N O T E

Genotyping of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Reveals the Presence of Two Genetic Groups in Sri Lanka[1]

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Whiteflies (Hemiptera: Aleyrodidae) are comprised of more than 1,300 species (Jones 2003, Eur. J. Plant Pathol. 109: 195–219). The sweetpotato whitefly, *Bemisia tabaci* (Gennadius), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), cause significant economic damage to many herbaceous and woody crops grown in warm climates and protected plants in temperate climates (Jones 2003). *Bemisia tabaci* has been a subject for several reviews after achieving international prominence in the early 1980s (De Barro et al. 2011, Annu. Rev. Entomol. 56: 1–19) as an agricultural pest and a vector of viral plant diseases. With global invasions of *B. tabaci*, it has been discovered that a substantial polymorphism occurs in different populations (Frohlich et al. 1999, Mol. Ecol. 8: 1683–1691; de Barro et al. 2011). In addition, genetically distinguishable biological forms of *B. tabaci* have been reported (Ashfaq et al. 2014, PLoS One 9: e104485). Based on numerous studies on phylogeny of *B. tabaci*, it is argued that *B. tabaci* is a species complex with at least 34 morphologically indistinguishable species (Boykin and De Barro 2014, Front. Ecol. Evol. 2: 45; De Barro et al. 2011).

More than 49 species of whiteflies have been reported from different parts of Sri Lanka (David 1993, FIPPAT Entomology Series. 3: 1–32) where *B. tabaci* is known to attack a variety of plants, including horticultural crops such as beans, brinjal, tomato, and cucurbits along with ornamental plants (Kumrasinghe et al. 2009, J. Plant Prot. Res. 49: 373–377; Marasinghe et al. 2017, J. Natl. Sci. Found. Sri. 45: 23–31). Some populations have reportedly developed resistance to several recommended insecticides (Marasinghe et al. 2017).

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Our objective in this study was to characterize *B. tabaci* populations from different agroclimatic zones in Sri Lanka by using the mitochondrial gene cytochrome oxidase subunit I (*cox1*). If, as stated previously, *B. tabaci* exists as species complex, then different genetic entities might have different host preferences, pathogen vectoring capacity, and levels of insecticide resistance. Hence, the presence of genetic groups would impact management of this pest.

Sampling locations were randomly selected among different agroecological zones and vegetable growing areas of Sri Lanka but also where commonly occurring whitefly types could be collected. Live whiteflies were aspirated from different types of vegetation in seven districts of Sri Lanka (Table 1; Fig. 1). Collected samples were categorized according to the collection site and host plants. Whiteflies were identified using published keys of Martin (2008, Trop Pest Manage. 33: 289–322) and Gregory et al. (2005, Florida Entomol. 88: 518–534). Collected adults were preserved in 70% ethyl alcohol and stored at −20°C for eventual molecular analysis.

DNA was extracted from individual whiteflies by using the DNeasy blood and tissue kit (Qiagen, CA, USA) following the manufacturer protocol. A portion of the *cox1* gene was amplified using the forward primer CI-J-1632 (5′ TGA TCA AAT TTA TAA T 3′) and the reverse primer CI-N-2191 (5′ GGT AAA ATT AAA ATA TAA ACT TC 3′) (Simon et al. 1994, Ann. Entomol. Soc. Am. 87: 651–701). For each amplification, PCR reactions were performed in a 25-μl volume that included 1 μl of DNA, each primer at 0.5 μM, 2.5 mM $MgCl_2$, 0.2 mM deoxyribonucleotide triphosphate mix, and 1.25 U *Taq* DNA polymerase in 1× PCR buffer (Bioline, UK). PCR parameters were 94°C for 4 min and 35 cycles of 94°C for 30 s, 50°C 30s, and 72°C for 45 s, followed by a final extension step of 72°C for 10 min. The amplified PCR products were purified using the Qiaquick PCR purification kit (Qiagen, CA, USA) using the manufacturer protocol and were sequenced at a sequencing facility at the University of Peradeniya, Sri Lanka, and M/s Macrogen, South Korea.

The sequences were manually edited in Finch TV (Geospiza, USA) and were aligned in Clustal W in MEGA 5.0 software (Tamura et al. 2011, Mol. Biol. Evol. 28: 2731–2739). The consensus sequence was obtained for each species. The aligned sequences for coding genes were translated into amino acid sequences by using the invertebrate mitochondrial coding to determine that they were coding and, therefore, unlikely to be nuclear pseudogenes. Once the alignment was completed, sequences were compared with the publicly available sequence data in GenBank using BLAST (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) and the BOLD interface (http://www.boldsystems.org) to confirm species identification.

