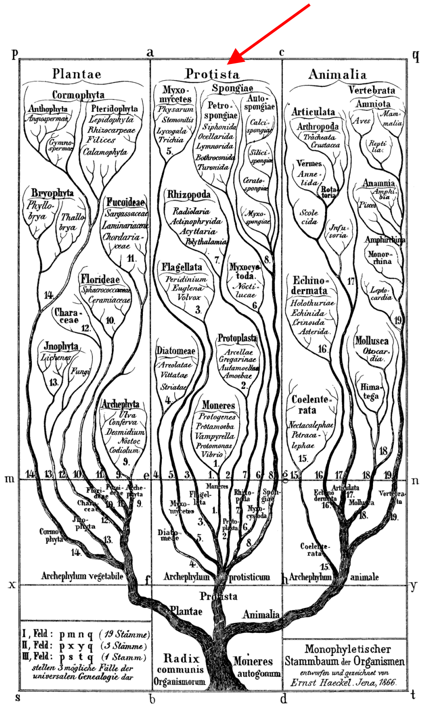
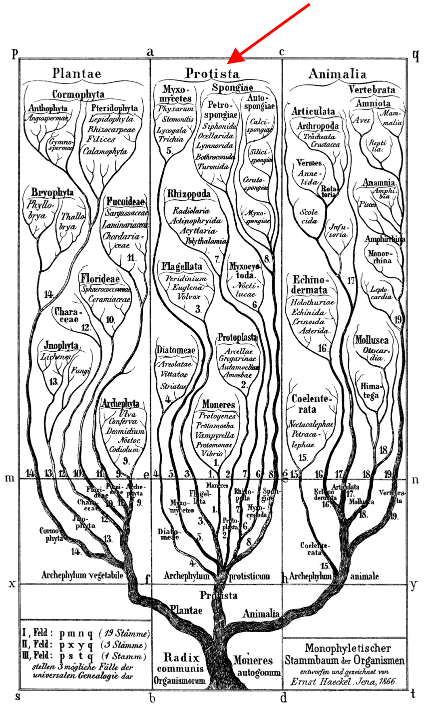
„secondary“ endosymbiosis
algae with rhodophyte-derived plastids

Eukaryotic tree of life

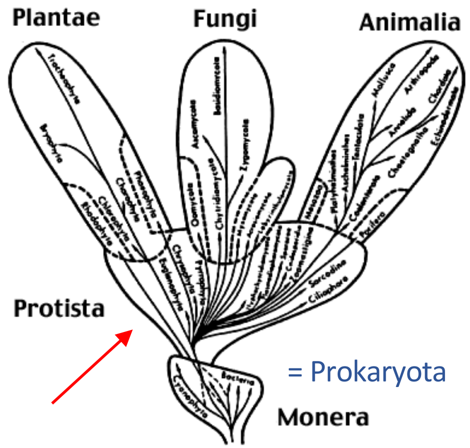


Haeckel (1866)

Eukaryotic tree of life

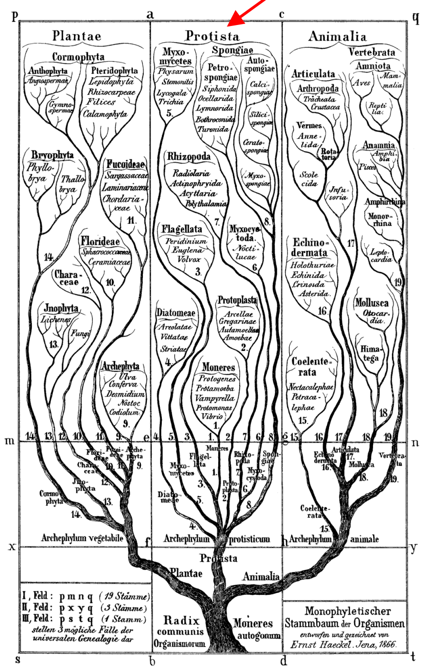


Haeckel (1866)

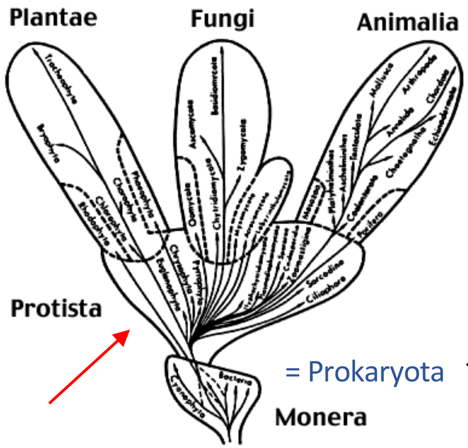


Whitaker (1969)

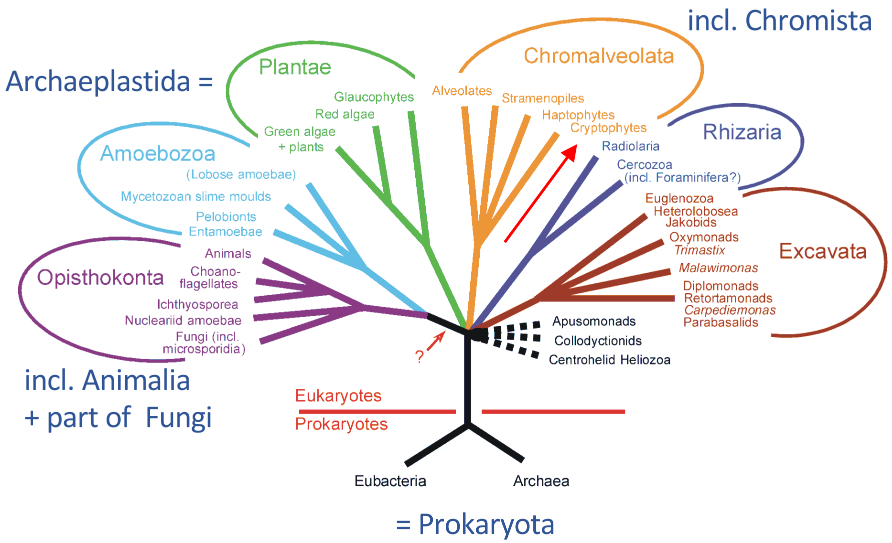
Eukaryotic tree of life



Haeckel (1866)



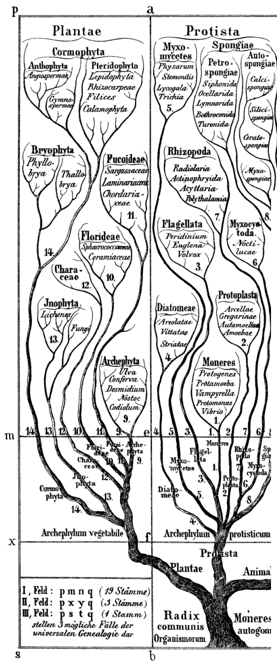
Whitaker (1969)



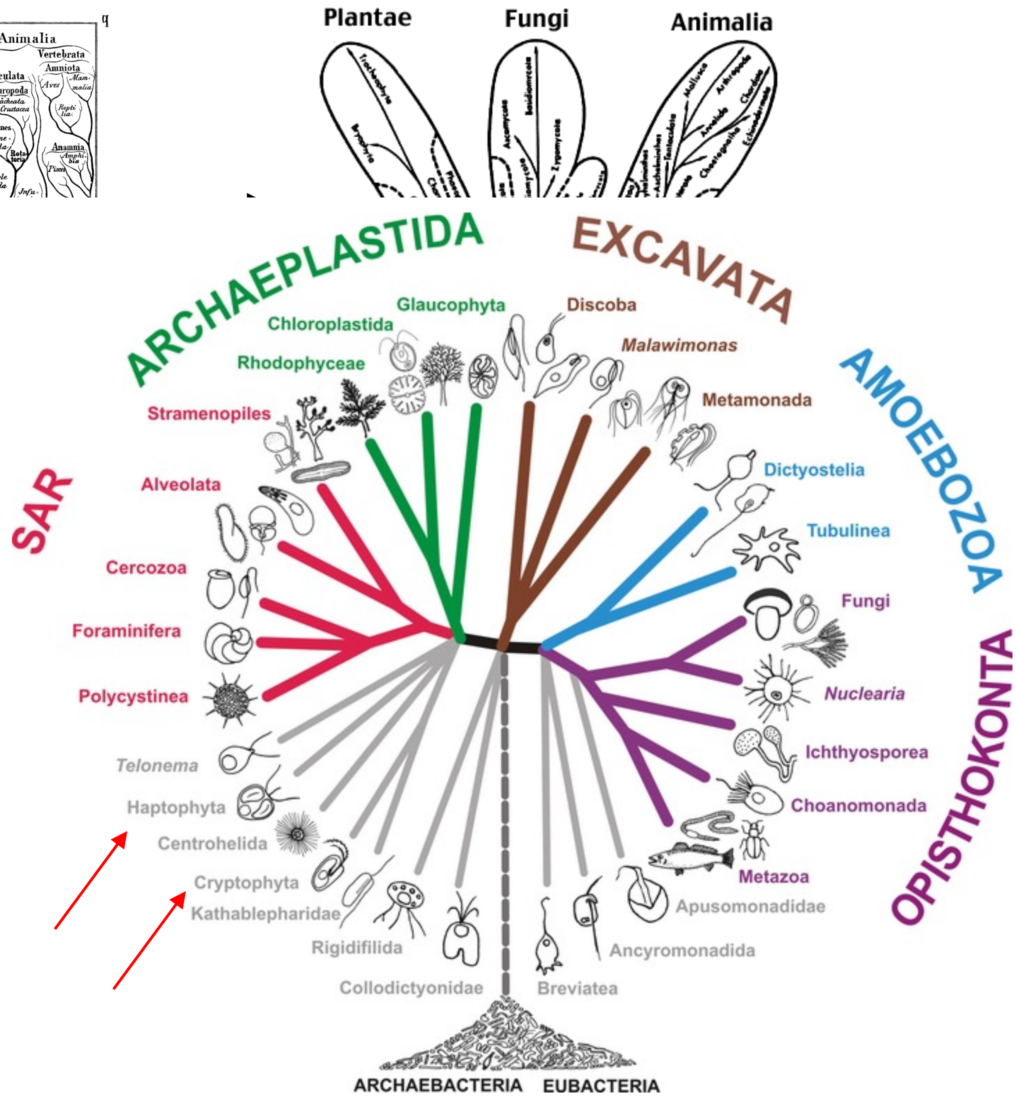
Simpson & Roger (2004)

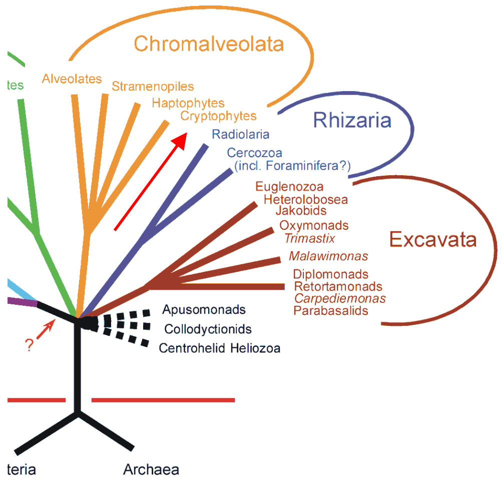
Classification: kingdoms

Eukaryotic tree of life



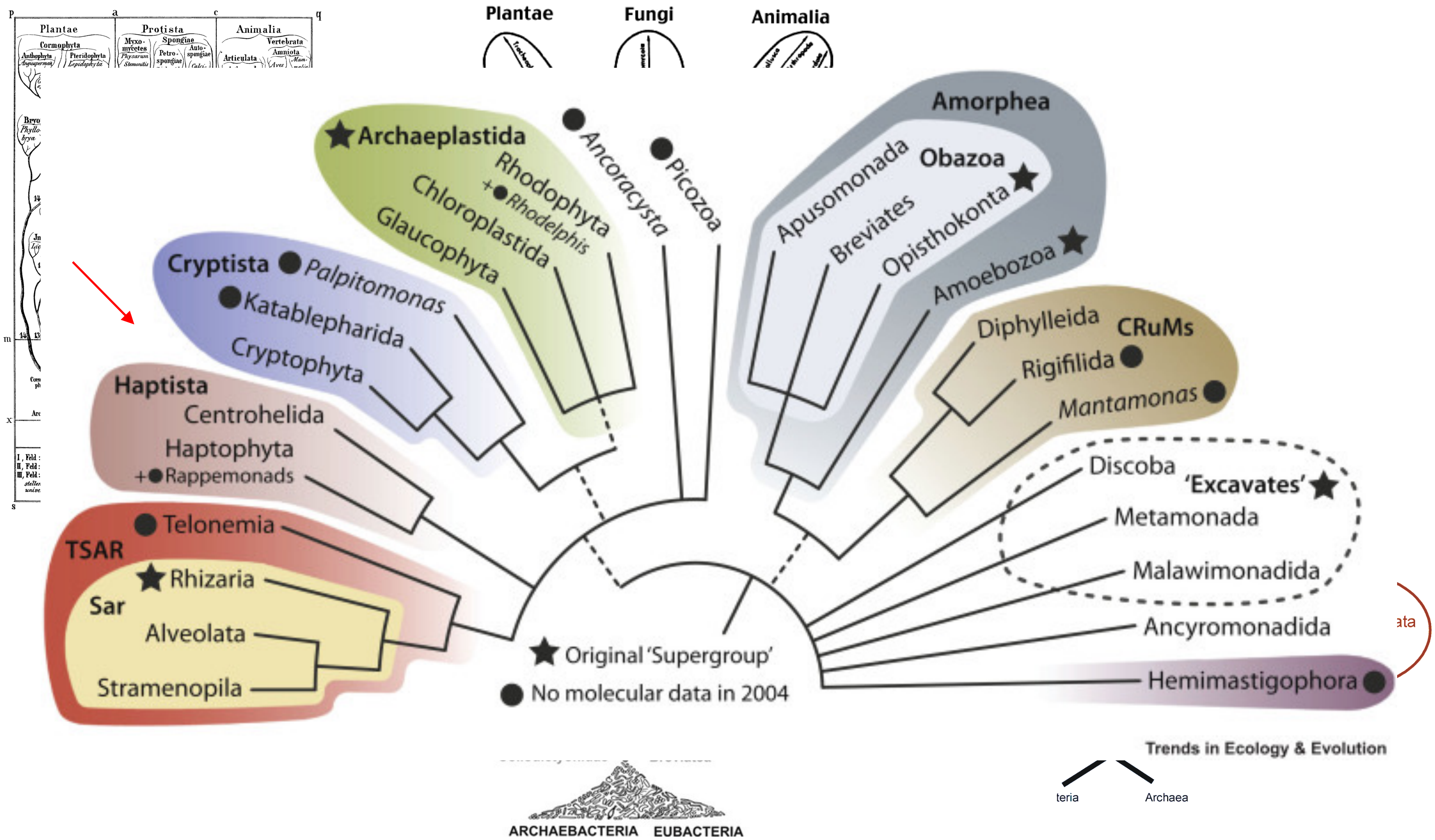
Haeckel (18





Adl et al. (2012)

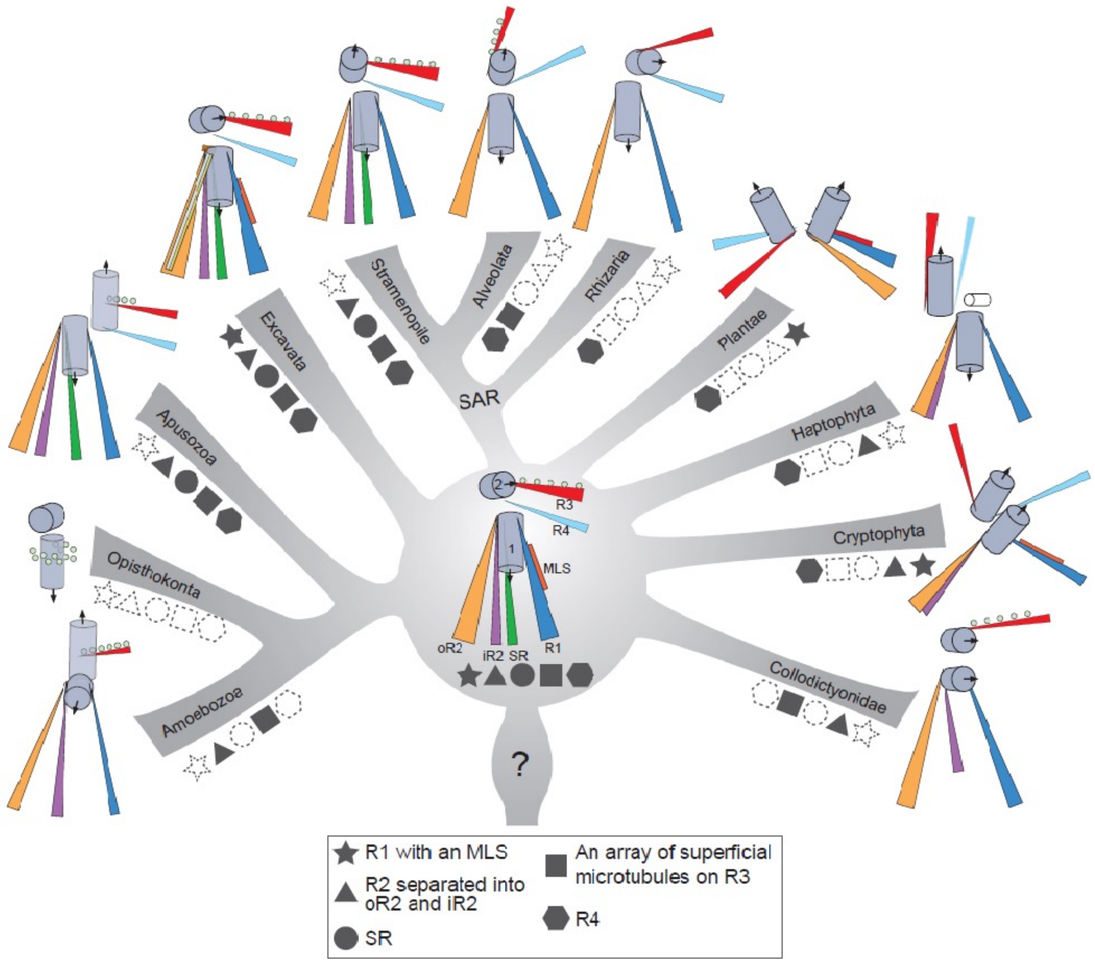
Eukaryotic tree of life



Burki et al. (2020)

Eukaryotic tree of life

- inferred ancestral traits for the flagellar apparatus
  (variations in the structure of cytoskeletal system (MTOCs) reflect phylogenetic relationships)
- LECA (last Eukaryotic common ancestor) = best represented by typical Excavate



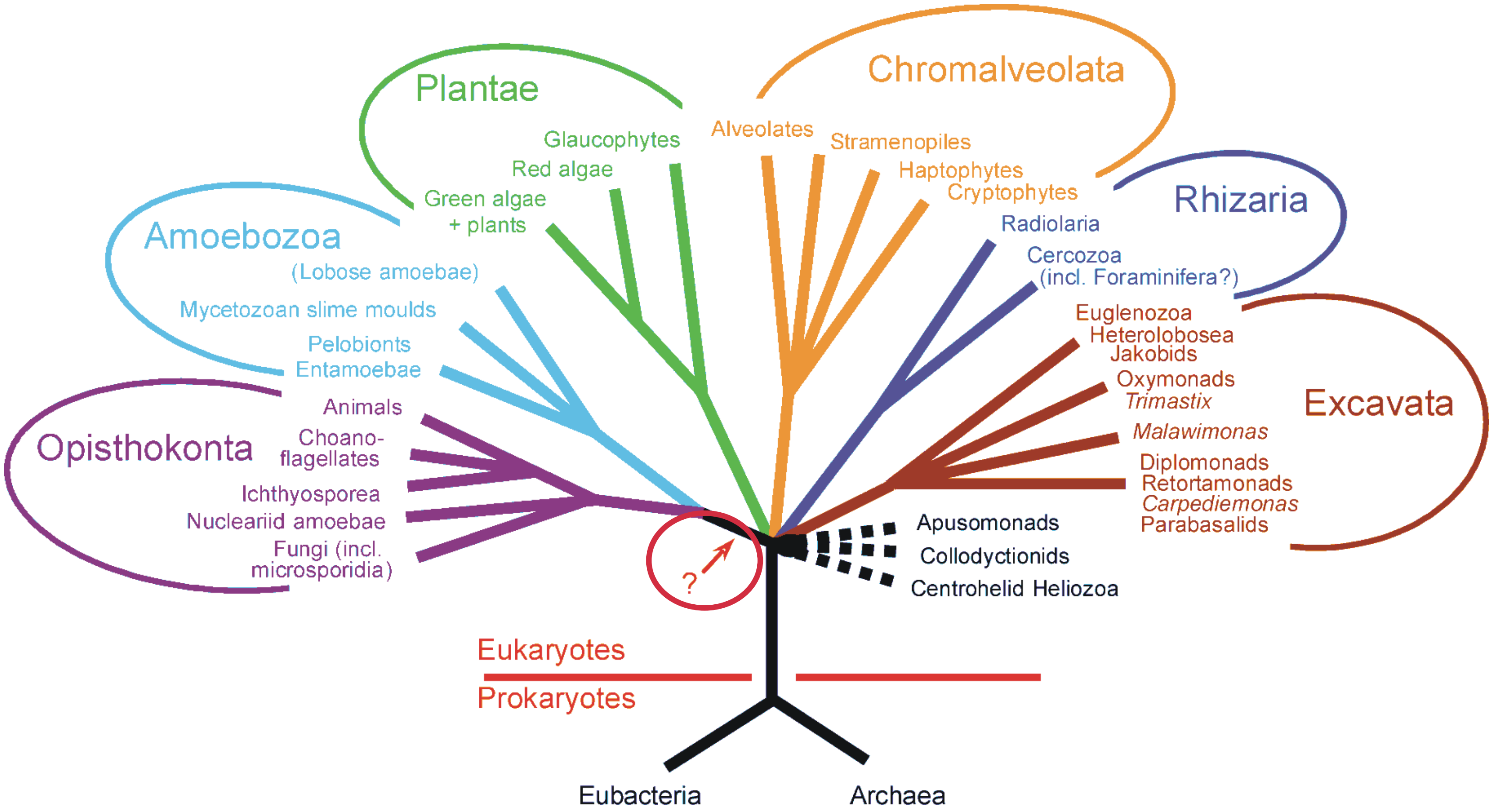
MTOC = microtubule organizing center, with few exceptions MTOCs consist of two basal bodies that anchor flagellar axonemes and different configurations of microtubular roots

MLS = multilayered structure
R1 = microtubule root 1
R2 = root involved with phagotrophy
R3 = superficial microtubules
SR = a singlet root (feeding groove)
R4 = microtubule root 4

solid lines = presence of trait
dashed outlines = loss of trait

Yukubi & Leander (2013), *Plant Journal*

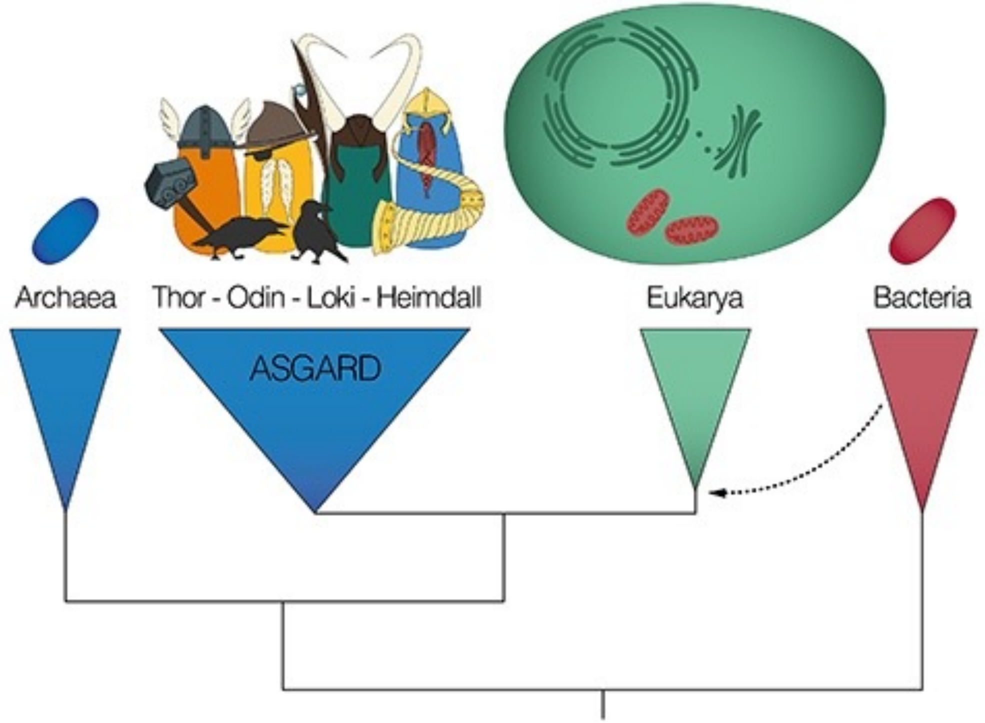
Eukaryotic tree of life



Simpson & Roger (2004)

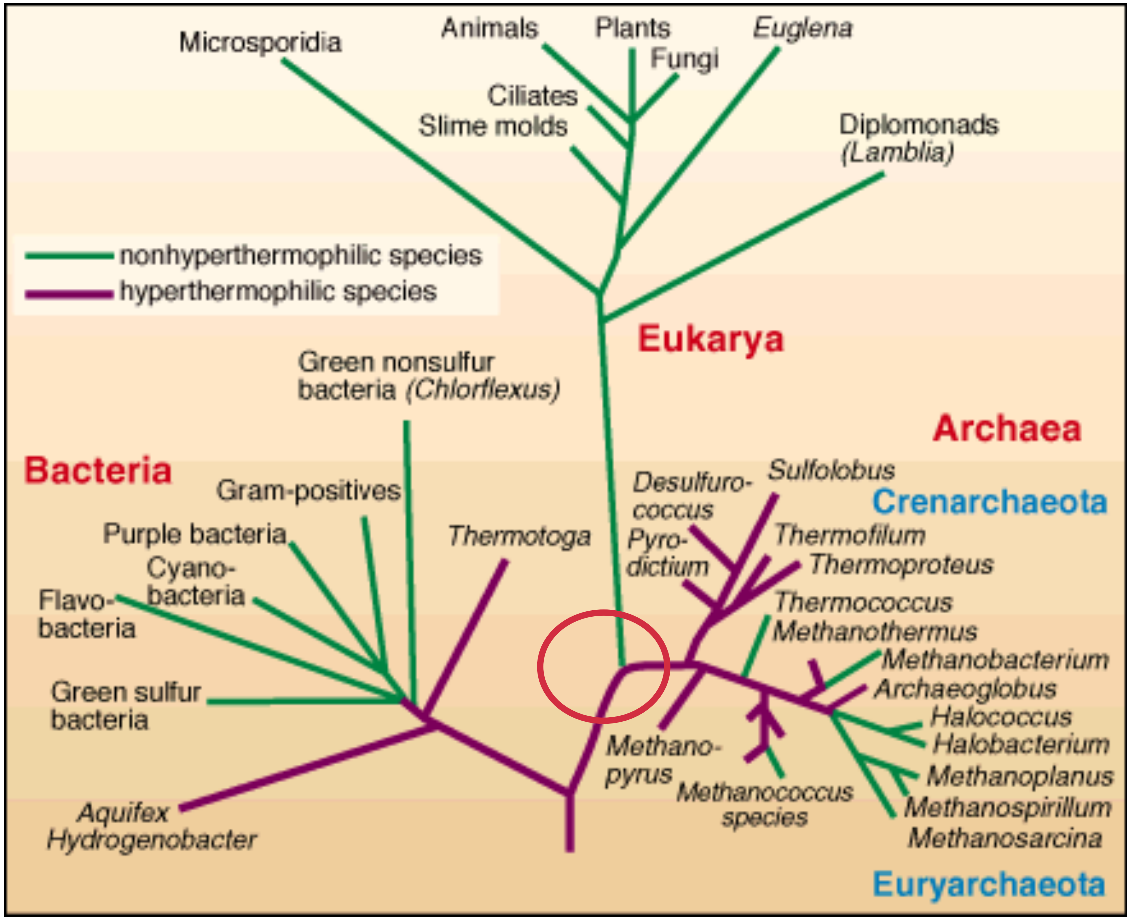
Eukaryotic tree of life

- Eukaryota nested in Archaea
- the Asgard superfylum: the closest prokaryotic relatives of eukaryotes
- their genomes are enriched for proteins formerly considered specific to eukaryotes -> the archaeal host cell already contained many key components that govern eukaryotic cellular complexity



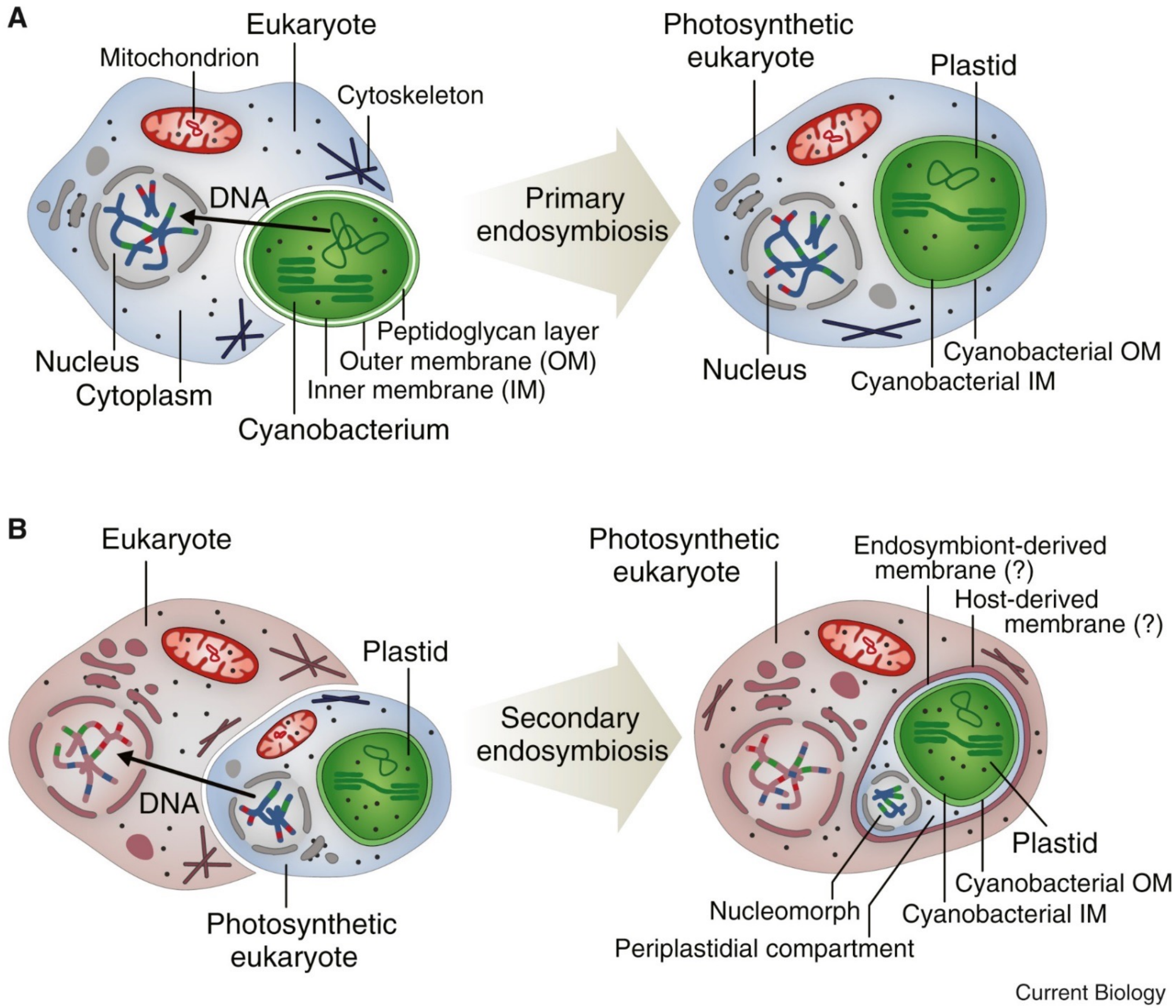
Zaremba-Niedzwiedzka et al. (2017), *Nature*

Eukaryotic tree of life



Woese & Fox (1977), *PNAS*

Endosymbioses



Primary and secondary endosymbiosis

Archibald (2015), *Current Biology Review*

Endosymbioses

Lineage	Belong to[a]	Plastid Membranes[b]	Putative Plastid Origin[c]
Glaucophytes	Archaeplastida	2 (with peptidoglycan)	Cyanobacteria
Red algae	Archaeplastida	2	Cyanobacteria
Green algae	Archaeplastida	2	Cyanobacteria
Paulinella chromatophora	Rhizaria	2 (with peptidoglycan)	Cyanobacteria
Chlorarachniophytes	Rhizaria	4	Green algae
Euglenids	Excavates	3	Green algae
Cryptophytes	Cryptista	4 (with ER connection)[d]	Red algae
Haptophytes	Haptista	4 (with ER connection)[d]	Red algae
Ochrophytes	Stramenopiles	4 (with ER connection)[d]	Red algae
Myzozoans	Alveolates	2–5	Red algae

[a]Main assemblage (no specific taxonomic rank) containing the plastid lineage.
[b]Number of membranes dividing the host cytosol from the plastid stroma.
[c]All plastids ultimately trace back to cyanobacteria, so this column only refers to the direct source of plastids in the lineage under consideration.
[d]The plastid outermost membrane is continuous with the ER in these three lineages.

