The extent of protist diversity

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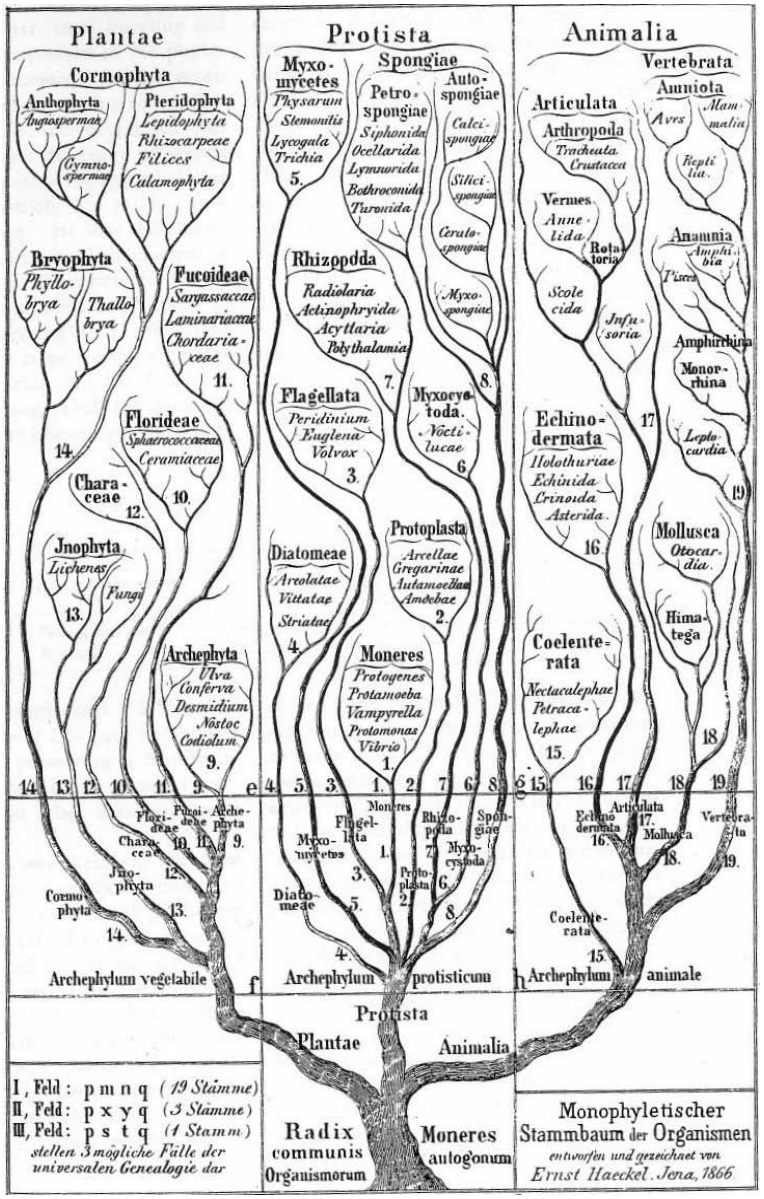
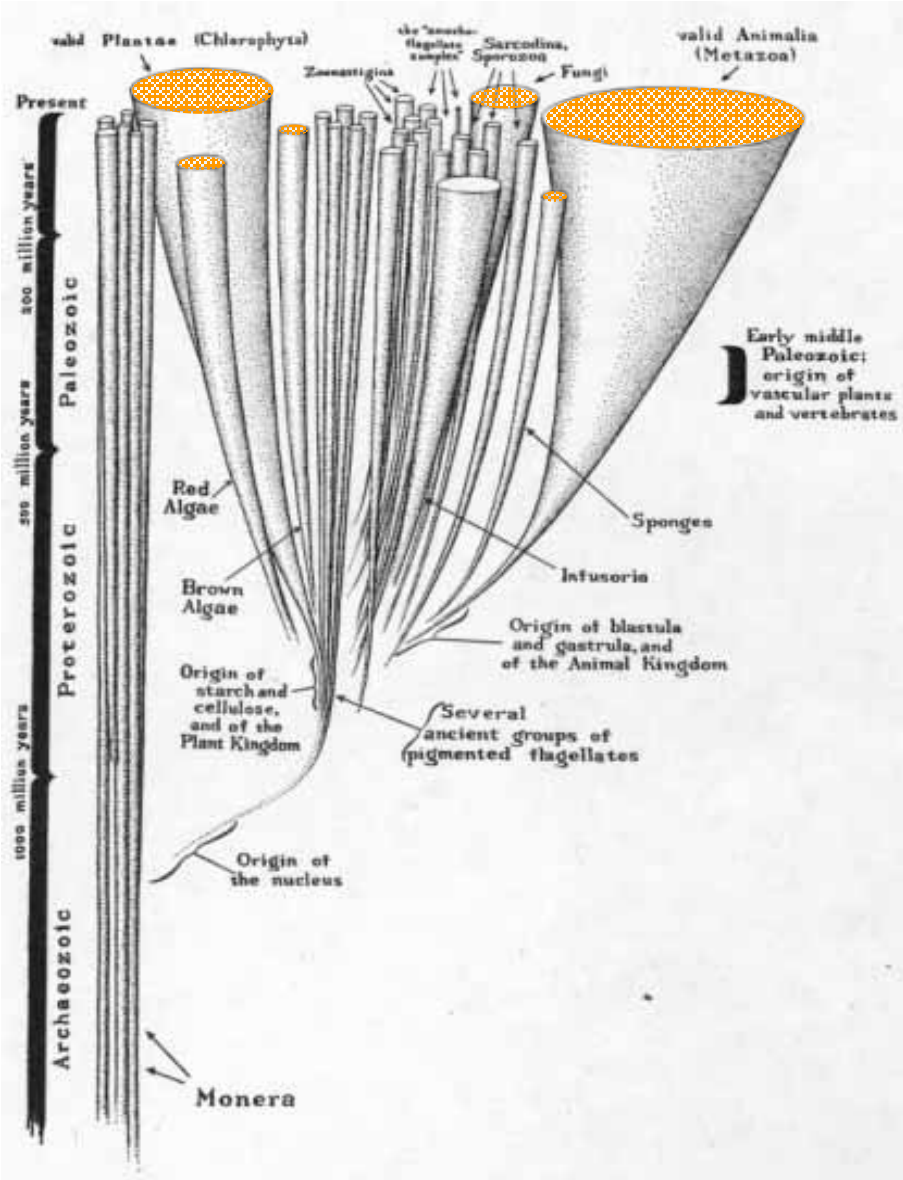
The first phylogenies



