

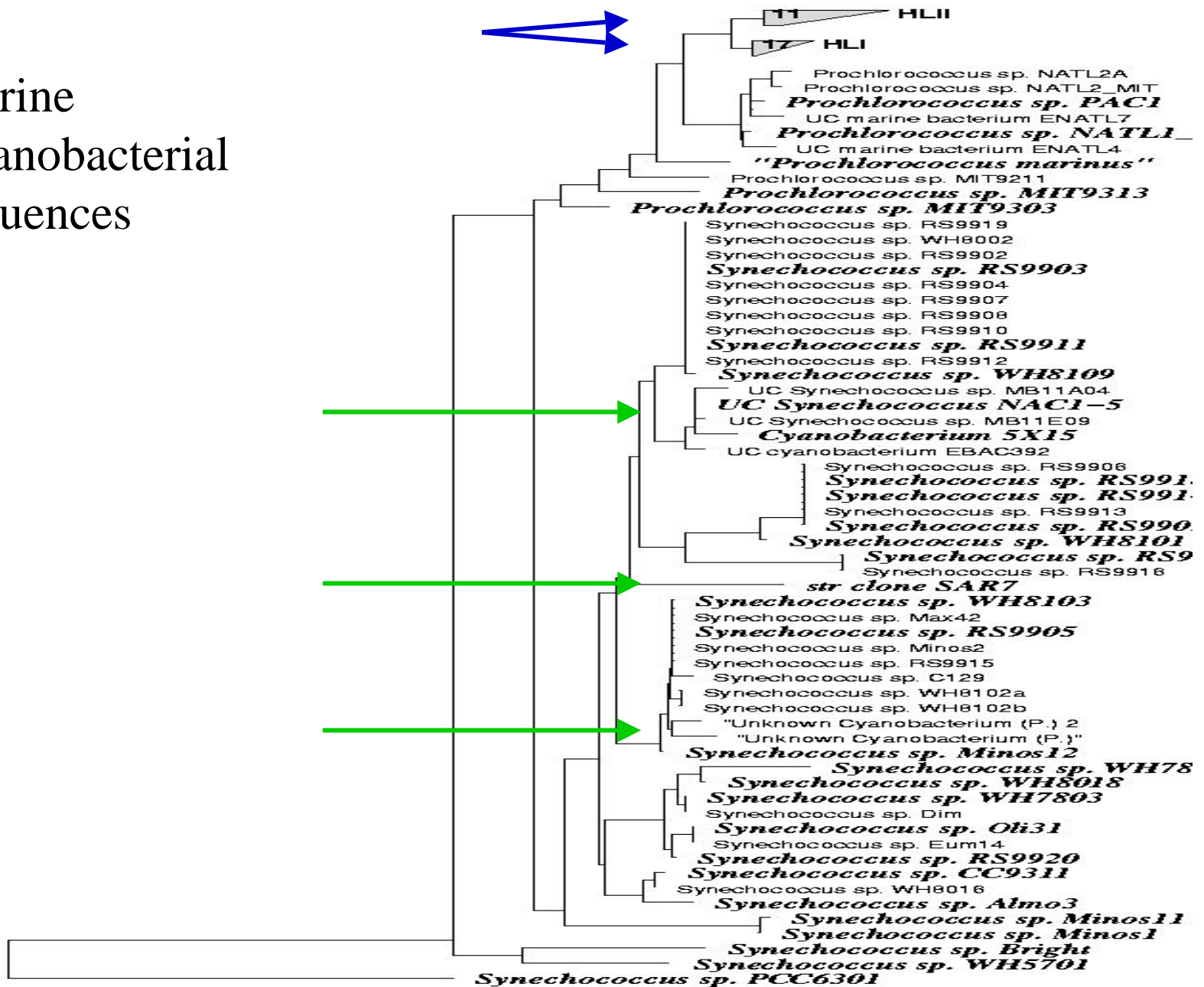
Analysis of Marine Cyanobacterial 16S rRNA Genes from European Coastal Environments

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Specific Aims

- Acquire picocyanobacterial SSU rDNA sequences from both cultures and natural samples
- Construct clone libraries to investigate picocyanobacterial diversity directly from *in situ* samples
- Develop clade-specific oligonucleotide probes recognising each picocyanobacterial group
- Utilise these probes by dot-blot hybridisation or FISH technology to assess the distribution temporally and spatially at three European coastal sites

Marine Cyanobacterial sequences



Oligonucleotide primers and probes used in dot blots analysis (and DGGE)

