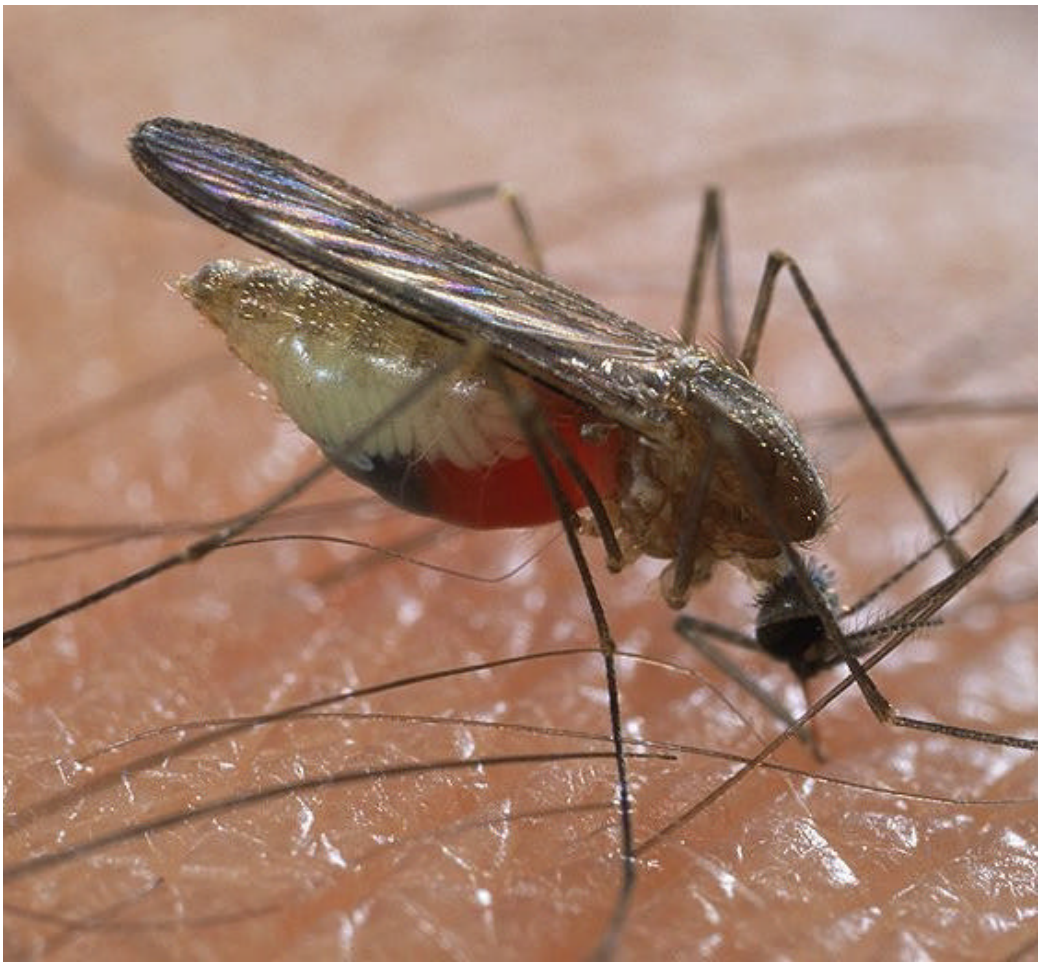
THE BIOLOGY AND VECTOR COMPETENCE

OF THE ANOPHELINE MOSQUITOES OF MYANMAR

WITH SPECIAL CONSIDERATION OF *ANOPHELES DIRUS*



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Referees: Prof. Dr. Volker Storch/Dr. Norbert Becker

Prof. Dr. Gabriele Elisabeth Pollerberg

CONTENTS

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Abstract

A brief critical review has been made of the material concerning malaria available from the second world war. In addition a short description has been given of the war-time anti-malaria organization in Myanmar. The physical features of Myanmar, in relation to the distribution of malaria in seven natural sub-divisions have been briefly discussed.

In part I: thirty-seven species of anopheline mosquitoes recorded in Myanmar have been considered in detail with particular reference to the distribution, breeding sites, seasonal prevalence, adult behaviour and their vector competence. Some general aspects of anopheline behaviour are discussed. Classification has been made of all recorded species in relation to malaria transmission and the distribution of malaria in Myanmar as follows: dissection records, feeding habits, distribution, seasonal prevalence, evidence from other sources. In Myanmar, the mosquitoes responsible for regular or annual malaria transmission (primary vectors) are (i) *Anopheles dirus* and (ii) *Anopheles minimus.* Predominatly cattle-feeders which may under abnormal conditions, feed on man. Often abundant and capable, therefore, of transmitting malaria (secondary vectors) are (iii) *Anopheles aconitus* (iv) *Anopheles annularis* (v) *Anopheles culicifacies* (vi) *Anopheles sinensis* (vii) *Anopheles jeyporensis* (viii) *Anopheles maculatus* (ix) *Anopheles philippinensis* (x) *Anopheles sundaicus.* Vector competence of these ten species are also described.

Part II: mainly concerns genetic studies of *Anopheles dirus* from the Mudon area. *An. dirus* normally occurs in the forest and forest fringes where it transmits malaria efficiently. Changes in the ecology of an area induced by new developments, like deforestation, construction of dams and irrigation projects may have profound or indirect effects upon vector occurrence because of the creation of suitable ecotypes for the completion of its life cycle. Thus, the dangerous vector *An. dirus* has invaded human settlements. This typical forest breeder could successfully adapt and spread all over Mudon. It is potentially hazardous to public health so as to provide knowledge for further research and control needed in this area.

Thus, the purpose of the study is to establish the degree of genetic divergence (similarity) between the three topographically different populations of *An. dirus: An. dirus* 1 (from forested areas), *An. dirus* 2 (from rubber plantation areas) and *An. dirus* 3 (from domestic areas). Field-collected specimens (both adults and larvae) were used in horizontal ultrathin agarose electrophoresis for identification. A more detailed analysis was performed in the present study using eleven gene enzyme systems (comprising twelve presumptive loci) to determine the degree of genetic differentiation among these three populations of *An. dirus* in

comparison with *An. maculipennis* from Mannheim, Germany and *An. stephensi* from Indonesia, provided by Bayer AG, Leverkusen (breeding stock). Based on the data obtained from the migration of enzymes, the following values were computed in each population: relative frequencies of alleles and genotypes, confidence and the variances of allele frequencies, conformity to expectations of the Hardy-Weinberg rule with X^2 (chi square) values, the degree of heterozygosity and polymorphism, Nei`s genetic distances and the values of genetic similarity between the species.

Phylogenetic relationship between the respective population pairs among these gene pools have been demonstrated by dendrogram using the Kitsch program. This dendrogram clustered the populations in two forms; three *An. dirus* population groups together in two groups. The first group (population 1: *An. dirus* 1 from forested area and population 2: *An. dirus* 2 from rubber plantation area) to be clustered are those with the smallest genetic distance. Populations of second group are developed from the first group and population 3 (*An. dirus* 3 from domestic area). These two groups of *An. dirus* are then combined and taken to be a single group. The populations of the third group entity are from another cluster for *An. dirus* combined group and population 4 (*An. stephensi* from Indonesia).

The genetic identity values between these three populations of *An. dirus* ranged from 0.9978 to 0.9999 which is the generally accepted range for conspecific populations. The high values of genetic similarity indices suggest that natural populations of *An. dirus* in Mudon area share an undifferentiated gene pool. The very low genetic distance D (between 0.0001 to 0.0022) also indicate that these three populations of *Anopheles dirus* from Mudon area are parts of a metapopulation without measureable adaptations due to selective conditions in ecologically different breeding sites.

1. General introduction

1. General introduction

In contrast to most of the other countries of Asia little information had been published on aspects of malaria and anopheline mosquitoes in Myanmar before Second World War. Only four references can be found in the literature (Stott, 1916; Christophers, 1933; Grewal, 1937; James, 1941). No references are available from Myanmar sources regarding the anopheline mosquitoes and/or the incidence of malaria in the country during the Japanese occupation from 1942 to 1945. It is presumed that malaria investigations were interrupted during that period. However, five references are avaliable from British military sources (to fill the knowledge gap concerning anopheline mosquitoes within Myanmar) during Second World War. These are the results of surveys carried out by the British Anti-malaria Unit in Upper Burma (now Myanmar) and Arakan (now Rakhine) fronts, during the Burma Campaign from 1943 to 1945. Of these five references, four are published as articles, and one is a Ph D thesis. Yofe and Fox (1946) dealt with entomological aspects, and Macan (1948, 1950) included epidemiological data accompanied by spleen surveys and mosquito dissection records in Burma. Tyssul-Jones (1959) furnished indirect epidemiological evidence to incriminate *Anopheles sundaicus* as one of the local vectors of malaria. Fox (1949) listed a total of 27 species of anophelines encountered in Burma up to the end of the Second World War in 1945, and reviewed all the available data accompanied by distribution maps and dissection records.

A malaria control project assisted by World Health Organization (WHO) was conducted in Lashio area in Northern Shan State, from October 1951 to March 1954, and subsequently in Maymyo (now Pyinoolwin) area, from April 1954 to December 1955, during which detailed studies were undertaken on the epidemiology of malaria and bionomics of the local vector *Anopheles minimus* (Weeks, 1955; Postiglone and Venkat-Rao, 1956; Tun Aung & Mya Maung, personal communication). A total of 24 anopheline species was encountered by national and WHO entomologists in the project area.

During the malaria eradication period (1970 and 1980), a vast amount of information has been accumulated on the biology of anopheline mosquitoes in Burma, with particular reference to their distribution, bionomics, malaria transmission and susceptibility to insecticides, as a result of routine entomological surveys undertaken on a country-wide basis, by Khin Maung Kyi (1970 a, b, 1971, 1972 a, b, c, 1975, 1976).

In addition there are some published and unpublished departmental reports held by the Department of Medical Research (DMR) and Department of Vector Borne Disease Control

(VBDC). These materials form the bulk of the general background information on the malaria incidence and status of anopheline mosquitoes in the country. The main focus of the studies was to determine the vector-competence of the various species. It is a critical survey of all availiable data regarding the species of anopheline mosquitoes so far recorded in Myanmar and their relation to malaria or medical importance.

1.1. Brief Geographic Description of Myanmar

1.1.1. Location and size

As the western segment of the Indochina Peninsula, the Union of Myanmar lies between South East Asia and Southern Asia. Latitudinally Myanmar extends from 9° 55´ to 28° 30´ North and longitudinally from 92° 10´ to 101° 9´ East. The Tropic of Cancer passes near Tiddim Town (Chin State), Tagaung Town (Sagaing Division) and Kutkai Town (Northern Shan State). Approximately one third of Myanmar lies outside the tropics and the remaining two-thirds belongs to the hot tropical area. Myanmar adjoins the Bay of Bengal in the west and the Andaman Sea (Katpatli Sea) in the south, both parts of the Indian Ocean.

It has a long land frontier (6,170.69 kilometers) that it shares with Bangladesh (258 kilometers) and India (1341 kilometers) on the west, China (2210 kilometers) on the north and northeast as well as Laos (226 kilometers) and Thailand (2097 kilometers) on the east. The country´s maximum east-west extention is 927 kilometers and maximum north-south 1935 kilometers. Regional political divisions are shown in Fig. 1.1.

Myanmar with a total area of 676,577 square kilometers, is the second largest country in the Association of Southeast Asian Nations (ASEAN), after Indonesia. It is about the same size of France and Britain together.

1.1.2. Physiography

The Union of Myanmar lies within the most distinctive physical environment in Asia with a single core area placed within a bounding framework of mountains. The country consists of a group of central lowland alluvial valleys ending in a broad delta opening to the Andaman Sea. The lowland is surrounded by a series of east and west mountainous uplands with few useful passes. In the north the landscape is high and rugged with very narrow valleys. To the south the mountain frame is open towards the Ayeyarwady-Sittoung delta and the Gulf of Martaban. It is an elongated region in which all essential units have a chiefly north-south axis.

Physically, the country falls into the following seven well-marked geomorphological regions, each of which will be described in more detail in Fig. 1.2. They are: (1) Rakhine Coastal Strip; (2) Western Hills; (3) Northern Hills; (4) Dry Zone; (5) Southern Plain and Delta (6) Shan Plateau and (7) Tanintharyi Coastal Strip.

Region 1- Rakhine Coastal Strip: This region, lying between the Western Hills and the sea, receives the heaviest rainfall in Myanmar. There are three natural sub-divisions: (a) A series of small mountain ranges, running parallel to the coast, covered by bamboo jungle on the lower slopes and forest on the upper. Perennial streams are scanty and the region is mostly uninhabited. (b) A hillock area, the formation of which is largely clay and shales, where perennial streams are more abundant and the population denser. (c) The coastal area which narrows from north to south and which is cut into by numerous tidal creeks which connect with the larger streams. The largest villages are to be found in this portion and rice is grown wherever salt water can be excluded by draining off the tidal creeks during the rains. This region includes Rakhine State (except mountainous region).

Region 2- Western Hills: These ranges arise from the vast mountain knot in the Tibetan-Chinese borderlands and swing round in a great arc of some 1129 kilometres along the coast to Cape Negrais, east of the Ayeyarwady delta. Due to the tectonic foldings, they consist of numerous north-south parallel ridges. Their height gradually decreases from 3048 meters in the north, to almost 2000 metres (m) in the centre, to the Rakhine Yoma ranges in the south which are less than 1000 m above sea level. Lying athwart the south-west monsoon winds, heavy rainfall is typical in this region varying from 2050 mm to 5100 mm per year with the consequent development of dense jungle. Further south, where the hills are less steep, the broad valleys with their numerous perennial streams are more densely populated. This region includes Chin State, the mountainous region of Rakhine State, the upper parts of Magwe Division and Sagaing Division.

Region 3- Northern Hills: This region consists of a series of small, roughly parallel mountain ranges decreasing in height as they descend from north to south. The Chindwin and Ayeyarwady rivers both rise in this region flowing through narrow, steep-sided valleys in the north which gradually broaden and become more fertile further south. The Mu river flows through the centre of the southern portion. In general the large river valleys are the most populated portions of this region. Rainfall is moderate, varying from 2050 mm to 2500 mm

per year. This region includes Kachin State, the upper and middle part of Sagaing Division and the upper part of Mandalay Division.

Region 4- Dry Zone: The dry zone is characterized by limited rainfall of only 510 mm to 1000 mm per year. Most of the Central Myanmar plain is located within this region which also includes the great east-west bend of the Ayeyarwady from Mandalay to the Chindwin confluence. The terrain consists of undulating plains and low hills, mainly of light sandy soils with alluvial deposits in the riverine areas. The vegetation is mainly thorny bushes and scrub. Dry crops comprise the greater portion of the agriculture. Rice is grown in scattered areas throughout narrow valleys or in areas adjacent to hills where the meagre water supply can be supplemented by perennial streams. This region includes Magwe Division, Mandalay Division and the lower part of Sagaing Division.

Region 5- The Southern Plain and Delta (Central Basin): West of this region lies the lower Ayeyarwady valley with its large delta. This central belt lies between the Shan Plateau and the Rakhine Yoma range, filled up by a great mass of sediment brought down by the rivers. Most of this region is flat, the largest rice-growing area in Myanmar and consequently the most densely populated. Rainfall is fairly heavy from 2050 mm to 2500 mm per year. This region includes Ayeyarwady, Yangon and Bago Divisions.

Region 6- Shan Plateau: This region consists of a vast plateau of limestone and crystalline rocks. The western slope rises steeply from the central and southern Myanmar plains, while on the east it merges with the mountains of western and southern Yunan. The average elevation is more than 900 metres above sea level and the plateau is dissected from north to south by the deep gorges of the Thanlwin river and its tributaries. The average rainfall in the area is 1025 mm to 2050 mm per year. This region includes Shan State, Kayah State and the upper part of Kayin State.

Region 7- Tanintharyi Coastal Strip: This consists of the narrow strip of land projecting southward and lying between the coast and numerous north-south, jungle-covered ranges consisting mainly of granite and limestone. It also contains the delta of the Thanlwin river. Rainfall is very heavy, usually exceeding 5000 mm per year. There are important mineral resources associated with the granite intrusions. Rice is grown in the flat area and there is also

extensive rubber cultivation.This region includes Mon State, Tanintharyi Division and the lower part of Kayin State.

1.1.3. Climate

The climate of Myanmar is largely the result of the great monsoon circulation system of Southern Asia. However, it varies extremely according to slope, latitude and elevation. Thus highland areas lying in low latitudes possess a moderate climate and some lowland areas in temperate latitudes have a tropical climate. In general, highlands have lower temperatures, as temperature decreases with the increasing altitude at a normal lapse rate of 1.4 °C per 300 m.

In the far north of Myanmar, snow covers the elevations above 3,000 m for nearly three months each year, and in limited areas above 4,500 m the snow lies all year round. Khakaborazi, on the border between Myanmar and Tibet, is the highest mountain in southeast Asia at 5881 m followed by Gamlang Razi at 5835 m, both are permanently snow-clad.

In the maritime areas such as the Rakhine coastal lowland, Ayeyarwady delta and Tanintharyi, the temperatures are higher and the range of temperature is smaller due to the maritime effect.

Myanmar has three quite distinct seasons; namely, summer (March to May), rainy (June to October) and cool (November to February) season.

The temperature is highest in the dry zone (Central Myanmar) during the summer months from late February to mid May being 37 °C in average, with peaks in late March and April reaching 41 °C. In the delta area temperatures vary between 30 to 37 °C and rarely exceed 37 °C. A milder climate prevails at this time of the year in the eastern region on the highland plateau with a temperature ranging from 28 to 32 °C.

With the periodical return of the southwest monsoon, the rainy season usually starts inthe third week of May. The total annual rainfall varies from place to place with a range of 600 to 5500 mm. It is abundant, occasionally with heavy squalls, in Rakhine and Tanintharyi regions amounting to over 5500 mm whereas the rain-shadow belt of Central Myanmar receives less than 800 mm and in some locations even less than 600 mm. Yangon, the capital city, lying in the southern part of Ayeyarwady deltaic region, has an annual rainfall of about 2400 mm. The peak monsoon period is from July to August.

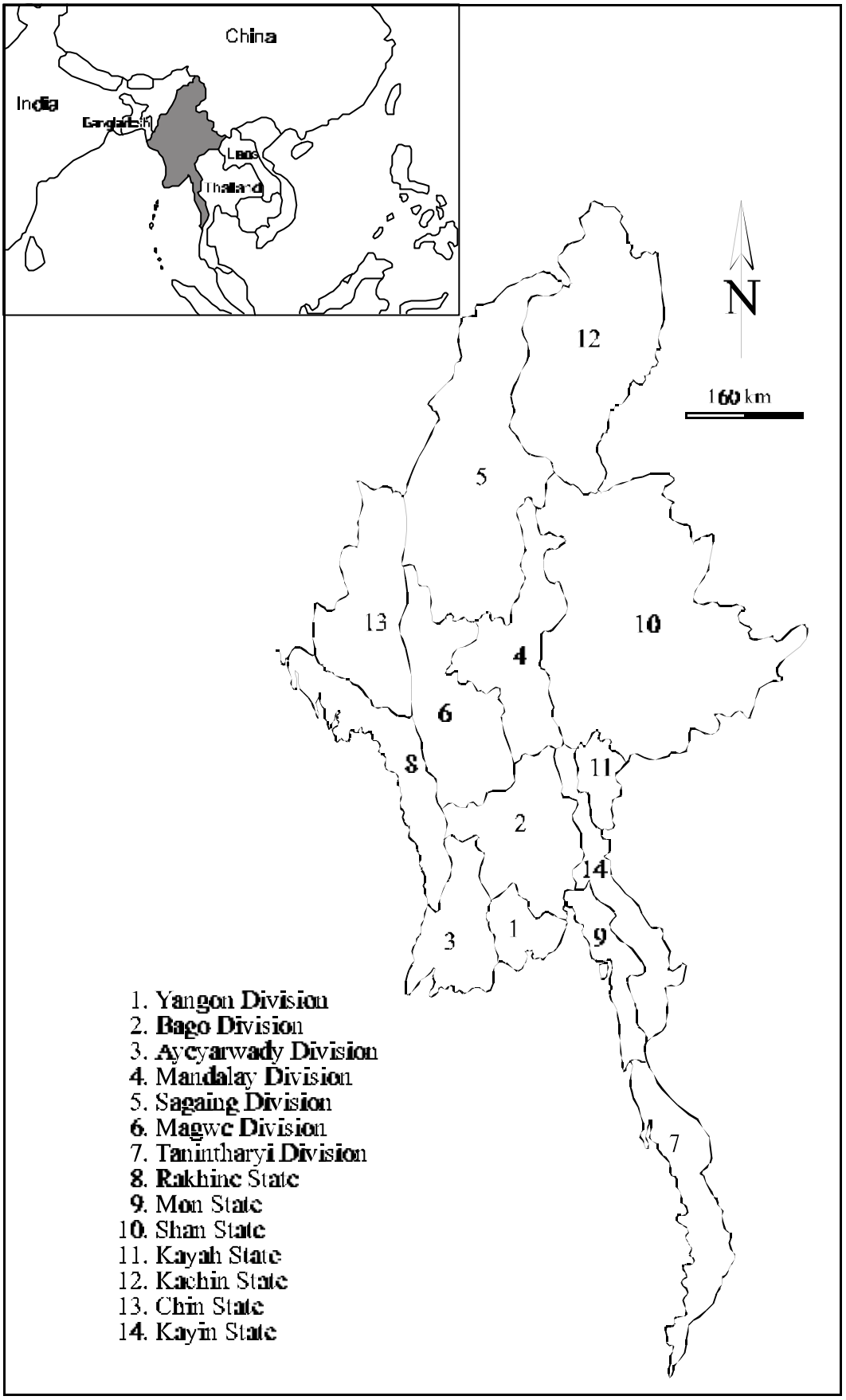

Fig. 1.1. Map of Myanmar showing political Divisions and States

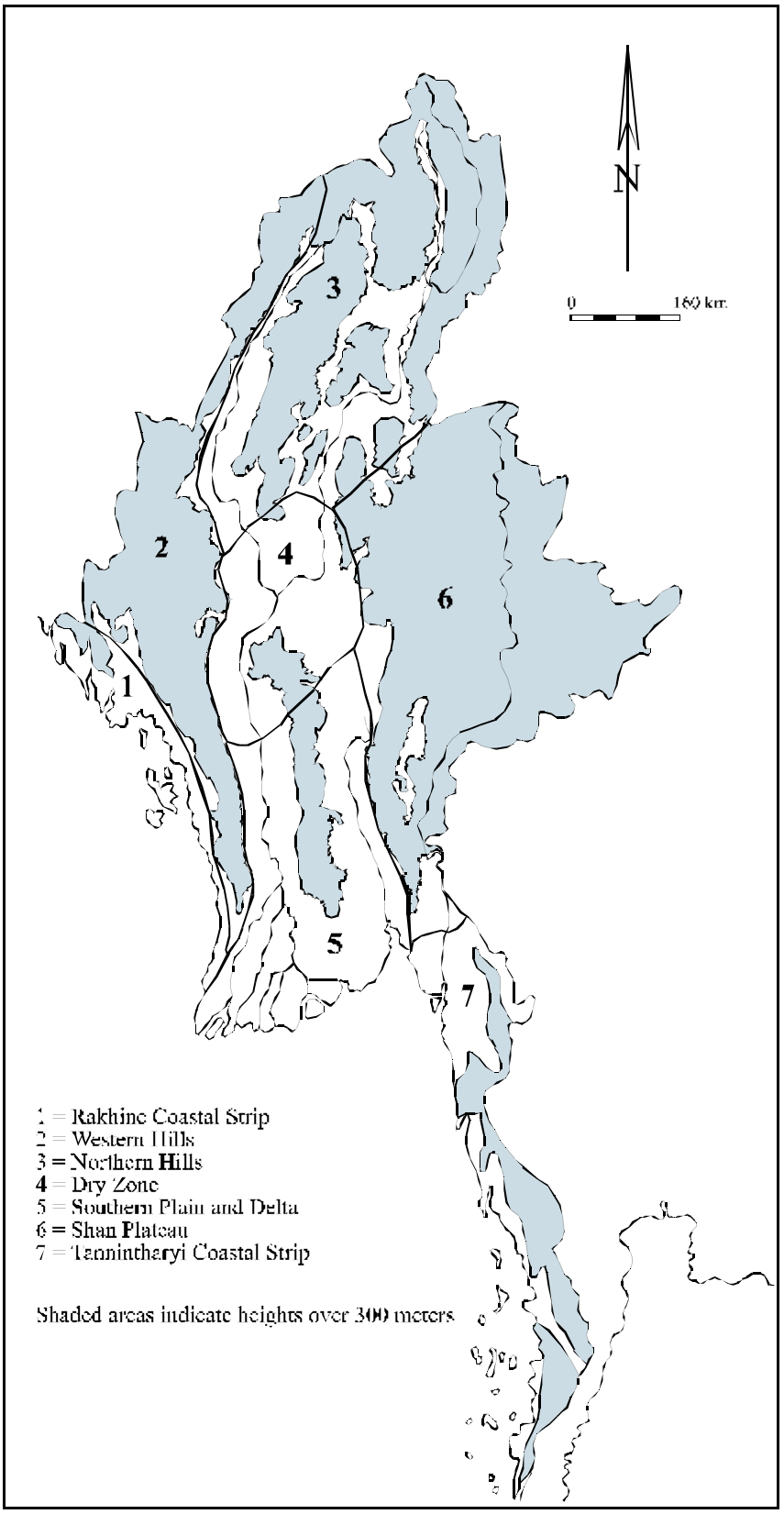

Fig.1.2. Map of Myanmar showing physiographic regions (modified after Fox, 1949)

2. The anopheline mosquitoes of Myanmar

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2.1. Morphology

2.1.1. Larva

The anopheline mosquito larva is divided into three distinct regions, the head, the thorax and the abdomen (Fig. 2.1).

The ***head*** bears a number of hairs, some of which on the dorsal surface are important in identification of the species (Fig. 2.2):

Inner anterior clypeal hairs (one pair) arising near the anterior edge of the frontoclypeus and projecting forward between the feeding brushes. These hairs may be placed close together (proximated) or wide apart and may be simple, finely or branched.

Outer anterior clypeal hairs (one pair) are placed external to the inner anterior clypeal hairs. They are sometimes obscured by the feeding brushes.

Posterior clypeal hairs (one pair) are placed immediately behind the anterior clypeal hairs.

The outer anterior clypeal hairs and posterior clypeal hairs may also be simple, frayed or branched.

*Frontal hairs***,** six in number, arise behind the posterior clypeal hairs. They are usually branched.

Inner sutural hairs (one pair) aries behind the frontal hairs on the inner side of the "suture". These may be simple bifid or branched.

Outer sutural hairs (one pair), arise on the outer side of the suture.

Antennae (one pair) arise from a slightly raised base on either side of the group of frontal hairs.

The ***thorax*** is the most conspicuous part of the larvae. Its cuticle is mainly or entirely membraneous, and during the growth of the instars it becomes increasingly larger relative to the head. As in the adult, the thorax consists of three segments called prothorax, mesothorax and metathorax. The segments are completely fused, their borders can only be determined by the arrangement of the setae in three distinct sets (submedian prothoracic hairs, metathoracic palmate hairs and pleural hairs), some of which are important for identification as shown below (Fig. 2.3):

Submedian prothoracic hairs consist of two groups, one on each side of the mid-thoracic line, near the anterior edge. The middle hair is usually branched while the inner one may be simple, bifid or branched and may arise from a conspicuous or inconspicuous tubercle.

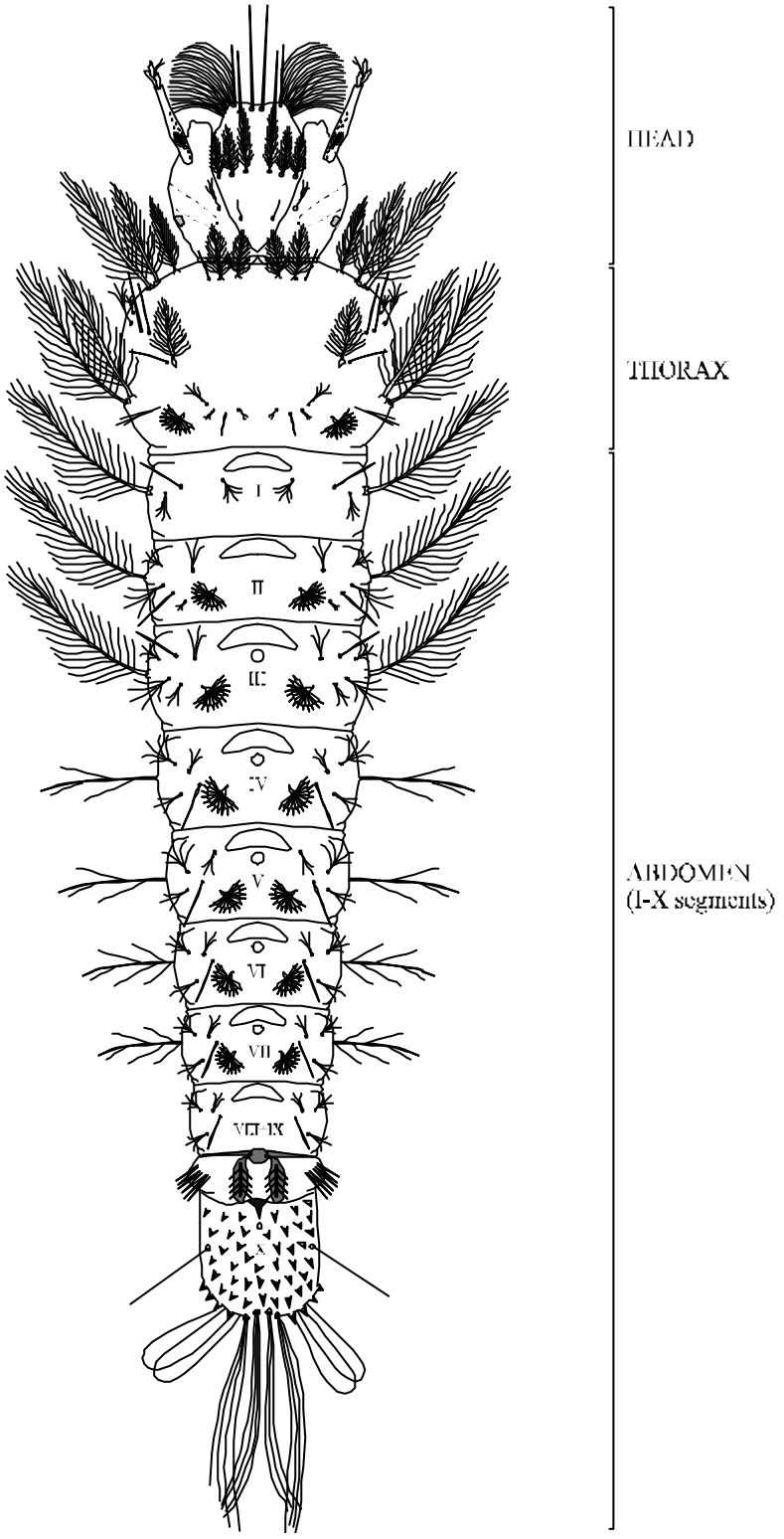
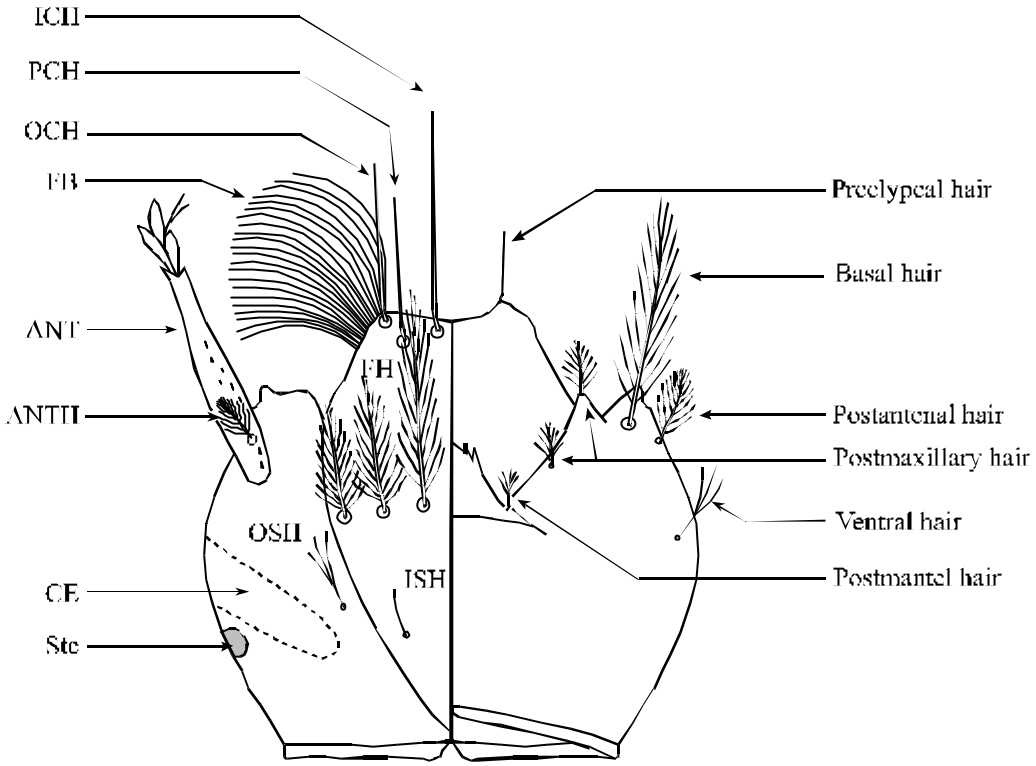


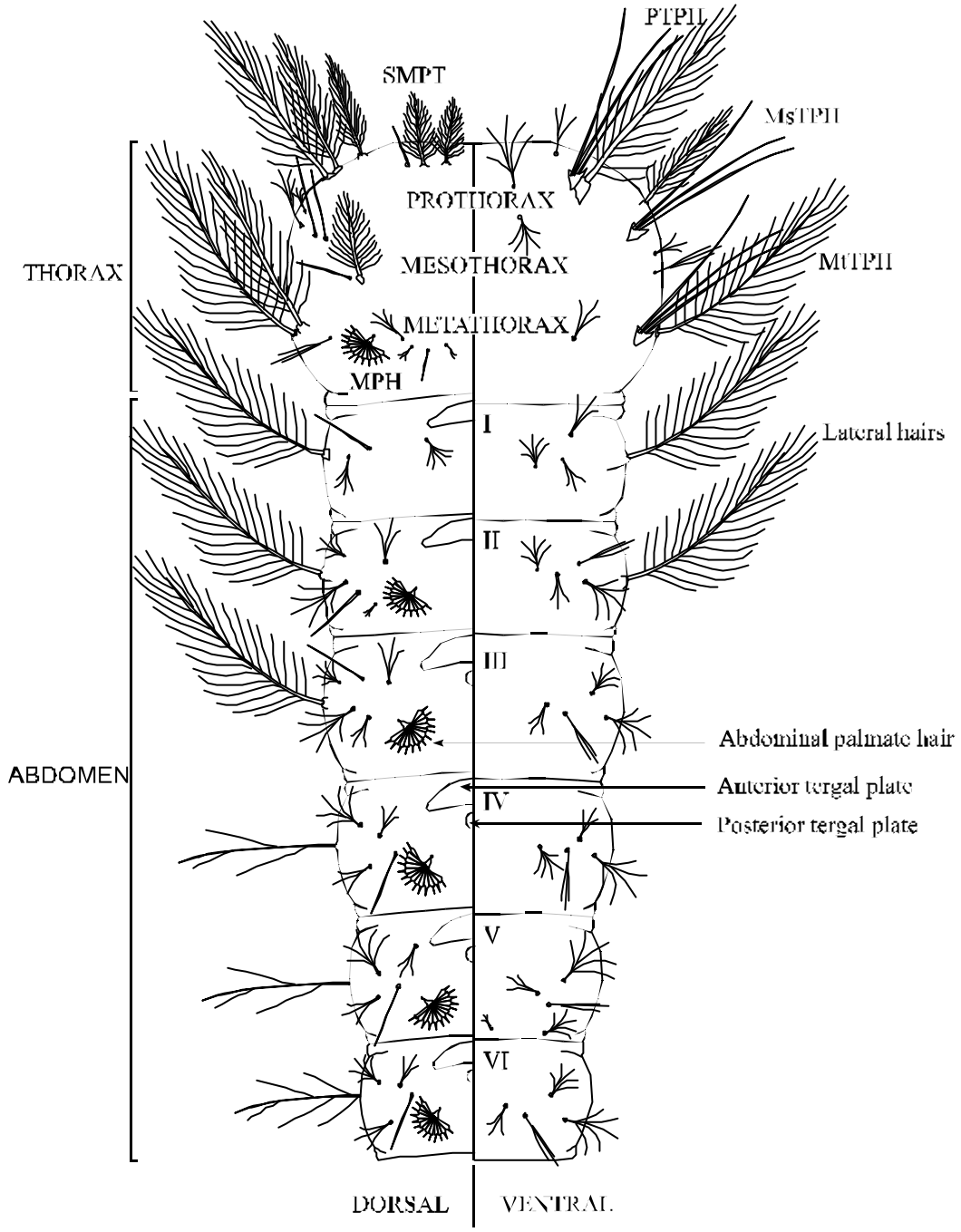
Fig. 2.1. Morphological features of anopheline mosquito larva

Metathoracic palmate hairs are the outermost of a group of four hairs, one on each side of the thorax. These may be short (as the other three) or long with flattened branches, forming a "palmate hairs".



ICH = Inner anterior clypeal hair
PCH = Posterior clypeal hair
OCH = Outer anterior clypeal hair
FB = Feeding brush
ANT = Antenna
ANTH = Antennal hair
FH = Frontal hairs
OSH = Outer sutural hair
ISH = Inner sutural hair
CE = Compound eye
Ste = Stemmata

Fig. 2.2. Fourth stage anopheline larva head; dorsal left, ventral right.



SMPT = Sub-medium prothoracic group of hairs
MPH = Metathoracic palmate hairs
PTPH = Prothoracic pleural hairs
MsTPH= Mesothoracic pleural hairs
MtTPH = Metathoracic pleural hairs

Fig. 2.3. Fourth stage anopheline larvae: Thorax and abdomen

Pleural hairs are situated on the ventral surface of the thorax near the edge and occur in three groups (prothoracic pleural hairs, mesothoracic pleural hairs and metathoracic pleural hairs) on each side, developing on prothorax, mesothorax and metathorax. These may be simple or branched.

The ***abdomen*** consists of ten segments, the first seven (counting from the thorax) closely resemble each other. The eighth and ninth segments are fused together to form the "spiracular apparatus" The tenth segment is very simple and bears the anal gills at its posterior end (Fig. 2.4):

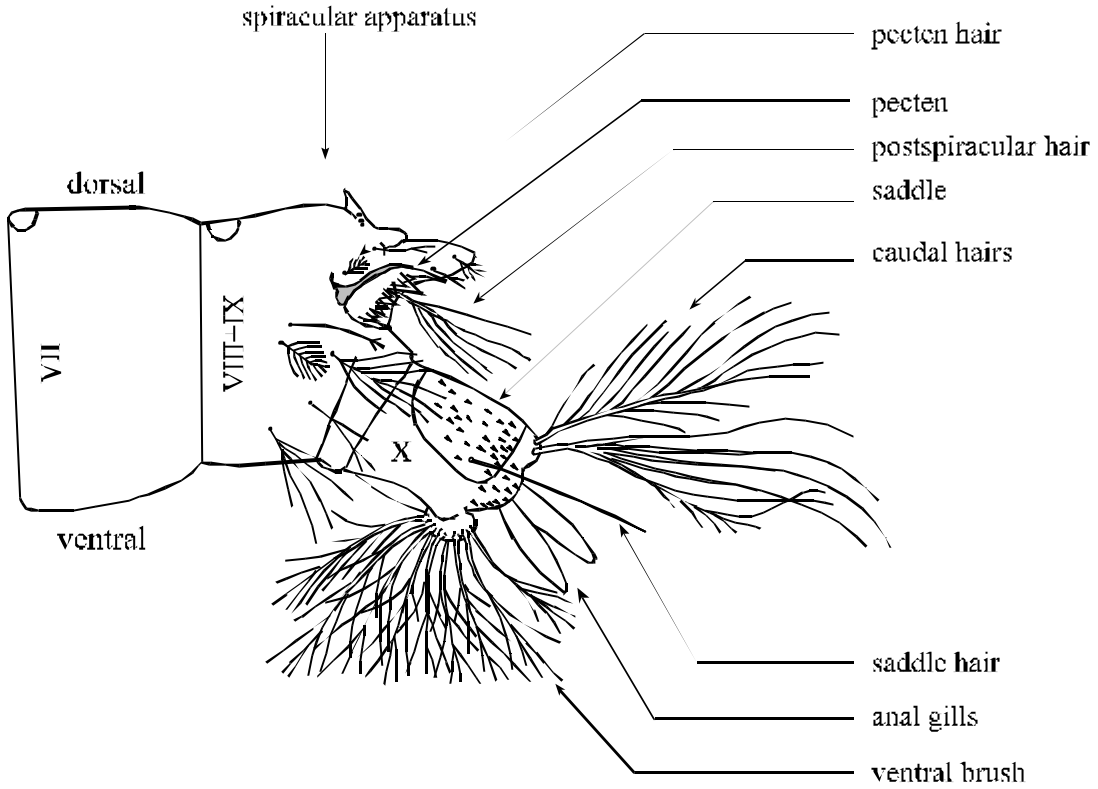


Fig. 2.4. Fourth stage anopheline larva abdomen

Tergal Plates: There are tergal plates on the first seven segments on the dorsal surface. Those which are close to the anterior border of an abdominal segment are the "anterior tergal plates" and the small rounded plates situated about the middle of the segment are the "posterior tergal plates". The anterior tergal plates may be large, enclosing the posterior tergal plates or may be smaller.

*Lateral hairs***:** **T**hese are one or two hairs on each side on the lateral surface of abdominal segments I to VII. The first two segments bear a pair of long, stout and branched lateral hairs, while on the other segments there are single hairs only.

*Palmate hairs***:** On each of the first seven segments on the dorsal surface, there are five hairs, of which the innermost is developed into a palmate hair in most species. In some species these palmate hairs are developed on all the seven segments, while in others they are developed on segments II or III to VII.

2.1.2. Adult

The body of the adult (Fig. 2.5) is divided into three well-marked regions, the head, the thorax and the abdomen.

The ***head*** (Fig. 2.5) bears, besides a pair of large compound eyes, a pair of hairy antennae, a pair of palps, a proboscis and a number of scales. The palps are about as long as the proboscis and lie above it, often close to each other, obscuring the proboscis when seen from above. In some species the palps are uniformly dark but in many they are ornamented by pale bands, the width and arrangement of which varies in different species. In many species, there are three pale bands, while in some there are four, or even five, pale bands. The band involving the extreme tip of the palp is referred to as the "apical" band and the one next to it as the "pre-apical" or "sub-apical" band. The proboscis is usually dark. However, in some species, there is what is termed "flavescence" on the apical portion of the proboscis, which gives it a yellow or golden colour.

The ***thorax*** (Fig. 2.6) is formed by the fusion of three segments, the prothorax, the mesothorax and metathorax. The wings are attached to the mesothorax. Three pairs of legs (the hind pair being the longest) are also attached to the thorax, one pair being borne on each segment. There are hairs and scales on the thorax, which are some times useful in identification of the species. A hair is round and tapers evenly from the base to the apex while a scale is flat, narrow at the base and widening out ditsally.

The ***wings*** (Fig. 2.7) consist of upper and lower epidermal layers, not fused along certain strengthening tubes, such as wing veins. The complete system of wing veins is called the venation. Beginning from the anterior margin of the mosquito wing, the first unbranched vein is the *costa* (C), which passes round the apex of the wing and forms its anterior margin. The *subcosta* (Sc) is located closely behind the costa and is also undivided. The *radius* (R) forks into an anterior branch R1 and a posterior branch, or radial sector Rs, which branches again into R2+3 and R4+5. The R2+3 divides once more into R2 and R3, while R4+5 remains unbranched. The fourth vein, or *media* (M), bifurcates apically into M1+2 and M3+4. Likewise, the fifth vein, or *cubitus* (Cu), divides into Cu1 and Cu2. Finally, there is one *anal vein* (A) present. The longitudinal veins may be connected by six different cross

veins (Becker et al., 2003). Two of them are situated close to the wing base, i.e., the *humeral vein* (h) stretches from C to Sc and the *arculus* (Ar) from R to M and Cu. The other four cross veins are displaced towards the wing apex. They are the *subcostal-radial* vein (sc-r) extending from Sc to R, the r1-rs from R1 to Rs, the *radio-medial* vein (r-m) from R4+5 to M and the *medio-cubital* vein (m-cu) from M to Cu1.

