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Analyses of the genetic structure of *Sargassum horneri* in the Yellow Sea: implications of the temporal and spatial relations among floating and benthic populations

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Abstract *Sargassum horneri* is one of the most common seaweeds in China growing on solid substrates in the sublittoral zones along the Chinese coast. Large-scale drifting biomass of *S. horneri* has been reported in the Yellow Sea since 2010. Huge amount of the stranded biomass of this alga on the cultivation rafts of *Pyropia yezoensis* has drawn wide attention after damaging the local *Pyropia* farming industry. The original sources of the drifting biomass in the Yellow Sea remain unknown. In this study, 16 populations including three benthic and 13 drifting ones were sampled from the Yellow Sea and the East China Sea. Results of microsatellite analyses revealed that there were significant genetic differentiations among most of the investigated populations. Cluster analysis demonstrated that the drifting individuals from the same year, rather than from the same sampling locations, were clustered together. The existence of marked admixture in six populations also implies that the same drifting population could be of different origins. These results provide evidence suggesting that the drifting populations, in particular the populations occurring in different years, originated from multiple sources. Satellite imagery observation combined with genetic analyses of more benthic and drifting populations in the future are expected to elucidate, on a clearer level, the exact locations of the origin of the drifting populations.

Introduction

Sargassum horneri (Turner) C. Agardh is a large conspicuous brown seaweed that usually forms submarine forest in the northwest Pacific coasts (Komatsu et al. 1982). It is one of the most common seaweeds in China with an extensive distribution ranging from Liaodong peninsula in the north to Guangdong province in the south (Tseng 2000). This alga has become the principal species of choice for reconstruction of seaweed beds in the Eastern Asian countries (Choi et al. 2003; Sun et al. 2009; Yoon et al. 2013). It plays important ecological roles in providing nursery sites for marine animals and contributes significantly to the removal of nutrients in the eutrophicated coastal water due to its large amount of biomass (Pang et al. 2009).

Early in the 1960s Yoshida (1963) reported floating *Sargassum* species in Japanese waters. These drifting seaweeds originally grew on the rocky bottom. In spring, when the growing sporophytes became larger, the increased buoyancy of the growing plants with hundreds of vesicles might exceed the fixation force of the small disc-like holdfast, thus detaching the plants from the

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substratum. The buoyancy provided by the vesicles could keep the plants floating after detachment, forming the drifting biomass (Yoshida 1963). Similar to their benthic counterpart, the drifting biomass plays important ecological roles in offshore waters, providing not only spawning media for flying fish and Pacific saury, but also nursery sites for juveniles of commercially important pelagic fishes such as jack mackerel (*Trachurus japonicus*) and yellow tail (*Seriola quinqueradiata*). Konishi (2000) firstly reported drifting seaweeds of *S. horneri* along the front between the Kuroshio Current and continental waters in the East China Sea. The same phenomenon was found in 2002 and 2004. The abundance of drifting *S. horneri* between continental shelf peripheral area and the Kuroshio oceanic front was estimated to be higher than in any other area within the eastern East China Sea in March and May (Komatsu et al. 2008). It was also found by GPS monitoring system that the drifting *S. horneri* released from Zhejiang province, China in March 2005 were transported to the eastern East China Sea in 2 months (Komatsu et al. 2007). These facts suggest that *S. horneri* originatimg from the Chinese coasts in March or months earlier could be transported to fringe area of the continental shelf and waters influenced by Kuroshio Current from March to May (Komatsu et al. 2007; Mizuno et al. 2014). In 2012, drifting plants of *S. horneri* were also found to be distributed in the Kuroshio Current and its outer waters, and massive plants were observed on the northern coast of Taiwan and on Tarama Island in the Ryukyu Archipelago (Komatsu et al. 2014b).

