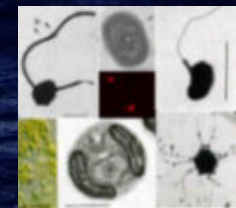


# Opening the black box of marine heterotrophic flagellates

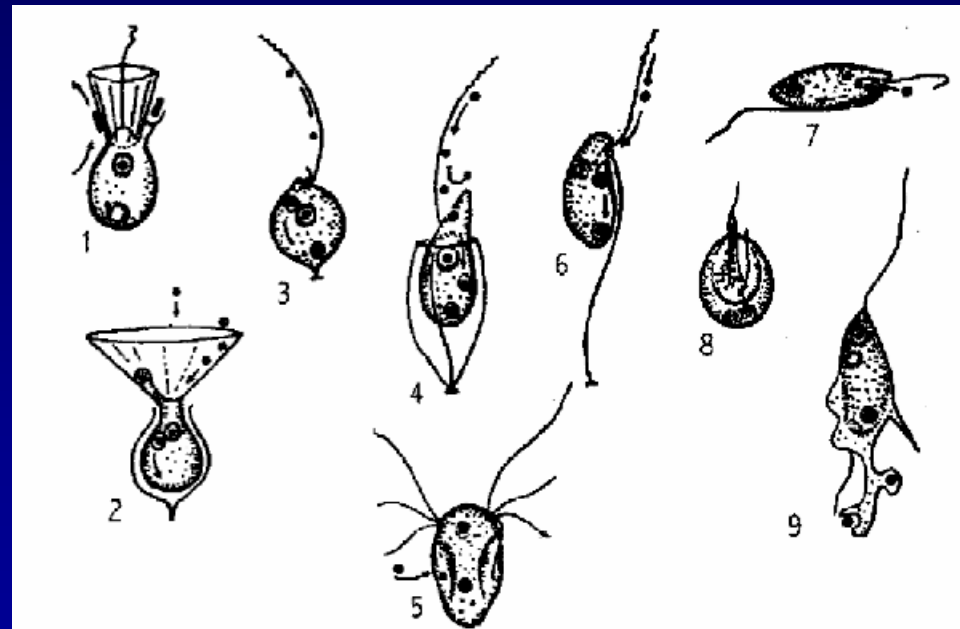


Ramon Massana

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## Marine heterotrophic flagellates

- Unpigmented protists with flagella



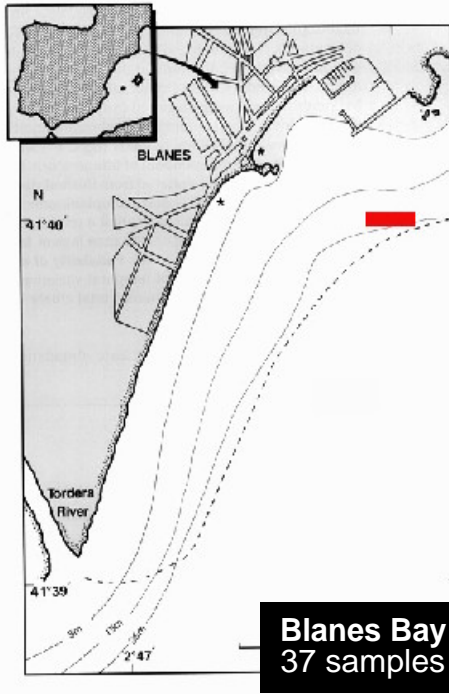
Boenigk & Arndt 2002

Large phylogenetic and functional diversity behind a "uniform" morphology

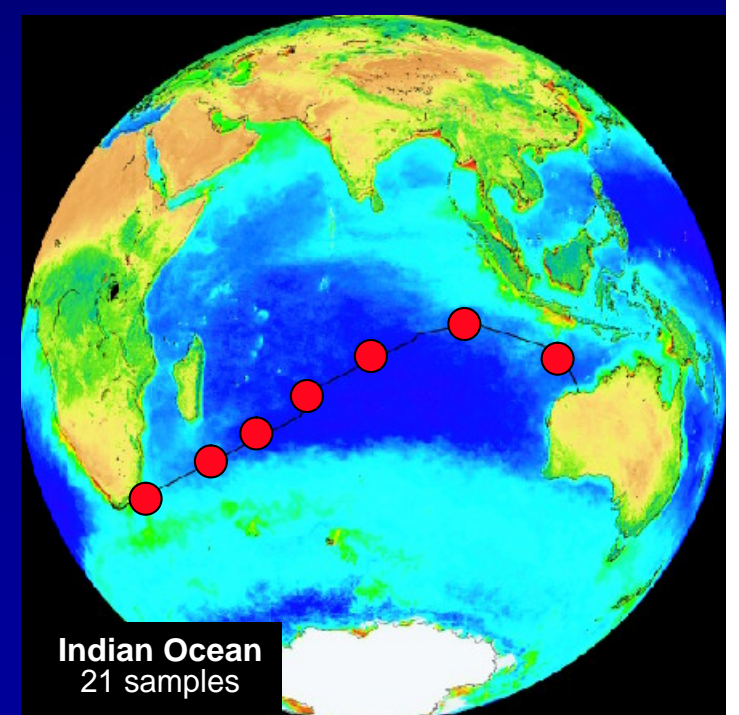
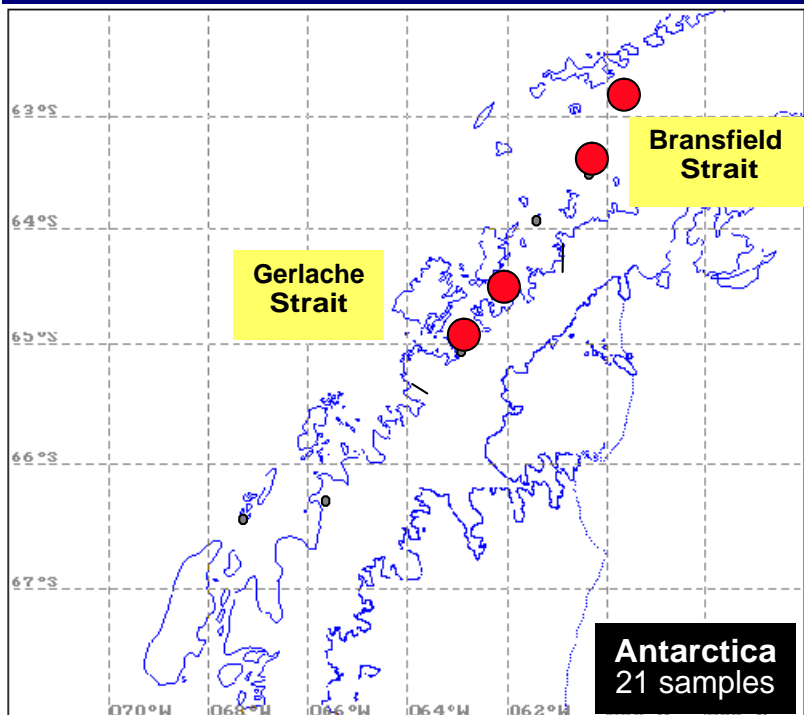
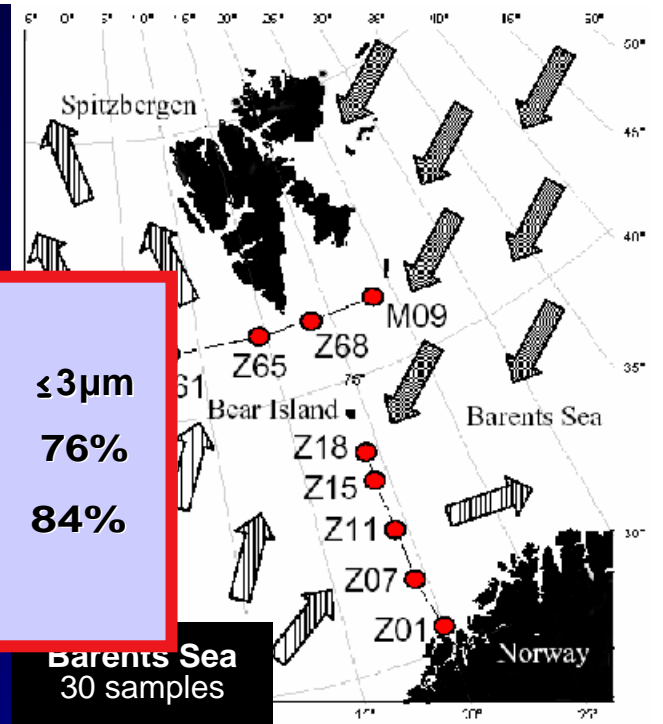
## Marine heterotrophic flagellates

