ORIGINAL ARTICLE

Natural Infestation of *Wolbachia* Strains in the Populations of *Ae. albopictus* in Subang Jaya, Malaysia : a Study Based on *wsp* and Mitochondrial *Co1* Sequence Analysis

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ABSTRACT

Introduction: : Maternally inherited endosymbiont bacterium *Wolbachia* A (*w*AlbA) and B (*w*AlbB) strains can naturally infect *Ae. albopictus*, resulting in cytoplasmic incompatibility (CI) that could suppress mosquito populations. Exploring the evolutionary relationship between *Ae. albopictus* and *Wolbachia* infestation can help elucidate the distribution and future expansion of the vector. **Methods:** *Cytochrome oxidase subunit 1* (*CO1*) gene and the *Wolbachia* surface protein (*wsp*) gene were employed as markers to determine patterns of infestation of local *Ae. albopictus* in 12 localities in Subang Jaya, Selangor, Malaysia. A total of 120 individual mosquitoes and 20 pooled samples were examined using the *CO1* and *wsp* genes, respectively. **Results:**The majority of the local *Ae. albopictus* shared the same genetic lineage with mosquitoes from other neighboring Asian countries as revealed by phylogenetic analysis. Genotypic detection of samples from all localities was positive for both *w*AlbA and *w*AlbB strains with no genetic polymorphism detected within the *wsp* gene, indicating the successful introduction of *Wolbachia* in the mosquito population in Subang Jaya. The high occurrence of *Wolbachia* infestation may result in the low genetic diversity of *Ae. albopictus* possibly due to the effects of CI**. Conclusion:** Findings from this study contributes to the existing body of knowledge regarding the existence of *Wolbachia* superinfection and polymorphism of *CO1* gene in local *Ae. albopictus*; paving the way for further research and potential development of *Wolbachia*-based strategies for vector control.

Keywords: *Aedes albopictus*, *Wolbachia, cytochrome oxidase 1*, *Wolbachia* surface protein, phylogenetic analysis

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INTRODUCTION

Aedes albopictus (Skuse) is an invasive species that was first reported by Skuse in 1894 (1). During the early 20th century, *Ae. albopictus* has extended its range from Southeast Asia, the Indian Ocean, and Western Pacific islands to Africa, Europe, Middle East, Caribbean and America (2). This species continued to spread globally and to date, many cases have been reported throughout European countries, North America, Middle East and West and Central Africa regions (3,4). This species, alongside *Ae. aegypti* is accountable for the emergence and spread of dengue endemicity (2). *Ae. albopictus* possesses strong ecological plasticity (5) which aids its adaptation and spread. It can tolerate climatic change through photoperiodic diapause (6) which intricates control and surveillance efforts.

The mitochondrially encoded *cytochrome oxidase subunit 1* (*CO1*) gene is a highly conserved phylogenetically informative genetic marker (7–9). The gene is often used to study the historical processes in female mosquitoes (10–15) and other insect species. The evolution of vectors have been known to influence the transmission efficiency of viral infections (16). Insights

into the genetic variableness among *Ae. albopictus* using *CO1* markers can help disclose the origin and plausible spread of the vector. In addition, such knowledge can help forecast its forthcoming expansion and could conceivably assist vector control approaches.

Wolbachia is an alpha-proteobacterium that is maternally transmitted by a wide range of arthropods, nematodes, isopods, and mites. The capacity of *Wolbachia* to influence various functional systems of its hosts as well as to induce cytoplasmic incompatibility (CI) have been documented in the literature (17). This intracellular bacteria functions as reproductive parasites that can be used as a tool to suppress the growth of *Ae. albopictus* population (18). As such, details regarding the prevalence of Wolbachia in *Ae. albopictus* in a natural setting can be used to evaluate its potential application for dengue prevention (19).