Phylogenetic relationships among *B. tabaci* from India, China, and The Philippines were inferred using the maximum likelihood (ML) method. The substitution model selection was also performed in MEGA (version 5) based on the lowest Bayesian information criterion value. The Hasegawa-Kishino-Yano model for *cox1* sequence data set was selected. Bootstrap (Felsenstein 1985, Evol. 39: 783–791) supports were based on 1,000 resampled datasets using MEGA (version 5). Samples identified as *T. vaporariorum* during the study, along with available GenBank sequences of *T. vaporariorum*, were used as the outgroup.

The outcomes of the phylogenetic analysis *cox1* sequences of identified genetic groups of *B. tabaci* of Sri Lanka were used for genetic diversity analysis. Number of

Table 1. Whitefly samples collected and identified from different host plants cultivated in different localities of Sri Lanka.

Locality	District	Agroclimatic Zone	Host Plant(s)	Whitefly Genetic Group/Biotype	Reference GenBank Accession Numbers
Marassana	Kandy	Mid-country intermediate zone 3 (IM3a)	Tomato, cucurbits	*B. tabaci* Asia I, *T. vaporariorum*	MF152900, MF152902
Sooriyawewa	Hambantota	Low-country dry zone 1 (DL1b)	Brinjal (egg plant)	*B. tabaci* Asia I	MF152894
Huruluwewa	Anuradhapura	Low-country dry zone 1 (DL1b)	Brinjal	*B. tabaci* Asia I	MF152885
Bandarawela	Badulla	Up-country intermediate zone 3 (IUe3)	Brinjal, bean, tomato,	*B. tabaci* Asia I	MF152860
Matale, Bandarapola	Matale	Mid-country Intermediate Zone 3 (IM3b, WM3b)	Brinjal	*B. tabaci* Asia I	MF152904
Kurunegala	Kurunegala	Low-country intermediate zone (IL1a)	Brinjal, cucurbits	*B. tabaci* Asia I, *B. tabaci* Asia II	MF152882, MF152880
Kalpitiya	Puttalam	Low-country dry zone 3 (DL3)	Brinjal, cucurbits	*T. vaporariorum*	MF152890
Jaffna	Jaffna	Low-country dry zone 3 (DL3 & DL4)	Brinjal	*B. tabaci* Asia I	MF152872

