

Phylogenetic Distributions of Phycocyanin-Rich *Synechococcus* spp. in the East China Sea in Summers

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Abstract

Synechococcus spp. have been suggested as the primary component of picophytoplankton in the East China Sea (ECS). However, the influences of sudden environmental changes on *Synechococcus* assemblage composition have not yet been investigated. In the summer of 2010, a disastrous flood occurred in the Changjiang River basin. To improve understanding of how this flood affected the *Synechococcus* ecology on the ECS surface, their assemblages and distributions have been described using 2-laser flow cytometry and phylogenetic analysis of the phycocyanin operon. During the non-flooding summer of 2009, phycoerythrin-rich (PE-rich) *Synechococcus* thrived near the outer boundary of the Changjiang River diluted water (CDW) coverage, while phycocyanin-rich (PC-rich) *Synechococcus* predominated inside the turbid CDW with a transparency of <80%. During the 2010 summer, flooding expanded the CDW coverage area to over half of the ECS. PE-rich cells showed a homogeneous distribution and a decline in abundance, while the spatial pattern of the PC-rich *Synechococcus* resembled the pattern from 2009. Based on the phycocyanin operon phylogeny, the *Synechococcus* in the ECS were categorized into 5 groups, ECS-1 to ECS-4 and ECS-PE, comprising a total of 19 operational taxonomic units. In the summer of 2009, ECS-2 dominated in the coast, and the ECS-3 and ECS-PE clades prevailed in the offshore waters. However, during the summer of 2010, ECS-4 and ECS-PE became the dominant strains. The injection of plentiful anthropogenic pollutants and the enhancement of transparency within the CDW expansion area appear to be the factors needed to transiently alter the ecology of *Synechococcus* after flooding.

Chung et al., 2013. Influence of the Changjiang River flood on *Synechococcus* ecology in the surface waters of the East China Sea. (Accepted by Microbial Ecology)

