Prevalence of a New Genetic Group, MEAM-K, of the Whitefly Bemisia tabaci (Hemiptera: Aleyrodidae) in Karnataka, India, as Evident from mtCOI Sequences

Authors: Roopa, H. K., Asokan, R., Rebijith, K. B., Hande, Ranjitha H., Mahmood, Riaz, et al.

Source: Florida Entomologist, 98(4) : 1062-1071

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.098.0409

Prevalence of a new genetic group, MEAM-K, of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Karnataka, India, as evident from *mtCOI* sequences

H. K. Roopa[1,], R. Asokan[1], K. B. Rebijith[1], Ranjitha H. Hande[1], Riaz Mahmood[2], and N. K. Krishna Kumar[3]*

Abstract

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an economically important agricultural pest globally that costs growers billions of dollars to control. Given the pest status of *B. tabaci* and its propensity for invasiveness, a detailed work to resolve the prevalence of *B. tabaci* genetic groups is warranted. Hence, we analyzed mitochondrial cytochrome oxidase I sequences from 71 samples of *B. tabaci* to determine the prevalence of genetic groups on various host plants in India. Results revealed the prevalence of the 4 previously existing genetic groups, namely Asia-I, Asia-II-7, Asia-II-8, and Middle East Asia Minor-1 (MEAM-1), and of a new group called Middle East Asia Minor-K, which is genetically close (92.6%) to MEAM-1.

Key Words: putative species; Middle East Asia Minor-K; mitochondrial cytochrome oxidase I

Resumen

La mosca blanca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) es una plaga económicamente importante para la agricultura a nivel mundial que cuesta miles de millones de dólares a los productores en controlar. Dado el estatus de plaga de *B. tabaci* y su propensión para invadir, se justifica un trabajo detallado para resolver la prevalencia de los grupos genéticos de *B. tabaci.* Por lo tanto, se analizaron las secuencias mitocondriales de citocromo oxidasa I de 71 muestras de *B. tabaci* para determinar la prevalencia de grupos genéticos en varias plantas hospederas en la India. Los resultados revelaron que la prevalencia de los 4 grupos genéticos ya existentes, Asia-I, Asia-II-7, Asia-II-8 y Asia de Medio Oriente Menor-I (MEAM-I) y de un nuevo grupo llamado Asia de Medio Oriente Menor-K, que es genéticamente cercano (92.6%) al MEAM-1.

Palabras Clave: especies putativas; Asia de Medio Oriente Menor-K; citocromo oxidasa I mitocondrial

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a pest of global importance that causes significant crop loss as a direct pest and as a vector of 120 geminiviruses, especially begomoviruses (Jones 2003). It is highly polyphagous, feeding on an estimated 900 hosts of agricultural, fiber, vegetable, and ornamental crops (Cahill et al. 1996; Jones 2003). It stands as one of the world's 100 invasive species (International Union for Conservation of Nature and Natural Resources [IUCN] list: http://www.issg.org). Emerging new genetic groups of *B. tabaci* increase the risk of transmission of geminiviruses to many crops worldwide and of development od high levels of resistance of this insect to various insecticides, especially neonicotinoids (Horowitz et al. 2003, 2004; Rauch &Nauen 2003). *Bemisia tabaci* is a species complex that contains morphologically indistinguishable biotypes or cryptic species or genetic groups (Dinsdale et al. 2010; De Barro et al. 2011; Liu et al. 2012). However, biotypes—as designated based on esterase banding pattern (Costa &Brown 1991)—and genotypes have recently been elevated to putative species of *B. tabaci* (Dinsdale et al. 2010; De Barro et al. 2011).

The nymphs and adults actively feed on the phloem sap and excrete copious amounts of honeydew, which supports the growth of sooty mold that in turn impedes photosynthesis (Byrne &Bellows 1991). Several studies examined the host-related phenotypic variations in *B. tabaci* and concluded that variations are evident not only among the populations on different hosts but also among individuals occurring on the same host (Mound 1963; Palaniswami et al. 1996; Lisha et al. 2003). Morphometric analysis of 4th instar nymphs revealed phenotypic variations corresponding to variations in the leaf anatomy of the host plant (Maruthi et al. 2007). *Bemisia tabaci* is known to be an aggressive colonizer of crops, with varying traits at the morphological (Bellows et al. 1994; Costa et al. 1995; Rosell et al. 1997), biochemical (Costa &Brown 1991; Brown et al. 2000; Perring 2001), and molecular levels (Gawel & Bartlett 1993; De Barro et al. 2005; Boykin et al. 2007).

Different geographical and ecological variations with host plant specialization offer an ideal system for the study of sympatric speciation in *B. tabaci*, direct damage to crops and vector ability (Chu

[1]Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta Lake P.O Karnataka, Bengaluru 560 089, India
[2]Department of Biotechnology and Bioinformatics, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shimoga577 451, India
[3]Division of Horticulture, ICAR, Krishi Anusandhan Bhawan - II, New Delhi 110 012, India
*Corresponding author; E-mail: roopa21hk@gmail.com
Supplementary material for this article in Florida Entomologist 98(4) (Dec 2015) is online at http://purl.fcla.edu/fcla/entomologist/browse

et al. 2007). De Barro et al. (2011) stated that asymmetrical mating interference provides a clear mechanism that could contribute to formation of global genetic structure. Genetic differentiation of different populations in the species complex was analyzed mainly based on the ribosomal internal transcribed spacer 1 (*rITS-1*) and mitochondrial cytochrome oxidase I (*mtCOI*) sequences worldwide (Simon et al. 1994; Hu et al. 2011; Zasada et al. 2014). Dinsdale et al. (2010) identified 3.5% pair-wise genetic divergence as the considered boundary for separating different species; this was further supported with either complete or partial mating isolation between a number of putative *B. tabaci* "species" (Xu et al. 2010; Wang et al. 2011). Considering all the past taxonomical approaches, Boykin et al. (2012) defined species in the *B. tabaci* species complex. In the present study, we examined the genetic variation in the *mtCOI* region of the genome of *B. tabaci*, collected on 30 different host plants from different locations in Karnataka, India, to assess the presence and prevalence of various genetic groups.

Materials and Methods

Nymphs and adults of *B. tabaci* were collected with a handheld aspirator from different locations in Karnataka, India, on 30 different hosts (Fig. 1 and Table 1) and preserved in 70% alcohol until further use.

GENOMIC DNA EXTRACTION AND AMPLIFICATION

The total DNA was extracted from individual adult *B. tabaci* specimens using DNAeasy Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol and stored at −80 °C until further use. Polymerase chain reaction (PCR) was performed with *mtCOI* primers F-C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and R-L2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon et al. 1994; Frohlich et al. 1999). The 25 µL PCR consisted of 50 ng/µL template,10 picomoles of each primer, 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 1 U Taq polymerase, and 2.5 µL Taq buffer (Fermentas GmBH, St. Leon-Rot, Germany). The PCR cycling was carried out in a thermal cycler (Applied Biosystems, Veriti 96 wells, USA) with the following parameters: 94 °C for 5 min as initial denaturation followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s, and extension at 72 °C for 50 s, and 72 °C for 10 min as the final extension. The amplified products were resolved in 1.0% agarose gel, stained with ethidium bromide (10 µg/mL), and visualized under UV light. Amplified PCR products were eluted using the gel extraction kit Nucleospin® Extract II (Macherey-Nagel, Duren, Germany) according to the manufacturer's protocol, and sequencing was performed in M13 forward and reverse directions.