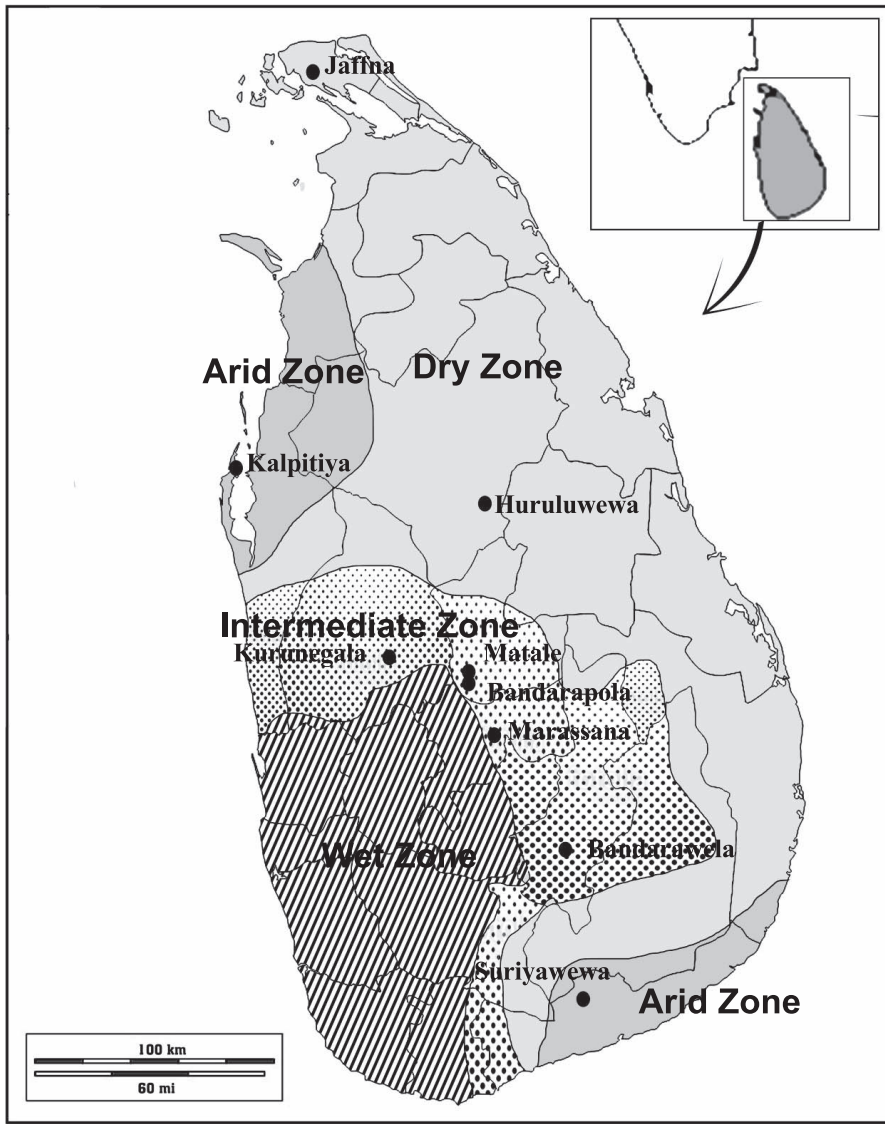

Fig. 1. Sri Lanka map showing whitefly samples collection localities from different agroclimatic zones.

haplotypes (h), genetic diversity indices (haplotype diversity index [Hd] and nucleotide diversity index [Pi]), and neutrality tests (Tajima's *D* and Fu's *F*) were performed in DnaSP version 5.1.10 (Librado and Rozas 2009, Bioinformatics. 25: 1451–1452). The pairwise fixation index (F_{ST}) for the identified genetic groups was determined in Arlequin 3.11 (Excoffier et al. 2007, Evol. Bioinform. 1: 47–50) and significance was evaluated based on 10,000 permutations.

A total of approximately 500 whitefly samples were collected from each study site. More than 75 randomly selected whitefly samples were morphologically screened for each study site. A total of 580 *B. tabaci* and 28 *T. vaporariorum* samples were morphologically identified during the study period. All whitefly adults were morphologically identified as *B. tabaci* except for some samples that were collected from Kalpitiya and Marassana in the Puttalam and Kandy districts, respectively, and identified as *T. vaporariorum*. The *cox1* sequences were used to confirm the morphological identifications of *B. tabaci* and *T. vaporariorum* and were submitted to Genbank (accession numbers for *cox1* sequences, MF152860–MF152907).

The 516-bp *cox1* sequences of 46 Sri Lankan *B. tabaci* samples representing all seven study sites were aligned along with GenBank entries for *B. tabaci* from India, China, Taiwan, and The Phillipines, representing Asia I and Asia II biotypes. The resulting ML tree with sample names and corresponding GenBank accession numbers is given in Fig. 2. The tree shows two distinct clades, namely Asia I and Asia II biotypes or genetic groups, for *B. tabaci* samples. The phylogenetic analysis revealed that two *cox1* sequences from Sri Lanka identified morphologically as *B. tabaci* clustered with GenBank entries for *B. tabaci* Asia II biotype groups. The remainder of the sequences grouped in a clade with Asia I biotype. Therefore, the phylogenetic analysis reveals for the first time that both the biotypes Asia I and Asia II of *B. tabaci* exist in Sri Lanka.

A total of 46 *cox1* sequences of *B. tabaci* representing a minimum of 4 sequences from each district were used for population genetic analysis. Translated amino acid sequences revealed that there are no frame shifts or stop codons in all the edited sequences. Irrespective of biotype groups, four different haplotypes (H1–H4) were identified among all 46 sequences, that is H1 with 34 sequences (GenBank accession number MF152860), H2 with 6 sequences (GenBank accession number MF152880), H3 with 5 sequences (GenBank accession number MF152895), and H4 with 1 sequence (GenBank accession number MF152900). The H1 was the dominant (73%) haplotype representing samples collected from all the localities and representing different agroclimatic zones. The haplotype diversities ($Hd = 0.434 \pm 0.082$) and nucleotide diversities ($Pi = 0.0296 \pm 0.009$) were low even with all *B. tabaci* samples (irrespective of biotypes). The neutrality test revealed that both Tajima's D (0.0603; $P > 0.1$) and Fu's and Li's F (1.555; $P > 0.05$) values were not significant for *B. tabaci* complex in Sri Lanka, indicating that the populations are evolving neutrally. These genetic diversity indices were not determined for each biotypes/genetic group of Sri Lanka as inferred in the ML phylogenetic tree, as only a single haplotype was found among 6 Asia II biotype/genetic group samples (GenBank accession number MF1528880) and 3 haplotypes (GenBank accession numbers MF152895 [5 samples], MF152860 [34 samples], and MF152900 [1 sample]) in 40 samples identified as Asia 1 biotype. However, the two biotype/genetic group samples of Sri Lanka had a pairwise F_{ST} value of 0.997 ($P > 0.01$), indicating the two biotype groups, even though sympatric, are genetically distinct and reproductively isolated.

