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AFLP analysis revealed a north to south genetic break in the brown alga *Sargassum thunbergii* along the coast of China

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Abstract

Sargassum thunbergii, a common brown macroalga that occurs in the Asian Northwest Pacific, has important ecological services and economic values. In recent years, the distribution range and biomass of wild *S. thunbergii* resources have declined rapidly due to severe anthropogenic impacts and habitat loss. To slow the decline of this natural resource by taking appropriate measures, it is very important to evaluate the population genetic structure and diversity of *S. thunbergii*. In this study, we used amplified fragment length polymorphism (AFLP) markers to investigate population genetic differentiation and diversity of *S. thunbergii* along the coast of China at a genome-scanning scale. Five primer combinations that yielded high polymorphisms were identified in ten populations of *S. thunbergii*. Genetic analyses revealed low genetic diversity within populations and high levels of genetic differentiation among populations. Moreover, apparent gene flow occurred between *S. thunbergii* populations in the Yellow-Bohai Sea, whereas no such exchange was detected in the populations of the East China Sea. Genetic clustering, principal coordinates analysis (PCoA), and pairwise F_{ST} estimates consistently indicated that *S. thunbergii* populations divided into two genetic groups, coinciding with their geographical distribution (Yellow-Bohai Sea vs. East China Sea). The detected genetic diversity of *S. thunbergii* along the coast of China provides important insights not only for selecting high-yielding varieties for commercial cultivation, but also for proposing strategic management plans to protect and restore declining natural resources under anthropogenic climate change.

Keywords Phaeophyceae · Molecular markers · Genetic diversity · Population structure

Introduction

Sargassum thunbergii (Fucales, Phaeophyta) is a common brown macroalga that occurs in the intertidal and shallow sublittoral zones of the Asian Northwest Pacific (Aral et al. 1985; Koh et al. 1993; Cho et al. 2012; Kim et al. 2016). As an important component of the summer algal flora in Japan, it is distributed throughout the Archipelago, which is located along the coasts of both the Japan Sea and the Pacific Ocean (Umezaki 1974). *Sargassum thunbergii*, which grows perennially on rocks near the low tide level along calm, open and sheltered coasts in Korea (Park et al. 2005), serves as a major habitat-forming species and can be used as an anthelmintic (Yang et al. 2016). In China, *S. thunbergii* occurs in warm and temperate waters from northern Liaodong Peninsula to southern Leizhou Peninsula (Tseng 1983). Similar to most other large brown algae, *S. thunbergii* plays essential roles in maintaining coastal ecosystem services and marine economic development. Its large biomass and high productivity allow *S. thunbergii* to form seaweed beds, providing feeding and sheltering habitats for invertebrates and fish in the intertidal

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zone (Liu et al., 2016a). Because of its capacity to absorb heavy metal ions, *S. thunbergii* can be used for bioremediation of ocean contamination (Davis et al. 2003, 2004). In the past decades, *S. thunbergii* has been a predominant seaweed resource of alginate and medical extracts (Park et al. 2005; Seo et al. 2007; Itoh et al. 1993; Zhuang et al. 1995) and has been widely used as animal feed in the aquaculture of holothurians or abalone in China (Liu et al., 2016a), Japan (Battaglene et al. 1999), and Korea (Seo et al. 2011).

In recent years, the rapid development of holothurians and abalone aquaculture in China has resulted in massive hand-harvesting of *S. thunbergii* used as aquaculture feed (Sun et al. 2007; Wang and Liu 2007), leading to extensive consumption of natural populations and susceptible units of *S. thunbergii*. In some areas along the coast of China, *S. thunbergii* populations are on the verge of depletion, with high risk of loss or extinction due to increased anthropogenic interference. Therefore, there is an urgent need to explore the population genetic diversity of *S. thunbergii* along the coast of China, with the aim of enhancing the protection of natural resources and selecting improved varieties for artificial cultivation (Liu et al. 2012; Liang et al. 2014).