SEQUENCE ANALYSES

All *mtCOI* sequences corresponding to different genetic groups of *B. tabaci* were downloaded from the National Center for Biotechnology Information (NCBI) GenBank (Suppl. Table 1, available online at http://purl.fcla.edu/fcla/entomologist/browse). Sequence alignment was performed employing MUSCLE implemented in Seaview (Thompson et al. 1994). Genetic divergence was calculated employing MEGA5 (Tamura et al. 2011). The nucleotide substitution model for the best fits and the model parameters were estimated using Akaike Information Criterion implemented in the program MODELTEST 3.7 (Posada &Crandall 1998) in conjunction with PAUP*. Maximum parsimony and maximum likelihood analyses were performed with PAUP* 4.0b10 (Swofford 1998), using the heuristic search procedure with 1,000 random additions of sequences and 10 trees held at each pseudo-replicate, and the tree bisection reconnection branch swapping method with all characters was treated as unordered and equally weighted. The chosen model with estimated parameters was used to derive the maximum likelihood tree in RaxML with the heuristic search settings. The same software was used to generate consensus trees using the CONSENSE program (http://evolution.genetics.washington.edu/phylip/doc/consense.html), and the tree was rooted with the outgroup *Bemisia afer* (Priesner & Hosny) (Hemiptera: Aleyrodidae). Phylogenetic analysis was performed using MrBayes (Huelsenbeck & Ronquist 2001). MrBayes 3.1 was run for 10 million generations by using 8 chains and sampled every 1,000 generations. All runs reached a plateau in the likelihood score, and the same was indicated by standard deviations of split frequencies (0.0023). Our 4 Markov Chain Monte-Carlo chains were converged, indicated by the potential scale reduction factor, which was close to one. The burn-in parameter was estimated employing Tracer version 1.5 (Rambaut & Drummond 2009), and the trees corresponding to the first 20% of generations were discarded. We performed 2 independent MrBayes runs to ensure the analyses were not trapped in the local optima, after which topologies and posterior probabilities (PP) from these 2 runs were compared for congruence purpose. The trees obtained were visualized using FigTree v1.3.1 (Rambaut 2009).

Results

The *mtCOI* gene was successfully amplified and sequenced from 71 individual adult *B. tabaci*. We analyzed 131 sequences, including the 71 generated from this study. The sequence analysis revealed that out of 816 bp, 332 nucleotides were conserved, 484 were variable, and 379 were parsimony informative. Absence of stop codons indicated that no pseudogenes were amplified within the sequences, and similar base composition indicated no indels. *Bemisia tabaci* nucleotide frequencies were 24.46% (A), 43.10% (T/U), 19.23% (C), and 13.22% (G). Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics in Table 2. The base composition of the *mtCOI* gene fragment was biased toward adenine (A) and thymine (T) with an overall 67.56%. The transition/transversion rate ratios were k_1 = 6.255 (purines) and k_2 = 6.190 (pyrimidines). The overall transition/transversion bias was R = 2.629. All sequences were deposited in the NCBI GenBank with the accession numbers KF790634 to KF790689 and HQ268814, HQ268813, HQ268812, HQ268811, HQ331246, HQ331245, and HQ331244.

PHYLOGENETIC ANALYSIS

Based on the phylogenetic analyses, the Indian *B. tabaci* samples were clustered into Asia-I, Asia-II-7, Asia-II-8, Middle East Asia Minor-1 (MEAM-1), and MEAM-K groups (Figs. 2a and 2b). The Asia-I genetic group was predominant representing 44 of the 71 (61.97%) samples sequenced. The Asia-I population had 17 different host plant species (Table 1), of which *Solanum lycopersicum* L., *Solanum melongena* L. (Solanales: Solanaceae), *Gossypium hirsutum* L., and *Abelmoschus esculentus* (L.) Moench (Malvales: Malvaceae) were the most frequent host plants and are widely distributed in Karnataka (Fig. 2b). Few samples collected from Karnataka clustered with Asia-II-7 and were found on 9 host plant species. The Asia-II-8 genetic group contained 5 samples, which had been collected on 5 different host plants. The most important genetic groups MEAM-1 and MEAM-2 formed 2 subclusters representing B and B2 genotypes. Here, MEAM-1 comprised 5 sequences, which had been collected on 3 host plants (mostly cabbage). However, 3 sequences from speci-

