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Geographic Genetic Contour of a Ground Beetle, *Scarites aterrimus* (Coleoptera: Carabidae) on the Basis of Mitochondrial DNA Sequence

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The *Scarites aterrimus* (Coleoptera: Carabidae), is one of the carabid beetles dwelling exclusively on coastal sandy dunes. Habitat deterioration and equivalent activity have greatly concerned population declines in several species dwelling on the coastal sandy dunes. As a first step to establish long-term conservation strategy, we investigated the nation-wide magnitude and nature of genetic diversity of the species. As a first step, we sequenced a portion of mitochondrial COI gene, corresponding to "DNA Barcode" region (658 bp) from a total of 24 *S. aterrimus* individuals collected over nine sandy dunes belonging to four Korean provinces. The sequence analysis evidenced moderate to low magnitude of sequence diversity compared with other insect species distributed in Korean peninsula (0.152% to 0.912%). The presence of closely related haplotypes and relatively high gene flow estimate collectively suggest that there had been no historical barriers that bolster genetic subdivision. Population decline was postulated on the basis of several missing haplotypes that are well found in the species with a large population size. This interpretation is consistent with field observation of small population size in the coastal sandy dune habitats. The highest genetic diversity estimates were found in the coastal sand dune population of Seogwipo, Jeju Island, justifying a prior attention to the population, in order to sustain overall genetic diversity of the species. Further scrutinized study might be required for further robust conclusion.

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Introduction

As has been known, world biodiversity in fact has greatly been diminished in several levels, such as community, species, geographic populations, and even in the genetic diversity (Soulé, 1986; Wilson, 1992). The main cause for such diminishment includes a massive destruction of habitat, urbanization, contamination and so on. In this regard, biodiversity in Korea also has greatly been exterminated for over the last 50 years and now more than 200 species (including 20 insect species) are endangered.

The coastal dunes also have long been subjected to construction, deterioration, and fragmentation, although these are important habitat for many wildlife including insects such as carabid beetles (Kim, 1980, 2003). In fact, several species of carabid beetles dwelling on coastal sandy beaches are reported to rarely be found and some of them are under the pressure of extinction. Thus, these species are considered to be the potential endangered species in Korea. In this circumstance, it is necessary to take some steps to rescue the carabid beetles for long-term conservation.

The ground beetle, *Scarites aterrimus* (Carabidae: Coleoptera), is one of the carabids well adapted to shore environment in littoral (Kawakami *et al*., 2004). The medium-sized (*e.g*., 20 mm in body length) *S. aterrimus* that occurs from May to September is distributed in sandy beaches in Korea, Japan, and China (Park and Paik, 2001). In Korea and Japan, the great population diminishment has been reported (personal communication, Cho; Kawakami and Sugiura, 2006).

In this study, we, thus, investigated genetic diversity,

Table 1. A list of trapping localities, animal numbers, mitochondrial COI haplotypes of *Scarites aterrimus*

Collecting locality (no. of individuals)	Collection date	Animal number	COI haplotype	Genbank accession number
1. Sand dune of Goraebul, Byeonggok-ri Gyeongsangbuk-do province (3)	2009.07.22	SA3570	BARSA06	JF713794
	2009.07.22	SA3571	BARSA03	JF713795
	2009.07.22	SA3572	BARSA02	JF713796
2. Sand dune of Dukcheon, Dukcheon-ri Gyeongsangbuk-do province (1)	2009.05.24	SA3573	BARSA03	JF713797
3. Sand dune of Songji, Songho-ri Jeollanam-do province (1)	2008.07.22	SA3574	BARSA09	JF713798
4. Sand dune of Buldeung, Tongho-ri Jeollanam-do province (2)	2008.07.22	SA3575	BARSA03	JF713799
	2009.07.23	SA3576	BARSA03	JF713800
5. Sand dune of Namryul, Namryul-ri Jeollanam-do province (3)	2009.05.23	SA3577	BARSA03	JF713801
	2009.05.23	SA3578	BARSA03	JF713802
	2009.05.23	SA3579	BARSA03	JF713803
6. Sand dune of Sohwang, Sohwang-ri Chungcheongnam-do province (6)	2008.07.15	SA3580	BARSA03	JF713804
	2008.07.15	SA3581	BARSA08	JF713805
	2009.05.22	SA3582	BARSA03	JF713806
	2009.05.22	SA3583	BARSA03	JF713807
	2009.05.22	SA3584	BARSA03	JF713808
	2009.05.22	SA3585	BARSA03	JF713809
7. Sand dune of Songseok, Songseok-ri Jeollanam-do province (4)	2009.05.22	SA3587	BARSA01	JF713810
	2009.05.22	SA3589	BARSA03	JF713811
	2009.05.22	SA3590	BARSA03	JF713812
	2009.05.22	SA3591	BARSA10	JF713813
8. Sand dune of Jangpo, Jangpo-ri Chungcheongnam-do province (1)	2008.07.15	SA3592	BARSA03	JF713814
9. Sand dune of Seogwipo, Seogwipo city Jeju-do province (3)	2004.05.18	SA3593	BARSA05	JF7137815
	2004.05.18	SA3594	BARSA07	JF7137816
	2004.05.18	SA3595	BARSA04	JF7137817

