Diversity and evolution of the endosymbionts of *Bemisia tabaci* in China

Xiao-Tian Tang [1, 2] , **Li Cai** [1] , **Yuan Shen** [3] , **Yu-Zhou Du** Corresp. [1, 4]

[1] School of Horticulture and Plant Protection & Institute of Applied Entomology, Yangzhou University, Yangzhou, Jiangsu, China

[2] Department of Entomology, Texas A&M University, College Station, TX, United States

[3] Agriculture and Forestry Bureau of Binhu District, Wuxi, China

[4] Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education, Yangzhou University, Yangzhou, Jiangsu, China

Corresponding Author: Yu-Zhou Du
Email address: yzdu@yzu.edu.cn

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a cryptic species complex, including members that are pests of global importance. This study presents a screening of *B. tabaci* species in China for infection by the primary endosymbiont, *Portiera aleyrodidarum*, and two secondary endosymbionts, *Arsenophonus* and *Cardinium* . The results showed that *P. aleyrodidarum* was detected in all *B. tabaci* individuals, while *Arsenophonus* was abundant in indigenous species of *B. tabaci* Asia II 1, Asia II 3 and China 1 but absent in the invasive species, Middle East-Asia Minor 1 (MEAM1); *Cardinium* presented in the Mediterranean (MED), Asia II 1 and Asia II 3 species but was rarely detected in the MEAM1 and China 1 species. Moreover, phylogenetic analyses revealed that the *P. aleyrodidarum* and *mtCO1* (mitochondrial cytochrome oxidase 1) phylograms were similar and corresponding with the five distinct cryptic species clades to some extent, probably indicating an ancient infection followed by vertical transmission and subsequent co-evolutionary diversification. In contrast, the phylogenetic trees of *Arsenophonus* and *Cardinium* were incongruent with the *mtCO1* phylogram, potentially indicating horizontal transmission in *B. tabaci* cryptic species complex. Taken together, our study showed the distinct infection status of endosymbionts in invasive and indigenous whiteflies; we also probably indicated the co-evolution of primary endosymbiont and its host as well as potential horizontal transfer of secondary endosymbionts.

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Xiao-Tian Tang [1, 3, *], Li Cai [1, *], Yuan Shen [1, 4], Yu-Zhou Du [1, 2* *]

[1]School of Horticulture and Plant Protection & Institute of Applied Entomology, Yangzhou University, Yangzhou, Jiangsu, 225009, China

[2]Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education, Yangzhou University, Yangzhou 25009, China.

[3]Current address: Department of Entomology, Texas A&M University, College Station, TX 77843, USA

[4]Agriculture and Forestry Bureau of Binhu District, Wuxi 214071, China

*These authors contributed equally to this work

**Corresponding author: School of Horticulture and Plant Protection & Institute of Applied Entomology, Yangzhou University, 48 Wenhui Road (East), Yangzhou, Jiangsu, 225009, China. Tel: 0086-514-87971854; Fax: 0086-514-87347537.

E-mail address: yzdu@yzu.edu.cn (YZD)

Abstract

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a cryptic species complex, including members that are pests of global importance. This study presents a screening of *B. tabaci* species in China for infection by the primary endosymbiont, *Portiera aleyrodidarum*, and two secondary endosymbionts, *Arsenophonus* and *Cardinium*. The results showed that *P. aleyrodidarum* was detected in all *B. tabaci* individuals, while *Arsenophonus* was abundant in indigenous species of *B. tabaci* Asia II 1, Asia II 3 and China 1 but absent in the invasive species, Middle East-Asia Minor 1 (MEAM1); *Cardinium* presented in the Mediterranean (MED), Asia II 1 and Asia II 3 species but was rarely detected in the MEAM1 and China 1 species. Moreover, phylogenetic analyses revealed that the *P. aleyrodidarum* and *mtCO1* (mitochondrial cytochrome oxidase 1) phylograms were similar and corresponding with the five distinct cryptic species clades to some extent, probably indicating an ancient infection followed by vertical transmission and subsequent co-evolutionary diversification. In contrast, the phylogenetic trees of *Arsenophonus* and *Cardinium* were incongruent with the *mtCO1* phylogram, potentially indicating horizontal transmission in *B. tabaci* cryptic species complex. Taken together, our study showed the distinct infection status of endosymbionts in invasive and indigenous whiteflies; we also probably indicated the co-evolution of primary endosymbiont and its host as well as potential horizontal transfer of secondary endosymbionts.

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48 **Introduction**

49 *Bemisia tabaci* is a cryptic species complex comprising a minimum of 40 morphologically
50 similar species (De Barro et al. 2011; Dinsdale et al. 2010; Hu et al. 2018; Wang et al. 2017).
51 Among members of the complex, the Middle East-Asia Minor 1 (MEAM1) and Mediterranean
52 (MED) groups (commonly known as the B and Q biotypes, respectively) have drawn much
53 attention due to their global invasion and vectoring important plant pathogens (e.g. Tomato yellow
54 leaf curl virus, TYLCV) (Brown 1994; Cohen & Nitzany 1966). In China, *B. tabaci* was not
55 considered as a serious pest until the arrival of the MEAM1 group in the mid-1990s (Qiu et al.
56 2007). In 2004, the MED group was detected, and rapidly became widely distributed in China,
57 causing considerable damage to a wide range of vegetables, fibers, and ornamental crops (Chu et
58 al. 2005). It is interesting that MED has been replacing the earlier invader MEAM1 as well as
59 several indigenous species of whiteflies (e.g. Asia II and China 1) in many regions (Liu et al. 2007;
60 Sun et al. 2013).

61 Associations between insects and endosymbionts are quite common in nature. It has been
62 estimated that at least 15−20% of all insect species live in symbiotic relationships with bacteria
63 (Douglas 1998; Gosalbes et al. 2010). Endosymbionts associated with insects can be classified into
64 primary endosymbionts (P-endosymbionts) and secondary endosymbionts (S-endosymbionts)
65 (Baumann 2005). The P-endosymbionts are obligate and usually have mutualist relationships with
66 their hosts. Besides, P-endosymbionts are generally localized in bacteriocytes of bacteriome
67 (Caspi-Fluger et al. 2011) and transmitted vertically from mother to progeny (Werren & O'Neill
68 1997). In contrast, the S-endosymbionts are usually facultative symbionts, and they could reside
69 in several host tissues such as gut, hemolymph, Malpighian tubules, salivary glands or ovarian
70 cells (Cicero et al. 2016; Cooper et al. 2014; Dobson et al. 1999; Zchori-Fein et al. 1998). Infection
71 of secondary endosymbionts can be either maternally inherited or horizontally-transmitted (Moran

& Baumann 2000). It has been discovered that the P-endosymbionts *Portiera aleyrodidarum* and S-endosymbionts such as *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Rickettsia*, and *Wolbachia* were infected in whiteflies (Bing et al. 2013; Chiel et al. 2007; Chu et al. 2011; Everett et al. 2005; Karut et al. 2017; Thao & Baumann 2004; Zchori-Fein et al. 2014). Previous studies have investigated the prevalence, diversity and evolution of endosymbionts in the *B. tabaci* species complex from different countries or regions (e.g. Turkey, China, Brazil and Africa) (Ahmed et al. 2010; Bing et al. 2013; Bing et al. 2014; Ghosh et al. 2015; Hashmi et al. 2018; Jahan et al. 2015; Karut et al. 2017; Marubayashi et al. 2014; Santos-Garcia et al. 2015; Sseruwagi et al. 2018; Thierry et al. 2011; Thierry et al. 2015). However, most of these reports only focused on the two invasive cryptic species MEAM1 and MED; furthermore, for studies from China, the sample size and distribution range were limited. For example, regarding prevalence of S-endosymbionts in *B. tabaci*, only the laboratory samples from two provinces were investigated (Bing et al. 2013); only one S-endosymbiont (*Wolbachia*) was explored from a slightly larger geographical scale (Bing et al. 2014).

