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Genetic Diversity and Population Dynamics of Invasive *Ascidiella aspersa*: Insights from Cytochrome Oxidase Subunit I and 18S rDNA Analyses in Korean and Global Populations

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Abstract: *Ascidiella aspersa*, originally native to the northeastern Atlantic, has emerged as a prolific invasive species in coastal waters worldwide. In 2010, it was identified as an alien species in Republic of Korea, rapidly colonizing artificial harbor structures and outcompeting native species. This study employs morphological analyses and genetic sequencing, focusing on mitochondrial DNA (cytochrome oxidase subunit I; mt-COI) and nuclear markers (18S rRNA), to unravel the genetic structure and haplotype diversity (Hd) of *A. aspersa* populations in Republic of Korea and globally. The analysis of 154 mt-COI and 127 18S rDNA global population sequences, as well as 80 mt-COI and 79 18S-rDNA Korean population sequences, revealed distinct genetic patterns. Among global populations, the mt-COI gene displayed significant genetic diversity, with 21 distinct haplotypes distributed across 41 polymorphic sites, which is indicative of extensive genetic variability. In contrast, the 18S rDNA marker exhibited limited diversity, with only four haplotypes identified at three polymorphic sites. In Korean populations, the mt-COI gene also exhibited substantial genetic diversity, with 14 distinct haplotypes displaying genetic variations at 29 polymorphic sites. Conversely, the 18S rDNA marker in Korean populations revealed a unique genetic pattern, with only one shared haplotype. These findings emphasize the complex genetic diversity within *A. aspersa* populations, both globally and in Republic of Korea. This genetic analysis provides valuable insights into the species' colonization history and adaptation mechanisms, shedding light on the factors shaping its genetic structure. Further research is warranted to elucidate the ecological implications of these genetic patterns in the context of invasion biology.

Keywords: invasive marine species; Tunicata; *Ascidiella aspersa*; genetic diversity; Republic of Korea population; global population

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1. Introduction

The rapid increase in the global proliferation of invasive marine species has negative impacts on endemic biodiversity and aquatic ecosystems [1,2]. Invasive marine organisms often possess competitive advantages over native species, allowing them to colonize new locations and artificial structures more abundantly [3–6]. This colonization signifies significant ecological changes within invaded areas. Among these invasive species, Ascidians, specifically *Ascidiella aspersa* (European sea squirt), have emerged as a significant issue, particularly in harbors where they attach to artificial underwater structures [7,8].

A. aspersa is native to Norway in the NE Atlantic [9–13] and has also been found in the northern Mediterranean. However, this species has since invaded various parts of the world, including Australia [4,14], Argentina [15,16], Iceland [17], India [18], Japan [19–21], Republic of Korea [22], New Zealand [23,24], the NW Atlantic including the USA [25,26], Canada [27,28], and South Africa [29]. It spreads through ballast water or attachments

to ship hulls, allowing it to easily migrate and colonize farming facilities, fishing gear, and port infrastructure, resulting in significant damage. *A. aspersa* has the potential to be a successful invader due to its rapid growth rate, short lifespan, production of large numbers of short-lived non-feeding planktonic larvae, lack of predators, and tolerance to a wide range of environmental conditions [8,19,30]. The biological advantages of *A. aspersa* may lead to the formation of large populations and subsequent high biomass of *A. aspersa*. Colonization by *A. aspersa* may provide them with a significant advantage in the competition for habitats against native species and other species [25,31]. For that reason, this species was registered in the Global Invasive Species Database (GISD) in 2010.

In Republic of Korea, *A. aspersa* was first confirmed in the Tongyong yacht marina in 2010 and has since spread along the eastern coast, including areas such as Gunsan, Jindo, Jeju Island, Wando (WD), and Yeosu (YS). Currently, it has invaded and inhabited the entire sea area, including coastal sites and Jeju Island. Monitoring efforts for *A. aspersa* have been ongoing since 2008, and several studies have been conducted to understand its morphology, phylogenetic relationships, risk assessment, damage analysis, and physiological and ecological aspects [7,22,32].

The present study focuses on evaluating the population genetic structures of *A. aspersa* in Korean and global populations using cytochrome oxidase subunit I (COI) data. The analysis included genetic mutations in the COI gene of sea squirts found along the coast of the Korean Peninsula. The haplotype analysis of mitochondrial DNA (mtDNA) sequences from populations in 10 countries provided insights into invasion pathways, potential sources of origin, and information for predicting the introduction time, location, and transport vectors of this species. This knowledge aids in estimating subsequent spread routes to other regions. Therefore, this study aimed to contribute to the understanding of population genetics in *A. aspersa* and provide valuable insights for predicting and managing the spread of this invasive species.