Drifting *Sargassum* biomass has also been found in nearshore coastal waters of the Yellow Sea from 2010 to 2016, often accompanying the outbreak of the floating biomass of the green-tide alga *Ulva prolifera*. At the end of 2016, the huge *Sargassum* biomass that was stranded on the cultivation rafts of *Pyropia yezoensis* caused enormous economic damage to the industry of this commercially important seaweed (http://www.js.xinhuanet.com/2017-01/11/c_1120284277.htm). The accumulation of drifting *Sargassum* biomass in this *Pyropia* farming area has become a disaster, very tricky to deal with. The dominant *Sargassum* species has been identified as *S. horneri* by determining ITS2 and 5.8S rDNA-ITS sequences (Cai et al. 2014; Chen et al. 2016). Since 2013 floating clusters of *S. horneri* have been constantly found to be stranded on long-lines that are used to grow kelp species at Rongcheng and Lvshun (S.J. Pang and T.F. Shan 2013–2017, personal observations). The original sources of this drifting biomass in the Yellow Sea still remain unclear.

The objective of the present investigation is to analyze the genetic relationship and structures between/among the samples that we have constantly sampled from various locations along the Chinese coast from 2012 to 2016 using the microsatellite markers available in *S. horneri* at present (Shan et al. 2015; Kubo et al. 2017).

Materials and methods

Sample collection and genomic DNA extraction

Two hundred and seven individual samples were collected from seven locations in China and two locations in Korea during the period from 2012 to 2016 (Table 1, Fig. 1). Benthic *Sargassum horneri* populations are very sparse in the north of China, with few distributed in small islands far from the mainland, making it very difficult to collect them. With dramatic disappearing of biomass, benthic populations are also difficult to find in the south of China. As a result, only three benthic populations (NJ12, NJ13, and DS16) are incorporated in this study, and the others are floating ones. The two benthic populations NJ12, NJ13 and the floating one NJ15 were sampled in very near sites from Nanji Island in 2012, 2013, and 2015, respectively and regarded as three temporal populations. In general, the population codes are named after the abbreviations of the sampling locations plus the sampling year. The physical distance between any two sampled individuals in one location was at least 5 m in order to avoid collecting related plants. After sampling, leaf tips were dried and preserved in silica gel for genetic analysis. Genomic DNA was extracted from the dry specimen by the use of DNeasy Plant Mini Kit (Qiagen, Germany).

Microsatellite genotyping

A pilot screening step was performed to select the polymorphic microsatellite loci that could be easily and unambiguously amplified in all the sampled individuals. Eight comparatively polymorphic microsatellite markers SarACA09, SarACA18, SarHGA05, SarHGA10, SarHGA18, SarHGA35 (Kubo et al. 2017), Shorn27, and Shorn47 (Shan et al. 2015) were finally singled out for use. Polymerase chain reaction (PCR) amplification was performed in 20 μL volume containing $1 \times$ PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM fluorescent-labeled (forward) and unlabeled (reverse) primers, 0.5 U Taq DNA polymerase (TAKARA Biotechnology, China), and 10 ng of genomic DNA. PCR thermal cycle comprised an initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 30 s, locus-specific annealing temperature for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 7 min. Genotyping was carried out on an ABI 3730XL automated sequencer (Applied Biosystems, USA), and allele sizes were determined with GeneMapper version 4.0.

Data analyses

Number of total and effective alleles (N_a and N_e, respectively) observed and expected heterozygosity (H_o and H_e) were computed using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Unbiased genetic distance (Nei 1978) between populations

Table 1 Sampling sites, codes, coordinates, dates, and sample size (N) of *Sargassum horneri*

