BIONOMICS OF *ANOPHELES* IN GRIK, HULU PERAK AND INSECTICIDE SUSCEPTIBILITY OF TWO *ANOPHELES* SPECIES FROM TWO LOCATIONS IN MALAYSIA

HO LAI YEE

UNIVERSITI SAINS MALAYSIA

2008

BIONOMICS OF *ANOPHELES* IN GRIK, HULU PERAK AND INSECTICIDE SUSCEPTIBILITY OF TWO *ANOPHELES* SPECIES FROM TWO LOCATIONS IN MALAYSIA

by

HO LAI YEE

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

JUNE 2008

ACKNOWLEDGEMENTS

All praise and glory be unto God Almighty for His blessings in the completion of my research work and thesis. I'm deeply thankful and grateful to my supervisor, Associate Professor Dr Zairi Jaal for his patience and constant guidance and teaching throughout my whole course of research. I would also like to thank Encik Adenan Che Rus for always availing himself to help and share with me his expertise and experience.

Special thanks and gratitude also to Encik Nasir, Pak Yah, Chooi Khim, Firdaus and the staff of Vector Control and Research Unit (VCRU), University Sains Malaysia who endured many long sleepless nights with me for my collections in Kampung Bongor, Grik. Not forgetting to thank also Encik Asmad bin Matusop, Mr. David Lubim, Mr. Litter Umul, Bohari, Main, Shamsuddin, Atien and the rest of the staff of the Entomology Unit of the Sarawak Health Department for helping me in my collections and testing in Kampung Tepin, Balai Ringin.

My gratitude also goes out to Pak Yah, Pak Jang and their respective families for their warm hospitality throughout my stay in Grik. Many thanks also to the Malaysian Meteorological Center for providing me with the necessary rainfall data that is needed for my analysis and writing. I would also like to thank the administration staff of VCRU and the School of Biological Sciences for their generous help.

I also appreciate the friendship and the ample amount of help and advices given to me by all my seniors and course mates in the VCRU lab. I am truly grateful for the financial support given to me that have helped me during the course of my research work and thesis writing. Finally, I would like to extend my love and appreciation for all the prayers, support and encouragement given to me by my family members, friends and loved ones.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

KAJIAN BIONOMIK *ANOPHELES* DI GRIK, HULU PERAK DAN KERENTANAN INSEKTISID BAGI DUA SPESIES *ANOPHELES* DARI DUA LOKASI DI MALAYSIA

ABSTRAK

Suatu penyelidikan telah dijalankan di Kampung Bongor, Grik yang terletak di bahagian timurbarat Hulu Perak, Malaysia untuk mengkaji bionomik populasi *Anopheles sp.* di kawasan tersebut. Dengan menggunakan kaedah CBT (*cow baited trap*), sebanyak 4,497 nyamuk *Anopheles* telah ditangkap sepanjang 14 bulan tempoh persampelan dari Januari 2005 hingga Februari 2006 dan 11 spesies telah dikenalpasti. Daripada kumpulan subgenus *Anopheles* (*Anopheles*) terdapat *An. barbirostris, An. umbrosus, An. nigerrimus* dan *An. separatus* dan dari kumpulan *Anopheles* (*Cellia*) pula terdapat *An. aconitus*, *An. maculatus*, *An. vagus*, *An. philippinensis, An. kochi*, *An. tessellatus* dan *An. subpictus*. Kelimpahan bermusim bagi kebanyakan populasi nyamuk *Anopheles* daripada Kampung Bongor dipengaruhi oleh kadar curahan hujan. Pada amnya, ketumpatan adalah lebih tinggi ketika musim hujan dan lebih rendah ketika musim kemarau. Walau bagaimanapun, kelimpahan bermusim populasi nyamuk *Anopheles* juga dipengaruhi oleh perubahan iklim dan geografi. Kitar gigitan nyamuk *Anopheles* bermula selepas senja dan aktiviti akan menurun sebelum matahari terbit. Puncak activiti biasanya adalah pada waktu tengah malam. Satu kajian juga telah dijalankan di Kampung Tepin, Balai Ringin, di Serian, Sarawak untuk melihat populasi nyamuk *Anopheles* yang terdapat di kawasan tersebut pada bulan Februari 2006. Kaedah HBT (*human baited trap*) dengan penangkapan nyamuk kaedah *human landing-biting catch* telah digunakan dalam persampelan populasi nyamuk *Anopheles* di Kampung Tepin. Sepanjang tempoh persampelan, sebanyak 2,654 *An. letifer* telah dikumpulkan. Pada amnya, aktiviti gigitan *An. letifer* bermula sejam selepas hari senja dan aktiviti mereka

meningkat dengan mendadak dalam masa dua jam. Satu kajian kerentanan insektisid dengan menggunakan kelengkapan ujian WHO dan kertas dengan 0.75 % permethrin telah dijalankan ke atas dua strain spesies *Anopheles* dari dua lokasi berbeza dalam Malaysia untuk melihat status kerentanan insektisid mereka. Keputusan yang diperolehi menunjukkan nilai KT_{50} dan KT_{95} bagi *An. maculatus* dari Kampung Bongor, Grik masing-masing ialah 28.09 minit dan 62.98 minit. *Anopheles letifer* dari Kampung Tepin, Balai Ringin pula memberikan nilai KT_{50} sebanyak 35.09 minit dan KT_{95} sebanyak 73.03 minit. Kedua-dua *An. maculatus* dan *An. letifer* menunjukkan 100 % mortaliti selepas 24 jam.

BIONOMICS OF *ANOPHELES* IN GRIK, HULU PERAK AND INSECTICIDE SUSCEPTIBILITY OF TWO *ANOPHELES* SPECIES FROM TWO LOCATIONS IN MALAYSIA

ABSTRACT

A study was conducted in the rural village of Kampung Bongor, Grik in the north eastern part of Hulu Perak, Malaysia to examine the bionomics of the population of *Anopheles sp.* in that locality. The mosquito collection was done using CBT (cow baited trap) method. A total of 4,497 *Anopheles* mosquitoes were caught during the 14 months collection period from January 2005 to February 2006 and 11 species were identified. They were *An. barbirostris, An. umbrosus, An. nigerrimus* and *An. separatus* from the *Anopheles* (*Cellia*) subgenus group, and *An. aconitus*, *An. maculatus*, *An. philippinensis*, *An. vagus*, *An. kochi*, *An. tessellatus* and *An. subpictus* from the *Anopheles* (*Anopheles*) subgenus group. The seasonal distribution of most of the population of *Anopheles sp.* mosquitoes is partly influenced by the rainfall pattern. The distribution is generally higher during heavy rainfall and lower during the drier months. However, environmental and geographical changes also affect the distribution pattern. Generally the biting rhythm begins after sunset and its activities decrease before sunrise with the peak of activity occurring around midnight. A survey was also carried out in Kampung Tepin, Balai Ringin, in the Serian district in Sarawak to look at the population of *Anopheles* mosquitoes available during the month of February 2006. The HBT (human baited trap) method by human landing-biting catches was used for the collection of mosquitoes in this locality. During the whole collection period, a total of 2,654 *An. letifer* were caught. Generally, the biting activity of *An. letifer* in Kampung Tepin begins an hour after sunset and its activities increases rapidly in two hours time. An insecticide susceptibility test using the WHO test kits and impregnated papers with 0.75 % permethrin was carried out on two

strains of *Anopheles* from two different locations in Malaysia to determine their insecticide resistance status. Results show that *An. maculatus* from Kampung Bongor, Grik recorded KT_{50} and KT_{95} value of 28.09 minutes and 62.98 minutes respectively. *Anopheles letifer* from Kampung Tepin, Balai Ringin recorded KT_{50} and KT_{95} value of 35.09 minutes and 73.03 minutes respectively. Both *An. maculatus* and *An. letifer* showed 100 % mortality after 24 hour holding period.

