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Institute of Chemistry, Technology and Metallurgy
National Institute of the Republic of Serbia

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BIOLAWEB

KICK-OFF MEETING

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Acronym: BIOLAWEB Boosting Institute of Chemistry, Technology and Metallurgy in Water Biomonitoring

Grant No: 101079234

Type of action: HORIZON Coordination and Support Actions (HORIZON - CSA)

Starting Date: 01/10/2022

Duration: 36 months

eDNA Workshop

Metabarcoding of macrophytes for biomonitoring

Belgrade, October 2023

BIOLAWEB
presentation

Funded by
the European Union

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See also

1st BIOLAWEB Workshop "Metabarcoding of diatoms and phytoplankton for biomonitoring“

https://youtube.com/playlist?list=PLdcHHeVPb7ARF--wPtfEGOgFnIOtm43aA

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Metabarcoding macrophytes

General introduction DNA

Idea: to develop a new tool for the monitoring of lakes based on eDNA = environmental DNA of macrophytes (Characeae)

Why environmental DNA = eDNA?

Are not the traditional methods used in monitoring studies enough (e.g., EU-WFD)?

- it is time consuming
- relatively expansive
- needs taxonomic knowledge

Why environmental DNA = eDNA?

using eDNA is using traces of organisms instead of whole organisms

Why environmental DNA = eDNA?

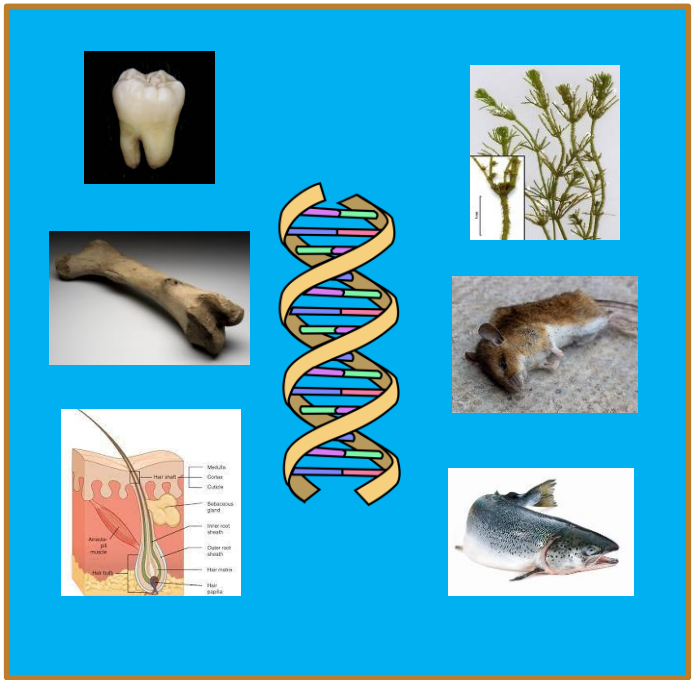
Why environmental DNA = eDNA?

The fingerprint  of all organisms in nature is:

= Environmental DNA (eDNA)

DNA: a tool for identification

- Living or dead organisms
- Cell remains
- fossils



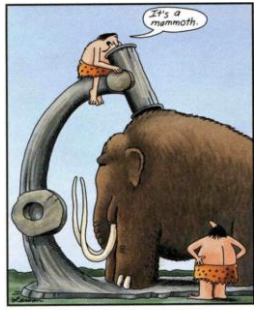
all organisms possess and leave behind traces of DNA from body particles (hair, skin particles, cells, free DNA etc.)

= **environmental DNA (eDNA)**

- Crime scenes

Advantages of using eDNA

- Potentially cheaper than traditional methods,
- Identification of a higher taxa number, compared to classical methods LM
- Detection of rare species (invasive species)



Early microscope

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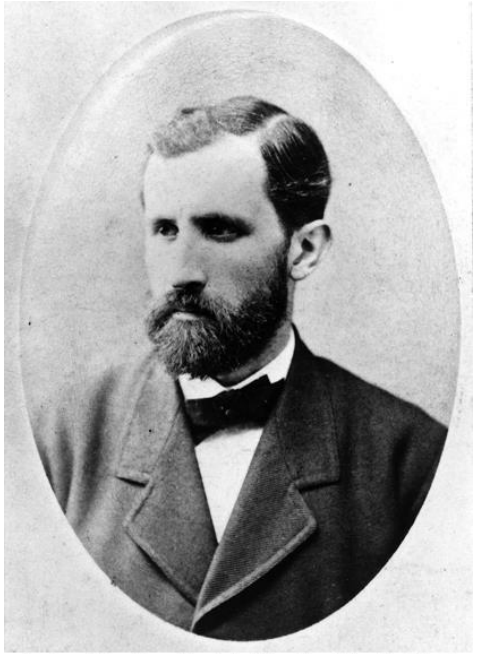
what is DNA?

DNA



Courtesy of Herrn Courvoisier, Portrait-Sammlung, University of Basel.
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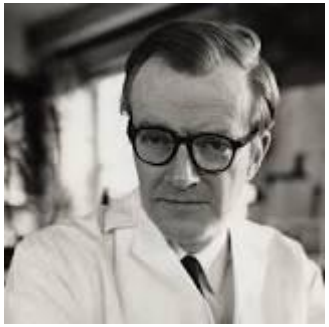
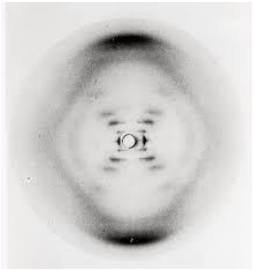
- Swiss chemist Friedrich Miescher first identified DNA in the 1860s,

DNA

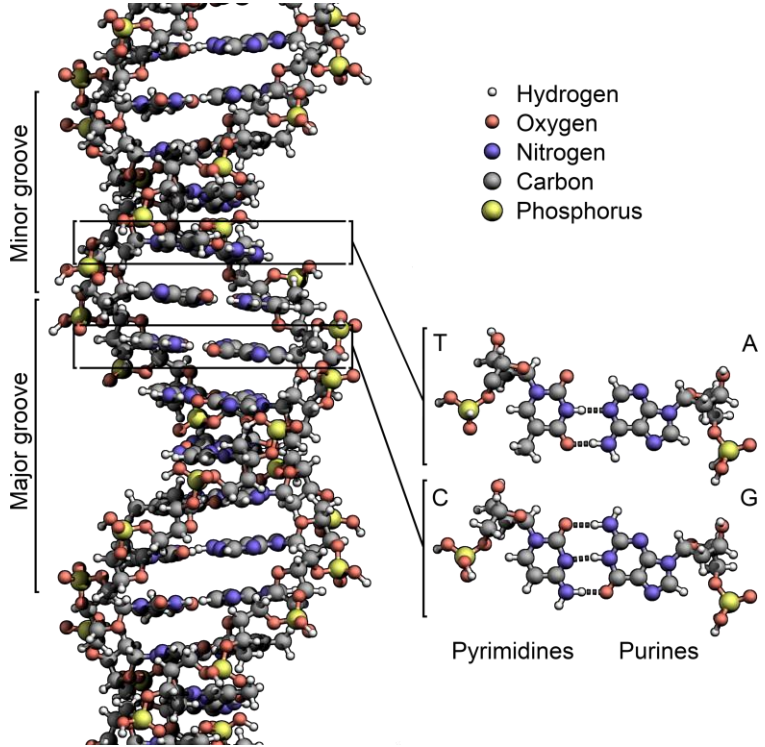


- James Watson, Francis Crick, officially discovered the double helix structure of DNA in 1953 (Nobel prize 1962) also for Maurice Wilkins)