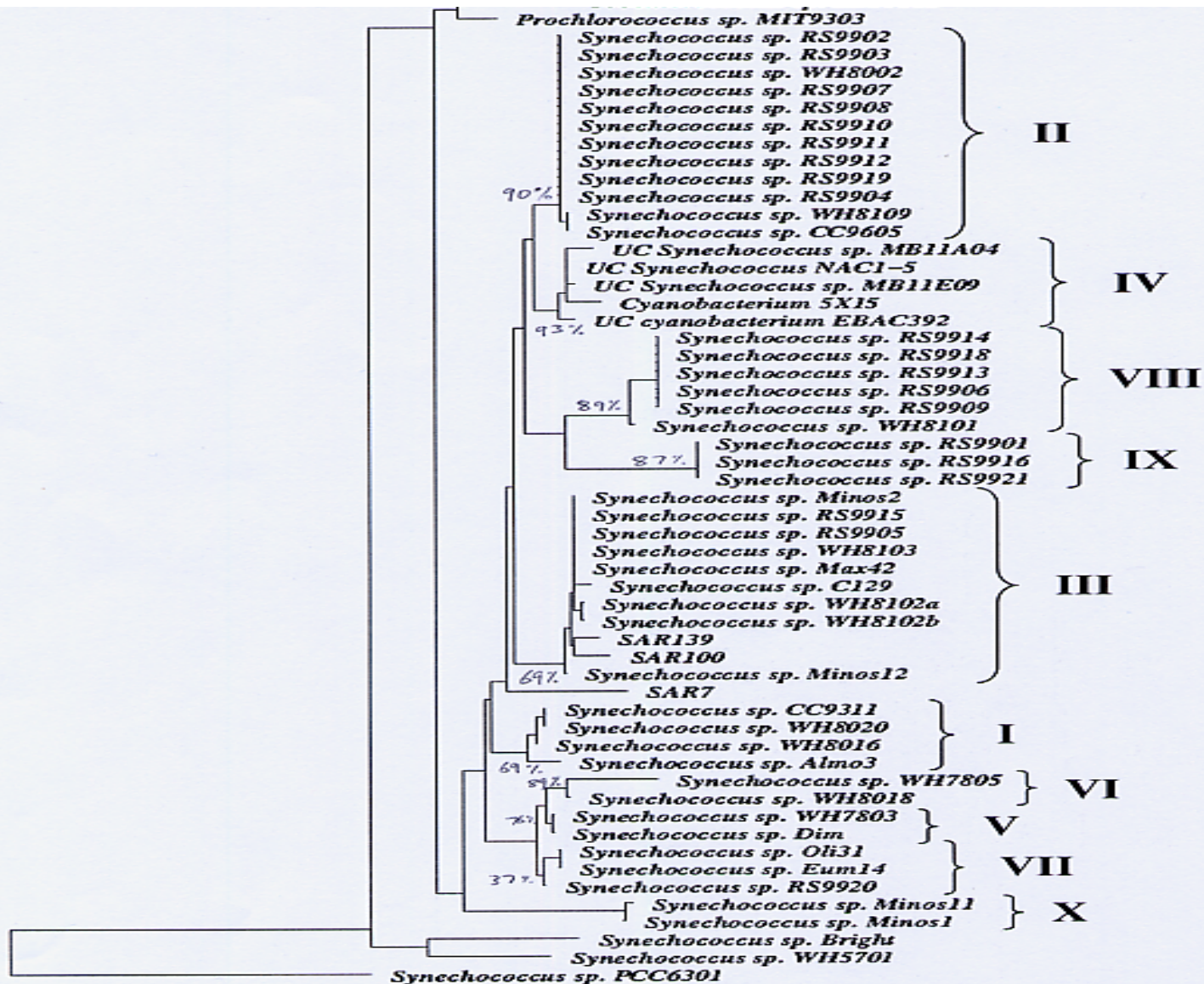
PCR Primers	Specific target DNA	Sequence 5'-3'
OXY107F	Oxygenic phototroph biased	GGA CGG GTG AGT AAC GC GTG R
OXY1313R	Oxygenic phototroph biased	CTT CAC GTA GGC GAG TTG CAG C
CYA359F	Oxygenic phototroph specific	GGG GAA TYT TCC GCA ATG GG
PRO1017R	<i>Prochlorococcus</i> , excluding MIT9303, MIT9313, MIT9302	TCC CGA AGG CAC CCT CWA AA
27F	Eubacterial	AGA GTT TGA TCM TGG CTC AG
1224R	Eubacterial	CAT TGT AGC ACG TGT GTA

