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Article

Molecular Variation of Endosymbiotic Bacteria *Wolbachia* in *Bemisia tabaci* and Related Whiteflies

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Abstract

Whiteflies harbor several secondary endosymbionts, which are maternally inherited from mother to offspring, that have major effects on host preferences, biology, and evolution. Here, we identified *Wolbachia* bacteria in sweetpotato whitefly (*Bemisia tabaci*) as well as whitefly popluations from other countries by comparison of 16S rDNA sequences. *Wolbachia* were detected in all tested indigenous *B. tabaci* populations (Bangladesh, Myanmar, Nepal, and the Philippines) as well as Q1 biotype of Korea, whereas they were absent from B biotype of Korea and Q biotype of China. *Wolbachia* were also detected in all five tested *Aleurodicus dispersus* populations as well as *Tetraleurodes acaciae*, whereas they were not detected in all seven *Trialeurodes vaporariorum* populatuions. In addiiton, *Wolbachia* were detected in parasitic wasp (*Encarsia formosa*) of *B. tabaci* as well as honeybee (*Apis mellifera*). Among the 19 whitefly populations from different countries, our analysis identified four phylogenetic groups of *Wolbachia*, thereby demonstrating the high diversity of this genus. *Wolbachia* phylogeny suggests a correlation of geographical range with ecological variation at the species level.

Keywords : Population diversity, Spiraling whitefly, Secondary endosymbiont, Sweetpotato whitefly

Introduction

Endosymbiotic bacteria are harbored within the cells or tissues of many arthropods, including insects (Buchner, 1965; Brown et al., 1995 Frohlich et al., 1999). The symbiotic relationships between insects and endosymbionts drive evolutionary interactions, resulting in broad ranging activities from neutralism to ammensalism (Moran, 2007; Moya et al., 2008).

Endosymbionts can be categorized as either primary or secondary endosymbionts according to their physiological roles. The primary endosymbiont *Portiera aleyrodidarum* is harbored within bacteriocytes and supplements *B. tabaci* with essential amino acids for growth and development (Moran and Telang, 1998; Thao et al., 2000; Baumann, 2005). Although secondary endosymbionts are not necessary for host survival, they may play important roles in their host's physiology, ecology, and evolution (Zchori-Fein and Brown, 2002; Chiel et al., 2007). Until now, six secondary endosymbionts have been identified in *B. tabaci,* namely *Arsenophonus, Cardinium, Fritschea, Hamiltonella, Rickettsia,* and *Wolbachia* (Baumann, 2005; Gottlieb et al., 2006; Chiel et al., 2007).

All these bacteria possess the ability to manipulate the physiological characteristics of their hosts. Specifically, *Wolbachia, Arsenophonus, Cardinium*, and *Rickettsia* can manipulate host reproduction (Duron et al., 2008; Werren et al., 2008), *Hamiltonella* can induce virus resistance in pea aphid (Oliver et al., 2002), and *Rickettsia* increases thermotolerance in *B. tabaci* (Brumin et al., 2011). Further, fluorescence *in situ* hybridization (FISH) has revealed *Arsenophonus* infection within bacteriocytes of *B. tabaci* (Gottlieb et al., 2008). However, these characteristics of endosymbionts can be highly differentiated by genetic variations in various region, host insects and ecosystems.

The objectives of this study were to determine the geographic distribution of *Wolbachia* infection in *B. tabaci* and other whiteflies as well as the relationship between *Wolbachia* infection and evolutionary linkage in whiteflies.

Materials and methods

Collection of whitefly samples

Adult individuals of *B. tabaci* and other whiteflies were collected from various host plants, such as tomato, pepper, ridge gourd, bean, okra, eggplant, and guava, grown in Bangladesh, Myanmar, Nepal, China, the Philippines, and Korea (Tables

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2 and 3). In Bangladesh,we collected bean, okra, and eggplant from the southern and northern parts of the country in 2010-2011, immediately preserved samples in 99% ethanol, and stored them at -20℃ forfurther analysis. Adults of *B. tabaci* B and Q biotypes were collected from cucumber, sweet melon, and tomato plants grown throughout Korea in 2010 in order to compare the morphologies and genetic sequences of foreign whiteflies.

