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Three different genetic lineages of the jewel beetle *Chrysochroa fulgidissima* (Buprestidae; Chrysochroinae) inferred from mitochondrial COI gene

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Abstract

The phylogenetic analysis was carried out to find out the validity of *Chrysochroa coreana* as a new species. The insect specimens were collected at Kaohsiung, Taiwan and Shizuoka, Japan. Partial region (532 bp) of COI was amplified and sequenced. The sequences were aligned and then analyzed. Based on the Kimura-2-parameter method, we calculated genetic distances among them. It indicated that the Korean individual of *C. fulgidissima* was closely related to Taiwan one with relatively low genetic distance (0.083). On the other hand, the Japanese individual was remotely related with those of Korean (0.192) and Taiwan (0.183) individuals. To clarify if the populations of *C. fulgidissima* from Korea, Taiwan, and Japan are different at the level(s) of subspecies, species, or genus, it is necessary that more samples of the members of the family Buprestidae should be collected and genetically analyzed.

Key words: *Chrysochroa fulgidissima*, genetic lineages, jewel beetle, phylogeny, subspecies

INTRODUCTION

The Korean jewel beetle, *Chrysochroa f. fulgidissima* is one of the most beautiful insect species living in Korean peninsula. It is registered as an endangered species by the Ministry of Environment of Korea and has been protected as a natural monument (no. 496) designated in 2008. Han et al. (2012) reported this insect as a new species, *C. coreana*, based upon the phylogenetic and morphological characteristics. However, this conclusion was purely drawn on the taxonomic basis, and the biological assessment on the possibility of reproduction among these species is unknown.

The ecological study carried out by Kwon (2013) resulted in acquiring several specimens of *C. fulgidissima* in Korean peninsula. The pre-acquired specimens of *C. fulgidissima* from Taiwan and Japan made it possible to carry out the similar study. In this paper, *C. coreana* (Han et al. 2012) was regarded as *C. fulgidissima* for the comparison among the samples from Taiwan and Japan. The phylogenetic analysis was carried out to find out the validity of *C. coreana* as a new species.

MATERIALS AND METHODS

Sample collection and total cellular DNA extraction

The jewel beetle *C. fulgidissima* examined in this study belongs to the family Buprestidae (the order Coleoptera). The insect specimens were collected at Wan-do, Ko-

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rea, Kaohsiung, Taiwan and Shizuoka, Japan. The genus *Chrysochroa* members are widespread in the Southeast Asian nations. Total cellular DNA was extracted from the tissue pieces of the sample using the DNeasy Blood & Tissue kit (QIAGEN, Valencia, CA, USA) by following the manufacturer's protocol.

PCR amplification and sequencing

The partial cytochrome C oxidase I gene (COI) which is 532 bp in length was amplified using a pair of primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al. 1994). PCR was performed in a volume of 20 μL containing 1 pg to 1 μg DNA, 2.5 mM of each dNTP, 10 pM of each primers, and 2.5 unit of DNA polymerase with the reaction buffer (iNtRON Biotechnnology, Sungnam, Korea); the cycle setting include a cycle of 5 min at 94℃ for initial denaturation, 34 cycles of 30 s denaturation at 94℃, 30 s annealing at 63℃ and 30 s extension at 72℃; and single extension cycle of 72℃ for 5 min. The PCR products were mixed with ExoSAP-IT endonuclease (USB, Santa Clara, CA, USA) and incubated for 15 min at 37℃ to remove any unused primers and nucleotides. The PCR fragments of the purified were sequenced in both directions with an ABI PRISM BigDye terminator system (Applied Biosystems, Foster City, CA, USA). The reactants were analyzed on an ABI3700 model automatic sequencer (GenoTech Co., Daejeon, Korea).

Sequence analyses

COI sequences were identified by using BLAST searches (www.ncbi.nlm.nih.gov/BLAST/) and edited by BioEdit software (Hall 1999). In addition to the BLAST search, identification of COI was conducted by aligning with that of the previously published *C. fulgidissima* mitochondrial genome sequences (NC_012765; Hong et al. 2009). For the following genetic and phylogenetic analyses, we made a multiple alignment of COI from those of the members of Elateriformia and Bostrichiformia Infraroders retrieved from GenBank (Table 1).