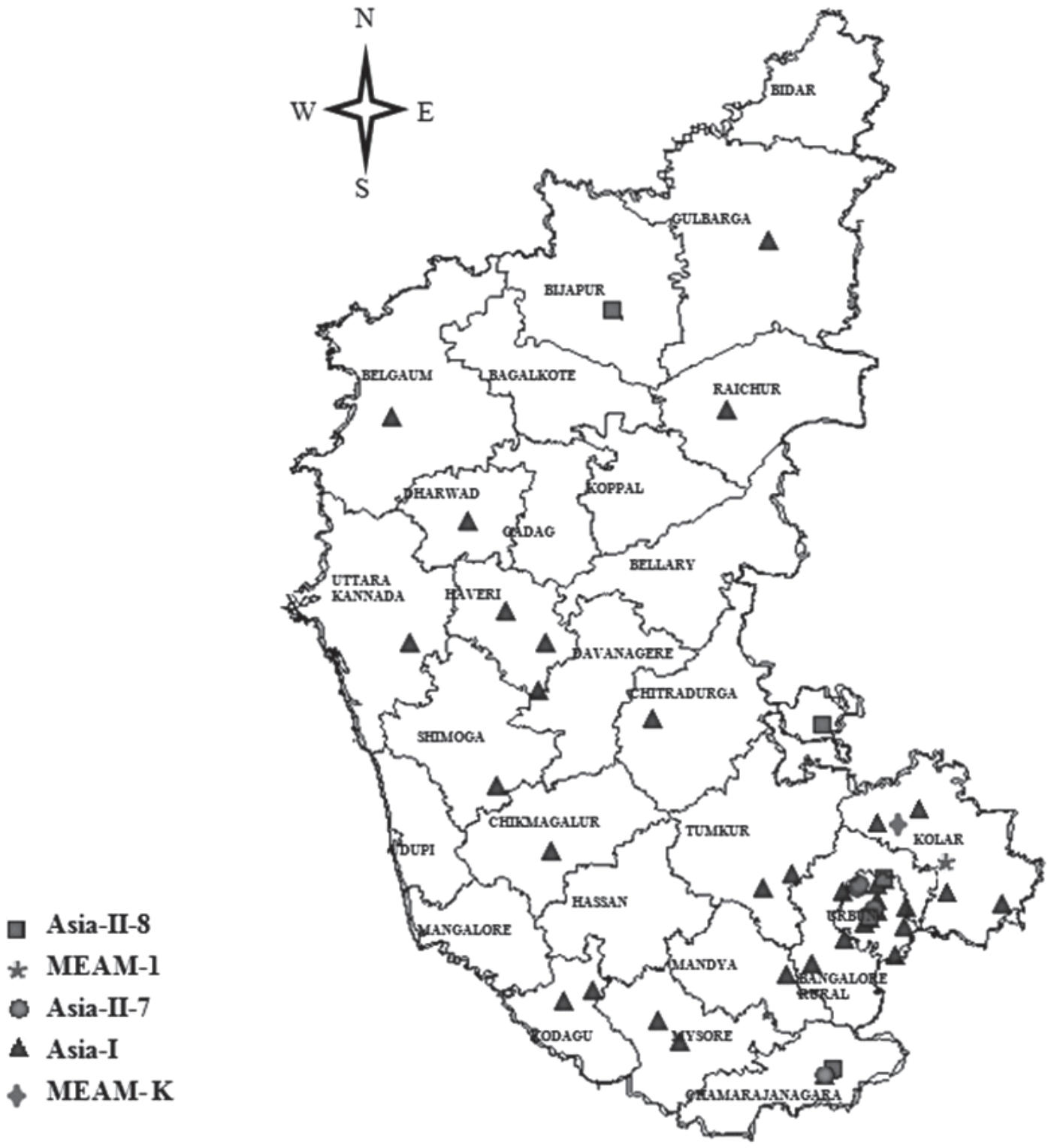

Fig. 1. Map indicating different locations of *Bemisia tabaci* sampling and distribution of genetic groups in Karnataka, India.

mens collected on *Phaseolus vulgaris* L. (Fabales: Fabaceae) at Kolar, H-Cross, and Malur (Karnataka, India) diverged from all the MEAM-1, MEAM-2, Indian Ocean, and Mediterranean groups. Thus, they formed an independent genetic group, which we named MEAM-K group. The genetic divergence ranged from 4.0 to 24.0% with an average of 15.7% (Table 3 and Fig. 3).

Discussion

"There is probably no other concept in biology that has remained as consistently controversial as the species concept" (Mayr 1982). This quote is remarkably true with *B. tabaci*, because this species is difficult

Table 1. Details of *Bemisia tabaci mtCOI* sequences generated for this study along with place of collection, accession number, voucher specimen number, respective genetic group, and host plants.

Sl no.	Host	Common name	Bangalore, Karnataka, India	Accession number	Latitude	Longitude	Genetic group	Voucher specimen	Year of collection
1	*Ocimum sanctum*	Tulsi	Malleshwarum	KF790634	12.98	77.57	Asia-II-7	HKR01	2012
2	*Parthenium hysterophorus*	Aster (white top weed)	Tarabanahalli	KF790635	13.11	77.47	Asia-II-7	HKR02	2012
3	*Raphanus sativus*	Radish	IIHR	KF790636	13.13	77.48	Asia-II-7	HKR03	2012
4	Unknown Ornamental	Ornamental	Malleshwarum	KF790637	12.98	77.57	Asia-II-7	HKR04	2012
5	*Manihot esculenta*	Tapioca	Malleshwarum	KF790638	12.98	77.57	Asia-II-7	HKR05	2012
6	*Hibiscus* cultivar	Hibiscus	Malleshwarum	KF790639	12.98	77.57	Asia-II-7	HKR06	2012
7	*Gossypium hirsutum*	Cotton	Hessaraghatta	KF790653	13.13	77.48	Asia-II-7	HKR20	2013
8	*Cucumis sativus*	Cucumber	Andaman	KF790667	11.68	92.77	Asia-II-7	HKR34	2012
9	*Solanum melongena*	Eggplant	Chamarajanagar	KF790678	11.56	77.00	Asia-II-7	HKR45	2011
10	*Brassica oleracea*	Cabbage	Rajankunte	KF790641	12.97	77.59	MEAM-1	HKR08	2012
11	*Musa acuminata*	Banana	IIHR	KF790663	13.13	77.48	MEAM-1	HKR30	2013
12	*Phaseolus vulgaris*	Common beans	Rajarajeshwarinagar	KF790664	12.80	77.51	MEAM-1	HKR31	2013
13	*Brassica oleracea*	Cabbage	Kolar	HQ268814	14.10	77.27	MEAM-1	NA	2010
14	*Brassica oleracea*	Cabbage	Kolar	HQ268811	14.10	77.27	MEAM-1	NA	2010
15	*Lablab purpureus*	Hyacintha beans	Banashankari	KF790659	12.93	77.55	Asia-II-8	HKR26	2013
16	*Abelmoschus esculentum*	Okra	Chikkajala	KF790661	13.16	77.63	Asia-II-8	HKR28	2013
17	*Citrullus lanatus*	Watermelon	Chamarajanagar	KF790679	11.56	77.00	Asia-II-8	HKR46	2011
18	*Lycopersicon seculentum*	Tomato	Kolar	HQ331244	14.10	77.27	Asia-II-8	NA	2010
19	*Gossypium hirsutum*	Cotton	Bijapur	KJ523177	16.83	75.71	Asia-II-8	HKR57	2013
20	*Lagenaria siceraria*	Bottle gourd	Bidadi	KF790640	12.80	77.40	Asia-I	HKR07	2012
21	*Lycopersicon esculentum*	Tomato	Rajarajeshwarinagar	KF790642	12.90	77.51	Asia-I	HKR09	2012
22	*Musa acuminta*	Banana	IIHR	KF790643	13.13	77.48	Asia-I	HKR10	2012
23	*Luffa* spp.	Ridge gourd	Bidadi	KF790644	12.80	77.40	Asia-I	HKR11	2012
24	*Abelmoschus esculentus*	Okra	Chikkajala	KF790645	13.16	77.63	Asia-I	HKR12	2012
25	*Helianthus annuus*	Sunflower	IIHR	KF790646	13.13	77.48	Asia-I	HKR13	2012
26	*Capsicum annuum*	Capsicum	IIHR	KF790647	13.13	77.48	Asia-I	HKR14	2012
27	*Cucurbita moschata*	Pumpkin	IIHR	KF790648	13.13	77.48	Asia-I	HKR15	2012
28	*Vigna unguiculata*	Cowpea	Golahalli	KF790649	13.13	77.59	Asia-I	HKR16	2013
29	*Citrullus lanatus*	Watermelon	IIHR	KF790650	13.13	77.48	Asia-I	HKR17	2013
30	*Daucus carota*	Carrot	Dommasandra	KF790651	12.88	77.75	Asia-I	HKR18	2013
31	*Brassica juncea*	Mustard	Ranjantunte	KF790652	12.97	77.59	Asia-I	HKR19	2013
32	*Crossandra infundibuliformis*	Crossandra	IIHR	KF790654	13.13	77.48	Asia-I	HKR21	2013
33	*Cucumis sativus*	Cucumber	IIHR	KF790655	13.13	77.48	Asia-I	HKR22	2013
34	*Gossypium hirsutum*	Cotton	Bangalore	KF790666	13.04	77.59	Asia-I	HKR33	2013
35	*Solanum melongena*	Eggplant	Bidadi	KF790656	12.80	77.40	Asia-I	HKR23	2013
36	*Ricinus communis*	Castor bean	Kadugedi	KF790657	12.99	77.76	Asia-I	HKR24	2013
37	*Cucumis melo*	Muskmelon	Banashankari	KF790658	12.93	77.55	Asia-I	HKR25	2013
38	*Gossypium hirsutum*	Cotton	Rajajinagar	KF790660	12.97	77.57	Asia-I	HKR27	2013
39	*Solanum tuberosum*	Potato	Anekal	KF790662	12.70	77.70	Asia-I	HKR29	2013
40	*Brassica rapa*	Turnip	Makali	KF790665	13.09	77.39	Asia-I	HKR32	2013
41	*Solanum melongena*	Eggplant	Andaman	KF790668	11.68	92.77	Asia-I	HKR35	2012
42	*Solanum melongena*	Eggplant	Gujarat	KF790669	23.21	72.68	Asia-I	HKR36	2011

