Research Article

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Genetic diversity of *Undaria pinnatifida* populations from China and their genetic relationship with those from Japan and Korea as revealed by mitochondrial and nuclear DNA sequences

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Abstract: Large-scale farming of *Undaria pinnatifida* is conducted in northern China. Conspicuous natural populations of this alga are distributed on rocky shores in this region. However, the genetic relationship between *U. pinnatifida* from China and native populations in other countries remains largely uncertain. We obtained sequences for the mitochondrial *cox3* and *tatC–tLeu* regions and the internal transcribed spacer one of nuclear ribosomal DNA from representative natural and farmed populations of *U. pinnatifida* in China. We analyzed genetic diversity, and evaluated the genetic relationship between Chinese populations and Japanese and Korean populations. The mitochondrial and nuclear DNA sequences revealed high genetic diversity in most Chinese populations. Unique mitochondrial haplotypes were detected in the Gouqi Island population consistent with historical records of a native population on the island. Phylogenetic analyses derived from the mitochondrial DNA sequences revealed that the Chinese samples were classifiable as the Continental and Northern Japan types. All natural populations from rocky reefs in northern China were grouped with the Continental type and all farmed populations with the Northern Japan type. Mitochondrial and nuclear DNA sequences revealed significant genetic differentiation between the farmed populations and adjacent natural populations from rocky reefs, in agreement with previous results obtained using microsatellites.

Keywords: brown algae; genetic connectivity; genetic diversity; kelp; seaweed.

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1 Introduction

Undaria pinnatifida (Harv.) Suringar is a kelp species indigenous to the northwestern Pacific coast. The species has recently become cosmopolitan owing to its worldwide spread driven by aquaculture and maritime transport, and it is regarded as invasive in many countries outside its native range (Epstein and Smale 2017). Phylogeographic analyses have shed light on the mechanism of its introduction and worldwide spread. Mitochondrial sequences of the intergenic spacers *atp8–trnS and trnW–trnI* have been used to compare genetic diversity and sequence divergence among 24 populations of *U. pinnatifida* from its native and introduced ranges (Voisin et al. 2005). The results suggest that aquaculture was a major vector of introduction and spread in Europe, and that maritime transport likely facilitated recurrent introductions to Australasia. Sequence data for the mitochondrial gene *cox3* and the noncoding region between the *tatC* and *tLeu* genes of 260 specimens of *U. pinnatifida* from Japan, Korea, China, and introduced populations were investigated previously, among which rich genetic diversity was detected in the samples from Japan (Uwai et al.

2006a,b). Samples of *U. pinnatifida* from its native range were classified into four genetic and biogeographical groups according to haplotypes of concatenated sequences of *cox3* and the *tatC–tLeu* region. The Japanese samples were divided into three types, namely the Northern Japan type, the Pacific central Japan type, and the Sea of Japan type. The Continental type was detected in the Chinese and Korean samples (Uwai et al. 2006a). Recently, microsatellites and single-nucleotide polymorphisms were used to analyze the distribution pattern of genetic diversity of *U. pinnatifida* (Graf et al. 2021; Shan et al. 2019). The genetic diversity in the native range of the species was generally higher than in the introduced regions, reflecting founder effects in the introduced populations.

Undaria pinnatifida is extensively farmed as an economically important alga in Japan, Korea, and China (Tseng 2001; Yamanaka and Akiyama 1993). Commercial farming began in the 1980s in China with the original cultivated stock introduced from Japan and Korea (Tseng 2001). Such introductions have subsequently been a frequent commercial strategy of Chinese seaweed-farming companies, aiming at improving the agronomical traits of the farmed populations (Qu 1993). Liaoning and Shandong provinces are the two most important farming regions. Conspicuous natural populations can be observed on rocky shores in northern China, among which the natural populations in Dalian and Qingdao originated from intentional transplantation of *U. pinnatifida* from Korea in 1930s, and those in Yantai and Weihai were secondary derivatives of individuals transplanted from Dalian and Qingdao (Li 1991). Native populations of *U. pinnatifida* in China are documented to be distributed in the Zhejiang and Fujian provinces (Tseng 2001), but individuals have seldom been observed recently in Fujian, which is possibly a result of global ocean warming. Populations of *U. pinnatifida* still prosper on small islands off the coast of Zhejiang province, such as Gouqi and Yushan islands. The distribution range of this alga in China is now generally restricted between the latitudes of 28° and 40° N.

Given the economic importance of *U. pinnatifida* and to facilitate its breeding and cultivation, it is necessary to understand the geographic distribution pattern of its genetic diversity in China and particularly the genetic relationship of Chinese populations with those from Japan and Korea. Microsatellites have been used previously to analyze the genetic diversity and connectivity of natural and farmed populations of *U. pinnatifida* in China (Li et al. 2020; Shan et al. 2018). However, Japanese and Korean populations were not included in those studies. Although native and introduced populations were investigated using mitochondrial DNA sequences by Voisin et al. (2005) and Uwai et al. (2006a), samples from only one provenance in China were included in each study, which is insufficient to reflect the overall genetic diversity in China. Hence, the genetic relationship of Chinese populations with Japanese and Korean populations remains largely uncertain. Given the informativeness of mitochondrial DNA sequences revealed in these two studies, sequences for samples from Chinese populations can be integrated with the previously analyzed data sets for comparison. Considering the larger data sets of Japanese and Korean samples of Uwai et al. (2006a), sequences of *cox3* and the *tatC–tLeu* region are expected to be more suitable for comparison of Chinese populations with Japanese and Korean populations. In addition, sequences of the internal transcribed spacer 1 (ITS1) of nuclear ribosomal DNA for some Japanese samples of *U. pinnatifida* have been obtained (Uwai et al. 2006b), which can also be exploited to study the genetic relationship between Chinese and Japanese populations.