Genetic distances based on the Kimura-2-parameter method were compared across the examined species listed in Table 1 using MEGA ver. 4.0 (Tamura et al. 2007). The neighbor joining method (NJ) implemented in MEGA ver. 4.0 (Tamura et al. 2007), maximum likelihood method (ML) with PhyML ver. 3.0 (Guindon and Gascuel 2003), and Bayesian inference (BI) method with MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001) were employed to resolve their relationships. The bootstrapping values representing for node confidence were obtained with 1,000 bootstrapping replicates in the NJ and ML analyses. In BI, Bayesian posterior probability (BPP) was presented for the node confidence values. The BI analysis was carried out with the following options: 1,000,000 generations, 4 chains and a burn-in step of the 1,000.

RESULTS AND DISCUSSION

Two individuals of the jewel beetle *C. fulgidissima* were collected from Kaohsiung, Taiwan and Shizuoka, Japan, respectively. Partial region (532 bp) of COI was amplified and sequenced. The sequences were aligned and then analyzed. Out of 532 nucleotides, 106 were variable sites (Fig. 1). Based on the Kimura-2-parameter method, we calculated genetic distances among them (Table 2).

It indicated that the Korean individual of *C. fulgidissima* was closely related to Taiwan one with relatively low ge-

Table1. List of used for comparisons of mitochondrial COI gene sequence

Infra order	Super family	Species	Accession No.	Reference
Elateriformia	Buprestoidea	*Chrysochroa fulgidissima* (Korea)	NC-012765	Hong et al. 2009
		Chrysochroa fulgidissima (Taiwan)		Present
		Chrysochroa fulgidissima (Japan)		Present
		Dicerca divaricata	AY165645	Hebert et al. 2003
		Acmaeodera sp.	NC-013580	Unpublished
	Elateroidea	*Chauliognathus opacus*	NC-013576	Unpublished
		Pyrophorus divergens	NC-009964	Arnoldi et al. 2007
		Pyrocoelia rufa	NC-003970	Bae et al. 2004
		Rhagophthalmus lufengensis	NC-010969	Li et al. 2007
		Rhagophthalmus ohbai	NC-010964	Li et al. 2007
	Scirtoidea	*Cyphon* sp.	NC-011320	Sheffield et al. 2008
Bostrichiformia	Bostrichoidea	*Apatides fortis*	NC-013582	Sheffield et al. 2009

