Probes targeting novel stramenopiles

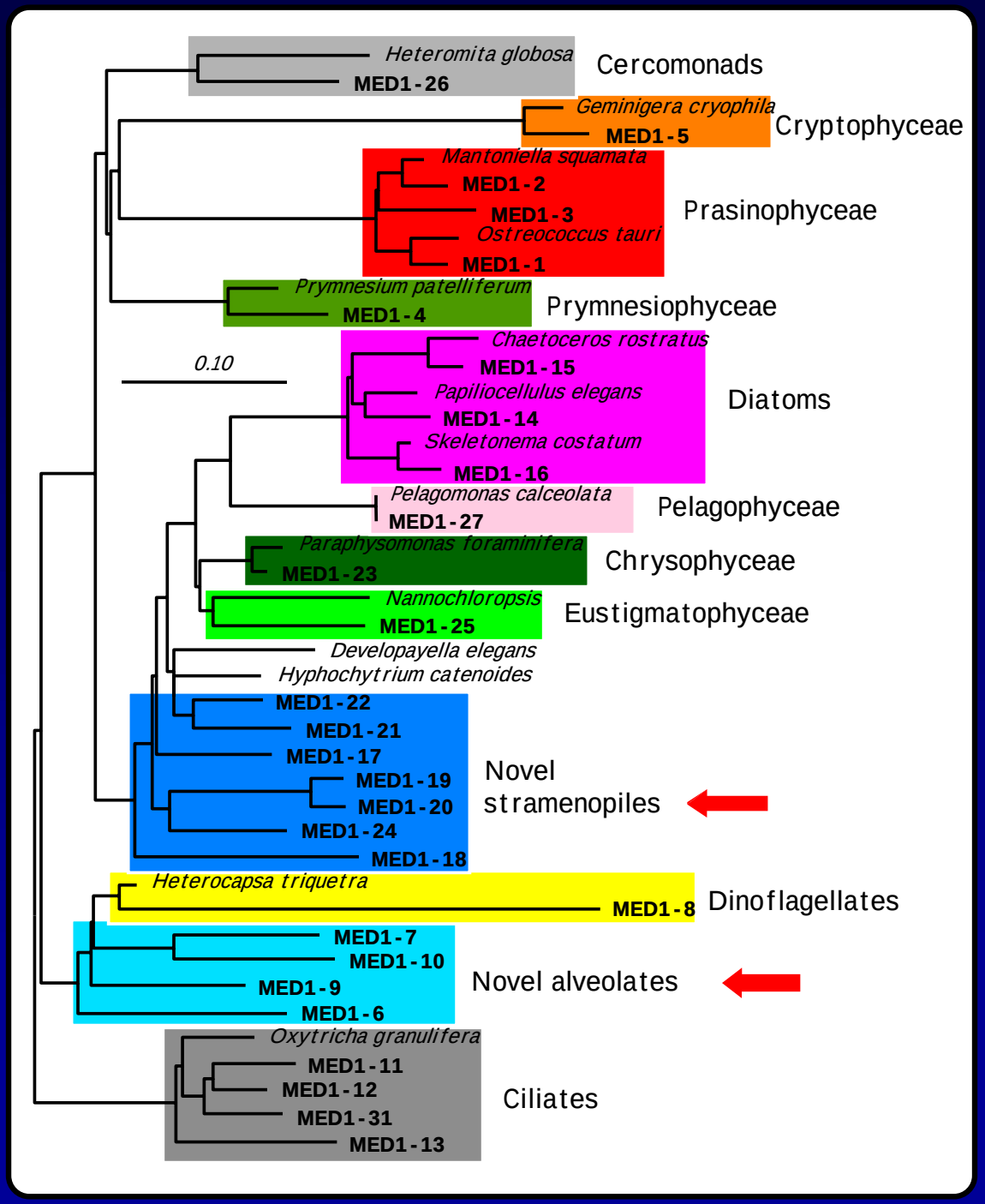
Oslo, March 2003. Last PICODIV Workshop

Results from the ICM partner (and data from other groups)

A background for a recently recognized group

Novel stramenopiles are . . .

