

Article

Population Genetic Structure of *Sargassum horneri*, the Dominant Species of Golden Tide in the Yellow Sea

Xiaoran Wang ^{1,†}, Weiqian Zhao ^{1,†}, Minmin Zhuang ^{1,2,†}, Tingjian Wu ^{1,3}, Chunyan Zhao ¹, Wei Dai ¹ and Jianheng Zhang ^{1,*} 

¹ College of Oceanography and Ecological Science, Shanghai Ocean University, Shanghai 201306, China; w19811796066@163.com (X.W.); 19121695051@163.com (W.Z.); mm_zhuang1992@163.com (M.Z.); wutingjian@4io.org.cn (T.W.); zhaochunyan980204@163.com (C.Z.); 19521463131@163.com (W.D.)

² State Key Laboratory of Estuarine & Coastal Research, East China Normal University, Shanghai 200062, China

³ Key Laboratory of Tropical Marine Ecosystem and Bioresource, Fourth Institute of Oceanography, Ministry of Natural Resources, Beihai 536015, China

* Correspondence: jh-zhang@shou.edu.cn

† These authors contributed equally to this work.

Abstract: *Sargassum horneri* golden tides are increasingly becoming a marine ecological problem in the Yellow Sea (YS) and East China Sea. To understand the genetic relationship between the attached *S. horneri* along the China coast and the floating biomass in the YS, we used partial *rbcL*, *ITS2*, *cox1*, *cox3*, and *cob-cox2* to analyze the population genetic evolution of 165 *Sargassum* samples. The results showed that all samples were a single species of *S. horneri*. Partial sequences of each gene had major haplotypes, and other haplotypes evolved from the occurrence of base mutations. The *cob-cox2* gene haplotype research showed only the attached samples from ZJ, LN, and KR contained Hap3, and the distribution proportions of Hap2 and Hap4 in SS and the YS were closest to the distribution of the attached samples from SD. These novel findings provided information about the genetic evolutionary relationship between attached *S. horneri* along the coast of China and floating *S. horneri* in the YS, as well as new ideas for tracing the source of floating *S. horneri* in the YS from a molecular biological perspective.

Keywords: aquaculture; genetic structure; macroalgal blooms; origin; *Sargassum horneri*; Yellow Sea



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1. Introduction

The genus *Sargassum* is a large group of 354 species of brown algae commonly found along tropical and temperate coasts. It is one of the most abundant genera in the Order Fucales, which is widely distributed throughout the world [1–4]. Eutrophication is caused by the enrichment of nitrogen and phosphorus nutrients in water [5,6] and can cause macroalgal blooms to develop rapidly in the sea [7,8]. Some species in the genus *Sargassum* have air sacs that allow the algae to float for a long time at the sea surface, where they can grow rapidly via vegetative propagation and subsequently form large floating biomass. These blooms form “Sargasso seaweed beds” or “golden tides” [9].

As an invasive species of macroalgae, *Sargassum* has invaded European shores and the Mediterranean, and even drifting individuals have been observed in the sea area near the Canary Islands, becoming a potential species responsible for the “*Sargassum* golden tide”. Golden seaweed tides are a global environmental and social problem, that have been occurring along Ireland’s eastern coastline since the 1990s [10]. Recently, significant accumulations of drifting *Sargassum* have been observed on the sea surface in specific regions such as the Gulf of Mexico in the Atlantic Ocean [6,11], the East China Sea and the Yellow Sea. In the Atlantic region, two species namely *S. natans* and *S. fluitans* dominate these accumulations [6], while in the East China and Yellow Seas only *S. horneri* is found. The proliferation of this invasive genus can lead to substantial modifications in the original

marine ecosystem [12], resulting in adverse effects on offshore tourism and maritime transport activities [13], as well as deterioration of seawater quality upon sinking and decomposition [11]. If an outbreak occurs within aquaculture areas, it can cause significant losses to local mariculture industries.

In 2008, during the monitoring of green tides caused by *Ulva prolifera*, a small amount of *Sargassum* was found to be entrapped in *U. prolifera*, but its biomass was small biomass and did not attract attention [14–16]. Researchers have identified the floating golden tide algae as a single ecotype of *Sargassum horneri* in the YS and ECS [17,18]. From March to May 2017, the largest distribution and coverage areas of golden tides in the YS and ECS reached 7700 km² and 188 km², respectively. Large amounts of biomass were stranded in the offshore region of Jiangsu Province, which is the traditional area for *Pyropia yezoensis* aquaculture in China, resulting in economic losses of hundreds of millions of yuan (information source: National Bureau of Oceans). Golden tide outbreaks also occurred in 2020 and 2021, which caused additional damage to *P. yezoensis* aquaculture in China. In recent years, however, the phenomenon of golden tides in the Yellow Sea (YS) and East China Sea (ECS) emerged and gradually began to show a regular trend.

Sargassum filicinum was defined by Harvey (1860) using Eastern Japanese coastal samples, and its reported major distribution areas are coastal zones of Japan, Korea, Philippines, Mexico (Pacific), and California in the USA [19]. In China, *S. horneri* is mainly distributed in warm temperate sea areas (Liaoning, Shandong, Zhejiang, Fujian, Guangdong Provinces) and in Taiwan, Macao, and Hong Kong [20–22].

The genus *Sargassum* is also an economically important alga, and it has strong ecological functions and bioresource utilization capabilities. In marine ecosystems, *Sargassum* can efficiently adsorb excess nitrogen, phosphorus, and carbon dioxide in seawater, and thus it is listed as a choice for algal bed reconstruction and marine ecological remediation because of its ability to improve the offshore ecological environment [23,24]. In the open sea, *Sargassum* can float on the sea surface in pieces due to its branches and air sacs, which creates a “sea forest” that provides organisms such as fish and turtles with a habitat for foraging and reproduction [25], thereby promoting marine biodiversity [26].