Identification of biotypes, endosymbionts, and TYLCV

Biotype of *B. tabaci* was determined by amplification of mitochondrial cytochrome oxidase subunit I (COI) gene fragments from extracted genomic DNA samples (Khasdan et al., 2005). The presence of *Wolbachia* in whiteflies was determined using specific primer sets for *Wolbachia* by amplification of 16S rDNA gene fragments (Chiel et al., 2007). The occurrence of *Tomato yellow leaf curl virus* (TYLCV) on cultivars of various horticultural crops was surveyed in different regions. TYLCV acquisition by *B. tabaci* was determined using a TYLCV-specific primer set that can amplify conserved intergenic sequences (Lee et al., 2010). Specific primer sets for biotypes, endosymbionts, and TYLCV are listed in Table 1. PCR reactions were performed in a 20 μl mixture containing 5× SuperTaq PCR buffer (10 mM Tris-HCL, 40 mM KCl, 1.5 mM $MgCl_2$, pH 9.0), 2.5 mM dNTPs, 0.5 μM of each primer, 1 unit of SuperTaq DNA polymerase (SuperBio Co, Korea), and 1 g of DNA as a template. The mixtures were amplified using a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with 3 min of initial denaturation at 95℃, 35 cycles of annealing (30 sec at 94℃, 30 sec at 55℃, 30 sec at 72℃), and 10 min of extension at 72℃.The PCR products were visualized on a 1.0% agarose gel containing ethidium bromide. Expected PCR products were excised from the gel and purified using the Wizard PCR preps DNA purification system (Promega, Madison, WI, USA) and sequenced either directly or by cloning into pGEM-T easy plasmid vector (Promega, Madison, WI, USA).

DNA sequence analysis

Sequences of the PCR products were determined using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and analyzed using a 3730XL DNA Sequencer (Applied Biosystems, Foster City, USA). Databases were searched using the BLAST algorithm (Altschul et al., 1997; Schäffer et al., 2001) in NCBI, and sequences were aligned using the MUSCLE program (Edgar, 2004). Mitochondrial COI sequences of whiteflies were analyzed using Bayesian MrBayes 3.0 software. Four Metropolises-coupled Markov Chain Monte Carlo (MCMC) chains were run until standard divergence of the split frequencies become lower than 0.01 (Ronquist and Huelsenbeck, 2003). All sequences were analyzed over 10 million generations, and four sequences were sampled every 100 generations. The first 25% of burn-in (SUMP and SUMT) cycles were discarded prior to the construction of consensus tree, which were visualized by MEGA 4.0 (Tamura et al., 2007).

Results

Identification of *Wolbachia* in *B. tabaci* populations

To establish the association between biotypes of *B. tabaci* and infection by different endosymbionts the presence of *Wolbachia* in indigenous whitefly populations from Bangladesh, Myanmar, Nepal, and Q biotype of China were examined by

Table 1. Nucleotide sequences of primers for *B. tabaci* and its biotypes. Secondary endosymbiont identification by PCR amplification

Observation	Primer Name	Primer Sequence (5' to 3')	Size (bp)	References	Anneal. Temp.
B. tabaci	C1-J-2195 L2-N-3014	F-TTGATTTTTTGGTCATCCAGAAGT R-TCCAATGCACTAATCTGCCATATTA	~860	Simon et al. 1994	52℃
B. tabaci	B. tab-Uni	F-GAGGCTGRAAAATTARAAGTATTTGG R-CTTAAATTTACTGCACTTTCTGCCAYATTAG	~748	Shatters et al. 2009	64℃
Biotype	LR-J-12887 LR-N-13398	F-CCGGTTTGAACTCAGATCATGT R-CGCCTGTTTAACAAAAACAT	~520	Simon et al. 1994	55℃
Wolbachia	16S rDNA	F-CGGGGGAAAAATTTATTGCT R-AGCTGTAATACAGAAAGTAAA	~650	Heddi et al, 1999	55℃
TYLCV	TYLCV-CP TYLCV-CP	F-TGGGGATTCACAAATGTTTTCT R-CTGAACTTCGACAGCCCAT	~1000	Shatters et al. 2009	50℃