- Abundant and widespread in genetic libraries of marine picoeukaryotes

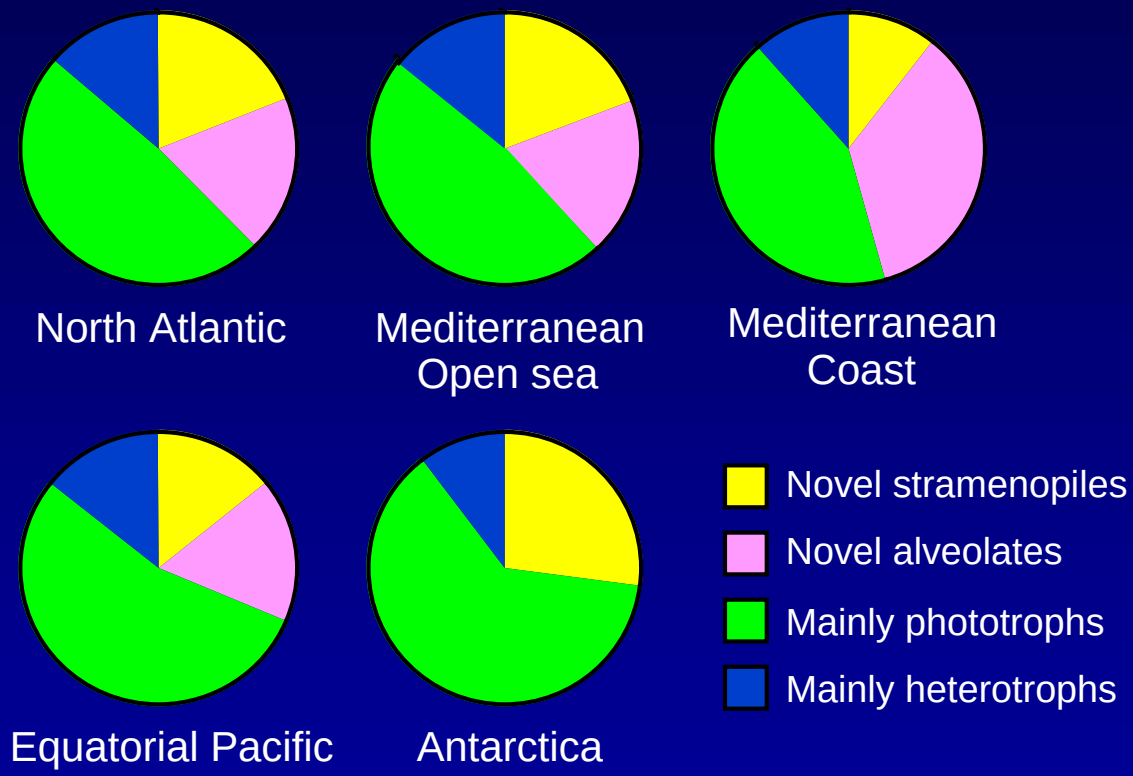


Genetic library of 18S rRNA genes

Sample analyzed
Alboran Sea (NW Med) - 5m
Fraction: 0.2 - 5 µm

Díez et al. 2001

Novel groups are abundant and widespread



Díez et al. 2001
López-García et al. 2001
Moon van der Staay et al 2001

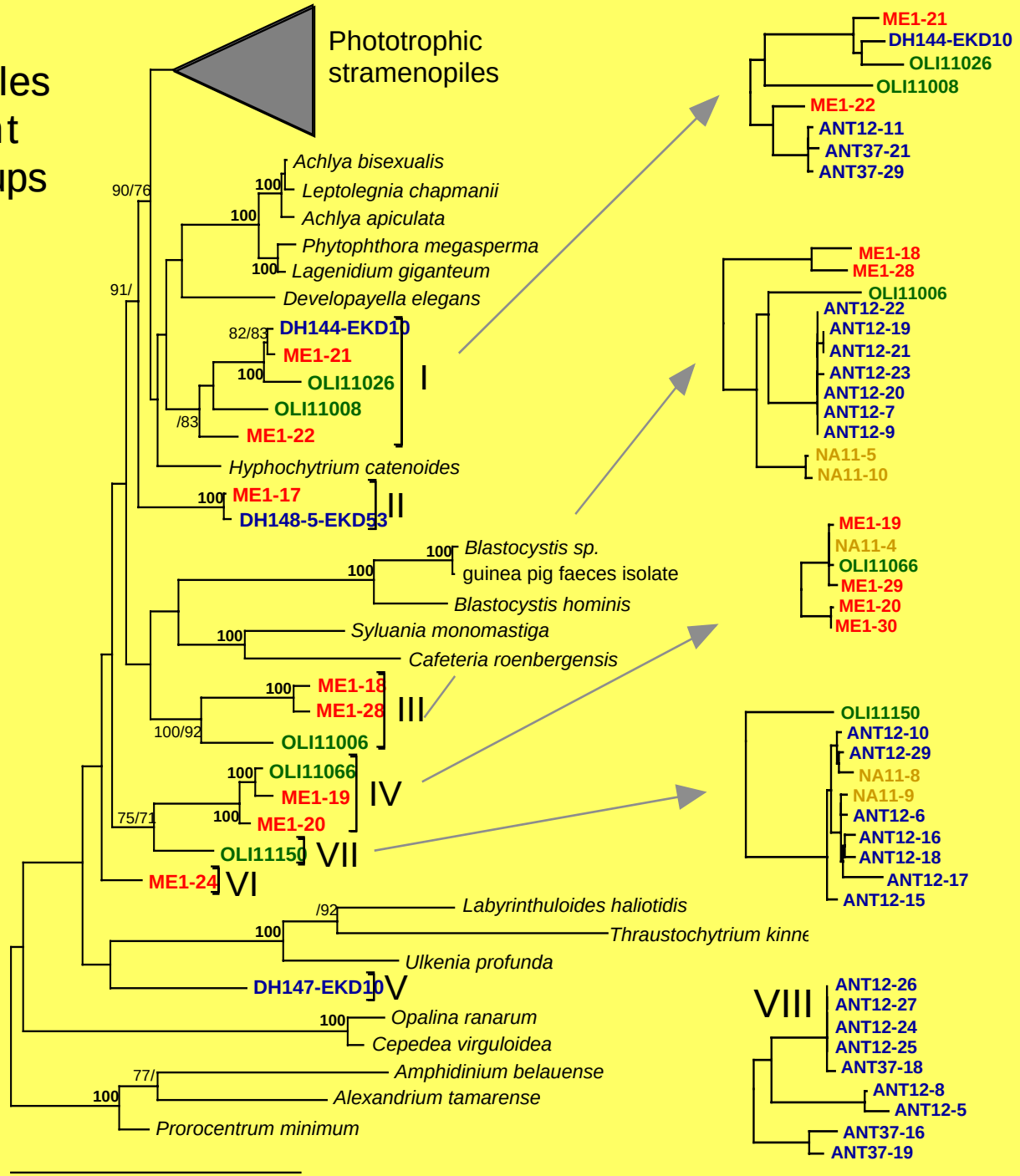
A background for a recently recognized group

Novel stramenopiles are . . .

- Abundant and widespread in genetic libraries of marine picoeukaryotes
- Phylogenetically very diverse, likely including different organisms

Libraries already analyzed (n=52)				
			TOTAL	Complete
	Mediterranean Sea	ME1	10	8
	Antartica	ANT12	22	0
	Antartica	ANT37	8	0
	Antartica	DH147	3	3
	North Atlantic	NA11	5	0
	Equatorial Pacific	OLI011	5	5

Novel stramenopiles include different phylogenetic groups



Massana et al. 2002

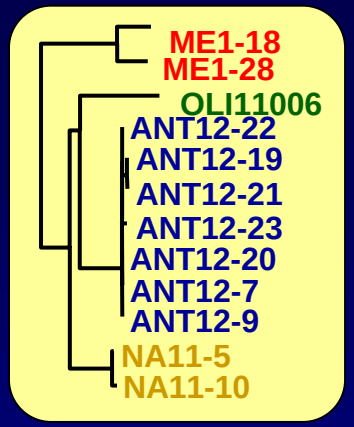
A background for a recently recognized group

Novel stramenopiles are . . .

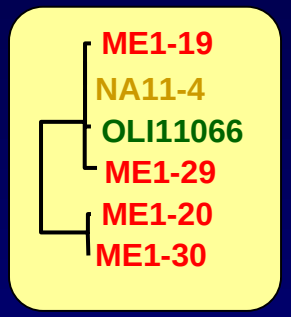
- Abundant and widespread in genetic libraries of marine picoeukaryotes
- Phylogenetically very diverse, likely including different organisms
- Some are bacterivorous heterotrophic flagellates