Table 1. (Continued) Details of *Bemisia tabaci mtCOI* sequences generated for this study along with place of collection, accession number, voucher specimen number, respective genetic group, and host plants.

Sl no.	Host	Common name	Bangalore, Karnataka, India	Accession number	Latitude	Longitude	Genetic group	Voucher specimen	Year of collection
			Karnataka Region						
43	*Solanum melongena*	Eggplant	Kodagu	KF790670	12.42	75.73	Asia-I	HKR37	2011
44	*Solanum melongena*	Eggplant	Maddur	KF790671	12.59	77.05	Asia-I	HKR38	2011
45	*Solanum melongena*	Eggplant	Hunasuru	KF790672	12.31	76.29	Asia-I	HKR39	2011
46	*Abelmoschus esculentum*	Bhendi	Mysore	KF790673	12.18	76.42	Asia-I	HKR40	2011
47	*Solanum melongena*	Eggplant	Mysore (*Periyapatna)	KF790677	12.18	76.42	Asia-I	HKR44	2011
48	*Solanum melongena*	Eggplant	Harangi	KF790675	12.49	75.90	Asia-I	HKR42	2011
49	*Solanum melongena*	Eggplant	Basavanahalli	KF790676	13.11	76.91	Asia-I	HKR43	2011
50	*Solanum melongena*	Eggplant	Channapattana	KF790674	12.65	77.20	Asia-I	HKR41	2011
51	*Solanum tuberosum*	Potato	Chamarajanagar	KF790680	11.56	77.00	Asia-I	HKR47	2011
52	*Cucumis sativus*	Cucumber	Chamarajanagar	KF790681	11.56	77.00	Asia-I	HKR48	2011
53	*Abelmoschus esculentum*	Bhendi	Chamarajanagar	KF790682	11.56	77.00	Asia-I	HKR49	2011
54	*Lycopersicon esculentum*	Tomato	Tumkur	KF790683	13.20	77.08	Asia-I	HKR50	2011
55	*Gossypium hirsutum*	Cotton	Rannibennur	KF790684	14.61	75.62	Asia-I	HKR51	2011
56	*Gossypium hirsutum*	Cotton	Davanagere	KF790685	14.31	75.58	Asia-I	HKR52	2011
57	*Solanum melongena*	Eggplant	Davanagere	KF790686	14.31	75.58	Asia-I	HKR53	2011
58	*Gossypium hirsutum*	Cotton	Chitradurga	KF790687	14.14	76.26	Asia-I	HKR54	2011
59	*Solanum melongena*	Eggplant	Sirsi	KF790688	14.61	74.82	Asia-I	HKR55	2011
60	*Gossypium hirsutum*	Cotton	Haveri	KF790689	14.80	75.39	Asia-I	HKR56	2011
62	*Brassica oleracea*	Cabbage	Kolar	HQ268812	13.01	78.33	Asia-I	NA	2010
63	*Gossypium hirsutum*	Cotton	Kolar	HQ331246	13.73	75.33	Asia-I	NA	2010
64	*Solanum melongena*	Eggplant	Kolar	HQ331245	13. 09	78.11	Asia-I	NA	2010
65	*Gossypium hirsutum*	Cotton	Gulbarga	KJ523182	17.33	76.83	Asia-I	HKR62	2013
66	*Solanum melongena*	Eggplant	Raichur	KJ523180	16.20	77.37	Asia-I	HKR60	2013
67	*Lycopersicon esculentum*	Tomato	Chikmangaluru	KJ523179	13.32	75.77	Asia-I	HKR59	2013
68	*Gossypium hirsutum*	Cotton	Dharward	KJ523181	15.45	75.00	Asia-I	HKR61	2013
69	*Solanum melongena*	Eggplant	Belgaum	KJ523178	15.85	74.50	Asia-I	HKR58	2013
70	*Phaseolus vulgaris*	Beans	Kolar (H Cross)	KJ787661	13.24	77.93	MEAM-K	HKR63	2014
71	*Phaseolus vulgaris*	Beans	Kolar (Malur)	KJ787662	13.02	77.93	MEAM-K	HKR64	2014
72	*Phaseolus vulgaris*	Beans	Kolar	HQ268813	13.13	78.13	MEAM-K	NA	2010

