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Population Genetic Structures and Demographic Expansion of the Exotic Jellyfish *Carybdea brevipedalia* in Korean Coasts Inferred from Mitochondrial *COI* Analysis

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Carybdea brevipedalia Kishinouye, 1891 is a poisonous jellyfish that usually occurs only in Japanese coastal regions. However, it was recently found on the Korean coast, thus expanding its known geographical range. In this study, we analyzed the population genetics and demographic histories of 113 *C. brevipedalia* specimens from the southern and eastern coastal regions of Korea by sequencing mitochondrial DNA cytochrome *c* oxidase subunit I (*COI*). We identified 42 *C. brevipedalia COI* haplotypes with high genetic diversity and a significant genetic structure. Populations were highly differentiated based on geographic location and distinctly divided into A and B clades. The results of Mantel tests indicated that geographic distance influenced the genetic distance between the two clades. Moreover, demographic analyses (neutrality tests) and the star-like profile of the Templeton, Crandall, and Sing (TCS) haplotype network indicated that *C. brevipedalia* had recently expanded into the southern and eastern coastal regions of Korea. These findings suggest that *C. brevipedalia* populations along the Korean coast have significant genetic differentiation that could be influenced by geographic isolation and subsequent adaptation to regional ecological conditions.

Key words: *Carybdea brevipedalia*, Cytochrome *c* oxidase subunit I (*COI*), Exotic species, Genetic differentiation, Population expansion.

BACKGROUND

The class Cubozoa, commonly called box jellyfish, is one of the smallest groups in the phylum Cnidaria; it comprises the monophyletic orders Carybdeida and Chirodropida and contains at least 49 known species (Collins and Jarms 2021). It has a metagenetic life cycle in which a benthic polyp can reproduce asexually by budding into multiple polyps, each of which metamorphoses into a single medusa (Werner et al. 1971; Toshino et al. 2018). The scientific community has recently become more interested in Cubozoans since their discovery in 1810 due to their unique characteristics such as complex eyes (Coates 2003; Nilsson et al. 2005), exceptional courtship and reproductive behavior (Lewis and Long 2005), superior swimming abilities (Gordon and Seymour 2008), and highly toxic venom (Chung et al. 2001; Kintner et al. 2005) that pose a serious threat to public health, particularly among fishermen and swimmers (Fenner et al. 1996). Various venomous species of box jellyfish such as *Chironex fleckeri* and *Chironex yamaguchii* are

largely restricted to the tropical Indo-Pacific region (Fenner and Hadok 2002; Lewis and Bentlage 2009). Nonetheless, various species of box jellyfish are widely distributed in tropical and subtropical oceans (Gershwin and Gibbons 2009; Straehler-Pohl et al. 2017).

Péron and Lesueur (1810) originally identified *Carybdea* as the first cubozoan, and numerous species of box jellyfishes were assigned to this genus after recent taxonomic revisions (Bentlage and Lewis 2012; Acevedo et al. 2019). Ten species in this genus (Collins and Jarms 2021) are characterized by a heart-shaped rhopaliar niche ostium (Bentlage et al. 2010; Acevedo et al. 2019). Most *Carybdea* species inhabit warm waters worldwide (Straehler-Pohl et al. 2017). Some species are globally distributed, whereas others are endemic to specific geographical regions (Acevedo et al. 2019). For example, *Carybdea rastoni* commonly inhabits the warm waters around Hawaii, Australia, Japan, and the Philippines (Scripps Institution of Oceanography 2021). In addition, *C. sivickisi* inhabits the western Pacific Ocean from Japan to New Zealand (Hoverd 1985). However, some *Carybdea* species are restricted to specific regions, such as *C. branchi* in the oceanic areas of Namibia and South Africa (Gershwin and Gibbons 2009; Branch et al. 2010), *C. brevipedalia* in Japan, and *C. marsupialis* in the Mediterranean Sea (Acevedo et al. 2019; Rodríguez-García et al. 2021).

