eDNA barcoding and metabarcoding
- General introduction -

Eva BELLEMAIN

Introduction

- Need for biodiversity assesment
- Environmental DNA (eDNA)
- Terminology

The eDNA barcoding and metabarcoding approaches

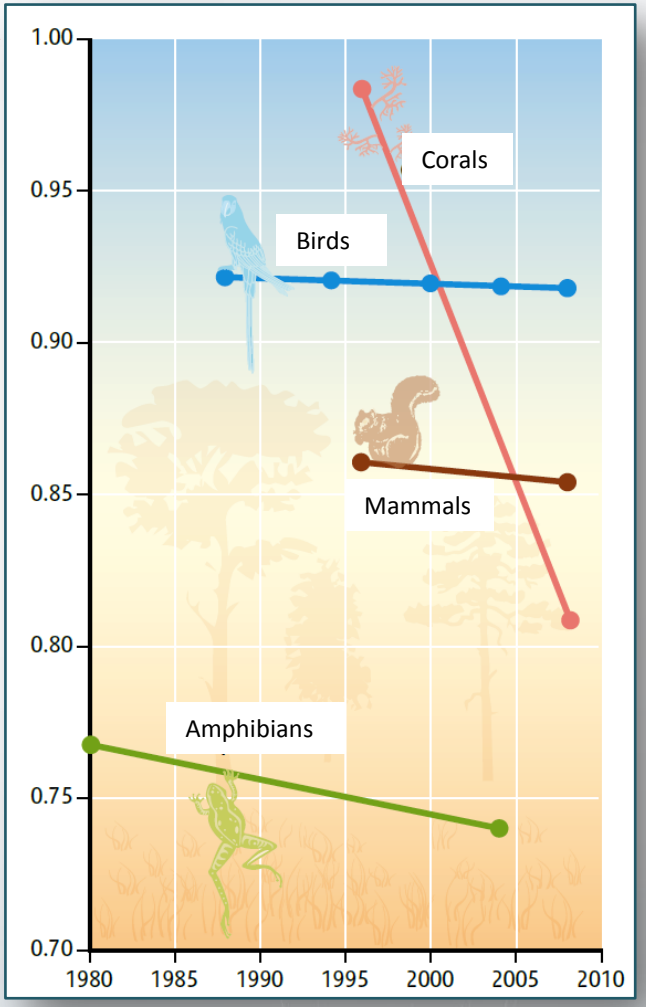
Case studies

- Terrestrial environments
- Aquatic environments
- Environmental samplers

Conclusion

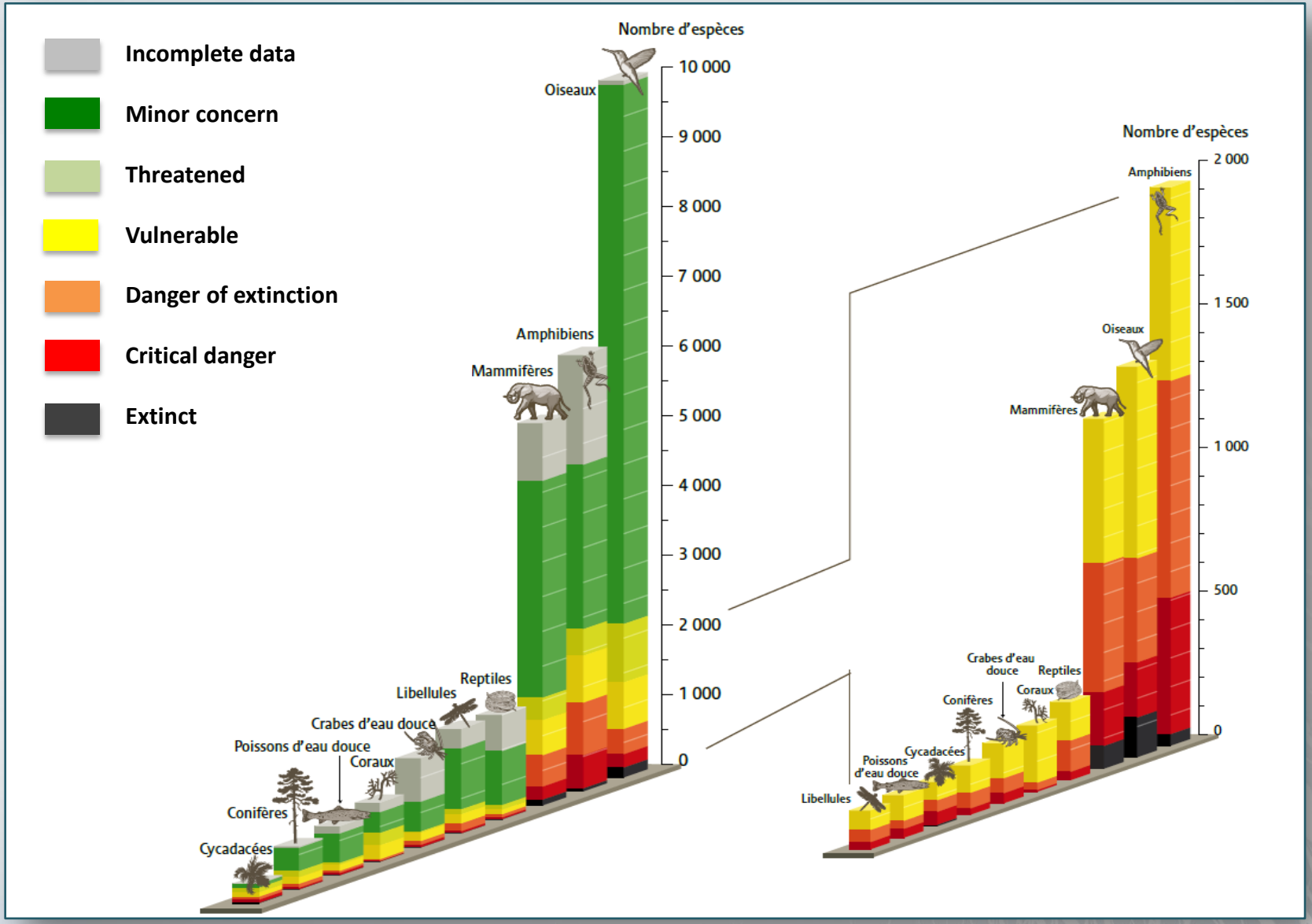
Loss of biodiversity at the world scale from several decades

Minor concern



Following how biodiversity evolves through time:
Red list index

Major concern: risk of extinction



e.g. 1/3 of more than 6000 recorded amphibian species are threatened by extinction

- Refers to DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organism

- Characterized by a **complex mixture** of genomic DNA from different organisms and by possible **degradation**

- Total eDNA contains **cellular DNA** (living cells or organisms) and **extracellular DNA** (resulting from natural cell death and subsequent destruction of cell structure)

- **Feces** contains DNA from all consumed items plus host DNA
- **Hairs**
- **Soil** contains:
  - Intracellular DNA mainly from bacteria, fungi, roots
  - Extracellular DNA from all organisms living around (bacteria, fungi, plants, animals, etc.)
- **Water** contains animal / plant / microorganism DNA
- Other sources: e.g. urine, tracks from soil or snow, **environmental samplers**

Terminology

Complexity of DNA extracted

Single specimen

Multiple specimens (bulk samples)

Environmental samples (air, water, soil, feces …)