CHAPTER ONE
GENERAL INTRODUCTION

1.0 Introduction

The genus *Anopheles* is from the subfamily Anophelinae belonging to the family Culicidae under the order Diptera. Under this genera, there are six subgenera which are divided into either New World form; namely the *Stethomyia* Theobald, 1902; *Kerteszia* Theobald, 1905; and *Nyssorhynchus* Blanchard, 1902; or Old World form; which are the *Lophopodomyia* Antunes, 1937; *Anopheles* Meigen, 1818 and Meigen, 1918; and *Cellia* Theobald, 1902 (Reid, 1968).

The adult *Anopheles* mosquitoes are easily recognizable from the pale white and dark markings on their wings and by its 45º angle resting position from the surface. The *Anopheles* larvae can be distinguished from the other Culicine larvae by their lack of siphon and their horizontal resting position on the water surface (Abu Hassan & Yap, 2003).

The *Anopheles* mosquitoes can be found in almost all regions of the world from as far north as latitudes 65º N in places in Alaska, Scotland, Sweden and Siberia to as far south as latitudes 45º S in Argentina and Tasmania, excluding New Zealand and the Pacific Islands (Reid, 1968).

Malaria is a well known parasitic disease transmitted by the *Anopheles* mosquitoes. However, there are some *Anopheles* species which serve as vectors of other disease agents as well, such as the O'nyong nyong virus and the filarial parasites *Wuchereria bancrofti* and *Brugia malayi*.

During the years 1959 to 1962, there was a major epidemic of a non-life threatening but extremely painful disease called the O'nyong nyong (ONN) fever. This disease which was first discovered in the northern part of Uganda is caused by a virus of the same name and is transmitted by the *Anopheles* mosquitoes.

The name O'nyong nyong originates from the African language which means 'joint-breaker' (Service, 1996). As the name goes, the affected persons suffer from extreme pain of the joints (arthralgia), fever and rashes. There were records of recurrence of the disease in the south western Uganda in 1996 to 1997 (Kiwanuka *et al*., 1999).

Besides being vectors of O'nyong nyong, *Anopheles* mosquitoes such as *An. aconitus*, *An. letifer* and *An. balabacensis* are also known to transmit human lymphatic filariasis (nocturnal periodic) which is caused by the parasitic filarial worms *Brugia malayi*, *B. timori* and *Wuchereria bancrofti* (Service, 1996).

Human lymphatic filariasis is endemic particularly in Africa (Figure 1.1) and it is thought that there are over 120 million people worldwide who are infected with lymphatic filariasis (TDR, 2005). The lymphatic system is damaged when these parasitic worms get lodged in the lymphatic nodes and vessel; thus causing painful and disfiguring swelling of the limbs, breasts or genitals. This swelling condition is also known as elephantiasis (TDR, 2005).

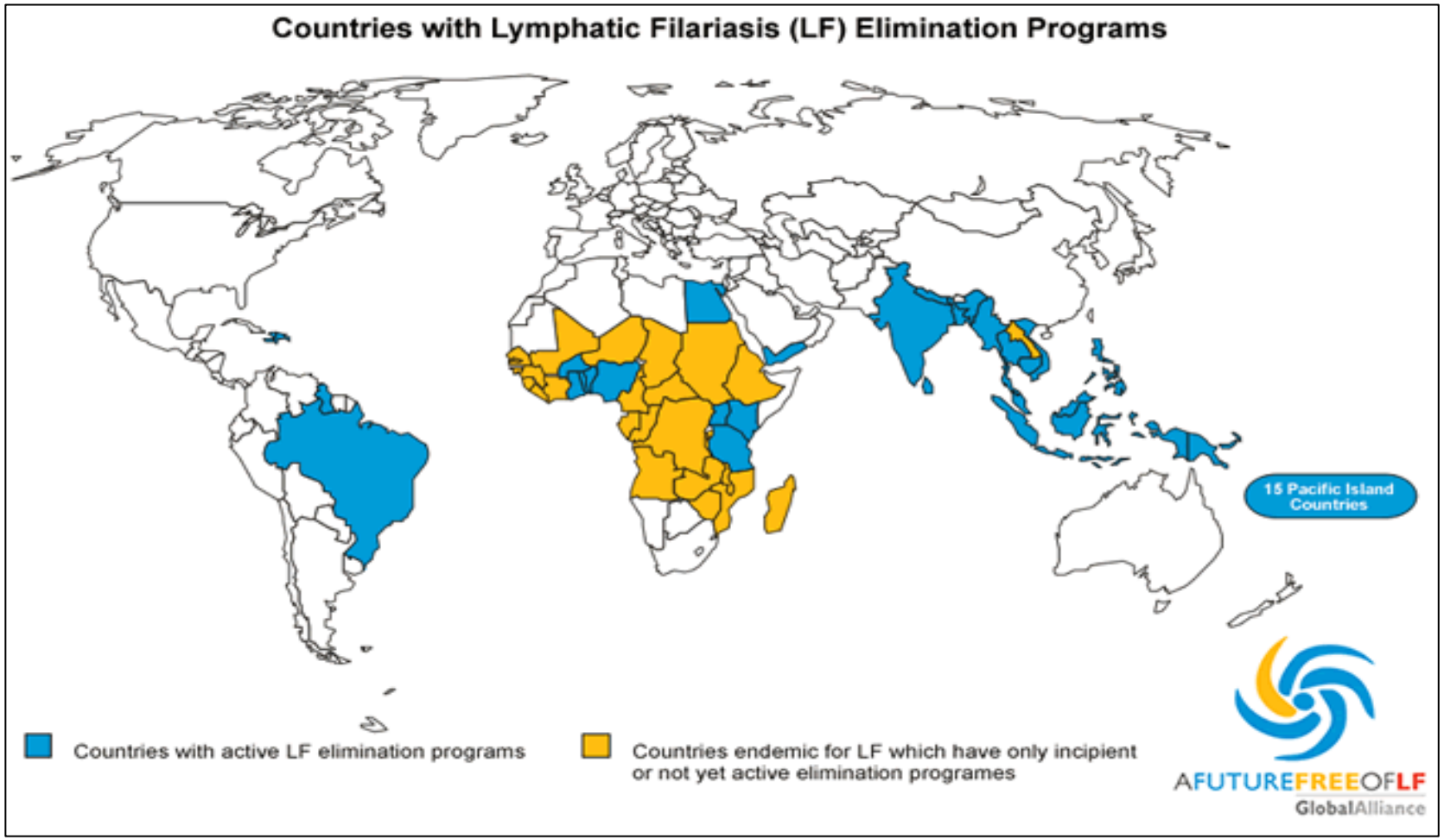


Figure 1.1 Map of Lymphatic Filariasis Endemic Countries
(Taken from Global Alliance, 2007)

Though rarely a life threatening disease, lymphatic filariasis causes chronic suffering, disfiguring and disabilities; incapacitating the affected persons from having a normal working and social life (TDR, 2005).

