



Diatom metabarcoding for biomonitoring : 1st part

F. Rimet

Reminders:

Biology

Taxonomy

Biomonitoring

The INRAE logo is located at the bottom left of the slide. It consists of the letters "INRAE" in a bold, teal-colored, sans-serif font. The letter "A" is stylized with a circular element on its right side. The logo is partially overlaid by a large, rounded, teal-colored hexagonal shape that is part of a decorative graphic on the left side of the slide.



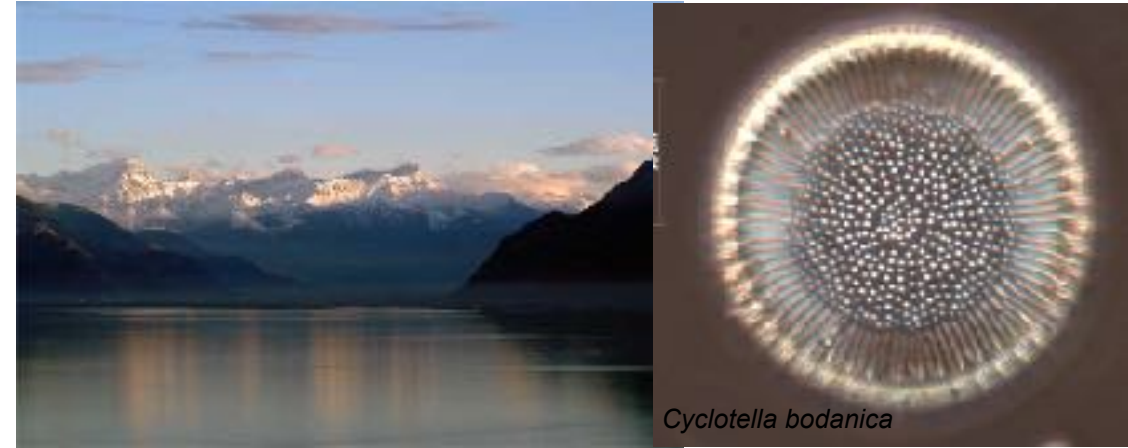
Reminders

- 1- Diatom biology
- 2- Taxonomy
- 3- Classical diatom biomonitoring



Diversity and importance in the biosphere

- 100 000 species (Mann & Vanormelingen 2013)
- 25% of the total biomass on earth (Werner 1977)
- Colonize all habitats:
lakes, rivers, oceans, soils,
wet walls, caves ...



Cyclotella bodanica



Sellaphora bacillum

Luticola ventricosa



Pinnularia brebissoni

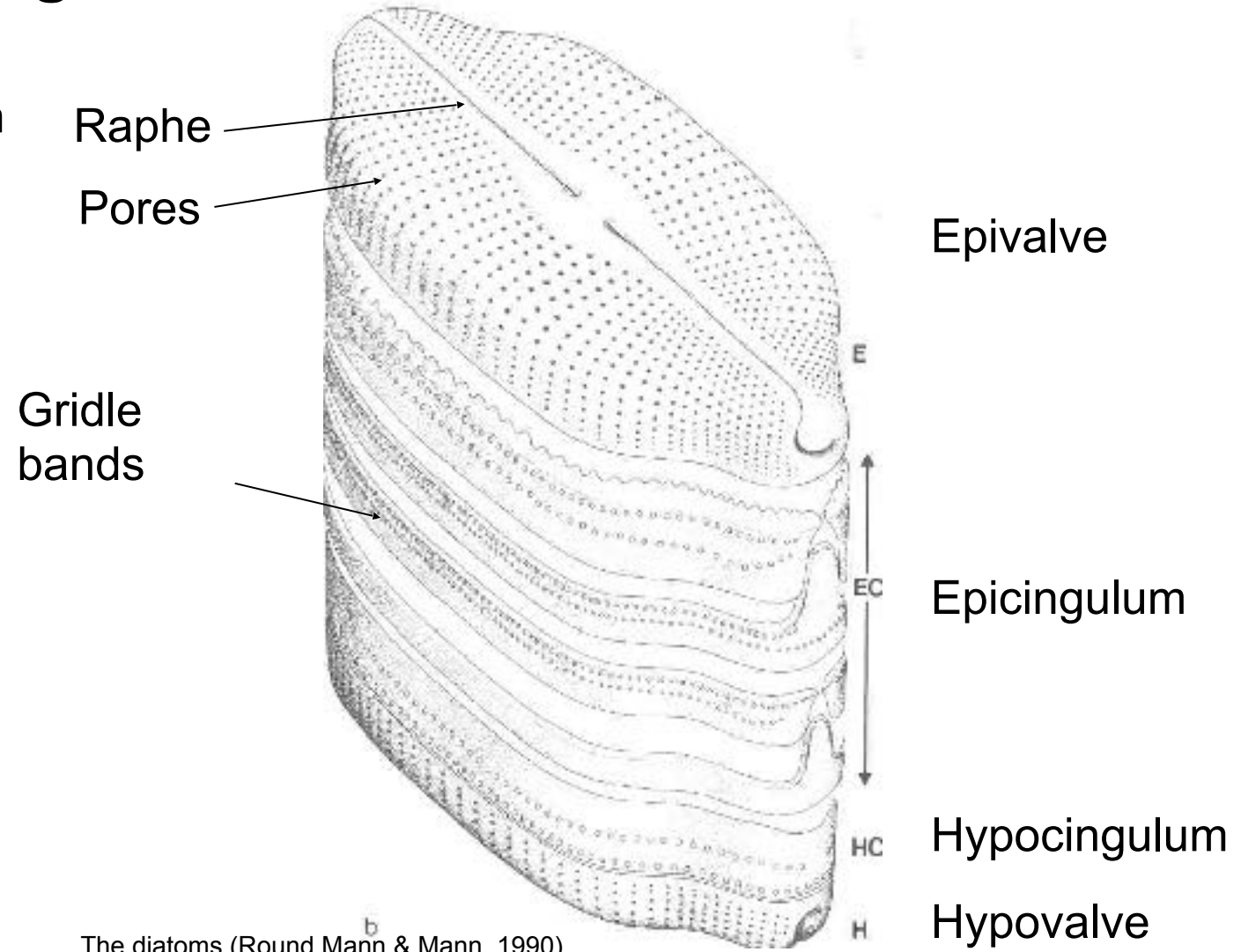
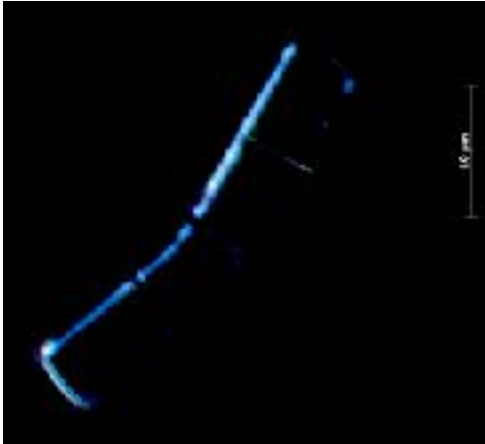


Cymbella erhenbergii



Cell organisation

Extracellular siliceous skeleton



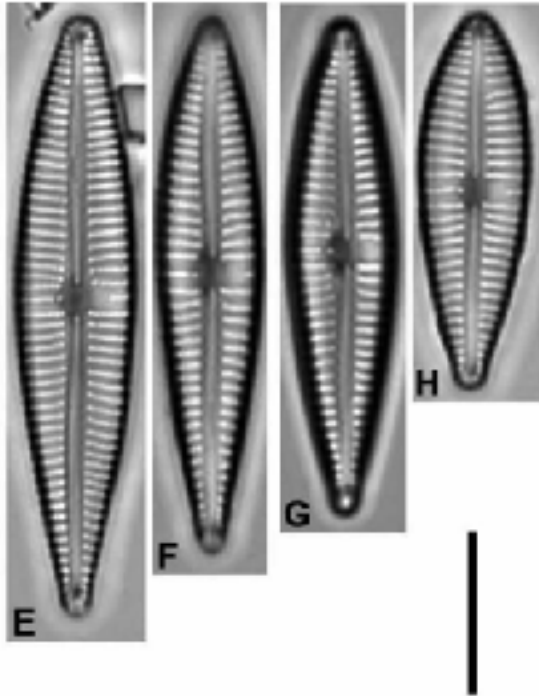
The diatoms (Round Mann & Mann, 1990)

Life cycle

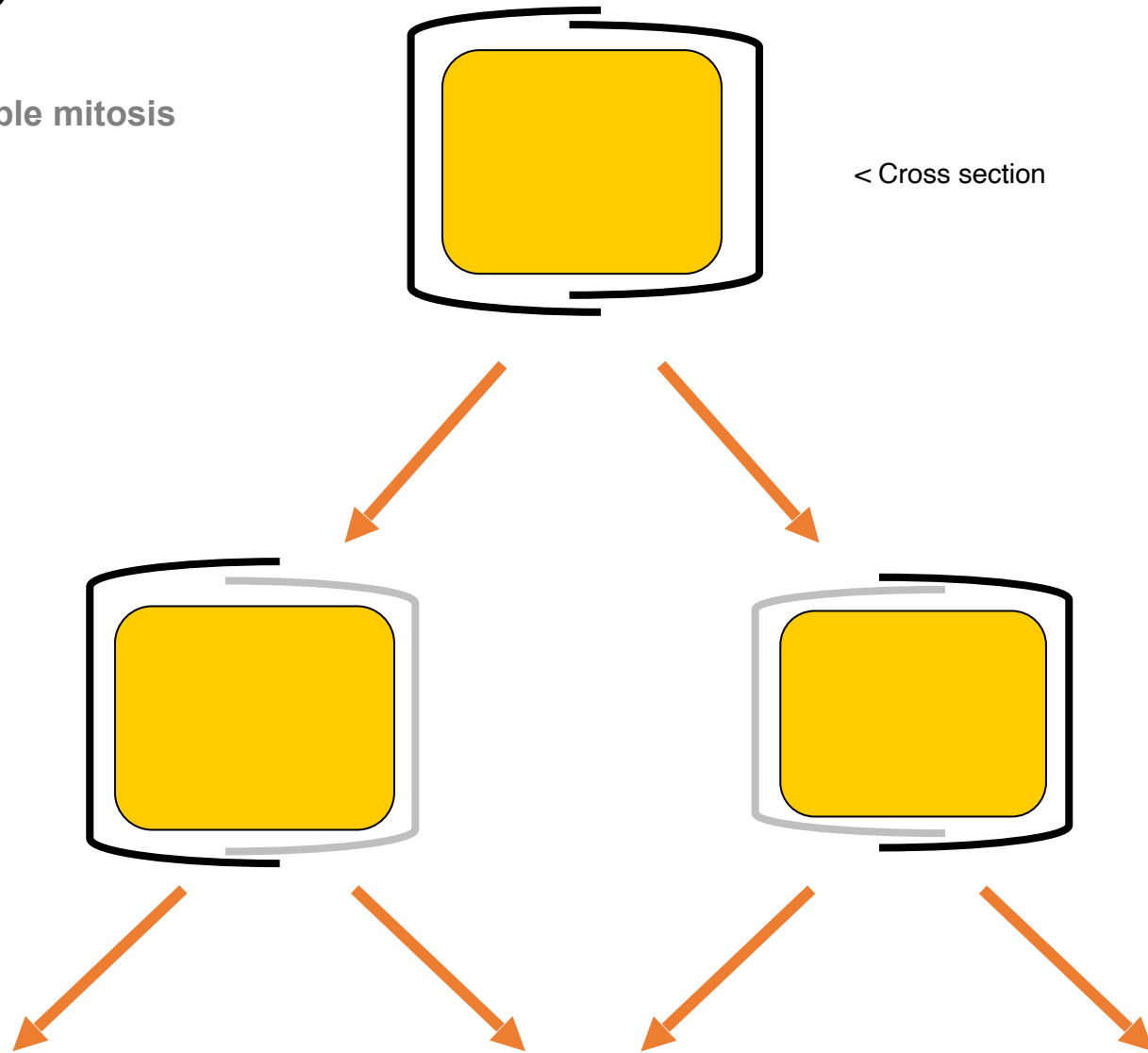
- Reproduction :

Vegetative reproduction: simple mitosis

Cell reduction



Gomphonema parvulum
(culture strain) Rose & Cox 2014



Life cycle

- Reproduction:

Sexual reproduction (Round, Crawford, Mann 1990)

Sexual reproduction
(Gametes mating)