Genetic diversity, the foremost of biodiversity (Hamrick et al. 1991), is highly correlated with germplasm resources and the sustainability of populations (Rao and Hodgkin 2002), showing important potential in evolution, breeding, and variety improvement (Yi et al. 2010). Evolutionary biological evidence illustrates that species with high genetic diversity are more likely to adapt to environmental changes (Lebret et al. 2012). Recently, population genetics of *Sargassum* species have been studied at different geographical scales. Phylogeographical studies of *Sargassum* species (e.g., *S. hemiphyllum*, *S. horneri*, *S. fusiforme*, and *S. thunbergii*) showed that oceanic surface currents and paleoclimate change made a major contribution to genetic population differentiation and biogeographical gradients in the northwestern Pacific (Cheang et al. 2010; Hu et al. 2011, 2013, 2017; Li et al. 2017). Yu et al. (2013) observed low genetic diversity in *S. horneri* along the coast of China by using inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP). Using sequences of mitochondrial *cox*3 (cytochrome c oxidase subunit 3) and *trn*W-I (mitochondrial tRNA W-I spacer), Bae et al. (2013) compared the population genetic diversity of *S. muticum* in Korea, Japan, USA, and Europe and suggested a native diversity center. Zhao et al. (2008) and Liu et al. (2013) detected low genetic variability of *S. muticum* populations in Shandong Peninsula, China, using random amplified polymorphic DNA (RAPD), ISSR markers, and three gene sequences. By applying RAPD and ISSR, Zhao et al. (2007) also assessed the population genetic variation of *S. thunbergii* in Shandong Peninsula, China, and reported a typical isolation by distance (IBD) model. However, population genetic structure and diversity at a wide regional scale in China remains unexplored.

Amplified fragment length polymorphism (AFLP) is characterized by sensitivity, efficiency, high stability polymorphisms, and reproducibility (Vos et al. 1995; Donaldson et al. 1998; Shan and Pang 2009; Liu et al. 2011) to detect subtle population genetic variation based on strict protocols (Vos et al. 1995). More importantly, the high reproducibility of AFLP has been verified in population genetics (Belaj et al. 2003; Pejic et al. 1998) and germplasm assessment (Powell et al. 1996; McGregor et al. 2000). In the past decades, AFLP has been applied in the fields of linkage mapping, population-based phylogenetic assessment (Albach 2007) and population genetic diversity of animals, plants, and algae (Donaldson et al. 2000; Li et al. 2013; Chen et al. 2015; Oliveira et al. 2015; Galaktionov et al. 2016; Yotsukura et al. 2016). In particular, AFLP markers have been proven to generate large numbers of polymorphic loci in multiple seaweed taxa (Donaldson et al. 1998; Müller et al. 2005; Shan and Pang 2009; Pang et al. 2010; Yi et al. 2010; Liu et al. 2011; Lebret et al. 2012; Li et al. 2013). For example, Shan and Pang (2009) detected subtle genetic variation in the brown alga *Undaria pinnatifida* using AFLP markers. Müller et al. (2005) reconstructed the phylogeny of *Chlorella vulgaris* by using AFLP datasets and found that the topological relationships were closer to the true organismal phylogeny than to phylogenies inferred from other molecular data. Liu et al. (2011) studied the genetic diversity of *Enteromorpha* (*Ulva*) *prolifera* and reported high reproducibility with AFLP markers. These studies demonstrated that AFLP markers are efficient and economical to assess population genetic diversity of target species.

In this study, we applied AFLP markers to evaluate the population genetic diversity and differentiation of *S. thunbergii* at ten geographical sites along the coast of China. The results can empirically supplement previous studies and can provide insights to protect and manage natural populations and/or slow the declining natural resources of *S. thunbergii* in China.

Materials and methods

Experimental materials

A total of 200 individuals of *Sargassum thunbergii* from ten localities were collected from the coast of China (Fig. 1 and Table 1). Twenty individuals were chosen from each locality at 10-m intervals along the transect to minimize the effect of asexual reproduction. Seaweed samples were washed with aseptic sea water and then preserved at −20 °C for genomic DNA extraction.