Design of probes and detection by FISH

Cluster 3

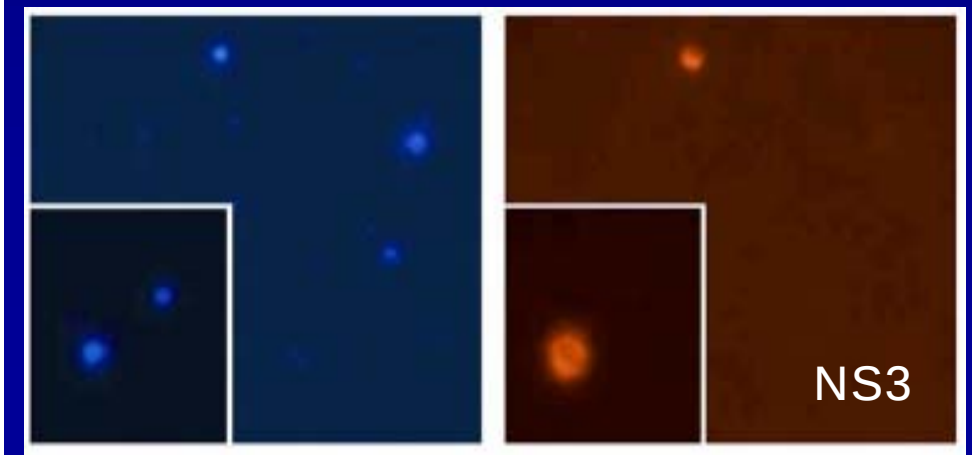


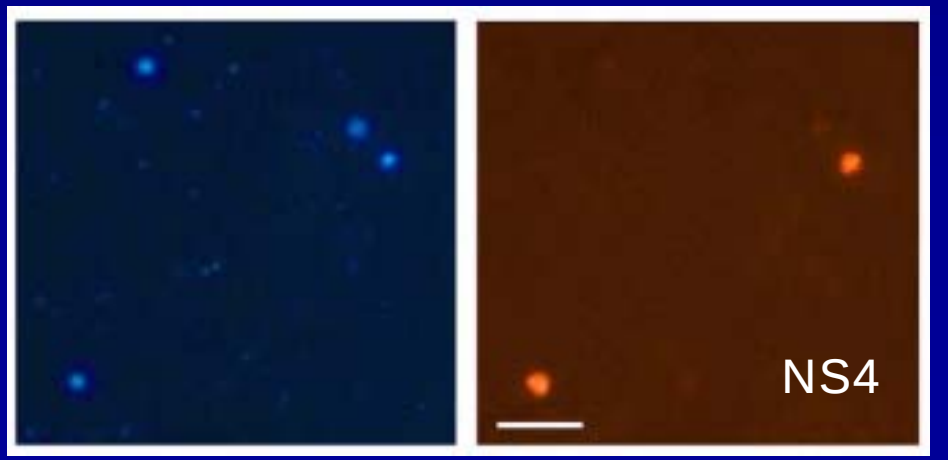
Cluster 4



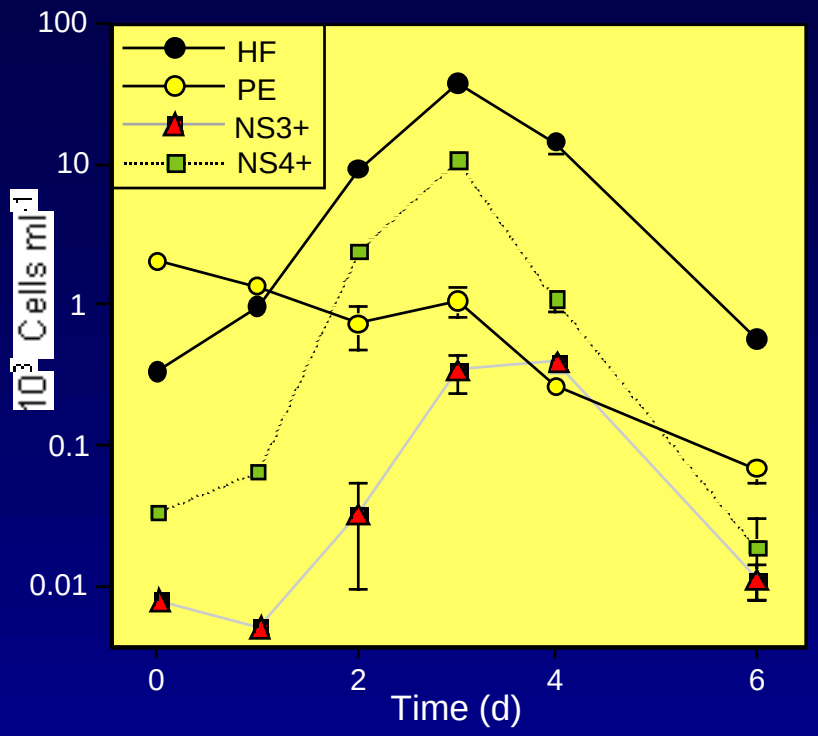
Cultures used as negative controls:

- Prasinophyte
- Prymnesiophyte
- Cryptophyte
- Stramenopiles (Eustigmatophyte, Chrysomonad, Bicosoecid)