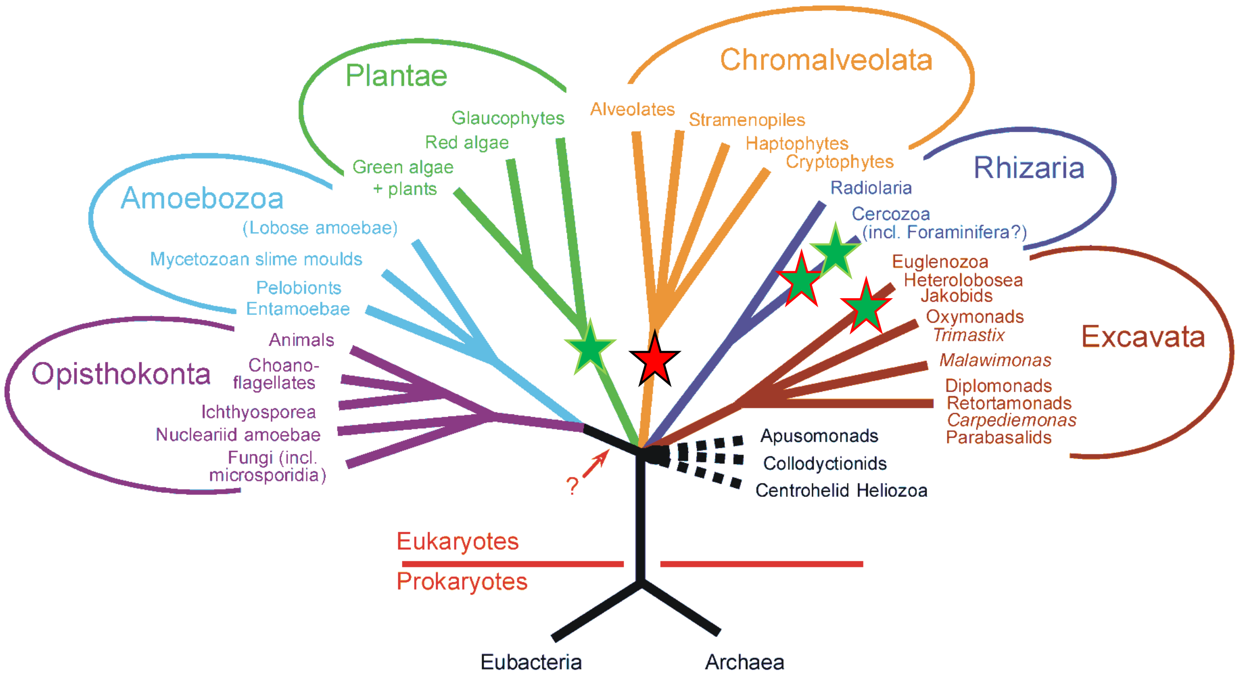
primary endosymbiosis

higher order endosymbioses

Burki (2017), *Advances in Botanical Research*

Endosymbioses

The Chromalveolate hypothesis (Cavalier-Smith 1999)



primary (cyanobacteria)

- Plantae (=Archaeplastida)
- *Paulinella chromatophora*

secondary (green alga)

- photosynthetic Euglenids
- Chlorarachniophytes

secondary (red alga)

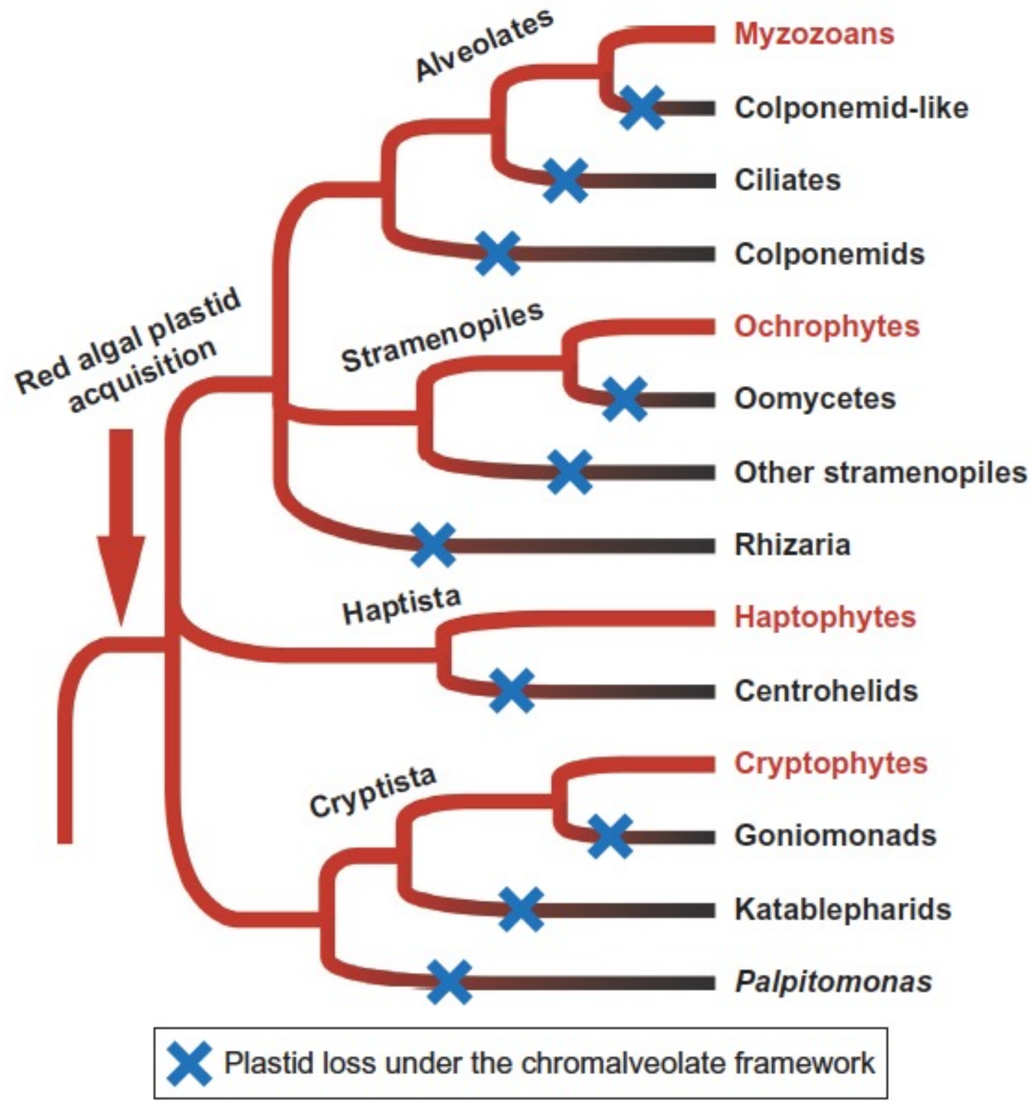
- ochrophytes
- photosynthetic dinoflagellates
- haptophytes
- cryptophytes

Simpson & Roger (2004)

Endosymbioses

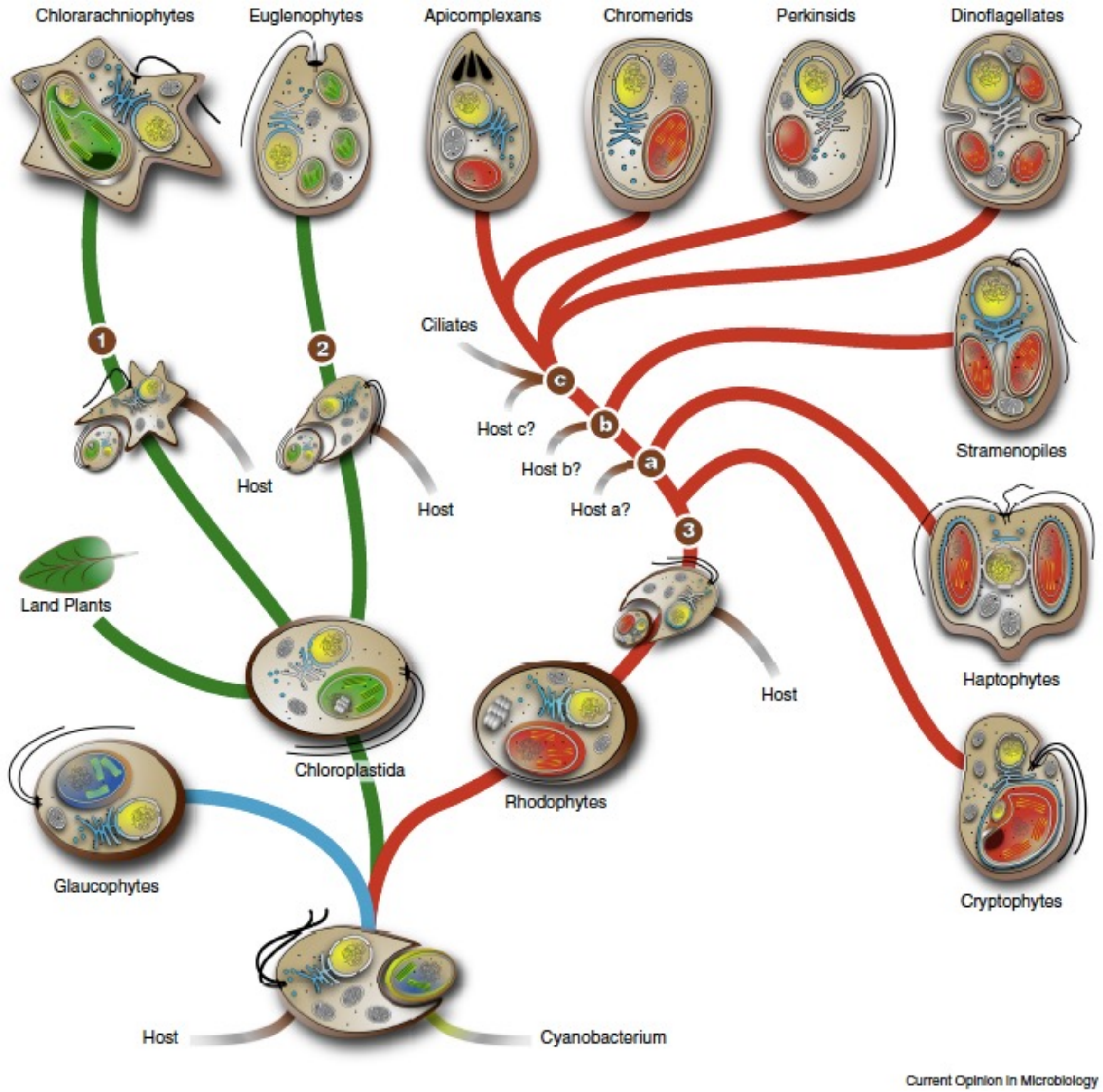
Hypothetical tree best fitting **chromalveolate hypothesis**

- the minimum count of plastid losses required is 10 (but in reality much more)
- an alternative better fitting model is needed



Burki (2017), *Advances in Botanical Research*

Endosymbioses

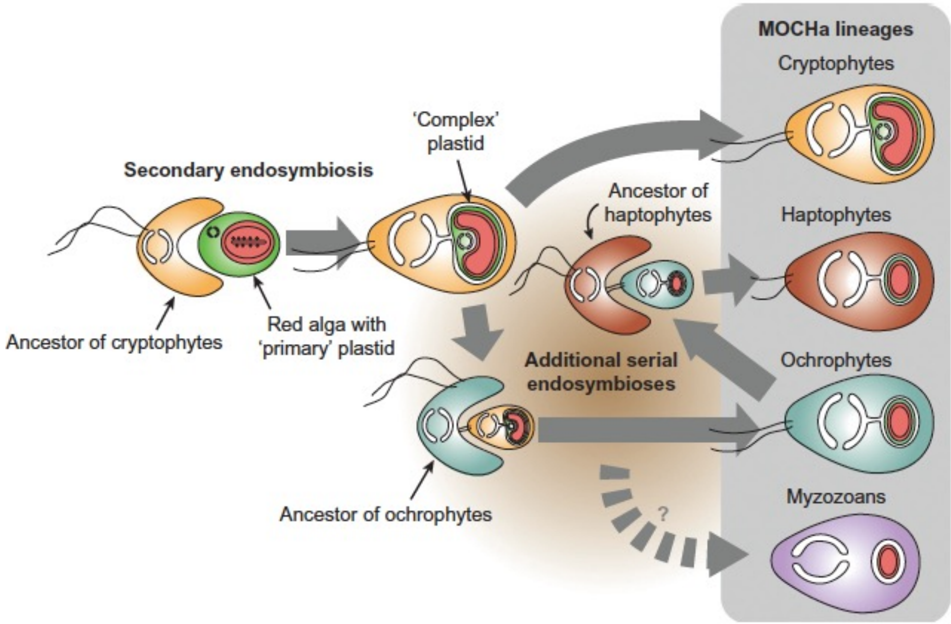


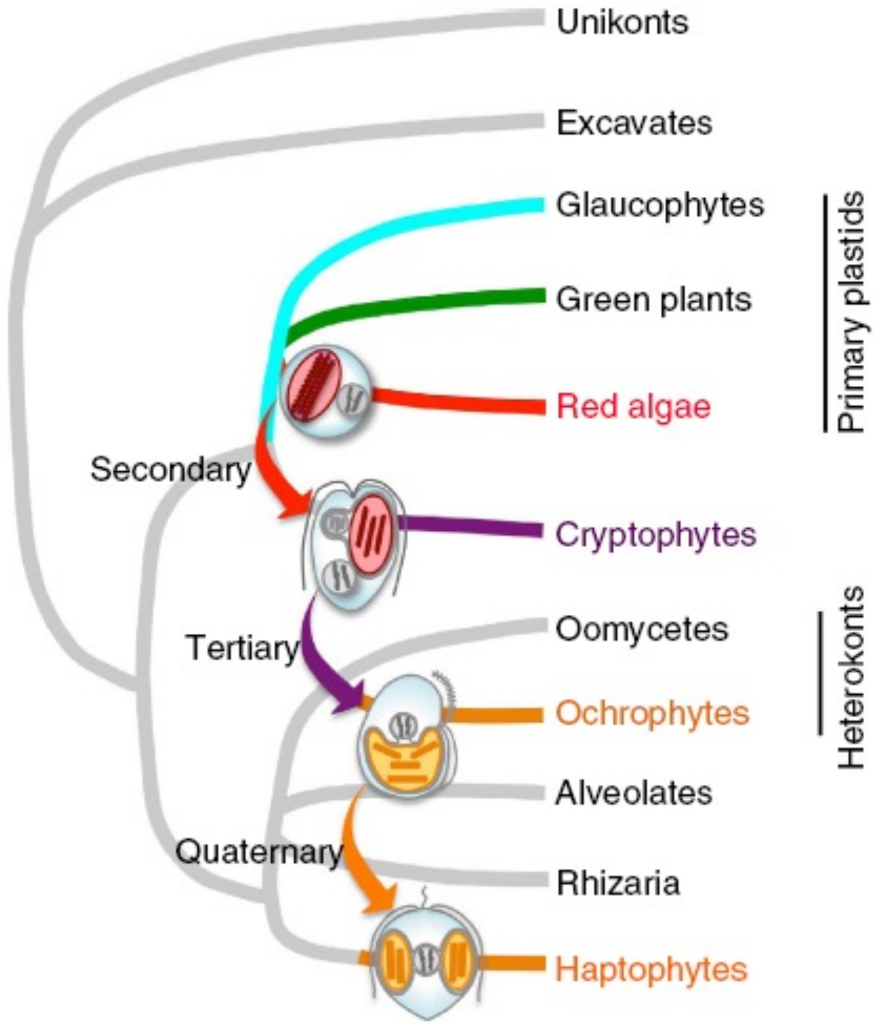
Complex endosymbiosis

Zimorski et al. (2014), *Current Opinion in Microbiol.*

Endosymbioses

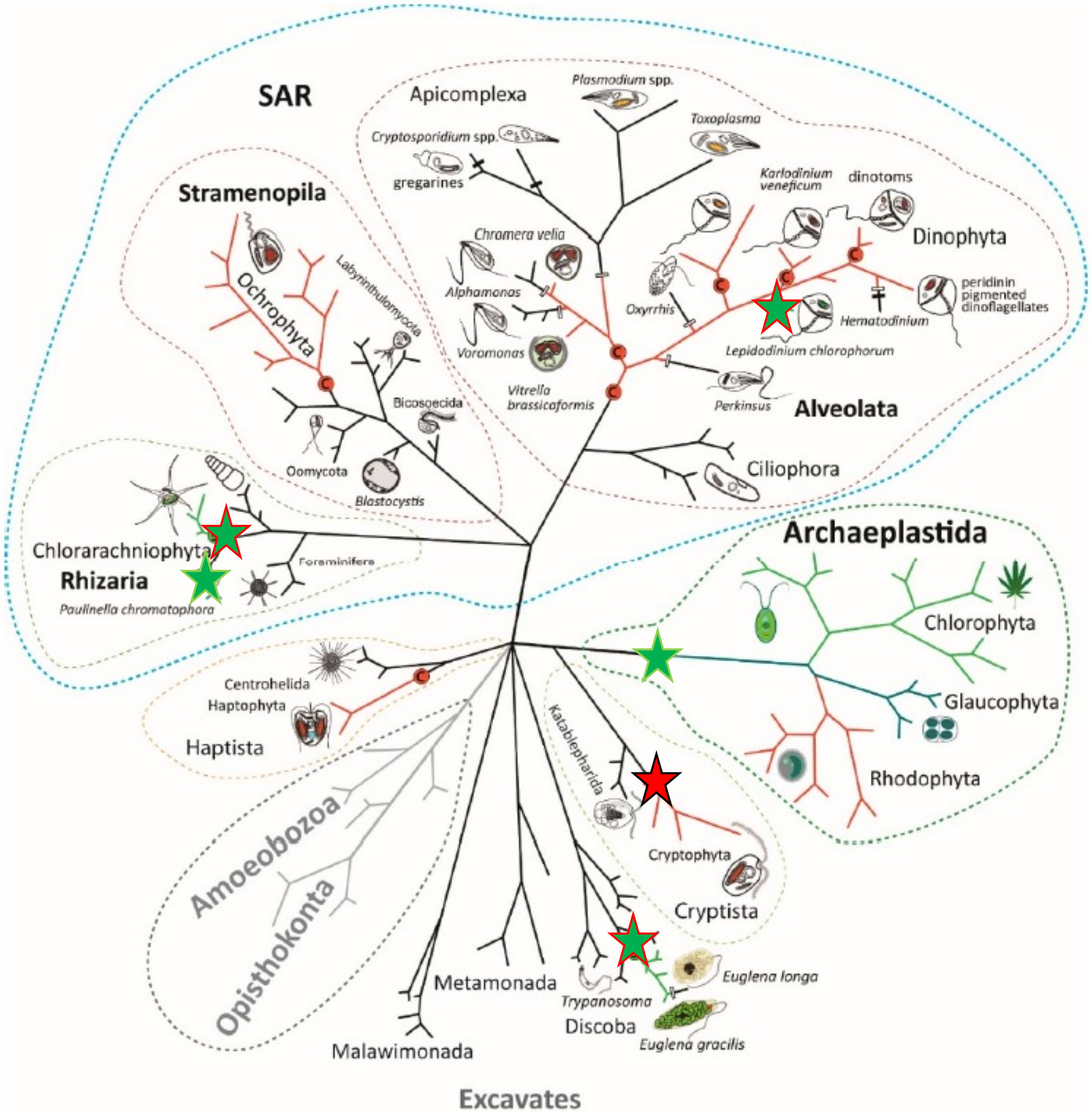
Serial eukaryote-to-eukaryote endosymbiosis

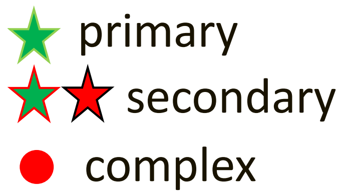




Burki (2017); Stiller et al. (2014), *Nature Communication*

Endosymbioses





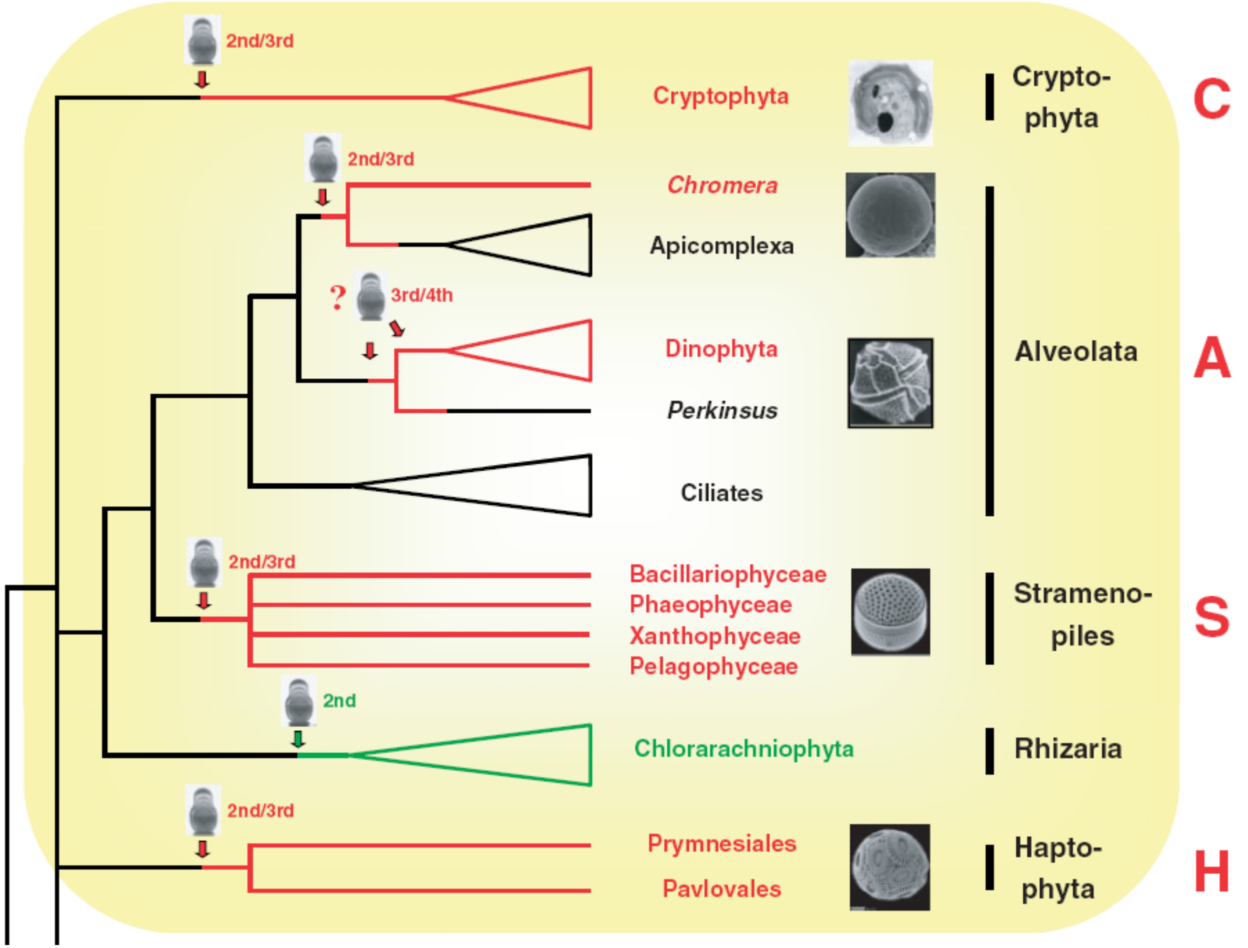
Complex endosymbiosis.

Oborník (2019), *Biomolecules*

Endosymbioses

The rhodoplex hypothesis

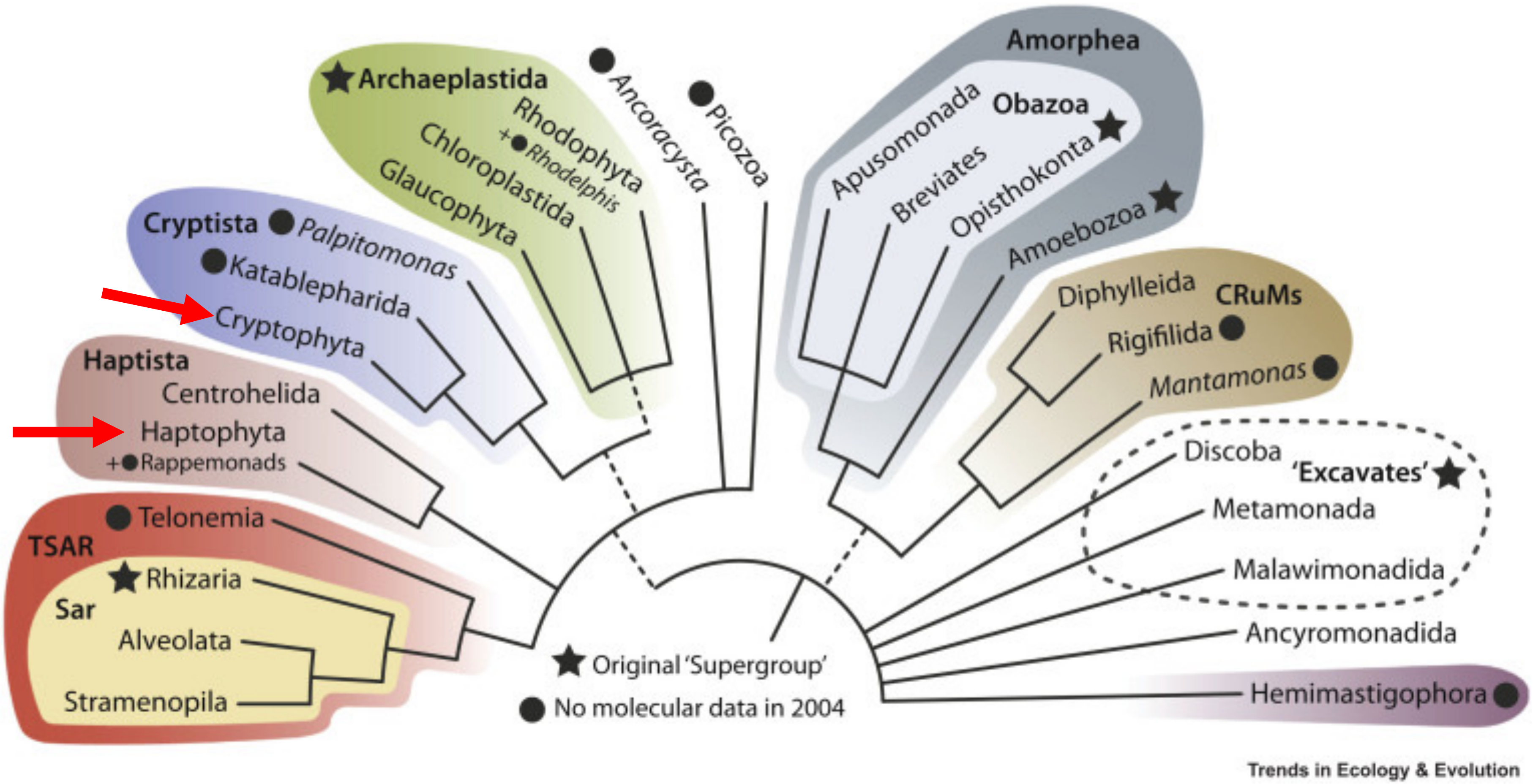
- the complex plastids from CASH originate from a single secondary endosymbiosis with a rhodophyte
- subsequent eukaryote-to-eukaryote endosymbioses are needed to explain the incongruence observed between plastid-encoded and nuclear-encoded markers



CASH = Cryptophytes, Alveolates, Stramenopiles, and Haptophytes; = complex algae with red plastids; = evolutionary independent groups, i.e. not chromalveolates

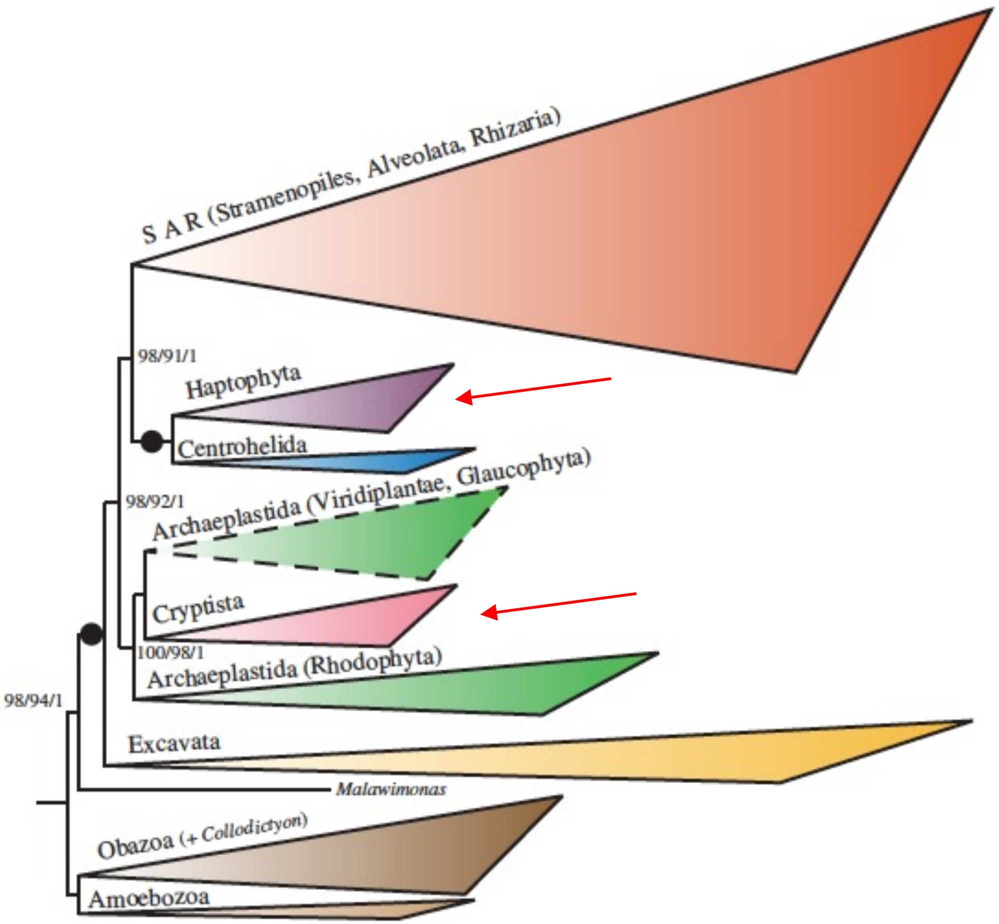
Petersen et al. (2014), *Genom. Biol. Evol.*

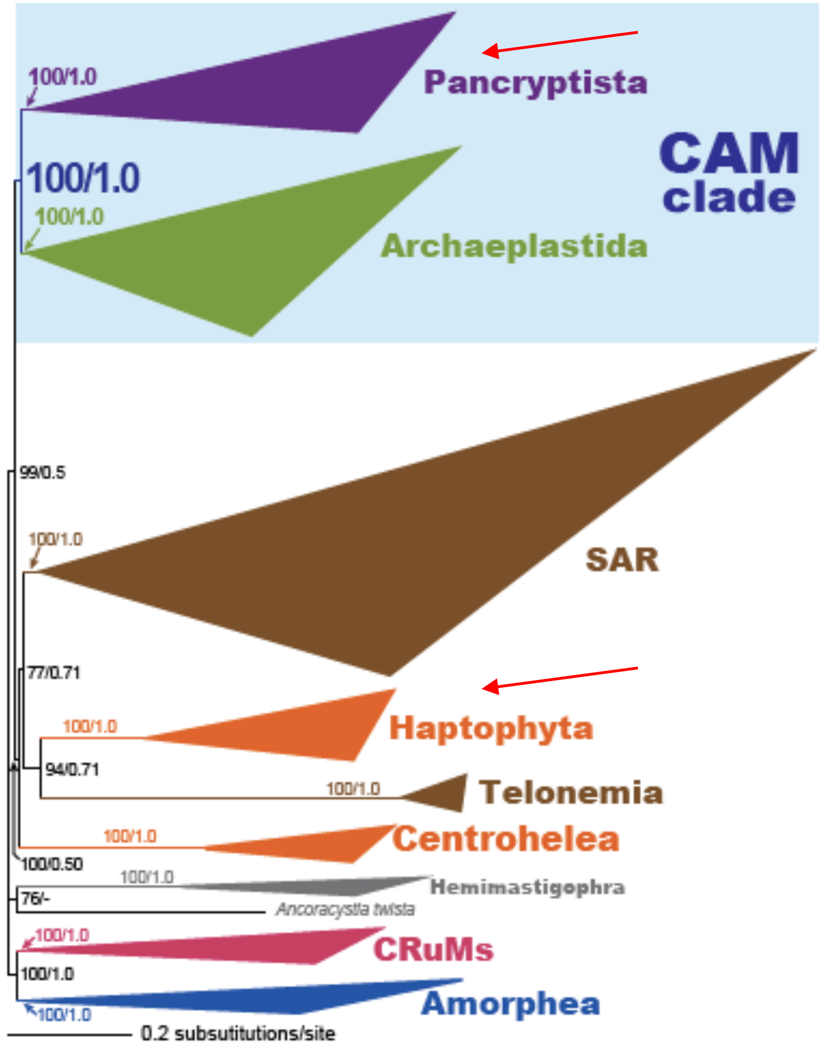
Haptophyta and Cryptophyta



Burki et al. (2020), *Trends in Ecology end Evolution*

Haptophyta and Cryptophyta





Burki et al. (2015); Yazaki et al. (in press)

Haptophyta and Cryptophyta

Algal group (chapter number)	*Photosynthetic pigments*[a]	*Chloroplast outer membranes*	*Thylakoid associations*	*Starch-like reserve*[b]	*External covering*[c]	*Flagella*
Cyanobacteria (3 & 4)	chl[a], PE, PC, APC	0	0	Cyanophycean	Pepitoglycan matrices or walls	0
Red algae (5)	chl[a], PE, PC, APC	2	0	Floridean	Walls with a galactose polymer matrix	0
Green algae (6–9)	chl[a, b]	2	2–6	True	Cellulosic walls, scales	0 – many
Euglenoid Algae (10)	chl[a, b]	3	3	Paramylon	Pellicle	1–2 emergent
Yellow–green and related algae (11)	chl[a, c]	4	3	Chrysolaminarin	Mostly cellulosic walls	2 unequal if present
Chrysophyte algae (12)	chl[a, c] fucoxanthin	4	3	Chrysolaminarin	None, scales, lorica	2 unequal
Haptophyte algae (13)	chl[a, c] fucoxanthin	4	3	Chrysolaminarin	Nonsiliceous scales	2 equal + haptonema
Synurophyte algae (14)	chl[a, c] fucoxanthin	4	3	Chrysolaminarin	Siliceous scales	2 unequal
Diatoms (15–19)	chl[a, c] fucoxanthin	4	4	Chrysolaminarin	Siliceous frustule	1, reproductive cells only
Dinoflagellates (20)	chl[a, c] peridinin	3	3	True	Theca	2 unequal
Cryptomonads (21)	chl[a, c] PC or PE	4	2	True	Periplast	2 equal
Brown algae (22)	chl[a, c] fucoxanthin	4	3	Laminarin	Walls with alginate matrices	2 unequal

Source: Various phycology textbooks (e.g., Sze, 1998; and Graham and Wilcox, 2000).

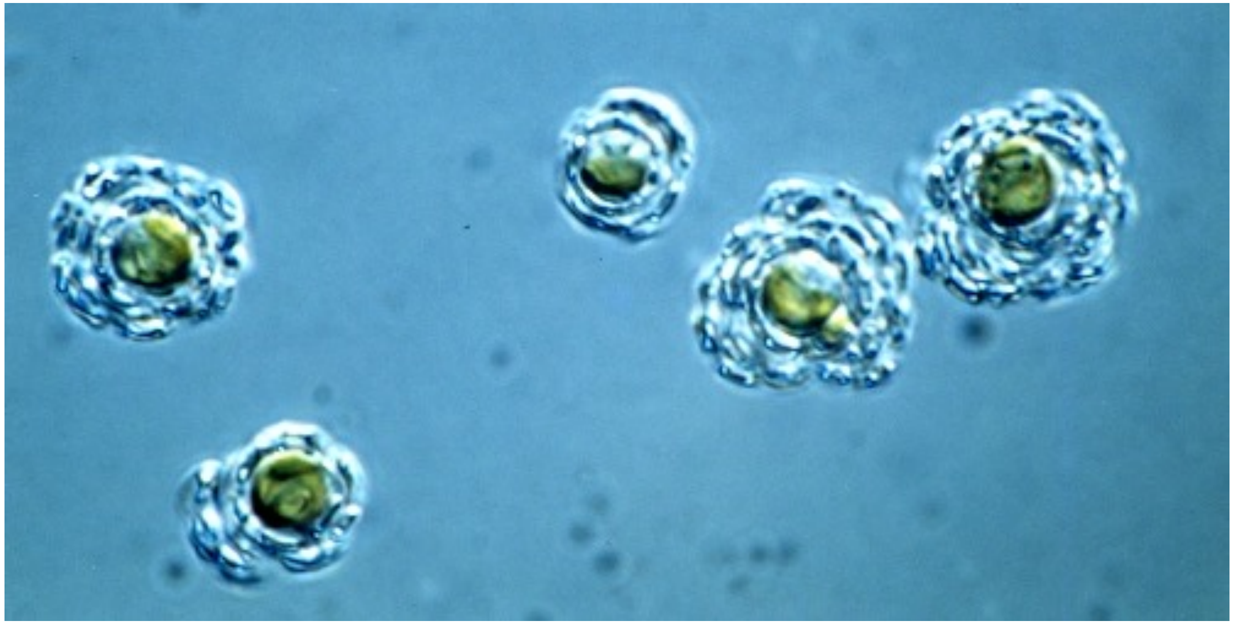
[a] chl = chlorophyll (green); PE = phycoerythrin (red); PC = phycocyanin (blue); APC = allophycocyanin (blue); fucoxanthin and peridinin (golden to brown).

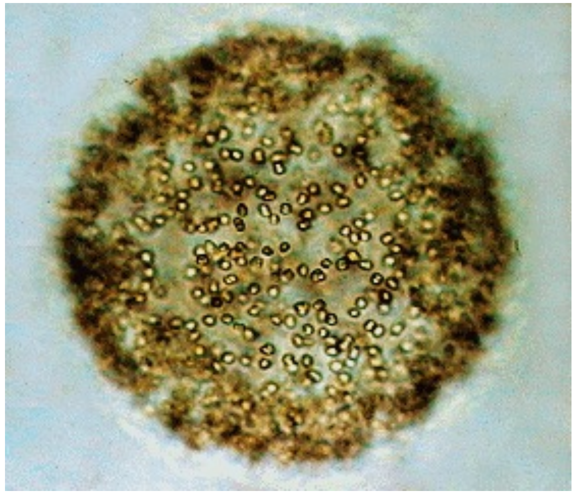
[b] All of the reserves are polymers of glucose. They differ by their linkages: cyanophycean and floridean α1, 4 and α1, 6 branches; true starch with amylose α1, 4 and amylopectin α1, 4 and α1, 6 branches; paramylon β1, 3; chrysolamin and laminarin β1, 3 and β1, 6 branches. Only true starch stains positively with iodine (purple to black).

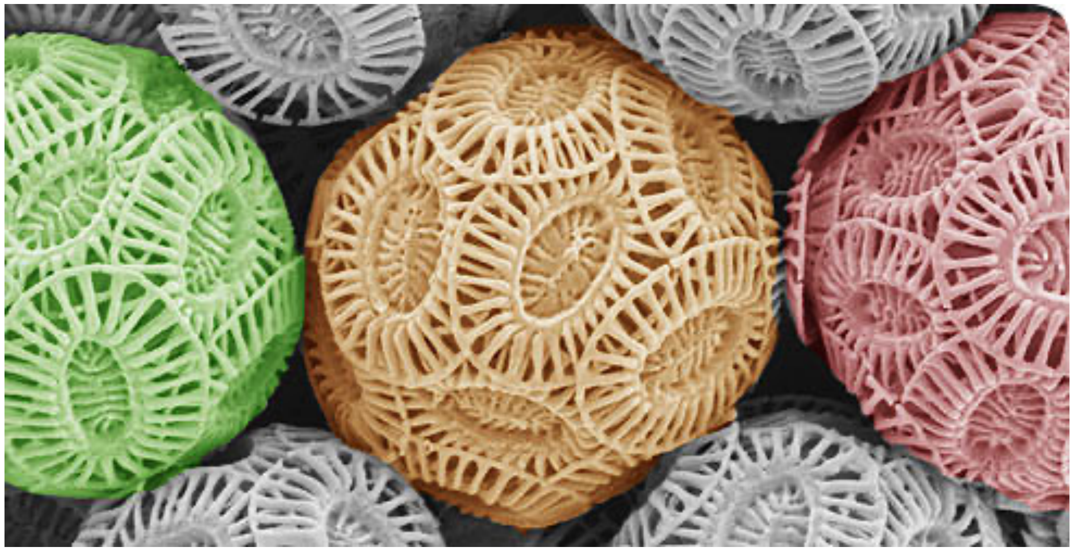
[c] Pellicle and periplast within plasma membrane; the rest are external to it.

