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Population genetic analysis of *Salurnis marginella* (Hemiptera: Flatidae)

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Abstract

Salurnis marginella Guérin−Méneville, 1829 (Hemiptera: Flatidae) is an invasive species first reported in 2003 in Iksan, which is located in the mid-western region of South Korea, and subsequently found in the nearby regions in 2005. However, molecular-perspective reports on their invasive characteristics are not yet available. In this study, population genetic characteristics of Korean *S. marginella* were evaluated using the mitochondrial *COI* region and sequencing 124 individual samples collected in 11 Korean localities. A total of 12 haplotypes were identified with a maximum sequence divergence of 1.368% (9 bp). Haplotype diversity was relatively higher than that of other insect species invaded into Korea, providing 2-6 haplotypes per populations, indicating that introduction to Korea may have happened rather extensively and consistently. Nucleotide diversity (π) was the highest in Iksan but owing to the limited sample size (three individuals) from this locality, additional studies are required for drawing conclusive inference regarding the place of entry to Korea. Ulsan, the easternmost population in the present study, revealed nearly the lowest diversity estimates, such as the lowest *H* and the second-lowest π; a unique haplogroup with a higher frequency; and an independent genetic cluster, suggesting that the introduction of *S. marginella* to Ulsan was an independent event. Further collection in Korea and neighboring countries, including the original distributional range is necessary to elucidate the invasive dynamics of *S. marginella*

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Introduction

Flatidae of Hemiptera is composed of 1,422 species belonging to 298 genera (Bourgoin 2016). Among these, four species are found in Korea: *Geisha distinctissima*, *Mimophantia maritima*, *Metcalfa pruinosa*, and *Salurnis marginella* (Kwon and Huh, 2001; Lee and Wilson, 2010). *S. marginella* Guérin-Méneville, 1829 was originally distributed in the southern and partial northern regions of China as well as in Laos and Thailand (Chou *et al.*, 1985). In Korea, the species was first observed in Iksan, which is located in the mid-western region, in 2013 and then spread southward and southeastward to several cities and counties including Ulsan, which is the easternmost locality (Lee *et al.*, 2016).

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This species reproduces twice per year in southern China, overwintering as eggs, but reproduces only once per year in Korea, overwintering as eggs (Kim *et al.*, 2016). Nymphs stages occur at the beginning of June to late July, and adults thrive from the end of July and September in Korea (Lee *et al.*, 2016). Nymphs are detected at the back of leaves near new shoots, but as they grow into adults, they are also found in the stems (Kim *et al.*, 2016). Nymphs secrete wax from their body, including from the end of the abdomen, and deposit the wax on host plants. This long, thick, and V-shaped wax accumulation is a characteristic feature of the species and sometimes becomes twice as long as their body length, but shortens after molting (Kim *et al.*, 2016).

Table 1. List providing details of collection locality, collection date, sample number, and *COI* haplotype of *Salurnis marginella*

Locality (no. of individuals)	Collected date	Sample number	Haplotype	GenBank number
1. Gwangju (20) Sinan-dong 35°10'20.6" N, 126°53'56.5" E	2018. 06. 22	CNU8296	SMBAR06	OL884228
		CNU8297	SMBAR01	OL884229
		CNU8298	SMBAR06	OL884230
	2018. 07. 16	CNU8303	SMBAR02	OL884231
		CNU8304	SMBAR03	OL884232
		CNU8305	SMBAR02	OL884233
		CNU8306	SMBAR06	OL884234
		CNU8307	SMBAR02	OL884235
		CNU8308	SMBAR02	OL884236
		CNU8316	SMBAR06	OL884237
Daechon-dong 35°05'20.1" N, 126°52'36.7" E	2018. 07. 23	CNU8328	SMBAR01	OL884238
		CNU8329	SMBAR08	OL884239
		CNU8330	SMBAR10	OL884240
		CNU8331	SMBAR02	OL884241
		CNU8332	SMBAR10	OL884242
		CNU8333	SMBAR02	OL884243
		CNU8334	SMBAR03	OL884244
		CNU8335	SMBAR02	OL884245
		CNU8336	SMBAR02	OL884246
		CNU8337	SMBAR02	OL884247
2. Haenam, JN (13) 34°30'04.7" N, 126°37'22.2" E	2018. 08. 08	CNU8367	SMBAR02	OL884248
		CNU8368	SMBAR04	OL884249
		CNU8369	SMBAR02	OL884250
		CNU8370	SMBAR02	OL884251
		CNU8371	SMBAR02	OL884252
		CNU8372	SMBAR02	OL884253
		CNU8373	SMBAR02	OL884254
		CNU8374	SMBAR02	OL884255
		CNU8375	SMBAR02	OL884256
		CNU8376	SMBAR02	OL884257
		CNU8377	SMBAR02	OL884258
		CNU8378	SMBAR02	OL884259
		CNU8379	SMBAR01	OL884260