- Unpigmented protists with flagella
- Very abundant in the plankton from the euphotic zone

# Epifluorescence counts in different systems



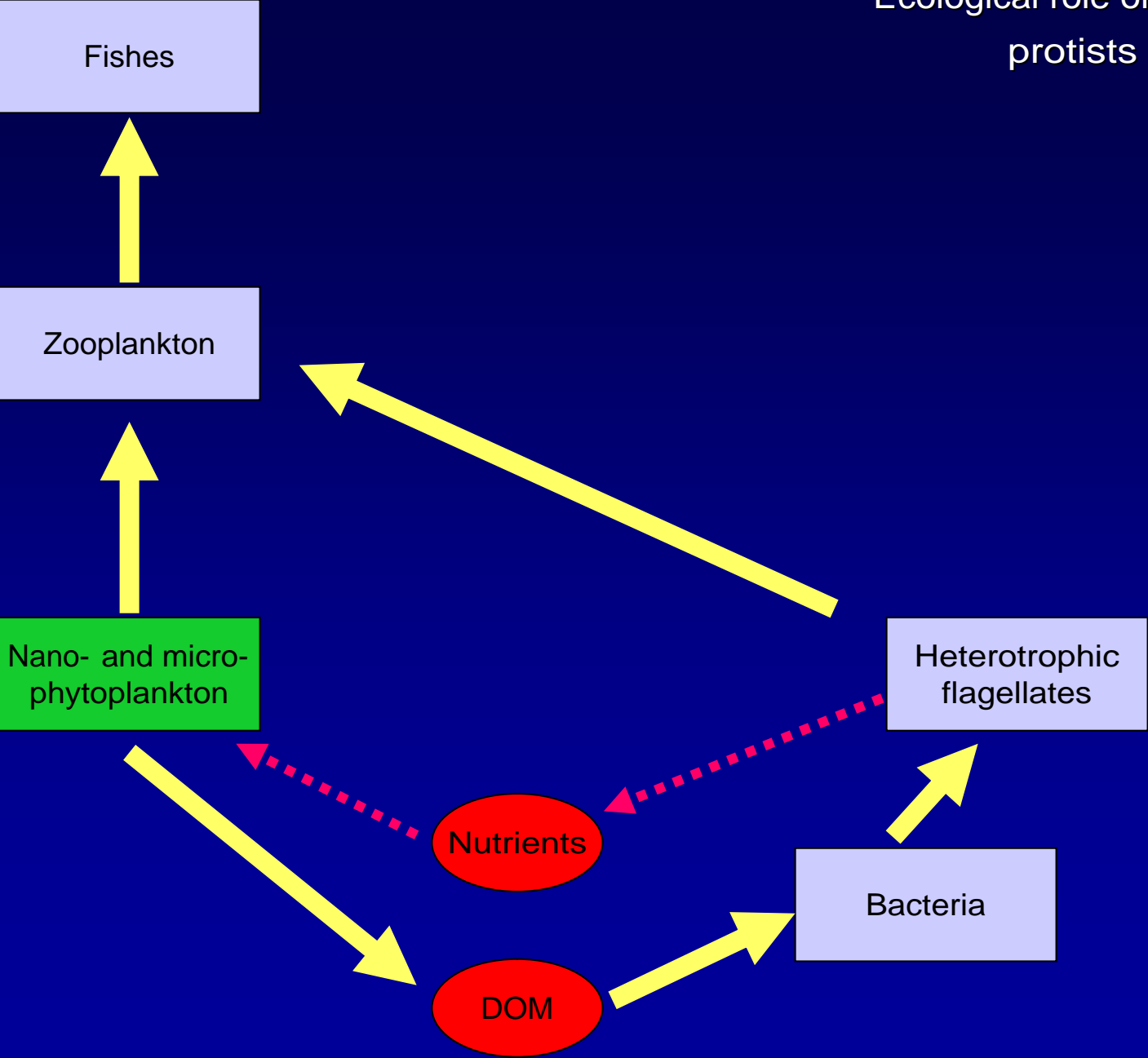
	Abundance cells ml <sup>-1</sup>	≤3μm
Heterotrophic flagellates	850	76%
Phototropic protists	5000	84%



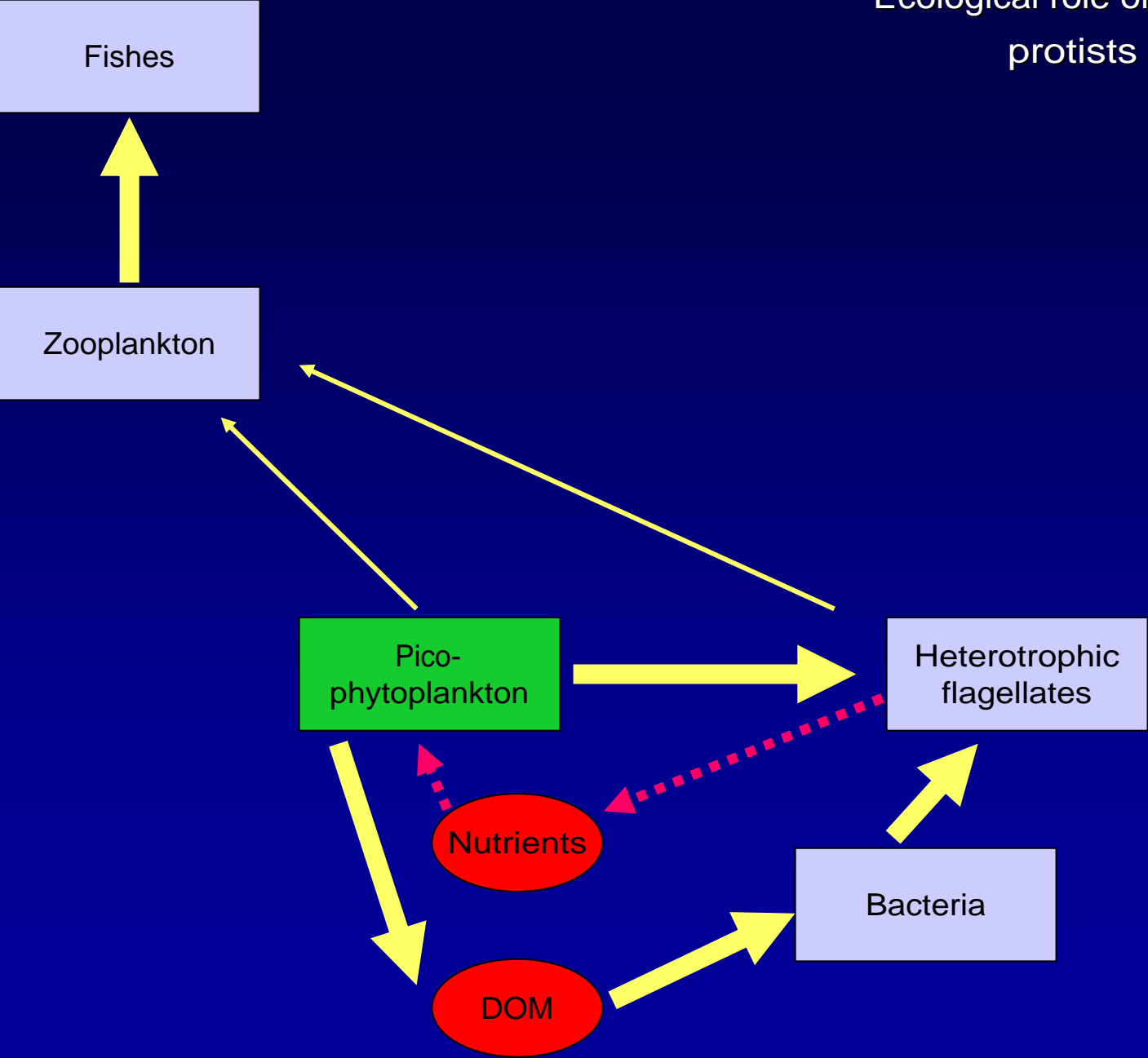
## Marine heterotrophic flagellates

- Unpigmented protists with flagella
- Very abundant in the marine plankton from the euphotic zone
- They play a very important ecological role in marine ecosystems

Ecological role of marine protists



Ecological role of marine protists



## Marine heterotrophic flagellates

- Unpigmented protists with flagella
- Very abundant in the marine plankton from the euphotic zone
- They play a very important ecological role in marine ecosystems
- But “in situ” diversity largely unknown !!!