Sampling sites	Codes	Type	Coordinates	Dates	N
Nanji Island, Zhejiang	NJ12	Benthic	27° 27′ N, 121° 05′ E	2012-04-10	8
Nanji Island, Zhejiang	NJ13	Benthic	27° 29′ N, 121° 04′ E	2013-03-01	10
Dalian, Liaoning	DL13	Floating	38° 47′ N, 121° 16′ E	2013-03-20	9
Rudong, Jiangsu	RD13	Floating	32° 22′ N, 121° 34′ E	2013-04-20	9
Qingdao, Shandong	QD13	Floating	36° 03′ N, 120° 22′ E	2013-06-10	4
Dalian, Liaoning	DL15a	Floating	38° 47′ N, 121° 16′ E	2015-03-07	5
Shinan Jido, Korea	K15	Floating	34° 28′ N, 126° 16′ E	2015-01-08	1
Jeju, Korea	K15	Floating	33° 30′ N, 126° 32′ E	2015-01-21	1
Jeju, Korea	K15	Floating	33° 31′ N, 126° 34′ E	2015-01-21	1
Shinan, Korea	K15	Floating	34° 48′ N, 126° 17′ E	2015-01-28	1
Shinan, Korea	K15	Floating	34° 45′ N, 126° 18′ E	2015-01-29	1
Lidao, Shandong	LD15a	Floating	37° 13′ N, 122° 36′ E	2015-04-12	21
Nanji Island, Zhejiang	NJ15	Floating	27° 27′ N, 121° 03′ E	2015-05-04	10
Lidao, Shandong	LD15b	Floating	37° 14′ N, 122° 35′ E	2015-06-05	10
Dalian, Liaoning	DL15b	Floating	38° 47′ N, 121° 15′ E	2015-06-16	12
Lidao, Shandong	LD15c	Floating	37° 09′ N, 122° 30′ E	2015-07-16	12
Yushan, Zhejiang	YS16	Floating	28° 53′ N, 122° 15′ E	2016-03-26	14
Shidao, Shandong	SD16	Floating	36° 52′ N, 122° 26′ E	2016-12-20	34
Dalian, Liaoning	DL16	Floating	38° 47′ N, 121° 17′ E	2016-12-12	30
Dalian, Liaoning	DS16	Benthic	38° 47′ N, 121° 17′ E	2016-12-12	14

was calculated and used to construct a dendrogram by POPULATIONS 1.2.31 software (http://bioinformatics.org/~tryphon/populations/) using the neighbor-joining (NJ) clustering approach. During bootstrapping, 1000 permutations were performed to evaluate the robustness of the clusters. Pairwise population genetic differentiation was assessed by F_{st} values, which were computed using ARLEQUIN version 3.11 with 1000 permutations (Excoffier et al. 2005).

A Bayesian model-based clustering analysis was performed by the use of STRUCTURE 2.3.4 software to estimate the most likely number of genetic clusters (Pritchard et al. 2000). This clustering method was used to identify genetically distinct subpopulations on the basis of allele frequencies. The admixture model was applied and the number of clusters (K value) was set from 2 to 16 with five independent runs for each fixed number K. Each run included a burn-in length of 100,000 followed by 1000,000 MCMC (Monte Carlo Markov Chain) repetitions. The most likely value of K was determined based on the method described in Evanno et al. (2005) by submitting all results files of $K = 2$ to 16 to STRUCTURE HARVESTER (Earl and vonHoldt 2011). Among the five independent runs, the one with the highest Ln Pr ($X|K$) value (log probability) was chosen and represented as bar plots (Sitther et al. 2014).

Results

A total of 61 alleles was detected at the eight microsatellite loci across all populations, with an average number of 7.6 alleles per locus, ranging from 4 at locus SarHGA05 to 15 at locus SarHGA18 (Fig. 2). The average value of N_a across all loci was highest in DS16 (3.4) and lowest in DL15a, DL15b, and NJ15 (1.5), while that of N_e highest in DS16 (2.3) and lowest in DL13 (1.4). The highest values of H_o and H_e were found in RD13 and NJ13 populations, and the lowest values of them in DL13 population, respectively. Rare allele that was defined as having a frequency below 0.05 was found in LD15a, DL15c, YS16, SD16, DL16, and DS16. In general, the floating populations collected in 2016 had a higher genetic diversity than those collected in 2013 and 2015 in terms of N_a, N_e as well as H_e values.

