The Raphidophytes – the enigmatic class!

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Raphidophytes

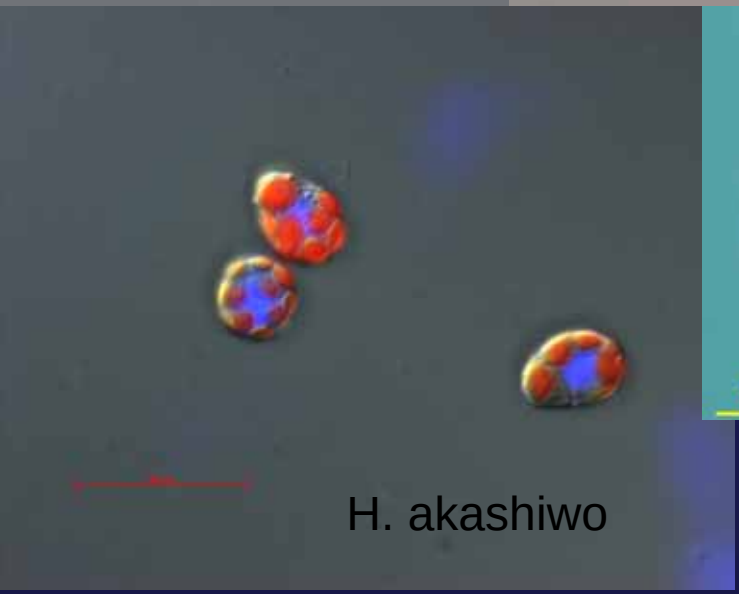
C. antiqua

C. ovata

Gonoystomum

C. marina

Fibrocapsa.



H. akashiwo

Haramonas

Vacuolaria

Kingdom – Chromista

Infrakingdom – Heterokonta

Phylum Ochrophyta

Class Raphidopyceae – M. Chadefaud ex P.C. Silva 1980

Orders – Raphidomonadales Norris 1982

Chattonellales Throndsen 1995

Systma Naturae 2000/Classification

Raphidophytes

Green Raphidophytes

Vaucheriaxanthin derivatives

Freshwater

Vacuolaria
- *V. virescence*
- *V. depressum*

Gonyostomum
- *G. semen*
- *G. latum*

Merotrichia
- *M. capitata*

Golden Brown Raphidophytes

Fucoxanthin dominated carotenoids

Brackish/Marine

Heterosigma
- *H. akashiwo = H. carterae*

Chattonella
- *C. subsalsa*
- *C. antiqua (C. harima??)*
- *C. ovata*
- *C. marina*
- *C. globosa*
- *C. minima*

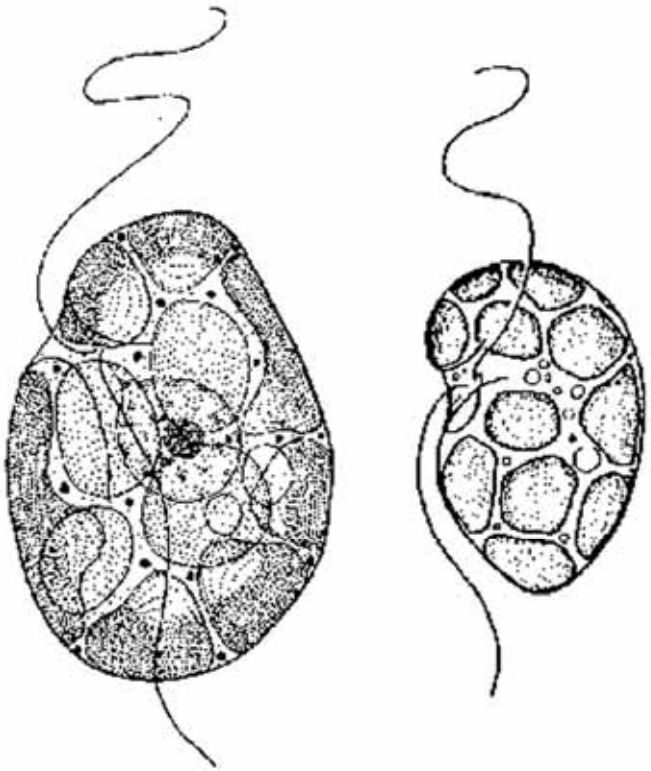
Fibrocapsa
- *F. japonica (Chattonella japonica)*

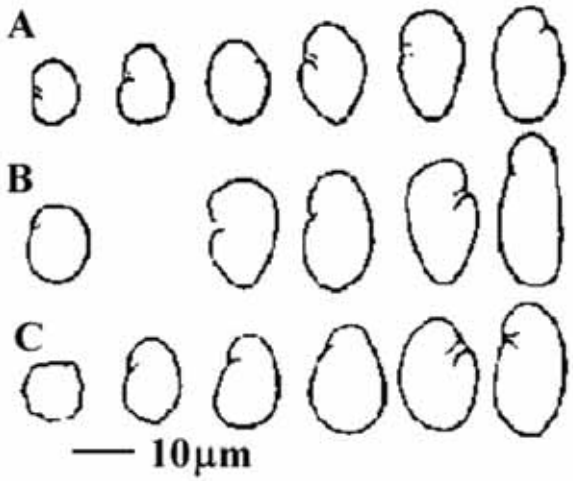
Haramonas
- *H. dimorpha*
- *H. viridis*

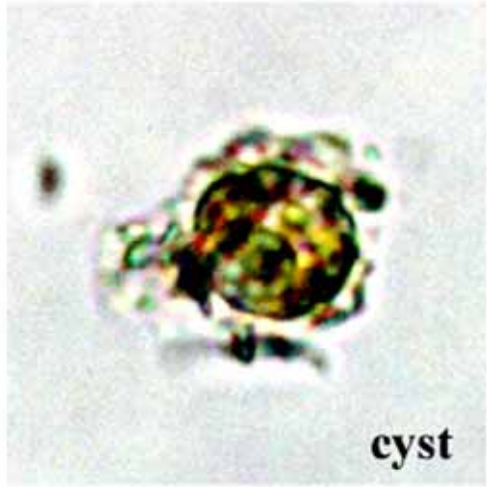
Vacuolaria

Gonyostomum

Merotrichia







Heterosigma akashiwo

(Hada) Hada

Photomicrographs by Ichiro Imai and drawings by Yoshiaki Hara and Mitsuo Chihara

- 3>25 µm long
- 4-18 µm wide
- Shape – varying
- 2 subapical heterokont flagella
- Chloroplasts 3 - ~ 27
- Forms benthic stage and cysts
- Accumulates in surface layers under strong light
- Produces allelopathic substances
- Toxins – reactive oxygen species, hemolytic substances, neurotoxins, ichthyotoxins and unsturated fatty acids

Heterosigma akashiwo - **What's in a name?**

Vulgar names –

Pot shard flagellate –Pratt for Narrangasett Bay

Potato – morphology within a sack of potatoes -Tomas

Corn Flake flagellate

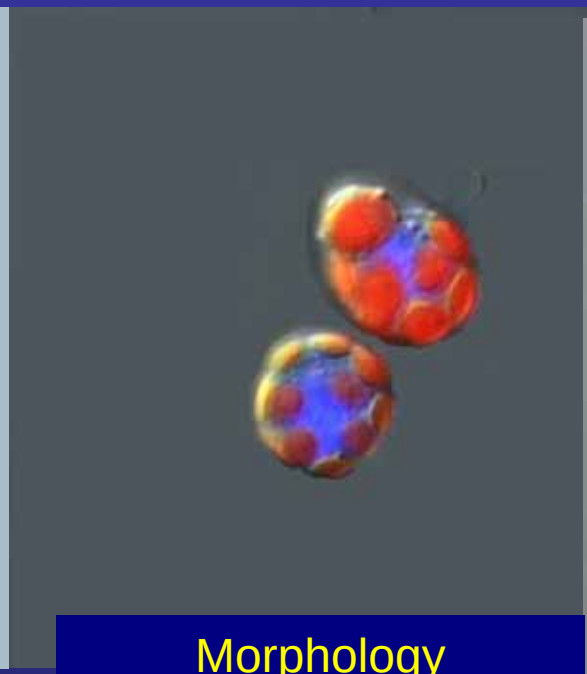
Pre 1980 - literature

For many years mistakenly called *Olsithodiscus luteus*

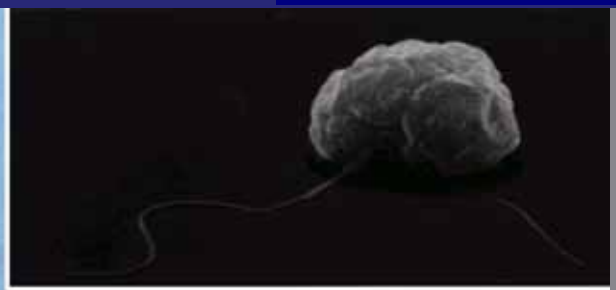
Plymouth Clone – 12A, 239

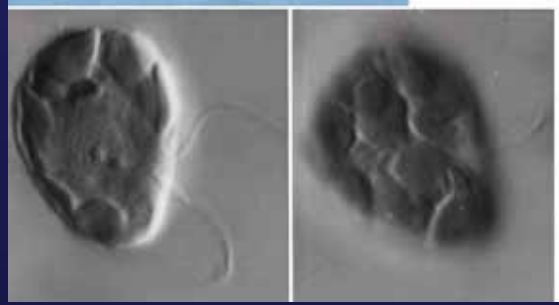
Guillard Collection (WHOI) Olisth

Narrangasett Bay Clones – OLMS, O. luteus



Morphology





Heterosigma akashiwo
(Hada) Hada

Photomicrographs
by Yoshiaki Hara

WESTPAC-HAB
IOC Harmful Algal Bloom Programme
T0007

Chattonella subsalsa

30-50 µm long

10-30 µm wide

Shape mostly pyriform

Flagella 2 heterodynamic emerging subapically in depression.

