

# Study of new phylogenetic lineages belonging to alveolate phylum

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# ALVEOLATES

All species classified as Alveolata possess a system of sacs beneath their cell membranes, called alveoli. The species that compose the Alveolata are very diverse and differ from one another greatly. The Alveolates are composed of three main groups of organisms with unique characteristics :

**Ciliates:** heterotrophs propelled by cilia that eat bacteria, algae, and other ciliates

**Dinoflagellates:** Dinoflagellates are important primary producers in both marine and freshwater environments. Half are photosynthetic while the other half are heterotrophic. Dinoflagellates are also predatorial and parasitic.

**Apicomplexa:** parasitic heterotrophs that form spores and cause human disease



**Recent publications analysed 18S ribosomal DNA genetic libraries from eukaryotic microbes collected in different marine systems :**

Lopez-garcia et al., 2001

Moon-van der staay et al., 2001

Diez et al., 2001

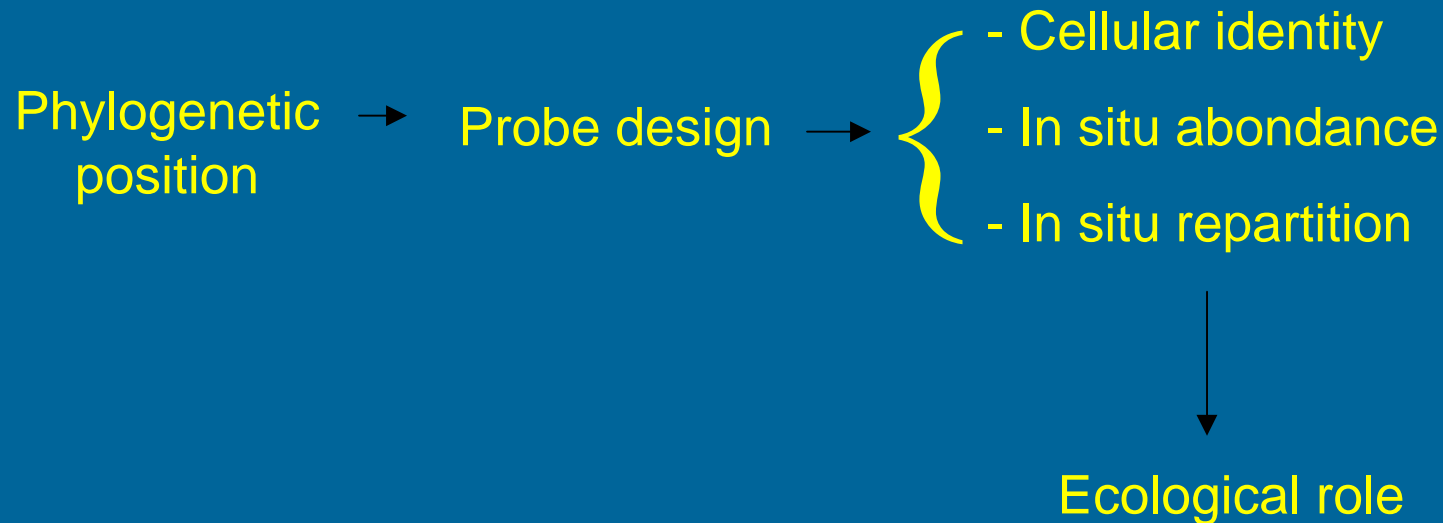
Edgcomb et al., 2002



**- High phylogenetic diversity**

**- Novel phylogenetic lineages within the stramenopiles and alveolate phyla**

# Study of new phylogenetic lineages belonging to alveolate phylum



## Available sequences :

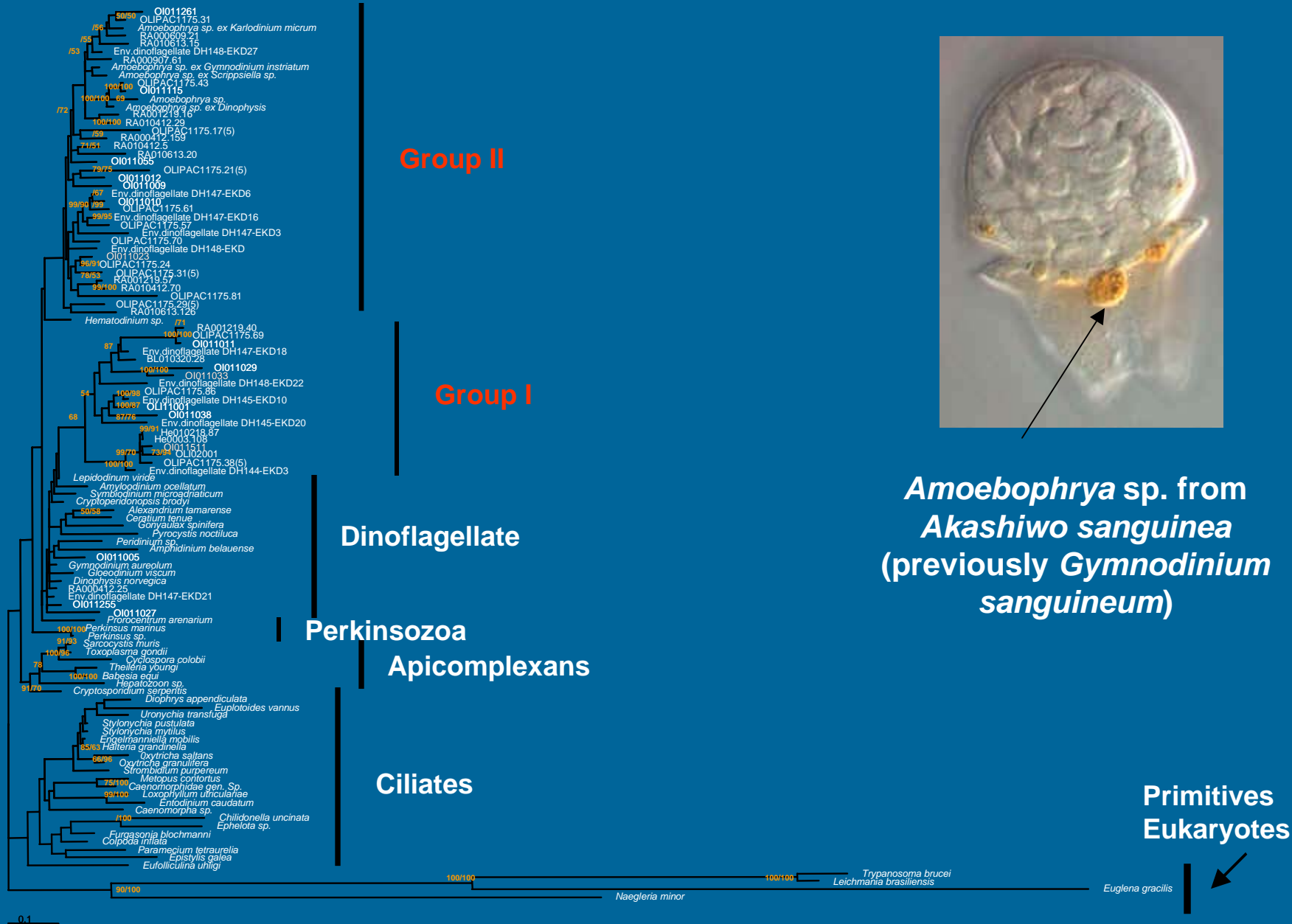
- 15 full group II and 11 full group I sequences
- 143 partial group II and 85 partial group I sequences

Strain/Lib	Clone	Class	Order	Done	By	Remark
HE0003xx	108	Alveolates	Group I	Done	Klaus	
HE010218	87	Alveolates	Group I	Done	Klaus	
BL010320	28	Alveolates	Group I	Done	Ramon	
OLIPAC 11.75	17(5)	Alveolates	Group II	Done	Agnès	
	21(5)	Alveolates	Group II	Done	Agnès	
	24	Alveolates	Group II	Done	Agnès	
	29(5)	Alveolates	Group II	Done	Agnès	
	31	Alveolates	Group II	Done	Agnès	
	31(5)	Alveolates	Group II	Done	Agnès	
	38(5)	Alveolates	Group I	Done	Agnès	
	43	Alveolates	Group II	Done	Agnès	
	45	Alveolates	Group I	Done	Agnès	OL11019 Group II
	57	Alveolates	Group I	Done	Agnès	
	61	Alveolates	Group II	Done	Agnès	
	69	Alveolates	Group I	Done	Agnès	
	70	Alveolates	Group II	Done	Agnès	
	86	Alveolates	Group I	Done	Agnès	
RA000412	25	Alveolates	Group I	Done	Agnès	Dinoflagellate
	159	Alveolates	Group II	Done	Agnès	
RA000609	21	Alveolates	Group II	Done	Agnès	
RA000907	61	Alveolates	Group II	Done	Agnès	
RA001219	16	Alveolates	Group II	Done	Agnès	
	40	Alveolates	Group I	Done	Agnès	
	57	Alveolates	Group II	Done	Agnès	
RA010412	5	Alveolates	Group II	Done	Agnès	
	29	Alveolates	Group II	Done	Agnès	
	70	Alveolates	Group II	Done	Agnès	
RA010613	15	Alveolates	Group II	Done	Agnès	
	20	Alveolates	Group II	Done	Agnès	
	126	Alveolates	Group II	Done	Agnès	