Carybdea brevipedalia Kishinouye, 1891 was originally identified in the western coastal waters of Japan (Kishinouye 1891; Uchida 1929 1970) and was misclassified as *C. rastoni* because of similar morphology (Kramp 1961; Ueno 2003; Nagai 2003). However, recent taxonomic and molecular findings of Cubozoa (Straehler-Pohl et al. 2017; Acevedo et al. 2019) have revealed that the *C. rastoni* in Japanese coastal waters should be regarded as *C. brevipedalia*. This species is considered one of the most problematic species among all coastal regions of Japan, especially for fishermen and swimmers (Uchida 1970; Ueno 2003). Their stings can cause severe pain that might be associated with a longer discharge of nematocyst tubules (Kitatani et al. 2015). The poison of this jellyfish has the high hemolytic activity of purified protein toxins (Nagai et al. 2000). *Carybdea brevipedalia* has historically been distributed only in the coastal waters of Japan (Ueno 2003; Bentlage and Lewis 2012; Acevedo et al. 2019). Despite being undetectable elsewhere, it was recently discovered in Korean coastal waters (Chae et al. 2017) when hundreds of people were stung on the southern coastline and Jeju Island (W. D. Yoon; personal communication). Since then, many such incidents have occurred annually in the southern coastal region (SCR). Although the Korean government recognized the need for studies on countermeasures (Chae et al.

2017), blooms appeared for the first time in the eastern coastal region (ECR) during 2020. This suggested that the geographical range of *C. brevipedalia* is expanding in Korea and probably elsewhere. In the face of increasing socio-economic damage, the *C. brevipedalia* population that has invaded the Korean coast should be investigated to reveal the genetic structure and determine connectivity between recently emerged populations in the Korean SCR and ECR. In addition, understanding the genetic structure and phylogeography of this jellyfish species is imperative to predict its origin and geographical expansion patterns for effective bloom management.

Mitochondrial genes such as cytochrome *c* oxidase subunit I (*COI*), cytochrome B (*cytB*), and 16S rDNA (Glynn et al. 2016; Seo et al. 2021a) have been investigated to determine the population genetics and phylogeography of jellyfish. In particular, *COI* is a powerful molecular marker of the genetic variation and structure of populations in various organisms (Palraju et al. 2018; Choi et al. 2020). This is because it has the advantages of rapid evolution, elevated polymorphism, and is easily amplified and sequenced (Hu et al. 2008; Xu et al. 2011; Palraju et al. 2018). To date, 89 DNA sequences identified from *Carybdea* nuclear rDNA and mitochondrial *COI* listed in the National Center for Biotechnology Information (NCBI) database have mostly been used for evolutionary studies of box jellyfish (Collins 2002; Bentlage et al. 2010). However, the population genetics of box jellyfish have not been investigated using these sequences.

Therefore, we analyzed the mitochondrial *COI* gene to reveal the population genetic structure and phylogeographic profiles of *C. brevipedalia* populations sampled from the SCR and ECR of Korea. We also assessed genetic relationships between native and recently emerged populations in the SCR and ECR. We resolved the expansion profiles and genetic differentiation among *C. brevipedalia* populations along the Korean coast.

MATERIALS AND METHODS

Sampling and morphological identification

Carybdea brevipedalia specimens were collected in August 2020 from four different coastal regions located in the southern coastal region (SCR; St. 1 and 2) and eastern coastal region (ECR; St. 3 and 4) of Korea (Fig. 1A). A total of 113 specimens (41 at St. 1, 12 at St. 2, 40 at St. 3, and 20 at St. 4) were collected using a hand-net, immediately fixed with 100% ethanol and transported to the laboratory where they were stored at

4°C for further analysis.

Morphological observations (Fig. 1B) were carried out following Chae et al. (2017). In particular, we observed the morphology using a high-resolution camera (D810; Nikon, Tokyo, Japan) with close-up lenses (Micro-Nikkor 60 mm f2.8; Nikon and Makro-planar 100 mm f2.0; Carl Zeiss, Oberkochen, Germany) and a close-up tube (PK-13, Nikon, Tokyo, Japan).

DNA extraction, amplification and sequencing

Preserved specimens were washed individually with distilled water to remove all ethanol, and then genomic DNA (gDNA) was extracted using the modified cetyltrimethylammonium bromide (CTAB) DNA extraction protocol (Richards et al. 1994).