But the most dangerous of all diseases which is carried by the *Anopheles* mosquitoes, is malaria. According to Michael Finkel (2007) in his article in the National Geographic, the name malaria comes from the Italian word *mal'aria*, meaning 'bad air'; as it was a common belief that swamp fumes had produced the illness which plagued Rome for centuries.

Anopheles mosquitoes are the main vectors that transmit malaria which is a disease caused by the infections of the protozoan parasites *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale*. This disease is endemic to 106 nations. Having over two billion people at risk of infection and with at least one million deaths each year, malaria is one of the most important tropical diseases in the world (TDR, 2005).

Being prominent vectors of malaria, the *Anopheles* mosquitoes are a great threat especially to the malarious prone countries. These mosquitoes are widely distributed around the world. Although there are more than 400 species of *Anopheles*, only around 40 of these are important vectors of malaria (Service, 1996).

The main *Anopheles* vectors of malaria disease differ according to the geographic regions (Figure 1.2). The principal malaria vectors include *An. funestus* and *An. gambiae* in tropical Africa; *An. culicifacies, An. minimus, An. annularis, An. dirus, An. fluviatilis, An. maculipennis, An. sacharovi, An. superpictus,* and *An. farauti* in

Asia; and *An. albimanus* (Central America) and *An. darlingi* (Amazon Basin) in The Americas (WHO, 2005). Some of the principal malaria vectors of Southeast Asia not mentioned are *An. aconitus*, *An. sundaicus* and *An. koliensis* which are found in Indonesia, (WHO, 1998a) and *An. maculatus*, *An. balabacensis* and *An. leucosphyrus* in Malaysia (Sulaiman, 2000).

Understanding the bionomics of *Anopheles* mosquitoes is important to help understand how they become the effective vectors of the diseases that they transmit. The data and information gathered can help better understand the prevalence of these mosquitoes, its biting behaviour, ability to carry and transmit diseases, and susceptibility to insecticides. These observations are necessary to assist in designing the appropriate control measures.

Bionomics is defined as a study of the relation between an organism and its environment. When studying the bionomics of a mosquito species, its biology and behaviour such as growth and development of the immature stages, the biting rhythm, oviposition sites and seasonal abundance are observed (WHO, 1975a). The study of bionomics provides essential information needed especially for vectors of diseases.

Changes in the environment may have direct or indirect impact on the behaviour and bionomics of mosquitoes (De Las Llagas, 1985). As such, ongoing entomological surveillance, continued improvements and implementations of control programmes are important in managing the spread of the diseases transmitted by *Anopheles* mosquitoes.

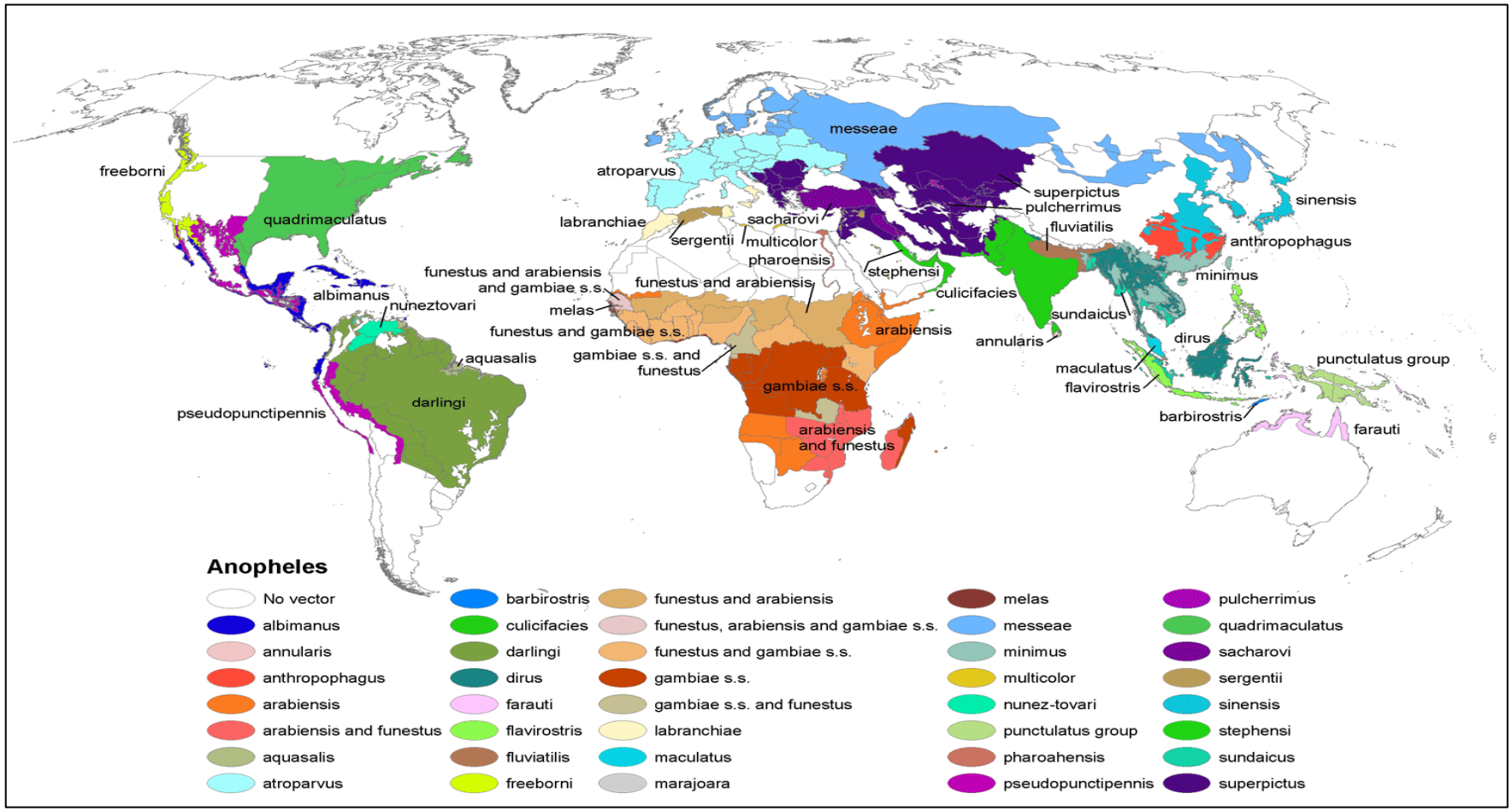


Figure 1.2 Global Distributions (Robinson Projection) of Dominant or Potentially Important Malaria Vectors
(Taken from Kiszewski *et al.*, 2004)

1.1 Research Objectives

i.) To look into and examine the changes in the current bionomics of the population of *Anopheles* mosquitoes in Grik, Hulu Perak in relation to human activities

ii.) To examine and identify the population of *Anopheles* mosquitoes in Balai Ringin, Serian, Sarawak

iii.) To test and determine the insecticide susceptibility status of *An. maculatus* from Grik, Hulu Perak and *An. letifer* from Balai Ringin, Serian using the WHO test kits

