



*Revealing the organisms behind novel  
eukaryotic phylogenetic lineages*

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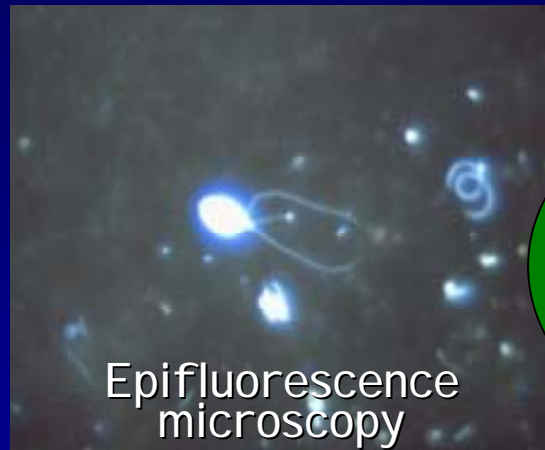
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# *Marine picoeukaryotes*

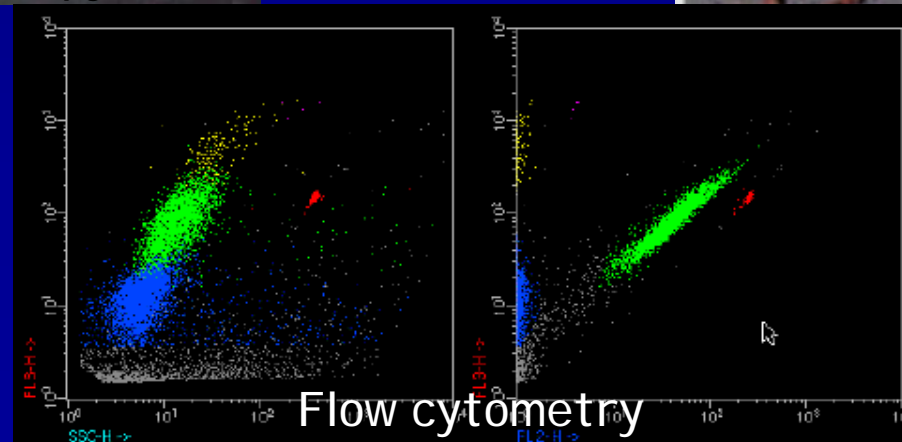
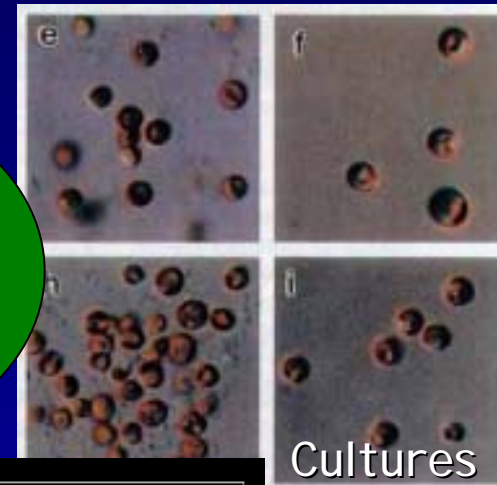
Ubiquitous and significant members of the plankton