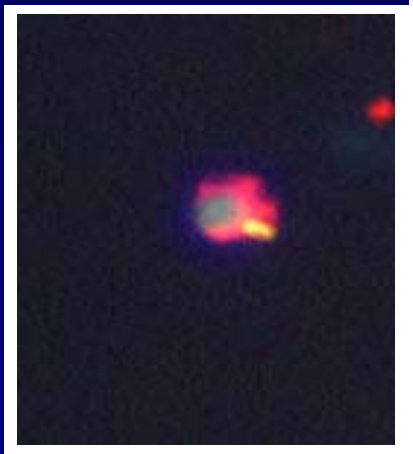




Dynamics during an enrichment culture

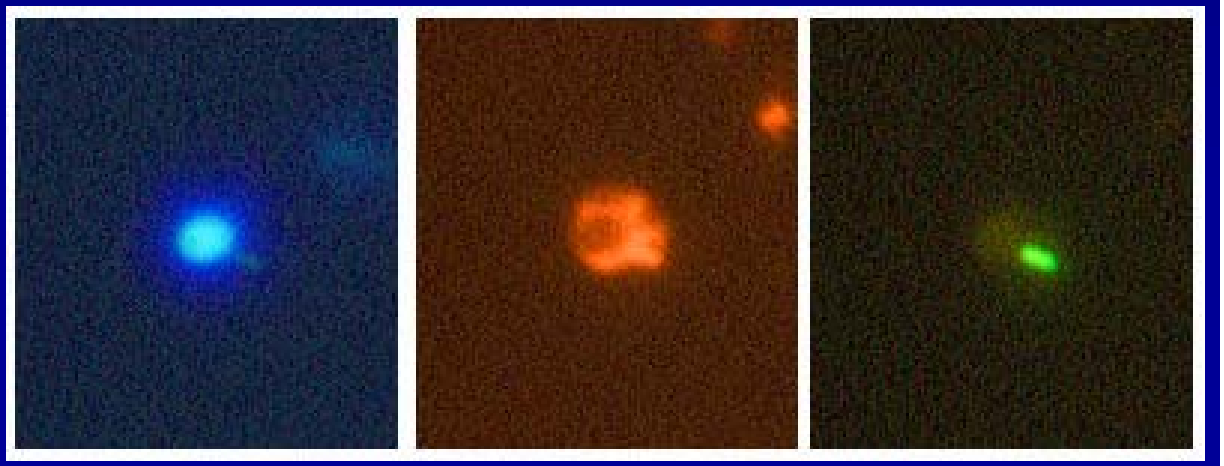


Blanes Coast sample
2 µm filtrate



Ingestion of FLB

10^6 FLB ml^{-1} for 2 h in the enrichment culture



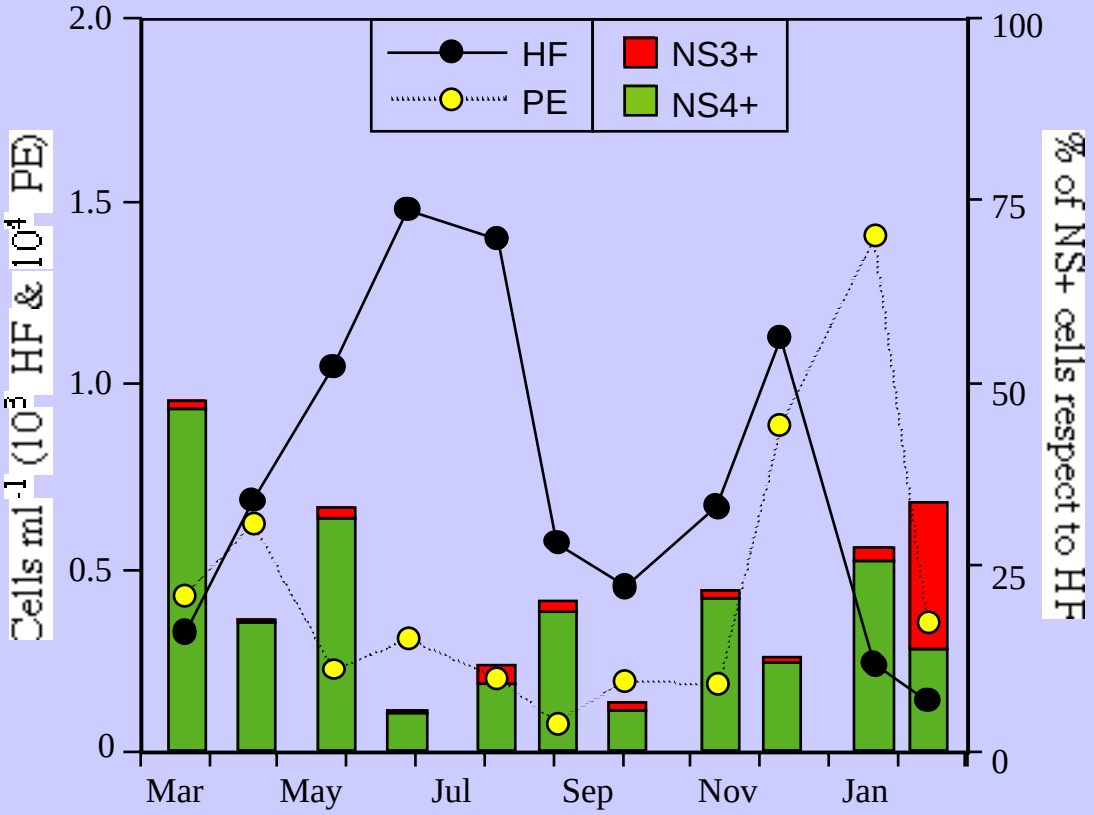
Massana et al. 2002

A background for a recently recognized group

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

Abundance in situ - Blanes Coast



Seasonal average

- NS3+ cells: 12 cells ml^{-1} (3% of HF)
- NS4+ cells: 120 cells ml^{-1} (20% of HF)

New data

Analysis of partial sequences from other systems

Complete sequencing of selected clones and phylogenetic analysis

Design more probes and evaluation of the already designed

More partial sequences ...

Libraries from PICODIV (n=123)		
Mediterranean Sea	BL000921	12
	BL001221	4
	BL010320	7
	BL010625	10
Equatorial Pacific	OLI	2
English Channel	RA000412	13
	RA000609	5
	RA000907	2
	RA001219	6
	RA010412	6
	RA010516	7
	RA010613	10
	RD010517	1
North Sea	He0003xx	1
	He000427	6
	He000803	6
	He001005	6
	HE001206	5
	HE010218	3
North Atlantic	Or0004xx	11

Libraries from other groups (n=71)		
Anoxic sediments		
Dawson & Pace 2002	BAQD	9
	BAQA	2
	BOLA	2
Guaymas Basin hydrothermal vent		
Edgcomb et al. 2002	CS_E0	8
	C1_E0	6
	C2_E0	9
	C3_E0	9
	A3_E0	26

Complete sequences

After an preliminar analysis with partial sequences, representative clones were chosen and completely sequenced:

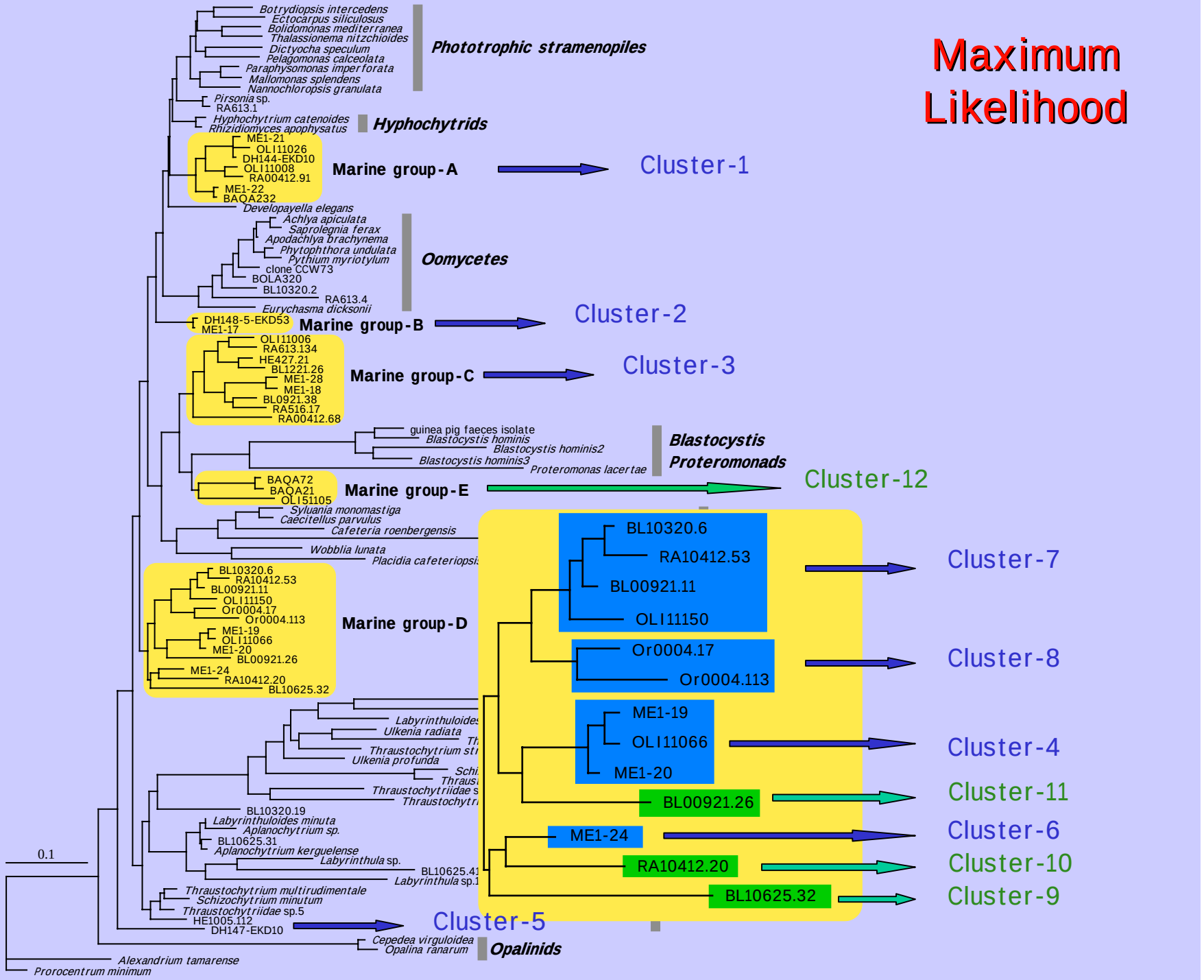
Blanes Coast	BL	10
Roscoff Coast	RA	8
Helgoland Coast	HE	2
Orkney Coast	OR	2