2. Materials and Methods

2.1. Sample Collection

2.1.1. Korean Coast Population

Between April and October 2021, we collected 80 European sea squirts from 10 harbors (or ports) along the coast of Republic of Korea (Figure 1, Table 1). The specimens were fixed in 96% ethanol solution at the collection site. The reproductive tracts were transported to the laboratory and subsequently immersed in 96% alcohol and stored until DNA extraction.

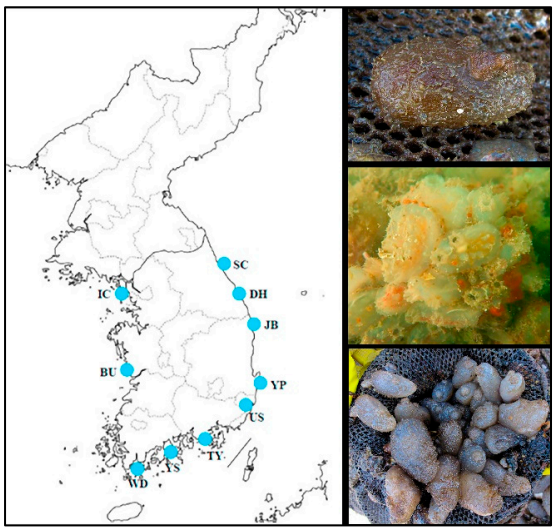

Figure 1. Overview of *Ascidiella aspersa* specimens collected from the Korean coast. Please see Table 1 for detailed information.

Table 1. Collection information, haplotype code, and GenBank accession Nos. of specimens in Republic of Korea used in this study.

Species	Locality Code	Locality	GPS	No. of Specimens	Haplotype Code	GenBank Accession Number	
				COI	COI	COI	18S
Ascidiella aspersa	IC	Incheon	37°46′49.83″ N, 126°62′26.88″ E	H_1, H_3, H_5, H_6, H_8	5, 2, 1, 1, 1	OR131201-OR131210	OR453015-OR453022
	BU	Bieung	35°93′66.81″ N, 126°52′76.53″ E	H_1, H_2, H_3, H_4, H_5	3, 1, 3, 1, 1	OQ722425-OQ722433	OR453000-OR453007
	WD	Wando	34°31′8761″ N, 126°75′32.31″ E	H_3, H_11, H_12, H_13	4, 1, 1, 1	OR131242-OR131248	OR453055-OR453062
	YS	Yeosu	34°74′26.76″ N, 127°75′46.28″ E	H_1, H_3, H_8, H_11, H_14	2, 3, 1, 1, 1	OR131257-OR131264	OR453071-OR453078
	TY	Tongyeong	34°82′80.55″ N, 128°43′64.44″ E	H_3, H_7, H_9, H_10	5, 1, 1, 1	OR131227-OR131234	OR453039-OR453046
	US	Ulsan	35°51′46.72″ N, 129°37′59.00″ E	H_1, H_3, H_5, H_11	1, 4, 1, 1	OR131235-OR131241	OR453047-OR453054
	YP	Yangpo	35°87′84.86″ N, 129°52′02.29″ E	H_3, H_5, H_8, H_9	2, 1, 3, 2	OR131249-OR131256	OR453063-OR453070
	JB	Jukbyeon	37°05′53.00″ N, 129°41′73.98″ E	H_3, H_5, H_7, H_9	4, 1, 1, 2	OR131211-OR131218	OR453023-OR453030
	DH	Donghae	37°49′06.97″ N, 129°12′498.08″ E	H_1, H_3, H_6, H_7	1, 4, 1, 1	OR131195-OR131200	OR453008-OR453014
	SC	Sokcho	38°19′79.70″ N, 128°59′30.33″ E	H_1, H_3, H_5, H_9	2, 2, 1, 3	OR131219-OR131226	OR453031-OR453038

2.1.2. Global Population

We sequenced and aligned partial mitochondrial COI sequences of 82 European sea squirts from Republic of Korea and 72 from the nine localities of *A. aspersa* retrieved from GenBank [6,22,33–38]. Similarly, 18S rDNA sequences were obtained from 83 sea squirts from Republic of Korea and 44 from the four localities of *A. aspersa* retrieved from GenBank [6,22,34]. Detailed information, including the collection localities and GenBank accession numbers, is provided in Table 1.