Table 2. Profile of secondary endosymbiotic bacteria in different biotypes of *B. tabaci* on different host plants

Biotype	Host Plants	Locations	TYLCV	Wolbachia
B	Cucumber	Goyang, Korea	-	-
Q1	Various	Various places, Korea	+/-	+/-
Q	Tomato	Qingdao, China	-	-
Indigenous	Bean	Patuakhali, Bangladesh	-	+
Indigenous	Bean	Patuakhali, Bangladesh	+	+
Indigenous	Eggplant	Patuakhali, Bangladesh	-	+
Indigenous	Eggplant	Patuakhali, Bangladesh	-	+
Indigenous	Eggplant	Kurigram, Bangladesh	+	+
Indigenous	Eggplant	Kurigram, Bangladesh	-	+
Indigenous	Okra	Kurigram, Bangladesh	-	+
Indigenous	Eggplant	Kyuktan, Myanmar	+	+
Indigenous	Ridge gourd	Kyuktan, Myanmar	+	+
Indigenous	Ridge gourd	Magway, Myanmar	-	+
Indigenous	Eggplant	Yangon, Myanmar	-	+
Indigenous	Marigold	Kathmandu, Nepal	-	+
Indigenous	Chili (Pepper)	Kathmandu, Nepal	-	+
Indigenous	Cucumber	Kathmandu, Nepal	-	+
Indigenous	Brinjal	Kathmandu, Nepal	+	+
Indigenous	Tomato	Kathmandu, Nepal	+	+

PCR analysis of 16S rDNA sequences (Table 2). *Wolbachia* were detected in all tested whitefly populations regardless of location, but not in Q biotype of China.

Wolbachia in other whiteflies

We collected many other genera of whiteflies from Korea, Bangladesh, Myanmar, Nepal, New Zealand, Japan, and the Philippines. We found that *Wolbachia* infection in spiraling whitefly (*Aleurodicus dispersus*) and acacia whitefly (*Tetraleurodes acaciae*) was very common (Table 3). On the other hand, *Wolbachia* infection was apparently absent from all examined greenhouse whitefly (*Trialeurodes vaporariorum*) populations.

Phylogenetic analysis of *Wolbachia*

Neighbour-joining phylogenetic tree reconstruction based on 19 16S rDNA sequences of *Wolbachia*, which were detected in *B. tabaci, T. acaciae, A. dispersus,* and *A. mellifera* from various countries, is shown in Figure 1. The results showed that the distribution of *Wolbachia* endosymbionts was highly diverse due to host and geographical variations. We observed high genetic variance among the 16S rDNA sequences of *Wolbachia* from different countries and established four distinct clades, namely W1 – W4. Diversified endosymbiont identification for *Wolbachia* was performed by sequencing the PCR products of individuals originating from Bangladesh, Myanmar, Nepal, the Philippines, and Korea.

Analysis of 16S rDNA gene sequences of *Wolbachia*

Nineteen 16S rDNA gene sequences of *Wolbachia* in whiteflies, parasitic wasp, and honeybee from different countries were analyzed. There were no nucleotide insertions or deletions found. The sequences showed A+T and G+C residue compositions of 52.8% and 47.2%, respectively, at the 3rd codon position. The average proportion of T: C: A: G was 22.5: 18.1: 30.3: 29.1 with a narrow standard error around the means, which indicated the base composition varied substantially within the sequences. Among these 523 nucleotides, 513 characters were conserved, 10 characters were variable, and nine characters were informative for parsimony analysis (Fig. 2 and Table 4). Sequence divergence in pairwise comparisons revealed that *Wolbachia* was not a diverse group compared with other

Table 3. Profile of secondary endosymbiotic bacteria in various whiteflies, parasitic wasp and honey bee from domestic and foreign countries