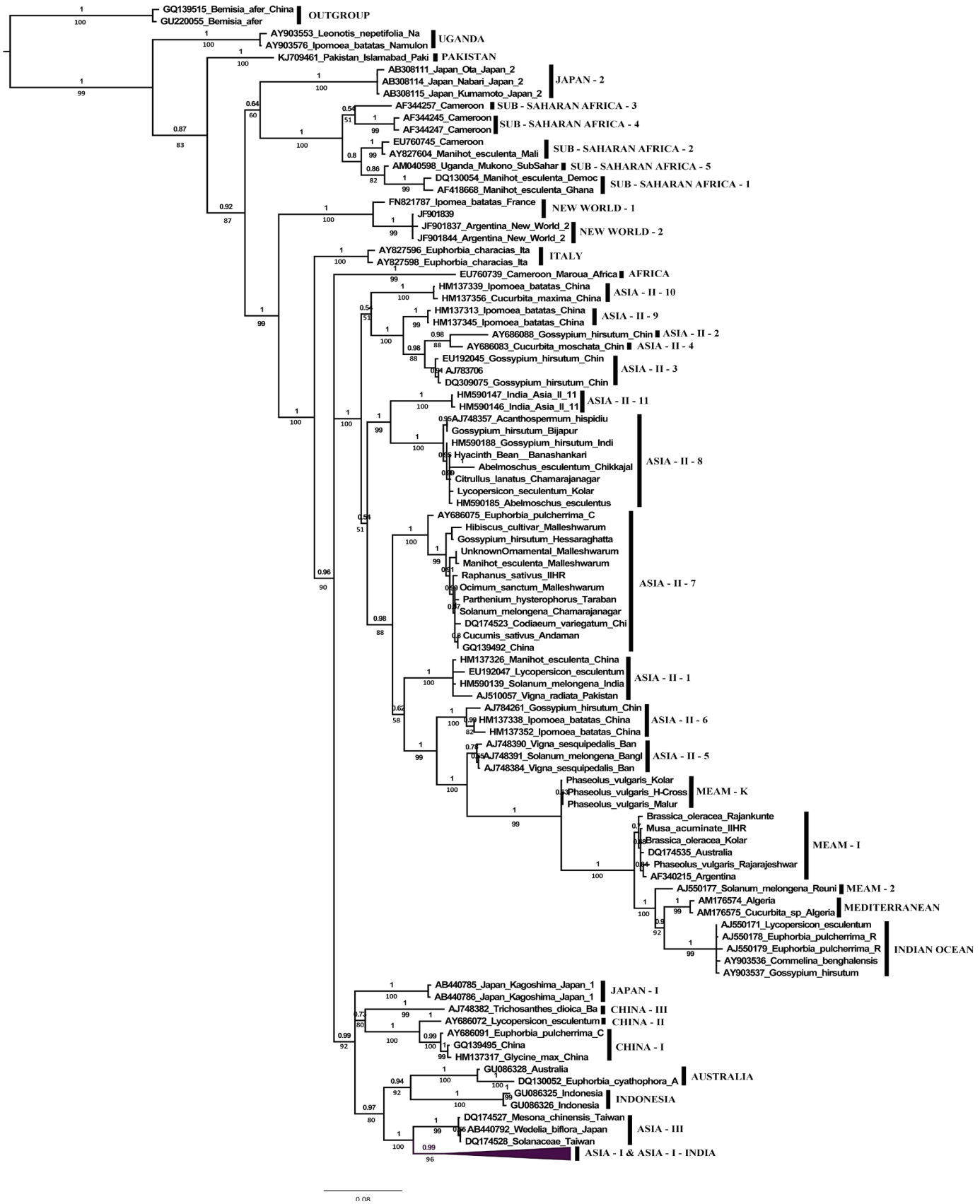

Fig. 2a. Phylogenetic tree showing the relationship of the *Bemisia tabaci*mt *mtCOI* sequences collected in this study to consensus sequences of Dinsdale et al. (2010). Bayesian analyses were performed employing MrBayes under the best-fit model GTR+I+G of molecular evolution for 20 million generations and 25% discarded as burn-in. Posterior probabilities are shown above the branches and maximum likelihood scores from RaxML indicated below the branches. Asia-I condensed is shown in Fig. 2b.

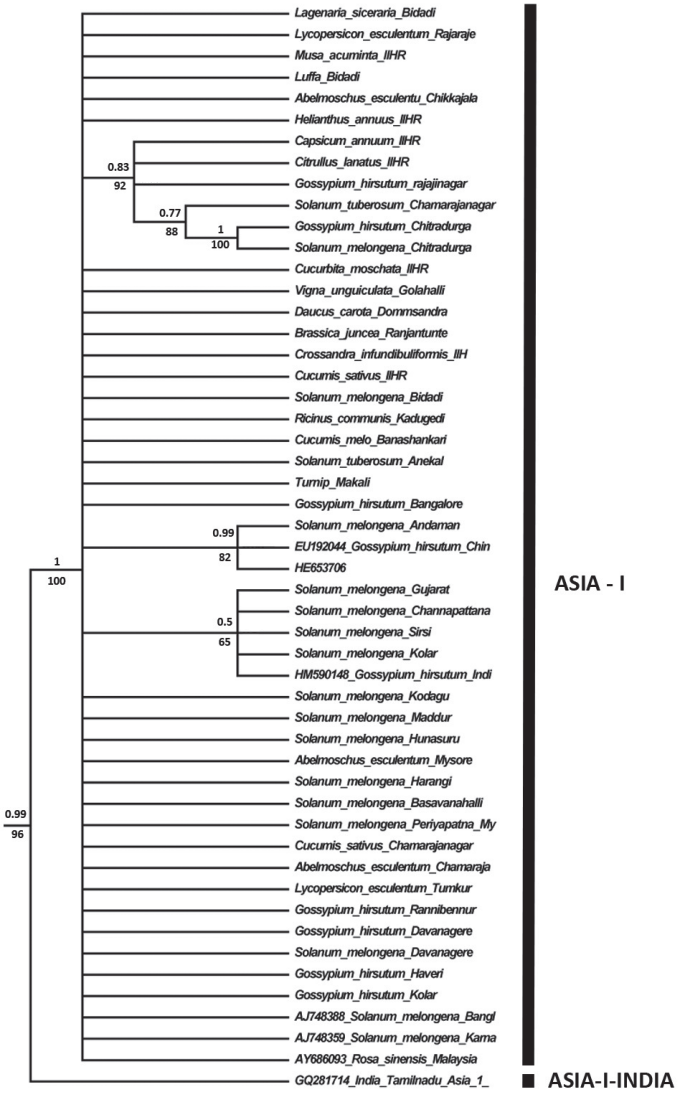

Fig. 2b. Phylogenetic tree indicating the Asia-I group, which was the most abundant genetic group in this study.

to be diagnosed using morphological, cytological, behavioral, molecular, and biochemical methods, and its identification is complicated further by reproductive isolation (Rosell et al. 1997; Maruthi et al. 2007). Molecular studies on various insects of agricultural importance have helped to identify new species (Ball &Armstrong 2006), biotypes (Perring 2001), cryptic species (Hebert et al. 2004), and haplotypes (Toda &Murai 2007), which are difficult to identify through morphology due to phenotypic plasticity and lack of distinguishing morphological features (Russell 1957; Mound 1963; Rosell et al. 1997; Maruthi et al. 2007).

Table 2. Maximum composite likelihood estimate of the pattern of nucleotide substitution from *Bemisia tabaci* populations collected on various host plants in Karnataka, India.

	A	T	C	G
A	—	*5.24*	*1.61*	**14.63**
T	*2.98*	—	**9.96**	*2.34*
C	*2.98*	**32.46**	—	*2.34*
G	**18.62**	*5.24*	*1.61*	—

Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Only entries within a row should be compared. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

Considering the vector potential of *B. tabaci*, it is necessary to analyze the molecular diversity of the same species or species complex collected on various host plants. In this regard, the resistant tomato varieties Arka Ananya (against tomato leaf curl virus) and Arka Abhay and Arka Anamika (against Bhendi yellow vein mosaic virus) have become susceptible again, and this breakdown of resistance may be correlated with the existence of various species of vectors or virus, but the exact plant physiological mechanism is still unknown. In 1991, the biotype nomenclature was introduced to the *B. tabaci* species complex based on esterase banding patterns but was no longer used after the advent of Random Amplified Polymorphic DNA–PCR and *mtCOI* and *ITS* sequencing (Perring 2001; Simon et al. 2003; Zang et al. 2006; Boykin 2013). In the discussion on *B. tabaci* nomenclature, it remains unclear whether to call a new biotype a genetic group or a putative species with novel binomial nomenclature, such as *Bemisia argentifolii* Bellows & Perring (De Barro et al. 2011; Boykin et al. 2013).

