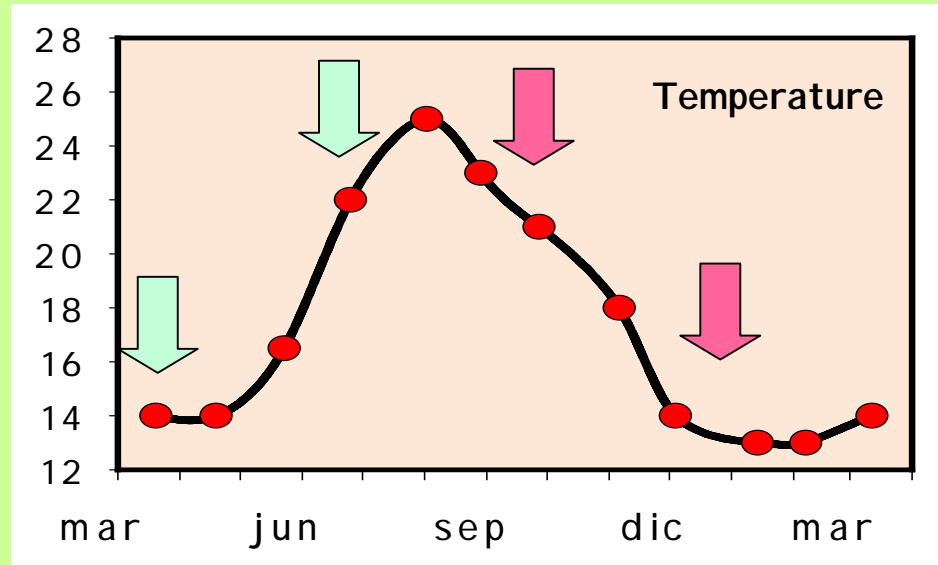


The four genetic libraries



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

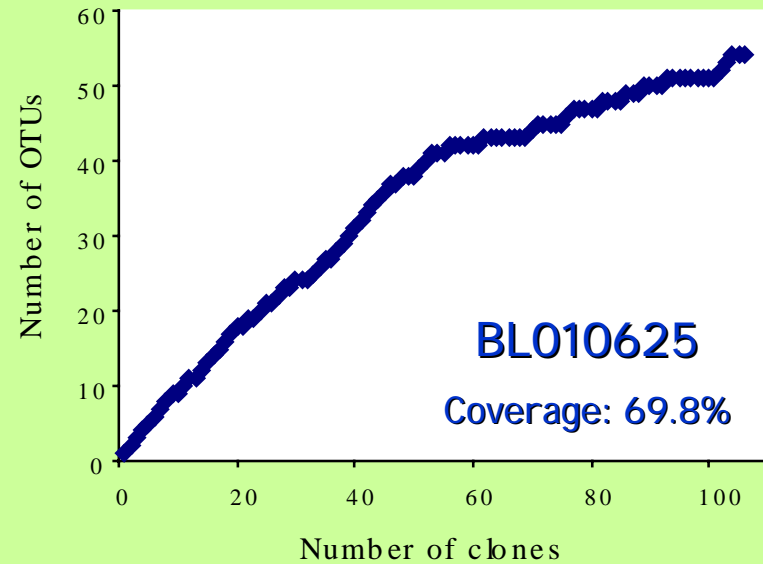
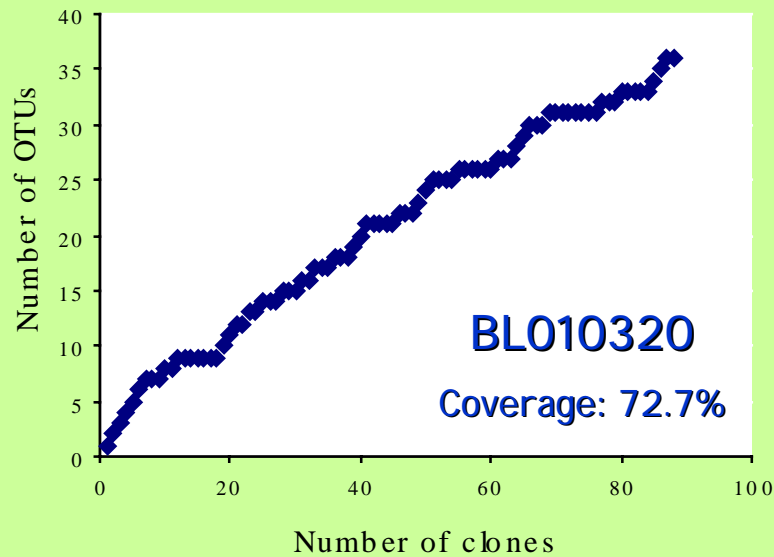
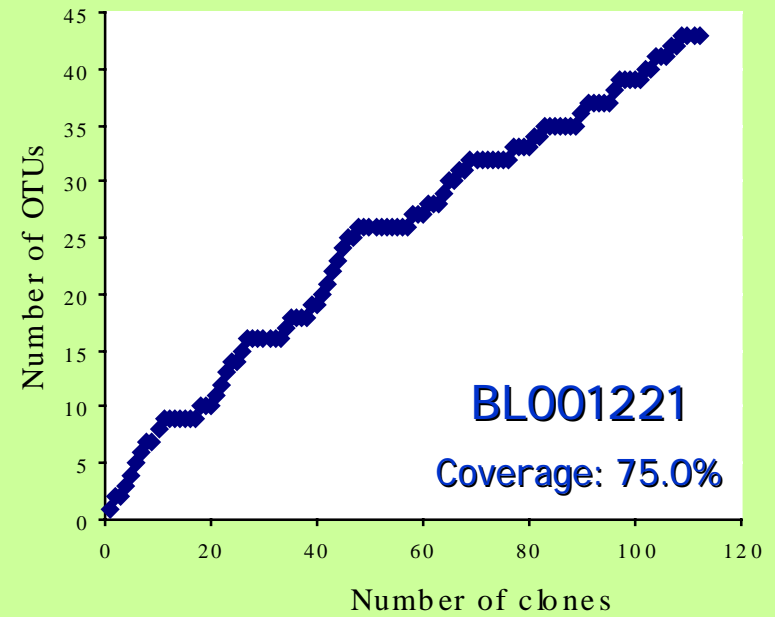
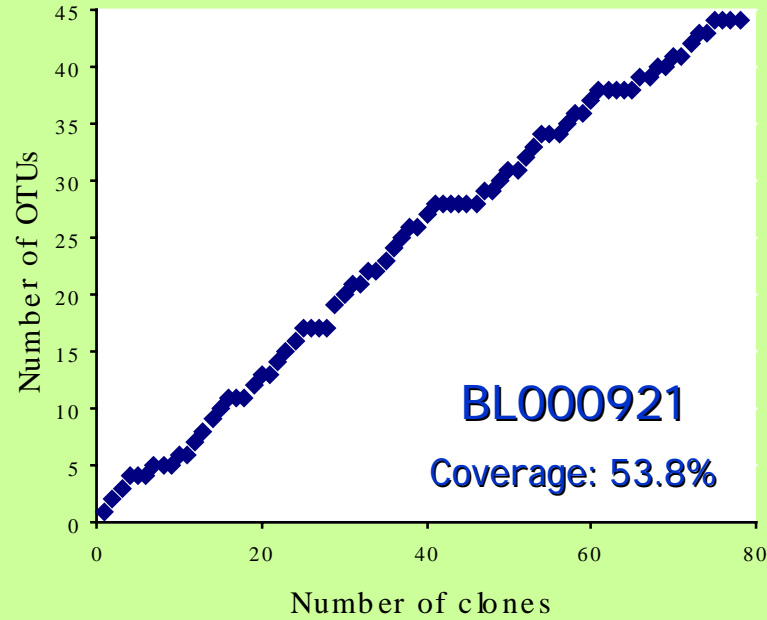
Fraction analyzed: 0.2 - 3 μ m

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

A2	BL000921.2	1	763	<i>Cryothecomonas longipes</i>	754/763	98.8	Cercozoa
A75	BL000921.43	1	726	BAQA80	690/725	95.2	Copepoda
A12	BL000921.7	1	836	<i>Teleaulax amphioxieia</i>	831/838	99.2	Cryptophyta
A56	BL000921.34	1	711	<i>Chroomonas</i> sp.	562/594	94.6	Cryptophyta
A72	BL000921.41	1	721	<i>Hemiselmis virescens</i>	576/606	95.0	Cryptophyta
A74	BL000921.42	2	766	<i>Teleaulax amphioxieia</i>	761/768	99.1	Cryptophyta
A52	BL000921.31	1	733	Un. chlorarachniophyte	578/616	93.8	Chlorarachniophyte-nucleus
A32	BL000921.20	1	770	<i>Monosiga brevicollis</i>	624/662	94.3	Choanoflagellate
A38	BL000921.24	1	774	OLI11013	762/776	98.2	Choanoflagellate
A51	BL000921.30	1	703	<i>Diaphanoeca grandis</i>	604/631	95.7	Choanoflagellate
A25	BL000921.17	1	710	C3_E031	692/710	97.5	Chrysophyceae
A7	BL000921.5	1	750	<i>Phaeothamnion confervicola</i>	570/604	94.3	Diatom
A37	BL000921.23	1	589	<i>Adenoides eludens</i>	484/517	93.6	Dinoflagellate
A10	BL000921.6	5	784	<i>Gyrodinium galatheanum</i>	768/784	98.0	Dinoflagellate
A23	BL000921.15	1	785	<i>Gymnodinium MUCC284</i>	778/785	99.1	Dinoflagellate
A49	BL000921.29	2	731	<i>Heterocapsa rotundata</i>	643/732	87.8	Dinoflagellate
A19	BL000921.12	1	739	<i>Bangia fuscopurpurea</i>	221/252	87.7	Inserta sedis
A20	BL000921.13	1	680	CS_E040	614/681	90.2	Novel alveolate-I
A1	BL000921.1	12	603	A1_E045	598/603	99.2	Novel alveolate-I
A4	BL000921.4	14	804	C1_E005	798/806	99.0	Novel alveolate-I
A54	BL000921.32	1	796	DH145-EKD10	769/797	96.5	Novel alveolate-I
A31	BL000921.19	1	593	OLI11023	518/556	93.2	Novel alveolate-II
A63	BL000921.38	1	732	CS_E041	567/717	91.9	Novel stramenopile-3
A27	BL000921.18	1	729	OLI11006	595/629	94.6	Novel stramenopile-3
A39	BL000921.25	1	725	OLI11006	641/670	95.7	Novel stramenopile-3
A42	BL000921.27	1	657	OLI11006	587/602	97.5	Novel stramenopile-3
A14	BL000921.9	1	735	OLI11066	715/735	97.3	Novel stramenopile-4
A24	BL000921.16	2	863	OLI11066	841/863	97.5	Novel stramenopile-4
A35	BL000921.22	1	799	OLI11066	775/797	97.2	Novel stramenopile-4
A60	BL000921.36	1	489	NA11-4	474/489	96.9	Novel stramenopile-4
A70	BL000921.40	1	784	OLI11066	752/784	95.9	Novel stramenopile-4
A16	BL000921.11	2	800	OLI11150	600/611	98.2	Novel stramenopile-7
A55	BL000921.33	1	803	OLI11150	797/803	99.2	Novel stramenopile-7
A40	BL000921.26	1	725	OLI11150	568/606	93.7	Novel stramenopile-11
A68	BL000921.39	1	777	<i>Pelagomonas calceolata</i>	777/777	100.0	Pelagophyceae
A15	BL000921.10	1	790	<i>Mantoniella squamata</i>	764/790	96.7	Prasinophyte
A22	BL000921.14	1	844	<i>Ostreococcus tauri</i>	824/844	97.6	Prasinophyte
A33	BL000921.21	1	746	<i>Phaeocystis cordata</i>	737/747	98.7	Prymnesiophyta
A43	BL000921.28	1	748	<i>Chrysochromulina scutellum</i>	723/748	96.7	Prymnesiophyta
A13	BL000921.8	1	737	<i>Apusomonas proboscidea</i>	573/616	93.0	Rhodophyta-env