Taberlet et al. 2012

Terminology

Identification level

Species level

Genus, Family or Order level

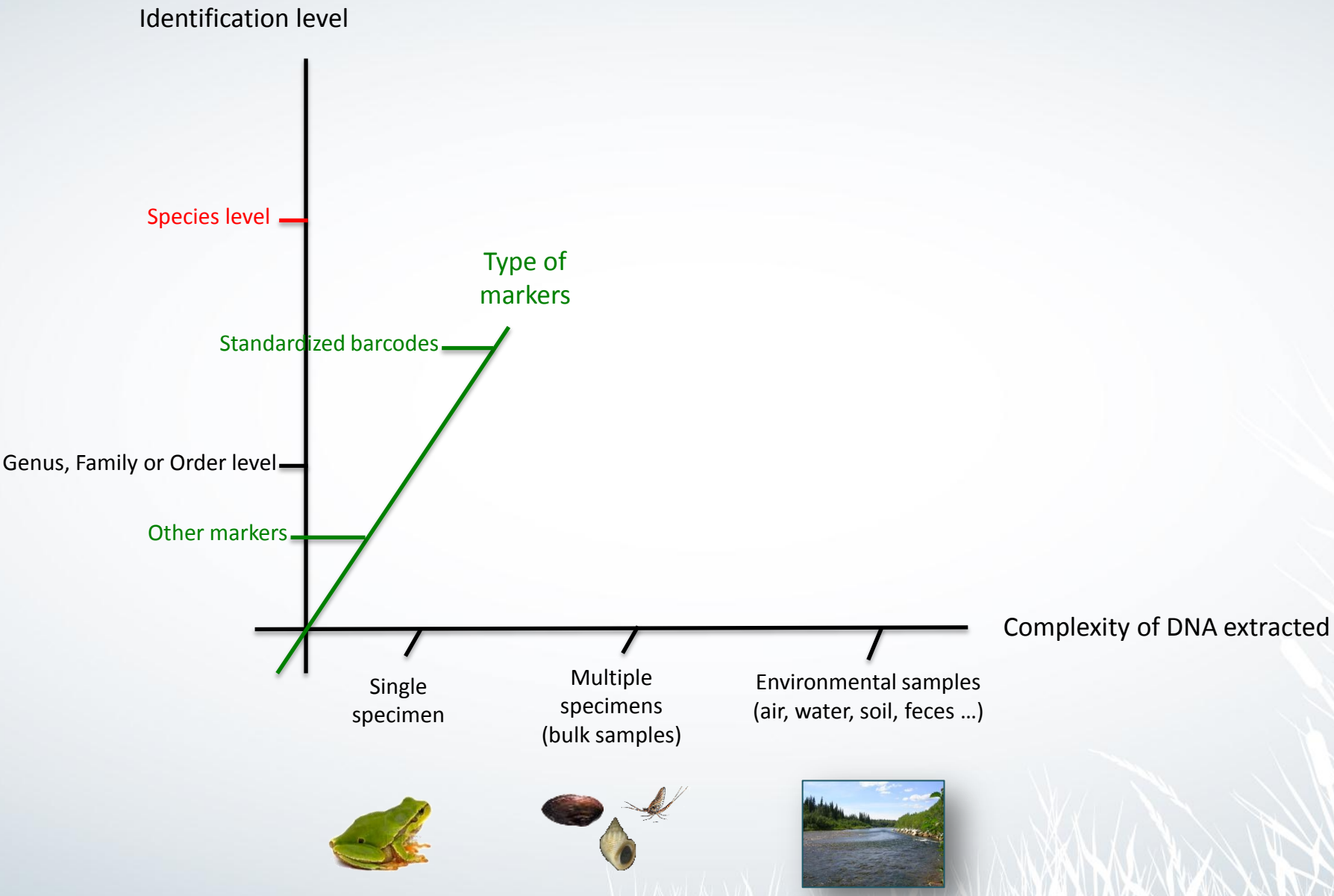
Complexity of DNA extracted

Single specimen

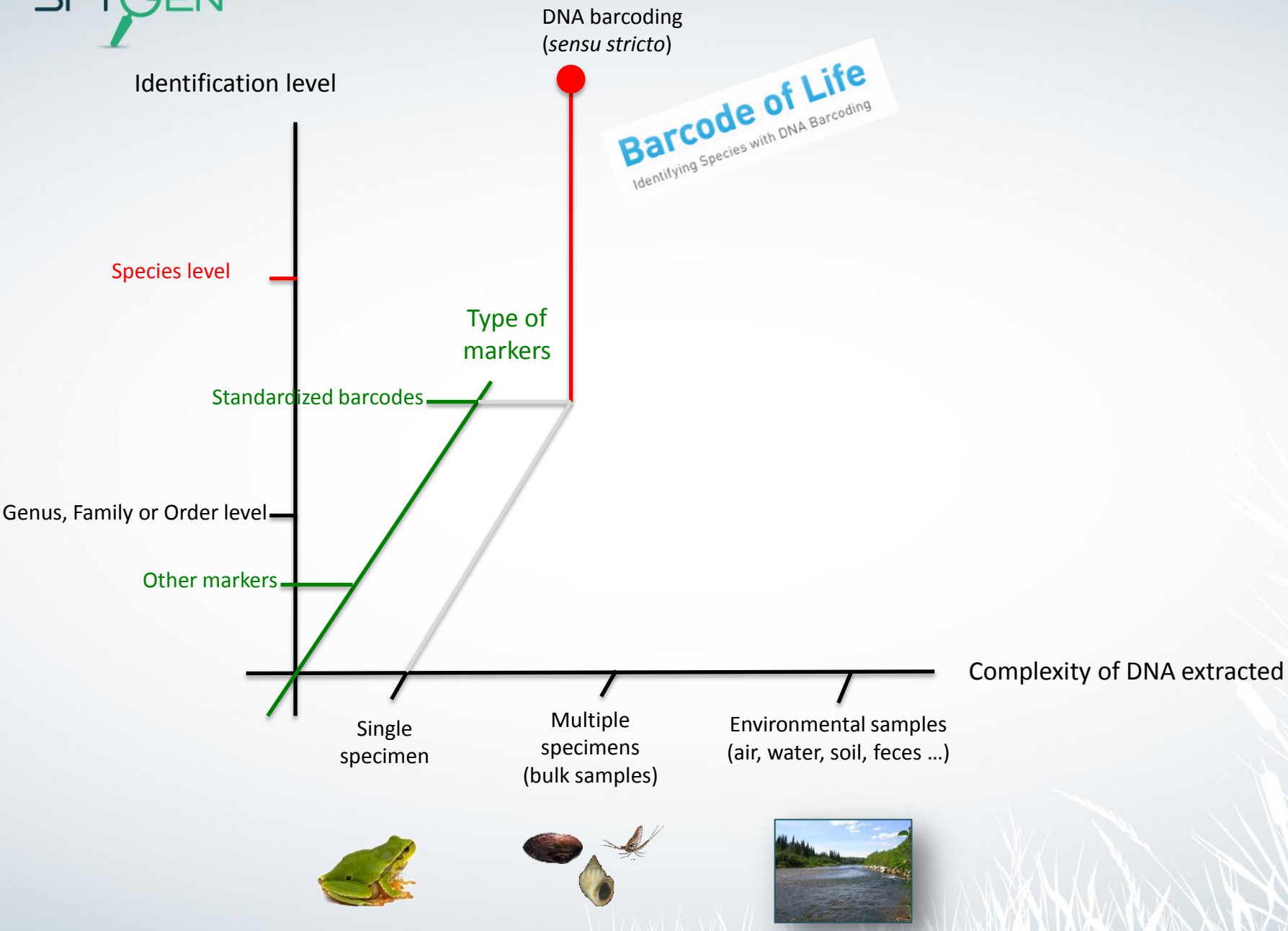
Multiple specimens (bulk samples)

Environmental samples (air, water, soil, feces …)

