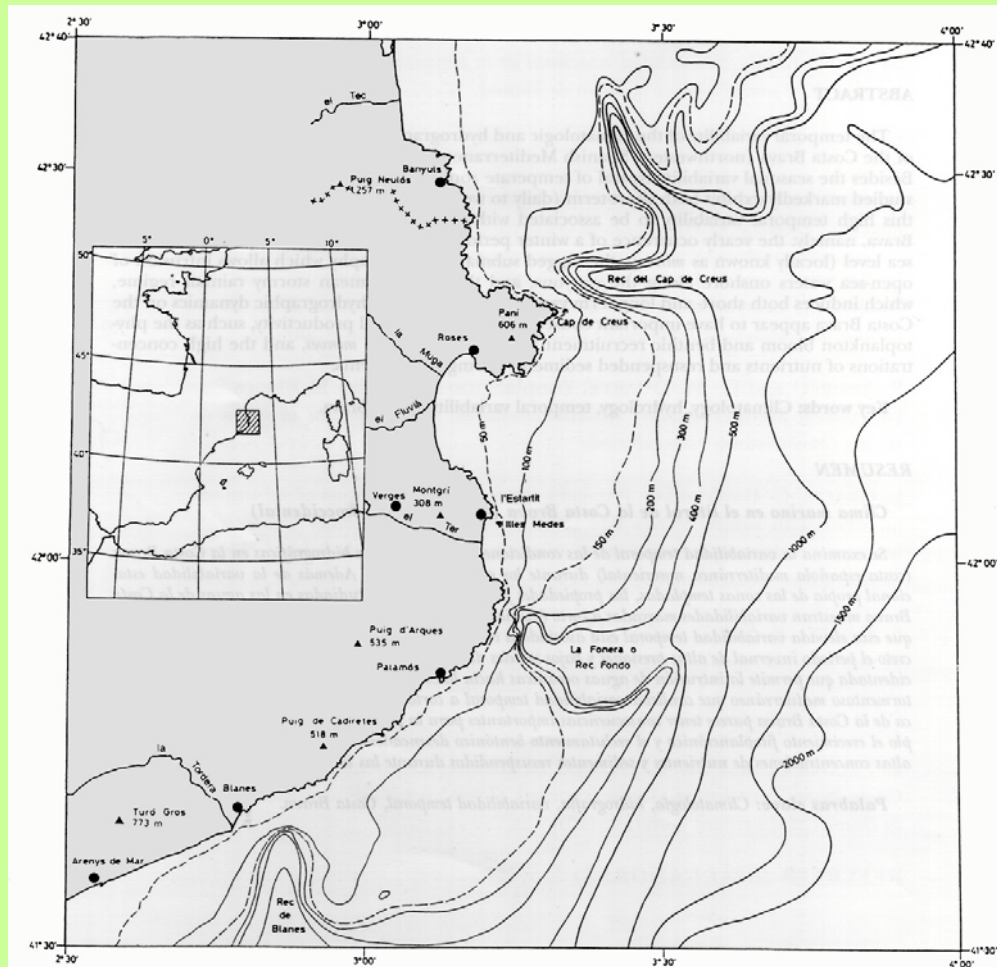


Picoeukaryotic diversity from Blanes

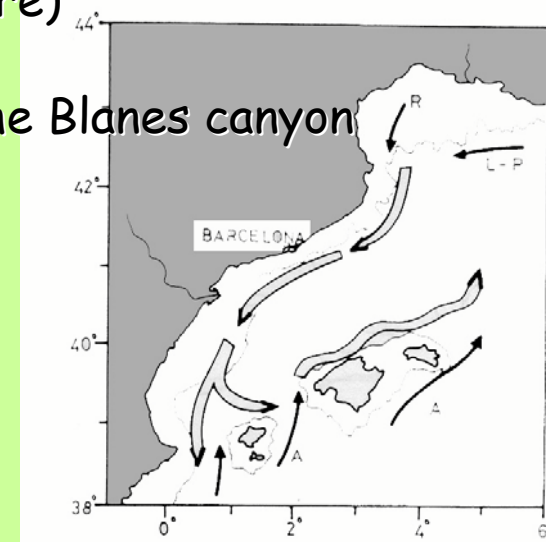
Results from clone libraries

ICM, Barcelona-July 2002

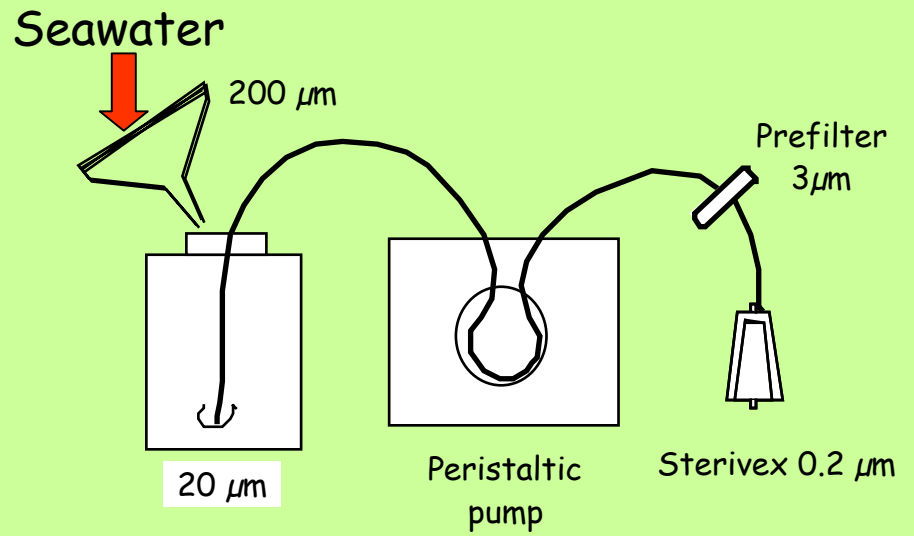


General characteristics of Blanes Bay

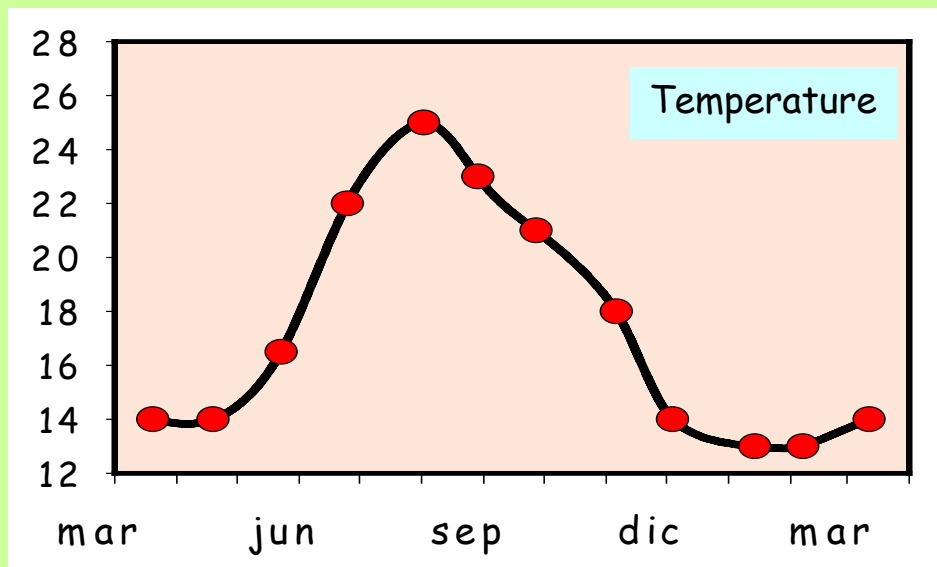
- Typical Mediterranean waters: warm, salty and nutrient-poor
- Oligotrophic coastal system (annual average chlorophyll of $0.54 \mu\text{g l}^{-1}$)
- Relatively unaffected by human influence
- Separated from oceanic waters by a southwest current associated with a front in the continental slope (10-20 miles offshore)
- Episodic intrusions of oceanic waters caused by the Blanes canyon



Sample collection



The four genetic libraries



Date	Temperature	Library	PF	HF
21-Sept-2000	22°C	BL_000921	-	-
21-Des-2000	14°C	BL_001221	-	-
20-Mar-2001	14°C	BL_010320	4200	330
20-Jun-2001	22°C	BL_010625	3120	1480