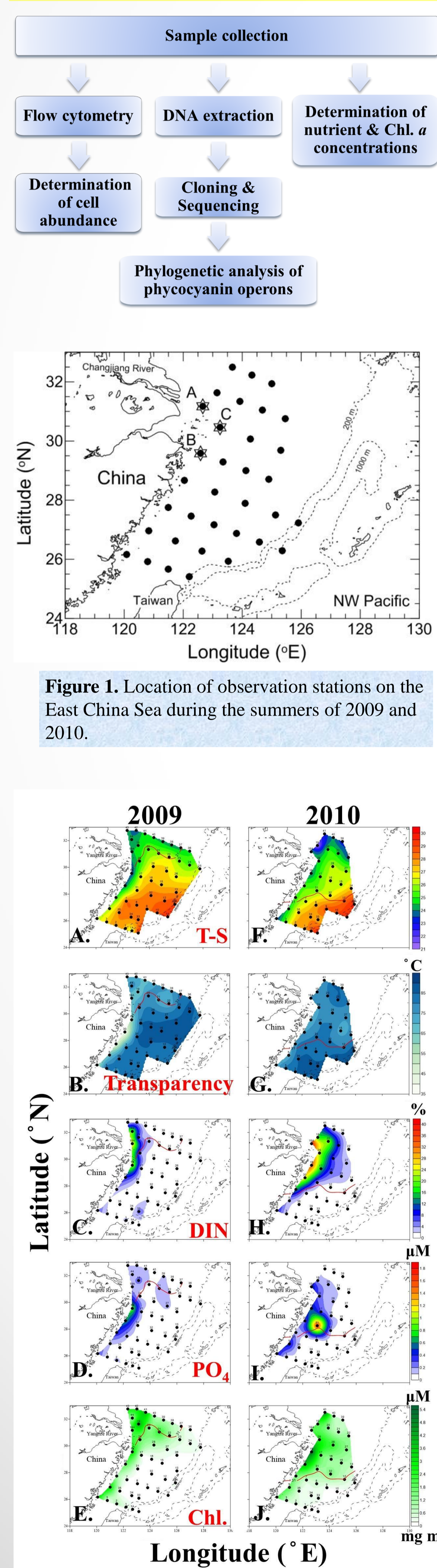


Table 1. Hydrographic features of the Changjiang dilution water coverage (salinity ≤ 31 PSU) during the summers 2009 and 2010. Each parameter is presented as the mean value ± 1 standard deviation. n: station number; DIN: dissolved inorganic nitrogen ($\text{NH}_4 + \text{NO}_2 + \text{NO}_3$); DIP: dissolved inorganic phosphate; Chl. *a*: chlorophyll *a*. **: significant difference at a level of $p < 0.01$ (Student's *t*-test).

	2009 (n=7)	2010 (n=20)
Coverage area ($\times 10^3$ km ²)	43	160
Temperature (°C)	25.0 \pm 0.9	24.9 \pm 1.6
Transparency (%)	69.9 \pm 4.9	78.4 \pm 4.4**
DIN (μM)	4.90 \pm 9.79	12.04 \pm 11.97
DIP (μM)	0.13 \pm 0.07	0.24 \pm 0.38
Chl. <i>a</i> (mg m^{-3})	2.51 \pm 1.69	2.10 \pm 1.56