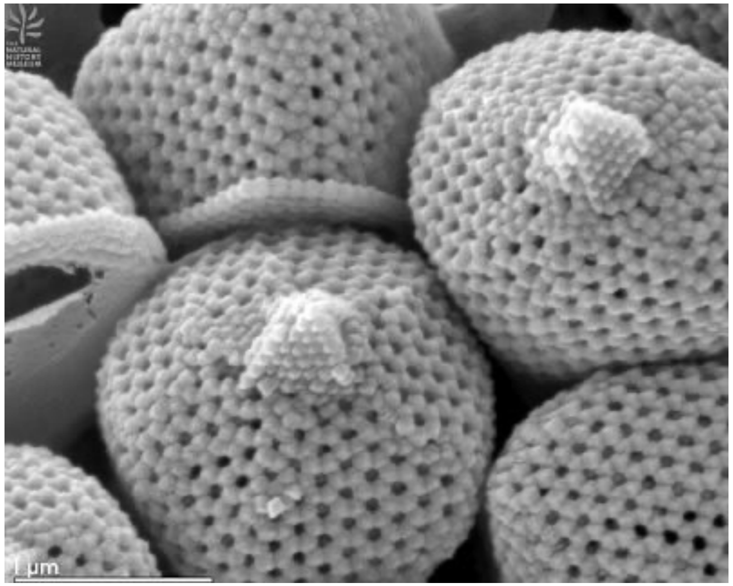
Wehr & Sheath (2003), *Freshwater Algae of North America,* Elsevier

Haptophyta / Prymnesiophyta



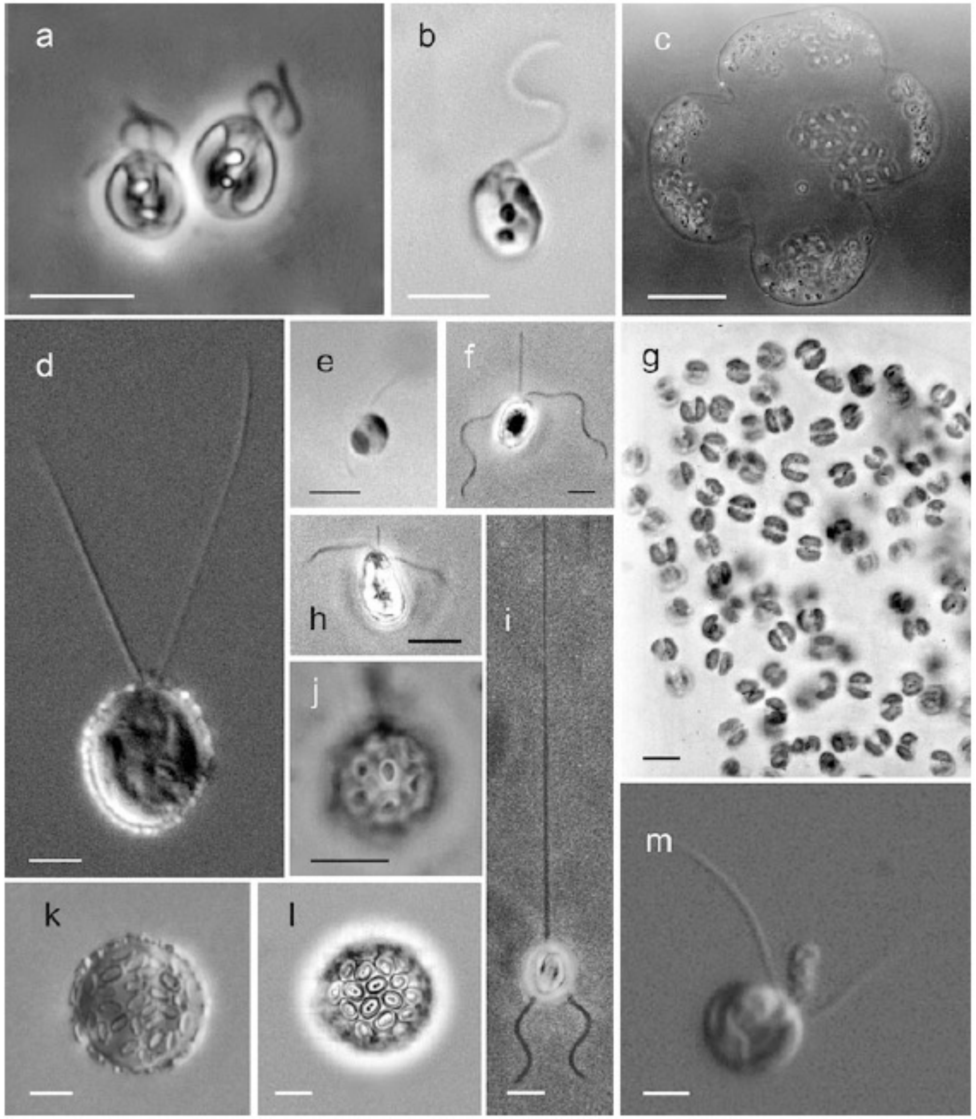






Haptophyta

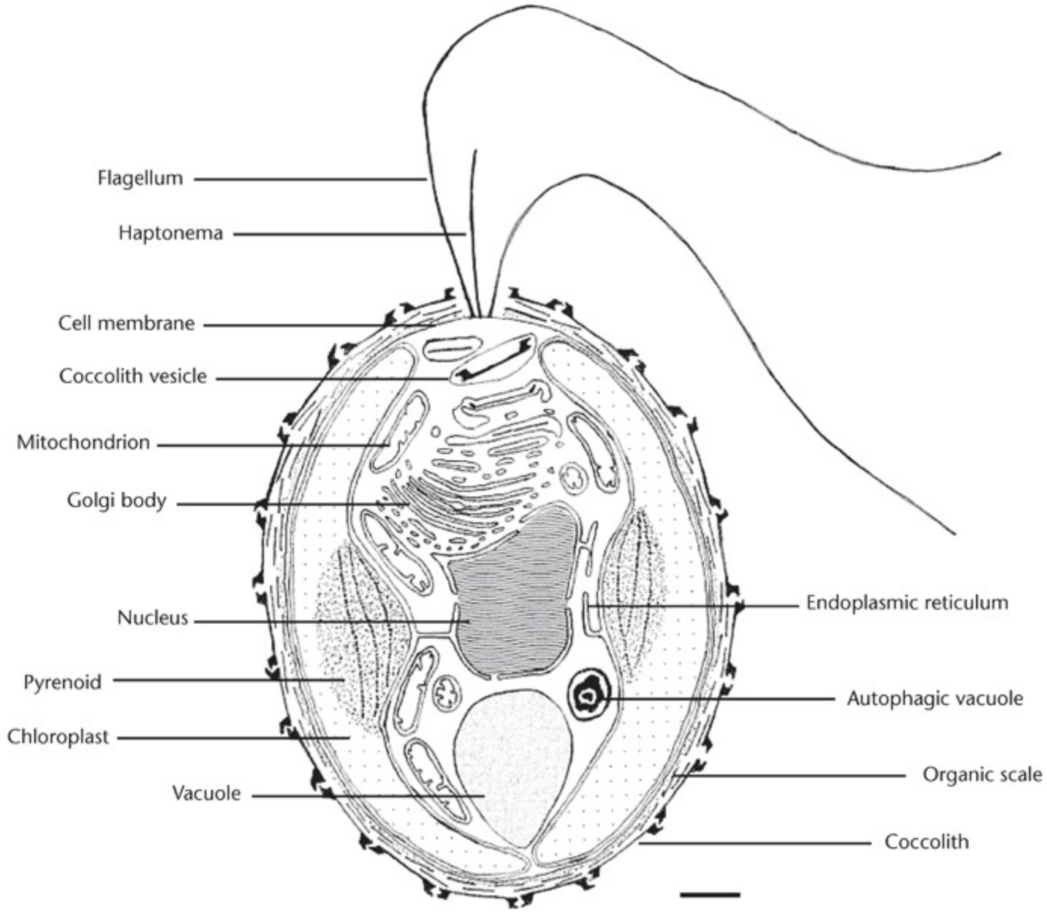
- unicellular free-living protists
- predominantly planktonic (picoplankton: < 3 µm and nanoplankton: 2-20 µm) and photoautotrophic [but many exhibit phagotrophy, some exclusively heterotrophic]
- main distribution in marine environments worldwide
- some form massive blooms (e.g., *Gephyrocapsa/Emiliania huxlei, Phaeocystis* spp.) and some are toxic (e.g., *Prymnesium parvum*)
- important in global carbonate cycle (photosynthesis – 30-50% of total chlorophyll *a* biomass in oceans; calcification of scales - coccolithophores)



Eikrem et al. (2017), *Handbook of the Protists*

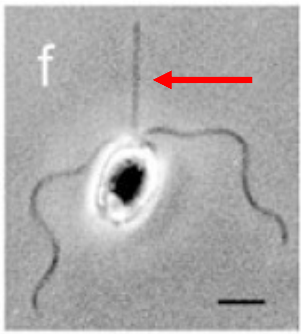
Haptophyta

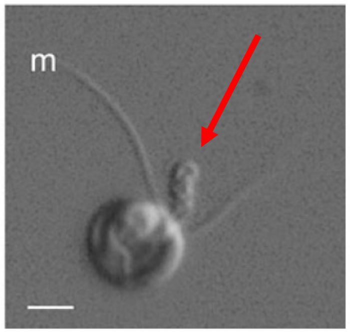
- two similar naked **flagella** [markedly unequal flagella with coverings in Pavlovophyceae, or missing in Coccolithophyceae]
- homo- or heterodynamic **flagella** (9+2 arrangement of microtubules)
- **haptonema**: thin organelle resembling the flagellum [can be reduced in many Coccolithophyceae]; (Greek "*hapto*" = to touch)
- **haptonema**: avoidance response, capturing and gathering food particles, adhering to the substratum

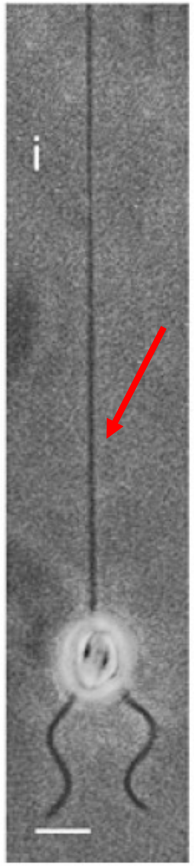


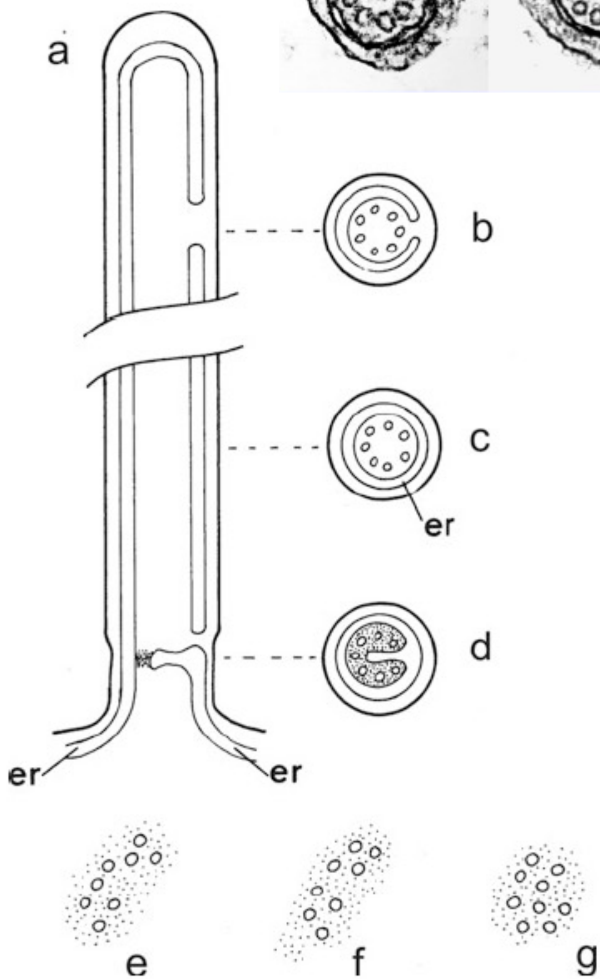
Haptophyta

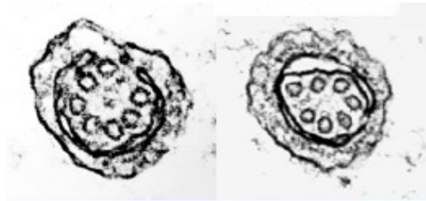
- **haptonema**: different inner structure; cross-section: 6-7 microtubules in the free part of the organelle and 8-9 MT at the base; MT are surrounded by the ER
- differ in length among taxa [degraded in some Isochrysidales species, without hapt. MT]
- behavior coordinated with flagella
- cannot beat like a flagellum does but can be bent or coil











(a) longitudinal section with endoplasmic reticulum (er), microtubuli not shown; (b–d) transverse section at indicated positions showing endoplasmatic reticulum and microtubuli; (e–g) microtubuli within the cell.

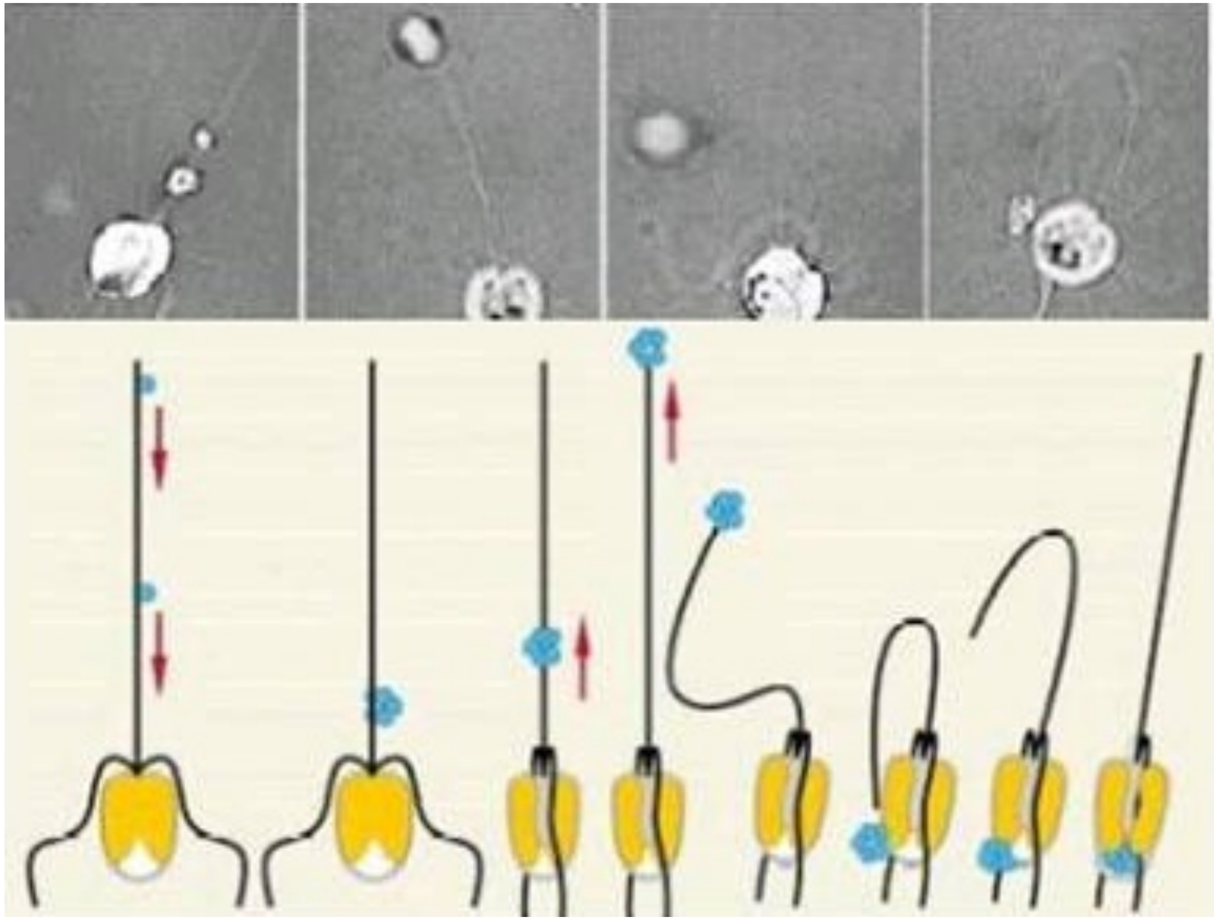
Eikrem et al. (2017), *Handbook of the Protists*

Haptophyta

- **ingestion** of bacteria and smaller eukaryotes (phagotrophy)
- **obstacle avoidance:** coiling in extremely short time + the beating of flagella changes to the opposite direction

https://bit.ly/3TsGZVX

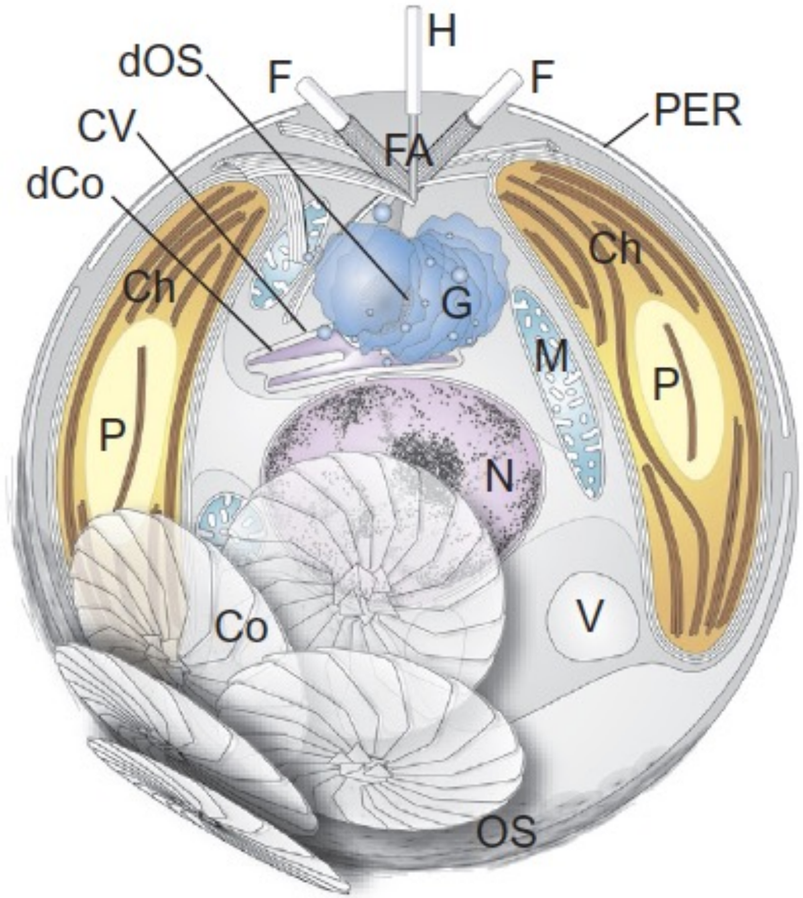
https://bit.ly/3Tt60jT



Chrysochromulina. Bacterial particles stick on the haptonema. They are transported to the aggregation center. The clump of bacteria is then transported to the end of haptonema, and delivered to the posterior cell end, where it is engulfed.

Haptophyta

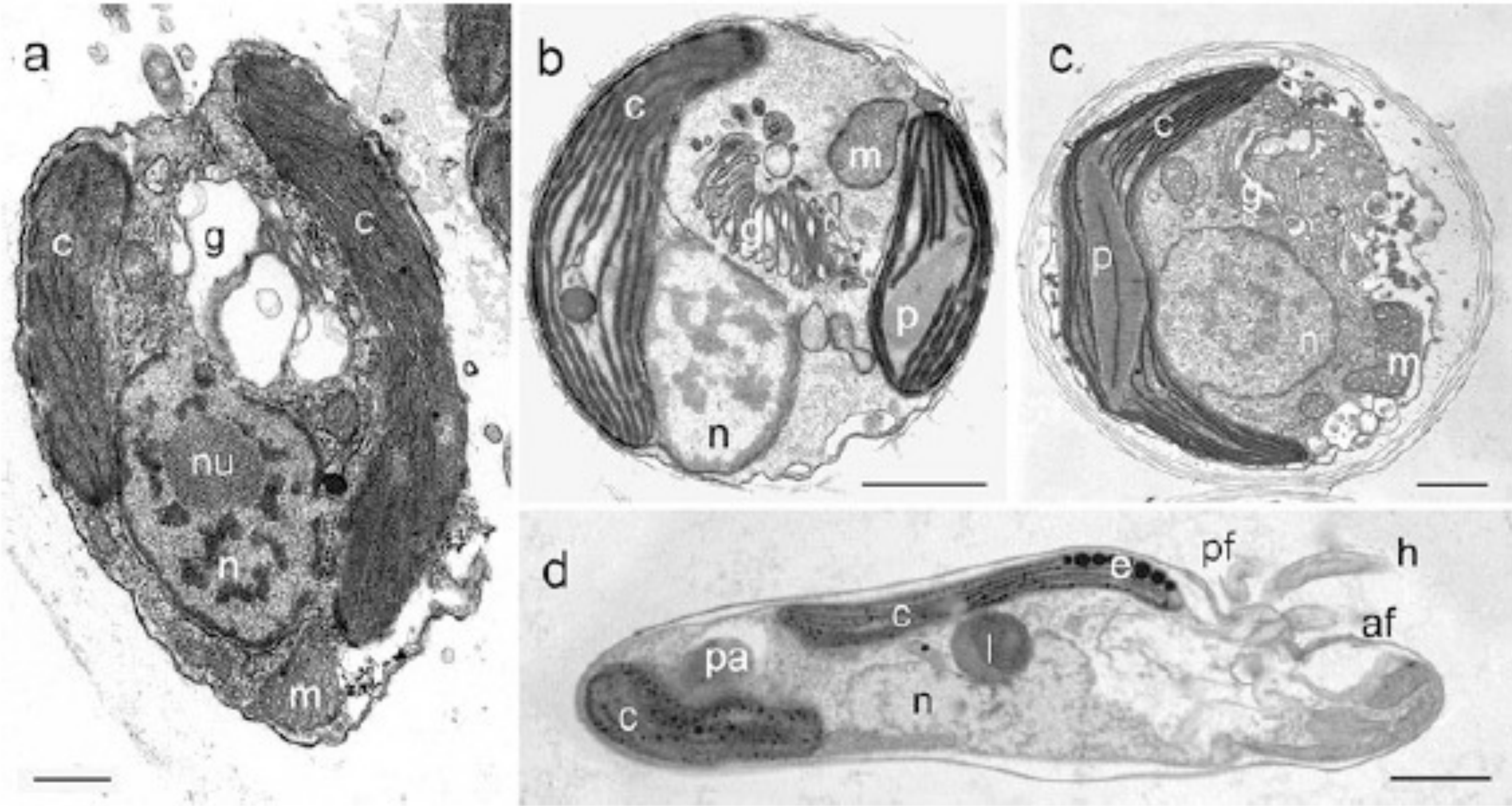
- cells are surrounded by a cytoplasmatic membrane and peripheral ER [no cell wall]
- one or two yellow/golden-brown red-algal derived chloroplasts (4 membranes, outermost membrane from ER)
- plastids: with pyrenoid; lamellae comprised of 3 thylakoids; no stigma [but occurs in many Pavlovaphyceae]; no girdle lamella
- chlorophyll *a* and c_2 + carotenoids (fucoxanthin derivates, beta-carotene)
- storage product: chrysolaminarin [paramylon in Pavlovales], lipid bodies
- mitochondria has tubular cristae



Ch - chloroplast; **Co** - coccolith; **CV** - coccolith forming vesicle; **dCo**- developing heterococcolith; **dOS** developing organic scale; **F** - flagellum; **FA** - flagellar apparatus; **G** - Golgi apparatus; **H** - haptonema; M - mitochondrion; **N** - nucleus; **OS -** organic scale; **P** - pyrenoid; **PER** - peripheral endoplasmic reticulum; **V** - vacuole.

Tsuji & Yoshida (2017)

Haptophyta

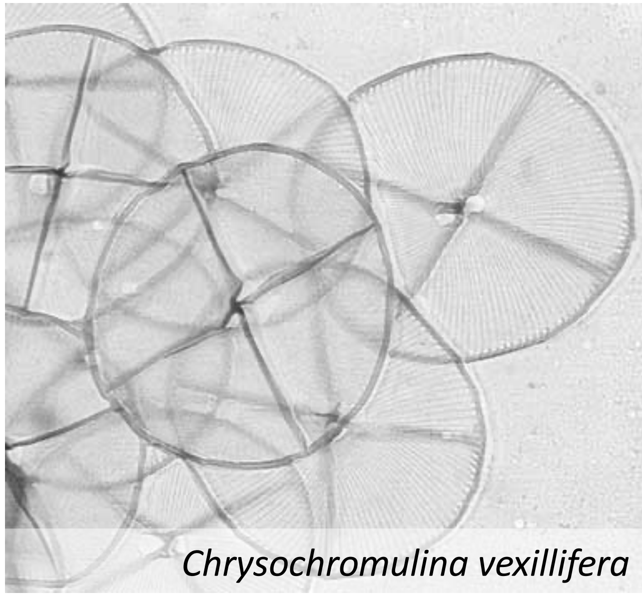


c - chloroplast; **n** - nucleus; **nu** - nucleolus; **pf** - posterior flagellum; **af** - anterior flagellum; **h -** haptonema; **p** - pyrenoid; **l** - lipid droplet; **m** - mitochondrion

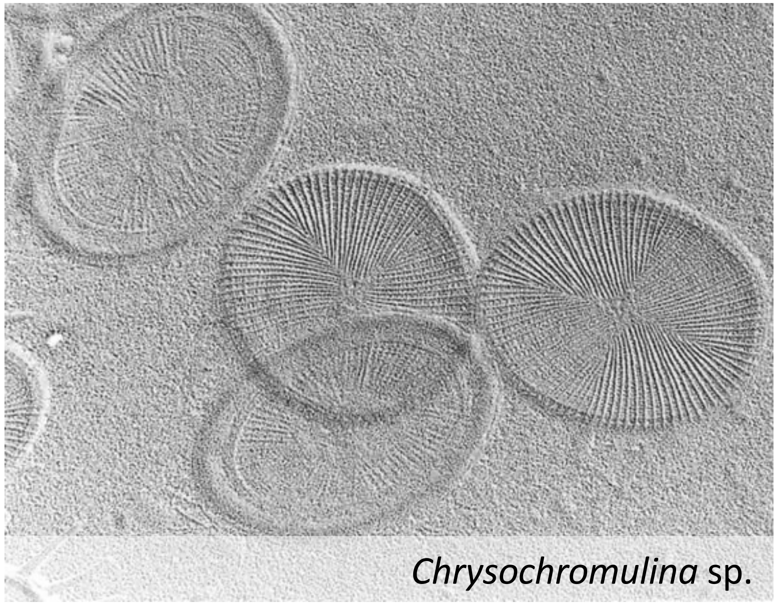
Eikrem et al. (2017), *Handbook of the Protists*

Haptophyta

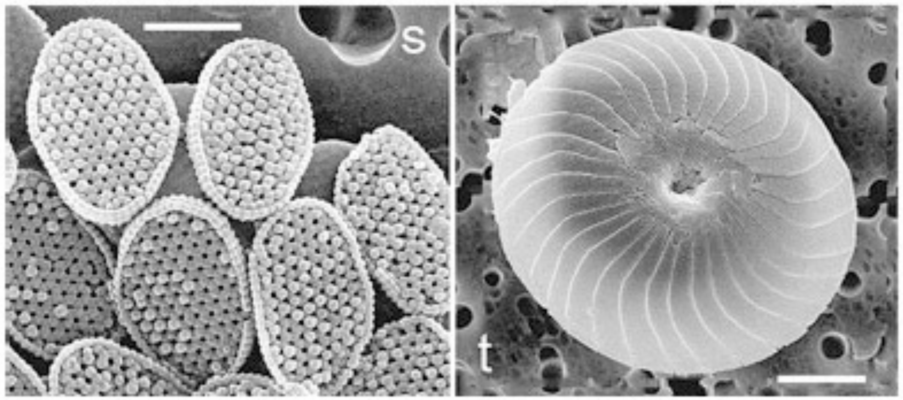
- usually covered with scales (production associated with Golgi vesicles)
- organic nonmineralized body scales (from carbohydrates; spiral microfibrils contain protein and cellulose)
- above body scales silicified (some Prymnesiales) or calcified scales = coccoliths (coccolithophores)
- scales: a diagnostic taxonomic character



Chrysochromulina vexillifera



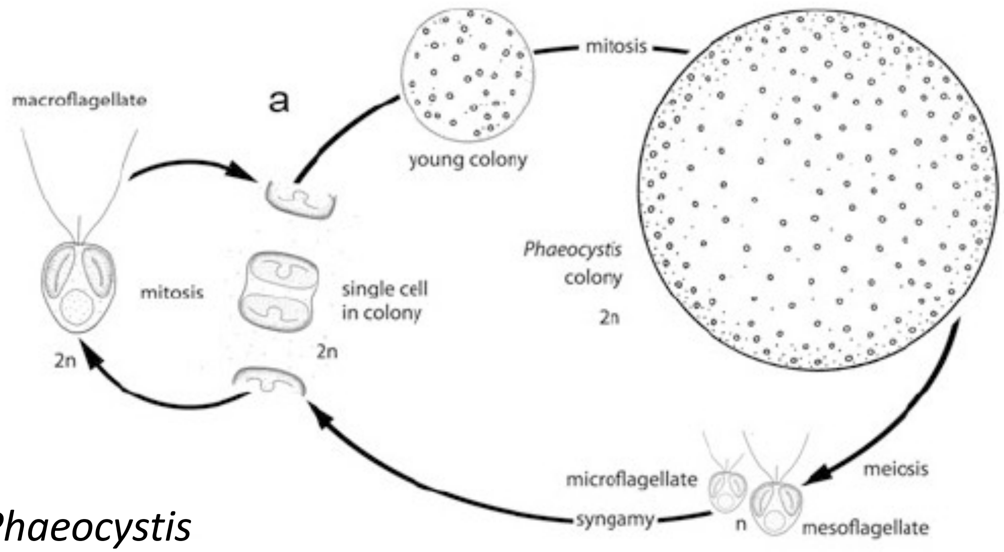
Chrysochromulina sp.



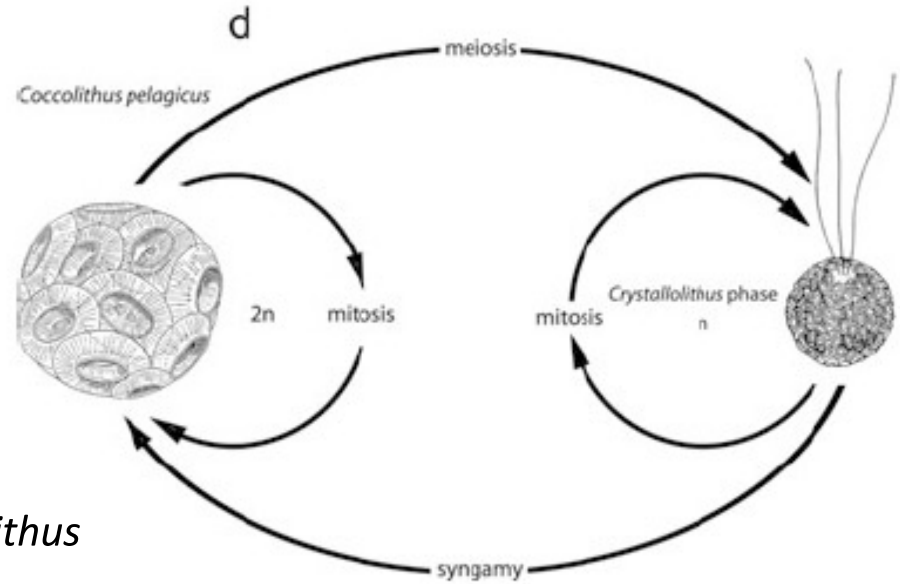
holococcoliths and heterococcolith

Haptophyta

- mitotic division: cell coverings are equally shared by daughter cells (new scales supply a deficiency after division)
- heteromorphic phases, alteration between motile and nonmotile forms
- alteration of a haploid stage with a diploid stage [so far not observed in Pavlovophyceae]
- haplo-diplontic life cycle: distinct genetics, morphology, and metabolism -> different ecological niche



Phaeocystis



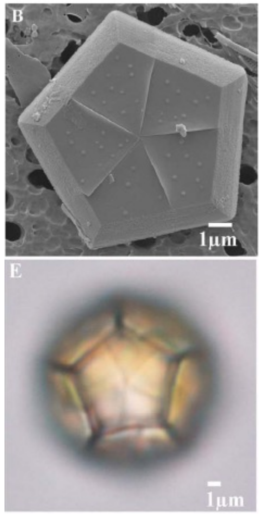
Coccolithus

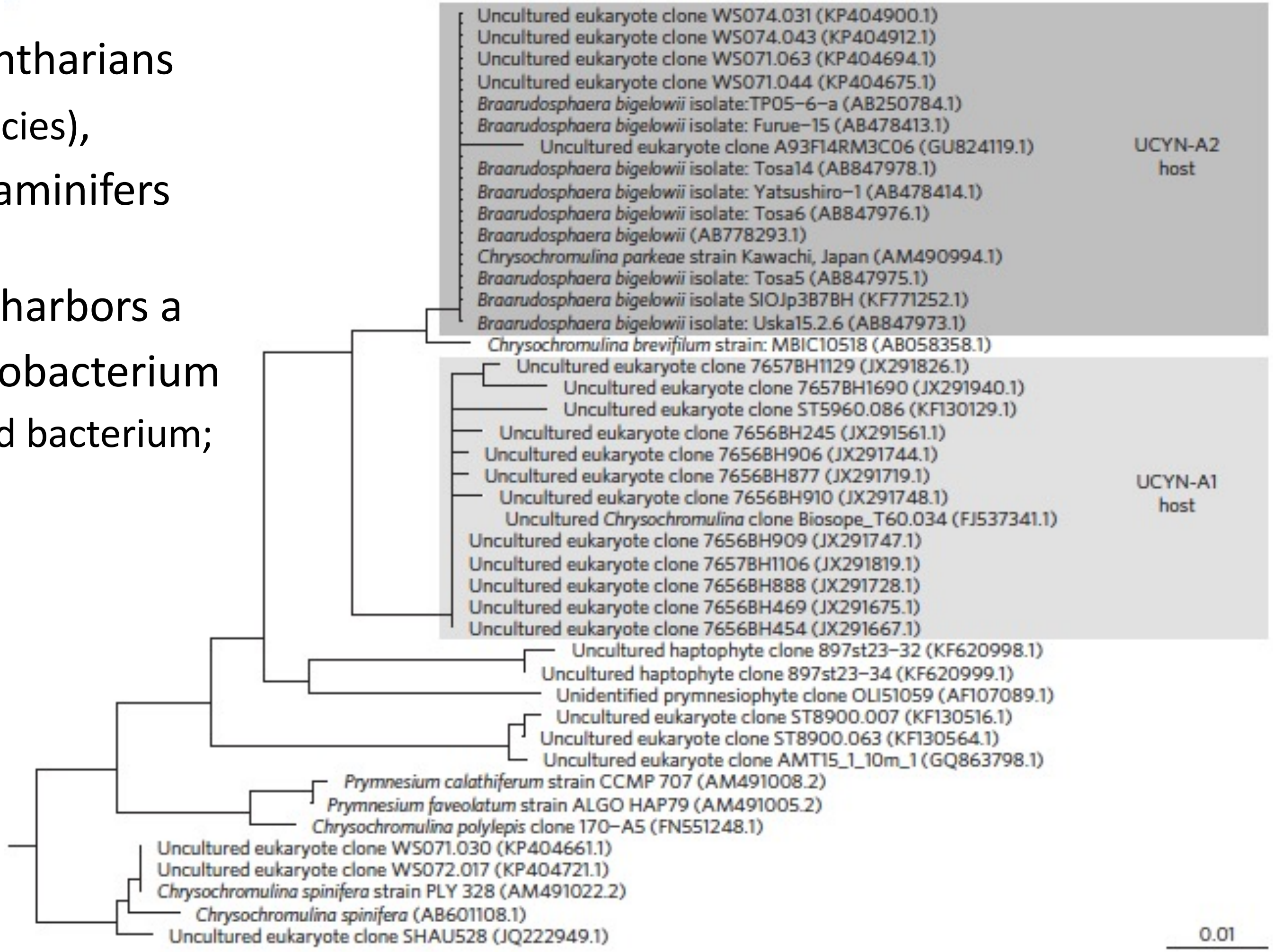
Eikrem et al. (2017), *Handbook of the Protists*

Haptophyta

- as symbionts of acantharians (some *Phaeocystis* species), radiolarians and foraminifers

- haptophyte lineage harbors a nitrogen-fixing cyanobacterium (UCYN-A = uncultivated bacterium; in spheroid bodies)



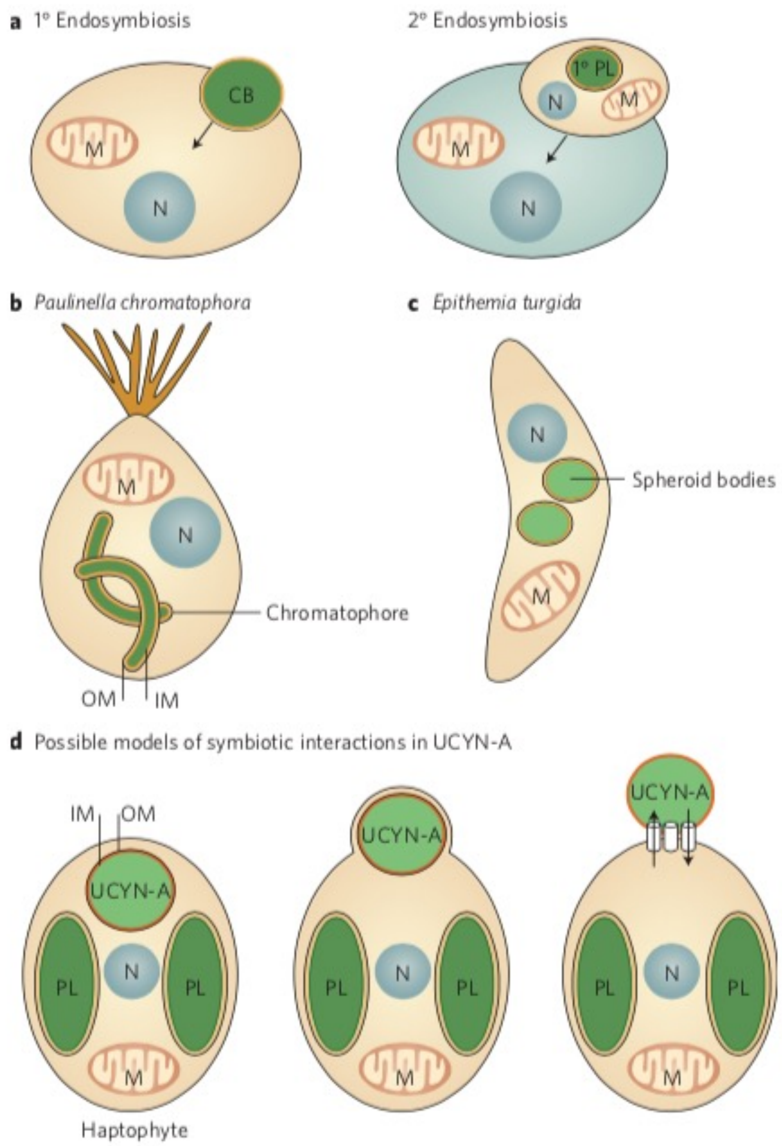


Braarrudosphaera bigelowii

Zehr et al. (2016), *Nature Microbiology*

Haptophyta

- symbiotic interactions between N_2-fixing prokaryotes and photosynthetic eukaryotes



d: Possible models of symbiotic interactions between UCYN-A and its haptophyte host.

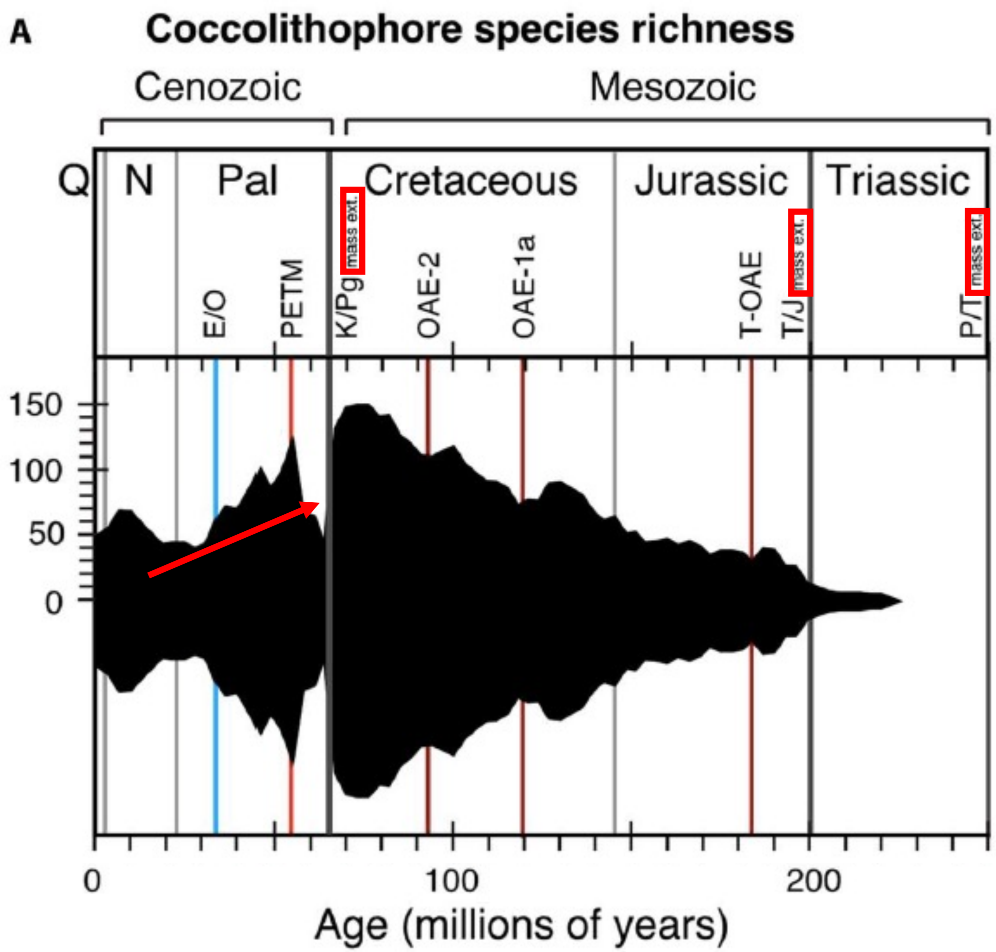
CB, cyanobacterium; N, host nucleus; PL, plastids; M, mitochondrion; OM, outer membrane; IM, inner membrane.