Impacts of these golden tides have been a paradox [27]. Floating *Sargassum* in oceanic water can increase the productivity and biodiversity, while large amount of biomass inundating neritic coasts caused serious economic losses in tourism, aquaculture and coastal ecology.

ITS2, *rbcL*, *cox1*, and *cox3* are commonly used DNA markers for population genetic analysis of *S. horneri* [7,28,29]. Sequencing of both mitochondrial and chloroplast genomes of *S. horneri* from the coasts of the YS and ECS was previously conducted [30–32]. These studies provided new perspectives for the development of molecular markers and the analysis of the genetic structure of *S. horneri* populations. As organelle gene sequences, both mitochondrial and chloroplast gene sequences are effectively haploid with no heterozygosity [30]. Thus, molecular markers of their DNA sequences may be an efficient way to trace floating *S. horneri* golden tides in the YS and ECS. Liu et al. [31] reported that the rate of gene variation in mitochondrial DNA was three times higher than that of chloroplast DNA, which indicates that mitochondrial DNA is more suitable for analyzing population genetic diversity of *S. horneri*.

To understand the population genetic diversity of attached *S. horneri* along the coast of China, to further analyze the population genetic relationship between floating *S. horneri* in the YS and attached *S. horneri* along the coast of China (Figure 1a–f), and to explore the source of floating *S. horneri* in the YS, we conducted large-scale spatiotemporal sampling. We collected the attached samples from five provinces along the Chinese and Korean coastlines and floating samples from the YS and Subei Shoal in 2017–2021. We evaluated the population genetic diversity of the samples using DNA markers of mitochondrial, chloroplast, and ribosomal DNA, and we also assessed golden tide drift paths in the YS using satellite remote sensing to further analyze the sources.

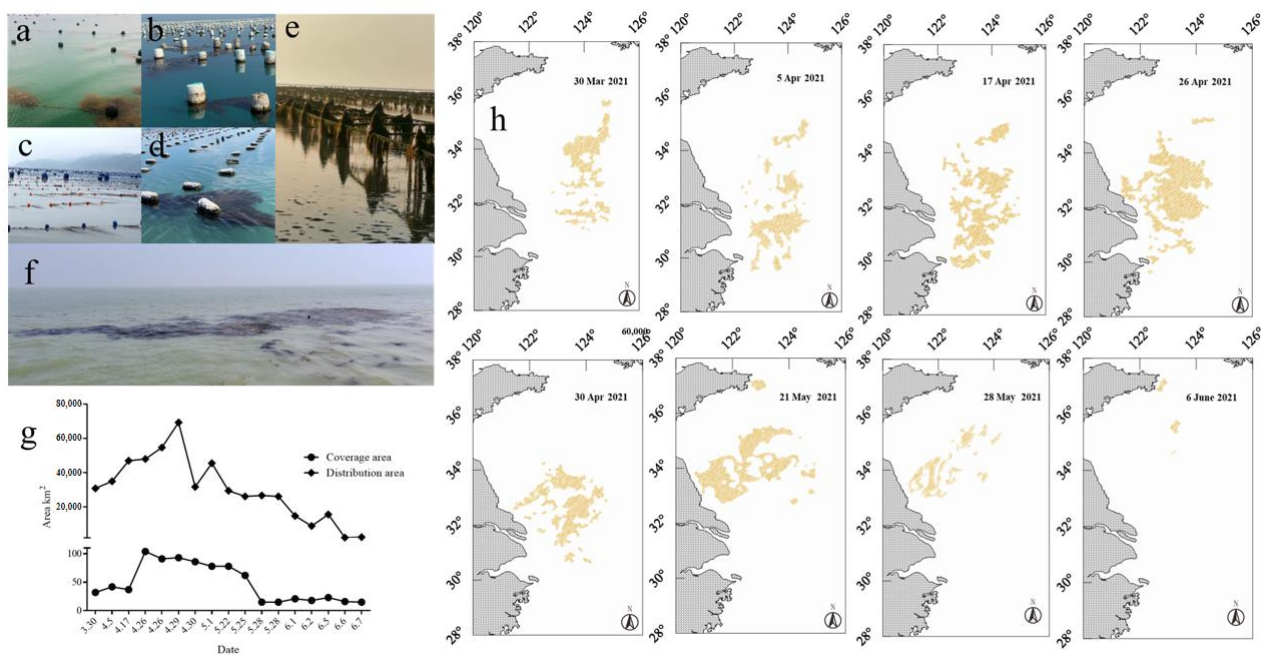


Figure 1. General situation of *Sargassum* blooms: (a–d) are, in order, the *S. horneri* biomass grown in coastal aquaculture areas of Shandong, Zhejiang, Fujian, and Guangdong Provinces; (e) *S. horneri* biomass stranded on *P. yezoensis* cultivation rafts located on Subei Shoal, and (f) drifting *S. horneri* biomass in the YS; (g) Long time-series trend of golden tides based on satellite remote sensing images in the Yellow Sea and the East China Sea in 2021; (h) The distribution and coverage areas of the golden tide that evolved over time in the YS and ECS in 2021.

2. Materials and Methods

2.1. Sample Collection

Of the 165 samples collected, the attached samples were collected from coastal areas of Liaoning (LN), Shandong (SD), Zhejiang (ZJ), Fujian (FJ), Guangdong (GD) Provinces of China and Jeju Island (KRJJ) from South Korea, and floating samples were collected from Subei Shoal (SS) and the YS (Table 1). The algal thalli from each site were sampled randomly and cleaned with distilled seawater to remove attached organisms and debris. The samples then were preserved at -20°C for DNA extraction.

Table 1. Information about the *S. horneri* samples collected from the coast of China, Subei Shoal, and the YS.