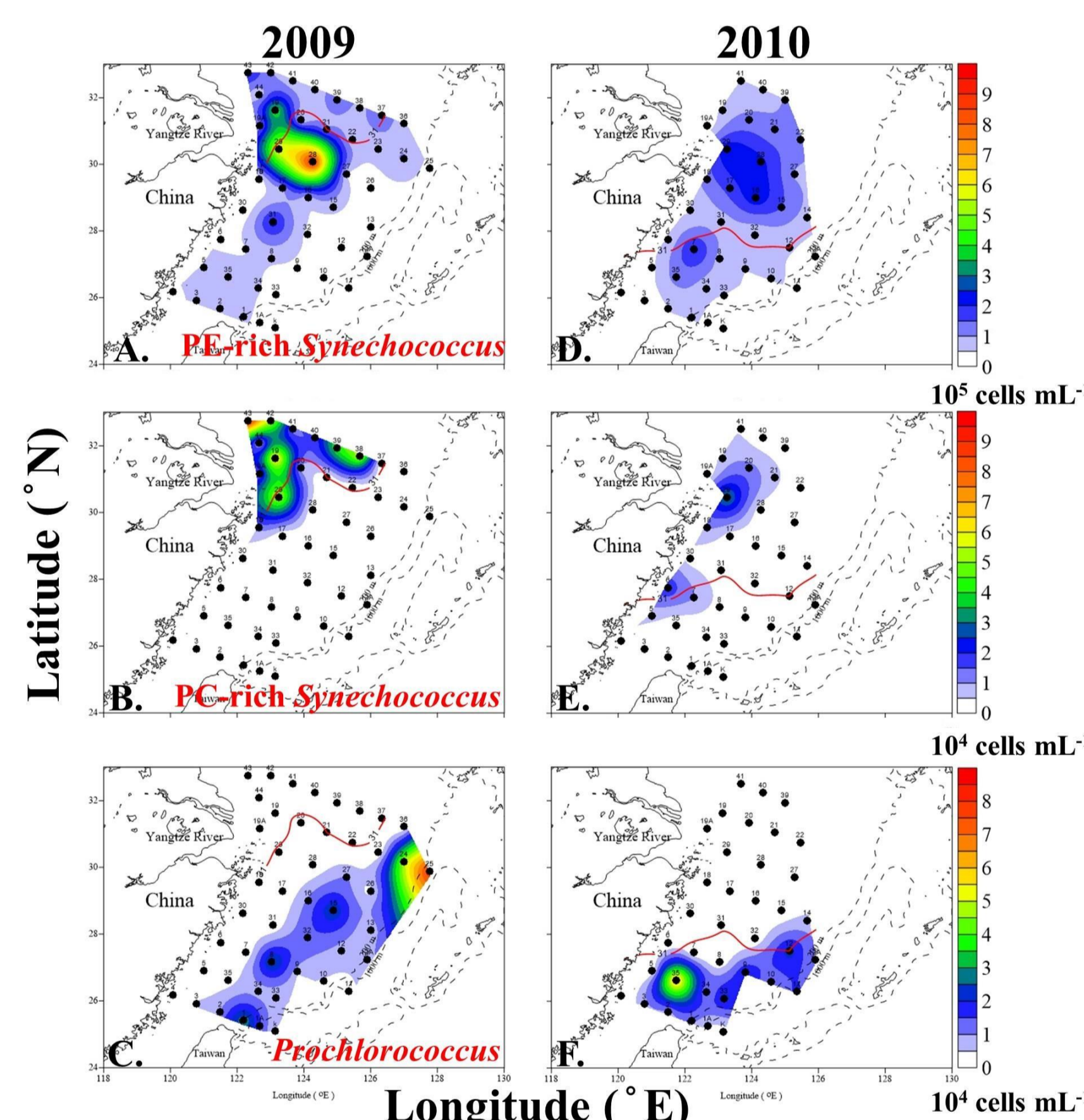


Figure 3. Distribution of picocyanobacterial abundance on the surface of the East China Sea: (A, D) Phycoerythrin-rich *Synechococcus*, (B, E) phycocyanin-rich *Synechococcus*, and (C, F) *Prochlorococcus*. The bold dashed lines represent the isohaline of salinity 31, indicating the outer boundary of the CDW-affected area.

Table 2. Pearson correlations observed between picocyanobacterial abundances and hydrographic factors during the summers of 2009 and 2010 (n=68). DIN: dissolved inorganic nitrogen ($\text{NH}_4 + \text{NO}_2 + \text{NO}_3$); DIP: dissolved inorganic phosphate.

	Temperature	Salinity	Transparency	DIN	DIP
<i>Syn.</i>					
PE-rich	-0.198	-0.130	-0.065	-0.003	-0.031
PC-rich	-0.404**	-0.260*	-0.290**	0.105	0.071
<i>Pro.</i>	0.290**	0.314**	0.293**	-0.236*	-0.218*

