UNIVERSITI TEKNOLOGI MARA

MOLECULAR CHARACTERIZATION OF *AEDES ALBOPICTUS* BASED ON THE MITOCHONDRIAL *CYTOCHROME OXIDASE 1* MARKER IN DENGUE INFESTED AREAS

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ABSTRACT

The global spread of *Ae. albopictus* from its native range in Southeast Asia has been implicated in the recent emergence of dengue endemicity in Malaysia. Maternally inherited endosymbiont bacteria *Wolbachia* A (*w*AlbA) and B (*w*AlbB) strains are known to naturally infect *Ae. albopictus* resulting in cytoplasmic incompatibility (CI) that could reduce mosquito populations. Studies focusing on genetic diversity of *Ae. albopictus* and *Wolbachia* infestation in local populations are currently lacking in the Malaysian setting, yet such studies are crucial to enhance current vector control programs. This study was conducted to determine the pattern of *Wolbachia* infection in the local population using the surface protein (*wsp*) gene and to establish the genetic variability of maternally inherited mitochondrial DNA encoding *cytochrome oxidase subunit 1* (*CO1*) gene in *Ae. albopictus.* Analysis from 12 localities in the Subang Jaya District based on temporal indices patterns were performed using 120 individual samples and 20 pooled samples for *CO1* and *wsp* genes, respectively. Genetic polymorphism and phylogenetic analysis were conducted to reveal the genetic variability and geographic origin of *Ae. albopictus* and *Wolbachia* populations. Haplotype network was mapped to determine the genealogical relationship of sequences among groups of population in the Asian region. Genotypic detection of all sampled localities were positive for both strains with no genetic polymorphism of the *wsp* gene, indicating the successful introduction of *Wolbachia* in the *Ae. albopictus* populations in Subang Jaya. Comparison of local *CO1* sequences with sequences derived from 5 Asian countries revealed genetically distinct intrapopulation of *Ae.albopictus*. Phylogenetic analysis of *Ae. albopictus* revealed that all local sequences descended from the same genetic lineage with the sequences from other Asian countries. Our study highlights the discovery of 20 novel haplotypes within the local population that has not been reported to date. High frequency of *Wolbachia* infections in Subang Jaya population may lead to low genetic diversity in *Ae. albopictus* due to the CI effect that may influence the genetic structure of the mosquitoes. As such, the co-existence of *Wolbachia* superinfection in *Ae. albopictus* is suggested to be beneficial towards the future development of a biological vector control agent that can be used in the local settings.

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CHAPTER ONE
INTRODUCTION

1.1 RESEARCH BACKGROUND

The Asian Tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) (*Ae. albopictus*) is one of the most invasive species in the world as listed in the Global Invasive Species Database (Lowe, Browne, Boudjelas, & De Poorter, 2000). The first entomological detection of *Ae. albopictus* was documented in the USA, which then spread to South East Asia and various parts of the world including South Pacific and Indian Ocean islands, Europe, Middle East, Caribbean, Central and South America, Central Africa and Australasia (Haddad, Harbach, Chamat, & Bouharoun-Tayoun, 2007; Scholte et al., 2007; Delatte et al., 2011; Vaux & Medlock, 2015). Upon adaptation to new environment, this competent vector continued to invade extensively and is responsible for the emergence of several pathogenic viruses including Chikungunya (CHIKV) (Reiter, Fontenille, & Paupy, 2006), Zika (ZIKV) (Attar, 2016) and dengue (DENV) (Rosen, 1987); causing concern worldwide. Although *Ae. albopictus* generally considered as a secondary vector for DENV, *Ae. albopictus* possesses strong ecological plasticity that could replace the primary vector, *Aedes aegypti* (*Ae. aegypti*) in the future (Paupy, Delatte, Bagny, Corbel, & Fontenille, 2009).

Aedes albopictus is a semi-container breeder and is usually found in tropical, subtropical and temperate regions within suburban, urban and forest areas (Rosen, 1987). It usually accommodates in artificial containers such as used tires, pots plant, bottles, tins, clogged rain drainages and buckets. Larvae and pupae can also be found in natural containers including tree holes, bamboo, rock holes, rock pools, coconut shells and leaf axils especially in the presence of vegetation (Centers for Disease Control and Prevention, 2010). The uncontrolled expansion of *Ae. albopictus* can be attributed to ecological plasticity, rapid urbanization, lack of public awareness, improper solid waste disposal, climatic change and increased international trade especially in used tires and lucky bamboo (Gratz, 2004; Paupy et al., 2009; Medlock et al., 2015). The control of *Ae. albopictus* has become more challenging in recent years in tandem with the increase of human populations (Padmanabha, Durham,