Phytoplankton metabarcoding 1st part

F. Rimet

1- Biology, diversity of phytoplankton
2- Classical methodology for phytoplankton biomonitoring

Schedule:

Reminders

1- Biology, diversity of phytoplankton
2- Classical methodology for phytoplankton biomonitoring

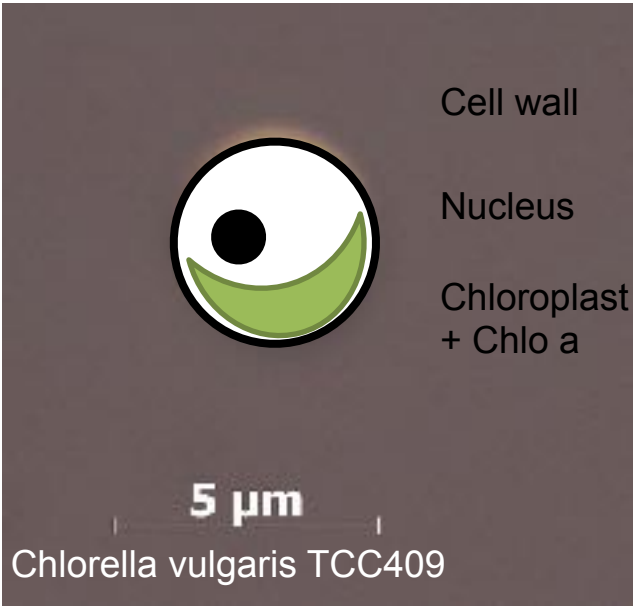
1- Biology, diversity of phytoplankton

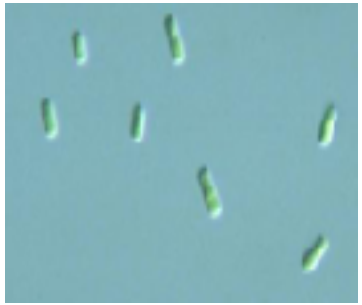
> Definition
> Diversity
> Endosymbiosis
> Taxonomy and phylogeny

Funded by European Union

Biology, diversity of phytoplankton

Definition a microalgae :
Photosynthetic organism
Simple vegetative unit (≠ higher plants, Embryophyta)