geographic variation, and populations genetic structure of the species collected from several sandy beaches of Korean coasts (East Sea, West Sea, and South Sea) and a remote island Jeju to accumulate fundamental information of the species for conservation purpose. As a first step, we sequenced a partial mitochondrial cytochrome *b* oxidase subunit I (COI) gene. This molecular maker has been utilized to illustrate several geographic genetic perspectives of insects occurring in Korean peninsula (e.g., Jeong *et al*., 2009; Kim *et al*. 2009).

Materials and Methods

Sampling

Adult *S. aterrimus* were sampled from nine Korean localities in 2008 and 2009. Sampling locality, number of individuals, date of collection, and GenBank accession numbers for the individuals of each population are provided in Table 1, and the locality map is shown in Fig. 1. The different sampling size may reflect the difference in population size at each locality, considering that similar effort for sample collection was made. The samples were deposited in 70% alcohol until being used in molecular analysis.

DNA extraction, primer, PCR, and sequencing

The total DNA was extracted using the Wizard Genomic DNA Purification Kit, in accordance with the manufacturer's instructions (Promega, USA). For the amplification of a portion of mitochondrial COI gene, corresponding to "DNA Barcode" region being utilized for global animal identification (Hebert *et al*., 2003) a pair of primer designed from Folmer *et al*. (1994) was applied, but not always successful. Thus, we designed another forward primer, named COLCOIF1, 5'-AAACTAW-TARCCTTCAAAG-3' from the alignment of several full-length mitochondrial genomes of Coleoptera. This primer

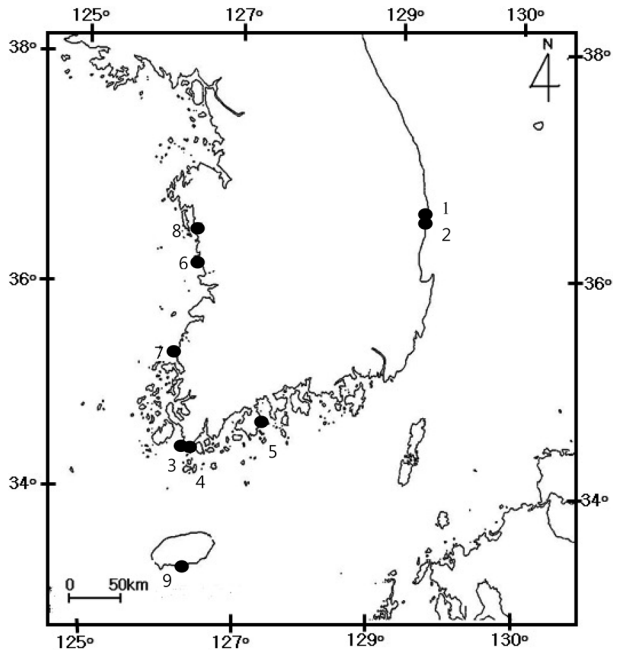

Fig. 1. Sampling location of *Scarites aterrimus* in Korea. General locality names are as follows: 1, Goraebul, Gyeongsangbukdo; 2, Duckcheon, Gyeongsangbukdo; 3, Songji, Jeollanamdo; 4, Buldeung, Jeollanamdo; 5, Namryul, Jeollanamdo; 6, Sohwang, Cungcheongnamdo; 7, Songseok, Jeollanamdo; 8, Jangpo, Chungcheongnamdo; and 9, Seowipo, Jejudo.

