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Genetic diversity and host relationships of endosymbiotic bacteria in the Asian cryptic species of *Bemisia tabaci* from Bangladesh

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Abstract

Endosymbiotic bacteria are common in many herbivorous insects. *Bemisia tabaci* is a phloem-sapping pest of various crop plants and is known to harbor at least five endosymbionts. This species is a complex of at least 40 genetically distinct but morphologically indistinguishable cryptic species worldwide. Endosymbiont composition has been studied in invasive cryptic species such as MEAM1 and MED, but little information exists regarding the indigenous genetic groups in Asia. Here, we determined the endosymbiont profiles of four indigenous Asian cryptic species (Asia I, Asia II 1, Asia II 5 and Asia II 10) of *B. tabaci* identified in Bangladesh. Overall, the infection rates of *Arsenophonus*, *Cardinium*, *Hamiltonella, Rickettsia*, and *Wolbachia* were 93%, 86%, 0%, 31%, and 88%, respectively. Phylogenetic analysis revealed two subgroups in *Arsenophonus* (A1, A2) and *Rickettsia* (R1, R2), but only one subgroup in *Cardinium* (C2) and *Wolbachia* (W1). Each endosymbiont showed varying rates of infection in the four cryptic species and most were co-infected with various combinations. The results of this study provide important information on the relationships between the endosymbionts and cryptic species of *B. tabaci* indigenous to Asia.

Keywords Co-infection · Cryptic species · Endosymbionts · Genetic diversity · Phylogenetics

1 Introduction

Endosymbionts are common in plant-sapping insects and they have important relationships with their host species (Baumann 2005). Primary endosymbionts coevolve with their hosts, becoming key to host survival by assisting in obtaining essential nutrients (Baumann 2005; Rosell et al. 2010). Secondary endosymbionts have a facultative relationship with their hosts, providing fitness benefits such as in reproduction, host plant specialization, and increased tolerance to thermal stress and parasites (Chiel et al. 2009; Feldhaar 2011; Kaiser et al. 2010; Montllor et al. 2002; Oliver et al. 2003; Sintupachee et al. 2006; Tsuchida et al. 2004). In particular, secondary endosymbionts provide pivotal roles in virus transmission for vectoring species such as *B. tabaci,* which is a unique vector species of begomoviruses (Czosnek and Ghanim 2011).

Bemisia tabaci is a polyphagous pest species that feeds on various horticultural crops, ornamental crops and weed species (Cahill et al. 1996; Jones 2003). This species harbors primary endosymbiont *Portiera aleyrodidarum* along with at least six secondary endosymbionts, viz., *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Rickettsia*, and *Wolbachia* (Bing et al. 2013a, b; Zchori-Fein et al. 2014). Recently, a new endosymbiont, Candidatus *Hemipteriphilus asiaticus*, was found to be a cryptic species of *B. tabaci* (Bing et al. 2013a, b). Secondary endosymbionts influence various biological characteristics, such as reproduction (Himler et al. 2011; Hunter et al. 2003; Zchori-Fein et al. 2001; Zchori-Fein and Perlman 2004), survival (Gottlieb et al. 2010; Kontsedalov et al. 2008; Liu et al. 2007; Thierry et al. 2011), insecticide resistance (Kontsedalov et al. 2008), and capacity for disease transmission to plants (Gottlieb et al. 2010). Given their various roles, endosymbionts are

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necessary for the modulation of host development and environmental impacts (Thao and Baumann 2004).

Bemisia tabaci is a species complex comprising at least 40 cryptic species that are morphologically indistinguishable, but genetically distinct in biological characteristics (De Barro et al. 2011). Among them, the Middle East-Asia Minor 1 (MEAM1, formerly B biotype) and Mediterranean (MED, formerly Q biotype) species are highly invasive and have dispersed to many countries in different continents (Dalton 2006; Horowitz et al. 2005; Pascual and Callejas 2004). In addition, many indigenous cryptic species of *B. tabaci* has been observed in Africa and Asia. To date, at least 23 indigenous cryptic species with different geographic distributions had been identified in Asia (Dinsdale et al. 2010; Ahmed et al. 2011; Hameed et al. 2012; Firdaus et al. 2013; Shah et al. 2013; Prasanna et al. 2015; Ellango et al. 2015; Hu et al. 2015, 2017; Götz and Winter 2016; Kumar et al. 2016; Jiu et al. 2017). Recently, Our previous study identified four indigenous cryptic species (Asia I, Asia II 1, Asia II 5 and Asia II 10) in Bangladesh (Khatun et al. 2018).

Each genetic group of *B. tabaci* exhibits a distinct composition of secondary endosymbionts. Several studies have investigated invasive cryptic species such as MEAM1 and MED from different geographic regions (Bing et al. 2013a, b; Chiel et al. 2007; Chu et al. 2011; Gueguen et al. 2010; Park et al. 2012; Skaljac et al. 2010, 2013). However, little information exists regarding the endosymbiont profiles of indigenous cryptic species in Asian countries.

The objective of this study was to determine the infection profiles of secondary endosymbionts in the cryptic species of *B. tabaci* from Bangladesh. Results from this study will improve our understanding of the composition of endosymbionts and their relationships with the Asian genetic groups of *B. tabaci*.

2 Materials and methods

2.1 Sample collection

Adult *B. tabaci* were collected from crop fields in different regions of Bangladesh from 2015 to 2017. Samples were preserved in 70% ethanol and kept at −20 °C for further analysis (Table S1). Identification and genetic diversity of four cryptic species of *B. tabaci* in these samples has been reported in a previous study (Khatun et al. 2018).

2.2 DNA extraction

Genomic DNA was extracted from a single adult *B. tabaci* using a pure link genomic DNA mini kit (Invitrogen, Carlsbad, CA, USA). The sample was placed in a 1.5 mL centrifuge tube containing 180 μL digestion buffer and 20 μL proteinase K (50 μg/mL) then incubated at 55 °C for 4 h. DNA samples were extracted and purified using genomic spin columns following the manufacturer's protocol. DNA concentration was determined using a NanoPhotometer™ (Implen GmbH, Schatzbogen, Germany).