Population NJ13 and SD16 had the highest average number of private alleles (0.9). All the populations of 2015 but LD15c had no private alleles. DL16 had no private alleles either. The genetic distance between populations ranged from zero to 1.356 (Table 2). Pairwise genetic distances between the populations of 2015 were very low, equal, or close to zero except for that between LD15a and others. The genetic distance between LD15a and YS16 was zero. The dendrogram, which was constructed by the use of the genetic distance matrix, demonstrated that two clusters were supported with bootstrap values above 60% (Fig. 3). One was comprised of the two benthic populations from Nanji Island (NJ12 and NJ13), and the other was comprised of LD15a, YS16, SD16, and DL16. The three floating populations of 2016 composed a subgroup with a bootstrap value of 84%. DL13 and DS16 were grouped together with a relatively low bootstrap value (51%). Most populations of 2015, including LD15b, DL15b, NJ15, and DL15a, were very close as shown by the topology
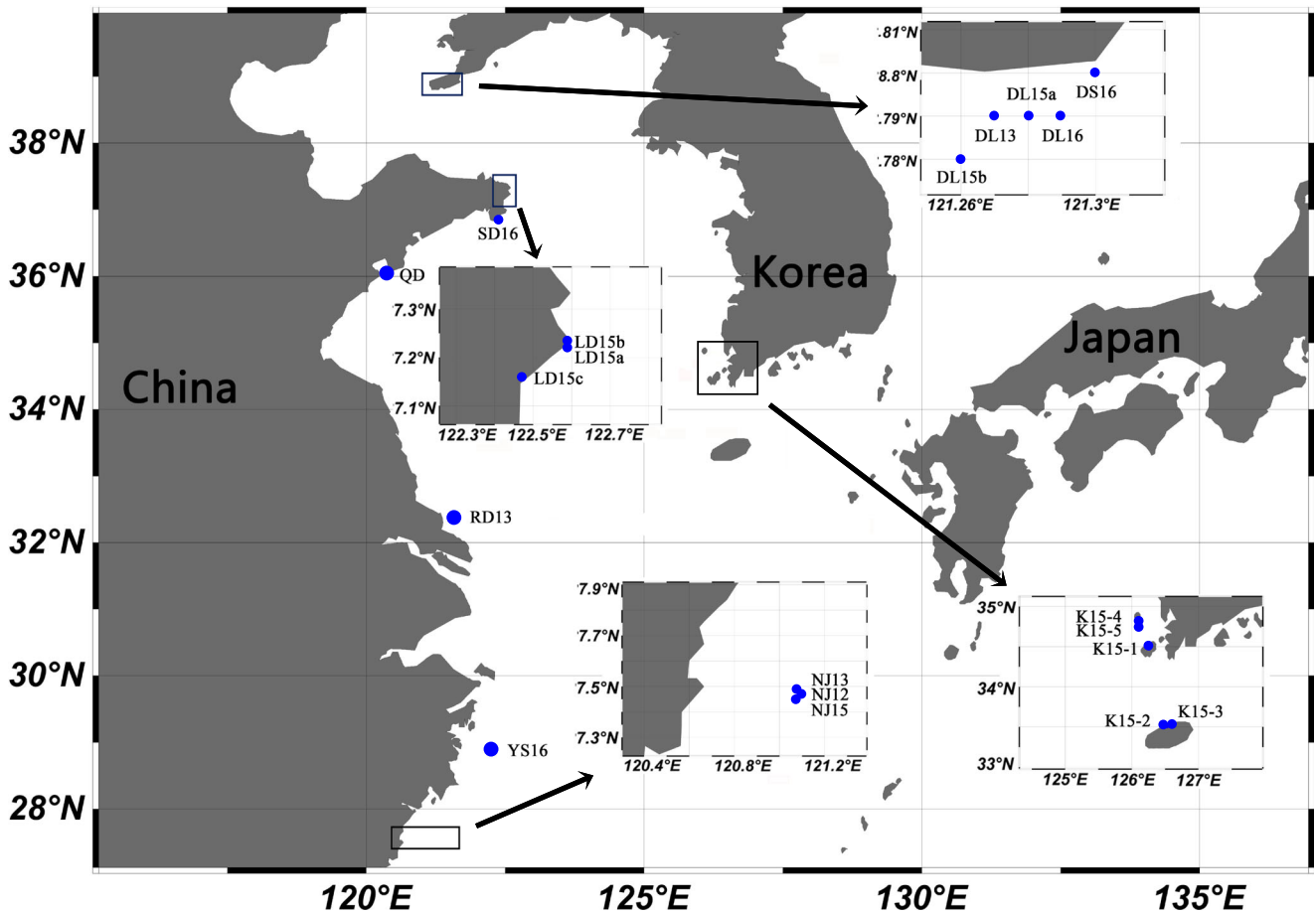
Fig. 1 A map of the sampling locations of the 16 populations of *Sargassum horneri* in the present study. The codes of each of the locations and the related information are shown in Table 1. NJ12, NJ13, and DS16 are benthic samples and the others floating ones