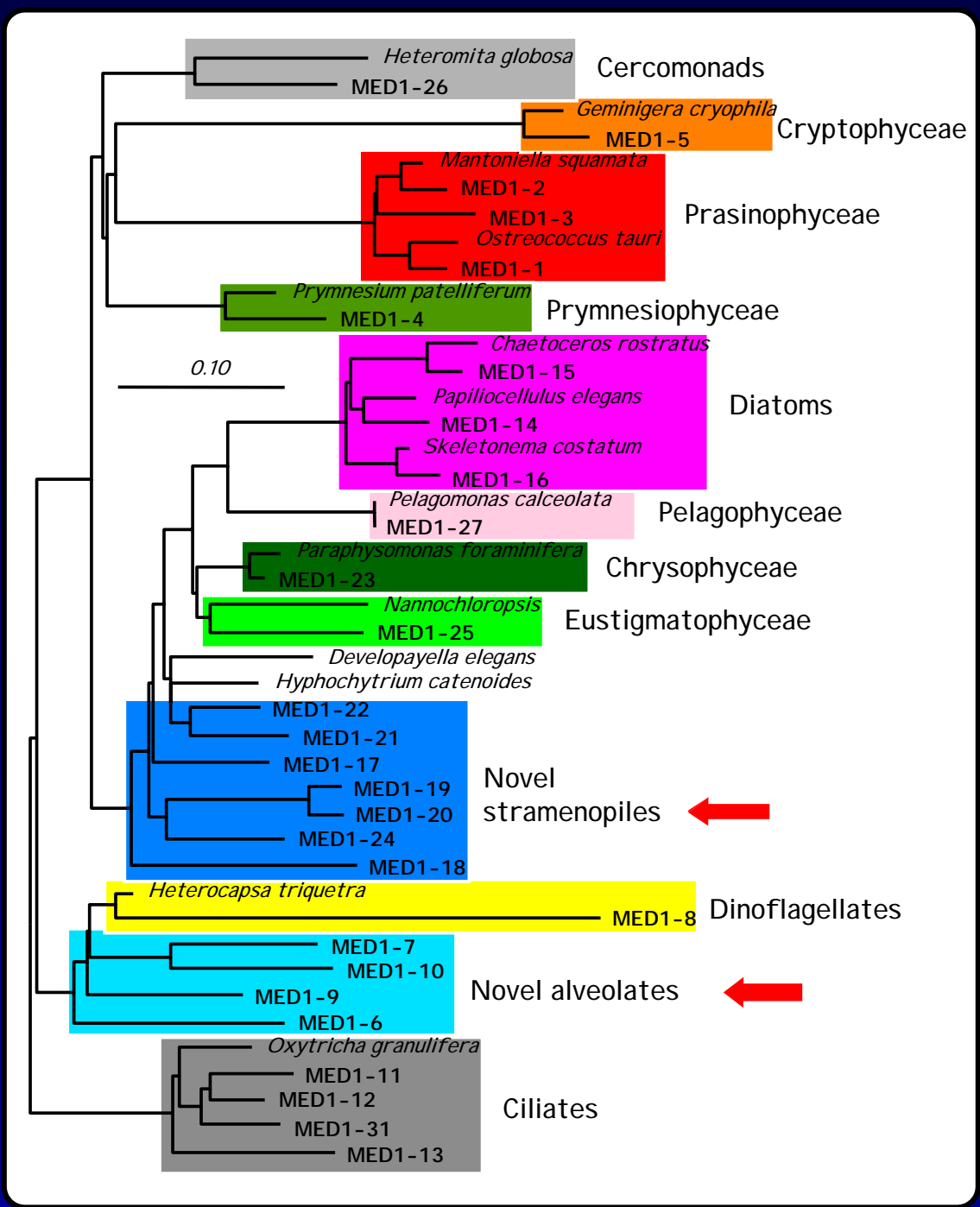
Phototrophic:  $10^3$ - $10^4$  cells ml<sup>-1</sup>