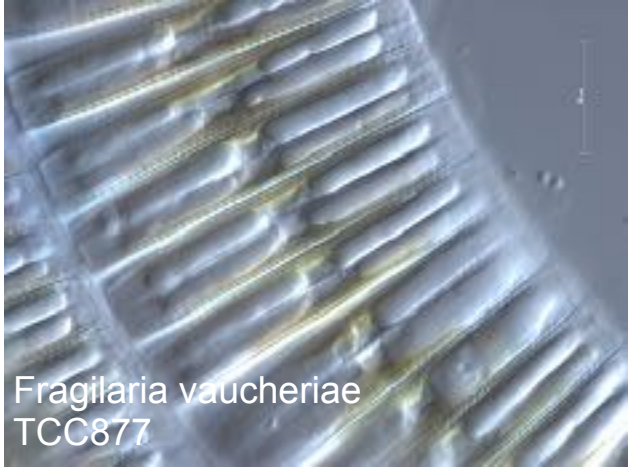




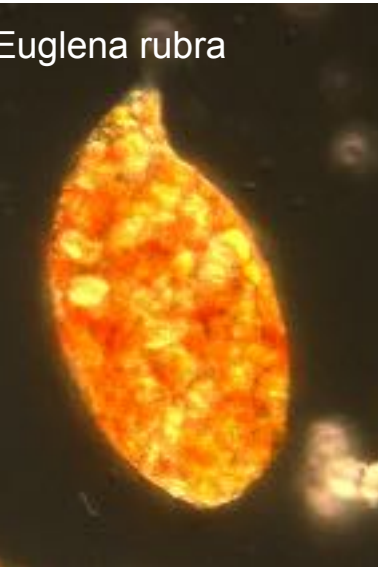
Synechococcus
No intracell organites

BIOLAWEB

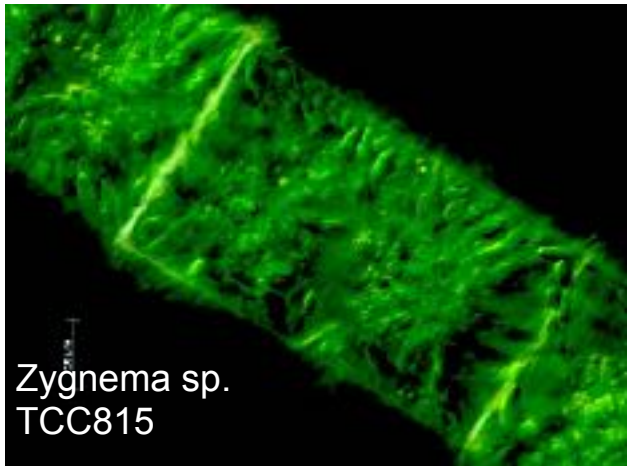
Diversity of shapes

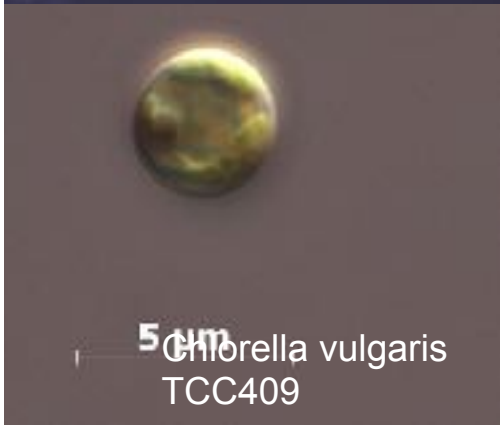

Fragilaria vaucheriae
TCC877


Ceratium cornutum
TCC827


Euglena rubra


Cryptomonas
TCC168


Zygnema sp.
TCC815

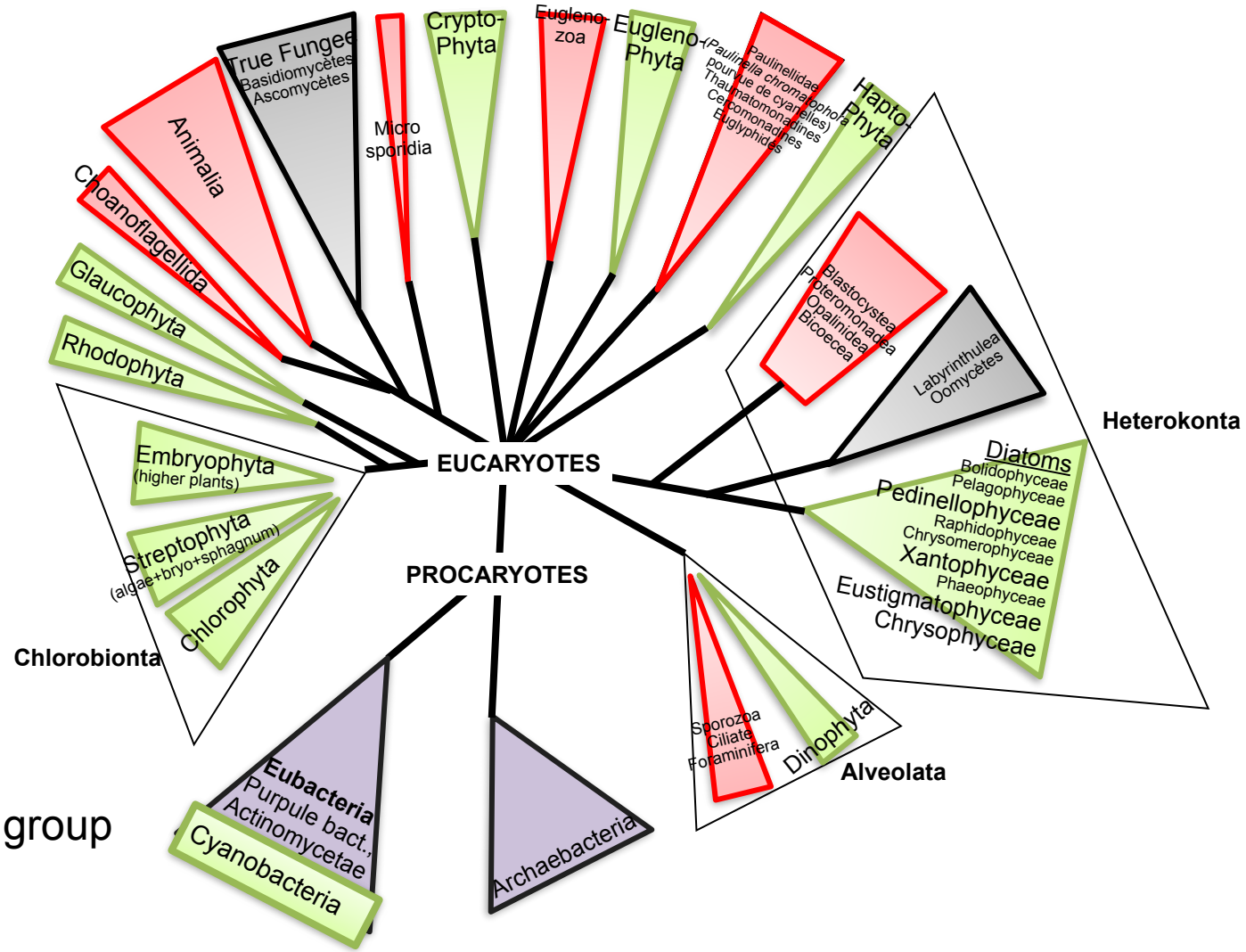


Chlorella vulgaris
TCC409

Biology, diversity of phytoplankton

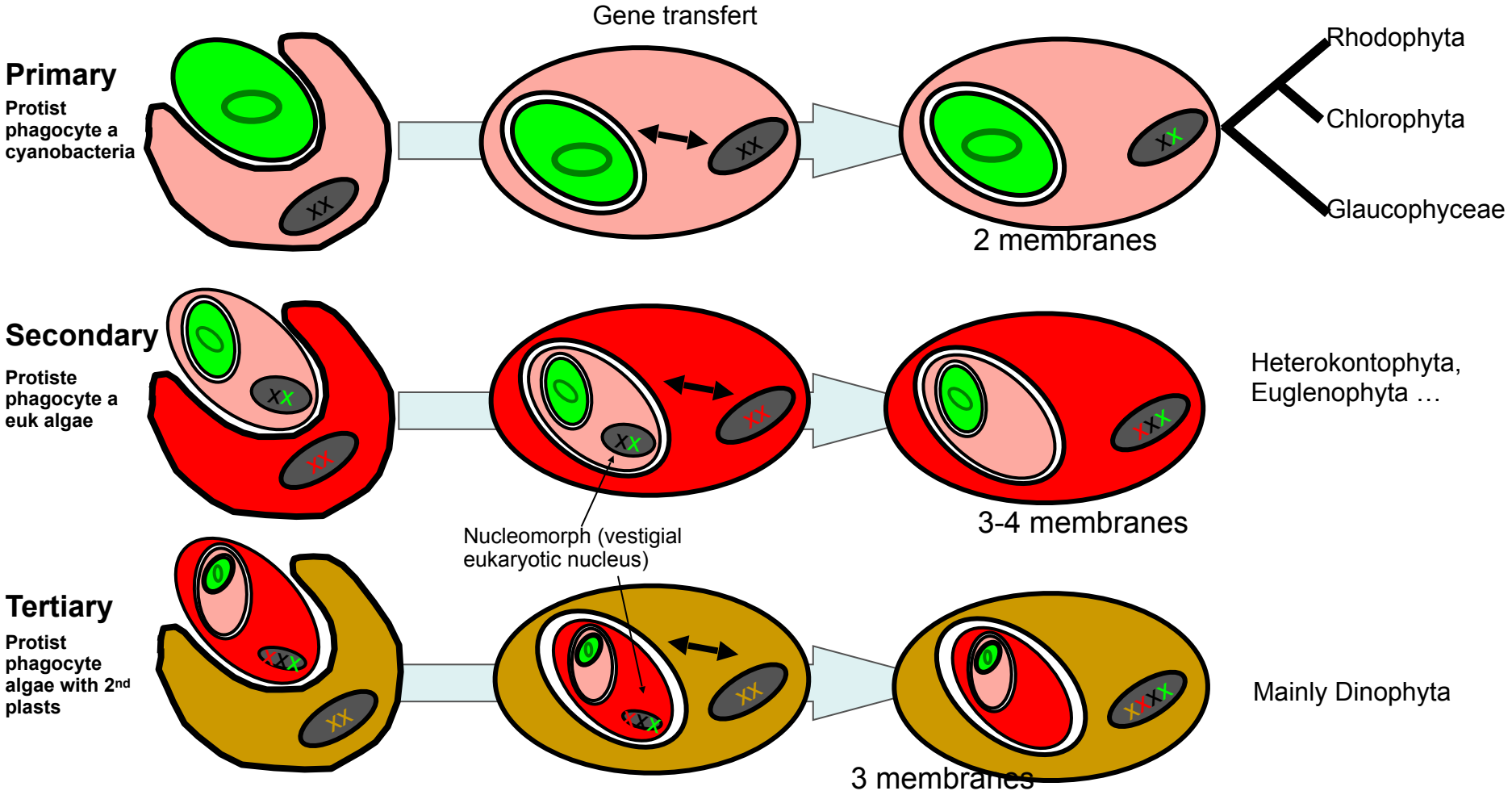
Where are they in the tree of life?

BIOLAWEB



Algae = polyphyletic group
> endosymbiosis

Endosymbiosis of the chloroplast in algae



Biology, diversity of phytoplankton

Important revision of eukaryotes taxonomy: Adl et al. 2019

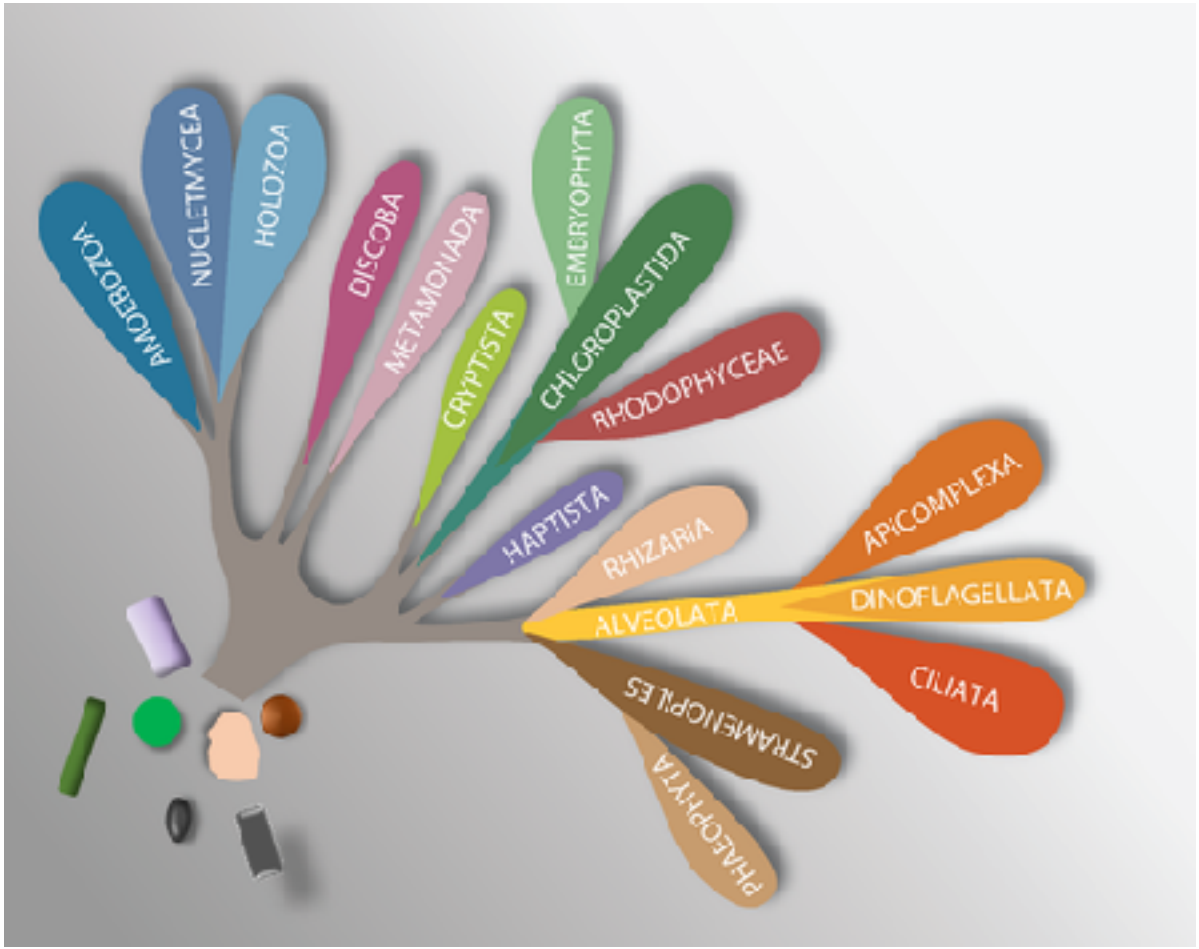
Journal of Eukaryotic Microbiology ISSN 1066-5234

