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#### **Short Communication**

# Molecular comparison of *Lycorma delicatula* (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan



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#### ABSTRACT

Lycorma delicatula (White) was recently introduced in Korea, acting as a grape insect pest. Since the introduction of this invasive insect, it initially spread rapidly throughout central and southern Korea, and is now distributed throughout the mainland. Here we developed new mitochondrial markers from NADH dehydrogenase subunit 2 and NADH dehydrogenase subunit 6 regions, and analyzed the regional isolates of *L. delicatula* collected from original locations in China, as well as invasive locations in Korea and Japan. All Korean and Japanese isolates were found to be genetically identical to those from Beijing, Tianjin, Qingdao, and Shanghai, China. Further isolates, from Zhejiang province, China, had two additional haplotypes.

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#### Introduction

The lantern fly, *Lycorma delicatula*, suddenly arrived in the Korean Peninsula, and is now distributed throughout the mainland (Han et al., 2008). This species appears to have become fully established in Korea due to the increase of winter temperature, spurred by the global warming effect (Lee et al., 2011). Egg masses are often found in host plants, and huge numbers of first instar nymphs can be observed every spring. Because of the importance of the species as an invasive insect pest, many researchers have studied *L. delicatula* in terms of its taxonomy, biology, ecology, ethology, and control, within Korea (Han et al., 2008; Park et al., 2009; Shin et al., 2010; Choi et al., 2011; Kim et al., 2011c).

In Korea, this species has become a serious pest of grapes, and now brings substantial economic losses by causing serious damages to grapevines (Shin et al., 2010; Lee et al., 2011). Lycorma delicatula primarily damages its host plants through the sucking of plant sap (Kim et al., 2011b), and secondarily, through spreading sooty mold disease (Lee et al., 2009). Because sooty mold disease disrupts photosynthesis, leading to a decline in the quality and yield of grapes, this

species is frequently controlled in vineyards through the use of chemical pesticides (Shin et al., 2010). Aside from grapes, this species can utilize 38 woody and four herbaceous species (Park et al., 2009). In fact, the main host of *L. delicatula* is the native Chinese tree called the tree of heaven, *Ailanthus altissima* Swingle, in which the lantern flies feed, mate, and overwinter as an egg form. This Chinese tree may contribute to the settlement and proliferation of *L. delicatula*, which has been already become widely distributed in Korea along roadsides (Lee et al., 2011). Based on the observed distribution patterns to date, it is unlikely that it will be possible to eradicate this species in order to protect the grapevines. Instead, it is necessary to understand the biology of *L. delicatula* to control it (Kim et al., 2011b).

The native range of *L. delicatula* was once contained within China, from the previous record (Liu, 1939). Until the 1930's, this species was distributed in only Shanxi, Shandong, and Hebei provinces in China (Liu, 1939). Recent studies of Chinese fauna have confirmed that this species has nation-wide coverage, being distributed in Anhui, Beijing, Guangdong, Henan, Hubei, Jiangsu, Shaanxi, Sichuan, Yunnan, and Zhejiang in China, and is also distributed in Taiwan, Vietnam, and India, where temperatures are relatively higher than those in northern China (Xiao, 1992; Hua, 2000). In Japan, this species was recorded in Okinawa, Honshu, and Kyushu since the 1930's. However, it was reportedly sporadically observed, and thereby, it's distribution throughout Japan was doubtful. More recently, mass occurrences of *L. delicatula* have been identified in Hakusan, Ishikawa Prefecture in 2008 where some

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**Table 1**Collection list of *Lycorma delicatula*. Abbreviation of each location in parenthesis. KOR; Korea. CHN: China. and IPN: Japan.

