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Insights on the *Sargassum horneri* golden tides in the Yellow Sea inferred from morphological and molecular data

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Abstract

Large-scale drifting *Sargassum* biomass, known as golden tides, has caused considerable damage to the local environment and economy associated with the Yellow Sea of China. To understand the reoccurrence of *Sargassum horneri* golden tides in the Yellow Sea, large-scale spatio-temporal sampling was performed across nine cruises and five coastal surveys. Morphological data indicated that the floating *S. horneri* thalli with differing reproductive timing coexisted in the Yellow Sea. A total of 196 *S. horneri* samples had identical sequences of partial *cox3* and *rbc*L-S spacer region, revealing very low genetic diversity in the floating biomass. A total of 19 haplotypes for partial *cox3* previously found in the Yellow Sea were not detected in our large-scale sampling. Based on four novel mtDNA markers, the 196 samples could be further distinguished into two forms, which varied in proportions at various locations, but coexisted in each of the spatio-temporal sampling. These results indicated that the floating *Sargassum* biomass in the Yellow Sea came from only two dominating haplotypes. The novel findings uncovered by this work will provide further insight into the underlying mechanisms of reoccurring golden tides in the Yellow Sea, and lead to the improved management of the *Sargassum* biomass.

The genus *Sargassum* C. Agardh (1820) is one of the most species-rich genera within the order Fucales containing 354 recognized species worldwide (Mattio and Payri 2011; Guiry and Guiry 2017). Some *Sargassum* species have an efficient vegetative growth rate in floating state, leading to the accumulation of large quantities of drifting biomass known as seaweed rafts and golden tides (Milledge and Harvey 2016; Amaral-Zettler et al. 2017). The past 30 yr have seen a global increase in the occurrence of golden tides (Smetacek and Zingone 2013). Floating *Sargassum* biomass has been reported in the Sea of Japan (Yoshida 1963; Yatsuya 2008), the East China Sea (Komatsu et al. 2007, 2008, 2014*a*,*b*; Qi et al. 2017), the West African coast (Gower et al. 2013), and the Sargasso Sea of the North Atlantic Ocean (Gower and King 2011; Hinds et al. 2016; Sissini et al. 2017), causing serious economic losses in fisheries and tourism, and posing a serious threat to the native biodiversity.

Recent years have seen *Sargassum* golden tides become commonplace along the Chinese coastline. In Rongcheng, Shandong, large-scale drifting *Sargassum horneri* biomass has been observed to become tangled on *Saccharina* cultivation rafts from December to May over the past few years. The invasion of such large biomass has had a serious negative impact on the kelp farming and fisheries industries (Liu Feng observation). The Sanya coasts of China have seen hundreds of tons of tropical *Sargassum* biomass including *Sargassum polycystum*, *Sargassum spinuligerum*, *Sargassum ilicifolium*, and *Sargassum aquifolium* accumulated along the coasts in spring and seriously affected local tourism as well as the native ecosystems (Liu et al. 2017). Recently, drifting *Sargassum* biomass was observed in the western Yellow Sea along the Jiangsu coasts from November 2016 to April 2017, a highly unusual sighting along this coastline (Xing et al. 2017). Biomass accumulation along the Jiangsu coastline peaked between early December 2016 and late January 2017. Large-scale drifting *S. horneri* biomass became stranded on *Pyropia*

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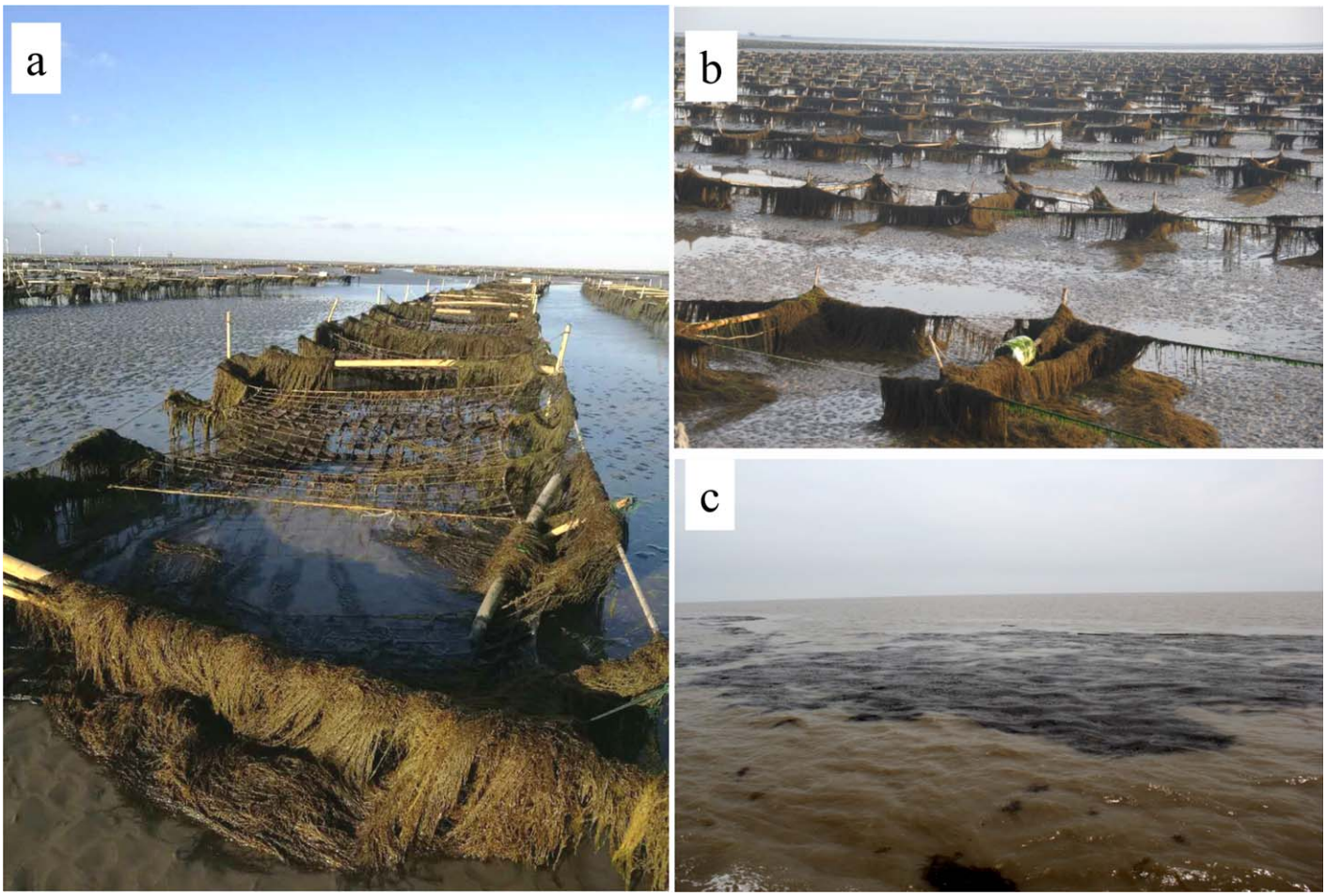

