



University of Naples "Federico II"

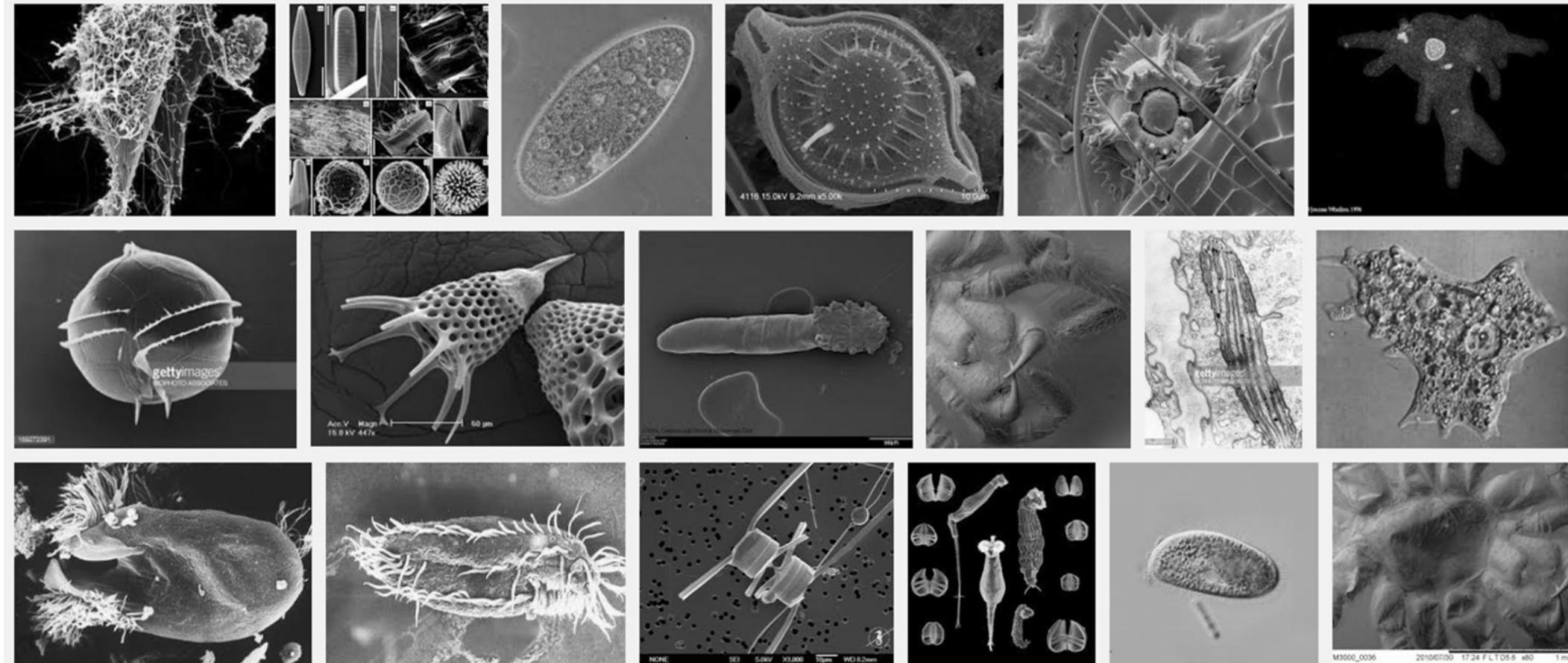
# Marine Microbial Diversity

aa 2020-2021

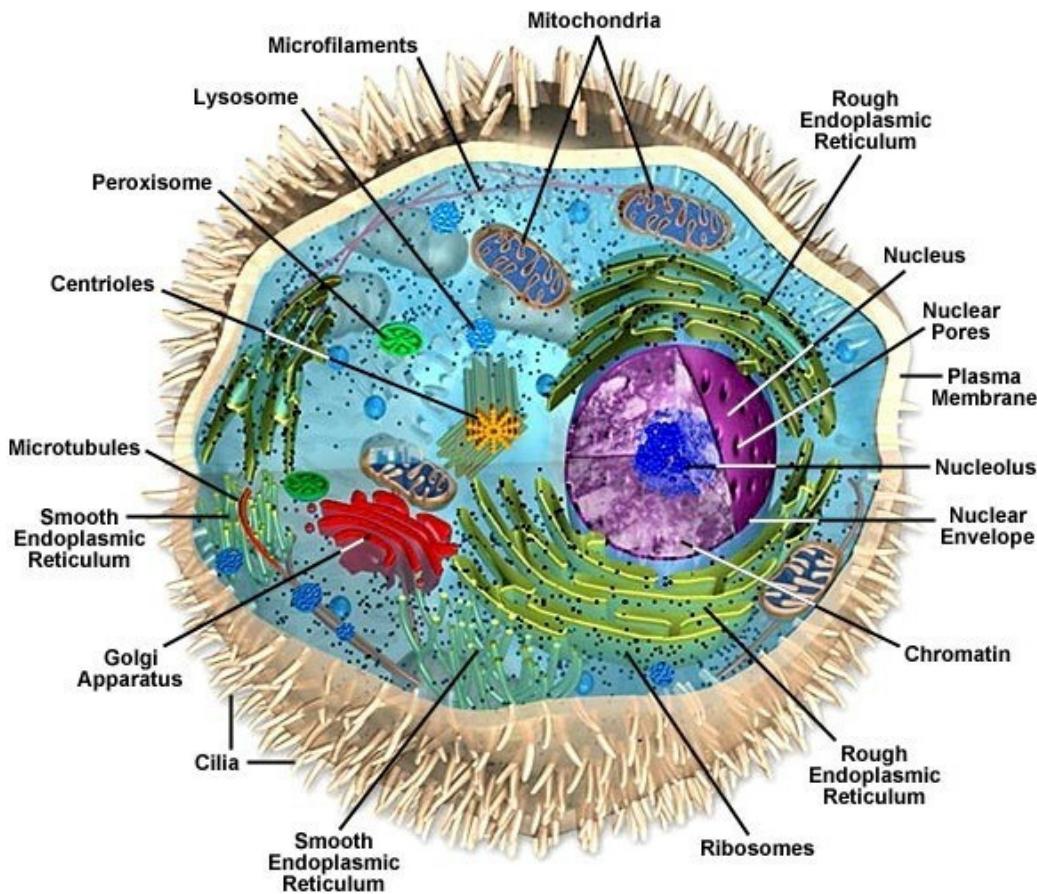
## Introduction 2 (aka why microbes?)

lecture 2

# Microbial Eukaryotes

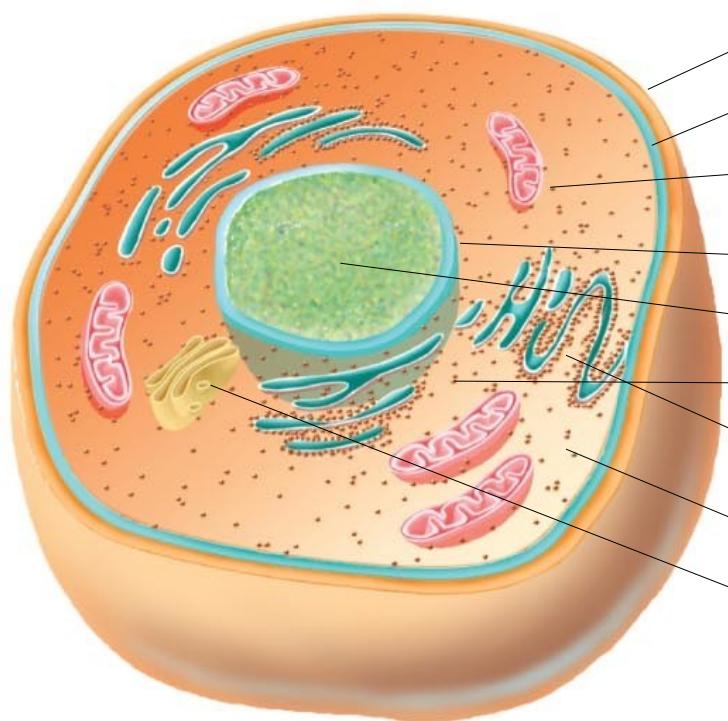


# Eukaryotic cell structures

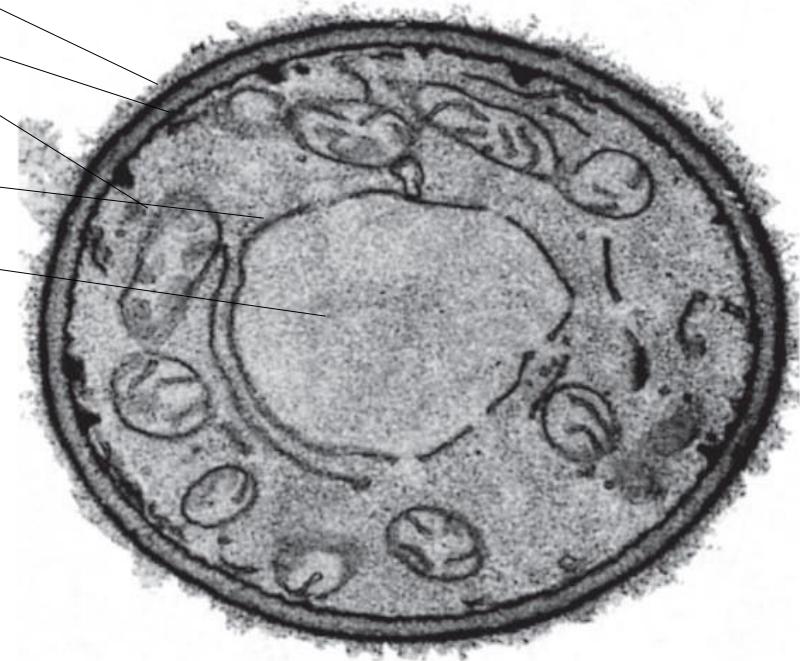


Compared with prokaryotic cells, microbial eukaryotes typically have structurally more complex and much larger cells

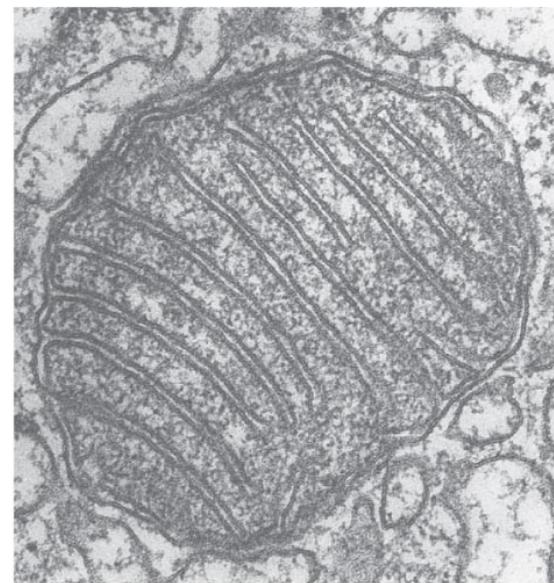
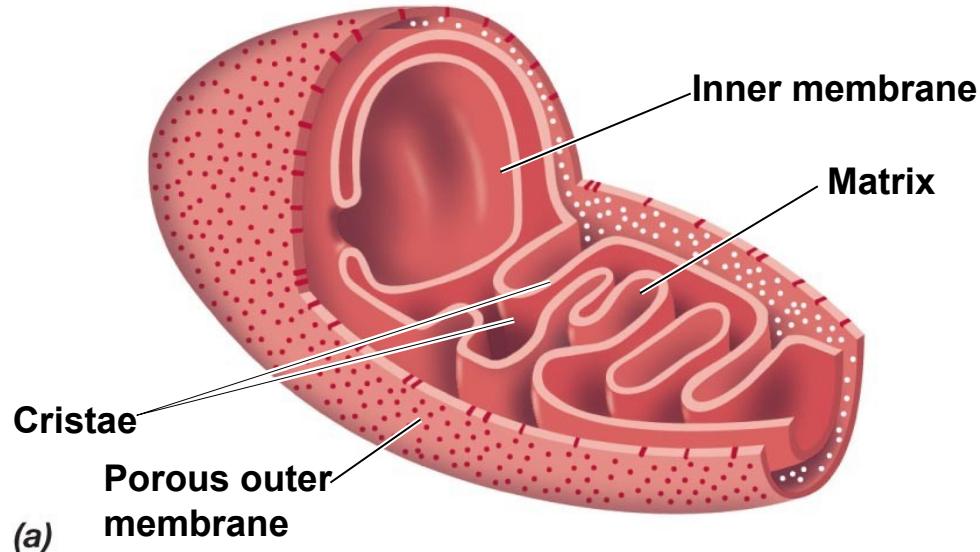
# Eukaryotic cell structures



(b)



# Mitocondria

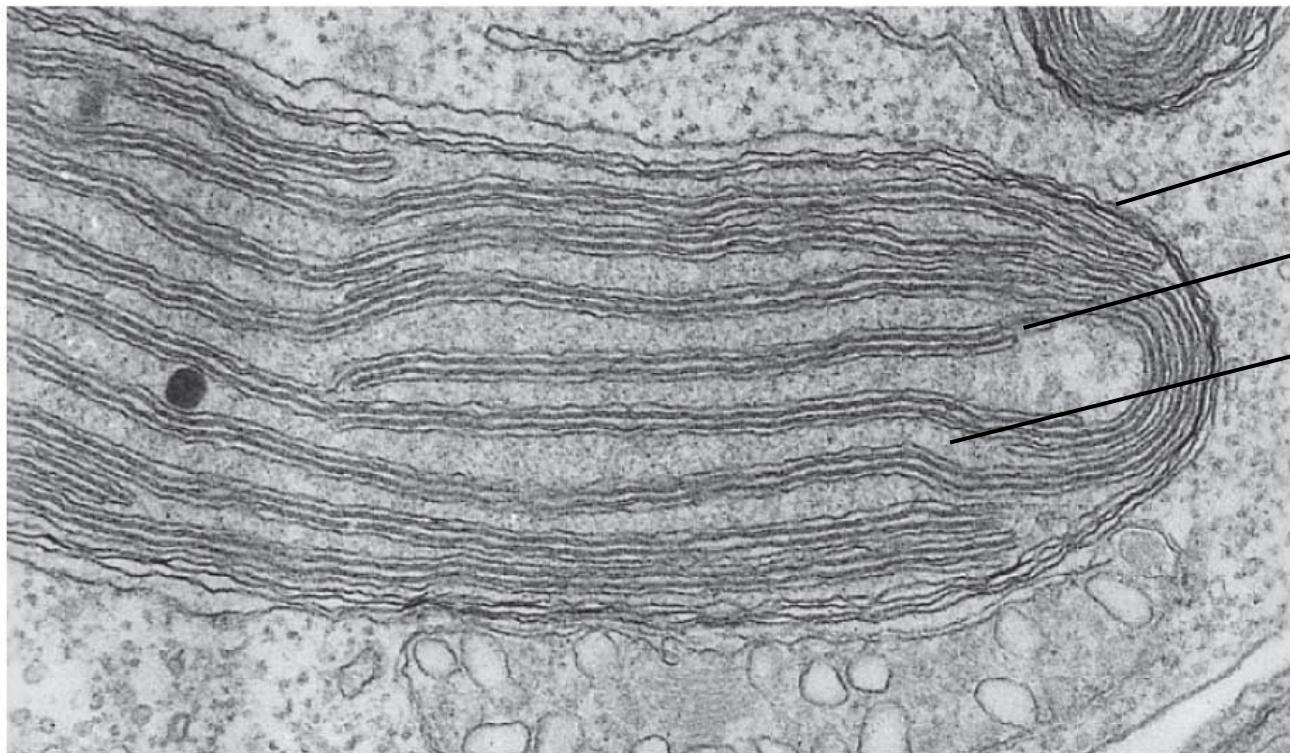


D. W. Fawcett



D. W. Fawcett

# Chloroplast

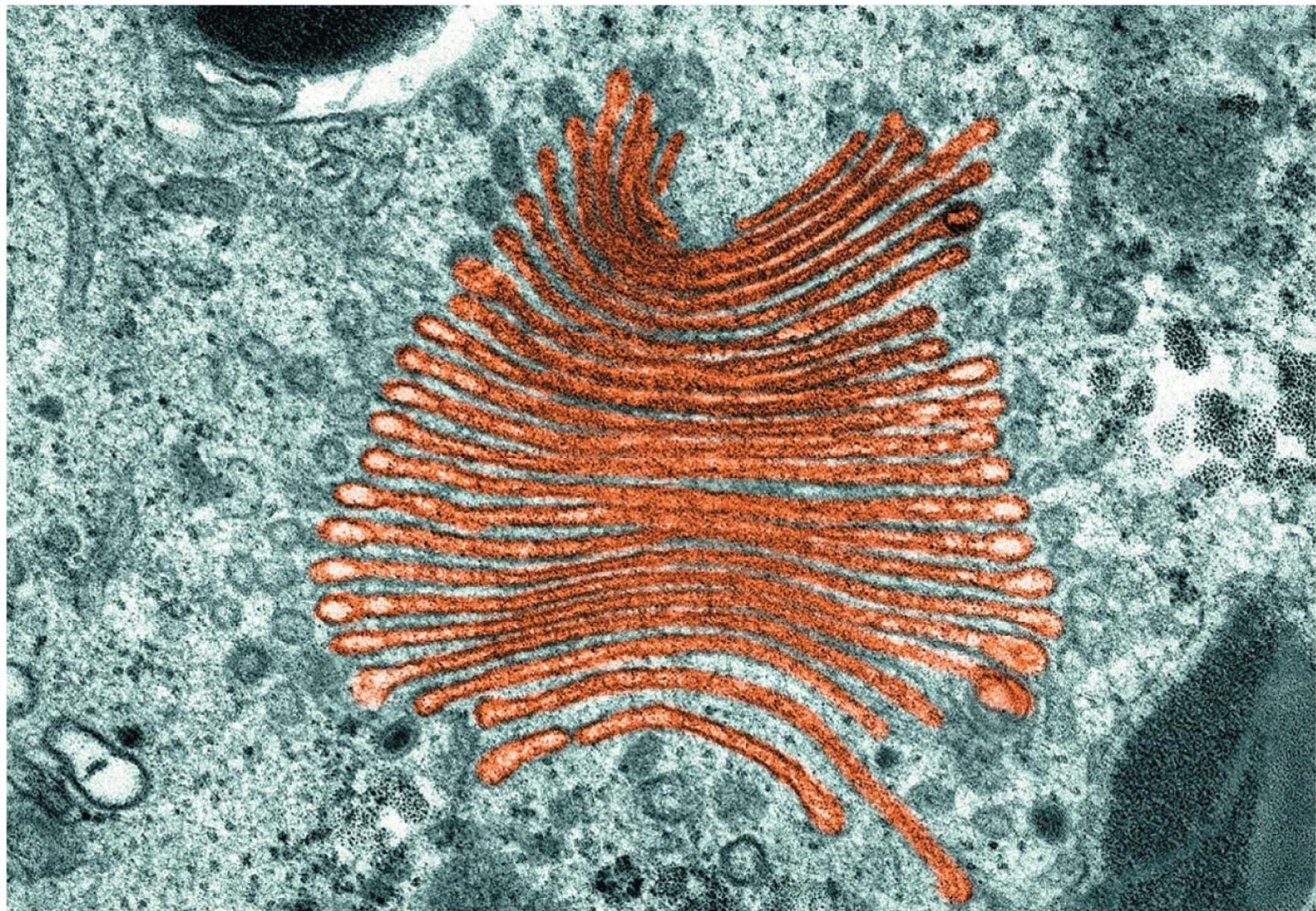


T. Slankis and S. Gibbs

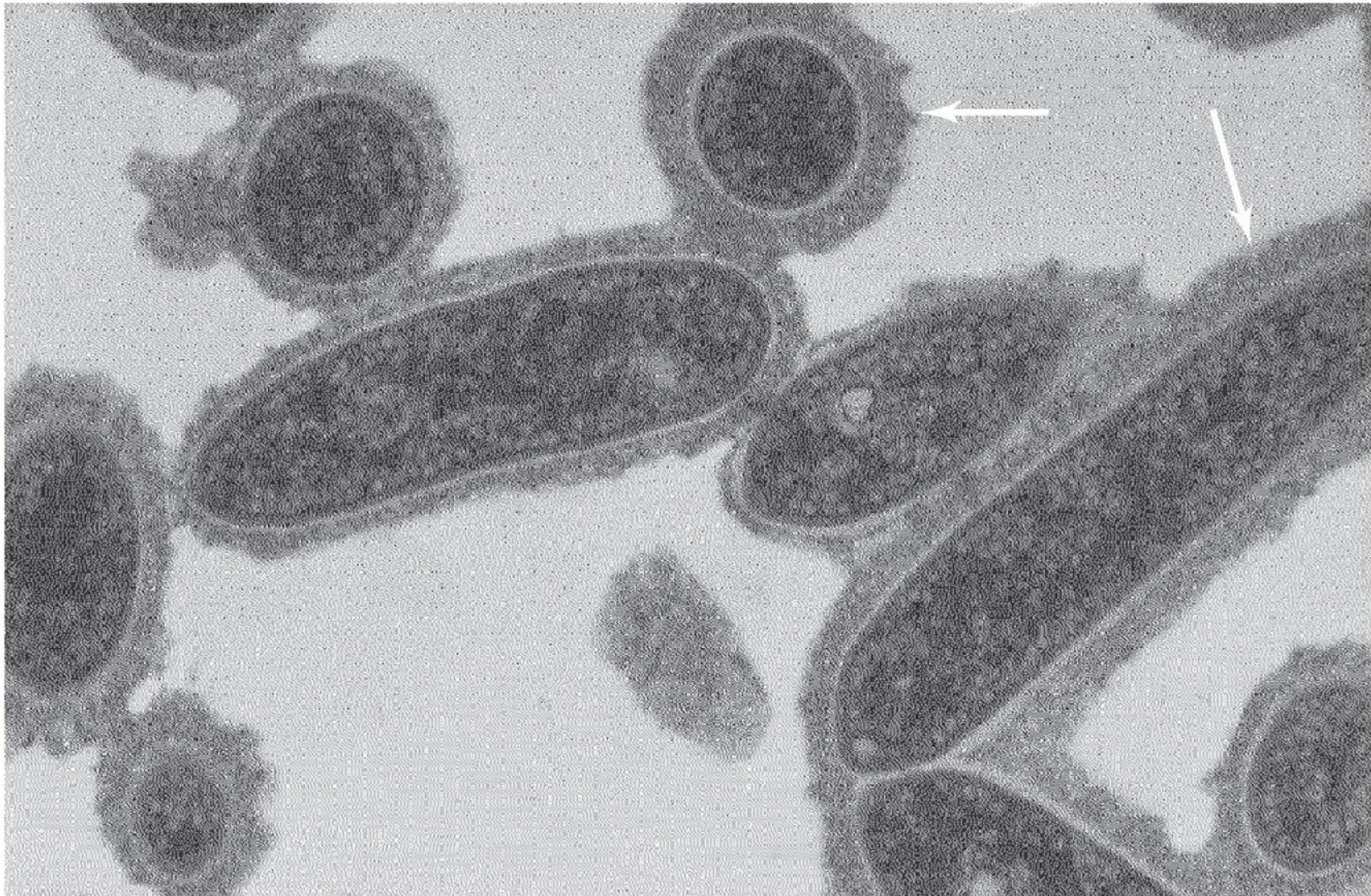
**Chloroplast**  
**Thylakoid**  
**Stroma**

(c)

# Golgi apparatus



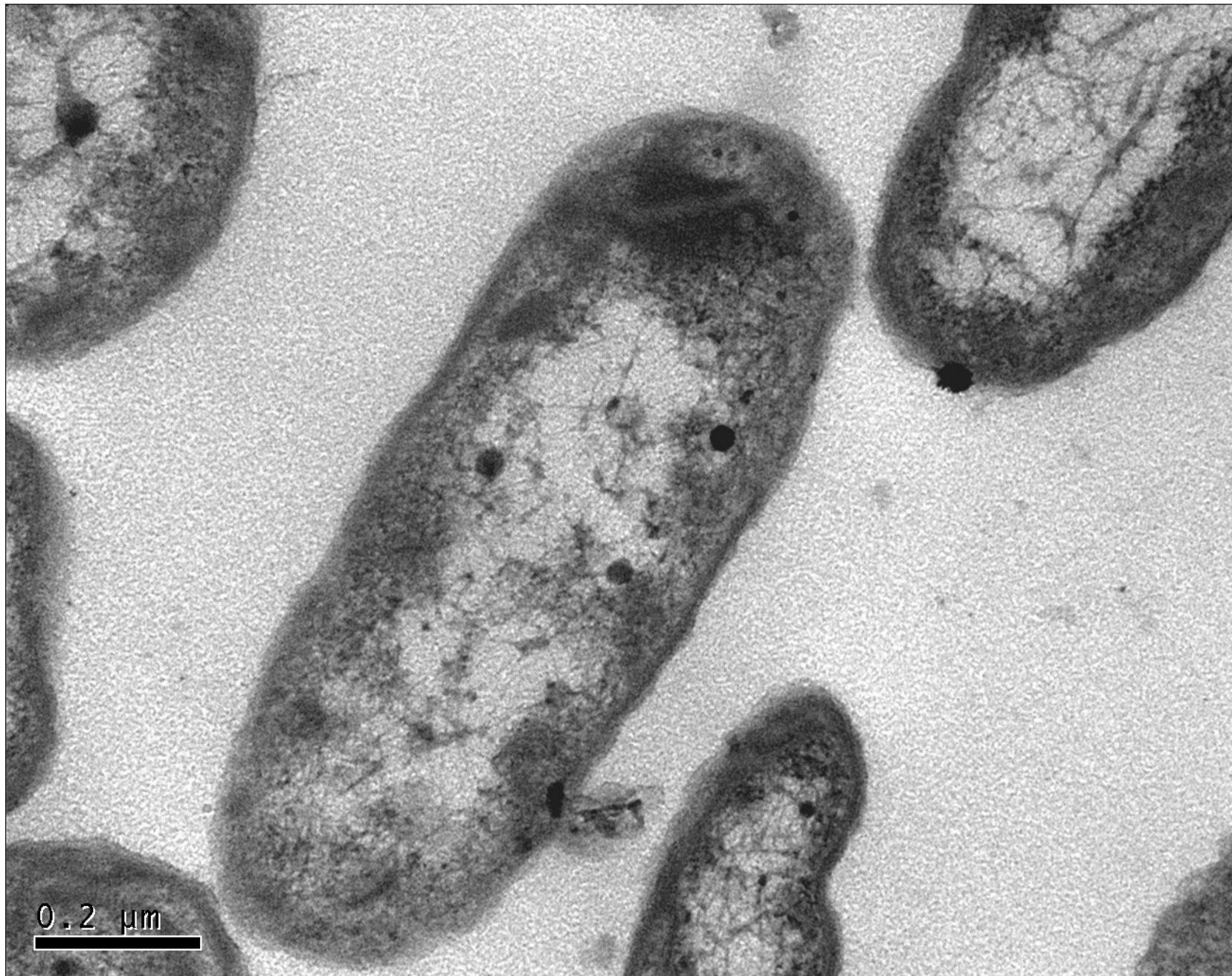
# Prokaryote or Eukaryote?