ORIGINAL ARTICLE

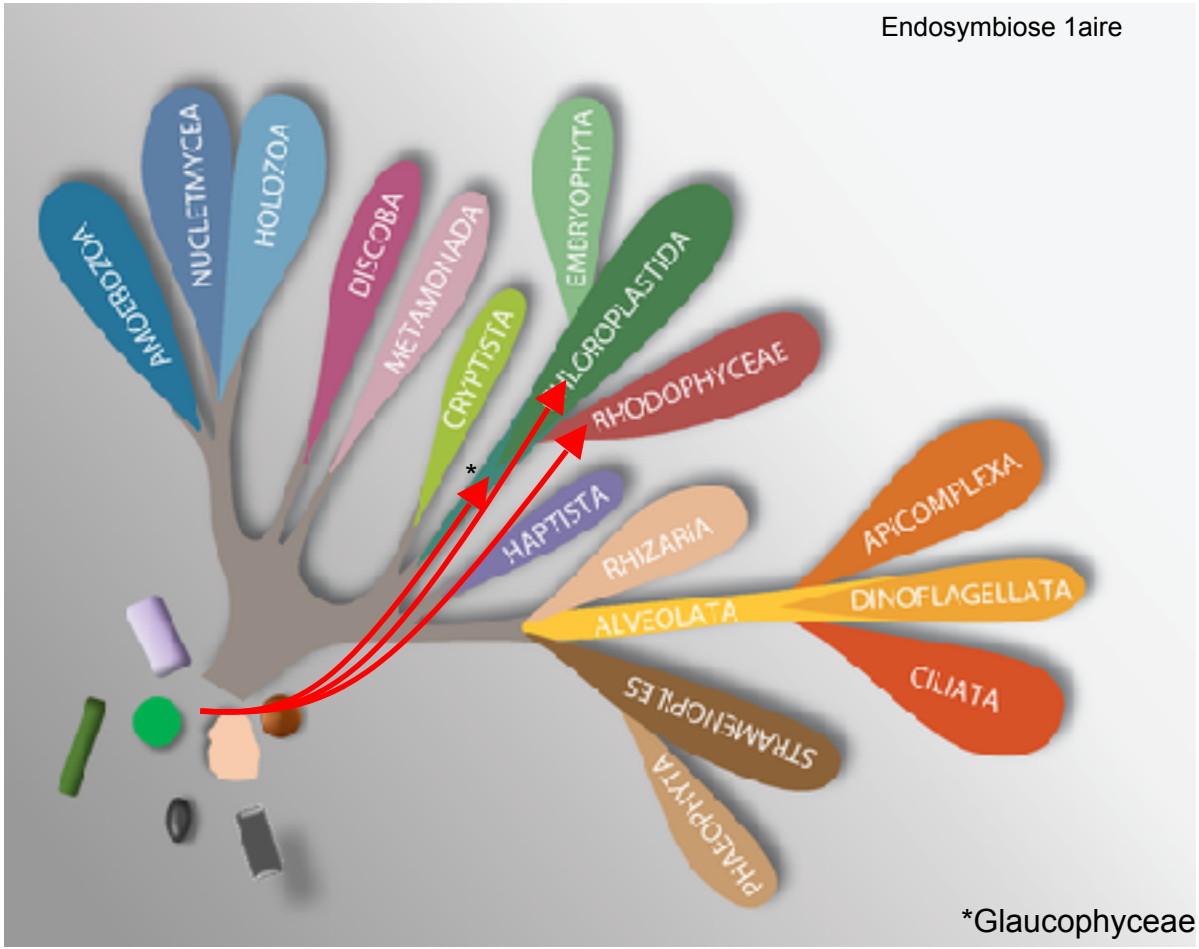
Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes

Sina M. Adl[a,*], David Bass[b,c], Christopher E. Lane[d], Julius Lukeš[e,f], Conrad L. Schoch[g], Alexey Smirnov[h], Sabine Agatha[i], Cedric Berney[j], Matthew W. Brown[k,l], Fabien Burki[m], Paco Cárdenas[n], Ivan Čepička[o], Lyudmila Chistyakova[p], Javier del Campo[q], Micah Dunthorn[r,s], Bente Edvardsen[t], Yana Eglit[u], Laure Guillou[v], Vladimír Hampl[w], Aaron A. Heiss[x], Mona Hoppenrath[y], Timothy Y. James[z], Anna Karnkowska[aa], Sergey Karpov[h,ab], Eunsoo Kim[x], Martin Kolisko[o], Alexander Kudryavtsev[h,ab], Daniel J.G. Lahr[ac], Enrique Lara[ad,ae], Line Le Gall[af], Denis H. Lynn[ag,ah], David G. Mann[ai,aj], Ramon Massana[q], Edward A.D. Mitchell[ak,al], Christine Morrow[al], Jong Soo Park[am], Jan W. Pawlowski[an], Martha J. Powell[ao], Daniel J. Richter[ap], Sonja Rueckert[aq], Lora Shadwick[ar], Satoshi Shimano[as], Frederick W. Spiegel[at], Guifré Torruella[ak], Noha Youssef[au], Vasily Zlatogursky[h,av] & Qianqian Zhang[aw]