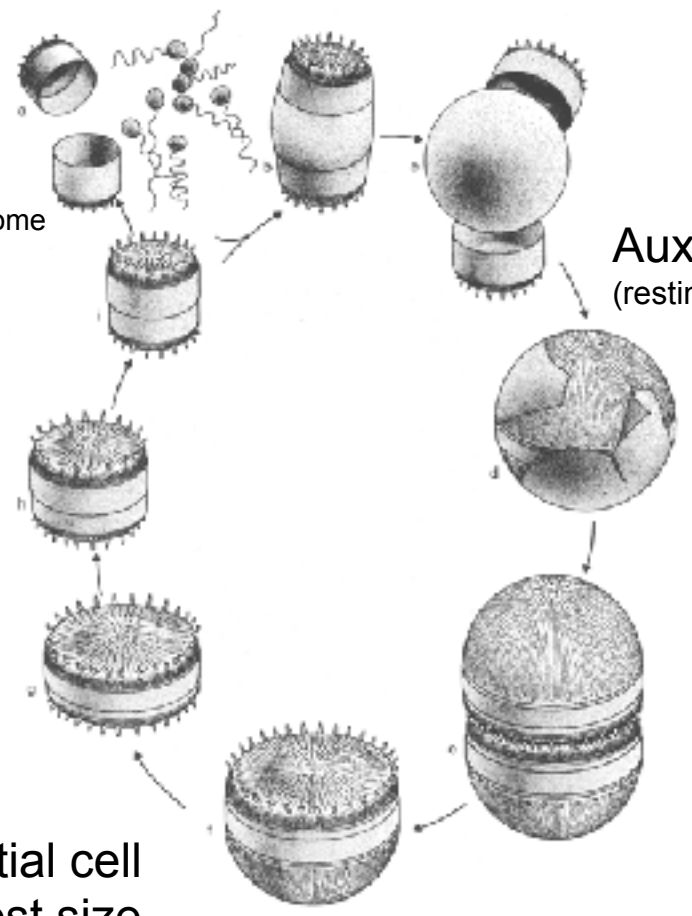
Meiosis
 $2n$ chromosomes \rightarrow n chromosome
Production of gametes

Auxospore
(resting spore)

Mitosis
Vegetative cell

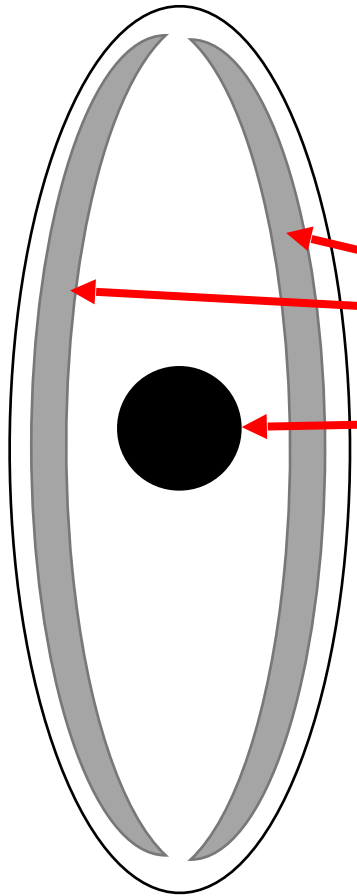
Mitosis

Initial cell
largest size



Cell organisation

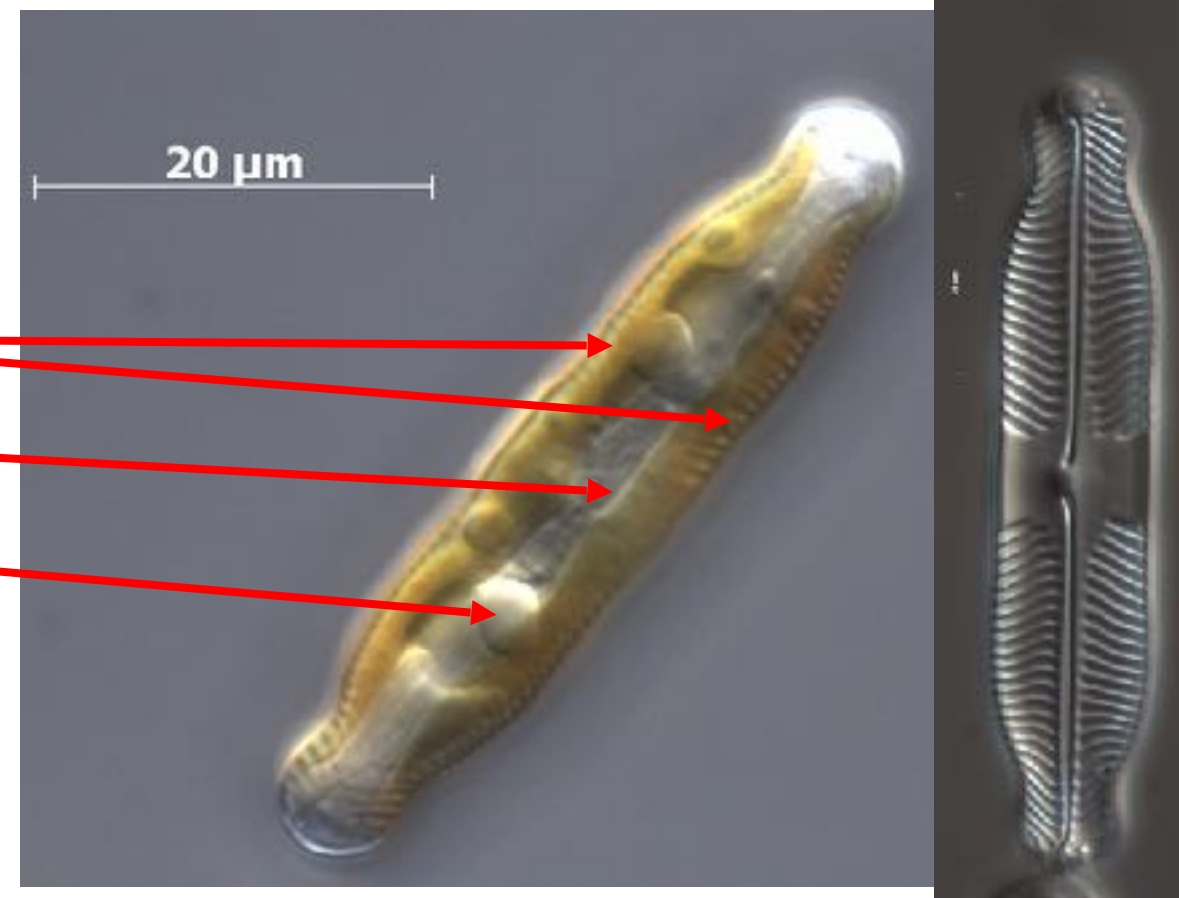
Intracellular organelles



Chloroplasts

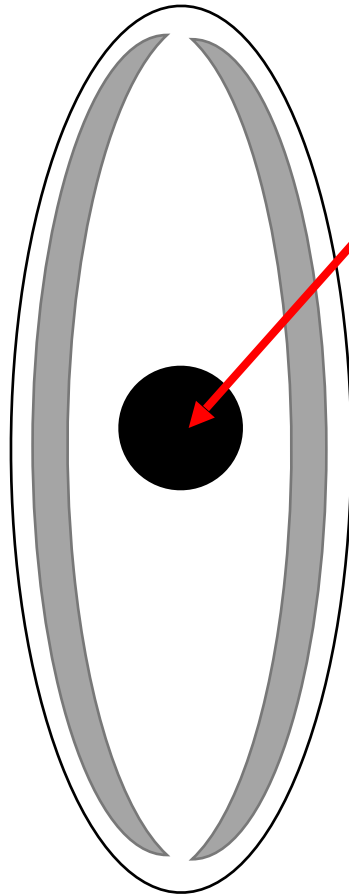
Nucleus

Lipidic drops



TCC879-*Pinnularia lundii* var. *linearis*

Cell organisation



Nucleus :

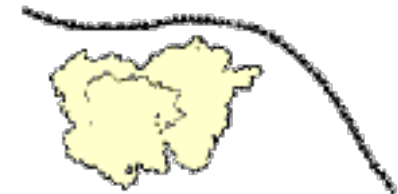
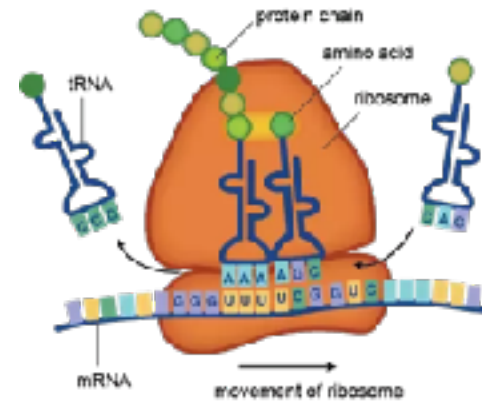
Several chromosomes (nucleus) have coding genes for ribosomal RNA.

rRNA > ribosome, composed by:

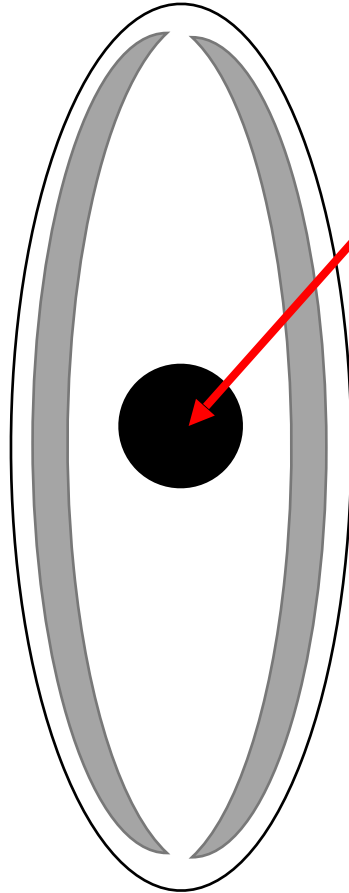
- Large sub-unit (60S) : 28S rRNA, 5,8S rRNA, 5S rRNA
- Small sub-unit (40S) : 18S rRNA

18S marker is widely used in biodiversity studies because:

- Variable sequences flanked by highly conserved regions allowing the use of universal primers.
- Numerous copies in the genome: present in large quantities



Cell organisation



Nucleus :

Several chromosomes (nucleus) have coding genes for ribosomal RNA.

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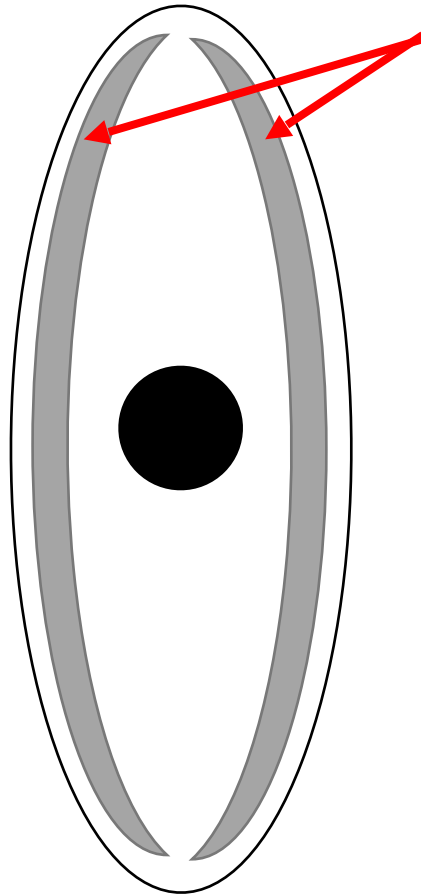
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- Numerous copies in the genome: present in large quantities



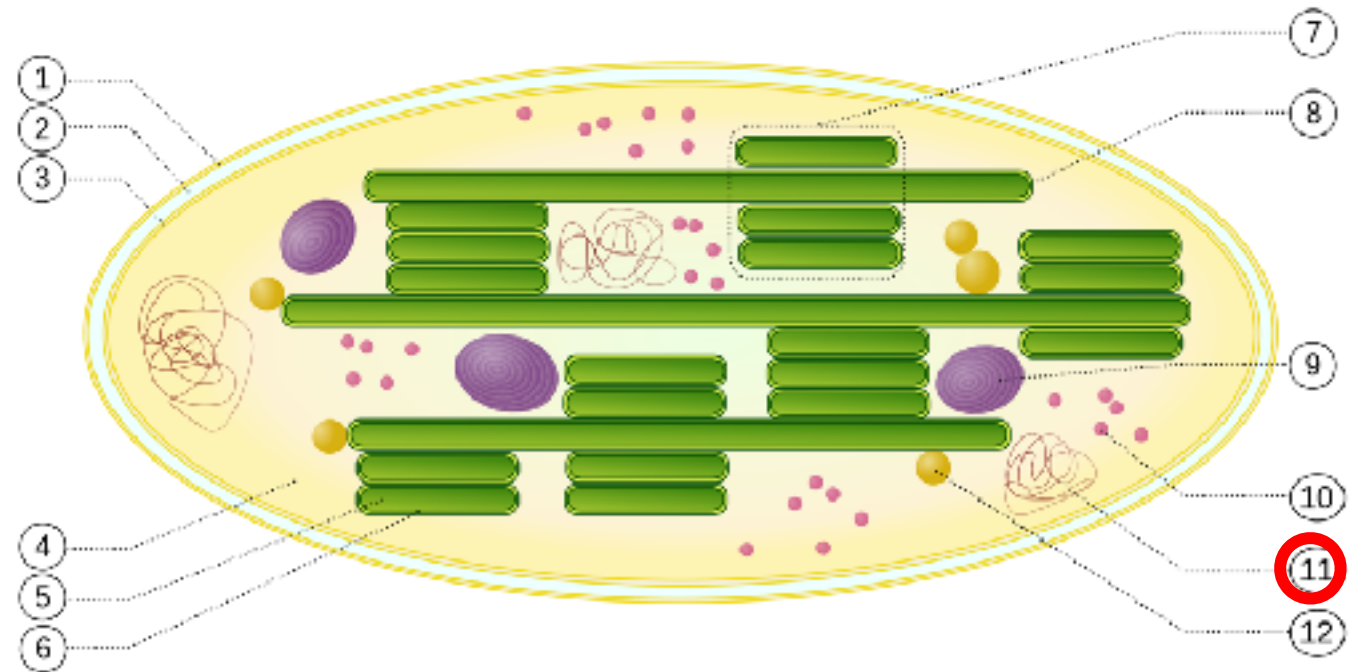
Protist Ribosomal Reference database (PR²)