Heterotrophic:  $10^2$ - $10^3$  cells ml<sup>-1</sup>



Observation and  
count

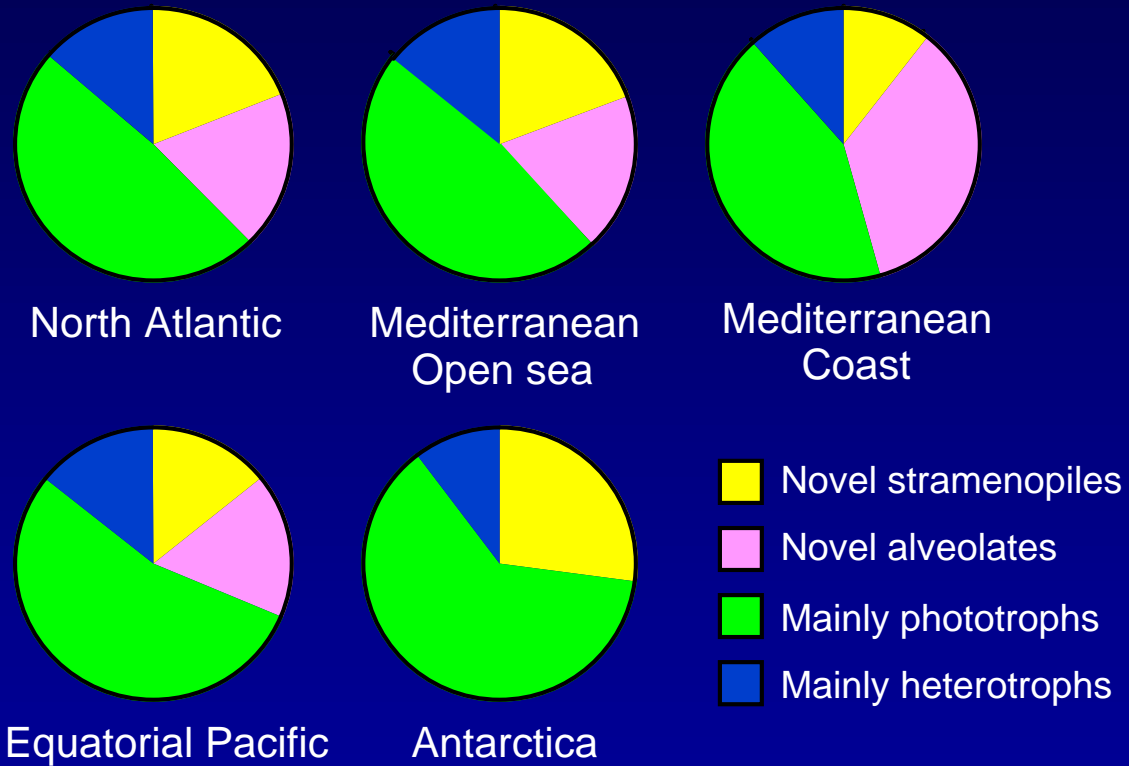




## Genetic library of 18S rRNA genes

Sample analyzed  
Alboran Sea (NW Med) - 5m  
Fraction: 0.2 - 5  $\mu$ m

## Novel groups are abundant and widespread



*Díez et al. 2001*

*López-García et al. 2001*

*Moon van der Staay et al 2001*



## Distribution of clones inside clusters

|      | Mediterranean | North Atlantic | Antarctica | Eq Pacific | Antarctica-deep | SUM |
|------|---------------|----------------|------------|------------|-----------------|-----|
| I    | 2             | 0              | 4          | 2          | 1               | 9   |
| II   | 1             | 0              | 0          | 0          | 1               | 2   |
| III  | 3             | 4              | 7          | 1          | 0               | 15  |
| IV   | 4             | 1              | 0          | 1          | 0               | 6   |
| V    | 0             | 0              | 0          | 0          | 1               | 1   |
| VI   | 2             | 0              | 1          | 0          | 0               | 3   |
| VII  | 0             | 2              | 8          | 1          | 0               | 11  |
| VIII | 0             | 0              | 14         | 0          | 0               | 14  |
| SUM  | 12            | 7              | 34         | 5          | 3               | 61  |

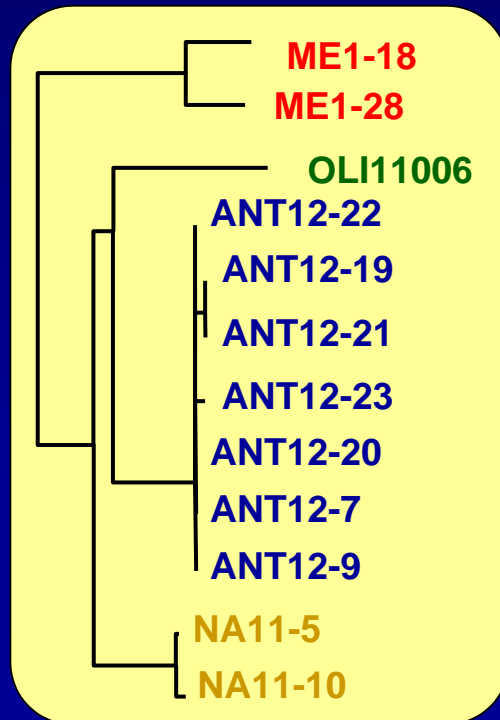
# Probes for novel stramenopiles

846 828

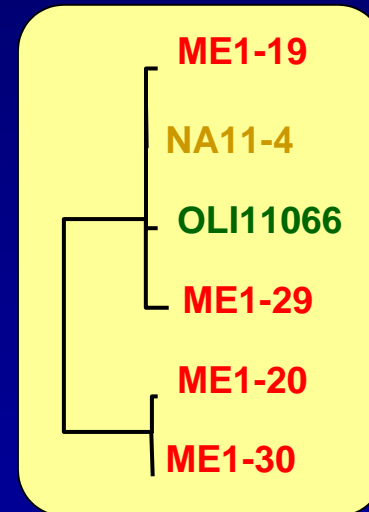
NS3 A T T A C C T T G G C C T C C - A A C

