BIOS3300/4300 - Marine Biology

Primary Production (Plankton)
Diversity of primary producers

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CNRS • SORBONNE UNIVERSITÉ
Station Biologique de Roscoff

UiO : University of Oslo

Station Biologique de Roscoff

Station Biologique de Roscoff

- 1872 - Antoine Lacaze-Duthiers (150 years ago)
- CNRS and Sorbonne Université
- Staff: 350
- Students : 1,000-2,000 per year

Ecologie of Marine Plankton team (ECOMAP)

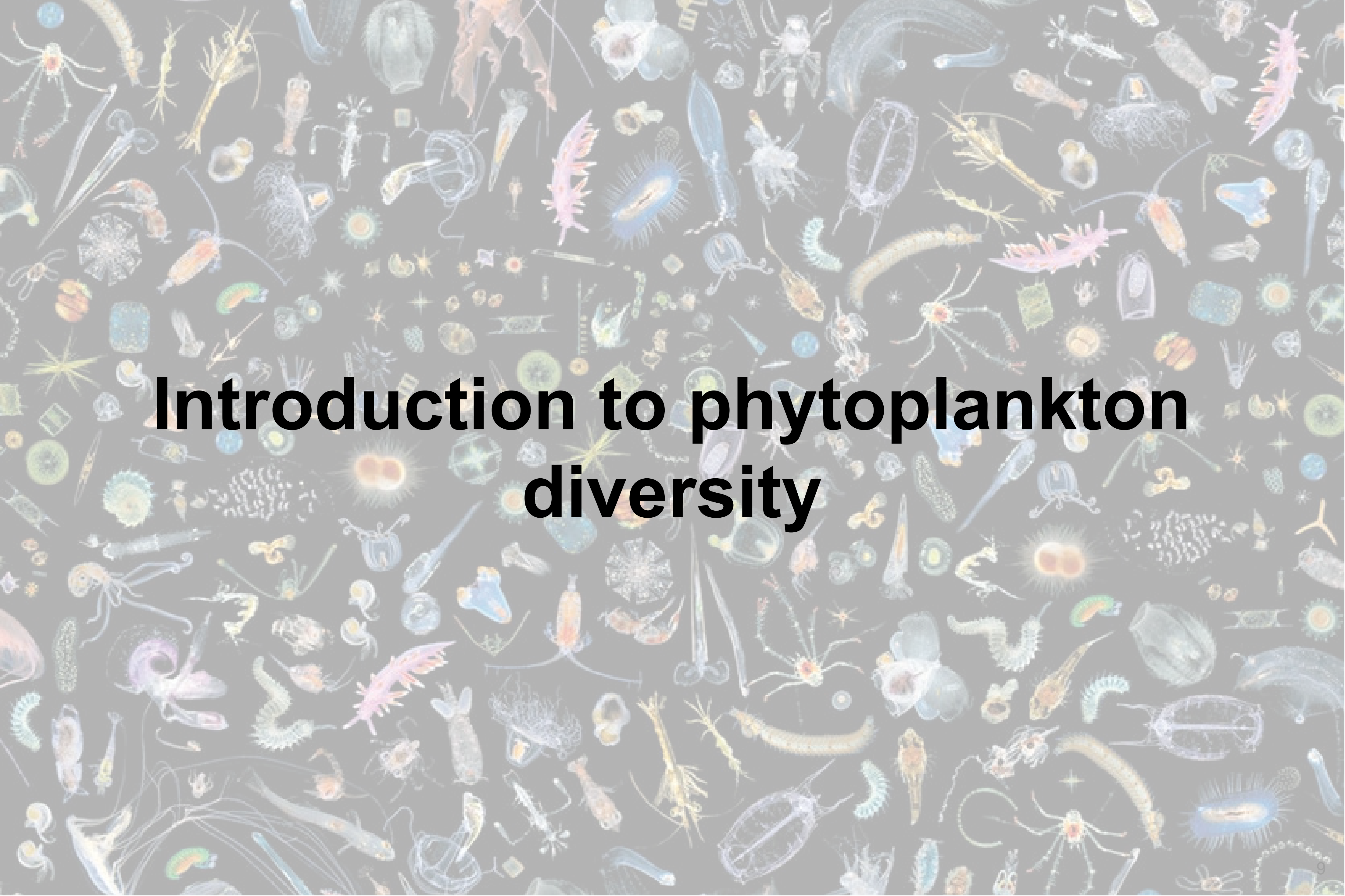
- Scientists: 10
- Staff: ~ 40
- Research themes:
  - Viruses
  - Bacteria
  - Cyanobacteria
  - Symbioses
  - Parasitism

Outline

- Introduction to phytoplankton diversity
- Methods for phytoplankton diversity
- Diatoms
- Dinoflagellates
- Haptophytes
- Green algae
- Cyanobacteria

Reference material

- Kaiser et al. Marine Ecology. 3rd ed. - 2020 - Chapter 2
- Not, F. et al. 2012. Diversity and ecology of eukaryotic marine phytoplankton. In Piganeau, G. [Ed.] Genomic Insights Gained into the Diversity, Biology and Evolution of Microbial Photosynthetic Eukaryotes. Elsevier, Amsterdam, pp. 1–53.