In the present study we obtained partial sequences for the *cox3*, *tatC–tLeu*, and ITS1 regions from the main natural and farmed populations of *U. pinnatifida* in China. We evaluated the genetic diversity, and compared the sequence divergence with Japanese and Korean populations using DNA sequences accessible in the GenBank database (https://www.ncbi.nlm.nih.gov/). The results are expected to guide future plans concerning the conservation of stock resources and the design of breeding programs for this commercially important alga.

2 Materials and methods

Natural and farmed *Undaria pinnatifida* populations, including the W17, QD16, SW-18, F1-15, and F2-15 populations from our previous studies (Li et al. 2020; Shan et al. 2018), and two additional intertidal natural populations on rocky reefs from Heishijiao, Dalian (designated HSJ17; 38.87° N, 121.56° E) and Gouqi Island (designated GQ21; 30.70° N, 122.77° E) sampled on April 10, 2017 and April 29, 2021, respectively, were investigated (Table 1). Genomic DNA (from 24 individuals per population) was extracted as described by Shan et al. (2018).

The extracted genomic DNA was used to amplify the partial *cox3* gene, the *tatC–tLeu* region, and ITS1 using the primers and PCR programs of Uwai et al. (2006a,b). The primers used were CAF4A (5′-ATGTTTACTTGGTGRAGRGA-3′) and CAR4A (5′-CCCCACCARTAWATNGTNAG-3′) for the *cox3* gene, tatCEF (5′-AAATAATATATTGAGATTTTAAGTCTATTCAT-3′) and tLeuR (5′-AACCTAAACACCGCGTGTATACC-3′) for the *tatC-tLeu* region, and Pha18EF (5′-AGGAAGGTGAAGTCGTAACAAGGTTT-3′) and Pha5.8ER (5′-AACAGACACTCCGACAAGCATGCTCCC-3′) for ITS1. The *Taq* Master Mix (Accurate Biology, China) was used for PCR amplification with a T-gradient thermocycler (Biometra, Germany). The PCR products were sequenced in both directions using an ABI 3730XL automated sequencer (Applied Biosystems, USA) by the Beijing Tsingke Biotechnology Co., Ltd.

The publicly available sequences for *cox3*, *tatC–tLeu*, and ITS1 of *Undaria* were downloaded from GenBank (Table 2) and used for

Table 1: Sampling information of the farmed and wild populations of *Undaria pinnatifida* from China.

Name	Type	Location	Collection date	Coordinate	Growing substrate	References
F1-15	Farmed	Dalian	April 13, 2015	38°47′N, 121°16′E	Longline	Shan et al. (2018)
F2-15	Farmed	Dalian	April 13, 2015	38°47′N, 121°16′E	Longline	Shan et al. (2018)
W17	Natural	Dalian	April 18, 2017	38°47′N, 121°16′E	Cultivation raft	Shan et al. (2018)
SW18	Natural	Dalian	May 10, 2018	38°47′N, 121°16′E	Rocky reef	Li et al. (2020)
HSJ17	Natural	Dalian	April 10, 2017	38°52′N, 121°34′E	Rocky reef	This study
QD16	Natural	Qingdao	April 10, 2016	36°03′N, 120°22′E	Rocky reef	Shan et al. (2018)
GQ21	Natural	Gouqi island	April 29, 2021	30°42′N, 122°45′E	Rocky reef	This study

alignment and comparative analysis with sequences newly obtained in the present study. The sequences were aligned with MUSCLE in MEGA X (Kumar et al. 2018). Sequences of the *cox3* and *tatC–tLeu* regions were first aligned separately, and the aligned sequences were then joined to form a concatenated alignment. Note that the length of the downloaded sequences differed, and their length was trimmed to conform with that of the shortest sequence during alignment. Haplotypes were identified using DnaSP 5.10 (Rozas et al. 2003). The number of haplotypes (N_h), haplotype diversity (h), and nucleotide diversity (π) were computed with ARLEQUIN 3.11 (Excoffier et al. 2005). The pairwise F_{st} values among Chinese populations were calculated using ARLEQUIN 3.11 with 1000 permutations to assess the levels of genetic differentiation.

Phylogenetic trees were constructed using the maximum likelihood (ML) method with MEGA X and the Bayesian inference (BI) method with MrBayes 3.2 (Ronquist et al. 2012). In the ML analysis, the nucleotide substitution model with the lowest Bayesian information criterion score was considered the best model. The best model was determined to be HKY + G (Hasegawa-Kishino-Yano + Gamma distribution) and JC (Jukes-Cantor) + G for the combined *cox3* and *tatC–tLeu* sequences, and ITS1 sequences, respectively. The Nearest-Neighbor-Interchange heuristic method was used for tree inference. The initial trees were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with the highest log-likelihood value. A bootstrap analysis with 2000 repetitions was applied to estimate the reliability of the inferred trees. For the BI method, four Markov chains were set to run up to 10 million generations, with a sample frequency of 100. The burnin fraction and the stopval were set at 0.25 and 0.01, respectively. The kelps *Lessoniopsis littoralis* (GenBank accession no. MZ156066; Starko et al. 2021) and *Alaria esculenta* (GenBank accession no. MH482485; Bringloe and Saunders 2019) were used as outgroups to root the phylogenetic tree of the combined *cox3* and *tatC–tLeu* region, and ITS1 sequences, respectively. Statistical parsimony network analyses were conducted to evaluate the relationships among haplotypes using TCS 1.21 (Clement et al. 2000). Each continuous gap was treated as a single block according to Uwai et al. (2006a) and considered the fifth character state for network estimation.