Bemisia tabaci can attack a wide range of host plants globally, and its species complex is composed of at least 34 morphologically indistinguishable species (Boykin et al. 2012, 2013; Boykin 2014). Different cropping patterns and diverse climatic conditions may be responsible for the apparent diversity in *B. tabaci*, which otherwise is grouped by geographic location (Rekha et al. 2005). The current study revealed the existence of 5 genetic groups within Karnataka State, India, namely Asia-I, Asia-II-7, Asia-II-8, MEAM-1, and MEAM-K, with the latter being a new group identified in this study.

The genetic divergence ranged from 4.0 to 24.0% with an average of 15.7%. According to Dinsdale et al. (2010), the genetic divergence among 198 *mtCOI* sequences of *B. tabaci* and sequences of the outgroup species *B. afer*, *B. atriplex* (Froggatt), and *B. subdecipiens* Martin (Hemiptera: Aleyrodidae) ranged from zero to 34%. However, Lee et al. (2013) analyzed the genetic divergence after excluding the outgroup sequences and concluded that it ranged from zero to 24.0%, which was on par with our study.

Of the reported 5 genetic groups, the major group was Asia-I with members collected on 19 different host plants although most had been collected on eggplant; this group was found previously to be prevalent across Asia (Boykin et al. 2007; Dinsdale et al. 2010; Hu et al. 2011). The eggplant-associated Asia-I *B. tabaci* can transmit several begomoviruses (Govindappa 2002; Muniyappa et al. 2003) and was shown to transmit eggplant yellow mosaic virus in Thailand (Green et al. 2003). In this study, we report another 17 host plants for Asia-I including cotton, pumpkin, ridge gourd, okra, capsicum, sunflower, potato, carrot, mustard, and tomato. However, a previous study showed that tomato is not a preferred host plant for Asia-I because it is associated with low fecundity (Chowda-Reddy et al. 2012). Host-associated variations in *B. tabaci* influence its rate of fecundity, which could be due to premating or post-mating selection against migrants and hybrid progeny (Liou & Price 1994; Brunner et al. 2004). However, the most to least preferred hosts, in terms of oviposition, were eggplant, cotton, pumpkin, tomato, and cassava (Venkatesh 2000). Our study also supported this finding, wherein eggplant and cotton were the most frequent host plants for Asia-I. Apart from the Jatropha genetic group, which occurs in a separate ecological niche, most of the putative species exhibit high levels of polyphagy (Burban et al. 1992; Brown et al. 1995). According to Chowda-Reddy et al. (2012), MEAM-1 has a wide host range and produces higher quantities of honeydew than other genetic groups. This is also true for genetic groups Asia-I, Asia-II-7, and Asia-II-8, of which Asia-I had the greatest number of host plants in our study.

We identified Asia-II-7 from Karnataka occurring on 9 different host plants, mostly on ornamental plants. Asia-II-7 was reported first in 1998 in India (Ramappa et al. 1998) and in China (Qiu et al. 2006). A previous study suggested that this putative species adapts readily

Table 3. Estimates of net evolutionary divergence between groups of sequences showing pair-wise genetic distances for *Bemisia tabaci* populations collected on various host plants in Karnataka, India.

Asia-II-7																															
Asia-I	0.139																														
MEAM-1	0.173	0.161																													
Asia-II-8	0.119	0.152	0.161																												
MEAM-1-Karnataka	0.153	0.164	0.074	0.157																											
Asia-III	0.135	0.078	0.174	0.163	0.175																										
Asia-II-10	0.109	0.137	0.172	0.120	0.155	0.139																									
Asia-II-1	0.092	0.157	0.172	0.133	0.160	0.158	0.131																								
Asia-II-2	0.111	0.144	0.129	0.120	0.130	0.145	0.108	0.067																							
Asia-II-3	0.122	0.176	0.174	0.118	0.165	0.166	0.107	0.133	0.075																						
Asia-II-4	0.129	0.169	0.160	0.142	0.166	0.158	0.119	0.125	0.051	0.048																					
Asia-II-5	0.109	0.173	0.172	0.117	0.095	0.168	0.139	0.106	0.107	0.136	0.133																				
Asia-II-6	0.096	0.159	0.171	0.136	0.124	0.147	0.116	0.107	0.118	0.136	0.141	0.067																			
Asia-II-9	0.108	0.169	0.169	0.118	0.155	0.160	0.098	0.121	0.091	0.050	0.080	0.120	0.126																		
Australia	0.156	0.117	0.163	0.155	0.153	0.124	0.154	0.148	0.130	0.152	0.142	0.133	0.150	0.147																	
Australia-Indonesia	0.178	0.146	0.174	0.176	0.166	0.139	0.170	0.179	0.151	0.176	0.180	0.172	0.174	0.163	0.136																
China-I	0.145	0.128	0.161	0.148	0.168	0.122	0.134	0.156	0.135	0.156	0.154	0.147	0.141	0.148	0.147	0.138															
Indian_Ocean	0.165	0.152	0.076	0.169	0.126	0.171	0.168	0.167	0.153	0.176	0.183	0.177	0.175	0.163	0.180	0.180	0.165														
Italy	0.124	0.145	0.153	0.128	0.155	0.131	0.131	0.140	0.134	0.134	0.148	0.142	0.129	0.123	0.131	0.122	0.123	0.153													
Mediterranean	0.170	0.171	0.052	0.164	0.110	0.185	0.177	0.179	0.150	0.182	0.186	0.187	0.171	0.165	0.191	0.180	0.168	0.070	0.161												
New-World-1	0.159	0.134	0.139	0.163	0.166	0.152	0.147	0.165	0.148	0.157	0.159	0.162	0.173	0.145	0.164	0.179	0.150	0.156	0.128	0.148											
Subgroup-Af-1	0.189	0.196	0.164	0.184	0.195	0.187	0.180	0.193	0.182	0.184	0.198	0.202	0.199	0.178	0.182	0.180	0.186	0.187	0.155	0.181	0.149										
Subgroup-Af-2	0.205	0.186	0.165	0.187	0.198	0.191	0.184	0.205	0.183	0.175	0.191	0.205	0.190	0.164	0.175	0.186	0.198	0.176	0.149	0.176	0.147	0.076									
Subgroup-Af-3	0.199	0.172	0.172	0.176	0.197	0.180	0.178	0.190	0.180	0.188	0.197	0.216	0.204	0.180	0.189	0.183	0.189	0.183	0.165	0.185	0.151	0.072	0.061								
Subgroup-Af-4	0.195	0.183	0.175	0.189	0.201	0.185	0.172	0.202	0.188	0.193	0.203	0.208	0.206	0.187	0.183	0.181	0.199	0.182	0.156	0.180	0.150	0.078	0.072	0.071							
Uganda	0.229	0.228	0.202	0.207	0.209	0.220	0.198	0.212	0.211	0.237	0.239	0.218	0.225	0.212	0.233	0.207	0.206	0.213	0.209	0.207	0.194	0.190	0.196	0.194	0.202						
MEAM-2	0.162	0.160	0.041	0.150	0.098	0.166	0.167	0.163	0.137	0.169	0.171	0.172	0.157	0.162	0.174	0.177	0.154	0.069	0.148	0.040	0.144	0.167	0.163	0.175	0.170	0.195					
China-II	0.135	0.127	0.153	0.154	0.144	0.130	0.134	0.157	0.139	0.158	0.152	0.142	0.145	0.144	0.146	0.142	0.044	0.160	0.121	0.173	0.144	0.190	0.195	0.189	0.204	0.209	0.159				
China-III	0.151	0.150	0.156	0.135	0.154	0.142	0.138	0.161	0.140	0.162	0.168	0.165	0.160	0.140	0.150	0.115	0.123	0.191	0.142	0.179	0.174	0.176	0.195	0.191	0.196	0.205	0.157	0.120			