Table 2. List of primers used to amplify and sequence the COI sequences of *Scarites aterrimus*

Fragment	Primer name	Sequence (5` - 3`)
F1	COLCOIF1	AAACTAWTARCCT-TCAAAG
	ST-INTER-R1	GATGATACAC-CAGCTAAATG
F2	ST-INTER-F1	GGGAGCACCAGACAT-AGC
	HCO2198	TAAACTTCAGGGTGAC-CAAAAATCA

amplified the "DNA Barcode" region in combination with the reverse primer, named HCO2198, 5'-TAAACTTCAGGGT-GACCAAAAAATAC-3', which was designed from Folmer *et al*. (1994). Nevertheless, the efficiency of the amplification was still not perfect enough. Therefore, we additionally designed two overlapping internal primers with different directions after several sequences of COI gene from *S. aterrimus* individuals were obtained. Each of these internal primers was in combination successfully used with each of the universal primers reported in Folmer *et al*. (1994). The primers information is listed in Table 2. After an initial denaturation step at 94°C for 7 min, a 35-cycle amplification (94°C for 1 min, 48~53°C for 1 min, and 72°C for 1 min) was conducted. The final extension step was continued for 7 min at 72°C. To confirm the successful DNA amplification, electrophoresis was carried out using 0.5× TAE buffer on 0.5% agarose gel. The PCR product was then purified using PCR purification Kit (QIAGEN, Germany). Each fragment of the gene was cloned into a pGEM-T Easy vector (Promega). For the cloning process, XL1-Blue competent cells (Stratagene, USA) were transformed with the ligated DNA, and the resultant plasmid DNA was isolated using a Wizard Plus SV Minipreps DNA Purification System (Promega). DNA sequencing was conducted using the ABI PRISM®-BigDye® Terminator ver. 3.1 Cycle Sequencing Kit with an ABI 377 Genetic Analyzer (PE Applied Biosystems, USA). All products were sequenced from both strands.

Sequence and phylogenetic analyses

Sequence alignment was performed using CLUSTAL X programs (ver. 1.8; Thompson *et al*., 1997). When homologous sequences from two individuals differed by ≥ one nucleotide base, the sequences were considered as different haplotypes. Haplotype designations were applied to new sequences as they were discovered (*i.e.*, BARSA01, BARSA02, BARSA03 and so forth).

Phylogenetic analysis was performed by maximum-parsimony (MP) method (Fitch, 1971) using PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver. 4.0b10 (Swofford, 2002). To root trees, the within-familial species of Carabidae, *Loricera pilicornis*, was utilized as an outgroup (GenBank accession number FN868618). The analysis was performed using an equal weighting of transitions and transversions by heuristic search. The reliability of the trees was tested by 1,000 iterations of bootstrapping (Felsenstein, 1985). With intraspecific mtDNA sequence data it often happens that parsimony analyses provide limited resolution because of polytomies, possibly caused by back mutations and parallel mutations. One solution, which we employed, is to prepare one-step median networks, which provide insight into probable relationships among closely related lineages (Bandelt *et al*., 1995).

Genetic diversity indices

For the genetic diversity indices and subsequent population genetic analyses, the populations with more than one haplotype were included in the analyses. Thus, four populations (localities 1, 3, 4, and 6) were selected by this criterion and the remaining populations that were represented either by single individual or single haplotype were excluded from the analyses. Genetic diversity estimates, such as haplotype diversity and nucleotide diver-