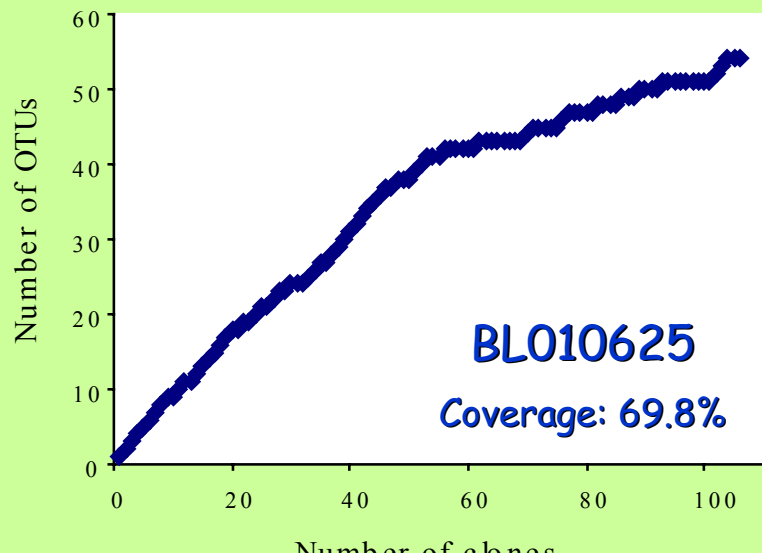
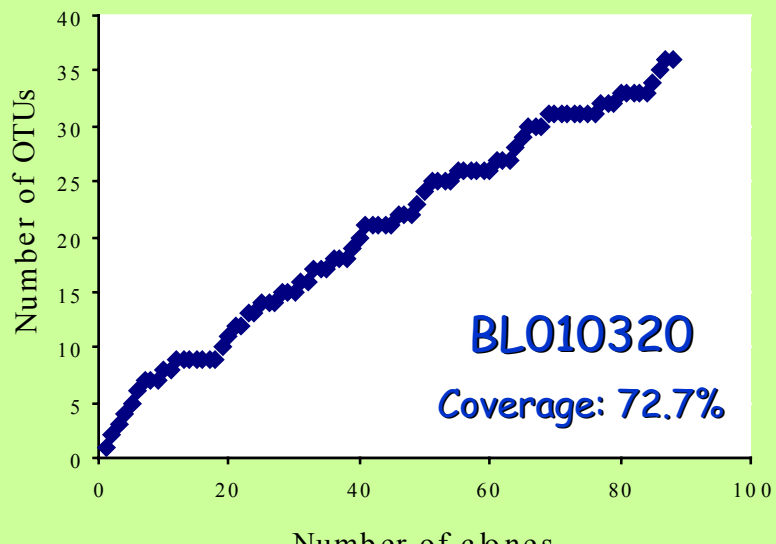
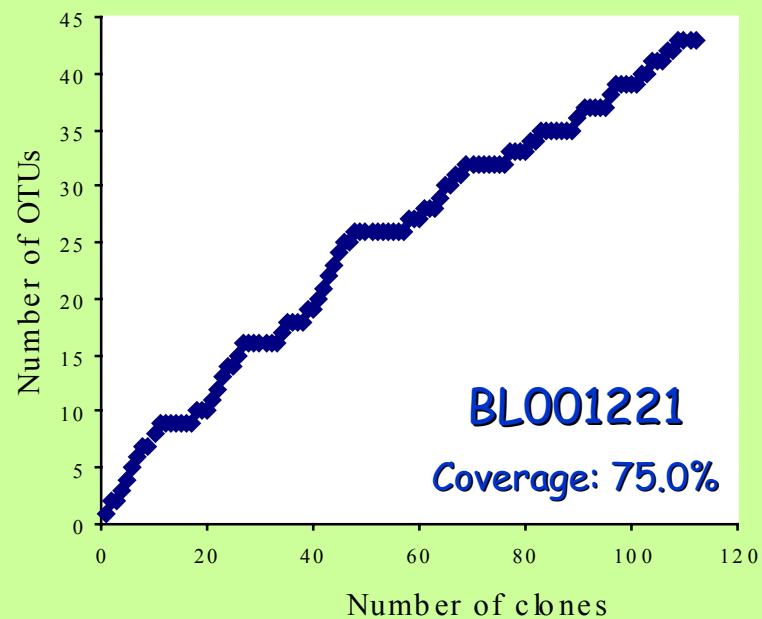
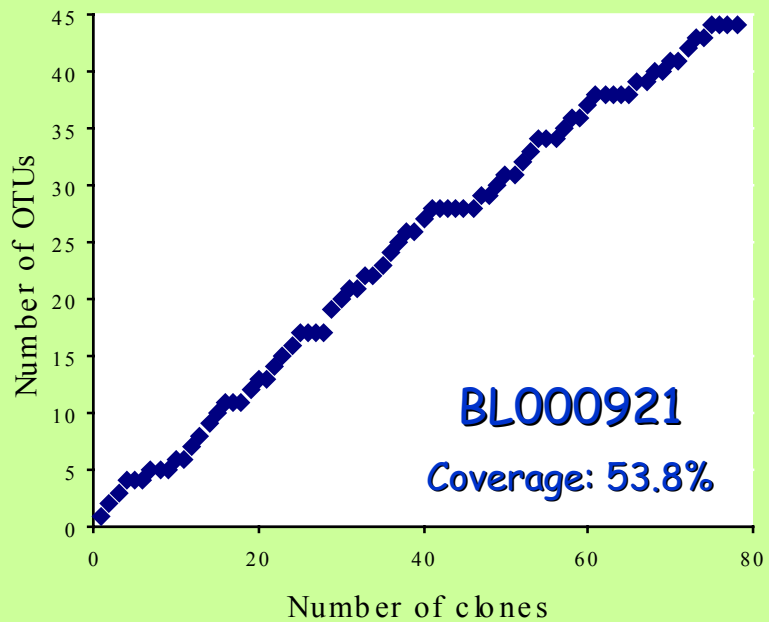
Fraction analyzed: 0.2 - 3 μm

Amplification: Primers EukA and EukB (Medlin et al. 1988)

Screening of libraries by RFLP

Library	Number of clones	Number of RFPLs	Number of sequences
BL000921	78	44	40
BL001221	112	43	43
BL010320	88	36	33
BL010625	106	54	53

Coverage of genetic libraries



BL000921

BL000921.4	14	C1_E005	99.0	Novel alveolate-I
BL000921.1	12	A1_E045	99.2	Novel alveolate-I
BL000921.6	5	<i>Gyrodinium galatheanum</i>	98.0	Dinoflagellate
BL000921.42	2	<i>Teleaulax amphioxeia</i>	99.1	Cryptophyta-nucleus
BL000921.29	2	<i>Heterocapsa rotundata</i>	87.8	Dinoflagellate
BL000921.11	2	OLI11150	98.2	Novel stramenopile
BL000921.16	2	OLI11066	98.3	Novel stramenopile
BL000921.23	1	<i>Adenoides eludens</i>	93.6	Apicomplexa
BL000921.5	1	<i>Phaeothamnion confervicola</i>	94.3	Bicosoecid
BL000921.2	1	<i>Cryothecomonas longipes</i>	98.8	Cercozoa
BL000921.7	1	<i>Teleaulax amphioxeia</i>	99.2	Cryptophyta-nucleus
BL000921.34	1	<i>Chroomonas</i> sp.	94.6	Cryptophyta-nucleus
BL000921.41	1	<i>Hemiselmis virescens</i>	95.0	Cryptophyta-nucleus
BL000921.31	1	Un. chlorarachniophyte	93.8	Chlorarachniophyte-nucleus
BL000921.20	1	<i>Monosiga brevicollis</i>	94.3	Choanoflagellate
BL000921.24	1	OLI11013	98.2	Choanoflagellate
BL000921.30	1	<i>Diaphanoeca grandis</i>	95.7	Choanoflagellate
BL000921.17	1	C3_E031	97.5	Chrysophyceae
BL000921.15	1	<i>Gymnodinium</i> MUCC284	99.1	Dinoflagellate
BL000921.12	1	<i>Bangia</i> sp.	87.7	Inserta sedis
BL000921.8	1	<i>Apusomonas proboscidea</i>	93.0	New clade
BL000921.13	1	CS_E040	90.2	Novel alveolate-I
BL000921.32	1	DH145-EKD10	96.5	Novel alveolate-I
BL000921.19	1	OLI11023	93.2	Novel alveolate-II
BL000921.38	1	CS_E041	91.9	Novel stramenopile
BL000921.9	1	OLI11066	97.3	Novel stramenopile
BL000921.18	1	OLI11006	94.6	Novel stramenopile
BL000921.22	1	OLI11066	98.2	Novel stramenopile
BL000921.25	1	OLI11006	95.7	Novel stramenopile
BL000921.26	1	OLI11150	93.7	Novel stramenopile
BL000921.27	1	OLI11006	97.5	Novel stramenopile
BL000921.33	1	OLI11150	99.2	Novel stramenopile
BL000921.36	1	NA11-4	96.9	Novel stramenopile
BL000921.40	1	OLI11066	95.9	Novel stramenopile
BL000921.39	1	<i>Pelagomonas calceolata</i>	100.0	Pelagophyceae
BL000921.10	1	<i>Mantoniella squamata</i>	96.7	Prasinophyte
BL000921.14	1	<i>Ostreococcus tauri</i>	97.6	Prasinophyte
BL000921.21	1	<i>Phaeocystis cordata</i>	98.7	Prymnesiophyta

BL001221

BL001221.9	37	C5_E023	98.9	Novel alveolate-I
BL001221.1	13	Dinophysis norvegica	98.1	Novel alveolate-I
BL001221.12	5	DH147-EKD21	99.7	Dinoflagellate
BL001221.15	4	OLI11225	98.9	Dinoflagellate
BL001221.10	4	Mantoniella squamata	97.0	Prasinophyte
BL001221.14	3	Pseudo-nitzschia multiseriis	98.9	Diatom
BL001221.25	2	Geminigera criophila	99.3	Cryptophyta-nucleus
BL001221.32	2	Chlorarachniophyte	93.5	Chlorarachniophyte-nucleus
BL001221.2	2	Nanochlorum eucaryotum	97.7	Chlorophyta
BL001221.31	2	Gymnodinium sp.	96.9	Dinoflagellate
BL001221.18	2	OLI11023	92.5	Novel alveolate-II
BL001221.26	2	OLI11006	96.2	Novel stramenopile
BL001221.4	2	Micromonas pusilla	96.9	Prasinophyte
BL001221.28	2	Mantoniella squamata	94.3	Prasinophyte
BL001221.22	1	Cryptosporidium muris	93.6	Apicomplexan
BL001221.5	1	C3_E037	98.3	Ciliate
BL001221.6	1	C3_E037	97.4	Ciliate
BL001221.11	1	DH147-EKD23	94.7	Ciliate
BL001221.33	1	C1_E042	97.7	Ciliate
BL001221.27	1	Falcomonas daucooides	98.0	Cryptophyta-nucleus
BL001221.34	1	Teleaulax amphioxeia	99.2	Cryptophyta-nucleus
BL001221.16	1	OLI11013	98.9	Choanoflagellate
BL001221.29	1	C2_E024	97.9	Diatom
BL001221.35	1	Dictyocha speculum	97.7	Dictyochales
BL001221.42	1	Gymnodinium sp.	97.5	Dinoflagellate
BL001221.21	1	Pentapharsodinium tyrrhenicum	97.0	Dinoflagellate
BL001221.36	1	Pentapharsodinium tyrrhenicum	97.8	Dinoflagellate
BL001221.38	1	Gymnodinium galatheanum	99.0	Dinoflagellate
BL001221.39	1	Gymnodinium sp.	97.1	Dinoflagellate
BL001221.43	1	Cryptococcus cellulolyticus	99.3	Fungi-Basidiomycota
BL001221.13	1	C1_E005	99.2	Novel alveolate-I
BL001221.24	1	C2_E017	98.2	Novel alveolate-I
BL001221.41	1	C5_E023	96.7	Novel alveolate-I
BL001221.3	1	DH147-EKD6	94.7	Novel alveolate-II
BL001221.19	1	DH147-EKD6	96.4	Novel alveolate-II
BL001221.40	1	OLI11023	95.0	Novel alveolate-II
BL001221.8	1	OLI11066	97.4	Novel stramenopile
BL001221.23	1	DH148-5-EKD53	98.9	Novel stramenopile

