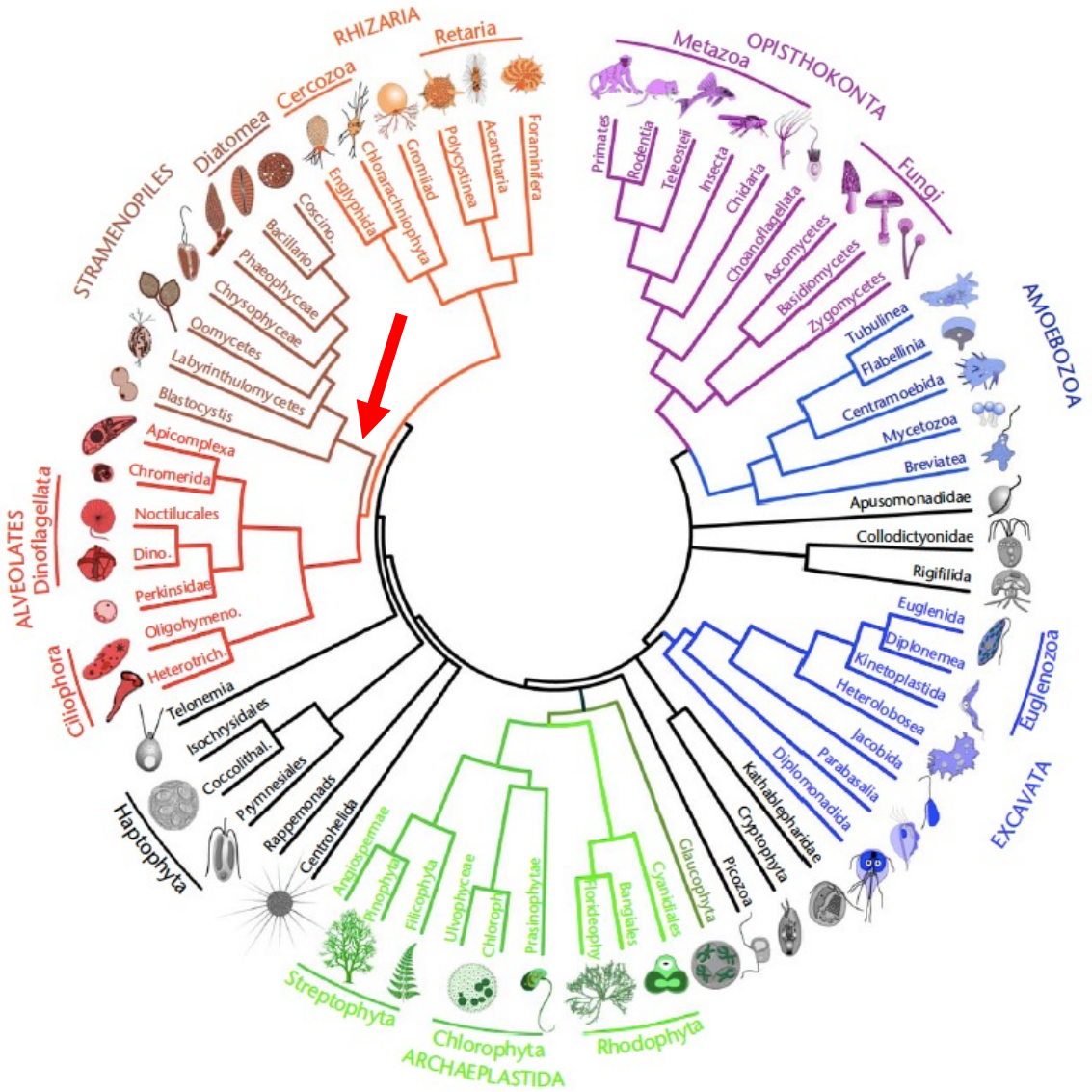
Stramenopiles / Heterokontophyta
clade Ochrophytes

Eukaryotic tree of life



Pawlowski (2014), *Prostist Evolution and Phylogeny, eLS*

Stramenopiles = Heterokonta

*historically recognized photosynthetic classes

Supergroups	Major taxa	Representative genera	Catalogued diversity
Stramenopiles	→ Bacillariophyta	*Thalassiosira, Nitschia*	20 000 *
	Bicosoecida	*Bicosoeca, Cafeteria*	72
	→ Chrysophyceae-Synurophyceae	*Chromulina, Ochromonas Synura*	1200 *
	→ Dictyochophyceae	*Dictyocha*	15
	→ Eustigmatales	*Vischeria*	15
	Hyphochytriales	*Hyphochytridium*	25
	Labyrinthulomycetes	*Labyrinthula, Amphitrema*	40
	Oomycetes	*Saprolegnia, Phytophthora*	676
	Opalinata	*Opalina*	400
	→ Pelagophyceae	*Aureococcus*	12
	→ Phaeophyceae	*Fucus, Laminaria*	1750 *
	→ Phaeothamniophyceae	*Phaeothamnion*	25
	→ Pinguiophyceae	*Pinguiochrysis*	5
	→ Raphidophyceae	*Goniostomum*	20
	→ Xanthophyceae	*Botrydium, Vaucheria*	6000.5 *

+ other less diversified classes

Bicosoecida: flagellates having tiny flagellar hairs, some genera encased in lorica; **Hyphochytriales:** flagellates having hypha-like structures; **Labyrinthulomycetes**: gliding flagellates producing a network of filaments, amobeoid; **Oomycetes = Peronosporomycetes**: fungus-like plant pathogens, parasites of both freshwater and marine algae; **Opalinata**: animal endobionts, flagellates mostly found in frog, with a flat shape like a slice, endocommensals.

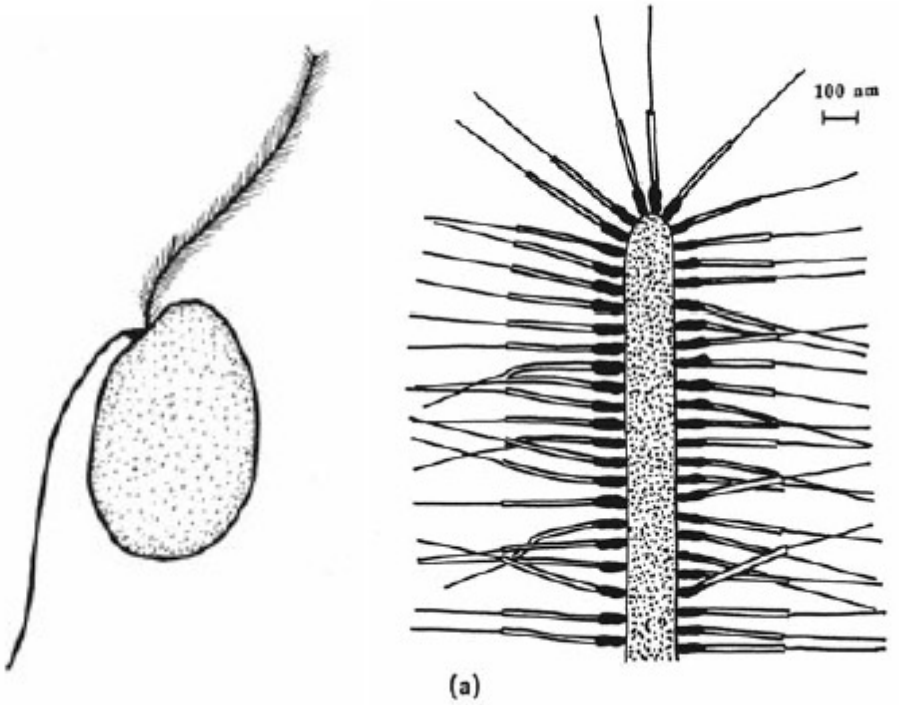
Photo/heterotrophy

Pawlovski (2014); *Protist Evolution and Phylogeny*, eLS

Stramenopiles = Heterokonta

- distinctive feature are flagella [if swimming cells are present in their life-cycle]: uneven length and functionally different = heterokont flagella
- tripartite tubular hairs on the long flagellum = stramenopiles; anterior position
- no mastigonemes on the short flagellum (smooth/naked flagellum); posterior position
- but in Pelagoph. only a single, long flagellum with bipartite hairs; in some Pinguioph. a single flagella with no hairs; in Bacillarioph. a single pleuronematic (i.e. with tripartite hairs) long flagellum

typical stramenopile flagella

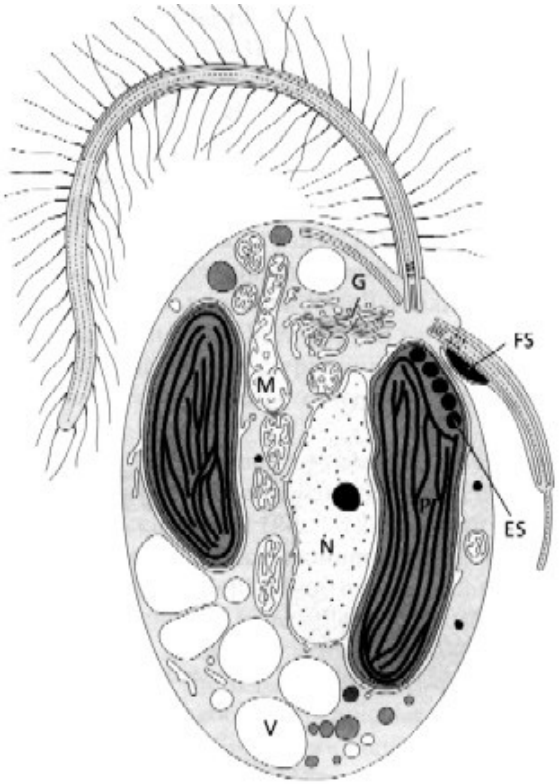


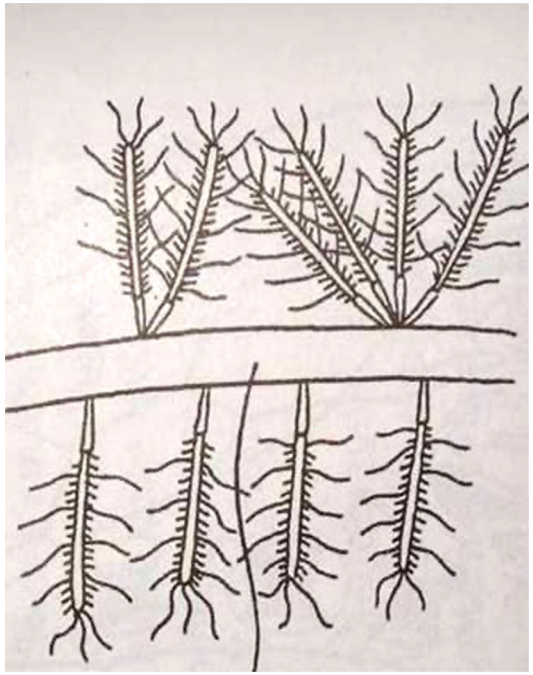
two rows of tripartite tubular hairs (basal part, microtubular part, a terminal hair or hairs), from glycoproteins, assembled in ER

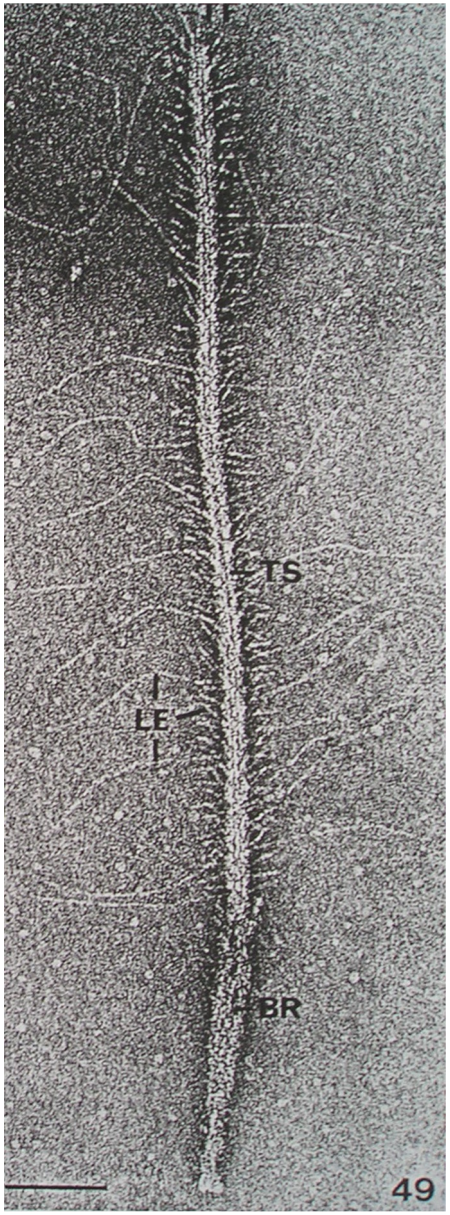
Lee et al. (2008); *Phycology*, Cambridge

Stramenopiles = Heterokonta

typical long stramenopile flagellum



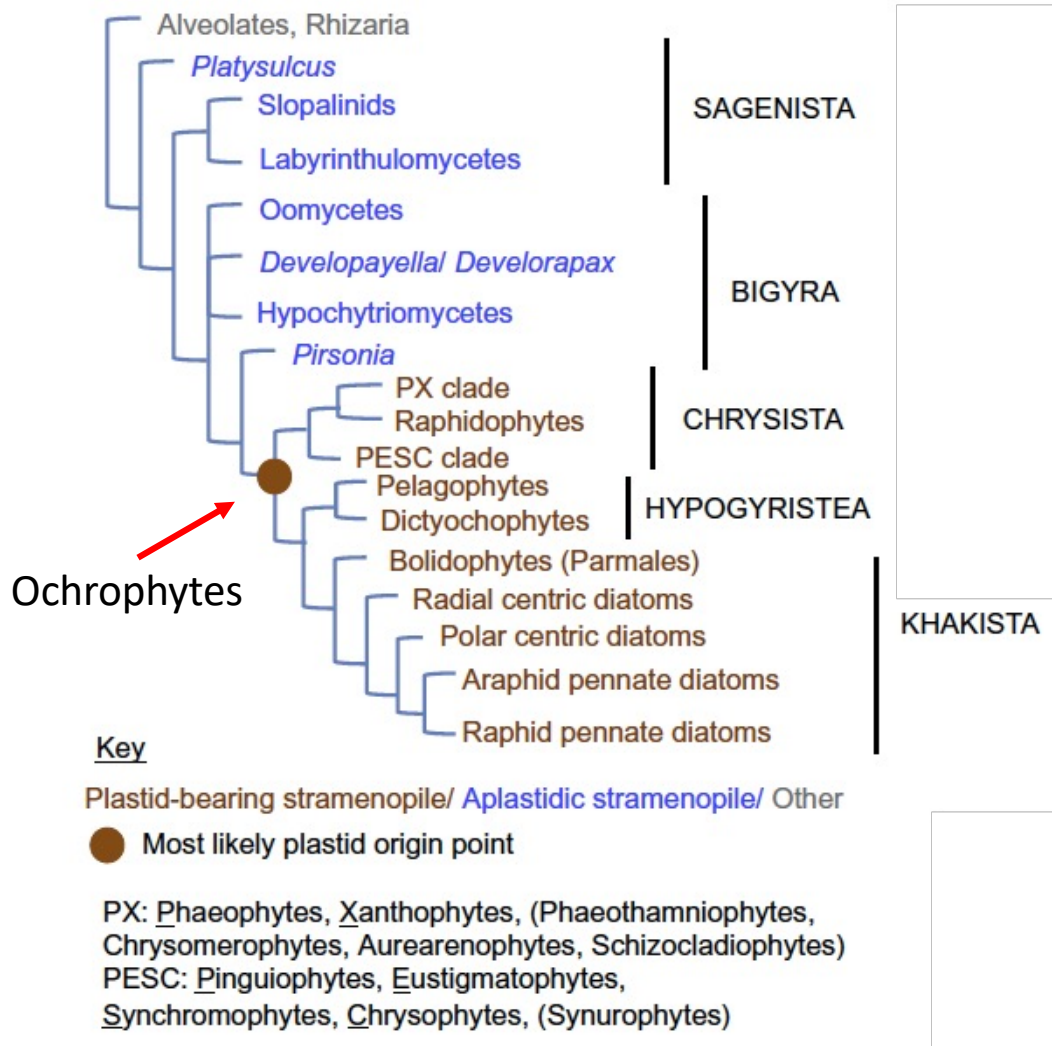




two rows of tripartite tubular hairs
BR: basal part, TS: tubular part, LE: terminal hairs

Ochrophytes = Chromophytes

- non-photosynthetic groups basally divergent
- a recent common ancestor of the extant photosynthetic lineages [secondary lost of photosynthesis in some lineages]

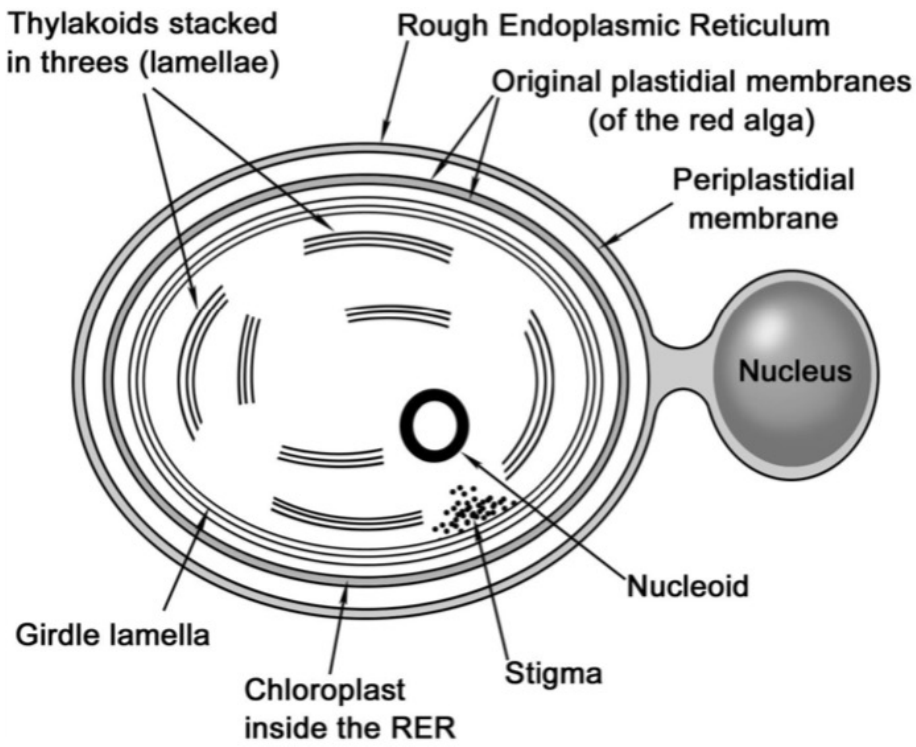


Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - plastids

- plastids: 4 membranes [unconventional outer membrane arrangement in Synchromoph. and Aurearenoph.; only two membranes in Chrysoparadoxoph.]; outer plastid ER membrane connected with outer nuclear envelope membrane
- chlorophylls *a* + *c1*, *c2*, and/or *c3* [*c* absent in Eustigmatoph., not detected in Aurearenoph.], various carotenoids including fucoxanthin [absent in Eustigm, Xanthoph.]
- unique thylakoid organization: girdle lamella [but not found in Eustigmatoph., Synchromoph., Aurearenoph.; some Pinguioph. and Raphidoph. species]

typical stramenopile chloroplast

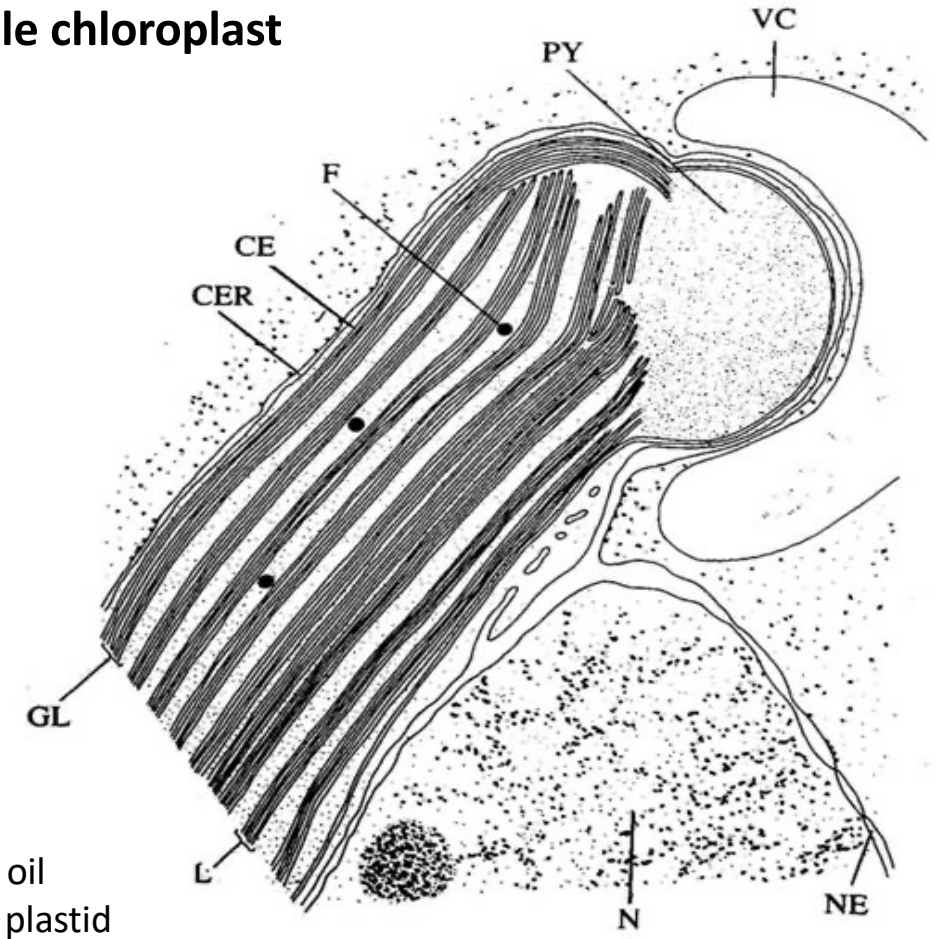


Sym & Maneveldt (2012), *Engineering in Life Sciences*

Ochrophytes - plastids

typical stramenopile chloroplast

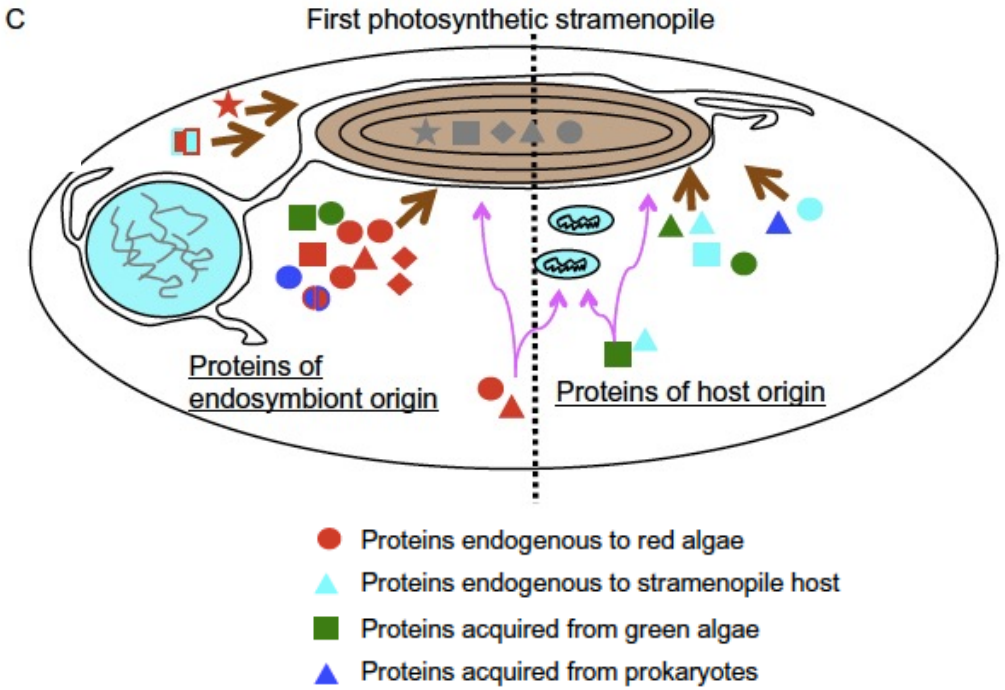
- chrysolaminaran and oil globules as a storage product (absence of starch which is characteristic for Archaeplastida)



VC: chrysolaminaran vacuole, PY: pyrenoid, F: oil globules, CE: 2 layered plastid envelope, CER: plastid ER, GL: girdle lamella, L: lamella (thylakoids stacked in triplets), N: nucleus, NE: nuclear envelope

Ochrophytes - plastids

- chimeric origins of plastids: genes of red algal origin + from prokaryotes + from green algae (footprint of a cryptic plastid?)
- physiological capacities known in green algae but not known in red algae: xanthophyll cycle (light-harvesting function and photoprotective role)
- fucoxanthin/chlorophyll pigment-binding proteins: both red and green origin

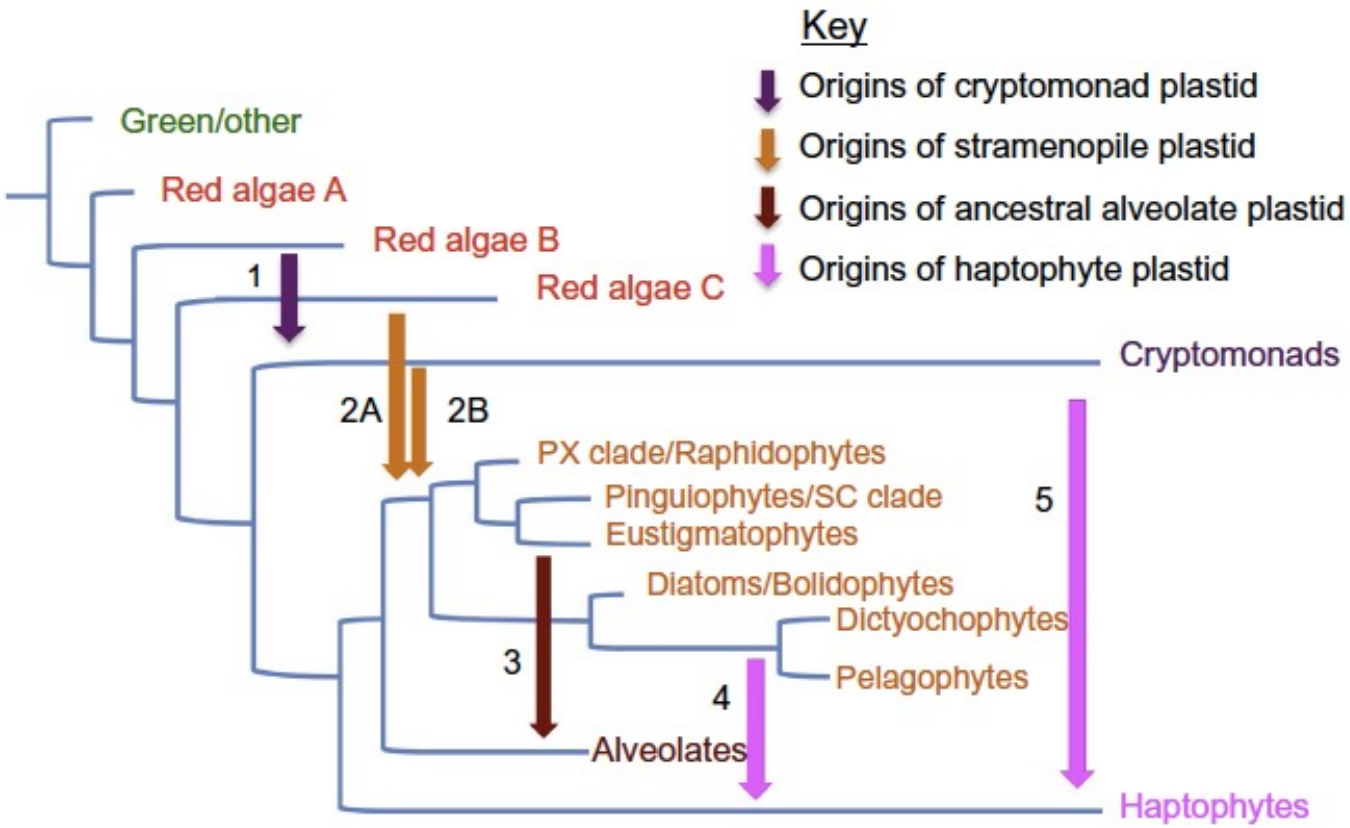


Mosaic origin of plastids, green alga and/or prokaryotic origin: i) acquired through lateral transfer events or through endosymbiosis; ii) transfer from green algae into red algae or other endosymbiont, which might be a possible vector for introducing green genes into the nucleus.

Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - plastids

Possible endosymbiotic connections between stramenopiles and other plastids of red origin.



plastids: secondary or more complex endosymbiosis of a red alga

Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - pigments

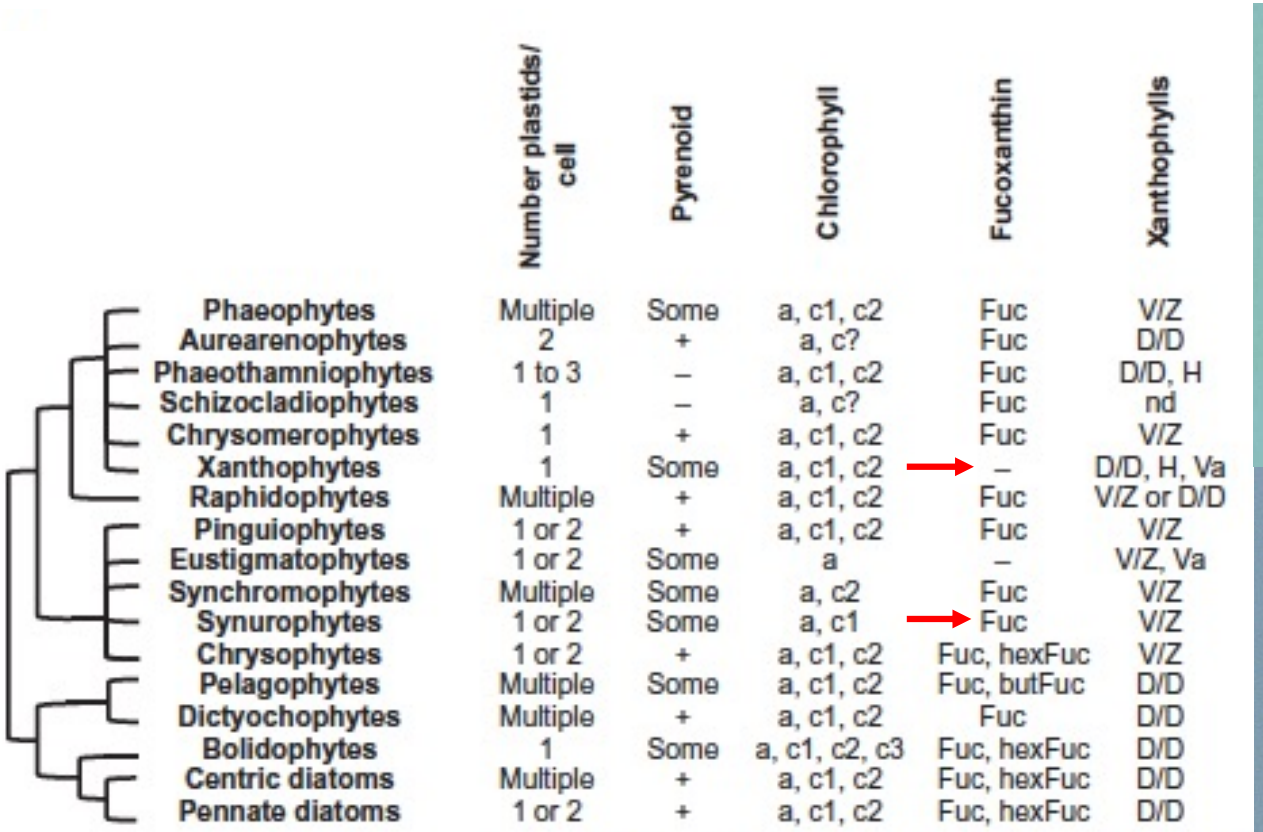
	Number plastids/ cell	Pyrenoid	Chlorophyll	Fucoxanthin	Xanthophylls	Girdle lamella	Plastid-nucleus membrane connection	Eyespot	ptDNA
Phaeophytes	Multiple	Some	a, c1, c2	Fuc	V/Z	+	+	Some	R
Aurearenophytes	2	+	a, c?	Fuc	D/D	–	+	+	nd
Phaeothamniophytes	1 to 3	–	a, c1, c2	Fuc	D/D, H	+	+	+	R
Schizocladiophytes	1	–	a, c?	Fuc	nd	+	+	+	R
Chrysomerophytes	1	+	a, c1, c2	Fuc	V/Z	+	+	+	R
Xanthophytes	1	Some	a, c1, c2	–	D/D, H, Va	+	+	Some	R
Raphidophytes	Multiple	+	a, c1, c2	Fuc	V/Z or D/D	Some	–	–	S
Pinguiophytes	1 or 2	+	a, c1, c2	Fuc	V/Z	Some	+	–	R/S
Eustigmatophytes	1 or 2	Some	a	–	V/Z, Va	–	+	+	R
Synchromophytes	Multiple	Some	a, c2	Fuc	V/Z	–	+	–	nd
Synurophytes	1 or 2	Some	a, c1	Fuc	V/Z	+	–	–	R
Chrysophytes	1 or 2	+	a, c1, c2	Fuc, hexFuc	V/Z	+	+	+	R
Pelagophytes	Multiple	Some	a, c1, c2	Fuc, butFuc	D/D	+	+	Some	S
Dictyochophytes	Multiple	+	a, c1, c2	Fuc	D/D	+	+	Some	S
Bolidophytes	1	Some	a, c1, c2, c3	Fuc, hexFuc	D/D	+	+	–	R
Centric diatoms	Multiple	+	a, c1, c2	Fuc, hexFuc	D/D	+	Some	–	R
Pennate diatoms	1 or 2	+	a, c1, c2	Fuc, hexFuc	D/D	+	Some	–	R

Fucoxanthins Fuc: fucoxanthin; hexFuc: 19′-hexanoyloxyfucoxanthin; butFuc: 19′-butanoyloxyfucoxanthin
Xanthophylls V/Z: violaxanthin/zeaxanthin; D/D: diatoxanthin/diadinoxanthin; H: heteroxanthin; Va: vaucherioxanthin
Plastid DNA R: ring genophore; S: scattered genophore

Ultrastructural and pigment characteristics.

Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - pigments



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Phaeothamniophytes	1 to 3	–	a, c1, c2	Fuc	D/D, H
Schizocladiophytes	1	–	a, c?	Fuc	nd
Chrysomerophytes	1	+	a, c1, c2	Fuc	V/Z
Xanthophytes	1	Some	a, c1, c2	–	D/D, H, Va
Raphidophytes	Multiple	+	a, c1, c2	Fuc	V/Z or D/D
Pinguiophytes	1 or 2	+	a, c1, c2	Fuc	V/Z
Eustigmatophytes	1 or 2	Some	a	–	V/Z, Va
Synchromophytes	Multiple	Some	a, c2	Fuc	V/Z
Synurophytes	1 or 2	Some	a, c1	Fuc	V/Z
Chrysophytes	1 or 2	+	a, c1, c2	Fuc, hexFuc	V/Z
Pelagophytes	Multiple	Some	a, c1, c2	Fuc, butFuc	D/D
Dictyochophytes	Multiple	+	a, c1, c2	Fuc	D/D
Bolidophytes	1	Some	a, c1, c2, c3	Fuc, hexFuc	D/D
Centric diatoms	Multiple	+	a, c1, c2	Fuc, hexFuc	D/D
Pennate diatoms	1 or 2	+	a, c1, c2	Fuc, hexFuc	D/D

Fucoxanthins Fuc: fucoxanthin; hexFuc: 19′-hexanoyloxyfucoxanthin; butFuc
Xanthophylls V/Z: violaxanthin/zeaxanthin; D/D: diatoxanthin/diadinoxanthin;
Plastid DNA R: ring genophore; S: scattered genophore

Xanthophytes, yellow-green

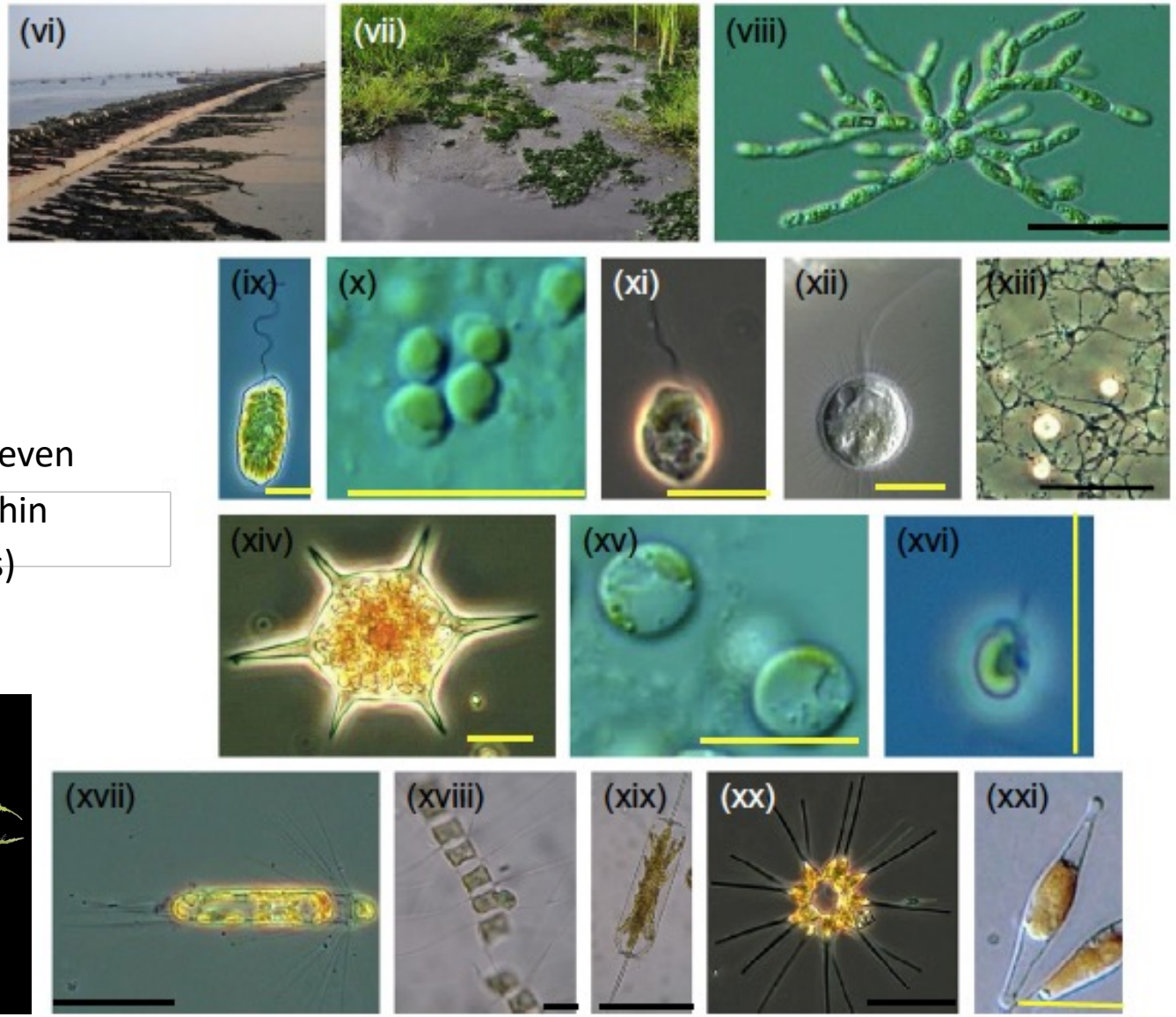
Synurophytes, golden-brown

Ultrastructural and pigment characteristics.

Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - diversity

- huge morphological, physiological and phylogenetic diversity (even rivalling that observed within the green algae and plants)



Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - trophy

- photoautotrophy
- photo-mixotrophy: supplementing the products of photosynthesis with phagotrophy (Chrysoph., Raphidoph.) or osmotrophy (Pelagoph., Bacillarioph.)
- secondary lost of photosynthesis (e.g., Chrysoph., Dictyochoph., Bacillarioph.; some of them have leucoplasts)

Dinobryon, Chrysophyta

Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophytes - symbioses

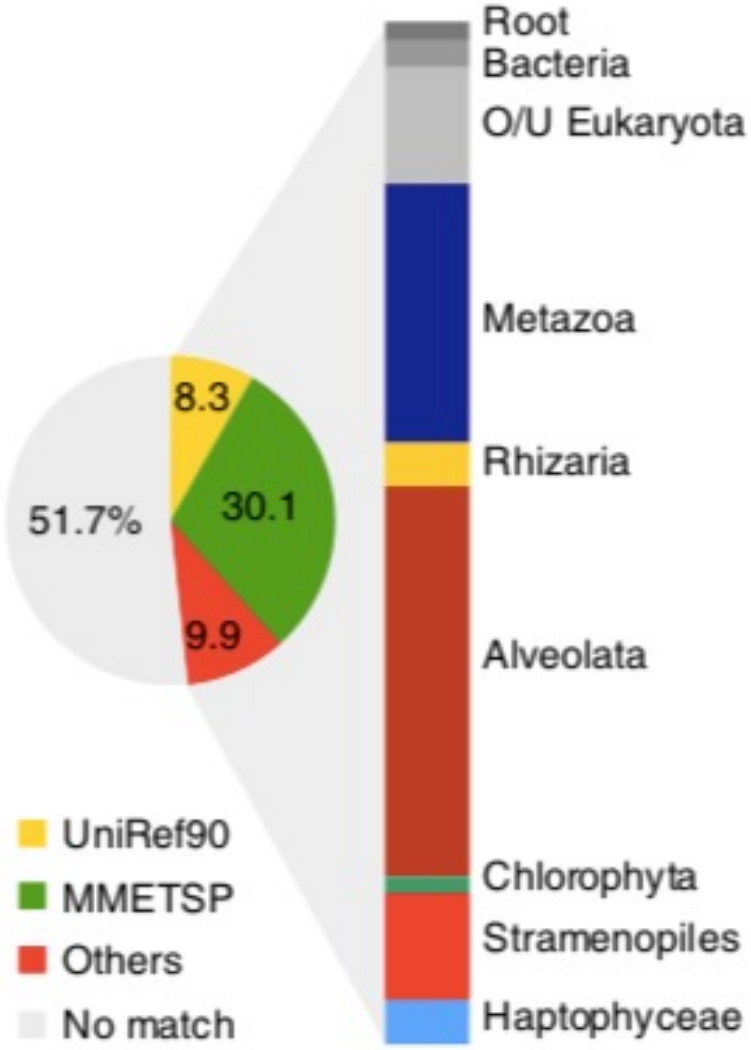
- plastids as transient symbionts by other eukaryotes = kleptoplasts: marine sea slug (*Elisia chlorotica* – from *Vaucheria*), dinoflagellates (from Dictyochoph.); foraminifera (from Bacillarioph.), ”marine lichen” (*Verrucaria*: fungi and phaeophyte alga)
- dinotoms: dinoflagellate algae with permanent plastids of diatom origin

Elisia chlorotica

Dorrell & Bowles (2014); *Advances in Botanical Research*

Ochrophyta – habitats

- high diversity and frequency of ochrophytes in marine habitats [but Xanthoph. and Eustigmatoph. predominantly freshwater or terrestrial]
- ecological function (food webs, biogeochemical cycles) of contemporary marine ecosystems are critically dependent on eukaryotic phytoplankton (despite they are numerically inferior to cyanobacteria)

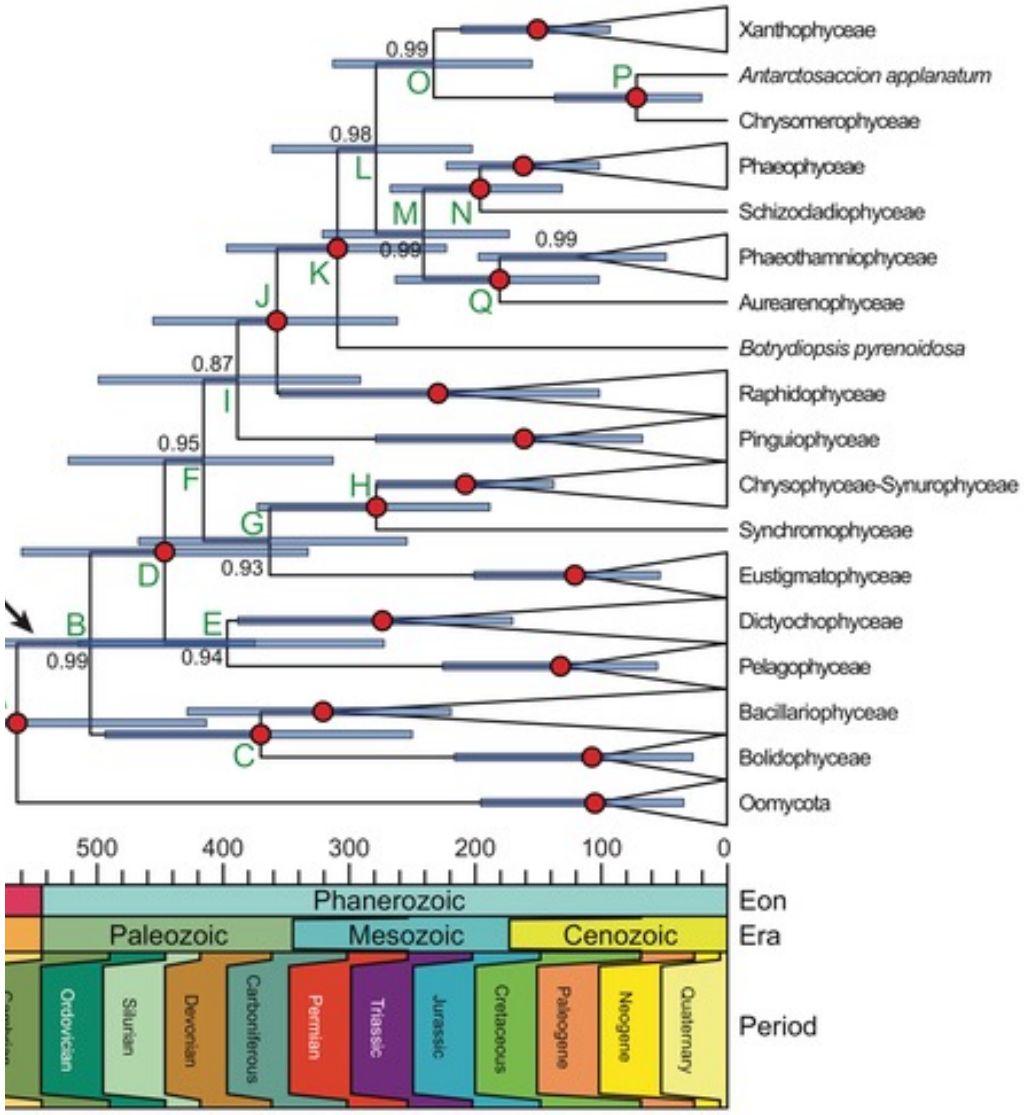


Carradec et al. (2018); *Nat. Commun.*

Ochrophytes - history

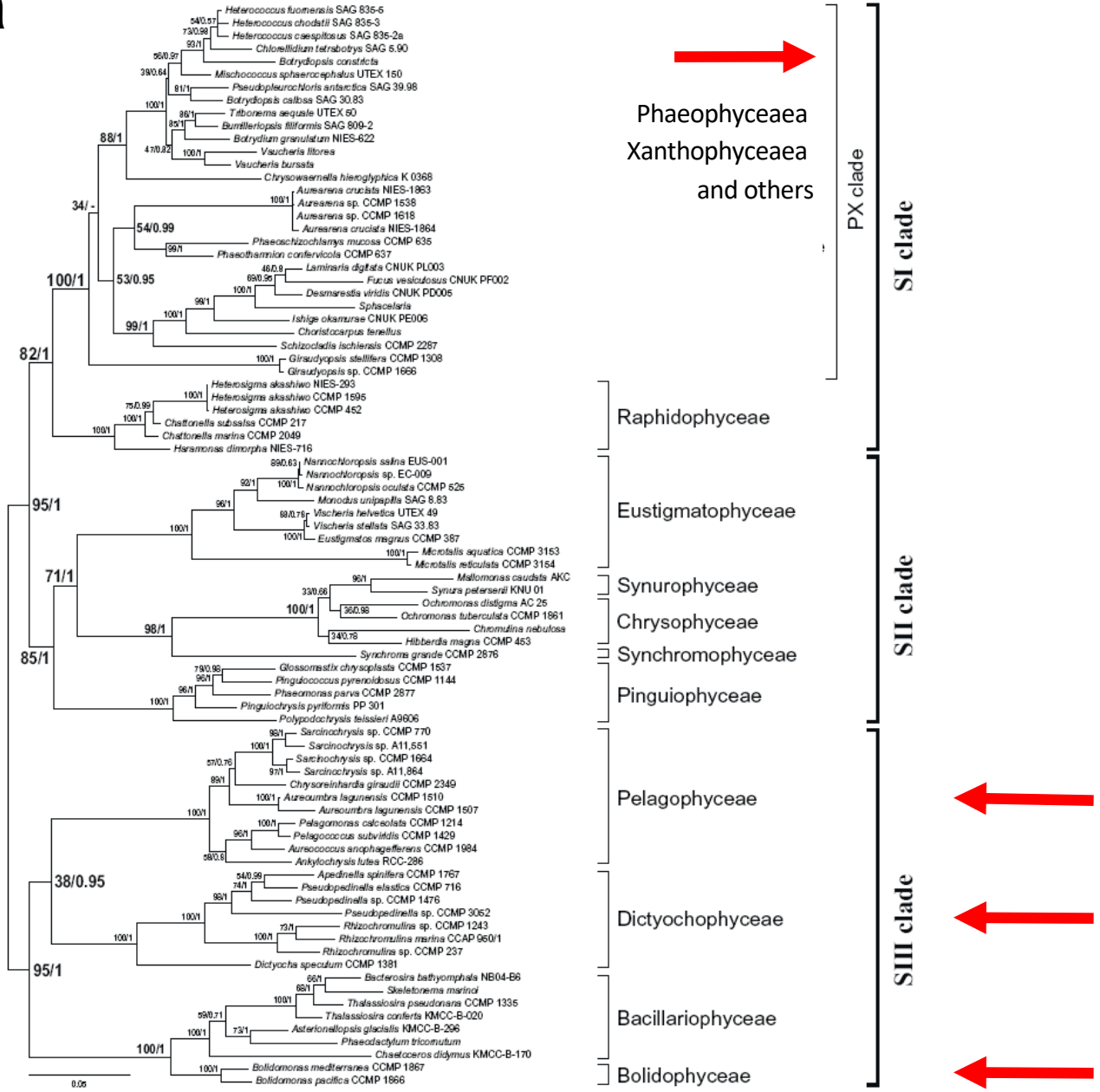
- the radiation of the majority of the known heterokont algal classes occurred throughout the Paleozoic and in the Triassic/Jurassic of the Mesozoic

Ochrophytes



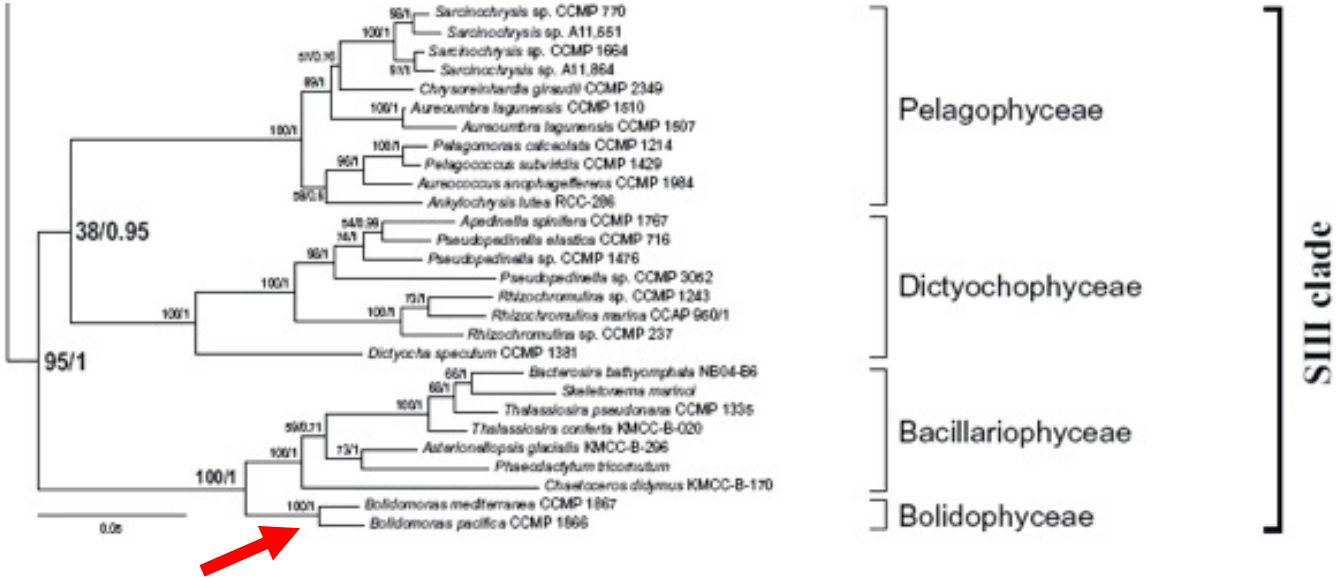
Brown & Sorhannus (2010); *Plos One*

Ochrophyta



Yang et al. (2012), *Protist*

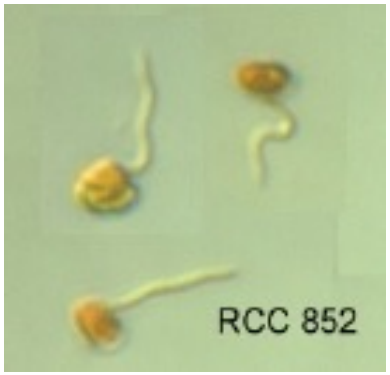
Bolidophyceae

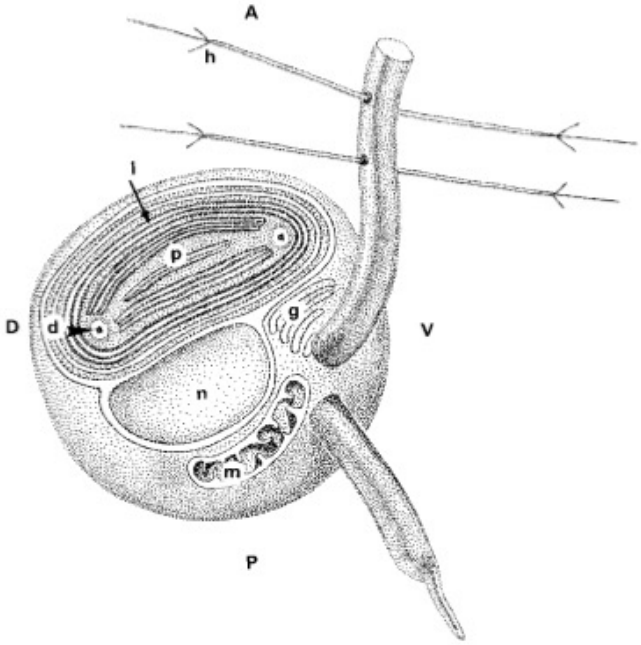


Yang et al. (2012), *Protist*

Bolidophyceae

- marine picophytoplankton (~1.2 um)
- heterokont naked flagellates
- typical stramenopile chloroplast; no eyespot; chlorophyll *a*, *c* + xanthophylls (including fucoxanthin)
- established in 1999, analyses of the SSU rDNA gene
- etymology: the rapid swimming behavior of the cells reminiscent of a racing car
- a sister group of diatoms





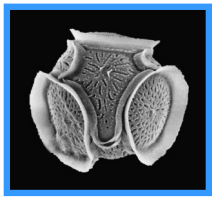
Bolidomonas mediterranea

(h) tubular flagellar hair, flagellum appears bipartite because their **basal section is reduced** to a basal disk. plastid (p), nucleus (n), Golgi body (g), mitochondrion (m). The plastid has a ring DNA genophore (d), girdle lamella (l). A = anterior, D = dorsal, P = posterior, V = ventral part

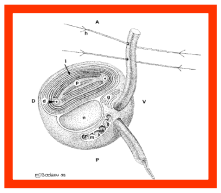
Guillou et al. (1999), *J. Phycol.*

Bolidophyceae ← Parmales

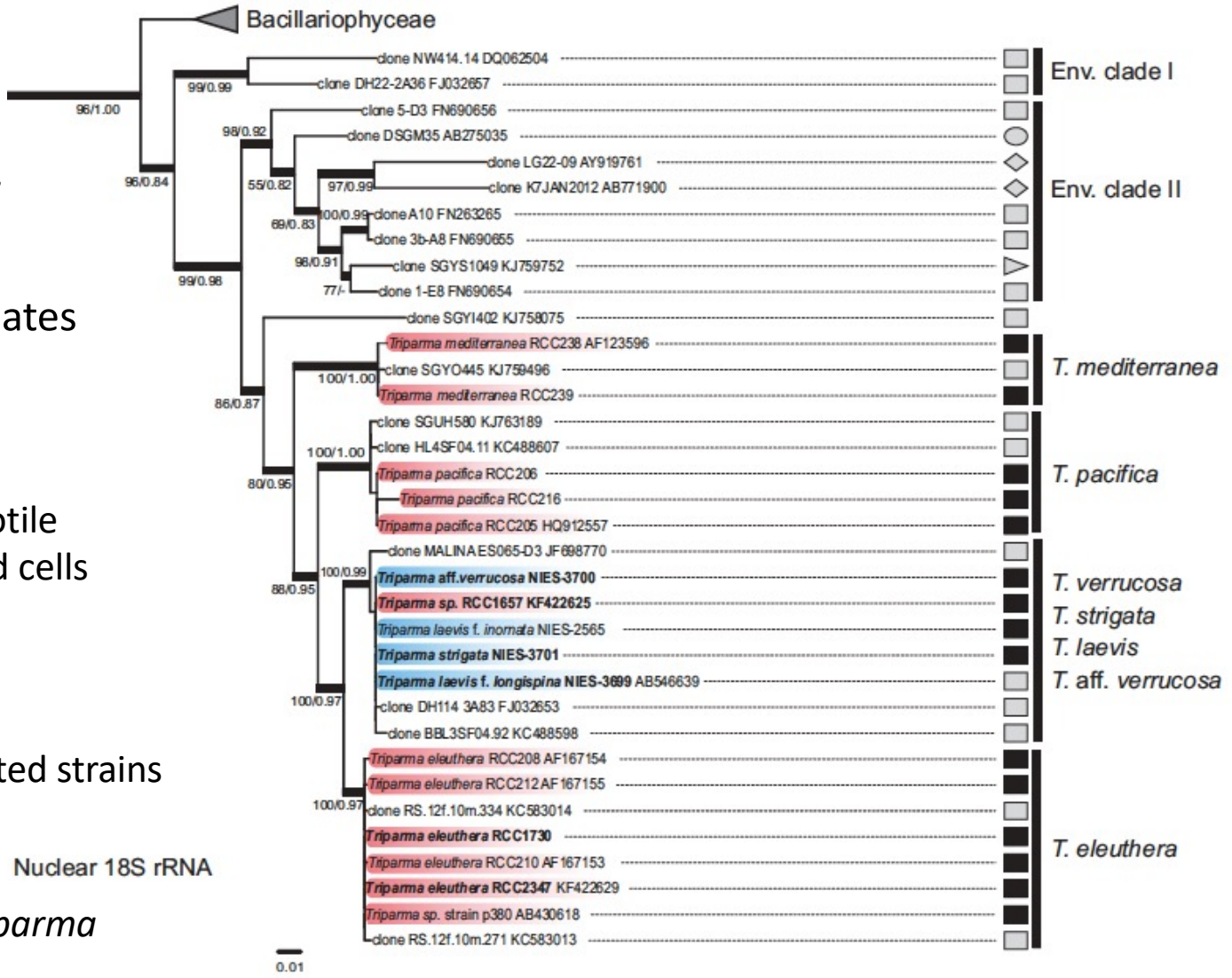
- Parmales (since 1987 within Chrysophytes) multipartite silica plates



non-motile silicified cells



motile flagellated strains



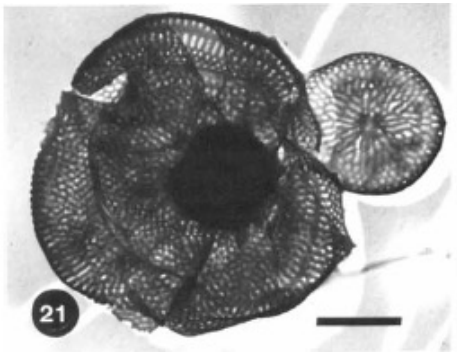
Nuclear 18S rRNA

Bolidomonas = Triparma

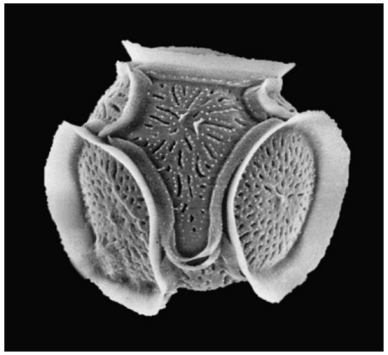
Ichinomiya et al. (2016), *The ISME Journal*

Bolidophyceae ← **Parmales**

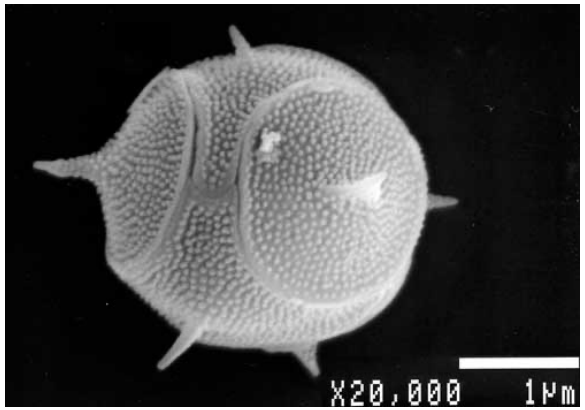
- marine nanophytoplankton (~2-5 um)
- coccoid non-motile forms
- abundant in polar and subpolar regions
- siliceous walls made up of plates (round, triradiate or oblong shape; often bearing ridges and spines and radiating lines of pores)
- etymology: small round shields

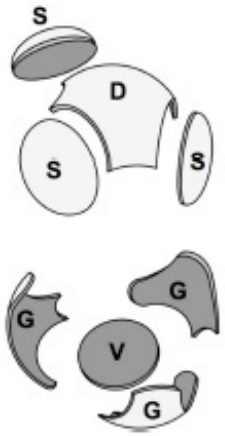


Triparma, TEM

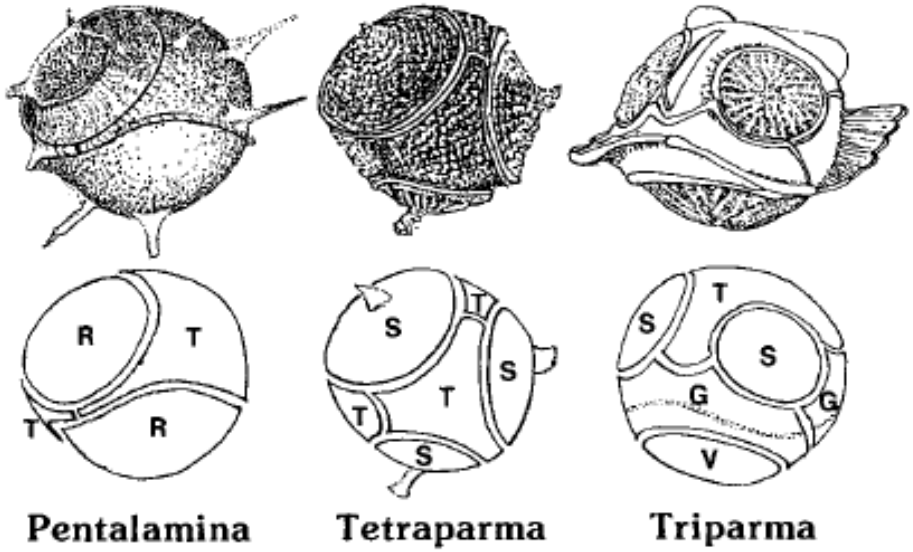


Tetraparma



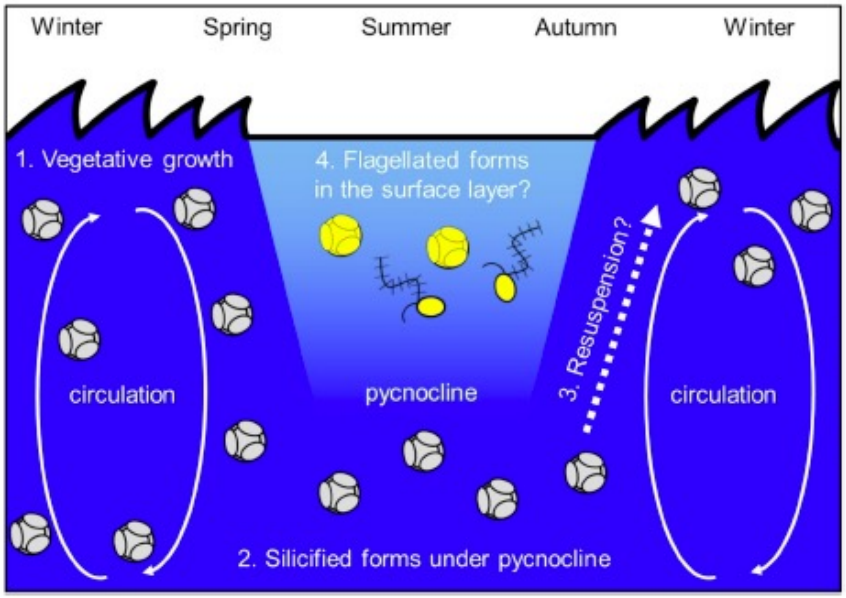


Tetraparma: 4 round and 4 triradiate plates



Bolidophyceae

- differences in ecological requirements between silicified and flagellated species
- the phylogenetically close relationship between silicified and naked strains (and recent occasional observation of both forms in cultures) -> different life cycle stages of the same species?