Species	Host plants / vectors	Locations	Wolbachia
Trialeurodes vaporariorum	Tomato	Euiseong, Korea	-
	Tomato	Gimcheon, Korea	-
	Cucumber	Sangju, Korea	-
	Tomato	Kathmandu, Nepal	-
	Brinjal	Kathmandu, Nepal	-
	Unknown	Japan	-
	Unknown	New Zealand	-
Tetraleurodes acaciae	Unknown	Calamba, Philippines	+
Aleurodicusdispersus	Eggplant	Patuakhali, Bangladesh	+
	Guava	Dumki, Bangladesh	+
	Guava	Magway, Myanmar	+
	Unknown	Calamba, Philippines	+
	Unknown	Calamba, Philippines	+
Encarsia formosa	*B. tabaci*	Daegu, Korea	+
Apis mellifera	-	Yecheon, Korea	+
Apis mellifera	-	Yecheon, Korea	+

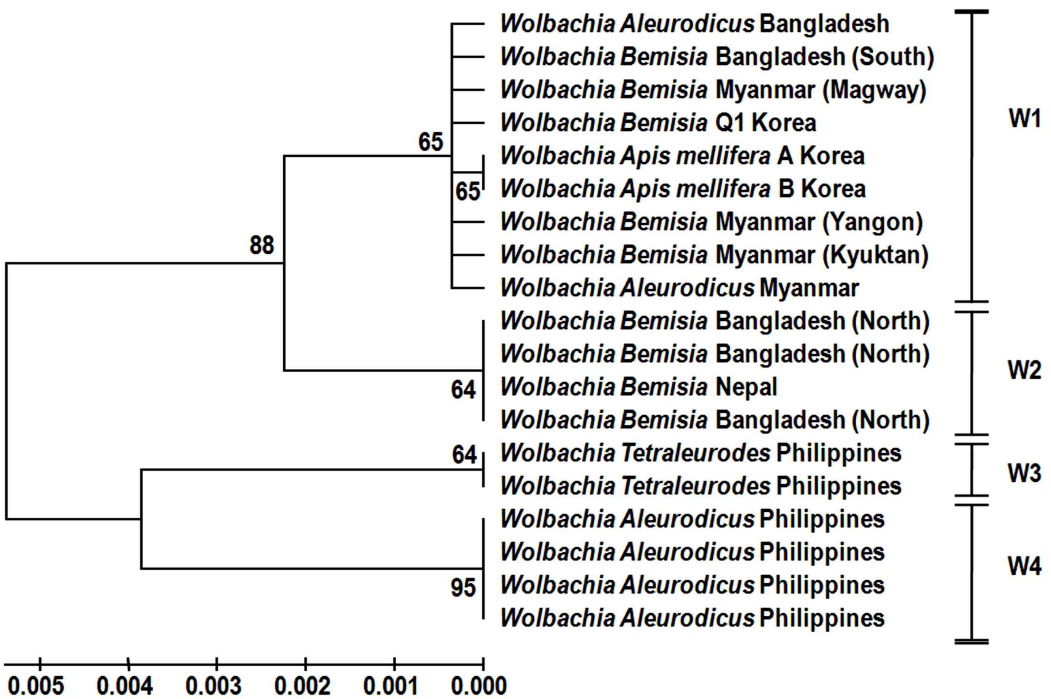

Fig 1. According to the Bayesian method, a neighbor-joining (NJ) tree was constructed based on a fragment (~625 bp) using Kimura two-parameter distances with complete deletion of gap/missing data using partial 16S rDNA sequences.

The number on each branch is the bootstrap support (1,000 replicates). Phylogenetic relationships among 16S rDNA sequences of *Wolbachia* in *B. tabaci* were compared with other *Wolbachia* in various species of whiteflies and honey bee.