Polymerase chain reaction (PCR) amplifications of *COI* fragments were done using a newly designed primer pair (Cb-F1 5'-GTTCTACAAACCAC AAAGATATAGG-3', and Cb-R1 5'-TATGGCTAACA

TAGCATAAACCAT-3'). In brief, the reaction solution (total volume: 20 μL) was prepared for the PCR amplification. It consisted of 2 μL template DNA, 2 μL Ex Taq buffer, 2 μL dNTP mix, 1 μL forward primer, 1 μL reverse primer, 0.2 μL Ex Taq Polymerase (TaKaRa Bio Inc, Shiga, Japan), and 11.8 μL of distilled water. PCR reaction conditions were as follows: 94°C for 3 min, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s, and finally 72°C for 5 min. All PCR amplified samples were confirmed in a 1% agarose gel using MIDORI green dye (Nippon Genetics Europe GmbH, Düren, Germany) as a fluorescent source. Confirmed PCR products were purified using the PCR Cleanup S & V Kit (Bionics, Seoul, Korea) according to the manufacturer's instructions.

Purified PCR amplicons were sequenced by Bionics Inc. (Seoul, Korea). DNA sequencing was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), and the synthesized sequences were analyzed using the
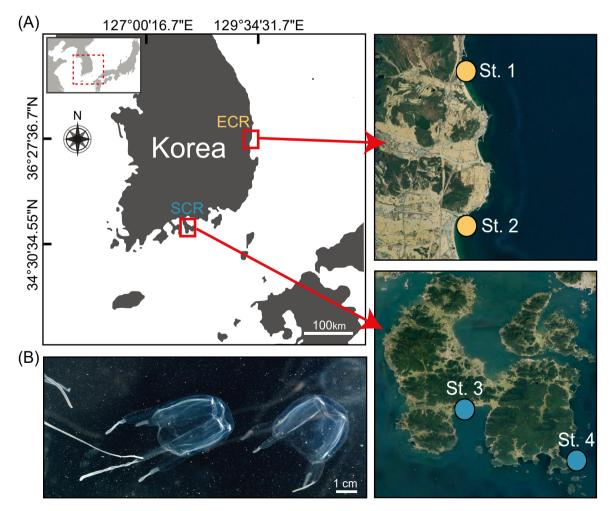

Fig. 1. A map showing four sampling locations (St. 1, St. 2, St. 3, and St. 4; (A) of *C. brevipedalia* specimens (B) collected from the southern coastal regions (SCR) and eastern coastal regions (ECR) of Korea.

Applied Biosystems™ 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Editing and contig assembly of the *COI* sequence fragments were carried out using Sequencher v5.1 (Gene Codes, Ann Arbor, MI). All the sequences determined in this study were deposited into the GenBank database with accession numbers (OM108321–OM108433).

Phylogenetic analysis

To determine the taxonomic relationship between *C. brevipedalia* and other jellyfishes, we constructed maximum likelihood (ML) and Bayesian inference (BI) analyses based on the 113 *COI* sequences determined in this study and 27 *COI* sequences of other medusozoans (including cubozoans, scyphozoans, and hydrozoans) retrieved from the GenBank database. The ML analysis was inferred using MEGA X (Kumar et al. 2018) with the General Time-Reversible model (GTR + I + G) and 1,000 bootstrap values. BI analyses were built with MrBayes v3.2.6 (Huelsenbeck and Ronquist 2001) using the GTR model with a gamma distribution for the remaining sites. One million generations were run until the standard deviation of the split frequencies was < 0.01. Trees were sampled every 1,000 generations, with a burn-in of 250 trees. The output file containing trees with posterior probabilities (PP) was shown in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/). The tree resulting from the ML analysis with bootstrap values (BS) was compared with the BI tree with posterior probability, and the 50% majority-rule consensus tree was summarized with PP and BS as nodal support. The tree was edited using Adobe Illustrator CS6 (Adobe Systems, San Jose, CA, USA). A list of all the sequences used for the phylogenic analysis is provided in supplementary material tables S1 and S3.

Estimates of genetic diversity and differentiation

The assembled 113 *COI* sequences (689 bp) of *C. brevipedalia* were used to calculate the standard genetic diversity indices, 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). In addition, neutrality, Tajima's D (Tajima 1989), and Fu's F_S tests (Fu 1997) were performed to reveal molecular evidence for past demographic changes using DnaSP v.6 (Rozas et al. 2017). To determine genetic variation among different clusters, the 113 *COI* sequences were imported into Arlequin v.3.5.2.2 where Analysis of Molecular Variance (AMOVA) was assessed. In addition, a pairwise F_{ST} statistical analysis (with 10,000 permutations; Wright 1969) was used to test for genetic differentiation among populations across two different regions.