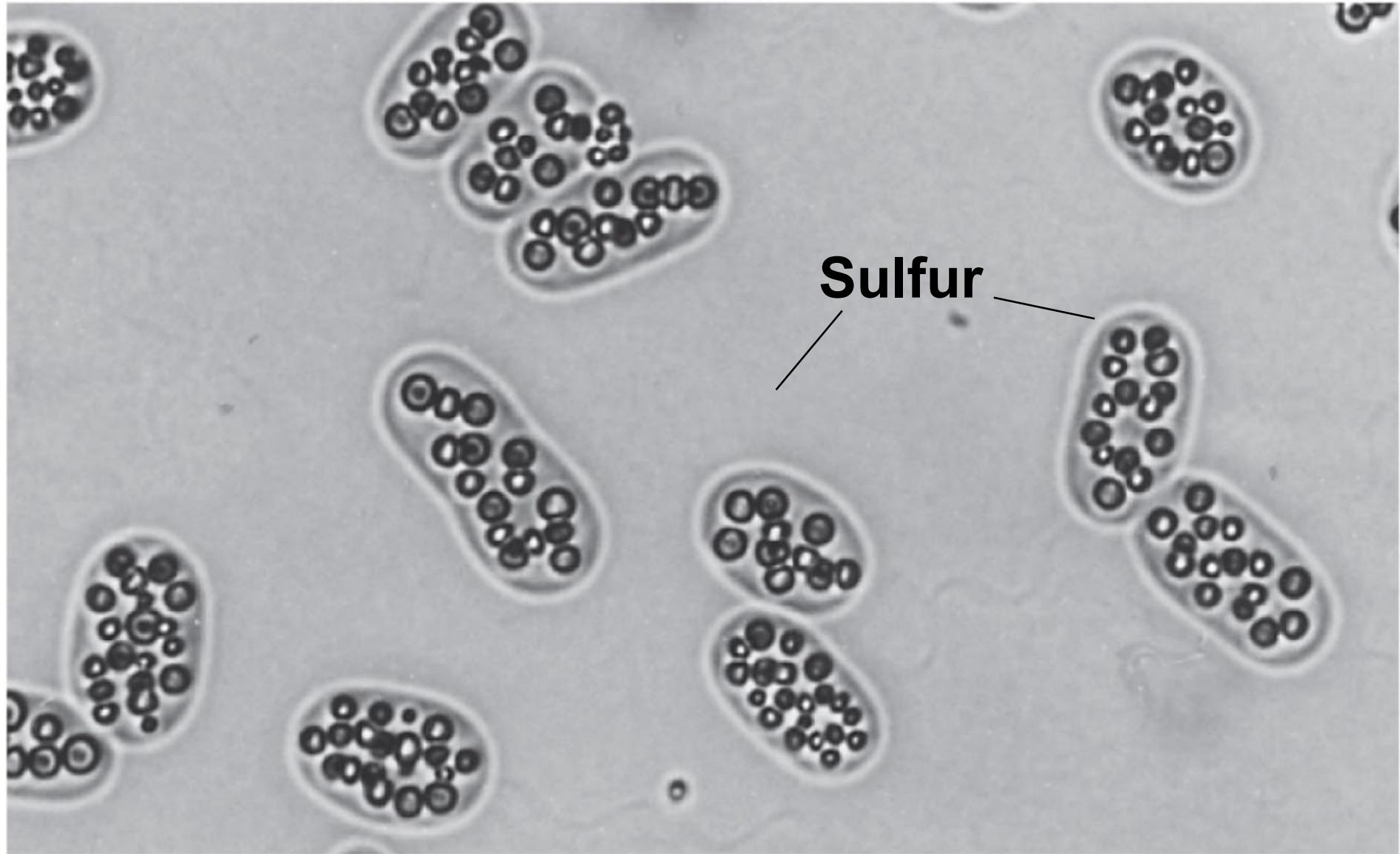
**(b)**

M.T. Madigan

# Prokaryote or Eukaryote?



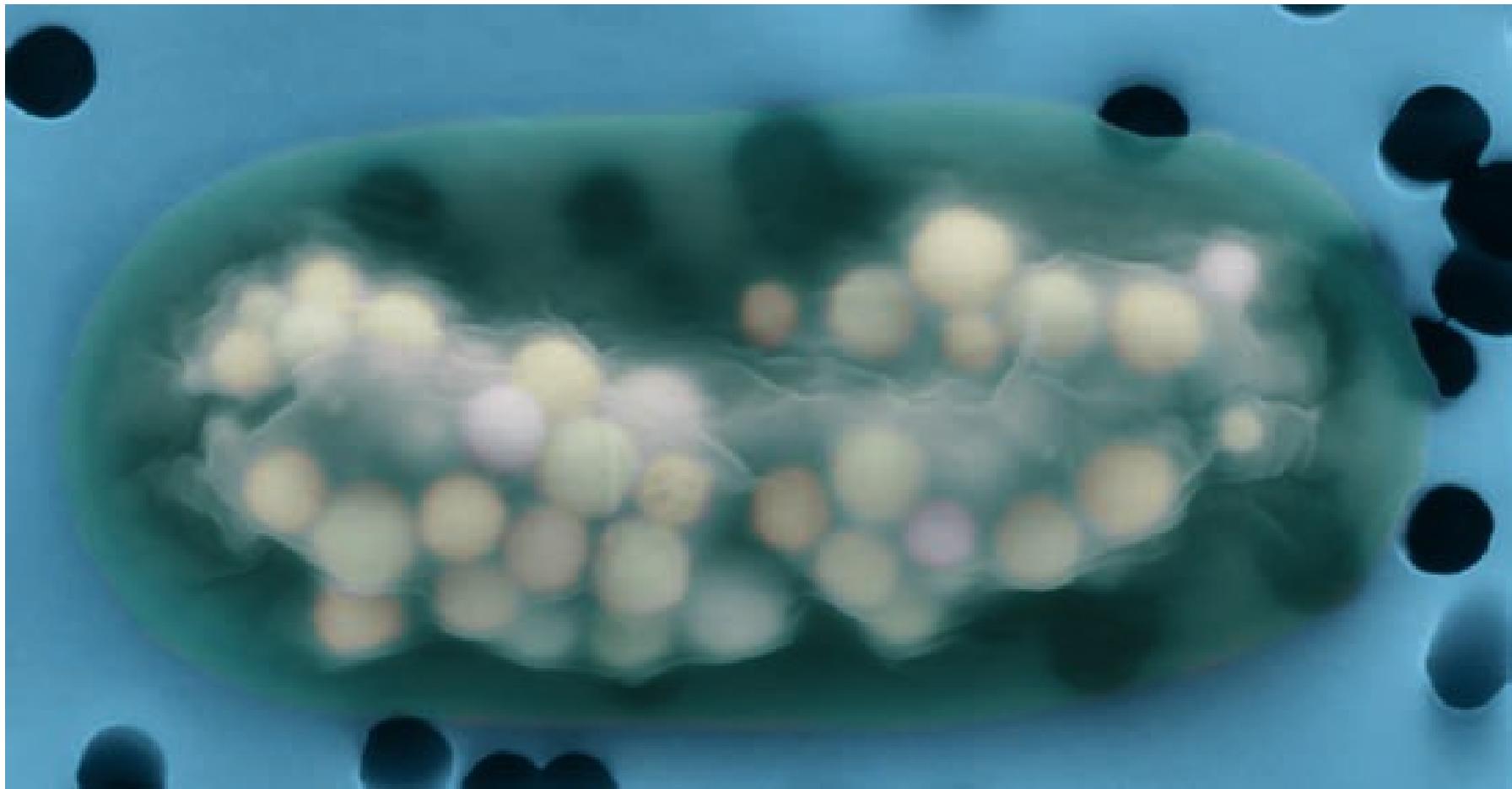
# Prokaryote or Eukaryote?



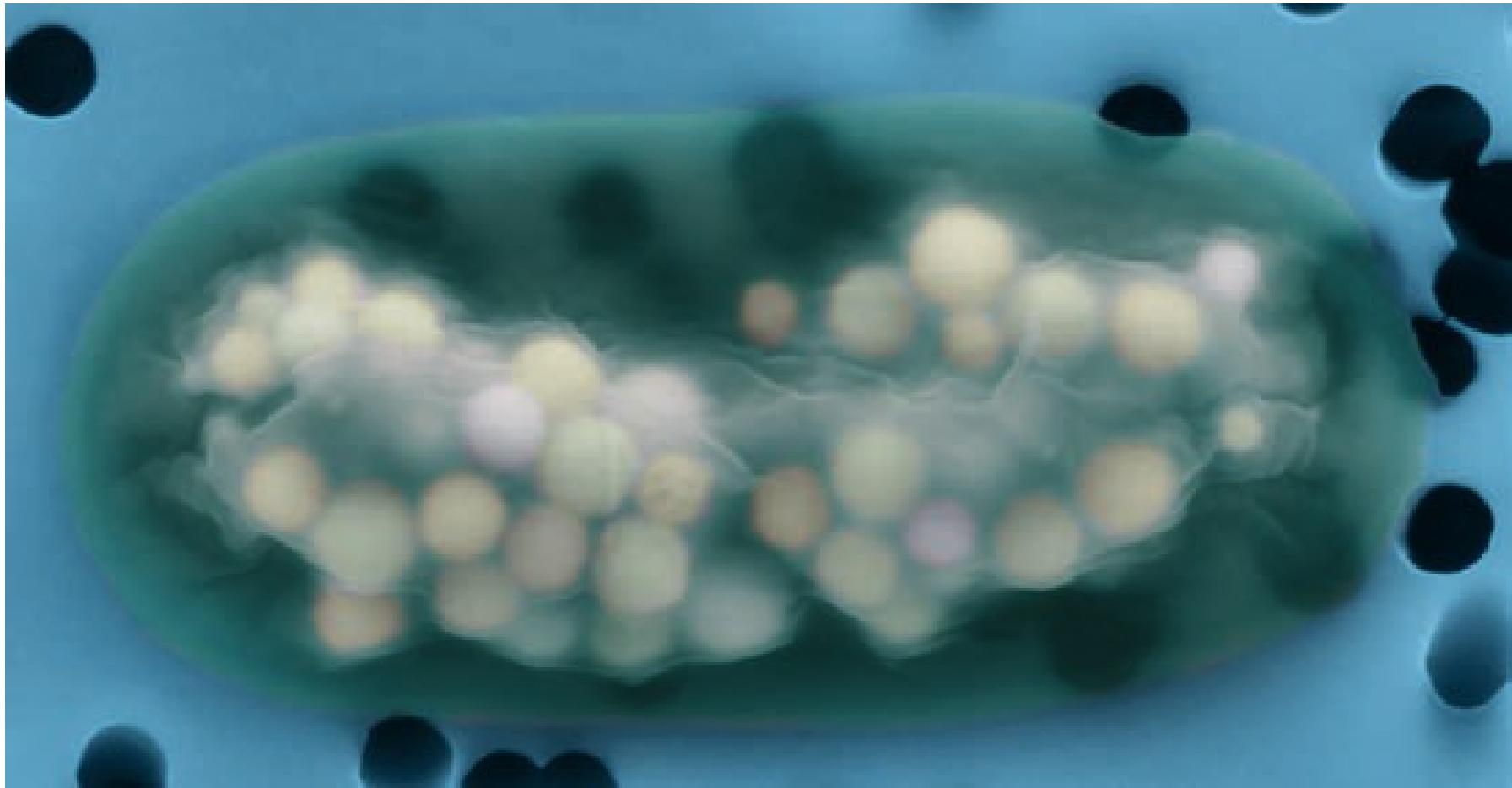
(b)

Norbert Pfennig

# Prokaryote or Eukaryote?

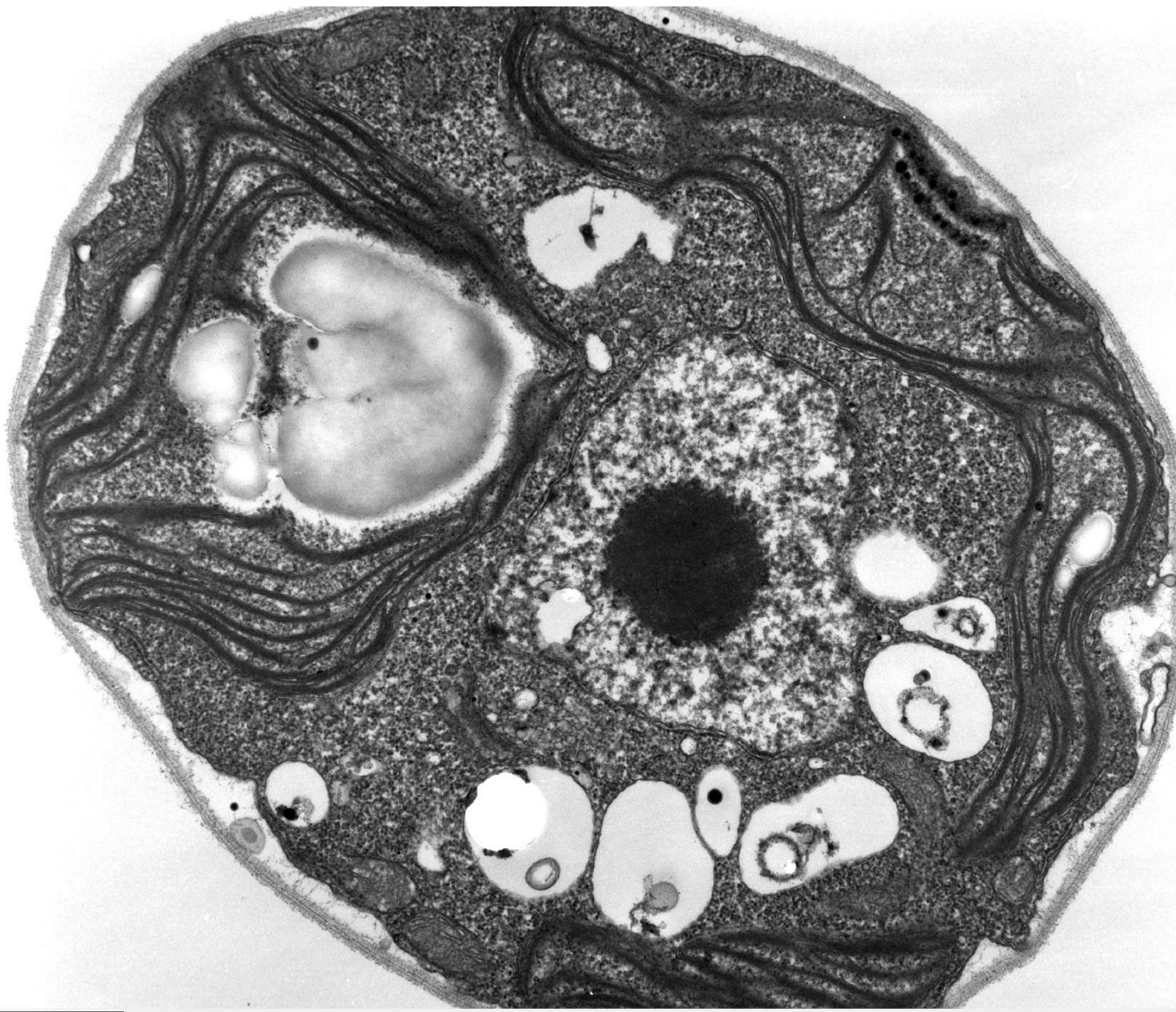


# Prokaryote or Eukaryote?

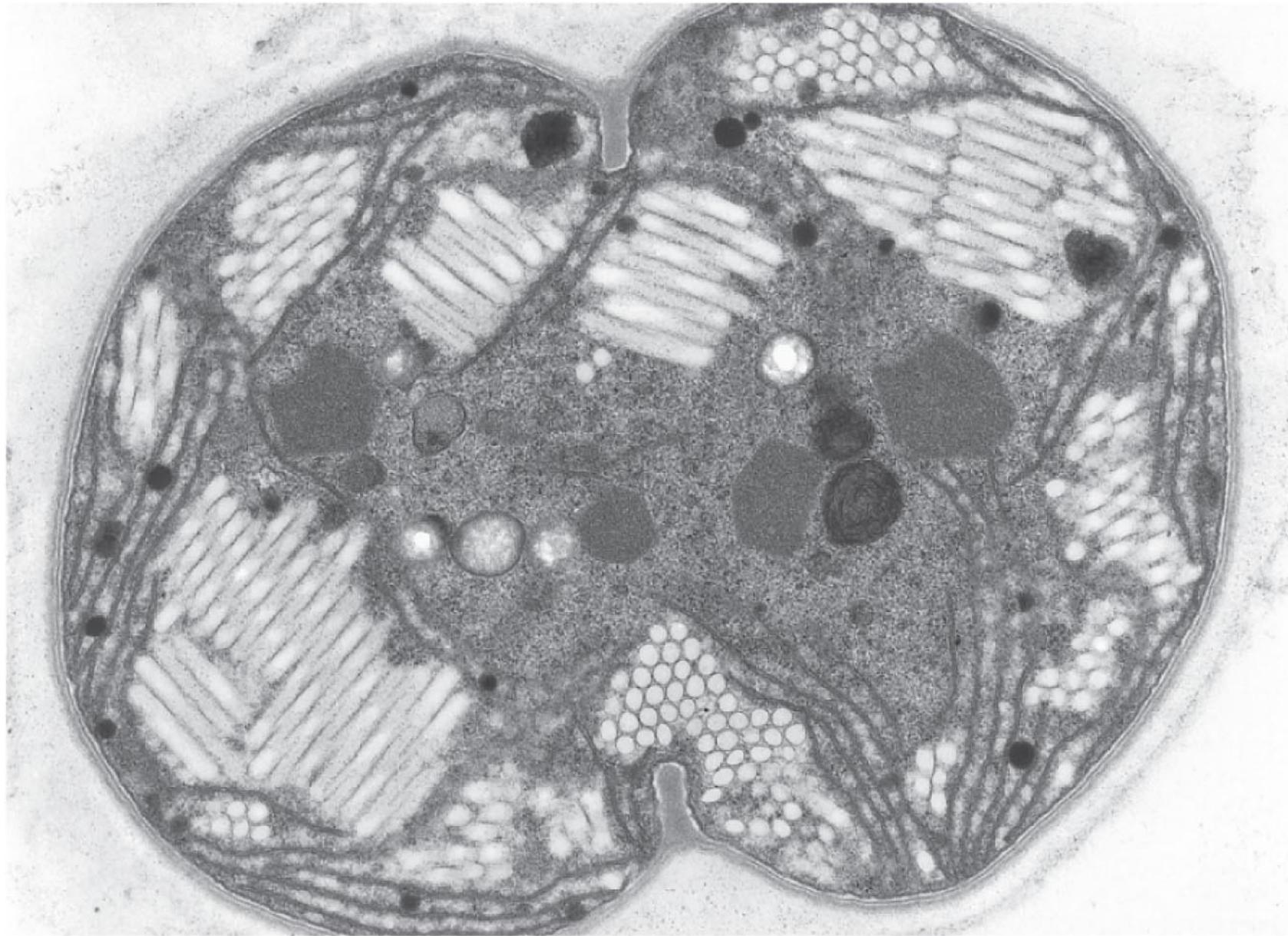


**Benstonite  $[(\text{Ba},\text{Sr},\text{Ca})_6\text{Mg}(\text{Co}_3)_{13}]$**

# Prokaryote or Eukaryote?



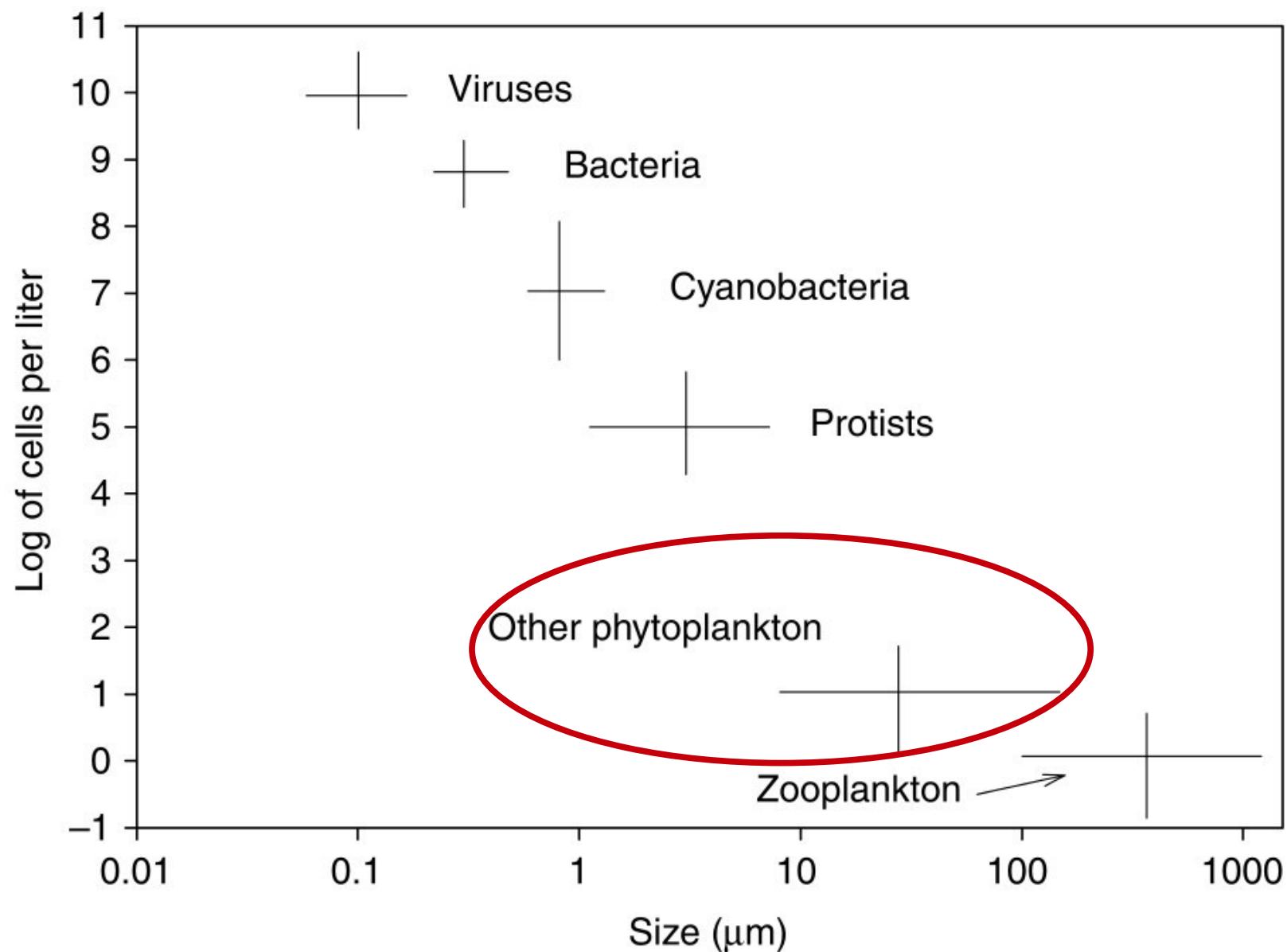
# Prokaryote or Eukaryote?



(b)

S. Pellegrini and M. Grilli Caiola

# Abundance and Size





# How did Eukaryotes form?

Symbiogenesis, or endosymbiotic theory (Mereschowsky 1910 and Lynn Margulis in 1967) is a theory on the evolution of eukaryotes.

Primary endosymbiosis is the process in which a eukaryote engulfs another living prokaryote.

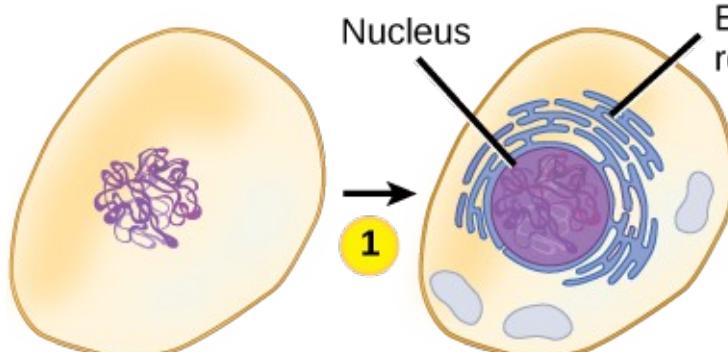
The mitochondria and plastids (for example chloroplasts), and possibly other organelles of eukaryote cells are thought to represent formerly free-living prokaryotes taken one inside the other in endosymbiosis around 1.5 billion years ago.

Molecular and biochemical evidence suggest that mitochondria developed from *Proteobacteria* (*Alphaproteobacteria*, like SAR11) and chloroplasts from *Cyanobacteria* (in particular, nitrogen-fixing filamentous cyanobacteria).

# Primary endosymbiosis

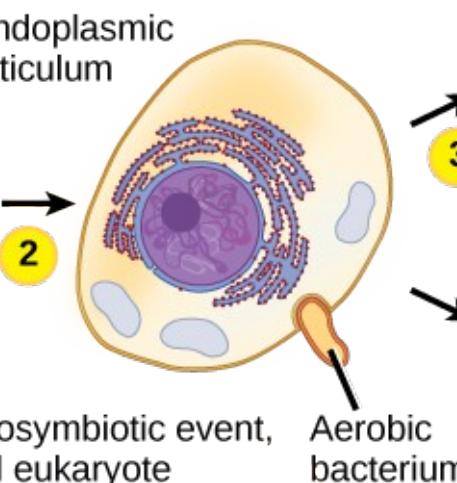
## The ENDOSYMBIOTIC THEORY

1 Infoldings in the plasma membrane of an ancestral prokaryote gave rise to endomembrane components, including a nucleus and endoplasmic reticulum.



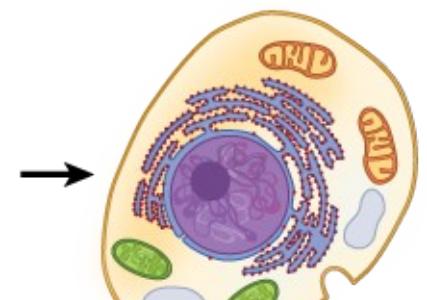
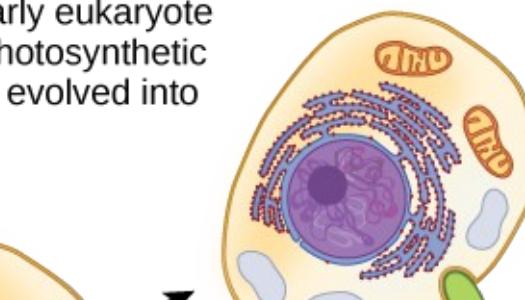
Proto-eukaryote

3 In a second endosymbiotic event, the early eukaryote consumed photosynthetic bacteria that evolved into chloroplasts.



Modern photosynthetic eukaryote

2 In a first endosymbiotic event, the ancestral eukaryote consumed aerobic bacteria that evolved into mitochondria.



Modern heterotrophic eukaryote

# Primary endosymbiosis: evidence

- Both mitochondria and chloroplasts can arise only from preexisting mitochondria and chloroplasts. They cannot be formed in a cell that lacks them because nuclear genes encode only some of the proteins of which they are made.
- Both mitochondria and chloroplasts have their own genome, and it resembles that of bacteria not that of the nuclear genome.
- Both genomes consist of a single circular molecule of DNA.
- There are no histones associated with the DNA.
- Both mitochondria and chloroplasts have their own protein-synthesizing machinery, and it more closely resembles that of bacteria than that found in the cytoplasm of eukaryotes, and are sensitive to a number of antibiotics (e.g. streptomycin, rifampicin).
- Genes for the functioning of both mitochondria and chloroplast are shared between their own genomes and the nuclear genomes.