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                                        30                                  60
BARSA01  AACTCTATAT  TTTATTTTTG  GAGCATGATC  AGGAATAGTA  GGGACTTCCT  TAAGTATATT
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ..........  ..........  ..........  ..........
BARSA05  ..........  ..........  ..........  ...G......  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  .......A..  ..........  ..........  ..........  ..........
BARSA08  ..........  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                        90                                 120
BARSA01  AATTCGAGCA  GAATTAGGAA  ATCCTGGATC  ATTAATTGGA  GATGATCAGA  TTTATAATGT
BARSA02  ..........  ..........  ..........  ..........  ........A.  ..........
BARSA03  ..........  ..........  ..........  ..........  ........A.  ..........
BARSA04  ..........  ..........  ..........  ...G......  ........A.  ..........
BARSA05  ..........  ..........  ..........  ..........  ........A.  ..........
BARSA06  ..........  ..........  ..........  ..........  ........A.  ..........
BARSA07  ..........  ..........  ..........  ..........  ...A....A.  ..........
BARSA08  ..........  ..........  ..........  ..........  ........A.  ..........
BARSA09  ..........  ..........  ..........  ..........  ........A.  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                       150                                 180
BARSA01  TATTGTTACT  GCCCATGCTT  TTATTATAAT  TTTTTTCATG  GTTATACCAA  TCATAATTGG
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ..........  ..........  ..........  ..........
BARSA05  ..........  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  ..........  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                       210                                 240
BARSA01  AGGATTTGGA  AATTGACTAG  TTCCTTTGAT  ATTGGGAGCA  CCAGACATAG  CTTTCCCACG
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  .......C..  ..........  ..........  ..........  ..........
BARSA05  ..........  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  .....C....  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                       270                                 300
BARSA01  AATAAATAAT  ATAAGTTTTT  GACTTTTACC  CCCTTCTTTA  ACTCTCCTTC  TAATGAGAAG
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ........T.  ..........  ..........  ..........
BARSA05  .........G  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  ..........  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
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Fig. 2. Sequencing alignment of ten haplotypes (designated as BARSA01 ~ BARSA10) obtained from 658-bp of COI gene sequence from *Scarites aterrimus*. Only nucleotide positions that differ from haplotype BARSA01 are indicated.

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                                        330                                     360
BARSA01  TTTAGTGGAA  AGAGGAGCCG  GAACAGGTTG  AACAGTTTAC  CCCCCCCTTT  CATCTGGAAT
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ..........  ..........  ..........  ..........
BARSA05  ..........  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  ...G......  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                        390                                     420
BARSA01  TGCCCATAGA  GGAGCATCTG  TAGATTTAGC  AATTTTTAGT  CTTCATTTAG  CTGGTGTATC
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ..........  ..........  ..........  ..........
BARSA05  .........G  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  ..........  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                        450                                     480
BARSA01  ATCAATTCTA  GGGGCAGTAA  ATTTTATTAC  TACAATTATT  AATATACGAT  CTGTAGGAAT
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA04  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA05  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA06  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA07  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA08  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA09  ..........  ..A.......  ..........  ..........  ..........  ..........
BARSA10  ..........  ..A.......  ..........  ..........  ..........  ..........
                                        510                                     540
BARSA01  TACTTTAGAA  CGAATACCCC  TATTTGTTTG  ATCTGTAGGA  ATTACTGCTT  TATTACTTTT
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ..........  ..........  ..........  ..........
BARSA05  ..........  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  ..........  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
                                        570                                     600
BARSA01  ATTATCCCTA  CCTGTTTTAG  CCGGAGCTAT  TACTATATTA  CTAACTGATC  GAAATTTAAA
BARSA02  ..........  ..........  ..........  ..........  ..........  ..........
BARSA03  ..........  ..........  ..........  ..........  ..........  ..........
BARSA04  ..........  ..........  ..........  ..........  ..........  ..........
BARSA05  ..........  ..........  ..........  ..........  ..........  ..........
BARSA06  ..........  ..........  ..........  ..........  ..........  ..........
BARSA07  ..........  ..........  ..........  ..........  ..........  ..........
BARSA08  ..........  ..........  ..........  ..........  ..........  ..........
BARSA09  ..........  ..........  ..........  ..........  ..........  ..........
BARSA10  ..........  ..........  ..........  ..........  ..........  ..........
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Fig. 2. continued.

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                                                630                                   658
BARSA01  TACTTCATTT  TTTGATCCGG  CAGGAGGGGG  AGATCCAATT  CTTTATCAAC  ATTTATTT
BARSA02  ..........  ..........  ..........  ..........  ..........  ........
BARSA03  ..........  ..........  ..........  ..........  ..........  ........
BARSA04  ..........  ..........  ..........  ..........  ..........  ........
BARSA05  ..........  ..........  ..........  ..........  ..........  ........
BARSA06  ..........  ....T.....  ..........  ..........  ..........  ........
BARSA07  ..........  ..........  ..........  ..........  ..........  ........
BARSA08  ..........  ..........  ..........  ..........  ..........  ........
BARSA09  ..........  ..........  .......A..  ..........  ..........  ........
BARSA10  ..........  ..........  ....G.....  ..........  ..........  ........
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Fig. 2. continued.

sity within each locality were obtained using Arlequin ver. 3.0 (Excoffier *et al*., 2005). On the other hand, maximum sequence divergence within population was obtained by extracting the estimate of unrooted pairwise distance within each population from PAUP (Swofford, 2002).