Cell organisation



Chloroplasts:

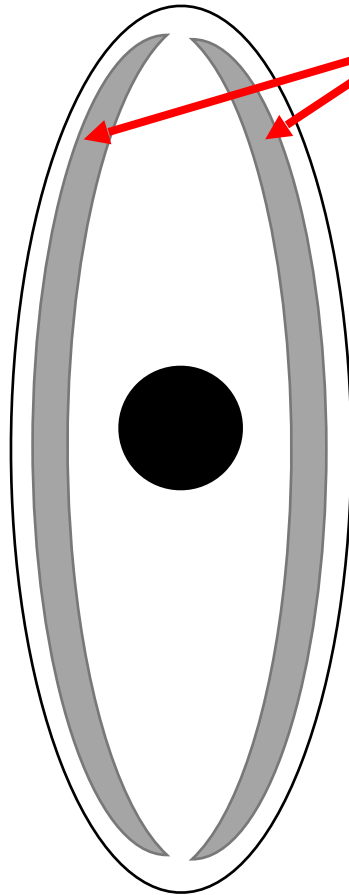
- (1) outer membrane; (2) intermembrane space; (3) Inner membrane
- (4) stroma; (5) lumen du thylakoïde; (6) membrane du thylakoïde;
- (7) granum (thylakoïdes stack(8))
- (9) amidon; (10) ribosome;
- (11) Chloroplastic DNA;
- (12) Lipidic droplet



Cell organisation

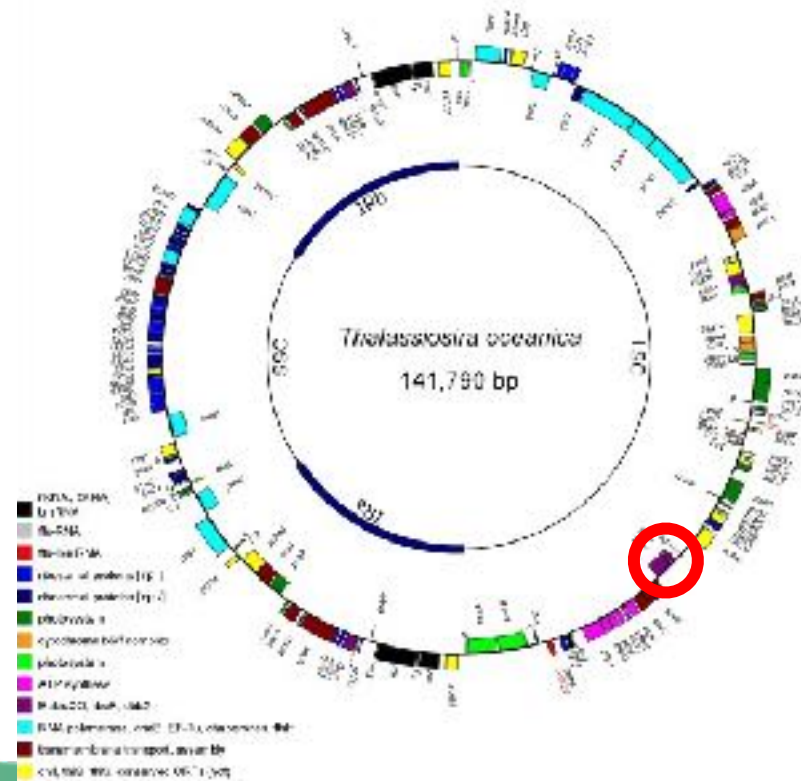


DIAT.BARCODE

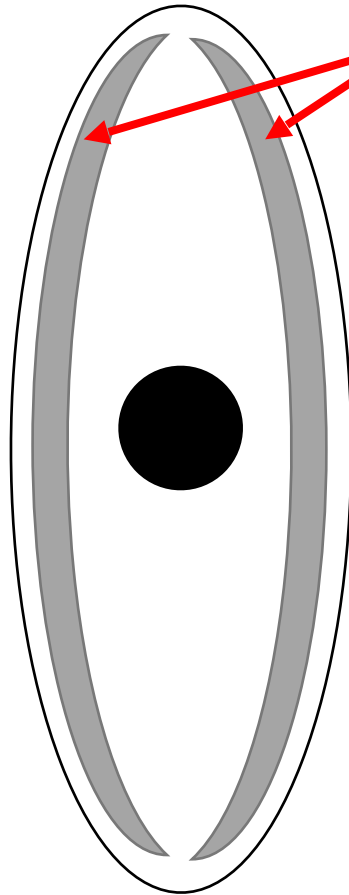


Chloroplasts:

In the chloroplast genome, an important marker: *rbcl*
 Coding the large sub-unit (« L ») of RuBisCo (« *rbc* »), key enzyme of photosynthesis.

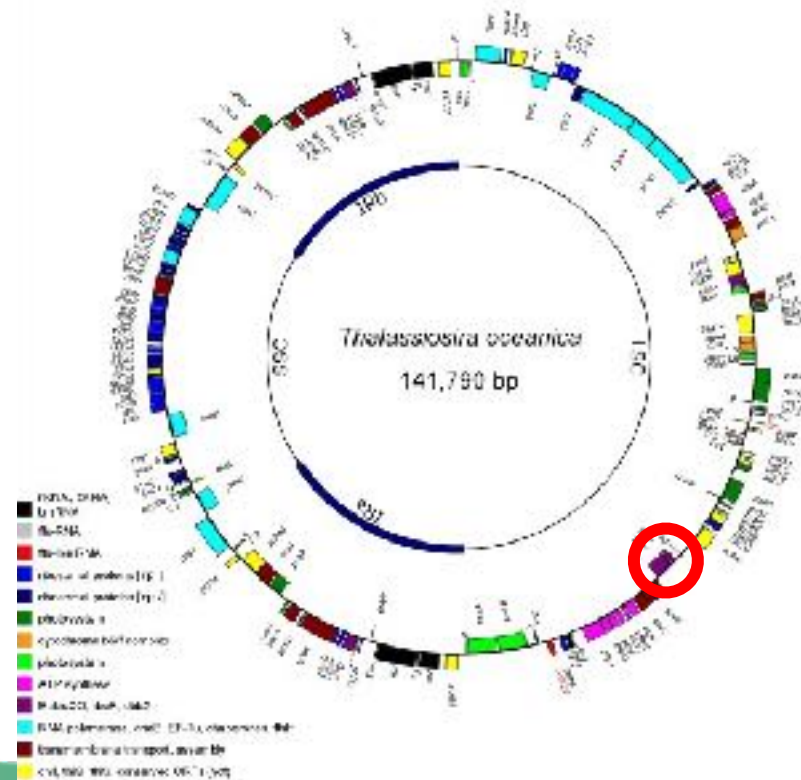


Cell organisation

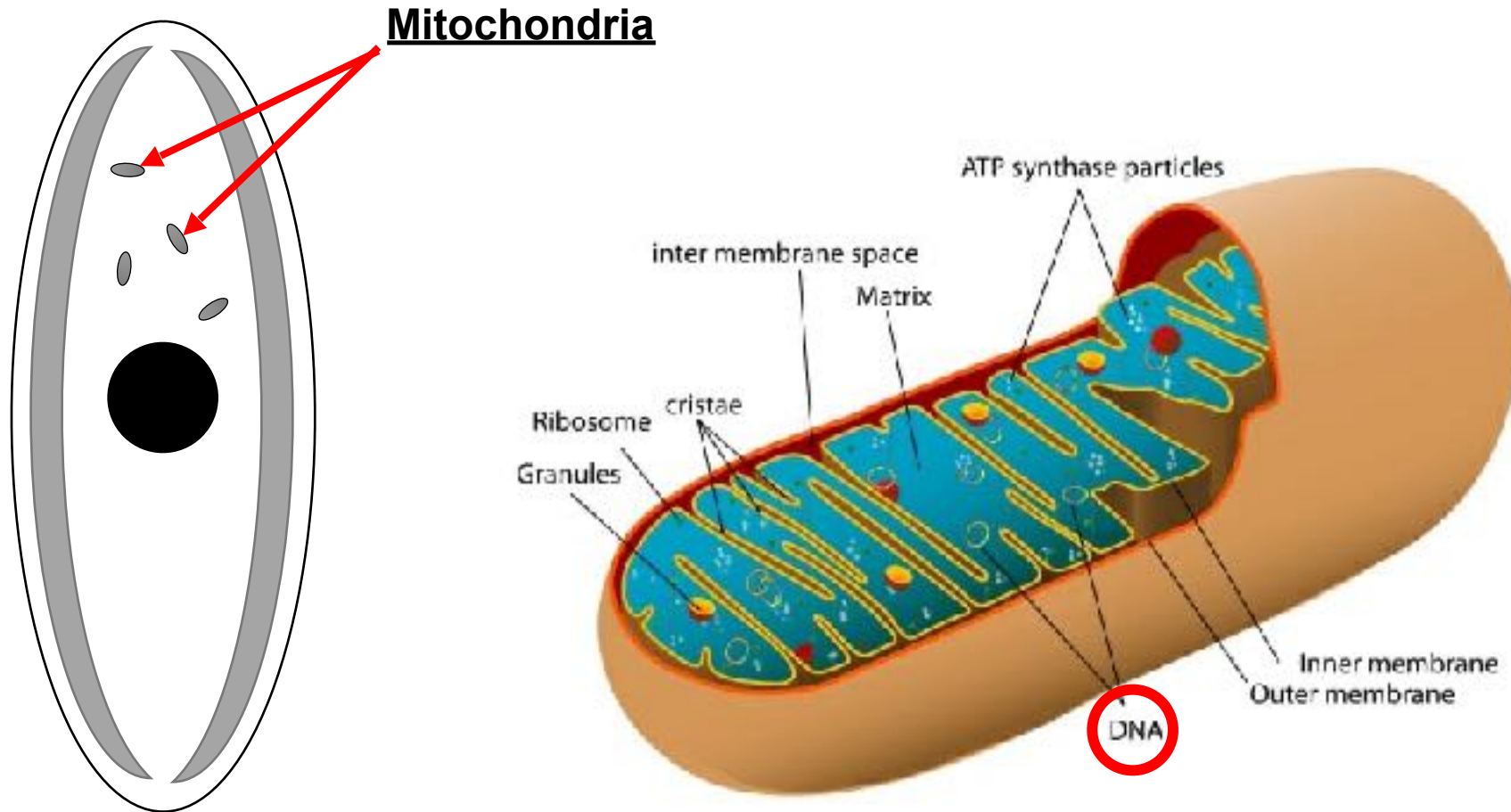


Chloroplasts:

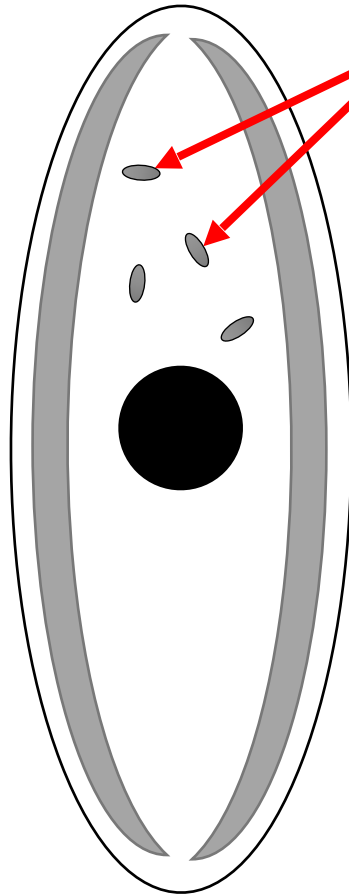
In the chloroplast genome, an important marker: *rbcl*
 Coding the large sub-unit (« L ») of RuBisCo (« *rbc* »), key enzyme of photosynthesis.



Cell organisation




Cell organisation



Mitochondria:

In the mitochondrial genome, an important marker : Cox1 encodes a subunit of Cytochrome c oxidase, which is involved in the respiration process. It is a key enzyme in aerobic respiration.





DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA

BOLD is a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada. It consists of four main modules: a data portal, an educational portal, a registry of BINs (putative species), and a data collection and analysis workbench.



DATA PORTAL

A data retrieval interface that allows for searching over 9.7M public records in BOLD using multiple search criteria including but not limited to geography, taxonomy and depository.



EDUCATION PORTAL

A custom platform for educators and students to explore barcode data and contribute novel barcodes to the BOLD database.