Table 4. Pairwise distance among 19 *Wolbachia* endosymbionts in various whiteflies from different countries based on 16S rDNA gene sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[1]		0.000	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.010	0.008	0.008	0.008	0.008	0.008	0.008	0.008
[2]	0		0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.010	0.008	0.008	0.008	0.008	0.008	0.008	0.008
[3]	4	4		0.000	0.000	0.000	0.012	0.012	0.012	0.014	0.012	0.012	0.012	0.012	0.012	0.012	0.012
[4]	4	4	0		0.000	0.000	0.012	0.012	0.012	0.014	0.012	0.012	0.012	0.012	0.012	0.012	0.012
[5]	4	4	0	0		0.000	0.012	0.012	0.012	0.014	0.012	0.012	0.012	0.012	0.012	0.012	0.012
[6]	4	4	0	0	0		0.012	0.012	0.012	0.014	0.012	0.012	0.012	0.012	0.012	0.012	0.012
[7]	4	4	6	6	6	6		0.000	0.004	0.002	0.000	0.000	0.000	0.004	0.004	0.004	0.000
[8]	4	4	6	6	6	6	0		0.004	0.002	0.000	0.000	0.000	0.004	0.004	0.004	0.000
[9]	4	4	6	6	6	6	2	2		0.006	0.004	0.004	0.004	0.000	0.000	0.000	0.004
[10]	5	5	7	7	7	7	1	1	3		0.002	0.002	0.002	0.006	0.006	0.006	0.002
[11]	4	4	6	6	6	6	0	0	2	1		0.000	0.000	0.004	0.004	0.004	0.000
[12]	4	4	6	6	6	6	0	0	2	1	0		0.000	0.004	0.004	0.004	0.000
[13]	4	4	6	6	6	6	0	0	2	1	0	0		0.004	0.004	0.004	0.000
[14]	4	4	6	6	6	6	2	2	0	3	2	2	2		0.000	0.000	0.004
[15]	4	4	6	6	6	6	2	2	0	3	2	2	2	0		0.000	0.004
[16]	4	4	6	6	6	6	2	2	0	3	2	2	2	0	0		0.004
[17]	4	4	6	6	6	6	0	0	2	1	0	0	0	2	2	2	

Total nucleotide differences (below diagonal) and mean character differences (above diagonal) was analysed using Kimura two-parameter distances between 16S rDNA genes of *Wolbachia*. Numbers indicated *Tetraleurodes acaciae* from the Philippines (1-2), *Aleurodicus dispersus* from the Philippines (3-6), Bangladesh (7), Myanmar (8), Indigenous *Bemisia tabaci* from Nepal (9), Bangladesh W1 (10), Korea (11), Myanmar (Kyuktan) (12), Myanmar (Kyuktan) (13), Bangladesh W2 (14-16), Myanmar (Yangon) (17).

endosymbionts. Although *Wolbachia* was characterized by low divergence and minimal variability, it was made into four phylogenetic clades in which nucleotides 1-7 were altered. The lowest distance was 0.002 and the highest was 0.014 among all examined *Wolbachia* sequences. *Wolbachia* in *B. tabaci* from Bangladesh (south) (BW1), Myanmar (Magway, Kyuktan, and Yangon), and Korea (Q1 biotype), *Apis mellifera* from Korea, and *A. dispersus* from Bangladesh and Myanmar constituted a single clade, W1, in the tree. Furthermore, *Wolbachia* in *B. tabaci* from Bangladesh (north) (BW2) and Nepal made another clade, W2. The remaining two clades, W3 and W4, consisted of *Wolbachia* in *T. acaciae* and *A. dispersus* from the Philippines, respectively (Fig. 1 and Table 5). The genetic relationships between the *Wolbachia* sequences were extracted using the same neighbor-joining method (NJ). Analysis runs were performed with Kimura 2-parameter distance model using the Mega program. The inferred phylogenetic topology based on the NJ tree did not exhibit diversity. Results showed that *Wolbachia* was divergent due to geographic but not host features. *Wolbachia* made the same clade even though it was harbored in different species (different hosts).

Discussion

This study was attemtped to identify and analyze *Wolbachia* infection in different hosts from Bangladesh, Myanmar, Nepal, the Philippines, and Korea. The presence of *Wolbachia* was very consistent among indigenous genetic groups of *B. tabaci, T. acaciae*, and *A. dispersus*, whereas it was slightly present in *B. tabaci* Q1 biotype from Korea. In support of our results, high rates of *Wolbachia* infection were reported in invasive and indigenous B biotypes as well as Q biotype from China (Li et al., 2007; Ahmed et al., 2009, 2010).

