

Synechococcus Diversity and Probe Development

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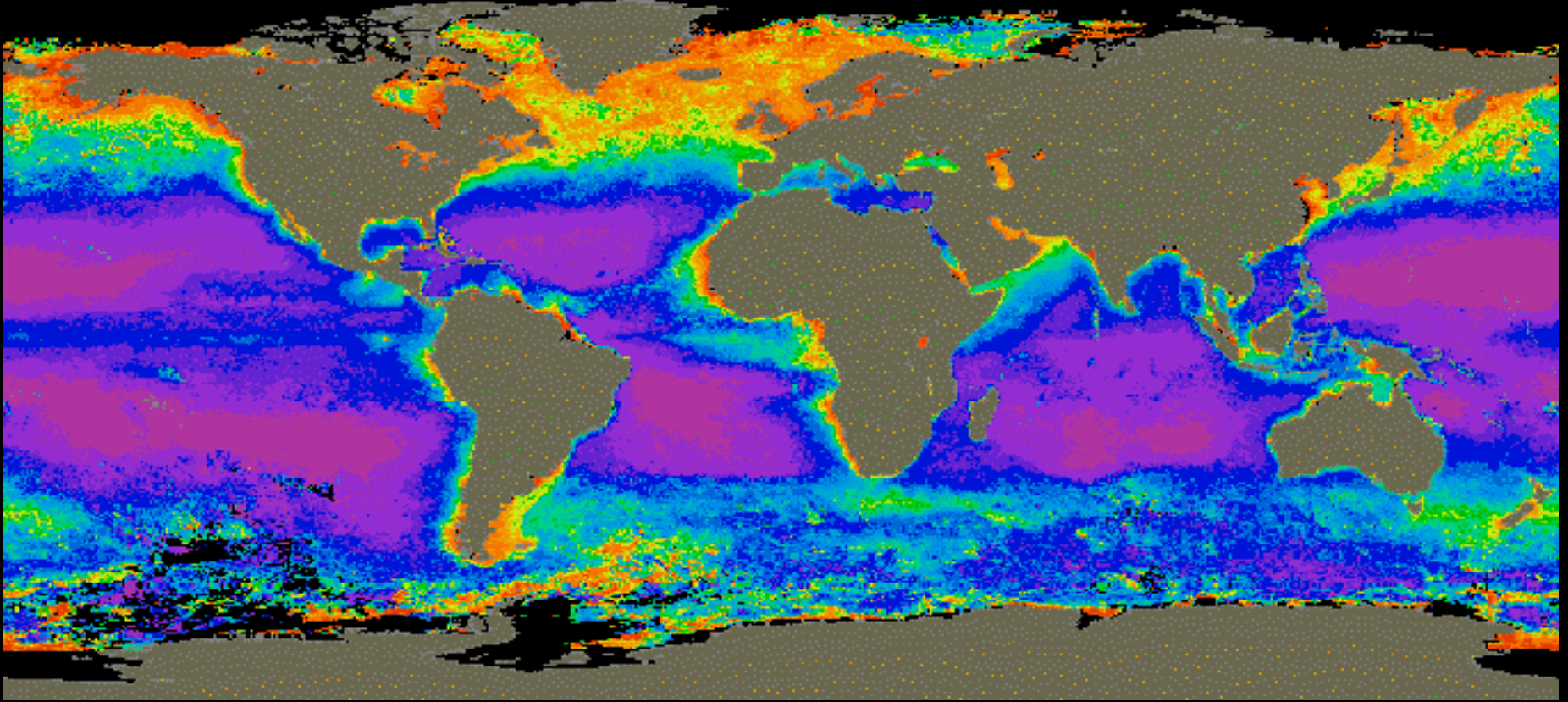


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Marine *Synechococcus*



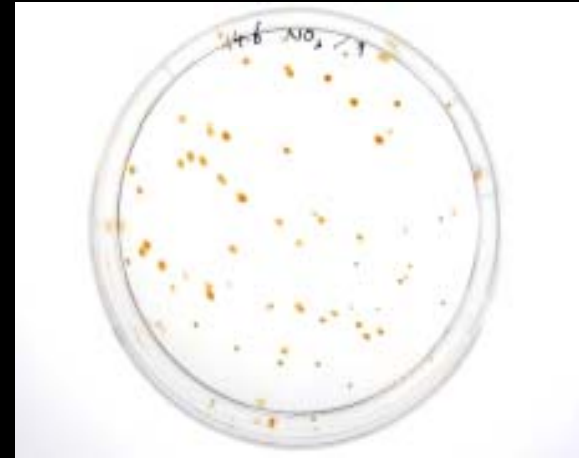
- Global distribution
- Mesotrophic-oligotrophic
- $10^4 - 10^5$ cells ml^{-1}
- Throughout euphotic zone
- Significant contribution to marine C-fixation

Aims

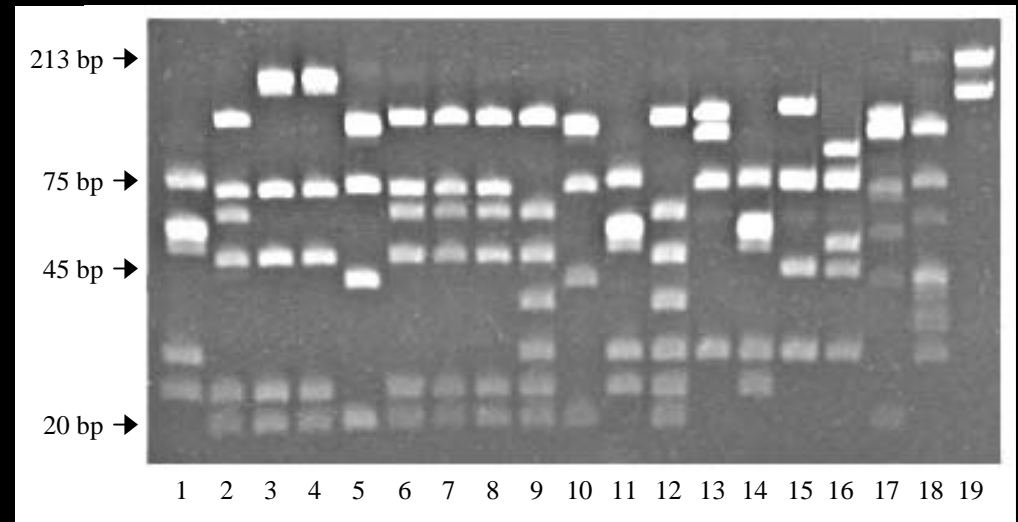
- Determine the diversity of marine *Synechococcus* using 16S rRNA
- Design oligonucleotide probes specific for the different *Synechococcus* clades
- Application of probes to marine systems

Strain isolation

Isolation & purification
on plates (250)



Screening isolates (*ntcA* RFLP)



