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Original article

Intraspecific diversity of *Monochamus saltuarius* (Gebler) based on DNA barcode analysis

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ABSTRACT

Monochamus saltuarius has a morphological polymorphism, but there is no standard phenotype to distinguish the differences in *M. saltuarius* species. To investigate molecular diversity of *M. saltuarius*, mitochondrial cytochrome c oxidase I 5' sequence were analyzed against specimens collected from Chungbuk, Gyeonggi, and Gangwon province. The DNA barcode results showed that the specimens make two groups with a 1.68%–3.1% K2P distance, but cannot find a specific phenotype difference among the specimens.

Introduction

The Cerambycidae are an abundant species in the family of Chrysomeloidea (Insecta: Coleoptera), and all members are normally known as longhorn beetles. The family consists of ~ 25,000 described species in nine subfamilies all over the world (Sama et al 2010; Bouchard et al 2011) and 357 species are known from the Korean fauna (Lee 1987; Danilevsky 1992; Paek et al 2010; Danilevsky 2013; Lim et al 2012, 2013a, 2013b; Oh 2013; Oh and Lee 2013; Jang et al 2015). Eight species in the genus *Monochamus* were recorded from Korea (Paek et al 2010). Among them, *Monochamus saltuarius* is an economic important pest of conifer in Korea, Japan, China, Taiwan, Western Europe, and Northern America regions (Dominik 1982; Shao et al 1988; Vallentgoed 1991; Morewood et al 2002). *M. saltuarius* occurred in *Pinus koraiensis* in the central area of Korea, Chungnam and Gangwon province (Lee and Lee 2000; Kwon et al 2006). Meanwhile, *Monochamus alternatus* had been collected at various sites covering the whole seaside area of Gyeongnam and Jeonnam province in the survey from 1989 to 1994 (Moon et al 1995).

In Korea, *M. alternatus* is known to be a vector of the pinewood nematode, *Bursaphelenchus xylophilus*, but *M. saltuarius* has the ability to be a vector of this nematode. According to a recent report, it is accepted that *M. saltuarius* transmits the pinewood nematode to Korean white pine trees at Gwangju, which is located in Gyeonggi province (Korea Forest Research Institute 2007; Han et al 2007).

M. saltuarius, as well as *M. alternatus*, are important pests because they transmit the pinewood nematode and cause serious damage to conifer species which are very abundant in Korean peninsula as well as Japan and North-Eastern China. Therefore, precise ecological study and taxonomic records are especially important to control the pests.

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Table 1. PCR primers used in this study.

Primer name	Primer sequence (5′–3′)	Primer source
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al (1994)
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al (1994)
MHemF1	GCATTYCCACGAATAAATAAYATAAG	Park et al (2011)
MHemR1	GGTGGATAAACTGTTCAWCC	Park et al (2011)

PCR = Polymerase chain reaction

M. saltuarius have morphological polymorphism, but there are no data to distinguish the diversity of the pest. Recently, molecular identification, so called DNA barcoding (Hebert et al 2003), is getting popular to discriminate species. In this study, we collected over a hundred specimens in the central region of Korean Peninsula and analyzed the molecular diversity of *M. saltuarius.*

Materials and methods

The specimens examined in this study were collected from five locations from 2011 to 2015 in Korea. Most of the specimens from the collection sites were collected mainly by sweeping nets in the field located in Gangwon, Gyunggi, and Chungbuk province (Table 2). All specimens were stored under air-dried conditions. The materials were preserved at the following institutions: Systematic Entomology Laboratory, Hannam University, Daejeon, Korea (SEL/HNU).

DNA was extracted from either dried or ethanol-fixed leg samples using a standard Glass Fiber extraction protocol (Ivanova et al 2006). Furthermore, genomic DNA extraction was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Mitochondrial cytochrome c oxidase I (COI) a 658-bp fragment was amplified using the primer set, LCO149/HCO2198 (Folmer et al 1994), LCO1490/MhemR1 or MhemF1/HCO2198 (Park et al 2011; Table 1). Polymerase chain reaction (PCR) amplifications were done in a 20-μL volume including 5 μL of 20% trehalose, 2.8 μL of distilled water, 2 μL of 10 × PCR buffer [20mM Tris-HCL (pH 8.0), 40mM NaCL, 2mM sodium phosphate, 0.1mM EDTA, 1mM DDT, stabilizers, 50% (v/v) glycerol], 2 μL of $MgCl_2$ (50mM), 2 μL of each primer (10μM), 2 μL of 25mM dNTP, 0.2 μL of Taq polymerase (Platinum® Taq, Invitrogen, Carlsbad City, CA, USA) and 2 μL of extracted DNA. PCR thermocycling was done under the following conditions: 5 minutes at 94°C; five cycles of 30 seconds at 94°C, 30 seconds at 45°C, 60 seconds at 72°C; 40 cycles of 30 seconds at 94°C, 30 seconds at 51°C, 60 seconds at 72°C; 7 minutes at 72°C; held at 4°C. Contigs were assembled using CodonCode aligner version 2.0.6 (CodonCode Co., Centerville City, MA, USA) and were subsequently aligned by the same software. A neighbor-joining (NJ) analysis (Saitou and Nei, 1987) was performed with MEGA 6.0 (Tamura et al 2013).