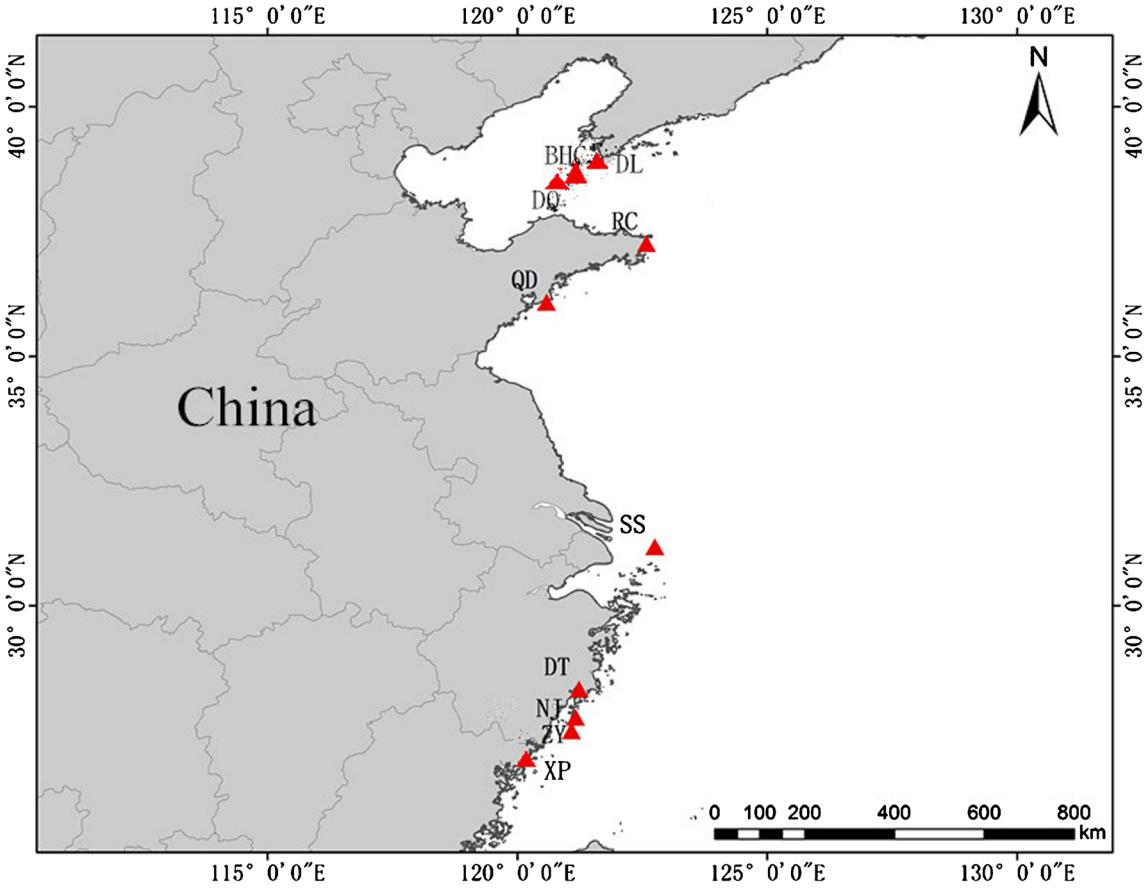

Fig. 1 Sampling locations of ten populations of *S. thunbergii* along the coast of China. Detailed locality information is shown in Table 1

DNA extraction and AFLP analysis

Thawed seaweed specimens were cleaned using distilled water and were dried with absorbent paper. Genomic DNA was extracted using the Plant Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. The size of the DNA was 23 kb, and the quality of the DNA was determined by checking the $OD_{260/280}$ (1.8–2.0).

Approximately 50 ng of DNA was double-digested with 8 U *Eco*R I (a relatively rare six-base cutter) and 4 U *Mse* I (a frequent four-base cutter) endonucleases (New England Biolabs, USA), 1× NEBuffer, and a total reaction mixture of 20 μL was incubated at 37 °C for 2 h, followed by 20 min at 65 °C to inactivate the endonucleases. After digestion, 10 μL of the reaction mixture (smeared size with 250–2000 bp) was checked using 1% agarose gels. The remaining 10 μL was ligated to 0.5 μM *Eco*R I and 5 μM *Mse* I adapters with 100 U of T4 DNA ligase (New England Biolabs) and 1× T4 DNA Ligase Reaction Buffer. Reaction mixtures (20 μL) were incubated overnight at 16 °C. Pre-amplifications were performed in a 25 μL reaction mixture containing 3 μL DNA for ligation, 10-μM primers with no selective bases (*Eco*R I + 0 and *Mse* I + 0; Vos et al. 1995), and 12.5 μL 2× Utaq PCR Master Mix. Based on the PCR protocol, reactions were denatured at 94 °C for 3 min, followed by 30 cycles with denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. Pre-amplification fragments (5 μL) were tested using 1% agarose gels (smeared sizes of 250–1000 bp). The pre-amplification products were diluted 100 times and were