## List of important marine heterotrophic flagellates

Common taxonomic groups	Common taxa	marine
Euglenozoans		
Euglenids	<i>Petalomonas, Peranema</i>	□
Kinetoplastids	<i>Bodo, Caecitellus</i>	□
Cryptomonads	<i>Goniomonas</i>	□
Stramenopiles		
Chrysomonads	<i>Spumella, Paraphysomonas</i>	■ ■ ■ □ □ □ ?
Bicosoecids	<i>Cafeteria, Bicosoeca</i>	■ ■ □ □ □ ? ?
Alveolates		
Dinoflagellates	<i>Gymnodinium, Katodinium</i>	■ ■ ■ ■ ■ ■ ■ □
Choanoflagellates	<i>Monosiga, Diaphanoeca</i>	■ ■ ■ □ □ □
Protista <i>incertae sedis</i>		
Cercomonads	<i>Cercomonas, Bodomorpha</i>	□
Kathablepharids	<i>Kathablepharis, Leucocryptos</i>	■ ■ □ □
Thaumatomonads	<i>Protaspis, Thaumatomonas</i>	□
Apusomonads	<i>Amastigomonas, Apusomonas</i>	□
Ebriids	<i>Ebria</i>	■ ■ ■ □
Ancyromonads	<i>Ancyromonas</i>	□
diverse groups of uncertain systematic affiliation	<i>Metromonas, Diphylleia, Metopion, Telonema</i>	■ ■ □ □

The heterotrophic flagellates in culture, represent those dominant in the sea?

Paraphysomonas

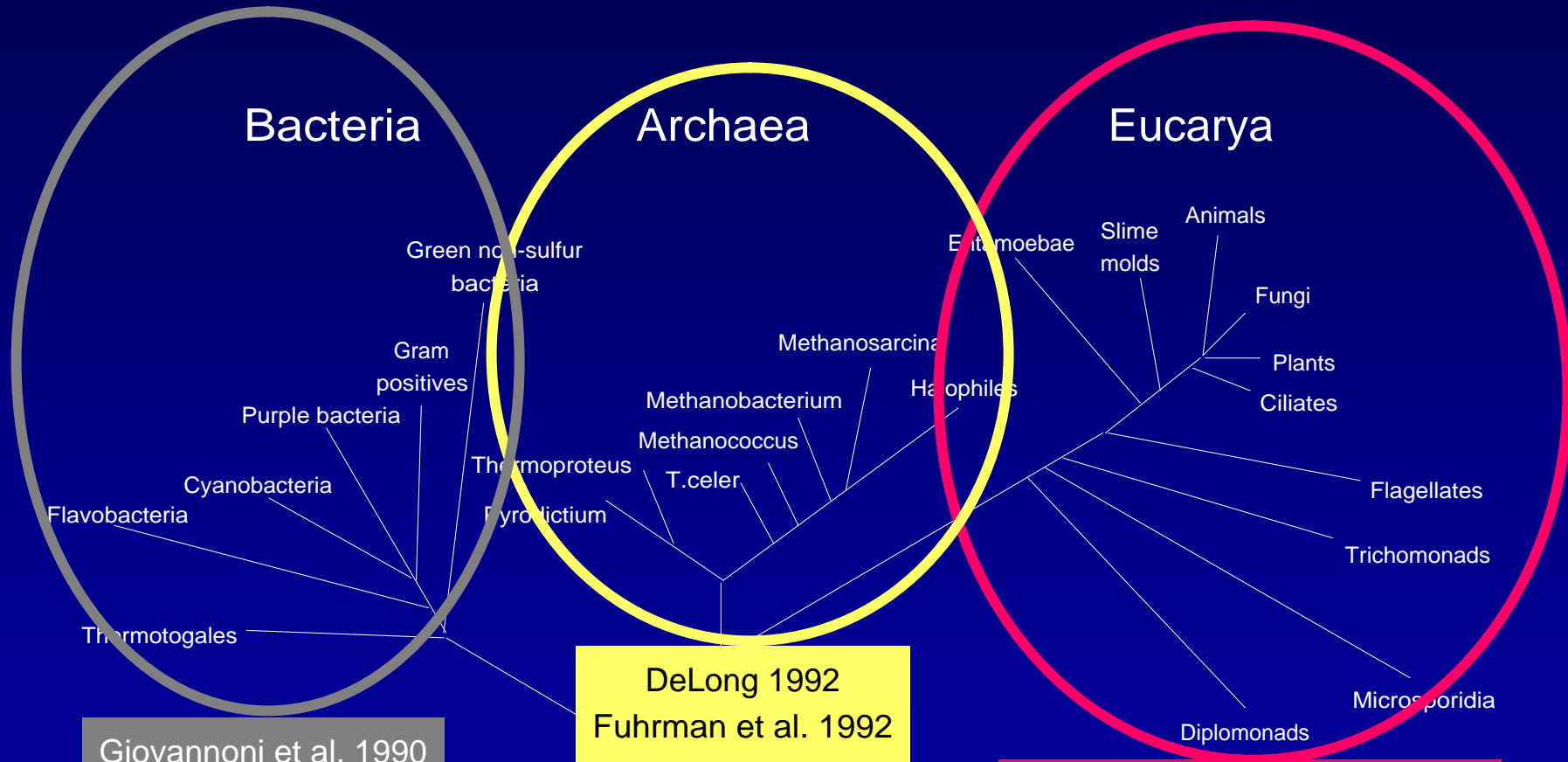
Dominates in 98% of the enrichments in  
US East Coast and Bermuda