Rosalind Franklin's X-ray diffraction

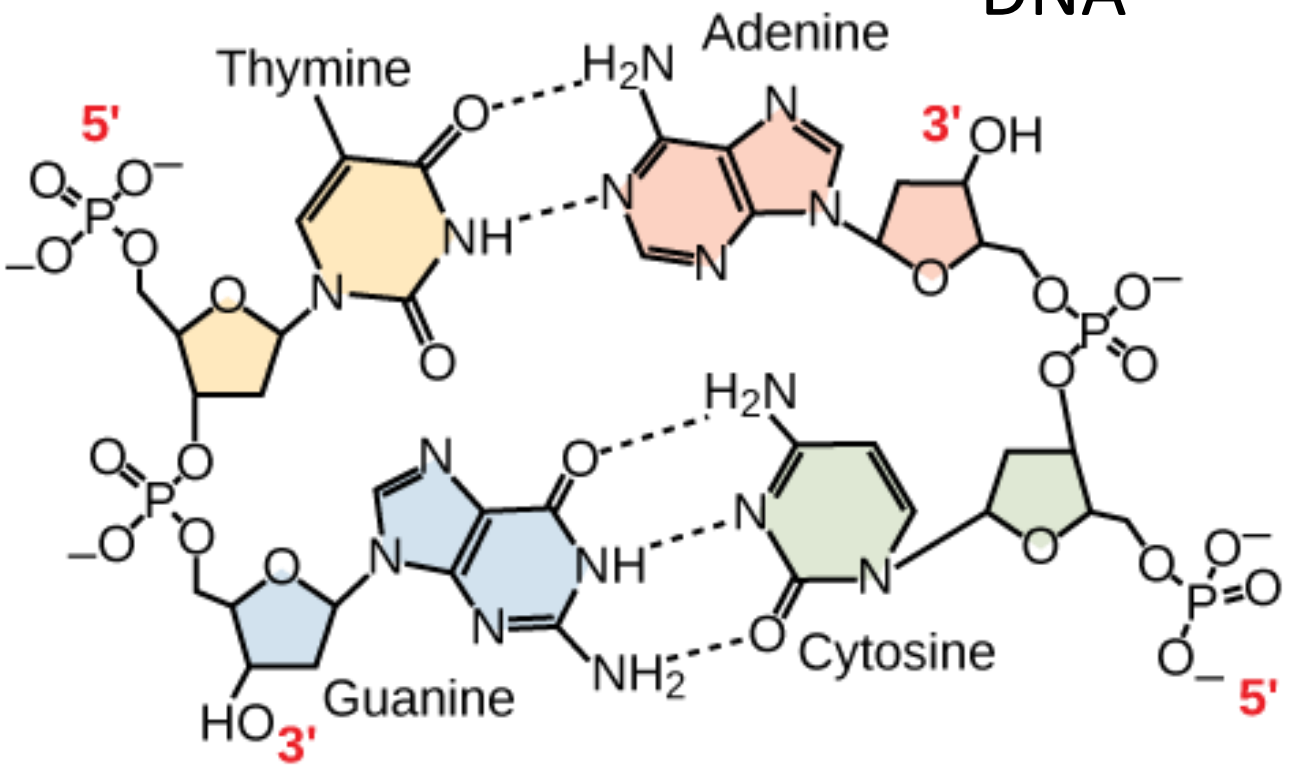


DNA



DNA: Deoxyribonucleic acid found in all living organisms as storage for genetic information

DNA



A, G Purin base

C, T Pyrimidin base

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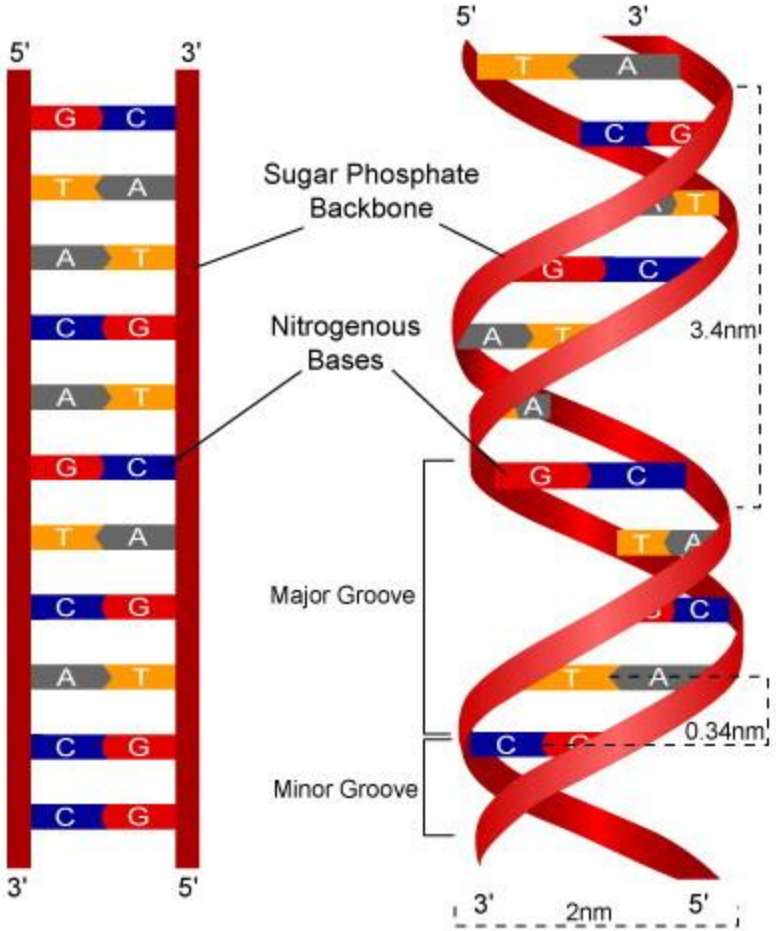
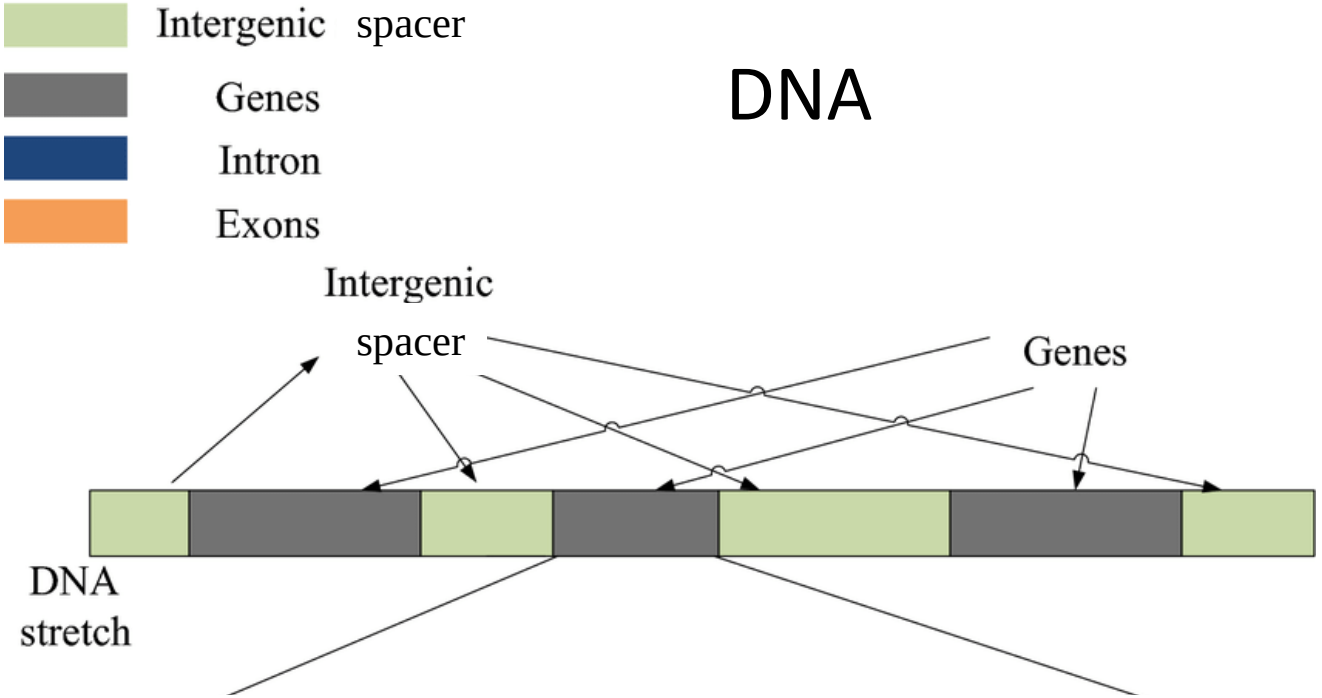
DNA