Table 2. Sample list of the *Monochamus saltuarius* used in the third study.

Group	Region	No.	Host plant	Date	Stage
A	Gangwon A	6		Jun 2011	Adults
	Gapyeong A	24	Nut pine	Jun 2014–Jun 2015	Adults
	Yangpyeong A	15	Nut pine	Jun 2014	Adults
B	Gangwon B	1		Jun 2011	Adults
	Chungbuk B	14	Rigida, nut pine, pine	Jun 2011	Adults
	Gyeonggi B	4	Breed	Aug 2011	Adults
	Gapyeong B	4	Nut pine	Jun 2014–Jun 2015	Adults
	Yangpyeong B	1	Nut pine	Jun 2014	Adults

Table 3. Pairwise distance of COI between populations.

Region	Intragroup		Intergroup	
	Min	Max	Min	Max
GWA	0.00	0.30	1.68	3.10
GWB	0.00	0.00	1.80	3.10
CBB	0.00	0.00	1.68	3.10
GGB	0.00	0.00	1.80	3.10
YPA	0.00	0.40	1.68	3.10
YPB	0.00	0.00	1.90	3.10
GPA	0.00	0.30	1.68	3.10
GPB	0.00	0.00	1.90	3.10
Average	0.00	0.15	1.76	3.10

COI = cytochrome c oxidase I; CBB = Chungbuk B group; GGB = Gyeonggi B group; GPA = Gapyeong A group; GPB = Gapyeong B group; GWA = Gangwon A group; GWB = Gangwon B group; Min = minimum; Max = maximum; YPA = Yangpyeong A group; YPB = Yangpyeong B group.

Results and discussion

A total of 69 DNA barcodes were secured among a total of 104 individual specimens. Phylogenetic analysis of the secured DNA barcode showed the specimens make two phylogenetic groups with an average K2P distance 1.68–3.1%. A group, included three sites including the Gangwon A group (GWA), Yangpyeong A group (YAP), and Gapyeong A group (GPA). B group was composed of the Gangwon B group (GWB), Chungbuk B group (GBB), Gyeonggi B group (GGB), Yangpyeong B group (YPB), and Gapyeong B group (GPB). In the COI sequences, the average nucleotide composition was as follows: A, 30.70%; T 38.45%; G, 15.05%; and C, 15.05% within the A Group taxa but A, 30.55%; T 38.75%; G, 15.81%; and C, 14.89% when the B Group taxa were included.

Diversity of intergroup specimens averaged 1.3% K2P distance, ranged from 1.68% to 3.1%, whereas it ranged from 0% to 0.4% in the intragroup, with an average of 0.15%. The two groups collected in Gangwon province, GWA and GWB showed 2.4% difference and it was 2.3% and 2.5% between GWA and CBB, GWA and GGB, respectively. Also, GBA and YPB showed 2.4% difference of K2P distance and it was 2.8% between GWA and GPB (Tables 3 and 4).

Average body lengths of Group A are 18.12 mm and 17.59 mm, respectively, for males and females, and it was 18.40 mm and 18.15 mm for males and females, respectively, in Group B specimen. However, there is no significant difference in morphological character by microscopic examination, we confirm the fact that A group sample's parameter length is a little shorter and wider. A and B groups are collected from each area and the regional difference is not considered to be shown.

The habitat distribution data showed that A group specimens were collected only in the Yangpyeong and Gapyeong region in Gangwon province whereas B group specimens were distributed most in the collection region (Figure 1).

In conclusion, it is clear that there are two genetically different groups of *M. saltuarius* with a different regional distribution. However, we cannot conclude where the two group of the pest distributed to a specific region because of limited specimen collection areas. Expanding the collection to the southern part of the Korean peninsula, Jeju Island, Japan, and East-Northern Asia is