Table 1. List providing details of collection locality, collection date, sample number, and *COI* haplotype of *Salurnis marginella* (Continued)

Locality (no. of individuals)	Collected date	Sample number	Haplotype	GenBank number
3. Jangheung, JN (10) 34°48'45.6" N, 126°54'07.3" E	2018. 08. 08	CNU8382	SMBAR02	OL884261
		CNU8383	SMBAR02	OL884262
		CNU8384	SMBAR04	OL884263
		CNU8385	SMBAR01	OL884264
		CNU8386	SMBAR01	OL884265
		CNU8387	SMBAR02	OL884266
		CNU8388	SMBAR11	OL884267
		CNU8389	SMBAR01	OL884268
		CNU8390	SMBAR02	OL884269
		CNU8391	SMBAR02	OL884270
4. Jangseong, JN (9) 35°24'41.1" N, 126°53'18.8" E	2018. 08. 02	CNU8358	SMBAR03	OL884271
		CNU8359	SMBAR01	OL884272
		CNU8360	SMBAR01	OL884273
		CNU8361	SMBAR01	OL884274
		CNU8362	SMBAR03	OL884275
		CNU8363	SMBAR01	OL884276
		CNU8364	SMBAR01	OL884277
		CNU8365	SMBAR03	OL884278
		CNU8366	SMBAR01	OL884279
5. Namwon, JB (4) 35°25'45.2" N, 127°21'37.1" E	2018. 08. 07	CNU8445	SMBAR03	OL884280
		CNU8446	SMBAR04	OL884281
		CNU8447	SMBAR04	OL884282
		CNU8448	SMBAR04	OL884283
6. Jeonju, JB (6) 35°51'21.0" N, 127°07'45.6" E	2018. 08. 02	CNU8340	SMBAR02	OL884284
		CNU8341	SMBAR04	OL884285
	2018. 08. 08	CNU8343	SMBAR01	OL884286
		CNU8344	SMBAR04	OL884287
		CNU8345	SMBAR03	OL884288
		CNU8346	SMBAR03	OL884289
7. Gimje, JB (20) 35°48'36.1" N, 126°53'57.3" E	2018. 08. 08	CNU8350	SMBAR03	OL884290
		CNU8351	SMBAR03	OL884291
		CNU8352	SMBAR01	OL884292
		CNU8353	SMBAR03	OL884293
		CNU8354	SMBAR11	OL884294
		CNU8355	SMBAR06	OL884295
		CNU8356	SMBAR09	OL884296
		CNU8357	SMBAR02	OL884297
	2018. 08. 20	CNU8394	SMBAR03	OL884298
		CNU8395	SMBAR03	OL884299
		CNU8396	SMBAR03	OL884300
	2018. 09. 14	CNU8423	SMBAR04	OL884301
		CNU8424	SMBAR01	OL884302
		CNU8425	SMBAR01	OL884303
		CNU8426	SMBAR03	OL884304
		CNU8427	SMBAR03	OL884305
		CNU8428	SMBAR03	OL884306
		CNU8429	SMBAR03	OL884307
		CNU8430	SMBAR03	OL884308
		CNU8431	SMBAR01	OL884309

Table 1. List providing details of collection locality, collection date, sample number, and *COI* haplotype of *Salurnis marginella* (Continued)