Table 2: Accession numbers of sequences for the *cox3*, *tatC-tLeu* and ITS1 of *Undaria* downloaded from GenBank and used for analyses in the present study.

Sequence	Accession nos.	References
cox3	AB213030-AB213038	Uwai et al. (2006b)
	AB240669-AB240673	Uwai et al. (2006a)
	AB889527-AB889535	Yoshinaga et al. (2014)
	LC185231-LC185232	Niwa et al. (2017)
	AB267269	Uwai et al. (2007)
	AB775240	Kawai et al. (2013)
	GQ368282	Silberfeld et al. (2010)
	KF319031	Li et al. (2015)
tatC-tLeu	AB240644-AB240668	Uwai et al. (2006a)
	LC185233-LC185234	Niwa et al. (2017)
	KF319031	Li et al. (2015)
ITS1	AB213040-AB213052	Uwai et al. (2006b)
	AF319008	Yoon et al. (2001)

3 Results

A total of 164 DNA samples (22–24 individuals per population) were successfully amplified for the three DNA regions. Together with the sequences downloaded from GenBank, the alignments for *cox3*, the *tatC–tLeu* region, and the combined data set consisted of 193, 189 and 197 sequences, leading to resolution of 22, 23 and 37 distinct haplotypes, respectively. The alignment of ITS1 comprised 178 sequences, of which 24 distinct ribotypes were resolved. For consistency with previous studies (Uwai et al. 2006a,b; Yoshinaga et al. 2014) and to avoid redundancy, if the haplotypes detected among the Chinese samples in the present study were identical to previously resolved haplotypes, we adopted the previously used haplotype names; otherwise, new haplotype names were designated. Only novel haplotypes were submitted to GenBank and allocated accession numbers.

3.1 Genetic diversity and haplotype distribution in Chinese populations

The aligned sequences of *cox3* comprised 431 bp. Four haplotypes were detected among the Chinese samples, of which two were identical to the HI/HVII/C1 and C2 haplotypes (accession nos. AB213030/AB213036/AB889527 and AB889528, respectively; Uwai et al. 2006b; Yoshinaga et al. 2014), and two detected in the GQ21 population were novel (accession nos. OL539399 and OL539400). Note that some

haplotypes were identical (e.g., HI and HVII with accession nos. AB213030 and AB213036) owing to the shorter alignment in the present study, and are indicated by a backslash (/) between the haplotypes throughout the text. The HI/HVII/C1 haplotype was the predominant haplotype and was detected in all Chinese populations (Supplementary Figure S1). One to three haplotypes were detected in each population, with h ranging from 0 to 0.514 ± 0.041 and π ranging from 0 to 0.0012 ± 0.0012 (Supplementary Table S1). Each of the two novel haplotypes was detected only in one individual of GQ21.

The aligned *tatC–tLeu* sequences comprised 410 bp and contained substitutions and insertions/deletions (indels). Most variation was detected in the intergenic spacer region between the *tTrp* and *tIle* genes. Six haplotypes were identified among the Chinese samples, of which two detected in the F1-15 and GQ21 populations were novel (accession nos. OL580828 and OL580829; Supplementary Figure S2). Each population contained one to four haplotypes, with h ranging from 0 to 0.598 ± 0.051 and π ranging from 0 to 0.0195 ± 0.0105 (Supplementary Table S1).

Consistent with the 33 haplotypes resolved by Uwai et al. (2006a), the concatenated *cox3* and *tatC–tLeu* sequence of cultivars one and two in Niwa et al. (2017) was designated H34, and a combination of the previously reported accession nos. LC185231/AB889528 of *cox3* and AB240652 of *tatC–tLeu* was designated H35 (Table 3). Alignment of the concatenated sequences (841 bp) of *cox3* and *tatC–tLeu* led to detection of 10 haplotypes among the Chinese samples. Six of these haplotypes (H1, 10/25, 11, 14, 34, and 35) were identified in previous studies. The other four were novel haplotypes (designated H36 to H39) that were only detected in the Chinese samples: H36 was detected in three individuals of F1-15, H37 in two individuals of GQ21, and H38 and H39 each in one individual of GQ21. One to six haplotypes were detected in each population, with h ranging from 0 to 0.634 ± 0.058 and π ranging from 0 to 0.0097 ± 0.0052 (Table 4). When all Chinese populations were considered as a whole, the h and π values were 0.810 ± 0.013 and 0.0118 ± 0.0060, respectively. The haplotypes H1 and H34 predominated with the same frequency in F1-15, whereas H34 accounted for more than 90% of the haplotypes in F2-15 (Figure 1A). Only H10/H25 was detected in QD16 and this haplotype was also predominant in GQ21. H14 was detected at high frequency in W17, SW-18, and HSJ17. In addition, H35 accounted for a large proportion of the haplotypes in SW-18 (Figure 1A).