2.3 Screening of secondary endosymbionts

Five known endosymbionts (*Arsenophonus, Cardinium*, *Hamiltonella*, *Rickettsia*, and *Wolbachia*) were detected through PCR using 16S or 23S rDNA primers (Table 1). The reaction (25 μL) contained 13 μL Smart-Taq Pre-Mix (Solgent Co., Daejeon, Korea), 1 μL of each primer (10 pmol/μL), and 5 μLtemplate DNA solution (40 ng). Amplicons were separated using 1% agarose gel electrophoresis, stained with ethidium bromide solution, and visualized under Ultraviolet (UV) light. After excision from the gel, the amplicons were purified using the Wizard® PCR Preps DNA Purification System (Wizard® SV Gel, Promega Co., Madison, WI). They were then either sequenced directly or via cloning into the T-Blunt™easy plasmid vector (Promega Co., Madison, WI).

2.4 DNA sequence analysis

Cloned amplicons were sequenced using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed with a 3100 Capillary DNA Sequencer (Applied Biosystems, Foster City, CA) at the Solgent

Table 1 Primers for amplifying DNA from secondary endosymbionts of *Bemisia tabaci*

Primers	Sequences (5′ → 3′)	Annealing temperature (°C)/size (bp)	Species/gene	References
Ars23S-1	CGTTTGATGAATTCATAGTCAAA	60/600	*Arsenophonus* 23S rDNA	Thao and Baumann 2004
Ars23S-2	GGTCCTCCAGTTAGTGTTACCCAAC			
CFB F	GCGGTGTAAAATGAGCGTG	58/400	*Cardinium* 16S rDNA	Weeks et al. 2003
CFB R	ACCTMTTCTTAACTCAAGCCT			
Hb F	TGAGTAAAGTCTGGAATCTG	58/700	*Hamiltonella* 16S rDNA	Zchori-Fein and Brown 2002
Hb R	AGTTCAAGACCGCAACCTC			
Rickettsia F	GCTCAFAACGAACGCTATC	60/900	*Rickettsia* 16S rDNA	Gottlieb et al. 2006
Rickettsia R	GAAGGAAAGCATCTCTGC			
Wolbachia F	CGGGGGAAAAATTTATTGCT	55/650	*Wolbachia* 16S rDNA	Gottlieb et al. 2008
Wolbachia R	AGCTGTAATACAGAAAGTAAA			

Sequencing Facility (Solgent Co., Daejeon, Korea). Sequences were obtained from one individual per primer set and BLASTed against the NCBI database (Schaffer et al. 2001).

2.5 Phylogenetic analyses of secondary endosymbionts

Endosymbiont 16S and 23S rDNA sequences were aligned in Clustal Omega for the construction of a maximum-likelihood (ML) phylogenetic tree in MEGA 6.0. The model was selected based on GTR + G for *Arsenophonus* and *Wolbachia*, HKY + G for *Cardinium* and HKY + I for *Rickettsia*. Phylogeny robustness was tested with 1000 bootstraps (Felsenstein 1985).

2.6 Correlation analysis

Genetic differentiation among the endosymbionts was determined in DnaSP version 5.10 (Librado and Rozas 2009; Tajima 1989). Pairwise genetic distance was generated from 1023 permutations with the K2P model in MEGA 6.0 (Kimura 1980). Geographic distances were calculated from GPS coordinates measured during *B. tabaci* sample collections. The correlation between endosymbiotic genetic variability and geographic distances was analyzed using IBM SPSS software, version 23.

3 Results

3.1 Endosymbiont profiles of *B. tabaci* in Bangladesh

Infection profiles of five endosymbionts were determined from 110 individuals representing four Asian cryptic species of *B. tabaci* (Asia I, Asia II 1, Asia II 5, and Asia II 10; Table S1). Overall, the infection rates of *Arsenophonus*, *Cardinium*, *Rickettsia*, and *Wolbachia* were 93.6%, 86.4%, 31.8%, and 88.2%, respectively, but *Hamiltonella* was not detected in any individuals in this study (Fig. 1, Tables 2 and 3). Phylogenetic analysis of 23S rDNA sequences showed that *Arsenophonus* belonged to A1 and A2, but not to the A3 and A4 subgroups (Fig. 2). Sequences of both A1 and A2 exhibited a 0.19–3.18% variation and differences between A1 and A2 subgroups were 2.25–3.18% (Table 4, Table S2). The A1 subgroup was detected in Asia I and Asia II 1, while A2 was present in all four cryptic species (Figs. 1 and 2). *Cardinium* was present in C2 but not in the C1 or C3 subgroups (Figs. 1 and 3). The C2 subgroup was detected in Asia I, Asia II 1, and Asia II 5 with a variation of 0.25–0.74% (Fig. 3, Table 4, Table S3). *Rickettsia* belonged to the R1 and R2 subgroups;16S rDNA sequence variation ranged from 0.11–13.37% and differences between the R1 and R2 subgroups ranged from 10.15–13.37% (Figs. 1 and 4; Table 4, Table S4). Both R1 and R2 were detected at different rates in Asia I, Asia II 1, and Asia II 5 (Fig. 4). *Wolbachia* was present in W1 but not in the W2 or W3 subgroups. W1 subgroup was detected in all four cryptic species with a variation of 0.17–0.68% (Figs. 1 and 5; Table 4, Table S5).

The four endosymbionts differed in infection rates depending on *B. tabaci* genetic groups (Table 2). *Arsenophonus* was 93.6% infected on average in all four cryptic species, with a range of 90.0–95.5%, except for in Asia II 10. Asia II 10 had 100% infection, but only one individual was examined in this group. The infection rate of A1 was higher than that of A2 in both Asia I and Asia II 1, but only A2 was infected into Asia II 5 and Asia II 10 (Fig. 1, Table 2). The infection rate of *Cardinium* was 80.6–100%, with the highest rate occurring in Asia II 5. The infection rate of *Rickettsia* was 13.6–50.0% with R1 being the highest in Asia II 1 (35%) and R2 being the highest in Asia I (25.4%). The infection rate of *Wolbachia* was 75–100% with the highest rate observed for Asia II 5 (Table 2). Otherwise, we did not find any significant relationships between the infection rates of endosymbionts and crop species infested by *B. tabaci* (Table S1).