Locality (no. of individuals)	Collected date	Sample number	Haplotype	GenBank number
8. Iksan, JB Seogwang-dong (1) 35°58'21.4" N, 127°01'02.9" E	2018. 08. 08	CNU8347	SMBAR04	OL884310 OL884311
Eoyang-dong (2) 35°56'57.1" N, 126°59'12.2" E		CNU8348	SMBAR09	OL884312
		CNU8349	SMBAR04	OL884313
9. Sacheon, GN (3) 35°04'24.2" N, 128°00'38.8" E	2018. 08. 20	CNU8397	SMBAR06	OL884314
		CNU8398	SMBAR01	OL884315
	2018 .09. 08	CNU8422	SMBAR01	OL884316
10. Jinju, GN (13) 35°09'12.6" N, 128°05'58.7" E	2018. 07. 15	CNU8323	SMBAR06	OL884317
		CNU8324	SMBAR03	OL884318
		CNU8325	SMBAR07	OL884319
		CNU8326	SMBAR03	OL884320
		CNU8327	SMBAR06	OL884321
	2018. 08. 06	CNU8437	SMBAR03	OL884322
		CNU8438	SMBAR07	OL884323
		CNU8439	SMBAR03	OL884324
		CNU8440	SMBAR03	OL884325
		CNU8441	SMBAR07	OL884326
		CNU8442	SMBAR07	OL884327
		CNU8443	SMBAR07	OL884328
		CNU8444	SMBAR12	OL884329
11. Ulsan (23) 35°30'55.2" N, 129°19'19.0" E	2018. 08. 21	CNU8399	SMBAR05	OL884330
		CNU8400	SMBAR05	OL884331
		CNU8401	SMBAR05	OL884332
		CNU8402	SMBAR04	OL884333
		CNU8403	SMBAR05	OL884334
	2018. 09. 07	CNU8404	SMBAR05	OL884335
		CNU8405	SMBAR05	OL884336
		CNU8406	SMBAR05	OL884337
		CNU8407	SMBAR02	OL884338
		CNU8408	SMBAR02	OL884339
		CNU8409	SMBAR05	OL884340
		CNU8410	SMBAR05	OL884341
		CNU8411	SMBAR05	OL884342
		CNU8412	SMBAR05	OL884343
		CNU8413	SMBAR05	OL884344
		CNU8414	SMBAR05	OL884345
		CNU8415	SMBAR05	OL884346
		CNU8416	SMBAR05	OL884347
		CNU8417	SMBAR05	OL884348
		CNU8418	SMBAR05	OL884349
		CNU8419	SMBAR05	OL884350
		CNU8420	SMBAR05	OL884351
		CNU8421	SMBAR05	OL884228

JN, Jeollanam-do Province, JB, Jeollabuk-do Province, and GN, Gyeongsangnam-do Province

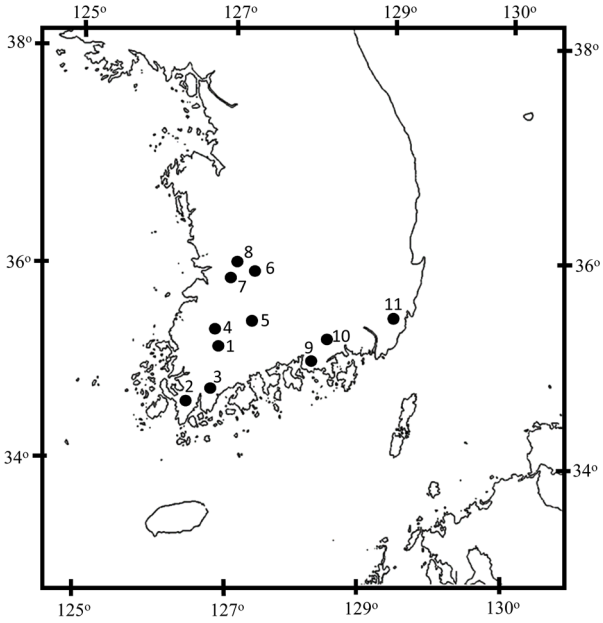

Fig 1. Sampling locations of *Salurnis marginella* in Korea. 1, Gwangju; 2, Haenam; 3, Jangheung; 4, Jangseong; 5, Namwon; 6, Jeonju; 7, Gimje; 8, Iksan; 9, Sacheon; 10, Jinju; and 11, Ulsan.

Typical of Flatidae species, nymphs and adults damage host plants by feeding on phloem sap, secreting wax and honeydew, inhibiting transpiration, and eventually forming sooty mold (Kim *et al.*, 2016).

In China, this species has been reported to damage various plants, including tea, orange, and mulberry (Zhang *et al.*, 2010). In Korea, they have been reported in seven species of four families, such as Korean chestnut *Castanea crenata*, sawtooth *Quercus acutissima*, and jolcham oak *Quercus serrata*, which belong to the Fagaceae family; *Prunus verecunda* (Koidz.) Koehne var. verecunda and *Prunus yedoensis* Matsum., which belong to the Rosaceae family; *Zanthoxylum schinifolium* Siebold & Zucc. of Rutaceae; and *Styrax japonicus* Siebold & Zucc. of Styracaceae (Kim *et al.*, 2016).

Considering the wide host range of Flatidae, including *S. marginella* (Kwon and Huh, 2001; (Kim *et al.*, 2016; Lee and Wilson, 2010), continuous monitoring of the species in terms of range expansion, damage, and host adaption is necessary. Furthermore, estimation of the population genetic characteristics, such as population structure, genetic diversity, and genetic relationships are important for devising control strategy for the invading species (Choi *et al.*, 2018; Jeong *et al.*, 2021; Lee *et al.*, 2021). In the first genetic-perspective study, the full-length mitochondrial genome (mitogenome) of this species has been sequenced (Kim *et al.*, 2021). To date, only four partial sequences of the mitochondrial (mt) genes have been registered in the GenBank, in contrast to other invaded species in Korea whose invasive characteristics were evaluated earlier (Jeong *et al.*, 2021; Lee *et al.*, 2021).