2.2. DNA Extraction, PCR Amplification, and Sequence Alignment

The total genomic DNA was extracted from the gonad tissue using the DNeasy® Tissue Kit (Qiagen, Düsseldorf, Germany). DNA fragments (mt-COI and 18S rRNA) were amplified via a polymerase chain reaction (PCR) using previously published primers (Table 2).

Table 2. Primers used for the amplification of 18S rDNA and mt-COI sequences.

Gene	Primer Name	Primer Sequence (5′–3′)	Reference
COI	LCO1490 HCO2198	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994 [39]
18S rDNA	18S-TF 18S-TR	AAACGGCTACCACATCCAAG AACTAAGAACGGCCATGCAC	Carreras-Carbonell et al. 2005 [40]

The PCR reaction mixture contained the AccuPower® Multiplex PCR PreMix with 3 µL of genomic DNA template, 10 mM of each primer, and 18 µL of ddH2O in a total volume of 20 µL. The PCR was performed using the AllInOneCycler™ PCR system (Bioneer, Daejeon, Republic of Korea). The PCR (mt-COI and 18S-rRNA) comprised an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 48–52 °C for 1 min, and elongation at 72 °C for 1 min 30 s, with a final elongation step

at 72 °C for 7 min. The PCR products were verified using 2% agarose gel electrophoresis and were sequenced by Cosmo Genetech (Seoul, Republic of Korea). These sequences were deposited in GenBank.

2.3. Phylogenetic Analyses and Genetic Diversity

The sequences of COI (657 bp) and 18S-rRNA (744 bp) segments were obtained from 80 and 79 sea squirts, respectively. All sequences were submitted to the GenBank database under accession numbers (OQ722425-OQ722433, OR131195-OR131264 for mt-COI, and OR453000-OR453078 for 18s-rRNA).

Alignment and sequence verifications for mt-COI and 18S-rRNA were performed using MAFFT v7.49 [41] in Geneious Prime 2019.2.1 [42] (Biomatters Ltd., Auckland, New Zealand) and by applying the L-INS-I setting (Scoring matrix: 200PAM/k = 2). All haplotypes were defined using DnaSP 6.0, and the specific genetic diversity indices for each population, including the number of polymorphic sites (S), nucleotide diversity (π), number of haplotypes (h), and haplotype diversity (Hd), were calculated using DnaSP 6.0 [43]. To evaluate the historical demography, Fu's Fs statistics and Tajima's D were calculated using Arlequin 3.5.2.2 with 1000 permutations to test for neutrality. The values of the fixation index (FST) between different populations and the analyses of molecular variance components (AMOVA) were also calculated using Arlequin 3.5.2.2. [44]. Genetic distances among the sampled locations were computed in MEGA 11.0 using the Pairwise Kimura 2-parameter (K2P) distance model of nucleotide substitution [45]. The haplotype networks were individually generated based on the mt-COI and 18S DNA gene sequences using the TCS plug-in [46] in PopART v. 1.7 [47] to evaluate the genealogy patterns of the haplotypes.

3. Results

3.1. Genetic Diversity and Population Structure of A. aspersa

3.1.1. mt-COI

In the Korean population, we collected samples from 80 European sea squirts across 10 harbors or ports, resulting in the identification of 14 distinct mt-COI haplotypes (Figure 2, Table S1).