Represent <1% in natural samples

*Lim et al. 1999*

# The molecular approach

Clone libraries with Domain-specific primers and community DNA



SSU rRNA gene

*From microorganisms  
to sequences*

Microorganisms

*Biomass concentration  
0.2 - 3  $\mu\text{m}$*

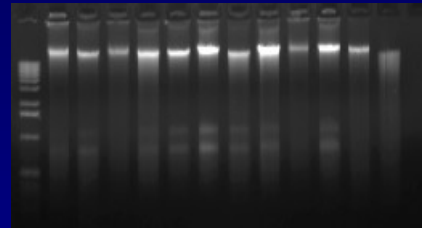
Microbial biomass

*DNA extraction*

Nucleic acids extract

*PCR amplification*

PCR product



*Community DNA*



*Community rRNA genes*

*From microorganisms  
to sequences*

Microorganisms

*Biomass concentration*

Microbial biomass

*DNA extraction*

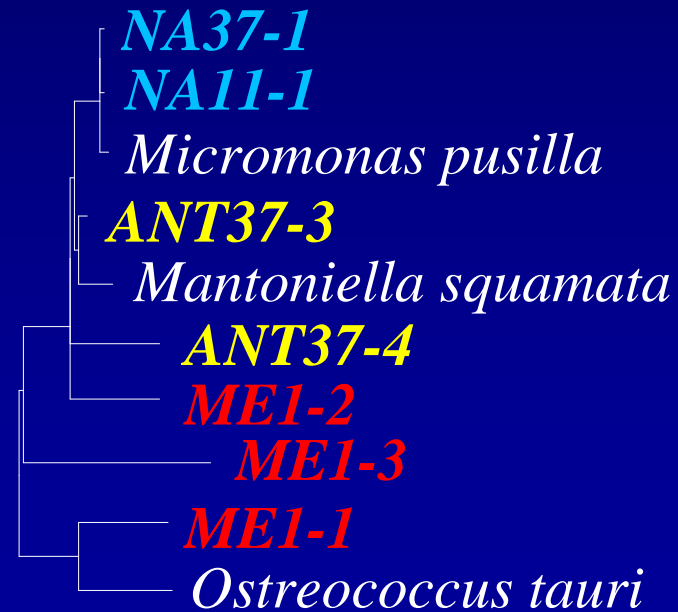
Nucleic acids extract

*PCR amplification*

PCR product

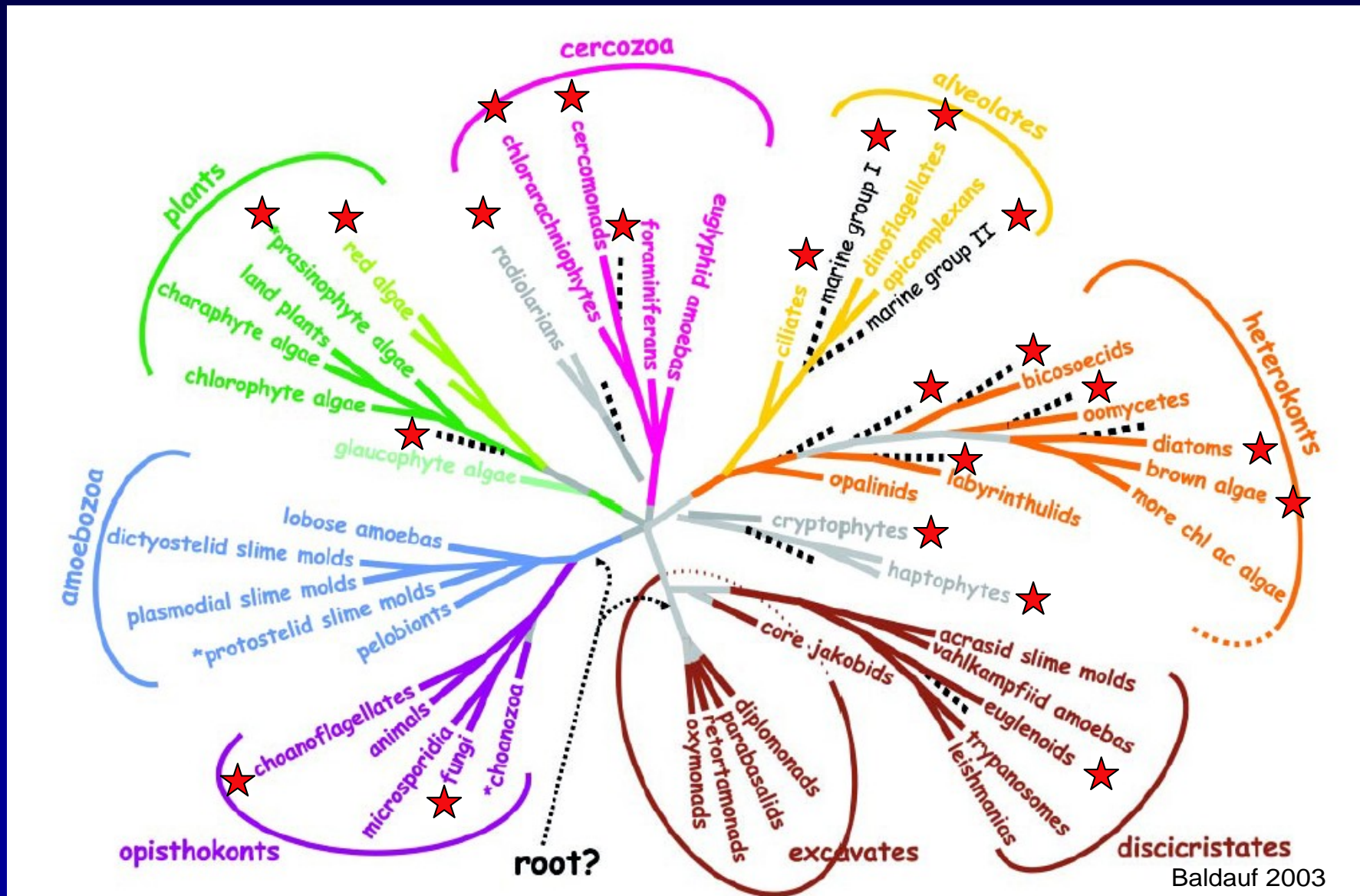
*Cloning and sequencing*

Genetic library



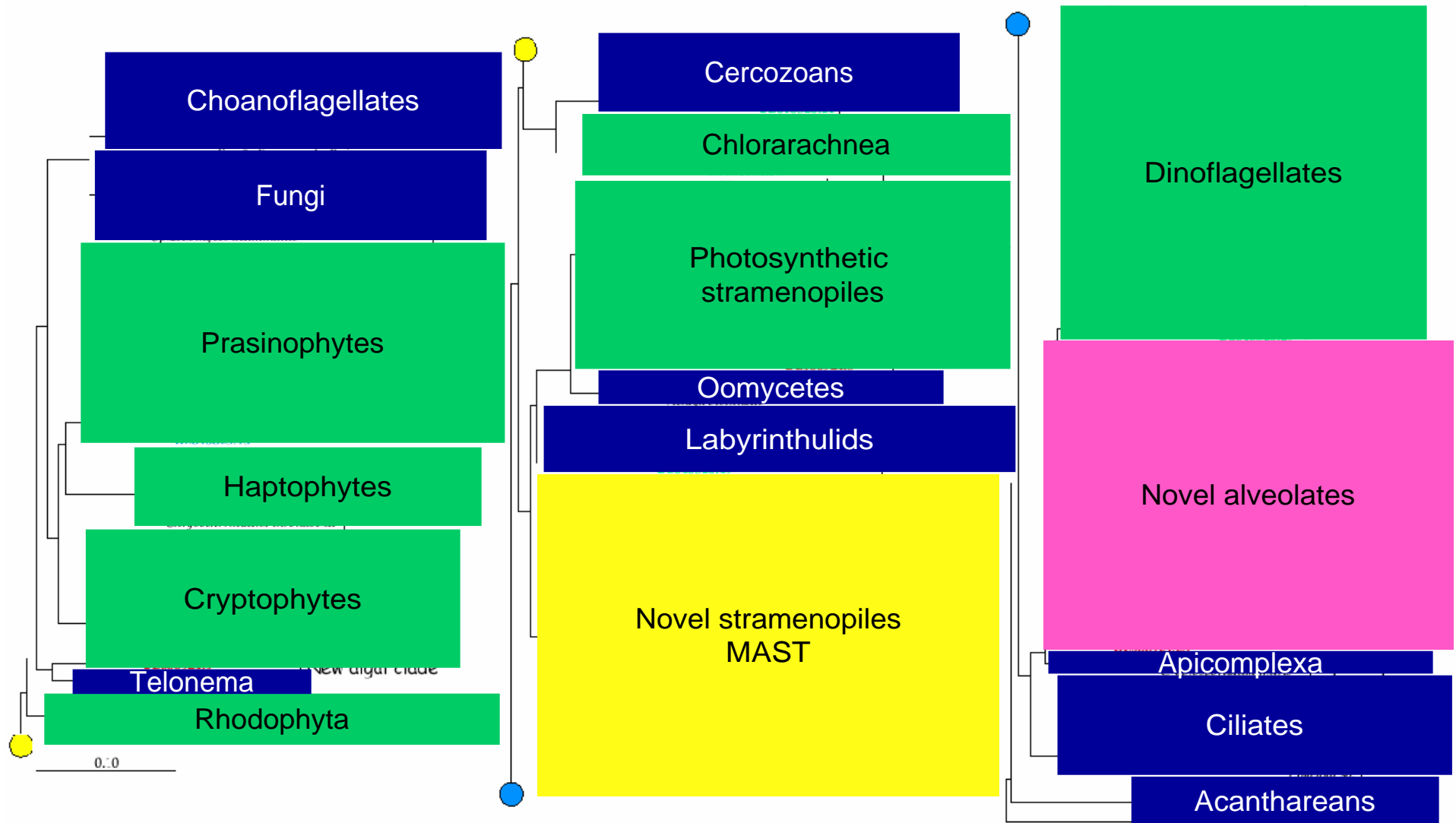
*Phylogenetic analysis*

# What have we found?

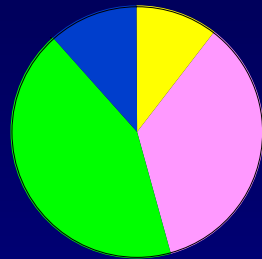


A very large phylogenetic diversity

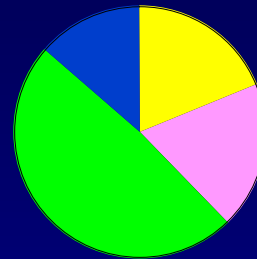
# Blanes Bay (Mediterranean Coast) as example



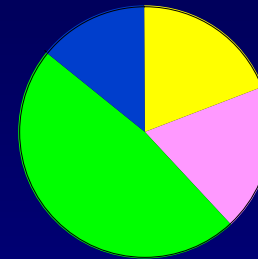
# How widespread are novel stramenopiles and novel alveolates?