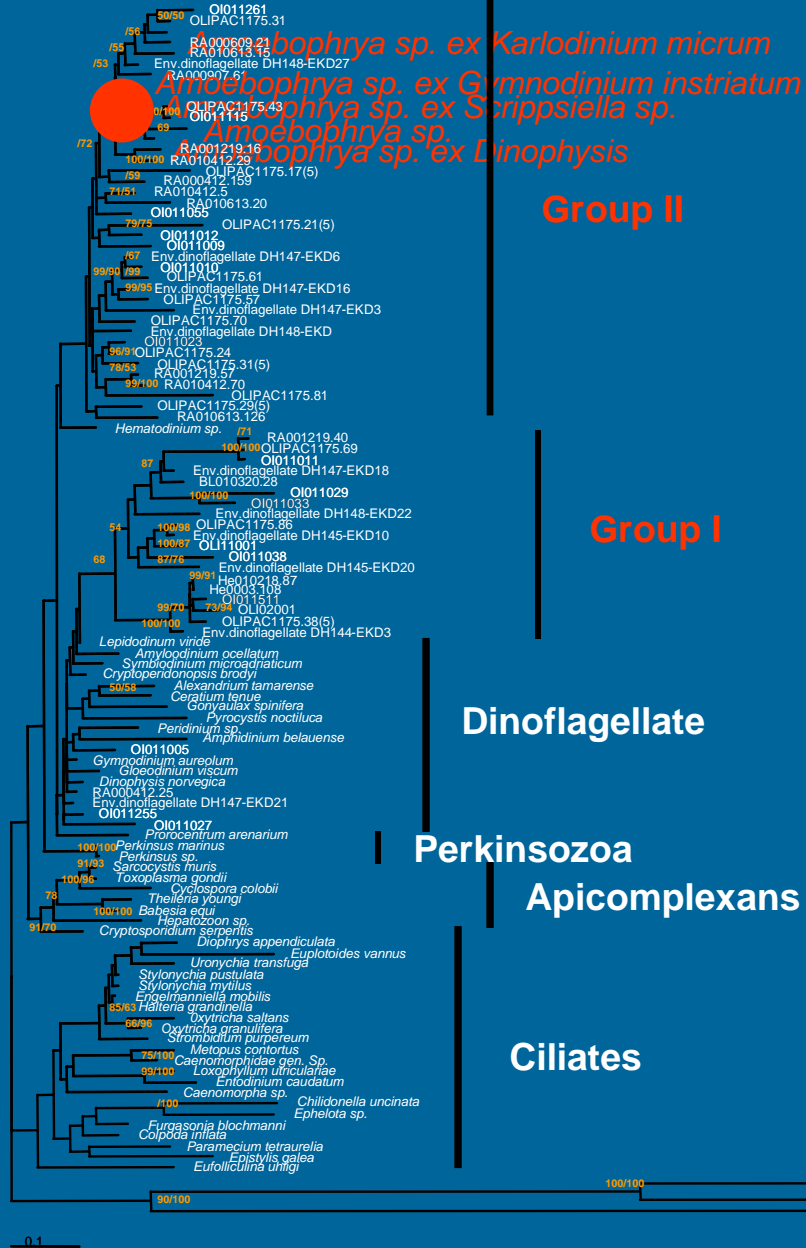
Full 18S rDNA  
sequences added :

- 21 group II

- 7 group I



Maximum likelihood phylogenetic tree with full 18S rDNA sequences of novel alveolates. The scale bar indicates 0,1% sequence divergence. Neighbor-joining and maximum-parsimony bootstrap values (left to right, respectively) are shown at the internal branches ( 100 replicates , values > 50% shown).



***Amoeboophrya sp.* from  
*Akashiwo sanguinea*  
 (previously *Gymnodinium sanguineum*)**

**Primitives  
 Eukaryotes**

Maximum likelihood phylogenetic tree with full 18S rDNA sequences of novel alveolates. The scale bar indicates 0,1% sequence divergence. Neighbor-joining and maximum-parsimony bootstrap values (left to right, respectively) are shown at the internal branches ( 100 replicates , values > 50% shown).

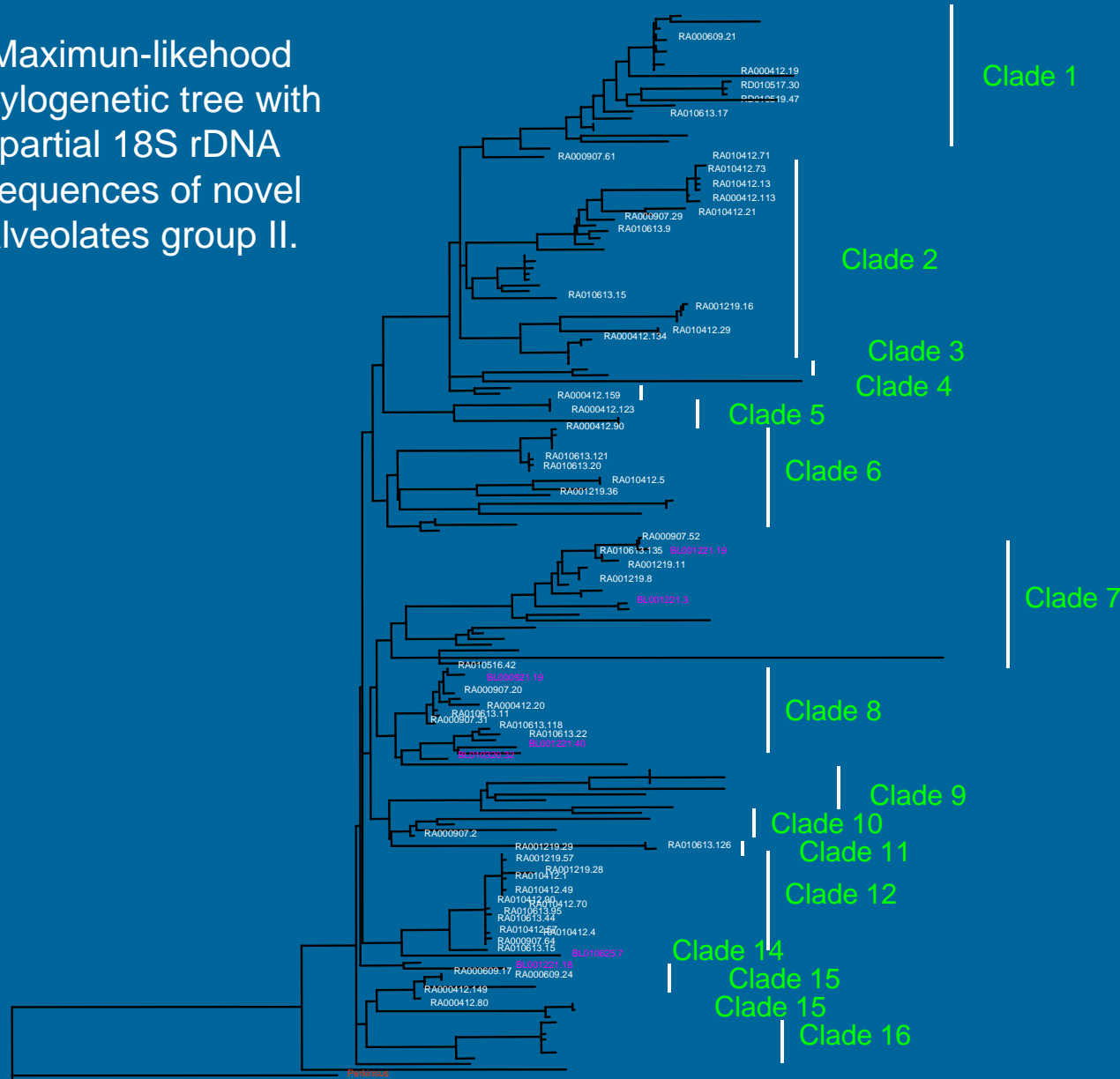
# Next stage : PROBE DESIGN

**Construction of phylogenetic group I and group II trees with all full and partial sequences available**

- **Probe design for whole group I and whole group II**
- **Define clades component group I and II**
- **Probe design for these clades**

Use of ARB design probe function and GENEDOC program

Maximun-likelihood  
phylogenetic tree with  
partial 18S rDNA  
sequences of novel  
alveolates group II.