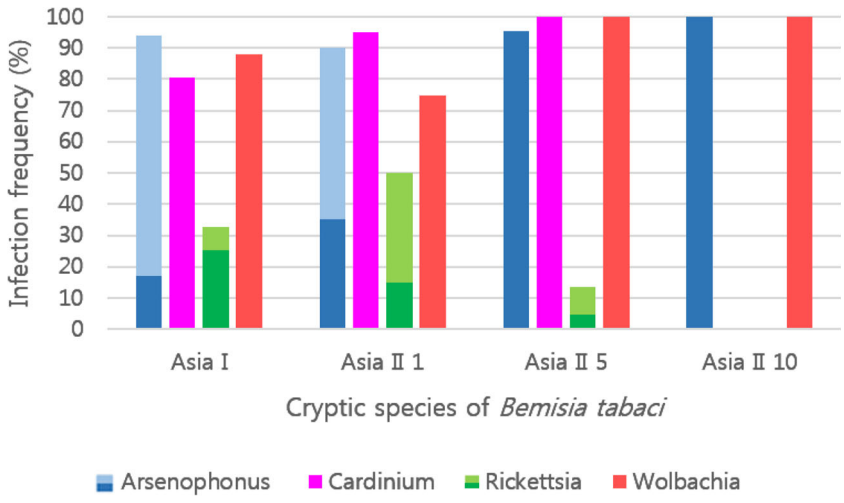

Fig. 1 Infection frequency (%) of secondary endosymbionts in four cryptic *Bemisia tabaci* species: Asia I, Asia II 1, Asia II 5, and Asia II 10. *Arsenophonus* and *Rickettsia* were divided into subgroups, indicated by different colors: A1 (bright blue), A2 (dark blue), R1 (bright green), and R2 (dark green)

Table 2 Infection rates of secondary endosymbionts in four cryptic species of *Bemisia tabaci* from Bangladesh

Secondary endosymbionts	Infection rates [% (individual numbers)] of each cryptic species					
	Sub-groups	Asia I	Asia II 1	Asia II 5	Asia II 10	Overall
Arsenophonus	A1	76.1% (51)	55.0% (11)	–	–	56.3% (62)
	A2	17.9% (12)	35.0% (7)	95.5% (21)	100% (1)	37.3% (41)
	A1 + A2	94.0% (63)	90.0% (18)	95.5% (21)	100% (1)	93.6% (103)
Cardinium	C2	80.6% (54)	95.0% (19)	100% (22)	–	86.4% (95)
Hamiltonella	–	–	–	–	–	–
Rickettsia	R1	7.5% (5)	35.0% (7)	9.1% (2)	–	12.7% (14)
	R2	25.4% (17)	15.0% (3)	4.5% (1)	–	19.1% (21)
	R1 + R2	32.8% (22)	50.0% (10)	13.6% (3)	–	31.8% (35)
Wolbachia	W1	88.1% (59)	75.0% (15)	100% (22)	100% (1)	88.2% (97)
Total number of individuals		67	20	22	1	110

3.2 Multiple infections of *B. tabaci* endosymbionts

All *B. tabaci* individuals were multiplied infected in various combinations by the four endosymbionts (Table 3). We identified 12 types of co-infection patterns, with multiple infections of 2, 3, or 4 species of endosymbionts. Single infections were identified only in A1 in 4.5% of Asia I and C2 in 10.0% of Asia II 1 cryptic species. *Arsenophonus* was the most common endosymbiont found in multiple infections. Infection patterns were more diverse in Asia I (8 patterns) and Asia II 1 (6 patterns) than in Asia II 5 (3 patterns). The most common combination was A1 + C2 + W1, which was the highest in Asia I (41.8%) and Asia II 1 (35.0%), whereas the A2 + C2 + W1 combination was only present in Asia II 5 at a high rate (86.4%). Among *Arsenophonus* and *Rickettsia* co-infections A1 was co-infected with R2 but A2 was co-infected with R1 (Table 3).

3.3 Correlation between genetic variation of endosymbionts and geographic distance of cryptic species

The variations in ribosomal DNA nucleotide sequences of *Arsenophonus*, *Cardinium*, and *Wolbachia* did not correlate with the geographic distribution of *B. tabaci* in Bangladesh. However, rDNA sequences of *Rickettsia* differed significantly and had a high distribution frequency ($r = 0.428$, $P = 0.023$; Table 5). Namely, the R1 subgroup was mostly present in the

Table 3 Multiple infection patterns of the secondary endosymbionts in four cryptic species of *Bemisia tabaci* from Bangladesh

Combinations of endosymbionts	Infection rates [% (individual numbers)] of each cryptic species			
	Asia I	Asia II 1	Asia II 5	Asia II 10
A1 + C2 + R2 + W1	25.4% (17)	–	–	–
A2 + C2 + R1 + W1	–	35.0% (7)	9.1% (2)	–
A1 + C2 + W1	41.8% (28)	35.0% (7)	–	–
A1 + R2 + W1	–	5.0% (1)	–	–
A1 + C2 + R2	–	10.0% (2)	–	–
A2 + C2 + W1	–	–	86.4% (19)	–
A2 + R1 + W1	4.5% (3)	–	–	–
A2 + C2 + R1	3.0% (2)	–	–	–
C2 + R2 + W1	–	–	4.5% (1)	–
A1 + C2	4.5% (3)	5.0% (1)	–	–
A2 + W1	10.4% (7)	–	–	100% (1)
C2 + W1	6.0% (4)	–	–	–
A1	4.5% (3)	–	–	–
C2	–	10.0% (2)	–	–
Total number of individuals	67	20	22	1