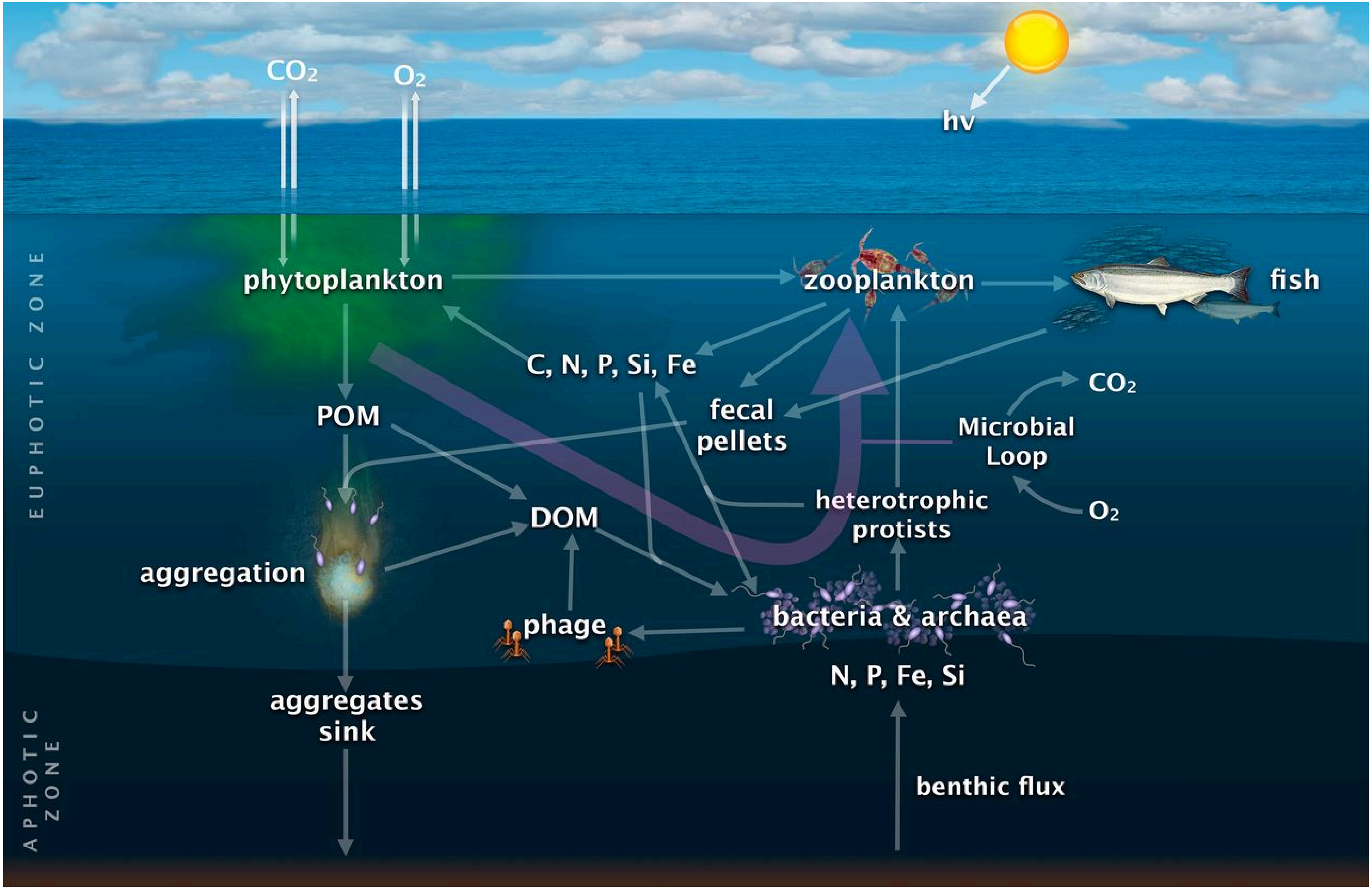


Introduction to phytoplankton diversity

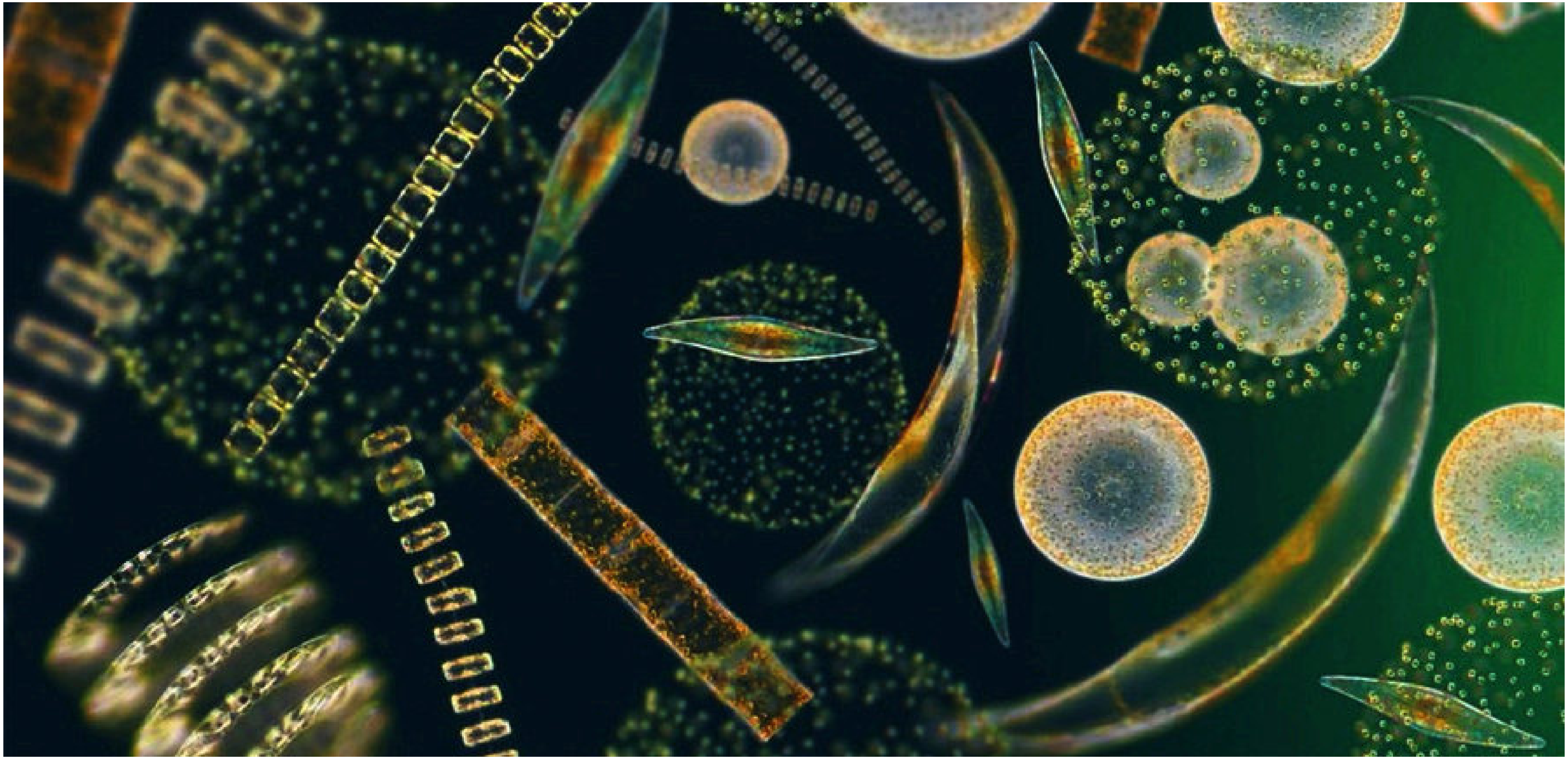
Plankton diversity

- **Phytoplankton**
- Zooplankton
- Bacteria
- Viruses

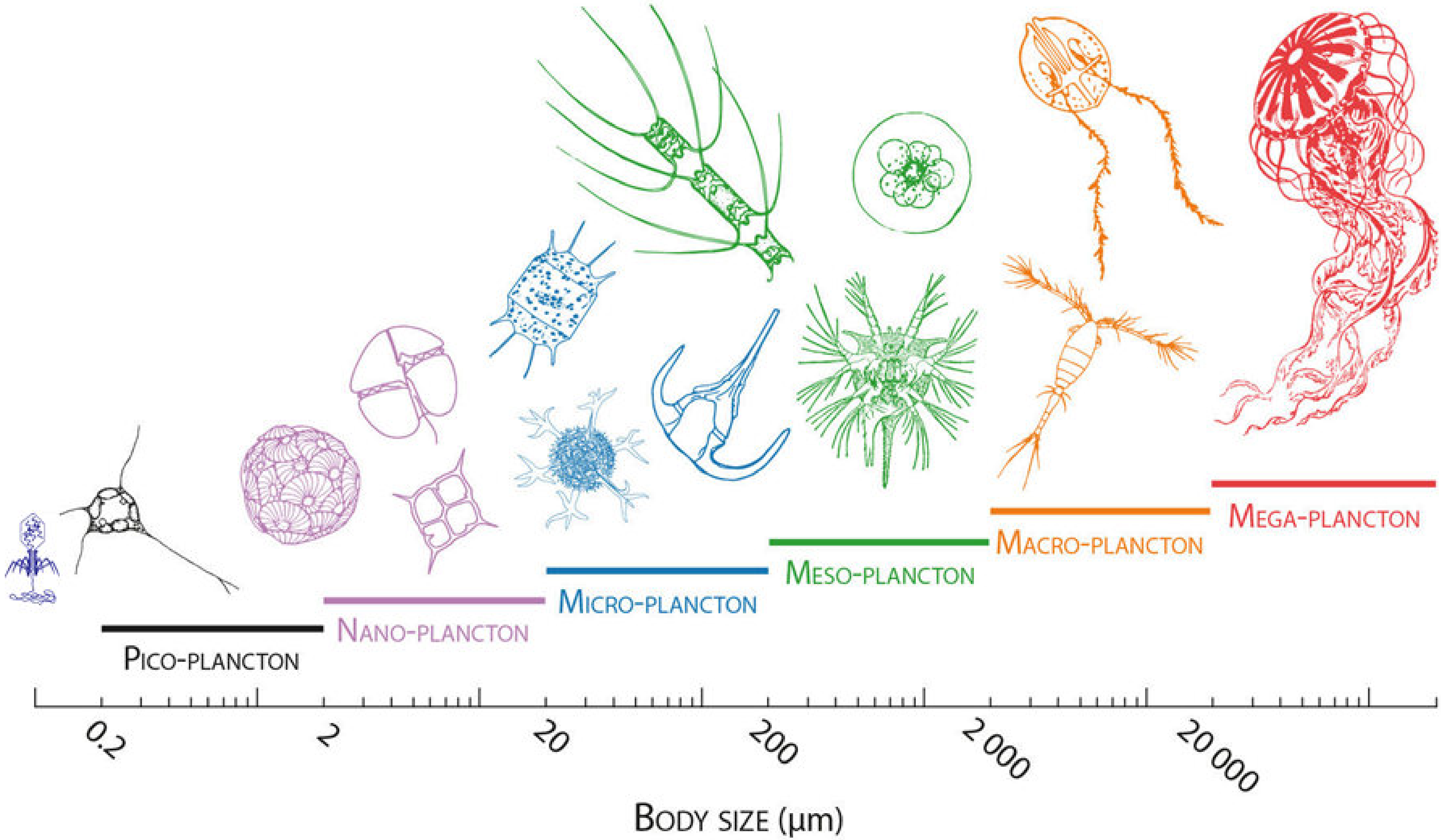
Marine food webs



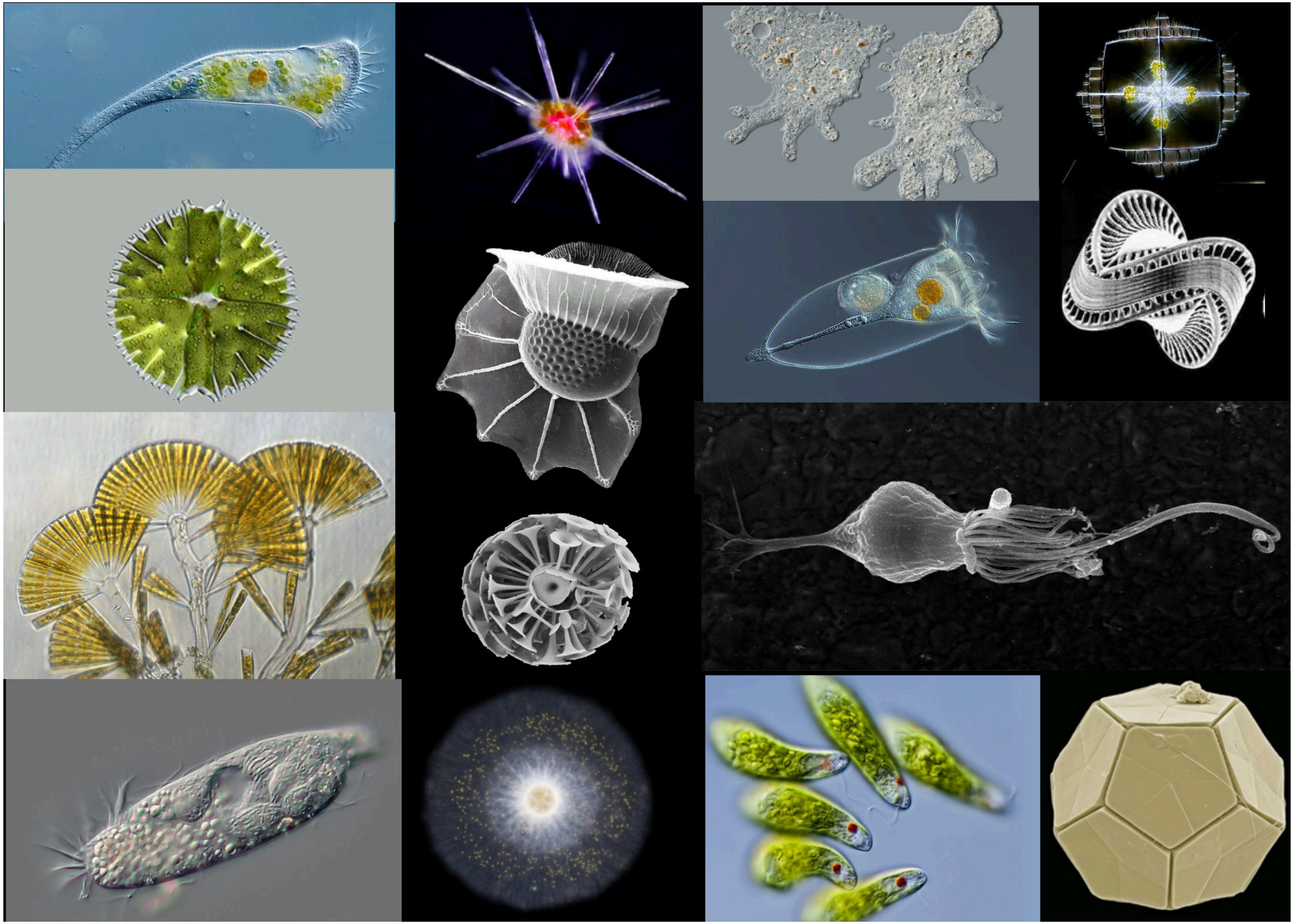
Phytoplankton



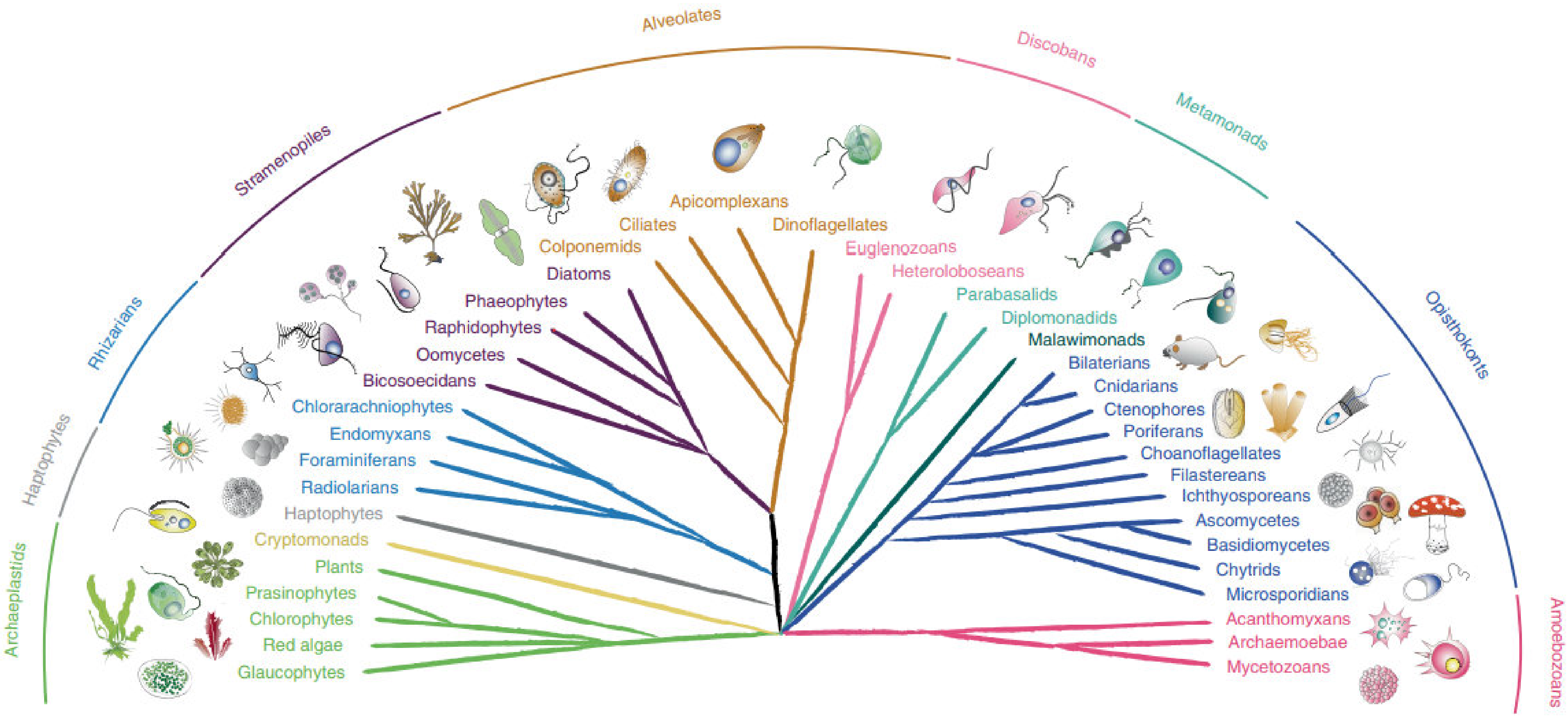
Size classes



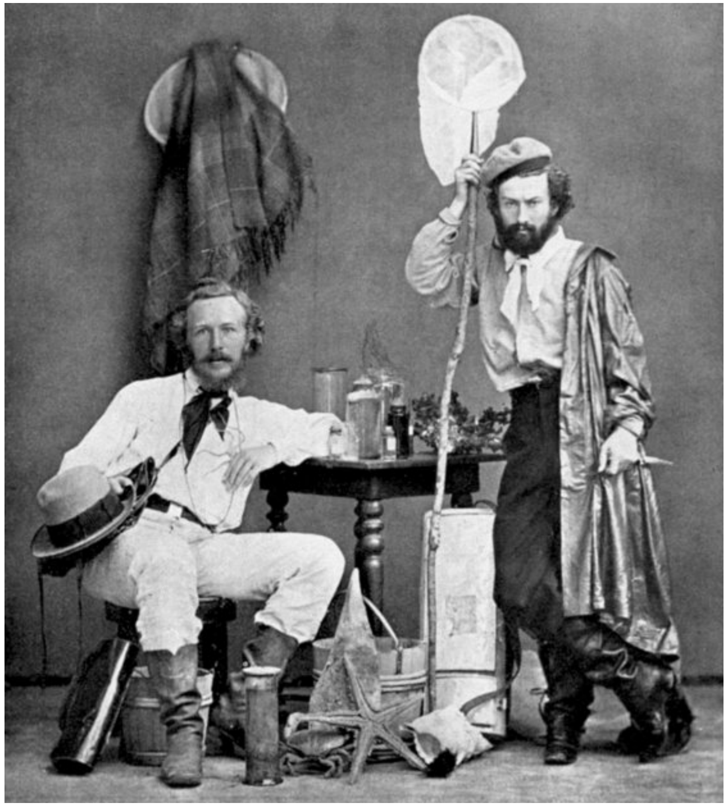
Form and function

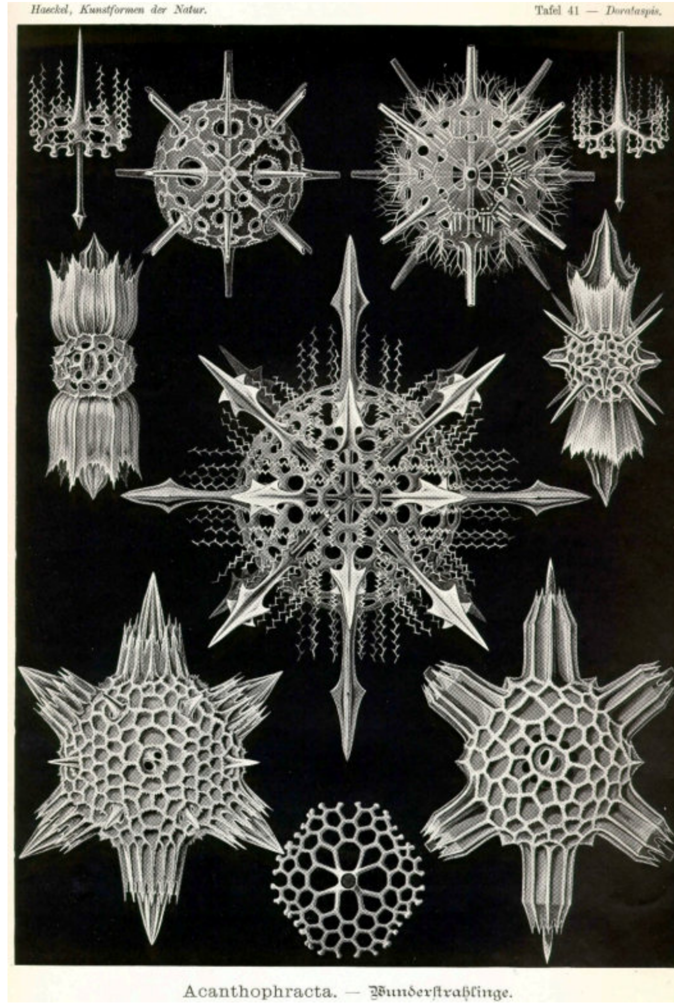


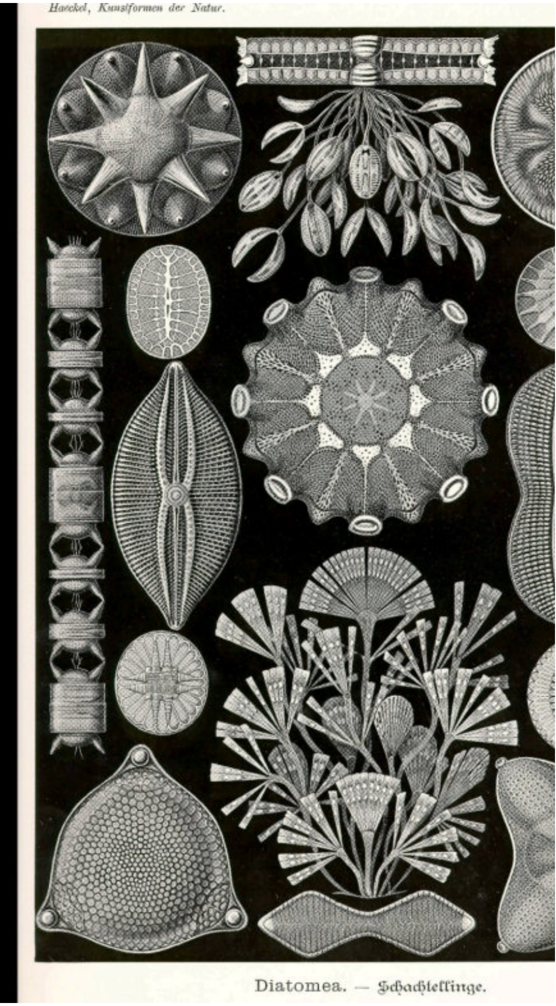
Phylogenetic diversity



Haeckel



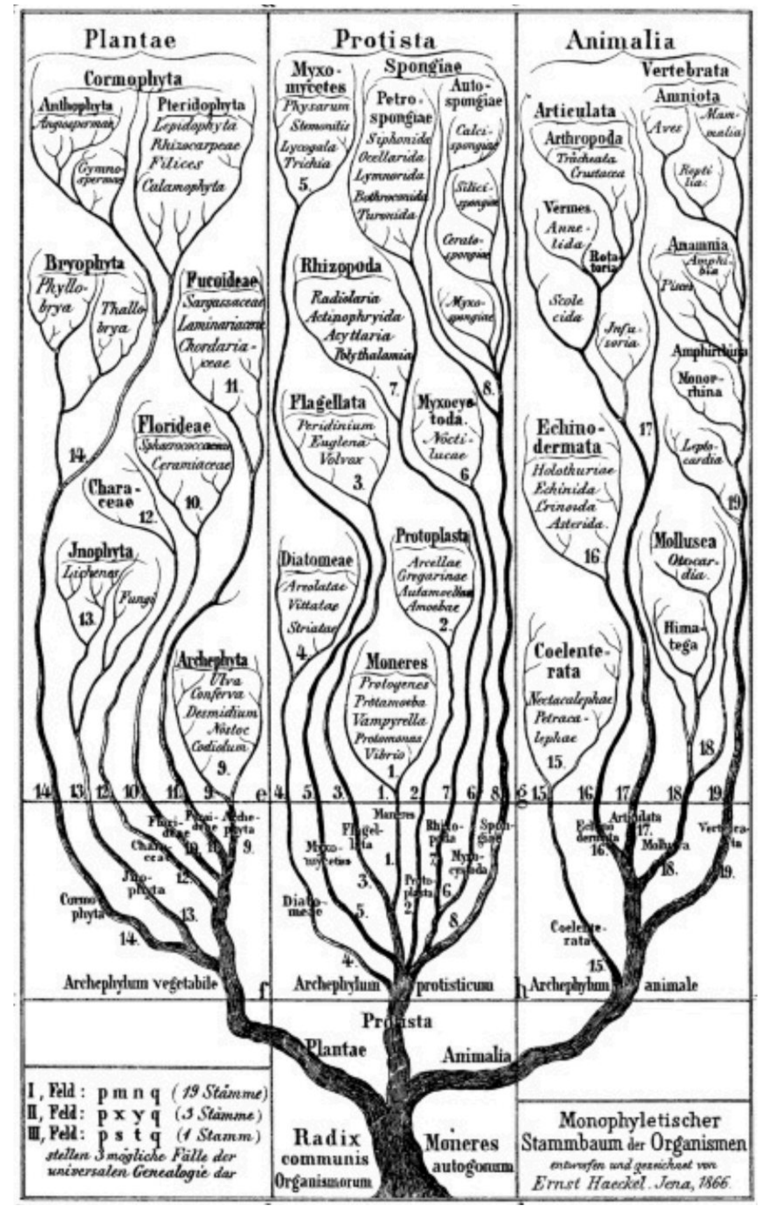

Haeckel, Kunstformen der Natur.
Tafel 41 — Dorataspis.
Acanthophracta. — Wunderstrahlinge.


Haeckel, Kunstformen der Natur.
Diatomea. — Schachtelinge.

Haeckel

Generelle Morphologie der Organismen - 1866

- Ernst Haeckel believed that the “natural system”, proposed by Darwin (1859), should be represented as a **genealogical tree**.
- Haeckel’s book Generelle Morphologie der Organismen (1866) provided major improvements to the theory of descent, including:
  - a large vocabulary of neologisms, some of which became successful, such as **phylogeny, monophyletic, and polyphyletic**
  - the term **protists** (“the first of all or primordial”) to distinguish unicellular organisms

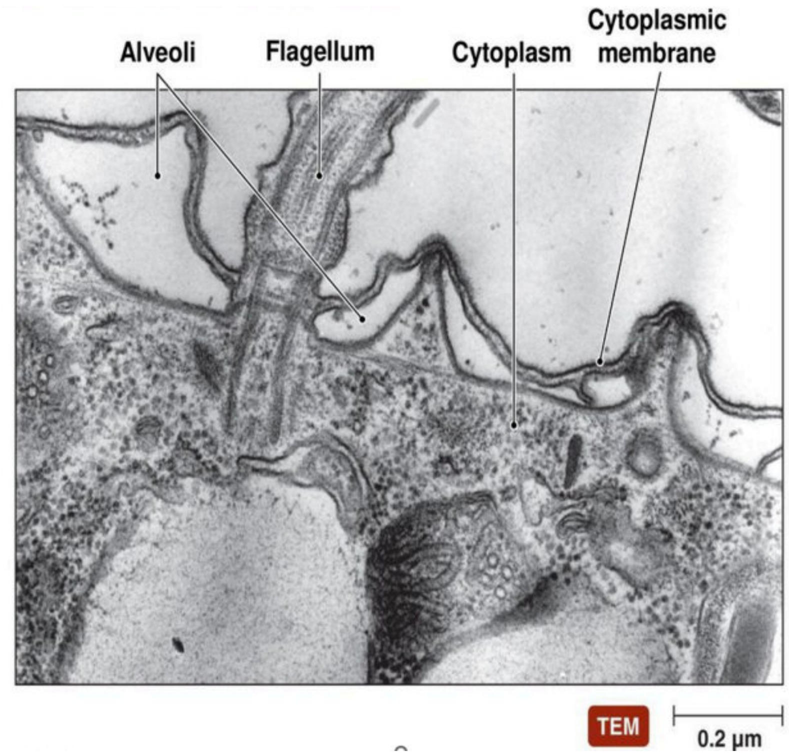


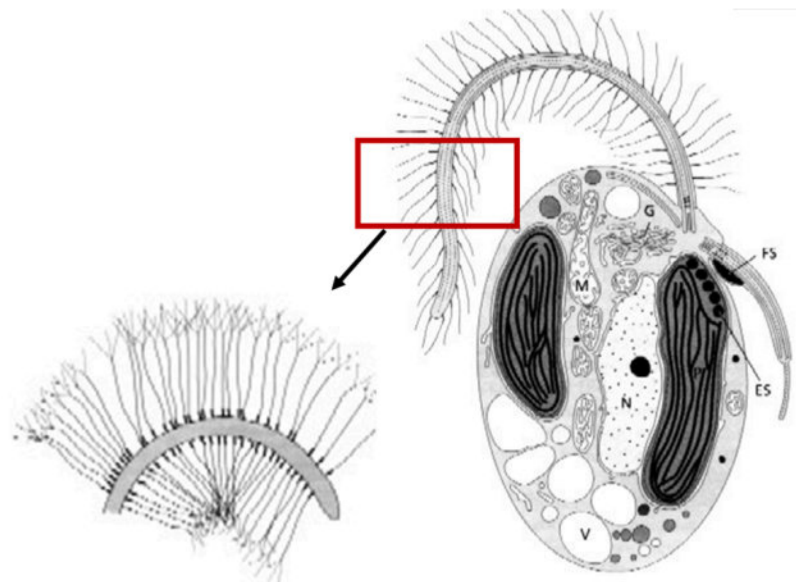
Ultrastructural data

- Hypotheses of relationships among unicellular eukaryotes emerged in 60s from ultrastructural data (electron microscopy). Some would be corroborated a few years later by molecular data

Examples

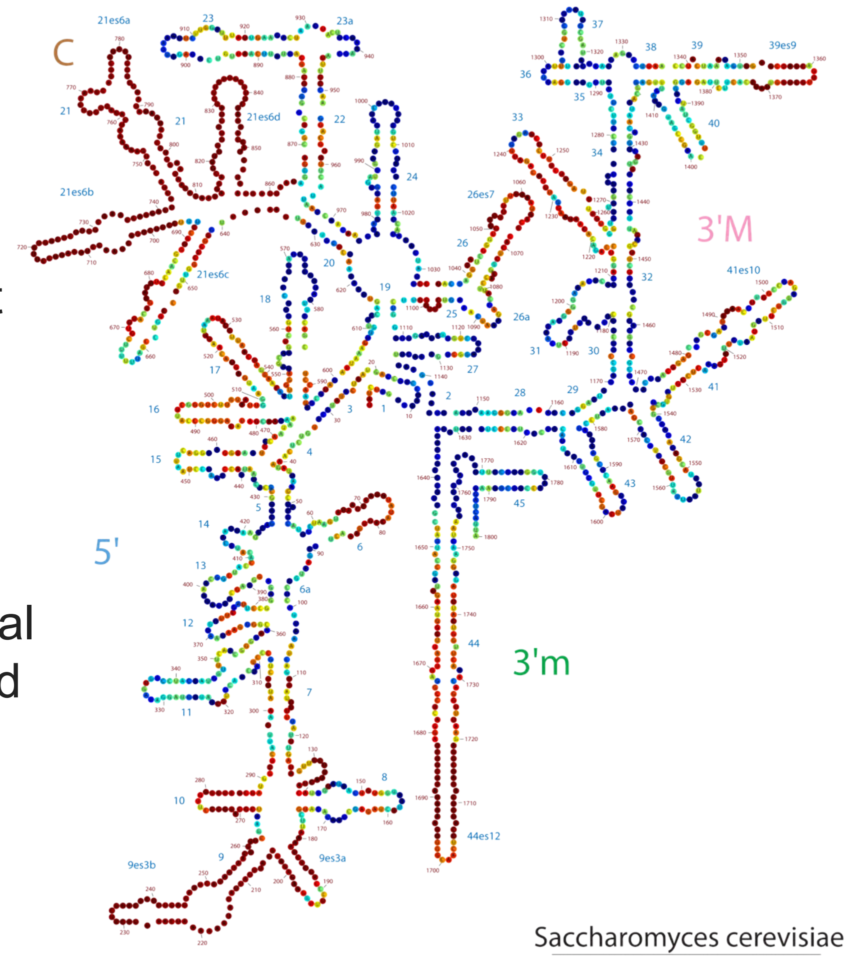
- Presence of **alveoli** (membrane bond sacs that form a continuous layer under the plasma membrane) in Alveolates lineage (ciliates,dinoflagellates and apicomplexans).
- Presence of **tripartite tubular hairs** (straminipilous) in Stramenopiles lineage (bicosoecids, labyrinthulids, oomycetes, diatoms, brown algae, silicoflagellates





Molecular phylogeny

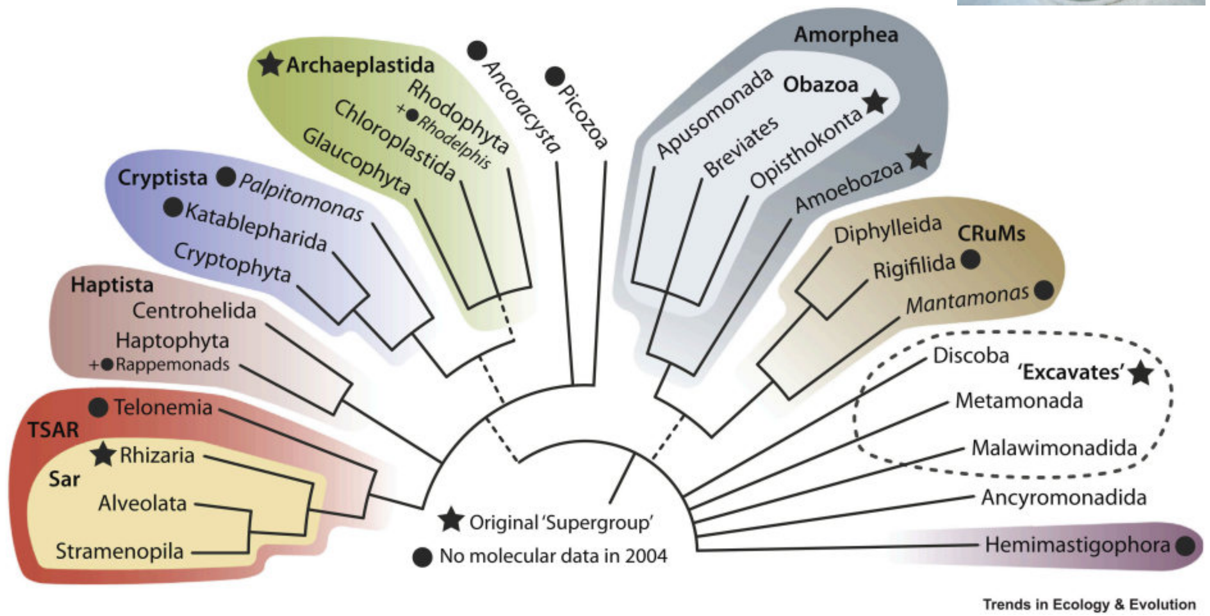
- With the advent of molecular phylogeny came high hopes to quickly resolve eukaryote relationships
- **18S rRNA gene** became the first golden standard for eukaryote phylogeny. Significant hypotheses were confirmed / revealed for the first time:
  - Most protists groups identified by morphology and physiology do not represent evolutionarily coherent entities.
  - **Algae, protozoa, protists are names without taxonomic value**. They do not represent any evolutionary relationship among the organisms.
  - Some lineages stablished based on of ultrastructural data (alveolates and stramenopiles) were confirmed



Saccharomyces cerevisiae
small subunit ribosomal RNA

The tree today

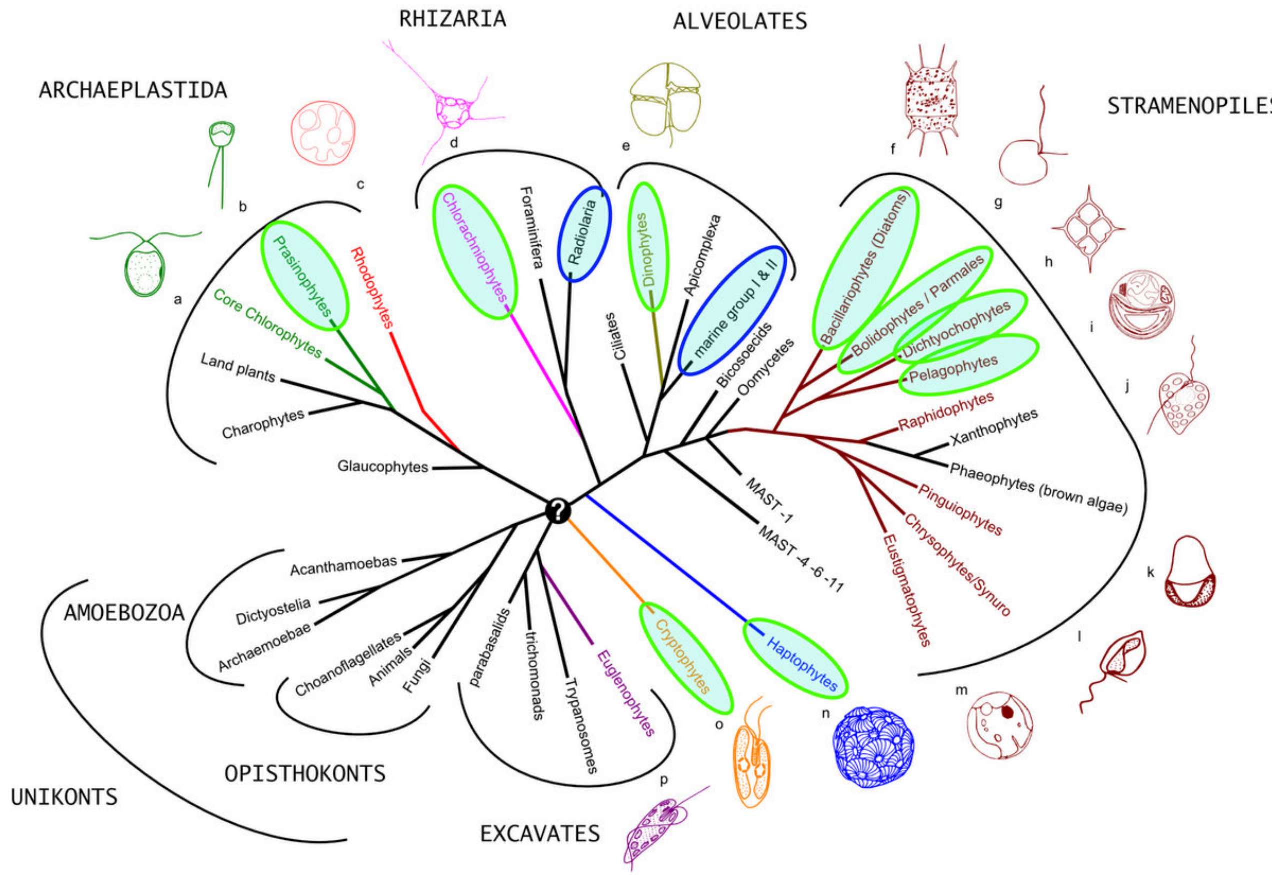
- Most recent iteration in 2020[1]
- Supergroups represent a set of eukaryotic species for which there are reasonable evidence that they form a **monophyletic** group.
- Most of supergroups have at least one distinctive biological characteristic that seems to define them ancestrally[2].
- The precise number and membership of the supergroups has varied, reflecting the rapid pace with which important taxa are being discovered and added to tree (broad molecular phylogenetic analyses).



1. Burki, F., Roger, A. J., Brown, M. W. & Simpson, A. G. B. The New Tree of Eukaryotes. Trends Ecol. Evol. 35, 43–55 (2020)

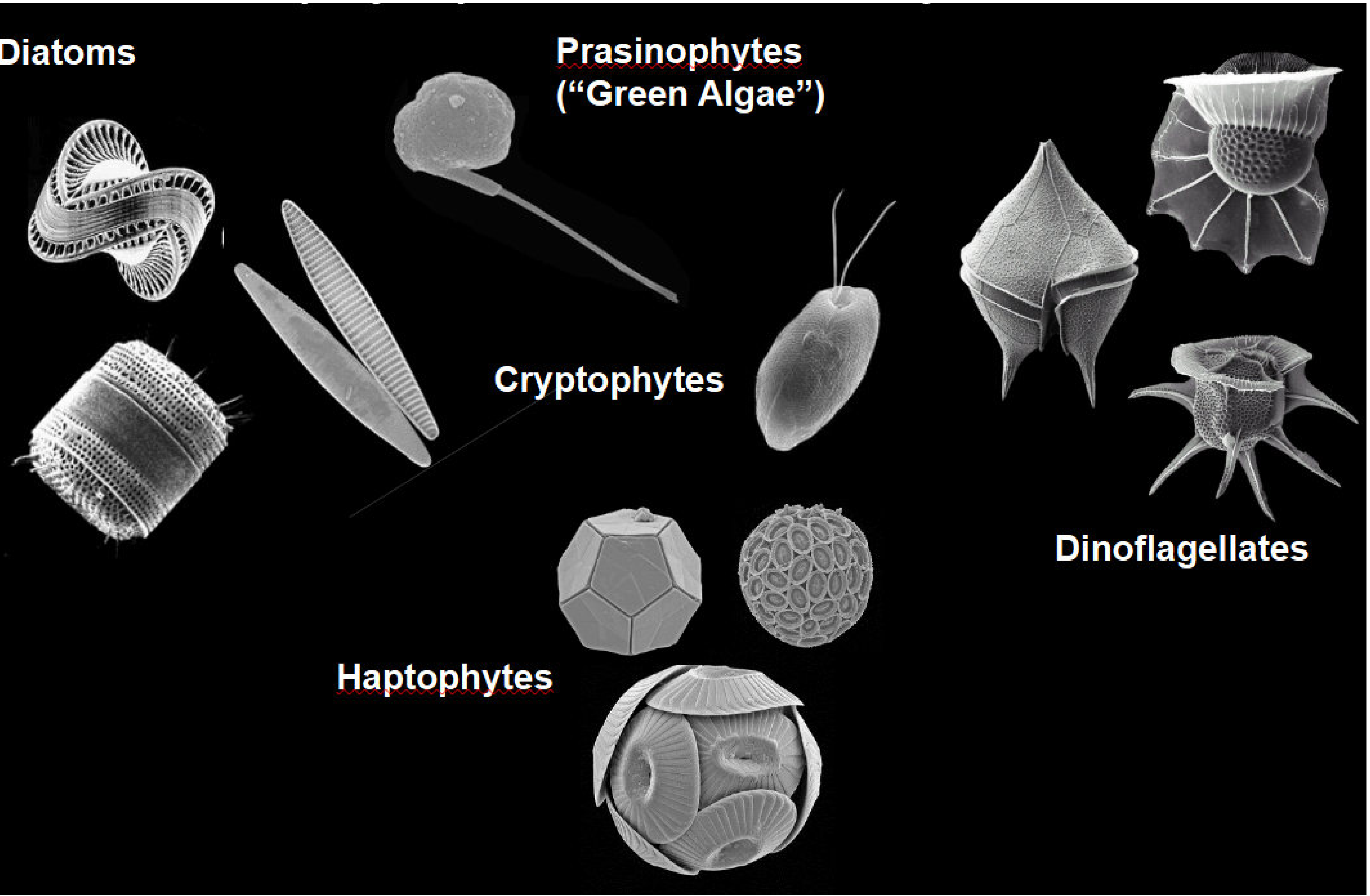
2. morphological **synapomorphy**: a characteristic present in an ancestral species and shared exclusively by its evolutionary descendants.

Phytoplankton is not monophyletic



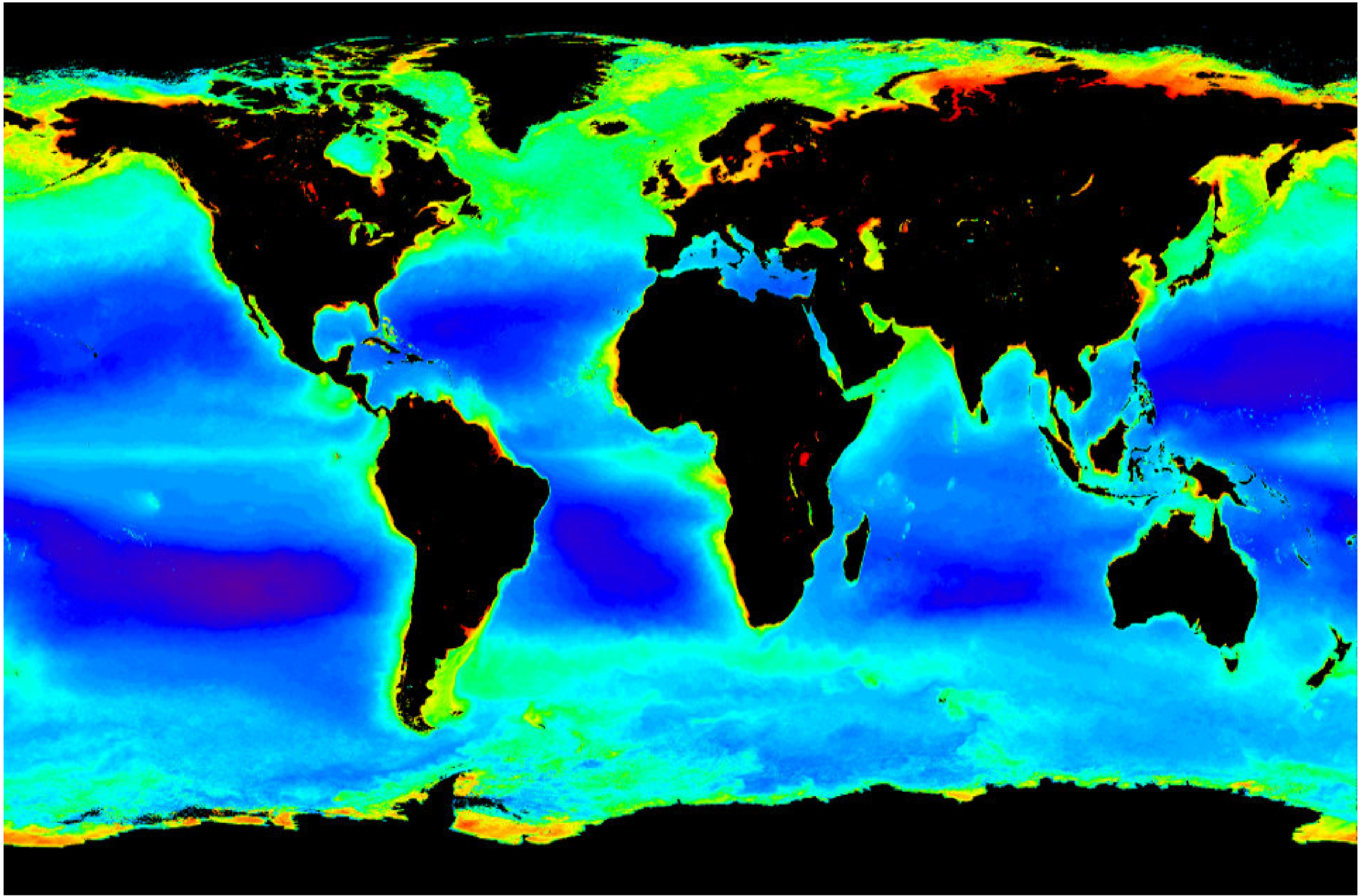
Not, F., Siano, R., Kooistra, W.H.C.F., Simon, N., Vaulot, D. & Probert, I. 2012. In Piganeau, G. [Ed.] Genomic Insights Gained into the Diversity, Biology and Evolution of Microbial Photosynthetic Eukaryotes. Elsevier.

