DISTRIBUTION OF *ANOPHELES* MOSQUITOES AND *PLASMODIUM KNOWLESI* TRANSMISSION IN KUDAT, SABAH BORNEO

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IJAZAH: **MASTER OF SCIENCE (MEDICAL SCIENCE)**

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DECLARATION

I hereby declare that this thesis is entirely my own work and that where material could be construed as the work of others, it is fully cited and referenced, and with appropriate acknowledgement given.

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ABSTRACT

The zoonotic malaria caused by *Plasmodium knowlesi* (*Pk*) threatens the rural residents in Sabah. This disease is transmitted by mosquitoes of genus *Anopheles*. A study was carried out with the main objective to collect distribution data of *Anopheles* in Kudat, to investigate whether land use or land cover (LULC) affects the mosquito distribution. Another objective is to examine the parity, proportion of mosquito infected with malaria parasites and estimate longevity of the main vector. Six LULC, namely clearing, forest, rubber, coconut, oil palm plantation and settlement were classified. Mosquito population was sampled from February 2015 to January 2016 in all LULCs. Outdoor human landing catch was performed in 1800-2400 hour to collect exophilic *Anopheles*. A total of 1,910 individuals, comprised of eight species were collected. The most abundant species was *Anopheles balabacensis* (85%), followed by *Anopheles donaldi* (6.23%), *An. barbumbrosus* (3.72%), *An. tessellatus* (1.52%), *An. maculatus* (1.52%), *An. barbirostris* (1.26%), *An. introlatus* (0.42%), and *An. umbrosus* group (0.58%). The highest peak of population was recorded in September 2015, with the peak biting time at 1800-2000 hour throughout the year. Generalized linear mixed model (GLMM) analysis, incorporating negative binomial distribution showed that the number of *An. balabacensis* collected at clearing (mean 2.98) was significantly lower than coconut (mean 7.12, z-ration=-2.89, p-value=0.045), oil palm plantation (mean 7.13, z=-3.04, p=0.03), forest (mean 7.99, z=-3.25, p=0.01) and settlement (mean 7.70, z=-3.21, p=0.02). Besides LULC, wind speed and rain fall are likely factors to affect mosquito caught. The pattern of mosquito distribution can be described as seasonal. It correlates positively (Kendall tau = 0.867, $p < 0.05$) to monthly rainfall up to the threshold level of 350mm. All *Anopheles* collected in Jun 2015 – January 2016 were dissected for the presence of *Plasmodium* sporozoite in salivary glands and oocytes on midgut wall. As a result, total of 36 individuals of *Anopheles balabacensis* were infected with malaria parasites, with the highest infection rate (5.45%, n=256) in September 2015. The parity rate of *An. balabacensis* fluctuated little (54.11±3%) in all LULC throughout the year. The highest entomological inoculation rate (EIR) was 0.14. The longevity of an infective *An. balabacensis* was estimated 5-6 days. Mosquito controls such as source reduction and fogging to be carried out at 1800-2000 hour are recommended during drought period.