Fig. 1. *S. horneri* biomass: (**a**) *S. horneri* biomass stranded on the *Pyropia* cultivation rafts settled on the Subei Shoal, photo taken on 17 January 2017, (**b**) *S. horneri* biomass stranded on the *Pyropia* rafts, photo taken on 25 May 2017, and (**c**) drifting *S. horneri* biomass in the western Yellow Sea, photo taken on 24 May 2017. [Color figure can be viewed at wileyonlinelibrary.com]

(nori) cultivation rafts and settled on sandy shoals, and destroyed the floating nori cultivation infrastructure (Fig. 1). A dangerous combination of wave and tide conditions led to an economic loss of at least ¥0.5 billion (Chinese yuan) in the nori industry of Dafeng in Jiangsu Province (China Xinhua News 2017).

S. horneri is an annual seaweed distributed in the Pacific Northwest coasts and functions as one of the important primary producers in the marine ecosystem (Hu et al. 2011). *S. horneri* grows on rocky coastal bottoms and forms underwater forests or meadows in sublittoral regions, serving as nursery habitats and spawning grounds for marine invertebrates and fish (Yatsuya 2008). The adult *S. horneri* thalli contain a large number of gas-filled vesicles which function as buoyancy aids and allow the algal thalli to stand upright from the bottom (Yoshida 1963). Algal thalli can become detached from the bottom by the sheer drag force of waves and currents, and their positive buoyancy provided by the gas-filled vesicles allow them to float to the water surface (Xu et al. 2016, 2018). The floating biomass can maintain its vegetative state in the intertidal and subtidal zones, and some biomass may even reach offshore waters via surface currents (Komatsu et al. 2007, 2008). In addition, research has shown *S. horneri* to expand its distribution from the coasts of East Asia to the American coasts, spreading along the coasts of southern California and down into Baja California, Mexico. This is most likely the result of natural dispersal methods such as seaweed rafts or due to human activities such as shipping (Miller et al. 2007; Kaplanis et al. 2016).

A sample of sessile *S. horneri* previously collected from the rocky shores of Xiaohuyu, Nanji Islands, Zhejiang Province, China, was identified to harbor a 34,606 bp mitochondrial genome (mtDNA, Liu et al. 2015) and a 124,068 bp plastid genome (ptDNA, Liu and Pang 2016). This genomic data provides a valuable source for the exploration of new DNA markers. One important advantage of organelle genome sequences (mtDNA and ptDNA) over the diploid nuclear genome in *Sargassum* is that organelle DNA sequences are

Table 1. Information of the 196 samples of *S. horneri* collected in the Yellow Sea.

Source ID	Sample number	Dates	Collection sites	Geographical coordinate	State
YSa (F)	10	16–26 May 2016	Yellow Sea (No. 1 Cruise via R/V Science III)	33°30′–34°30′N, 121°00′–122°30′E	Floating
RCa (B)	5	16–17 Jan 2017	Sanggou bay, Rongcheng, Weihai	37°09′N, 122°30′E	Stranded on beaches
SSa (R)	2	26 Feb 2017	Subei Shoal (No. 2 Cruise via R/V Yuzheng-32505)	32°35′N, 121°10′E	Stranded on *Pyropia* cultivation rafts
SSb (F)	15	04–12 Mar 2017	Subei Shoal (No. 3 Cruise via R/V Sutongyu-01026)	32°40′–34°00′N, 120°30′–121°45′E	Floating
SSb (BM)	10				Sank to the bottom
SSc (F)	6	18–21 Apr 2017	Subei Shoal (No. 4 Cruise via R/V Suruyuyun-288)	32°40′–33°40′N, 120°40′–121°30′E	Floating
YSb (F)	27	20 Apr 2017–23 May 2017	Yellow Sea (No. 5 Cruise via R/V Science III)	32°30′–36°00′N, 121°00′–124°00′E	Floating
RZ (B)	5	29–30 Apr 2017	Wanpingkou, Rizhao	35°24′N, 119°33′E	Stranded on beaches
SSd (F)	20	20–26 May 2017	Subei Shoal (No. 6 Cruise via R/V Suruyuyun-288)	32°20′–33°40′N, 120°40′–121°45′E	Floating
SSd (BM)	15				Sank to the bottom
SSd (R)	9				Stranded on *Pyropia* cultivation rafts
YSc (F)	23	08–22 Jun 2017	Yellow Sea (No. 7 Cruise via R/V Science III)	33°30′–36°00′N, 120°30′–124°00′E	Floating
QD (B)	8	01–03 Jul 2017	Huiquan bay and Shilaoren, Qingdao	36°03′–09′N, 120°20–48′E	Stranded on beaches
YT (B)	8	14–15 Nov 2017	Laishan, Yantai	37°29′N, 121°26′E	Stranded on beaches
RCb (R)	20	13–16 Nov 2017	Sanggou bay, Rongcheng, Weihai	37°07–08′N, 122°32–34′E	Stranded on *Saccharina* cultivation rafts
YSd (F)	8	27–28 Nov 2017	Yellow Sea (No. 8 Cruise via R/V Science III)	37°00′N, 123°31′E	Floating
YSe (F)	5	01–02 Dec 2017	Yellow Sea (No. 9 Cruise via R/V Sutongyu-01026)	34°09′–34°40′N, 121°09′–121°49′E	Floating

effectively haploid, with no heterozygosity. This may be the key that will allow for the identification of suitable markers to track the source of a golden tide (Liu et al. 2017). Considering that mtDNAs had an approximately threefold greater mutation rate than ptDNAs in *Sargassum* species, the mtDNA markers would be more suitable than ptDNA markers for investigating the population genetic diversity (Liu et al. 2018).