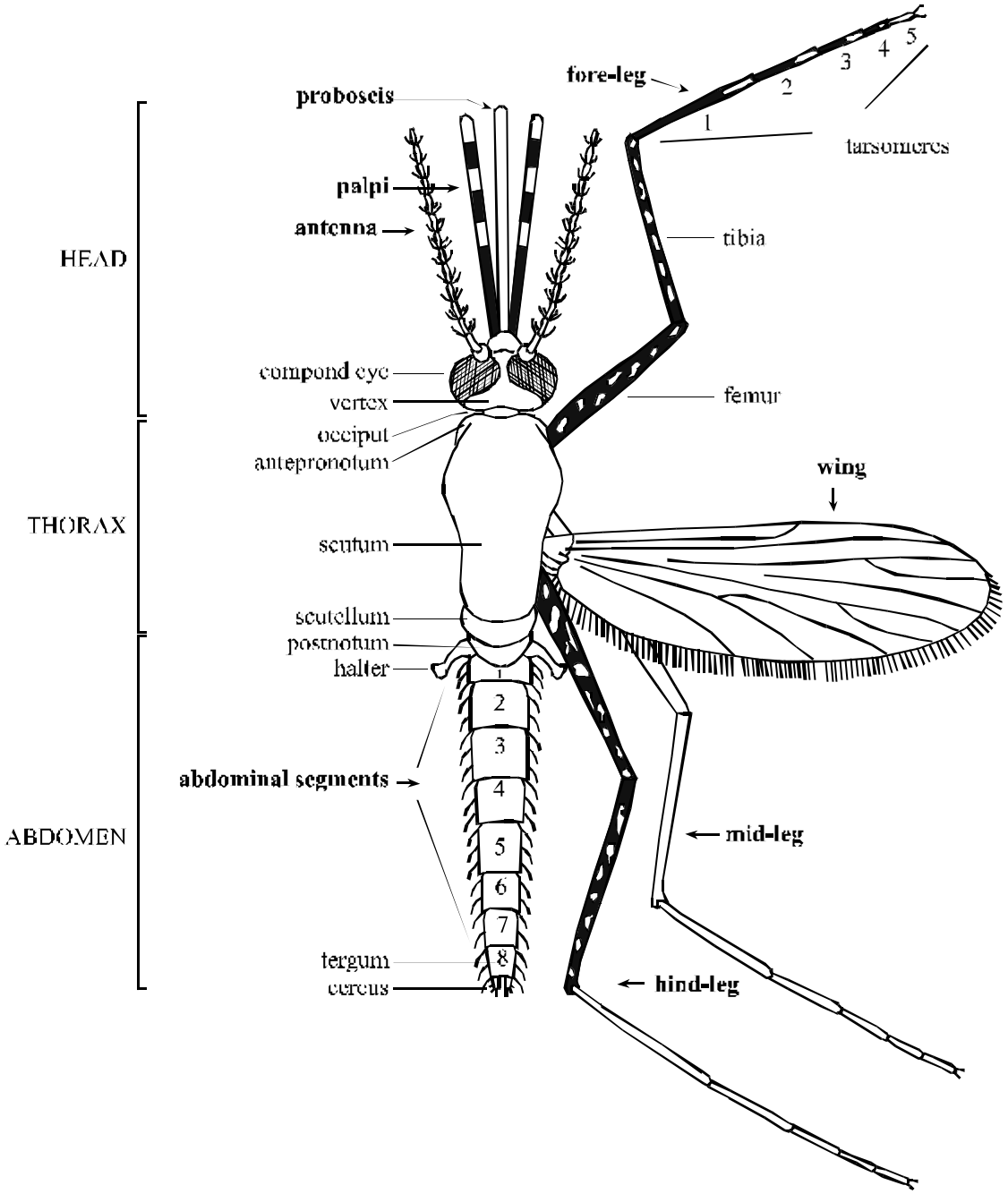
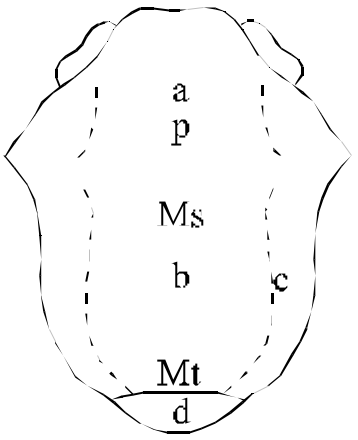


Fig. 2.5. Morphology of adult *Anopheles* mosquito (female)

The wings are usually ornamented, giving them the “spotted” appearance, consisting of alternate dark and pale scales on the veins. The cross veins are without scales. Along the posterior border of the wing is a fringe of scales (called the wing fringe) with some definite pale areas or “fringe-spots”.



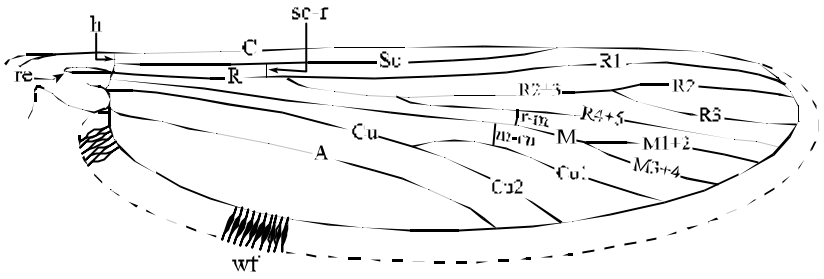
a – Anterior area of mesonotum
b – Median area of mesonotum
c – Lateral area of mesonotum
d – Scutellum

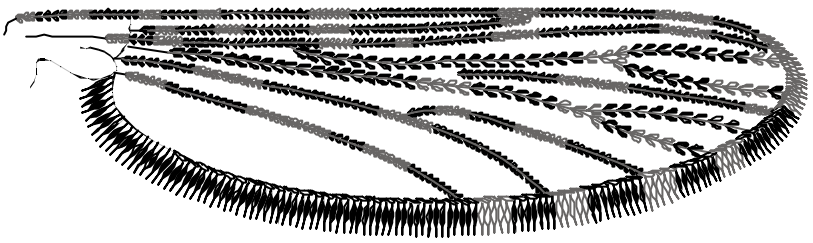
P – Prothorax
Ms – Mesothorax
Mt – Metathorax

Fig. 2.6. Thorax (adult)

The ***leg*** **(**Fig.2.5) consists of the “coxa”, the trochanter”, the “femur”, the “tibia” and the “tarsomere”. The tarsomere (tarsus) is formed of five tarsal segments, the first or that nearest to the tibia being the longest and terminal one being the shortest. The legs are covered with scales. If the scales are of varying colours, ornamentation of the leg is produced. The most important ornamentation is “speckling” of the femora and tibiae, which is produced by the presence of alternating dark and pale scales in the form of well-defined white or creamy spots. This should not be confused with “mottling”, which consists of indefinite extensive pale areas.

The tarsal segments may be uniformly dark or contain pale bands at the joints. The latter are called “banded tarsi”. Of the hind tarsal segments, one or more terminal ones may be completely white in some species and uniformly dark in others.





C = Costa
Sc = Subcosta
R = Radius
R1 = 1st longitudinal vein
R2+3 = 2nd longitudinal vein
R4+5 = 3rd longitudinal vein
M = Media or 4th longitudinal vein
Cu = Cubitus or 5th longitudinal vein
A = Anal vein or 6th longitudinal vein
wf = Wing fringe
h = Humeral crossvein
re = Remigium
r-m = Radiomedial crossvein
m-cu = Mediocubial crossvein
sc-r = Subcostal-radial vein

Fig. 2.7. Wing veins

The ***abdomen*** (Fig. 2.5) consists of a series of similar segments, the first segment being that nearest to the thorax. The last two segments are termed the male and female genitalia or terminalia. The abdomen is of interest in identification chiefly for the scales, sometimes forming to “tufts” which are present in some species and also for the fact that certain species like *Anopheles aitkeni* and *Anopheles insulaeflorum* can be separated only by differences in the male genitalia.

The palps, wings and legs are the organs which are mostly used for the purpose of identification and thus should be studied carefully.

2.2. Keys for identification of anophelines

The first taxonomic key for *Anopheles* mosquitoes of Myanmar (Burma) can be found in the "Fauna of British India including Ceylon and Burma" Vol. IV by Christophers (1933). "A guide for the identification of the full-grown larvae and imagines of the anophelines of Burma" by Venkat Rao and Frederick Delphin, became available in 1957 but this book is now out of print. It is felt that a new guide should be prepared because some new species have been recorded and the manner of presenting the subject matter requires some alteration. In the present study, 2.2.1. identifications of anopheline larvae and 2.2.2. identifications of female anopheline mosquitoes are modified by Thin Thin Oo, after Delphin and Rao (1957).

2.2.1. Identifications of anopheline larvae

1 Inner anterior clypeal hairs more or less proximate (Fig. 2.8) **2**

Inner anterior clypeal hairs wide apart (Fig. 2.9) **7**

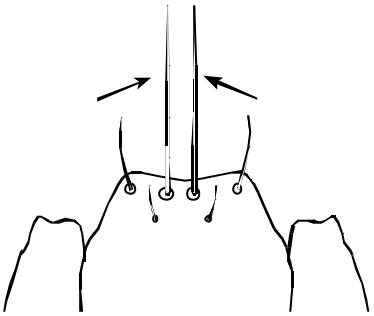


Fig. 2.8

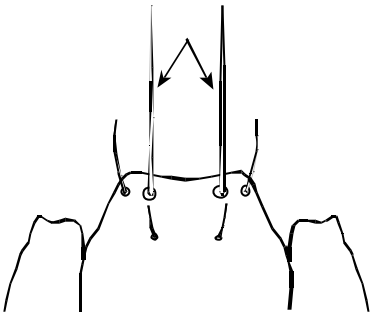


Fig. 2.9

2 (1) Inner anterior clypeal hairs simple **3**

Inner anterior clypeal hairs branched **5**

3 (2) Outer anterior clypeal hairs simple **4**

Outer anterior clypeal hairs bushy **6**

Outer anterior clypeal hairs split distally into two or three branches; posterior clypeal hairs branched (Fig. 2.10) ..... ***An. gigas***

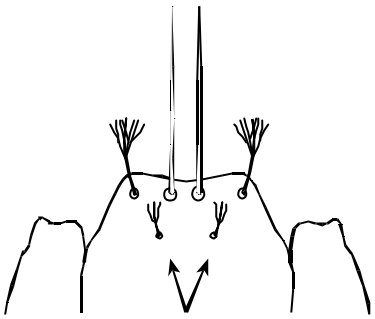


Fig. 2.10

4 (3) Posterior clypeal hairs simple (Fig. 2.11) ..... ***An. lindesayi***

Posterior clypeal hairs branched (Fig. 2.12) .....

…… ***An. insulaeflorum*** and ***An. kyondawensis**[1]**

*[1] see Appendix A.

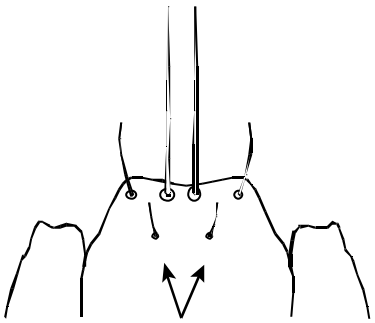


Fig. 2.11

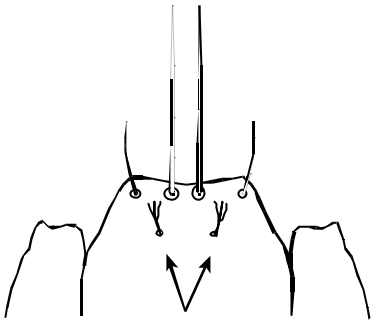


Fig. 2.12

5 (2) Inner anterior clypeal hairs split into two branches a little above base (Fig. 2.13) ..... ***An. aitkeni***

Inner anterior clypeal hairs split into three or six branches about the middle (Fig. 2.14)..... ***An. bengalensis***

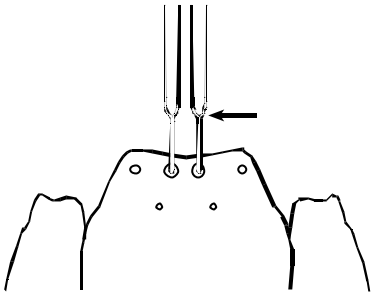


Fig. 2.13

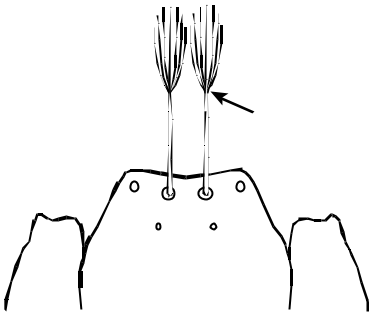


Fig. 2.14

6 (3) Innermost submedian prothoracic hair simple or bifid (Fig. 2.15) .... ***An. hyrcanus group****[2]

Innermost submedian prothoracic hair with many branches (Fig. 2.16) .... ***An. barbirostris***

*[2] see 2.2.3. Key for identification of *Anopheles hyrcanus* group

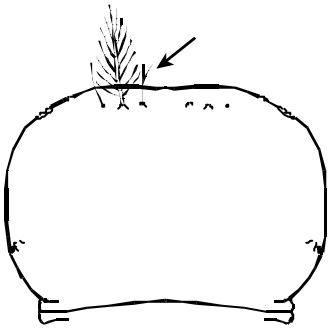


Fig. 2.15

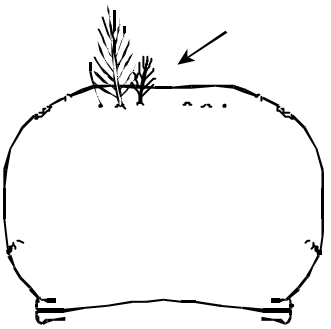


Fig. 2.16

7 (1) Anterior tergal plates on abdominal segments III to VII very large enclosing posterior tergal plates (Fig. 2.17) **8**

Anterior tergal plates not very large and not enclosing posterior tergal plates (Fig. 2.18) **10**

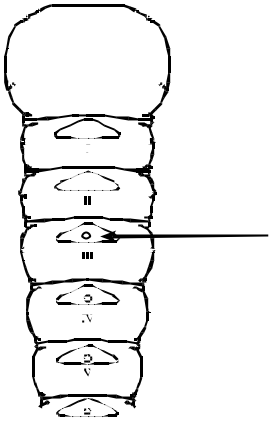


Fig. 2.17

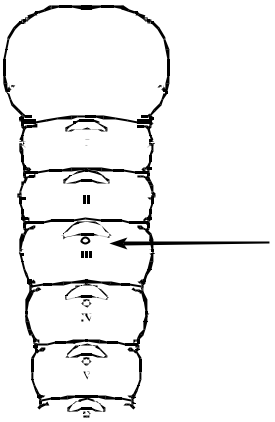


Fig. 2.18

8 (7) All clypeal hairs simple **9**

Inner anterior clypeal hairs and outer anterior clypeal hairs with short scattered branches (Fig. 2.19)..... ***An. aconitus***

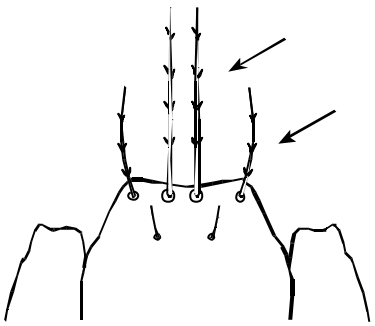


Fig. 2.19

9 (8) Minute 'O' hair arising on tergal plate of abdominal segments II-VIII on each side (Fig. 2.20)..... ***An. varuna***

Minute 'O' hair arising external to the tergal plate (Fig. 2.21)….. ***An. minimus****[3]

..... ***An. fluviatilis****[3]

*[3] Larvae of these two species are indistinguishable.

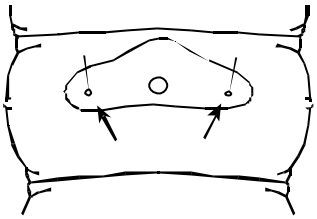


Fig. 2.20

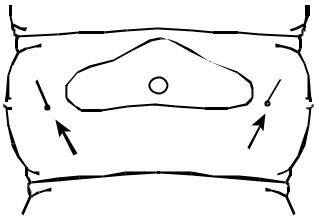


Fig. 2.21

10 (7) Posterior clypeal hairs placed interior to inner anterior clypeal hairs (Fig. 2.22) . . ..***An. vagus***

Posterior clypeal hairs not placed so..... **11**

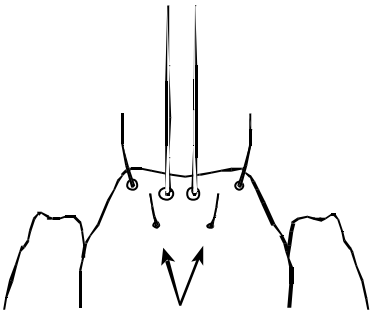


Fig. 2.22

11 (10) Inner anterior clypeal hairs and outer anterior clypeal hairs either simple or with fine inconspicuous fraying..... **12**

Inner anterior clypeal hairs and outer anterior clypeal hairs with conspicuous lateral branches **22**

12 (11) Palmate hairs on abdominal segments I-VII..... **13**

Palmate hair on abdominal segment I absent..... **17**

13 (12) All thoracic pleural hairs simple (Fig. 2.23). Inner anterior clypeal hairs faintly frayed and about four times the length of the outer anterior clypeal hairs ....***An. kochi***

Some thoracic pleural hairs pectinate **14**

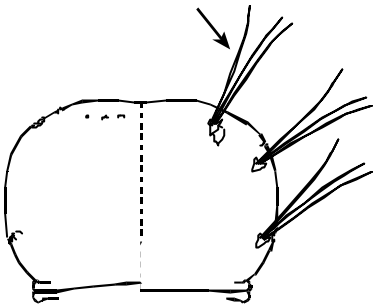


Fig. 2.23

14 (13) Both long mesothoracic pleural hairs simple but only one metathoracic pleural hair pectinate (Fig. 2.24). **15**

Both long mesothoracic pleural hairs simple and both long metathoracic pleural hairs pectinate (Fig. 2.25). **16**

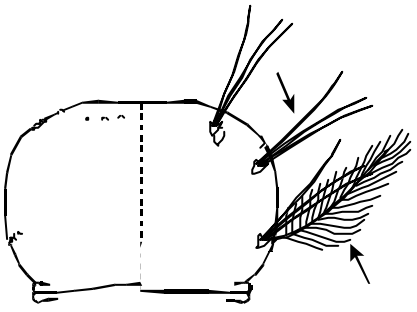


Fig. 2.24

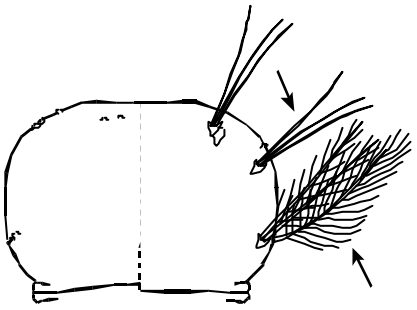


Fig. 2.25

15 (14) Filaments of abdominal palmate hairs about half as long as the blades (Fig. 2.26a).....***An. culicifacies***

Filaments of abdominal palmate hairs only about 1/4 the length of the blades (Fig. 2.26b).....***An. majidi***

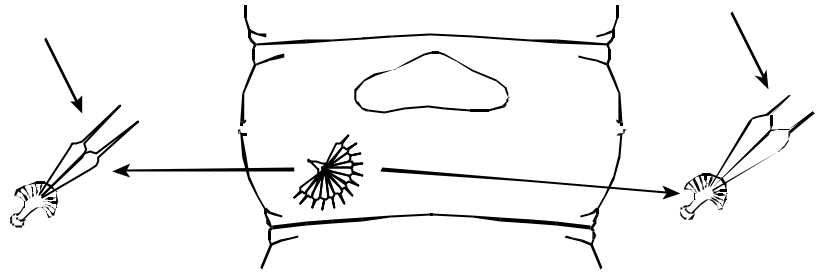


Fig. 2.26a Fig. 2.26b

16 (14) Setae on tenth abdominal segment coarse, pigmented and cone-shaped (Fig. 2.27); post-spiracular hair with eight or nine branches.....***An. sundaicus***
Setae on tenth abdominal segment slender, not pigmented and almost flattened at base (Fig. 2.28); post-spiracular hair with four or five branches.....***An. subpitus***

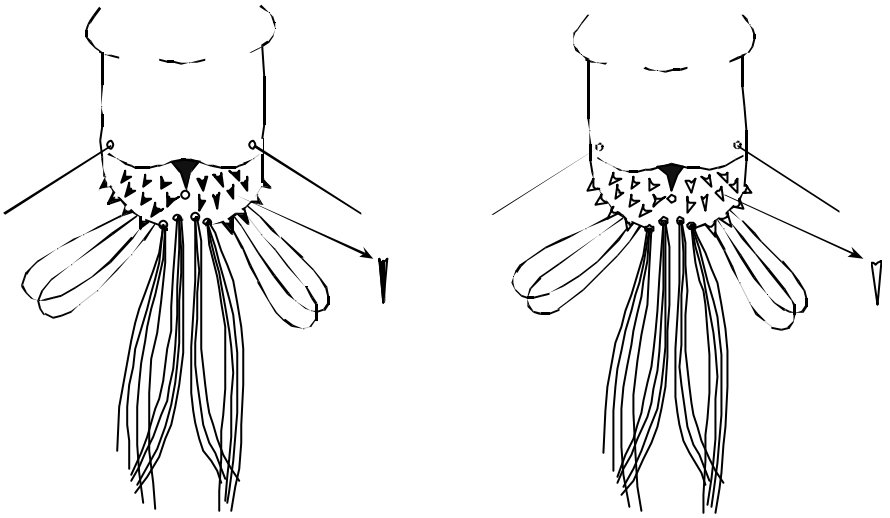


Fig.2.27 Fig.2.28

17 (12) One of the long mesothoracic pleural hairs pectinate..... **18**
All thoracic pleural hairs simple **21**

18 (17) Both outer anterior clypeal hairs and inner anterior clypeal hairs not frayed..... **19**
Both inner anterior clypeal hairs and outer anterior clypeal hairs finely frayed. **20**

19 (18) Second hair on first abdominal segment with three to five branches (Fig. 2.29) ...
.....***An. stephensi***

20 (18) Second hair on first abdominal segment with six to eight branches.(Fig. 2.30)…….. ..... ***An. maculatus*** and ***An. willmori****[4]
.....***An. theobaldi****[5]
*[4] see Appendix B.
*[4] and *[5] Larvae of these three species are indistinguishable.

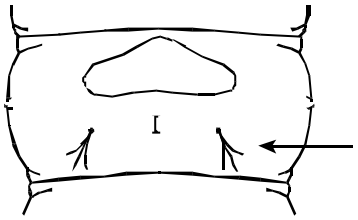


Fig. 2.29

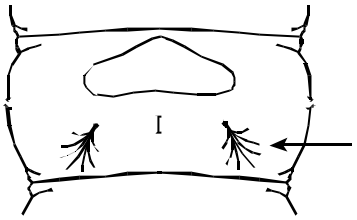


Fig. 2.30

21 (17) Innermost submedian prothoracic hair with only two to four branches arising from an inconspicuous root..... ***An. tessellatus***
Innermost submedian prothoracic hair with more than four branches arising from a large root .....***An. dirus***

22 (11) Outer anterior clypeal hairs with long lateral branches **23**
Outer anterior clypeal hairs with short lateral branches **26**

23 (22) Inner sutural hairs simple or bifid (Fig. 2.31)..... **24**
Inner sutural hairs branched from base (Fig. 2.32)..... **25**

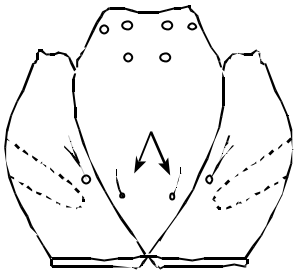


Fig. 2.31

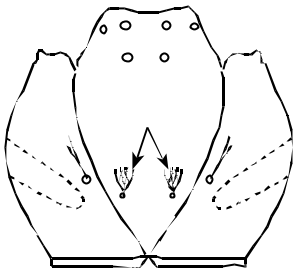


Fig. 2.32

24 (23) Palmate hair on abdominal segment I well developed ..... ***An. annularis***
Palmate hair on abdominal segment I not well developed .....***An. jamesii***

25 (23) Posterior clypeal hairs with two to five branches .....***An. pallidus***
Posterior clypeal hairs with seven to ten branches .....***An. philippinensis***

26 (22) ..One of the long metathoracic pleural hairs simple **27**
Both the long metathoracic pleural hairs branched..... **28**

27 (26) Outer anterior clypeal hairs with a large number of branches from the base; anterior tergal plates rather large and concave…….....***An. jeyporiensis***

28 (26) Inner anterior clypeal hairs long; palmate hair on abdominal segment II very poorly developed (Fig. 2.33)..... ***An. pseudojamesi***
Inner anterior clypeal hairs normally long; palmate hair on abdominal segment II well developed (Fig.2.334) **29**

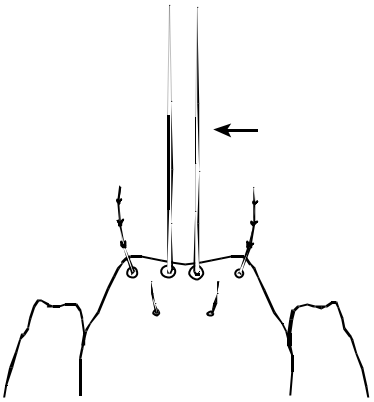


Fig. 2.33

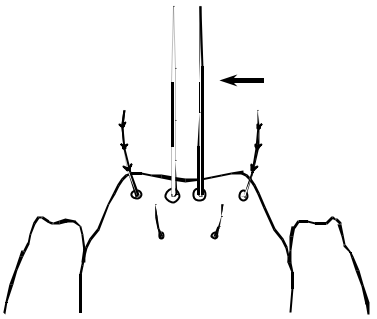


Fig. 2.34

29 (28) Inner sutural hair simple ..... ***An. karwari***

Inner sutural hair split into two or four branches..... ***An. splendidus***

2.2.2. Indentifications of female anopheline mosquitoes

1 Wings not spotted .....***An. aitkeni*** *[1]

.....***An. bengalensis****[1]

.....***An. insulaflorum****[1]

Wings spotted **2**

*[1] These three species can be distinguished from each other in the larval stage and by the examination of the male genitalia.

2 (1) Fewer than four dark areas involving both the costa and first longitudinal vein of wing (Fig. 2.35) **3**

At least four dark areas involving both the costa and first longitudinal vein of wing (Fig. 2.36) **7**

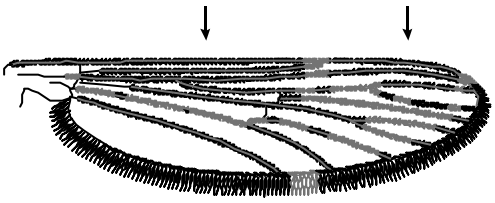


Fig. 2.35

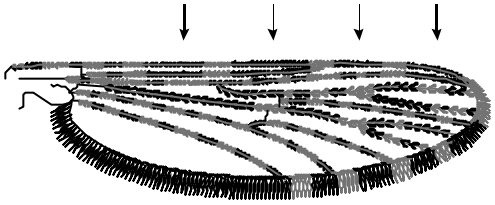


Fig. 2.36

3 (2) Inner quarter of costa mainly pale .....***An. gigas***

Inner quarter of costa mainly dark **4**

4 (3) Hind femur with a broad white band, about the middle (Fig. 2.37) ..***An. lindesayi***

Hind femur without a broad white band; a prominent tuft of scales on ventral surface of abdominal segment VII (Fig. 2.38) **5**

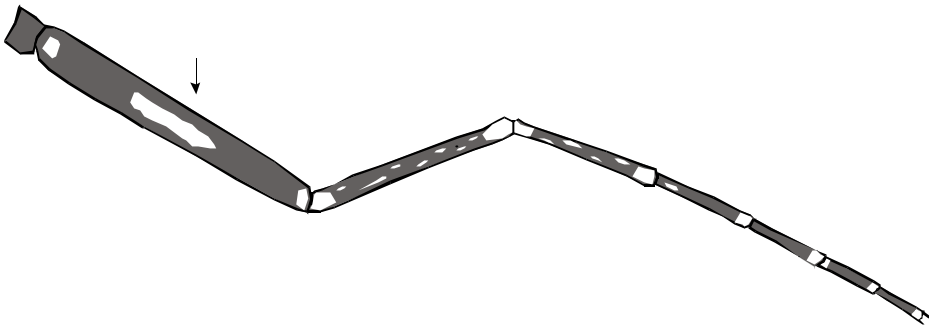


Fig. 2.37

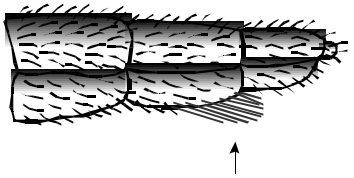


Fig. 2.38

5 (4) Palps mainly dark and basal half of vein 6 with some black scattered scales ....***An. barbirostris***

Palps with some narrow pale bands; basal half of vein 6 with only a few black scales near the base **6**

6 (5) Tarsal segment IV of hind leg pale distally only; sub-costal pale area involving vein 1 also.....***An. hyrcanus* group*[2]**

*[2] see 2.2.3. Key for identification of *Anopheles hyrcanus* group.

7 (2) Last tarsomeres of hind legs white **21**

Last tarsomeres of hind leg dark..... **8**

8 (7) Femora and tibiae speckled (Fig. 2.39) **17**
Femora and tibiae not speckled **9**

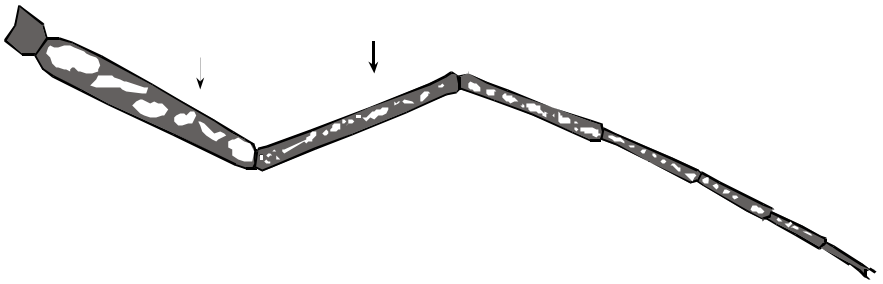


Fig. 2.39

9 (8) Joints between tarsal segments of forelegs with basal and apical broad pale bands..... **10**
Joints between tarsal segments without pale bands or only with basal and apical narrow pale bands..... **11**

10 (9) Palps of female with a dark pre-apical band equal or nearly equal to the apical pale band (Fig. 2.40) .....***An. subpictus***
Palps of female with a dark pre-apical band not more than half the length of the apical band (Fig. 2.41).....***An. vagus***

Fig. 2.40 Fig. 2.41

11 (9) Third longitudinal wing vein mainly or entirely dark. Inner quarter of costa with a pale interruption and with an opposing dark area on the first longitudinal vein (Fig. 2.42) ..... ***An. culicifacies***

Third longitudinal wing vein mainly or entirely pale (Fig. 2.43) **12**

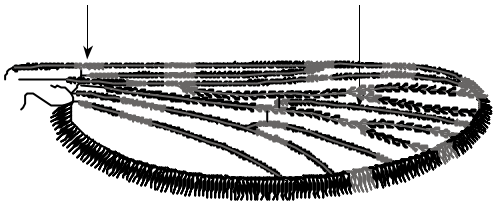


Fig. 2.42

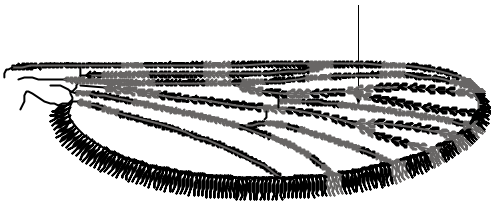


Fig. 2.43

12 (11) Palps with two pale distal bands separated by an intervening dark band much longer than the apical pale band (Fig. 2.44) **16**

Palps with two pale distal bands separated by an intervening dark band about equal to or narrower than the apical pale band (Fig. 2.45) **13**

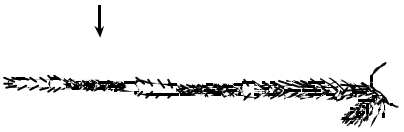


Fig. 2.44

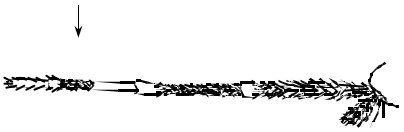


Fig. 2.45

13 (12) With a fringe spot at the termination of vein 6 (Fig. 2.46) **14**

Without a fringe spot at the termination of vein 6 ..... **15**

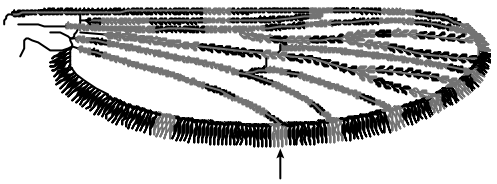


Fig. 2.46

14 (13) Pre-apical dark band on palp very narrow (Fig. 2.47); tarsal joints of hind and fore-legs without white bands..... ***An. aconitus***

Fig. 2.47

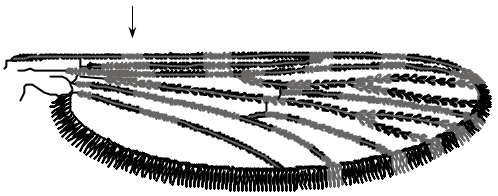


Fig. 2.48

15 (13) Basal area of costa with a pale interruption, or an indication of such at least on one wing; proboscis without flavescence (Fig. 2.48)..... ***An. minimus***
Basal area of costa without any pale interruption, proboscis with flavescence all round the distal half..... ***An. varuna***

16 (12) Tarsal joints of hind and fore-legs with narrow but distinct white bands; inner quarter of costa with at least one interruption and fringe spot at termination of vein 6..... ***An. jeyporiensis***
Tarsal joints of hind and fore-legs without distinct white bands; inner quarter of costa without any interruption and no fringe spot at termination of vein 6. .....
..... ***An. fluviatilis***

17 (8) Front tarsal joints without broad pale bands **20**
Front tarsal joints with broad pale bands **18**

18 (17) Palps with three pale bands, dark pre-apical band equal or nearly equal to the apical pale band (Fig. 2.40) ..... ***An. sundaicus***
Palps with four pale bands (Fig. 2.49) and vein 6 with five or six dark areas.... **19**

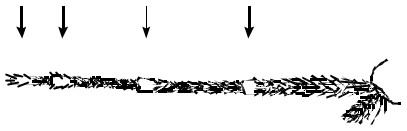


Fig. 2.49

19 (18) Tibio-tarsal joint of hind leg with a broad and conspicuous white band (Fig. 2.50)..... ***An. dirus***
Tibio-tarsal joint of hind leg without such band (Fig. 2.51)..... ***An. tessellatus***

20 (17) Sixth vein with not more than three dark areas; the pre-apical dark band on palpi much narrower than the apical and basal pale bands and palpi usually speckled...
…….. ***An. stephensi***

21 (7) Femora and tibiae not speckled **22**
Femora and tibiae speckled **26**

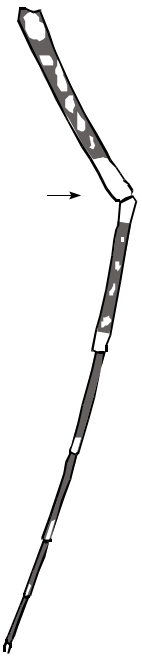


Fig. 2.50

Fig. 2.51

22 (21) At least two terminal tarsal segments completely white **23**
Only the terminal tarsal segment completely white with two broad white bands above this **25**

23 (22) Fifth vein mainly dark with a dark area near the fork (Fig. 2.52) ... ***An. annularis***
Fifth vein mainly pale without a dark area near the fork (Fig. 2.53)..... **24**

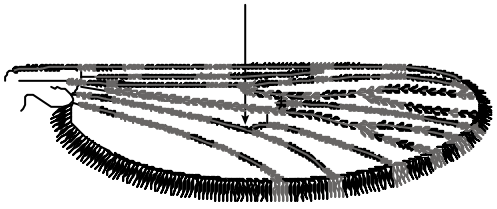


Fig. 2.52

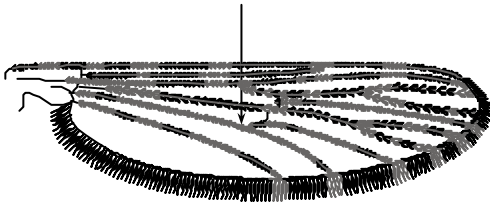


Fig. 2.53

24 (23) End of first tarsal segment never picked out with white (Fig. 2.54) ..***An. pallidus***
End of first tarsal segment usually picked out in some degree with white (Fig. 2.55).....***An. philippinensis***

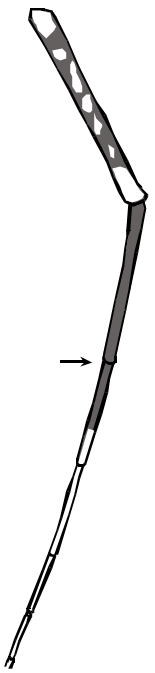


Fig. 2.54

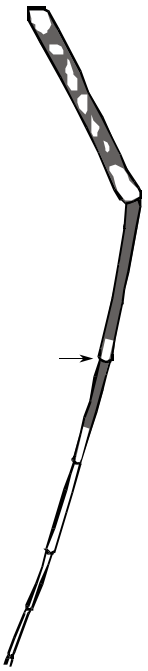


Fig. 2.55

25 (22) Palps with two narrow and two broad white bands, including the apical band (Fig. 2.56).....***An. karwari***
Palps with only one narrow and two broad white bands, including the apical white band (Fig. 2.57).....***An. majidi***

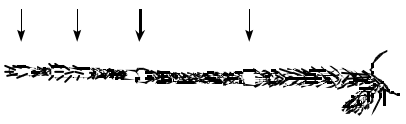


Fig. 2.56

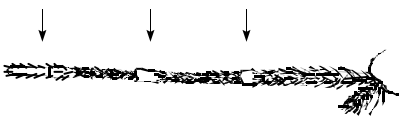


Fig. 2.57

26 (21) Abdomen with a row of conspicuous tufts of black scales on ventral surface (Fig. 2.58) ..... ***An. kochi***
Abdomen without such tufts **27**

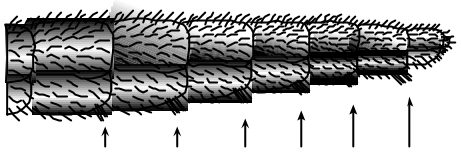


Fig. 2.58

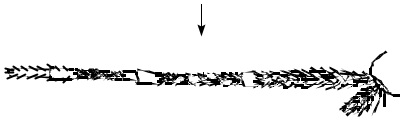


Fig. 2.59

27 (26) Palps speckled (Fig. 2.59) ..... ***An. splendidus***
Palps not speckled **28**

28 (27) Less than three terminal tarsal segments completely white **29**
Three terminal tarsal segments completely white **30**

29 (28) Whole of the terminal tarsomere white and the next tarsomere with two broad pale bands (Fig. 2.60) ..... ***An. maculatus*** and ***An. willmori***
Two terminal tarsomere completely white and the next tersomere with one broad pale band above this (Fig. 2.61) ..... ***An. theobaldi***

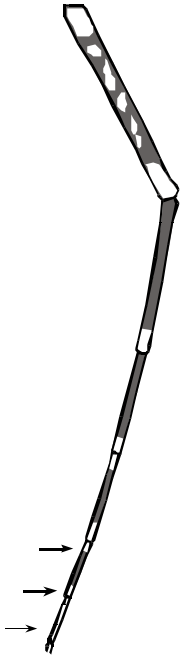


Fig. 2.60

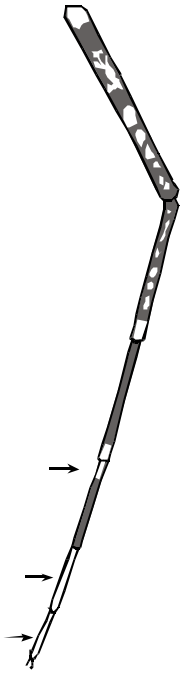


Fig. 2.61

30 (28) Inner quarter and outer third of costa mainly pale (Fig.2.62).....***An. jamesii***

Inner quarter and outer third of costa mainly dark (Fig.2.63) ... ***An. pseudojamesi***

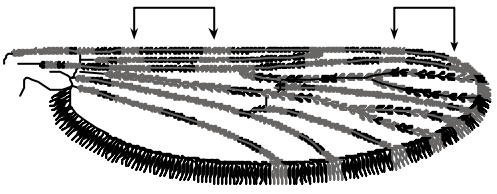


Fig. 2.62

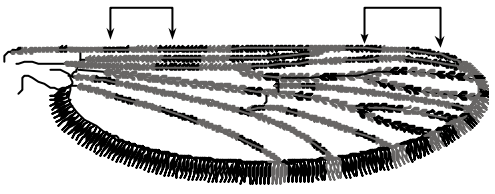


Fig. 2.63

2.2.3. Key for identification of *Anopheles hyrcanus* group

Key for identification of *Anopheles hyrcanus* group (larvae and adult) was illustrated by Reid (1953).

Larvae

1 Mesothoracic hair 5 small, with sinuate horizontally spreading branches arising together from the base……...…….……...…….***An. paditaeniatus***
Mesothoracic hair 5 not so, the branches straight, stiff and more or less erect ….**2**

2 (1) Sutural hair with numerous branches (13-23), commonly 17 (Fig. 2.64). Antennal shaft rather slender, usually with rather large, coarse, erect teeth. Tergal plate on abdomen VII between two-thirds and three-quarters as long as wide, usually tapering posteriorly more or less in the form of a truncated wedge. Pigmentation of palmate hairs usually uniform and rather dence. Saddle hair strong, at least as long as the width of segment VIII. Usually seven long teeth on the pecten, rather fewer…….….. ***An. argyropus***
Without this combination of characters. If the sutural hair has more than 12 branches, then either tergal plate VII is large and transversely rectangular, less than two-thirds as long as wide, and the palmate hairs are large with the pigmentation generally less dense and not uniform, paler towards the base of the leaflets (*nigerrimus*), or the saddle hair is weak, less than the width of segment VIII, and the pecten has more than six long teeth (*nitidus*)……...…… …..**3**

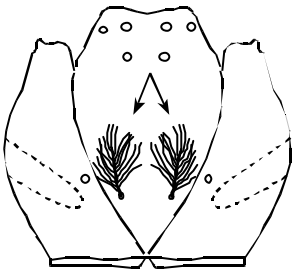


Fig. 2.64

3 (2) Palmate hairs large, the pigmentation often not uniform nor very dense, but paler at the base, often extending well into the tips of the leaflets. Tergal plate VIII transverse, usually less than two-thirds as long as wide. Spiracles large **4**
Palmate hairs somewhat smaller, pigmentation commonly uniform and dense and not extending much into the tips of the leaflets. Tergal plate VIII usually less transverse, two-thirds or more as long as wide. Spiracles smaller **5**

4 (3) Abdomen VI, hairs 5 and 9 with 2-5 branches, usually three or four. Sutural hair with 12-24 branches, average 17 ..... ***An. nigerrimus***
Abdomen VI, hairs 5 and 9 with 6-11 branches, average eight (Fig. 2.65). Sutural hair with 8 to 13 branches, average 11 ..... ***An. sinensis***

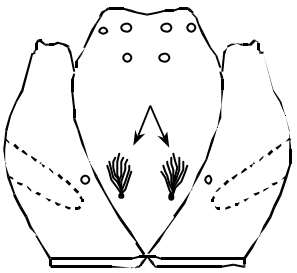


Fig. 2.65

5 (3) Sutural hair with 11-17 branches, average 13. Abdomen III, hair 9, 10-16 branches. Saddle hair weak, not as long as the width of segment VIII (Fig. 2.66). Pecten seldom with more than six long teeth..... ***An. nitidus***

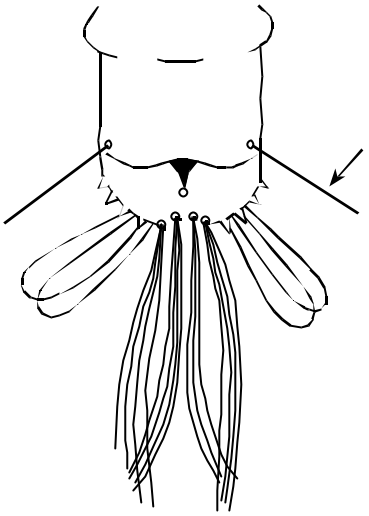


Fig. 2.66

Adult

The *Anopheles hyrcanus* group belongs to the series Myzorhynchus and is distinguished from most other species by the presence in the female: (i) of a tuft of dark scales on the clypeus on each side; (ii) more readily observed pale bands on the palps (usually four) one of which is always apical; (iii) ventral tuft of dark scales on the seventh abdominal segment; (iv) fifth hind tarsus not all white (rare exceptions occur in *An. argyropus*) and (v) stem of vein five always with a well-defined dark mark towards the base.