Zehr et al. (2016), *Nature Microbiology*

Haptophyta

- first appear in the fossil of the Late Triassic (~ 220 million years ago)
- the higher abundance during the Late Cretaceous (~95 mya)
- ~80 % of all coccolithophorids went extinct during the Cretaceous-Paleogene (K-Pg /K-T) event at the end of the Cretaceous


white chalk cliffs, England



Q - Quaternary; **N** - Neogene; **Pal** - Paleogene; **E/O** - Eocene/Oligocene glacial onset event; **PETM** - Paleocene/ Eocene thermal maximum warming event; **K/Pg -** Cretaceous/ Paleogene; **OAE -** oceanic anoxic event; **T-OAE -** Toarcian oceanic anoxic event; **T/J -** Triassic/Jurassic; **P/T -** Permian/ Triassic

Monteiro et al. (2016), *Science Advances*

REPORT

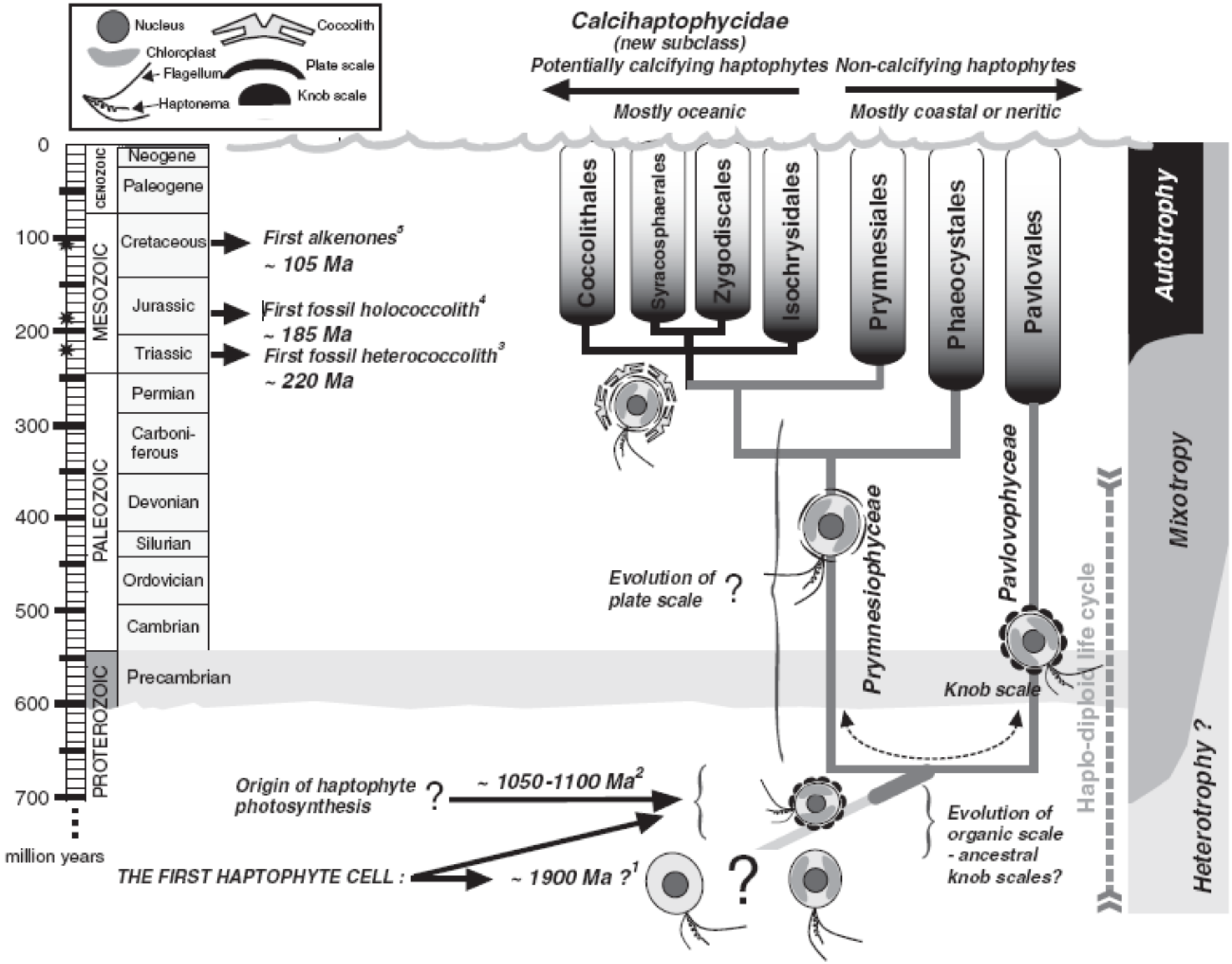
FOSSIL RECORD

Global record of "ghost" nannofossils reveals plankton resilience to high CO_2 and warming

Sam M. Slater[1]*, Paul Bown[2], Richard J. Twitchett[3], Silvia Danise[4], Vivi Vajda[1]

Predictions of how marine calcifying organisms will respond to climate change rely heavily on the fossil record of nannoplankton. Declines in calcium carbonate ($CaCO_3$) and nannofossil abundance through several past global warming events have been interpreted as biocalcification crises caused by ocean acidification and related factors. We present a global record of imprint—or "ghost"—nannofossils that contradicts this view, revealing exquisitely preserved nannoplankton throughout an inferred Jurassic biocalcification crisis. Imprints from two further Cretaceous warming events confirm that the fossil records of these intervals have been strongly distorted by $CaCO_3$ dissolution. Although the rapidity of present-day climate change exceeds the temporal resolution of most fossil records, complicating direct comparison with past warming events, our findings demonstrate that nannoplankton were more resilient to past events than traditional fossil evidence suggests.

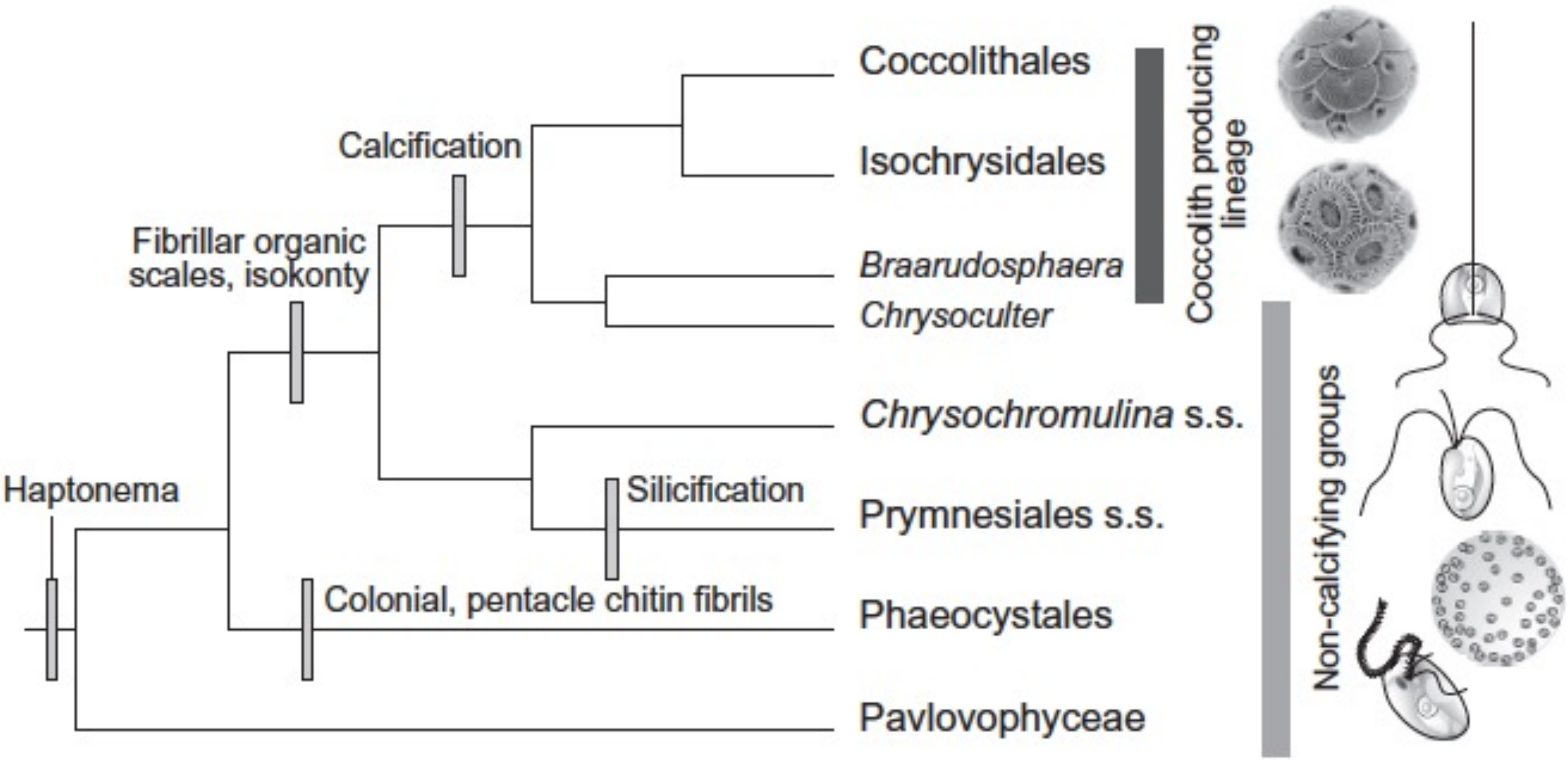
Haptophyta



Evolutionary history

de Vargas et al. (2007), *Evolution of Primary Producers in the Sea*

●**Haptophyta**
●●**Pavlovophyceae**
●●●**Pavlovales** (e.g. *Pavlova*, *Rebecca*)
●● **Coccolithophyceae** (=*Prymnesiophyceae*)
●●●**Phaeocystales** (*Phaeocystis*)
●●●**Isochrysidales** (e.g. *Isochrysis*, *Emiliania*)
●●●**Coccolithales** (e.g. *Coccolithus*, *Syracosphaera*)
●●●**Prymnesiales** (e.g. *Prymnesium*, *Chrysochromulina*)



Tsuji & Yoshida (2017)

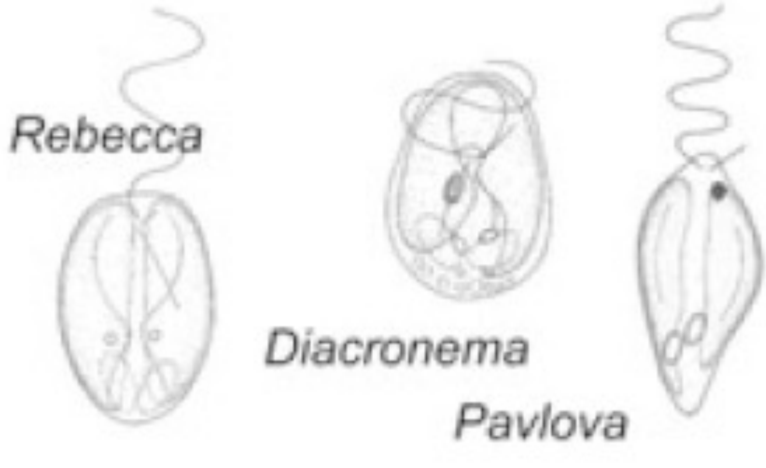
Haptophyta

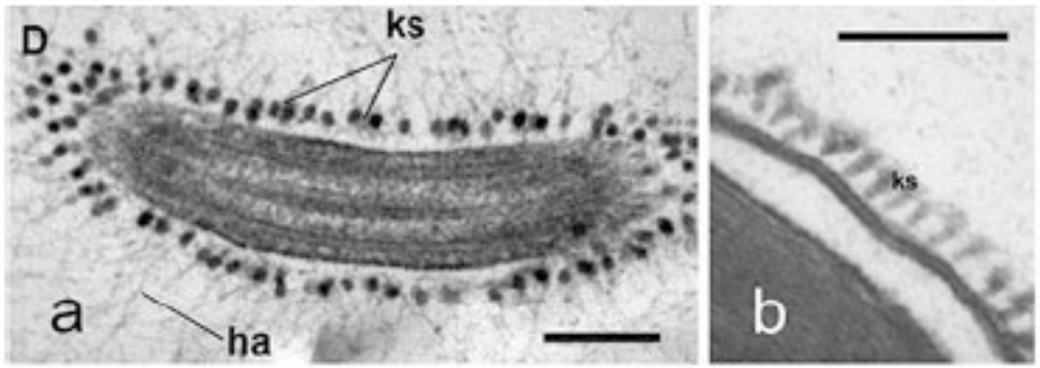
Phylum Haptophyta	Cells with haptonema
Class: Pavlovophyceae	Cells with knob scales
Order: Pavlovales	Motile cells with two unequal flagella, the longer with an investment of small knob scales and fine hairs and the shorter sometimes vestigial. Haptonema present but reduced (e.g., *Diacronema*, *Pavlova*)
Class: Coccolithophyceae (Prymnesiophyceae)	Cells with organic scales, with or without coccoliths
Order: Isochrysidales	Motile cells with two equal or subequal flagella, haptonema reduced or absent (e.g., *Ruttnera*, *Emiliania*, *Isochrysis*)
Order: Coccolithales	Cells coccolith bearing many genera with a polymorphic life cycle. Haptonemata recorded in several genera (e.g., *Acanthoica*, *Braarudosphaera*, *Calyptrosphaera*, *Chrysotila*, *Coccolithus*, *Corisphaera*, *Crenalithus*, *Cyclolithella*, *Discosphaera*, *Helicosphaera*, *Laminolithus*, *Rhabdosphaera*, *Syracosphaera*, *Umbellosphaera*, *Umbilicosphaera*)
Order: Prymnesiales	Motile cells with two (rarely four) equal or subequal flagella and usually an obvious haptonema which may be very long and coiling (e.g., *Chrysochromulina*, *Haptolina*, *Prymnesium*)
Order: Phaeocystales	Motile cells with two flagella and short haptonema. Flagellate alternating with colonial stage (*Phaeocystis*)

Eikrem et al. (2017), *Handbook of the Protists*

Pavlovales

- flagella are markedly different in length (the more posteriorly inserted one is much shorter than the anterior flagellum)
- the longer flagellum usually carries a covering of fine hairs and knob-like scales [in some species scales may also be found also on the cell body]
- nonmotile cells may be invested with layers of mucilage
- a stigma or “eyespot” consisting of a single layer of lipid globules
- 4 genera and 13 species (Bendif et al. 2011)





(a–b) Pavlovophyceae: (a) *Pavlova pinguis*, knob scales on flagella; (b) cell surface with knob scales in *Rebecca salina*.

Eikrem et al. (2017), *Handbook of the Protists*

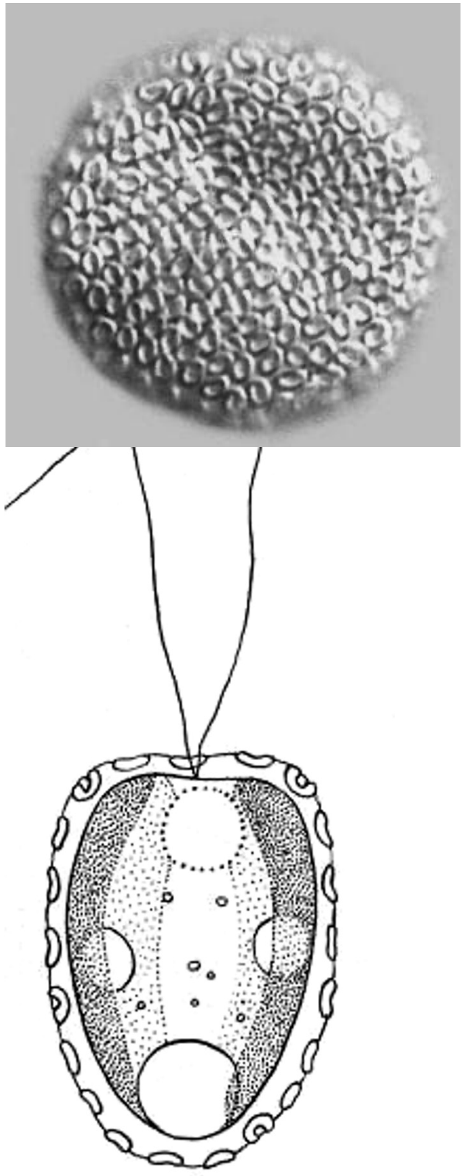
Pavlovales and Prymnesiales

- Pavlovales: common in nearshore coastal environments
- only a few species of Haptophyta have been reported from freshwater environments (lineages in blue) -> representatives of Pavlovales (*Diacronema/ Exanthemachrysis*, *Pavlova*) and Prymnesiales (*Hymenomonas*, *Prymnesium*, *Chrysochromulina*)

Schalchian et al. (2011), *J. Eukaryot. Microbiol.*

Prymnesiales

- *Hymenomonas roseola*, calcified scales
- freshwater in metaphyton of clear, oligotrophic waters

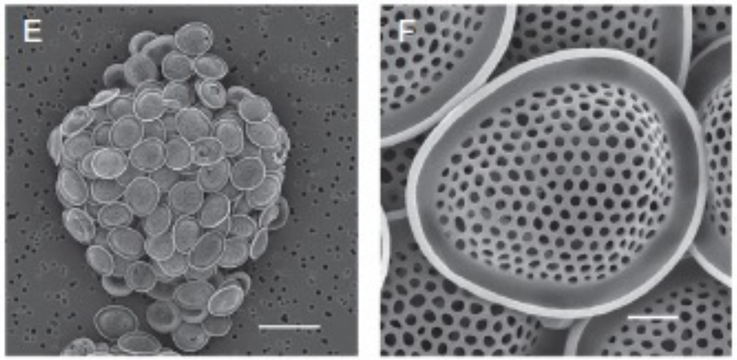


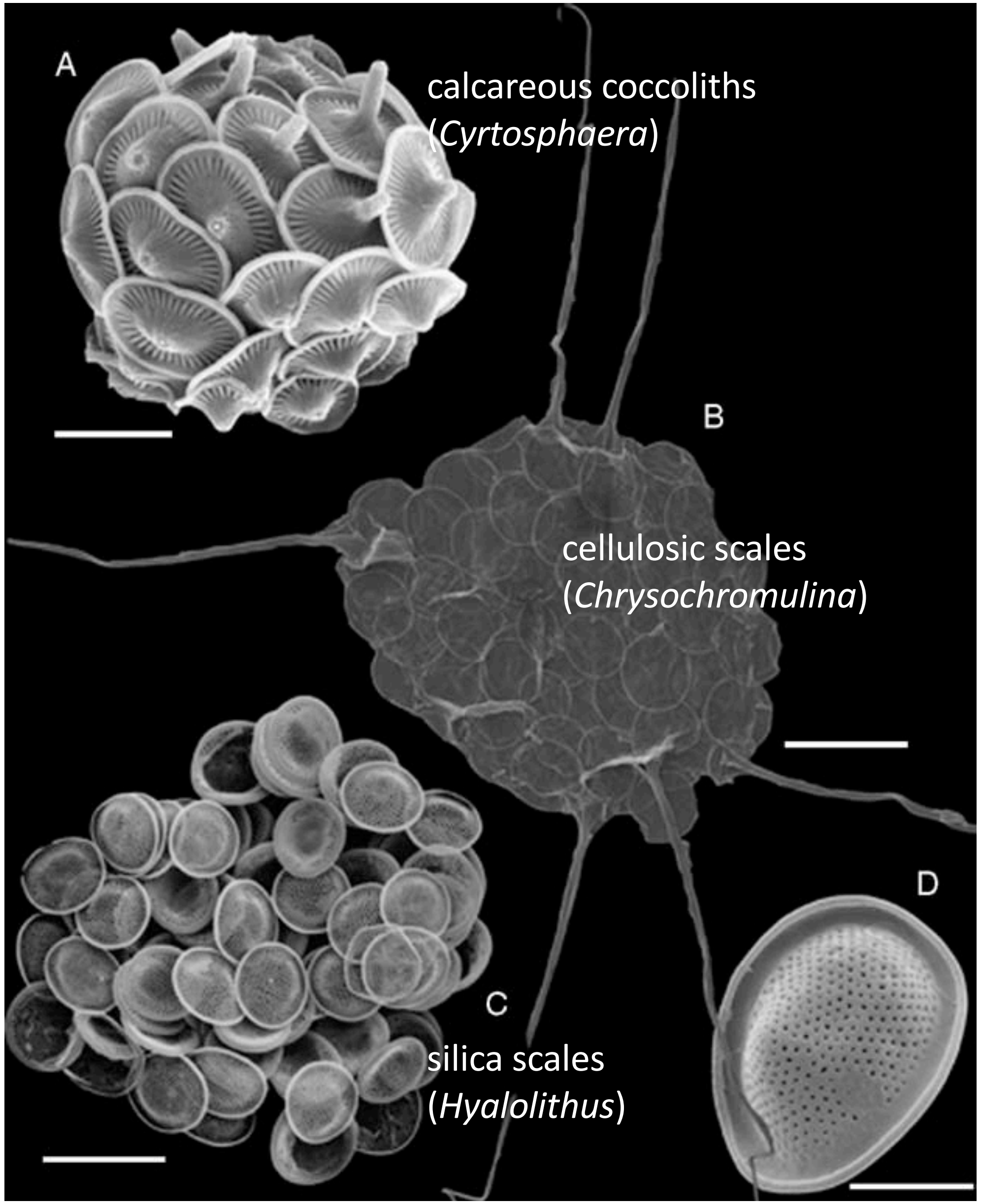
C

Schalchian et al. (2011), *J. Eukaryot. Microbiol.*

Prymnesiales

- very few species in Prymnesiales use silica to mineralize their scales
- production in SDV (silica deposition vesicles)
- small pores, deposited on cells in several layers
- *Prymnesium parvum* cysts are composed of layers of scales with siliceous material

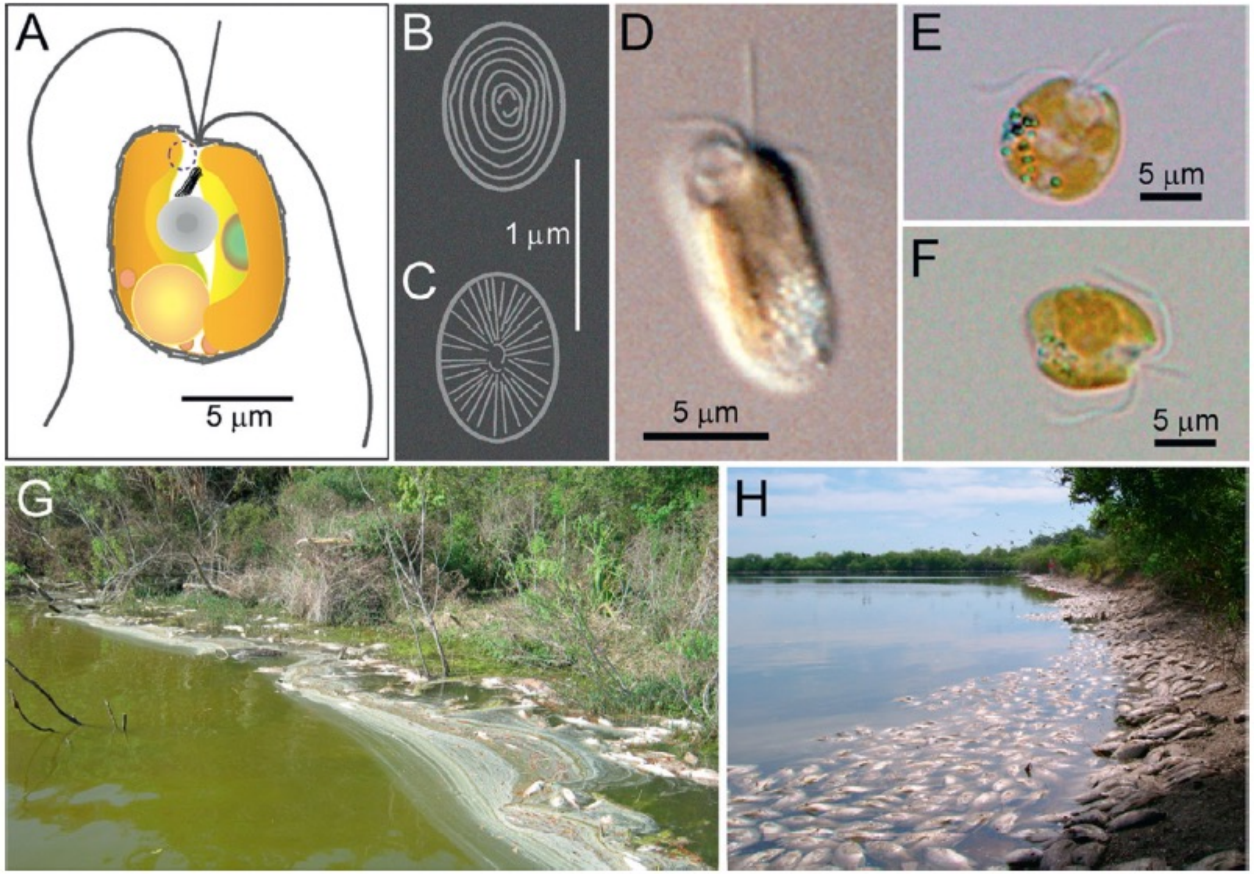




de Vargas et al. (2007); Eikrem et al. (2017)

Prymnesiales

- *Prymnesium parvum,* mixotrophic, flagellates with organic scales
- toxic episodes in coastal area and freshwaters (toxins prymnesins, massive fish kills - ichtyotoxin)
- salinity appears to have been the primary factor controlling distribution of this species

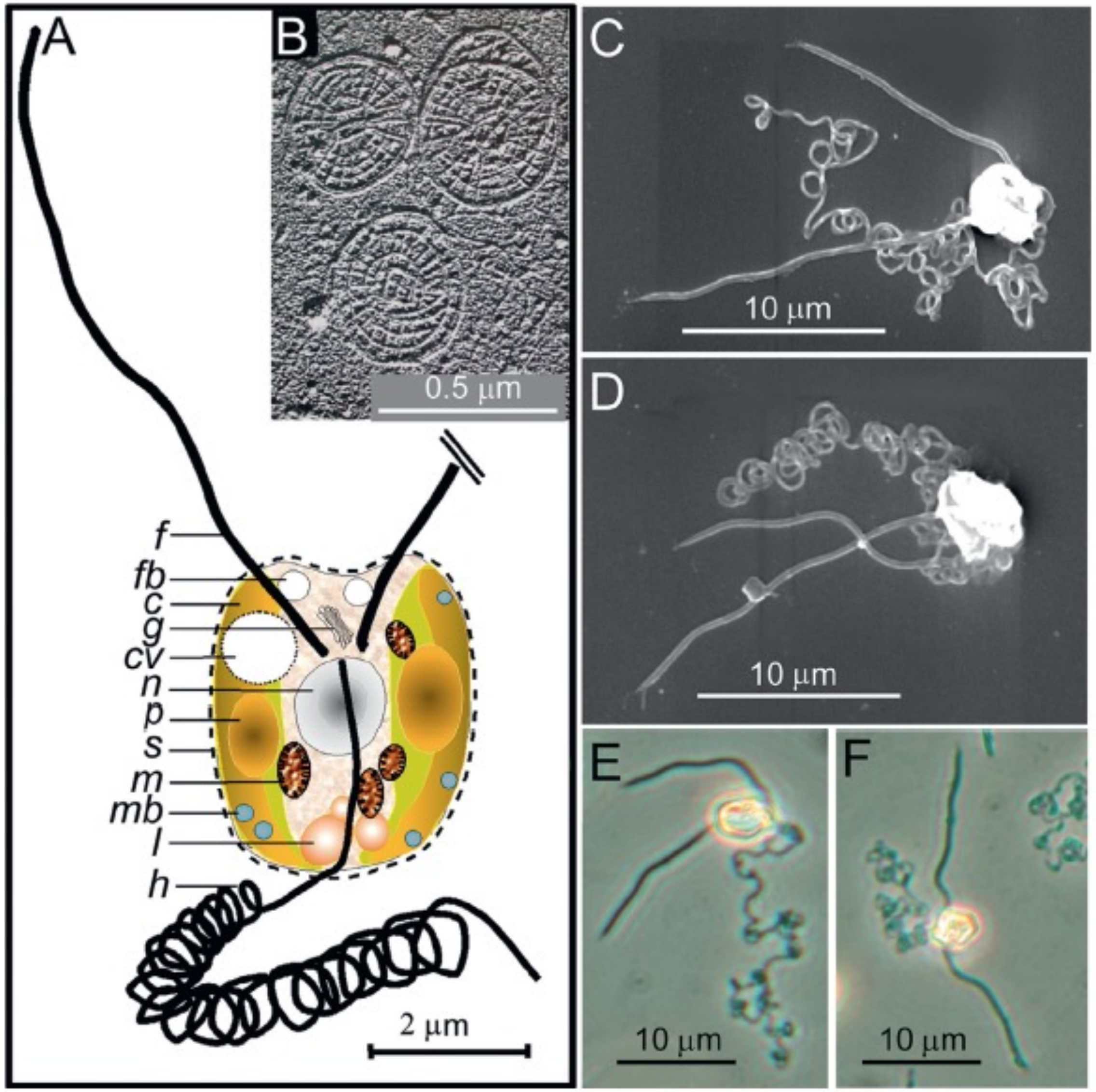


Nicholls (2015), *Freshwater Algae of North America*, Elsevier

Prymnesiales

- *Chrysochromulina parva,* only organic scales
- widely distributed in freshwaters
- from eutrophic small ponds and rivers to large, deep, and cold lakes

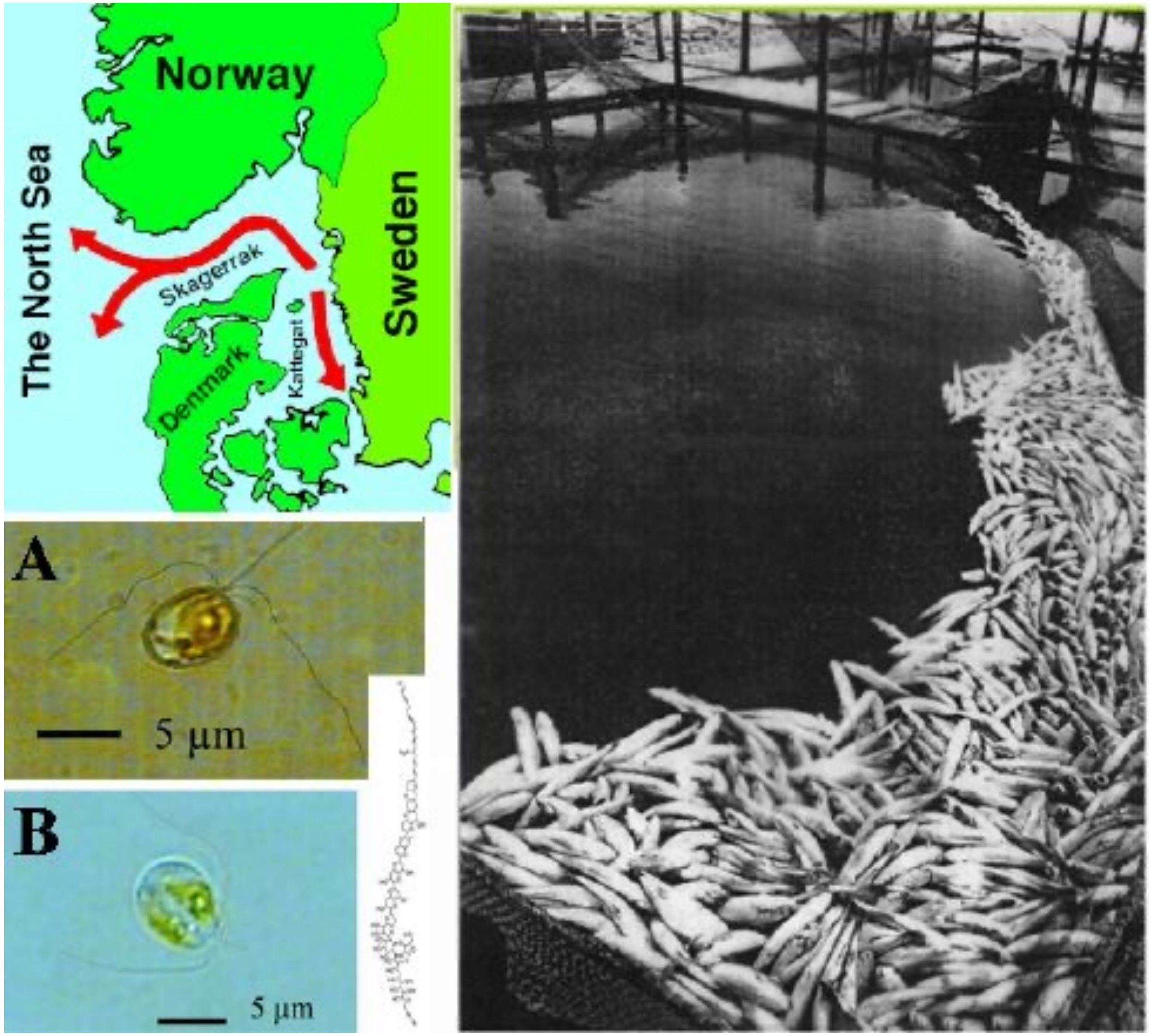
f - flagellum; **fb** - lipid globule; **c** - chloroplast; **g** - Golgi body; **cv** - contractile vacuole; **n** - nucleus; **p** - pyrenoid; **s** - scale layer; **m** - mitochondrion; **mb** - muciferous body; **l** - leucosin vesicle; **h** - haptonema



Nicholls (2015), *Freshwater Algae of North America*, Elsevier

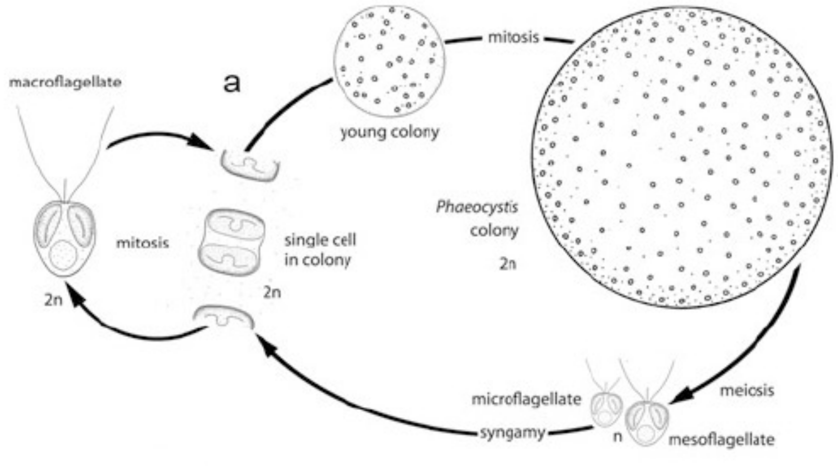
Prymnesiales

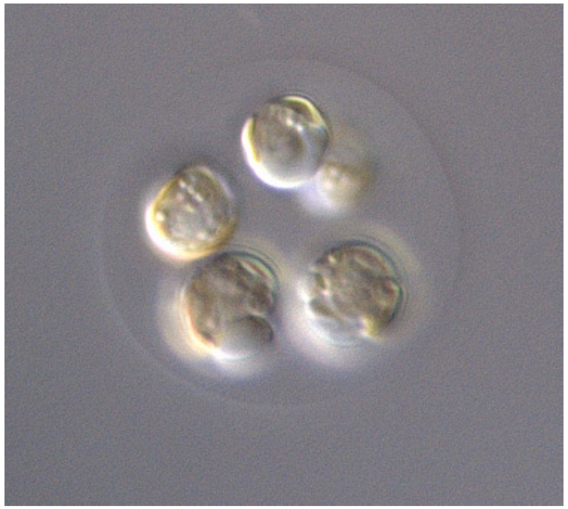
- *Chrysochromulina polylepis*
- production of ichtyotoxins

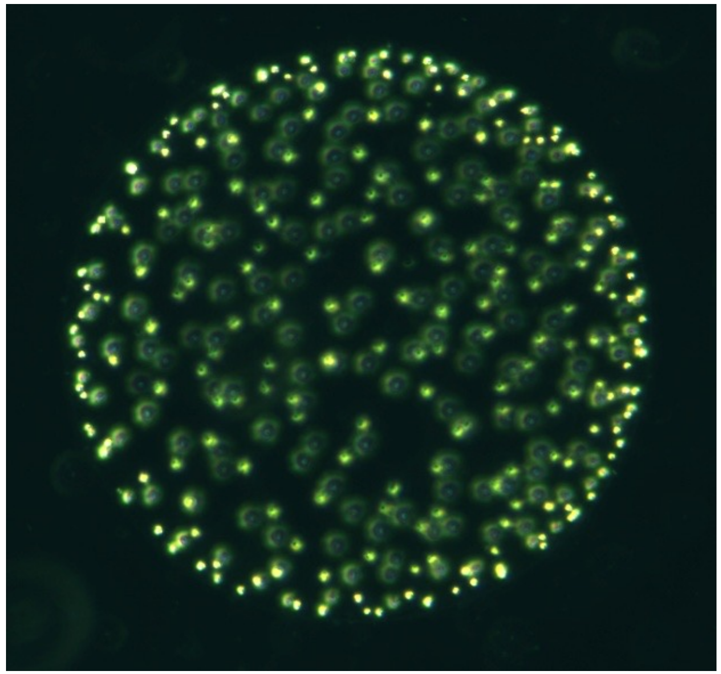


Phaeocystales

- *Phaeocystis pouchetii*: flagellates (3-8 µm) in large (up to a few mm or cm) mucilaginous colonies (pallmeloid stage)