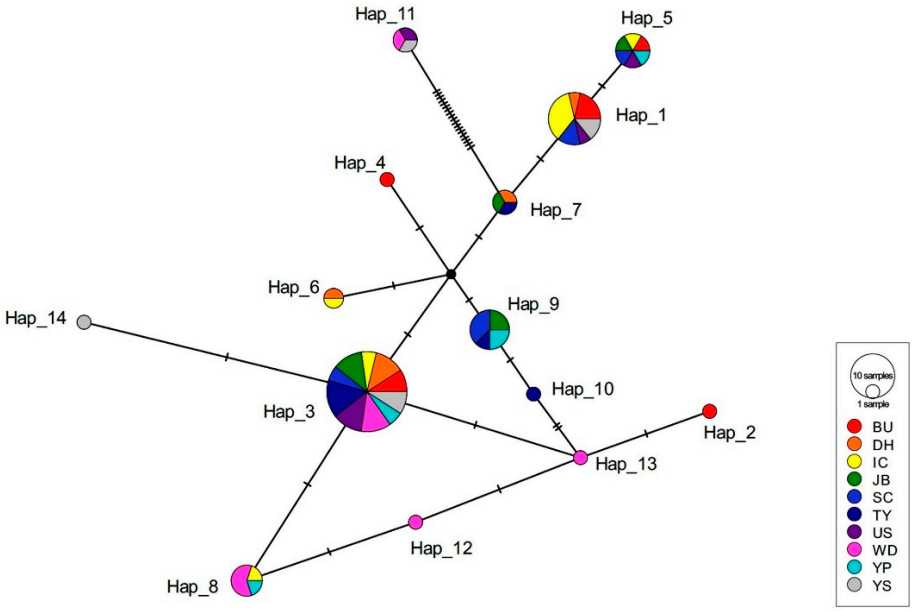

Figure 2. TCS networks of *Ascidiella aspersa* cytochrome oxidase I (mt-COI) haplotypes in the Korean population. Areas of circles are proportional to the frequency of each haplotype in the dataset. Small black circles on the branches indicate hypothetical intermediate haplotypes that were not observed.

These haplotypes exhibited variations at 29 polymorphic sites. The Hd was 0.785, and the nucleotide diversity (π) was 0.00853. The most prevalent haplotype, Hap_3, was present in all 10 populations, with 33 sea squirts (41.25%) matching this haplotype. Six haplotypes were unique to their respective populations (Figure 2, Table 3).

Table 3. Collection information, haplotype code, and GenBank accession Nos. of specimens in the global population used in this study.

Species	Locality Code	Locality/State and Country	Haplotype Code		No. of Specimens		GenBank Accession Number		Reference
			COI	18S-rDNA	COI	18S-rDNA	COI	18S-rDNA	
Ascidiella aspersa	KO	Korea	H_1, H_2, H_3, H_4, H_8, H_10, H_11, H_12, H_21	H_1, H_5	23, 36, 1, 2, 6, 9, 1, 1, 3	81, 2	OQ722425-OQ722433, JQ742948-9	OR453000-OR453078, JN573230-JN573233	This study Pyo, et al., 2012 [22]
	JA	Japan	H_1, H_2, H_3, H_10	H_1	1, 4, 2, 1	8	AB794912-19	AB811877-AB811884	Nishikawa et al., 2014 [34]
	SP	Spain	H_2, H_ H_3, H_4, H_10, H_13, H_14, H_15, H_16, H_17, H_18, H_19, H_20	H_1, H_2	6, 7, 1, 3, 3, 6, 1, 4, 1, 1, 1, 1	21,1	AB794920-41 KF309529 KF309533 KF309534 KF309555 KF309559 KF309562 KF309568 KF309594 KF309606 KF309617 KF309631 KF309637 KF309653 KF309661	AB811885-AB811906	Nishikawa, et al., 2014 [34] López-Legentil, Susanna, et al., 2015 [6]
	EN	England	H_1, H_2, H_5, H_6	H_1, H_3, H_4	2, 8, 1, 1	10,1,1	AB794942-53	AB811907-AB811918	Nishikawa, et al., 2014 [34]
	SW	Sweden	H_1, H_10	H_1	1, 1	2	AB794954 AB794955	AB811919-AB811920	Nishikawa, et al., 2014 [34]
	US	USA	H_1, H_2	-	2, 8	-	KF886702 MW872258 MW872260 MW872267 MW872271 MW872272 MW872276 MW872277 MW872307 MW872313	-	Nichols, et al., 2023 [37]
	IN	India	H_9	-	1	-	KJ725163	-	Sathish et al., 2014 [48]
	FR	France	H_2, H_3, H_7	-	1, 1, 1	-	AY116600 MN064594 MN064595	-	Stach, & Turbeville, 2002 [33]; Couton, et al., 2019 [35]
	CA	Canada	H_1	-	1	-	MN718193	-	LeBlanc et al., 2020 [36]

Yeosu had the highest Hd of 0.8571 $\pm$ 0.1083 and nucleotide diversity (π) of 0.0102 $\pm$ 0.0061, whereas the Tongyeong (TY) population exhibited the lowest haplotype and nucleotide diversities (0.6429 $\pm$ 0.1841 and 0.0022 $\pm$ 0.0017, respectively) (Table 4).

Table 4. Summary statistics of mt-COI genetic variations in *Ascidiella aspersa* along the coast of Republic of Korea.