DNA


Intergenic spacer
Genes
Intron
Exons
Intergenic
spacer
Genes
DNA
stretch

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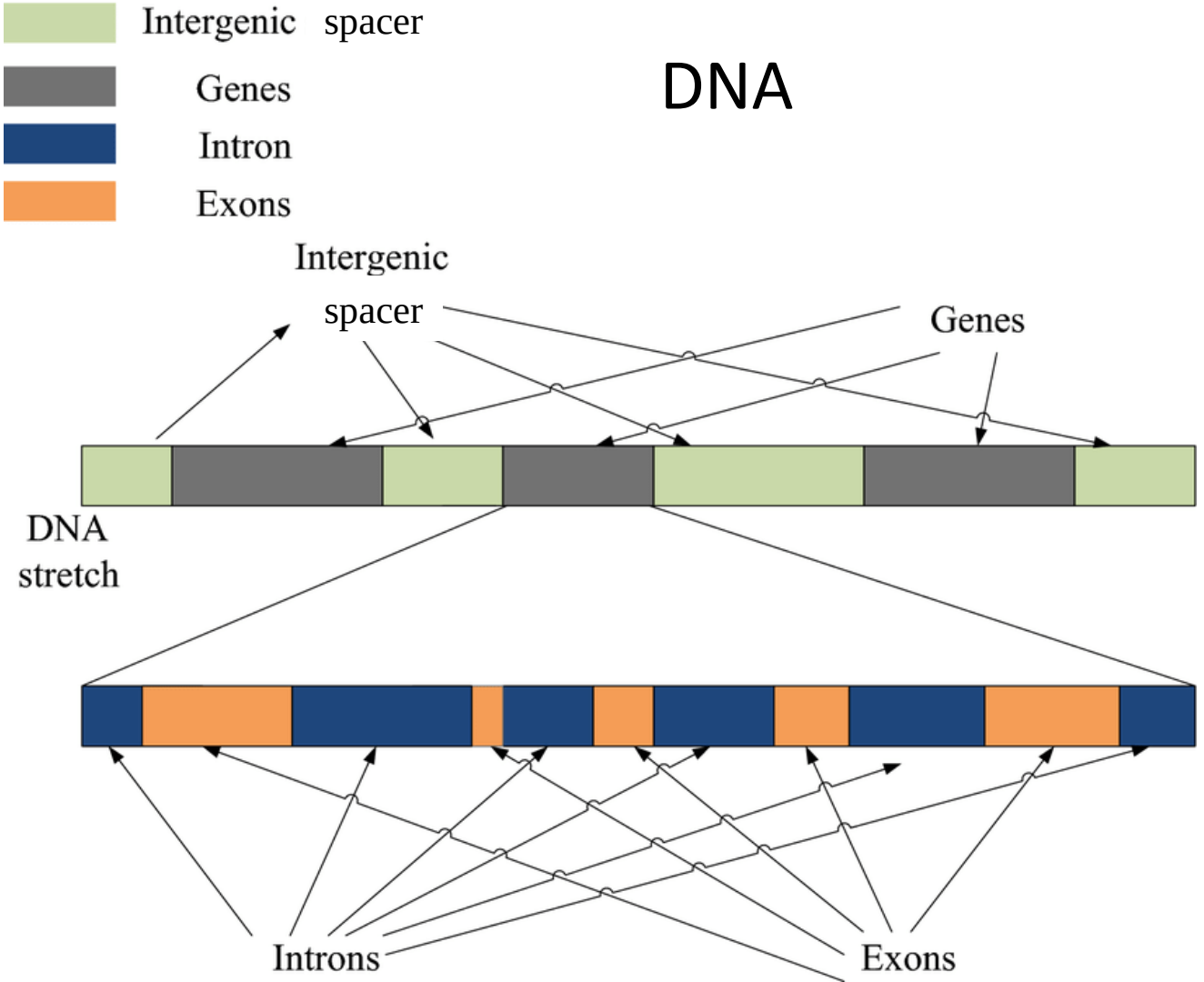
DNA



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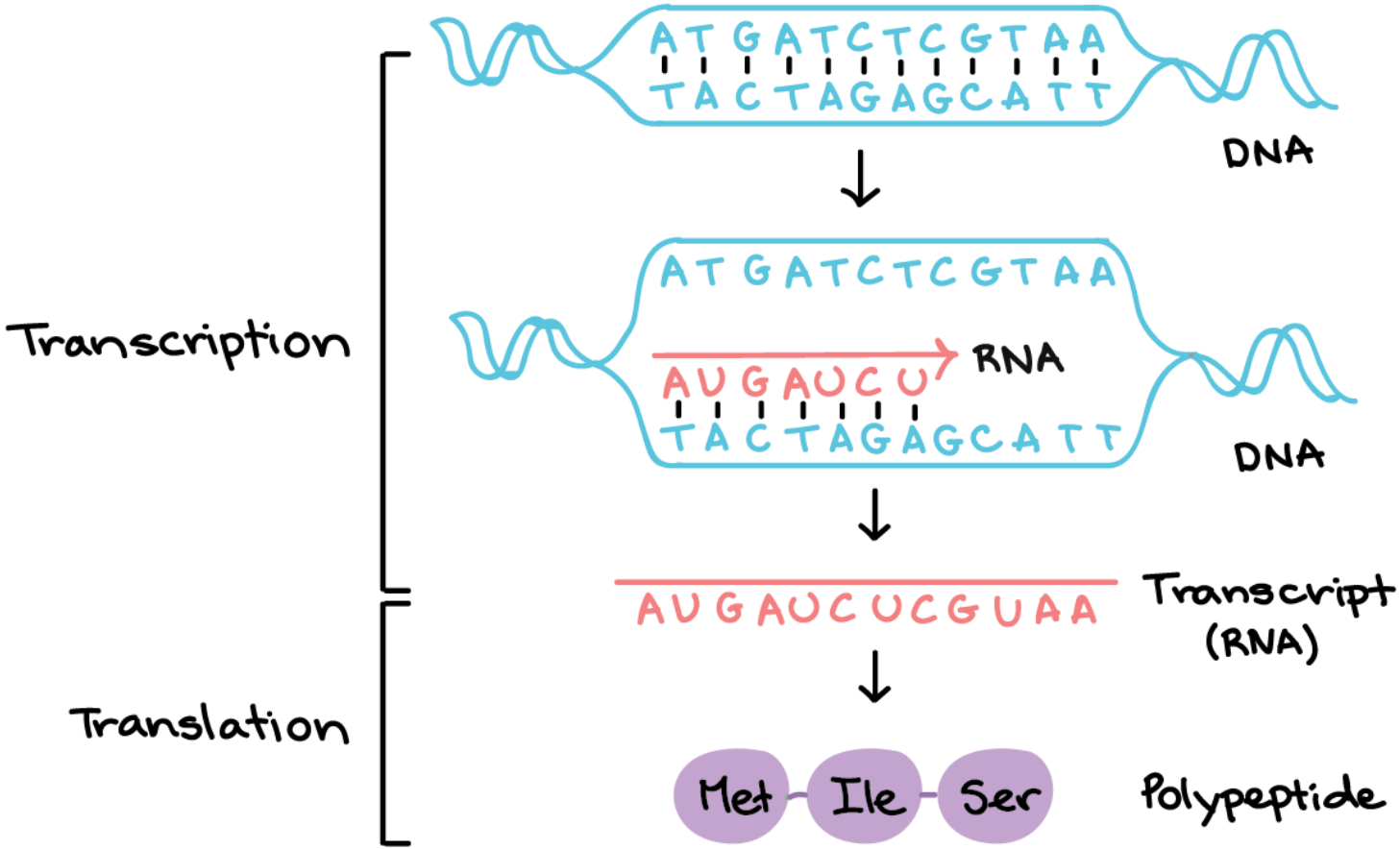
DNA