1 Pale bands on hind tarsi narrow, fourth segment without basal pale band……...**2**

Pale bands on hind tarsi moderately broad to very broad, fourth segment with basal pale band…….…….**4**

2 (1) Apical fringe spot not very short, extending at least from 2.1 to 3. Basal dark mark on 5 short, separated by its own length or more from the upper mark on 6. Coxites of the male genitalia with pale scales……..……....**3**

3 (2) Wing pattern blurred. Tip of vein 1 dark apical fringe spot longer commencing at or above vein 1, fringe spot usually present at 5.2, apical dark mark on 6 longer than that on 5.2, some pale scales on 1 between subcostal and preapical pale spots ……..*****An. sinensis***

4 (1) Wing pattern bright, the dark marks mostly short and well defined. Basal half of the costa always with some pale scales (Fig. 2.67), basal dark mark on 5 separated by its own length or more from the upper mark on 6. Seldom more than four propleural setae ……..***An. nitidus***

Wing pattern darker, more or less blurred. Basal dark mark on 5 approaching to within its own length or less of the upper mark on 6.Basal half of the costa without pale scales, except in *An. nigerrimus* which seldom has fewer than seven propleural setae……...……..……..……**5**

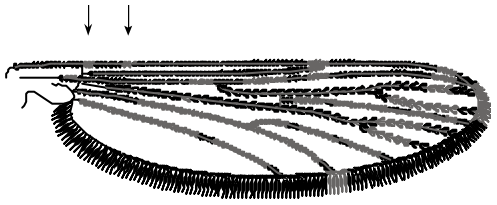


Fig. 2.67

5 (4) Third hind tarsal pale band seldom longer than the fifth segment. Costa usually with one or two pale scales toward the base, often a fringe spot at 5.2, tip of the abdomen on each side (eight tergite) usually with a few narrow scales ……...…. ……...……..……..***An. nigerrimus***

Third hind tarsal pale band longer than the fifth segment. Basal half of costa without pale scales, no fringe spot at 5.2. Seldom any scales at the tip of the abdomen ……...……..……..**6**

6 Hind tarsal pale bands very broad, third band more than three-quarters the length of the fourth segment (Fig. 2. 68); mid-tarsal bands narrow, the third band about one-quarter the length of the third segment. Wing dark; no pale scales on vein 1 between subcostal and preapical pale spots……..…….……***An. argyropus***

Hind tarsal pale bands not so broad, third band usually less than three-quarters as long as the fourth segment; mid-tarsal bands broad, the third band one-third or more as long as the segment. Wing lighter, with pale scales usually numerous, on vein 1 between subcostal and preapical pale spots…… ……***An. peditaeniatus***

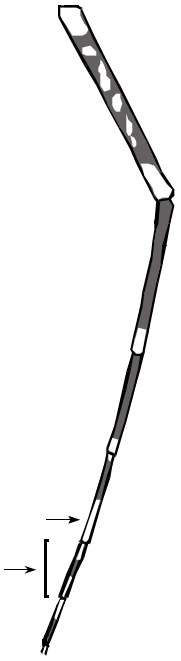


Fig. 2.68

2.3. Studies of the ecology of anophelines

2.3.1. Study areas

The locations where anophelines were collected, are as indicated in the following (brackets show the number of sampling sites):

(1) Yangon Division, Fig. 2.69

(a) Forest area

(1) Yesitkan village (Yangon water supply area), Taikkyi Township area - reservoir and catchment area with thick forests and low foothills with some cultivation nearby.

(2) Leinmawchan village, Taikkyi Township area - low foothills with thick vegetation and forest.

(3) Yenetgyi village, Hlegu Township area (road construction camp) - near forest and low foothills.

(4) Phaunggyi village, Hlegu Township area - near forest and low foothills.

(b) Rice cultivated area

(5) Kyaukain village, Hlegu Township area - paddy cultivating plain.

(c) Urban area

(6) Insein Strand Road area, Insein Township area - collection site in cattle-shade near a pond. Semi-urban, lower and middle class residential area.

(7) Ayeyar 15th Street, North Okkalapa Township area - near paddy fields and small ponds.

(8) Ngadatkyi Pagoda Road area, Bahan Township area - many small ponds and streams blocks to store water for gardening. Residential area.

(9) Thiri Avenue, Pyay Road, Mayangon Township area - high class residential area, one kilometre (km) from Inya lake.

(2) Bago Division, Fig. 2.70

(a) Forest area

(10) Kaingdawsu village, Okpo Township area - situated between paddy fields and streams near foothills.

(11) Yebya timber extraction camp, Okpo Township area - deep forest timber extraction camp on western slope of Bago Yoma range with many streams.

(12) Myochaung village, Kyauktagar Township area - foothill village with paddy fields on one side.

(13) Htiphado, Kyauktagar Township area - deep forest wood-cutting camp in Bago Yoma range, foothill village of Bago Yoma range.

(14) Phado and Kyarkyaungthaik villages, Kyauktagar Township area - temporary settlements on edge of forested foothills.

(15) Katsene camp, Kyauktagar Township area - timber extraction camp in Bago Yoma range. Deep forest.

(16) Thabyewa village, Oktwin Township area - village surounded by teak forest reserve and teak plantations. Situated in a valley in Bago Yoma range at an altitude of 213.4 m above sea level.

(17) Hsachaung village, Letpadan Township area - near foothill with light scrub jungle.

(18) Khintan village, Pyu Township area - foothill area with paddy fields nearby.

(b) Rice cultivated area

(19) Hlelangu village, Thayawady Township area - near scrub jungle with paddy fields nearby.

(20) Ngokto and Gwegone villeges, Kyauktagar Township area - villages within 2.4 km of forested foothill with paddy fields on one side.

(21) Bwechin village, Kyauktagar Township area.

(22) Taungoo Town, Taungoo Township area.

(23) Sezongone village, Yedashe Township area.

(24) Bago Town, Bago Township area.

Locations 21 to 24 are paddy cultivation plain.

(c) Urban area

(25) Thayarwady Town, Thayarwady Township area - edge of town, about 0.3 km from paddy fields.

(26) Shwekuyad, Htanpauk and Kyauklongyi villages, Pyay Township area - upper delta plain neighbouring foothills of Bago Yoma range.

(27) Gonemingone and Taunglae villages, Paukhaung Township area

(28) Shwedaung Town, Shwedaung Township area

Locations 27 and 28 situated in the lower Ayeyarwady valley in the neighbouring foothills of Bago Yoma range.

(3) Ayeyarwady Division, Fig. 2.71

Rice cultivation area

(29) Magyipin village, Myaungmya Township area.

(30) Seikkyi village, Chaungthar Township area.

(31) Chaungthar village, Chaungthar Township area.

Locations 29 to 31 are lower Ayeyarwady delta (plain).

(32) Hinthada Town, Hinthada Township area.

(33) Thabyuchaung and Thalatkhwar villages, Pathein Township area.

(34) Kaditchaung village, Ngaputaw Township area.

Locations 32 to 34 are in the lower Ayeyarwady delta adjoining sea coast.

(4) Mandalay Division, Fig. 2.72

(a) Irrigated plain area

(35) Sedawgyi Town, Madaya Township area - near foothills and forest, irrigation scheme area.

(36) Botegone and Zeephyupin villages, Madaya Township area

(37) Mandalay Town, Mandalay Township area

(38) Shwesaryan and Tawsu villages, Patheingyi Township area

(39) Sargalein and Nyaungnibin villages, Amarapura Township area

(40) Nweyone and Shwepyi villages, Singu Township area

Locations 36 to 40 are irrigated plains close to foothill.

(41) Sezone and Badaquinn villages, Sintgaing Township area

(42) Aungpintha village, Meiktila Township area

(43) Lunkyaw village, Kyaukse Township area

Breeding sites 41 to 43 are plains with extensive irrigation.

(44) Legwa village, Kyaukpadaung Township area

(45) Shwehlaing village, Nyaungoo Township area

(46) Semikan and Magyibin villages, Thaungtha Township area

Locations 44 to 46 are irrigated plains at the foot of Mount Popa (dead volcanic mountain).

(b) Hilly area

(47) Tagaung and Taungtalone villages, Thabeikkyin Township area - narrow valley.

(48) Shwenyaungbin village, Mogok Township area - narrow valley.

(49) Kabaing village, Mogok Township area - hilly area with forest at an altitude of about 1219.5 m above sea level.

(c) Plateau area

(50) Wetwun village, Pyinoolwin Township area - plateau area about 914.6 m altitude, farming area.

(d) Forest area

(51) Moeswe and Aungthukha villages, Pyinmana Township area - timber extraction area.

(52) Mandalay-Pyinoolwin area - foothills.

(e) Plain area

(53) Wundwinvillage, Meiktila Township area

(54) Indaingtha village, Thazi Township area

Locations 53 and 54 are plains, close to Shan Plateau.

(55) Kinywa-indaing village,Yamethin Township area

(56) Chinsu and Kantha villages, Lewe Township area

Locations 55 and 56 are lowlands interspersed by foothills.

(5) Sagaing Division, Fig. 2.73

(a) Valley area

(57) Khamaya 368 (Military camp), Mawleik Township area - Chindwin valley and foothill area.

(58) Kabaw valley, Kale Township area

(59) Kale valley, Kale Township area

Locations 58 and 59 are narrow jungle valley areas.

(60) Tamu Town and Bokekan village, Tamu Township area - narrow valley adjoining Indian-border.

(61) Myothit Quarter, Sagaing Township area

(62) Monywa Town, Monywa Township area

Locations 61 and 62 situated in Chindwin valley.

(63) Bonchaung and Paygone villages, Wuntho Township area

(64) Nabar village, Inndaw Township area

Locations 63 and 64 are valley in hill ranges with forests and irrigated tracts.

(65) Katha Town, Katha Township area - valley in hill ranges.

(b) Plain area

(66) Shwebo Town, Shwebo Township area - irrigated plains.

(6) Magwe Division, Fig. 2.74

Plain area

(67) Ywathitgyi village,Yenangyaung Township area

(68) Mingyan village, Chauk Township area

Locations 67 and 68 are oilfields.

(69) Lingadaw village, Myaing Township area

(70) Yinkekaung village, Saw Township area

(71) Khaungtone village, Gangaw Township area

(72) Sinlan village, Pakoku Township area

(73) Koeidaung village, Seikpyu Township area

Locations 69 to 73 are lowlands and Ayeyarwady valley. Some districts are close to the Western Range.

(74) Kamontaung and Thabyepin villages, Taungdwingyi Township area

(75) Khinkaung village, Natmauk Township area

(76) Yeayetaing village, Myothit Township area

(77) Tegyigone village, Magwe Township area

(78) Mezali Town, Mezali Township area

(79) Zitaw village, Yesagyo Township area

(80) Mogaung village, Myayde Township area

(81) Ywathit village, Thayet Township area

(82) Gyopin village, Kama Township area

(83) Nyaungbintha village, Sinbaungwe Township area

(84) Zeedaw village, Mindon Township area

(85) Thanatwa village, Pwinbyu Township area

(86) Minbu Town, Minbu Township area

(87) Sagu Town, Sagu Township area

(88) Linzin village, Salin Township area

(89) Sedoktaya Town, Sedoktaya Township area

(90) Padan village, Ngape Township area

All locations (74 to 90) are irrigated plains close to foothills in the dry Zone of Central Myanmar.

(7) Tanintharyi Division, Fig. 2.75

(a) Forest area

(91) Shwedu Hydroelectric Station area, Myeik Township area - forested hill area with a small stream.

(92) Hangadaing camp, Bokpyin Township area - deep forest timber extraction camp near sea with jungle streams under dense shade.

(93) Namtun border camp, Kawthaung Township area - deep forest timber extraction camp adjacent to Itshmus of Kra on Thailand. Hilly topography with many perennial jungle streams.

(b) Urban area

(94) Sanchi village, Dawei Township area - coastal urban area.

(c) Rural area

(95) Monsu village, Myeik Township area - small village near foothills and rubber plantations.

(8) Rakhine State, Fig. 2.76

(a) Plain area

(96) Sittwe town, Sittwe Township area - coastal urban area with many small ponds for growing watercress.

(97) Kyaukpyu Town, Kyaukpyu Township area - low-lying land which forms north-east point of Ramree Island area.

(98) Myebon Town, Myebon Township area - plain close to foothills.

(99) Gwa Town, Gwa Township area - coastal plain near foothills.

(b) Hillock area

(100) Myanmar-Bangladesh border, Taungpyo Township area - mountain and wet hillock area.

(101) Taungpyo Town, Taungpyo Township area

(102) 10^{th} miles, Maungdaw Township area

(103) Minbya Town, Minbya Township area

Locations 101 to 103 are mountains and wet hillock areas adjoining sea coast.

(104) Shwegudaung village, Myohaung Township area

(105) Kardi village, Buthedaung Township area

(c) Foothill area

(106) Ann Town, Ann Township, foothill area.

(9) Mon State, Fig. 2.77

Plain area

(107) Taungwaing and Innwaing villages, Mawlamyine Township area

(108) Tagukkana village, Chaungzone Township area

(109) Wetlay village, Thanbyuzayat Township area

(110) Kalinekaning village, Kyaikmaraw Township area

(111) Kawkalok village, Mawlamyine Township area

Locations 107 to 111 have topography ranging from coastal plain through valleys to foothills.

(112) Mudon Town, Mudon Township area - edge of town near rubber plantation.

(113) Thinhtaw village, Thaton Township area

(114) Botayza village, Belin Township area

(115) Kinmonchaung village, Kyaikto Township area

Locations 113 to 115 are plains.

(10) Shan State, Fig. 2.78

Plateau area

(116) Khalaya 223 (Military camp) - Momeik Township area.

(117) Hsipaw Town, Hsipaw Township area.

(118) Mansan fall, Lashio Township area.

(119) Mabein Town, Mabein Township area.

(120) Quarter (9), Kyaukme Town, Kyaukme Township area.

(121) Loilem Town, Loilem Township area.

(122) Kunlong Town, Kunlong Township area.

(123) Myanmar-China border, Chinshwehor Township area.

(124) Kengtung Town, Kengtung Township area.

(125) Tachileik Town, Tachileik Township area.

(126) Heho Town, Heho Township area.

(127) Hopone Town, Hopone Township area.

(128) Yutsawk Town, Yutsawk Township area.

(129) Aungban Town, Aungban Township area.

(130) Thayetgon village, Pindaya Township area.

(131) Nyaungshwe Town, Nyaungshwe Township area.

(132) Myogyi village, Ywangan Township area.

(133) Nanpandit village, Kalaw Township area.

(134) Namsam Town, NamsamTownship area.

(135) Phetmun village, Shwenyaung Township area.

(136) Aungthapye village, Sesai Township area.

(137) Indaw Town, Indaw Township area.

(138) Yebu village, Taunggyi Township area.

All areas situated in Shan Plateau with an average elevation of 900 m Topography ranges from foothills through narrow valleys to areas with terraced rice cultivation.

(11) Kayah State, Fig. 2.79

Valley area

(139) Mawchi mines, Mawchi Township area.

(140) Myenigone village, Demoesoe Township area.

(141) Nwalasoe village, Loikaw Township area.

All areas (locations 139 to 141) comprised with Shan Plateau. Most of the areas lie between 450 and 900 m above sea level.

(12) Kachin State, Fig. 2.80

Foothill area

(142) Mawphaung village, Myitkyina Township area - upper Ayeyarwady valley.

(143) Aungthapye village, Mohnyin Township area - foothills with forests.

(144) Lawa village, Kamaing Township area.

(145) Minekune village, Tanaing Township area.

(146) Kyuntaw village, Moegaung Township area.

Locations 144 to 146 are valleys with forests.

(147) Bhamo Town, Bhamo Township area.

(148) Myothit Town, Myothit Township area.

Locations 147 and 148 are foothills with forest.

(13) Chin State, Fig. 2.81

Hilly area (southern part)

(149) Panunchaung village, Falam Township area.

(150) Htaungza village, Paletwa Township area.

(151) Mindat Town, Mindat Township area.

(152) Syawlaung village, Kanpetlet Township area.

Locations 149 and 152 are hilly areas with forest.

(14) Kayin State, Fig. 2.82

Foothill area

(153) Thandaung Town, Thandaung Township area - foothill with forests.

(154) Khamaya 231 (Military camp) - Kawkareik Township area.

(155) Kyaikdon village, Kyaraingseikkyi Township area.

Locations 154 and 155 are foothills and valleys.

(156) Kamanoung village, Pyapon Township area.

(157) Haungkhayaing village, Hpaan Township area.

Locations 156 and 157 are foothills.

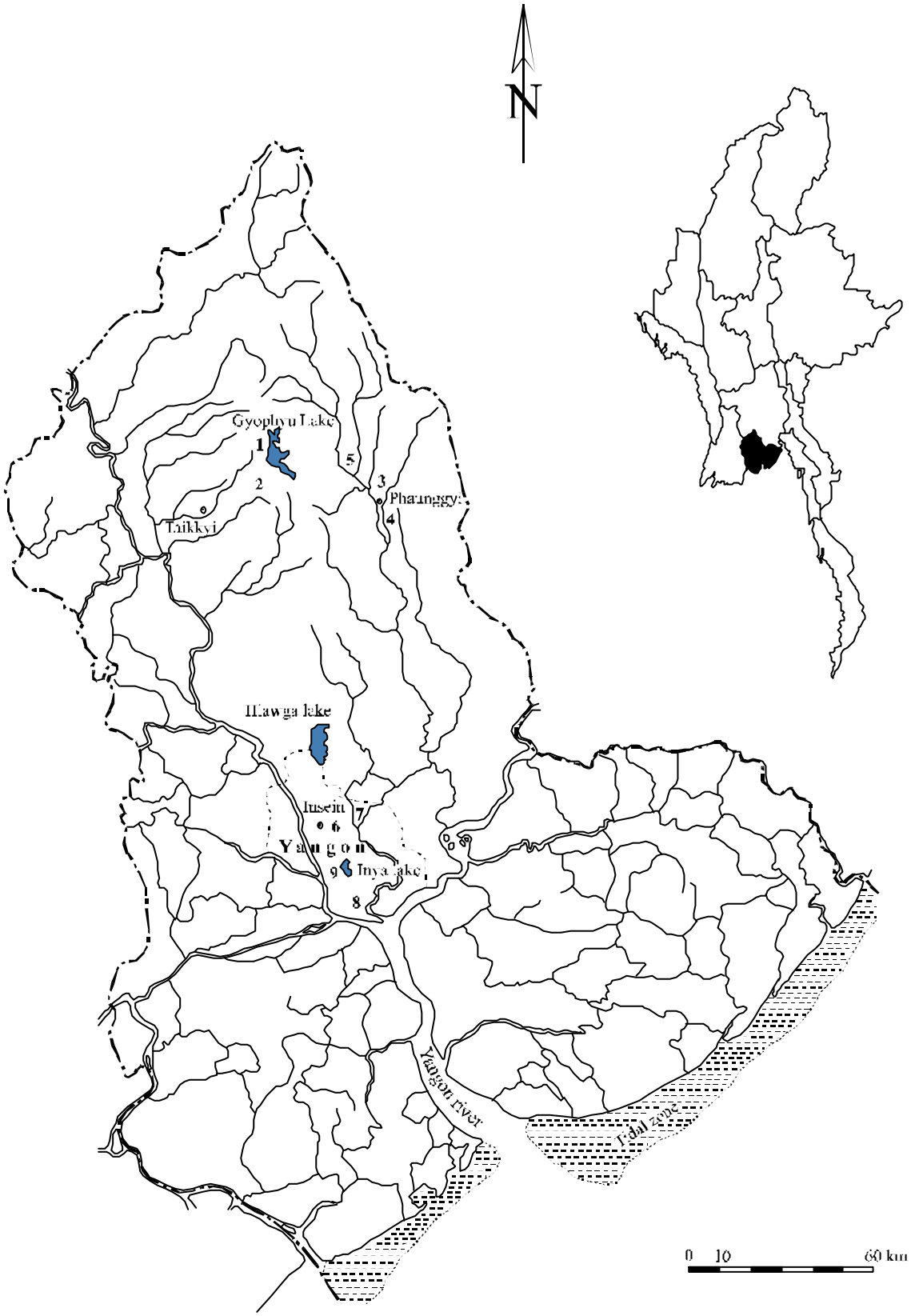


Fig. 2.69. Yangon Division
1 to 9 – locations where anophelines were collected

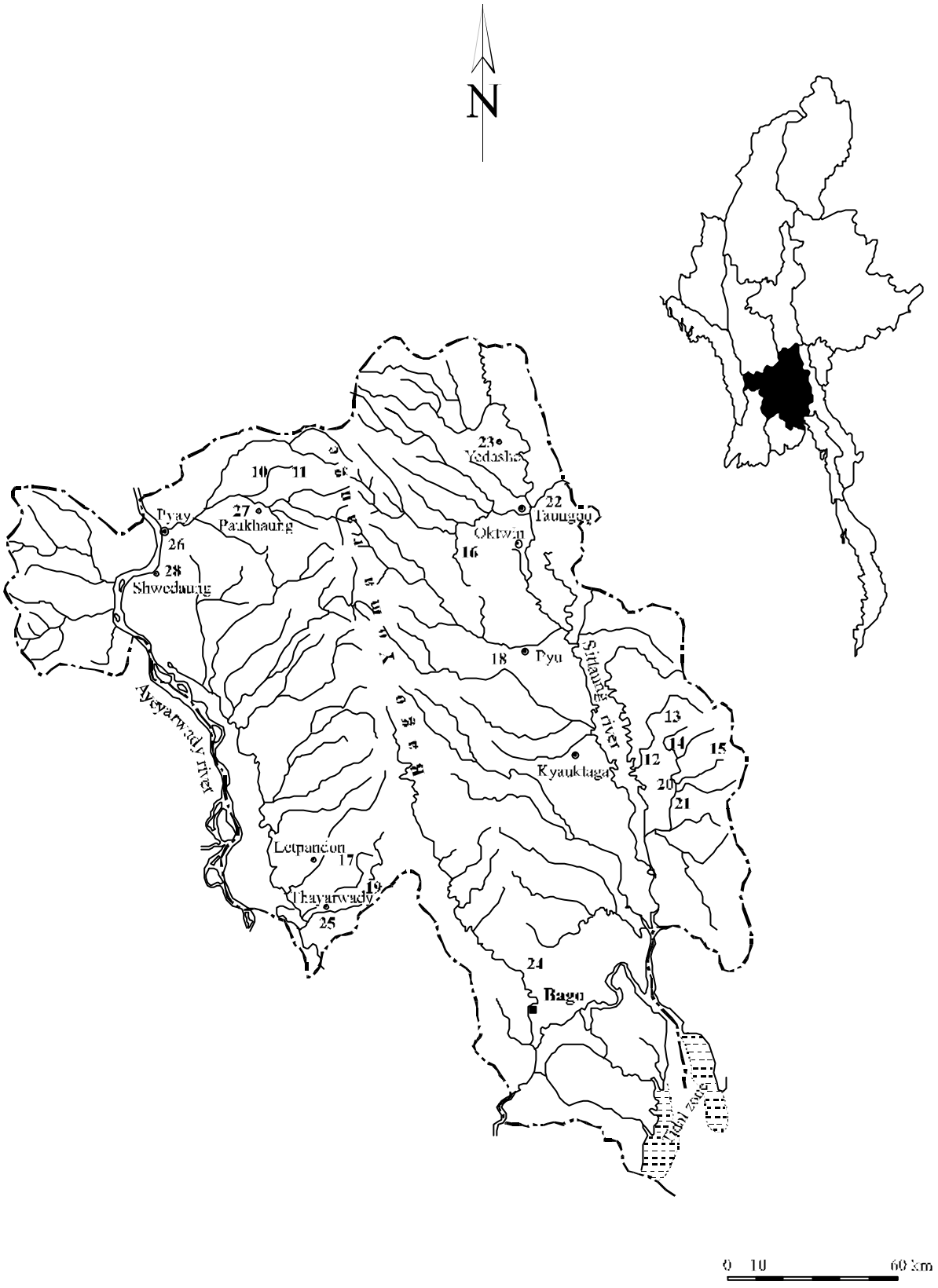


Fig. 2.70. Bago Division
10 to 64 – locations where anophelines were collected

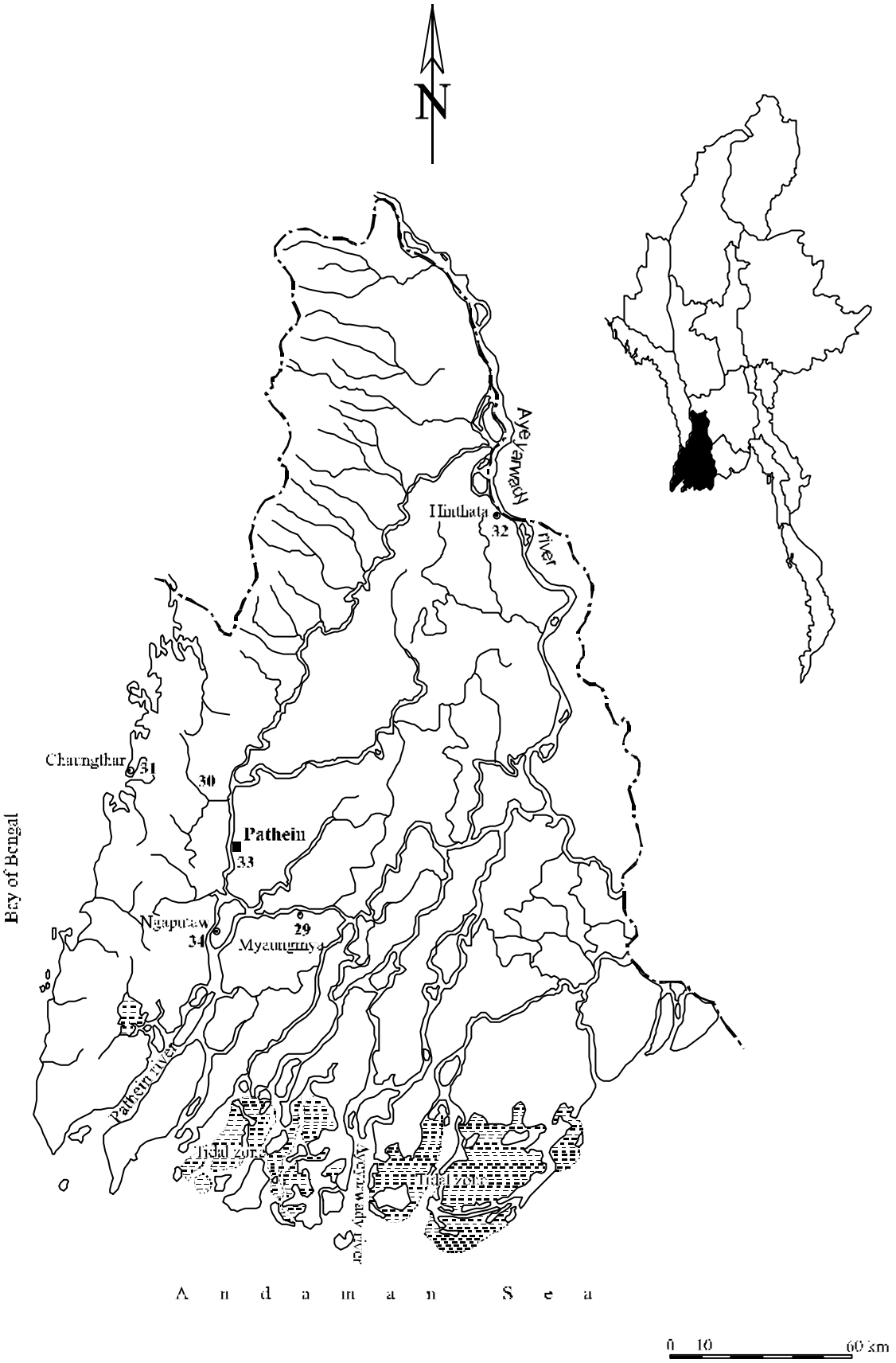


Fig. 2.71. Ayeyarwady Division
29 to 34 – locations where anophelines were collected

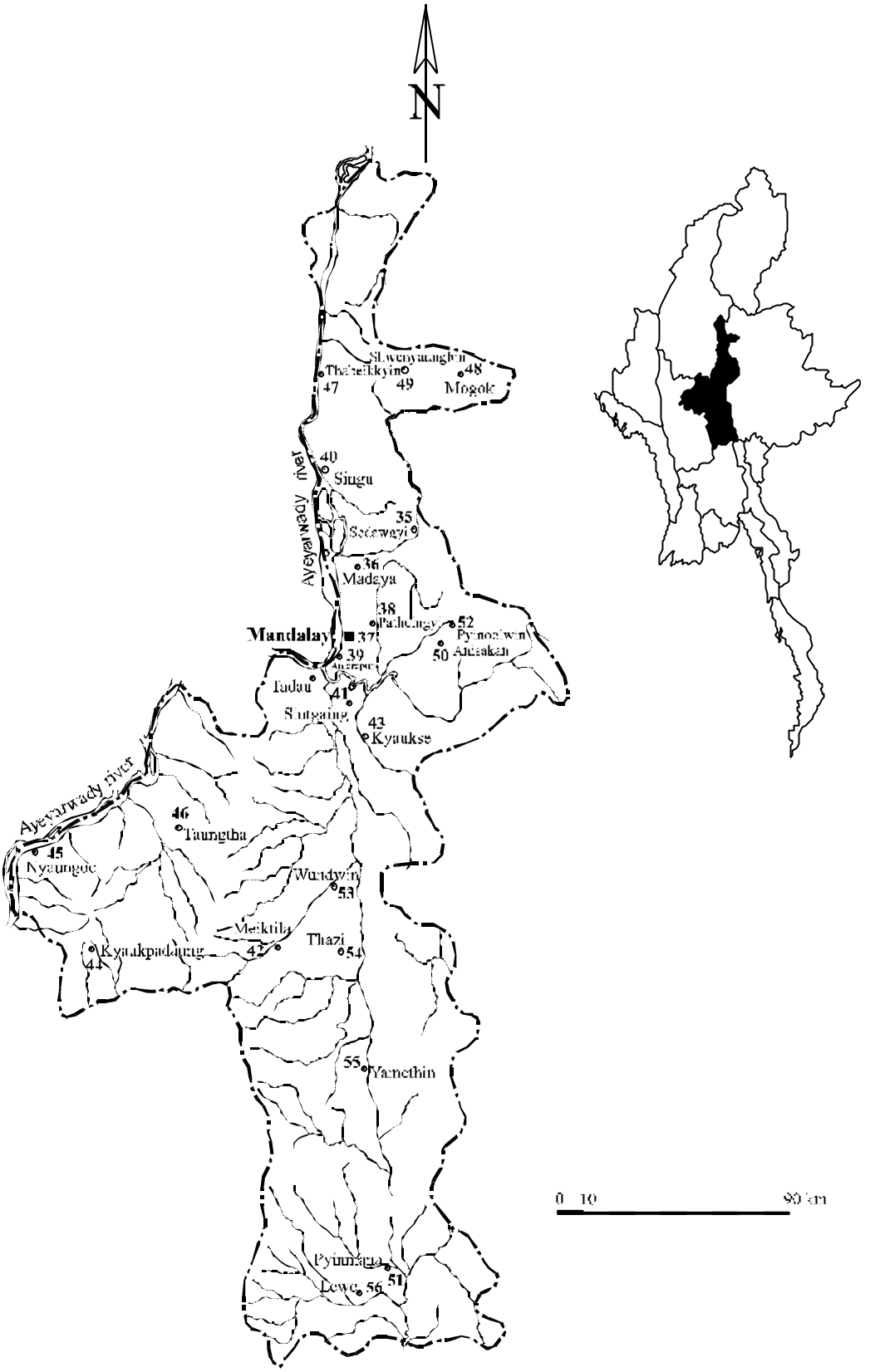


Fig. 2.72. Mandalay Division
35 to 56 = locations where anophelines were collected

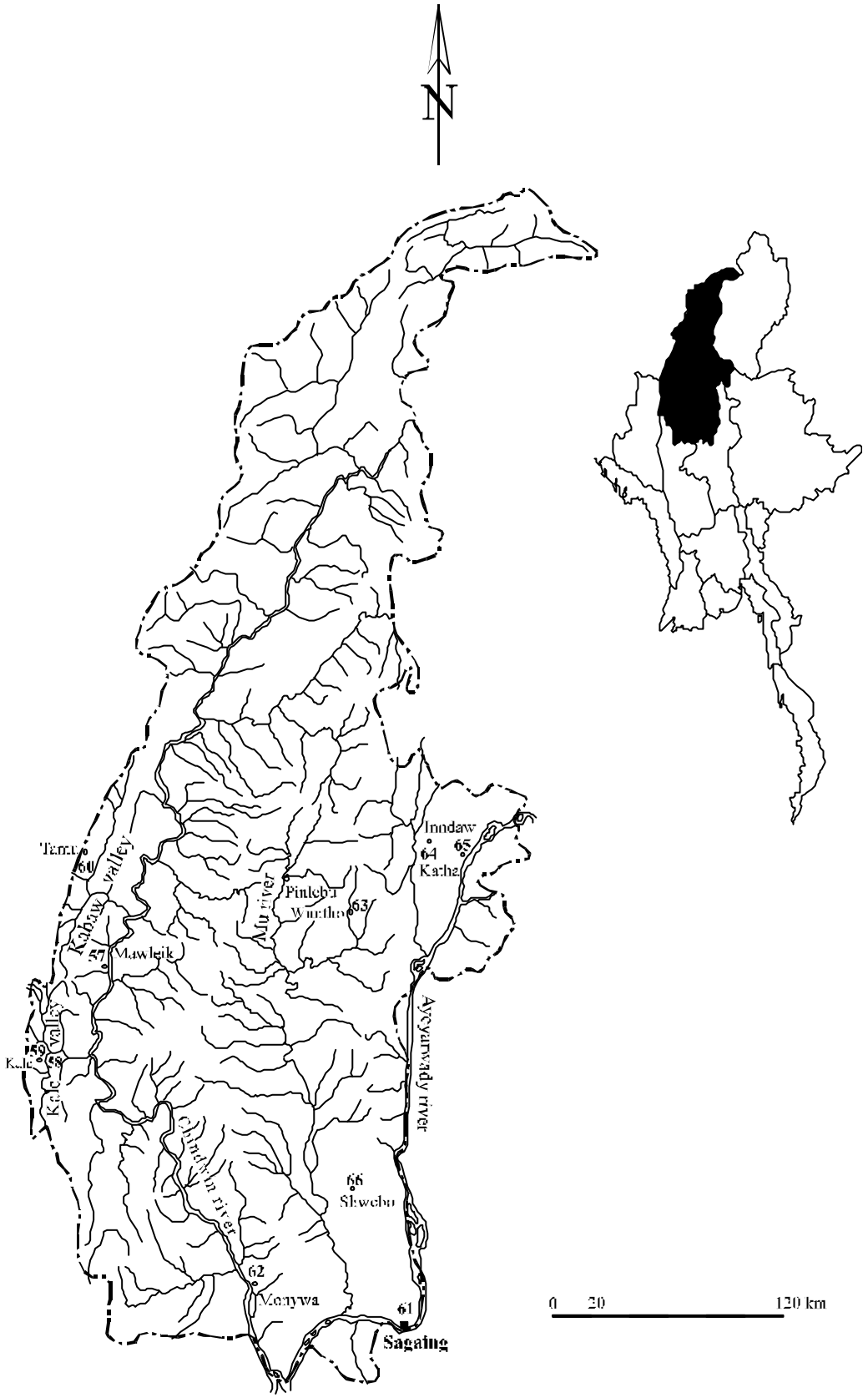


Fig. 2.73. Sagaing Division
57 to 66 = locations where anophelines were collected

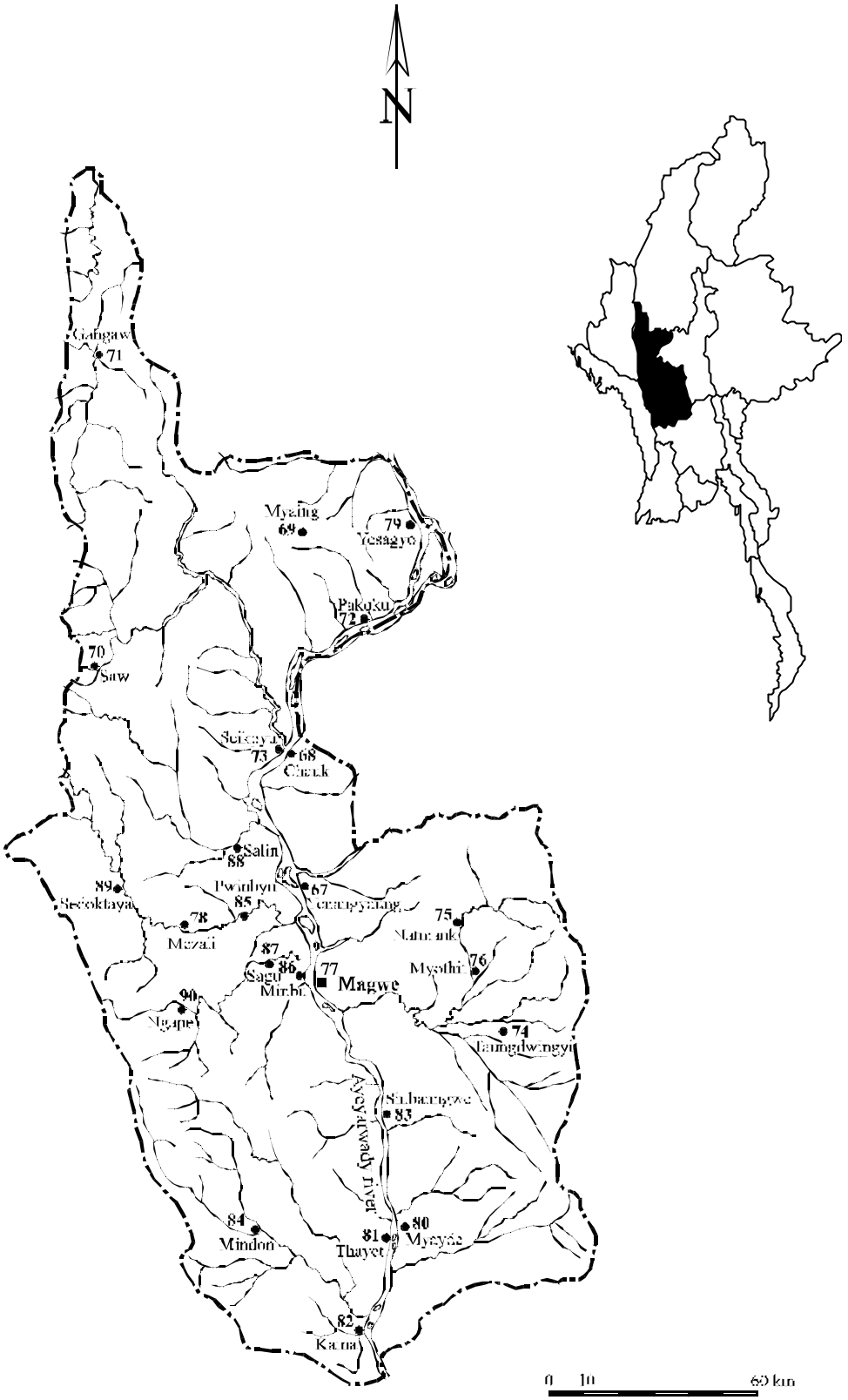


Fig. 2.74. Magwe Division
67 to 90 = locations where anophelines were collected

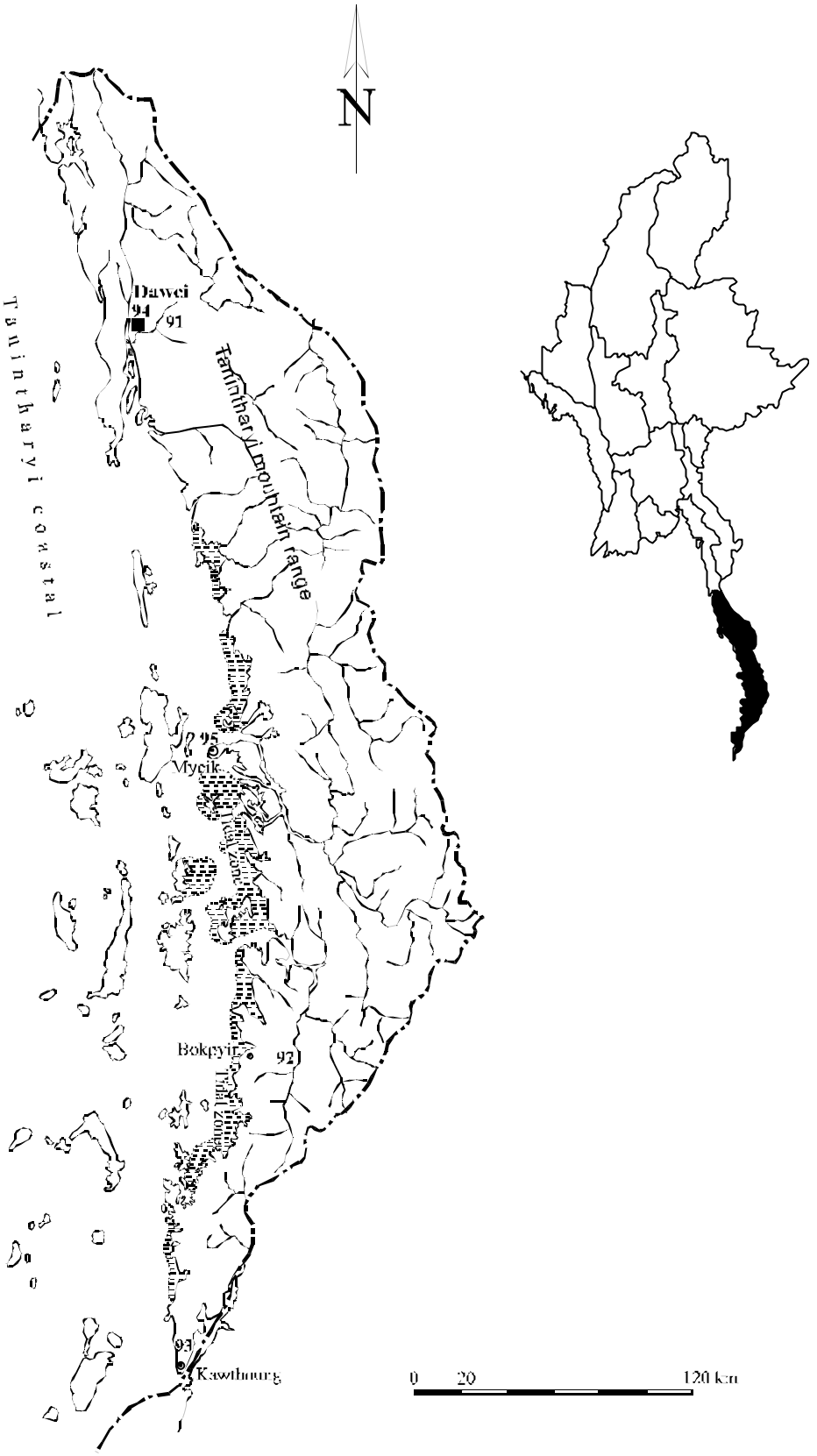


Fig. 2.75. Tanintharyi Division
91 to 95 = locations where anophelines were collected