In this study, we sequenced the mt *COI*, which corresponds to the DNA barcoding region, from 124 individuals of *S. marginella* collected from 11 Korean localities to accumulate genetic data and understand their genetic diversity, population structure, and phylogenetic relationships among *S. marginella* haplotypes. Combined with the additional data derived from samples obtained from the original distributional range, the findings of this study will provide a basis for further comprehensive studies on the invasive history of *S. marginella*.

Materials and methods

Sample collection

A total of 124 adults and nymphs of *S. marginella* were collected across 11 South Korean localities from June to September 2019 (Table 1; Fig. 1). Manually or using insect nets, *S. marginella* were collected from nine plants belonging to six families, namely common mulberry *Morus alba* of Moraceae; Korean autumn olive *Elaeagnus umbellata* var. coreana (H. Lév.) of Elaeagnaceae; Japanese wisteria *Wisteria floribunda, Sawtooth* oak *Quercus acutissima*, and lack locust *Robinia pseudoacacia* of Fabaceae; common camellia *Camellia japonica* of Theaceae; Chinese quince *Cydonia sinensis* of Rosaceae; and oriental persimmon *Diospyros kaki* of Ebenaceae. The samples were stored at –70°C immediately after being transported to the laboratory.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from the thoracic tissues using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. The *COI* sequences were amplified using primers designed via alignment of the complete mitochondrial genome sequences of *Geisha distinctissima* (Song and Liang, 2009) and *Metcalfa pruinosa* (Kim *et al.*, 2021). The primer sequences were as follows: Ppyojock-F, 5′–TATCAACAAATCACAAAGACATCGG-3′ for forward direction and Ppyojock-L, 5′–TATACTTCTGGATGACCAAAAAATCA-3′

Table 2. Pairwise comparisons among 12 *COI* haplotypes of *Salurnis marginella*

No.	Haplotype	1	2	3	4	5	6	7	8	9	10	11	12
01.	SMBAR01	-	0.456	0.152	0.456	0.456	0.304	0.608	1.064	0.608	0.760	0.456	0.456
02.	SMBAR02	3	-	0.304	0.608	0.608	0.456	0.76	0.608	0.456	0.304	0.608	0.608
03.	SMBAR03	1	2	-	0.304	0.304	0.152	0.456	0.912	0.456	0.608	0.304	0.304
04.	SMBAR04	3	4	2	-	0.304	0.152	0.456	1.216	0.760	0.912	0.304	0.304
05.	SMBAR05	3	4	2	2	-	0.152	0.456	1.216	0.760	0.912	0.304	0.304
06.	SMBAR06	2	3	1	1	1	-	0.304	1.064	0.608	0.760	0.152	0.152
07.	SMBAR07	4	5	3	3	3	2	-	1.368	0.912	1.064	0.152	0.456
08.	SMBAR08	7	4	6	8	8	7	9	-	1.064	0.304	1.216	1.216
09.	SMBAR09	4	3	3	5	5	4	6	7	-	0.76	0.760	0.760
10.	SMBAR10	5	2	4	6	6	5	7	2	5	-	0.912	0.912
11.	SMBAR11	3	4	2	2	2	1	1	8	5	6	-	0.304
12.	SMBAR12	3	4	2	2	2	1	3	8	5	6	2	-

The numbers above the diagonal are the mean distance values; the numbers below the diagonal are the absolute distance values.

for reverse direction. Using the primer sets, 658 bp of *COI* was amplified. PCR amplification was conducted using AccuPower PCR PreMix (Bioneer, Seoul, Korea) with the following steps: initial denaturation for 5 min at 95°C; followed by 35 cycles of 1 min of denaturation at 95°C, 1 min of annealing at 56°C, and 1 min of extension at 72°C; and a final extension for 7 min at 72°C. The success of PCR amplification was verified by performing electrophoresis using 1 × TAE buffer on 1% agarose gel. PCR amplicons were purified using an AccuPrep PCR Purification Kit (Bioneer). Both strands of the PCR amplicons were cycle-sequenced using an ABI Prism BigDye Terminator ver. 3.1 Cycle Sequencing Kit, and subsequently electrophoresed in each direction on an ABI 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

Sequence analysis

Individual sequences of both strains were aligned using the Clustal Omega program (Sievers *et al.* 2011; http://www.ebi.ac. uk/Tools/msa/clustalo) to obtain a finalized sequence for each individual. Nucleotide sequences were translated based on invertebrate mt DNA to check for the presence of pseudogene sequences; however, none of the sequences contained any pseudogene sequence. When homologous sequences from two individuals differed by at least one nucleotide, the sequences were considered to belong to different haplotypes. Haplotypes were designated to the new sequences in order of their discovery (i.e., SMBAR01, SMBAR02, SMBAR03, SMBAR04, and so forth).

