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Genetic diversity and biogeography of native and introduced populations of *Ulva pertusa* (Ulvales, Chlorophyta)

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SUMMARY

Genetic diversity of native and introduced populations of *Ulva pertusa* (Ulvales, Chlorophyta) was examined using genetic markers of chloroplast, mitochondria and nuclear non-coding region sequences. In the preliminary investigations to genetically identify the species for further analyses, *U. pertusa* was found only from temperate coasts of the more extensive collection sites including tropical coasts suggesting that it is a temperate species and basically not distributed in tropical regions. For chloroplast and mitochondrial sequences, repeating patterns of short tandem repeat sequences and nucleotide substitutions were used to recognize the haplotypes (genetic types). A total of 48 haplotypes based on combinations of chloroplast and mitochondrial haplotypes were recognized in the 244 specimens collected in the presumptive native range (Northeast Asia) and introduced populations (North America, Australia, New Zealand, Chile and Europe). Among them, 46 haplotypes (H1–H8 and H11–H48) were recognized in Northeast Asia, whereas only 1–5 haplotypes were recognized in the other areas. Nuclear microsatellite sequences were also analyzed. The lengths of the PCR products including the nuclear microsatellite region of 234 specimens were determined, and a total of 17 genotypes were recognized. Among them, 14 genotypes were found in Northeast Asia, whereas 1–7 genotypes were recognized in the other areas. Based on the results, the hypothesis that the native range of the species is in Northeast Asia was supported, and the populations outside this range were concluded to be non-indigenous populations.

Key words: biogeography, genetic marker, haplotype.

INTRODUCTION

The globalization of ship transport systems, fisheries and aquaculture activities has increased trans-oceanic introductions of marine organisms, posing a threat to coastal ecosystems. In particular, both the discharge from large bulk carriers of ballast water containing various planktonic microorganisms, as well as ship hulls acting as vectors for attached organisms, have been implicated in trans-oceanic introductions. Globally, more than one hundred seaweed species are believed to have been spread outside their native ranges by human-mediated means (Farnham & Irvine 1973; Rueness 1989; Curiel *et al.* 1998; Fletcher & Farrell 1998; Rueness & Rueness 2000; Boudouresque & Verlaque 2002; Smith *et al.* 2002; Kim *et al.* 2004; Streftaris *et al.* 2005).

Ulva pertusa Kjellman is one of the commonest intertidal macroalgae in Japan. It was originally described from Japan (Kjellman 1897), and its native range is also considered to include Northeast Asia (Okamura 1921; Nagai 1940; Tokida 1954; Tseng 1984; Huang 2000; Lee & Kang 2001). However, recently the species has been reported from various regions outside Northeast Asia: Indonesia, Singapore and the Philippines (Silva *et al.* 1996), France (Verlaque 2001; Verlaque *et al.* 2002), Italy (Wolf *et al.* 2012), The Netherlands (Stegenga *et al.* 2007), Spain (Couceiro *et al.* 2011, as *U. australis* Areschoug), Canada and the USA (Hayden & Waaland 2004; Hofman *et al.* 2010; Saunders & Kucera 2010), Mexico (Aguilar-Rosas *et al.* 2008), Yemen, Kenya, Mauritius and Tanzania (Silva *et al.* 1996), New Zealand (Heesch *et al.* 2009), Australia (Kraft *et al.* 2010, as *U. australis*), and was explained as a non-intentional introduction associated with aquaculture (probably associated with young oysters) or associated with maritime activities. *Ulva* spp. are frequent fouling species, and have also been reported from ballast waters of trans-ocean shipping (Flagella *et al.* 2007; H. Kawai, unpublished data).

However, the species-level identification of *Ulva pertusa* is rather difficult because of their simple morphology and great morphological plasticity, and therefore identification solely based on morphology is not very reliable. Kawai *et al.* (2007) reported the occurrence, based on genetic identifications, of several *Ulva* species that had not been previously reported in Japan, although dominating the coastal macroalgal flora. López *et al.* (2007) and Couceiro *et al.* (2011) reported that the distribution of the introduced *U. pertusa* (as *U. australis*) in the northwestern Iberian Peninsula was considerably broader than that formerly estimated from morphological studies. On the other hand, *U. pertusa* was also recorded in New Zealand in a biogeographical survey of *Ulva* and related taxa featuring genetic identifications (Heesch *et al.* 2007, 2009). However, due to the common occurrence of the species including on remote coastlines distant from apparent human modifications, the authors did not regard the species as non-indigenous taxon.

