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Population genetic structure of the exotic smooth cordgrass Spartina alterniflora and its invasion into Korean coasts

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Abstract

The smooth cordgrass, *Spartina alterniflora* Loisel, is an aggressive invasive species that causes a significant negative impact on coastal ecosystems worldwide. The species is native to the eastern United States, but has spread rapidly along East Asian coasts and was unintentionally introduced into Korea, leading to the devastation of native ecosystems. Here, we examined the population genetic structure and invasion pathway of *S. alterniflora* along Korean coasts using chloroplast DNA (cpDNA). Our results revealed that the *S. alterniflora* population in Korea has a strong genetic structure, primarily consisting of two geographically isolated haplotypes (C1 and C4). Furthermore, two individuals were found to have haplotype N, which might be a newly discovered haplotype in this study. The haplotypes C1 and C4 are widely distributed in the native population as well as in the introduced population in East Asia. Thus, the presence of these two haplotypes in different localities in Korea suggests multiple invasion events. Comparisons of the cpDNA data of this study with the available GenBank data from the native range in the U.S. and the invasive range in East Asia (China and Japan) revealed that the East Asian population (China, Japan, and Korea) is nearly homogeneous (AMOVA; Fst = 0.06871, p = 0.5) with no restricted gene flow. Our study suggests that the C1 and C4 haplotypes might have been introduced into Korea multiple times particularly from China, possibly *via* natural dispersal or transport vectors. These emphasize the importance of strengthening coastal management to prevent further spread.

Introduction

The introduction of invasive species is a critical concern for native ecosystems, particularly when these organisms establish themselves and rapidly expand their range (Pyšek and Richardson 2010). Among invasive species, aquatic plants present significant threats to both local biodiversity and the overall functioning of ecosystems. Cordgrasses in the genus *Spartina*, for example, are one of the most aggressive invaders in coastal ecosystems worldwide, spreading across five continents (Strong and Ayres 2013). A remarkable instance of a massive *Spartina* invasion is recorded in China, where *Spartina alterniflora* Loisel (smooth cordgrass) has spread rapidly, leading to the disruption of the ecosystem and resulting in substantial economic losses amounting to millions of dollars (Zuo et al. 2012; Strong and Ayres 2013).

Initially, *Spartina alterniflora* (native to North America) was introduced into China in 1979 from Florida, Georgia, and North Carolina in the United States for the purpose of coastal restoration (Blum et al. 2007). However, the species quickly became invasive, expanded rapidly in China, and spread into other East Asian regions, such as Taiwan and Hong Kong (Guo et al. 2015). Recently, *S. alterniflora* has been discovered on the coasts of Japan and South Korea (Kim et al. 2015; Maebara et al. 2020). In Korea, the presence of *Spartina* species was first recorded on Ganghwa Island in 2012 (Kim et al. 2015). Since then, the species have colonized several regions along Korean coastlines, leading to the devastation of Korea's native ecosystems causing huge economic loss (An et al. 2020). As a result, the Korean Ministry of Oceans and Fisheries designated all *Spartina* species, including *S. alterniflora*, as invasive alien species in 2016 (Korea Marine 2021), and the government has made intensive efforts to control and/or eradicate

this species. However, in order to effectively control the invasive *S. alterniflora* and prevent its continued spread, it is crucial to gain a comprehensive understanding of its current status in Korea using a population genetic approach.