Genetic distance and migration estimate

Genetic distance and migration rate were estimated from mitochondrial DNA sequences and subroutines in the Arlequin ver. 3.0 (Excoffier *et al*., 2005). Population pairwise genetic distance (F_{ST}) and a permutation test of the significant differentiation of the pairs of localities (1,000 bootstraps) were obtained following the approach described in Excoffier *et al*. (1992) and the distance between DNA sequences were calculated by the Kimura 2-parameters method (Kimura, 1980). Pairwise F_{ST} values were used to estimate per generation migration rate, *Nm* (the product of the effective population size *Ne* and migration rate, *m*) based upon the equilibrium relationship: $F_{ST} = 1/(2Nm + 1)$.

Genetic structure

Genetic relationships among populations and sets of populations were assessed by the Holsinger and Mason-Gamer (H-MG) method (1996). A detailed rationale of this method is described in the original study of Holsinger and Mason-Gamer (1996) and other reports, which utilized this method (Kim *et al*., 1998). Unlike other variance analyses, this approach generated the hierarchical relationships of the groups without specifying the hierarchical structure of the populations before the analysis (Holsinger and Mason-Gamer, 1996). Therefore, any structure present in the dataset emerged naturally.

Finally, to determine whether any isolation-by-distance (IBD) effect is present in *S. aterrimus* populations, matrices of genetic distance data [$F_{ST}/(1-F_{ST})$] and the logarithms of geographical distance data (*ln* km) were constructed among available populations. These matrices were then analyzed to determine their degree of correlation via a Mantel test, with significance tests conducted over 10,000 randomizations (Mantel, 1967). The analysis was conducted using the Isolation-By-Distance software package, with the negative genetic distance set to zero (Bohonak, 2002).

Results and Discussion

Genetic diversity

A total of ten haplotypes (BARSA01 – BARSA10) was obtained by sequencing 658-bp of COI gene from 24 individuals of *S. aterrimus* (Fig. 2). Sequence alignment revealed 15 variable nucleotides: 12 transitions (three T→C and nine G→A) and three transversions (two T→A and

Table 3. Type of substitution in the COI sequences of *Scarites aterrimus*

Nucleotide position	Nucleotide substitution	Amino acid substitution
18	T A	TTT (Phe) TAT (Tyr)
34	A G	-
94	A G	-
104	G A	GAT (Asp) AAT (Asn)
109	G A	-
186	T C	TTT (Phe) TCT (Ser)
198	T C	CTA (Leu) CCA (Pro)
250	T G	AAT (Asp) AAG (Lys)
269	C T	CCC (Pro) TCC (Ser)
304	A G	-
370	A G	-
433	G A	-
615	A T	GAT (Asp) GTT (Val)
625	A G	-
628	G A	-

-, no amino acid substitution.

Table 4. Pairwise comparisons among ten haplotypes obtained from COI gene of *Scarites aterrimus*

	1	2	3	4	5	6	7	8	9	10	11
1. BARSA01	-	0.00152	0.00304	0.0076	0.0076	0.00456	0.00608	0.00608	0.00456	0.00304	0.15957
2. BARSA02	1	-	0.00152	0.00608	0.00608	0.00304	0.00456	0.00456	0.00304	0.00456	0.15805
3. BARSA03	2	1	-	0.00456	0.00456	0.00152	0.00304	0.00304	0.00152	0.00304	0.15653
4. BARSA04	5	4	3	-	0.00912	0.00608	0.0076	0.0076	0.00608	0.0076	0.15957
5. BARSA05	5	4	3	6	-	0.00608	0.0076	0.0076	0.00608	0.0076	0.16109
6. BARSA06	3	2	1	4	4	-	0.00456	0.00456	0.00304	0.00456	0.15805
7. BARSA07	4	3	2	5	5	3	-	0.00608	0.00456	0.00608	0.15957
8. BARSA08	4	3	2	5	5	3	4	-	0.00456	0.00608	0.15653
9. BARSA09	3	2	1	4	4	2	3	3	-	0.00456	0.15502
10. BARSA10	2	3	2	5	5	3	4	4	3	-	0.15957
11. *Loricera pilicornis*	105	104	103	105	106	104	105	103	102	105	-

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

one T→G) (Table 3). These caused seven amino acid replacements as shown in Table 3.