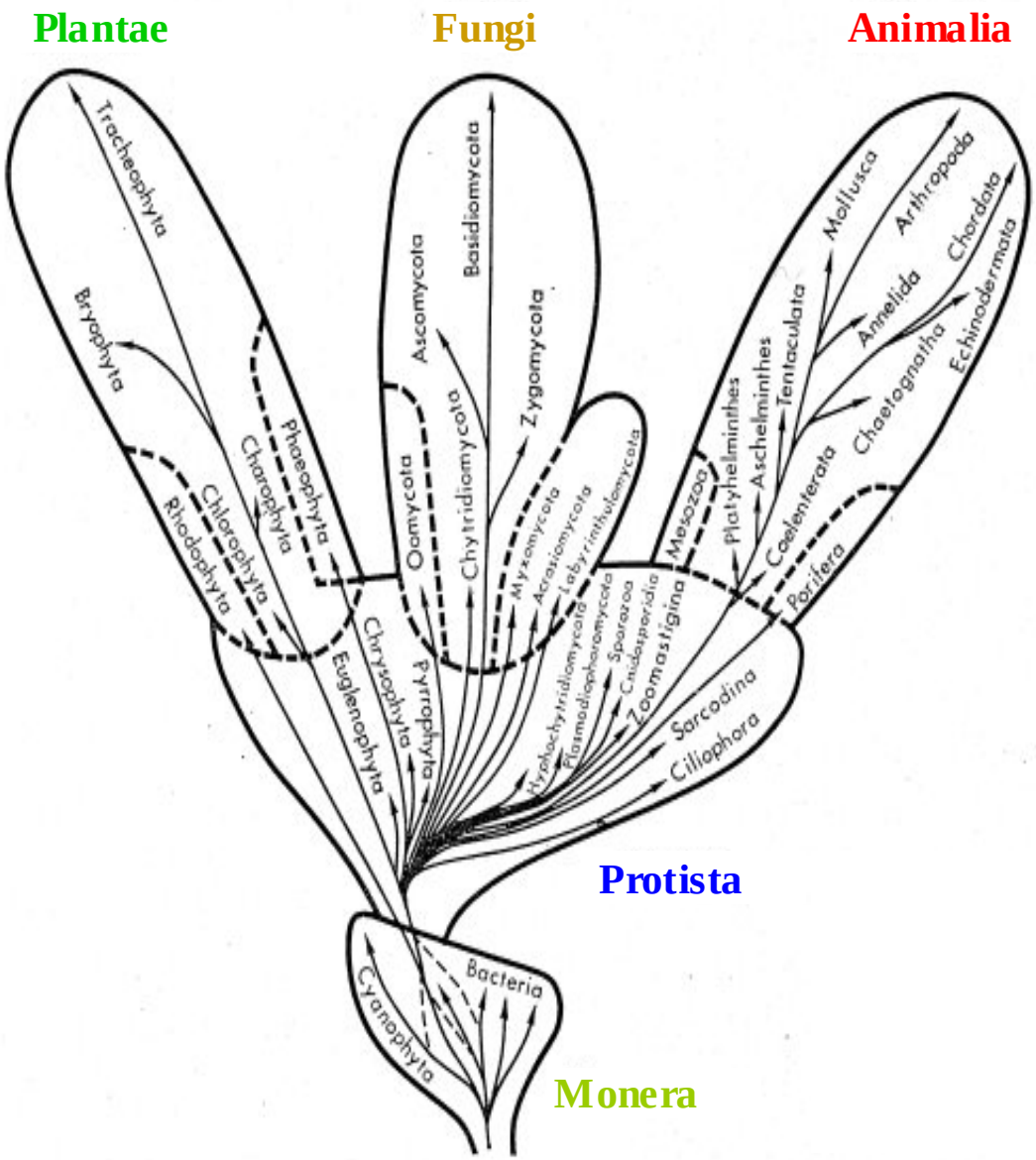
Haeckel, 1866

The first phylogenies



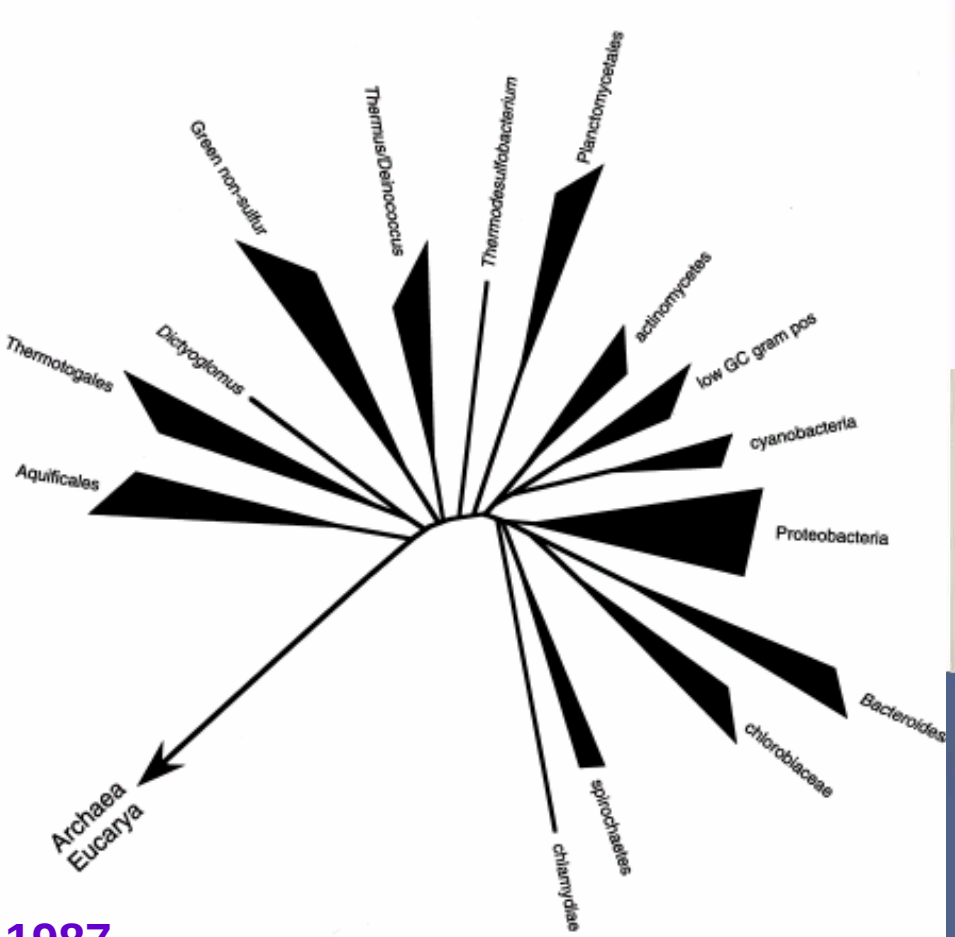
Copeland, 1938

The first phylogenies

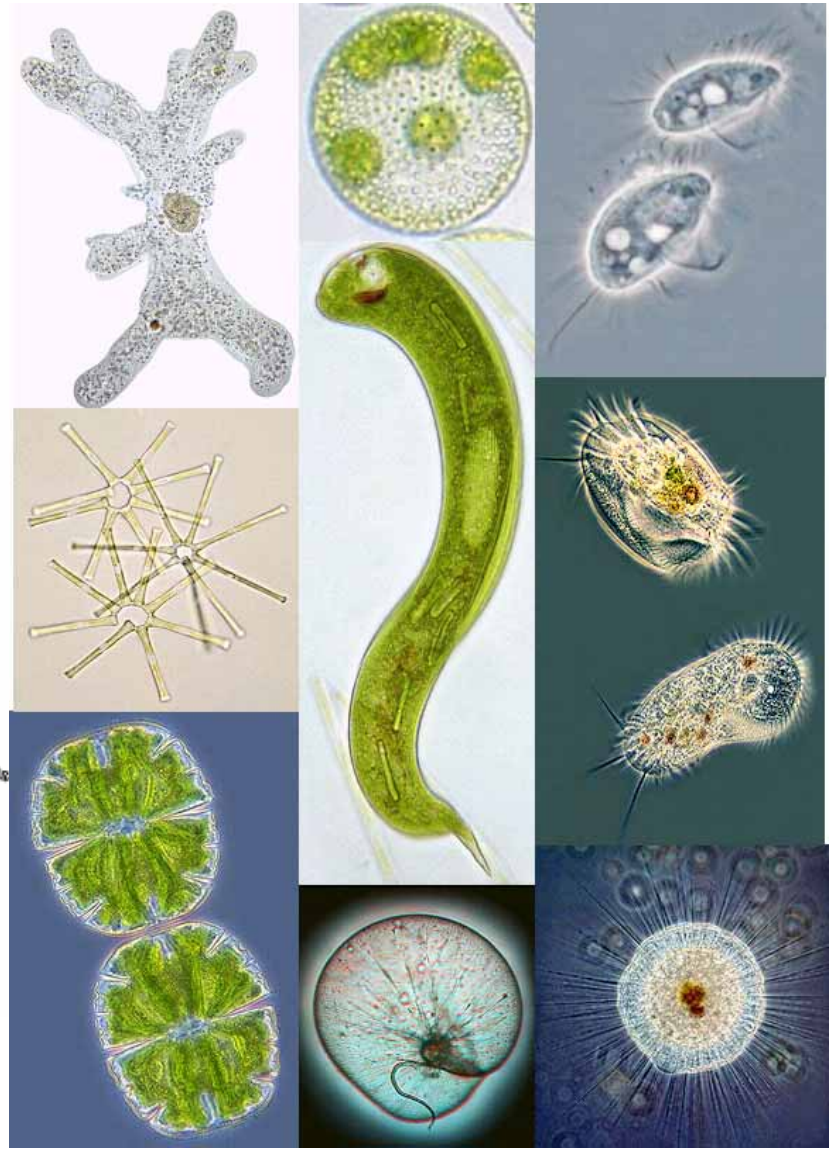


Whittaker, 1969

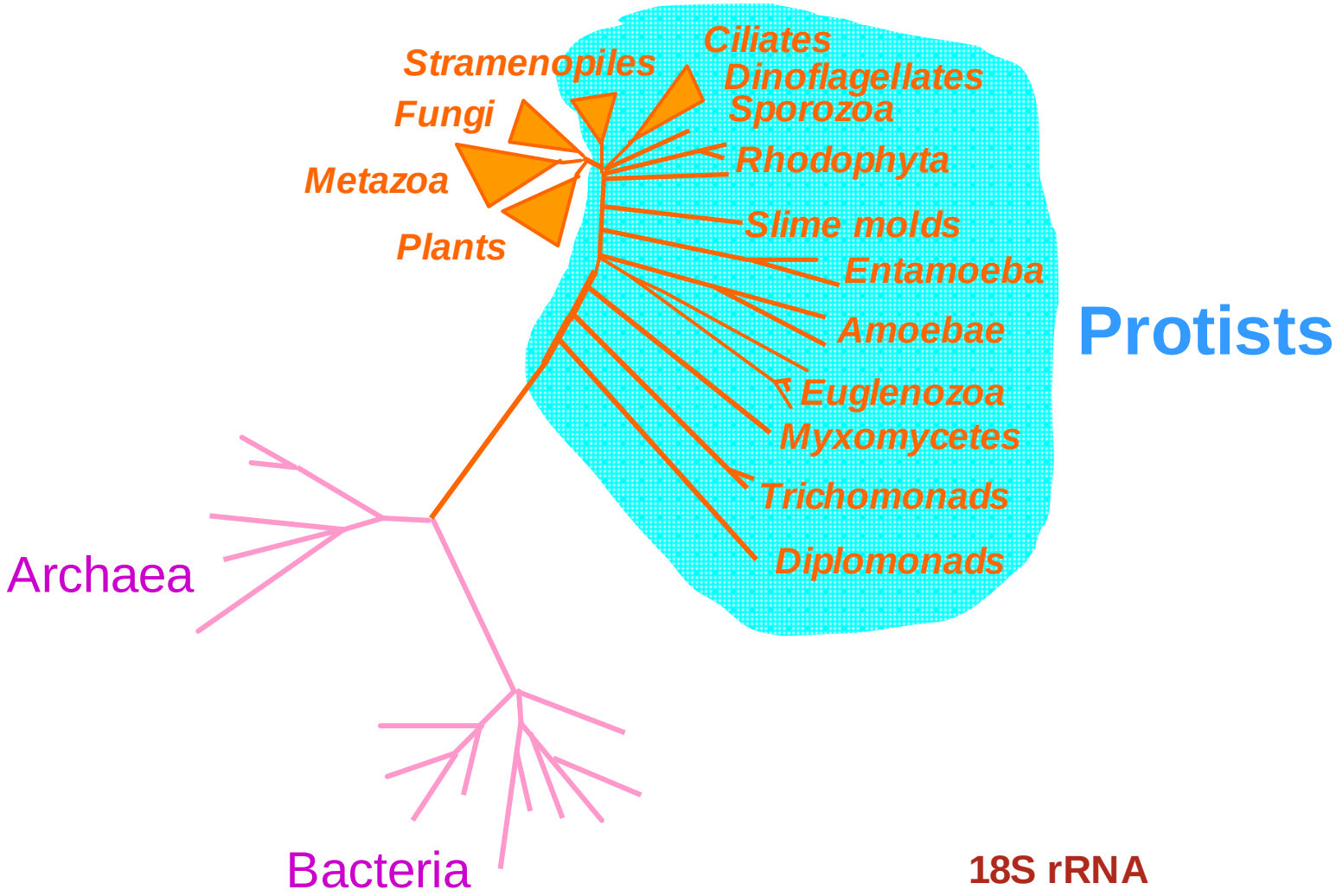
The advent of molecular phylogeny



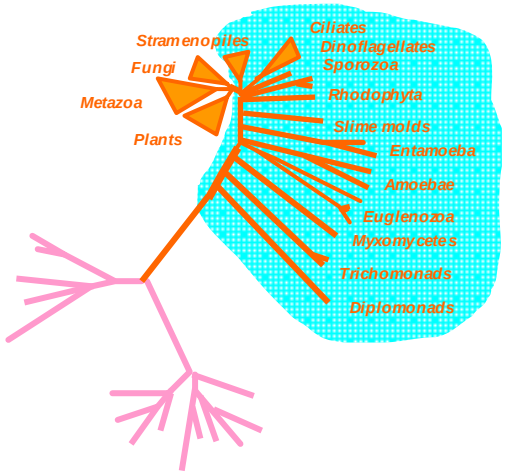
1987

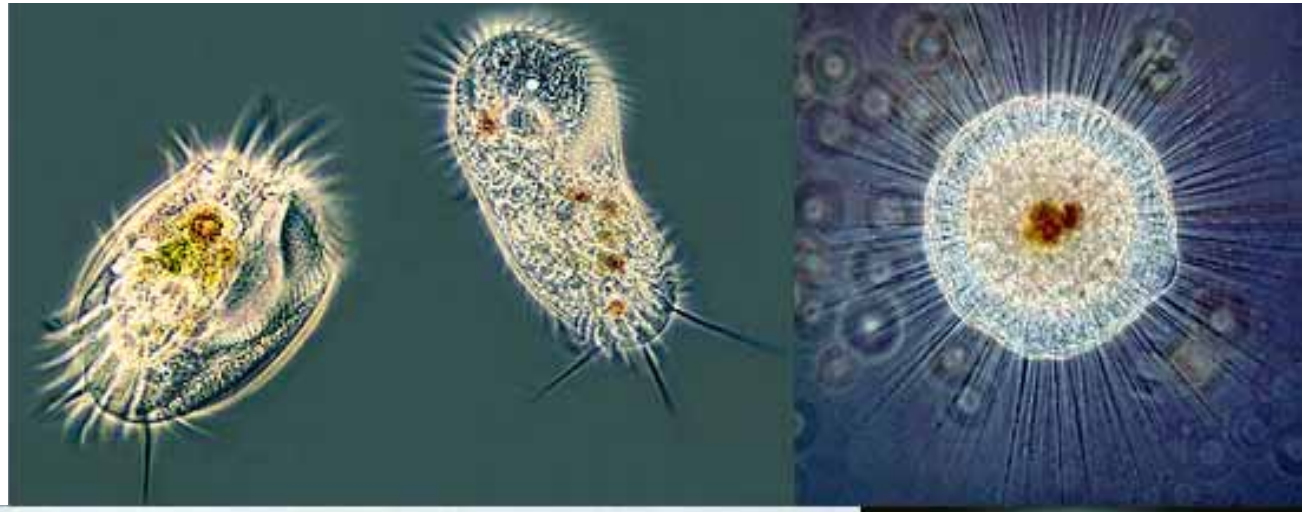


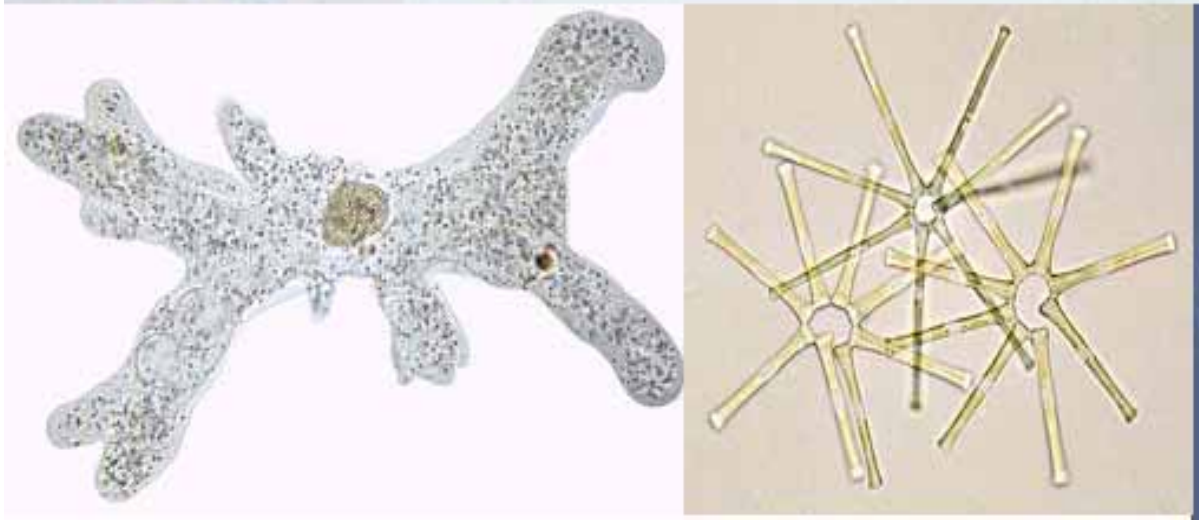
The advent of molecular phylogeny

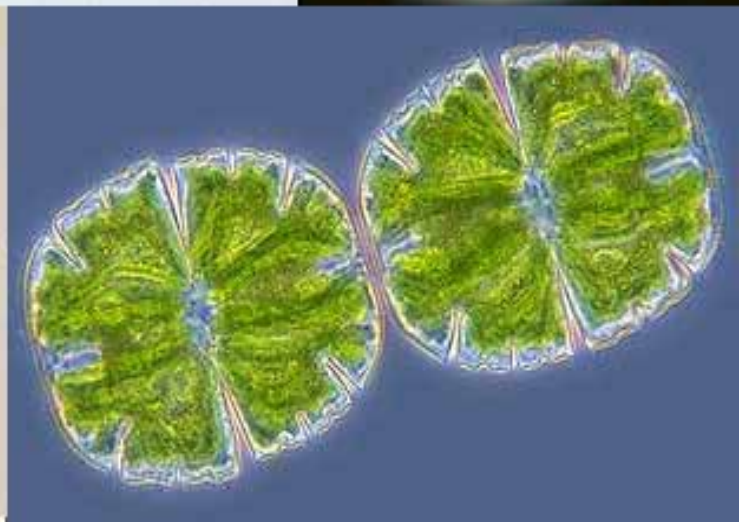


Sogin, 1991

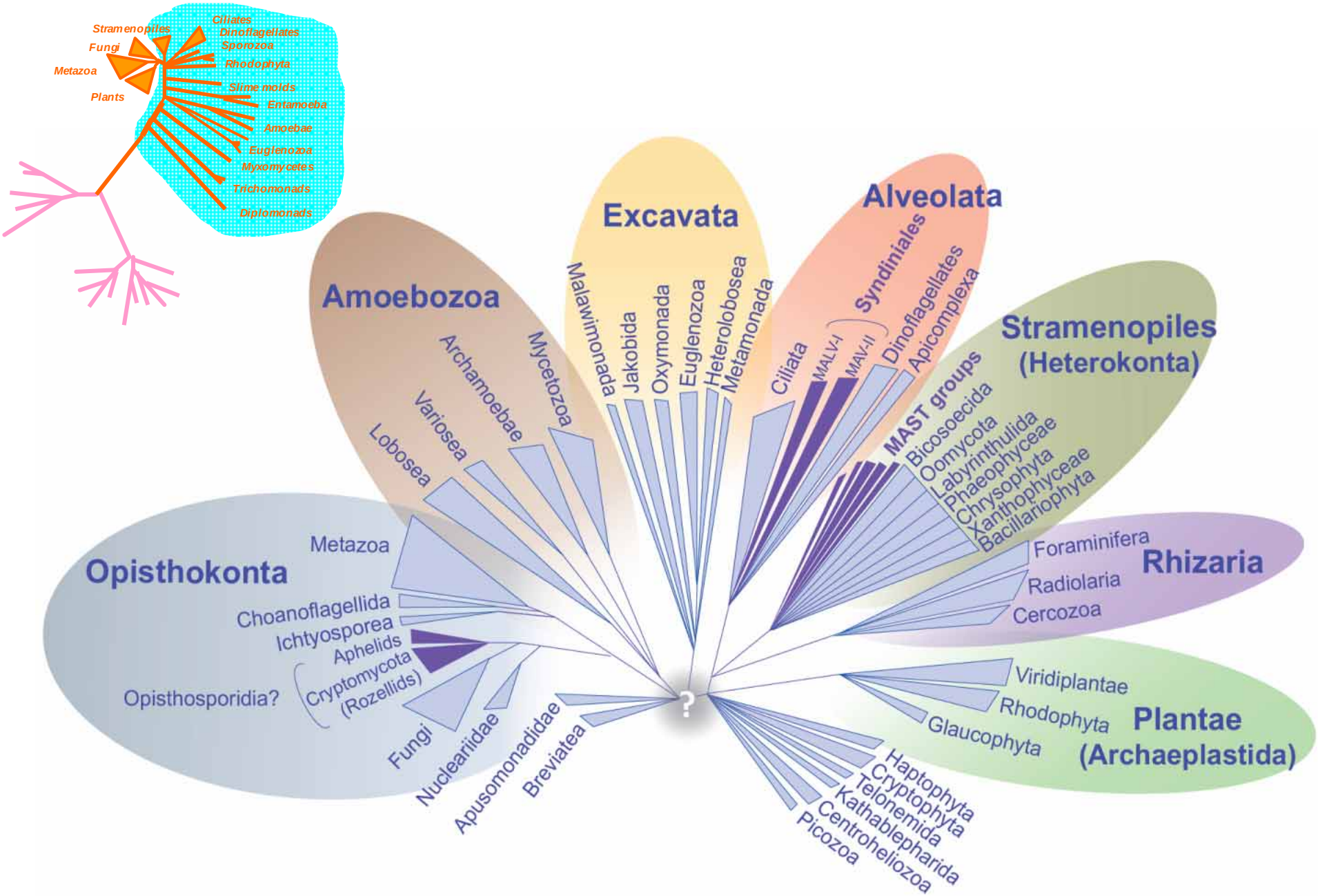

Stramenopiles
Fungi
Metazoa
Plants
Ciliates
Dinoflagellates
Sporozoa
Rhodophyta
Slime molds
Entamoeba
Amoebas
Euglenozoa
Myxomycetes
Trichomonads
Diplomonads







Molecular phylogeny of eukaryotes



Are all eukaryotes already known?