Chloroplasts – many, golden brown

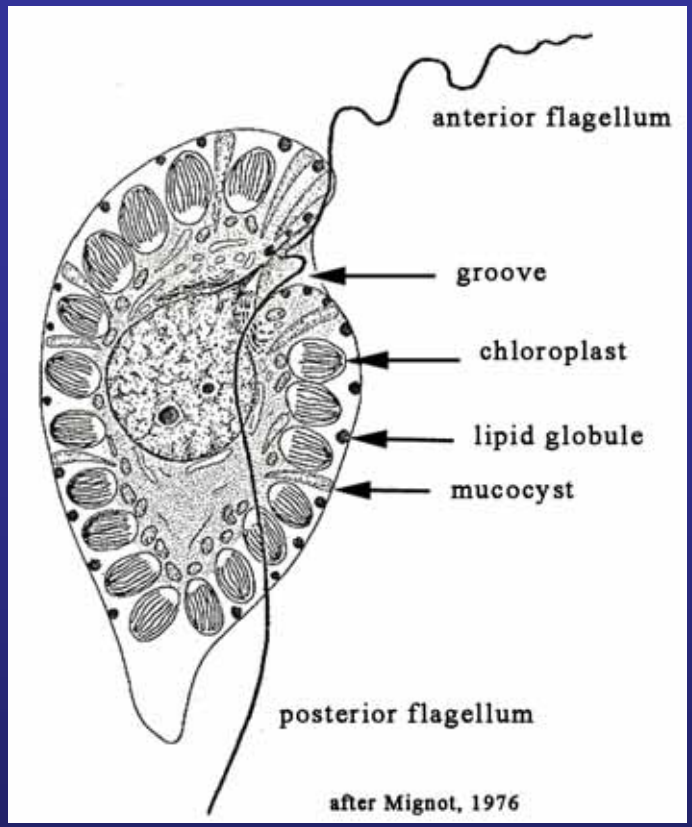
Cyst formation?

Toxiciy unknown –

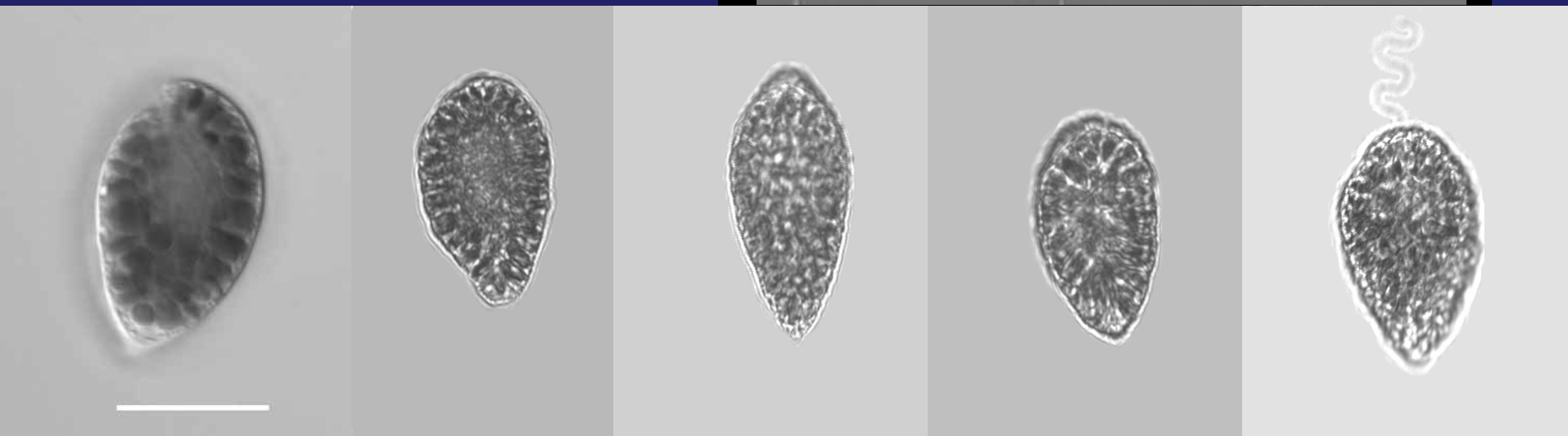
Can be confused with *Chattonella marina* although EM features differ.

Most common bloom species in coastal U.S. often associated with other raphidophytes.

Separated from C. marina, C. antiqua and C. ovata by molecular sequences.