BL001221

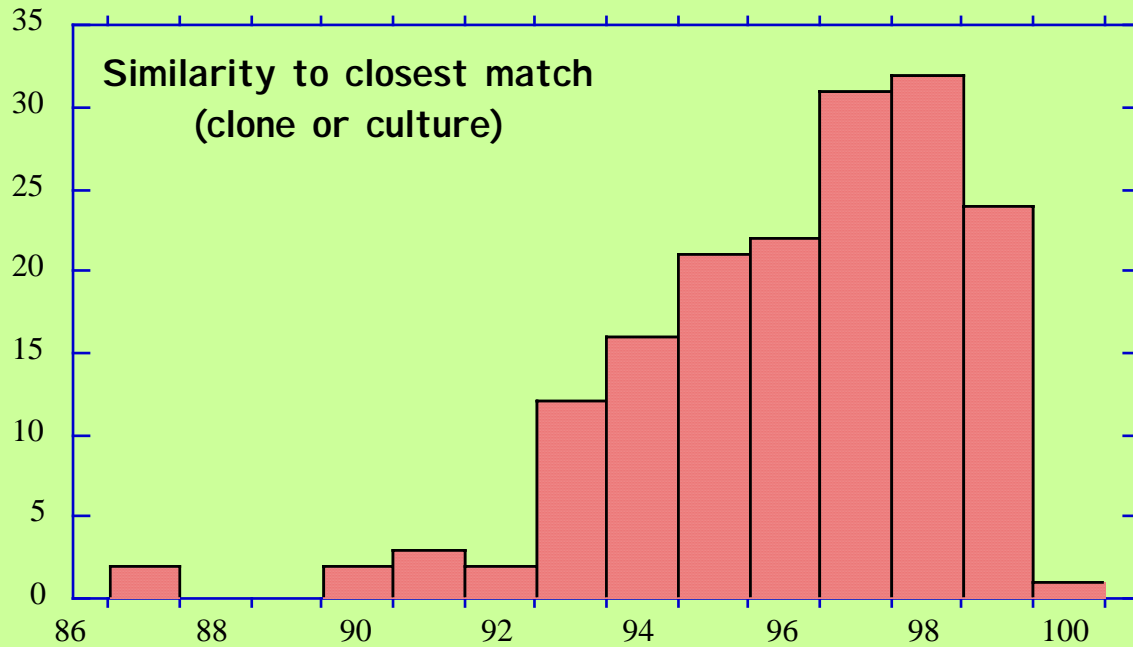
Clon	Name	n	read	Closest match	similarity	%	Phylogenetic group
B43	BL001221.22	1	822	Cryptosporidium muris	465/497	93.6	Apicomplexan
B92	BL001221.37	1	711	Oikopleura sp	555/564	98.4	Appendicularia
B8	BL001221.7	1	817	Elliptio complanata	258/263	98.1	Bivalva
B6	BL001221.5	1	754	C3_E037	740/753	98.3	Ciliate
B7	BL001221.6	1	740	C3_E037	723/742	97.4	Ciliate
B21	BL001221.11	1	843	Bresslaua vorax	456/478	95.4	Ciliate
B78	BL001221.33	1	753	C1_E042	596/610	97.7	Ciliate
B34	BL001221.17	2	759	Muggiaea sp.	756/759	99.6	Cnidaria
B47	BL001221.25	2	696	Teleaulax acuta	690/695	99.3	Cryptophyta
B59	BL001221.27	1	753	Falcomonas daucoides	744/759	98.0	Cryptophyta
B82	BL001221.34	1	843	Teleaulax amphioxieia	838/845	99.2	Cryptophyta
B70	BL001221.32	2	687	NA37-5	480/489	98.2	Chlorarachnniophyte
B2	BL001221.2	2	514	Nanochlorum eucaryotum	495/514	96.3	Chlorophyta
B27	BL001221.16	1	788	OLI11013	779/788	98.9	Choanoflagellate
B24	BL001221.14	3	749	Pseudo-nitzschia multiseriis	741/749	98.9	Diatom
B65	BL001221.29	1	761	C2_E024	746/762	97.9	Diatom
B84	BL001221.35	1	787	Dictyocha speculum	625/640	97.7	Dictyochales
B108	BL001221.42	1	798	Gymnodinium sp.	778/798	97.5	Dinoflagellate
B22	BL001221.12	5	681	DH147-EKD21	679/681	99.7	Dinoflagellate
B26	BL001221.15	4	844	OLI11225	835/844	98.9	Dinoflagellate
B42	BL001221.21	1	845	Pentapharsodinium tyrrhenicum	820/845	97.0	Dinoflagellate
B68	BL001221.31	2	513	Gymnodinium sp.	497/513	96.9	Dinoflagellate
B91	BL001221.36	1	805	Pentapharsodinium tyrrhenicum	788/805	97.9	Dinoflagellate
B97	BL001221.38	1	845	Gymnodinium galatheanum	837/845	99.0	Dinoflagellate
B98	BL001221.39	1	760	Gymnodinium sp.	738/760	97.1	Dinoflagellate
B1	BL001221.1	13	570	Dinophysis norvegica	559/570	98.1	Dinoflagellate
B110	BL001221.43	1	775	Cryptococcus cellulolyticus	770/775	99.3	Fungi
B11	BL001221.9	37	681	CS_E023	674/681	98.9	Novel alveolate-I
B23	BL001221.13	1	757	C1_E005	752/758	99.2	Novel alveolate-I
B46	BL001221.24	1	805	C2_E017	445/453	98.2	Novel alveolate-I
B105	BL001221.41	1	760	CS_E023	735/760	96.7	Novel alveolate-I
B4	BL001221.3	1	718	DH147-EKD6	680/718	94.7	Novel alveolate-II
B35	BL001221.18	2	803	OLI11023	745/805	92.5	Novel alveolate-II
B39	BL001221.19	1	840	DH147-EKD6	812/842	96.4	Novel alveolate-II
B103	BL001221.40	1	763	OLI11023	725/763	95.0	Novel alveolate-II
B44	BL001221.23	1	795	DH148-5-EKD53	786/795	98.9	Novel stramenopile-2
B49	BL001221.26	2	701	OLI11006	535/556	96.2	Novel stramenopile-3
B66	BL001221.30	1	771	OLI11006	601/626	96.0	Novel stramenopile-3
B10	BL001221.8	1	766	OLI11066	746/766	97.4	Novel stramenopile-4
B41	BL001221.20	1	792	Hippospongia communis	787/793	99.2	Porifera
B5	BL001221.4	2	804	Micromonas pusilla	781/806	96.9	Prasinophyte
B18	BL001221.10	4	836	Mantoniella squamata	811/836	97.0	Prasinophyte
B62	BL001221.28	2	801	Mantoniella squamata	761/807	94.3	Prasinophyte