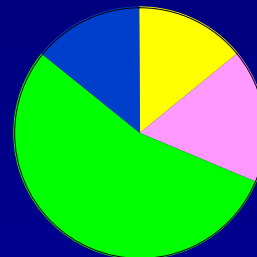
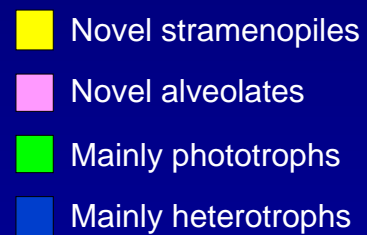
Blanes Bay



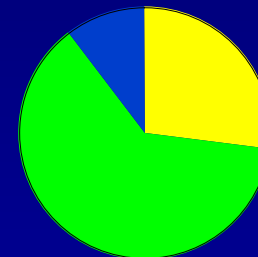
North Atlantic



Mediterranean sea



Equatorial Pacific



Antarctica

Massana et al. 2004

Díez et al. 2001

Moon van der Staay et al. 2001

## And what about the "known" heterotrophic flagellates?

Common taxonomic groups	Common taxa	marine
Euglenozoans		
Euglenids	<i>Petalomonas, Peranema</i>	□
Kinetoplastids	<i>Bodo, Caecitellus</i>	□
Cryptomonads	<i>Goniomonas</i>	□
Stramenopiles		
Chryomonads	<i>Spumella, Paraphysomonas</i>	■ ■ ■ □ □ □ ?
Bicosoecids	<i>Cafeteria, Bicosoeca</i>	■ ■ □ □ □ ? ?
Alveolates		
Dinoflagellates	<i>Gymnodinium, Katodinium</i>	■ ■ ■ ■ ■ ■ □
Choanoflagellates	<i>Monosiga, Diaphanoeca</i>	■ ■ ■ □ □ □
Protista <i>incertae sedis</i>		
Cercomonads	<i>Cercomonas, Bodomorpha</i>	□
Kathablepharids	<i>Kathablepharis, Leucocryptos</i>	■ ■ □ □
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Ancyromonads	<i>Ancyromonas</i>	□
diverse groups of uncertain systematic affiliation	<i>Metromonas, Diphyllia, Metopion, Telonema</i>	■ ■ □ □

Blanes libraries

1 clones

0 clones

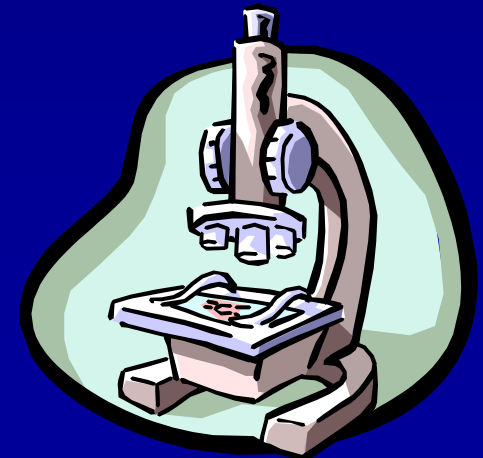
5 clones

9 clones

Novel stramenopiles  
(MAST): 34 clones

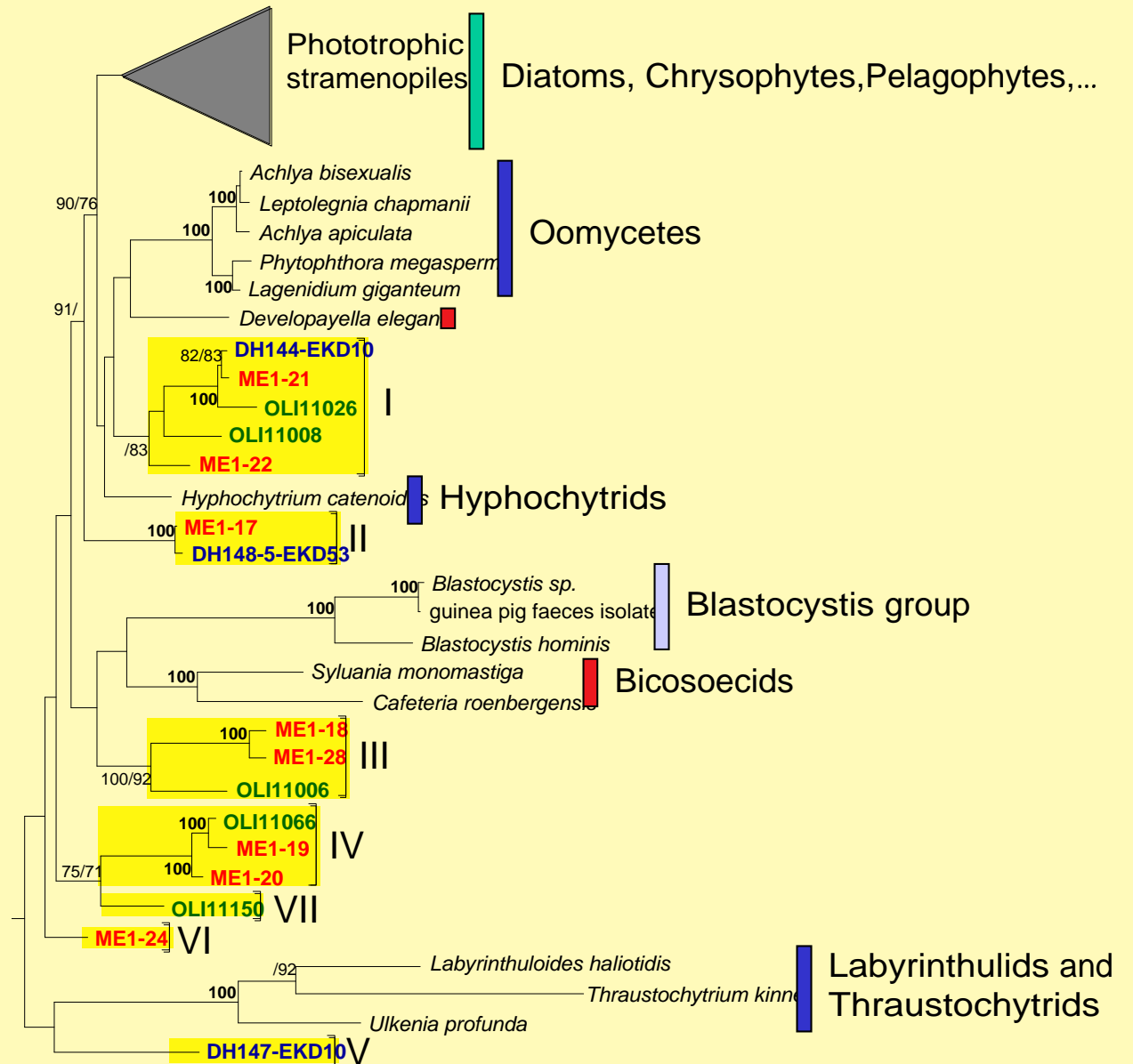
Novel alveolates:  
139 clones

A closer look at Novel Marine Stramenopiles  
(MAST)