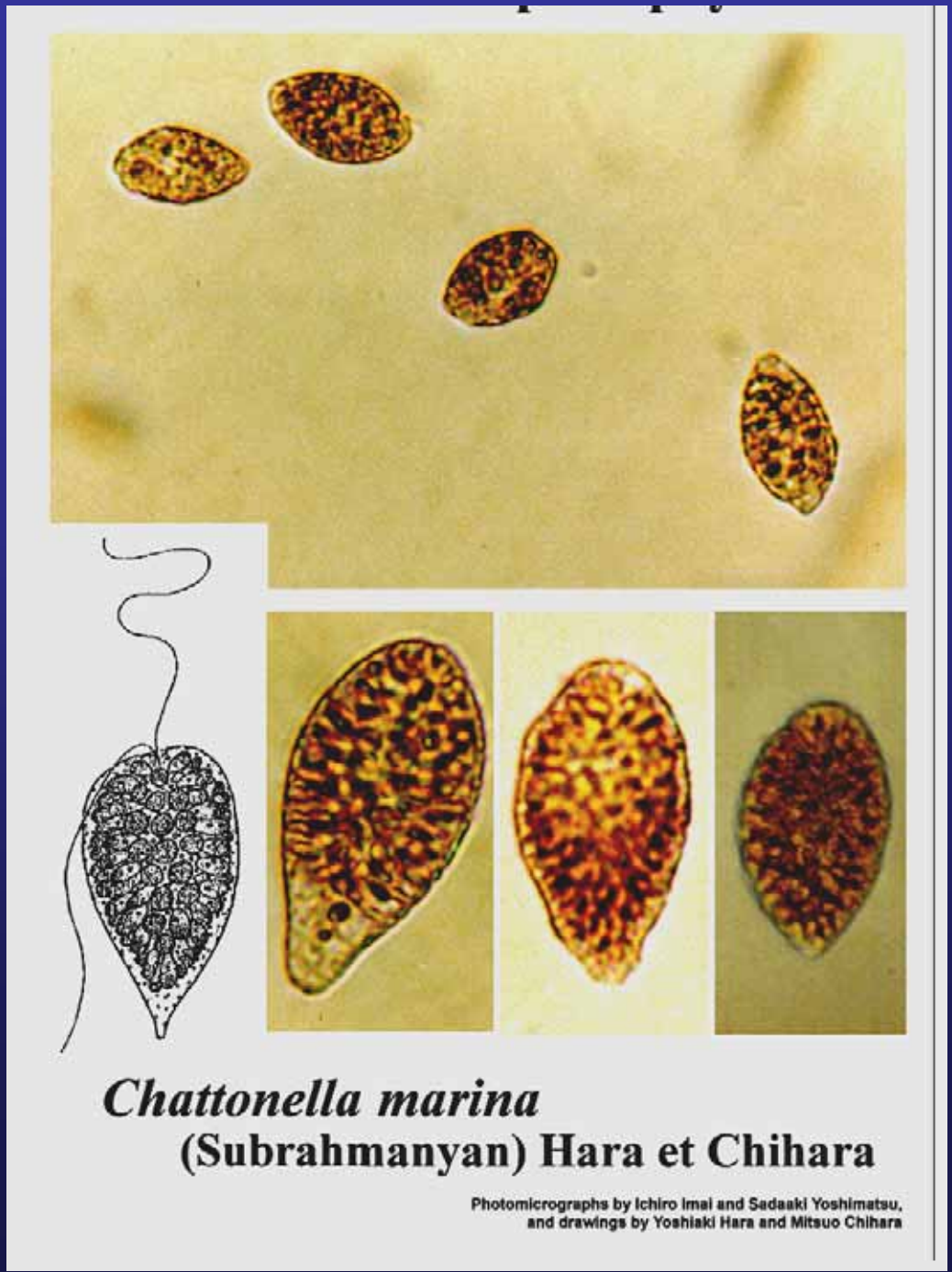
Chattonella subsalsa




10 µm


10 µm


20 µm



Chattonella marina

30-70 µm long

20-30 µm wide

2 flagella

Toxins – neurotoxins

ROS

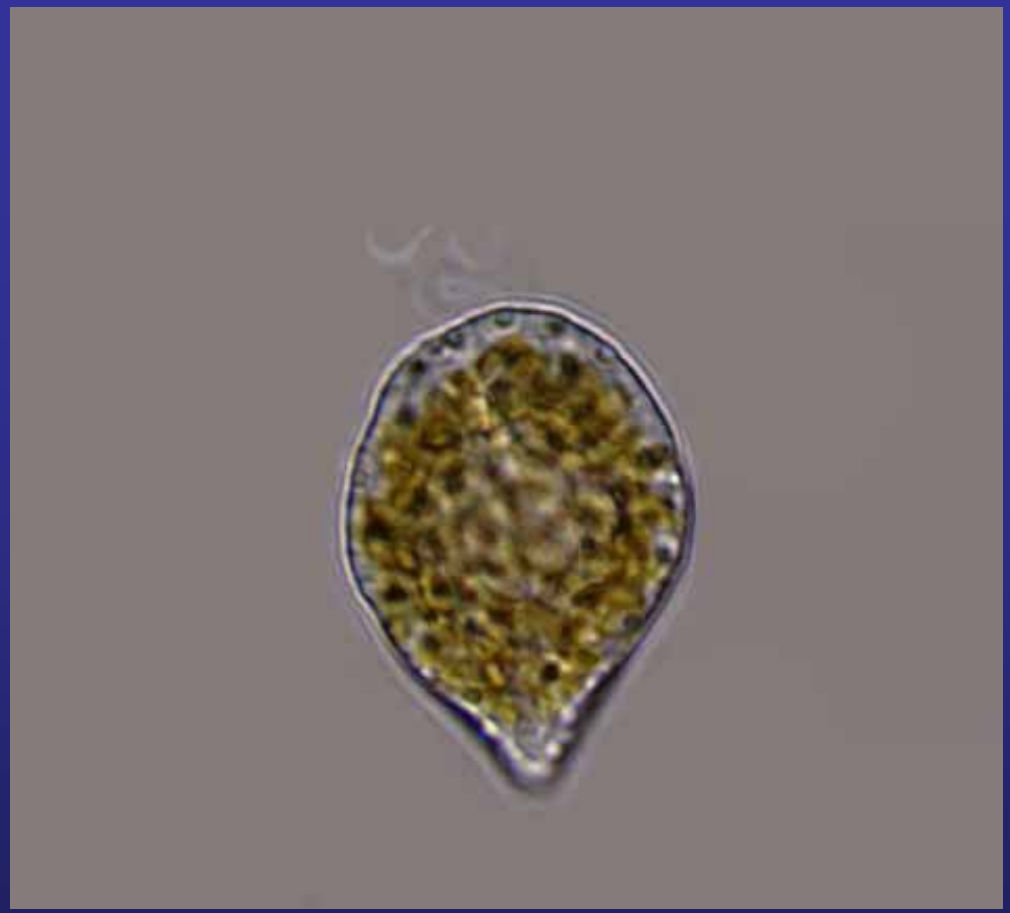
Hemolytic Agents

20 µm

Chattonella marina

Chattonella marina

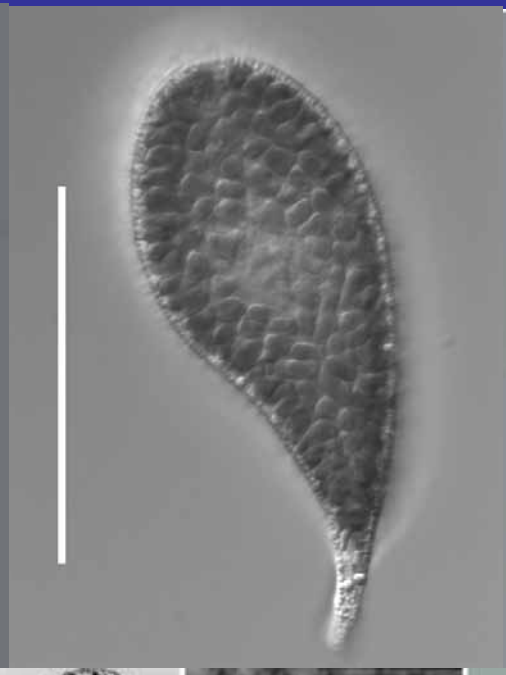
C. marina

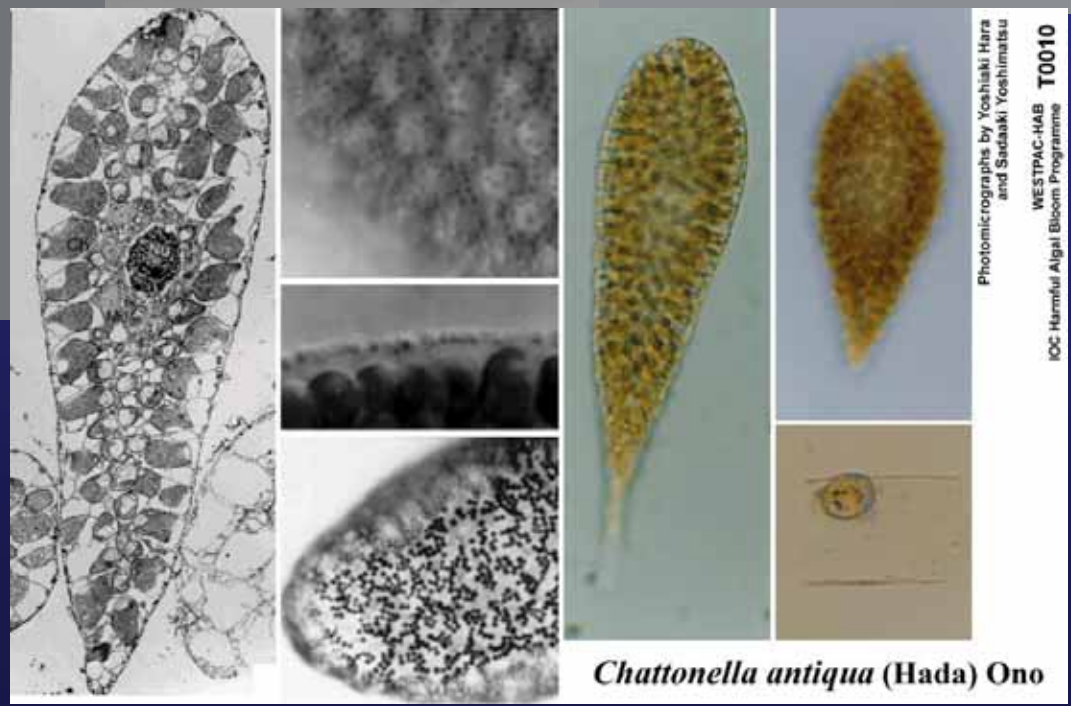


C. subsalsa


CCMP2050
20 µm

Chattonella antiqua





Yellow Tail – Seto Inland Sea, Japan

Red tide by *Chattonella antiqua* and consequent mass mortality of aquaculture fish, *Seriola quin.* (Seto Inland Sea, August 1977)
WESTPAC-HAB R0003

Cultured tuna – South Australia

WESTPAC-HAB R0004-2

Mass mortality of yellowtail, *Seriola quinqueradiata*, cultured in cages by a red tide of rahydoflagellate *Chattonella antiqua* (Seto Inland Sea, Aug.1977)


20 µm

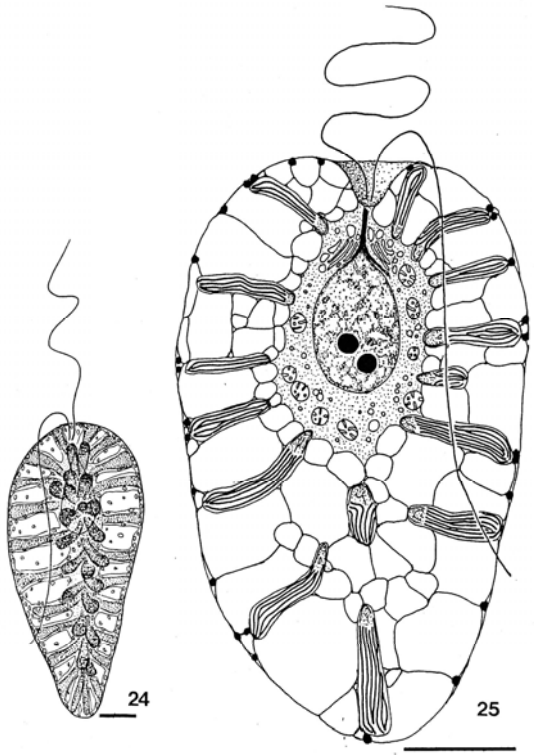
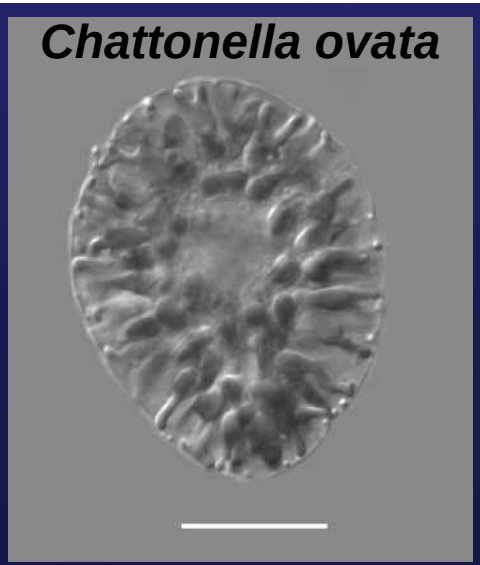
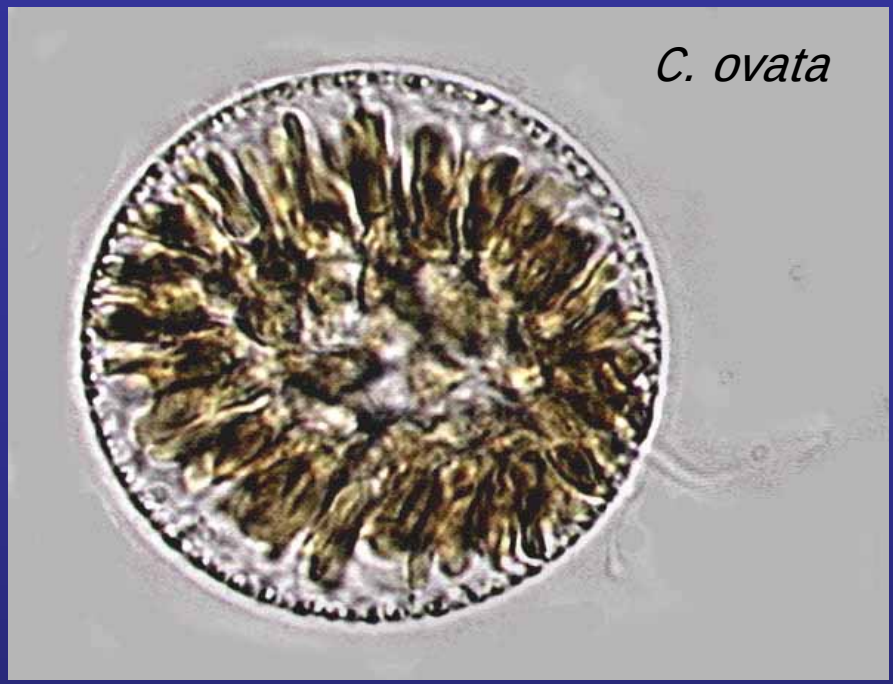
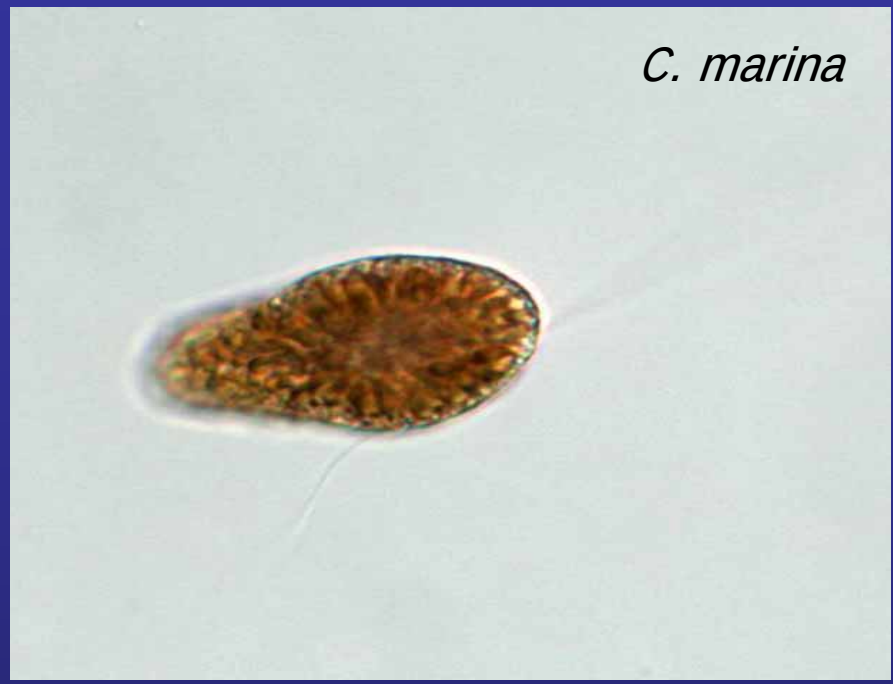