Bacteria

Eukaryotes

Archaea

LUCA



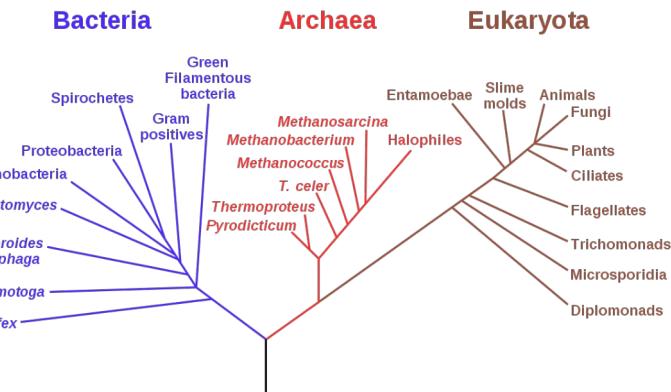
nature  
International journal of science

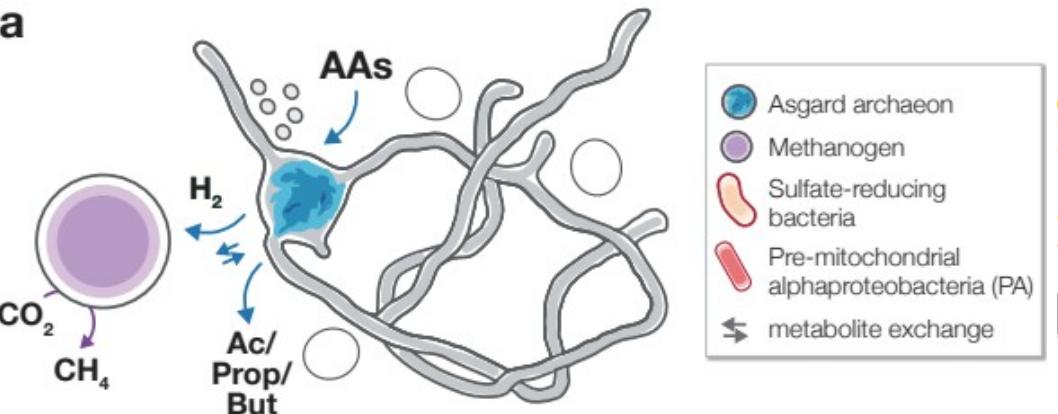
Article | Published: 06 May 2015

# Complex archaea that bridge the gap between prokaryotes and eukaryotes

Anja Spang, Jimmy H. Saw, Steffen L. Jørgensen, Katarzyna Zaremba-Niedzwiedzka, Joran Martijn,  
Anders E. Lind, Roel van Eijk, Christa Schleper, Lionel Guy & Thijs J. G. Ettema

Nature 521, 173–179 (14 May 2015) | Download Citation ↴



**a**

**Pre-G.O.E. (2.7 Ga~)**  
ox. of  $\text{S}^{2-}$  to  $\text{SO}_4^{2-}$

Loki/Thor/Hel/  
Odin ancestor

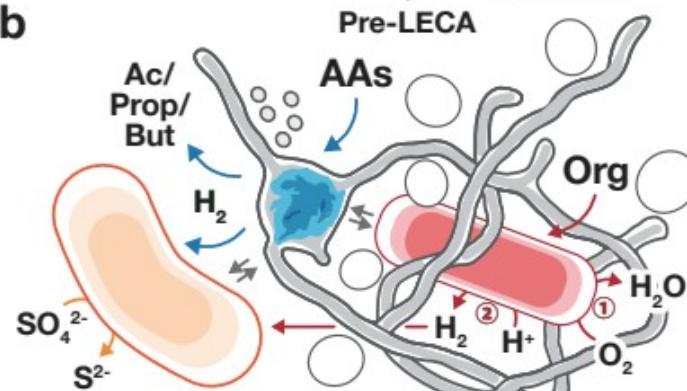
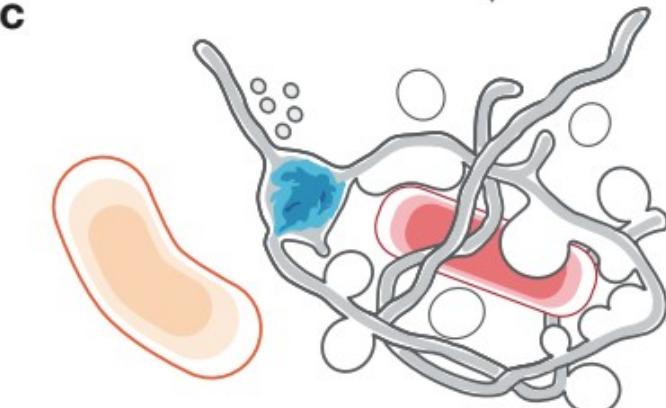
Heimdall-Eukarya  
ancestor

Heimdall  
ancestor

Pre-LECA

High organics &  $\text{SO}_4^{2-}$   
availability at sediment  
surface, but aero-  
tolerance necessary  
=

Parallel interaction  
with SRB and  
pre-mitochondrial  
alphaproteobacterium  
(PA)

**b****c**

Transition towards a  
more aerobic lifestyle  
requires heavier  
interaction with PA  
=

Protrusion-vesicle  
fusion to increase  
intimacy

## Isolation of an archaeon at the prokaryote-eukaryote interface

Hiroyuki Imachi, Masaru K. Nobu, Nozomi Nakahara, Yuki Morono, Miyuki Ogawara, Yoshihiro Takaki, Yoshinori Takano, Katsuyuki Uematsu, Tetsuro Ikuta, Motoo Ito, Yohei Matsui, Masayuki Miyazaki, Kazuyoshi Murata, Yumi Saito, Sanae Sakai, Chihong Song, Eiji Tasumi, Yuko Yamanaka, Takashi Yamaguchi, Yoichi Kamagata, Hideyuki Tamaki, Ken Takai  
doi: <https://doi.org/10.1101/726976>

This article is a preprint and has not been certified by peer review [what does this mean?].

Abstract

Full Text

Info/History

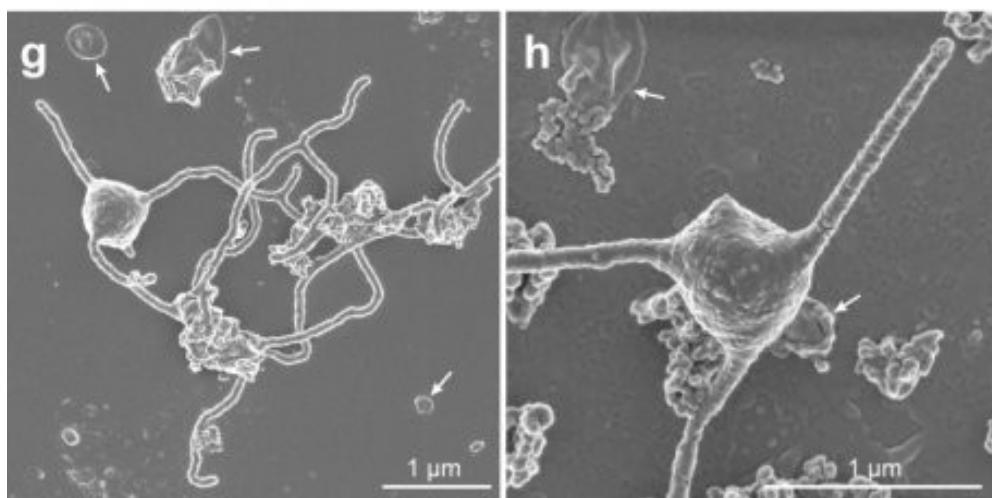
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Preview PDF

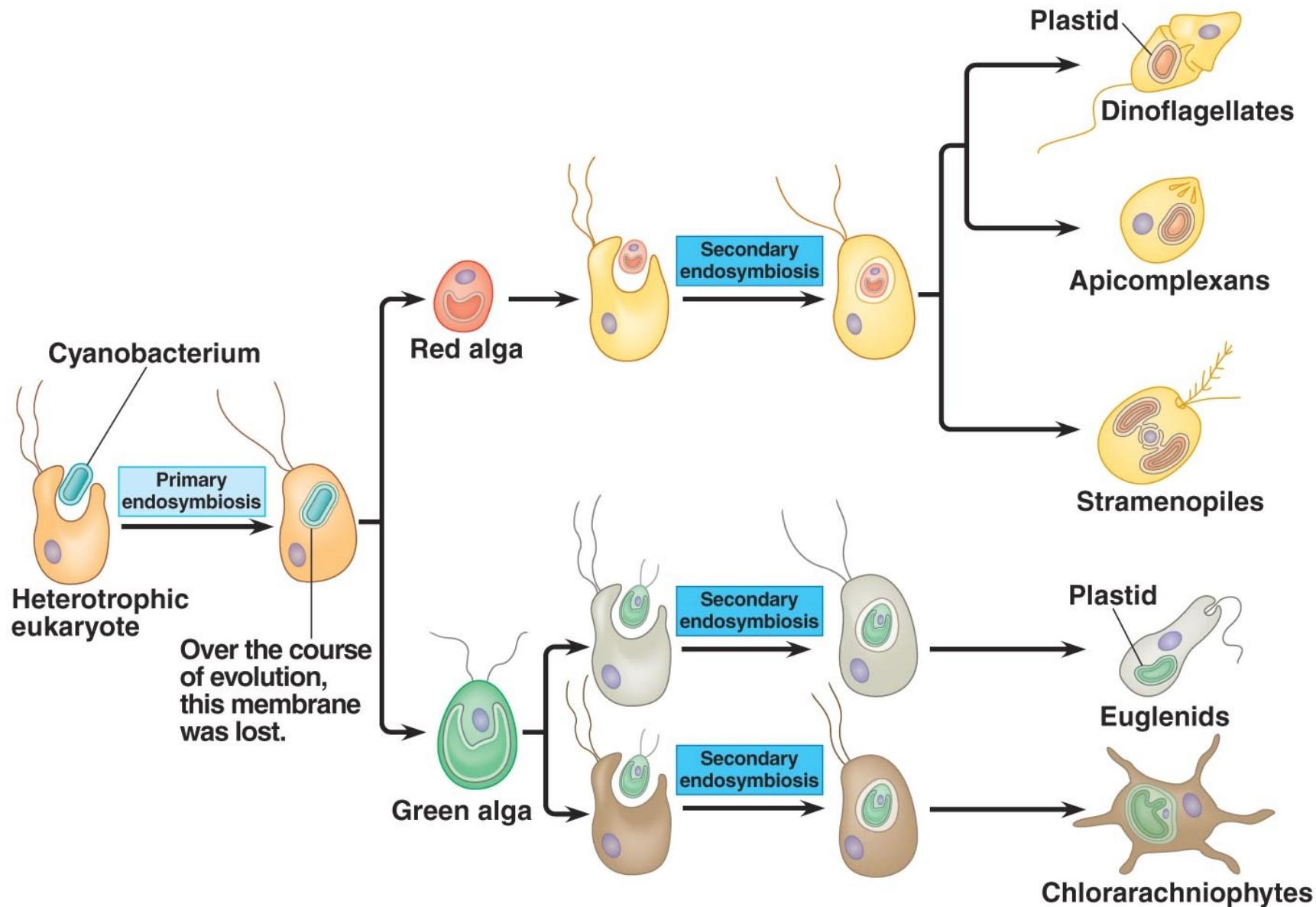
### Abstract

The origin of eukaryotes remains enigmatic. Current data suggests that eukaryotes may have risen from an archaeal lineage known as “Asgard archaea”. Despite the eukaryote-like genomic features found in these archaea, the evolutionary transition from archaea to eukaryotes remains unclear due to the lack of cultured representatives and corresponding physiological insight.

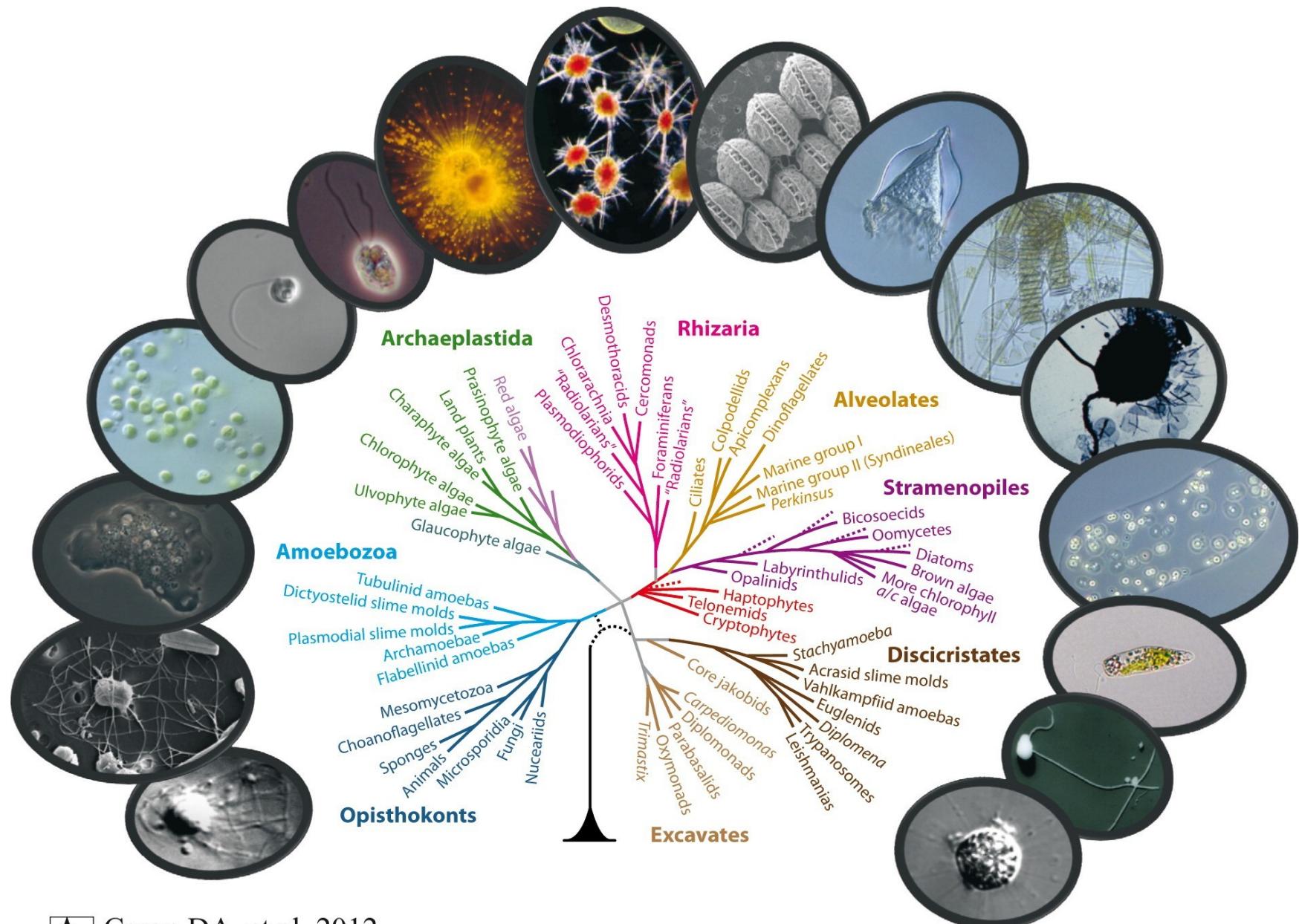
Here we report the decade-long isolation of a Lokiarchaeota-related Asgard archaeon from deep marine sediment. The archaeon, *Candidatus Prometheoarchaeum syntrophicum* strain MK-D1, is an anaerobic, extremely slow-growing, small cocci (~550 nm), that degrades amino acids through syntrophy. Although eukaryote-like intracellular complexities have been proposed for Asgard archaea, the isolate has no visible organella-like structure. *Ca. P. syntrophicum* instead displays morphological complexity – unique long, and often, branching protrusions. Based on cultivation and genomics, we propose an “Entangle-Engulf-Enslave (E<sup>3</sup>) model” for eukaryogenesis through archaea-alphaproteobacteria symbiosis mediated by the physical complexities and metabolic dependency of the hosting archaeon.



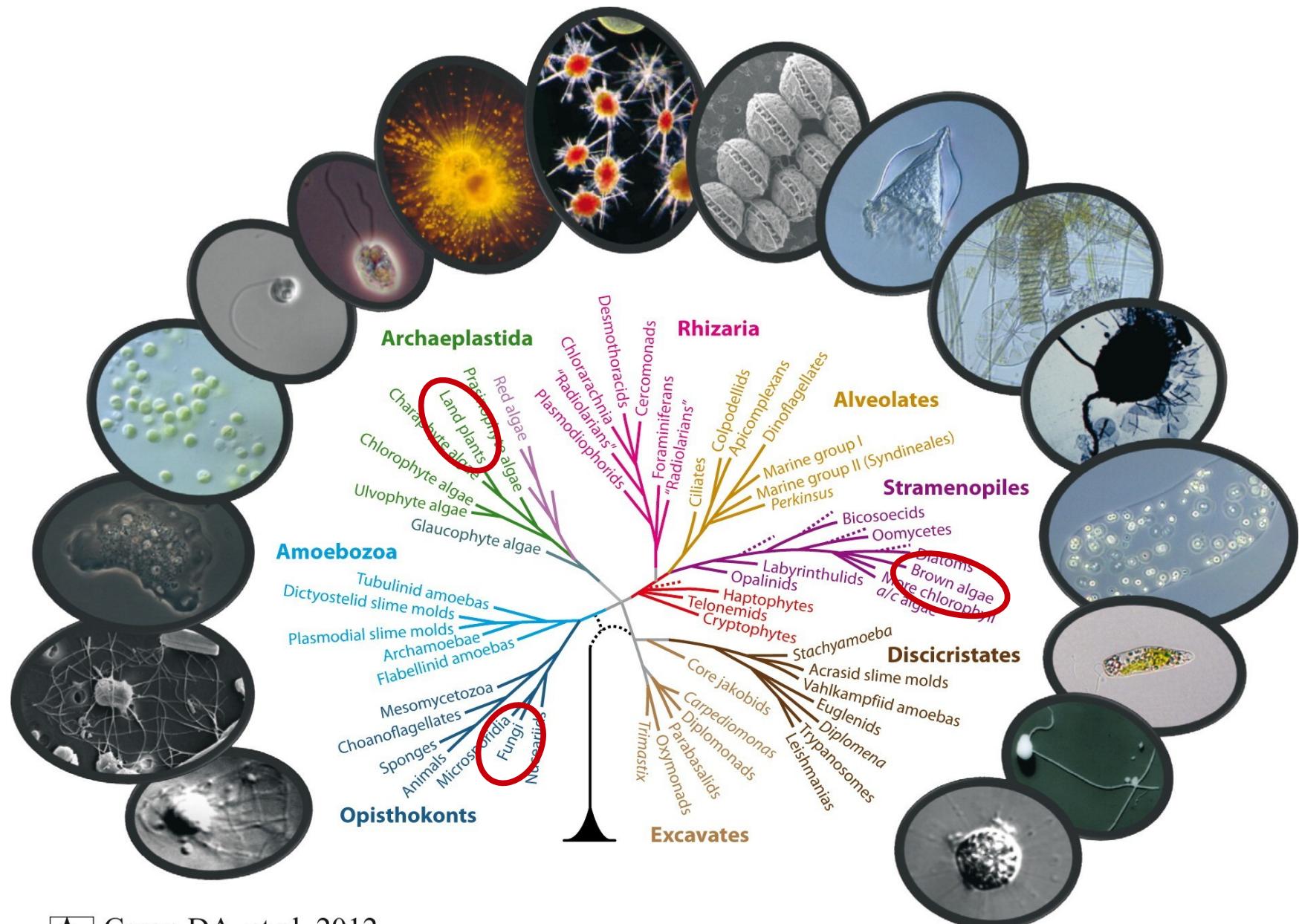
# Secondary endosymbiosis



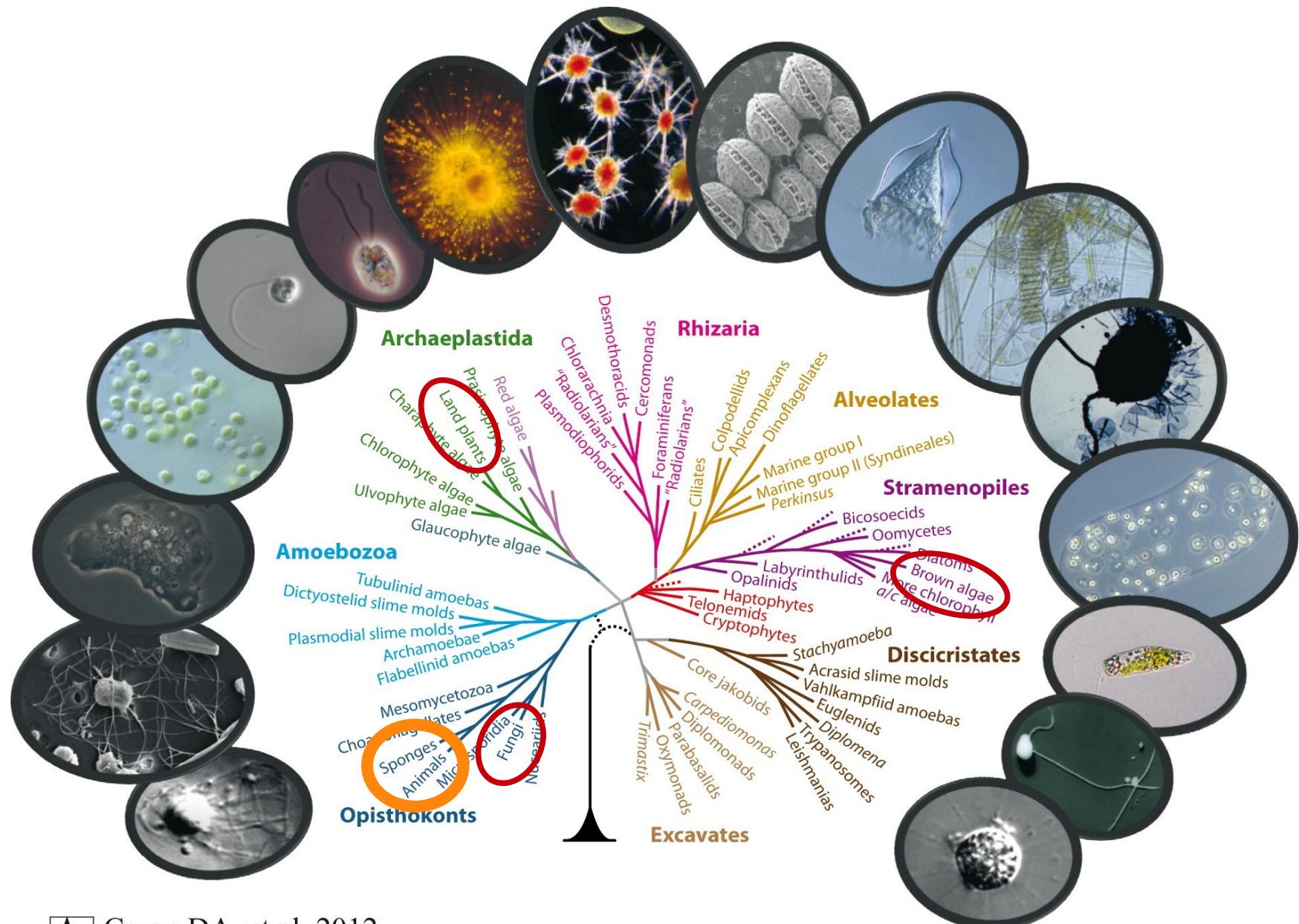
# Protist



# Protist



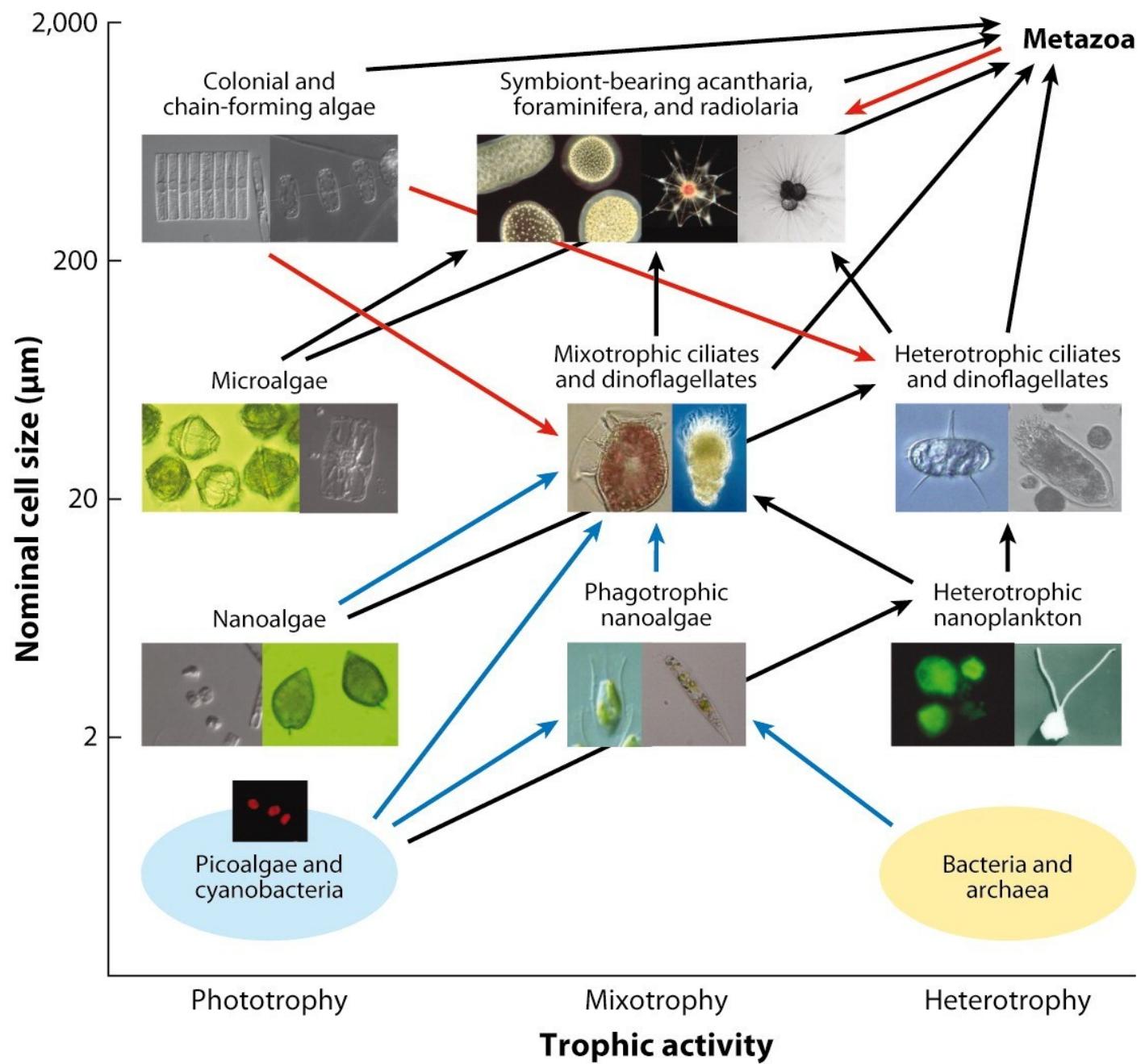
# Protist



# Protist

- Protists have fascinated microbiologists since their discovery nearly 350 years ago
- Single-celled, eukaryotic species span an incredible range of sizes, forms, and functions and, despite their generally diminutive size, constitute much of the genetic diversity within the domain Eukarya
- Complex taxonomy with numerous paraphyletic groups
- Protists in marine ecosystems play fundamental ecological roles as primary producers, consumers, decomposers, and trophic links in aquatic food webs

# Protist: why important?



# Brief overview of protist diversity

# Foraminferans

- Characters: Calcium carbonate shell, possess pseudopodia mainly benthonic
- 100 µm to 20 cm (!) long
- Both heterotroph and phototroph thanks to symbiosis with algae
- Use reticulopodia (like pseudopodia) to move and feed
- Shells of bottom-living foram used for paleoclimate and paleoceanography



# Radiolarian

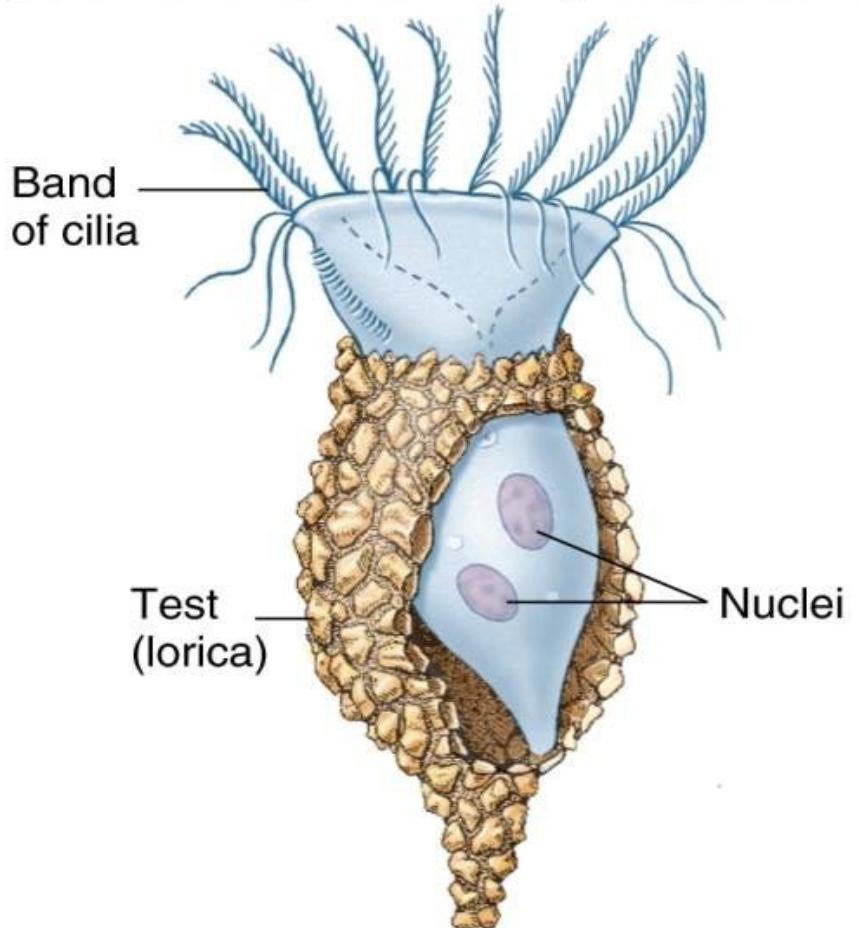
- Planktonic marine protozoans
- Siliceous skeletons
- 100-200 µm
- Inhabit in open ocean throughout the world
- Shell and pseudopodia