Therefore, although substantial datasets regarding endosymbionts prevalence of *B. tabaci* species complex are present, their current situation in whole China is still not very clear and further investigation is essential. In the present study, our first goal is to investigate the prevalence and diversity of the P-endosymbionts *P. aleyrodidarum* and two common S-endosymbionts *Arsenophonus* and *Cardinium* (as representatives of S-endosymbionts) within *B. tabaci* in wider range of China. We also aim to explore the evolutionary relationships between these three endosymbionts and their host based on phylogenetic analyses of 16S and 23S ribosomal DNA (*rDNA*) (from endosymbionts) as well as mitochondrial cytochrome oxidase 1 (*mtCO1*) gene (from *B. tabaci*). Our study will not only uncover the current status of endosymbionts infection within *B. tabaci* in China, but also greatly provide a supplement for studies of *B. tabaci* endosymbionts worldwide.

Materials and Methods

99 **Sample collection**

100 Totally 1,510 *B. tabaci* individuals were collected from 71 geographical locations, including
101 19 provinces and four municipalities in China (Fig. 1). At each location, *B. tabaci* was collected
102 from different leaves of separate plants. The collection details, geographical sites and host plants
103 were described in Table S1.

104

105 **DNA extraction and gene amplification**

106 Total DNA was extracted from individual whitefly as described in Luo et al. (2002). The
107 primers of *mtCO1* gene was used for whitefly species identification. 16S *rDNA* primers were used
108 to detect *P. aleyrodidarum* and *Cardinium*, and for *Arsenophonus*, 23S *rDNA* primers were
109 utilized. The primers, annealing temperature, and predicted PCR products size were shown in
110 Table 1. The PCR reaction mixture contained 1 U Taq DNA polymerase, 5 µl (10×) reaction buffer,
111 3 µl $MgCl_2$ (final concentration of 25 mmol/L), 2 µl dNTPs (10 mmol/L), 2 µl of forward and
112 reverse primers (20 µmol/L each) and 2 µl of template DNA (Simon et al. 1994). PCR products
113 were visualized by 1.5% agarose gels and sequenced by IGE Biotechnology Co., Ltd (Guangzhou,
114 China). The sequences were deposited in Genbank under accession numbers KP137471 to
115 KP137491 for *B. tabaci mtCO1* gene, KP201110 to KP201126 for *P. aleyrodidarum* 16S *rDNA*,
116 KP201103 to KP201109 for *Arsenophonus* 23S rDNA, and KP201127 to KP201134 for
117 *Cardinium* 16S *rDNA* (Table 2).

118

119 **Sequence alignment and phylogenetic analysis**

120 Sequence fragments were assembled using ContigExpress and aligned using the Clustal X 1.83
121 program (Chenna et al. 2003). The GenBank database was searched for homologous sequences of
122 *mtCO1*, 16S rDNA and 23S rDNA using the Basic Local Alignment Search Tool (BLAST).
123 Phylogenetic trees were constructed using MrBayes 3.2.1 (Ronquist & Huelsenbeck 2003). The
124 best-fit substitution model for each of the aligned sequences was selected with the program
125 Modeltest 3.7 (Posada & Crandall 1998). All the trees were constructed using the GTR+I+G

model. The Metropolis-coupled Markov chain Monte Carlo algorithm was conducted using four chains. Analyses were initiated with random starting trees, processed for 3×10^6 generations, and sampled every 1,000 generations. For the burn-in period, we discarded 100,000 generations. Posterior clade probabilities obtained from the analysis were used to assess nodal support. Tree information was visualized and edited using FigTree ver. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Molecular identification of *B. tabaci* individuals

Analyses of *mtCO1* sequences indicated that our 1,510 *B. tabaci* samples comprised of two invasive species (MEAM1 and MED) and three indigenous species (Asia II 1, Asia II 3 and China 1). Among the individuals tested, 36.4% (550/1,510) and 39.7% (600/1,510) were identified as MEAM1 and MED, respectively. The remained 4.6%, 9.3% and 9.9% insects were identified as Asia II 1 (70/1,510), Asia II 3 (140/1,510) and China 1 (150/1,510), respectively (Fig. 1A; Table S1). Moreover, both the MEAM1 and MED whiteflies were widely-distributed across China, whereas the three indigenous species (Asia II 1, Asia II 3 and China 1) were relatively less detected and mainly distributed in southeastern part of China (Fig. 1B).

Prevalence of endosymbionts among five species of *B. tabaci*

As expected, *P. aleyrodidarum* was detected in all whitefly individuals and species (Fig. 2A); 21.7% (327/1,510) individuals harbored S-endosymbiont (*Arsenophonus* or *Cardinium*); 5.6% (84/1,510) whiteflies were co-infected with both, and the remained majority (72.8%; 1,099/1,510) lacked an infection with either of the two S-endosymbionts. Infection frequencies with S-endosymbionts varied across *B. tabaci* species. In detail, *Arsenophonus* was abundant in the indigenous species (Asia II 1, Asia II 3 and China 1), with infection rates ranging from 50.7-55.7%; however, it was infrequent in MED (11.5%) and MEAM1 (0.0%) (Fig. 2B). *Cardinium* was moderately common in MED, Asia II 1 and Asia II 3 populations with frequencies of 20.3-40.7%,

153 but rarely detected in MEAM1 (0.5%) and not found in China 1 (Fig. 2C).

154

155 **Genetic diversity of *B. tabaci* and its endosymbionts**

156 Aligned sequences from *B. tabaci* (813 bp, *mtCO1* gene), *P. aleyrodidarum* (886 bp, 16s
157 *rDNA*), *Arsenophonus* (551 bp, 23S *rDNA*), and *Cardinium* (460 bp, 16S *rDNA*) were used to
158 analyze the genetic variation of whitefly and its endosymbionts. The results showed that 21
159 haplotypes were identified in whiteflies based on *mtCO1* sequences, while 17, 7 and 8 symbiont
160 haplotypes were defined based on analysis of *P. aleyrodidarum*, *Arsenophonus* and *Cardinium*
161 sequences, respectively (Table 2). These haplotypes sequences were used to construct the
162 phylogenetic trees.