Source ID	Sample Number	Dates	Collection Sites	Geographical Coordinate	State
2017-A-ZJZS	2	26 November 2017	ZJ	122°45' E 30°43' N	Attached
2018-A-ZJNJ	5	23 December 2018	ZJ	120°83' E 27°32' N	Attached
2018-A-ZJZS	9	11 November 2017–24 May 2018	ZJ	122°45' E 30°43' N	Attached
2019-A-ZJZS	3	22 January–18 October 2019	ZJ	122°43' E 30°43' N	Attached
2019-A-SDWH	5	10 January–31 October 2019	SD	122°34' E 37°17'8"–37°17'11" N	Attached
2019-A-KRJJ	1	30 April 2019	KR	126°46' E 33°51' N	Attached

Table 1. Cont.

Source ID	Sample Number	Dates	Collection Sites	Geographical Coordinate	State
2019-F-YS	3	3–4 June 2019	YS	121°08′–121°20′ E 30°43′–33°04′ N	Floating
2019-F-JSNT	1	4 June 2019	SS	120°58′ E 32°36′ N	Floating
2020-A-ZJZS	3	27 July–17 November 2020	ZJ	122°43′ E 30°43′ N	Attached
2020-A-SDQD	5	1 May 2020	SD	120°21′ E 36°02′ E	Attached
2020-F-YS	11	1 May–7 June 2020	YS	121°43′–120°56′ E 35°13′–35°57′ N	Floating
2020-F-JSYC	5	12 May 2020	SS	33°53′ N 120°52′ E	Floating
2020-F-JSNT	11	17 January–13 May 2020	SS	120°59′ E 33°12′ N	Floating
2021-A-SDWH	7	15 January–29 April 2021	SD	122°34′ E 37°17′8″–37°17′11″ N	Attached
2021-A-FJND	6	10–12 April 2021	FJ	119°44′–119°46′ E 26°36′–26°35′ N	Attached
2021-A-FJPT	5	13 April 2021	FJ	25°15′ N 119°28′ E	Attached
2021-A-FJFZ	5	11 April 2021	FJ	26°21′ N 119°42′ E	Attached
2021-A-FJZZ	5	14–15 April 2021	FJ	23°34′ N 117°22′ E	Attached
2021-A-GDCZ	5	14 May 2021	GD	117°03′ E 23°33′ N	Attached
2021-A-GDST	2	15 May 2021	GD	116°56′ E 23°26′ N	Attached
2021-A-ZJZS	41	24 June–25 October 2021	ZJ	122°45′ E 53°43′ N	Attached
2021-F-YS	9	6–9 May 2021	YS	119°45′–122°58′ E 33°58′–35°14′ N	Floating
2021-F-JSNT	13	25 March–30 April 2021	SS	120°59′–121°39′ E 32°05′–32°36′ N	Floating
2021-F-LNDL	3	7 July 2021	LN	121°34′ E 38°52′ N	Attached

2.2. Satellite Remote Sensing

Satellite remote sensing is one of the most efficient tools for natural hazard monitoring and assessment. The Haiyang-1C (HY-1C) satellite, which was launched in September 2018, has a spatial resolution of 50 m and revisiting frequency of 3 days. In this study, images from the HY-1C were used qualitatively to provide a time-series view of the *Sargassum* distribution pattern. The HY-1C satellite images were provided by the East China Sea Environmental Monitoring Center of State Oceanic Administration (Shanghai) to monitor drifting seaweeds in 2021.

2.3. Molecular Identification of *Sargassum* Samples

Total DNA was extracted using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The regions selected for polymerase chain reaction (PCR) amplification and automated sequencing were as follows: (1) partial *rbcL* in the chloroplast genome, (2) partial *ITS2* in ribosomal DNA, and (3) partial *cox1*, *cox3*, and *cob-cox2* in the mitochondrial genome. Table 2 shows information about the primers used. The PCR cycling procedures for *ITS2* and *cob-cox2* were described previously [33,34]. Amplification was initiated with denaturing at 94 °C for 4 min, followed by 38 cycles of

94 °C for 45 s, annealing at 50 °C for *rbcL*, *cox1*, and *cox3* for 45 s, and 72 °C for 90 s, and then a final extension at 72 °C for 10 min. Neighbor-joining analyses of the aligned sequences were performed with MEGA 5.05 [35] and the reliability of each branch was tested with 1000 bootstrap replications. All samples were identified as *S. horneri*.

Table 2. Information about the five marker genes in chloroplasts (*rbcL*), ribosomes (*ITS2*), and mitochondria (*cox*) used in this study.

Primers	Sequences (5'-3')	Size of Products (bp)	References [28–30,34–37]
<i>rbcL</i> -F	5'-TTAGGGTTATTTGTAAATGGATGCG-3'	700	This study
<i>rbcL</i> -R	5'-ACATCCTTGTGTAAAGTCTCATTACT-3'		
<i>ITS2</i> -F	5'-CGATGAAGAACGCAGCGAAATGCGAT-3'	582	Yoshida et al., 2000 [36]
<i>ITS2</i> -R	5'-TCCTCCGCTTAGTATATGCTTAA-3'		
<i>cox1</i> -F	5'-CAGCGATGTCTGTTCTTATAAGG-3'	1027	This study
<i>cox1</i> -R	5'-TAATAAGTATCGTGAAGAGCAATATCTACAC-3'		
<i>cob-cox2</i> -F	5'-GTTACCTTTTTTAATTACGGGTAT-3'	890	Liu et al., 2018 [29]
<i>cob-cox2</i> -R	5'-AAGAAATAATACCTTCCATAATCGG-3'		
<i>cox3</i> -F	5'-GCGGTTCAACAAGGTTTGC-3'	469	This study
<i>cox3</i> -R	5'-GACGACTAAAATGATCTAGATATAACCGAAA-3'		

2.4. Haplotype Statistics and Analysis of *cob-cox2* and *cox3* Partial Sequences

We used the Alignment Editor of BioEdit7.0 sequence software to optimize the gene sequences of the samples that we collected from eight collection sites [37]. Of the available sequences from the eight collection sites (Table 1), we counted and analyzed haplotypes using DNASP5.0 software [38]. The network diagrams of *cob-cox2* and *cox3* partial sequences and pedigrees obtained in this study were constructed using PopART1.7 software [39].