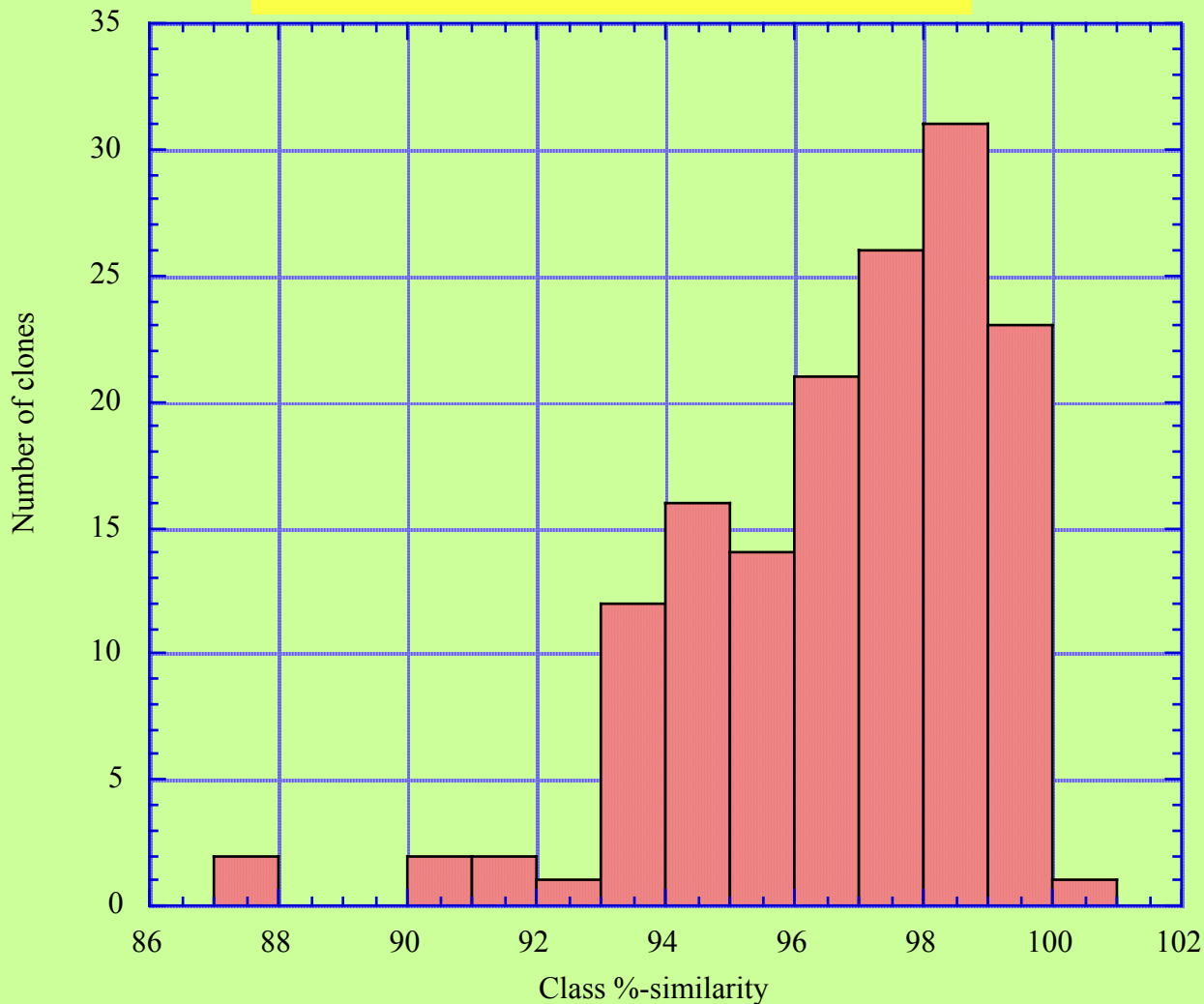
BL010320

Sequence	n	Closest match	% similarity	Phylogenetic group
BL_010320.3	28	C1_E005	99.8	Novel alveolate-I
BL_010320.12	10	C5_E023	98.6	Novel alveolate-I
BL_010320.9	3	C3_E010	96.8	Acantharea
BL_010320.13	3	Pentapharsodinium sp.	98.0	Dinoflagellate
BL_010320.33	3	EMPE7	95.9	Fungi
BL_010320.2	3	A3_E008	97.9	Novel stramenopile
BL_010320.1	3	Mantionella squamata	96.9	Prasinophyte
BL_010320.21	2	Cryothecomonas longipes	95.2	Cercozoa
BL_010320.8	2	Unidentified euk LKM46	96.2	Fungi
BL_010320.6	2	OLI11150	98.4	Novel stramenopile
BL_010320.10	1	C3_E010	96.9	Acantharea
BL_010320.18	1	Cryothecomonas longipes	98.3	Cercozoa
BL_010320.34	1	Unnamed chlorarachniophyte	94.5	Chlorarachniophyte-nucleus
BL_010320.5	1	Chaetocerus rostratus	98.2	Diatom
BL_010320.7	1	Rhizosolenia setigera	94.6	Diatom
BL_010320.16	1	Rhodotorula glutinis	99.3	Fungi
BL_010320.36	1	BAQA128	95.4	Fungi
BL_010320.19	1	Labyrinthuloides minuta	95.0	Labyrinthulid
BL_010320.26	1	C1_E009	99.5	Labyrinthulid
BL_010320.27	1	Karena brevis	90.4	Novel alveolate-I
BL_010320.29	1	Pfiesteria piscicida	93.6	Novel alveolate-I
BL_010320.15	1	C2_E017	99.3	Novel alveolate-I
BL_010320.20	1	C2_E017	98.7	Novel alveolate-I
BL_010320.25	1	C2_E017	99.3	Novel alveolate-I
BL_010320.28	1	A1_E039	94.9	Novel alveolate-I
BL_010320.35	1	C2_E017	99.3	Novel alveolate-I
BL_010320.32	1	OLI11023	95.2	Novel alveolate-II
BL_010320.14	1	C3_E008	97.9	Novel stramenopile
BL_010320.17	1	OLI11006	93.4	Novel stramenopile
BL_010320.22	1	OLI11150	97.7	Novel stramenopile
BL_010320.30	1	Mantionella squamata	94.2	Prasinophyte
BL_010320.4	1	Lithophyllum incrustans	99.1	Rhodophyta

BL010625

BL010625.7	10	DH147-EKD16	91.5	Novel alveolate-II
BL010625.15	9	C5_E023	98.4	Novel alveolate-I
BL010625.12	5	Unnamed chlorarachniophyte	93.8	Chlorarachniophyte-nucleus
BL010625.17	5	Pentapharsodinium sp.	95.2	Novel alveolate-I
BL010625.49	3	Cryothecomonas longipes	96.2	Cercozoa
BL010625.16	3	Teleaulax amphioxeia	99.1	Cryptophyta-nucleus
BL010625.34	3	Pentapharsodinium sp.	96.7	Dinoflagellate
BL010625.45	2	Ephelota	93.8	Ciliate
BL010625.24	2	Chroomonas sp.	94.8	Cryptophyta-nucleus
BL010625.41	2	Labyrinthula sp.	95.3	Labyrinthulid
BL010625.5	2	DH147-EKD21	98.3	Novel alveolate-I
BL010625.19	2	BAQA72	97.8	Novel stramenopile
BL010625.33	2	OLI11006	96.4	Novel stramenopile
BL010625.35	2	OLI11006	99.0	Novel stramenopile
BL010625.1	2	Prasinophyte symbiont	98.6	Prasinophyte
BL010625.10	2	Chrysochromulina trondsenii	98.7	Prymnesiophyte
BL010625.27	1	Cryothecomonas longipes	97.5	Cercozoa
BL010625.54	1	Cryothecomonas longipes	97.3	Cercozoa
BL010625.38	1	C3_E037	96.3	Ciliate
BL010625.51	1	C2_E022	94.3	Ciliate
BL010625.4	1	Teleaulax amphioxeia	97.2	Cryptophyta-nucleomorph
BL010625.36	1	Monosiga brevicollis	94.6	Choanoflagellate
BL010625.6	1	Rhizosolenia setigera	96.6	Diatom
BL010625.13	1	Pfiesteria-like dino	97.3	Dinoflagellate
BL010625.20	1	Lepidodinium viride	99.9	Dinoflagellate
BL010625.46	1	Amphidinium longum	97.3	Dinoflagellate
BL010625.52	1	Lepidodinium viride	98.4	Dinoflagellate
BL010625.53	1	Lepidodinium viride	98.5	Dinoflagellate
BL010625.31	1	Labyrinthuloides yorkensis	99.3	Labyrinthulid
BL010625.25	1	Neocallimastix sp.	94.3	New clade
BL010625.3	1	DH147-EKD21	98.0	Novel alveolate-I
BL010625.42	1	C2_E017	97.8	Novel alveolate-I
BL010625.44	1	OLI11005	95.3	Novel alveolate-I
BL010625.32	1	C3_E007	98.5	Novel stramenopile
BL010625.21	1	BOLA515	96.3	Novel stramenopile
BL010625.8	1	OLI11006	93.1	Novel stramenopile
BL010625.30	1	OLI11006	96.8	Novel stramenopile
BL010625.14	1	ME1-24	95.7	Novel stramenopile
BL010625.2	1	Prasinophyte symbiont	98.9	Prasinophyte
BL010625.18	1	Pyramimonas australis	97.5	Prasinophyte

AVERAGE SIMILARITY: 96.7



44% of clones have an environmental clone as closest match

General analysis of libraries - I

Significant presence of metazoans

Appendicularia, Bivalvia, Cnidaria, Copepoda, Polychata, Porifera

17 RFLP patterns

32 clones

8.6% of clones

General analysis of libraries - II

Dominant groups (>3%)

Phylogenetic group	Number clones	%-clones	Clones/RFLP	
Novel alveolate-I	145	42.6	6.0	Present in 4 libraries
Novel stramenopile	38	11.2	1.3	Present in 4 libraries
Dinoflagellate	35	10.3	1.9	Present in 4 libraries
Prasinophyte	18	5.3	1.8	Present in 4 libraries
Novel alveolate-II	17	5.0	2.4	Present in 4 libraries
Cryptophyta-nucleus	14	4.1	1.6	Present in 3 libraries