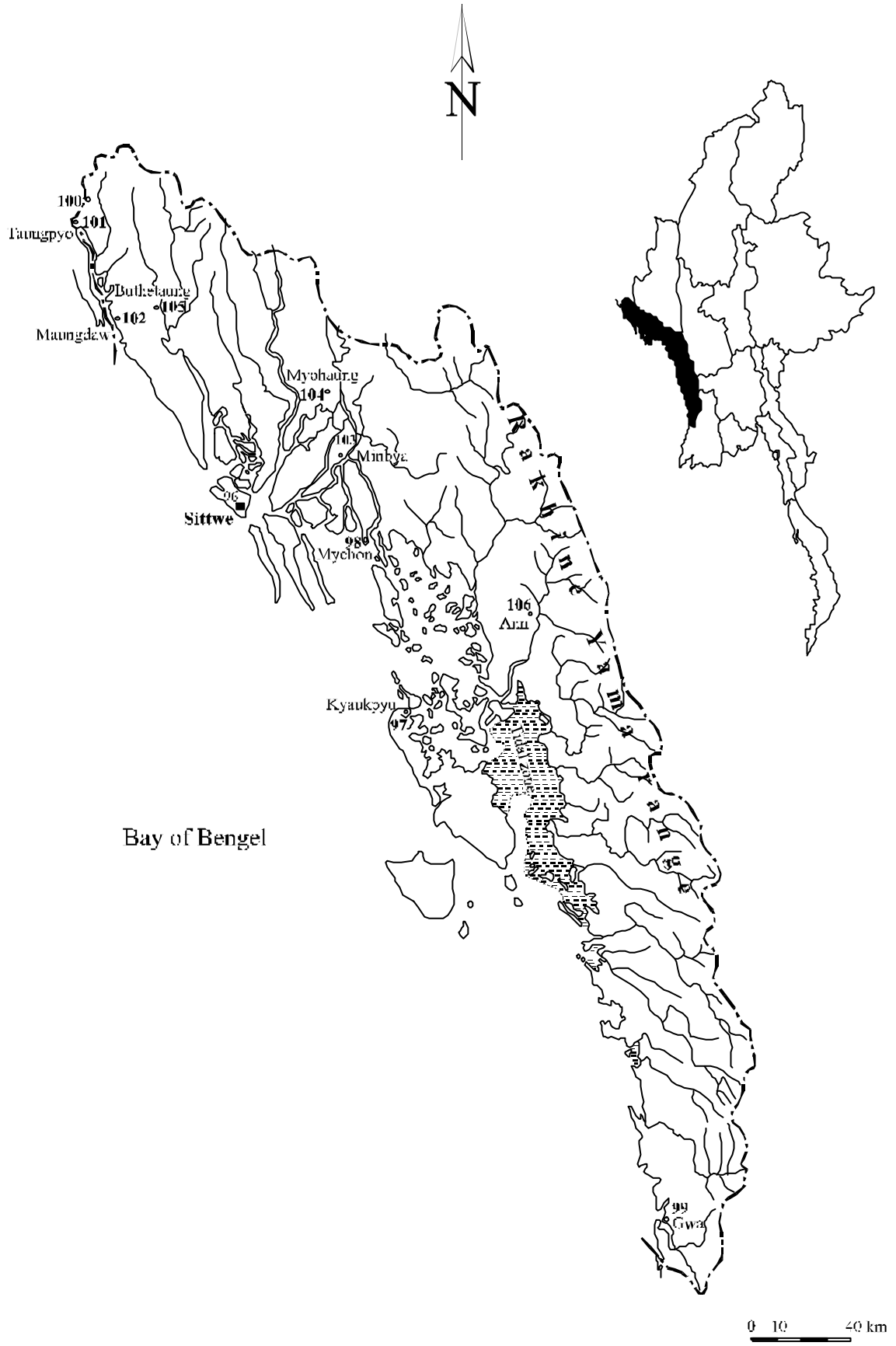


Fig. 2.76. Rakhine State
96 to 106 – locations where anophelines were collected

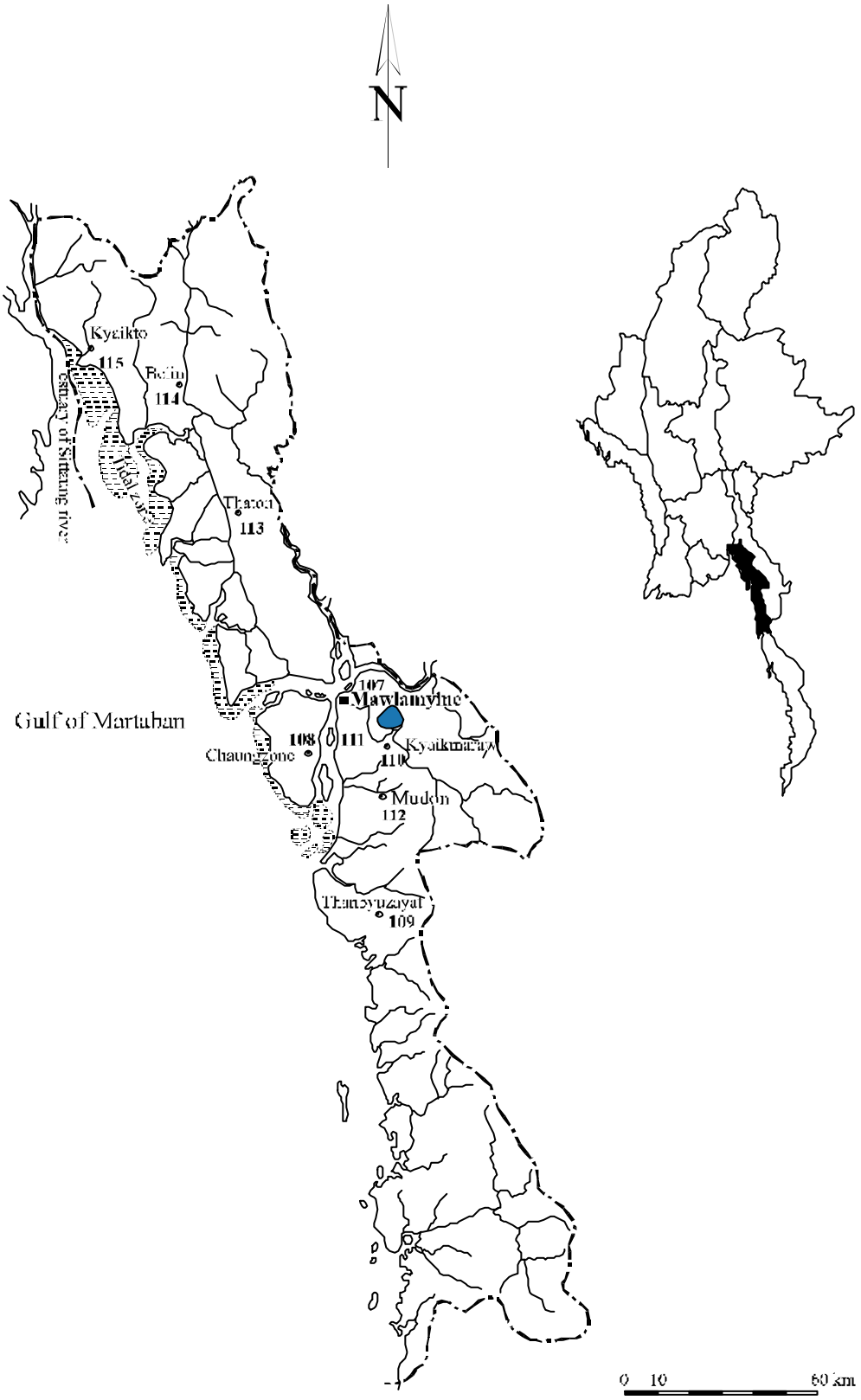


Fig. 2.77. Mon State
107 to 115 = locations where anophelines were collected

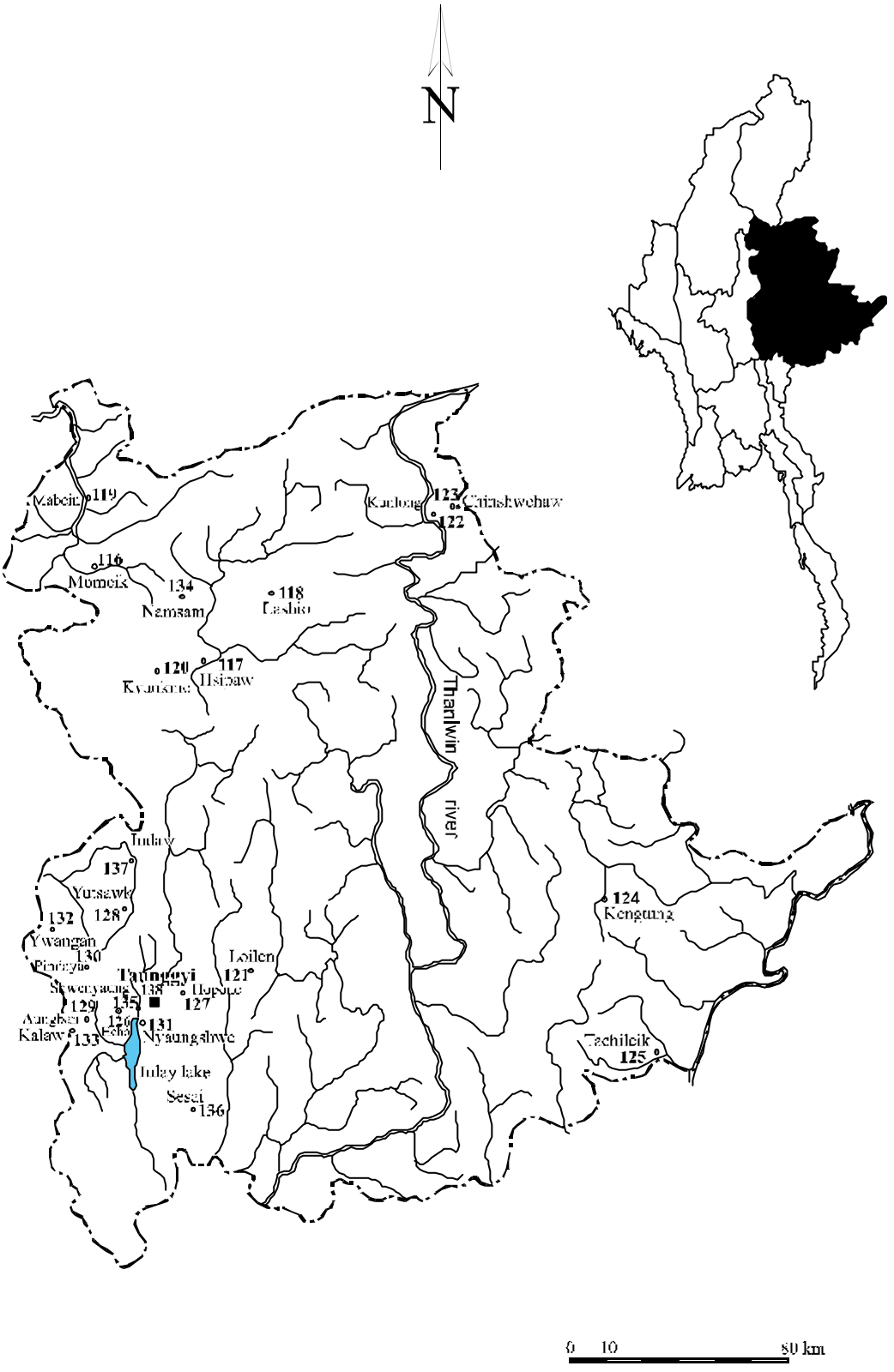


Fig. 2.78. Shan State
116 to 138 = locations where anophelines were collected

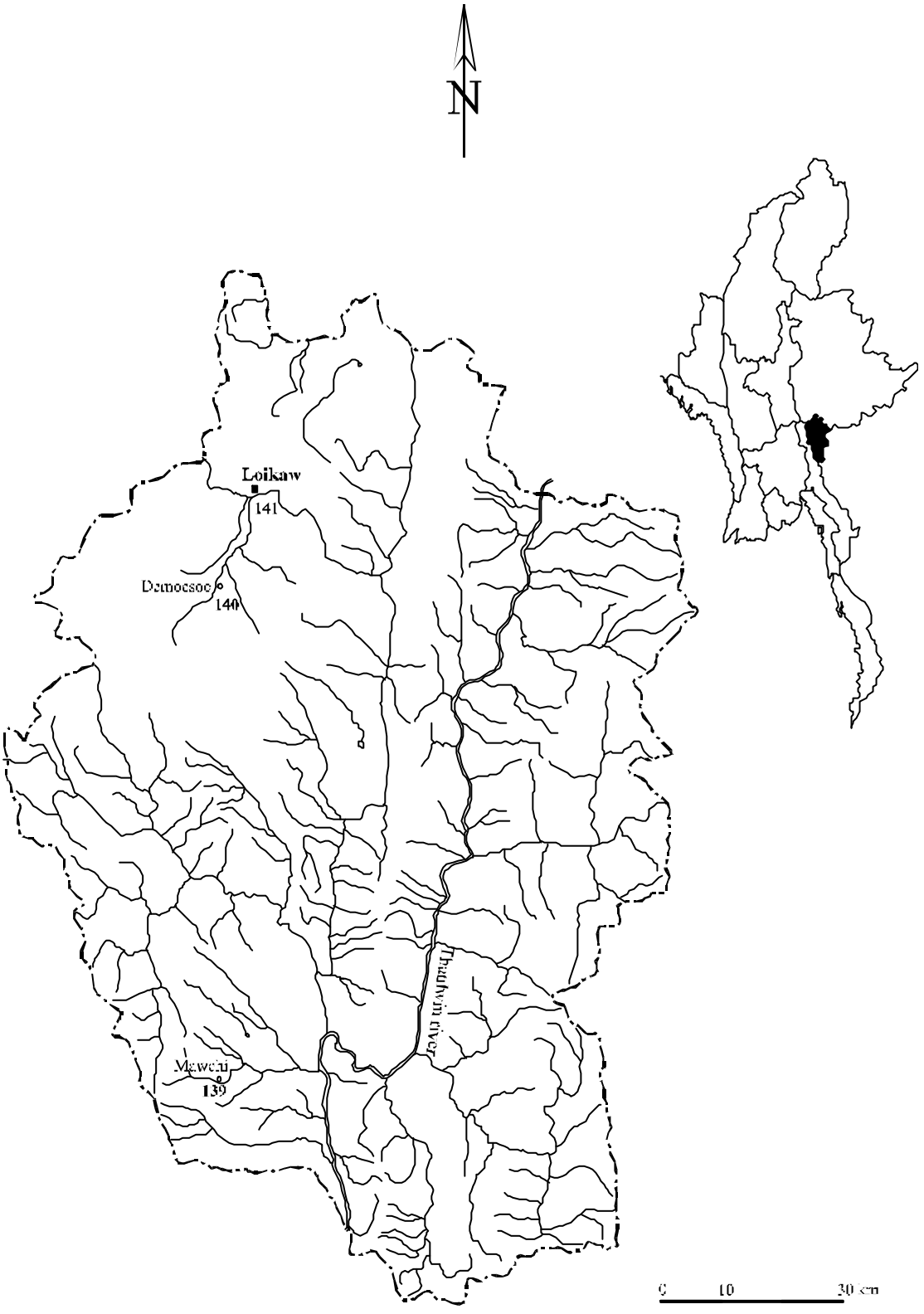


Fig. 2.79. Kayah State
139 to 141 = locations where anophelines were collected

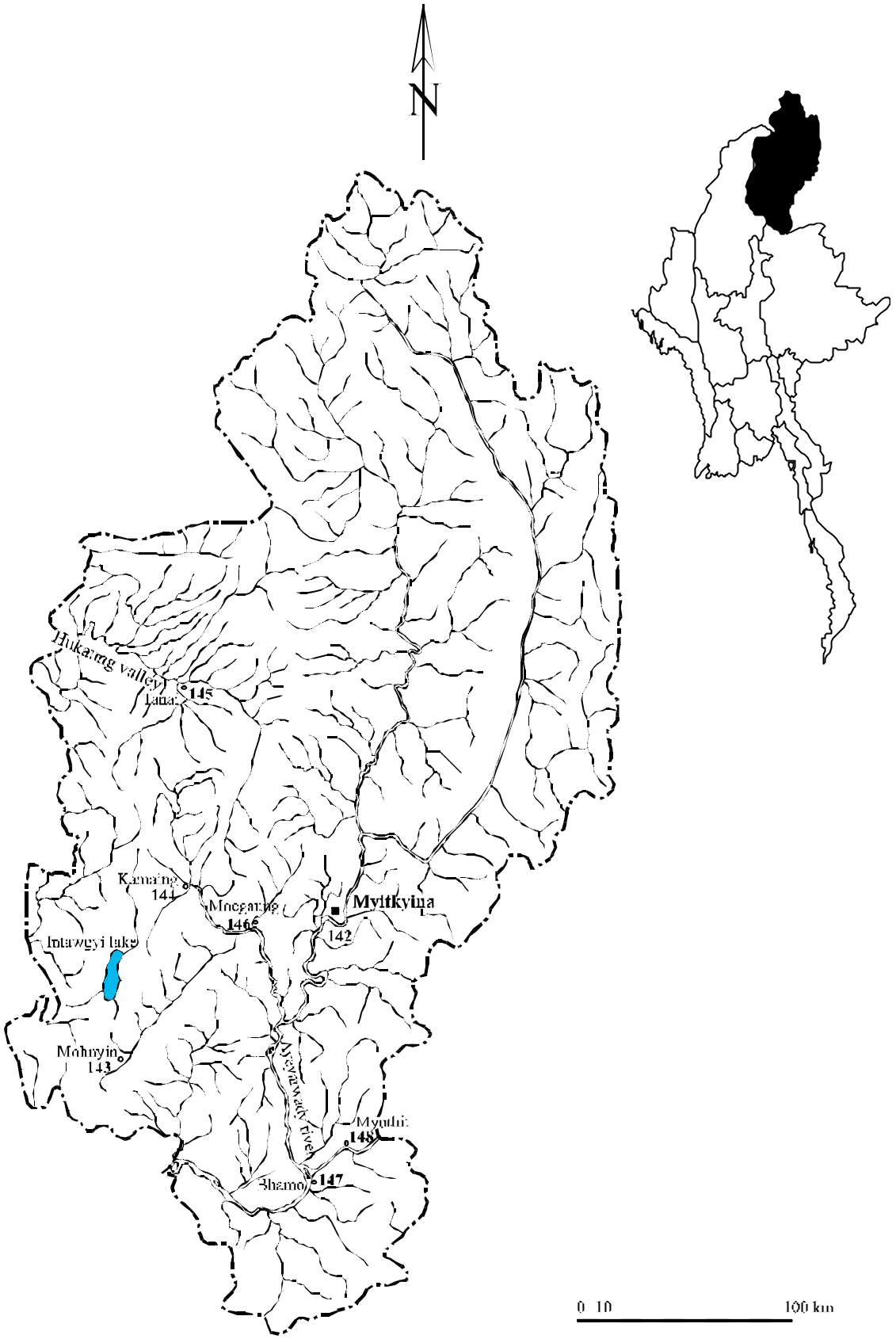


Fig. 2.80. Kachin State
142 to 149 = locations where anophelines were collected

Fig.2.81. Chin State
150 to 151 = locations where anophelines were collected

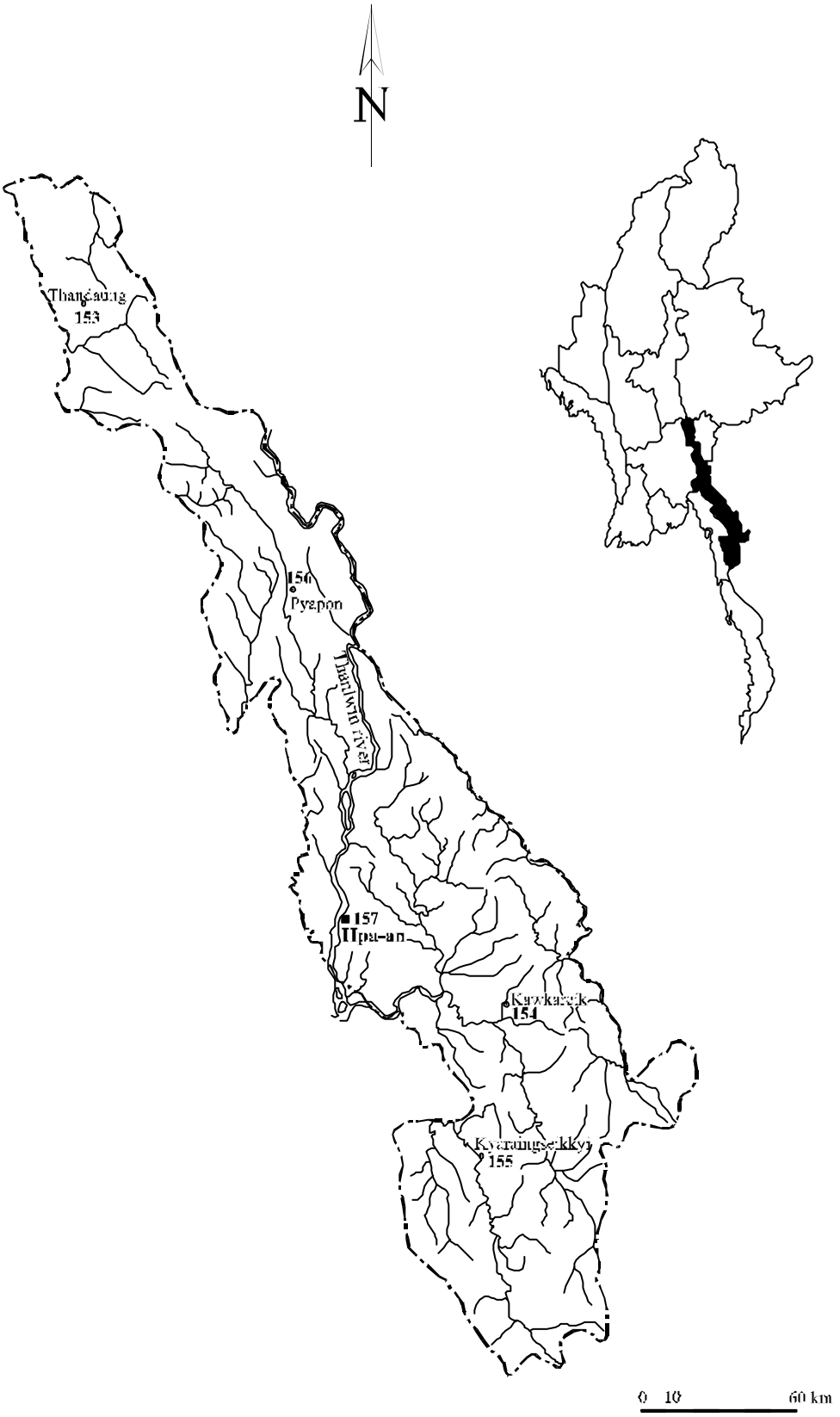


Fig. 2.82. Kayin State
152 to 155 = locations where anophelines were collected

2.3.2. Material and Methods

2.3.2.1. Methods for collecting larvae

Larvae were sampled at mosquito breeding sites in the study areas (section 2.3.1) on a weekly basis and appropriate data such as type of breeding sites, abundance of certain species, co-breeders, water conditions, aquatic vegetation and other conditions were recorded from May 1998 to March 2000. Larval sampling was carried out using a dipper, larval net, well net and a small spoon or pipette.

2.3.2.1.1. Use of the dipper: Various kinds of dippers were used, including small frying pans, soup ladles and photographic dishes (Fig. 2.83). It was important to use the right type and size for each breeding sites. A white enamelled dipper was preferred, because this allows easy recognition of larvae. The dipper was gently moved into the water at an angle of about 45°, until one side was just below the surface.

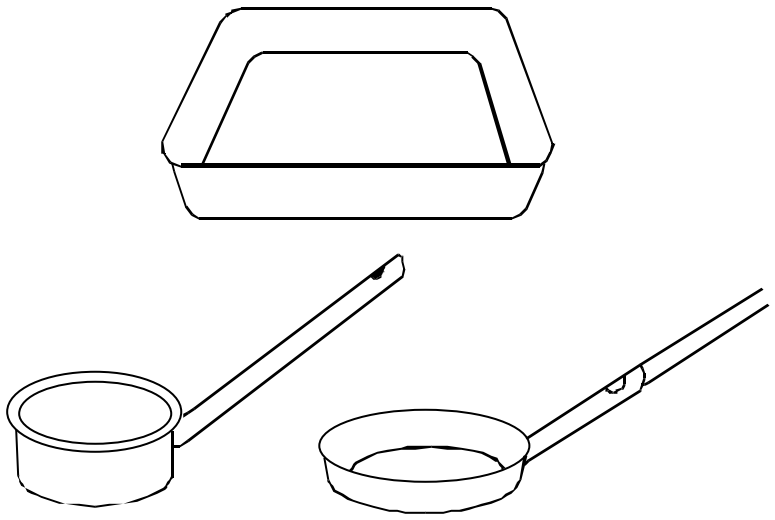


Fig. 2.83. Types of larval dipper

While dipping, care was taken not to disturb the larvae and thus cause them to swim downwards. If they were disturbed, dipping was continued after a minute or two when the larvae came up to the surface again. Moving along the breeding site, the surface of the water was skimmed with the

dipper. The dipper was carefully lifted out of so that the larvae and pupae were not spilled out with the water. The dipper was held steady until the larvae and pupae rose to the surface of the water. Then they were collected by means of a pipette and transferred to a bottle or vial.

When the area was thickly surrounded by vegetation, the dipper was pressed through the plants and lowered into the water allowing the water to flow in. Then the dipper was taken out of the water and larvae and pupae were collected as indicated above.

2.3.2.1.2. Use of the larval net: A larval net for collecting larvae and pupae in ponds and lakes consists of a fine mesh net which has a plastic bottle or tube tied to one end and is mounted to a wooden handle (Fig. 2.84). In order to collect larvae and pupae, the water surface was swept by holding the net at an angle and moving it through the water. Larvae and pupae on the water surface were swept into the net and collected in the plastic bottle or tube. Alternatively, a simple net with no attached bottle or tube could be used. After sweeping, the net was inverted into a bowl of water and its contents were dislodged. The water in the bowl was then searched for larvae and pupae, which were picked up and transferred to a bottle or vial by means of a pipette.

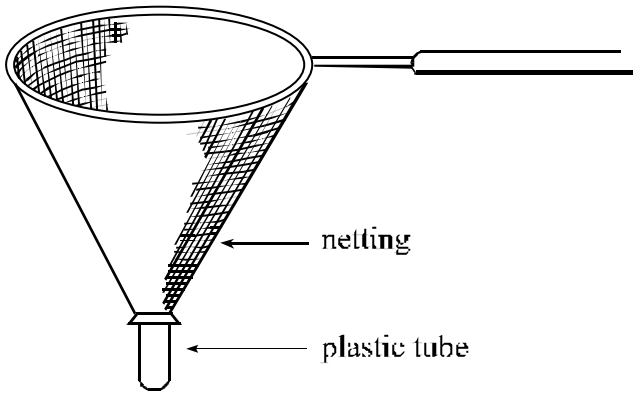


Fig. 2.84. Larval net

2.3.2.1.3. Use of the well net: A well net (conical shaped, white cloth dipper with a diameter of 35 cm and a length of 46 cm) is held at an angle by four strings and could be handled by a long string or rope (Fig. 2.85 and Photo 2.1). The net was introduced into the well so that the lower side of the net

was just under the surface of the water and its opening was at an angle of about 45°. After waiting 2-3 minutes to allow the disturbed larvae to return to the water surface, the net was dragged slowly two or three times around the border of the well. Then the net was withdrawn and inverted into a bowl of water. The water in the bowl was then searched for larvae and pupae, which were picked up and transferred to a bottle or vial using a pipette.

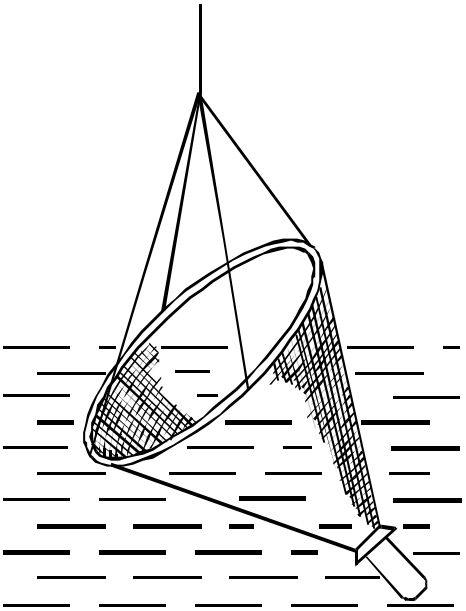


Fig. 2.85. Well net

2.3.2.1.4. Using a small spoon or pipette: A small spoon or wide-mouth pipette was used for collecting larvae and pupae from small collections of water, e.g. the gum collecting bowls attached to rubber trees (Photo 2.2), bamboo stumps, in a tin can or a hoof-print.

2.3.2.1.5. Maintaining accurate records of collections: A sketch-map was drawn to show the positions of the breeding sites relative to villages and settlements. Each breeding place was numbered and the location, type of breeding site, number of dips made or time spent sampling and date was recorded.

Photo 2.1. Using a well net for larval sampling from well.

Photo 2.2. Examining larval breeding in a gum collecting bowl on a rubber tree.

2.3.2.1.6. Transporting live larvae and pupae to the laboratory: The bottles or vials which contained larvae or pupae, were only by ? filled with water to provide enough oxygen for larvae and pupae. Each bottle or vial was tightly closed with a stopper. Where the laboratory was more than two hours away, the stoppers were removed every two hours to provide the specimens with fresh air.

2.3.2.1.7. Killing and preserving larvae and pupae: In the laboratory, larvae and pupae were killed by placing them in warm water, i.e. at 60°C. The larvae and pupae were removed from the water and put into labelled specimen tubes containing 70% alcohol.

Another commonly used method of killing and preserving larvae and pupae in the field was to place the larvae in vials containing 2% formalin solution.

2.3.2.2. Methods for collecting adult mosquitoes

Fixed mosquito catching stations were chosen in the study areas (section 2.3.1) and mosquito collections were conducted during May 1998 to March 2000. All catches followed the same procedure:

(i) Human bait catches (both indoors and outdoors, night collection) with glass text-tubes and sucking tubes (aspirators) were conducted from 18:00 to 06:00 on the next day according to WHO (1992) instructions.

(ii) Animal-baited trap-net catches (night collections) were conducted with sucking tubes starting from 18:00 to 06:00 on the next day. The mosquitoes were collected every three hours, for instance at 21 h, 24 h, 03 h and sunrise.

(iii) Daytime outdoor and indoor resting collections were carried out with a sucking tube from 07:00 to 09:00.

(iv) Transport to the laboratory

(v) Processing the collected samples of mosquitoes (identification etc.).

(vi) Incrimination of vector: gut and salivary gland were dissected to assess the rate of infections with *Plasmodium* sporozoites according to the WHO method (1975).

(vii) Malaria situation was obtained from local township medical officer and from the current Vector Borne Disease Control (VBDC) Departmental Reports.

Test-tubes: 150 mm long and 16 mm in diameter and smaller ones (100 mm x 10 mm or 60 mm x 10 mm) were commonly used. Small tubes were useful for collecting single specimens or when specimens were to be kept for some time before being examined and processed.

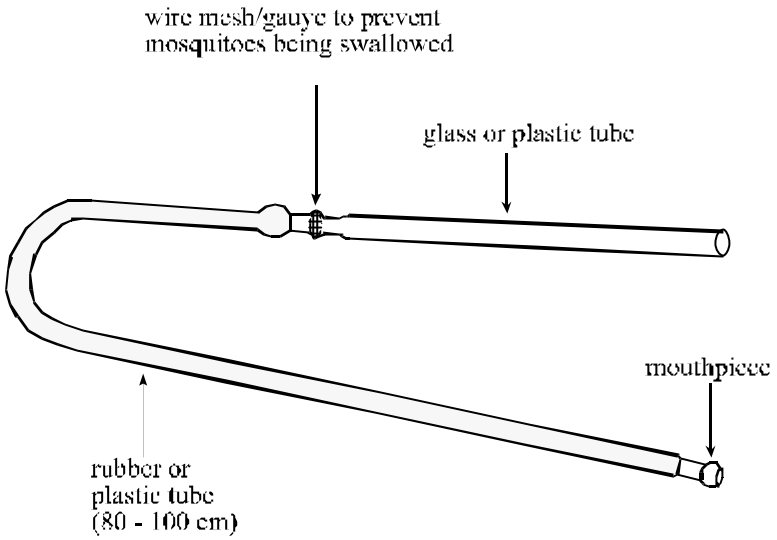


Fig. 2.86. Sucking tube or aspirator

The type of **sucking tube** (aspirator), which was used, is illustrated in Fig. 2.86. Sucking tube and test-tubes were always kept clean and dry.

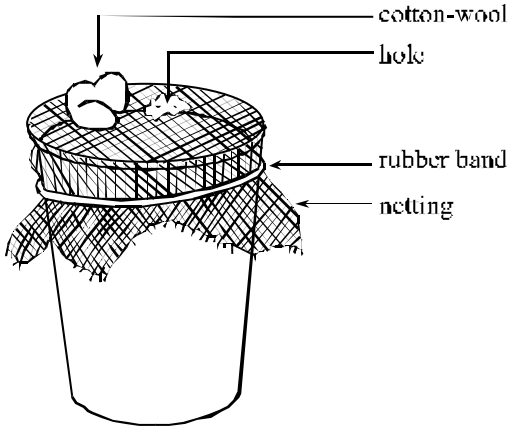


Fig. 2.87. Paper cup prepared as a mosquito container

A **paper cups** containers were made easily as shown in Fig. 2.87. They consist of: an unwaxed paper cup (capacity 250-300 ml), a square of mosquito netting with a hole for insertion of the sucking tube, a rubber band and a piece of cotton wool as a stopper.

2.3.2.2.1. Daytime collections

(1) Daytime outdoor collection of mosquitoes

(a) Collecting mosquitoes with a sucking tube: A sucking tube was used to collect mosquitoes from vegetation. The mosquitoes were transferred into a paper cup. Then the location and the total time for searching mosquitoes were recorded. The same technique was used to examine shelters for mosquito.
(b) Collecting mosquitoes using a hand net: A hand net or sweep net (Fig. 2.88) was used to collect mosquitoes resting on vegetation. The hand net was moved swiftly above grasses or close to the ground around bushes. The number of collections and the total time spent collecting were recorded.

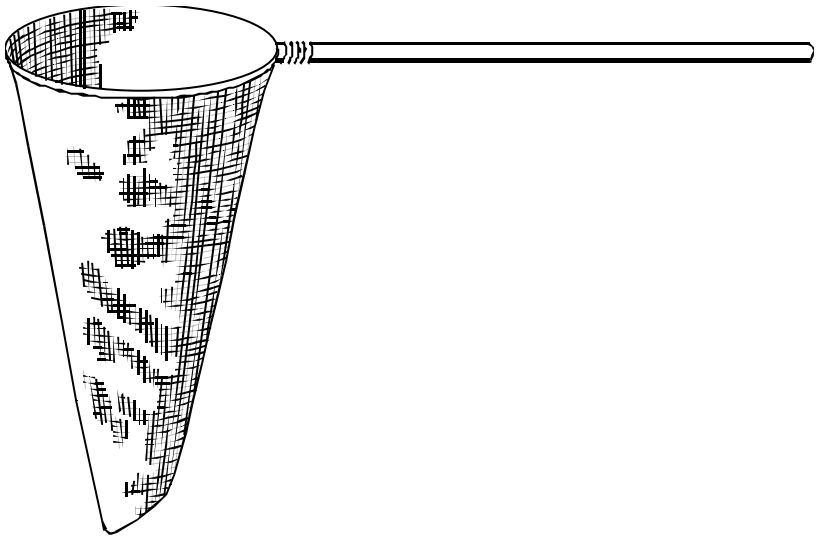
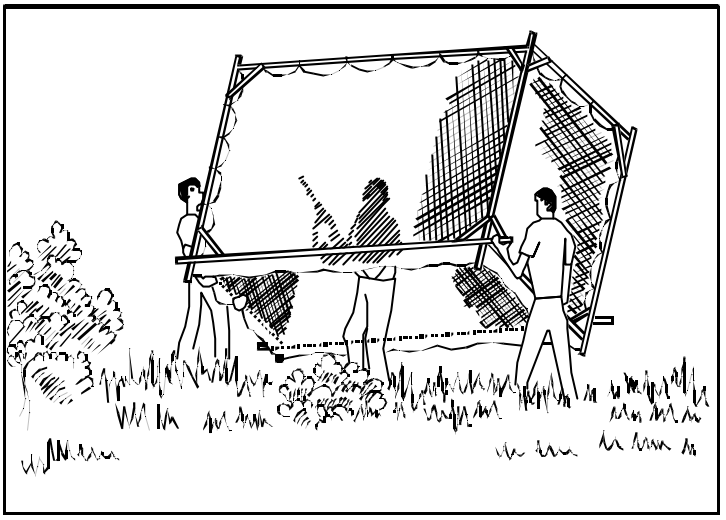
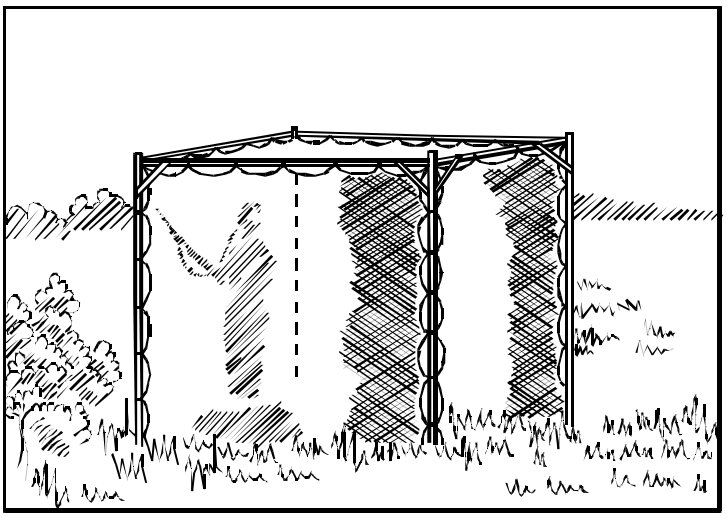


Fig. 2.88. Hand net

(c) Collecting mosquitoes using a drop net: A drop net (Fig. 2.89) consists of a light, collapsible wooden frame measuring about 2m x 2m x 2m, with its top and sides covered by a mosquito net. The drop net was placed over grass or low vegetation. Any mosquito beneath it was disturbed with a stick so that it flew up and rested on the net. The mosquitoes were collected with a sucking tube and transferred to a paper cup. The number of times the drop net was positioned for collecting was recorded.



Net being lowered



Net in place

Fig. 2.89. Drop net for collecting mosquitoes from grass

All paper cup containers were labelled with the following information: location, method of collection, data and time of collection, whether the nearest village was treated with insecticide, and if so when.

The data derived from outdoor collections were used to calculate the following indices: (i) number of mosquitoes collected per person per hour, based on outdoor collection with a sucking tube or a hand net and (ii) number of mosquitoes collected per unit area, based on outdoor collection with a drop net.

(2) Daytime hand collection of indoor-resting mosquitoes

In each village about 10 houses were normally examined in order to provide a representative sample. The selection of houses for routine collecting was a complex statistical procedure. In the case of a single visit, the houses selected were scattered throughout the village. It was often advantageous to select the poorest and worst-ventilated houses because they usually contained the largest numbers of mosquitoes. Houses on the fringe of a village or near known breeding sites often yielded more day-resting mosquitoes.

The collection of mosquitoes in a house took place early in the morning. The whole house was usually examined, though if it was too large, was restricted to 15 minutes searching each room. Special attention was paid to those parts of the house most likely to yield vector mosquitoes, including rooms in which people slept the previous night, the washing room, and any other areas suggested by experience. Those rooms with few external openings were selected.

The search for mosquitoes on walls, ceilings or the roof was aided by a torch. It was searched systematically, starting from the door and moving clockwise around the inside of the house. Furnishings such as wall hangings and curtains, behind and under furniture, and inside large pots and jars were thoroughly investigated. No more than five mosquitoes were collected in the sucking tube before they were transfered to a paper cup.

During hand collecting, a separate paper cup was used for each house. The cup was clearly labelled with at least the following essential information: location, date and time of collection, time spent on collecting (minutes), house number or householder's name, type of structure (house, animal shelter, store, etc.), whether sprayed, and if so, when.

2.3.2.2.2. Night collections

(1) Human bait: (a) Indoors: A house was selected in the area of the village with the greatest number of cases of malaria. A house with more than one room was preferred, allowing the usual residents to sleep in one room while another was used by the collector.

(b) Outdoor: The collection of mosquitoes from the body was a common way of obtaining biting specimens (Photo 2.3), especially of *Anopheles*. Outdoor collecting from human bait was conducted to fit in with the normal resting and sleeping habits of the local people. The collections were often made during the entire period from dusk to dawn.

The paper cups containing the hourly collections were labelled with location, date, whether and when the location was last sprayed, type of bait, site of collection (indoors or outdoors), hour of collection (e.g., 18:00 to 19:00) and the collector's name.

(2) Collecting by means of animal-baited trap nets: A tame animal was selected from the village, usually a cow or water buffalo. An animal-baited trap net was sited close to where the animal is customarily kept overnight. Before sunset, the animal was tied securely using a short tether attached to wooden or metal pegs driven firmly into the ground. At sunset, the mosquitoes were collected by means of a torch and sucking tube (Photo 2.4) in a three hourly interval, for instance at 18:00, 21: 00, 24:00, 03:00, and sunrise. Each hour's collection was kept in a separate paper cup. The animal bait collection was carried out in the same location and at the same time.

2.3.2.2.3. Transport to the laboratory

It was necessary to take the following steps: pieces of cotton wool were soaked in 5-8% sugar solution and squeezed out any excess sugar solution. This cotton wool was placed over the tops of the cups. Cups holding mosquitoes were placed upright in an insulated picnic box. Newspaper or other material was placed between the cups to minimize movement. The cups were covered with a damp towel. The towel was kept damp until the mosquitoes reached the laboratory. A number of test-tubes with specimens from a single house were held together with a rubber band and transported in an insulated picnic box. The box was closed as tightly as possible to prevent loss of moisture. During the period of transportation, the mosquitoes were not exposed to direct sunlight and heat.

Photo 2.3. Human bait hand catches (outdoor) with sucking tube.

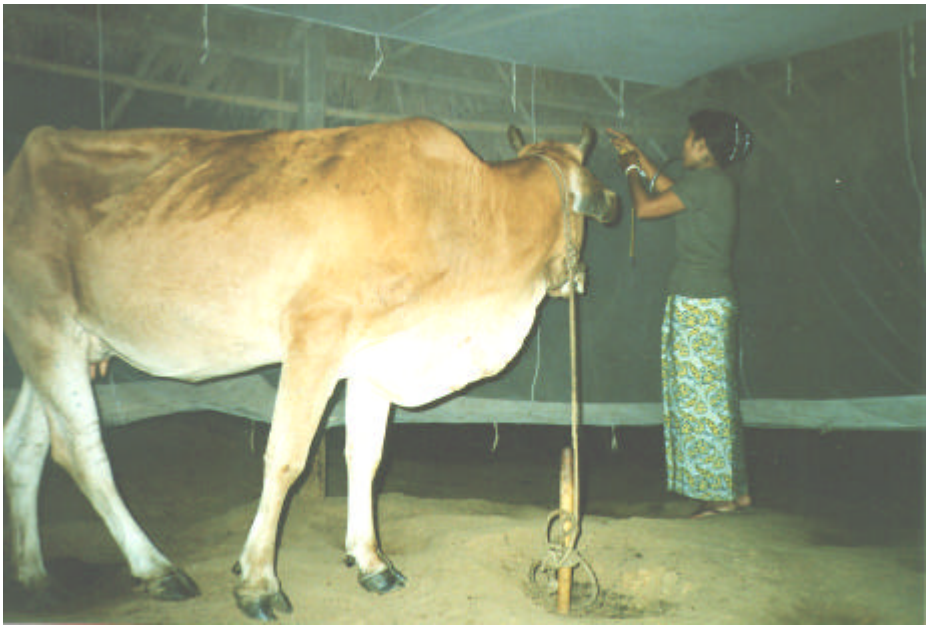


Photo 2.4. Collecting by means of cattle bait and a big bed net.

2.3.2.3. Processing the collected samples of mosquitoes

2.3.2.3.1. Identification of species based on the external morphology

A hand lens was used which was necessary not only for species identification but also for classification of the physiological stages. Some species, especially the large ones were recognized with the naked eye.

2.3.2.3.2. Dissection of mosquitoes

The choice of which organ had to be dissected, depends on the type of data required. The stomach was dissected to investigate the presence of oocysts, their number, stage of development, and positioning on the midgut. If oocysts were observed, the mosquito was considered "infected". However, in order to determine whether a mosquito was infective, the salivary glands were dissected and examined for the presence of sporozoites. This was carried out routinely for establishing the parity, sporozoite and oocyst rate.

Preparation of the mosquitoes for dissection: Live mosquitoes were killed either with chloroform or ether or carbon dioxide. After immobilization, the insect was held by one wing to remove the legs and afterwards pulled off the wings. The insect was then placed on a dry slide and arranged in a more suitable position for dissection of the gland or stomach, as described in the following.

(1) Stomach dissection (to estimate the oocyst rate)

The mosquito was placed on a microscope slide with the apex of the abdomen to the right. The abdomen was separated from the thorax by a cut, leaving part of the metanotum attached to the abdomen (Fig. 2.90.a). The left dissecting needle was fixed in the attached part of the thorax (Fig. 2.90.b). With the right needle, a small cut was made in the integument on each side of the seventh abdominal segment. A small drop of physiological saline was placed at the tip of the abdomen. The abdomen was held using the left needle, and the apex of the abdomen was pulled until the ovaries, malpighian tubes and the stomach were gradually drawn out (Fig. 2.90.c & d). The malpighian tubes from around the stomach were cut as close as possible to their insertion without tearing the gut wall. The gut was then drawn out completely from the abdomen and the rectum was cut off from the stomach just below the pyloric ampulla (Fig. 2.90.e). The stomach was removed and transferred to the slide. Another drop of 0.65% physiological saline was added and the specimen was covered with a coverslip.