Genetic diversity, population structure, and gene flow

To obtain an estimate of sequence divergence among haplotypes, unordered pairwise comparisons were performed using PAUP ver. 4.0b (Swofford, 2002). Haplotype diversity and nucleotide diversity per population were obtained using Arlequin ver. 3.5 (Excoffier and Lischer, 2010). For phylogenetic analyses using the maximum-likelihood method, 1,000 nonparametric iterations were run with the GTR model using Modeltest ver. 3.7 (Posada and Crandall, 1998) with default parameter settings. A co-familial species, *Poekilloptera phalaenoides* (Unpublished, GenBank Acc. no. MN621004), was included as an outgroup. The generated tree was viewed with FigTree ver. 1.4.2 (http://tree.bio.ed.ac.uk/softw are/figtree).

Population pairwise genetic distances (F_{ST}) were determined and a permutation test of the significant differentiation (1,000 bootstraps) of the pairs of populations was performed following the approach described by Excoffier *et al.* (1992) using Arlequin ver. 3.5 (Excoffier and Lischer, 2010). Pairwise F_{ST} values were used to estimate the per generation migration rate, *Nm* (the product of the effective population size *Ne* and the migration rate *m*), based on the equilibrium relationship [$F_{ST} = 1/(2Nm + 1)$] following the Kimura 2-parameters method (Kimura, 1980).

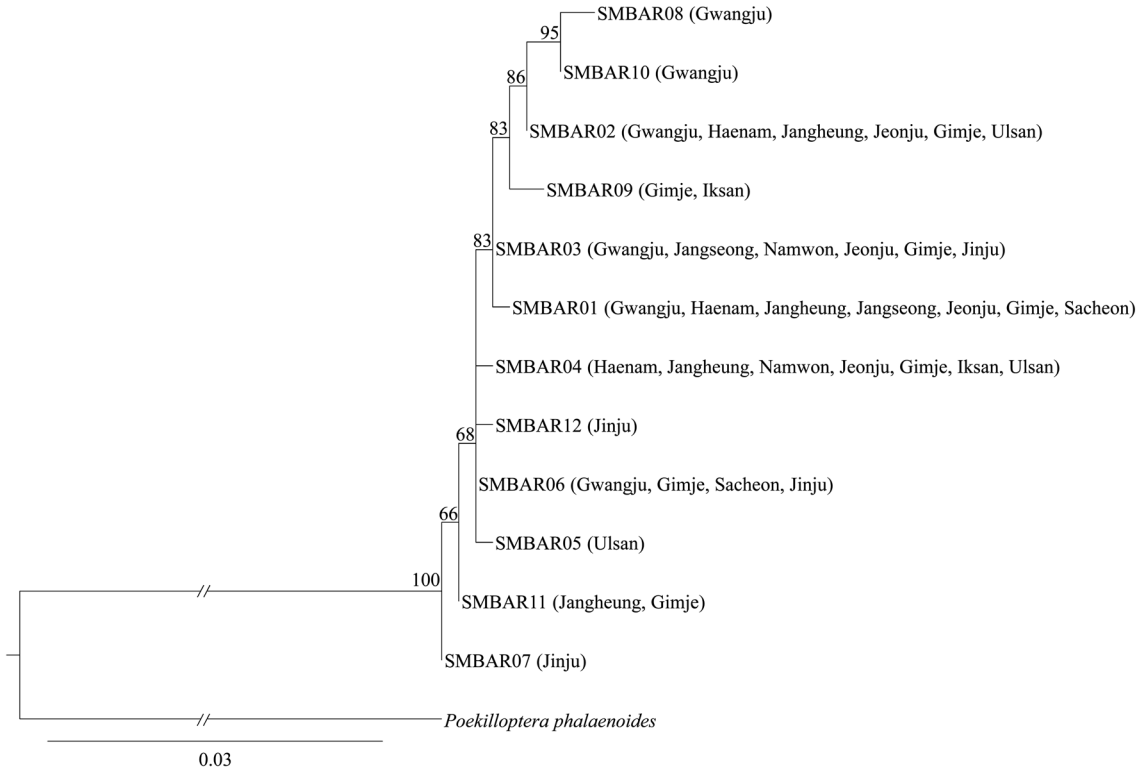

Fig. 2. Maximum-likelihood tree for *Salurnis marginella* haplotypes. Values on the nodes indicate bootstrap percentages of 1,000 pseudoreplicates. Within-parentheses indicate localities where haplotypes were found. The scale bar indicates the number of substitutions per site. *Poekilloptera phalaenoides* was utilized as an outgroup.