Major groups of Phytoplankton

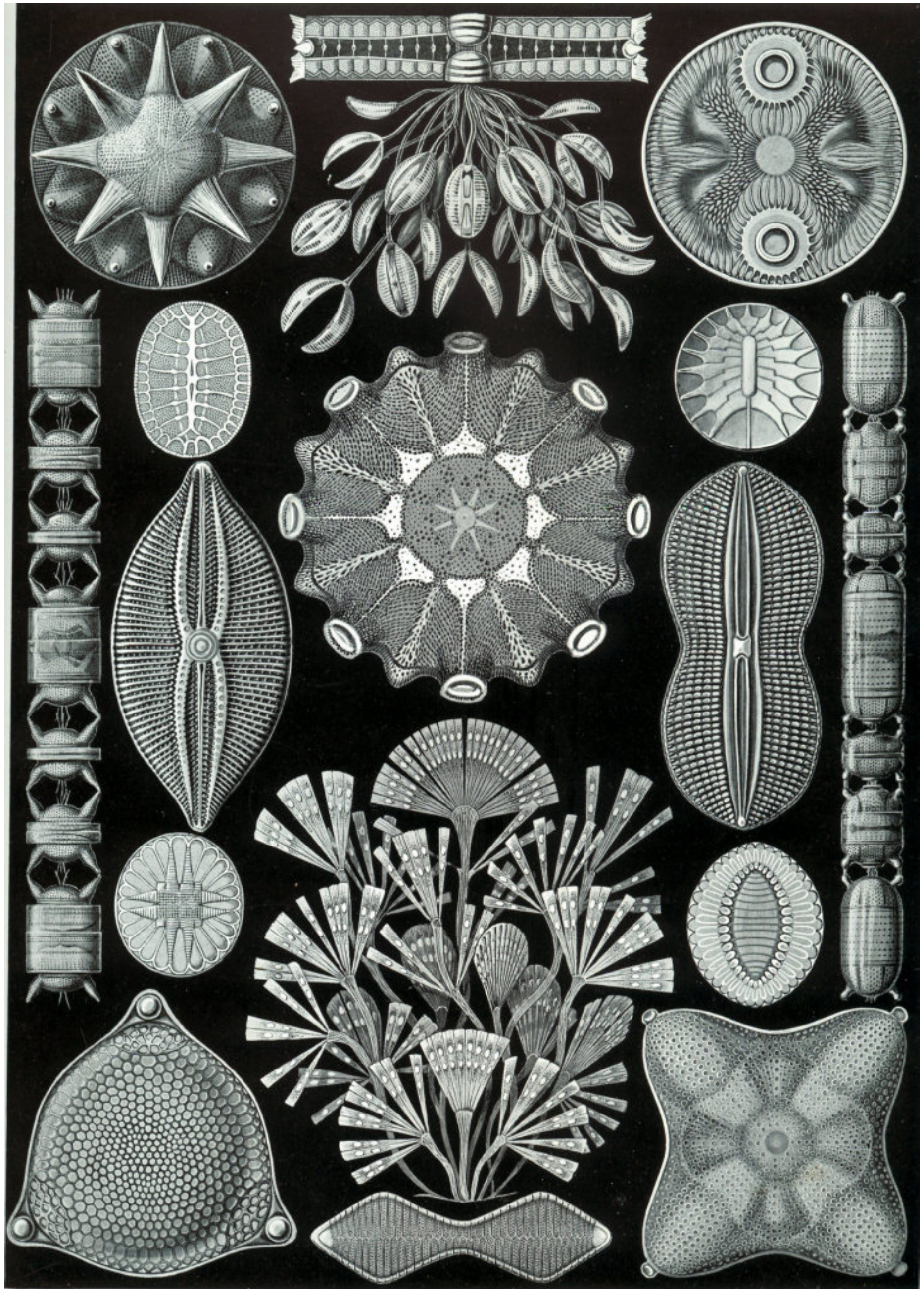


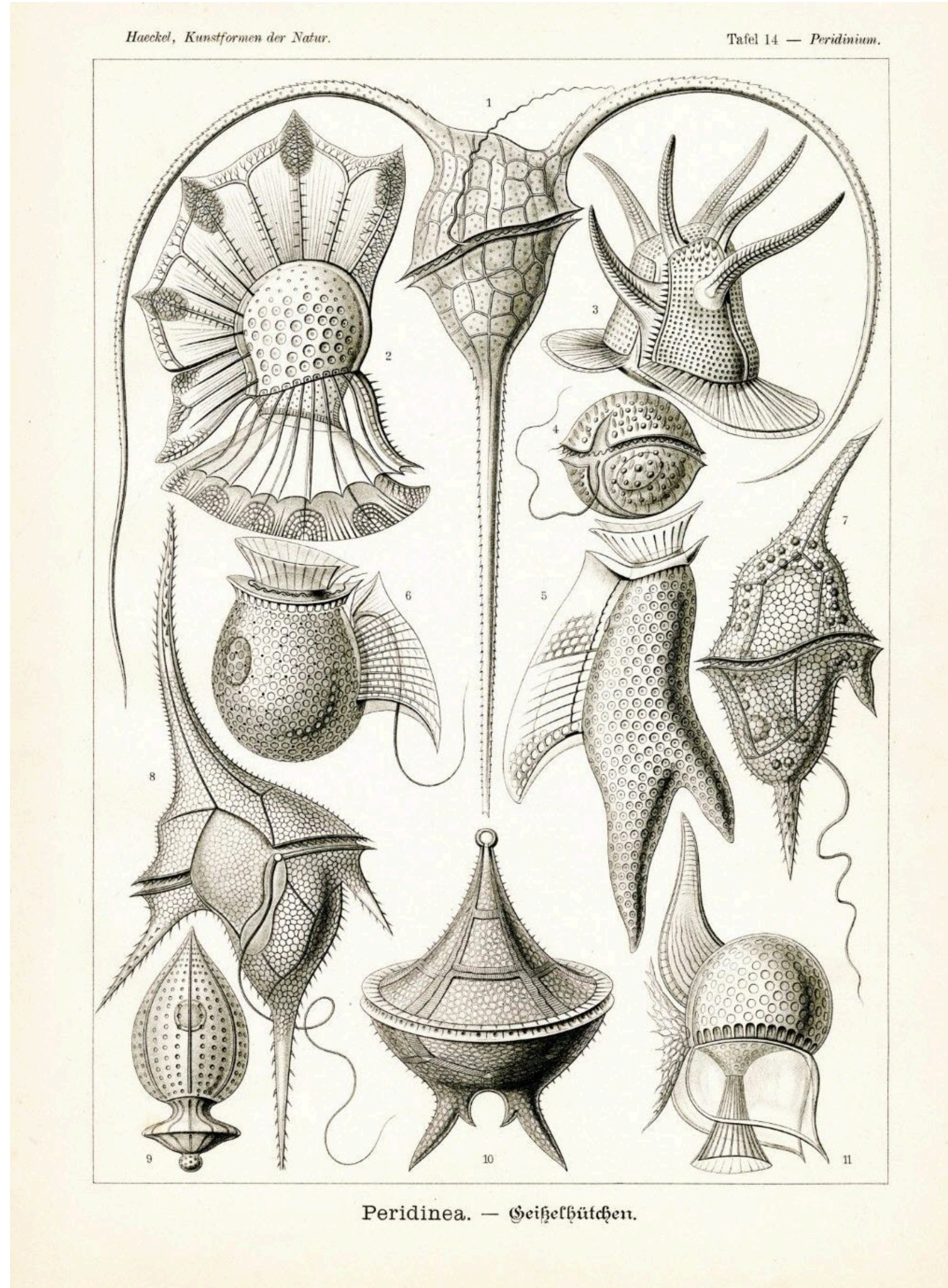
Phytoplankton rich regions

- Chl *a* estimated from satellite

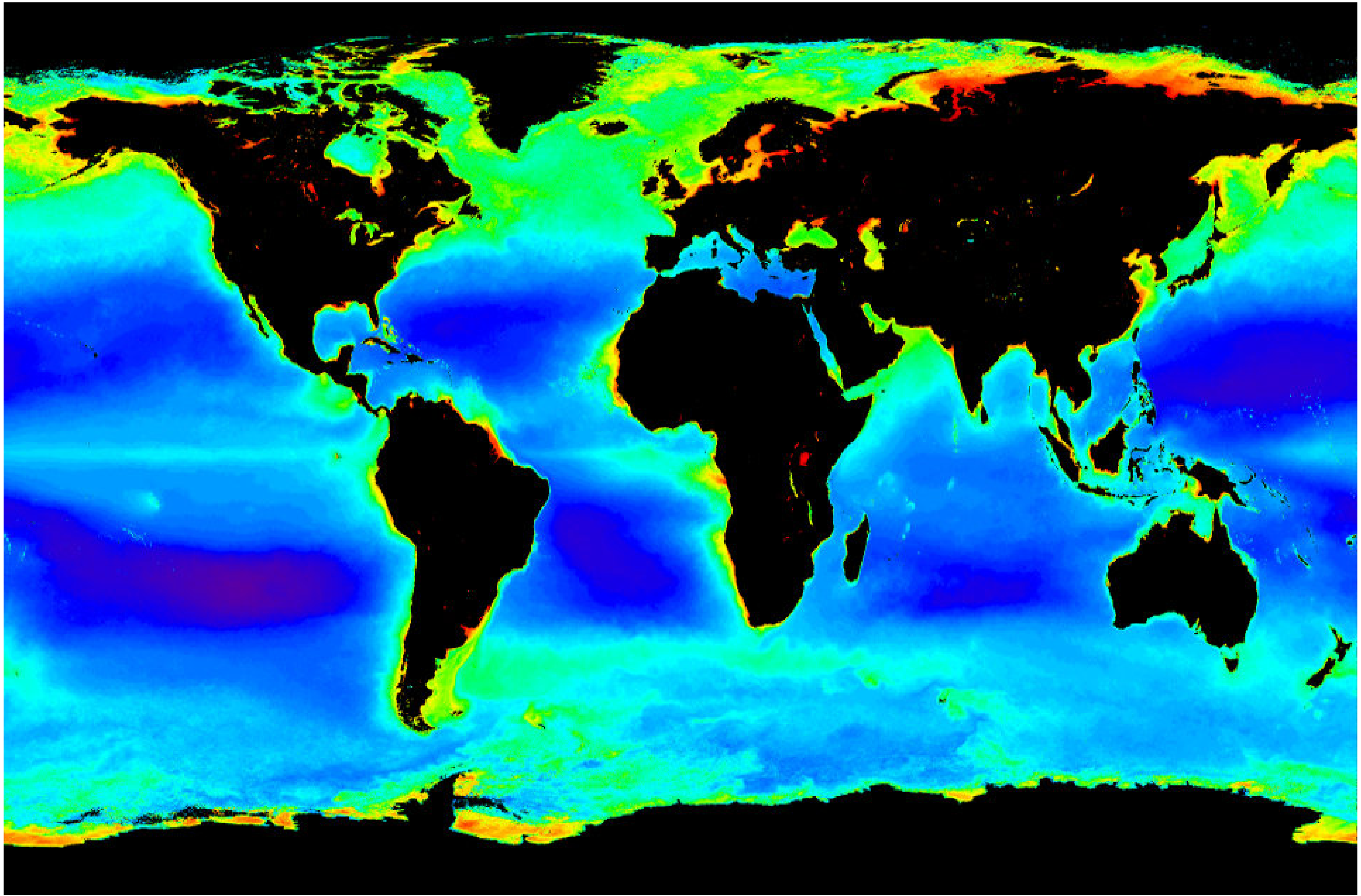


Diatoms and dinoflagellates: 20-200 µm



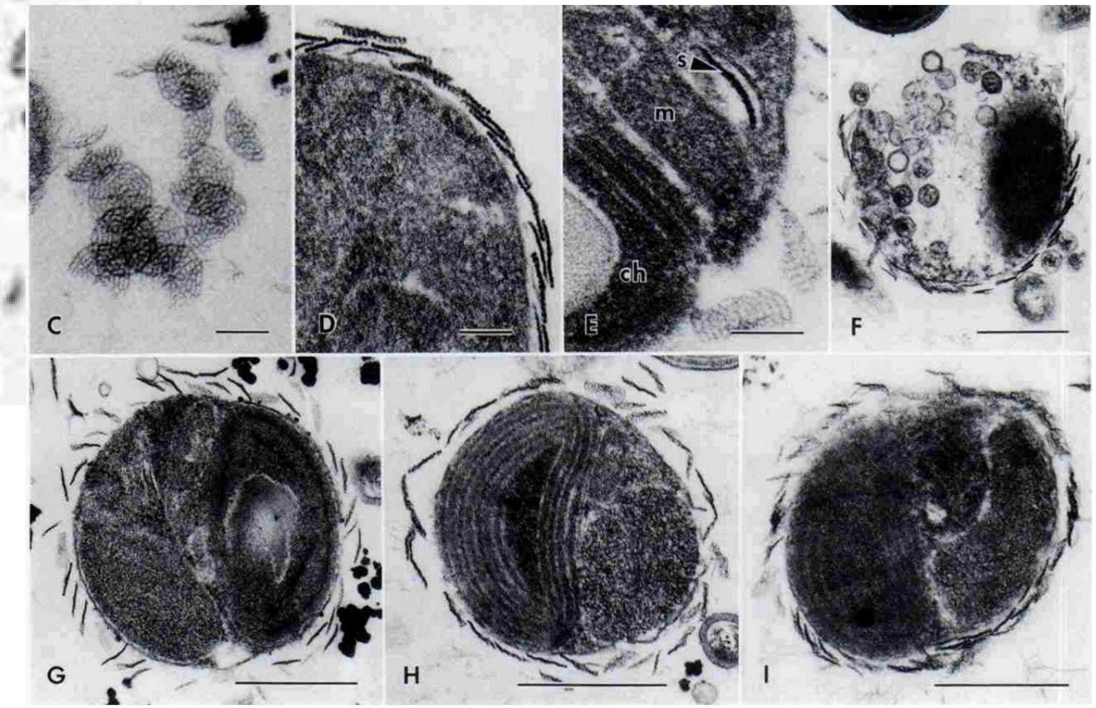


Oceanic deserts



Picoplankton

1982 - John Sieburth - Electron microscopy

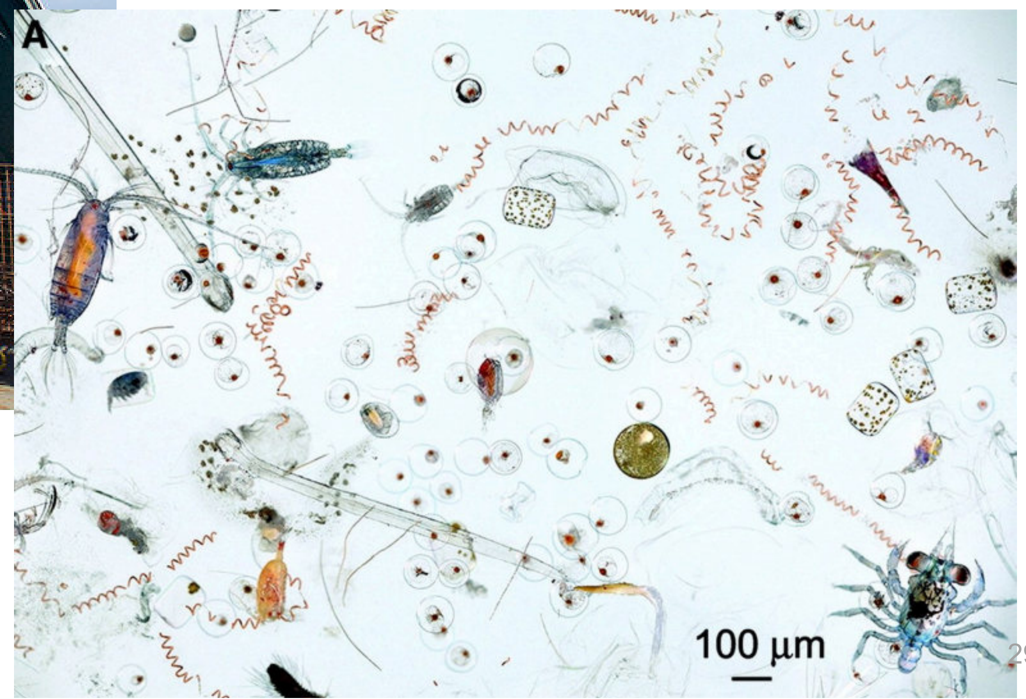


Johnson, P.W. & Sieburth, J.M. 1982. J. Phycol. 18:318–27.

Methods for phytoplankton diversity

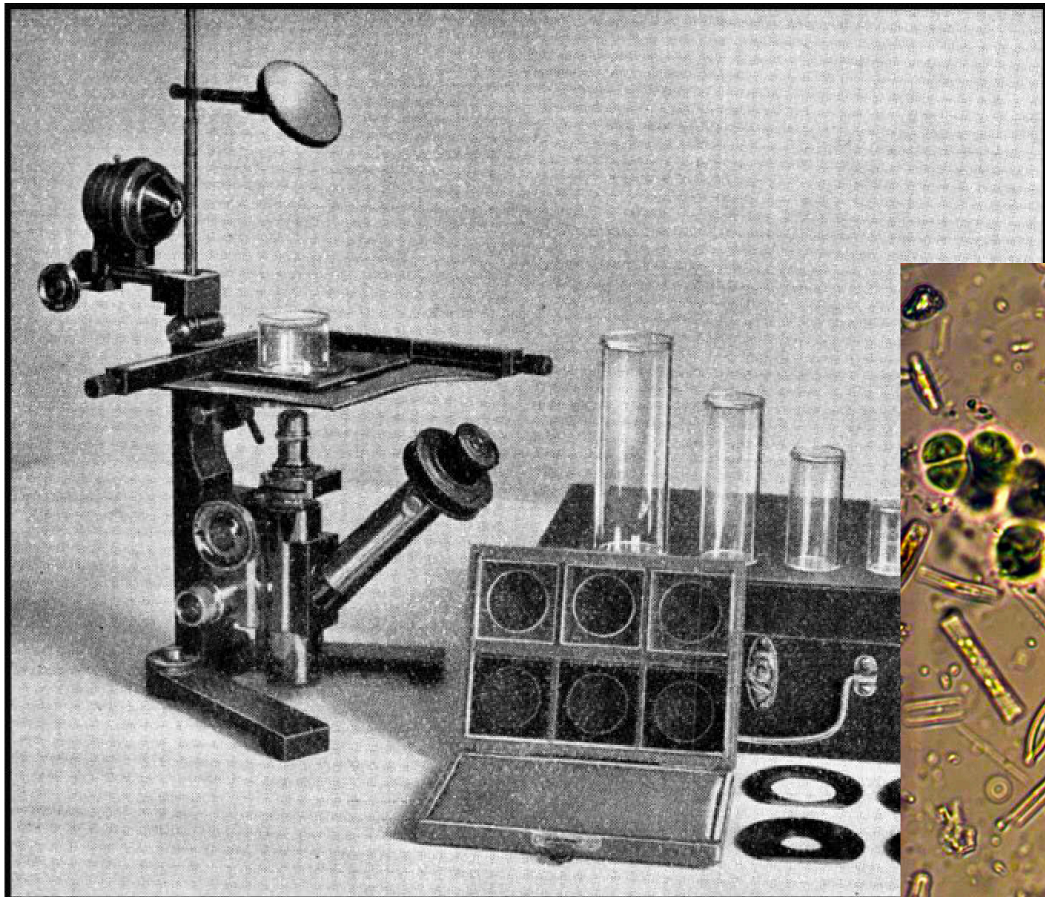
Microscopy

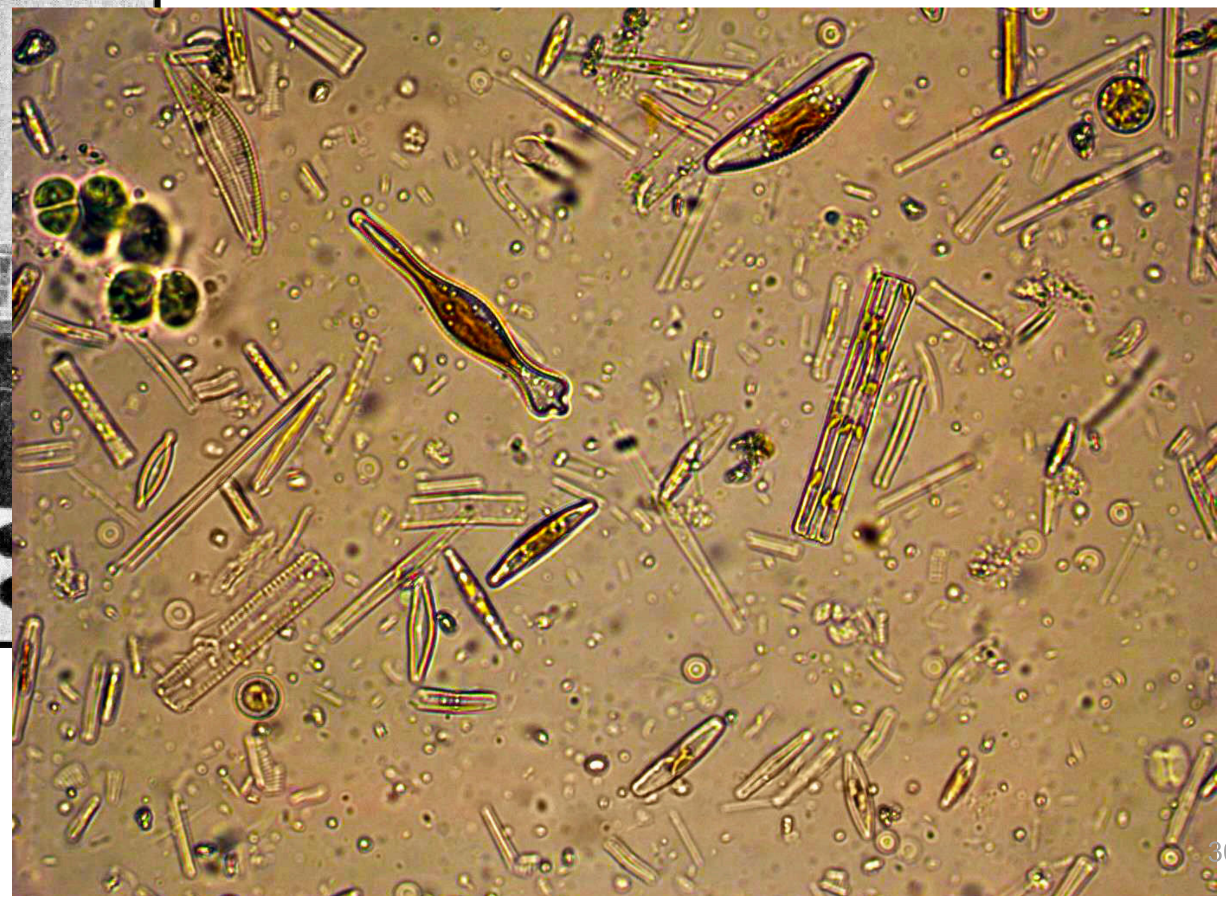
- Plankton Net - qualitative



Microscopy

- Utermohl's method - quantitative

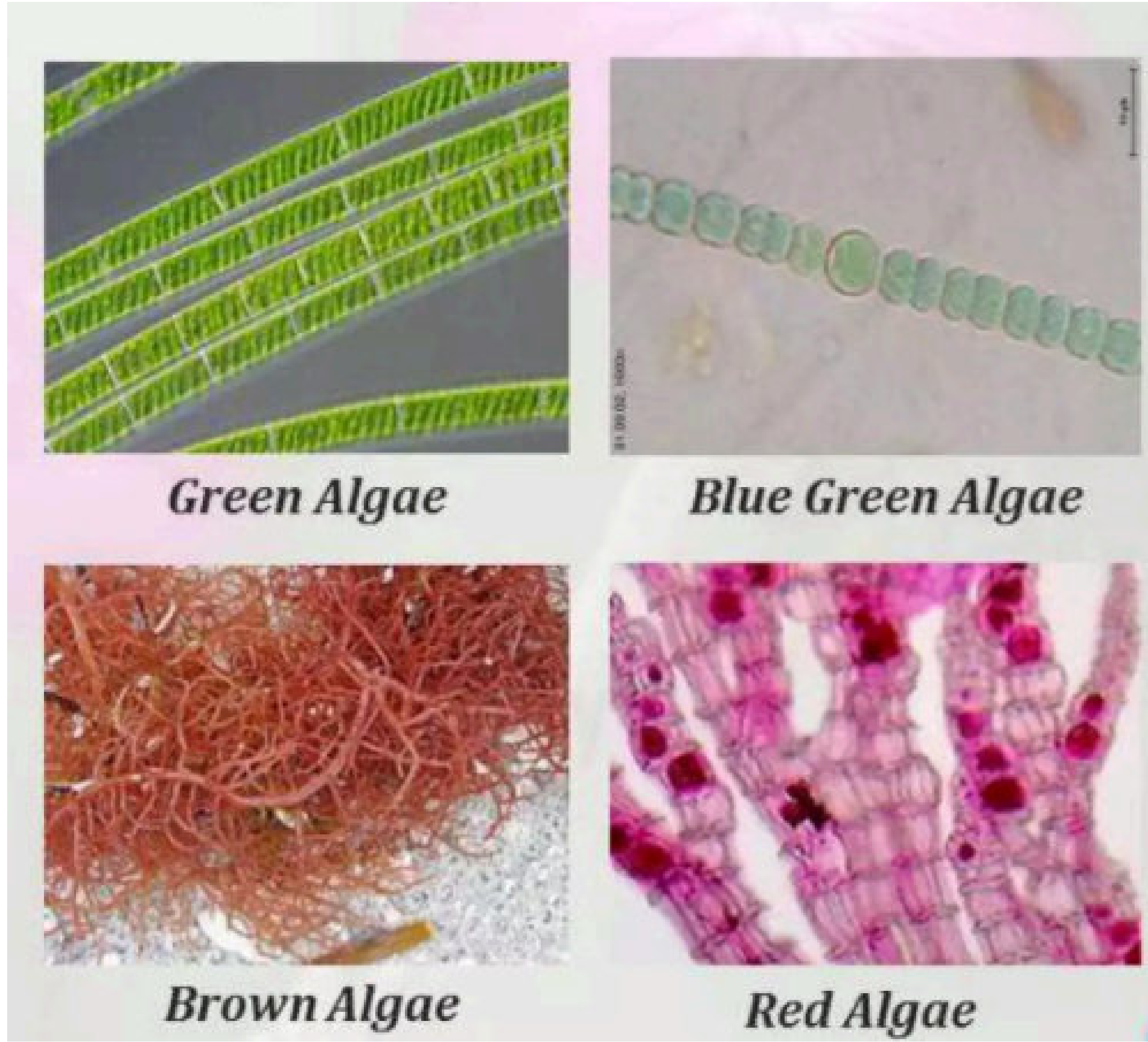




Pigment composition

Three types of pigments

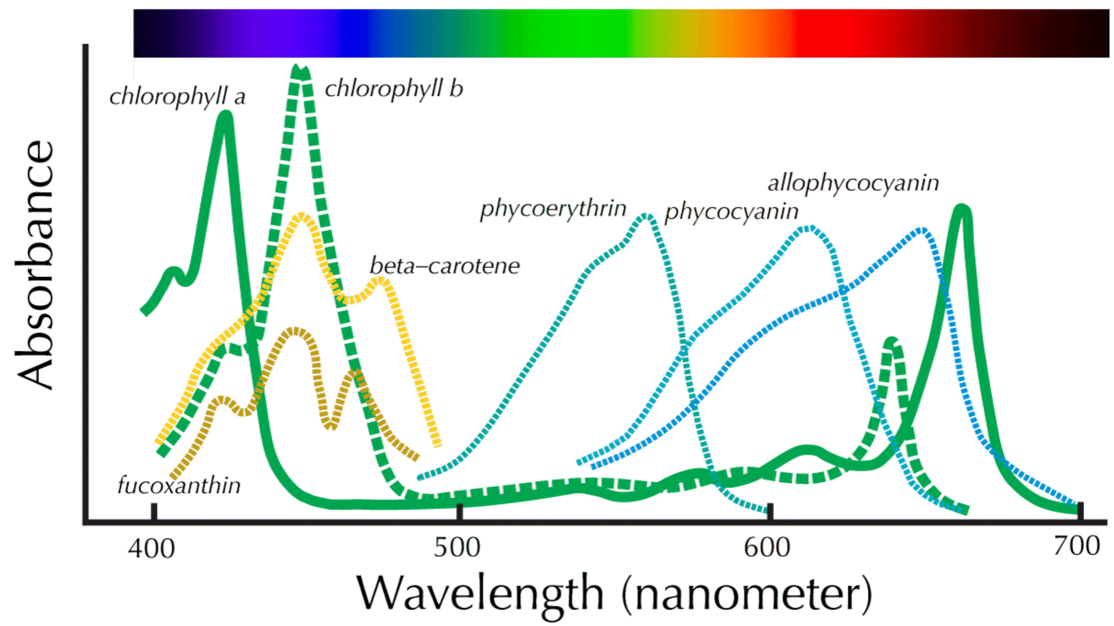
- Chlorophylls
- Carotenoids
- Phycobiliproteins