ABSTRAK

TABURAN NYAMUK ANOPHELES DAN PENYEBARAN MALARIA PLASMODIUM KNOWLESI DI KUDAT, SABAH BORNEO

Penyakit malaria zoonotik yang disebabkan oleh protozoa Plasmodium knowlesi telah mengancam kesihatan penduduk luar bandar di Sabah. Malaria ini dibawa oleh nyamuk genus Anopheles. Kajian ini bertujuan untuk mengumpul maklumat taburan Anopheles di daerah Kudat, mengenalpasti kesan gunaan tanah terhadap taburan nyamuk. Objektif yang kedua adalah mengkaji keberkesanan Anopheles sebagai pembawa malaria di Kudat. Populasi nyamuk disampel dari bulan Februari 2015 sehingga Januari 2016 di enam jenis gunaan tanah, iaitu tanah kosong, hutan, ladang kelapa, kelapa sawit, getah dan penempatan. Anopheles yang bersifat "exophilik" ditangkap dengan kaedah tangkapan berumpan manusia ("human landing catch") dari jam 1800-2400. Sebanyak 1,910 individu nyamuk, terdiri daripada sembilan spesis telah diperolehi. Spesis yang paling banyak termasuk Anopheles balabacensis (85%), seterusnya An. donaldi (6.23%), An. barbumbrosus (3.72%), An. tessellatus (1.52%), An. maculatus (1.52%), An. barbirostris (1.26%), An. introlatus (0.42%), dan An. umbrosus group (0.58%). Keputusan menunjukan bahawa populasi nyamuk yang tertinggi di bulan September 2015, serta puncak masa menggigit dari jam 1800-2000 sepanjang tahun. Model Linear umum campuran bersama dengan taburan negatif binomial menunjukkan bahawa bilangan An. balabacensis terdapat di tanah kosong (min 2.98) adalah ketara berbeza dengan ladang kelapa (min 7.12, z-ration=-2.89, p-value=0.045), kelapa sawait (min 7.13, z=-3.04, p=0.03), hutan (min 7.99, z=-3.25, p=0.01) dan petempatan (min 7.70, z=-3.21, p=0.02). Selain gunaan tanah, kelajuan angin dan hujan merupakan faktor yang menpengaruhi populasi nyamuk. Taburan nyamuk adalah berbentuk musim dan berkelompok. Perubahan populasi berhubungkait secara positif dengan hujan sehingga tahap ambang sebanyak 350mm. Setiap individu nyamuk dibedah untuk identifikasi kewujudan sporozoite di dalam kelenjar air liur, serta oosit pada permukaan usus tengah. Hanya 27 individu An. balabacensis dijangkiti, dengan kadar jangkitan yang paling tinggi (8.82%) dalam bulan Mei 2015. Kadar pariti An. balabacensis kurang berubah di semua gunaan tanah sepanjang tahun (54.81±5.6%). Kadar inokulasi entomologi harian ialah 0.09± 0.05. Hayat hidup nyamuk terjangkit dijangka selama 5-6 hari. Kaedah pengawalan nyamuk yang dicadang adalah pengurangan tapak pembiakan serta semburan kabus ("fogging") pada jam 1800-2000 dalam musim kering.

TABLE OF CONTENTS

		Page
TITLE		i
DECLARATION		ii
CERTIFICATION		iii
ACKNOWLEDGEMENT		iv
ABSTRACT		v
ABSTRAK		vi
TABLE OF CONTENTS		vii
LIST OF FIGURES		x
LIST OF TABLES		xi
LIST OF PHOTO		xii
LIST OF ABBREVIATIONS		xiii
LIST OF APPENDICES		xiv
CHAPTER 1:	**INTRODUCTION**	1
1.1	Background	1
1.2	Significance of study	4
1.3	Objectives	4
CHAPTER 2	**LITERATURE REVIEW**	5
2.1	Biology of Mosquitoes	5
	2.1.1 Morphology	5
	2.1.2 Classification	7
	2.1.3 Life Cycle of *Anopheles* Mosquitoes	11
	2.1.4 Feeding	12
2.2	Medical Importance	13
2.3	Malaria	15
	2.3.1 Life Cycles of *Plasmodium* species	15
	2.3.2 Human Malaria	18
	2.3.3 Geographical Distribution	20

2.3.4 Effect of Environmental Change on Malaria 21
2.3.5 Malaria Control 22

CHAPTER 3 METHODOLOGY 24

3.1 Study Design 24
3.1.1 Study Site 24
3.1.2 Criteria for Site Selection 25
3.1.3 Land Use and Land Cover (LULC) 25
3.2.4 Mapping 27
3.2 Sampling Design 29
3.2.1 Sampling Method 29
3.2.2 Environmental Conditions 31
3.3 Mosquito Dissection 32
3.3.1 Midgut (Stomach) Dissection 32
3.3.2 Salivary Gland Dissection 34
3.4 Data Analysis 35
3.4.1 Man-biting Rate (Ma) 35
3.4.2 Sporozoite Rate (SR) 35
3.4.3 Entomological Inoculation Rate (EIR) 35
3.4.4 Longevity and Infectivity 36
3.4.5 Statistical Analysis 36
3.5 Ethical Approval 37