It is imperative to elucidate the distribution of *Wolbachia* infection in the local settings and to unravel its genetic characteristics. Currently, the natural prevalence of *Wolbachia* in *Ae. albopictus* population in Subang Jaya is unknown. Hence, this study was performed to elucidate the genetic association of local *Ae. albopictus* in Subang Jaya based on the *CO1* marker. Besides, the infection frequencies and spread of *Wolbachia* in this setting are currently unknown, yet can be elucidated by screening and genotyping using the surface protein (*wsp*), *w*AlbA and *w*AlbB genes (20).

MATERIALS AND METHODS

Mosquito sampling

Mosquito eggs were sampled from 12 dengue cluster areas within Subang Jaya, Selangor based on previously described temporal indices (21,22), and described in Table I. Eggs were collected using dark plastic ovitraps from suburban residential areas from September 2013 to February 2014. Eggs were reared until adulthood in an insectary within a week of sample collection. All mosquitoes were being kept at temperature of 28±2°C, relative humidity of 70±10% and a photoperiod of 12:12h (light:dark), as described by Gerberg (23). The adults were maintained on ad libitum 10% (w/v) sucrose solution. A total of 130 female mosquitoes were identified based on morphological characteristics and standard pictorial keys (24). Ten individual mosquitoes from each localities, including USM laboratory strain (LS); F135 were fixed in 95% ethanol until being used for molecular analysis.

Mitochondrial Analysis

Genomic DNA was isolated from 10 individual mosquitoes per locality using the DNeasy Blood & Tissue kit (Qiagen, Germany), following manufacturer's instructions. A set of primers adopted from Porretta et al. (14); CO1F: TTATTACACAAGAAAGAGGAAAAA and CO1R: CATTGCACTAATCTGCCATA were used to amplify a 780 bp region of the *CO1* gene. Polymerase chain reaction (PCR) was performed using 200ng/µL

Table I. The 12 localities used in the placement of ovitraps based on temporal indices, coordinates, housing patterns and geographical descriptions.

Temporal Indices	Localities	Coordinates	Housing Pattern	Geographical descriptions
Frequency Index	PJS7	3°04′03.2″N 101°37′01.6″E	Flat	Flat houses located near to temple, private institutions, highway and medium cost residential areas
	PJS9	3°04′25.3″N 101°36′39.7″E	Terrace	A medium cost residential houses located near to the construction site
	Taman Puchong Perdana (TPP)	3°00′26.6″N 101°36′05.8″E	Flat	A low cost residential houses located near to the main road, LRT constructions and surround with shaded vegetative area
	Taman Universiti Indah (TUI)	3°00′44.8″N 101°41′18.9″E	Terrace	Terrace houses surrounded with vegetation areas
	USJ11	3°02′26.0″N 101°34′43.8″E	Terrace	Terrace houses near to the main road and shaded vegetation areas
Duration Index	USJ6	3°03′21.3″N 101°35′28.9″E	Flat	flat houses surrounded with shaded vegetation areas
	Taman Bukit Kinrara (TBK)	3°02′35.2″N 101°38′52.7″E	Terrace	Terrace houses surrounded with vegetation areas, and near to air force military camp
	Taman Puncak Kinrara (TPK)	3°02′20.5″N 101°38′07.2″E	Flat	Medium cost apartment surrounded with vegetation areas, and located near to industrial sites with improper waste disposal
	Taman Subang Mas (TSM)	3°03′14.4″N 101°33′26.8″E	Apart-ment	Low cost residential (flat houses) surrounded with several construction sites and improper waste disposal management
Intensity Index	SS14	3°03′59.3″N 101°35′23.8″E	Terrace	Terrace houses located near to highways
	Taman Kota Perdana (TKP)	2°59′30.4″N 101°39′36.6″E	Apart-ment	Apartment surrounded by vegetation areas near to the creek, improper waste management, and many automotive workshops in vicinity with several abandoned cars and motorcycles
	Taman Sungai Besi Indah (TSBI)	3°01′51.3″N 101°43′26.6″E	Flat	Flat and terrace residential with improper waste management, and near to highways

genomic DNA, 2.5 units Top Taq DNA Polymerase, 10 µM primers, 1x PCR buffer with 1.5 mM $MgCl_2$ and 200 µM each dNTPs. The PCR cycling conditions consisted of an initial denaturation at 95°C for 15 minutes, followed by 35 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The final extension step was performed at 72°C for 10 minutes.