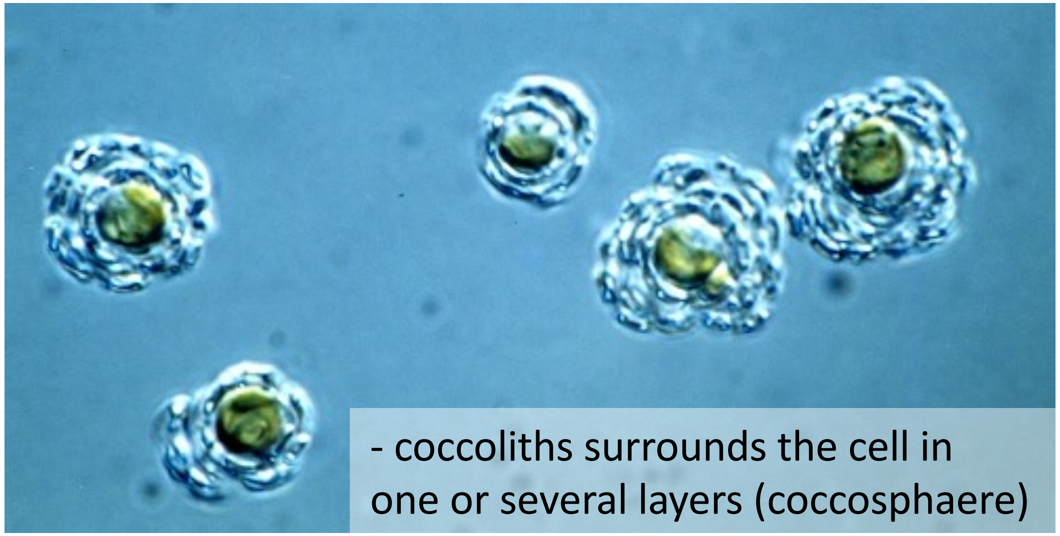


Phaeocystales

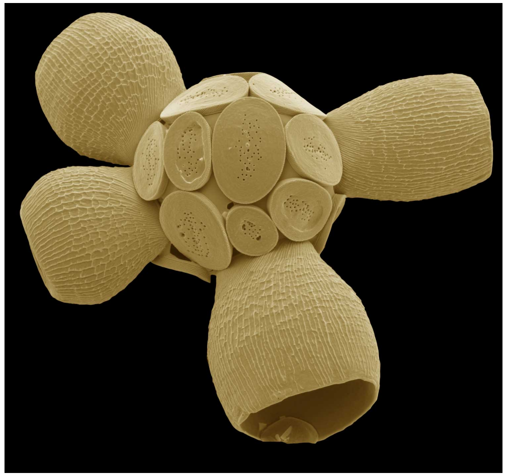
- *Phaeocystis* nuisance and ?toxic bloom, foam accumulated on beaches

coccolithophores

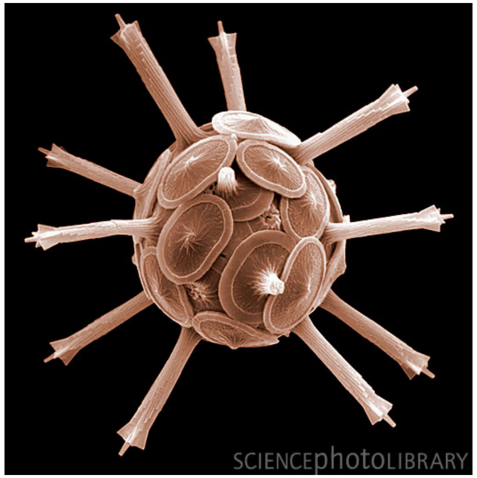
- coccolith-bearing haptophytes
- *kokkos* = berry, grain
  lithos = stone (Huxley 1858)
- coccoliths especially in **Isochrysidales** and **Coccolithales**



Scyphosphaera



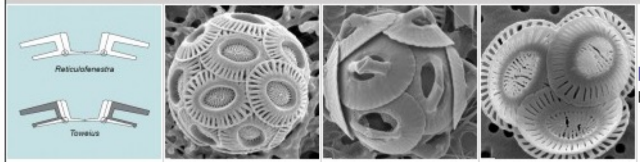
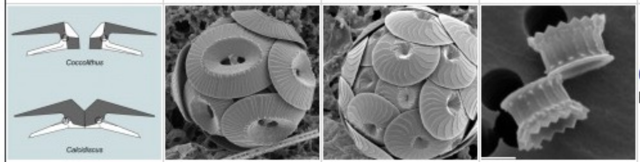
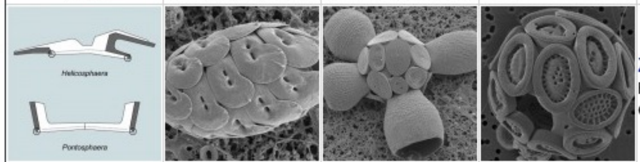
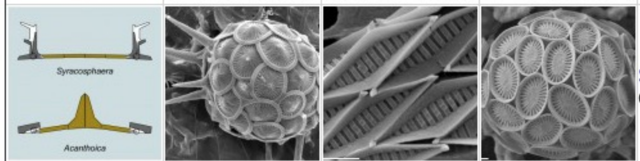
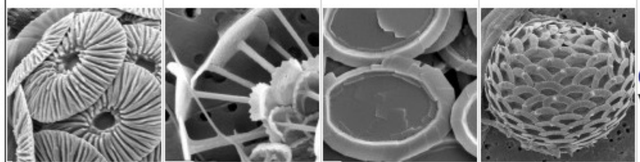
Rhabdosphaera clavigera



Syracosphaera anthos

- a huge variety of ornamented calcified scales

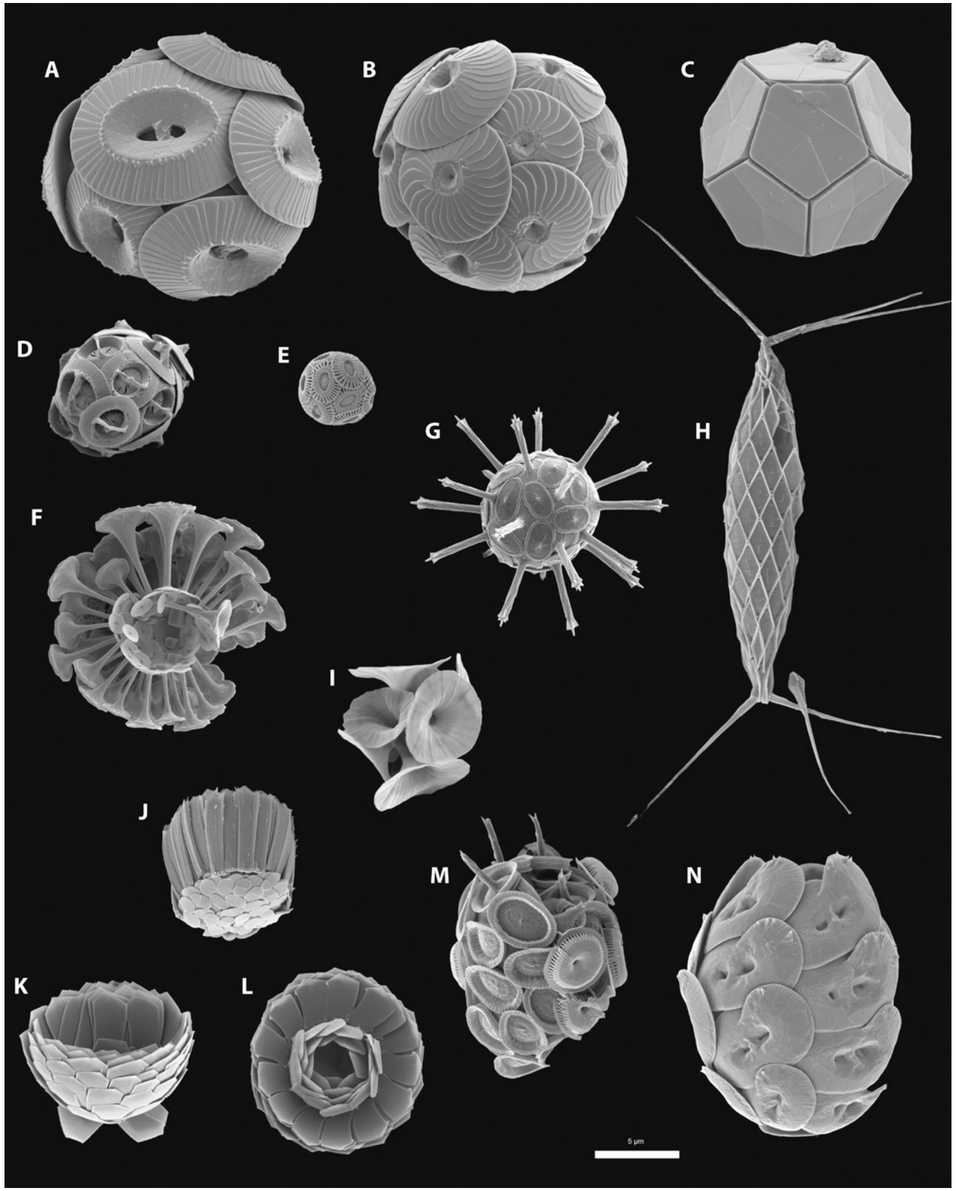
Coccolithophores

Daughter taxa: (blue => in age window 0-800Ma)		Granddaughter taxa
	Heterococcoliths	
	Isochrysidales Heterococcoliths mostly placoliths with R-unit dominant. Motile phases with vestigial haptonema.	Noelaerhabdaceae Prinsiaceae Isochrysidaceae
	Coccolithales Mostly placolith heterococcoliths, with V-unit forming the distal shield; R-unit the proximal shield.	Coccolithaceae Calcidiscaceae Hymenomonadaceae Pleurochrysidaceae
	Zygodiscales Heterococcoliths with V-units forming upper/outer cycle of imbricated elements and R-units forming basal plate and central mass of irregular elements	Helicosphaeraceae Pontosphaeraceae Zygodiscaceae
	Syracosphaerales Coccoliths with radial lath cycle of T-units, and disjunct, often complex, axial structure, coccospheres often polymorphic	Calciosoleniaceae Rhabdosphaeraceae Syracosphaeraceae
	Coccolith families inc sed Various heterococcolith groups not obviously related to the well-defined orders	Alisphaeraceae Papposphaeraceae Umbellosphaeraceae narrow rimmed placoliths Coccolith genera inc sed unidentified

Cenozoic and Modern Coccolithophores

http://ina.tmsoc.org/Nannotax3/index.php?dir=Coccolithophores&top=34&base=300

Coccolithophores

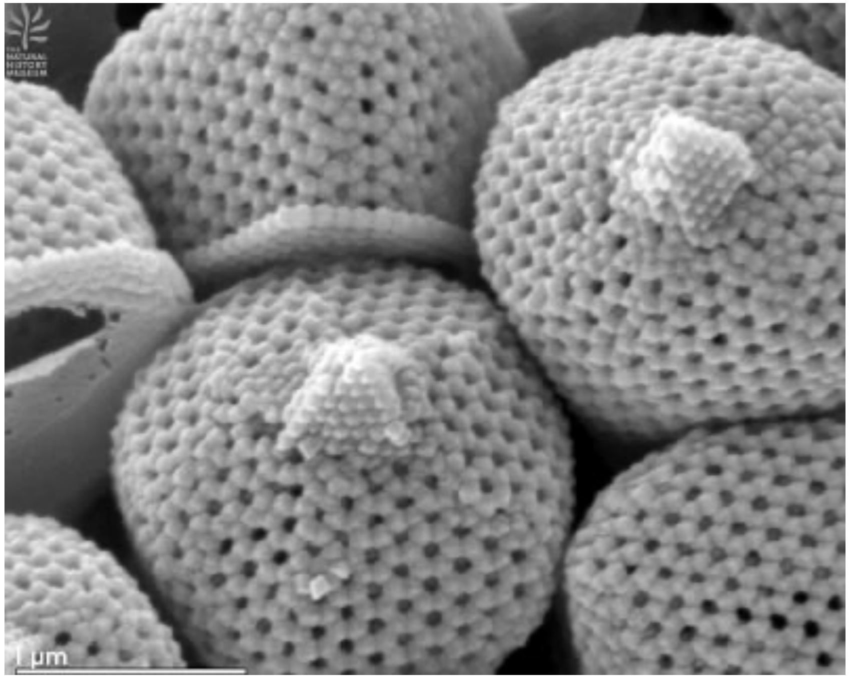


(A) *Coccolithus pelagicus*, (B) *Calcidiscus lepto*porus, (C) *Braarudosphaera bigelowii*, (D) *Gephyrocapsa oceanica*, (E) *E. huxleyi*, (F) *Discosphaera tubifera*, (G) *Rhabdosphaera clavigera*, (H) *Calciosolenia murrayi*, (I) *Umbellosphaera irregularis*, (J) *Gladiolithus flabellatus*, (K and L) *Florisphaera profunda*, (M) *Syracosphaera pulchra*, and (N) *Helicosphaera carteri*.
Scale bar, 5 µm.

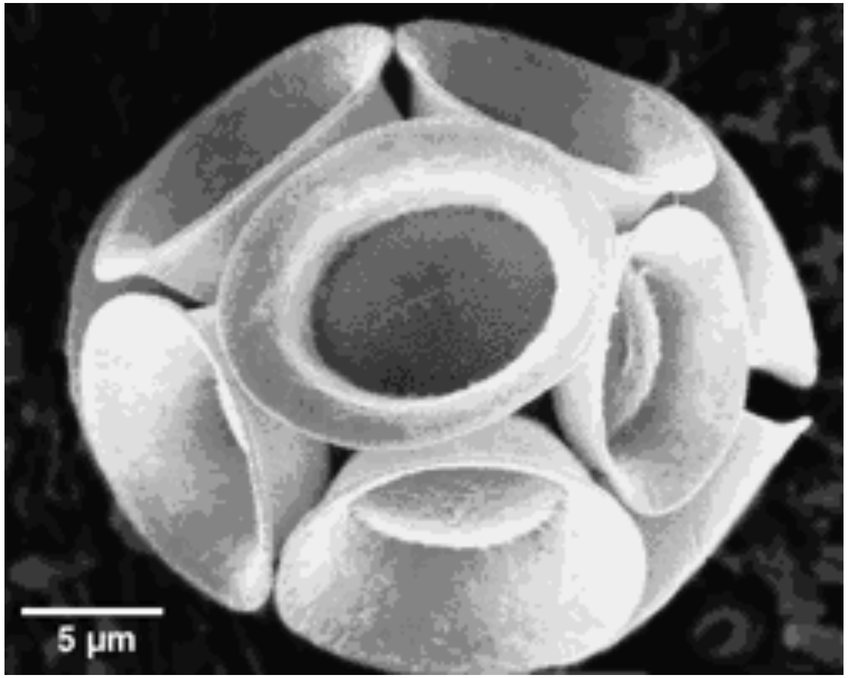
Monteiro et al. (2016), *Science Advances*

Coccolithophores

Holococcoliths: haploid cells; individual crystals have simple morphologies, no distinction between the rim and central area

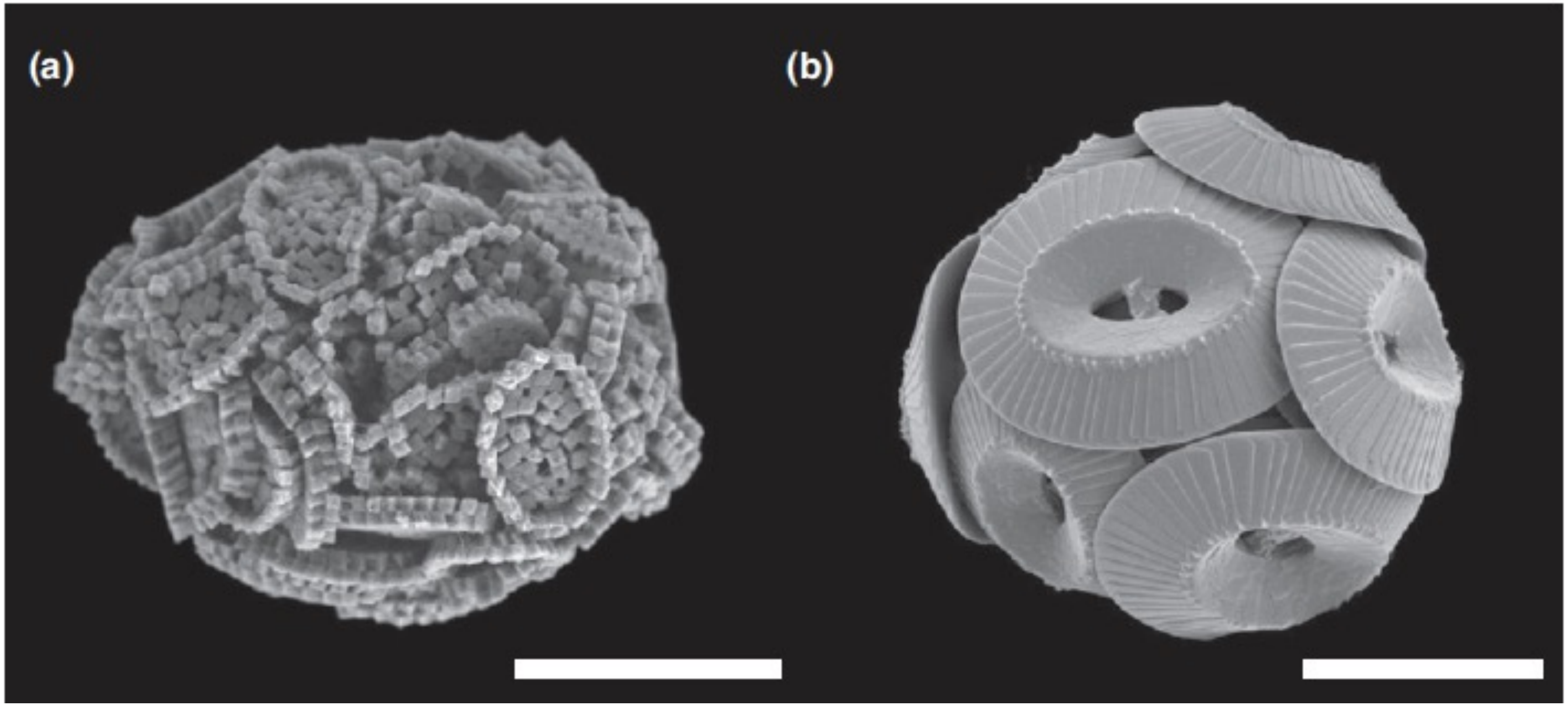


Heterococcoliths: diploid cells; complex morphologies, a rim of radial crystal units surrounding the central area



Pontosphaera syracusana

Coccolithophores

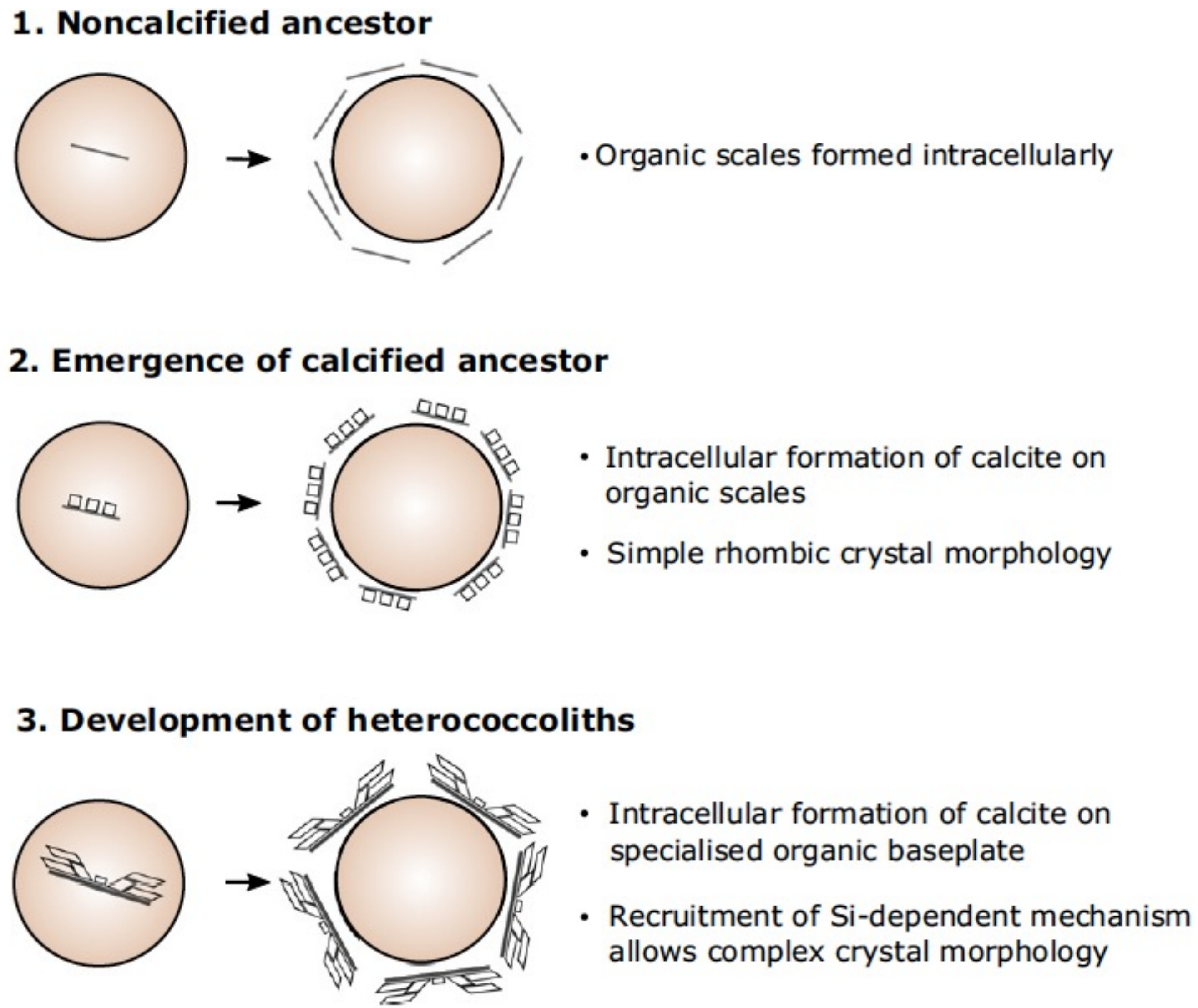


Coccolithus braarudii

Holococcoliths are comprised of identical rhombic crystals, whereas heterococcoliths are comprised of a variety of crystal sizes and shapes.

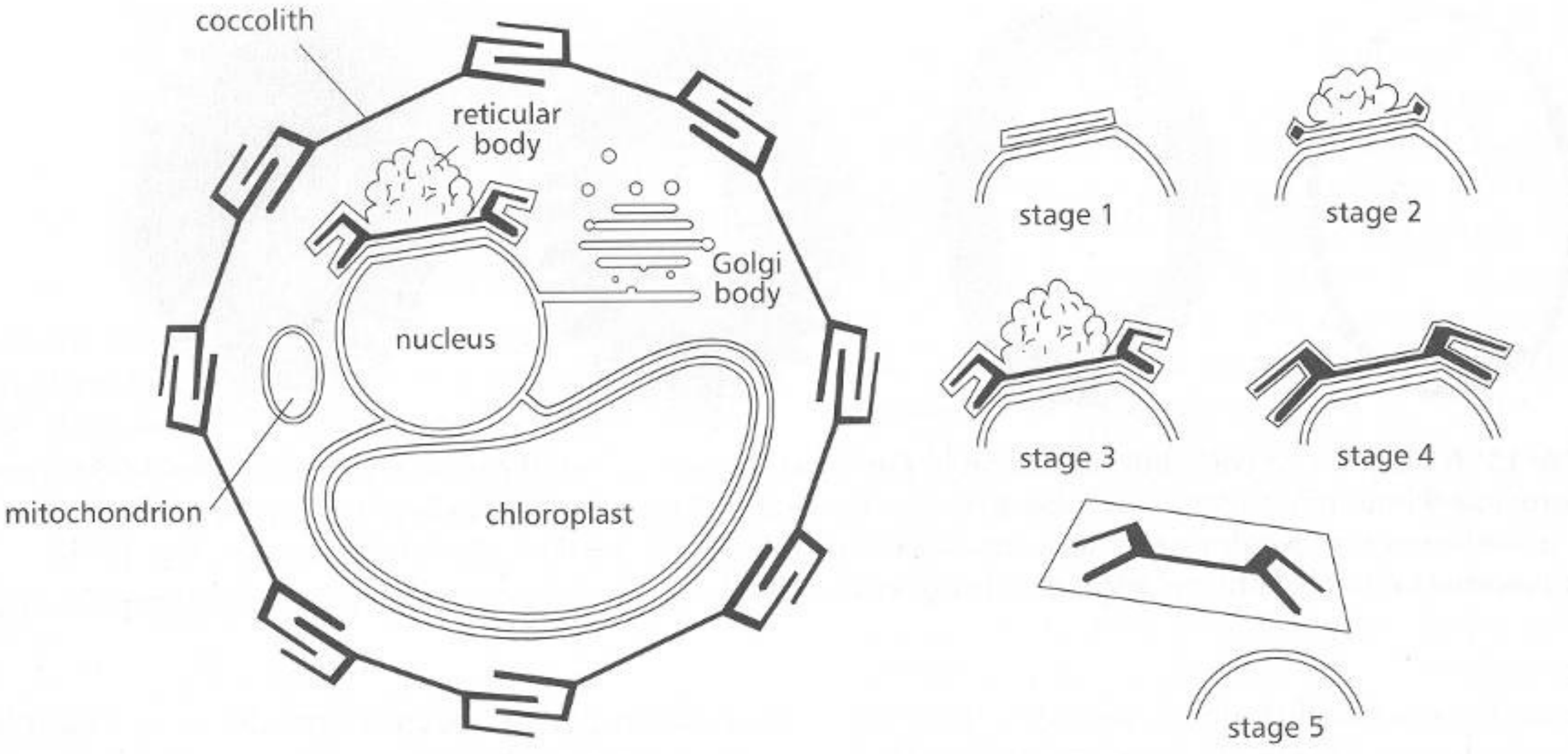
Langer et al. (2021), *New Phytologist*

Coccolithophores



Langer et al. (2021), *New Phytologist*

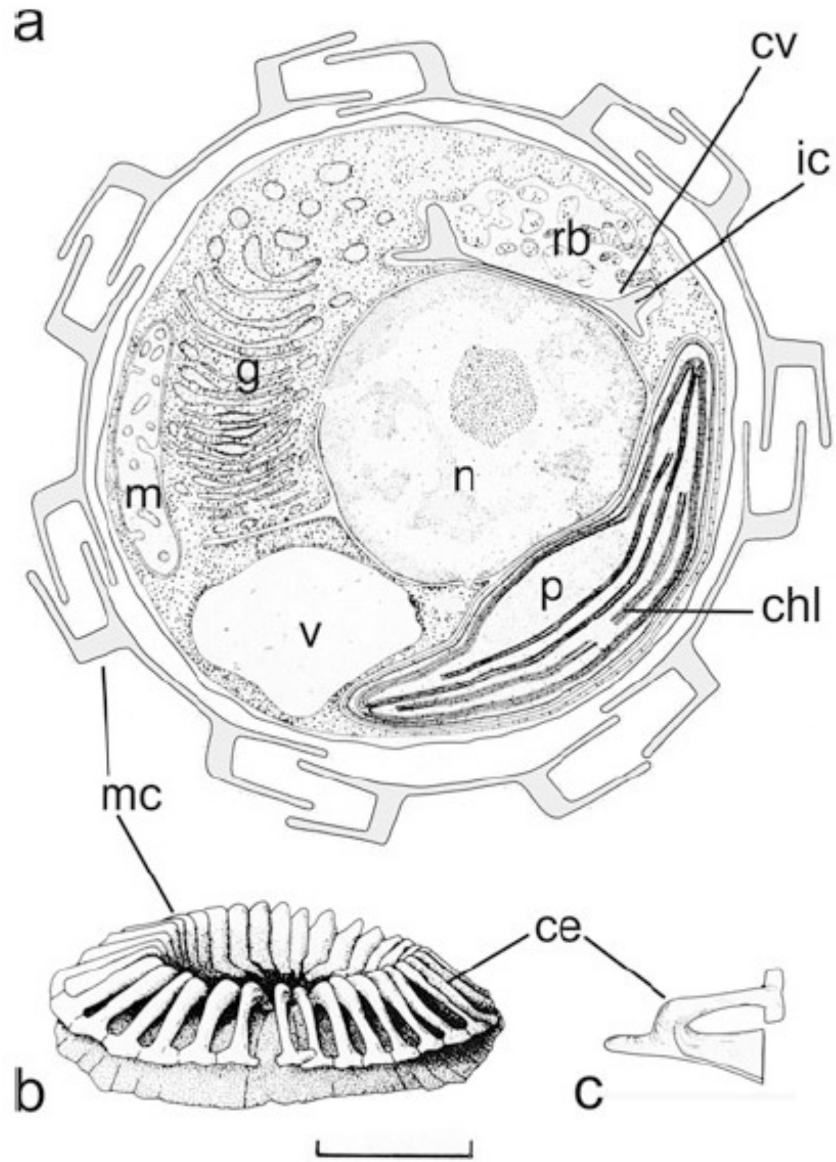
Coccolithophores



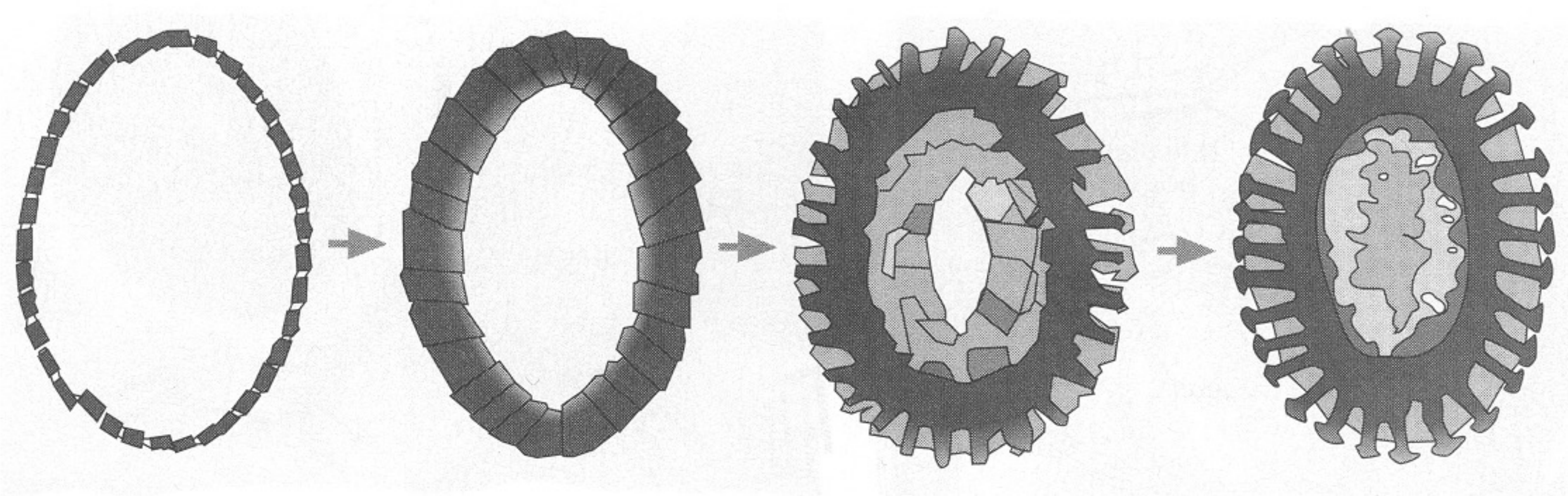
Biosynthesis of heterococcoliths: (1) Golgi vesicles fuse to form one big vesicle attached to the nuclear membrane, (2) thin organic lamella is formed inside the vesicle, reticular body (microtubular net) is attached outside, (3) calcification, (4) disappearing of reticular body, (5) complete coccolith is transferred to the cell surface and released outside.

Coccolithophores

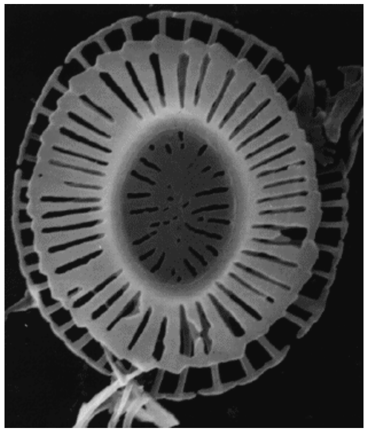
Schematic drawing *Emiliania huxleyi*: (a) transverse section of whole cell showing the chloroplast (chl), coccolith vesicle (cv), Golgi body (g), immature coccolith (ic), mature coccolith (mc), mitochondrion (m), nucleus (n), pyrenoid (p), reticulate body (rb), vacuole (v), (b) single mature coccolith showing elements arranged in an oval ring, (c) calcite element (ce) of a coccolith. Scalebar 1 µm

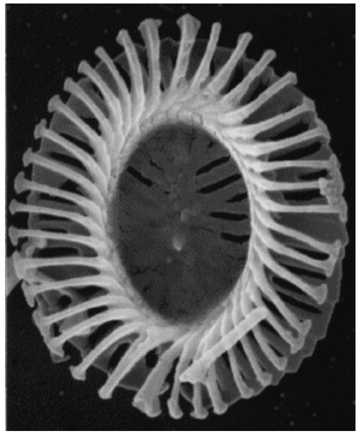


Coccolithophores



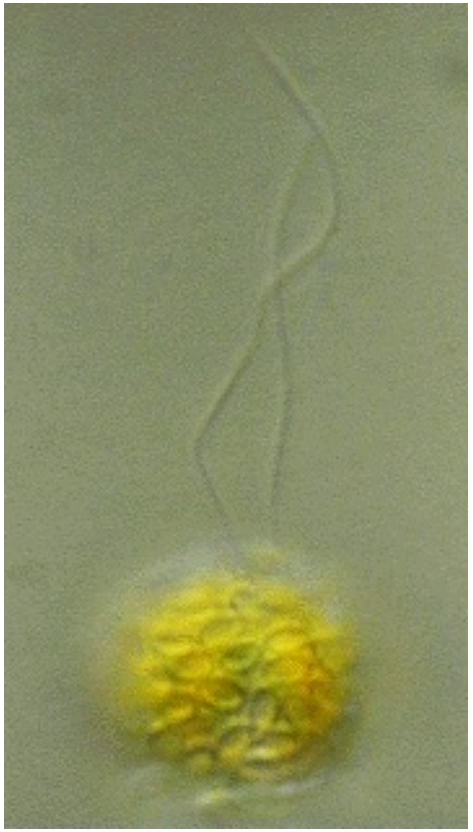
Biosynthesis of heterococcoliths
(*Emilianea huxleyi*)

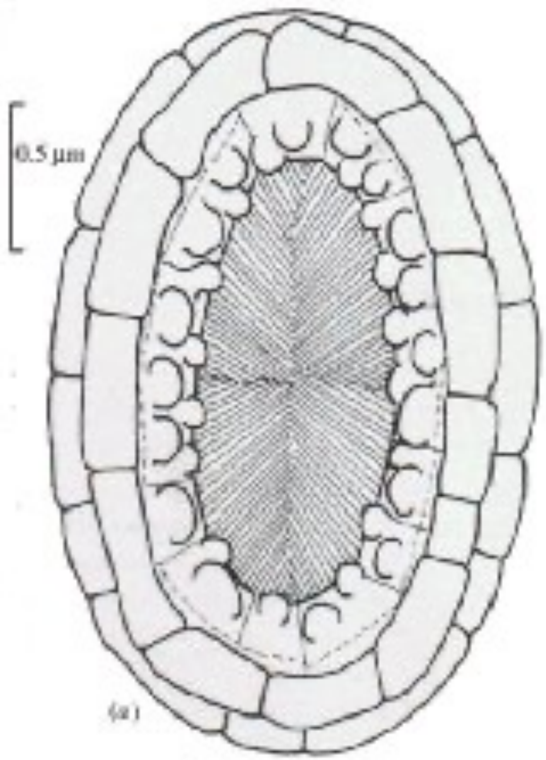




Coccolithophores

- calcified scales are usually formed in addition to underlying organic scales

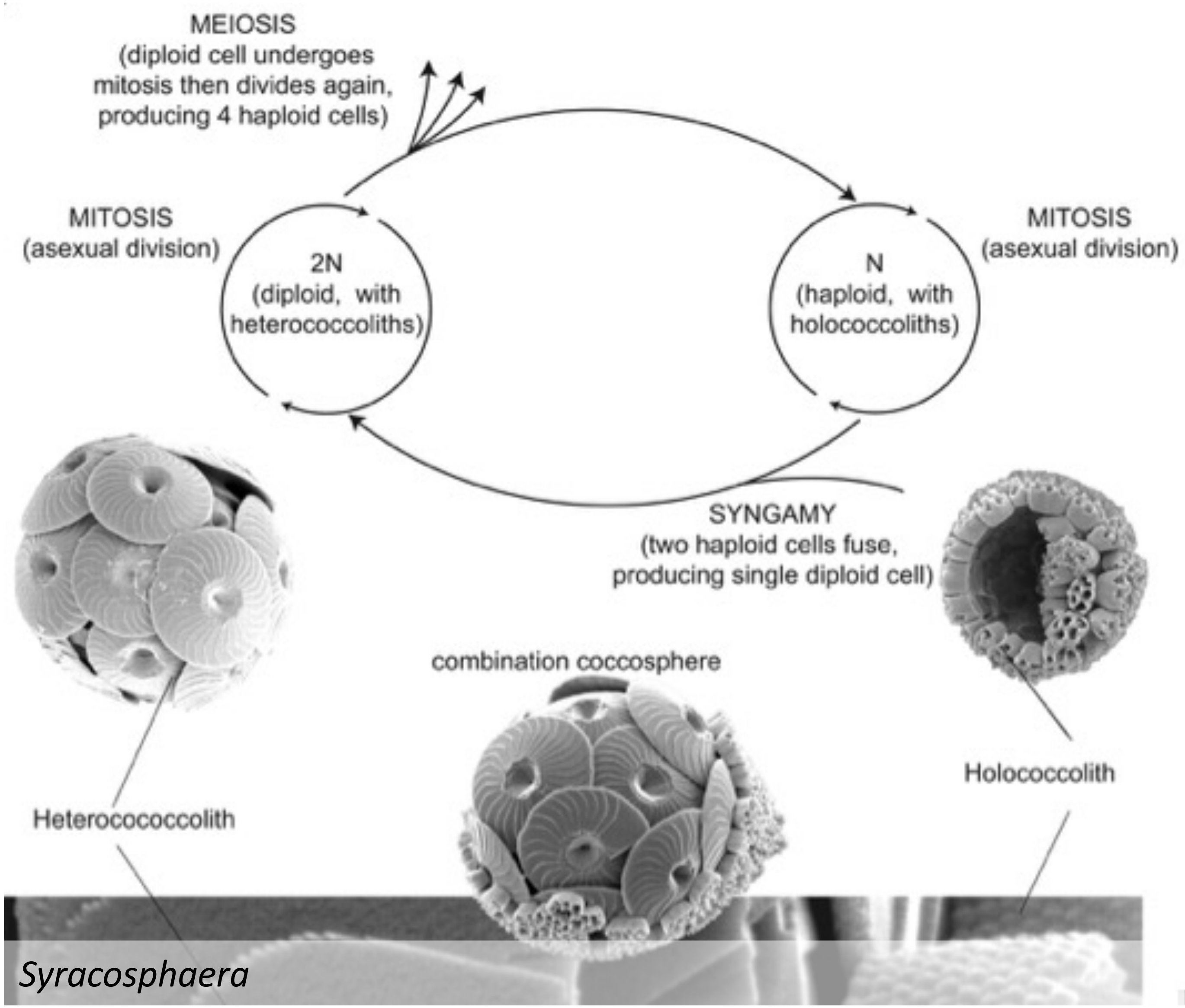




two calcite layers surround the polysaccharide scale

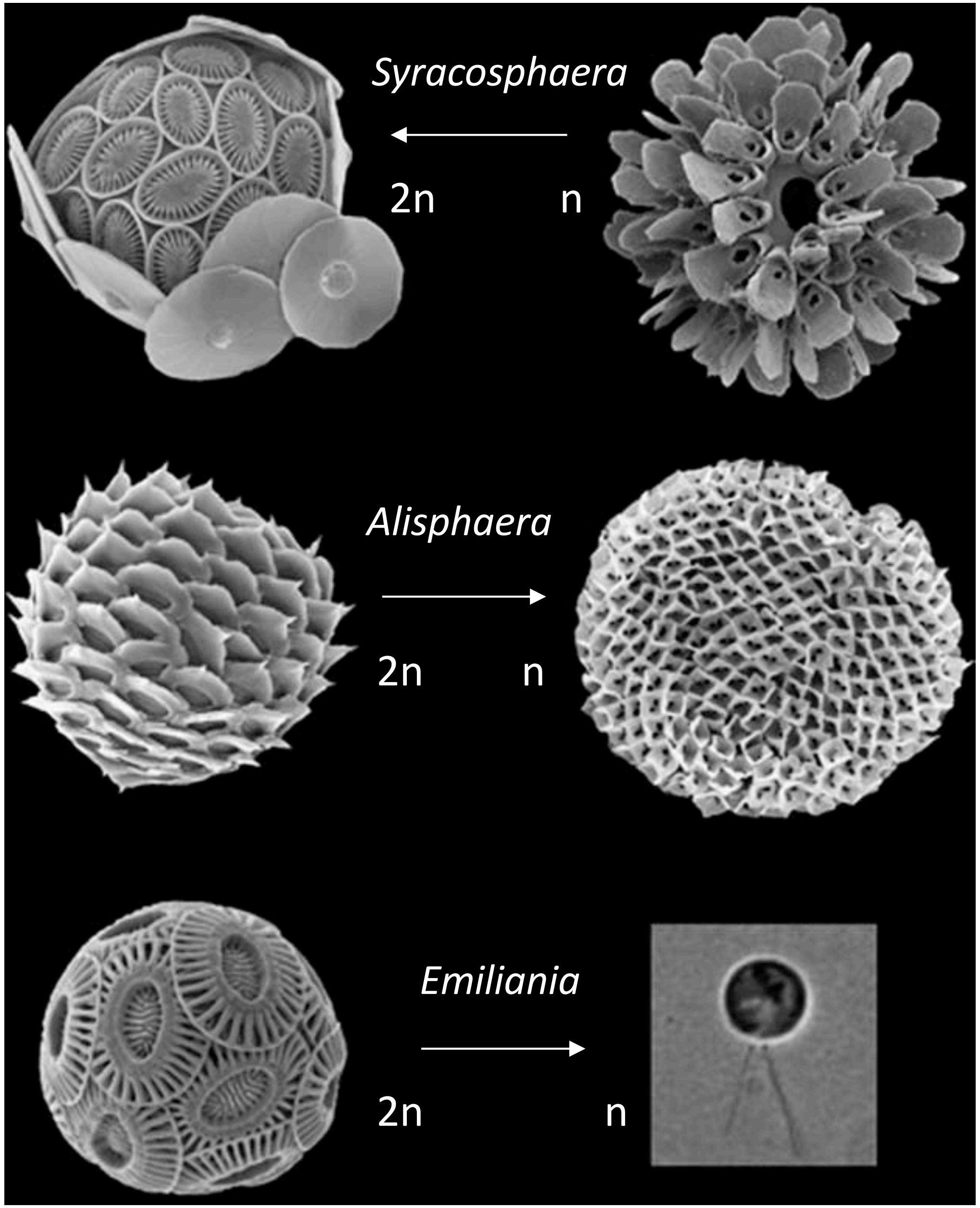
unmineralised scales are composed of microfibrils

