Three Species of the Bemisia tabaci (Hemiptera: Aleyrodidae) Complex in the Republic of Korea; Detection by an Extensive Field Survey Combined with a Phylogenetic Analysis

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THREE SPECIES OF THE BEMISIA TABACI (HEMIPTERA: ALEYRODIDAE) COMPLEX IN THE REPUBLIC OF KOREA; DETECTION BY AN EXTENSIVE FIELD SURVEY COMBINED WITH A PHYLOGENETIC ANALYSIS

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ABSTRACT

Field surveys for the Bemisia tabaci complex were conducted from 2009 to 2013 in Korea, and the results were compared with published data of the B. tabaci complex. Three species, MED, MEAM1, and JpL, were collected from several provinces. The MED was mainly collected in greenhouses, displacing the earlier invasive species, MEAM1, and the JpL species was collected in the field. JpL is newly confirmed as a unique species of B. tabaci species complex in Korea and Japan.

Key Words: biotype B, haplotype, JpL, MEAM1, MED, JpL, molecular identification

RESUMEN

Se realizó un sondeo de campo sobre el complejo de Bemisia tabaci desde el 2009 hasta el 2013 en Corea, y se comparó los resultados con los datos publicados del complejo de B. tabaci. Se recolectaron tres especies, MED, MEAM1 y JPL de varias provincias. Se recogió la MED principalmente en invernaderos, la MEAM1 que reemplazó la que se invadió anteriormente y la especie JPL que fue recolectada en el campo. La JPL es recién confirmada como una especie única del complejo de B. tabaci en Corea y Japón.

Palabras Clave: biotipo B, haplotipo, JPL, MEAM1, MED, JpL, identificación molecular
to understand the current status of the \textit{B. tabaci} complex in Korea.

Recently, the number of invasive alien species has continuously increased in Korea because of increased global trade and developments in transportation (Hong et al. 2012). Thus, possibly, other species of the \textit{B. tabaci} complex may have invaded Korea. Currently there are 6 species in Japan (\textit{Asia I}, \textit{Aisa II}, \textit{China}, \textit{JpL}, MED and \textit{MEAM1}) (Ueda et al. 2008), and 14 species in China (\textit{Asia I}, \textit{Asia II 1-4}, \textit{Asia II 6-7}, \textit{Asia II 9-10}, \textit{China I-3}, MED, and \textit{MEAM1}) (Hu et al. 2011). It is necessary to determine the distribution of other species (excluding \textit{MED} and \textit{MEAM1}) of the \textit{B. tabaci} complex in Korea. Thus, in this study, we examined the distribution and diversity of the \textit{B. tabaci} complex through a large-scale survey.

Sampling was conducted from Dec 2009 to Jul 2013 throughout 7 provinces of Korea: Gyeonggi-do (GG), Jeollanam-do (JN), Chungcheongbuk-do (CB), Chungcheongnam-do (CN), Gyeongsangnam-do (GN), Gyeongsangbuk-do (GB), and Jeju-do (JJ). Adults, nymphs, and eggs were collected from vegetables, ornamental plants and weeds, and from urban as well as agricultural landscapes. Collection details, geographical locations, host plants and dates of collection are summarized in Table 1. A total of 276 whitefly adults, nymphs, and/or eggs were collected, and individual samples were preserved in 99% ethanol. Voucher specimens are deposited in the collection of the Institute of Insect Sciences at the National Academy of Agricultural Science, Korea.

Genomic DNA extraction was performed using DNEasy® Blood & Tissue Kit (QIAGEN Inc., Dusseldorf, Germany), according to the manufacturer’s protocol. Each sample for extraction consisted of a single individual from the same colony. PCR amplification was conducted with one primer set, C1-J-2195 (5'-TIGATTTTTTGGTACATCCAAGTG-3') and TL2-N-3014 (5'-TCGAATGCACATATCGGATATA-3') (Simon et al. 1994), using AccuPower® PCR PreMix (Bi-oneer, 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 52 °C, 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). Resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs in SeqManTMPro (version 7.1.0, 2006; DNASTarInc., Madison, Wisconsin, USA). The sequences were visually checked individually for protein coding frame-shifts to avoid pseudogenes (Zhang & Hewitt 1996). Consensus files were aligned using Clustal X 1.83 (Thompson et al. 1997). All sequences are deposited in the GenBank (accession numbers given in Table 1). These sequences are not unique to previously reported \textit{COI} sequences of \textit{B. tabaci}.