How many protist species?

ENVIRONMENTAL MICROBIOLOGY
VIEWPOINT

Global Dispersal of Free-Living Microbial Eukaryote Species

Small size = ubiquity = low genetic diversity = few species?

sented with the opportunity of evolutionary diversification because they were never restricted by geographical barriers.

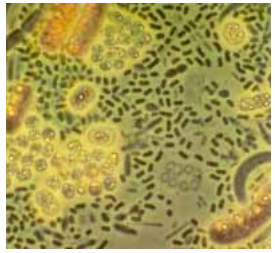
BLAND J. FINLAY[1]* AND TOM FENCHEL[2]

~1 mm

log(Length)

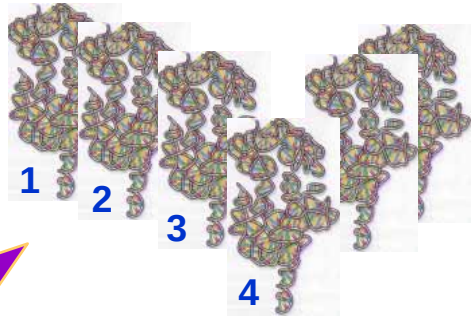
How to study microbial diversity?

The problem...

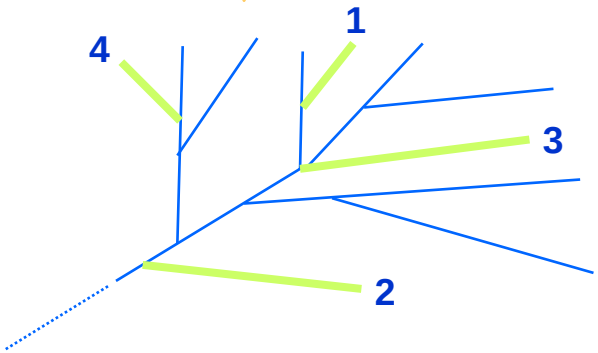


"The Great Plate Count Anomaly"

A solution...



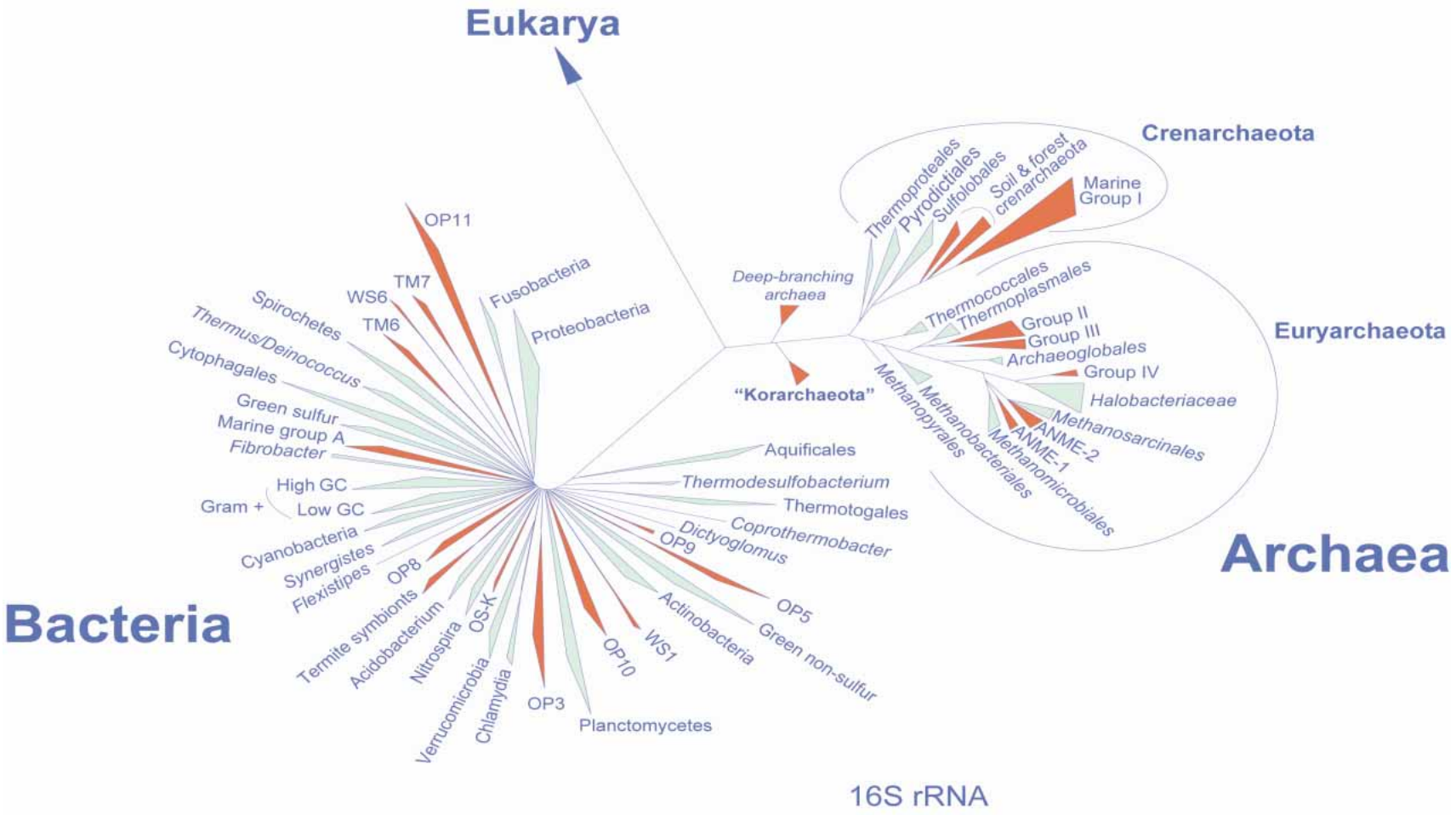
PCR amplification of rRNA genes directly from environmental samples



Sequence comparison
Construction of phylogenetic trees

Molecular surveys of microbial diversity

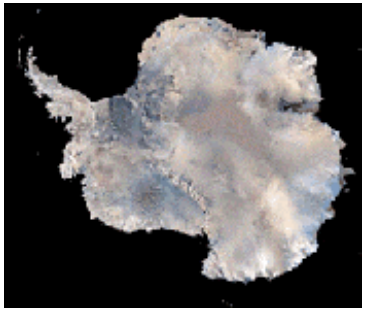
rRNA gene sequencing from non-cultured species