NS4 T A C T T C G G T C T G C A A A C C

### Cluster III



### Cluster IV

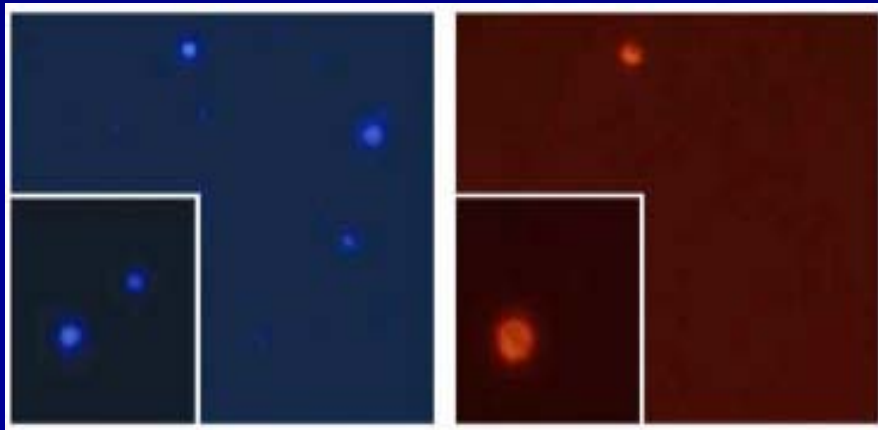


# Detection of novel stramenopiles by FISH

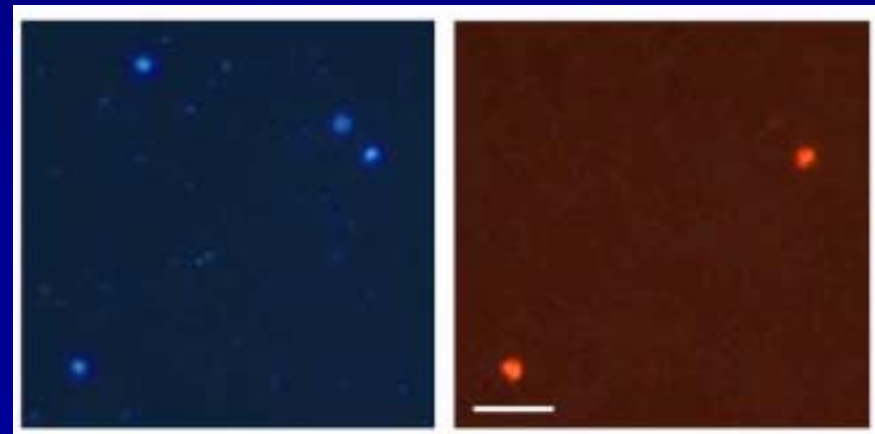
Cultures used as negative controls:

- Prasinophyte
- Prymnesiophyte
- Cryptophyte
- Stramenopiles
  - Eustigmatophyte
  - Chrysoomonad
  - Bicosoecid