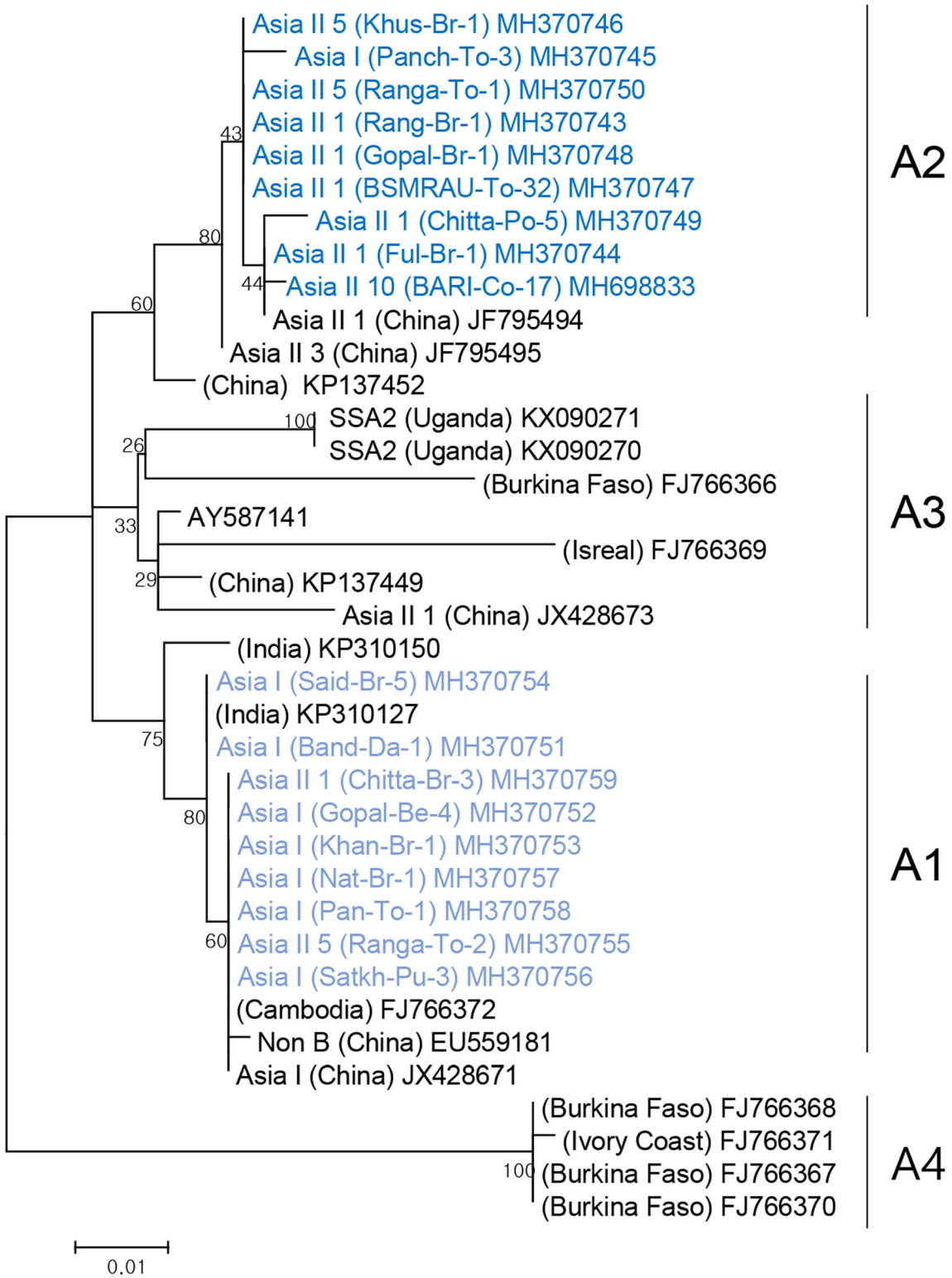

Fig. 2 Maximum-likelihood phylogenetic tree of 23S rDNA in *Arsenophonus* (600 bp) in infected *Bemisia tabaci*, using a GTR + G substitution model. Evolutionary analyses were conducted in MEGA 6. Sequences from GenBank are indicated in black color and the name of the samples from this study are shown in bright and dark blue color and include the genetic group, collection site, and accession number in Bangladesh

north, while R2 was found in the southern regions of Bangladesh (Fig. 6). Thus, *Rickettsia* subgroups had different distributions in Bangladesh, but the distributions of the other endosymbionts were not related to geographic distribution.

Table 4 Comparison of the genetic variation in secondary endosymbionts in cryptic species of *Bemisia tabaci* in Bangladesh

Secondary endosymbionts	Subgroups	Genetic variation (%) within groups or subgroups	Genetic variation (%) between subgroups	
Arsenophonus	A1	0.19–0.37		
	A2	0.19–0.94		
	A1 + A2	0.19–3.18	A1: A2	2.25–3.18
Cardinium	C2	0.25–0.74		
Rickettsia	R1	0.11–2.05		
	R2	0.11–0.22		
	R1+ R2	0.11–13.37	R1: R2	10.15–13.37
Wolbachia	W1	0.17–0.68		

4 Discussion

Our study showed that profiles of the five species of endosymbionts in the indigenous Asian cryptic species of *B. tabaci* identified in Bangladesh were highly variable. Overall, the infection rate of *Arsenophonus* was the highest (93.6%), followed by *Cardinium* and *Wolbachia* (86.4% and 88.2%, respectively). *Rickettsia* only showed a moderate level of infection (31.8%). *Hamiltonella* was not detected in any individuals. These infection rates were complex and varied among the four Asian cryptic species (Asia I, Asia II 1, Asia II 5 and Asia II 10) of *B. tabaci.*

Arsenophonus was the most prevalent among the four Asian cryptic species. This is consistent with the findings of previous studies (Bing et al. 2013a, b; Ansari et al. 2017; Hashmi et al. 2018). *Arsenophonus* was detected in all indigenous genetic groups identified from China and India, such as Asia 1, Asia II 1, Asia II 5, and Asia II 7. In India, among all Asian cryptic species, *Arsenophonus* infection rate was higher in Asia 1 (70.0%) (Ansari et al. 2017; Hashmi et al. 2018). The infection rate of *Arsenophonus* was higher in Asia II 1 (78.9%) than in Asia II 3 and Asia II 7 from China (Bing et al. 2013a, b). *Arsenophonus* was also abundant in China I, but had not been previously detected in China (Bing et al. 2013a, b; Tang et al. 2018). Many studies have shown that *Arsenophonus* infection rates in invasive species are low in MEAM1 (Chiel et al. 2007; Chu et al. 2011; Gottlieb et al. 2008; Gueguen et al. 2010; Marubayashi et al. 2014; Skaljac et al. 2010; Thierry et al. 2011) or absent in MEAM1 and MED (Bing et al. 2013a, b). However, some MED populations from the USA, Montenegro (Q1), Israel (Q2), and Burkina Faso (Q3) were highly infected with *Arsenophonus* (Bing et al. 2013a, b; Gueguen et al. 2010; Skaljac et al. 2010; Skaljac et al. 2013). Therefore, these findings suggest that *Arsenophonus* is the most prevalent endosymbiont in Asian cryptic species, which is unlike the profiles of invasive species MEAM1 and MED of *B. tabaci*.