For identifying samples, a neighbor-joining tree was constructed based on 47 new but not unique \textit{COI} sequences together with 212 \textit{COI} sequences of \textit{B. tabaci} (including 31 species) from the GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and 4 \textit{COI} sequences of \textit{B. aitripex}, \textit{B. subdecipiens}, and \textit{B. afer}, as an outgroup. Alignments of nucleotide sequences were performed using CLUSTALX with default conditions. A neighbor-joining (NJ) analysis was conducted for the combined data set, in MEGA 5.0 (Tamura et al. 2011). Intra-specific genetic divergences were calculated by using a K2P distance model (Kimura 1980) of MEGA 5.0.

In the NJ tree, the 47 \textit{COI} sequences were categorized into 3 species, \textit{MED}, \textit{MEAM1}, and \textit{JpL} (Fig. 1). Among the 47 \textit{COI} sequences, 29 \textit{COI} sequences belonged to the \textit{MED} species, with no genetic variations, while the 17 \textit{COI} sequences belonged to the \textit{JpL} species, in which divergences ranged from 0.0% to 0.2%. The one remaining \textit{COI} sequence was referred to as \textit{MEAM1}. Among the 33 reported haplotype of \textit{MED} (Fig. 1), the 29 \textit{COI} sequences from Korea were identical to \textit{COI} sequences reported from China, Croatia, Taiwan (Dinsdale et al. 2010), France (Dalmon et al. 2008), Greece (Tsagkarakou et al. 2007), Japan (Ueda 2006; Boykin et al. 2007), North America (Mckenzie et al. 2012), Spain, U.S.A. (Shatters et al. 2009), and Uganda (Sseruwagi et al. 2005). Among the 5 haplotypes of \textit{JpL} (Fig. 1), the 17 \textit{COI} sequences from Korea were identical to either of 2 types, AB308114 and AB308116 of Japan (Ueda et al. 2008), and among the 28 haplotypes of \textit{MEAM1} (Fig. 1), the one \textit{COI} sequence was identical to the \textit{COI} sequence from USA, Spain, Australia, China, Colombia, Dominican Republic, France, Guadeloupe, India, Italy, Sicily, Saudi Arabia (Dinsdale et al. 2010), Israel (Hsieh et al. 2006), Reunion (Delatte et al. 2006), and Argentina (Viscarret et al. 2003).

From the large scale sampling, we observed that \textit{MED} is widely distributed across Korea, being found in 7 of the country’s 9 provinces, GG, GB, GN, JJ, JN, CB, CN. Also \textit{JpL} was detected from GG, JJ, and JB (Fig. 2A). On the other hand, \textit{MEAM1} was only detected in GG. We compared our results with prior research papers (Lee et al. 2000; Lee et al. 2005; Park et al. 2010; Lee et al. 2012) and confirmed that there has been a considerable change in the relative abundance of \textit{MEAM1} and \textit{MED} (Fig. 2) in that \textit{MED} has been displacing the earlier invader, \textit{MEAM1}. The displacement of an earlier invasive \textit{B. tabaci} race by a new invasive race has been reported in several countries such as China (Liu et al. 2007) and Aus-
Table 1. Collection of *Bemisia tabaci* samples in the Republic of Korea from 2009 to 2013.