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Related to the phylogeography of *U. pertusa*, the Sea of Japan was almost closed during the Last Glacial Maximum (LGM), and many marine organisms were believed to have become extinct by the considerably reduced salinity of the surface water and hypoxia in the deeper water (Tada 1994; Amano 2004). In a study of the geographical distributions of macroalgae haplotypes in Japan, Uwai *et al.* (2006) showed remarkably smaller genetic divergences of *Undaria pinnatifida* (Harvey) Suringar in the Sea of Japan populations, compared with those on the Pacific coast of Japan. However, there have been limited studies on the geographical distributions of haplotypes of *Ulva* and related taxa. Blomster *et al.* (1998) and Leskinen *et al.* (2004) examined the haplotype distributions of *U. compressa* L. and *U. intestinalis* L. in Northern Europe, but there has been no report on *U. pertusa*. The aim of this study was to identify *Ulva pertusa*-like species using multiple genetic markers, to elucidate the genetic diversities of the species worldwide, to consider their native and introduced distributional ranges, and to examine the evidence available for estimating the origins of the introduced populations.

MATERIALS AND METHODS

Specimens of *Ulva* spp. were collected from various localities worldwide: East Asia, Southeast Asia, Europe, North America, South America, Australasia, and South Africa. For this study, *U. pertusa* was preliminarily identified from the collections based on the rDNA ITS region sequences following Kawai *et al.* (2007). Based on the genetic identifications, 244 *U. pertusa* specimens from 145 localities were analyzed in the present study (Table S1 in the Supporting Information).

For the genetic study, a portion of each newly collected specimen was quickly dried in silica gel. Genomic DNA was extracted from the specimens using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. PCR was carried out with a GeneAmp PCR Cycler 9700 (Applied Biosystems, CA, USA) and a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio, Kusatsu, Japan) using a TaKaRa ExTaq (TaKaRa Bio) reaction kit or KOD FX (ToYoBo, Osaka, Japan). After PEG purification (Lis 1980), PCR products were sequenced using a CE DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) and a CEQ8000 Genetic Analysis System (Beckman Coulter) according to the manufacturer's instructions. Determined ITS2 sequences were compared to that of *U. pertusa* from Mikawa Bay, Japan (AB280825, Kawai *et al.* 2007). Among the collections, 244 specimens from 145 localities (10 countries and regions) were identified as *U. pertusa* (Table S1 in the Supporting Information) and used for the following analyses.

Genetic diversities of local populations of *Ulva pertusa* were investigated using chloroplast, mitochondrial, and nuclear DNA sequences. The intergenic region of chloroplast *atp*I and *atp*H genes (*atp*I–H) and mitochondrial *trn*A and *trn*N genes (*trn*A–N) were amplified using newly designed primers based on the sequences of *Oltmannsiellopsis viridis* (Hargraves et Steele) Chihara et Inouye (DQ291132 and DQ365900, Pombert *et al.* 2006a,b) and *Pseudendoclonium akinetum* Tupa (AY835431 and AY359242, Pombert *et al.* 2004, 2005) (Fig. S1 in the Supporting Information). Organelle genomes are basically inherited to offspring by uniparental inheritance, and rarely recombine (Birky 2001). Therefore we assumed that chloroplast and mitochondria genomes share the history, and the haplotypes based on combined sequences of chloroplast and mitochondrial nucleotide sequences can be used for accessing the genetic divergence as those based on either mitochondrial or chloroplast gene sequences. A nuclear microsatellite maker was developed according to Lian *et al.* (2006). The nucleotide sequence flanking the compound microsatellite region was determined and the primer was designed (s2-3_P1: 5-′TCACGAAAAGG-CAGCACAGAG-3′. Compound microsatellite primer (5′-TCTCTCTCTCTCACACACACAC-3′) and s2-3_P1 were used for PCR and the genotype of each specimen was determined using a CEQ8000 Genetic Analysis System and a CEQ DNA Size Standard-400 (Beckman Coulter). Haplotype diversity was examined following Nei (1987). Pairwise F_{st} was estimated using Arlequin v. 2 (Schneider *et al.* 2000).