3. Results

3.1. Blooming Process of Drifting *Sargassum* Species in 2021

The evolution of the golden tides was depicted by a long time-series based on satellite remote sensing images in the YS and ECS in 2021 (Figure 1h). The *Sargassum* rafts generally drifted southwards and expanded from March to April, and then they moved northwards and gradually decayed in June. On 5 April, drifting algae were distributed from the ECS to the south YS. *Sargassum* bloom patches began to congregate to form a much larger bloom from 5 April to 26 April, with a cover of up to 104 km². The maximum distribution area of 69,285 km² occurred on 9 April (Figure 1g). A large biomass of floating *Sargassum* was stranded on *P. yezoensis* cultivation rafts located on Subei Shoal during a coastal survey in May (Figure 1e). These patches then moved northwards. Between 30 April and 6 June, algal cover gradually decreased, fragmented into several small patches, and then disappeared in the YS.

3.2. Molecular Analysis Based on Marker Genes in Ribosomes, Mitochondria, and Chloroplasts

Comparison of the partial sequences of *ITS2*, *rbcL*, *cox1*, *cob-cox2*, and *cox3* revealed genetic variation sites in all five genes (Figure 2). We identified 10 variant sites in the *ITS2* partial sequences (two insertion mutations), as well as seven haplotypes and 10 single nucleotide polymorphisms (SNPs). We found three variant sites in the *rbcL* partial sequences (one mutation type with insertion and one with deletion), four haplotypes, and three SNPs. Seven variant sites were present in the *cox1* partial sequences (one deletion mutation), with four haplotypes and seven SNPs. We identified 12 variant sites in the *cob-cox2* partial sequences (including one deletion mutation), with 12 haplotypes and 12 SNPs present. Finally, we found 16 variant sites (no insertion or deletion mutations), 10 haplotypes, and 16 SNPs in the *cox3* partial sequences. These results showed that the gene sequence polymorphism was higher on mitochondria than on ribosomes or chloroplasts. Among the five genes analyzed, the partial sequences of the mitochondrial *cob-cox2* and *cox3* had high

polymorphisms with more variation sites, so they are more suitable for further population diversity analysis.

ribosomal marker gene (<i>ITS2</i>)								chloroplast marker gene (<i>rbcL</i>)				
Position	Hap1	Hap2	Hap3	Hap4	Hap5	Hap11	Hap12	Position	Hap1	Hap3	Hap5	Hap6
45	G	G	G	A	G	G	G	8	A	A	A	-
57	C	C	C	A	C	C	C	212	G	A	G	G
70	A	A	A	C	A	A	A	553	-	-	T	-
90	G	G	G	G	G	G	C	mitochondrial marker gene (<i>cox1</i>)				
146	C	T	C	C	T	T	T	Position	Hap1	Hap2	Hap5	Hap8
148	T	T	C	T	T	T	T	46	A	A	A	-
194	G	G	G	G	G	G	C	80	C	C	C	T
405	-	-	-	-	-	-	C	134	T	T	G	T
410	-	-	-	-	-	C	-	210	T	T	G	T
603	G	G	G	G	C	G	G	237	G	G	T	G
								428	T	T	G	T
								530	G	C	G	G
mitochondrial marker gene (<i>cox3</i>)												
Position	Hap1	Hap3	Hap5	Hap6	Hap9	Hap11	Hap15	Hap16	Hap23	Hap24		
119	G	T	T	T	T	T	T	T	T	T	T	T
122	G	G	G	G	G	G	G	G	G	G	A	
144	T	T	T	A	T	C	T	T	T	T	T	
148	G	G	G	A	G	C	A	G	G	G	G	
158	C	G	G	G	G	G	G	G	G	G	G	
247	G	T	T	T	T	T	T	G	T	T	T	
250	A	T	T	T	T	T	T	T	T	T	T	
257	G	G	A	G	G	G	G	G	G	G	G	
276	T	T	T	T	T	T	G	T	T	T	T	
283	A	A	A	A	A	A	C	A	A	A	A	
326	G	G	G	G	G	G	A	G	G	G	G	
331	T	T	T	T	T	T	C	T	T	T	T	
338	G	G	G	G	G	G	C	G	G	G	G	
395	C	C	C	C	C	C	C	C	T	C	C	
460	A	G	G	G	A	A	T	G	G	G	G	
468	A	T	T	T	T	T	T	T	T	T	T	
mitochondrial marker gene (<i>cob-cox2</i>)												
Position	Hap1	Hap2	Hap3	Hap4	Hap8	Hap18	Hap20	Hap21	Hap22	Hap23	Hap24	Hap25
41	A	A	A	A	A	A	A	A	-	A	A	A
53	C	C	C	C	C	T	C	C	C	C	C	C
65	T	C	T	T	T	C	C	C	T	C	C	T
143	T	T	T	T	C	T	T	T	T	T	T	T
152	T	T	T	T	T	T	T	T	C	T	T	T
404	A	A	A	A	A	G	G	G	A	G	G	A
548	G	T	T	T	T	T	T	T	G	T	T	T
601	C	C	C	C	C	G	C	C	C	C	C	C
624	T	T	C	T	T	T	T	T	T	T	T	T
735	G	G	G	G	G	G	G	G	G	A	G	G
776	A	A	A	A	A	A	T	A	A	A	A	A
800	T	T	T	T	T	T	T	T	T	T	G	G

Figure 2. Comparison of haplotype sequence polymorphisms of ribosome (ri), chloroplast (cp), and mitochondrial (mt) marker genes.