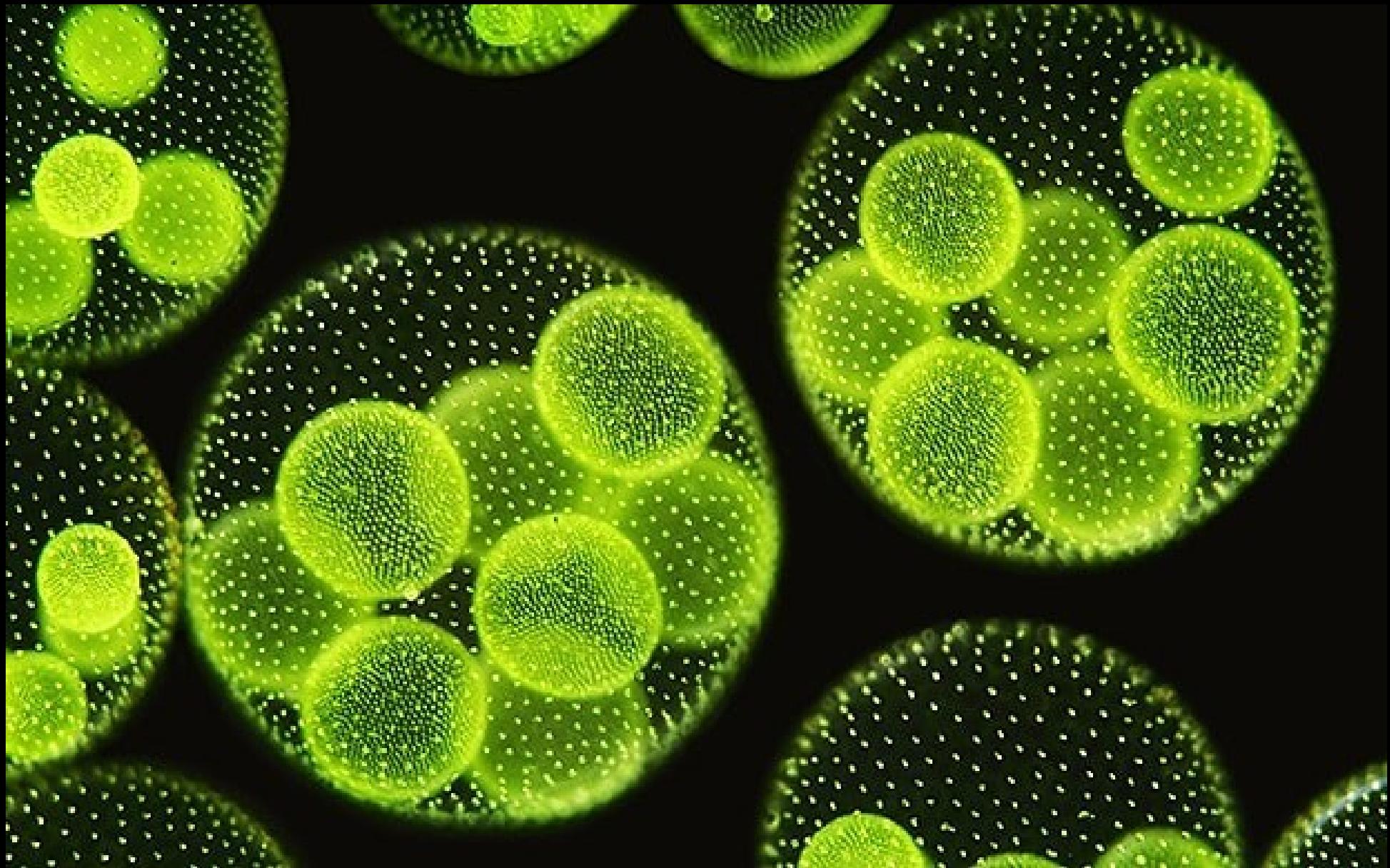
# Ciliates

- Cilia for locomotion and feeding
- Abundant in open waters, in bottom sediments, in the intestine and skin of marine animals
- Important in the microbial loop of open-water; facilitate energy flow

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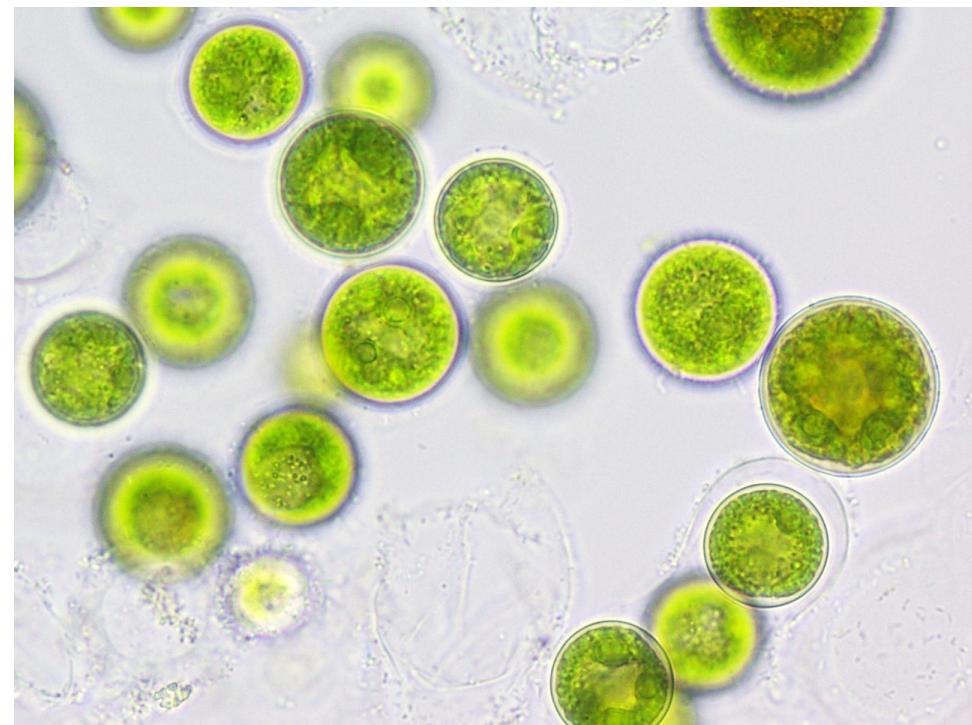


# Green Algae



# Green Algae (Chlorophytes)

- Paraphyletic group (excludes the *Plantae*)
- 2 to 40 µm diameters
- Can form large colonies
- Include some symbionts (in ciliates, hydra and others)
- Over 7,000 species
- Economically important as sources of industrial products, biofuels and food

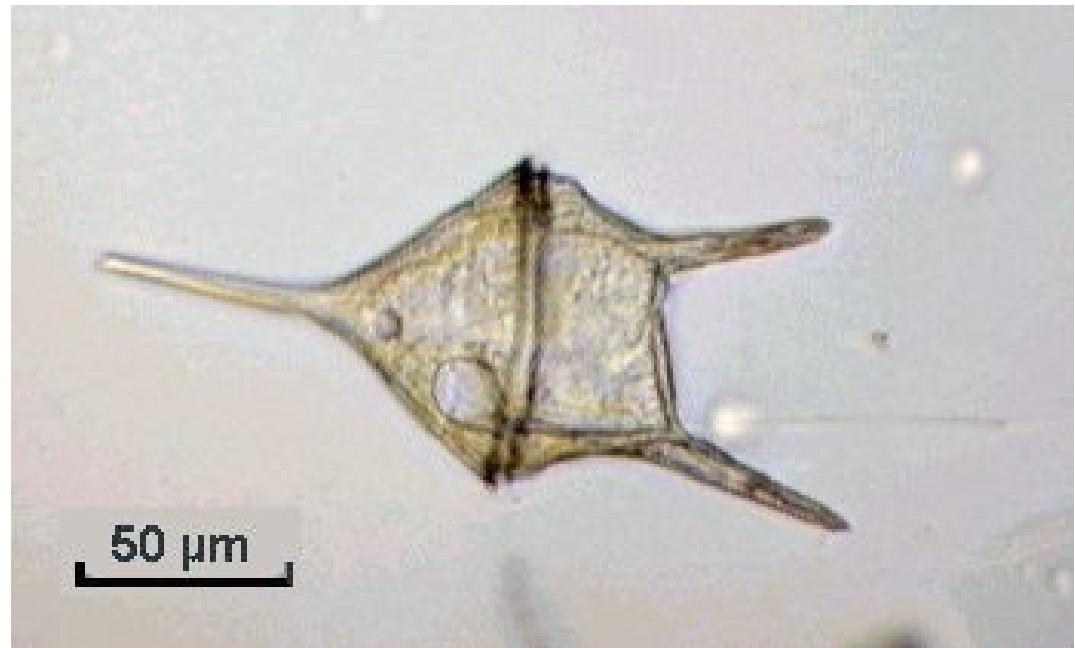


# Dinoflagellates

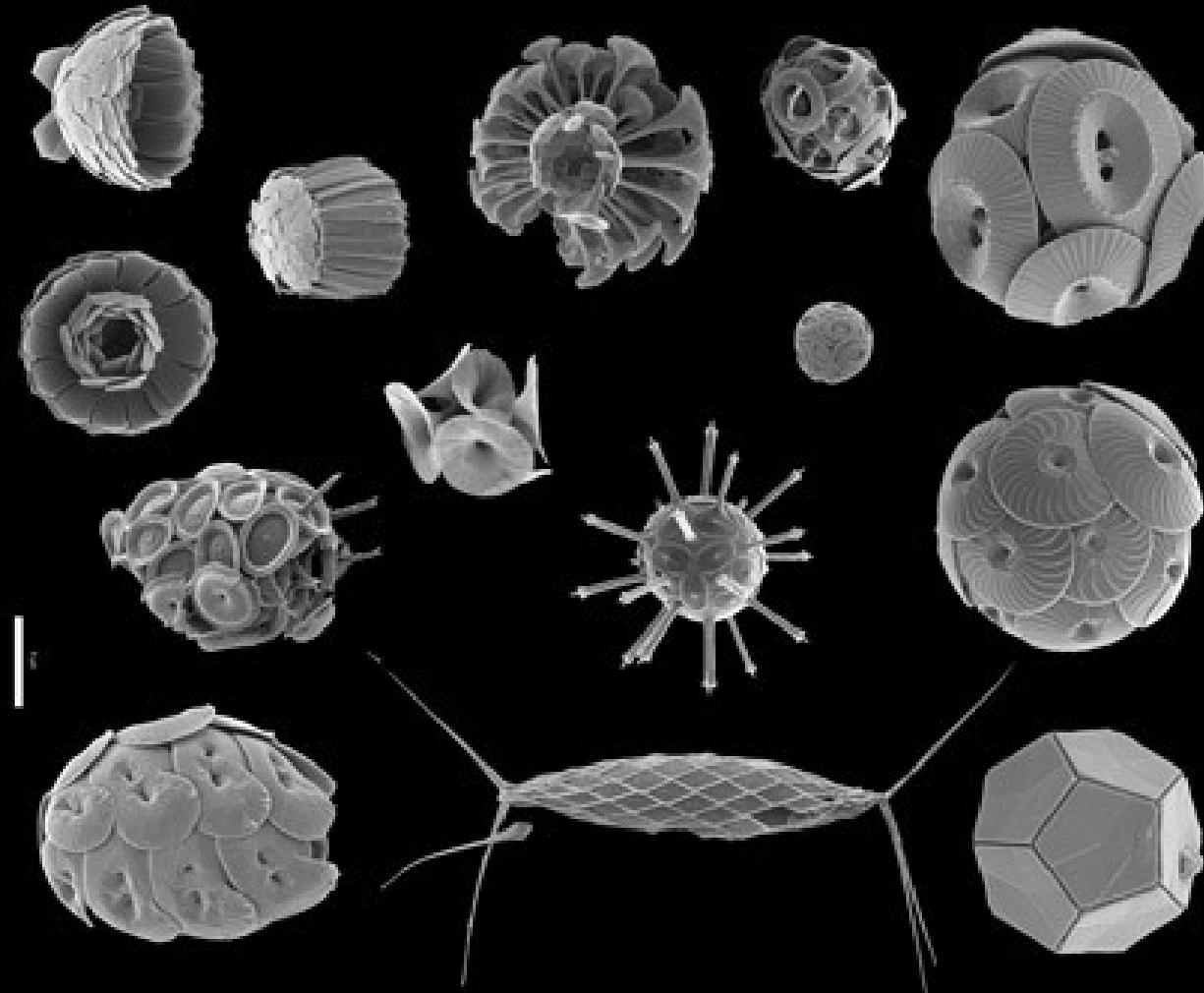


# Dinoflagellates

- 20-150 µm in size
- The theca is made of cellulose plates
- 90% are marine plankton
- Include symbiont (zooxanthellae) and few parasites
- Over 2,000 species
- Potential health hazard (ciguatera and PSP, *Pfiesteria piscicida*)
- $\frac{1}{2}$  are photosynthetic
- Some are bioluminescent (e.g. *Noctiluca*)

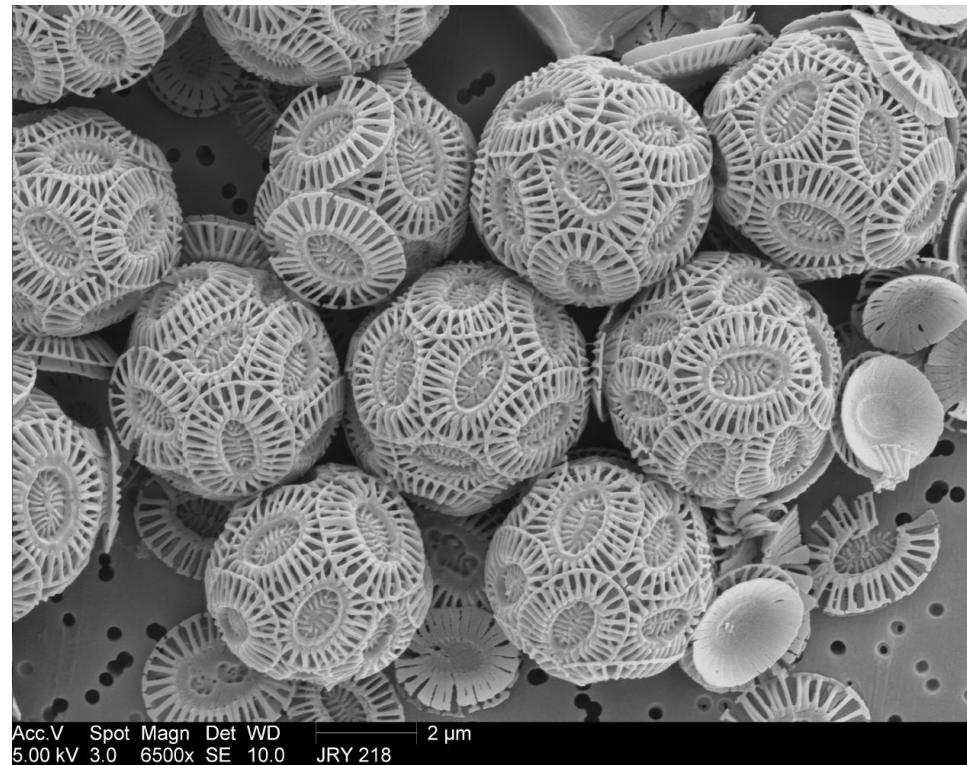


# Coccolithophores

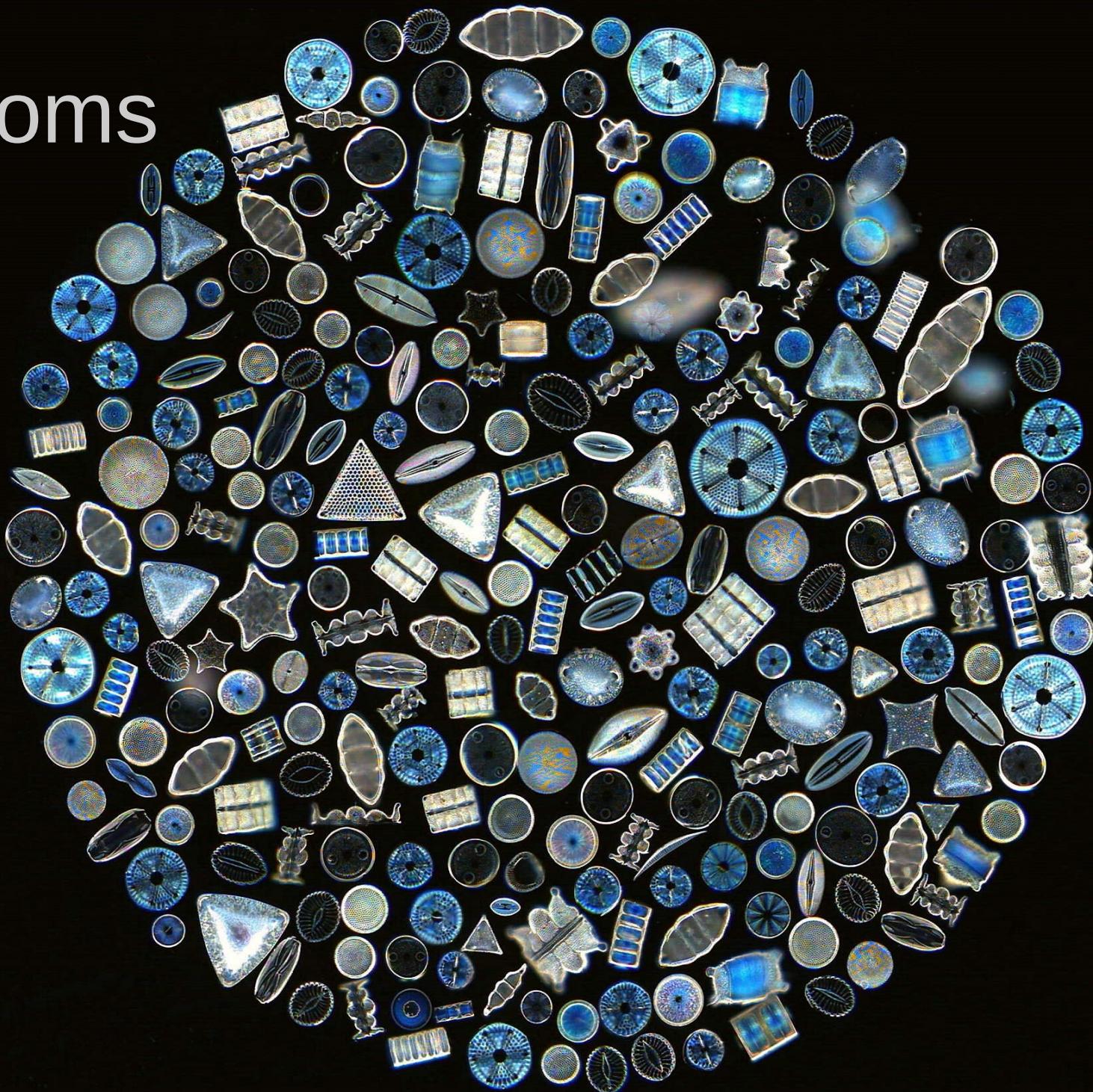


# Coccolithophores

- Free drifting photosynthetic phytoplankton
- Diameter of 5-10 µm
- Major calcifying organism in the phytoplankton
- Built of calcium carbonate scales (coccoliths)
- Produce massive blooms in favorable conditions (white waters)
- Mostly distributed in sub polar region in upper water column
- Nutrient poor and mild temperatures waters
- Major players in biological carbon pump (as we will see later)
- Affected by ocean acidification

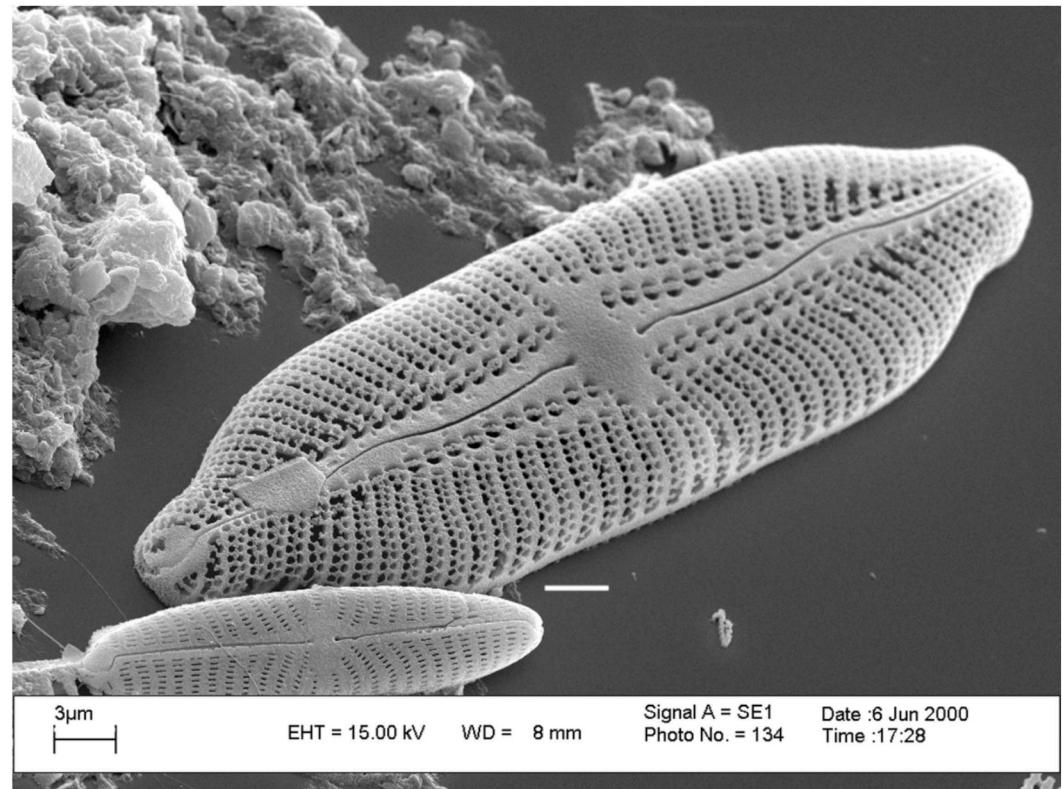


# Diatoms

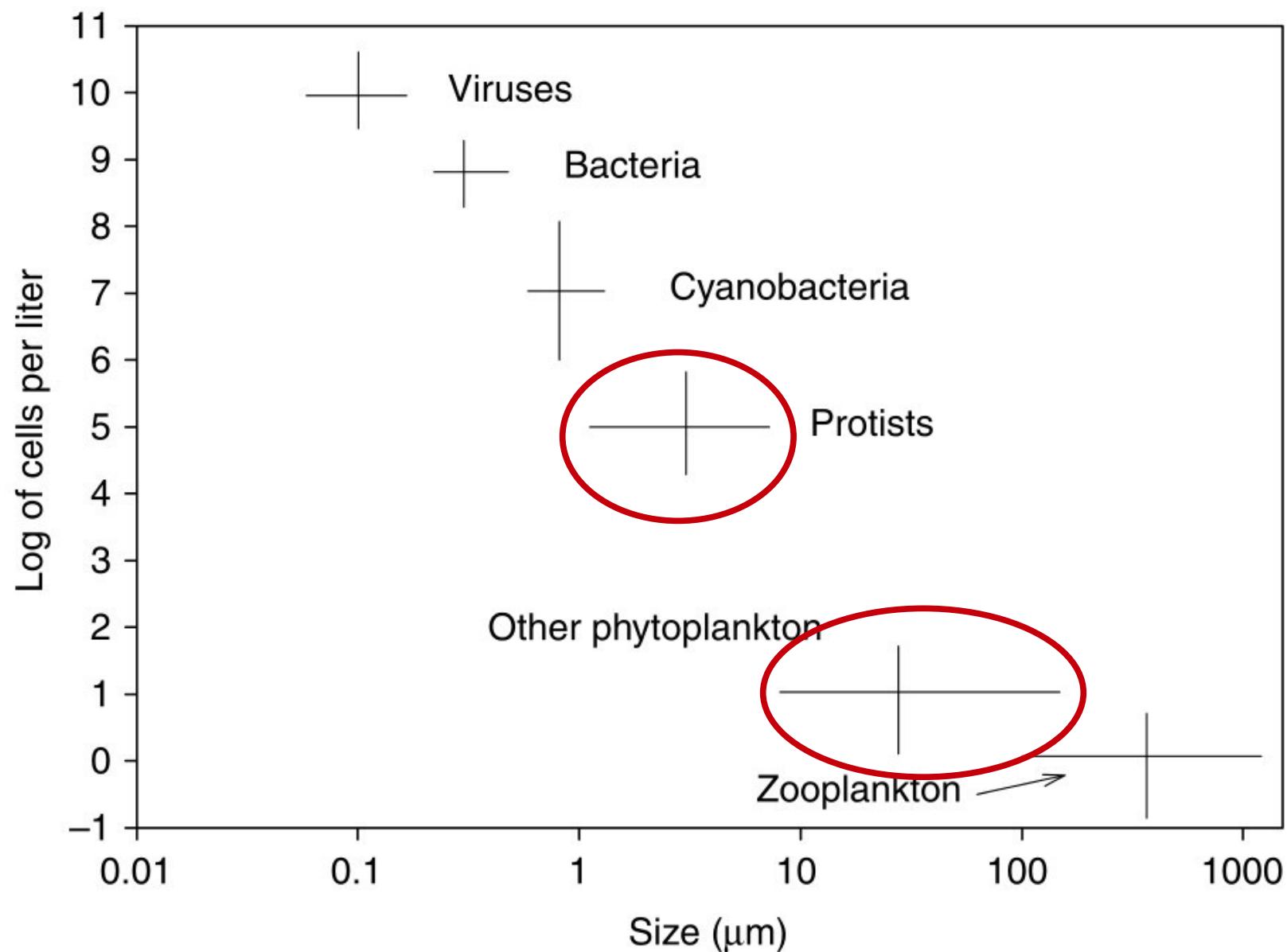


# Diatoms

- Solitary or colonial
- 2 to 500 µm in size
- Made of Siliceous shells (frustule)
- Planktonic and Benthic forms
- Over 60,000 species
- Can be found in almost any moist place
- Potential health hazard
- Dominate phytoplankton populations (responsible for 20-25% of all carbon fixation)