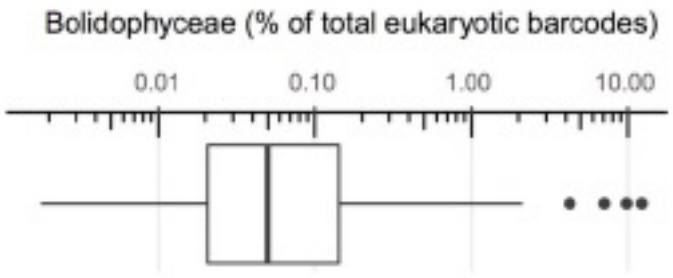


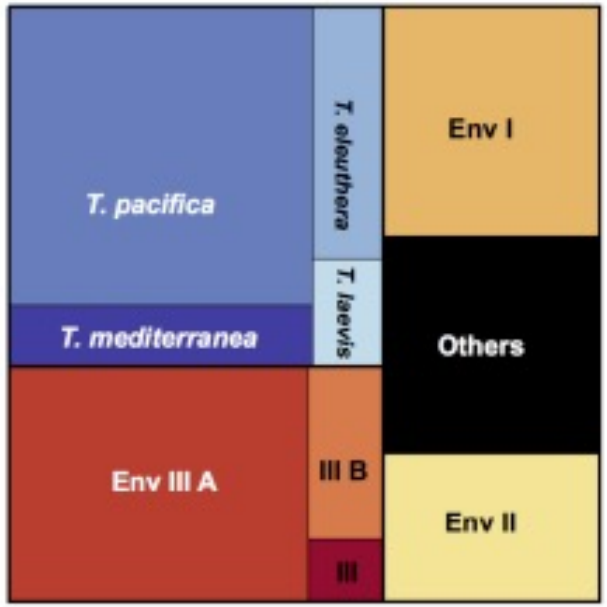
Properties	Bolidophyceae	
	Silicified species	**Flagellated species**
Size (µm)	2–5	1–1.7
Level of organization	Unicellular	Unicellular
Silicified cell wall	Yes	No
Flagellate form	Yes	Yes
Number of species	12	3
Oceanic distribution	Ubiquitous, but minor	
Main habitat	Cold eutrophic water (Polar and subpolar region)	Warm oligotrophic water (Tropical or subtropical)

Kuwata et al. (2018), *Frontiers in Martine Science*

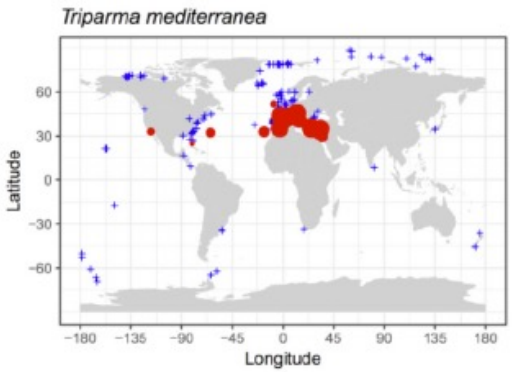
Bolidophyceae

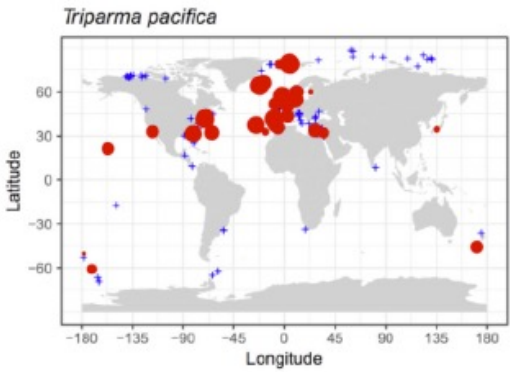
- the average contribution of Bolidoph. to total metabarcodes was 0.23% (highest, up to 12%, in both Arctic and Antarctic regions as well as around the European coast)





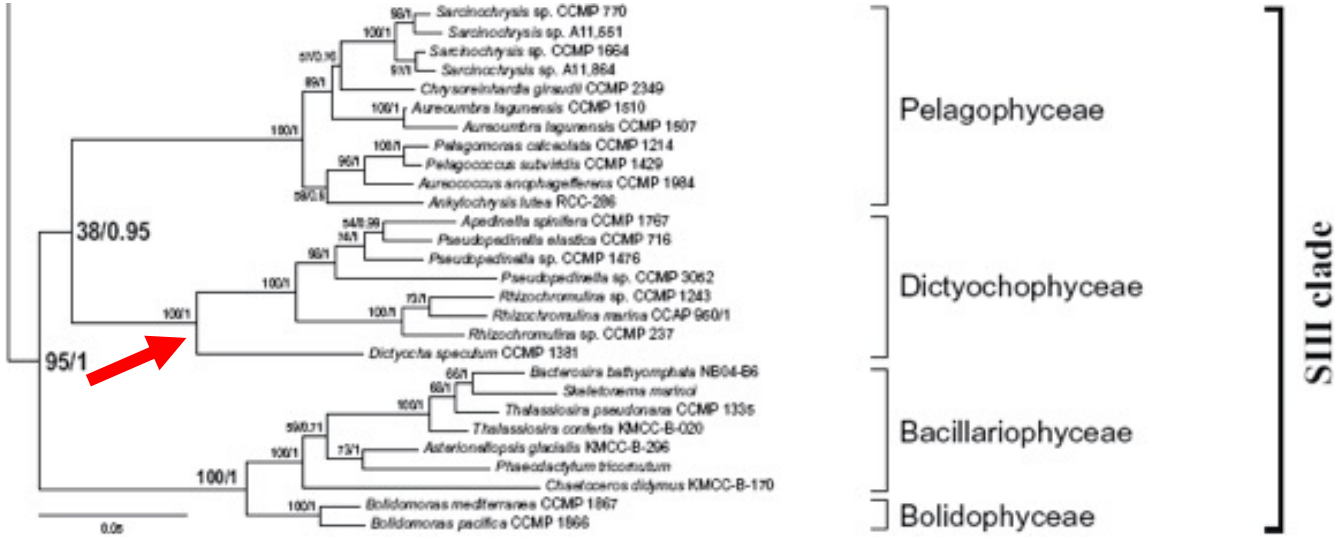
- *Triparma pacifica* was most abundant followed by *T. mediterranea* (both were originally described as naked, i.e. *Bolidomonas*)





Kuwata et al. (2018), *Frontiers in Martine Science*

Dictyochophyceae



Yang et al. (2012), *Protist*

Dictyochophyceae

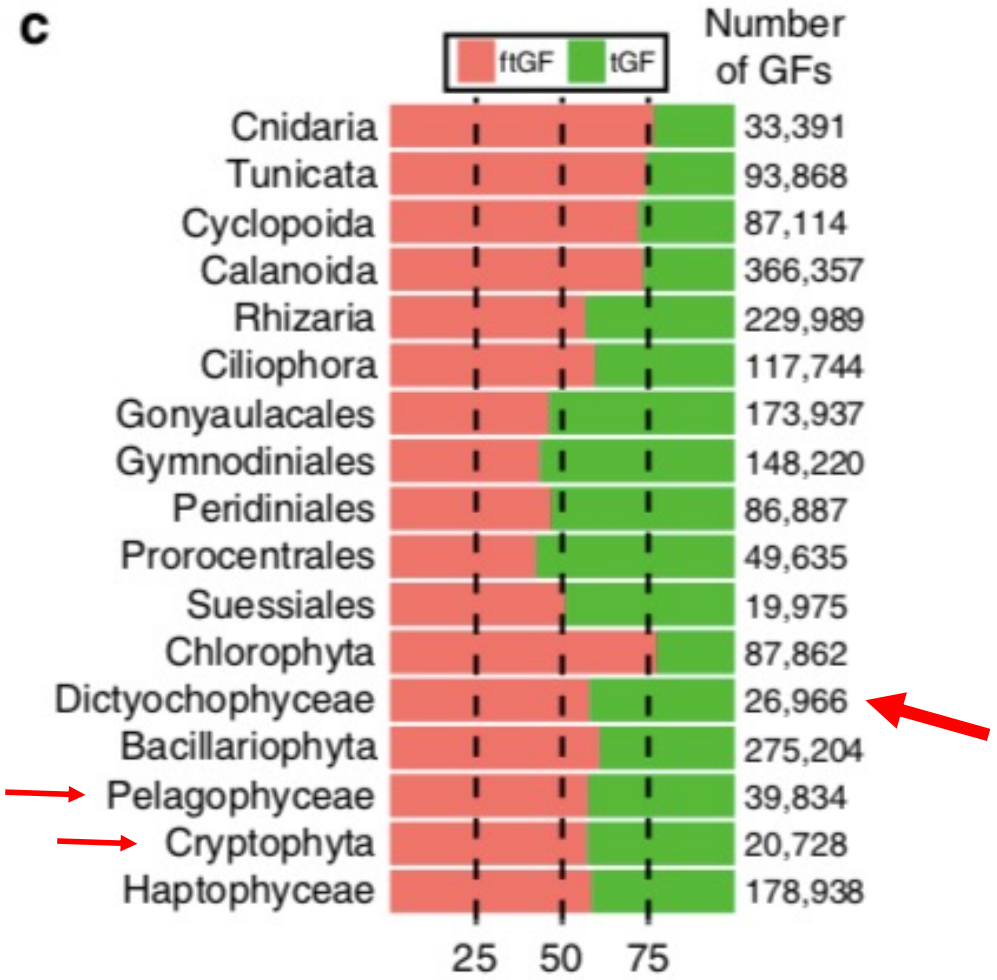
- freshwater and marine environments
- as planktonic autotrophs (some are mixotrophs, and non-photosynthetic bacterivores)
- single cells or colonial; basically amoeboid cells (tentacles/rhizopodia/pseudopodia)
- usually with one flagellum with mastigonemata
- cells naked, with organic scales or with siliceous skeleton
- typical stramenopile chloroplast; chlorophylls *a*, *c1, c2*, xanthophylls (including fucoxanthin)
- previously classified in the Chrysophyceae (1950-1986)

Dictyocha speculum

Apedinella

Dictyochophyceae

- during the TARA Oceans expedition (to generate a global ocean reference catalog of genes from planktonic eukaryotes) show that Dictyochophyceae is quite abundant and diverse in global oceans, possibly being important planktonic primary producers

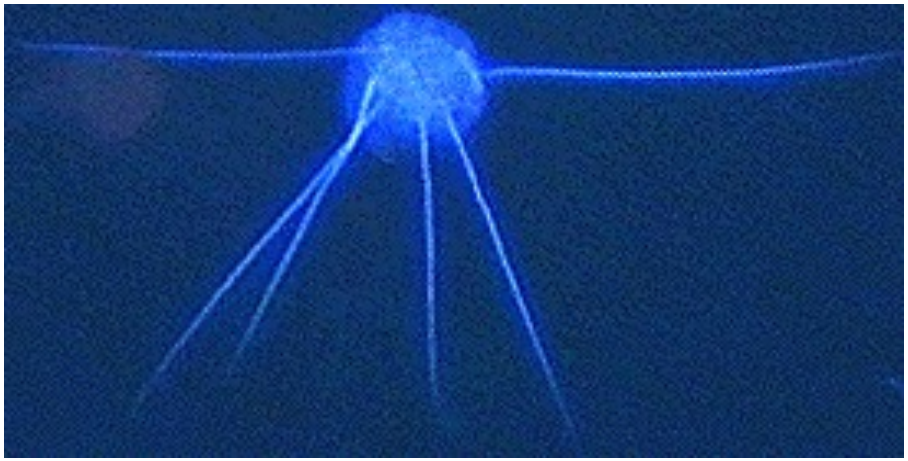


percentage of unigenes; ftGF: functional and taxonomical assigned groups; tGF: taxonomically assigned.

Carradec et al. (2018); *Nat. Commun.*

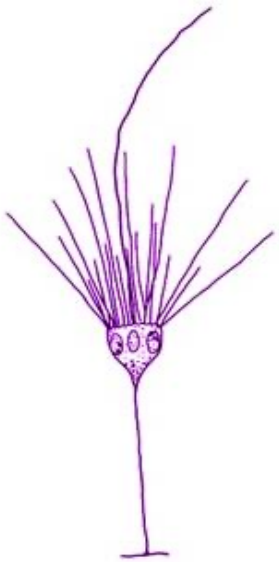
Dictyochophyceae – **Pedinellales**

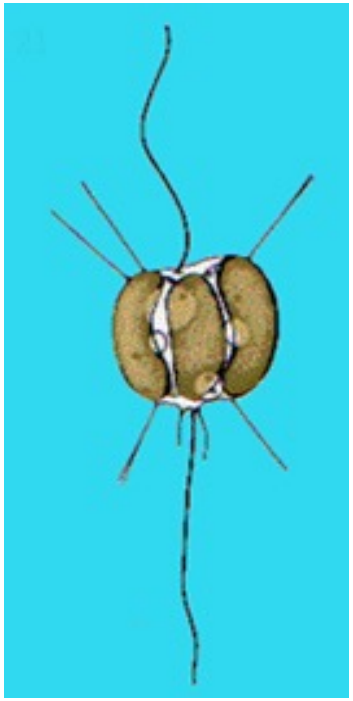
- marine and freshwater
- mixotrophic or heterotrophic (phagotrophy of bacteria, *Actinomonas*, *Pteridomonas* - detection of a leucoplast)
- unicells with a long anterior flagellum (a second flagellum reduced to a basal body)
- usually three to six chloroplasts (if chloroplasts are present)
- organically scaled (*Pedinella*, *Apedinella*) or loricate cells

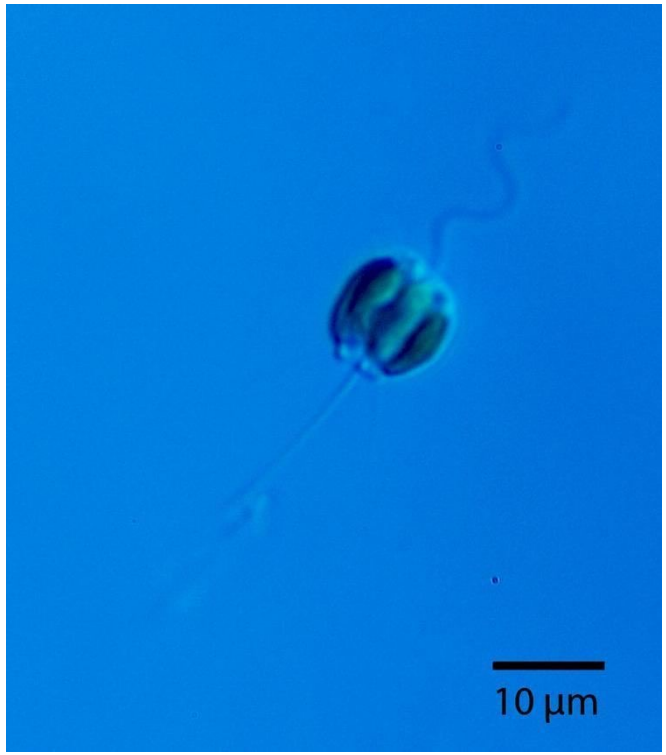


Apedinella - photoautotrophy

Dictyochophyceae – **Pedinellales**



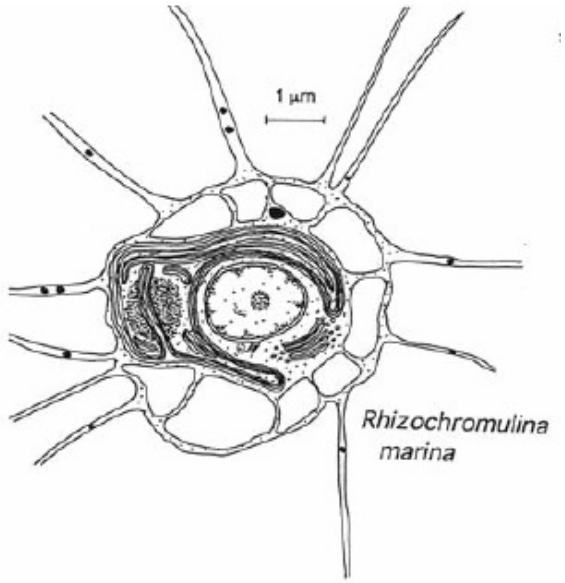




- *Pedinella*: mixotrophy, a posterior sticky stalk (cells rotate while swimming, trailing the stalk behind, often adhere a swimming cell to a substrate)

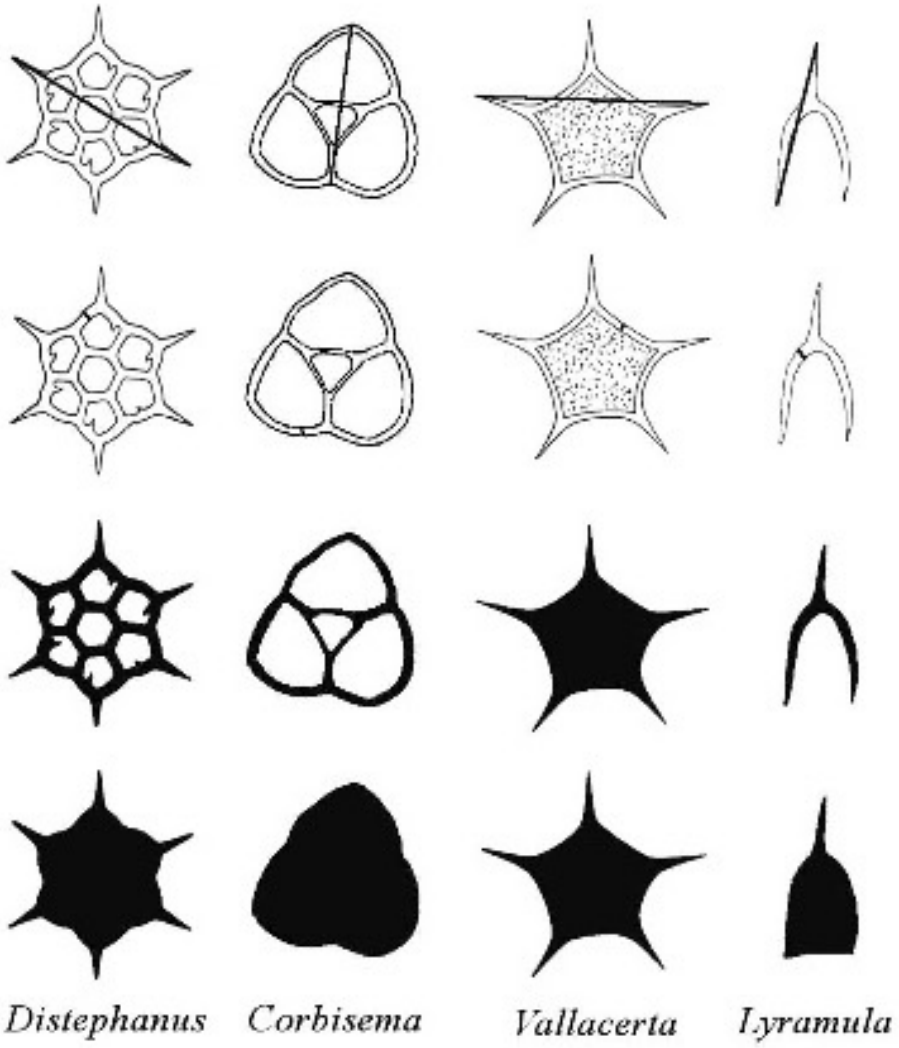
Dictyochophyceae – **Rhizochromulinales**

- marine (puddles and tidal zones) and freshwater
- amoeboid vegetative cells, fine beaded-filipodia (pseudopods)
- swimming cells with a single flagellum (a second basal body in protoplasm)
- a single golden-brown chloroplast (*Rhizochromulina marina*) or heterotrophic taxa (*Ciliophrys* spp.)



Dictyochophyceae – **Dictyochales**

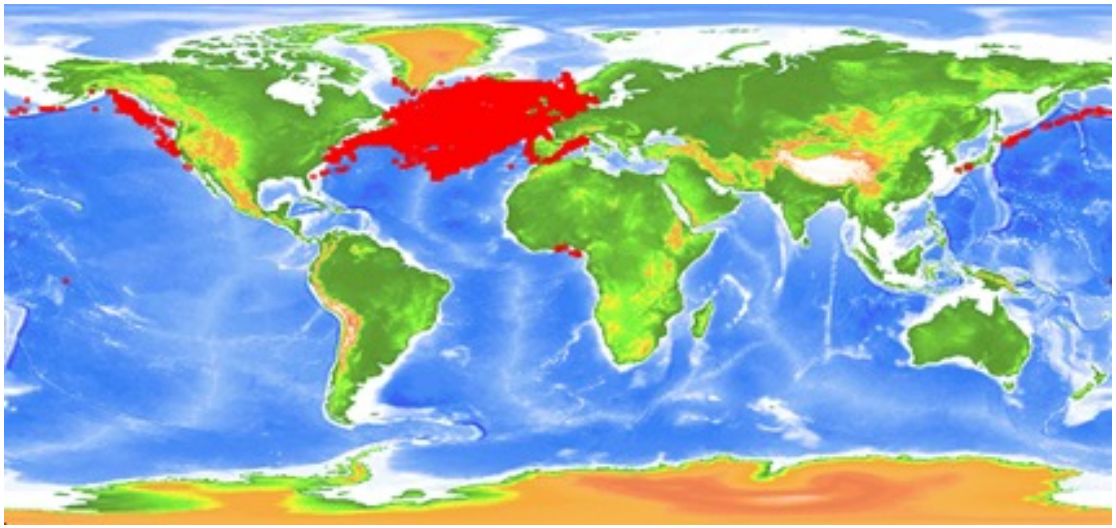
- so-called silicoflagellates
- marine habitats, phytoplankton
- external silica skeleton present on at least one life stage [but *Vicicitus globosus*]
- predominantly fossil (first appearing in the Early Cretaceous, currently only four extant siliceous species, genus *Dictyocha*); indication of seawater temperature (predominantly in cold waters)

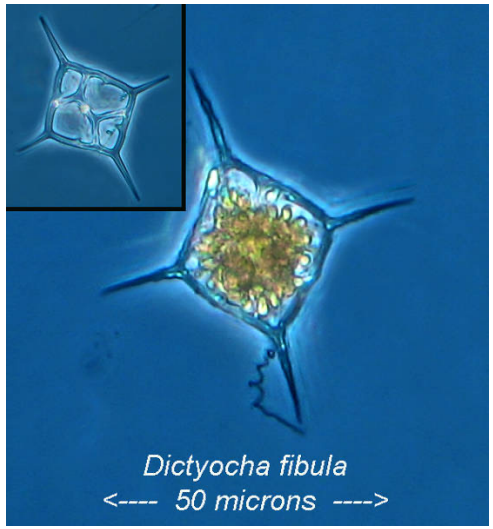


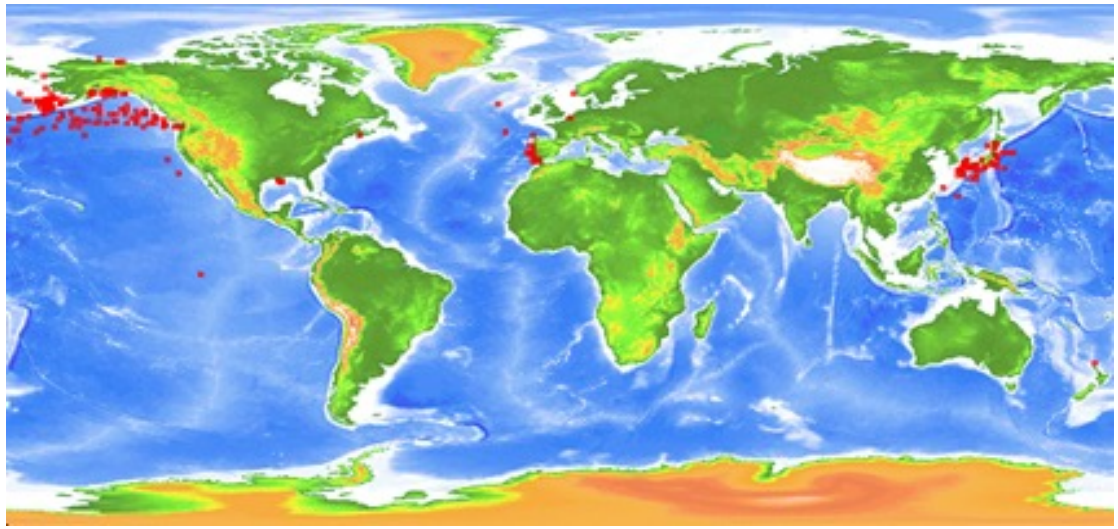
fossil genera, Dictyochales

Dictyochophyceae – **Dictyochales**

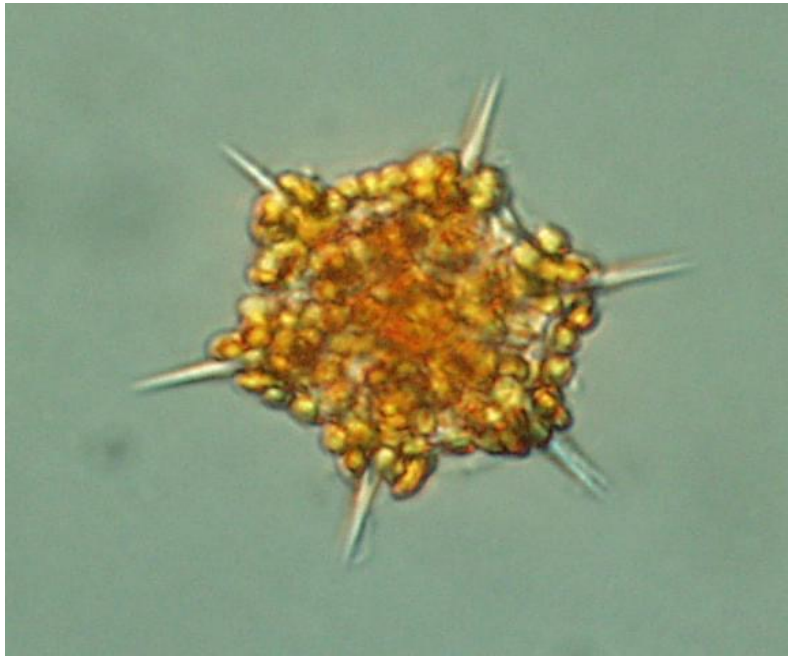
Dictyocha speculum



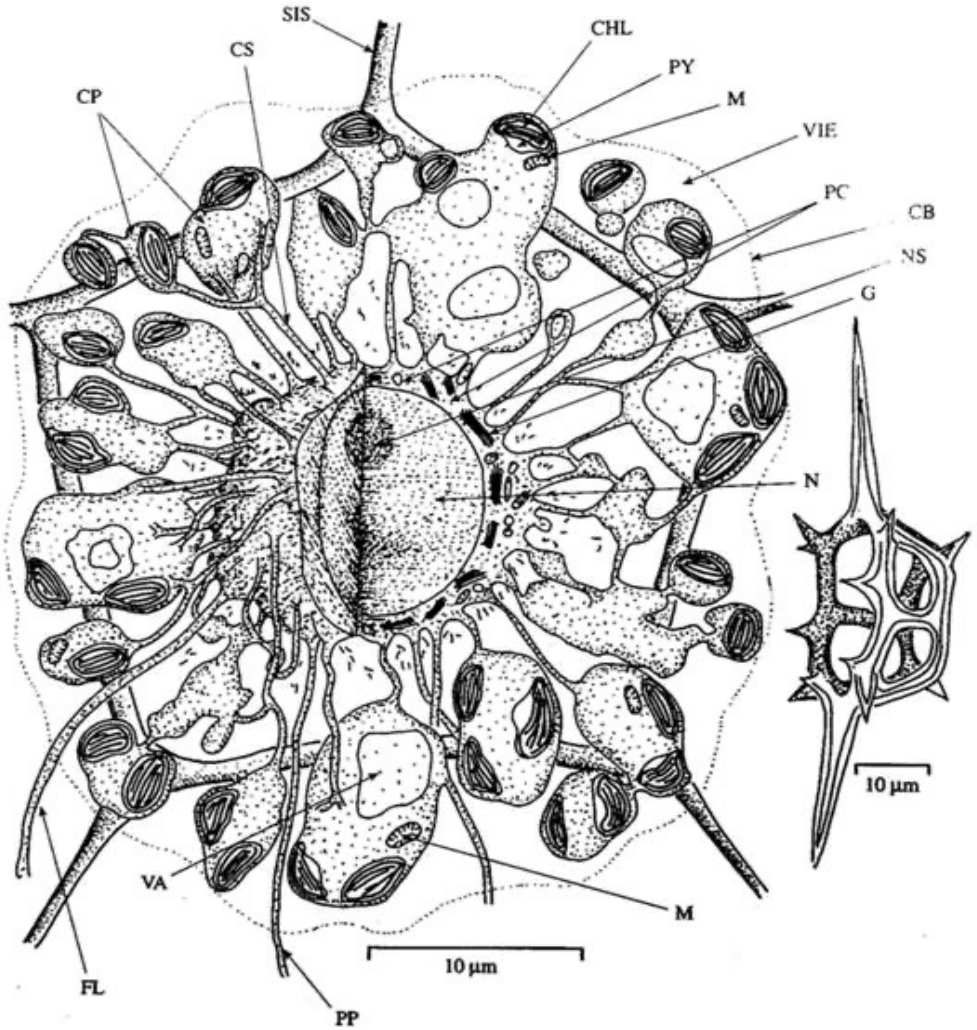




Dictyochophyceae – **Dictyochales**

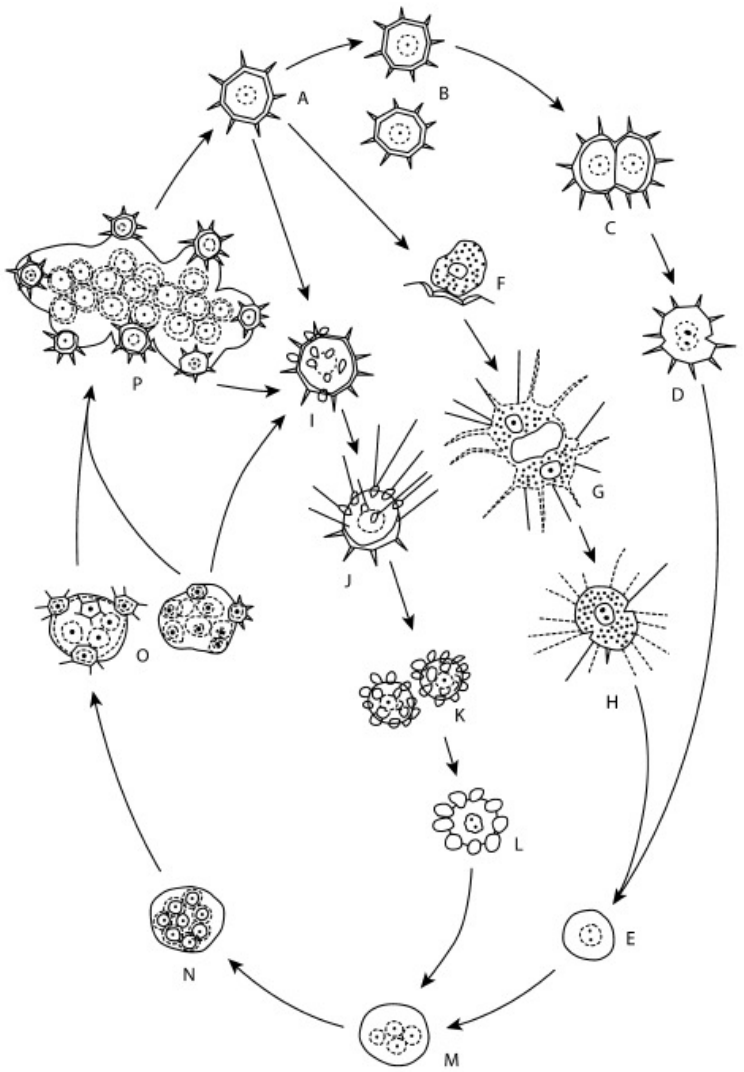


Dictyocha



FL: flagellum; **M**: mitochondria, **N**: nucleus; **CHL**: chloroplast; **PY**: pyrenoid; **SIS**: siliceous skeleton; **PP**: pseudopodium; **CB**: cell boundary; **VIE**: mucilaginous envelope

Dictyochophyceae – **Dictyochales**



Sexual reproduction of *Dictyocha octonaria*.

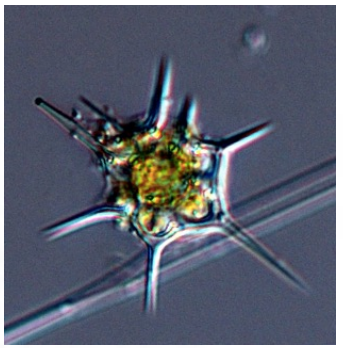
A–E + I. Skeletonbearing cells. **F.** Transformation of a skeletonbearing to amoeboid cell. **G–H**. **Amoeboid cells J.** Some cells started to show long **mucus filaments** on mucocysts before moving away from the parent skeleton. **K–L.** Fusion of two gamete-like, mucocyst-bearing cells to form a zygote. **M–O. Multinucleate cells P. Flagellated** daughter cells pushed their way through the membrane of the massive **plasmodium-like aggregate**.

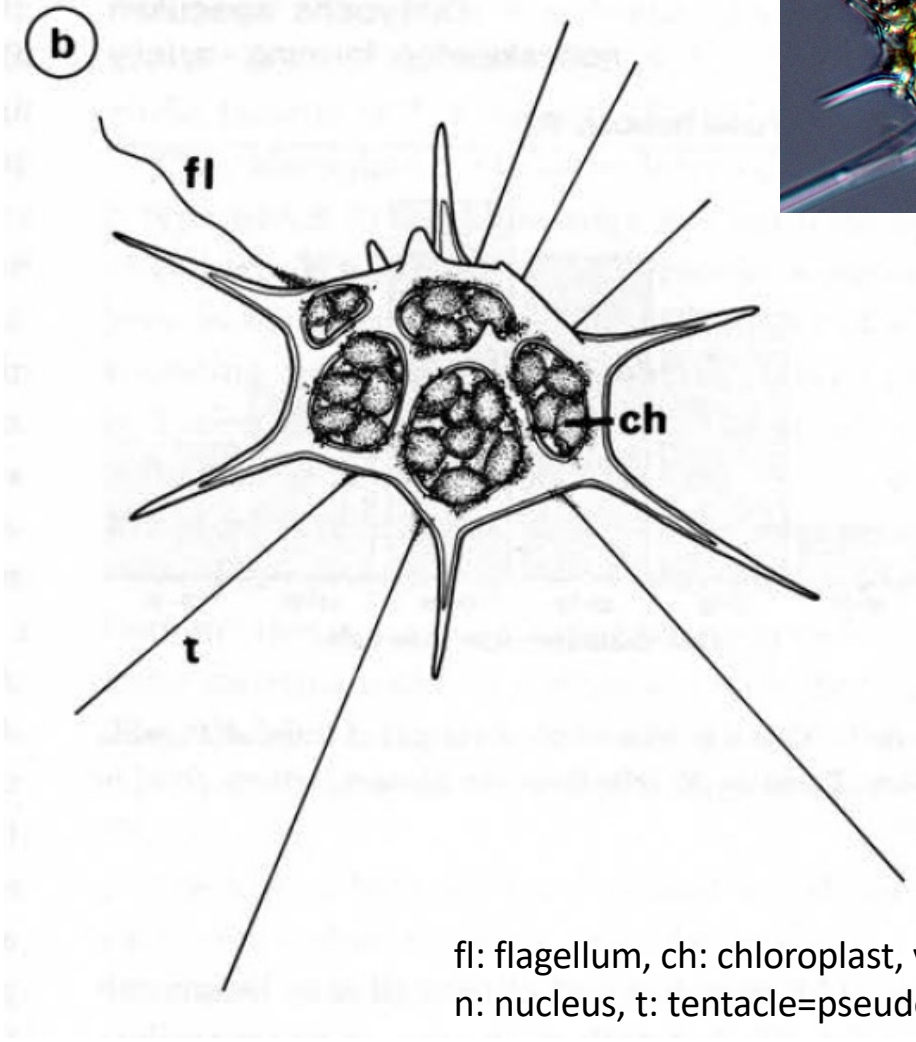
With the exception of the zygote, which is in diplophase, all the rest, from uninucleate, gamete-like cells, to daughter cells formed within the multinucleate/parent and plasmodium-like cells, are in haplophase.

