BIODIVERSITAS
Volume 25, Number 11, November 2024
Pages: 4304-4314

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251130

Diversity of bacterial endosymbionts of *Bemisia tabaci* from some regions in Indonesia and the genetic diversity of *Wolbachia* endosymbiont

NADIYAH HIDAYATI, ALAN SOFFAN, TRIWIDODO ARWIYANTO, ARMAN WIJONARKO♥
Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.
Tel.: +62-274-523926, ♥email: arman_w@ugm.ac.id

Manuscript received: 15 June 2024. Revision accepted: 18 November 2024.

Abstract. *Hidayati N, Soffan A, Arwiyanto T, Wijonarko A. 2024. Diversity of bacterial endosymbionts of* Bemisia tabaci *from some regions in Indonesia and the genetic diversity of* Wolbachia *endosymbiont. Biodiversitas 25: 4304-4314. Bemisia tabaci* Gennadius 1889 (Hemiptera) is an insect that associates with endosymbiont bacteria to meet nutritional needs lacking in its food. The species has both primary and secondary endosymbionts, with infections showing significant dynamism among populations. *Wolbachia* is a facultative endosymbiont that infects 66% of all insect species, including *B. tabaci*, which is a key focus of this study. The study aimed to investigate the diversity of bacterial endosymbiont infections in several *B. tabaci* populations collected from Java and Sumatra, Indonesia, to explore the genetic diversity of *Wolbachia* endosymbiont. The *B. tabaci* population was collected from 17 districts in Java and Sumatra, and to determine the presence of bacterial endosymbiont was determined through molecular analysis using specific primers. The study involved a comprehensive methodology, including the construction and comparison of multiple alignment sequences, phylogenetic analysis, and genetic differentiation of *Wolbachia* in 10 representative populations of *B. tabaci*, which were then compared with *Wolbachia* sequences from other countries and arthropods. The results showed the presence of various bacterial endosymbionts found among the *B. tabaci* populations, including *Candidatus portiera, Arsenophonus* sp., *Cardinium* sp., *Hamiltonella* sp., *Wolbachia* sp., and *Rickettsia* sp. *Wolbachia* was found in all *B. tabaci* samples, while other endosymbiont bacteria varied quite widely among all *B. tabaci* population samples. The genetic diversity of *Wolbachia* of *B. tabaci* from Indonesia showed close relatedness and has a different clade from *Wolbachia* from other countries and arthropods. The population structure of *Wolbachia* populations from Java was genetically related and similar to that of *Wolbachia* from Sumatra.

Keywords: *Bemisia tabaci*, endosymbiont, genetic diversity, phylogenetic, *Wolbachia*

INTRODUCTION

Bemisia tabaci Gennadius 1889 (Hemiptera: Aleyrodidae) is a cryptic whitefly species complex with a global distribution that has become one of the most economically important crop pests with an extensive range of host plants (Kanakala and Ghanim 2019). The species causes severe economic damage by directly sucking plant saps and indirectly by transmitting plant viruses of the genera *Begomovirus, Crinivirus, Carlavirus, Torradovirus, Ipomovirus, Polerovirus,* and *Cytorhabdovirus* (Ghosh and Ghanim 2021; Temaja et al. 2022). *B. tabaci* feeds exclusively on plants' phloem sap, which is generally rich in carbohydrates but lacks essential amino acids (Sani et al. 2020). This unbalanced diet can be compensated by bacterial communities residing in the insect, commonly called bacterial endosymbionts (Milenovic et al. 2022).

Plant-sucking insects, such as *B. tabaci*, generally interact with bacterial endosymbionts. Insect endosymbiont bacteria usually have two types, namely primary and secondary endosymbionts. Primary endosymbionts synthesize nutrients their host lacks and requirements (Hu and Tsai 2020). In contrast, secondary endosymbionts have roles that vary depending on the type of bacteria, like increased fitness, and might also contribute to the sexual selection of the insect host (Zhou and Li 2016). Primary endosymbiont bacteria infect every individual of any *B. tabaci* population and are usually vertically transmitted from mother to offspring (Hu and Tsai 2020). In contrast, the frequency of infection from secondary endosymbionts varies widely among *B. tabaci* populations and can be transmitted vertically or horizontally (Liu and Guo 2019). *Candidatus portiera aleyrodidarum* (Thao and Baumann) is the only primary endosymbiont of *B. tabaci*. At the same time, some confirmed secondary endosymbionts are the genera *Rickettsia* sp., *Cardinium* sp., *Hamiltonella* sp., *Arsenophonus* sp., *Wolbachia* sp., and *Fritschea* sp. (de Marchi and Smith 2020; Lestari et al. 2021).

Secondary endosymbionts, such as *Wolbachia*, play significant roles in the biology of their hosts. *Wolbachia* is a facultative endosymbiont that infects most Arthropods and filarial nematodes (Li et al. 2017). Approximately 40% of terrestrial Arthropod species and 66% of all insects are infected with *Wolbachia*, so it has become the most widely studied endosymbiont (Bing et al. 2014). The spread of *Wolbachia* is mainly achieved by manipulating reproduction, including cytoplasmic incompatibility, causing parthenogenesis, feminizing genetic males, and killing male progenies (Zhong and Li 2014; Zhou and Li 2016). Cytoplasmic incompatibility has attracted much attention,

making it possible for similar biocontrol strategies using *Wolbachia* as the biological control agent of insect pests, especially in *B. tabaci* (Lv et al. 2023).

The interactions between *B. tabaci* and its endosymbionts are complex and can influence the pest's adaptability and resistance to control measures. Recent studies have highlighted the importance of endosymbiont diversity in shaping the ecological and evolutionary dynamic of *B. tabaci* populations (Milenovic et al. 2022). Indonesia has a varied geography with distinct ecological zones, providing a natural laboratory for examining how different environmental conditions and agricultural practices influence endosymbiont diversity and host interaction. This collaborative study, involving researchers from various fields, is crucial for devising targeted and effective pest management strategies for specific regions and crop systems. The infection dynamics of secondary endosymbionts in *B. tabaci* are usually related to species or biotype, sex, host plant, and geographical location (Pan et al. 2012). This study aimed to investigate the diversity of bacterial endosymbiont infections in several *B. tabaci* populations collected from Java and Sumatra, Indonesia, and to explore the genetic diversity of *Wolbachia* endosymbiont of *B. tabaci* with other groups from different countries and arthropods. Our study improves the late understanding of the bacterial endosymbionts of *B. tabaci* in Indonesia and the genetic variation of *Wolbachia* endosymbionts. The result will contribute to a better understanding of the ecological and evolutionary processes driving the success and adaptability of *B. tabaci*, offering new perspectives for sustainable pest management.