Wolbachia Genotyping

Twenty pooled adult mosquitoes were used to screen *w*AlbA and *w*AlbB strains, either being singly-infected or super-infected in *Ae. albopictus* from the 12 localities. The pooled samples were extracted using the DNeasy Blood & Tissue kit (Qiagen, Germany), following manufacturer's instructions. Firstly, *Wolbachia* infection was screened using the wsp gene. Genotyping was then performed using *Wolbachia*-specific *w*AlbA and *w*AlbB primers described by Zhou et al. (20). All genes were amplified in a 50 µl reaction mixture using similar PCR reaction and amplification conditions as described in the mitochondrial analysis of *CO1* gene

Data Analysis

PCR amplicons were confirmed via electrophoresis, purified using a QIAquick PCR purification kit using standard protocols and sequenced using an ABI Big Dye Terminator. Multiple sequence alignments were edited using Chromas Lite v.2.1.1 and ClustalX v2.1 (25) programs. DnaSP v.5.10.1 software (26) was used to analyze the nucleotide (ϖ) and haplotype diversities (Hd) of the 130 aligned sequences of the *CO1* gene. Phylogenetic trees were constructed using MEGA6 (27), inferred by MrBayes v.3.2.4 (28). Analysis were based on local sequences as well as ones retrieved from the NCBI GenBank database (Table II). *w*AlbA and wAlbB genes were used to produce a phylogenetic tree alongside *w*Mel and *w*Pip sequences from the NCBI database (Table III). Four combined methods were applied namely Neighbor-Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian method. The Markov chains were sampled every 1000 generations and only ≥50% of the bootstrap value was shown in every branch.

Table II: *CO1* gene sequences retrieved from GenBank utilized in this study.

Regions	Location	Accession numbers
South-Eastern Asia	Vietnam (Hanoi 7)	JQ436997
	Vietnam (Hanoi 5)	JQ436996
	Vietnam (Hanoi 3)	JQ436995
	Vietnam (Vinh Phuc 12)	JQ436994
	Vietnam (Vinh Phuc 10)	JQ436993
	Vietnam (Vinh Phuc 5)	JQ436992
	Thailand (Lampang 3)	JQ437005
	Thailand (Lampang 4)	JQ437006
	Thailand (Lampang 8)	JQ437007
	Thailand (Chiang Mai 6)	JQ437003
	Thailand (Chiang Mai 13)	JQ437004
	Thailand (Songkhla 2)	JQ437008
	Thailand	KM613116
Southern Asia	Bhutan (Gelephu 9)	JQ437002
	Bhutan (Gelephu 8)	JQ437001
	Bhutan (Phuntsholing 2)	JQ436998
	Bhutan (Phuntsholing 3)	JQ436999
	Bhutan (Phuntsholing 5)	JQ437000
	Pakistan (Punjab Lahore)	KF406579
	Pakistan (Punjab Faisalaba)	KF406432

continued

Table II: *CO1* gene sequences retrieved from GenBank utilized in this study (cont.)

