

Sequence comparison and phylogenetic analysis of UPA gene sequences in *Gracilaria edulis*

Xu Rui^{1,2}, Wang Yun^{1*}

(1. Key Laboratory of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangdong Guangzhou 510300,
2. Shanghai Ocean University, Shanghai, 200120; *Corresponding author e-mail: wangyun@scsfri.ac.cn)

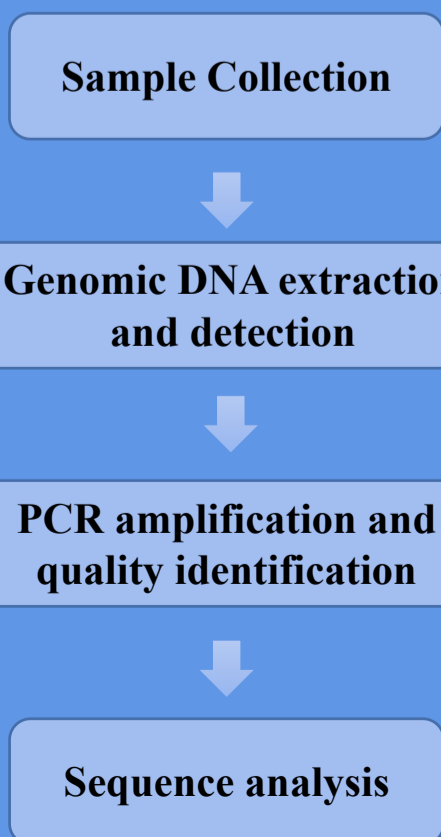
Introduction

Gracilaria edulis, a large marine economic algae, widely distributed in the South China Sea. *Gracilaria* is rich in colloids, polysaccharides, crude fiber and protein, and it's an important raw material for the production of agar and health food. Recently, with the increasing use of *Gracilaria*, it is crucial to carry out the research on its germplasm resources and genetic diversity of *Gracilaria* to better develop and utilize this kind of seaweed resources.

Universal Plastid Amplicon(UPA) is often used as a molecular marker in the molecular identification of algae, which is widely used in the study of red algae species identification, phylogeny and population genetic diversity. In this study, we applied the UPA gene sequence of *G. edulis* sampled from Hainan province sea area, and analyzed the genetic distance and phylogenetic relationship of *G. edulis* with other algae. The results of this research will help to explore the status of population germplasm resources and genetic diversity of *G. edulis* in Hainan province sea area.



Materials and Methods



Result

Tab.1 Variable sites of UPA gene fragments from different haplotypes of *G. edulis*

Nucleotide polymorphic sites	22233333 717912244 79182382318
hap1	GAAGGGGAAAA
hap2	CCC...CTTCC
hap3C
hap4	...AA.....

Notes: Numbers above the table indicate the location of the variable sites in the UPA gene sequences;
" ." means the same base with the first line