The presence of *Wolbachia* was not consistent among *B. tabaci* populations. *Wolbachia* was only present in *B. tabaci* Q biotype from Israel, whereas it was absent from B biotype (Chiel et al., 2007). Gueguen et al. (2010) showed that *Wolbachia* is present in Q1 and Q2 biotypes, but not in B or Q3 biotype. Our results further show that *Wolbachia* infection was very common in all collected indigenous biotype populations from different countries, except for B biotype from Korea. This

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Indigenous Bangla     AGCTAGTTGGTGGAGTAATAGCCTACCAAGGCAATGATCTATAGCTGATCTGAGAGGATGAT   62
Indigenous Myanmar    ............................................................   62
Indigenous Nepal      ............................................................   62
Q-biotype  Korea      ................-.........-...............................   60

Indigenous Bangla     CAGCCACACTGGAACTGAGATACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG  124
Indigenous Myanmar    ............................................................  124
Indigenous Nepal      ............................................................  124
Q-biotype  Korea      ............................................................  122

Indigenous Bangla     GACAATGGGCGAAAGCCTGATCCAGCTATGCCGCATGAGTGAAGAAGGCCTTTGGGTTGTAA  186
Indigenous Myanmar    ............................................................  186
Indigenous Nepal      ............................................................  186
Q-biotype  Korea      ........................-...................................  183

Indigenous Bangla     AGCTCTTTTAGTGAAGAAGATAATGACGGTACTCACAGAAGAAGTCCTGGCTAACTCCGTGC  248
Indigenous Myanmar    ..............G.............................................  248
Indigenous Nepal      ..............G.............................................  248
Q-biotype  Korea      ..............G.............................................  245

Indigenous Bangla     CAGCAGCCGCGGTAATACGGAGAGGGCTAGCGTTATTCGGAATTATTGGGCGTAAAGGGCGC  310
Indigenous Myanmar    ............................................................  310
Indigenous Nepal      ............................................................  310
Q-biotype  Korea      ............................................................  307

Indigenous Bangla     GTAGGCTGATTAATAAGTTAAAAGTGAAATCCCGAGGCTTAACCTTGGAATTGCTTTTAAAA  372
Indigenous Myanmar    ............................................................  372
Indigenous Nepal      ............................................................  372
Q-biotype  Korea      ............................................................  369

Indigenous Bangla     CTATTAATCTAGAGATTGAAAGAGGATAGAGGAATTCCTGATGTAGAGGTAAAATTCGTAAA  434
Indigenous Myanmar    ............................................................  434
Indigenous Nepal      ............................................................  434
Q-biotype  Korea      ............................................................  431

Indigenous Bangla     TATTAGGAGGAACACCAGTGGCGAAGGCGTCTATCTGGTTCAAATCTGACGCTGAGGCGCGA  496
Indigenous Myanmar    ............................................................  496
Indigenous Nepal      ............................................................  496
Q-biotype  Korea      ............................................................  493

Indigenous Bangla     AGGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTATAAACGAT         550
Indigenous Myanmar    ...................................................            550
Indigenous Nepal      ...................................................            550
Q-biotype  Korea      ...................................................            547
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Fig 2. Sequence alignments of *Wolbachia* in *B. tabaci* from different countries using partial 16S rDNA gene sequences (5'-3') by the ClustalW2 program

finding contradicts a study by Li et al. (2007) and Ahmed et al. (2009, 2010). They showed a high rate of *Wolbachia* infection in invasive and indigenous B biotypes as well as Q biotype from China. Our sequence analysis shows that *Wolbachia* diverged into four clades, each having a different host. Recently, a high level of genetic diversity was reported for *Wolbachia*, with 36 unique strains detected (Ros et al., 2012). Similarly, 19 allelic profiles and six phylogenetic groups were obtained for the endosymbiont *Arsenophonus* among 152 individuals, demonstrating this bacterium's high diversity (Mouton et al., 2012). It is estimated that about 66% of all insects are infected with *Wolbachia* (Hilgenboecker et al., 2008). This diverse genus