To understand the genetic diversity of the golden tide-forming alga *S. horneri* in the Yellow Sea, and as part of an effort to track the source of the floating biomass, this study performed large-scale spatio-temporal sampling during nine cruises in the Yellow Sea as well as five coastal surveys, and analyzed morphological features as well as the genetic diversity of *S. horneri* inferred from multiple organelle DNA markers.

Materials and methods

Algal sampling and DNA extraction

The vegetative thalli of floating and benthic *S. horneri* samples in the Yellow Sea were collected using ring nets with a diameter of 0.55 m and bottom drag nets with the width of 4 m during nine separate cruises, organized to survey the abundance of the floating biomass of macroalgae in the Yellow Sea of China in 2016–2017 (Table 1). Additional thalli samples of *S. horneri* were collected from biomass stranded on cultivation rafts of seaweeds (*Saccharina japonica* in Shandong Province and *Pyropia* spp. in Jiangsu Province), as well as collected manually from beaches in January to November 2017. A total of 196 samples were collected in this study.

Algal branches with vesicles and blades for each sample were cut and kept in coolers and then transported to the laboratory to be kept at −80°C in a freezer. Unialgal tissue frozen for less than two weeks was ground to a fine powder in liquid nitrogen for DNA extraction. Total genomic DNA was extracted using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturers' instructions. The concentration and quality of isolated DNA was evaluated via electrophoresis on a 1.0% agarose gel.

Table 2. The primers designed in this study to amplify the sequences of four novel mitochondrial DNA markers.

Primers	Sequences (5′-3′)	Location (5′-3′)	Size of products (bp)
rnl-atp9 region-F	5′-GGACCAGGAAAACTCGATCCCT-3′	*rnl* (2458 to 2479)	625
rnl-atp9 region-R	5′-TAGACCAATTGTAGCTAGACCAGC-3′	*atp9* (31 to 54)	
rps12-rps7 region-F	5′-AGACCAAATATTACTCAATGGTTT-3′	*rps12* (7 to 30)	514
rps12-rps7 region-R	5′-CCATTTTTCATTAAAAGATTAATCA-3′	*rps7* (116 to 140)	
rpl5-rps3 region-F	5′-GTTAAAAGAATGCCGCGGAAGTA-3′	*rpl5-trnG* spacer	1053 (form I), 1054 (form II)
rpl5-rps3 region -R	5′-TACTTGGAAATACCAAATAGGTC-3′	*rps3* (699 to 721)	
cob-cox2 region-F	5′-GTTACCTTTTTTAATTACGGGTAT-3′	*cob* (555 to 578)	890
cob-cox2 region -R	5′-AAGAAATAATACCTTCCATAATCGG-3′	*cox2* (133 to 157)	