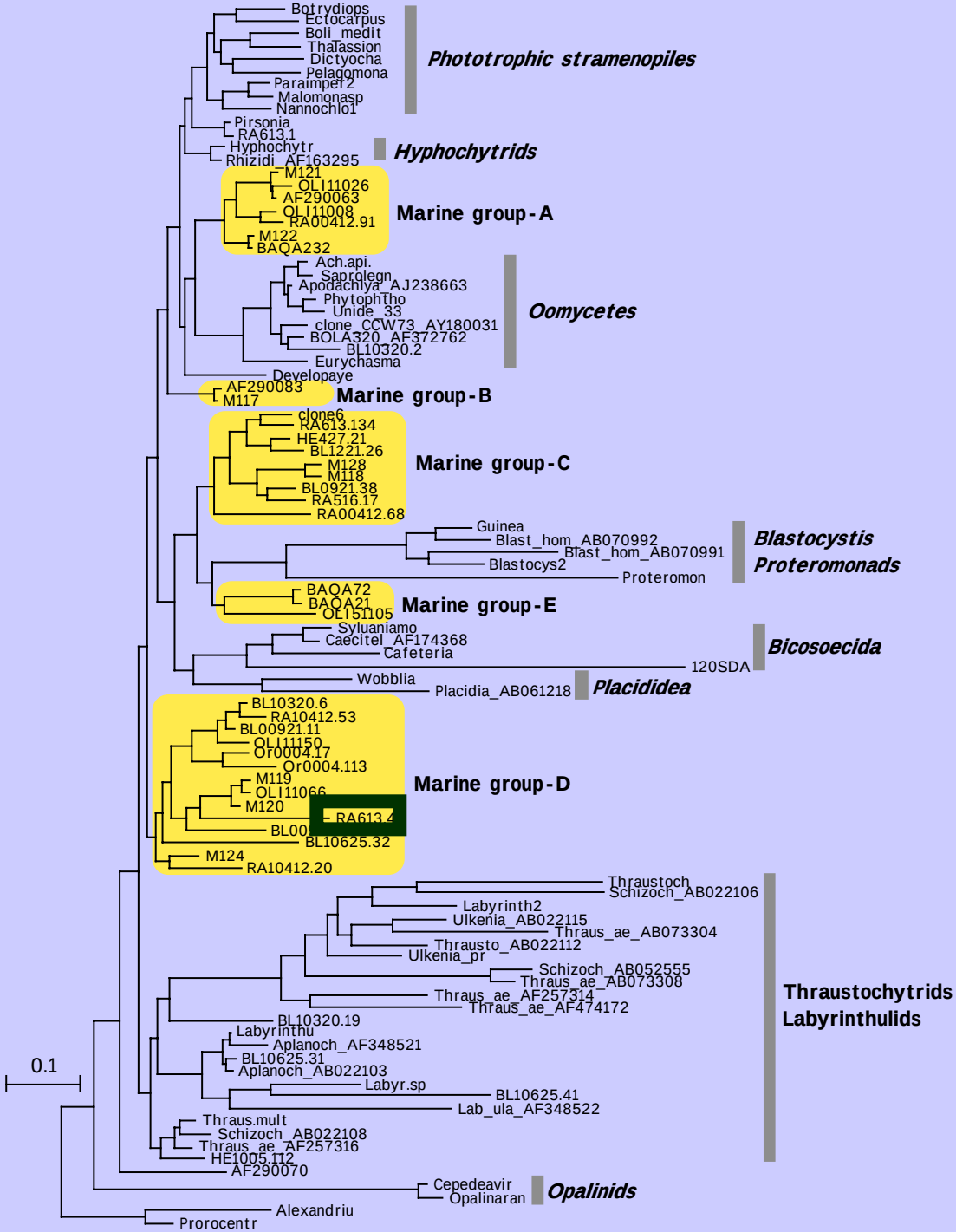
Previous complete sequences

Mediterranean	ME1	8
Pacific Ocean	OLI	6
Antarctica	DH	3

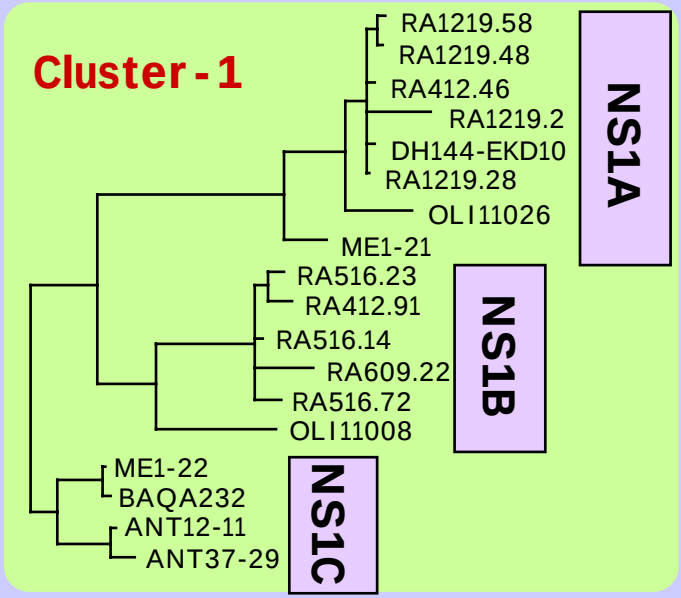
Phylogenetic analysis with complete sequences

- Search for relevant sequences within basal heterotrophic stramenopiles
- Automatic alignment using Clustalw 1.82
- Automatic deletion of non-informative regions with Gblocks (Castresana 2000)
- Maximum Likelihood analysis with Paup
- Bayesian phylogeny with MrBayes


Maximum Likelihood
Botrydiopsis intercedens
Ectocarpus siliculosus
Bolidomonas mediterranea
Thalassionema nitzchioides
Dictyocha speculum
Pelagomonas calceolata
Paraphysomonas imperforata
Mallomonas splendens
Nannochloropsis granulata
Phototrophic stramenopiles
Pirsonia sp. RA613.1
Hyphochytrium catenoides
Rhizidiomyces apophysatus
Hyphochytrids
ME1-21
OLI11026
DH144-EKD10
OLI11008
RA00412.91
ME1-22
BAQA232
Marine group-A
Cluster-1
Developayella elegans
Achlya apiculata
Saprolegnia ferax
Apodachlya brachynema
Phytophthora undulata
Pythium myriotylum
clone CCW73
BOLA320
BL10320.2
RA613.4
Eurychasma dicksonii
Oomycetes
DH148-5-EKD53
ME1-17
Marine group-B
Cluster-2
OLI11006
RA613.134
HE427.21
BL1221.26
ME1-28
ME1-18
BL0921.38
RA516.17
RA00412.68
Marine group-C
Cluster-3
guinea pig faeces isolate
Blastocystis hominis
Blastocystis hominis2
Blastocystis hominis3
Proteromonas lacertae
Blastocystis
Proteromonads
BAQA72
BAQA21
OLI51105
Marine group-E
Cluster-12
Syluania monomastiga
Caecitellus parvulus
Cafeteria roenbergensis
Wobblia lunata
Placidia cafeteriopsis
BL10320.6
RA10412.53
BL00921.11
OLI11150
Or0004.17
Or0004.113
ME1-19
OLI11066
ME1-20
BL00921.26
ME1-24
RA10412.20
BL10625.32
Marine group-D
Labyrinthuloides
Ulkenia radiata
Thraustochytrium sp.
Ulkenia profunda
Schizochytrium
Thraustochytrium
Thraustochytriidae sp.
Thraustochytrium
BL10320.19
Labyrinthuloides minuta
Aplanochytrium sp.
BL10625.31
Aplanochytrium kerguelense
Labyrinthula sp.
BL10625.41
Labyrinthula sp.1
Thraustochytrium multirudimentale
Schizochytrium minutum
Thraustochytriidae sp.5
HE1005.112
DH147-EKD10
Cluster-5
Cepedea virguloidea
Opalina ranarum
Opalinids
Alexandrium tamarense
Prorocentrum minimum
0.1
BL10320.6
RA10412.53
BL00921.11
OLI11150
Cluster-7
Or0004.17
Or0004.113
Cluster-8
ME1-19
OLI11066
ME1-20
Cluster-4
BL00921.26
Cluster-11
ME1-24
Cluster-6
RA10412.20
Cluster-10
BL10625.32
Cluster-9