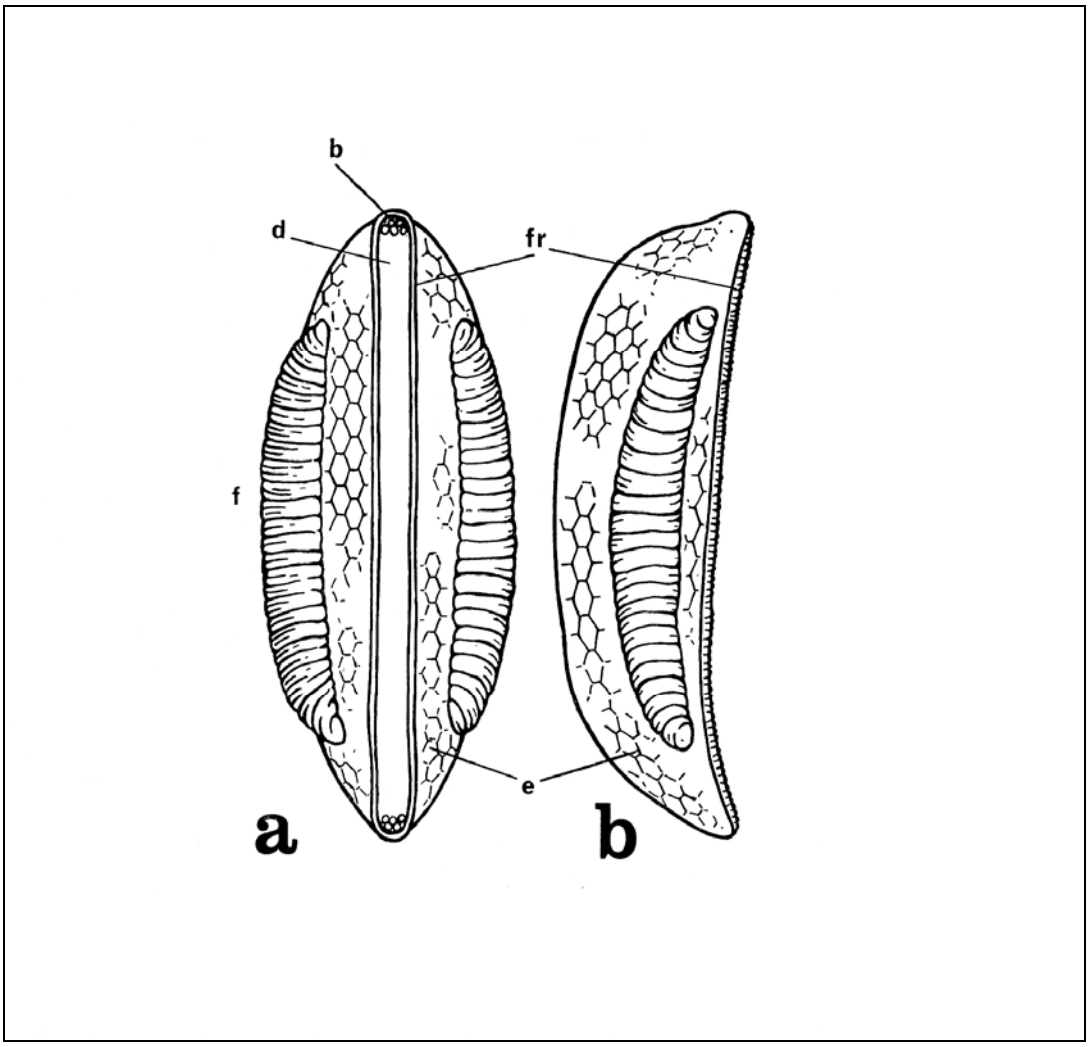
CHAPTER TWO
LITERATURE REVIEW

2.1 The Life Cycle of *Anopheles* Mosquitoes

2.1.1 Oviposition and Biology of the Eggs

After mating and obtaining blood meals, the gravid female *Anopheles* mosquito will look for a suitable water habitat as their oviposition site. The average female can lay some 50 – 150 eggs at one time (Abu Hassan & Yap, 2003). The *Anopheles* eggs are laid singly on the water surface. Each egg is provided with a pair of lateral air-filled floats on either side which keeps it floating on the water surface (Figure 2.1).

They are brown or blackish in colour and are boat-shaped, measuring around 0.4 – 0.6 mm in length depending on the species (Sulaiman, 2000). Unlike the *Aedes* eggs, the *Anopheles* eggs cannot withstand desiccation. These *Anopheles* eggs usually hatch within two to three days in tropical countries, but in colder temperate climates, the egg hatching only occurs after about two to three weeks depending on the temperature (Service, 1996).



Label	Detail
a	Dorsal View
b	Lateral View
b	Bosses
d	Deck
e	Exochorion (With Polygonal Pattern)
f	Float
fr	Frill

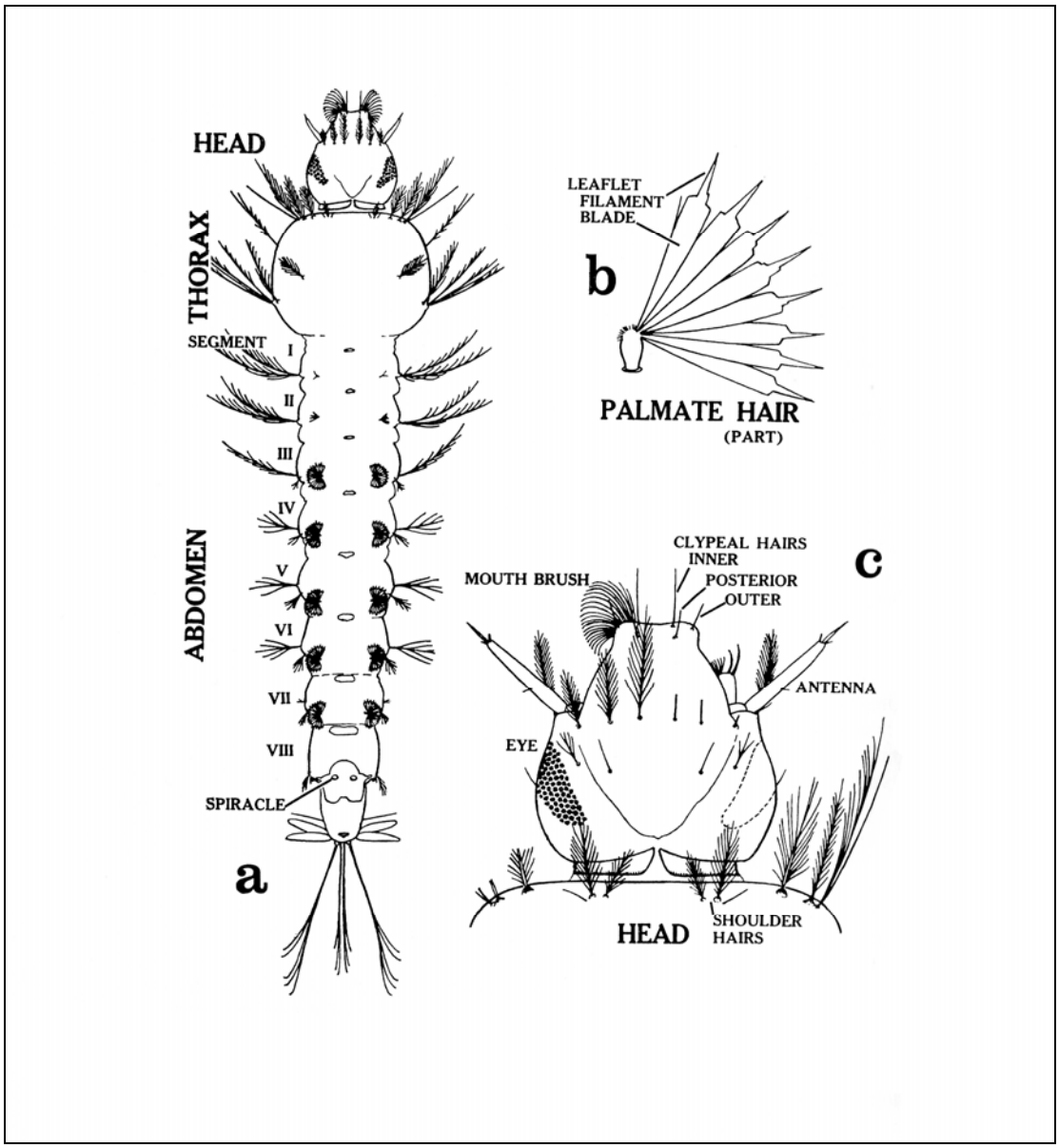
Figure 2.1 Egg of *Anopheles* (*Myzorhynchus* series, subgenus *Anopheles*); Anterior is at the Top of the Figure
(Taken from Reid, 1968)