At least 60 prokaryotic phyla, half of them without cultured representatives

Schloss & Handelsman, MMBR 2004

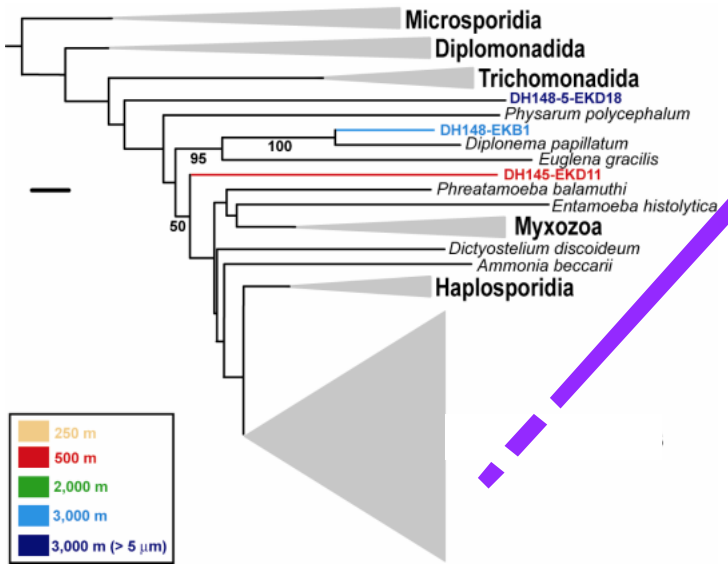
Marine protist diversity

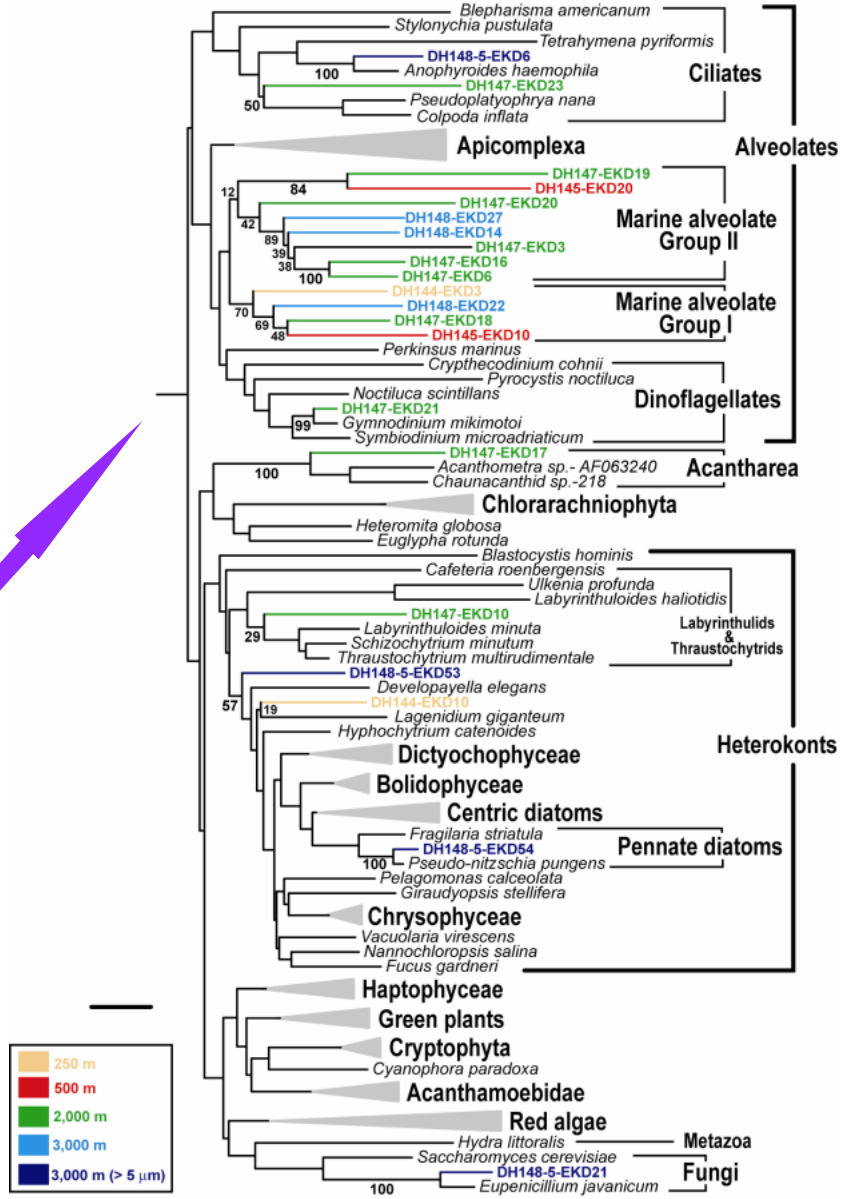


Plankton <5 µm

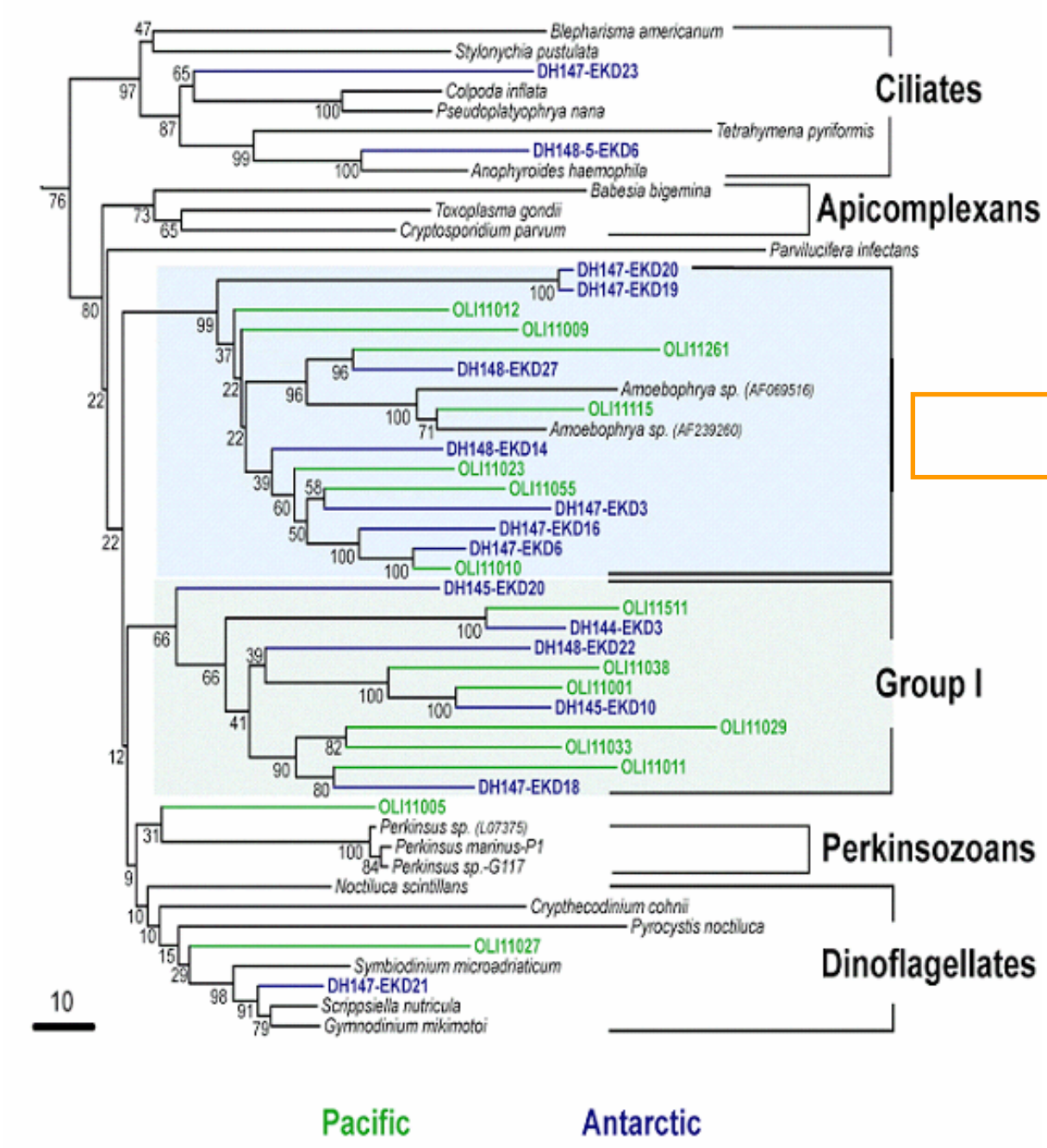
250 - 3,000 m

18S rDNA



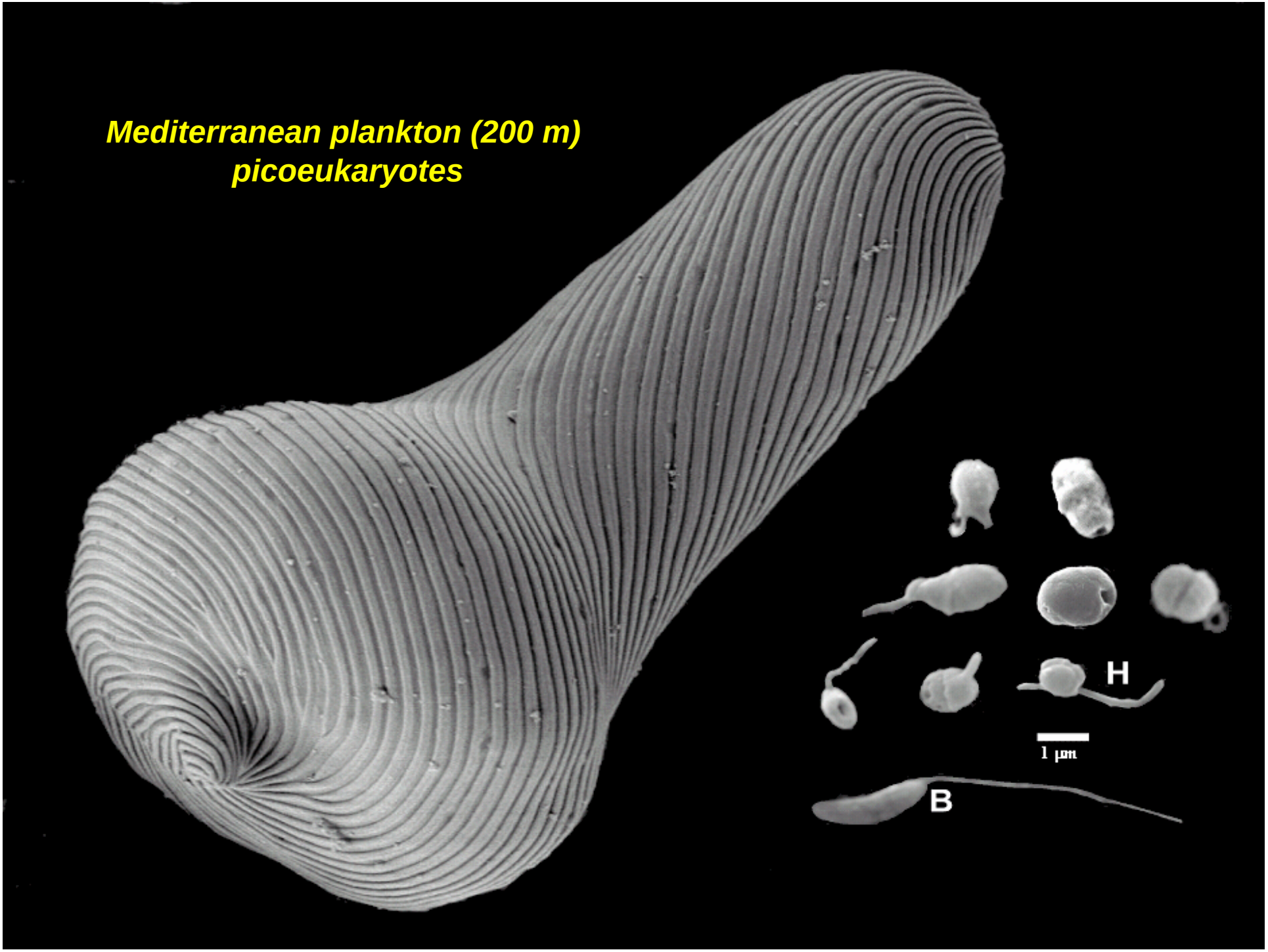


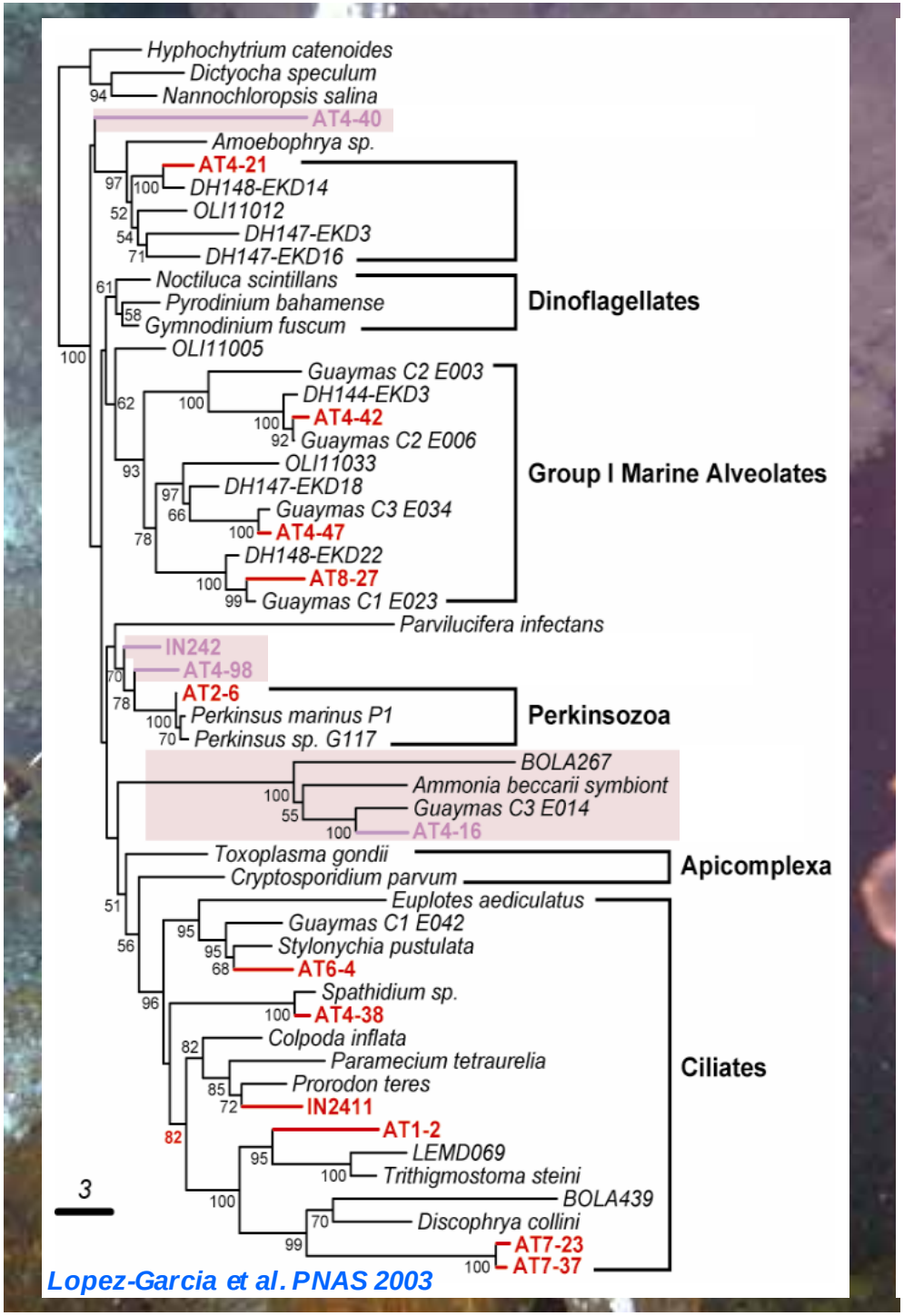
Lopez-Garcia et al. Nature 2001



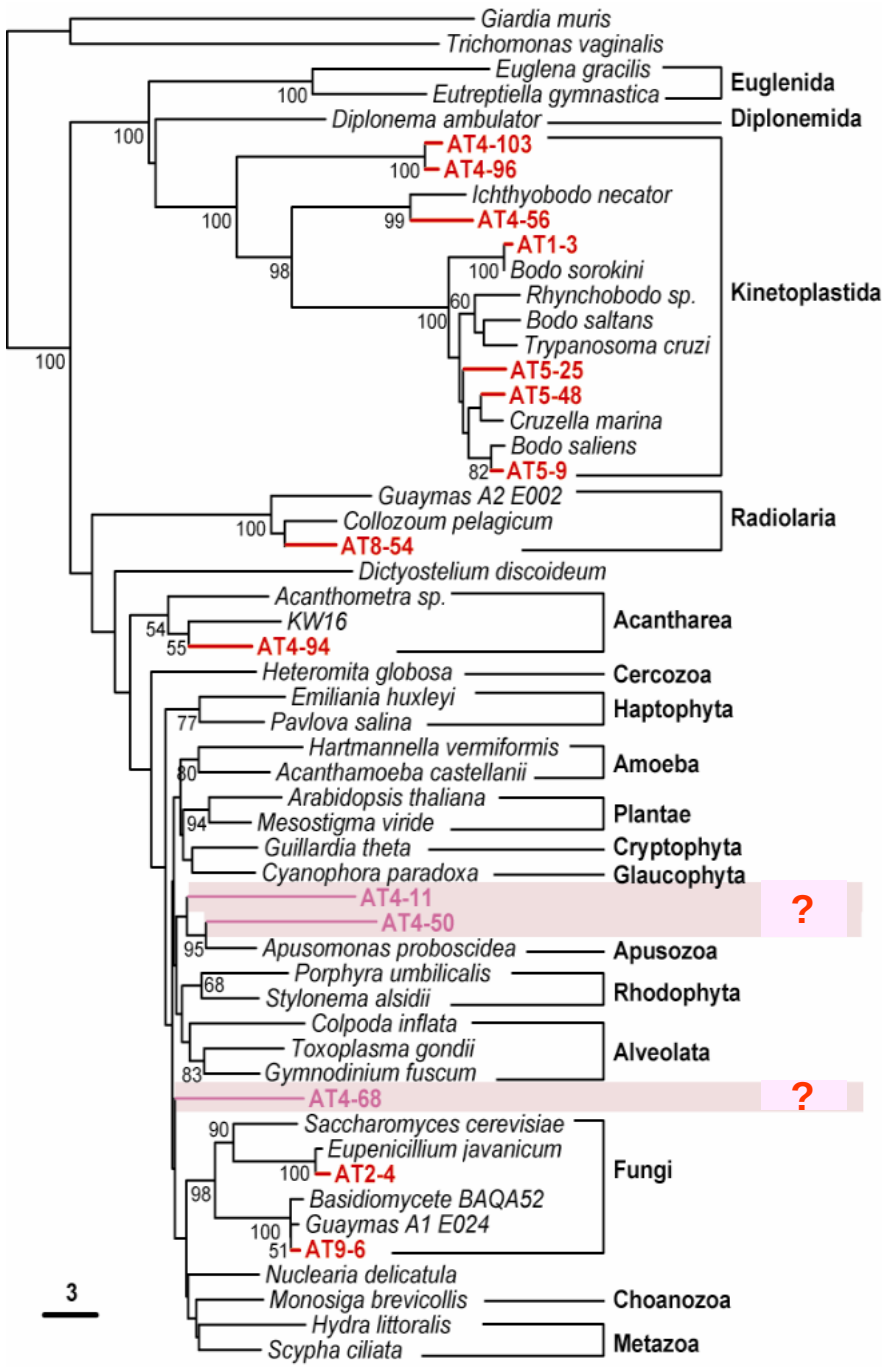
***Amoebophrya* sp.**

Moreira & Lopez-Garcia, Trends Microbiol 2002

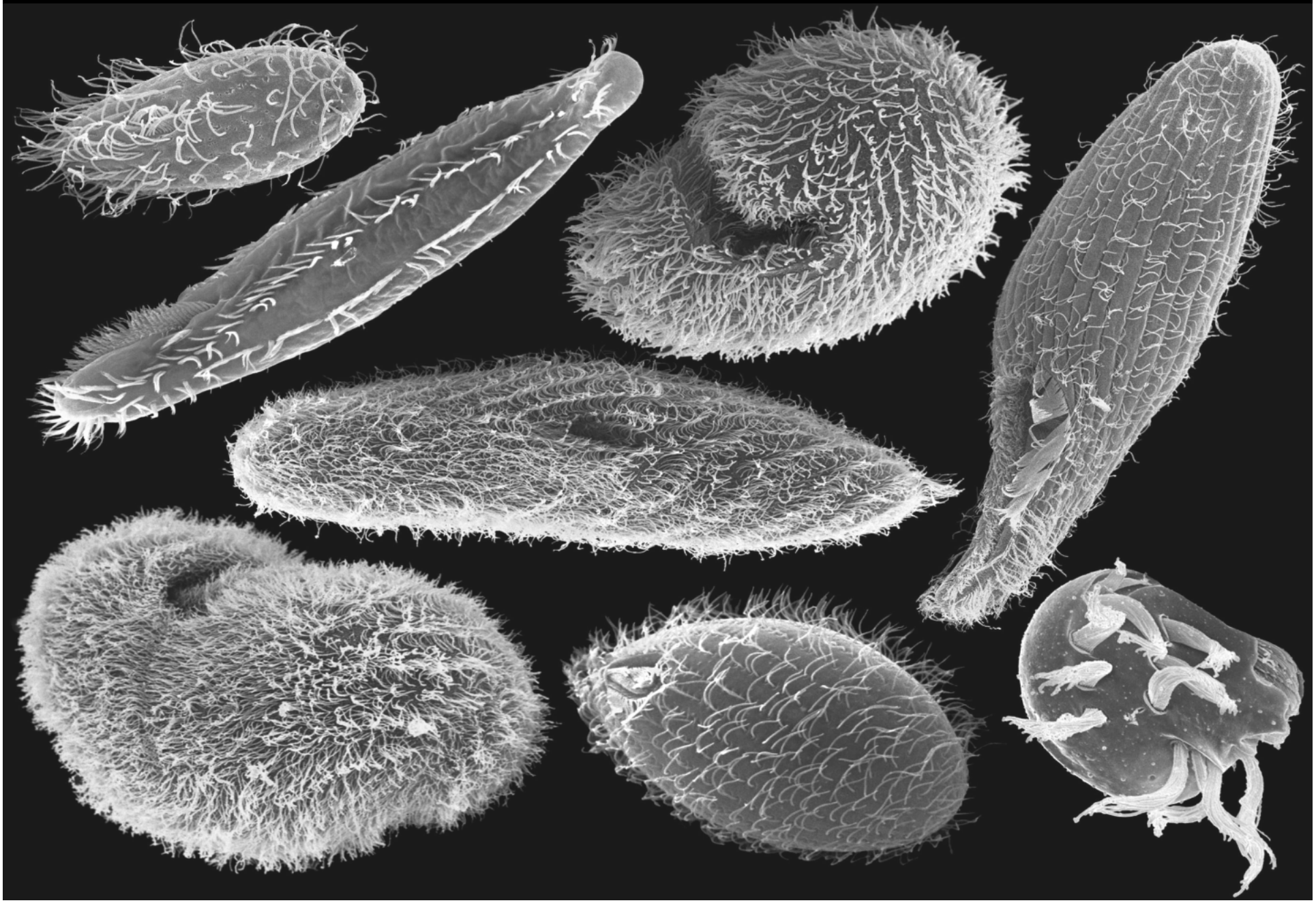

Mediterranean plankton (200 m)
picoeukaryotes
H
1 µm
B



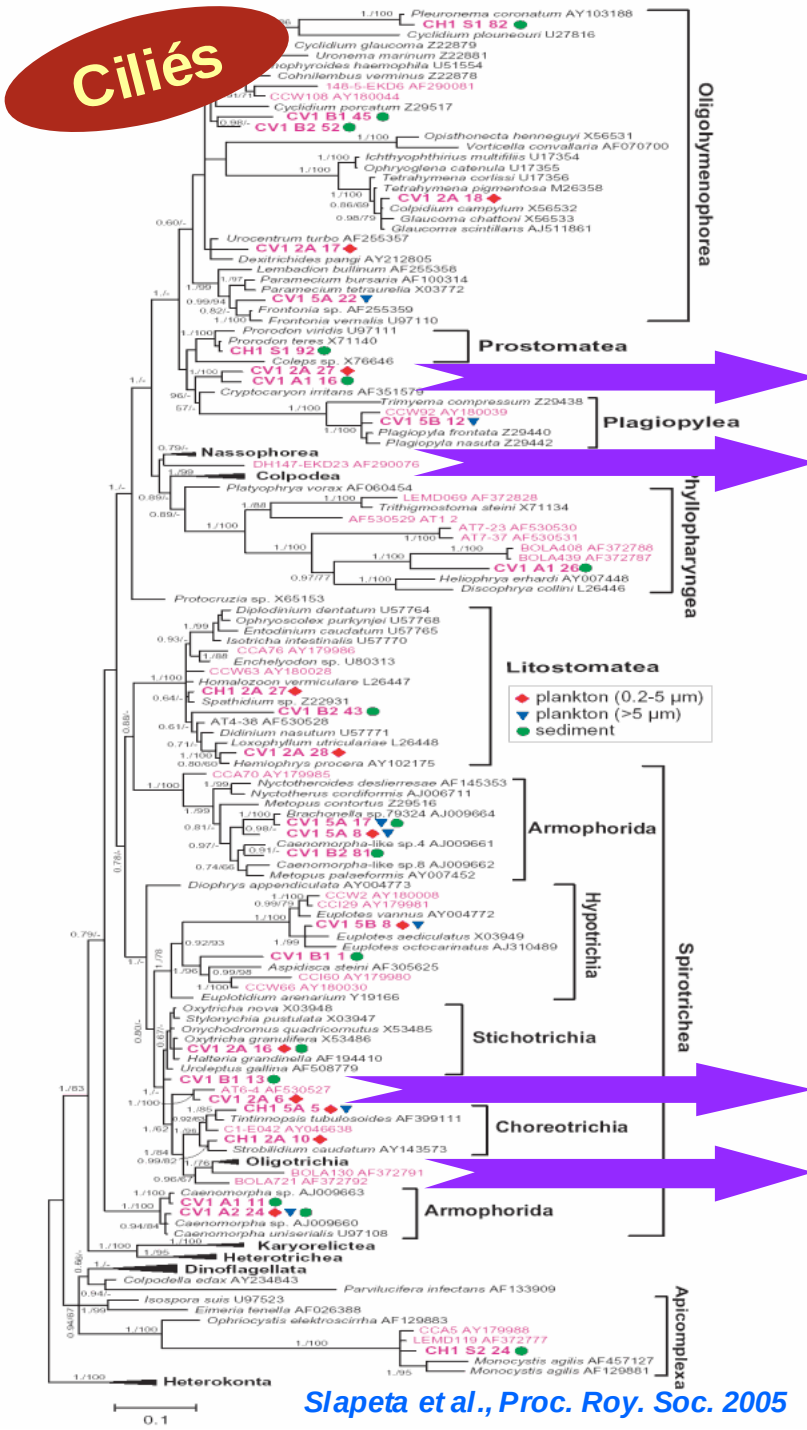
Lopez-Garcia et al. PNAS 2003



Ciliates



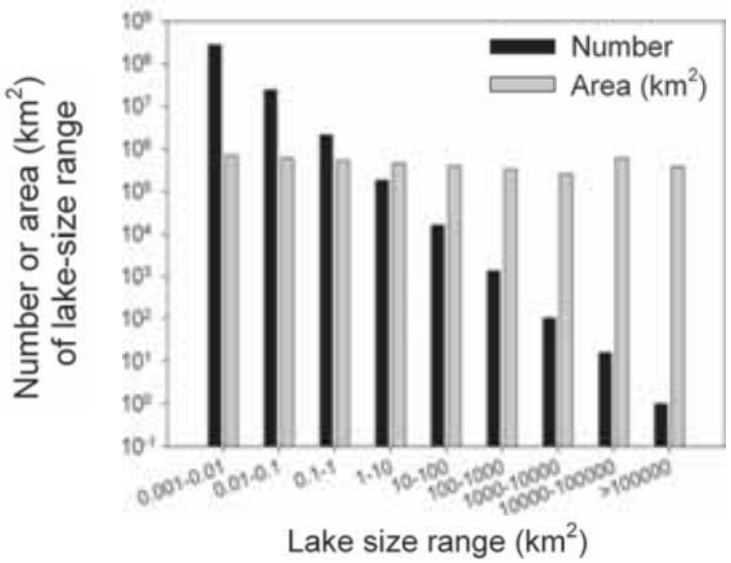
Ciliés



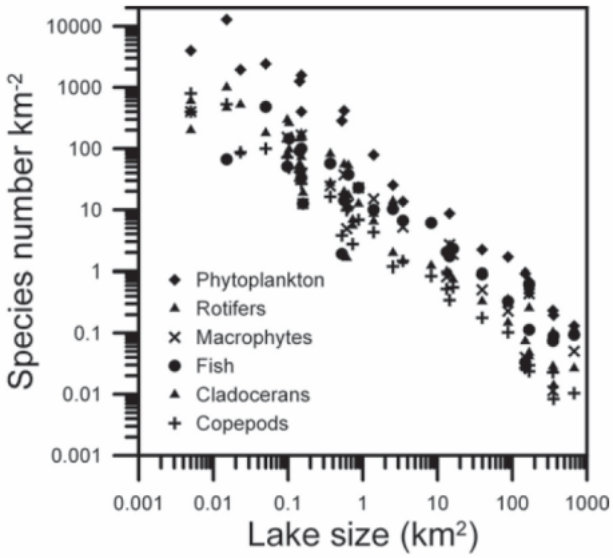
Slapeta et al., Proc. Roy. Soc. 2005

Small freshwater systems

- ~ 3 billion lakes 100-1000 m^2
- Highly diverse
- Little inertia – strong influence of environmental conditions
- Ecologically important – very active, CO_2 fixation

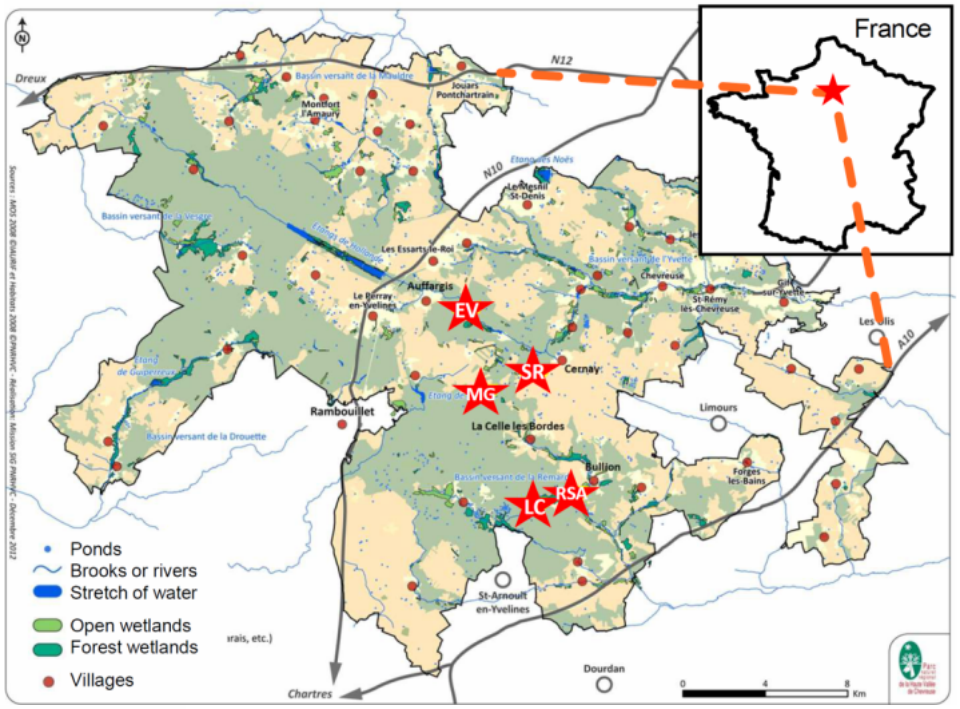