Location	n	S	h	Hd	π	Pi	SSD	SSD *p*-Value	Rag	Rag *p*-Value	Fu's Fs Statistic	Fu's Fs *p*-Value	Tajima's D	Tajima's D *p*-Value
Incheon (IC)	10	6	5	0.7556 ± 0.1295	0.00325 ± 0.00222	3.00000	0.06623	0.23	0.17333	0.31	**−0.45535**	0.32800	0.02422	0.53700
Bieung (BU)	9	7	5	0.8333 ± 0.0980	0.00389 ± 0.00261	3.40000	0.02991	0.37	0.08642	0.45	**−0.30568**	0.36000	**−0.03464**	0.50300
Wando (WD)	7	23	4	0.7143 ± 0.1809	0.01029 ± 0.00633	11.66667	0.05838	0.58	0.09977	0.83	2.42842	0.87200	**−1.58023**	0.01900
Yeosu (YS)	8	23	5	0.8571 ± 0.1083	0.01017 ± 0.00611	9.80000	0.06050	0.39	0.11224	0.57	1.42961	0.75000	**−1.29548**	0.09600
Tongyeong (TY)	8	4	4	0.6429 ± 0.1841	0.00223 ± 0.00171	2.16667	0.09059	0.19	0.30867	0.25	**−0.46960**	0.25500	**−0.22175**	0.44300
Ulsan (US)	7	23	4	0.7143 ± 0.1809	0.01116 ± 0.00682	11.66667	0.12542	0.29	0.22676	0.35	2.62696	0.88800	**−1.23635**	0.28000
Yangpo (YP)	8	6	4	0.8214 ± 0.1007	0.00343 ± 0.00239	3.16667	0.00591	0.93	0.02934	0.99	0.39513	0.57000	**−0.12902**	0.48200
Jukbyeon (JB)	8	5	4	0.7500 ± 0.1391	0.00294 ± 0.00212	2.66667	0.19217	0.03	0.72832	0.03	0.08149	0.47000	0.00046	0.53900
Donghae (DH)	7	4	4	0.7143 ± 0.1809	0.00246 ± 0.00189	2.16667	0.08696	0.12	0.29478	0.25	**−0.53807**	0.21900	**−0.03984**	0.47300
Sokcho (SC)	8	5	4	0.8214 ± 0.1007	0.00348 ± 0.00242	2.83333	0.03841	0.20	0.11607	0.50	0.42753	0.56100	0.84031	0.82100

The analysis of variance (ANOVA) revealed that 4.39% of the genetic variation was attributed to differences among the 10 populations, with the remaining 95.61% within the populations. The overall Fst value was 0.04394 ($p = 0.09271 \pm 0.01575$; $p > 0.05$), indicating high genetic variation within populations (Table S2). Pairwise genetic distances (K2P) within populations ranged from 0.0022 to 0.0119, whereas distances among populations ranged from 0.0024 to 0.0099 (Table S3). The largest genetic differences were observed between Donghae (DH) and TY, whereas the smallest differences were observed between WD and YS. The haplotype network demonstrated the diversity indices and results of the AMOVA, featuring one common haplotype and six unique haplotypes across the 10 populations (Figure 2). In the 18S-rDNA analysis, 79 sea squirts shared a single haplotype. The presence of unique mutations and low sequence divergence suggests the potential for rapid population expansion.

Regarding the global population, we analyzed mt-COI sequences from 154 sea squirts across nine countries, including two sea squirts from the Korean population. This resulted in the identification of 21 mt-COI haplotypes, which varied at 41 polymorphic sites (Figure 3, Table S4).

The global population exhibited Hd of 0.781 and nucleotide diversity (π) of 0.00601. The dominant haplotypes were hap_2 (matched by 63 sea squirts; 40.9%), hap_1 (matched by 30 individuals; 19.48%), and hap_10 (matched by 14 sea squirts; 9.09%) (Table S4). The Spanish population exhibited the highest haplotype and nucleotide diversities (Hd = 0.9524 ± 0.0955; $\pi = 0.0073 \pm 0.0052$), whereas the USA population had the lowest haplotype and nucleotide diversities (Hd = 0.3556 ± 0.1591; $\pi = 0.00173 \pm 0.00160$), except for populations with a size of one (Figure 3, Table 5). The pairwise genetic distances within populations ranged from 0.0017 to 0.0053, and the distances among populations ranged from 0.0012 to 0.0784 (Table S6). The ANOVA results indicated that 27.69% of the genetic variation was among the nine populations, whereas 72.31% was within the populations. The overall Fst value was 0.2769 ($p = 0.00098 \pm 0.00098$; $p > 0.05$), indicating high genetic variation within the populations (Table S2).