| Region         | Host plant          | Collection sites | Collection date | Status       | No. of collection | Voucher number | Species | Accession number |
|----------------|---------------------|------------------|-----------------|--------------|-------------------|----------------|---------|------------------|
| JJ, Jeju-si    | Lamium amplexicaule | greenhouse       | 2009-12-08      | Adult        | 4                 | 091208GS-C1    | MED     | KF468455         |
| JJ, Jeju-si    | Conyza sumatranseis | near greenhouse  | 2009-12-08      | Adult        | 2                 | 091208GS-C10   | MED     | KF468456         |
| JJ, Jeju-si    | Lycopersicon esculentum | greenhouse       | 2009-12-08      | Adult        | 15                | 091208GS-C12   | MED     | KF468457         |
| JJ, Jeju-si    | Lamium amplexicaule | near greenhouse  | 2009-12-08      | Adult        | 5                 | 091208GS-C14   | MED     | KF468458         |
| JJ, Jeju-si    | Conyza sumatranseis | outdoors         | 2010-01-27      | 3rd nymph    | 17                | 100127GS-C15   | MED     | KF468459         |
| JJ, Jeju-si    | Malva pusilla       | near greenhouse  | 2009-12-08      | Adult        | 1                 | 091208GS-C18   | MED     | KF468460         |
| JJ, Jeju-si    | Lamium amplexicaule | near greenhouse  | 2009-12-08      | Adult        | 5                 | 091208GS-C19   | MED     | KF468461         |
| JJ, Jeju-si    | Lycopersicon esculentum | greenhouse       | 2009-12-08      | Adult        | 4                 | 091208GS-C2    | MED     | KF468462         |
| JJ, Jeju-si    | Leonurus sibiricus  | near greenhouse  | 2009-12-08      | Adult        | 4                 | 091208GS-C20   | MED     | KF468463         |
| JJ, Jeju-si    | Conyza sumatranseis | greenhouse       | 2009-12-08      | Adult        | 5                 | 091210GS-C22   | MED     | KF468464         |
| JJ, Jeju-si    | Lycopersicon esculentum | greenhouse       | 2009-12-08      | Adult        | 5                 | 091208GS-C3    | MED     | KF468465         |
| JJ, Jeju-si    | Conyza sumatranseis | greenhouse       | 2009-12-08      | Adult        | 4                 | 091208GS-C4    | MED     | KF468466         |
| JJ, Jeju-si    | Veronica persica    | near greenhouse  | 2009-12-08      | Adult        | 1                 | 091208GS-C6    | MED     | KF468467         |
| JJ, Jeju-si    | Lycopersicon esculentum | greenhouse       | 2009-12-08      | Adult        | 5                 | 091208GS-C7    | MED     | KF468468         |
| JJ, Jeju-si    | Malva pusilla       | near greenhouse  | 2009-12-08      | Adult        | 1                 | 091208GS-C8    | MED     | KF468469         |
| JJ, Jeju-si    | Lactuca indica var. laciniata | greenhouse       | 2009-12-08      | Adult        | 2                 | 091208GS-C9    | MED     | KF468470         |
| GN, Hapcheon-gun | Lycopersicon esculentum | greenhouse | 2010-06-29      | Adult        | 8                 | 100629GS-B1    | MED     | KF468471         |
| GN, Uiryeong-gun | Lycopersicon esculentum | greenhouse | 2010-06-30      | Adult        | 8                 | 100630GS-B12   | MED     | KF468472         |
| GB, Gyeongju-si | Lycopersicon esculentum | greenhouse | 2010-07-01      | Adult        | 8                 | 100701GS-B5    | MED     | KF468473         |
| GB, Andong-si  | Lycopersicon esculentum | greenhouse      | 2010-07-02      | Adult        | 12                | 100702GS-B6    | MED     | KF468474         |
| GN, Damyang-gun | Lycopersicon esculentum | greenhouse | 2010-10-01      | Adult        | 12                | 101001GS-D1    | MED     | KF468475         |
| CB, Okcheon-gun | Lycopersicon esculentum | greenhouse | 2010-10-25      | Adult        | 6                 | 101025GS-D7    | MED     | KF468476         |
| JB, Iksan      | Lonicera japonica   | outdoors         | 2012-06-04      | 4th nymph    | 1                 | 120604GS-434   | JpL     | KF468477         |
| JB, Iksan      | Lonicera japonica   | outdoors         | 2012-06-04      | nymph        | 11                | 121024GS-435   | JpL     | KF468478         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-24      | Adult        | 1                 | 121024GS-710   | JpL     | KF468479         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-24      | Adult        | 1                 | 121024GS-711   | JpL     | KF468480         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-24      | 3rd nymph    | 4                 | 121024GS-719   | JpL     | KF468481         |
| JJ, Jeju-si    | Perilla frutescens var. japonica | outdoors | 2012-10-24      | Adult        | 2                 | 121024GS-723   | MED     | KF468482         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-25      | 3rd nymph    | 2                 | 121025GS-755   | MED     | KF468483         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-25      | 3rd nymph    | 1                 | 121025GS-771   | JpL     | KF468484         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-25      | 3rd nymph    | 1                 | 121025GS-757   | MED     | KF468485         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-25      | 4th nymph    | 1                 | 121025GS-760   | JpL     | KF468486         |
| JJ, Jeju-si    | Lonicera japonica   | outdoors         | 2012-10-25      | 4th nymph    | 1                 | 121025GS-761   | MED     | KF468487         |