163

164 **Phylogenetic analysis of *B. tabaci* and endosymbionts**

165 Phylogenetic trees were constructed for *B. tabaci*, *P. aleyrodidarum*, *Arsenophonus* and
166 *Cardinium* based on *mtCO1* gene, 16s *rDNA*, 23S *rDNA* and 16s *rDNA* sequences of haplotypes,
167 respectively. We also pulled the related sequences from NCBI to explore the phylogenetic status
168 of our haplotypes. The GenBank number of those related sequences could be found in phylogenetic
169 trees. Based on the *mtCO1* gene, we found five distinct genetic groups of *B. tabaci*, which was
170 corresponding with the five cryptic species including the MEAM1, MED, Asia II 1, Asia II 3 and
171 China 1 (Fig. 3). We can also find that all of our MED individuals belonged to Q1 subclade (Chu
172 et al. 2011). For phylogenetic trees of endosymbionts, several distinct bacterial strains existed
173 within individual bacterium. It is interesting that the phylogenetic tree of *P. aleyrodidarum* were
174 similar to the *mtCO1* tree and exhibited four groups corresponding to MEAM1+MED, Asia II 1,
175 Asia II 3 and China 1 clades to some extent (Fig. 4). While there are still several differences. For
176 example, the sequences of *P. aleyrodidarum* of China 1 are not well clustered and *P.*
177 *aleyrodidarum* from *B. tabaci* species MED (KP201113) was also present in that China 1 clade.
178 In addition, the MEAM1+MED clade (Fig. 4) contained sequences of *P. aleyrodidarum* from both
179 MEAM1 and MED; however, it was separated into two distinct clades in the *B. tabaci* tree (Fig.

3). In contrast, the phylogenetic trees for *Arsenophonus* and *Cardinium* were totally incongruent with the *mtCO1* tree, exhibiting four (A1-A4) and three groups (C1-C3), respectively (Fig 5 and 6).

Discussion

In this study, we conducted an extensive screening of *B. tabaci* for the presence one P-endosymbiont of two common S-endosymbionts, along with phylogenetic analyses of these symbionts to compare with host species from the cryptic *B. tabaci* complex. The reason we chose *Arsenophonus* and Cardinium as representatives of S-endosymbionts because they are the very common S-endosymbionts in whiteflies, and *Wolbachia* has been thoroughly investigated in the study of Bing et al. (2014). The results showed that P-endosymbiont *P. aleyrodidarum* was detected in all whitefly individuals while S-endosymbionts infection were varied in species. The variation in the prevalence of endosymbionts could be influenced by numerous factors such as host, environmental conditions, geographical location or even climate (Chu et al. 2011; Karut & Tok 2014; Morag et al. 2012; Skaljac et al. 2010). In our study, *Arsenophonus* was abundant in Asia II 1, Asia II 3 and China 1 species but absent in the invasive species MEAM1, which is exactly consistent with previous studies (Bing et al. 2013; Karut et al. 2017); *Cardinium* was present in the MED, Asia II 1and Asia II 3 species (20.3-40.7%) but was rarely detected in MEAM1 and not detected in China 1. Taken together, it seemed that these two S-endosymbionts had high prevalence in native species rather than invasive species, which is consistent with another S-endosymbiont *Wolbachia* but in contrast to *Hamiltonella*; *Hamiltonella* was found abundant in invasive species rather than native species (Bing et al. 2013).

Previous studies showed that *B. tabaci* could be co-infected with particular pairs of S-endosymbionts, including *Rickettsia* and *Hamiltonella*, *Hamiltonella* and *Cardinium*, or *Rickettsia* and *Arsenophonous*; others were less common, such as *Cardinium* and *Rickettsia*, *Hamiltonella* and *Arsenophonous*, *Cardinium* and *Wolbachia*, and even three or four endosymbionts together (Gueguen et al. 2010; Karut & Tok 2014; Marubayashi et al. 2014; Pan et al. 2012; Skaljac et al.

2010). In our study, we found evidence for a low rate (5.6%) of co-infection with *Arsenophonous* and *Cardinium* in four *B. tabaci* species (MEAM1 was the exception since *Arsenophonous* was not detected in this species), which has also been reported before (Bing et al. 2013; Chu et al. 2011; Parrella et al. 2014; Zchori-Fein et al. 2014). However, the reason of so few rate of co-infection by *Arsenophonous* and *Cardinium* is that *Arsenophonous* and *Cardinium* are potential reproductive manipulators that compete for resources inside the bacteriocytes, thus compromising the fitness of host (Gottlieb et al. 2008). We have one plausible explanation for the co-infection status, that is *Cardinium* is not restricted to the bacteriocytes (Skaljac et al. 2010), and perhaps the non-overlapping niche makes co-infection of *Arsenophonous* and *Cardinium* possible. In addition, the co-infection symbiont system in whiteflies may indicate the roles of dual endosymbionts: work as important mutually dependents to provide full complement of nutrients to their host (Rao et al. 2015) or affect the fitness and biology of the *B. tabaci* (Ghosh et al. 2018).

Our phylogenetic analyses indicated that *B. tabacia* mt*CO1* sequences could be assigned to five distinct clades, which conformed to existing MEAM1, MED, Asia II 1, Asia II 3, and China 1 clades. Similarly, the sequences of the P-endosymbionts *P. aleyrodidarum* were assigned to their own clade and the phylogeny was similar with that of *B. tabaci* genetic groups to some extent. This may potentially indicate an ancient infection followed by vertical transmission and subsequent co-evolutionary diversification (Baumann 2005). Meanwhile, it is important to note that the correlation was not perfect: sequences of *P. aleyrodidarum* from MEAM1 and MED were assigned to the same clade instead of the two distinct clades presented in the mt*CO1* tree. The reason could be the dissemination of the MEAM1 and MED species; furthermore, the spread of these two invasive species in China has been frequently associated with founder effects that fix specific mtDNA variation(s) along with particular endosymbionts (Chu et al. 2011; Gueguen et al. 2010). Taken together, although there was similarity between the two trees of *P. aleyrodidarum* and *B. tabaci*, the genetic variation of primary symbiont might not be an ideal reflecting the genetic variation of the cryptic *B. tabaci*.

The S-endosymbionts, *Arsenophonus* and *Cardinium,* both showed a lack of congruence with

234 the *B. tabaci mtCO1* phylogram. This is consistent with the finding from Ahmed et al. (2013), who
235 provided evidence for horizontal transmission of S-endosymbionts in the *B. tabaci* cryptic species
236 complex based on phylogenies studies. There are substantial phylogenetic evidences showing that
237 S-endosymbionts such as *Wolbachia* and *Arsenophonus*, undergoing horizontal transfer among
238 host arthropod species (Ahmed et al. 2013; Chrostek et al. 2017; Kolasa et al. 2017; Li et al. 2017;
239 Russell et al. 2003; Vavre et al. 1999). In some cases, the mechanisms for horizontal transmission
240 of S-endosymbionts are already known, including transferring through parasitoid wasps (Ahmed
241 et al. 2015; Gehrer & Vorburger 2012), plants (Caspi-Fluger et al. 2012; Li et al. 2017) or even
242 sexual transmission (Moran & Dunbar 2006). Therefore, the potential horizontal transfer of S-
243 endosymbionts in our samples could be one or combination of the above ways.