Downing, 2010

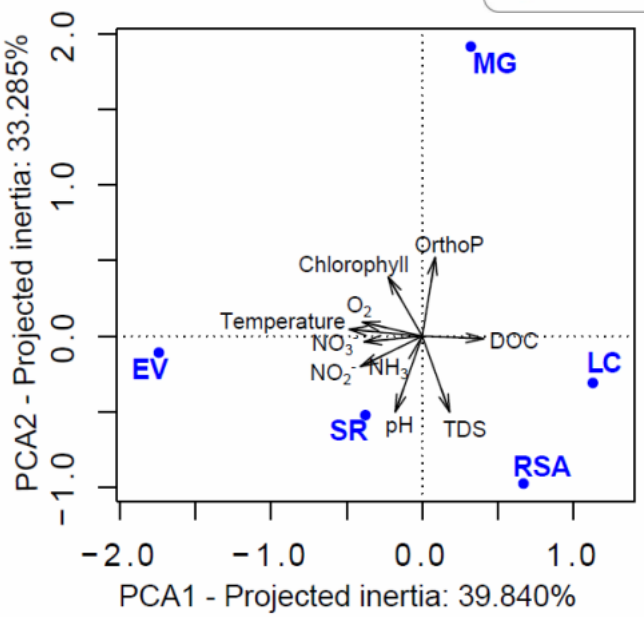


Contrasted shallow freshwater ecosystems

Parc naturel régional de la Haute Vallée de Chevreuse



distances between 2 and 9.5 km



Simon et al., in press

Methods

Water samples → **Physico-chemical parameters**

5 ecosystems, monthly, 2 years

- *In situ:* pH, diss. O_2, salinity, temperature
- Lab: DOC, chlorophyll a, NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+
- Climate variables at regional scale

↓

Sequential filtration – 30 µm, 5 µm, 0.22 µm

↓

DNA purification

↓

PCR amplification 18S rRNA gene fragments

↓

454 pyrosequencing Roche FLX Titanium → CGTAATCGTAACTCGTC

0.2-5 µm
(sometimes also 5-30 µm

↓

Local bioinformatic pipeline (highly stringent):

- Quality check (short sequences, errors in MIDs or primers)
- AmpliconNoise (Quince et al, 2011 – remove additional errors)
- Clustering: operational taxonomic units - **OTUs** (98% id.)
- Distribution of OTUs among samples
- Phylogenetic affiliation (SILVA 111 and RP2 databases)
- Chimera check (various, including semi-manual assessment)