Fig. 24–25. *Chattonella ovata*. 24: Drawing of a motile cell. 25: Drawing showing the features of a motile cell based on the observations using electron microscopy. Scale bars = 10 μm in Figs. 24, 25.



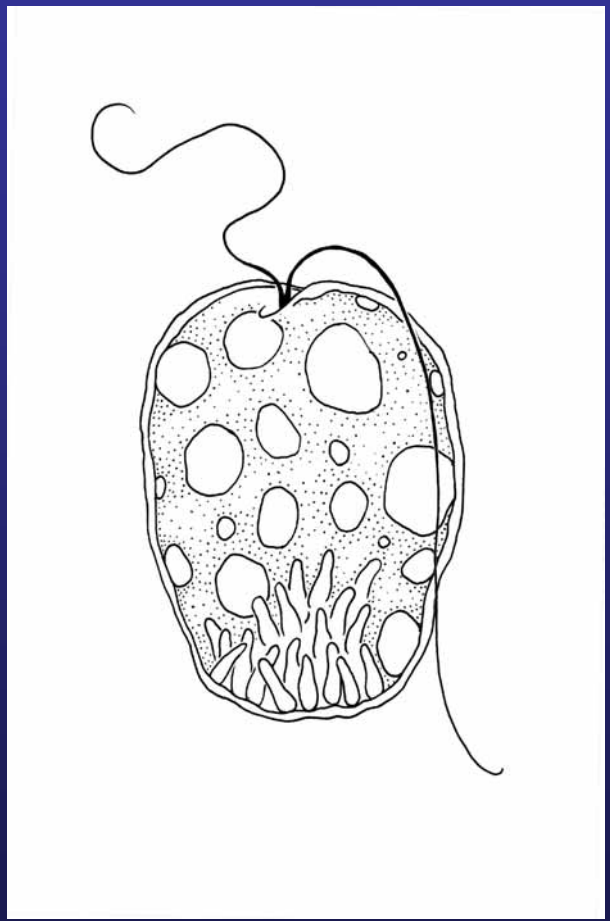

C. ovata


C. marina


C. antiqua


C. subsalsa

Fibrocapsa japonica



20 – 30 µm Long

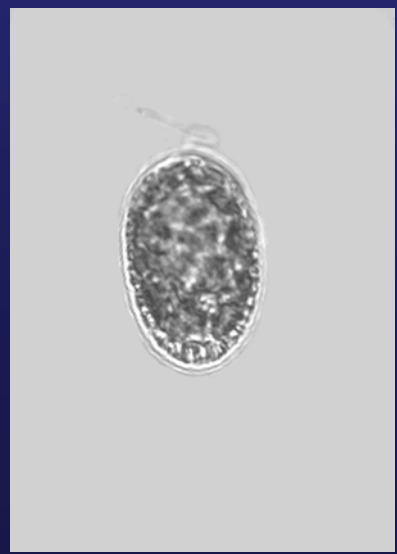
30 – 50 µm Wide

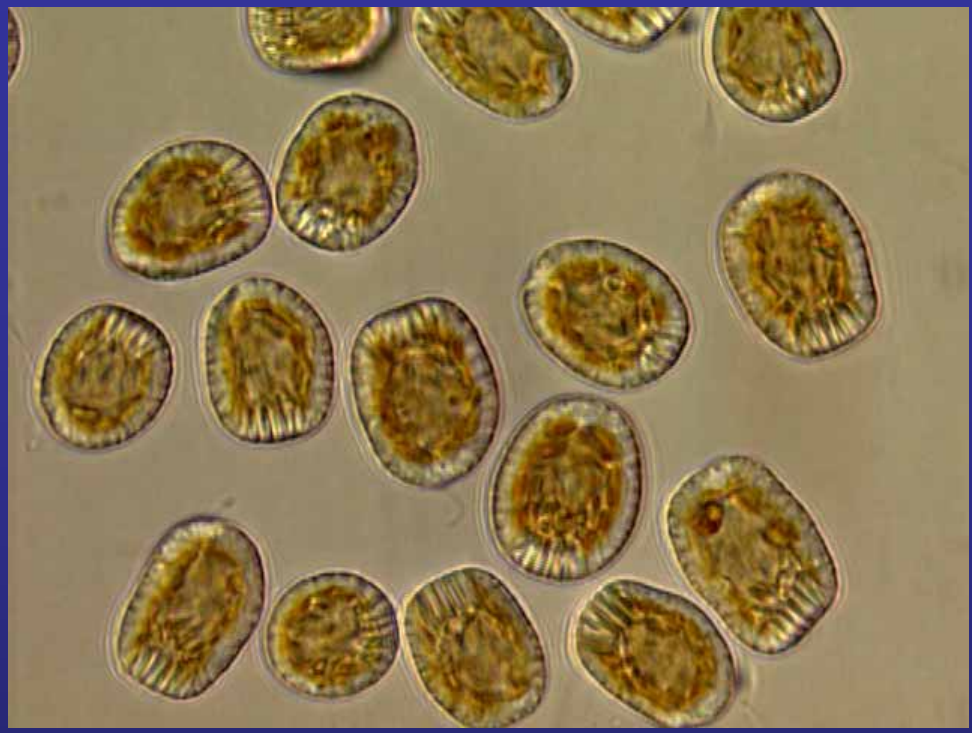
Shape – flattened, ovate, oval or round

Flagella – 2 heterokont – one long posterior and shorter anterior

Emerge from anterior furrow.

Mucocysts – prominent particularly in posterior of cell





Fibrocapsa japonica

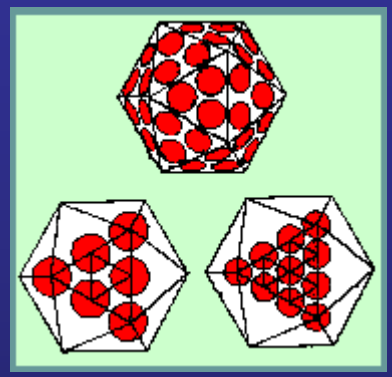
Haramonas dimorpha

Heterosigma or Chattonella????

Which species???

Does this help??

So much for morphology!!!!



What about pigments????

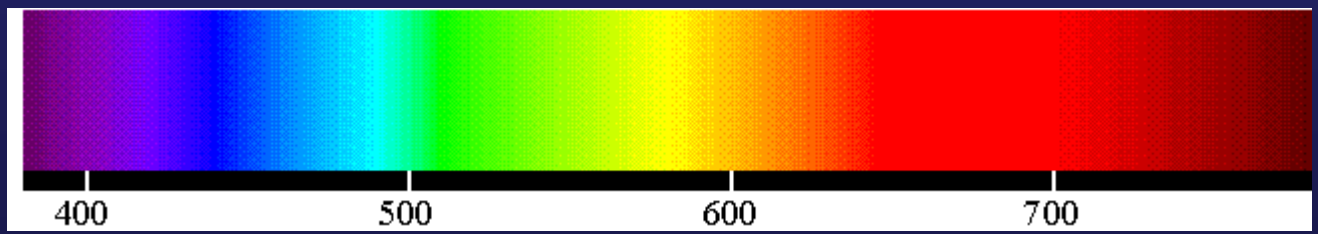
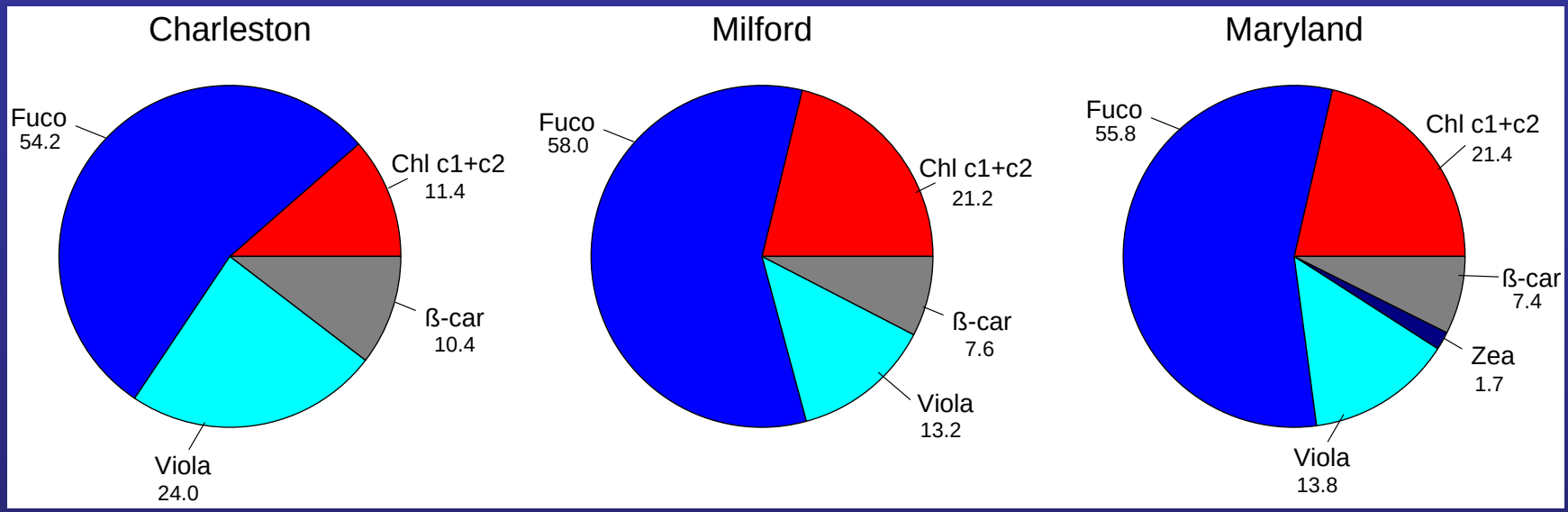