BL010320

Clon	Name	n	read	Closest match	similarity	%	Phylogenetic group
C12	BL_010320.9	3	869	C3_E010	843/871	96.8	Acantharea
C20	BL_010320.10	1	881	C3_E010	856/883	96.9	Acantharea
C38	BL_010320.18	1	885	<i>Cryothecomonas longipes</i>	871/886	98.3	Cercozoa
C43	BL_010320.21	2	863	<i>Cryothecomonas longipes</i>	580/609	95.2	Cercozoa
C21	BL_010320.11	3	744	<i>Calanus pacificus</i>	721/744	96.9	Copepoda
C92	BL_010320.34	1	872	Unnamed chlorarachniophyte	580/614	94.5	Chlorarachniophyte
C5	BL_010320.5	1	820	<i>Chaetocerus rostratus</i>	719/732	98.2	Diatom
C7	BL_010320.7	1	823	<i>Rhizosolenia setigera</i>	635/671	94.6	Diatom
C24	BL_010320.13	3	866	<i>Pentapharsodinium</i> sp.	852/869	98.0	Dinoflagellate
C63	BL_010320.27	1	878	<i>Karena brevis</i>	795/879	90.4	Dinoflagellate
C71	BL_010320.29	1	875	<i>Gymnodinium</i> sp.	453/488	92.8	Dinoflagellate
C10	BL_010320.8	2	835	EMPE7	258/265	97.4	Fungi
C87	BL_010320.33	3	853	EMPE7	819/854	95.9	Fungi
C32	BL_010320.16	1	907	<i>Rhodotorula glutinis</i>	895/908	98.6	Fungi
C94	BL_010320.36	1	861	BAQA128	823/862	95.4	Fungi
C41	BL_010320.19	1	874	<i>Labyrinthuloides minuta</i>	358/377	95.0	Labyrinthulida
C57	BL_010320.26	1	893	C1_E009	890/894	99.5	Labyrinthulida
C3	BL_010320.3	28	831	C1_E005	829/831	99.8	Novel alveolate-I
C22	BL_010320.12	10	800	CS_E023	789/800	98.6	Novel alveolate-I
C29	BL_010320.15	1	877	C2_E017	871/877	99.3	Novel alveolate-I
C42	BL_010320.20	1	868	C2_E017	857/868	98.7	Novel alveolate-I
C53	BL_010320.25	1	920	C2_E017	914/920	99.3	Novel alveolate-I
C70	BL_010320.28	1	848	A1_E039	808/851	94.9	Novel alveolate-I
C93	BL_010320.35	1	885	C2_E017	751/756	99.3	Novel alveolate-I
C83	BL_010320.32	1	822	OLI11023	841/883	95.2	Novel alveolate-II
C34	BL_010320.17	1	886	OLI11006	833/892	93.4	Novel stramenopile-3
C6	BL_010320.6	2	891	OLI11150	601/611	98.4	Novel stramenopile-7
C48	BL_010320.22	1	904	OLI11150	597/611	97.7	Novel stramenopile-7
C26	BL_010320.14	1	859	C3_E008	841/859	97.9	Novel stramenopile-9
C2	BL_010320.2	3	861	A3_E008	843/861	97.9	Oomycetes
C1	BL_010320.1	3	850	<i>Mantionella squamata</i>	826/852	96.9	Prasinophyte
C72	BL_010320.30	1	919	<i>Mantionella squamata</i>	869/922	94.2	Prasinophyte
C4	BL_010320.4	1	778	<i>Lithophyllum incrustans</i>	771/778	99.1	Rhodophyta

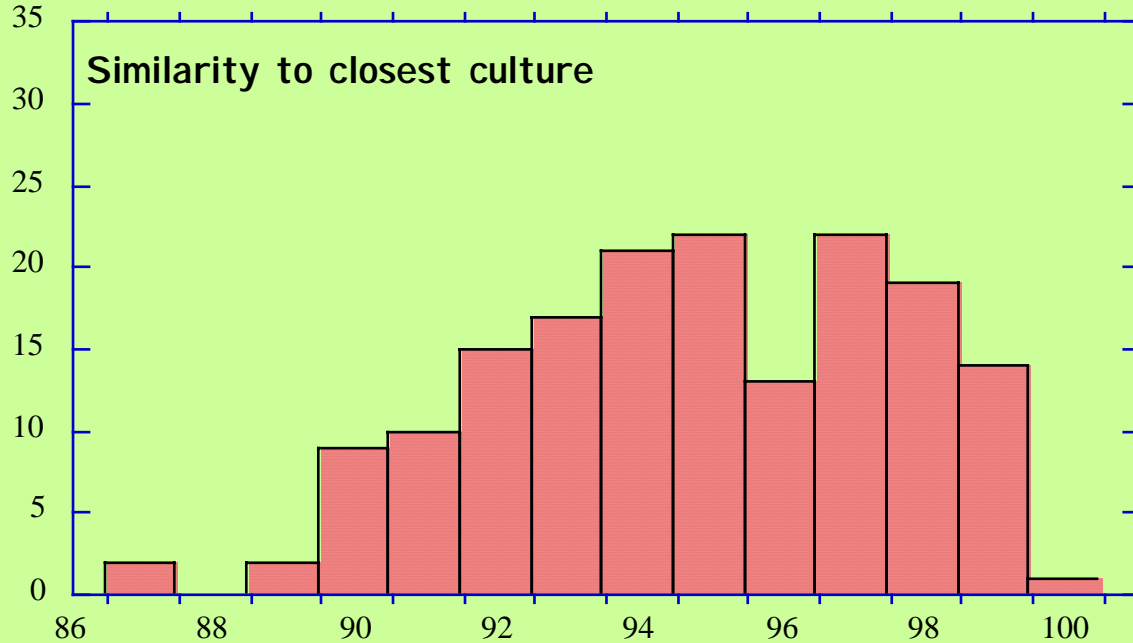
BL010625

Clon	Name	n	read	Closest match	similarity	%	Phylogenetic group
D37	BL010625.28	1	730	Acila castrensis	701/731	95.9	Bivalvia
D38	BL010625.29	5	722	Acila castrensis	706/722	97.8	Bivalvia
D27	BL010625.22	3	726	Brachiopod sp.	514/564	91.1	Brachiopoda
D35	BL010625.27	1	732	Cryothecomonas longipes	629/645	97.5	Cercozoa
D86	BL010625.49	3	761	Cryothecomonas longipes	734/763	96.2	Cercozoa
D104	BL010625.54	1	823	Cryothecomonas longipes	801/823	97.3	Cercozoa
D48	BL010625.38	1	807	C3_E037	780/810	96.3	Ciliate
D71	BL010625.45	2	803	Ephelota sp.	558/595	93.8	Ciliate
D93	BL010625.51	1	695	C2_E022	527/559	94.3	Ciliate
D34	BL010625.26	2	730	A2_E042	682/732	93.1	Copepoda
D18	BL010625.16	3	786	Teleaulax amphioxeia	781/788	99.1	Cryptophyta
D30	BL010625.24	2	718	Chroomonas sp.	543/573	94.8	Cryptophyta
D4	BL010625.4	1	729	Teleaulax amphioxeia	703/723	97.2	Cryptophyta-nucleomorph
D14	BL010625.12	5	729	Unnamed chlorarachniophyte	559/596	93.8	Chlorarachniophyte
D45	BL010625.36	1	734	Monosiga brevicollis	558/590	94.6	Choanoflagellate
D6	BL010625.6	1	734	Rhizosolenia setigera	712/735	96.6	Diatom
D15	BL010625.13	1	671	Pfiesteria-like dino	653/671	97.3	Dinoflagellate
D24	BL010625.20	1	801	Lepidodinium viride	800/801	99.9	Dinoflagellate
D43	BL010625.34	3	794	Pentapharsodinium sp.	768/794	96.7	Dinoflagellate
D76	BL010625.46	1	816	Amphidinium longum	795/817	97.3	Dinoflagellate
D102	BL010625.52	1	833	Lepidodinium viride	820/833	98.4	Dinoflagellate
D103	BL010625.53	1	794	Lepidodinium viride	782/794	98.5	Dinoflagellate
D3	BL010625.3	1	564	DH147-EKD21	554/565	98.0	Dinoflagellate
D5	BL010625.5	2	717	DH147-EKD21	705/717	98.3	Dinoflagellate
D19	BL010625.17	5	483	Pentapharsodinium sp.	460/483	95.2	Dinoflagellate
D40	BL010625.31	1	546	Labyrinthuloides yorkensis	542/546	99.3	Labyrinthulida
D53	BL010625.41	2	712	Labyrinthula sp.	467/490	95.3	Labyrinthulida
D62	BL010625.43	1	683	Cephalothrix rufifrons	670/684	97.9	Nemertea
D56	BL010625.42	1	741	C2_E017	725/741	97.8	Novel alveolate-I
D70	BL010625.44	1	729	OLI11005	695/729	95.3	Novel alveolate-I
D17	BL010625.15	9	776	C5_E023	757/769	98.4	Novel alveolate-I
D7	BL010625.7	10	576	DH147-EKD16	411/449	91.5	Novel alveolate-II
D8	BL010625.8	1	705	OLI11006	658/707	93.1	Novel stramenopile-3
D39	BL010625.30	1	741	OLI11006	579/598	96.8	Novel stramenopile-3
D42	BL010625.33	2	745	OLI11006	622/645	96.4	Novel stramenopile-3
D44	BL010625.35	2	735	OLI11006	728/735	99.0	Novel stramenopile-3
D16	BL010625.14	1	725	ME1-24	556/580	95.7	Novel stramenopile-3
D41	BL010625.32	1	734	C3_E007	723/734	98.5	Novel stramenopile-9
D22	BL010625.19	2	725	BAQA72	507/518	97.8	Novel stramenopile-12
D25	BL010625.21	1	737	BOLA515	612/635	96.3	Oomycete
D9	BL010625.9	3	723	Protodrilus purpureus	540/572	94.4	Polychaeta
D12	BL010625.11	3	697	Protodrilus purpureus	526/551	95.4	Polychaeta
D51	BL010625.39	1	734	Protodrilus purpureus	557/584	95.3	Polychaeta
D52	BL010625.40	2	733	Protodrilus purpureus	557/584	95.3	Polychaeta
D77	BL010625.47	1	761	Protodrilus purpureus	589/614	95.9	Polychaeta
D82	BL010625.48	1	740	Protodrilus purpureus	555/592	93.7	Polychaeta
D1	BL010625.1	2	731	Prasinophyte symbiont	714/731	97.7	Prasinophyte
D2	BL010625.2	1	755	Prasinophyte symbiont	740/755	98.0	Prasinophyte
D20	BL010625.18	1	525	Pyramimonas australis	512/525	97.5	Prasinophyte
D89	BL010625.50	1	797	Chrysochromulina hirta	756/798	94.7	Prymnesiophyta
D11	BL010625.10	2	793	Chrysochromulina trondsenii	783/793	98.7	Prymnesiophyte
D33	BL010625.25	1	725	Neocallimastix sp.	543/576	94.3	Telonema



