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First Report of the Fall Armyworm, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera, Noctuidae), a New Migratory Pest in Korea

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한국에서 새로운 비래해충 열대거세미나방, *Spodoptera frugiperda* (Smith) 최초 보고

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ABSTRACT: The fall armyworm, *Spodoptera frugiperda* (Smith, 1797), originated from tropical and subtropical America is one of sporadic agricultural pests in the world. Since the moth has high migration capacity, it rapidly expanded the world distribution such as Africa in 2016, India in 2018, and East-Asian countries in 2019. In Korea, this species was firstly found at maize fields of Jeju Island, in early June 2019, and subsequently detected at many counties of Jeolla-do and Gyeongsang-do in June and July 2019. The first invaded populations of *S. frugiperda* in Korea were genetically confirmed as one species, *S. frugiperda* by using a mitochondrial *cytochrome oxidase subunit I (COI)* gene, and analyzed to be comprised of two haplotypes (hap-1 and hap-2) each belonging to different clades. Among 31 *COI* sequences, the hap-1 sequence was predominant, accounting for 93.5%.

Key words: *Spodoptera frugiperda*, Maize, Invasive, Migratory, Korea

초 록: 열대 및 아열대 아메리카 지역이 원산지인 열대거세미나방(신칭; *Spodoptera frugiperda* (Smith, 1797))은 최근 전세계적에서 돌발적으로 문제가 되고 있는 농업 해충이다. 높은 비행능력을 가진 열대거세미나방은 2016년 아프리카를 시작으로 2018년 인도, 2019년 동남아시아에서 발견되어 확산 속도가 매우 빠르다. 한국에서 열대거세나방은 2019년 6월 13일 제주도 옥수수 재배 농가포장에서 처음 발견되었고, 그 후 2019년 7월 초까지 전라도, 경상남도의 여러 시/군에서 추가로 발견되었다. 한국에서 최초 침입집단을 미토콘드리아 *COI*유전자를 이용하여 열대거세미나방임을 유전적으로 동정하였고, 서로 다른 분기군에 속하는 2개의 haplotypes(hap-1, hap-2)으로 구성됨을 확인하였다. 분석된31개의 *COI* 염기서열 중 hap-1 이 93.5%로 우점하였다.

검색어: 열대거세미나방, 옥수수, 침입, 비래, 한국

The fall armyworm, *Spodoptera frugiperda* (Smith, 1797), is one of the most important noctuid moth pests in the world and be known to damage economically important cultivated rice, maize, sorghum, cabbage, beet, peanut, soybean, alfalfa, onion, cotton, pasture grasses, millet, tomato and potato (Chapman et al., 2000; Montezano et al., 2018). Until now, it has a broad host range and attacks more than 350 species of plants (Montezano et al., 2018), but it prefers maize sometimes resulting in yield losses (>70%) when it outbreaks (Johnson, 1987).

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This species is native to tropical and subtropical regions of the Americas (Goergen et al., 2016). Since *S. frugiperda* was first described in 1797, its outbreaks have occurred irregularly in USA with severe damages in 1870, 1912 to 1920, and 1975 to 1977 (Sparks, 1986). After it invaded into Nigeria in West Africa in 2016, it covered through about 40 countries in sub-Saharan Africa during one year (Nagoshi et al., 2018). In July 2018, it was firstly found in India, and then has spread rapidly to other Asian countries including Bangladesh, China, Laos, Myanmar, Sri Lanka, Thailand, and Vietnam (Ma et al., 2019).

In Korea, *S. frugiperda* was firstly found on 13 June 2019 at a maize field of Gujwa, Jeju-si, Jeju Island by experts of Jeju Agricultural Extension and Service Center. The life stages of samples were 2nd to 3rd instars and crop damage rates were measured as about 5% (per 100 plants). This moth was further found at three maize fields adjacent to the first founding location of Jeju Island (Fig. 1). The subsequent surveys by Rural Development Administration (RDA) and the provincial Agricultural Research & Extension Services showed that many maize fields had been infested by this moth and damage rates of the maize fields were less than 1% on many counties of Jella-do and Gyeongsang-do in June and July 2019.

In this study, we analyzed a mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene about the first invaded populations of *S. frugiperda*, and the larval samples collected at several counties in Korea to confirm genetically as one species *S. frugiperda* and found several populations collected in Korea were separated into two clades (A and B) based on *COI* sequences.