CHAPTER 4 RESULTS 38

4.1 Land Use and Land Cover (LULC) in Study Site 38
4.2 Species Composition and Abundance of *Anopheles* Mosquitoes 39
4.3 Distribution of *Anopheles balabacensis* 42
4.3.1 Mosquito Biting Time 42
4.3.2 Distance, Temperature, and Wind Speed in Mosquito Samplings 43
4.3.3 Relationship between Rainfall and *An. balabacensis* caught 47
4.3.4 Distribution of *An. balabacensis* on Land Use and Land Cover 48

4.4 Vector Efficacy of *Anopheles balabacensis* 50

4.4.1 Parous Rate of *Anopheles balabacensis* 50

4.4.2 Oocyst and Sporozoite Rate of *Anopheles balabacensis* 49

4.4.3 A Comparison of Entomological Inoculation Rate and Longevity in Different Land Use and Land Cover 52

CHAPTER 5 DISCUSSION 54

5.1 Diversity of *Anopheles* Mosquitoes in Kudat 54

5.2 The Effect of Land Use and Land Cover on the Distribution of *Anopheles balabacensis* 56

5.3 Environmental Factors Affecting Mosquito Distribution 58

5.3.1 Rainfall 58

5.3.2 Wind speed 59

5.3.3 Temperature and Distance to the Nearest Settlement 60

5.4 Transmission Efficacy of Malaria 61

5.4.1 Malaria Vector 61

5.4.2 Biting time of *Anopheles balabacensis* 62

5.4.3 Infectivity of *Anopheles balabacensis* 63

5.5 Limitations of study 65

CHAPTER 6 CONCLUSION 66

REFERENCES 68

APPENDIX 77

LIST OF FIGURES

		Page
Figure 2.1:	Morphology of adult mosquito	6
Figure 2.2:	Head of Culicine and Anopheline mosquitoes	7
Figure 2.3:	Resting posture of Anopheline and Culicine mosquitoes	9
Figure 2.4:	Life cycle of *Plasmodium* parasite	16
Figure 2.5:	The global distribution of malaria from 1900-2002	21
Figure 3.1:	Location of Kudat in Sabah, Borneo Island	26
Figure 3.2:	Drone map of sampling site	27
Figure 4.1:	Percentage coverage of land uses and land covers in study area	38
Figure 4.2:	The abundance of *Anopheles* spp. caught from Feb'15-Jan'16	39
Figure 4.3:	Man-biting/man/night of *An. balabacensis* in Feb'15-Jan'16	41
Figure 4.4:	Plot of the biting time of *An. balabacensis* caught from 1800-2400h per night per person	42
Figure 4.5:	Pairplot of the number of *An. balabacensis* caught and other environmental factors: distance, wind speed, temperature	44
Figure 4.6:	Plot of *An. balabacensis* caught from Jun'15-Jan'16 against distance from the nearest settlement	44
Figure 4.7:	Plot of *An. balabacensis* caught from Jun'15-Jan'16 against the ambient temperature	46
Figure 4.8:	Plot of *An. balabacensis* caught from Jun'15-Jan'16 against wind speed	46
Figure 4.9:	Monthly rainfall (mm) and *An. balabacensis* in fixed sites in a lagged time of one month	47

