

# Mitochondrial COI sequence-based population genetic analysis of the grasshopper, *Patanga japonica* Bolívar, 1898 (Acrididae: Orthoptera), which is a climate-sensitive indicator species in South Korea

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## Abstract

*Patanga japonica* Bolívar, 1898 (Orthoptera: Acrididae) is listed as a climate-sensitive indicator species in South Korea and is called southern group of insects in that the main distributional range is southern region of South Korea and Asian continent. In South Korea, thus, the species was distributed mainly in southern region of South Korea including southward a remote Jeju Island, but recently the species has often been detected in mid to northern region of South Korea, implying northward range expansion in response to climate change. Understanding the characteristics of the changes in genetic diversity during range expansion in response to climate change could be a foundation for the understanding of future biodiversity. Thus, in this study, we attempted to understand the changing pattern of the genetic diversity of the *P. japonica* in newly expanded regions. For the purpose of study, we collected 125 individuals from seven localities throughout South Korea including two newly distributed regions (Pyeongtaek and Yeongwol at ~37° N). These were sequenced for a segment of mitochondrial cytochrome oxidase subunit I (COI) and analyzed for genetic diversity, haplotype frequency, and population genetic structure among populations. Interestingly, northward range expansion accompanied only haplotypes, which are most abundant in the core populations, providing a significant reduction in haplotype diversity, compared to other populations. Moreover, genetic diversity was still lower in the expanded regions, but no genetic isolation was detected. These results suggest that further longer time would take to reach to the comparable genetic diversity of preexisting populations in the expanded regions. Probably, availability of qualified habitats at the newly expanded region could be pivotal for successful northward range expansion in response to climate change.

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## Introduction

*Patanga japonica* (Orthoptera: Acrididae), which is an univoltine grasshopper overwinters as adults, mating occurs during April and May, eggs are produced during May and June, and adults of new generation appear during September and October (Nam, 1996; Tanaka and Okuda, 1996). The species lives on the grass at the flatland and edge of forest in South Korea (Nam, 1996). Distributed in China, Taiwan, and Vietnam, Japan and South Korea (Cigliano *et al.*, 2022) the northern limit of its distributional range corresponds approximately to 36° of the north latitude. In South Korea, indeed, the species was distributed mainly in southern region (below 36° N) and a remote Island, Jeju, which is located ~100 km southward from the Korean peninsula, and was occasionally detected in the middle region at about 36° of the north latitude, but never reported in further northern region (Nam, 1996). Due to its southerly limited distribution (Nam, 1996; Cigliano *et al.*, 2022) *P. japonica* is classified as southerly distributed insects in South Korea, naming southern species. However, *P. japonica* recently has shown further northward range expansion up to 38° of the north latitude at the east side and 37° of the north latitude at the west side, obviously showing northward expansion in response to global warming (Shin *et al.*, 2022).

There has been abundancy in prediction study for future biodiversity under global warming. One of the influential predictions has shown that one out of six species are predicted to extinct and such disaster will be global, not confined to specific biological group and continent under the representative concentration pathway 8.5 scenario (Thomas *et al.*, 2004; Urban, 2015). On the other hand, a diverse type of organismal response and adaptation in response to ongoing global warming has also been reported (Thomas *et al.*, 2006; Wilson *et al.*, 2007; Canale and Henry, 2010; Vandewoestijne and Van Dyck, 2010; Maggini *et al.*, 2011; Lourenço-de-Moraes *et al.*, 2019; Adhikari *et al.*, 2020; McCain and Garfinkel, 2021; Zografou *et al.*, 2021). One of the such responses include expansion of current distributional range toward higher latitude and elevation (Wilson *et al.*, 2007; McCain and Garfinkel, 2021).

Under such expansion scenario it has been concerned about the change in genetic diversity in the newly expanded areas. This is particularly because genetic diversity within species

is a fundamental source of all biological diversity, such as species diversity and ecosystem diversity and is the primary basis for adaptation to future environmental uncertainty (Frankham, 1995, 1996; Hoffmann and Sgrò, 2011; Lanfear *et al.*, 2014). Moreover, reduction in genetic diversity within populations potentially will cause deleterious effects by breeding (Templeton, 1998). Consequently, many of previous studies focused on the relationships between genetic diversity and range expansion in response to global warming (Barton, 2000; Beaumont and Balding 2004; McInerny *et al.*, 2009; Woolbright *et al.*, 2014; Rödder *et al.*, 2021; Zografou *et al.*, 2021).

One exemplar study has shown that newly expanded leading edge allows individual dispersal, but the leading edge may not yet suitable enough to establish new populations due to heterogeneous environment, limiting adaptation to new habitats (Woolbright *et al.*, 2014). Consequently, the edge populations will have only a part of genetic constituents of long-sustained core populations, due to the genetic drift driven by founder effect and bottlenecks (Woolbright *et al.*, 2014). Moreover, it has been predicted that the populations in leading edge will be genetically isolated from core populations due to reduced genetic diversity (Woolbright *et al.*, 2014). On the other hand, somewhat opposite results such that an adaptation to new environment in the leading edge and an increase in newly adapted alleles by selective sweep have been suggested (Barton, 2000; Beaumont and Balding, 2004). However, at the same time, the diminished genetic diversity in neutral genes and consequent genetic isolation between core and edge populations have also been predicted (Barton, 2000; Beaumont and Balding, 2004). On another hand, it has been suggested by simulation study that dispersal to new habitat will cause founder effect, reducing genetic diversity, but continual founding to new areas has been suggested to generate newly adapted lineage and alleles, which will consequently increase the genetic diversity at the leading edge comparable enough to core areas (McInerny *et al.*, 2009). Although a substantial study on the change on genetic diversity in response to global warming is undergoing still more studies on local scales and a diverse species are required to have further general inference (Stefanescu *et al.*, 2011; Swaegers *et al.*, 2013; Rödder *et al.*, 2021; Zografou *et al.*, 2021).

In South Korea, 100 species have been designated as climate-sensitive indicator species, which require scrutinized

monitoring of their change in distributional range in response to climate change and this chart includes the *P. japonica* as one of the 15 insect species (National Institute of Biological Resources, 2017). However, no study has been made on the change of genetic diversity in these northward expanding species including the *P. japonica*. In this study, as a preliminary study, we collected a total of 125 individuals of *P. japonica* from seven localities in South Korea including two newly distributed regions (Pyeongtaek and Yeongwol at ~37° N) and sequenced for a partial mitochondrial COI to understand the characteristics of the changes in genetic diversity among regions, particularly between previously distributed region and recently occupied regions. Understanding such pattern could provide us further specific direction against global warming in terms of researches and policy establishment.