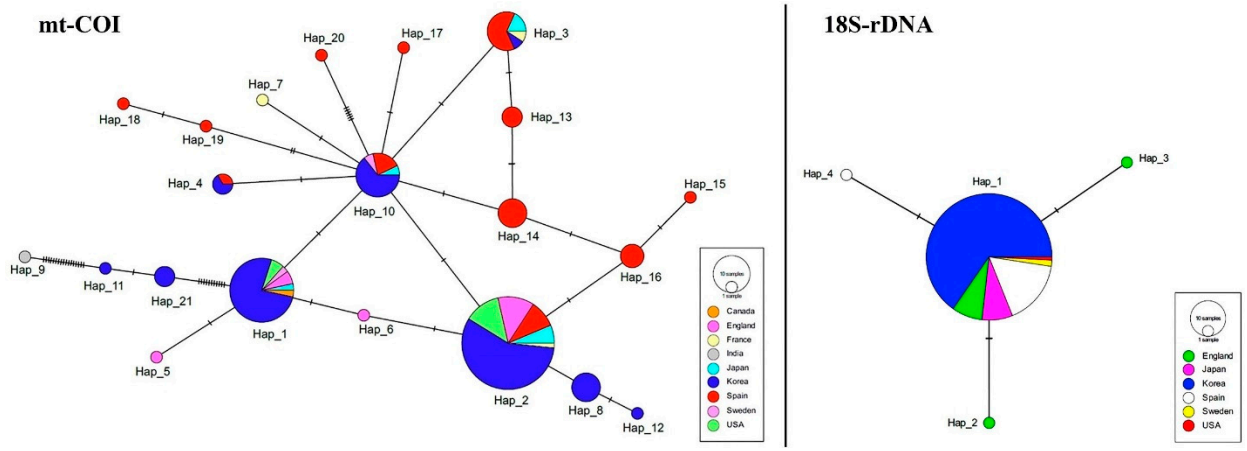

Figure 3. TCS networks of *Ascidiella aspersa* mitochondrial cytochrome oxidase I (mt-COI) and 18S-rDNA haplotypes in the world population. Areas of circles are proportional to the frequency of each haplotype in the dataset, and differing shading indicates the nine different geographic regions.

Table 5. Summary statistics of mt-COI genetic variations in *Ascidiella aspersa* in the global population.

Location	n	S	h	Hd	π	Pi	SSD	SSD *p*-Value	Rag	Rag *p*-Value	Fu's Fs Statistic	Fu's Fs *p*-Value	Tajima's D	Tajima's D *p*-Value
Incheon (IC)	10	6	5	0.7556 ± 0.1295	0.00325 ± 0.00222	3.00000	0.06623	0.23	0.17333	0.31	**−0.45535**	0.32800	0.02422	0.53700
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Wando (WD)	7	23	4	0.7143 ± 0.1809	0.01029 ± 0.00633	11.66667	0.05838	0.58	0.09977	0.83	2.42842	0.87200	**−1.58023**	0.01900
Yeosu (YS)	8	23	5	0.8571 ± 0.1083	0.01017 ± 0.00611	9.80000	0.06050	0.39	0.11224	0.57	1.42961	0.75000	**−1.29548**	0.09600
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Ulsan (US)	7	23	4	0.7143 ± 0.1809	0.01116 ± 0.00682	11.66667	0.12542	0.29	0.22676	0.35	2.62696	0.88800	**−1.23635**	0.28000
Yangpo (YP)	8	6	4	0.8214 ± 0.1007	0.00343 ± 0.00239	3.16667	0.00591	0.93	0.02934	0.99	0.39513	0.57000	**−0.12902**	0.48200
Jukbyeon (JB)	8	5	4	0.7500 ± 0.1391	0.00294 ± 0.00212	2.66667	0.19217	0.03	0.72832	0.03	0.08149	0.47000	0.00046	0.53900
Donghae (DH)	7	4	4	0.7143 ± 0.1809	0.00246 ± 0.00189	2.16667	0.08696	0.12	0.29478	0.25	**−0.53807**	0.21900	**−0.03984**	0.47300
Sokcho (SC)	8	5	4	0.8214 ± 0.1007	0.00348 ± 0.00242	2.83333	0.03841	0.20	0.11607	0.50	0.42753	0.56100	0.84031	0.82100