TOTAL

151 RFLPs

340 clones

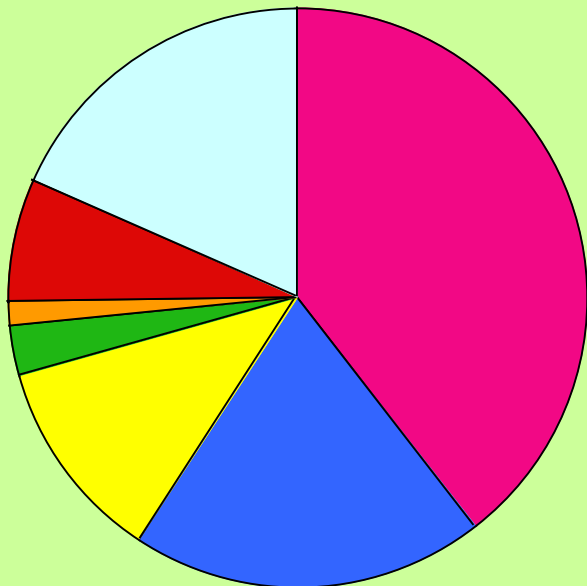
Present in 4 libraries
Present in 3 libraries
Present in 2 libraries
Present in 1 libraries

General analysis of libraries - III

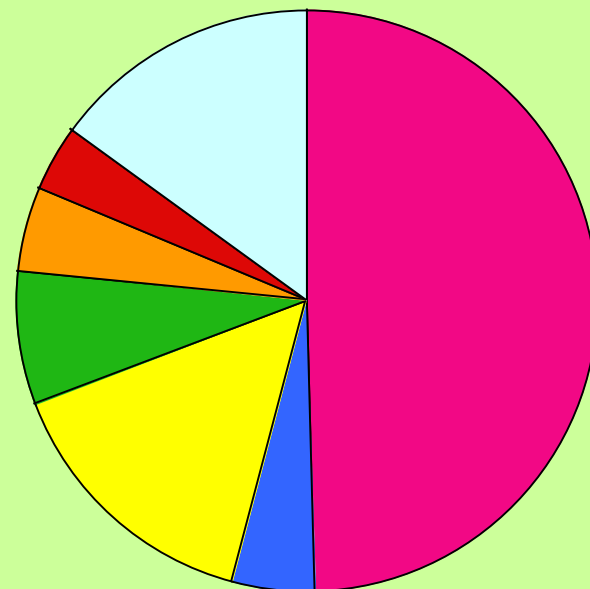
Minoritary groups (>3%)

Phylogenetic group	Number clones	%-clones	Clones/RFLP	
Cercozoa	9	2.6	1.5	Blue
Chlorarachniophyte	9	2.6	2.3	Black
Ciliate	8	2.4	1.1	Orange
Fungi	8	2.4	1.6	Orange
Diatom	7	2.1	1.4	Blue
Choanoflagellate	5	1.5	1.0	Blue
Labyrinthulid	5	1.5	1.3	Orange
Prymnesiophyta	5	1.5	1.3	Orange
Acantharea	4	1.2	2.0	Yellow
Apicomplexa	2	0.6	1.0	Orange
Chlorophyta	2	0.6	2.0	Yellow
Rodophyta-environmental	2	0.6	1.0	Orange
Bicosoecid	1	0.3	1.0	Yellow
Cryptophyta-nucleomorph	1	0.3	1.0	Yellow
Chrysophyceae	1	0.3	1.0	Yellow
Dictyochales	1	0.3	1.0	Yellow
Inserta sedis	1	0.3	1.0	Yellow
Pelagophyceae	1	0.3	1.0	Yellow
Rhodophyta	1	0.3	1.0	Yellow

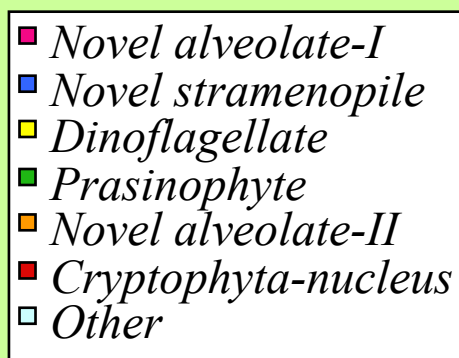
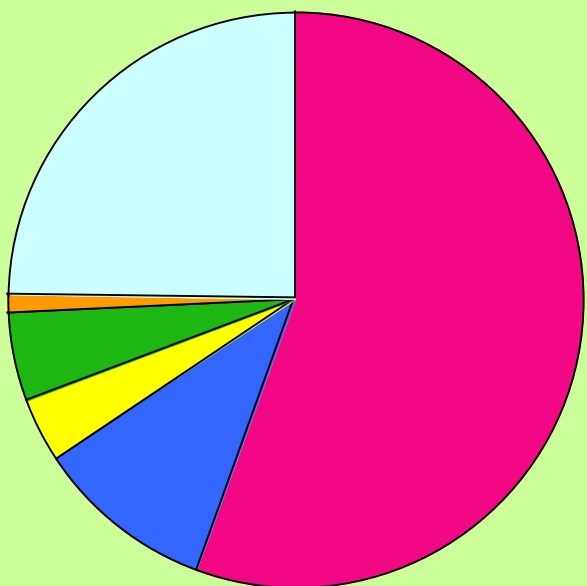
BL00921