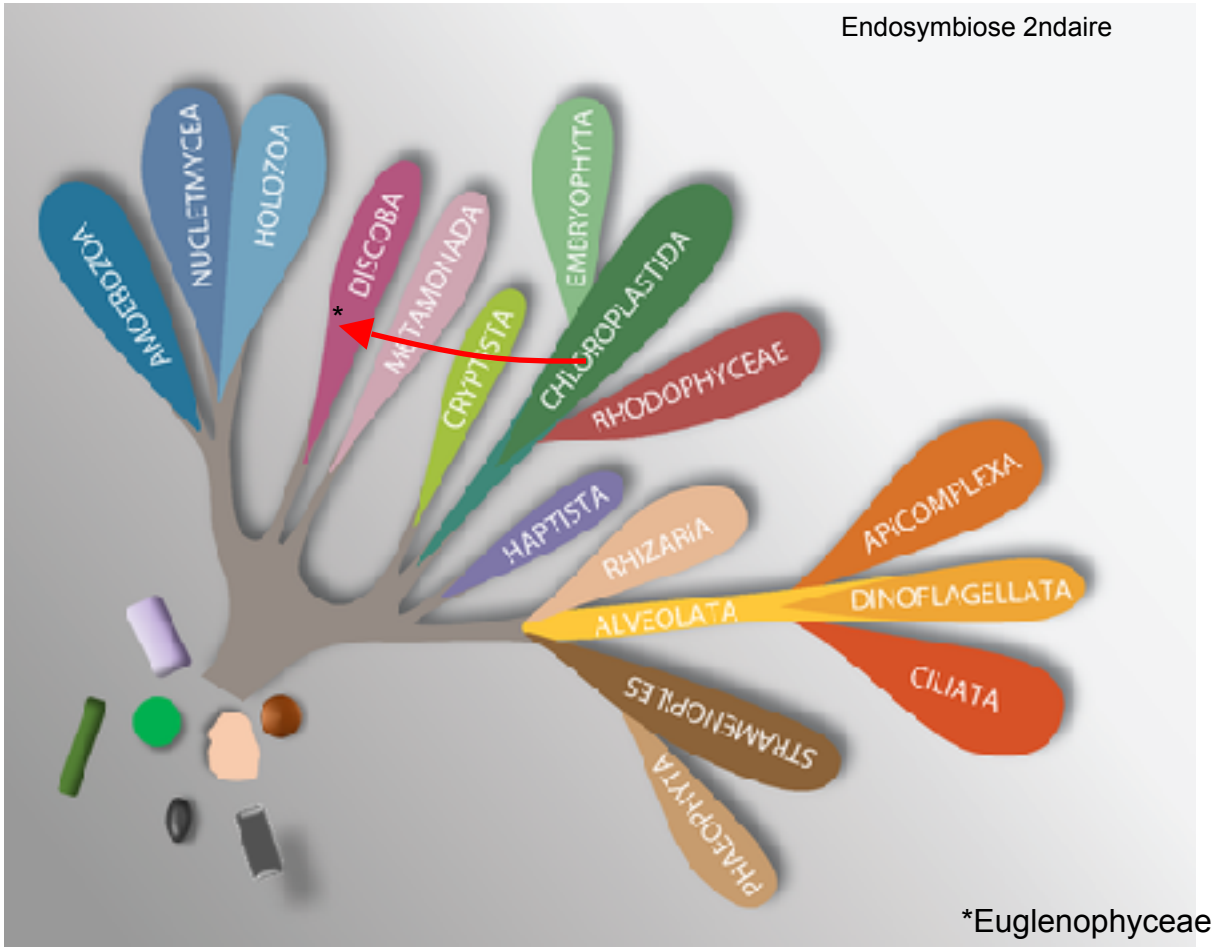
Funded by European Union



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

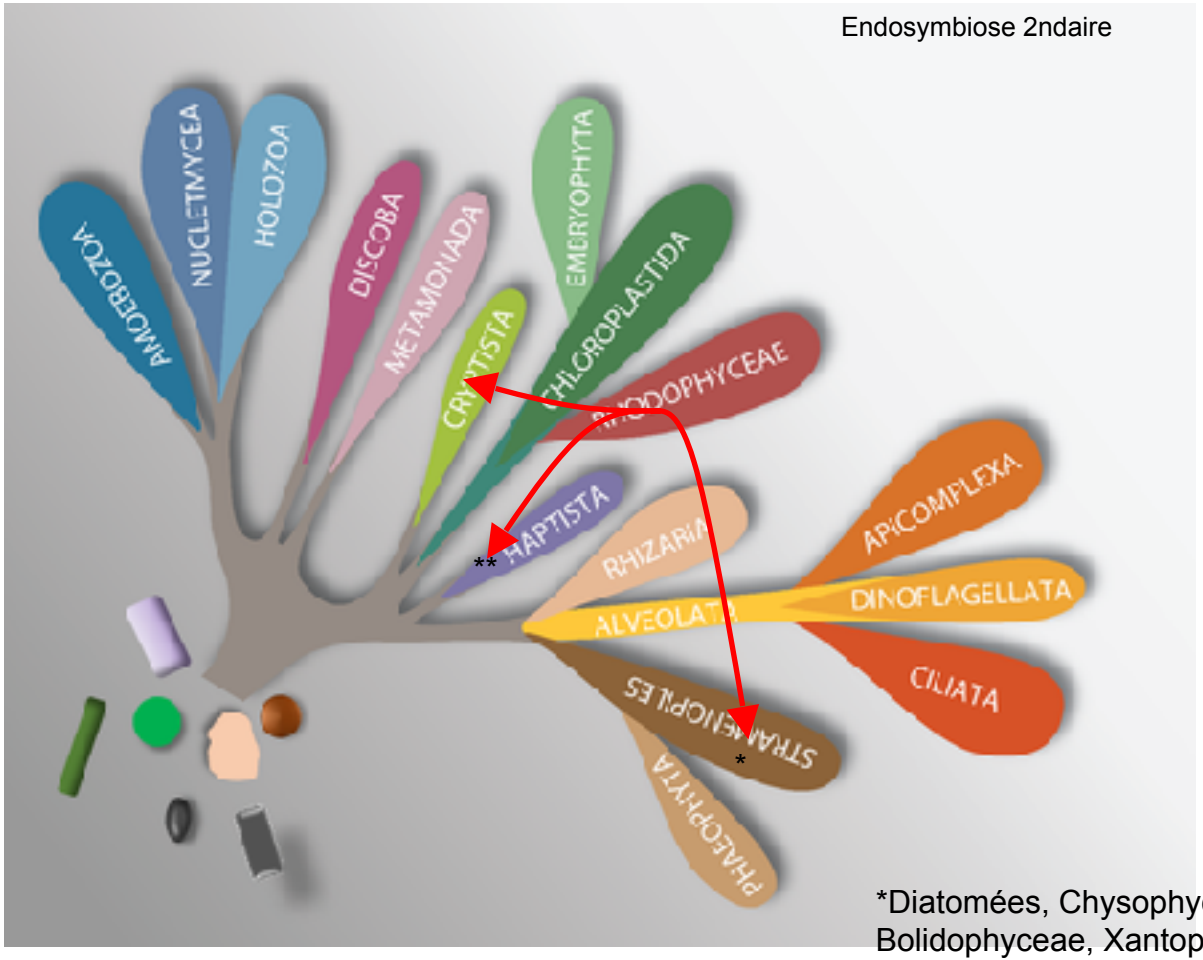


Adl et al. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. JEM



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

Endosymbiose 2ndaire



*Diatomées, Chysophyceae Bolidophyceae, Xantophyceae

**Erkenia (Coccolitophorideae)

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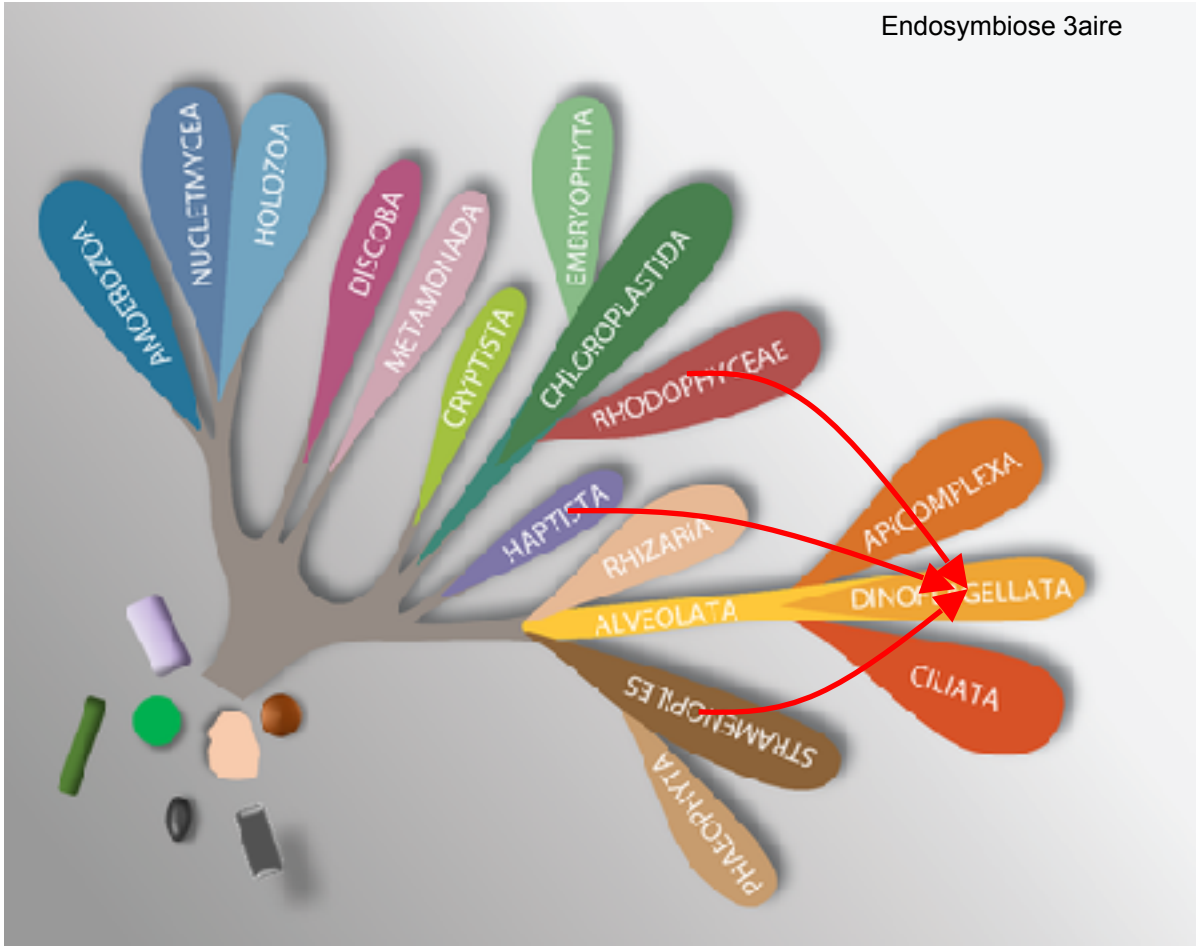


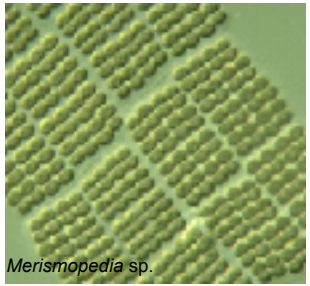
Adl et al. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. JEM

Biology, diversity of phytoplankton

Overview of the main algal groups

Funded by European Union

❖ Cyanobacteria: more than 2000 species



No intracellular organelle (prokaryotes)

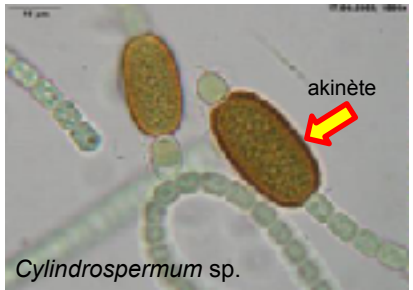
No flagella

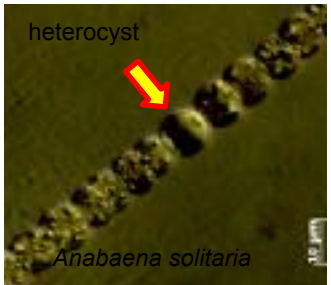
Mode of division: mitosis

Presence of akinetes (resting spore)