Cardinium infection rates were high in Asia I, Asia II 1 and Asia II 5 from Bangladesh. In particular, *Cardinium* infected all investigated individuals ($n = 22$) of Asia II 5. High infection rates of *Cardinium* were also reported in Asia II 1 from India (Ansari et al. 2017; Hashmi et al. 2018) and Asia II 3 and Asia II 7 from China (Bing et al. 2013a, b). Otherwise, *Cardinium* infection is rare in MEAMI and MED (Chu et al. 2011; Gueguen et al. 2010; Thierry et al. 2011). Similar to *Arsenophonus*, *Cardinium* had high infection rates in the Asian genetic groups, but low infection rates in the invasive genetic groups.

Hamiltonella was absent in all Asian cryptic species from Bangladesh, and these results are consistent with the reports of previous studies (Bing et al. 2013a, b; Singh et al. 2012). *Hamiltonella* is known to infect only two cryptic species, MEAM1 and MED and these species are highly invasive worldwide (Bing et al. 2013a, b; Chu et al. 2011; Gottlieb et al. 2008; Gueguen et al. 2010; Marubayashi et al. 2014; Skaljac et al. 2017; Thierry et al. 2011). *Hamiltonella* infection in MED (previously Q-biotype) is variable in the subgroups of this genetic group. For example, *Hamiltonella* has high infection rates in Q1 populations in China, but is absent in Q2 and ASL of MED (Bing et al. 2013a, b; Chu et al. 2011; Gottlieb et al. 2008; Gueguen et al. 2010; Marubayashi et al. 2014; Skaljac et al. 2017; Thierry et al. 2011). *Hamiltonella* is also absent in the sub-Saharan Africa 1–5 cryptic species of *B. tabaci* (Ghosh et al. 2015).

Rickettsia was identified in MEAM1 of *B. tabaci,* which is the first recorded occurrence in an insect (Gottlieb et al. 2006). Analysis of its genome sequence revealed that it grouped together with *R. bellii* (Rao et al. 2012). Our study showed that *Rickettsia* was detected in three cryptic species in Bangladesh. Its infection rate was moderate, but variable with the highest infection rate in Asia II 1 (50.0%), a moderate infection rate in Asia I (32.8%), and the lowest infection rate in Asia II 5 (13.6%). However, its infection rate was higher in Asia I than in Asia II 1 and Asia II 7 in Indian populations (Hashmi et al. 2018). *Rickettsia* was also detected in Asia II 3, Asia II 7, China 1, and MEAM1, but was absent from Asia II 1 and MED in

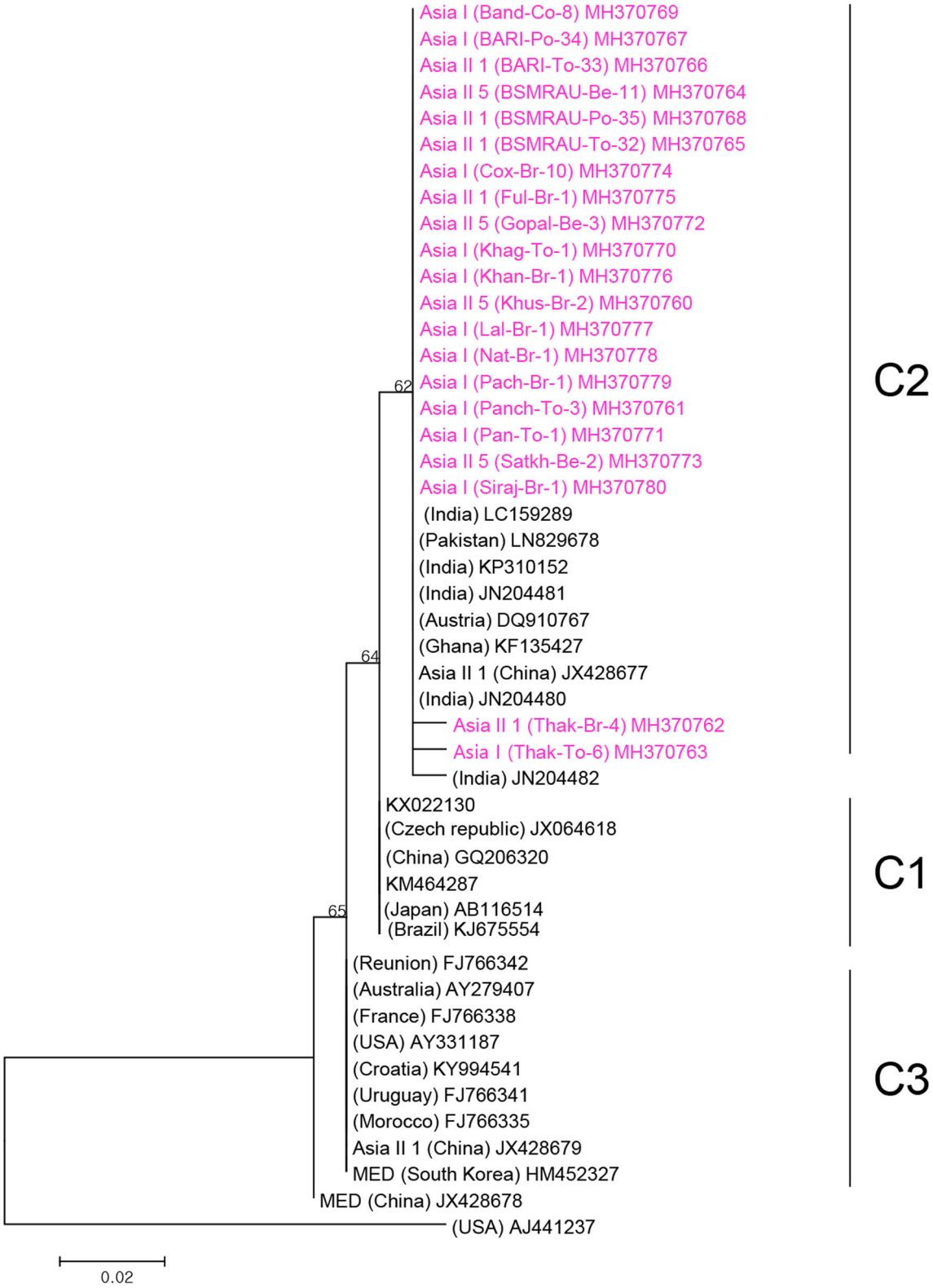

Fig. 3 Maximum-likelihood phylogenetic tree of 16S rDNA in *Cardinium* (400 bp) in infected *Bemisia tabaci*, using a HKY + G substitution model. Evolutionary analyses were conducted in MEGA 6. Sequences from GenBank are indicated in black color and the name of the samples from this study are shown in pink color and include the genetic group, collection site, and accession number in Bangladesh