6 RFLP types; 21 clonal *Synechococcus* strains

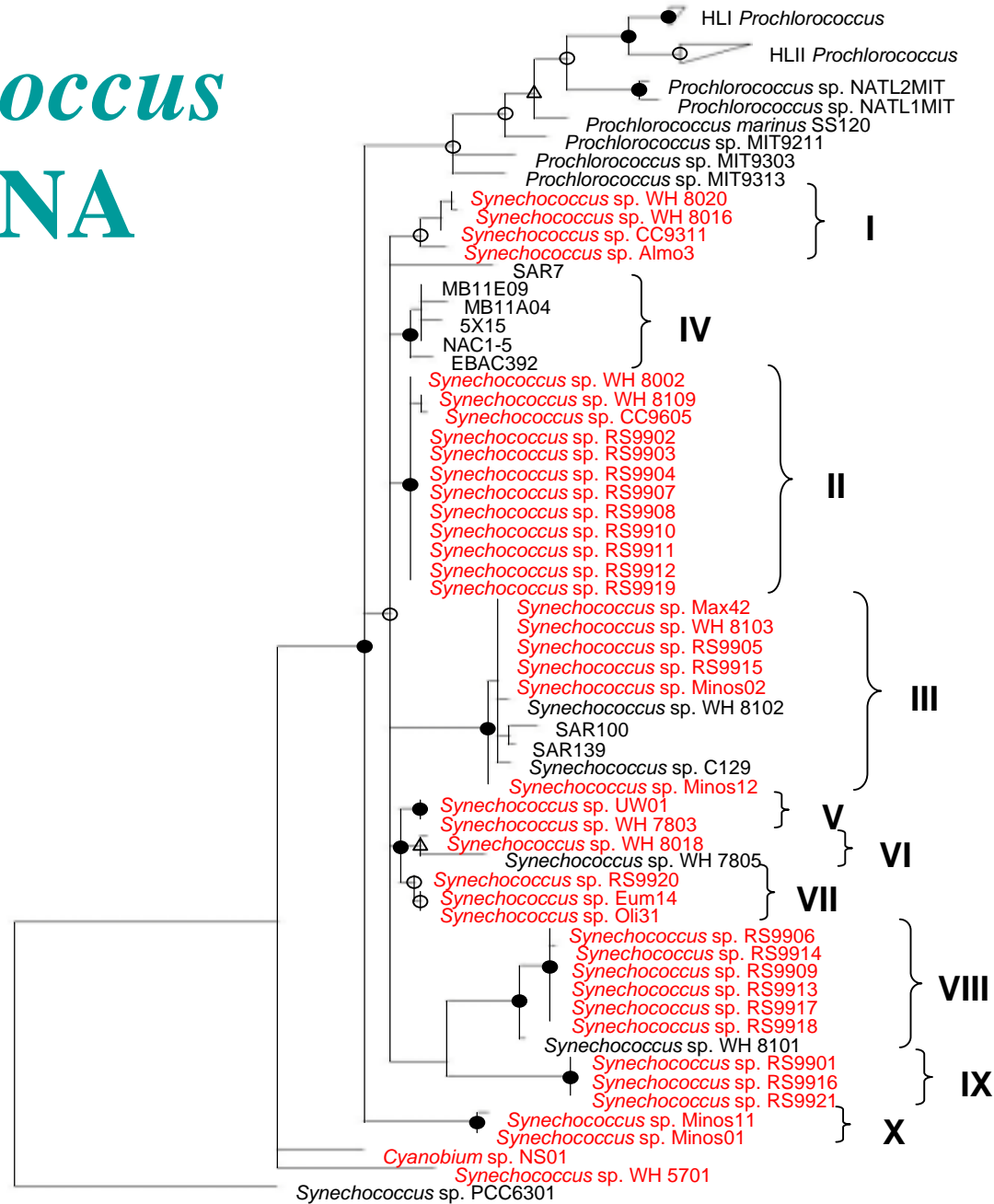
Red Sea *Synechococcus* strains

Strain	Depth m	Date of Isolation	PUB/PEB ratio	Obligately marine	Growth on N source	ntcA RFLP type
RS9901	1	29.3.99	0.7	+	NO ₃ ⁻ /NH ₄ ⁺	I
RS9902	1	29.3.99	1.4	+	NO ₃ ⁻ /NH ₄ ⁺	II
RS9903	10	11.5.99	2.0	+	NO ₃ ⁻ /NH ₄ ⁺	III
RS9904	10	14.6.99	1.5	+	NO ₃ ⁻ /NH ₄ ⁺	III
RS9905	10	18.7.99	1.0	+	NO ₃ ⁻ /NH ₄ ⁺	IV
RS9906	10	23.8.99	NA	-	NO ₃ ⁻ /NH ₄ ⁺	N/A
RS9907	10	23.8.99	0.5	+	NO ₃ ⁻ /NH ₄ ⁺	II
RS9908	10	7.9.99	0.5	+	NO ₃ ⁻ /NH ₄ ⁺	II
RS9909	10	7.9.99	NA	-	NO ₃ ⁻ /NH ₄ ⁺	N/A
RS9910	10	7.9.99	0.5	+	NO ₃ ⁻ /NH ₄ ⁺	II
RS9911	10	11.5.99	0.8	+	NO ₃ ⁻ /NH ₄ ⁺	V
RS9912	10	23.8.99	0.5	+	NO ₃ ⁻ /NH ₄ ⁺	N/A
RS9913	10	7.9.99	NA	-	NH ₄ ⁺	N/A
RS9914	10	18.10.99	NA	-	NH ₄ ⁺	N/A
RS9915	10	18.10.99	0.8	+	NO ₃ ⁻ /NH ₄ ⁺	IV
RS9916	10	22.11.99	0.7	+	NO ₃ ⁻ /NH ₄ ⁺	I
RS9917	10	22.11.99	NA	-	NH ₄ ⁺	N/A
RS9918	10	22.11.99	NA	-	NO ₃ ⁻ /NH ₄ ⁺	N/A
RS9919	50	22.11.99	1.8	+	NO ₃ ⁻ /NH ₄ ⁺	V
RS9920	150	22.11.99	1.7		NO ₃ ⁻ /NH ₄ ⁺	VI
RS9921	150	22.11.99	0.7	+	NO ₃ ⁻ /NH ₄ ⁺	I