These pairwise F_{ST} values were used in principal coordinate analysis (PCoA; Orlóci, 1978) to detect and plot the relationships among populations using GenAlEx ver. 6.5 with default parameters (Peakall and Smouse, 2012). Bayesian Analysis of Population Structure (BAPS) was performed to uncover the distribution of genetic diversity as well as relationships among populations using BAPS ver. 6.5 (Corander and Tang, 2007). The analysis was performed by clustering with a linked locus module and a codon model. In this process, mixture analysis was performed with *K* values ranging from 1 to 20 to identify optimal clusters based on the maximum log marginal likelihood values.

Results

Mitochondrial DNA variability

The analysis of 658-bp *COI* from 124 adult individuals revealed 12 haplotypes (SMBAR01– SMBAR12), with a maximum sequence divergence of 1.368% (9 bp) (Table 2). Twelve haplotypes presented 14 substitutions, of which 11 were transitions (8 A↔G and 3 T↔C) and three were transversions (2 A↔T and 1 T↔G). In terms of distribution, both SMBAR01 and SMBAR04 showed the widest distribution, accounting for 7 of 11 localities, accounting for 15.3% (19 individuals) and 8.9% (11 individuals), respectively, of the samples utilized in this study (Table 3). Similarly, both SMBAR02 and SMBAR03 were found in six localities. However, SMBAR05, SMBAR07, SMBAR08, SMBAR10, and SMBAR12 were found only in one locality each (Table 3). Thus, the distribution of *S. marginella* haplotypes can be summarized as a wide distribution of a few haplotypes and a restricted distribution of the other haplotypes.

Phylogenetic analysis

Phylogenetic analysis using 12 haplotypes of *S. marginella* showed that all haplotypes were well interrelated without any divergent subgroup (Fig. 2). In particular, the comparatively divergent SMBAR05, which provided the largest divergence when it was compared to SMBAR07 (Table 2), was well interrelated to the other haplotypes, forming a monophyletic origin.

Table 3. Locality-based relative frequencies of *COI* haplotypes of *Salurnis marginella*

Haplotype	Locality											
	1. Gwangju (20)	2. Haenam (13)	3. Jangheung (10)	4. Jangseong (9)	5. Namwon (4)	6. Jeonju (6)	7. Gimje (20)	8. Iksan (3)	9. Sacheon (3)	10. Jinju (13)	11. Ulsan (23)	Total (124)
1. SMBAR01	0.100 (2)	0.077 (1)	0.300 (3)	0.667 (6)		0.167 (1)	0.200 (4)		0.667 (2)			15.3% (19)
2. SMBAR02	0.450 (9)	0.846 (11)	0.500 (5)			0.167 (1)	0.050 (1)				0.087 (2)	23.4% (29)
3. SMBAR03	0.100 (2)			0.333 (3)	0.250 (1)	0.333 (2)	0.550 (11)			0.385 (5)		19.4% (24)
4. SMBAR04		0.077 (1)	0.100 (1)		0.750 (3)	0.333 (2)	0.050 (1)	0.667 (2)			0.043 (1)	8.9% (11)
5. SMBAR05											0.870 (20)	16.1% (20)
6. SMBAR06	0.200 (4)						0.050 (1)		0.333 (1)	0.154 (2)		6.5% (8)
7. SMBAR07										0.385 (5)		4.0% (5)
8. SMBAR08	0.050 (1)											0.8% (1)
9. SMBAR09							0.050 (1)	0.333 (1)				1.6% (2)
10. SMBAR10	0.100 (2)											1.6% (2)
11. SMBAR11			0.100 (1)				0.050 (1)					1.6% (2)
12. SMBAR12										0.077 (1)		0.8% (1)

Numbers in parentheses indicate sample size collected from each locality.

Genetic diversity

The within-locality diversity estimates of the haplotype diversity (*H*), maximum sequence divergence, mean number of pairwise differences, and nucleotide diversity (π) are presented in Table 4. In a range of 0~1 for *H*, Jeonju (locality 6) was the highest at 0.8667, followed by Jangheung (locality 3) at 0.7111, whereas Ulsan (locality 11) was the lowest at 0.2451 and the second-lowest was Haenam (locality 2) at 0.2949. In terms of π, Iksan (locality 8), the place where *S. marginella* was first reported, was the highest (0.005066), whereas Ulsan was the lowest (0.001249).