Table 5. Percentages of nucleotide frequencies in variable DNA sites of *Wolbachia* endosymbiont in various whiteflies from different countries based on 16S rDNA gene sequences

Wolbachia	Nucleotide composition (%)				Conserved sites (%) (513/523)				Variable sites (%) (10/523)				Parsim-info sites (%) (9/523)				Total
	T	C	A	G	T	C	A	G	T	C	A	G	T	C	A	G	
Ta-P	22.6	18.0	30.4	29.1	22.6	17.9	30.6	28.8	20.0	20.0	20.0	40.0	22.2	22.2	22.2	33.3	523
Ad-P	22.8	17.8	30.6	28.9	22.6	17.9	30.6	28.8	30.0	10.0	30.0	30.0	33.3	11.1	33.3	22.2	523
Ad-B	22.4	18.2	30.2	29.3	22.6	17.9	30.6	28.8	10.0	30.0	10.0	50.0	11.1	33.3	11.1	44.4	523
Ad-M	22.4	18.2	30.2	29.3	22.6	17.9	30.6	28.8	10.0	30.0	10.0	50.0	11.1	33.3	11.1	44.4	523
Bt-N2	22.2	18.4	30.4	29.1	22.6	17.9	30.6	28.8	0.0	40.0	20.0	40.0	0.0	44.4	22.2	33.3	523
Bt-BW1	22.4	18.2	30.4	29.1	22.6	17.9	30.6	28.8	10.0	30.0	20.0	40.0	11.1	33.3	11.1	44.4	523
Bt-K	22.4	18.2	30.2	29.3	22.6	17.9	30.6	28.8	10.0	30.0	10.0	50.0	11.1	33.3	11.1	44.4	523
Bt-M15	22.4	18.2	30.2	29.3	22.6	17.9	30.6	28.8	10.0	30.0	10.0	50.0	11.1	33.3	11.1	44.4	523
Bt-M16	22.4	18.2	30.2	29.3	22.6	17.9	30.6	28.8	10.0	30.0	10.0	50.0	11.1	33.3	11.1	44.4	523
Bt-BW2	22.2	18.4	30.4	29.1	22.6	17.9	30.6	28.8	0.0	40.0	20.0	40.0	0.0	44.4	22.2	33.3	523
Bt-M18	22.4	18.2	30.2	29.3	22.6	17.9	30.6	28.8	10.0	30.0	10.0	50.0	11.1	33.3	11.1	44.4	523
Am-K	22.6	18.2	30.0	29.3	22.6	17.9	30.6	28.8	20.0	30.0	0.0	50.0	22.2	33.3	0.0	44.4	523
Avg.	22.5	18.1	30.3	29.1	22.6	17.9	30.6	28.8	14.2	26.8	16.8	42.1	15.8	29.8	18.1	36.3	523

Samples were colledted from *Tetraleurodes acaciae* from the Philippines (Ta-P), *Aleurodicus dispersus* from the Philippines (Ad-P), Bangladesh (Ad-B), Myanmar (Ad-M), *Bemisia tabaci* from Nepal (Bt-N2), Bangladesh (Bt-W1, W2), Korea (Bt-K), Myanmar (Bt-M15, 16, 18), *Apis mellifera* from Korea (Am-K).

is subdivided into 11 "supergroups" (A-K) on the basis of molecular phylogenetic analysis (Bandi et al., 1998; Bordenstein and Rosengaus, 2005; Casiraghi et al., 2005; Ros et al., 2009). *Wolbachia* and *Cardinium* have been found to co-infect the same host species (Duron et al., 2008).

In conclusion, the present study shows that all of the *B. tabaci* populations collected from host plants in Bangladesh, Myanmar, and Nepal were infected by *Wolbachia*, whereas the Q biotype population from China was not. According to the 16S rDNA sequence analysis, we identified four phylogenetic clades, illustrating the divergence of *Wolbachia* endosymbionts.

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