Coccolithophores



Coccolithophores

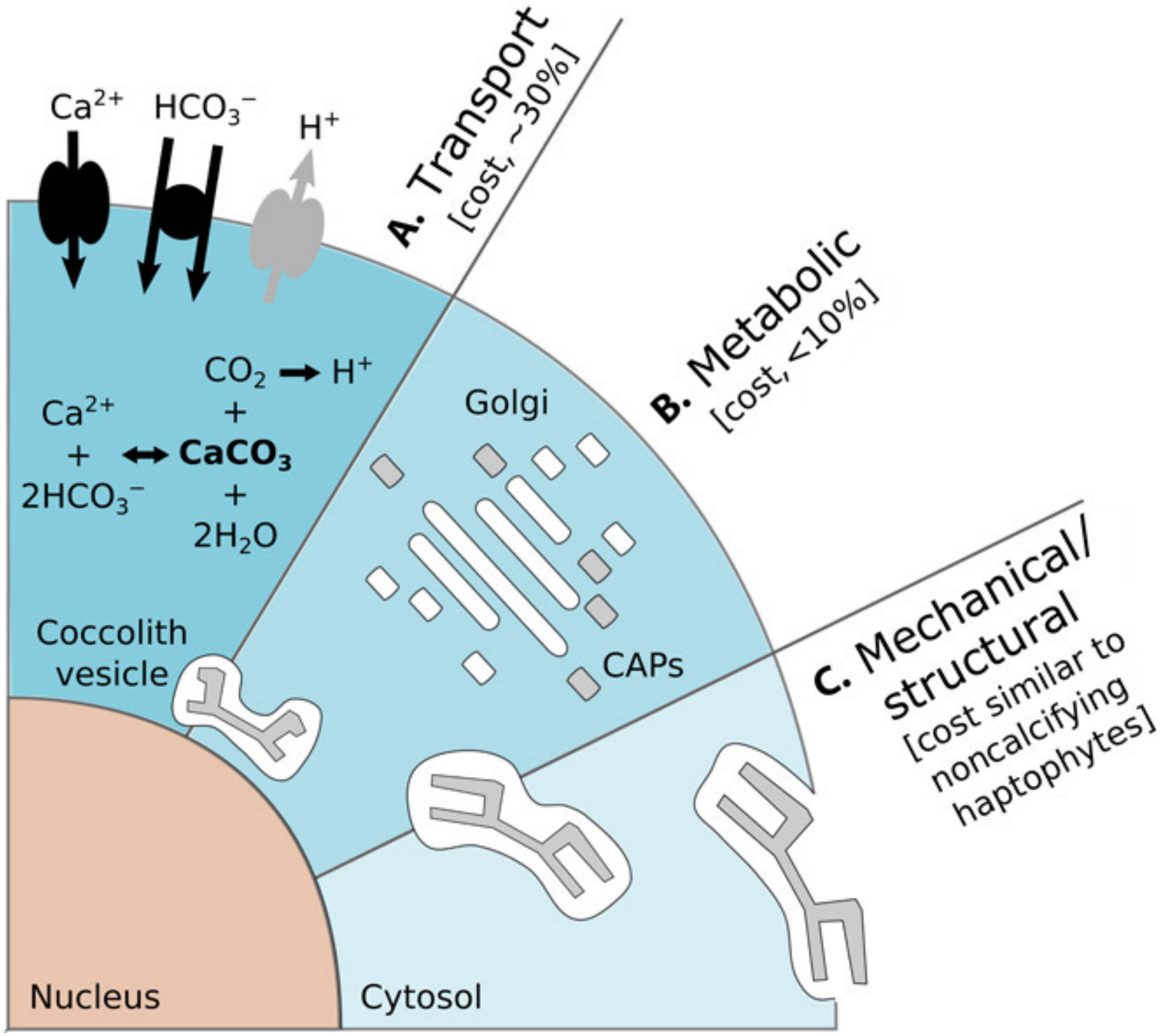
- holococcoliths and heterococcoliths occur on alternate phases of the life-cycle of single species
- it reflects a haplodiplontic life-cycle, with holococcoliths consistently occurring in the haploid phase
- nomenclature problems: a single species was occasionally described as separate species



de Vargas et al. (2007), *Evolution of Primary Producers in the Sea*

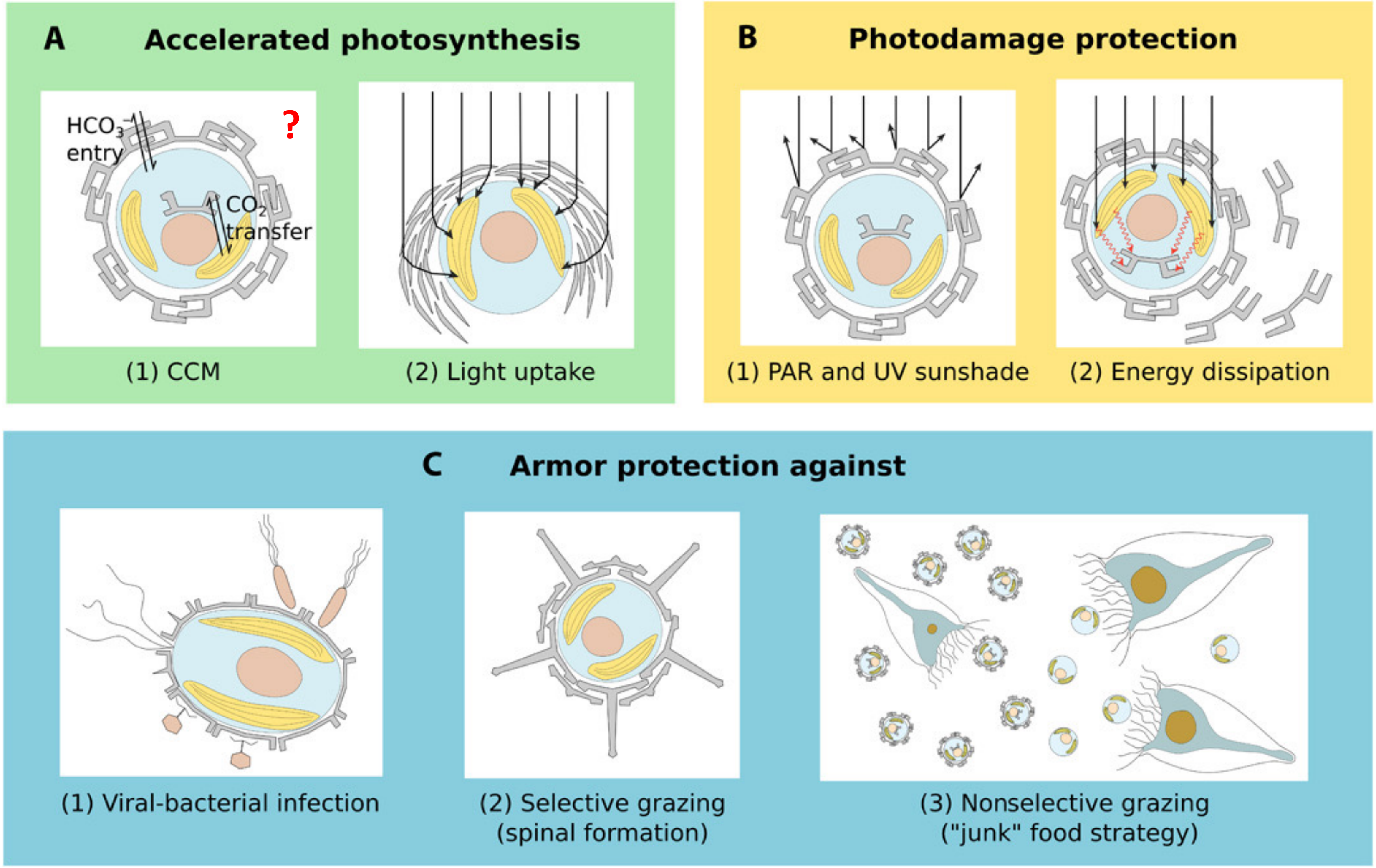
Coccolithophores

Schematic of the cellular processes associated with calcification and the approximate energetic costs of a coccolithophore cell.



Monteiro et al. (2016), *Science Advances*

Coccolithophores



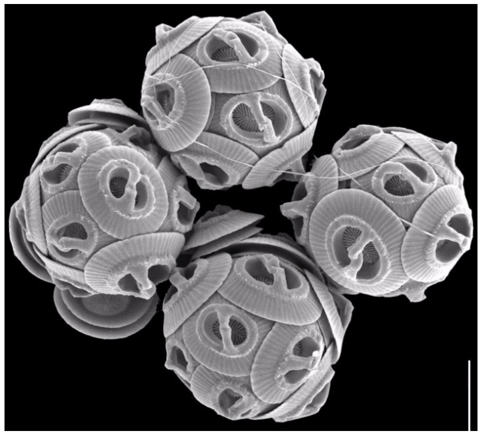
Proposed main benefits of calcification in coccolithophores.

Monteiro et al. (2016), *Science Advances*

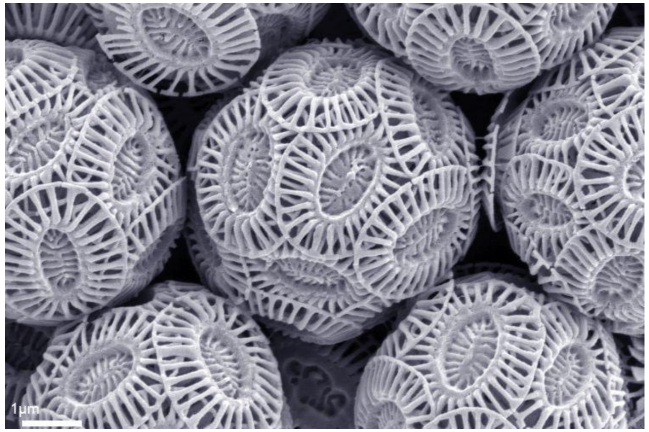
Coccolithophores

- global carbon cycle: fixing dissolved inorganic carbon (DIC) through photosynthesis and calcification
- sequester atmospheric CO_2 as limestone (Ca_2CO_3)
- white tides: blooms that frequently cover >100,000 km^2 in temperate and boreal regions

Satellite image of a bloom in the English Channel



Gephyrocapsa oceanica



Gephyrocapsa huxleyi

Coccolithophores

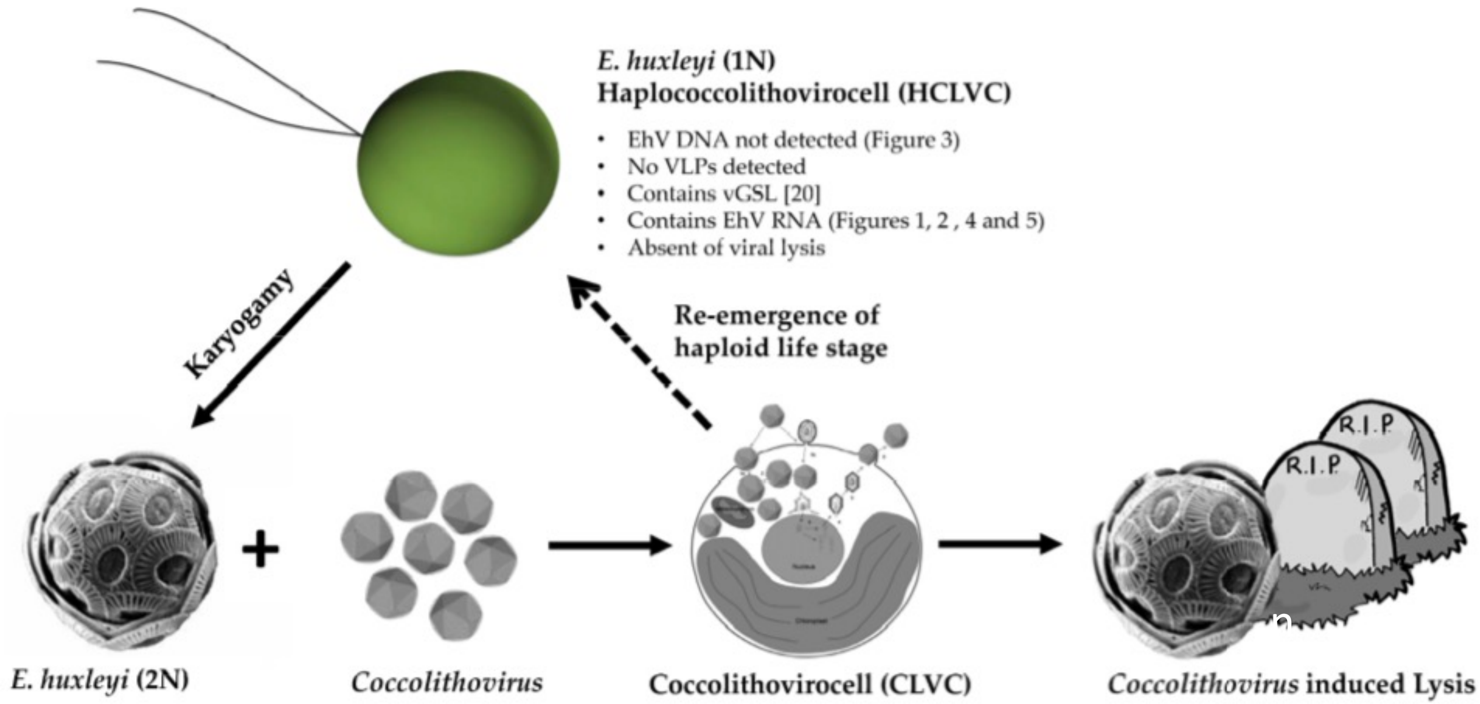
- *E. huxleyi*: photo- and phagotrophy, multilayers and monomorphic coccoliths; no trace of haptonema
- tend to bloom in the stratified surface layers in the Earth's oceans (not inhibited by high irradiances)
- white tide blooms follow seasonal succession cycles: after diatom bloom (low nutrient and silicate concentrations)
- *E. huxleyi*: tolerates nitrogen deprivation much better than diatoms; able to substitute Fe-containing enzymes
- mature blooms are typically lysed by specific viruses (phyco-dna-viruses)

- blooms change optical properties: coccoliths or entire coccospheres are shed and scatter (by the $CaCO_3$ crystals) the incoming light (turning surface waters turbid and milky)

Gephyrocapsa huxleyi

Coccolithophores

- *Emiliania huxleyi*: resistance to viral attack
- Cheshire cat escape strategy: haploid cells are thought to resist infection -> population as a new founding population
- Schrödinger's Cheshire Cat: the presence of viral RNA in the haploid cells -> an alternative stage of the virus life cycle, no evidence of viral cell lysis

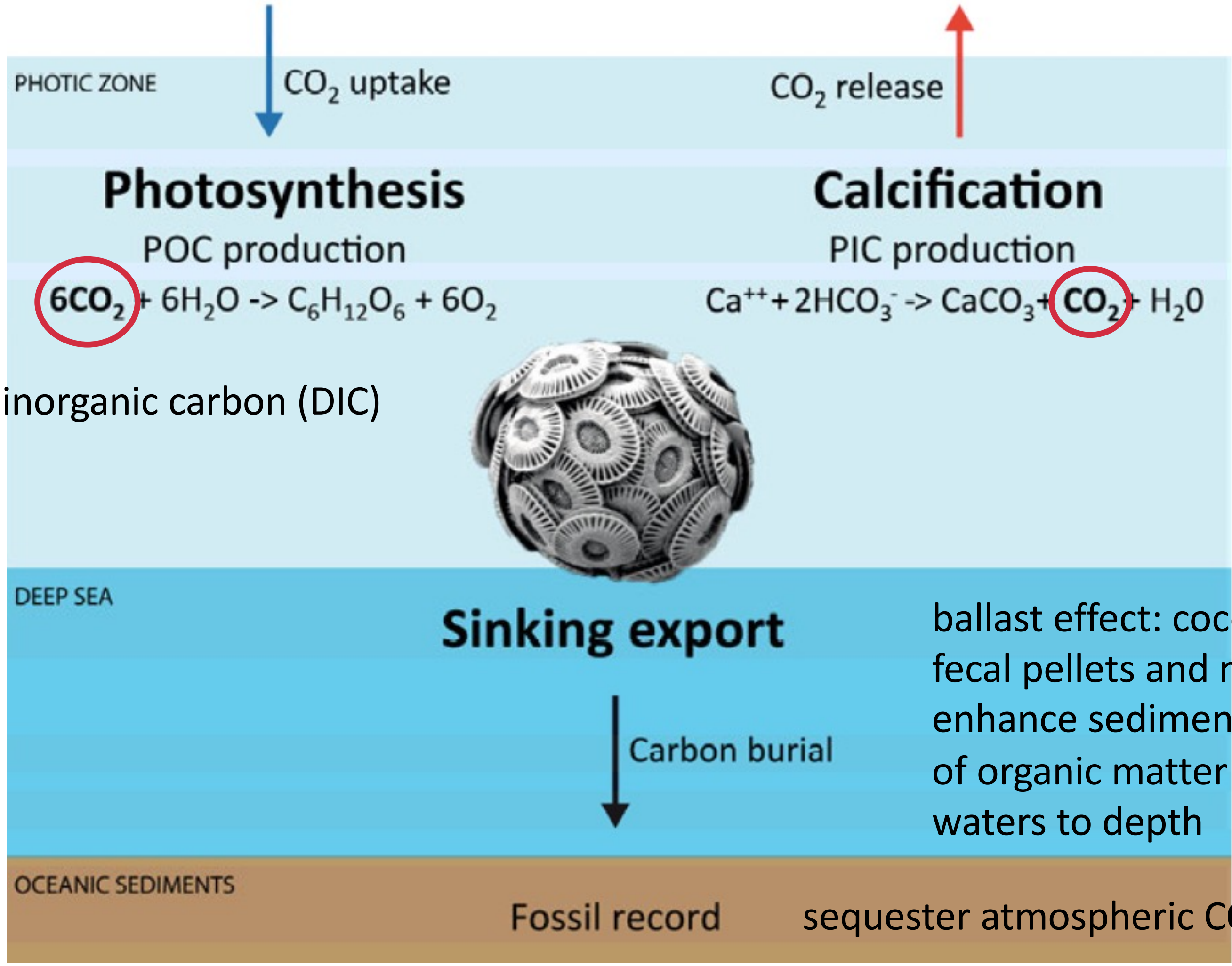


Mordecai et al. (2017), *Viruses*

Coccolithophores

organic carbon pump

carbonate counter pump



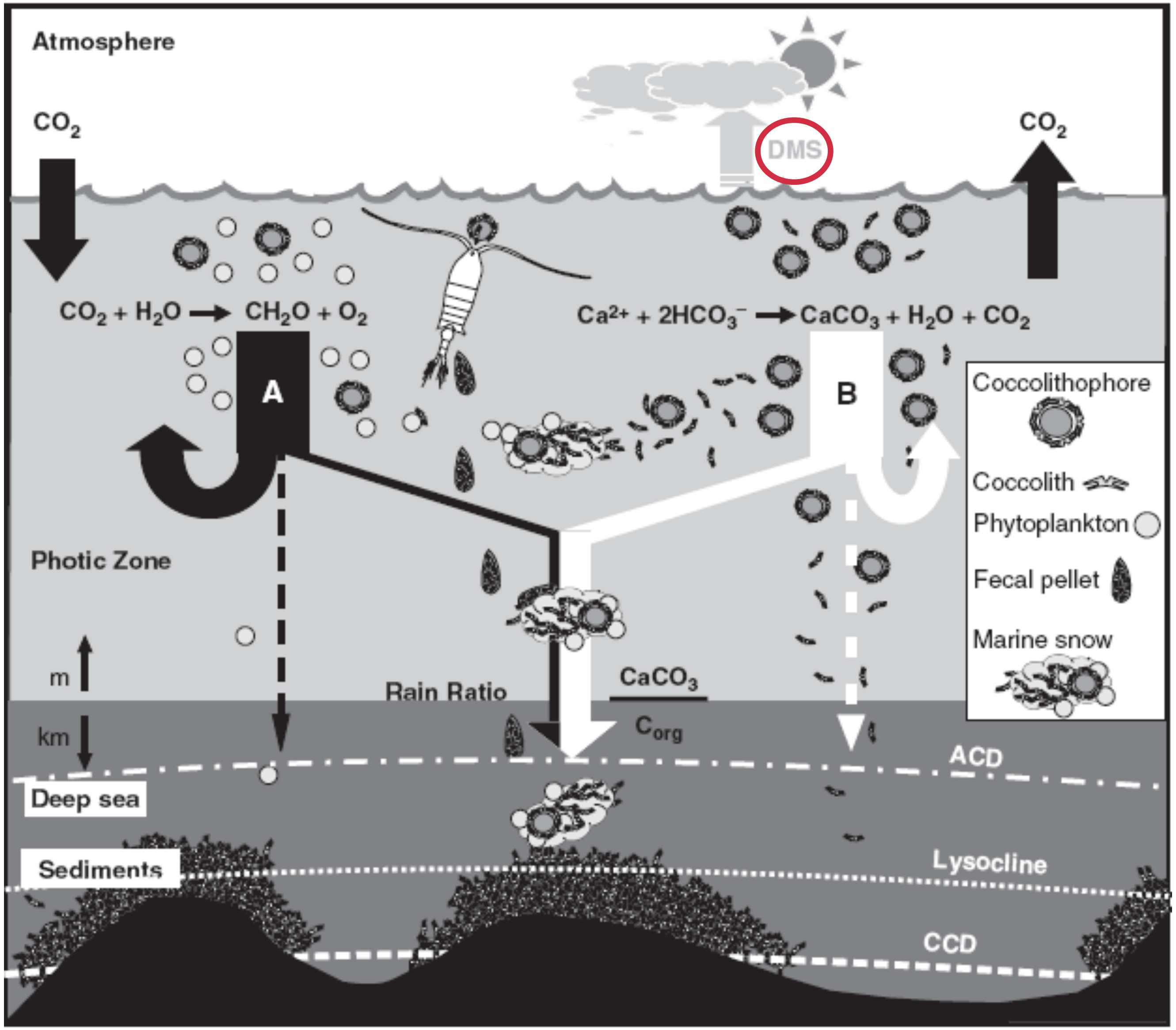
as dissolved inorganic carbon (DIC)

ballast effect: coccoliths in fecal pellets and marine snow enhance sedimentation rate of organic matter from surface waters to depth

sequester atmospheric CO_2 as limestone

Šupraha (2006), *Dissertation*

Coccolithophores



de Vargas et al. (2007), *Evolution of Primary Producers in the Sea*

DMS(P)

- marine phytoplankton: producers of dimethylsulfoniopropionate (DMSP)
- the most likely function of DMSP is related to the osmotic protection of the cell + antioxidant function + lysis of DMSP may be an effective chemical defense against grazing
- DMSP: degraded into dimethyl sulfide (DMS)
- DMS oxidation products act as (CCN) cloud condensation nuclei (atmospheric cloud formation)

TABLE 2. Intracellular concentration of DMSP in marine phytoplankton

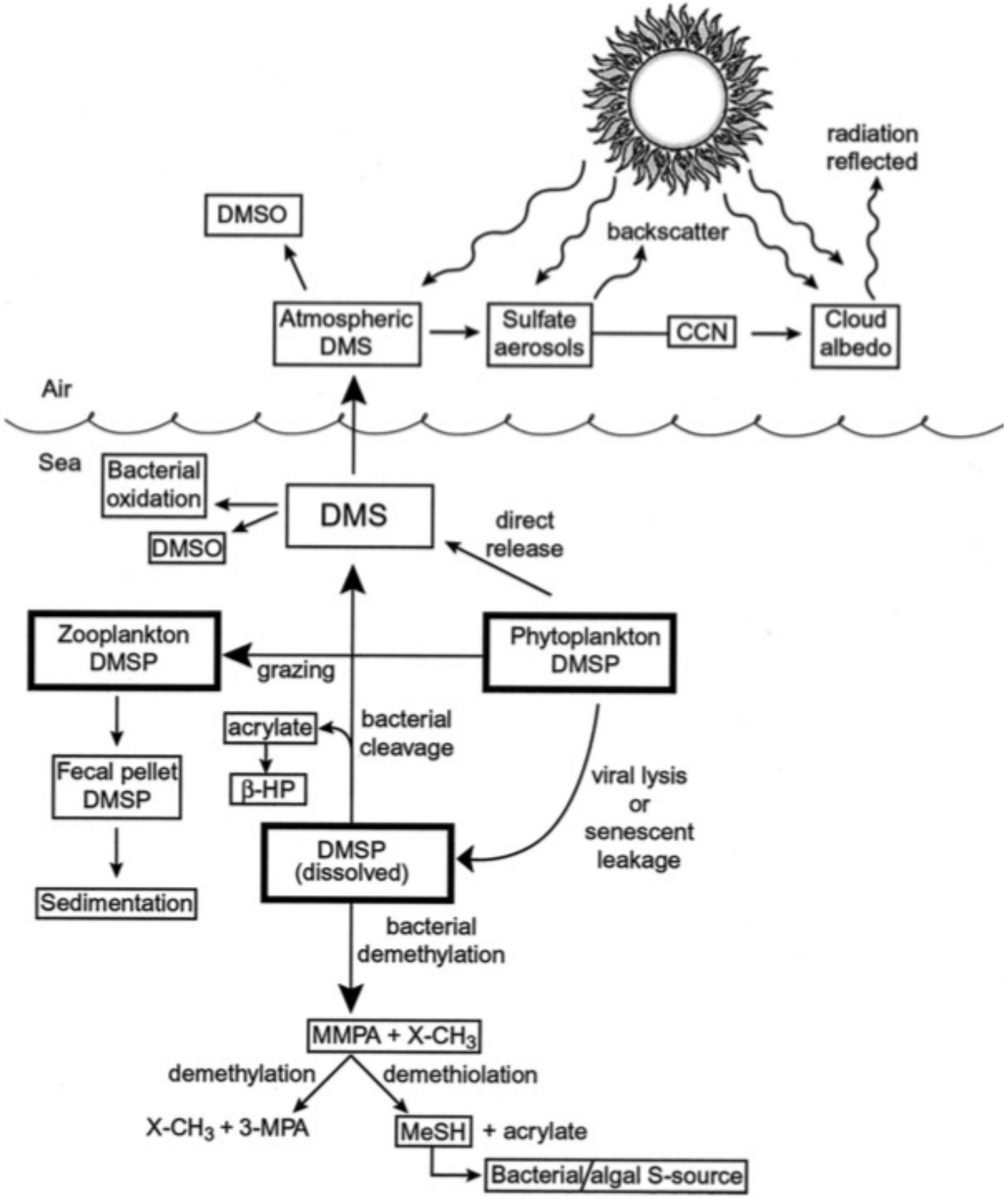
Organism	Cytosolic DMSP[a] (mM)	Reference
Hymenomonas carterae	120	Vairavamurthey et al. (120)
Gymnodinium nelsoni	280	Dacey and Wakeham (30)
Platymonas subcordiformis	170	Dickson and Kirst (40)
Phaeocystis sp.	71–169	Stefels and van Boekel (109)
Melosira numuloides (Diatoms)	264	Keller et al. (55)
Chrysameoba sp. (chyrosphyte)	596	
Ochromonas sp. (chrysophyte)	529	
Prorocentrum sp. strain IIB_2b_1 (dinoflagellate)	1,082[b]	
Emiliania huxleyi BT6 (coccolithophore)	166	
Diatoms (from sea water)	38 ± 18	Turner et al. (119)
<10-μm flagellates (from sea water)	125	Belviso et al. (15)
	650 ± 429	Turner et al. (119)
Dinoflagellates (from sea water)	640	Belviso et al. (15)
	70 ± 30	Turner et al. (119)

[a] Per liter of cell volume.
[b] A reviewer of this article pointed out that "seawater osmolarity is about 0.7 M, and DMSP is never the only osmolyte, so its intracellular concentrations can't be >1 M."

Yoch (2002), *Applied Environmental Microbiology*

DMS(P)

- DMSP cleavage to DMS plays an important biogeochemical role in the global sea-to-land transfer of sulfur
- it has a potential effect on remote oceanic weather patterns due to cloud formation -> reflection of solar radiation
- white tides: increasing of planetary albedo (i.e., increased light reflection) -> cooling down the Earth

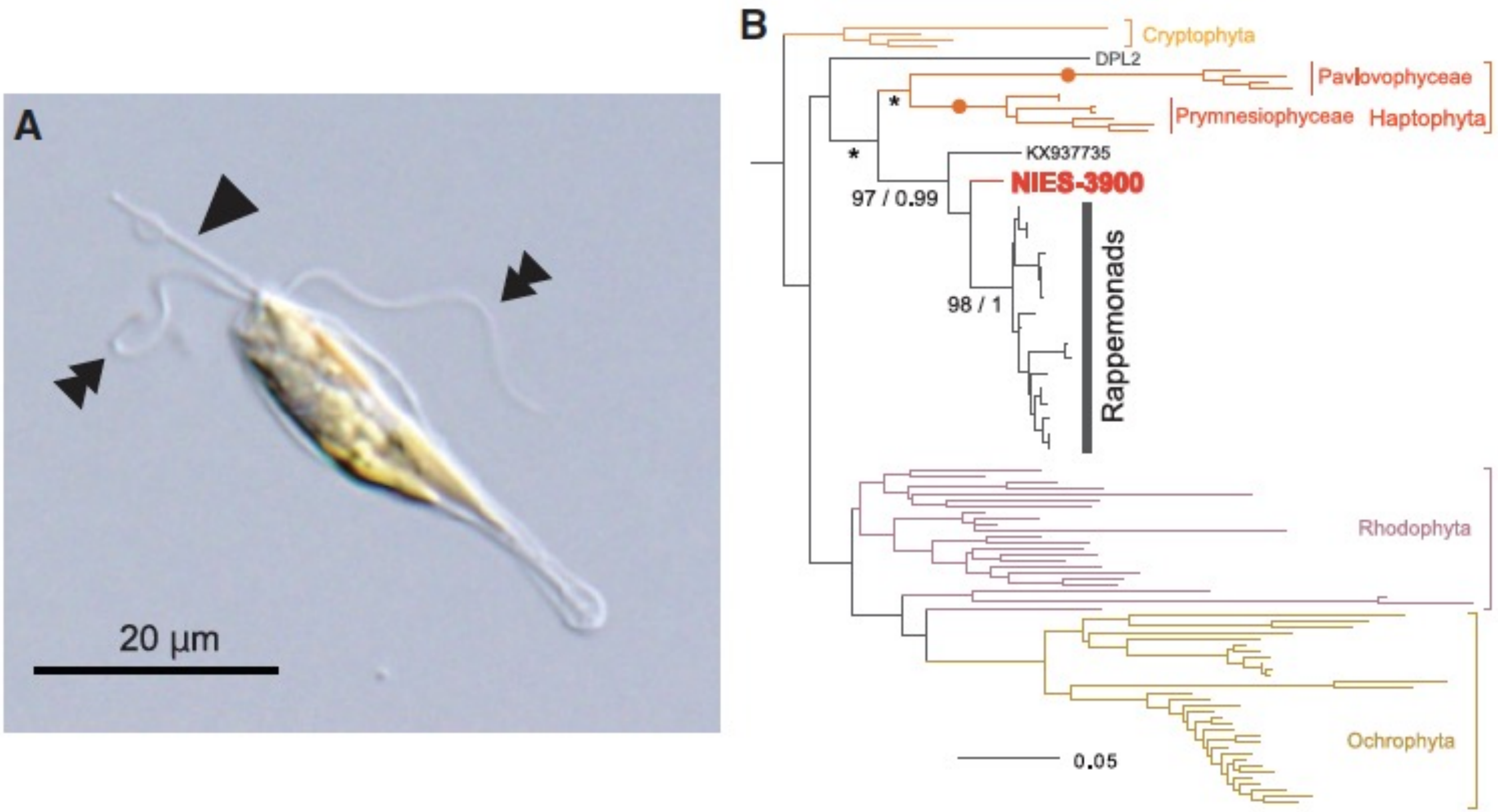


Yoch (2002), *Applied Environmental Microbiology*

Haptophyta

- feeding of copepods, shellfish, aquaculture of bivalves, crustaceans, and fish
- nuisance or toxic blooms: serious economic losses
- paleolimnology: past climatic conditions, sedimentary rocks and ocean sediments composed of coccoliths
- climatic feedbacks? may affect planetary albedo and concentration of CO_2