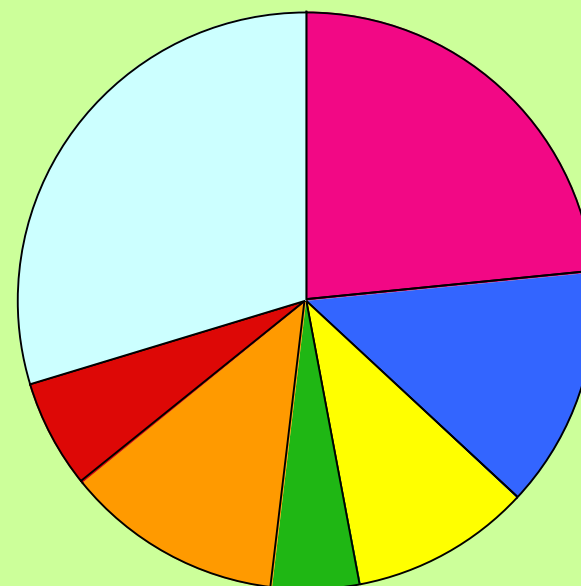
BL001221

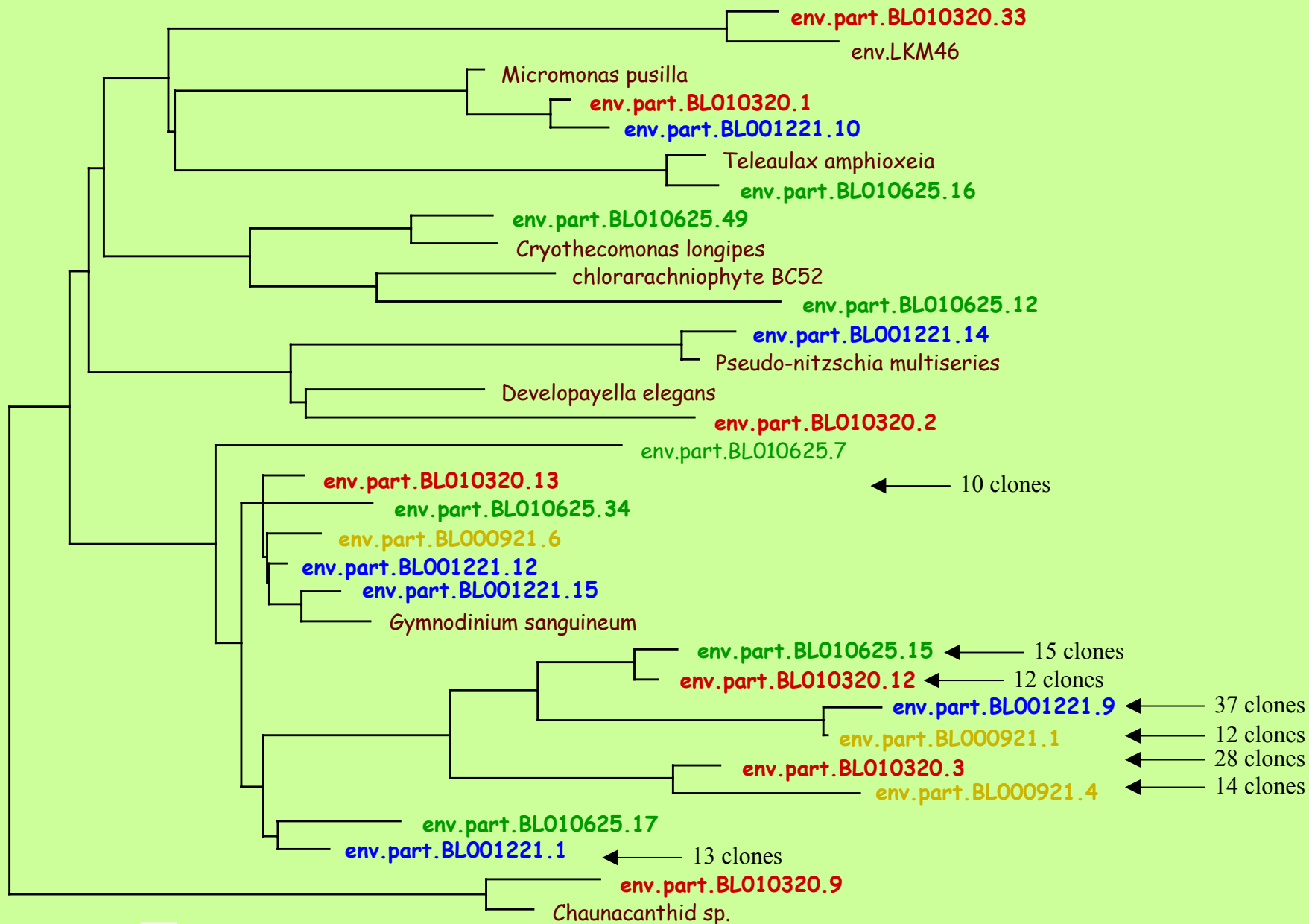


BL010320



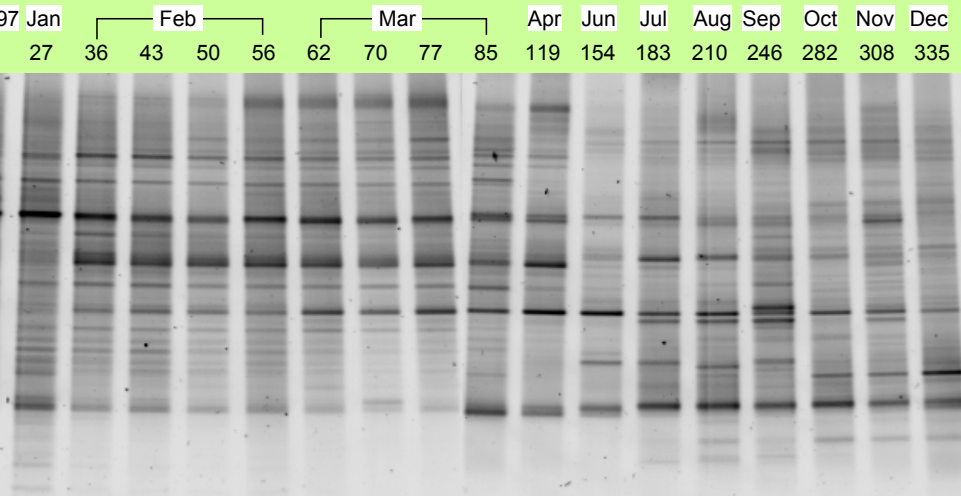
BL010625



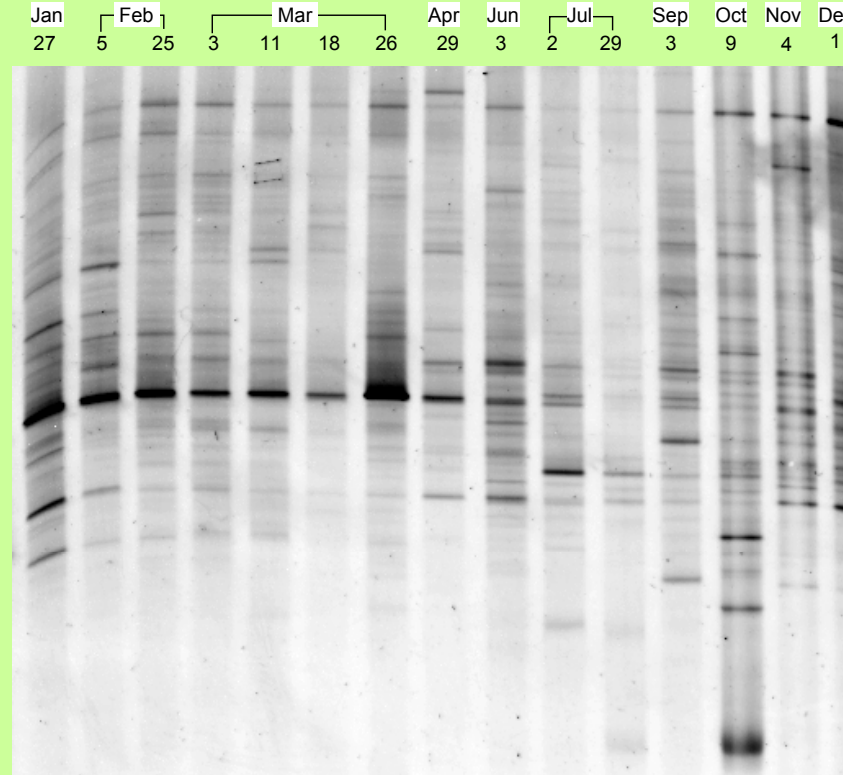


0.1

Temporal changes during year 1998



Bacteria



Picoeukaryotes