AVERAGE SIMILARITY: 96.7

45% of sequences have an environmental clone as closest match



AVERAGE SIMILARITY: 95.1

General analysis of libraries - I

Significant presence of metazoans

Appendicularia, Bivalvia, Brachiopoda, Cnidaria, Copepoda,
Nemertea, Polychaeta, Porifera

18 RFLP patterns (10.2%)

34 clones (8.6%)

General analysis of libraries - II

Dominant groups (>3%)

Phylogenetic group	Number clones	%-clones	Clones/RFLP	
Novel alveolate-I	122	36.0	6.8	Present in 4 libraries
Dinoflagellate	59	17.4	2.4	
Novel stramenopile	34	10.0	1.3	
Prasinophyte	18	5.3	1.8	
Novel alveolate-II	17	5.0	2.4	
Cryptophyta	14	4.1	1.6	Present in 3 libraries

TOTAL

150 RFLPs

339 clones

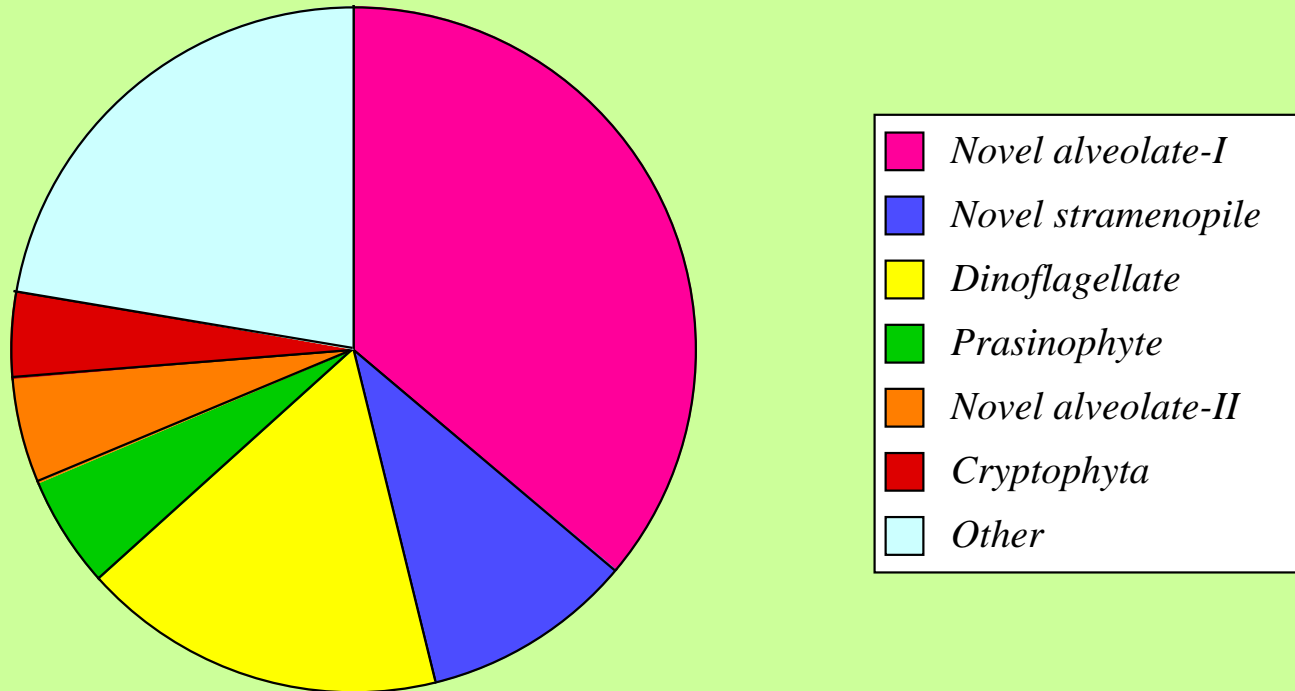
Present in 4 libraries
Present in 3 libraries
Present in 2 libraries
Present in 1 libraries

General analysis of libraries - III

Minoritary groups (>3%)

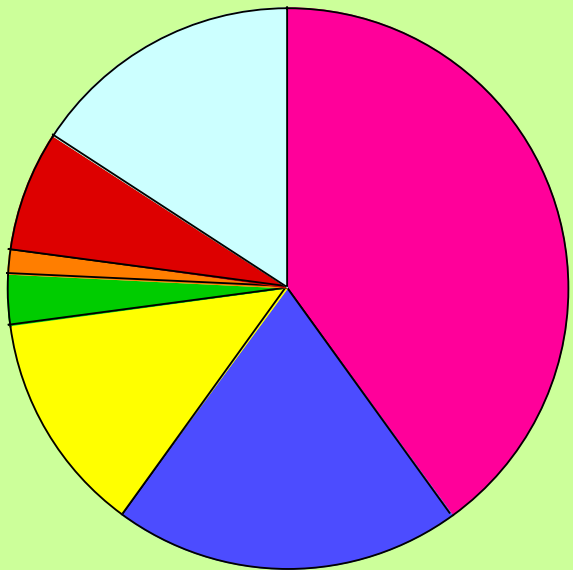
Phylogenetic group	Number clones	%-clones	Clones/RFLP	
Chlorarachniophyte	9	12.0	2.3	Black
Cercozoa	9	12.0	1.5	Blue
Diatom	8	10.7	1.3	Black
Fungi	8	10.7	1.6	Orange
Ciliate	8	10.7	1.1	Orange
Choanoflagellate	5	6.7	1.0	Blue
Prymnesiophyta	5	6.7	1.3	Orange
Labyrinthulida	5	6.7	1.3	Orange
Oomycetes	4	5.3	2.0	Orange
Acantharea	4	5.3	2.0	Yellow
Chlorophyta	2	2.7	2.0	Yellow
Apicomplexan	1	1.3	1.0	Yellow
Telonema	1	1.3	1.0	Yellow
Rhodophyta	1	1.3	1.0	Yellow
Rhodophyra-env	1	1.3	1.0	Yellow
Pelagophyceae	1	1.3	1.0	Yellow
Dictyochales	1	1.3	1.0	Yellow
Chrysophyceae	1	1.3	1.0	Yellow
Crypt-nucleomorph	1	1.3	1.0	Yellow

Summary of clones

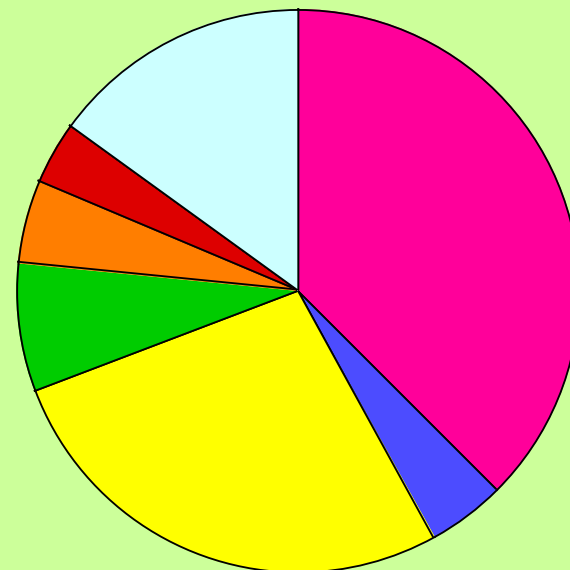


339 sequences of picoeukaryotes
from Blanes

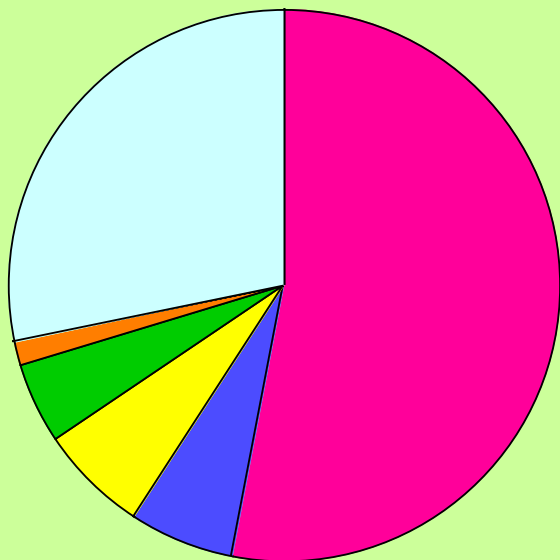
BL00921



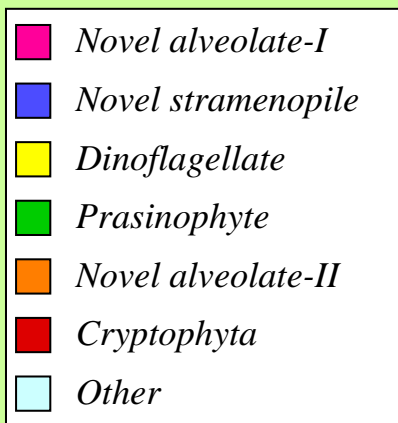
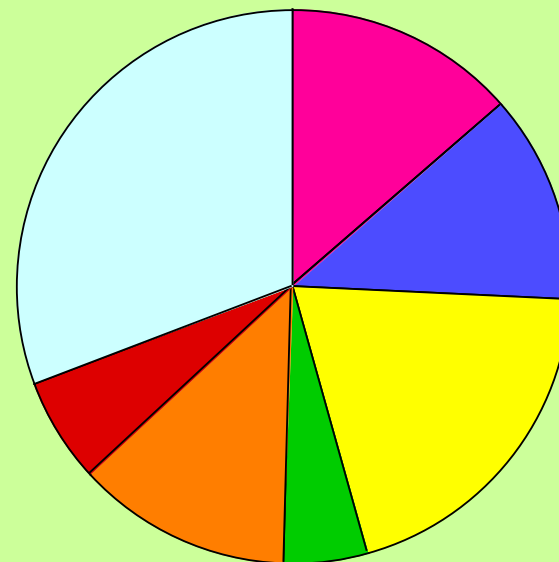
BL001221