Table 1 Sampling locations of ten populations of *S. thunbergii* along the coast of China

Sampling location	Longitude (E)	Latitude (N)
DL (Dalian, Liaoning)	121° 22′ 21	38° 49′ 20
BHC (Beihuangcheng, Yantai)	120° 90′ 29	38° 38′ 45
DQ (Daqin Island, Yantai)	120° 83′ 44	38° 30′ 31
RC (Rongcheng, Weihai)	122° 35′ 16	37° 15′ 07
QD (Qingdao, Shandong)	120° 35′ 33	36° 04′ 87
SS (Shengsi, Zhoushan)	122° 45′ 37	30° 71′ 43
DT (Dongtou, Wenzhou)	121° 14′ 08	27° 80′ 13
ZY (Zhuyu Island, Wenzhou)	121° 10′ 23	27° 46′ 28
NJ (Nanji Island, Wenzhou)	121° 05′ 04	27° 30′ 21
XP (Xiapu, Fujian)	120° 06′ 25	26° 89′ 24

used as templates for selective amplification. Subsequently, selective amplification was performed with five *Mse* I primers containing three selective nucleotides and two fluorescently labeled *Eco*R I primers containing two selective nucleotides (Table S1), which yielded ten primer combinations (*Eco*R I-*Mse* I). The PCR reaction was carried out in a volume of 25 μL containing 2 μL DNA template, 10 μM *Eco*R I primer, 10 μM *Mse* I primer, and 12.5 μL 2× Utaq PCR Master Mix using the following protocol: 3 min at 94 °C, 12 cycles of 30 s at 94 °C, 30 s at 65 °C (lowering the temperature by 0.7 °C per cycle), and 1 min at 72 °C, followed by 24 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, with a final step at 72 °C for 5 min before holding at 4 °C. In order to test the reliability and reproducibility of the AFLP procedure, two DNA isolations (replications) from each population of *S. thunbergii* were assayed using the five primer combinations, which yielded the average reproducibility of 96.9%.

Selective PCR products were separated by capillary electrophoresis using an ABI Prism 3730XL automatic sequencer (Applied Biosystems) with the GeneScan-500 LIZ size standard to create a multilocus genotype from a presence-absence binary matrix using GeneMarker v2.2.0 software (SoftGeneticsUSA).

Data analysis

AFLP loci with lengths between 50 and 500 bp were scored using GeneMarker and the analysis parameters referred to in the application notes (www.softgenetics.com/GeneMarker.php). We used the normalization implemented by GeneMarker during data collection to reduce gross variation in peak heights among the AFLP fingerprints. In addition, we set a locus selection threshold of 10% to obtain the number of retained loci. GenAlEx 6.5 (Peakall and Smouse 2006) was used to estimate multiple parameters related to population genetic variation and diversity, including the percentage of polymorphic loci (*PPL*), Nei's gene diversity, and Shannon's information index. Analysis of molecular variance (AMOVA) was conducted to detect the distribution of genetic variation within populations, among populations within grouped locations and among grouped locations using 999 permutations.

GenAlEx was also used to evaluate the genetic differentiation coefficient (F_{ST}); a value exceeding 0.25 indicates high genetic differentiation among populations (Balloux and Lugon-Moulin 2002). With respect to gene flow (*Nm*), if $Nm < 1$, there is a low gene flow between two populations, which cause genetic differentiation. If $Nm > 1$, there is a gene exchange between two populations (Slatkin 1985). If $Nm > 4$, gene flow is high and may prevent genetic differentiation to a certain extent (Whitlock and Mccauley 1999). Nei's genetic distance between populations (Nei 1972), a principal coordinates analysis (PCoA) based on Nei's genetic distance, and the Mantel test between genetic distance and geographic distance were also evaluated using GenAlEx.