An analysis of the percentage of haplotypes composition of the partial sequences of the five genes revealed the presence of major haplotypes (high percentages) in all of them (Figure 3). In the partial sequence of the *ITS2* gene, the major haplotype was Hap1 and the minor haplotype was Hap2, and the total proportion of both was 94.48%. Among the partial *rbcL* sequences, Hap1 accounted for 97.93% of the cases. Hap1, Hap3, Hap4, and Hap21 shared some proportion of the distribution among the partial *cob-cox2* sequences; except for 35.92% of Hap2, the analysis of partial *cob-cox2* showed high richness and diversity. Of the *cox1* and *cox3* partial sequences, the major haplotypes were Hap1 (86.90%) and Hap3 (75.86%), respectively. In addition, Hap2 (11.72%) was a minor haplotype in *cox1*, and Hap9 and Hap23 (9.66% for both) were minor haplotypes in *cox3*. The genetic evolutionary relationship between major and minor haplotypes requires further analysis.

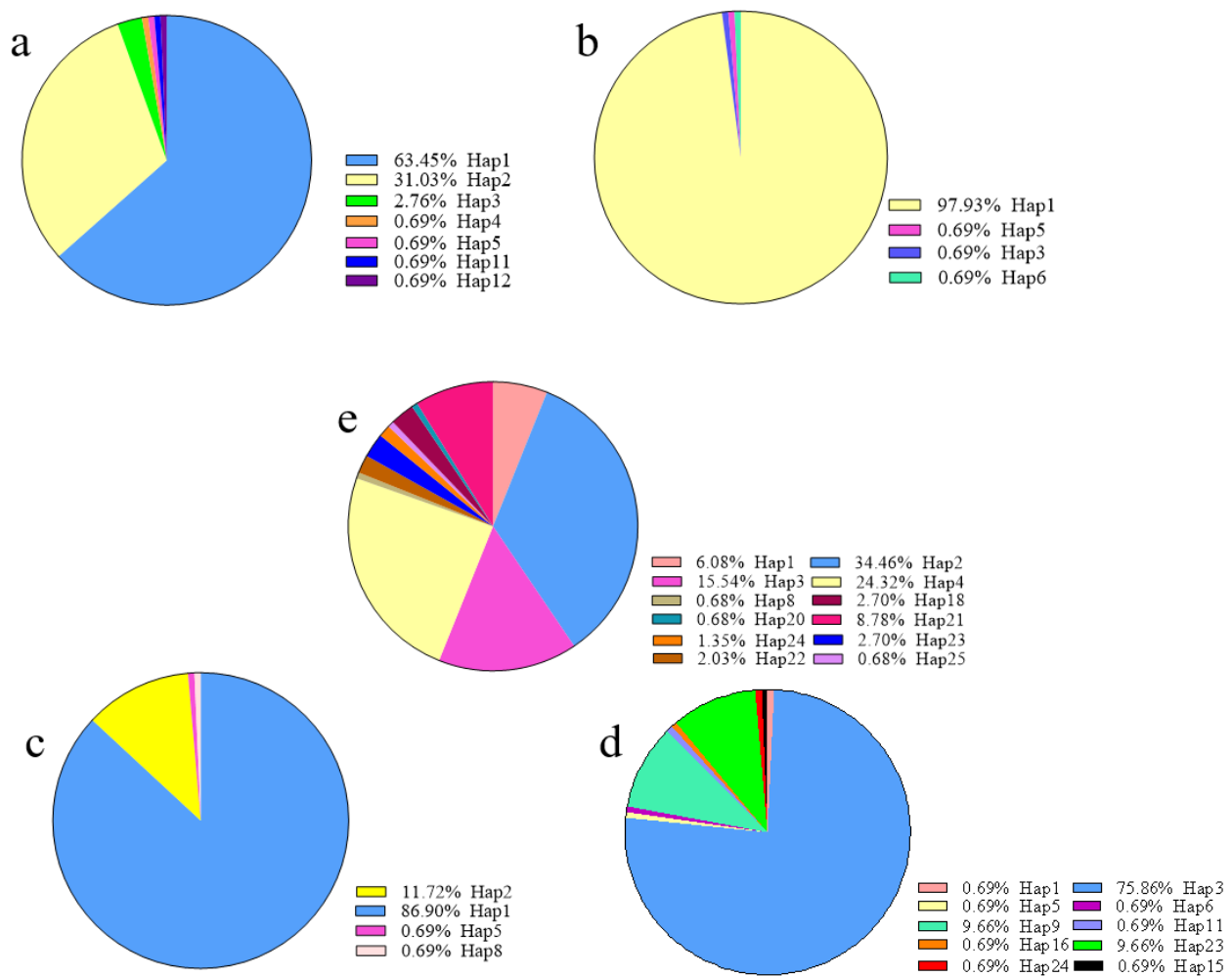


Figure 3. Composition percentage (%) of haplotypes in the *S. horneri* samples collected in this study based on five marker genes ((a): *ITS2*, ribosome (ri), (b): *rbcL*, chloroplast (cp); and (c): *cox1*; (d): *cox3*; and (e): *cob-cox2*, mitochondrial (mt)).

The relationships among the major haplotypes and other haplotypes were further analyzed by generating a NJ phylogenetic evolution tree for the partial sequences of the *ITS2*, *rbcL*, *cox1*, *cob-cox2*, and *cox3* genes (Figure 4). The *ITS2* partial sequence tree showed that the major haplotype, Hap1, was at the start of the main clade of the evolutionary tree, the minor haplotype Hap2 was on a branch of this main clade, and both floating and attached samples were clustered in Hap1 and Hap2 (Figure 4). Notably, in the evolutionary trees for the *rbcL*, *cox1*, *cob-cox2*, and *cox3* partial sequences, both floating and attached samples were clustered simultaneously only in the main haplotypes (▲), and all were at the position where the evolutionary tree branching began. In addition, many of the haplotypes that accounted for a relatively small proportion of the total were derived from the major haplotypes, and there was a relatively close genetic evolution relationship with the major haplotypes.