Examination of the stomach for oocysts: The stomach was examined from the posterior end to the anterior part by means of a binocular. In moderate infections, the oocysts were located in the posterior half of the stomach (Fig. 2.91). Young oocysts were easy to detect because of their refractiveness and the presence of characteritsic malaria pigment. Both sides of the stomach was examined.

(2) Dissection of the salivary glands (to establish the sporozoite rate)

The mosquito was prepared in the same way as for stomach dissection. The mosquito was arranged on the slide with the head pointing to the right. The left needle was inserted gently into the thorax just below the region where the glands lie (Fig. 2.92.a). The neck close to the head was cut with the right needle (Fig. 2.92.b). A drop of saline (the size of a pinhead) was placed close to the neck section. Then the right needle was pressed gently on the thorax and the salivary glands were pulled out from the thorax with the left-hand needle.

The salivary glands were covered with a coverslip (Fig. 2.92.e) and then transfered to the platform of the microscope to examine the specimen. The coverslip was pressed gently with the needle in order to disrupt the cells to free the sporozoites. The sporozoites were easily recognized (even inside the salivary gland cells) as very minute needle like forms.

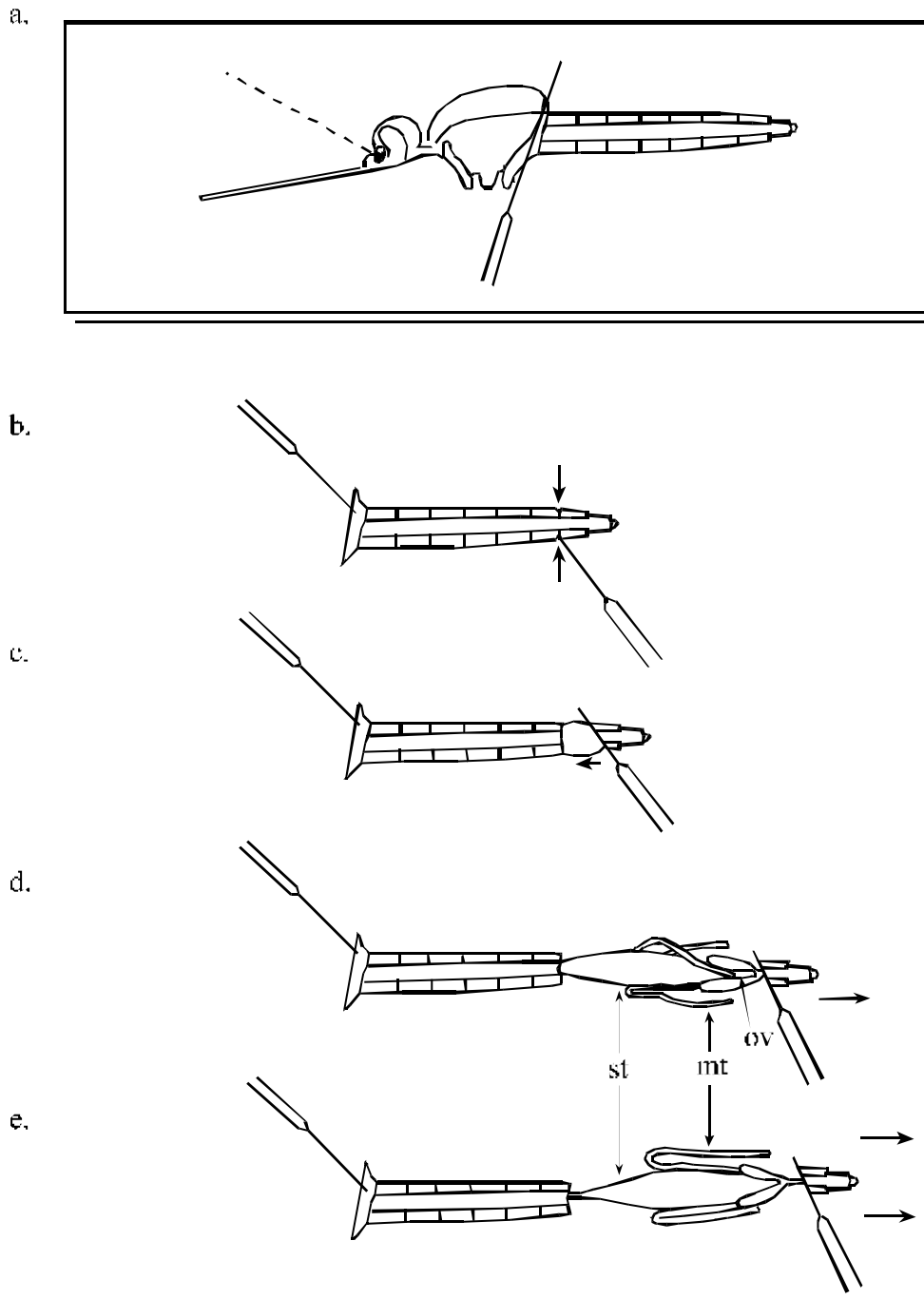


Fig. 2.90. Extraction of stomach.
st = stomach, mt = malpighian tubes, ov = ovary

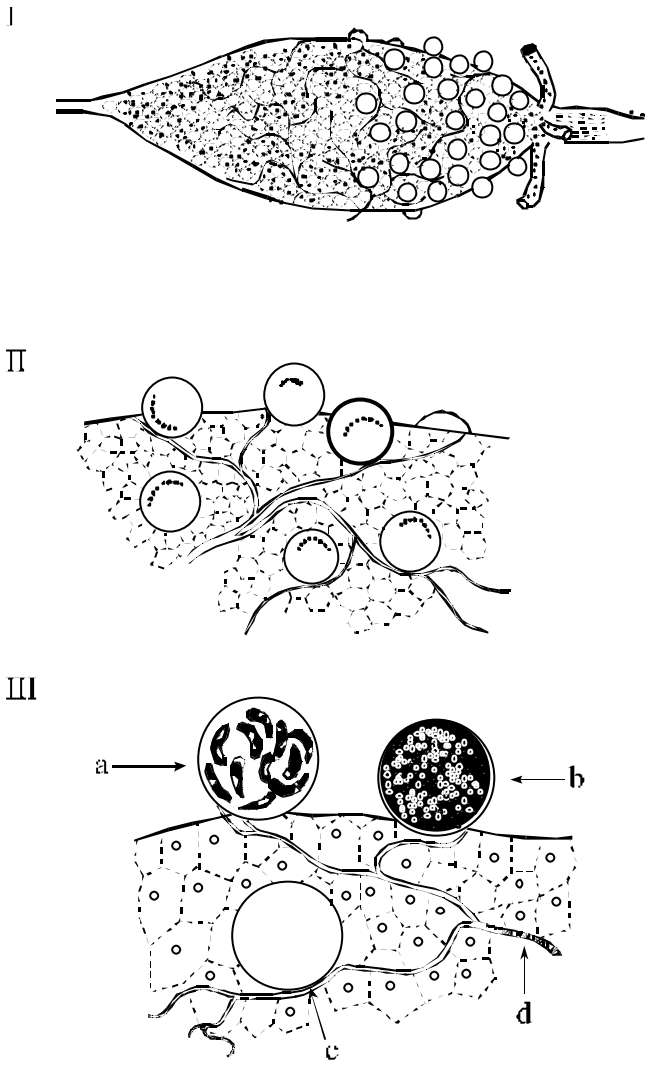


Fig. 2.91. Examination of stomach for oocysts.

I. Stomach with fully developed oocysts.

II. Young oocysts, 3-4 days old (*Plasmodium falciparum*).

III. a = black spores of Ross

b = black spores of Ross: entirely chitinised oocyst,

c = normal oocyst

d = trachea

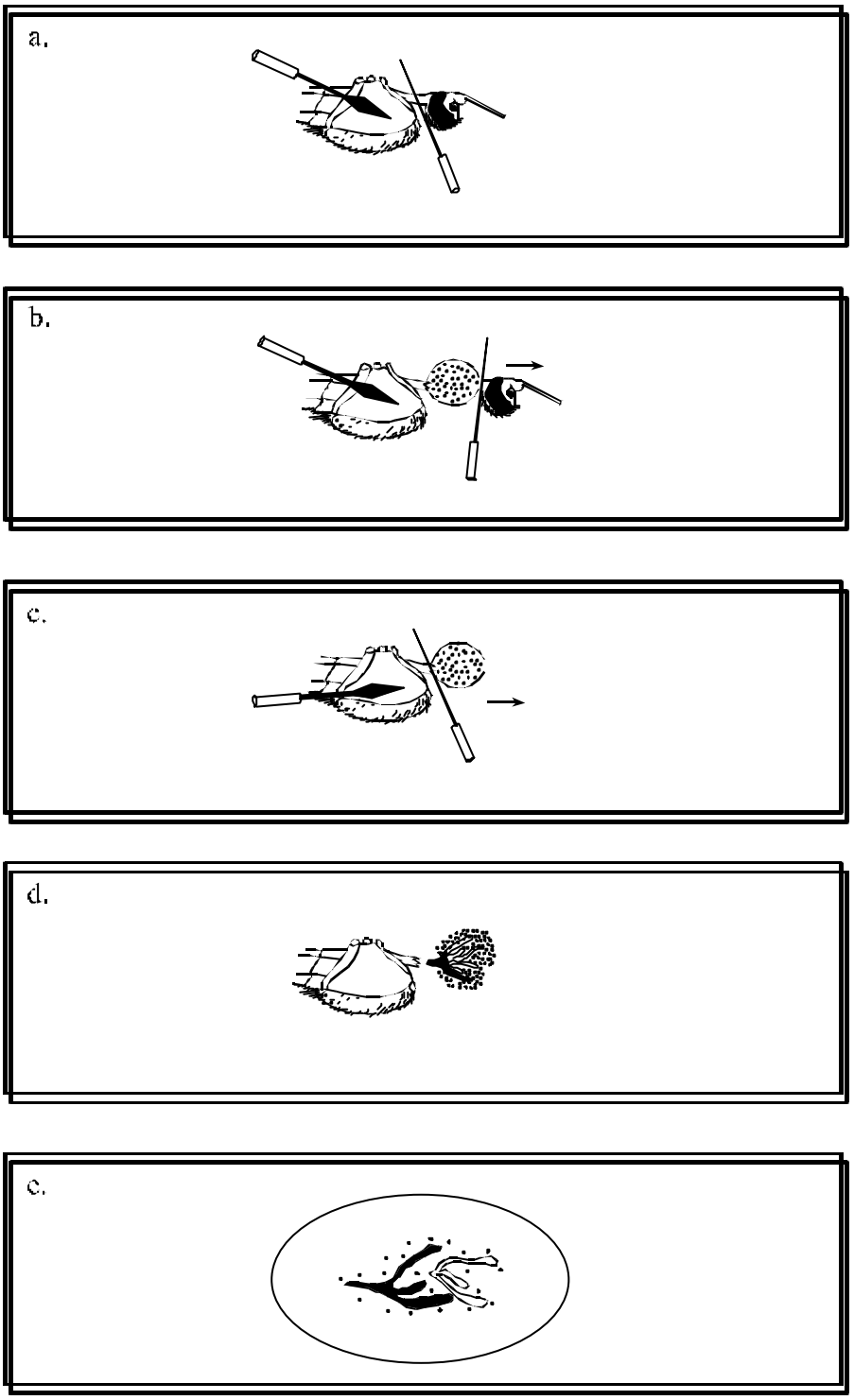


Fig. 2.92. Extraction of salivary glands

2.3.3. Results

2.3.3.1. List of species of *Anopheles* fauna in Myanmar

1. *Anopheles aconitus* Döenitz, 1902
2. *Anopheles aitkeni*i James, 1903
3. *Anopheles annularis* Van der Wulp, 1884
4. *Anopheles argyropus* (Swellengrebel), 1914
5. *Anopheles barbirostris* Van der Wulp, 1884
6. *Anopheles bengalensis* Puri, 1930
7. *Anopheles culicifacies* Giles, 1901
8. *Anopheles dirus* Peyton and Harrison, 1979
9. *Anopheles fluviatilis* James, 1902
10. *Anopheles gigas* Giles, 1901
11. *Anopheles sinensis* Weidemann, 1828
12. *Anopheles nitidus* Harrisson, Scanlon and Reid, 1973
13. *Anopheles insulaeflorum* (Swellengrebel and Swellengrebel de Graaf), 1920
14. *Anopheles jamesii* Theobald, 1901
15. *Anopheles jeyporiensis* James, 1902
16. *Anopheles karwari* (James), 1903
17. A*nopheles kochi* Döenitz, 1901
18. *Anopheles kyondawensis* Abraham, 1947
19. *Anopheles lindesayi* Giles, 1900
20. *Anopheles maculatus* Theobald, 1901
21. *Anopheles willmori* (James), 1903
22. *Anopheles majidi* Young and Majid, 1928
23. *Anopheles minimus* Theobald, 1901
24. *Anopheles nigerrimus* Giles, 1900
25. *Anopheles pallidus* Theobald, 1901
26. *Anopheles peditaeniatus* (Leicester), 1908
27. *Anopheles philippinensis* Ludlow, 1902
28. *Anopheles pseudojamesi* Strickland and Chowdhury, 1927
29. *Anopheles splendidus* Koidzumi, 1920
30. *Anopheles stephensi* Liston, 1901
31. *Anopheles subpictus* Grassi, 1899

32. *Anopheles sundaicus* (Rodenwaldt), 1925

33.*Anopheles tessellatus* Theobald, 1901

34. *Anopheles theobaldi* Giles, 1901

35. *Anopheles vagus* Döenitz, 1902

36. *Anopheles varuna* Iyengar, 1924

2.3.3.2 Larval ecology

The distribution of the species is shown in Appendix C.

(1) *Anopheles aconitus* Döenitz, 1902

In Bago Division and Rakhine State, *An. aconitus* larvae are found most commonly in swamps, ponds, grassy pools with dense submerged vegetation, pits, stagnant roadside drains and stagnant water of rice fields when the young rice plants are a few inches above water.

However, it is not entirely a stagnant water breeder. In Mandalay Division, *An. aconitus* larvae are also collected along the grassy edges of streams and irrigation channels, associated with certain types of aquatic plants including water hyacinth (*Eichhornia speciosa*). The larvae prefer clear water and do not tolerate heavy pollution.

(2) *Anopheles aitkeni* James, 1903

During the study period, (i) in Mogok Township area, Mandalay Division, only three *An. aitkeni* larvae were recorded, from a jungle stream well away from human habitations; (ii) in Northern Chin Hills area, a total of 13 larvae were collected from slow running streams close to villages and (iii) in Southern Chin Hills area, a total of 5 larvae were recorded from streams close to Kanpetlet Town.

(3) *Anopheles annularis* Van der Wulp, 1884

Breeding sites of *An. annularis* are mainly in collections of stagnant water with thick grassy edges such as in ponds, pits, tanks, swamps, stagnant drains and rice fields.

In Kyaukpyu area, Rakhine State, *An. annularis* mainly breeds in rice fields, pools and streams and is associated with larvae of *An. aconitus, An. sinensis* and *An. jamesii*. In Sittwe area, the main breeding sites of *An. annularis* are small ponds, used for growing watercress for human consumption.

In Sagaing Division, (i) Kabaw and Kale valleys, *An.annularis* breeds mainly in association with larvae of *An. sinensis* and *An. philippinensis*, in derelict rice fields and (ii) in

Pwinbyu area, *An. annularis* larvae were observed in pools where algae and aquatic vegetation were present.

In Bago Division, *An. annularis* was found breeding in small ponds near rice fields and swamps near villages, mainly in association with *An. philippinensis* and *An. vagus* larvae.

In Mandalay Division, *An. annularis* was most abundant in weed-choked ponds in association with *An. sinensis, An. barbirostris* and *An. jamesii* larvae.

In Yangon Division, Taikkyi area, *An. annularis* was found growing in rice fields, in association with *An. culicifacies* larvae.

The aquatic plants which are most abundant in favourable breeding sites are the aquatic herbs; *Hydrilla verticillata, Najas bengalensis* and *Ceratophyllum demersum*.

(4) *Anopheles argyropus* (Swellengrebel), 1914

During the study period, only four larvae of *An. argyropus* were collected from Tamu, which adjoins the Chin-Assam border between India and Myanmar. Breeding sites are rice fields and swamps.

(5) *Anopheles barbirostris* Van der Wulp, 1884

An. barbirostris breeds in stagnant water especially associated with thick aquatic vegetation. It is found most commonly in swamps, ponds, pits and shady pools.

An. barbirostris is the most common species in rice fields during the early part of the rice-growing season. It prefers a certain degree of pollution in the water. It was often found in association with the species of *An. hyrcanus* group (*An. nitidus, An. nigerrimus, An. peditaeniatus*), *An. annularis* and *An. philippinensis.*

(6) *Anopheles bengalensis* Puri, 1930

During the study period, only two larvae of *An. bengalensis* were collected, from a slow running stream in Northern Chin Hills. Dead leaves and floating vegetation debris were found in the breeding site, otherwise the water was unpolluted.

(7) *Anopheles culicifacies* Giles, 1901

The common breeding sites of *An. culicifacies* are (i) in fairly fresh (unpolluted) water, either stagnant or gently moving, with vegetation; (ii) in clear sandy or rocky pools in streams beds; (iii) in pits; (iv) unpolluted rainwater pools; (v) irrigation

channels, both sandy or pebbly with some grassy vegetation and (vi) in paddy fields during the early part of the rains before the paddy has grown to any height.

This species can also breed in artificial collections of water such as ornamental waters and unused swimming pools. The rain-filled pits, dug on the side of roads and railways are well established breeding sites. *An. culicifacies* is most frequently associated with the larvae of *An. subpictus*.

(8) *Anopheles dirus* Peyton and Harrison, 1979

An. dirus larvae were found in the following type of breeding sites during the study period.

(a) Mudon Town, Mon State: Table 2.1.1 shows the percentage distribution of wells positive for *An. dirus* breeding in Mudon Town, Mon State, during pre-monsoon (May), post-monsoon (October), cool-dry month (January) and hot-dry month (March) for two years. The highest numbers of *An. dirus* larvae and pupae were found during pre-monsoon and post-monsoon, whereas the lowest numbers were recorded during cool-dry and hot-dry months. Because the average number of *An. dirus* larvae and pupae per dip per well showed a definite increase in pre-monsoon and post-monsoon time periods, a strong correlation with rainfall could be demonstrated (Table 2.1.2 and Fig. 2.93).

Table 2.1.1: Percentage distribution of wells positive for *An. dirus* breeding in Mudon Town

Period	Percentage of wells positive for *An. dirus* breeding in Quarters				
	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Total
1998					
May	55.5 (5/9)*	28.5 (2/7)	60.0 (3/5)	77.7 (7/9)	56.6 (17/30)
October	66.6 (6/9)	28.5 (2/7)	80.0 (4/5)	88.8 (8/9)	66.6 (20/30)
1999					
January	33.3 (3/9)	14.2 (1/7)	80.0 (4/5)	77.7 (7/9)	50.0 (15/30)
March	22.2 (2/9)	14.2 (1/7)	60.0 (3/5)	66.6 (6/9)	40.0 (12/30)
May	44.4 (4/9)	28.5 (2/7)	80.0 (4/5)	88.8 (8/9)	60.0 (18/30)
October	55.5 (5/9)	28.5 (2/7)	80.0 (4/5)	88.8 (8/9)	63.3 (19/30)
2000					
January	44.4 (4/9)	28.5 (2/7)	80.0 (4/5)	66.6 (6/9)	53.3 (16/30)
March	44.4 (4/9)	14.2 (1/7)	40.0 (2/5)	66.6 (6/9)	43.3 (13/30)

N.B. *(x/y) = x, positive wells and y, investigated wells

Table 2.1.2: Correlation with rainfall and average number of *An. dirus* larvae-pupae/dip/well in Quarters 1,2,3 and 4

Period	average number of larvae-pupae/dip/well				Rainfall (mm)
	Quarter1	Quarter2	Quarter3	Quarter4	
1998					
May	20.7	20.1	34.1	35.2	288.46
October	28.9	22.5	50.0	39.2	415.12
1999					
January	9.3	8.9	15.5	18.2	12.82
March	2.5	4.5	8.0	3.5	0.00
May	21.7	23.4	38.2	38.0	303.84
October	29.9	29.8	55.7	58.4	436.41
2000					
January	10.0	11.2	21.3	22.1	0.51
March	3.5	3.1	7.5	8.9	2.56

Table 2.1.3: Mosquito species associated with *An. dirus* in investigated wells, during the rainy season, July 1999

Site	Total number of larvae collected	*An. dirus*	Co-breeder	
			Species	Number
Quarter 4	302	272	*Culex malayi*	25
			An. vagus	1
			An. maculatus	3
			An. barbirostris	1
Quarter 3	255	237	*Culex malayi*	18
Quarter 2	5	5	0	0
Quarter 1	15	15	0	0

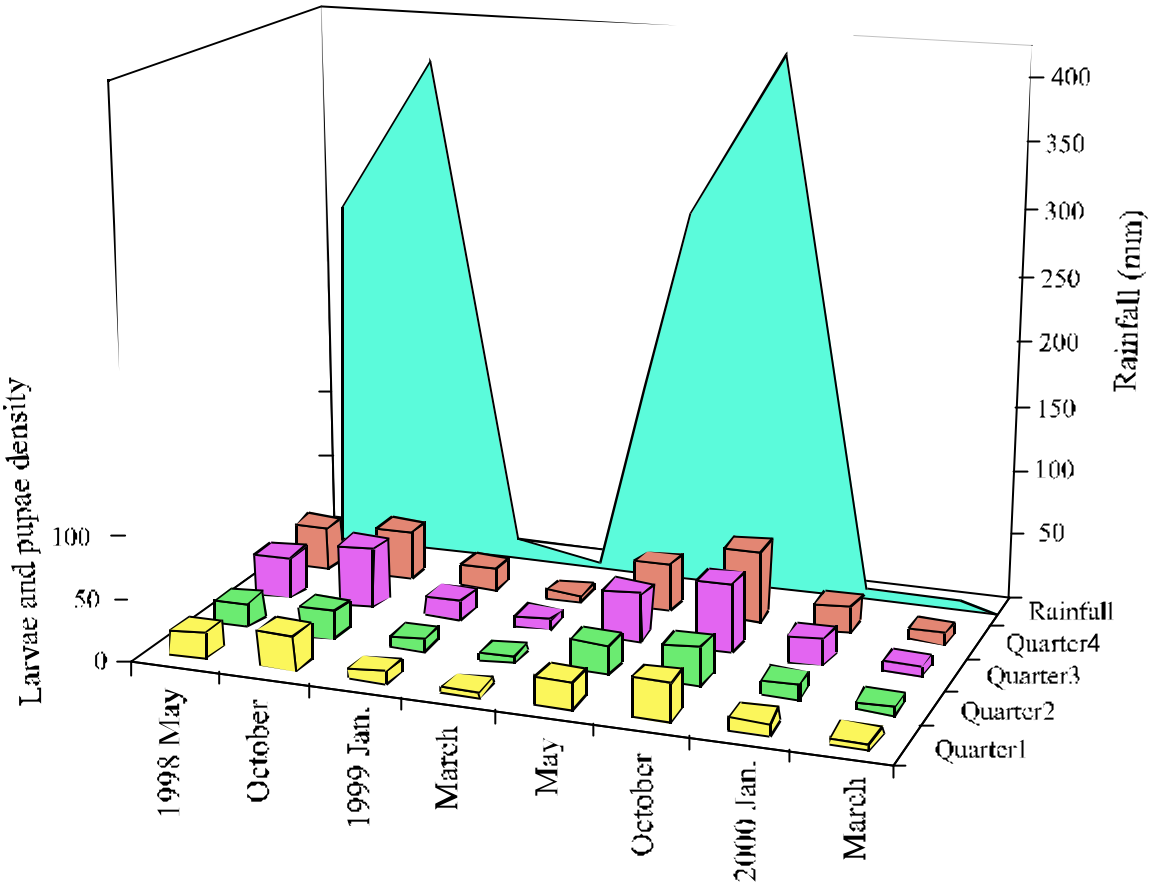


Fig. 2.93. Correlation with rainfall and average number of *An. dirus* larvae and pupae/dip/well in Quarters 1,2,3 and 4 during study period (after Oo et al., 2002)

In addition to *An. dirus*, other mosquitoes included *An. vagus*, *An. maculatus* and *An. barbirostris* and *Culex malayi*. *Cx. malayi* accounted for about 80% of the co-breeders, the other species were found in smaller numbers (Table 2.1.3).

Almost all wells harboured at least one or more predators. The most common predators were Notonectidae (backswimmer), Agrionidae (damselfly larvae), Nepidae (water scorpion) and *Orthetrum sabina* (dragonfly). Carnivorous fish, catfish (*Ophicephalus sp.*), and *Panchax* (endemic species), were also found in some wells.

All the wells inhabited by the immature stages of *An. dirus* were partially or totally shaded and contained debris and vegetation. None were exposed to direct sunlight.

The majority of wells (66%) were situated in compounds, where cattle, fowl, dogs and cats were present.

(b) Phado area, Kyauktagar Township, Bago Division: In the Phado wood-cutting camp, situated in a deep forest, *An. dirus* larvae were found breeding in rocky pools along the bank of densely shaded streams (Table 2.1.4).

(c) Thabyewa area, Oktwin Township, Bago Division: (i) *An. dirus* larvae were found in rocky pools shaded by trees inside a dense teak forest area and (ii) bamboo stumps wereinhabited by numerous larvae of *An. dirus* in this area. Water in these bamboo stumps contained decayed leaves and co-breeders were the larvae of *Aedes albolateralis* (Table 2.1.4).

Table 2.1.4: The breeding site conditions of *An. dirus* in Bago Division, during 1998 , 1999

Localities	Type of breeding-sites	Co-breeder	Water condition	Other conditions
Bago Division				
Phado area	Rocky pools	*Culex malayi*	Clear	Deep forest under heavy shade, the pool contains decayed leaves at the bottom
Thabyewa area	Rocky pools	*Culex malayi*	Clear	Shaded by trees inside a dense teak forested area
	Bomboo-stumps	*Aedes albolateralis*	Clear	Shaded by trees inside a dense teak forested area, the water contained decayed leaves

(9) ***Anopheles fluviatilis*** James, 1902

During the study period, in Rakhine State, Shan State and Sagaing Division, a very few larvae of *An. fluviatilis* were found in streams, canals, ponds with grassy edges and in clear rainwater pools.

(10) ***Anopheles gigas*** Giles, 1901

An. gigas is only found at high altitudes. During the study period, in Mogok Township, Mandalay Division, six larvae of *An. gigas* were recorded in a forest stream. In

Falam Township area, Northern Chin Hills, a total of 25 larvae were sampled in pools situated along slow running streams.

(11) *Anopheles sinensis* Weidemann, 1828

An. sinensis is predominatly a stagnant water breeder. In Katha Township, Sagaing Division, it breeds in tanks, swamps, pits, rice fields and other stagant collections of water invariably in association with thick vegetation. In Mogok Township, Mandalay Division, *An. sinensis* is also found among grass and weeds along the edges of slow flowing streams or irrigation channels. It is frequently found to breed in association with *An. barbirostris.*

In Pyay Township, Bago Division, *An. sinensis* larvae are also commonly found in field channels closely associated with rice fields and associated with larvae of *An. culicifacies*, *An. subpictus* and *An. pallidus.*

(12) *Anopheles nitidus* Harrison, Scanlon and Reid, 1973

The breeding sites are similar to those of *An. nigerrimus*. *An. nitidus* is predominantly a stagnant water breeder. In Taungoo Township area, Bago Division, *An. nitidus* larvae are commonly found in rice fields and associated with larvae of *An. barbirostris*, *An. annularis* and *An. nigerrimus.*

In Mawlaik Township, Sagaing Division, it breeds in tanks, swamps, pits, paddy-fields and other stagnant collections of water invariably in association with thick aquatic vegetation and associated with larvae of *An. nigerrimus* and *An. barbirostris.*

In Mudon Township, Mon State, *An. nitidus* were recorded in unused shallow wells filled with decaying vegetation and also associated with larvae of *An. barbirostris*, *An. annularis* and *An. nigerrimus.*

(13) *Anopheles insulaeflorum* (Swellengrebel and Swellengrebel de Graaf), 1920

During the study period, in Mabein Township, Shan State, only five larvae of *An. insulaeflorum* were found in shady pools along forest streams.

(14) *Anopheles jamesii* Theobald, 1901

An. jamesii breeds mainly in stagnant water. In Kengtung and Lashio Townships, Shan State, it has been found in grassy edged slow-flowing streams with aquatic vegetation.

In Bago, Mandalay and Sagaing Divisions, *An. jamesii* have been recorded in pools, swamps, pits, stagnant drains and unused shallow wells always in association with thick aquatic vegetation.

(15) *Anopheles jeyporiensis* James, 1902

Breeding sources of *An. jeyporiensis* are mainly in slow-moving streams and channels with grassy edges and associated with larvae of *An. aconitus.* Rice fields are also attractive breeding sites for *An. jeyporiensis* when fallow but become rapidly unfavourable as the plants grow.

In the Rakhine State, the larvae of *An. jeyporiensis* were found in slow moving streams with a marshy bottom and a dense growth of sedge (grass) and other emergent plants. They are associated with the larvae of *An. aconitus*.

In Kabaw valley, Sagaing Division, *An. jeyporiensis* larvae breed in stagnant water, mainly swamps, particularly where there was dense emergent vegetation.

(16) *Anopheles karwari* (James), 1903

In Kyaukpyu, Rakhine State, *An. karwari* breeds in standing water of rice fields. On all other occasions this species was found in hill streams either along grassy edges or in sandy pools in the bed of the stream.

(17) *Anopheles kochi* Döenitz, 1901

An. kochi is essentially a stagnant-water breeder. In Rakhine, Kachin, Kayah and Kayin States, the larvae of *An. kochi* were found mainly in muddy pools, roadside drains and puddles, cart tracks, uncultivated rice fields and swamps. However, in Mandalay and Sagaing Divisions, it has also been found occasionally in irrigation channels and in sandy beds of streams fully exposed to sunlight, often in association with the larvae of *An. vagus*.

(18) *Anopheles kyondawensis* Abraham, 1947

During the study period, only one larva of *An. kyondawensis* was sampled in Mawlamyine Township area, Mon State. It was found in shady pools along forest stream associated with the larvae of *An. insulaeflorum*.

(19) *Anopheles lindesayi* Giles, 1900

During the study period, in Chin and Shan States, the larvae of *An. lindesayi* were caught from shaded margins of a jungle streams close to the villages. They were associated with the larvae of *An. aikeni*.

(20) *Anopheles maculatus* Theobald, 1901 and

The preferred breeding sites of *An. maculatus* are in rocky or sandy pools of hill streams, or in trickles of water among pebbles along the edge of streams. The larvae prefer open areas in direct sunlight and are not found in the heavily shaded patches of streams. The presence or absence of vegetation does not appear to be important. These species are rarely found in stagnant water and have been recorded only very occasionally from pits and fresh pools in uncultivated rice fields. The larvae do not tolerate any degree of pollution.

In Tanintharyi Division, (a) Kyawthaung Township area, (i) the larvae of *An. maculatus* group were found breeding in rocky streams under partial shade together with the larvae of *An. minimus* and (ii) these larvae were also gathered under rocks and pebbles along the edges of the stream; (b) in Meik Township, forested hilly area, (i) this group was collected in a water overflow stream (from a hydroelectric station) between rocks under direct sunlight and (ii) the *An. maculatus* group breeds in very sluggishly flowing streams with plenty of vegetation.

(21) *Anopheles willmori* (James), 1903

The breeding sites are similar to those of *An. maculatus*.

In Bago Division, Kyauktagar Township area, (i) in the foothill villages of Ngokto and Gwegon, the *An. willmori* was sampled in rice fields in association with the larvae of *An. minimus* and (ii) in the teak forests of Thabyewa village, the larvae of *An. willmori* were found breeding in jungle streams under partial shade.

(22) *Anopheles majidi* Young and Majid, 1928

During the study period, only adults were collected and no larvae of *An. majidi* were recorded.

(23) *Anopheles minimus* Theobald, 1901

During the study period, *An. minimus* larvae were found in following types of breeding sites:

(a) In Phado village, Kyauktagar Township, Bago Division, (i) *An. minimus* larvae were sampled at the edge of slow-running clear streams together with larvae of *An. maculatus*; (ii) during the dry season when water flow stopped, *An. minimus* larvae were found in small water collections and puddles in the stream bed associated with the larvae of *An. maculatus* and (iii) at the end of monsoon, *An. minimus* breeds at the edge of rice fields and water collections near rice fields.

(b) In Mandalay Division, Pyinoolwin Township, Sedawgyi area and Mogok Township, the larvae of this species were collected in small running streams where water flow was extremely sluggish and the edges of the stream covered with vegetation.

(c) In Hlegu Township area, Yangon Division, *An. minimus* larvae were sampled in stagnant pools with unpolluted water and associated with the larvae of *An. maculatus.*

(d) In Oktwin Township area, Bago Division and Magway Township area, Magway Division, the larvae of *An. minimus* were collected along the grassy edges of small forest streams which were partially shaded. The breeding sites were either inside or very close to deep forest.

(e) In Kawthaung and Bokpyin Township deep forest areas, Tanintharyi Division, *An. minimus* was most abundant in small clear water streams with rocks and grassy edges together with the larvae of *An. maculatus.*

(24) *Anopheles nigerrimus* Giles, 1900

The breeding sites are similar to those of *An. nitidus*. *An. nigerrimus* is predominantly a stagnant water breeder. In Taungoo Township area, Bago Division, *An. nigerrimus* larvae are commonly found in rice fields and associated with larvae of *An. barbirostris*, *An. annularis* and *An. nitidus*.

In Mawlaik Township, Sagaing Division, it is sampled in tanks, swamps, pits, rice fields and other stagnant collections of water, invariably in association with thick aquatic vegetation and associated with larvae of *An. nitidus* and *An. barbirostris.*

In Mudon Township, Mon State, *An. nigerrimus* was breeding in unused shallow wells filled with decaying vegetation and and also associated with larvae of *An. barbirostris*, *An. annularis* and *An. nitidus*.

(25) *Anopheles pallidus* Theobald, 1901

An. pallidus is a stagnant water breeder. In Shwebo area, Sagaing Division, this species have been collected in rice fields and in stagnant collections of water, always in association with thick vegetation. In Pyapon area (Kayin State), Taungoo area (Bogo

Division) and Mawlamyine Township area (Mon State), the larvae of *An. pallidus* have been found in swamps, ditches, pits, and other stagnant water, always in association with thick vegetation and often grossly polluted.

(26) *Anopheles peditaeniatus* (Leicester), 1908

In Insein Township, Yangon Division, *An. peditaeniatus* breeds in rice fields in association with the larvae of *An. nigerrimus*. In Ayewarwady, Bago, Mandalay Divisions and Mon State, the larvae of this species were sampled from the rice fields together with the larvae of *An. nigerrimus*.

(27) *Anopheles philippinensis* Ludlow, 1902

An. philippinensis is predominatly a stagnant water breeder especially in wide stretches of water with developing vegetation. It is often found breeding with the larvae of *An. aconitus, An. annularis* and *An. sinensis*. It is particularly associated with areas of derelict or cultivated rice fields but is also found in tanks, ponds, pits or swamps with grassy edges or with emergent vegetation. In hilly areas it has been found along the grassy edges of streams and in pools. It is frequently found in water collections covered by a dense growth of green algae.

Many types of aquatic vegetation are favourable sites. The aquatic plants which are most frequently associated are *Hydrilla*. It appears that certain plants such as *Lemna* (duck weed) and *Eichhornia* (water hyacinth) are inimical to this species. The dense growth of the water hyacinth controlled the breeding of this species effectively. The effect of shade seemed to be an indirect one affecting algal growths, particularly of green algae which provided the main food for the larvae and favoured the development of the larvae.

(28) *Anopheles pseudojamesi* Strickland and Chowdhury, 1927

During the study period, only five larvae of *An. pseudojamesi* were recorded (June to November 1998) from Kyaukpyu Township, Rakhine State (Western coastal strip). The larvae were found among water hyacinth in a slow flowing stream.

(29) *Anopheles splendidus* Koidzumi, 1920

In Mandalay Division, Meiktila and Pyinoolwin Township areas, the larvae of *An. splendidus* breed mainly in irrigation channels and also in grassy edged pits. In Bago Division, Taungoo, Yemethin and Pyay Township, and Pyinmana Township, Mandalay

Division, the larvae of *An. splendidus* were found in the beds of hill streams and unpolluted jungle pools.

(30) *Anopheles stephensi* Liston, 1901

In foothill areas, such as Meiktila and Mandalay-Pyinoolawin Townships, Mandalay Division, *An. stephensi* has been found in backwaters along the edges of rocky hill streams and in pools, often in association with thick vegetation. However, in a few urban areas, e.g. Mawlaik Township (Sagaing Division), Pyay Township (Bago Division) and Magwe Township (Magwe Division), larvae have been recorded from cisterns, shallow cemented wells and domestic water vessels inside houses, while in rural areas they are found in pools, stream beds and irrigation channels. *An. stephensi* is able to tolerate a moderate amount of pollution and prefers sunlit to shaded stretches of water.

(31) *Anopheles subpictus* Grassi, 1899

An. subpictus larvae are found in association with *An. vagus* larvae in all types of stagnant water collections such as muddy pools, roadside puddles, pits, drains and rice fields. The larvae of this species is able to tolerate a good deal of water pollution.

(32) *Anopheles sundaicus* (Rodenwaldt), 1925

In coastal area, *An. sundaicus* was found to be breeding mainly in the lakes. It was also found in tanks, and ponds with heavy aquatic vegetation. Situated close the lakes were the chief inshore breeding sitess with a mixture of saline and freshwater.

In Rakhine State; (i) in Kyaukphyu Township area, *An. sundaicus* was sampled in brackish ponds and also in shallow water in rice fields and (ii) on the Myanmar-Bangladesh border, the larvae were recorded from brackish pools in the intertidal zones near the seawater inlet.

In Ayeyarwady Division, this species was collected in rice fields, close to seawater inlets.

There appears to be an association of *An. sundaicus* breeding with the growth of both vegetation and algae. Several types of algae including *Lyngbya, Anabena* and *Spirogyra* were linked to the breeding of this species. Floating or submerged vegetation including ferms like *Najas, Ceratophylum* and *Hydrilla* are also favourable. A dense cover of water hyacinth, however, could prevent breeding of *An. sundaicus*.

(33) *Anopheles tessellatus* Theobald, 1901

Breeding sites of *An. tessellatus* are mainly in stagnant water. It has been found in tanks, ditches, drains and pits, always with thick vegetation. *An. tessellatus* is able to tolerate some degree of pollution. In Bago Township area, Bogo Division, the larvae was sampled in muddy pools together with the larvae of *An. vagus*. In Kyaukse Township, Mandalay Division, *An. tessellatus* larvae were breeding in clear sandy streams.

(34) *Anopheles theobaldi* Giles, 1901

Around Lashio Town, Shan State, *An. theobaldi* breeds in rocky or sandy pools in the beds of hill streams. The larvae prefer open areas in direct sunlight. The presence or absence of vegetation does not appear to be important. *An. theobaldi* has been recorded only in Lashio Town, Shan State.

(35) *Anopheles vagus* Döenitz, 1902

The larvae of *An. vagus* was found in all types of stagnant water, both polluted and unpolluted. It is very common in muddy pools, pits, stagnant drains, marshes and rice fields. *An. vagus* is found in association with the larvae of *An. subpictus* in almost all types of stagnant water. It has also been recorded occasionally from the edges of streams and irrigation channels.

(36) *Anopheles varuna* Iyengar, 1924

The breeding sites of *An. varuna* are mainly in slow-running, grass-edged streams which are exposed to sunlight and contain clear water. However, in hill tracts such as near Myitkyina Town, Kachin State, *An. varuna* were found in rice fields, exposed to sunlight.

2.3.3.3. Adult bionomics

The distribution of the species is shown in Appendix C.

(1) *Anopheles aconitus* Döenitz, 1902

Seasonal prevalence: In Rakhine State, *An. aconitus* is fairly abundant from October to February with a peak in November. It is very rarely recorded in March and not at all between April to September. In Mandalay Division, Mandalay-Pyinoolwin foothill area, *An. aconitus* first appears in October and rapidly increases in numbers until the third week of November. It is not recorded from April to August. In some areas, however, *An. aconitus* appears later, in Bago and Sagaing Divisions for instance adults are more prevalent in April and May than in the earlier months of the year. In almost all areas *An. aconitus* is very scarce during the monsoon. The seasonal abundance of *An. aconitus* at various catching stations is shown in Table 2.2.1.

Table 2.2.1: Number of *An. aconitus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	October	November	December	January	February	March	April	May
Rakhine State									
Sittwe	1998	73	401	208	101	43	22	0	0
	1999	69	380	198	112	32	19	0	0
Kyaukpyu	1998	84	165	92	52	30	19	1	0
	1999	76	148	98	62	41	14	1	0
Mandalay Division									
Mandalay-Pyinoolwin foothill area	1998	182	480	301	89	18	8	0	0
	1999	112	453	230	78	22	10	0	0
Bago Division									
Taungoo	1998	15	18	12	15	18	18	49	65
	1999	16	16	19	22	23	32	54	69
Bago area	1998	22	29	28	30	28	32	55	68
	1999	28	29	31	34	31	35	48	52

Resting habits: Very few *An. aconitus* mosquitoes were found resting in houses or stables during daytime catching attempts. In some areas *An. aconitus* rests outdoors in bushes. Table 2.2.2 shows the results of day and night collections of *An. aconitus* in various catching stations during 1998, 1999.

Host preference: An. aconitus was predominantly a cattle feeder, but adults attack man if cattle are not available or very scarce (Table 2.2.2).

Table 2.2.2: Number of *An. aconitus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An.acontius* collected (day)	Total no. of *An.acontius* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Kawkalok	Nov.-Dec., 98	8	0	118	1	2	4	0	12	37	54	8
Mudon	Nov.-Dec., 98	8	0	106	9	17	32	6	6	11	21	4
Mandalay Div.												
Kyaukse	October, 1998	4	0	96	0	4	5	0	10	25	45	7
Mandalay-Pyinoolwin area	October, 1998	4	0	182	0	5	5	0	17	65	81	9
Rakhine State												
Myohaung	January,1999	4	0	103	2	6	9	0	16	25	34	11
Bago Division												
Paukhaung	Jan.-Dec., 98	8	0	135	1	6	8	0	12	43	55	10

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Biting rhythm: The results in Table 2.2.2 show *An. aconitus* to be an early feeder. The invasion started as early as 18:00 and ended by 01:00 with a peak at 00:30.

Medical importance: The dissection records for *An. aconitus* are shown in Table 2.2.3. In Taungoo Township, Bago Division, 65 specimens were dissected with negative results. In Mon State, 102 specimens were dissected and none were found to be positive. In Rakhine State, (i) Sittwe Township area, 350 specimens of *An. aconitus* were dissected, out of which one had a gland infection and (ii) in Kyaukpyu and Myohaung Township areas no gut and salivary gland infections were found. In Ayeyarwady Division, Seikkyi village, *An. aconitus* had an observed 0.2 percent (1/350) infection rate.

Distribution: An. aconitus is widely distributed and recorded in most types of locality especially in hilly tracts, foothills and also in the plains of Central and Southern Myanmar.

Table 2.2.3: Dissection records of *An. aconitus* infection rates in different localities

Localities	No. of *An. aconitus* dissected	No. of *An. aconitus* with gut infection	No. of *An. aconitus* with gland infection	Infection rate (%)
Sagaing Division				
Kabaw valley	121	0	0	0
Mon State				
Mawlamyine area	102	0	0	0
Rakhine State				
Sittwe	350	0	1	0.2 (1/350)
Kyaukpyu	160	0	0	0
Myohaung	240	0	0	0
Mandalay Division				
Mandalay-Pyinoolwin	78	0	0	0
Kyaukse area	150	0	0	0
Bago Division				
Taungoo	65	0	0	0
Ayeyarwady Division				
Sagagyi village	350	0	1	0.2 (1/350)
Hinthada	85	0	0	0

(2) *Anopheles aitkeni* James, 1903

Seasonal prevalence: During the study period, *An. aitkeni* was collected only in November and April (Table 2.2.4).

Resting habits: An. aitkeni has never been recorded resting in houses during daylight hours (Table 2.2.5).

Host preference: An. aitkeni was caught only by cattle bait at night (Table 2.2.5).

Biting rhythm: An. aitkeni was collected from cattle bait in the first quarter of the night (Table 2.2.5).

Medical importance: An. aitkeni is a wild species and plays no role in malaria transmission.

Distribution: An. aitkeni has a very restricted distribution in Myanmar. The altitude where this species was recorded in this country ranges from 1036 to 1890 m above sea level.