244 In summary, this study reported the varied prevalence of three endosymbionts within five *B.*
245 *tabaci* cryptic species. The P-endosymbiont *P. aleyrodidarum* was detected in all whitefly
246 individuals; the S-endosymbionts *Arsenophonus* was abundant in native species while *Cardinium*
247 was common in the invasive species. In addition, the phylogenetic relationships between
248 endosymbionts and their hosts *B. tabaci* probably indicated the vertical transmission and co-
249 evolution of *P. aleyrodidarum* and *B. tabaci*; meanwhile, horizontal transfer of *Arsenophonus* and
250 *Cardinium* may happen in our collecting samples. Our study not only reported current infection
251 status of endosymbionts within *B. tabaci* populations in China, but also demonstrated that S-
252 endosymbionts genetic variation may not reflect host genetic variation and should not be used to
253 infer taxonomic relationships within the host species complex. If funding allows, more
254 endosymbionts should be investigated and future investigations could be the contribution of
255 endosymbionts to invasiveness, population expansion, or even competitiveness of whitefly
256 species.

257

258 **Acknowledgements**

259 We express our deep gratitude to the Testing Center of Yangzhou University. We sincerely
260 thank Dr. Kerry M. Oliver from Department of Entomology, University of Georgia for polishing

261 the manuscript and providing valuable comments.

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454

455

456 **Figure Captions**

457 **Figure 1. The quantity and distribution of *B. tabaci* cryptic species in China.** (A) The quantity
458 of each *B. tabaci* cryptic species based on molecular identification. (B) The locations of the *B.*
459 *tabaci* cryptic species populations in China. Names of locations are given in Table S1. Maps were
460 created using Esri's ArcGIS platform (http://www.esri.com/software/arcgis).

461

462 **Figure 2. Infection frequency of endosymbionts in five *B. tabaci* cryptic species.** (A) *P.*
463 *aleyrodidarum*; (B) *Arsenophonus*; (C) *Cardinium*. Number above bars indicate the number of
464 infection.

465

466 **Figure 3. The Bayesian phylogenetic tree of *B. tabaci* cryptic species based on *mtCOI***
467 **sequences**. The value beside the nodes are posterior probabilities. *Trialeurodes vaporariorum*
468 (AF418672) is used as outgroup. Accession numbers for *mtCOI* sequences submitted to GenBank
469 are KP137471-KP137491. All *mtCOI* sequences of *B. tabaci* cryptic species used in this study
470 were clustered with other related references sequences from GenBank and their accession numbers
471 are also indicated in the tree. Bold dots indicate the sequences from the present study.

472

473 **Figure 4. The Bayesian phylogenetic tree of *P. aleyrodidarum* based on 16S *rDNA* sequences.**
474 The value beside the nodes are posterior probabilities. *Trialeurodes vaporariorum* (AY266113) is

used as outgroup. Accession numbers for 16S *rDNA* sequences submitted to GenBank are KP201110-KP201125. All 16S *rDNA* sequences of *P. aleyrodidarum* used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. Bold dots indicate the sequences from the present study. Dotted boxes indicate imperfect cluster of each *B. tabaci* cryptic species.

Figure 5. The Bayesian phylogenetic tree of *Arsenophonus* based on 23S *rDNA* sequences. The value beside the nodes are posterior probabilities. *Aleurodicus dispersus* (AY264664) is used as outgroup. Accession numbers for 23S *rDNA* sequences submitted to GenBank are KP201103-KP201109. All 23S *rDNA* sequences of *Arsenophonus* used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. A1-A4 indicate the four clusters.

Figure 6. The Bayesian phylogenetic tree of *Cardinium* based on 16S *rDNA* sequences. The value beside the nodes are posterior probabilities. *Paralvinella palmiformis* (AJ441237) is used as outgroup. Accession numbers for 16S *rDNA* sequences submitted to GenBank are KP201127-KP201134. All 16S *rDNA* sequences of *Cardinium* used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. C1-C3 indicate the three clusters.

Figure 1

The quantity and distribution of *B. tabaci* cryptic species in China.

(A) The quantity of each *B. tabaci* cryptic species based on molecular identification. (B) The locations of the *B. tabaci* cryptic species populations in China. Names of locations are given in Table S1. Maps were created using Esri's ArcGIS platform (http://www.esri.com/software/arcgis) .

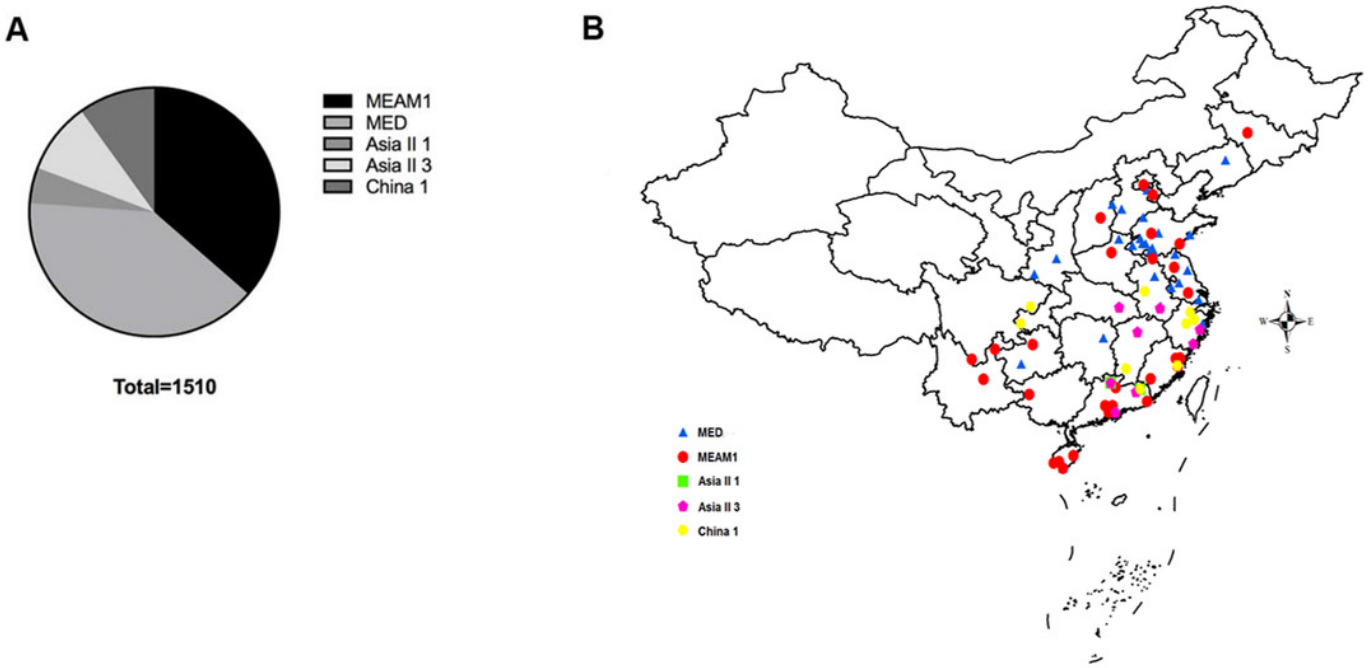

Figure 2

Infection frequency of endosymbionts in five *B. tabaci* cryptic species.

(A) *P. aleyrodidarum*; (B) *Arsenophonus*; (C) *Cardinium*. Number above bars indicate the number of infection.