↓

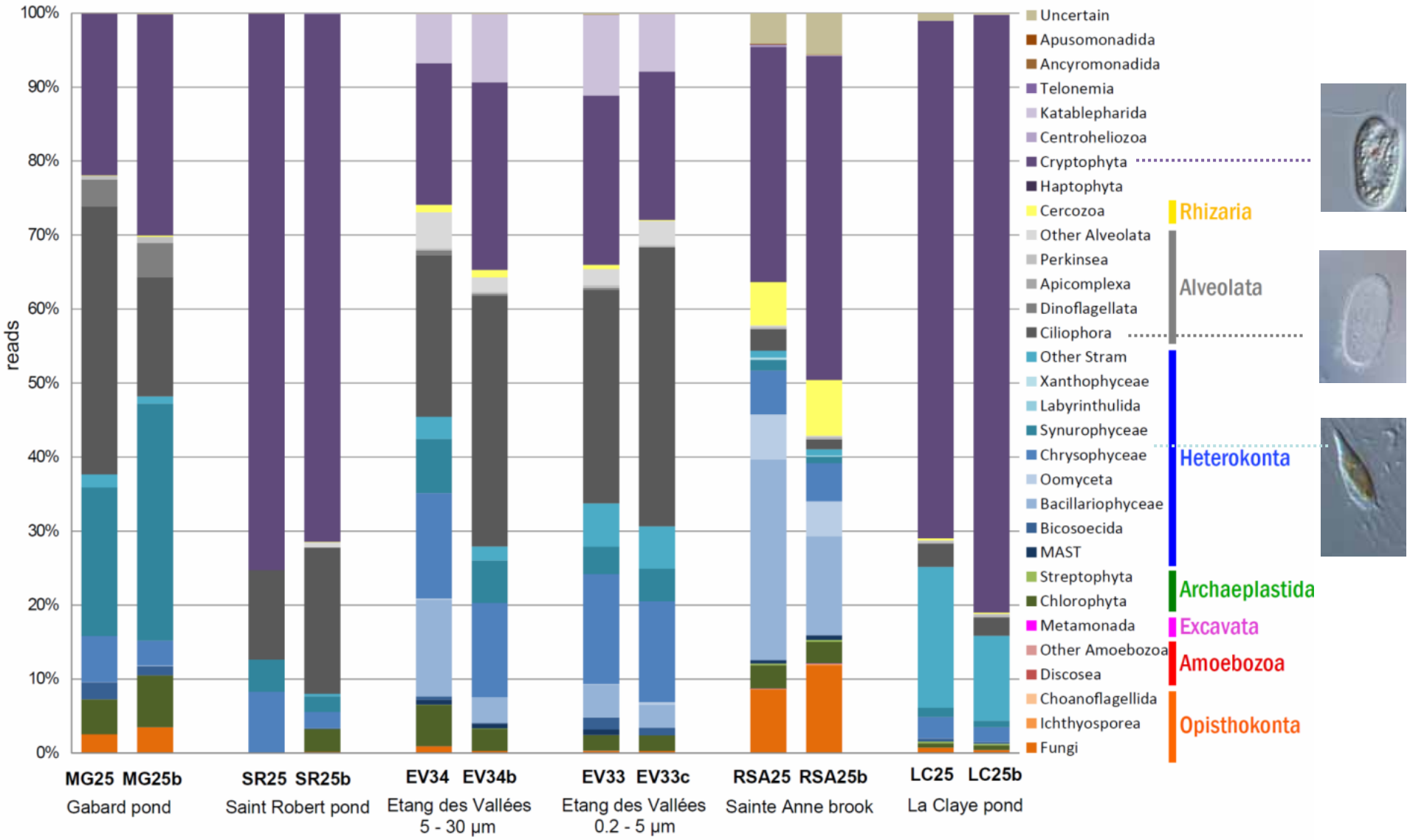
Community composition

↓

Multivariate statistical analyses

	April 2012	2 years
Reads	265,899	1,521,380
Retained	146,549	1,272,748
OTUs	812	3,742

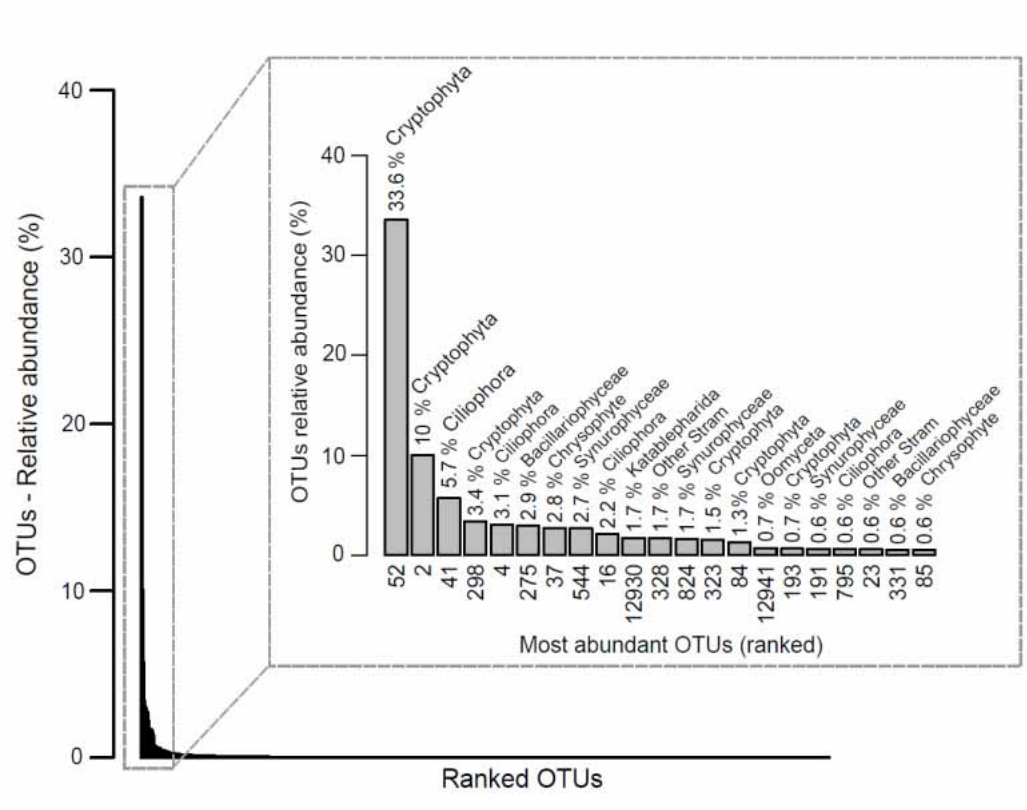
Spatial distribution of small protists (<5 µm) – April 2012

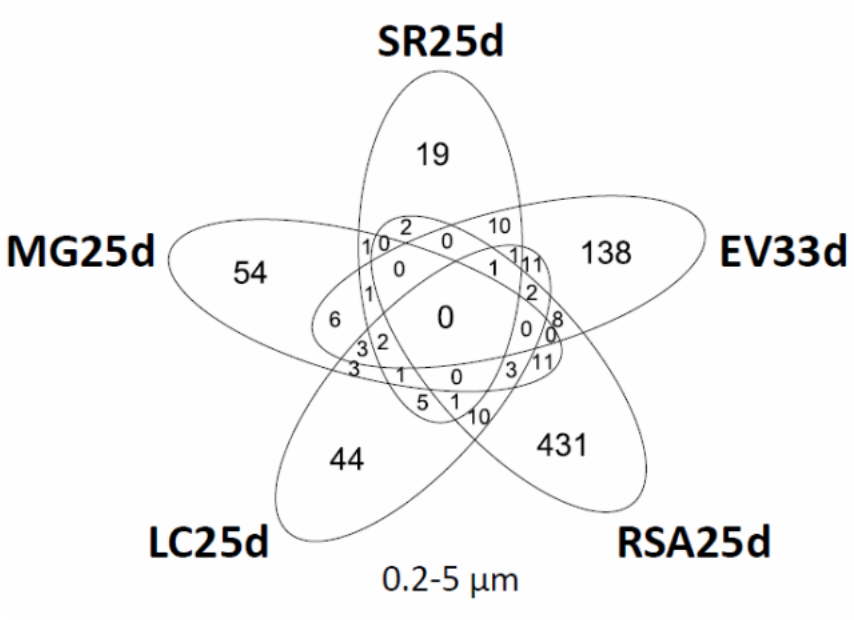


Simon et al., in press

Spatial distribution of small protists – April 2012

812 OTUs (total)



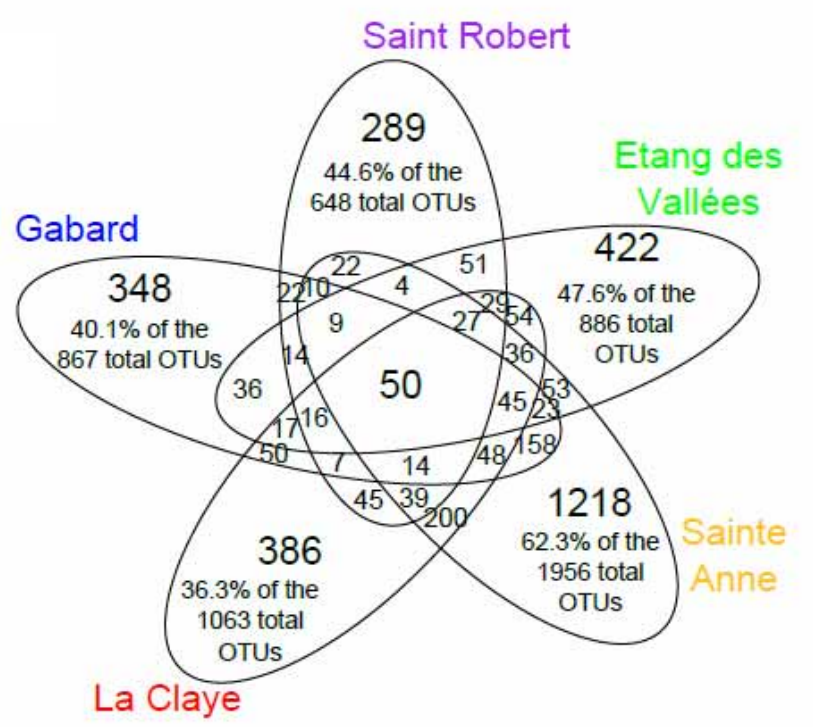


Highly specific communities

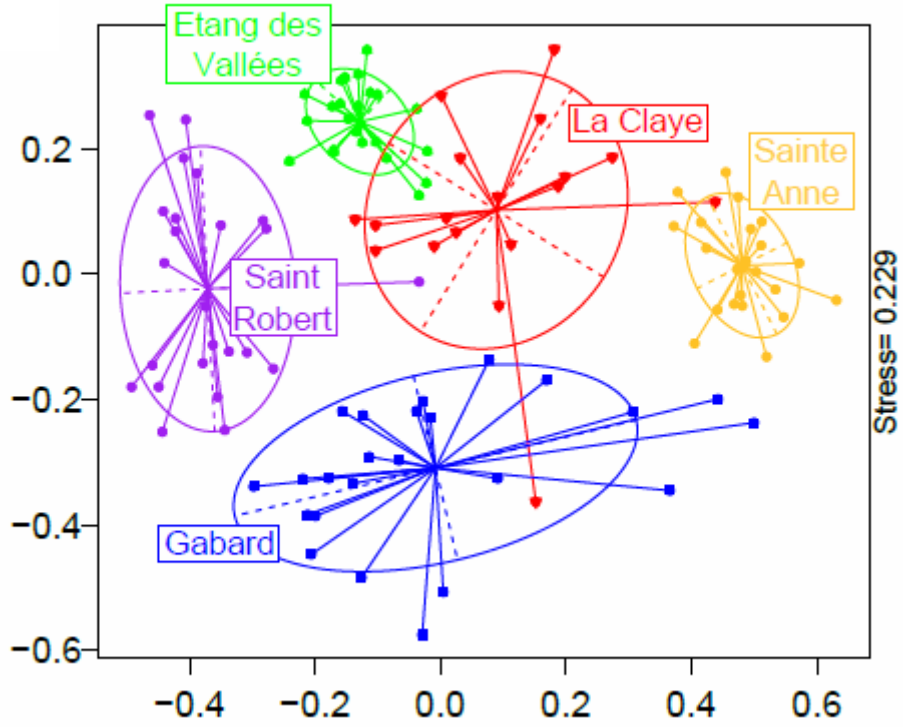
Simon et al., in press

24 months: contrasting communities

3,742 OTUs (total)