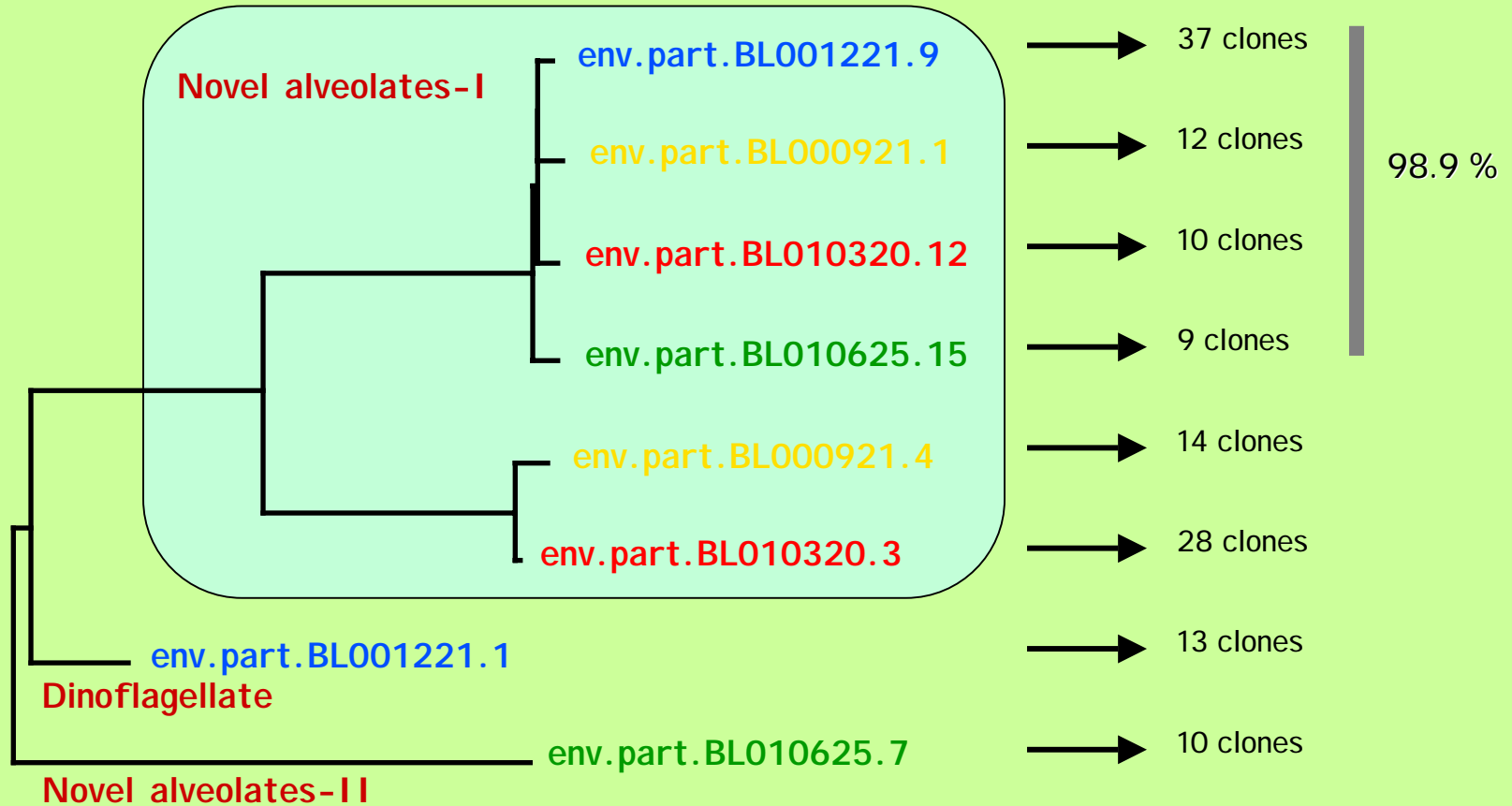
BL010320



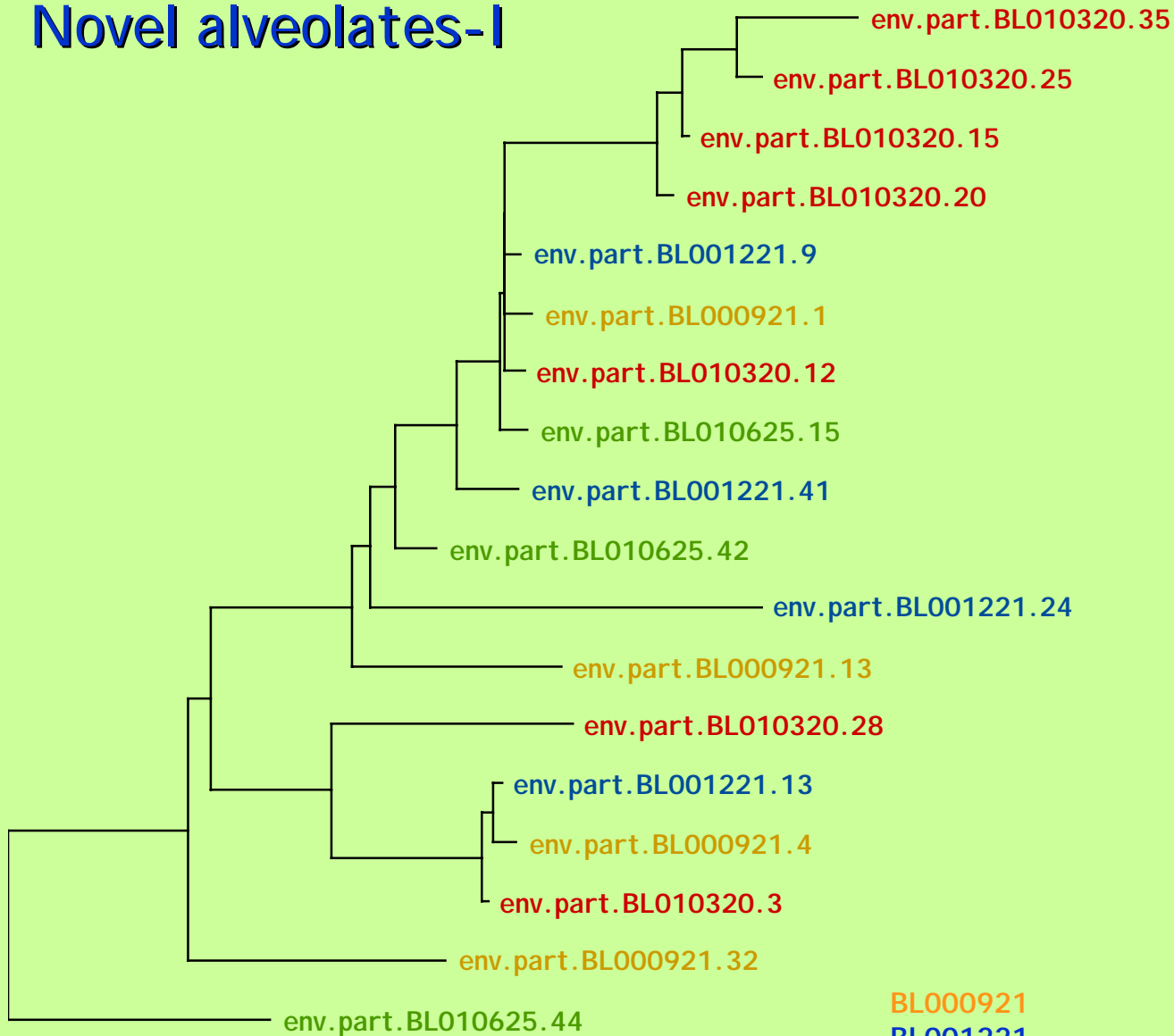
BL010625



The two more abundant clones in each library

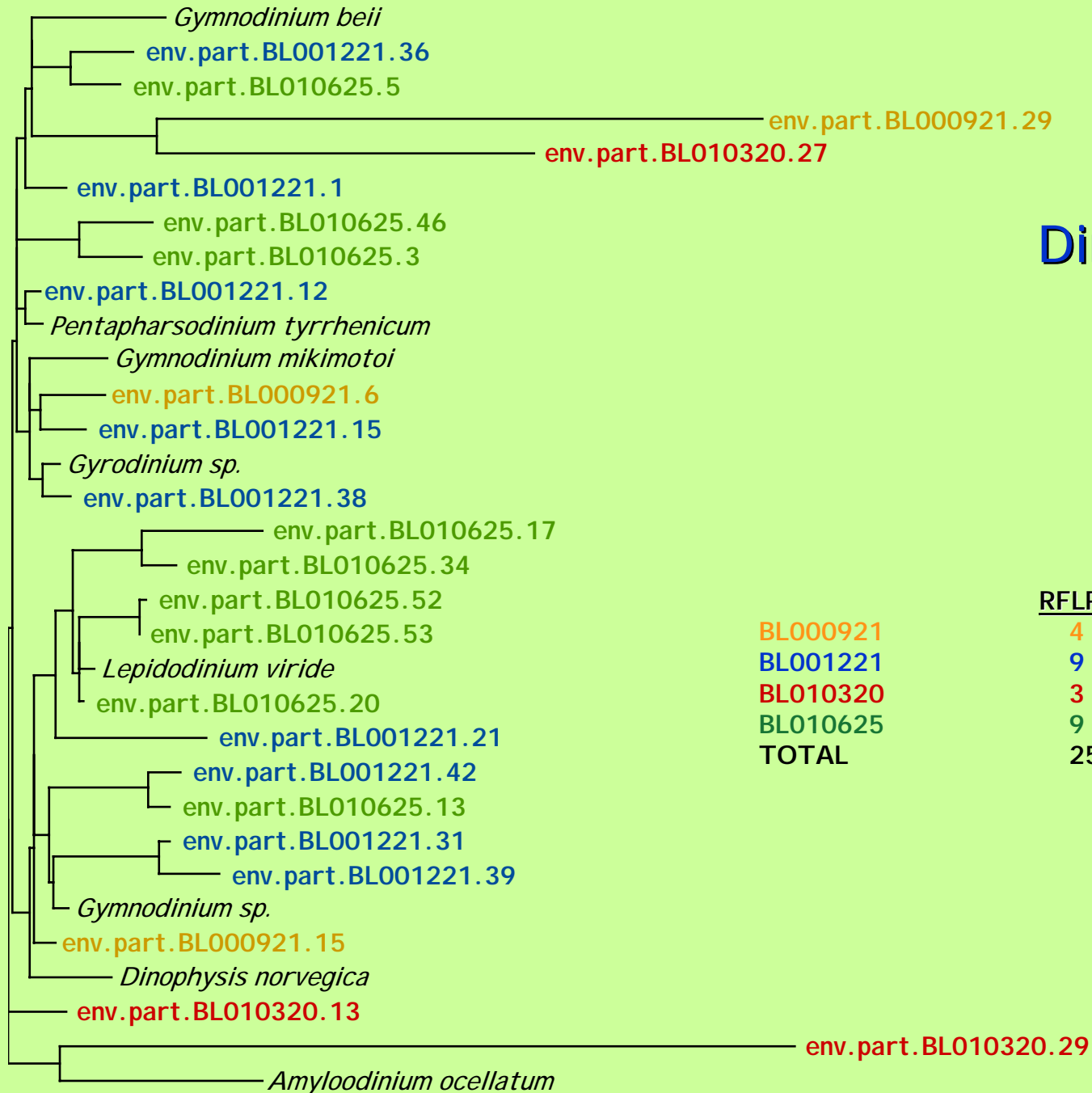