Regions	Location	Accession numbers
Eastern-Asia	Japan (Saga)	DQ397911
	Japan (Tanega Shima 1)	JQ436984
	Japan (Tanega Shima 3)	JQ436985
	Japan (Choral Uji 1)	JQ436981
	Japan (Choral Uji 3)	JQ436982
	Japan (Choral Uji 6)	JQ436983
	Japan (Okinawa 4)	JQ436987
	Japan (Fukushima)	DQ397912
	Japan (Nagasaki 2)	JQ436986
	Taiwan (Taipei)	AY072044
	China (Shantou 3)	JQ436989
	China (Hangzhou 6)	JQ436988
	China (Taoyuan 4)	JQ436990
	China (Taoyuan 10)	JQ436991
Northern America	USA (Los Angeles H37)	KC690932
	USA (Los Angeles H17)	KC690912
	USA (Los Angeles H22)	KC690917
	USA (ATCC)	HM102286
Western-Asia	Turkey (Edirne Ipsala 12-E12)	JQ412505
	Turkey (Edirne Ipsala 12-E09)	JQ412506
Southern Europe	Italy (Lab strain)	AF253022
	Italy (Alessandria 3)	JX679379
	Greece (Athens H1)	JF810659
Western Europe	Netherlands (6289079)	KM457562
	Netherlands (CMV06)	KM457564
	Germany (Weil am Rhein)	JQ388786
Caribbean	Puerto Rico (M163)	DQ181458
	Puerto Rico (M162)	DQ181457
Oceana	Australia (Torres Strait)	GQ143719
Sub-Sahara, Africa	Madagascar (Morondava)	JN406675

RESULTS

Phylogenetic Analysis of *CO1* Gene

The final sequence alignment of the *CO1* gene with the USM LS which utilised 689 nucleotides from 130 individual mosquitoes produced 26 novel haplotypes, (GenBank accession numbers KP896550-KP896575).

Table III. ***wsp*** **sequences retrieved from the GenBank that were used in this study.**

Wolbachia supergroup	*Wolbachia* strain	Host	Country	Accession numbers
A	**Mel**	*Drosophila melanogaster*	Shandong, China	EU395833
		Drosophila melanogaster	Uman, Ukraine	HM775090
	Riv	*Drosophila simulans* (Riverside strain)	Riverside, USA	AF020070
	Haw	*Drosophila simulans* (Hawaii strain)	Hawaii	DQ235408
	AlbA	*Ae. albopictus*	Kuala Lumpur, Malaysia	JX129187
		Ae. albopictus (Houston strain)	Houston, USA	AF020058
B	**Pip**	*Culex pipiens*	California, USA	AF301010
		Culex pipiens quinquefasciatus	Florida, USA	AF301012
		Ae. albopictus	Kuala Lumpur, Malaysia	JX129186
		Ae. albopictus	Taiwan	AY462863

Overall nucleotide and haplotype diversity estimates were 0.022 and 0.83, respectively. A phylogenetic tree was built using molecular data derived from NJ, ML, MP and Bayesian methods. Tamura-3-parameter with Gamma distribution (T92+G) had been chosen as the best fit model in this analysis based on the lowest value of AIC and BIC in MEGA6 software. The tree was structured with an overall average value of 0.321 genetic distance, inferred with 1,000,000 generations and converged with every 500 generations of sampling. The resulting phylogenetic tree (Fig.1) showed that the haplotypes from local *Ae. albopictus* were well distributed and merged with sequences from Asian countries. The tree topology was clustered into five groups with well-defined subgroups (2a-2j) branches supported with bootstrap values of >50%.

The construction of a phylogenetic tree combining the *CO1* gene sequence data from other countries provides an overview of the distribution pattern of *Ae. albopictus* in Subang Jaya, Malaysia. Five groups were observed; Group 1 contains sequences from South-Eastern Asia and Caribbean Islands; Group 2 comprises of several sequences from South-Eastern Asia, Southern Asia, Eastern Asia, Northern America and Southern Europe; Groups 3 and 4 are derived from local samples (South-Eastern Asia); and Group 5 consists of sequences from Oceania, Southern Europe, Western Europe, Western Asia, Sub-Saharan Africa and Northern America.