This is the first report on genotyping of Sri Lankan *B. tabaci*, a major vector of viral diseases of horticultural crop plants, using *cox1* gene. The study further reveals for the first time that two Asia biotypes, namely Asia I and Asia II, are

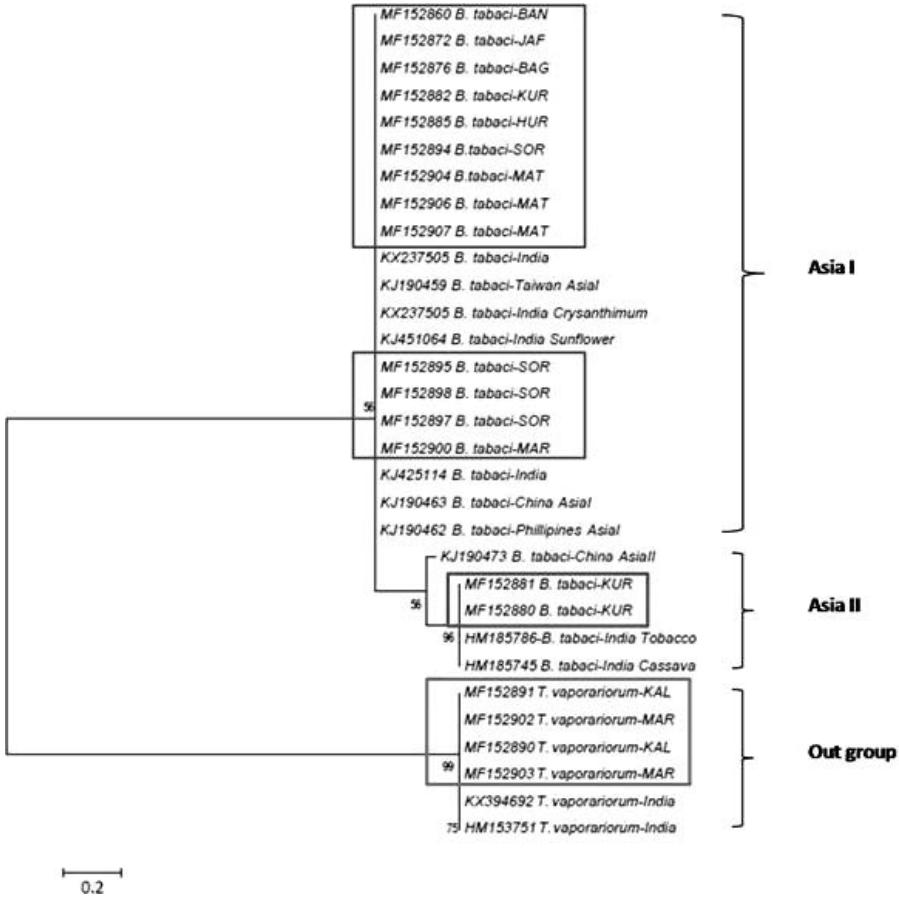

Fig. 2. Phylogenetic analysis based on *cox1* sequence having 516 positions in the data set and constructed using the maximum likelihood method using Hasegawa_Kishino-Yano model, showing bootstrap values >55%. All Sri Lankan samples are boxed and represented by GenBank accession numbers. Asia I biotype clade consists of all specimens identified as *B tabaci* except a few samples that were included in the Asia II clade and collected from Kurunagala district. Samples identified as *T. vaporariorum* and collected from Puttalam and Kandy districts along with other GenBank entries were used as out-groups.

present in Sri Lanka. Asia I genetic group is widespread in the country occupying different agroclimatic zones that include low-country dry zone (DL1b, DL3, and DL4), up-country intermediate zone (IUe3), mid-country wet zone (WM3b), and mid-country intermediate zone (IM3a and IM3b). However, Asia II is only confined to low-country intermediate zone (IL1a) (Table 1). Along with *B. tabaci*, *T. vaporariorum* is also present in Sri Lanka sharing the same host plant species. Sri Lankan

Asia I genetic group/biotype was collected from brinjal (eggplant), tomato, bean, and cucurbits, whereas Asia II was from brinjal and cucurbits (Table 1).

Bemisia emilieae Corbett collected from Sri Lanka in 1912 was recently identified as *B. tabaci* Asia II-7 based on next-generation sequencimg analysis (Tay et al. 2017, Sci. Rep. 7: 429). It has been reported that an invasion of a new genetic group can displace another genetic group due to intergenetic group competition (Liu et al. 2007, Science 318: 1769–1772), making a direct impact on pest-vectored disease management (Ashfaq et al. 2014). Therefore, further molecular characterization studies are warranted to determine the presence of different genetic groups of *B. tabaci* in Sri Lanka to monitor their prevalence and spread in different agroclimatic zones to develop effective whitefly control strategies.

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