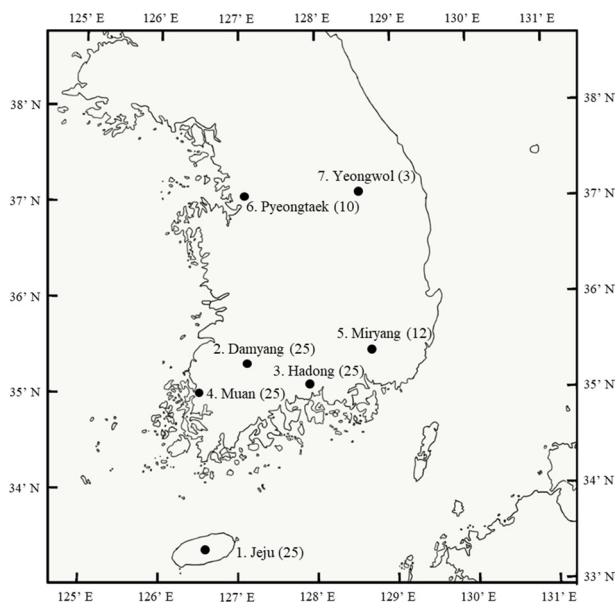
## Materials and Methods

### Sampling, molecular experiment, and sequencing

A total of 125 individuals of *P. japonica* were sampled from seven Korean localities from 2019 to 2022 (Fig. 1 and Table 1). Jeju, Damyang, and Hadong are previously known localities with collection record, but the remaining four localities are relatively new localities. In particular, two middle localities

(Pyeongtaek and Yeongwol at ~37° N) have never been reported for the occurrence of *P. japonica* (Nam, 1996) and are presumed to be newly expanded region in response to global warming. After live samples were transferred to the laboratory, identification to species was performed by one of the present authors (H. C. Jeong) through examination of the external morphology. Collected samples were then stored at -70°C until molecular work is performed.

Total DNA was extracted from one leg using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), in accordance with the manufacturer's instructions. The mitochondrial (mt) COI gene sequences, corresponding to a DNA barcoding region (658 bp), were amplified under the following conditions: an initial denaturation step at 94°C for 4 min, a 30-cycle amplification (94°C for 1 min, 53°C for 1 min, and 72°C for 1 min), and a final extension step of 1 min at 72°C. The primers were designed using the complete mt genome sequences from *Nomadacris japonica* (GenBank acc. no. MK059455; Unpublished), *Nomadacris septemfasciata* (KY980908, Song *et al.*, 2017), *Schistocerca nitens* (KY980904, Song *et al.*, 2017), *Schistocerca centralis* (KY980898, Song *et al.*, 2017), and *Austracris guttulosa* (MG993415; Song *et al.*, 2018). These were PJ-LCO (5'- TTT TCA ACA AAC CAT AAG GAT ATT GG -3'), and PJ-HCO (5'- GTA AAC CTC CGG ATG CCC AAA TCA -3'). Purification of the PCR product was performed using a PCR Purification Kit (Qiagen, Germany). To confirm successful DNA amplification electrophoresis was carried out in 0.5 × TAE buffer on 1% agarose gels. DNA sequencing for both strains was conducted using the ABI PRISM BigDye Terminator ver. 3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). To obtain finalized individual sequences the Clustal Omega program was used for sequence alignment (<http://www.ebi.ac.uk/Tools/msa/clustalo/>; Sievers *et al.*, 2011). Nucleotide sequences were translated based on the invertebrate mt DNA genetic code to check for the presence of any pseudogene sequences. Haplotype designations were applied to new sequences, which differed by ≥1 nucleotide (i.e., PJHAP01, PJHAP02, PJHAP03, PJHAP04 and so forth) after alignment using PAUP ver. 4.0b (Swofford, 2002).



**Fig. 1.** Collection localities of *Patanga japonica*. The number in parentheses indicates the number of individuals sequenced in this study.

### Haplotype relationships

Phylogenetic analysis to infer haplotype relationship was

**Table 1.** List of *Patanga japonica* samples used in this study.

Locality (no. of individuals)	Collection date	Sample animal number	COI haplotype (658 bp)	GenBank Accession no.
1. Jeju, Jeju (25)	2019.03.25	CNU10656	PJHAP01	OR672604
"	-	CNU10660	PJHAP01	OR672605
"	"	CNU10661	PJHAP02	OR672606
"	"	CNU10662	PJHAP01	OR672607
"	"	CNU10663	PJHAP03	OR672608
"	"	CNU10664	PJHAP01	OR672609
"	"	CNU10665	PJHAP01	OR672610
"	"	CNU10666	PJHAP03	OR672611
"	2019.08.07	CNU12925	PJHAP01	OR672612
"	"	CNU12926	PJHAP03	OR672613
"	"	CNU12927	PJHAP01	OR672614
"	"	CNU12928	PJHAP03	OR672615
"	"	CNU12929	PJHAP03	OR672616
"	"	CNU12930	PJHAP03	OR672617
"	"	CNU12931	PJHAP03	OR672618
"	"	CNU12932	PJHAP01	OR672619
"	"	CNU12933	PJHAP03	OR672620
"	"	CNU12934	PJHAP03	OR672621
"	"	CNU12935	PJHAP03	OR672622
"	"	CNU12936	PJHAP03	OR672623
"	"	CNU12937	PJHAP03	OR672624
"	"	CNU12938	PJHAP03	OR672625
"	"	CNU12939	PJHAP03	OR672626
"	"	CNU12940	PJHAP03	OR672627
"	"	CNU12941	PJHAP01	OR672628
2. Damyang, Jeollanamdo (25)	2019.09.27	CNU12942	PJHAP04	OR672629
"	"	CNU12943	PJHAP05	OR672630
"	"	CNU12944	PJHAP05	OR672631
"	"	CNU12945	PJHAP05	OR672632
"	"	CNU12946	PJHAP06	OR672633
"	"	CNU12947	PJHAP05	OR672634
"	"	CNU12948	PJHAP03	OR672635
"	"	CNU12949	PJHAP05	OR672636
"	"	CNU12950	PJHAP05	OR672637
"	"	CNU12951	PJHAP05	OR672638
"	"	CNU12952	PJHAP05	OR672639
"	"	CNU12953	PJHAP05	OR672640
"	"	CNU12954	PJHAP06	OR672641
"	"	CNU12955	PJHAP05	OR672642