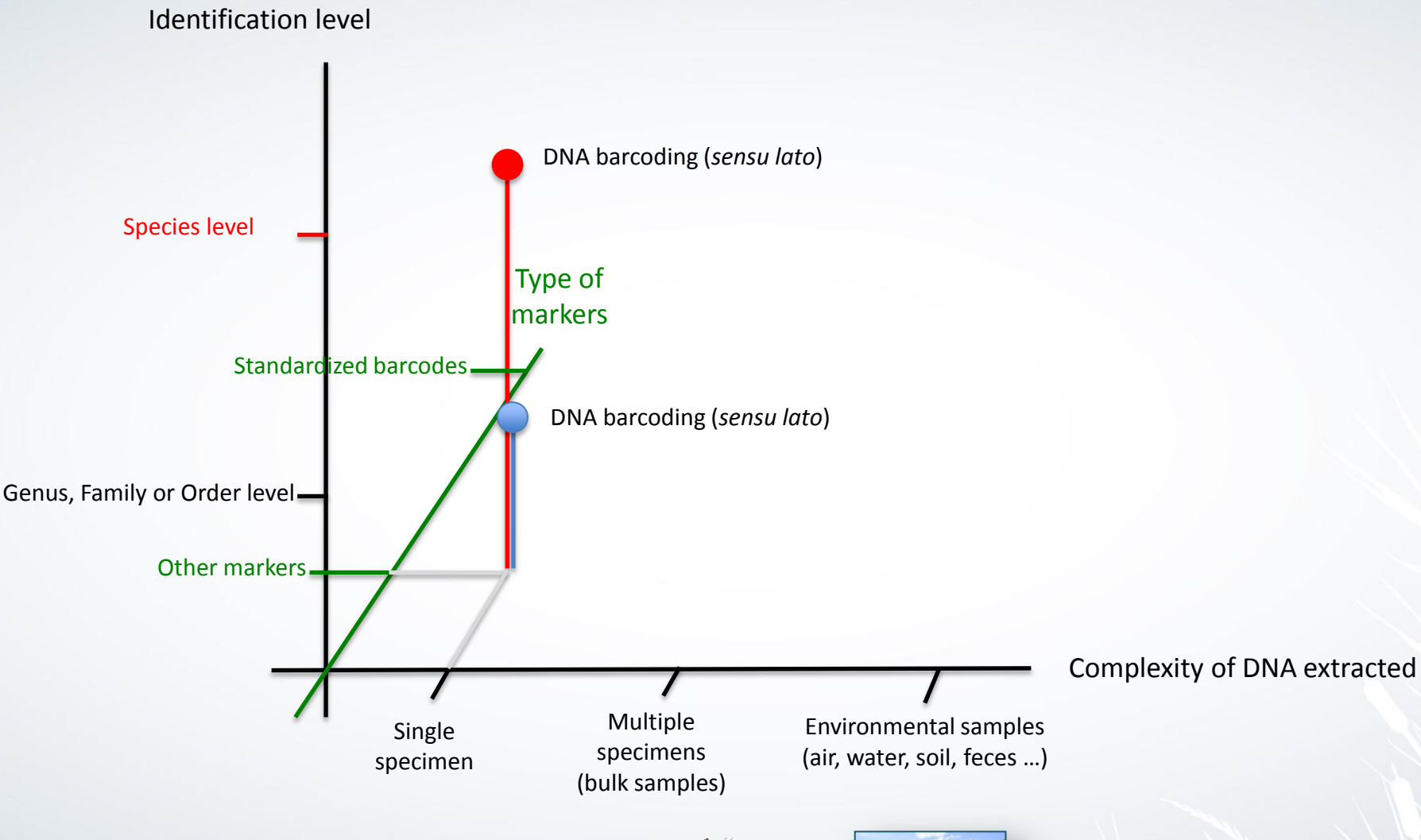
Taberlet et al. 2012



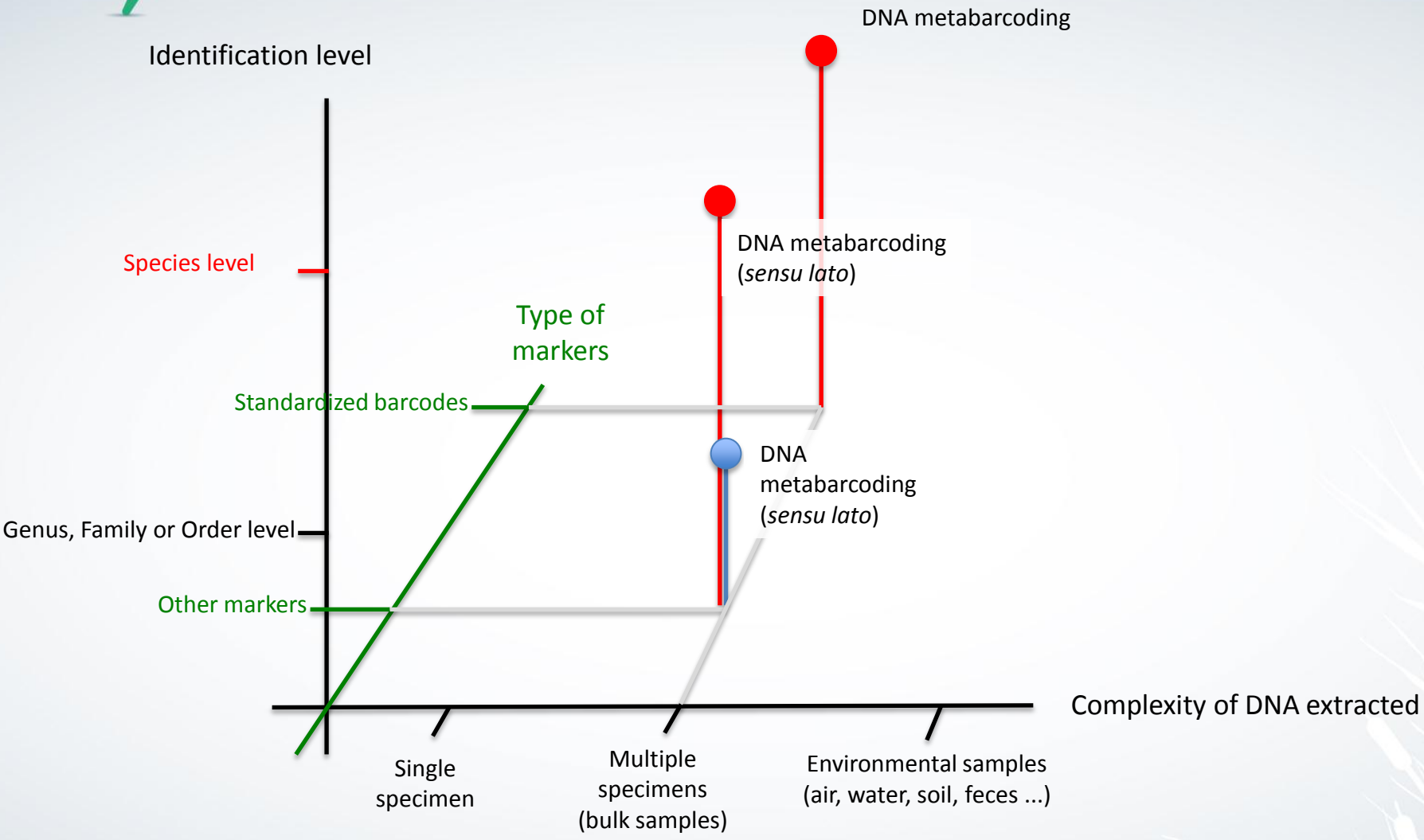
Taberlet et al. 2012



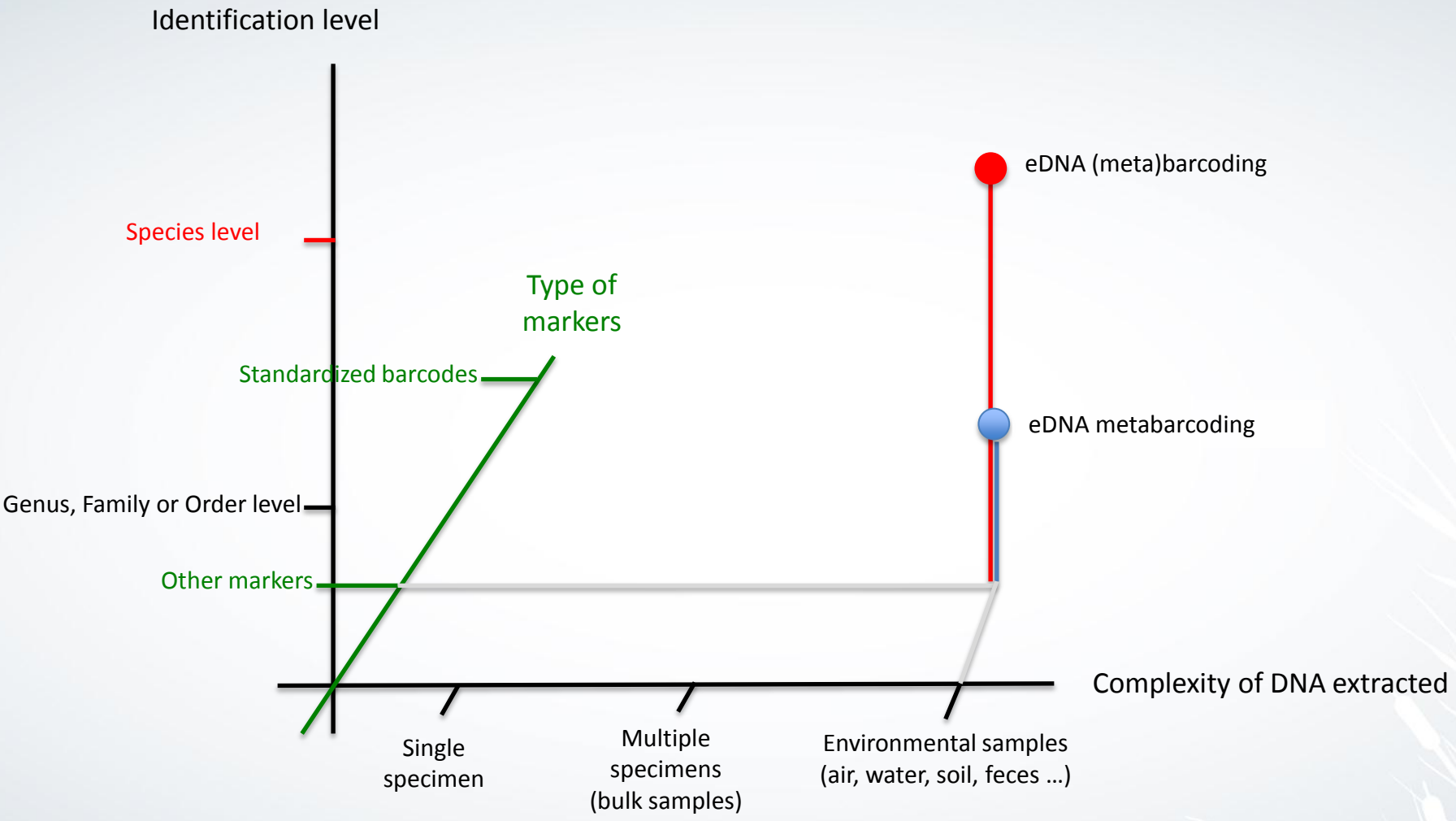
Taberlet et al. 2012



Taberlet et al. 2012

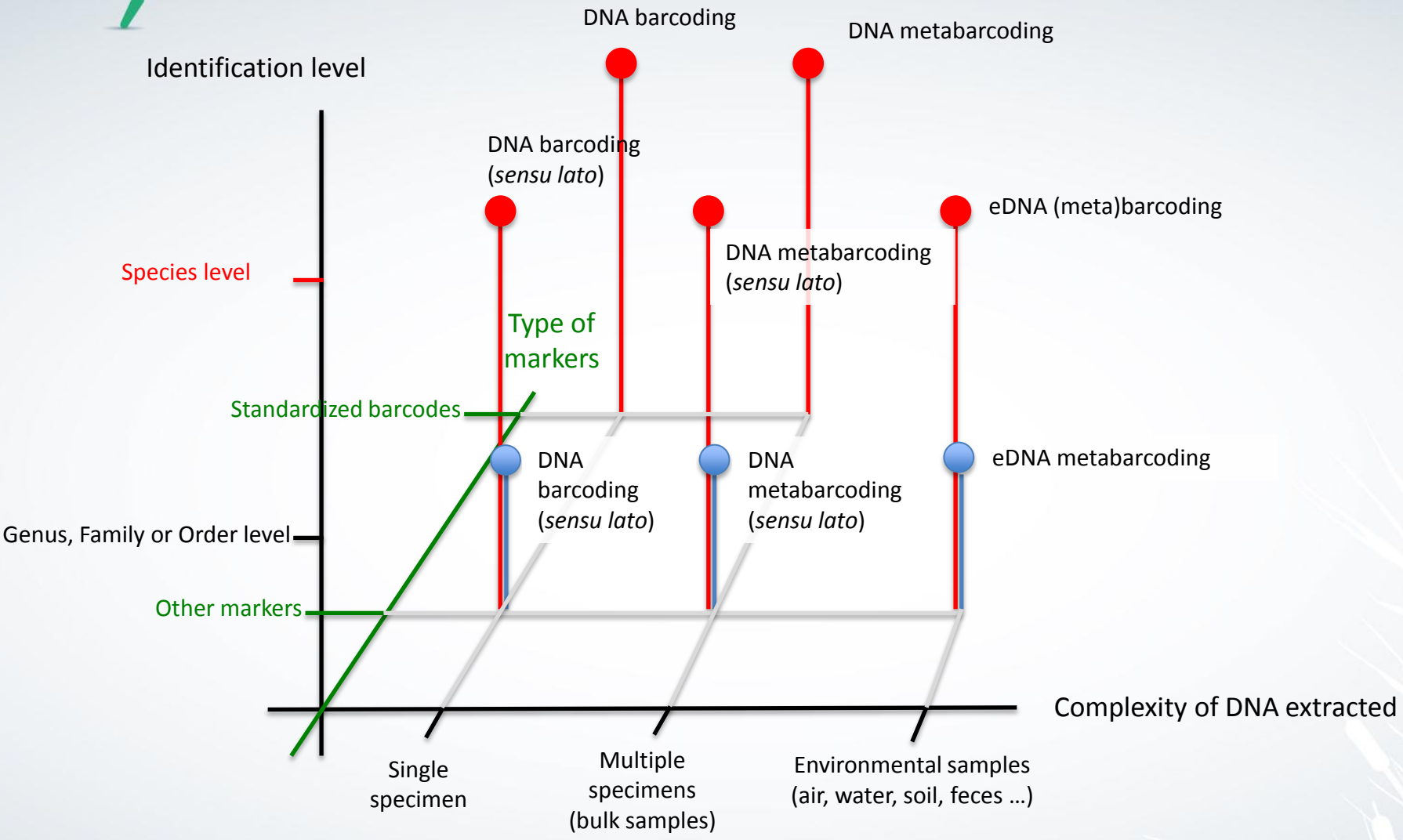


Taberlet et al. 2012



Taberlet et al. 2012

Terminology



Taberlet et al. 2012

DNA Barcoding

DNA extraction

Amplification using universal primers COI

Sanger sequencing

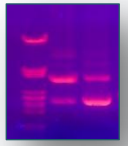
Hyla arborea

eDNA Barcoding

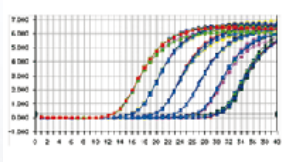
DNA extraction

Classical PCR

Electrophoresis



qPCR



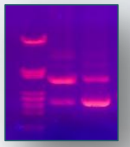
(SYBRgreen or TaqMan)

using specific primers for *Hyla arborea* (<100 pb)

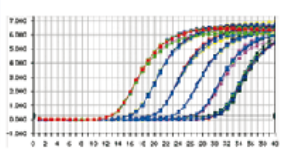
eDNA Barcoding

DNA extraction

Classical PCR → Electrophoresis



qPCR



(SYBRgreen or TaqMan)

using specific primers for *Hyla arborea* (<100 pb)

eDNA Metabarcoding

DNA extraction

Amplification using **amphibian** primers < 100 pb

Next Generation Sequencing

Comparison with reference databases

Standard barcodes

- 658 bp of Cytochrome Oxydase I (**CO1**) for animals
- ~500 bp of ***rbc*L** or ~800 bp of ***mat*K** for plants
- **ITS2** for fungi

Not usable with eDNA because:

- The amplified fragments are too long for degraded DNA
- CO1: strong bias when amplifying a mixture of species
  (Coding regions: 3rd base highly variable)

eDNA metabarcodes

- Must amplify short DNA fragments
- Must be adapted for the different taxonomic groups
- Must be highly versatile (to equally amplify the different target DNAs)