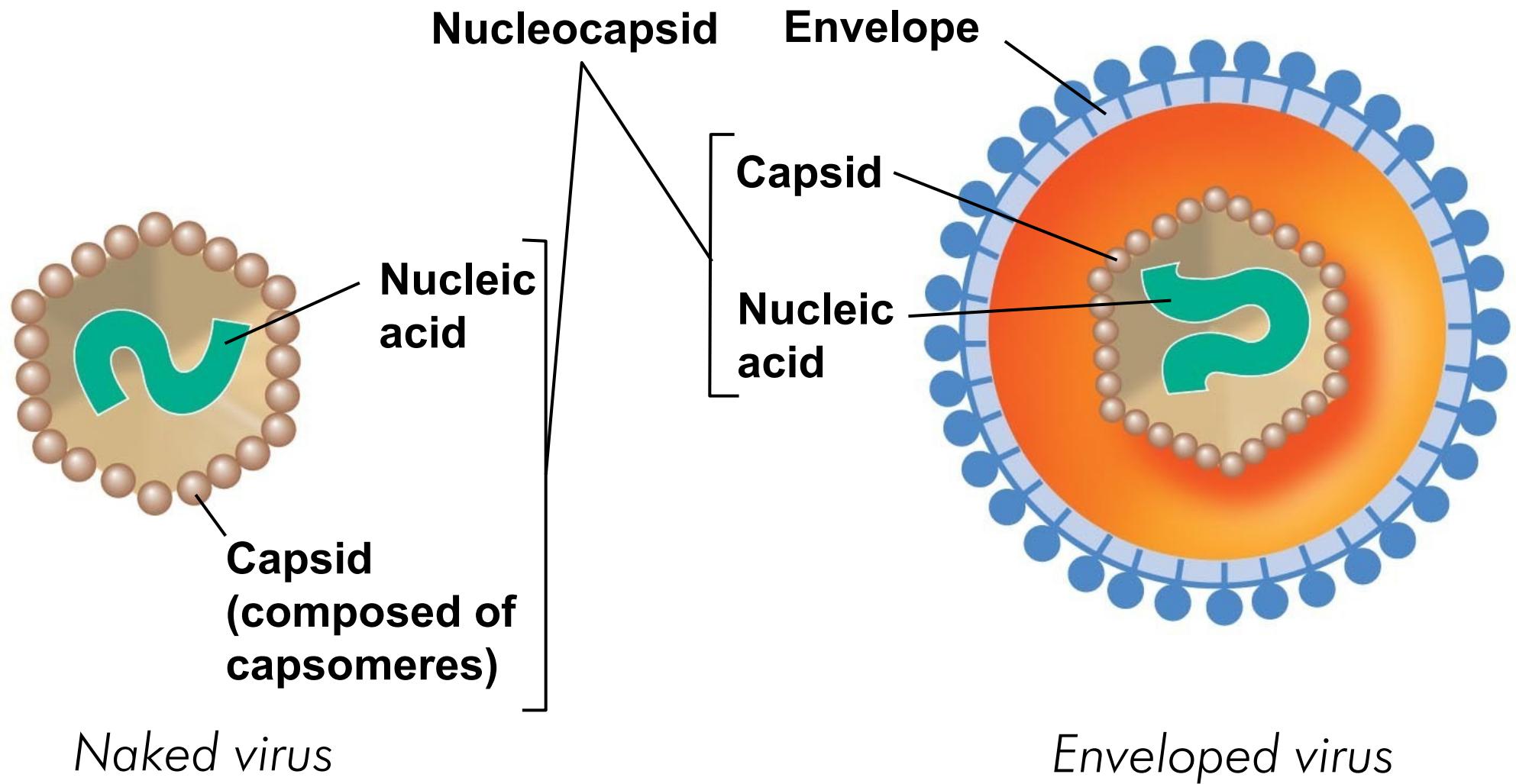
# Abundance and Size



# What is a virus

- A **virus** is a genetic element that can replicate only inside a living cell, called the host cell
- The word virus comes from the Latin, poison (syn. Venum)
- Viruses can only replicate themselves by infecting a host cell and therefore cannot reproduce on their own
- At the most basic level, viruses consist of genetic material contained within a **protective protein coat** called a **capsid**. They infect a wide variety of organisms: both eukaryotes and prokaryotes
- A virus that infects bacteria is known as a **bacteriophage**, often shortened to phage
- The study of viruses is known as virology, and those who study viruses are known as virologists
- Simply stated a virus is a “piece of bad news wrapped in a protein case”

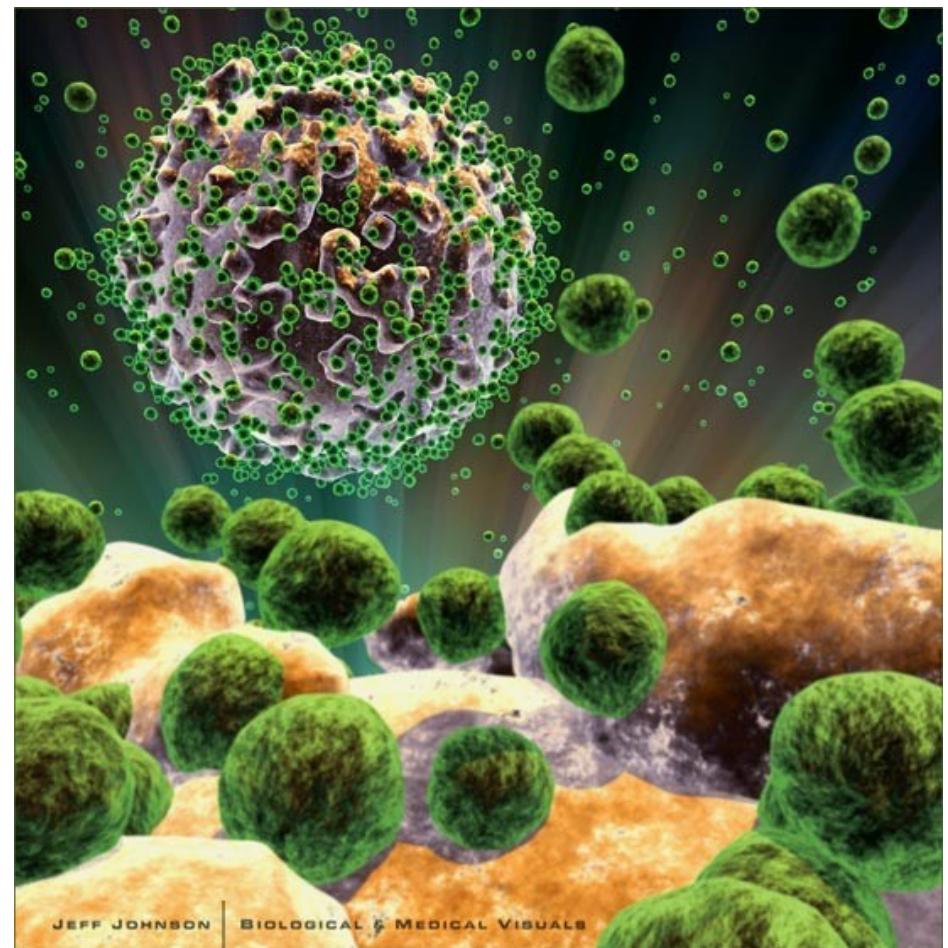
# Viral structure



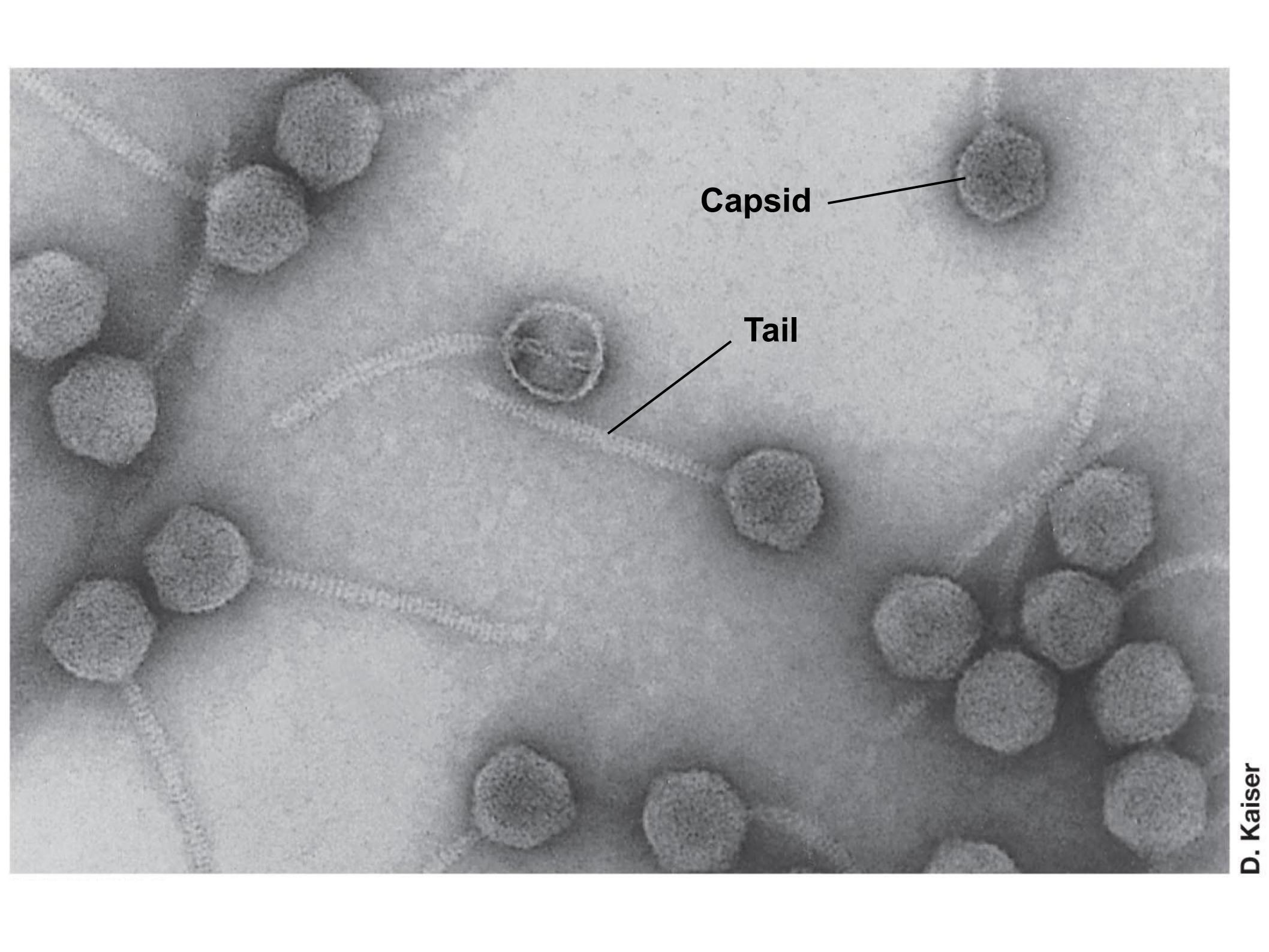
# An Ocean of Viruses

There are an estimated  $10^{31}$  viruses on Earth.

The sheer number of viruses and their intimate relationship with microbial life suggest that viruses play a critical role in the planet's biosphere.



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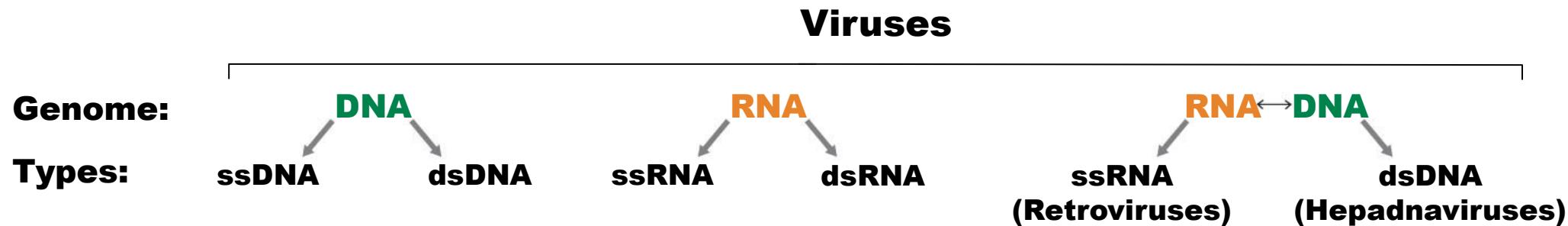


Capsid

Tail



# Virus: Structure and Morphology



All cells contain double-stranded DNA genomes.

By contrast, viral genomes consist of either DNA or RNA and are further subdivided based on whether the genome is single-stranded or double-stranded.

# Virus: Structure and Morphology

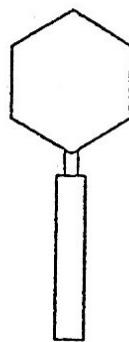
## **ssDNA**



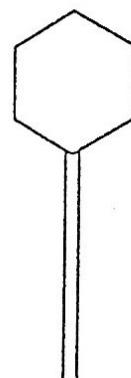
## **Microviridae**

## Inoviridae

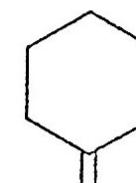
## dsDNA



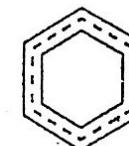
## Myoviridae



## Siphoviridae



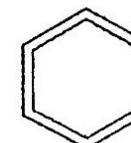
### **Podoviridae**



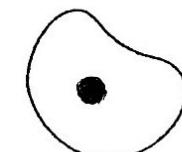
## Corticoviridae



## Fuselloviridae



## Tectiviridae



Plasmaviridae

A bundle of four thin, light-colored fibers, likely glass fibers, arranged in a fan-like shape. The fibers are slightly curved and have a fine, granular texture.

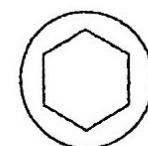
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## **ssRNA**



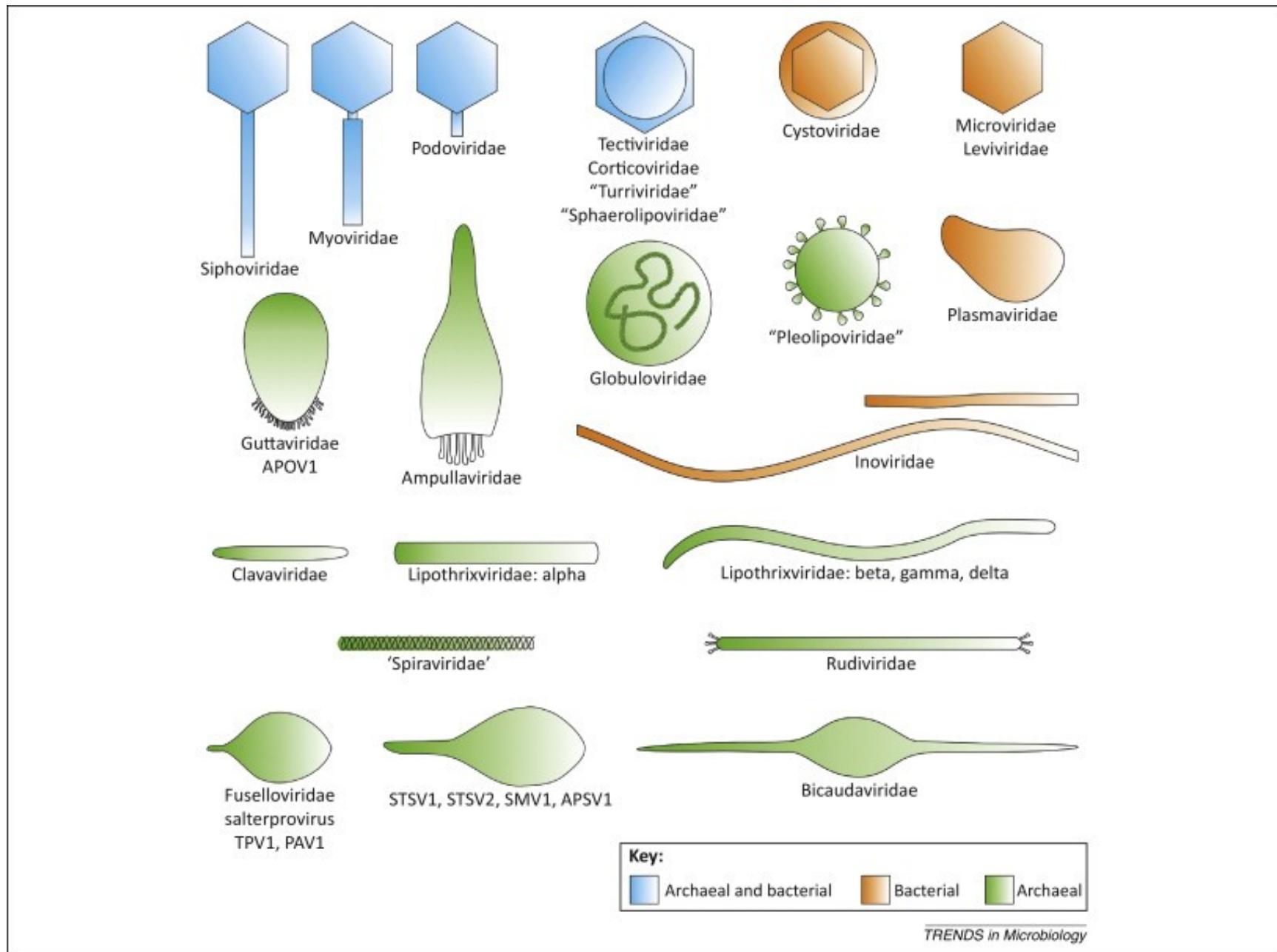
## Leviviridae

## dsRNA



## Cystoviridae

# Virus: Structure and Morphology



**Key:**

■ Archaeal and bacterial    ■ Bacterial    ■ Archaeal

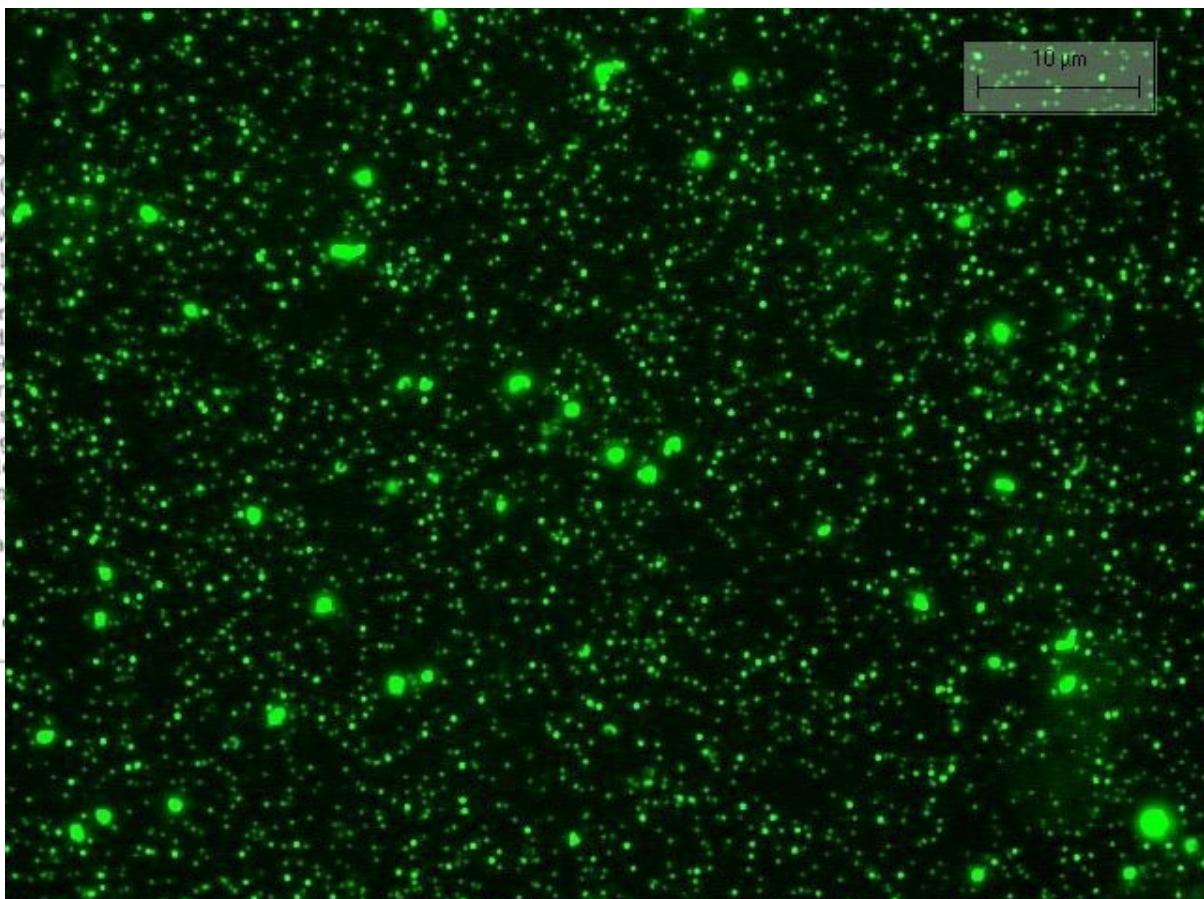
# Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria

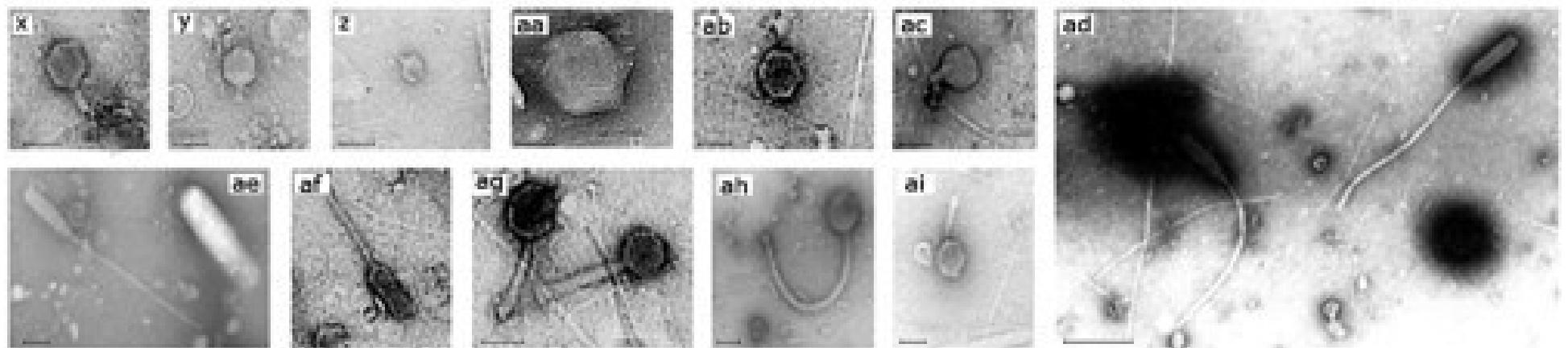
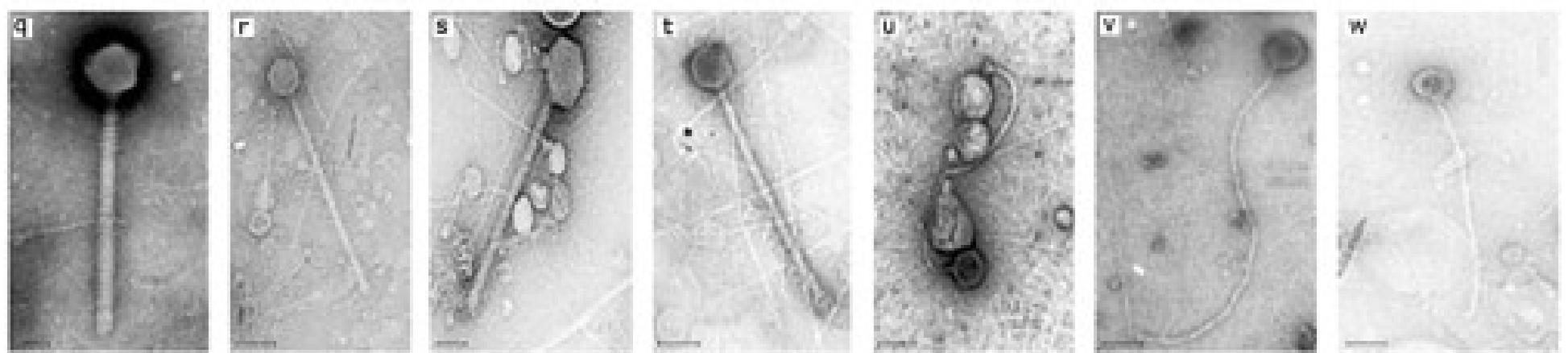
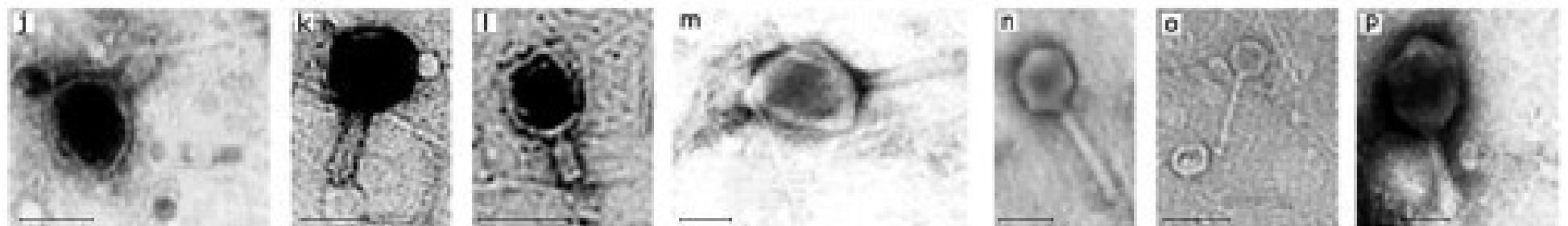
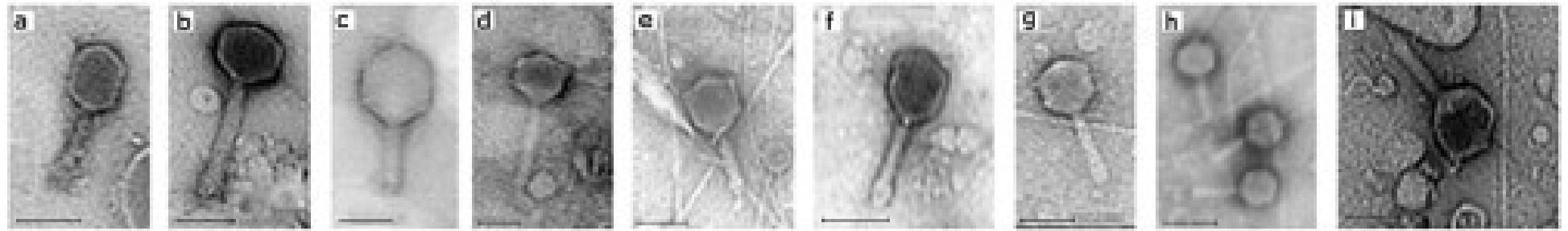
Rachel T. Noble\*, Jed A. Fuhrman

University of Southern California, Department of Biological Sciences, AHF 107, University Park, Los Angeles, California 90089-0371 USA

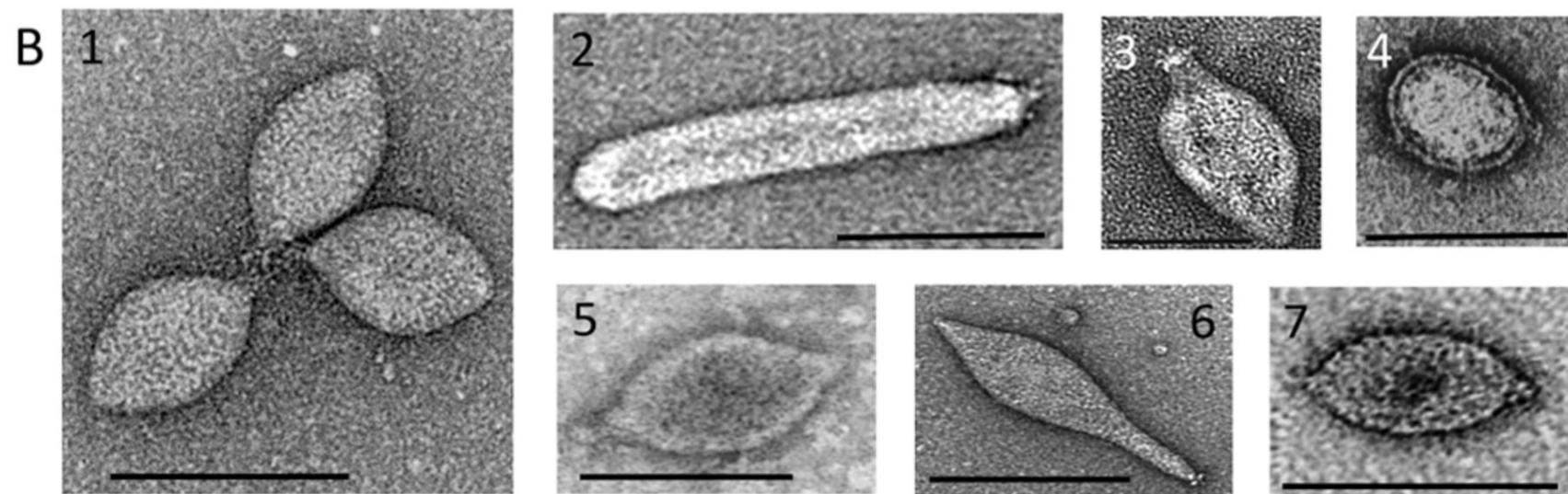
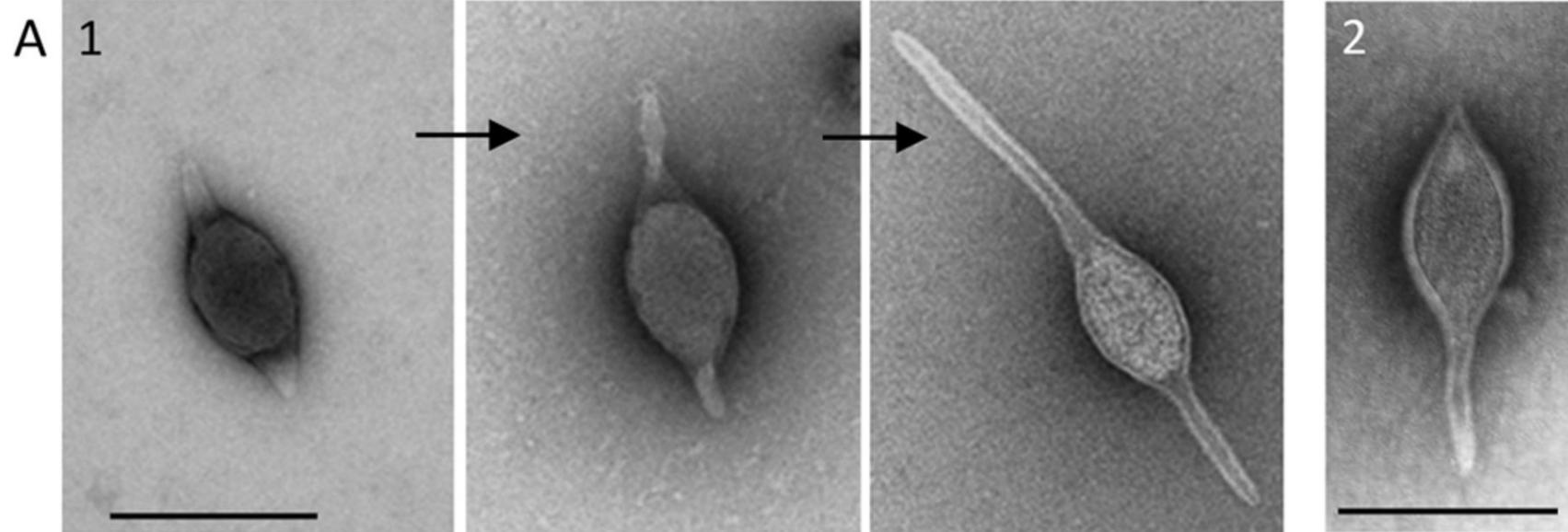
**ABSTRACT:** A new nucleic acid stain, SYBR Green I, can be used to estimate viral and bacterial abundances in diverse marine samples. Preserved samples of coastal water and also from depth profiles (shore, by filtering a few ml onto 0.02 µm pore-size filters and counting bacterial counts to those made with acridine orange (AO) and transmission electron microscopy (TEM) showed very strong correlation. SYBR Green I were indistinguishable and almost perfectly correlated, from 0.03 to  $15 \times 10^7$  virus ml<sup>-1</sup>. Virus counts by SYBR Green I were as accurate as those made by TEM, and a SYBR Green I versus TEM plot yielded a linear relation between the two with an  $r^2$  value of 0.99. The method was the same as that for TEM, with coefficients of variation for viruses and bacteria are intensely stained and easy to distinguish and newer generation epifluorescence microscopes. Detritus is a problem if alternative dye YoPro I is used, so this approach may be suitable for samples need no desalting or heating, can be fixed with formalin, and preparation time is 15 min (resulting in a total preparation time of less than 1 h). This method may form the basis for most aquatic microbiology laboratories.