MATERIALS AND METHODS

Research materials

Adult *Bemisia tabaci* were collected from various crops (chili, eggplant, cucumber, and tomato) in several regions in Java and Sumatra, Indonesia using aspirators (Figure 1). The samples were preserved in 70% alcohol in separate vials based on the collection site. At least 20-30 individuals from each site were collected by random sampling. The samples were stored at -20°C until further analysis.

Procedures

DNA extraction

Genome DNA was extracted from adult *B. tabaci* following the procedure provided by the DNeasy Genomic DNA Mini Kit (Geneaid, Taiwan). A total of 5 imagos of *B. tabaci* from each preserved population were placed into a 1.5 mL microcentrifuge tube containing 200 µL digestion buffer and 20 µL proteinase K, then crushed with a micropestile until smooth. The quality and concentration of the extracted DNA were measured with a spectrophotometer (BioDrop, DKSH, Switzerland).

Detection of bacterial endosymbiont

Detection of endosymbiont bacterial diversity in the total genome extracted from each population of *B. tabaci* samples was performed using PCR T100 Thermal Cycler (Bio-Rad USA) with endosymbiont-specific primers (Table 1). Amplification was carried out to obtain and multiply the number of 16S or 23S rDNA gene fragments of endosymbiont bacteria with a total volume of 10 µL consisting of 5 µL MyTaq Red Mix, 2 µL Nuclease-Free Water (NFW), 1 µL for each primer, and 1 µL DNA sample. The PCR program protocol consisted of pre-denaturation at 95°C for 1 minute, 35 cycles of denaturation at 95°C for 15 seconds, annealing temperature adjusting the primers for 15 seconds, and extension at 72°C for 45 seconds. It ended with a final extension at 72°C for 10 minutes. The PCR products were separated using 1.5% agarose gel electrophoresis, dissolved in 1× TBE buffer, and stained with florosafe (1st BASE Singapore) as a gel colorant. Each sample was electrophoresed at 50 volts for approximately 40 minutes. Visualization of DNA bands formed was carried out using a UV transilluminator.

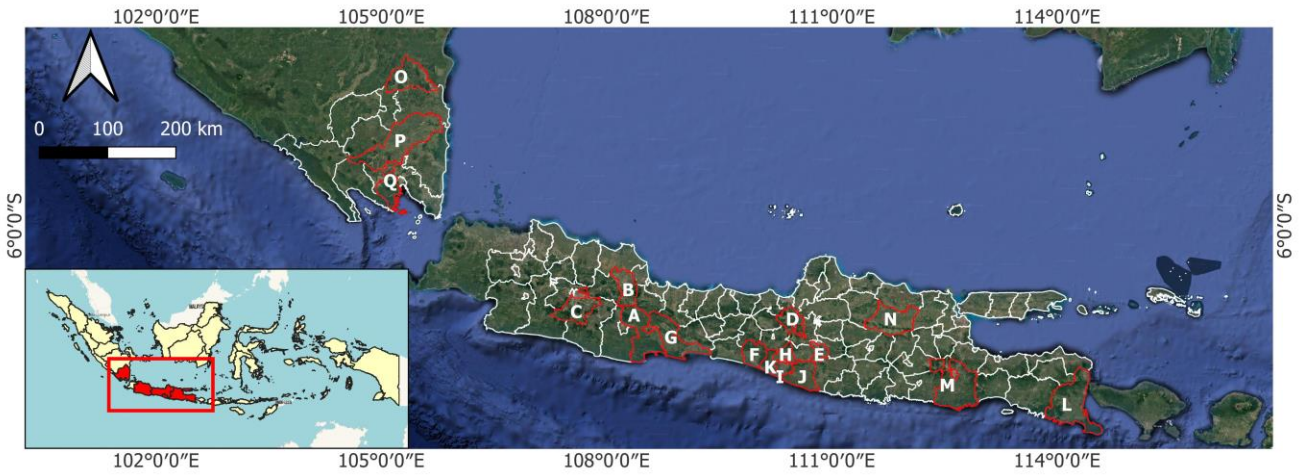


Figure 1. Sampling locations of *Bemisia tabaci* in Java (West Java Province: A. Ciamis, B. Majalengka, C. Bandung; Central Java Province: D. Semarang, E. Sukoharjo, F. Purworejo, G. Cilacap; Yogyakarta Province: H. Sleman, I. Bantul, J. Gunung Kidul, K. Kulonprogo; East Java Province: L. Banyuwangi, M. Malang, N. Bojonegoro) and Sumatra (Lampung Province: O. Mesuji, P. Central Lampung, Q. Pesawaran)

DNA sequences

One of the endosymbionts detected in all sampled *B. tabaci* populations, namely *Wolbachia*, was selected for sequence analysis. Two representative areas were taken from each province regarding distance and altitude. PCR amplification results from the representative populations were sent and analyzed for sequences to PT Genetica Science Indonesia.

Data analysis

The nucleotide sequences of *Wolbachia* from each representative area were analyzed using BioEdit v7.0.5.3 software. This powerful tool was used to generate consensus sequences, which were then compared with the database in GenBank using NCBI Blast (https://blast.ncbi.nlm.nih.gov). Twenty sequences of *Wolbachia* in *B. tabaci* were collected from various countries around the world, and 10 *Wolbachia* sequences from different Arthropod species were also collected. The alignment of all sequences was performed with ClustalW BioEdit v7.0.5.3 software. Phylogenetic trees were constructed using the Maximum Likelihood phylogenetic tree method in MEGA 11.0 software based on genetic *Wolbachia* from different locations and species. We used 1000 bootstrap replicates to test the robustness of the phylogeny, ensuring the reliability of our results and instilling confidence in our methodology.

Ten sequences of *Wolbachia* in *B. tabaci* from several districts in Indonesia were aligned using ClustalW in the MEGA 11.0 software application. The genetic structure of *Wolbachia* sequences from 10 populations of *B. tabaci* was estimated using DnaSP v. 6.12.03 software, considering several parameters, including the number of haplotypes, haplotype diversity, and nucleotide diversity. Pairwise genetic distance (Fst) values, which determine the genetic differentiation, were also calculated using DnaSP v.6.12.03.

RESULTS AND DISCUSSION

Detection of bacterial endosymbiont in *B. tabaci* population

The presence of 6 endosymbionts in 17 tested populations of *B. tabaci* was examined by PCR analysis of 16S or 23S rDNA sequences. The PCR detection result showed that *Wolbachia* was the most common endosymbiont detected in all the tested populations. However, other endosymbionts, such as *Candidatus portiera*, *Arsenophonus* sp., *Cardinium* sp., *Hamiltonella* sp., and *Rickettsia* sp., were found to be various infections in each sample (Table 2).