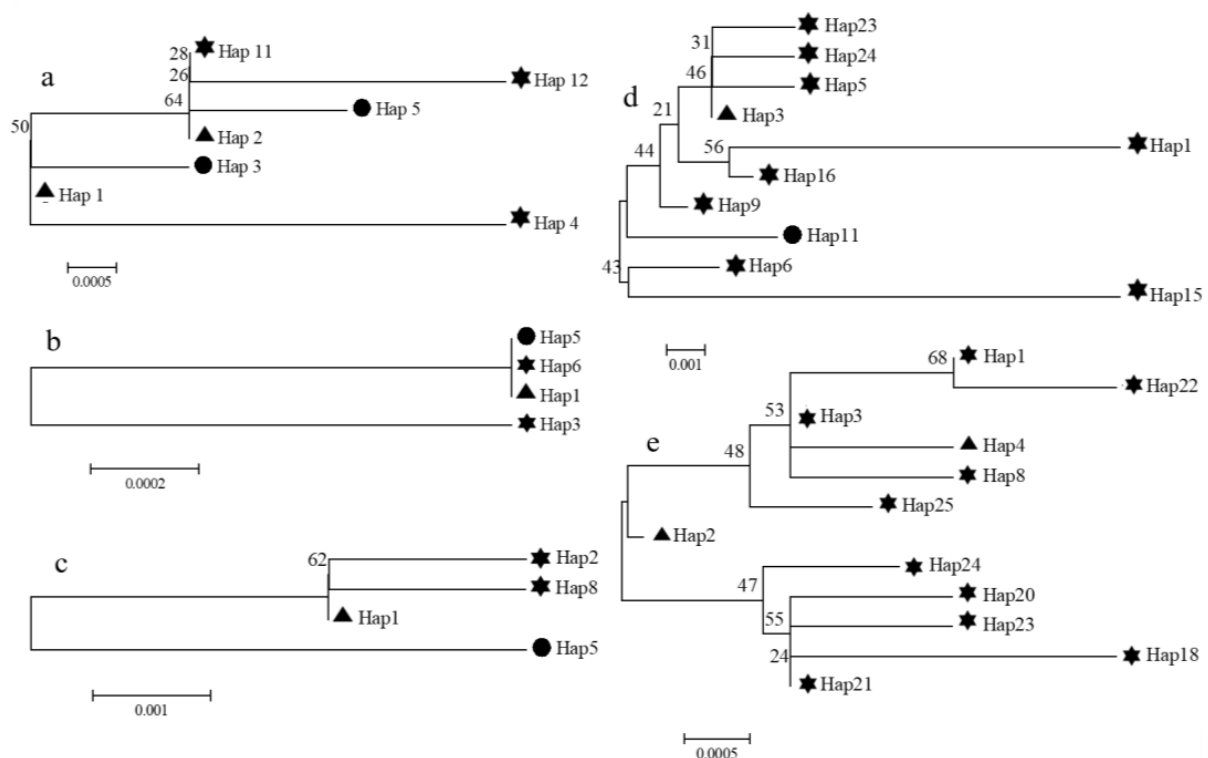


Figure 4. Neighbor-joining phylogenetic tree of haplotypes in the *S. horneri* samples collected in this study based on five marker genes ((a): *ITS2*, ribosome (ri), (b): *rbcL*, chloroplast (cp); and (c): *cox1*; (d): *cox3*; and (e): *cob-cox2*, mitochondrial (mt)). Floating and attached samples (▲), attached samples only (★), and floating samples only (●).

3.3. Haplotype Network Analysis Based on the Partial *cob-cox2* and *cox3* Sequences

The haplotype network map of *cob-cox2* showed that the attached samples from the Zhejiang (ZJ) coast had the most haplotypes with more abundant population diversity compared to the other sampling locations. Of concern were samples from ZJ that shared Hap circles with samples from all regions and that shared common haplotypes. The attached samples from ZJ, Liaoning (LN), and Jeju Island from South Korea (KRJJ) shared Hap3 circles, which were also the only Hap circles present in the South Korea (KR) samples. Except for the attached samples from LN and KRJJ, other regional samples were present in the two main haplotypes Hap2 and Hap4 (Figure 5a).

The *cox3* gene haplotype network map also showed that the attached samples from the ZJ coast had more haplotypes that were not found in samples collected in other regions. Some attached samples from LN were genetically distant from other regional samples. In addition, the attached samples from along the coast of ZJ and Shandong (SD) shared Hap9 and were connected to Hap11, which was unique to the attached samples from KRJJ. The genetic evolution between the samples from KR and the attached samples from other regions of China (CN) was relatively distant (Figure 5b).

Analysis of the population diversity and genetic evolutionary relationships of the 2021 samples based on partial sequences of *cob-cox2* (Figure 5c) and *cox3* (Figure 5d) revealed the same regularity as those of previous years' samples, with more haplotypes and more abundant population diversity in the attached samples from the ZJ coast.