# Marine stramenopiles MAST

- Phototrophic
- Fungi-like cells
- Parasites
- Free-living flagellates



Phylogenetic diversity  
Biogeography



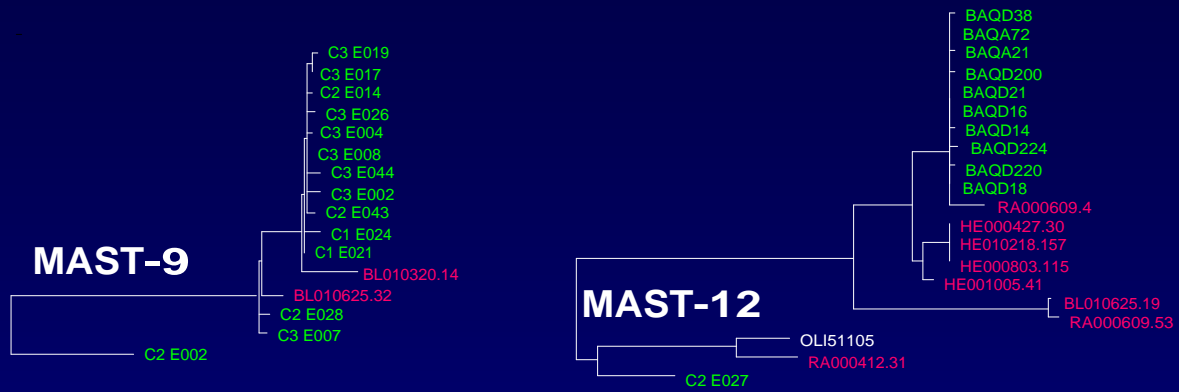
Genetic libraries



**MASTs**

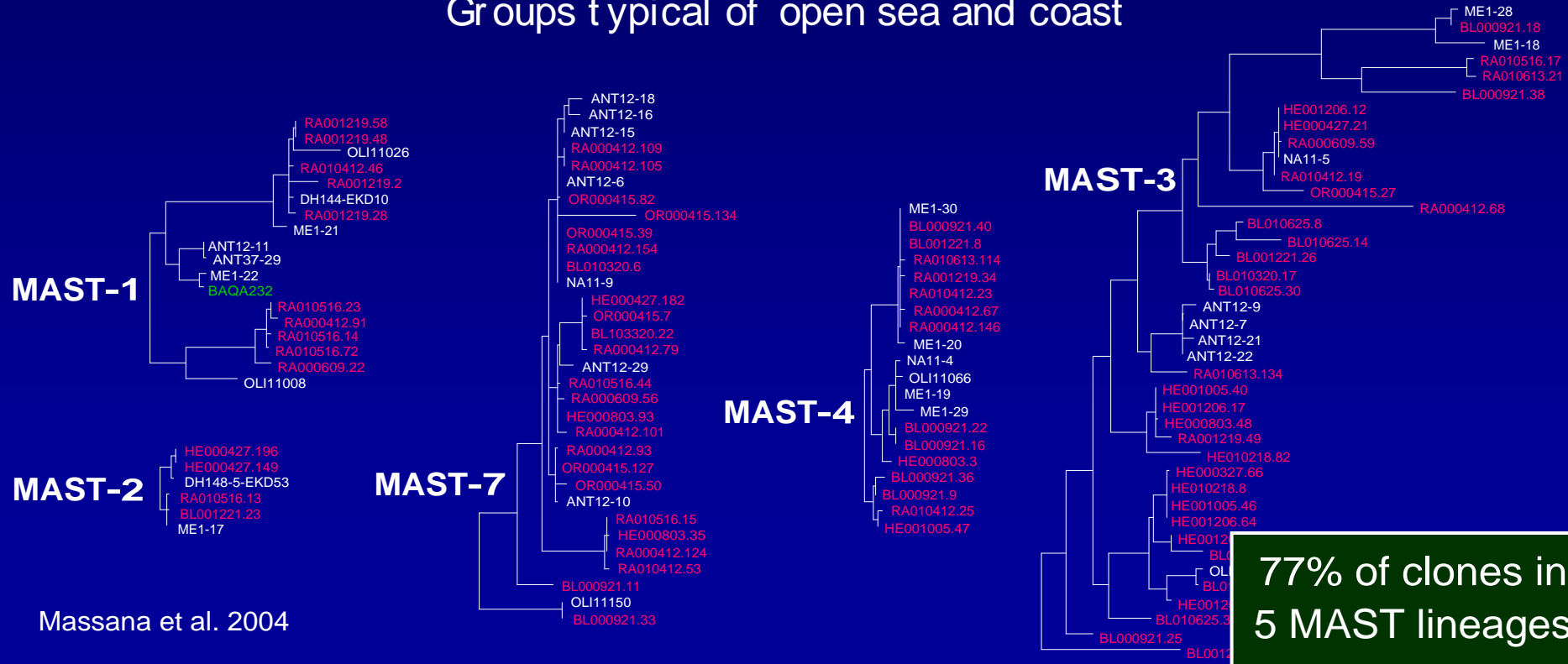
# Groups typical of anoxic systems (and coastal)