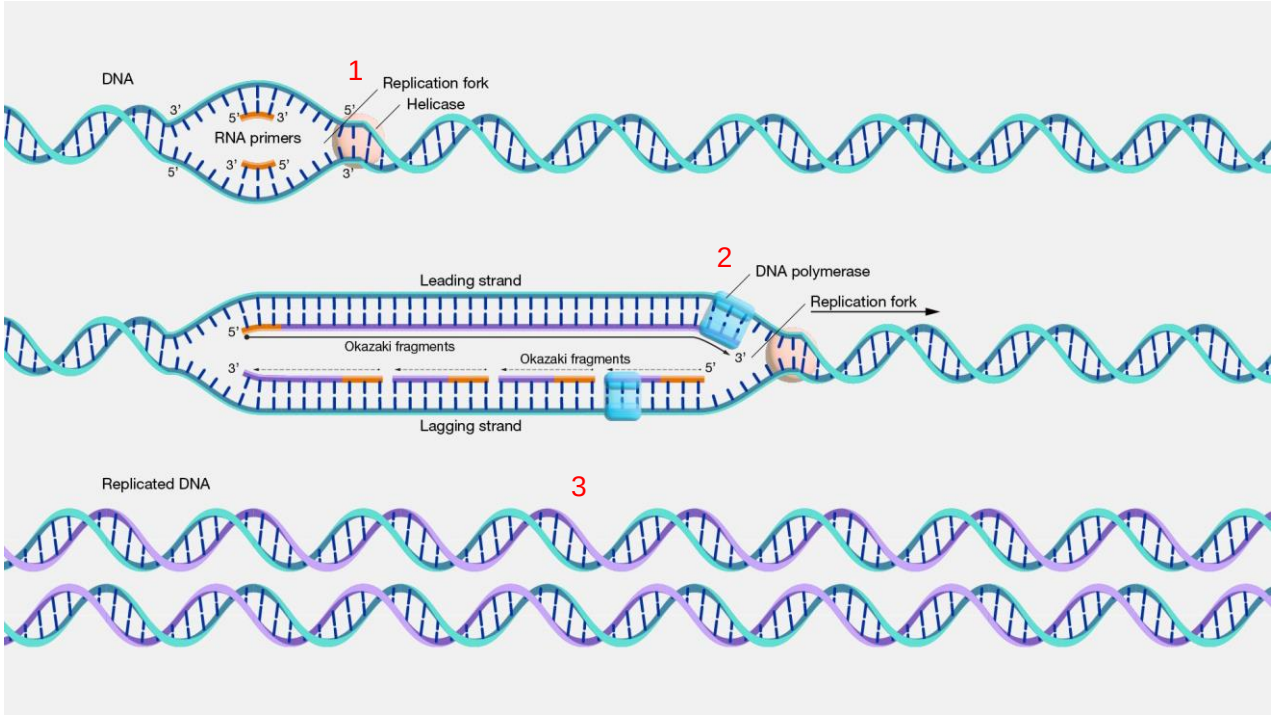
DNA

DNA replication

1 initiation

2 elongation

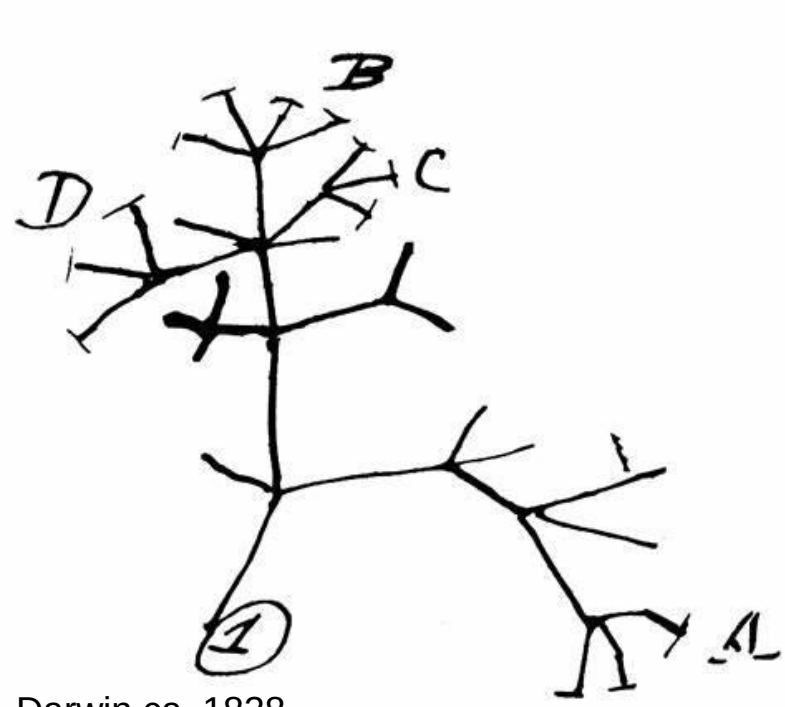
3 termination



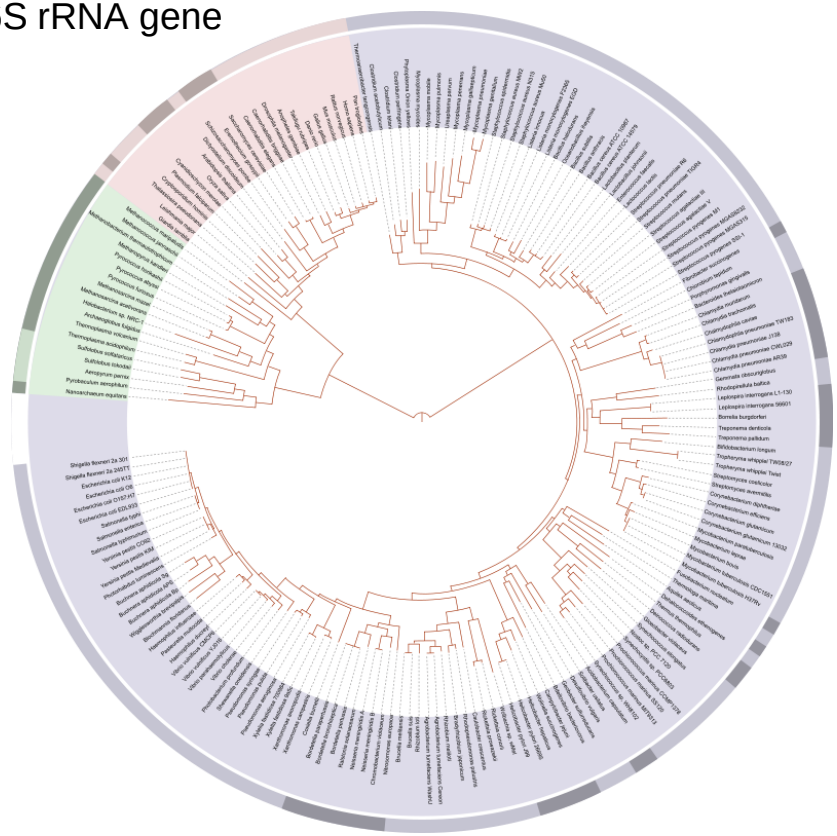
- DNA replication takes place in the cytoplasm in prokaryotes and in the nucleus in eukaryotes.
- a natural process, at body temperature