Clade 1

Clade 2

Clade 3

Clade 4

Clade 5

Clade 6

Clade 7

Clade 8

Clade 9

Clade 10

Clade 11

Clade 12

Clade 14

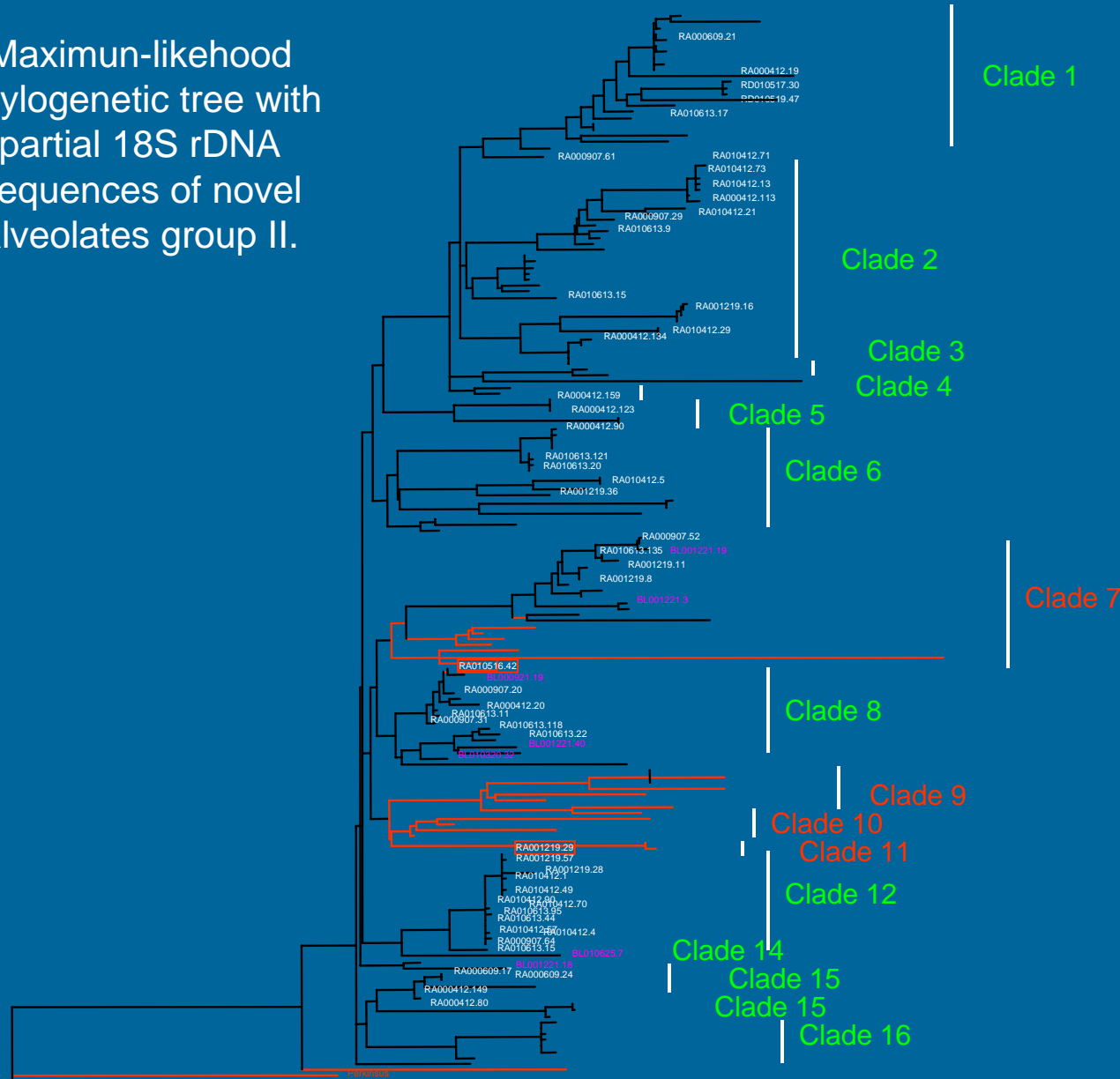
Clade 15

Clade 15

Clade 16

Only sequences  
from Roscoff and  
Blanes are retained

Maximum-likelihood phylogenetic tree with partial 18S rDNA sequences of novel alveolates group II.



Clade 1

Clade 2

Clade 3  
Clade 4

Clade 5

Clade 6

Clade 7

Clade 8

Clade 9  
Clade 10  
Clade 11

Clade 12

Clade 14

Clade 15

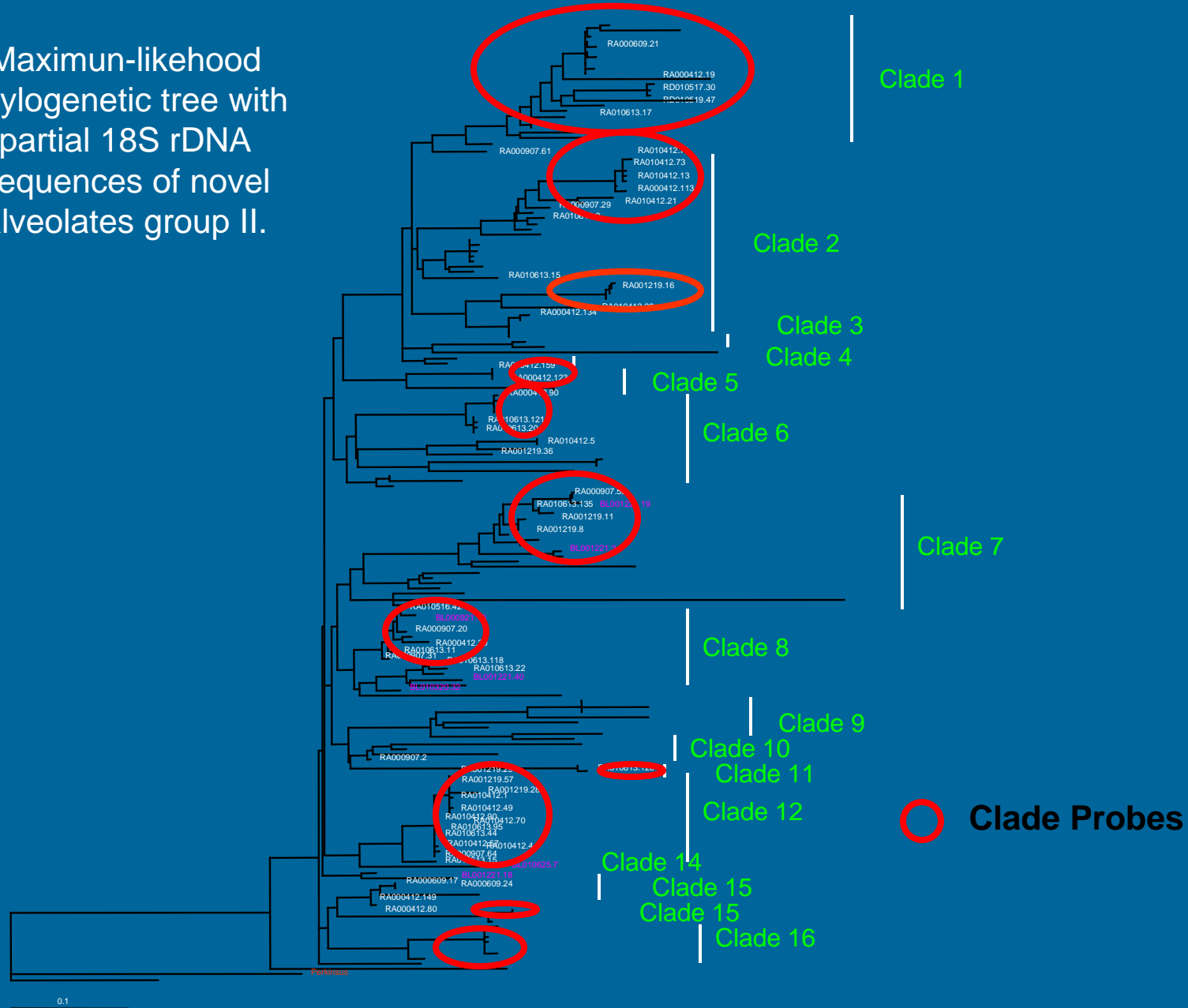
Clade 15

Clade 16

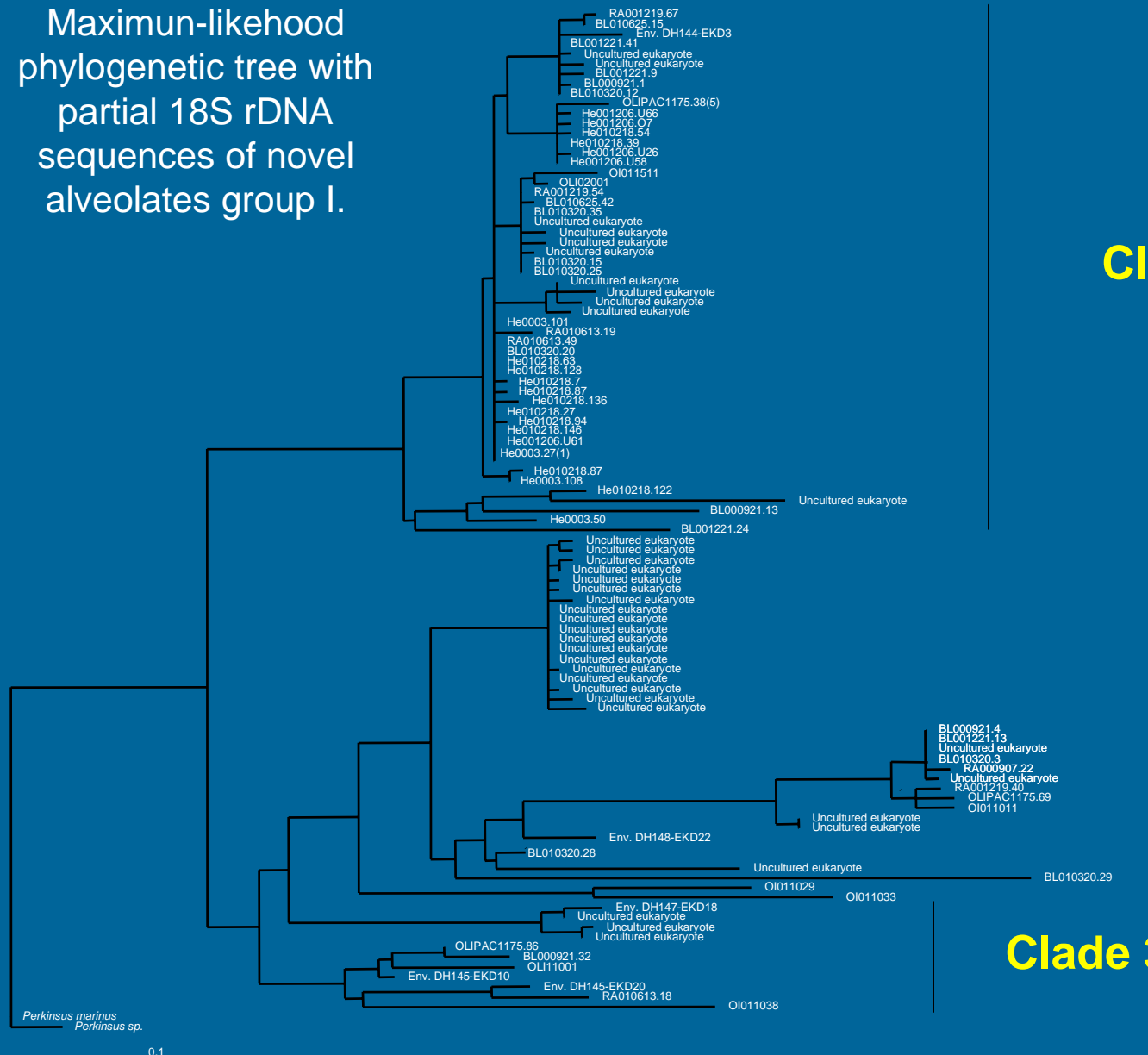
ALVO1  
probe

0.1

Maximum-likelihood phylogenetic tree with partial 18S rDNA sequences of novel alveolates group II.