Population structure

Examination of the likelihood scores from 10 replicate runs

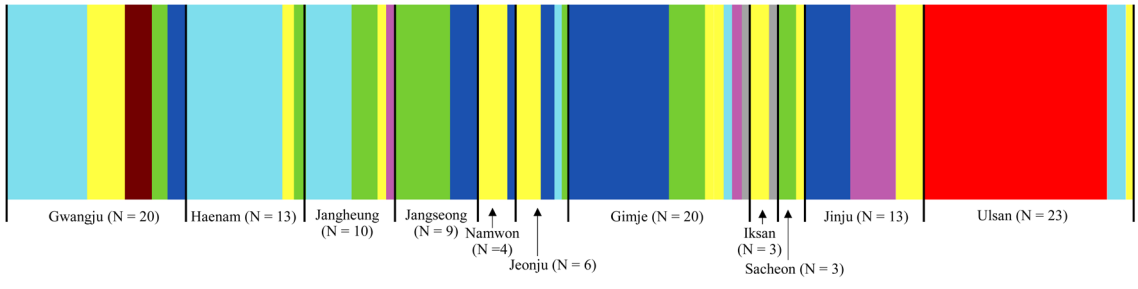

Fig. 3. Bayesian clustering (BAP) analysis of 124 individuals of *Salurnis marginella*. The optimum number of clusters (*K*) was 8.

Table 4. Within-locality diversity estimates of *Salurnis marginella*

Locality	SS[a]	NH[b]	*H*[c]	NP[d]	MSD[e] (%)	MPD[f]	π[g]
1. Gwangju	20	6	0.7632±0.0781	8	1.06	2.273684±1.302596	0.003455±0.002211
2. Haenam	13	3	0.2949±0.1558	5	0.61	1.025641±0.731486	0.001559±0.001250
3. Jangheung	10	4	0.7111±0.1175	6	0.61	2.333333±1.389579	0.003546±0.002388
4. Jangseong	9	2	0.5000±0.1283	1	0.15	0.500000±0.466504	0.000760±0.000804
5. Namwon	4	2	0.5000±0.2652	2	0.30	1.000000±0.829646	0.001520±0.001506
6. Jeonju	6	4	0.8667±0.1291	5	0.61	2.066667±1.338324	0.003141±0.002349
7. Gimje	20	7	0.6789±0.1024	8	0.76	1.294737±0.845489	0.001968±0.001435
8. Iksan	3	2	0.6667±0.3143	5	0.76	3.333333±2.323107	0.005066±0.004403
9. Sacheon	3	2	0.6667±0.3143	2	0.30	1.333333±1.098339	0.002026±0.002082
10. Jinju	13	4	0.7308±0.0787	4	0.46	1.692308±1.057439	0.002572±0.001806
11. Ulsan	23	3	0.2451±0.1126	5	0.61	0.822134±0.610598	0.001249±0.001035

[a]Sample size
[b]Number of haplotype for mitochondrial genes
[c]Haplotype diversity
[d]Number of polymorphic sites
[e]Maximum sequence divergence
[f]Mean number of pairwise differences
[g]Nucleotide diversity

across *K* values from 1 to 10 indicated that the optimal *K* value was 8, suggesting that the *S. marginella* collected from 11 localities were composed of eight haplotype clusters, hereafter referred to as haplogroups (Fig. 3). The assignment results of *K* = 8 showed that each population had two (Jangseong, Namwon, Iksan, and Sacheon) to six haplogroups (Gimje). Most of the haplogroups were found in ≥ 2 localities, except for one haplogroup (dark brown) found in Gwangju as three individuals and another haplogroup (red) found abundantly in Ulsan as 20 individuals.

PCoA among populations was performed to further examine population relationships (Fig. 4). The first and second components

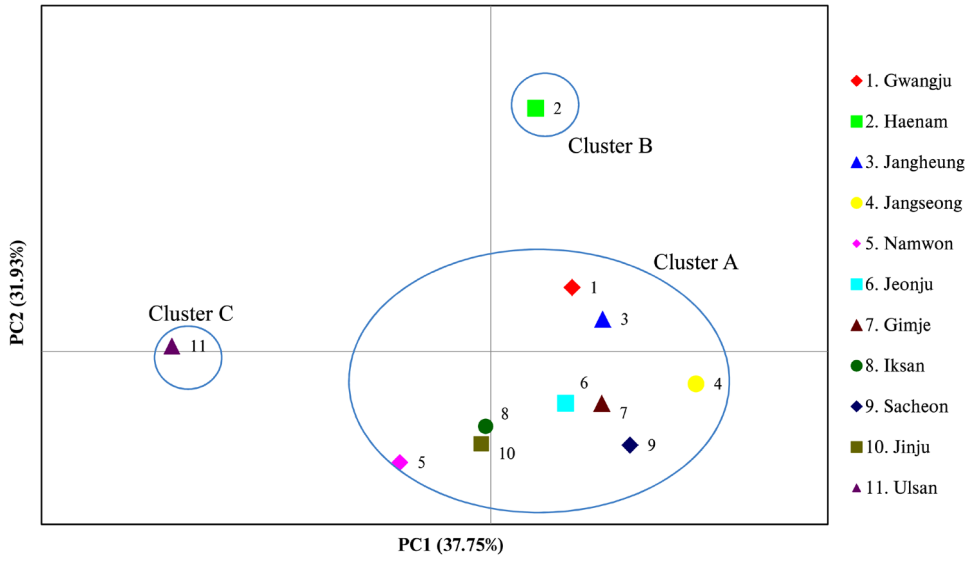

Fig. 4. Results of principal coordinates analysis based on pairwise F_{ST} values. The percentage of variation explained by the first and second principal components (PC1 and PC2, respectively) are indicated.