How can we use the DNA

A number of genes is found in all living organisms e.g. 16S rRNA gene



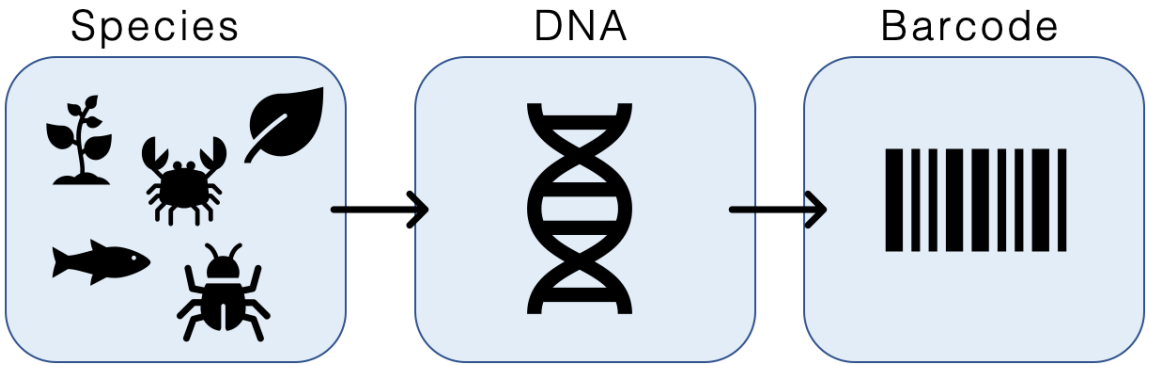
Darwin ca. 1838



Based on whole genomes: Ciccarelli et al 2006

DNA

Identification tool ⟶ DNA Barcoding/Metabarcoding



DNA Barcoding

THE ROYAL SOCIETY

Received 29 July 2002
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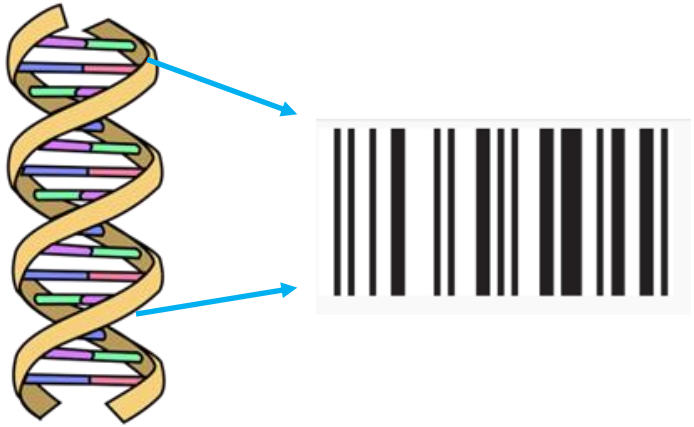
Published online 8 January 2003

Biological identifications through DNA barcodes

Paul D. N. Hebert*, Alina Cywinska, Shelley L. Ball and Jeremy R. deWaard

Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada



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Mitochondrial gene: cytochrom c oxidase subunit 1

As barcode: a 658 bp long region of this gene

Can serve as a core of a global bioindication system for animals

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A DNA based taxonomic ID tool for animal species

A tool for sharing taxonomic expertise, overcoming the future lack of experts

A standardised method using specific information present:
- in all species
- In all tissues
- at all life stages

DNA Barcoding

Goal: Identification to species level using a single universal marker for barcoding off all organisms

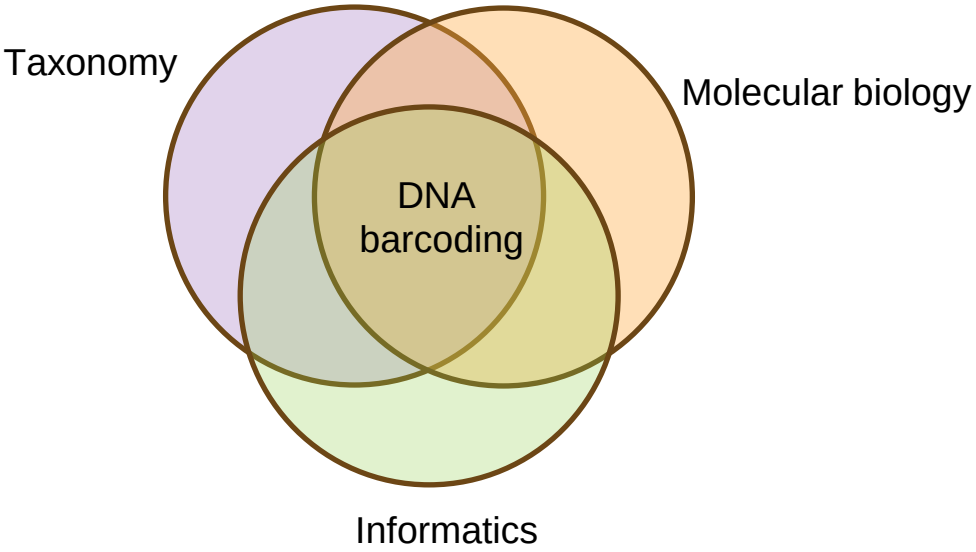
Was not achievable

Upto date

- Metazoens: **COI** (mitochondrial genes)
- Plants: **matK +rbcl** (chloroplast genes) + (atpF–atpH, psbK–psbI, trnH–psbA spacers)
- Fungi: **ITS** (Internal transcribed spacer, between rRNA genes

DNA Barcoding

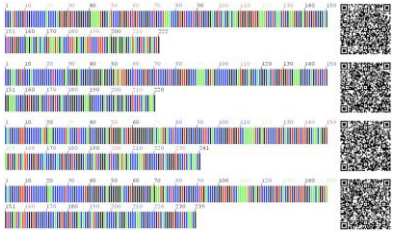
DNA barcoding: a tool for the taxonomic identification of organisms at the crossroads of several disciplines



DNA Barcoding

Taxonomists

Analytical facility
Molecular biologists



Reference library

End users

DNA barcoding

Specimen

Metadata

Tissue sample

Foto

Reference database

library

DNA extraction

PCR amplification

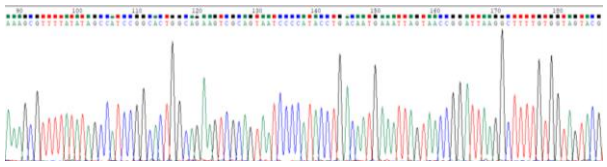
Sequencing

DNA barcoding

- Invasive alien species
- Rare endangered species
- Indicator species
- Morphologically unidentifiable organisms (tissue, fragments,larvae, egg etc.)

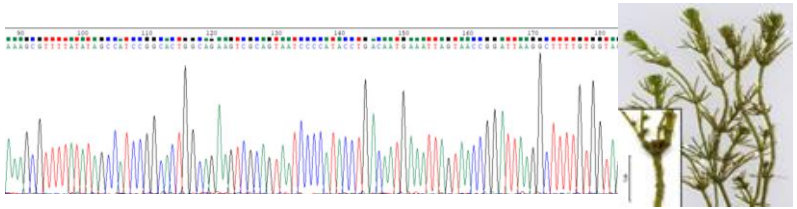
DNA barcoding → metabarcoding

- **DNA barcoding (Hebert et al. 2003)**
- a DNA based standardized identification tool
- Barcode: short DNA fragment
- Taxa specific
- Easy to sequence



- **Metabarcoding (Taberlet 2012)**
- Extending the barcoding concept to natural samples
- Identification of organisms in a community
- Use of next generation, high throughput sequencing