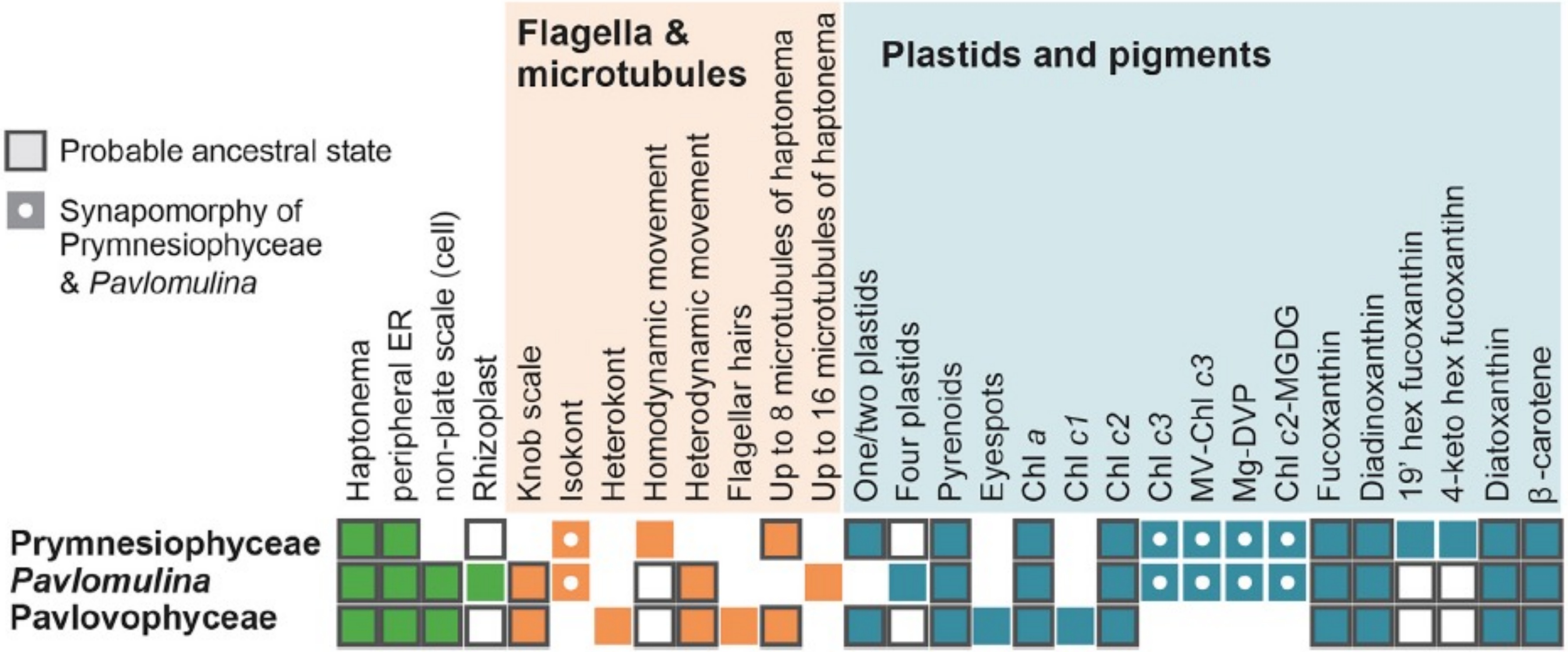
+ class Rappephyceae



NIES-3900 = *Pavlomulina ranunculiformis*

Kawachi et al. (2021), *Current Biology*

Rappephycea

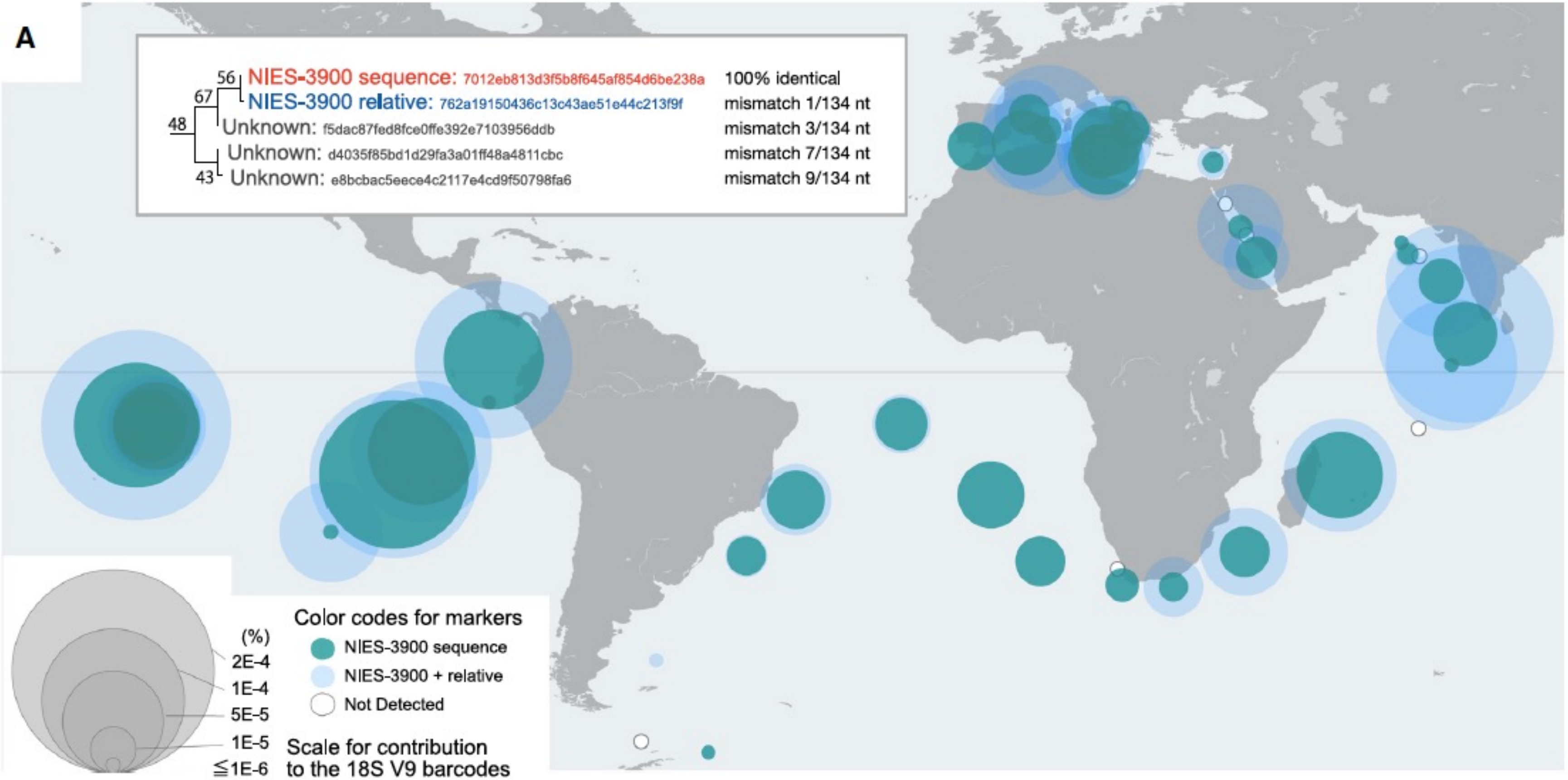


-> class: Rappephyceae

Kawachi et al. (2021), *Current Biology*

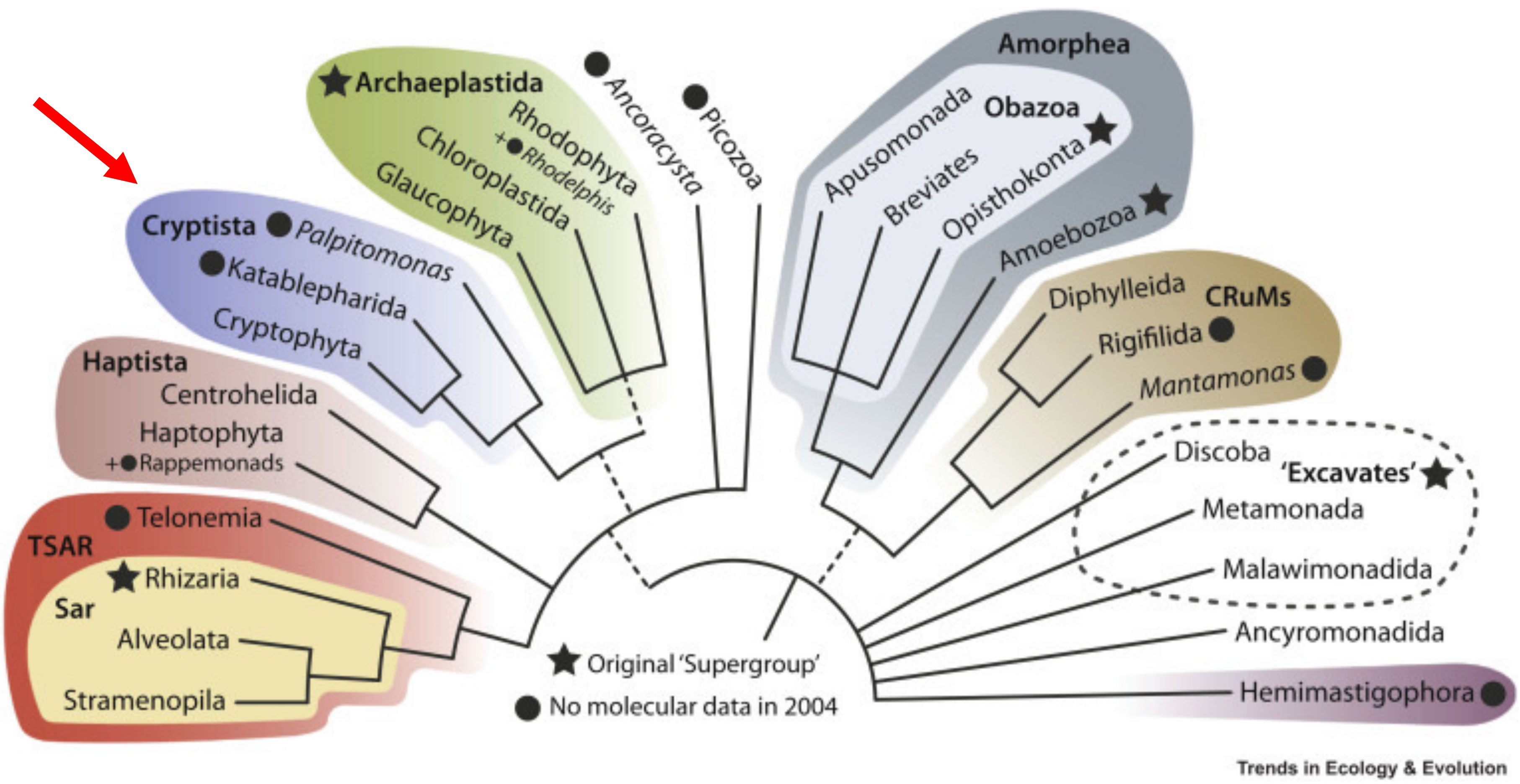
Rappephycea

NIES-3900 = *Pavlomulina ranunculiformis*



Rappephyceae have a cosmopolitan distribution, and an abundance comparable to or greater than the two previously known haptophyte classes.

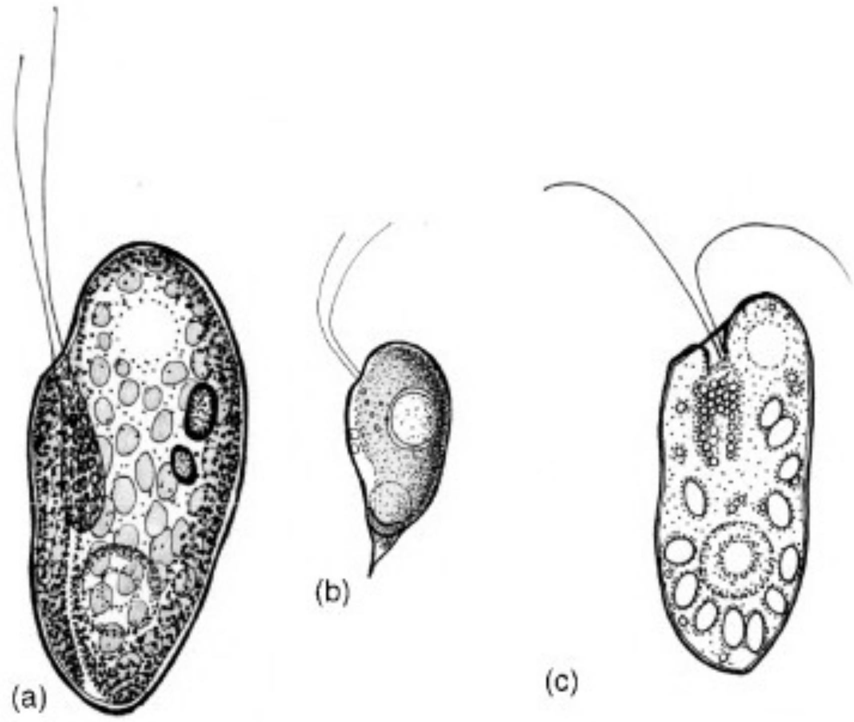
Cryptista

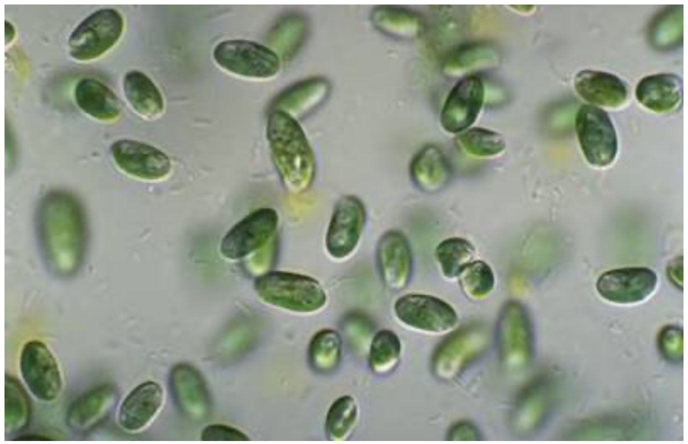


Sister taxa

Burki et al. (2020), *Trends in Ecology end Ecolution*

Cryptomonads





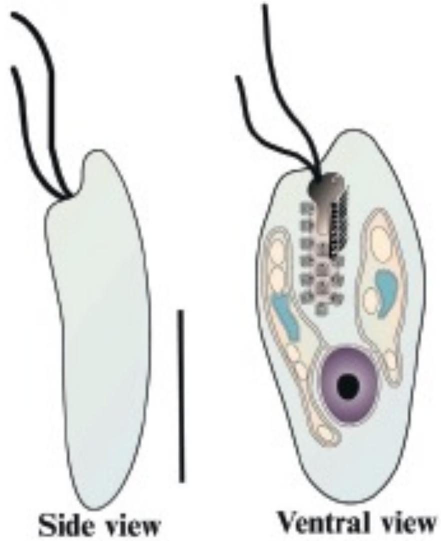
https://m.youtube.com/watch?v=g6kXduiDrF0

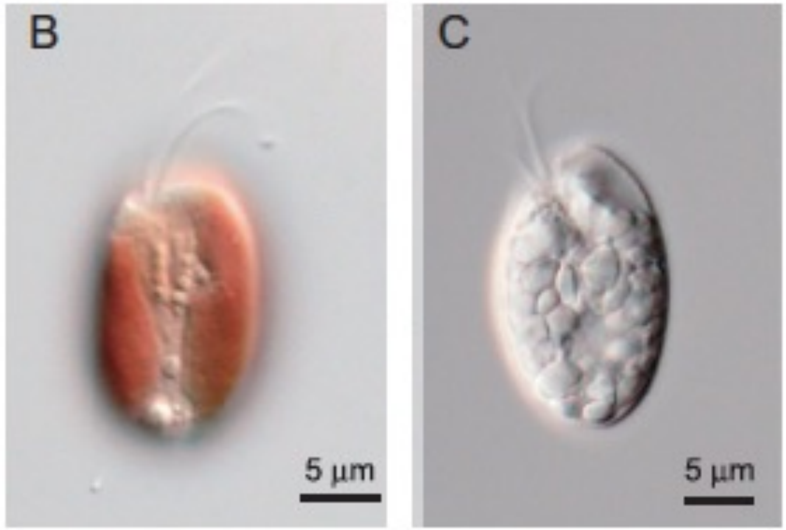
https://m.youtube.com/watch?v=iiMaUefa_3g

Cryptista – Cryptophyta - Cryptophyceae

Cryptophyta

- unicellular free-living flagellates [palmeloid forms are also known]
- planktonic and photoautotrophic [may be mixotrophic, some exclusively heterotrophic with leucoplast], vertical migration, low-light specialists
- ubiquitous in the earth's hydrosphere: marine, brackish, and freshwater
- flattened asymmetric shape of cells, distinctive swimming motion (the cells rotates about its longitudinal axis), refractile ejectosomes (light microscopically visible)
- diverse coloration (chocolate brown, olive brown, bright brown, tomato red, dark orange, blue green, sky blue)





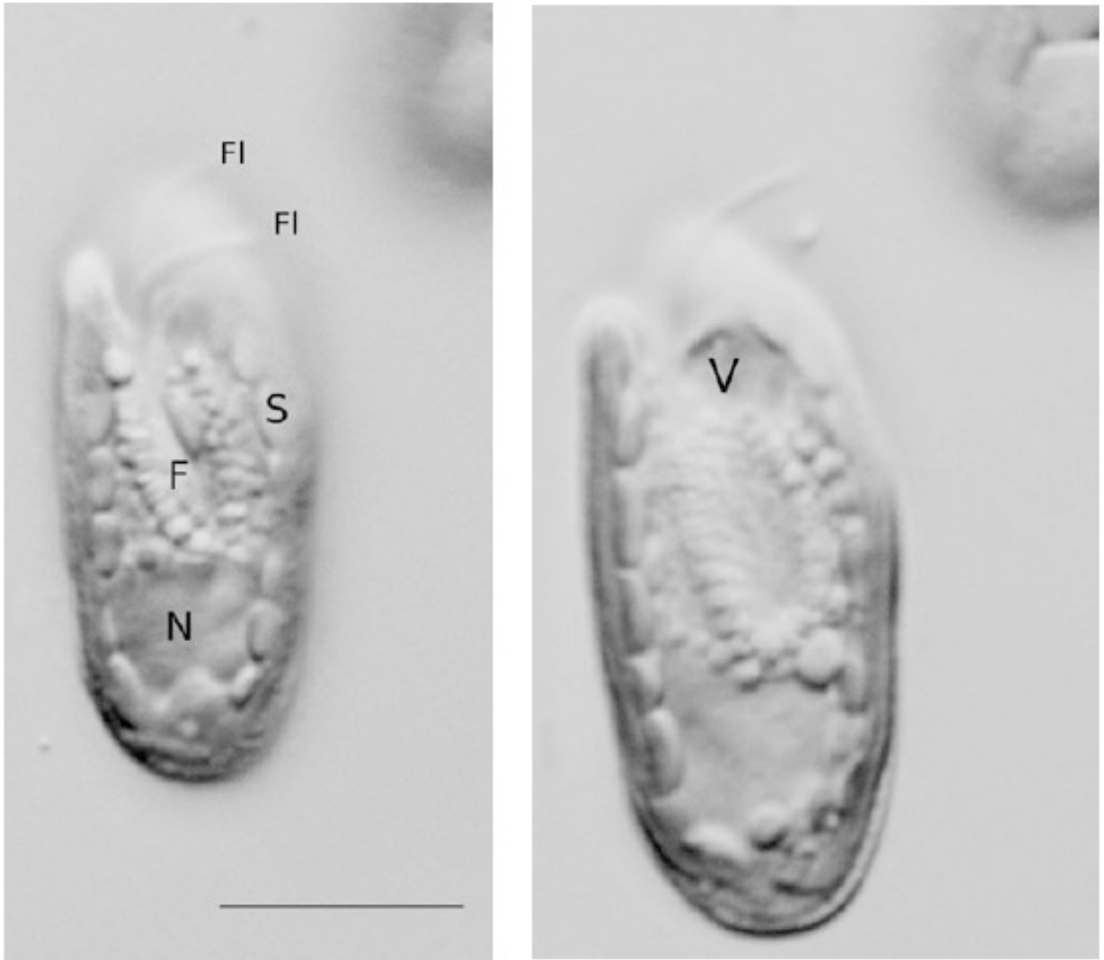
*Cryptomonas curva*ta and *C. paramecium*

Tanijufi & Onodera (2017), *Advances in Botanical Research*

Cryptophyta

LEFT image: the ventral side of the cell, showing the elongate open furrow and approximate insertion site of the two flagella. The elliptical platelets lining the cell periphery are starch grains.

RIGHT image: plane of focus in the cell middle: the ejectosome-lined gullet.

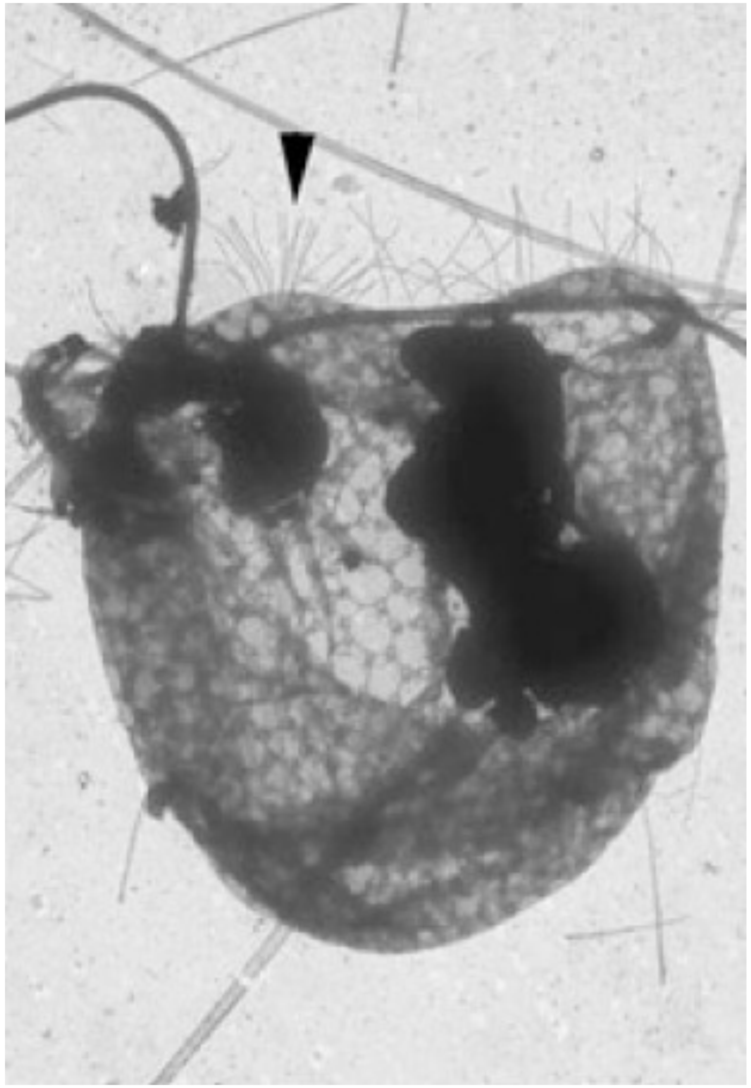


Cryptomonas borealis, the same cell*:* **Fl** - flagella; **S -** starch grain; **F –** furrow-gullet system; **N** - nucleus; **V** - contractile vacuole

Hoef-Emden & Archibald (2017), *Handbook of the Protists*

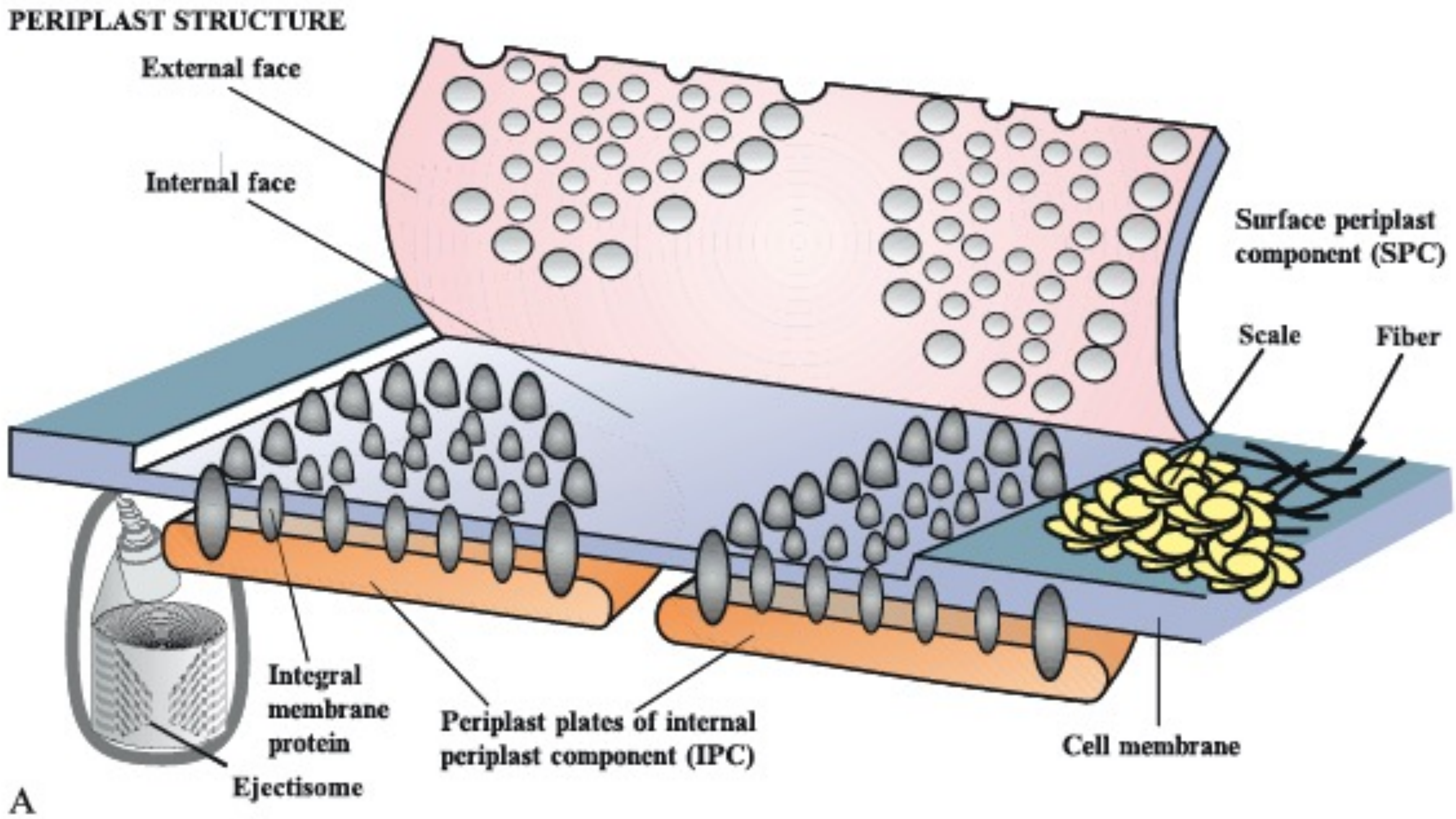
Cryptophyta

- **periplast**: composed of proteinaceous inner (IPC) and surface (SPC) layers that sandwich the plasma membrane (no true cell wall)
- the surface periplast component (SPC): covered by congruent plates, rosulate scales, and/or a fibrous coat
- the inner periplast component (IPC): hexagonal, polygonal, rectangular, or elongate plates; or may be continuous and sheetlike
- scales also in association with flagella



Hoef-Emden & Archibald (2017), *Handbook of the Protists*

Cryptophyta

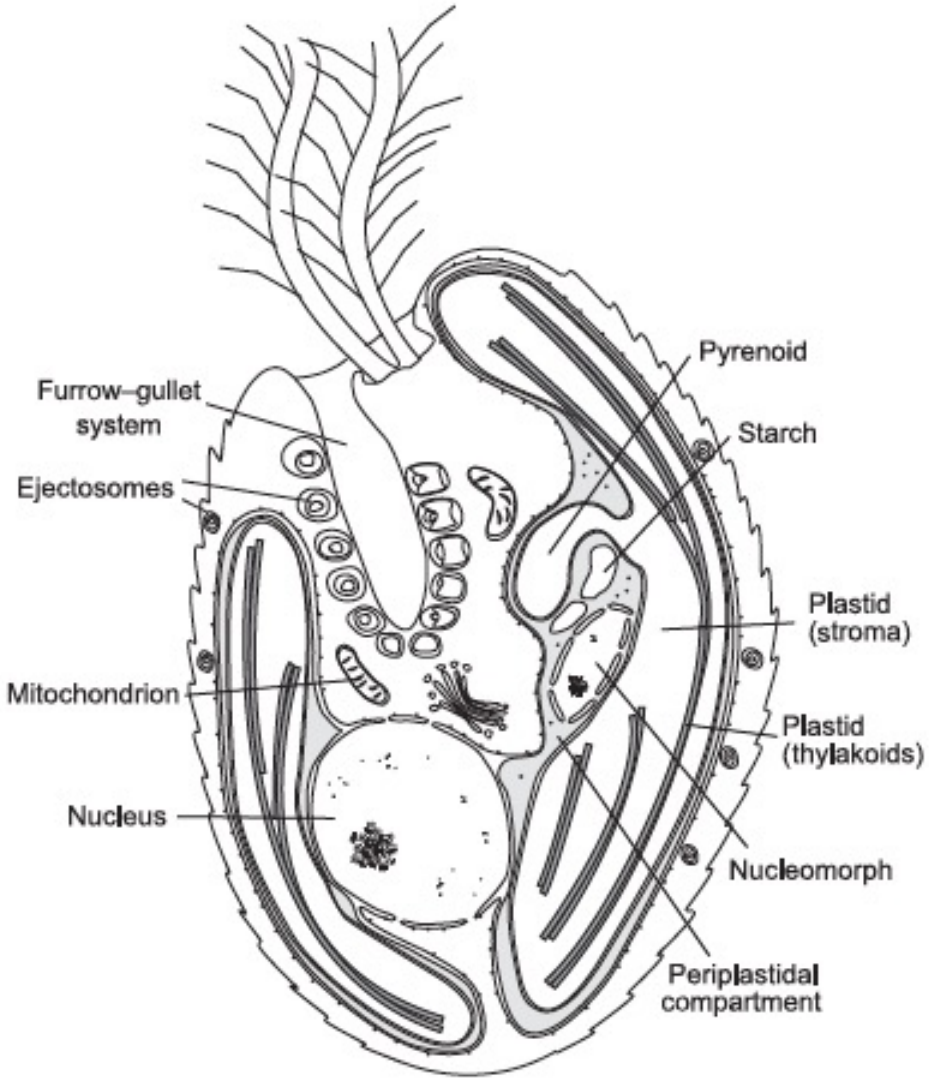


Periplast

Clay (2015), *Freshwater Algae of North America*, Elsevier

Cryptophyta

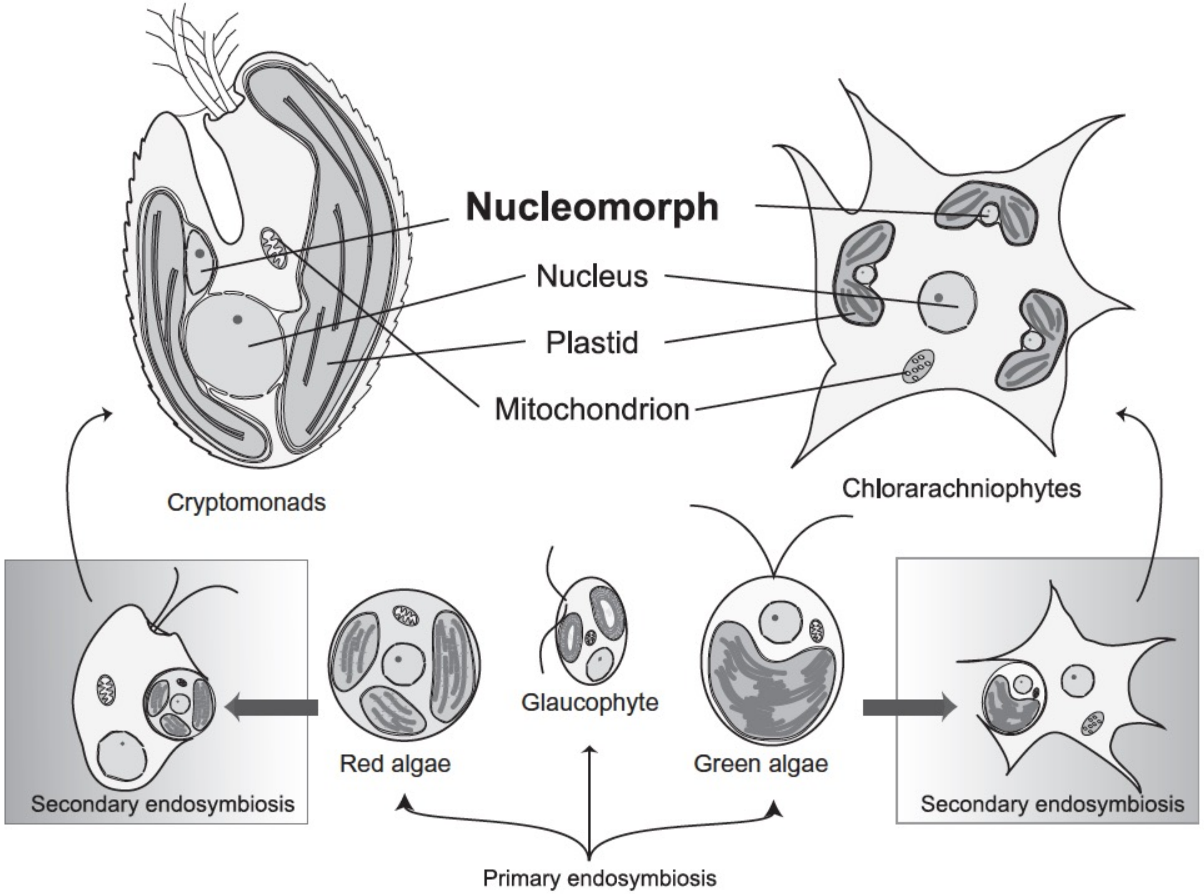
- two slightly unequal flagella in the vestibulum of an invagination = the furrow-gullet system; covered with bipartite flagellar hairs
- usually one H-shaped plastid per cell, a single pyrenoid [missing on some species]
- red-algal derived plastid; four membranes (the inner pair - stroma and the outer pair - the plastid ER, which is continuous with the nuclear envelope)
- expanded space = periplastidial compartment (inside: starch grains, pyrenoid, 80S ribosomes, nucleomorph)



Tanijufi & Onodera (2017), *Advances in Botanical Research*

Cryptophyta

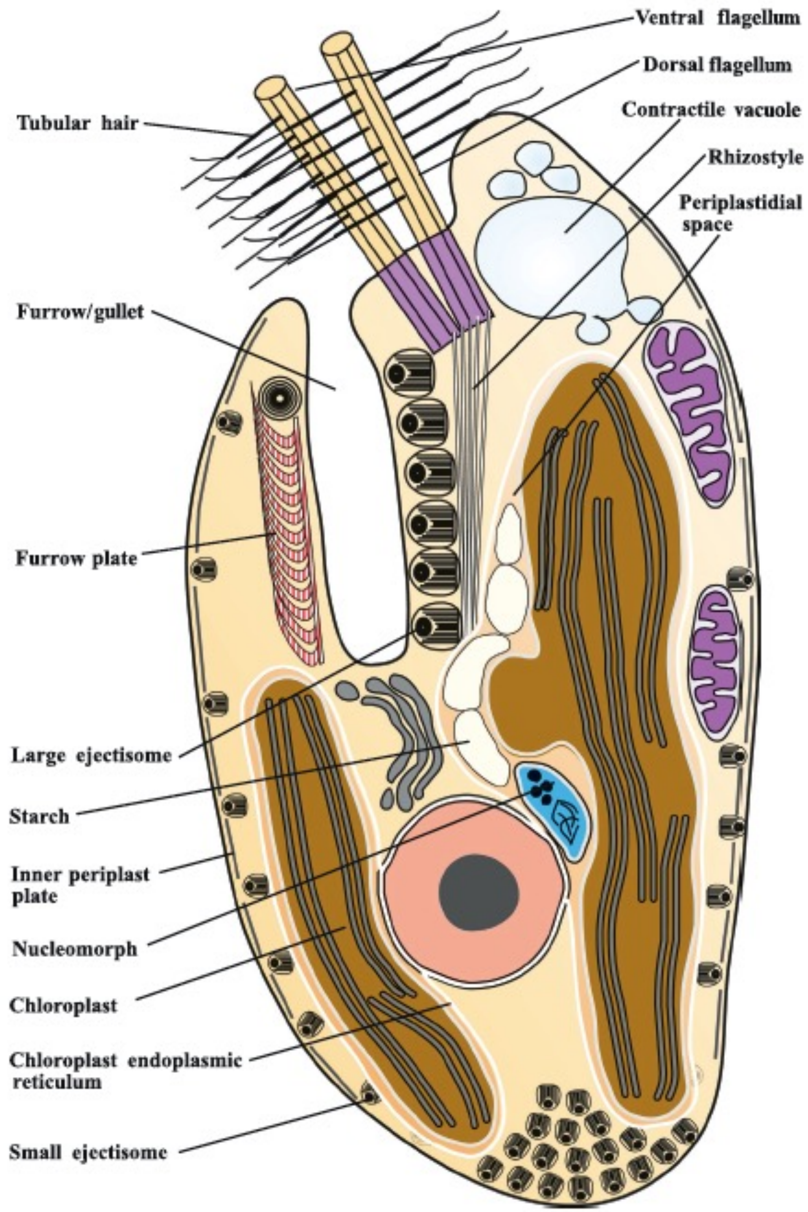
- **nucleomorph**: a residual nucleus belonging to a red algal endosymbiont
- often attached to pyrenoids
- two membranes
- presence of the nucleomorph: slow evolution or recent acquisition?



Tanijufi & Onodera (2017), *Advances in Botanical Research*

Cryptophyta

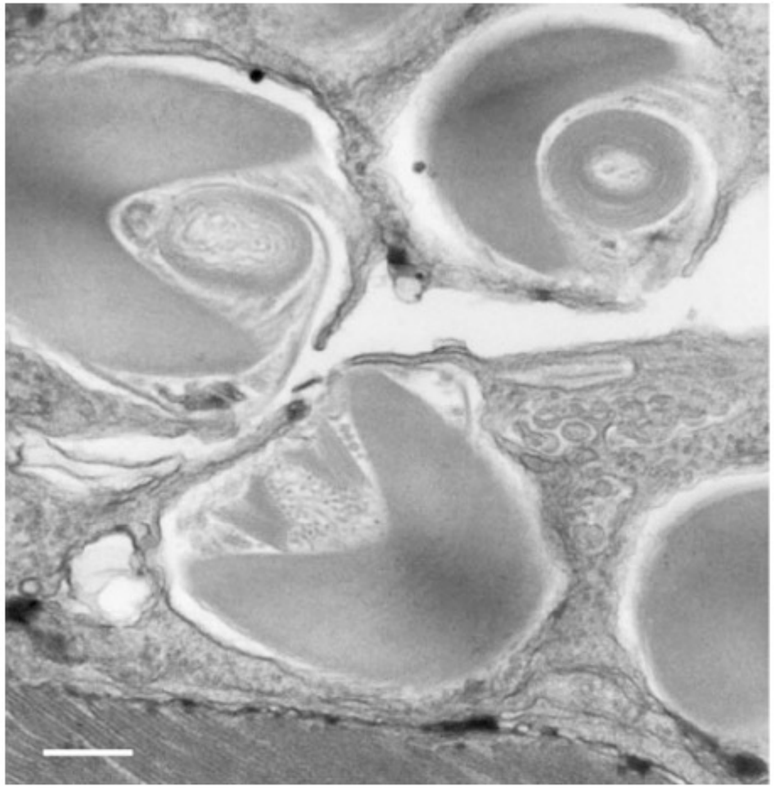
- plastids with lamellae comprised of 3 thylakoids, no girdle lamela
- diverse pigments: chlorophyll *a* and c_2 + carotenoid alloxanthin + one type of phycobiliprotein per cell: blue = phycocyanin or red = phycoerytrin (both evolved from red algal phycoerytrin)
- phycobiliproteins in thylakoid lumen (not aggregated in phycobilisomes as in Cyanobacteria, red algae, Glaucophyta)
- an eyespot consisting of carotene globules in the plastid (some *Chroomonas* species)
- mitochondria: fingerlike cristae
- starch and lipids as storage material



Clay (2015), *Freshwater Algae of North America*, Elsevier

Cryptophyta

- **ejectosomes**: explosive organelles; large along furrow-gullet system and small ejectosomes near the periplast
- undischarged ejectosome is tightly coiled ribbon-like structure; enclosed within vesicles
- two connected ribbons of different sizes, that are rolled up and under tension
- discharged upon mechanical or chemical stress irritating the cells



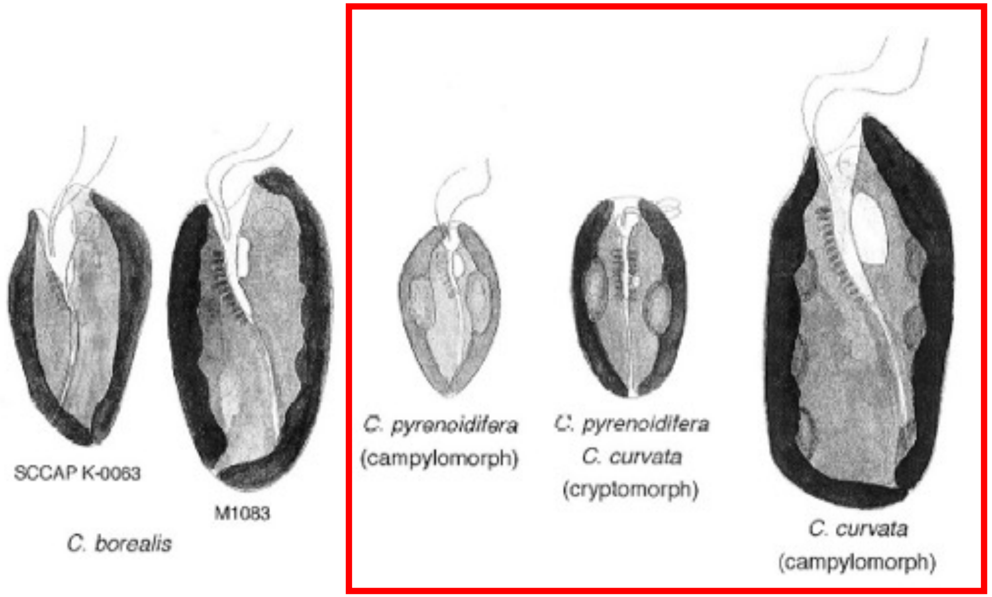
large ejectosomes along furrow-gullet system (transmission electron microscopy)

Hoef-Emden & Archibald (2017), *Handbook of the Protists*

Cryptophyta

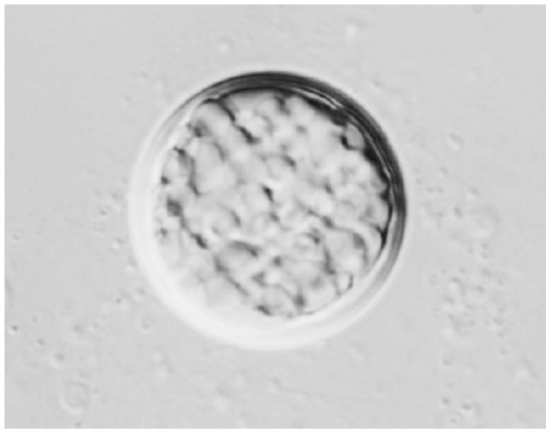
- mitosis and cytokinesis (schizotomy) + indirect evidence for sexual reproduction: cell fusion (isogamy), four-flagella zygotes (planozygotes)
- cellular dimorphism: most likely haploid and diploid stage

- some form thick-walled cysts as resting stages (no ejectosomes, no furrow-gullet system)



Cryptomonas spp.