RESULTS

Based on the nuclear ITS2 sequence, 244 specimens out of approximately 350 specimens morphologically assigned to *Ulva pertusa* were identified as *U. pertusa* and used for the analyses in the present study. The other specimens (~100) differed from *U. pertusa* by 30–60 bp in the sequenced region. Fifteen haplotypes (cH1–cH15) were recognized for the chloroplast *atp*I–H region sequences in the 244 specimens examined (Fig. S2 in the Supporting Information). There were insertions or deletions among haplotypes, but nucleotide substitution was not present. In this region, there were repeats of AAGATCATTAG or a part of the sequence (Fig. S3 in the Supporting Information), and the total length of the repeated sequences ranged from 68 bp to 218 bp (Fig. S3 and Table S2 in the Supporting Information). All of the 15 haplotypes (cH1–cH15) were found in Northeast Asia, but only 1 to 2 haplotypes were found in North America (cH3), Chile (cH3 and cH4), Australia (cH3), New Zealand (cH3) and Europe (cH3 and cH11). In Northeast Asia, two major haplotypes of cH3 and cH11 were recognized. The haplotype cH3 was distributed in 13 localities in the Pacific coast of Japan. The haplotype cH11 was distributed in 26 localities mostly in the Sea of Japan and the Seto Inland Sea. Haplotype diversity was 0.76 in Northeast Asia, but was 0.00 in New Zealand (Table 1).

Twenty-seven haplotypes (mH1–mH27) were recognized for the mitochondrial *trn*A–N intergenic region sequences in the 233 specimens examined. In this region, nine polymorphic sites (nucleotide substitution and/or insertion/deletion) were found (Fig. S4 and Table S3 in the Supporting Information). Twenty-five haplotypes (mH1–mH3 and mH6–mH27) were found in Northeast Asia. One to five haplotypes were found in North America (mH3), Chile (mH3), Australia (mH3), New Zealand (mH1–mH5) and Europe (mH3 and mH25). The haplotypes mH4 and mH5 were only found in New Zealand. The geographical distribution of the haplotypes is shown in Figure S5 in the Supporting Information. Haplotype diversity was 0.95 in Northeast Asia and was 0.22 in New Zealand.

By combining the chloroplast and mitochondria haplotype datasets, we determined a total of 48 combined haplotypes

Table 1. Genetic diversity based on the chloroplast *atp*I-H region (central), mitochondrial *trn*A-N region (right) and combined chloroplast and mitochondrial regions (left) for Northeast Asia and New Zealand

Region	Combined (chloroplast and mitocondria)				Chloroplast *atp*I-H				Mitocondrial *trn*A-N			
	*N*ind	*N*pop	*N*hap	*H* (SD)	*N*ind	*N*pop	*N*hap	*H* (SD)	*N*ind	*N*pop	*N*hap	*H* (SD)
Northeast Asia†	97	51	46	0.93 (±0.01)	100	51	15	0.76 (±0.03)	97	51	25	0.95 (±0.01)
New Zealand	118	77	5	0.22 (±0.05)	124	83	1	0.00 (±0.00)	118	77	5	0.22 (±0.05)

H, haplotype diversity; *N*ind, number of individuals; *N*pop, number of populations; *N*hap, number of haplotypes.
†Population in Vladivostok is included.

(H1–H48) for 233 specimens (Table S4 in the Supporting Information). The geographical distribution of the combined haplotypes is shown in Figure 1. Among them, 46 haplotypes (H1–H3, H5–H7 and H9–H48) were recognized in Northeast Asia (Fig. 1A), whereas 1–5 haplotypes were recognized in North America (H5), Chile (H5), Australia (H5), New Zealand (H4–H8) and Europe (H5 and H42) (Fig.1 B,C). Haplotype diversity was 0.93 in Northeast Asia and was 0.22 in New Zealand.