DNA barcoding → metabarcoding



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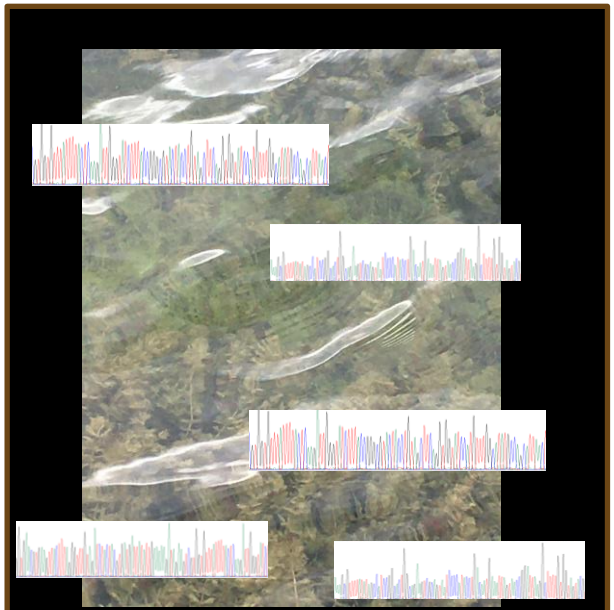
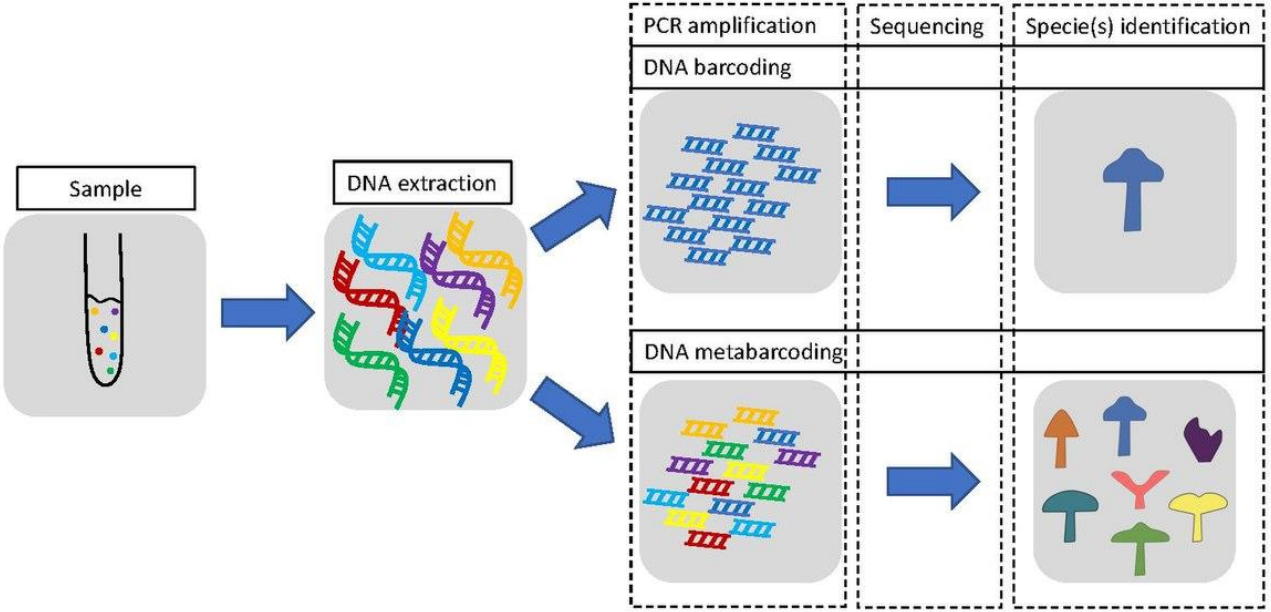


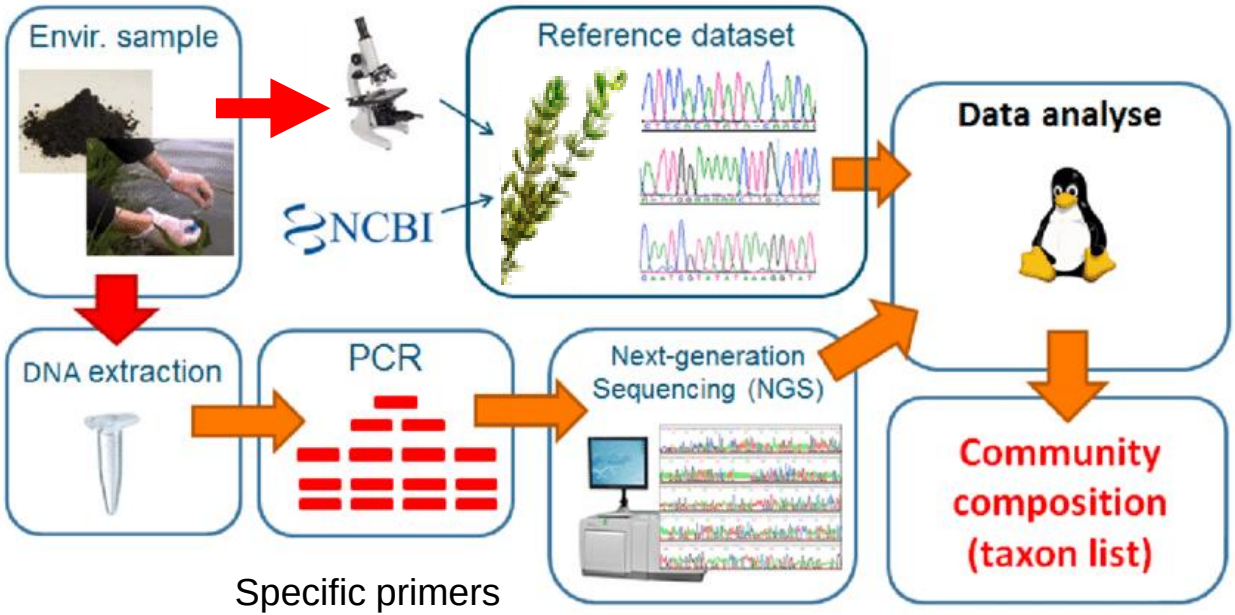
- **Metarbarcoding (Taberlet 2012)**
- Extending the barcoding concept to natural samples
- Identification of organisms in a community
- Use of next generation, high throughput sequencing

