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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 replazo 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

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a globally distributed species complex, which includes several cryptic species (Brown et al. 1995; De Barro et al. 2000; De Barro et al. 2011). This species complex has been known to damage commercially important plants by direct feeding (Byrne & Bellows 1991) or by the transmission of begomoviruses (Geminiviridae) (Brown 2000). Currently, because of the lack of morphological characters, which can be used for distinguishing the 31 species of the *B. tabaci* complex (De Barro et al. 2011), several species of the complex have been mainly distinguished based on a threshold of genetic differentiation in one mitochondrial gene, *cytochrome oxidase subunit I* (*COI*) (Dinsdale et al. 2010), and a genetic differentiation of 3.5% has been used as the species genetic boundary. However, Lee et al. (2013) revealed that the

species boundary is changeable with increasing reports of *COI* sequences and suggested a new genetic boundary of 4.0%.

Until now, 2 tentative species, *Middle East-Asia Minor 1* (*B* biotype; *MEAM1*) and *Mediterranean* (*Q* biotype; *MED*), have been recorded in Korea: the *MEAM1* species, which was identified based on 2 mitochondrial genes (*large subunit ribosomal RNA* (*lrRNA*) and *small subunit ribosomal RNA* (*srRNA*)), was reported in 2000 (Lee & Paul 2000), while the *MED*, identification based on *lrRNA*, was reported in 2005 (Lee et al. 2005). Until now, some research papers dealing with the *B. tabaci* complex have been published; however, these were mostly concentrated on responses to insecticides (Lee et al. 2012) and/or transmitted viruses (Lee et al. 2010; Park et al. 2012). As a result, information is not sufficient

to understand the current status of the *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 *B. tabaci* complex may have invaded Korea. Currently there are 6 species in Japan (*Asia I*, *Aisa II*, *China*, *JpL*, *MED* and *MEAM1*) (Ueda et al. 2008), and 14 species in China (*Asia I*, *Asia II 1-4*, *Asia II 6-7*, *Asia II 9-10*, *China 1-3*, *MED*, and *MEAM1*) (Hu et al. 2011). It is necessary to determine the distribution of other species (excluding *MED* and *MEAM1*) of the *B. tabaci* complex in Korea. Thus, in this study, we examined the distribution and diversity of the *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'-TTGATTTTGTGTCATCCAGAAGT-3') and TL2-N-3014 (5'-TC-CATGCACTAATCTGCCATATTA-3') (Simon 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 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 SeqManTMPPro (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 & Hewitt 1996). Consensus files were aligned us-

ing 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 *COI* sequences of *B. tabaci*.