The lengths of PCR products including the nuclear microsatellite region of 237 specimens were determined, and a total of 17 genotypes (G1–G17) and nine alleles (165–181) were recognized (Tables S1, S5 in the Supporting Information). The geographical distributions of the nuclear genotypes in Japan and New Zealand are shown in Figure 2. Fourteen genotypes (G1–G13 and G16) and nine alleles were found in Northeast Asia (Fig. 2A, Table S1 in the Supporting Information), whereas 1–7 genotypes and 1–5 alleles were recognized in North America (G1, G6 and G17), Chile (G4), Australia (G4 and G5; 173 and 175), New Zealand (G1, G2, G4, G5, G11, G14 and G15; 165, 167, 169, 173 and 175) and Europe (G1, G5 and G12; 167, 175 and 177) (Fig. 2B, C, Table S1 in the Supporting Information).

Pairwise F_{st} value estimates among seven regions in Northeast Asia are summarized in Tables 2, 3. The differentiation between region two (Pacific coast of Central Japan) and the other six regions was positive. All pairwise F_{st} values, except for the value between region two and region seven (Vladivostok) based on *trn*A–N, were statistically significant. On the other hand, all pairwise F_{st} values between region seven and the other six regions based on *trn*A–N were not significant.

DISCUSSION

Although relatively intensive collections of *Ulva* specimens, especially those with gross morphology similar to *U. pertusa*, were done in Malaysia and Taiwan, all of the specimens from these areas were genetically identified as *U. rigida* C. Agardh and *U. ohnoi* M. Hiraoka & S. Shimada (data not shown). In Japan, *U. pertusa* is widely distributed in the temperate regions, but rarely collected in subtropical regions such as the Ryukyu Islands. Therefore, although there have been many reports of *U. pertusa* from tropical regions (*e.g.*, Indonesia, Singapore, the Philippines; for details see the Introduction), we consider that these identifications are doubtful and a genetic reexamination of these specimens is needed to confirm the taxonomy.

We have recognized a total of 48 *U. pertusa* haplotypes worldwide in the combined haplotypes deduced from chloroplast and mitochondrial genetic markers (repeating pattern of a selected short sequence, and nucleotide substitutions) and also recognized a total of 17 genotypes (nine alleles) worldwide in the microsatellite region. Among them, 46 haplotypes and 14 genotypes (nine alleles) were found in Northeast Asia, supporting the notion that the native distributional range of *U. pertusa* is in this region. In contrast, few of the haplotypes (1–5 haplotypes) and about half or less genotypes and alleles (1–7 genotypes, 1–5 alleles) were found outside Northeast Asia. Those haplotypes and genotypes (alleles) corresponded to those commonly found in Northeast Asia, and suggest that the populations in this study outside Northeast Asia are non-indigenous, introduced from Northeast Asia. The haplotypes H4 and H8 were found only in New Zealand. There was only one nucleotide substitution between H4 and H5, and only one nucleotide insertion/deletion was present between H7 and H8. It is assumed that these results proceeded from insufficient sampling in native range. However, we cannot contradict the possibility that these mutations had happened after introduction in New Zealand. In Europe, two haplotypes (H5 and H42) were recognized. These two haplotypes indicated distinct distributions in Northeast Asia; H5 were found in the Pacific coast (S17, S19, S24 and S53) in Japan, whereas H42 were found in the Seto Inland Sea (S39) and Korea (S60). These results suggest that introduction events have occurred repeatedly (Table 3).

Three heterozygous genotypes (G14 and G15 in New Zealand and G17 in North America) found in the introduced populations were not found in the native range of Northeast Asia. This result may suggest the occurrence of sexual reproductions in the introduced populations. In the North American populations, G17 (allele: 175, 177) and G6 (allele: 177) were found, whereas G5 (allele: 175) was not found. In addition, in the European populations, G12 (allele: 167, 177) and G1 (allele: 167) were found, whereas G5 (allele: 175) was not found. This may be explained as the result of the limited samplings in the regions. On the other hand, the combinations of haplotype and genotype were sometime different between the native and introduced populations. For example, the two specimens showing G11 from New Zealand had the haplotype H5. In contrast, the three specimens showing G11 from Northeast Asia had H18, H21 or H32. These results may suggest the occurrence of introgression in the introduced populations or may be attributed to high mutation rate in microsatellite region.