**Table 1.** Continued

Locality (no. of individuals)	Collection date	Sample animal number	COI haplotype (658 bp)	GenBank Accession no.
"	"	CNU12956	PJHAP06	OR672643
"	"	CNU12957	PJHAP05	OR672644
"	"	CNU12958	PJHAP05	OR672645
"	"	CNU12959	PJHAP05	OR672646
"	"	CNU12960	PJHAP05	OR672647
"	"	CNU12961	PJHAP05	OR672648
"	"	CNU12962	PJHAP06	OR672649
"	"	CNU12963	PJHAP05	OR672650
"	"	CNU12964	PJHAP03	OR672651
"	"	CNU12965	PJHAP05	OR672652
"	"	CNU12966	PJHAP03	OR672653
3. Hadong, Gyeongsangnamdo (25)	2019.09.30	CNU12970	PJHAP03	OR672654
"	"	CNU12971	PJHAP05	OR672655
"	"	CNU12972	PJHAP03	OR672656
"	"	CNU12973	PJHAP05	OR672657
"	"	CNU12974	PJHAP05	OR672658
"	"	CNU12975	PJHAP07	OR672659
"	2019.10.05	CNU12976	PJHAP05	OR672660
"	"	CNU12977	PJHAP02	OR672661
"	"	CNU12978	PJHAP05	OR672662
"	"	CNU12979	PJHAP08	OR672663
"	"	CNU12980	PJHAP02	OR672664
"	"	CNU12981	PJHAP03	OR672665
"	"	CNU12982	PJHAP02	OR672666
"	"	CNU12983	PJHAP02	OR672667
"	"	CNU12984	PJHAP05	OR672668
"	"	CNU12985	PJHAP05	OR672669
"	"	CNU12986	PJHAP02	OR672670
"	"	CNU12987	PJHAP02	OR672671
"	"	CNU12988	PJHAP02	OR672672
"	"	CNU12989	PJHAP05	OR672673
"	"	CNU12990	PJHAP05	OR672674
"	"	CNU12991	PJHAP02	OR672675
"	"	CNU12992	PJHAP03	OR672676
"	"	CNU12993	PJHAP05	OR672677
"	"	CNU12994	PJHAP02	OR672678
4. Muan, Jeollanamdo (25)	2019.12.18	CNU13001	PJHAP09	OR672679
"	"	CNU13002	PJHAP10	OR672680
"	"	CNU13003	PJHAP03	OR672681

Table 1. Continued

Locality (no. of individuals)	Collection date	Sample animal number	COI haplotype (658 bp)	GenBank Accession no.
"	"	CNU13004	PJHAP03	OR672682
"	"	CNU13005	PJHAP03	OR672683
"	"	CNU13006	PJHAP11	OR672684
"	"	CNU13007	PJHAP05	OR672685
"	"	CNU13008	PJHAP03	OR672686
"	"	CNU13009	PJHAP05	OR672687
"	"	CNU13010	PJHAP03	OR672688
"	"	CNU13011	PJHAP12	OR672689
"	"	CNU13012	PJHAP03	OR672690
"	"	CNU13013	PJHAP05	OR672691
"	"	CNU13014	PJHAP11	OR672692
"	"	CNU13015	PJHAP05	OR672693
"	"	CNU13016	PJHAP03	OR672694
"	"	CNU13017	PJHAP05	OR672695
"	"	CNU13018	PJHAP03	OR672696
"	"	CNU13019	PJHAP05	OR672697
"	"	CNU13020	PJHAP05	OR672698
"	"	CNU13021	PJHAP05	OR672699
"	"	CNU13022	PJHAP05	OR672700
"	"	CNU13023	PJHAP05	OR672701
"	"	CNU13024	PJHAP03	OR672702
"	"	CNU13025	PJHAP13	OR672703
5. Miryang, Gyeongsangnamdo (12)	2021.08.26	CNU15145	PJHAP05	OR672704
"	"	CNU15146	PJHAP03	OR672705
"	"	CNU15147	PJHAP05	OR672706
"	"	CNU15148	PJHAP05	OR672707
"	"	CNU15149	PJHAP05	OR672708
"	"	CNU15150	PJHAP05	OR672709
"	"	CNU15151	PJHAP05	OR672710
"	"	CNU15152	PJHAP03	OR672711
"	"	CNU15153	PJHAP05	OR672712
"	"	CNU15154	PJHAP02	OR672713
"	"	CNU15155	PJHAP05	OR672714
"	"	CNU15156	PJHAP03	OR672715
6. Pyeongtaek, Gyeonggido (10)	2021.11.16	CNU15373	PJHAP05	OR672716
"	"	CNU15374	PJHAP05	OR672717
"	"	CNU15375	PJHAP05	OR672718
"	"	CNU15376	PJHAP05	OR672719



Table 1. Continued

Locality (no. of individuals)	Collection date	Sample animal number	COI haplotype (658 bp)	GenBank Accession no.
"	"	CNU15377	PJHAP05	OR672720
"	"	CNU15378	PJHAP05	OR672721
"	"	CNU15379	PJHAP05	OR672722
"	"	CNU15380	PJHAP05	OR672723
"	"	CNU15381	PJHAP05	OR672724
"	"	CNU15382	PJHAP05	OR672725
7. Yeongwol, Gangwondo (3)	2022.05.03	CNU15405	PJHAP03	OR672726
"	"	CNU15406	PJHAP05	OR672727
"	"	CNU15407	PJHAP05	OR672728

performed via the Bayesian inference (BI) method. The GTR+I+G was selected as the best substitution models using Modeltest ver. 3.7 (Posada and Crandall, 1998) within the IQ-TREE web server (Trifinopoulos *et al.*, 2016). BI analysis was performed using MrBayes ver. 3.2.7 (Ronquist *et al.*, 2012), which is incorporated into the CIPRES Portal ver. 3.1 (Miller *et al.*, 2010). Two separate runs of an incrementally heated Markov Chain Monte Carlo, consisting of four chains (one cold chain and three hot chains) were conducted. These runs were carried out simultaneously for one million generations, with sampling performed every 100 generations. Trace plots and convergence diagnostics were examined using MrBayes and Tracer ver. 1.7 (Rambaut *et al.*, 2018) to ensure that the Markov chains achieved stationarity and converged on parameter estimates and tree topology. This assessment included the verification of the standard deviation of split frequencies ( $< 0.01$ ) and the effective sample size ( $> 200$ ) after the burn-in phase, which was set at 25%. The confidence values are expressed as Bayesian posterior probabilities (BPPs) in percentages. To root trees, homologous region of within-familial species *Valanga irregularis* was downloaded from GenBank (acc. no. HQ969563; Unpublished) and utilized as an outgroup. The generated tree was viewed using FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>). To further scrutinize haplotype relationships, network analysis was performed using Network 10.2.0.0 (Bandelt *et al.*, 1999).