Oligonucleotide Probes	Specific target DNA	Sequence 5'-3'
S1PRO634R	HLI <i>Prochlorococcus</i>	GCC GAT CAG TTT CCA CTG
S2PRO634R	HLII <i>Prochlorococcus</i>	GCC TTT CAG TTT CCA CTG
DPRO634R	LL <i>Prochlorococcus</i> (except SS120)	GCC AAT CAG TTT CCA CTG
SARG634R	SS120	GCC CTT CAG TTT CCA CTG
MIT1023R	MIT9303	TGC GTT CCC AAA GGC ACT
EUB338	Eubacteria	GCT GCC TCC CGT AGG AGT
PRO444R	<i>Prochlorococcus</i> , excluding MIT9303, MIT9313	TAT TCC TCA AGT ACC GTC ATA
MAR572R	Marine picophytoplankton clade biased	GCC GCC TGC GGA CGC TTT

Cyanobacterial-specific oligonucleotide probes.

Probe	<i>E. coli</i> posit.	T_d, °C	Probe Sequence 5'-3'	<i>Synechococcus</i> clade
SYN1006	1006	44	CTC TCA AGT TTC CAA GAG	I (SynCC9311 et al.)
SYN1006RS	1006	42	CTC TCC TGT TTC CAA GAG	II (Syn RS9902 et al.)
SYN262	262	53	GAT GCC TTG GTA GGC CTT	III (SynWH8103 et al.)
SYN635	635	50	AAG CCC CTC AGT TTC CAC	IV (5X15 et al.)
SYN1280	1280	50	GAG CCA CGG TTT ATG AGA	V, VI & VII (SynWH7803, SynWH7805 & SynRS9920 et al.)
SYN620	620	51	CAC TGC CAC GAT GGA GTT	VIII (SynRS9918 et al.)
SYN1000	1000	45	GGT TTC CCA GAA ATT CGC	IX (SynRS9901 & SynRS9916)
SYN1007	1007	48	ACC CTC CGG TTT CCC AGA	X (SynMinos 11 et al.)
SYN1258	1258	47	TTG TCC TCG CGA ACT TGC	I - X (not VIII & IX) All marine <i>Synechococcus</i> except RS9918 et al. & RS9901 & RS9916; no <i>Prochlorococcus</i> except MIT9303

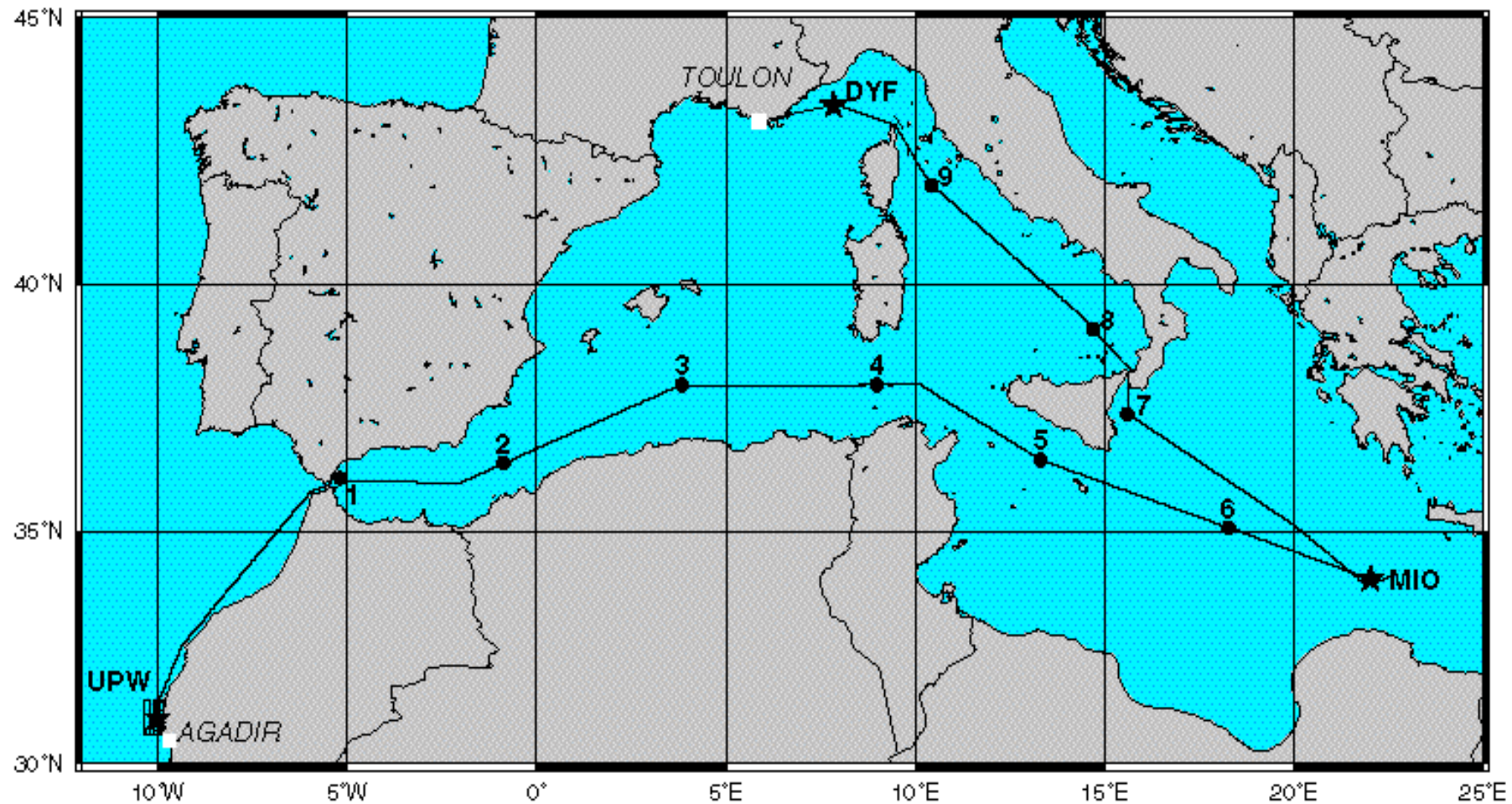
Cyanobacterial 16S rDNA phylogeny & clade-specific probes