Maximum-likelihood phylogenetic tree with partial 18S rDNA sequences of novel alveolates group I.



Clade 1

Clade 2

Clade 3



# PROBE TEST

- Define the optimal protocol for probe test (Temperature, % formamide, TSA or CY3)
- Probe test on different cultures (negatif control)
- Probe test on enrichment from Roscoff
- Probe test on environment samples from Blanes and Roscoff

## Available filters for blanes and Roscoff

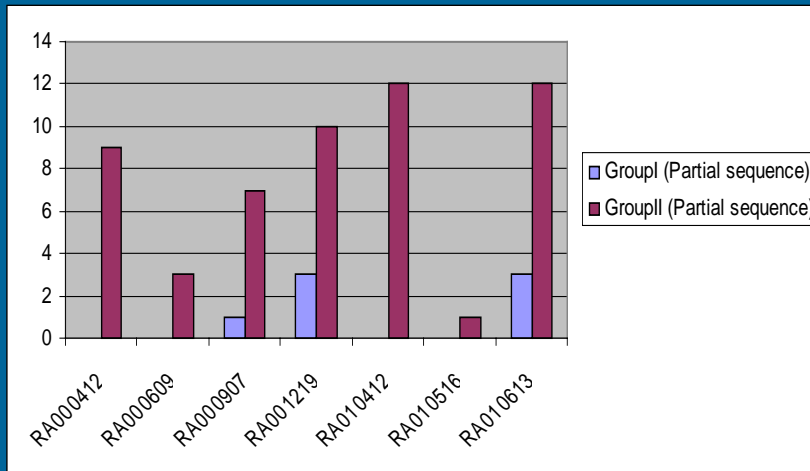
### \*Roscoff filtration method

BL010320 / RA010319	BL020114 / RA020108
BL010419 / RA010412	BL020312 / RA020303
BL010523 / RA010530	BL020415 / RA020422
BL010625 / RA010626	BL020627 / RA020618
BL010802 / RA010731	BL020724 / RA020719
BL010829 / RA010831	BL020814 / RA020810
BL010927 / RA010926	BL020926 / RA020816
BL011106 / RA011112	BL021023 / RA021029
BL011204 / RA011207	

**+ 25 others dates for roscoff**

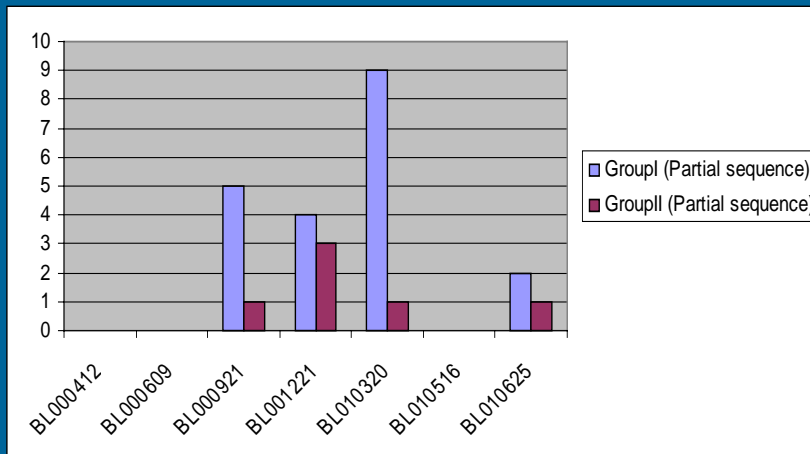
# Number of alveolate partial sequences obtained by libraries over 14 months

A  
S  
T  
A  
N  
  
B  
L  
A  
N  
E  
S



Prevailing group II

These two sites were selected for analysis

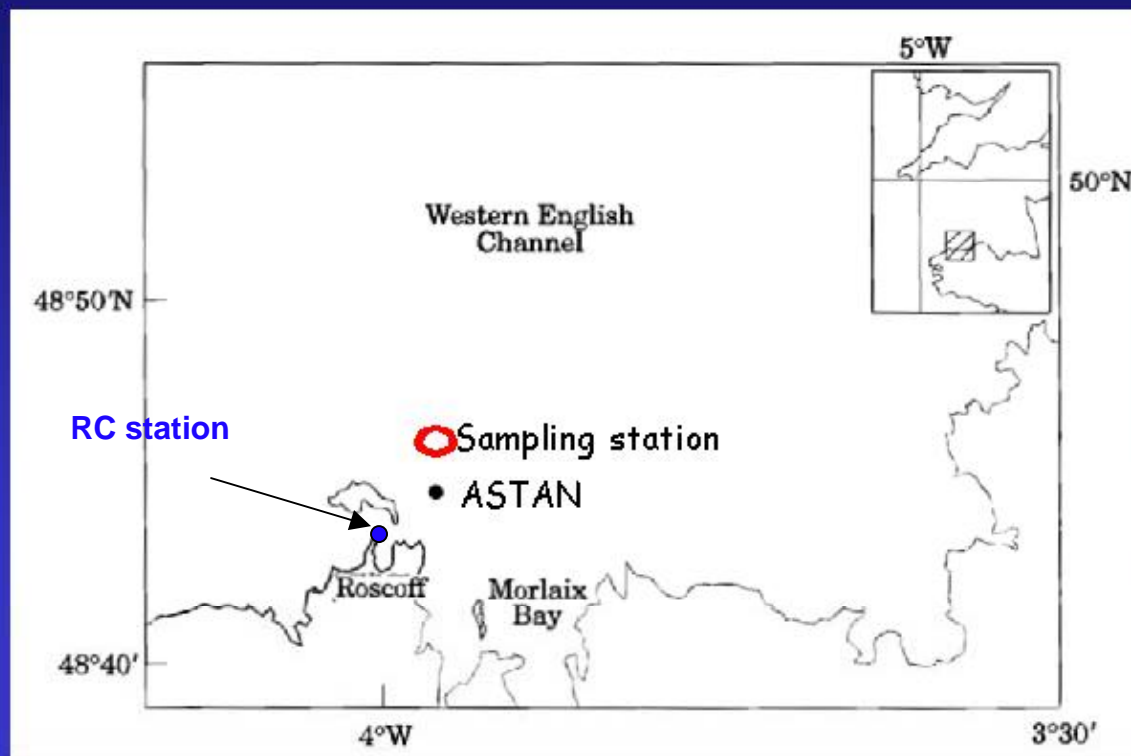


Prevailing group I

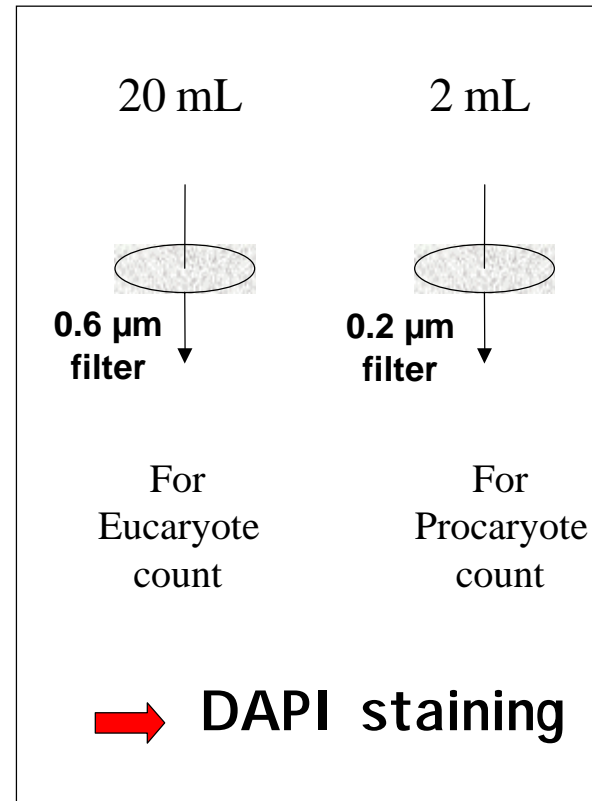
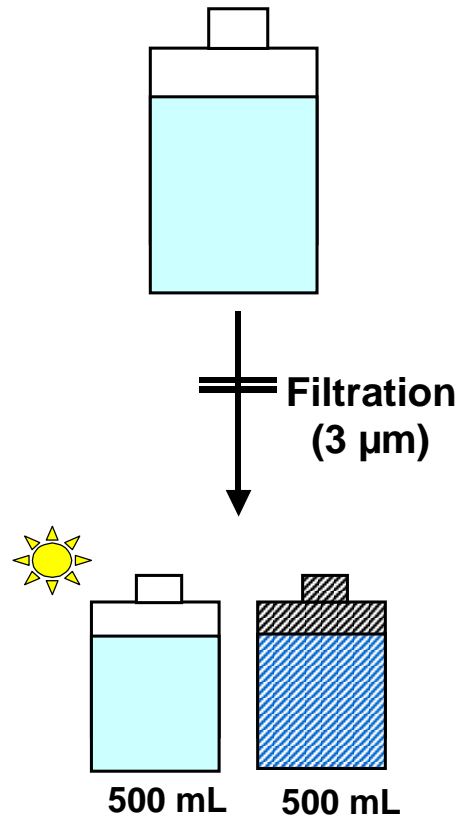
Remark : No correlation between dates and clades