2.1.2 Larval Stage

2.1.2.1 External Morphology

As in all mosquito larvae, the *Anopheles* larvae have a well developed dark brown or blackish sclerotized head; which bears a pair of antennae, a pair of compound eyes and prominent mouthbrushes which serve to sweep water containing minute food particles into the mouth (Figure 2.2). The thorax is roundish with numerous simple and branched hairs and a pair of thoracic palmate hairs (Service, 1996).

Out of the nine-segmented abdomen of the *Anopheles* larvae, the first six or seven of the segments usually have a pair of palmate hairs situated at the dorsal part of the abdomen (Abu Hassan & Yap, 2003). The palmate hairs serve to maintain the larvae in a horizontal position at the water surface (Figure 2.3). The first eight (8 + 9) abdominal tergites of the *Anopheles* larvae also have tergal plates on the dorsal surface. These tergal plates are small, sclerotized light or dark brown structures which vary in shapes and sizes according to species (Service, 1996).



Label	Detail
a	Whole Larva from Above
b	Part of an Abdominal Palmate Hair
c	Head and Front of Thorax, Mouth Brush on Right Side Omitted

Figure 2.2 General Appearance of the *Anopheles* Larvae (subgenus *Cellia*)
(Taken from Reid, 1968)

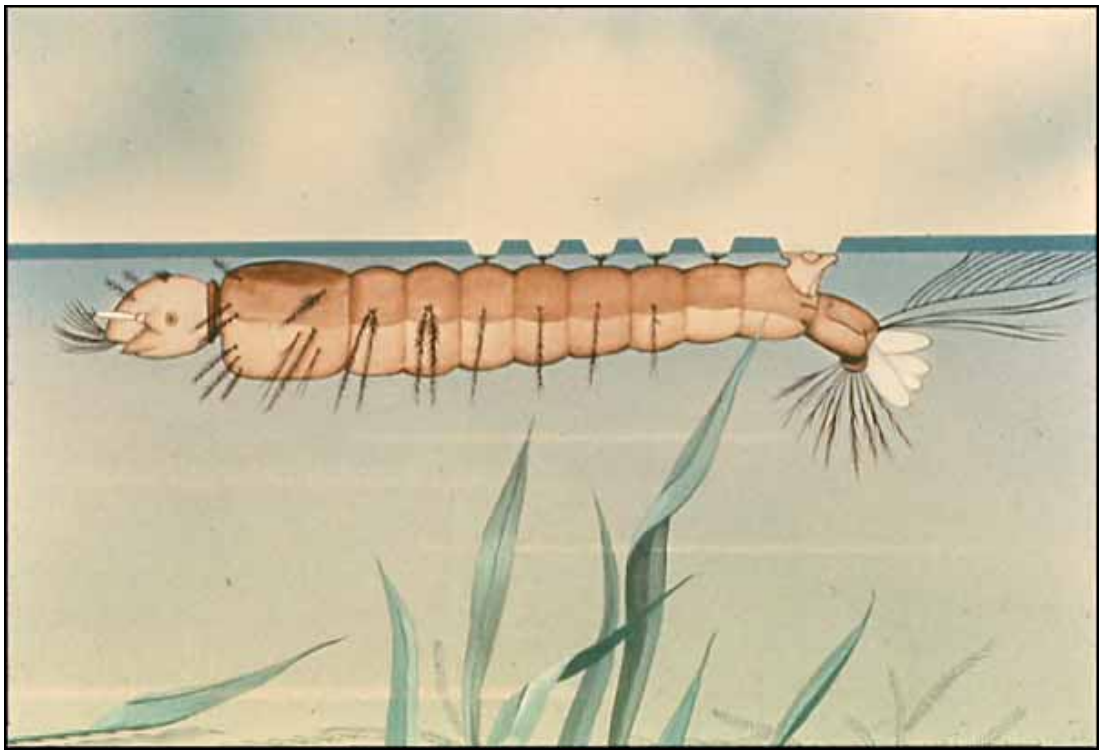


Figure 2.3 Characteristic Horizontal Position of the *Anopheles* Larvae
(Taken from Centennial Museum, 2006)

As in all mosquitoes, the *Anopheles* larvae go through four larval instar stages during their larval development phase. In tropical countries, the larval duration is usually about seven to eight days, but in cooler climates the larval duration is about two to four weeks (Service, 1996). The *Anopheles* larvae are filter-feeders, remaining at the water surface unless disturbed, rotating their heads through 180º so that their ventrally positioned mouthbrushes can sweep the underside of the water surface. They usually feed on yeasts, bacteria, protozoa, rotifers and numerous other plant and animal microorganisms (Abu Hassan & Yap, 2003).

2.1.2.2 Habitat and Distribution

The *Anopheles* larvae can be found in various types of habitats; from large or small to permanent or temporary collections of water (Sulaiman, 2000). Their larval habitats range from fresh and saltwater marshes to mangrove swamps, grassy ditches, ricefields, edges of streams and rivers, burrow pits, puddles, hoof prints and even in a variety of natural water containing habitats such as water filled treeholes in some species. In the Neotropical regions of Central and South America and the West Indies, few *Anopheles* larvae are found breeding in water collections in leaf axils of epiphytic plants growing on tree branches such as bromeliads (Service, 1996).

According to Sandosham (1984), the distribution of *Anopheles* mosquitoes, especially the vectors of malaria disease, differ according to different ecological zones. Malaysia, a country which is situated on the Equatorial zone with a constant high temperature and humidity, provides a suitable environment for the breeding of *Anopheles* mosquitoes and transmission of malaria (Sandosham, 1970). Its ecological distribution can be classified into three main zones; which are the brackish water zone, the coastal plain zone and the hills and mountains zone (Figure 2.4).

Under the brackish water zone, the untouched mangrove swamps along the coast are unsuitable for *Anopheles* larvae. However, when the mangrove is cleared and the tidal waters are allowed to come in contact with collections of fresh water exposed to sunlight, larvae of *An. sundaicus* Rodenwalt, 1925 and *An. subpictus* Grassi, 1899 will be found breeding abundantly.