A dendrogram showing the genetic relationships of ten populations was constructed based on Nei's genetic distance, using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA 6.0 (Tamura et al. 2013). Genetic structure was estimated from up to 20 individuals from each population using STRUCTURE 2.3.4; the process was conducted based on the method described by Evanno et al. (2005).

Results

AFLP polymorphism and genetic diversity

Five primer combinations yielding highly polymorphic loci were obtained in all ten populations after screening all ten pair of primers. In total, 832 loci were scored in 10 populations of *S. thunbergii* (200 individuals) by 5 primer combinations (Table 1, Table 2, and Table S1). After setting the locus threshold, 828 (99.5%) were polymorphic, and the number of loci generated by each primer combination ranged from 147 to 202. The loci generated by the primer combination *Eco*R I+AG/*Mse* I+ CAG were 100% polymorphic (Table 2). The percentage of polymorphic loci in each population ranged from 62.50–84.62%. The Beihuangcheng (BHC) population was characterized by the highest percentage of polymorphic loci, whereas the Shengsi (SS) population showed the lowest polymorphic value (Table 3).

The values of Nei's gene diversity ranged from 0.181 to 0.279, and those of Shannon's information index ranged from

Table 2 The five primer combinations used for AFLP analysis and associated polymorphisms in ten populations of *S. thunbergii* along the coast of China

Primer combinations	Total loci numbers	Polymorphic numbers	Proportion of polymorphism (%)
*Eco*R I + AA/*Mse* I + CAT	161	160	99.38
*Eco*R I + AA/*Mse* I + CAA	202	201	99.50
*Eco*R I + AA/*Mse* I + CCA	160	159	99.37
*Eco*R I + AG/*Mse* I + CAC	147	146	99.32
*Eco*R I + AG/*Mse* I + CAG	162	162	100.00
Total	832	828	99.52

Table 3 Genetic diversity index of *S. thunbergii* populations along the coast of China

	PPL	Nei's gene diversity	Shannon's information index
DL	80.29	0.242 ± 0.006	0.373 ± 0.008
BHC	84.62	0.279 ± 0.006	0.424 ± 0.008
DQ	73.08	0.236 ± 0.007	0.359 ± 0.009
RC	72.96	0.227 ± 0.006	0.348 ± 0.009
QD	77.52	0.234 ± 0.006	0.360 ± 0.009
SS	62.50	0.181 ± 0.006	0.281 ± 0.009
DT	79.45	0.260 ± 0.006	0.395 ± 0.009
ZY	63.22	0.209 ± 0.007	0.315 ± 0.010
NJ	72.60	0.226 ± 0.006	0.346 ± 0.009
XP	64.66	0.185 ± 0.006	0.288 ± 0.009
Mean	73.09	0.228 ± 0.002	0.349 ± 0.003

Polymorphic loci indicate the loci with the exception of common loci; common loci indicate the loci shared by all individuals within a population

PPL percentage of polymorphic loci

0.281 to 0.424, with the highest and lowest values detected in the BHC and SS populations, respectively (Table 3). Except for the Dongtou (DT) population, the percentage of polymorphic loci, Nei's gene diversity, and Shannon's information index for other populations exhibited comparable trends, with the northern populations exhibiting higher values than the southern populations (Table 3).

Genetic relationships and structure

AMOVA results revealed limited variation (17.49%) among grouped locations (Table 4), and the remaining 24.28 and 58.23% variations were found among populations within grouped locations and within populations, respectively, with all *F* values being statistically significant ($P < 0.001$, Table 4). The pairwise F_{ST} value revealed high levels of genetic differentiation in *S. thunbergii* (91.1% for $F_{ST} > 0.25$, $P < 0.001$, Table S2). *Nm* ranged from 0.538 to 6.558 among the ten populations; the highest value was found between the BHC and Daqin Island (DQ) populations, whereas the lowest *Nm* value was found between the Qingdao (QD) and SS populations (Table 5).