12 MAST lineages



Open sea  
Coast  
Anoxic systems

# Groups typical of open sea and coast

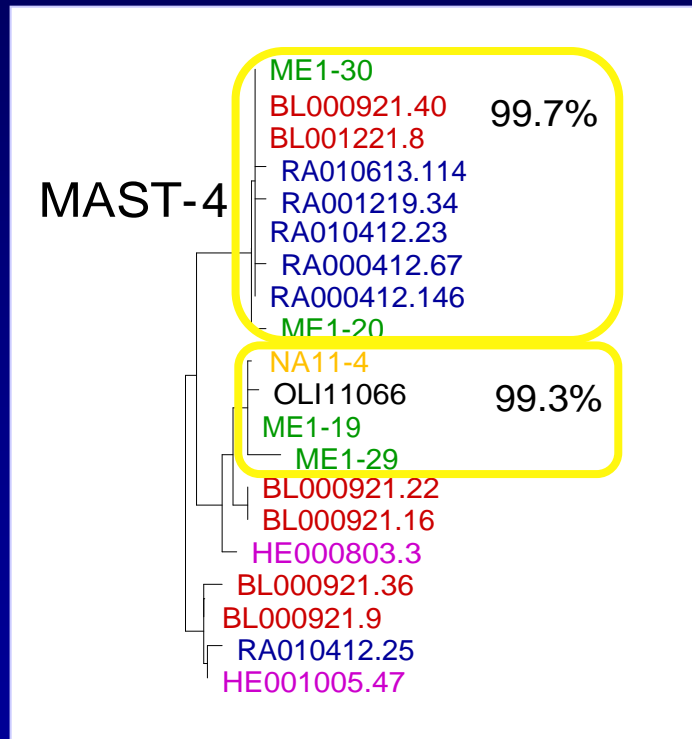


77% of clones in 5 MAST lineages

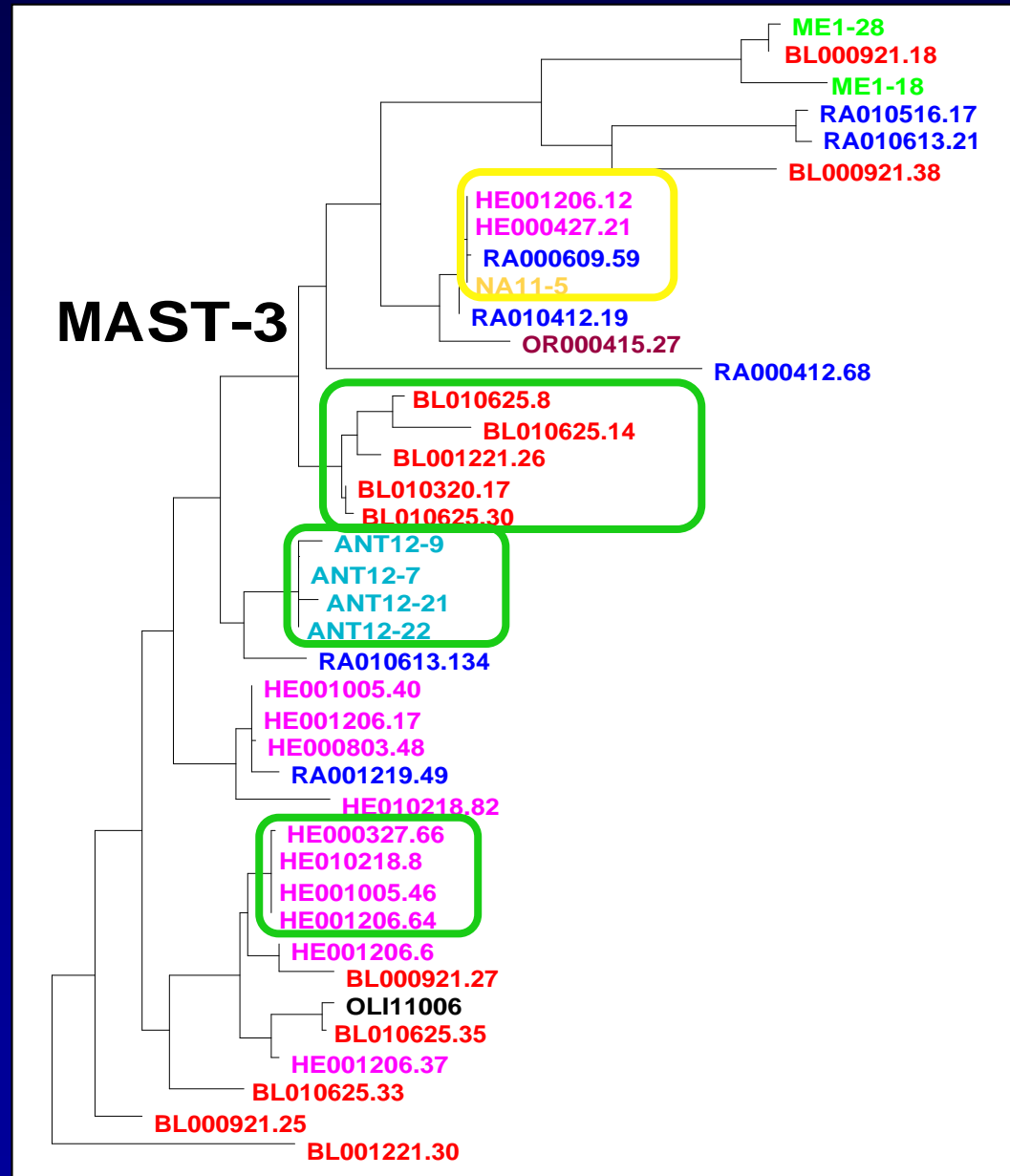
Microdiversity

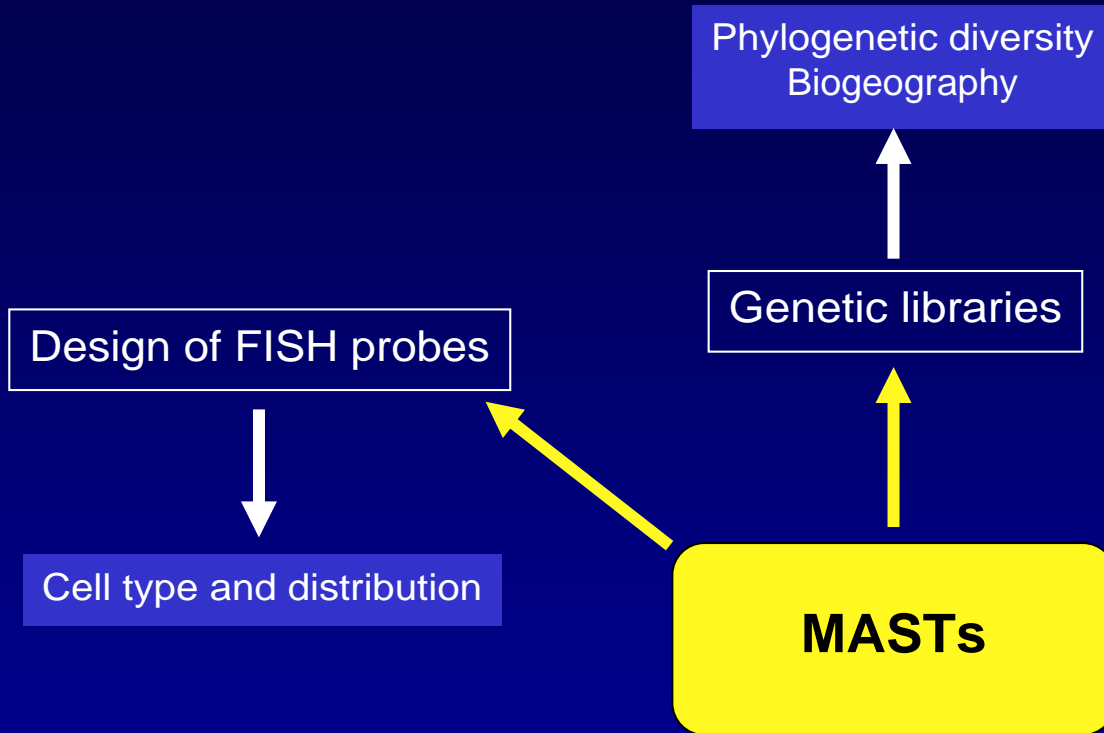
Cosmopolitanism

Endemism

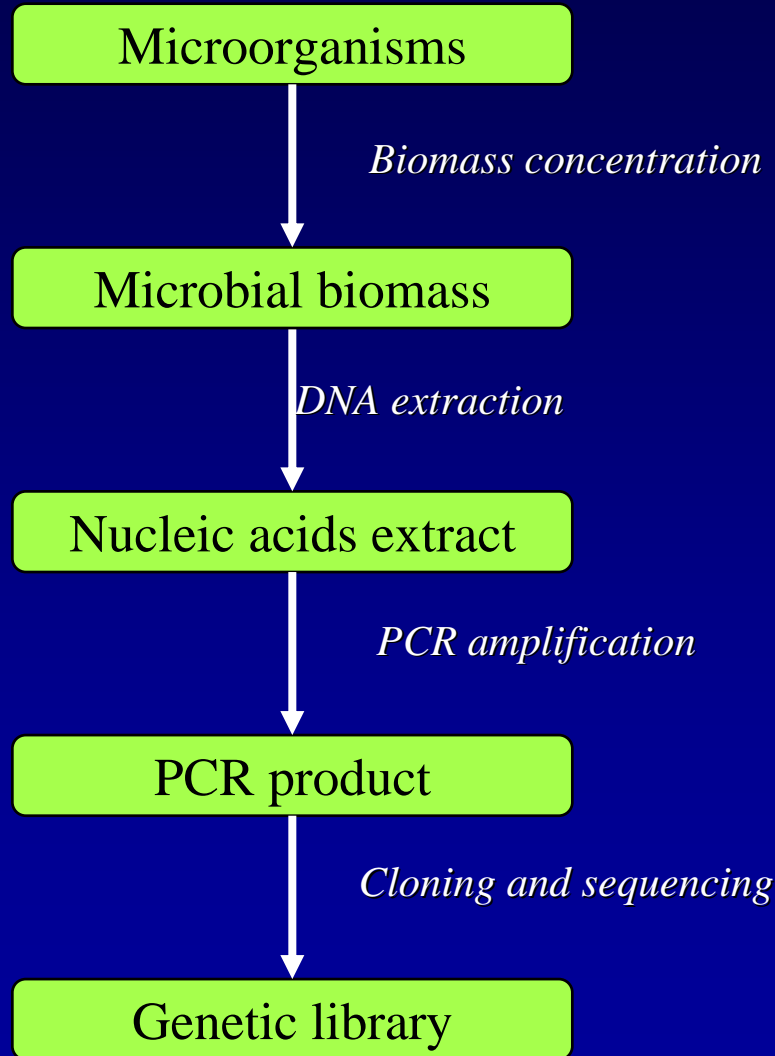


Open questions

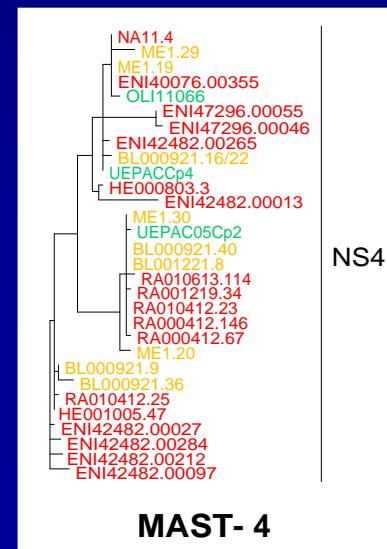
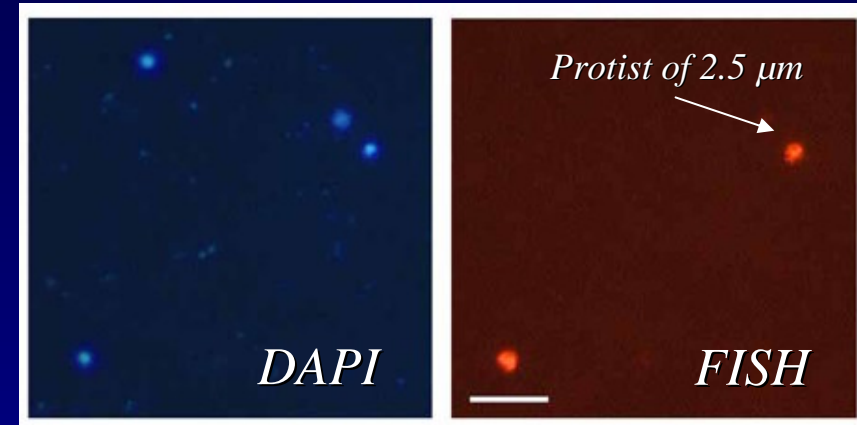