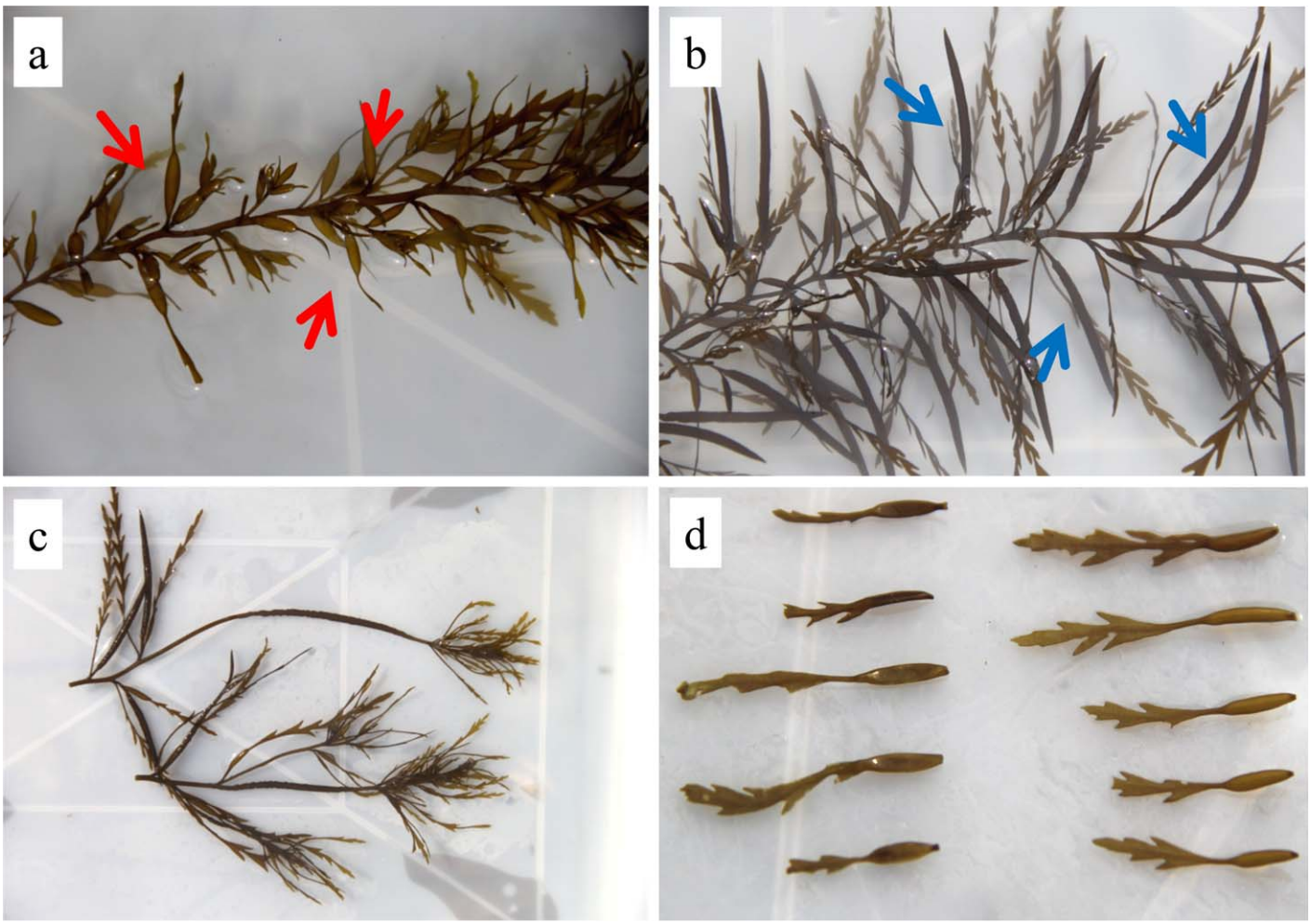

Fig. 2. Morphology of *S. horneri*: (**a**) *S. horneri* thalli with many vesicles (red arrows), (**b**) *S. horneri* thalli with receptacles (blue arrows), (**c**) the stem-like receptacles, and (**d**) the morphology of vesicles on floating thalli (gas-filled vesicles, right) and on the thalli collected on the bottom of sea (shriveled vesicles, left). [Color figure can be viewed at wileyonlinelibrary.com]

Field measurements and statistical analyses

Field measurements were performed to compare morphological features between benthic and floating *S. horneri* thalli during two cruises (no. 3 and 6) in early March and late May. The algal thalli were randomly sampled during the two cruises, and the number of thalli with receptacles was recorded to calculate the proportion of maturation. Small branches broken from the thalli were not counted. Five morphological parameters including the diameter of the main axes, vesicle density and length, and receptacle density and

Table 3. Morphological characteristics of the *S. horneri* thalli in the Subei Shoal during two cruises (no. 3 and 6) in early March and late May.

Source ID	Individual number (*n*)*	Thalli with receptacles (%)	Diameter of main axes (mm)†	Vesicle density (10 cm^{-1})	Vesicle length (mm)	Receptacle density (10 cm^{-1})‡	Receptacle length (mm)
SSb (F)	27	70.37	3.8 ± 0.4	24.9 ± 5.7	11.1 ± 2.5	19.8 ± 6.4	38.6 ± 7.5
SSb (BM)	25	88.00	3.9 ± 0.3	14.3 ± 3.3	13.4 ± 3.6	24.9 ± 5.8	45.8 ± 9.2
SSd (F)	38	47.37	3.3 ± 0.5	32.0 ± 7.8	11.7 ± 3.1	17.8 ± 5.7	46.2 ± 12.1
SSd (BM)	27	92.59	3.1 ± 0.6	12.4 ± 5.8	10.8 ± 2.4	25.3 ± 4.5	53 ± 13.8

* Small branches broken from the thalli were not counted.
† Vesicle density referred to the maximum number of vesicles per 10 cm algal thalli.
‡ Receptacle density referred to the maximum number of receptacles per 10 cm algal thalli.

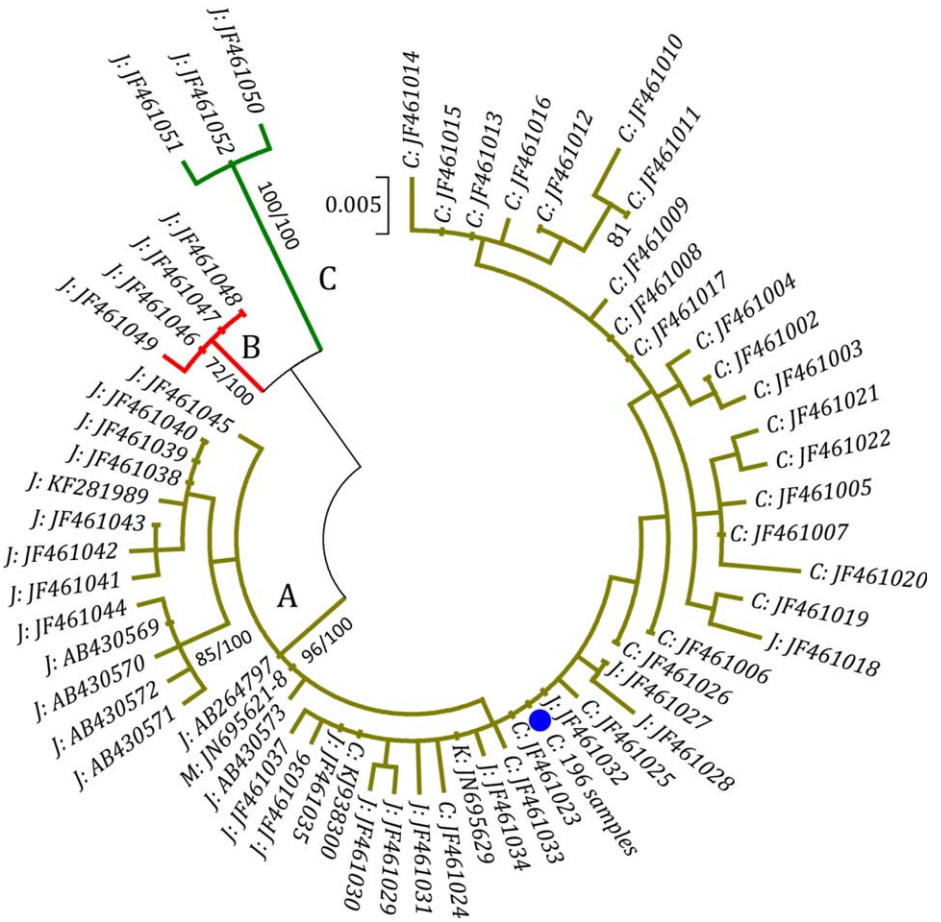

Fig. 3. Unrooted phylogenetic tree constructed from analysis of partial mitochondrial *cox3* gene sequences of the 196 samples as well as the GenBank data for *S. horneri*. Numbers in each branch (ML/BI) indicated maximum likelihood (ML) bootstrap values and Bayesian Inference (BI) posterior probability values, respectively. The letter before the accession number indicated the countries in which sample was collected, including China (C), Japan (J), South Korea (K), and Mexico (M). [Color figure can be viewed at wileyonlinelibrary.com]

length were measured and counted on the deck after algal collection. Vesicle or receptacle density referred to the maximum number of vesicles or receptacles per 10 cm of algal thalli in this study. Statistical analyses were performed using the SPSS. 13.0 program (SPSS, Chicago, U.S.A.). Morphological data as response variables were initially tested for normal distributions using Shapiro-Wilk's test and for homogeneity of variance using Levene's test. Significant differences were