Analyses were conducted using the maximum composite likelihood model (Tajima & Nei 1984). The analysis involved 135 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 620 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

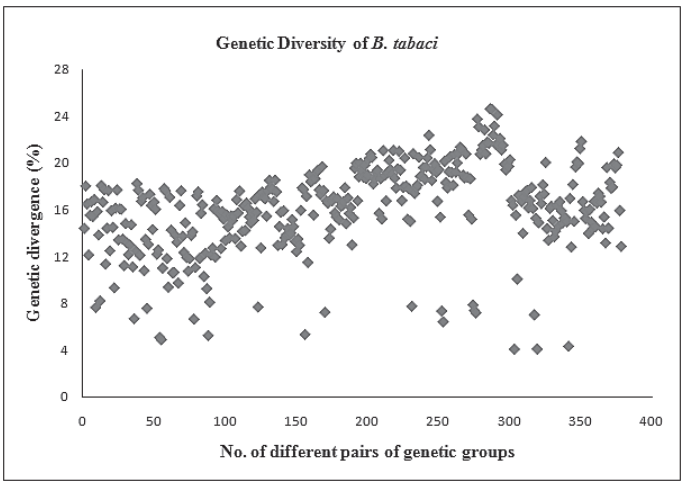

Fig. 3. Graph indicating net evolutionary divergence between groups of sequences.

to ornamental plants rather than vegetables (Shah et al. 2013). The 3rd genetic group identified in this study was Asia-II-8, which was recently named as *B. gossypiperda* (Boykin 2014) and was collected on 5 different host plants including cotton (Chowda-Reddy et al. 2012). Significantly, we here report the occurrence of a previously unreported subclade for Middle East genetic groups (MEAM-1 and 2). We named it MEAM-K because the samples were collected on *P. vulgaris* from Kolar, Karnataka, the native location for MEAM-1. For Kolar and nearby areas where both species have become established, our study raises questions about the relative pest status of MEAM-1and MEAM-K. We do not know to what extent these genetic groups differ with respect to important factors such as their ability in feeding and reproduction, their efficiency as vectors, or their susceptibility to insecticides. These high levels of molecular and ecological resolution will be needed to mitigate quarantine disputes arising from the detection of morphologically indistinguishable members of the *B. tabaci* genetic groups. Thus, our study may stand as a link to detect and identify *B. tabaci* genetic groups, determine areas of occurrence, identify areas of invasion, and design management strategies.

In conclusion, the current study revealed the existence of 5 genetic groups of *B. tabaci* in Karnataka, India, identified as Asia-I, Asia-II-7, Asia-II-8, MEAM-1, and a previously unreported genetic group, MEAM-K. Thus, our work will help in rapid and accurate identification of these putative genetic groups of *B. tabaci*, which in turn will help in further elucidating the epidemiology and management of geminiviruses and be of value in the operation of quarantines.

Acknowledgments

This paper is part of the doctoral degree work of the senior author. We gratefully acknowledge the financial support received for the "Outreach Programme on Management of Sucking Pests in Horticultural Crops" from the Indian Council for Agricultural Research (ICAR), New Delhi, India. We are thankful to the director at the Indian Institute of Horticultural Research (IIHR) for the support and facilities.

References Cited

Ball SL, Armstrong KF.2006. DNA barcodes for insect pest identification: a test case with tussock moths (Lepidoptera: Lymantriidae). Canadian Journal of Forest Research 36: 337-350.

Bellows TS, Perring TM, Gill RJ, Headrick DH. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Annals of the Entomological Society of America 87: 195-206.

Boykin LM. 2014. *Bemisia tabaci* nomenclature: lessons learned. Pest Management Science 70: 1454-1459.

Boykin LM, Shatters Jr RG, Rosell RC, McKenzie CL, Bagnall RA, De Barro PJ, Frohlich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. Molecular Phylogenetics and Evolution 44: 1306-1319.

Boykin LM, Armstrong KF, Kubatko L, De Barro PJ. 2012. Species delimitation and global biosecurity. Evolutionary Bioinformatic Online 8: 1-37.

Boykin LM, Bell CD, Evans G, Small I, De Barro PJ. 2013. Is agriculture driving the diversification of the *Bemisia tabaci* species complex (Hemiptera: Sternorrhyncha: Aleyrodidae)? Dating, diversification and biogeographic evidence revealed. BMC Evolutionary Biology 13: 228.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex. Annual Review of Entomology 40: 511-534.

Brown JK, Perring TM, Cooper AD, Bedford ID, Markham PG. 2000. Genetic analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations by isoelectric focusing electrophoresis. Biochemical Genetics 38: 13-25.

Brunner PC, Chatzivassiliou EK, Katis NI, Frey JE. 2004. Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. Heredity 93: 364-370.

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel JC. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). Journal of Applied Entomology 113: 416-423.

Byrne DN, Bellows Jr TS. 1991. Whitefly biology. Annual Review of Entomology 36: 431-457.

Cahill M, Denholm I, Ross G, Gorman K, Johnston D. 1996. Relationship between bioassay data and the simulated field performance of insecticides against susceptible and resistant adult *Bemisia tabaci*. Bulletin of Entomological Research 86: 109-116.

Chowda-Reddy RV, Kiran Kumar M, Seal SE, Muniyappa V, Valand GB, Govindappa MR, Colvin J. 2012. *Bemisia tabaci* phylogenetic groups in India and the relative transmission efficacy of tomato leaf curl Bangalore virus by an indigenous and an exotic population. Journal of Integrative Agriculture 11: 235-248.

Chu D, Jiang T, Liu GX, Jiang DF, Tao YL, Fan ZX, Zhou HX, Bi YP. 2007. Biotype status and distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Shandong Province of China based on mitochondrial DNA markers. Environmental Entomology 36: 1290-1295.

Costa HS, Brown JK. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* (Genn.) and the association of one population with silver leaf symptom development. Entomologia Experimentalis et Applicata 61: 211-219.

Costa HS, Westcot DM, Ullman DE, Rosell R, Brown JK, Johnson MW. 1995. Morphological variation in *Bemisia* endosymbionts. Protoplasma 189: 194-202.

De Barro PJ, Trueman JWH, Frohlich DR. 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetics differentiation of *B. tabaci* populations around the world. Bulletin of Entomological Research 95: 193-203.