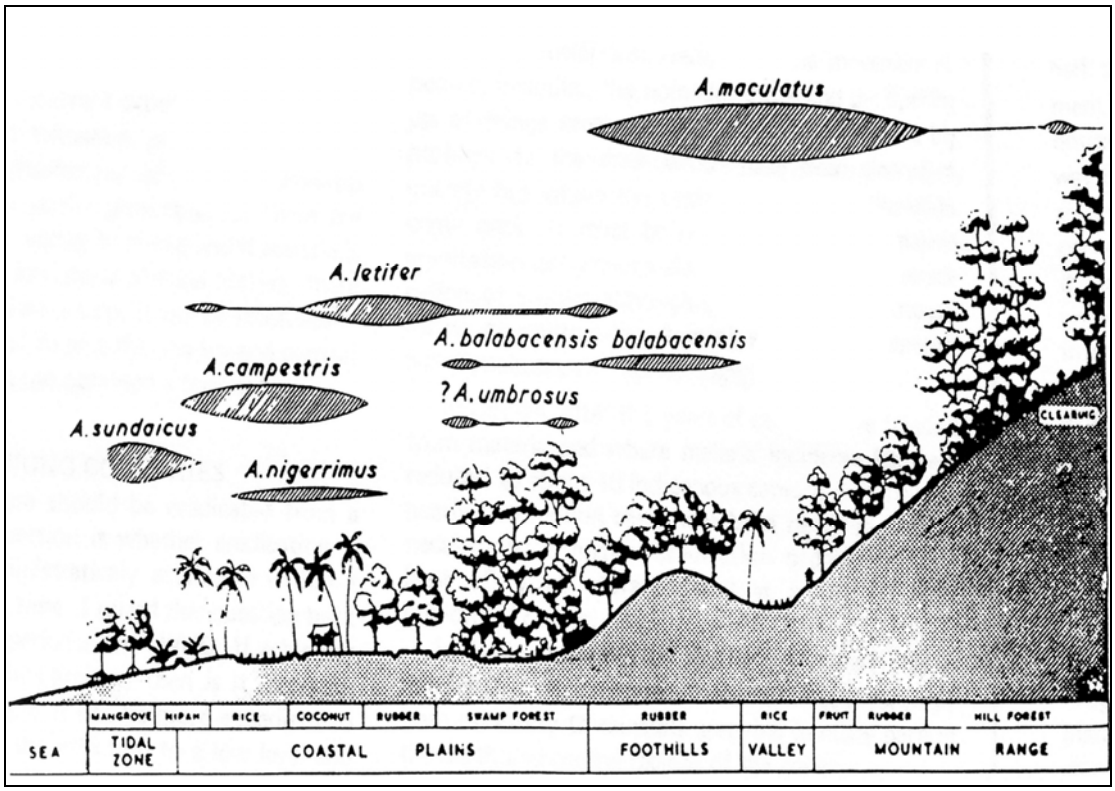


Figure 2.4 Ecological Distribution Zones
(Taken from Sandosham, 1984)

The coastal plain zone which extends from the tidal zone to the foothills is breeding grounds for *An. umbrosus* Theobald, 1903. There are also *An. balabacensis balabacensis* Baisas, 1936 found breeding in swamps and hill forests in the north eastern part of Peninsular Malaysia. Open swamps or rice fields from cleared areas of the coastal plains are breeding grounds for *An. campestris* Reid, 1962 and *An. nigerrimus* Giles, 1900. *Anopheles barbirostris* Van der Wulp, 1884 larvae have also been found in these types of habitats such as marshes and swamps (Rattanarithikul *et al*., 1994).

Larvae of *An. aconitus* Dönitz, 1902 and *An. philippinensis* Ludlow, 1902 are typically found along the grassy edges of rice fields (Reid, 1968). If the area is cleared to build a village or to cultivate coconut or rubber trees, then *An. letifer* Sandosham, 1944 and *An. separatus* Leicester, 1908 will be the prominent breeders. If the area is close to cleared hill forests, then it will be a breeding ground for *An. maculatus* Theobald, 1901.

The hills and mountains zone which forms the backbone of Peninsular Malaysia is usually sparsely populated by the nomadic aboriginals and jungle tribes. Mostly found breeding are members of the *An. leucosphyrus* Dönitz, 1901 group and the *An. umbrosus* group. However, *An. maculatus* usually breeds readily and forms establishments along the streams and seepages in hill slope plantations or clearings prepared by the Aborigines for their shifting cultivation (Sandosham, 1984).

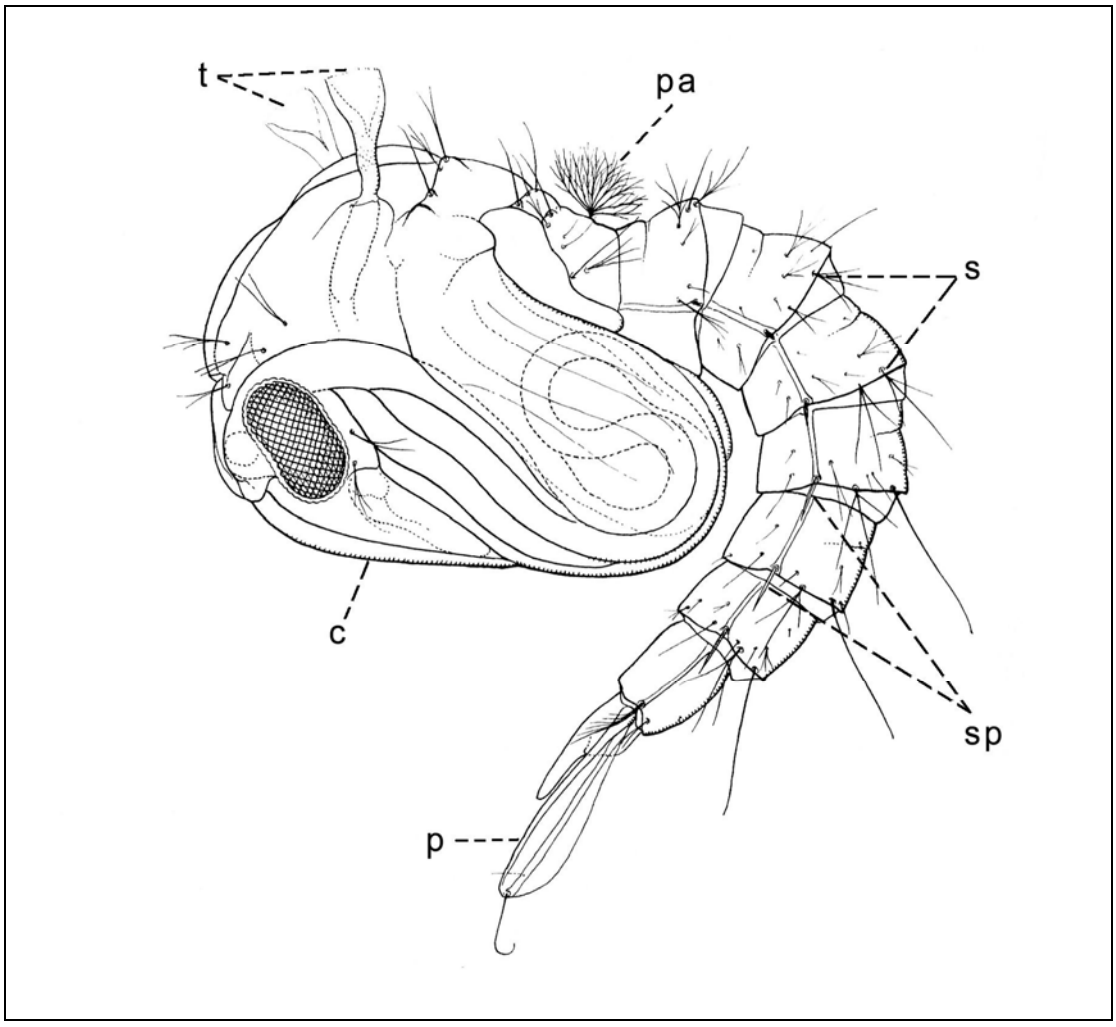
Besides natural environments, some species of *Anopheles* larvae are also found in man-made water containing habitats. Reid (1968) reported finding the larvae of *An. tessellatus* Theobald, 1901 in wells; *An. kochi* Dönitz, 1901 larvae in small muddy collections of water such as ruts, hoof marks, buffalo wallows, and pools in trampled rice fields just after the harvest; and *An. vagus* Dönitz, 1902 larvae in open muddy pools and in hoof marks and ditches. Generally *Anopheles* larvae prefer clean and unpolluted water (Abu Hassan & Yap, 2003), and are usually absent from habitats containing decaying plants or faeces (Service, 1996).