The smallest Nei's genetic distance (0.047) occurred between the BHC and DQ populations, whereas the largest variation (0.314) occurred between the QD and SS populations (Table 5). The UPGMA dendrogram based on genetic distance showed that the ten populations of *S. thunbergii* from the coast of China were grouped into two major clusters. The Dalian (DL), BHC, DQ, Rongcheng (RC), and QD populations from the Yellow-Bohai Sea formed one cluster, while the SS, DT, Zhuyu Island (ZY), Nanji Island (NJ), and Xiapu (XP) populations from the East China Sea formed another cluster (Fig. S1). This result was visible in the PCoA results, in which there was a clear genetic break between populations from the northern Yellow-Bohai Sea and the southern East China Sea, (Fig. 2). The PCoA results of 200 individuals showed that genetic variation resolved the grouping of *S. thunbergii* along two axes (Fig. 2). The first and second axes contributed to 11.51 and 6.58% of the total variation, respectively. Furthermore, Mantel's test indicated that Nei's genetic distances were closely correlated with the geographic distances among the ten populations of *S. thunbergii* ($P < 0.02$) (Fig. 3). In addition, the population genetic divergence of *S. thunbergii* along the coast of China was further evidenced by STRUCTURE analysis. The results showed that $K = 2$ was the best fit value (Fig. S2), indicating that *S. thunbergii* populations were grouped into two genetic clusters. The five populations from the Yellow-Bohai Sea (DL, BHC, DQ, RC, QD) formed cluster I (green), while the other five populations from the East China Sea formed cluster II (red) (Fig. 4a). In comparison, there was more subdivided genetic structure between populations of *S. thunbergii* from the East China Sea when *K* was set to 3 (Fig. 4b).

Discussion

In this study, we used AFLP markers to analyze the population genetic structure of *S. thunbergii* along the coast of China. The high polymorphic loci detected by each primer combination suggest that AFLP markers are technically efficient to explore the population genetic diversity and differentiation of *S. thunbergii* and can be extended to assess germplasm

Table 4 Analysis of molecular variance (AMOVA) for *S. thunbergii* populations along the coast of China

Source of variation	d.f.	SSD	Variance components	% variation	*F* value
Among regions	1	3132.105	29.987	17.49	$\Phi_{CT} = 0.1749$**
Among populations within regions	8	7456.44	41.6125	24.28	$\Phi_{SC} = 0.2943$**
Within populations	190	18,963.050	99.806	58.23	$\Phi_{ST} = 0.4177$**
Total	199	21,551.595	171.4055		

d.f. degrees of freedom, *SSD* sum of squares

**$P < 0.001$

Table 5 Pairwise *Nm* values (above diagonal) and Nei's genetic distances (below diagonal) between *S. thunbergii* populations along the coast of China

Population	DL	BHC	DQ	RC	QD	SS	DT	ZY	NJ	XP
DL		1.641	1.149	1.304	1.094	0.722	0.989	0.866	0.863	0.661
BHC	0.141		6.558	1.291	1.422	0.934	1.188	1.208	1.262	0.832
DQ	0.175	0.047		1.022	1.207	0.708	0.949	0.764	1.076	0.673
RC	0.151	0.169	0.188		1.409	0.566	0.818	0.599	0.675	0.653
QD	0.182	0.157	0.163	0.137		0.538	0.951	0.600	0.743	0.623
SS	0.234	0.202	0.235	0.290	0.314		1.156	1.092	1.541	0.874
DT	0.218	0.202	0.224	0.254	0.222	0.154		0.839	1.954	1.150
ZY	0.213	0.170	0.238	0.300	0.370	0.139	0.233		0.927	0.668
NJ	0.226	0.172	0.177	0.280	0.258	0.106	0.109	0.189		1.035
XP	0.261	0.230	0.251	0.252	0.271	0.159	0.157	0.156	0.231	

resources, molecular diversity, and the evolution of other *Sargassum* species.

The mean Nei's gene diversity and Shannon's information index values are relatively low in *S. thunbergii* populations along the coast of China. This pattern is consistent with previous findings. For instance, Zhao et al. (2007) found that the mean Nei's gene diversity and Shannon's information index values were 0.2729 and 0.4118, respectively, in four populations of *S. thunbergii* from Shandong Peninsula, China, using RAPD and ISSR markers. Recently, Li et al. (2017) detected low levels of genetic diversity in most *S. thunbergii* populations in the Yellow-Bohai Sea by using ITS2 (nuclear internal transcribed spacer-2) and *cox* 3 sequences. The low levels of genetic diversity in *S. thunbergii* populations along the coast of China may stem from vegetative reproduction and restricted genetic exchange. Sexual reproduction plays an important role in maintaining and increasing genetic diversity of the existing seaweed populations (Olsen et al. 2004). However, vegetative reproduction can promote homozygous genotypes within a population and may result in low population genetic diversity (Liu et al. 2016b). Considering the dominant selfing between identical genotypes or asexual individuals in *S. thunbergii* (Tseng and Lu 2000; Wang et al. 2006), we can speculate that the population genetic homogeneity observed in *S. thunbergii* may stem from vegetative reproduction. Moreover, low levels of gene flow can cause limited genetic exchange among *S. thunbergii* populations along the coast of China, leading to low population-level genetic diversity.