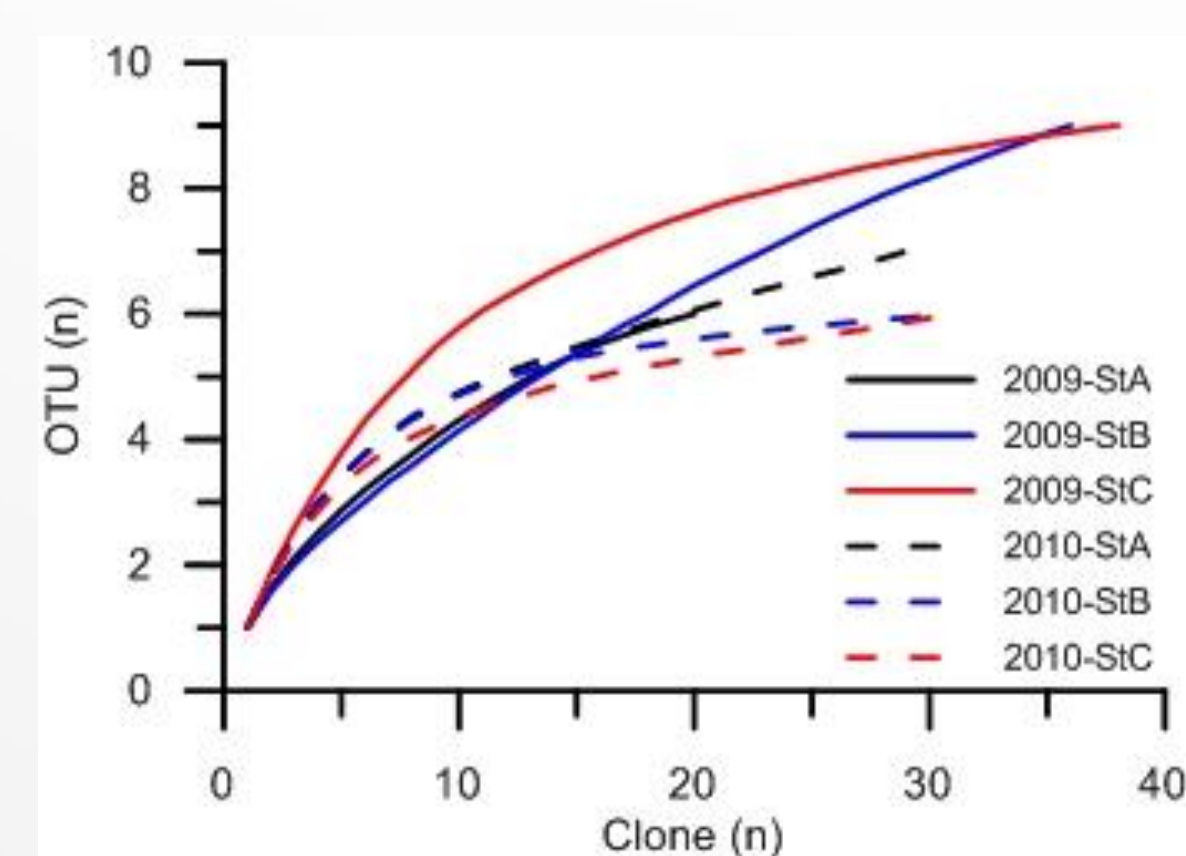


Figure 4. Rarefaction curves inferred from *cpcBA*-ITS and *rpcBA*-ITS sequences obtained from the surface of the East China Sea during the summers of 2009 and 2010. DNA samples were collected from stations A, B, and C (Fig. 1). Operational taxonomic units (OTU) were defined at the level of 90% sequence identity.