TCS haplotype network and phylogenetic analyses

To explore the phylogeographic patterns of the *C. brevipedalia* haplotypes in the Korean coasts, we constructed a Templeton, Crandall, and Sing (TCS) haplotype network (Clement et al. 2002) and a Maximum-Likelihood (ML) phylogenetic tree for the 113 *COI* sequences. The haplotype network was constructed using PopART v.1.7 (Leigh and Bryant 2015) with default settings, while the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees were constructed in MEGA X and MrBayes v3.2.6, respectively, with a General Time-Reversible model (GTR + I + G) and 1000 bootstrap values. The tree was visualized with Mega X Tree Explorer and was edited in Adobe Illustrator CS6 (Adobe Systems, San Jose, CA). The haplotype frequency of the 42 unique haplotypes is presented in supplementary table S2.

Furthermore, we used the non-parametric Mantel test to investigate the relationship between genetic distance and geographic distance (Peakall and Smouse 2012). It was evaluated using two distance measures between all 42 distinct haplotype pairs. In the Mantel test, the pairwise genetic distance between specimens was calculated as the number of nucleotide differences between the *COI* sequences. In addition, the pairwise geographic distance was calculated as a direct physical distance based on their longitudinal and latitudinal coordinates that were transformed logarithmically. The Mantel test was conducted using GenAlEx v.6.5 with statistical significance derived based on 1,000 permutations (Peakall and Smouse 2012).

RESULTS

Identification and phylogenetic relationships of *C. brevipedalia*

We confirmed the identities of 113 box jellyfish (*C. brevipedalia*) collected from four sites in the SCR and ECR of Korea according to their evident morphology (Fig. 1B). A sharp cylindrical bell with an almost flat apex was slightly narrower and rounder towards the end. The box-shaped part of the bell in adult specimens averaged 3.3 (2.6–4.2) cm in height and four tentacles were attached to each corner.

The molecular identity of *COI* fragments was impossible to determine because *C. brevipedalia COI*

sequences were not available in any public databases. Therefore, we sequenced 113 *COI* fragments of 689-bp from *C. brevipedalia* for the first time and inferred phylogenetic relationships among cubozoans, with an emphasis on *Carybdea* species (Fig. 2). Our phylogenetic tree showed that the cubozoans were separated from Scyphozoans and Hydrozoans and that they formed a monophyletic clade with 87% bootstrap support, but not by posterior probability (PP) < 50%. The order Carybdeida in the Cubozoan clade formed polyphyletic clades with the order Chirodropida nested between the Carybdeida clades. The *Alatina*, *Carybdea*, *Morbakka*, and *Tamoya* genera differed from one another. The *C. brevipedalia* sequences determined herein clustered together and were clearly separated from those of the *Carybdea* species, *C. arborifera*, and *C. xaymacana*, with moderate support (PP 69% and BS

55%).

Genetic diversity and neutrality test

The genetic diversity assessed in 113 *COI* sequences identified 53 polymorphic sites (Ps) and 42 unique haplotypes across all populations (Table 1). The estimated haplotype (h) and nucleotide (π) diversities were 0.8428 ± 0.0248 and 0.005139 ± 0.002915, respectively. The genetic diversity (h and π) was higher for SCR than ECR (0.6944 and 0.004132 *vs.* 0.6691 and 0.001573, respectively).

The neutrality of the *COI* haplotypes of all populations was tested. The Tajima D (-2.03, $p = 0.003$) and Fu F_S (-26.092, $p = 0.000$) were negative and statistically significant for the overall population. The Tajima D values and Fu F_S scores for the ECR and SCR
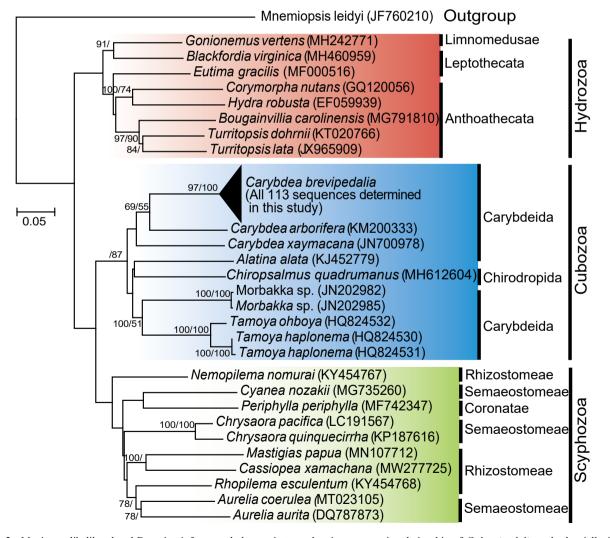