Pairwise comparison between pairs of haplotypes was performed to know about the divergence and relationships among haplotypes (Table 4). The divergence among ten haplotypes ranged from 0.152% to 0.912% (one ~ six nucleotides), indicating moderate to low sequence divergence in this species. In other similar studies, it showed approximately ≤ 1.0% in a diverse taxonomic group, where homologous region of mitochondrial genome was utilized (Bae *et al*., 2001; Jeong *et al*., 2009; Kim *et al*., 2000; Kim *et al*., 2007; Kim *et al*., 2008; Kim *et al*., 2009; Li *et al*., 2006). Thus, the magnitude of sequence divergence of the *S. aterrimus* shows moderate to low compared with other insect species.

With regard to distribution BARSA03 showed the widest distribution, accounting for seven of nine localities, composed of 62.5% (15 of 24) of samples utilized in this study (Table 1). Except for this haplotype the others are distributed each in one locality represented as a single individual (Table 1). Thus, distribution of *S. aterrimus* haplotypes can be summarized as a restricted local distribution in most haplotypes, except for one haplotype.

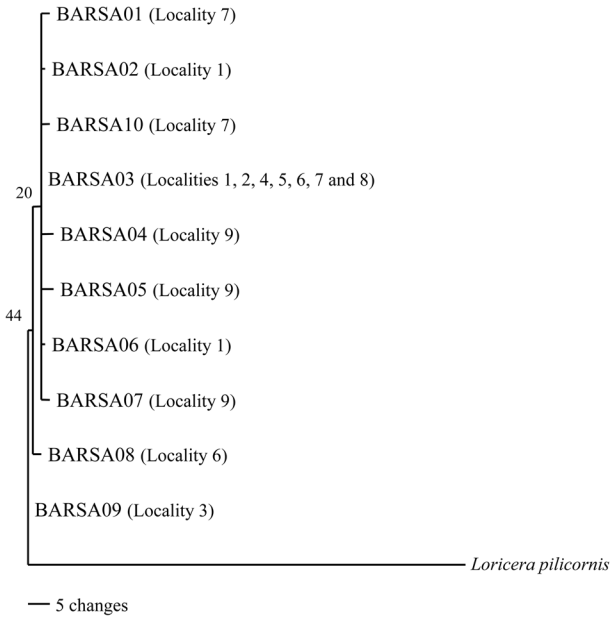

Fig. 3. Phylogenetic analysis of ten haplotypes obtained from mitochondrial COI gene sequence of the *Scarites aterrimus*. The tree was acquired via the MP method incorporated in PAUP (Phylogenetic Analysis Using Parsimony and Other) ver. 4.0b10 software (Swofford, 2002). *Loricera pilicornis* was used as an outgroup in order to root tree. The numbers on the branches represent bootstrap values of 1,000 replications.

Phylogenetic and network analyses

Phylogenetic analysis was investigated to know evolutionary relationships among haplotypes and to detect any discernable group in connection with geographic distribution (Fig. 3). PAUP analysis by maximum parsimony method showed that all haplotypes were weakly associated or unresolved, with relatively low node support (≤ 44%). The network analysis showed that several haplotypes were very closely related to BARSA03 (Fig. 4), evidencing "star phylogeny". Both analyses suggest that *S. aterrimus* haplotypes do not have phylogenetically distinct or divergent haplotype or haplotype group and this, in turn, suggests that the species occurring in Korean peninsula did not experience historical biogeographic barriers that bolster genetic subdivision.

According to Neigel and Avise (1993), the variance of geographic distribution of a mitochondrial DNA lineage is expected be proportional to its age under a simple isolation by distance model. If this theory is applied to *S. ater-*

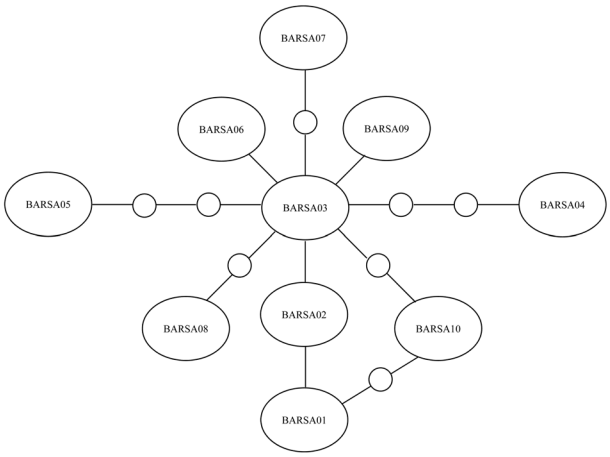

Fig. 4. Parsimonious one-step median networks analysis among ten mitochondrial COI haplotypes of *Scarites aterrimus*. Each bar represents a single nucleotide difference from the neighboring haplotype. The empty circles indicate missing haplotypes that are not found in this study.