BIN DATABASE

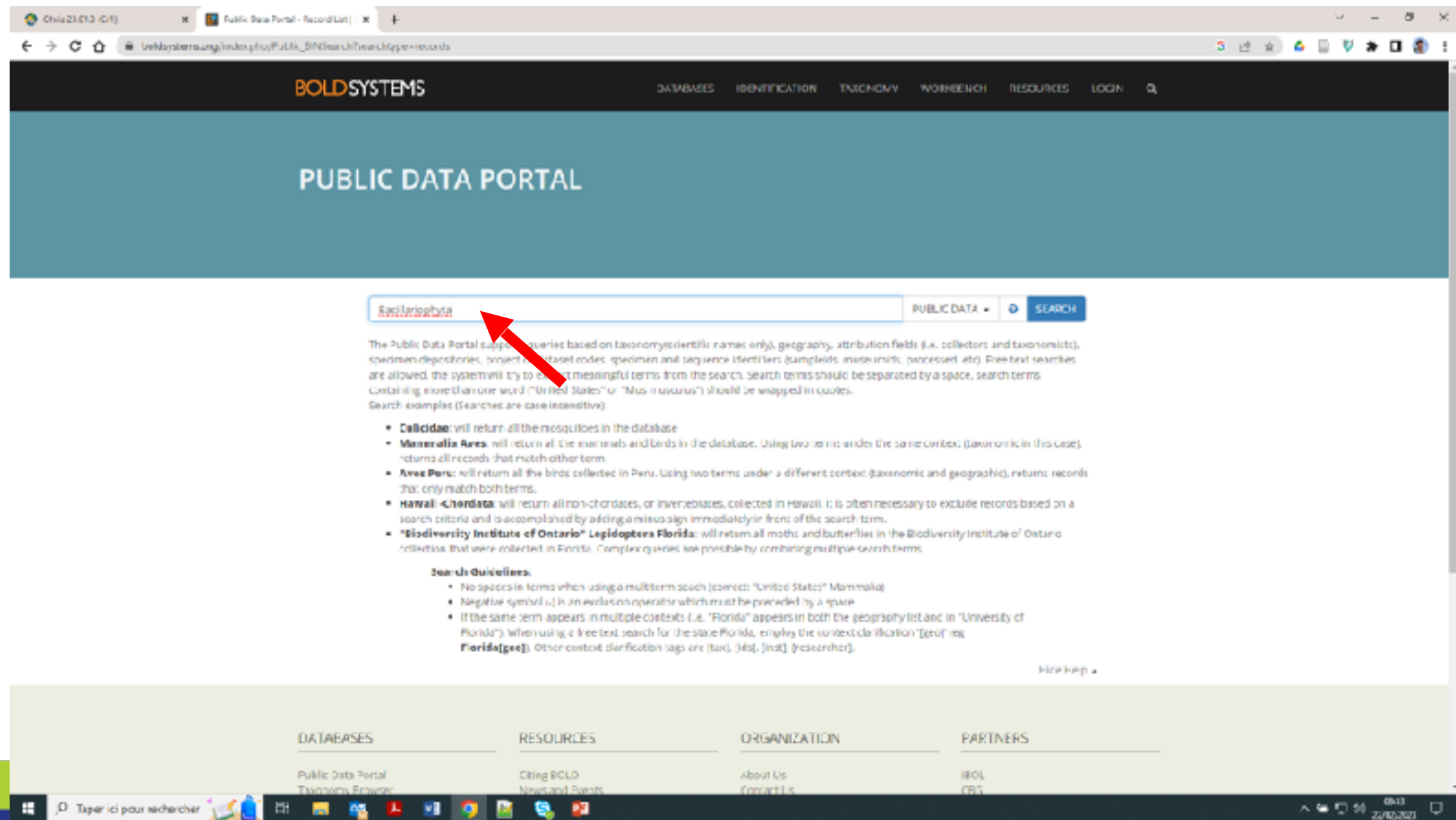
A searchable database of Barcode Index Numbers (BINs), sequence clusters that closely approximate species.



WORKBENCH

A data collection and analysis environment that supports the assembly and validation of DNA barcodes and other sequences.





Chila 23.11.3 (C1)

Public Data Portal - Record List

urlidsystems.org/index.php/PuLk_BIN/Search?searchtype=records

BOLDSYSTEMS DATABASES IDENTIFICATION TAXONOMY WORKBENCH RESOURCES LOGIN

PUBLIC DATA PORTAL

PUBLIC DATA SEARCH

The Public Data Portal supports queries based on taxonomy (scientific names only), geography, attribution fields (i.e. collectors and taxonomists), specimen depositories, project and dataset codes, specimen and sequence identifiers (sampleids, accessionids, processed, etc). Free-text searches are allowed, the system will try to extract meaningful terms from the search. Search terms should be separated by a space, search terms containing more than one word ("United States" or "Mus musculus") should be wrapped in quotes. Search examples (Searches are case insensitive):

- **Culicidae**: will return all the mosquitoes in the database
- **Mammalia Aves**: will return all the mammals and birds in the database. Using two terms under the same context (taxonomic in this case), returns all records that match either term.
- **Aves Peru**: will return all the birds collected in Peru. Using two terms under a different context (taxonomic and geographic), returns records that only match both terms.
- **Hawaii -Chordata**: will return all non-chordates, or invertebrates, collected in Hawaii. It is often necessary to exclude records based on a search criteria and is accomplished by adding a minus sign immediately in front of the search term.
- **"Biodiversity Institute of Ontario" Lepidoptera Florida**: will return all moths and butterflies in the Biodiversity Institute of Ontario collection that were collected in Florida. Complex queries are possible by combining multiple search terms.

Search Guidelines:

- No spaces in terms when using a multiterm search (correct: "United States" Mammalia)
- Negative symbol (-) is an exclusion operator which must be preceded by a space
- If the same term appears in multiple contexts (i.e. "Florida" appears in both the geography list and in "University of Florida") when using a free text search for the state Florida, employ the context classification [geo]:req **Florida[geo]**. Other context identification tags are [tax], [ids], [inst] [researcher].

Help

DATABASES **RESOURCES** **ORGANIZATION** **PARTNERS**

Public Data Portal
Taxonomy Browser

Citing BOLD
News and Events

About Us
Contact Us

IBOL
CBG

Tiper ici pour rechercher

22/10/2021



Chia 23.013 (C1) | Record List | Public Data Portal | unihydra.org/index.php/PuLiH_SearchForm

BOLD SYSTEMS DATABASES IDENTIFICATION TAXONOMY WORKBENCH RESOURCES LOGIN

Search: PUBLIC DATA SEARCH

Specimens: [DVC](#) [XML](#) [TSV](#)
 Sequences: [FASTA](#) [TRACT](#)
 Combined: [XML](#) [TSV](#)
 MISC:

Records: 1 to 100 Page: 1 2 3 4 5 6 7 8 9 10 Results Per Page: 100


- ACH0040-09 - Bacillarietes (C010P.030)**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Bacillarietes
 Identifier: ACH0040_09[sampled], 80746dd
 Depository: Université Paris, Biogéochimie, écologie des milieux continentaux
 Collected in: France
- ACH0001-16 - Achnanthesidum [180:129,215801,rvd,1-04]**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Achnanthes, Achnanthesidaceae, Achnanthesidum
 Identifier: SPITS_16_ARMAS[sampled], PVD09[museumid]
 Depository: Ghent University Protistology and Acoustic Ecology
 Collected in: Norway, Svalbard, Sørøstberg
- ACH0004-13 - Achnanthesidum [180:129,215801,rvd,1-04]**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Achnanthes, Achnanthesidaceae, Achnanthesidum
 Identifier: SPITS_M3_10[sampled], PVD01[museumid]
 Depository: Ghent University Protistology and Acoustic Ecology
 Collected in: Norway, Svalbard, Sørøstberg
- ACH0003-16 - Achnanthesidum [180:129,215801,rvd,1-01]**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Achnanthes, Achnanthesidaceae, Achnanthesidum
 Identifier: SPITS_M3Aplud_14[sampled], PVD671[museumid]
 Depository: Ghent University Protistology and Acoustic Ecology
 Collected in: Norway, Svalbard, Sørøstberg
- ACH0004-15 - Achnanthesidum [180:129,215801,rvd,1-04]**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Achnanthes, Achnanthesidaceae, Achnanthesidum
 Identifier: SPITS_M3Aplud_10[sampled], PVD67[museumid]
 Depository: Ghent University Protistology and Acoustic Ecology
 Collected in: Norway, Svalbard, Sørøstberg
- ACH0005-15 - Achnanthesidum [180:129,215801,rvd,1-04]**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Achnanthes, Achnanthesidaceae, Achnanthesidum
 Identifier: SPITS_M3_10[sampled], PVD451[museumid]
 Depository: Ghent University Protistology and Acoustic Ecology
 Collected in: Norway, Svalbard, Sørøstberg
- ACH0006-15 - Achnanthesidum [180:129,215801,rvd,1-04]**
 Taxonomy: Bacillariophyta, Bacillariophyceae, Achnanthes

Results Summary

Found **4,310** published records, with **4,410** records with sequences forming **233** OTUs clusters, with specimens from **09** countries, deposited in **22** institutions.

Of these records, **3,646** have species names, and represent **738** species.

Specimen Distribution



Data Summary

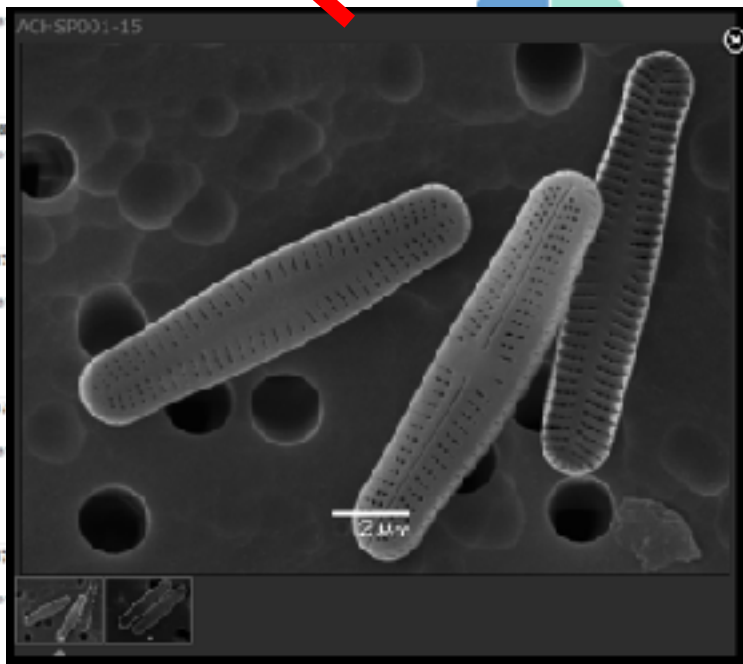


BOLD SYSTEMS DATABASES IDENTIFICATION TAXONOMY WORKBENCH RESCUE

0/16

- ASTBR002-13 - Asterionella sp. tenuisilica [185:374, rbcL:711]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragiliales, Fragilariaceae, Asterionelloidea
Identifiers: IC_MFA4:2[smpyeid], MB2[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR003-13 - Asterionella sp. tenuisilica**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragilales, Asterionelloidea
Identifiers: IC_MFA4:3[smpyeid], MB5[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR004-13 - Asterionella sp. tenuisilica [ITS:70]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragilales, Asterionelloidea
Identifiers: IC_MFA4:4[smpyeid], MB7[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR005-13 - Asterionella sp. tenuisilica [185:374, rbcL:711]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragilales, Asterionelloidea
Identifiers: IC_MFA4:5[smpyeid], MB8[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR006-13 - Asterionella sp. tenuisilica [185:374, rbcL:711]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragilales, Asterionelloidea
Identifiers: IC_MFA4:6[smpyeid], MB9[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR007-13 - Asterionella sp. tenuisilica [185:374, rbcL:711]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragilales, Asterionelloidea
Identifiers: IC_MFA4:7[smpyeid], MB10[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR008-13 - Asterionella sp. tenuisilica [185:374, ITS:748, rbcL:711]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragiliales, Fragilariaceae, Asterionelloidea
Identifiers: IC_MFA4:8[smpyeid], MB11[fieldid]
Repository: Research Collection of I. Kacmarška
Collected in: Canada, New Brunswick, Bay of Fundy
- ASTBR009-13 - Asterionella sp. tenuisilica [185:374, ITS:748, rbcL:711]**
Taxonomy: Bacillariophyta, Eucalariophyceae, Fragiliales, Fragilariaceae, Asterionelloidea
Identifiers: IC_MFA4:9[smpyeid], MB12[fieldid]

ACH-SPO31-15



Families

- Naviculites: 1632
- Thalassiosira: 619
- Bacillariales: 480
- Chaetocerales: 229
- Fragilariales: 221
- Cymbelales: 139
- Tricraterales: 90
- Frustulales: 43