The primary endosymbiont *C. portiera* was detected in all populations except Mesuji and Sukoharjo. Our results did not agree with previous studies showing that *C. portiera* was a primary endosymbiont detected in all *B. tabaci* populations. Hashmi et al. (2019) stated that there was a 100% occurrence of *C. portiera* primary endosymbiont, meaning that all the samples observed contained *C. portiera* endosymbiont. Hu and Tsai (2020) also reported that *C. portiera*, as a primary endosymbiont, plays a vital role in synthesizing essential amino acids and carotenoids lacking from the sap phloem feed of *B. tabaci*. The absence of *C. portiera* in Mesuji and Sukoharjo populations could have significant implications for the metabolic functions of *B. tabaci*, as these populations may lack the essential amino acids and carotenoids synthesized by *C. portiera*. This contrast was made possible due to the genomic degradation of the *C. portiera* sequence from Mesuji and Sukoharjo *B. tabaci* populations. According to Andreason et al. (2020), an endosymbiont may become so degraded and effectively becomes an organelle, such as *Carsonella*, the p-endosymbiont of psyllids. It may be possible because many of its genes are transferred to the host genome, and the products of these genes are shipped back to the symbiont. *C. portiera* genome has been reduced during co-evolution with its host by being approximately 350,000 bp long and containing only about 300 genes (Zhu et al. 2019). This reduction has been described as extreme even for endosymbionts, as components of the citrate cycle and glycolysis pathway are absent (Opatovsky et al. 2018). Rao et al. (2015) stated that the *C. portiera* genome is highly reduced in the synthesis of certain vitamins, cofactors, and some essential amino acids, suggesting that there is a metabolic niche that secondary endosymbionts could fill.

Table 1. A specific primer was used to identify *Bemisia tabaci* endosymbiont

Target gen	Primer sequences (5'' - 3'')	Temp (°C)	Product size (bp)	References
C. portiera 16S rDNA	F-TGCAAGTCGCGGCATCAT	60	1000	Singh et al. (2012)
	R-CCGCCTTCTGCGTTGGCAACT			
Arsenophonus 23S rDNA	F-CGTTTGATGAATTCATAGTCAAA	50	630	Singh et al. (2012)
	R-GGTCCTCCAGTTAGTGTTACCCAAC			
Cardinium 16S rDNA	F-GCGGTGTAAAATGAGCTTG	53	440	Weeks et al. (2003)
	R-ACCTCTTCTTTAACTCAAGCCT			
Hamiltonella 16S rDNA	F-TGAGTAAAGTCTGGAATCTGG	53	700	Zchori-Fein and Brown (2002)
	R-AGTTCAAGACCGCAACCTC			
Rickettsia 16S rDNA	F-GCTCAGAACGAACGCTGG	55	800	Gottlieb et al. (2006)
	R-GAAGGAAAGCATCTCTGC			
Wolbachia 16S rDNA	F-CGGGGGAAAATTTATTGCT	50	650	Singh et al. (2012)
	R-AGCTGTAATACAGAAAGGAAA			

Table 2. Endosymbiont infection of various *Bemisia tabaci* populations

Sample ID	Collection sites	Province	Coordinates	Alt (m asl)	Host plants	Endosymbiont						*Wolbachia* GenBank accession
						P	A	C	H	R	W	
Bj	Bojonegoro	East Java	7°17'58.8"S, 111°58'07.5"E	60	*Solanum melongena* L.	+	+	+	+	+	+	-
By	Banyuwangi	East Java	8°31'23.2"S, 114°15'26.5"E	35	*Solanum melongena* L.	+	+	-	+	+	+	OR856518
M	Malang	East Java	7°55'28.6"S, 112°33'21.5"E	756	*Solanum melongena* L.	+	+	+	+	+	+	OR856519
lok 1	Purworejo	Central Java	7°52'08.1"S, 109°59'25.2"E	14	*Solanum lycopersicum* L.	+	+	+	+	+	+	-
lok 29	Cilacap	Central Java	7°40'48.7"S, 109°02'01.4"E	12	*Cucumis sativus* L.	+	+	+	+	+	+	-
wf 22	Semarang	Central Java	7°13'59.0"S, 110°20'51.7"E	894	*Capsicum annuum* L.	+	+	+	+	+	+	OR856520
19	Sukoharjo	Central Java	7°46'01.0"S, 110°44'27.5"E	101	*Solanum melongena* L.	-	-	-	-	-	+	OR856521
G5	Gunungkidul	Yogyakarta	8°8'57.05"S, 110°44'44.25"E	243	*Solanum melongena* L.	+	-	-	+	+	+	-
K5	Kulonprogo	Yogyakarta	7°46'40.1"S, 110°12'54.6"E	90	*Solanum melongena* L.	+	-	-	+	+	+	OR856522
S2	Sleman	Yogyakarta	7°37'57.5"S, 110°25'30.2"E	551	*Solanum melongena* L.	+	+	+	+	+	+	OR856523
B1	Bantul	Yogyakarta	7°53'52.1"S, 110°22'40.2"E	45	*Solanum melongena* L.	+	+	-	+	+	+	-
lok 3	Ciamis	West Java	7°12'54.3"S, 108°11'59.4"E	448	*Cucumis sativus* L.	+	+	+	+	+	+	OR856525
lok 4	Majalengka	West Java	6°47'55.4"S, 108°17'30.3"E	99	*Solanum lycopersicum* L.	+	+	+	+	+	+	OR856524
L	Bandung	West Java	6°49'21.5"S, 107°37'59.1"E	1233	*Capsicum annuum* L.	+	+	-	+	-	+	-
Lamteng	Central Lampung	Lampung	5°11'24.0"S, 104°58'10.0"E	117	*Capsicum annuum* L.	+	+	+	+	+	+	-
Msj	Mesuji	Lampung	4°05'45.5"S, 105°19'37.0"E	17	*Solanum melongena* L.	-	-	-	-	-	+	OR856526
Psw	Pesawaran	Lampung	5°32'38.1"S, 105°06'33.5"E	629	*Solanum lycopersicum* L.	+	+	+	+	+	+	OR856527

Note: P: *C. portiera*; A: *Arshenophonus* sp.; C: *Cardinium* sp.; H: *Hamiltonella* sp.; R: *Rickettsia* sp.; W: *Wolbachia* sp.; +: present; -: absent

The secondary endosymbionts *Arshenophonus* sp., *Rickettsia* sp., *Cardinium* sp., and *Hamiltonella* sp. were highly variable in the observed samples. In contrast, *Wolbachia* was the only one found in all *B. tabaci* samples. Our results demonstrate both single and dual infection of *Wolbachia* and other endosymbionts. A single infection of *Wolbachia* was reported in *B. tabaci* samples from Mesuji and Sukoharjo. This study supported Lestari et al. (2021) that *Wolbachia* was the most prevalent endosymbiont and was detected in many *B. tabaci* populations collected in Indonesia. The prevalence of *Wolbachia* in various species of *B. tabaci* is possible due to several *Wolbachia* acquisitions and horizontal transfers (Kanakala and Ghanim 2019). Li et al. (2017) demonstrated that *Wolbachia* persisted in the plant leaves for at least 50 days and transmitted horizontally. When the *Wolbachia*-free whiteflies fed on the infected plant leaves, the majority of them became infected with the symbiont and vertically transmitted it to their progeny. Ahmed et al. (2015) also demonstrated that *Wolbachia* can be transmitted from the whitefly host to the parasitoid wasps *Eretmocerus* sp. nr *furuhashii* through a non-lethal interaction that occurs after 48 h of contact with *Wolbachia*-infected whiteflies. *Wolbachia* is one of the endosymbiont bacteria naturally harbored in *B. tabaci*, which can potentially be a pest control agent because of its ability to manipulate its host reproduction system (Atikah et al. 2019). This finding opens up new possibilities for pest control strategies. However, in some cases, *Wolbachia* is not found in certain biotypes of *B. tabaci*. Kareem et al. (2020) reported that no *Wolbachia* symbiont was found for all the samples *B. tabaci* tested. This occurs because secondary symbionts are usually considered non-essential to their hosts. Hence, their presence between and within the population can be variable (Chiel et al. 2007).