LIST OF TABLES

		Page
Table 2.1:	Classification of Culicidae	10
Table 2.2:	Distribution of the Major Mosquito-borne Diseases	14
Table 2.3:	Features of human malaria	19
Table 2.4:	Principles of comprehensive malaria control	23
Table 3.1:	Categories of each LULC are listed as followed	28
Table 4.1:	Correlation between different variables and *An. balabacensis*	46
Table 4.2:	Correlation between monthly rainfall (mm) and *An. balabacensis* in a lagged time of one month	47
Table 4.3:	Results of generalized linear mixed models tested with various models	49
Table 4.4:	Comparison of *An. balabacensis* collected in different LULC	49
Table 4.5:	Parous rate of *An. balabacensis* in Jun'15 – Jan'16	51
Table 4.6:	Infection of *An. balabacensis* by *Plasmodium* spp. caught in Jun'15-Jan'16	51
Table 4.7:	Sporozoite & oocyst rate, entomological inoculation rate, parous rate, life expectancy and vectorial capacity of *An. balabacensis* during Jun'15 – Jan'16	53

LIST OF PHOTO

		Page
Photo 3.1:	Human landing catch performed by two volunteers	30

LIST OF ABBREVIATIONS

AIC	-	Akaike information criterion
ANOVA	-	Analysis of variance
CL	-	clearing
CO	-	coconut plantation
EIR	-	entomological inoculation rate
FO	-	forest
GLMM	-	generalised linear mixed model
HLC	-	human landing catch
hrs	-	hours
kpg.	-	village (="kampung")
LULC	-	Land use and land cover
IRS	-	indoor residue spray
ITN	-	insecticide-treated net
Ma	-	Man-biting Rate
MOH	-	Ministry of Health
OP	-	oil palm plantation
RU	-	rubber plantation
SE	-	settlement
SR	-	sporozoite rate
spp.	-	species
UAV	-	unmanned aerial vehicle
WHO	-	World Health Organisation

LIST OF APPENDICES

		Page
Appendix I	National Institutes of Health Approval for Conducting Research	77
Appendix II	*Borang Persetujuan Bermaklumat*	79
Appendix III	GLMM Analysis	82
Appendix IV	Analysis of Kendall's Rank Correlation	85
Appendix V	Microscopic View of Mosquito Salivary Gland and Midgut	86

CHAPTER 1

INTRODUCTION

1.1 Background

Malaria is a global infectious disease that is caused by parasitic protozoa of the genus *Plasmodium*, family Plasmodiidae. An estimated 3.2 billion of people worldwide remain at risk of malaria (WHO, 2015). In 2015, malaria killed about 438,000 individuals of all ages. Of these, 306,000 were children under five year old and almost 838 children died every day, even though the number of malaria cases globally has declined about 18%, from an estimated 262 million in 2000 to 214 million in 2015 (WHO, 2015). The Millennium Development Goal 6 of United Nations was achieved which is to halt global malaria cases in 2015 and begin to reverse the incidence of malaria (UN Millennium Project, 2005). But the war is far from over as millions of people in South Africa are still out of the servicing boundaries to prevent and treat malaria (WHO, 2015).

Human malarial protozoa are transmitted by mosquitoes of the genus *Anopheles*, which includes 472 formally recognised species and more than 50 unnamed members of species complex (Harbach, 2013). Of these about 20% or less are considered as vectors of malaria transmission (Gilles, 2002). The vector mosquito species varies with geographical regions (Harbach, 2013): In the Afrotropical Region, the most important malaria vectors include *Anopheles arabiensis* Patton, *An. funestus* Giles, and *An. gambiae* Giles. In the Australasian Region, there are *An. farauti* Laveran and *An. punctualatus* Donitz complexes. In the Middle East and Indian Subcontinent, there are *An. sergentii* Theobald and *An. stephensi* Liston. In the

Oriental Region, there are *An. balabacensis* Baisas, *An. latens* Sallum and Peyton, and *An. dirus* Peyton and Harrison which are members of the Leucosphyrus Group.

The global trend shows mosquito distribution increases towards tropics or equator (Foley *et al.*, 2007). In the Oriental Region, countries of Southeast Asia, such as Malaysia, Indonesia, and Thailand have the highest number of recorded mosquito species. Among them, Malaysia has the highest densities of total species and endemic species (Foley *et al.*, 2007). The high humidity and temperature contribute to the growth of mosquito population and thus the transmission of malaria.