Fig. 2. Maximum likelihood and Bayesian inference phylogenetic tree, showing taxonomic relationship of *C. brevipedalia* and other jelly fish species. Numbers separated by a slash above each branch are posterior probabilities (on the left side) followed by bootstrap values (on the right side). Nodal support of less than 50% is not shown.

were significantly negative (Table 1).

Genetic differentiation

The population differentiation was determined using analyses of molecular variance (AMOVA). Most genetic variation (59.95%, $p < 0.001$) occurred between the regions, whereas 40.05% occurred within the entire population (Table 2). The fixation index (F_{ST}), calculated to assess population differentiation caused by genetic structure, was 0.599 ($p = 0$).

Phylogeographic structures of *C. brevipedalia*

The phylogenetic profiles of *C. brevipedalia COI* haplotypes on the Korean coast were determined using the TCS haplotype network and phylogenetic analysis (Fig. 3). The results showed that the 42 unique haplotypes detected herein were differentiated into supported clades A and B based on their geographical locations. The TCS network revealed that clade A comprised 18 haplotypes unique to the SCR, with C01 being the most frequent in 33 individuals (Fig. 3A). Clade B contained 19 haplotypes, of which 17 and 2 (C23 and C24) were unique to the ECR and SCR, respectively. Haplotype C25 in clade B served as the intermediate haplotype connecting the two clades and was shared between the SCR and ECR. Four haplotypes (C19–22) unique to SCR were separated from both clades by 4–8 mutational steps and did not belong to either. Clades A and B were shaped like stars in the TCS network, in which most haplotypes were linked to those that were the most frequent (C01 in SCR and C30 in ECR) with one or two mutational steps, indicating recent population expansion. The unrooted phylogenetic tree similarly revealed two distinct clades with four intermediate haplotypes (CB19–22) that were clearly distinguishable (Fig. 3B). Table S2 shows the frequency and distribution of the 42 unique haplotypes.

Mantel correlation analyses of genetic and geographic distances uncovered a significant positive correlation ($R^2 = 0.128$, $p = 0.001$; Fig. 4).

DISCUSSION

Genetic structures of *C. brevipedalia*

Several outbreaks of jellyfish and the organisms that caused them to spread globally due to ocean currents and anthropogenic activities have recently been reported (Condon et al. 2012). For example, *C. brevipedalia* is a toxic jellyfish species native to Japanese coastal waters that has recently been identified in Korean coastal waters where it has caused socio-economic damage (Chae et al. 2017; Acevedo et al. 2019). Population genetic analyses can help to determine the invasion and transmission routes of harmful marine organisms such as jellyfish *Aurelia* and ascidians (Dawson 2005; Zhan et al. 2010 2015). This is fundamental to understanding the population dynamics of certain species (Kingsford et al. 2021). We found high genetic diversity with significant differentiation in the overall *C. brevipedalia* population along the

Table 1. Genetic diversity indices and neutrality test for mitochondrial *COI* of 113 specimens of *Carybdea brevipedalia* populations

Location	N	Nh	Ps	Tajima's D	Fu's F_S	h	π
Eastern coastal region (ECR)	53	18	20	-2.38***	-18.56***	0.6691 ± 0.0709	0.001573 ± 0.001166
Southern coastal region (SCR)	60	25	39	-2.182**	-16.763***	0.6944 ± 0.0682	0.004132 ± 0.002446
Total	113	42	53	-2.03**	-26.092***	0.8428 ± 0.0248	0.005139 ± 0.002915

Abbreviations: N, Number of sample; Nh, Number of haplotype; Ps, Number of polymorphic site; D, Tajima's D; Fs, Fu's F_S; h, Haplotype diversity; π, Nucleotide diversity; **, $p < 0.05$; ***; $p < 0.001$.