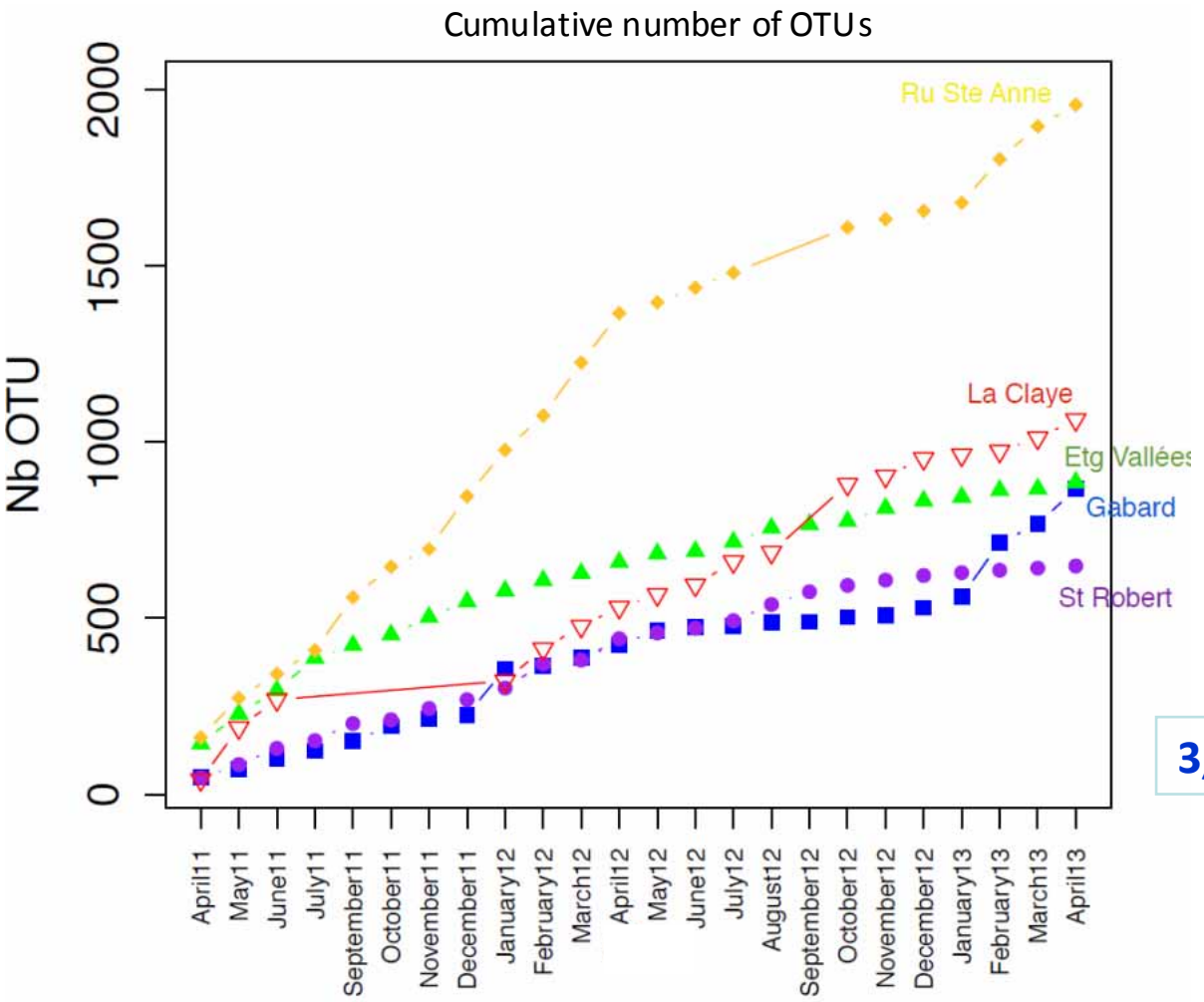


Non-metric MultiDimensional Scaling



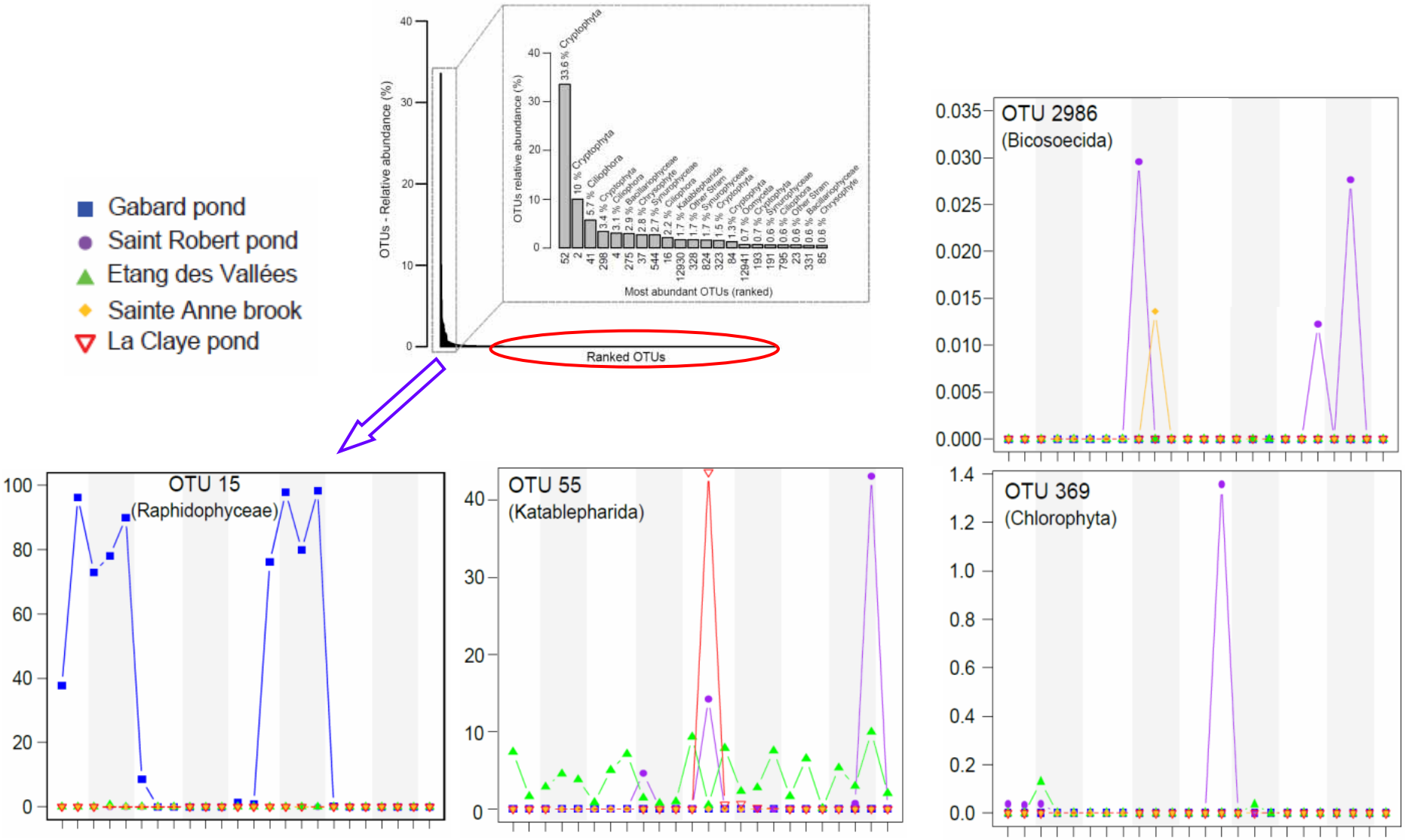
Simon et al., submitted

Large unexplored diversity



3,742 OTUs (total)

Highly variable taxon dynamics



Conclusions from molecular surveys:

- **Few sequences defining "new phyla"**
- **Huge diversity within many groups**

Small size = ubiquity = low genetic diversity = ~~few species~~?

How many protist species?

ENVIRONMENTAL MICROBIOLOGY
VIEWPOINT

Global Dispersal of Free-Living Microbial Eukaryote Species

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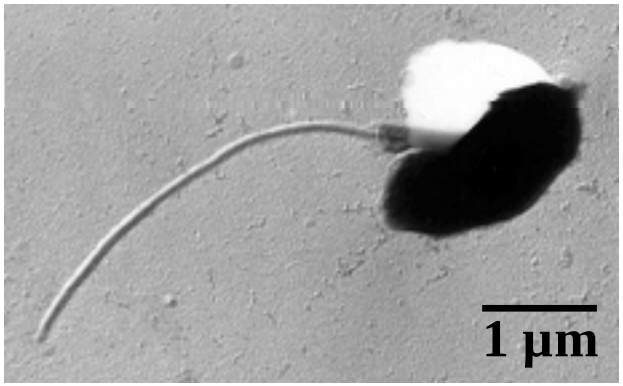
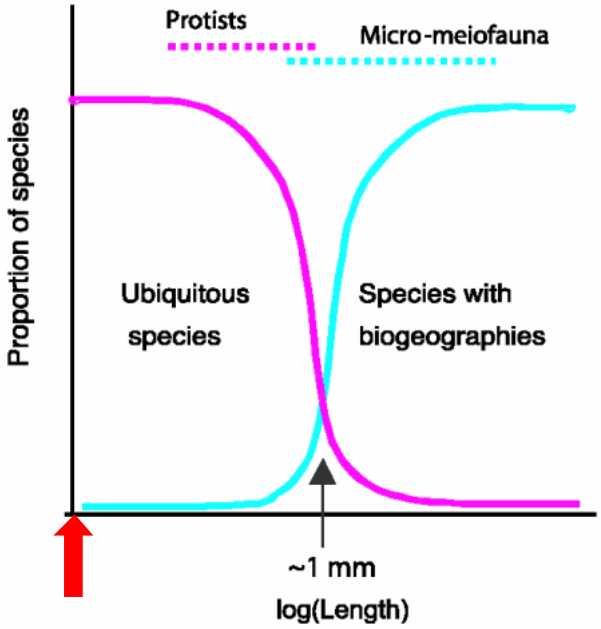
BLAND J. FINLAY[1]* AND TOM FENCHEL[2]

~1 mm

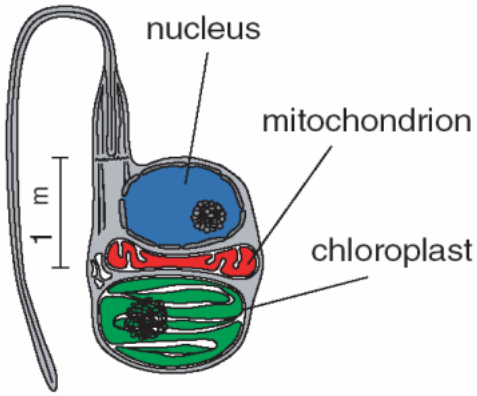
log(Length)

A case study: *Micromonas pusilla*





Multi-locus sequencing of 17 strains



SSU rDNA

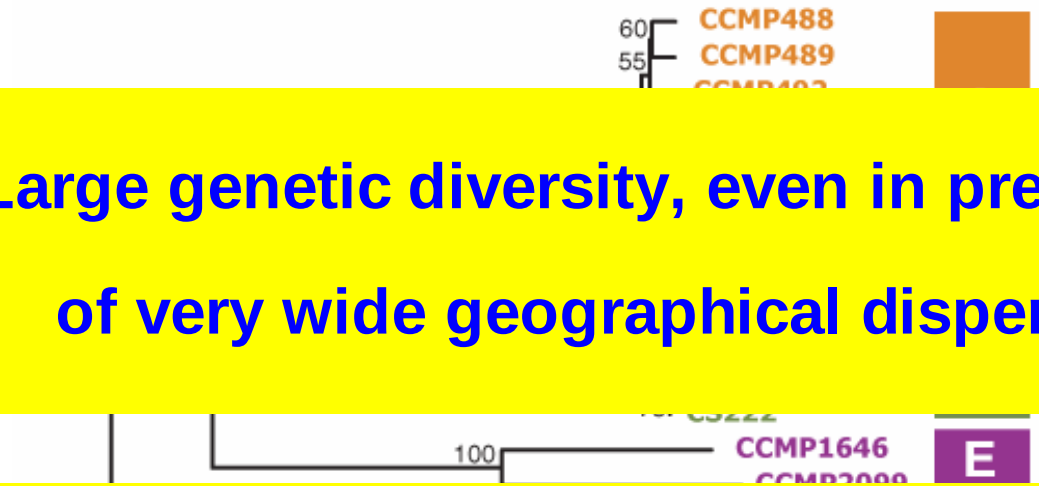
rDNA ITS

β-tubulin (+2 introns)

coxI

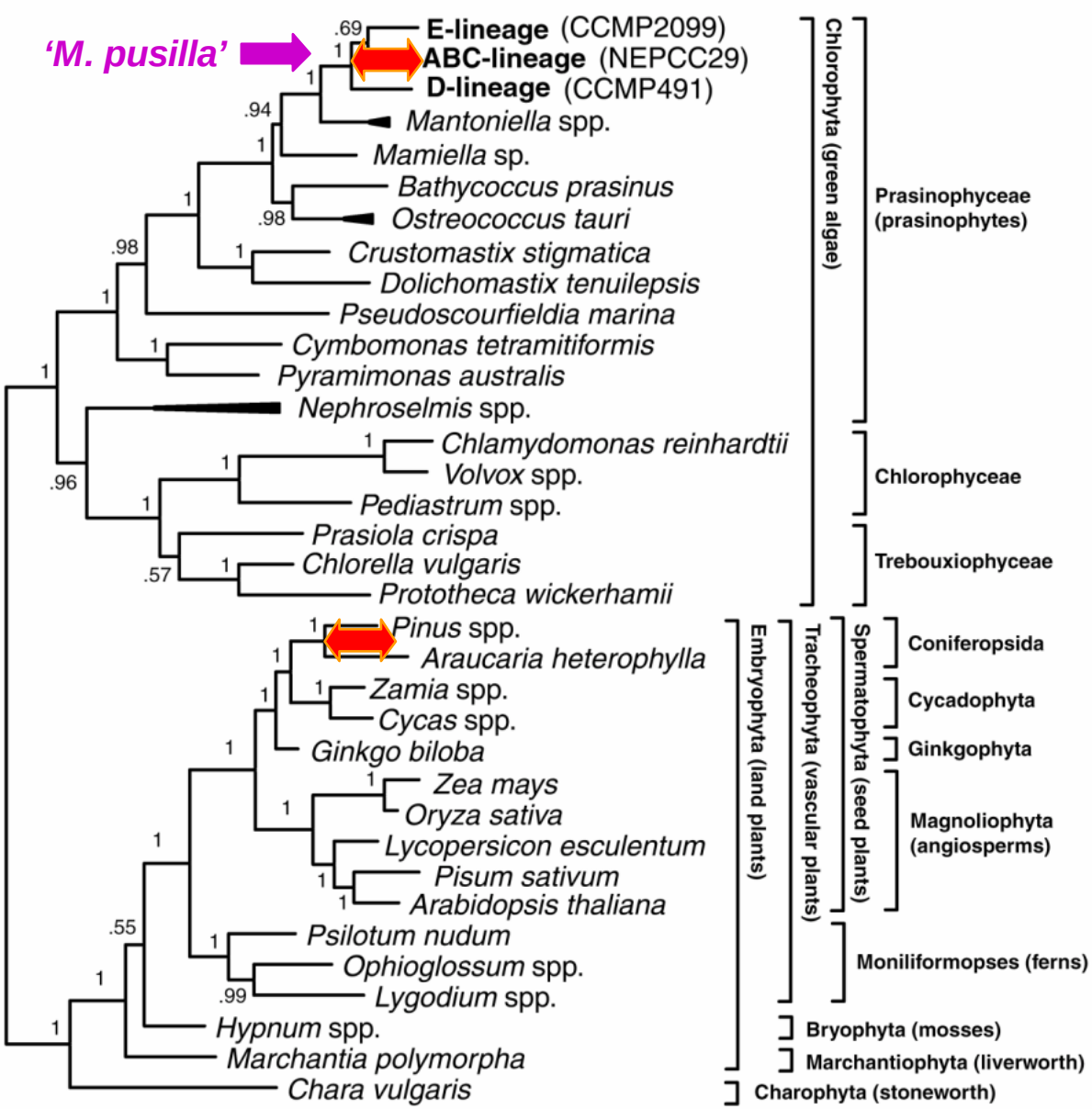
rbcL

rDNA+*coxI*+*rbcL*



Large genetic diversity, even in presence of very wide geographical dispersal

Morphology may be a poor criterion to estimate the real diversity of microbial eukaryotes : many cryptic species exist