Malaria is a major public health problem in Malaysia since 1900's. In 1967, before the launching of Malaria Eradication Programme, the number of cases was estimated 300,000 in Peninsular Malaysia, 100,000 in Sabah and 1,500 in Sarawak (MOH, 2015; Rahman *et al.* , 1997). In 1991, the total number of reported cases had greatly decreased to 50,500 (MOH, 2013). A decade later the reported cases fell to 12,705. In 2012, the reported cases had been reduced to 4,725. Of these, 1,097 cases in Peninsular Malaysia, 1,571 cases in Sarawak and 2,052 cases in Sabah (MOH, 2013).

In 2004, a zoonotic malaria caused by *Plasmodium knowlesi* was identified by Singh *et al.* (2004). This malaria is a simian malaria and the natural host is macaque (principally long-tailed macaque, *Macaca fascicularis*). Previously, it was observed that it is not likely to infect human because its natural vector, *Anopheles hacker* Edwards is zoophilous which only feeds on animals (Warren and Wharton, 1963). After several investigation were done in different areas of Southeast Asia, e.g. in Thailand (Jongwutiwes *et al.,* 2004), *P. knowlesi* has alarmed the health authorities of the world. As there are already four human malarias, *P. falciparum, P. vivax, P. ovalae,* and *P. malariae, P. knowlesi* was declared as the fifth human malaria by White (2008).

Unlike the other human malaria, the *P. knowlesi* malaria produces high parasite counts in blood quickly and thus likely to cause fatality (Bartoloni and Zammarchi, 2012; Cox-Singh *et al.* , 2008). Besides it can be misdiagnosed by microscopy as *P. malariae* malaria due to similar morphology (Singh *et al.*, 2004; Lee *et al.*, 2009), which indicates the failure of microscopic identification for *P. knowlesi* (Barber *et al.* , 2013). Misdiagnosis could be very dangerous to patients from the medical aspect as the *P. malariae* is a mild and non-fatal malaria. With the aid of molecular identification, many cases reported as *P. malariae* were actually *P. knowlesi* (Cox-Singh *et al.*, 2008). However, the *P. knowlesi* notifications have shown increasing in recent year (William *et al.*, 2013) due to the higher rate of transmission by vectors, probably as a result of the changing landscape due to deforestation (Fornace *et al.*, 2016).

Prevention is always the priority rather than the treatment. Vector controls have been carried out as a frontline prevention by breaking the malaria cycle. In 2011, National Strategic Plan for Elimination of Malaria was launched by Malaysia government with a target of "malaria free" status by 2020 (MOH, 2013). To achieve this target, vector control was one of the most important components. The existing front line vector control measures had successfully reduced the malaria cases but these intervention were insufficient to eliminate the transmission (Ferguson *et al.*, 2010).

The rate of malaria transmission depends on the vectorial capacity, longevity, and sporozoite rate of the mosquito (Snow and Gilles, 2002). Application of control method should vary with species because of the variation in behaviour (Ferguson *et al.* , 2010). Insecticide-treated nets (ITNs) and indoor residue spray (IRS) are effective in controlling the indoor-resting or indoor-biting vectors but not the outdoor biting mosquitoes. For example, *Anopheles balabacensis* and *An. minimus* Theobald are highly exophilic and exophagic species that are difficult to be controlled by using IRS (Zeitschrift *et al.* , 2014). Thus, the knowledge of vector ecology is critical for integrated vector control in order to achieve national plan of malaria eradication by 2020.

1.2 Significance of study

Deforestation and associated environmental changes have been blamed as one of the key drivers in the malaria epidemiology in Kudat (Fornace *et al.*, 2016). The changes of land use are favourable to the development of mosquito larvae (Munga *et al.*, 2006; Srivastava *et al.*, 2013). However, there is lack of entomological data support this hypothesis. Previous researches on mosquito bionomics in Malaysia largely focused on larval bionomics and little was done on adult distribution due to the limited resources such as manpower and finance. This study is thus important as a preliminary study focusing on adult *Anopheles* distribution in different habitats by using random sampling method.