Table 2. Analysis of molecular variance (AMOVA) for populations of *Carybdea brevipedalia* in Korean coasts based on mitochondrial *COI*

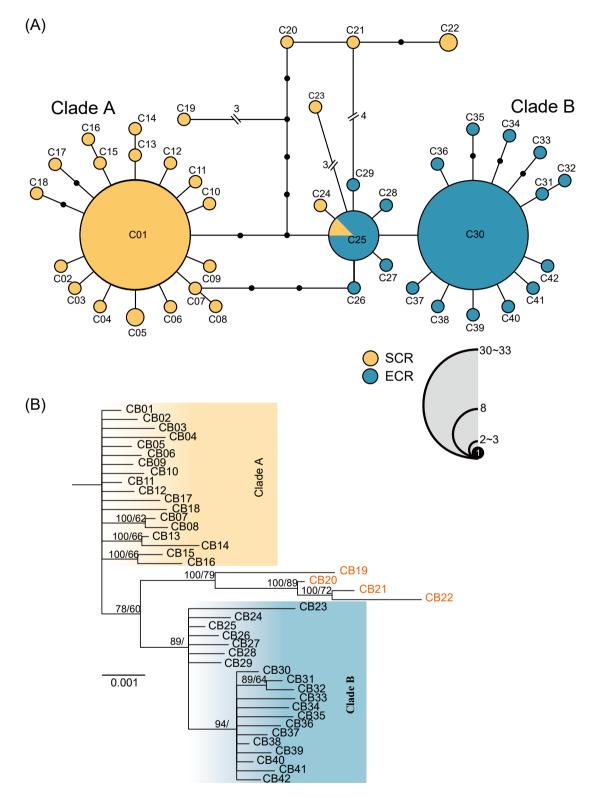| Source	$d.f.$	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among Populations	1	86.507	1.51897	59.95%	$F_{ST} = 0.59947$ ($p = 0$)
Whole Populations	111	112.652	1.01488	40.05%	

Fig. 3. The haplotype TCS network (A) and Bayesian inference and maximum likelihood phylogenetic tree (B) show phylogeography and relationships among the 42 *COI* haplotypes of *C. brevipedalia* collected from the southern (SCR; orange colors) and eastern coastal regions (ECR; blue colors) of Korea. 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. Small black dots indicate unsampled haplotypes. On the phylogenetic tree, numbers on branches are Bayesian posterior probabilities/bootstrap support values.

Korean coast. This is consistent with previous findings, which showed that genetic differentiation is commonly intraspecific in marine jellyfish (*e.g.*, Holland et al. 2004; Dawson 2005; Ramšak et al. 2012; Lee et al. 2013). Similar *COI* genetic variation and significant genetic structure were also found in the jellyfish *Aurelia coerulea* (Seo et al. 2021a b) and the sea squirt *Ciona savignyi* (Yi and Kim 2020). Our results showed that the Korean *C. brevipedalia* population also consists of two genetically distinct populations.

Genetic differentiation among relatively distant populations is primarily caused by geographical isolation and adaptation to the ecological conditions of specific regions (Coyne and Orr 2004; Zhan et al. 2009; Liu et al. 2019). The Mantel test results of the *COI* sequences (Fig. 4) showed that genetic differentiation and gene flow between the populations might be greatly influenced by the geographical distance between the Korean SCR and ECR. This agrees with the findings of other Korean marine organisms, such as the Asian shore crab (Hong et al. 2012), ascidians (Kim et al. 2012; Yi and Kim 2020), barnacles (Yoon et al. 2013), and disk abalone (Nam et al. 2021). The Asian shore crab (*Hemigrapsus sanguineus*), for example, lacks geographically-associated haplotypes and a genetic structure within and among populations in the Korean coasts. However some degree of genetic differentiation occurred between populations of the ECR and other coastal regions (Hong et al. 2012). A population of disk abalone (*Haliotis discus*) sampled from the ECR was also genetically separated from those in the SCR and in western coastal regions (WCR) (Nam et al. 2021). These findings suggested strong genetic structures among marine creatures, including *C. brevipedalia* along the Korean coast (Hong et al. 2012; Kim et al. 2012; Yoon

et al. 2013; Yi and Kim 2020, and the present study). In addition, genetic structures might have been influenced by geographical distance and other environmental factors, such as ocean current, temperature, and salinity (Hong and Cho 1983; Rebstock and Kang 2003).