The aligned ITS1 sequences comprised 239 bp, and included indels and partial 5.8S rDNA. For the convenience of comparison and subsequent analysis, the 13 ribotypes resolved by Uwai et al. (2006b) were designated R1–R13 (accession nos. AB213040 to AB213052) in the present study. A total of 16 ribotypes were identified among the Chinese samples; five ribotypes were identical to R3–R5, R8, and R12 of Uwai et al. (2006b), 10 were novel (accession nos. OL584517 to OL584526, designated R14–23), and one was identical to accession no. AF319008 (designated R24) (Figure 2A). One to seven ribotypes were observed in each population, with h ranging from 0 to 0.775 ± 0.063, and π ranging from 0 to 0.01451 ± 0.0086 (Table 4). The ribotype R8 was the most common and was detected in all populations (85 individuals), followed by R14 detected in three populations (37 individuals). R14 was the most common of the novel ribotypes. All populations except QD16 harbored at least one novel ribotype.

Table 3: Novel haplotypes of the combined sequences of *cox3* and *tatC-tLeu* compared to Uwai et al. (2006a), with the corresponding combination of the accession nos. of *cox3* and *tatC-tLeu* sequences.

Haplotype	*cox3* gene	*tatC-tLeu* region
H34	LC185231/AB889528	LC185233/AB240644
H35	LC185231/AB889528	AB240652
H36	AB213030	OL580828
H37	AB213030	OL580829
H38	OL539399	AB240644
H39	OL539400	AB240652

There were 33 haplotypes in Uwai et al. (2006a), and hence the six novel haplotypes were designated H34–H39.

Table 4: Genetic diversity of Chinese *Undaria pinnatifida* populations estimated using the combined *cox3* and *tatC-tLeu* sequences and ITS1 sequences.

Population	N	The combined *cox3* and *tatC-tLeu*				ITS1			
		N_p	N_h	h	π ($\times 10^{-2}$)	N_p	N_h	h	π ($\times 10^{-2}$)
HSJ17	24	3	4	0.533 ± 0.105	0.1168 ± 0.0913	9	7	0.775 ± 0.063	1.4508 ± 0.8571
SW-18	24	3	4	0.634 ± 0.058	0.1409 ± 0.1043	9	4	0.562 ± 0.092	0.8742 ± 0.5659
W17	23	23	6	0.569 ± 0.114	0.8009 ± 0.4370	11	5	0.668 ± 0.079	1.1185 ± 0.6914
QD16	24	0	1	0.000 ± 0.000	0.000 ± 0.000	0	1	0.000 ± 0.000	0.000 ± 0.000
GQ21	24	23	5	0.580 ± 0.099	0.9693 ± 0.5195	9	2	0.083 ± 0.075	0.3191 ± 0.2719
F1-15	23	2	3	0.632 ± 0.052	0.0915 ± 0.0780	13	6	0.672 ± 0.070	1.1942 ± 0.7296
F2-15	22	1	2	0.173 ± 0.101	0.0211 ± 0.0324	6	4	0.593 ± 0.089	1.1470 ± 0.7079

N, number of individuals; N_p, number of polymorphic sites; N_h, number of haplotypes; h, haplotype diversity; π, nucleotide diversity.

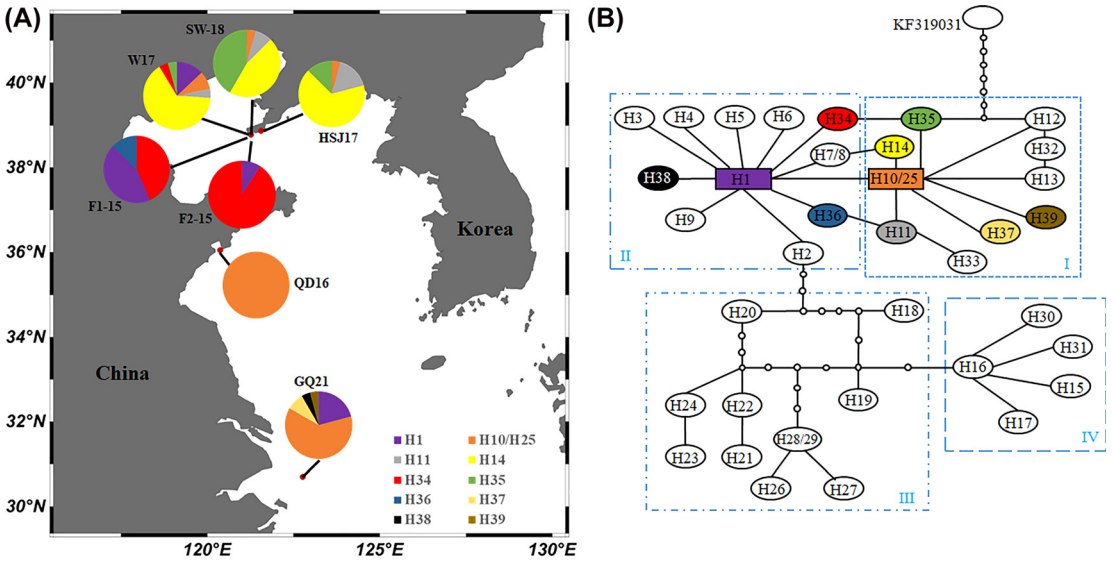

Figure 1: Geographic distribution of haplotypes in natural and farmed populations of *Undaria pinnatifida* from China (A) and statistical parsimony network (B) of the combined *cox3* and *tatC–tLeu* sequences. The color areas in the pie charts are proportional to the haplotype frequency in the map. Refer to Uwai et al. (2006a) and Table 3 for explanation of the haplotype names and classification of the clades I to IV, which are enclosed by boxes with lines of different patterns. Small circles indicate undetected haplotypes. Each line connecting haplotypes represents one base mutation. The haplotypes detected in the Chinese samples in the present study are indicated in the haplotype network with the same colors as those in the map.