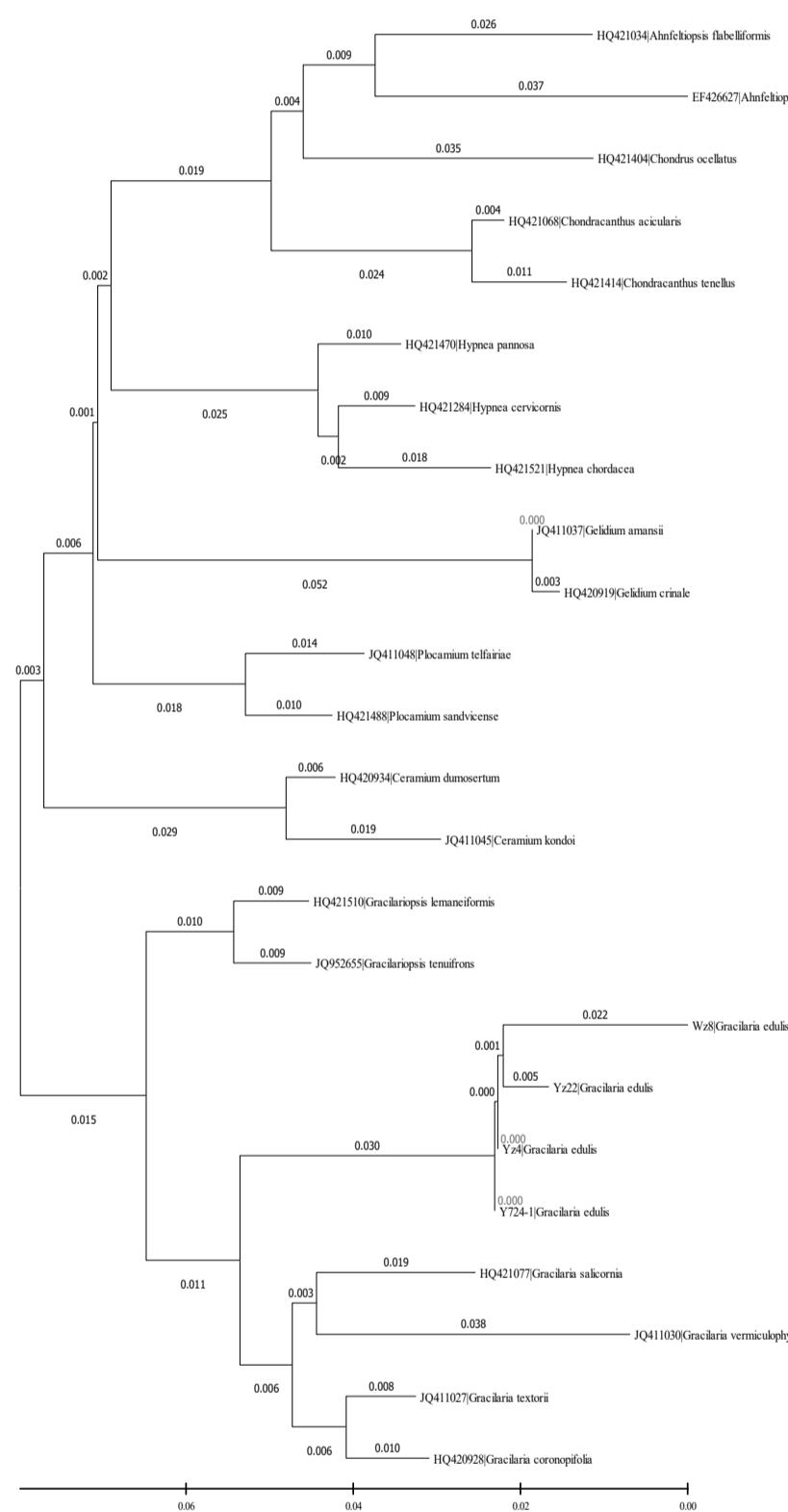


Fig.1 NJ phylogenetic tree based on UPA gene fragments

Tab.2 Genetic diversity parameters of UPA gene fragments of *G. edulis*

Gene types	Number of samples (N)	Number of variable sites (P)	Number of haplotypes (H)	Haplotype diversity (H _d)	Average	
					number of nucleotide differences (K)	Nucleotide diversity (P _i)
UPA	27	11	4	0.214	0.815	0.00229

Tab.3 Kimura-2-parameter genetic distances based on the UPA gene fragments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1	0.000																								
2	0.006	0.006																							
3	0.021	0.021	0.027																						
4	0.066	0.066	0.073	0.086																					
5	0.047	0.047	0.053	0.069	0.034																				
6	0.083	0.083	0.090	0.107	0.057	0.057																			
7	0.043	0.043	0.050	0.066	0.043	0.018	0.053																		
8	0.132	0.132	0.139	0.157	0.128	0.110	0.132	0.107																	
9	0.154	0.154	0.161	0.179	0.135	0.139	0.147	0.132	0.063																
10	0.059	0.059	0.066	0.082	0.053	0.043	0.076	0.046	0.104	0.103															
11	0.069	0.069	0.076	0.093	0.053	0.053	0.076	0.056	0.093	0.093	0.018														
12	0.086	0.086	0.093	0.110	0.093	0.103	0.121	0.100	0.111	0.096	0.083	0.086													
13	0.100	0.100	0.107	0.124	0.104	0.110	0.121	0.100	0.125	0.107	0.096	0.100	0.027												
14	0.083	0.083	0.090	0.106	0.093	0.096	0.118	0.100	0.104	0.086	0.090	0.018	0.034												
15	0.093	0.093	0.100	0.117	0.097	0.094	0.112	0.093	0.083	0.090	0.077	0.073	0.066	0.079	0.066										
16	0.086	0.086	0.093	0.110	0.097	0.090	0.108	0.086	0.083	0.097	0.077	0.066	0.073	0.086	0.066	0.025									
17	0.132	0.132	0.135	0.157	0.121	0.110	0.139	0.110	0.063	0.087	0.100	0.093	0.093	0.089	0.082	0.100	0.100								
18	0.121	0.121	0.128	0.145	0.107	0.107	0.132	0.096	0.070	0.080	0.083	0.079	0.083	0.086	0.079	0.076	0.083	0.067							
19	0.132	0.132	0.139	0.157	0.110	0.110	0.139	0.107	0.073	0.073	0.086	0.083	0.096	0.103	0.097	0.083	0.086	0.077	0.015						
20	0.100	0.100	0.107	0.124	0.093	0.083	0.107	0.089	0.103	0.117	0.066	0.066	0.062	0.089	0.082	0.076	0.073	0.096	0.103	0.110					
21	0.117	0.117	0.124	0.141	0.106	0.099	0.124	0.106	0.113	0.127	0.082	0.082	0.089	0.096	0.089	0.086	0.083	0.106	0.113	0.120	0.024				
22	0.117	0.117	0.124	0.141	0.125	0.117	0.139	0.121	0.107	0.128	0.104	0.107	0.089	0.103	0.086	0.086	0.079	0.107	0.104	0.107	0.089	0.096			
23	0.120	0.120	0.128	0.145	0.128	0.121	0.143	0.124	0.110	0.132	0.107	0.111	0.093	0.107	0.089	0.090	0.083	0.110	0.107	0.111	0.083	0.089	0.003		
24																									