The colonization of a new area by invasive species can occur through either a single introduction or multiple introductions of individuals (Dlugosch and Parker 2008; Zalewski et al. 2010). When the number of founding individuals is small, it often leads to bottlenecks and founder events. As a result, low genetic diversity is expected across the expanding range of invasive species (Roman and Darling 2007), such as that found in the Japanese population (Maebara et al. 2020). The low genetic diversity may reduce the invasion success of the introduced species by reducing their ability to adapt to the new environment (Roman and Darling 2007). However, through multiple introductions or novel genetic admixture, invasive species can overcome these negative effects by mitigating founder effects, increasing genetic diversity and structuring, and facilitating adaptation in the new range (Zalewski et al. 2010; Guo et al. 2018). For instance, the invasion success of *S. alterniflora* in China was shown to be due to genetic admixture, which increases genetic diversity and facilitates rapid adaptation (Qiao et al. 2019). Hence, effective mitigation of ongoing invasions and management of invasive species require information about the colonization history, invasion processes, and population structuring (Zalewski et al. 2010). In a similar way, *S. alterniflora* has colonized several regions in Korea; however, to date, the invasion pathway and population genetic structure of this species are mostly unknown. We hypothesized that *S. alterniflora* could have been introduced into Korea multiple times through natural dispersal or trade from China. Much of this critical information can be obtained by examining the genetic variability and genetic structure of this invasive species, which is critical for preventing its spread.

In the present study, we assessed the genetic structure of the invasive *S. alterniflora* along Korean coasts using chloroplast genomic markers. We compared the genetic variation between the populations in the native region in the U.S. and the introduced (China) and invaded (Japan and Korea) regions on the East Asian coasts. This could provide significant information on the genetic structure and invasion mechanisms of *S. alterniflora* in Korea. This, in turn, could greatly benefit the management strategies and improve control and/or eradication efforts.

Materials and Methods

Field sample collection and DNA extraction

Based on our preliminary molecular analyses and the reported distribution of *Spartina alterniflora* in Korea, we collected *S. alterniflora* samples across its current distribution along the Koreans coasts (**Supplementary Fig. 1**). A total of 48 individuals were collected at three geographically different sites (Jindo, Gimje, and Seosan). At each site, five to ten young leaves were sampled from each individual that were at least 2.5 m apart (Blum et al. 2007). Leaves were preserved in 70% ethanol at room temperature until DNA extraction. Genomic DNA was isolated using the cetyltrimethylammonium bromide (CTAB) method (Richards et al. 1994).

PCR amplification and sequencing of the chloroplast DNA

We used the universal primers listed in Taberlet et al. (1991) to amplify and sequence the chloroplast *trnT-trnF* intergenic spacer region for 48 samples from three sites. The *trnT-trnF* region was used considering this spacer was also used across the native population in the U.S. (Blum et al. 2007) and the invasive population in China and Japan (Qiao et al. 2019; Maebara et al. 2020), which provided detailed cpDNA haplotype information. For polymerase chain reaction (PCR) amplification, the *trnT-trnF* region was amplified in three sections using the primer pairs Tab A (5'-CATTACAAATGCGATGCTCT-3') and Tab B (5'-TCTACCGATTTCGCCATATC-3') to recover the *trnT-trnL* region. Tab C (5'-CGAAATCGGTAGACGCTACG-3') and Tab F (5'-ATTTGAACTGGTGACACGAG-3') were used to recover *trnL-trnF* region. In addition, we used Tab C (5'-CGAAATCGGTAGACGCTACG-3') and Tab D (5'-GGGGATAGAGGGACTTGAAC-3') to recover the missing part between *trnL* (5' exon) and *trnL* (3' exon; Taberlet et al. 1991).

All PCR volumes were 20 µL, which included 2 µL template DNA, 2 µL Ex Taq buffer, 2 µL 2',3'-dideoxynucleotide 5'-triphosphates (dNTP) mix, 1 µL forward primer, 1µL reverse primer, 0.2 µL Ex Taq Polymerase (TaKaRa Bio Inc., Shiga, Japan), and 11.8 µL distilled water. PCR cycling was performed in an iCycler (Bio-Rad, Hercules, CA) with the following reaction conditions: 94°C for 3min, followed by 40 cycles of 94°C for 30s, 55°C for 30s, 72°C for 1min, and 72°C for 5min. PCR results were confirmed on a 1% agarose gel using MIDORIGreen (Nippon Genetics Europe GmbH, Germany) as a fluorescent source. The confirmed samples were purified using a PCR Cleanup S & V Kit (Bionics, Daejeon, Korea). Purified PCR products were sequenced by Bionics Co., Ltd. (Seoul, Korea) using the same primers that were used in PCR. Sequencing data were assembled with Sequencher v5.1 (Gene Codes Corporation, Ann Arbor, MI). The ambiguous regions were manually checked and removed.