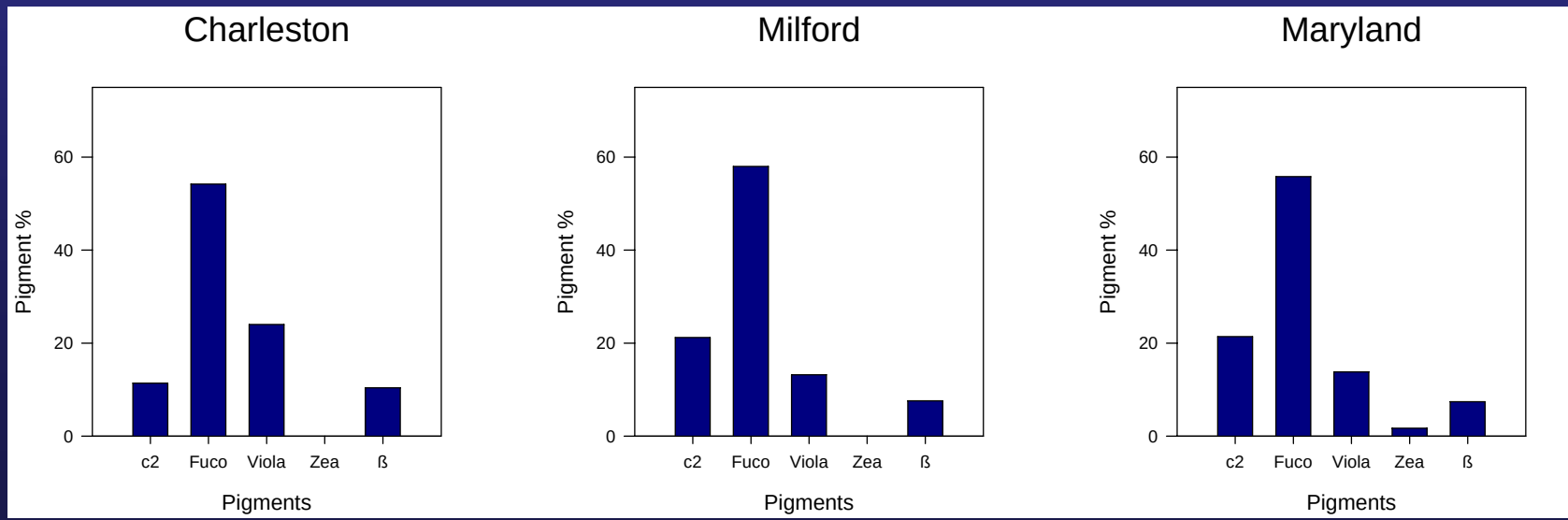
Table 3. Pigment comparison of Chloromorum toxicum with other Xanthophyceae, Raphidophyceae and Dictyochophyceae. Pigments are in order of retention in chromatograms. All species contain Chlorophyll *a* and is not indicated

Class	Species	Culture origin	Chl c_1+c_2	Chl c_3	Hetero	19' But	Fuco	19' Hex	Viola	Dd	Diato	Morum	Vaucher	Zea	β,β-carot
Xanthophyceae	*Vaucheria germinata*	CCMP	+		+					+ + +	+		+ +		+
	Vaucheria bursata	CCMP	+		+					+ + +	+		+ +		+
	Tribonema bombicina	CCMP	+		+					+ + +	+		+ +		+
	Tribonema aequale	CCMP	+		+					+ + +	+		+ +		+
Green Raphidophyceae	*Vacuolaria virescence*	UTEX2237	+		+					+			++**		
	Gonyostomum semens	CMSTAC	+		+					+			++**		
Brown Raphydophyceae	*Chatonella antiqua*	Kagawa	+ +				+ +		+ +	+				+	+
	Chatonella ovata	Kagawa	+ +				+ + +		+ +	+				+	+
	Chatonella marina	Kagawa	+ +				+ + +		+ +	+				+	+
	Chatonella subsalsa Texas	CMSTAC	+ +				+ + +		+	+				+	+
	Chatonella subsalsa SING	CMSTAC	+ +				+ + +		+	+				+	+
	Chatonella subsalsa DEL	CMSTAC	+ +				+ + +		+	+				+	+
	Chatonella subsalsa SS	CMSTAC	+ +				+ + +		+	+				+	+
	Haramonas dimorpha	CCMP	+ +			+ + +	+ + +		+						+
	Fibrocapsa japonica HH	CMSTAC	+ +				+ + +		+ +						+
	Heterosigma akaskivo Texas	CMSTAC	+ +				+ + +		+ +					+	+
	Heterosigma akaskivo CRW	CRW005	+ +				+ + +		+ +					+	+
	Heterosigma akaskivo MIL	Milford	+ +				+ + +		+ +					+	+
Dictyocophyceae	*Verrucophora verruculosa*	Kagawa		+ +		+ +	+ + +			+ +	+				+
	Verrucophora farcimens	Goebel		+ +		+ +	+ + +			+ +	+				+

Pigment designations: + =Area % peak < 5; + + =Area % peak 5 -15; + + + =Area % peak > 15. Pigment abbreviations: Chl = Chlorophyll; Hetero = Heteroxanthin; But = 19'-butanoyloxyfucoxanthin; Fuco = Fucoxanthin; Hex = 19'-hexanoyloxyfucoxanthin; Viola = Violaxanthin; Dd = Diadinoxanthin; Morum = Morumxanthin; Diato = Diatoxanthin; Vaucher = Vaucheriaxanthin; Zea = Zeaxanthin; β, β-carotine.
* Morumxanthin = 3-0-acetyl-19'-0-hexanoyl Vaucheriaxanthin;** 3-acetate 19'-decanoate Vaucheriaxanthin.

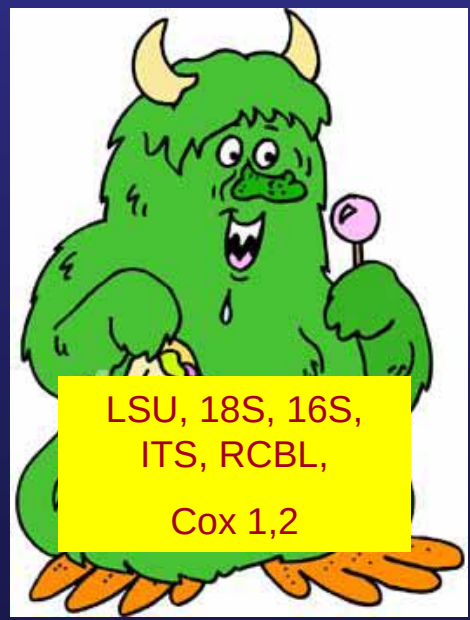
HPLC Carotenoid Pigment Comparison of three *Heterosigma akashiwo* clones