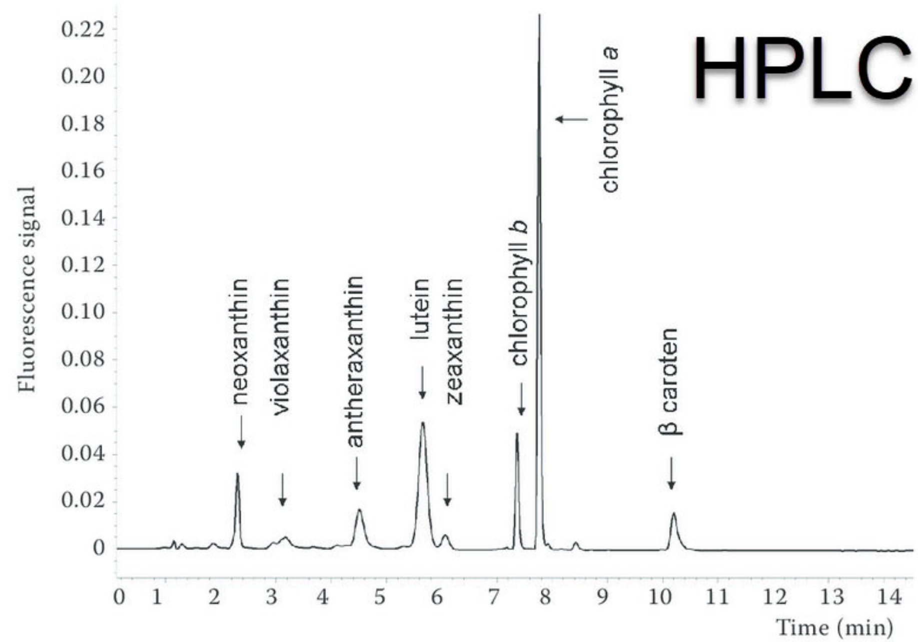


https://easybiologyclass.com/pigments-in-algae-key-points-with-ppt/

Pigment composition

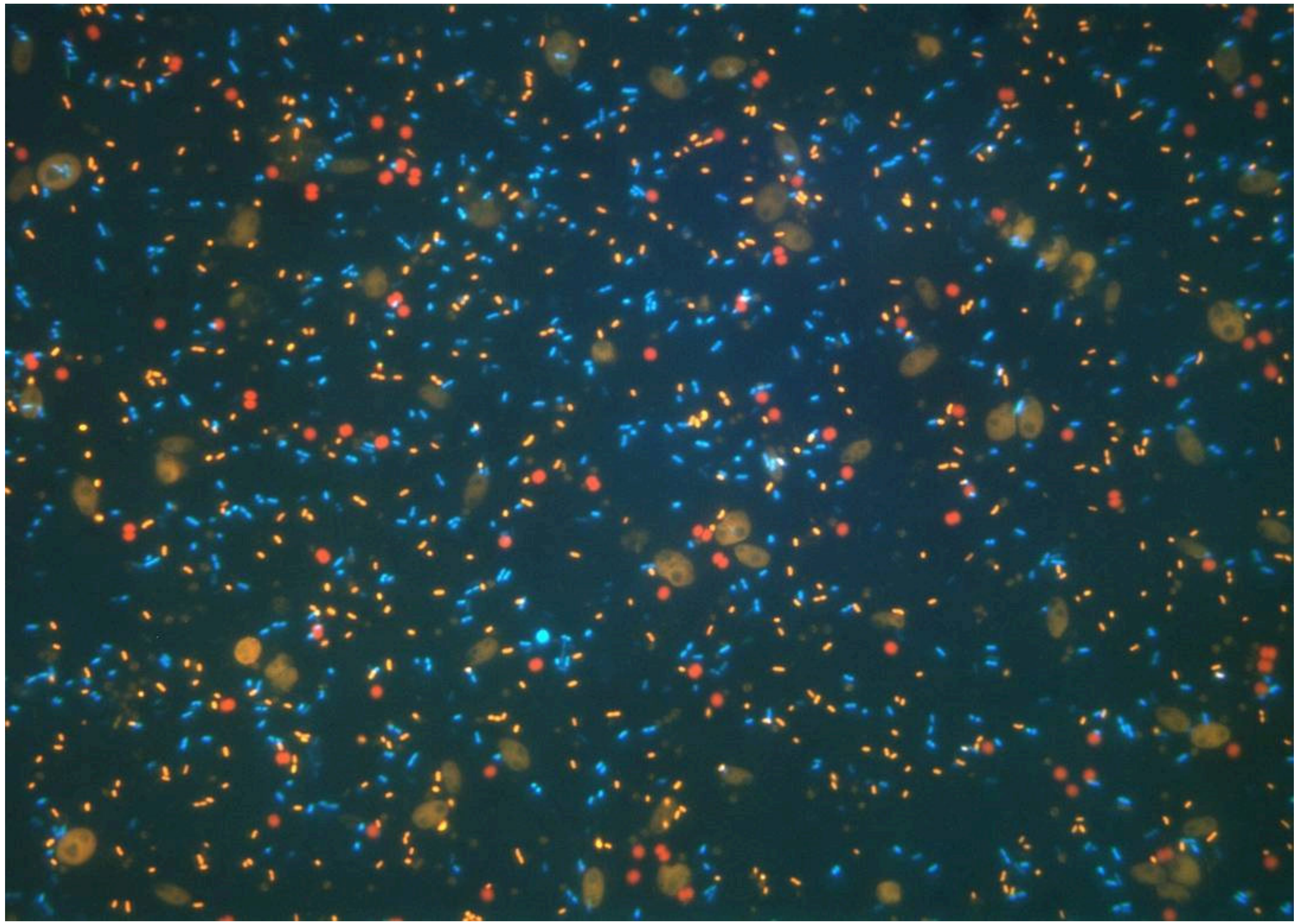
- Different taxonomic groups of algae have different pigment composition
- Link to color but also absorption spectrum
- Can be measured by HPLC (High precision Liquid Chromatography)





Flow cytometry

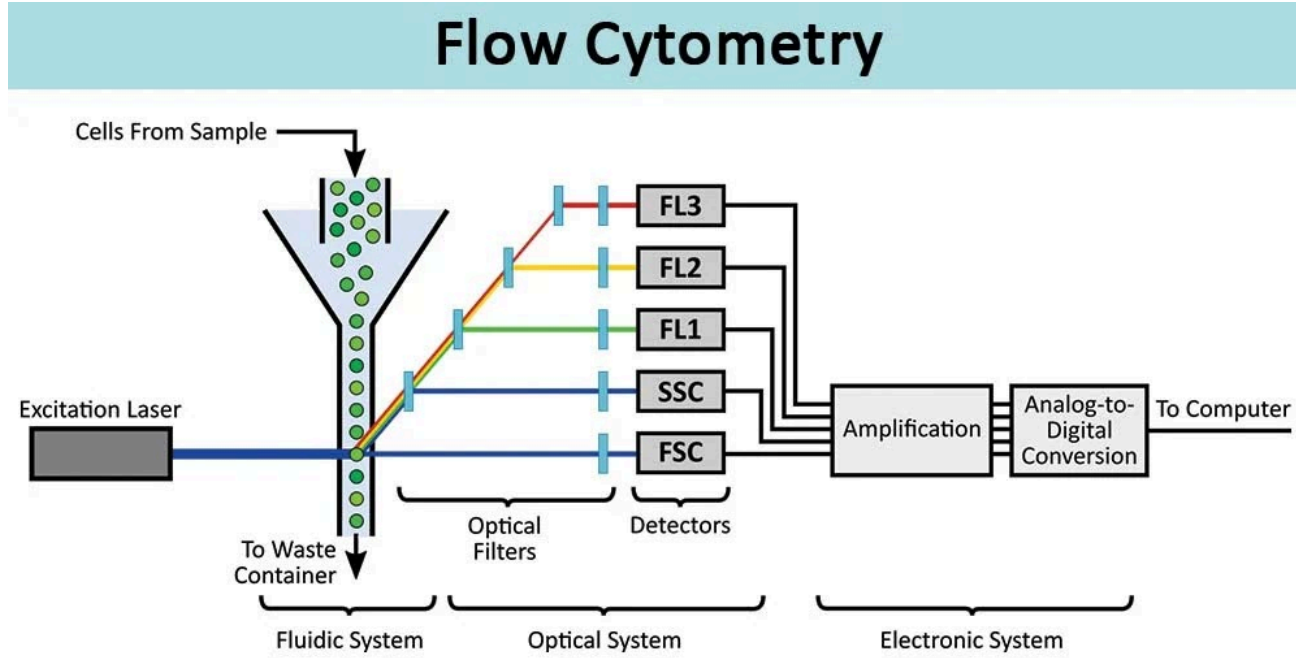
- Based on fluorescence
- Natural fluorescence - Chlorophyll
- Induced fluorescence - DNA



Flow cytometry

- Align cells in flow
- Use laser (488 nm)
- Record scatter and fluorescence

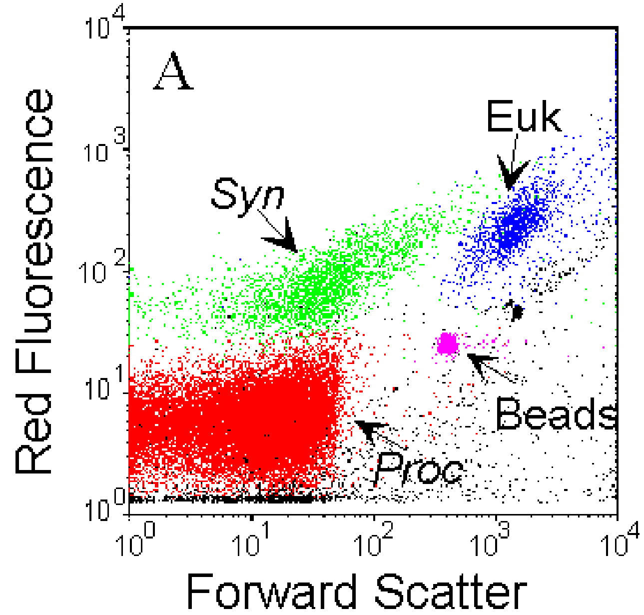
- Many instruments

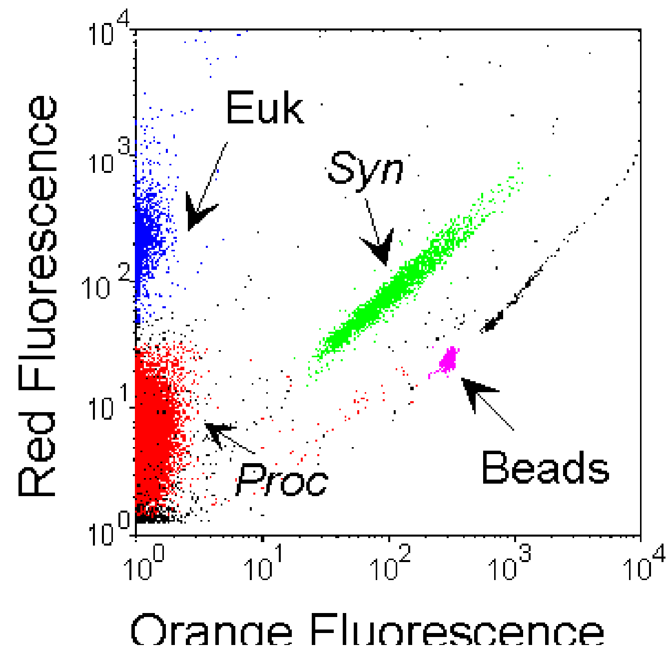


Flow cytometry

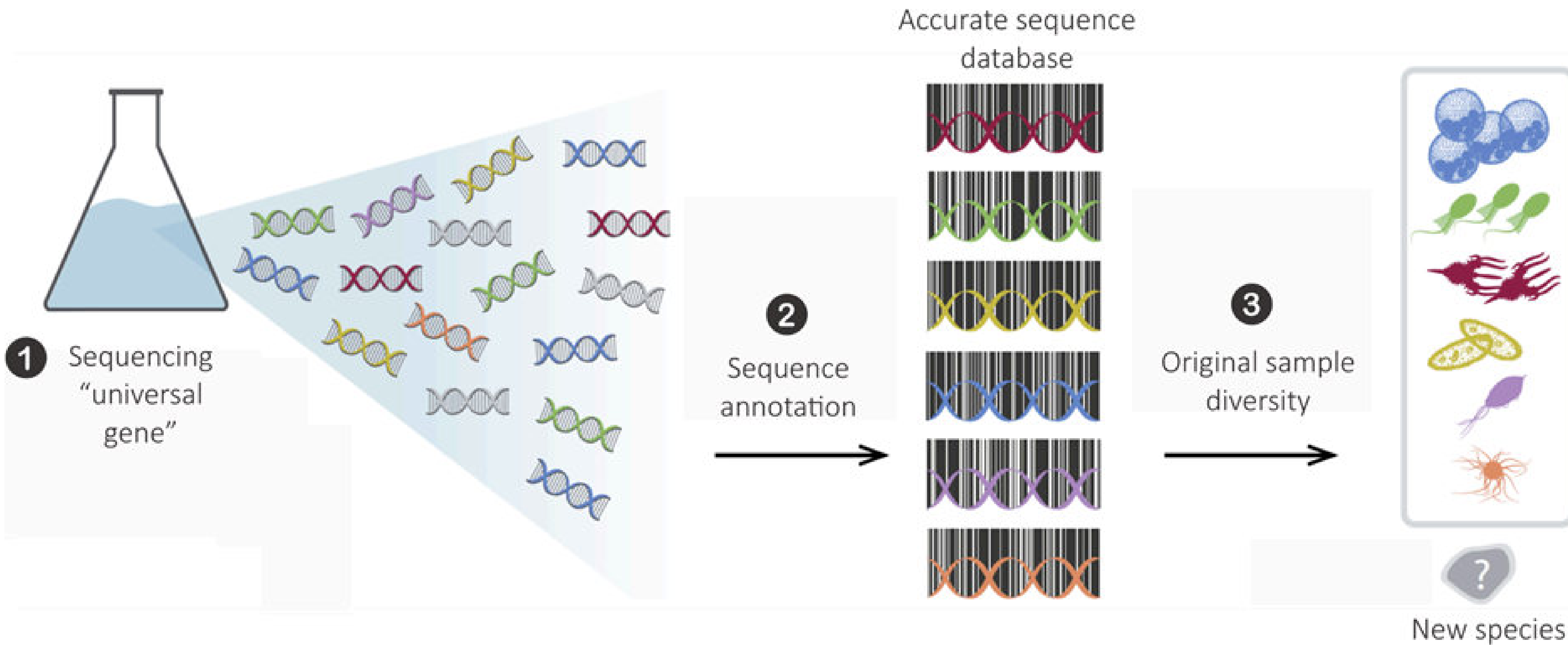
- Discriminate on basis of:
  - Size
  - Pigment fluorescence

- Can only resolve broad groups
  - Pico vs Nano
  - Cyanobacteria vs Eukaryotes

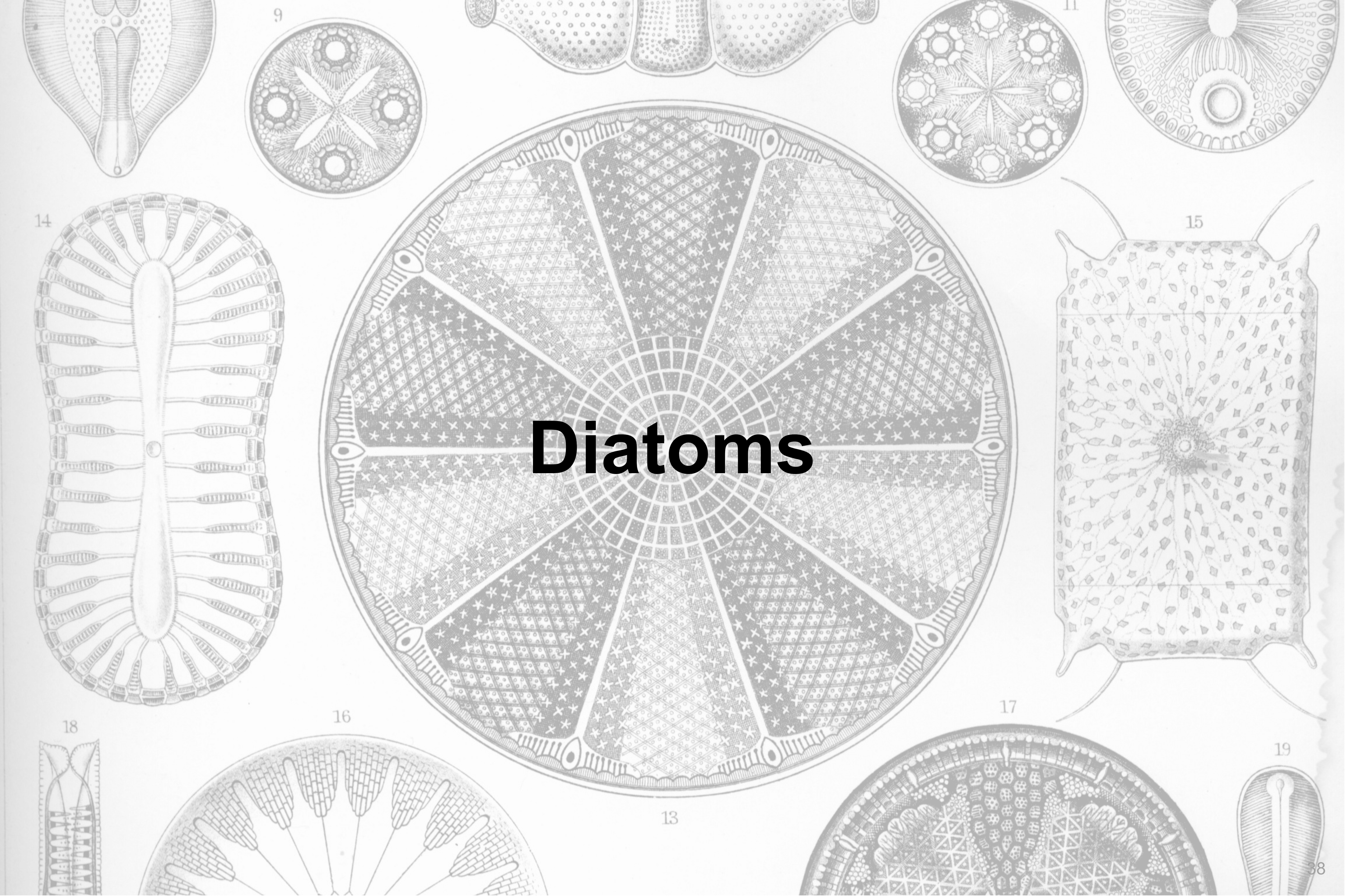


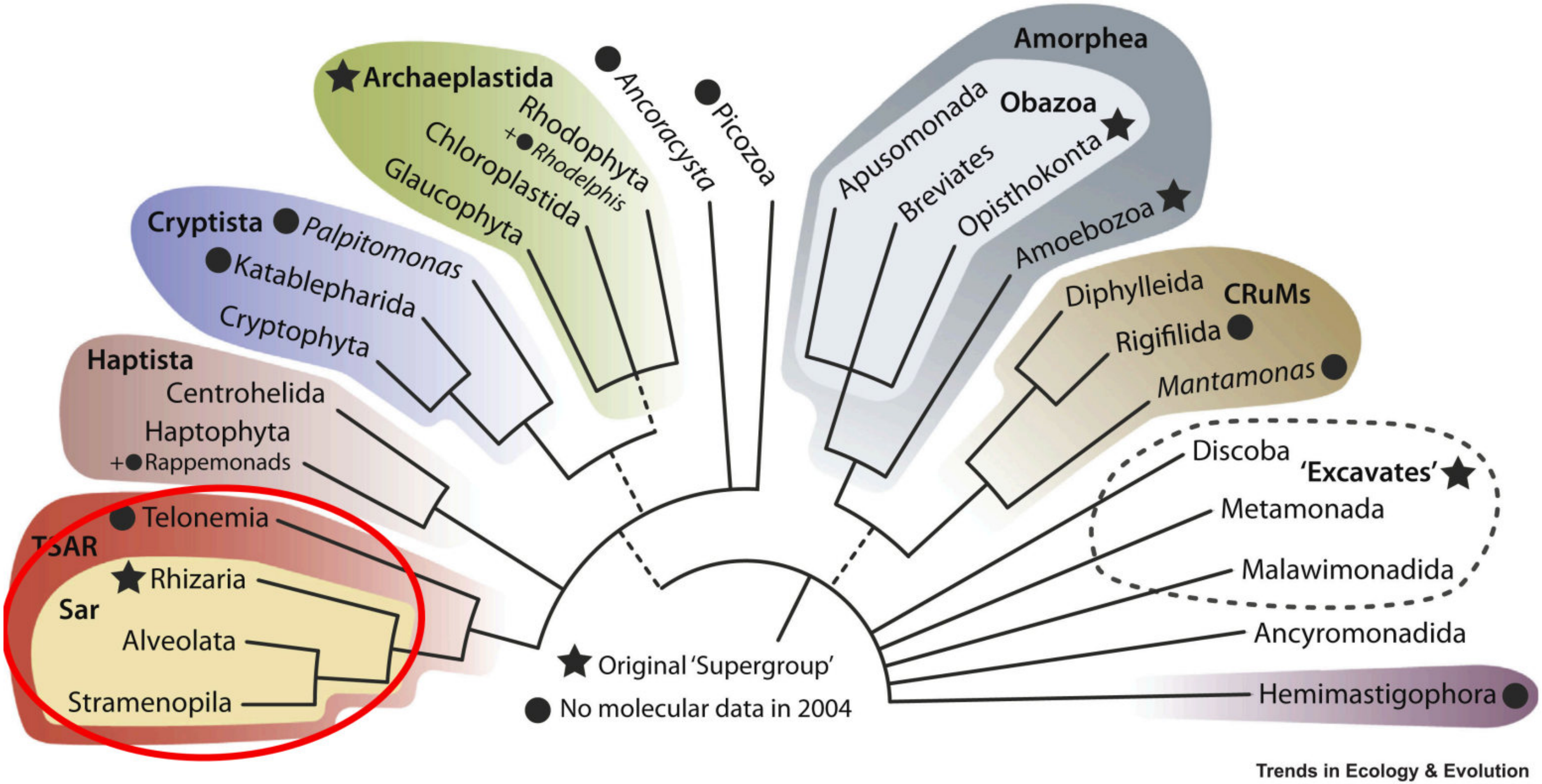


Molecular methods - Metabarcoding



Diatoms

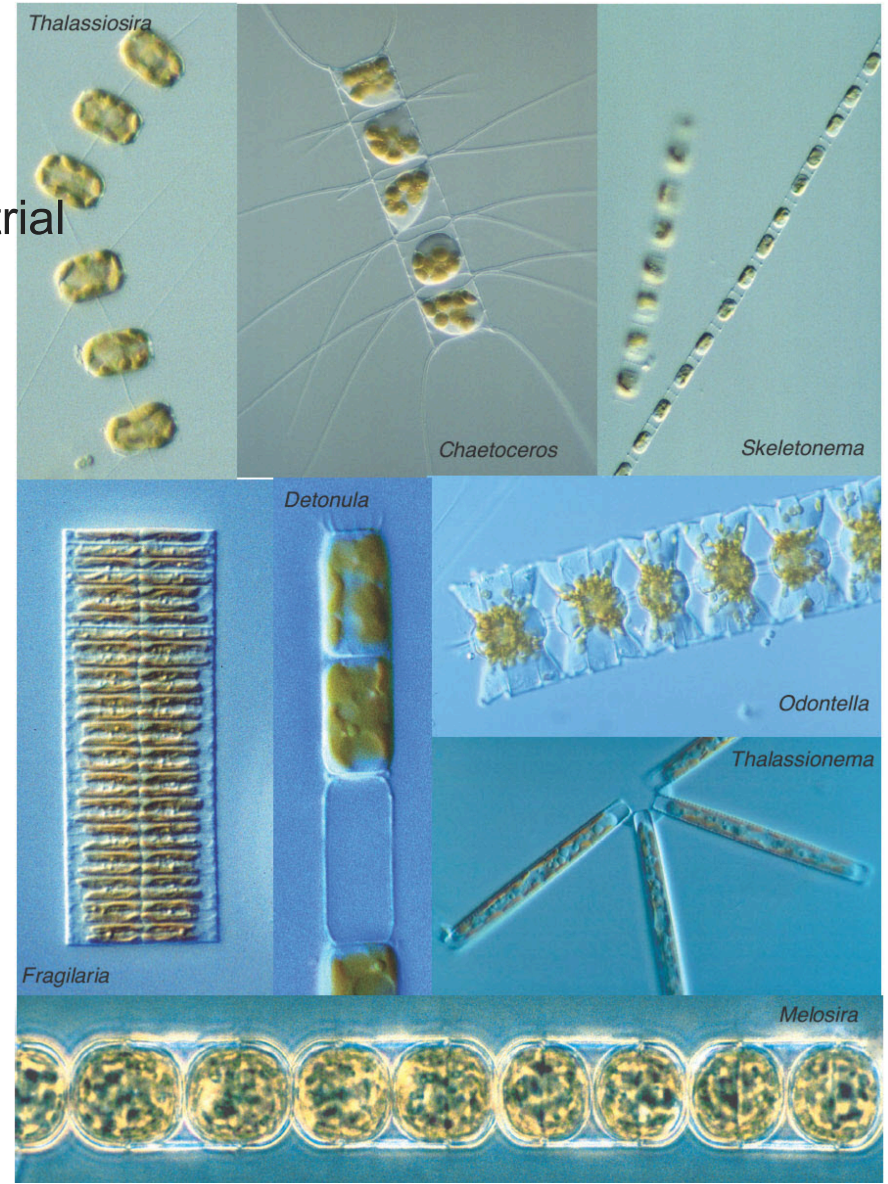


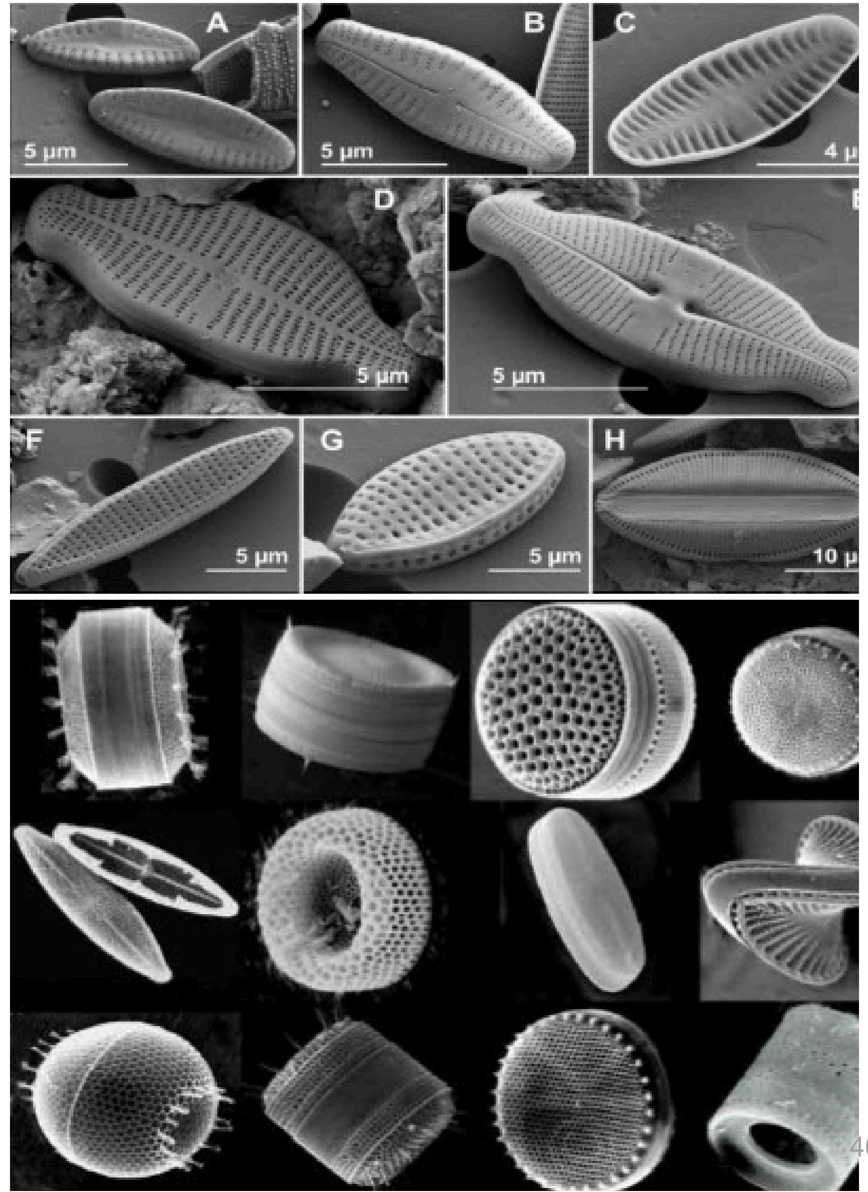

Archaeplastida
Rhodophyta
+Rhodelphis
Chloroplastida
Glaucophyta
Ancoracysta
Picozoa
Amorphea
Obazoa
Apusomonada
Breviates
Opisthokonta
Amoebozoa
Cryptista
Palpitomonas
Katablepharida
Cryptophyta
Haptista
Centrohelida
Haptophyta
+Rappemonads
TSAR
Telonemia
Sar
Rhizaria
Alveolata
Stramenopila
CRuMs
Diphylleida
Rigifilida
Mantamonas
Discoba
'Excavates'
Metamonada
Malawimonadida
Ancyromonadida
Hemimastigophora
Original 'Supergroup'
No molecular data in 2004
Trends in Ecology & Evolution