Data analyses

The assembled sequences of the three chloroplast fragments of the *trnT-trnF* region for each individual were combined as a single matrix (1,800 bp), aligned in MEGA X (Kumar et al. 2018), and used for subsequent data analyses. The sequences of the 48 individuals determined in this study were used to calculate the standard genetic diversity indices of the Korean population, including the number of haplotypes (Nh), the number of polymorphic sites (Ps), haplotype diversity (h), and nucleotide diversity (π), using Arlequin v.3.5.2.2 (Excoffier and Lischer 2010). The phylogeographic patterns of chloroplast haplotypes of the Korean *S. alterniflora* were explored by constructing a haplotype network using the pegas and ape packages in R v.4.3.0 (R Core Team 2022). Principal Component Analysis (PCA) was employed to assess and visualize geographic genetic variation, which was calculated in Paleontological Statistics Software Package (PAST) v. 3.25 (Hammer et al. 2001).

We compared genetic variation between populations in both native and introduced regions to determine the haplotype of the *trnT-trnF* sequences obtained in this study. Specifically, we retrieved all the available *trnT-trnF* sequences of *S. alterniflora* from the NCBI database, which were previously published for the U.S., Chinese (GenBank acc. numbers: MG201945-MG201973, Qiao et al. 2019), and Japanese populations (GenBank acc. number: LC565815, Maebara et al. 2020). The sequences were aligned (1,365

bp) in MEGA X, and a haplotype network was constructed in R software. Furthermore, Analysis of Molecular Variance (AMOVA) was conducted in Arlequin to examine the population differentiation between native (U.S.) and invasive regions (China, Japan, and Korea), within invasive regions (China, Japan, and Korea), and within the Korean population (Seosan, Gimje, and Jindo). In addition, neutrality tests; Tajima's *D* (Tajima 1989), and Fu's *F*s tests (Fu 1997) were performed in DnaSP v.6 (Rozas et al. 2017) to reveal if there is any population expansion in the invasive regions.

Results

Population genetic structure and genetic diversity of S. alterniflora along Korean coasts

In this study, the total length of the sequenced *trnT-trnF* region was determined to be 1,800 bp. Three distinct haplotypes were identified by analyzing base-pair polymorphism and indel variation at positions 124 and 1,624. Among these haplotypes, two were identified as haplotypes C1 and C4, based on the 29 previously reported haplotypes (Qiao et al. 2019). The remaining haplotype was not matched with any of the previous haplotypes, and was tentatively designated as "N" (Fig. 1A). All the individuals in the Jindo coastal region were C1 haplotypes (GenBank No. OR130493), while the individuals at Seosan and Gimje were C4 haplotype (OR130494). Furthermore, two individuals from the Gimje coastal regions were found to have the N haplotypes (Fig. 1B; OR130495). The geographic genetic variation of these haplotypes is shown in Fig. 1C.

The genetic diversity assessment revealed that only the Gimje population has haplotype (h) and nucleotide (π) diversities of 0.8428 ± 0.0248 and 0.005139 ± 0.002915, respectively (Table 1). Similarly, the neutrality test, Tajima *D* (-0.44823, p = 0.2), and Fu's *F*s (0.08323, p = 0.2) revealed that the Gimje population experienced a slight but statistically insignificant population expansion. Overall, there was low genetic diversity within Korean populations.

Table 1
Genetic diversity and neutrality test chloroplast DNA of *Spartina alterniflora* population in Korean coasts.