- Benefit reduced costs, time and easier intercalibration

DNA barcoding → metabarcoding

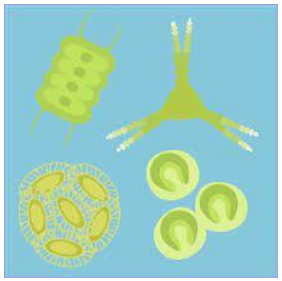


DNA metabarcoding



Metabarcoding: DNA

organismal DNA



Organisms
Tissue
Gamets
cells

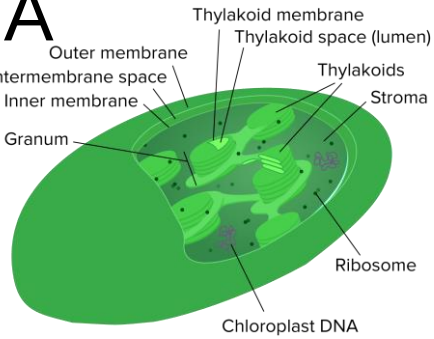
Metabarcoding: DNA

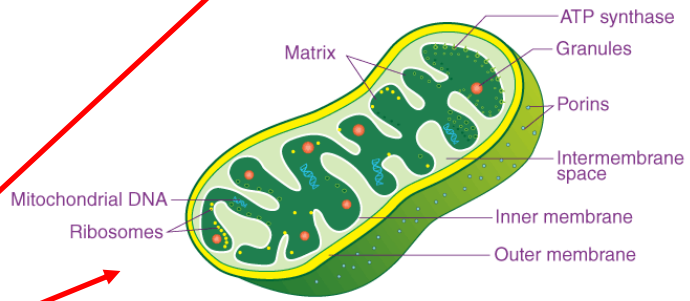
Organismal DNA

Organisms
Tissue
Gamets
cells

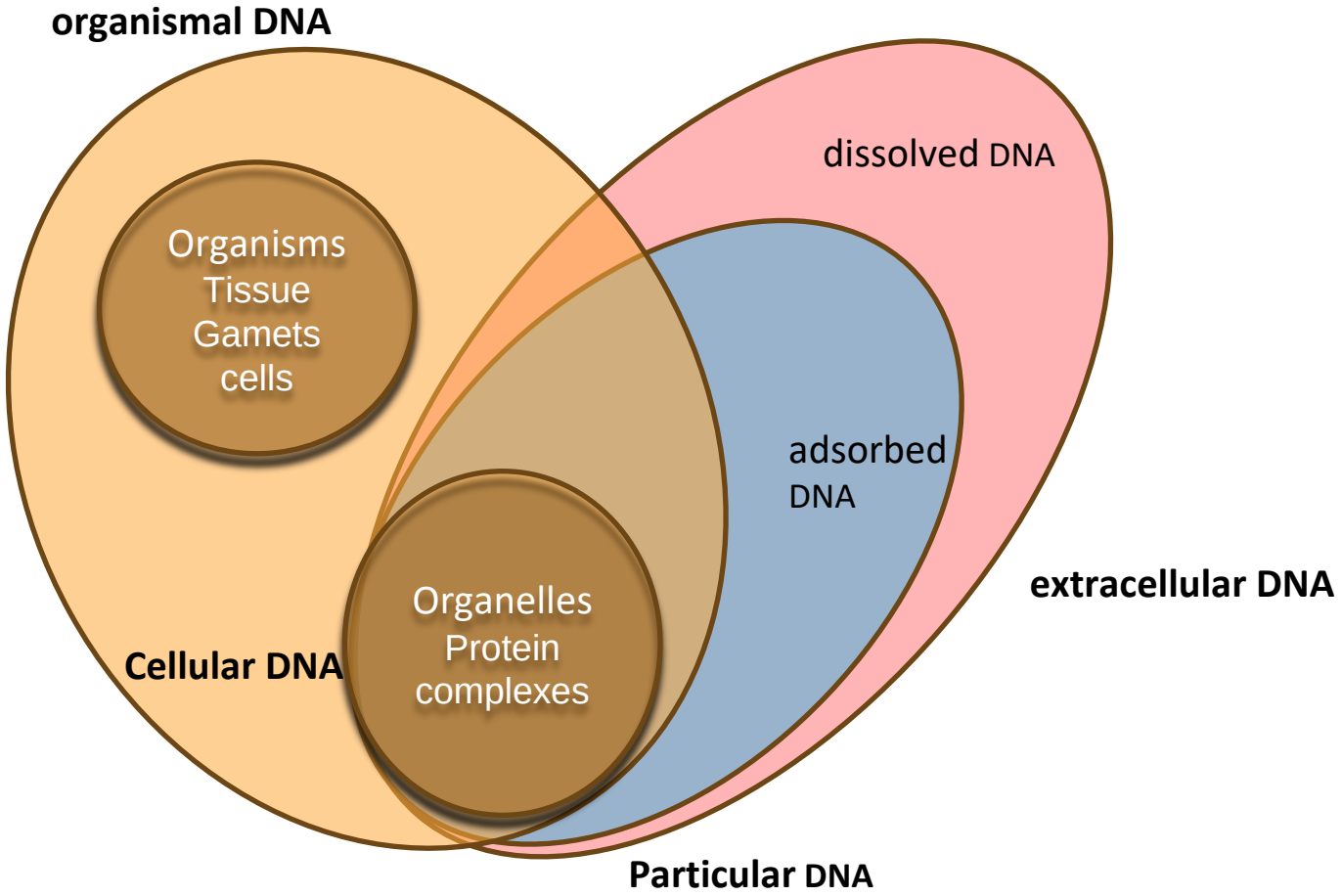
Cellular DNA

Organelles
Protein
complexes

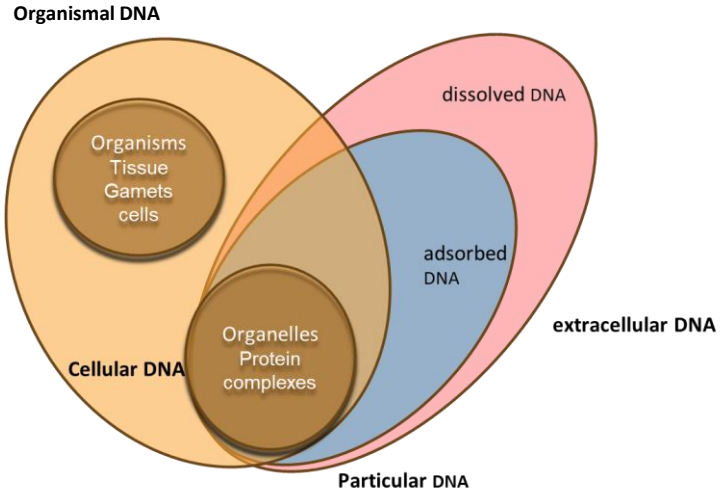




Metabarcoding: DNA

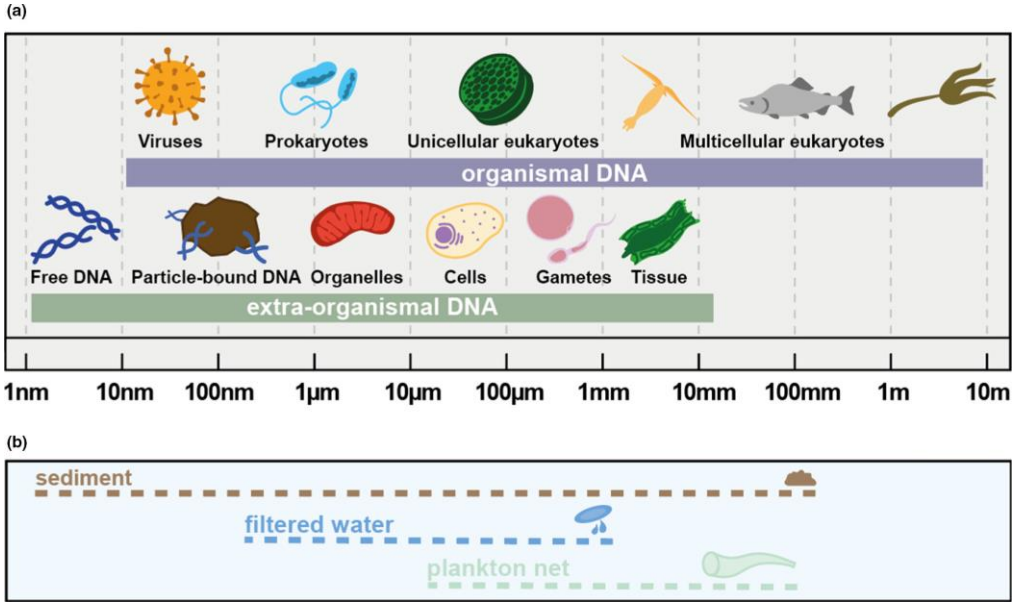


Metabarcoding: DNA



Environmental DNA = eDNA

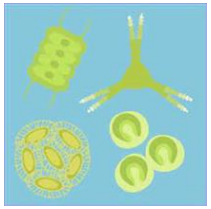
- **In a broader view all DNA in a sample**
- **In a strict view extracellular DNA only**