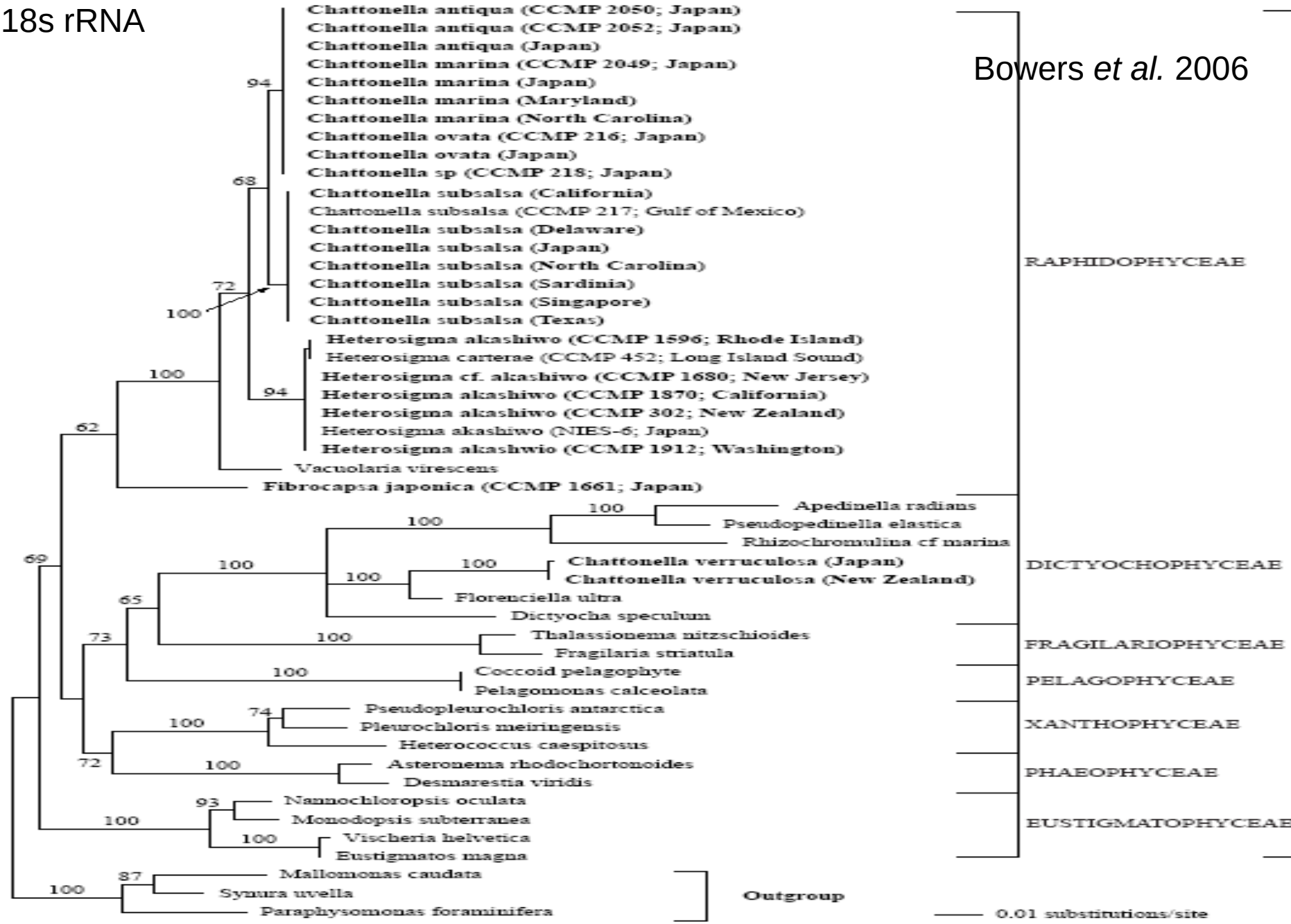
"green" *H. akashiwo/Charleston* **"golden brown" *Heterosigma*** **"green" *H. akashiwo/Maryland***

Candy’s dandy but isn’t molecular better?



Molecular Troll

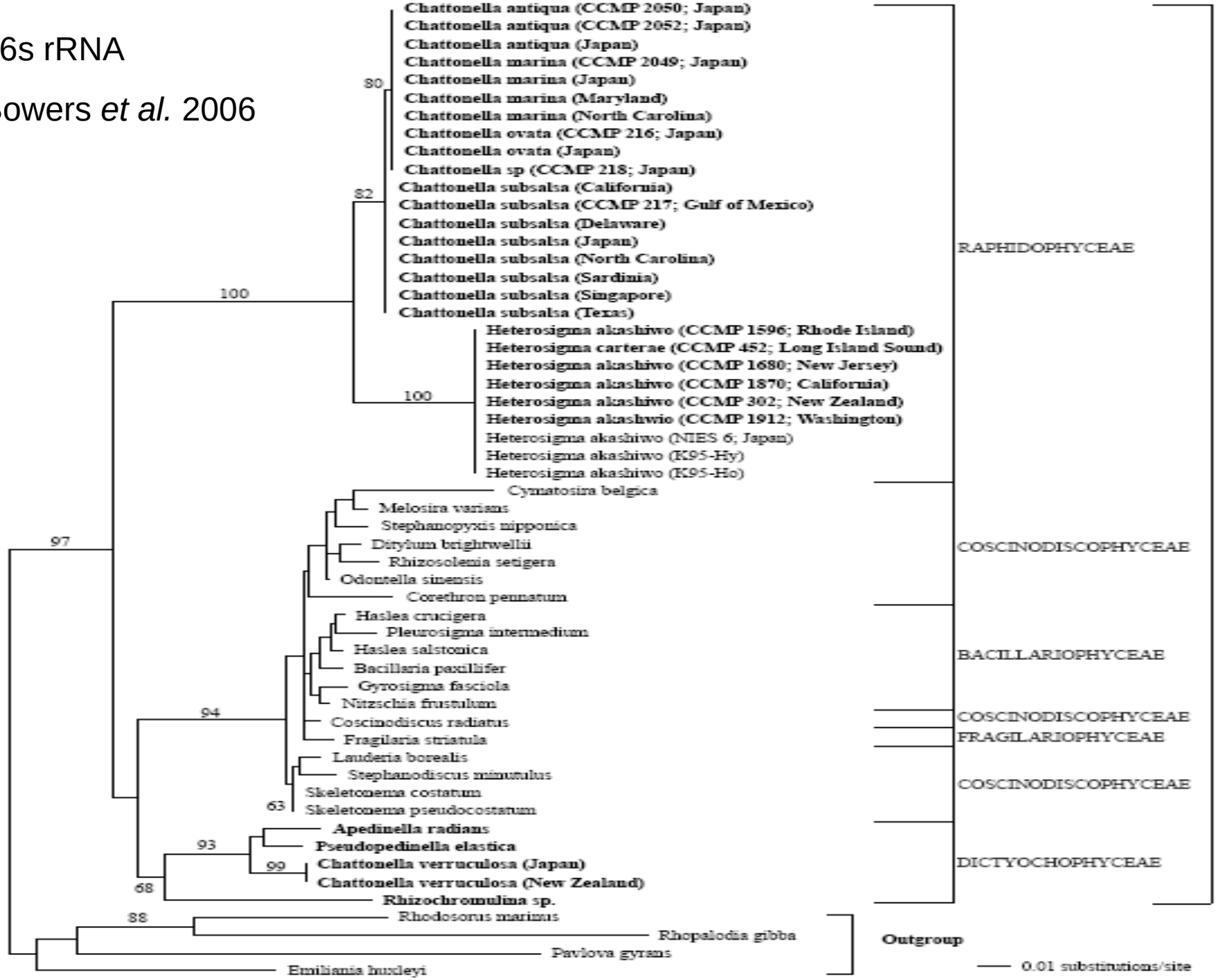
18s rRNA

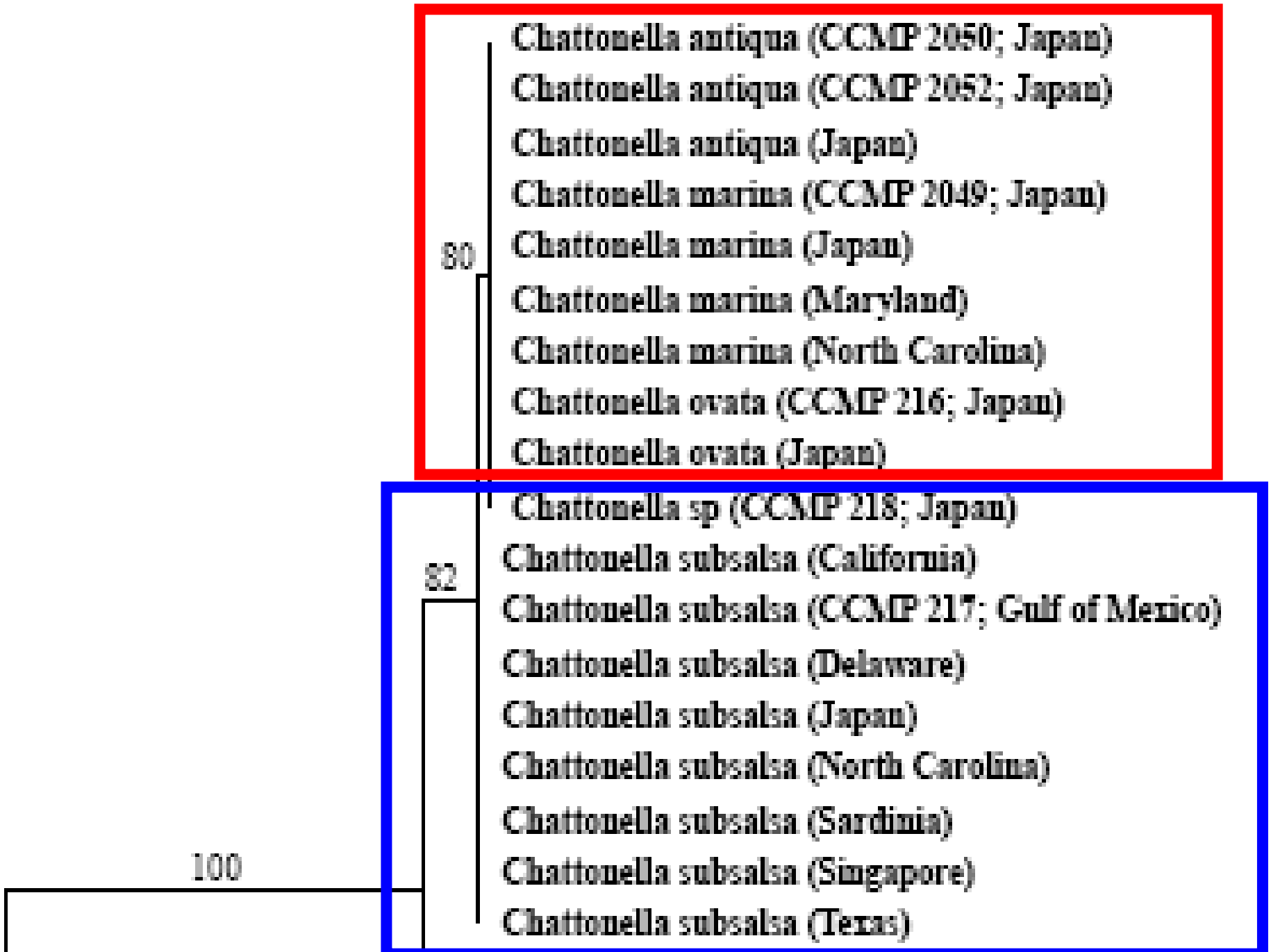


Bowers *et al.* 2006

16s rRNA

Bowers *et al.* 2006




Chattonella antiqua (CCMP 2050; Japan)
Chattonella antiqua (CCMP 2052; Japan)
Chattonella antiqua (Japan)
Chattonella marina (CCMP 2049; Japan)
Chattonella marina (Japan)
Chattonella marina (Maryland)
Chattonella marina (North Carolina)
Chattonella ovata (CCMP 216; Japan)
Chattonella ovata (Japan)
Chattonella sp (CCMP 218; Japan)
Chattonella subsalsa (Californuia)
Chattonella subsalsa (CCMP 217; Gulf of Mexico)
Chattonella subsalsa (Delaware)
Chattonella subsalsa (Japan)
Chattonella subsalsa (North Carolina)
Chattonella subsalsa (Sardinia)
Chattonella subsalsa (Singapore)
Chattonella subsalsa (Texas)
80
82
100

