACTAGCTCGATTATAGCTACGATG

Sequenced DNA is compared with plants in a barcode database

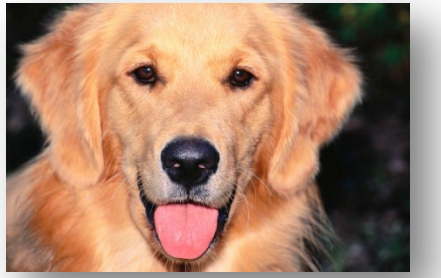
How many species can you name?

How many Animals did you name?
How many mammals?
How many plants?
How many insects?



“Cat”
Felis catus

“Dog”
Canis lupus familiaris



“Oak Tree”
Quercus alba

“Shark”
Ginglymostoma cirratum

“Beetle”
Popillia japonica

Problem 1: No one know how many species there are.

Vertebrates	Species
Mammals	5,490
Birds	9,998
Reptiles	9,084
Amphibians	6,433
Fishes	31,300
Total	62,305

Invertebrates	Species
Insects	1,000,000
Mollusks	85,00
Crustaceans	47,000
Corals	2,175
Arachnids	102,248
Total (+others)	1,305,250

Plants	Species
Angiosperms	281,821
Gymnosperms	1,021
Ferns and Allies	12,000
Mosses	16,236
Green and Red Algae	10,134
Total	321,212

•There are currently between 1.5 and 2 million described species

•It is estimated that this number may represent as little as half of the true number of species

• Perhaps more than 1/3 of all species are threatened

(IUCN Red list version 2010.1)

Problem 2: Even though there are millions of species, there is also a lack of agreement on what a “species” means.

Canis lupus

Canis lupus (familiaris)

Anas platyrhynchos

Defining what species are is a complex task

Dependent on many factors

- Interbreeding capabilities
- Morphological variation
- Ecological context
- Genetic similarities

Problem 3: Current taxonomic methods may be inadequate (or at least too slow) to capture vanishing biodiversity

Classical taxonomy is steeped in terminology that can act barrier to understanding and reduce the number of persons who are qualified to describe biodiversity

Leaves alternate proximally, opposite and ultimately decussate distally, 6–16 × 4–13 cm; petiole ca. as long as blade, winged, base clasping, basal lobes stipulate, growing as extensions of wings, less than 1 mm wide; blade 5–7-veined, ovate, glabrous, base typically sagittate, margins entire, apex acute to acuminate. Staminate inflorescences axillary, 1–2 per axil, paniculate, fasciculate; panicles bearing flowers singly,bracteolate, in a zigzag pattern along rachis, internodes less than 2 mm; rachis to 25 cm, secondary axes 1–3(–6), fasciculate, less than 3 cm, each subtended by deltate-ovate bracteole shorter than 1 mm. *Pistillate* inflorescences solitary, 4–8(–20)-flowered, 6–35 cm, internodes ca. 1 cm

The body form ranges from hemispherical (e.g., *Cleidostethus*) to elongate oval (e.g., *Clypastraea*) to latridiid-like (e.g., *Foadia*). Corylophids are typically dull brown, but some species have contrasting yellowish-brown patches on the pronotum or elytra. The integument is often densely punctured and may be glabrous or bear short, fine recumbent setae. Most corylophid adults can be diagnosed using the following morphological features: Maxilla with single apical lobe; Mesotrochanter short and strongly oblique; Head usually covered by pronotum; Frontoclypeal suture absent; Antennae elongate with 3-segmented club; Procoxal cavities closed externally; Tarsal formula 4-4-4; Pygidium exposed

Adding to the complexity, if the specimen to be identified is immature in its development or damaged and incomplete, identification may be impossible.