- Must have a good taxonomic resolution (ideally to the species level)

Studying plant biodiversity

Above ground surveys

eDNA sampling

→ Amplification using *trn*L fragment (gh primers)

Yoccoz et al. 2012

Studying plant biodiversity

Size of the pictures proportional to ….

… species density

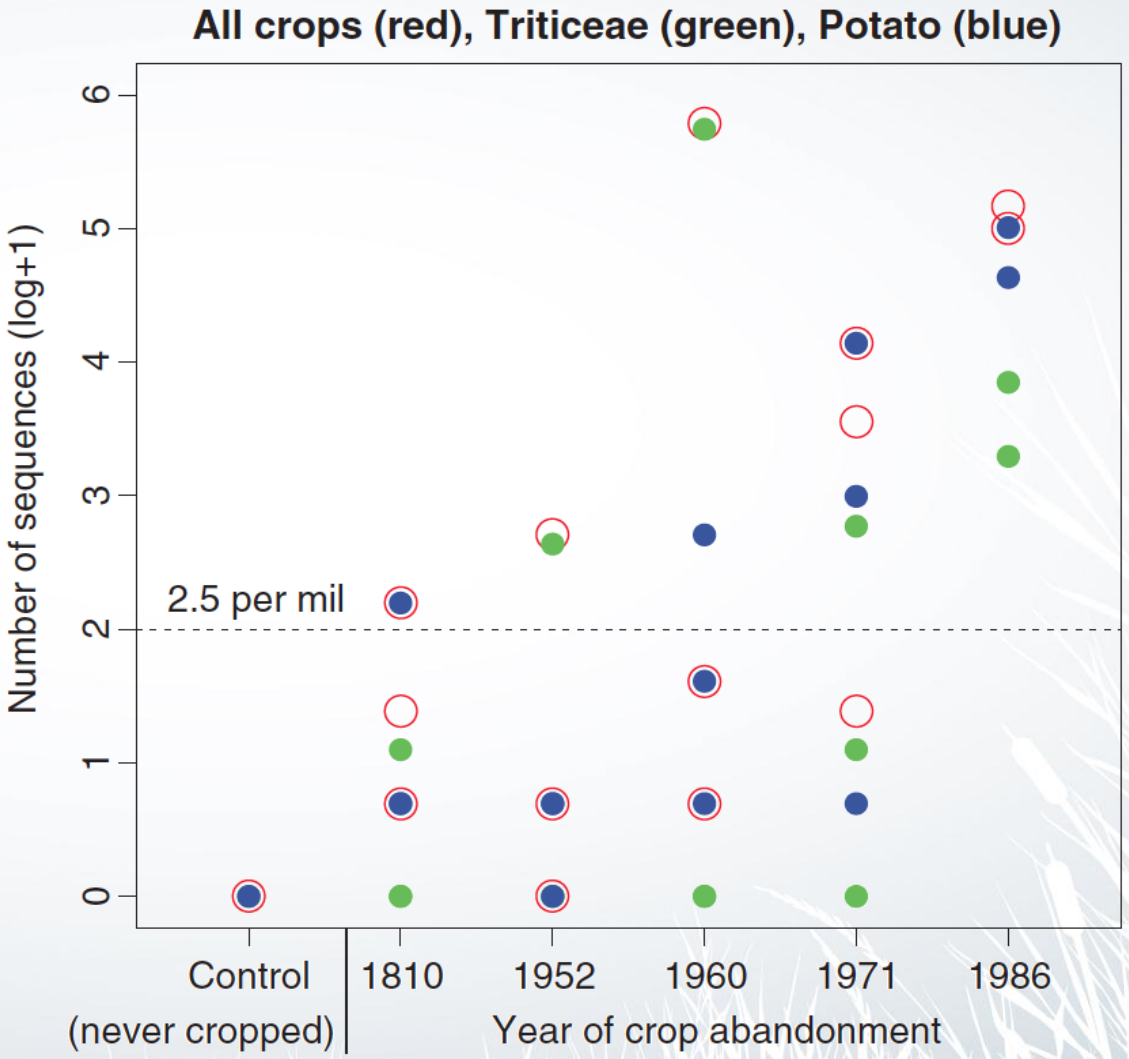
… number of sequences

Above-ground surveys

Field sampling

Yoccoz et al. 2012

How long does a DNA molecule persist in soil?



Yoccoz et al. 2012

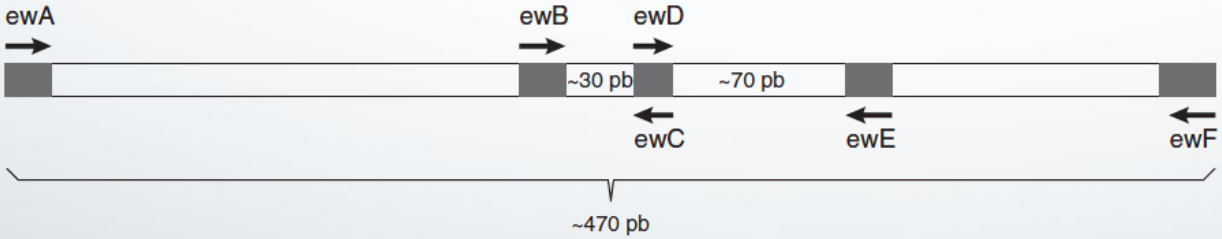
Earthworm diversity: eDNA-based approach vs traditional handsorting method

Earthworms → important role with the functioning of an ecosystem

Their diversity can be used as an **indicator of ecosystem health**

Conventional extraction methods laborious and time consuming

Reference database: 14 species from french Alps → Design new primer pairs (16S)



Bienert et al. 2012

Earthworm diversity : eDNA-based approach vs traditional handsorting method

	Chartreuse			Grenoble		
	DNA (no. of sequence reads)			DNA (no. of sequence reads)		
Species	ewB/ewC	ewD/ewE	Handsorting (no. of individuals)	ewB/ewC	ewD/ewE	Handsorting (no. of individuals)
Allobophora chlorotica	—	—	—	95 149	3918	—
Aporrectodea cupulifera	—	—	—	472 702	84 217	5
Aporrectodea icterica	1 486 631	123 684	—	2 193 386	95 841	—
Aporrectodea longa	—	—	—	516 596	52 225	—
Aporrectodea rosea	2106 (?)	17 017	—	—	—	—
Aporrectodea sp.	107 789	—	32	—	—	9
Lumbricus castaneus	—	—	—	(?)	(?)	4
Lumbricus friendi	—	—	7	—	—	—
Lumbricus terrestris	—	—	—	449 025	77 425	116
Octolasion cyaneum	472 285	29 482	12	—	—	—
Octolasion tyrtaeum	306 476	14 430	—	—	—	—