The objective of study is to provide essential data for the malaria transmission dynamics and vector bionomics in response to different land use and land cover (LULC). The data of vectorial spatio-temporal distribution is critical to show the relationship between environment and health. The data can be also used for computer modelling to predict the mosquito population.

1.3 Objectives

This study aims to collect distribution data of *Anopheles* mosquitoes and use it to estimate the risk of *Plasmodium* malaria exposure in different land use and land cover (LULC). The specific objectives of study are as follows:

- **a.** To study the spatio-temporal distribution of *Anopheles* mosquitoes in different land use and land cover (LULC) types.
- **b.** To determine the proportion of *Anopheles* positive with *Plasmodium.*
- **c.** To investigate the longevity and parous rate of *Anopheles balabacensis*.

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of Mosquitoes

2.1.1 Morphology

Mosquitoes are slender, long-legged flies with a clear, long scaled mouth-form proboscis projecting forwards (Figure 2.1). The head, thorax and abdomen are covered with scales and setae. The legs, hind wing margins and wing veins are also clothed with scales and some with patterns. Clothed scales can be shining to give mosquito physical coloration under different light source. The setae are always different in length, shape and coloration which are genus and species-specific taxonomy important (Becker *et al.*, 2010).

Crane flies (Family Tipulidae), *Culicoides* biting midges (Family Ceratopogonidae) and sandflies (Family: Phlebotominae) are always wrongly recognised as mosquitoes with naked eye (Becker *et al.*, 2010). Crane flies have similar mouth-form proboscis and similar in size but its wings are scaleless and wing patterns are different. Sandflies are smaller than most mosquitoes, its proboscis is shorter and the wings are hairy instead of scaleley. Biting midges are even smaller than sandfly. Its proboscis and legs are shorter compared to mosquitoes.

Mosquitoes attain sexual dimorphism as female and male are having different characteristics beyond the difference of sexual organ (Becker *et al.*, 2010). Body size of male tends to be larger than female (Becker *et al.*, 2010). The male has plumose antennae and long, hair maxillary palps (Figure 2.2). The first 12 flagellomeres of male antenna bear longer and denser setae than that of female (Becker *et al.*, 2010). Culicine male has long maxillary palpi which are almost the same length with its proboscis. Whereas the female has significant short maxillary palpi (Figure 2.2). Both *Anopheles* male and female have long maxillary palpi which are almost same length with its proboscis. Setae on male maxillary palpi are denser than that of female (Figure 2.2).