As to the genetic divergence of local macroalgal populations in Northeast Asia, Uwai *et al.* (2006) studied a kelp

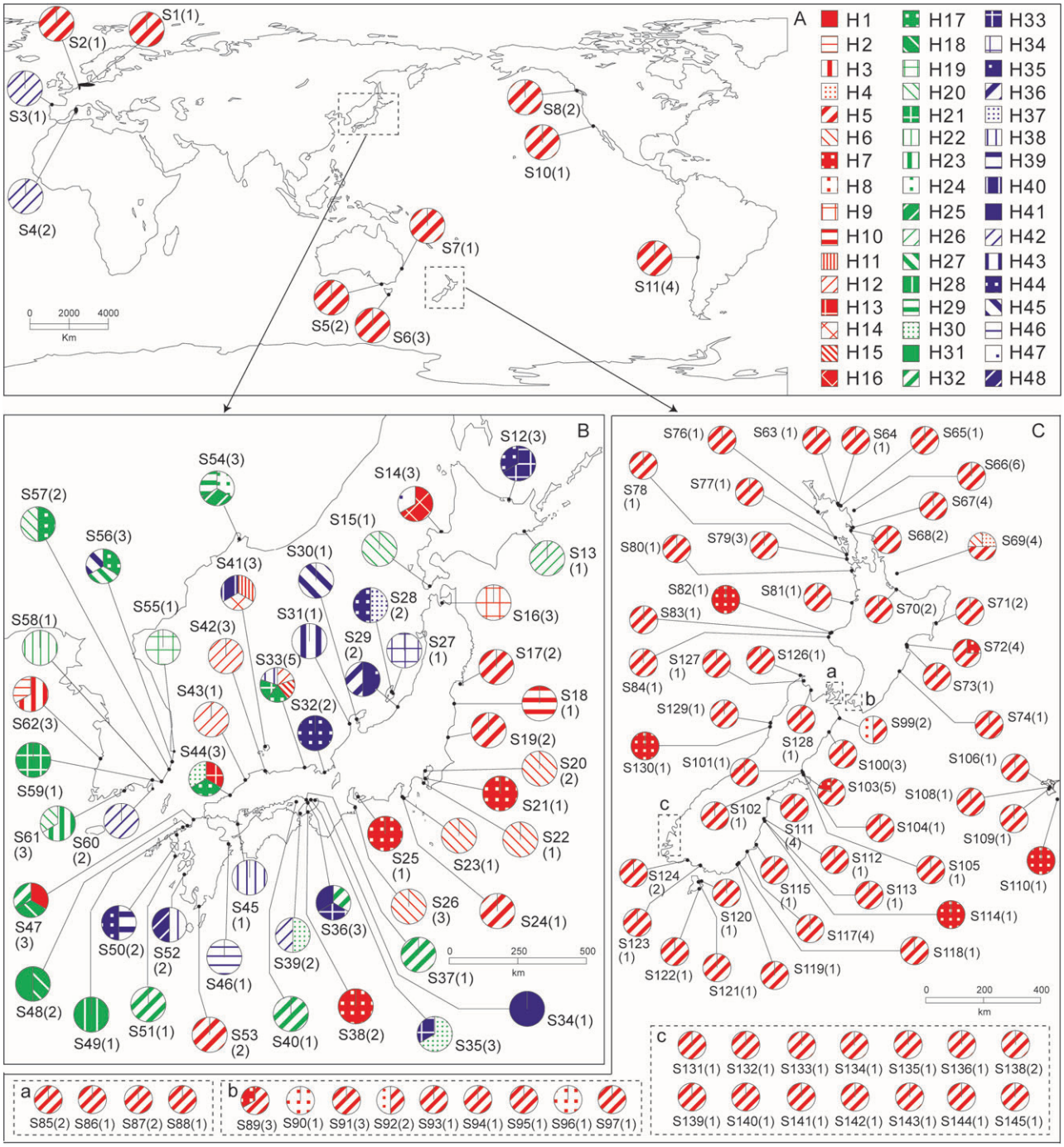

Fig. 1. Collection sites of the *Ulva pertusa* specimens examined in the present study (S1–S145), and the distributions of the combined haplotypes (H1–H48). Numbers in parentheses indicate the number of the specimens analyzed from each site. A: Map of the world. B: Map of Northeast Asia. C: Map of New Zealand. The haplotypes in three areas (a, b and c) in New Zealand are shown in the dotted line boxes.