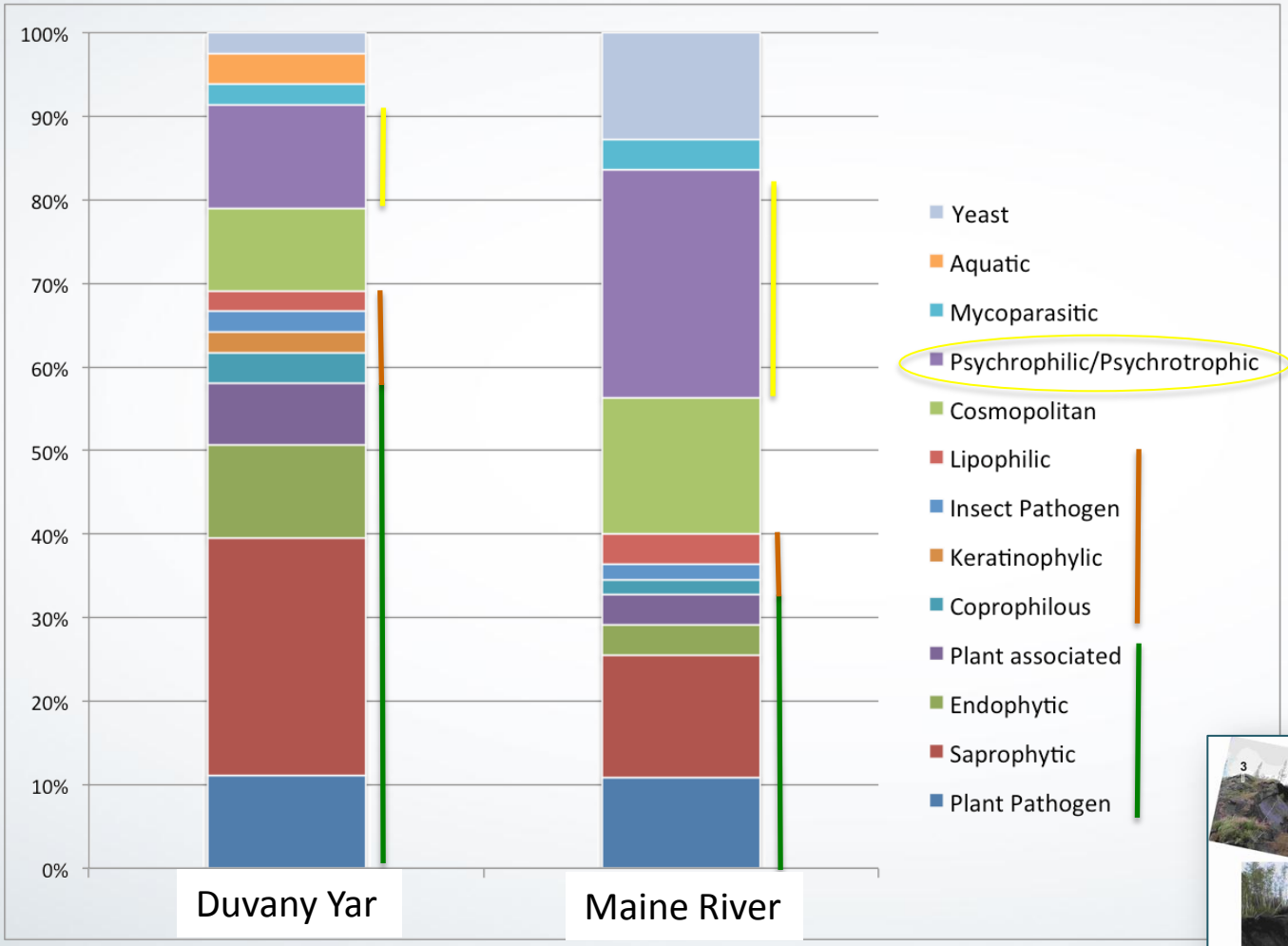
Endogeic species

Epigeic species

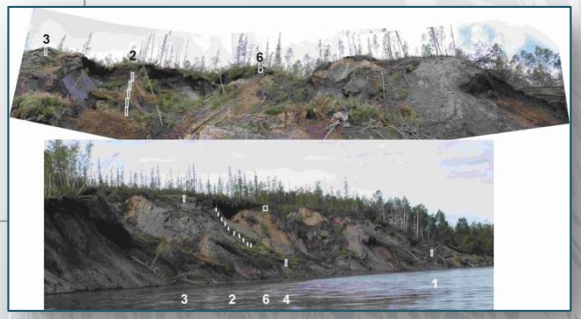
→ Better representation of earthworm diversity could be achieved by taking larger samples

Bienert et al. 2012

Fungi diversity in permafrost samples



79 permafrost cores (16,000-32,000 years-old) from Siberia



Proportion of OTUs identified according to their putative ecological groups

Bellemain et al. 2012

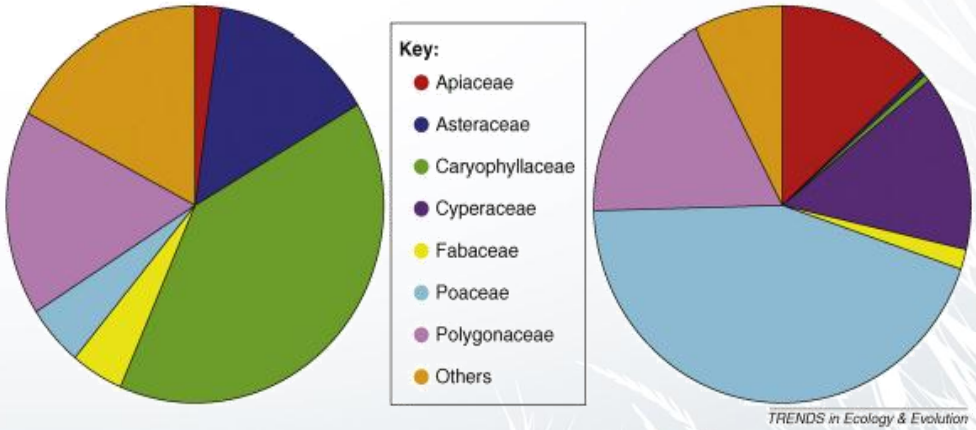
Brown bear (*Ursus arctos*) and golden marmot (*Marmota caudata*) plant diets in the Himalayan environment.

Deosai National Park, Pakistan

Golden marmot

Brown bear

- 12 feces from each species
- Universal plant primers
- 454 sequencing



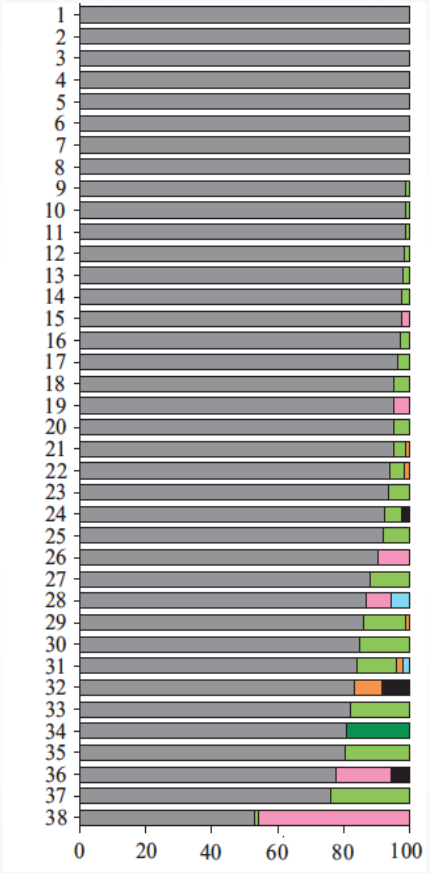
→ Different diets of the 2 species: bears prefer *Poaceae,* whereas marmots prefer *Caryophyllaceae*

Valentini et al. 2009

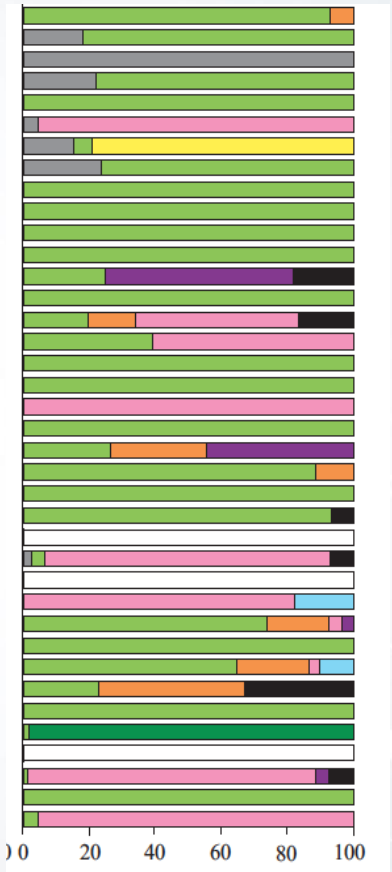
Leopard cat diet
(carnivore)