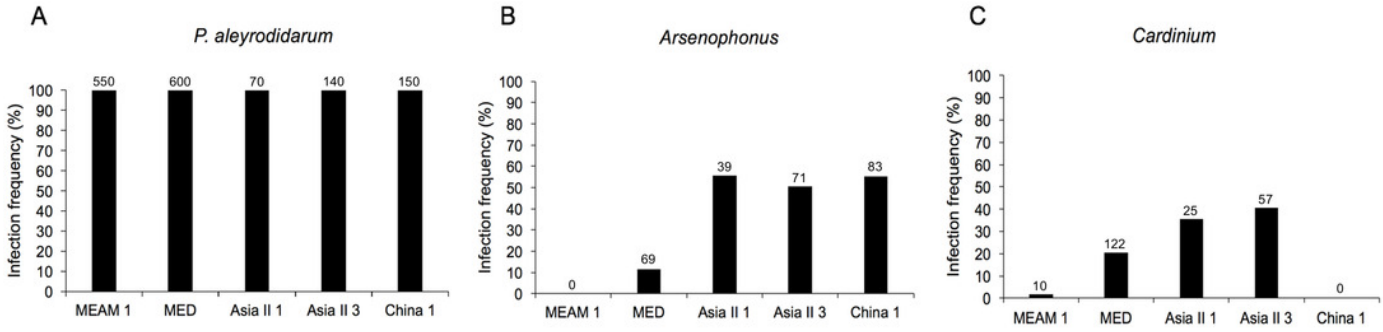

Figure 3

The Bayesian phylogenetic tree of *B. tabaci* cryptic species based on *mtCOI* sequences.

The value beside the nodes are posterior probabilities. *Trialeurodes vaporariorum* (AF418672) is used as outgroup. Accession numbers for *mtCOI* sequences submitted to GenBank are KP137471-KP137491. All *mtCOI* sequences of *B. tabaci* cryptic species used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. Bold dots indicate the sequences from the present study.

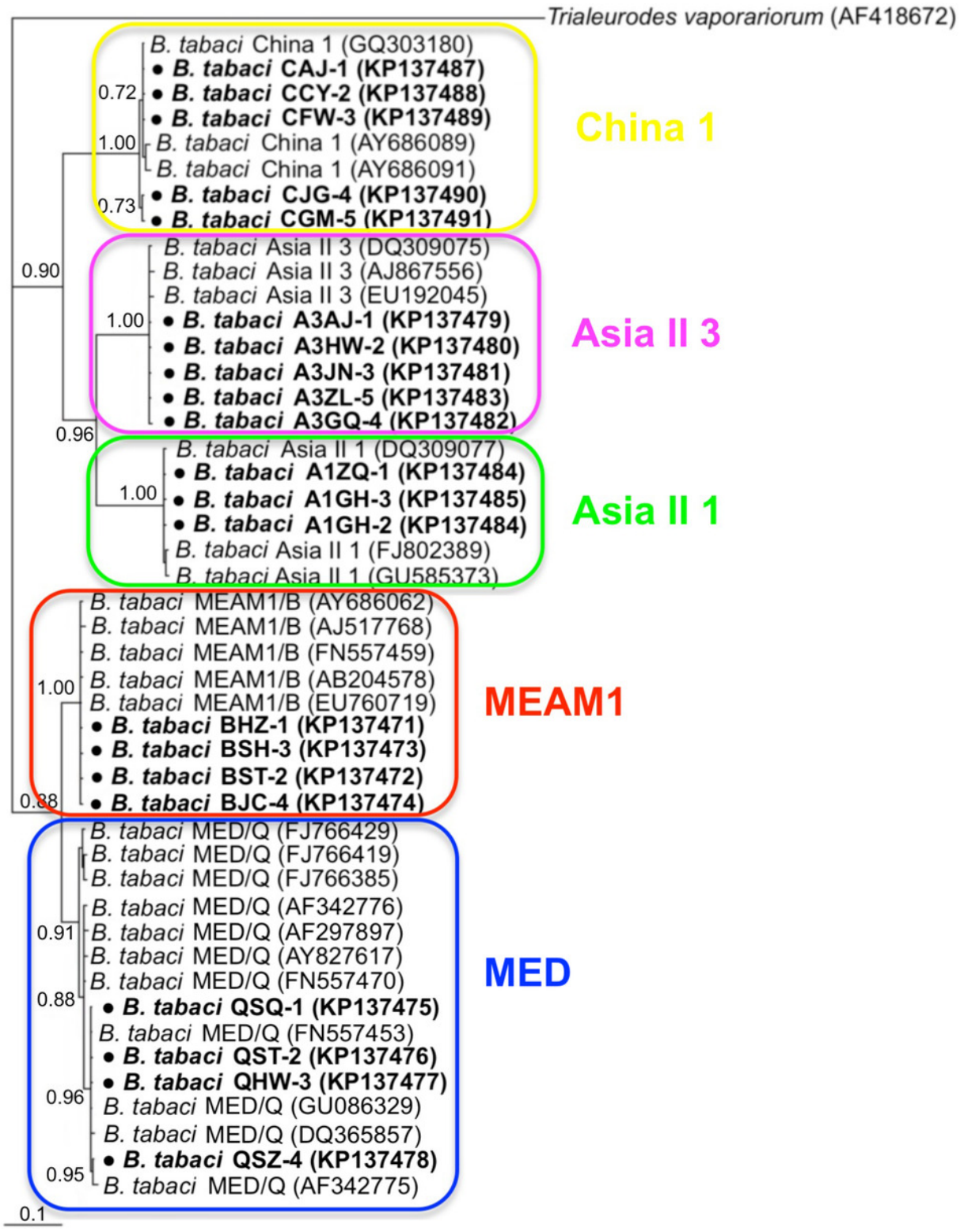
Trialeurodes vaporariorum (AF418672)
B. tabaci China 1 (GQ303180)
• B. tabaci CAJ-1 (KP137487)
• B. tabaci CCY-2 (KP137488)
• B. tabaci CFW-3 (KP137489)
B. tabaci China 1 (AY686089)
B. tabaci China 1 (AY686091)
• B. tabaci CJG-4 (KP137490)
• B. tabaci CGM-5 (KP137491)
China 1
B. tabaci Asia II 3 (DQ309075)
B. tabaci Asia II 3 (AJ867556)
B. tabaci Asia II 3 (EU192045)
• B. tabaci A3AJ-1 (KP137479)
• B. tabaci A3HW-2 (KP137480)
• B. tabaci A3JN-3 (KP137481)
• B. tabaci A3ZL-5 (KP137483)
• B. tabaci A3GQ-4 (KP137482)
Asia II 3
B. tabaci Asia II 1 (DQ309077)
• B. tabaci A1ZQ-1 (KP137484)
• B. tabaci A1GH-3 (KP137485)
• B. tabaci A1GH-2 (KP137484)
B. tabaci Asia II 1 (FJ802389)
B. tabaci Asia II 1 (GU585373)
Asia II 1
B. tabaci MEAM1/B (AY686062)
B. tabaci MEAM1/B (AJ517768)
B. tabaci MEAM1/B (FN557459)
B. tabaci MEAM1/B (AB204578)
B. tabaci MEAM1/B (EU760719)
• B. tabaci BHZ-1 (KP137471)
• B. tabaci BSH-3 (KP137473)
• B. tabaci BST-2 (KP137472)
• B. tabaci BJC-4 (KP137474)
MEAM1
B. tabaci MED/Q (FJ766429)
B. tabaci MED/Q (FJ766419)
B. tabaci MED/Q (FJ766385)
B. tabaci MED/Q (AF342776)
B. tabaci MED/Q (AF297897)
B. tabaci MED/Q (AY827617)
B. tabaci MED/Q (FN557470)
• B. tabaci QSQ-1 (KP137475)
B. tabaci MED/Q (FN557453)
• B. tabaci QST-2 (KP137476)
• B. tabaci QHW-3 (KP137477)
B. tabaci MED/Q (GU086329)
B. tabaci MED/Q (DQ365857)
• B. tabaci QSZ-4 (KP137478)
B. tabaci MED/Q (AF342775)
MED
0.72
1.00
0.73
0.90
1.00
0.96
1.00
1.00
0.88
0.91
0.88
0.96
0.95
0.1

Figure 4

The Bayesian phylogenetic tree of *P. aleyrodidarum* based on 16S *rDNA* sequences.