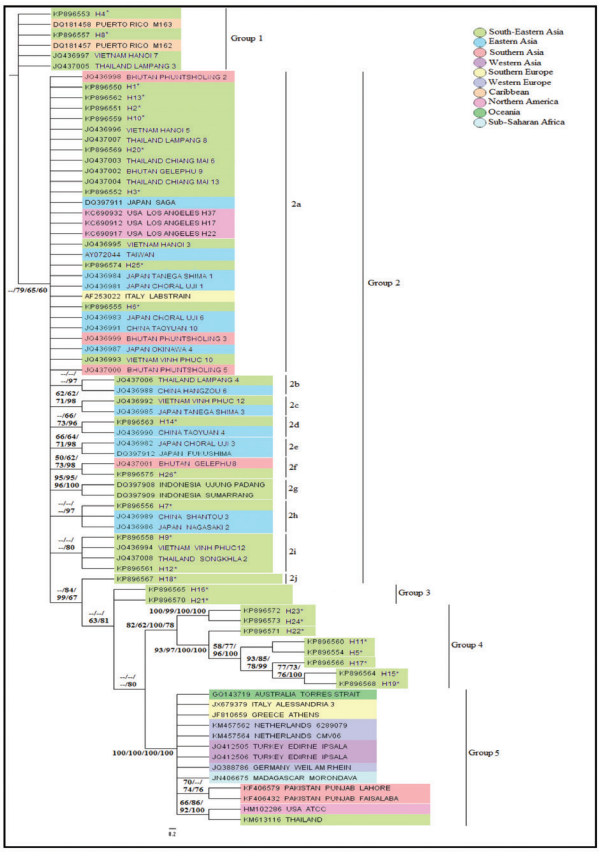

Fig. 1. Bayesian phylogram consensus tree (50% cut-off value) represents the phylogenetic relationship among ***Ae. albopictus*** **based on partial** ***CO1*** **gene sequences. Bootstrap values (expressed as percentages of 1000 replicates and 1,000,000 generations) from NJ (1st score), ML (2nd score), MP (3rd score), and Bayesian posterior probabilities (4th score); (--): no support value.**

Group 1 consists of sequences from South-Eastern Asia (Malaysia, Vietnam, and Thailand) and the Caribbean (Puerto Rico) with the lowest genetic divergence within the tree. Interestingly, sequences from the most frequent haplotype, H4 are clustered in Group 1 together with haplotypes from Puerto Rico (M163 and M162), Vietnam (Hanoi7) and Thailand (Lampang3).

Group 2 is the largest group; which encompasses sequences from most Asian continents including South-Eastern Asia (Malaysia, Indonesia, Vietnam, and Thailand), Eastern Asia (Japan, Taiwan and China) and Southern Asia (Bhutan). Three sequences from Northern America (USA: Los Angeles) and one from Southern Europe (Italy: LS) are also clustered in Group 2. This group contains 10 subgroups defined by the different lengths of tree branches where most of the haplotype sequences from local *Ae. albopictus* were assembled. The remaining sequence haplotypes are clustered in Group 3 and 4 were segregated into 3 lineages.

Wolbachia Genotyping

All positive samples were verified as *Wolbachia* by BLAST analysis using the NCBI database. Interestingly, our results as shown in Table IV revealed the USM LS had been superinfected by both *w*AlbA and *w*AlbB strains. Numerous field strains of local *Ae. albopictus* from 7

Table IV. Summary of *Wolbachia* infection status in *Ae. albopictus* from 12 localities and USM LS samples.

No.	Localities	Positive *Wolbachia* infections
1	USM LS	*w*AlbA+*w*AlbB
2	PJS7	*w*AlbA+*w*AlbB
3	PJS9	*w*AlbA+*w*AlbB
4	TPP	*w*AlbA
5	TUI	*w*AlbA
6	USJ11	*w*AlbA+*w*AlbB
7	USJ 6	*w*AlbA
8	TBK	*w*AlbA
9	TPK	*w*AlbA+*w*AlbB
10	TSM	*w*AlbA+*w*AlbB
11	SS14	*w*AlbA+*w*AlbB
12	TKP	*w*AlbB
13	TSBI	*w*AlbA+*w*AlbB

tree. Tamura-3-parameter (29) with discrete Gamma distribution (T92+G) was found to be the best nucleotide substitution model based on the lowest value of Bayesian Information Criterion (BIC) and corrected Akaike Information Criterion (AIC). All gaps and missing data were eliminated (27,30). The phylogenetic tree was inferred using MrBayes with 1000 generations (sampling every 500 generations) to allow sufficient time for convergence.