of the dendrogram. Similarly, there was no genetic differentiation ($F_{st} = 0$) among four floating populations of 2015, LD15b, DL15a, DL15b, and NJ15. The F_{st} value between

LD15a and YS16 was also zero. Although pairwise F_{st} values were higher than zero, the genetic differentiation among DL15a, K15, and LD15c was not significant ($P > 0.05$).
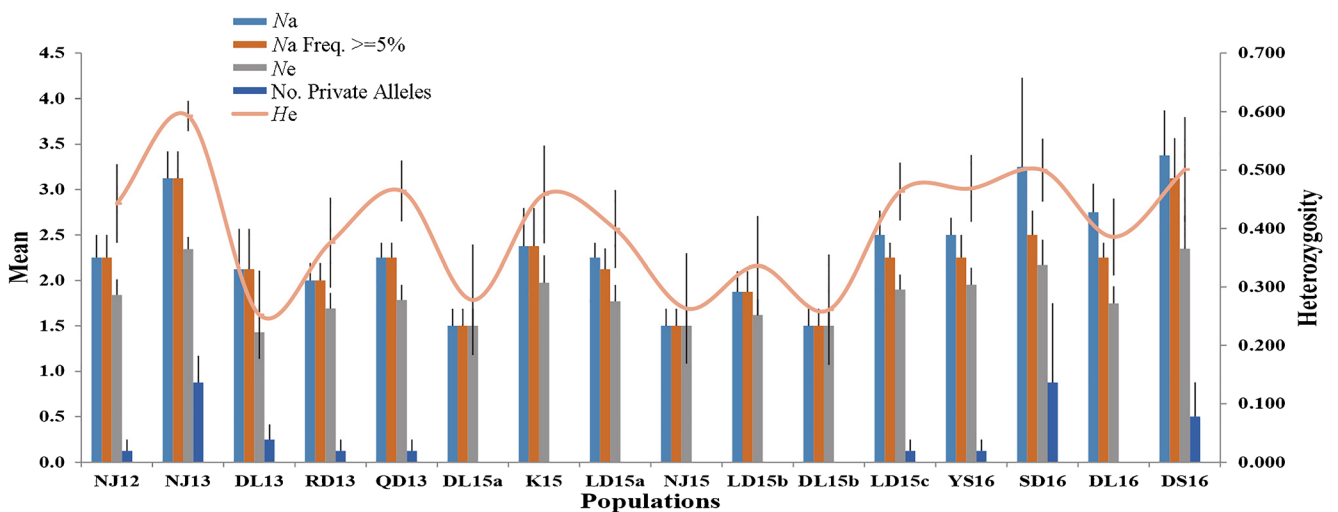