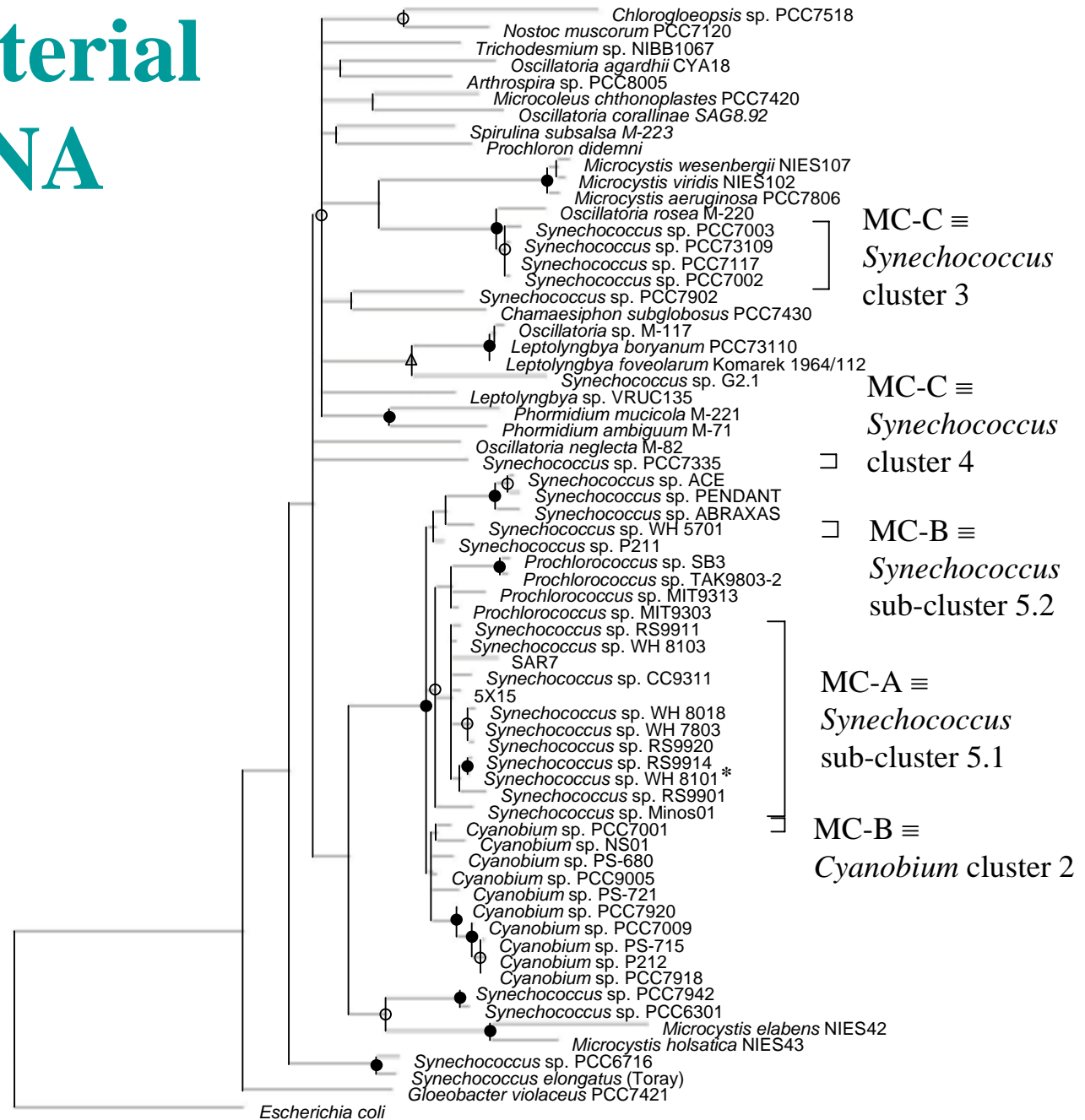
Other *Synechococcus* strains

Strain	Isolation Location	Depth m	Isolation Date	PUB/PEB ratio	Isolated by
Almo3	Mediterranean Sea	0	1.5.91	0.4	Partensky
NS01	central North Sea			NA	Nieuwland
UW01	North Sea			0.5	Peeters
Eum14	Tropical Atlantic	105	1.10.91	2.2	Partensky
Max42	Sargasso Sea	120	9.10.87	1.4	Vaulot & Courties
Minos12	Mediterranean Sea	20	19.6.96	1.8	Vaulot
Minos02	Mediterranean Sea	15	26.5.96	1.6	Partensky
Oli31	Equatorial Pacific	70	11.11.94	1.7	Vaulot
Minos11	Mediterranean Sea	20	19.6.96	0.7	Vaulot
Minos01	Mediterranean Sea	15	26.5.96	0.7	Partensky
53/4	Mediterranean Sea	5	19/9/99		Partensky & LeGall
77/2	Mediterranean Sea	5	20.9.99		Partensky & LeGall
117/	Mediterranean Sea	5	27.9.99		Partensky & LeGall
153	Mediterranean Sea	25	30.9.99		Partensky & LeGall
157/13	Mediterranean Sea	15	30.9.99		Partensky & LeGall
157/21	Mediterranean Sea	15	30.9.99		Partensky & LeGall
BL8	Mediterranean Sea	0	21.9.00		Guillou
C129	Gulf of Aqaba	20	9.93	0.9	Lindell & Post
CC9311	California Current	95	1993	1.16	Palenik
CC9605	California Current		1996	2.1	Palenik
WH5701	Long Island South		1957	NA	Waterbury
WH7803	Sargasso Sea		1.7.78	0.5	Waterbury
WH8002	Gulf of Mexico		15.4.80	0.48	Waterbury
WH8016	Woods Hole		6.80	0.40	Waterbury
WH8018	Woods Hole		6.80	No PUB	Waterbury
WH8020	Sargasso Sea	50	26.6.80	0.78	Waterbury
WH8103	Sargasso Sea		17.3.81	1.3	Waterbury
WH8109	Sargasso Sea		6.81	0.89	Waterbury