Introduction and demographic expansion of *C. brevipedalia* on Korean coasts

The first blooms of *C. brevipedalia* in Korea were recently identified in the SCR (Chae et al. 2017). However, whether the species was recently introduced or native to the Korean coast has remained unknown. In general, a significant geographic structure is considered a major distinguishing factor between native and recently introduced populations (Hellberg et al. 2002; Geller et al. 2010; Zhan et al. 2010). Geographically differentiated populations can reflect species persistence in a region, which would allow for sufficient gene flow isolation and the accumulation of distinct genetic variability (Avise et al. 1987; Yi and Kim 2020), whereas the introduced population is usually genetically homogeneous (Zhan et al. 2010). However, diverse sources might result in genetic variations within introduced populations (Pineda et al. 2016). Here, we applied the Tajima D and Fu F_S neutrality tests to test the hypothesis that *C. brevipedalia* has recently emerged and expanded along the Korean coast. These tests are generally based on the distribution of pairwise differences between sequences within populations and have been implemented to detect population growth (Ramos-Onsins and Rozas 2002; De Jong et al. 2011). Positive and negative values indicate bias towards intermediate-frequency and rare alleles, respectively, and the negative value is a sign of recent population expansion (Tajima 1989; Fu 1997). The Tajima D and Fu F_S values determined herein were negative in the overall population and significantly so in the SCR and ECRs. These findings implied recent population expansion in the overall population, as well as in both SCR and ECR.

The recent demographic expansion closely corresponded with the *COI* haplotype network. The profiles of the SCR and ECR haplotypes (Fig. 3A) were both star-shaped and their compositions were similar. The star-like patterns reflect a recent appearance and rapid population growth (Slatkin and Hudson 1991; Rogers and Harpending 1992; Avise 2000; Froufe et al. 2016). The phylogenetic tree (Fig. 3B) was also consistent with the haplotype network that showed the connectivity in the population and that the ancestral clade was A. In addition, the high haplotype (0.6691) and lower nucleotide diversity (0.001573) in the ECR indicated that the populations might have rapidly
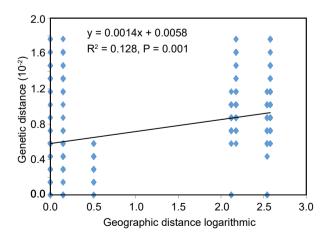

Fig. 4. Mantel test showing relationships between genetic distance and the logarithm of geographic distance between all pairs of 42 *COI* haplotypes of *C. brevipedalia* populations from two coastal regions of Korea.

emerged from a small effective ancestral population (Grant and Bowen 1998; Avise 2000; De Jong et al. 2011). The profiles of population expansion were similar in the Asian shore crab *Hemigrapsus sanguineus* along the Korean coast (Hong et al. 2012). Considering these findings, our results suggested that *C. brevipedalia* populations recently appeared and thrived in Korean coastal regions, thus increasing population in these regions (Avise 2000; Froufe et al. 2016).

Inflow pathway of *C. brevipedalia* into Korean coasts

The genetic relationships between the Korean and Japanese *C. brevipedalia* populations remain unknown because *COI* sequences for the latter are not available. However, considering the present results, oceanographic characteristics, and earlier findings (Hong et al. 2012; Kim et al. 2012; Yoon et al. 2013; Yi and Kim 2020; Nam et al. 2021), the emergence and expansion of *C. brevipedalia* in the Korean coasts could be explained as follows (Fig. 5). The Tsushima Warm Current branches off the Kuroshio Current (Fig. 5A) and is a factor for the emergence and/or expansion of marine species. It is characterized by high water temperatures and high densities (Rebstock and Kang 2003). Some parts of the
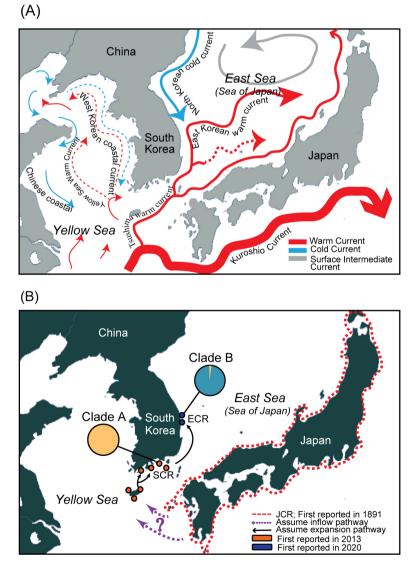