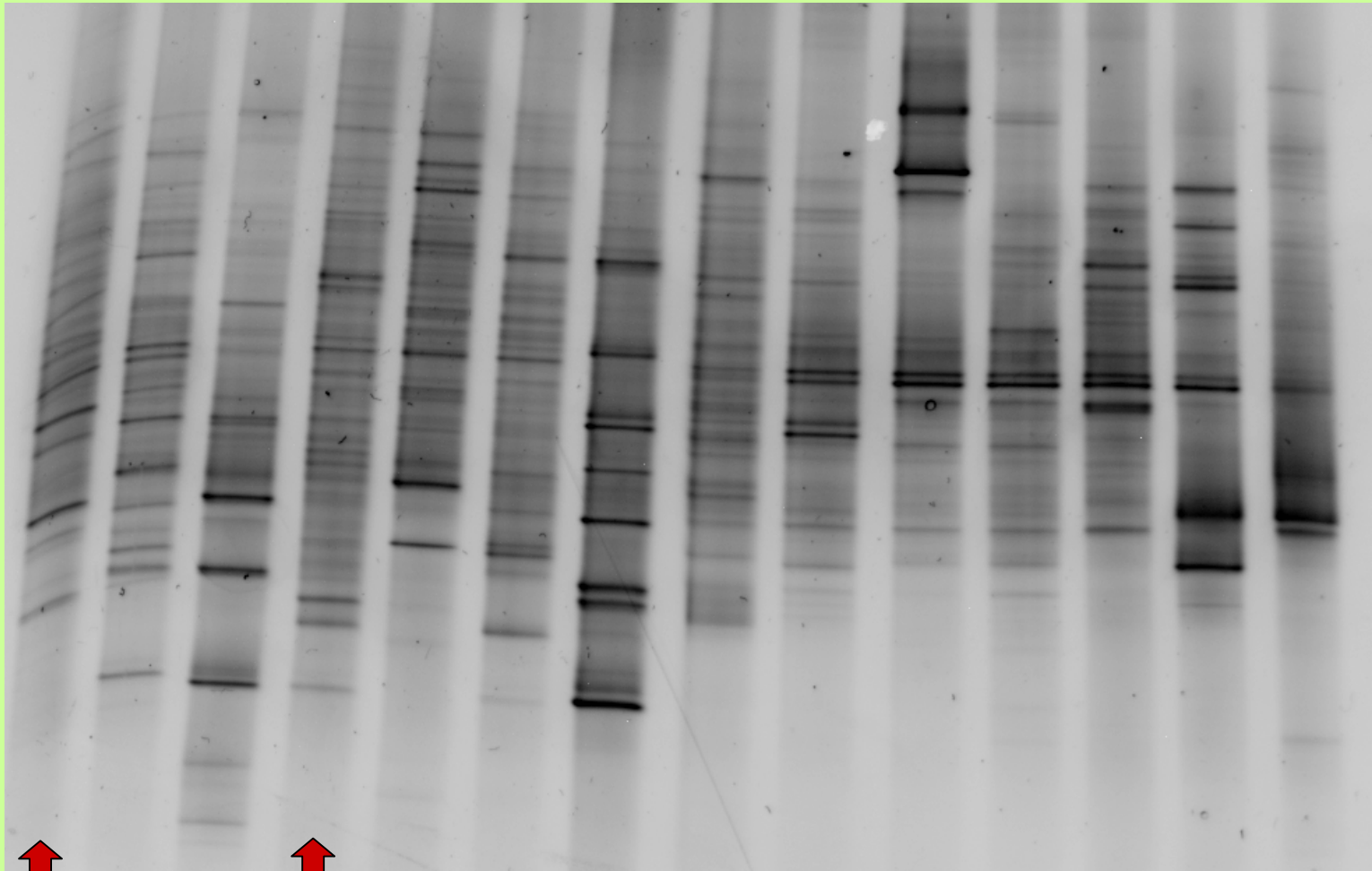
0.2 - 5 μm

Temporal changes during PICODIV sampling

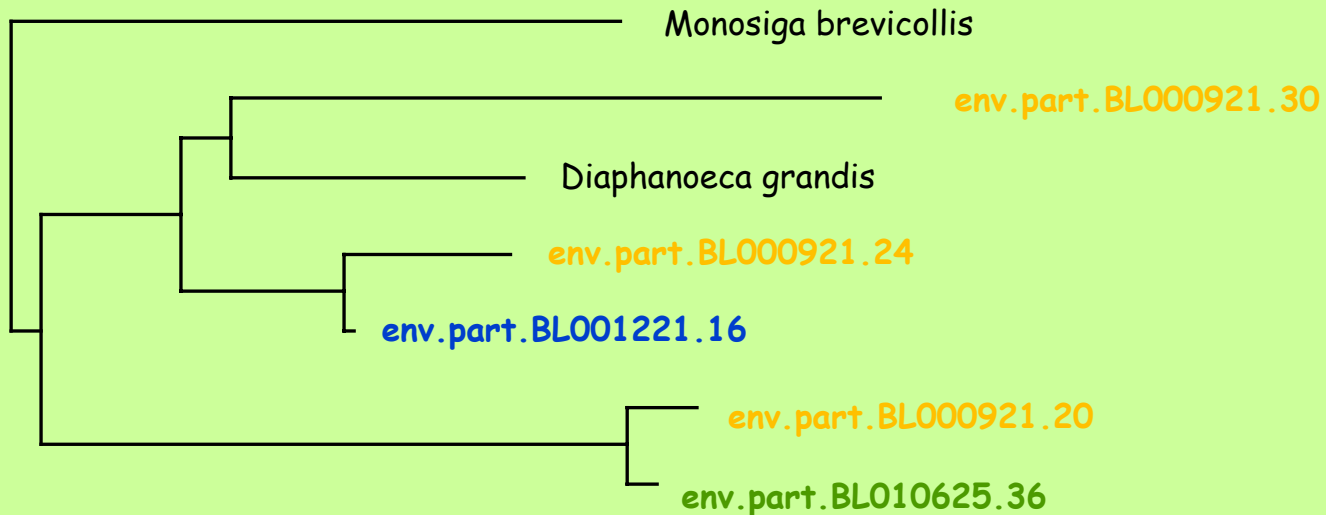
2001

2002

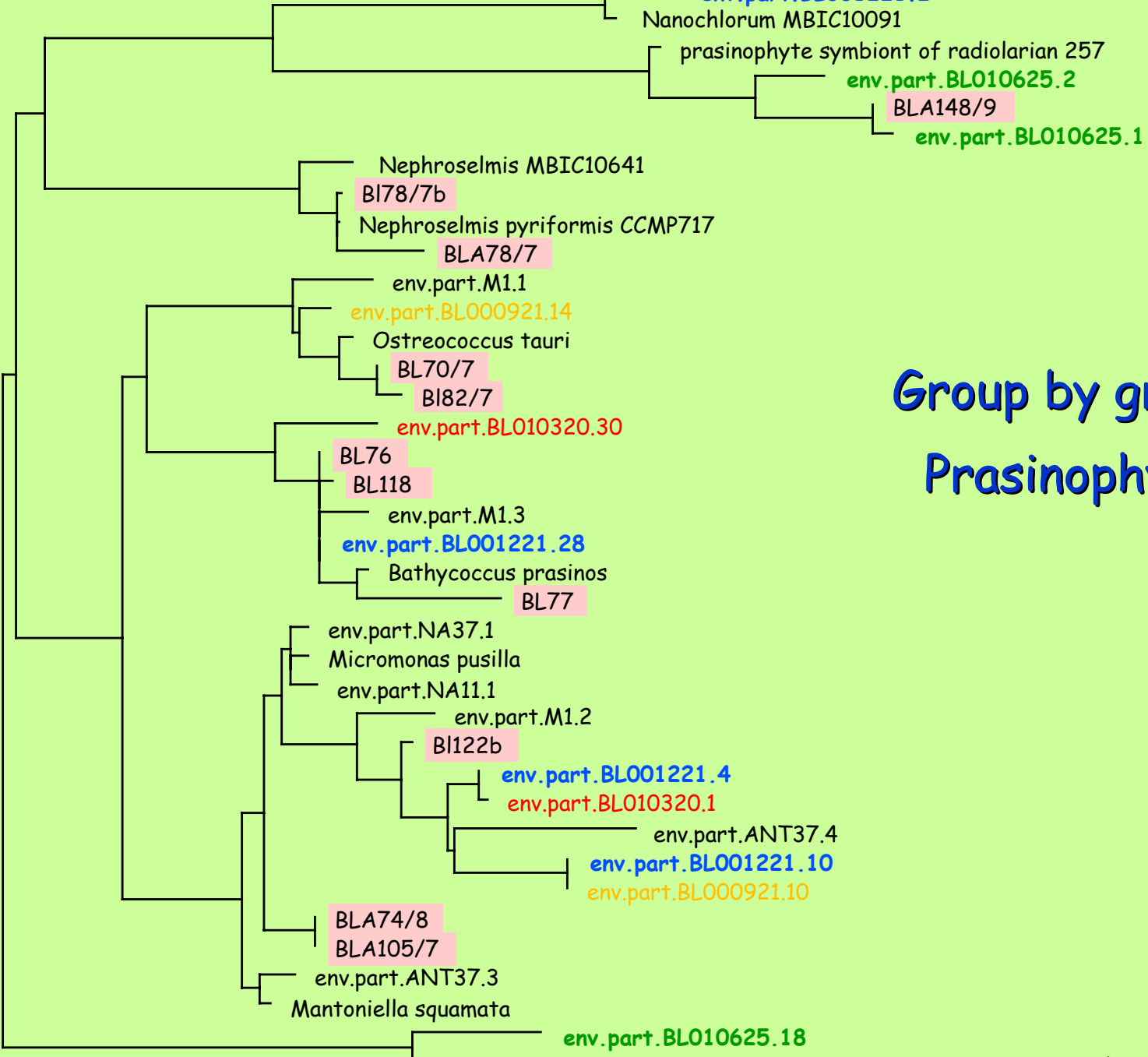
20/3 19/4 23/5 25/6 2/8 29/8 27/9 6/11 4/12 14/1 7/2 12/3 15/4 22/5