### Genetic diversity indices

Haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) per population were estimated according to Nei (1987) using

Arlequin ver. 3.5 (Excoffier and Lischer, 2010). Within-locality maximum sequence divergence was extracted from within-locality unrooted pairwise distance using PAUP (Swofford, 2002). Considering population-level analysis requires at least two haplotypes per unit the Pyeongtaek, which provided only a single haplotype (PJHAP05) was either combined with the data of Yeongwol, which is located to the Pyeongtaek most closely or separately (Fig. 1 and Table 1).

### Genetic distance and migration estimate

Genetic distance and migration rate were estimated using the Arlequin ver. 3.5 (Excoffier and Lischer, 2010). Population pairwise genetic distance ( $F_{ST}$ ) and migration rate ( $Nm$ ) were calculated by the Kimura 2-parameters method (Kimura, 1980) and a permutation test of the significant differentiation of the pairs of populations (1,000 bootstraps) were obtained following the approach described in Excoffier *et al.* (1992). Pairwise  $F_{ST}$  values were used to estimate per generation migration rate,  $Nm$  (the product of the effective population size  $N_e$  and migration rate,  $m$ ) based upon the equilibrium relationship:  $F_{ST} = 1/(2Nm + 1)$ . The degree of population differentiation was visualized as a heat map2 ([https://rdocumentation.org/packages/gplots/versions/3.1.3/topics/heatmap.2.](https://rdocumentation.org/packages/gplots/versions/3.1.3/topics/heatmap.2)) in the Galaxy web platform ver. 3.0.1 (; The Galaxy Community, 2022). The genetic structure was further analyzed to detect distribution, relationships, and abundance of gene pools using Bayesian Analysis of Population Structure (BAPS) ver. 6.0 (Corander and Tang, 2007). The analysis was performed using clustering, with a linked locus module and a codon model. In this process, mixture analysis was performed with  $K$ -values

**Table 2.** Relative frequencies of haplotypes.

Haplotype	Locality							Total (125)
	Jeju (25)	Damyang (25)	Hadong (25)	Muan (25)	Miryang (12)	Pyeongtaek (10)	Yeongwol (3)	
PJHAP01	0.36 (9)							0.072 (9)
PJHAP02	0.04 (1)		0.36 (9)		0.08 (1)			0.088 (11)
PJHAP03	0.60 (15)	0.12 (3)	0.16 (4)	0.36 (9)	0.25 (3)		0.33 (1)	0.280 (35)
PJHAP04		0.04 (1)						0.008 (1)
PJHAP05		0.68 (17)	0.40 (10)	0.40 (10)	0.66 (8)	1.00 (10)	0.66 (2)	0.456 (57)
PJHAP06		0.16 (4)						0.032 (4)
PJHAP07			0.04 (1)					0.008 (1)
PJHAP08			0.04 (1)					0.008 (1)
PJHAP09				0.04 (1)				0.008 (1)
PJHAP10				0.04 (1)				0.008 (1)
PJHAP11				0.08 (2)				0.016 (2)
PJHAP12				0.04 (1)				0.008 (1)
PJHAP13				0.04 (1)				0.008 (1)

Numbers in parentheses indicate number of individuals.

ranging from 1–10, and optimal clusters were identified based on the maximum log marginal likelihood values.

### Test of isolation-by-distance

To test for correlation between geographic and genetic distances, a Mantel test (Mantel, 1967) using isolation-by-distance (IBD), which compares the matrices of pairwise genetic distance [ $F_{ST}/(1 - F_{ST})$ ] and the logarithms of geographical distance data (km) was performed, with the significance test conducted over 10,000 randomizations (Mantel, 1967). The analysis was conducted using the IBD software package, with the negative genetic distance set to zero (Bohonak, 2002). With the consideration that Jeju is an island that may not have an equivalent effect of geographic distance to inland populations IBD analysis was performed both including and excluding Jeju.

## Results

### Sequence analysis

A total of 13 haplotypes were obtained by sequencing

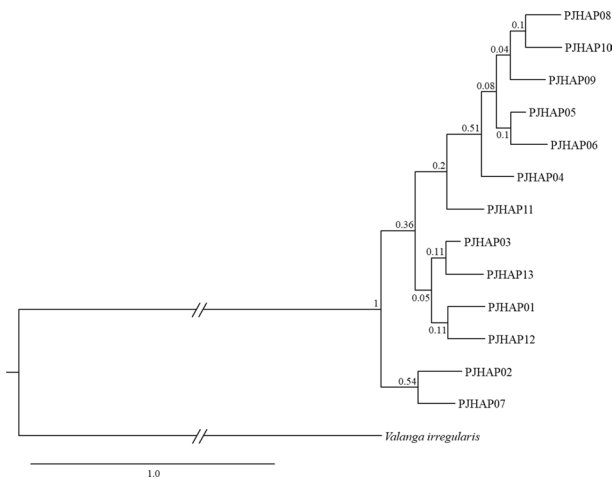
658 bp of COI gene from 125 individuals of *P. japonica* collected in seven localities in South Korea (Fig. 1 and Table 1). Three haplotypes shared among localities (PJHAP02, PJHAP03, and PJHAP05), whereas the rest of them were locality-specific (Table 2). PJHAP05 showed the highest frequency at 45.6% (57 individuals) and this haplotype was found in all localities, except for Jeju (Table 2). Further, the frequency of this haplotype was always the highest at each inland population, where it is detected, but is absent in Jeju. On the other hand, the PJHAP03, which ranked the second highest at 28% (35 individuals) mostly ranked as the second or third highest frequency at each inland population, but was the highest in Jeju (15 among 25 individuals). The third highly frequent PJHAP02 has 8.8% of frequency (11 individuals) and found more frequently in inland populations, particularly in Hadong (locality 3), but was found only as a single individual in Jeju. The fourth frequent PJHAP01 had the 7.2% of frequency (9 individuals), and found only in Jeju, but not in inland populations. Collectively, haplotype distribution is somewhat different between Jeju Island and the inland populations.