For identifying samples, a neighbor-joining tree was constructed based on 47 new but not unique *COI* sequences together with 212 *COI* sequences of *B. tabaci* (including 31 species) from the GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and 4 *COI* sequences of *B. atriplex*, *B. subdecipiens*, and *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 *COI* sequences were categorized into 3 species, *MED*, *MEAM1*, and *JpL* (Fig. 1). Among the 47 *COI* sequences, 29 *COI* sequences belonged to the *MED* species, with no genetic variations, while the 17 *COI* sequences belonged to the *JpL* species, in which divergences ranged from 0.0% to 0.2%. The one remaining *COI* sequence was referred to as *MEAM1*. Among the 33 reported haplotypes of *MED* (Fig. 1), the 29 *COI* sequences from Korea were identical to *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 *JpL* (Fig. 1), the 17 *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 *MEAM1* (Fig. 1), the one *COI* sequence was identical to the *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 *MED* is widely distributed across Korea, being found in 7 of the country's 9 provinces, GG, GB, GN, JJ, JN, CB, CN. Also *JpL* was detected from GG, JJ, and JB (Fig. 2A). On the other hand, *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 *MEAM1* and *MED* (Fig. 2) in that *MED* has been displacing the earlier invader, *MEAM1*. The displacement of an earlier invasive *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	<i>Lamium amplexicaule</i>	greenhouse	2009-12-08	Adult	4	091208GS-C1	MED*	KF468455
JJ, Jeju-si	<i>Conyza sumatrensis</i>	near greenhouse	2009-12-08	Adult	2	091208GS-C10	MED	KF468456
JJ, Seogwipo-si	<i>Lycopersicon esculentum</i>	greenhouse	2009-12-08	Adult	15	091208GS-C12	MED	KF468457
JJ, Seogwipo-si	<i>Lamium amplexicaule</i>	near greenhouse	2009-12-08	Adult	5	091208GS-C14	MED	KF468458
JJ, Seogwipo-si	<i>Conyza sumatrensis</i>	outdoors	2010-01-27	3rd nymph	17	100127GS-C15	MED	KF468459
JJ, Seogwipo-si	<i>Malva pusilla</i>	near greenhouse	2009-12-08	Adult	1	091208GS-C18	MED	KF468460
JJ, Seogwipo-si	<i>Lamium amplexicaule</i>	near greenhouse	2009-12-08	Adult	5	091208GS-C19	MED	KF468461
JJ, Jeju-si	<i>Lycopersicon esculentum</i>	greenhouse	2009-12-08	Adult	4	091208GS-C2	MED	KF468462
JJ, Seogwipo-si	<i>Leonurus sibiricus</i>	near greenhouse	2009-12-08	Adult	4	091208GS-C20	MED	KF468463
JJ, Jeju-si	<i>Conyza sumatrensis</i>	greenhouse	2009-12-10	Adult	5	091210GS-C22	MED	KF468464
JJ, Jeju-si	<i>Lycopersicon esculentum</i>	greenhouse	2009-12-08	Adult	5	091208GS-C3	MED	KF468465
JJ, Jeju-si	<i>Conyza sumatrensis</i>	greenhouse	2009-12-08	Adult	4	091208GS-C4	MED	KF468466
JJ, Seogwipo-si	<i>Veronica persica</i>	near greenhouse	2009-12-08	Adult	1	091208GS-C6	MED	KF468467
JJ, Seogwipo-si	<i>Lycopersicon esculentum</i>	greenhouse	2009-12-08	Adult	5	091208GS-C7	MED	KF468468
JJ, Jeju-si	<i>Malva pusilla</i>	near greenhouse	2009-12-08	Adult	1	091208GS-C8	MED	KF468469
JJ, Jeju-si	<i>Lactuca indica</i> var. <i>lacinata</i>	near greenhouse	2009-12-08	Adult	2	091208GS-C9	MED	KF468470