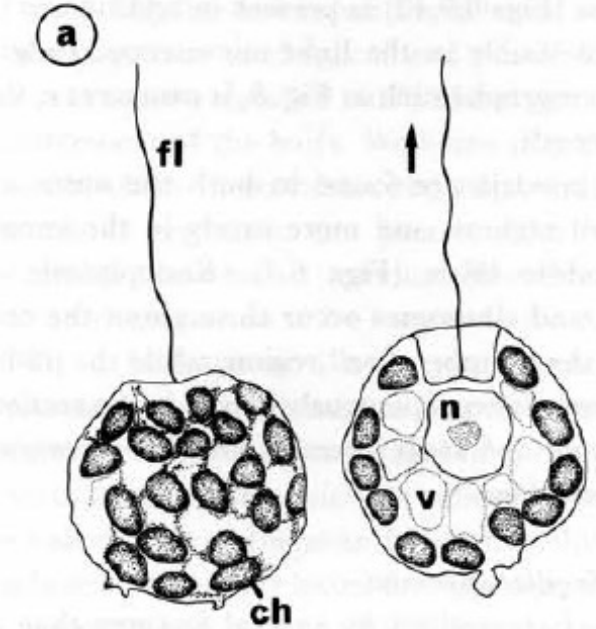
Chang et al. (2015), *Phycological Research*

Dictyochophyceae – **Dictyochales**

- microtubule-supported pseudopodia





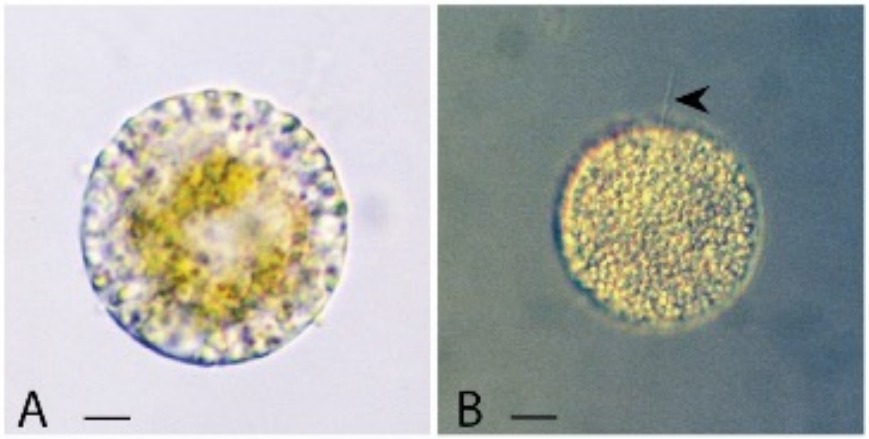


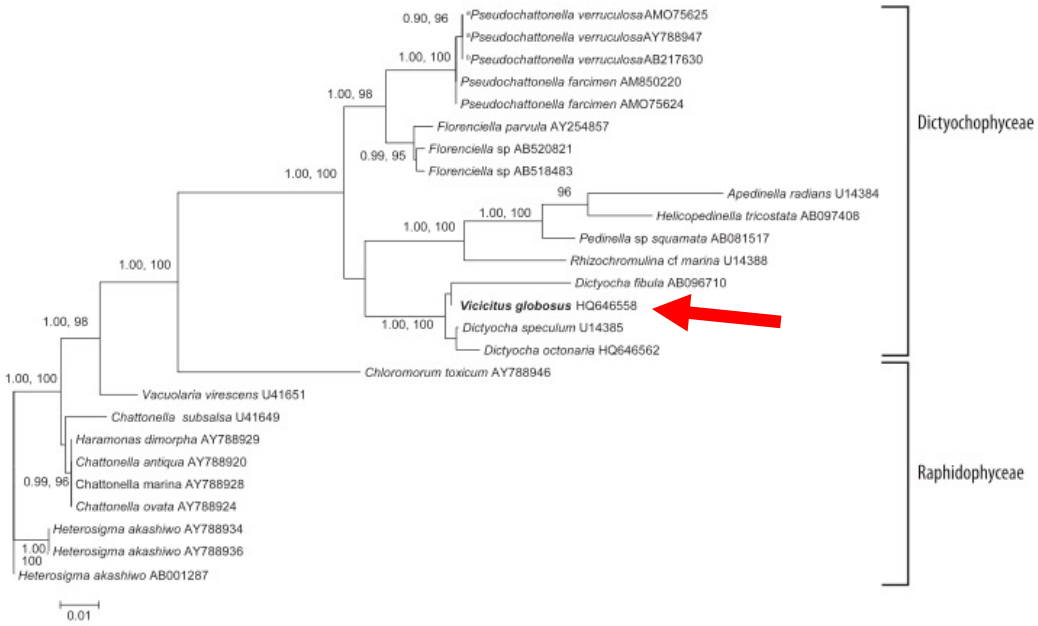
fl: flagellum, ch: chloroplast, v: vacuole,
n: nucleus, t: tentacle=pseudopodium

the naked form and skeleton bearing form, *Dictyocha speculum*

Dictyochophyceae – **Dictyochales**

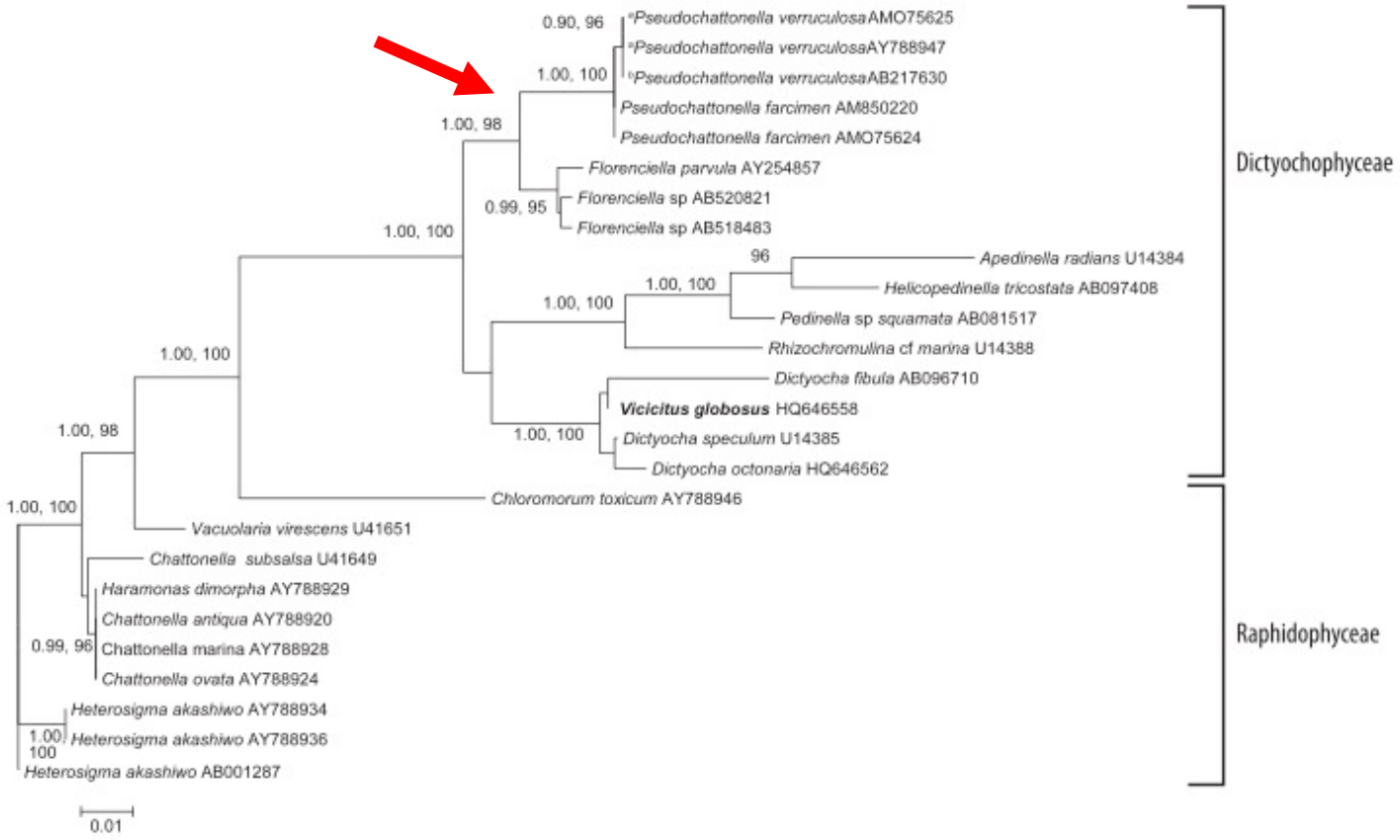
- *Vicicitus globosus* – previously in Raphidophyceae (as *Chatonella globosa*)
- no siliceous skeleton-bearing stage observed in the life history
- mucocysts protruding evenly over the cell membrane (swimming cells can transform swiftly from regular globular shape to amoeboid form in a matter of seconds)





Hoe Chang et al. (2012); *Phycologia*

Dictyochophyceae – **Florenciellales**



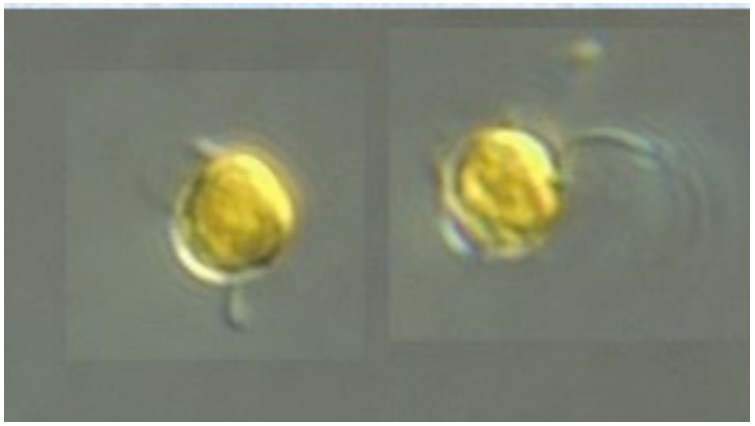
Pseudochatonella ← *Chatonella aff. verruculosa*

- confusion with ichtyotoxic Raphidoph.

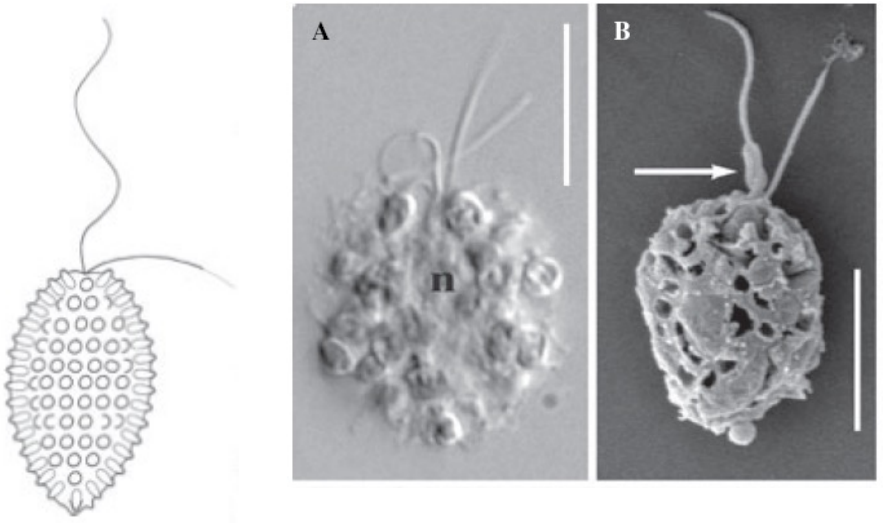
Hoe Chang et al. (2012); *Phycologia*

Dictyochophyceae – **Florenciellales**

- marine environment
- picoflagellate *Florenciella parvula,* described in 2004 (English Channel)
- *Verrucophora* spp. (previously as *Pseudochatonella* ← *Chatonella aff. verruculosa*) a bloom forming flagellate in cold waters (Norway, Japan, Chile, New Zealand)



Florenciella parvula

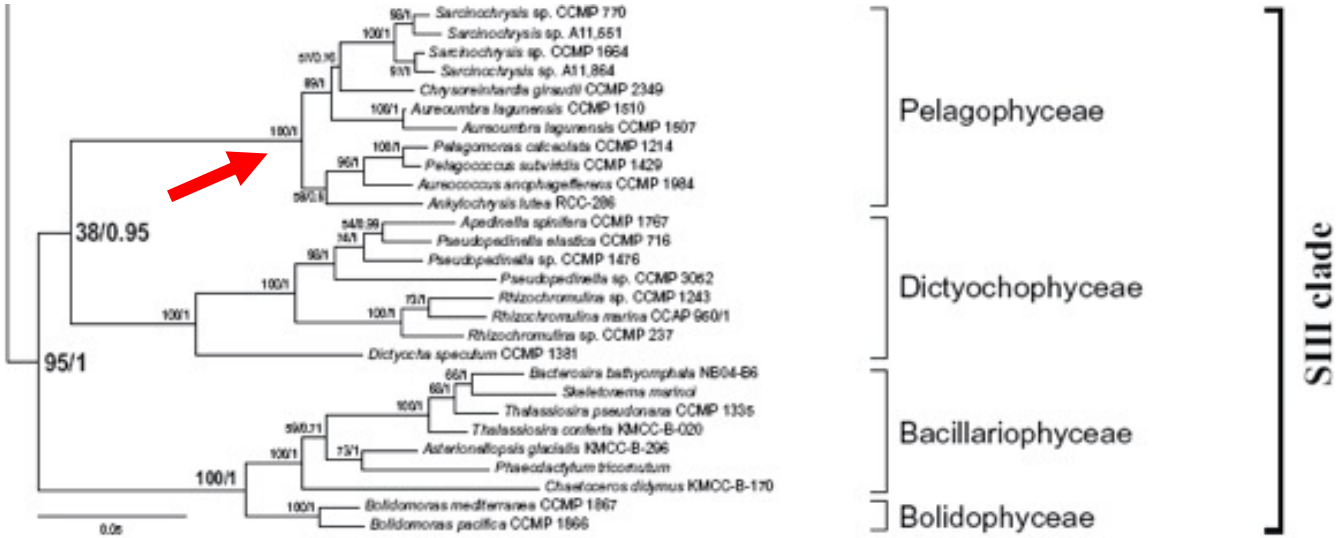


Verrucophora farcimen

mucocysts evenly distributed on the cell surface – secretion of mucus, fish kills

Edvardsen et al. (2007), *Journal of Phycology*

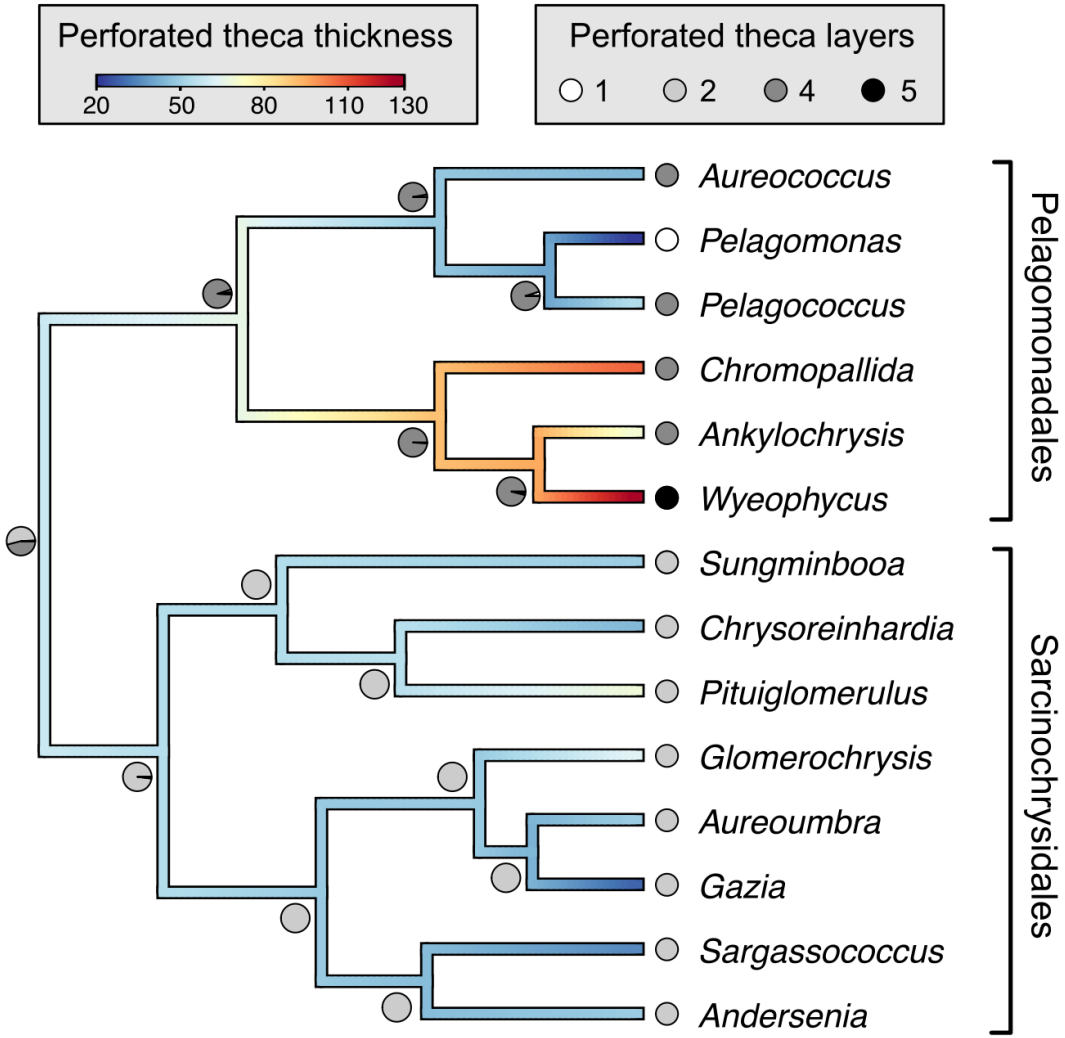
Pelagophyceae

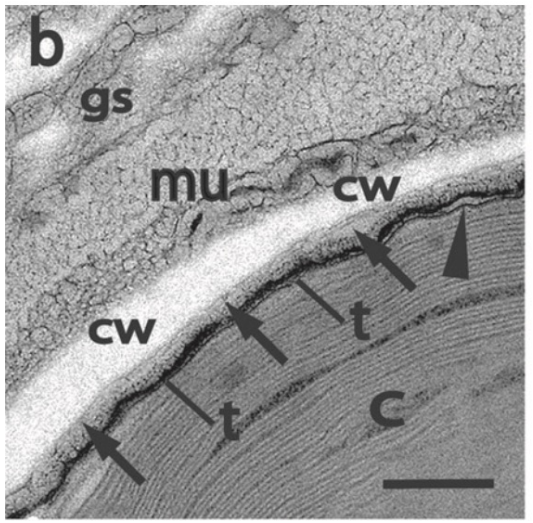


- variable forms: ciliated, coccoid, sarcinoid, capsoid, filamentous
- swimming cells with one or two flagella (bipartite or tripartite tubular hairs)
- typical stramenopile chloroplast; no eyespot;
  chlorophylls *a*, *c* + xanthophylls (including fucoxanthin)

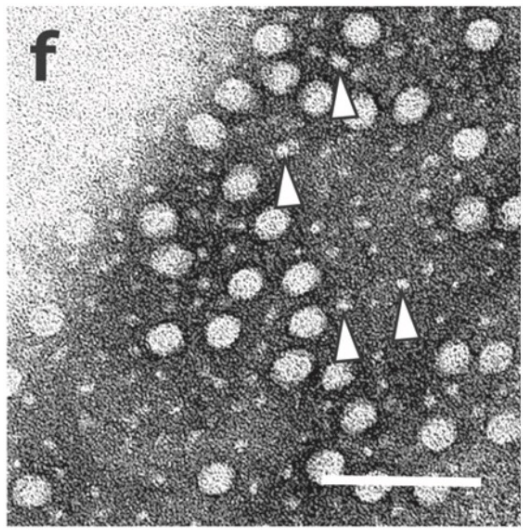
Yang et al. (2012), *Protist*

Pelagophyceae





cw: cell wall, **t: theca**, arrowheads: plasma membrane, gs: gel sheat, mu and arrows: mucilaginous layers

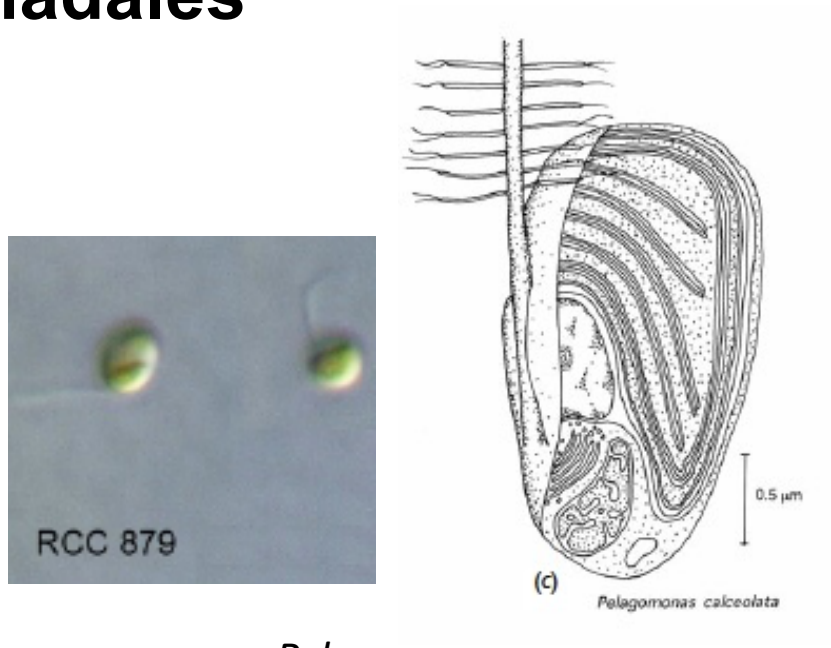


macropores and micropores

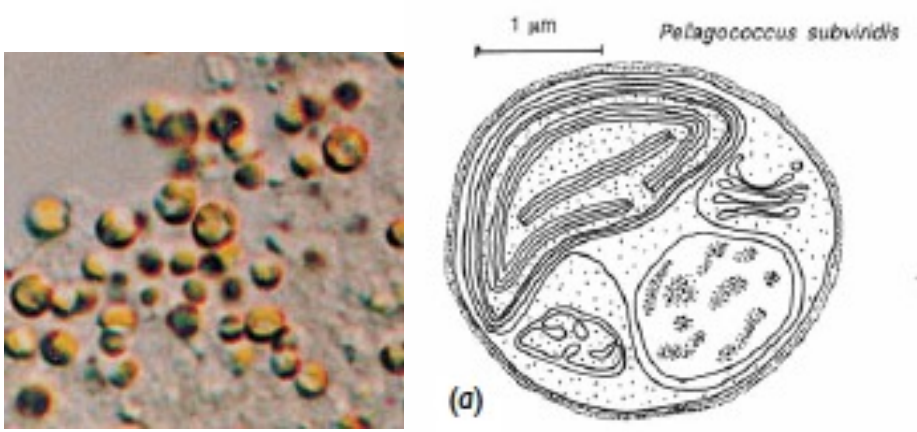
Wetherbee et al. (2021, 2023), *Journal of Phycology*

Pelagophyceae – **Pelagomonadales**

- marine picoplankton
- *Pelagomonas:* monadoid (a single flagellum with bipartite hairs, a second basal body absent); a thin organic theca surrounds most of the cell
- *Pelagococcus:* free living or symbiont of foraminifera



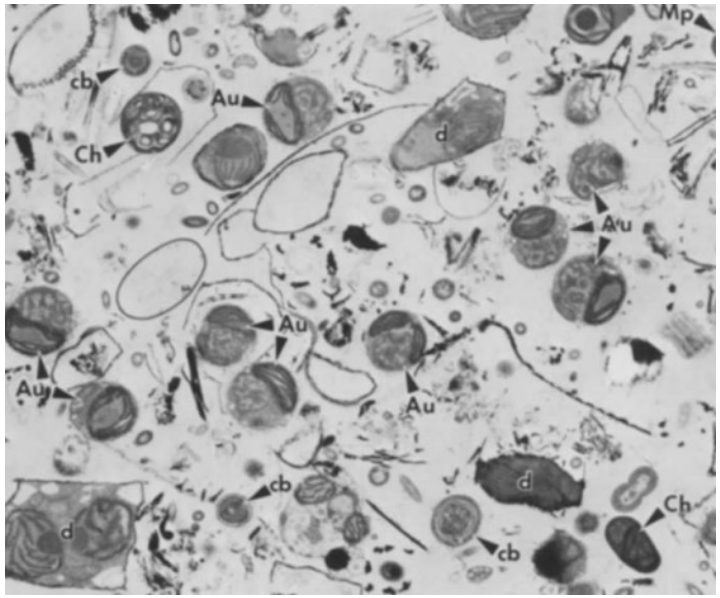
Pelagomonas



Pelagococcus

Pelagophyceae – **Pelagomonadales**

- *Aureococcus anophagefferens:* able to grow at low temperature and survive long periods of darkness
- brown-tides (along the eastern coast of the United States, South Africa), harmful for *Zostera marina* meadows (reduced light penetration) and shellfish (reduced growth rate of filter feeders, unavailable *Zostera* habitat)
- blooms: the importance of mixotrophy (metabolism of dissolved organic carbon) + cells are coated by polysaccharide material (inhibits filtration by grazing animals)
- etymology: golden yellow sphere



Au = *A. anophagefferens*; cb = the cyanobacterium *Synechococcus*; Ch = *Chlorella*-like species; d = diatom

Sieburt et al. (1988), *J. Phycol.*

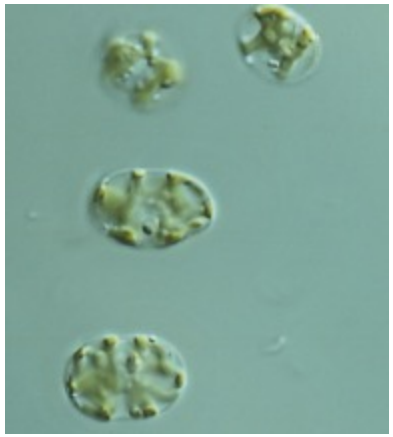
Journal of Applied Phycology (2022) 34:965–983
https://doi.org/10.1007/s10811-021-02677-9

Inhibition of harmful algal blooms caused by *Aureococcus anophagefferens* (Pelagophyceae) using native (*Gracilaria tikvahiae*) and invasive (*Dasysiphonia japonica*) red seaweeds from North America

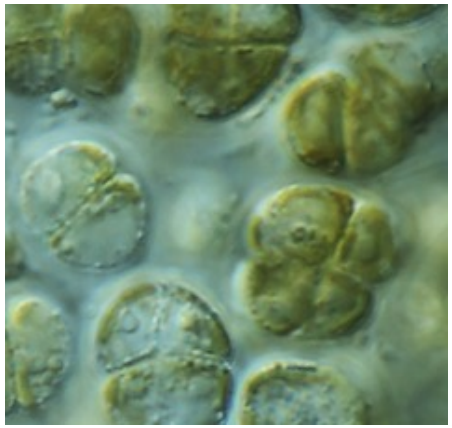
Colin Benitt[1] · Craig S. Young[1] · Laine H. Sylvers[1] · Christopher J. Gobler[1]

Pelagophyceae – **Sarcinochrysidales**

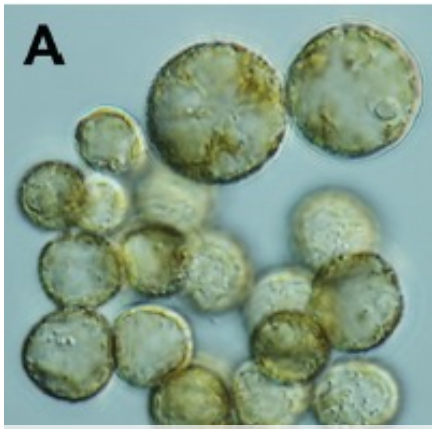
- single cell, filament, sarcinoid colony, clusters of cells
- all the Sarcinochrysidales species are capable of producing gel
- originally an order of Chrysophyceae



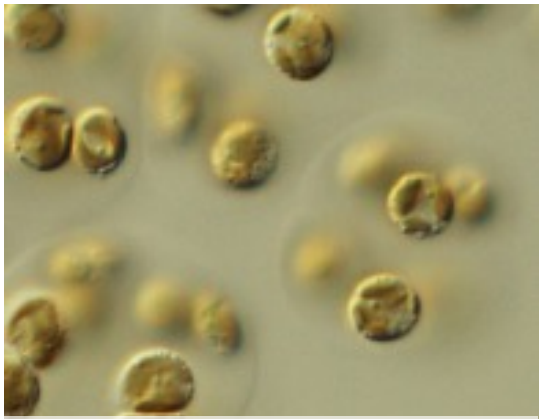
Arachnochrysis



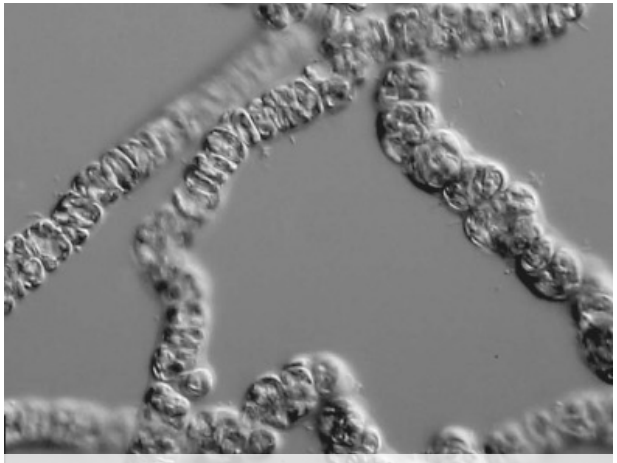
Chrysoreinhardtia



Sargasococcus



Sungminbooa

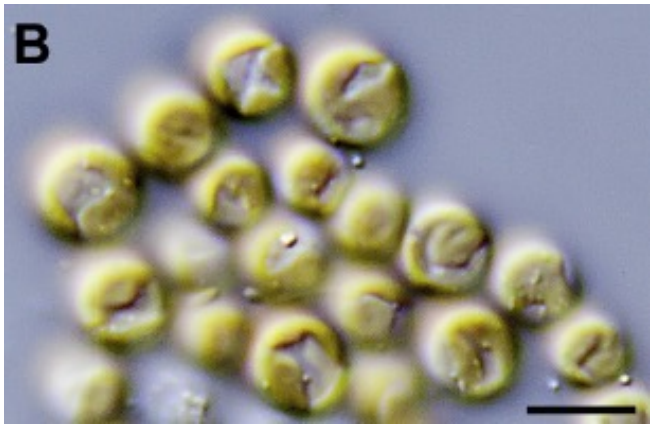


Andersenia

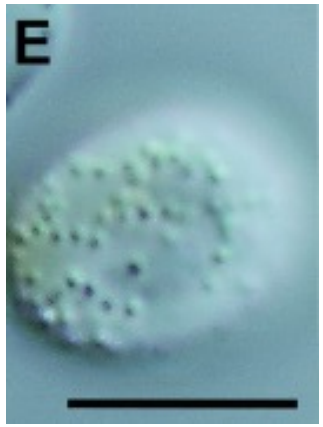
Han et al. (2018), *Protist*

Pelagophyceae – **Sarcinochrysidales**

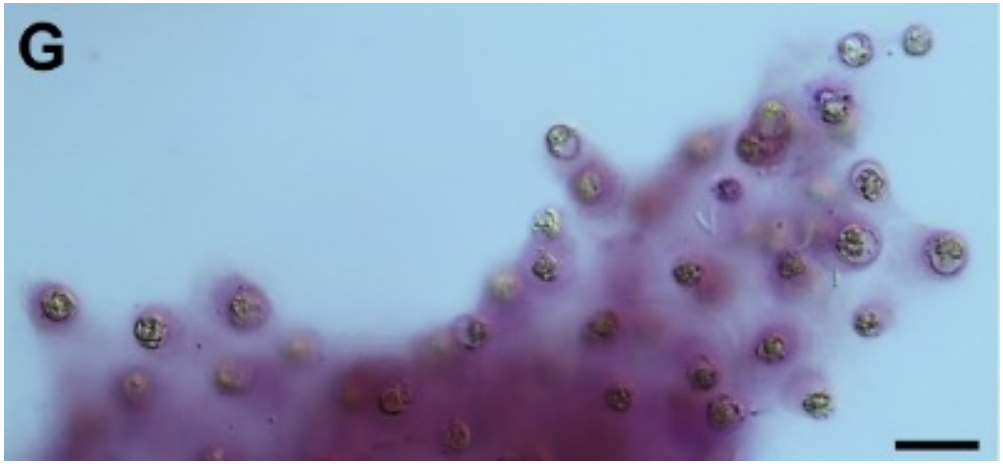
- *Aureoumbra lagunensis*: nonmotile coccoid cells that are covered with a slime layer that reduces predation
- survive and grow in hypersaline conditions
- massive blooms = brown tides
- *Aureoumbra geitlerii*: described in 2018, Canary Islands



Auroeumbra geitlerii



peripheral granules

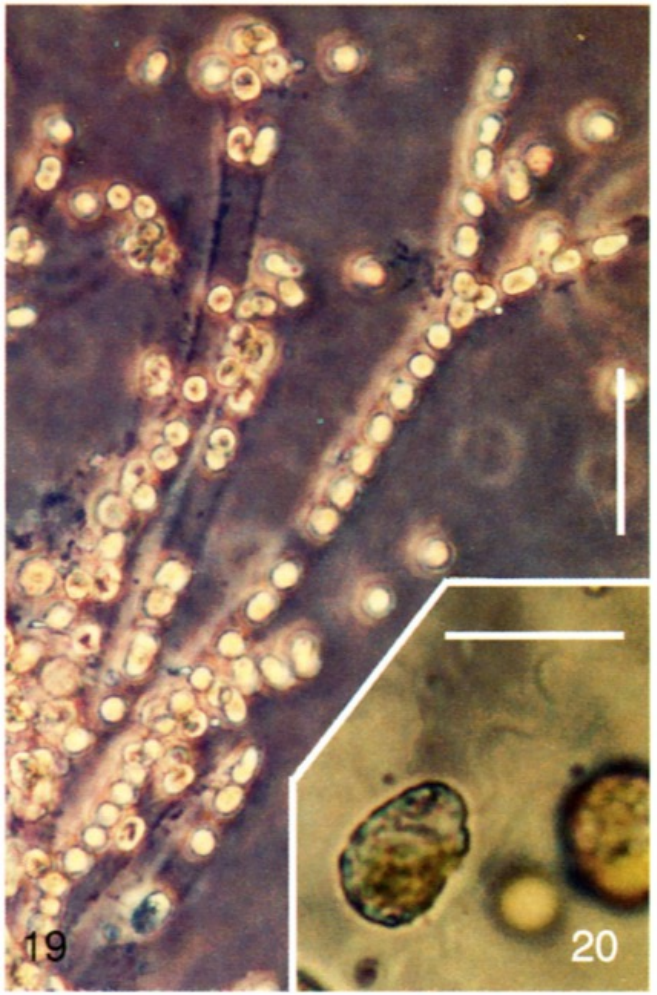


Auroeumbra geitlerii - gelatinous envelope

Han et al. (2018), *Protist*

Pelagophyceae – **Sarcinochrysidales**

- *Chrysocystis* (noodle alga)
- colonies forming sacs, cells in a layer of amorphous mucilage
- extensive nuisance blooms on benthic substrates in marine environments



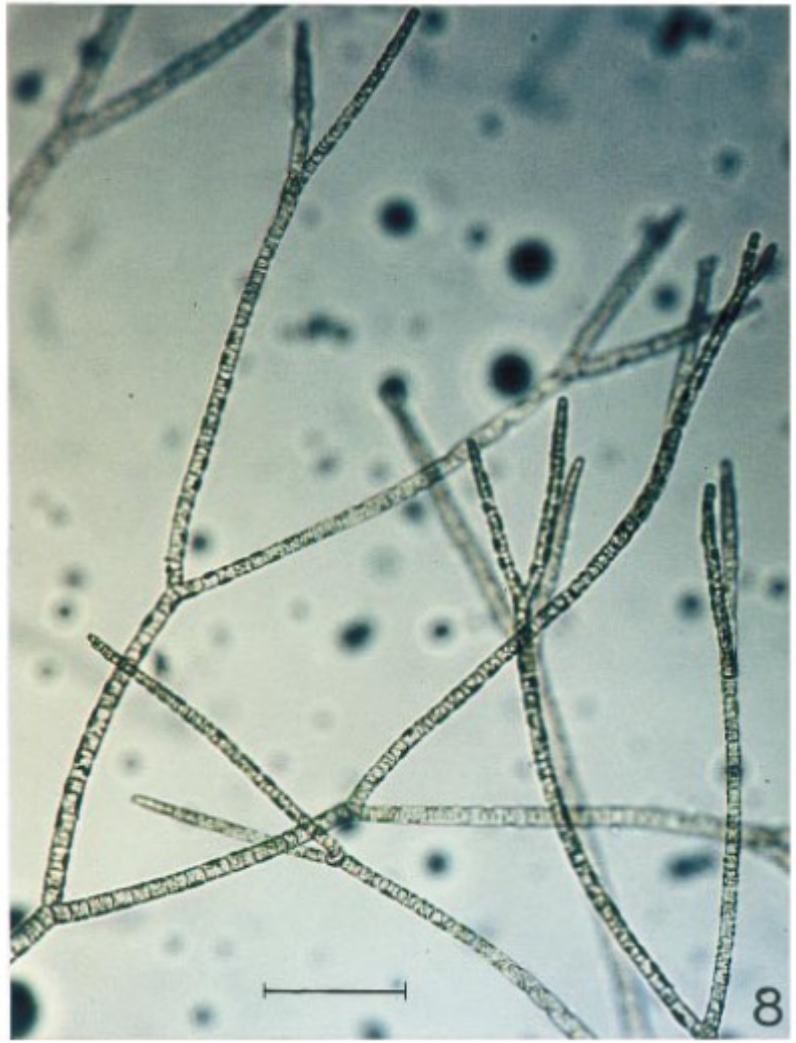
Lobban et al. (1995), *Micronesica*

Pelagophyceae – **Sarcinochrysidales**

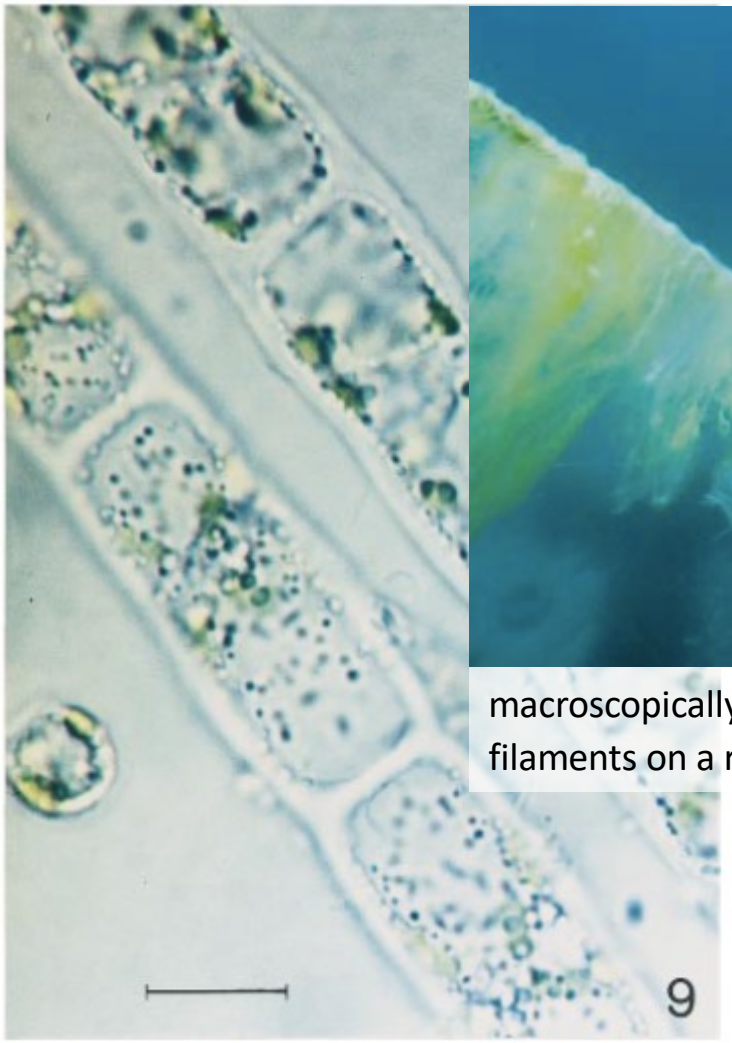
- *Chrysophaeum taylorii*: macroscopic gelatinous colonies (free-living cells randomly embedded in the mucilage)
- benthic; invasive spread in the Mediterranean Sea (from subtropic and tropic oceans)