# ENRICHMENTS

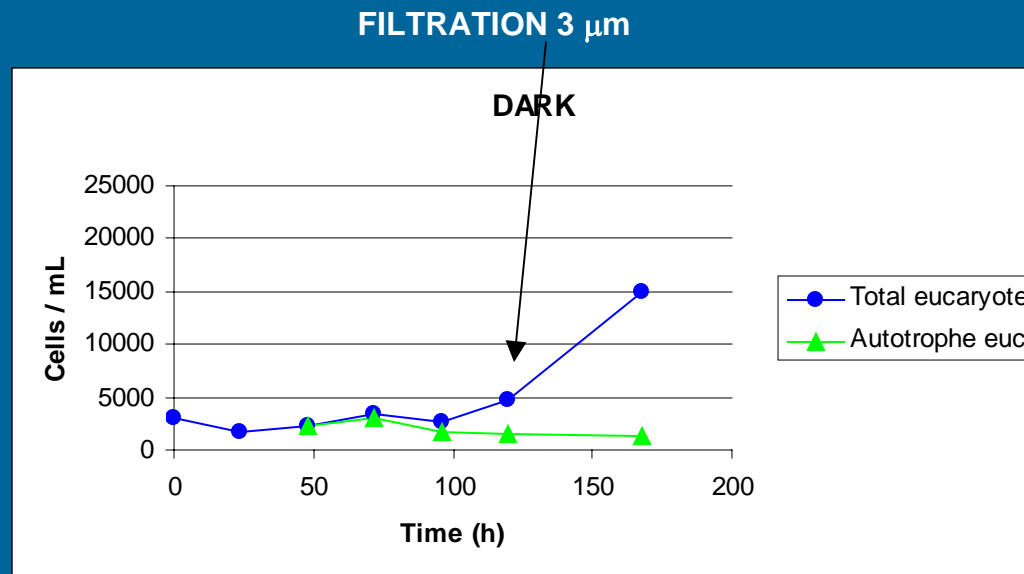
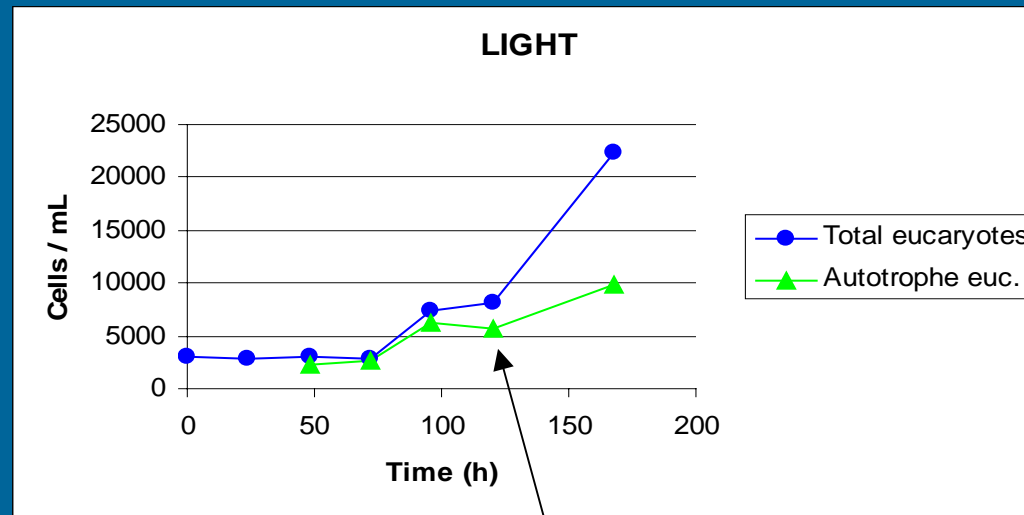
## The Sampling Station



# Enrichment cultures with Roscoff seawater



# Enrichment results of Roscoff coating sample RC030210



# Tested conditions in FISH-TSA with ALV01

% Formamide	Temperature hybridation/Washing
0	
20	35/37 ou
30	42/46
40	



Choice :

42/46°C

40%Formamide

\* DNA is labelled by Propidium Iodure (red coloration)

Tested RCC cultures :

*Symbiomonas scintillans* RCC25

*Thalassiosira weissflogii* RCC76

*Emiliana huxleyi* RCC173

*Micromonas* BL122 RCC434

*Colpodea* RCC590

*Prorocentrum nux* RCC303

*Alexandrium tumarensense* RCC84

*Bolidomonas mediterranea* RCC238

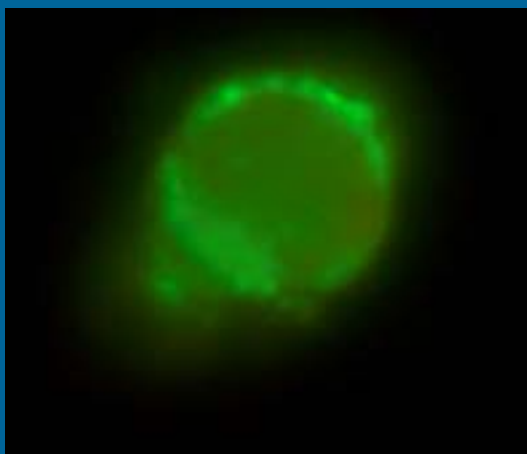
*Pelagomonas calceolata* RCC100

*Gymnodinium sanguinea* RCC89

**RCC89**



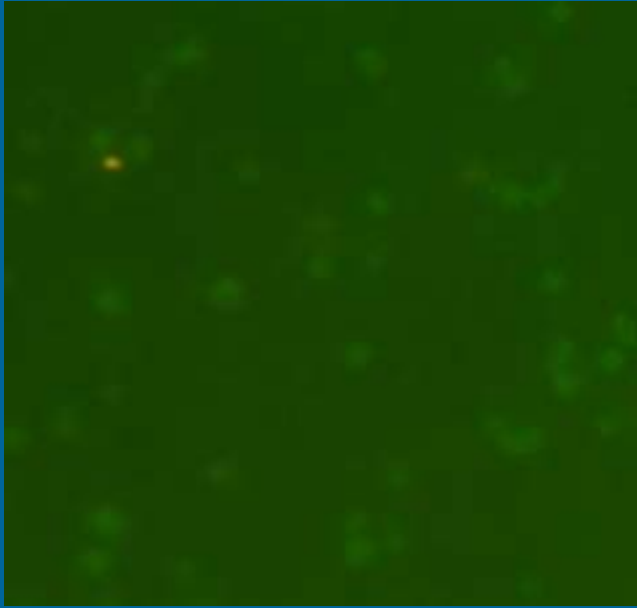
**ALVO1**



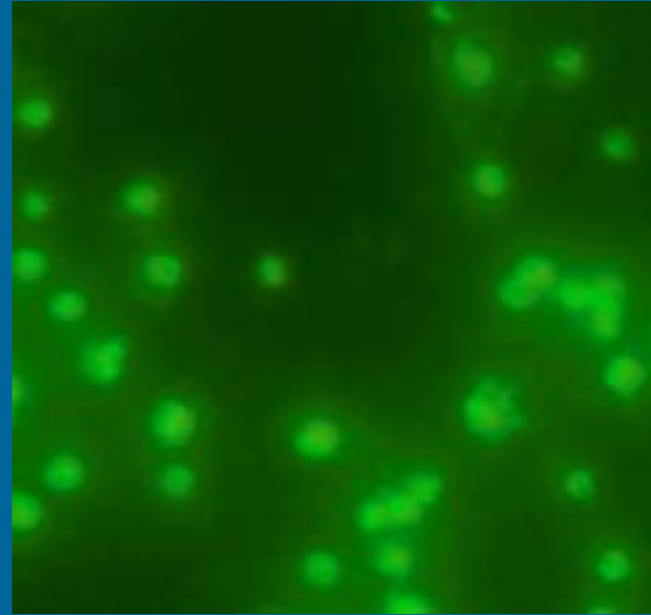
**NCHLO01**



**EUK1209+CHLO01  
+NCHLO01**



ALV01 probe



CHLO02 probe

**Micromonas BL122 culture**

Tested bacteria cultures :