GN, Hapcheon-gun	<i>Lycopersicon esculentum</i>	greenhouse	2010-06-29	Adult	8	100629GS-B1	MED	KF468471
GN, Uiryeong-gun	<i>Lycopersicon esculentum</i>	greenhouse	2010-06-30	Adult	8	100630GS-B12	MED	KF468472
GB, Gyeongju-si	<i>Lycopersicon esculentum</i>	greenhouse	2010-07-01	Adult	8	100701GS-B5	MED	KF468473
GB, Andong-si	<i>Lycopersicon esculentum</i>	greenhouse	2010-07-02	Adult	12	100702GS-B6	MED	KF468474
GN, Damyang-gun	<i>Lycopersicon esculentum</i>	greenhouse	2010-10-01	Adult	12	101001GS-D1	MED	KF468475
CB, Okcheon-gun	<i>Lycopersicon esculentum</i>	greenhouse	2010-10-25	Adult	6	101025GS-D7	MED	KF468476
JB, Iksan	<i>Loniceria japonica</i>	outdoors	2012-06-04	4th nymph	1	120604GS-434	JpL	KF468477
JB, Iksan	<i>Loniceria japonica</i>	outdoors	2012-06-04	nymph	11	121024GS-435	JpL	KF468478
JJ, Jeju-si	<i>Loniceria japonica</i>	outdoors	2012-10-24	Adult	1	121024GS-710	JpL	KF468479
JJ, Jeju-si	<i>Loniceria japonica</i>	outdoors	2012-10-24	Adult	1	121024GS-711	JpL	KF468480
JJ, Jeju-si	<i>Loniceria japonica</i>	outdoors	2012-10-24	3rd nymph	4	121024GS-719	JpL	KF468481
JJ, Jeju-si	<i>Perilla frutescens</i> var. <i>japonica</i>	outdoors	2012-10-24	Adult	2	121024GS-723	MED	KF468482
JJ, Jeju-si	<i>Loniceria japonica</i>	outdoors	2012-10-25	3rd nymph	2	121025GS-755	JpL	KF468483
JJ, Jeju-si	<i>Loniceria japonica</i>	outdoors	2012-10-25	3rd nymph	1	121025GS-771	JpL	KF468484
JJ, Seogwipo-si	<i>Lactuca indica</i> var. <i>lacinata</i>	outdoors	2012-10-25	3rd nymph	1	121025GS-757	MED	KF468485
JJ, Seogwipo-si	<i>Loniceria japonica</i>	outdoors	2012-10-25	4th nymph	1	121025GS-760,	JpL	KF468486
JJ, Seogwipo-si	<i>Loniceria japonica</i>	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	<i>Lonicera japonica</i>	outdoors	2012-10-24	Egg	3	121024GS-763	JpL	KF468488
JJ, Jeju-si	<i>Lonicera japonica</i>	outdoors	2012-10-24	Egg	3	121024GS-764	JpL	KF468489
CB, Goesan-gun	<i>Lonicera japonica</i>	outdoors	2012-10-19	4th nymph	1	121019GS-770	JpL	KF468490
GG, Ansan-si	<i>Lactuca indica</i> var. <i>laciniata</i>	outdoors	2012-09-26	4th nymph	2	120926GS-663	JpL	KF468491
GG, Namyangju-si	<i>Lonicera japonica</i>	outdoors	2012-09-27	4th nymph	6	120927GS-665	JpL	KF468492
GG, Namyangju-si	<i>Lonicera japonica</i>	outdoors	2012-09-27	3rd nymph	2	120927GS-669	JpL	KF468493
GG, Namyangju-si	<i>Lonicera japonica</i>	outdoors	2012-09-27	1st nymph	2	120927GS-671	JpL	KF468494
GG, Namyangju-si	<i>Lonicera japonica</i>	outdoors	2012-09-27	4th nymph	2	120927GS-673	JpL	KF468495
GG, Namyangju-si	<i>Lonicera japonica</i>	outdoors	2012-09-27	2nd nymph	8	120927GS-677	JpL	KF468496
GG, Goyang-si,	<i>Euphorbia milii</i> var. <i>splendens</i>	greenhouse	2013-07-04	Adult	23	130704GS-001	MEAM1 ^b	KF468497
CN, Buyeo-gun	<i>Lycopersicon esculentum</i>	greenhouse	2013-07-11	Adult	15	130711GS-002	MED	KF468498
CN, Cheongyang-gun	<i>Duranta erecta</i>	greenhouse	2013-07-11	Adult	30	130711GS-003	MED	KF468499
GG, Yongin-si	<i>Lycopersicon esculentum</i>	greenhouse	2013-07-18	Adult	12	130718GS-004	MED	KF468500
GG, Suwon-si	<i>Lycopersicon esculentum</i>	greenhouse	2013-07-19	Adult	7	130719GS-005	MED	KF468501