Fig. 2 Allelic patterns and the heterozygosity across the populations of *Sargassum horneri*. N_a, N_e, and H_e indicate the number of alleles, the number of effective alleles, and the expected heterozygosity,

respectively. Vertical bars indicate standard errors. The codes of each of the locations and the related information are shown in Table 1. NJ12, NJ13, and DS16 are benthic samples and the others floating ones

Table 2 Pairwise genetic distance (below diagonal) and F_{st} (above diagonal) between different populations of *Sargassum horneri*

	NJ12	NJ13	DL13	RD13	QD13	DL15a	K15	LD15a	NJ15	LD15b	DL15b	LD15c	YS16	SD16	DL16	DS16
NJ12		0.126*	0.490**	0.439**	0.350***	0.463**	0.349**	0.453**	0.498**	0.430**	0.507***	0.372**	0.404**	0.404**	0.496**	0.421**
NJ13	0.161		0.456***	0.361***	0.309***	0.384***	0.299***	0.404***	0.429***	0.375***	0.441***	0.324***	0.357***	0.376***	0.469**	0.360**
DL13	0.685	0.943		0.314***	0.373*	0.373***	0.223**	0.401***	0.386***	0.360***	0.388***	0.322***	0.379***	0.421**	0.555***	0.276**
RD13	0.773	0.751	0.232		0.160***	0.094***	0.098***	0.149***	0.126***	0.122***	0.132***	0.154***	0.174***	0.294***	0.470***	0.220***
QD13	0.581	0.769	0.313	0.132		0.129**	0.051	0.146*	0.172***	0.143***	0.181***	0.096	0.131*	0.220***	0.375***	0.222***
DL15a	0.721	0.774	0.237	0.055	0.073		0.002	0.155*	0.000	0.0000	0.000	0.063	0.189***	0.338***	0.510***	0.249**
K15	0.574	0.684	0.135	0.072	0.047	0.000		0.135*	0.039***	0.044***	0.046***	0.001	0.124***	0.244***	0.400***	0.148**
LD15a	0.882	0.936	0.437	0.120	0.123	0.114	0.112		0.182***	0.174***	0.188***	0.137***	0.223**	0.144***	0.304***	0.218**
NJ15	0.731	0.784	0.247	0.065	0.083	0.000	0.001	0.124		0.000	0.000	0.096*	0.223**	0.362***	0.527***	0.285**
LD15b	0.636	0.704	0.269	0.079	0.094	0.000	0.018	0.135	0.000		0.000	0.084*	0.201**	0.339***	0.499***	0.279**
DL15b	0.732	0.786	0.248	0.067	0.085	0.000	0.002	0.126	0.000	0.000		0.103*	0.231***	0.369***	0.531***	0.295**
LD15c	0.679	0.742	0.312	0.142	0.096	0.043	0.001	0.122	0.053	0.059	0.055		0.123***	0.209***	0.344***	0.212**
YS16	0.850	0.938	0.448	0.171	0.141	0.171	0.132	0.000	0.181	0.187	0.182	0.130		0.076***	0.215***	0.198**
SD16	0.994	1.162	0.740	0.436	0.320	0.512	0.370	0.149	0.522	0.525	0.523	0.287	0.081		0.036	0.300**
DL16	1.092	1.356	1.034	0.799	0.515	0.873	0.597	0.330	0.884	0.873	0.885	0.460	0.214	0.025		0.426**
DS16	1.084	1.093	0.269	0.255	0.321	0.276	0.181	0.247	0.286	0.333	0.288	0.288	0.265	0.559	0.814	