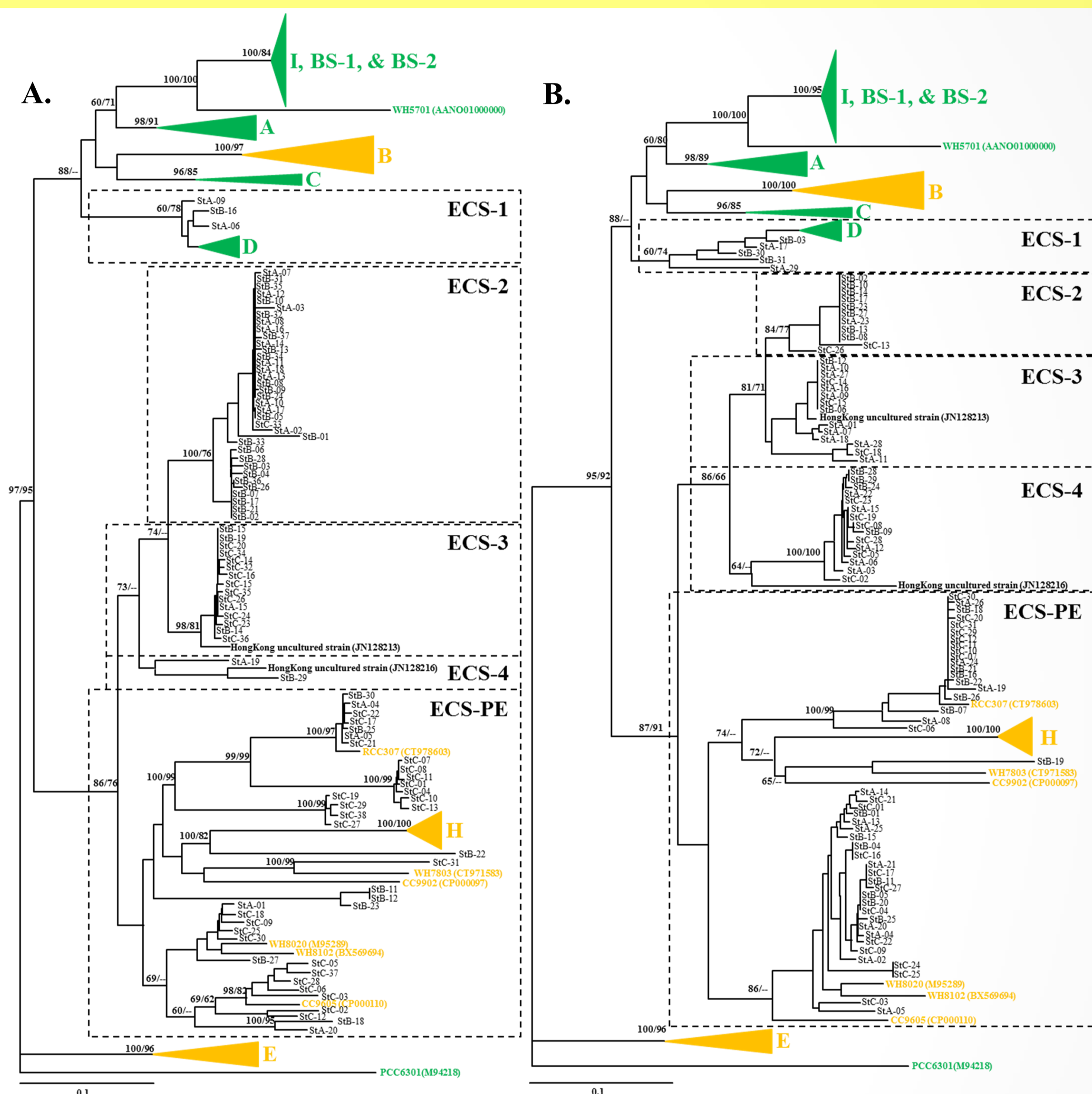


Figure 5. Neighbor-joining tree inferred from *cpcBA*-ITS and *rpcBA*-ITS sequences obtained from the East China Sea (ECS) during the summer of 2009 (A) and 2010 (B). DNA samples were collected from stations A, B, and C (Fig. 1). The effects of sampling error on tree inference and the stability of branch nodes were tested by a bootstrap analysis with 1000 repetitions. Bootstrap values of ≥ 60 are displayed on the branches. The freshwater PC-rich strain of *Synechococcus* PCC6301 served as the root. The length of the scale bar on the lower-left indicates a 0.1 nucleotide substitution unit.

Table 3. Distribution of *Synechococcus* analyzed by the phylogeny of the phycocyanin operon (as shown in Figures 6 and 7) on the surface of the East China Sea during the summers of 2009 and 2010. The percentage of phycocyanin operon sequences belonging to a specific clade is included in parentheses.

Station	Salinity (PSU)	Transparency (%)	<i>cpcBA</i> -ITS				<i>rpcBA</i> -ITS	Total clones
			ECS-1	ECS-2	ECS-3	ECS-4	ECS-PE	
2009								
A	30.38	62.5	2 (10%)	12 (60%)	1 (5%)	1 (5%)	4 (20%)	20 (100%)
B	31.25	60.4	1 (3%)	23 (64%)	3 (8%)	1 (3%)	8 (22%)	36 (100%)
C	32.10	77.2	0	1 (3%)	11 (29%)	0	26 (68%)	38 (100%)
2010								
A	27.84	75.4	2 (7%)	1 (4%)	9 (31%)	5 (17%)	12 (41%)	29 (100%)
B	24.98	69.9	3 (10%)	8 (26%)	2 (6%)	4 (13%)	14 (45%)	31 (100%)
C	25.44	75.0	0	2 (6%)	3 (10%)	6 (19%)	20 (65%)	31 (100%)

Conclusion

In comparison with previous studies, this study was the first to completely describe the distributions of both PE- and PC-rich *Synechococcus* in the ECS by flow cytometric enumeration and molecular phylogenetic analysis. Furthermore, PE- and PC-rich *Synechococcus* ecology was affected by a well-described disastrous flood that happened in the Changjiang River basin. Based on our findings, we suggest that anthropogenic pollutants carried in with the floodwater and increased transparency within the CDW expansion appear to be the factors that transiently changed the *Synechococcus* ecology after the flood.