*Mediterranean and Middle East-Asia Minor 1*
**Table 1. (Continued) Collection of *Bemisia tabaci* samples in the Republic of Korea from 2009 to 2013.**

| Region | Host plant          | Collection sites | Collection date | Status  | No. of collection | Voucher number | Species  | Accession number |
|--------|---------------------|------------------|-----------------|---------|------------------|----------------|----------|------------------|
| JJ, Jeju-si | *Lonicera japonica* | outdoors         | 2012-10-24      | Egg     | 3                | 121024GS-763  | JpL      | KF468488         |
| JJ, Jeju-si | *Lonicera japonica* | outdoors         | 2012-10-24      | Egg     | 3                | 121024GS-764  | JpL      | KF468489         |
| CB, Goesan-gun | *Lonicera japonica* | outdoors         | 2012-10-19      | 4th nymph | 1          | 121019GS-770  | JpL      | KF468490         |
| GG, Ansan-si | *Lactuca indica var. laciniata* | outdoors | 2012-09-26      | 4th nymph | 2         | 120926GS-663  | JpL      | KF468491         |
| GG, Namyangju-si | *Lonicera japonica* | outdoors         | 2012-09-27      | 4th nymph | 6         | 120927GS-665  | JpL      | KF468492         |
| GG, Namyangju-si | *Lonicera japonica* | outdoors         | 2012-09-27      | 3rd nymph | 2         | 120927GS-669  | JpL      | KF468493         |
| GG, Namyangju-si | *Lonicera japonica* | outdoors         | 2012-09-27      | 1st nymph | 2         | 120927GS-671  | JpL      | KF468494         |
| GG, Namyangju-si | *Lonicera japonica* | outdoors         | 2012-09-27      | 4th nymph | 2         | 120927GS-673  | JpL      | KF468495         |
| GG, Namyangju-si | *Lonicera japonica* | outdoors         | 2012-09-27      | 2nd nymph | 8         | 120927GS-677  | JpL      | KF468496         |
| GG, Goyang-si, | *Euphorbia milii var. splendens* | greenhouse | 2013-07-04      | Adult   | 23          | 130704GS-001  | MeAM1    | KF468497         |
| CN, Buyeo-gun | *Lycopersicon esculentum* | greenhouse    | 2013-07-11      | Adult   | 15          | 130711GS-002  | MED      | KF468498         |
| CN, Cheongyang-gun | *Duranta erecta* | greenhouse    | 2013-07-11      | Adult   | 30          | 130711GS-003  | MED      | KF468499         |
| GG, Yongin-si | *Lycopersicon esculentum* | greenhouse | 2013-07-18      | Adult   | 12          | 130718GS-004  | MED      | KF468500         |
| GG, Suwon-si | *Lycopersicon esculentum* | greenhouse | 2013-07-19      | Adult   | 7          | 130719GS-005  | MED      | KF468501         |

*Mediterranean and Middle East-Asia Minor 1*
Fig. 1. Neighbor-joining tree based on 259 COI sequences of *Bemisia tabaci*.
Florida entomologist (De Barro et al. 2011). JpL had been reported only in Japan only until now (Ueda et al. 2008). In this survey, JpL was recorded in Korea for the first time. In Japan (Ueda et al. 2008) and Korea (in this study), most of the JpL samples were collected on the Japanese honeysuckle, Lonicera japonica Thunb. (Dipsacales: Caprifoliaceae). This is a native plant in temperate eastern Asia regions including Japan and Korea (Williams et al. 2001), suggesting that JpL may be mainly distributed in the East Asian region.

Recently, Lee et al. (2010) reported that the Korean MED had the same tRNA sequence as those from Iran (AF247525) and Nigeria (AF247526), suggesting that this species was introduced either from Africa or the Near East to Korea. However, because these are unpublished sequences, this finding is not conclusive. In this study, we observed that the 29 COI sequences of the MED species from Korea were identical to those from wide areas of the world (including China, Croatia, France, Greece, Japan, North America, Spain, Taiwan, U.S.A., and Uganda), indicating that the place of the origin of this putative species is unsettled.

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**Fig. 2. Distribution of 3 species of the Bemisia tabaci complex in Korea from 2000 to 2013. A, Mediterranean, Middle East-Asia Minor 1, and JpL from 2009 to 2013. B, Mediterranean from 2005 to 2012. C, Middle East-Asia Minor 1 from 2000 to 2012.**

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