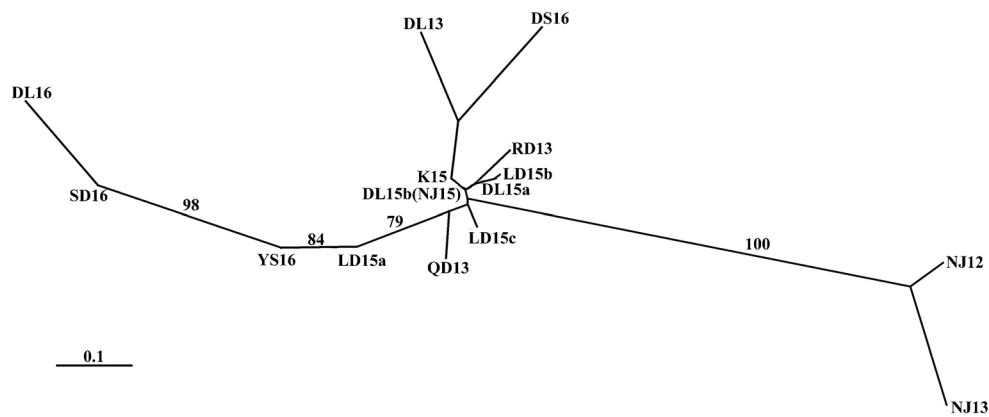*indicates significance at $P < 0.05$, **indicates significance at $P < 0.01$ for F_{st} values

Fig. 3 The neighbor-joining dendrogram based on unbiased genetic distances among different populations of *Sargassum horneri*. Bootstrap values greater than 60% are indicated. The scale bar indicates the genetic distance. The codes of each of the locations and the related information are shown in Table 1. NJ12, NJ13, and DS16 are benthic samples and the others floating ones

There was also no significant genetic differentiation between QD13 and K15, between QD13 and LD15c, or between SD16 and DL16. Significant genetic differentiations were detected among all the other populations ($P < 0.05$).

The most likely number of K was determined to be 4 by using STRUCTURE HARVESTER based on the ΔK value. Ninety-one percent of the individuals were assigned to one of the four clusters with high proportion of membership ($Q > 0.8$); however, marked admixture was found in populations QD13, K15, LD15a, LD15c, YS16, and SD16. NJ12 and NJ13 formed the first cluster with an average Q value of 0.988 (100% of them with Q value > 0.8). The second cluster mainly consisted of DL13 and DS16 with an average Q value of 0.927 (91.3% of them with Q value > 0.8). The third cluster was primarily comprised of RD13, DL15a, NJ15, LD15b, and DL15b with an average Q value of 0.951 (95.6% of them with Q value > 0.8). The fourth cluster was mainly represented by DL16 with an average Q value of 0.828 (Fig. 4).

Discussion

The most important discovery of this investigation is the finding that the drifting populations from the same year, rather than from the same sampling location, were clustered together. For example, drifting populations from Dalian, Lidao (LD15b) and Nanji in 2015 were clustered together, whereas

drifting populations from Dalian collected in three different years were of different ancestries. The existence of marked admixture in six populations (QD13, K15, LD15a, LD15c, YS16, and SD16) also implies that the same drifting population was of different origins. What is more is that the drifting populations from the same year (2015 and 2016) were originated from very limited number of sources. Combined these results provide evidence implying that the drifting populations in the Yellow sea, in particular the populations occurring in different years, originated from multiple sources. Accumulation of floating *S. horneri* populations near Korea has also drawn much attention from Korean people (E. K. Hwang, personal communication, 2016–2017), who are wondering where the floating algae come from. Limited by the international transportation, only five individuals were included in this study. Their similarity to some Chinese populations such as QD13, DL15a, and LD15c, however, preliminarily suggests they might be of the same origins.