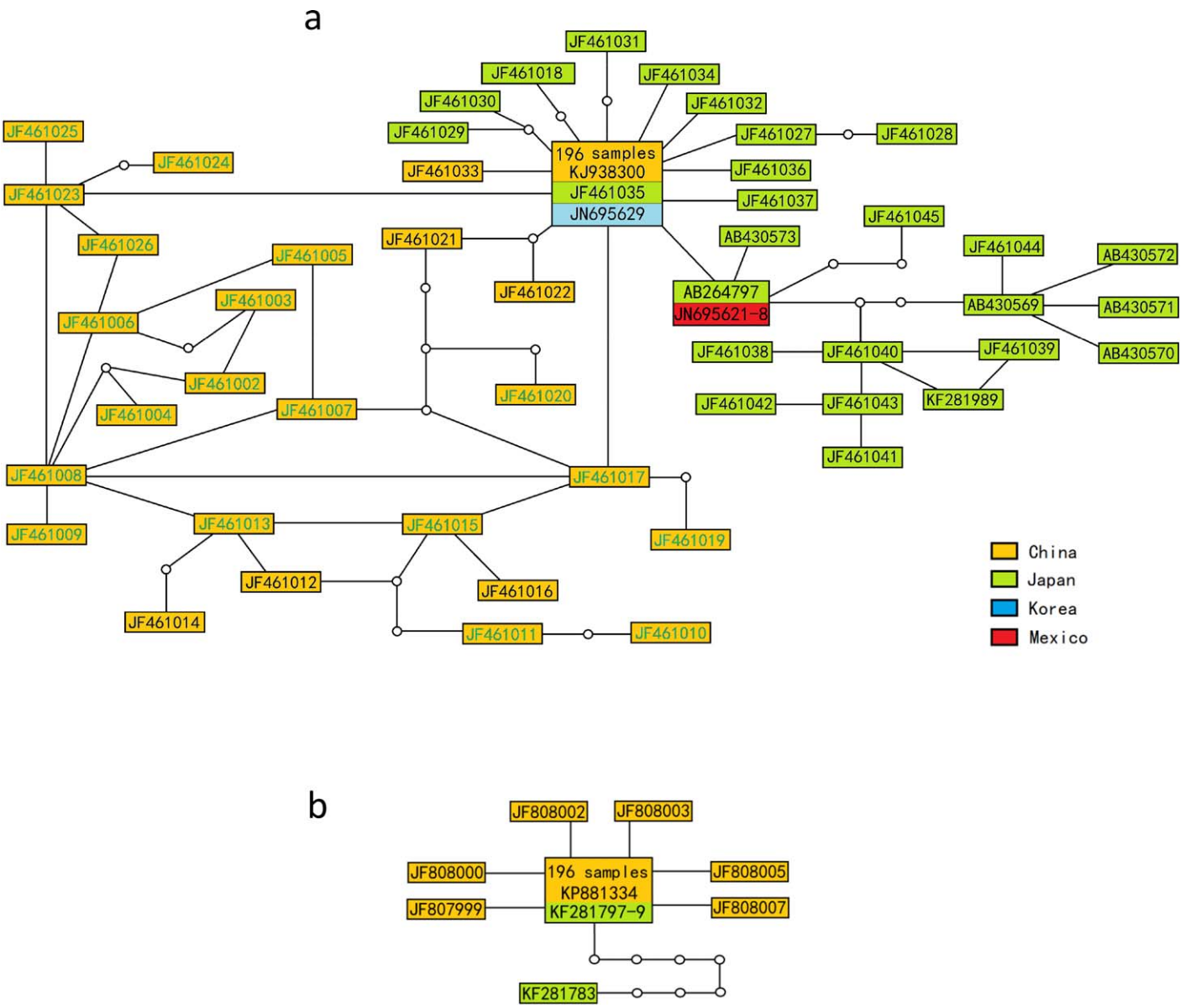

Fig. 4. Haplotype networks based on aligned sequences of (**a**) partial *cox3* sequences, and (**b**) *rbc*L-S spacer sequences from the 196 *S. horneri* samples as well as the GenBank data. Each line between two connecting haplotypes corresponds to one base substitution. Haplotypes are colored according to the sources. The letters in green color indicated the 19 haplotypes previously found in the Yellow Sea. [Color figure can be viewed at wileyonlinelibrary.com]

analyzed by a one-way analysis of variance (ANOVA) with Duncan's multiple range test. All values cited in this paper were obtained from fully independent samples.

PCR amplification and sequencing

Two DNA markers often used in phylogenetic or population genetic studies in *Sargassum* were employed for this study: (1) the partial *cox3* in mitochondrial genomes and (2) the *rbc*L-S spacer region (with partial *rbc*L and *rbc*S genes) in plastid genomes (e.g., Uwai et al. 2009; Hu et al. 2011). The sequences of partial *cox3*, and *rbc*L-S spacer region in the 196 *S. horneri* samples were amplified using primers published in Kogame et al. (2005) and Mattio et al. (2008), respectively. To further investigate the genetic diversity of floating *S. horneri* populations, four novel mitochondrial DNA markers including regions of *rnl-atp9*, *rps12-rps7*, *rpl5-rps3*, and *cob-cox2*, were developed in this study (Table 2), based on the complete mtDNA sequence of *S. horneri* (KJ938300) (Liu et al. 2015). These markers exhibit high resolution at the intraspecific level.