The value beside the nodes are posterior probabilities. *Trialeurodes vaporariorum* (AY266113) is used as outgroup. Accession numbers for 16S *rDNA* sequences submitted to GenBank are KP201110-KP201125. All 16S *rDNA* sequences of *P. aleyrodidarum* used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. Bold dots indicate the sequences from the present study. Dotted boxes indicate imperfect cluster of each *B. tabaci* cryptic species.

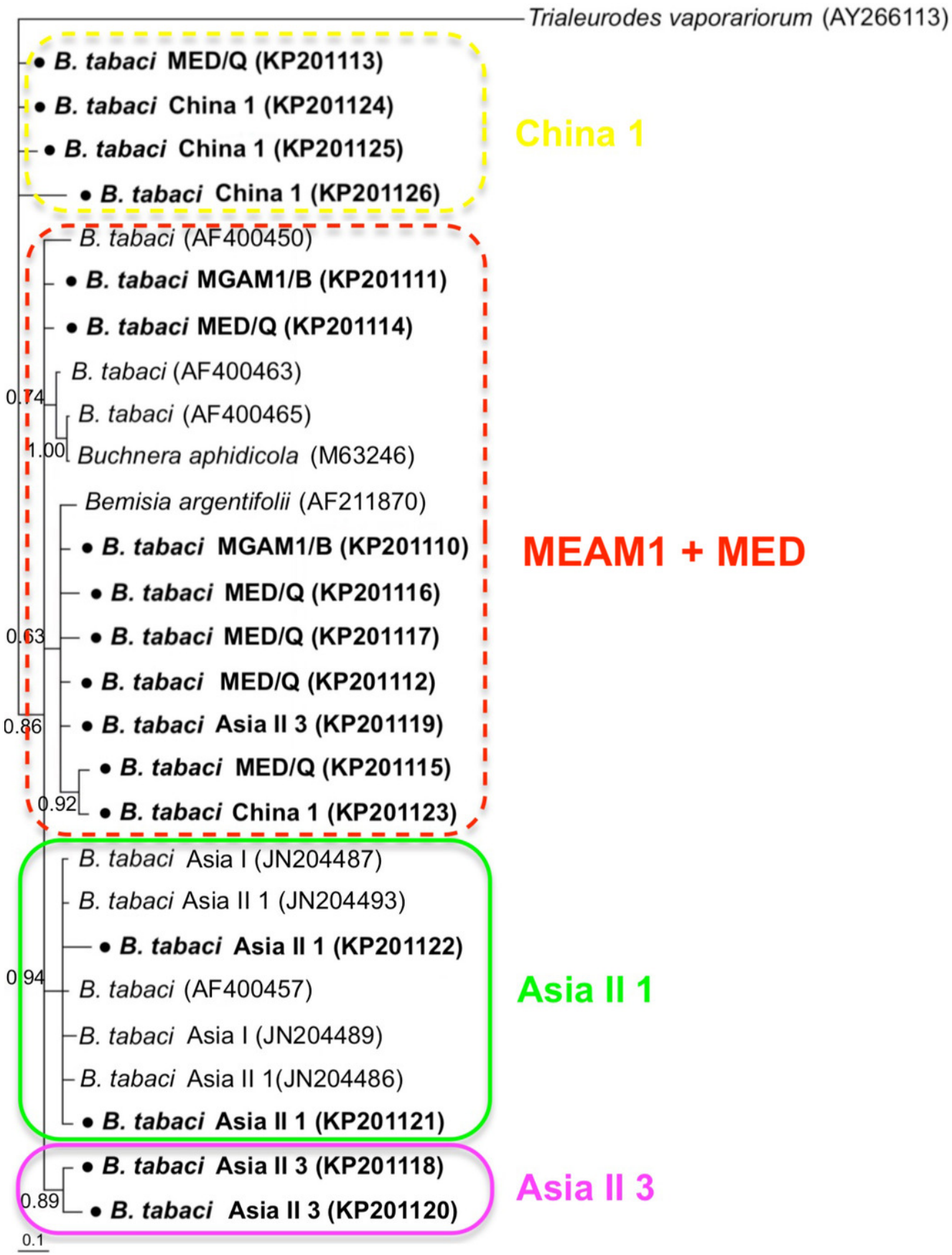
Trialeurodes vaporariorum (AY266113)
B. tabaci MED/Q (KP201113)
B. tabaci China 1 (KP201124)
B. tabaci China 1 (KP201125)
B. tabaci China 1 (KP201126)
China 1
B. tabaci (AF400450)
B. tabaci MGAM1/B (KP201111)
B. tabaci MED/Q (KP201114)
B. tabaci (AF400463)
0.74
B. tabaci (AF400465)
1.00
Buchnera aphidicola (M63246)
Bemisia argentifolii (AF211870)
B. tabaci MGAM1/B (KP201110)
B. tabaci MED/Q (KP201116)
0.63
B. tabaci MED/Q (KP201117)
B. tabaci MED/Q (KP201112)
0.86
B. tabaci Asia II 3 (KP201119)
B. tabaci MED/Q (KP201115)
0.92
B. tabaci China 1 (KP201123)
MEAM1 + MED
B. tabaci Asia I (JN204487)
B. tabaci Asia II 1 (JN204493)
B. tabaci Asia II 1 (KP201122)
0.94
B. tabaci (AF400457)
B. tabaci Asia I (JN204489)
B. tabaci Asia II 1(JN204486)
B. tabaci Asia II 1 (KP201121)
Asia II 1
B. tabaci Asia II 3 (KP201118)
0.89
B. tabaci Asia II 3 (KP201120)
Asia II 3
0.1

Figure 5

The Bayesian phylogenetic tree of *Arsenophonus* based on 23S *rDNA* sequences.

The value beside the nodes are posterior probabilities. *Aleurodicus dispersus* (AY264664) is used as outgroup. Accession numbers for 23S *rDNA* sequences submitted to GenBank are KP201103-KP201109. All 23S *rDNA* sequences of *Arsenophonus* used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. A1-A4 indicate the four clusters.