Regions	N	Nh	Ps	D	Fs	h	π
SS	5	1	0	0	0	0	0
GJ	16	2	1	-0.44832*	0.08323*	0.2333 ± 0.1256	0.000134 ± 0.000183
JD	27	1	0	0	0	0	0

Abbreviations: N, Number of samples; Nh, Number of haplotypes; Ps, Number of polymorphic sites; D, Tajima's D; Fs, Fu's FS; h, Haplotype diversity; π, Nucleotide diversity. *p = 0.2

Geographic distribution and genetic relationship of S. alterniflora

In order to investigate the geographic distribution and genetic relationship between the identified haplotypes in Korea and previously reported haplotypes in both native and invasive regions, a haplotype network was constructed, including all 29 previously published haplotypes. The haplotype network revealed that the Korean populations were categorized into two haplotypes: C1 and C4. The haplotype N found at Gimje was clustered with the C4 haplotype group. This was based on the fact that the polymorphic site (site 1,624) observed in the N haplotype fell outside the aligned length (1,365 bp) of all the previously known haplotypes. Consequently, the C4 haplotypes encompassed populations of *S. alterniflora* from the U.S., China, Japan, and Korea (Seosan and Gimje; Fig. 2a). On the other hand, the C1 haplotype consisted of populations from the U.S., China, and Korea (Jindo).

Population differentiation of S. alterniflora

The population differentiation and hierarchical distribution of genetic variation among *S. alterniflora* populations were determined using analyses of molecular variance (Table 2; AMOVA). The majority of the genetic differentiation between native and invasive populations was found within the population (72.4%). However, the value of partitioned genetic variance was significant (Fst = 27598, $p < 0.001$). Similarly, in the invasive regions (China, Japan, and Korea), most of the variation was found within the population (93.13%), with no significant genetic variation existing between the three regions (Fst = 0.06871, $p = 0.5$), highlighting that there was no restricted gene flow between the regions and that the populations are homogeneous. When the Korean populations were analyzed separately, the majority of the genetic variation was observed among the population (95.77%), with significant haplotype differentiation between the regions (Fst = 0.91854 $p = 0.0$). This highlights the strong genetic structure, which is consistent with the haplotype network, suggesting that there were at least two local populations of *S. alterniflora* in Korea.

Table 2
Analysis of molecular variance (AMOVA) for populations of *Spartina alterniflora* in the U.S., China, Japan, and Korea based on chloroplast dataset.

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*
Native (USA) and invasive region (China, Japan, and Korea)						
Among populations	3	298.415556	10.25949	27.6	*F*st = 0.27598	< 0.001
Within populations	70	1884.112	26.91588	72.4		
Total	73	2182.52756	37.17537	100		
Invasive region (China, Japan, and Korea)						
Among populations	2	1.144	0.02935	6.87	*F*st = 0.06871	> 0.1 (NS)
Within populations	15	5.967	0.39778	93.13		
Total	17	7.111	0.42713	100		
Korea (Seosan, Gimje, and Jindo)						
Among populations	2	23.792	0.87965	95.77		
Within populations	45	1.75	0.03889	4.23	*F*st = 0.91854	0.00
Total	47	25.542	0.91854	100		

In addition, we used the neutrality test to examine the population expansion in the invasive regions (Table 3). Tajima's *D* was not significant in the Chinese population; however, Fu's *F*s value (-4.2606 $p = 0$) indicated that the Chinese population had recently expanded. However, in the Japanese and Korean populations, Tajima's *D* value was zero with a non-significant Fu's *F*s value. The zero value of Tajima's *D* indicated that the populations were evolving as per mutation-drift equilibrium.

Table 3
Neutrality test based on chloroplast DNA of *Spartina alterniflora* for the population of China, Korea, and Japan.