Notes:

1.*Gracilaria edulis* hap1, 2.*Gracilaria edulis* hap2, 3.*Gracilaria edulis* hap3, 4.*Gracilaria edulis* hap4, 5.*Gracilaria salicornia*, 6.*Gracilaria textorii*, 7.*Gracilaria vermiculophylla*, 8.*Gracilaria coronopifolia*, 9.*Ahnfeltiopsis flabelliformis*, 10.*Ahnfeltiopsis concinna*, 11.*Gracilariopsis lemneiformis*, 12.*Gracilariopsis tenuifrons*, 13.*Hypnea cervicornis*, 14.*Hypnea chordacea*, 15.*Hypnea pannosa*, 16.*Plocamium telfairiae*, 17.*Plocamium sandvicense*, 18.*Chondrus ocellatus*, 19.*Chondracanthus acicularis*, 20.*Chondracanthus tenellus*, 21.*Ceramium dumosertum*, 22.*Ceramium kondoi*, 23.*Gelidium amansii*, 24.*Gelidium crinale*

Tab.4 Species used for analysis and their GenBank serial numbers

Genus	Species	GenBank accession number
	<i>Gracilaria salicornia</i>	HQ421077
<i>Gracilaria</i>	<i>Gracilaria textorii</i>	JQ411027
	<i>Gracilaria vermiculophylla</i>	JQ411030
	<i>Gracilaria coronopifolia</i>	HQ420928
<i>Gracilariopsis</i>	<i>Gracilariopsis lemneiformis</i>	HQ421510
	<i>Gracilariopsis tenuifrons</i>	JQ952655
<i>Ahnfeltiopsis</i>	<i>Ahnfeltiopsis flabelliformis</i>	HQ421034
	<i>Ahnfeltiopsis concinna</i>	EF426627
<i>Hypnea</i>	<i>Hypnea cervicornis</i>	HQ421284
	<i>Hypnea chordacea</i>	HQ421521
	<i>Hypnea pannosa</i>	HQ421470
<i>Plocamium</i>	<i>Plocamium telfairiae</i>	JQ411048
	<i>Plocamium sandvicense</i>	HQ421488
<i>Chondrus</i>	<i>Chondrus ocellatus</i>	HQ421404
<i>Chondracanthus</i>	<i>Chondracanthus acicularis</i>	HQ421068
	<i>Chondracanthus tenellus</i>	HQ421414
<i>Ceramium</i>	<i>Ceramium dumosertum</i>	HQ420934
	<i>Ceramium kondoi</i>	JQ411045
<i>Gelidium</i>	<i>Gelidium amansii</i>	JQ411037
	<i>Gelidium crinale</i>	HQ420919

Conclusion

1.The results of the study showed that the average content of T, C, A, and G of the UPA gene were 24%, 17.1%, 31.0%, and 27.9%, respectively, and the average content of A+T base was 55%, which was higher than C+G base content. From 358 bp of UPA gene sequences, 4 haplotypes and 11 polymorphic sites were detected, and they were all singleton variable sites.

2.The UPA gene fragments can distinguish different genera. The dendrogram results show that *G. edulis* form independent branches earlier, and *Gracilaria* and *Gracilariopsis* are relatively closely related, were first merged into a branch. The results of phylogenetic tree analysis were consistent with the results of traditional taxonomy.