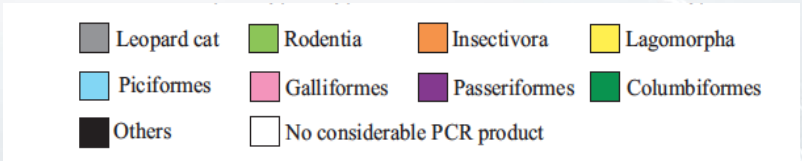
- 38 feces
- Universal vertebrate primers
- Illumina sequencing

Universal vertebrate primers



Universal vertebrate primers
+ blocker for leopard cat





Shehzad et al. 2012

First study showing species detection using eDNA from water samples

Bullfrog
(*Lithobates catesbianus*)

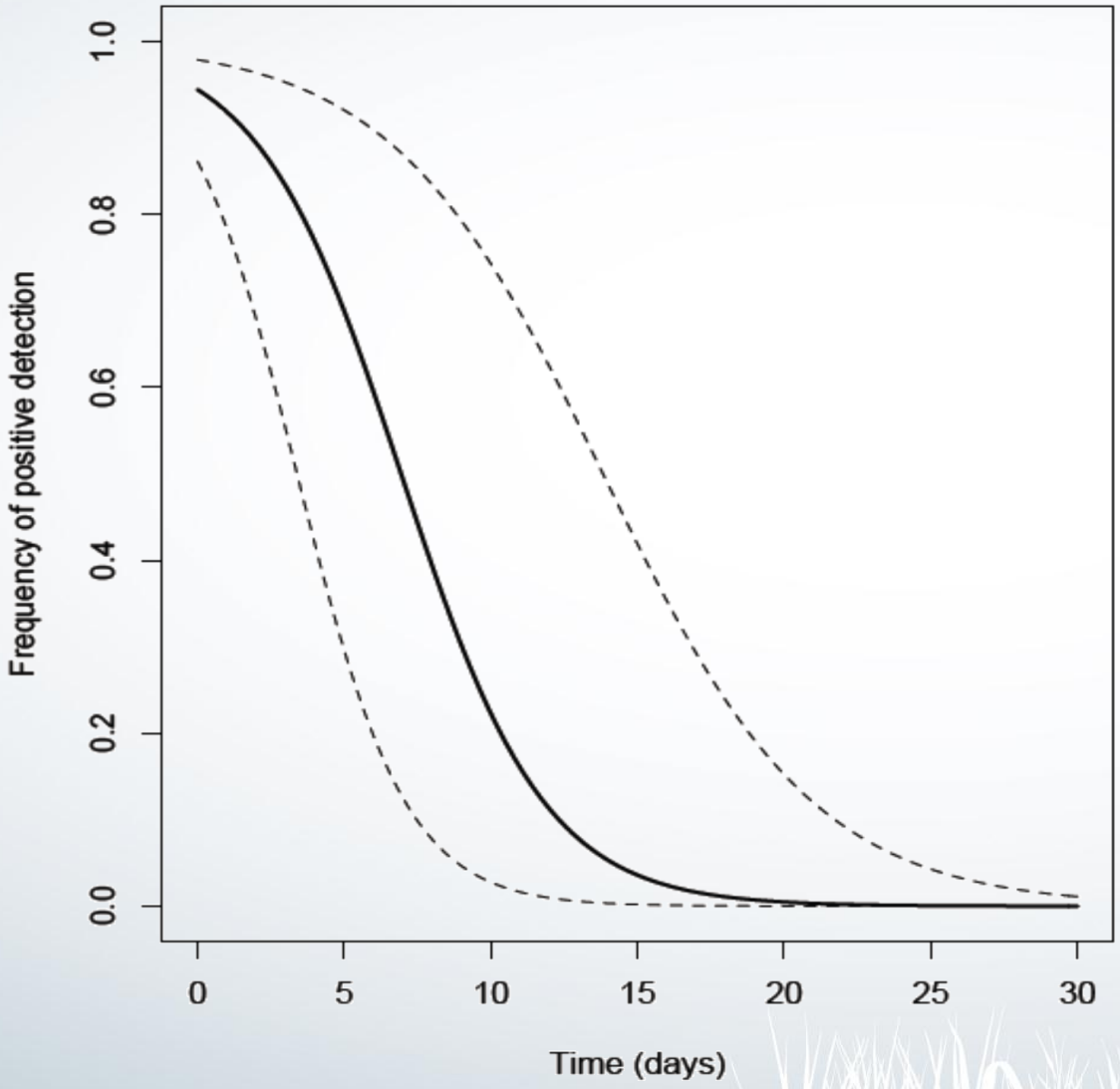
Sampling of water (15 ml * 3 tubes per sampling location) over 9 ponds

Detection

- High density 
- Low density 
- Absence 

Ficetola et al. 2008

How long does a DNA molecule persist in water?