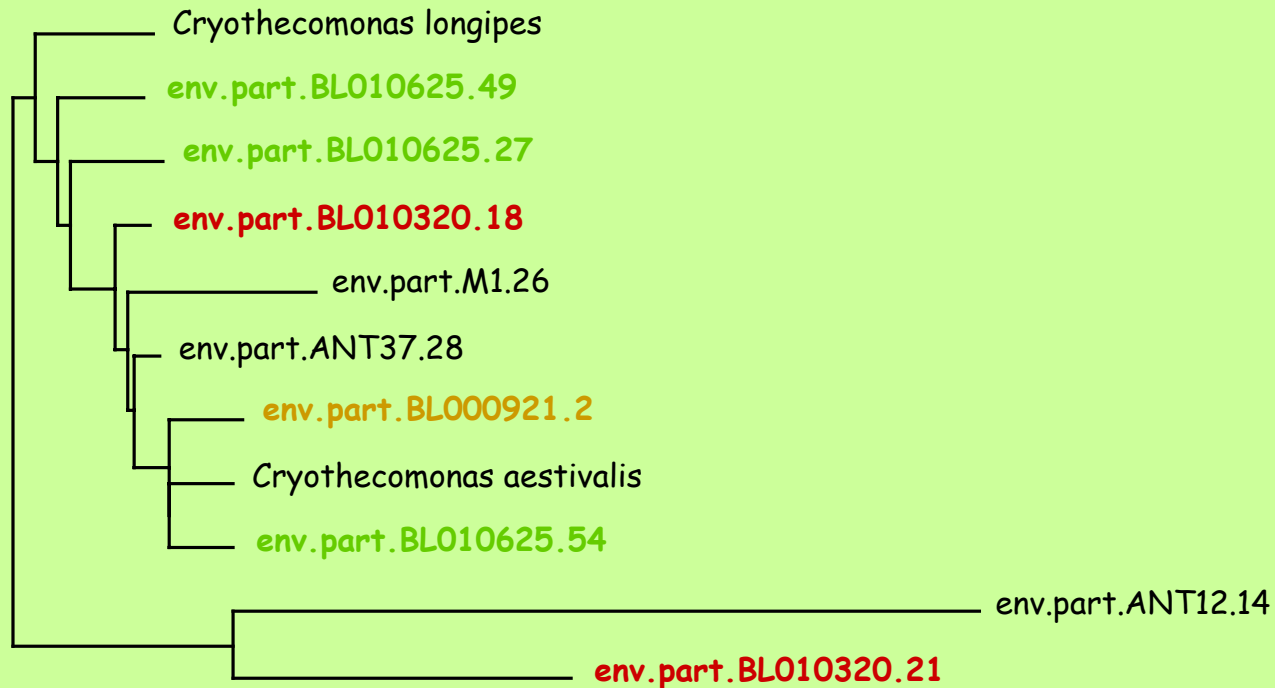
Group by group - Choanoflagellates



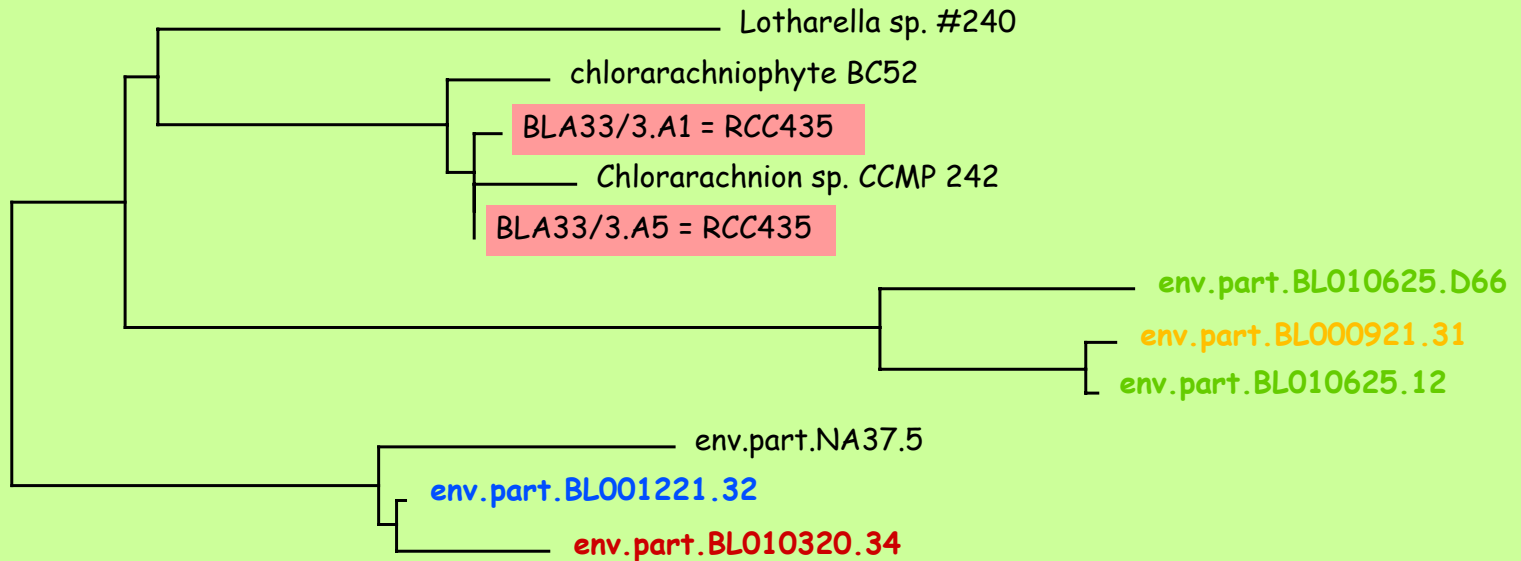
Group by group - Prasinophytes



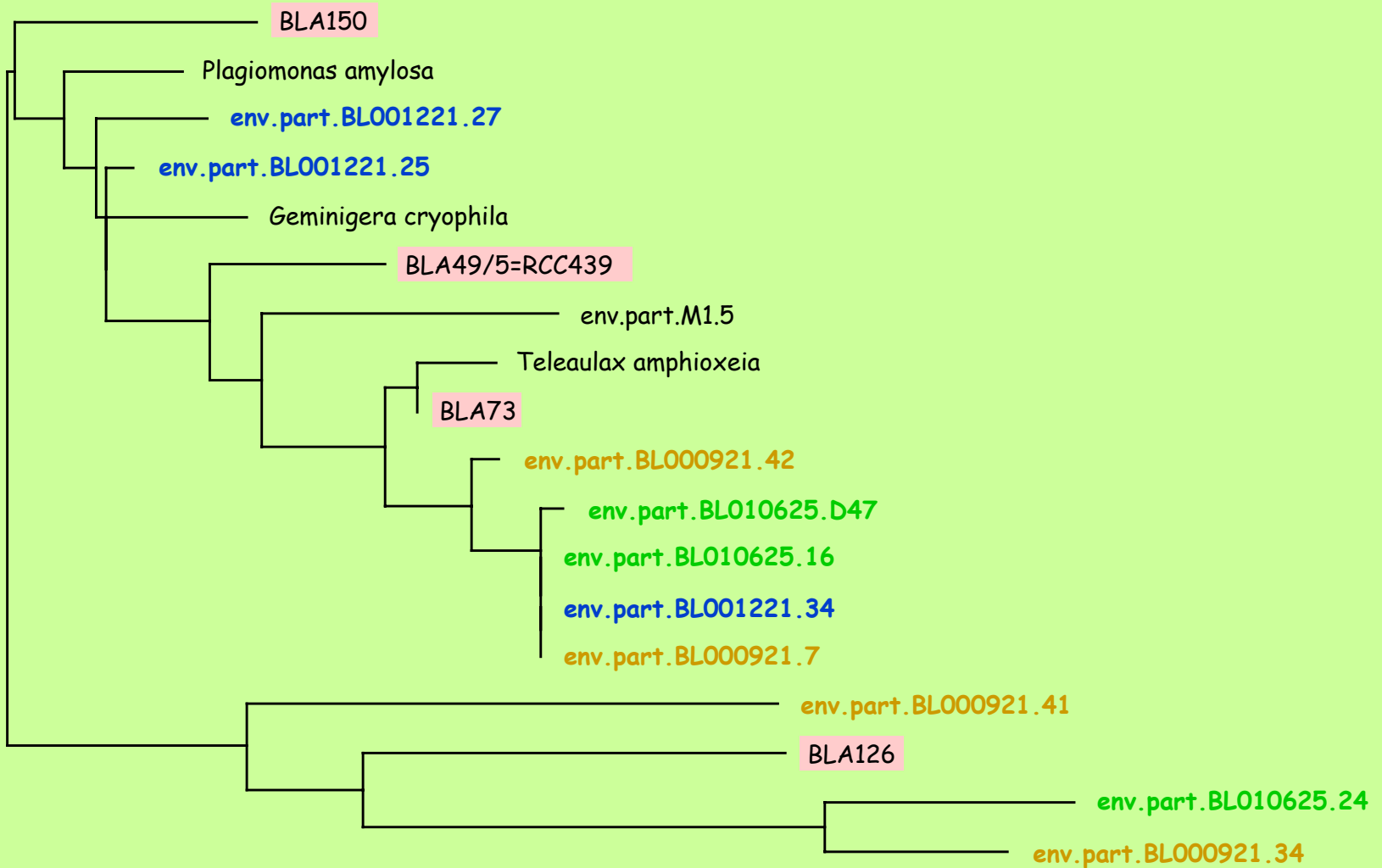
Group by group - Cercozoans

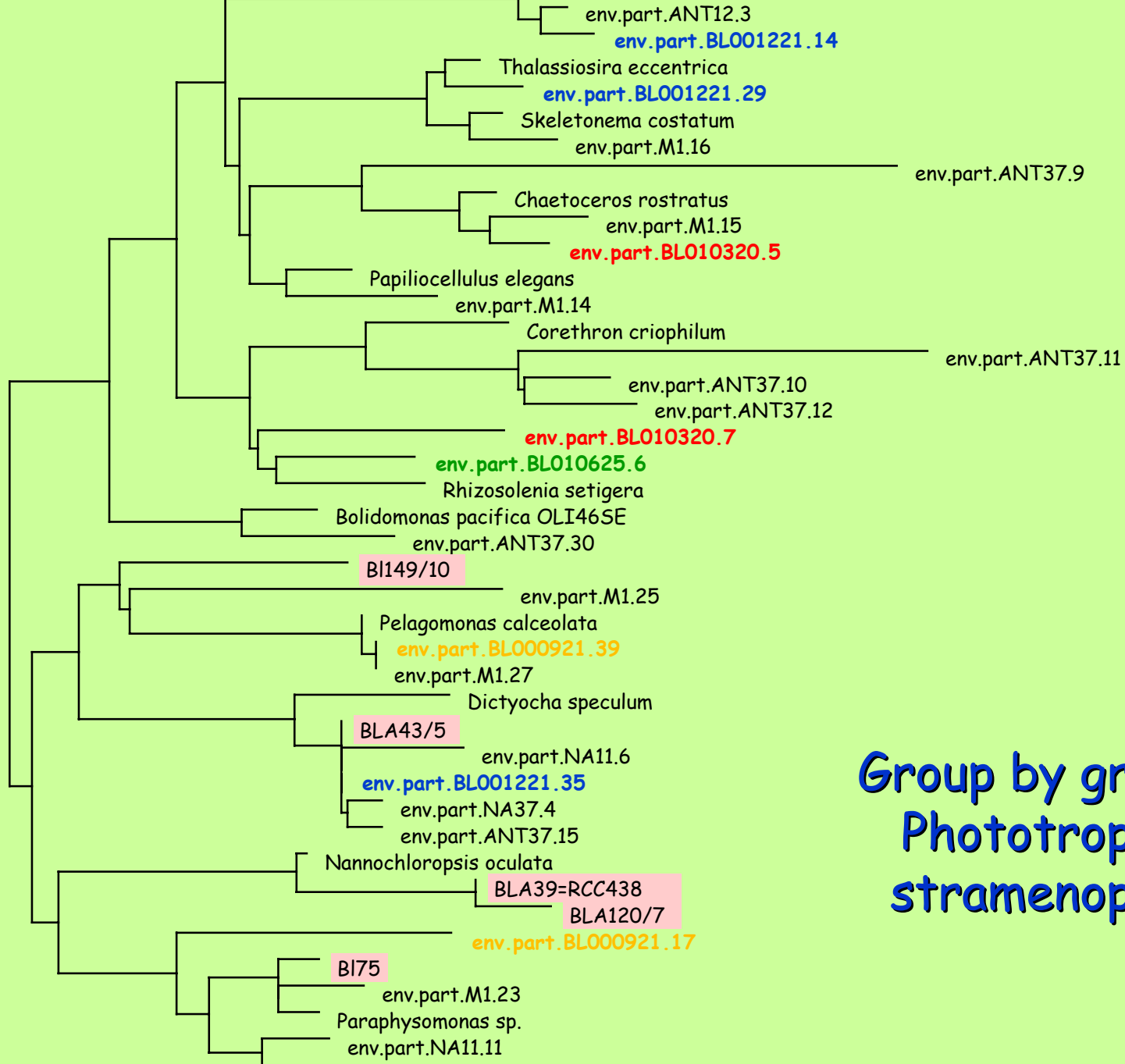


Group by group - Chlorarachniophytes



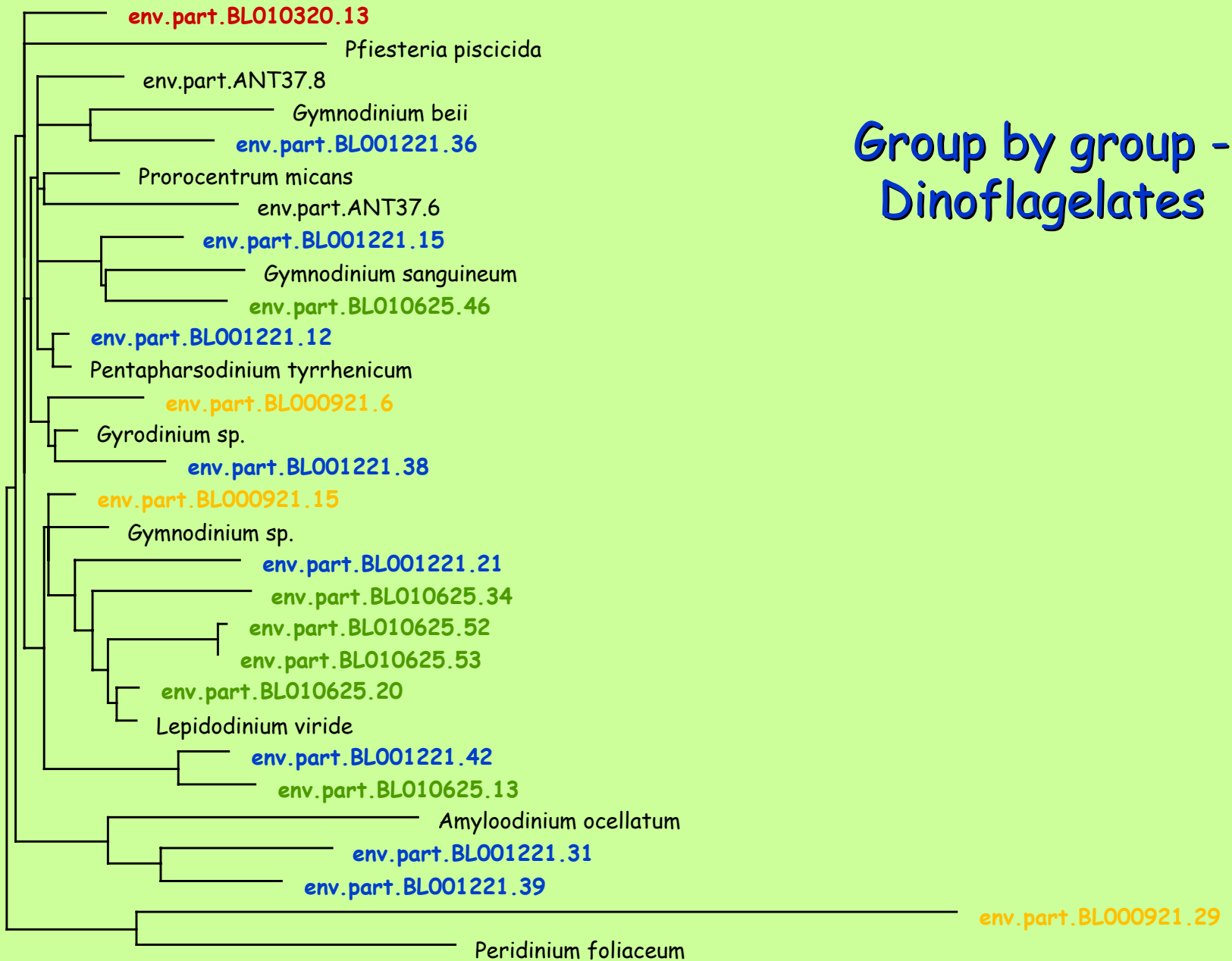
Group by group - Cryptophytes



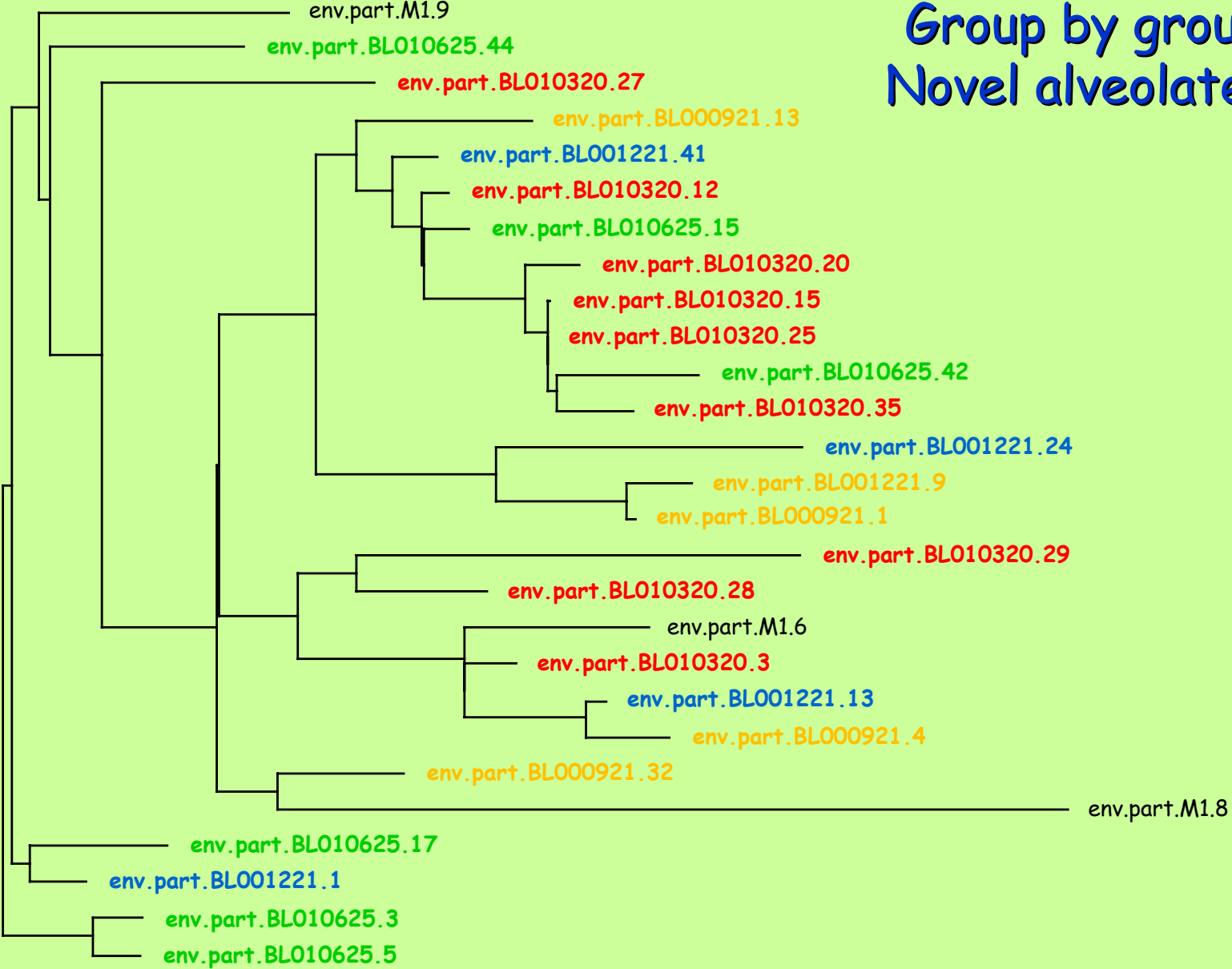


Group by group -
Phototrophic
stramenopiles