Novel alveolates-I



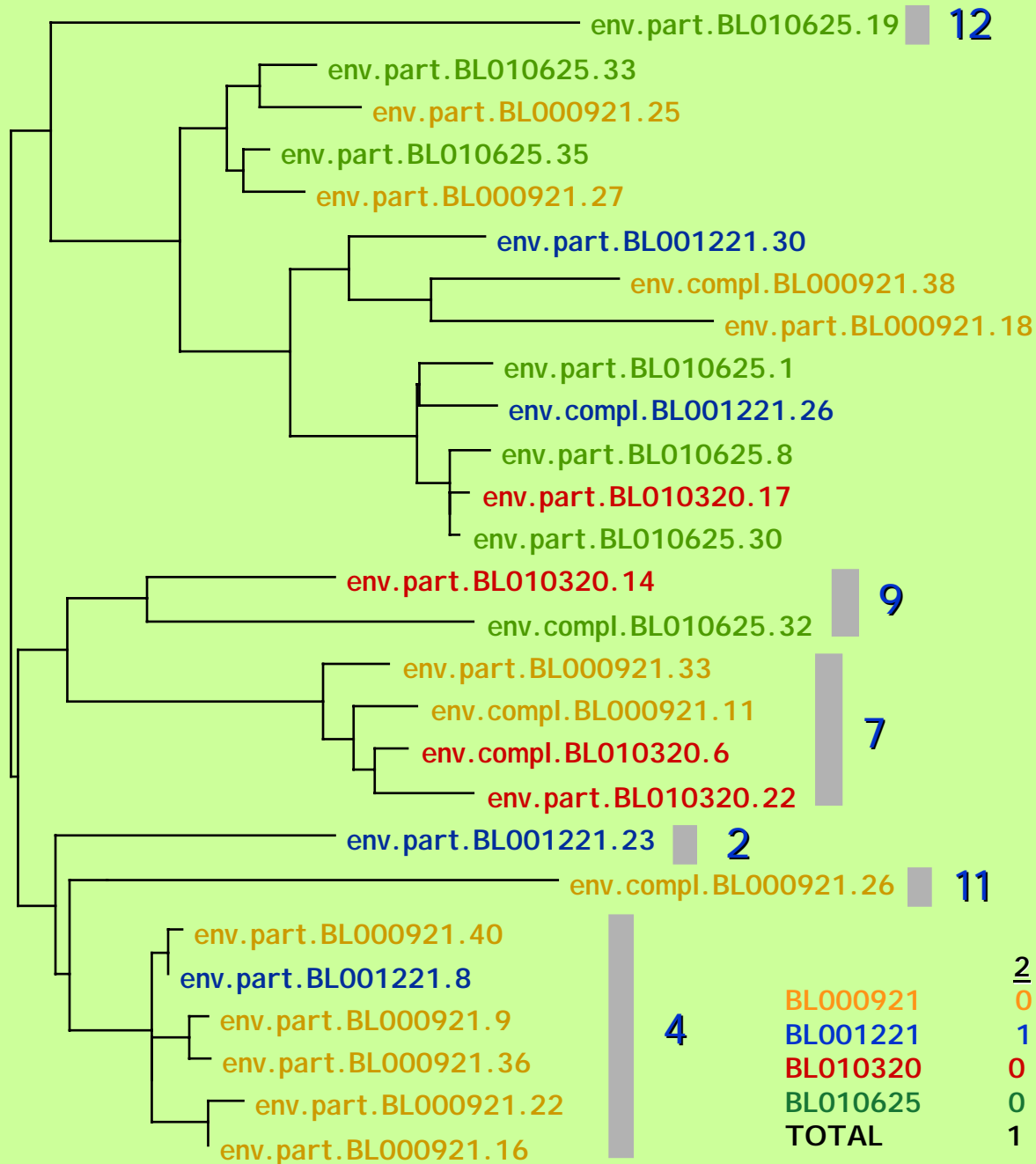
	<u>RFLPs</u>	<u>Clones</u>
BL000921	4	28
BL001221	4	40
BL010320	7	43
BL010625	3	11
TOTAL	18	122