*Pseudomonas aeruginosa* NCIMB8295

*Alteromonas macleodii* NCIMB1963

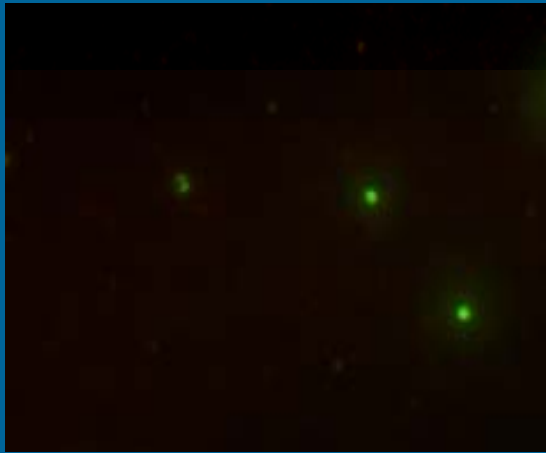
*Roseobacter denitrificans* NCIMB2176

*Vibrio mediterranei* NCIMB13228

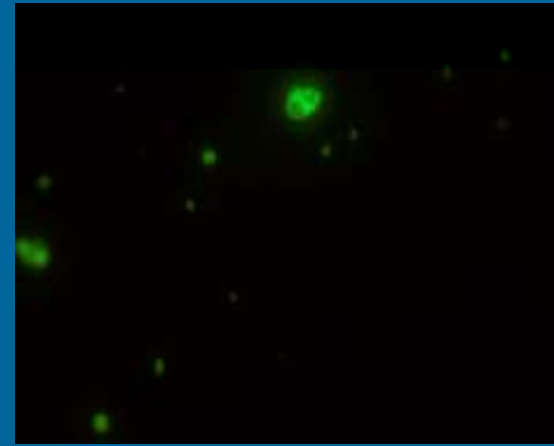
**NCIMB8295**



**ALV01**

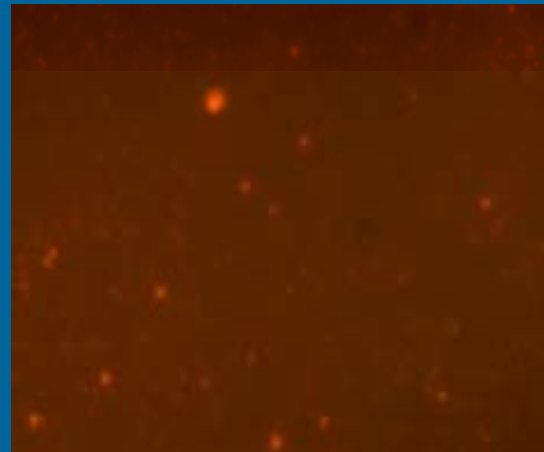


NCHLO01

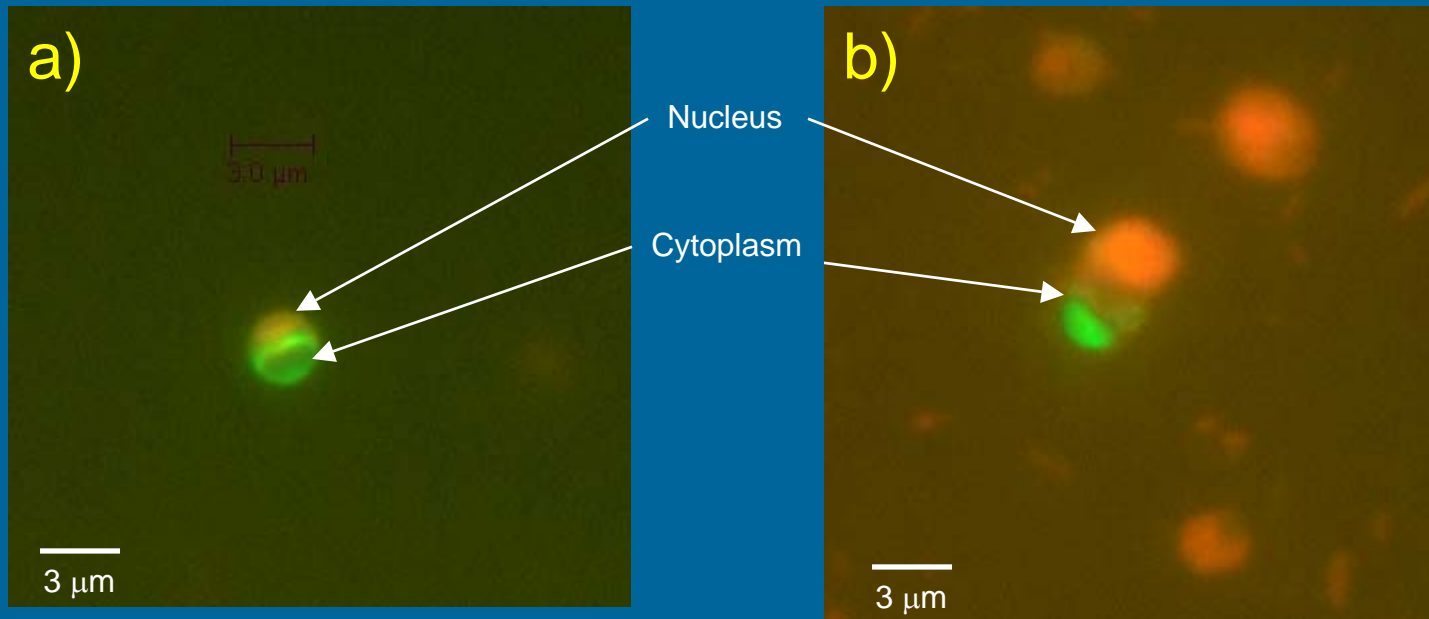


EUK1209+CHLO01  
+NCHLO01

ALVO1



**Enrichment RC030215 in light**



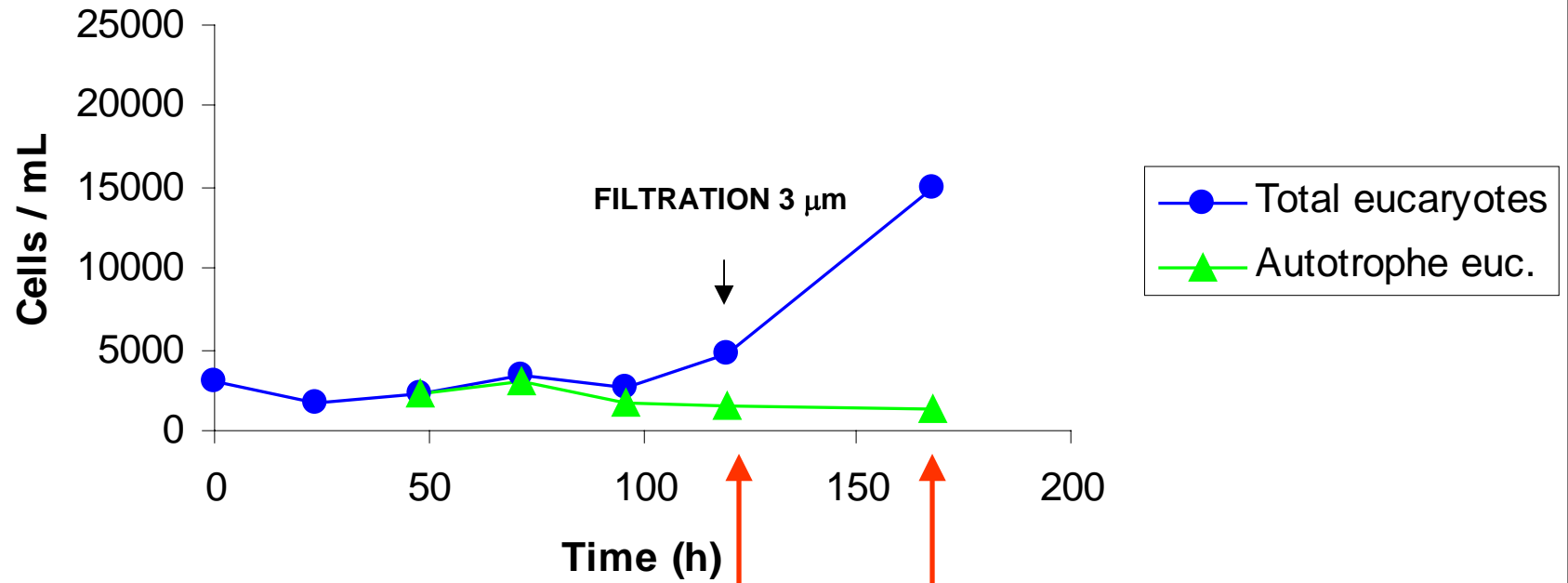
Enrichment : RC03/02/15 in dark



Two cellular types targetted with ALV01 probe  