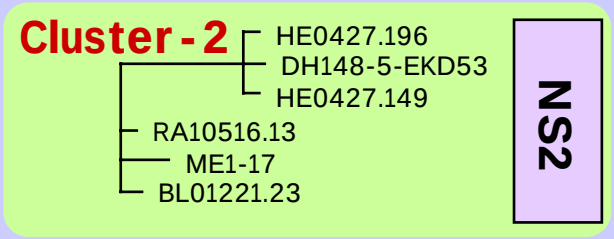

Bayesian phylogeny
Botrydiops
Ectocarpus
Boli_medit
Thalassion
Dictyocha
Pelagomona
Paraimper2
Malomonasp
Nannochlo1
Phototrophic stramenopiles
Pirsonia
RA613.1
Hyphochytr
Rhizidi_AF163295
Hyphochytrids
M121
OLI11026
AF290063
OLI11008
RA00412.91
M122
BAQA232
Marine group-A
Ach.api.
Saprolegn
Apodachlya_AJ238663
Phytophtho
Unide_33
clone_CCW73_AY180031
BOLA320_AF372762
BL10320.2
Eurychasma
Oomycetes
Developaye
AF290083
M117
Marine group-B
clone6
RA613.134
HE427.21
BL1221.26
M128
M118
BL0921.38
RA516.17
RA00412.68
Marine group-C
Guinea
Blast_hom_AB070992
Blast_hom_AB070991
Blastocys2
Proteromon
Blastocystis
Proteromonads
BAQA72
BAQA21
OLI51105
Marine group-E
Syluaniamo
Caecitel_AF174368
Cafeteria
120SDA
Bicosoecida
Wobblia
Placidia_AB061218
Placididea
BL10320.6
RA10412.53
BL00921.11
OLI11150
Or0004.17
Or0004.113
M119
OLI11066
M120
RA613.4
BL00
BL10625.32
M124
RA10412.20
Marine group-D
Thraustoch
Schizoch_AB022106
Labyrinth2
Ulkenia_AB022115
Thraus_ae_AB073304
Thrausto_AB022112
Ulkenia_pr
Schizoch_AB052555
Thraus_ae_AB073308
Thraus_ae_AF257314
Thraus_ae_AF474172
BL10320.19
Labyrinthu
Aplanoch_AF348521
BL10625.31
Aplanoch_AB022103
Labyr.sp
BL10625.41
Lab_ula_AF348522
Thraustochytrids
Labyrinthulids
0.1
Thraus.mult
Schizoch_AB022108
Thraus_ae_AF257316
HE1005.112
AF290070
Cepedeavir
Opalinaran
Opalinids
Alexandriu
Prorocentr

Marine group-A



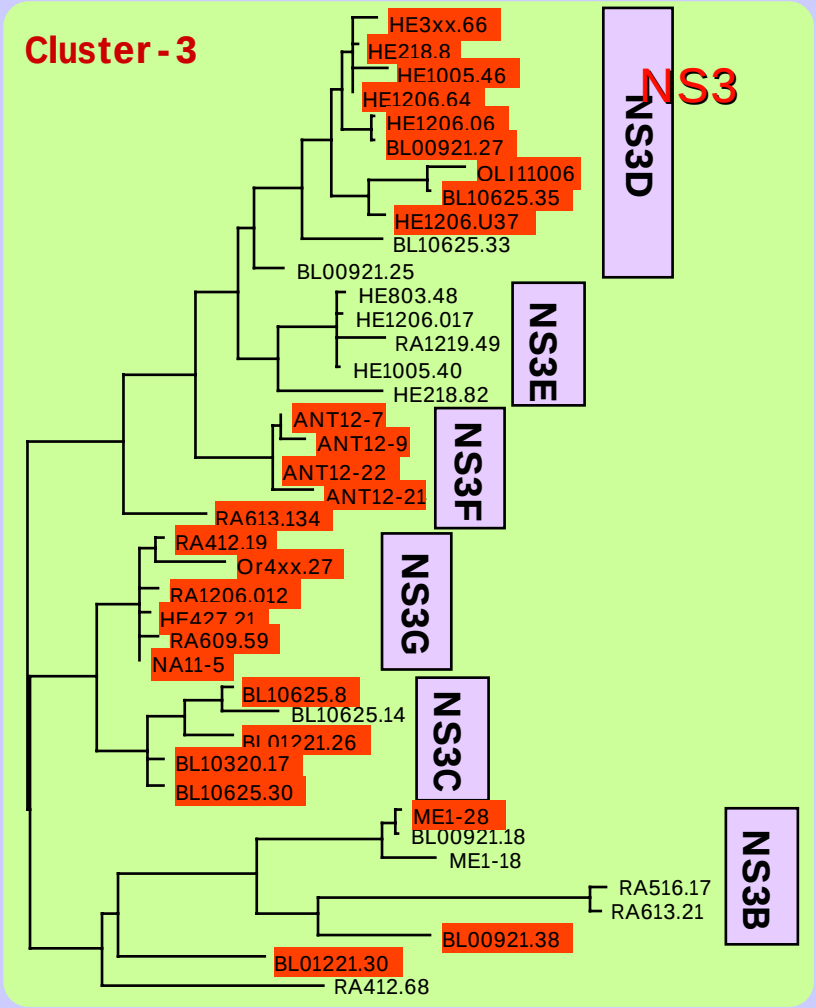
Coast	10		Open Sea	8		Sediments	1
Blanes	0		Mediterranean	2		Anoxic sediments	1
Roscoff	10		Pacific	2		Hydrothermal vent	0
Helgoland	0		North Atlantic	0			
			Orkney Islands	0			
			Antarctica	4			