Slapeta et al. Mol. Biol. Evol. 2006

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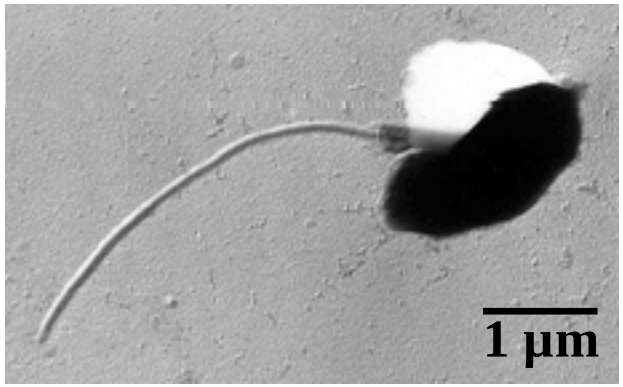
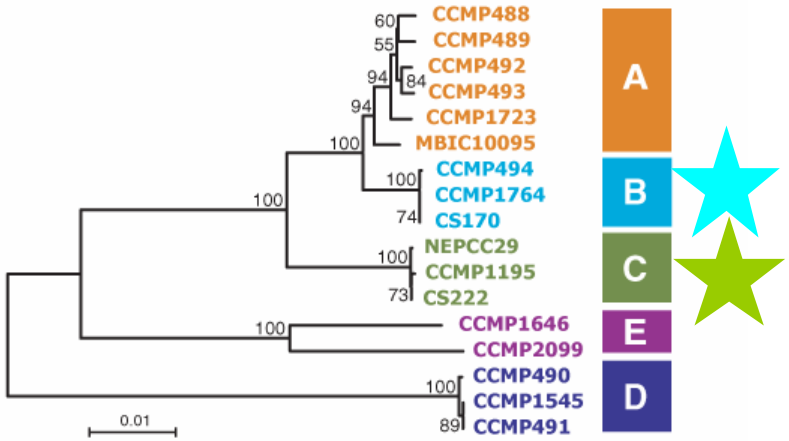
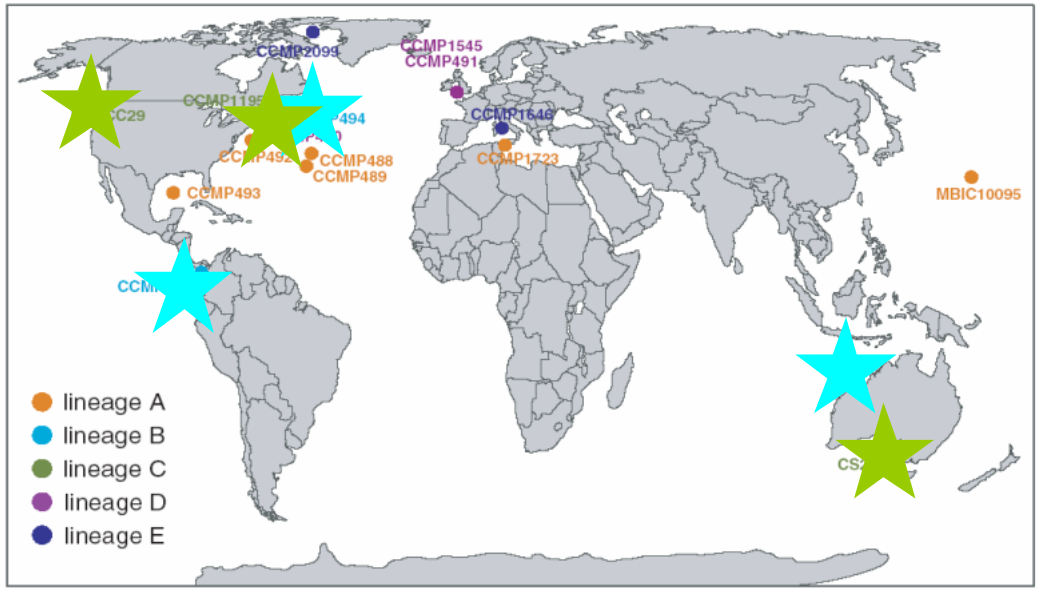
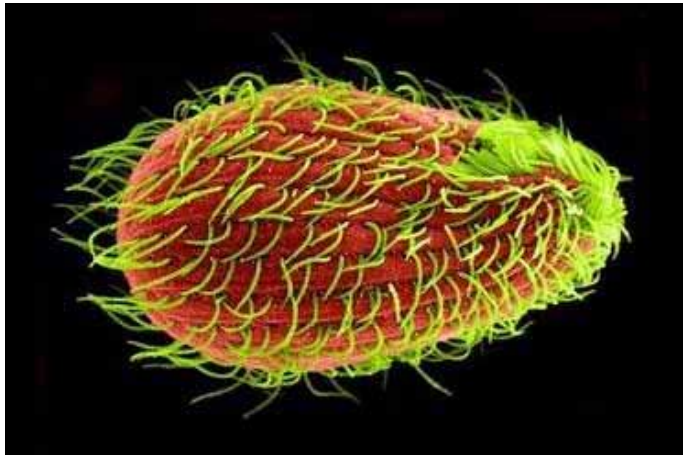






Widespread species do exist. What is really difficult is to prove is that a protist species is absent from a particular geographical region

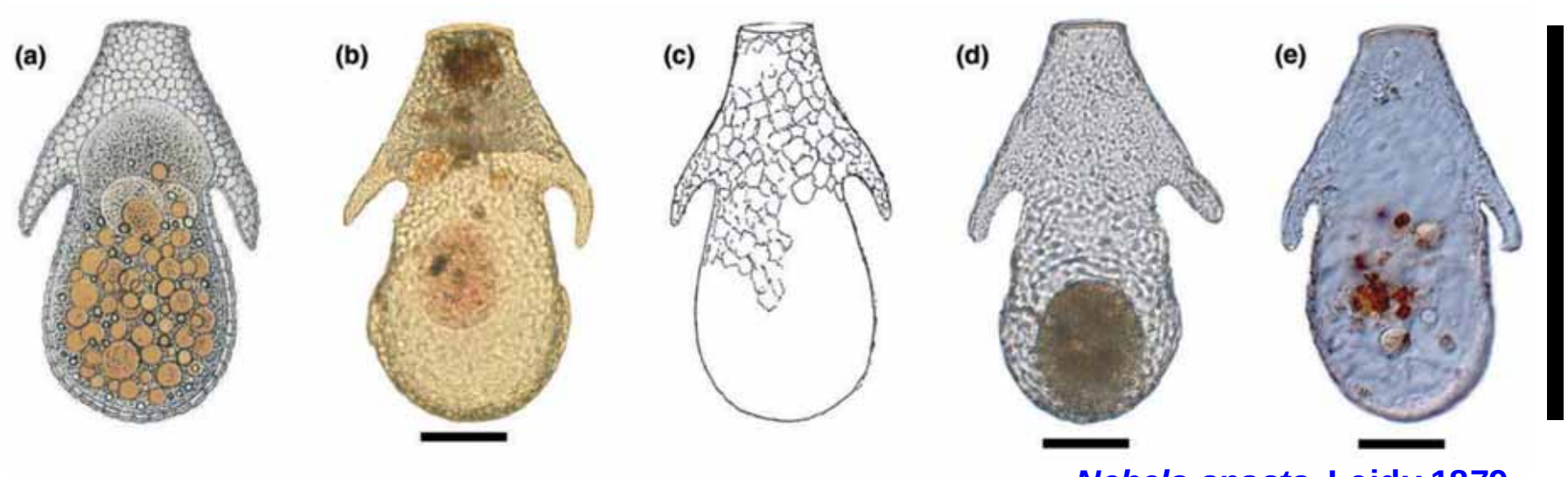
Endemic species? Looking for "flagship" species



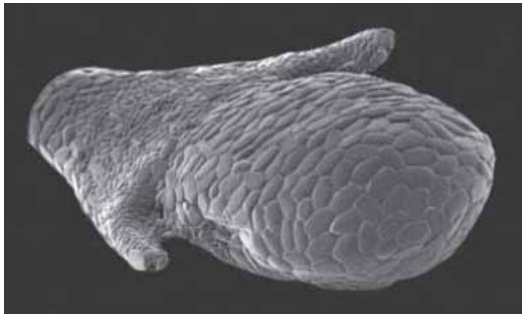
Tetrahymena thermophila

Zufall et al., 2013

Endemic species?



***Nebela ansata*, Leidy 1879**



Heger et al., 2011

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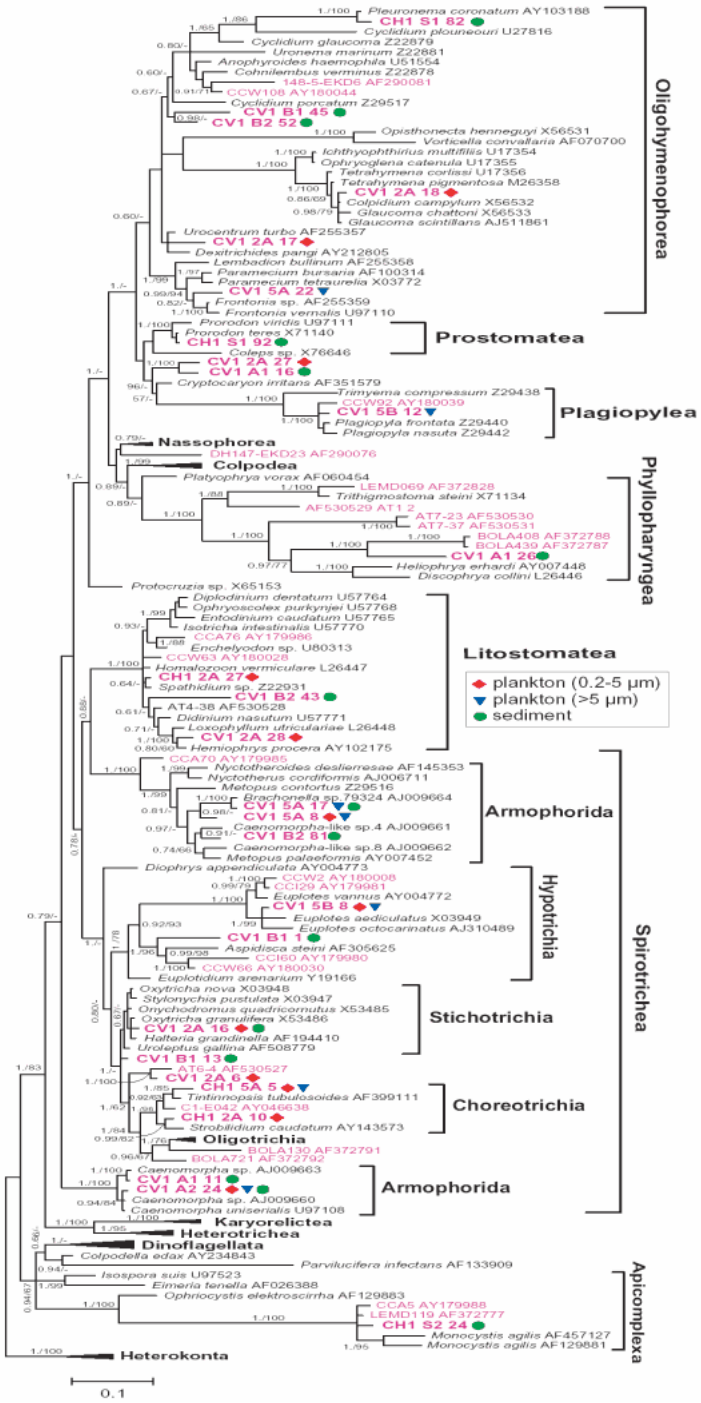

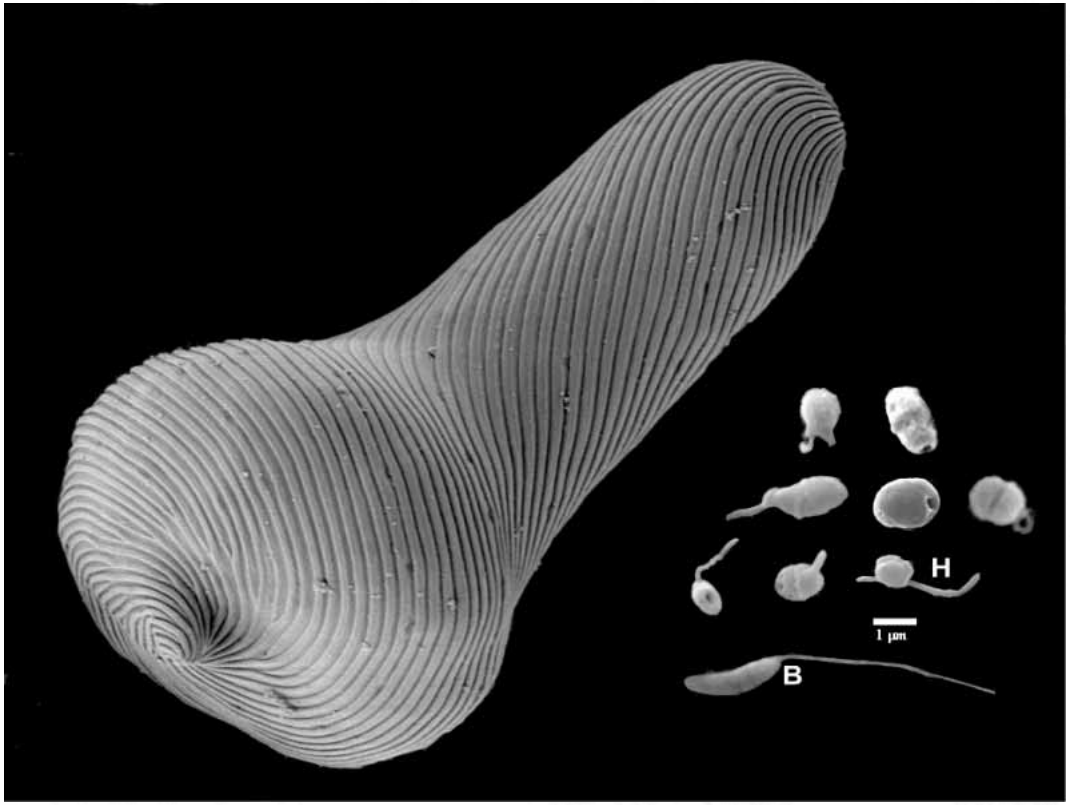

There is a huge diversity of unicellular eukaryotes that remains to be explored

Conclusions

- Most of the genetic diversity of eukaryotes is found among protists

- Known species only represent a small fraction of the whole diversity (e.g., many picoeukaryotes unknown)

- A single morphospecies may be composed of a large number of cryptic species