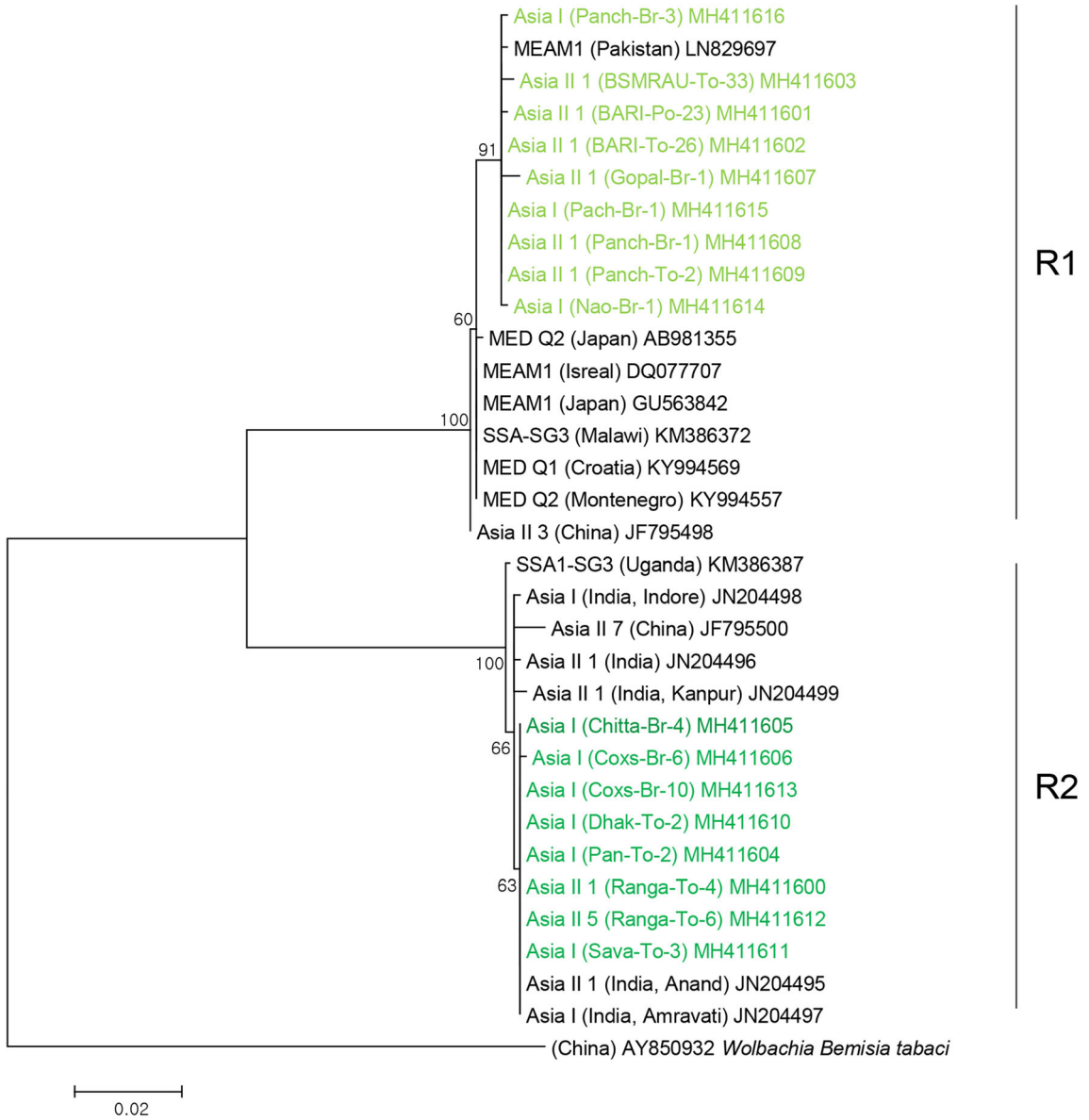

Fig. 4 Maximum-likelihood phylogenetic tree of 16S rDNA in *Rickettsia* (900 bp) in infected *Bemisia tabaci*, using a HKY + I substitution model. Evolutionary analyses were conducted in MEGA 6. Sequences from GenBank are indicated in black color and the name of the samples from this study are shown in bright and dark green color and include the genetic group, collection site, and accession number in Bangladesh

China (Bing et al. 2013a, b; Chiel et al. 2007; Chu et al. 2011; Gueguen et al. 2010; Thierry et al. 2011). This suggests that the infection rate of *Rickettsia* is variable in different genetic groups and geographic regions. For example, our study showed that its infection rate in Asia II 1 in Bangladesh had a significantly different geographic distribution than those in China and India.

Wolbachia is one of the most common insect endosymbionts, with various infection rates for different genetic groups (Correa and Ballard 2016). Our study showed that *Wolbachia* was highly prevalent in all cryptic species of *B. tabaci* from Bangladesh, with a 100% infection rate in Asia II 5. High infection rates of *Wolbachia* were reported in Asia II 1, Asia II 7, and China 1, but *Wolbachia* was absent in Asia II 3 from China (Bing et al. 2013a, b). However, its infection rate was moderate in Asia 1 and Asia II 7 and low in Asia II 1 from India (Hashmi et al. 2018). This suggests that *Wolbachia* infection rates vary regardless of the genetic groups of *B. tabaci*. Otherwise, *Wolbachia* infection was high in Bangladesh and China, but low in India. However, this difference may not be associated with geographic distribution. In fact, the *Wolbachia* infection rate is highly variable across regions and populations. For example, the *Wolbachia* infection rate in both MED and MEAM1 ranges from 0% to 100% throughout China (Bing et al. 2013a, b, 2014; Pan et al. 2012). In Europe and western Africa, *Wolbachia* infection of MEAM1