Synechococcus 16S rRNA

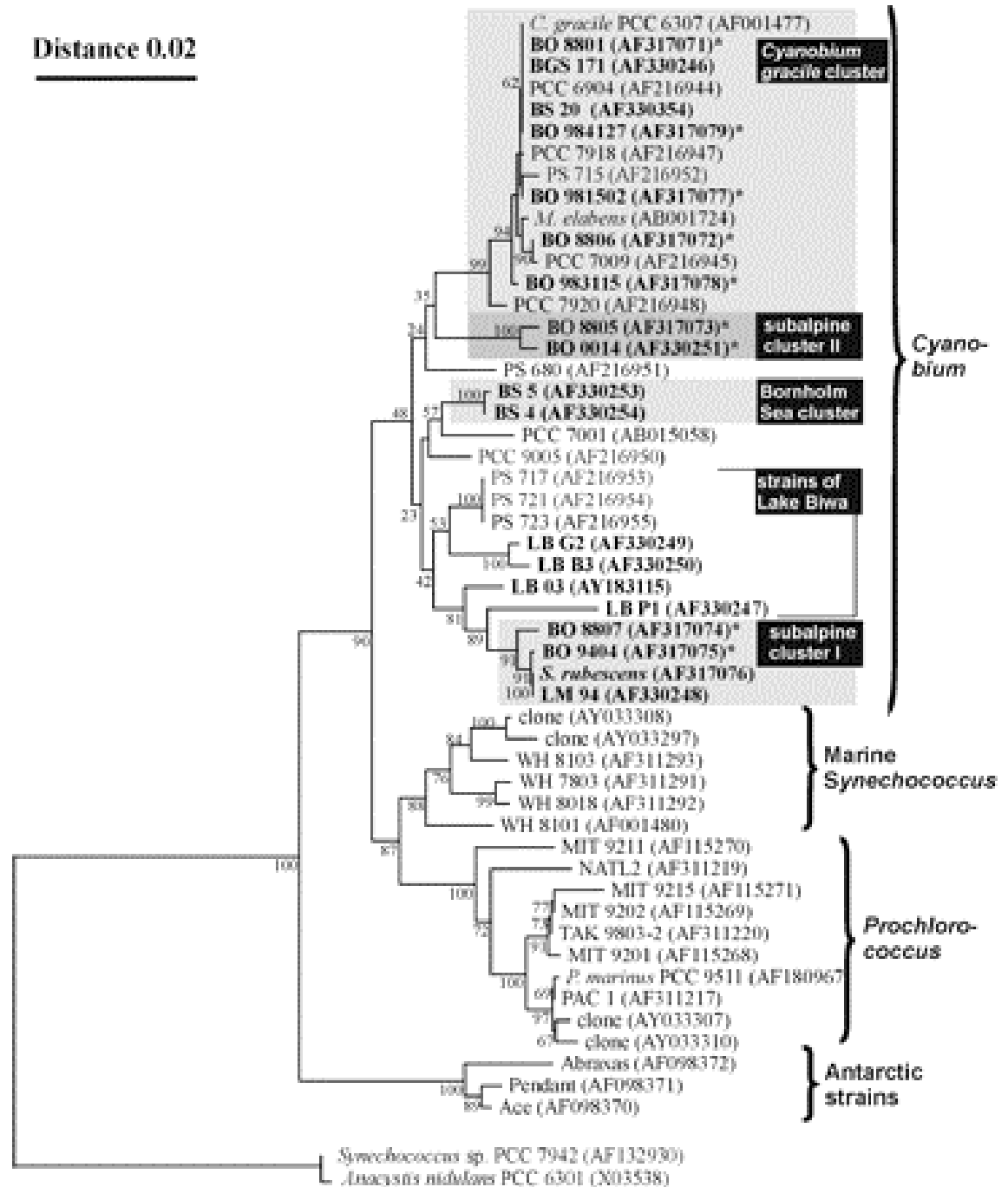


Cyanobacterial 16S rRNA



Cyanobium 16S rRNA

Distance 0.02

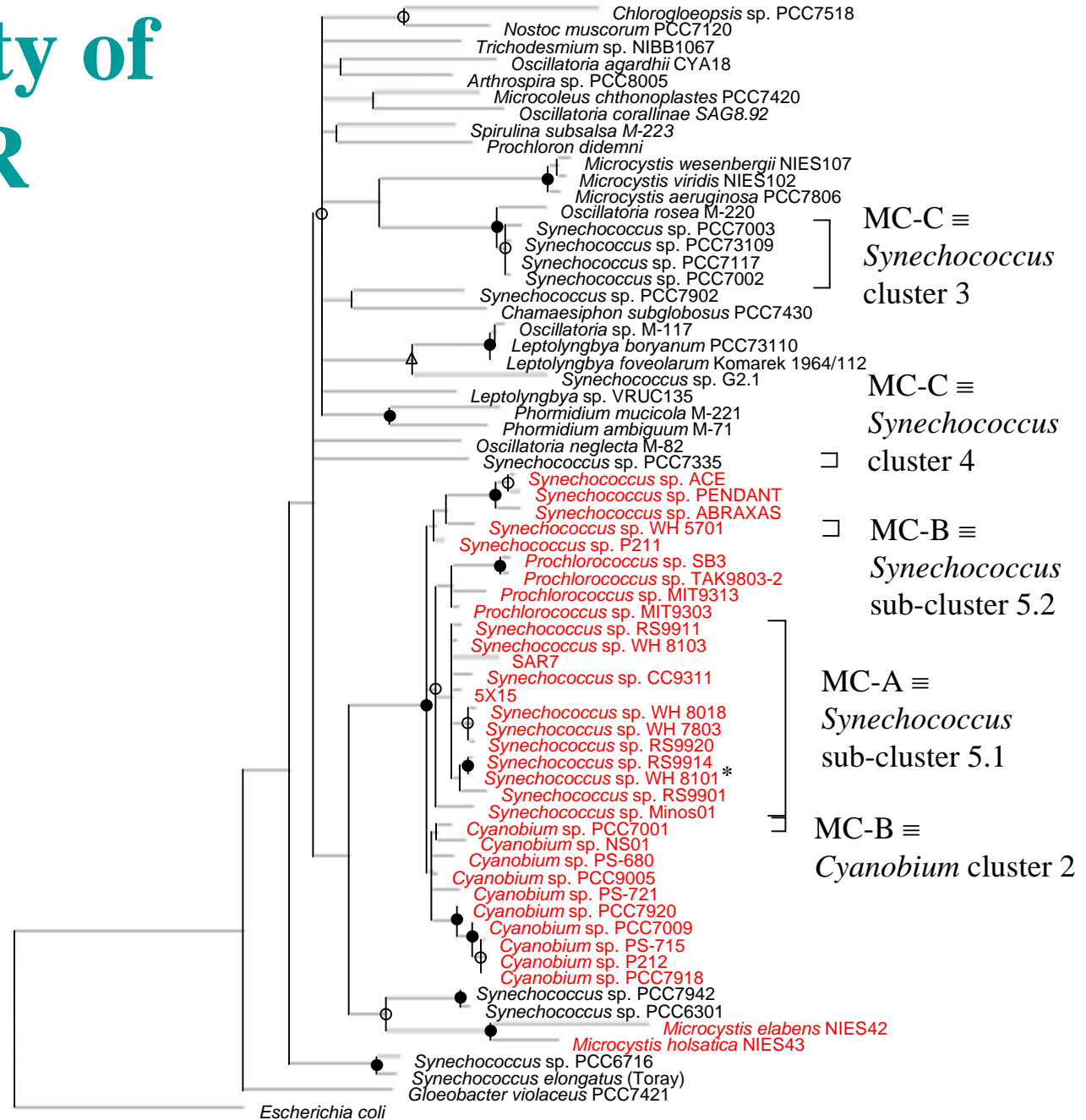


Ernst *et al.* (2003).

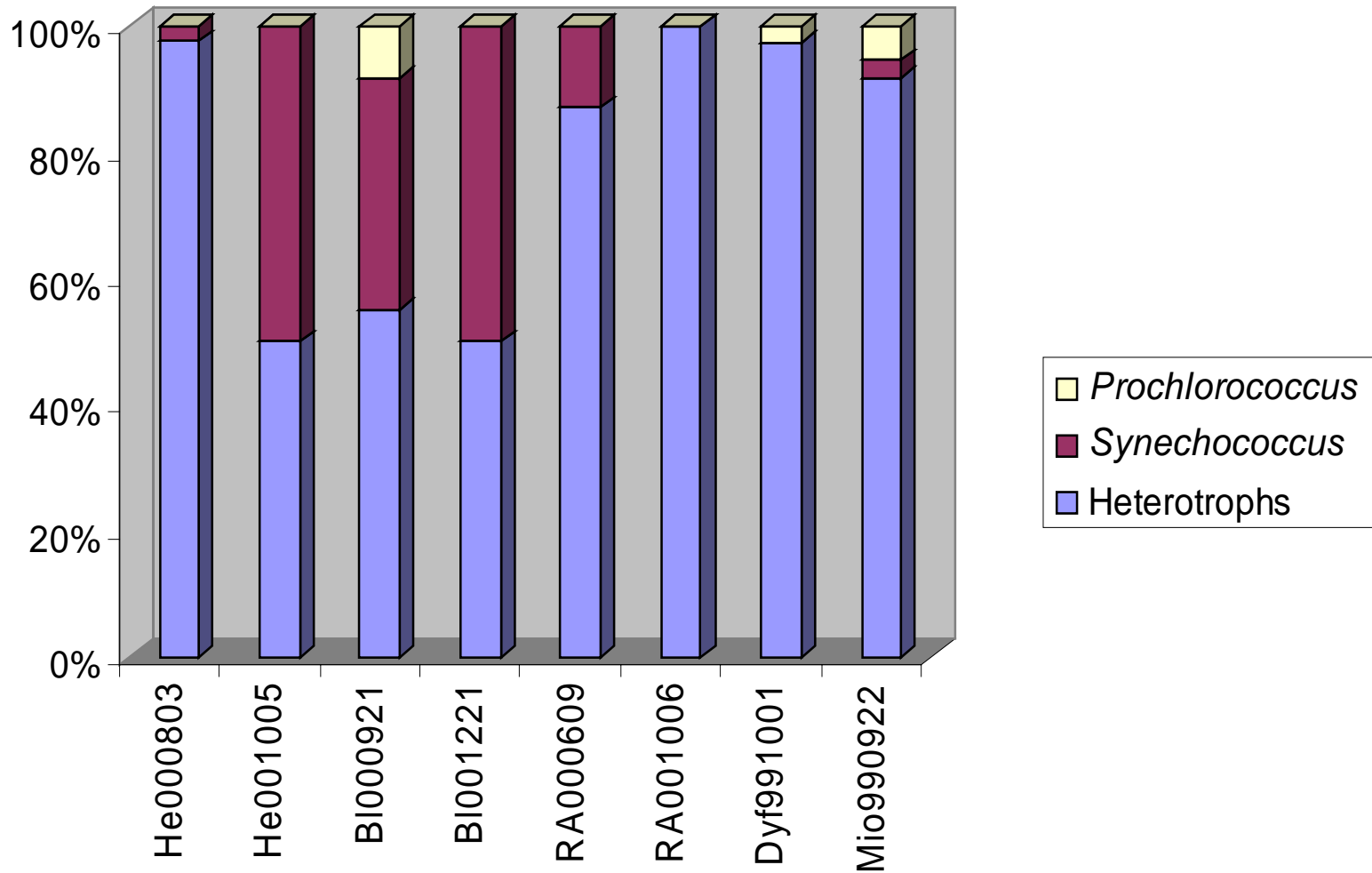
Bacterial clone libraries

- Roscoff RA000609 RA001006
 - Blanes B1000921 B1001221
 - Helgoland He000803 He001005
-
- Constructed using primers 107f and 1038r