species *Undaria pinnatifida* (Laminariales, Phaeophyceae), which has a similar native distributional range to *Ulva pertusa*, and also lacks buoyancy that would allow long distance dispersal of detached individuals by currents. Interestingly, the distributional patterns of haplotypes in the native ranges were significantly different between the two species. In the native range (Northeast Asia), *Un. pinnatifida* showed very low genetic diversity in the Sea of Japan area, compared to those on the Pacific coast (1–2, S3, S4 in the Supporting Information). This may be explained by the relatively short history of the macroalgal populations in the Sea of Japan after recovery from the extinctions during the LGM (Oba *et al.* 1991; Oba & Irino 2012). In this period (~18 000–15 000 years B.P.), the connections of the Sea of Japan to the Pacific Ocean (Tsushima Strait, Tsugaru Strait, Soya Strait and Miyama Strait) were closed, and the organisms in the Sea of Japan

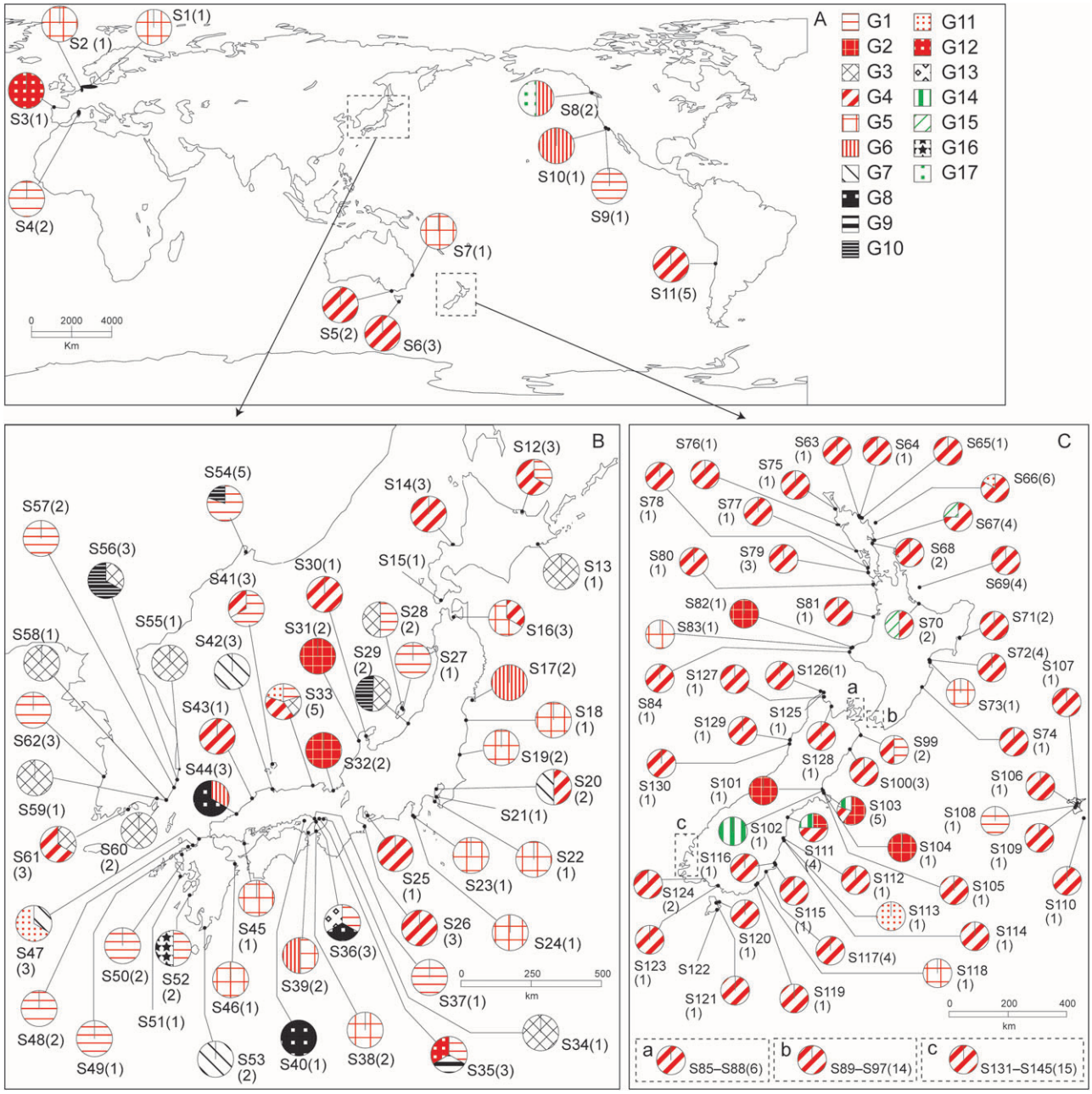

Fig. 2. Distribution of the microsatellite genotypes (G1–G17). Numbers in parentheses indicate the number of samples analyzed for each site. Black, red, and green colors show the genotypes found in only the native area, the genotypes found in both native and introduced areas, and the genotypes found in only introduced areas, respectively. A: Map of the world. B: Map of Northeast Asia. C: Map of New Zealand. The haplotypes in three areas (a, b and c) in New Zealand are shown in the dotted line boxes.