Various endosymbiont combinations were obtained in each observed sample in this study. However, there needed to be a clear pattern of exact endosymbiont bacterial community combinations between sites, as there seemed to be little difference. El Hamss et al. (2022) showed that secondary endosymbiont diversity and composition were not significantly different across sites. It could be related to the adopted sampling where samplings were carried out in one country (Uganda). In a previous study, the presence of different endosymbionts across sites is complex to analyze as it depends on many factors, such as the horizontal transfer of these bacteria, the interaction between each endosymbiont and the host, and the environment in which the host is located (Singh et al. 2012; Karut et al. 2020). Several studies have also suggested that the dynamics of secondary endosymbiont diversity in *B. tabaci* are usually related to species or biotype, sex, host plant, and geographic location (Pan et al. 2012). A little or no variation in these 17 samples is also possible because the composition of this bacterial community is a very complex and dynamic process, and it suggests studying in more detail and comprehensively. Studying more about the interaction between endosymbionts and the host insect, especially *B.tabaci*, which becomes most of the vectors of many viruses, has the potential to understand biological control (Bigiotti et al. 2019).

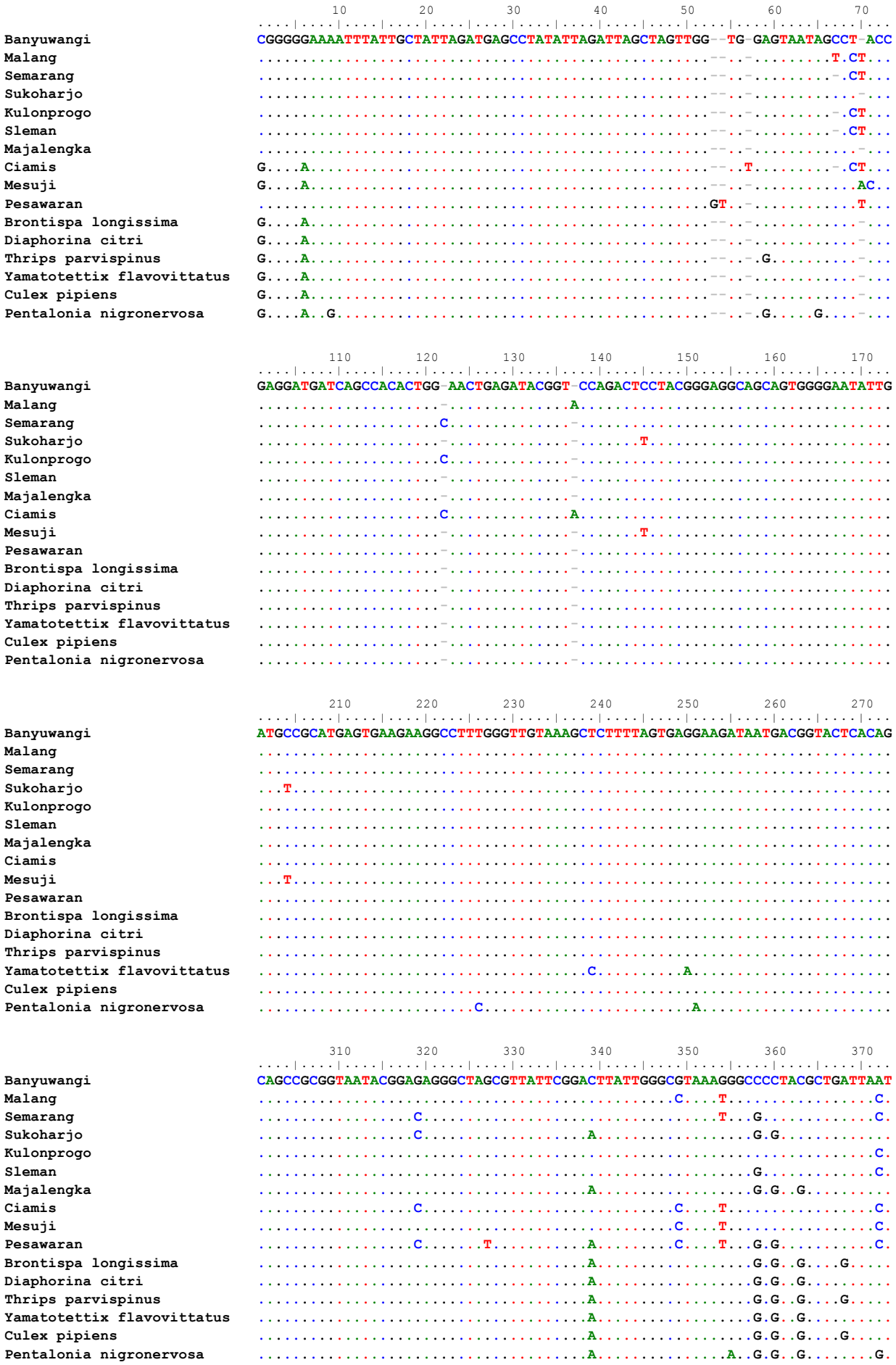
Genetic variation and phylogenetic analysis of *Wolbachia* endosymbiont

Wolbachia sequence analysis was carried out on 10 representative population samples (Table 2). According to BLAST on NCBI, *Wolbachia* sequence of 10 representative *B. tabaci* populations from Java and Sumatra have high similarity with *Wolbachia* endosymbiont of *B. tabaci* from India and *Wolbachia* endosymbiont of *Hishimonoides sellatiformis* Ishihara 1965 from Japan (Table 3). *B. tabaci* and *H. sellatiformis* are insects from the order Hemiptera; they are also known as sucking insects that cause plant damage either directly by sucking plant fluid or indirectly as disease vectors. However, the host preferences of the two insects are different. *H. sellatiformis* are known to be vectors of mulberry dwarf (MD) phytoplasma, and not obtained any evidence of reproductive alterations, including cytoplasmic incompatibility, feminization, parthenogenesis, or male-killing, by these *Wolbachia* in the leafhoppers (Mitsuhashi et al. 2002).