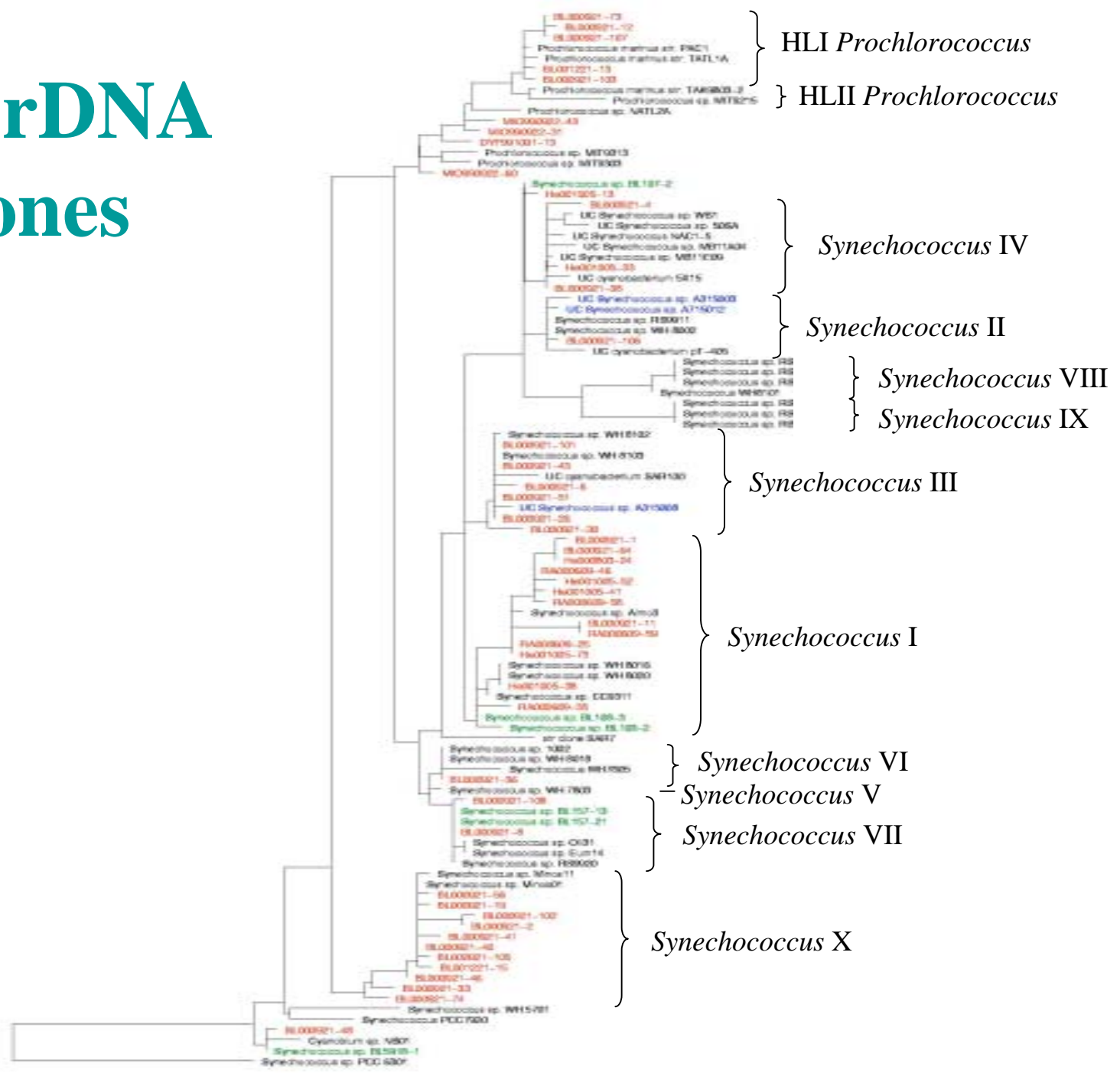
Specificity of 1038R



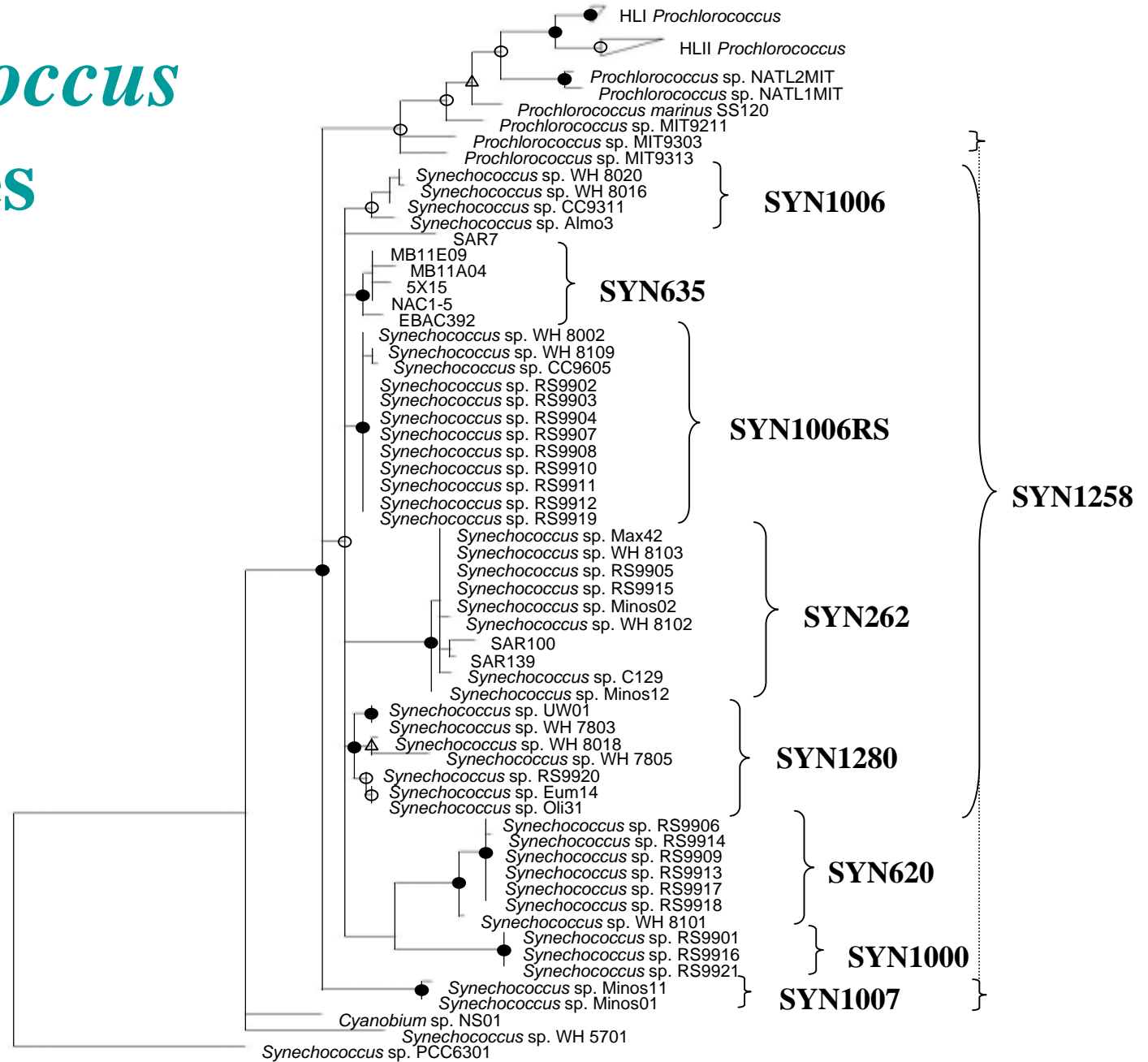
Clone sequence identities



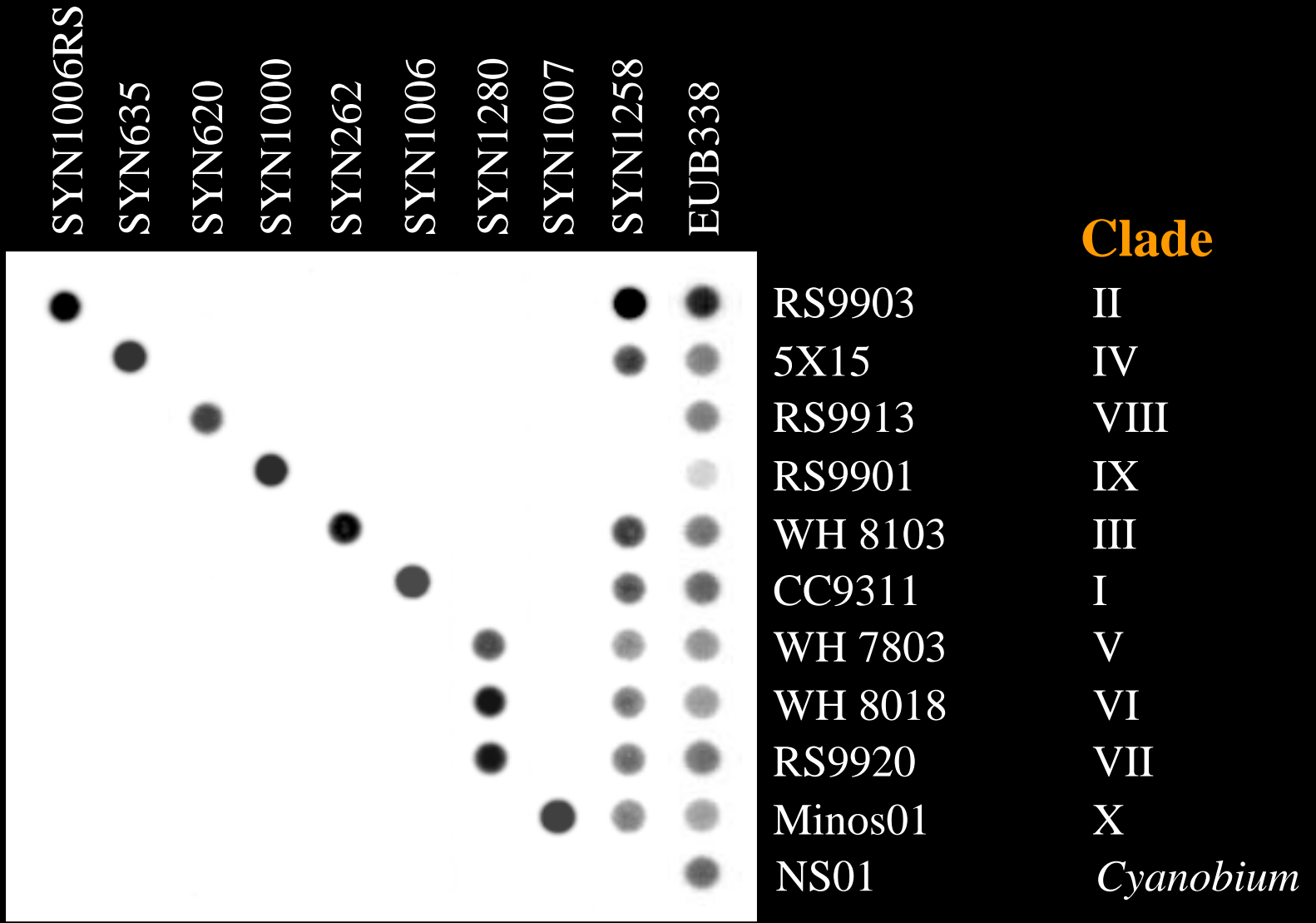
16S rDNA clones



Synechococcus probes



Probe specificity



Clade

- II
- IV
- VIII
- IX
- III
- I
- V
- VI
- VII
- X
- Cyanobium*

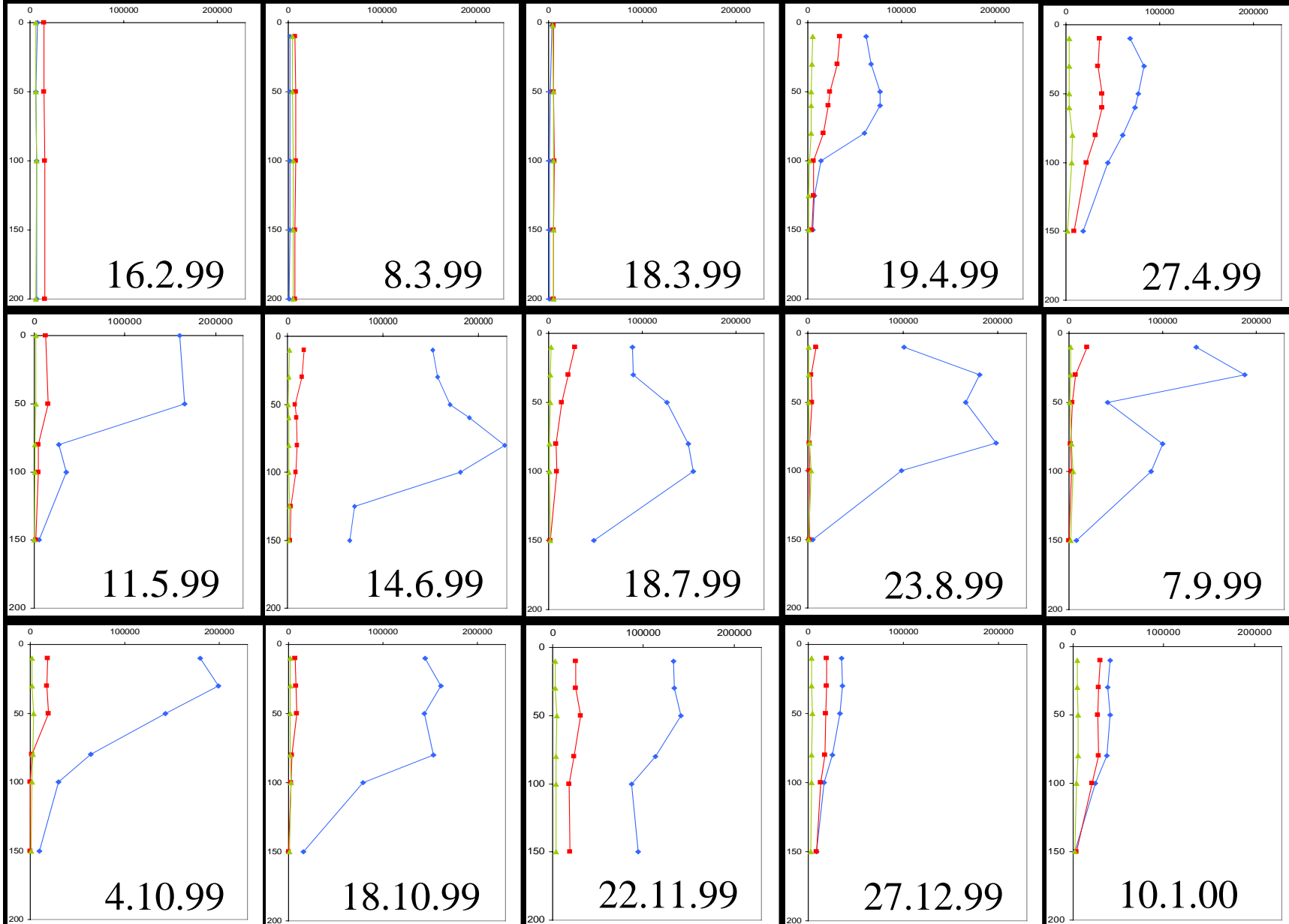
The Gulf of Aqaba



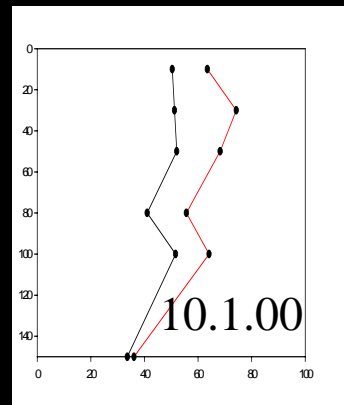
