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Note

Molecular phylogenetic analysis of attached Ulvaceae species and free-floating *Enteromorpha* from Qingdao coasts in 2007*

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Abstract Based on the sequence data of the nuclear ribosomal DNA internal transcribed spacer (ITS) 1, 5.8 S, and ITS 2, the molecular phylogeny was analyzed on Ulvaceae species collected from Qingdao coasts in summer of 2007, including 15 attached *Ulva* and *Enteromorpha* samples from 10 locations and 10 free-floating *Enteromorpha* samples from seven locations. The result supported the monophyly of all free-floating *Enteromorpha* samples, implying the unialgal composition of the free-floating *Enteromorpha*, and the attached Ulvaceae species from Qingdao coasts were grouped into other five clades, suggesting that they were not the biogeographic origin of the free-floating *Enteromorpha* in that season.

Keyword: free-floating; *Enteromorpha*; *Ulva*; ITS; rDNA; Qingdao

1 INTRODUCTION

Formation of a mass of free-floating green macroalgae has been recorded for decades in both marine and estuary environment (Fletcher, 1996; Hernández et al., 1997; Charlier et al., 2007). Despite serious influence on tourism, accumulation of free-floating green macroalgae leads to negative ecological consequences to the benthos due to shading and destroying-induced anaerobic environment (Charlier et al., 2007).

The great majority of the reported species of free-floating green macroalgae mass belong to just two genera of the Ulvaceae, *Ulva* and *Enteromorpha* (Fletcher, 1996). Due to their flexibility between tubular and blade morphologies in culture (Gayral, 1959, 1967; Føyn, 1960, 1961; Bonneau, 1977), and newly found ecological forms with significant shape variation (Malta et al., 1999; Blomster et al., 2002), great challenge has been brought on morphological identification for species in these two genera (Hayden et al., 2003). To address this problem, the DNA sequence data, especially the nuclear ribosomal DNA internal transcribed spacer (ITS) and ribulose 1,5-bisphosphate carboxylase large subunit gene (*rbcL*), have been generally employed on phylogeny reconstruction (Leskinen and Pamilo, 1997; Tan et al., 1999; Blomster, 2000; Hayden et al., 2003), species identification (Blomster et al., 1998, 1999; Hiraoka et al., 2003a, 2003b), and phylogeography analysis (Shimada et al., 2003, 2008) in *Ulva* and *Enteromorpha*.

In summer of 2007, both attached Ulvaceae species and free-floating *Enteromorpha* samples were collected from Qingdao coasts. A molecular phylogenetic analysis was performed based on the full-length sequence data of ITS 1, 5.8 S, and ITS 2.

2 MATERIALS AND METHODS

2.1 Algal sampling and genome DNA preparation

From July to August 2007, totally 15 attached

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Ulva and *Enteromorpha* samples fixed to intertidal zone substrate from 10 locations, 10 samples of free-floating green macroalgae from seven locations were collected along the coasts of Qingdao (Fig.1). All samples were washed with cold boiled seawater for several times, and immersed in 0.7% KI for 5 minutes before genome DNA extraction using the modified protocol from Doyle and Doyle (1990).

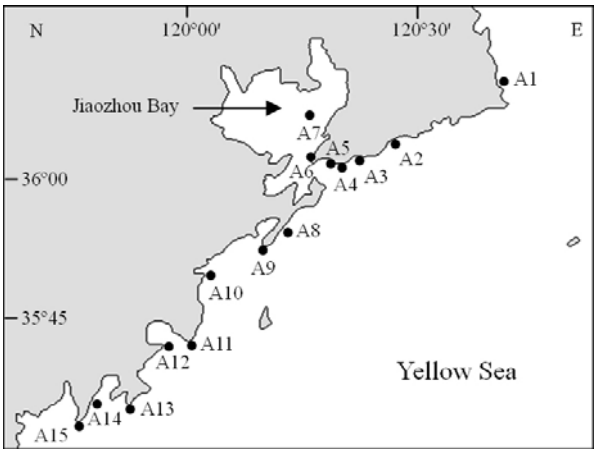

Fig.1 Sampling location along Qingdao coasts

2.2 ITS sequences amplification, determination and phylogenetic analysis

The primers for rDNA ITS were synthesized by Shanghai Sangon Corp. as the following sequence, FW: 5'-TCGTAACAAGGTTTCCGTAGG-3' and RV: 5'-TTCCTTCCGCTTATTGATATGC-3', and used for Polymerase Chain Reaction (PCR) profile from Leskinen and Pamilo (1997). The amplified bands were recovered and sequenced by National Human Genome Center at Shanghai. Based on the sequence alignments of about 500 bp, both the Maximum Parsimony (MP) and Neighbor-Joining (NJ) phylogenetic trees were constructed by bootstrapping with 1 000 replications of the data (software Mega 4.0), with *Blidingia minima* and *Monostroma grevillei* from the same order (Ulvales) served as outgroup taxa. The sequence divergency was calculated using Kimura 2-parameter model.

3 RESULTS AND DISCUSSIONS

All free-floating macroalgae samples were detected as highly branched linear thalli, tubular and hollow with monostromatic walls, matching the morphological criteria for *Enteromorpha*.

According to the phylogenetic tree, the monophyly of 10 free-floating samples was supported; all were grouped to one cluster (Fig.2), including a GenBank data for *E. linza* (AJ000203). Bootstrap support for the assemblage was 94% by MP method and 99% by NJ method. Further works on morphological taxonomy and culture experiments would be necessary to identify the species of free-floating samples. The 15 attached samples were resolved into other five clades. Sequence divergencies among all free-floating samples are very low (0.0%–2.5%), sequence divergency between free-floating samples and attached samples are much higher, ranging from 7.6% to 24.4% (Table 1).