Chrysophaeum taylorii

Pelagophyceae – **Sarcinochrysidales**



Chrysonephos lewisii

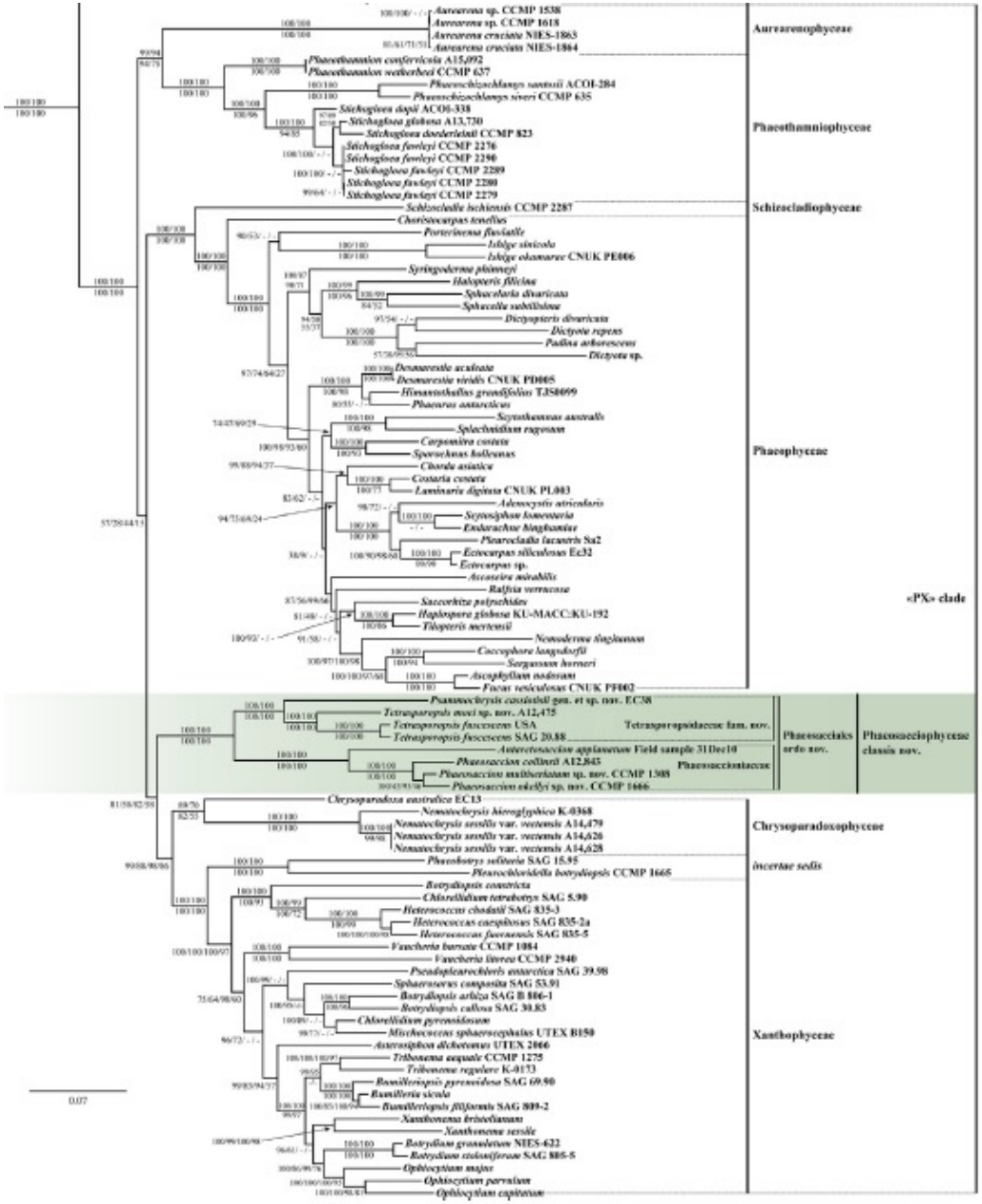


Nematochrysopsis marina (Tribonema marinum)

macroscopically visible filaments on a rope

Hoffman et al. (2000), *Botanica Marina*

PX clade



Graf et al. (2020), *Protist*

PX clade

Table 2. Comparison of selected characters among the members of the PX clade

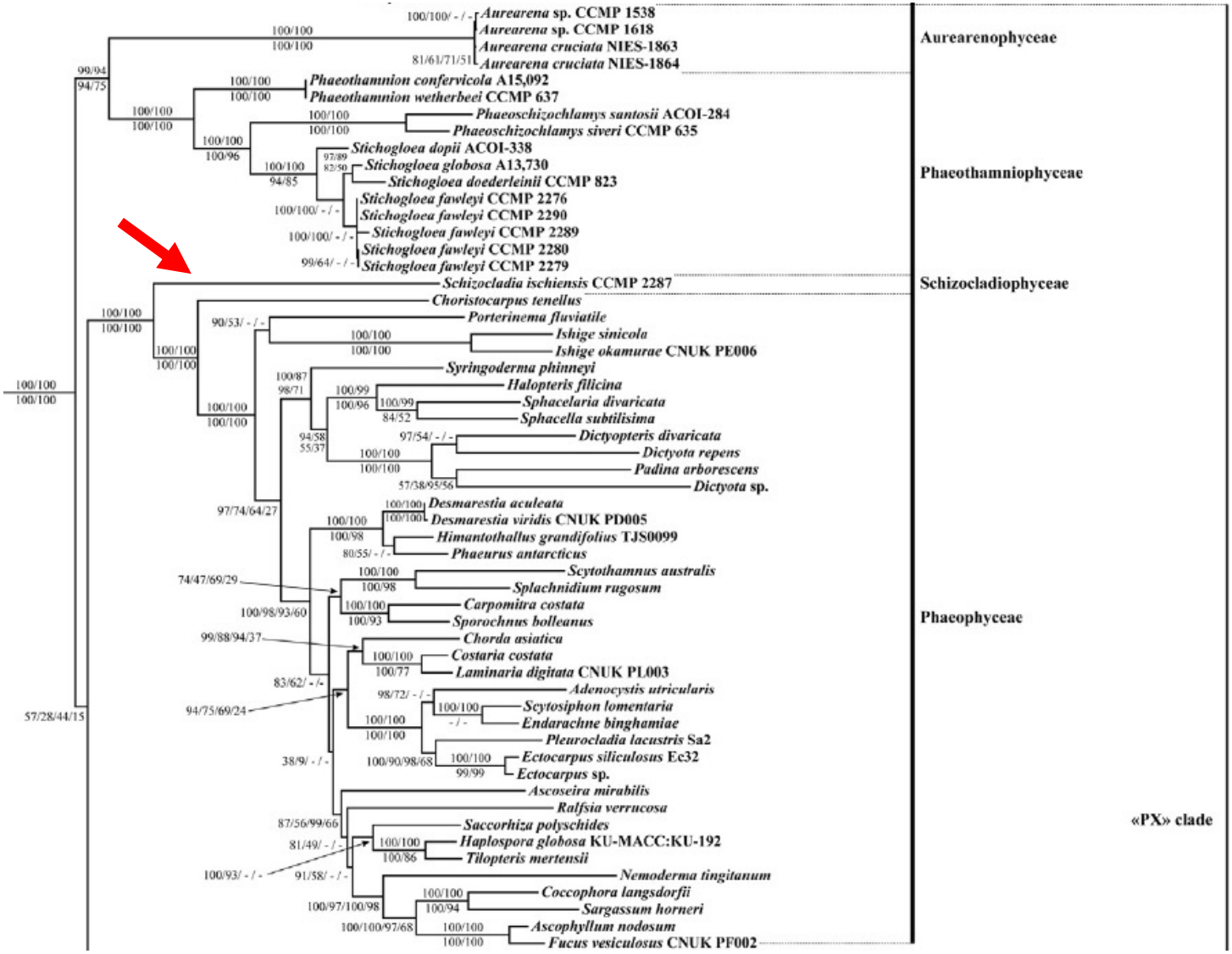
	Organization	Habitat	PO	CF	GL	PN	TF	TH	BR	RH	Chl.c	Fuc	Dia	Vio	Het	Vau
Aurearena	FL–CC	M	+	+	–	S	3	+	–	+	–	+	+	+	–	–
Phaeothamniophyceae s.s.	CC, FI	F	+	–	+	R	1	+	–	–	+	+	+	–	+	–
Xanthophyceae	CC, FI, SI	F (M)	–	–	+ (–)	R (S)	2	+	–	+	+*	–	+	–	+	+
Pleurochloridella	CC	F	+	?	?	?	?	?	?	?	+*	+	+	–	+	–
Chrysomerophyceae	FI	M	–	–	+	R	3	+	+	–	+	+	–	+	–	–
Tetrasporopsis	CC	F	+	–	+	?	?	?	?	?	?	?	?	?	?	?
Schizocladiophyceae	FI	M	–	–	+	R	?	+	?	–?	+	+	?	?	?	?
Phaeophyceae	MC	M (F)	+	–	+	R	1–3	–	+	+	+	+	– (+)	+	–	–

FL = flagellate, CC = coccoid, FI = filamentous, SI = siphonous, MC = multicellular, M = marine, F = freshwater, PO = periplasmic opaque vesicle, CB = chloroplast boundary, GL = girdle lamella, PN = plastid nuceoid (R = ring type, S = scattered type), TF = no. of terminal filaments of mastigoneme, TH = transitional helix, BR = bypassing root, RH = rhizoplast, Fuc = fucoxanthin, Dia = diatoxanthin/diadinoxanthin, Vio = violaxanthin/antheraxanthin/zeaxanthin, Het = heteroxanthin, Vau = vaucheriaxanthin, asterisks mean very small amount, exceptional example in parentheses.

- very diverse life forms and habitats
- emergence of true multicellularity in Phaeophyceae
- absence of flagellate (FL) cells in the vegetative phase
- all members of the PX clade posses a cell wall in the vegetative phase
- presence of electron-opaque vesicles (PO) beneath the plasma membrane

Kai et al. (2008), *Protist*

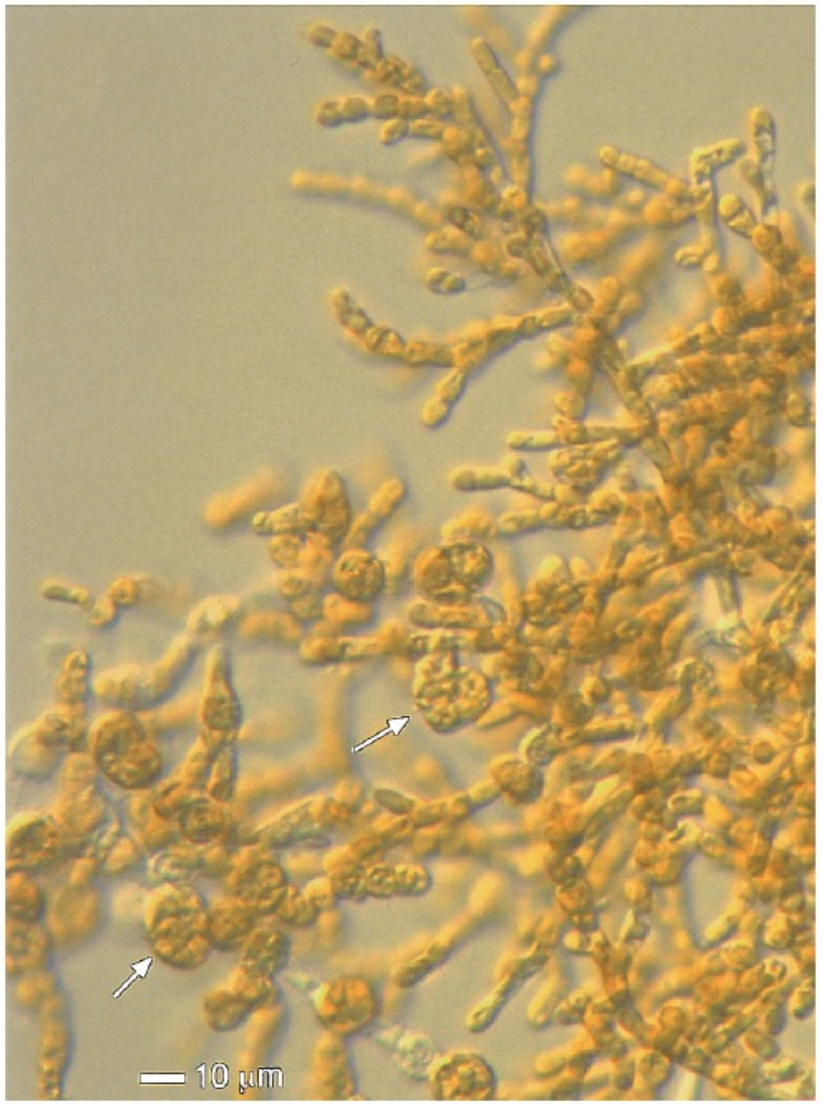
Schizocladiophyceae



Graf et al. (2020), *Protist*

Schizocladiophyceae

- a monotypic class, *Schizocladia ischiensis*
- the closest relative of Phaeophyceae, but probably not a direct ancestor
- branched filamentous algae
- marine habitats
- described in 2003 (type locality: Naples, Italy)

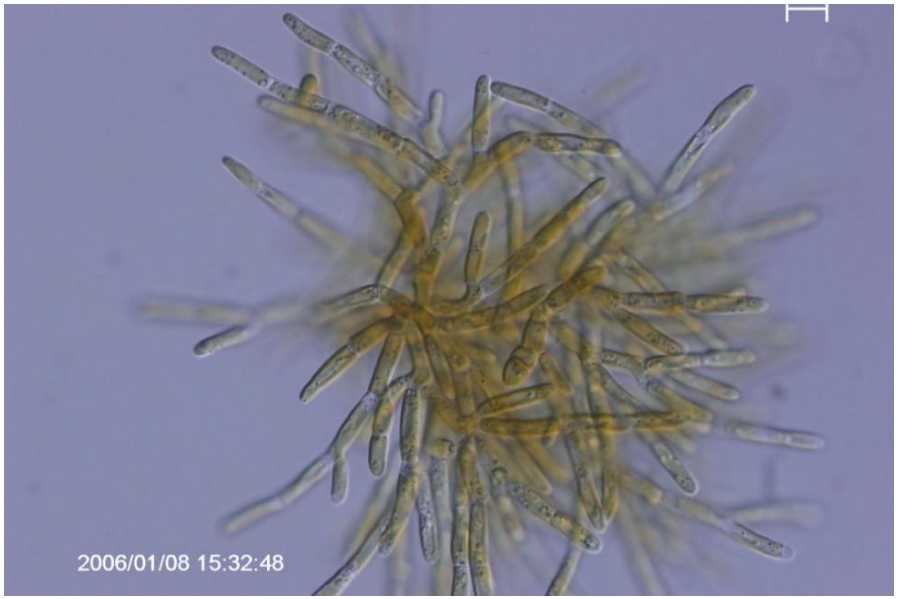


Schizocladia ischiensis, vegetative filaments and swollen reproductive cells (arrows).

Kawai et al. (2003), *Protist*

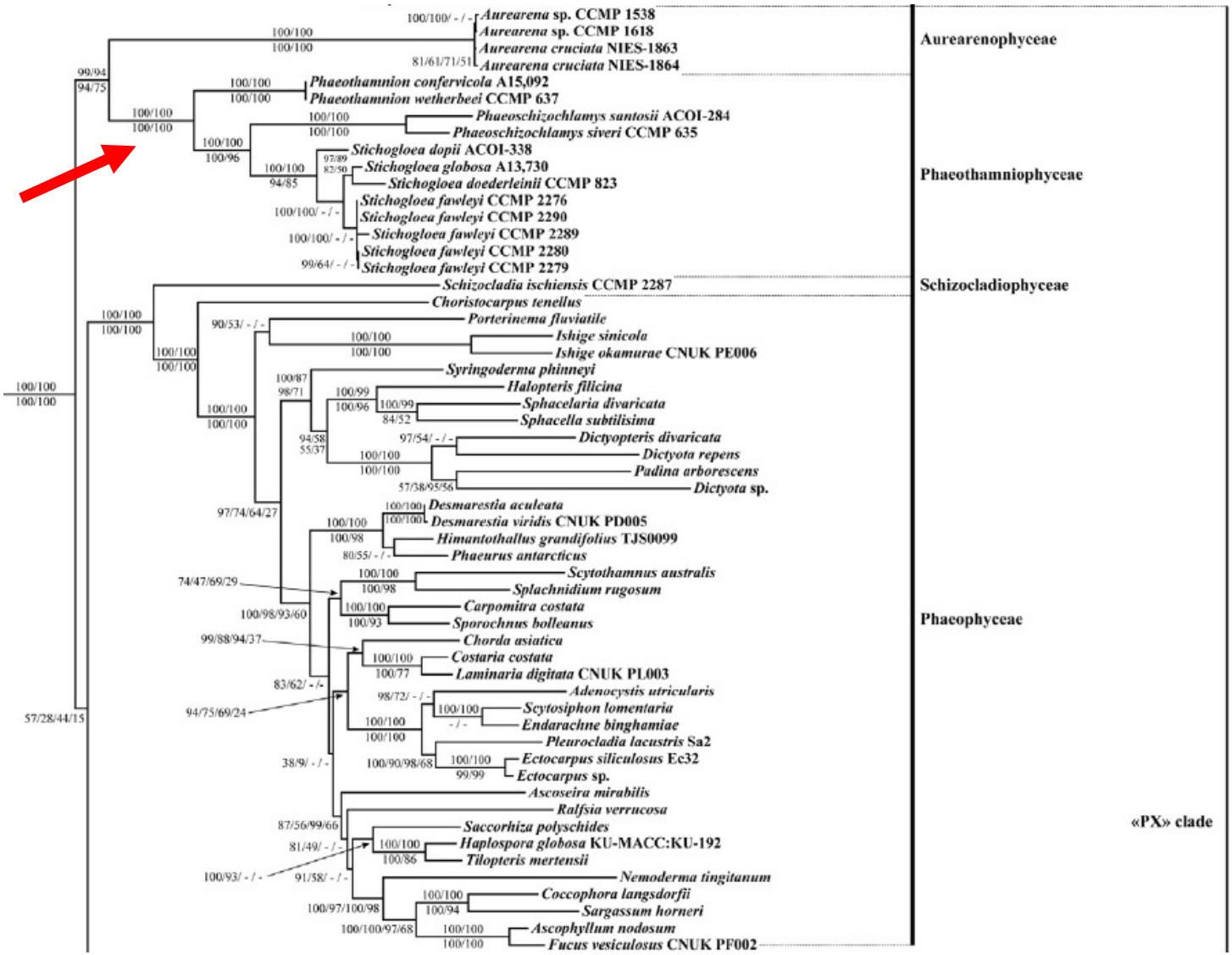
Schizocladiophyceae

- cell wall containing alginates (but lacking cellulose and plasmodesmata, that are present in Phaeoph.)
- 1-2 plastids, chlorophyll *a*+*c*, fucoxanthin, no pyrenoid
- heterokont zoospores with eyespot (sexual reproduction not detected)



Kawai et al. (2003), *Protist*

Phaeothamniophyceae



Graf et al. (2020), *Protist*

Phaeothamniophyceae

- established in 1998 from genera formerly classified in the class Chrysophyceae (but some taxa erroneously classified – polyphyly of Phaeothamniophyceae)
- filamentous, capsoid, palmelloid, ?ciliated, or coccoid
- swimming cells heterokont flagella
- chloroplast with girdle lamella, chlorophyll a, c + xanthophylls (including fucoxanthin)
- alginates present in the cell walls

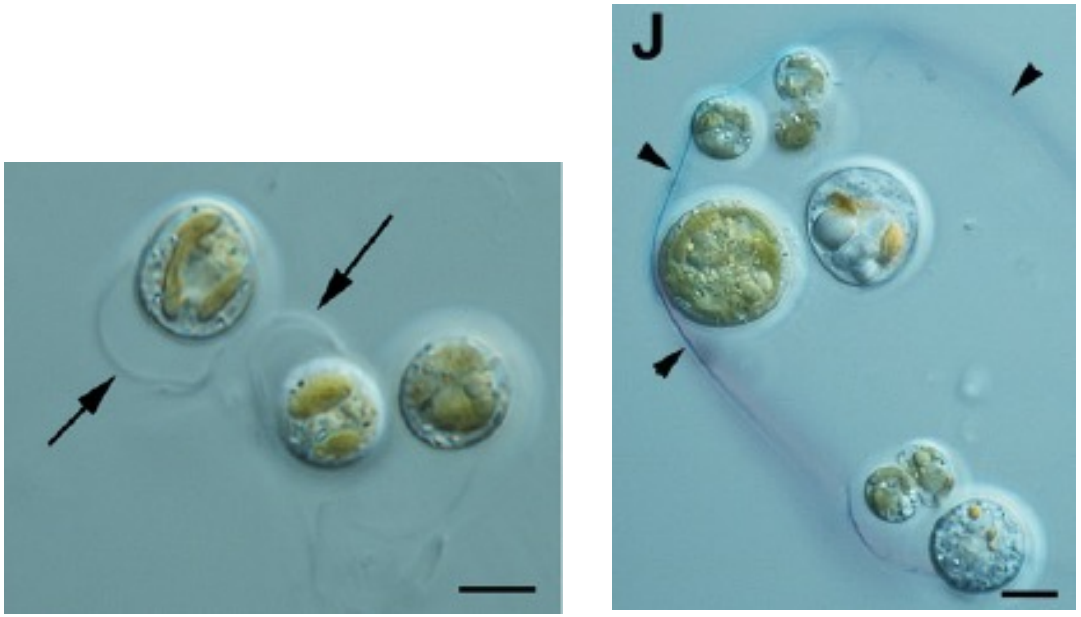
Phaeothamniophyceae

- *Phaeothamnion* spp.
- freshwater bodies, pools and ponds; widely distributed
- filaments with branches, a basal cell attached to substratum (tree like colonies)
- ellipsoidal cells with one or more chloroplasts (no pyrenoid)
- production of heterokont zoospores

Phaeothamniophyceae

Phaeoschizochlamys

- freshwater and brackish waters (pools, lakes)
- colonial within a mucilage
- remnant empty cell walls (cup-like in appearance)
- one or two plastids
- heterokont zoospores



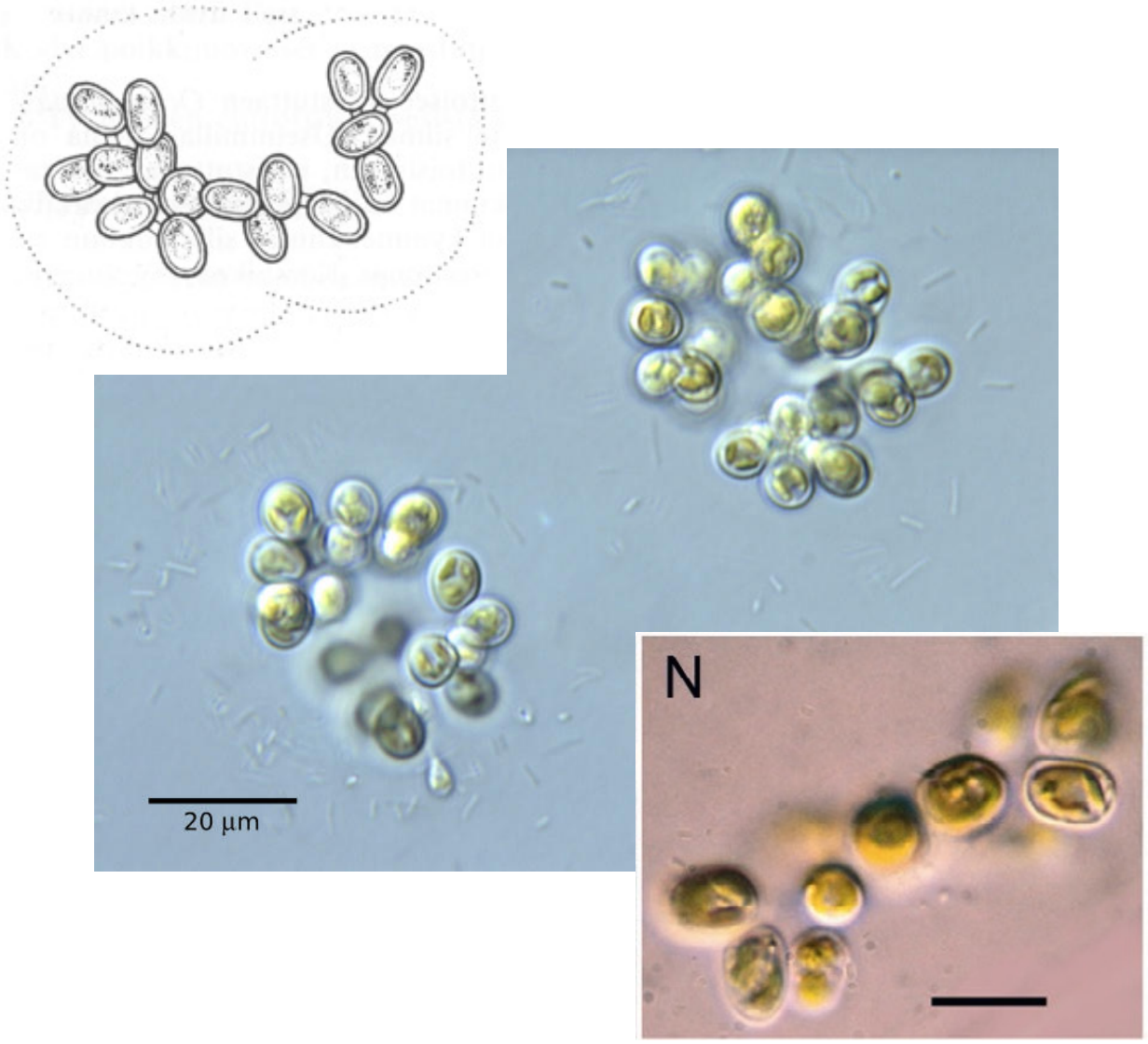
Phaeoschizochlamys santosii

Graf et al. (2020), *Journal of Phycology*

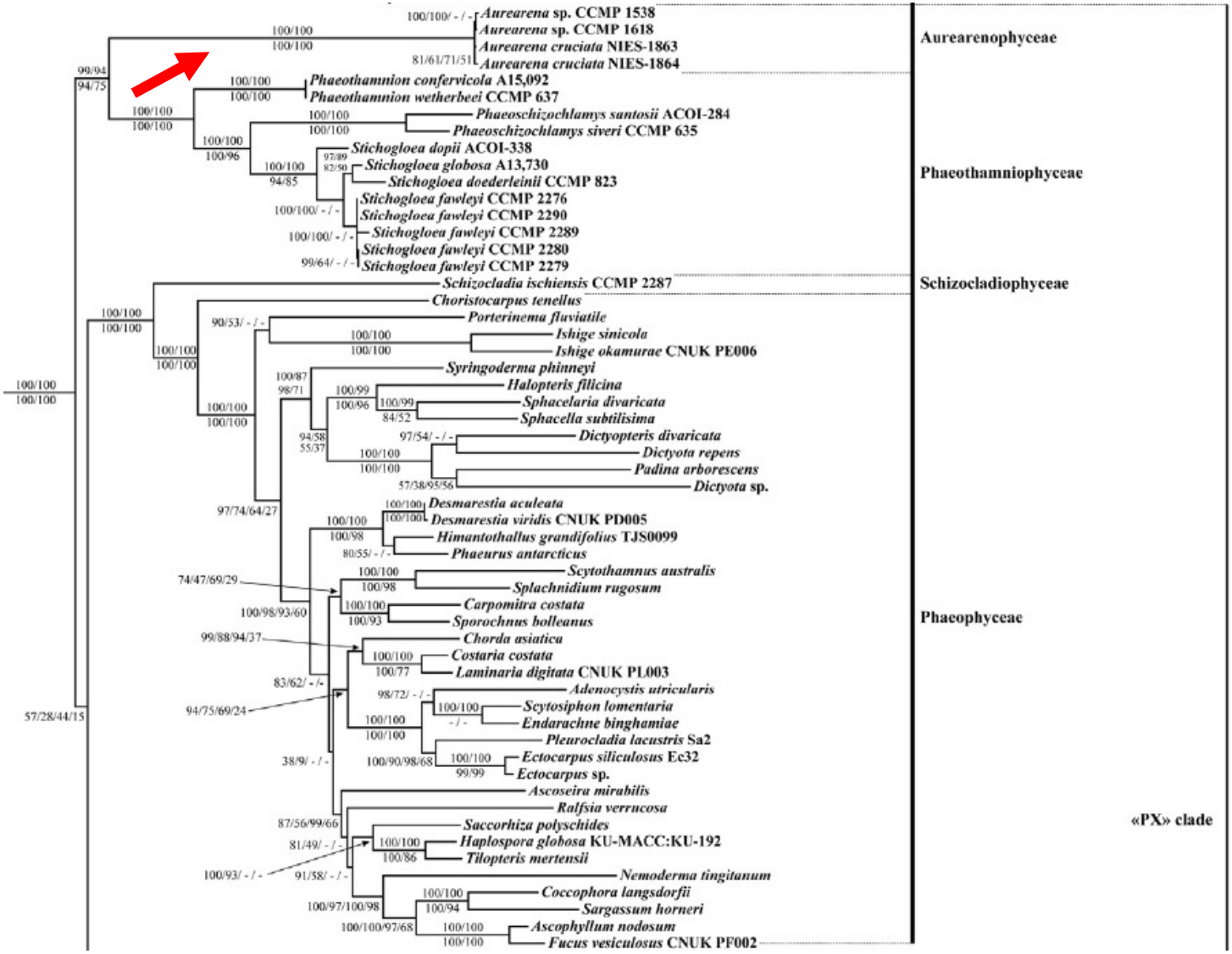
Phaeothamniophyceae

Stichogloea spp.

- freshwater plankton, alpine and northern lakes
- mucilaginous free-floating colonies (4 and 4 cells together)
- 1-2 parietal chloroplasts



Aurearenophyceae

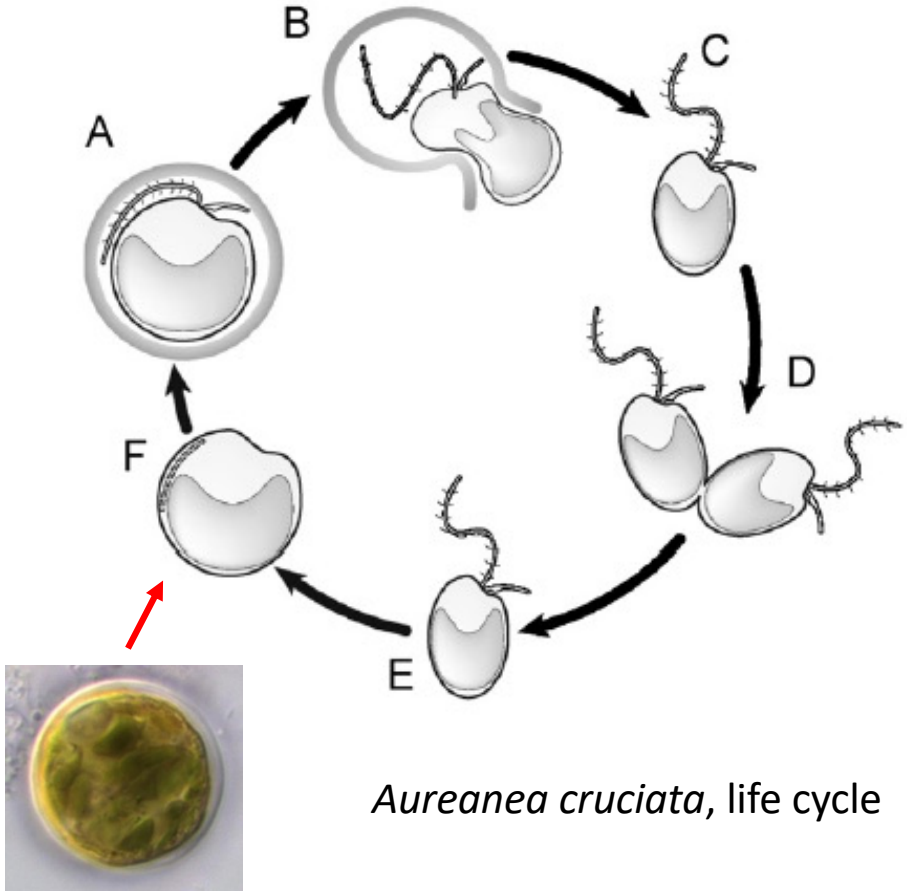


Graf et al. (2020), *Protist*

Aurearenophyceae

- a monotypic class, *Aureanea cruciata* (described in 2008; type locality: sandy beaches in Japan, etymology: golden sand)
- walled non-motile coccoid cells (flagella underneath the cell wall: not seen elsewhere); swimming cells lack cell walls
- heterokont flagella; golden-yellow chloroplast; with stigma and pyrenoid; lamellae of three thylakoids (no girdle lamella)
- chlorophyll *a* (chl *c* not detected), beta-carotene, various xanthophylls (including fucoxanthin)
- unconventional outer membrane arrangement of plastids

A. A flagellate cell inside a cell wall. B. Naked swimming cell emerging through a pore in the cell wall. D. Cytokinesis proceeding in a swimming cell. E. Swimming cell absorbing flagella into the cell. F. Non-motile cell after absorption of flagella.