3.1.2. 18S-rDNA

Conversely, the 18S rDNA marker exhibited a more constrained genetic diversity among global populations. We identified five haplotypes, differing at only three polymorphic sites. The Hd was 0.0774, and the nucleotide diversity (π) was 0.00008. The most common haplotype, Hap_1, was found in all regions, with 124 sea squirts matching this haplotype. Four haplotypes were unique to their respective populations (Table S6). The English population exhibited the highest haplotype and nucleotide diversities (Hd = 0.9524 ± 0.0955; π = 0.0073 ± 0.0052) (Figure 2, Table 6). In contrast, despite comprising the largest number of sea squirts, the Korean population exhibited only two haplotypes (Hap_1 and Hap_5), signifying a notably low genetic diversity within this population (Hd = 00.0476 ± 0.0321; π = 0.000081 ± 0.000220) (Table 6). It is noteworthy that this outcome incorporated data from four sea squirts (JN573230-JN573233) from a prior study [22]. Remarkably, the sea squirts obtained in the current study

shared only one (Hap_1) of these two haplotypes (Table S6). The AMOVA results revealed that 1.39% of the genetic variation was attributed to differences among the five populations, whereas 98.61% was within the populations (Table S7). The overall Fst value was 0.01390 ($p = 0.06940 \pm 0.00772$; $p > 0.05$, indicating high genetic variation within the populations. The pairwise genetic distances (K2P) within the populations ranged from 0.000000 to 0.000570, whereas distances among the populations ranged from 0.000000 to 0.0003614 (Table S8).

Table 6. Summary statistics of 18S-rDNA genetic variations in *Ascidiella aspersa* in the global population.

Location	n	S	h	Hd	π	Pi	SSD	SSD *p*-Value	Rag	Rag *p*-Value	Fu's Fs Statistic	Fu's Fs *p*-Value	Tajima's D	Tajima's D *p*-Value
Korea (KO)	83	0	2	0.04761 ± 0.0321	0.000081 ± 0.000220	0.04761	0.00001	0.083	0.82091	0.93000	**−1.32483**	0.06600	0.00000	1.00000
England (EN)	12	2	3	0.3182 ± 0.1637	0.000568 ± 0.000684	0.33333	0.00283	0.132	0.22658	0.78300	**−1.32484**	0.02500	**−1.45138**	0.05800
Japan (JA)	8	0	1	0.000000 ± 0.000000	0.000000 ± 0.000000	0.00000	0.00000	0.000	0.00000	0.00000	0.00000	N.A.	0.00000	1.00000
Spain (SP)	22	1	2	0.0909 ± 0.0809	0.000155 ± 0.000320	0.00019	0.00004	0.117	0.67769	0.92400	**−0.95676**	0.06500	**−1.16240**	0.15800
Sweden (SW)	2	0	1	0.000000 ± 0.000000	0.000000 ± 0.000000	0.00000	0.00000	0.000	0.00000	0.00000	0.00000	N.A.	0.00000	1.00000

The findings regarding the genetic diversity and population structure of *A. aspersa* indeed offer valuable insights into its colonization history and potential for population expansion. The stark differences in genetic diversity between the Korean population and the global population, as indicated by the 18S-rDNA and mt-COI markers, provide clues about the species' history and dynamics. The low genetic diversity observed in the 18S-rDNA marker within the Korean population, with only one haplotype being detected, suggests a relatively recent colonization event. This could mean that *A. aspersa* was introduced to Korean waters more recently compared to other regions where it exhibits higher genetic diversity in the mt-COI marker. Conversely, the higher genetic diversity observed in the mt-COI marker globally, with 21 haplotypes being identified across various regions, could imply that *A. aspersa* has been present in waters in other parts of the world for a longer time. This extended presence might have allowed for more mutations to accumulate in the COI gene, resulting in a more diverse set of haplotypes.

3.2. Neutrality Tests and Mismatch Distribution Analysis

3.2.1. mt-COI

In the Korean populations, both Fu's Fs statistic test and Tajima's D test yielded negative values for the Bieung (BU), TY, and DH populations. This suggests that these populations likely experienced population selection or expansion events. Conversely, the Yangpo (YP) and Sokcho (SC) populations did not exhibit significant deviations in these tests. Mismatch distribution analysis, which estimates the congruence between observed and expected distributions under a sudden expansion model, revealed statistically significant differences between the TY and DH populations based on the sum of square deviations (SSD). This further supports the notion that the BU population experienced an expansion phase. The raggedness index (Rag) varied among populations without statistical significance, except for in the YP population, which indicates the population expansion of *A. aspersa* within the Korean population (Table 4).