Materials and Methods

Sample Collection

Sampling was conducted from June to July 2019 throughout four provinces of Korea: Jeollanam-do (JN), Jeollabuk-do (JB), Gyeongsangnam-do (GN), and Jeju-do (JJ). After crop damage was observed by naked eyes, larvae (Fig. 2) were collected from maize fields using larval tweezer. Collection details, geographical locations, host plants, and dates of collection are summarized in Table 1.

A total of 31 larvae were collected, and individual samples were preserved in 99% ethanol. Voucher specimens were deposited in the insect collection of the National Institute of Agricultural Sciences, Korea.

DNA Extraction, Amplification, and Sequencing

Genomic DNA extraction was performed using DNeasy® Blood & Tissue Kit (QIAGEN Inc., Dusseldorf, Germany) according to the manufacturer's protocol. Samples for extraction consisted of a single individual from the same colony. PCR amplification was conducted with one primer set, LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994), using AccuPower® PCR PreMix (Bioneer, Seoul, Korea) with the following thermal cycle parameters for 20 amplification reactions: initial denaturation for 5 min at 94°C, followed by 34 cycles of 1 min each at 94°C, 1 min at 45.2°C,

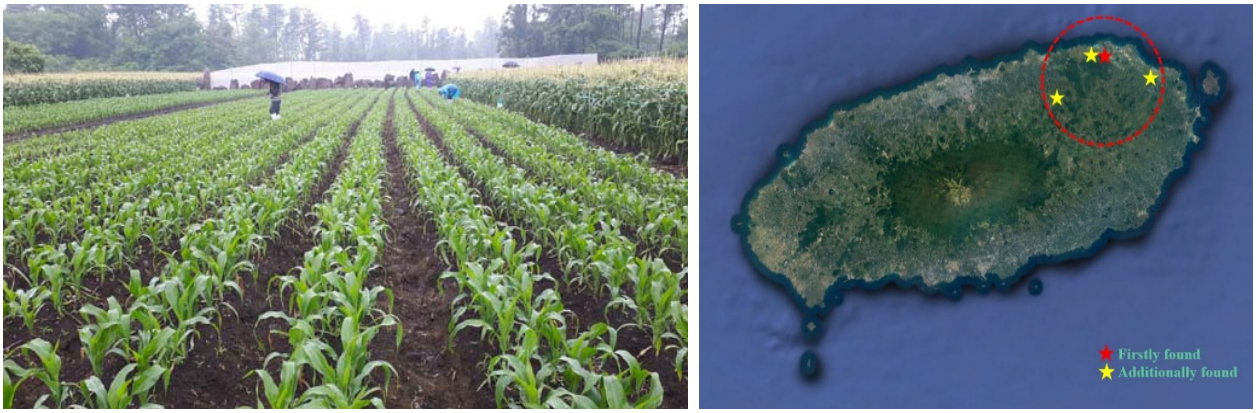

Fig. 1. A maize field where *Spodoptera frugiperda* was first found, and its distribution in Jeju Island (13-15 June 2019).

Fig. 2. Larvae of *Spodoptera frugiperda* and its damage to maize in Korea.

Table 1. Collection information about *Spodoptera frugiperda* firstly invaded into Korea

Provinces	Counties	Abbreviation	Collection Date	No. of sample	Larval Instar
Jeju	Gujwa	JJ_GJ1	2019-06-14	2	1-2
	Jocheon	JJ_JC1	2019-06-15	2	2-3
Jeonnam	Boseong1	JN_BS1	2019-06-18	2	3
	Boseong2	JN_BS2	2019-06-18	2	3
	Yeongam	JN_YA1	2019-06-18	2	3
	Muan1	JN_MA1	2019-06-20	2	5
	Muan2	JN_MA2	2019-06-20	2	5
	Haenam1	JN_HN1	2019-06-21	2	3
	Haenam2	JN_HN2	2019-06-28	2	5
	Yeosu1	JN_YS1	2019-06-21	2	3
	Yeosu2	JN_YS2	2019-07-01	2	4-5
	Gangjin1	JN_GJ1	2019-07-08	1	6
	Gangjin2	JN_GJ2	2019-07-12	2	6
Jeonbuk	Gochang1	JB_GC1	2019-06-21	2	3
	Gochang2	JB_GC2	2019-06-24	2	4-5
Gyeongnam	Milyang	GN_MY1	2019-06-28	2	6

and 1 min at 72°C, with a final extension for 5 min at 72°C. PCR products were visualized on agarose gels after electrophoresis. Single bands were purified using a QIAquick PCR purification kit (QIAGEN, Dusseldorf, Germany). PCR products were sequenced in both directions by ABI 3730xl sequencer (Applied Biosystems). The resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs in SeqManTMPro (version 7.1.0, 2006; DNAStar, Inc., Madison, Wisconsin, USA). The

sequences were visually checked individually for protein coding frame-shifts to avoid pseudogenes (Zhang and Hewitt, 1996). Consensus files were aligned using Clustal X 1.83 (Thompson et al., 1997). All sequences are deposited in the GenBank.