Presence of heterocysts (N2 fixation)

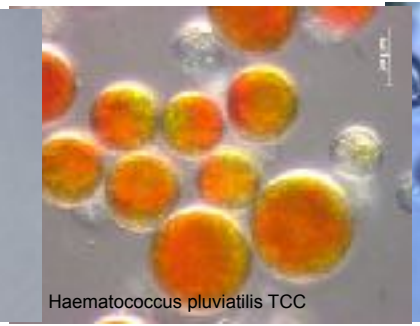
Presence of aerotopes





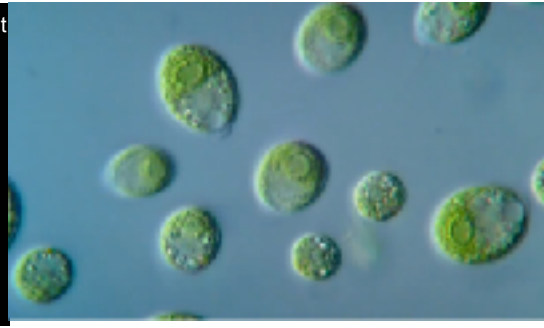
❖ Chlorophyta: more than 6000 species

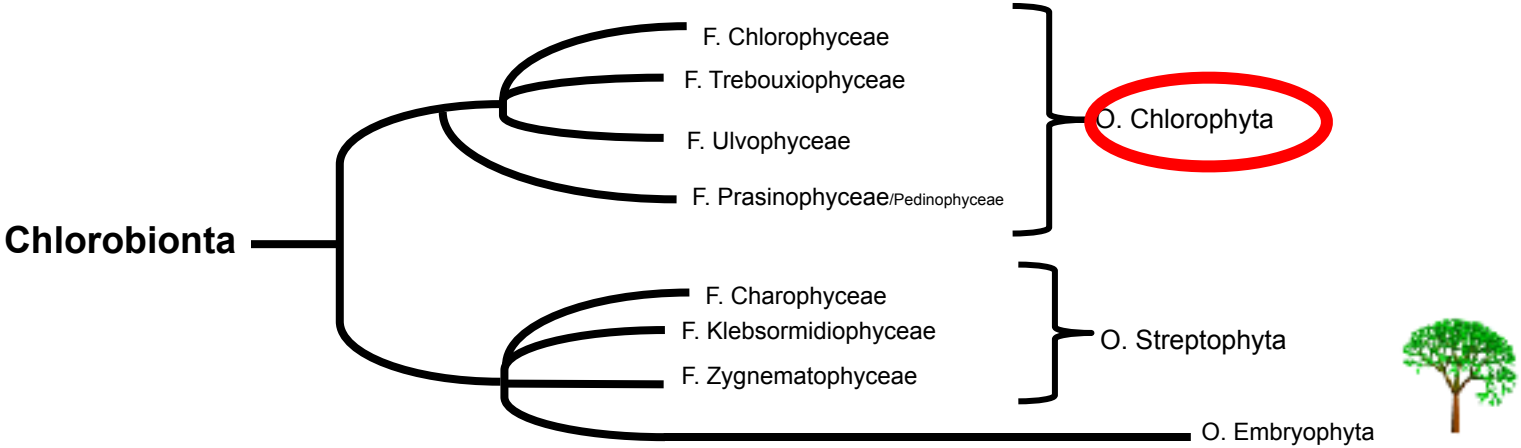

Tetraselmi sp.


Haematococcus pluviatilis TCC

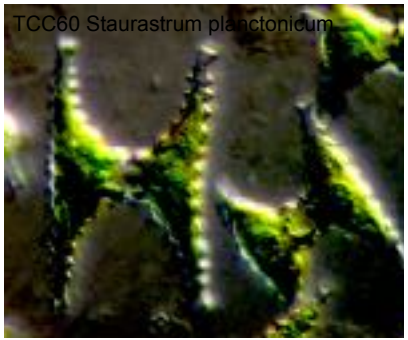

Pediastrum simplex


Pandorina morum (Bourget 2009)





❖ **Streptophyta: more than 4000 species**



Chlorobionta

- F. Chlorophyceae
- F. Trebouxiophyceae
- F. Ulvophyceae
- F. Prasinophyceae/Pedinophyceae

→ O. Chlorophyta

- F. Charophyceae
- F. Klebsormidiophyceae
- F. Zygnematophyceae

→ O. Streptophyta

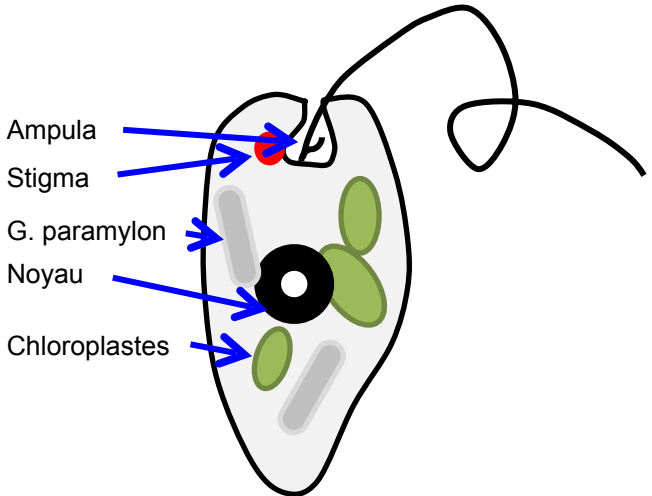
O. Embryophyta

❖ **Euglenophyta: more than 1200 species**

Mixotrophic, mobile

Several chloroplasts/cell

1 apical flagellum



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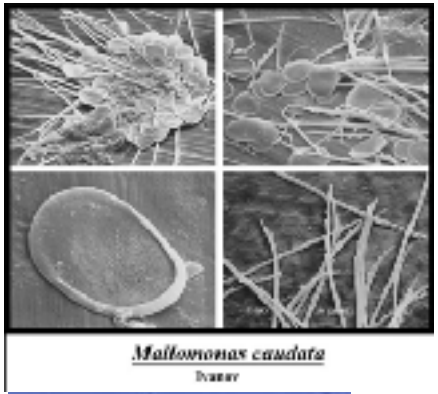
❖ Chrysophyta: more than 1000 species

Produce siliceous cysts, brown color, often mobile

Colonial or unicellular, presence of two unequal flagella

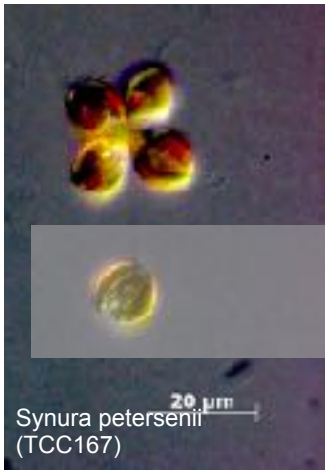
Dinobryon divergens (Bourget n9-2009)



Mallomonas caudata

Ivanov

Mallomonas sp.

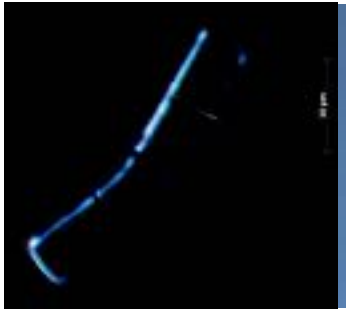


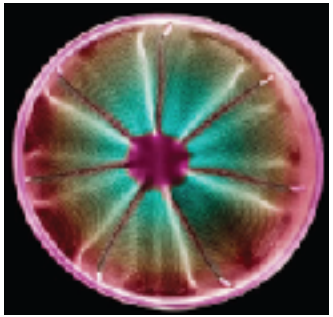
Synura petersenii (TCC167)

BIOLAWEB

❖ Diatoms: 100.000 species

(Vanormelingen & Mann 2013)