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Complex and Somewhat objective

>Dioscorea alata (matK) gene, partial

ATTTAAATTATGTGTCAGATATATTAATACCCCATCCCATCCATCTGGAAATCCTGGTTCAAATACTTCAATGCTGGACTCAAGATGTTTCCTCTT
TGCATTTATTGCGATTCTTTCTCCACGAATATCATAATTCGAAT AGTTTCATTACTCCGAAAAAACCTATTTACGTGATTTCAATTTCAAAAGAAA
ATAAAAGATTTTTTCGAT TCCTATATAATTCTTATGTATTTGAATGTGAATTTGTATTAGTTTTTTTTCATAAGCAATCCTCTTATTT ACGATCAA
GGTCCTCTGGAGTCTTTCTTGAGCGAACACATTTCTATGGAAAAATGGGGCATTTTTTAGTAGTGTGTTGTAATTATTTTCAGAAGACCCAATG
GTTCTTCAAAGATCCTTTTCTGCATTATGTTCGATATC AAGGAAAAGCAATTCTGGTGTCAAAGGGAACTCGTCTTTTGATGAGGAAATGGAGA
TCTTACCTTGTCCATTTTTGGCAATATTATTTTCAATTTTGGTCTCATCCGCATAGGATTCATATAAACCAATTATCAAATTATTCCTTCTGTTTTC
TGGGTTATCTTTCAAATGTACTAATAAATTTTTCCGTGGTAAGGAGTCAAATGTTAGAAAATTCATTTGTAATAGATACTCTTACTAAGAAATT
TGATACCAGAGTTTCAGTTATTGCTCTTATTCG ATCATTGTCTAAAGCGAAATTTTGTACCGTATCCGGGCATCCTATTAGTAAGTCAATATGGA
CAAATTTC TCAGATTTGGATATTATTCATCGATTTGGTTGGATATGTAGAA

Simple (A,T,G, or C) and more Reliably objective

Choosing a DNA barcode

There are many criteria that go in to selecting an appropriate region that can serve as a DNA barcode.

Three of them include:

- Universality
- Robustness
- Discrimination

Why are these three criteria important?

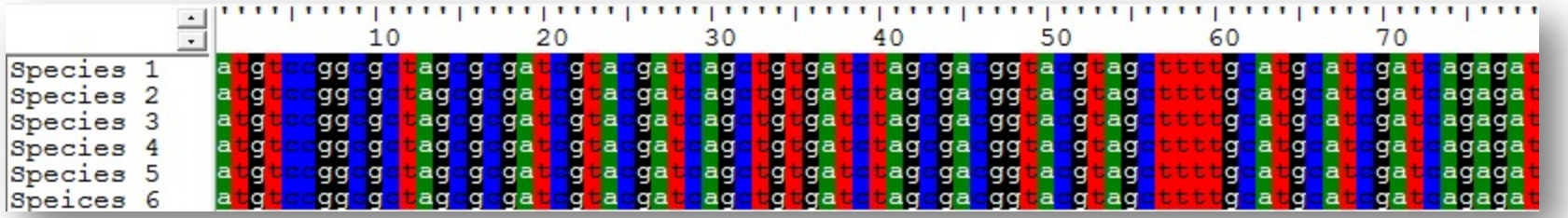
Discrimination

Barcoding regions must be different for each species. Ideally you are looking for a single DNA locus which differs in each species.

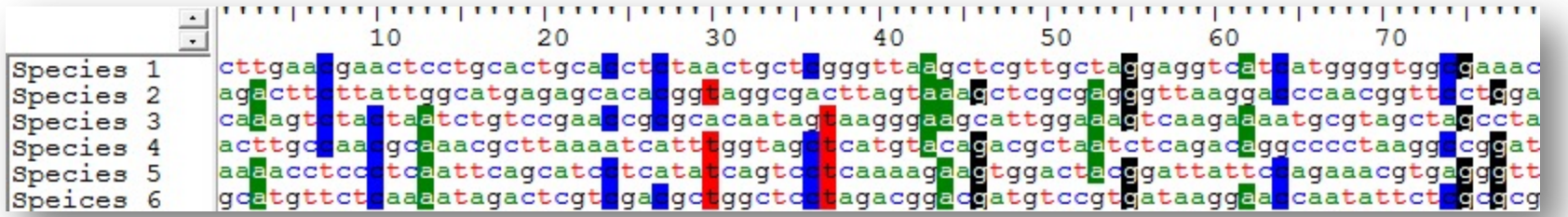
Oppositional Goals:

- Each loci must be different for each species
- Although loci must be different, they must be similar enough that they can be amplified by PCR, aligned and compared

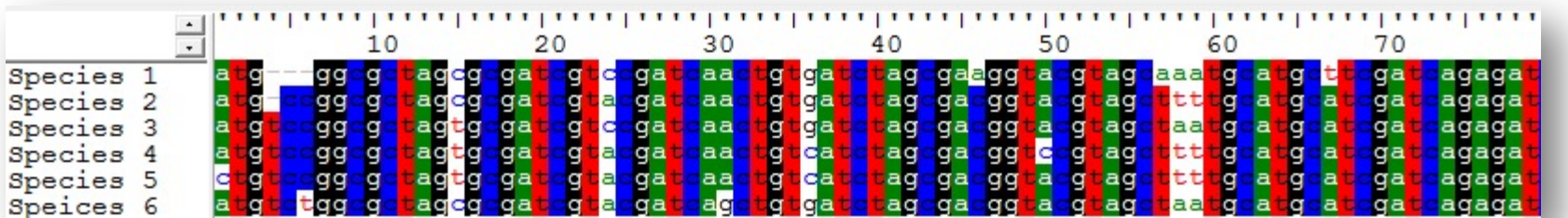
Fail: Sequence is completely conserved, good for PCR, but uninformative as barcode



Fail: Sequence shows no conservation, impossible for PCR, but good as barcode



Win: Sequence shows some (ideally ~70%) conservation, good for PCR, good as barcode



Universality

Since barcoding protocols (typically) amplify a region of DNA by PCR, you need primers that will amplify consistently.

• Once you have a candidate locus (loci) that seem discriminatory, do these loci (possibly genes, but possibly non-coding DNA) exist in in virtually all of the species you wish to barcode?

• Will you be able to find PCR primers that can amplify across many species, despite mismatches?

Robustness

Since barcoding protocols (typically) amplify a region of DNA by PCR, also need to select a locus that amplifies reliably, and sequences well.

•PCR is very sensitive to the chemistry involved (types of enzymes, concentration of reagents, cycling parameters, etc.

•The amplified PCR product must also be sequenced. Sanger sequencing is sensitive to highly repetitive DNA.

DNA Barcoding
Plants vs. Animals

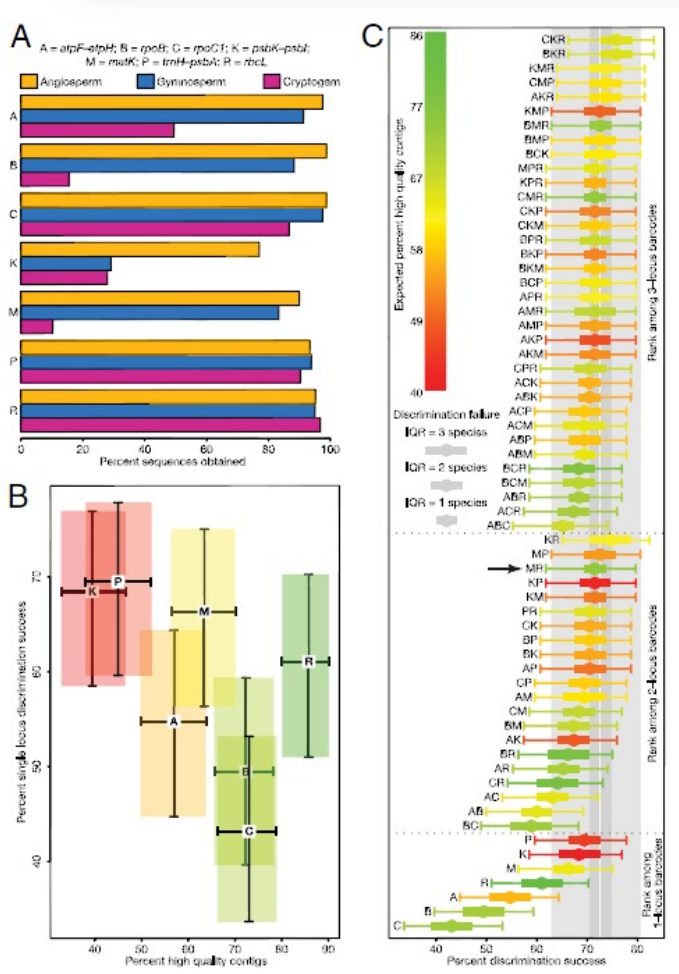
Finding a DNA locus that possesses all of these qualities(Discrimination, Universality, Robustness) was relatively easy in animals.