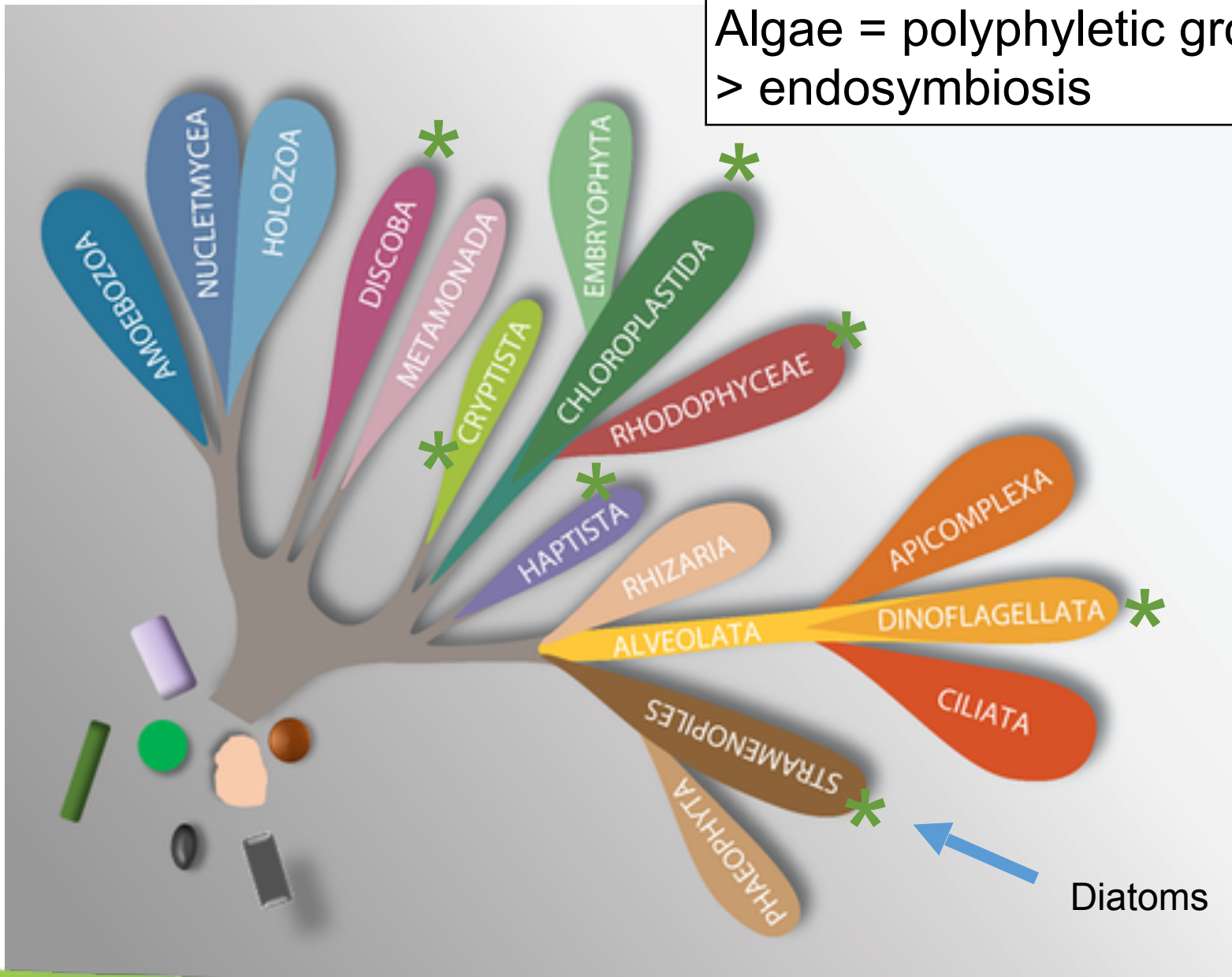


Reminders

- 1- Diatom biology
- 2- Taxonomy ←
- 3- Classical diatom biomonitoring



Algae = polyphyletic group
> endosymbiosis



Adl et al. 2019.
Revisions to the
Classification,
Nomenclature,
and Diversity of
Eukaryotes. JEM

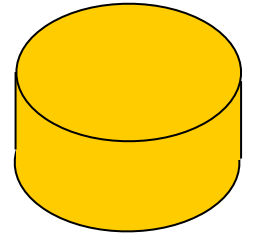


Taxonomy

- Classification: 4 classes
 - Coscinodiscophyceae
 - Mediophyceae
 - Fragilapiophyceae
 - Bacillariophyceae

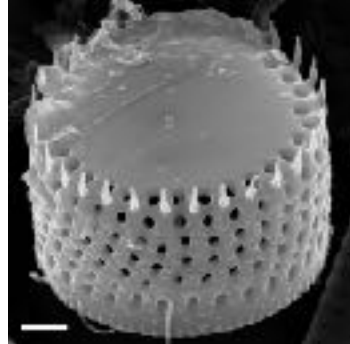


Taxonomy

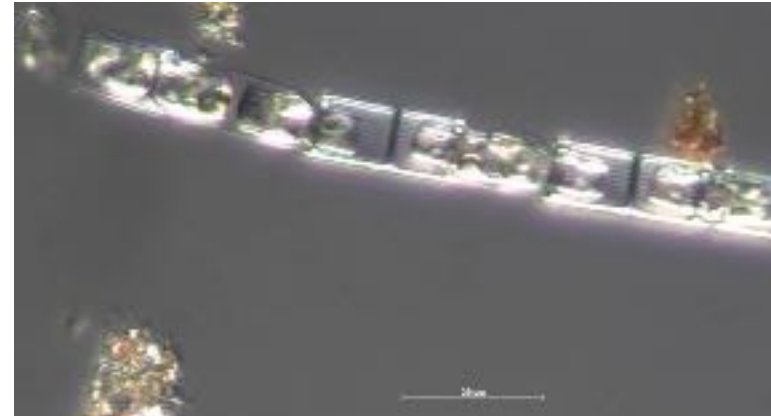


- **Coscinodiscophyceae :**

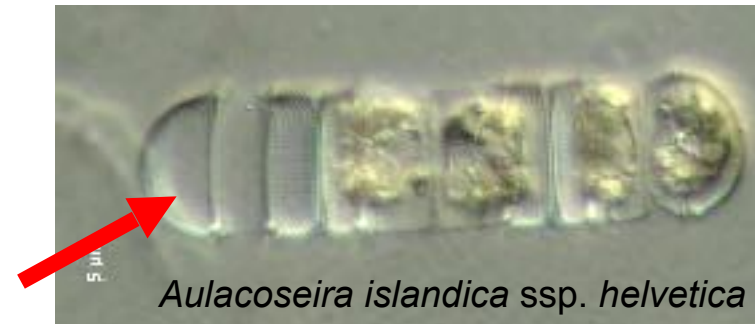
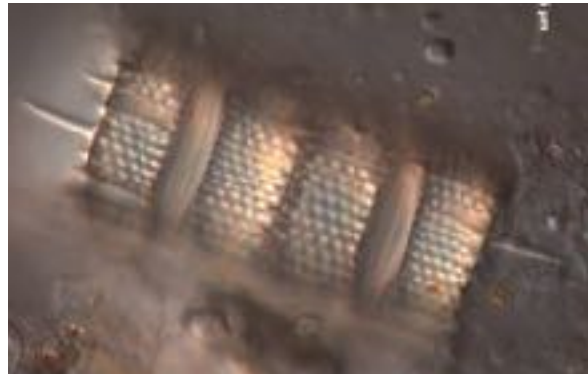
- *Aulacoseira* : filamentous, pores on the mantle, Spines between valve/mantle
- *A. alpigena*



Aulacoseira islandica (Leman fev 2010)



- *Aulacoseira muzzanensis* (Canal Marne à Maixe 30-07-09)



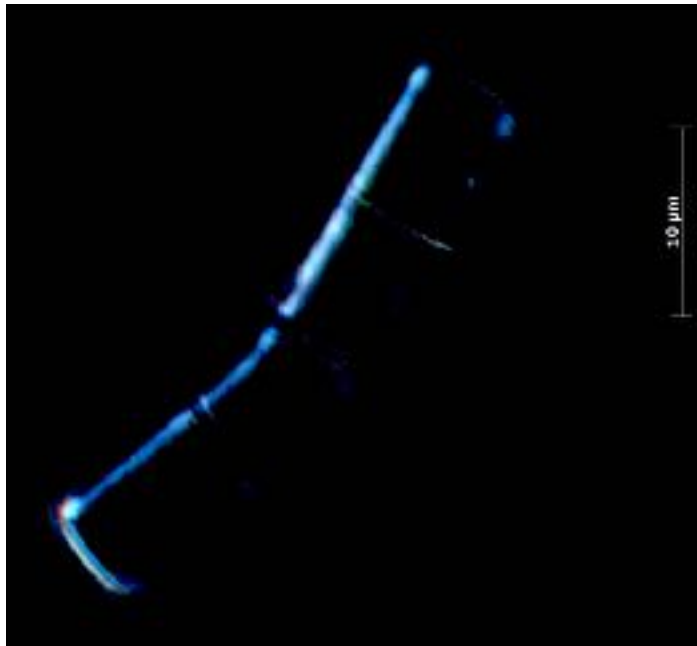
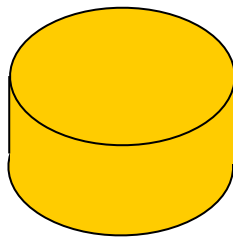
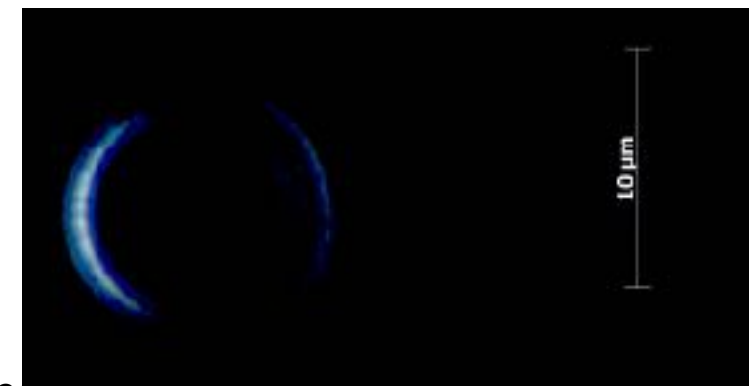
Aulacoseira islandica ssp. *helvetica*

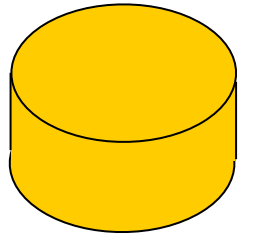
Taxonomy

- Coccinodiscophyceae :