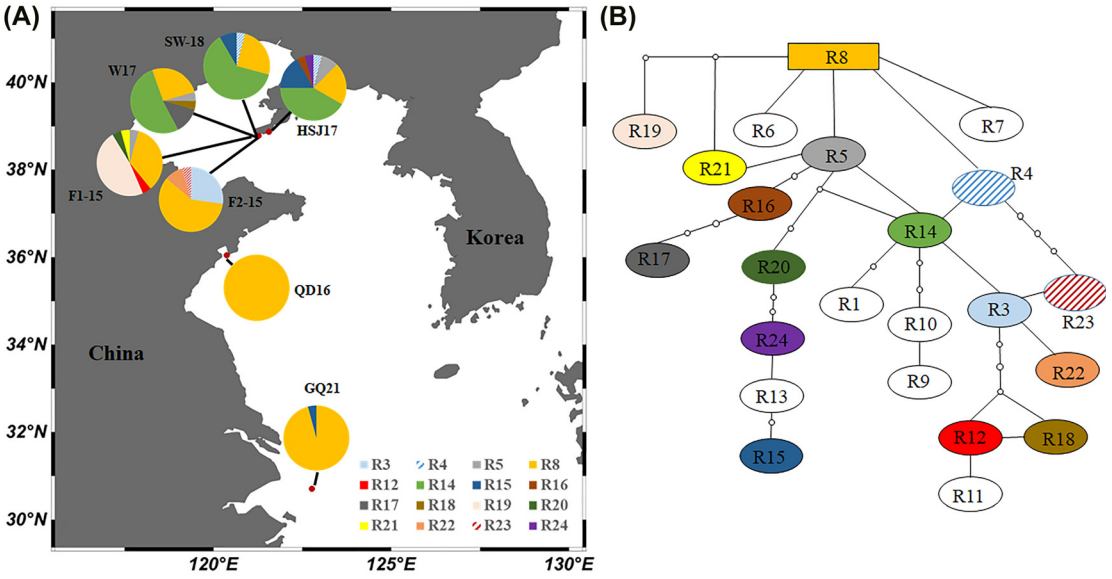

Figure 2: Geographic distribution of haplotypes in natural and farmed populations of *Undaria pinnatifida* from China (A) and statistical parsimony network (B) of ITS1 sequences. The color areas in the pie charts are proportional to the ribotype frequency in the map. Small circles indicate undetected ribotypes. Each line connecting ribotypes represents one base mutation. The ribotypes detected in the Chinese samples in the present study are indicated in the ribotype network by the same colors as those in the map.

3.2 Phylogenetic relationships among the haplotypes

The phylogenetic trees constructed from the combined *cox3* and *tatC–tLeu* sequences using the ML and BI methods showed similar topologies (Figure 3). The haplotypes were generally clustered into three clades, corresponding to the Continental type and the Northern Japan type (I and II), the Pacific central Japan type (III), and the Sea of Japan type (IV) of Uwai et al. (2006a). Clade I and II was supported by a

bootstrap value of 77% and a posterior probability of 0.91. All haplotypes detected among the Chinese samples, including the newly designated and detected haplotypes (H34–H39), were placed in clades I and II. Haplotypes H15–H17, H30, and H31 were grouped in clade IV with bootstrap support of 72% and a posterior probability of 0.87. Most of the other haplotypes were grouped in clade III. The statistical parsimony network of the combined *cox3* and *tatC–tLeu* sequences revealed that the samples were generally resolved into four groups corresponding to types I to IV (Figure 1B). Again, all haplotypes identified among the Chinese samples were grouped into types I and II.

Similar topologies were obtained in the phylogenetic trees inferred from ITS1 ribotypes with the ML and BI methods (Figure 4). However, no large clade was supported by both high bootstrap and posterior probability values. In the statistical parsimony network inferred from ITS1 ribotypes, R8 was placed at the base of the network and the other ribotypes were derived from R8 via at least one base mutation (Figure 2B).

3.3 Genetic differentiation among Chinese populations

Similar patterns of genetic divergence among Chinese populations were revealed by the combined *cox3* and *tatC–tLeu* sequences and the ITS1 sequences (Table 5). In general, limited genetic differentiation was detected among the three natural populations from Dalian (SW-18, W17, and HSJ17), except for that between SW-18 and W17 with a significant F_{st} value of 0.13 (adjusted p = 0.004), on the basis of the combined *cox3* and *tatC–tLeu* sequences. GQ21 was significantly differentiated from all other populations except W17 and QD16 on the basis of the combined *cox3* and *tatC–tLeu* sequences. With regard to the ITS1 sequences, no genetic differentiation between QD16 and all other populations was observed. On the basis of the combined *cox3* and *tatC–tLeu* sequences, however, QD16 was significantly differentiated from the two farmed populations F1-15 and F2-15 because no haplotypes were shared between QD16 and the latter two populations. F1-15 and F2-15 were not only significantly differentiated from each other, but also from all other populations, on the basis of the mitochondrial and nuclear DNA sequences.