Dejean et al. 2012

Distribution of the Asian carp in the U.S

1997

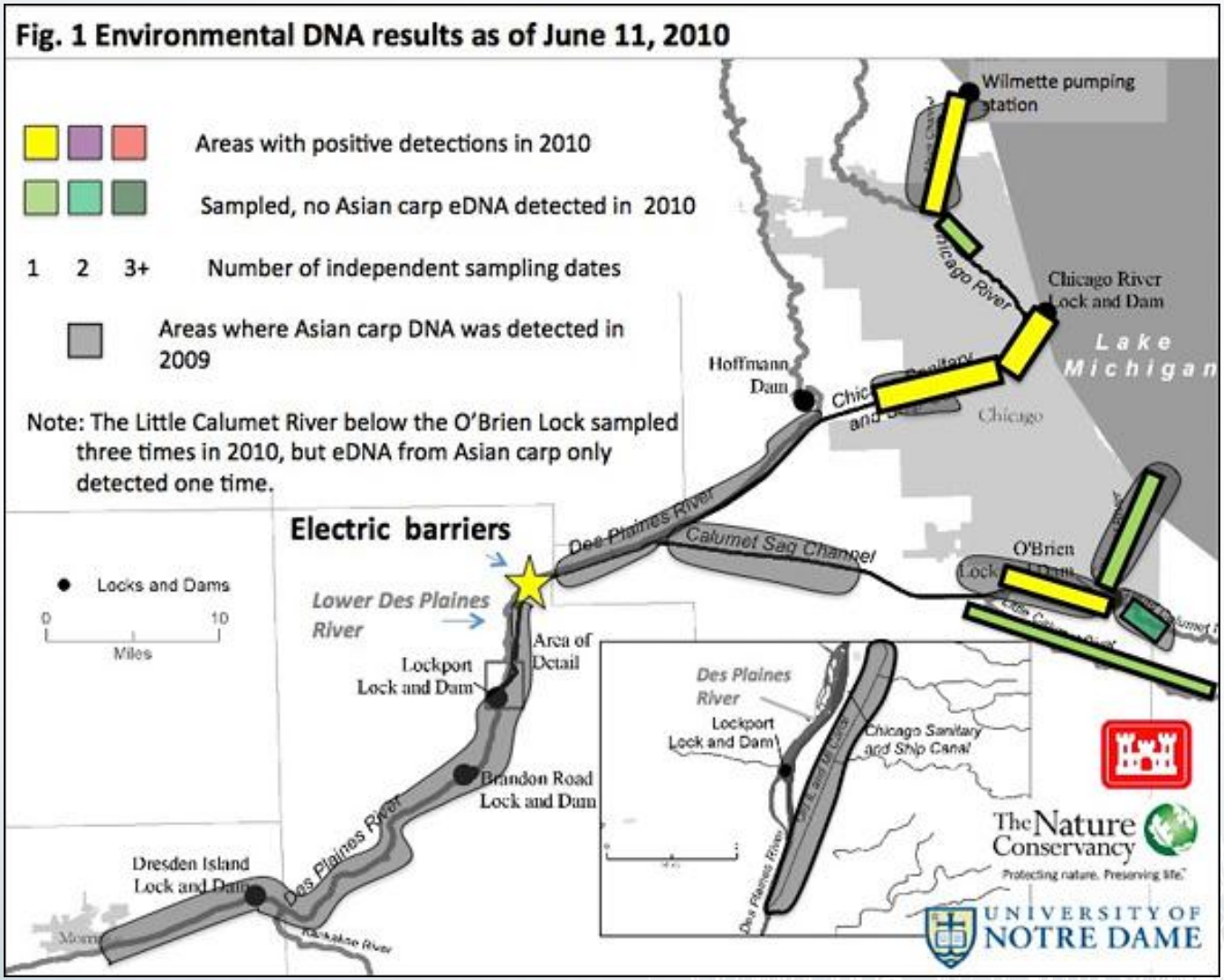
2004

Bighead Carp Distribution

Silver Carp Distribution

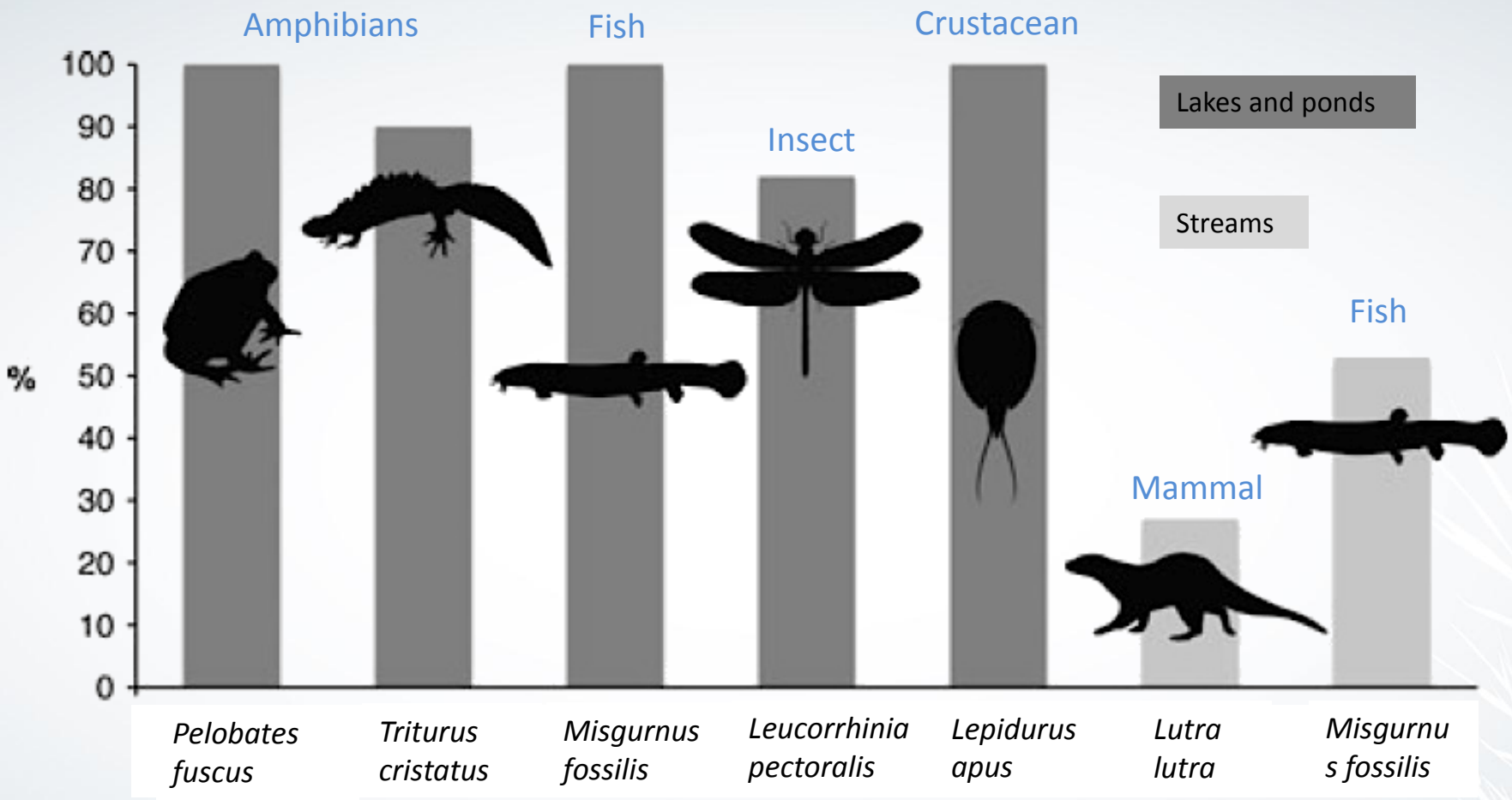
Jerde *et al.* 2011

Distribution of the Asian carp in the U.S



Jerde *et al.* 2011

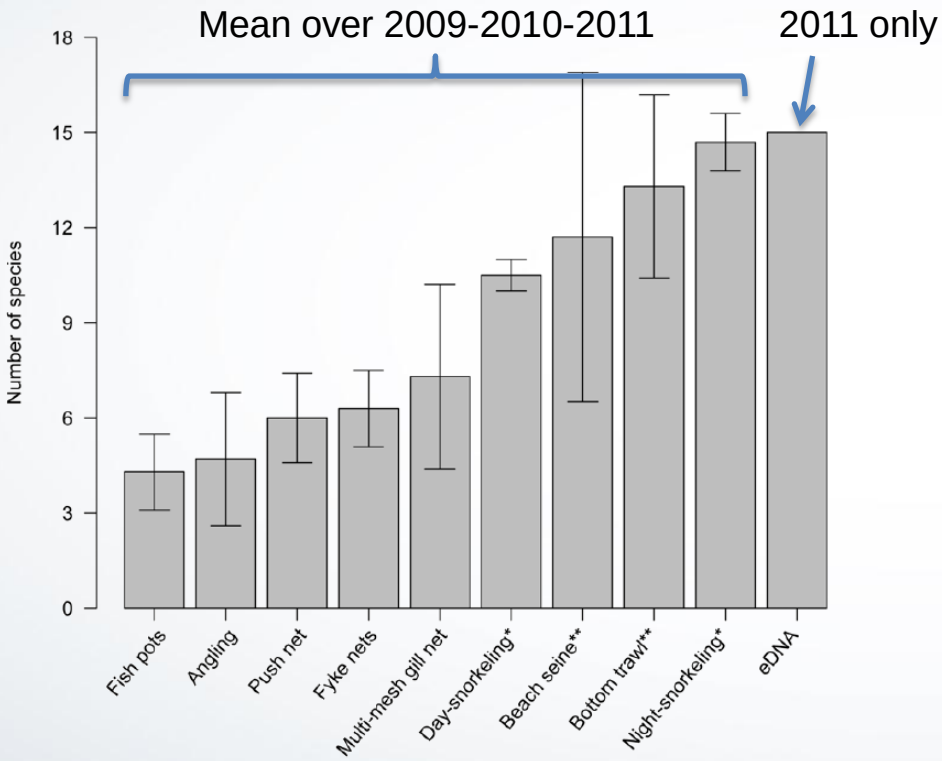
Case study: running waters



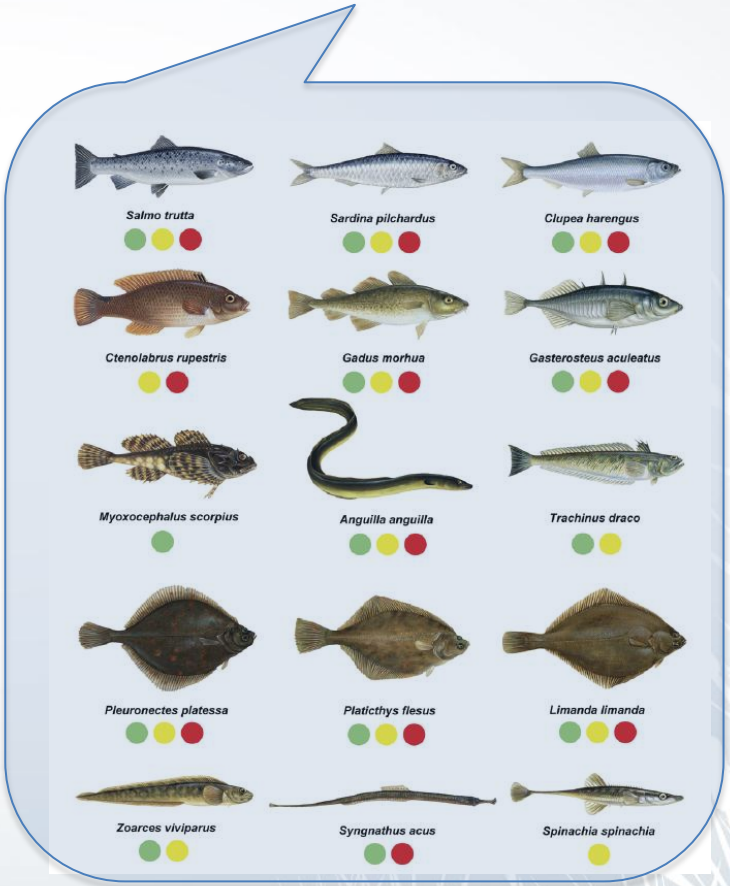
Environmental DNA detection rates by qPCR in natural freshwater ponds (% positive localities out of the total number of localities surveyed for each species).

Thomsen et al. 2012

Detection of marine fishes using eDNA from seawater samples (1st study)



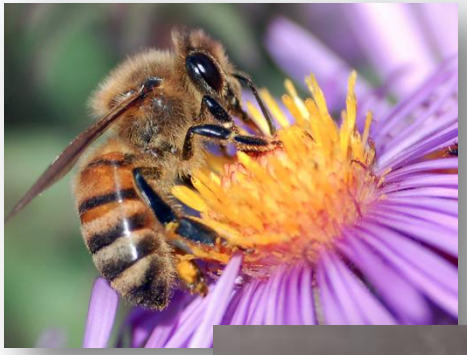
From 0.5 liter of seawater



Number of fish species recorded by 9 different conventional survey methods and eDNA at The Sound of Elsinore, Denmark.