In the global population, negative values were observed in the Korean and Spanish populations in both Fu's Fs test and Tajima's D test. However, only the Spanish population exhibited a significant deviation in Tajima's D test, whereas England, France, Japan, and Spain exhibited significant deviations in Fu's Fs test (Table 4). This suggests that these populations, including those in Korea, England, and the USA, may have experienced population selection or expansion events. The SSD ranged from 0.00594 to 0.2907, and statistically significant differences ($p > 0.05$) were observed between the Korean, English, and USA populations. This indicates that the Spanish and French populations were not at equilibrium and were undergoing expansion. The Rag varied among populations without

statistical significance, except for in the Spanish population (Table 5), which suggests the population expansion of *A. aspersa* in the global population.

3.2.2. 18S-rDNA

In the global population, both Fu's Fs statistic test and Tajima's D test yielded negative values for the English and Spanish populations. However, only the English population exhibited a significant deviation in Tajima's D test, whereas the Korean population exhibited significant deviations in Fu's Fs statistic test. These results suggest that the Korean population has recently undergone a demographic expansion event. The SSD ranged from 0.0001 to 0.67769, and the Rag varied among populations from 0.22658 to 0.82091. Statistically significant differences ($p > 0.05$) were observed between the Korean, English, and Spanish populations (Table 6).

These results obtained from neutrality tests and mismatch distribution analyses provide insights into the population dynamics, selection, and expansion events in both the Korean and global populations of *A. aspersa*.

4. Discussion

The genetic analysis conducted in this study provides valuable insights into the population dynamics and colonization history of *Ascidiella aspersa* in Korean and global populations. Notably, our findings reveal differences in the distinct genetic patterns between global and Korean populations of *A. aspersa*, with implications for understanding its spread and potential for population expansion.

The genetic diversity patterns observed in this study are consistent with the notion of recent colonization and population expansion in both the global and Korean populations of *A. aspersa*. In particular, the high genetic diversity observed in the COI gene marker globally, with 21 haplotypes being identified, suggests a longer presence of *A. aspersa* in other regions, allowing for more mutations to accumulate over time. In contrast, the low genetic diversity observed in the 18S rDNA marker in the Korean population, where only one haplotype was detected, suggests a lack of genetic variation within this marker within the Korean population. This is indicative of either a population bottleneck or a recent colonization event where a small number of individuals with similar genetic makeups established the population. When comparing these findings with those of previous studies, several key points come to light. First, the genetic diversity patterns in *A. aspersa* are consistent with those observed in other invasive marine species. For instance, similar genetic homogeneity has been reported in invasive ascidians in non-native environments [49]. Second, the presence of a single haplotype in the Korean population for the 18S rDNA marker aligns with the findings of previous studies on introduced populations of marine species [50]. This pattern is often associated with founder effects or bottleneck events during colonization. The genetic patterns observed here highlight the complex dynamics of invasive species, where rapid population expansion can contribute to the successful colonization of new areas by an invasive species. The wide distribution range and genetic connectivity of *A. aspersa* among populations, despite geographic distances, underscore its invasive potential.

In conclusion, this study contributes to our understanding of the genetic diversity and population dynamics of *A. aspersa* and sheds light on its invasive potential and population expansion patterns. The distinct genetic patterns observed among global and Korean populations highlight the complexity of invasive species dynamics. To further enhance our knowledge, it is essential to conduct future research with larger sample sizes and broader geographic ranges, thereby providing more comprehensive insights into the genetic diversity and invasion scenarios of *A. aspersa* populations. These expanded research efforts may help clarify the species' colonization history, adaptive mechanisms, and factors that lead to successful invasions in various regions. It may also contribute to more effective management and conservation strategies to address the challenges posed by invasive marine organisms such as *A. aspersa*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15223886/s1, Table S1: Haplotype distribution of *Ascidiella aspersa* inferred from 80 COI sequences in Korea population. Each column represents the sampling location, and the rows refer to the 14 haplotypes; Table S2. Results of mt-COI analysis of molecular variance (AMOVA) for *Ascidiella aspersa* along the coast of Korea and global population; Table S3. The pairwise genetic distances (K2P) mt-COI results between Korean populations; Table S4. Haplotype distribution of *Ascidiella aspersa* inferred from 154 mt-COI sequences in the global population. Each column represents the sampling location, and the rows refer to the 21 haplotypes in global population; Table S5. The pairwise genetic distances (K2P) mt-COI results between Global populations; Table S6. Haplotype distribution of *Ascidiella aspersa* inferred from 124 18S-rDNA sequences in the global population. Each column represents the sampling location, and the rows refer to the 5 haplotypes in global population; Table S7. Results of 18S-rDNA analysis of molecular variance (AMOVA) for *Ascidiella aspersa* along the coast of Korea and global population; Table S8. The pairwise genetic distances (K2P) among populations 18S-rDNA results between Global populations.

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