Fig. 5. A major ocean current flow pattern in Korean waters (A) and a possible scenario for the introduction and expansion of *C. brevipedalia* in the Korean coasts (B). The big cycles represent the haplotype compositions sampled in the present study, while the small cycles indicate the current distribution of *C. brevipedalia* in Korean waters. The big orange cycle represents clade A haplotypes while the blue cycle represents clade B haplotypes. Moreover, the orange color in clade B represents the shared haplotypes between southern coastal regions (SCR) and eastern coastal regions (ECR). JCR; Japanese coastal regions.

current run into the Yellow Sea, and the main part flows into the East Sea along the Korean Peninsula (Hong and Cho 1983; Rebstock and Kang 2003). The Tsushima Warm Current might have transported *C. brevipedalia* from its native habitat in Japan to Jeju Island and to the Korean SCR (Fig. 5B). However, we inferred from the present findings that the dispersal and gene flow of *C. brevipedalia* from the Korean SCR to the ECR are highly restricted. Nevertheless, due to the genetic drift (founder effect) caused by water currents and/or human-related vectors, new populations were established in the ECR, most likely by haplotype C25, which was shared between the SCR and ECR and intermediate between the two populations. As a result, haplotype C30 adapted to the ECR then rapidly disseminated.

The ECR population was located at the sub-polar front, where the North Korean cold (low temperature with relatively low salinity) and East Korean warm (high temperature, high salinity) currents converge (Rebstock and Kang 2003). Temperature and salinity influence the metamorphosis of polyps into medusae in *Carybdea* spp. (Canepa et al. 2014; Toshino et al. 2018). Thus, the balanced effects of cold and warm water currents in the Eastern Sea might explain the genetic differentiation of *C. brevipedalia* between the ECR and SCR populations. Such hydrographic differentiation profiles can change the dispersal and population structures of *C. brevipedalia* and other marine species on the Korean coast (Hong et al. 2012; Kim et al. 2012; Yoon et al. 2013; Nam et al. 2021).

Our demographic history results support the recent appearance and rapid expansion of *C. brevipedalia* populations in Korean coastal waters. We presented a possible scenario for the introduction and profile of the gene flow of this species in these environments. However, this perspective remains ambiguous, and genetic parameters could not be directly compared because molecular data for *C. brevipedalia* from its native region are not available. Further sampling from Japan is necessary to clarify the historical influences and to determine the population connectivity and inflow pathways of this species in Korean coastal waters. Understanding population connectivity is critical for risk assessment and to design appropriate management strategies for invasive species (Hampton et al. 2004).

CONCLUSIONS

We analyzed the population genetic structure and demographic history of *Carybdea brevipedalia* populations using specimens that have massively bloomed in Korean coastal waters. Our results revealed high genetic diversity and strong genetic differentiation between geographically distant populations (SCR and ECR), most likely caused by geographical isolation and other ecological and hydrographic factors. The demographic history indicated that the *C. brevipedalia* populations in Korea underwent a recent population expansion. Our findings of the first genetic analysis of *C. brevipedalia* populations could serve as baseline data for future phylogeographic and demographic studies, especially in the native and other recently emergent regional populations. Furthermore, our findings provide a more in-depth understanding of the population structure of this species in Korea, thus aiding in the management of these potentially lethal organisms. Future studies should broaden the sampling to include native populations to compare genetic characteristics, which could provide useful information about how the species was introduced and its general gene flow profiles.

Authors' contributions: Muhammad BL: analyses, writing original draft, writing-review & editing. Seo Y: conceptualization, investigation, analyses. Chae J: Investigation. Ki JS: Conceptualization, Writing - review & editing.

Competing interests: The authors declare no conflict of interest.

Availability of data and materials: All the data presented in this study are available in the supporting information (Supplementary materials). All sequences determined were deposited into the GenBank database under the accession numbers OM108321–OM108433.

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Supplementary materials

Table S1. List of all the *Carybdea brevipedalia COI* sequences determined in this study. (download)

Table S2. Frequency and distribution of the 42 unique haplotypes detected in the Eastern coastal region (ECR) and Southern coastal region (SCR) of South Korea. (download)

Table S3. List of other jellyfish species accessions retrieved from the GenBank database and used in the phylogenetic analysis. (download)