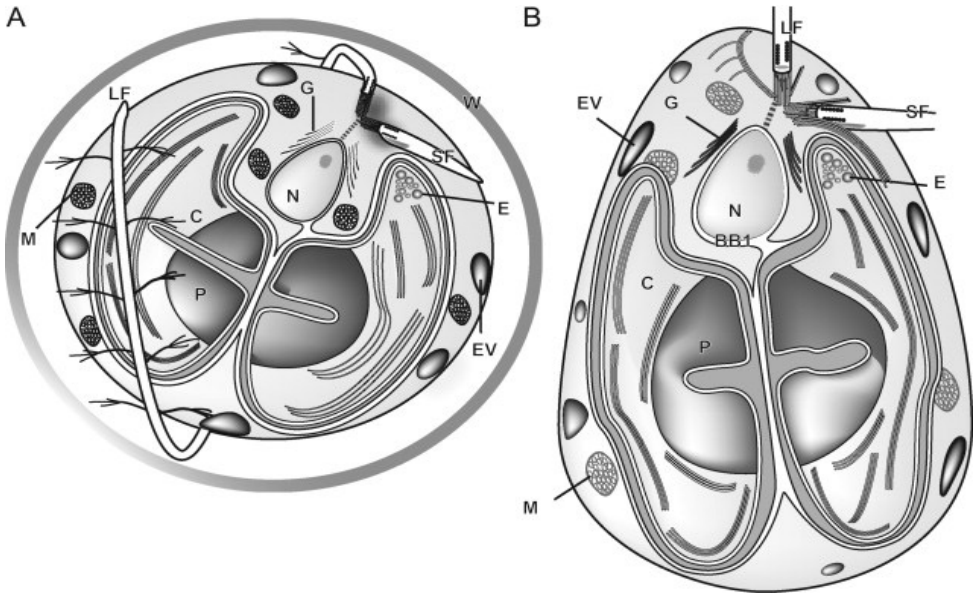
Aureanea cruciata, life cycle

Kai et al. (2008), *Protist*

Aurearenophyceae

- a single chloroplast is a consortium of multiple plastids, each surrounded by three inner membranes, is enclosed by a shared outer membrane complex
- this membrane topology has not been reported before

Aureanea cruciata

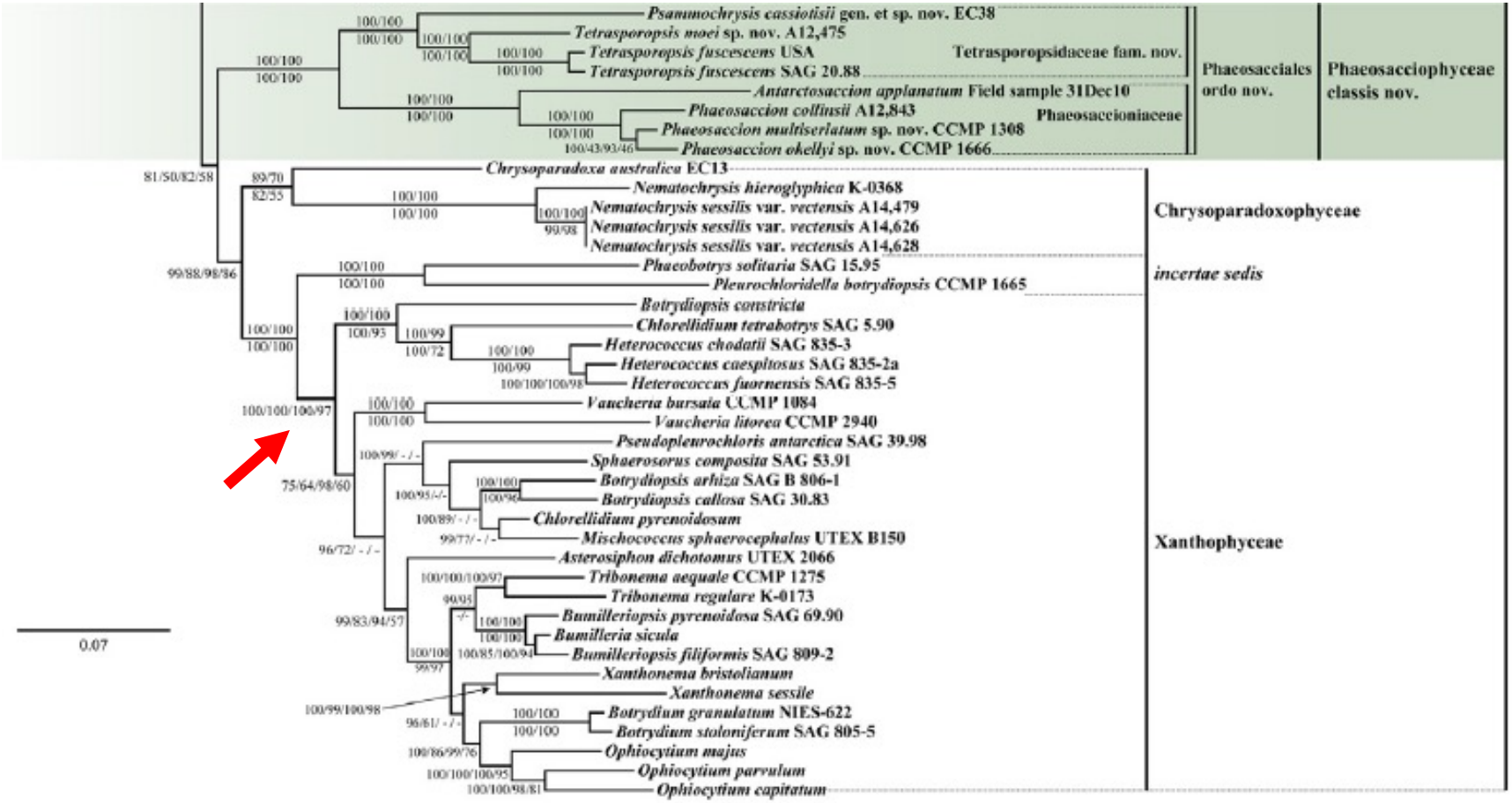


A. Walled cell. B. Swimming cell.

C: chloroplast with cross-shapes furrow (longitudinal = two chloroplast membranes + one ER; transverse = two chloroplast membrane); **E:** eyespot; **G:** Golgi body; **M:** mitochondrion; **N:** nucleus; **P:** central pyrenoid; **W:** cell wall; **EV:** electron opaque vesicle; **BB:** basal body; **LF:** long anterior flagellum.

Kai et al. (2008), *Protist*

Xanthophyceae



Graf et al. (2020), *Protist*

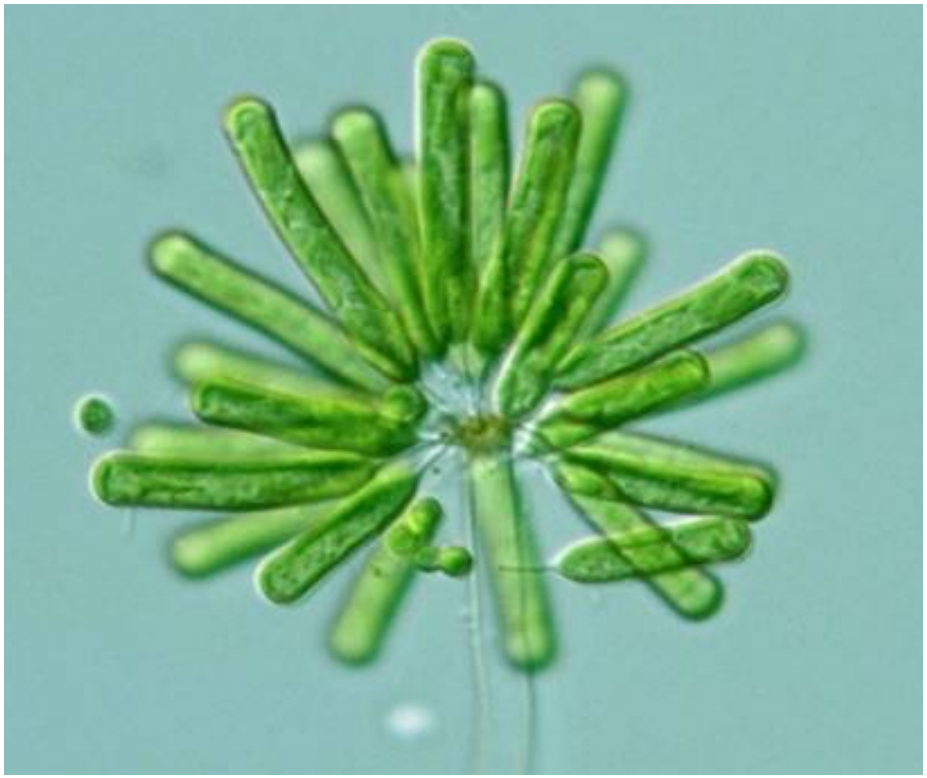
Xanthophyceae (yellow-green algae)

- predominately coccoid or filamentous, (?amoeboid, ?ciliated or ?capsoid)
- swimming cells with heterokont flagella
- cell walls (cellulose in *Vaucheria;* alginates in the cell walls of *Botrydiopsis*, *Botrydium*, *Tribonema* and *Vaucheria;* cell wall may be impregnated by silica)
- either entire cell wall or two overlapping halves (bipartite cell wall, H-shaped pieces)
- usually several chloroplasts; typical stramenopile ultrastructure; chlorophylls *a*, *c1*, *c2*; xanthophylls

Tribonema, H-shaped pieces of bipartite cell wall

Xanthophyceae (yellow-green algae)

- green to yellow-green color: absence of fucoxanthin (golden/brown color cased by fucoxanthin), chrysolaminaran as a storage product
- chloroplast color: confusions with Chlorophyta (difference: presence of chlorophyll *b* and starch as a storage product) and Eustigmatophyceae (different xanthophyll composition, absence of chlorophyll *c* and fucoxanthin)
- about 600 species, over 90 genera (however, unclear number of true members)



Ophiocythium sp.

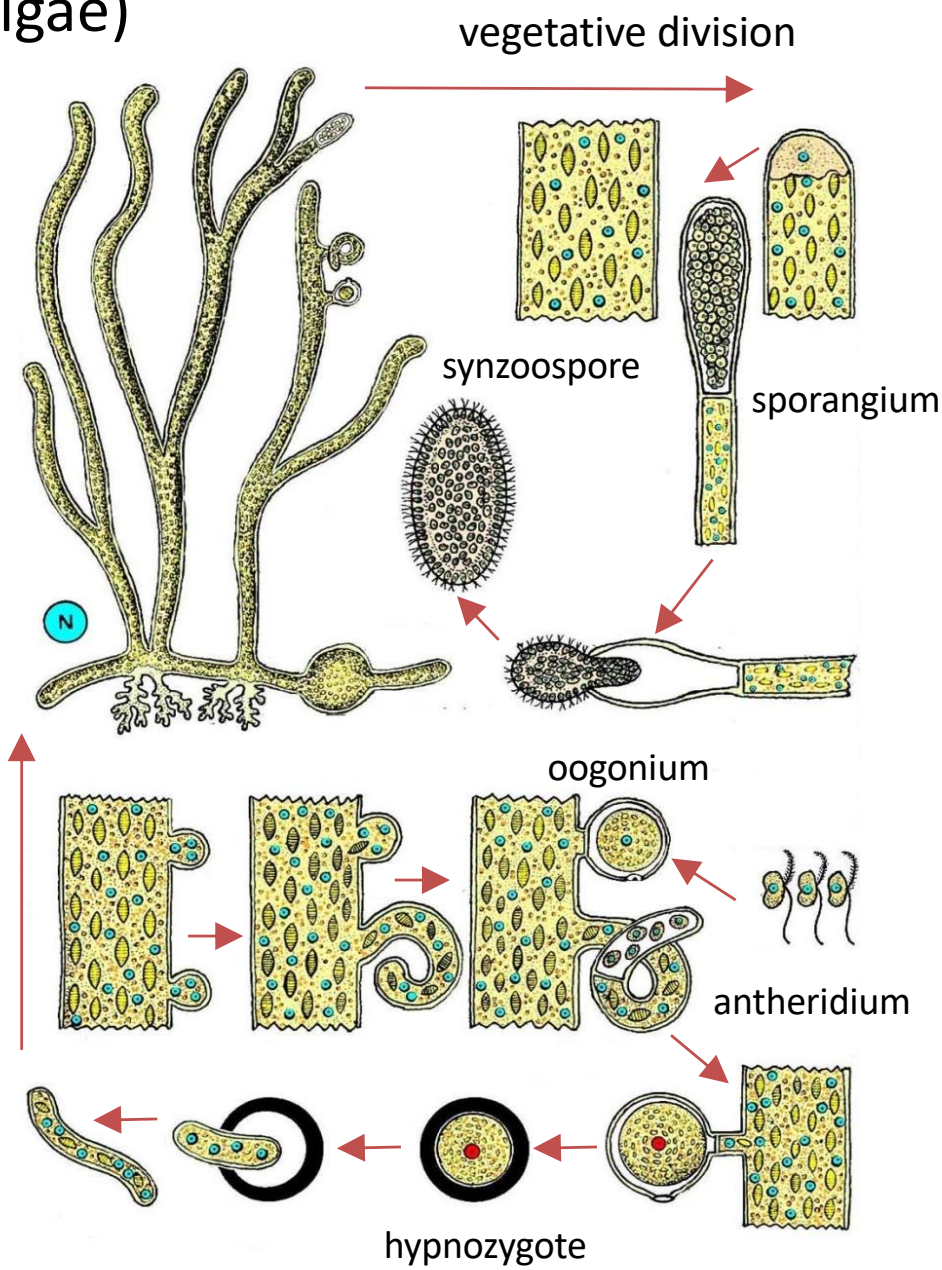
Xanthophyceae (yellow-green algae)

- a majority of xanthophyceaens reproduce asexually, divide bilaterally (mitotic cell division);
- produce endogenous cysts (impregnated by silica, from two unequal parts) or the akinete (a single celled spore, cell wall from thick parent cell wall)
- zoospores, hemiautospores (without mastigonemata), aplanospores (without flagella); and autospores (small replicates of the parent cell)

Tribonema, filaments and zoospores

Xanthophyceae (yellow-green algae)

- sexual reproduction observed in *Vaucheria:* oogamy (anteridium: producing a large number of colorless spermatozoids, oogonium: uninucleate, a single oosphere) -> a thick-walled resting zygote (oospore = hypnozygote) -> meiosis, a new siphonous filament
- *Botrydium*: isogametes or anisogametes
- *Tribonema*: isogamous



Vaucheria,
sexual reproduction

Xanthophyceae (yellow-green algae)

- from tropics to polar regions
- predominantly in freshwater as phytoplankton and periphyton + terrestrial habitats [*Vaucheria* in marine/brackish habitats]
- their habitat appear somehow as exceptions (together with Eustigmatoph.) among the Ochrophytes [most groups with red-algal derived plastids dominate in the oceans]
- they resemble ecologically and morphologically Chlorophyta: evolutionary parallelism
- most taxa only rarely (and in small numbers) but some commonly form visible growths (filaments of *Vaucheria* and *Tribonema*) and some are macroscopic (Botrydium, Asterosiphon)

Botrydium, macroscopic thallus

Xanthophyceae – **systematics**

- traditional classification does not reflect phylogeny: poly- or paraphyletic taxa (order, families, genera)
- coccoid and filamentous taxa with cell wall confirmed as Xanthophyte members
- other species are doubtful and may represent new stramenopile lineages with uncertain phylogenetic position

Xanthophyceae – **systematics**

- **doubtful orders of Xanthophytes**: absence of the cell wall

- Heterochloridales = Chloramoebales: unicellular naked flagellates (constant shape or amoeboid); one to several chloroplasts, palmeloid stages, usually freshwater (also found in marine and brackish waters), *Chloromeson* –> unavailable molecular data; some representatives transferred to Chrysophyta

Ott et al. (2015)

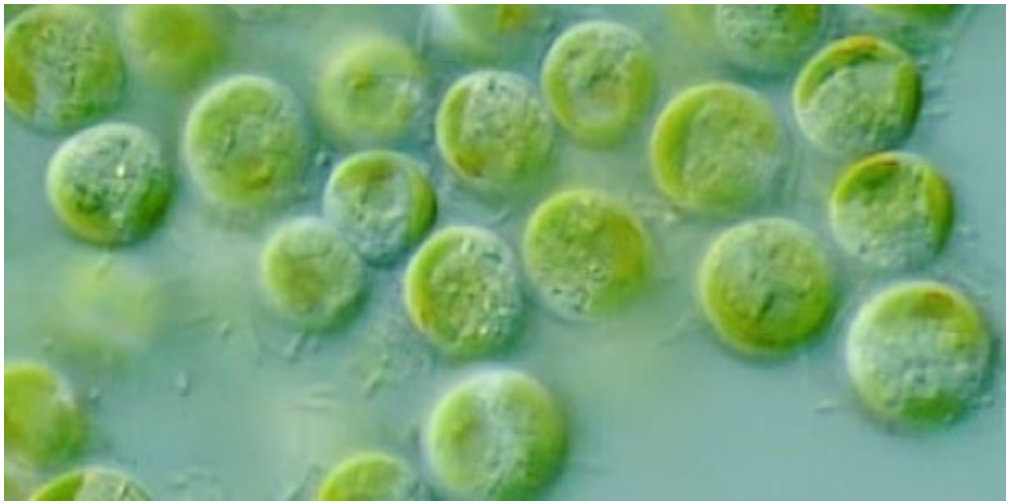
Xanthophyceae – **systematics**

- **doubtful orders of Xanthophytes**: absence of the cell wall
- Heterochloridales = Chloramoebales: unicellular naked flagellates
- Heterogloeales: capsoid (= palmeloid) forms (non-motile cells without wall, surrounded by mucilage or not), a former member *Pleurochloridella*: phylogenetically not included in the class Xanthophyceae

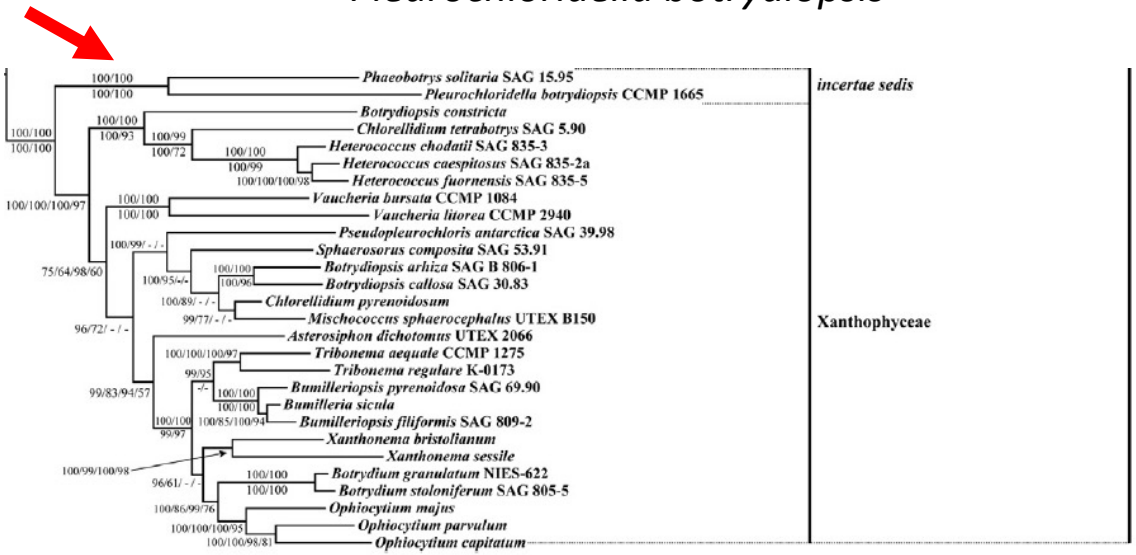
Ott et al. (2015)

Pleurochloridella

- *Pleurochloridella botrydiopsis*: marine representative (molecular data not available for a freshwater species *P. vacuolata*)
- described in 1938 as Xanthoph. -> moved to Phaeothamnioph. (1998) on the basis of pigment analysis -> phylogenetically related to Xanthophytes (not to Phaeothamnioph.)
- differs from Xanthophytes by possessing fucoxanthin



Pleurochloridella botrydiopsis



Kai et al. (2008), *Protist*

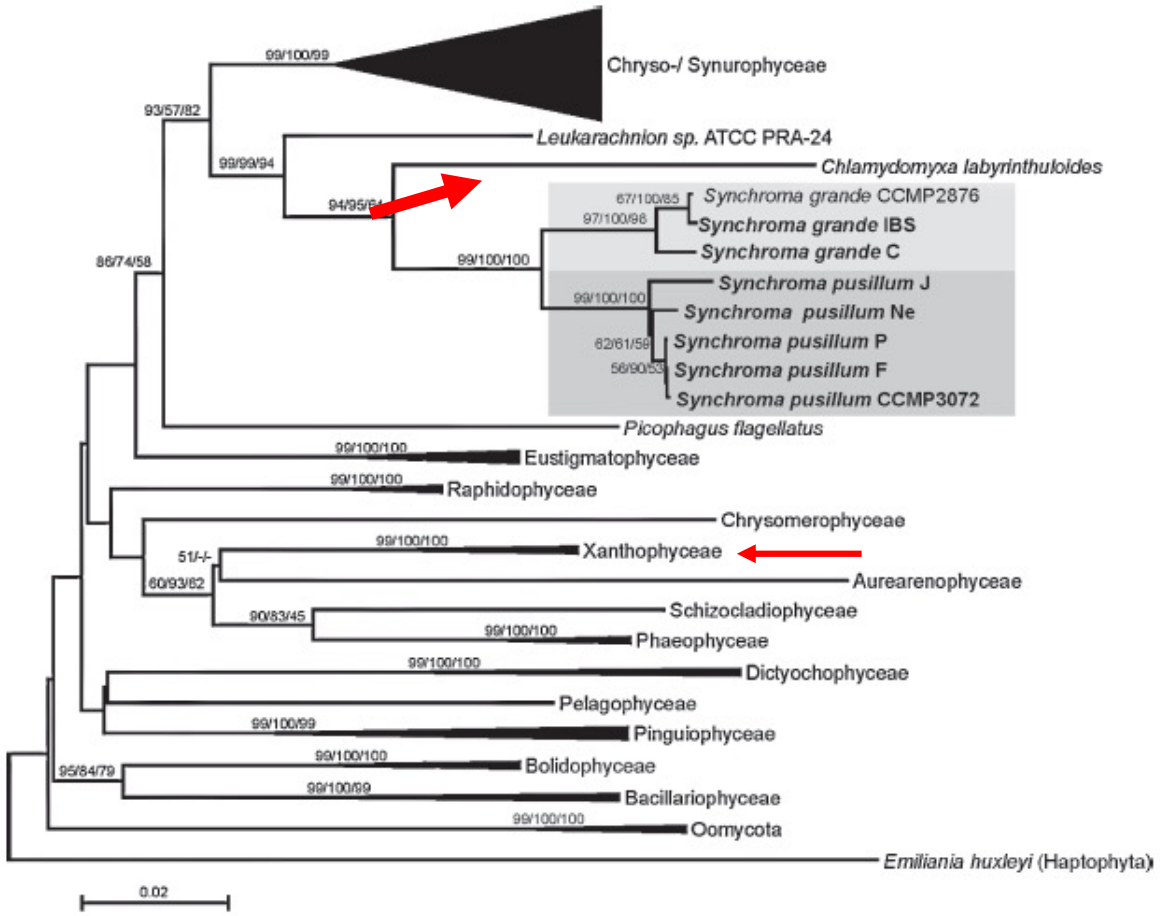
Xanthophyceae – **systematics**

- **doubtful orders of Xanthophytes**: absence of the cell wall

- Heterochloridales = Chloramoebales: unicellular naked flagellates
- Heterogloeales: capsoid (= palmeloid) forms
- Rhizochloridales: rhizopodial forms (naked amoeboid cells with rhizopods); colonial, free living or attached (sometimes loricate); one or more chloroplasts; endogenous cysts; freshwater, marine or brackish; *Rhizochloris* (heterotrophic) and *Myxochloris* (autotrophic): within *Sphagnum* water-holding cells; *Stipitococcus, Stipitoporos:* freshwater, live epiphytically on filamentous algae –> a former member *Chlamydomyxa labyrinthuloides*: phylogenetically closely related with the class Synchromophyceae (SII clade)

Ott et al. (2015)

Chlamydomyxa labyrinthuloides



Ch. labyrinthuloides, plasmodium, on Sphagnum and other water plants

- chlorophyll a, c + fucoxanthin, violaxanthin

Schmidt et al. (2012), Protist

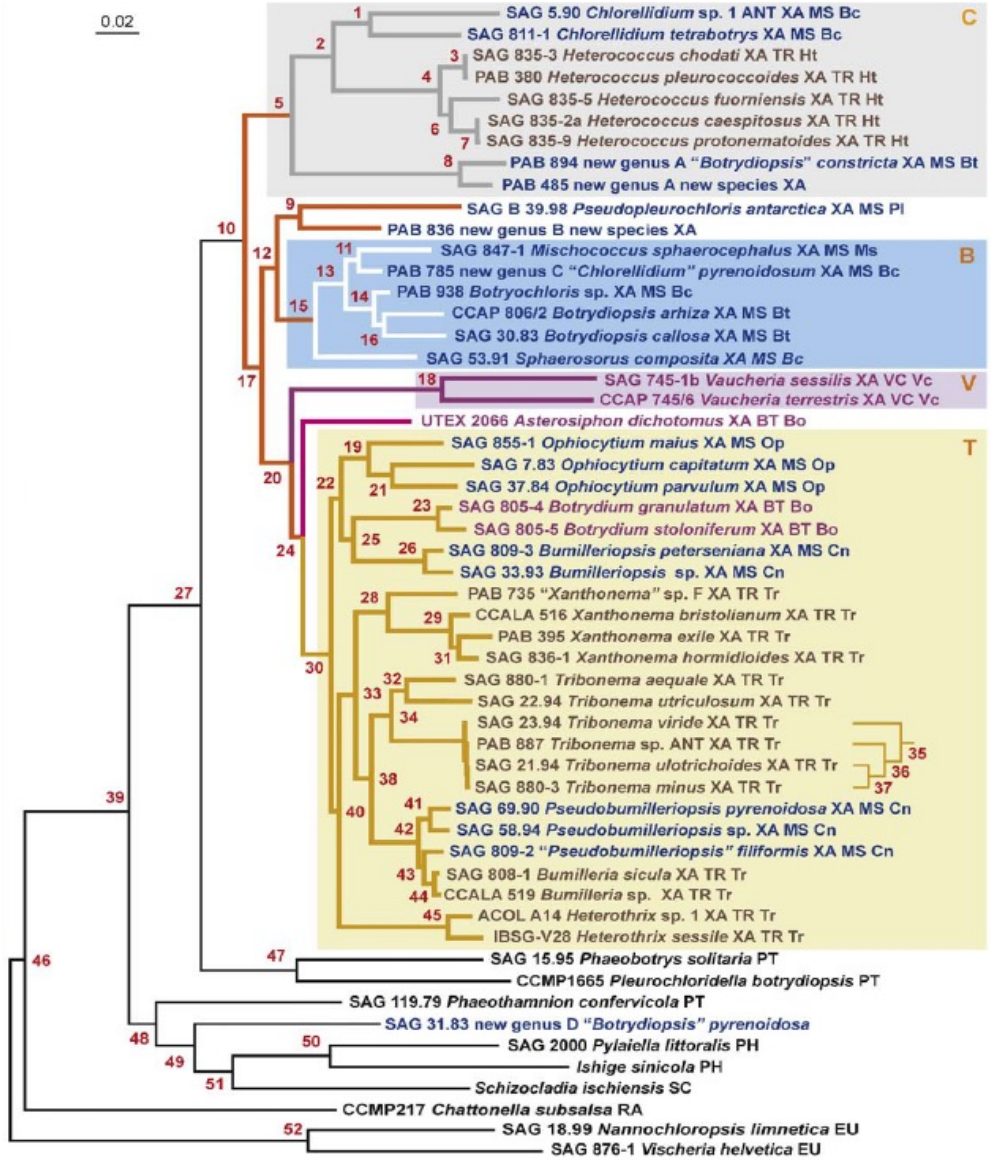
Xanthophyceae – **systematics**

- **approved representatives of Xanthophytes but polyphyletic orders**

- Mischococcales: coccoid forms
- Tribonematales: filamentous (branched and unbranched forms)
- Botrydiales: siphonous forms
- Vaucheriales: siphonous filaments

- orders and some genera are not monophyletic
- some representatives of Mischococcales are actually Eustigmatophyceae (*Goniochloris, Characiopsis*)
- *Botrydium pyrenoidosa* form a new lineage **outside** of Xanthoph.

Xanthophyceae – **systematics**



Chlorellidium and others
- coccoid and heterotrichal

Botrydiopsis and others
- most of the coccoid forms included in Mischococcales

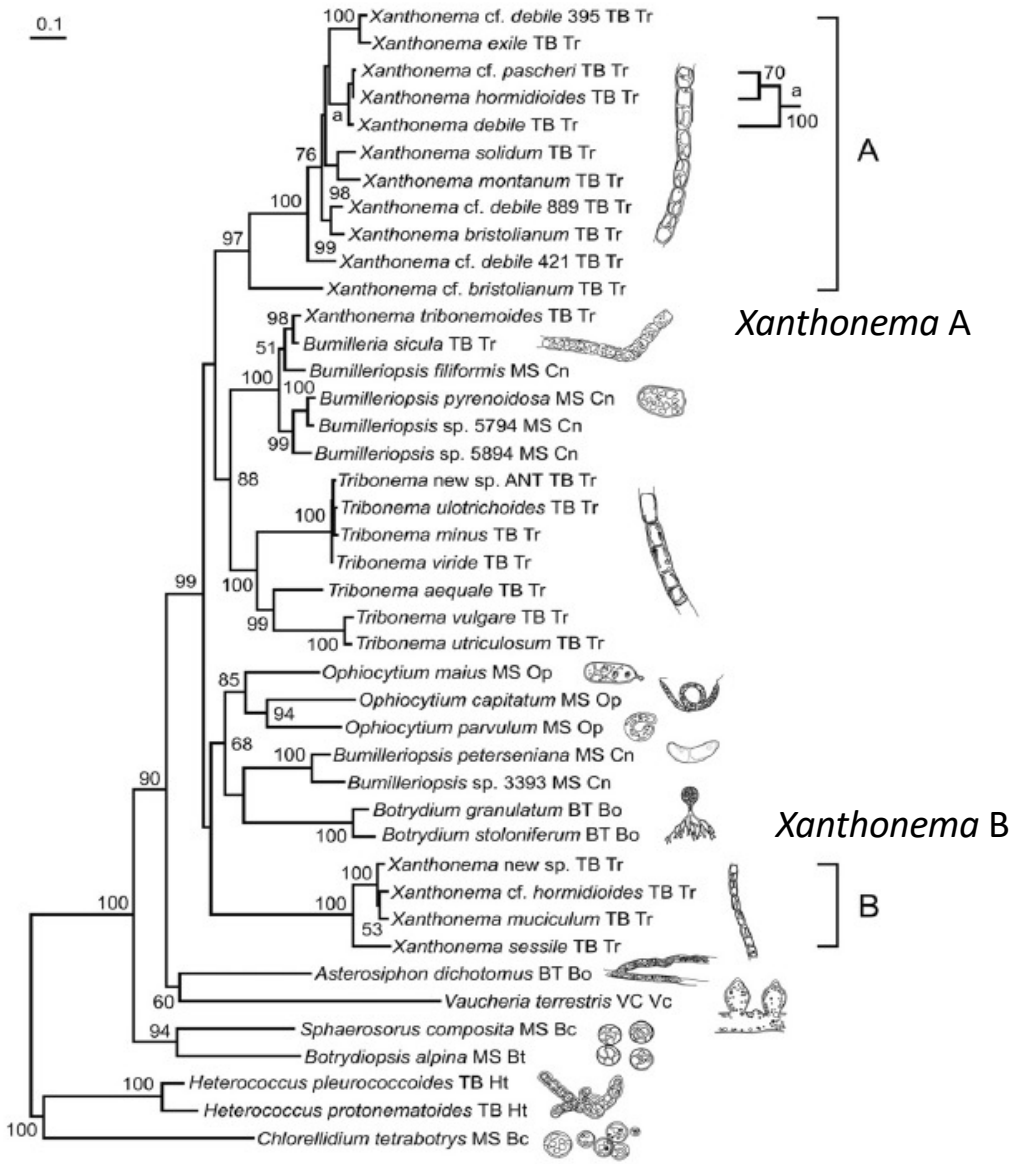
Vaucheria - siphonous

Tribonema and others
- coccoid, unbranched filaments, siphonous thalus

4 major clades

Maistro et al. (2009), *Protist*

Xanthophyceae (yellow-green algae)



Xanthonema

Bumilleria

Tribonema

Maistro et al. (2012), *Mol. Phyl. Evol.*

Xanthophyceae (yellow-green algae)

a split of the filamentous Xanthophyceae in two independent subclades:
1) bipartite cell walls of H-shaped pieces; 2) cell walls of one piece