The animal barcode of choice
Is the mitochondrial gene
cytochrome *c* oxidase I (COI).

A DNA barcode for land plants

CBOL Plant Working Group[1]

Communicated by Daniel H. Janzen, University of Pennsylvania, Philadelphia, PA, May 27, 2009 (received for review March 18, 2009)



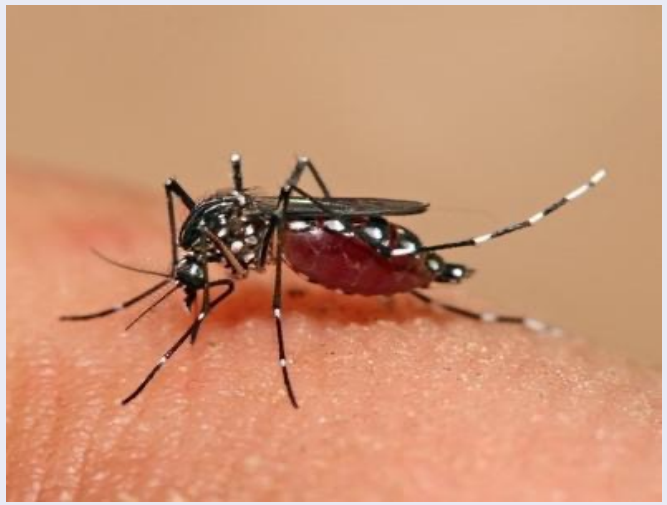
Based on recommendations by a barcoding consortium (Consortium for the Barcode of Life, plant working group) the chloroplast genes *rbcL* and *matK* come very close to being ideal candidates for universal plant barcodes.

Like any barcode loci that could be chosen, there will is always a possibility of failure to make a reasonably definitive identification of a particular specimen.

Example Barcoding Experiment

Can we tell the difference between larvae that look (nearly) identical?

Aedes adult



By Muhammad Mahdi Karim - Own work, GFDL 1.2, https://commons.wikimedia.org/w/index.php?curid=11185617

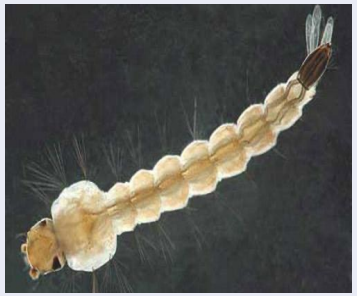
Anopheles adult

By Jim Gathany - (PHIL), ID #5814. https://commons.wikimedia.org/w/index.php?curid=799284

Culex adult

By Muhammad Mahdi Karim - Own work, GFDL 1.2, https://commons.wikimedia.org/w/index.php?curid=7673048

Aedes larva



Photograph by Michele M. Cutwa, University of Florida.

Anopheles larva

Culex larva

Photograph by Michelle Cutwa-Francis, University of Florida.


Aedes
Anopheles
Culex
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Why does this matter?

***Aedes*:**

- Chikungunya
- Dengue fever
- Lymphatic filariasis
- Rift Valley fever
- Yellow fever
- Zika

***Anopheles*:**

- Malaria
- Lymphatic filariasis

***Culex*:**

- Japanese encephalitis
- Lymphatic filariasis
- West Nile fever

Why does this matter?