Dinoflagelates

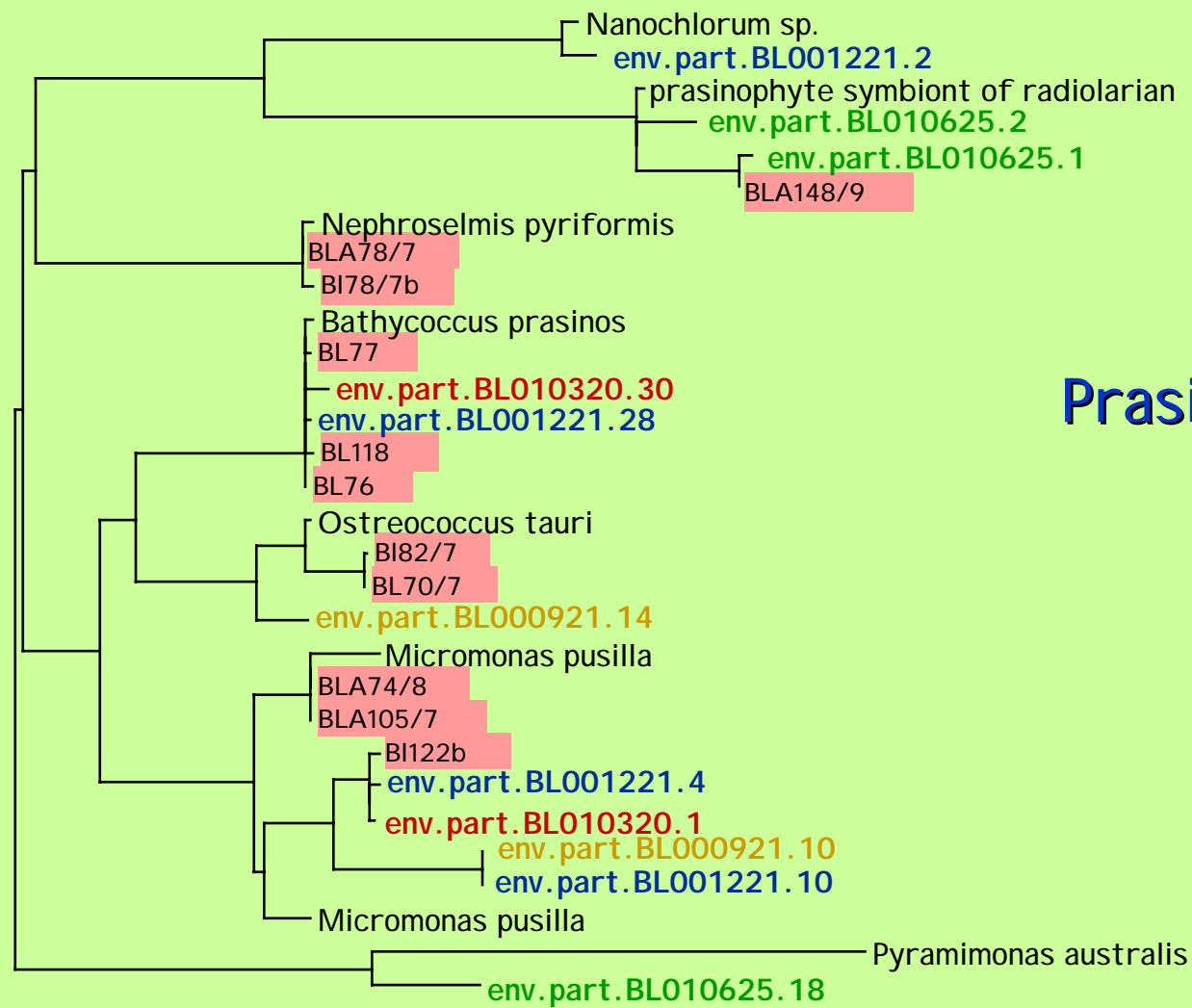


	RFLPs	Clones
BL000921	4	9
BL001221	9	29
BL010320	3	5
BL010625	9	16
TOTAL	25	59

Novel Stramenopiles



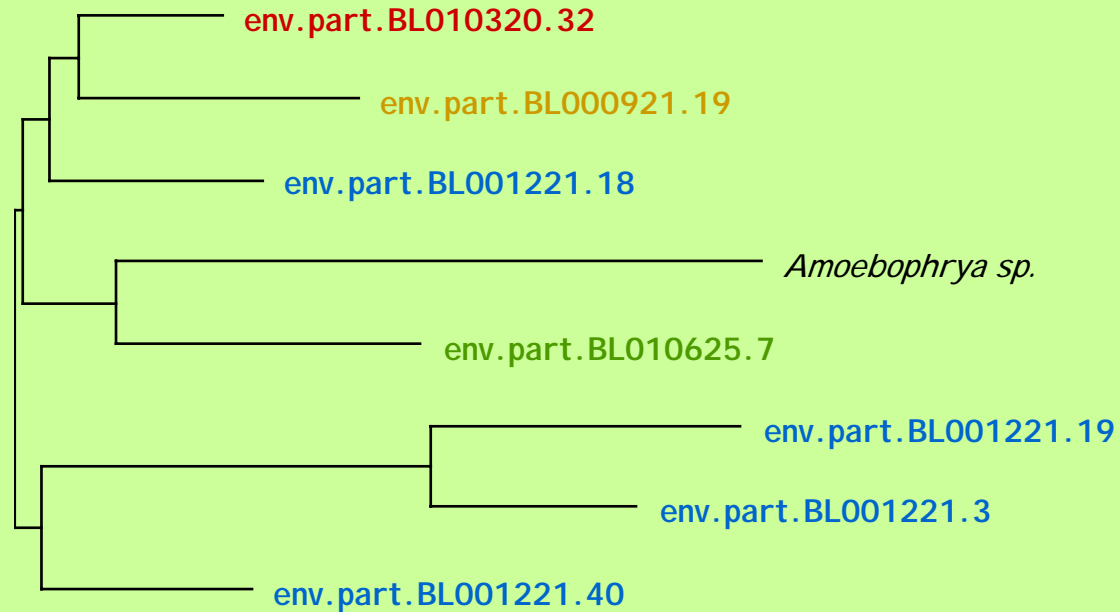
	<u>2</u>	<u>3</u>	<u>4</u>	<u>7</u>	<u>9</u>	<u>11</u>	<u>12</u>
BL000921	0	4	6	3	0	1	0
BL001221	1	3	1	0	0	0	0
BL010320	0	1	0	3	1	0	0
BL010625	0	7	0	0	1	0	2
TOTAL	1	15	7	6	2	1	2



Prasinophytes

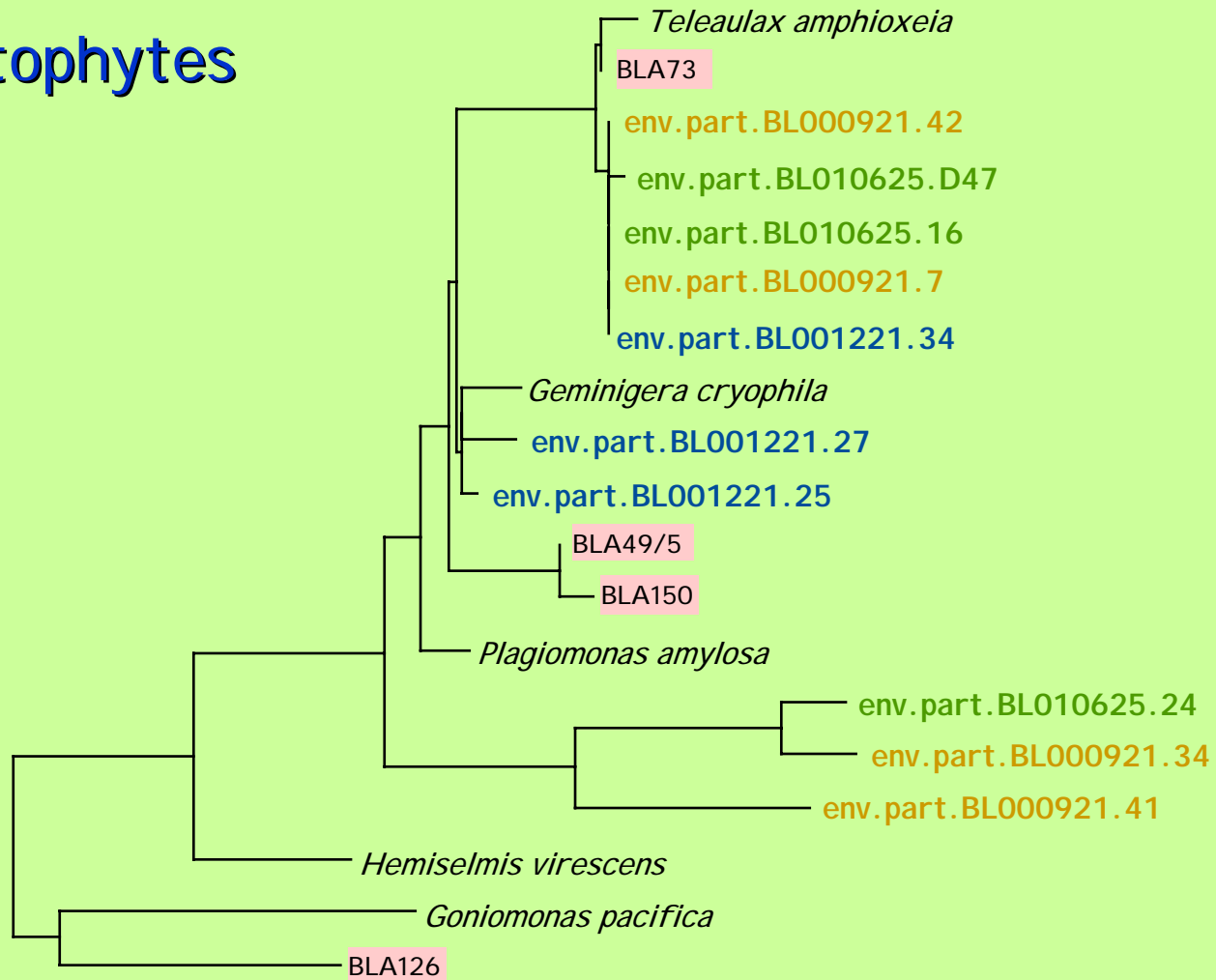
	<u>RFLPs</u>	<u>Clones</u>
BL000921	2	2
BL001221	3	8
BL010320	2	4
BL010625	3	4
TOTAL	10	18

Novel alveolates-I I

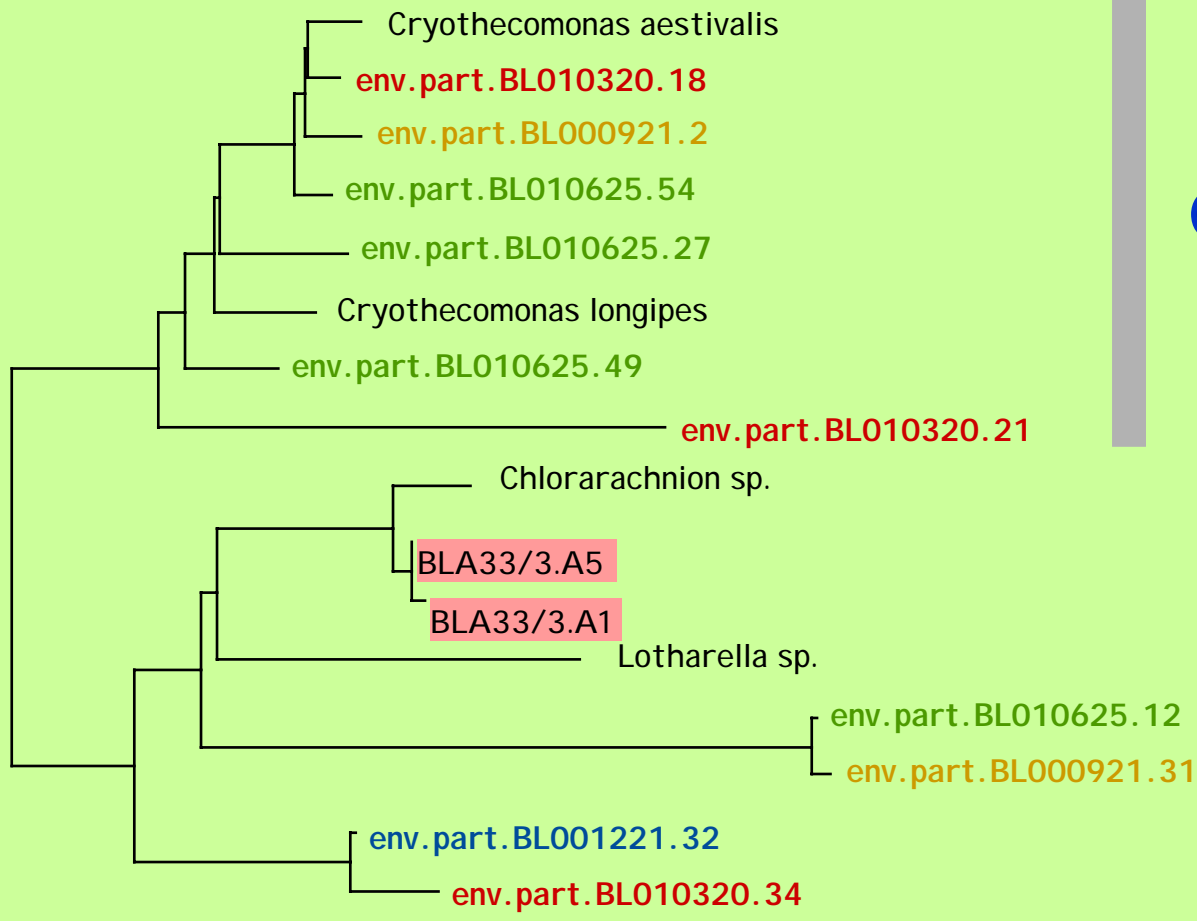


	<u>RFLPs</u>	<u>Clones</u>
BL000921	1	1
BL001221	4	5
BL010320	1	1
BL010625	1	10
TOTAL	7	17

Cryptophytes



	<u>RFLPs</u>	<u>Clones</u>
BL000921	4	5
BL001221	3	4
BL010320	0	0
BL010625	2	5
TOTAL	9	14