Equally important is the finding that drifting individual of this species could complete its life cycle, shed zygotes into the surrounding waters, and eventually form sessile populations as has been discovered in this study. DL13 and DS16 were clustered together. DL13 was a drifting population collected in a seaweed farm adjacent to a project site where artificial concrete reefs were constructed by filling up the shallow nearshore waters. This project was completed in 2013. Sessile *S. horneri* were thereafter discovered at this location in 2016
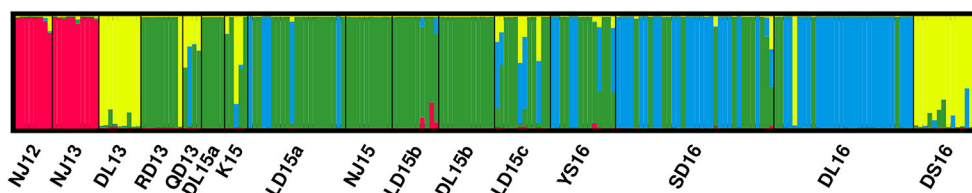

Fig. 4 Estimated genetic structure resulted from the Bayesian model-based analysis by using STRUCTURE 2.3.4 for populations of *Sargassum horneri*. Each individual is represented by a vertical colored bar, and the proportion of the color in each bar represents the probability of membership in the relevant cluster. The codes of each of the locations and the related information are shown in Table 1. NJ12, NJ13, and DS16 are benthic samples and the others floating ones

(DS16). The close genetic relationship of these two populations suggests that the drifting population could generate offspring colonizing the waters it passes by as long as the environment is suitable.

Disappearance of the once benthic seaweed species coincident with the unusual distribution of the drifting populations in the Yellow Sea and the East China Sea has drawn much attention from academics (Komatsu et al. 2014a). Although benthic *S. horneri* populations were reported to be distributed all along the Chinese coast ranging from Liaoning to Guangdong province, prominent biomass has been rarely observed in the north of China. Natural populations of *S. horneri* have been reported to decline in some of the southern islands over the past 10 years (Sun et al. 2008). Benthic *S. horneri* now can hardly be observed on Nanji Island, an important natural conservation area in which conspicuous *S. horneri* beds used to exist (Q.H. Sun observation, 2014–2017). The two benthic populations (NJ12 and NJ13) sampled from Nanji Island in 2012 and 2013 were found to be closely related to each other, but different from the floating population (NJ15) collected from the near site. This suggests that is was unlikely that NJ15 had originated from the benthic populations in the near region.

Previous study has revealed that the drifting individuals of *S. horneri* originating from the sessile populations in Zhejiang province could be transported to the eastern East China Sea under the influence of the Kuroshio Current (Komatsu et al. 2007). This fact indicates that it is also possible that the drifting plants of *S. horneri* in the Yellow Sea are derived from the benthic populations living in the southern coastal waters of China. The decrease of the benthic populations of *S. horneri* in Zhejiang province correlated well with the recently observed increase of the drifting populations in the eastern East China Sea since 2000 (Komatsu et al. 2007). Benthic populations of *S. horneri* in northern China (from Liaoning to Shandong provinces) have been seldom investigated or reported. No reference samples in this region were obtained except the one collected from Lvshun (DS16). We do not know whether decrease of benthic populations in northern China exists. Thus, a systematic investigation on the benthic resources of *S. horneri* in northern China is needed in the future.

Never in history has the south of the Yellow Sea experienced a damaging "attack" of the drifting *Sargassum* biomass as large as the one in 2016. A few successful examples of using satellite image to track the source and scale of drifting *Sargassum* biomass suggest that the same method be applied in China in order to localize the right original sources of the drifting biomass of *S. horneri*. This could allow us to know what has been happening in the once "home" of this species. In 2011, satellite image analysis showed that the *Sargassum* raiding the Caribbean islands and the West Africa originated from the northern coast of Brazil (Gower et al. 2013; Smetacek and Zingone 2013). The same technique has helped

people to understand the full distribution and movement of the population of *Sargassum* in the Gulf of Mexico and western Atlantic in the years from 2002 to 2008 (Gower and King 2011).

Based on the results of these investigations, a more extensive sampling of both benthic and drifting populations along the Chinese coast should be performed and samples should be submitted to genetic analysis. In combination with the satellite image information, the original niches of the drifting biomass are expected to be elucidated.

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