rimus BARSA03 that was found most widely (seven among nine localities with 62.5% of frequency) seems to be ancestral, and the remaining haplotypes (BARSA01 ~ BARSA02 and BARSA04 ~ BARSA10) found in a single locality might be ones derived from the BARSA03 (Fig. 4). With the consideration of this aspect, it is noteworthy to mention that several haplotypes that are immediately close to BARSA03 were not found in this study, except for three haplotypes, BARSA02, BARSA06, and BARSA09 (Fig. 4). This result indicates that several individuals possessing those missing haplotypes may have not been collected in this study. However, considering that we were unable to collect enough individuals from several localities even with similar trapping effort it is highly likely that those individuals possessing the undiscovered haplotypes may have already been extinct after they have been evolved from the individuals possessing BARSA03. This explanation is plausible in that species that forms a large population size often shows the "star phylogeny", filled with immediately close haplotypes. For example, the mason bee, *Osmia cornifrons*, has shown a "star phylogeny" fully filled with seven immediately close haplotypes with the centered one that are found most widely and abundantly (Kim *et al*., 2008). Identically, the bumble bee, *Bombus ardens*, has also shown a "star phylogeny" fully filled with seven immediately close haplotypes (Kim *et al*., 2009). In fact, Kim (2003) has investigated species and population diversity of insects dwelling on sand dune habitats in Korean peninsula for 24 years and found only 11 individuals of *S. aterrimus* from four among 16 sand dune areas. This field observation strongly supports a small population of the species and is consistent with the network data

Genetic diversity indices

The within-locality diversity estimates in terms of haplotype diversity (*H*), maximum sequence divergence (MSD), mean number of pairwise differences (MPD), and nucleotide diversity (π) are presented in Table 5. In a range of 0

Table 5. Within-locality diversity estimates of *Scarites aterrimus*

Locality	SS[a]	NH[b]	*H*[c]	NP[d]	MSD[e] (%)	MPD[f]	π[g]
1. Goraebul	3	3	1.0000	2	0.304	1.333333	0.002026
2. Dukcheon	1	1	1.0000	0	-	-	-
3. Songji	1	1	1.0000	0	-	-	-
4. Buldeung	2	1	0.0000	0	-	-	-
5. Namryul	3	1	0.0000	0	-	-	-
6. Sohwang	6	2	0.3333	2	0.304	0.666667	0.001013
7. Songseok	4	3	0.8333	3	0.304	1.666667	0.002533
8. Jangpo	1	1	1.0000	0	-	-	-
9. Seogwipo	3	3	1.0000	8	0.912	5.333333	0.008105

[a]Sample size
[b]Number of haplotypes
[c]Haplotype diversity
[d]Number of polymorphic sites
[e]Maximum sequence divergence
[f]Mean number of pairwise differences
[g]Nucleotide diversity
-, unavailable.

~ 1 in *H*, the sand dunes of Seogwipo (locality 9) and that of Goraebul (locality 1) was highest as 1. In terms of π, Seogwipo (locality 9) was highest (π = 0.008105) and the estimates of Goraebul (locality 1; π = 0.002026) and Songseok (locality 7; π = 0.002533) were as low as nearly one quarter of that obtained from Seogwipo (locality 9). Taking these diversity estimates into consideration, the populations in Jeju Island (locality 9) is highest, suggesting that this area supports a larger population than any other regions in Korean peninsula for *S. aterrimus*. For the long-term conservation purpose, this region can be served as a genetic source population to rescue others, which show lower genetic diversity. However, for further decisive conclusion, more scrutinized sampling and molecular analysis involving further rapidly evolving gene may be required.

