FINAL REPORT FOR RESEARCH ACCULTURATION COLLABORATIVE EFFORT GRANT SCHEME (RACE)

PROJECT TITLE:

MOLECULAR CHARACTERIZATION OF *AEDES ALBOPICTUS* (DIPTERA:CULICIDAE) FROM HOT SPOT DENGUE-INFESTED AREAS

PROJECT LEADER:

SITI NAZRINA CAMALXAMAN (174664)

PROJECT MEMBERS:

PROF. ABU HASSAN AHMAD (USM)

PROF MADYA RODZIAH ISMAIL (091569)

DR. NAZRI CHE DOM (230090)

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INTRODUCTION

Laboratory work and thesis writing has been completed within one and a half years. The aim of this study is to characterize the genetic diversity and evolutionary relationship of *Ae. albopictus* collected from dengue cluster areas based on the *CO1* gene as a genetic marker. Local sequences will be amplified and compared, results obtained will help determine the genetic diversity, geographic origin and future expansion of *Ae. albopictus*. The distribution and infection frequencies of *Wolbachia* in local *Ae. albopictus* is currently unknown also forms the basis of this study. Broadly, findings from this study are expected to provide a new perspective aimed to improve current vector control and surveillance program.

One of the approaches that could mitigate the spread and threat of dengue would be by improving the current understanding of the vector biology and enhancing vector control strategies. This in turn necessitates the need to characterize the vector itself, and the determination of the prevalence of *Wolbachia* in these vectors for biological control based approaches (Bonizzoni, Gasperi, Chen, & James, 2013). *Wolbachia* is an endosymbiotic alpha-proteobacteria that infects more than 70% of insect species worldwide including a variety of arthropods (Miller, 2013). The potential of *Wolbachia* to manipulate various functional systems of its hosts affects several aspects of host biology, physiology, immunity, ecology and evolution as well as reproductive properties including feminization, parthenogenesis, male killing and most commonly, cytoplasmic incompatibility (CI) (Werren, 1997). This intracellular bacteria functions as reproductive parasites and has been proposed for the future development of a symbiont-based control approach to tackle *Ae. albopictus* population (Bourtzis et al., 2014). In tandem, data regarding the natural infection frequency of *Wolbachia* in *Ae. albopictus* and the effectiveness of its transmission rates are essential to evaluate its use as a candidate vehicle to modify vector populations (Mains, Brelsfoard, Crain, Huang, & Dobson, 2013).

Morphological characterization using DNA barcoding methods that utilize *cytochrome oxidase subunit I* (*CO1*) gene has been widely used in the past to attain information regarding mosquito taxonomy (Besansky, Severson, & Ferdig, 2003; Meier & Zhang, 2008). In addition, it has also been used for diagnostic purposes of specific target species. Most research to date focuses on spatial distribution and abundance of *Aedes* vectors, but scarce information exists regarding the phylogenetic and evolutionary relationship among the vectors at specific localities in Malaysia. Recently, a temporal model using climate variables was developed to forecast dengue cases in Subang Jaya (Dom, Ahmad, Latif, & Ismail, 2013). This model proved to be useful in predicting dengue cases and reinforces previous studies using different modalities and risk assessment methods (Dom, Ahmad, Latif, Ismail, & Pradhan, 2012a; Dom, Latif, Ahmad, Ismail, & Pradhan, 2012b). Such hotspots described by Dom et al. (2013) could now serve as a platform to determine the prevalence and distribution of both the DENV and the vector of choice.

OBJECTIVES

General Objective

To characterize *Ae. albopictus* collected from 12 dengue cluster areas in the Subang Jaya Municipality (MPSJ), Selangor.

1.3.2 Specific Objectives

1. To screen the frequency of *Wolbachia* infection in *Ae. albopictus* using the *wsp*, *w*AlbA and *w*AlbB genes.
2. To characterize *Ae. albopictus* collected from the dengue cluster areas based on the *cytochrome oxidase 1* (*CO1*) gene.
3. To determine the genetic relationships and distribution of *Ae. albopictus* in both local and other neighboring Asian countries based on haplotype networking and phylogenetic tree.
4. To determine the phylogenetic relationships of local haplotype sequences and the sequences retrieved from GenBank.

1) SAMPLE COLLECTION AND MOSQUITO REARING

Field work started on September 29th, 2013. The ovitraps used in this study were prepared manually (Appendix 1) and placed at 12 localities in Subang Jaya Municipal Areas (Appendix 2). Mosquito eggs were collected after four days of initial sampling (Appendix 3), and reared into adult forms in specialized insectariums at the Vector Lab (Appendix 4). The total number of eggs and adult mosquitoes reared per locality were individually enumerated; results of which are shown in Table 1. Preliminary morphological analysis of the samples was performed using compound microscope. Samples were fixed in 75% (v/v) ethanol prior to extraction (Appendix 5).

Table 1:
Mosquito rearing analysis collected from 12 localities representing the Subang Jaya Municipality.

No.	Locality	Field Collected (Wild Strain) Mosquito			
		Total no of eggs	Total no of larvae	Total no of adult (*Ae. albopictus*)	Total no of adult (*Ae. aegypti*)
1	PJS7	204	184	174	-
2	PJS9	197	162	153	-
3	TPP	187	152	139	-
4	TUI	217	188	172	-
5	USJ11	253	239	224	-
6	USJ6	235	217	205	-
7	TBK	238	215	208	-
8	TPK	216	173	161	-
9	TSM	450	432	417	-
10	SS14	213	196	188	-
11	TKP	302	275	259	-
12	TSBI	283	251	237	-

(-) No *Ae. aegypti* found in all 12 localities.