DNA metabarcoding applications

Sampling
DNA extraction

DNA amplification
Sequencing
bioinformatics



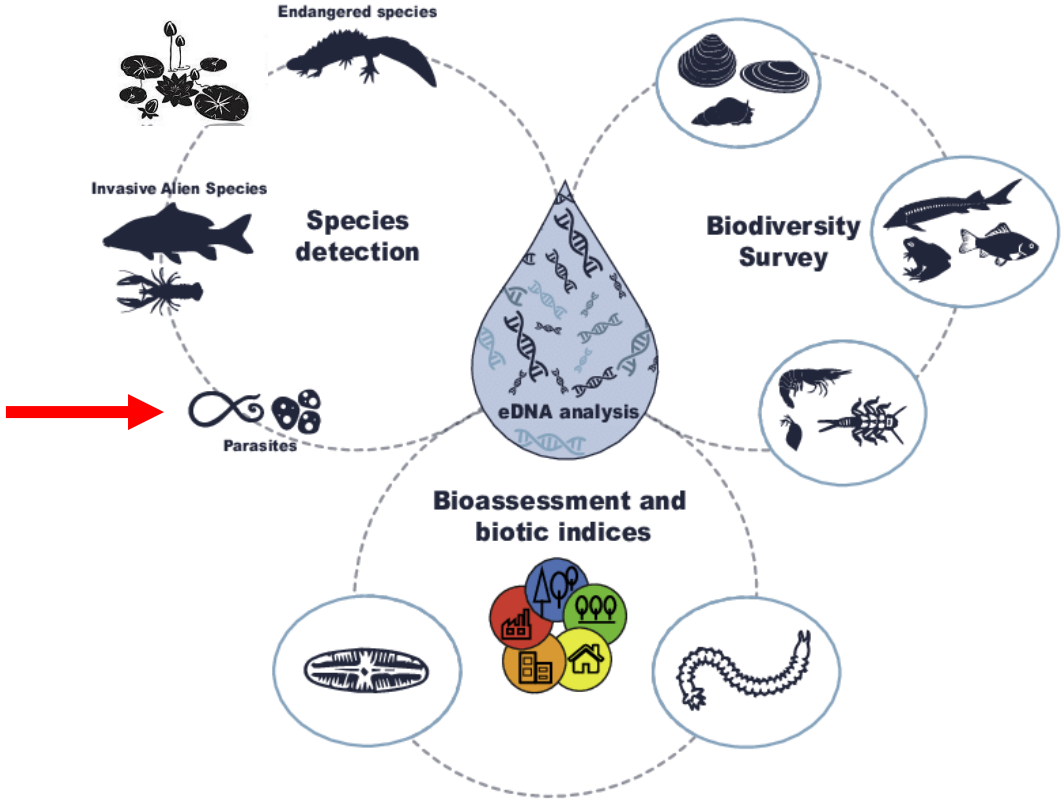
Biomonitoring
Status assessment

Fossils
Ancient ecosystems

Taxa diversity
Biomonitoring
Rare species
Invasive species

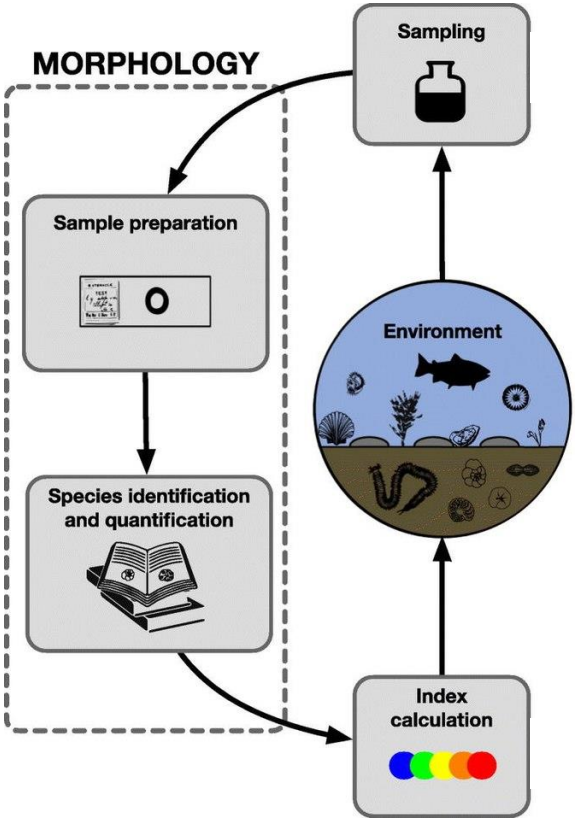
Crime scenes

DNA metabarcoding applications lakes and rivers



DNA metabarcoding applications lakes and rivers

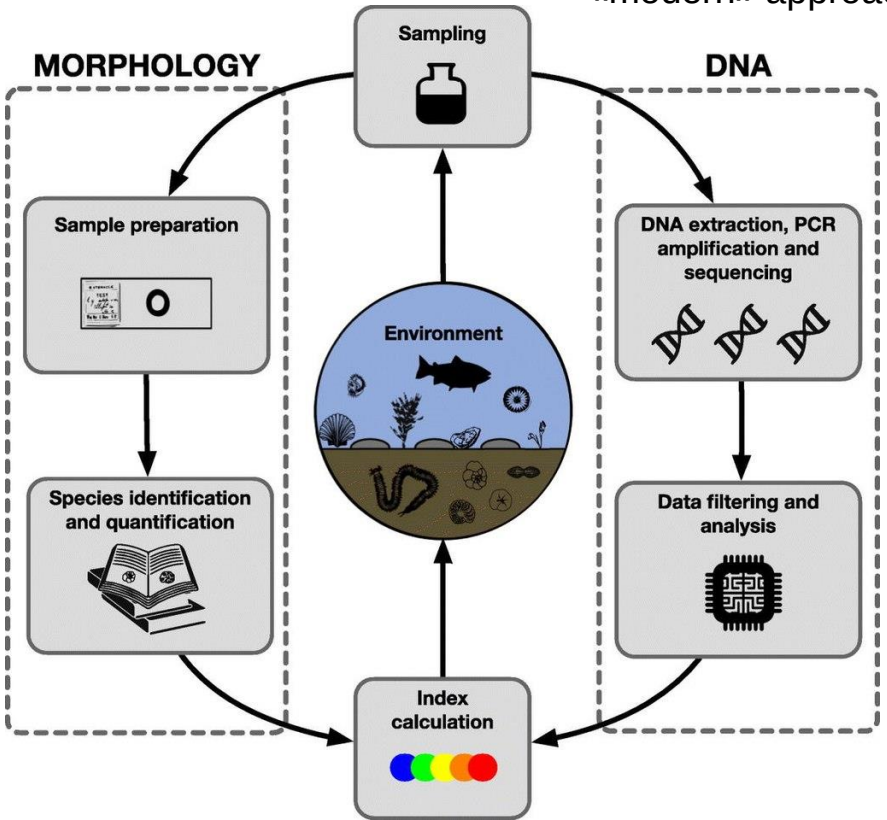
«Classical» approach



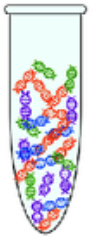
DNA metabarcoding applications lakes and rivers

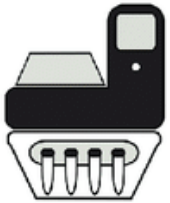
«Classical» approach

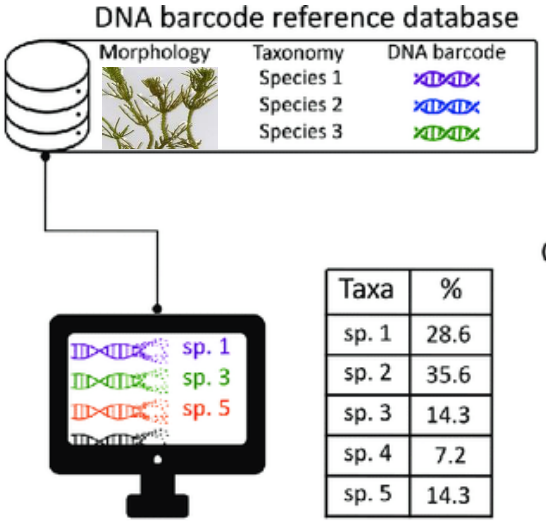
«modern» approach



DNA metabarcoding applications lakes and rivers







Taxa	%
sp. 1	28.6
sp. 2	35.6
sp. 3	14.3
sp. 4	7.2
sp. 5	14.3

Water sample for macrophytes

Total DNA extraction

Barcode PCR amplification

High-Throughput Sequencing

Bioinformatics treatments

Taxonomic list (species)

Ecological status (quality indices)

Relative abundance!

Take home message

DNA related methods are cheaper and faster in the long run

However, a cooperation between taxonomists and molecular biologist is also a must in the future even with a good reference library

If not the risk of misinterpretations of data is high

Acknowledgement

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Funded by the European Union

Thank you for your attention!

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