Basin were greatly influenced by freshwater and ice, dominated by much colder water masses with anaerobic bottom-water conditions. A large proportion of the marine vegetation became extinct. However, it has been controversial whether the Sea of Japan was completely isolated, without any connections to North Pacific warm water, or whether there remained a narrow channel allowing the inflow of the Tsushima warm current (Park *et al.* 2000), resulting in the survival of some marine species that had a high tolerance to ice and low salinity.

By the end of glacial period, the vegetation recovered due to the influence of the northward Tsushima warm current, a branch of Kuroshio (the Japan Current). Therefore, reflecting the short history of the Sea of Japan after the glacial period, the genetic divergences of the native macroalgae in the region are relatively small compared to the Pacific side (Uwai *et al.* 2006). In contrast, the genetic diversity of *U. pertusa* in the Sea of Japan was comparable to specimens collected from the Pacific coast. Both *Undaria* and *Ulva* lack buoyancy, but they differ in life history characteristics (heteromorphic by

Table 2. Pairwise F_{st} estimates among seven region of *Ulva pertusa* in East Asia, on the basis of haplotype frequencies (above diagonal) and genotype frequencies (below diagonal)

Region	1	2	3	4	5	6	7
1. North area of Japan	–	0.204***	0.071*	0.081*	0.064*	0.072*	0.058
2. Pacific coast of Central Japan	0.049	–	0.185***	0.195***	0.136*	0.189***	0.211
3. Sea of Japan coast of Honshu	0.081*	0.144***	–	0.094***	0.051*	0.055*	0.053
4. Seto Inland Sea area	0.159*	0.078*	0.084*	–	0.049*	0.095***	0.075
5. West area of Japan	0.329***	0.347***	0.136*	0.153*	–	0.063***	0.033
6. Korea	0.191*	0.275***	0.071*	0.124*	0.174*	–	0.049
7. Vladivostok	0.403*	0.431***	0.202*	0.186*	0.016	0.175	–

1: S12–S16, 2: S17–S26, 3: S27–S33, S41–S44, 4: S34–S40, S45–S46, 5: S47–S53, 6: S55–S62, 7: S54.
P-value was generated by 10 100 times of permutation (*$P < 0.05$ and ***$P < 0.001$).