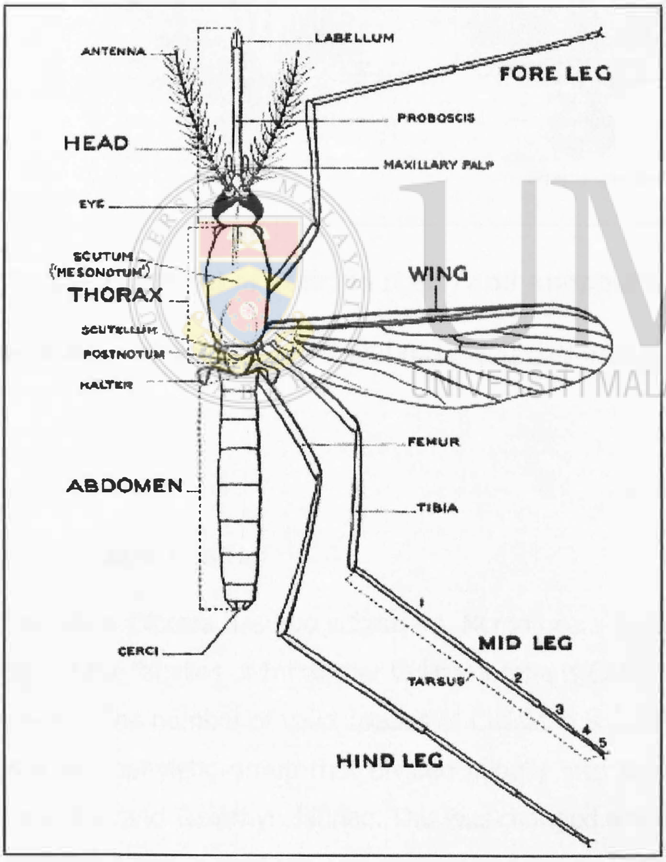

Figure 2.1: Morphology of adult mosquito

Source : Reid, 1968

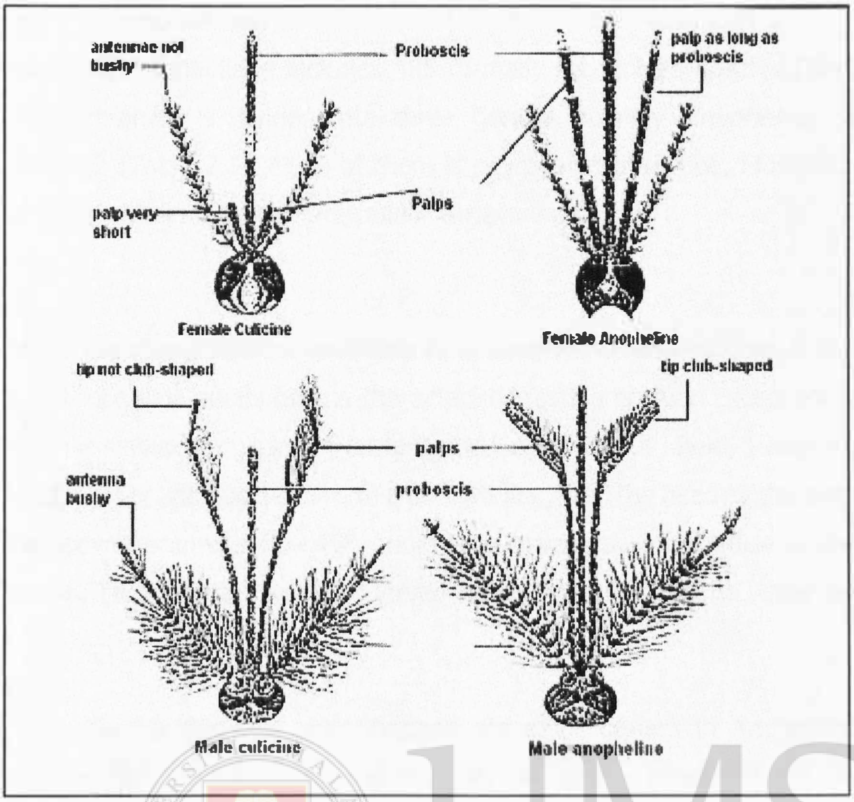

Figure 2.2: Head of Culicine (Left) and Anopheline (Right) mosquitoes.

Source : http://wikieducator.org/Lesson_1:Introduction_To_Malaria

2.1.2 Classification

The order Diptera has two suborders, Nematocera and Brachycera. In Nematocera, one of the families of Infraorder Culicomorpha is Culicidae, to which the mosquitoes belong. The number of valid species of Culicidae is 3,549 (Harbach, 2013). Culicidae is a monophyletic group that divided initially into three subfamilies: Anophelinae, Culicidae, and Toxorhynchitinae. This was changed when Harbach and Kitching (1998) suggested maintaining the Anophelinae and Culicinae as subfamilies but lowered the Toxorhynchitinae to tribal rank within the Culicinae (Table 2.1).

(a) Anophelinae

Subfamily Anophelinae includes 485 formally recognised species (Harbach, 2013). This subfamily is divided into three genera, namely *Anopheles*, *Bironella*, and *Chagasia* (Table 2.1). None of them is segregated into tribe. Mosquitoes belonging to subfamily Anophelinae are called "anophelines".