Diatom diversity

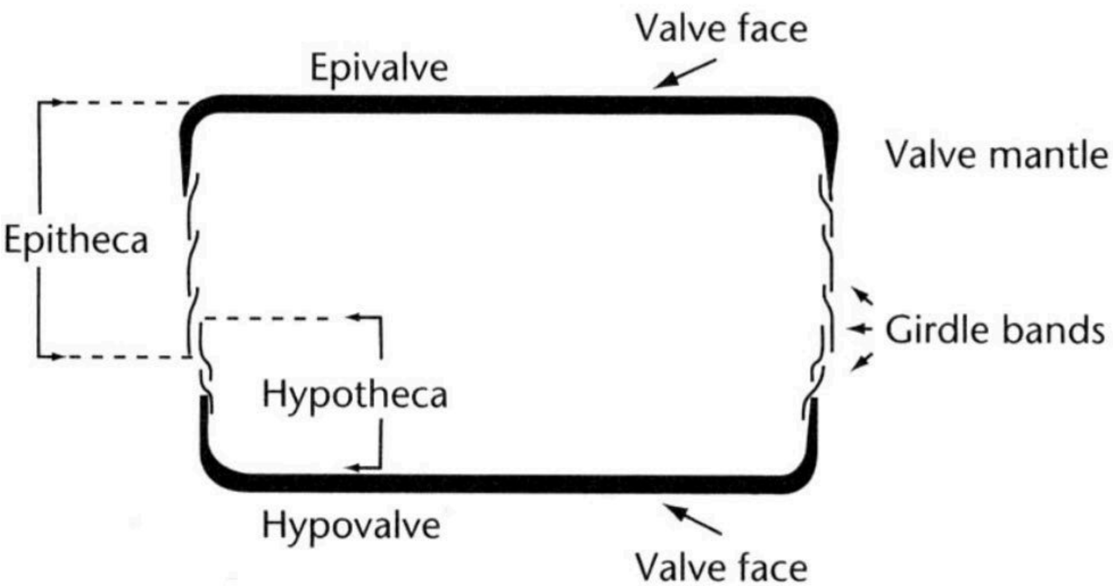
Habitats

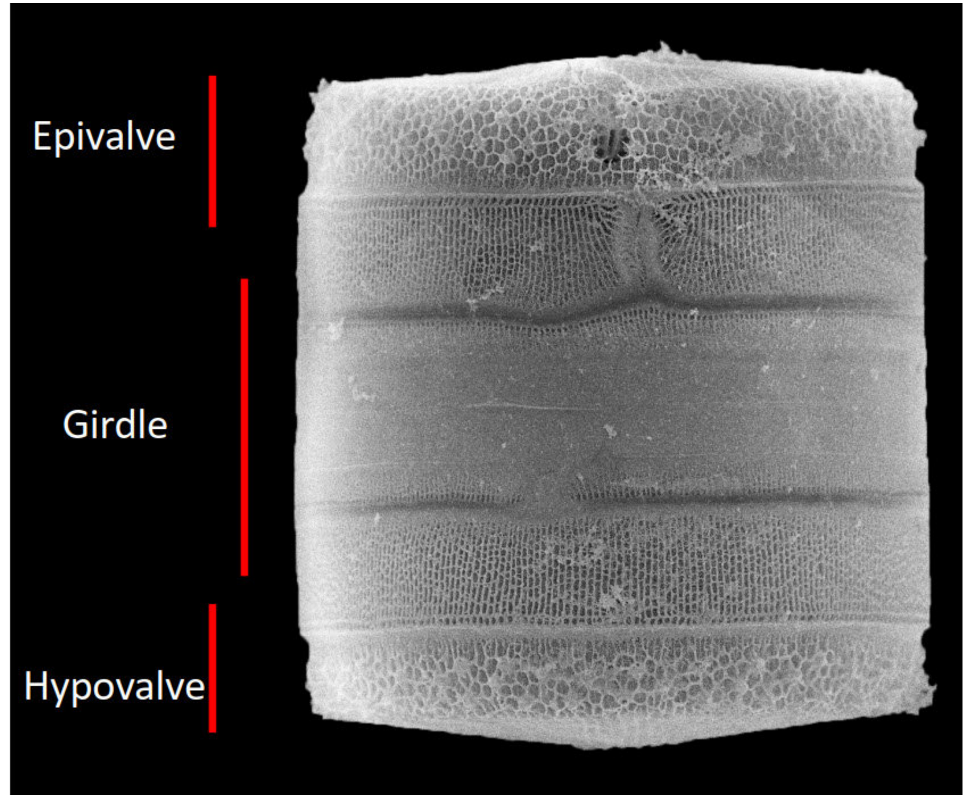
- Ocean
- Lakes
- Ice
- Terrestrial





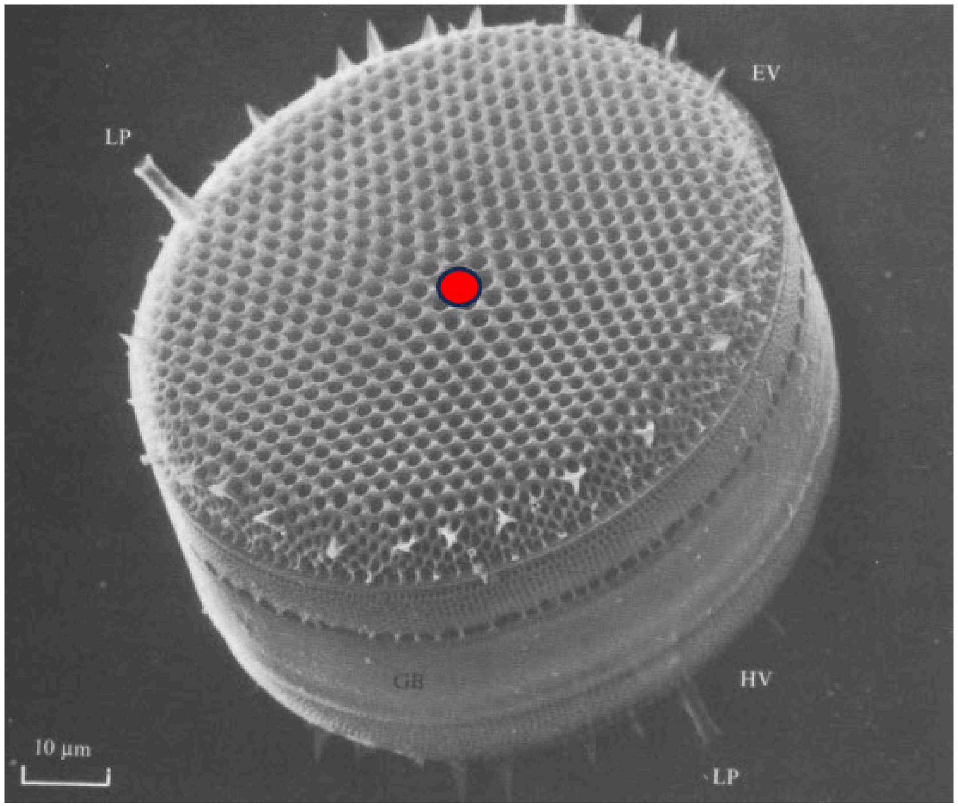
Silica frustule

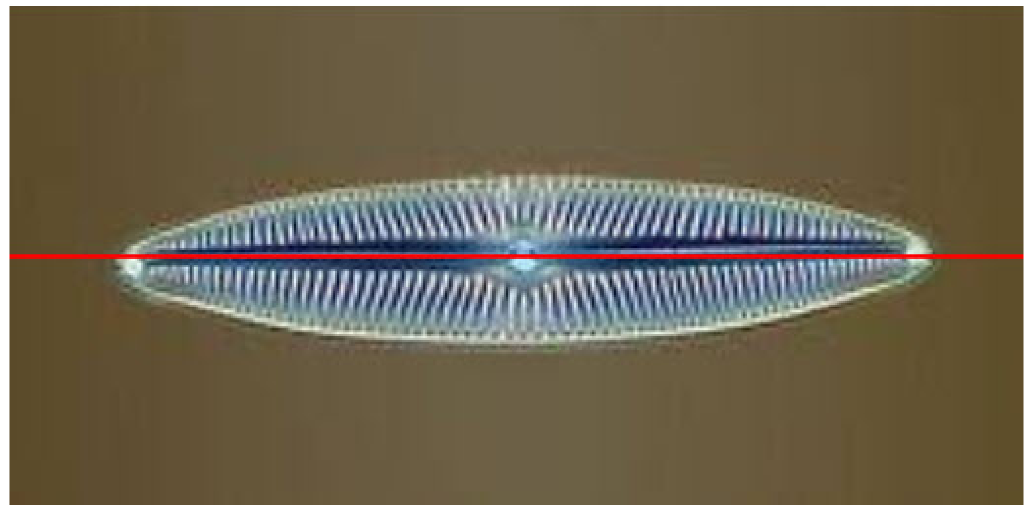




Centric vs Pennate

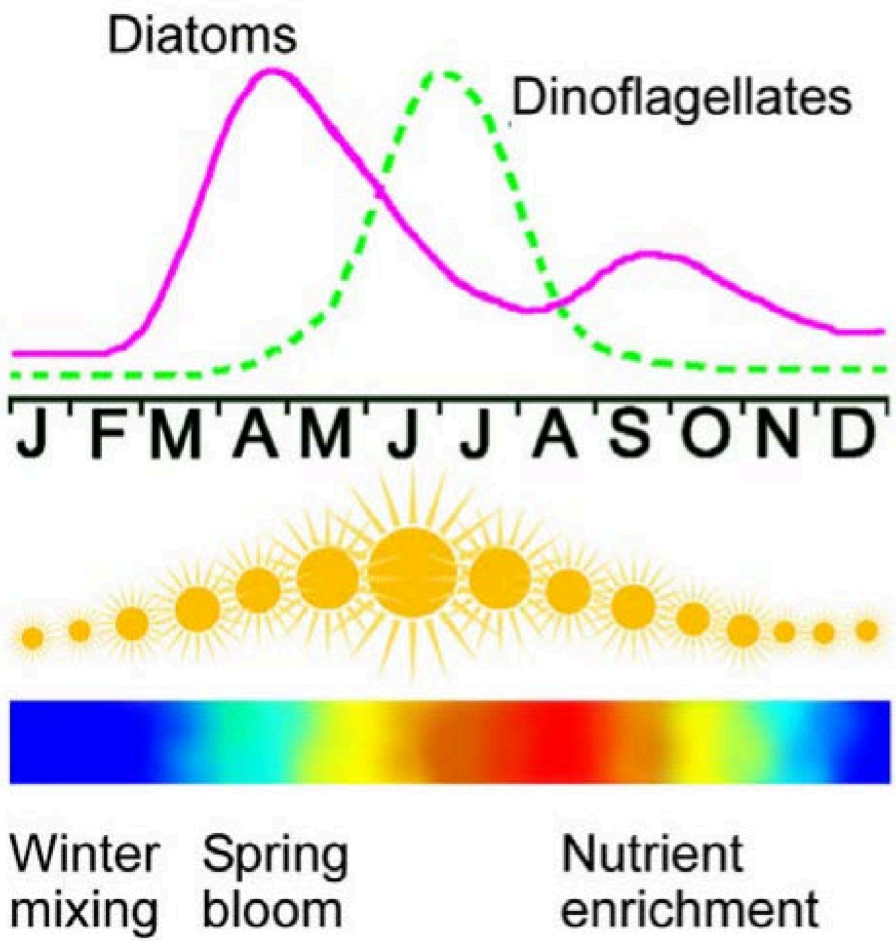
- centric: radial symmetry
- pennate: bilateral symmetry

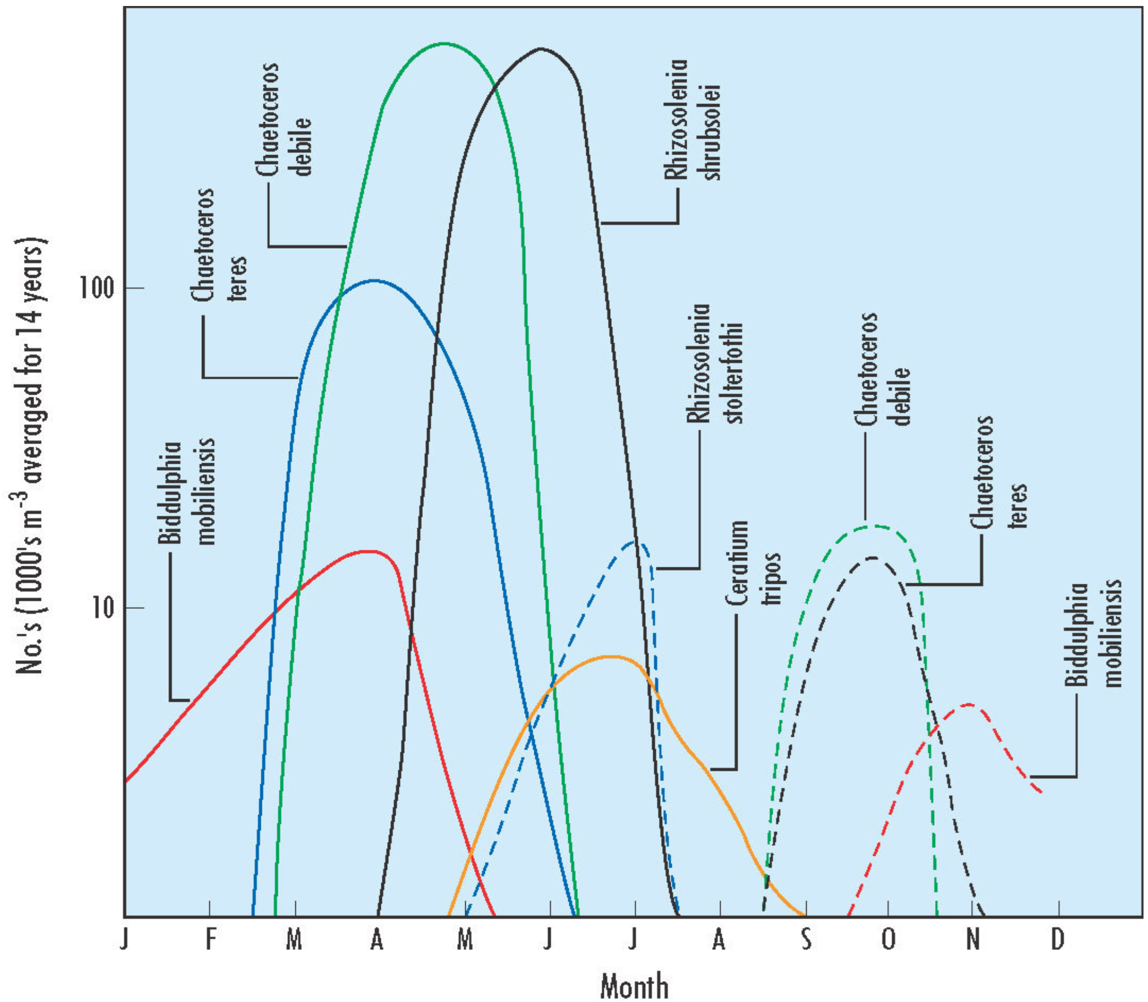




Spring bloom

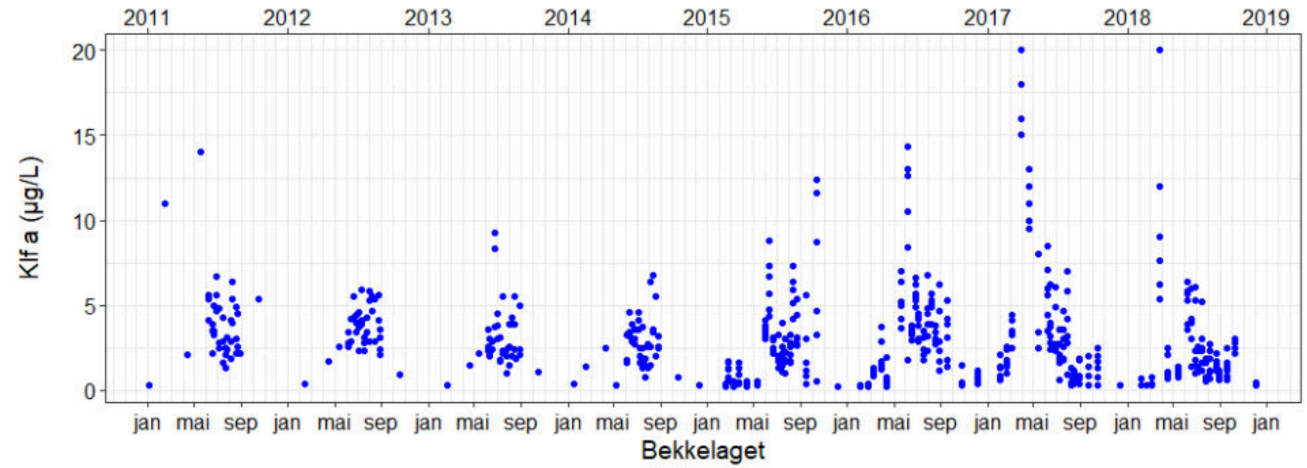
- Triggered by increase in light and temperature
- Species succession

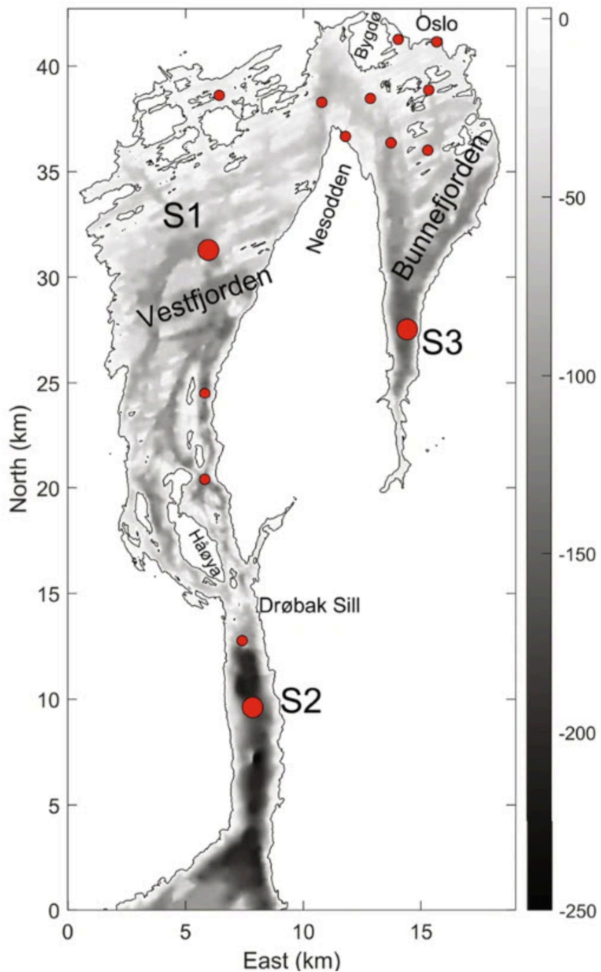


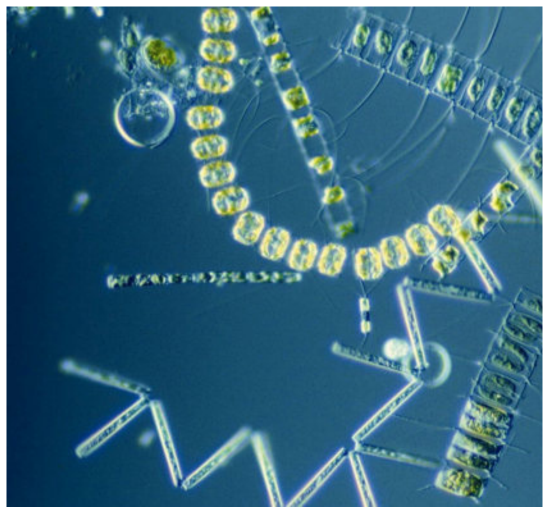


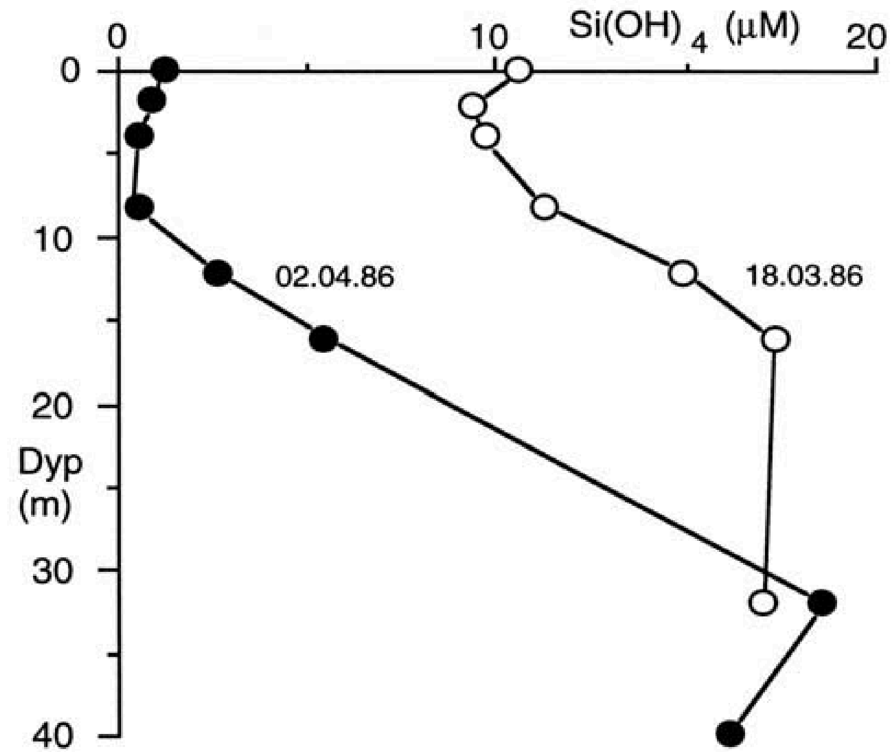
Spring bloom

- Oslo fjord
- Silica becomes depleted

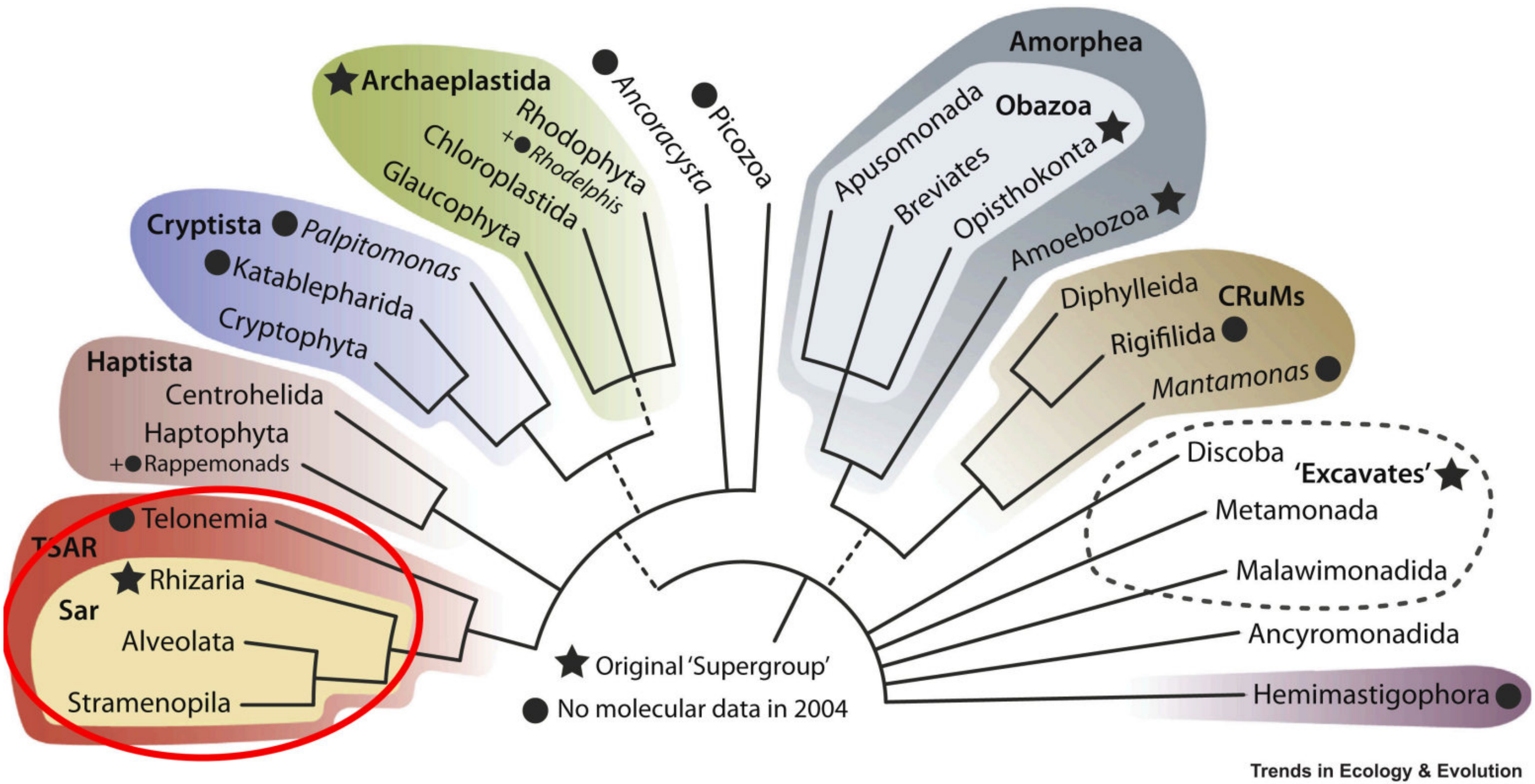








Dinoflagellates


Amorphea
Obazoa
Apusomonada
Breviates
Opisthokonta
Amoebozoa
Archaeplastida
Rhodophyta
+ Rhodelphis
Chloroplastida
Glaucophyta
Ancoracysta
Picozoa
Cryptista
Palpitomonas
Katablepharida
Cryptophyta
Haptista
Centrohelida
Haptophyta
+ Rappemonads
Telonemia
TSAR
Rhizaria
Sar
Alveolata
Stramenopila
Diphylleida
CRuMs
Rigifilida
Mantamonas
Discoba
'Excavates'
Metamonada
Malawimonadida
Ancyromonadida
Hemimastigophora
Original 'Supergroup'
No molecular data in 2004
Trends in Ecology & Evolution