KEY WORDS: Virus · Epifluorescence · SYBR Green I · Marine

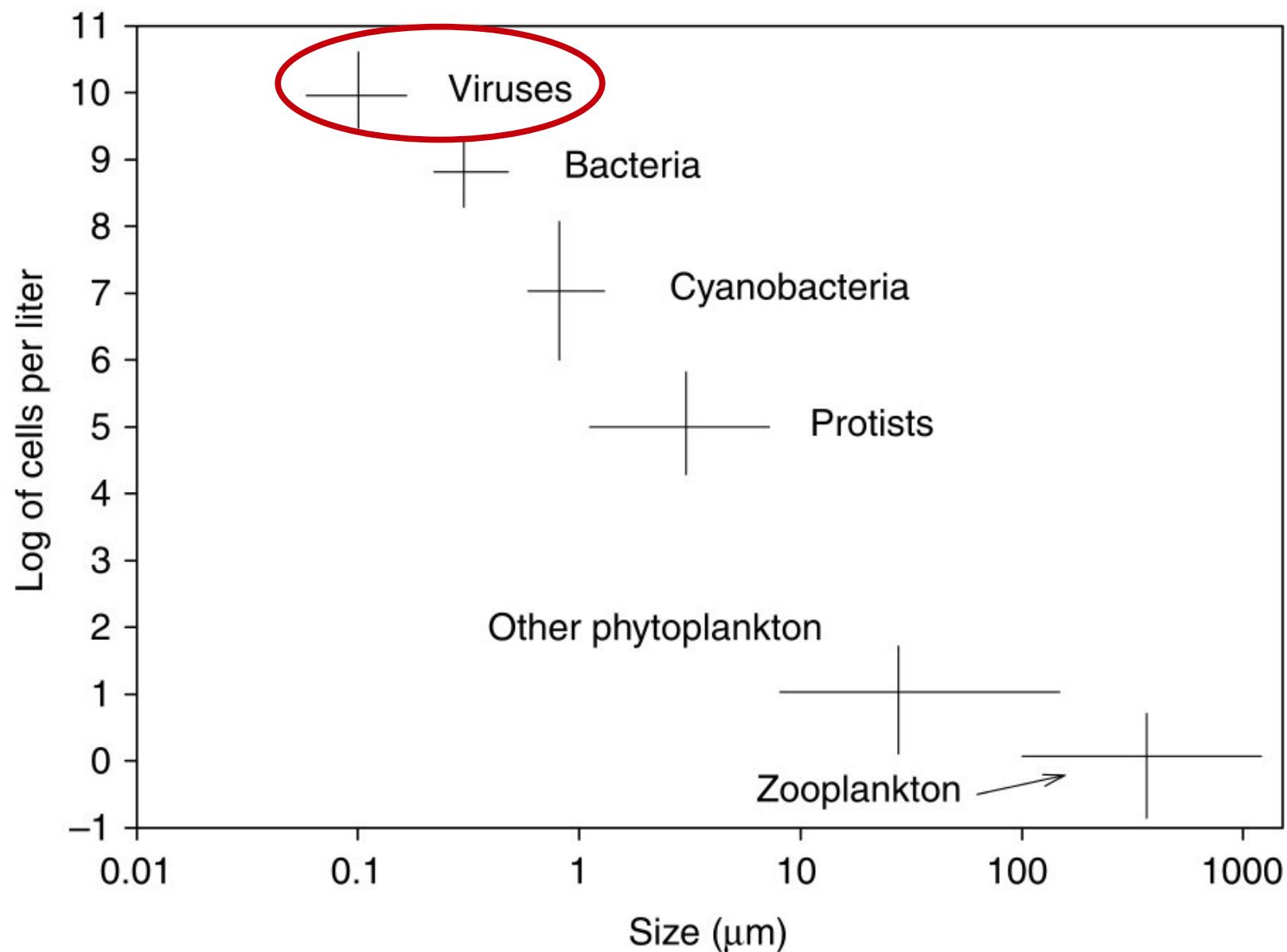




# Virus: Archaeal Virus

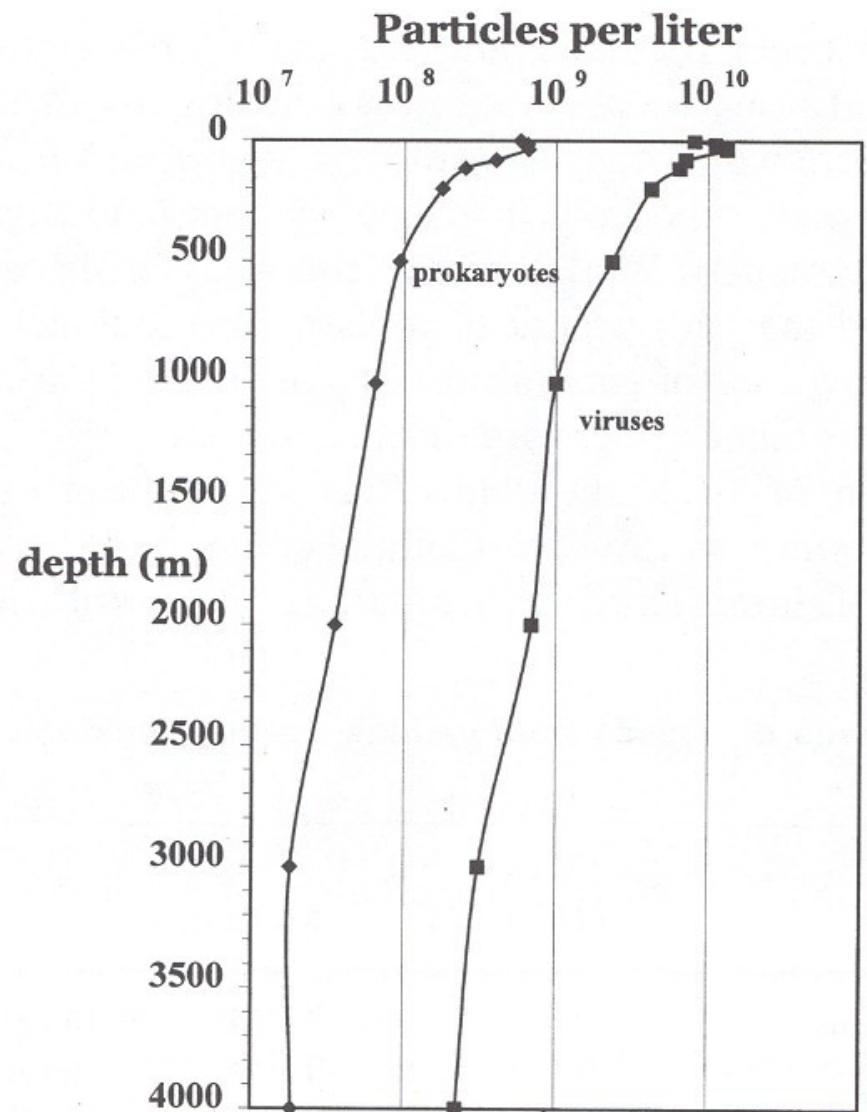


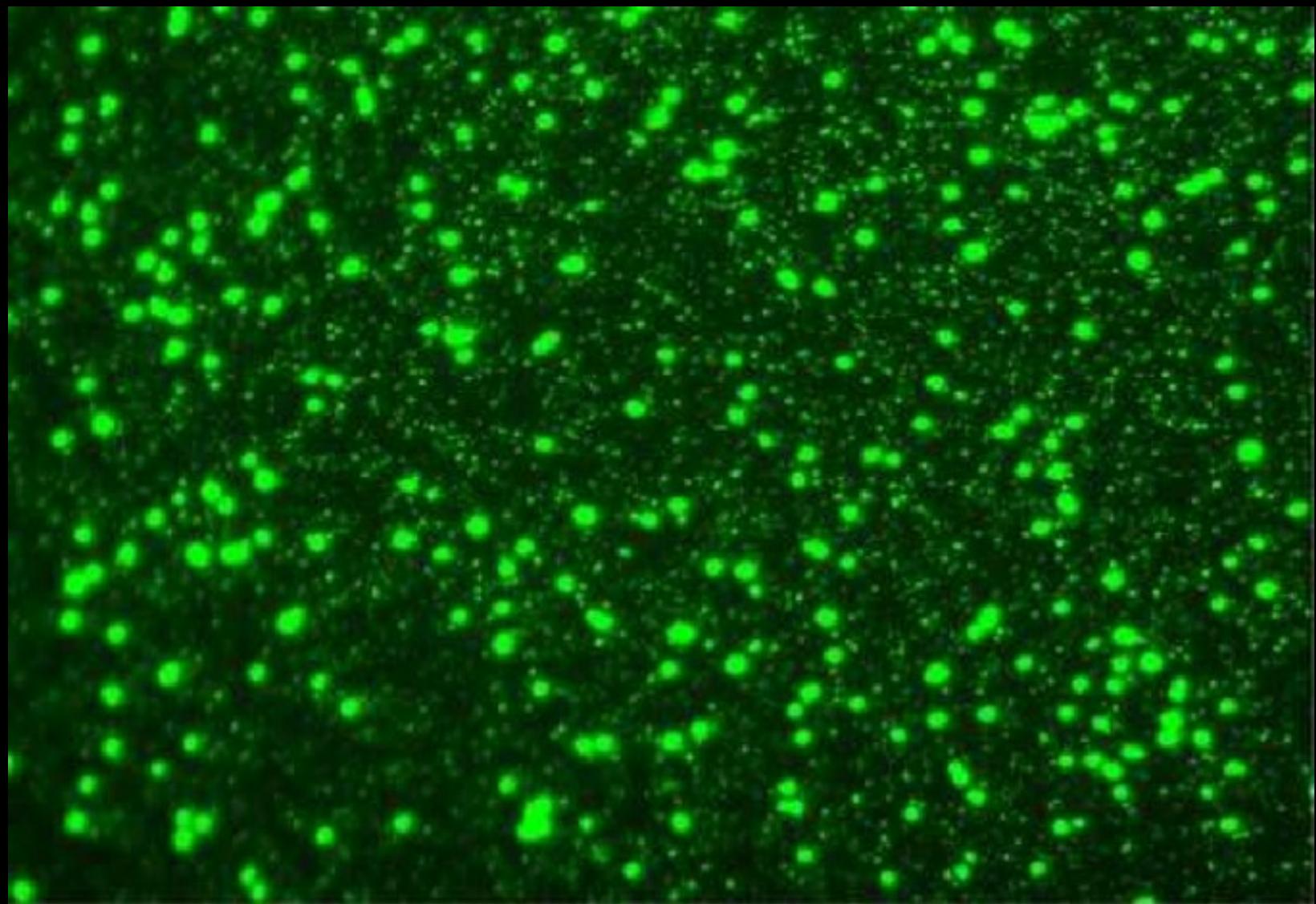
# *Abundance and Size*



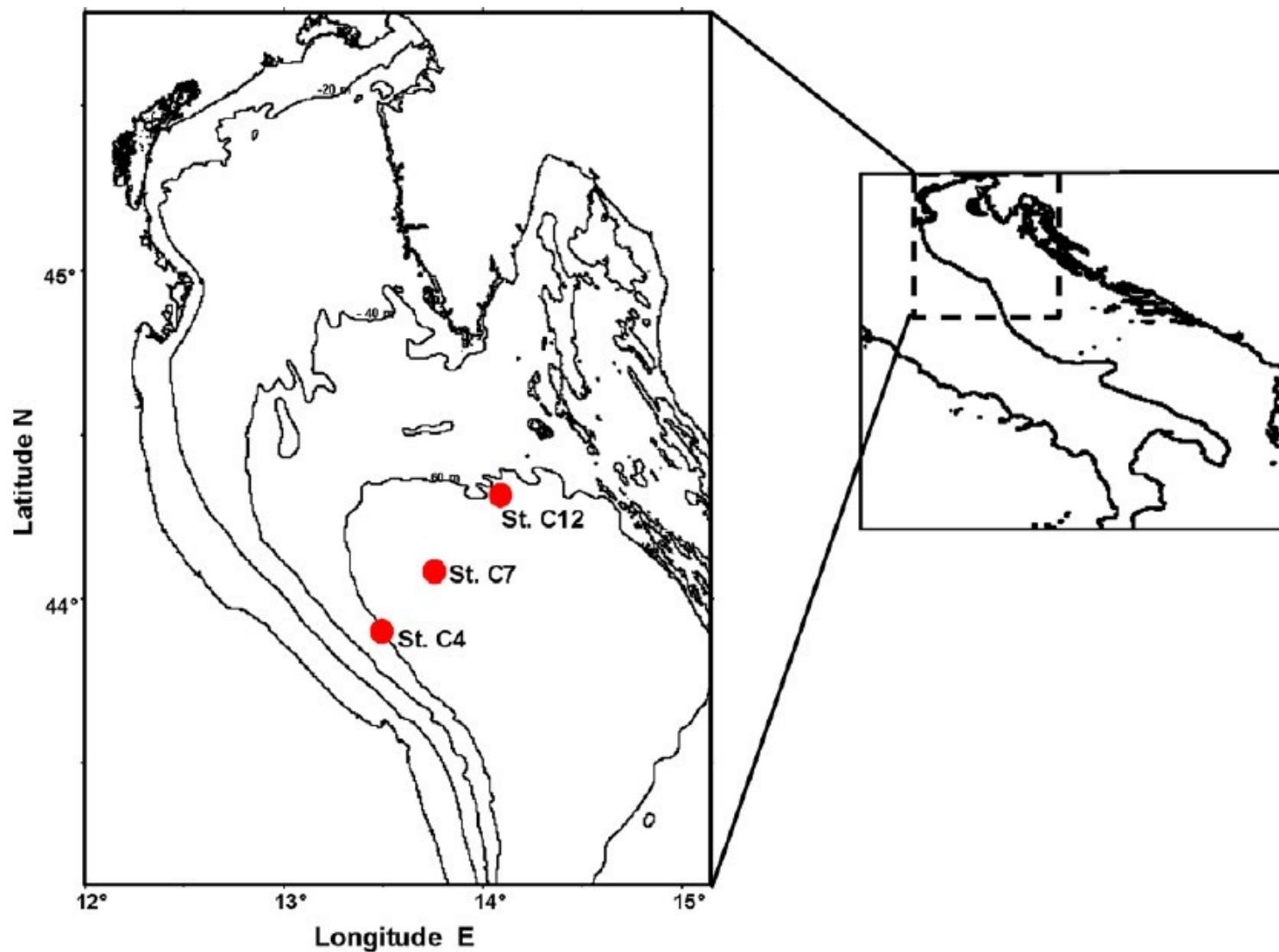
# Abundance

- Viruses are *highly abundant* in ocean ( $\sim 10^7 \text{ ml}^{-1}$ )
- In sediment they reach  $10^8\text{-}10^9 \text{ ml}^{-1}$
- 10x average bacterial abundance
- Mobilize via passive diffusion, hence need an abundant host so most are believed to *infect* prokaryotes

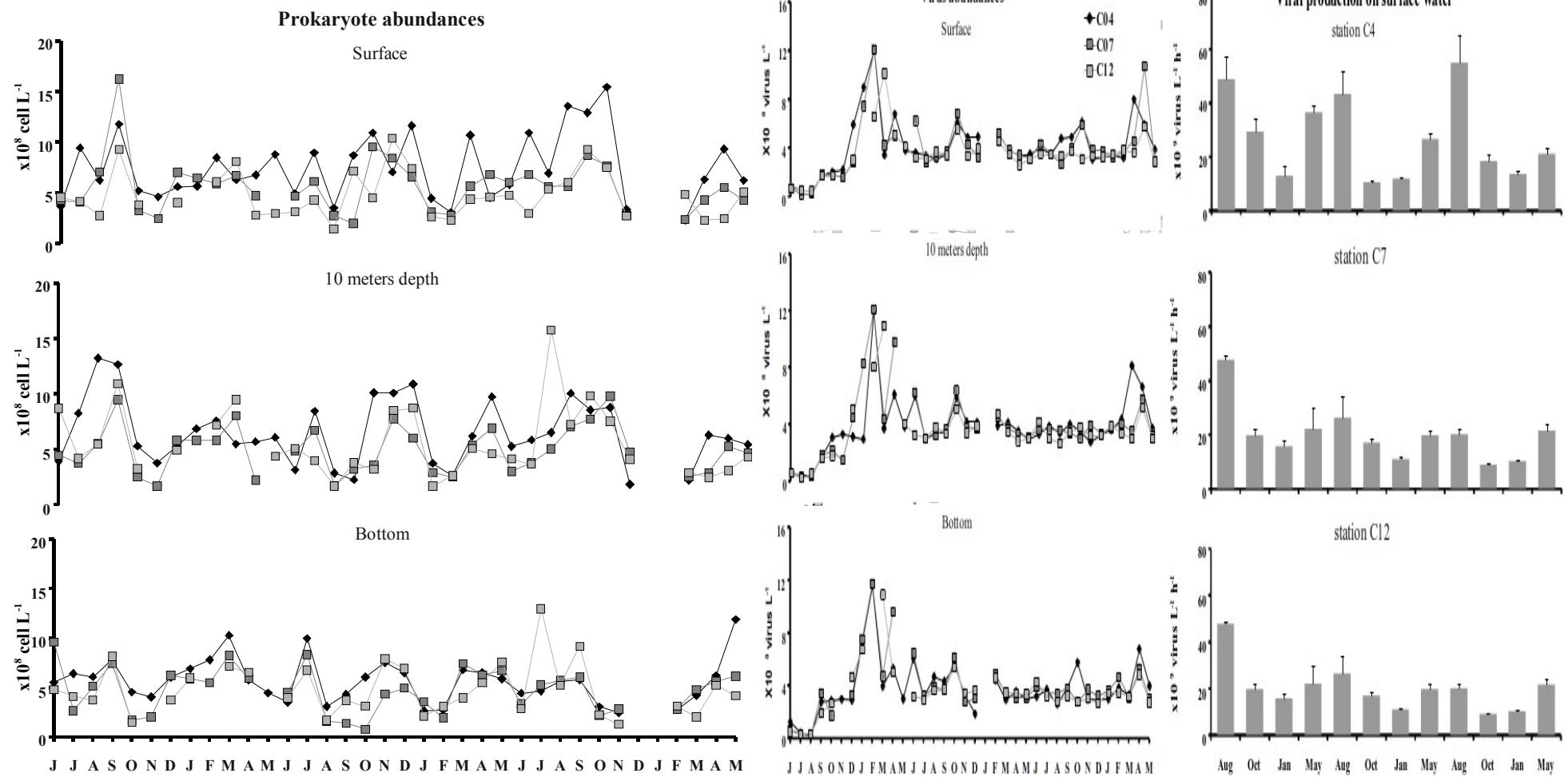




# Abundance: case study

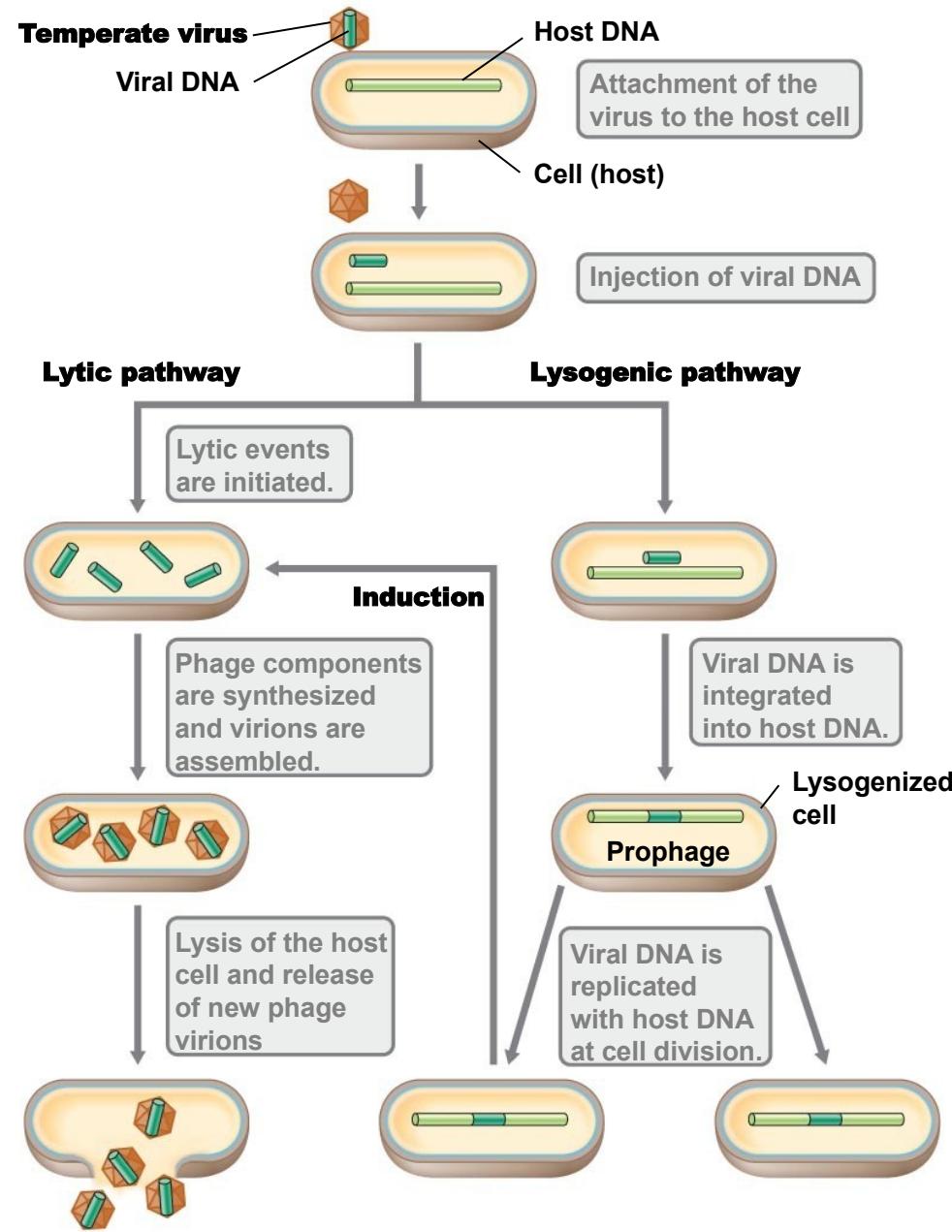


# Abundance



*Viral dynamic largely follow bacteria and Chl-a*

# Virus Life Cycle



# Virus Life Cycle



Viruses

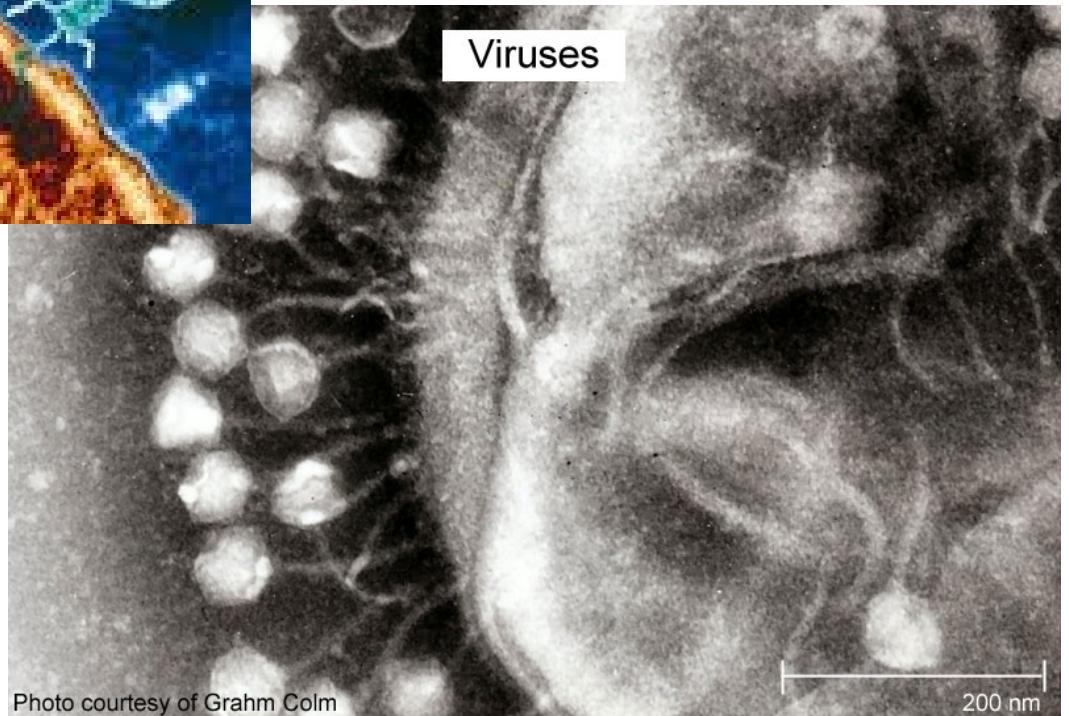
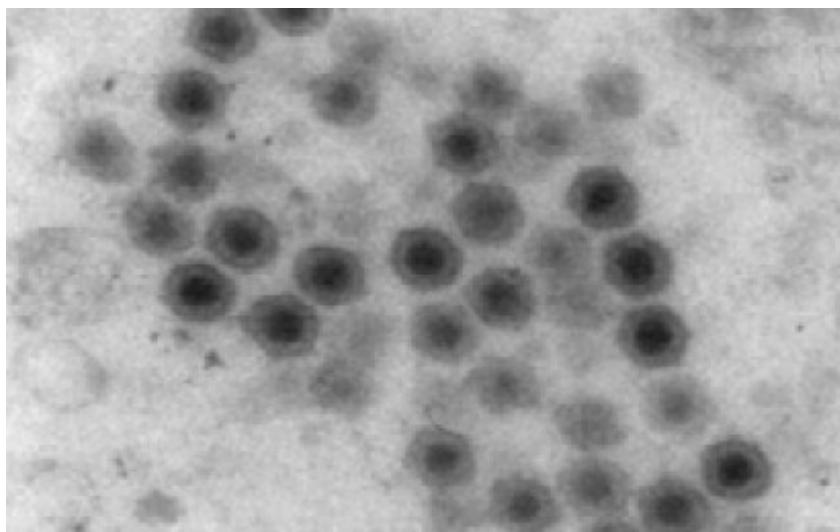