One of the characteristics to differentiate anophelines and culicines is shown in Figure 2.3. Anopheline adults have a characteristic resting position except for the *Chagasia*, which is similar to culicines (Harbach and Howard, 2009; Reid, 1968). The proboscis, head, thorax and abdomen form a straight line, with the head as the point on surface, the body is inclined at 30 - 45°. Anopheline larvae have no spiracle tube or respiratory siphon. They usually can be observed resting in parallel to the water surface.

Genus *Bironella* and *Chagasia* are small genera in Anophelinae. *Bironella* includes eight species and *Chagasia* with five species (Harbach and Howard, 2009; Harbach, 2013). The former is confined to New Guinea and the latter to tropical America (Reid, 1968). *Bironella* and *Chagasia* are not known to transmit pathogen to human (Harbach, 2013).

Genus *Anopheles* is the largest genus in Anophelinae which includes 472 valid species. It is further divided into eight subgenera: *Anopheles* (183 species), *Baimaia* (1), *Cellia* (224), *Christya* (2), *Kerteszia* (12), *Lophopodomyia* (6), *Nyssorhynchus* (35) and *Stethomyia* (5). Of these, *Anopheles, Cellia* and *Nyssorhynchus* are further segregated into Sections, Series, and Groups (Harbach, 2013). *Anopheles* is the only genus to transmit human malaria.

(b) Culicinae

Subfamily Culicinae includes 3,064 formally recognised species (Harbach, 2013). This subfamily has 109 genera segregated into 11 tribes, namely Aedeomyiini, Aedini, Culicini, Culisetini, Ficalbiini, Hodgesiini, Mansoniini, Orthopodomyiini, Sabethini, Toxorhynchitini, and Uranotaeniini (Table 2.1). Mosquitoes belong to this subfamily are called "culicines".

Culicine adults can be differentiated by their resting position. They rest with the body parallel on the surface, unlike the inclined body of anophelines. Culicine larvae have a spiracle tube or siphon. They usually can be observed resting in slanting position on the water surface.

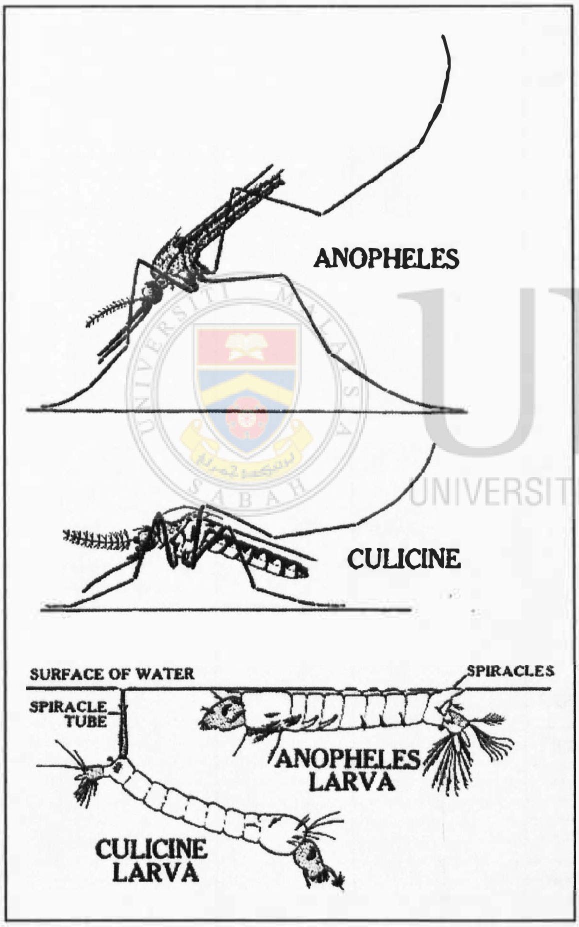

Figure 2.3: Resting posture of Anopheline and Culicine mosquitoes.

Source : Reid, 1968