- *Melosira* : filaments, no pores

M. varians (TCC538), lentic rivers, shore of calme lakes

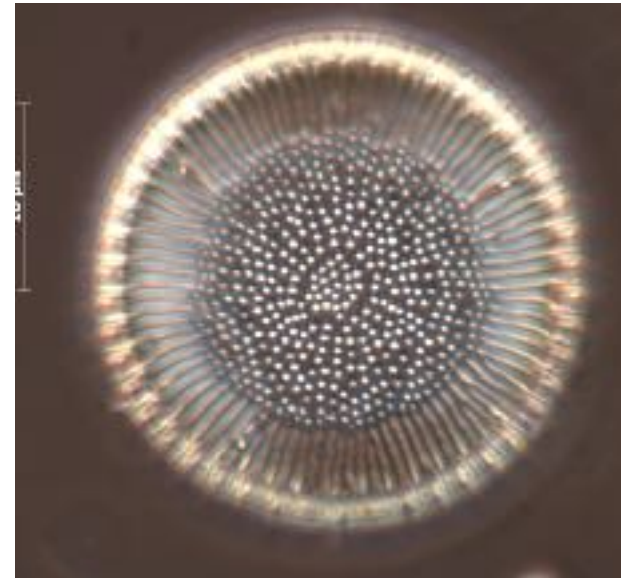




- **Mediophyceae :**
 - *Cyclotella* : single cell or in chains, planktonic



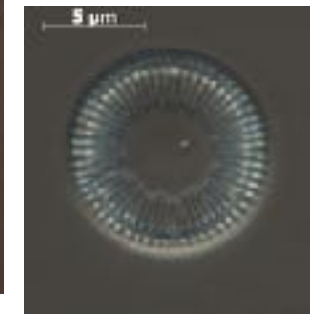
Cyclotella meneghiniana TCC358



Cyclotella lemanensis

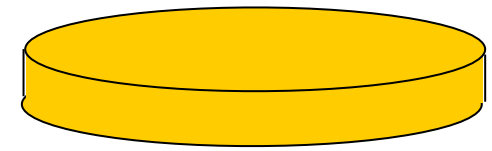


Cyclotella comensis

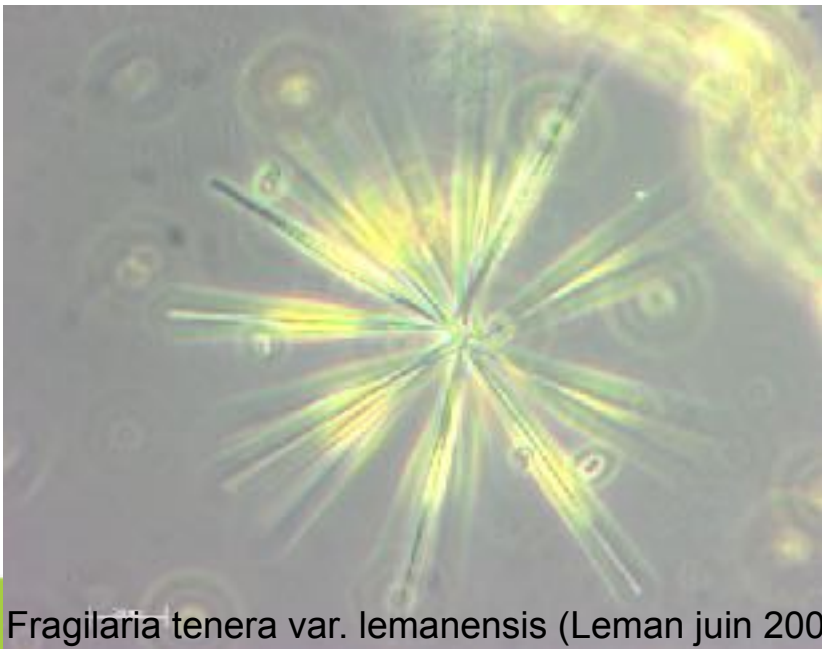


Cyclotella costei

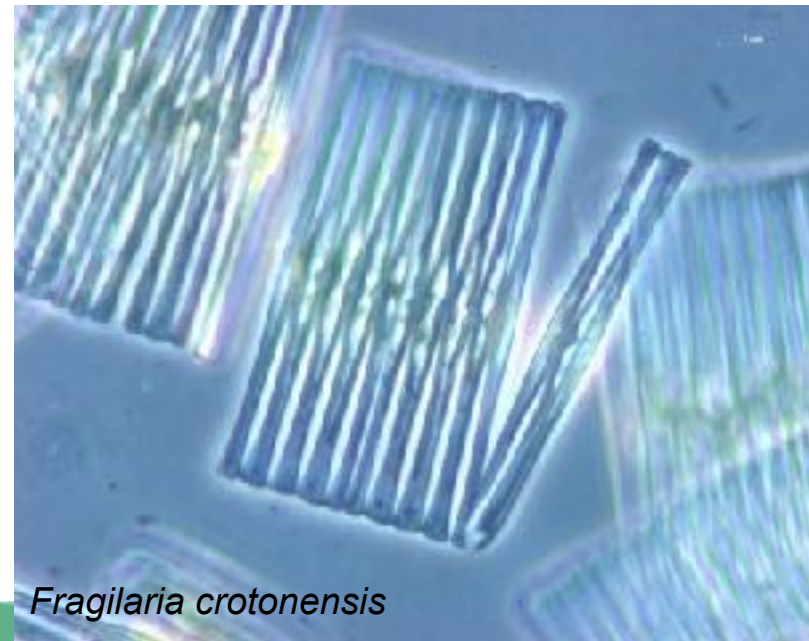
Taxonomy



- **Fragilariophyceae** : F. Fragilariaceae
- No raphe in this family
- *Fragilaria/Ulnaria* : presence of a pseudo-raphe

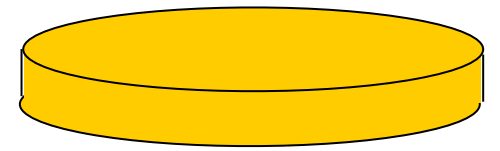


Fragilaria tenera var. *lemanensis* (Leman juin 2008)



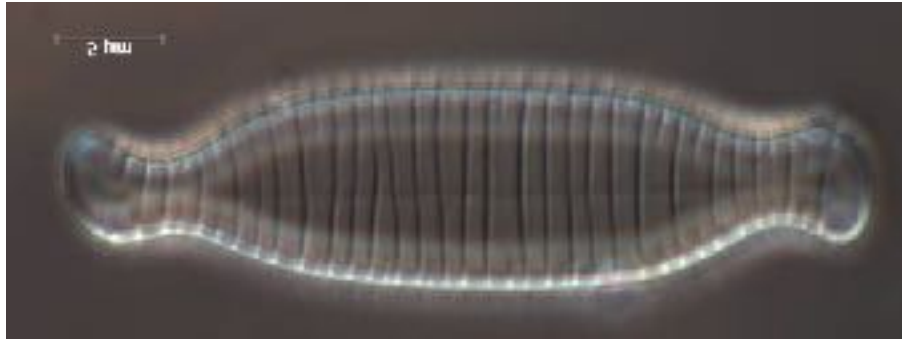
Fragilaria crotonensis

Taxonomy



- **Fragilariophyceae** : F. Fragilariaceae

Diatoma: strong transversal costae

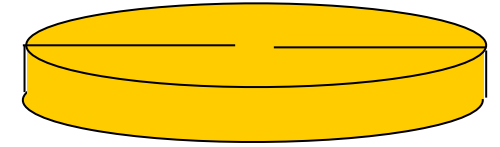


D. ehrenbergii

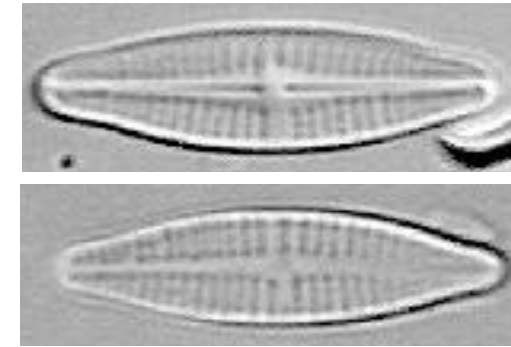
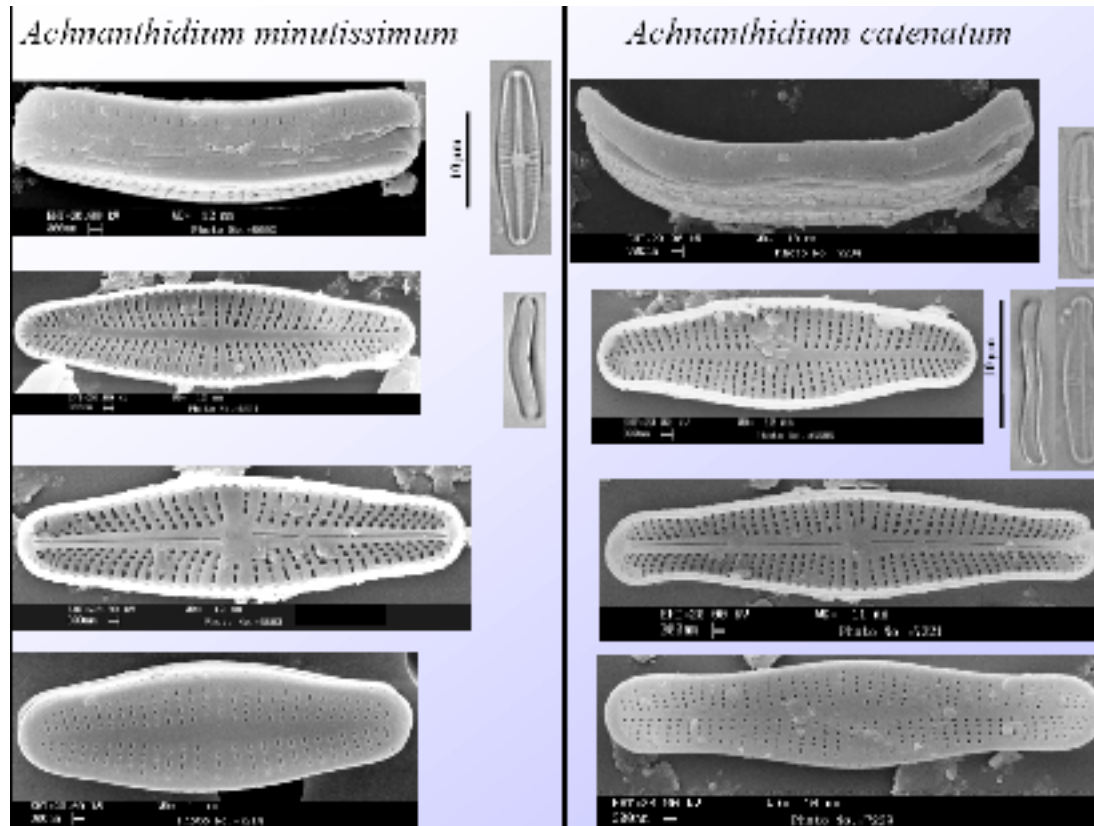


D. elongatum (Bourget 2009)

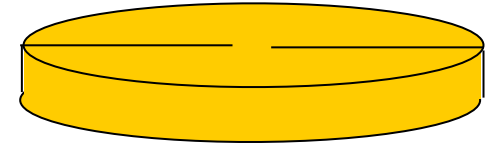
Taxonomy



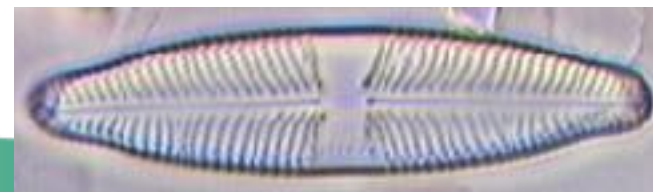
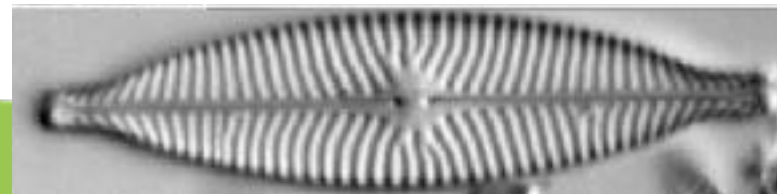
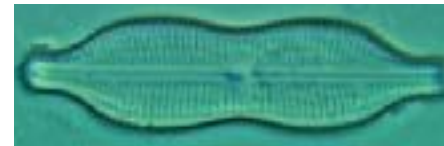
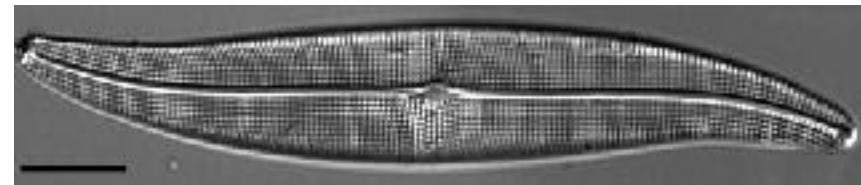
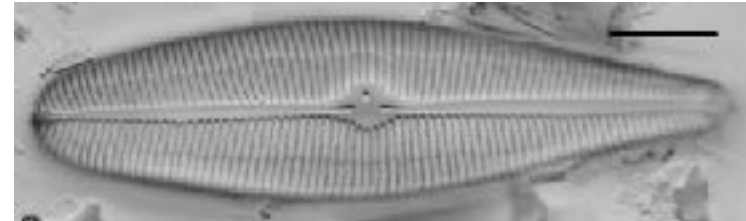
- **Bacillariophyceae:** F. Achnantheaceae, Achnanthidiaceae
 Raphe present only on one valve (Monoraphids)



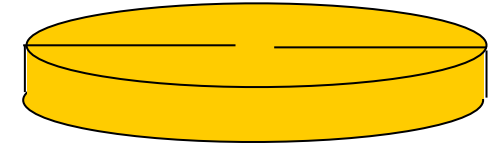
Taxonomy



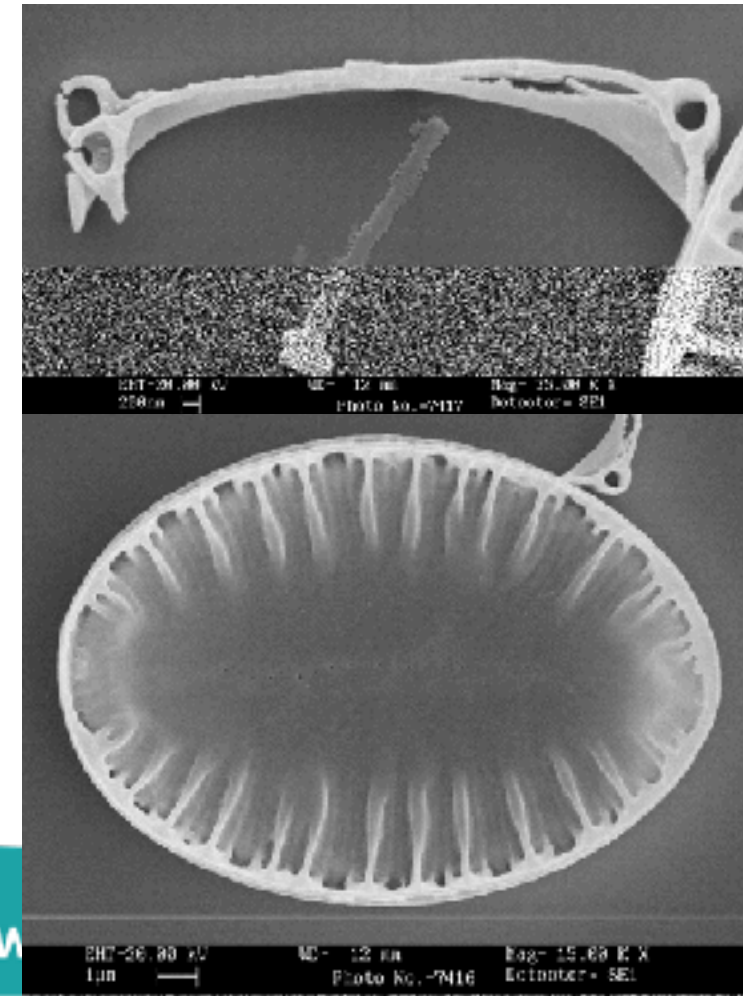
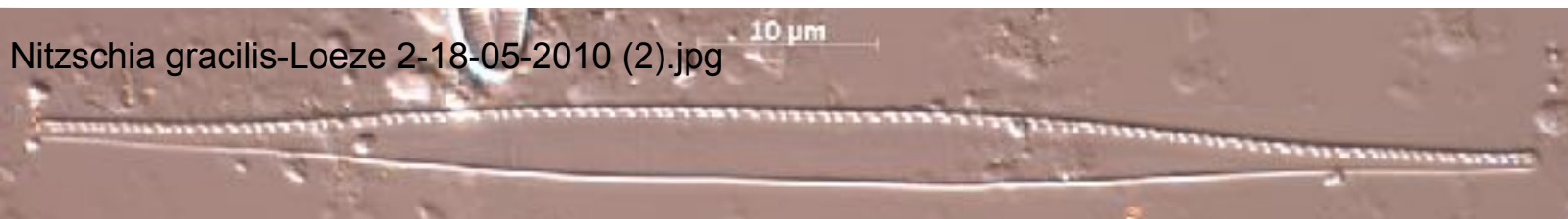
- **Bacillariophyceae** : Biraphids, raphe on the 2 valves
- Heteropolar valves:
- Isopolar valves:
 - Curved raphe
 - Sigmoid raphe
 - Straight raphe
 - Raphé avec 2 crochet au centre : Neidium
 - Cotes lisses : Pinnularia
 - Aire centrale en stauros : Stauroneis
 - Stries irrégulières, très fines : Brachysira
 - Navicula sensu stricto