Table 2.2.4: Number of *An. aitkeni* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Sagaing Division												
Mawlaik	1998	0	2	0	0	0	0	0	0	5	0	0
	1999	0	3	0	0	0	0	0	0	4	0	0
Shan State												
Lashio	1998	0	5	0	0	0	0	0	0	5	0	0
	1999	0	4	0	0	0	0	0	0	5	0	0
Kayah State												
Mawchi	1998	0	8	0	0	0	0	0	0	7	0	0
	1999	0	6	0	0	0	0	0	0	6	0	0
Chin State												
Mindat	1998	0	7	0	0	0	0	0	0	5	0	0
	1999	0	6	0	0	0	0	0	0	6	0	0

Table 2.2.5: Number of *An. aitkeni* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. aitkenii* collected (day)	Total no. of *An. aitkenii* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Sagaing Div.												
Mawlaik	Nov., 1998	4	0	5	0	0	0	0	5	0	0	0
Shan State												
Lashio	Nov., 1999	4	0	5	0	0	0	0	5	0	0	0
Kayah State												
Mawchi	April, 1998	4	0	8	0	0	0	0	7	1	0	0
Chin State												
Mindat	April, 1999	4	0	6	0	0	0	0	6	0	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

(3) *Anopheles annularis* Van der Wulp, 1884

Seasonal prevalence: The prevalence of *An. annularis* in different parts of Myanmar is found to vary quite markedly as the rainfall pattern differs: (i) in coastal regions, such as Rakhine State and Tanintharyi Division, where rainfall is exceptionally heavy (3846 mm to 5128 mm annually), *An. annularis* is first noted in October, numbers rapidly increase in subsequent

months to a maximum in December through January to February, after which gradually a decrease occurred; (ii) in Bago Division, Ayeyarwady Division and Kayah State, where rainfall is fairly heavy (2051 mm to 3846 mm annually), *An. annularis* occurs in considerable numbers throughout the year; (iii) in Kachin and Shan States, with moderate rainfall (1025 mm to 2051 mm annually) and winters are severe, adults of *An. annularis* are first noted in April, density rises from June with its peak during September and October, after which a decrease was noted; (iv) in the dry zone, Central Myanmar, including Mandalay and Magwe Divisions, where rainfall is extremely limited (1025 mm or less annually), *An. annularis* is most abundant in the cold season, October to December. The population gradually increased until February after which *An. annularis* numbers gradually diminish but adults could be captured until May. Table 2.2.6 shows the seasonal abundance of *An. annularis* at various catching stations during 1998, 1999.

Table 2.2.6: Number of *An. annularis* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.
Rakhine State													
Myohaung	1998	4	32	57	80	68	59	28	18	8	2	0	0
	1999	8	45	52	89	72	48	31	21	9	3	0	0
Bago Division													
Taungoo area	1998	10	12	8	9	11	12	10	8	9	8	7	8
	1999	14	14	9	11	9	16	10	12	13	14	18	11
Kachin State													
Kamaing	1998	63	68	47	34	24	10	3	22	26	36	39	42
	1999	69	79	51	41	29	18	3	23	23	39	43	52
Mandalay Divi.													
Kyaukse area	1998	18	29	30	30	22	17	15	12	8	0	0	2
	1999	22	34	38	41	30	23	18	12	10	0	0	1

Resting habits: The resting habit of *An. annularis* varies from one locality to another and shows some seasonal variation (see Table 2.2.7). In Rakhine State for example, in Kyaukpyu, Sittwe and Myohaung areas, a few *An. annularis* were found resting in houses and cattlesheds. In Mon State, adults fed on cattle at night but were never found in houses or cattlesheds by day. In the Mandalay-Pyinoolwin foothill area, however, during the period October to December, although 147 adults of *An. annularis* were taken in night catches, not a single one was found resting in houses by day. During December 1998 and 1999, in Seasone village from Kyaukse area, Mandalay Division, *An. annularis* was noted resting by day on

small plants about one meter in height, but not a single specimen was observed resting in cattlesheds.

Table 2.2.7: Number of *An. annularis* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. annularis* collected (day)	Total no. of *An. annularis* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Innwaing	Nov.,1998	4	0	179	15	6	1	1	58	45	31	22
Mudon,(Q.4)	Nov.,1998	4	0	161	32	3	1	1	52	58	42	30
Mudon,(Q.3)	Nov.,1998	4	0	178	30	12	5	2	32	50	41	6
Mandalay Div.												
Kyaukse	Dec.98 to Dec.,99	12	1**	218	1	0	0	0	97	72	45	2
Rakhine State												
Myohaung	Oct.-Nov.,99	8	20	89	16	7	0	0	30	16	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
**Daytime outdoor collection

Host preference: The results in Table 2.2.8 show that *An. annularis* has a high preference for cattle blood in almost all areas.

Biting rhythm: The biting time of *An. annularis* can be illustrated by the results in Table 2.2.8. The greatest biting activity was recorded during the first and second quarter of the night on cattle bait. The biting activity gradually diminished from 22:30 to 03:00, after which a sharp decline occurred. The proportion of *An. annularis* feeding on cattle during the first and second quarter of the night ranged from 80 to 90 percent of the total night catches at different catching stations.

Medical importance: The dissection records on *An. annularis* are shown in Table 2.2.8. In Taungoo, Bago Division, 85 specimens were dissected with negative results. In Mon State, 100 specimens were dissected without any positive finding. In Rakhine State (i) Kyaukpyu area 700 specimens of *An. annularis* were dissected, from which one was found with oocysts

(gut infection); (ii) in Sittwe 350 specimens were dissected with an observed 0.2 percent infection rate.

Table 2.2.8: Dissection records of *An. annularis* infection rates in different localities

Localities	No. of *An. annularis* dissected	No. of *An. annularis* with gut infection	No. of *An. annularis* with gland infection	Infection rate (%)
Sagaing Division				
Kabaw valley	221	0	0	0
Shan State				
Lashio	300	0	0	0
Mon State				
Mawlamyine area	90	0	0	0
Rakhine State				
Sittwe	350	1	0	0.2 (1/350)
Myohaung	450	1	0	0.2 (1/450)
Kyaukpyu	700	1	0	0.1 (1/700)
Mandalay Division				
Kyaukse area	160	0	0	0
Bago Division				
Taungoo	85	0	0	0

Distribution: An. annularis is very widely distributed and has been found in all regions. It has been recorded from Kachin State in the north to the Tanintharyi coastal strip in the south, Shan State in the east to Rakhine coastal area adjoining Myanmar-Bangladesh border in the west.

(4) *Anopheles argyropus* (Swellengrebel), 1914

Seasonal prevalence: During the study period, only three specimens (adults) have been recorded, in October and November, 1998 (Table 2.2.9).

Resting habits: No specimen was recorded in daytime catches (Table 2.2.9).
Host preference: These three specimens (*An. argyropus*) were collected by cattle bait at night (Table 2.2.9).

Biting rhythm: An. argyropus is early feeder, it was only collected by cattle bait during the first quarter of the night (Table 2.2.9).

Medical importance: An. argyropus is not a vector of malaria.

Table 2.2.9: Number of *An. argyropus* caught by different techniques in various catching station from Tamu Township, Sagaing Division, during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. argyropus* collected (day)	Total no. of *An. argyropus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Sagaing Div.												
Tamu	Oct., 1998	4	0	2	0	0	0	0	2	0	0	0
	Oct., 1999	4	0	1	0	0	0	0	1	0	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Distribution: *An. argyropus* is recorded only in Tamu Township area, Sagaing Division.

(5) *Anopheles barbirostris* Van der Wulp, 1884

Seasonal prevalence: In almost all areas, *An. barbirostris* is abundant in the post-monsoon months, with a peak in October and November. Table 2.2.10 shows the seasonal abundance of *An. barbirostris* at various catching stations during the study period. In Bago, Ayeyarwady and Tanintharyi Divisions, *An. barbirostris* was found throughout the year, reaching a peak in November.

Resting habits: In most areas, *An. barbirostris* did not occur in daytime catches. However, a small number of adults were recorded in daytime catches e.g. (i) in Mandalay-Pyinoolwin foothill area, although 807 *An. barbirostris* were found during night catches, only 9 were found resting in houses by day; (ii) in Magwe and Bago Divisions, a few *An. barbirostris* specimens were found in morning catches. Table 2.2.10 shows the results of day and night catches at various catching stations during 1998, 1999.

Host preference: An. barbirostris feeds mainly on cattle although small numbers will enter houses and bite man especially if cattle are not available (Table 2.2.11).

Biting rhythm: An. barbirostris is a early feeder with a maximum of biting activity during the second quarter of the night; 21:00 to 24:00 (Table 2.2.11).

Medical importance: An. barbirostris is not a vector of malaria.

Table 2.2.10: Number of *An. barbirostris* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Ayeyarwady Division													
Myaungmya	1998	7	8	8	9	10	10	12	12	15	18	22	20
	1999	9	9	9	10	10	12	13	18	18	20	25	24
Bago Division													
Taungoo	1998	11	14	10	14	18	20	28	34	38	42	55	40
	1999	13	18	19	20	19	22	30	34	40	58	78	45
Magwe Division													
Pyintpyu	1998	19	18	22	31	32	40	45	45	48	50	59	52
	1999	18	22	30	40	44	45	49	49	49	51	68	53
Mandalay Division													
Kyaukpadaung	1998	12	17	18	20	20	32	36	40	47	101	120	83
	1999	10	10	13	22	30	31	30	42	50	100	109	74
Sagaing Division													
Wuntho	1998	11	20	20	22	22	28	31	30	38	42	45	40
	1999	8	10	11	20	21	22	38	39	40	42	52	42

Table 2.2.11: Number of *An. barbirostris* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An.barbirostris* collected (day)	Total no. of *An.barbirostris* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Mandalay-Pyinoolwin area	Oct.-Dec. 1998	12	9	807	9	12	3	2	357	363	46	15
Kyaukpadung	Oct.-Dec. 1998	12	0	304	8	10	3	3	102	140	27	11
Bago Division												
Pyay	Jul.-Oct. 1998	16	7	126	2	2	0	0	51	65	5	1
Bago	Apr.-June 1999	12	1	10	1	1	0	0	2	6	0	0
Magwe Division												
Magwe	Apr.-June 1999	12	2	21	1	1	0	0	7	8	3	1
Sagaing Div.												
Kalemyo	Sep.-Nov. 1999	12	0	17	1	1	0	0	4	6	3	2
Katha	Sep.-Nov. 1999	12	0	214	3	4	1	0	92	101	10	3

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Distribution: An. barbirostris is the most widely distributed species in Myanmar and found in every locality surveyed.

(6) *Anopheles bengalensis* Puri, 1930

Seasonal prevalence: During the study period, *An. bengalensis* was collected only in November and April (Table 2.2.12).

Resting habits: An. bengalensis has never been recorded resting in houses by day (Table 2.2.13).

Host preference: An. bengalensis is predominantly a cattle feeder (Table 2.2.13).

Table 2.2.12: Number of *An. bengalensis* females caught over four days (daytime and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Mandalay Division												
Mogok	1998	0	4	0	0	0	0	0	0	8	0	0
	1999	0	6	0	0	0	0	0	0	7	0	0
Chin State												
Falam	1998	0	8	0	0	0	0	0	0	10	0	0
	1999	0	10	0	0	0	0	0	0	11	0	0
Kanpetlet	1998	0	12	0	0	0	0	0	0	13	0	0
	1999	0	11	0	0	0	0	0	0	11	0	0

Biting rhythm: An. bengalensis is an early feeder and was collected from cattle bait in the first quarter of the night (Table 2.2.13).

Medical importance: An. bengalensis is a wild species and plays no part in malaria transmission.

Distribution: An. bengalensis is not widely distributed in Myanmar. The altitude where this species is recorded ranges from 1036 to 1890 m above sea level.

Table 2.2.13: Number of *An. bengalensis* caught by different techniques in various catching stations during 1998, 1999

Catching Stations	Period	No. of catches	Total no. of *An. bengalensis* collected (day)	Total no. of *An. bengalensis* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Mogok	Nov., 98	4	0	8	0	0	0	0	8	0	0	0
Chin State												
Falam	Nov., 99	4	0	11	0	0	0	0	10	1	0	0
Kanpetlet	April, 98	4	0	12	0	0	0	0	11	1	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

(7) *Anopheles culicifacies* Giles, 1901

Seasonal prevalence: Table 2.2.14 shows the seasonal occurence of *An. culicifacies* in various parts of Myanmar. In the irrigated plain areas of Mandalay Division such as Kyaukse and Myittha Townships, *An. culicifacies* appears in May and rapidly increases in numbers during the subsequent months. The maximum abundance of *An. culicifacies* is in August and September, after which numbers decrease suddenly. During October, the number of specimens captured is small, while none could be found from November to March.

The seasonal abundance of *An. culicifacies* in Shwebo and Monywa Townships from Sagaing Division, is similar to that found in the irrigated plain areas of Mandalay Division. However, in the same Division, Katha and Mawlaik Townships, *An. culicifacies* appears in April and May. After a slight decline in June to August, it increases in numbers during September and reaches its highest abundance in October. Thereafter numbers gradually fall from November to April.

In Bago Division, Taungoo and Bago Townships, the number of adults rises from May and falls in June, especially after the onset of early rains. *An. culicifacies* is entirely absent during the end of September to early February. However, in Pyay Township it is noticed only in moderate numbers in April and May.

In Ayeyarwaddy Division, a few *An. culicifacies* have so far been recorded only in Myanaung Township. The seasonal abundance of *An. culicifacies* is high during the pre-monsoon months of February to May. It has never been found in other rice-growing areas of this Division.

Table 2.2.14: Number of *An. culicifacies* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Mandalay Division										
Kyaukse	1998	0	182	238	257	504	398	126	0	0
	1999	0	101	192	203	401	426	148	0	0
Bago Division										
Tounggoo	1998	0	284	262	172	149	35	0	0	0
	1999	0	286	147	119	101	58	0	0	0
Saging Division										
Katha	1998	131	147	99	106	126	249	343	190	92
	1999	109	132	101	104	108	238	369	210	101

An. culicifacies is recorded only near Thandaung Township, Kayin State, during the period of March to May and is found in moderate numbers. It has never been recorded from the southern part of this State, which is adjoining Thaninlaryi Division (Fig. 1.1).

An. culicifacies is very rare in Kachin State. It is recorded only from a few villages close to Myitkyina and Bhamo towns during the pre-monsoon months of May and June.

It has never been encountered in Chin State and Tanintharyi Division.

Resting habits: An. culicifacies is predominantly a domestic species. Adults prefer to rest in cattlesheds and houses during the day, but *An. culicifacies* may take shelter in paddy-sheds, heaps of fire-wood and piles of straw near the stables and outside houses. *An. culicifacies* have also been captured from dense vegetation and under bushes. *An.culicifacies* has a tendency to rest inside domestic utensils such as pots, umbrellas, hanging clothes and under furniture. The abundance of *An. culicifacies* in daytime catches of different catching stations are shown in Table 2.2.15.

During the survey, more adults were caught during daytime than in night catches (Table 2.2.15 and 2.2.16). Cattlesheds generally yielded a large number of resting adults than human dwellings. *An. culicifacies* also shelters in large grain storage containers and also uses the ceilings and thatched roofs of houses as its daytime resting sites; (i) in Taungoo Township, Bago Division, a large number of specimens were observed resting under the thatched roof of houses; (ii) in Kyaukse area, Mandalay Division, many *An. culicifacies* were found resting under and inside the roofs of open cattlesheds. These cattlesheds roofs are made of palm leaves in this area, which provide suitable daytime shelter for this species; (iii) in Seazone village in Kyaukse Township, Mandalay Division, *An. culicifacies* used outdoor shelters such as paddy sheds (rice barns) where paddy and other crops are stored, as daytime resting sites.

Table 2.2.15: Number of *An. culicifacies* females caught over four days (daytime) per month in various catching stations during 1998, 1999

Catching stations	Year	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Mandalay Division										
Kyaukse	1998	0	101	150	157	442	320	103	0	0
	1999	0	70	230	240	366	346	100	0	0
Bago Division										
Taungoo	1998	0	260	188	110	89	20	0	0	0
	1999	0	243	101	99	80	32	0	0	0
Saging Division										
Katha	1998	90	99	70	73	89	209	313	101	66
	1999	89	93	68	71	72	211	340	188	72

Host preference: An. culicifacies is mainly a zoophilic species. In almost all areas in Central Myanmar, cattle bait catches at night yielded moderate numbers of specimens and very few were caught by human bait. Table 2.2.16 shows the results of daytime and night collections of *An. culicifacies* in various catching stations.

Biting rhythm: The time of feeding varies from place to place and from season to season (Table 2.2.16). In Kyaukse Township, Mandalay Division, *An. culicifacies* began to feed on both human and animals mostly around midnight and very few were biting at 03:00 and none at 05:00 to 06:00. Subsequent work carried out in another village in the same Township, showed a similar trend. In Kyaukpadaung Township, Mandalay Division, it was observed that few mosquitoes were biting before 01:00, then there was a peak until 04:00, after which it gradually decreased and no specimen was found biting after 05:30.

Medical Importance: Dissections were made during the study period and the locality results are shown in Table 2.2.17.

Distribution: The distribution of *An. culicifacies* is mainly confined to plain and irrigated areas, especially in the Central Dry Zone. Although it is a plain species, it was found at moderate altitudes such as on the Shan Plateau (Shan State) in the eastern part of Myanmar. It has been recorded occasionally in the south, especially in Mon State, though only very few specimens have been observed.

Table 2.2.16: Number of *An. culicifacies* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An.culicifacies* collected (day)	Total no. of *An.culicifacies* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Kyaukse	June, 98	4	150	88	0	2	5	0	9	38	34	0
Kyautpadaung	June, 98	4	101	97	0	1	3	0	8	54	30	1
Bago Div.												
Taungoo	June, 98	4	188	74	0	2	5	0	4	42	18	3

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Table 2.2.17: Dissection records of *An. culicifacies* infection rates in different localities

Localities	No. of *An. culicifacies* dissected	No. of *An. culicifacies* with gut infection	No. of *An. culicifacies* with gland infection	Infection rate (%)
Sagaing Division				
Katha	120	0	0	0
Mawlaik	32	1	0	3.1 (1/32)
Shan State				
Hsipaw	113	1	0	0.8 (1/113)
Lashio	57	0	0	0
Shwenyaung	50	1	0	2.0 (1/50)
Myanmar-China border	26	1	0	3.8 (1/26)
Magwe Division				
Pwinbyu	66	1	0	1.5 (1/66)
Mezali	41	1	0	2.4 (1/41)
Mandalay Division				
Sezone, Kyaukse area	160	0	0	0
Pyinoolwin	108	0	0	0
Kyaukpadaung area	651	0	0	0
Mandalay area	35	0	0	0
Yamethin area	35	0	0	0
Bago Division				
Yedashe	35	0	0	0

(8) *Anopheles dirus* Peyton and Harrison, 1979

Seasonal prevalence: This species is abundant in the monsoon months and the peak density is obtained during September and October. Table 2.2.18 shows man-biting rate per hour (mbr/h) of *An. dirus* at Phado, foothill village of Bago Yoma range.

There is a definite relationship between *An. dirus* man-biting rate per hour (mbr/h) and the distance of the catching station from the forest (Table 2.2.19). The highest mbr`s were observed at Htiphado wood cutting camp. The next highest rate was at Phado and Kyarkyaungthaike, temporary settlements at the edge of forested foothills. At Ngokto village about 0.5 kilometer from the forest edge, *An.dirus* was collected in small numbers, while at Gwegon village, situated about 2.4 kilometers away from the forest, the density of *An. dirus* recorded was much lower.

Table 2.2.18: Average man-biting rates of *An. dirus* at Phado, Bago Division during 1998 1999

Period	man biting rate (mbr/h)	Rainfall (mm)
1998		
September	1.14	346
October	1.8	112
November	0.44	0
December	0.95	0
1999		
January	0.25	0
February	0	0
March	0	0
April	0	14
May	0.45	92
June	0.73	371
July	0.91	537
August	1.36	1039
September	1.04	276

Resting habits: An. dirus was found resting in the crevices and vegetation around the inner walls of the domestic wells during daytime at Mudon Town, Mon State (Table 2.2.20).

Daytime indoor resting catches carried out in various areas did not yield any *An. dirus.* Adults could be collected however, resting below banana leaves at Yasitkan village, Taikkyi Township, Yangon Division. According to the results, this species is highly exophilic (Table 2.2.21).

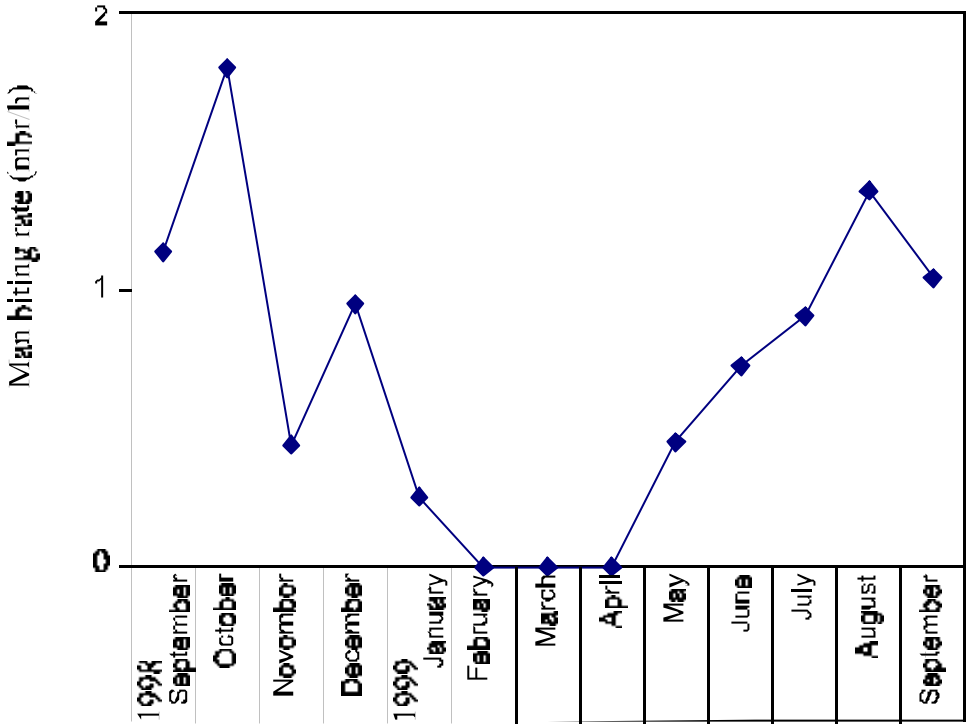


Fig. 2.94. Average man-biting rates of *An. dirus* at Phado, Bago Division during 1998, 1999

Table 2.2.19: Relationship between An. dirus man-biting rate per hour (mbr/h) and the distance of the catching station from the forest

Localities	Township	mbr/h	Remarks on location
Bago Division			
Htiphado	Kyauktagar	2.5	Deep forest wood-cutting camp in Bago Yoma range
Phado	Kyauktagar	1.36	Temporary settlement on edge of forested foothill
Kyarkyaungthaik	Kyauktagar	1.32	Temporary settlement on edge of forested foothill
Ngokto	Kyauktagar	0.83	Village, 0.5 kilometer from the forest edge
Gwegon	Kyauktagar	0.4	Village, 2.4 kilometers of forested foothill

Table 2.2.20: Results of the resting behaviour and physiological stage of *An. dirus* in ten wells from Mudon Town, Mon State (07:00 to 09:00)

Period	Physiological stage of mosquito						
	UF	F	HG	G	Male	Female	Total
1998							
May	1	3	3	0	2	7	9
October	1	4	3	1	2	9	11
1999							
January	1	3	1	0	0	5	5
March	0	2	0	0	0	2	2
May	0	4	3	1	1	8	9
October	1	4	5	1	3	11	14
2000							
January	1	4	1	0	1	6	7
March	0	3	1	0	1	4	5
Total	5	27	17	3	10	52	62

N.B.
UF = Unfed, F = Fully fed, HG = Half gravid, G = Gravid

Table 2.2.21.: Number of *An. dirus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. dirus* collected (day)	Total no. of *An. dirus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Mandalay area	May-Dec. 1998	32	0	264	1	79	108	34	1	6	33	0
Sedawgyi area	Dec., 1998	4	0	5	0	2	2	0	0	0	1	0
Yamethin	Nov., 1998		0	5	0	1	1	1	1	1	0	0
Sagaing Div.		4										
Katha	Apr.-Dec. 1999	36	0	34	9	8	13	3	0	1	0	0
Mon State												
Mawlamyine area	Oct., 1999	4	2**	71	8	14	29	6	0	3	9	0
Thanbyuzayut	Oct., 1999	4	0	62	5	18	20	4	2	5	7	1
Kawkalok	Oct., 1998	4	0	10	1	3	2	0	2	1	0	1
	Oct., 1999	4	0	9	3	2	0	1	1	1	0	1

N.B.
* Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Host preference: The preference of *An. dirus* for human blood is well documented by the results collected during different periods in various areas, as shown in Table 2.2.21. However, a similar study conducted at Mudon, Mon State, revealed that *An. dirus* can be more zoophilic although the breeding sites are situated very close to human dwellings (Table 2.2.22).

Table 2.2.22: Occurrence of *An dirus* in three different ecological areas of Mudon, caught by different techniques

Period	Locality/type of collections and number of mosquitoes collected/hour					
	Domestic area		Rubber plantation area		Forest area	
	Human bait	Cattle bait	Human bait	Cattle bait	Human bait	Cattle bait
1998						
May	0.8	1.6	0.2	0.5	0.2	0.6
October	0.9	2.5	0.4	0.5	0.3	0.5
1999						
January	0.6	0.8	0.0	0.1	0.0	0.1
March	0.4	1.0	0.0	0.0	0.1	0.1
May	0.9	1.0	0.4	0.6	0.2	0.1
October	1.1	2.8	0.4	0.6	0.4	0.8
2000						
January	0.5	1.0	0.1	0.3	0.2	0.5
March	0.1	0.6	0.2	0.5	0.1	0.5

Table 2.2.23: Dissection records of *An. dirus* infection rates in different localities

Localities	No. of *An. dirus* dissected	No. of *An. dirus* with gut infection	No. of *An. dirus* with gland infection	Infection rate (%)
Mandalay Division				
Zeephyupin village	250	1	2	1.2 (3/250)
Bago Division				
Htiphado village	250	1	6	2.8 (7/250)
Phado village	250	1	4	2.0 (5/250)
Kyarkyaungthaik village	250	1	3	1.6 (4/250)
Ngokto village	250	0	3	1.2 (3/250)
Gwegon village	250	0	3	1.2 (3/250)
Mon State				
Innwaing village	200	0	0	0
Thanbyuzayut	250	0	1	0.4 (1/250)
Tanintharyi Division				
Namtun camp	250	1	3	1.6 (4/250)

Biting rhythm: In the present study the outdoor biting peak occurred in the second and third quarter of the night; 21:00 to 03:00 (Table 2.2.21).

Medical importance: Table 2.2.23 shows the results of the dissection for assessment of *An. dirus* infection rates in different localities.

Distribution: An. dirus is mostly associated with forested foothills and deep forests (Oo et al., 2002). However, the forest breeder *An. dirus* can obviously become a peridomestic-breeder (wells in Mudon Town, Mon State) (section 3.1.1.).

(9) *Anopheles fluviatilis* James, 1902

Seasonal prevalence: In Shwebo Township, Sagaing Division, *An. fluviatilis* is abundant from June to December and the peak density is obtained during October and November. In Taunggyi Township, Shan State and Katha and Indaw Townships, Sagaing Division, it is recorded only during October to December. Elsewhere this species is extremely rare. Table 2.2.24 shows the seasonal abundance of *An. fluviatilis* at various catching stations during 1998,1999.

Resting habits: In almost all areas, no specimen was seen resting indoors during the daytime. Table 2.2.25 shows the results of daytime and night collections of *An. fluviatilis* from different catching stations.

Table 2.2.24: Number of *An. fluviatilis* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	June	July	August	September	October	November	December
Sagaing Division								
Shwebo	1998	14	32	34	43	99	98	70
	1999	12	42	48	53	102	101	82
Katha	1998	0	0	0	0	12	48	49
	1999	0	0	0	2	18	58	51
Inndaw	1998	0	0	0	1	20	38	31
	1999	0	0	0	0	28	30	31
Shan State								
Taunggyi	1998	0	0	0	1	8	11	9
	1999	0	0	0	0	4	12	9

Table 2.2.25: Number of *An. fluviatilis* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	no. of catches	Total no. of *An. fluviatilis* collected (daytime)	Total no. of *An. fluviatilis* collected (night)	Collected by different techniques*							
					Human Bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Division												
Mandalay-Pyinoolwin foothill area	Oct.-Dec., 1998	12	9	807	9	12	3	2	357	363	46	15
Kyaukse area	Oct.-Dec., 1998	12	0	304	8	10	3	3	102	140	27	11
Bago Division												
Pyay	July-Oct., 1998	12	7	126	2	2	0	0	51	65	5	1
Bago	Apr.-June, 1999	12	1	10	1	1	0	0	51	65	5	1
Magwe Division												
Magwe area	Apr.-June, 1999	12	2	21	1	1	0	0	7	8	3	1
Sagaing Division												
Kale area	Sep.-Nov., 1999	12	0	17	1	1	0	0	4	6	3	2
Katha	Sep.-Nov., 1999	12	0	214	3	4	1	0	92	101	10	3

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Host preference: The results of Table 2.2.25 show that *An. fluviatilis* has a high preference for cattle blood.

Biting rhythm: The main biting period was during the first and second quarter of the night (Table 2.2.25).

Medical importance: An. fluviatilis cannot be considered as a vector of malaria.

Distribution: An. fluviatilis shows a limited distribution in Myanmar.

(10) *Anopheles gigas* Giles, 1901

Seasonal prevalence: During the study period, *An. gigas* was collected only in March and April (Table 2.2.26).

Table 2.2.26: Number of *An. gigas* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	February	March	April	May	June
Shan State						
Lashio	1998	0	4	6	0	0
	1999	0	6	6	0	0
Chin State						
Falam	1998	0	15	17	0	0
	1999	0	12	14	0	0
Mandalay Division						
Mogok	1998	0	3	3	0	0
	1999	0	3	4	0	0

Resting habits: No specimen was found in houses or cattlesheds in daytime.

Host preference: An. gigas was only collected in shaded jungle areas where it attempted to bite man during the daytime (cattle were not present).

Biting rhythm: No information.

Medical importance: An. gigas is not important for the transmission of malaria because it is a wild species.

Distribution: An. gigas occurs only in hilly areas and is normally found at high altitudes.

(11) *Anopheles sinensis* Weidemann, 1828

Seasonal prevalence: In almost all areas, this species was found from July to December with a peak in August. In Katha, Kachin State, *An. sinensis* is recorded only in August and September after which no specimen was found. Table 2.2.27 shows the seasonal abundance of *An. sinensis* at various catching stations during the study period.

Resting habits: No specimen was found during daytime (Table 2.2.28).

Host preference: It is a cattle feeder. Very small numbers have been caught biting on human bait at night (Table 2.2.28).

Table 2.2.27: Number of *An. sinensis* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	June	July	August	September	October	November	December
Sagaing Division								
Kabaw	1998	0	2	8	6	5	3	2
	1999	0	2	10	6	4	4	2
Kachin State								
Katha	1998	0	0	21	20	0	0	0
	1999	0	0	18	11	0	0	0
Myitkyina	1998	0	22	107	98	12	0	0
	1999	0	18	99	82	20	0	0
Mandalay Division								
Mogok	1998	0	10	31	31	22	20	3
	1999	0	12	32	31	28	13	2

Biting rhythm: The peak biting activity of *An. sinensis* was recorded during the first and second quarter of the night (Table 2.2.28).

Medical importance: The dissection records on *An. sinensis* are shown in Table 2.2.29. In Shan State, Myanmar-China border area, 300 specimens of *An. sinensis* were dissected, out of which 2.3% (6/300) were infected.

Distribution: An. sinensis shows a fairly limited distribution in Myanmar.

Table 2.2.28: Number of *An. sinensis* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	no. of catches	Total no. of *An. sinensis* collected (daytime)	Total no. of *An. sinensis* collected (night)	Collected by different techniques*							
					Human Bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Division												
Mogok	Aug.,99	4	0	32	9	2	0	0	14	7	0	0
Sagaing Division												
Kabaw	Aug.,98	4	0	8	2	1	0	0	3	2	0	0
Kachin State												
Myitkyina	Aug.,98	4	0	107	16	8	0	0	67	16	0	0
Katha	Aug.,99	4	0	21	3	2	0	0	11	5	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Table 2.2.29: Dissection records of *An. sinensis* infection rates in different localities

Localities	No. of *An. sinensis* dissected	No. of *An. sinensis* with gut infection	No. of *An. sinensis* with gland infection	Infection rate (%)
Shan State				
Myanmar-China border	300	3	4	2.3 (6/300)
Sagaing Division				
Katha	45	0	0	0
Kabaw	50	0	0	0
Yangon Division				
Yesitkan	45	0	0	0
Kachin State				
Myitkyina	45	0	0	0
Mandalay Division				
Mogok	30	0	0	0

(12) *Anopheles nitidus* Harrison, Scanlon and Reid, 1973

Seasonal prevalence: In almost all areas, the maximum abundance of *An. nitidus* occurs in October and November (Table 2.2.30).

Resting habits: No adults were found in houses or stables during daytime (Table 2.2.31). Daytime resting places were in rice fields and bushes.

Table 2.2.30: Number of *An. nitidus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Sagaing Division													
Mawlaik	1998	20	13	11	0	0	0	0	0	80	109	148	122
	1999	13	11	10	0	0	0	0	0	75	99	122	103
Mon State													
Mudon	1998	8	7	8	2	3	21	20	24	30	45	31	9
	1999	6	6	7	6	4	18	20	23	38	54	41	8
Bago Division													
Taungoo	1998	20	11	22	29	60	68	78	101	109	118	121	104
	1999	16	18	21	30	54	69	79	99	111	138	139	109

Table 2.2.31: Number of *An. nitidus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. nitidus* collected (day)	Total no. of *An. nitidus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Bago Division												
Taungoo	Oct.,99	4	8**	130	0	0	0	0	79	49	2	0
Mon State												
Mudon	Oct.,99	4	5**	49	0	0	0	0	29	18	2	0
Sagaing Division												
Mawlaik	Oct.,98	4	4**	144	0	0	0	0	98	43	3	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Host preference: An. nitidus is predominantly a cattle feeder (Table 2.2.31).

Biting rhythm: An. nitidus bites as early as 18:00 and showed a peak biting time around 21:00 (Table 2.2.31).

Medical importance: An. nitidus is zoophilic and plays no part in malaria transmission.

Distribution: An. nitidus is widely distributed and found in almost every locality of Myanmar.

(13) *Anopheles insulaeflorum* (Swellengrebel and Swellengrebel de Graaf), 1920

During the study period, only larvae could be collected and no adults of *An. insulaeflorum* have been recorded.

No information on the seasonal prevalence, resting habits, host preference and medical importance are available.

Distribution: *An. insulaeflorum* is only recorded from northern Shan State.

(14) *Anopheles jamesii* Theobald, 1901

Seasonal prevalence: In Rakhine State, *An. jamesii* occurs during January to August and could not be found during September to December. In Taungoo Township, Bago Division and Hinthada Township, Ayeyarwady Division, *An. jamesii* could be collected during the months of March and April, whereas in Mawlamyine Township, Mon State, it is recorded the whole year. Table 2.2.32 shows the seasonal abundance of *An. jamesii* at various catching stations during the study period.

Resting habits: An. jamesii was found resting in bushes and undergrowth during daytime collections. Table 2.2.33 shows the results of day and night catches at various catching stations.

Host preference: An. jamesii is a cattle feeder. Very small numbers have been caught biting on human bait at night (Table 2.2.33).

Biting rhythm: An. jamesii feeds mainly during the first and second quarter of the night with the highest activity around 20:00 (Table 2.2.33).

Medical importance: An. jamesii cannot be regarded as a vector.

Distribution: An. jamesii has been found on few occasions.

Table 2.2.32: Number of *An. jamesii* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Mon State													
Chaungzone area	1998	10	12	11	14	32	88	45	30	14	11	32	8
	1999	23	20	12	13	52	97	41	34	12	7	68	9
Bago Division													
Taungoo	1998	20	22	40	42	33	28	10	2	8	10	18	15
	1999	25	28	48	45	32	30	11	4	8	12	11	10
Rakhine State													
Myanmar-Bangladesh													
border	1998	22	30	56	62	48	20	8	7	0	0	0	0
	1999	18	31	52	50	43	28	9	9	0	0	0	0

Table 2.2.33: Number of *An. jamesii* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. jamesii* collected (day)	Total no. of *An. jamesii* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Chaungzone	June,99	4	1**	96	7	2	0	0	36	29	12	10
Rakhine State												
Myanmar-Bangladesh border	April,98	4	0	62	1	1	0	0	23	17	11	9
Bago Division												
Taungoo	March,99	4	1**	47	1	1	0	0	20	12	9	4
Yangon Division												
Yesitkan	April,98	4	0	42	2	1	0	0	16	10	8	5

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

(15) *Anopheles jeyporiensis* James, 1902

Seasonal prevalence: In Rakhine State, *An. jeyporiensis* is abundant during the pre-monsoon period of March and April. A small number of this species may be caught from May to November (monsoon and post-monsoon) but none in December. However, in Katha and Mawlaik Townships, Sagaing Division, it is abundant during September and October. In Myitkyina and Bahmo Townships, Kachin State, and in northern Shan State, it occurs during August. In Bago, Taungoo and Pyay Townships, Bago Division, *An. jeyporiensis* is present in moderate numbers from September to November with a peak in October. Table 2.2.34 shows the seasonal abundance of *An. jeyporiensis* at various catching stations during 1998, 1999.

Resting habits: An. jeyporiensi was not recorded at any time during the day (Table 2.2.35).

Host preference: In Katha Township (Sagaing Division) and Taungoo and Bago Townships (Bago Division), this species was caught mostly by human bait, but in Mandalay and Kyaukse

Townships, Mandalay Division, it was caught on both human and cattle bait. The results show that *An. jeyporiensis* feeds on man rather than cattle (Table 2.2.35).

Biting rhythm: The main biting period was between 23:30 and 03:00 (Table 2.2.35).

Table 2.2.34: Number of *An. jeyporiensis* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Rakhine State											
Sittwe	1998	48	48	32	28	22	20	15	12	8	0
	1999	38	50	38	31	20	19	17	11	4	0
Bago Division											
Pyay	1998	4	4	5	7	8	11	28	32	25	10
	1999	1	4	4	9	9	12	20	28	24	9
Shan State											
Taunggyi area	1998	1	8	8	7	27	45	39	21	18	2
	1999	0	10	9	11	22	38	25	20	14	3
Kachin State											
Myitkyina	1998	3	10	12	18	24	40	32	28	18	9
	1999	8	12	20	32	41	50	35	23	13	8
Sagaing Division											
Kabaw valley	1998	0	8	9	15	25	32	56	58	38	12
	1999	0	10	10	12	21	31	42	48	28	10

Table 2.2.35: Number of *An. jeyporiensis* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. jeyporiensis* collected (day)	Total no. of *An. jeyporiensis* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Sagaing Div.												
Kabaw valley	Sep.-Oct.,98	8	0	114	2	13	34	10	5	13	25	12
Shan State												
Taunggyi	Jul.-Oct.,98	12	0	132	9	19	35	9	3	16	33	8
Rakhine State												
Sittwe	Mar.-Apr.,99	8	0	96	2	9	38	10	6	9	13	9
Kachin State												
Myitkyina	Jul.-Aug.,99	8	0	91	8	10	21	9	4	8	23	8

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Medical importance: The results of infection rates are shown in Table 2.2.36. At Myanmar-Bangladesh border, Rakhine State, 500 specimens of *An.jeyporiensis* were dissected and four gland infections were found, thus showing a infection rate of 0.8 percent. At Myanmar-China border (Shan State), 500 specimens of *An. jeyporiensis* were dissected with a 1.2% (6/100) infection rate.

Table 2.2.36: Dissection records of *An. jeyporiensis* infection rates in different localities

Localities	No. of *An. jeyporiensis* dissected	No. of *An. jeyporiensis* with gut infection	No. of *An. jeyporiensis* with gland infection	Infection rate (%)
Rakhine State				
Myanmar-Bangladesh boder	500	0	4	0.8 (4/500)
Sittwe	80	0	0	0
Kyaukpyu	86	0	0	0
Shan State				
Myanmar-China border	500	1	5	1.2 (6/500)
Bago Division				
Taungoo	120	0	0	0
Yedashe	132	0	0	0
Sagaing Division				
Kabaw valley	105	0	0	0
Kale valley	256	0	1	0.3 (1/256)

Distribution: An. jeyporiensis has a very limited distribution: this species occurs in hilly tracts, foothill areas and also on coastal plains. It is abundant in only two localities i.e., Rakhine State and Kabaw valley, Sagaing Division. Elsewhere *An. jeyporiensis* is found in very small numbers.

(16) ***Anopheles karwari*** (James), 1903

Seasonal prevalence: In Rakhine State, *An. karwari* is recorded between June and September, but none are seen between November and May. In Katha, Sagaing Division, it is recorded mostly in July to September, but none in March and April. In Mon State, *An. karwari* is found mostly from May to July. Table 2.2.37 shows the seasonal abundance of *An. karwari* at various catching stations during the study period.

Resting habits: No adults were collected in houses, though a few were recorded from cattlesheds (Table 2.2.38).

Host preference: Almost all specimens were caught from cattle or near cattle at night. *An. karwari* can be considered to be a cattle feeder (Table 2.2.38).

Biting rhythm: The greatest number of *An. karwari* was recorded during the early part (first quarter) of the night (Table 2.2.38).

Table 2.2.37: Number of *An. karwari* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	March	April	May	June	July	August	Sept.	Oct.	Nov.
Rakhine State										
Kyaukpyu	1998	0	0	0	18	30	42	53	10	0
	1999	0	0	0	20	42	48	48	18	1
Sagaing Division										
Katha	1998	0	0	1	1	12	14	13	8	2
	1999	0	0	2	1	18	13	13	4	1
Mon State										
Chaungzone	1998	0	0	2	6	3	3	7	6	8
	1999	0	0	1	4	4	3	9	9	10

Table 2.2.38: Number of *An. karwari* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. karwari* collected (day)	Total no. of *An. karwari* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Chaungzone	Nov.,99	4	1	9	1	0	0	0	4	3	2	1
Rakhine State												
Kyaukpyu	Sep.,98	4	1	52	1	0	0	0	23	19	6	4
Sagaing Div.												
Katha	Aug.,98	4	0	14	0	0	0	0	8	4	2	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Medical importance: An. karwari is not a vector of malaria because it is a cattle feeder.

Distribution: The distribution of *An. karwari* is very limited: it has been found on few occasions.

(17) *Anopheles kochi* Döenitz, 1901

Seasonal prevalence: In almost all areas, *An. kochi* is abundant from June to October. This species is recorded throughout the year in Katha and Mawlaik Townships (Sagaing Division), Mandalay Township (Mandalay Division), and southern Shan State. Table 2.2.39 shows the seasonal abundance of *An. kochi* at various catching stations during the study period.

Table 2.2.39: Number of *An. kochi* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Sagaing Division													
Katha	1998	10	11	12	18	22	39	39	40	42	48	23	19
	1999	9	9	21	25	30	32	40	51	50	52	28	20
Tamu	1998	10	13	20	25	25	32	40	42	44	45	38	23
	1999	12	12	19	24	30	38	43	42	45	54	31	20
Mandalay Division													
Yemathin	1998	1	2	2	4	4	6	6	8	8	8	6	3
	1999	2	2	3	2	5	7	7	8	10	9	7	2

Resting habits: Table 2.2.40 shows the results of day and night collections of *An. kochi* in various catching stations during the study period. *An. kochi* was recorded in very small numbers in daytime catches. In Myintkyina Township (Kachin State), Mawchi mines (Kayah State) and Tamu Township (Sagaing Division), it was found in small numbers resting in houses. In Mawlaik Township (Sagaing Division) and Papun Township (Kayin State) this species could not be found in houses but records show them to rest in vegetation and scrub jungle.

Host preference: An. kochi was recorded in Tamu and Katha Townships, Sagaing Division, as biting on humans during the study period. Elsewhere it was not recorded (Table 2.2.40).

Biting rhythm: An. kochi bites from dusk to midnight (first and second quarter) with a peak particularly during the first quarter of the night (Table 2.2.40).

Medical importance: An. kochi could not be regarded as a vector of malaria.

Distribution: This species is mainly found in foothills and narrow river valleys in association with jungle.

Table 2.2.40: Number of *An. kochi* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. kochi* collected (day)	Total no. of *An. kochi* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Sagaing Div.												
Katha	Oct.,98	4	1	47	1	1	0	0	27	10	9	0
Tamu	Oct.,99	4	1	53	2	0	0	0	31	18	2	0
Mandalay Div.												
Yamethin	Sep.,99	4	0	10	0	0	0	0	8	2	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

(18) *Anopheles kyondawensis* Abraham, 1947

During the study period, only larvae of this species could be collected. No information is available on the seasonal prevalence, resting habits, host preference and medical importance.

Distribution: *An. kyondawensis* is only recorded in Mawlamyine Township area, Mon State.

(19) *Anopheles lindesayi* Giles, 1900

Seasonal prevalence: During the study period, *An. lindesayi* could be found only in March and April (Table 2.2.41).