Wolbachia from different groups were clustered into two supergroups (A and B) (Fig. 2). Supergroup A consisted of AlbA, Riv, Haw and Mel *Wolbachia* while supergroup B comprised Pip *Wolbachia* according to Zhou et al. (20). All sequences were well clustered according to the group of *Wolbachia* strains. From the results above, the same length of branches on each *w*AlbA and *w*AlbB sequences indicated that there are no differences in their genetic distance among all *Wolbachia* positive localities and reference sequences from the GenBank (Table III).

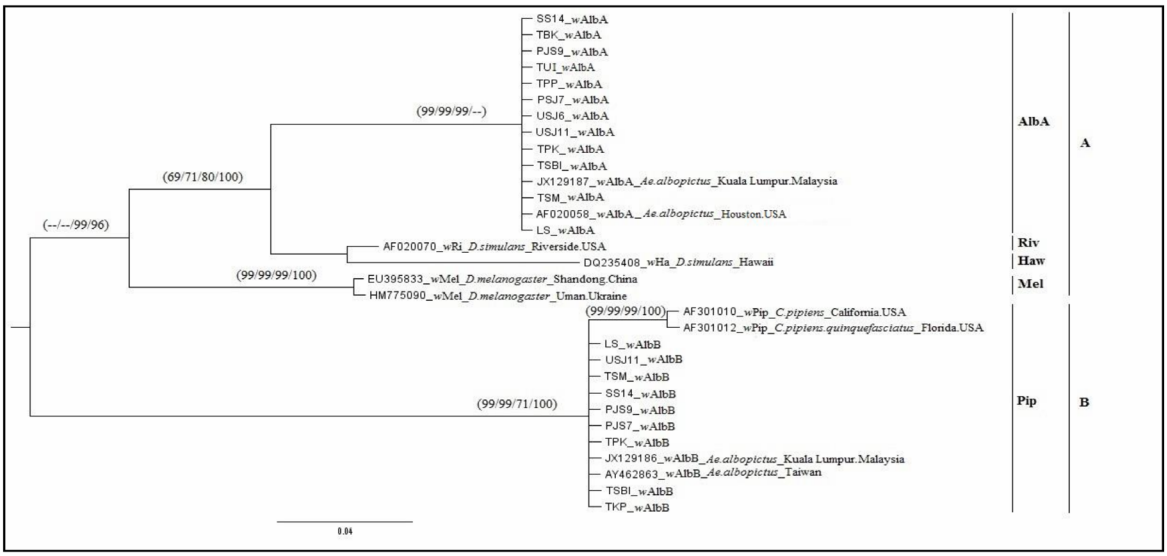

Fig. 2. Bayesian phylogram consensus tree (50% cut-off value) representing the phylogenetic relationship among Wolbachia strains based on partial wsp gene sequences. Bootstrap values (expressed as percentages of 1000 replicates) from neighbor-joining (1st score), maximum likelihood (2nd score), maximum parsimony (3rd score), and Bayesian posterior probabilities (4th score); (--): no support value.

localities (TPK, PJS7, PJS9, USJ11, SS14, TSBI and TSM) were also superinfected with both *Wolbachia* strains. Four localities (TPP, TUI, USJ6, and TBK) were singly infected with *w*AlbA strain while only one locality (TKP) was positive for a single infection with *w*AlbB strain. Overall, these results indicate that *Ae. albopictus* from 8 /13 localities were infected with both *w*AlbA and *w*AlbB strains. Only a few localities contain mosquitoes that were singly infected with either *w*AlbA or *w*AlbB strains. The *wsp* sequences of 12 *w*AlbA and 11 *w*AlbB obtained in this study were deposited into the GenBank database with the accession numbers KX573017-KX573037 respectively.