Data Analysis

For identifying 31 moth samples, a neighbor-joining (NJ) tree was constructed based on 31 new *COI* sequences analyzed in this study, together with 27 *COI* sequences of *S. frugiperda* from the GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Alignments of nucleotide sequences were performed using CLUSTALX with default conditions. A NJ analysis was conducted for the combined data set, in MEGA 5.0 (Tamura et al., 2011). Pairwise sequence divergences between the 58 *COI* sequences for each were calculated using a Kimura's 2-parameter (K2P) distance model (Kimura, 1980) in MEGA 5.0 (Tamura et al., 2011). Descriptive statistics (number of variable sites and number of haplotypes,) were calculated using DNASP ver. 5.0.

Results

Amplification Result and Sequence Information

A total of 31 *COI* sequences were successfully amplified from the 31 specimens and obtained bands of approximately 650 bp on the gel. We aligned the sequence once the PCR products were sequenced. Several base pairs were removed because of ambiguous alignment, which resulted in a final count of 546 bp. From the combined dataset (new 31 *COI* sequences + 27 *COI* sequences of the GenBank), we determined 29 variable sites at nucleotide positions 16, 51, 84, 133, 138, 140, 172, 176, 185, 247, 286, 288, 294, 316, 324, 369, 406, 420, 444, 450, 468, 486, 504, 513, 514, 543, and 546.

Genetic Diversity and Distances

Totally, 27 haplotypes of *S. frugiperda* were detected from the 58 *COI* sequences in the world. Among the 27 haplotypes, most of samples collected in Korea (29 out of the 31 *COI* sequences) belonged to the hap-1; whereas, the hap-2 was detected from samples which were collected in JN province. The genetic distances among the 27 haplotypes were ranged from 0.20% to 2.20%, and the hap-1 and hap-2 populations detected in Korea revealed a 1.90% genetic distance.

Phylogenetic Analysis of *S. frugiperda*

The NJ tree was generated based on the aligned dataset of 58 *COI* sequences of *S. frugiperda* and one *COI* sequence of *Spodoptera litura* as an outgroup (Fig. 3). The NJ tree revealed

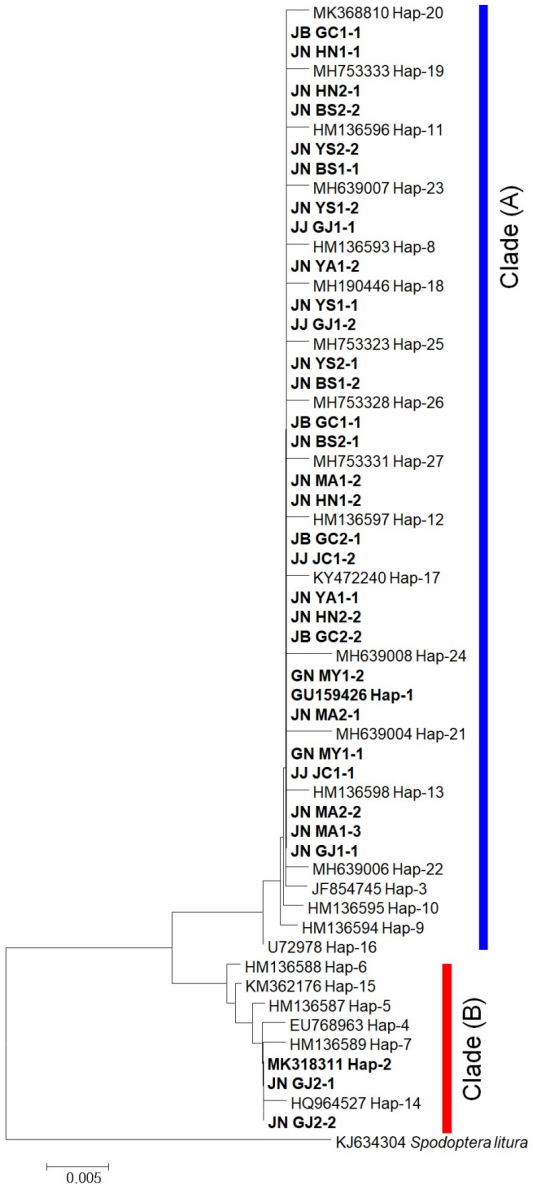

Fig. 3. A phylogenetic tree constructed by NJ analysis based on 31 *COI* sequences of *Spodoptera frugiperda* populations in Korea along with 27 *COI* haplotypes recovered from GenBank.