Table 3 Pairwise F_{st} estimates among seven region of *Ulva pertusa* in East Asia, on the basis of the chloroplast *atp*I–H region (above diagonal) and mitochondrial *trn*A–N region (below diagonal)

Region	1	2	3	4	5	6	7
1. Northern Japan	–	0.611***	0.125*	0.188*	0.117*	0.077	0.132
2. Pacific coast of Central Japan	0.133*	–	0.513***	0.707***	0.417***	0.717***	0.639***
3. Sea of Japan coast of Honshu	0.040	0.219***	–	0.211*	0.098*	0.176*	0.130
4. Seto Inland Sea area	0.017	0.175***	0.059*	–	0.195*	0.040	0.356*
5. Western area of Japan	0.007	0.166*	0.011	–0.001†	–	0.207*	0.098
6. Korea	0.048*	0.200***	0.032	0.056*	0.040*	–	0.297*
7. Vladivostok	0.028	0.246	0.069	0.056	0.050	0.033	–

1: S12–S16, 2: S17–S26, 3: S27–S33, S41–S44, 4: S34–S40, S45–S46, 5: S47–S53, 6: S55–S62, 7: S54.
P-value was generated by 10 100 times of permutation (*$P < 0.05$ and ***$P < 0.001$).
†Because parameter estimates will often deviate from the true value, a small negative estimate of F_{st} could be obtained if the true parameter value is zero.

oogamy *vs.* isomorphic by isogamy), time required to reach fertility (3–4 months for *Undaria* sporophytes *vs.* 2 to 3 weeks for *Ulva*), habitat (upper subtidal *vs.* subtidal to intertidal), tolerance to low salinity (~24–27 *vs.* <20; Baba 2008; Choi *et al.* 2010). Therefore, it is possible that *U. pertusa* populations in the Sea of Japan could have survived the glacial period, or have higher genetic diversity in the Sea of Japan area as the result of significant and frequent dispersal.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Position and sequence of the primers in the chloroplast genome (a) and mitochondrial genome (b). Bold characters indicate primers for PCR.

Fig. S2. Distribution of the chloroplast haplotypes (cH1–cH15). Black and red colors show the haplotypes found in only native areas and the haplotypes found in both native and introduced areas, respectively. A: Map of the world. B: Map of Northeast Asia. C: Map of New Zealand. The haplotypes in three areas (a, b and c) in New Zealand are shown in the dotted line boxes.

Fig. S3. Alignment of 15 chloroplast haplotypes.

Fig. S4. Sequence of mitochondrial haplotype 1, and the polymorphic positions among haplotypes (a–i). (a): repeat number of 'T'(T5 or T6), (b): 1-ACAAACAATTCCAA, 2-ACAATTCCAA, 3-ACAAACAA, (c): presence (+) or absence (–) of five nucleotides 'TAAAA', (d): repeat number of 'T', (e): differences of repeat number of 17 nucleotides 'TACAAAATACCTTAATG', (d): 1-CAGG, 2-CCGG, 3-CGG, (f):, 1-CAGG, 2-CCGG, 3-CGG, (g): presence (+) or absence (–) of four nucleotides 'ATAT', (h): Repeat number of 'T', (i): 1-TCTTA, 2-TCTTATCTTA, 3-TTTTA.

Fig. S5. Distribution of the mitochondrial haplotypes (mH1–mH27). Numbers in parentheses indicate the number of samples analyzed for each site. Black, red, and green colors show the haplotypes found in only native areas, the haplotypes found in both native and introduced areas, and the haplotypes found in only introduced areas, respectively. A: Map of the world. B: Map of Northeast Asia. C: Map of New Zealand. The haplotypes in three areas (a, b and c) in New Zealand are shown in the dotted line boxes.

Table S1. Origin of the specimens of *Ulva pertusa* in the present study, and their haplotypes, genotypes, and DDBJ accession numbers.

Table S2. Length variation among the chloroplast haplotypes, and specimen number of each haplotypes and/or regions.

Table S3. Differences among the mitochondria haplotypes, and specimen number of each haplotypes and/or regions.

Table S4. Differences among the combined haplotypes (chloroplast and mitochondria haplotypes) and specimen number of each haplotypes and/or regions.

Table S5. Differences among the microsatellite genotypes and specimen number of each genotypes and/or regions.

Table S6. Pairwise Fst estimates among seven region of Ulva pertusain East Asia, on the basis of chloroplast haplotype frequencies (above diagonal) and mitochondrial haplotype frequencies (below diagonal).