Phylogenetic and Genetic Distance Analysis of *Wolbachia* Strains

Previously aligned sequences from the different groups of *Wolbachia* were used to construct the phylogenetic

The average percentage of genetic distance within and amongst strains in Wolbachia genus was measured using MEGA6 based on the Kimura (K80) model (31) to determine the probabilistic relatedness among sampled population. In general, no significant genetic distance was detected for all tested Wolbachia within the same strain. This result indicates that the samples were identical and closely related to each other. However, the low average percentage of genetic distance was observed among Wolbachia strains. All Wolbachia strains including reference sequences were derived from a common ancestor within the same species indicated by the value of genetic distance that is close to 0.00; the average being 0.134 for all samples. The average percentage of genetic differences among the groups showed a value of >0.2 which forms the basis of phylogeny and PCR-based classification of Wolbachia strains A and B as described by Zhou et al. (20). The

Table V. Average percentage of K80 distance values within and among strains in *Wolbachia* genus based on *wsp* gene sequences.

Strain	*w*AlbA	*w*Ri	*w*Ha	*w*Mel	*w*AlbB	*w*Pip
*w*AlbA (n=14)	**0.000**					
*w*Ri (n=1)	0.074	**NA**				
*w*Ha (n=1)	0.116	0.079	**NA**			
*w*Mel (n=2)	0.119	0.086	0.154	**0.000**		
*w*AlbB (n=11)	0.237	0.201	0.252	0.223	**0.000**	
*w*Pip (n=2)	0.252	0.216	0.257	0.238	0.021	**0.000**

n; samples size for each strain. Bold; the average percentage of genetic distance within strains. NA; not applicable.

analysis of the 31 nucleotide sequences is summarised in Table V.

DISCUSSION

The construction of the phylogenetic tree combining the *CO1* gene sequence data from other countries provides an overview of the distribution pattern of *Ae. albopictus* in Subang Jaya. The local *Ae. albopictus* strain was closely related to the sequences from Asian regions suggesting continuing gene flow amongst them. All 12 sampled localities have similar temperate climates and average annual rainfall, yet harboured different housing patterns and population densities. Most of the localities were in the vicinity of construction sites and vegetative areas, providing excellent breeding sites for *Ae. albopictus* to breed. These environmental factors may affect the dispersal of *Ae. albopictus*, as well as the genetic diversity of this species (32,33). Our results signify that the sequences of the local *Ae. albopictus* revealed similarities with sequences from other Asian regions, possibly as a result of movement of people, migration and tourism (34). Environmental conditions and climate change may have favoured the infestation of *Ae. albopictus* in tropical and subtropical countries (3,5,35). In addition, vector control activities such as source reduction and the use of insecticides have contributed to the reduction of *Aedes* mosquito populations (14). This resulted in closely related genetic relationships of the studied *Ae. albopictus* populations which have been observed in this current study. *Aedes* mosquitoes may retain less genetic variation when subjected to less variation in environmental conditions. A similar study conducted by Kamgang et al. (13) in Cameroon revealed weak genetic variation, which could be due to the small size of founding populations.

wsp gene is useful for *Wolbachia* strain typing, phylogenetic and evolutionary studies as it provides characters for fine-scale phylogeny (36). It was able to identify 100% infection either singly or superinfected among *Ae. albopictus* populations in Subang Jaya. This study revealed that superinfection was more predominant in the Subang (PJS7, PJS9, USJ11, TSM, and SS14) compared to the ones in Puchong, Kinrara and Sri Kembangan populations. However, superinfection was not consistent throughout Subang Jaya Municipality. Out of 12 studied localities, 7 were superinfected with both strains, 5 were singly infected with *w*AlbA strain and only 1 was infected by *w*AlbB (TKP).

In response to the stressed environment, the singly infected *w*AlbB strain in *Ae. albopictus* populations could be lost, thus superinfection is required for sustainability in the mosquito populations (37) through generations without being affected by both biological and environmental factors. The findings are consistent with other previous studies (38–44), all of which described either singly or/and superinfection of Wolbachia is common across the geographical region. Since most of *Ae. albopictus* in Subang Jaya is superinfected with *w*AlbA and *w*AlbB, it is possible to introduce the transgene blocking mechanism through the infection of the host with *w*Mel (39,44). This could subsequently lead to a more robust pathogen blocking capacity with strong CI induction of the injected strain (44). This initiative can be applied in the local vector control regimen in tandem with adulticide-based approaches.