**Table 3.** Pairwise comparisons.

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13
1. PJHAP01	-	0.61	0.15	0.46	0.30	0.46	0.46	0.46	0.46	0.46	0.30	0.30	0.30
2. PJHAP02	4	-	0.46	0.76	0.61	0.76	0.46	0.76	0.76	0.76	0.61	0.61	0.61
3. PJHAP03	1	3	-	0.30	0.15	0.30	0.30	0.30	0.30	0.30	0.15	0.15	0.15
4. PJHAP04	3	5	2	-	0.15	0.30	0.61	0.30	0.30	0.30	0.30	0.46	0.46
5. PJHAP05	2	4	1	1	-	0.15	0.46	0.15	0.15	0.15	0.15	0.30	0.30
6. PJHAP06	3	5	2	2	1	-	0.61	0.30	0.30	0.30	0.30	0.46	0.46
7. PJHAP07	3	3	2	4	3	4	-	0.61	0.61	0.61	0.46	0.46	0.46
8. PJHAP08	3	5	2	2	1	2	4	-	0.30	0.30	0.30	0.46	0.46
9. PJHAP09	3	5	2	2	1	2	4	2	-	0.30	0.30	0.46	0.46
10. PJHAP10	3	5	2	2	1	2	4	2	2	-	0.30	0.46	0.46
11. PJHAP11	2	4	1	2	1	2	3	2	2	2	-	0.30	0.30
12. PJHAP12	2	4	1	3	2	3	3	3	3	3	2	-	0.30
13. PJHAP13	2	4	1	3	2	3	3	3	3	3	2	2	-

Numbers above the diagonal are percent distance values; numbers below the diagonal are absolute distance values.



**Fig. 2.** Phylogeny of *Patanga japonica* haplotypes. The numbers at each node specify Bayesian posterior probabilities (BPP). The scale bar indicates the number of substitutions per site. Tree was truncated into one-third between outgroup and ingroup.

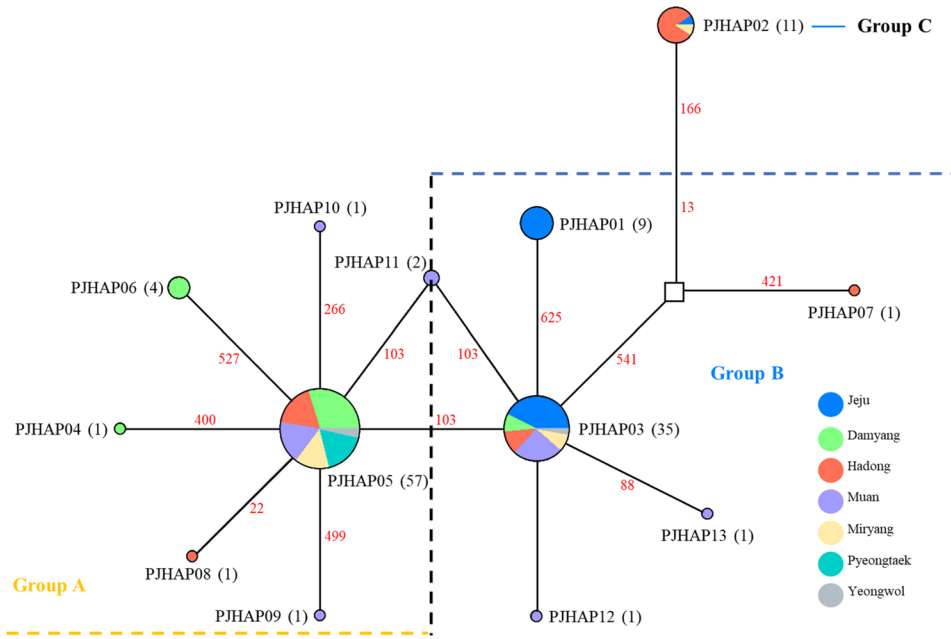
### Haplotype relationships

Pairwise comparison showed the sequence divergence, ranging from 0.15% (1 bp) to 0.76% (5 bp), providing relatively low divergence among them (Table 3). The maximum sequence divergence was detected when PJHAP02 (one from Jeju, nine from Hadong, and one from Miryang) was compared to PJHAP04 (one from Damyang),

PJHAP06 (four from Damyang), PJHAP08 (one from Hadong), PJHAP09 (one from Muan), and PJHAP10 (one from Muan).

Phylogenetic analysis among haplotypes showed no discernible group, forming a large one group (Fig. 2). Only PJHAP02 (one from Jeju, nine from Hadong, and one from Miryang) and PJHAP07 (one individual from Hadong) formed relatively inclusive group, with the BPP only at 0.54.

For further scrutinized analysis for haplotype relationship network analysis was performed (Fig. 3). PJHAP05, which was detected as the highest frequency (45.6%) and most widely (but excluding Jeju), and PJHAP03, which was detected as the second highest frequency (28%) and almost all mainland localities (excluding Pyeongtaek) and also in Jeju were located each in the center of the star-like phylogeny. From each centered-haplotype several haplotypes appear to have been derived. Finally, PJHAP02, which was most distant to other haplotypes in the pairwise comparison (Table 3) and found in two inland populations (Hadong and Miryang) and Jeju formed somewhat distant group alone. Although a clear subdivision among haplotypes was not detected haplotypes can somewhat arbitrary be dividable into three groups from the network: PJHAP05-derived group A (all mainland localities, but not



**Fig. 3.** Haplotype network of *Patanga japonica*. Each node represents a single nucleotide change. Circle size is proportional pertaining given haplotypes, with the actual number written within parentheses and circle color represents locality. Rectangular box indicates the hypothetical haplotypes that were not found in this study. Number on branch indicates the substitution site in the 658 bp of COI.