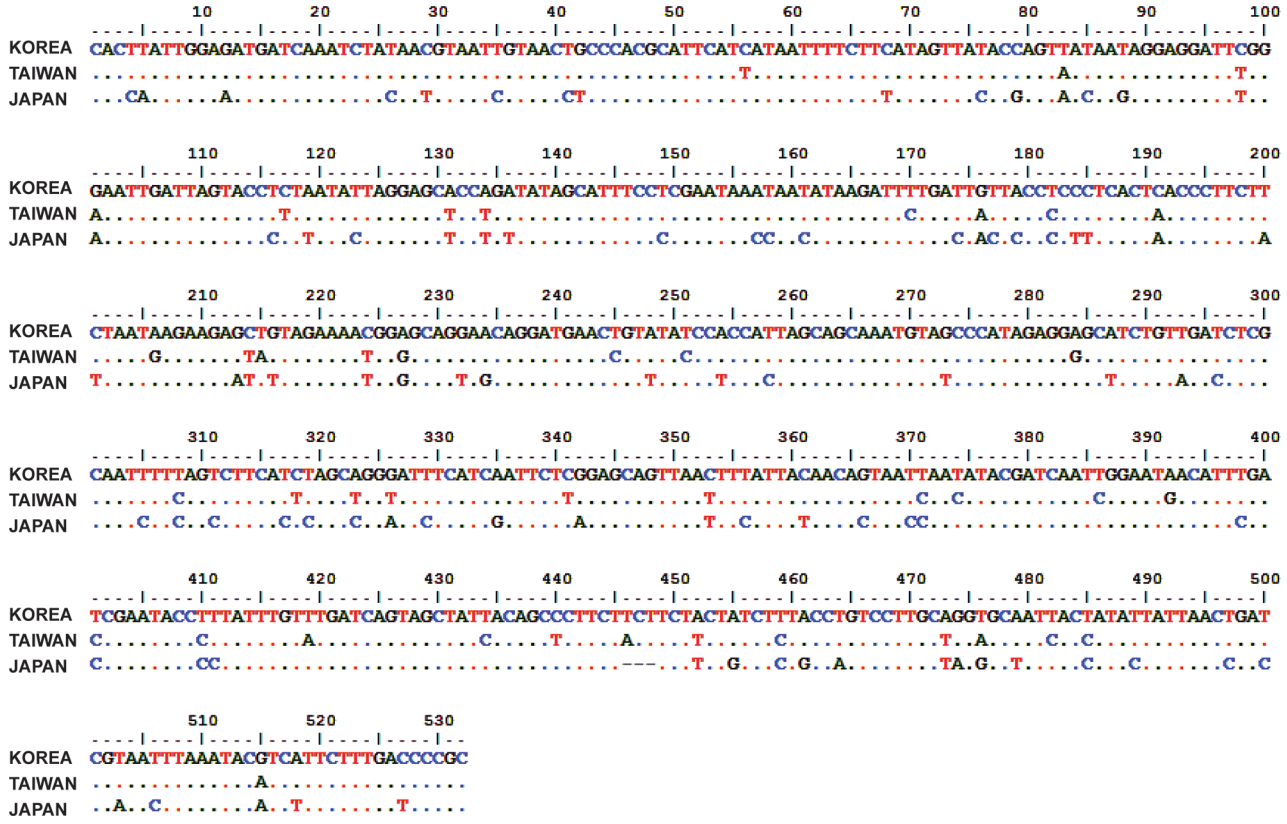

Fig. 1. Polymorphic sites shown among three different genetic lineages of *Chrysochroa fulgidissima*. Dots indicate nucleotide shared with the first sequence. Dashes indicate gaps in alignment.

netic distance (0.083). On the other hand, the Japanese individual was remotely related with those of Korean (0.192) and Taiwan (0.183) individuals. Genetic distance between *Rhagophthalmus lufengensis* and *R. ohbai* belonging to the superfamily Elatoroidea was only 0.065 even though they are different species. The genetic distance (0.083) between Korean and Taiwan individuals is bigger than 0.065 between the two *Rhagophthalmus* species. Those between Japanese and the Korean/Taiwan individuals are far bigger almost to the genus level.

Phylogenetic analyses using ML, BI, and NJ were coincident with the result of the genetic distance comparison (Fig. 2). It showed that Korean and Taiwan individuals consistently formed a sister group in all the three different

Table 2. Genetic distances based on the Kimura-2-parameter method were compared across the examined species

Taxons	1	2	3	4	6	8	9	10	11	12	13
1. *Acmaeodera* sp.											
2. *Chauliognathus opacus*	0.283										
3. *Chrysochroa fulgidissima* (KOREA)	0.211	0.241									
4. *Chrysochroa fulgidissima* (TAIWAN)	0.221	0.254	0.083								
6. *Chrysochroa fulgidissima* (JAPAN)	0.267	0.301	0.192	0.183							
8. *Pyrophorus divergens*	0.258	0.265	0.242	0.242	0.289						
9. *Pyrocoelia rufa*	0.324	0.226	0.263	0.255	0.336	0.292					
10. *Rhagophthalmus lufengensis*	0.286	0.183	0.208	0.227	0.290	0.286	0.205				
11. *Cyphon* sp.	0.242	0.256	0.250	0.253	0.292	0.262	0.285	0.288			
12. *Rhagophthalmus ohbai*	0.290	0.205	0.224	0.240	0.296	0.286	0.223	0.065	0.287		
13. *Apatides forti*	0.242	0.300	0.227	0.214	0.286	0.260	0.295	0.295	0.292	0.299	

Note: The number in each column corresponds to the species in each row.