Marine group-B



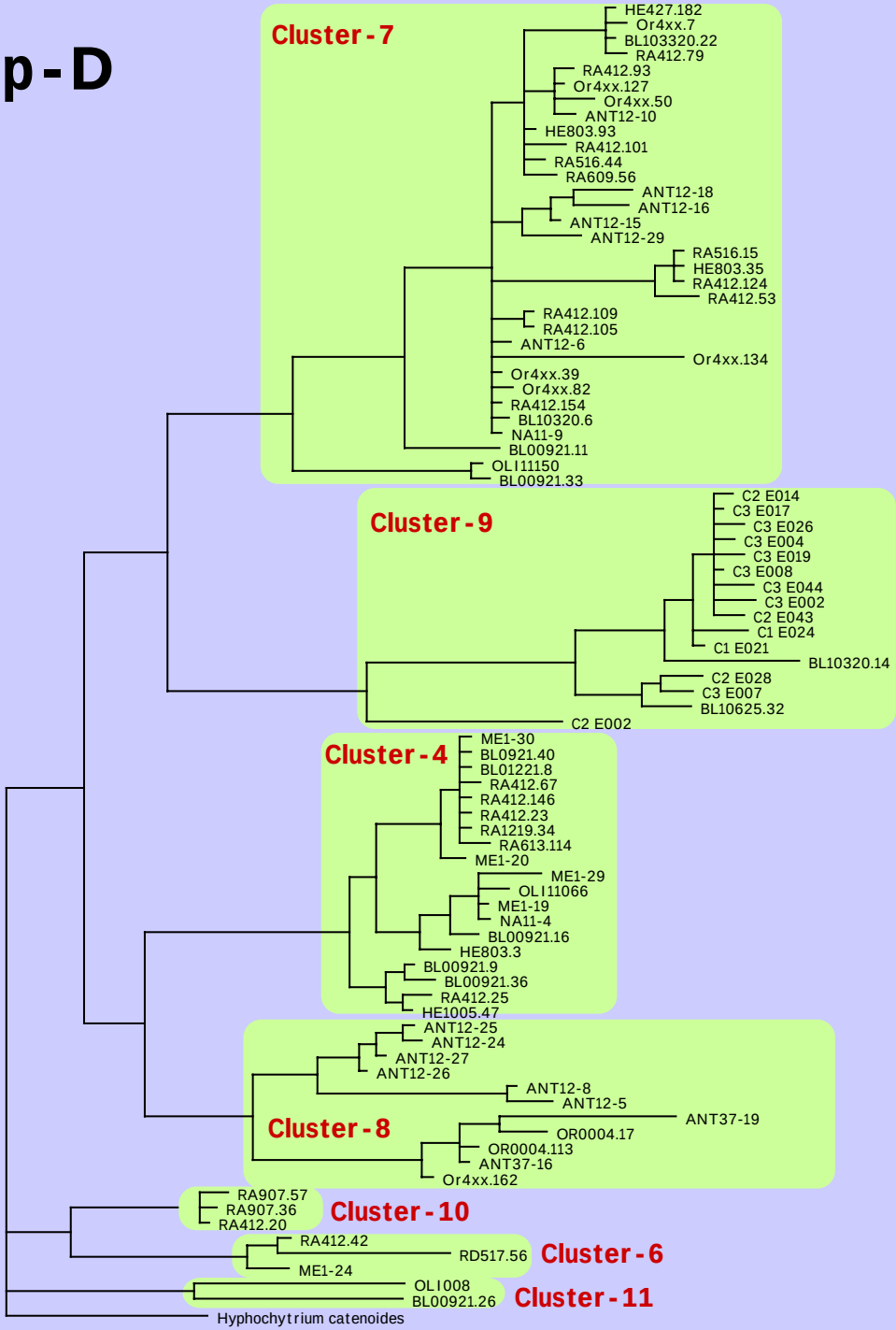
Coast	4		Open Sea	2		Sediments	0
Blanes	1		Mediterranean	1		Anoxic sediments	0
Roscoff	1		Pacific	0		Hydrothermal vent	0
Helgoland	2		North Atlantic	0			
			Orkney Islands	0			
			Antarctica	1			

Marine group-C

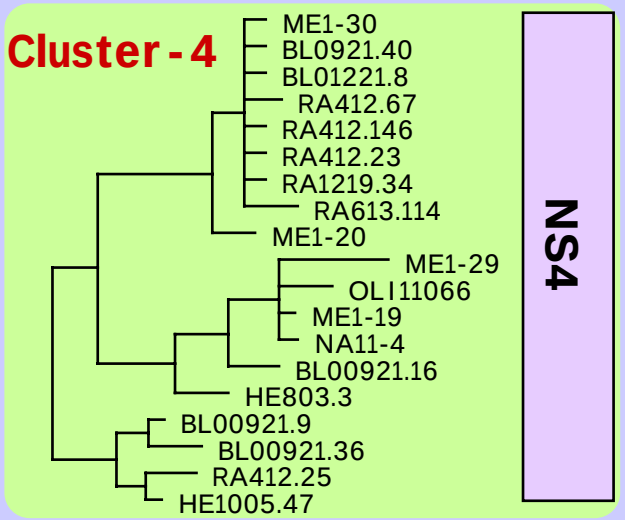


Coast	31		Open Sea	13		Sediments	0
Blanes	12		Mediterranean	2		Anoxic sediments	0
Roscoff	7		Pacific	1		Hydrothermal vent	0
Helgoland	12		North Atlantic	2			
			Orkney Islands	1			
			Antarctica	7			