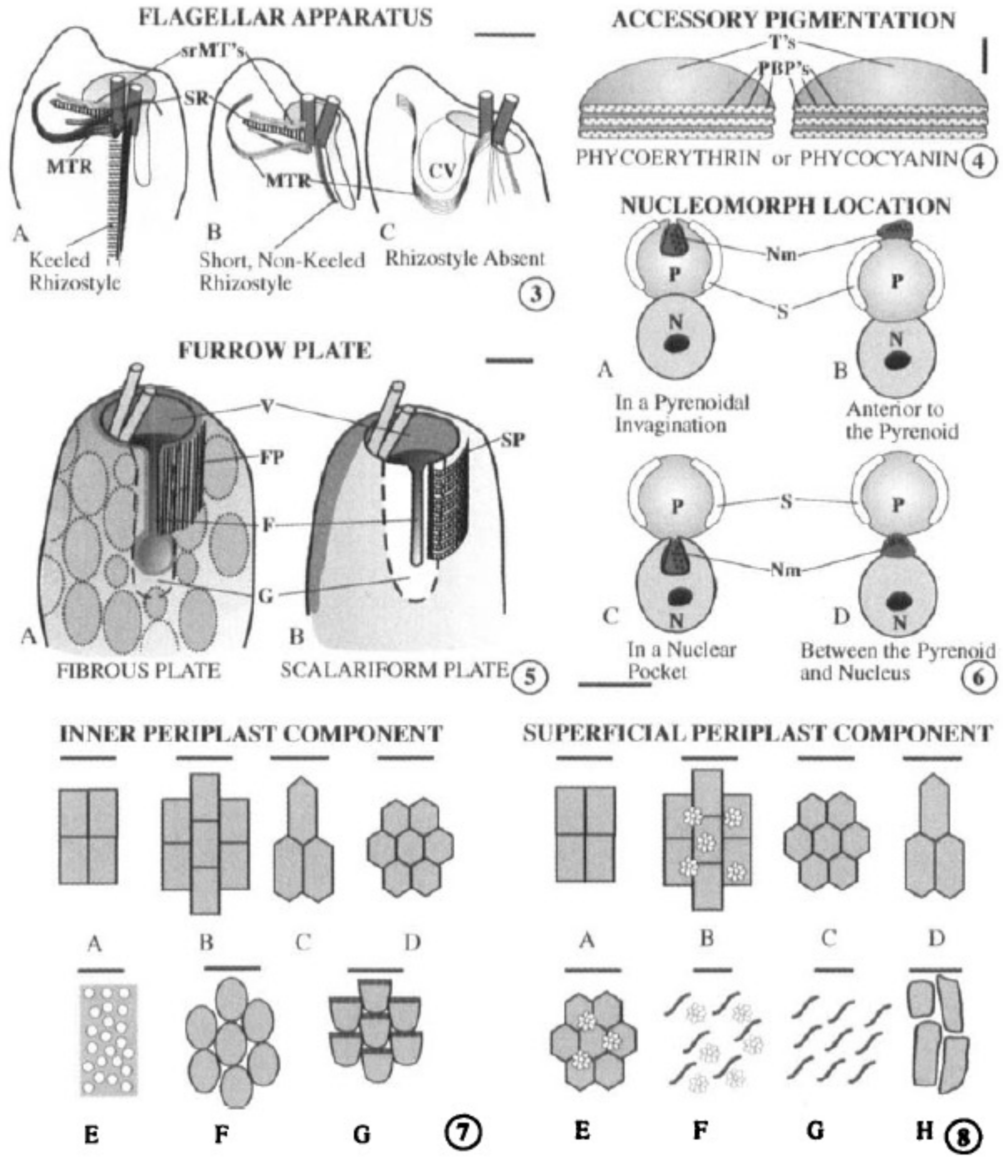
resting cyst,
Cryptomonas curvata



Hoef-Emden (2003); Hoef-Emden & Archibald (2017)

Cryptophyta

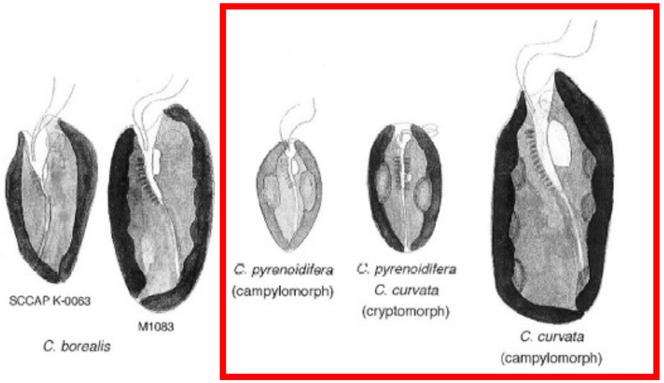
- fine-scale features used for classification: biliprotein type, plastid shape and number, position of nucleomorph, pyrenoid structure, flagellar apparatus, morphology of the furrow-gullet system, and ultrastructure of the periplast

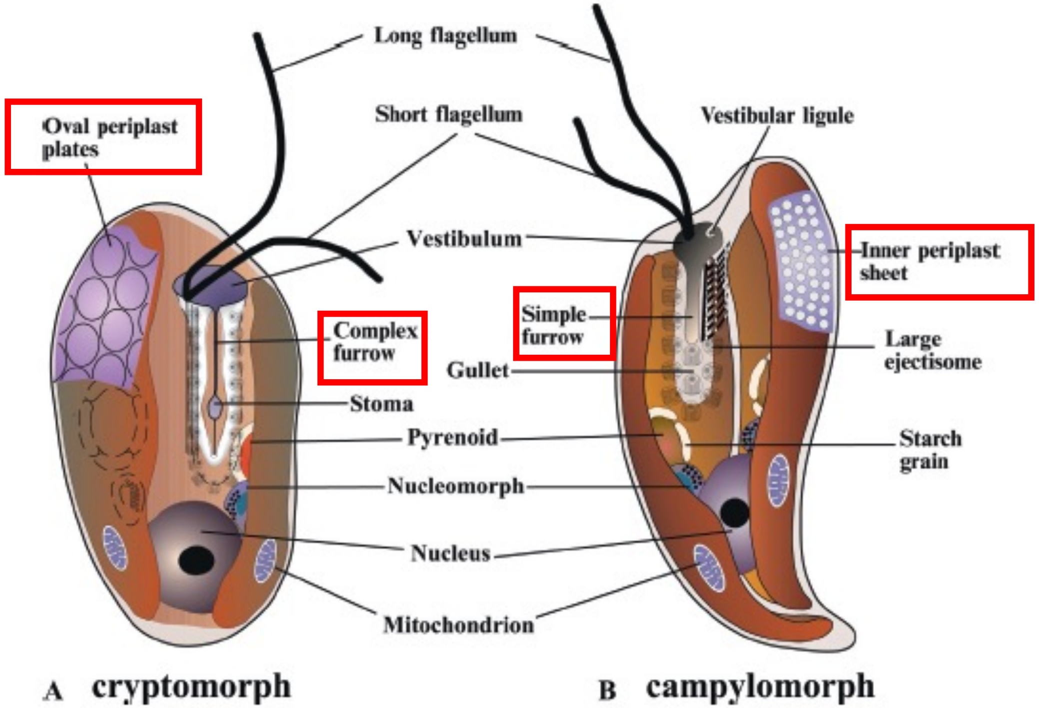


Clay et al. (1999), *Botanical Journal of Linnean Society*

Cryptophyta

- some characters are not stable within strains, problems with cell dimorphism





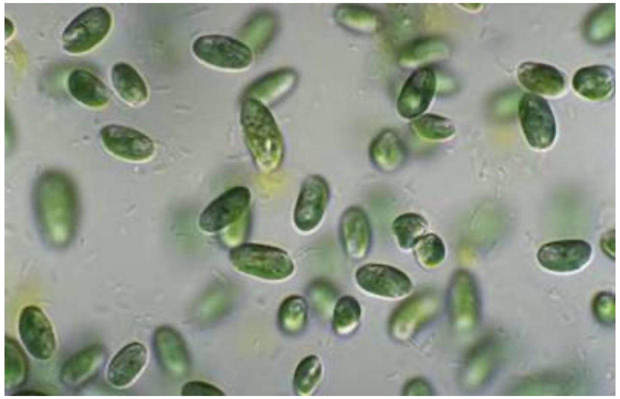
cell dimorphism (haplo/diplontic life cycle?)

Clay (2015), *Freshwater Algae of North America*, Elsevier

Cryptophyta

- unrealibility of color classification, because cell's color can change depending on the cell's condition
- a spectrogram analysis of pigment composition is helpful to recognize genera: eight types of distinct phycobiliproteins

Cryptomonas, Campylomonas (brown)



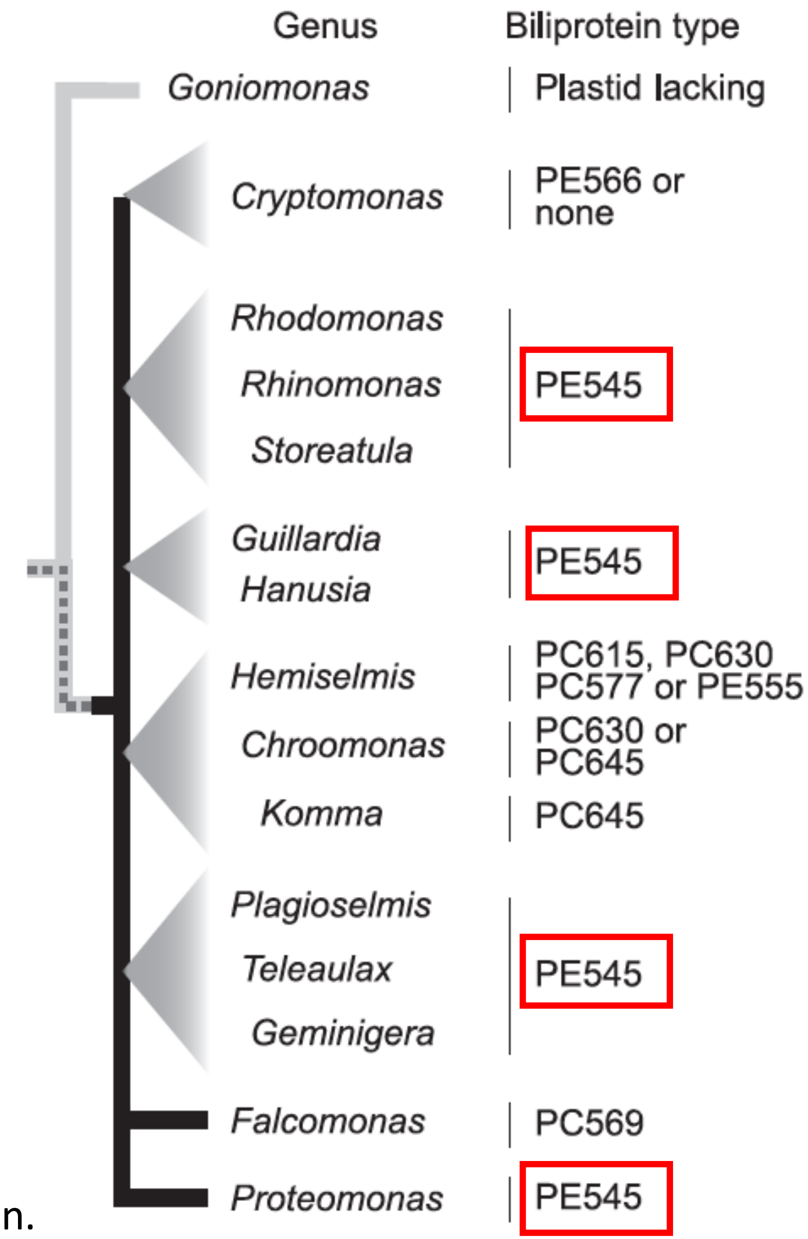
Chroomonas (blue)

Chilomonas (colourless)

Rhodomonas (red)

Cryptophyta

- a spectrogram analysis of phycobiliproteins
- different wavelength of their absorption maxima (e.g. phycoerytrin 545-PE545 = orange-red; phycocyanin 630-PC630)
- but some different genera share the same type of biliprotein or close relatives contain different types

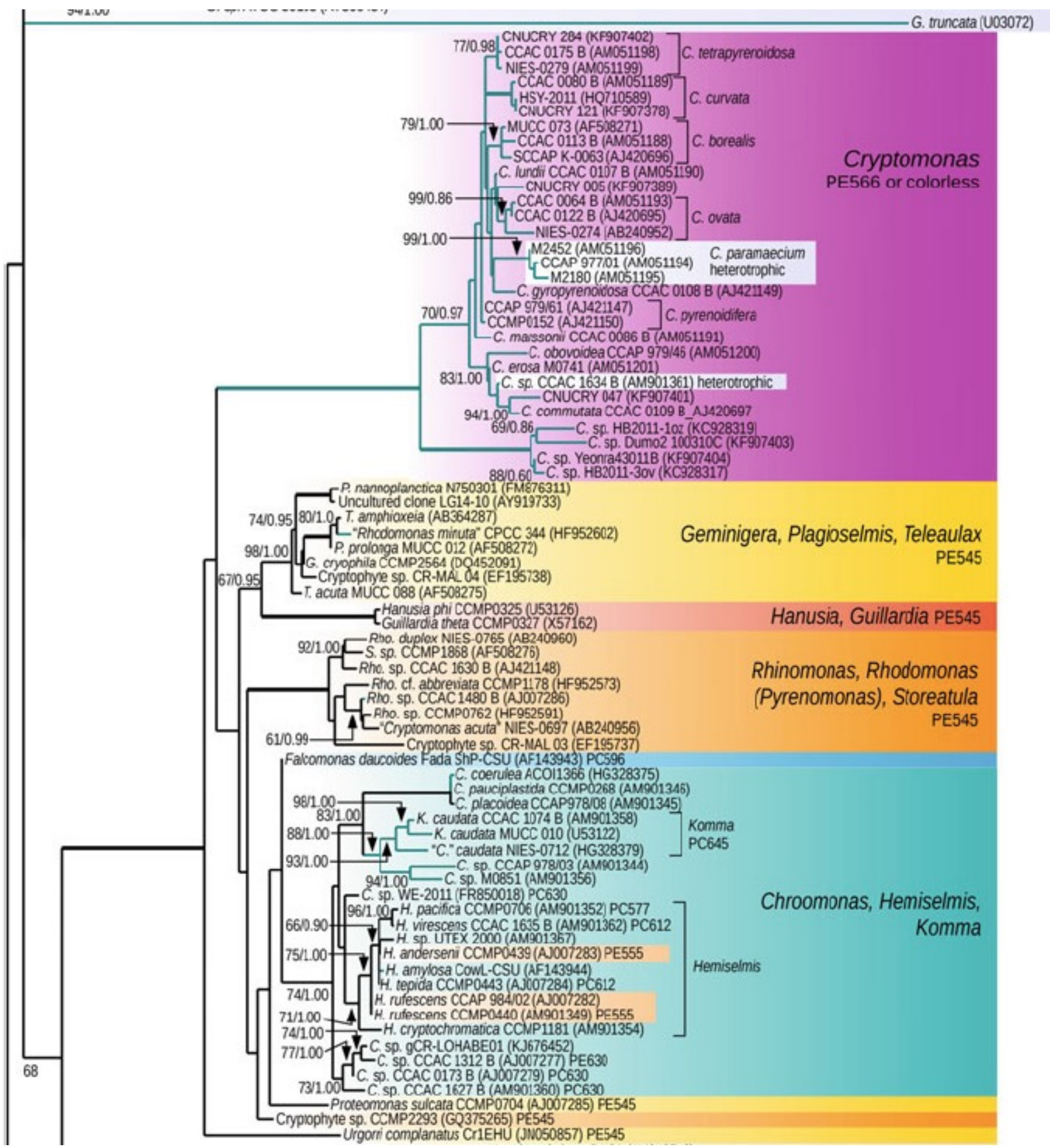


Schematic phylogeny (18S rDNA) of the cryptomonads with pigment information.

Tanijufi & Onodera (2017), *Advances in Botanical Research*

Cryptophyta

- morphological classification incongruent with reconstruction of phylogeny (polyphyletic or paraphyletic genera, genetically (almost) identical strains classified as different genera)
- *Cryptomonas* – PE566 or colorless, exclusively freshwater
- lack of species specific morphological characters
- revisiting the classification of cryptomonads is necessary



Hoef-Emden & Archibald (2017), *Handbook of the Protists*

Cryptophyta

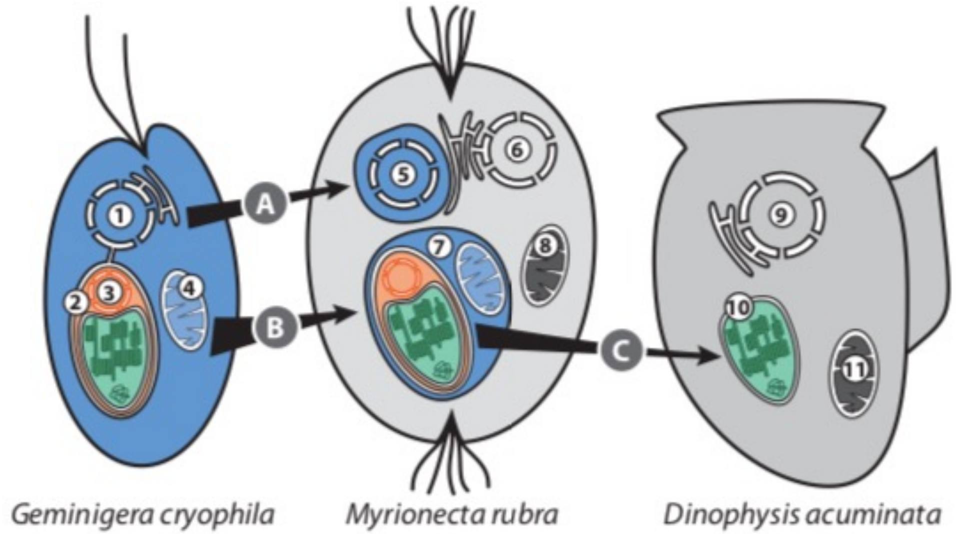
- nontoxic [*Chilomonas* may produce ichtyotoxin], easy to mass-cultivate + contain polyunsaturated fatty acid: as food for copepods in fish farming

- biliprotein pigments may be used as fluorescent dyes

- understanding the process of plastid acquisition: i) (non)photosynthetic plastids in cryptomonads; ii) apoplastic phagotrophic sister taxa (+ *Hatena arenicola* temporarily retain an engulfed green algal cell); iii) used as a temporary plastid = kleptoplastid (engulfed by dinophyte, ciliates)

- *Guillardia theta* – known plastid genome

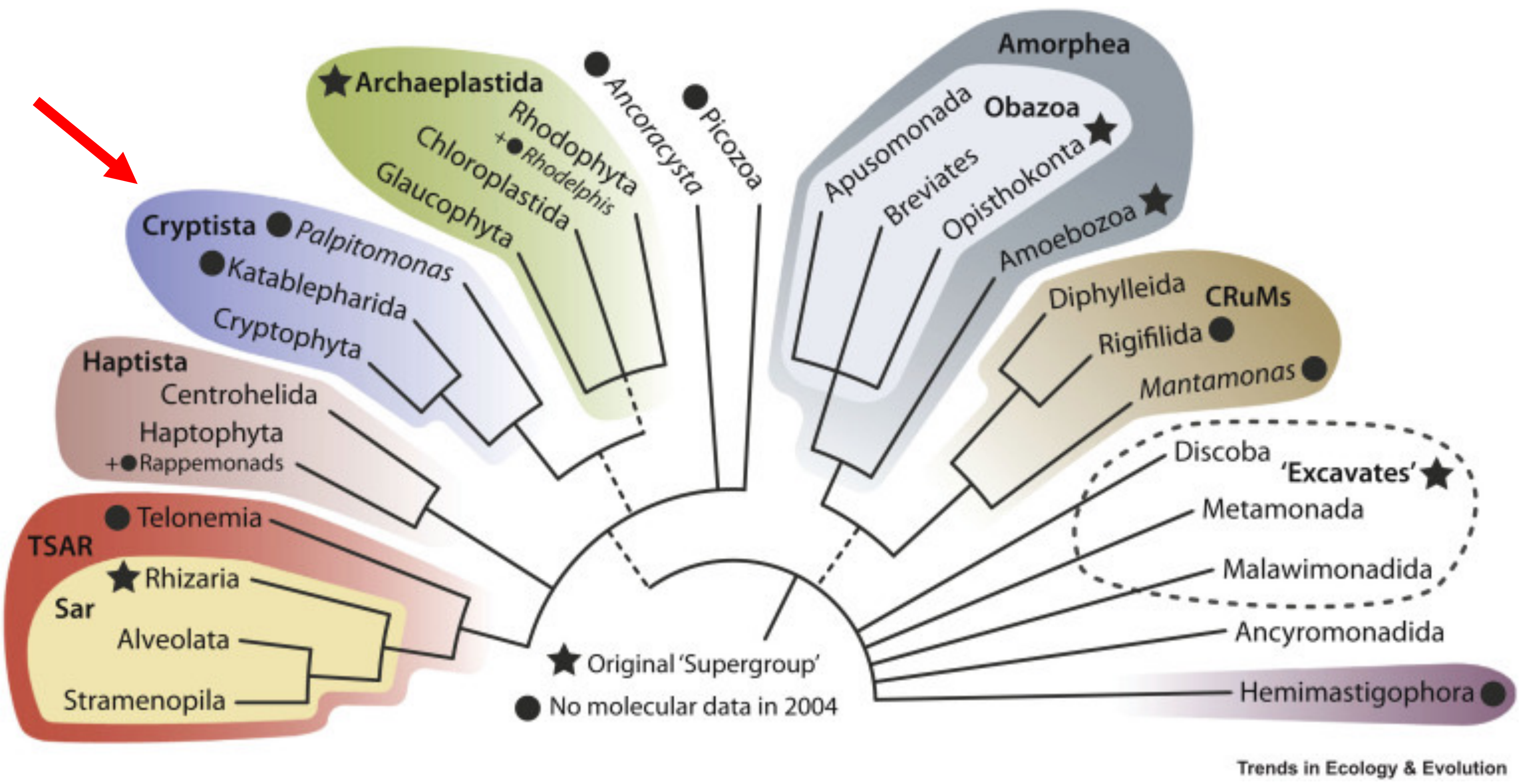
cryptophyte biliproteins, from left to right: PC630, PC577, PE555, PE566



kleptoplast acqusition

Wisecaver & Hackett (2010), *BMC Genomics*

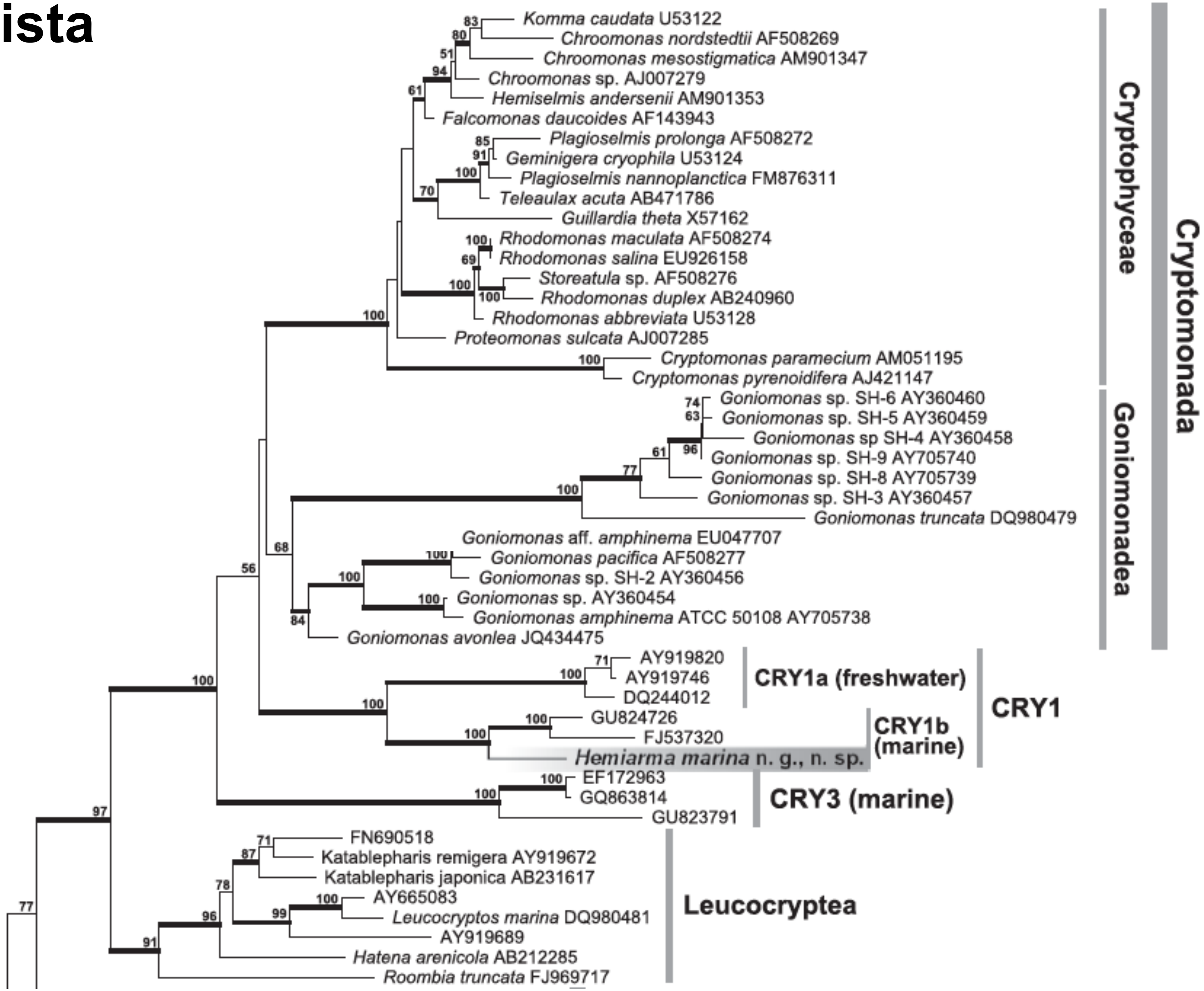
Cryptista



Sister taxa

Burki et al. (2020), *Trends in Ecology end Ecolution*

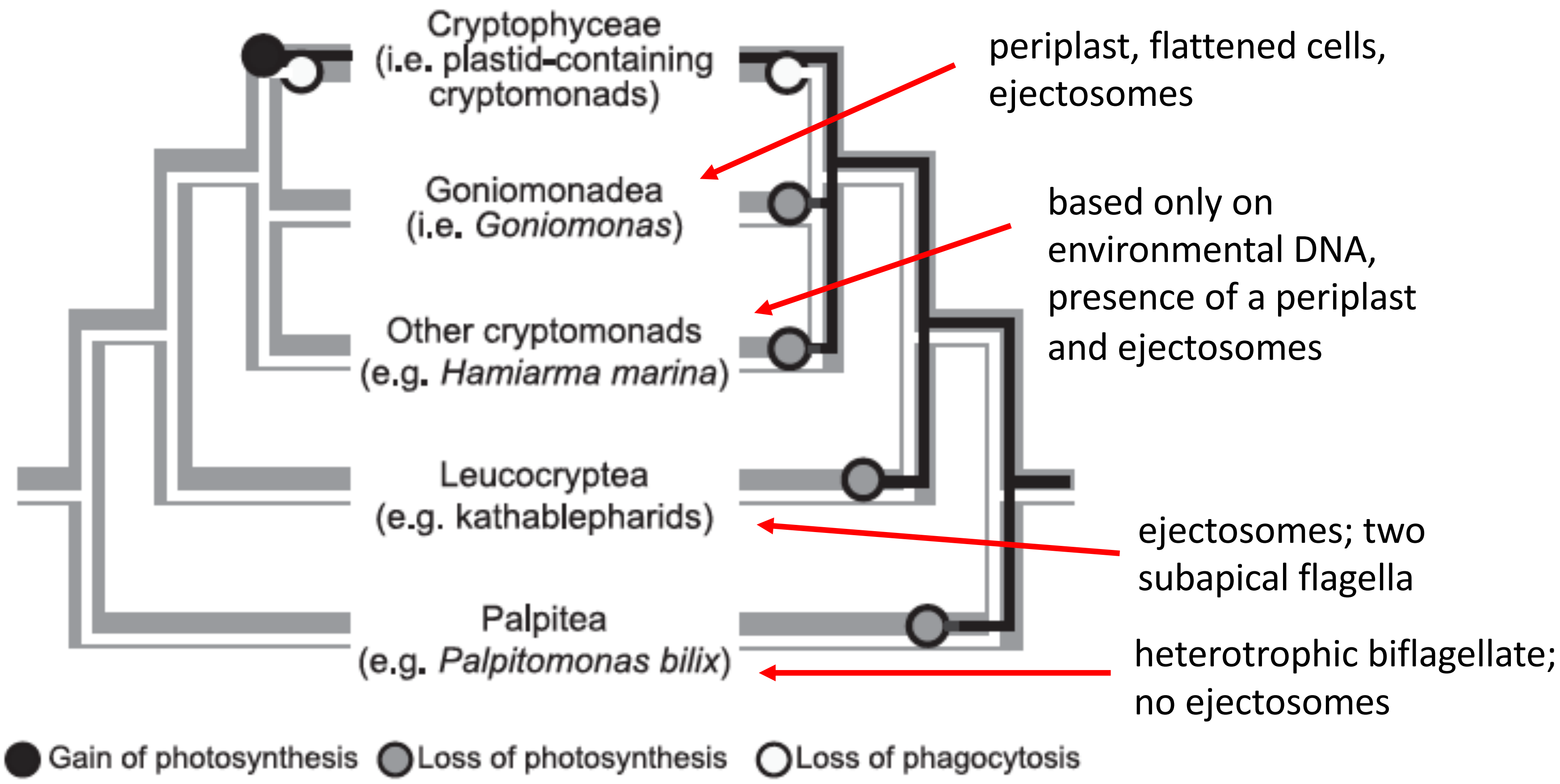
Cryptista



Classification

Shiratori & Ishida (2016), *Journal of Eukaryotic Microbiology*

Cryptista

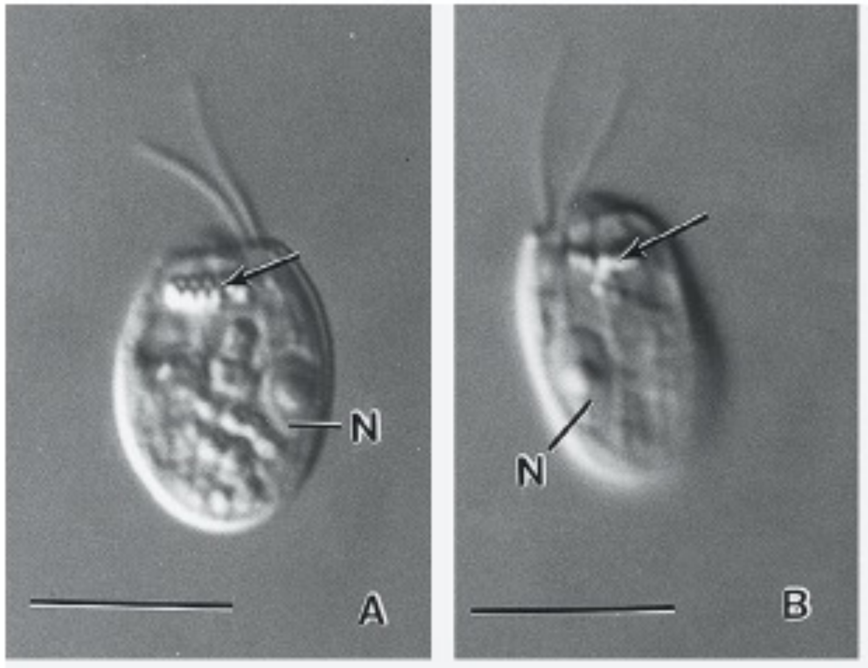


Two possible scenarios of the evolution of plastid acquisition or loss in Cryptista.

Tanijufi & Onodera (2017), *Advances in Botanical Research*

Goniomonadea

- freshwater habitats, seems to be benthic (prefer gliding along substrates)
- different in shape: markedly flattened with an obliquely truncate anterior
- apoplastic genus, phagotrophy (ingulf bacteria)
- different organization of ejectosomes (see black arrows in figures)
- light microscopically visible striation on the cell surface: large rectangular periplast plates
- long thin filaments cover both flagella

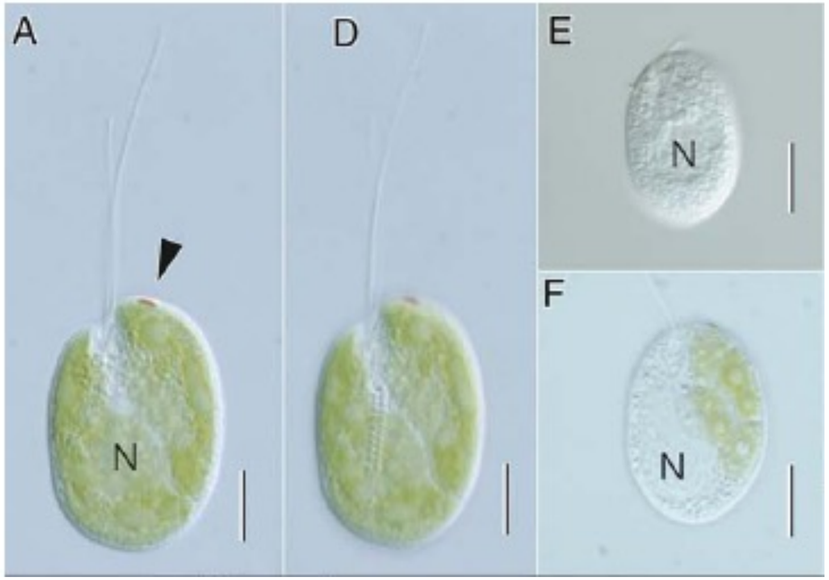


Goniomonas truncata

Clay (2015), *Freshwater Algae of North America*, Elsevier

Katablepharids

- understudied group
- marine and freshwater habitats
- heterotrophic flagellates with ejectosomes
- *Hatena arenicola* harbors a *Nephroselmis* symbiont
- the symbiont plastid is selectively enlarged, while other structures such as the mitochondria, Golgi body, cytoskeleton, and endomembrane system are degraded

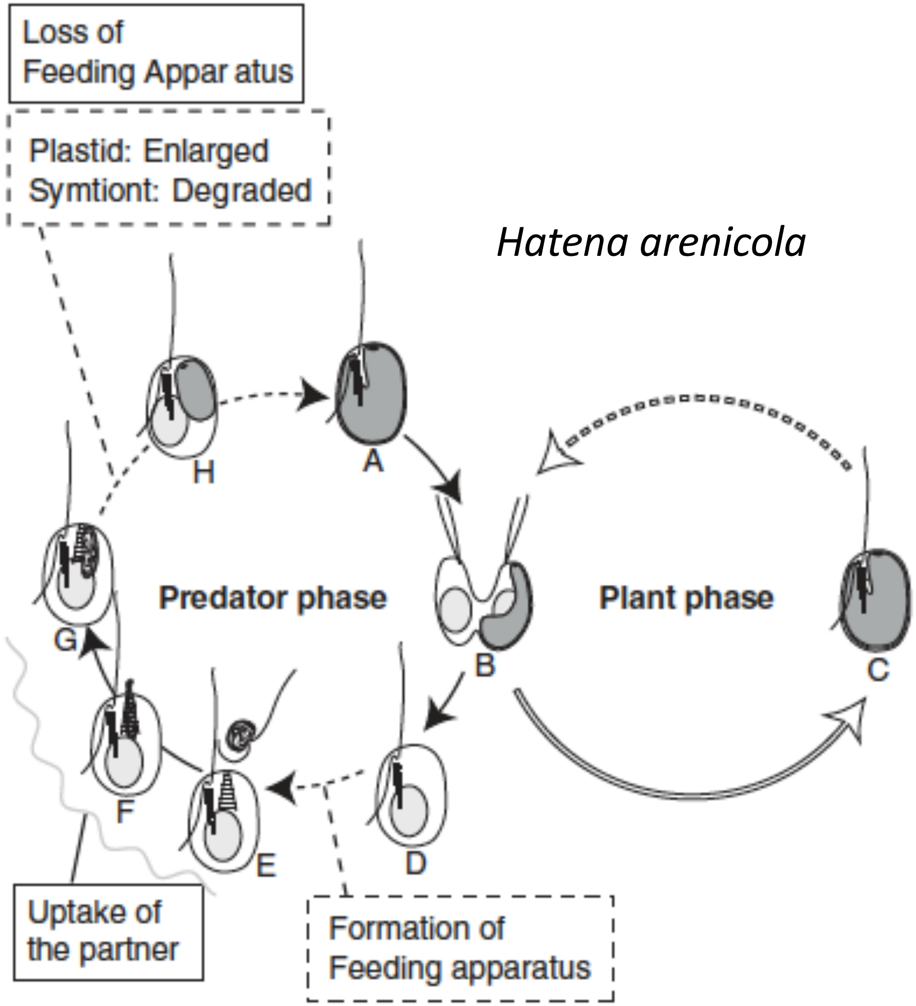


Hatena arenicola

Okamoto & Inouye (2006), *Protist*

Katablepharids

Half-plant, half-predator-hypothesis. The solid line indicates a witnessed process, and the broken line indicates a hypothetical process. A-D: A green cell with the symbiont, lacking the feeding apparatus (A) divides (B) into one green (C) and one colorless cell (D). E-G: The colorless cell should form a feeding apparatus de novo and engulfs a *Nephroselmis* cell. G-H: The symbiont plastid selectively grows in the host cytoplasm. Because cell division of a colorless cell or a cell with an ‘‘immature’’ symbiont (H) has never been observed, uptake and the subsequent changes in both host and symbiont apparently occur within one generation.



Okamoto & Inouye (2006), *Protist*