PCR amplification was carried out using Tag PCR Master Mix (Tiangen Biotech, China). Amplification was initiated

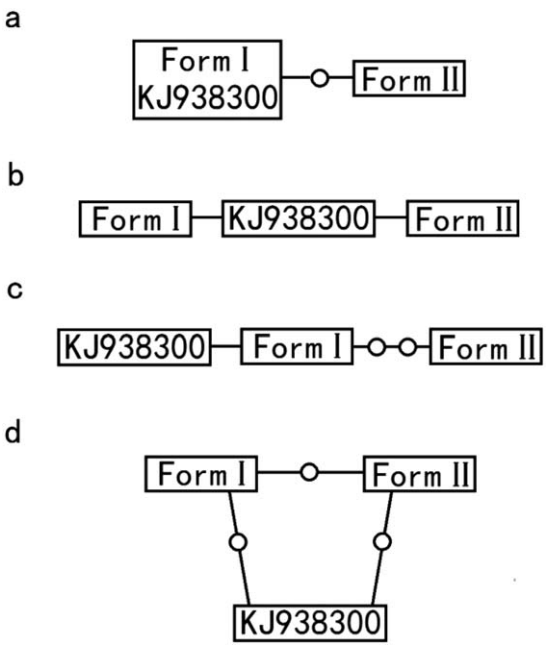

Fig. 5. Haplotype networks based on aligned sequences of (**a**) *rnl-atp9*, (**b**) *rps12-rps7*, (**c**) *rpl5-rps3*, and (**d**) *cob-cox2* regions from the *S. horneri* samples. Each line between two connecting haplotypes corresponds to one base substitution.

with denaturing at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing at 50°C for *cox3* and *rbc*L-S and 55°C for all four novel markers for 35 s, and 72°C for 40 s and then a final extension at 72°C for 10 min. Amplified products using different DNA markers were cut from the gel and purified using a DNA Gel Extraction Kit (Bio Basic, Canada). Sequencing reactions were performed from both sides using ABI 3730 XL automated sequencers (Applied Biosystems, U.S.A.) by Shanghai Personal Biotechnology, Ltd. When necessary, the sequencing reaction would be conducted twice to ensure the accuracy of the DNA sequences.

Phylogenetic and TCS analyses

DNA sequences of the 196 *S. horneri* samples in this study, as well as all available data from GenBank (Supporting Information Tables S1, S2), were subjected to concatenated alignments using ClustalX 1.83 with the default settings (Thompson et al. 1997). The phylogenies inferred from the partial *cox3* gene (469 bp) were constructed with the maximum likelihood (ML) method using MEGA v.7.0 (Kumar et al. 2016) and the Bayesian inference (BI) method using MrBayes v.3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The ML tree was obtained with 1000 bootstrap replicates based on the Kimura two-parameter model (Kimura 1980). For the BI tree, four Markov chains were run for 1,000,000 generations to provide adequate time for convergence (sampling every 1000 generations). The 10% of sampled trees were discarded as burn-in and the remaining trees were used to estimate the 50% majority rule consensus tree and the Bayesian posterior probabilities. Positions containing gaps and missing data were eliminated in DNA sequences. The computer software TCS v1.21 was utilized to construct the haplotype network for each DNA marker dataset (Clement et al. 2000). Final results were presented using gaps as fifth state in DNA sequences.

Table 4. Composition percentage (%) of two forms (I and II) in the *S. horneri* samples collected in the Yellow Sea based on the novel mitochondrial DNA markers.

	n			Composition percentage (%)	
Source ID*	**Total**	**Form I**	**Form II**	**Form I**	**Form II**
YSa (F)	10	4	6	40.00	60.00
RCa (B)	5	3	2	60.00	40.00
SSa (R)	2	1	1	50.00	50.00
SSb (F)	15	8	7	53.33	46.67
SSb (BM)	10	7	3	70.00	30.00
SSc (F)	6	4	2	66.67	33.33
YSb (F)	27	15	12	55.56	44.44
RZ (B)	5	3	2	60.00	40.00
SSd (F)	20	13	7	65.00	35.00
SSd (BM)	15	9	6	60.00	40.00
SSd (R)	9	7	2	77.78	22.22
YSc (F)	23	15	8	65.22	34.78
QD (B)	8	5	3	62.50	37.50
YT (B)	8	3	5	37.50	62.50
RCb (R)	20	15	5	75.00	25.00
YSd (F)	8	1	7	12.50	87.50
YSe (F)	5	3	2	60.00	40.00
Total	196	116	80	59.18	40.82

* Please refer to Table 1 on the abbreviation of sources ID.

Results

Morphological observation

In the Yellow Sea, the drifting seaweed rafts observed in 2016 and 2017 were composed almost totally of one species, *S. horneri* (Figs. 1, 2). Although the drifting thalli of *Sargassum confusum* had been found in the Subei Shoal, it accounted for a very small proportion of the drifting biomass (Liu Feng observation). A high proportion of floating *S. horneri* thalli (70.37%) bore receptacles in early March, while the floating thalli with receptacles only accounted for 47.37% of the samples in late May. The plants carrying receptacles occupied a higher percentage in bottom-collected thalli when compared to floating thalli in two bottom trawl surveys (Table 3).