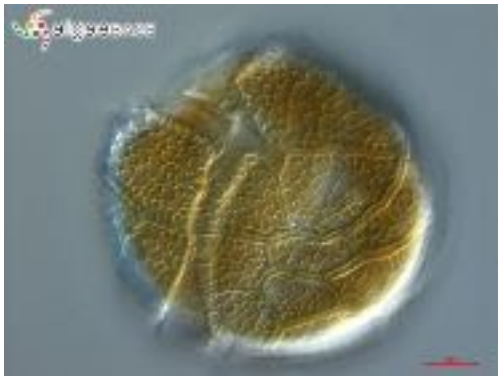


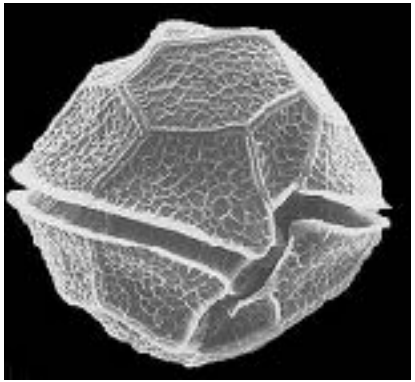


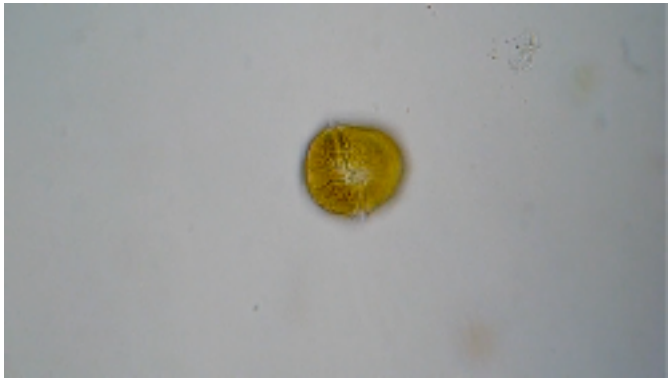
❖ Dinophyta: more than 3000 species

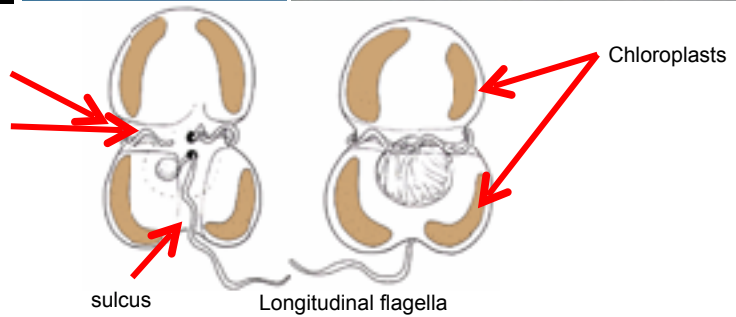
Unicellular, cell surrounded by a cellulose wall composed of plates

Chlorophyll a, c, beta-carotene, peridinin > brown









2- Phytoplankton biomonitoring in lakes

Funded by European Union

Phytoplankton biomonitoring in lakes

The French phytoplankton index in lakes: IPLac

Aims: - to assess the trophic level of lake (nutrient concentration)
- to be representative of the overall lake quality

Ecological Indicators 69 (2015) 686–700

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Performance of the Phytoplankton Index for Lakes (IPLAC): A multimetric phytoplankton index to assess the ecological status of water bodies in France

Christophe Laplace-Treyture*, Thibaut Feret

Carte 1: Inventaire des échantillonnages "phytoplancton" sur les

Phytoplankton biomonitoring in lakes

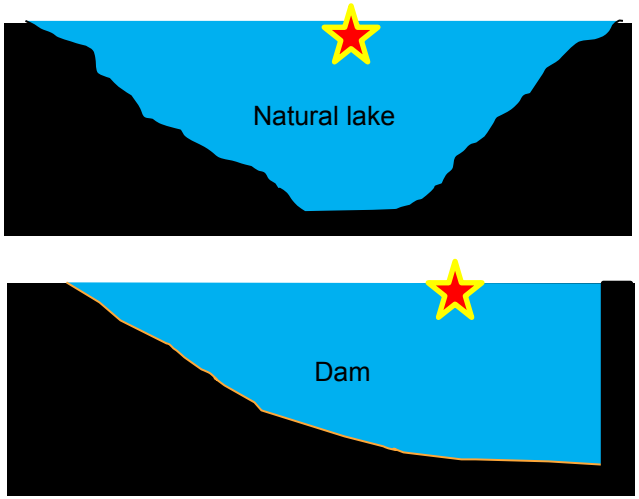
The French phytoplankton index in lakes: IPLac

Shedule:

- Field sampling (frequency, position, depth)
- Lab procedure for microscopy
- Estimation of biovolumes
- Metrics calculation

Field sampling

- **Position of the sampling site**
  - Far from tributaries
  - Above the deepest point of the lake
  - Distance from the shore must be enough to avoid contamination with littoral and benthic species



Field sampling

- **Sampling frequency**
  - For the WFD in France:
  - 4 samplings per year, every 6 year:
    - Late winter, 1st biomass development, (Feb-March)
    - During spring, when the thermocline appears (May-June)
    - Summer, during the 2nd biomass development (Jul-Aug)
    - End of summer stratification, when the epilimnion has a maximum depth (Sept-Oct)

Field sampling

❖ Sampling layer

- ❖ Euphotic zone (=layer that extends to a depth residual light intensity = 1% of surface light)
- ❖ Euphotic zone corresponds to 2.5 x Secchi depth
- ❖ Use of Integrated Water Sampler: collect regularily water along a depth transect

Mechanical:
Based on sampler shape and pressure

Programmable:
microprocessor, electric engine, pressure sensor

Phytoplankton biomonitoring in lakes

The French phytoplankton index in lakes: IPLac

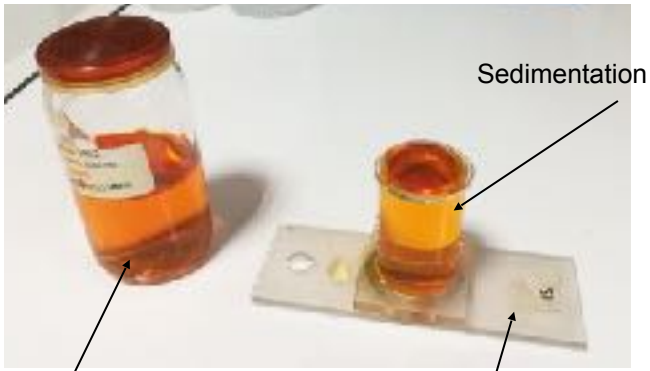
- Field sampling (frequency, position, depth)
- Lab procedure for microscopy
- Estimation of biovolumes
- Metrics calculation

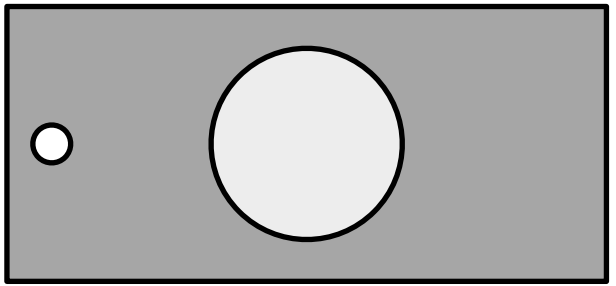
Funded by European Union

Lab procedure for microscopy

❖ Use of a microscope sedimentation chamber

- ❖ Utermohl method (1958), standard in 2005 by the CEN (European Committee for Standardisation)

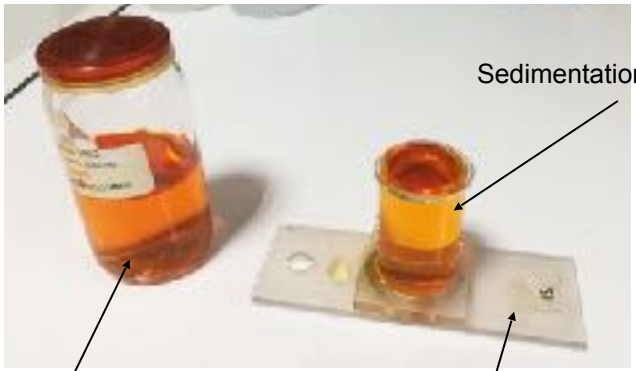


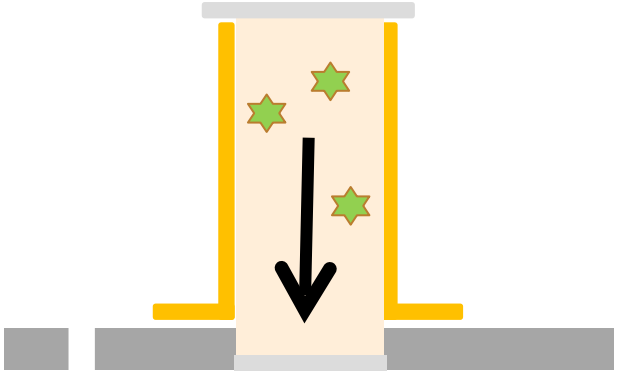


Lab procedure for microscopy

❖ Use of a microscope sedimentation chamber

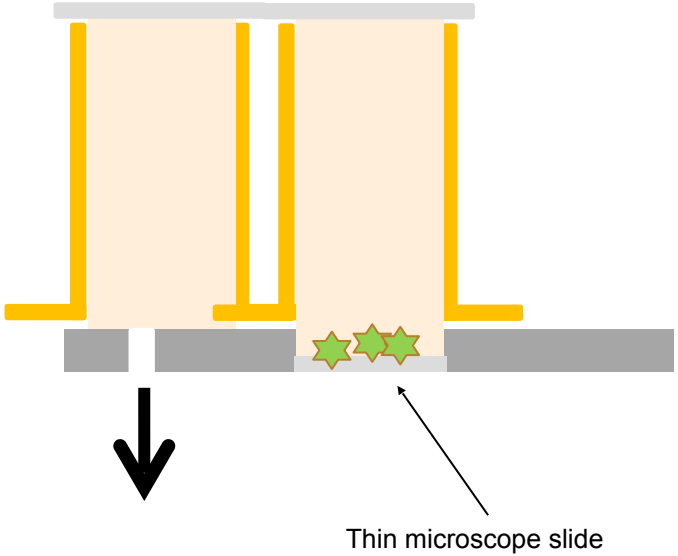
- ❖ Utermohl method (CEN standard in 2005)
- ❖ Sedimentation of a known volume