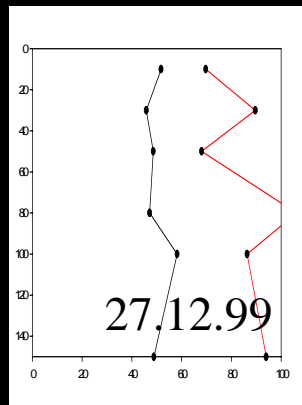
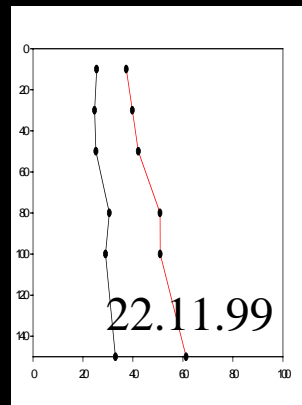
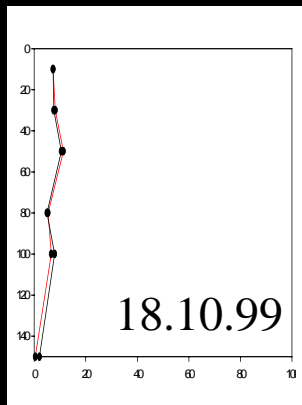
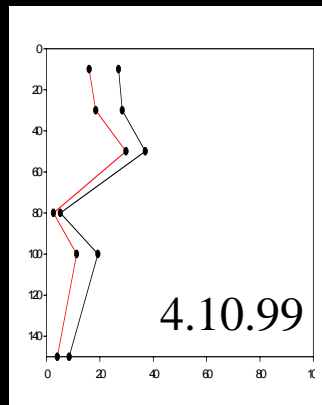
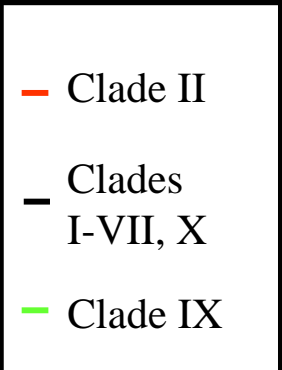
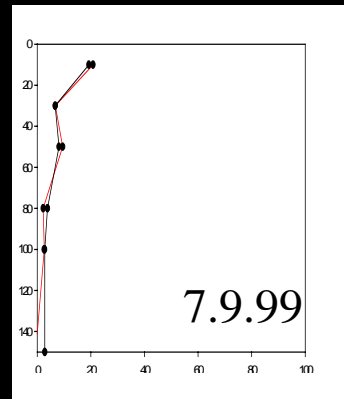
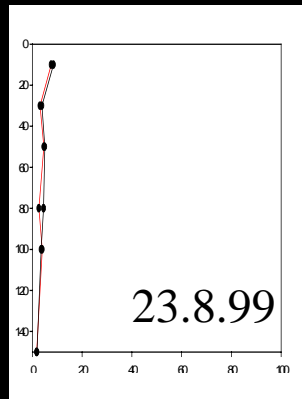
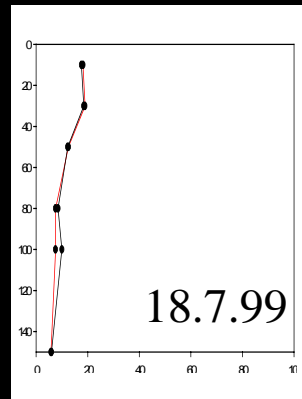
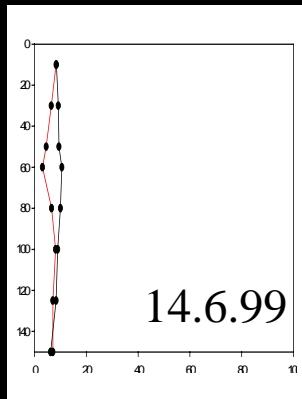