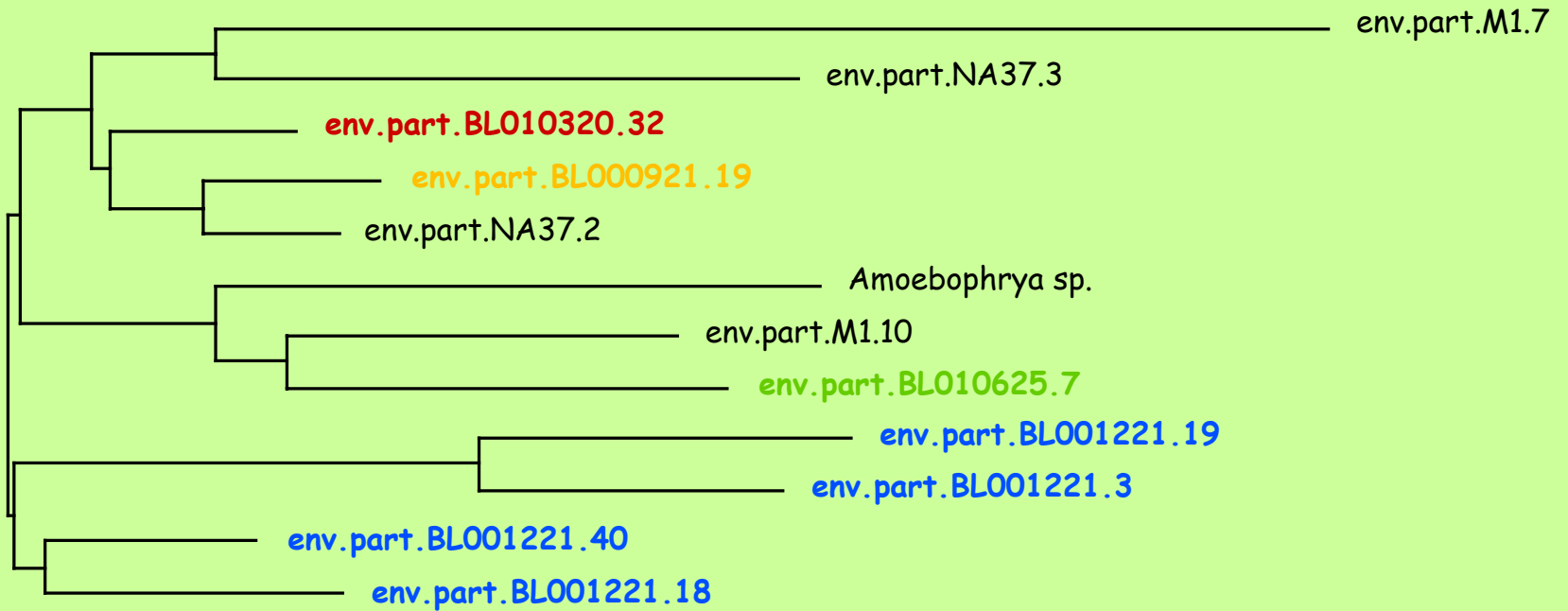
Group by group - Dinoflagelates



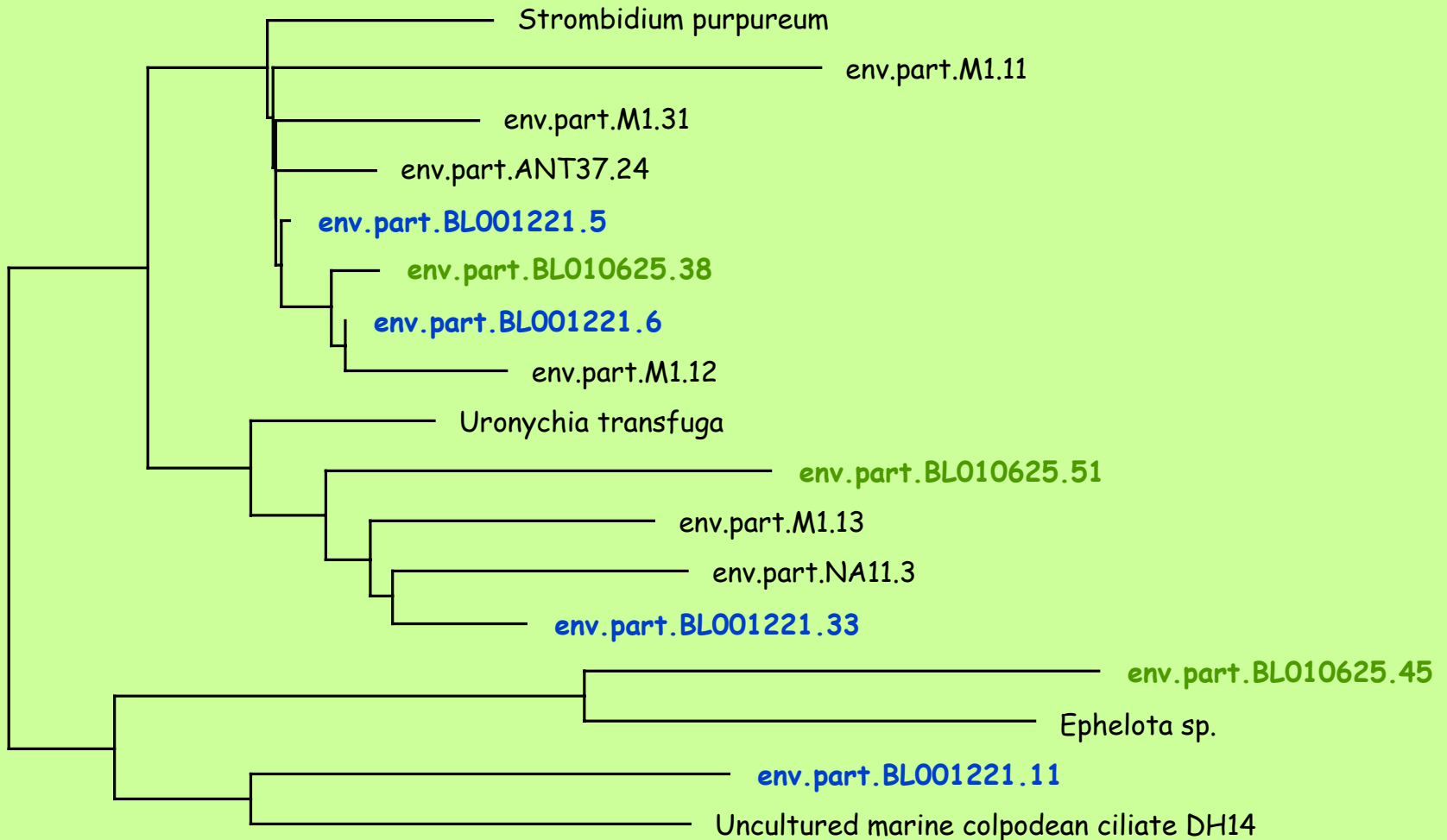
Group by group - Novel alveolates-I



Group by group - Novel alveolates-II



Group by group - Ciliates



Summary

Very large genetic diversity of picoeukaryotes

Novel alveolates-I dominate year round in Blanes libraries

Large variability during the year

Very few populations are found in different libraries

Some groups are well represented by cultures