Statistics	China	Korea	Japan
Tajima's D test			
Tajima's *D*	0.93302	0	0
p	0.254	1	1
Fu's FS test			
*F*s	-4.2606	0.09021	0
p	0	0.302	

Discussion

The invasion of *Spartina alterniflora* has posed a serious threat to native fauna and has caused severe biodiversity challenges, necessitating nationwide control and management (Zuo et al. 2012; Strong and Ayres 2013). The identification of the source of its invasive populations and the reconstruction of invasion histories can play a crucial role in identifying appropriate biological control agents to mitigate the spread of this species. (Blum et al. 2007). In addition, understanding the levels of genetic diversity and structure in both the native and exotic ranges of *S. alterniflora* can advance our knowledge of the processes underlying early demographic establishment and evolutionary adaptation that occur after invasion (Guo et al. 2015). The present study provides insights into the genetic structure and invasion mechanisms of the *S. alterniflora* population that was recently introduced unintentionally into Korea.

The genetic analysis of the chloroplast sequence in this study demonstrated that the invasive population of *S. alterniflora* in Korea exhibits a strong genetic structure, indicating genetic heterogeneity. The population primarily consisted of two distinct haplotypes: haplotype C1, which was found in Jindo, and haplotype C4, which was observed in Gimje and Seosan. In addition, our study identified a new haplotype (N) formed by two individuals in Gimje. This haplotype could be a novel one discovered in this study, highlighting the gradual genetic differentiation within the Gimje population, which may be attributed to population expansion (Avise 2000). However, *S. alterniflora* was recently introduced into Korea (Kim et al. 2015); it is possible that the new haplotype observed in this study could be one of the existing haplotypes in the East Asian regions. Unfortunately, the sequence length of the reported haplotypes utilized in this study (1,365 bp; Qiao et al. 2019) was insufficient to determine the exact origins of the N haplotype (1,800 bp). Nonetheless, this discovery highlights the strong genetic structure of *S. alterniflora* in Korea, and such a genetic structure was confirmed by AMOVA, which revealed that most of the genetic variation was among the sampling locations. This indicates that the genetic differentiation among these populations can be mainly attributed to their different origins (Zalewski et al. 2010). Therefore, based on our data, we suggest that independent colonization events of *S. alterniflora* occurred on Korean coastal regions.

S. alterniflora was intentionally introduced into China for land reclamation from multiple areas of the Atlantic coast of the U.S., such as North Carolina, Georgia, and Florida (see Fig. 2b; Blum et al. 2007; Qiao et al. 2019). However, the spread of *S. alterniflora* into other East Asian regions, such as Taiwan, Japan, and Korea, was unintentional (Guo et al. 2015, Kim et al. 2015; Maebara et al. 2020). Among the identified haplotypes of *S. alterniflora*, specifically C1 and C4 were observed to be widely distributed in both the native regions in the U.S. and the introduced regions in China (Qiao et al. 2019). Notably, the C4 haplotype was found to be the most prevalent in China (Fig. 2b) and the only haplotype in Taiwan (Guo et al. 2015). Similarly, Maebara et al. (2020) also found only the C4 haplotype in Japan. Their results suggest that the C4 haplotype was probably introduced into Japan multiple times *via* international trade from China. Consistent with this, we observed both C1 and C4 haplotypes in Korea. Our haplotype network and AMOVA analyses showed that there was no significant genetic differentiation between the *S. alterniflora* populations in China (C1 and C4) and the invasive populations of Japan and Korea. Considering these results, historical evidences, and the high frequency of the C1 and C4 haplotypes in East Asia, we suggest that the two haplotypes of *S. alterniflora* likely independently invaded Korean coastal regions from other East Asian countries, particularly China, possibly *via* natural dispersal or transport vector (Hulme et al. 2008).