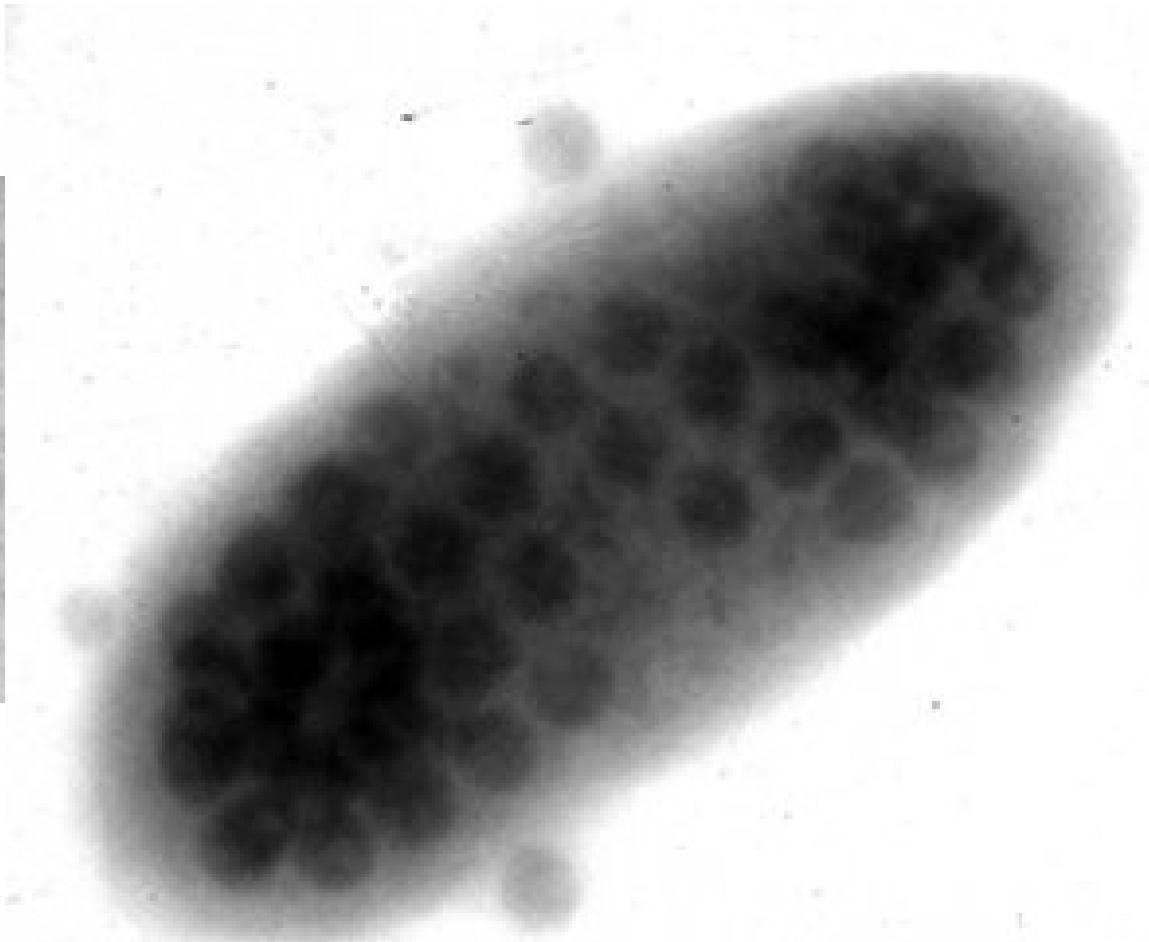
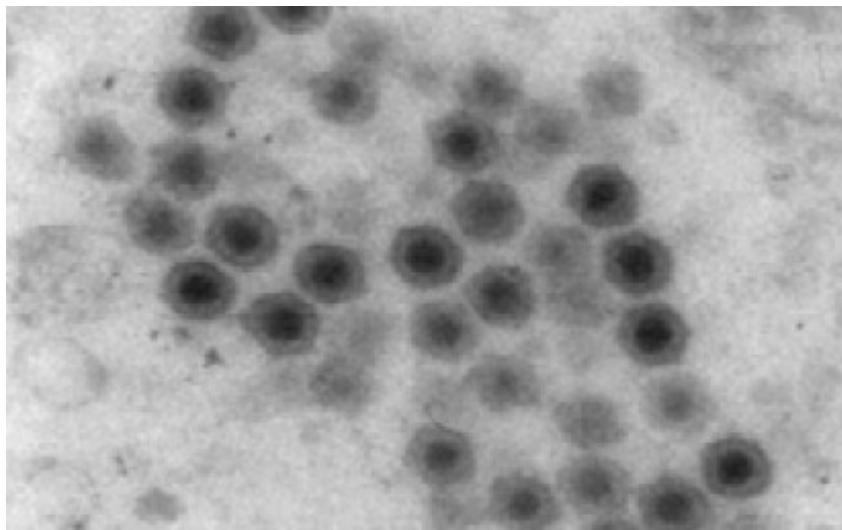
Photo courtesy of Graham Colm

200 nm

# *Virus Life Cycle*

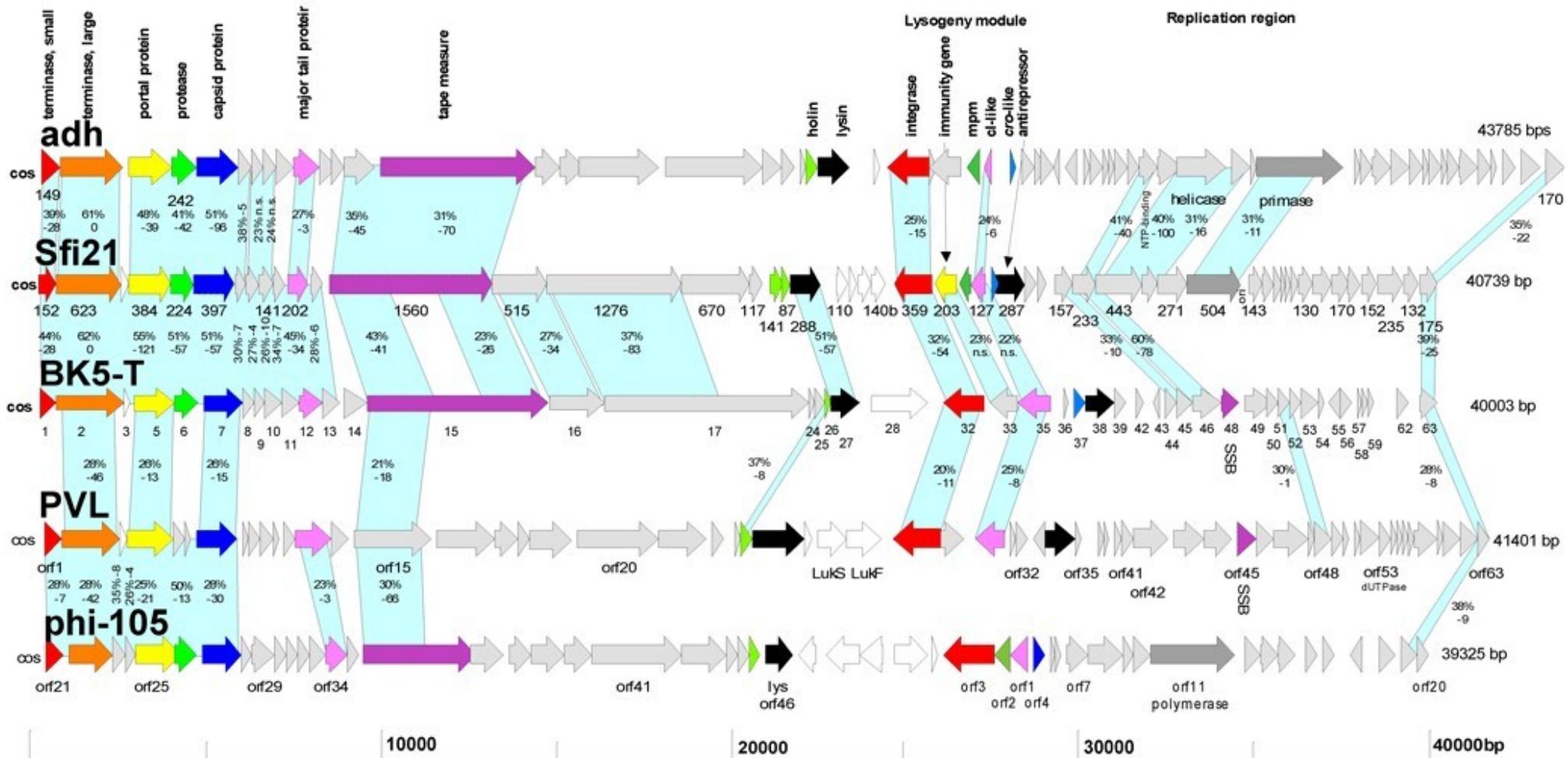


# Virus Life Cycle



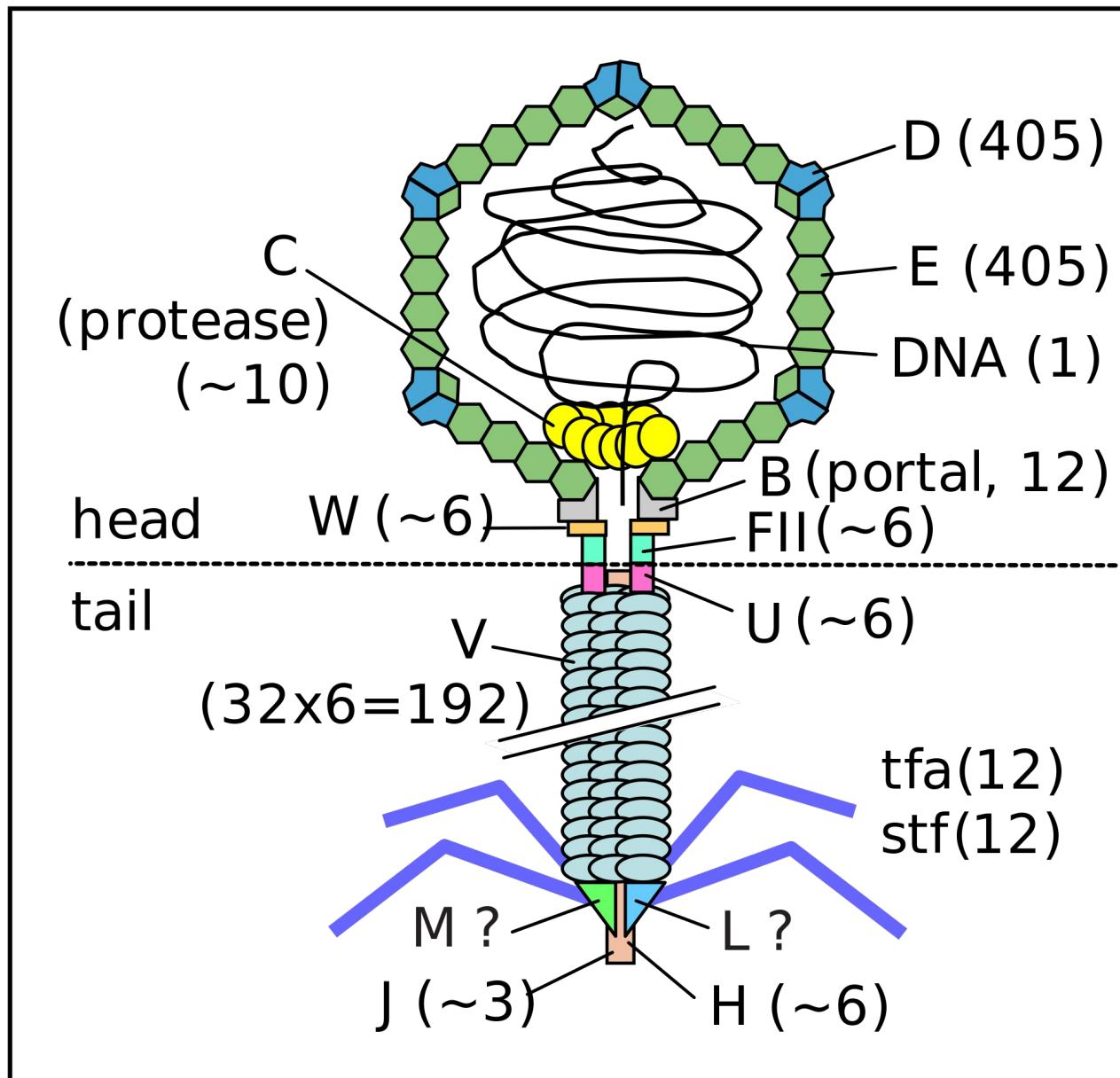
An important modeling parameter: **Burst Size**  
*i.e. the average number of virus released by a cell*

# Viral Genomes



20,000-100,000 bp in size (30-100 genes) on average

# Viral Genomes



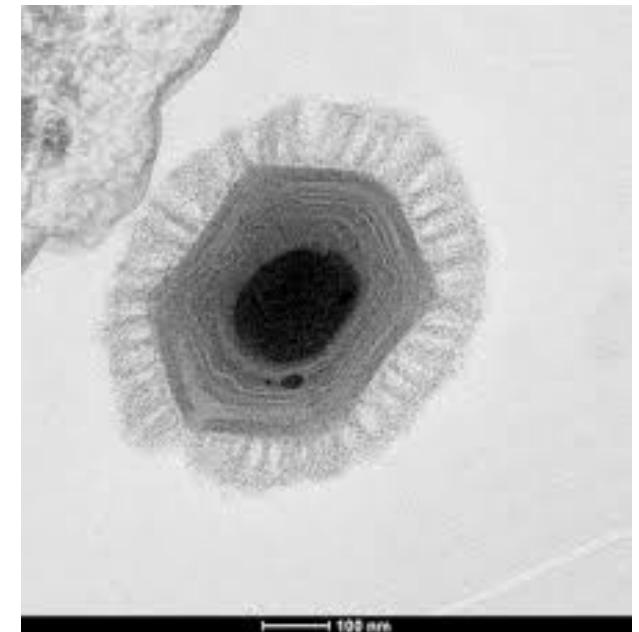
# *Viral diversity in the marine environment*

- Highest diversity of unknown genes with no matches in databases
- Highest number of new viral families
- Lowest number of “cultured” types available, mostly from model organisms



# Megavirus, and emerging group

- Capsid cross-sections that can exceed 500 nm, an order of magnitude larger than prototypical viruses
- Giant viruses infect eukaryotic hosts, including the protists and unicellular green algae
- Carry genomes larger than nearly all previously identified viral types, in some cases upwards of 1 million base pairs
- In both marine and non-marine contexts, researchers have even identified viruses that can infect giant viruses, the so-called **virophages**



# *Effect on biogeochemistry*

Ocean viruses may turn over as much as 150 gigatons of carbon per year—more than 30 times the standing abundance of carbon in marine plankton (**Viral shunt**).

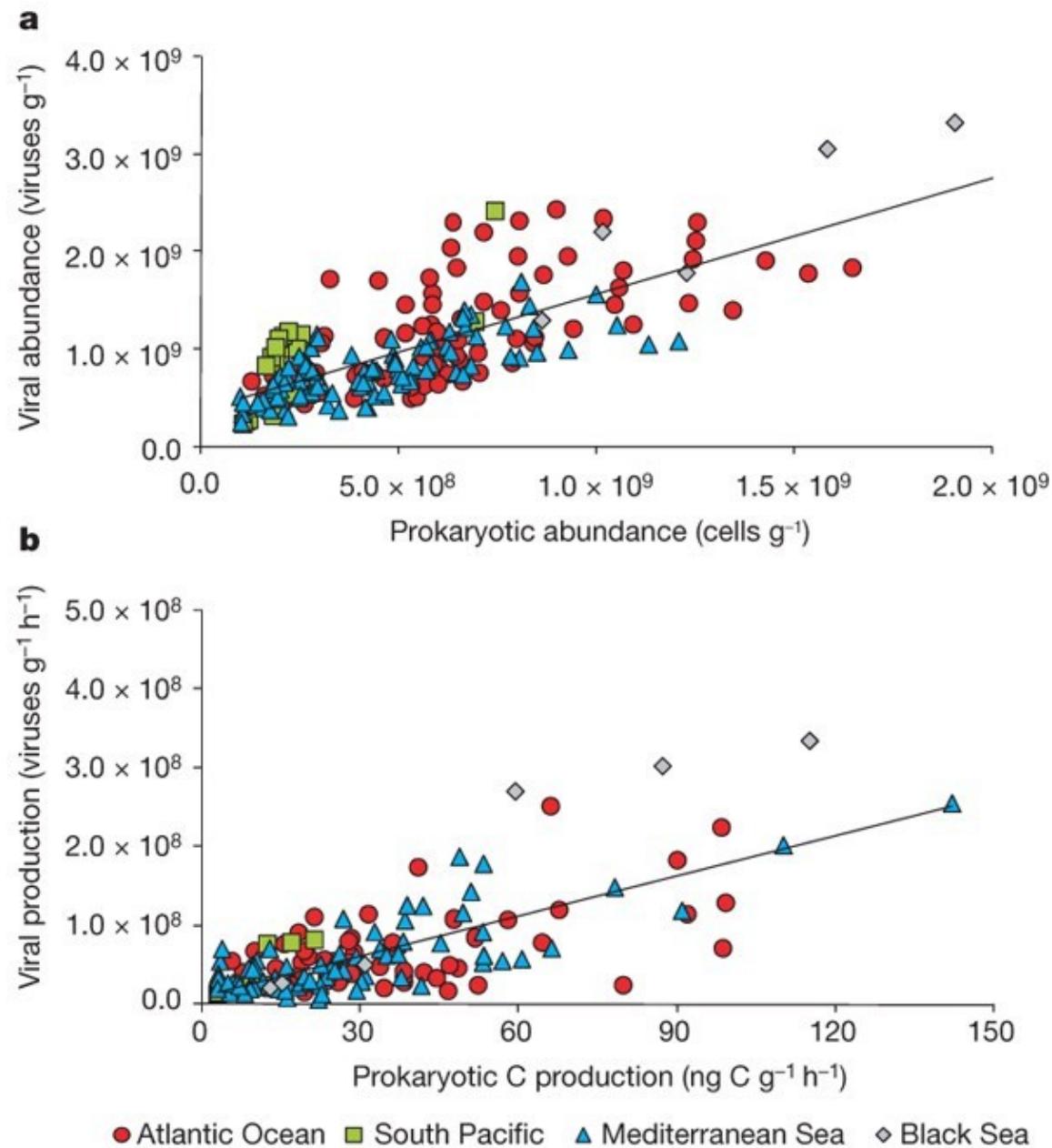
Top-Down control of prokaryotic and phytoplankton populations, and effect on community structure (**Killing the winner hypothesis**)

Evolutionary drivers (**Red queen hypothesis**), transduction, horizontal gene transfer

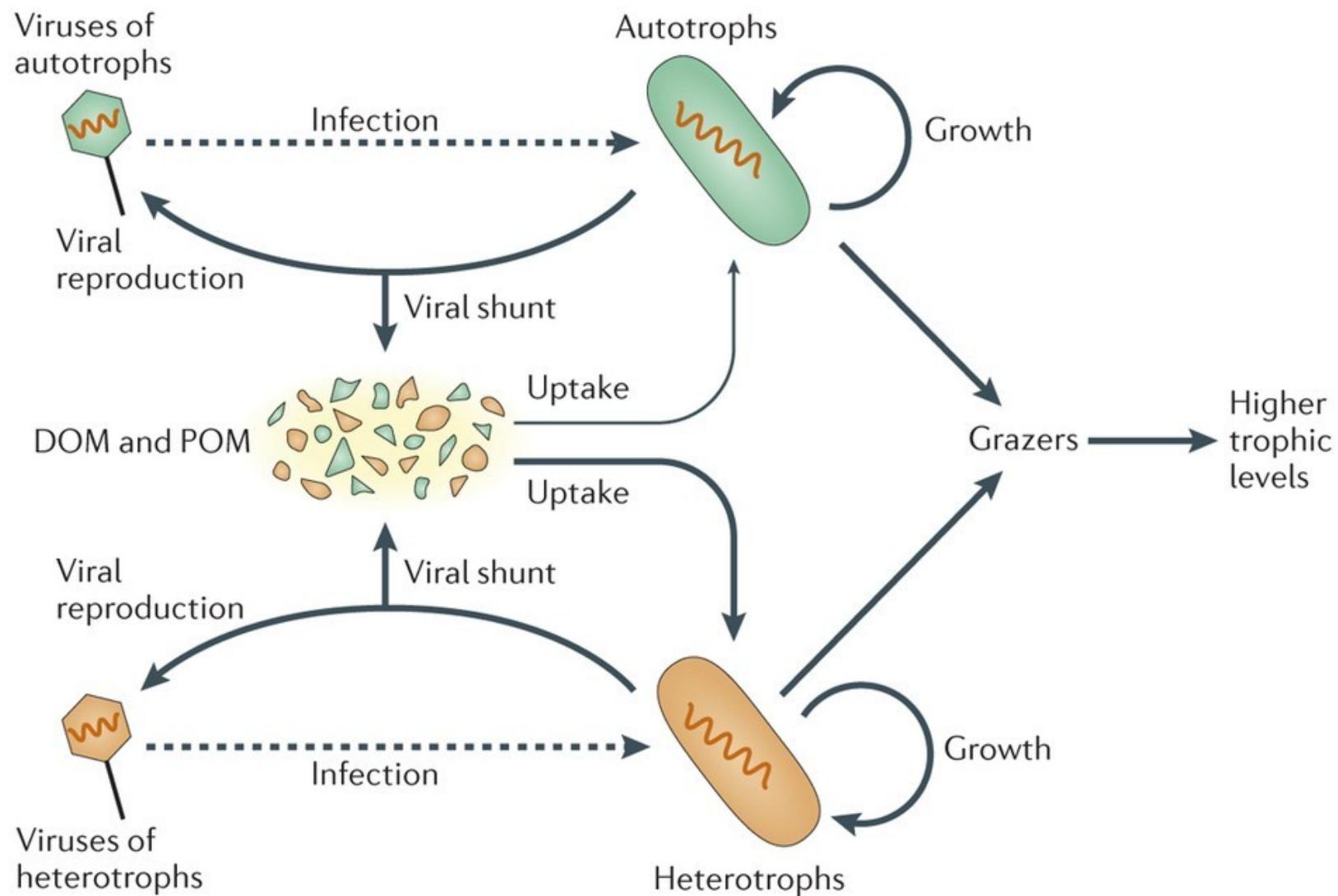
# Effect on biogeochemistry

Viral induced turnover is extremely high in the water column can reach 50% of the PP

In the deep-sea can be as high as 90% of prokaryotic biomass (sediments) is recycled through the viral shunt



# Ecological Role: Viral Shunt



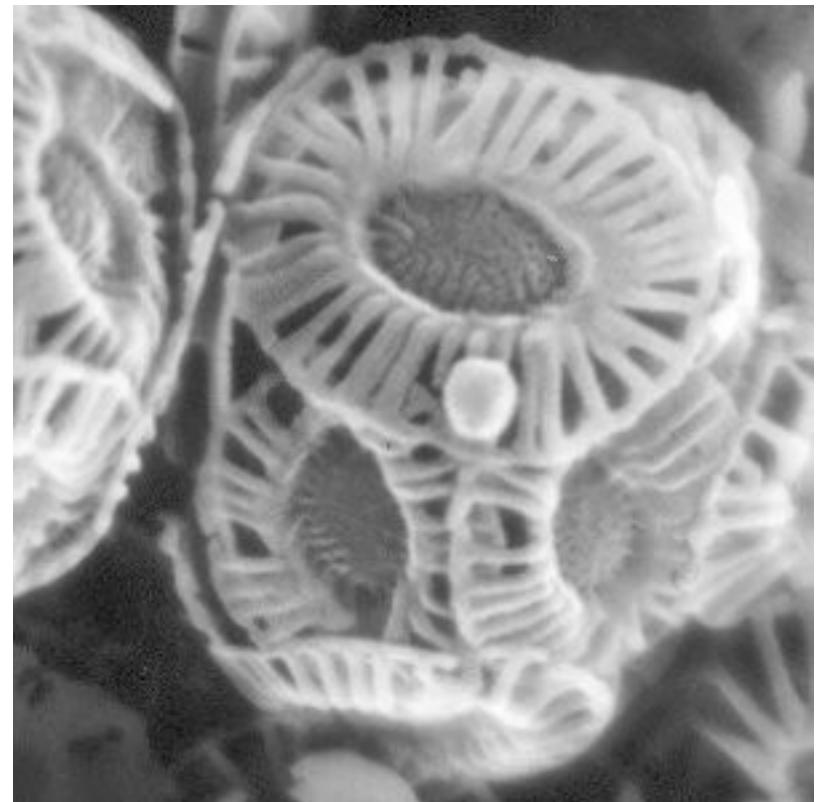
# *How virus influence climate*



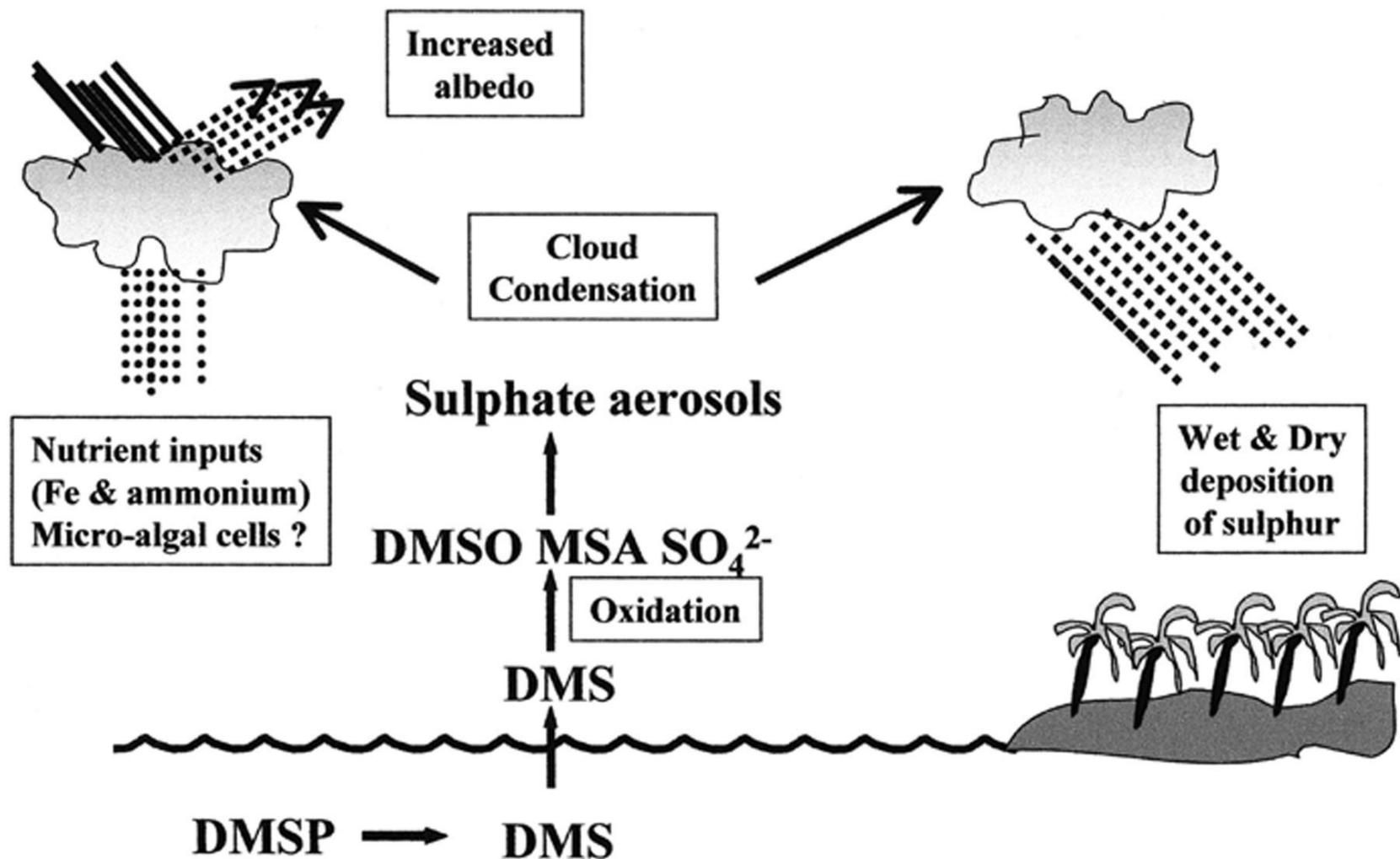
# How virus influence climate

- Virus infect phytoplankton
- Since their infection is controlled by random contact (they are not motile), most abundant organisms are more likely to be infected
- This can trigger bloom termination due to viral infection