Cluster III

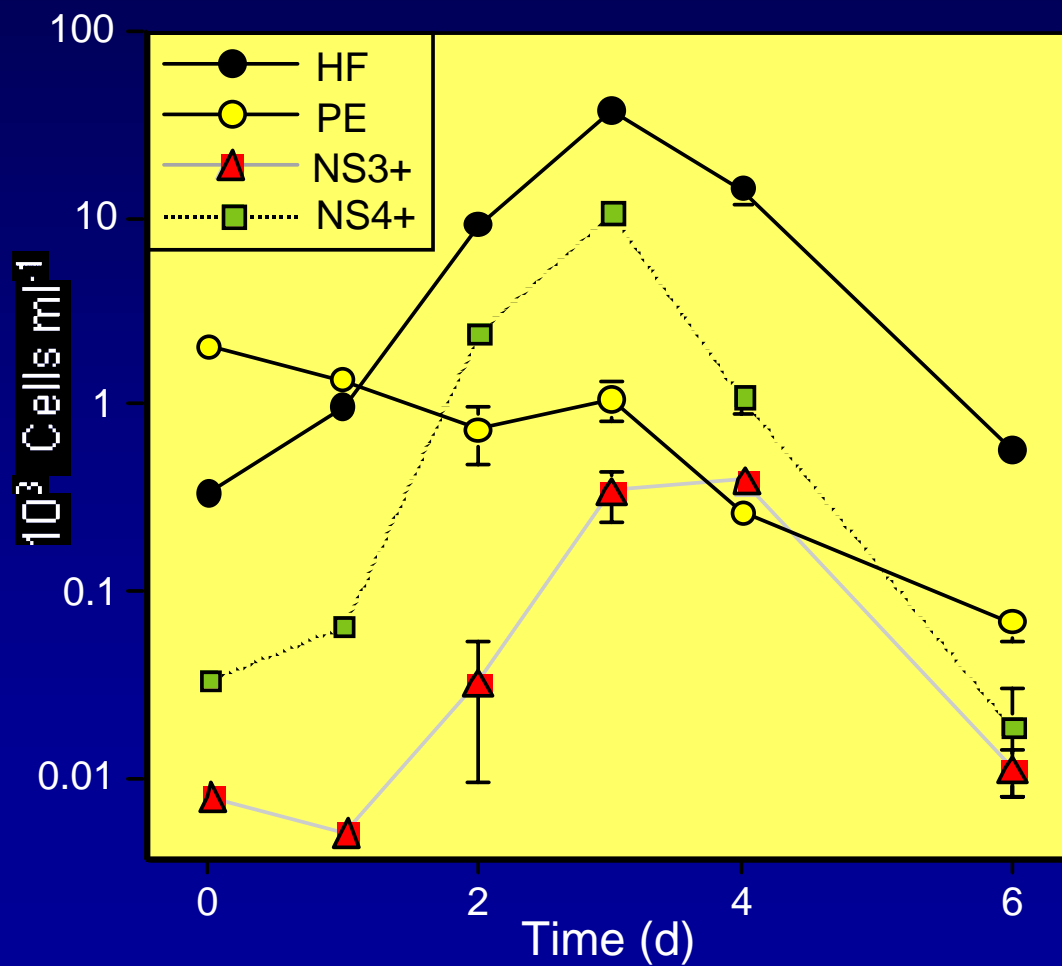


Cluster IV



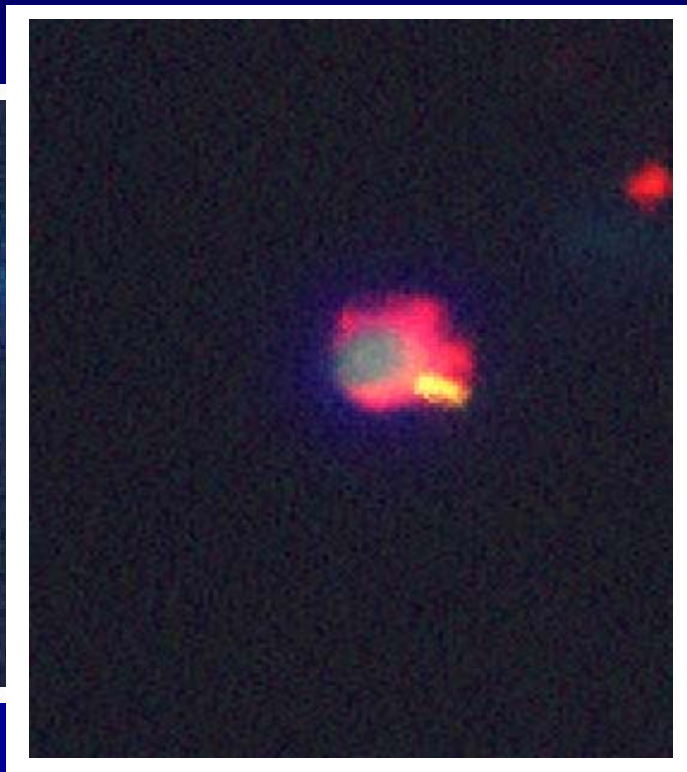
# Dynamics during an enrichment culture

Blanes Coast sample  
2  $\mu\text{m}$  filtrate

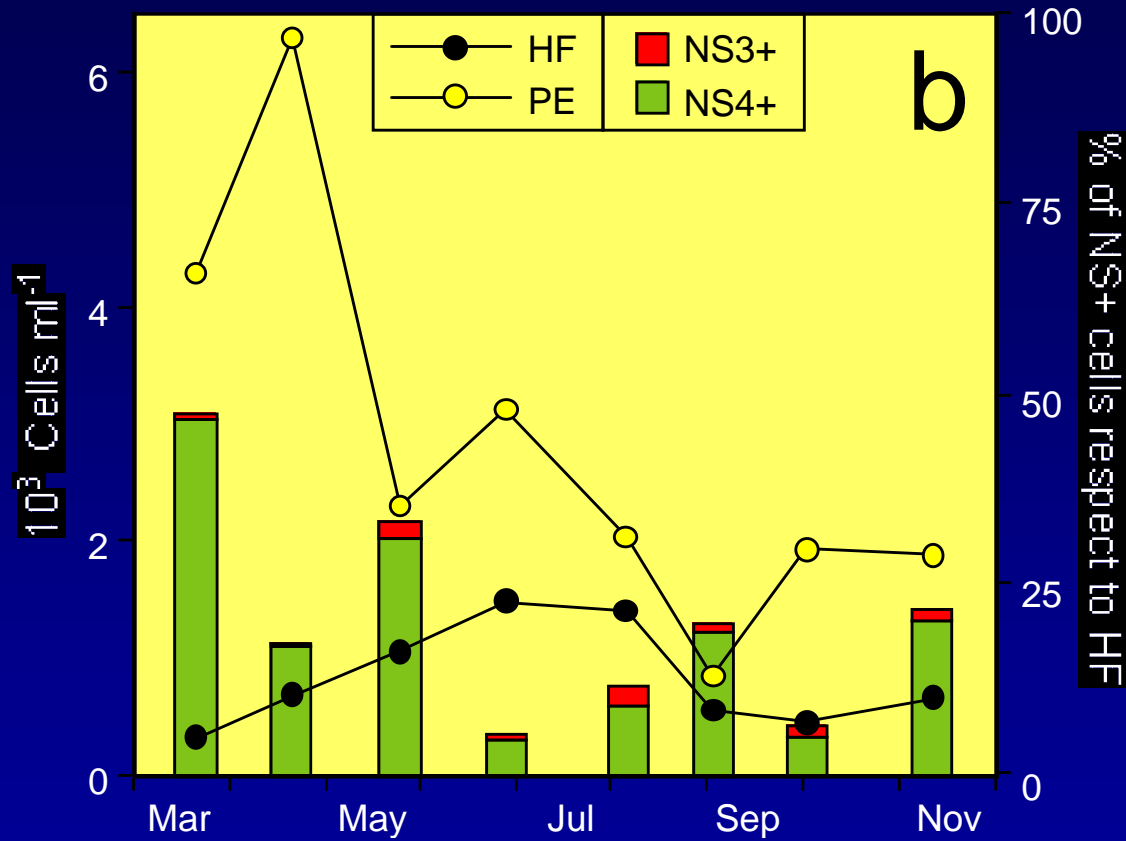


## Experiment of bacterivory

Ingestion of FLB  
 $10^6$  FLB ml<sup>-1</sup> in the enrichment culture for  
2 hours



# Abundance in situ - Blanes Coast



Seasonal average

- NS3+ cells: 12 cells ml<sup>-1</sup> (3% of HF)
- NS4+ cells: 120 cells ml<sup>-1</sup> (20% of HF)

## Summary

Novel stramenopiles are . . .

- Abundant and widespread in genetic libraries of marine picoeukaryotes
- Phylogenetically very diverse, likely including different organisms
- Some are bacterivorous heterotrophic flagellates
- They comprise a significant fraction of natural HF assemblages



# Detection of novel stramenopiles by FISH

Cluster III

Cluster IV