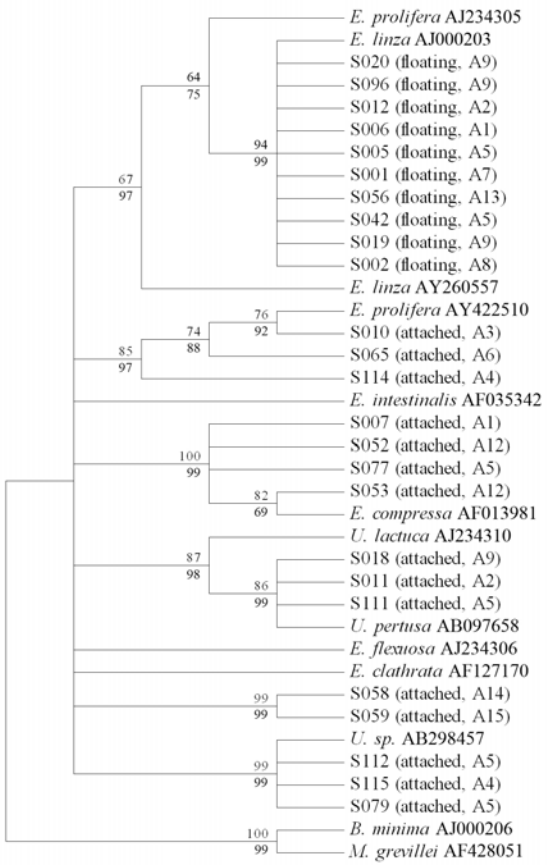

Fig.2 Phylogenetic tree of ITS sequences constructed by maximum-parsimony method

U.: *Ulva*; *E.*: *Enteromorpha*; *B.*: *Blidingia*; *M.*: *Monostroma*; floating: free-floating samples; attached: attached samples; A1—A15: sampling location; *E. clathrata*: homotypic synonym to *E. muscoides*; Bootstrap support (percentage of 1 000 replications) for MP (above) and NJ (below) is indicated at nodes

The results of molecular data analysis indicated that the free-floating *Enteromorpha* was unialgal, and the attached Ulvaceae species from Qingdao coasts in summer 2007 were not the biogeographic origin of the free-floating one.

Table 1 Sequence divergency matrix of the alignable, conserved regions of ITS 1, 5.8 S, ITS 2 for the samples

Sample	S001	S002	S006	S096	S005	S020	S019	S042	S012	S056	S010	S065	S114	S058	S059	S079	S112	S115	S011	S018	S111	S077	S052	S007	S053
S001																									
S002	0.013																								
S006	0.013	0.000																							
S096	0.013	0.000	0.000																						
S005	0.013	0.000	0.000	0.000																					
S020	0.013	0.000	0.000	0.000	0.000																				
S019	0.013	0.000	0.000	0.000	0.000	0.000																			
S042	0.025	0.015	0.015	0.015	0.015	0.015	0.015																		
S012	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.015																	
S056	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000																
S010	0.090	0.080	0.080	0.080	0.080	0.080	0.080	0.083	0.080	0.080															
S065	0.086	0.076	0.076	0.076	0.076	0.076	0.076	0.078	0.076	0.076	0.006														
S114	0.090	0.081	0.081	0.081	0.081	0.081	0.081	0.083	0.081	0.081	0.024	0.017													
S058	0.208	0.194	0.194	0.194	0.194	0.194	0.194	0.197	0.194	0.194	0.183	0.184	0.173												
S059	0.208	0.194	0.194	0.194	0.194	0.194	0.194	0.197	0.194	0.194	0.183	0.184	0.173	0.000											
S079	0.173	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.159	0.154	0.165	0.117	0.117										
S112	0.171	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.152	0.163	0.117	0.117	0.002									
S115	0.176	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.157	0.168	0.117	0.117	0.002	0.002								
S011	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.104	0.104	0.115	0.164	0.164	0.140	0.140	0.140							
S018	0.133	0.133	0.133	0.133	0.133	0.133	0.133	0.133	0.133	0.133	0.120	0.121	0.126	0.176	0.176	0.157	0.157	0.157	0.014						
S111	0.192	0.192	0.192	0.192	0.192	0.192	0.192	0.195	0.192	0.192	0.167	0.168	0.173	0.210	0.210	0.182	0.180	0.182	0.012	0.021					
S077	0.198	0.190	0.190	0.190	0.190	0.190	0.190	0.195	0.190	0.190	0.220	0.210	0.212	0.251	0.251	0.237	0.241	0.240	0.142	0.154	0.192				
S052	0.198	0.190	0.190	0.190	0.190	0.190	0.190	0.195	0.190	0.190	0.220	0.210	0.212	0.251	0.251	0.237	0.241	0.240	0.142	0.154	0.192	0.000			
S007	0.198	0.190	0.190	0.190	0.190	0.190	0.190	0.195	0.190	0.190	0.220	0.210	0.212	0.251	0.251	0.237	0.241	0.240	0.142	0.154	0.192	0.000	0.000		
S053	0.244	0.235	0.235	0.235	0.235	0.235	0.235	0.241	0.235	0.235	0.262	0.251	0.253	0.300	0.300	0.277	0.281	0.281	0.204	0.217	0.250	0.051	0.051	0.051	

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