**only in DARK**

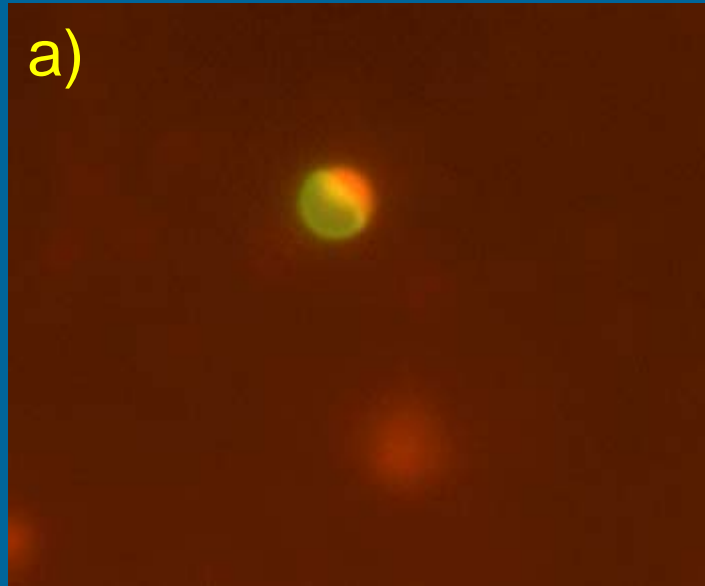
Number type b > type a

**DARK**



**RA030215**

**RA030217**



Enrichment : RC03/02/17 in Dark

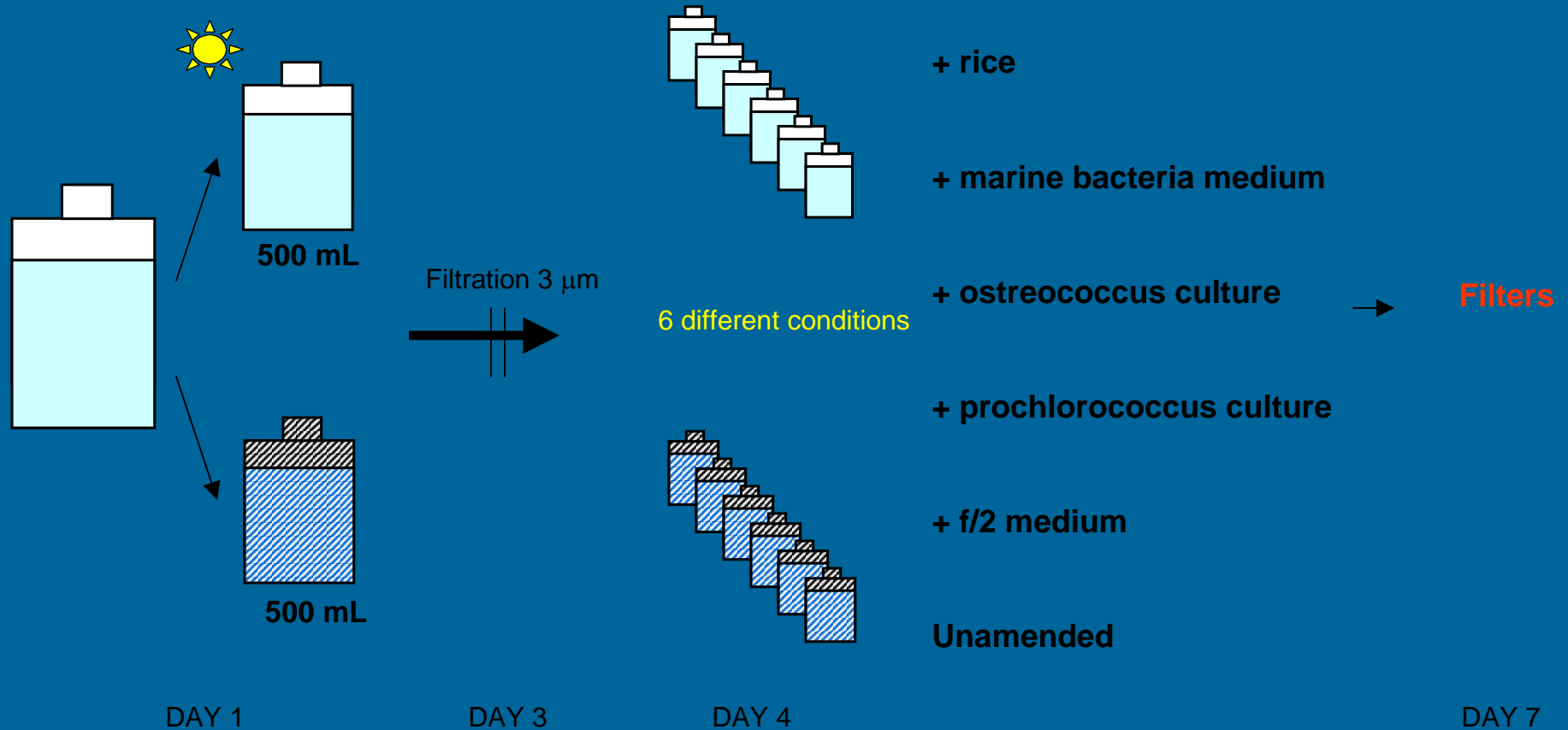


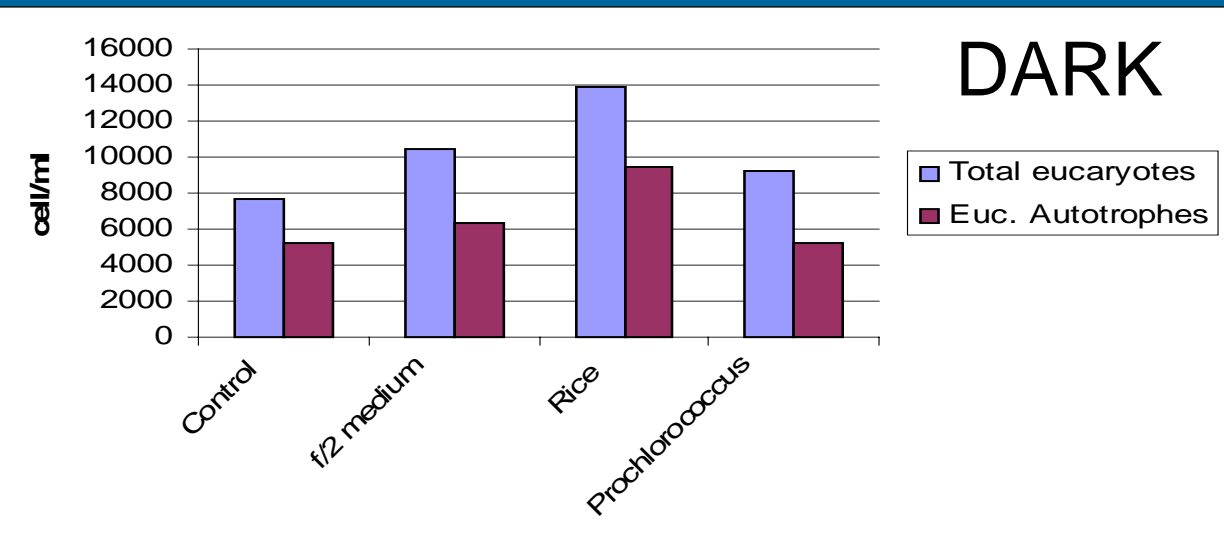
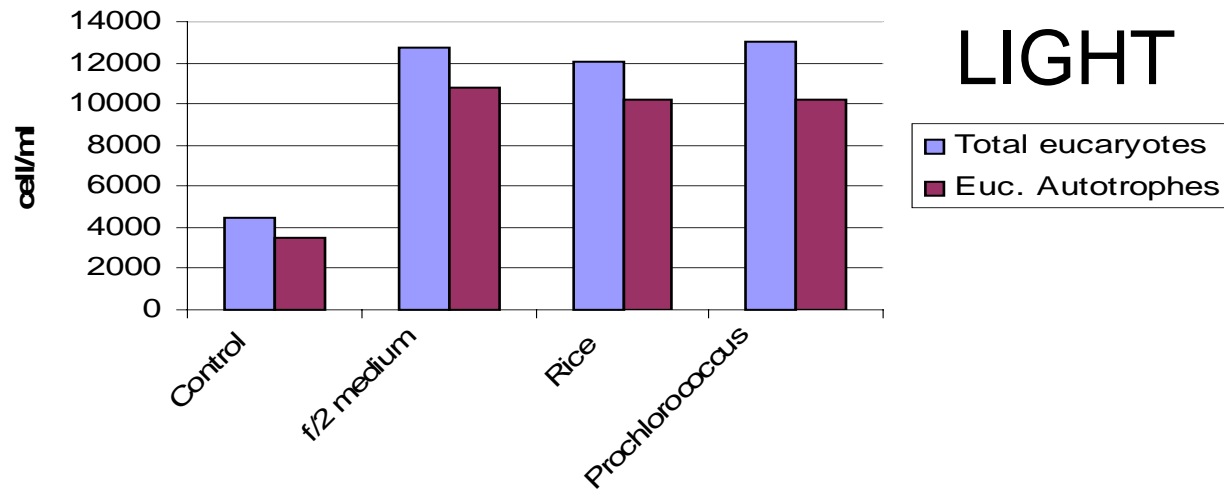
One cellular type targetted with ALV01 probe

Type b eliminated « by filtration »

Increase of type a number

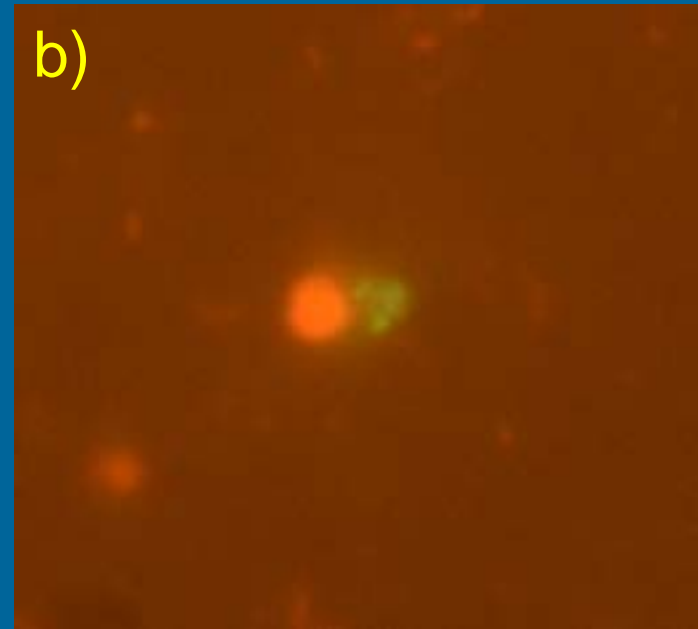
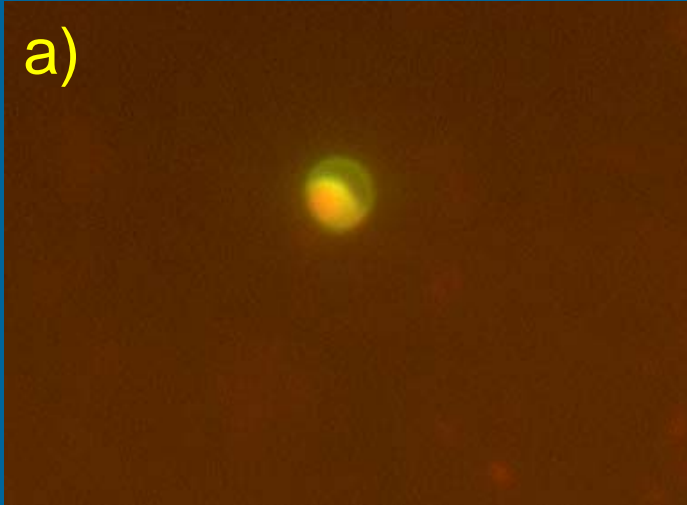
# Second enrichment