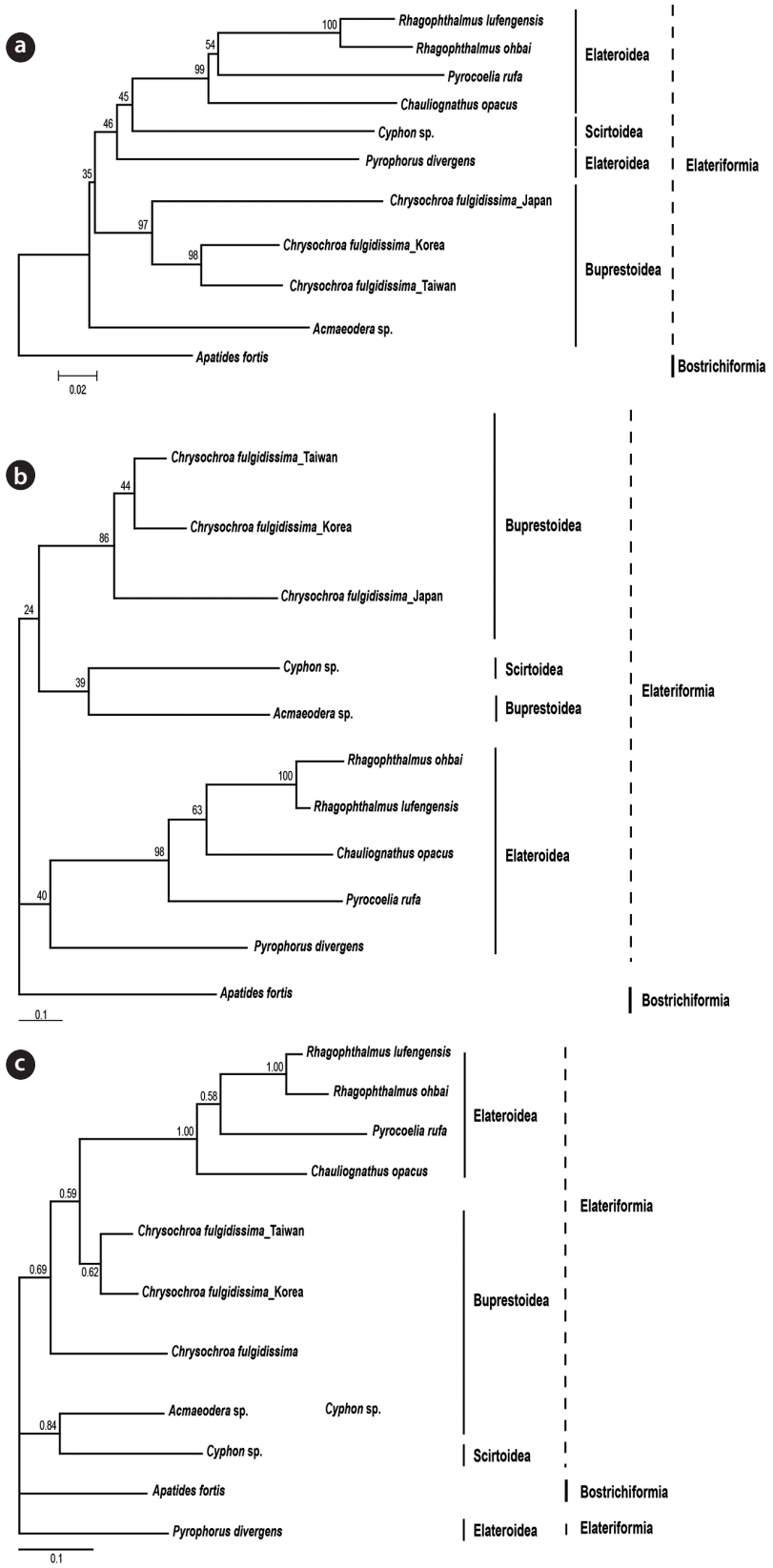

Fig. 2. Phylogenetic trees for 11 Elateriformia species and the three different populations of *Chrysochroa fulgidissima* based on COI sequences analyzed by (a) NJ (neighbor joining), (b) ML (maximum likelihood), and (c) BI (Bayesian inference) methods. Numbers above the branches in (a) and (b) indicate bootstrap supports of nodes, in (c) indicate Bayesian posterior probability.

trees (BP 98% in NJ, BP 44% in ML, BPP 62% in BI). Japanese one appeared to be a sister of the clade of Korean and Taiwan individuals in NJ (BP 97%) and ML (BP 86%). Unexpectedly, the Japanese individual in BI tree was not clustered with the remaining Korean and Taiwan *C. fulgidissima*.

The present results strongly suggested that the three different populations of *C. fulgidissima* may be different subspecies or species and also Korean and Taiwan populations are genetically more closely related than that of Japan. This is a contradicting to the result of the study by Han et al. (2012) where the Japanese and Taiwan populations were clustered together. To clarify if the populations of *C. fulgidissima* from Korea, Taiwan, and Japan are different at the level(s) of subspecies, species, or genus, it is necessary that more samples of the members of the family Buprestidae should be collected and genetically analyzed.

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