**Table 4.** Within-locality diversity estimates of *Patanga japonica*.

Locality	SS <sup>1</sup>	NH <sup>2</sup>	$h^3$	NP <sup>4</sup>	MSD <sup>5</sup> (%)	MPD <sup>6</sup>	$\pi^7$
1. Jeju, Jeju-do	25	3	0.5300±0.0642	4	0.608	0.720000±0.557067	0.001094±0.000943
2. Damyang, Jeollanamdo	25	4	0.5167±0.1049	3	0.304	0.580000±0.483910	0.000881±0.000820
3. Hadong, Gyeongsangnamdo	25	5	0.7100±0.0529	6	0.760	2.133333±1.227466	0.003242±0.002079
4. Muan, Jeollanamdo	25	7	0.7267±0.0614	5	0.456	0.913333±0.654248	0.001388±0.001108
5. Miryang, Gyeongsangnamdo	12	3	0.5303±0.1359	4	0.608	0.984848±0.714740	0.001497±0.001223
7. Yeongwol, Gangwondo	3	2	0.6667±0.3143	1	0.152	0.666667±0.666667	0.001013±0.001264
8. Pyeongtaek + Yeongwol, Gyeonggido + Gangwondo	13	2	0.1538±0.1261	1	0.152	0.153846±0.228664	0.000234±0.000391

<sup>1</sup> Sample size

<sup>2</sup> Number of haplotype for mitochondrial genes

<sup>3</sup> Haplotype or sequence type diversity

<sup>4</sup> Number of polymorphic sites

<sup>5</sup> Maximum sequence divergence

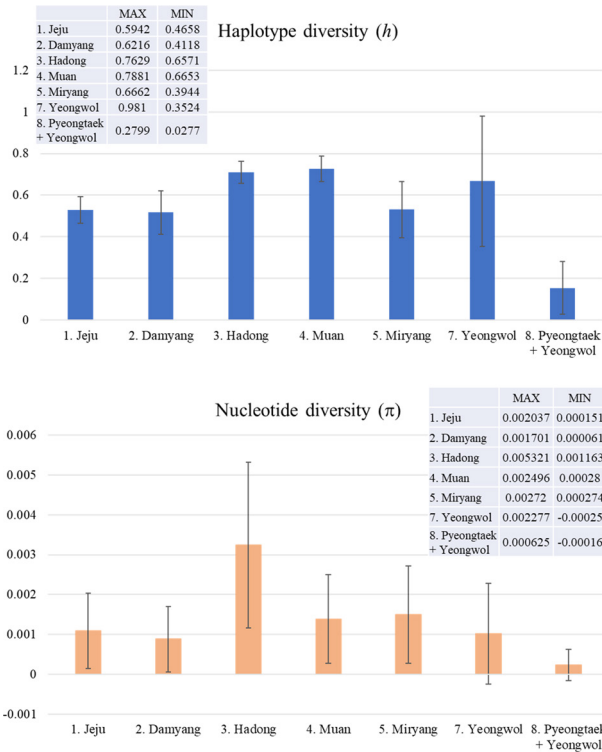
<sup>6</sup> Mean number of pairwise differences

<sup>7</sup> Nucleotide diversity

in Jeju), PJHAP03-derived group B (most abundant in Jeju and most inland populations, but excluding Pyeongtaek), and PJHAP02 alone as group C (only one from Jeju and a few from two inland populations, Hadong and Miryang).

### Genetic diversity

Haplotype diversity ( $h$ ) ranged from 0 to 0.7267, showing the lowest  $h$  in Pyeongtaek + Yeongwol and the highest  $h$  in Muan, excluding Pyeongtaek, which provided a single haplotype (Table 4). Excluding Pyeongtaek nucleotide

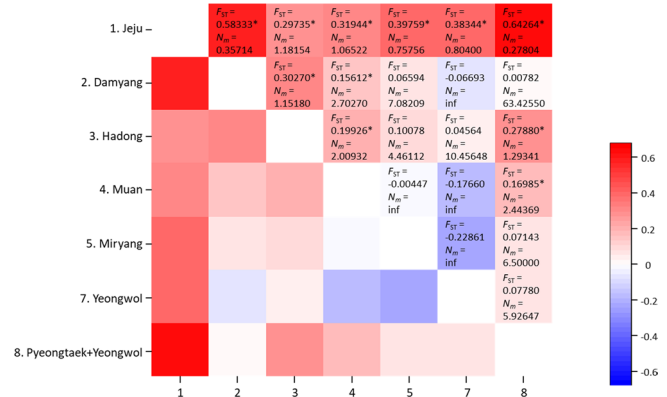


**Fig. 4.** Within-locality haplotype diversity and nucleotide diversity of *Patanga japonica* using COI with the consideration of standard deviation. Vertical bars indicate the standard deviation. Note that Pyeongtaek and Yeongwol populations were combined.

diversity ( $\pi$ ) was the lowest in Pyeongtaek + Yeongwol ( $\pi = 0.000234$ ) and the second lowest in Damyang ( $\pi = 0.000881$ ), whereas it was the highest in Hadong ( $\pi = 0.003242$ ), showing that the two newly expanded localities are lower in  $\pi$  (Table 4). Although the  $\pi$  did not show any significance among populations when standard error (SE) is considered  $h$  showed a significant difference between Pyeongtaek + Yeongwol and the remaining populations (Fig. 4).

### Genetic distance

Genetic distance ( $F_{ST}$ ) between 21 pairs of populations (including Pyeongtaek + Yeongwol as a single population, but excluding Pyeongtaek) showed a significant distance in 11 comparisons (Fig. 5). Jeju showed a significant divergence to all inland populations. The remaining populations had one to four populations with statistical significance ( $p < 0.005$ ). Miryang and Yeongwol had the least number of populations with statistical significance, whereas Hadong and Muan had the highest number of populations with statistical significance among the inland populations (Fig. 5). Conversely, Jeju showed