Thomsen et al. 2012

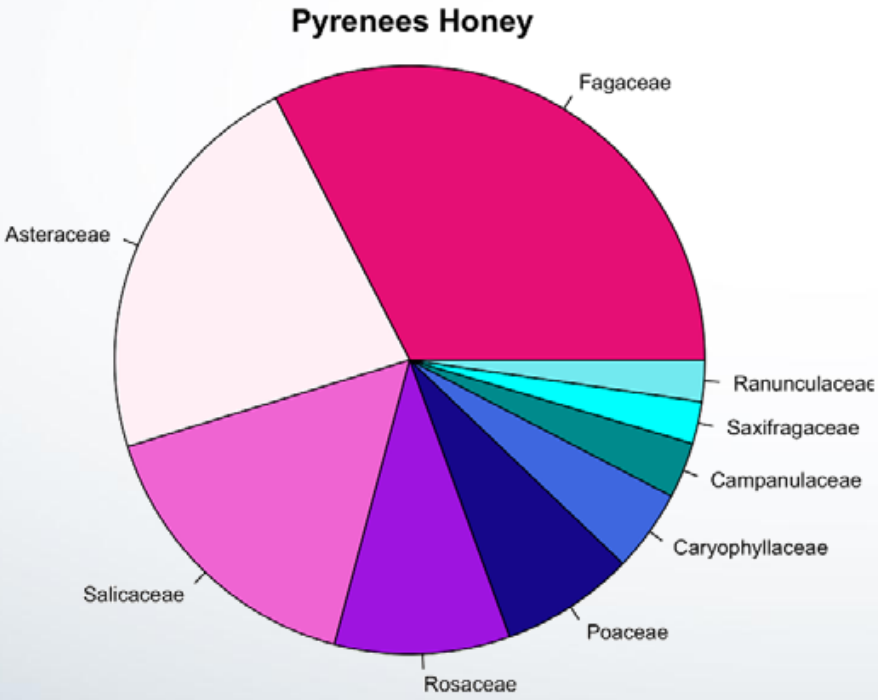
Environmental samplers

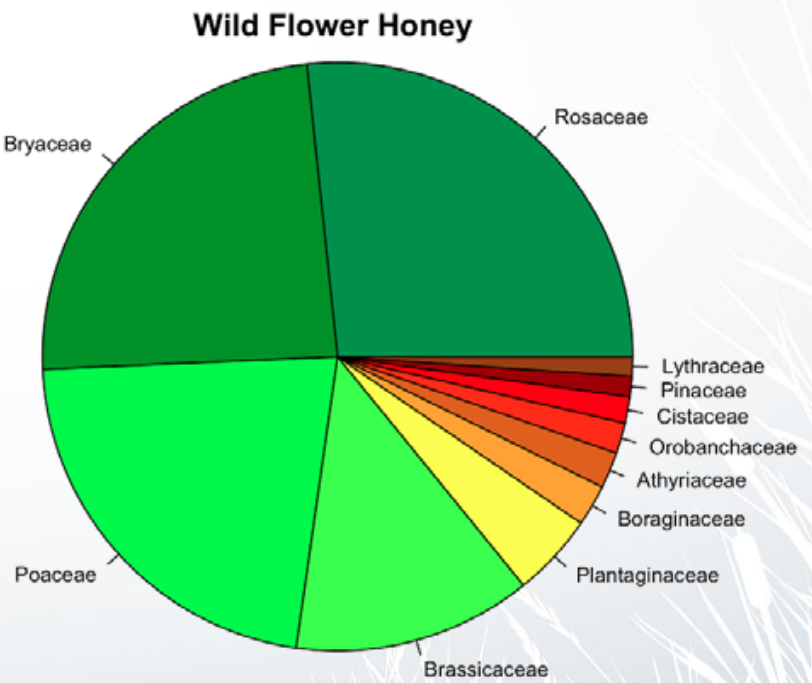


Plants

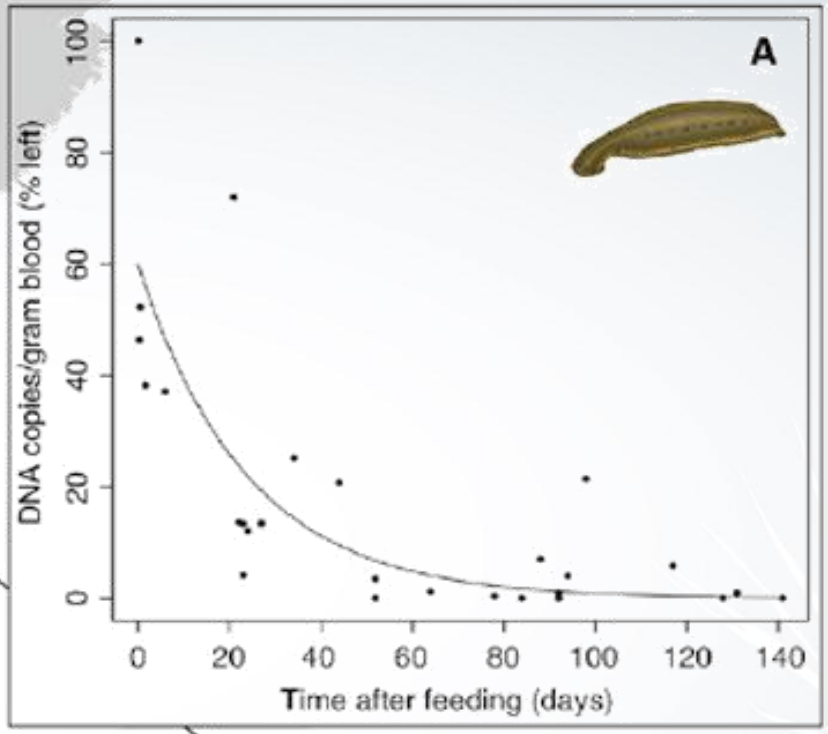
Mammals

Environmental samplers

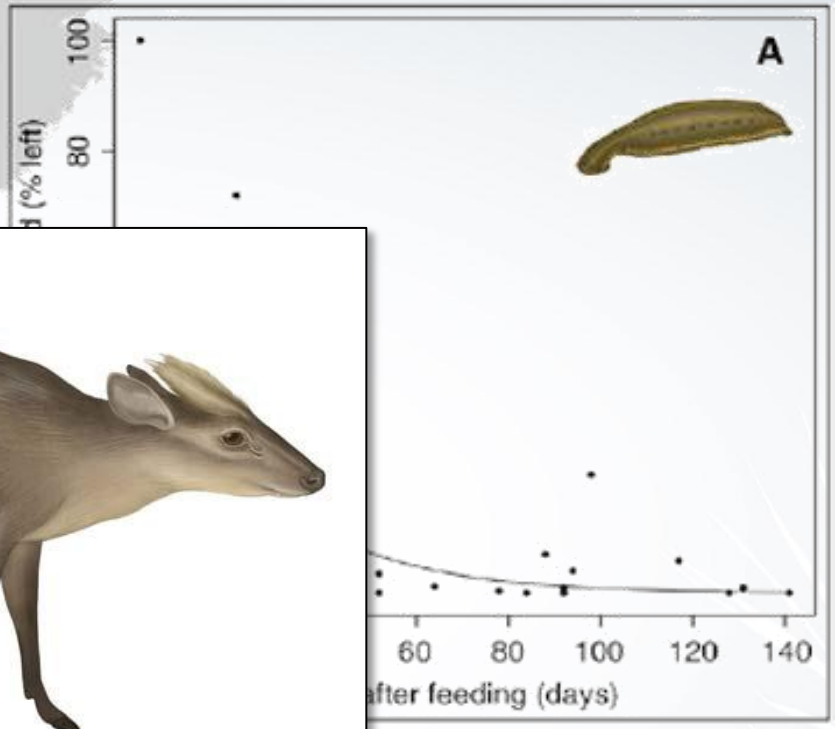


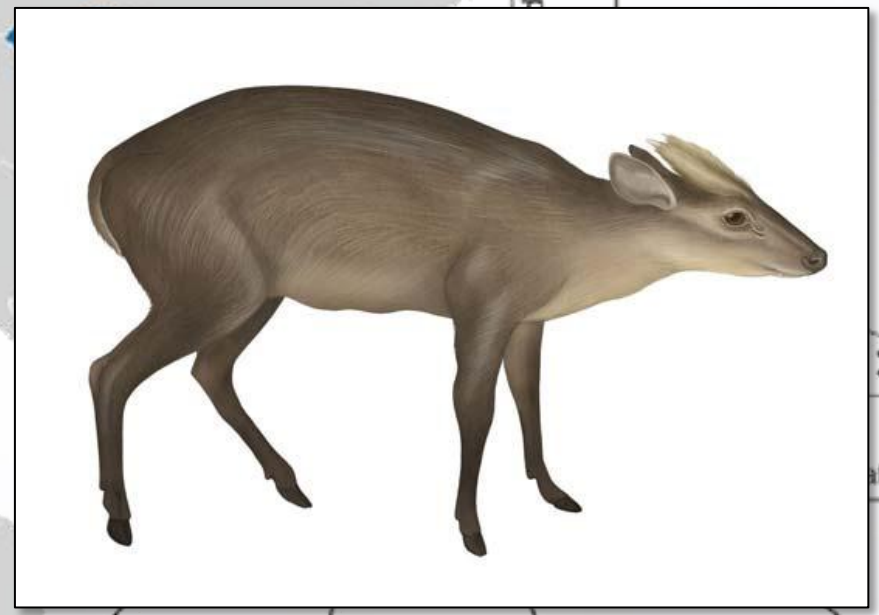


Valentini *et al.* 2010



Schnell *et al.* 2012





The Truong Son munjtac (*Muntiacus truongsonensis*)

Elusive mammalian species

Schnell *et al.* 2012

Environmental samplers

Calvignac-Spencer *et al.* 2013

Cephalophus jentinki

Philantomba maxwellii

Hexaprotodon liberiensis

Hyemoschus aquaticus

Atherurus africanus or *Hystrix cristata*

Crocidura sp.

Bycanistes sp.

Arthroleptis sp.

Cercocebus atys

Cercopithecus diana

Cercopithecus campbelii

Piliocolobus badius badius

Colobus polykomos

Cercopithecus nictitans

Myonycteris torquata

Hypsignathus monstrosus

Calvignac-Spencer *et al.* 2013

- Meaningful results when studying plant, animal and fungi DNA for different applications (Diet analysis, Detection of target species, Biodiversity analyses) and from different sources (Feces, soil, water, eDNA samplers)

→ Sampling and DNA extraction validated

- Challenges: need for further optimizations at different levels:

- Sampling strategy
- Laboratory
- Markers
- Reference databases
- Bioinformatics / Biostatistics

Thanks for your attention!

More studies in the special issue of *Molecular Ecology* (vol 21, issue 8)