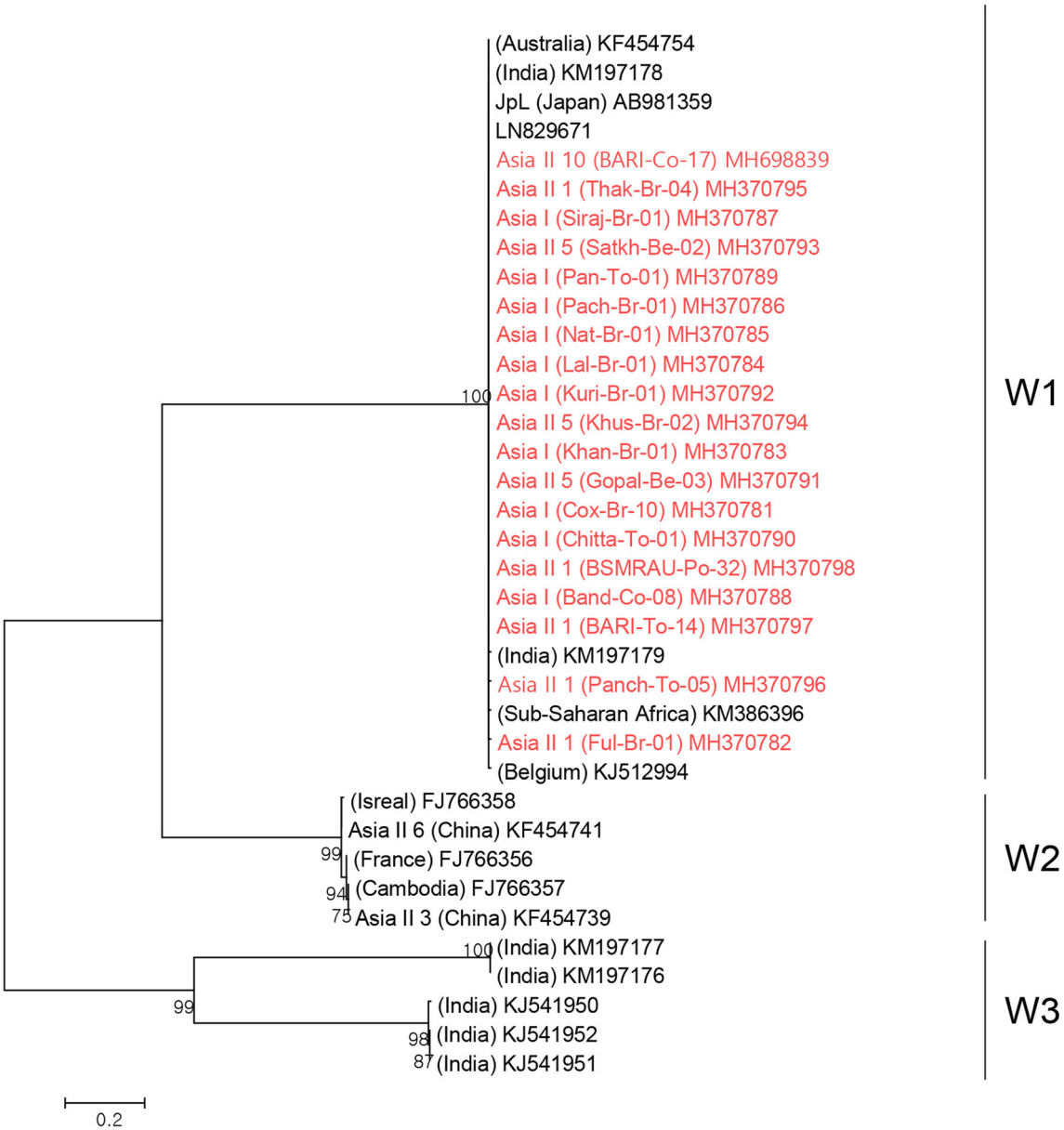

Fig. 5 Maximum-likelihood phylogenetic tree of 16S rDNA in *Wolbachia* (650 bp) in infected *Bemisia tabaci*, using a GTR + G substitution model. Evolutionary analyses were conducted in MEGA 6. Sequences from GenBank are indicated in black color and the name of the samples from this study are shown in red color and include the genetic group, collection site, and accession number in Bangladesh

and MED was 0–8.3% and 0–33% (Chiel et al. 2007; Chu et al. 2011; Gnankine et al. 2013; Gueguen et al. 2010; Nirgianaki et al. 2003; Skaljac et al. 2010; Thierry et al. 2011).

Phylogenetic analyses showed that both *Arsenophonus* and *Rickettsia* were detected in two subgroups, but *Cardinium* and *Wolbachia* were detected in a single subgroup in Bangladesh.

Table 5 Correlations between genetic variation in endosymbionts and geographic distance of cryptic species of *Bemisia tabaci* from Bangladesh

Endosymbionts	N*	Correlation (r)	Lower 95% CI	Upper 95% CI	*P* value
Arsenophonus	46	−0.016	−0.364	0.333	0.9279
Cardinium	43	−0.159	−0.503	0.185	0.3543
Rickettsia	26	0.428	0.064	0.792	0.0230
Wolbachia	43	−0.279	−0.614	0.055	0.0990