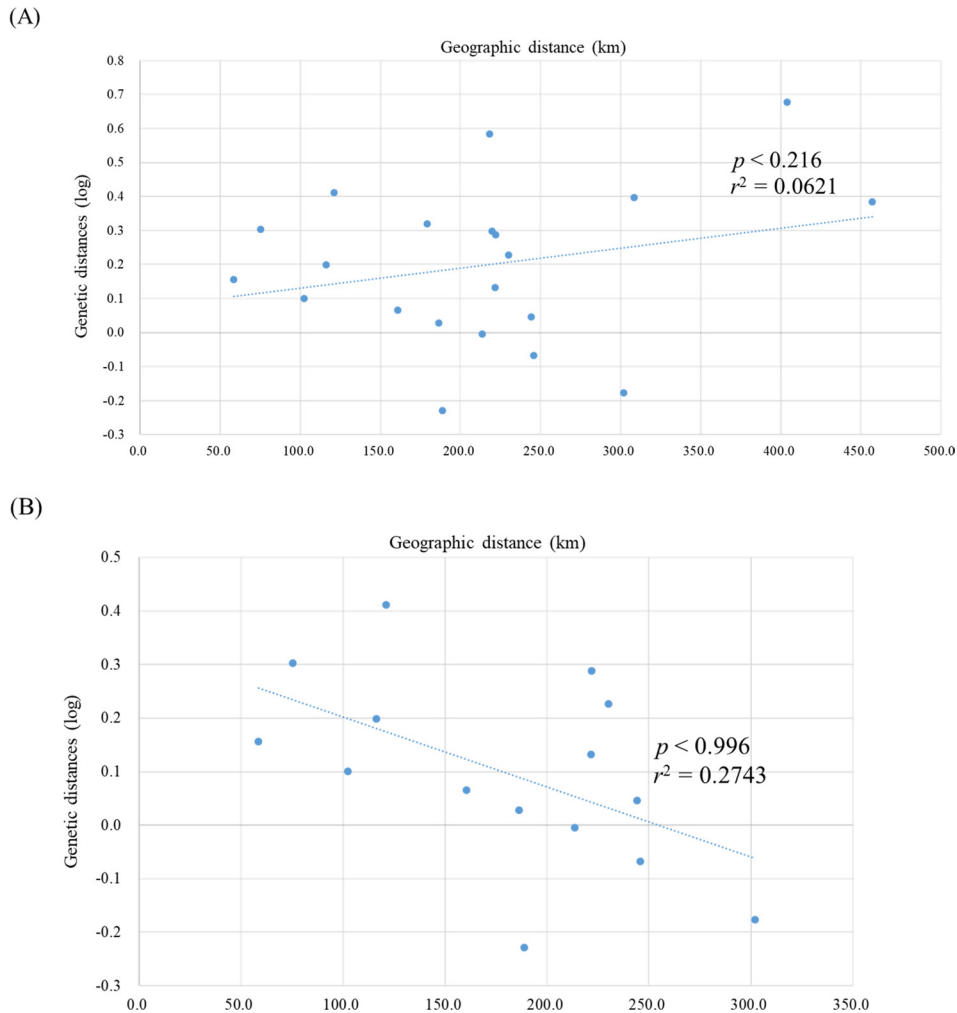


**Fig. 5.** Matrix of genetic distance ( $F_{ST}$ ) and migration rate ( $N_m$ ) between pairs of populations. \* $p < 0.05$ . inf, infinite.

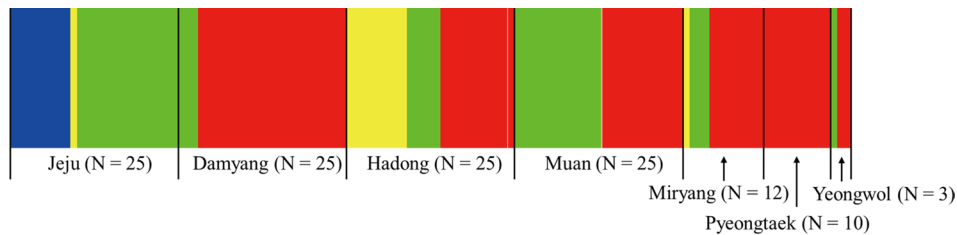
the least gene flow ( $N_m$ ) to other populations, ranging from 0.278 to 1.182, showing at most one individual female migrant per generation. On the other hand, infinite gene flow was detected in the comparisons among Damyang, Muan, Miryang, and Yeongwol (Fig. 5). The pattern of genetic distance appears not to be consistent with geographic distance considering that, for example, the geographically closer localities such as Damyang and Muan (~70 km) shows a significant genetic distance, whereas distant locality pair such as Damyang and Miryang (~240 km) does not show any statistically significant genetic distance. Indeed, the results of the Mantel test (10,000 randomizations) showed no statistically significant IBD ( $p < 0.216$ ), providing a correlation coefficient ( $r^2$ ) of 0.0621, indicating no positive correlation between geographic distance and genetic distance (Fig. 6A). Even excluding the island Jeju, no positive correlation was detected ( $p < 0.996$ ,  $r^2 = 0.2743$ ; Fig. 6B).

### BAPS analysis

An examination of the likelihood scores from 10 replicate runs across  $K$  values from 1 to 20 for the BAPS analysis revealed that *P. japonica* individuals were divided into four optimal haplotype cluster (Fig. 7,  $K = 4$ , hereafter referred to as haplogroup). Jeju, Hadong, and Miryang have each three haplogroups, whereas Damyang, Muan, and Yeongwol have each two haplogroup, and Pyeongtaek has a single haplogroup. Although Jeju, Hadong, and Miryang have three haplogroups, the constitution differs among localities. Jeju has a unique haplogroup, blue, and this haplogroup is not found in any inland populations. On the other hand, yellow haplogroup found in Jeju is also found at the south-eastern Hadong and Miryang, but is not found at the south-



**Fig. 6.** Isolation by distance accessed by plotting  $F_{ST}/1 - F_{ST}$  against geographic distance, along with corresponding coefficient of determination ( $R^2$ ),  $p$  value, and slope of the regression line. (A) All populations and (B) All, excluding Jeju population



**Fig. 7.** Bayesian clustering (BAPS) analysis. The optimum number of clusters ( $K$ ) was 4.

western Damyang and Muan, and absent in newly expanded Pyeongtaek and Yeongwol. The green haplogroup found in Jeju dominantly is found also in Damyang, Hadong, Muan, Miryang, and Yeongwol, but not in Pyeongtaek. The newly expanded Pyeongtaek and Yeongwol each has red and both green and red, respectively, and the two populations share the red haplogroup, which is the most dominant haplogroup in inland populations.

## Discussion

Understanding the pattern of genetic diversity in response to climate change could provide us an important chance for long-term conservation of biodiversity, particularly for genetic diversity, which is the most fundamental level of biodiversity. Current study is the first that examined the population genetic contour of

the climate-sensitive indicator insect in South Korea under the climate change, as far as we know. However, this study has a limitation in that the marker used has limited variation and reflects neutral variation, instead of functional adaptability of *P. japonica*. Nevertheless, our results could provide some valuable insight under the global warming situation and the ground to consider further scrutinized and extensive study.