No.	Date	Location	GPS-N	GPS-E
1	2010-06-28	Seoul, KOR (SL)	37.33.42.6	126.56.39.3
2	2010-07-03	Incheon, KOR (IC)	37.47.29.9	126.16.14.4
3	2010-08-17	Suwon, KOR (SW)	37.16.07.7	126.59.06.7
4	2010-08-19	Cheonan, KOR (CA)	36.44.01.6	127.15.08.1
5	2010-09-14	Cheongyang, KOR (CY)	36.26.15.8	126.46.05.0
6	2010-08-20	Gochang, KOR (GC)	35.25.50.8	126.43.09.7
7	2010-08-20	Buan, KOR (BA)	35.40.36.9	126.44.24.8
8	2010-07-31	Andong, KOR (AD)	36.32.31.1	128.47.49.4
9	2010-08-06	Yechoen, KOR (YC)	36.39.56.0	128.31.12.0
10	2010-09-29	Nonsan, KOR (NS)	36.13.20.6	127.0.55.1
11	2009-07-22	Beijing, CHN (BJ)	39.54.16.8	116.24.29.5
12	2010-07-05	Tianjin, CHN (TJ)	39.07.15.1	117.12.54.1
13	2011-08-15	Qingdao, CHN (QD)	36.19.21.6	120.23.36.9
14	2010-09-04	Shanghai, CHN (SH)	31.37.23.4	121.23.50.2
15	2010-09-05	Ningbo, CHN (NB)	29.47.42.4	121.47.37.4
16	2010-09-06	Tiantai, CHN (TT)	29.03.45.7	121.02.45.1
17	2010-09-07	Linan, CHN (LA)	30.14.01.9	119.43.29.0
18	2010-09-15	Hakusan, JPN (HS)	36.35.40.8	136.37.32.1

individuals were collected in this study (see Materials and methods). The occurrence of sudden outbreaks of *L. delicatula* in Japan is very similar to that of Korea, which has sparked our interest in an epidemiological investigation between the three adjacent countries, in order to determine whether this species has been naturally or artificially transferred.

Despite its relevance as an insect pest of grape, the sources of sudden emergences of *L. delicatula*, as well as the origin of the insects occurring in Korea, are, as yet, unknown. Like other invasive species, *L. delicatula* has rapidly spread throughout central and southern Korea over the past five years (Park et al., 2009). Recently, it is highly suggestive that *L. delicatula* was introduced from China, but this has not been scientifically proven. In addition, there is a question as to whether it could arrive from overseas by flight, because its morphology and wing structure are not appropriate for long distance migration, such as that exhibited by many other fulgorid species. An alternative theory regarding its invasion,

and one which is, in many respects, more believable, is that they were artificially transferred through the import of products, via transportation as adults or egg masses. However, it is still not known where the invasive population originated, and genetic comparisons between source and invasive populations have not previously been performed. A better understanding of its genetic structures is also important for the effective control in grape vineyards, as well as for the establishment of quarantine policy for *L. delicatula*.

In this study, we aim to confirm the genetic relationships between different *Lycorma* isolates from Korea, China, and Japan. Here, we developed new mitochondrial markers from NADH dehydrogenase subunit 2 and NADH dehydrogenase subunit 6 regions, and then compared the regional isolates of *L. delicatula* collected from some original and invasive locations in Korea, China, and Japan.

#### Materials and methods

Taxon sampling

We collected 18 *Lycorma delicatula* isolates from various locations in Korea, China, and Japan (Table 1). These individual samples were collected from 10 central and southern locations of Korea, seven eastern and eastern costal locations of China, and from one emerging location in Japan (Fig. 1). Except for two samples from Shanghai, samples from China were located more than 50 km apart, so as to take a more representative sample, and increase the possibility of confirming the origin of invasive populations in Korea and Japan. All individuals were collected as female adults from the main host, *Ailanthus altissima*. All samples were stored in 80% ethanol as voucher specimens, and their genomic DNA was preserved at  $-20\,^{\circ}$ C. All samples and voucher specimens were preserved in Kunsan National University, Republic of Korea.

#### Development of DNA markers

We developed two new markers based on the complete mitochondrial genome sequences of *L. delicatula* (Song et al., 2012), which was retrieved from GenBank (accession number EU909203). Among all

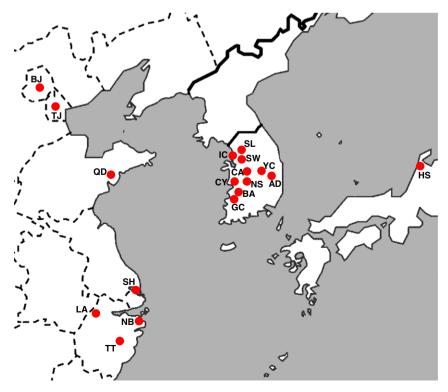


Fig. 1. Approximate locations of collection sites listed in Table 1.

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