**N* sample size; *CI* confidence intervals

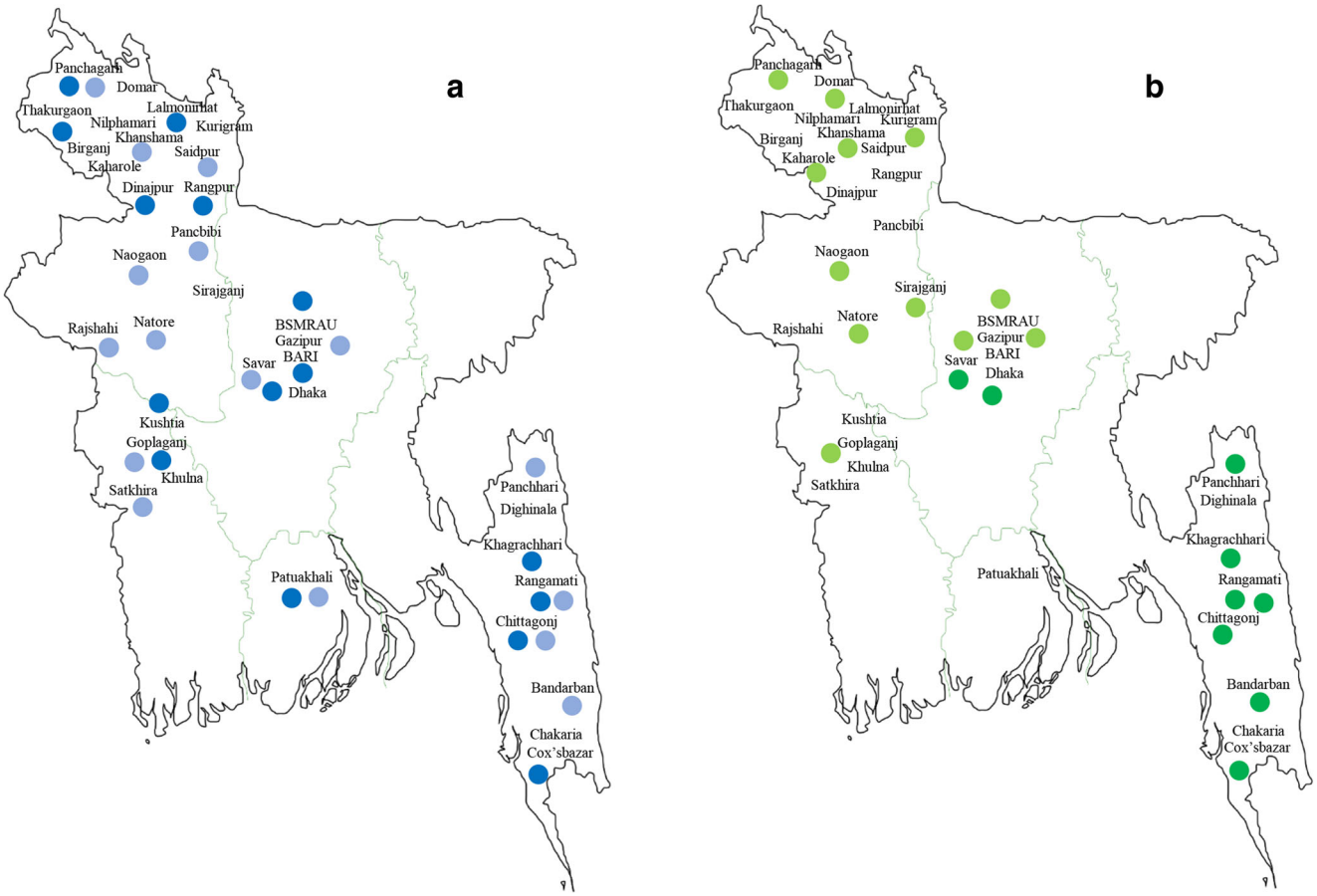

Fig. 6 Geographic distribution of *Arsenophonus* (**a**) and *Rickettsia* (**b**) subgroups of *Bemisia tabaci* in Bangladesh. Two subgroups of both *Arsenophonus* and *Rickettsia* are shown in bright blue (A1), dark blue (A2), bright green (R1), and dark green (R2)

Arsenophonus was infected into all four cryptic species. Our study showed that A2 infected all four cryptic species, but A1 infected only in Asia I and Asia II 1. This result suggests that *Arsenophonus* infection depends on the genetic group of *B. tabaci*. Similarly, two *Rickettsia* subgroups (R1, R2) were detected in three cryptic species in Bangladesh with different infection rates; R1 was higher in Asia I and R2 was higher in Asia II 1 and Asia II 5. The presence of two *Rickettsia* subgroups in *B. tabaci* has been reported in previous studies (Singh et al. 2012; Bing et al. 2013a, b; Ghosh et al. 2015). Singh et al. (2012) observed RI1 and RI2 strains in Indian populations. Bing et al. (2013a, b) reported that one subgroup detected in MEAM1 and Asia II 3 is widely separated from the other group found in Asia II 7 and China 1. Ghosh et al. (2015) described two clusters that had more than 8.5% nucleotide distance in various genetic groups of *B. tabaci* infesting cassava in Africa. Our results showed that 16S rDNA sequence variation was low within subgroups but subgroups were highly distinct, with differences of 10.15–13.37%. This suggests that there are at least two genetically distinct *Rickettsia* species in the *B. tabaci* species complex. Further, the two subgroups were present in the same cryptic species of *B. tabaci,* although the ratios were different. This suggests that each subgroup of *Rickettsia* is not confined within certain genetic groups of *B. tabaci*. This characteristic is also found in *Rickettsia* infection in the African genetic groups of *B. tabaci* (Ghosh et al. 2015). For example, sub-Saharan Africa 1- subgroup 3 (SSA 1-SG3) is infected by two groups of *Rickettsia* at different rates.

Our study also showed that there is a relationship between genetic variation of *Rickettsia* and geographic distribution, contrary to other endosymbionts. Namely, R1 subgroup was mostly present in the northern region, while R2 subgroup was distributed in the southern region of Bangladesh. Similarly, Singh et al. (2012) identified two subgroups of *Rickettsia* in *B. tabaci* collected from various regions of Central and Northern India. R1 was detected in the northern region, but R2 was detected in the central region of India. Our phylogenetic analysis showed that the R2 subgroup in Bangladesh was clustered with R2 strains collected from Akola and Indore, which are in the central region of India. Thus, the geographic distributions of the two *Rickettsia* subgroups are distinct in these two countries. Results from this study also showed that the distribution of the two *Rickettsia* subgroups was not associated with genetic groups of *B. tabaci,* because each Asian cryptic species was infected by both subgroups of

Rickettsia at various rates. Further analysis is required to determine the influence of geographic distribution.

Multiple infections of various secondary endosymbionts are common in most host species of insects (Zchori-Fein et al. 2014). We identified 12 types of co-infection patterns among four endosymbionts, particularly among two subgroups of *Arsenophonus* and *Rickettsia*. Every individual in this study was infected by at least one endosymbiont. *Arsenophonus* was the dominant endosymbiont in most co-infections. Interestingly, the combination of *Arsenophonus* and *Rickettsia* subgroups was always present as A1 + R2 or A2 + R1. Similarly, data from Israel and Burkina Faso demonstrated A1 + R co-infection in MED (Q2) and A2 + R in MED (Q3), respectively (Gueguen et al. 2010). *Arsenophonus* in MED (Q1 and Q2) is the R1 subgroup (Skaljac et al. 2017). Together, these data suggest that Asian cryptic *B. tabaci* may have a unique characteristic that is different from other cryptic species such as MED.

In conclusion, our results revealed the complexity of endosymbiont profiles in four cryptic *B. tabaci* species from Bangladesh. Specifically, each cryptic species exhibited characteristic patterns of infection rates and combinations of endosymbionts. Notably, *Arsenophonus*was a major endosymbiont but *Hamiltonella* was absent from all Asian cryptic species of *B. tabaci*. Two genetically distinct subgroups of *Rickettsia* were associated with geographic distribution. This study provides important insights that can help improve the control techniques of whiteflies.

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