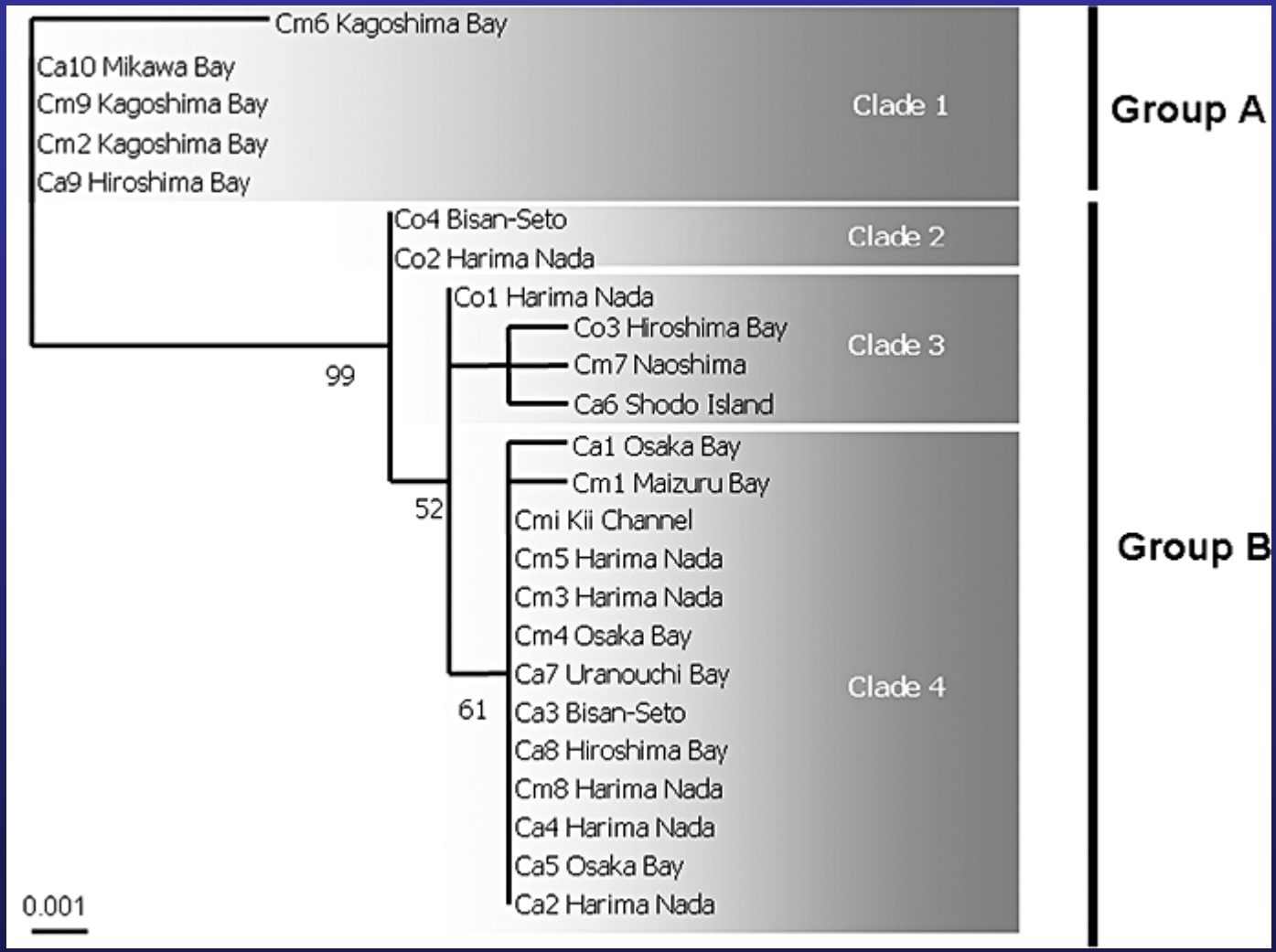
Genetic Analysis
Bowers *et al.* 2006

- "We sequenced the nuclear encoded 18s locus of a panel of raphidophyte cultures…Isolates of *C. antiqua*, *C. ovata*, *C. marina*, and *C.* sp. had 100% sequence similarity and shared 99% similarity to the *C. subsalsa* isolates."
- "We partially sequenced the plastid encoded 16S locus of several raphidophyte…cultures. The members of the *C. marina/antiqua/ovata/ sp.* complex were identical in the 16S plastid locus."

Genetic Analysis
Kamikawa *et al.* 2007

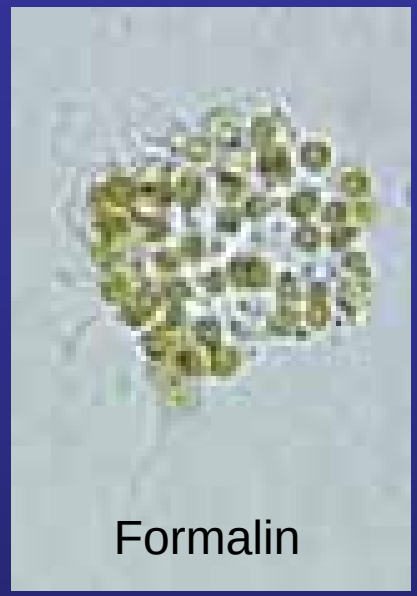
- "When all 24 sequences were compared, a total of 16 variant sites were detected in 939 bp of *cox2*-coding, *cox1*-coding, and intergenic regions…High variation in mtDNA was revealed, and sequence analysis of the mt genome data from *C. antiqua*, *C. marina*, [and] *C. ovata*… demonstrated considerable intraspecific divergence among the [three] species."

Mitochondrial Genetic Homology



KAMIKAWA, R, MASUDA, I, OYAMA, K, YOSHIMATSU, S & SAKO, Y Genetic variation in mitochondrial genes and intergenic spacer region in harmful algae *Chattonella* species. Fi*sheries Science* **73** (4), 871-880.

Preserved Samples – So who are they?



Take home message!!!!

Hint #1. Look at live cells!

Hint #2. Culture unknown cells if possible!

Raphidophyte – imposters

Olisthodiscus luteus ???? *Heterosigma akashiwo*????

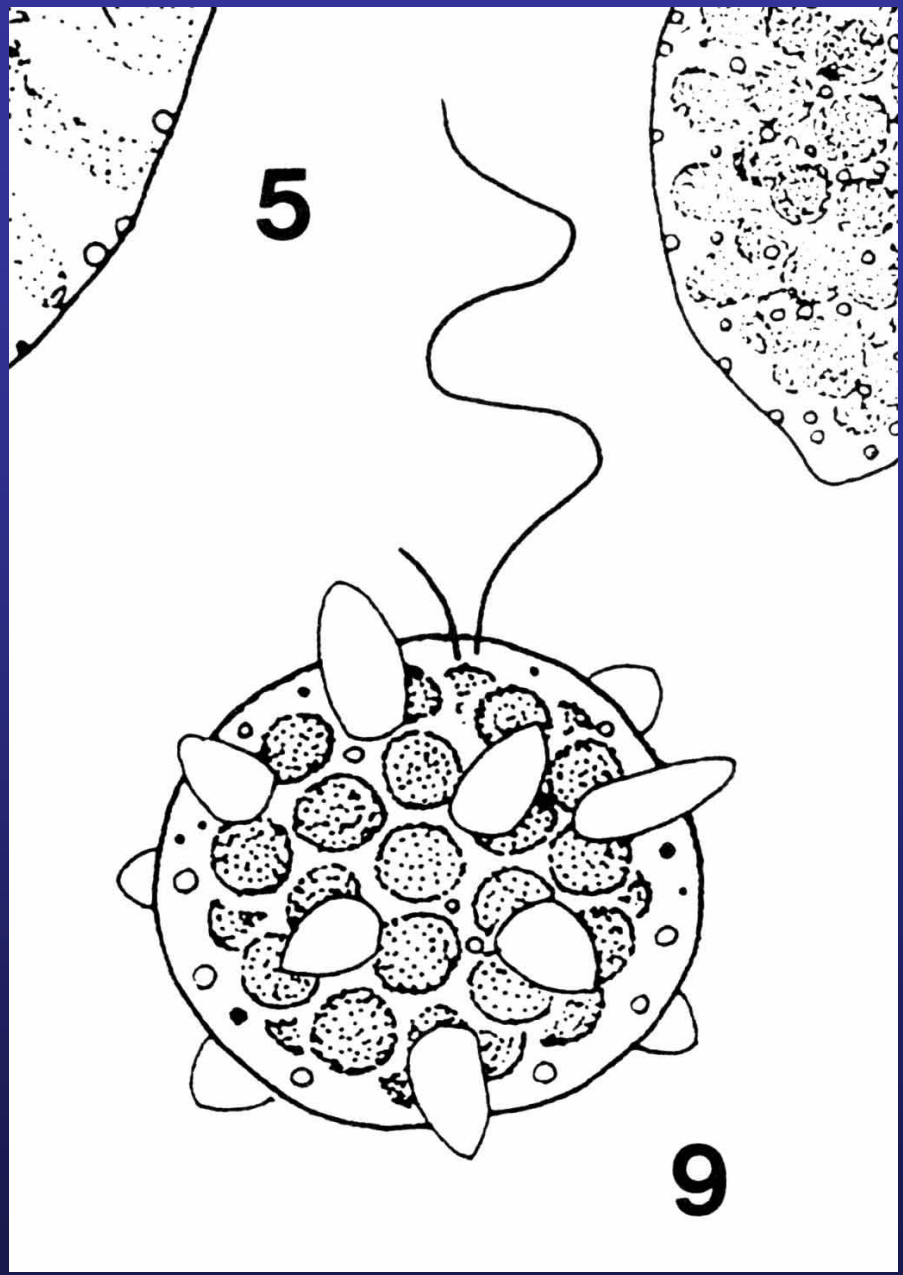
O. luteus = H. akashiwo in literature prior to 1984.