Gene flow and population structure

Genetic distance (F_{ST}) and per-generation migration rates (*Nm*) between pairs of populations are shown in Table 6. Test of statistical significance of pairwise F_{ST} estimates has shown that only population pair, locality 6 (Sohwang) and locality 9 (Seogwipo), was statistically significant ($p < 0.05$), but other pairs of populations, instead, has shown substantial female migration (*Nm* = 2.43902 ~ infinite). The hierarchical relationships among localities indicated that only locality 6 (Sohwang) has statistically differentiated from the group composed of locality 1 (Goraebul) and locality 9 (Seogwipo), but, in large, these groups are not statistically differentiated from the most basal locality, Songseok (locality 7) (Fig. 5). The results of the Mantel test (10,000 randomizations) for the detection of IBD provided an *r* (correlation coefficient) value of 0.776 without statistical significance ($p = 0.89$), indicating no positive correlation between geographic distance and genetic distance (Fig. 6). This result can be interpreted as a result of gene flow among *S. aterrimus* populations in Korean peninsula. Collectively, most *S. aterrimus* populations in Korean peninsula do not show genetic structure and are not genetically differentiated, although some divergence of Sohwang population (locality 6) is noteworthy.

Table 6. Fixation indices (F_{ST}) and migration rate (*Nm*) between pairs of populations of *Scarites aterrimus*

	6	7	9
1. Goraebul	F_{ST} = 0.06897 *Nm* = 6.75000	F_{ST} = -0.01099 *Nm* = inf	F_{ST} = 0.00000 *Nm* = 3752999
6. Sohwang		F_{ST} = 0.17012 *Nm* = 2.43902	F_{ST} = 0.18182* *Nm* = 2.25000
7. Songseock			F_{ST} = 0.10048 *Nm* = 4.47619
9. Seogwipo			

*$p < 0.05$.
inf, infinite.

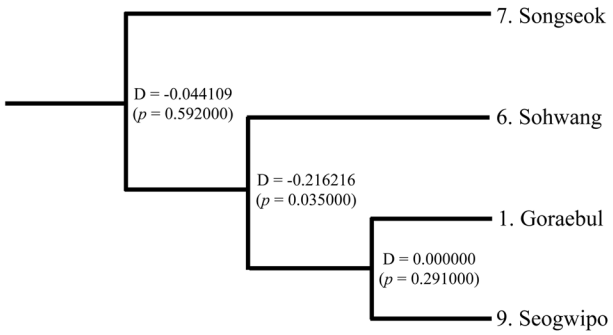

Fig. 5. Hierarchical relationships among localities analyzed using Holsinger and Mason-Gamer's method (1996). The D value is the distance between its two daughter nodes and the *p* value is the significance of differentiation (based on 10,000 random resamplings).

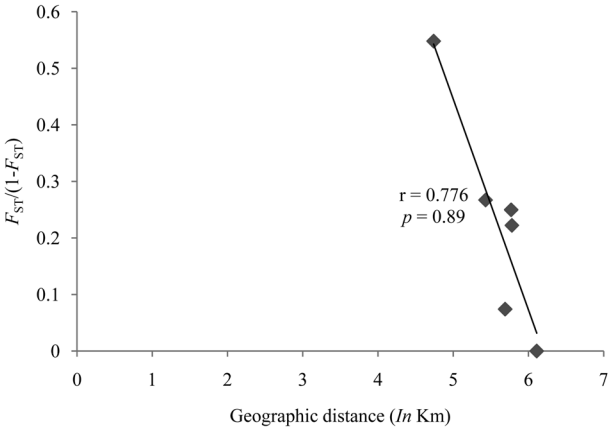

Fig. 6. Scatter plots of genetic distance vs. geographical distance (*ln*) for pairwise *Scarites aterrimus* population comparisons based on COI gene sequence.

Summarized, we collected a total of 24 individuals of *S. aterrimus* from nine Korean localities and sequenced 658 bp of the mitochondrial COI gene. The sequence analysis of *S. aterrimus* provided moderate to low genetic diversity compared with other insect species occurring in Korean peninsula. In terms of geography, Seogwipo population (locality 9) has shown the highest genetic diversity estimates, justifying a prior attention to sustain overall genetic diversity of the species. Considering the result of gene flow and hierarchical population structure, it appears that the geographic populations of *S. aterrimus* evidence a high ratio of gene flow, without long-term zoogeographic barriers. The haplotype network data that provide several undiscovered haplotypes and field observation, such as

rarity of the species in all sand dunes collectively suggest that the species may have been subjected to severe population decline. For further decisive conclusion, more scrutinized sampling and analysis with further rapidly evolving gene may be required.

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