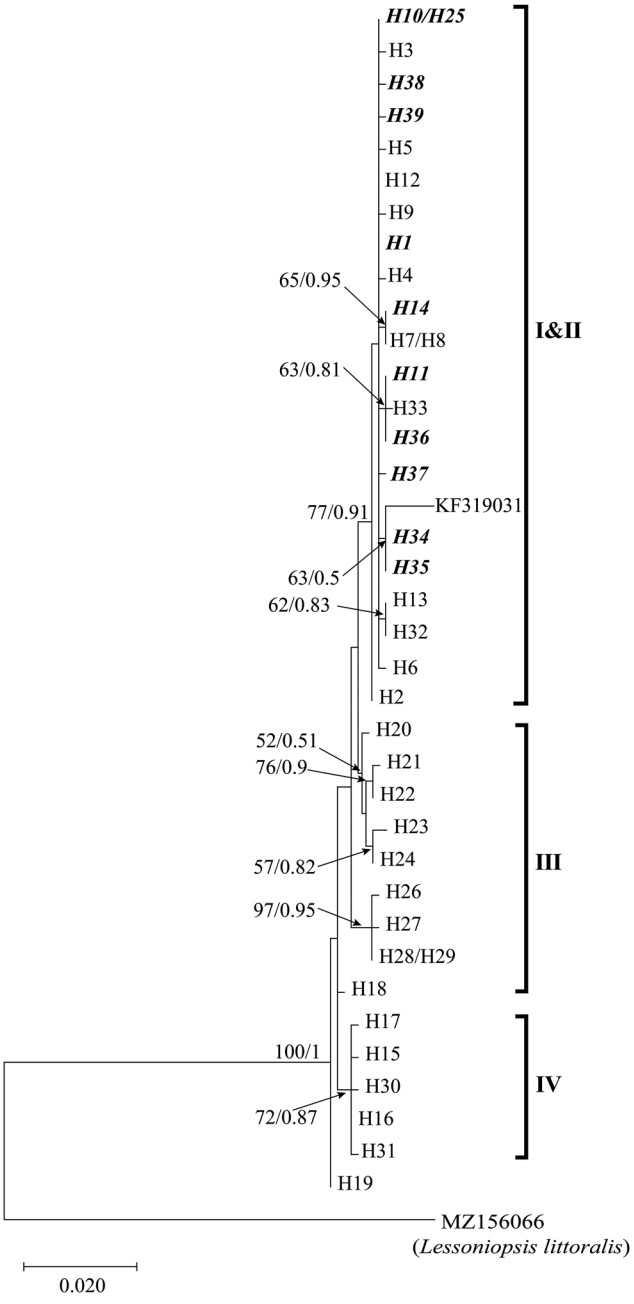

Figure 3: Maximum likelihood phylogenetic tree inferred from the alignment of the combined *cox3* and *tatC–tLeu* sequences. Bootstrap values and Bayesian posterior probabilities >50% are shown, and "-" indicates a value <50%. The branch length is proportional to the sequence divergence indicated by the scale bar (substitutions per site). Refer to Uwai et al. (2006a) for explanation of the haplotype names and classification of the clades I to IV. The haplotypes detected in the Chinese samples in the present study are indicated with bold italicized fonts. *Lessoniopsis littoralis* was used as an outgroup to root the tree.

4 Discussion

In the present study, the combined *cox3* and *tatC–tLeu* sequences and ITS1 sequences were both informative for detection of intraspecific genetic diversity in *U. pinnatifida* from China. High genetic diversity was revealed by these sequences in most Chinese populations as demonstrated