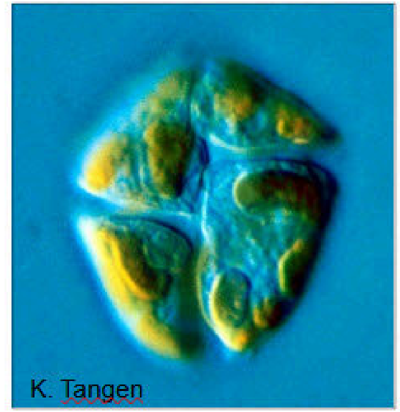
Dinoflagellate diversity

prorocentroid
Prorocentrum

dinophysoid
Dinophysis

peridinoid
Protoperidinium

gymnodinoid
Karenia



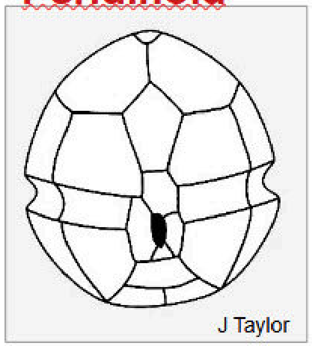
gonyaulacoid
Protoceratium

suessioid
Polarella

noctilucoid
Noctiluca

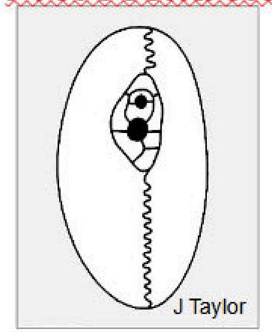
Dinoflagellate diversity

Peridinoid

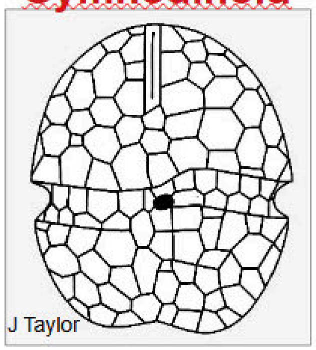


Cell types

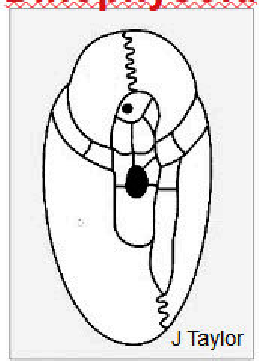
Prorocentroid



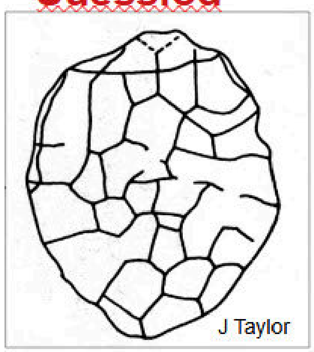
Gymnodinoid



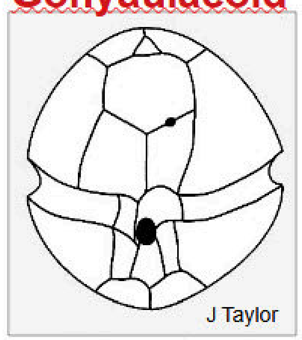
Dinophysoid



Suessiod



Gonyaulacoid



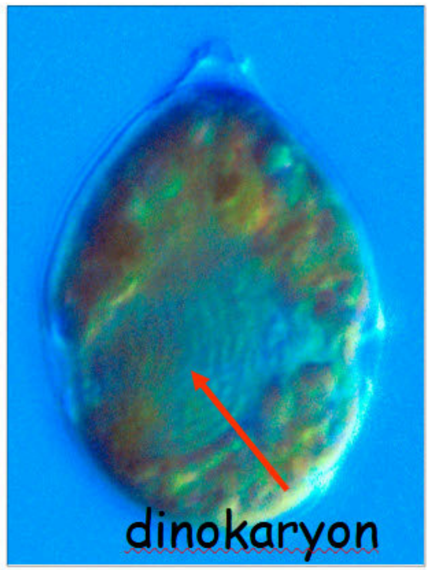
Dinoflagellate characters

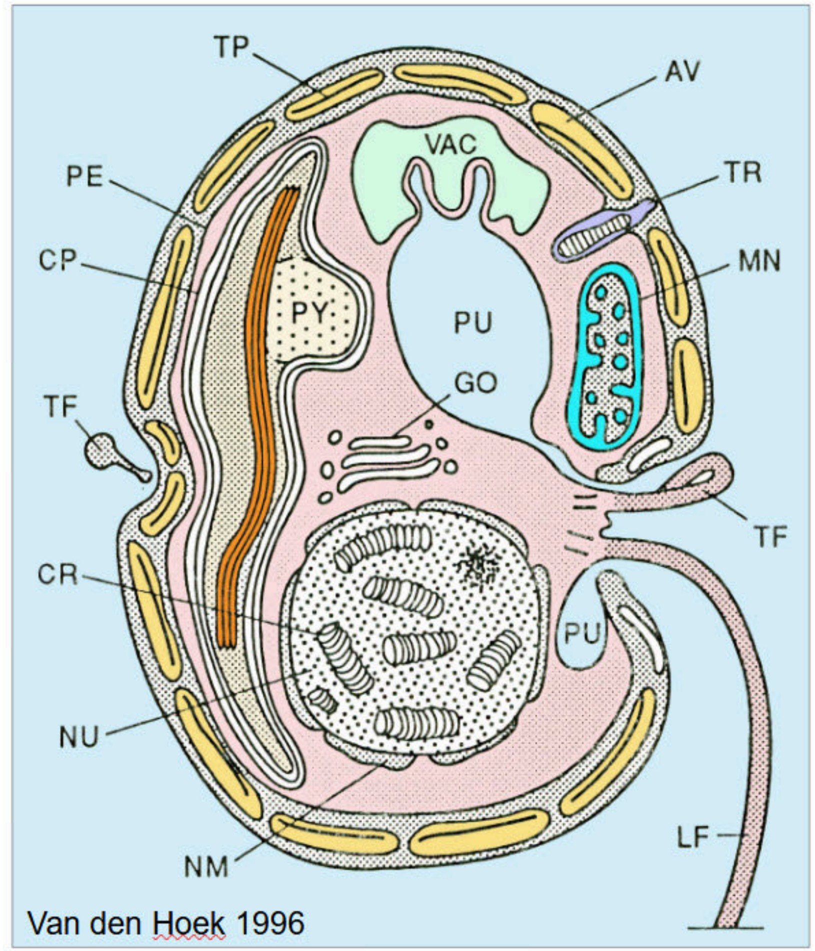
Cell structure

- naked vs. armed
  - cellulose plates
- 2 flagella
  - transverse
- dinokaryon
  - condensed chromosomes

Trophic status

- Autotrophs – ca 50%
- Heterotrophs – ca 50%
- Free-living – most
- Symbionts – few, but important
- Parasites – many



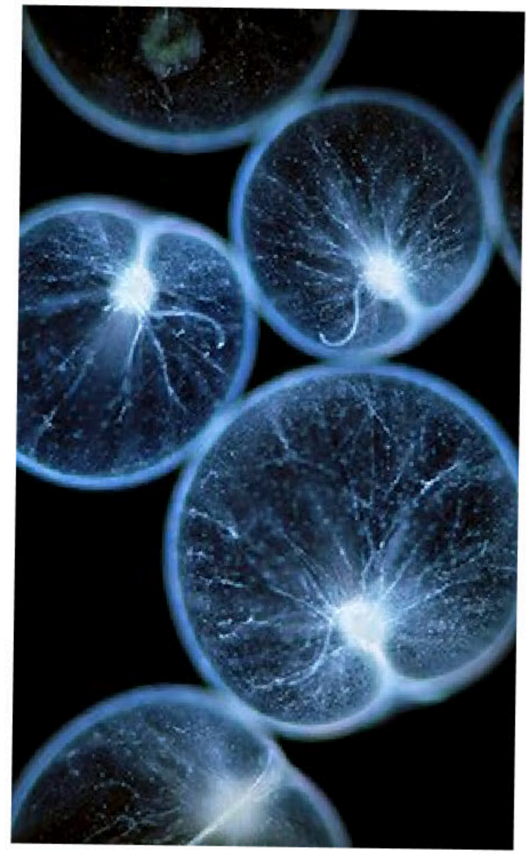


Dinoflagellate blooms

- Bioluminescent: *Noctiluca*

Fluorescent Hong Kong seas indicates harmful algae

The Associated Press
Published Thursday, January 22, 2015 8:26PM EST



This Thursday, Jan. 22, 2015 photo made with a long exposure shows the glow from a Noctiluca scintillans algal bloom along the seashore in Hong Kong. The luminescence, also called Sea Sparkle, is triggered by farm pollution that can be devastating to marine life and local fisheries, according to University of Georgia oceanographer Samantha Joye. (AP Photo/Kin Cheung)

Dinoflagellate blooms

- Toxic: *Karlodinium*

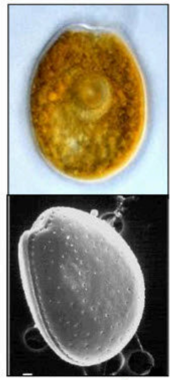
Last summer's fish kill was by a toxic dinoflagellate: emerging algal toxins in co Finnish waters

Scientists at SYKE Marine Research Centre have related the fish ki near Tammisaari last July to a dinoflagellate species, Karlodinium previously known to form blooms in Finnish waters.

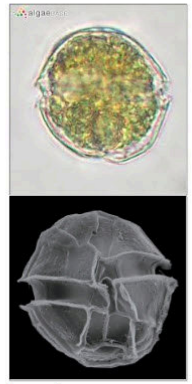
Water samples taken at the time of the fish kill in Ersöströmmen had a brown color, which according to microscopic analysis conducted at SYKE MRC was due to unusually high concentrations (over 10 million cells/L) of a small dinoflagellate of ca.

Karlodinium veneficum

Azadinium spinosum

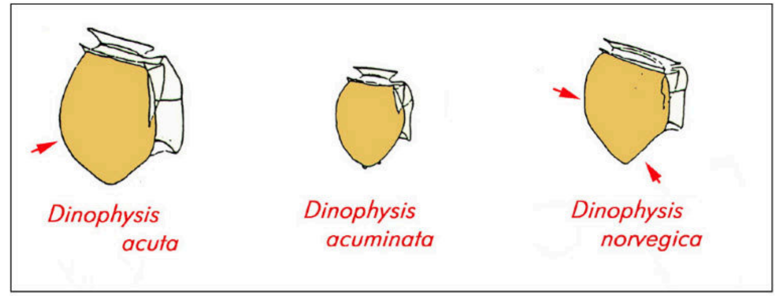


Prorocentrum lima

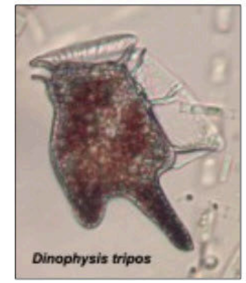


Alexandrium tamarense

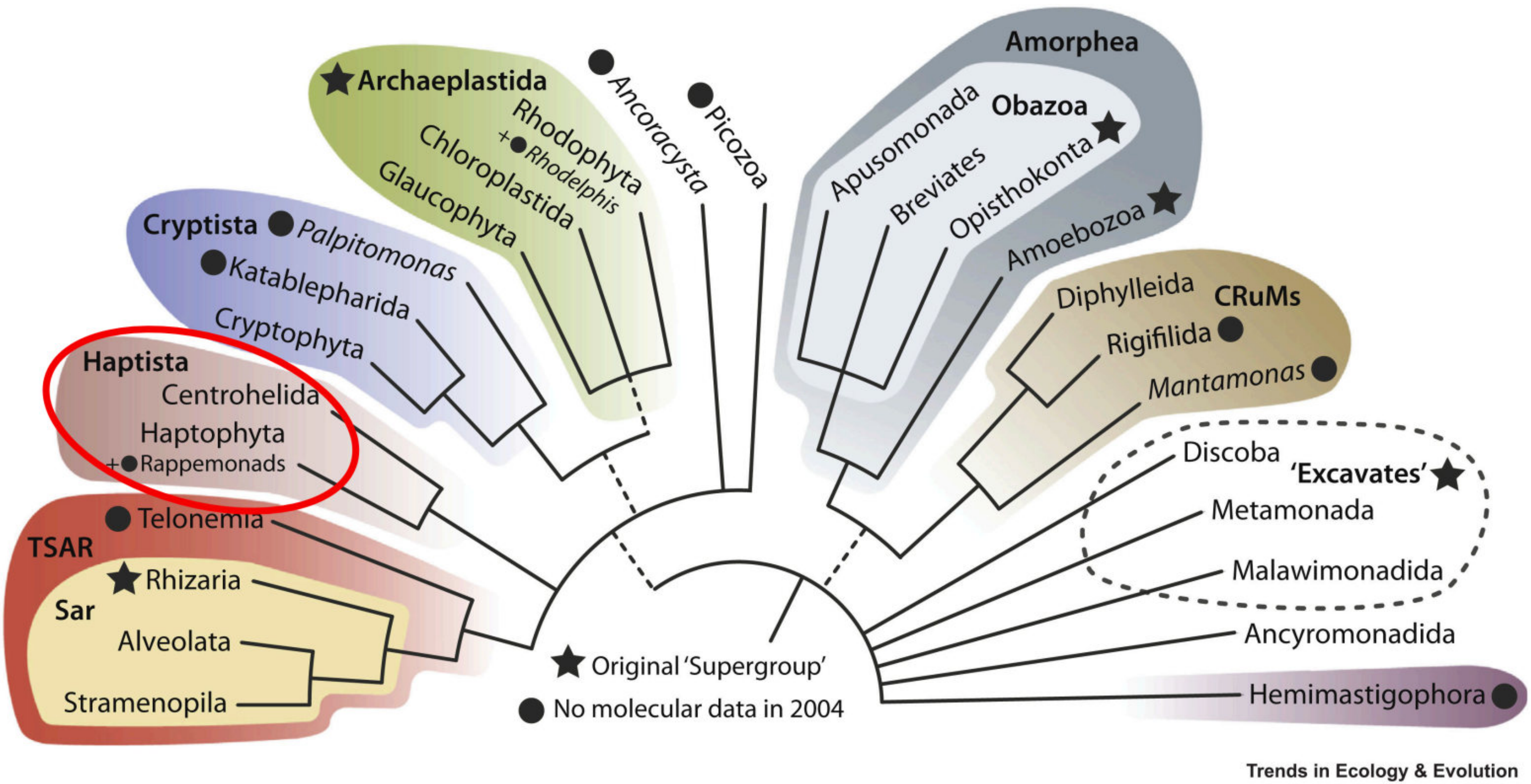
Examples of toxic dinoflagellates



Dinophysis acuta



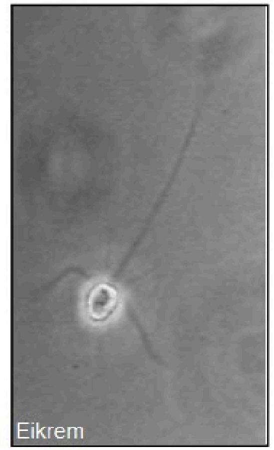
Haptophytes


Archaeplastida
Rhodophyta
+Rhodelphis
Chloroplastida
Glaucophyta
Ancoracysta
Picozoa
Amorphea
Obazoa
Apusomonada
Breviates
Opisthokonta
Amoebozoa
Cryptista
Palpitomonas
Katablepharida
Cryptophyta
Haptista
Centrohelida
Haptophyta
+Rappemonads
Telonemia
TSAR
Rhizaria
Sar
Alveolata
Stramenopila
Diphylleida
CRuMs
Rigifilida
Mantamonas
Discoba
'Excavates'
Metamonada
Malawimonadida
Ancyromonadida
Hemimastigophora
Original 'Supergroup'
No molecular data in 2004
Trends in Ecology & Evolution

Main characters

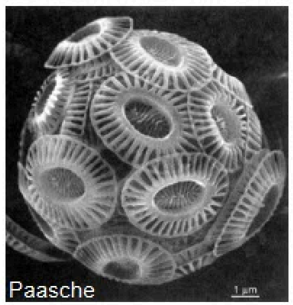
- Mostly phototrophic
- Mostly marine
- Mostly nanoplankton (5-20
- Two flagella + haptonema
- Cosmopolitan distribution
- Can form massive blooms
- Some are toxic

Prymnesium

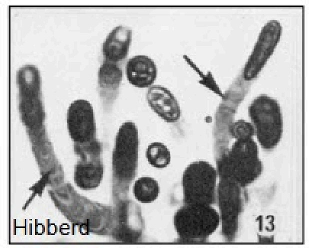


Chrysochromulina

Phaeocystis

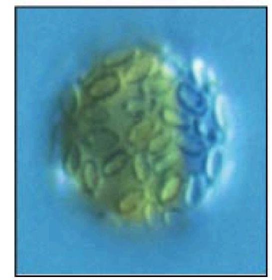


Emiliania



Chrysotila

Calcidiscus



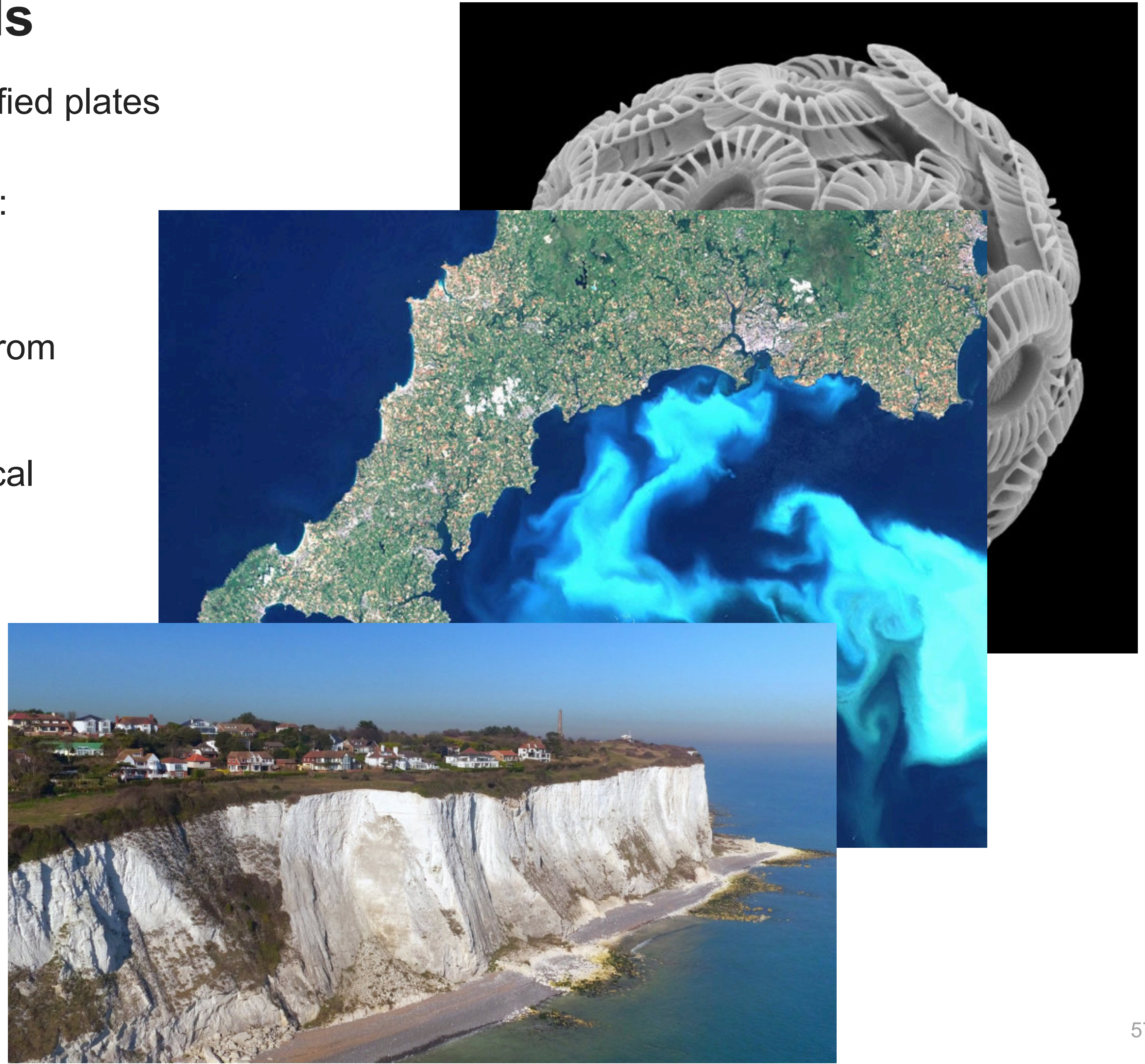
Chrysotila

Coccolithophorids

- Cells are covered of calcified plates (coccoliths)
- Most widespread species: *Gephyrocapsa huxleyi*

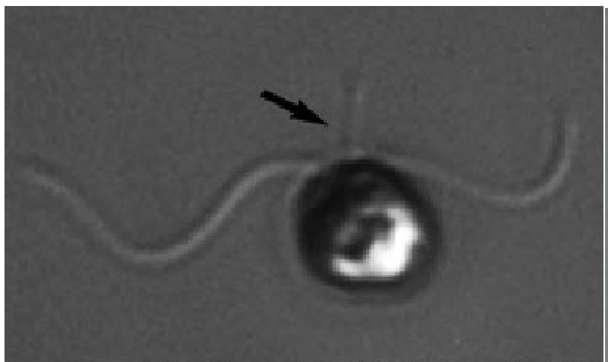
- Can form blooms seen from satellites

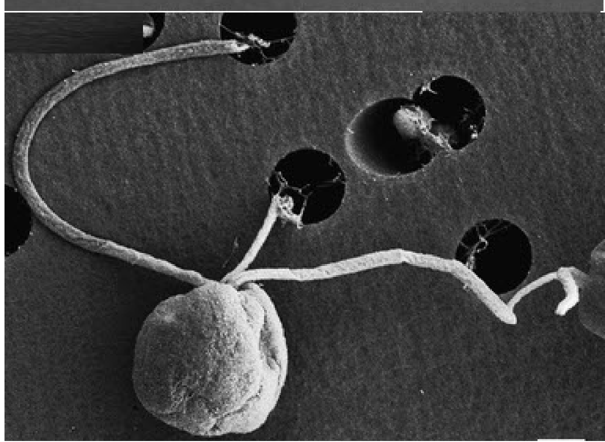
- Responsible for geological formations

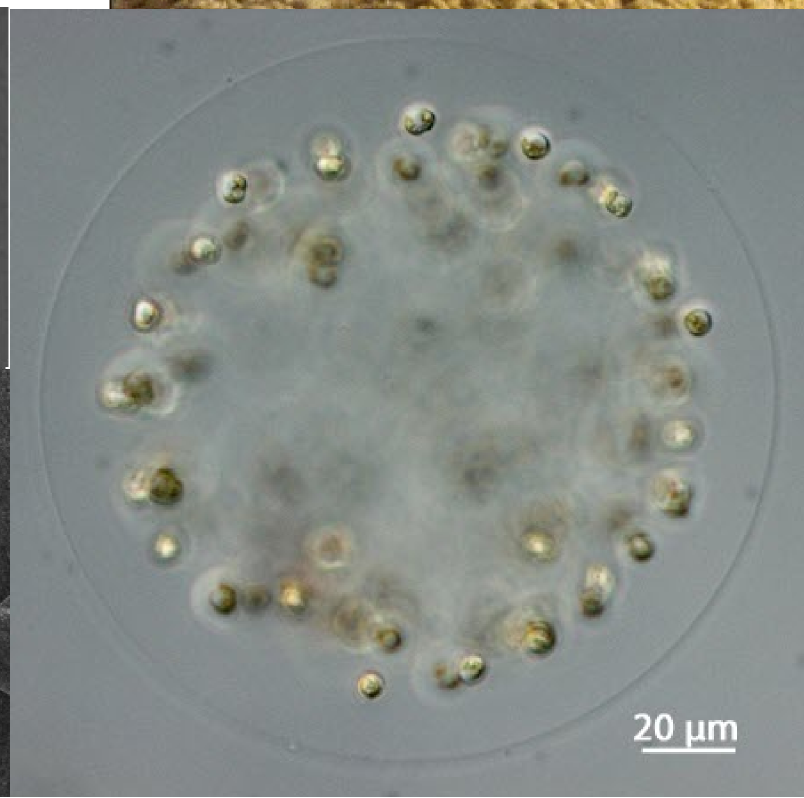


Phaeocystis

- Forms massive blooms
- Colonial form
- Flagellate form
- From tropics to poles







Chrysochromulina leadbeateri

- Killer alga

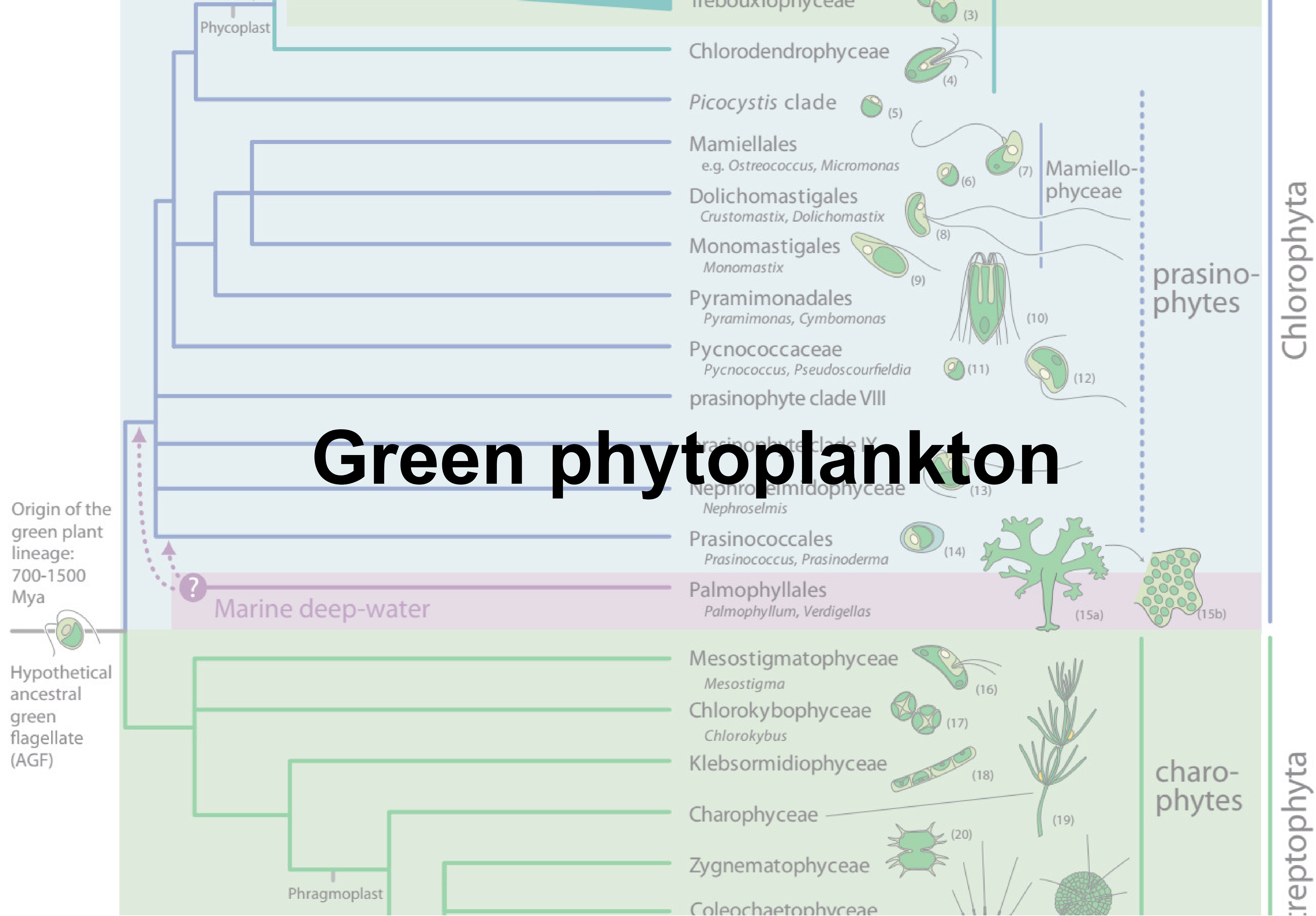
What we know about the so-called "killer alga" in northern Norway