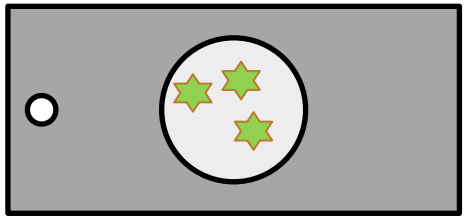
Lab procedure for microscopy

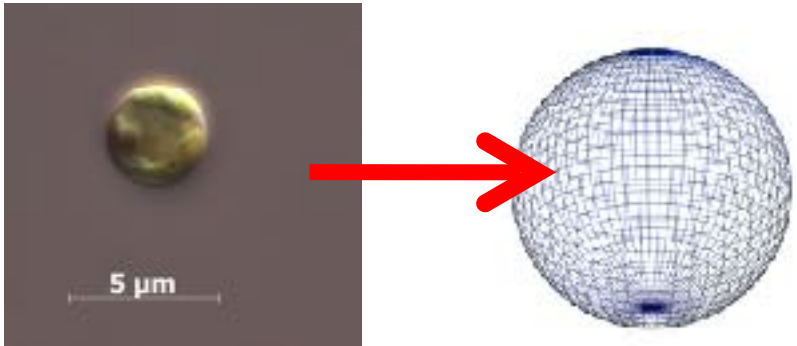
- **Use of a microscope sedimentation chamber**
  - Utermohl method (CEN standard in 2005)



Lab procedure for microscopy

- **Obervation under inverted micrscope**
- Determination under microscope (x40)
- Count of at least 400 cells (or algal objects) for a known volume of sample
- For each cell the specific biovolume must be known





Phytoplankton biomonitoring in lakes

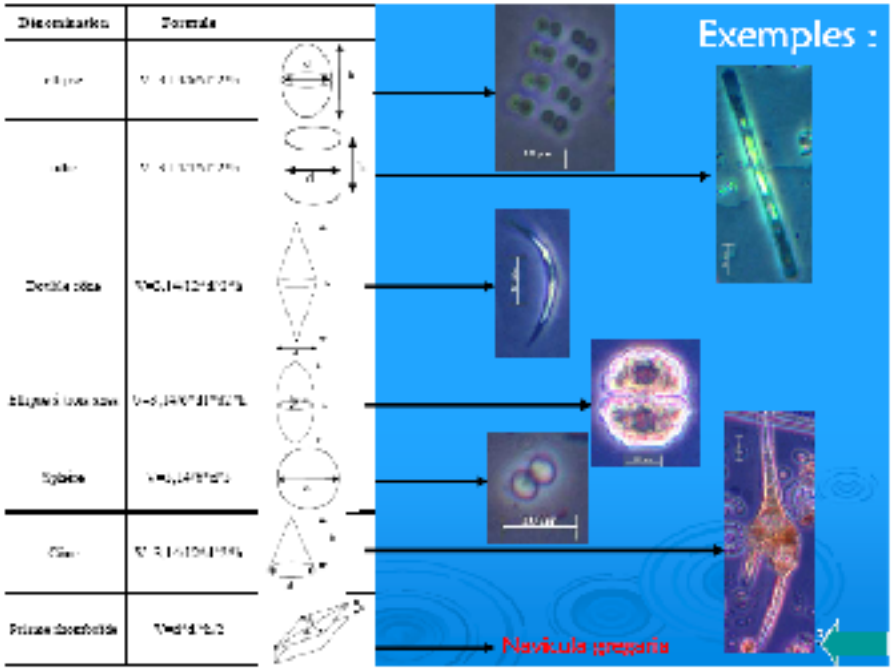
The French phytoplankton index in lakes: IPLac

- Field sampling (frequency, position, depth)
- Lab procedure for microscopy
- Estimation of biovolumes
- Metrics calculation

Estimation of biovolumes

For each species: a specific biovolume has to be defined

Cell measures + assignation of a geometric shape



Final result: a floristic list with biovolume for each taxon

Species name	Nb of cells / ml of sample	µm3 of algae / ml of sample
Diatoma elongatum	87,3	53 000
Aphanizomenon gracile	110,1	70 000
Ceratium hirundinella	3,8	149 000

Implementation

The French phytoplankton index in lakes: IPLac

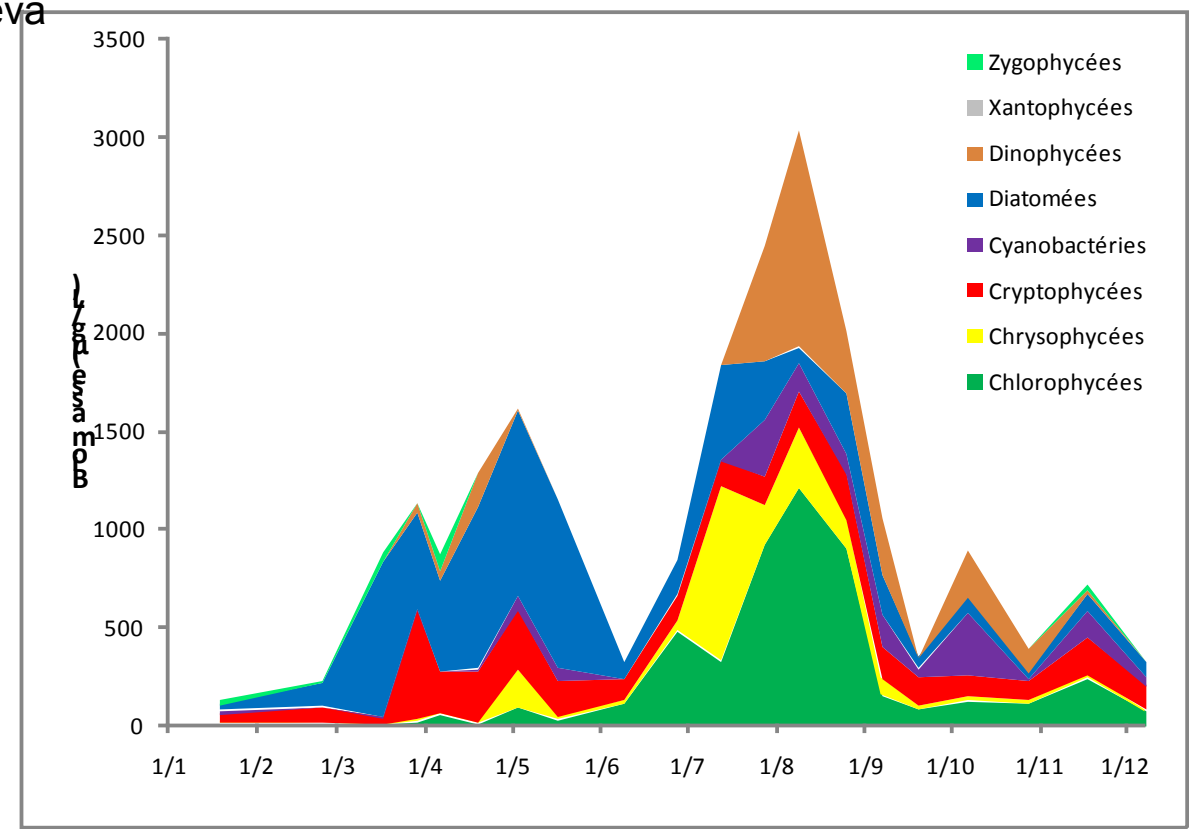
- Field sampling (frequency, position, depth)
- Lab procedure for microscopy
- Estimation of biovolumes
- Metrics calculation

Metrics calculation

❖ **Before explaning the calculation: some basic about phytoplancton communities**