### Difference between Jeju Island and mainland in haplotype distribution pattern

One of the main finding from this study includes that the current climate change has not yet overridden the historical pattern of genetic distribution of *P. japonica* completely, particularly considering that there is somewhat different distributional pattern between Jeju and mainland. There were two most frequent haplotypes, PJHAP05 (the highest frequency and most widespread in mainland, but not found in Jeju) and PJHAP03 (the second highest frequency and almost all mainland localities, excluding Pyeongtaek, but was found in Jeju), but their frequency differs between Jeju and the mainland. Further, Jeju has an abundant PJHAP01, which is not found in any mainland locality (Fig. 3 and Table 2). Due to such difference  $F_{ST}$  consistently indicates an isolation of Jeju from all mainland population (Fig. 5) and BAPS analysis indicates possession of a unique haplogroup (blue) only in Jeju (Fig. 7). Such separation between the two regions and the uniqueness in Jeju may be explained by historical event in the Korean peninsula including Jeju. The last glacial advance at about 20,000 years ago (BP) lowered sea level (estimated at  $80 \pm 5$  m), enabled the Korean peninsula to join to the present Jeju Island (Ohshima, 1990; Park, 1988) and allowed admixture of the populations in mainland and Jeju Island, particularly at Jeju Island. However, later Jeju Island became such about 12,000 BP when the sea level rose to its present state (Ohshima, 1990). Since then, Jeju Island may have accumulated an independent genetic composition different from the mainland. Consequently, the two geographic regions may have similar but somewhat differentiated genetic composition. Due to such history, indeed, a diverse level of divergence between the two geographic regions has often been reported in a diverse organism including insects (e.g., different genetic composition, isolation, and even speciation) (Lee *et al.*, 2003; Lee *et al.*, 2016; Jo *et al.*, 2017; Park *et al.*, 2019). Nevertheless, for further robust interpretation further variable molecular marker

will be necessary. Probably as climate change continues further complicate distributional pattern of genetic diversity than current observation will be generated.

### Haplogroup share between Jeju and south-eastern populations

The BAPS analysis indicated that Jeju shares the yellow haplogroup, with Hadong and Miryang, which are located more easterly, whereas does not share the haplogroup with Damyang and Muan, which are located more westerly and closer to Jeju (Fig. 7). This observation is interesting, but is not well explained solely by geographic distance either as on-going and historical event, particularly considering that haplotypes with the lowest frequency in Jeju shares with inland Miryang population. Maybe historical dispersal that facilitated dispersal to eastern part was stronger than that of western part, but no clear explanation for this observation could be obtained until increased samples and further variable molecular markers are employed.

### Haplotype diversity in newly dispersed regions

Available collection data (Nam, 1996; Shin *et al.*, 2022) including our effort to collect *P. japonica* suggest that Pyeongtaek and Yeongwol are obviously the places, where *P. japonica* have recently been expanded, although further scrutinized census data are not available. It could be important to know who is the dispersers that expanded their range in response to climate change. Among the 13 haplotypes only two haplotypes were found in the two regions (PJHAP03 in Yeongwol and PJHAP05 in Pyeongtaek and Yeongwol) and these are the two found with the highest frequency in all samples (Table 2). The distribution pattern of mitochondrial DNA lineages has been suggested to be proportional to their age under a simple isolation-by-distance model (Neigel and Avise, 1993). Thus, the oldest are most widespread, whereas their progeny are expected to exist closer to the areas, where they originated (Watterson and Guess, 1977). Consequently, the network can arrange the oldest haplotypes in the center of the network, while their progeny with limited distribution are expected to position derived from the oldest haplotypes, creating a star-like phylogeny. If this theory is applied, PJHAP05 and PJHAP03 are obviously the “oldest” haplotypes in that they are most widespread, with higher frequency, placed each at the center of the star-like-phylogeny, being the

source of several one-locality-confined haplotypes (Fig. 3). Thus, these two might be major sources of range expansion. In particular, we collected ten individuals from Pyeongtaek, but all individuals shared an identical haplotype PJHAP05, which is most widespread with the highest frequency in the mainland.

On the other hand, our data for genetic distance (Fig. 5) and BAPS analysis (Fig. 7) did not support the prediction that leading edge will be genetically isolated from core populations (Woolbright *et al.*, 2014) in that the two newly expanded populations mostly did not show any statistically significant genetic distance to other populations, except for Jeju when the two populations were combined (Pyeongtaek + Yeongwol population). We interpreted this observation three different ways. 1) Maybe relatively shorter latitudinal length of South Korea may not really be a suitable geographic region to test for such effect. 2) Or maybe current marker used in this study did not provide enough resolution for such test. 3) Or maybe current progress of climate change is not severe yet enough to test such isolation or times for such observation may not yet have been elapsed. We think that all these possibilities could be valid until more progressed results from expanded studies are available.

Although we did not detect any genetic isolation of the newly expanded regions genetic diversity was notably different in the newly expanded regions (Fig. 4). Nucleotide diversity ( $\pi$ ), which reflect sequence divergence, along with number of haplotypes did not show any significance, but the haplotype diversity ( $h$ ), which reflects only haplotype diversity showed a significant difference between the Pyeongtaek + Yeongwol populations and the remaining populations (Fig. 4). This finding is somewhat consistent with the previous prediction that only a part of genetic constituents of long-sustained core populations is able to disperse to new areas (Woolbright *et al.*, 2014). In this case IBD should be detected, but our IBD analysis, even excluding Jeju did not show any positive IBD (Fig. 6). Thus, it is assumed that dispersal is substantial to new areas (Wright, 1978) or equilibrium in newly expanded regions may not have been achieved. For this, also, additional expanded study is required.

In summary, our population genetic analysis of *P. japonica* using mitochondrial COI sequences provided a minimal, but a few noteworthy information that can be referable under climate changing situation. First, the climate change at this point did not yet completely overridden the historical distributional

pattern, particularly between Jeju Island and inlands, although inlands themselves appear to have been influenced. Second, newly expanded regions have the haplotypes that are “oldest” are involved. Thirds, number of haplotypes in the expanded areas are limiting compared to core populations. Finally, genetic isolation of the newly expanded region was not observable. Considering *P. japonica* is expanding its range northward available habitats at the potential expanded regions could be pivotal for its range expansion. Therefore, climate-sensitive species including current *P. japonica* may require monitoring of available habitats at the already expanded and potential regions will be needed. For further scrutinized understanding on climate-sensitive species including *P. japonica* further expanded studies are essential for further robust conclusion and inference for future conservation of biodiversity in the Korean peninsula under climate changing circumstances.

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