Cercozoans

Chlorarachniophytes

Cercozoans

RFLPs

Clones

BL000921
BL001221
BL010320
BL010625
TOTAL

1
0
2
3
6

1
0
3
5
9

Chlorarachniophytes

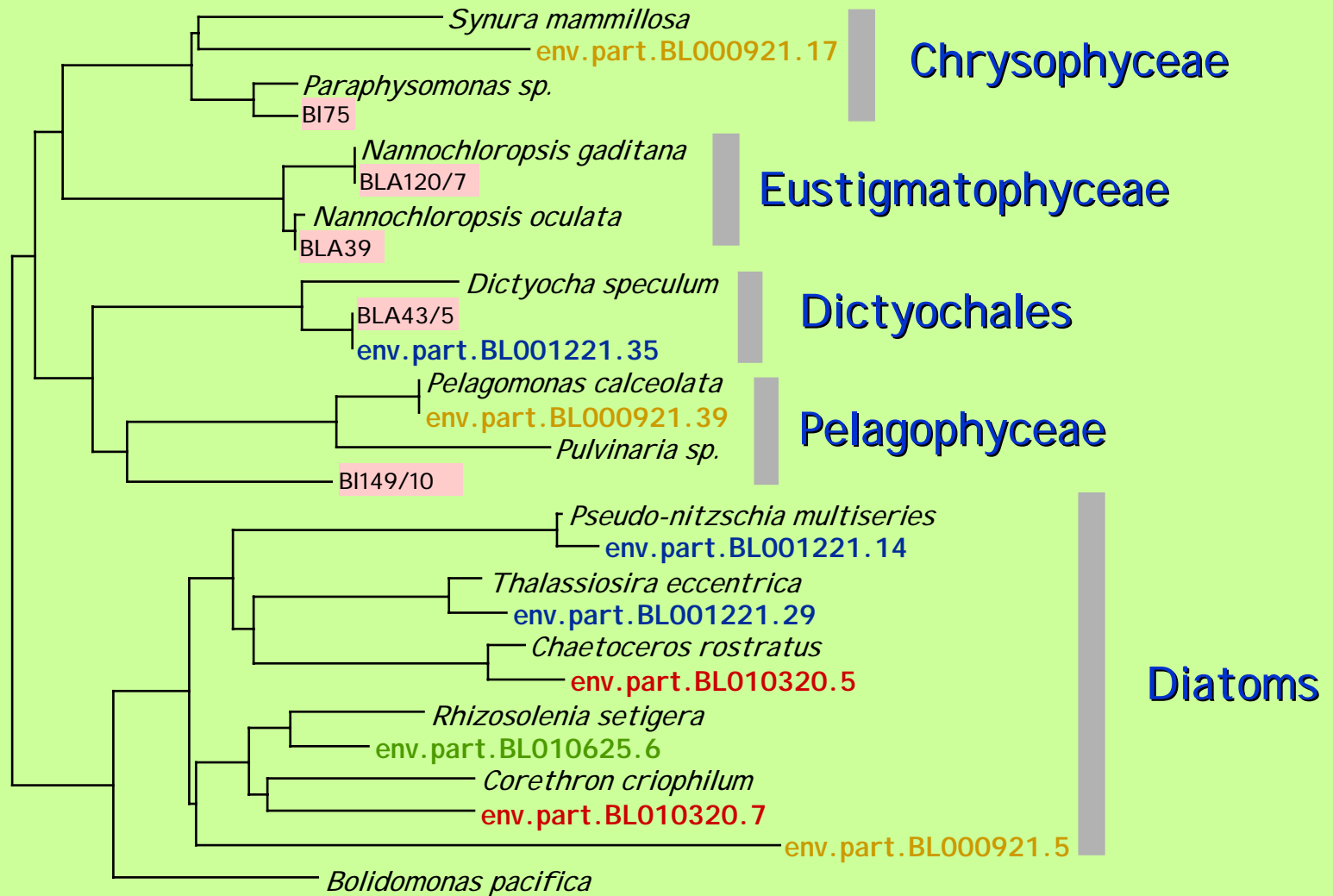
RFLPs

Clones

BL000921
BL001221
BL010320
BL010625
TOTAL

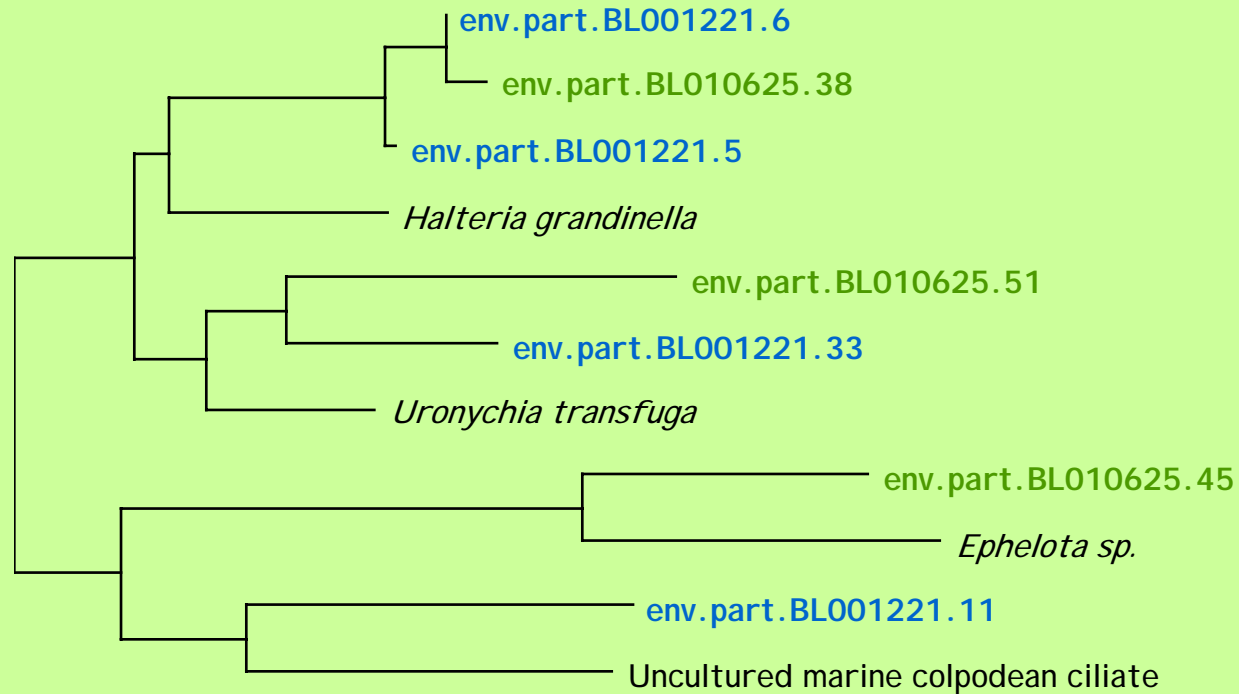
1
1
1
1
4

1
2
1
5
9



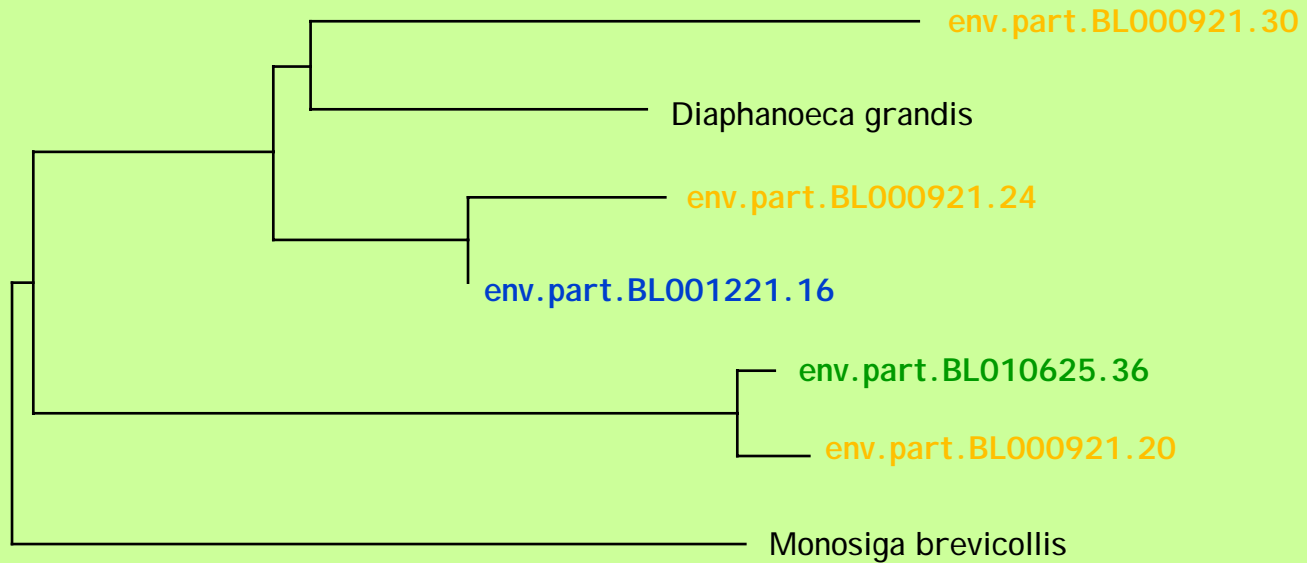
	<u>RFLPs</u>	<u>Clones</u>
BL000921	3	3
BL001221	3	5
BL010320	2	2
BL010625	1	1
TOTAL	9	11

Ciliates



	<u>RFLPs</u>	<u>Clones</u>
BL000921	0	0
BL001221	4	4
BL010320	0	0
BL010625	3	4
TOTAL	7	8

Choanoflagellates



	<u>RFLPs</u>	<u>Clones</u>
BL000921	3	3
BL001221	1	1
BL010320	0	0
BL010625	1	1
TOTAL	5	5

Summary

Very large genetic diversity of picoeukaryotes

Novel alveolates-I dominate year round in Blanes libraries

Large variability during the year

Few populations are found in different libraries

Some groups are well represented by cultures