The alignment sequence results showed that 10 samples of *Wolbachia* sequences had many nucleotide bases that have similarities, but there were also some variations in certain sequences (Figure 2). According to the p-distance, *Wolbachia* sequences that have the most similarities are those from Banyuwangi and Majalengka. The most noticeably different *Wolbachia* sequences are from Ciamis with Sukoharjo and Majalengka (Table 4). Meanwhile, when compared with *Wolbachia* sequences from other insect species, the 10 samples of *Wolbachia* sequences of *B.tabaci* also have high-similarity nucleotides (Figure 2).

Table 3. BLAST results of 10 *Wolbachia* sequences on NCBI

Sample	Blast species	Country	Identical (%)
Banyuwangi	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	98.26
Malang	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	92.51
Semarang	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	96.35
Sukoharjo	*Wolbachia* endosymbiont of *Hishimonoides sellatiformis*	Japan	97.46
Kulonprogo	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	98.23
Sleman	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	97.48
Majalengka	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	99.53
Ciamis	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	94.65
Mesuji	*Wolbachia* endosymbiont of *Hishimonoides sellatiformis*	Japan	95.71
Pesawaran	*Wolbachia* endosymbiont of *Bemisia tabaci*	India	94.03


Banyuwangi
Malang
Semarang
Sukoharjo
Kulonprogo
Sleman
Majalengka
Ciamis
Mesuji
Pesawaran
Brontispa longissima
Diaphorina citri
Thrips parvispinus
Yamatotettix flavovittatus
Culex pipiens
Pentalonia nigronervosa

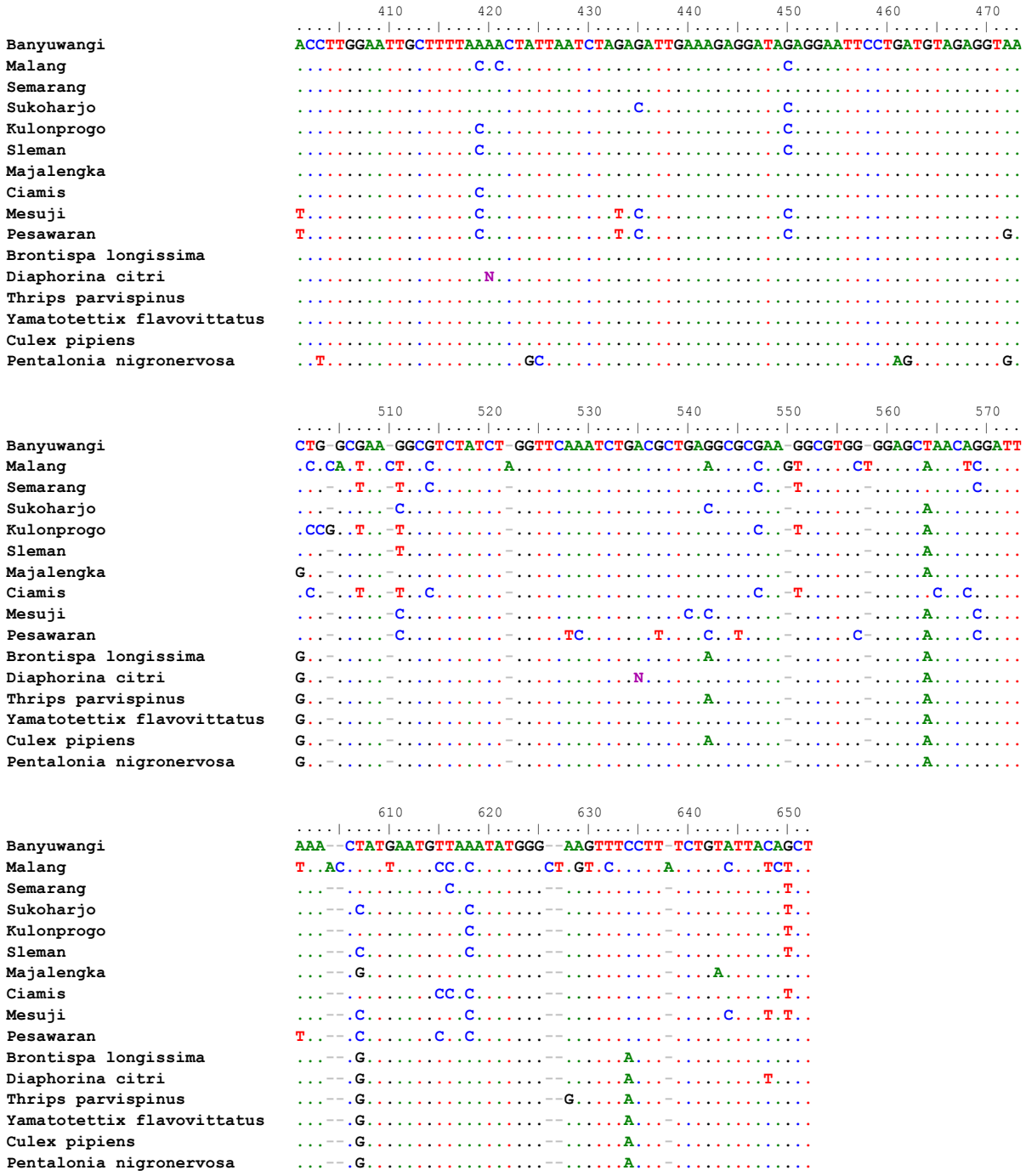


Figure 2. The full-length nucleotide sequence alignment of 650 bp *Wolbachia* endosymbiont of *Bemisia tabaci* with other arthropods

Table 4. The genetic differences (p-distance) of *Wolbachia* endosymbiont sequence from *B. tabaci* population samples in Indonesia

Location	Banyuwangi	Malang	Semarang	Sukoharjo	Kulonprogo	Sleman	Majalengka	Ciamis	Mesuji	Pesawaran
Banyuwangi	0.0000									
Malang	0.7009	0.0000								
Semarang	0.5886	0.6461	0.0000							
Sukoharjo	0.0301	0.7025	0.5870	0.0000						
Kulonprogo	0.5744	0.6349	0.1738	0.5728	0.0000					
Sleman	0.0934	0.6933	0.5197	0.1013	0.5869	0.0000				
Majalengka	0.0158	0.7057	0.5934	0.0316	0.5791	0.0965	0.0000			
Ciamis	0.7848	0.7132	0.7599	0.7896	0.7649	0.7884	0.7896	0.0000		
Mesuji	0.7484	0.3297	0.7235	0.7453	0.5804	0.7508	0.7532	0.6483	0.0000	
Pesawaran	0.7073	0.7107	0.6935	0.7009	0.6792	0.7170	0.7120	0.6950	0.7476	0.0000