# RESULTS :

We have found cellular type a and b only in **f/2** and **unamended media**



# Results of FISH-TSA with ALV01 on environment samples from Roscoff

## Analysed dates:

RA010319

RA010731

RA011207

RA020422

RA010412

RA010831

RA020108

RA020523

RA010529

RA010912

RA020207

RA020618

RA010628

RA011112

RA020302

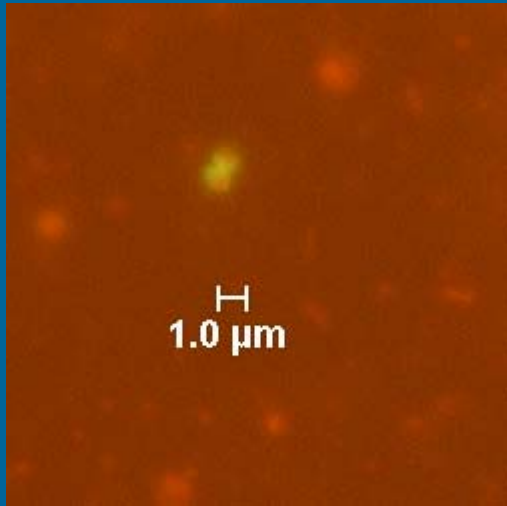
RA020819

## Results:

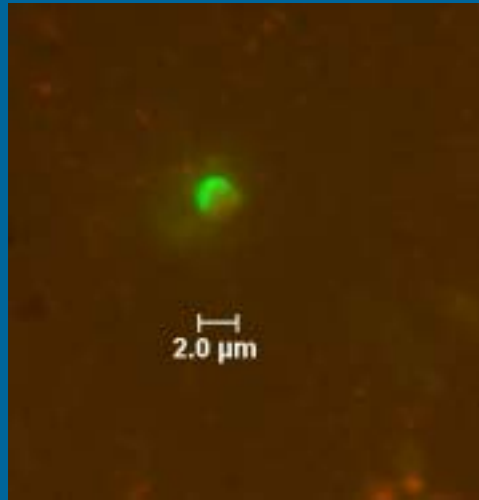
- Nothing for the majority of filters
- Any type a
- May be rare type b



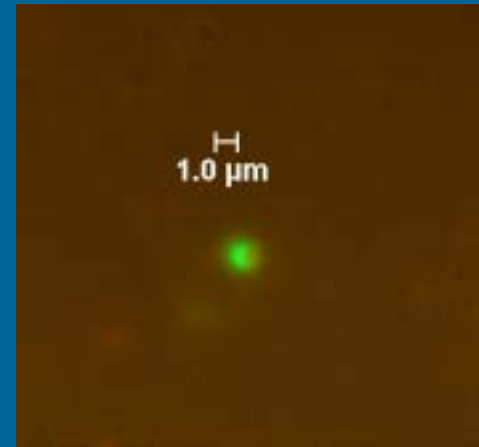
According with the results obtained with ALV01 on  
first day of two enrichments



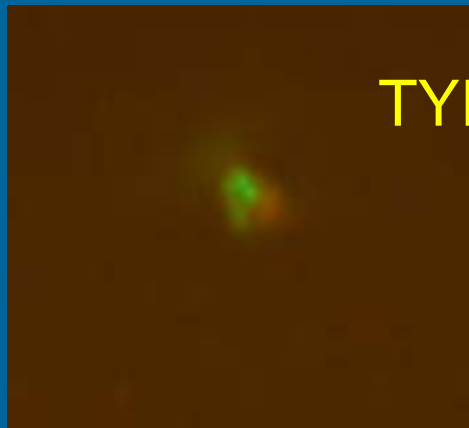
RA010319



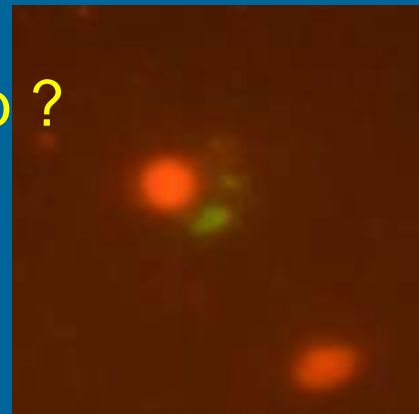
RA010412



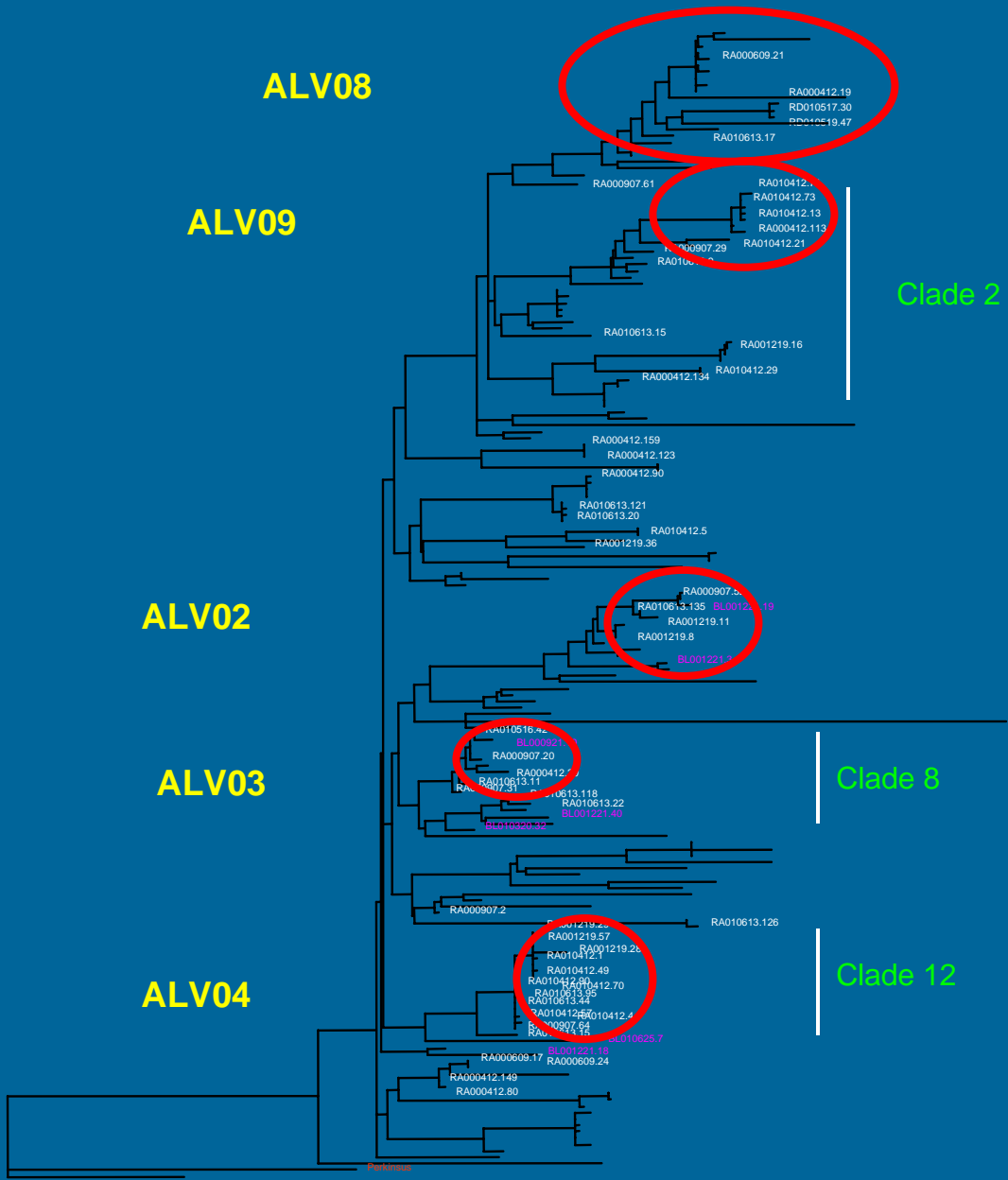
RA010912



RA011207



RA020618



ALV08

ALV09

ALV02

ALV03

ALV04

Clade 1

Clade 2

Clade 7

Clade 8

Clade 12

Where are cellular types a and b ?

Are there others cellular types ?

ALV01 more specific ?

# CONCLUSIONS

Group II :

- 1 « general » probe ALV01
- 11 clade probes on 16 clades

Group I :

- no general probe
- 1 probe for each clade 1, 2 and 3

Tested :

ALV01 on enrichment and environment samples from Roscoff



**Two cellular types detected only in enrichments unamended**

# PERSPECTIVES

Agnes , Antoine

- **Define the optimal protocol for all probes**
- **Probes test on different cultures (negatif control)**
- **Probes test on enrichment**

Agnes

**Probes test on environment samples from Blanes and Roscoff :**

- Cellular identity
- In situ abundance
- In situ repartition

Pb :With the available filters of Roscoff is no possible to differenciate photo and heterotrophes (ethanol fixation) .