accounted for 37.75% and 31.93% of the variation, respectively. Overall, the following three clusters were detected: Cluster A, comprising the majority of populations; Cluster B, comprising solely of the Haenam population; and Cluster C, comprising solely of the Ulsan population.

Discussion

Compared with that of insect species in Korea, the sequence divergence of *S. marginella* (1.368%) is not substantial (Kang *et al.*, 2012). However, it is notably higher than that of other invaded species in Korea, such as yellow-legged hornet *Vespa velutina nigrithorax* (Hymenoptera: Vespidae), flatid planthopper *Metcalfa pruinosa* (Hemiptera: Flatidae), and lantern fly *Lycorma delicatula* (Hemiptera: Fulgoridae) (Kim *et al.*, 2013; Jeong *et al.*, 2021; Lee *et al.*, 2021). *V. v. nigrithorax* recorded only a single *COI* haplotype based on 238 individuals collected from 11 Korean and two Japanese localities, *M. pruinosa* recorded three haplotypes with the maximum sequence divergence of 0.46% based on 493 individuals collected from 23 localities, and *L. delicatula* recorded a single haplotype both in *ND2* and *ND6* based on 10 samples collected from 10 localities. In addition, the highest number of haplogroups were detected in *S. marginella* (eight haplogroups) compared with the other invaded species, which had only one to two haplogroups, as analyzed using *COI* (Kim *et al.*, 2013; Jeong *et al.*, 2021; Lee *et al.*, 2021). Thus, the introduction of *S. marginella* in Korea seems to be extensive compared with other invaded species, although the divergence of the species in the native distributional range should be compared before further conclusive inference can be drawn. Furthermore, the sample size in Jangseong, Namwon, Iksan, and Sacheon was relatively small (three to nine individuals), but those populations had two haplogroups each, totaling four out of eight haplogroups (Fig. 3).

Considering that *S. marginella* was first detected in Iksan (Lee *et al.*, 2016), this or immediately closer regions could be ascribed as the first place of entry to Korea, like other invaded species, such as *M. pruinosa* (Lee and Wilson, 2010) and *V. v. nigrithorax* (Kim *et al.*, 2006). However, our current data are not conclusive for such inference due to the limited sample size from this locality (three individuals). Furthermore, invaded species typically show higher genetic diversity at the place of their entry or nearby regions, if no major invasive and biological characteristics perturb the original distributional characteristics (Lee and Wilson, 2010). However, Iksan showed only the 6th highest estimate in *H* among 11 localities, although the π was the highest (Table 4). Therefore, nearby harbors, such as Gunsan and Mokpo, could be possible points of entry, and this speculation should be tested using larger samples and variable markers.

Ulsan, the easternmost locality among our sampling sites, is unique as it showed the lowest diversity estimates in both *H* and π (Table 4), suggesting that this locality was founded with a relatively small number of *S. marginella*. This is particularly likely because 20 of 23 samples collected in Ulsan possessed the identical haplotype, SMBAR05 (Table 3). Furthermore, Ulsan had a unique haplogroup (red) that was not detected in any other localities (Fig. 3), standing alone as an independent cluster (Fig. 4). Collectively, this population may have been established with a somewhat different background, such as a different invasion event or different place of entry to Korea, although the latter is more likely because no other population had this haplogroup. This inference may be further supported by the observation that the Ulsan is the easternmost location among our sampling sites and *S. marginella* was suddenly detected in this locality during a field investigation in 2016, although no occurrence was reported up to 2015 (Lee *et al.*, 2016; Kim *et al.*, 2016). Thus, these field observations, along with the finding of the unique haplogroup (red), suggest that the introduction of *S. marginella* to Korea was an independent event. The Busan Port in Korea (https://busanpa.com), which is located in the southeast region close to Ulsan, is a likely place of entry, considering that this is the largest trading port in the country and handles an enormous quantity of agricultural products transported from diverse countries, including China, which falls in the distributional range of *S. marginella*. Our study focused only on Korean populations and was based on *COI* analysis. Thus, further sample collection in Korea from wider distributional range and analysis using microsatellite DNA or single nucleotide polymorphism are required to further elucidate the invasive dynamics of *S. marginella*, including the origin of the species, places of entry to Korea, and severity of introduction.

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