Notes: *: Lower p-distance values indicate a closer genetic relationship between sequences

The *Wolbachia* sequences from 6 other insects are most closely related to *Wolbachia* sequences from the *B. tabaci* Majalengka population (Table 5). The number of p-distances in Tables 4 and 5 showed that *Wolbachia* sequences among *B. tabaci* populations are highly divergent compared to *Wolbachia* from other insects. *B. tabaci* is a complex species with many different biotypes and clades, which often exhibit high genetic variation, including variation in their endosymbionts such as *Wolbachia* (Khatun et al. 2018). The possibility of local adaptation of *Wolbachia* to specific conditions within the host or environment (Bing et al. 2014) could explain this divergence between strains within different populations of *B. tabaci* compared to strains from other insects. The small number of differences in *Wolbachia* gene sequences between *B. tabaci* and other insects suggests the possibility of horizontal transfer of *Wolbachia* in other insects of different species. Our results are consistent with previous studies by Ahmed et al. (2015) and Li et al. (2017), which also suggested a horizontal transfer of *Wolbachia* in other insects that feed on the leaf surface and *B. tabaci* parasitoids. The results showed that the *Wolbachia* sequences of all representative populations of *B. tabaci* and some insects were very closely related but not identical. These findings have significant implications for our understanding of the genetic relationships and potential horizontal transfer of *Wolbachia* in *B. tabaci*.

The Maximum Likelihood phylogenetic tree showed that *Wolbachia* of *B. tabaci* from Indonesia was located in the same clade with a bootstrap value of 94%, which indicates strong support for the branch and a high confidence in the validity of the clade (group of *Wolbachia* from *B. tabaci* Indonesia) (Figure 3.A). Our result supported Lestari et al. (2021) that all *B. tabaci* samples in Indonesia in their research were infected by one *Wolbachia* subgroup, which is included in super subgroup B and is spread throughout Java. The phylogenetic tree also showed that *Wolbachia* of *B. tabaci* from Indonesia is located in a different clade from *Wolbachia* from other countries and Arthropods (Figure 3). *Wolbachia* is naturally and widely distributed among invertebrates. This finding has potential implications for biocontrol strategies, as the unique genetic makeup of the *Wolbachia* in *B. tabaci* from Indonesia could be leveraged for targeted interventions. The presence of *Wolbachia* in *B. tabaci* immediately has sparked interest in understanding the possibility of similar biocontrol of *B. tabaci* population. However, *Wolbachia* isolated from *B. tabaci* has different genetics from *Wolbachia* isolated from *Culex pipiens* Linnaeus 1758, which is capable of causing cytoplasmic incompatibility but provides fitness benefits for its host (Xue et al. 2012). Indeed, a *Wolbachia* strain isolated from the parasitic wasp *Scleroderma guani* is capable of causing CI in *B. tabaci* when artificially injected and transmitted to its offspring (Zhong and Li 2014).

Gene flow and genetic structure of *Wolbachia* sequences

The divergence of haplotype sequences of *Wolbachia* from 10 populations of *B. tabaci* representing 5 provinces in Indonesia consists of 10 haplotypes, with each province having two types of haplotypes (Table 6). The haplotype diversity value for all samples is also high, this is due to the small number of gene sequences compared in each province. Meanwhile, the highest nucleotide diversity is *Wolbachia* from East Java, and the lowest is Yogyakarta. However, because the nucleotide diversity compared in each province has a value of less than 0.1, this shows that there is little nucleotide variation in each province. According to Khatun et al. (2018), there is a significant relationship between genetic variation and geographic distribution of several endosymbionts that infect *B. tabaci*. This includes how different geographic conditions play a crucial role in forming different nucleotide variations at the level of endosymbiont bacteria.

Based on the Fst values obtained, most *Wolbachia* sequences from East Java, Central Java, West Java, and Yogyakarta have genetic similarities (Table 7). In contrast, they have differences genes with *Wolbachia* sequences from Lampung, Indonesia. A negative or very small Fst value indicates no divergence between the sequences. Therefore, the data obtained also shows a relatively strong relationship between the phylogenetic groupings of a strain based on geographic location (Irawan et al. 2023). Results showed that the *Wolbachia* population structure from Java (East Java, Central Java, West Java, and Yogyakarta, Indonesia.) was genetically related and similar to that of *Wolbachia* from Lampung.

Table 5. The genetic differences (p-distance) of *Wolbachia* endosymbiont sequence and other arthropods Sequences

	Culex pipiens	***Brontispa longissima***	***Diaphorina citri***	***Thrips parvispinus***	***Yamatotettix flavovittatus***	***Pentalonia nigronervosa***
Banyuwangi	0.02532	0.02532	0.02222	0.03006	0.02215	0.04905
Malang	0.07278	0.07278	0.07302	0.07753	0.07911	0.09810
Semarang	0.04596	0.04596	0.04293	0.05071	0.04279	0.06815
Sukoharjo	0.03323	0.03323	0.03492	0.03797	0.03797	0.05538
Kulonprogo	0.05329	0.05329	0.05031	0.05799	0.05016	0.07524
Sleman	0.03925	0.03925	0.03622	0.04396	0.03611	0.06122
Majalengka	0.01266	0.01266	0.00952	0.01741	0.00949	0.03639
Ciamis	0.05538	0.05538	0.05238	0.06013	0.05222	0.07753
Mesuji	0.05055	0.05055	0.04913	0.05529	0.05529	0.06951
Pesawaran	0.05854	0.05854	0.05714	0.06329	0.06013	0.07753

Notes: *: Lower p-distance values indicate a closer genetic relationship between sequences

Table 6. Genetic structure of *Wolbachia* sequences among the five provinces

Location	n	Hn	Hd	π
East Java	2	2	1	0.06200
Central Java	2	2	1	0.03975
Yogyakarta	2	2	1	0.01590
West Java	2	2	1	0.04928
Lampung	2	2	1	0.04293
All populations	10	10	1	0.04045

Notes: n: number of samples; Hn: number of haplotypes; Hd: haplotype diversity; π: nucleotide diversity

Table 7. Population structure analysis (Fst*) of *Wolbachia* sequences

Province	East Java	Central Java	Yogyakarta	West Java	Lampung
East Java	-	-	-	-	-
Central Java	-0.15315	-	-	-	-
Yogyakarta	-0.04255	-0.06061	-	-	-
West Java	-0.27273	-0.33333	-0.20588	-	-
Lampung	0.02222	0.00952	0.30841	0.12782	-

Notes: *: The greater Fst means that they have very different genes between sequences