The potential importance of *w*AlbA and *w*AlbB strains in *Ae. albopictus* remains to be further explored. Efforts to understand the mechanism of CI in *Ae. albopictus* is attributed to the *Wolbachia*-based population modification strategies. Previous studies by Bian et al. (45) and Ruang-Areerate and Kittayapong (46) reported that *Wolbachia* is unable to interrupt the viral replication in *Ae. albopictus*, but can instead inhibit the viral dissemination via the CI effect in *Ae. agypti* transinfected with *w*AlbB. Findings from Mousson et al. (47) suggests the role of *Wolbachia* in naturally limiting the transmission of DENV in *Ae. albopictus* by reducing viral infection in the salivary glands, thus highlighting the impacts of Wolbachia-mediated viral interference.

Phylogenetic findings of the *wsp* gene validate the transmission of *Wolbachia* infections in *Ae. albopictus* populations in Subang Jaya. High genetic divergence was identified between the *w*AlbA and *w*AlbB sequences resulting in subdivision into two groups. The *w*AlbA sequences are found to be identical and clustered in *Wolbachia* Supergroup A with the *w*AlbA sequences from Kuala Lumpur and Houston, USA alongside with Riv, Haw and Mel strains. Riv and Haw strains share the same branch with wAlbA strain supported by strong bootstrap values of 69 (NJ), 71 (ML), 80 (MP) and 100 (Bayesian) at each node with 1000 replicates. Although wMel shares a common ancestor with *w*AlbA, Riv and Haw strains, it is more genetically distinct when compared to each other. In this study, *w*AlbB sequences

were grouped in Pip strain together with *w*Pip sequences in *Wolbachia* Supergroup B. *w*AlbB sequences from this current study are similar to the *w*AlbB sequences from Kuala Lumpur and Taiwan.

The genetic diversity of *Wolbachia* is found to be identical to each other as well as to their respective group. No polymorphism is detected in both groups (*w*AlbA and *w*AlbB). This is in agreement with results from previous findings, in which 99% of homology with *Wolbachia* sp in *Ae. albopictus* is reported from different geographical regions of India, Taiwan, China (42), USA, France, Korea (41) and United Kingdom. According to Braig et al. (36), sequence variability in the outer membrane of intracellular bacterium might reflect a functional difference in terms of CI phenotypic effect, adaptation and specialization to the intimate interaction with the host cell suggesting stability and species specificity of *w*AlbA and *w*AlbB. This result can be further exploited in future studies, possibly towards the control of vector-borne diseases as described in a study by Nazni et al. (49). The impacts of *Wolbachia* release programs through the transfection of *w*AlbB strain in *Ae.aegypti* population in Kuala Lumpur in reducing viral transmission could be further explored (38,40,45,46).

Our findings suggests that the lack of genetic variability in *w*AlbA and *w*AlbB could be due to the effects of CI (48,49). In addition, findings obtained from the genetic diversity in wsp gene are in line with previous research findings utilizing *Ae. albopictus* sampled from different geographical regions (36,38,40,42). However, the small sample size of mosquitoes used in this study may have also contributed to the low average genetic diversity of *Wolbachia*. Extensive sampling covering all states in Malaysia should be performed in future studies to provide a more comprehensive insight into the ecology and evolutionary patterns of *Wolbachia.*

CONCLUSION

In conclusion, this study corroborates naturally infestation of *Wolbachia* superinfection in the local *Ae. albopictus* population, and warrant the reliability of *CO1* to identify distinct lineages of this vector species. This is in turn becomes a cornerstone in improving the existing vector control strategies to help mitigate the risk and spread of dengue, primarily in Malaysia.

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