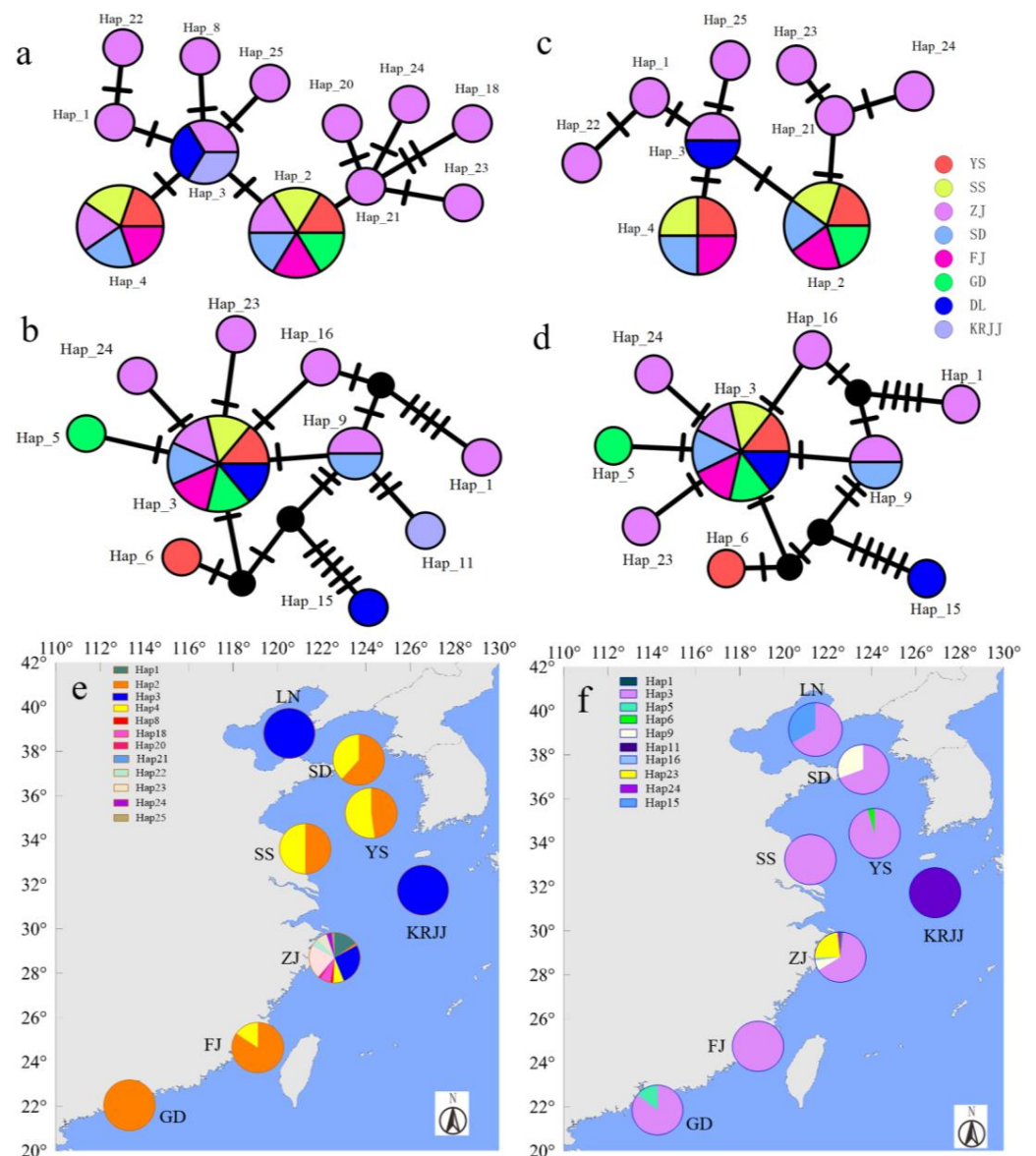


Figure 5. Haplotype network and composition percentage (%) of the *S. horneri* samples collected in the large-scale spate-temporal sampling along the coast of China. Samples from the Yellow Sea and Subei Shoal were floating samples, all others were attached samples. (a) *cob-cox2* and (b) *cox3* in 2017–2021; (c) *cob-cox2* and (d) *cox3* in 2021; (e) *cob-cox2* and (f) *cox3* in 2017–2021.

3.4. Analysis of Different Haplotype Occupancy in Different Geographic Regions Based on the Partial *cob-cox2* and *cox3* Sequences

In the regional percentage distribution of partial *cob-cox2* sequences (Figure 5e), the attached samples from ZJ had more haplotypes, with the most diverse sites of genetic variation. The attached samples from LN did not share two haplotypes (Hap2 and Hap4) that were characteristic of samples from the China coast, and the proportion of Hap2 showed a decreasing trend from north to south. In addition, only the attached samples from ZJ, LN, and KR contained Hap3. The distribution proportions of Hap2 and Hap4 in SS and the YS were closest to the distribution of the attached samples from SD.

In the regional percentage distribution of partial *cox3* sequences (Figure 5f), Hap3 was predominant in all regional haplotype compositions along the coast of China. The results of the analysis of the attached samples from ZJ were the same as those for *cob-cox2*, with more haplotypes and the most diverse sites of gene variation. Hap9 was only present in

the attached samples from the ZJ and SD regions, and this haplotype was absent in other regions. In addition, four regional samples (LN, Guangdong (GD), YS, and KRJJ) had region-specific haplotypes.

4. Discussion

Floating *S. horneri* has been present in the western Pacific Ocean for many years, but it was not a research focus because of its small biomass and range in early years [14–16]. However, a large outbreak of floating *S. horneri* biomass in the YS and ECS in 2017 resulted in stranded algae in the raft area of *P. yezoensis* aquaculture in Jiangsu Province, which caused direct economic losses as high as \$78 million [29].

S. horneri is widely distributed all over the world [1–3], especially in the western Pacific [20]. According to the incomplete statistics from <https://www.gbif.org> (accessed on 17 January 2022), *S. horneri* was observed 145 times in Japan, 14 times in South Korea, and 16 times in China between January 2000 and September 2021 along the coast of China, Korea, and Japan. We reinvestigated the distribution of *S. horneri* along the coast of China and found both attached and floating *S. horneri* in Dalian, Shandong, Zhejiang, Fujian, and Guangdong Provinces. Our survey added 34 distribution sites along the coast of China to the list on the GBIF site (Figure 6a).