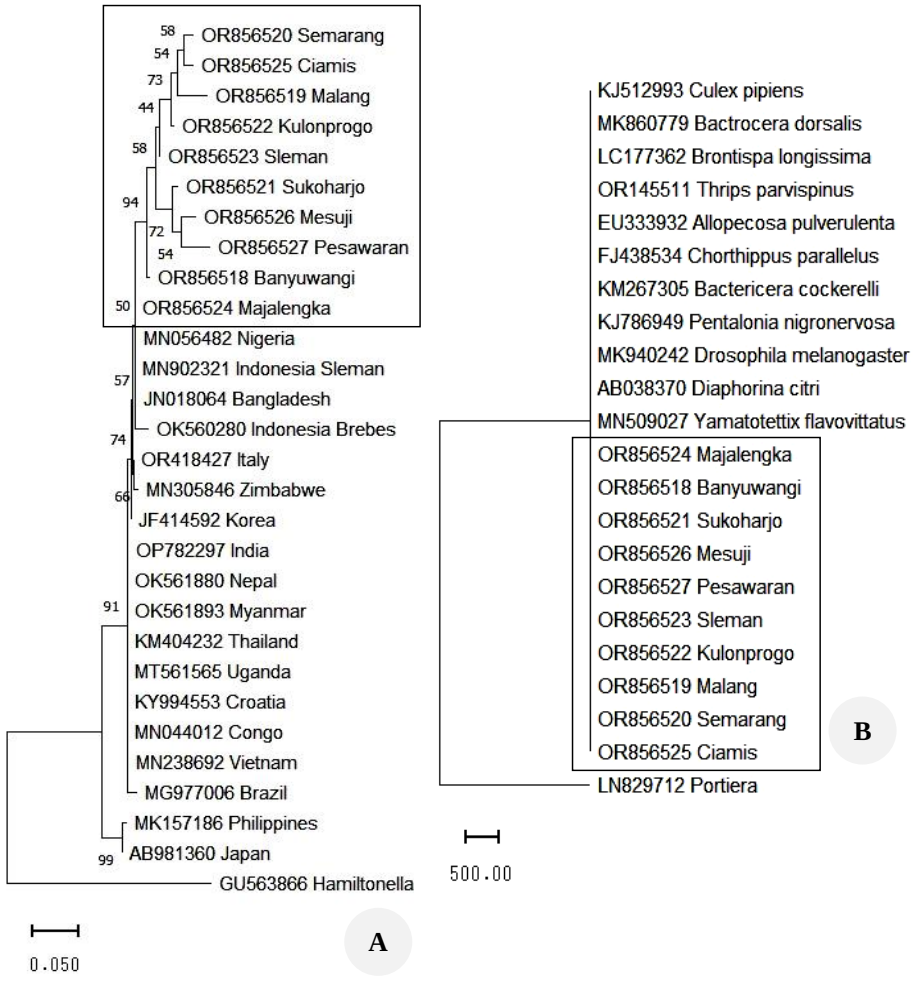


Figure 3. Phylogenetic tree based on *Wolbachia* endosymbiont sequences from *B. tabaci*. The tree was produced using the maximum likelihood method A. The 20 partial *Wolbachia B. tabaci* sequences were obtained from GenBank; B. The 11 partial *Wolbachia B. tabaci* sequences from other Arthropods were obtained from GenBank

In conclusion, the bacterial endosymbionts found among the *B. tabaci* populations were *C. portiera*, *Arshenophonus* sp., *Cardinium* sp., *Hamiltonella* sp., *Wolbachia* sp., and *Rickettsia* sp. The genetic diversity of *Wolbachia* of *B. tabaci* from Indonesia showed close relatedness and has a different clade from *Wolbachia* from other countries and Arthropods. The population structure of *Wolbachia* populations from Java was genetically related and similar to that of *Wolbachia* from Lampung, Sumatra.

ACKNOWLEDGEMENTS

This study has been funded by the research grant of the Indonesia Endowment Fund for Education (LPDP), Ministry of Finance, Indonesia. We thank Uci Agustina and Didik, assistants of Plant Pest Laboratory Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia, for technical support during laboratory research. We also thank Nandini, a magister student at Plant Pest Science UGM, for collecting samples.

REFERENCES

Ahmed MZ, Li S-J, Xue X, Yin X-J, Ren S-X, Jiggins FM, Greeff JM, Qiu B-L. 2015. The intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. PLoS Pathogens 11 (2): e1004672. DOI: 10.1371/journal.ppat.1004672.

Andreason SA, Shelby EA, Moss JB, Moore PJ, Moore AJ, Simmons AM. 2020. Whitefly endosymbionts: Biology, evolution, and plant virus interactions. Insects 11: 775. DOI: 10.3390/insects11110775.

Atikah A, Subandiyah S, Hartono S. 2019. Detection and Molecular Identification of *Wolbachia* Endosymbions in *Bemisia tabaci* (Hemiptera Aleyrodidae). [Thesis]. Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Bigiotti G, Pastorelli R, Belcari A, Sacchetti P. 2019. Symbiosis interruption in the olive fly: Effect of copper and propolis on *Candidatus erwinia* dacicola. J Appl Entomol 143 (4): 357-364. DOI: 10.1111/jen.12614.

Bing X-L, Xia W-Q, Gui J-D, Yan G-H, Wang X-W, Liu S-S. 2014. Diversity and evolution of the *Wolbachia* endosymbionts of *Bemisia* (Hemiptera: Aleyrodidae) whiteflies. Ecol Evol 4 (13): 2714-2737. DOI: 10.1002/ece3.1126.

Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M. 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. Bull Entomol Res 97 (4): 407-413. DOI: 10.1017/S0007485307005159.

de Marchi BR, Smith HA. 2020. Bacterial endosymbiont diversity among *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations in Florida. Insects 11 (3): 179. DOI: 10.3390/insects11030179.

El Hamss H, Maruthi MN, Ally HM, Omongo CA, Wang H-L, Brunschot S, Colvin J, Delatte H. 2022. Spatio-temporal changes in endosymbiont diversity and composition in the African cassava whitefly, *Bemisia tabaci* SSA1. Front Microbiol 13: 986226. DOI: 10.3389/fmicb.2022.986226.

Ghosh S, Ghanim M. 2021. Factors determining transmission of persistent viruses by *Bemisia tabaci* and emergence of new virus-vector relationships. Viruses 13 (9): 1808. DOI: 10.3390/v13091808.

Gottlieb Y, Ghanim M, Chiel E, Gerling D, Portnoy V, Steinberg S, Tzuri G, Horowitz AR, Belausov E, Mozes-Daube N, Kontsedalov S, Gershon M, Gal S, Katzir N, Zchori-Fein E. 2006. Identification and localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). Appl Environ Microbiol 72 (5): 3646-3652. DOI: 10.1128/aem.72.5.3646-3652.2006.

Hashmi TR, Devi SR, Ahmad A, Meshram NM, Prasad R. 2019. Genetic status and endosymbionts diversity of *Bemisia tabaci* (Gennadius) on hosts belonging to family Malvaceae in India. Neotrop Entomol 48: 207-218. DOI: 10.1007/s13744-018-0639-y.