Pairwise F_{ST} values revealed high genetic differentiation among the ten populations of *S. thunbergii*, which might be related to phenological variation in terms of geographical distribution. First, the reproductive seasons of *S. thunbergii* vary among different geographical populations. For example, populations in the Yellow-Bohai Sea reproduce during June and October, whereas populations in the East China Sea reproduce during April and July (Zheng and Chen 1993; Yu et al. 2012). The different reproductive seasons might hinder genetic exchange between *S. thunbergii* populations in the Yellow-Bohai Sea and the East China Sea. Second, seaweeds are characterized by relatively short-lived spores and gametes (Santelices 1990; Billot et al. 2003). The sperm and zygotes of *Sargassum* can only spread over a short distance (< 1 m) before they settle down (Kendrick and Walker 1995). The eggs of *S. thunbergii* adhere to the surface of female

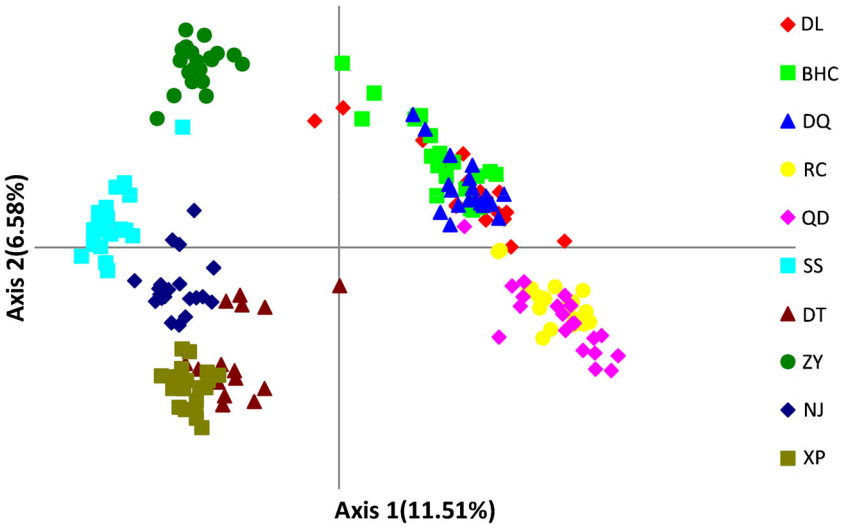

Fig. 2 Principal coordinates analysis (PCoA) of *S. thunbergii* populations along the coast of China

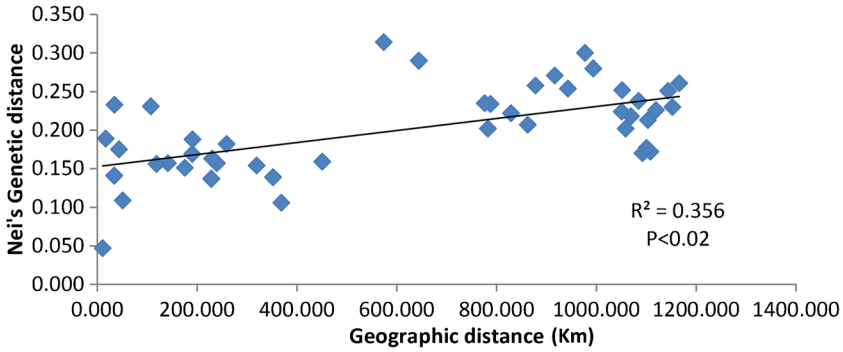

Fig. 3 Mantel's test of *S. thunbergii* along the coast of China

receptacles after release, and sperm swims around to form zygotes (Wang et al. 2006), making it difficult for long-distance dispersal; hence, low levels of genetic exchange have been detected between non-adjacent populations (Zhao et al. 2007). In fact, similar patterns have been reported in several marine benthic fauna, whose populations with low dispersal capability have high levels of genetic differentiations (Hellberg 1994, 1995; Féral 2002). Therefore, geographical isolation may hinder genetic exchange between *S. thunbergii* populations from the Yellow-Bohai Sea and the East China Sea, leading to high levels of genetic differentiation.