Full length clone libraries generated : pA (8-28) pHr (1542-1522)

Site	Primers	Identifier
HE000803	pA/pHr	He (H) 25/02/01
Mio (Prosope cruise)	pA/pHr	Mio (M) 25/02/01
Dyf (Prosope cruise)	pA/pHr	Dyf (D) 25/02/01

Prosopé cruise track



Analysis of full length environmental clone libraries

Sample Name	DYF	MIO	Helgoland
Number of clones	70	70	75
% Containing Insert	95.7	98.6	98.6
% Amplified with Primer couple 359F/1313R (oxygenic-phototroph specific)	72.9	72.9	61.3
% Amplified with Primer couple 359F/1038R (1038r amplifies all marine <i>Syns</i> , <i>Pros</i> , ACE/PS680 cluster, CC9005 = marine cyano-specific)	14.3	22.9	9.3

Sequence analysis of Cyanobacterial/plastid sequences from full length clone libraries

Sequence	Closest BLAST match	identity
DYF13	<i>Prochlorococcus</i> ENATL4, PAC1	99%
DYF33	<i>Prochlorococcus</i> ENATL4, PAC1	99%
HE24	env.SAR7; <i>Synechococcus</i>	
MIO12	<i>Prochlorococcus</i> MIT9211	99%
MIO31	<i>Prochlorococcus</i> ENATL4, PAC1	99%
MIO43	<i>Prochlorococcus</i> ENATL4, PAC1	99%
MIO60	<i>Prochlorococcus</i> MIT9211; MIT9313	99%
DYF18	env. Plastid seqs. OCS20; OM283 (cryptomonad)	99%
DYF25	env. Plastid seqs. OCS50, OM153; (Haptophyte)	97%
DYF38	<i>Skeletonema pseudocostatum</i> , uncult. Bacillariophyte env. Plastid seq. Diatom	96%
DYF45	<i>Chrysochromulina polyepsis</i> , env. Plastid OCS31; HE3	98%
HE3	<i>Chrysochromulina polyepsis</i> , env. Plastid OCS31; DYF45	98%
HE12	env. Seq. OCS50; <i>E. huxleyi</i> plastid	100%
HE17	env. Plastid seq. OM81; <i>Ochromonas</i> sp. plastid	93%
MIO26	env. Plastid OCS31, OM21;	98%

overhead

Analysis of 107F-1038R environmental clone libraries

Site	Total no. of clones	No. of unique RFLP types (<i>Hae</i> III + <i>Eco</i> RI)	No. of unique clones	Coverage (%)
HE000803	67	8	5	92.5
HE001005	100	34	24	76.0
RA000609	80	20	11	86.25
RA001006	72	13	7	90.3
BL000921	82	13	8	90.2
BL001221	82	16	9	89.0

Results of PCR-screening clone libraries for potential DNA sequence data

Clone Library	Clones 107/1038 positive	% of clones 359/781 positive	N° of potential DNA sequences
He 000803	71	82	58
He 001005	100	62	62
B1 000921	80	50	40
B1 001221	82	28	23
RA 000609	73	11	15
RA 001006	80	93	74
Total	486		272

Phylogeny of Cyanobacterial 16S rDNA retrieved from Marine Environment