Chattonella verruculosa

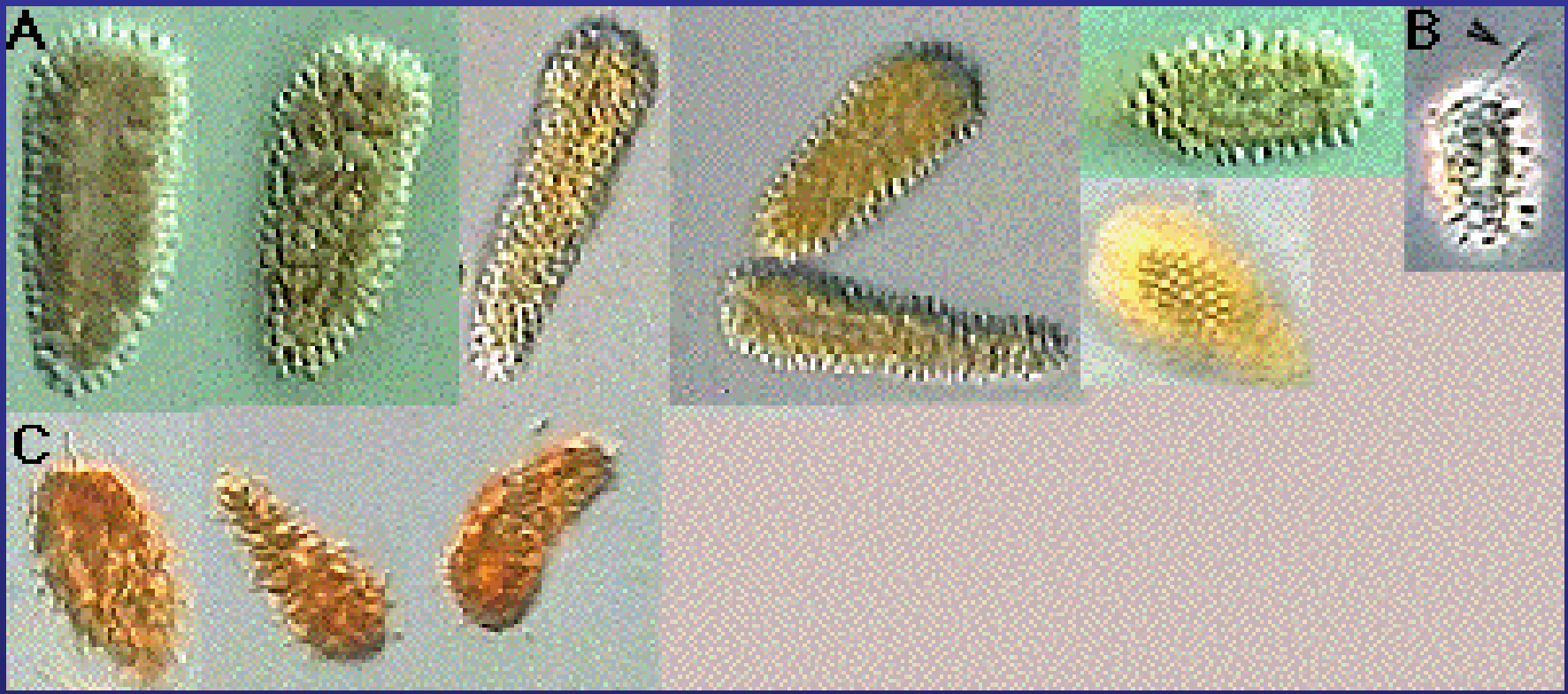
Japan – Pseudochattonella

Japan – Verrucophora verruculosa

North Sea – Verrucuphora farsimens



***Chattonella verruculosa* Hara et Chihara**
(spherical form)



Chattonella aff. verruculosa – Kattegat-Skagerrak area

Swedish, Norwegian Coastal Areas and North Sea

Photo's by Mats Kuylenstierna

Swedish Meterological and Hydrological Institute Reports Oceanography 32

***Chattonella aff. verruculosa* now redescribed as *Verrucophora farcimen* Eikrem, Edvardsen et Throndsen, gen et sp. nov.**

Chattonella verruculosa – Japan

Chattonella verruculosa – New Zealand

Chattonella aff. verruculosa – North Sea (Skagerrak)

} Dictyocophyceae

Chattonella cf. verruculosa – Delaware, USA (not C.v. – C. Tomas)

Recent publications:

Pseudochattonella verruculosa –

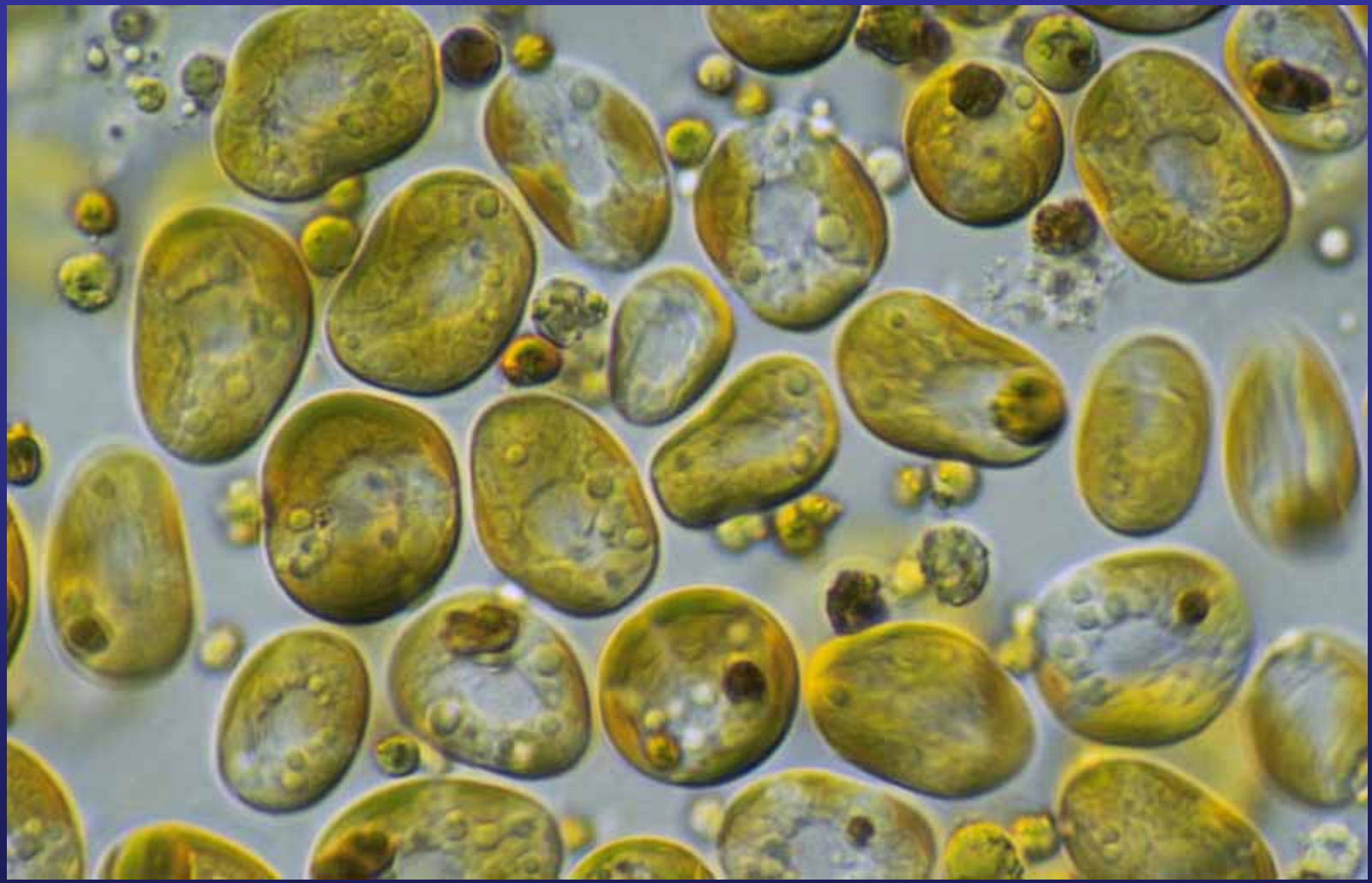
Hosoi-Tanabe, Shoko; Honda, Daiske; Fukaya, Sachiko; Otake, Isamu; Inagaki, Yuji; Sako, Yoshihiko. 2007. *Phycological Research.* 55:185-192(8)

Verrucophora farcimens

Verrucophora verruculosa

} **B. Edvardsen, W. Eikrem, K. Shalchian-Tabrizi, I. Riisberg, G. Johansen, L. Naustvoll and J. Throndsen, 2007. *Journal of Phycology.* 1054-1070.**

Olisthodiscus luteus



Olisthodiscus luteus – not a raphidophyte.

Bald Eagle Creek, Rehoboth Bay, Delaware
August 2000