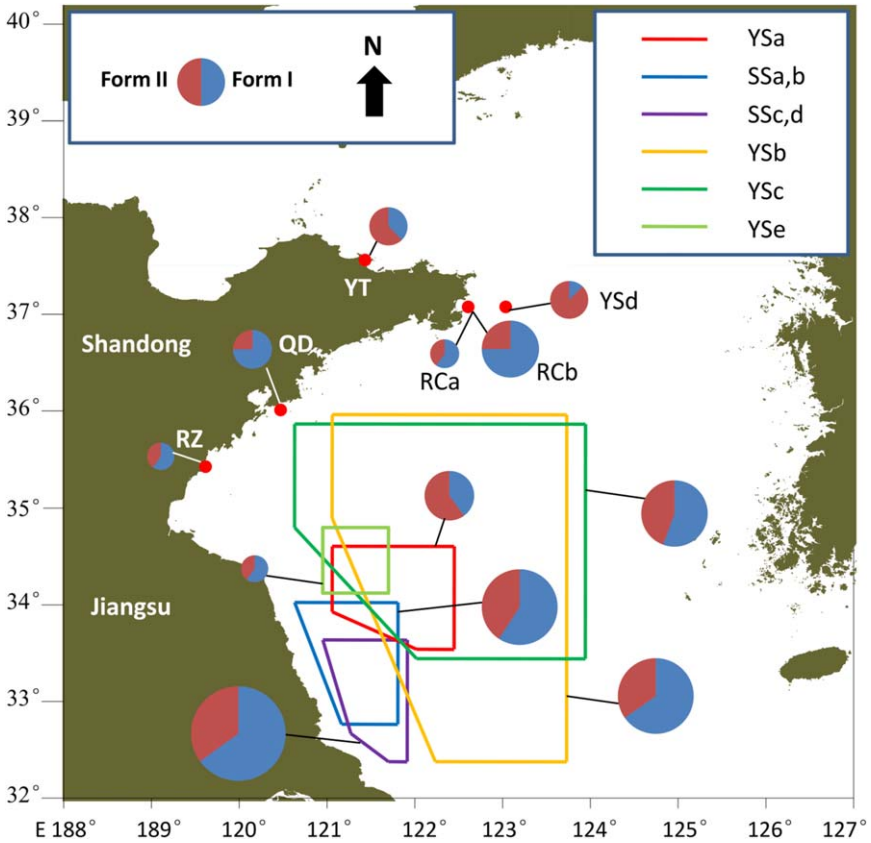

Fig. 6. The composition of two forms (form I in blue color and form II in red) of *S. horneri* in the large-scale spatio-temporal sampling. Circles are sized proportionally to frequency of occurrence in the Yellow Sea. The line in different colors represents our sampling ranges during cruises. [Color figure can be viewed at wileyonlinelibrary.com]

Five morphological parameters were compared between the drifting thalli and the bottom-collected thalli of *S. horneri* for each cruise (Table 3). No difference was observed in the diameter of main axes, vesicle length, and receptacle density and length. It is important to note that the floating thalli contained a large number of gas-filled vesicles, while the bottom-collected thalli only had a few shriveled or incomplete vesicles (Fig. 2d), if any at all as most of the vesicles appeared to have fallen off. The vesicle density of the drifting thalli was 24.9 ± 5.7 per 10 cm in March and 32.0 ± 7.8 per 10 cm in May. This was significantly higher than that of the bottom-collected thalli ($p \leq 0.001$. Supporting Information Table S3), which was at 14.3 ± 3.3 per 10 cm in March and 12.4 ± 5.8 per 10 cm in May.

Molecular analysis based on partial *cox3* and *rbcL*-S spacer

Alignments of partial *cox3* (469 bp), and *rbc*L-S spacer (597 bp) datasets from the 196 *S. horneri* samples and additional data from GenBank showed no difference in sequences of both partial *cox3* and *rbc*L-S spacer region among the 196 *S. horneri* samples as well as the benthic sample (KJ938300 and KP881334) from Nanji Island in China. This suggests a very low genetic diversity of the floating biomass of *S. horneri* in the Yellow Sea.

Based on the ML and BI methods implemented, the topological tree of partial *cox3* sequences of *S. horneri* was found to be polyphyletic (Fig. 3). The *cox3* dataset was divided into three clades which contained 51 (in clade A), 4 (B) and 3 (C) haplotypes, respectively, with high support values (72–100%). Two basal clades (B and C) consisted of specimens only from Japan. The network of the 51 haplotypes in clade A display the relationship and genetic variation of the samples from China, Japan, Korea, and Mexico (Fig. 4a). The samples from the Yellow Sea had the same *cox3* sequence as JF461035 from Japan and JN695629 from South Korea and were positioned in the junction between specimens previously collected from Chinese coastlines and those from Japanese lineages. However, a total of 19 haplotypes in partial *cox3* sequences previously reported in the Yellow Sea were not found in this study (Supporting Information Tables S1, S2. Hu et al. 2011, Wang et al. 2015). The haplotype network for the *rbc*L-S spacer sequence showed that the samples from the Yellow Sea were connected to other specimens previously collected from Chinese coastlines (Fig. 4b).

Molecular analysis based on novel mitochondrial DNA markers

Four novel mitochondrial DNA markers from regions of *rnl-atp9* (625 bp), *rps12-rps7* (514), *rpl5-rps3* (1054/1053), and *cob-cox2* (890) were designed to understand the genetic diversity of the floating *S. horneri*. The results of molecular typing using each of the four DNA markers were consistent for all four markers. The 196 *S. horneri* samples collected in the Yellow Sea could be distinguished into two forms (I and II) (Fig. 5). A total of 116 samples were identified as form I, occupying 59.18% of all *S. horneri* samples, and the remaining 80 samples belonged to form II (Table 4). Three DNA markers including *rnl-atp9*, *rps12-rps7*, and *cob-cox2* regions, had two variable nucleotide sites in the alignment between the two forms, while three sites were detected in the *rpl5-rps3* region, including two base substitutions and one insert/deletion mutation of A in the *trnG-orf129* intergenic spacer. These two forms were different from the benthic *S. horneri* sample collected at Nanji Islands in the East China Sea (Fig. 5).

Both forms of *S. horneri* were observed to have a large-scale distribution in the seaweed rafts found in the Yellow Sea in 2016 and 2017, the biomass stranded on the beaches of Yantai, Rongcheng, Qingdao, and Rizhao, as well as on the macroalgal cultivation rafts found at the *Saccharina* farms in Rongcheng and the *Pyropia* farms in the Subei Shoal (Table 4). Although the two forms varied in proportions at the various locations, it is worth noting that they coexisted in each of the spatio-temporal samples (Fig. 6). These results indicated that the floating *Sargassum* biomass in the Yellow Sea most likely came from two dominant haplotypes in the Chinese populations of *S. horneri*.

Discussion

The unusual floating *Sargassum* biomass in winter

In the past, floating *S. horneri* biomass was frequently observed to appear in the Subei Shoal and the Yellow Sea in spring or early summer, but not in winter. This is the first report of large-scale floating mass of *S. horneri* appearing in winter in this region. Massive drifting *Sargassum* biomass was stranded on *Pyropia* cultivation rafts and sandy shores from December 2016 to May 2017 (Fig. 1). Due to wave and tide action, the floating mass destroyed *Pyropia* cultivation infrastructure, leading to an economic loss of at least ¥0.5 billion (Chinese yuan) in the nori industry in Jiangsu Province (China Xinhua News 2017). In late spring, drifting biomass was observed to move southeastward from the Subei Shoal based on satellite data and field investigation.