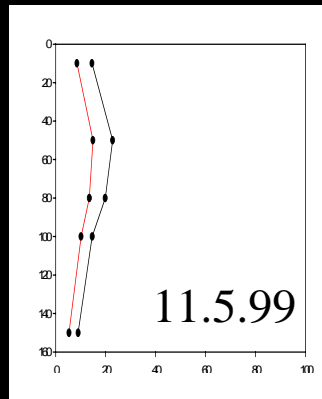
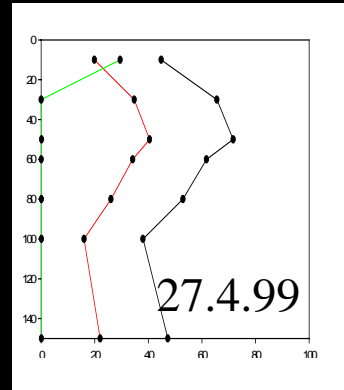
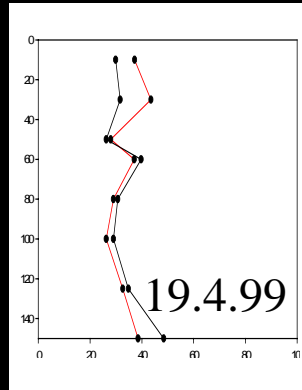
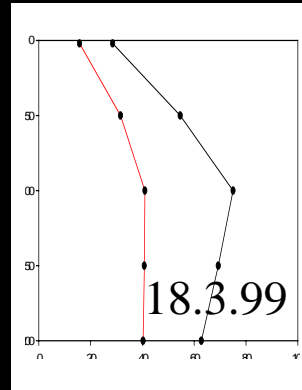
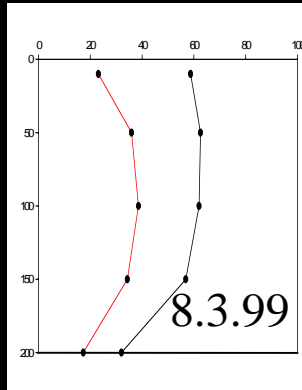
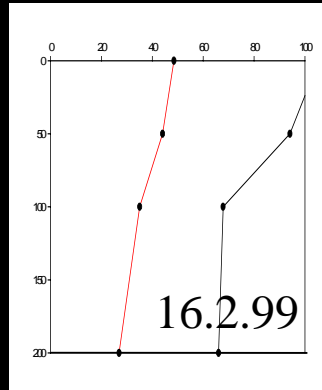
- Section of rift valley, up to 1800 m deep
- Shallow southern sill (250 m)
- No rivers
- Low biomass & chl *a* → deep light penetration
- Strong seasonal changes