Usually, when colonizing new habitats, invasive species can experience low genetic diversity compared to their native origins due to founder effects (Dlugosch and Parker 2008). The reduced genetic diversity can limit the evolutionary potential and invasion success of the introduced species (Roman and Darling 2007; Dlugosch and Parker 2008). In the present study, we found low cpDNA diversity in the *S. alterniflora* populations in Korea, implying that the founder effect might have occurred in the Korean population. This is consistent with the Japanese *S. alterniflora* population, which also exhibited a low level of genetic diversity (Maebara et al. 2020). However, the introduced population in China exhibited high genetic diversity, with no significant loss in genetic variation compared with the native range (Guo et al. 2015; Qiao et al. 2019). The high genetic diversity facilitated the invasion success of *S. alterniflora* in China, which was shown to be due to the multiple introductions from various sources and subsequent genetic admixture, which increases the genetic diversity and facilitates rapid adaptation (Qiao et al. 2019). Hence, despite the low genetic diversity observed in the Korean population, distinct genetic differences were observed among populations based on their geographical locations, likely resulting from multiple introductions. Therefore, the potential for genome exchange between regions, the continuous introduction of *S. alterniflora* individuals, and the possibility of population expansion may further increase the genetic diversity within the Korean population. This, in turn, can facilitate rapid adaptation, enhance invasion success, and further promote the spread of the species. Therefore, this observed genetic composition in the Korean population might warrant the most attention for coastal management to prevent the further spread of this invasive species.

In conclusion, this study revealed the population genetic structure of the invasive *S. alterniflora* on the Korean coasts. The population had low genetic diversity, with two haplotypes (C1 and C4) that were geographically isolated. Molecular comparisons revealed that East Asian populations are homogeneous. Our findings suggest that the *S. alterniflora* population experienced independent introduction events

along Korean coasts and that the populations might have been introduced unintentionally into Korea from China. The observed genetic structure in Korea may increase the adaptation and invasion success of *S. alterniflora*. Thus, it is important to strengthen coastal management to prevent further spread in Korea and other countries around the world.

Declarations

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Author contributions Conceptualization, molecular work, data analysis, and writing-original draft, review and editing were performed by BLM. Conceptualization, review and editing were performed by KL, WY and JC. Conceptualization, supervision, writing-review and editing by JSK.

Ethical approval

This research does not involve ethical research.

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Data availability The chloroplast DNA haplotypes sequences generated during current study are available in the GenBank with the following accession (C1, OR130493; C4, OR130494; N, OR130495) The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest The authors declare that they have no conflicts of interest.