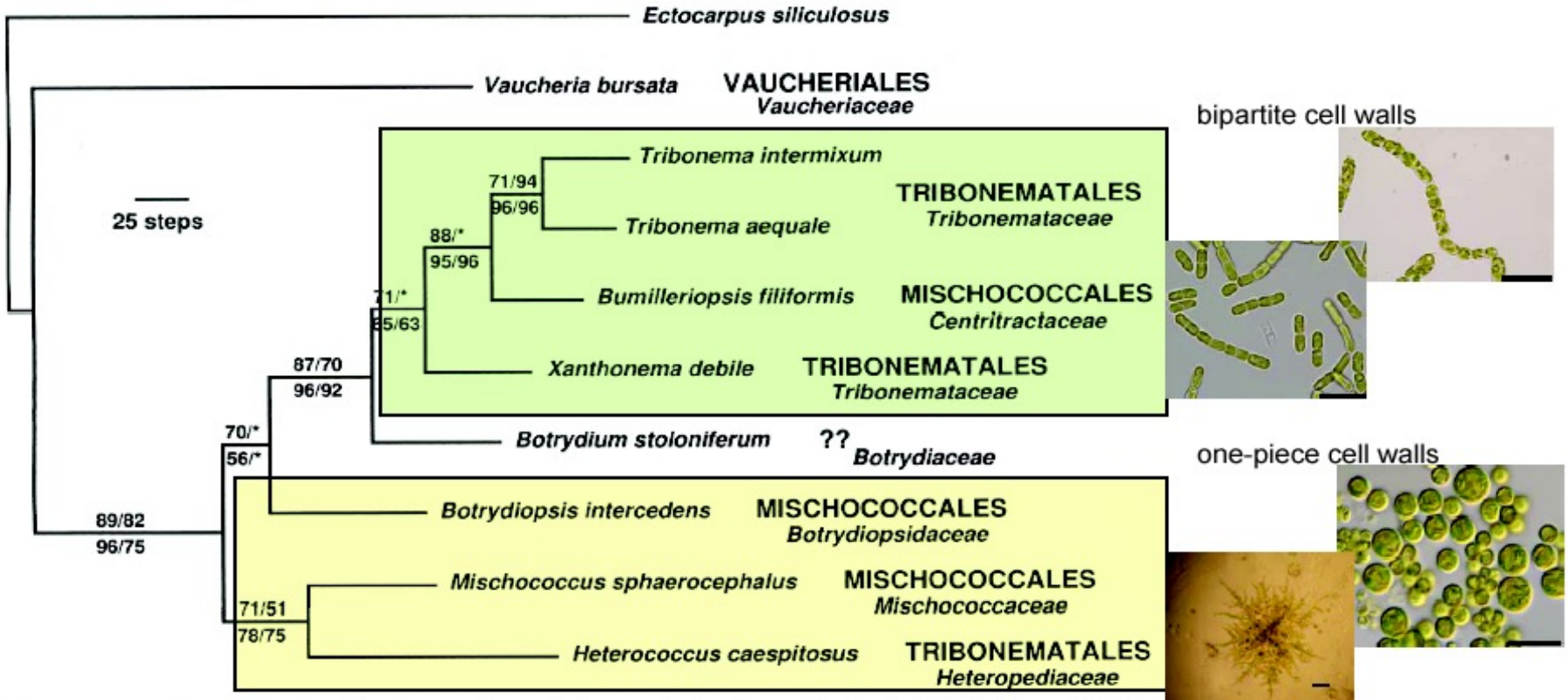
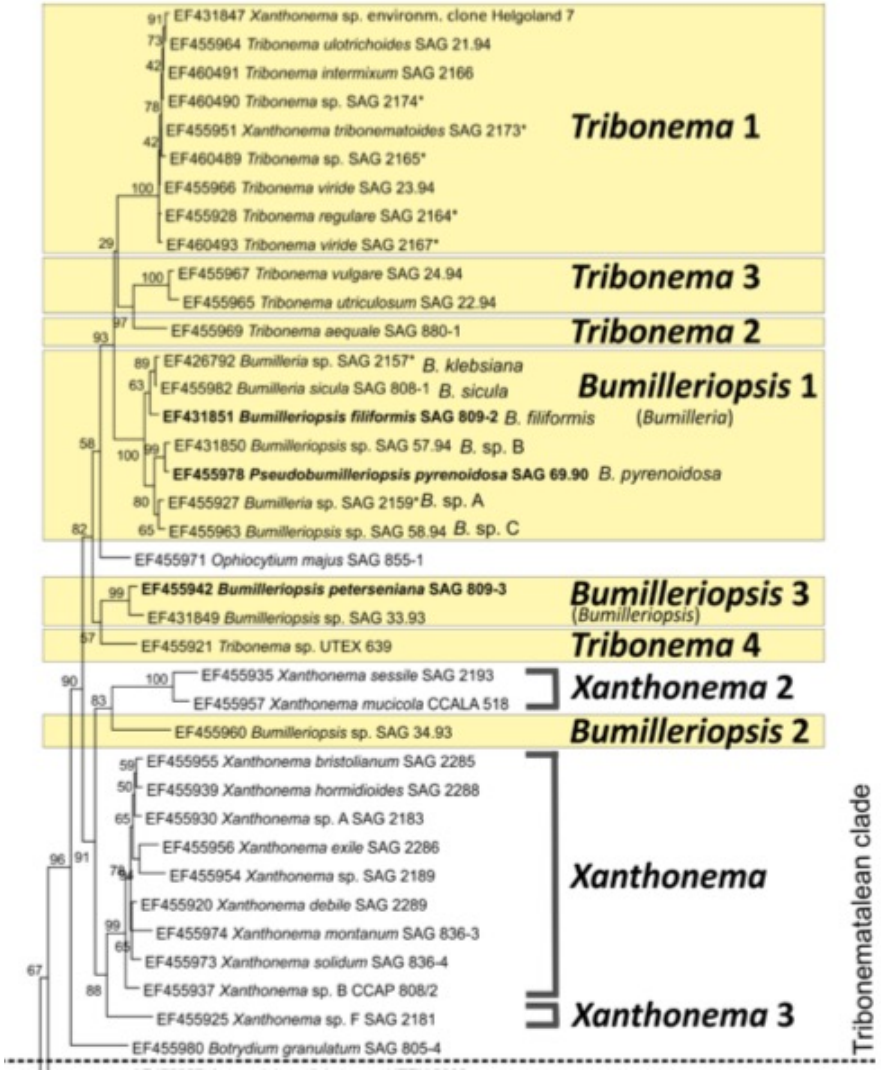
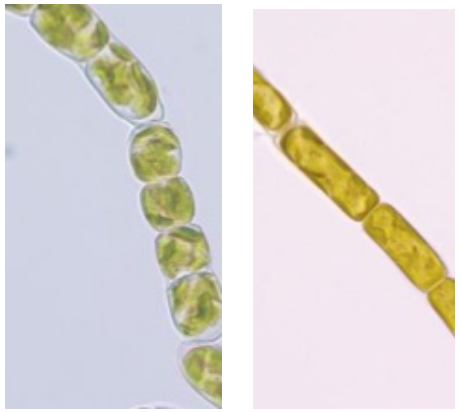


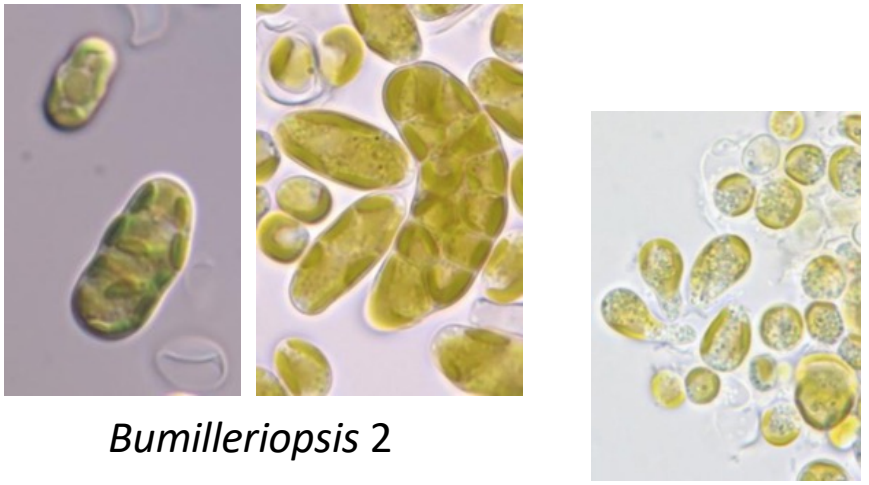
Fig. 3. Phylogram depicting relationships inferred among nine xanthophyte species based on combined analysis of plastid-encoded *rbc*L and nuclear-encoded 18S rRNA gene sequences. Bootstrap values above branches correspond to separate parsimony analyses of the *rbc*L and 18S rRNA data, respectively; values shown below branches are derived from parsimony/ML analyses of the combined data set. *, bootstrap value < 50%; ??, *Botrydium* (Botrydiaceae) has been classified in the Botrydiales (Ettl 1978), Mischococcales (Christensen 1980), and Vaucheriales (Bold & Wynne 1985; Hibberd 1990).

Xanthophyceae – ***Bumilleriopsis***





Bumilleriopsis 1 (*Bumilleria*)



Bumilleriopsis 2

bipartite cells

Bumilleriopsis 2 (new genus)

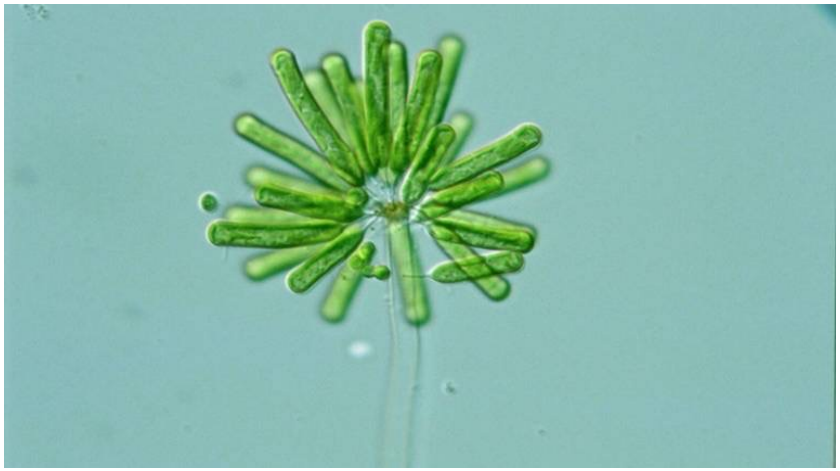
Xanthophyceae – ***Bumilleria***

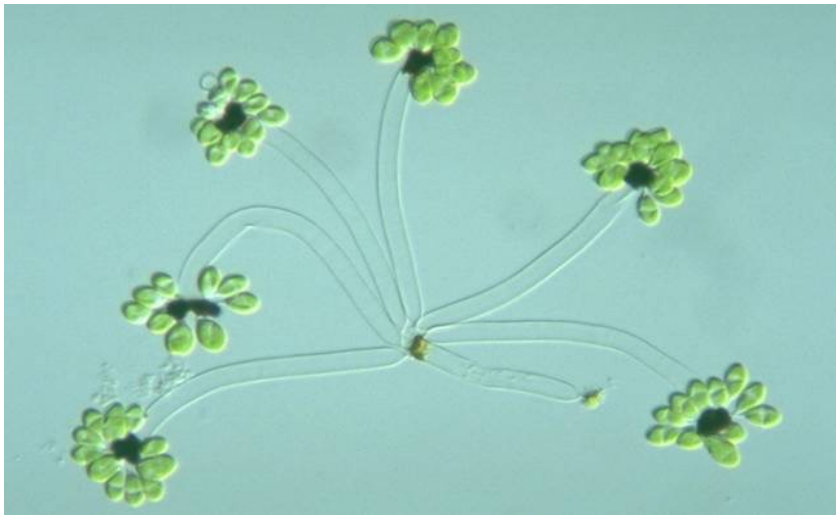
- unbranched filaments, cylindrical to quadrate cells
- fragmentation of filaments, two H-shaped pieces
- reproduction by zoospores
- freshwater; tychoplanktonic and metaphytic (among other filamentous algae) in creeks, rivers, wetlands, and ponds
- *Tribonema* clade

fragmentation of filaments

Xanthophyceae - ***Ophiocytium***

- freshwater standing waters (euplanktonic, tychoplanktonic, metaphytic, epiphytic)
- solitary or colonial
- elongate-cylindrical cells
- bipartite cell wall; several to many plastids
- autospores, zoospores, aplanospores
- *Tribonema* clade

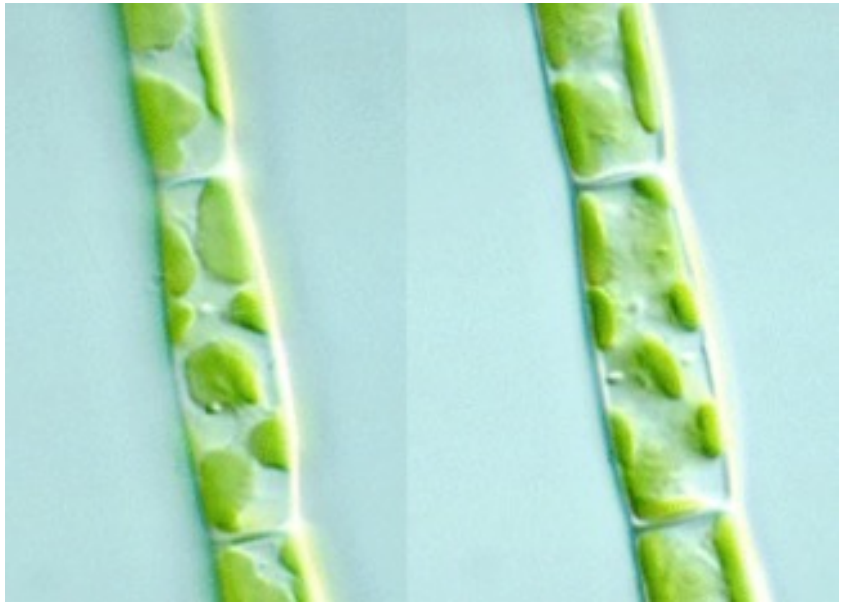




spines on the cell

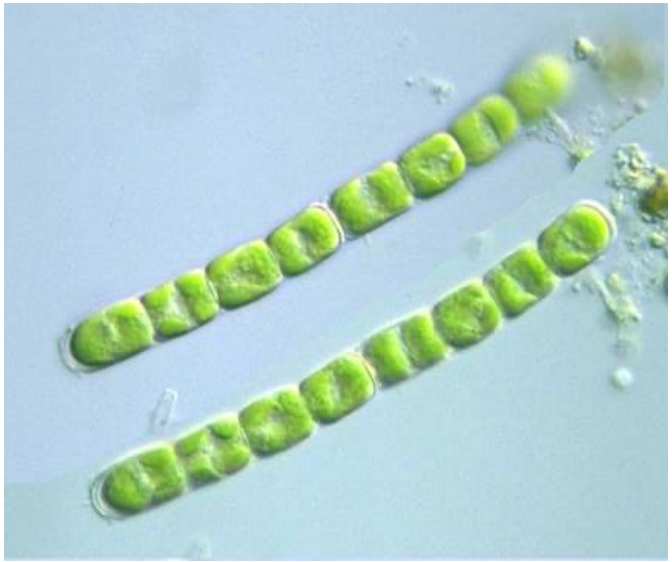
Xanthophyceae - ***Tribonema***

- frequently in clear, slightly acidic [some may be found up to pH = 9] freshwaters (standing and running waters, tychoplanktonic and metaphytic)
- unbranched uniseriate filaments; bipartite cell wall (H-shaped pieces), long, cylindrical cells
- one to many chloroplasts, zoospores, aplanospores, resting cysts
- polyphyletic genus
- *Tribonema* clade



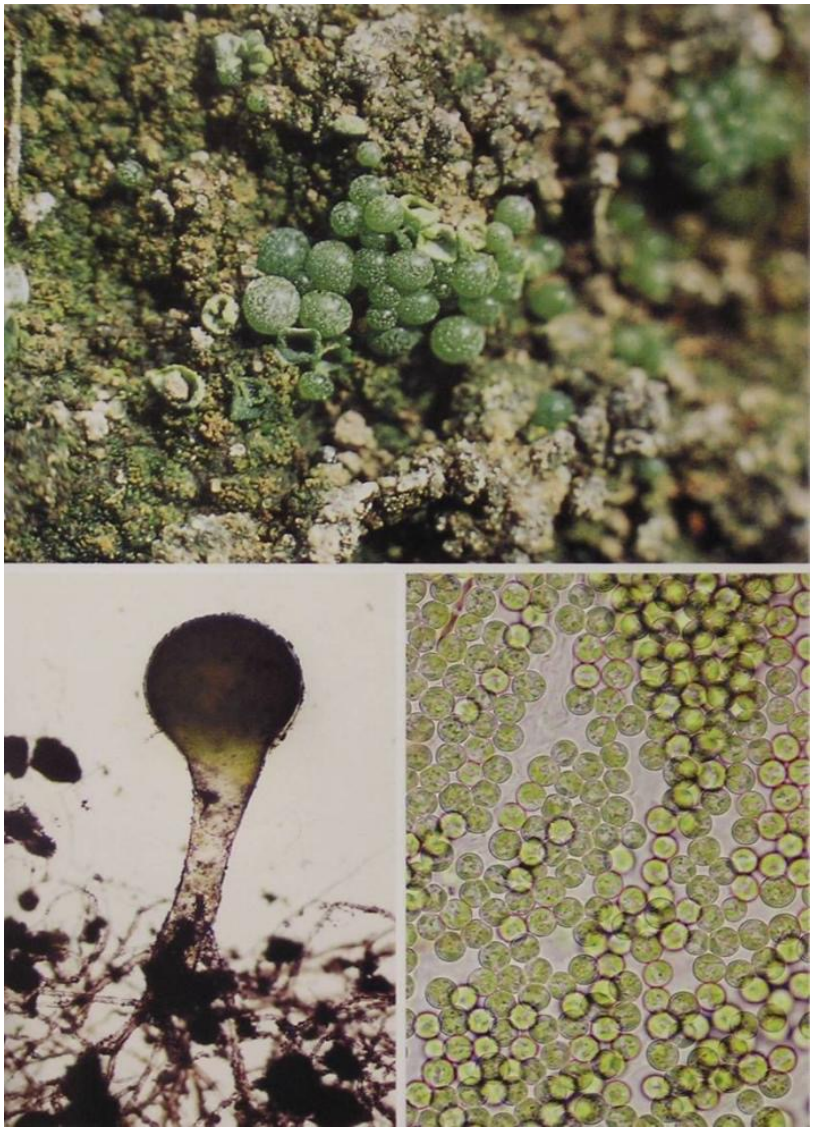
Xanthophyceae - ***Xanthonema***

- frequent soil alga, also euplanktonic and metaphytic in freshwater standing waters
- unbranched uniseriate filaments; fragmentation of filaments
- bipartite cell wall (H-shaped pieces), one or two chloroplasts
- polyphyletic genus
- *Tribonema* clade



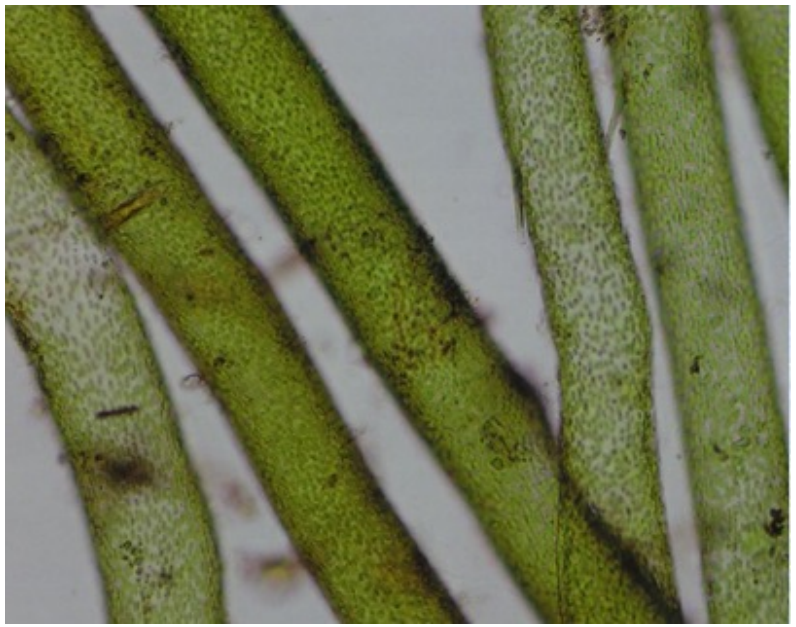
Xanthophyceae - ***Botrydium***

- macroscopic sack like vesicles
- aerial habitats (damp soil, drying mud in freshwater environments)
- siphonal thallus: large number of nuclei and chloroplasts, and subterranean colorless branched rhizoids
- zoospores, aplanospores; gametes from undifferentiated vegetative cells
- *Tribonema* clade

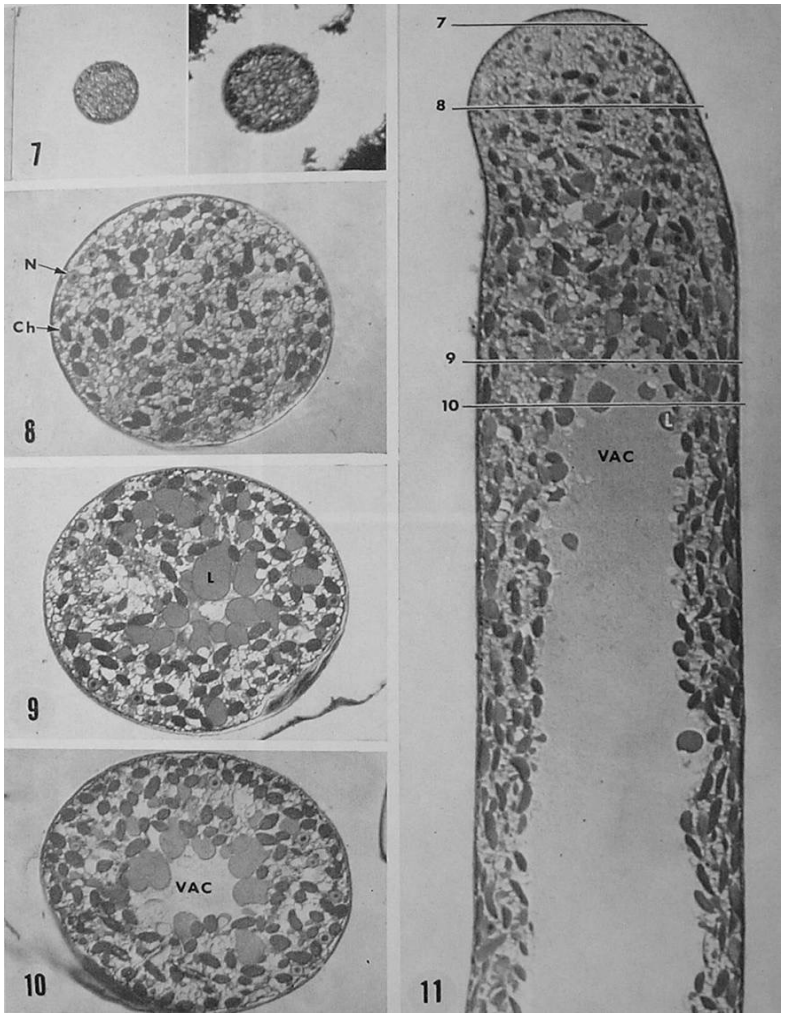


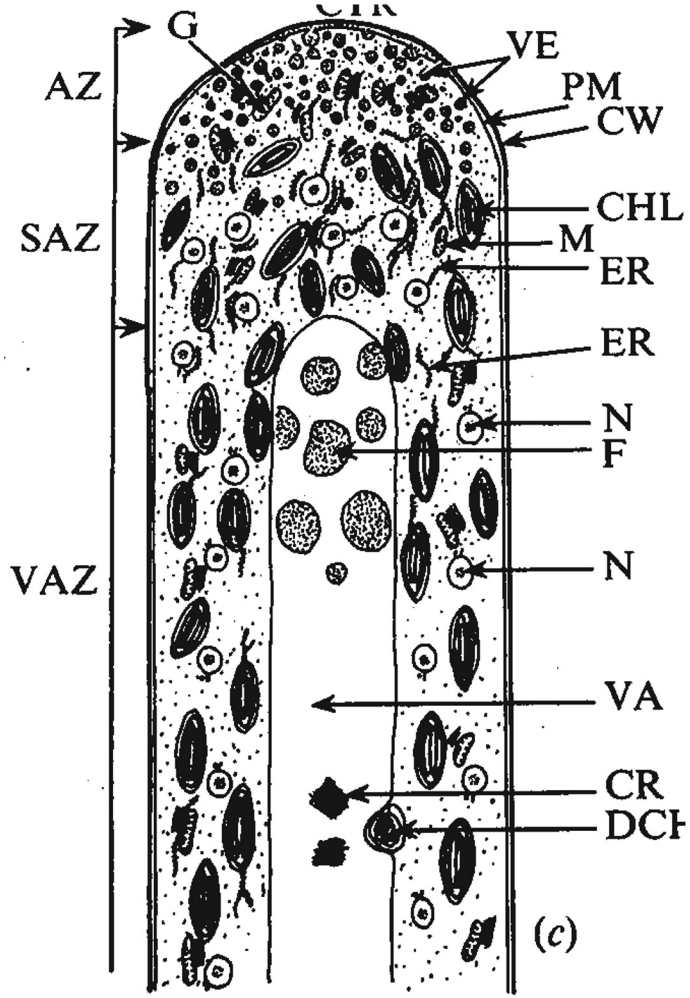
Xanthophyceae - ***Vaucheria***

- branched siphonous filaments (unlimited apical growth, cross walls during reproduction)
- large numbers of chloroplasts and nuclei in peripheral cytoplasmatic layer surrounding a central vacuole
- extensive growths on moist soil (stabilization of soils) or grow submerged (many species amphibious)
- freshwater, marine, and brackish
- *Vaucheria* clade



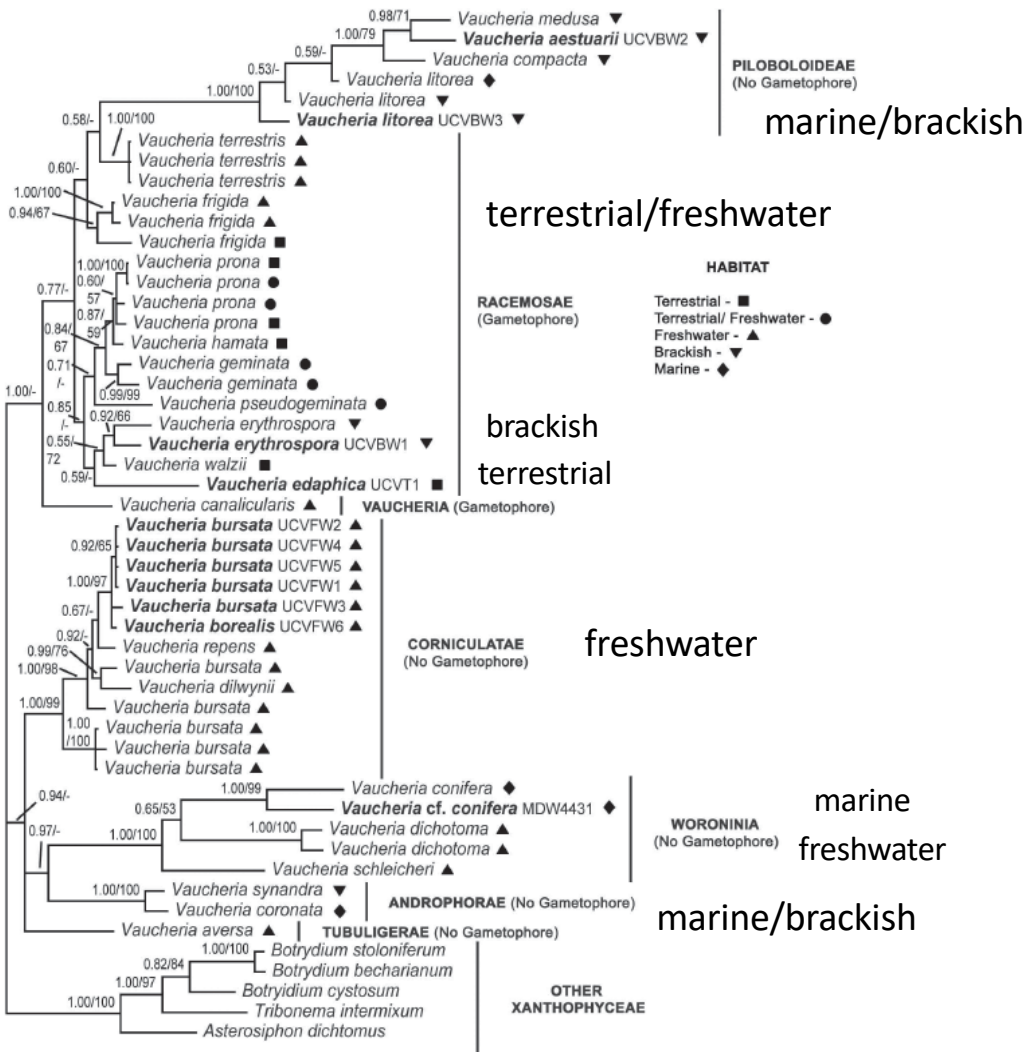
Xanthophyceae - ***Vaucheria***

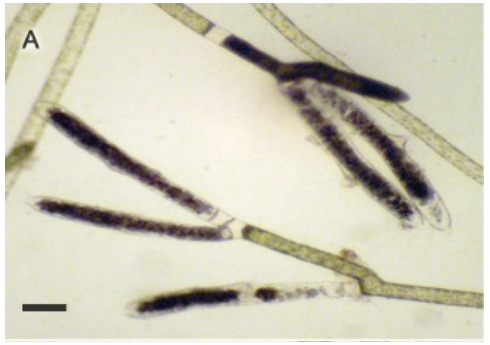


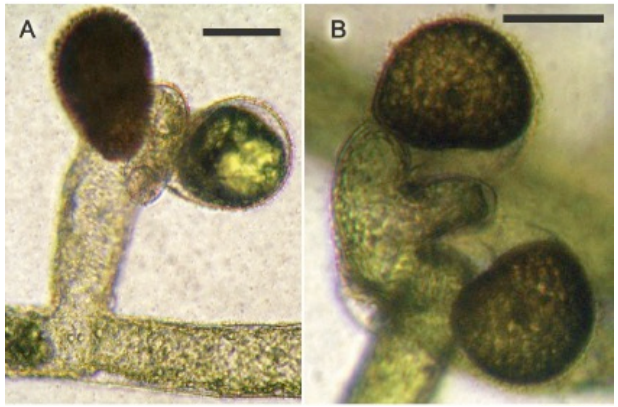


AZ: apical zone, SAZ: subapical zone, VAZ: vacuolised zone, M: mitochondria, VA: vacuole, CHL: chloroplast, N: nucleus, SW: cell wall, PM: plasma membrane, M: mitochondria, ER: endoplasmatic reticulum

Xanthophyceae - ***Vaucheria***







Habitat origin and gametophore formation mapped on phylogeny

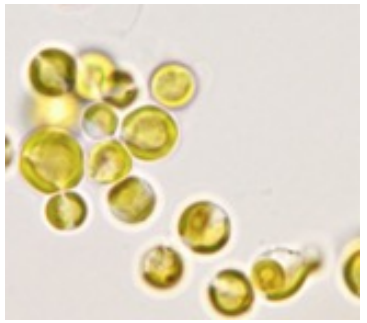
Muralidhar et al. (2014)

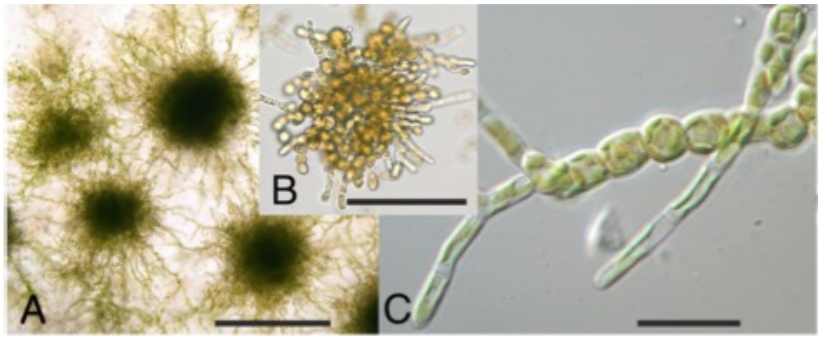
Xanthophyceae - ***Asterosiphon***

- filaments as *Vaucheria*
- regular branching forming rosettes (up to 1 cm)
- moist soil
- most likely its phylogenetic position outside the Vaucherialean clade

Xanthophyceae - ***Heterococcus***

- common and widespread in soils of cold regions [some are freshwater: epiphytic in dystrophic pools]
- unicellular coccoid cells (that are weakly connected) in field samples; branched uni- to multi-seriate filaments (pseudoparenchymatous basal and erect filamentous part) on agar plates
- one-piece cell wall, a single chloroplast; production of zoospores and aplanospores
- *Heterococcus caespitosus*: photobiont of lichen *Verrucaria*
- *Chlorellidium* clade

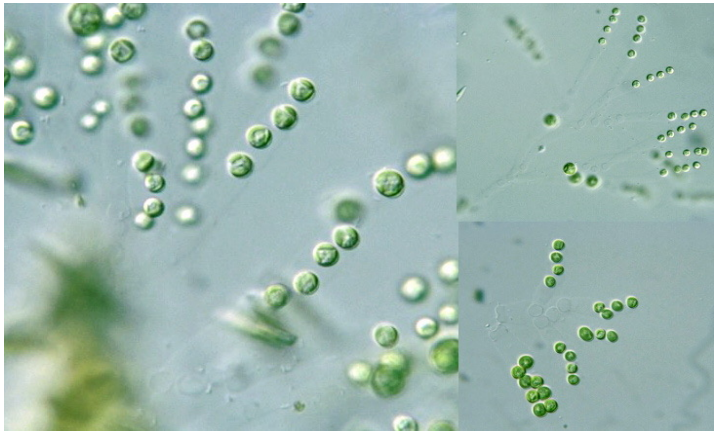


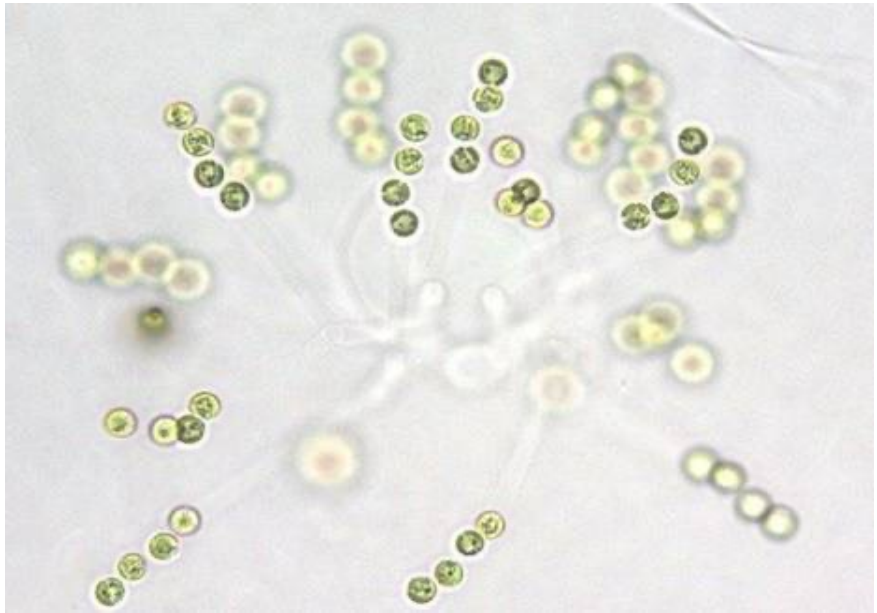


Rybalka et al. (2013), *BMC Evolutionary Biology*

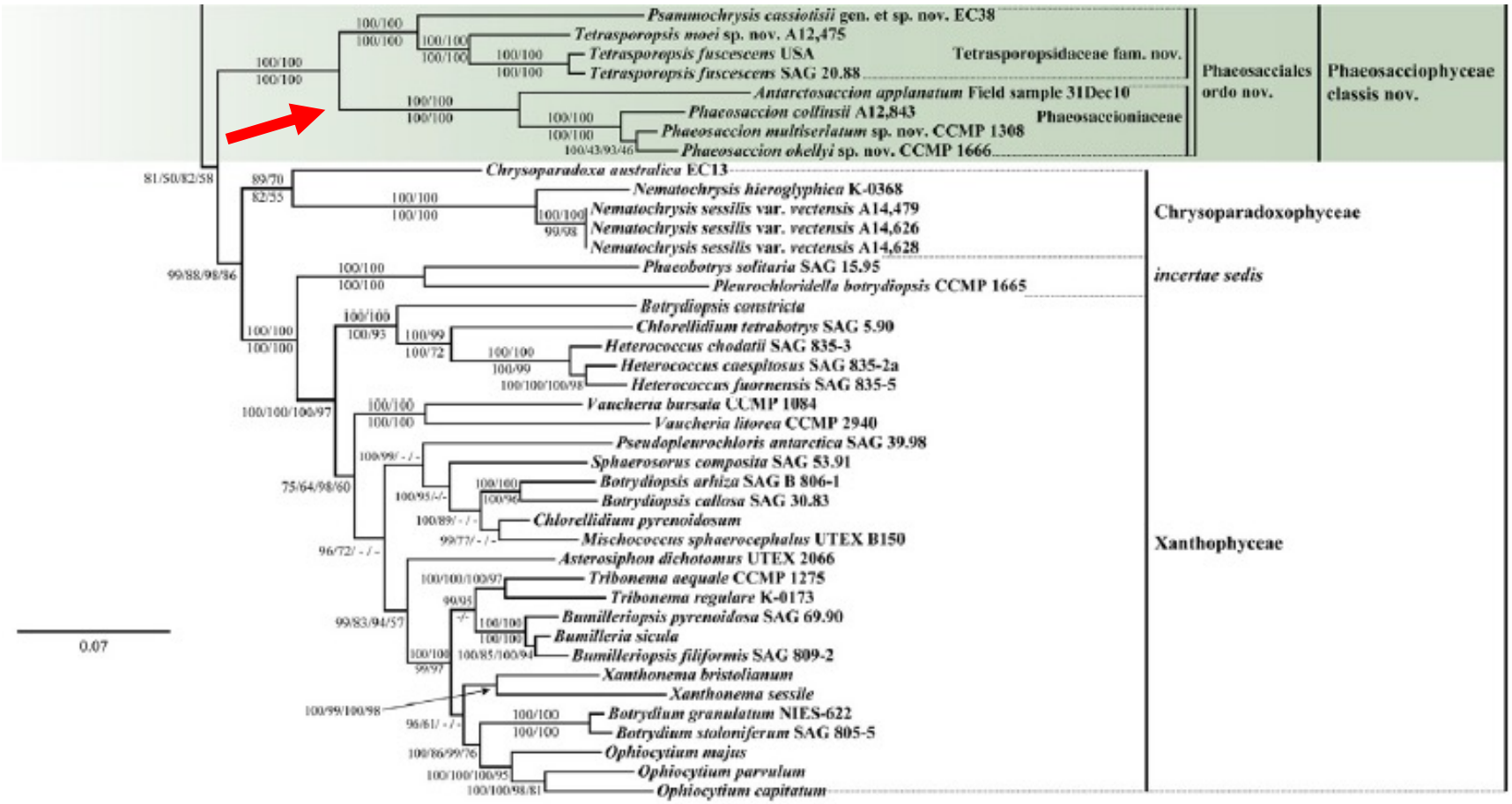
Xanthophyceae - ***Mischococcus***

- freshwater (epiphytic or tychoplanktonic)
- colonies on mucilaginous stipes = treelike colonies (2 or 4 cells at the ends of branches)
- one or more plastids
- one-piece cell wall
- autospores, zoospores
- *Botrydiopsis* clade