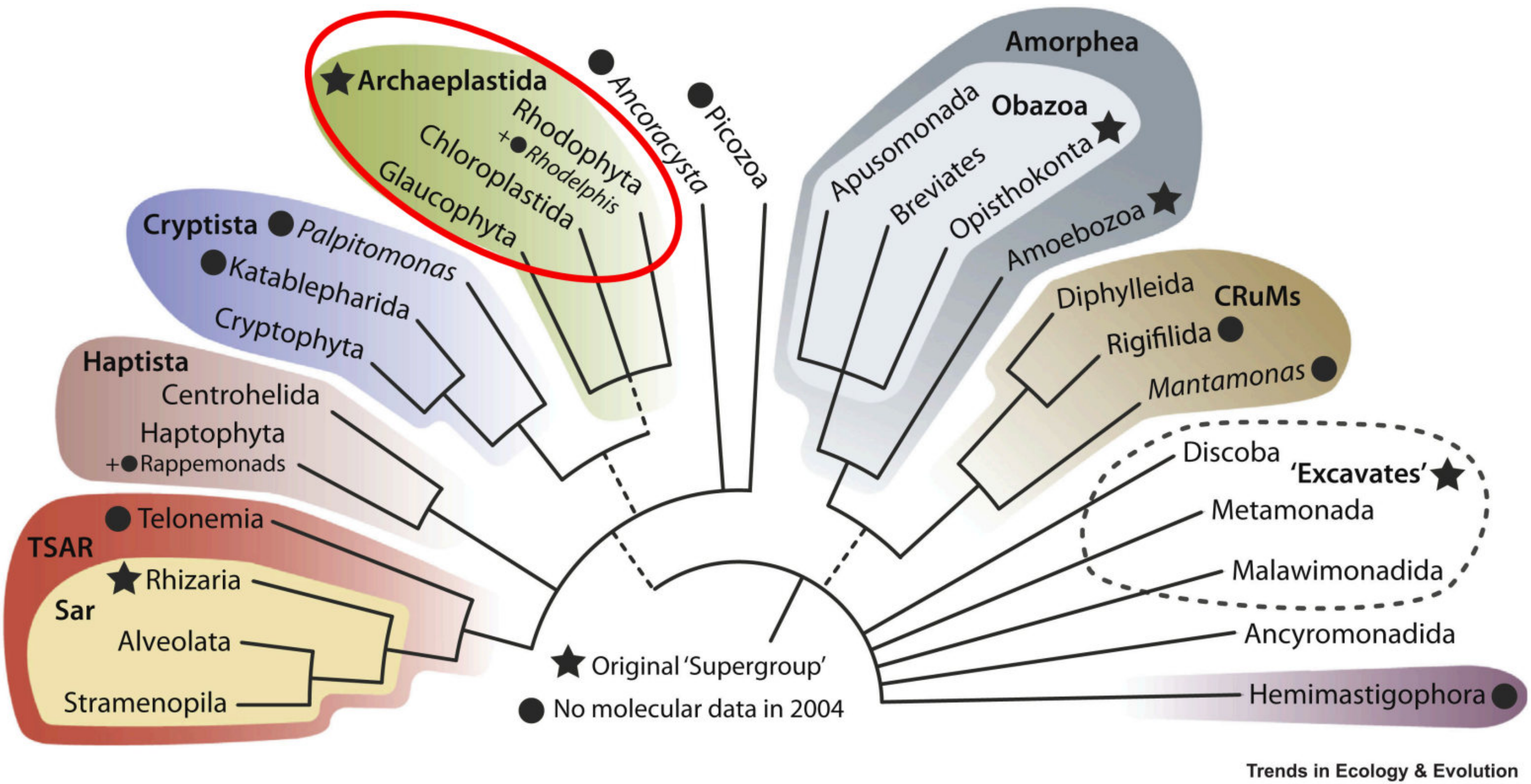
The salmon farm Ballangen sjøfarm was hit hard by the algae bloom.
Photo: Ballangen sjøfarm (with permission)

Harmful blooms of *Chrysochromulina leadbeateri* have led to the death of salmon in the counties of Nordland and Troms. This species of alga is common along the Norwegian coast.

IMR web site

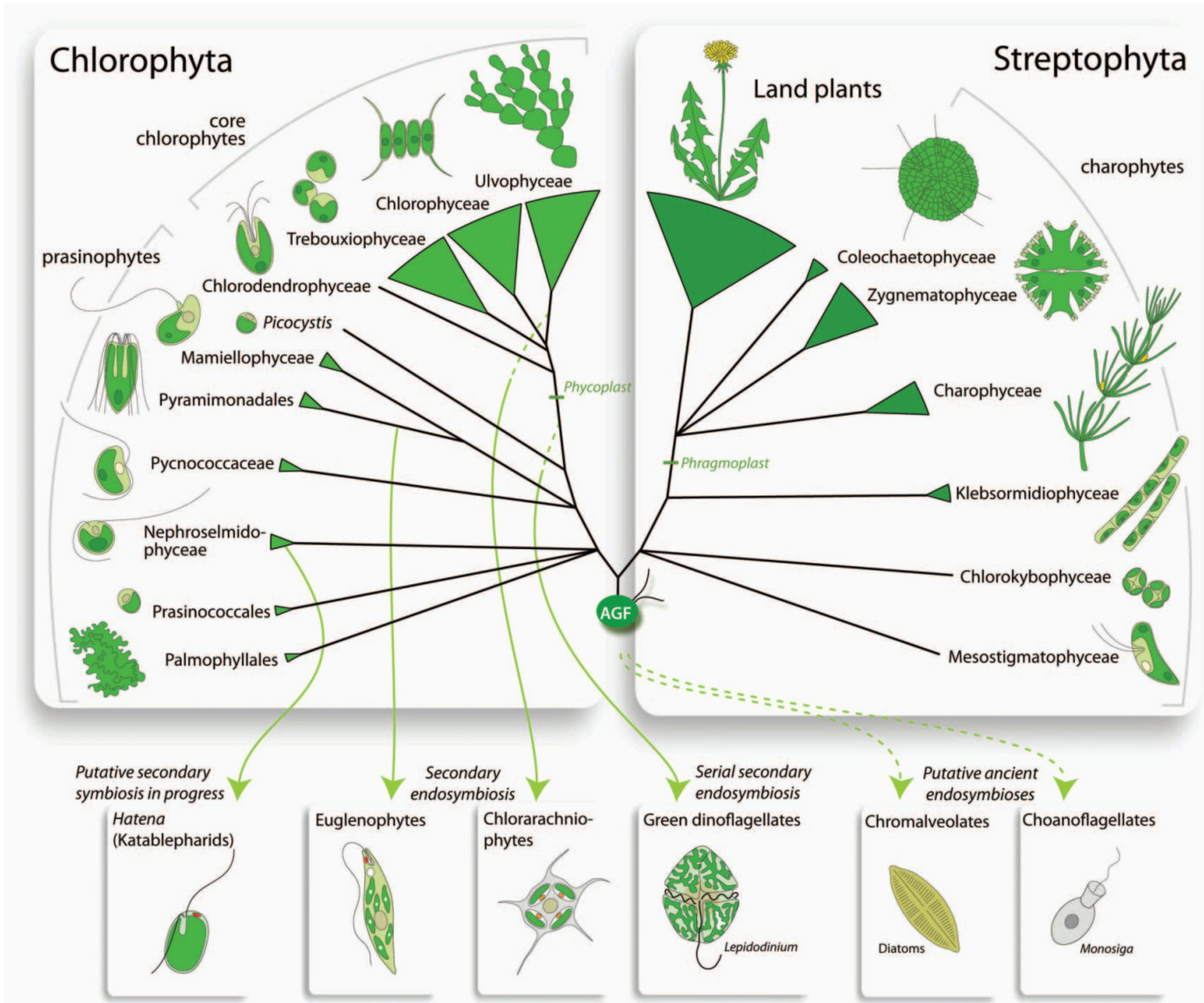
Green phytoplankton




Archaeplastida
Rhodophyta
+ Rhodelphis
Chloroplastida
Glaucophyta
Ancoracysta
Picozoa
Cryptista
Palpitomonas
Katablepharida
Cryptophyta
Haptista
Centrohelida
Haptophyta
+ Rappemonads
Telonemia
TSAR
Rhizaria
Sar
Alveolata
Stramenopila
Amorphea
Apusomonada
Obazoa
Breviates
Opisthokonta
Amoebozoa
Diphylleida
CRuMs
Rigifilida
Mantamonas
Discoba
'Excavates'
Metamonada
Malawimonadida
Ancyromonadida
Hemimastigophora
Original 'Supergroup'
No molecular data in 2004
Trends in Ecology & Evolution

The green lineage

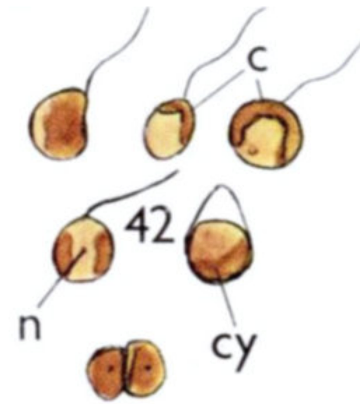
- Streptophyta
  - Land plants
- Chlorophyta
  - Core chlorophytes
  - “Prasinophytes”
    - **Mamiellophyceae**



Micromonas

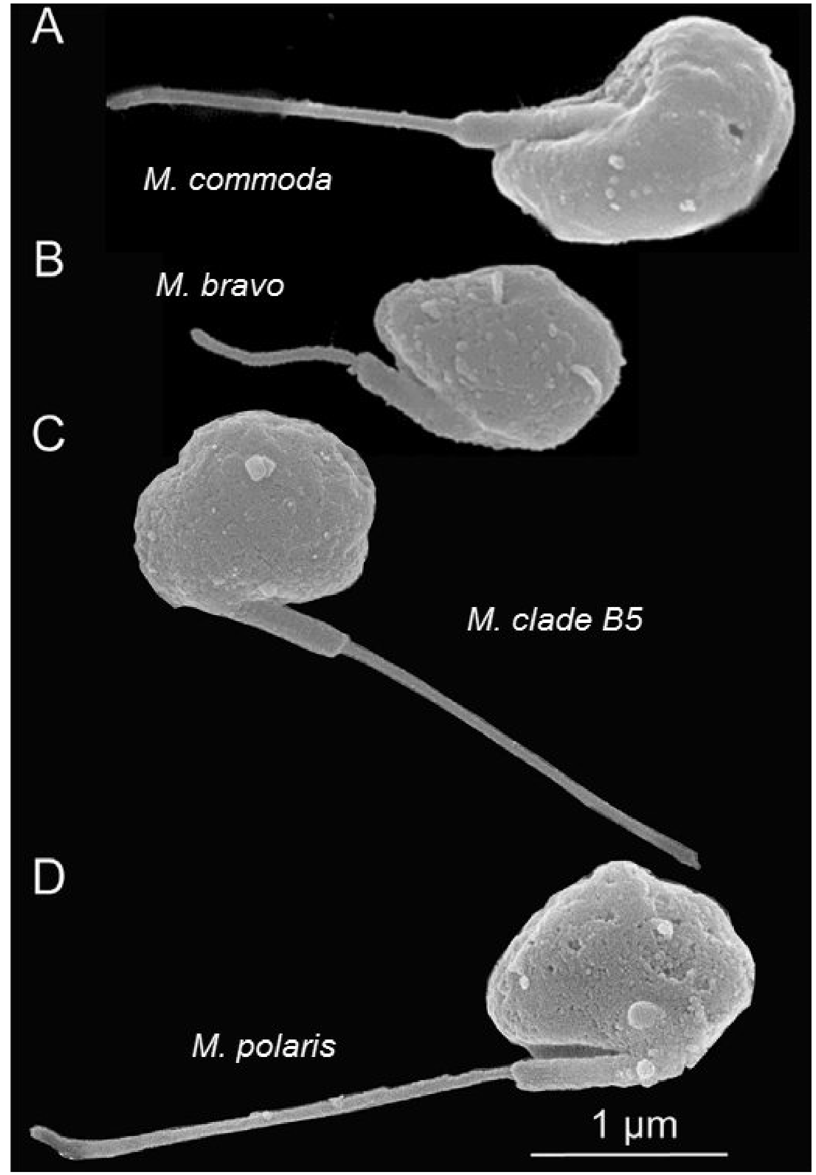
First picoplankton species described in 1952

- *M. pusilla* (*Chromulina pusilla*)
- 1.5 µm
- One flagellum
- Ubiquitous genus
  - From tropics to pole



Three more species described in 2017

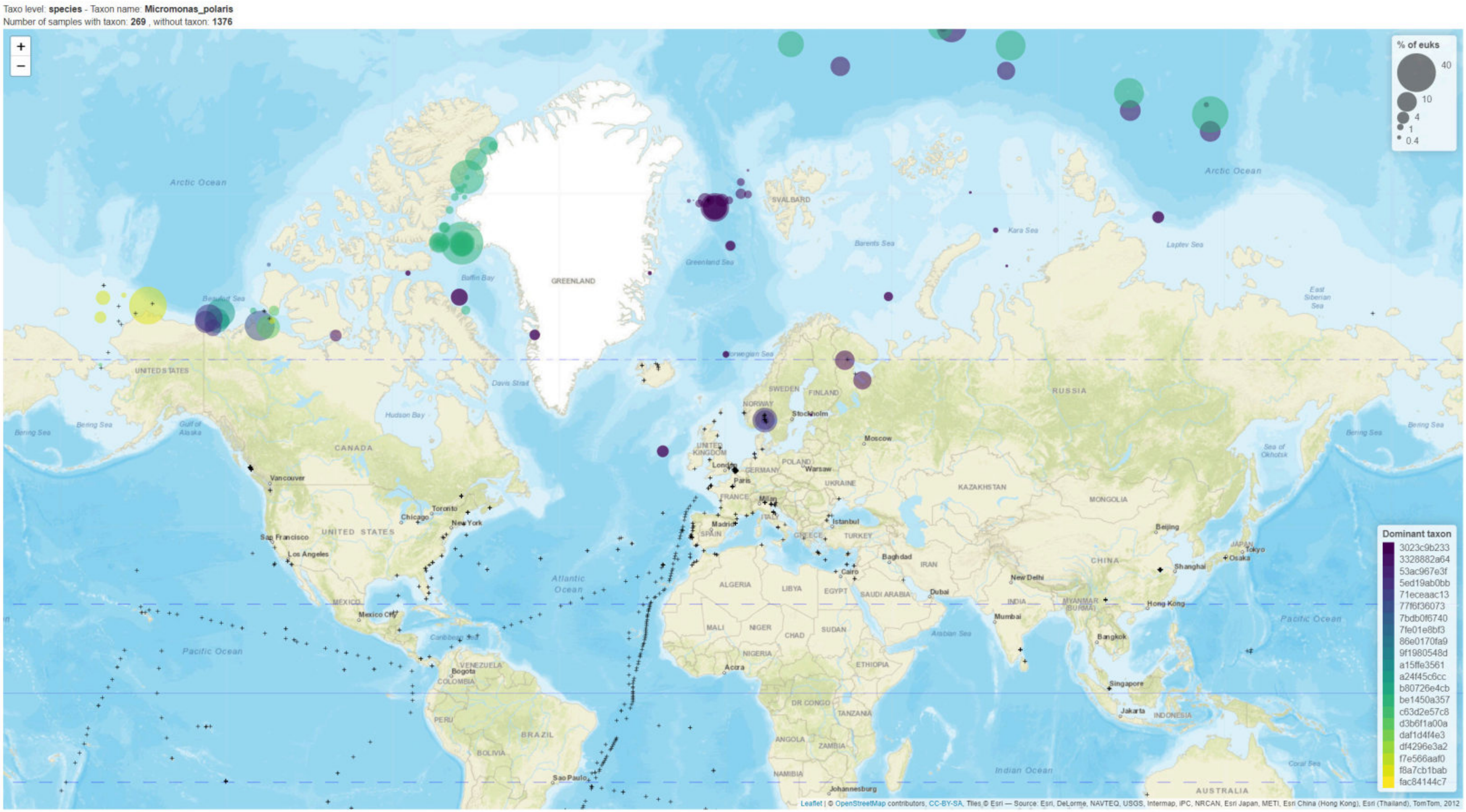
- *M. commoda*
- *M. bravo*
- *M. polaris*



Butcher, R.W. 1952. J. Mar. Biol. Assoc. U.K. 31:175–91.
Simon, N., Foulon, E., Grulois, D., Six, C., Desdevises, Y., Latimier, M., Le Gall, F. et al. 2017. Protist. 168:612–35.

Micromonas

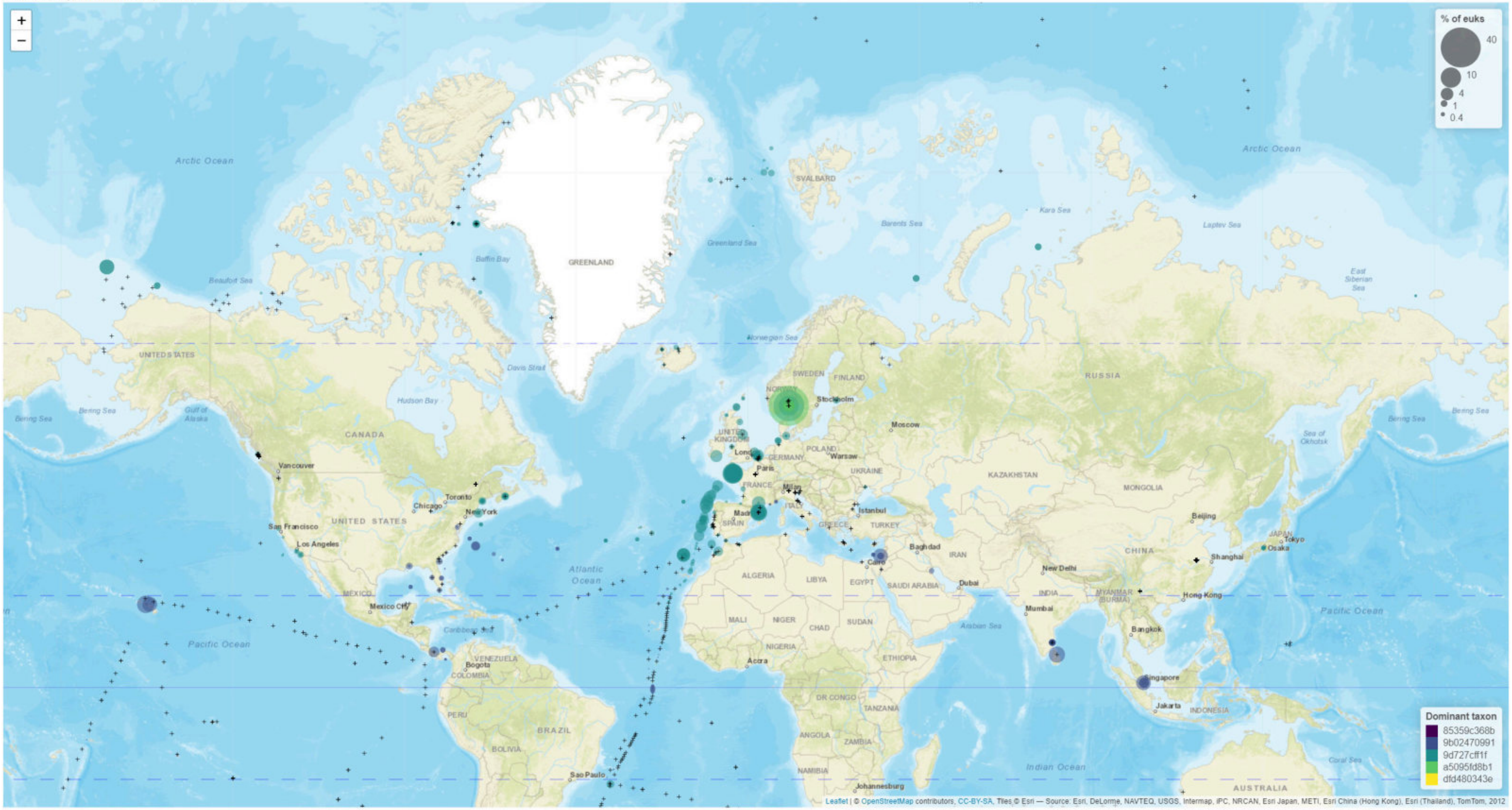
M. polaris only found in polar waters



Micromonas

M. commoda only found in coastal temperate waters

Taxo level: **species** - Taxon name: **Micromonas_commoda_A1;Micromonas_commoda_A2**
Number of samples with taxon: **788** , without taxon: **857**

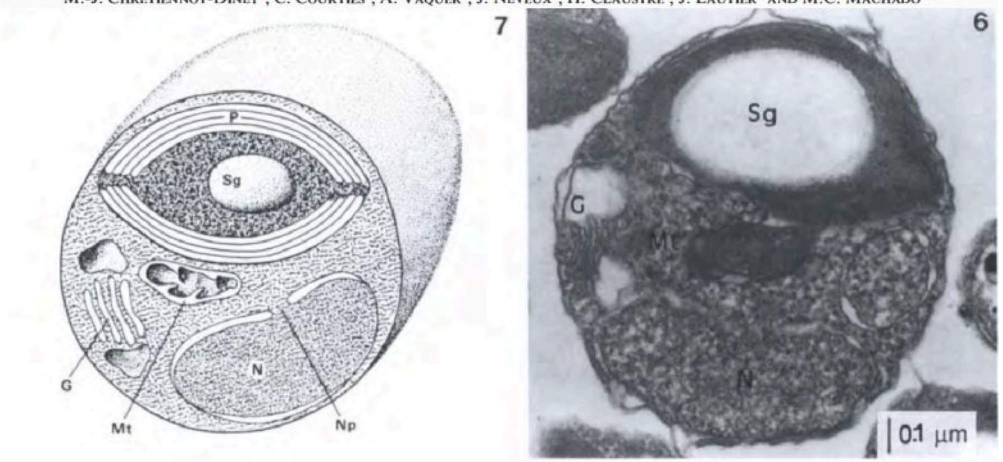


Ostreococcus

- Discovered in 1994
- Using flow cytometry
- 0.6 µm
- Several species
- Widespread except poles

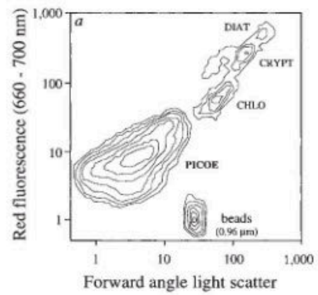
A new marine picoeucaryote: *Ostreococcus tauri* gen. et sp. nov. (Chlorophyta, Prasinophyceae)

M.-J. Chrétiennot-Dinet[1], C. Courties[2], A. Vaquer[2], J. Neveux[1], H. Claustre[3], J. Lautier[2] and M.C. Machado[1]*



Smallest eukaryotic organism

SIR — Autotrophic communities of picoplankton (cell size less than 2 µm diameter), known to be dominated by prokaryotes[1], are essential in the carbon cycle of estuaries[2] and oceans[1,3]. Hall and Vincent report that in other nutrient-rich ecosystems, the eukaryotic forms of the picoplankton "can play a major role in generating new production"[4]. That seems to be the case for the marine Mediterranean Thau lagoon (France, 43°24′ N–3°36′ E) where, using flow cytometry, we have discovered a photosynthetic picoeukaryote which is the main component of the phytoplankton. This picoplankter is classified as a green alga and named *Ostreococcus tauri* gen. et sp. nov. (C.C. and M.J.C.-D., manuscript submitted).



O. tauri, barely visible by epifluorescence microscopy, is detected by flow cytometry as shown in the figure (*a*), using low-forward-angle light scatter (related to cell size) and low red fluorescence (660–700 nm) due to their chlorophyll content. Electron microscopy reveals their extremely simple ultrastructure as detailed in *b*. The mean cell length and width are 0.97 ± 0.28 and 0.70 ± 0.17 µm, respectively, and the DNA content per cell is 33.31 ± 2.13 fg. *O. tauri* is thus the smallest eukaryote yet described.

Pigments are characterized by the presence of the three *a*-, *b*- and *c*-like chlorophylls, with a *b*/*a* ratio of 0.9 and *c*/*a* ratio near 0.1. The *c*-like chlorophyll was identified as the pigment Mg 3,8-divinylpheoporphyrin a_5 which is common to some photosynthetic eukaryotes (Prasinophyceae[5]) and prokaryotes (*Prochlorococcus marinus*[6]). Carotenoids are those of Chlorophyceae, with an unusually high violaxanthin content (3.05 fg per cell). *O. tauri*, counted bimonthly over one year, always appears as the main component of the phytoplankton in the lagoon. Cell abundances range between 10^7 and 2×10^8 cells per litre, which corresponds over the year to an average of 86 per cent of total phytoplankton cells. They are one or two orders of magnitude more abundant in Thau waters than picoeukaryote abundances reported by Hall and Vincent[4] in upwelling areas. The annual average chlorophyll *a* biomass related to *O. tauri* is 0.51 µg l^{-1}, which accounts for 28 per cent of the total biomass. In summer, when *O. tauri* is most abundant, carbon assimilation varies from 3.8 to 21.0 mg C mg(Chl*a*)$^{-1}$ h^{-1}. These rates are 1.3–5.4 times higher than those related to larger cells (over 2 µm diameter).

Such consistently high abundance and production raise several questions regarding the trophodynamic role of *O. tauri*. Why does this picoeukaryote species dominate the phytoplankton in the Thau

always more than 10 per cent of the incident radiation. Another hypothesis is related to the intensive oyster culture in the lagoon (25,000 tons produced per year). The oysters excrete large amounts of ammonium ion (1.04 µmol in the oyster beds and 0.35 µmol outside), which favours picoplankton over the larger size classes[9]. Also, oysters preferentially retain large cells[10] more than 2 µm in diameter, which could encourage the development of the cells smaller than 1 µm.