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Figures

Fig 1

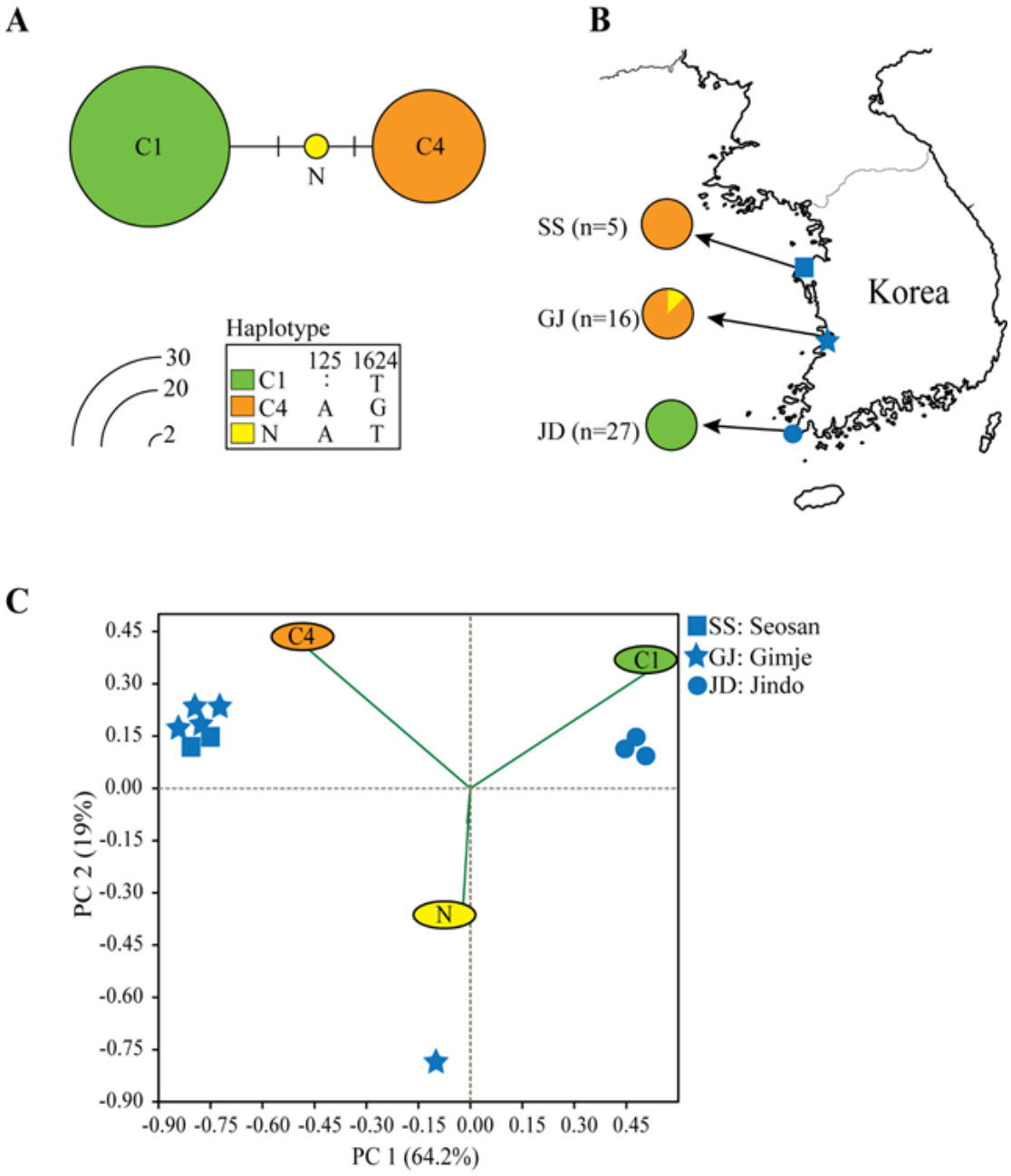


Figure 1

The TCS haplotype network of *trnT-trnF* cpDNA haplotypes exhibited by *Spartina alterniflora* sampled from Korea (**A**). Each circle on the haplotype network represents individual haplotypes, and the size of the cycle is the proportion of haplotype frequency. The colors indicate the geographical origin of the haplotypes. Each line between haplotypes represents a 1-nucleotide mutational change. The box contained haplotypes and the positions of indel variation. Geographic distribution of the haplotypes

along Korean coasts (**B**; SS; Seosan, Gj: Gimje, JD; Jindo; n = number of individuals sampled per location). PCA biplot showing the geographic genetic variation of *S. alterniflora*haplotypes (**C**).