Taxonomy



- **Bacillariophyceae** : Biraphidées, raphé sur les 2 valves
- Heteropolar valves:
- Isopolar valves:
 - Curved raphe
 - Sigmoid raphe
 - Straight raphe
 - Raphé avec 2 crochet au centre : Neidium
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 - Stries irrégulières, très fines : Brachysira
 - Navicula sensu stricto
 - Canal raphe





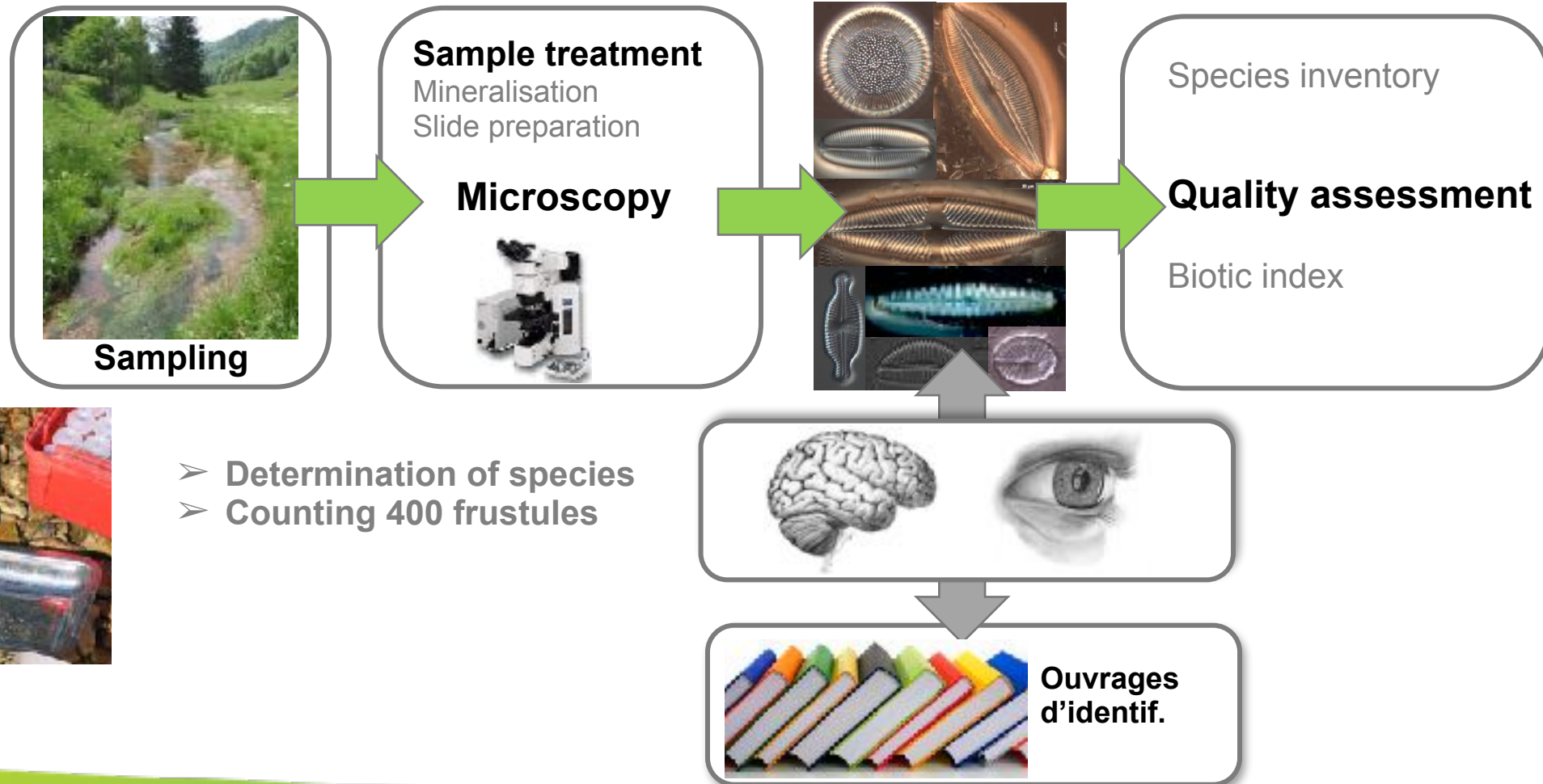
Reminders

- 1- Diatom biology
- 2- Taxonomy
- 3- Classical diatom biomonitoring ←



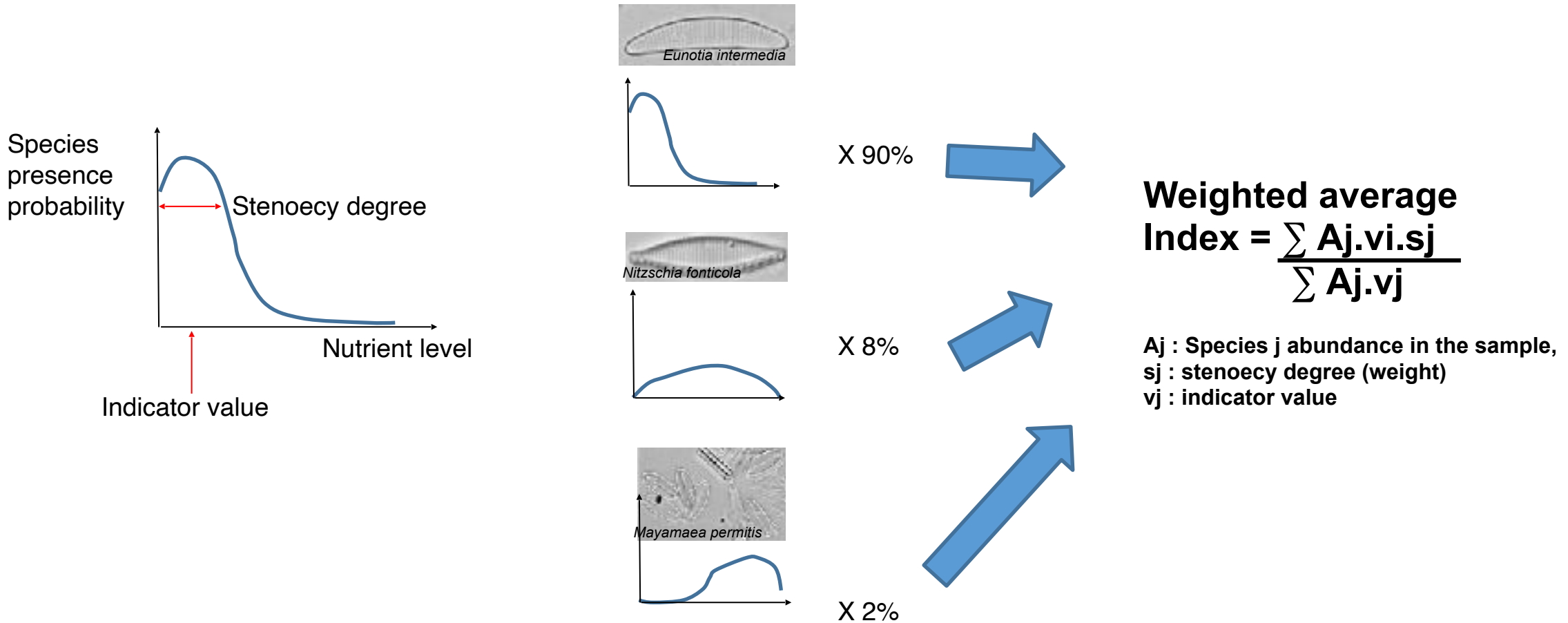
Usual methodology for diatom biomonitoring

❖ Standardised methodology: microscopy



Principle of a biotic index

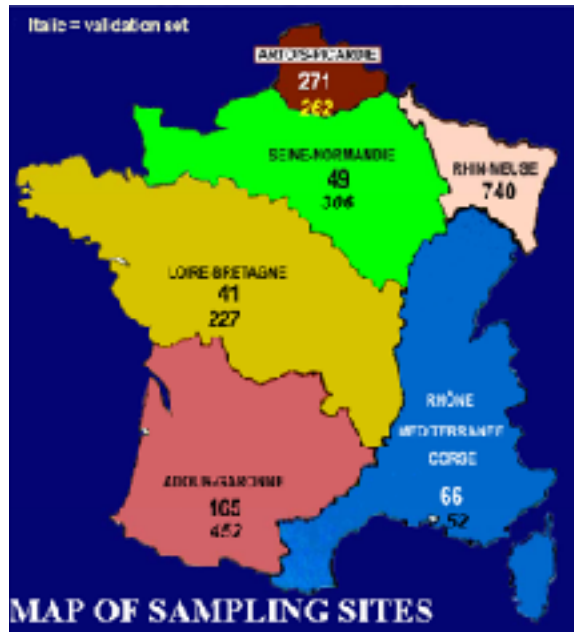
Based on the formula of Zelinka & Marvan (1961): ecological profiles are weighted by the abundance of the species in the sample



Which biotic index is used in France?

IBD: Indice Biologique Diatomée

Index developed in the framework of the WFD, first publication in 2000, and also standardised in 2000 (Afnor 2000).
New versions in 2009 and 2019.



How IBD is used to assess river ecological status?