2.1.3 Pupal Stage

As in all mosquito pupae, the comma-shaped *Anopheles* pupal stage is the third and final aquatic phase of the life cycle (Figure 2.5). The head and thorax are combined to form the cephalothorax, and dorsally situated is a pair of short, cone-like and trumpet-shaped breathing tubes with distally broad openings (Sulaiman, 2000). Positioned laterally at the cephalothorax, just below these respiratory trumpets is a pair of palmate hairs which aids the pupae to remain floating at the water surface.

The pupae have 10-segmented abdomen though only eight segments are visible, and each segment has numerous short hairs termed setae, with the last segment terminates into a pair of oval and flattened paddles. The most distinguishing characteristic of the *Anopheles* pupae is the presence of distinct, short peg-like spines on the second or third to the seventh abdominal segments (Service, 1996).

The pupae do not feed but normally spend most of their time floating at the water surface and breathing in air through the respiratory trumpets. If they are disturbed, they will swim vigorously up and down in a jerky manner to the bottom. In tropical countries, the pupation period is about two to three days but in cooler temperate countries, the pupation period can last as long as one to two weeks (Service, 1996).



Label	Detail
c	Cephalothorax
p	Paddles
pa	Palmate Hairs
s	Setae
sp	Spines
t	Breathing Trumpets

Figure 2.5 Pupa of the *An. gambiae* group
(Taken from Reid, 1968)

2.1.4 Adult Stage

2.1.4.1 Morphology

After emerging from the pupae, adult mosquitoes will remain resting on the water surface until the wings and legs are sufficiently hardened. The most obvious characteristic that allows it to be readily identified is that the *Anopheles* adults usually rest with their bodies at an angle to any surface; with the proboscis and abdomen in a straight line (Figure 2.6). However, according to Service (1996), there are some species that rest at almost right angles to the surface, whereas there are some species in which the angle they rest is much smaller, for example the Indian mosquito *An. culicifacies* Giles, 1900.

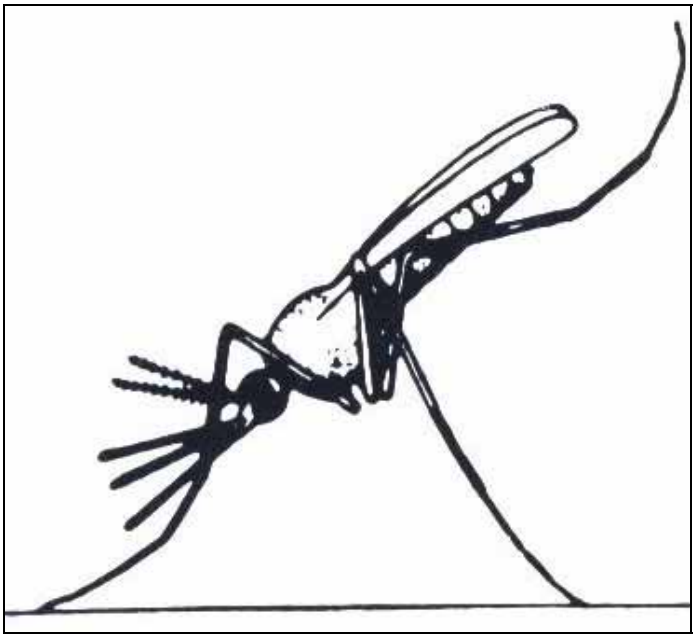


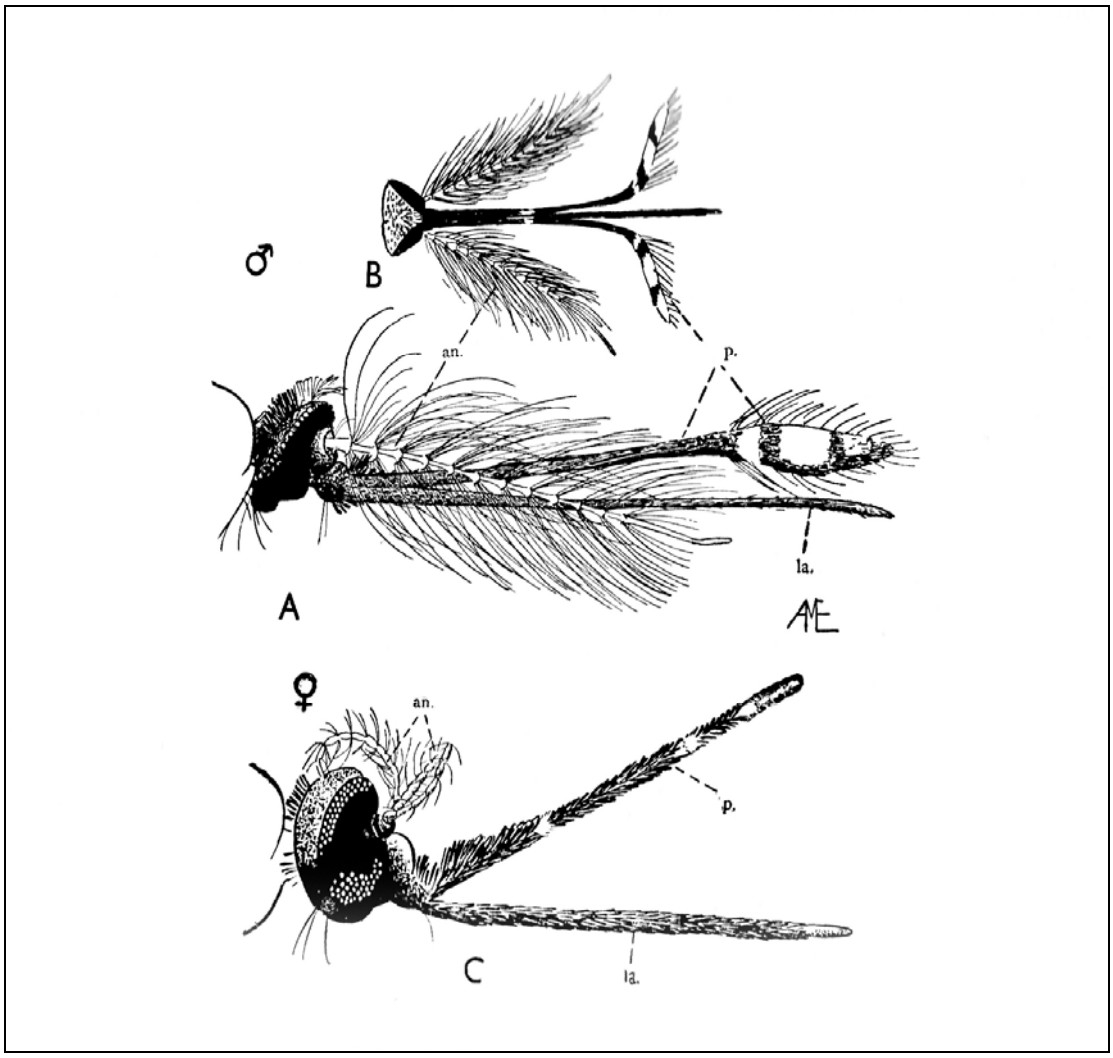
Figure 2.6 Adult *Anopheles* Resting at an Angle
(Taken from CDC, 2006)

In most *Anopheles* mosquitoes, dark or blackish, and pale, whitish or creamy whitish scales arranged in distinct spotted-looking ‘blocks’ are present on or at specific areas on the wing veins. The number, length and arrangement of these spotted patterns differ from species to species. There are few species, for example the European *An. claviger* Meigen, 1804 have dark or brownish scales covering the veins almost uniformly (Service, 1996).