Marine group-D

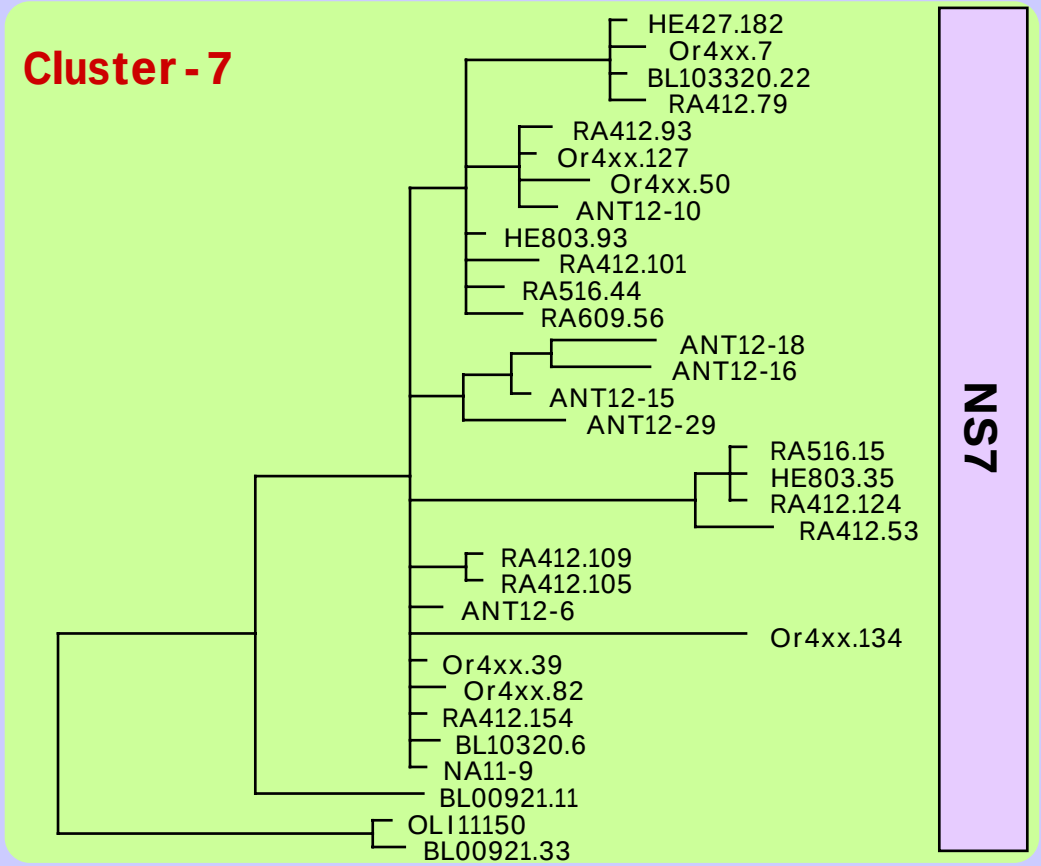


Marine group-D



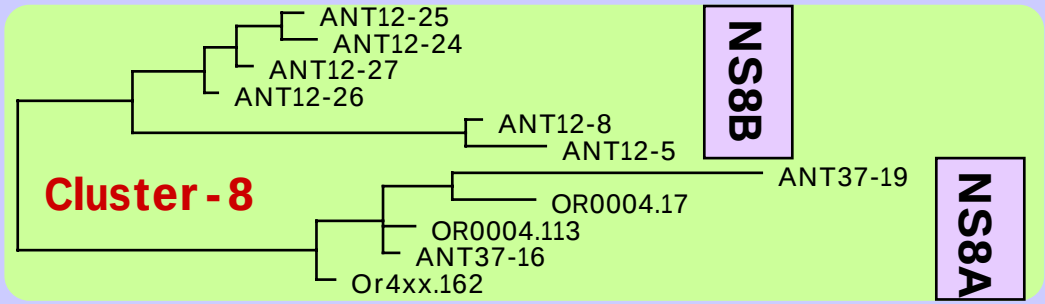
Coast	14		Open Sea	6		Sediments	0
Blanes	6		Mediterranean	4		Anoxic sediments	0
Roscoff	6		Pacific	1		Hydrothermal vent	0
Helgoland	2		North Atlantic	1			
			Orkney Islands	0			
			Antarctica	0			

Marine group-D



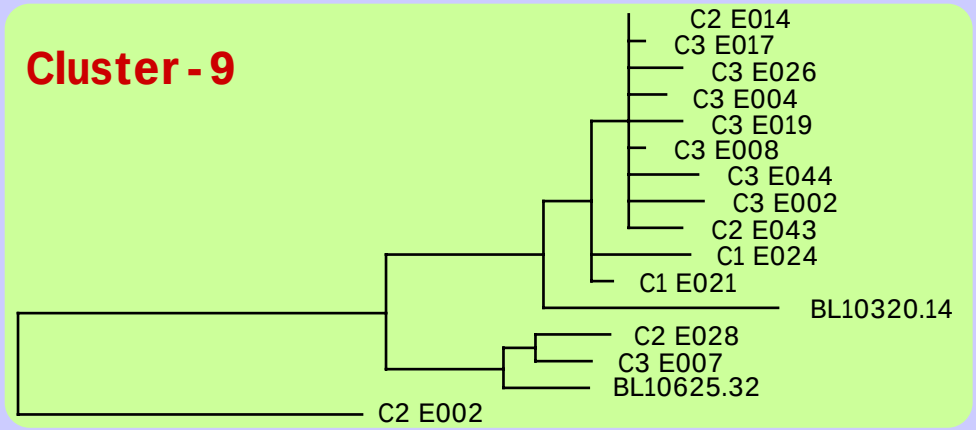
Coast	18		Open Sea	17		Sediments	0
Blanes	4		Mediterranean	0		Anoxic sediments	0
Roscoff	11		Pacific	1		Hydrothermal vent	0
Helgoland	3		North Atlantic	2			
			Orkney Islands	6			
			Antarctica	8			

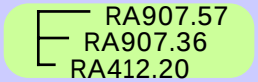
Marine group-D



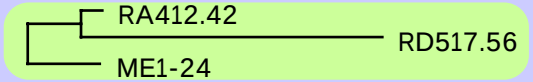
Coast	0		Open Sea	16		Sediments	0
Blanes	0		Mediterranean	0		Anoxic sediments	0
Roscoff	0		Pacific	0		Hydrothermal vent	0
Helgoland	0		North Atlantic	0			
			Orkney Islands	4			
			Antarctica	12			

Marine group-D

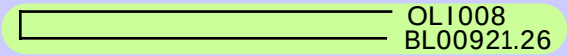




Cluster-10



Cluster-6



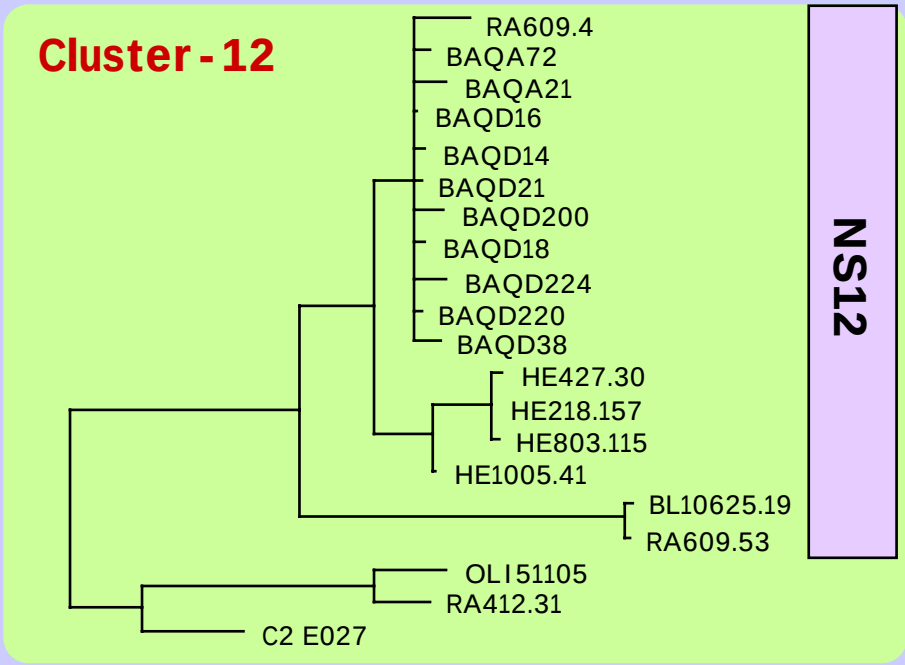
Cluster-11

	Cluster-6	Cluster-9	Cluster-10	Cluster-11
Coast	2	2	3	1
Blanes	0	2	0	1
Roscoff	2	0	3	0
Helgoland	0	0	0	0

	Cluster-6	Cluster-9	Cluster-10	Cluster-11
Open Sea	0	0	0	1
Mediterranean	0	0	0	0
Pacific	0	0	0	1
North Atlantic	0	0	0	0
Orkney Islands	0	0	0	0
Antarctica	0	0	0	0

	Cluster-6	Cluster-9	Cluster-10	Cluster-11
Sediments	0	14	0	0
Anoxic sediments	0	0	0	0
Hydrothermal vent	0	14	0	0

Marine group-E



Coast	8		Open Sea	1		Sediments	11
Blanes	1		Mediterranean	0		Anoxic sediments	10
Roscoff	3		Pacific	1		Hydrothermal vent	1
Helgoland	4		North Atlantic	0			
			Orkney Islands	0			
			Antarctica	0			

Other marine stramenopiles

	Oomycetes	Labyrinthulids		Oomycetes	Labyrinthulids		Oomycetes	Labyrinthulids
Coast	**6**	**8**	Open Sea	**0**	**0**	Sediments	**33**	**12**
Blanes	**2**	**4**	Mediterranean	0	0	Anoxic sediments	2	0
Roscoff	**2**	**2**	Pacific	0	0	Hydrothermal vent	31	12
Helgoland	**2**	**2**	North Atlantic	0	0			
			Orkney Islands	0	0			
			Antarctica	0	0			

Future work

Test probes

Apply probes