Example with lake Geneva

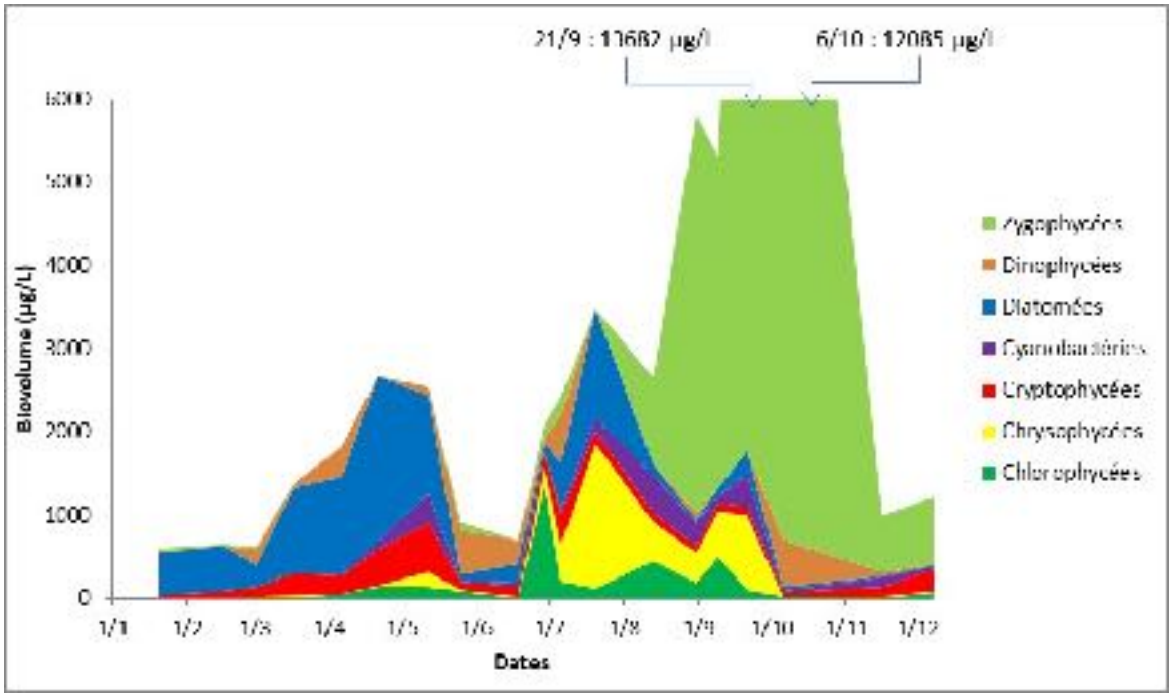
Seasonal biomass dynamic for Lake Geneva in 2010 (SHL2)



Metrics calculation

❖ **These dynamics change from a year to another**

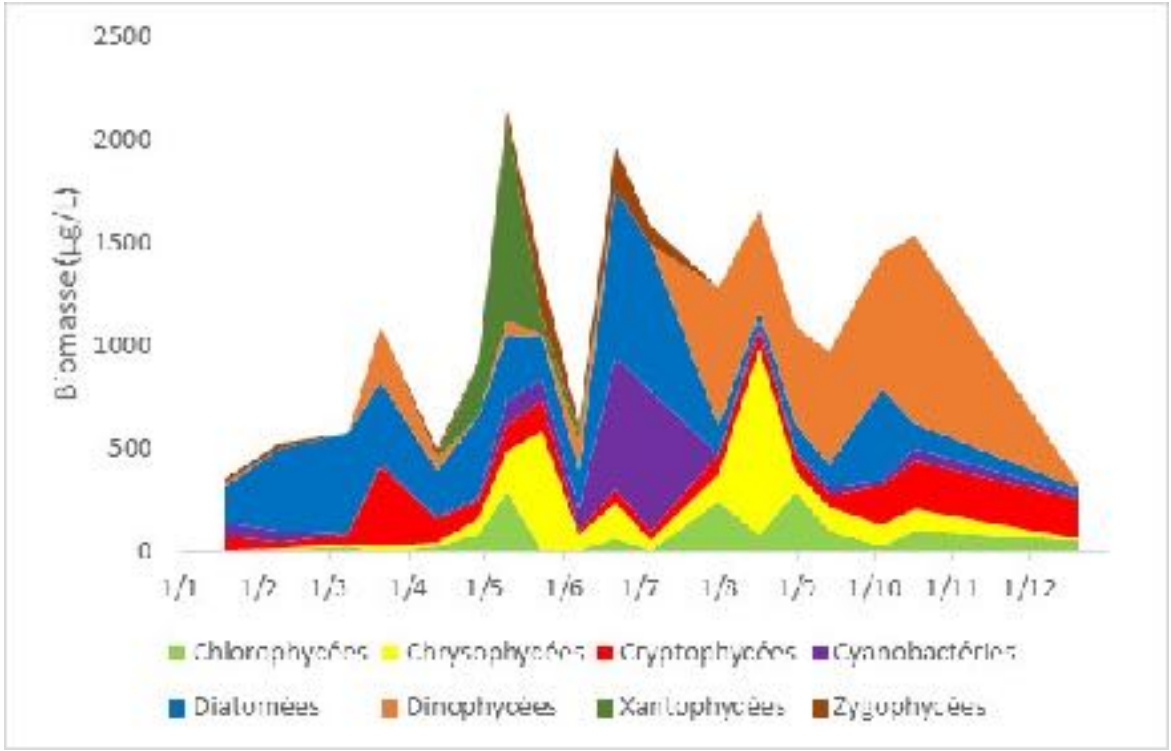
Seasonal biomass dynamic for Lake Geneva in 2009 (SHL2)



Metrics calculation

❖ **These dynamics change from a year to another**

Seasonal biomass dynamic for Lake Geneva in 2022 (SHL2)



Metrics calculation

- **It is important to capture this seasonal and interannual variability to assess correctly the ecological quality of the lake**
- **IPLac is based on 3 phytoplankton analyses**

- Spring
- Summer
- Autumn

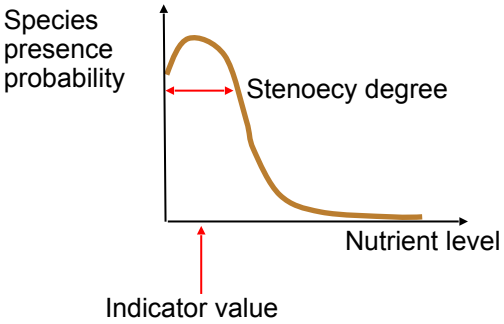
- **IPLac is measured every 6 years**

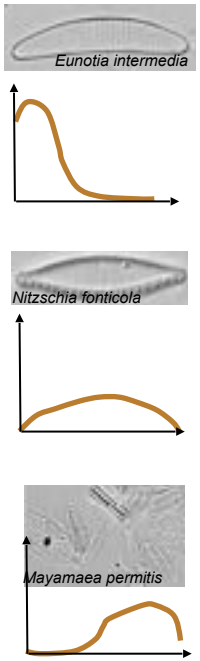
- **2 metrics are calculated in the IPLac**

- Specific composition
- Algal Biomass

Specific composition (MCS: Métrique de Composition Spécifique)

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





Weighted avearge = 4,5

$$\text{Index} = \frac{\sum Aj.vi.sj}{\sum Aj.vj}$$

Aj : Species j abundance in the sample,
sj : stenoecy degree (weight)
vj : indicator value

165 taxa, abundant in French lakes are integrated in the calculation of IPLac

MCS year = average (MCS spring + MCS summer + MCS autumn)

Algal Biomass (MBA: Métrique de Biomasse Algale)

Based on Chlorophyll A concentration in the sample

MBA year = average (MBA spring + MBA summer + MBA autumn)

Final calculation of IPLac

$$IPLac = 0.7 \times MCS_{nEQR} + 0.3 \times MBA_{nEQR}$$

Remark: before this calculation, MCS and MBA are transformed en normalized EQR:

Each metric is compared to the reference of the lake:

- MBA ref -> depend of the lake depth
- MCS ref -> depend on lake depth and altitude

Each metric is transformed into an EQR: where High/Good boundary was defined as the 25% quantile of the reference range

Then the results are normalized (variation between 0-1)

Objective of using DNA metabarcoding for phytoplankton biomonitoring?

$$IPLac = 0.7 \times MCS_{nEQR} + 0.3 \times MBA_{nEQR}$$

Specific composition

Challenging to get with microscopy:

- Time-consuming analysis
- Taxonomist experts are rare

We make the assumption that DNA metabarcoding can ease this analysis based on the experience we had with diatoms

?

Algal Biomass

- Not possible to assess with DNA metabarcoding.
- Chlorophyll-a analyses are cheap and easy to do.
- No need to develop a new methodology

BIOLAWEB

Questions ?