Other features that distinguish the adult *Anopheles* mosquitoes from the adult Culicine mosquitoes are that both the male and female adult *Anopheles* mosquitoes have palps and proboscis of about the same length, the scutellum is rounded posteriorly with the presence of setae along the entire edge, and the middle lobe of the salivary glands is considerably shorter than the other two outer lobes. In addition to that, only one spermotheca is present in the female *Anopheles* mosquitoes (Service, 1996).

2.1.4.2 The Male and Female Adult *Anopheles* Mosquitoes

The males usually reach maturity first and will remain near the breeding place. The male mosquitoes have plumose antennae, a distinguishing characteristic that separates it from the non-plumose antennae of the female (Figure 2.7). The palps of the female *Anopheles* are usually blackish with broad or narrow rings of pale scales, especially on the apical half, whereas the palps of the male *Anopheles* are swollen or club-shaped at the ends and may also have apically positioned rings of pale scales (Service, 1996).



Label	Detail
A	Male; Side View
B	Male; Dorsal View
C	Female; Side View
an.	Antenna
la.	Labium (Proboscis)
p.	Palp

Figure 2.7 Differences of Male and Female Adult *Anopheles* Mosquitoes (Taken from Reid, 1968)

The *Anopheles* adult mosquitoes seek a mate at first chance, shortly after emergence. If emergence occurred during the afternoon, mating activities will happen in a few hours, but if emergence occurred during the night, mating activities might only happen during the following evening (Reid, 1968). During the mating and insemination process which only happens once during a female's lifetime, the spermatozoa passed from the male will be kept in the spermotheca of the female, serving to fertilize all eggs produced throughout its life (WHO, 1982).

The male mosquitoes do not require blood meals and are unable to bite due to their insufficiently developed mouthparts. Although the male have a conspicuous proboscis, their mandibles and maxillae are usually reduced in size or absent, thus they are incapable of piercing the skin for blood feeding. They usually feed on sugar nectar of flowers and other sugary secretions from plants. However, females may also feed on sugary secretions to obtain energy for flight and dispersal.

The blood feeding habit is only developed in the female, which seeks their first blood meal 12 to 24 hours after emergence and mating. With a few exceptions, the female must go through the anautogenous development; in which she seeks and obtain a blood meal to provide the necessary nutrients for the development of her eggs in the ovaries.

Some species are able to go through the autogenous development; in which the female can develop her first batch of eggs without a blood meal. In tropical countries, the blood meal is digested in 30 – 72 hours, during which time the ovary is developing. In cooler temperate countries, the blood digestion process can be from 7

– 14 days. The fully gravid female then seeks a suitable water habitat for oviposition (Service, 1996). The female *An. maculatus*, for example, will usually be ready for oviposition about 48 hours after the blood-meal, unless in its first gonotrophic cycle where two blood meals are required for eggs formation and maturing (Reid, 1968).

2.2 The Bionomics of *Anopheles* Mosquitoes

Bionomics is as defined by World Health Organization (WHO) (1975a), 'that part of biology (often called autecology) which deals with the relationships of a given species and its environment'. Study on the bionomics of mosquitoes includes the growth and development of the immature stages, and life and behaviour of the adults under the effects of environmental conditions.

The bionomics and behaviour of mosquitoes can be affected by the changes in the environment (De Las Llagas, 1985), which may lead to malaria outbreaks if the condition is well suited for the development of the vectors (Sandosham, 1970). An understanding of the bionomics of mosquitoes, especially of the vector species, is important in the epidemiology of mosquito-borne diseases and in implementing effective vector control and management strategies (WHO, 1982).

2.2.1 Dispersal

The *Anopheles* adult mosquitoes usually do not fly far. The males which are usually the weaker fliers compared to the females, tend to remain close by to the breeding site and in outdoor shelters (WHO, 1975a). Thus, presence of large numbers of adult male mosquitoes at a particular area generally indicates that the breeding place is nearby (WHO, 1982).

The flight range for most *Anopheles* mosquitoes from the tropics is about one to three kilometres, though there are some species from temperate regions which can fly as far as four to five kilometres. The dispersal range differs according to species (Abu Hassan & Yap, 2003). However, the dispersal of mosquitoes can sometimes also be assisted by air currents, and through human and or even vehicles (WHO, 1982).

2.2.2 Biting Behaviour

Both the biting places and resting places of *Anopheles* mosquitoes differ according to the species. Some species of mosquitoes that enter houses to feed are termed as endophagic in their feeding behaviour. In contrast, the species that feed on their hosts outdoors are termed exophagic. Those species of mosquitoes which rest indoors after their blood meals are called endophilic, whereas those that rest outdoors are called exophilic (Abu Hassan & Yap, 2003).

Anopheles mosquitoes can be found both before and after their blood meals resting indoors or outdoors. Indoors; for example in the upper part of walls of houses, or outdoors; in a variety of natural shelters, such as amongst vegetation, in rodent burrows between plants, in termite mounds, in crack and crevices in trees, under bridges, in culverts, in cracks and holes in the ground, in caves and among rock fissures (Service, 1996).

Blood-fed female mosquitoes will usually leave the post-biting resting place in search of a daytime resting place. *Anopheles* mosquitoes of Malaya and Borneo are normally found resting amongst shady vegetation near the ground during the daytime (Reid, 1968).

In their book, Sandosham and Thomas (1983) recorded the daytime resting places of *An. aconitus* and a number of other *Anopheles* species taken from a rubber estate in the Tampin district of Negeri Sembilan. These adults were found in grassy, thick and close to ground vegetations, with sunlight cover and shade from mature rubber trees and easy access to human and cow population. However, there are also some species which rest indoors during daytime, for example *An. campestris* could be caught resting on walls and clothing inside houses during the day.

Similarly, the host preferences of the *Anopheles* mosquitoes also differ according to the species. Species that usually feed on human hosts are called anthropophagic in their feeding habits, whereas those that feed mainly on animal hosts are called zoophagic. The female *Anopheles* mosquitoes locate, recognize and are attracted to suitable blood sources by the various stimuli emitted by the suitable hosts, such as carbon dioxide, body odour, moisture and heat (Reid, 1968).

The main vector of malaria in Peninsular Malaysia, the *An. maculatus* (Chooi, 1985), is both zoophagic and anthropophagic. Although preferring to feed on animal rather than man, there have been results that showed that the *An. maculatus* strain in Malaysia seems to have a greater relative preference for man in comparison with