De Barro PJ, Liu SS, Boykin LM, Dinsdale A. 2011. *Bemisia tabaci*: a statement of species status. Annual Review of Entomology 56: 1-19.

Dinsdale A, Cook L, Riginos C, Buckley YM, De Barro PJ. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase I to identify species level genetic boundaries. Annals of the Entomological Society of America 103: 196-208.

Frohlich DR, Torres-Jerez I, Bedford ID, Markham PG, Brown JK. 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Molecular Ecology 8: 1683-1691.

Gawel NJ, Bartlett AC. 1993. Characterization of differences between whiteflies using RAPD-PCR. Insect Molecular Biology 2: 33-38.

Govindappa MR. 2002. Detection and transmission of tomato leaf curl virus and interactions studies with host plants and whitefly *Bemisia tabaci* Genn. in relation to epidemics. Ph.D. thesis, University of Agricultural Sciences, Bangalore, India.

Green SK, Tsai WS, Shih SL.2003. Molecular characterization of a new begomovirus associated with tomato yellow leaf curl and eggplant yellow mosaic diseases in Thailand. Plant Disease 87: 446.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the USA 101: 14812-14817.

Horowitz AR, Gorman K, Ross G, Denholm I. 2003. Inheritance of pyriproxyfen resistance in the whitefly, *Bemisia tabaci* (Q biotype). Archives of Insect Biochemistry and Physiology 54: 177-186.

Horowitz AR, Kontsedalov S, Ishaaya I. 2004. Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). Journal of Economic Entomology 97: 2051-2056.

Hu J, De Barro PJ, Zhao H, Wang J, Nardi F, Liu SS. 2011. An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. PLoS One 6: e16061.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.

Jones DR. 2003. Plant viruses transmitted by whiteflies. European Journal of Plant Pathology 109: 195-219.

Lee W, Park J, Lee GS, Lee S, Akimoto SI. 2013. Taxonomic status of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. PLoS One 8: e63817.

Liou LW, Price TD. 1994. Speciation by reinforcement of premating isolation. Evolution 48: 1451-1459.

Lisha VS, Antony B, Palaniswami MS, Henneberry TJ. 2003. *Bemisia tabaci* (Homoptera: Aleyrodidae) biotypes in India. Journal of Economic Entomology 96: 322-327.

Liu SS, Colvin J, De Barro PJ. 2012. Species concepts as applied to the whitefly *Bemisia tabaci* systematics: How many species are there? Journal of Integrative Agriculture 11: 176-186.

Maruthi MN, Rekha AR, Sseruwagi P, Hillocks RJ. 2007. Mitochondrial DNA variability and development of a PCR diagnostic test for populations of the whitefly *Bemisia afer* (Priesner and Hosny). Molecular Biotechnology 35: 31-40.

Mayr E. 1982. The Growth of Biological Thought. Harvard University Press, Cambridge, Massachusetts, USA.

Mound AL. 1963. Host-correlated variations in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Proceedings of the Royal Entomological Society of London, Series A, General Entomology 38: 171-180.

Muniyappa V, Maruthi MN, Babitha CR, Colvin J, Briddon RW, Rangaswamy KT. 2003. Characterization of pumpkin yellow vein mosaic virus from India. Annals of Applied Biology 142: 323-331.

Palaniswami MS, Nair RR, Pillai KS, Thankappan M. 1996. Whiteflies on cassava and its role as vector of cassava mosaic disease in India. Journal of Root Crops 22: 1-8.

Perring TM. 2001. The *Bemisia tabaci* species complex. Crop Protection 20: 725-737.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Qiu BL, Ren SX, Mandour NS, Wen SY. 2006. Population differentiation of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) by DNA polymorphism in China. Journal of South China Agricultural University 27: 29-34.

Ramappa HK, Muniyappa V, Colvin J. 1998. The contribution of tomato and alternative host plants to tomato leaf curl virus inoculum pressure in different areas of South India. Annals of Applied Biology 133: 187-198.

Rambaut A. 2009. FigTree Version 1.3.1, http://tree.bio.ed.ac.uk/software/figtree/ (last accessed 6 Mar 2015).

Rambaut A, Drummond AJ. 2009. Tracer Version 1.5, http://beast.bio.ed.ac.uk/Tracer (last accessed 6 Mar 2015).

Rauch N, Nauen R. 2003. Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). Archives of Insect Biochemistry and Physiology 54: 165-176.

Rekha AR, Maruthi MN, Muniyappa V, Colvin J. 2005. Occurrence of three genotypic clusters of *Bemisia tabaci* and the rapid spread of the B biotype in South India. Entomologia Experimentalis et Applicata 117: 221-233.

Rosell RC, Bedford ID, Frohlich DR, Gill RJ, Brown JK, Markham PG. 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). Annals of the Entomological Society of America 90: 575-589.

Russell LM. 1957. Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Bulletin of the Brooklyn Entomological Society 52: 122-123.

Shah SHJ, Malik AH, Qazi J. 2013. Identification of new genetic variant of *Bemisia tabaci* from Pakistan. International Journal of Entomological Research 1: 16-24.

Simon B, Cenis JL, Demichelis S, Rapisarda C, Caciagli P, Bosco D. 2003. Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Italy with the description of a new biotype (T) from *Euphorbia characias*. Bulletin of Entomological Research 93: 259-264.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651-701.

Swofford DL. 1998. PAUP* Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinaue Associates, Sunderland, Massachusetts, USA.

Tajima F, Nei M. 1984. Estimation of evolutionary distance between nucleotide sequences. Molecular Biology and Evolution 1: 269-285.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739.

Thompson JD, Higgins DG, Gibson JJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 11: 4673-4680.

Toda S, Murai T. 2007. Phylogenetic analysis based on mitochondrial COI gene sequences in *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in relation to reproductive forms and geographical distribution. Applied Entomology and Zoology 42: 309-316.

Venkatesh HM. 2000. Studies on tomato leaf curl geminivirus and *Bemisia tabaci* (Gennadius): molecular detection, farmers' perception and sustainable management. Ph.D. thesis, Department of Plant Pathology, University of Agricultural Sciences, Bangalore, India.

Wang P, Sun D, Qiu B, Liu SS. 2011. The presence of six cryptic species of the whitefly *Bemisia tabaci* complex in China as revealed by crossing experiments. Insect Science 18: 67-77.

Xu J, De Barro PJ, Liu SS. 2010. Reproductive incompatibility among genetic groups of *Bemisia tabaci* supports the proposition that the whitefly is a cryptic species complex. Bulletin of Entomological Research 100: 359-366.

Zang LS, Chen WQ, Liu SS. 2006. Comparison of performance on different host plants between the B biotype and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. Entomologia Experimentalis et Applicata 121: 221-227.

Zasada IA, Peetz A, Howe DK, Wilhelm LJ, Cheam D, Denver DR, Smythe AB. 2014. Using mitogenomic and nuclear ribosomal sequence data to investigate the phylogeny of the *Xiphinema americanum* species complex. PLoS One 9: e90035.