Fig. 2

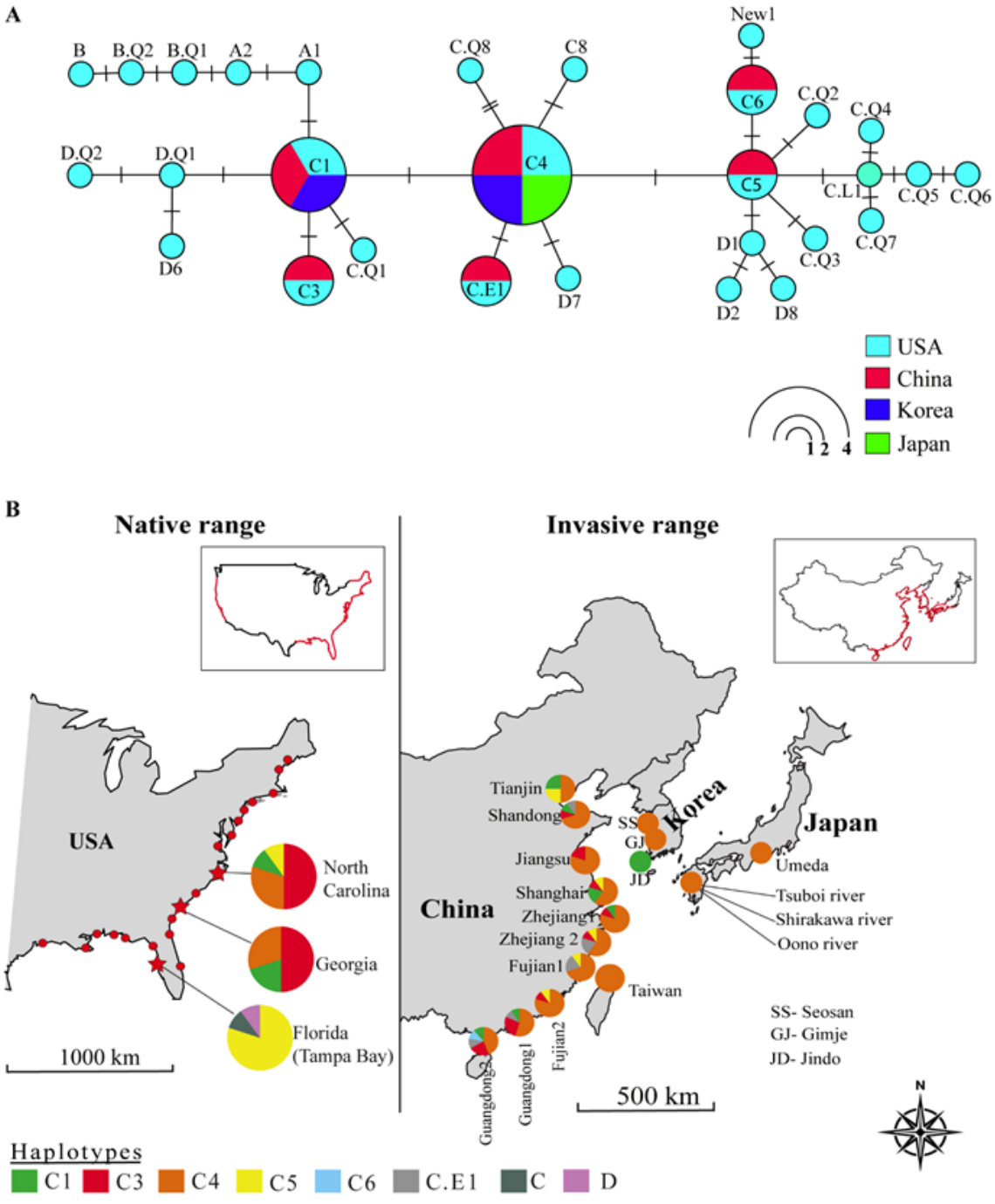


Figure 2

The TCS haplotype network of chloroplast DNA (cpDNA) haplotypes of the United States, China (Qiao et al. 2019), Japan (Maebara et al. 2020), and Korea (**A**; this study). Each circle on the haplotype network

represents individual haplotypes, and the size of the cycle is the proportion of haplotype frequency. The colors indicate the country of origin of the haplotypes. Each line between haplotypes represents a 1-nucleotide mutational change. Frequency and distribution of haplotypes in the regions of origin (the eastern United States) and in the regions where *Spartina alterniflora* had been introduced intentionally (China) or unintentionally (Japan and Korea; **B**). The red dots along eastern U.S. are the reported distribution of *S. alterniflora*, while the red star indicates the regions where *S. alterniflora* was initially introduced into China (Bernik et al. 2016).

Supplementary Files

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