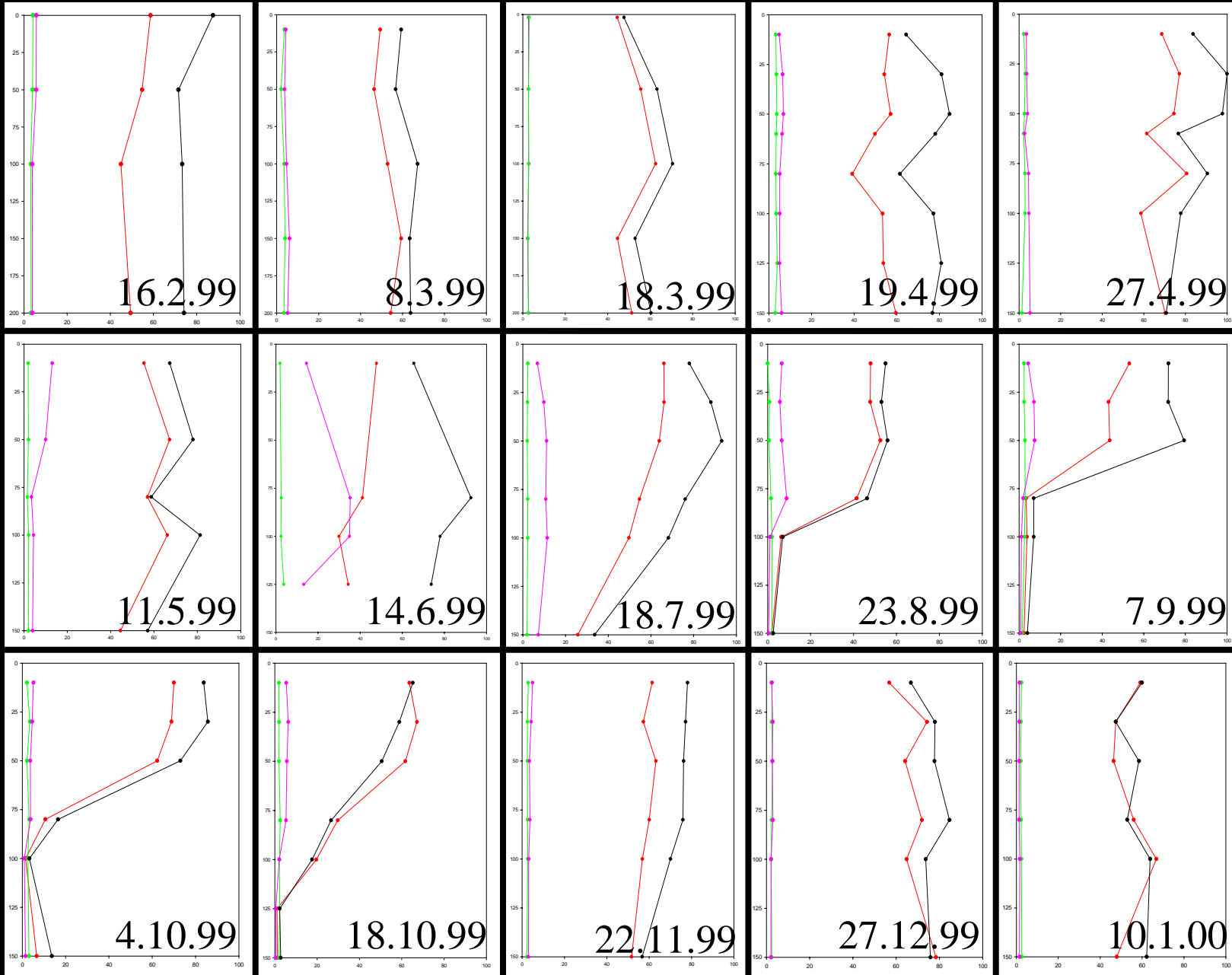
Cell counts



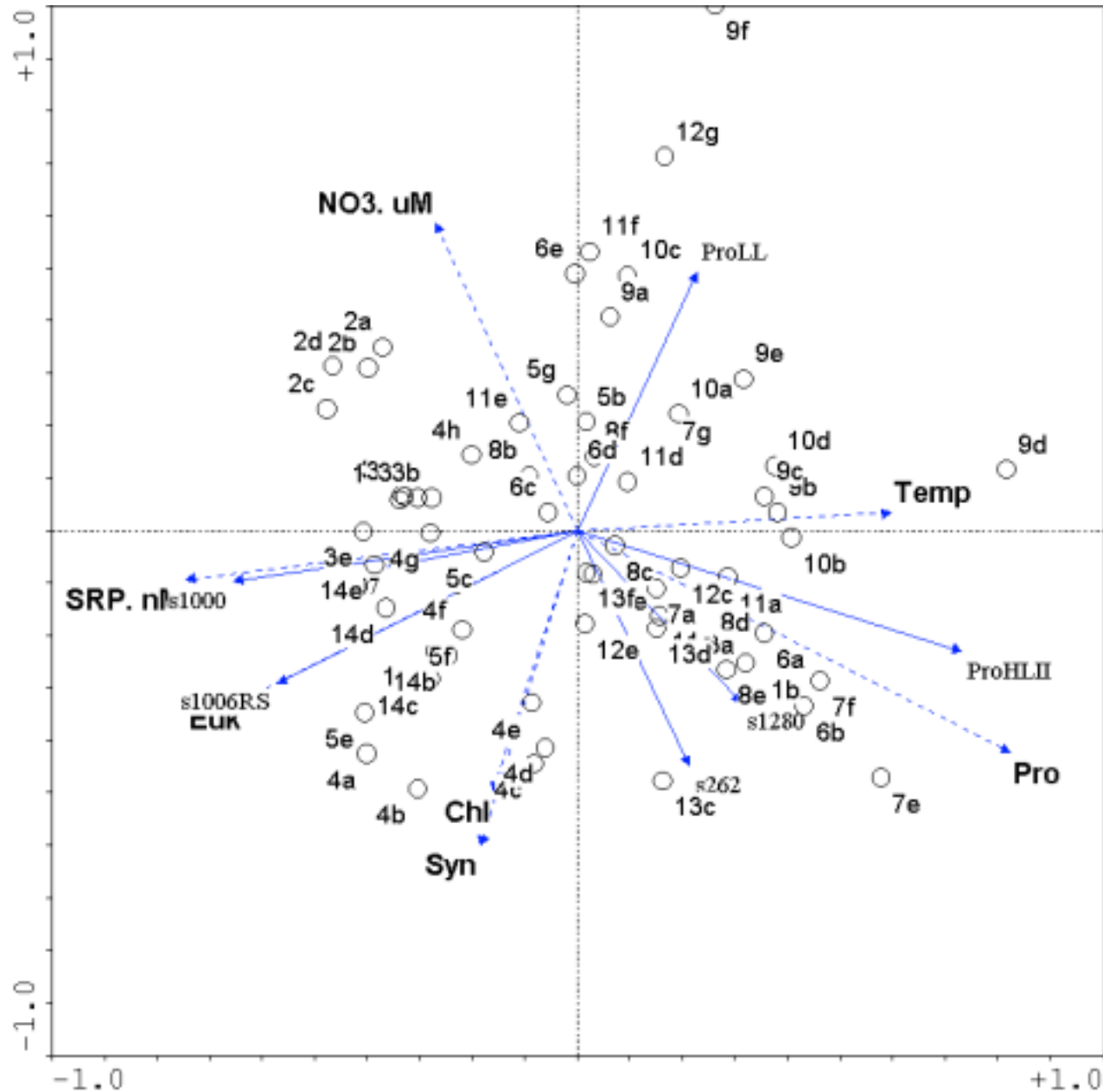
Synechococcus (107F)



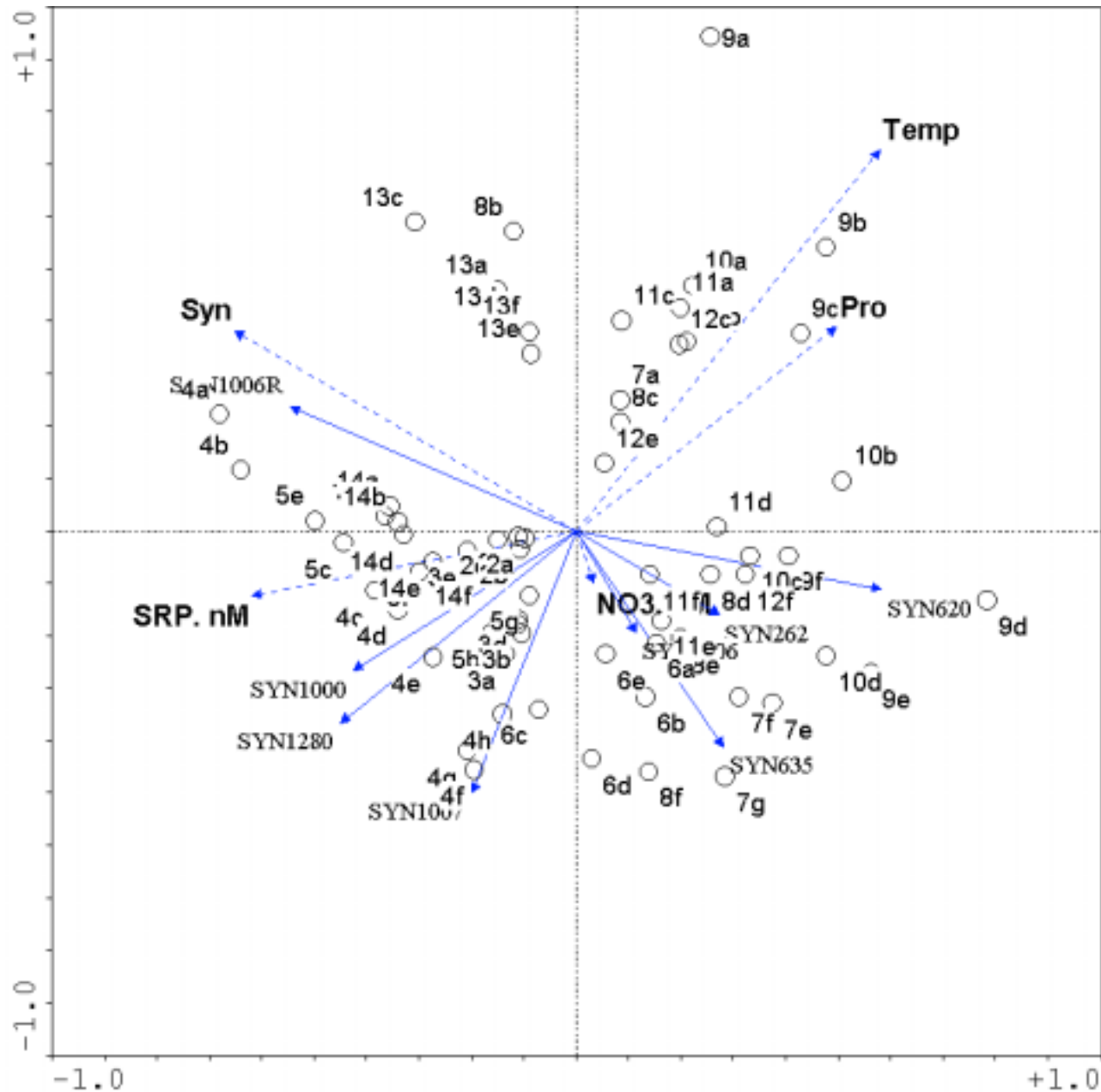
Synechococcus (172F)



Multivariate analysis – 107F



Multivariate analysis – 172F



Summary

- *ntcA* RFLP provided a rapid method for screening *Synechococcus* isolates
- 16S rDNA sequences identified 10 distinct clades of MC-A *Synechococcus*, including 3 novel clades
- Coastal libraries identified *Synechococcus* clones from 7 of the 10 clades, but no new clades
- Clade-specific probes showed the predominance of a single *Synechococcus* clade (II) in the Red Sea over an annual cycle
- Correlations between *Synechococcus* clades and environmental variables have been initiated by multivariate statistical analyses

Acknowledgements

Roscoff:

Dominique Marie -
Fred Partensky -

Flow cytometry

Flow cytometry

Synechococcus isolates

Daniel Vaultot -

Synechococcus isolates

Laure Guillou -

Synechococcus isolates

Eilat:

Asaph Rivlin -

STD data

Tanya Rivlin -

Nutrient analysis

Members of Post lab