On the side: EhV1 infecting the coccolithophore *Emiliania huxleyi*



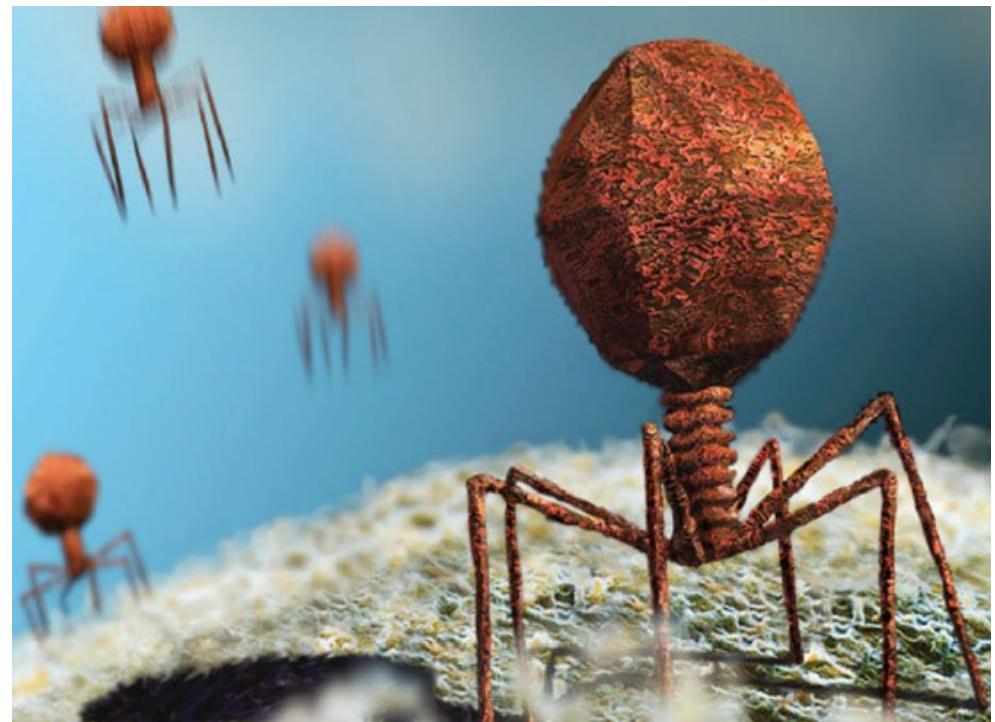
# How virus influence climate



Virus affect the amount of released DMSP in the Oceans, thus affecting climate

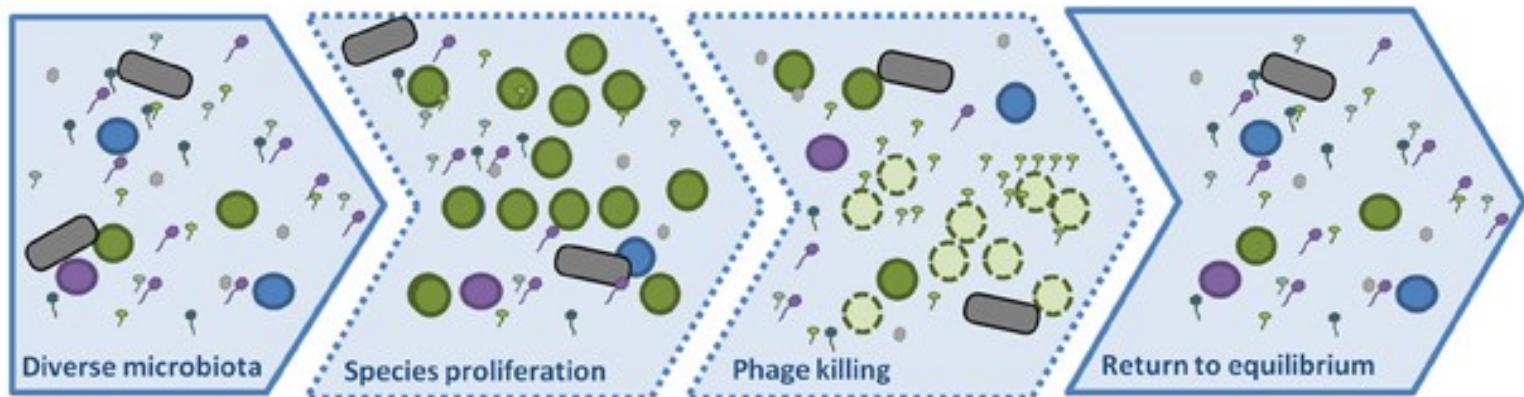
# *Killing the Winner Hypothesis*

- *The theory states that virus kill the most abundant organisms in a population (frequency dependent selection)*
- Supported by both experimental and modeling data
- Alternative models are possible



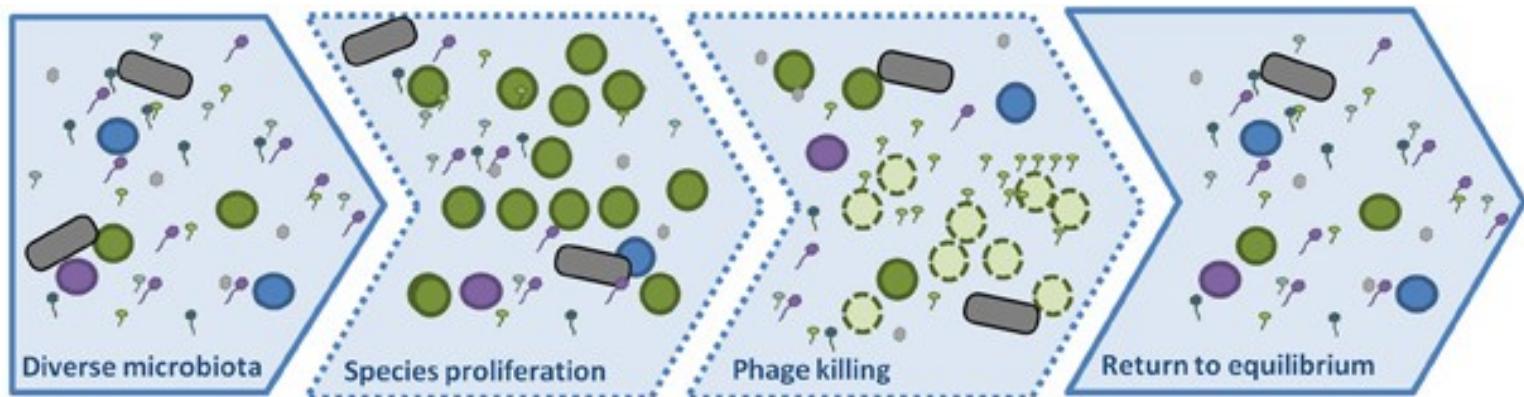
**A**

Kill the  
winner

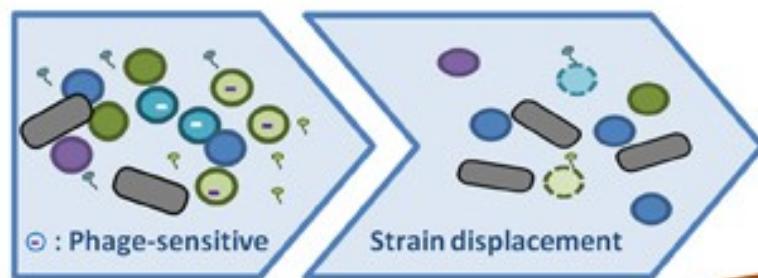


**A**

Kill the  
winner

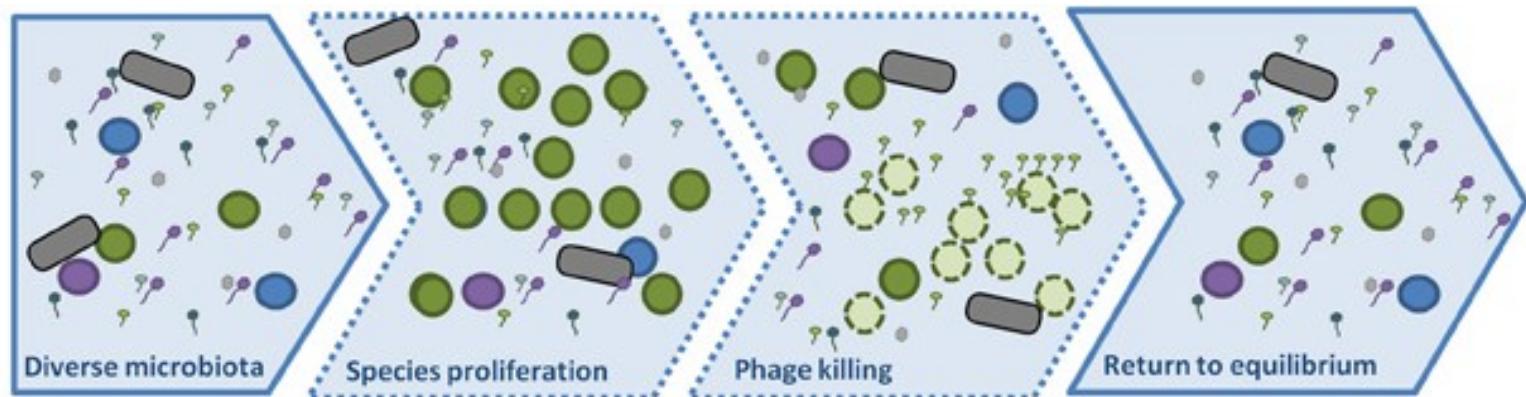
**B**

Kill the  
relative

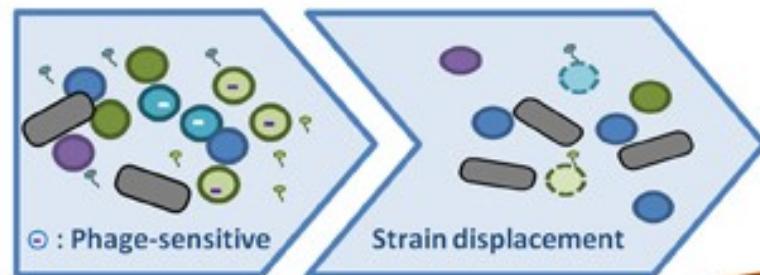


**A**

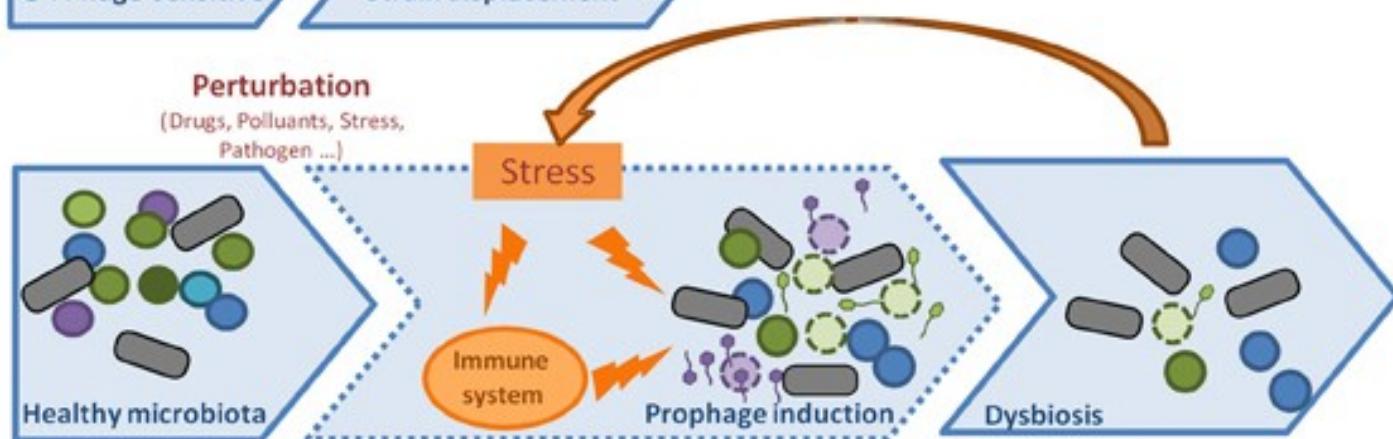
Kill the  
winner

**B**

Kill the  
relative

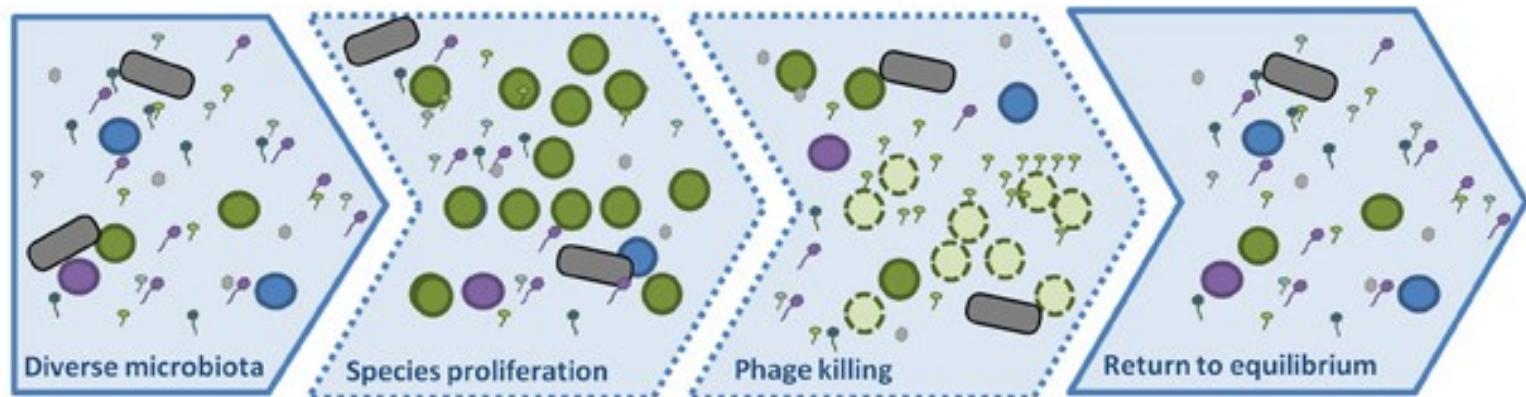
**C**

Community  
shuffling

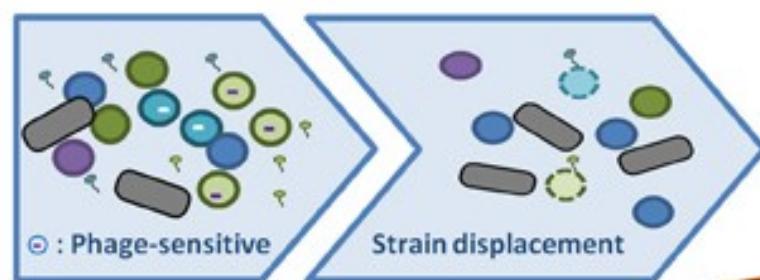


**A**

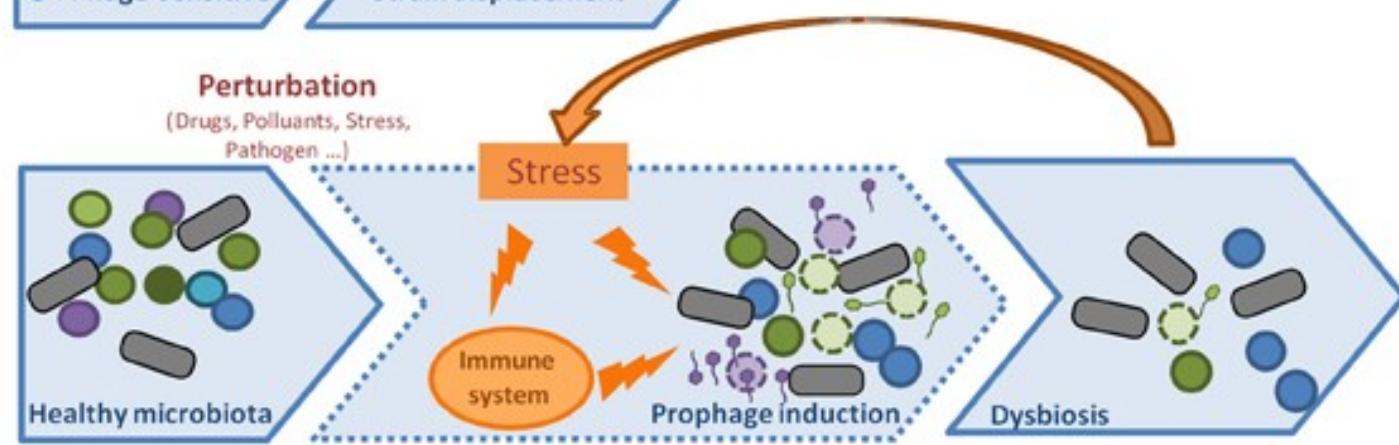
Kill the  
winner

**B**

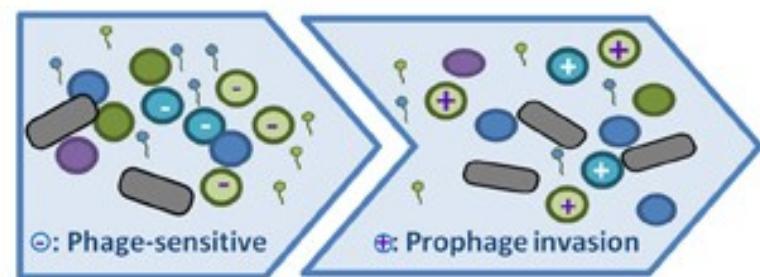
Kill the  
relative

**C**

Community  
shuffling

**D**

Invoke the  
relative



# The Red Queen Hypothesis

The Red Queen hypothesis is an evolutionary hypothesis which proposes that organisms must constantly adapt, evolve, and proliferate not merely to gain reproductive advantage, but also simply to survive while pitted against ever-evolving opposing organisms in an ever-changing environment, and *intends* to explain two different phenomena

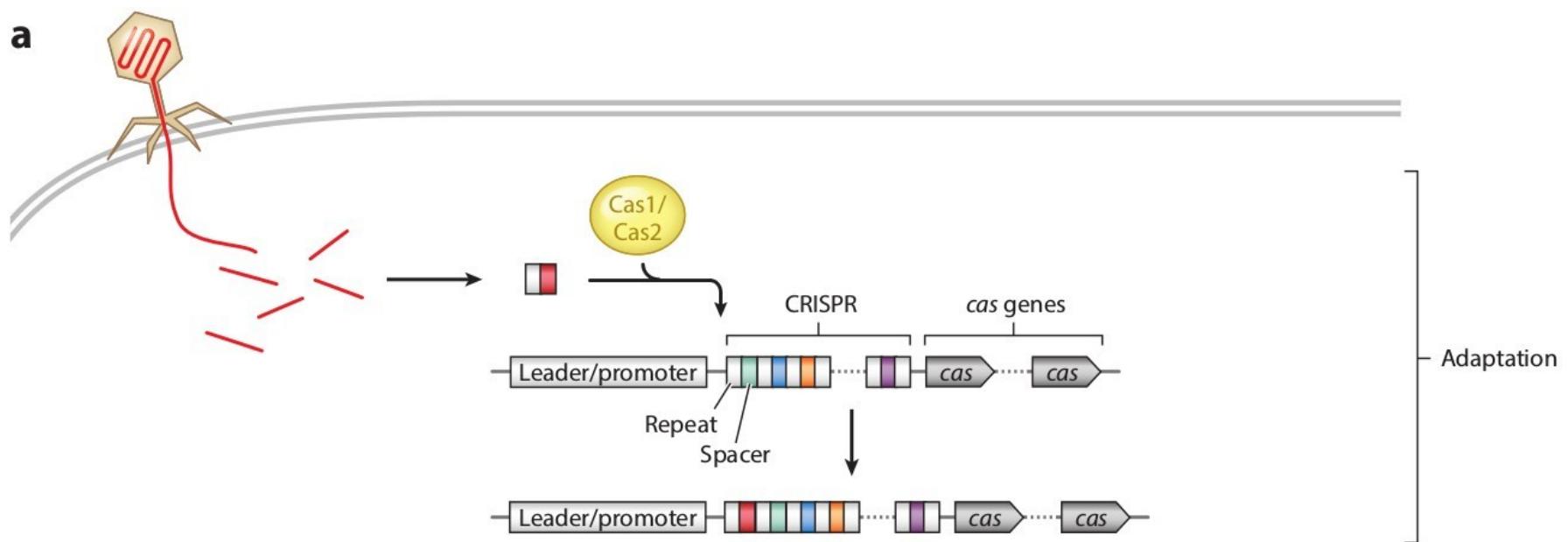


"The Red Queen has to run faster and faster in order to keep still where she is. That is exactly what you all are doing!"

# CRISPR-Cas System

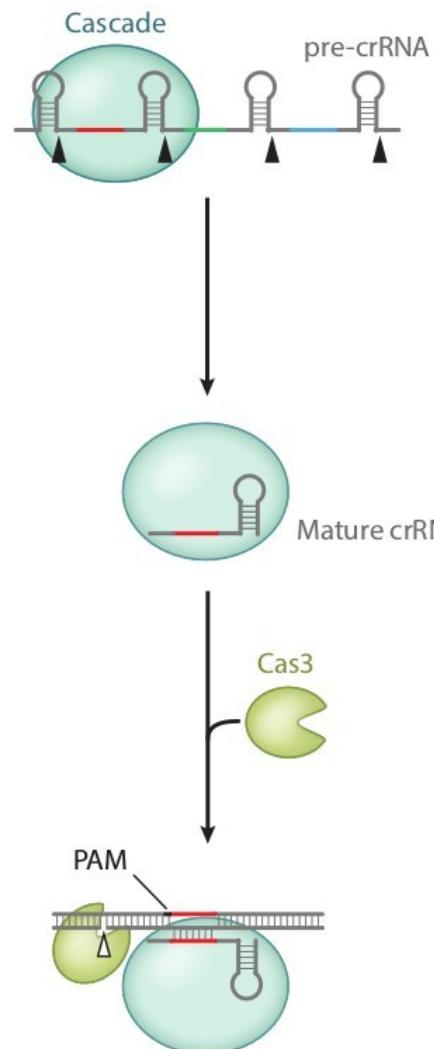
Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated Cas proteins function as an adaptive, small-RNA-based immune system that protects prokaryotes from infectious viruses and plasmids.

CRISPR-Cas immunity develops in three phases:

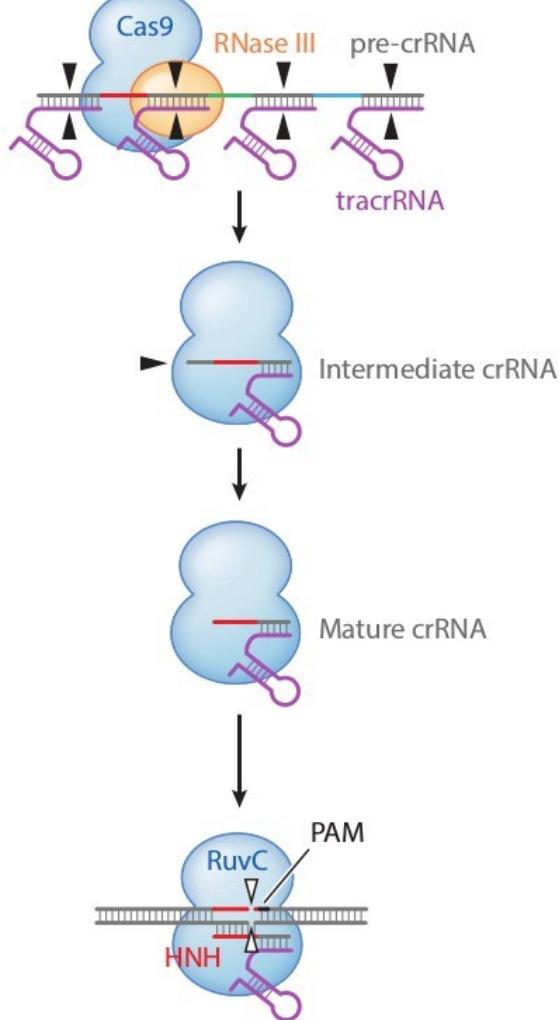


# CRISPR-Cas System

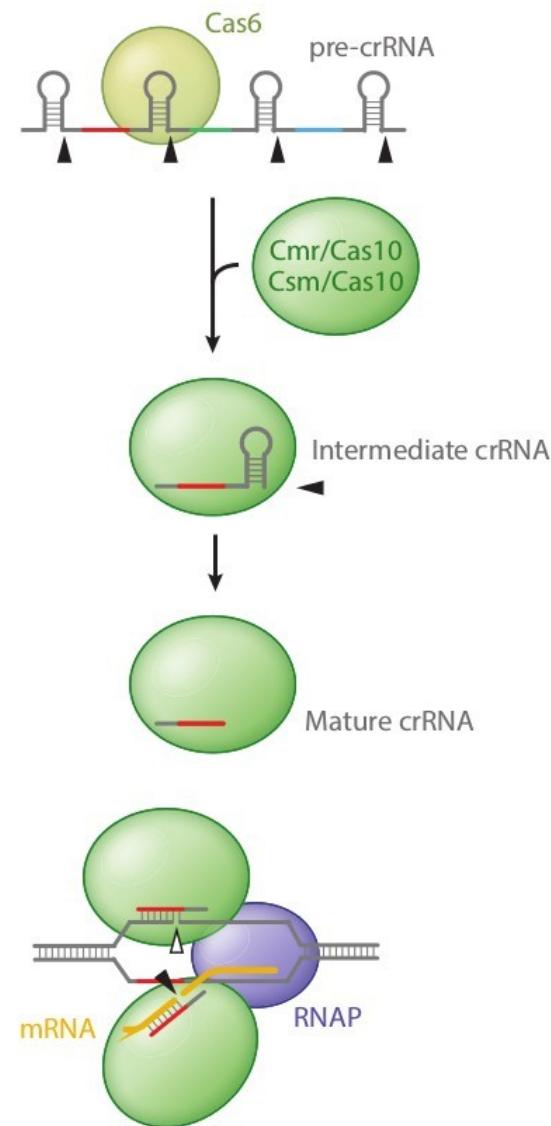
**b** Type I



**c** Type II



**d** Type III



crRNA biogenesis

Targeting

# *Virus role in controlling metabolism*

*Infection of microbes can alter host metabolism.*

*Virus-induced changes in host metabolism make it biochemically and metabolically a very different cell.*

## **Examples:**

*Phage-infected cyanobacteria exhibit a higher rate of photosynthesis than their noninfected counterparts.*

*Bacterial cells undergoing active phage infections can also have altered distributions of other major elements, such as nitrogen and phosphorus, making them biochemically unique.*

# Two “specials”: Viroids and Prions

**Viroids** are particle exclusively constituted by RNA (240-400 bp). Those are copied by the host RNA polymerase and proliferate inside the nucleus where they interfere with gene regulations

**Prions:** Infective particles made exclusively by a single protein (208 aa). They accumulate inside cells preventing correct folding of host protein and accumulate cell damage.

# This week read

C.A. Suttle, "Marine viruses - major players in the global ecosystem," *Nat Rev Microbiol*, 5:801-12, 2007

Gregory, A. C., Zayed, A. A., Conceição-Neto, N., Temperton, B., Bolduc, B., Alberti, A., et al. (2019). Marine DNA Viral Macro- and Microdiversity from Pole to Pole. *Cell* 177, 1109-1123.e14.  
doi:10.1016/j.cell.2019.03.040

Dion, M. B., Oechslin, F., and Moineau, S. (2020). Phage diversity, genomics and phylogeny. *Nature Reviews Microbiology* 18, 125–138. doi:10.1038/s41579-019-0311-5