River status is based on 3 quality parameters

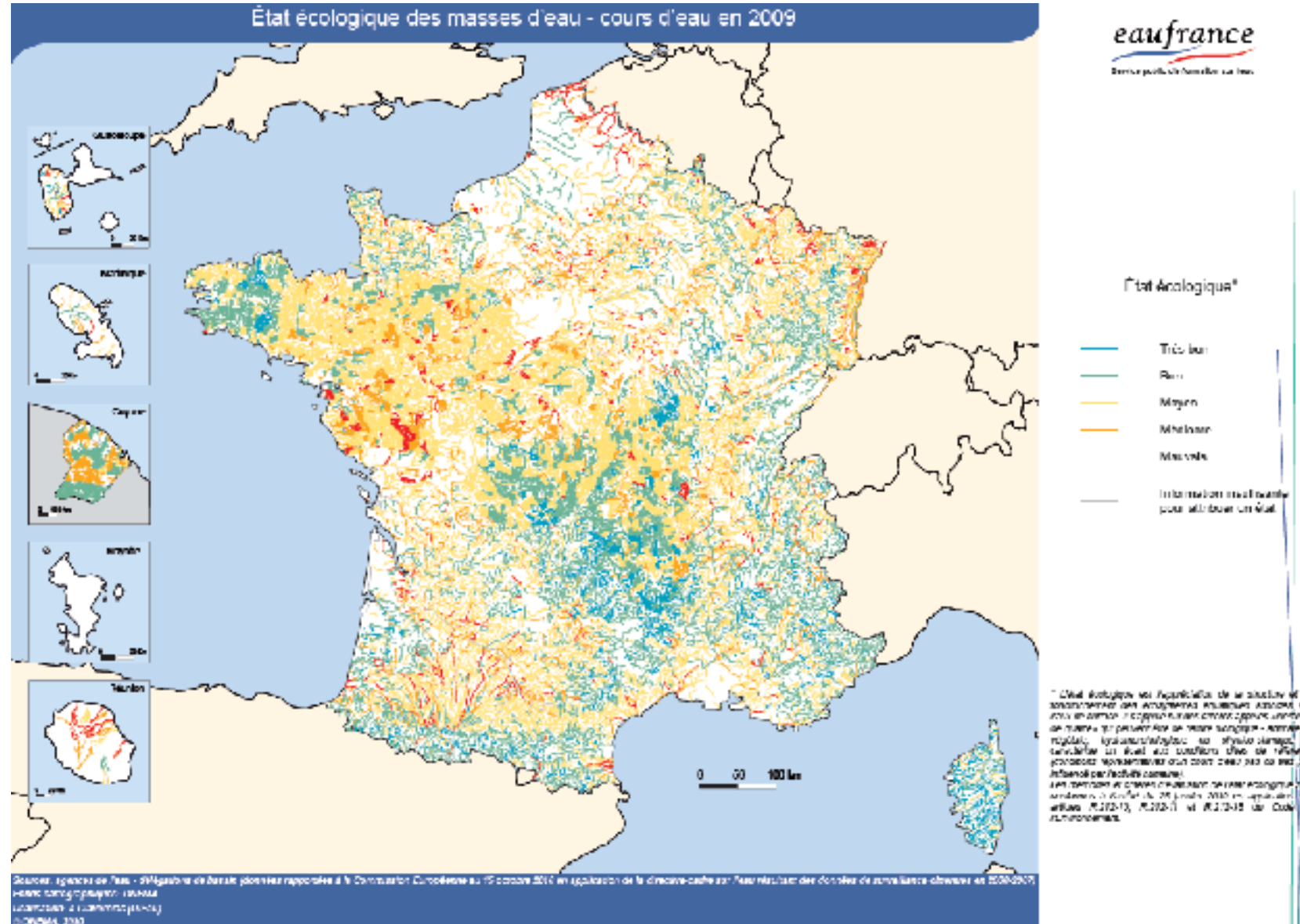
Hydro-morphological quality

Physico-chemical quality

Biological quality

- Diatoms
- Macroinvertebrates
- Fish
- Macrophytes

Ecological status of rivers in 2009



3 quality parameters

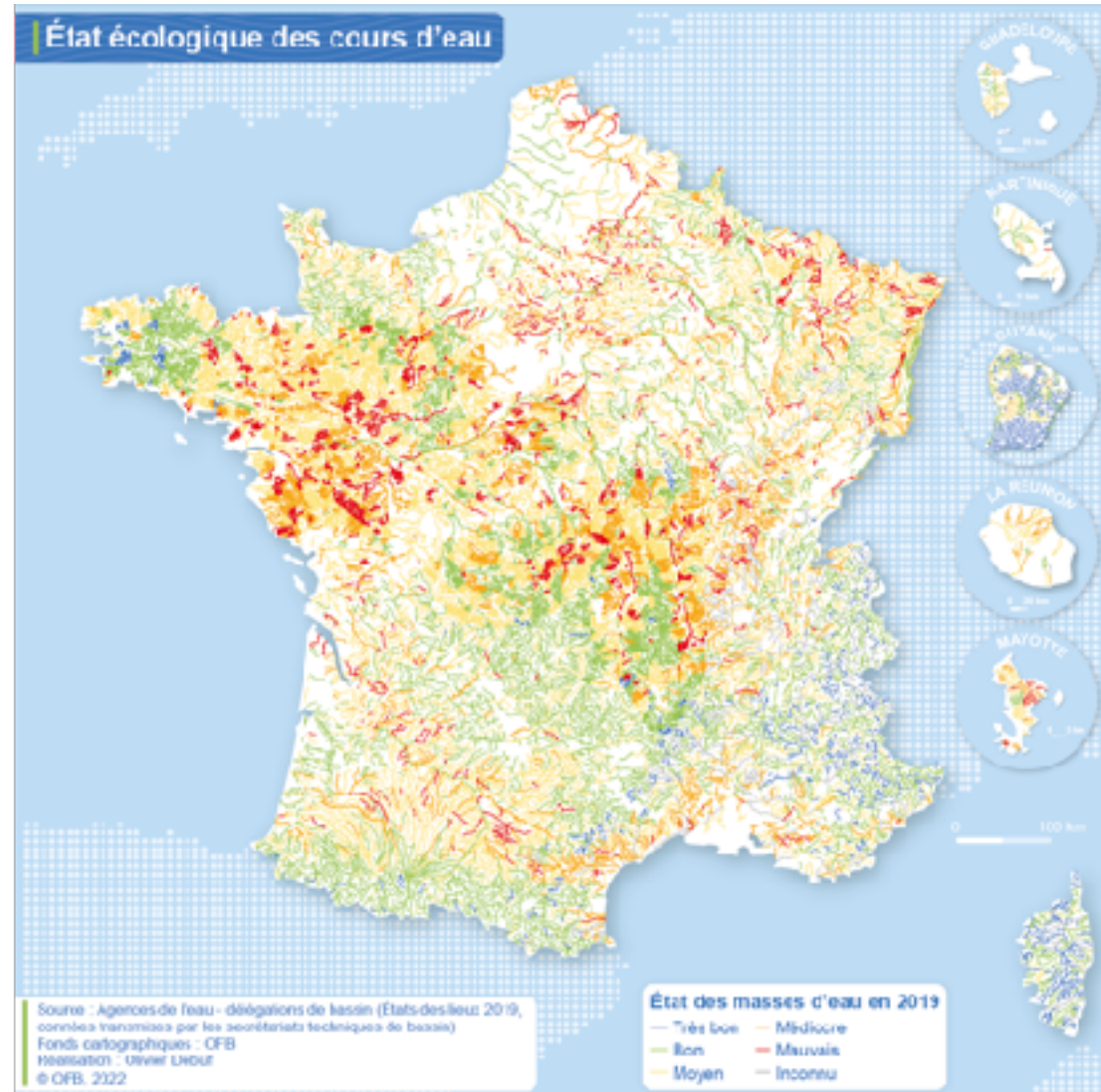
Hydro-morphological quality

Physico-chemical quality

Biological quality

Diatoms
Macroinvertebrates
Fish
Macrophytes

Ecological status of rivers in 2019



Ecological status of rivers in 2009

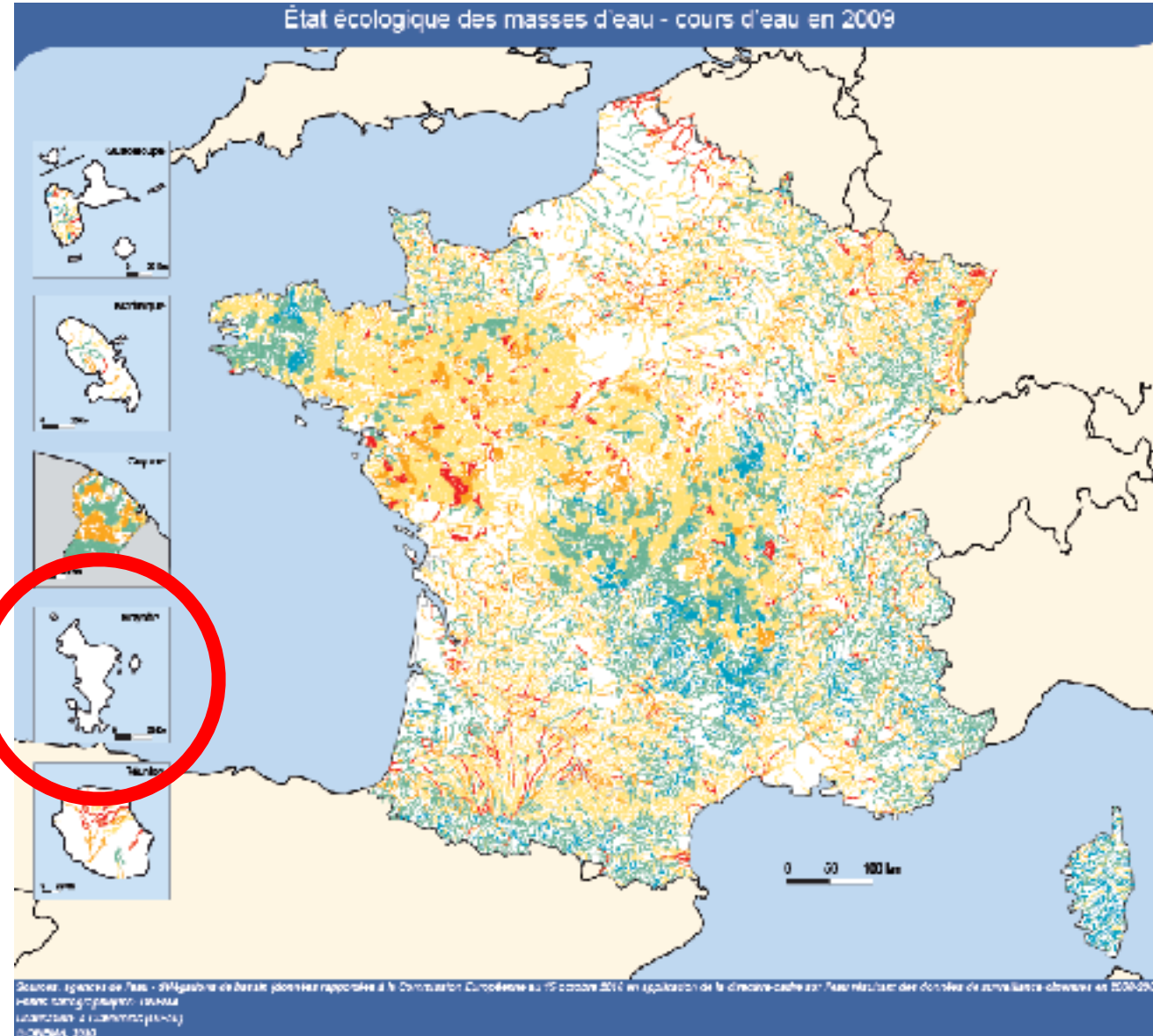
3 quality parameters

Hydro-morphological quality

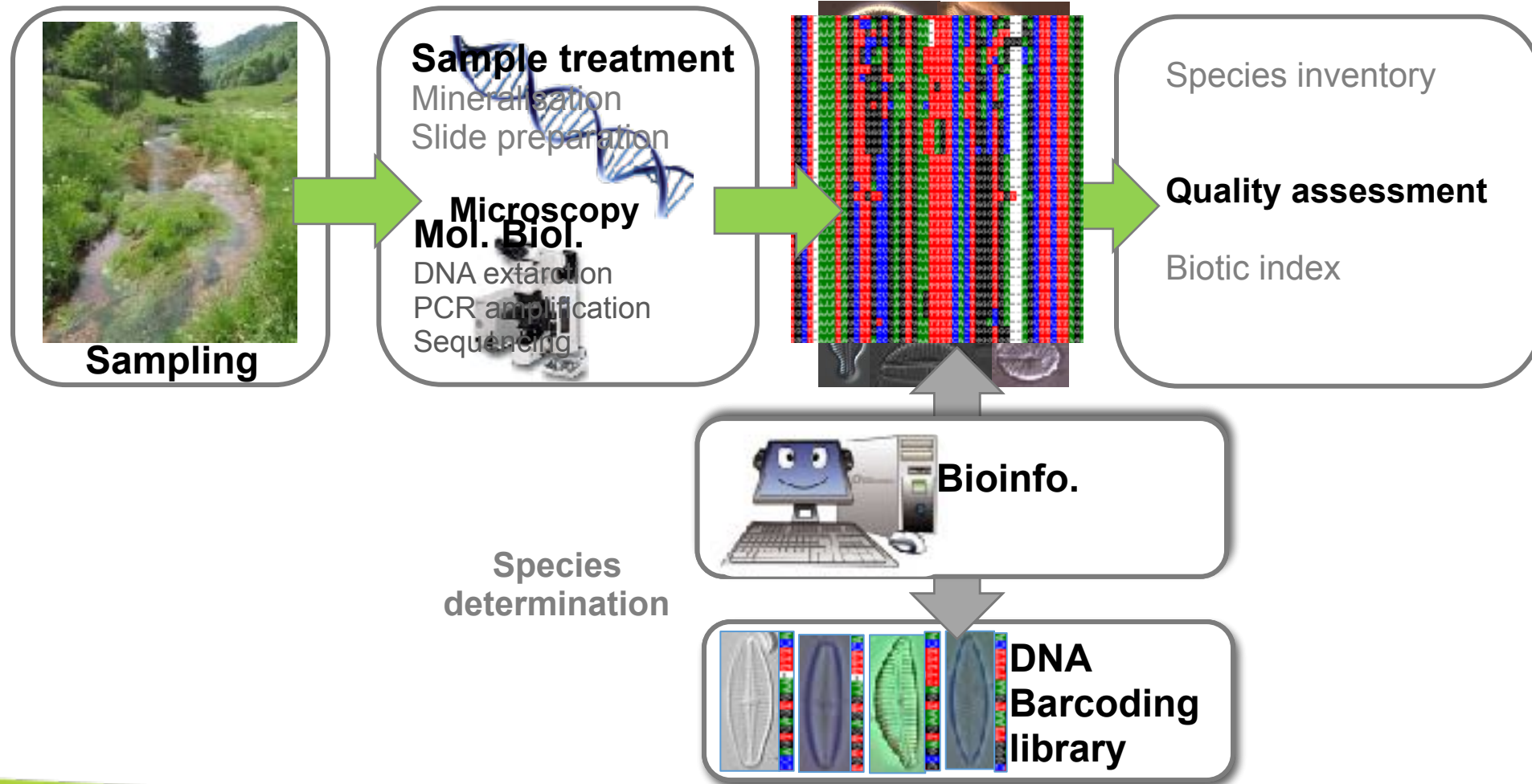
Physico-chemical quality

Biological quality

Diatoms
 Macroinvertebrates
 Fish
 Macrophytes



Objective: replace microscope determination and counting with DNA metabarcoding



Questions?