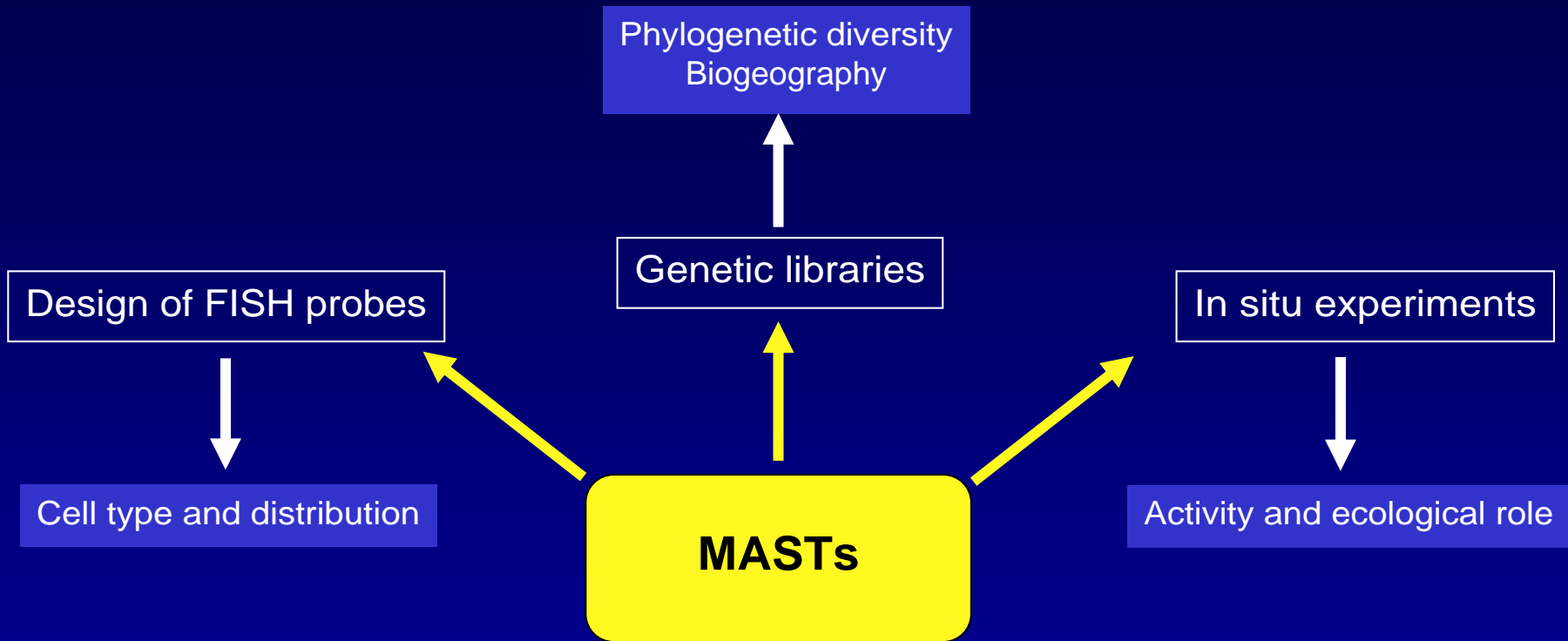
*From microorganisms  
to sequences*



*From sequences  
to microorganisms*

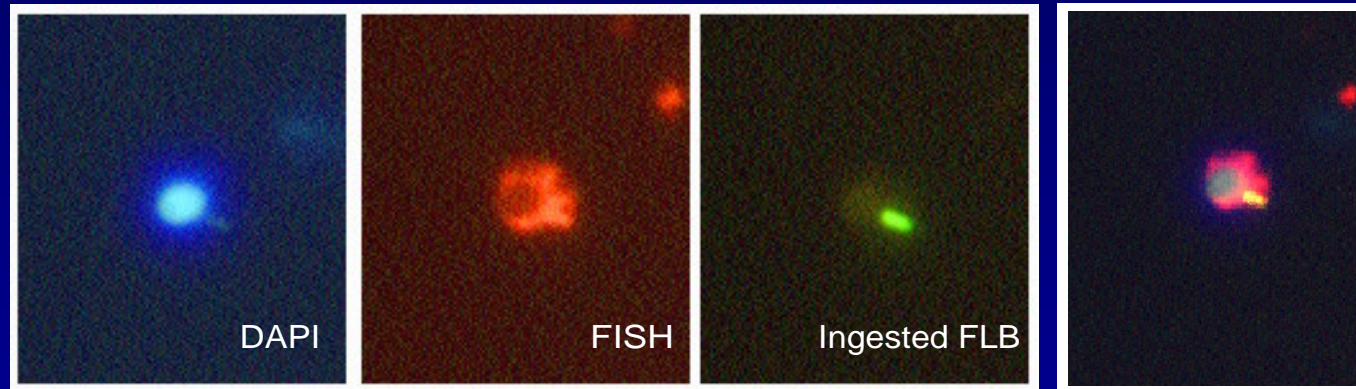


*Design of a  
phylogenetic  
probe*



# Bacterivory experiments with FLBs

## MAST-4



Phylogenetic diversity  
Biogeography

Genetic libraries

In situ experiments

Design of FISH probes

**MASTs**

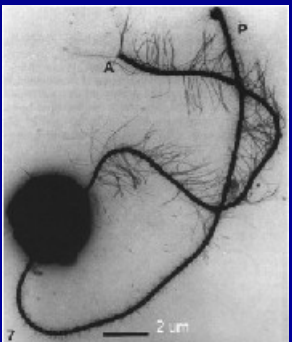
Activity and ecological role

Cell type and distribution

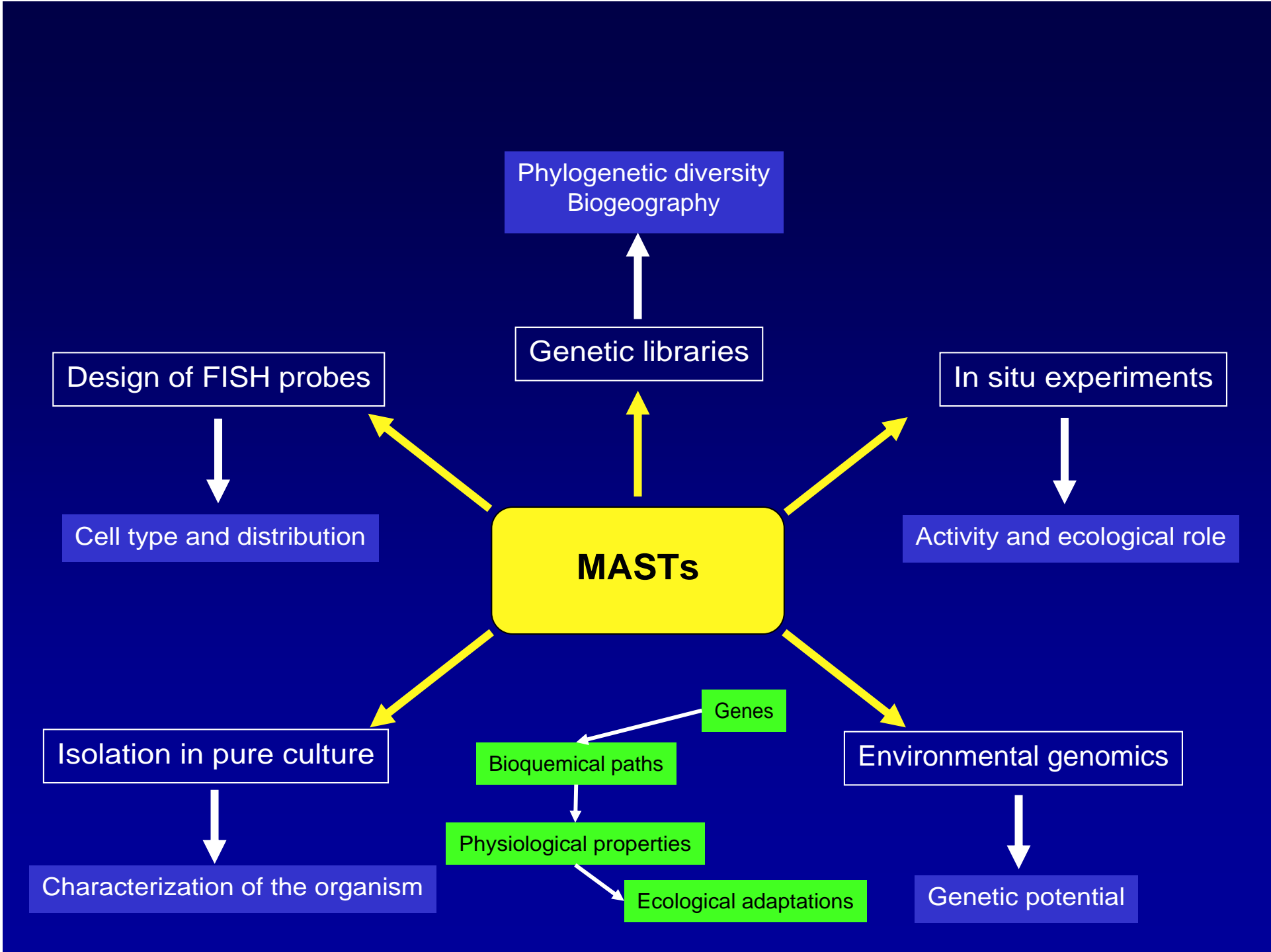
Pirsonia

Isolation in pure culture

- RA613.2
- RA613.1
- RA613.36
- Pirsonia sp.
- Pirsonia sp.
- Pirsonia sp.
- Pirsonia sp.



Characterization of the organism



# Summary

- Marine protists are important members of the euphotic zone plankton
- Its diversity is large and hides many uncharacterized organisms
- MASTs account for many unknown sequences in molecular surveys
- Each MAST group corresponds to a different cell type
- MASTs form a significant fraction of heterotrophic flagellates