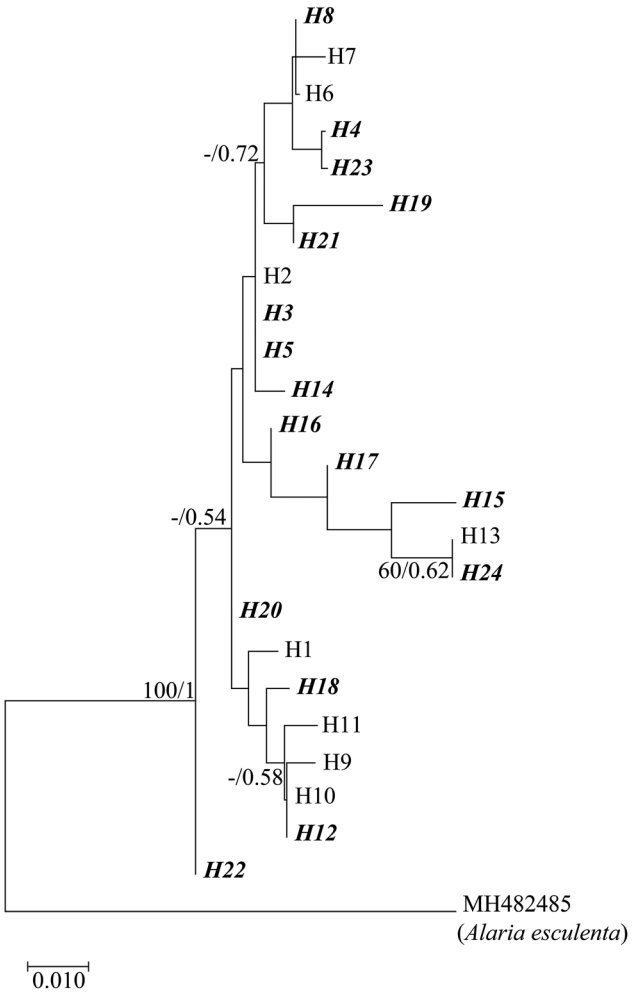

Figure 4: Maximum likelihood phylogenetic tree inferred from the alignment of ITS1 sequences. Support values are shown as in Figure 3. The ribotypes detected in the Chinese samples in the present study are indicated with bold italicized fonts. *Alaria esculenta* was used as an outgroup to root the tree. The branch length is proportional to the sequence divergence indicated by the scale bar (substitutions per site).

by the N_h, h, and π values. Among the Chinese natural populations, only the population from Gouqi Island harbored novel and unique haplotypes of the combined *cox3* and *tatC–tLeu* sequences. When the haplotypes detected in the present study were integrated with haplotypes detected in previous studies, a clear genetic relationship between Chinese populations and Japanese and Korean populations was revealed. Mitochondrial and nuclear DNA sequences also revealed significant genetic differentiation between the farmed populations and the adjacent natural populations from rocky reefs. These results provide valuable information for conservation of stock resources and design of breeding programs for *U. pinnatifida*.

4.1 Comparison of genetic diversity and haplotype distribution between Chinese populations and Japanese and Korean populations

Twenty-seven haplotypes of the combined *cox3* and *tatC–tLeu* sequences were identified in the native populations (Uwai et al. 2006a). In comparison, 10 haplotypes were detected in the Chinese populations in this study. Despite the fewer number, novel haplotypes of *cox3* and *tatC–tLeu* were detected in the Chinese populations, and analysis of the combined *cox3* and *tatC–tLeu* sequences led to the resolution of four novel haplotypes (H36–H39) only present in the Chinese populations: three (H37–H39) were detected in GQ21 and one (H36) in F1-15. Given that the sequence length was trimmed to that of the shortest sequence downloaded from GenBank, the mutations between H7 and H8, between H10 and H25, and between H28 and H29 were not recovered in this study. In total, five haplotypes of the combined *cox3* and *tatC–tLeu* sequences were detected in GQ21; except for the three unique haplotypes (H37–H39), the other two haplotypes were H1 and H10/H25, which were the same as those detected at Kuko Island (supposedly the same island as Gouqi Island) by Uwai et al. (2006a). The detection of three novel haplotypes (H37–H39) unique to GQ21 is

Table 5: Pairwise genetic differentiation coefficient F_{st} among *Undaria pinnatifida* populations from China (values above the diagonal were computed from concatenated sequences of *cox3* and *tatC-tLeu*, and those below from ITS1 sequences).

Populations	SW-18	HSJ17	W17	F1-15	F2-15	QD16	GQ21
SW-18		0.08	0.13[a]	0.95[a]	0.97[a]	0	0.24[a]
HSJ17	0.01		0.11	0.96[a]	0.97[a]	0	0.25[a]
W17	0.002	0.04		0.79[a]	0.81[a]	0	0.03
F1-15	0.37[a]	0.32[a]	0.34[a]		0.32[a]	0.96[a]	0.71[a]
F2-15	0.25[a]	0.17[a]	0.26[a]	0.29[a]		0.99[a]	0.73[a]
QD16	0	0	0	0	0		0
GQ21	0.38[a]	0.31[a]	0.36[a]	0.28[a]	0.27[a]	0	

[a]Significant at $p = 0.05$ after being adjusted for multiple comparisons with "BH" method.

consistent with historical records of a native *U. pinnatifida* population on this island (Tseng and Zhang 1952). Long-term independent evolution of *U. pinnatifida* at this island may have resulted in divergence of the unique haplotypes. In contrast, the single novel haplotype (H36) observed in F1-15 might exist in Japan or Korea although it has not yet been detected, because the farmed populations in China are documented to have originated from Japan and Korea (Tseng 2001). At least 15 batches of *U. pinnatifida* were successfully introduced from Japan, mainly from the Sanriku region, to Dalian between 1982 and 1993 (Qu 1993). H35 is also possibly a haplotype that has not yet been detected in Japan because it is composed of previously reported *cox3* and *tatC–tLeu* haplotypes (LC185231/LC185232 and AB240652; Niwa et al. 2017; Uwai et al. 2006a). No unique haplotypes of combined *cox3* and *tatC–tLeu* sequences were detected in the natural populations of northern China, in agreement with records that the natural populations in Dalian and Qingdao originated from Korea in 1930s and the native populations of *U. pinnatifida* only exist in Zhejiang and Fujian provinces in China (Li 1991). Although 10 novel ITS1 ribotypes were detected only in Chinese populations, it cannot be affirmed that they are unique to China because only 13 ribotypes of ITS1 were available from a limited number (42) of Japanese individuals (Uwai et al. 2006b), and these are insufficient to represent the overall genetic diversity in Japan.