Hu F-Y, Tsai C-W. 2020. Nutritional relationship between *Bemisia tabaci* and its primary endosymbiont, *Portiera aleyrodidarum*, during host plant acclimation. Insects 11: 489. DOI: 10.3390/insects11080498.

Irawan A, Ali S, Miroslav Ç, Çiğdem G, Serçe U, Ertunç F. 2023. Molecular analysis of prune dwarf virus reveals divergence within non- Turkish and Turkish viral populations. J Plant Pathol 105 (3): 943-954. DOI: 10.1007/s42161-023-01412-2.

Kanakala S, Ghanim M. 2019. Global genetic diversity and geographical distribution of *Bemisia tabaci* and its bacterial endosymbionts. PLoS One 14 (3): e0213946. DOI: 10.1371/journal.pone.0213946.

Kareem AA, Logan SA, Port G, Wolff K. 2020. *Bemisia tabaci* in Iraq: Population structure, endosymbiont diversity and putative species. J Appl Entomol 144 (4): 297-307. DOI: 10.1111/jen.12736.

Karut K, Castle SJ, Karut ŞT, Karaca MM. 2020. Secondary endosymbiont diversity of *Bemisia tabaci* and its parasitoids. Infec Genet Evol 78: 104104. DOI: 10.1016/j.meegid.2019.104104.

Khatun MF, Jahan SMH, Lee S, Lee KY. 2018. Genetic diversity and geographic distribution of the *Bemisia tabaci* species complex in Bangladesh. Acta Trop 187: 28-36. DOI: 10.1016/j.actatropica.2018.07.021.

Lestari SM, Hidayat P, Hidayat SH, Shim JK, Lee KY. 2021. *Bemisia tabaci* in Java, Indonesia: Genetic diversity and the relationship with secondary endosymbiotic bacteria. Symbiosis 83: 317-333. DOI: 10.1007/s13199-021-00752-w.

Li S-J, Ahmed MZ, Lv N, Shi P-Q, Wang X-M, Huang J-L, Qiu B-L. 2017. Plant mediated horizontal transmission of *Wolbachia* between whiteflies. ISME J 11 (4): 1019-1028. DOI: 10.1038/ismej.2016.164.

Liu X-D, Guo H-F. 2019. Importance of endosymbionts *Wolbachia* and *Rickettsia* in insect resistance development. Curr Opin Insect Sci 33: 84-90. DOI: 10.1016/j.cois.2019.05.003.

Lv N, Peng J, He Z-Q, Wen Q, Su Z-Q, Ali S, Liu C-Z, Qiu B-L. 2023. The dynamic distribution of *Wolbachia* and *Rickettsia* in AsiaII1 *Bemisia tabaci*. Insects 14 (4): 401. DOI: 10.3390/insects14040401.

Milenovic M, Ghanim M, Hoffmann L, Rapisarda C. 2022. Whitefly endosymbionts: IPM opportunity or tilting at windmills. J Pest Sci 95 (2): 543-566. DOI: 10.1007/s10340-021-01451-7.

Mitsuhashi W, Saiki T, Wei W, Kawakita H, Sato M. 2002. Two novel strains of *Wolbachia* coexisting in both species of mulberry leafhoppers. Insect Mol Biol 11 (6): 577-584. DOI: 10.1046/j.1365-2583.2002.00368.x.

Opatovsky I, Santos GD, Ruan Z, Lahav T, Ofaim S, Mouton L, Barbe V, Jiang J, Zchori-Fein E, Freilich S. 2018. Modeling trophic dependencies and exchanges among insects' bacterial symbionts in a host-simulated environment. BMC Genom 19: 402. DOI: 10.1186/s12864-018-4786-7.

Pan H, Li X, Ge D, Wang S, Wu Q, Xie W, Jiao X, Chu D, Liu B, Xu B, Zhang Y. 2012. Factors affecting population dynamics of maternally transmitted endosymbionts in *Bemisia tabaci*. PLoS One 7 (2): e30760. DOI: 10.1371/journal.pone.0030760.

Rao Q, Rollat-Farnier P-A, Zhu D-T, Santos-Garcia D, Silva FJ, Moya A, Latorre A, Klein CC, Vavre F, Sagot M-F, Liu S-S, Mouton L, Wang X-W. 2015. Genome reduction and potential metabolic complementation of the dual endosymbionts in the whitefly *Bemisia tabaci*. BMC Genomics 16: 226. DOI: 10.1186/s12864-015-1379-6.

Sani I, Ismail SI, Abdullah S, Jalinas J, Jamian S, Saad N. 2020. A review of the biology and control of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), with special reference to biological control using entomopathogenic Fungi. Insects 11 (9): 619. DOI: 10.3390/insects11090619.

Singh ST, Priya NG, Kumar J, Rana VS, Ellango R, Joshi A, Priyadarshini G, Asokan R, Rajagopal R. 2012. Diversity and phylogenetic analysis of endosymbiotic bacteria from field caught *Bemisia tabaci* from different locations of North India based on 16S rDNA library screening. Infec Genet Evol 12 (2): 411-419. DOI: 10.1016/j.meegid.2012.01.015.

Temaja IGRM, Selangga DGW, Phabiola TA, Khalimi K, Listihani L. 2022. Relationship between viruliferous *Bemisia tabaci* population and disease incidence of Pepper yellow leaf curl Indonesia virus in chili pepper. Biodiversitas 23: 5360-5366. DOI: 10.13057/biodiv/d231046.

Weeks AR, Robert V, Richard S. 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. Proc R Soc Biol Sci 270 (1526): 1857-1865. DOI: 10.1098/rspb.2003.2425.

Xue X, Li S-J, Ahmed MZ, De Barro PJ, Ren S-X, Qiu B-L. 2012. Inactivation of *Wolbachia* Reveals Its Biological Roles in Whitefly Host. PLoS One 7 (10): e48148. DOI: 10.1371/journal.pone.0048148.

Zchori-Fein E, Brown JK. 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Ann Entomol Soc Am 95: 711-718. DOI: 10.1603/0013-8746(2002)095[0711:DOPAWB]2.0.CO;2.

Zhong Y, Li Z-X. 2014. Bidirectional cytoplasmic incompatibility induced by cross-order transfection of *Wolbachia*: Implications for control of the host population. Microb Ecol 68: 463-471. DOI: 10.1007/s00248-014-0425-2.

Zhou X-F, Li Z-X. 2016. Establishment of the cytoplasmic incompatibility-inducing *Wolbachia* strain wMel in an important agricultural pest insect. Sci Rep 6: 39200. DOI: 10.1038/srep39200.

Zhu D-T, Zou C, Ban F-X, Wang H-L, Wang X-W, Liu Y-Q. 2019. Conservation of transcriptional elements in the obligate symbiont of the whitefly *Bemisia tabaci*. Peer J 7: e7477. DOI: 10.7717/peerj.7477.