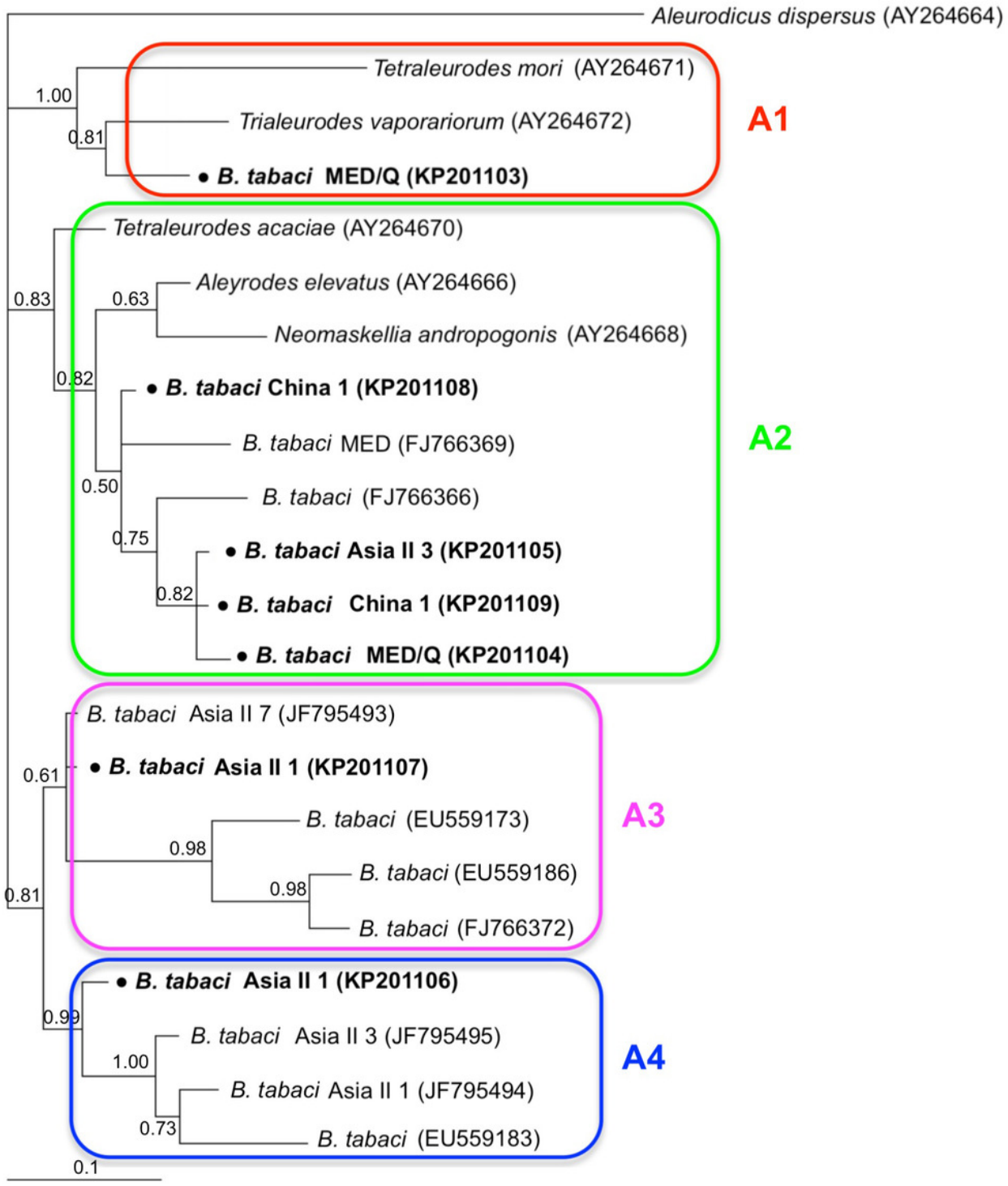
Aleurodicus dispersus (AY264664)
Tetraleurodes mori (AY264671)
1.00
Trialeurodes vaporariorum (AY264672)
0.81
• B. tabaci MED/Q (KP201103)
A1
Tetraleurodes acaciae (AY264670)
Aleyrodes elevatus (AY264666)
0.83
0.63
Neomaskellia andropogonis (AY264668)
0.82
• B. tabaci China 1 (KP201108)
B. tabaci MED (FJ766369)
A2
0.50
B. tabaci (FJ766366)
0.75
• B. tabaci Asia II 3 (KP201105)
0.82
• B. tabaci China 1 (KP201109)
• B. tabaci MED/Q (KP201104)
B. tabaci Asia II 7 (JF795493)
• B. tabaci Asia II 1 (KP201107)
0.61
B. tabaci (EU559173)
0.98
B. tabaci (EU559186)
0.81
0.98
B. tabaci (FJ766372)
A3
• B. tabaci Asia II 1 (KP201106)
0.99
B. tabaci Asia II 3 (JF795495)
1.00
B. tabaci Asia II 1 (JF795494)
A4
0.73
B. tabaci (EU559183)
0.1

Figure 6

The Bayesian phylogenetic tree of *Cardinium* based on 16S *rDNA* sequences.

The value beside the nodes are posterior probabilities. *Paralvinella palmiformis* (AJ441237) is used as outgroup. Accession numbers for 16S *rDNA* sequences submitted to GenBank are KP201127-KP201134. All 16S *rDNA* sequences of *Cardinium* used in this study were clustered with other related references sequences from GenBank and their accession numbers are also indicated in the tree. C1-C3 indicate the three clusters.