In the field bottom trawl survey, we observed that thousands of tons of *S. horneri* biomass sank to the bottom of the Yellow Sea. The thalli that sank to the bottom of the sea carried more receptacles than floating thalli. It seemed that a large number of receptacles increased the density rather than the buoyancy, causing thalli carrying many receptacles to sink to the bottom. Furthermore, we observed that the bottom biomass carried fewer vesicles than the floating thalli and many vesicles had fallen off from the branches. This was probably caused by the harsh environmental conditions as the muddy seawater severely reduced the availability of light. The decrease in vesicle density combined with the muddy seawater would make it impossible for these benthic *S. horneri* thalli to float to the surface again. Although most of thalli that sank to the bottom were fertile, it is highly unlike for them to complete their life cycle under such stressful conditions.

The variation in maturation of *S. horneri* along the different coastal sites may be due to differences in water temperature (Yoshida et al. 2001). Natural *S. horneri* thalli can produce zygotes in spring and autumn in Japan (Uchida and Arima 1993). The spring-fruiting type of *S. horneri* is found in the southern and central part of Japan and the autumn-fruiting type is distributed along the northern Japanese coasts (Yoshida et al. 1998). Our field studies showed that most thalli floating in the Subei Shoal, as well as those that sank to the bottom, had receptacles in spring (March to May), indicating that the floating *S. horneri* biomass resembled the spring-fruiting type. A considerable proportion of floating thalli with no receptacles and thalli stranded on *Pyropia* cultivation rafts continued to maintain a vegetative growth state in late May, indicating that the floating *S. horneri* populations was made up of plants with temporal differences in sexual reproduction.

Low genetic diversity of the floating *S. horneri* in the Yellow Sea

The study showed that the floating *S. horneri* in the Yellow Sea displayed a very low genetic diversity. Previous studies reported 19 haplotypes of *S. horneri* in the Yellow Sea identified using partial *cox3* sequences (Hu et al. 2011; Wang et al. 2015). Despite the large-scale spatio-temporal sampling and the use of multiple DNA markers in this study, only two forms (haplotypes) of *S. horneri* were identified in the floating *S. horneri* thalli in the Yellow Sea.

Recent years have seen phenomena such as green tides and jellyfish blooms (Dong et al. 2010; Liu et al. 2013) become the norm in the Yellow Sea. Furthermore, the past 11 yr have seen the reoccurrence of large-scale drifting *Ulva* biomass in the Yellow Sea exhibiting similar features including a low genetic diversity in the bloom-forming alga *Ulva prolifera* (Liu et al. 2013). The emergence of different ecological disasters alarms us to the rapid change in the ecosystem of the Yellow Sea. More research is needed to determine the relationship between the appearance of large-scale seaweed biomass and possible changes in environmental factors such as temperature, nutrients, and currents.

Substratum and freshwater discharge from the Yangtze River estuary could provide a phylogeographical barrier for

rocky shore species such as *S. horneri* (Wang et al. 2015). However, the fact that *S. horneri* can be transported long distances when in its floating state by currents might contradict the phylogeographical barrier theory. The long-distance drifting capacity of the floating biomass of *S. horneri* could explain the extensive distribution of *S. horneri*, including its appearance on the North American coastline (Marks et al. 2015; Kaplanis et al. 2016), which may lead to intra-specific competition and crosses between different populations, as well as the shift in genetic structure of native populations.

Source of the floating *Sargassum* biomass in the Yellow Sea

Tracking the source of the drifting *Sargassum* biomass is the key to understanding the reoccurrence of golden tides (Laffoley et al. 2011). Floating *S. horneri* biomass originates from attached thalli in the subtidal zones. The *Sargassum* beds grow on deeper and more stable substrata than kelp beds (Terawaki et al. 2003). Most thalli detach before maturation due to the positive buoyancy of the vesicles. In the East China Sea, *S. horneri* was found to detach from the Chinese coasts of Zhejiang Province between January and March, and the floating biomass transported to the fringes of the continental shelf towards waters influenced by the Kuroshio Current from March to May (Komatsu et al. 2007, 2008; Lin et al. 2017; Qi et al. 2017).

Satellite data indicated that the initial site of floating *Sargassum* in the Yellow Sea was near the eastern end of the Shandong Peninsula, China, after which it moved southward with the current and wind (Xing et al. 2017). At present, there is no direct evidence to show large *S. horneri* beds distributed along the Shandong coasts. However, there are more than 10,000 hectares of *Saccharina* cultivation sites along the eastern coasts of Shandong Peninsula. Large numbers of floating *S. horneri* thalli have been observed on the *Saccharina* cultivation rafts in winter and spring. These stranded *Sargassum* thalli can maintain a relative vegetative growth rate of 2–5% d^{-1}, accumulating into floating masses of seaweed.

The floating *S. horneri* identified in the Yellow Sea represented only two haplotypes, but where along the extensive Chinese coastlines the young seedlings came from (i.e., where they are found attached to the rocky substrates by a perennial holdfast) remained a mystery for both haplotypes. Despite the distinct difference, based on the novel DNA markers, between the two floating *S. horneri* forms and the benthic sample at Nanji Island of Zhejiang Province, there was no evidence to rule out their distribution in the southern coasts of China.

Due to the limitations of field investigations, we still know little about the natural distribution scale of attached *S. horneri* along the Chinese coastline and how, when and where the observed floating *S. horneri* thalli complete its life cycle. Furthermore, the sheer length of time (from November 2016 to July 2017) that the floating *S. horneri* biomass was observed, as well as the interannual variation of ocean currents in the Yellow Sea and East China Sea which played a key role in transporting the floating biomass, means emphasis must be placed on implementing an interdisciplinary approach to further understand the mechanisms of the golden tides occurring in the Yellow Sea and East China Sea. This will be the key to minimizing future economic losses and environmental damage by learning to manage and control the *Sargassum* golden tides.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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