Resting habits: None were found in houses or cattlesheds during daytime catches (Table 2.2.42).

Host preference: An. lindesayi is a cattle feeder (Table 2.2.42).

Biting rhythm: An. lindesayi is a early feeder (Table 2.2.42).

Medical importance: An. lindesayi is not a vector.

Distribution: An. lindesayi is a hill species occurring only at high altitudes.

Table 2.2.41: Number of *An. lindesayi* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	February	March	April	May	June
Shan State						
Kalaw	1998	0	1	2	0	0
	1999	0	1	0	0	0
Chin State						
Mindat	1998	0	2	2	0	0
	1999	0	1	2	0	0
Kanpetlet	1998	0	8	9	0	0
	1999	0	7	5	0	0

Table 2.2.42: Number of *An. lindesayi* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. lindesayi* collected (day)	Total no. of *An. lindesayi* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Shan State												
Kalaw	April, 1998	4	0	2	0	0	0	0	2	0	0	0
Chin State												
Mindat	April, 1999	4	0	2	0	0	0	0	2	0	0	0
Kanpetlet	April, 1998	4	0	9	0	0	0	0	8	1	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

(21) *Anopheles maculatus* Theobald, 1901

Seasonal prevalence: Table 2.2.43 shows the seasonal abundance of *An. maculatus* at various catching stations during the study period. The maximum prevalence of this species in almost all areas occurs during January (cold dry season). Numbers start to increase at the end of monsoon in early October and it is rare during the monsoon seasons. In Thabyewa village, Bago Division, it was collected in very large numbers during January. In the southern areas of Thanintharyi Division, *An. maculatus* could be found in high densities in January. There is only one exception in Mandalay Division, where *An. maculatus* was collected in large numbers during September, about the end of the rainy season.

Table 2.2.43: Number of *An. maculatus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	August	September	October	November	December	January	February
Bago Division								
Thebyewa	1998	31	38	84	86	92	482	230
	1999	48	58	92	90	98	397	210
Htiphado	1998	42	63	78	366	369	498	281
	1999	41	52	98	298	304	482	270
Thanintharyi Division								
Kawthaung	1998	34	36	32	34	81	582	89
	1999	22	28	29	35	78	597	82
Myeik	1998	14	14	25	22	91	409	75
	1999	13	24	27	25	97	401	77
Mandalay Division								
Mogok	1998	98	491	402	201	103	78	25
	1999	88	502	472	290	98	84	32
Sedawgyi	1998	102	540	528	119	84	69	18
	1999	94	483	509	201	103	72	20

Resting habits: It has never been found resting indoors during the day, even though many houses in the foothill areas are enclosed only on three sides. However, at times of peak densities, a few *An. maculatus* could be collected in cattlesheds (Table 2.2.44).

Host preference: An. maculatus feeds on both humans and animals. Table 2.2.44 shows the results of day and night collections in various catching stations. In deep forest timber extraction camps (where there are few cattle and other animals) such as Nammtun camp (Kawthaung,

Thanintharyi Division) and Htiphado village (Bago Division), *An. maculatus* readily attacks man. In these locations more were caught on human bait rather than cattle bait. In almost all areas including Kabaing (Mandalay Division) and Myeik (Thanintharyi Division), it was collected equally from human and cattle bait. That shows this species accepted human and cattle blood meals without preference. However, in Thabyewa village (Bago Division), cattle bait yielded the highest number of this species.

Table 2.2.44: Number of *An. maculatus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. maculatus* collected (day)	Total no. of *An.maculatus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Kabing	Sept.,98	4	0	491	114	99	30	3	98	104	37	6
Thanintharyi Div.												
Kawthaung	January,98	4	2**	580	132	148	74	46	80	72	21	7
Myeik	January,99	4	0	597	112	110	68	9	109	103	72	14
Bago Division												
Thebyewa	January,98	4	1	481	68	55	21	10	141	108	58	20
Htiphado	January,99	4	1	497	129	128	52	21	89	67	10	1

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Table 2.2.45: Dissection records of *An. maculatus* infection rates in different localities

Localities	No. of *An. maculatus* dissected	No. of *An. maculatus* with gut infection	No. of *An. maculatus* with gland infection	Infection rate (%)
Sagaing Division				
Kabaw	110	0	0	0
Mawlaik	35	0	0	0
Shan State				
Lashio	55	0	0	0
Bago Division				
Okpo	78	0	0	0
Mandalay				
Kabaing	180	1	0	0.5 (1/180)
Thanintharyi Division				
Myeik (monsu)	180	1	0	0.5 (1/180)
Kawthaung (namtun camp)	200	1	0	0.5 (1/200)

Biting rhythm: An. maculatus feeds mainly during the first and second quarter of the night, 18:00 to 24:00 (Table 2.2.44).

Medical importance: Table 2.2.45 shows the dissection records for *An. maculatus* infection rates in different localities. In Phado, Kyauktagar Township, Bago Division, *An. maculatus* was found to have no gut and salivary gland infection. However, in Kabaing, Mogok Township, Mandalay Division, *An. maculatus* was observed to have a 0.5 percent (1/180) total infection rate. In Thanintharyi Division, i.e., in Monsu village (Myeik Township) and Kawthaung (Namtun camp), this species had a 0.5 percent (1/180) gut infection rate.

Distribution: An. maculatus is primarly recorded from forested foothills, deep forest camps and in rocky mountainous areas about 1200 m above sea level. It is not found in lowlying areas far from the foothills.

(21) *Anopheles willmori* (James), 1903

Seasonal prevalence: An. willmori is abundant in the pre-monsoon months with a peak in March and April. Table 2.2.46 shows the seasonal abundance of this species at various catching stations during the study period.

Table 2.2.46: Number of *An. willmori* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Sagaing Division													
Mawlaik	1998	28	29	89	91	31	12	8	8	21	35	48	32
	1999	31	32	80	84	42	13	9	10	28	38	52	30
Katha	1998	19	39	52	58	40	32	25	13	21	45	43	28
	1999	26	32	59	64	51	48	38	18	25	39	41	31
Shan State													
Kalaw	1998	8	9	11	10	9	4	5	4	9	10	11	9
	1999	10	10	12	18	10	5	5	5	9	11	13	10
Lashio	1998	8	8	13	18	11	8	4	2	5	12	11	9
	1999	10	9	15	21	13	8	6	3	4	18	21	10

Table 2.2.47: Number of *An. willmori* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. willmori* collected (day)	Total no. of *An. willmori* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Sagaing Div.												
Mawlaik	April,98	4	8	83	0	0	0	0	39	41	3	0
Katha	Mar.,99	4	9	50	1	1	0	0	22	24	2	0
Shan State												
Kalaw	April,99	4	6	12	1	0	0	0	5	5	1	0
Lashio	April,99	4	4	17	0	0	0	0	7	9	1	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Resting habits: A few specimens were found in houses during the day (Table 2.2.47).

Host preference: As evidenced from the results in Table 2.2.47, *An. willmori* has a high preference for cattle blood in all areas.

Biting rhythm: An. willmori feeds mainly during the first and second quarter of the night, i.e., 18:00 to 24:00 (Table 2.2.47).

Medical importance: It is not a vector of malaria.

Distribution: An. willmori is mainly confined to foothills and mountain areas. It is rarely recorded in the plains.

(22) *Anopheles majidi* Young and Majid, 1928

Seasonal prevalence: An. majidi is recorded only during the beginning of monsoon period (June and July). Table 2.2.48 shows the seasonal abundance of *An. majidi* at various catching stations during the study period.

Resting habits: An. majidi was never found indoors during the day (Table 2.2.49).

Table 2.2.48: Number of *An. majidi* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	June	July	August	September	October	November	December
Mon State								
Mudon, Quarter 4	1998	4	2	0	0	0	0	0
	1999	2	0	0	0	0	0	0
Tanintharyi Division								
Dawei	1998	2	2	0	0	0	0	0
	1999	2	2	0	0	0	0	0
Kayah State								
Loikaw	1998	2	2	0	0	0	0	0
	1999	2	2	0	0	0	0	0
Shan State								
Lashio	1998	2	3	0	0	0	0	0
	1999	2	2	0	0	0	0	0

Table 2.2.49: Number of *An. majidi* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. majidi* collected (day)	Total no. of *An. majidi* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Mudon	June,98	4	0	4	0	0	0	0	4	0	0	0
Tanintharyi Div.												
Dawei	June,98	4	0	2	0	0	0	0	2	0	0	0
Kayah State												
Loikaw	July,98	4	0	2	0	0	0	0	2	0	0	0
Shan State												
Lashio	July,98	4	0	2	0	0	0	0	2	0	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Host preference: An. majidi is predominatly a cattle feeder (Table 2.2.49).

Biting rhythm: The biting time of *An. majidi* is illustrated in Table 2.2.49. It was collected only during the first quarter of the night.

Medical importance: An. majidi is not a vector of malaria, because it feeds only on cattle.

Distribution: An. majidi is extremely scarce in Myanmar.

(23) *Anopheles minimus* Theobald, 1901

Seasonal prevalence: *An. minimus* is abundant throughout the whole year in many locations, although densities vary seasonally. The maximum prevalence of *An. minimus* in almost all areas is during the post-monsoon months of October to December. Table 2.2.50 shows the monthly man-biting rate per hour (mbr/h) of *An. minimus* at Phado, foothill village of Bago Yoma range, Bago Division. It is collected throughout the year, although peak mbr/h rates were obtained from September to December.

Table 2.2.50: Average man-biting rates of *An. minimus* at Phado village, Bago Division during 1998, 1999

Period	Man biting rate (mbr/h)
1998	
September	9.08
October	8.69
November	9.95
December	7.44
1999	
January	1.0
February	1.61
March	3.30
April	4.90
May	4.70
June	0.36
July	2.30
August	3.82
September	2.73

There is only one exception in the Rakhine State, where the species is not abundant. There most specimens of *An. minimus* were recorded during the pre-monsoon period March to April. During the rainy season, *An. minimus* is absent and reappears in very small numbers during October and November.

There is a definite relationship between the mbr/h of *An. minimus* and the distance of the catching station from the forest (Table 2.2.51). Highest mbr`s were observed at Kyarkyaungthaike, a temporary settlement at the edge of forested foothills. The next highest rate was at Ngokto village, situated about 0.5 kilometers (km) away from the forest, though *An. minimus* numbers were reduced. At Gwegon village, situated about 2.4 km away from the forest, the density of *An. minimus* recorded was much lower.

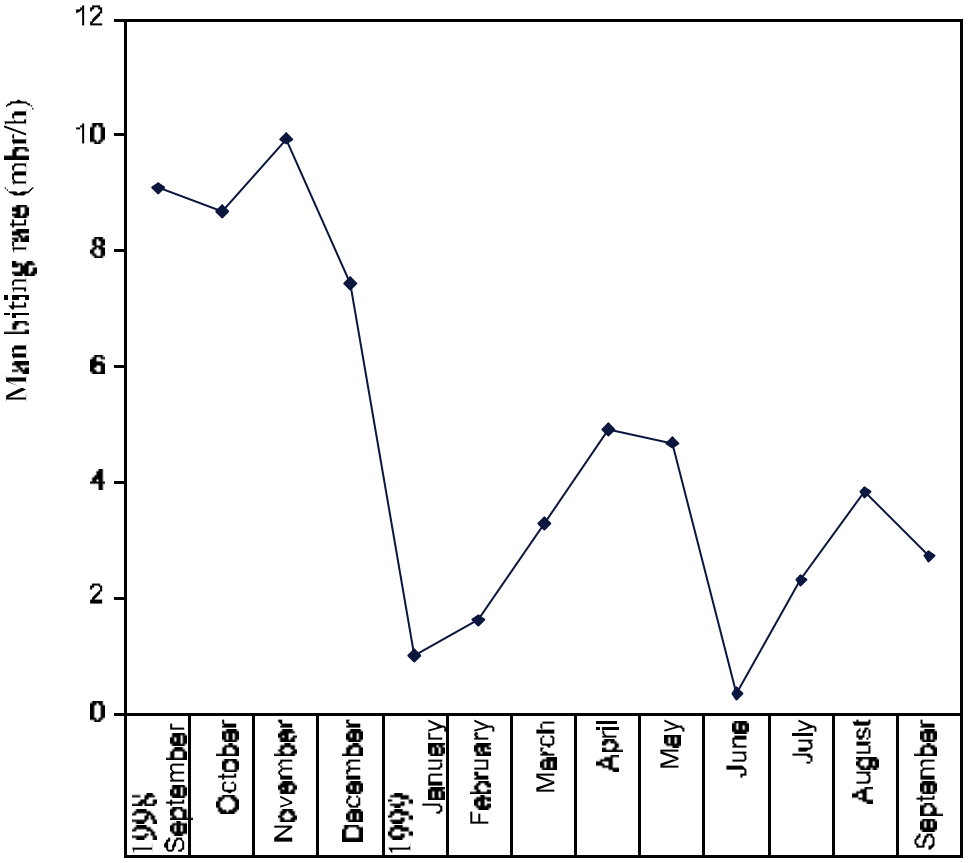


Fig. 2.95. Average man-biting rates of *An. minimus* at Phado, Bago Division during 1998, 1999

Table 2.2.51: Relationship between *An. minimus* man-biting rate per hour (mbr/h) and the distance of the catching station from the forest

Localities	Township	mbr/h	Topography & remarks on location
Bago Division			
Kyarkyaungthaik	Kyauktagar	2.73	Temporary settlement on edge of forested foothill
Ngokto	Kyauktagar	1.00	Village 0.5 kilometer from the forest edge
Gwegon	Kyauktagar	0.35	Village 2.4 kilometers of forested foothill

Resting habits: *An. minimus* is predominately a domestic species. Adults prefer to rest in houses and cattlesheds during daytime. The great proportion of adults left the house after feeding and sought shelter outdoors. The abundance of *An. minimus* in day catches in different catching stations are shown Table 2.2.52.

Table 2.2.52: Results of the daytime collections of *An. minimus* in houses during 1999

Localities	Total anophelines caught	Total *An.minimus*	Percentage of *An.minimus*
Mandalay Division			
Mandalay-pyinoolwin area	412	391	94.9
Mogok area	570	367	64.3
Kyaukse	387	270	69.7
Shan State			
Lashio	920	702	76.3
Shwenyaung	3,002	2,85	94.9
Bago Division			
Taungoo	260	201	77.3

Host preference: The preference of *An. minimus* for human blood is well documented by the results recorded during different periods in various areas, as shown in Table 2.2.53. Even when cattle are present, only a very small proportion of the mosquitoes deviated from biting human beings.

Table 2.2.53: Number of *An. minimus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period of observation	No. of catches	Total no. of *An. minimus* collected (night)	Collected by different techniques*							
				Human bait				Cattle bait			
				I	II	III	IV	I	II	III	IV
Mandalay Division											
Mandalay-Pyinoolwin foothill area	Oct.-Dec., 98	12	324	74	102	80	48	5	7	5	3
Kyaukse	April-Oct., 98	28	211	50	90	38	23	0	8	2	0
Rakhine State											
Maungdaw	Mar.-April, 99	8	84	20	38	18	7	0	1	0	0
Kakhin State											
Myitkyina	October, 99	4	65	15	35	10	5	0	0	0	0
Sagaing Division											
Kabaw Valley	Sept.-Oct., 98	8	317	88	108	71	50	0	0	0	0
Kale Valley	Nov.-Dec., 99	8	350	99	109	80	62	0	0	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Biting rhythm: An. minimus feeds mainly during the early hours, i.e., about 21:00. The biting peak occurs just before or just after midnight (Table 2.2.53). However, in the main season, when its

prevalence is high *An. minimus* is found to bite throughout the night (both outdoors and indoors) with a high density during the first quarter and with a gradual decrease till 06:00.

Medical importance: The infection rates of *An. minimus* in different locations are given in Table 2.2.54. In Kabaw valley, 500 specimens were dissected and 15 infections were found (3% infection rates). In Thebyewa village, Bago Division, a total of 60 *An. minimus* were dissected and only one was found with a gut infection.

Distribution: An. minimus has been recorded on many occasions in different parts of Myanmar. It is essentially a mosquito of hilly regions, either low rolling foothills or narrow river valleys in mountain ranges. When found in plains, it is always in association with extensive irrigation systems. *An. minimus* has not been recorded in locations over 915 m above see level.

Table 2.2.54: Dissection records of *An. minimus* infection rates in different localities

Localities	No. of *An.minimus* dissected	No. of *An.minimus* with gut infection	No. of *An.minimus* with gland infection	Infection rate (%)
Rakhine State				
Sittwe	70	0	1	1.4 (1/70)
Sagaing Division				
Kabaw valley	500	1	14	3.0 (15/500)
Katha	90	0	1	1.1 (1/90)
Bago Division				
Thabyewa	60	1	0	1.6 (1/60)

(24) *Anopheles nigerrimus* Giles, 1900

Seasonal prevalence: In several localities, such as Bago Division, Yangon Division and Mon State (Mawlamyine), *An. nigerrimus* has been recorded all the year round. The maximum density is found in October, at the end of the monsoon. Table 2.2.55 shows the seasonal abundance of *An. nigerrimus* at various catching stations during the study period.

Table 2.2.55: Number of *An. nigerrimus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Mandalay Division.													
Mandalay-Pyinoolwin foothill	1998	25	20	18	0	0	0	0	0	120	501	257	152
	1999	25	25	22	0	0	0	0	0	90	408	280	120
Rakhine State													
Sittwe	1998	0	0	0	0	0	21	20	20	28	43	0	0
	1999	0	0	0	0	0	17	18	28	41	54	0	0
Bago Division													
Taungoo	1998	28	12	28	30	71	71	82	120	121	128	128	118
	1999	34	10	13	16	68	68	70	89	180	208	198	102

Resting habits: No adults were found in day catches (Table 2.2.56). Daytime resting places were in rice fields, bushes and undergrowth close to the villages.

Table 2.2.56: Number of *An. nigerrimus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. nigerrimus* collected (day)	Total no. of *An. nigerrimus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Mandalay-Pyinoolwin foothill area	Sept.-Dec., 98	12	0	1030	15	5	3	2	705	201	87	12
Sagaing Division												
Shwebo	Mar.-April, 99	8	1**	119	1	0	0	0	71	35	8	3
Bago Division												
Taungoo	Jul.-Dec., 99	24	2**	290	53	14	4	1	176	34	5	1
Rakhine State												
Sittwe	Oct. 99	4	0	54	0	0	0	0	44	5	3	2

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Host preference: Comparative figures for *An. nigerrimus* taken by human bait and cattle bait during the night are shown in Table 2.2.56. It can be seen that *An. nigerrimus* was predominatly a cattle feeder.

Biting rhythm: An. nigerrimus feeds mainly during the first quarter of the night (Table 2.2.56).

Medical importance: An. nigerrimus is mainly zoophilic and plays no part in malaria transmission.

Distribution: An. nigerrimus shows a wide distribution and is found in every locality of Myanmar.

(25) *Anopheles pallidus* Theobald, 1901

Seasonal prevalence: In Sagaing and Bago Divisions, *An. pallidus* is recorded only in January to March. In Kayin and Mon States, *An. pallidus* is found during January to October with a peak in September and October. Table 2.2.57 shows the seasonal abundance of *An. pallidus* at various catching stations during 1998, 1999.

Table 2.2.57: Number of *An. pallidus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.
Bago Division											
Taungoo	1998	12	14	12	0	0	0	0	0	0	0
	1999	16	15	15	0	0	0	0	0	0	0
Sagaing Division											
Shwebo	1998	21	20	18	0	0	0	0	0	0	0
	1999	19	19	18	0	0	0	0	0	0	0
Kayin State											
Pyapon	1998	8	8	8	9	12	14	17	17	21	21
	1999	10	10	12	14	14	13	14	14	19	18
Mon State											
Mawlamyine	1998	8	9	9	8	8	2	1	5	12	17
	1999	10	11	10	7	7	5	5	5	14	14

Resting habits: An. pallidus had never been taken in day catches (Table 2.2.58).

Host preference: The adults were caught only by cattle bait at night. *An. pallidus* is a cattle feeder (Table 2.2.58).

Biting rhythm: An. pallidus feeds during the first quarter of the night (Table 2.2.58).

Medical importance: An. pallidus is not regarded as a malaria vector.

Distribution: An. pallidus has been found on very few occasions.

Table 2.2.58: Number of *An. pallidus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. pallidus* collected (day)	Total no. of *An. pallidus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Mawlamyine	Oct., 1999	4	0	17	0	0	0	0	16	1	0	0
Kayin State												
Pyapon	Oct., 1999	4	0	18	0	0	0	0	16	2	0	0
Sagaing Division												
Shwebo	Jan., 1998	4	0	21	0	0	0	0	20	1	0	0
Bago Division												
Bago	Jan., 1998	4	0	16	0	0	0	0	15	1	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

(26) *Anopheles peditaeniatus* (Leicester), 1908

Seasonal prevalence: The maximum incidence of *An. peditaeniatus* occurs at the end of the monsoon and during the early part of winter, i.e., October to November. Table 2.2.59 shows the seasonal abundance of *An. peditaeniatus* at various catching stations during the study period.

Resting habits: Blood-fed specimens were observed only in cattlesheds during the day (Table 2.2.60).

Host preference: An. peditaeniatus feeds only on cattle (Table 2.2.60).

Biting rhythm: An. peditaeniatus is a early feeder and biting activity occurs during first and second quarter of the night (Table 2.2.60).

Medical importance: An. peditaeniatus takes no part in malaria transmission because it is a predominatly a cattle feeder.

Table 2.2.59: Number of *An. peditaeniatus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Ayeyarwady Div.													
Hinthada	1998	22	18	18	22	20	20	12	12	31	52	50	48
	1999	12	20	20	24	24	30	31	32	42	60	58	58
Mandalay Division													
Madaya	1998	20	20	20	19	22	22	32	32	38	48	44	45
	1999	12	12	12	22	32	39	40	41	50	62	45	45
Bago Division													
Pyay	1998	20	22	27	28	28	31	48	51	50	72	68	65
	1999	25	31	31	41	40	39	38	45	52	69	69	68

Table 2.2.60: Number of *An. peditaeniatus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. peditaeniatus* collected (day)	Total no. of *An. peditaeniatus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Div.												
Madaya	Oct.,99	4	1**	61	0	0	0	0	32	28	1	0
Ayeyarwady Div.												
Hinthada	Oct.,99	4	2**	58	0	0	0	0	49	9	0	0
Bago Division												
Pyay	Oct.,98	4	2**	70	0	0	0	0	39	30	1	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Caught from cattlesheds

Distribution: An. peditaeniatus has been found on very few occasions.

(27) *Anopheles philippinensis* Ludlow, 1902

Seasonal prevalence: Table 2.2.61 shows the seasonal abundance of *An. philippinensis* at various catching stations during the study period: (i) in Sittwe and adjoining the Bangladesh border, Rakhine State, *An. philippinensis* is encountered in small numbers between March to May. No adults are seen during the months of June and July due to heavy rains, but it reappears in subsequent months from August to September with its maximum prevalence during October, after which there is a gradually decreases; (ii) in Mandalay-Pyinoolwin foothill area, Kyaukse and Meiktila Townships, Mandalay Division, where rainfall is very scanty, it is very rarely encountered during the months of April to August. This species appears in September and is very abundant during October and November, but diminishes gradually from December to February; (iii) in Katha and Mawlaik Townships, Sagaing Division, *An. philippinensis* is encountered in considerable numbers from March to August but gradually increases from September to October. The peak of its prevalence is noted during December; (iv) it is one of the most prevalent anopheline species in Shan plateau. Moderate numbers are found almost all the year round in this region. The maximum prevalence of *An. philippinensis* in Shan plateau is during the monsoon months of May to October.

Resting habits: This species was not found resting in houses and cattlesheds during daytime. A few specimens were recorded from bushes and tree-holes in some areas in Sagaing Division. However in Bhamo, Kachin State, *An. philippinensis* has only been found resting in houses during morning collections (Table 2.2.62).

Host preference: Table 2.2.62 shows the results of day and night collections of *An. philippinensis* in various catching stations during their peak season. *An. philippinensis* is a zoophilic species and feeds mainly on cattle under natural conditions, in most parts of Myanmar. In areas where cattle are either scarce or absent, this species has readily diverted to man. This can be illustrated in the following two areas of Myanmar (Table 2.2.63). The first area is Innwaing village in Mawlamyine Township, Mon State. Cattle are very scarce in this area. The important vector species of the area is *An. dirus*. *An. philippinensis* is also captured in large numbers, during the post-monsoon months from September to November by human bait at night.

Another station is Patheingyi Township, which is situated at the foothill area in Mandalay Division. No cattle are present at all in this area. The inhabitants are all monks. The present principal vector is *An. minimus*, but *An. philippinensis* is also collected in large numbers from October to November, by human bait.

Table 2.2.61: Number of *An. philippinensis* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	March	April	May	August	September	October	November	December
Rakhine State									
Myanmar-Bangladesh									
border	1998	4	5	4	40	72	98	64	53
	1999	8	7	7	38	70	89	61	58
Mandalay Division									
Mandalay-Pyinoolwin									
foothill area	1998	6	5	8	20	60	183	182	158
	1999	7	8	8	24	59	145	151	148
Sagaing Division									
Mawlaik	1998	2	8	12	34	60	83	92	101
	1999	4	6	9	29	61	78	88	98
Bago Division									
Taungoo	1998	14	12	22	25	80	84	82	40
	1999	13	13	23	27	71	78	79	40
Mon State									
Mawlamyine	1998	21	20	20	18	53	62	40	22
	1999	19	21	23	20	50	58	43	28
Kachin State									
Bhamo	1998	8	11	14	20	51	89	40	23
	1999	7	18	18	23	49	88	42	30
Chin State									
Paletwa	1998	4	8	8	7	8	16	53	44
	1999	3	3	5	4	8	11	45	40

Biting rhythm: An. philippinensis was found to bite mostly early in the night about 19:00 to 23:00. The species is almost always collected by outdoors where biting occurs. It was very rarely encountered during the third and fourth quarter of the night (Table 2.2.62 and 2.2.63).

Medical importance: Table 2.2.64 shows the dissection records on *An. philippinensis* from different catching areas; (i) in Katha, Sagaing Division, 497 specimens were dissected out of which one was found with gut infection; (ii) in Lashio and Taunggyi areas, Shan State, there were no gut

and salivary gland infections; (iii) in Bhamo Township, Kachin State, 80 specimens were dissected and the total infection rate was 1.2 percent (1/80).

Table 2.2.62: Number of *An. philippinensis* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. philippinensis* collected (day)	Total no. of *An. philippinensis* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Division												
Kyaukpadaung area	Oct.,98	4	0	205	0	1	0	0	82	99	20	3
Sedawgyi	Oct.,98	4	0	137	0	2	0	0	65	62	5	3
Mandalay area	Oct.,98	4	0	150	1	1	0	0	84	60	3	1
Sagaing Division												
Katha area	Dec.,99	4	1**	195	1	1	0	0	100	89	3	1
Kachin State												
Bhamo area	Oct., 98	4	10	98	0	1	1	0	42	28	5	2
Myintkyina area	Oct.,99	4	0	99	0	2	0	0	61	32	3	1

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Table 2.2.63: Number of *An. philippinensis* caught by different techniques in Mawlamyine and Patheingyi areas during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. philippinensis* collected (day)	Total no. of *An. philippinensis* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mon State												
Mawlamyine area												
(Innwaing)	Oct.,98	4	0	62	24	17	3	0	17	10	1	0
	Oct.,99	4	0	104	32	27	4	1	24	12	3	1
Mandalay Division												
Patheingyi area	Oct.,98	4	0	123	42	40	12	2	12	11	3	1
	Oct.,99	4	0	140	45	48	13	2	13	14	3	2

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Distribution: An. philippinensis is widespread throughout Myanmar and has been recorded from all parts of the country and all types of terrain except in Tanintharyi Division.

Table 2.2.64: Dissection records of *An. philippinensis* infection rates in different localities

Localities	No. of *An. philippinensis* dissected	No. of *An. philippinensis* with gut infection	No. of *An. philippinensis* with gland infection	Infection rate (%)
Sagaing Division				
Kathe area	497	1	0	0.2 (1/497)
Mawlaik	98	0	0	0
Shan State				
Lashio	305	0	0	0
Taunggyi area	327	0	0	0
Kachin State				
Bhamo	80	0	1	1.2 (1/80)
Myintkyina	98	0	0	0
Rakhine State				
Myanmar-Bangladesh border	100	1	0	1.0 (1/100)
Sittwe	98	0	0	0
Mon State				
Mawlamyine area	90	0	0	0
Mandalay Division				
Patheingyi	108	0	0	0

(28) *Anopheles pseudojamesi* Strickland and Chowdhury, 1927

During the study period, only larvae could be collected and no adults of *An. pseudojamesi* have been recorded.

No information on the seasonal prevalence, resting habits, host preference and medical importance are available.

Distribution: *An. pseudojamesi* is only recorded in Rakhine State.

(29) *Anopheles splendidus* Koidzumi, 1920

Seasonal prevalence: In all areas, the maximum incidence of *An. splendidus* is recorded during the pre-monsoon months of February to May and it reappears again during the months of August and September. Very few specimens have been recorded during October to December (Table 2.2.65).

Table 2.2.65: Number of *An. splendidus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Mon State													
Mudon	1998	0	5	5	6	5	0	0	4	4	3	2	2
	1999	0	4	5	5	6	0	0	3	3	2	2	0
Mandalay Division													
Pyinoolwin	1998	0	9	12	12	13	0	0	8	5	5	5	4
	1999	0	10	11	11	12	0	0	9	7	6	6	5
Bago Division													
Pyay	1998	0	8	9	9	9	0	0	4	5	3	3	2
	1999	0	9	10	10	12	0	0	5	5	4	4	4

Table 2.2.66: Number of *An. splendidus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. splendidus* collected (day)	Total no. of *An. splendidus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Division												
Pyinoolwin	May,98	4	1**	12	0	0	0	0	11	1	0	0
Bago Division												
Pyay	May,99	4	1**	11	0	0	0	0	11	0	0	0
Mon State												
Mudon	April,98	4	1**	5	0	0	0	0	5	0	0	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Resting habits: No specimen was seen resting in houses during the day. A few were recorded from bushes which are close to cattlesheds. Table 2.2.66 shows the results of daytime and night collections of *An. splendidus* in various catching stations during the study period.

Host preference: An. splendidus is a cattle feeder. No specimens have been seen biting on humans at night (Table 2.2.66).

Biting rhythm: An. splendidus bites as early as 18:00 to 21:00, in the first quarter of the night (Table 2.2.66).

Medical importance: An. splendidus plays no part in the transmission of malaria because this species is a cattle feeder.

Distribution: An. splendidus has a limited distribution in Myanmar. The localities which have been recorded, are all either foothill areas or river valleys in association with jungle.

(30) *Anopheles stephensi* Liston, 1901

Seasonal prevalence: In Magwe and Bago Divisions, *An. stephensi* is abundant from January to March and only a few specimens are recorded in June and July. Later no specimens are seen from September to December. In Mandalay and Sagaing Divisions, *An. stephensi* is abundant from April to June. Then, it diminishes in subsequent months and reappears again in January. In Ayeyarwady Division, it is recorded only during the pre-monsoon months of April and May. Table 2.2.67 shows the seasonal abundance of *An. stephensi* at various catching stations during the study period.

Table 2.2.67: Number of *An. stephensi* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Magwe Division													
Magwe	1998	54	58	59	40	32	18	11	5	0	0	0	0
	1999	52	52	58	50	35	24	20	9	0	0	0	0
Mandalay Division													
Amarapura	1998	25	32	40	58	59	62	28	13	10	8	0	0
	1999	31	35	38	62	62	68	32	18	12	10	0	0
Sagaing Division													
Monywa	1998	18	28	34	48	55	65	32	20	12	4	0	0
	1999	13	25	32	50	58	59	40	31	22	10	0	0
Ayeyarwady Div.													
Hinthada	1998	0	12	48	50	0	0	0	0	0	0	0	0
	1999	0	18	54	50	0	0	0	0	0	0	0	0
Bago Division													
Shwedaung	1998	40	42	45	35	30	12	10	5	0	0	0	0
	1999	50	50	52	48	42	18	12	8	0	0	0	0

Table 2.2.68: Number of *An. stephensi* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. stephensis* collected (day)	Total no. of *An. stephensis* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Mandalay Division												
Amarapura	June,99	4	0	68	2	1	1	0	48	10	6	0
Bago Division												
Shwedaung	March,99	4	0	52	1	1	0	0	35	9	6	0
Magwe Division												
Magwe	March,98	4	4	55	2	1	0	0	36	10	6	0
Sagaing Division												
Monywa	June,98	4	0	65	2	2	1	0	41	11	8	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Resting habits: An. stephensi was not recorded resting in human habitations during the day (Table 2.2.68).

Host preference: Most specimens were taken by cattle bait at night and *An. stephensi* is a cattle-feeder (Table 2.2.68).

Biting rhythm: An. stephensi was recorded biting from 18:00 to 01:00 and with a peak from 18:00 to 21:00 (Table 2.2.68).

Medical importance: An. stephensi cannot be recorded as playing an important part in the transmission of malaria because *An. stephensi* is a cattle feeder (Table 2.2.70).

Distribution: An. stephensi has been recorded mainly from the hill tracts and foothill areas close to the Central Dry Zone.

(31) *Anopheles subpictus* Grassi, 1899

Seasonal prevalence: In the southern part of the country such as Bago, Ayeyarwady and Tanintharyi Divisions, *An. subpictus* has been recorded in small numbers throughout the year with an increased prevalence in September and October. In the dry zone, Mandalay, Magwe

and Sagaing Divisions and in the north, Kachin State, *An. subpictus* is found mainly in May to November with a peak in October. In Shan State *An. subpictus* is most prevalent at the beginning of the monsoon month of June. Table 2.2.69 shows the seasonal abundance of *An. subpictus* at various catching stations during the study period.

Resting habits: An. subpictus was found to rest in houses and cattlesheds by daytime (Table 2.2.70).

Host preference: An. subpictus is predominantly a cattle feeder because it was mainly caught by cattle bait at night (Table 2.2.70).

Biting rhythm: Intense biting on cattle was observed during the first quarter of the night, 18:00 to 21:00 (Table 2.2.70).

Table 2.2.69: Number of *An. subpictus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Magwe Division													
Magwe	1998	3	9	10	12	22	62	68	77	80	88	14	5
	1999	8	8	11	21	25	68	70	75	76	77	28	11
Kachin State													
Bahmo	1998	8	8	11	18	22	49	51	51	60	64	30	12
	1999	9	19	21	20	21	47	48	50	51	58	29	18
Sagaing Division													
Katha	1998	5	5	8	10	21	48	50	68	71	84	11	3
	1999	5	5	9	11	18	42	48	62	64	68	12	2
Ayeyarwady Diision													
Myaungmya	1998	91	78	71	65	63	57	47	38	148	152	120	98
	1999	84	81	77	70	64	62	58	47	162	160	101	91
Mon State													
Thaton	1998	37	31	26	25	21	20	21	22	59	62	47	41
	1999	47	45	46	42	40	41	37	31	60	68	52	48
Bago Division													
Pyay	1998	82	80	74	67	64	51	40	31	288	341	180	101
	1999	72	71	66	59	58	42	42	40	321	304	203	99

Table 2.2.70: Number of *An. subpictus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. subpictus* collected (day)	Total no. of *An. subpictus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Bago Division												
Pyay	Sept.,98	4	21	267	32	2	0	0	138	82	11	2
Ayeyarwady Div.												
Myaungmya	Sept.,98	4	18	120	8	3	0	0	79	22	7	1
Magwe Division												
Magwe	Oct.,99	4	5	72	6	1	0	0	32	23	8	2
Sagaing Division												
Katha	Oct.,99	4	7	61	4	2	0	0	32	14	6	3

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Medical importance: An. subpictus plays no part in the transmission of malaria.

Distribution: An. subpictus has a patchy distribution in Myanmar but has been found in all types of terrain. In Ayeyarwady Division, Bago Division and Rakhine State, it is recorded as being very prevalent. It has also been recorded in almost every district in Shan State.

(32) *Anopheles sundaicus* (Rodenwaldt), 1925

Seasonal prevalence: Table 2.2.71 shows the seasonal abundance of *An. sundaicus* at various catching stations during 1998, 1999. In almost all areas, the number of *An. sundaicus* increases between May to July and again in October to February. In Ayeyarwady Division, the highest densities are recorded in October and November. In Kyaukpyu Township, Rakhine State, the maximum prevalence of *An. sundaicus* is between December to January. In Sittwe, in the same Division, the highest numbers are obtained during December.

Resting habits: During the study period, *An. sundaicus* was recorded in moderate numbers from houses and cattlesheds by daytime collections (Table 2.2.72).

Host preference: The host preference of *An. sundaicus* was studied in detail during the study

period in Rakhine State and Ayeyarwady Division. The results indicate that *An. sundaicus* feeds indiscriminately on man or cattle (Table 2.2.72).

Table 2.2.71: Number of *An. sundaicus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	May	June	July	October	November	December	January	February
Rakhine State									
Sittwe	1998	25	31	22	35	40	51	43	28
	1999	34	35	28	28	41	45	39	20
Kyaukpyu	1998	21	23	19	30	32	38	54	25
	1999	18	12	12	20	22	30	38	20
Ayeyarwady Division									
Seikkyi village	1998	64	72	22	70	92	68	60	32
	1999	54	62	30	64	79	61	50	34
Chaungthar	1998	43	50	41	60	65	62	40	25
	1999	38	59	50	62	70	62	38	20

Table 2.2.72: Number of *An. sundaicus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. sundaicus* collected (day)	Total no. of *An. sundaicus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Ayeyarwady Div.												
Seikkyi village	May-July, 98	12	10	158	34	21	10	2	35	30	21	5
Chaungthar	May-July, 98	12	12	134	37	29	11	10	23	20	3	1
Magyipi village	May-July, 98	12	18	165	33	26	8	3	45	29	12	9
Rakhine State												
Buthedaung	Oct.-Dec., 99	12	10	126	28	12	10	2	34	25	9	6
Kyaukpyu	Oct.-Dec., 99	12	12	154	45	22	12	7	32	18	12	6
Gwa	Oct.-Dec., 99	12	12	169	38	20	10	8	41	26	18	8

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Biting rhythm: As evidenced from the results in Table 2.2.72, *An. sundaicus* feeding takes place more during the first quarter (18:00 to 20:00) of the night than the second quarter (21:00 to 24:00). In Sittwe and Kyaukpyu Townships, Rakhine State, however, most specimens were taken during the second quarter.

Table 2.2.75: Dissection records of *An. sundaicus* infection rates in different localities

Localities	No. of *An. sundaicus* dissected	No. of *An. sundaicus* with gut infection	No. of *An. sundaicus* withgland infection	Infection rate (%)
Rakhine State				
Myanmar-Bangladesh boder	550	0	1	0.1 (1/550)
Sittwe	500	0	1	0.2 (1/500)
Myohaung	240	0	0	0
Kyaukpyu	350	0	1	0.2 (1/350)
Ayeyarwady Div.				
Sagagyi village	230	0	1	0.4 (1/230)
Hinthada	132	0	0	0
Chaungthar	220	0	1	0.4 (1/220)

Medical importance: The results of dissection records on *An. sundaicus* infection rates in different locations are shown in Table 2.2.73. In Chaungthar and Seikgyi areas, Ayeyarwady Division, *An. sundaicus* was found to have a 0.4 percent infection rate (1/220 and 1/230) respectively. At the Myanmar-Bangladesh border, Rakhine State, a total of 202 *An. sundaicus* were dissected out of which one was found with a gland infection.

Distribution: An. sundaicus is confined to coastal areas such as Rakhine State, Tanintharyi Division and the lower reaches of the Ayeyarwady Division (delta) where the creeks are subject to tidal influence.

(33) *Anopheles tessellatus* Theobald, 1901

Seasonal prevalence: In almost all areas *An. tessellatus* is recorded either during or just following the monsoon, i.e., June to beginning of November. *An. tessellatus* is scarce during the winter and pre-monsoon months. In Mandalay-Pyinoolwin foothill area, Mandalay Division, it is prevalent during September and October. In Taungoo and Bago Townships, Bago Division, *An. tessellatus* is mainly found between July to September. Table 2.2.74 shows the seasonal prevalence of *An. tessellatus* at various catching stations during the study period.

Resting habits: An. tessellatus was not observed in human dwellings during the day, however, some adults were collected from tree-holes in Kyaukse Township and Mandalay-Pyinoolwin

foothill area, Mandalay Division, during September, 1998. Table 2.2.75 shows the results of day and night time collections of *An. tessellatus* catches in various catching stations during the study period.

Table 2.2.74: Number of *An. tessellatus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Bago Division											
Taungoo	1998	0	0	5	12	14	12	6	4	3	0
	1999	0	0	3	18	16	15	8	5	3	0
Bago	1998	0	0	4	21	20	20	5	3	2	0
	1999	0	0	5	20	21	19	20	4	3	0
Mandalay Division											
Mandalay-Pyinoolwin											
foothill area	1998	0	11	62	64	92	130	132	101	22	0
	1999	0	10	59	70	89	108	112	94	11	0
Mon State											
Mawlamyine	1998	0	0	6	7	6	7	8	12	1	0
	1999	0	0	5	6	6	8	10	14	2	0

Table 2.2.75: Number of *An. tessellatus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. tessellatus* collected (day)	Total no. of *An. tessellatus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Bago Division												
Taungoo	August,99	4	0	16	0	1	2	0	1	2	9	1
Bago	August,99	4	0	21	0	1	1	0	1	5	11	2
Mandalay Division												
Mandalay-Pyinoolwin												
foothill area	Oct.,98	4	1*	131	0	0	1	1	12	21	81	15
Kyaukse	Oct.,98	4	2*	61	0	2	4	0	6	14	32	3

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00
** Daytime outdoor collection

Host preference: In Bago Division (Taungoo and Bago Townships) and Mandalay Division (Mandalay-Pyinoolwin foothills area), the great numbers of *An. tessellatus* was caught by

cattle bait. In Mandalay, Kyaukse and Katha Townships (Mandalay Division), a few were collected by human bait, rather than from cattle. *An. tessellatus* prefers cattle blood (Table 2.2.75).

Biting rhythm: An. tessellatus invaded late and the peak biting period was between 24:00 and 03:00 (Table 2.2.75).

Medical importance: An. tessellatus feeds mainly on cattle and it could not be recorded as a vector of malaria.

Distribution: An. tessellatus is not widely distributed in Myanmar, and has never been found in abundance. Although it has been recorded in hilly and forested areas, it is mainly found in plains, especially in Ayeyarwady Delta.

(34) *Anopheles theobaldi* Giles, 1901

Seasonal prevalence: During the study period, only two specimens (adults) were recorded once in the Lashio area, Shan State, in April, 1998.

Resting habits: No information.

Host preference: These two specimens (*An. theobaldi*) were collected in the shade of the jungle attempting to bite man (no cattle were available).

Biting rhythm: No information.

Medical importance: *An. theobaldi* is a wild species and is not a vector of malaria.

Distribution: *An. theobaldi* is recorded only in Lashio Township area in Northern Shan State.

(35) *Anopheles vagus* Döenitz, 1902

Seasonal prevalence: In many areas, especially in Southern Myanmar (Bago, Ayeyarwady and Tanintharyi Divisions) *An. vagus* has been recorded throughout the year with a peak

during and just after the monsoon, i.e., June to October. In the Central Dry Zone, *An. vagus* is found mainly during September and October. Table 2.2.76 shows the seasonal abundance of *An. vagus* at various catching stations during the study period.

Table 2.2.76: Number of *An. vagus* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Yangon Division													
Yaesitkan	1998	29	18	15	12	19	38	32	30	35	35	13	11
	1999	31	22	18	11	24	35	35	38	40	42	19	18
Bago Division													
Taungoo	1998	32	40	48	52	81	185	204	301	380	322	94	48
	1999	30	34	49	60	78	148	209	289	321	320	89	50
Mandalay Division													
Mandalay-Pyinoolwin area	1998	77	52	50	39	34	80	99	101	134	132	110	98
	1999	65	62	62	35	28	90	92	93	120	132	111	90

Table 2.2.77: Numbers of *An. vagus* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. vagus* collected (day)	Total no. of *An. vagus* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Bago Division												
Taungoo	Sept.,98	4	48	332	2	32	12	2	8	122	109	45
Rakhine State												
Kyaukpyu	Sept.,99	4	87	88	0	3	2	0	0	44	35	4
Mandalay Division												
Mandalay-Pyinoolwin foothill area	Sept.,98	4	5	129	1	3	2	1	0	68	49	5
Magwe Division												
Magwe	July,99	4	2	44	0	2	0	0	1	31	9	1

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Resting habits: In all areas, *An. vagus* has been frequently found in day catches in both houses, paddy sheds and cattlesheds. Table 2.2.77 shows the results of day and night collections in various catching stations during the study period.

Host preference: An. vagus was observed in almost every locality of the country, being mainly found on cattle bait or near cattle at night rather than on human bait. *An. vagus* feeds mainly on cattle (Table 2.2.77).