two distinct clades: clade (A) consisted of 19 haplotypes (hap-1, 3, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27) and 48 *COI* sequences (including 25 *COI* sequences of the JB, JN, JJ, GN populations), and clade (B) consisted of seven haplotypes (hap-2, 4, 5, 6, 7, 14, and 15) and 9 *COI* sequences (including two *COI* sequences of the JN populations). In the clade (A), no genetic differences among the JB, JN, JJ, and GN populations were observed (Fig. 3).

Discussion

Until early April 2019, *S. frugiperda* has been known to be distributed in the America, sub-Saharan Africa, and Asia (including Bangladesh, China, India, Myanmar, Sri Lanka, Thailand, and Yemen) (CABI, 2019). In this study, we firstly found the occurrences of *S. frugiperda* on maize fields in Korea (Fig. 2) and examined their 31 *COI* sequences together with the 27 *COI* sequences from the Genbank. From the 58 *COI* sequences, the first invaded Korean populations were comprised of two haplotypes, hap-1 and hap-2, and the phylogenetic tree revealed that *S. frugiperda* was separated into two clades (A and B) with the hap-1 and hap-2 of the Korean populations were included in each clade.

In this study, we checked distribution countries of the 27 haplotypes based on each of haplotype sequence data from GenBank (Table 2). In the phylogenic tree, 29 *COI* sequences from Korea (hap-1) were identical to native ones from Brazil, Canada, Costa Rica, Dominica, and USA, as well as invasive ones from Ghana, Kenya, Nigeria, South Africa, Uganda, China, India, and Vietnam; whereas, two *COI* sequences from Korea (hap-2) were identical to native ones from Brazil, Canada, Mexico, Puert Rico, and USA, as well as invasive ones from Ghana, Kenya, Sao Tome, Uganda, and India. It means that two haplotypes (hap-1 and hap-2) among the 27 haplotypes have been involved mostly in intra- and intercontinental dispersal, including Africa and Asia.

According to Nagoshi et al. (2019), the expansion of *S. frugiperda* in Africa can be explained by a single introduction, showing low numbers of haplotypes, regional similarities in haplotype composition, and regional differences in haplotype frequencies. If the long-distance migration of *S frugiperda* is one of reasons for the rapid invasion into Korea, the migration source, as estimated by Ma et al. (2019), might be the southern or middle regions in China. Zhang et al. (2019) recently reported that two haplotypes, hap-1 and hap-2, were found in the southern regions in China, which were the same haplotypes in Korea, until early June 2019, and the hap-1 was predominant in number of sequences (> 96%) in China, It was similar that the hap-1 was also predominant in Korea, accounting for 93.5%.

S. frugiperda has a remarkable dispersal capacity and this feature is understood to have evolved as part of its life history strategy (Jonhson, 1987). Considering its high spreading performance, large reproductive capacity (Murúa and Virla, 2004), absence of diapause (Jonhson, 1987), and wide host plant range, it is likely that the pest will be able to become one of important migratory insect pests in most of Korea. So, there is an urgent

Table 2. Distribution countries of *S. frugiperda* based on *COI* haplotype sequences in the world

Haplotype	Countries*
hap-1	(Nr, America) Brazil, Costa Rica, Dominica, (Ir, America) USA, Canada; (Ir, Africa) Ghana, Kenya, Nigeria, South Africa, Uganda; (Ir, Asia) China, India, Vietnam, Korea
hap-2	(Nr, America) Brazil, Mexico, Puerto Rico, (Ir, America) Canada, USA; (Ir, Africa) Ghana, Kenya, Sao Tome, Uganda; (Ir, Asia) India, Korea
hap-3	(Nr, America) Brazil
hap-4~hap-16	(Ir, America) USA
hap-17	(Ir, Africa) Ghana
hap-18	(Ir, Africa) Kenya
hap-19~hap-27	(Ir, Asia) India

*Native region (Nr); Invasive region (Ir).

need for developing pest control methods and detection tools to mitigate the impact of the pest in Korea. In addition, further studies about migration behavior using combined molecular markers should be conducted to estimate the source areas or migration times.

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모든 저자는 원고를 읽고 투고에 동의하였음

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