The overall haplotype diversity of the combined *cox3* and *tatC–tLeu* sequences was slightly lower among the Chinese samples than that observed among samples from Japan, Korea, and China in the previous study (0.810 ± 0.013 vs. 0.8687 ± 0.025), whereas the nucleotide diversity was higher in the former than in the latter (0.0118 ± 0.0060 vs. 0.0062 ± 0.0033) (Uwai et al. 2006a). The haplotype diversity at the population level was high in China except for QD16, in which only one haplotype/ribotype was detected for each DNA region. For other populations, at least two haplotypes/ribotypes were detected in each population for the combined *cox3* and *tatC–tLeu* sequences and the ITS1 region, and as many as six haplotypes were observed in W17 for the former and up to seven ribotypes were detected in HSJ17 for the latter. Most of these populations had an h value higher than 0.5, except F2-15 for the combined *cox3* and *tatC–tLeu* sequences ($h = 0.173$) and GQ21 for ITS1 ($h = 0.083$). This finding is in agreement with the high genetic diversity revealed in the same populations by microsatellite analysis, in which the average number of alleles was more than seven and the expected heterozygosity was greater than 0.7 (Li et al. 2020; Shan et al. 2018). However, microsatellites also revealed high genetic diversity in QD16, suggesting that the multi-allelic microsatellites are more informative in revealing intra-population genetic diversity than single gene sequences.

4.2 Phylogenetic relationship between Chinese populations and Japanese and Korean populations

Consistent with the results of Uwai et al. (2006a), phylogenetic trees and the statistical parsimony network derived from the combined *cox3* and *tatC–tLeu* sequences in the present study supported categorization of the samples into four groups. Interestingly, all haplotypes, including the novel ones, identified in the Chinese samples were classified as the Continental type and the Northern Japan type (I and II). Further, all haplotypes in the three natural populations sampled from rocky shores in northern China (HSJ17, SW-18, and QD16) were classified as the Continental type, and all haplotypes detected in the two farmed populations (F1-15 and F2-15) belonged to the Northern Japan type. The classification of the farmed populations was consistent with the fact that Chinese seaweed-farming companies used to buy seedlings from the Sanriku region of northern Japan, and the classification of the natural populations HSJ17, SW-18, and QD16 accords with records that the founding material originated from Korea (Li 1991). Other Chinese populations harbored both types I and II. In type I, H10/H25 connected other haplotypes detected in the Chinese populations via a one-mutation step. With regard to type II, H1 was located in the center of the haplotype network, connecting other haplotypes by a one-mutation step (Figure 1B).

The phylogenetic relationships suggested by the ITS1 sequences were less informative than those suggested by the combined *cox3* and *tatC–tLeu* sequences, which was likely due to the fewer available sequences of ITS1 from Japan and Korea. Although no clear relationship was revealed, the ITS ribotypes R3–R5, R8, and R12 were detected in the Chinese populations, and all of these ribotypes except R3 were detected in Hokkaido and Aomori (Uwai et al. 2006b), suggesting that the Chinese samples were similar to northern Japanese populations.

4.3 Genetic relationship between the farmed populations and the adjacent natural populations from rocky reefs in China

We previously observed significant genetic differentiation between the farmed populations and the adjacent subtidal natural population of *U. pinnatifida* (F1-15 and F2-15 vs. SW-18) using microsatellites, which suggested limited genetic connectivity between the populations (Li et al. 2020; Shan et al. 2018). This conclusion is supported by the analysis of mitochondrial and nuclear DNA sequences in the present

study. No haplotypes of the combined *cox3* and *tatC–tLeu* sequences were shared between the farmed populations and the natural populations growing on the rocky reefs (F1-15 and F2-15 vs. SW-18 and HSJ17), resulting in extreme F_{st} values higher than 0.9. Significant genetic differentiation was also revealed between these population groups by analysis of ITS1 sequences, although some ITS1 ribotypes were shared. W17 (naturally occurring on the cultivation raft) was revealed to be genetically closer to the natural populations on the rocky reefs (SW-18 and HSJ17) than the farmed populations. There was a temporal gap of 2–3 years between the sampling of the farmed and natural populations included in this study. Importantly, annual genetic turnover has been observed in cultivated populations of *U. pinnatifida* in France and Korea (Graf et al. 2021; Guzinski et al. 2018). Thus, such genetic turnover should not be ignored when assessing the genetic differentiation between farmed and natural populations. The farming practices applied in F1-15 and F2-15 differ from those in France and Korea. The farmers usually select hundreds to thousands of mature sporophytes from the farmed cultivars of the previous year and use them for seedling production. The larger number of parents possibly alleviates the effects of genetic drift and thus limited genetic turnover is expected in the farmed populations from China, which is supported by the results of cluster analyses in our previous study (Li et al. 2020). The two farmed populations (F1-15 and F2-15) also showed significant genetic divergence from each other as revealed by mitochondrial and nuclear DNA sequences. These two populations were derived by consecutive artificial selection from stock of different origins. Intentional separation of these two populations during seedling production and farming process has maintained the genetic divergence between them. Despite the limited connectivity detected between the farmed and natural populations, we cannot rule out the possibility that escapes happened in the past from the farmed populations to the wild to form new natural populations or that introgression into the natural populations had already occurred because our sampling might not encompass the long-term effect of small rates of escapes from farms to natural populations.

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Author contribution: TS and SP conceived the study. TS and YL extracted the DNA, conducted PCR, analyzed the genetic diversity and performed the phylogenetic analyses. TS wrote the manuscript draft and SP revised it. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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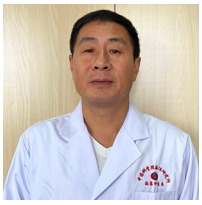

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