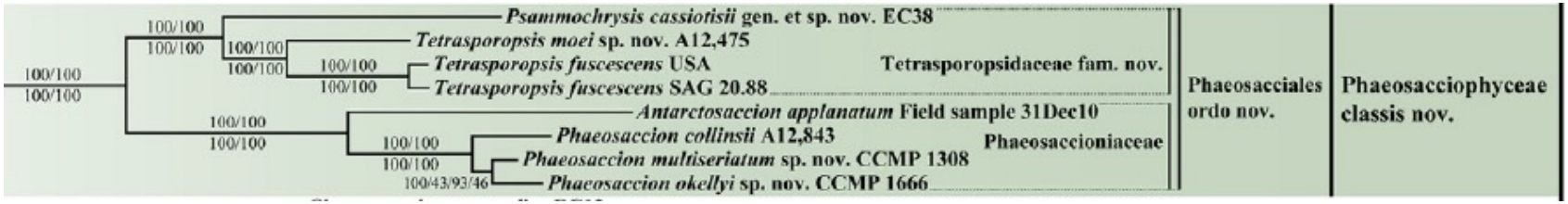


Phaeosacciophyceae



Graf et al. (2020), *Protist*

Phaeosacciophyceae



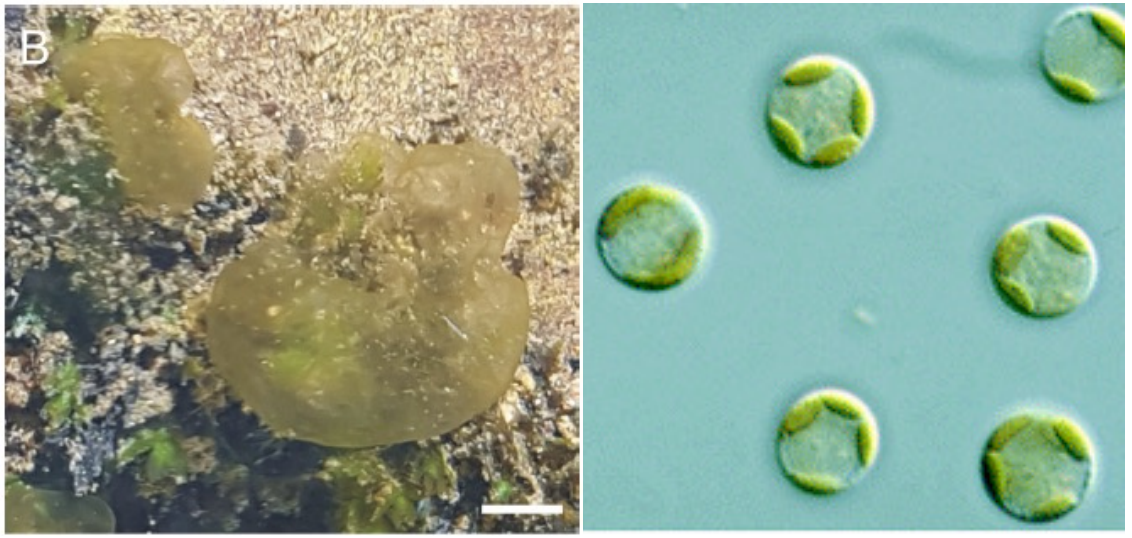
- unicellular, colonial, filamentous (uniseriate or branched multi-seriate) or thallic forms
- cells typically with cell wall
- chloroplasts one to two per cell: three thylakoids per lamella plus a girdle lamella; eyespot
- zoospores heterokont-like
- flagellar apparatus similar to brown algae and xanthophyte algae

Graf et al. (2020), *Protist*

Phaeosacciophyceae

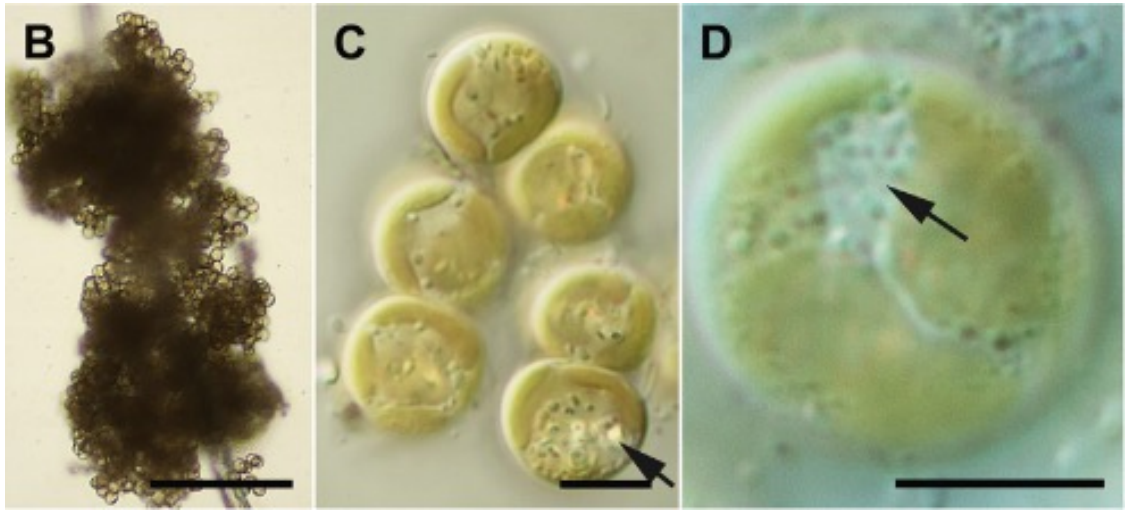
Tetrasporopsis fuscescens

- freshwater environments
- cool and running waters
- colonial in mucilage
- previously within Phaeoph., Chrysoph. and Phaeothamnioph.



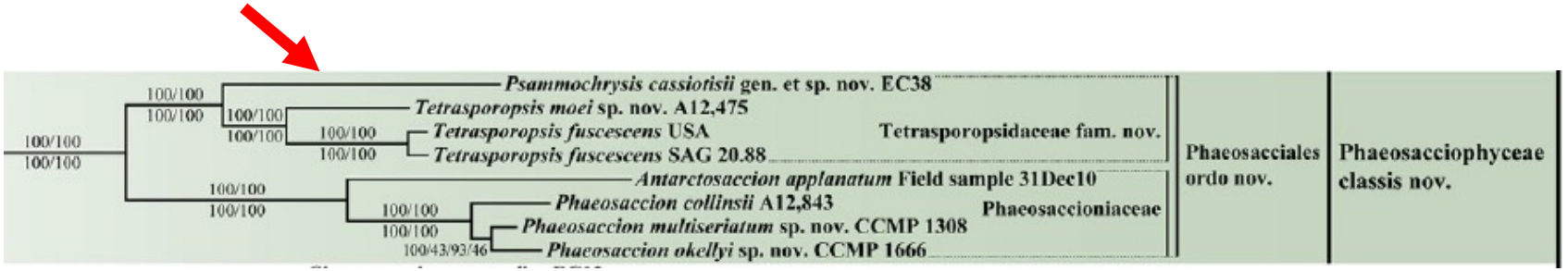
Tetrasporopsis moei

- freshwater (small pool)
- dancing particles located between the chloroplasts
- colonial (irregular cluster of cells)



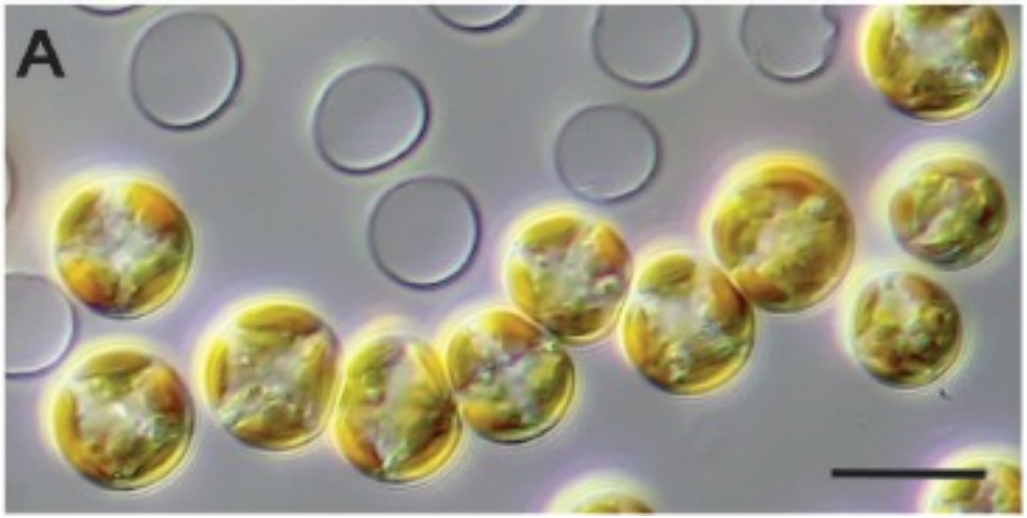
Graf et al. (2020), *Protist*

Phaeosacciophyceae



Psammochrysis cassiotissii

- rounded flattened unicells (as gold coins) adhered to sand grains
- a thick adhesive cell walls
- chloroplasts with 4 lobes
- marine (intertidal pool near Australia)

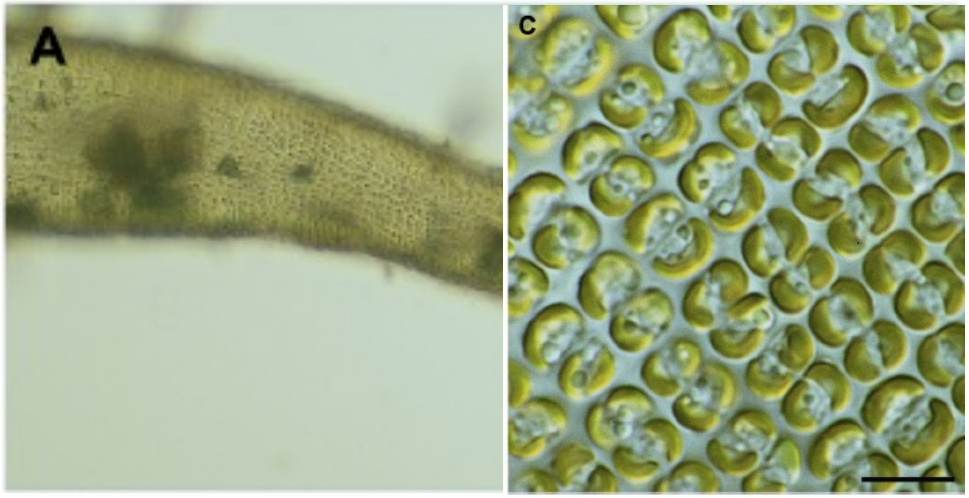


Graf et al. (2020), *Protist*

Phaeosacciophyceae

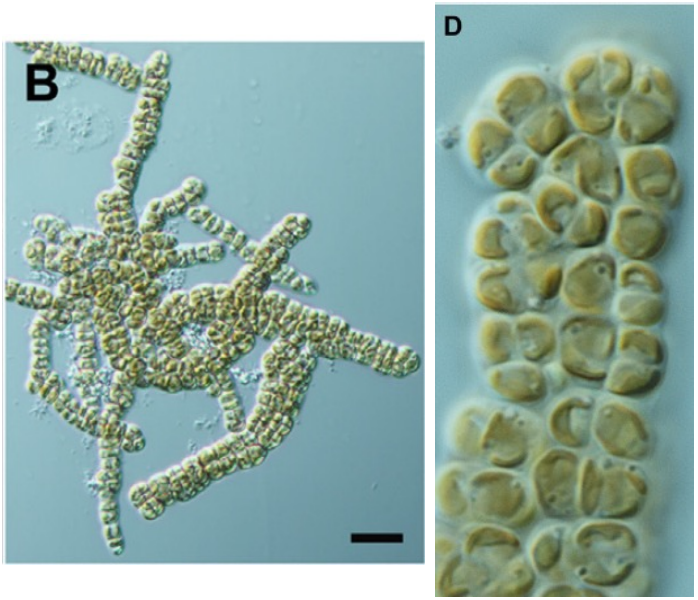
Phaeosaccion collinsii

- macroscopic hollow tubes attached to *Zostera marina*
- block-shaped cells; division in two directions



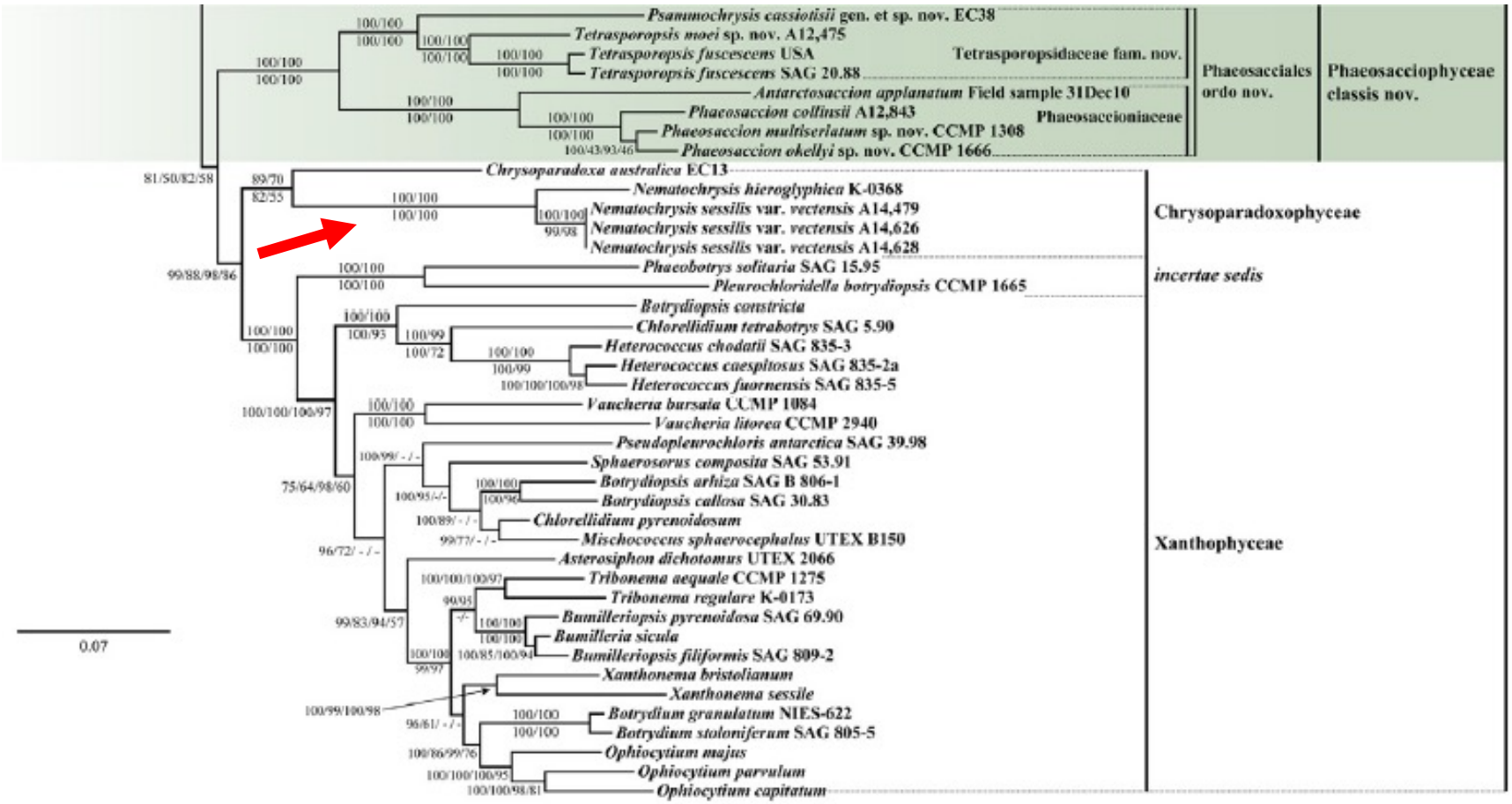
Phaeosaccion multiseriatum

- marine
- uniseriate and multiseriate branched filaments
- block-shaped cells; division not precise (along three axes)



Graf et al. (2020), *Protist*

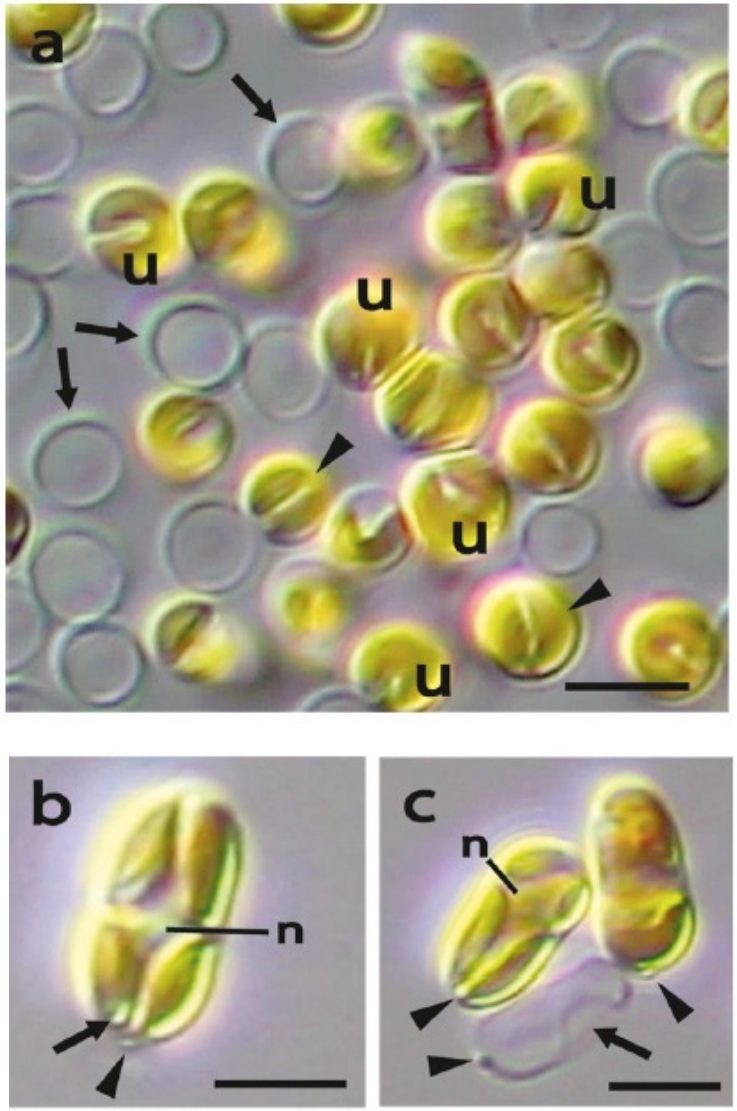
Chrysoparadoxophyceae



Graf et al. (2020), *Protist*

Chrysoparadoxophyceae

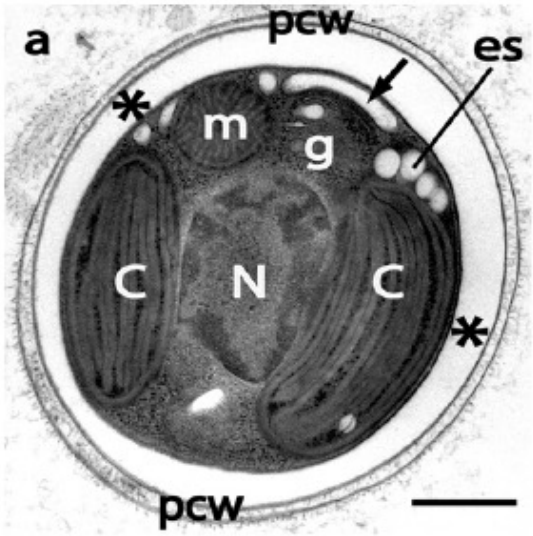
- *Chrysoparadoxa australica*
- marine, sand dwelling (benthic, attached to the substratum by a complex adhesive plug)
- unicellular, and surrounded by a multilayered cell wall
- etymology: Chryso- = golden; a paradox = chloroplasts are surrounded by only two membranes
- described in 2019, type locality: intertidal pool, Australia



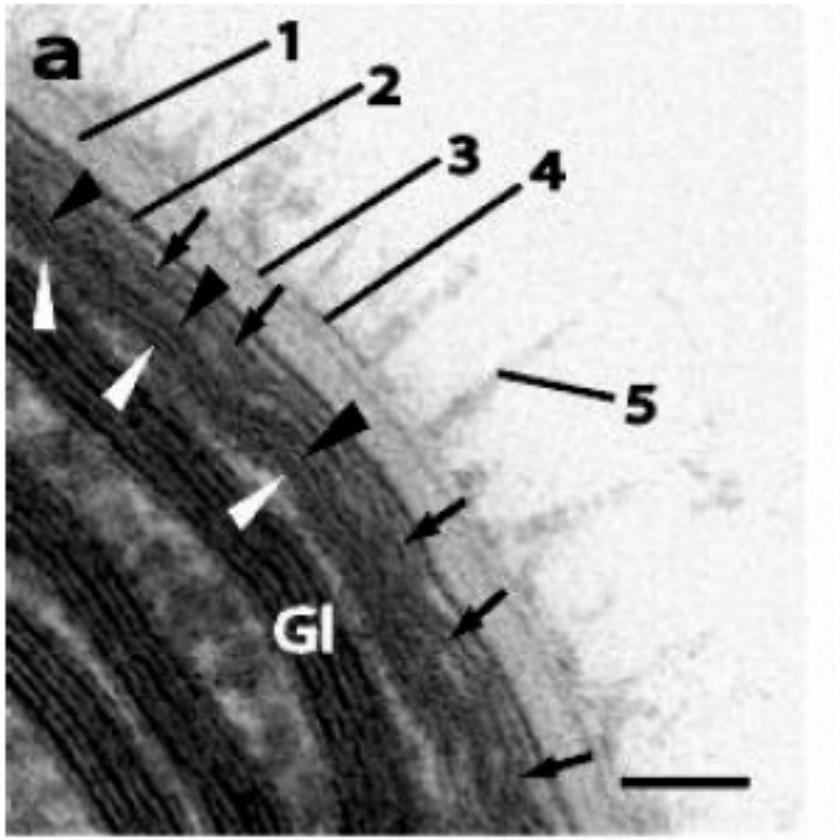
Wetherbee et al. (2019), *Journal of Phycology*

Chrysoparadoxophyceae - ***Chrysoparadoxa***

- multilayered cell wall
- primary and secondary cell wall



pcw: primary cell wall; * the secondary wall; es: eyespot; m: mitochondria; g: Golgi stack; V: a vesicle; N: nucleus; C: chloroplast



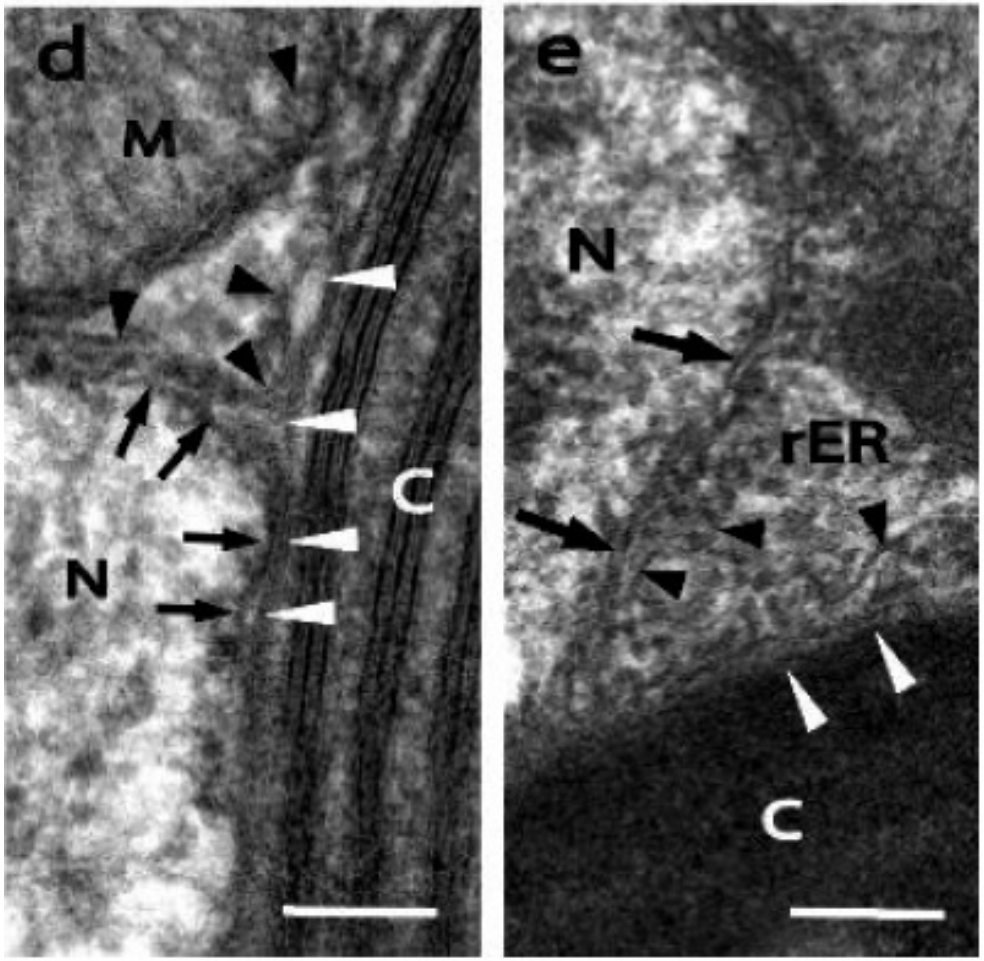
The inner chloroplast membrane (white arrowheads) and outer chloroplast membrane (black arrowheads) lie beneath the PM (arrows). The pcw is multilayered (#1–#5).

Wetherbee et al. (2019), *Journal of Phycology*

Chrysoparadoxophyceae - ***Chrysoparadoxa***

- 2 chloroplasts, surrounded by only two membranes (the inner membrane of the nuclear envelope and the inner chloroplast membrane)
- chlorophyll *a, c1, c2*; xanthophylls (including fucoxanthin); presence of pyrenoid, girdle lamella

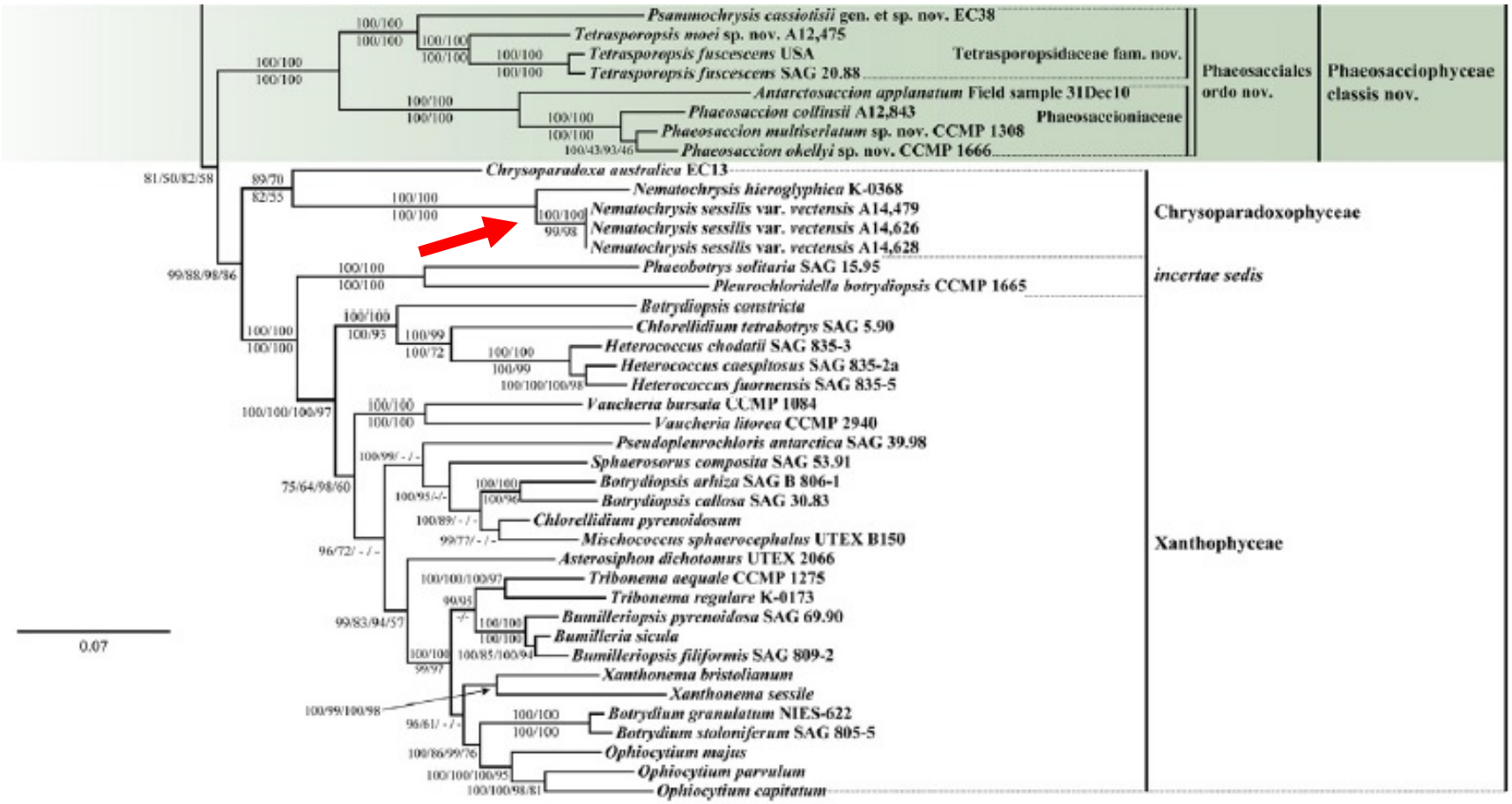
the chloroplast – nucleus interface



The outer chloroplast membrane is continuous with the outer membrane of the nuclear envelope (black arrowheads). The inner membrane of the nuclear envelope (arrows) and the inner chloroplast membrane (white arrowheads) separate the two organelles at their interface.

Wetherbee et al. (2019), *Journal of Phycology*

Chrysoparadoxophyceae



Graf et al. (2020), *Protist*

Chrysoparadoxophyceae

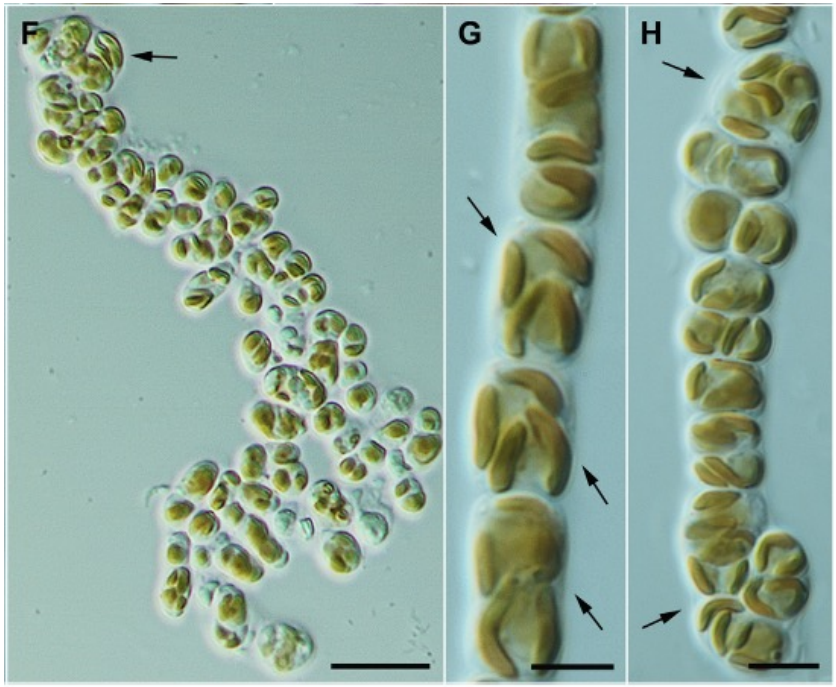
- there is doubt about the inclusion of *Nematochrysis* in this class

Nematochrysis sessilis

- originally described by Pasher (1914) [found in a Prague tank that was filled with water collected from the Adriatic Sea]
- aquarium tanks and estuaries [requirement for ammonia except nitrate]

N. (*Chrysowaernella*) *hieroglyphica*

- unbranched filaments, uni- to pluri-seriate; surrounded by mucilage
- epiphytic, brackish areas
- previously in Chrysophyta, Sarcinochrysidales - Pelagophyceae, Chrysomerophycea



N. sessilis var. *vectensis*

Graf et al. (2020), *Protist*