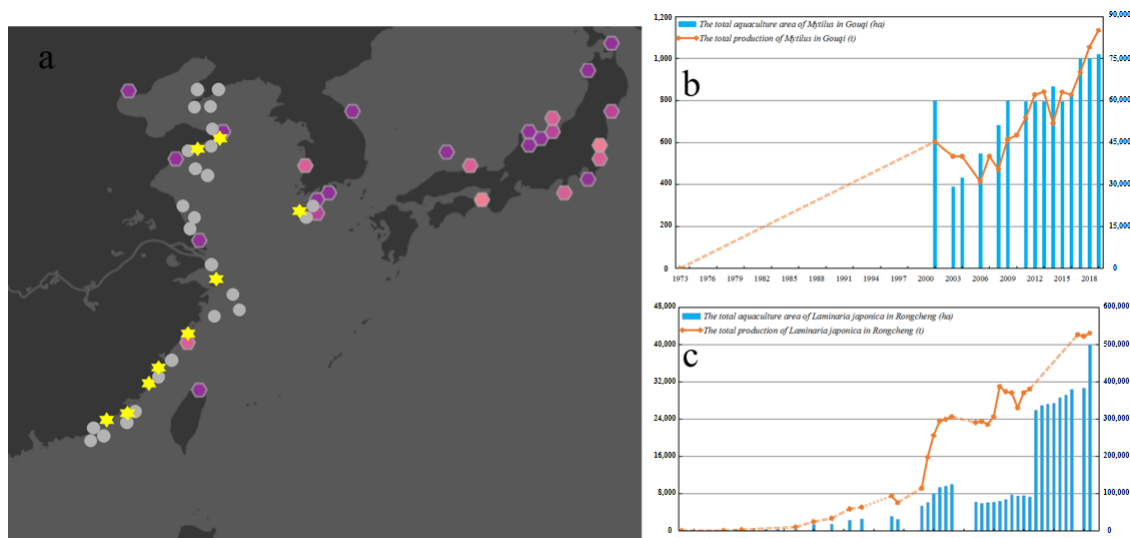


Figure 6. Distribution of *S. horneri* along the coast of China, South Korea, and Japan from January 2000 to present (●: floating samples found during reinvestigation; ★: attached samples found during reinvestigation) (a) and an overview of culture area and total production of mussels and *S. japonica* along the coast of Zhejiang (b) and Shandong Provinces (c) over successive years.

S. horneri mainly grows on rocks or stone marshes in the intertidal and subtidal zones under natural conditions [19]. However, large biomass of *S. horneri* have a strong relationship with aquaculture along the coast of China, and materials such as cables and drift in raft areas contribute greatly to the attached growth of *S. horneri*. Studies have found that the body length and biomass of intertidal *S. horneri* are much lower than those of *S. horneri* on rafts in aquaculture areas [24,40]. As the number and density of air sacs increases, the buoyancy of air sacs may exceed the attachment ability of their holdfasts. With the combined action of wind, waves, and currents, the ultra-long thalli in aquaculture areas are easily detached [10]. Large amounts of *S. horneri* in aquaculture areas are not welcomed by farmers because algal growth and surface coverage in raft areas affect the growth of cultured species, and fishers cut them off and discard them at will. Large scale floating accumulation of *S. horneri* on the sea surface is caused by the combined effects of the alga itself, natural conditions, and human factors [24].

In recent years, the area of aquaculture along the coast of China has gradually increased. The water in the culture area is highly eutrophic and suitable for the growth and reproduction of *S. horneri*. *S. horneri* absorbs nutrients from water, causing a large-scale outbreak, which inhibits the nutrient absorption of aquatic products and indirectly affects the local economic development. It will also destroy the local ecological environment and have a negative impact on the local economy, tourism and transportation. The mussel cultivation industry in China is mainly concentrated around Gouqi Island in Zhejiang Province, and the local cultivation area has shown a steadily increasing trend (remained above 1000 ha after 2015) from year to year (Figure 6b). Since 2005, there has been a dramatic increase in the area of cultivation of the brown alga *Saccharina japonica* in Rongcheng City of Shandong Province, which reached 4000 ha by 2016 (Figure 6c). Some studies have shown that the source of golden tides is located along the coast of Shandong and Zhejiang Provinces [13,18,41]. Specifically, the fast-growing aquaculture industry provides more sessile substrate for the attached growth of *S. horneri*, which in turn provides more biomass for floating golden tide outbreaks and may be the key to such outbreaks in the YS and ECS in recent years. In addition, the aquaculture areas along the coast of southern Korea and Japan are showing an increasing trend and may also supply considerable biomass to the floating golden tides in the YS and ECS.

Studies of the origin of golden tides in the YS and ECS have mainly focused on morphology, physical oceanography, satellite remote sensing, and molecular identification. Floating *S. horneri* can be transported into the ECS and even coastal Japan by the Kuroshio current [42] and further into the YS [17]. The YS floating *S. horneri* mainly originates from the coast of Zhejiang Province, but we cannot ignore the possibility of multiple sources along the coast in Shandong Province of China and southern Korea [18,29]. Xing et al. (2017) predicted the source region of floating *S. horneri* from October 2016 to January 2017 to be Rongcheng City of Shandong Province. In summary, there may be dual sources of floating golden tides in the YS and ECS, and their origin could be season dependent.

Population diversity of floating *S. horneri* in the YS was low [30]. We evaluated several partial sequences of *ITS2*, *rbcL*, *cox1*, *cob-cox2*, and *cox3*, which revealed a higher genetic diversity in attached *S. horneri* populations along the coast of China than in floating samples from the YS.

Although floating golden tides have serious impacts on the aquaculture industry along the coast of the western Pacific, the role of *S. horneri* in ecological remediation, resource-based utilization, and marine biodiversity conservation cannot be ignored [43,44]. From a dialectical perspective, we first need to rationally protect and support the unique ecosystem of the floating Sargasso seaweed bed and then we need to actively develop golden tide warning systems in order to prepare for the inshore drift of large amounts of biomass.

5. Conclusions

Compared with previous studies, the investigation and sampling area of *S. horneri* in this study was the widest, and it was the first comprehensive investigation and community genetic structure analysis of *S. horneri* along the coast of China. The analysis of *cox3* showed the attached samples from along the coast of ZJ and Shandong (SD) shared Hap9 and were connected to Hap11, which was unique to the attached samples from KRJJ. The genetic evolution between the samples from KR and the attached samples from other regions of China (CN) was relatively distant. The source of floating *S. horneri* golden tides in the YS was traced, which suggested the possibility of two sources for *S. horneri* golden tides, and the huge attached biomass in local aquaculture of Zhejiang and Shandong Provinces of China may provide a source for golden tides in the YS. In addition, researchers should think from a dialectical perspective about the prevention and management of *S. horneri*.

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