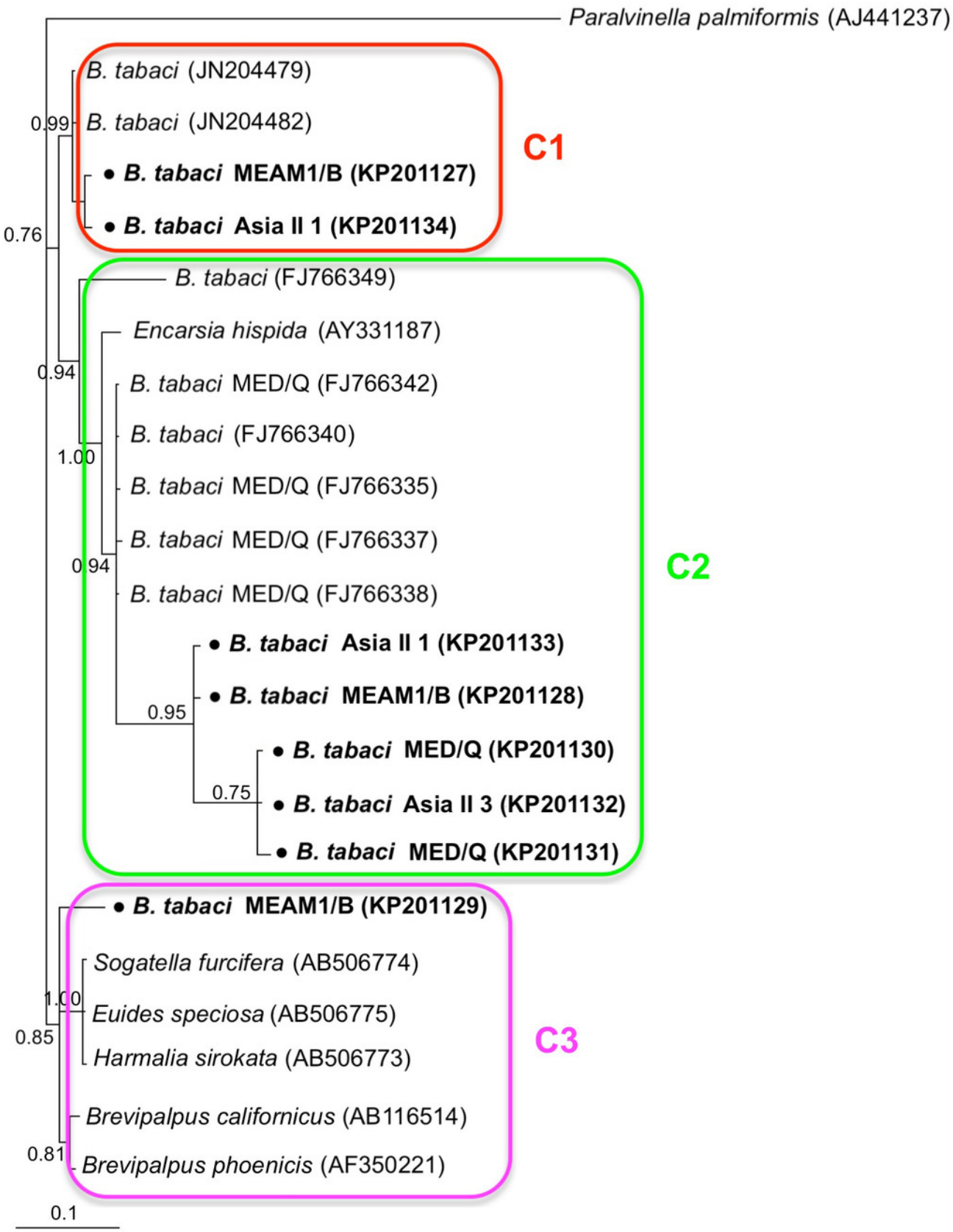
Paralvinella palmiformis (AJ441237)
B. tabaci (JN204479)
B. tabaci (JN204482)
B. tabaci MEAM1/B (KP201127)
B. tabaci Asia II 1 (KP201134)
C1
B. tabaci (FJ766349)
Encarsia hispida (AY331187)
B. tabaci MED/Q (FJ766342)
B. tabaci (FJ766340)
B. tabaci MED/Q (FJ766335)
B. tabaci MED/Q (FJ766337)
B. tabaci MED/Q (FJ766338)
B. tabaci Asia II 1 (KP201133)
B. tabaci MEAM1/B (KP201128)
B. tabaci MED/Q (KP201130)
B. tabaci Asia II 3 (KP201132)
B. tabaci MED/Q (KP201131)
C2
B. tabaci MEAM1/B (KP201129)
Sogatella furcifera (AB506774)
Euides speciosa (AB506775)
Harmalia sirokata (AB506773)
Brevipalpus californicus (AB116514)
Brevipalpus phoenicis (AF350221)
C3
0.99
0.76
0.94
1.00
0.94
0.95
0.75
1.00
0.85
0.81
0.1

Table 1(on next page)

The sequences, annealing temperature, product size and references of primers used in this study.

1 **Table 1. The sequences, annealing temperature, product size and references of primers used in this study.**

2

Gene	Primer sequence(5'→3')	Annealing temperature	Product size(bp)	References
B. tabaci mtCOI	C1-J-2195: TTGATTTTTTGGTCATCCAGAAGT L2-N-3014: TCCAATGCACTAATCTGCCATATTA	50℃	800	Frohlich et al., 1999
P. aleyrodidarum 16S *rDNA*	Pro-F: TGCAAGTCGAGCGGCATCAT Pro-R: AAAGTTCCCGCCTTATGCGT	59℃	1000	Zghori-Fein and Brown, 2002
Cardinium 16S rDNA	Ch-F: TACTGTAAGAAATAAGCACCGGC Ch-R: GTGGATCACTTAACGCTTTCG	57℃	400	Zchori-Fein et al., 2004
Arsenophonus 23S rDNA	Ars-F: CGTTTGATGAATTCATAGTCAAA Ars-R: GGTCCTCCAGTTAGTGTTACCCAAC	60.5℃	900	Thao and Baumann, 2004

Table 2(on next page)

Haplotype information of whitefly, *P. aleyrodidarum*, *Arsenophonus* and *Cardinium*.

1 **Table 2 Haplotype information of whitefly, *P. aleyrodidarum*, *Arsenophonus* and *Cardinium*.**

		mt COI			*P.aleyrodidarum*				*Arsenophonus*				*Cardinium*			
Species	n.	Re.Seq.	Acc. no.	Per.(%)	n.	Re.Seq.	Acc. no.	Per.(%)	n.	Re.Seq.	Acc. no.	Per.(%)	n.	Re.Seq.	Acc. no.	Per.(%)
MEAM1/B	4	BHZ-1	KP137471	85.1	2	BST-CA1	KP201110	89.1					3	BGD-CR1	KP201127	92.5
		BST-2	KP137472	8.2		BGW-CA2	KP201111	10.9						BHC-CR2	KP201128	2.5
		BSH-3	KP137473	4.9										BSRF-CR3	KP201129	5
		BJC-4	KP137474	1.8												
MED/Q	4	QSQ-1	KP137475	90.3	6	QBJ-CA1	KP201112	88.8	2	QHS-AR1	KP201103	89.7	2	QSH-CR1	KP201130	96.2
		QST-2	KP137476	0.5		QAH-CA2	KP201113	5.8		QHL-AR2	KP201104	10.3		QSZ-CR2	KP201131	0.4
		QHW-3	KP137477	5.8		QSL-CA3	KP201114	1.0								
		QSZ-4	KP137478	3.3		QSJ-CA4	KP201115	2.0								
						QSH-CA5	KP201116	1.8								
						QAH-CA6	KP201117	0.5								
Aisa Ⅱ 3	5	A3AJ-1	KP137479	86.9	3	A3ZW-CA1	KP201118	86.2	1	A3ZL-AR1	KP201105	100.0	1	A3ZL-CR1	KP201132	100.0
		A3HW-2	KP137480	1.5		A3ZL-CA2	KP201119	9.2								
		A3JN-3	KP137481	0.8		A3GQ-CA3	KP201120	4.6								
		A3GQ-4	KP137482	6.2												
		A3ZL-5	KP137483	4.6												
Aisa Ⅱ 1	3	A1ZQ-1	KP137484	81.4	2	A1GQ-CA1	KP201121	94.3	2	A1ZL-AR1	KP201106	92.3	2	A1GQ-CR1	KP201133	92.0
		A1GH-2	KP137485	12.9		A1JS-CA2	KP201122	5.7		A1GS-AR2	KP201107	7.7		A1SL-CR2	KP201134	8.0
		A1GH-3	KP137486	5.7												
China 1	5	CAJ-1	KP137487	94.7	4	CCY-CA1	KP201123	2.7	2	CCY-AR1	KP201108	97.6				
		CCY-2	KP137488	2.0		CZS-CA2	KP201124	90.7		CAJ-AR2	KP201109	2.4				
		CFW-3	KP137489	0.7		CZH-CA3	KP201125	5.3								
		CJG-4	KP137490	1.3		CZJ-CA4	KP201126	1.3								
		CGM-5	KP137491	1.3												
TOTAL	**21**				**17**				**7**				**8**			

2 n.: Haplotype number. Re. Seq.: Representative Sequence. Acc. no.: Accession number. Per.: Percentage in each cryptic species.

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