Genetic exchange between populations can prevent genetic differentiation among populations (Slatkin 1987). Our results revealed frequent genetic exchange among five populations of *S. thunbergii* in the Yellow-Bohai Sea. This pattern may be associated with the predominant southward-running sea waves in spring, summer, and autumn in the Yellow-Bohai Sea (Zhang et al. 2013). On the contrary, limited genetic exchange was found among the populations in the East China Sea, despite their close geographical proximities. The underlying mechanisms are unclear, but the isolation of islands may act as geographical barriers for genetic exchange (Travis et al. 2010). The germlings of *Sargassum* can be transported over long distances by means of floating mats driven by ocean currents (Komatsu et al. 2014; Mizuno et al. 2014). We thus speculate that fragmented *S. thunbergii* in the East China Sea could be transported from Fujian province (e.g., XP) to Zhejiang province (e.g., NJ and DT) by the northward flowing South China Sea Warm Current in summer (Ichikawa and Beardsley 2002; Hu et al. 2011). At the same time, fragmented *S. thunbergii* in the Yellow-Bohai Sea could be transported to the northern border of the East China Sea in autumn and winter by the China Coastal Current, leading to high genetic connectivity between the BHC and SS populations (Ichikawa and Beardsley 2002; Hu et al. 2011).

In summary, our genetic evidence inferred from AFLP data revealed a clear north to south genetic break of *S. thunbergii* along the coast of China, which geographically corresponds to the distribution range in the Yellow-Bohai Sea and the East China Sea. This finding can help policymakers to understand the current status of *S. thunbergii* genetic resources along the coast of China, identify ecological conservation and management units and regions, and select new varieties with different gene pools for germplasm collection and commercial cultivation under global climate change. Next, we will focus on identifying the ecological and physiological differences between the two genetic groups of *S. thunbergii* to better understand spatial adaptation and evolution of seaweeds.

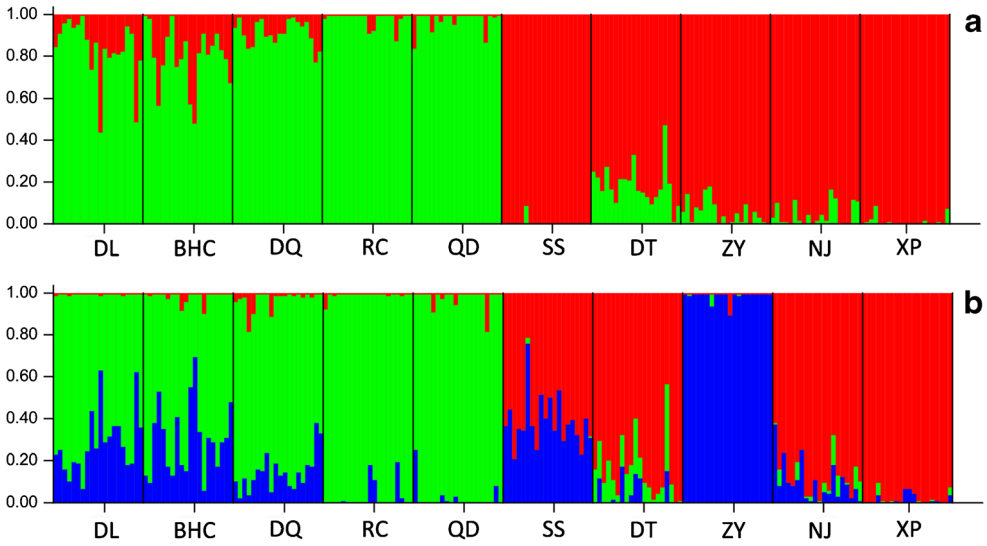

Fig. 4 Population structure of ten populations of *S. thunbergii* along the coast of China with $K = 2$ (**a**) and $K = 3$ (**b**)

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