^aMediterranean and ^bMiddle East-Asia Minor 1

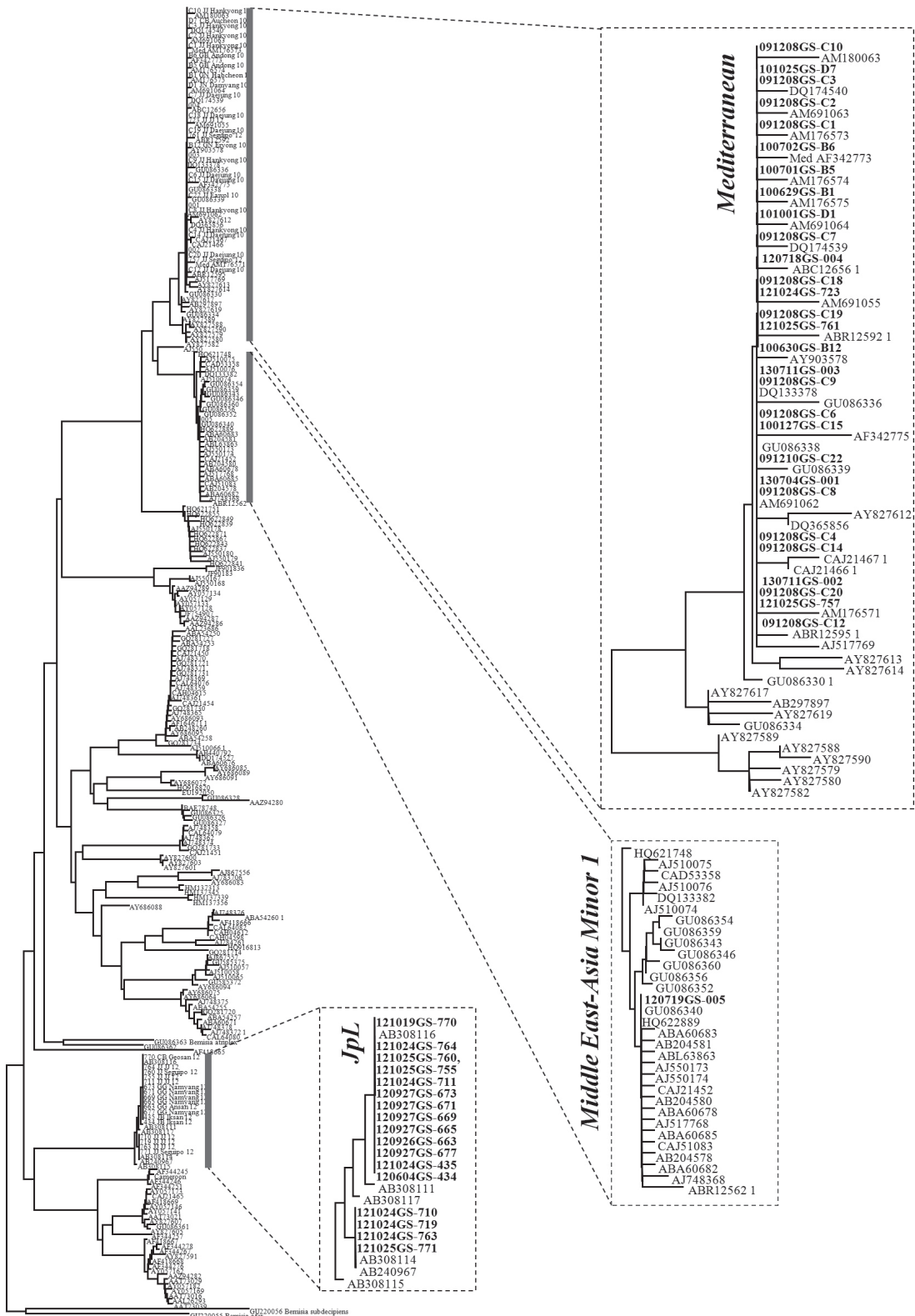


Fig. 1. Neighbor-joining tree based on 259 *COI* sequences of *Bemisia tabaci*.

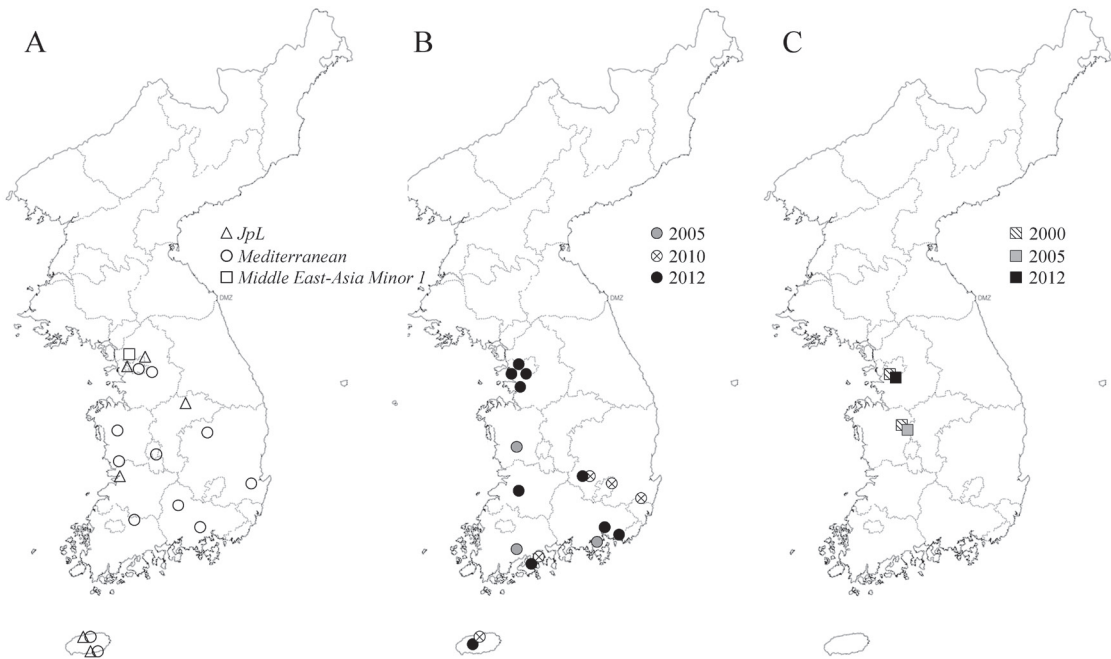


Fig. 2. Distribution of 3 species of the *Bemisia tabaci* complex in Korea from 2000 to 2013. A, *Mediterranean*, *Middle East-Asia Minor 1*, and *JpL* from 2009 to 2013. B, *Mediterranean* from 2005 to 2012. C, *Middle East-Asia Minor 1* from 2000 to 2012.

tralia (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 *lrRNA* 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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