We are now examining how common this alga is, to discover whether these organisms represent a substantial but overlooked contribution to primary production in marine coastal waters.

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André Vaquer
Marc Troussellier
Jacques Lautier
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Hervé Claustre
Laboratoire de Physique et Chimie Marines, La Darse, BP 8, 06230 Villefranche-sur-Mer, France

1. Stockner, J. G. & Antia, N. J. *Can. J. Fish. Aquat. Sci.* **43**, 2472–2503 (1986).
2. Malone, T. C., Ducklow, H. W., Peele, E. R. & Pike, S. E. *Mar. Ecol. Prog. Ser.* **78**, 11–22 (1991).
3. Platt, T. & Li, W. K. W. (eds) *Photosynthetic Picoplankton (Ottawa) Can. Bull. Fish. Aquat. Sci.* **214** (1986).
4. Hall, J. A. & Vincent, W. F. *Mar. Biol.* **106**, 465–471 (1990).
5. Ricketts, T. R. *Phytochemistry* **5**, 223–229 (1966).
6. Goericke, R. & Repeta, D. J. *Limnol. Oceanogr.* **37**, 425–433 (1992).
7. Stockner, J. G. & Shortreed, K. S. *Revue ges. Hydrobiol.* **76**, 581–601 (1991).
8. Lawlor, D. W. in *Photosynthesis* (ed. Lawlor, D. W.) 31–51 (Longman, Harlow, 1993).
9. Chisholm, S. W. in *Primary Production and Biogeochemical Cycles in the Sea* (eds Falkowski, P. G. & Woodhead, A. D.) 213–237 (Plenum, New York, 1992).
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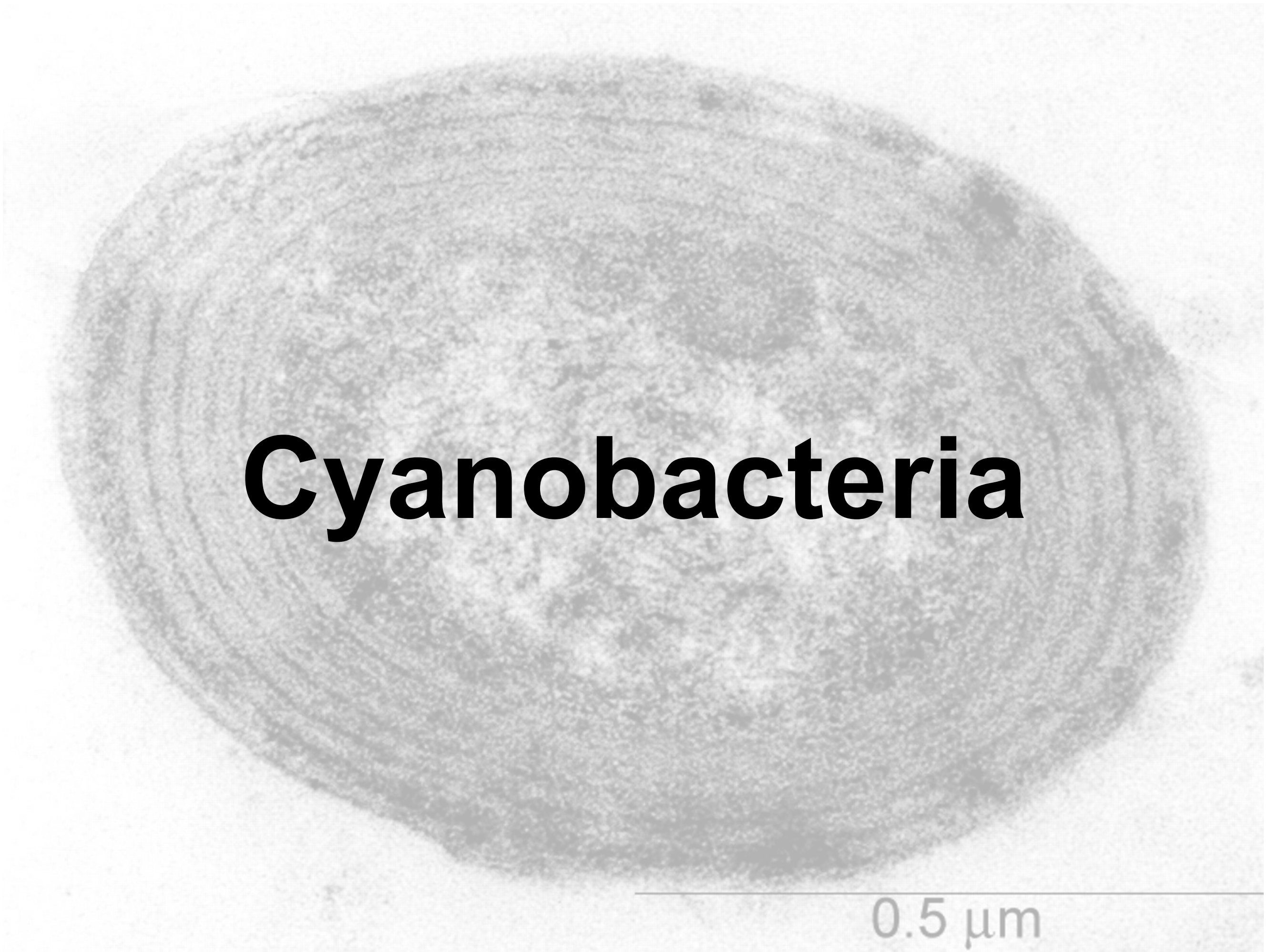
Genetic dyet?

Courties et al. 1994. Smallest eukaryotic organism. Nature. 370:255–255.

Cyanobacteria



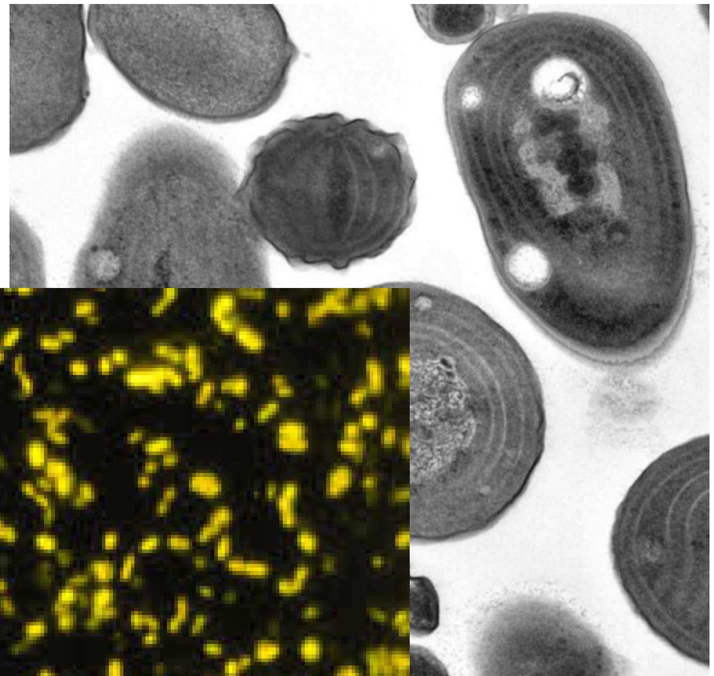
Synechococcus

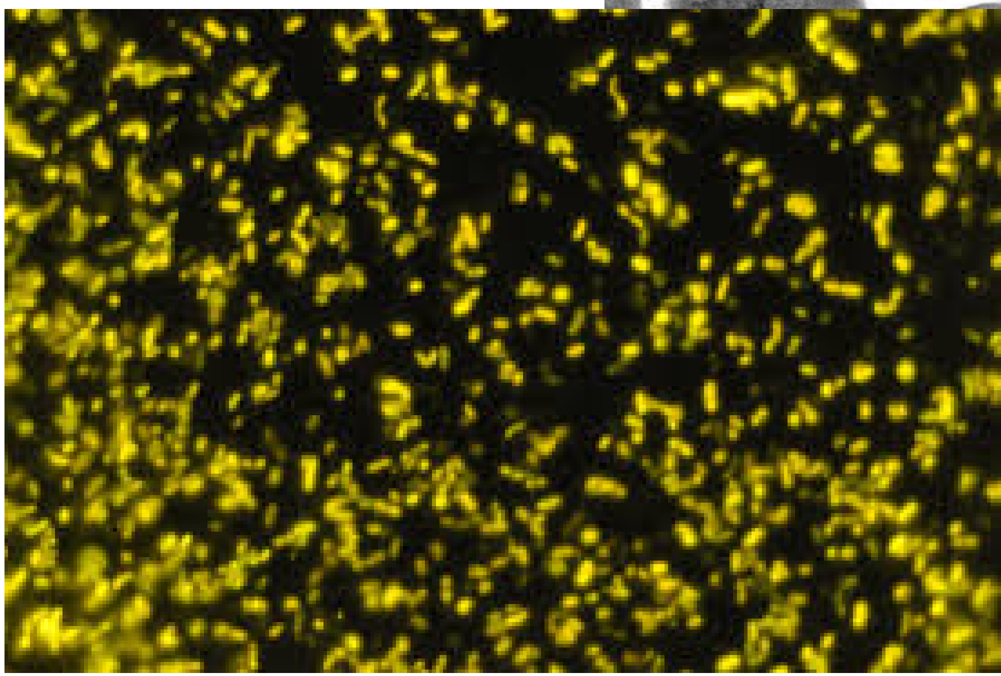
- Discovered in 1979
  - 1-2 µm
  - Main pigment phycoerythrin
  - Orange fluorescence

Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium

IN marked contrast to their freshwater counterparts, marine planktonic cyanobacteria are restricted to a few nostocalean genera, of which only *Trichodesmium* is capable of forming extensive water blooms[1–3]. We report here the widespread occurrence of a small, marine, chroococcalean cyanobacterium belonging to the genus *Synechococcus*.

Natural water samples were filtered through 0.2 µm Nuclepore filters, counterstained with Irgalan black[4]. The filters were examined with a Zeiss Standard microscope equipped with Neofluar objectives and an epifluorescent illumination system containing a 100-W halogen lamp, a BP 450–500 excitation filter, a LP 528 barrier filter and a FT 510 chromatic beam splitter. Using this system, phycoerythrin-containing cyano-





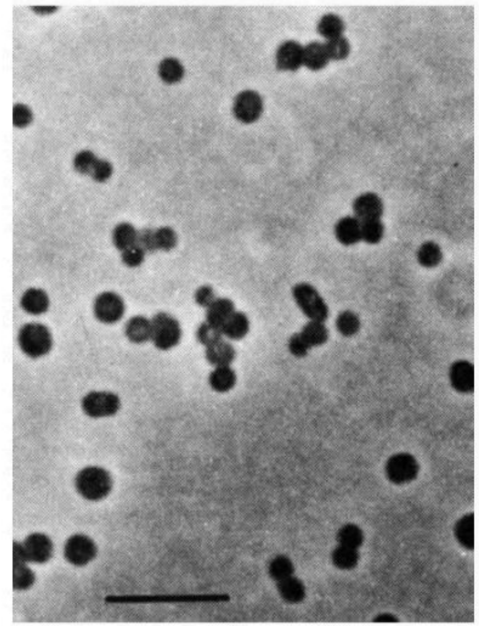


Fig. 1 Phase contrast photomicrograph of *Synechococcus* sp. (strain Syn-48) illustrating general cell morphology (scale bar, 5.0 µm).

Waterbury et al. 1979. Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature. 277:293–4.

Prochlorococcus

- Discovered in 1987
- Using flow cytometry

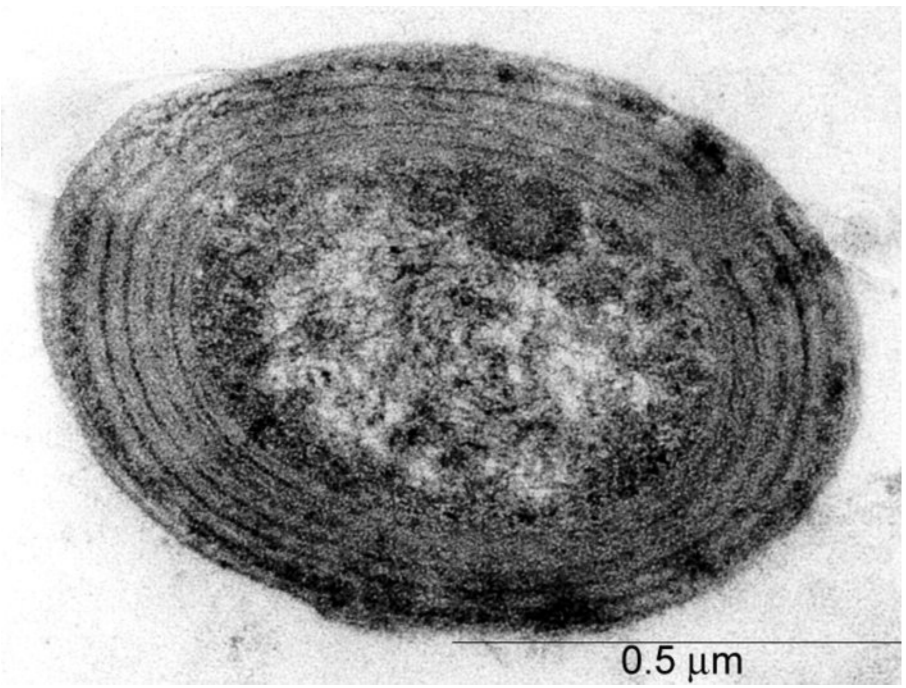
- 0.5 µm
- Do not contain phycoerythrin
- Chlorophyll *b*

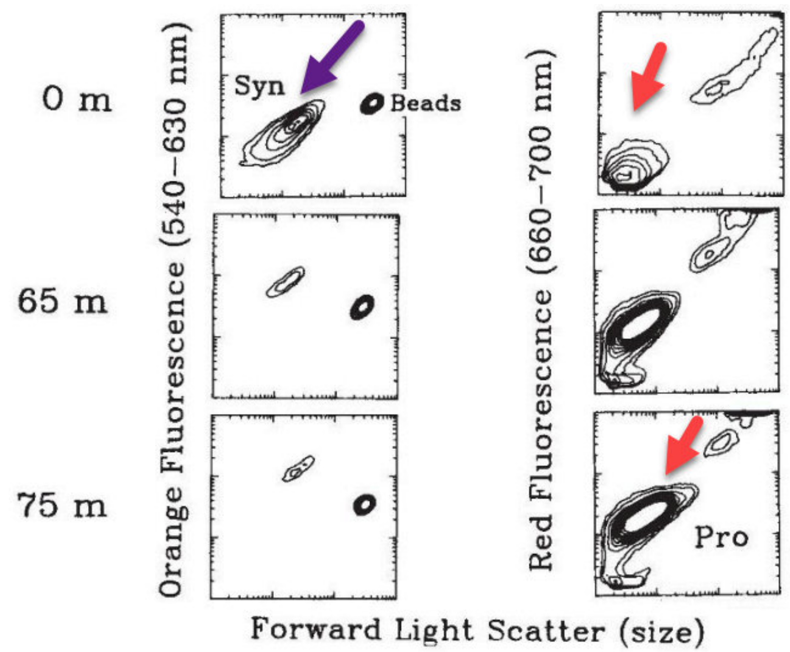
A novel free-living prochlorophyte abundant in the oceanic euphotic zone

Sallie W. Chisholm, Robert J. Olson*, Erik R. Zettler*, Ralf Goericke†, John B. Waterbury* & Nicholas A. Welschmeyer†

48-425 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
* Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
† Harvard University, Cambridge, Massachusetts 02138, USA

The recent discovery of photosynthetic picoplankton has changed our understanding of marine food webs[1]. Both prokaryotic[2,3] and eukaryotic[4,5] species occur in most of the world's oceans and account for a significant proportion of global productivity[6]. Using shipboard flow cytometry, we have identified a new group of pico-

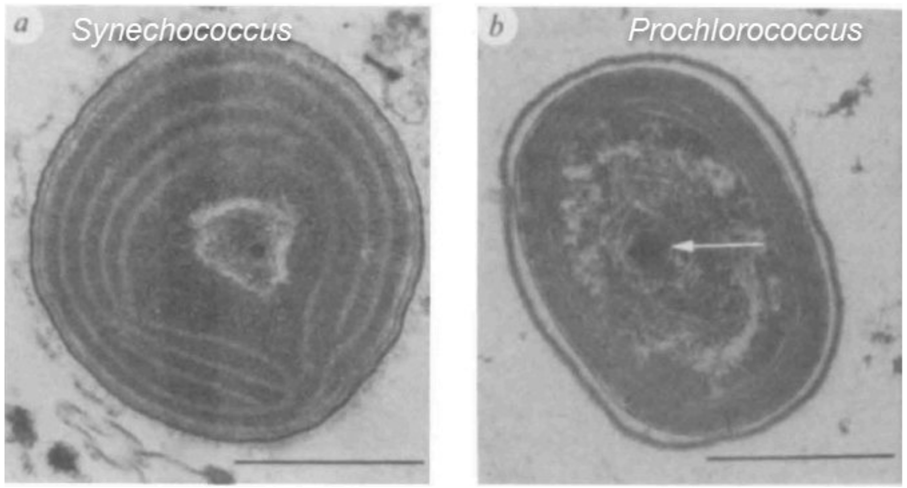


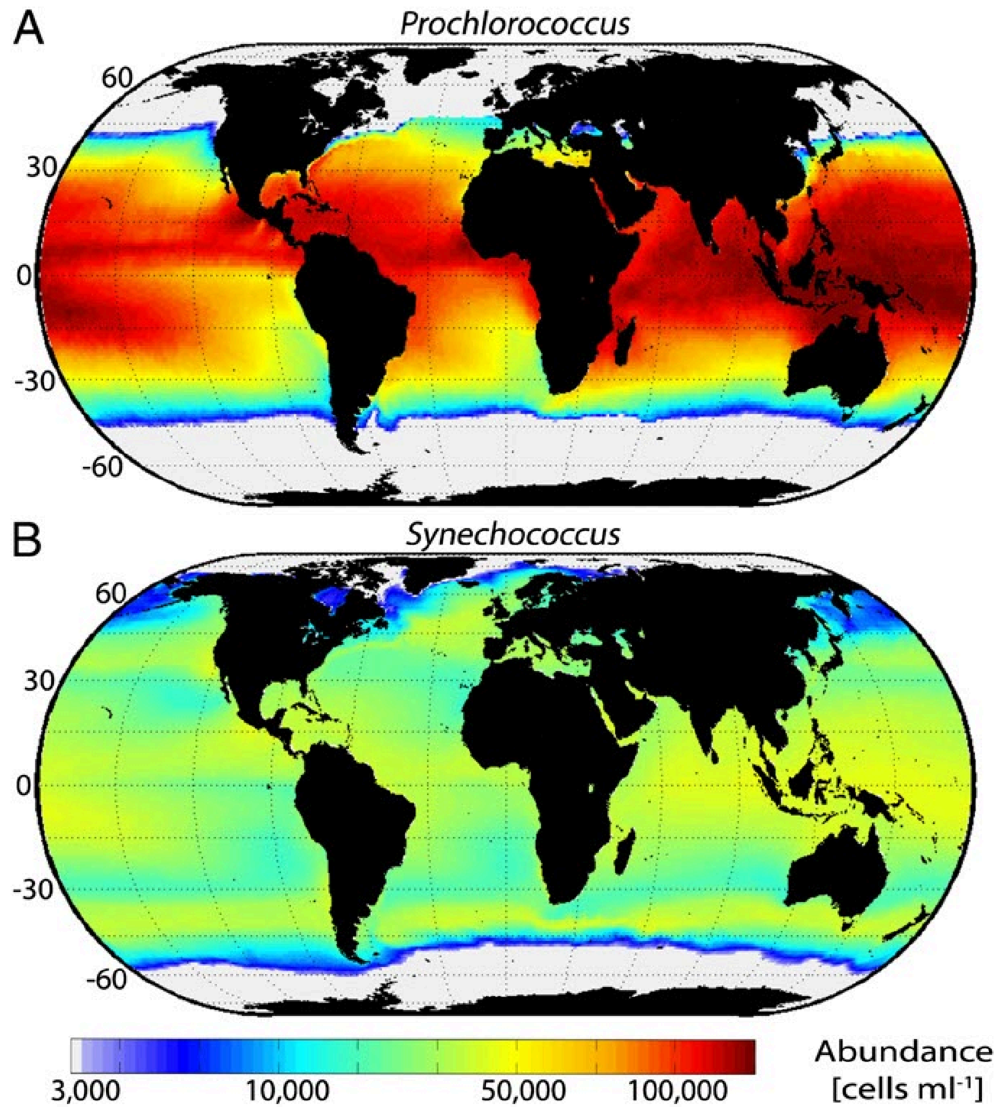


Chisholm et al. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature. 334:340–3

Prochlorococcus vs *Synechococcus*

- *Prochlorococcus* restricted to tropics
- *Synechococcus* everywhere except polar regions

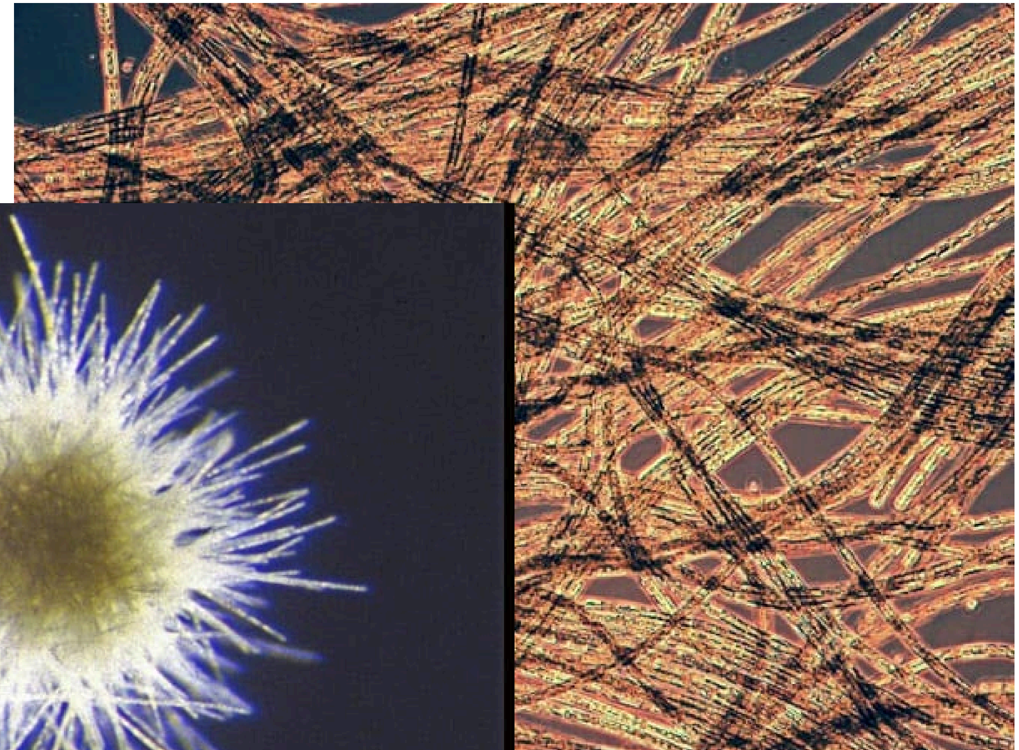


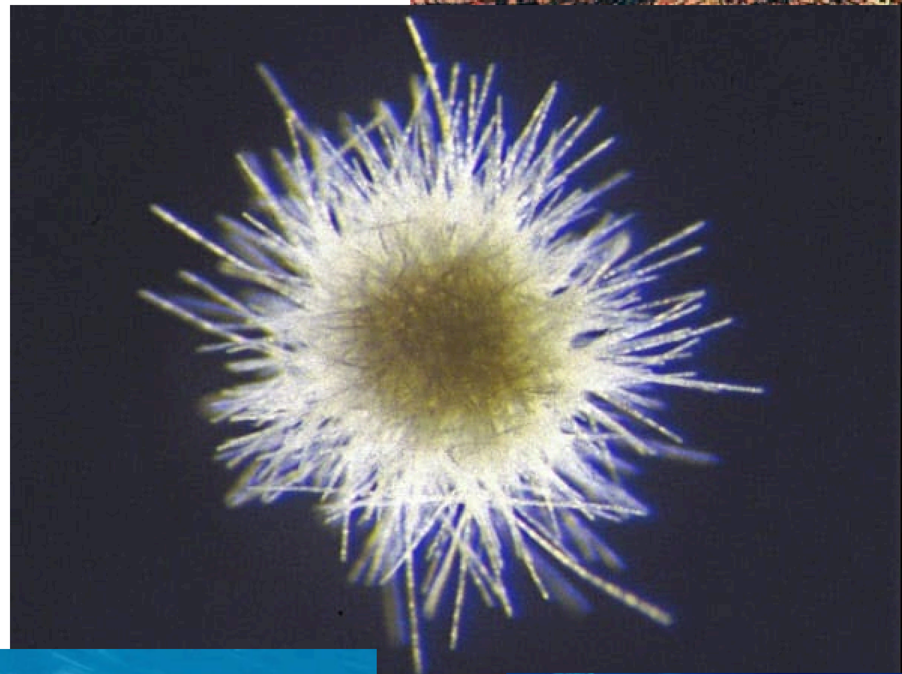


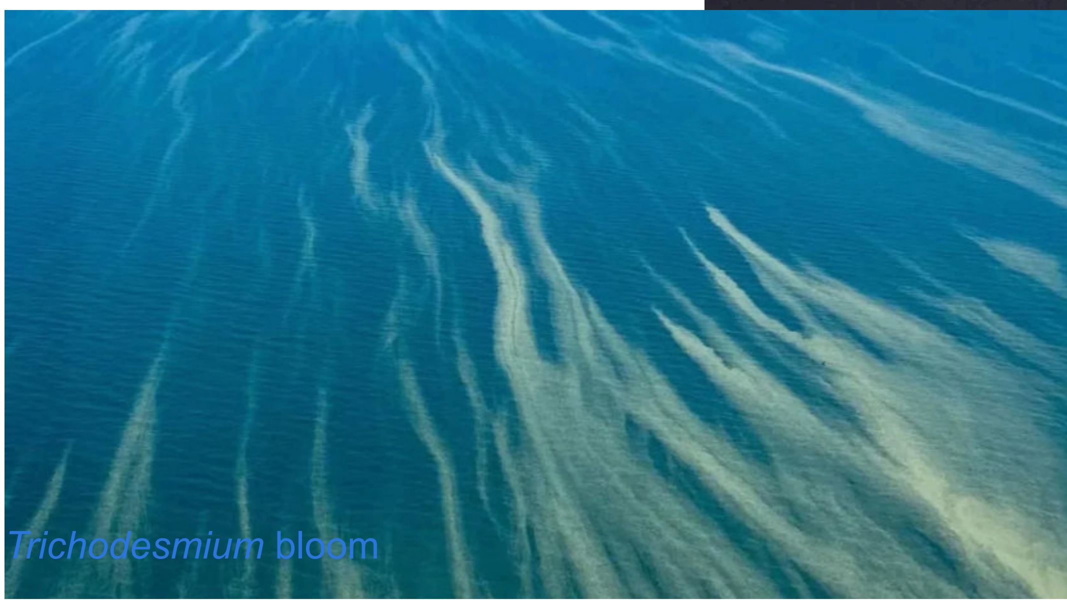
Flombaum et al. 2013. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. PNAS USA 110:9824–9.

Trichodesmium

- Nitrogen fixing
- Filamentous
  - Forms “colonies”
  - Can form blooms visible from space







Trichodesmium bloom

Take home messages

- Phytoplankton is not monophyletic
- Many groups have both autotrophic and mixotrophic/heterotrophic species
- Recent methods in particular metabarcoding are very useful to map species distribution
- However cultivation and traditional taxonomy remain of critical importance

Questions ?