Biting rhythm: The peak biting time of *An. vagus* was about midnight, 24:00 (Table 2.2.77).

Medical importance: An. vagus plays no parts in malaria transmission because of its cattle feeding habit.

Distribution: An. vagus is widely distributed throughout the country. It is found in all types of topography, but most abundant in the plains and the Ayeyarwady delta.

(36) *Anopheles varuna* Iyengar, 1924

Seasonal prevalence: An. varuna is recorded from April to December, with a peak in November. Table 2.2.78 shows the seasonal abundance of *An. varuna* at various catching stations during the study period.

Resting habits: An. varuna has never been recorded resting in houses in the day (Table 2.2.79).

Table 2.2.78: Number of *An. varuna* females caught over four days (day and night) per month in various catching stations during 1998, 1999

Catching stations	Year	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Yangon Division												
Yaesitkan	1998	5	8	12	10	15	13	20	22	25	12	1
	1999	7	9	14	17	17	18	21	24	28	13	2
Kachin State												
Myitkyina	1998	1	3	4	4	8	9	9	11	18	8	0
	1999	2	5	5	9	9	10	11	20	21	9	0
Rakhine State												
Minbya	1998	1	21	25	41	48	59	68	101	301	54	0
	1999	2	32	33	45	49	61	69	99	203	48	0

Table 2.2.79: Number of *An. varuna* caught by different techniques in various catching stations during 1998, 1999

Catching stations	Period	No. of catches	Total no. of *An. varuna* collected (day)	Total no. of *An. varuna* collected (night)	Collected by different techniques*							
					Human bait				Cattle bait			
					I	II	III	IV	I	II	III	IV
Sagaing Division												
Kalemyo-Tamu area	Nov.,98	4	0	18	0	0	0	0	8	9	1	0
Rakhine State												
Minbya	Nov.,98	4	0	301	1	0	0	0	138	139	23	0
Kachin State												
Myitkyina	Nov.,99	4	0	21	0	0	0	0	9	10	2	0
Yangon Division												
Yesitkan	Nov.,99	4	0	28	0	0	0	0	12	14	2	0

N.B.
*Roman numerals indicate first, second, third and fourth quarters of the night catch; 18:00-21:00, 21:00-24:00, 24:00-03:00 and 03:00-06:00

Host preference: An. varuna is a cattle feeder (Table 2.2.79).

Biting rhythm: An. varuna was caught by cattle bait usually between 18:00 to 24:00 (first and second quarter). Table 2.2.79 shows the results of day and night collections of *An. varuna* in various catching stations during the study period.

Medical importance: An. varuna plays no part in the transmission of malaria because this species is a cattle feeder.

Distribution: An. varuna has a patchy distribution in Myanmar but has been found in all types of terrain.

2.3.4. Conclusion and discussion

2.3.4.1. Conclusion

During the study period, a vast amount of information has been accumulated on the larval and adult ecology of anopheline mosquitoes in Myanmar, with particular reference to their distribution, bionomics, medical importance, as a result of routine entomological surveys undertaken on a countrywide basis.

Various aspects of how the study was organised and carried out need to be noted: (i) Most of the surveys described were carried out in the large centres of population and relatively few in the smaller villages. Some records were continuous observations over the study period of several months, while other areas were revisited at different periods during the year. Thus, a nearly complete picture is obtained of the seasonal incidence of malaria and of seasonal fluctuation in the anopheline population in different parts of Myanmar. (ii) The study of anopheline breeding sites and habits has been more extensive than the study of adult anopheline mosquitoes. (iii) Where adult mosquitoes were studied, they were caught resting in houses and cattle sheds during the day. Although this resulted, on occasion, in the identification of certain species as vectors, the technique of “morning collections” has certain limitations which will be discussed further below. (iv) It was a critical approach to decide which species of anopheline mosquitoes is directly concerned in malaria transmission during different seasons of the year in various parts of Myanmar. If a certain species had been identified or implicated as a vector before and after the Second World War (until the study period), it was automatically regarded as a suspect when it occurred in any area of Myanmar whether or not malaria was shown to be present in that area. This section reviews critically all the available data regarding the various species of anopheline mosquitoes so far recorded in Myanmar. For the purpose of uniformity the following sub-headings have been used for each species:

1. Larval ecology

(A) Breeding sites:

Anopheles larvae inhabit a great variety of breeding sites:

(i) *Natural:* Lakes, swamps, river margins, riverbed pools, streams, seepages, springs,

marshes, tidal creeks, ground pools both in open land and forests, footprints of animals such as elephants, cattle etc., tree holes, coconut shells, bamboo stumps, etc.

(ii) *Semi-natural:* Large reservoirs for irrigation or for water supply, tanks, ponds, canals, ditches, channels and irrigation drains, paddy fields and other agriculture fields with standing or slow flowing water, collection bowls for gum from rubber trees, domestic and irrigation wells, excavation sites, etc.

(iii) *Artificial:* Metal, plastic, glass, ceramic or earthen containers, metal or cement domestic water tanks, barrels, pots and other vessels, broken bottles, discarded tyres, decorative projections above windows and doors which hold rainwater, roof gutters, containers made of wood, rubber, paper, etc.

Though no larvae of *Anopheles* species are found in undiluted seawater, *Anopheles sundaicus* breeds in highly brackish waters in lakes and creeks which are subject to tidal influence.

Some species show wide diversity in the choice of breeding sites while others are found only in a few special places. Many species, however, are found in more than one type of breeding place.

With special reference to the Myanmar anophelines, some breeding sites and the species typically found in them can be detailed as follows:

1. Marshes in jungles, animal footprints with rotting leaves and peaty water, slow running streams in shaded jungles: *Anopheles aitkeni*i, *Anopheles bengalensis*, *Anopheles insulaeflorum*, *Anopheles lindesayi.*
2. Jungle and mountain streams: *Anopheles gigas*, *Anopheles splendidus*, sometimes *Anopheles fluviatilis*, *Anopheles minimus.*
3. Streams, irrigation channels, river margins etc., with flowing water, springs and seepages : *Anopheles culicifacies, Anopheles fluviatilis, Anopheles minimus, Anopheles barbirostris, Anopheles majidi*, A*nopheles kochi*, *Anopheles theobaldi, Anopheles stephensi, Anopheles subpictus, Anopheles vagus.*
4. River and streambed pools: *Anopheles culicifacies, Anopheles subpictus, Anopheles vagus.*
5. Rain or irrigation water-filled pits, ground pools, etc., stagnant water without much vegetation except grasses: *Anopheles culicifacies, Anopheles subpictus, Anopheles vagus, Anopheles jeyporensis,* A*nopheles kochi*, *Anopheles nigerrimus, Anopheles barbirostris,*

tessellatus, Anopheles stephensi, Anopheles pallidus, Anopheles splendidus, Anopheles jamesii, Anopheles pseudojamesi, Anopheles philippinensis.

6. Pits, tanks, ponds, pits, swamps with stagnant water and abundant vegetation: *Anopheles aconitus, Anopheles annularis, Anopheles culicifacies, Anopheles jamesii, Anopheles jeyporensis, Anopheles karwari, Anopheles nigerrimus, Anopheles pallidus, Anopheles philippinensis, Anopheles pseudojamesi, Anopheles subpictus, Anopheles varuna, Anopheles vagus.*
8. Trenches and channels, with shade: *Anopheles aitkenii, Anopheles insulaeflorum, Anopheles barbirostris, Anopheles minimus.*
9. Paddy fields (fallow or just planted): *Anopheles culicifacies, Anopheles subpictus, Anopheles pallidus.*
10. Paddy fields (growing): *Anopheles nigerrimus, Anopheles sinensis, Anopheles philippinensis, Anopheles pallidus* (sometimes *Anopheles fluviatilis, Anopheles minimus* and uncommonly *Anopheles culicifacies*).
11. Domestic wells: *Anopheles dirus.*
12. Brackish water swamps and lakes, coastal saltwater: *Anopheles sundaicus.*
13. Pools in sandy soil: *Anopheles culicifacies, Anopheles subpictus.*
14. Artifical containers, cisterns, fountains: *Anopheles stephensi, Anopheles kochi, Anopheles subpictus.*

While the above summary generally holds good for most breeding sites in Myanmar, there are several variations and the selection of any breeding sites by a species is not absolute, and unchanging. Many of mosquitoes breed occasionally in atypical breeding sites, particularly in adverse seasons. For example, *Anopheles fluviatilis* though normally preferring to breed in stream channels and paddy fields may occasionally be found in tanks and ponds. *Anopheles nigerrimus* normally found in paddy fields may also be found in channels and swamps. *Anopheles minimus* is sometimes found in paddy fields and pits or holes. Such variation in distribution may be the result of unusual factors forcing the gravid females to deposit eggs in atypical breeding sites or the larvae might be carried to such places by water currents. However, even recognizing that there is no such thing as an absolutely fixed behaviour in nature, there are certainly preferred types of water for most species. Some species may be catholic and some may be very selective.

(B) Larval environment:

A few of the anopheline larva habitats that have been considered in detail for the individual species are discussed in more general terms below:

(a) **Shade**: Many species of anophelines, such as *Anopheles culicifacies, Anopheles subpictus, Anopheles vagus,* etc., breed and survive both in open waters with very little or no shade and also in places with some shade. However, species like *Anopheles minimus* and *Anopheles fluviatilis* are found usually in slightly shaded breeding sites either under shade from overhanging trees and bushes or from thick growths of grass. Deep shade is, however, not suitable for the larvae of *Anopheles minimus.* Some species such as *Anopheles balabacensis, Anopheles lindesayi,* etc., breed only in dense shade of forest or plantation. Shade might also have an indirect adverse effect on the growth of plankton including algae which may prohibit influence breeding.

(b) **Movement of water**: Slowly flowing water is a requisite for several species, particularly *Anopheles minimus* and *Anopheles fluviatilis.* These two species are generally found in streams, channels and other breeding sites such as paddy fields in which there is a perceptible current. However, generally the larvae of these species do not continue to occur in places where weeding has been carried out. There are many species which are unaffected by water flow. Species such as *Anopheles culicifacies* and *Anopheles subpictus* breed as readily in stagnant as in flowing water. *Anopheles annularis, Anopheles jamesii, Anopheles pallidus, Anopheles nigerrimus* and several other *Anopheles* species are predominantly breeders in stagnant water.

(c) **Floral and fauna**: It is well known that certain species can breed in natural waters in which there is no visible flora and fauna, and others only in the presence of various kinds and degrees of growth of vegetation. It would not be correct to say that in the former case there would be no flora and fauna at all because natural waters usually contain many types of unicellular organisms, particularly diatoms, in the plankton without which anopheline larvae would not be able to obtain the food required for their growth.

In nature the conditions are very different. Species such as *Anopheles subpictus, Anopheles vagus,* and *Anopheles culicifacies* often grow in temporary pools of water without any visible growth of vegetation. In certain situations, however, as in long standing breeding sites such as in riverbed pools or excavation pits, they thrive equally well in the presence of filamentous green algae such as *Spirogyra spp.* Some of these pools or pits have many planktonic organisms including green

algae such as *Chorella spp., Chlamydomons spp., Volvox spp., Euglena spp., etc.* However, when blue green algae appear, *Anopheles culicifacies* begins to disappear.

At the other extreme are species such as *Anopheles annularis, Anopheles jamesii, Anopheles pallidus, Anopheles sundaicus, Anopheles philippinensis, Anopheles nigerrimus* and others, which thrive only in waters rich in vegetation. Vegetation of such waters includes:

(i) Planktonic forms such as unicellular algae, protozoans, diatoms and bacteria.

(ii) Lower vegetation forms particularly multicellular algae including filamentous green and blue green algae, some of the latter forming thick mucilagenous floating masses such as *Lyngbia spp.*

(iii) higher vegetation including:

- floating vegetation, such as *Lemna spp., Eichhornia spp.,* etc.
- emergent vegetation such as various types of grasses, bullrushes etc.
- submerged vegetation such as *Hydrilla spp., Vallesneria spp.,* etc.

While moderate growth of vegetation can provide optimum conditions for growth of larvae, abundance of some of the floating forms actually lessens the intensity of breeding, as in the case of water hyacinth and *Lemna* with regard to *Anopheles philippinensis.* Thick growth of vegetation, apart from grasses, tends to inhibit the breeding of species like *Anopheles culicifacies, Anopheles fluviatilis, Anopheles minimus* and *Anopheles vagus.*

Mountain streams are also generally free of rich growths of higher vegetation. However, the rocks and pebbles in flowing water are generally covered by slimy masses of algae. Species which breed in such places include (for example) *Anopheles minimus* and *Anopheles fluviatilis*.

The association of *Anopheles nigerrimus* with growing paddy fields has been noted all over Myanmar. What makes this habitat particularly attractive to this species, whether it is the rice plants themselves or other related vegetation, is not known. The mechanical obstruction provided by paddy plants which inhibit *Anopheles culicifacies* breeding is discussed in the section referring to that species.

Regarding aquatic fauna, it should be noted that apart from providing food, as in the case of protozoans, flagellates and ciliates, some of them are inimical to anopheline larvae. When there are heavy growths of *Vorticella spp.* they smother the larvae. The predatory effect of certain insects and higher forms of animals, such as fish, are also well known.

(C) Seasonal prevalence:

Regional surveys provide very useful information on the distribution and prevalence of anopheline species. The results of such surveys also offer valuable information on the malaria vectors and their prevalence. Physiography and climate play an important role in the seasonal prevalence of most species which will be discussed further below and in Chapter 4. General Conclusion.

The seasonal fluctuations in the numbers of each of the various species would seem to be closely related to two climatic factors: temperature and rainfall.

(a) **Temperature**: Throughout the northern regions of Myanmar winter temperature are usually sufficiently low to check the rate of development considerably and all species become scarcer at this time of the year. This is not the case in southern Myanmar where breeding continues throughout the winter, for instance *Anopheles dirus* breeds throughout the year in wells from Mon State, in southern parts of Myanmar.

(b) **Rainfall**: The monsoon affects different species (for instance, *An. dirus* and *An. philippinensis*) in quite different ways according to the nature of their various breeding places. Three main classes of breeding place may be distinguished;

(i) *Running water*: These breeding sites are subject to sharp spates brought on by each fall of rain and the monsoon is, therefore, a time when stream breeders are scarce. They are at their greatest abundance after the monsoon when many streams are flowing but fluctuations in the water level are small. *Anopheles minimus* and *Anopheles maculatus* are typical examples of stream-breeders affected in this way. On the other hand, the stream-breeding *Anopheles stephensi* is at its greatest abundance during the months just preceding the monsoon.

(ii) *Pools and puddles*: Species which breed in pools, puddles and other small stagnant areas of water are at their maximum during the monsoon, when there is a rapid extension of suitable breeding places. Conversely these species are scarce during the dry season. Example of pool breeders are *Anopheles dirus, Anopheles vagus, Anopheles subpictus* and *Anopheles kochi.*

(iii) *Marshes and paddy fields*: Continued heavy rain seems to affect adversely those species which breed in the larger stretches of stagnant water but the light, intermittent rain at the beginning and end of the monsoon favours them. Their season usually extends well into the post-monsoon months. Species which fall into this class are *Anopheles philippinensis, Anopheles annularis* and *Anopheles aconitus.*

The abundance of any species depends more upon the availability of breeding sites than season. The monsoon and the immediate post-monsoon months provide the best opportunity for many species of *Anopheles* to live and multiply. In certain other areas, such as irrigated areas, the season of irrigation, which may or may not coincide with the monsoon months, provides optimum conditions for high anopheline densities, for instance *Anopheles maculatus, Anopheles annularis* and *Anopheles philippinensis.* This finding is in agreement with Fox (1949) and Khin Maung Kyi (1971).

In certain riverine areas, particularly in central Myanmar where there are sharp differences between the monsoon months and pre-monsoon dry months, the pools in beds of rivers and streams provide favourable breeding sites for certain species like *Anopheles culicifacies*. There are probably, other factors influencing the seasonal abundance of anopheline mosquitoes, but no definite evidence has been found in the present study.

2. Adult bionomics

(A) Resting habits:

Throughout this study period, the techniques of "morning collections" and "night collections" of adult mosquitoes were used. The latter method, involves the collection of adult mosquitoes from various situations (inside and outside walls of houses or cattle sheds, on both humans and animals and on vegetation) at intervals throughout the night and is in marked contrast to the normal method of "morning collections" of adult mosquitoes resting in houses or cattle sheds. The only information obtainable from "morning collections" is that certain species of anopheline mosquitoes rest in houses or in cattle sheds by day. As, in the majority of cases, only a limited number of the total species present do rest indoors by day, it will be seen that an incomplete picture of the anopheline population is obtained.

In contrast, with a suitably conducted series of "night collections" it is possible to obtain information on all of the following points: (i) The total number of anopheline species present. (ii) The relative density of each species. (iii) The time of prevalence of each species throughout the night. (iv) The preferential feeding habits of each species. (v) The behaviour of certain species both before and after feeding. The real importance of this mosquito collection method lies in the fact that it makes possible the study, by direct observation, of the habits of adult *Anopheles* under natural conditions. The relationship between anopheline mosquitoes and malaria in different localities depends on the

behaviour of the various species as it is affected by variables such as seasonal influence and by the habits of man himself. A closer understanding of adult anopheline behaviour will inevitably lead to improved methods of malaria control.

The majority of anopheline species in Myanmar appear not to rest either in houses or in cattle sheds by day, although the evidence with regard to some of them is inconclusive. Species which can definitely be classed as house resters include: *Anopheles culcifacies, Anopheles minimus, Anopheles sundaicus, Anopheles subpitus* and *Anopheles vagus* (Table 2.2.16, Table 2.2.52, Table 2.2.72,Table 2.2.70 and Table 2.2.77). It will be recalled that the first three are malaria vectors (Table 2.2.17, Table 2.2.54 and Table 2.2.73) whereas the other two are purely cattle feeders (Table 2.2.70 and Table 2.2.77).

The resting habits of adults *Anopheles stephensi* varied in each locality in accordance with the type of breeding place adopted by this species. In urban areas, where breeding occurred in domestic water containers adults were found in houses by day. In rural areas, on the other hand, where breeding took place in streams, adults did not rest indoors.

It is possible that, in some cases, climatic conditions were responsible for the variations shown by some species in the daytime resting habits. Thus, both the study of Khin Maung Kyi (1971) and this study (1988 to 2000), at Katha area, Sagaing Division, recorded that *Anopheles culicifacies* adults were more prevalent in houses during the winter months. Similarly, with *Anopheles maculatus* in the Kabaw valley, Fox (1949) and Khin Maung Kyi (1971) found large numbers of adults in houses by daytime during January to March whereas in this study, not a single specimen was recorded during September to November although prolific breeding was occurring at the time. The resting habits of some species were found to vary widely in different localities. Thus, *Anopheles philippinensis* was only found resting in houses during morning collections on one occasion. That was at Bhamor township, Kachin State, and it is interesting to note that this was the only time during the survey, in which *Anopheles philippinensis* was found to be infected.

For *Anopheles annularis*, both the study of Khin Maung Kyi (1971) and this study, only at Myohaung township, Rakhine State, show this species resting in houses and cattle sheds. In many other areas of Myanmar no adults could be found indoors although larvae were very plentiful.

Whereas Khin Maung Kyi (1971) found adults of *Anopheles barbirostris* in great numbers at Wuntho area, Sagaing Division, in this study (1998) adult only be found in small numbers. The variation might be a result of the different climatic conditions occurring during the two surveys.

It is apparent that, between the two extremes of definite houses resting species on the one hand and those species which never enter houses on the other hand, there lies a large group of anopheline species the resting habits of which tend to vary from one locality to another, and also from one season of the year to another. The factors causing these variations are obscure and much further work remain to be done on this aspect of anopheline behaviour.

(B) Host preference (feeding habits):

The estimation of the preferential feeding habits of any particular anopheline species has, in the past, always been done by carrying out an extensive series of precipitin tests on blood from the gut of freshly-fed specimens caught during routine morning collections. The method is laborious and requires special equipment. Facilities for doing precipitin tests were not available in Myanmar.

During the study period, the feeding habits of each species have been assessed, wherever possible, by comparing the numbers of each species taken during night collections in human habitations with the numbers taken during similar collections on or near cattle during the same period in each locality. Provided the numbers to be considered are large enough then the latter method is just as reliable as the former and has the added advantage of requiring no special equipment. Moreover species which do not rest in houses are not liable to be overlooked.

With regard to their feeding preferences the anopheline species of Myanmar can be roughly divided into the following three groups (excluding wild species, the feeding habits of which are unknown).

Firstly, a small group comprising species which feed almost entirely on man and only to a limited extent on cattle. Into this group fall *Anopheles minimus, Anopheles jeyporiensis* and *Anopheles dirus* and all three are vectors of malaria in Myanmar (Table 2.2.23, 2.2.36 and 2.2.55).

Secondly, another small group made up of species which feed almost entirely on cattle Members of this group are *Anopheles jamesii, Anopheles pallidus, Anopheles subpitus, Anopheles vagus, Anopheles varuna* and *Anopheles stephensi* (Table 2.2.33, 2.2.58, 2.2.70, 2.2.77 and 2.2.79).

Thirdly, the largest group, into which the majority of the species fall. These anophelines appear, under normal conditions, to feed mainly on cattle but also to a small extent on man, for instance *Anopheles philippinensis, Anopheles annularis, Anopheles aconitus* and *Anopheles sinensis*. During abnormal circumstances, and especially if the man/cattle ratio is upset for any

season, these species will attack man readily (for example *Anopheles annularis*, *Anopheles aconitus)*. Out of this group, therefore, will come the sporadic malaria vectors such as *Anopheles sundaicus* and *Anopheles culcifacies* and also those species which, on account of their numerical abundance, could possibly transmit malaria.

At this stage it is worth recalling the points raised in the introduction regarding the technique of night collection of adult mosquitoes. It will be remembered that it was argued that night collections were superior to morning collections because the former produced direct evidence regarding the feeding habits of all species present, whereas the latter only gave information about the daytime resting places of certain species.

It will be clear from the preceeding paragraphs that, whereas all vectors must feed on man they do not all rest in houses by day. There can be no question, therefore, as to which technique of adults collection is likely to yield the more valuable information. These findings is in agreement with Fox (1949) and Khin Maung Kyi (1971).

(C) Biting rhythm: Anopheline mosquitoes, except for a few species occurring in the deep shade of the forest, take their blood meals at night time. They feed on man, cattle and other domestic animals. Many investigations have been made on the time of feeding (biting rhythm) and there are considerable variations in the recorded observations. Female anophelines bite mainly at night but occasional observations have been made of them biting in daylight hours also. The species differ considerably in their biting rhythms. Though biting may occur throughout the night on a small scale, there are distinct peak times. Some species bite early and others bite late. Some are exclusively crepuscular.

An. annularis seems to bite mostly in the earlier part of the night prior to midnight though some degree of biting continues throughout the night (Table 2.2.7). *An. sundaicus* also was found to bite mostly during the first quarter of the night in almost all study areas (Table 2.2.74). This observation somewhat differs from that made in Sittwe and Kyaukpyu Townships, Rakhine State, however, where biting takes place mostly during the second quarter. There can be real differences in the biting rhythms because of the existence of different biological strains. The present findings are similar to those of Khin Maung Kyi (1971). There are innate differences between species but the behaviour of the same species can also be influenced by seasonal factors.

Observations have been made on *Anopheles philippinensis,* mostly in other countries of Southeast Asia, particularly in Malaysia. Reid (1968) found the biting rhythm of this species to commence at about 21:00 and to go on throughout the night. In Myanmar, Fox (1949), Khin Maung Kyi (1971) and in this study as well found peak biting activity between 19:00 to 23:00. It was very rarely encountered during the third and fourth quarter of the night up to 23:00 (Table 2.2.62 and Table 2.2.63).

3. Medical importance (relation to malaria)

The infection rate, that is, the frequency of female anopheline mosquitoes with oocysts in their ovary and sporozoites in their salivary glands, is a very useful parameter for ascertaining the dynamics of malaria transmission in a given area. If oocysts were observed, the mosquito was considered "infected". To determine whether a mosquito was infective, the salivary glands were dissected and examined for the presence of sporozoites. The sporozoite rate can serve as an indicator of the efficacy of control programs based on mass drug administration, use of biological or chemical insecticides, integrated pest management schemes, or the much-anticipated antimalaria vaccines.

An attempt has been made to assess the importance of each species in relation to the transmission of malaria in Myanmar. As far as possible only the evidence recorded in Myanmar has been taken into account for this purpose. However, in a few instances, it has been considered justifiable to include observations made in areas closely adjacent to the borders of Myanmar such as the Myanmar-Bangladesh border portion of Rakhine State, and Myanmar-China border portion of Shan State.

For instance, regarding *Anopheles jeyporiensis,* Macan (1944) and Fox (1949) concluded that this species was an important vector in Rakhine State. In the Kabaw valley, Macan (1944) commented that its role as a vector could not be determined on the evidence available. Robertson (1941) concluded that this species is second in importance only to *Anopheles minimus* as a vector in Western Yunan (Myanmar-China border). Although, on account of its limited distribution, *Anopheles jeyporiensis* cannot be classed as a major vector in Myanmar it has been shown to feed readily on man. In view of the infection rates (Table 2.2.36) found on the Myanmar-China and Myanmar-Bangladesh borders during the study period, this species must be regarded with suspicion wherever it occurs in any abundance. This finding is in agreement with Fox (1949).

4. Distribution

An attempt is made to show whether the particular species confines itself to any particular type of terrain, e.g. coastal areas, foothills, jungle or irrigated plain, or whether it is ubiquitous in its distribution.

Anopheline mosquitoes are found in all parts of Myanmar from sea level up to an altitude of 1890 m, for instance, Kyin Maung Kyi (1971) and this study found *An. aitkenii* and *An. bengalensis* at heights between 1036 to 1890 m above sea level.

There are records of mosquitoes found in deep mines particularly *Anopheles annularis, Anopheles culicifacies, Anopheles maculatus* and *Anopheles bengalensis*. This finding is similar to those of Khin Maung Kyi and Win (1976).

Generally, the number of species at the sea coast or in the plains are smaller than in the hills and foothills. Most of the rare species occur in the hills, for example *Anopheles karwari* and *Anopheles splendidus* (compare with the observation of Khin Maung Kyi, 1971).

Anopheles sundaicus is confined to coastal areas and the lower reaches of the Ayeyarwady delta where the creeks are subject to tidal influence. This finding is similar to those of Fox (1949) and Khin Maung Kyi (1971).

2.3.4. 2. Discussion

The earliest references to Myanmar mosquitoes were written by Stott (1916) and Christophers (1933). Stott was dealing mainly with the clinical aspects of malaria especially in the Mandalay area. Christophers basically wrote a taxonomical monograph that included valuable information on *Anopheles* biology. He recorded 26 species of anophelines in Myanmar. Fox (1949) listed a total of 27 species of anopheline encountered in Myanmar up to the end of the Second World War in 1945, and reviewed all the available data accompanied by distribution maps and dissection records.

There were 33 species and varieties of anophelines in Myanmar, recorded by 1954 by national and WHO entomologists. This list did not include *An. kyondawensis* and *An. barbirostris* var. *ahomi* (only larvae of *An. kyondawensis* found in Kyondow village near Moulmein Township, Mon State and *An. barbirostris* var. *ahomi* was found in Southern Shan State by Abraham in 1947, unpublished).

An. kyondawensis was also described by Rao and Delphin (1957) and Khin Maung Kyi (1971) in the same region noted by Abraham. During my study period, in Innwaing area (near Kyondow village), Moulmein Township, Mon State, only one larva of *An. kyondawensis* was found in a shady pool along forest stream associated with larvae of *An. insulaeflorum*. *An. barbirostris* var. *ahomi* has only been described by Abraham in Myanmar.

Khin Maung Kyi (1971) has been stated a total of 37 species of anopheline encountered in Myanmar. In my study period, the same species that has been stated by Khin Maung Kyi (1971), were also observed. However, the following names are not avaliable for use nowadays (Oo et al., 2003 b).

(i) *An. indiensis* was synonymized under *nigerrimus* by Harrison et al., (1973) and the species called *indiensis* by Reid in Malaysia and Thailand was renamed *nitidus*. Thus, *nitidus* Harrison, Scanlon and Reid , would be the correct name for this species in Myanmar.

(ii) *An. jeyporiensis* var. *candidiensis* was synonymized under *jeyporiensis* by Harrison (1980). Thus, *jeyporiensis* James, is the correct name for this species in Myanmar.

(iii) *An. maculatus* var. *willmorei* was elevated to full species by Rattanarithikul and Green (1986) and the spelling was corrected to *willmori*. Thus, *An. willmori* (James) is the correct name for this species in Myanmar.

(iv) *An. ramsayi* was found to be a junior synonym of *An. pseudojamesi* by Nurul Huda and Harrison (1985). Thus, *pseudojamesi* Strickland and Chowdhury, is the correct name for this species in Myanmar.

Thus, there are 36 anopheline species recorded in my study period (section 2.3.3.1. List of species of *Anopheles* in Myanmar).

An. pampani is listed in "A cataloge of the mosquitoes of the world" by Knight and Stone (1977). However, this species has never been found in Myanmar. In 1989, the Medical Entomology Research Division, Department of Medical Research (DMR), published "New records of mosquitoes from Myanmar". In this publication, three new *Anopheles* species were listed; *Anopheles (Anopheles) nitidus, Anopheles (Cellia) nivipes* and *Anopheles sawadwongporni*.

A single male specimen of *Anopheles (Anopheles) nitidus* was collected only onece by Myo Paing in December 1987, resting on an orchid plant at 05.30 in his compound, Nga-dat-kyi Pagoda Road, Bahan Township, Yangon. The identification was made on the basis of the male hypopygium. Further collections did not yield any more specimens.

Anopheles (Cellia) nivipes was collected by the DMR survey team during July 1987 in Thabyewa village, Bago Division. It was identified according to Reid (1967) from the adult male characters, pupal and larval skin morphological characters. This species was collected only during the monsoon season. It is mainly zoophilic and feeds only on cattle. *Anopheles (Cellia) nivipes* was always associated with forest and foothills. This species could not be found during my study period.

Anopheles sawadwongporni is a new species of the *An. maculatus* group in Myanmar. Adult specimens were collected from Kyauktagar Township, Bago Division, forested foothill of Bago Yoma range during December 1985 by the DMR servey team. This species could not be recorded during the study period.

Certain other species occur both in Thailand and India which also may be expected in Myanmar such as *Anopheles barbumbrosus, Anopheles craefordi* and *Anopheles roperi* (Rao, 1984). However, these species have not been found so far in Myanmar.

Previously *An. leucosphyrus* was described as a *Leucosphyrus* group of species in Myanmar. However, Khin Maung Kyi (1971) examined specimens of *An. leucosphyrus* from the collections at the Malarie Institute of Myanmar and identified them as *An. balabacensis balabacensis,* which he regarded as an important vector of malaria in Myanmar. When the DMR initiated studies on malaria vectors in 1983, the prevalence of *An. dirus* was recognized for the first time in Myanmar. Subsequent surveys by DMR teams in various parts of the country did not yield *An. balabacensis balabacensis.* DMR teams investigated its bionomic and relationship to malaria transmission under varied ecological conditions. In addition to detailed morphological studies, cytogenetics and isoenzyme studies were undertaken to differentiate sibling species. DMR confirmed the species as *An. dirus*.

An. dirus Peyton and Harrison (= *dirus* A), which currently has *dirus* B, *dirus* C, and *dirus* D confirmed as distinct species (but still unnamed). Baimai et al., (1988) have identified only *An. dirus* species D from materials originating in Southern Myanmar (well-breeding *An. dirus*) and in Bago Division (forest-breeding *An. dirus*). Actually, the most common *An. dirus* in Myanmar is almost certainly *An. dirus* D, which is the primary species (and vector) in Bangladesh (Baimai, unpublished data). The distribution of *An. dirus* extends into eastern Myanmar, but *An. dirus* D is probably the more common member of this complex in Myanmar.

Concerning malaria vectors, Fox (1949) provided information as follow:

(1) Primary vectors: (a) Responsible for regular, annual malaria transmission: *An. minimus, An. leucosphyrus and An. jeyporiensis.* (b) Responsible for sporadics of malaria: *An. culicifacies* and *An. sundaicus.*

(2) Secondary or occasional vectors: *An. aconitus, An. annularis, An. hyrcanus* var. *sinensis* and *An. philippinensis.*

However, Khin Maung Kyi (1971) has provided information about major malaria vectors such as: (i) *An. minimus:* many positive dissection results were obtained in many localities; (ii) *An. balabacensis:* a major vector during monsoon in thickly forested areas of Myanmar; (iii) *An. annularis:* this species is a secondary vector, several positive dissections were obtained; (iv) *An.culicifacies:* it is responsible for local outbreaks; (v) Members of the *An. hyrcanus* group, particularly *An. sinensis*: probable, a secondary vector and should not be ignored; (vi) *An. jeyporiensis:* high infection rates of this species were found in both Indo-Burma border and Burma-China border; it should be regarded as a vector wherever it occurs in numbers; (vii) *An. philippinensis:* this species is considered as not important except in the Bhamo region and (viii) *An. sundaicus:* suspected vector.

According to my results (e.g. dissection records), the vector competence of the species can be catagorised (in Chapter 4. General Conclusion) as follow:

(1) Primary vectors: (i) *An. minimus* and (ii) *An. dirus*.

(2) Secondary vectors: (iii) *An. aconitus:* secondary vector in some isolated localities; (iv) *An. annularis:* in a few localities, high density of adult results in high infection rates; (v) *An.culicifacies:* this species is a suspected vector of importance in Central Myanmar, especially in the irrigated areas; (vi) *An. sinensis:* secondary vector only on Myanmar-China border when its density is high; (vii) *An. jeyporiensis:* it is a secondary vector on the Myanmar-Bengladesh border near Rakhine and on Myanmar-China border when its density is high; (viii) *An. maculatus:* this species is a primary and a secondary vector depending on the condition of the locations, especially in Tanintharyi Division; (ix) *An. philippinensis:* it is a local vector of minor importance on the Myanmar-Bengladesh border and (x) *An. sundaicus:* it is a secondary vector only in coastal regions. My conclutions are in agreement with Khin Maung Kyi (1971).

Regarding the vector control, the use of chemical insecticides play an important role in the control of disease vectors. However, there are many constraints such as the spread of vector resistance, enviromental pollution and rising operational costs. The high costs of new insecticides

poses a problem especially for developing countries. In addition chemical insecticides also have undesirable adverse effects on humans, domestic animals, plants and wild life. Under such situations, there is a great need to use other control strategies and environmentally safe biological control agents in integrated vector control programmes (WHO, 1975). The importance and necessity of microbial control of insects have been recognized and emphasized in recent years.

Entolomologists from DMR in their search for larvicide have tested *Bacillus sphaericus* (2362 and 2297) and *Bacillus thuringiensis* varity *israelensis* H-14 strain against malaria vector anophelines in the laboratory as well as in field trials (Myo Paing et al., 1987). Laboratory studies have been carried out against I-IV instar larvae of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles* group. Laboratory studies revealed that a concentration of 1 mg per litre of water (1 ppm) induced hundred percent mortality within a period of 24 hours exposure (Myat Myat Thu, 1990). Field trials have been undertaken since 1983 with a spore forming (*B.s*) bacterium, against anopheline larvae and culicine larvae, breeding in polluted ground water pits and water drains. Results of small field trials obtained show that a little higher dosage viz. 3 litre per hectare than the optimum recommended dosage of 2.5 litre per hectare, could induce 66 and 72 percent mortality in anopheline and culicine larvae respectively (Myo Paing et al., 1987).

Results obtained from laboratory trials show that microbial control agents are very useful against *Anopheles*. For practical use in control programmes, the larval breeding habitats of the target *Anopheles* species have to be known. For instance, *An. dirus*, the major vector of malaria in Myanmar, was found to breed in small rock pools in deep forests, in domestic wells and bamboo stumps (Myo Paing et al., 1987). In widely distributed small breeding sources, the practically use of microbial control agents, no matter however effective, is almost impossible. However, in domestic areas they could be used in wells to control *An. dirus*.

Another example is *An. annularis*. This species breeds predominatly in ponds used to grow watercress at Rakhine coastal region (Chapter 2). Here, it is an important vector of malaria and is highly resistant to insecticide (VBDC report, 1997). In such a situation, it could be possible to use microbial control agents as an alternative larvicide. Moreover, this mosquitoe breeds in large numbers in pools of stagnant water on sand banks in this area. The water of the breeding sites is not deep and the bottom is not muddy. In such a situation, application of microbial control agents will have a very favourable impact as part of a integrated vector control programme.

3. Biochemical studies on *Anopheles dirus* collected from Mudon

3. Biochemical studies on *Anopheles dirus* collected from Mudon

3.1. Introduction

The species group of *Anopheles* (Cellia) has long been regonized as of major public health significance in the Southeast Asia and the Indian subregions of the Orient (Reid, 1968). At least three species are known to be primary vectors of human malaria parasites. They are: Firstly *An. balabacensis balabacensis* Baisas, 1936, from East Malaysia and several islands of the Philippines (Balbac, Palawan) and Indonesia (Java, Kalimantan); Secondly *An. dirus* (Peyton and Harrision, 1979), in all mainland of Southeast Asian countries and from Nepal to West Malaysia, Thailand, Myanmar, and Vietnam; and thirdly *An. leucosphyrus* Dönitz, 1901, from Indonesia (Sumatra, Kalimantan). *Anopheles dirus* is an important vector of malaria in Myanmar (Vector-Borne Disease Control, VBDC report, 1990, Myo Paing et al., 1989.a). It normally occurs in the forest and forest fringes where it transmits malaria efficiently (Khin Maung Kyi, 1970, 1975, Khin Maung Kyi and Winn, 1976). Changes in the ecology of an area induced by new development projects, like deforestation, construction of dams and irrigation projects have profound or indirect effects on vector occurrence because of the creation of suitable ecotypes for the completion of their life cycle. Thus, the dangerous vector *An. dirus* could invade human settlements. This typical forest breeder could successfully adapt and spread all over the Mudon region.

Two main questions can be posed: (i) Is this *An. dirus* in the process of mutating and undergoing a certain degree of genetic divergence?; (ii) Has this condition facilitated their adaptation to breed in wells? In the present study three groups of *An. dirus* (breeding areas from domestic, rubber plantation and forest locations) were identified by allozyme variation.

In taxonomy, population genetics and evolutionary research, protein electrophoresis is a widely used and highly efficient technique. For this, homogenates of animal or organ samples are applied to a gel (polyacrylamide, agarose or starch) and exposed to an electrical field under ionic buffer conditions. The electrophoretic migration of solved proteins is determined by their net charge, size and shape. Depending on this, they segregate to bands in the gel and then are visualised by unspecific or, in case of enzymes, by substrate transformation coupled staining. Different migration of proteins depending on the same gene locus is the result of a charge of their amino acid sequence. Proteins are primary gene products, so changes of their amino acid sequence are caused by changes in the underlying DNA-sequence, i.e., different alleles exist on their gene locus. When different species possess different alleles exclusively, these fixed alleles may be used as genetic markers and aid the

diagnoses of species by morphological traits. By investigating several proteins, the data obtained can be used to quantify the degree of genetic variation within populations, detection of species boundaries, phylogenetic relatio nships and evolutionary processes.

Thus, the purpose of the present study is to establish the degree of genetic divergence (similarity) between the three topographically different populations of *An. dirus.* Protein electrophoresis was chosen as a means of assessing genetic relations among these populations. The second goal was to obtain estimates of levels of genetic heterozygosity within and amounts of variation between natural populations of *An. dirus*, a question about which little is presently known in Myanmar. The main aim of this study is to characterize the enzyme patterns of *An. dirus* collected from three different ecological areas of Mudon.

A more detailed analysis was performed in the present study using 11 enzyme systems to determine the degree of genetic differentiation among the three populations of *An. dirus* from Mudon Township (from domestic, rubber plantation and forested areas) in comparison with *An. maculipennis* from Mannheim, Germany and *An. stephensi* from Indonesia, provided by Bayer AG, Leverkusen (breeding stock).

3.1.1. Description of Mudon (study) area

Mudon, a coastal township in Mon State, covers an area of 23.96 square kilometres, which is divided into four Quarters, bordering the Gulf of Martaban, part of the Andaman Sea (Fig. 3.1). It is situated between latitudes 16° and 16° 25´ north and longitudes 97° and 97° 53´ east, bounded by Azin Dam and Azin Creek in the north. A central agricultural area (26.9 hectares) and rubber plantation (236.3 hectares) are located north of the Azin Creek. Kyonpike Creek marks the boundary in the south and Mawlamyine-Ye railway track in the west. Pastures and paddy fields dominate the area between the railway track and the Andaman Sea. Tanintharyi Yoma mountain range borders the eastern side of Mudon (Fig. 3.2).

3.1.2. Condition of wells: The wells are of varying size, depth and shape, approximately 4.5-12 m deep, either circular or square in shape. Most of the wells were dug under the shade of coconut palms, mango, banana, tamarind, horseradish (drum-stick), rubber and jackfruit trees. The coconut and tamarind trees mostly provide the shade. These wells are usually located within compounds and near houses. The soil is friable with porous soft lateritic rocks, dried fairly rapidly by percolation (high soil infiltration rate) and evaporation. For this reason most of the wells dry up during summer.

The lateritic rock lining these wells is a residual clay enriched with ferric hydroxide as a result of chemical weathering in the tropics (Mineral Development Corporation, Mudon, 1984).

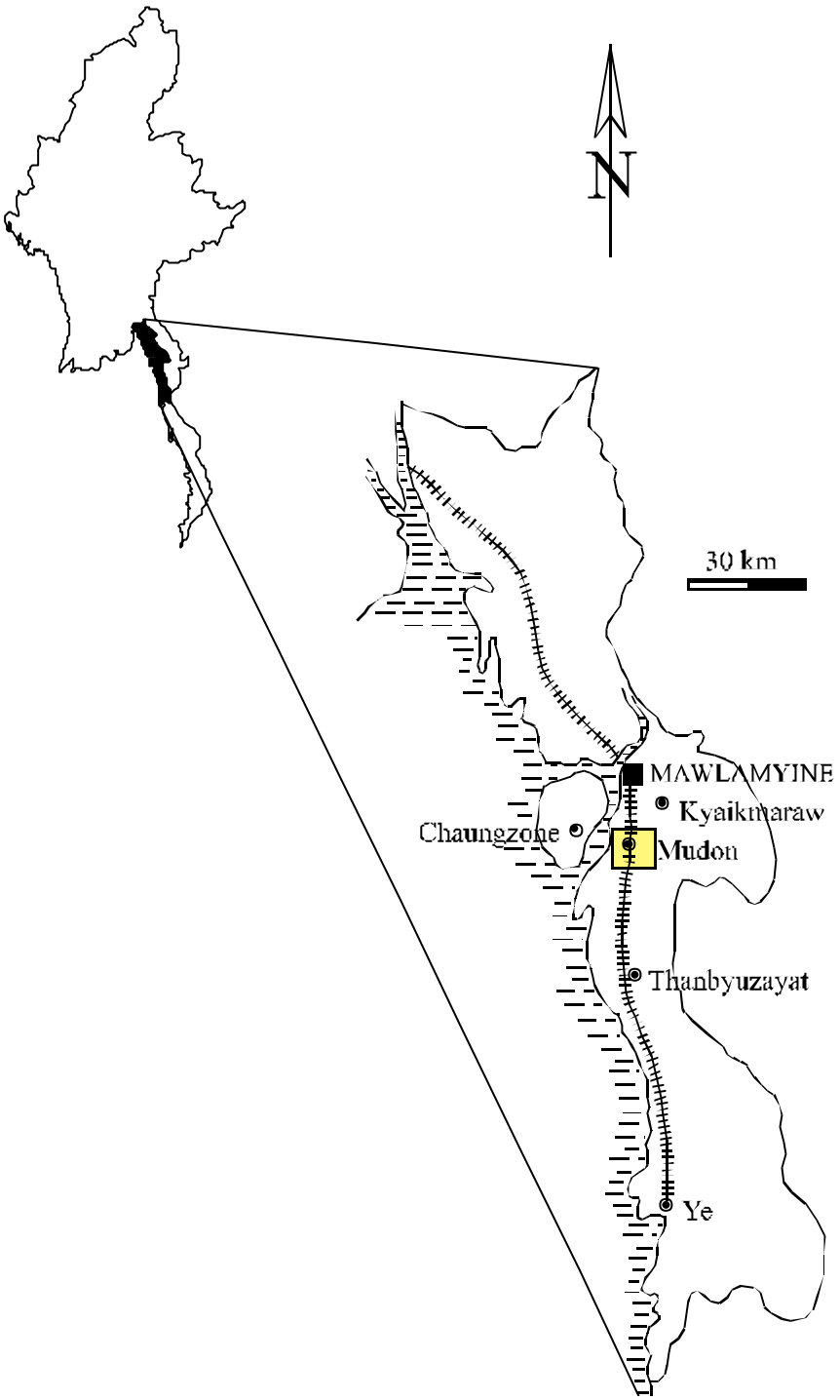


Fig. 3.1. Location map of Mudon (study) area.

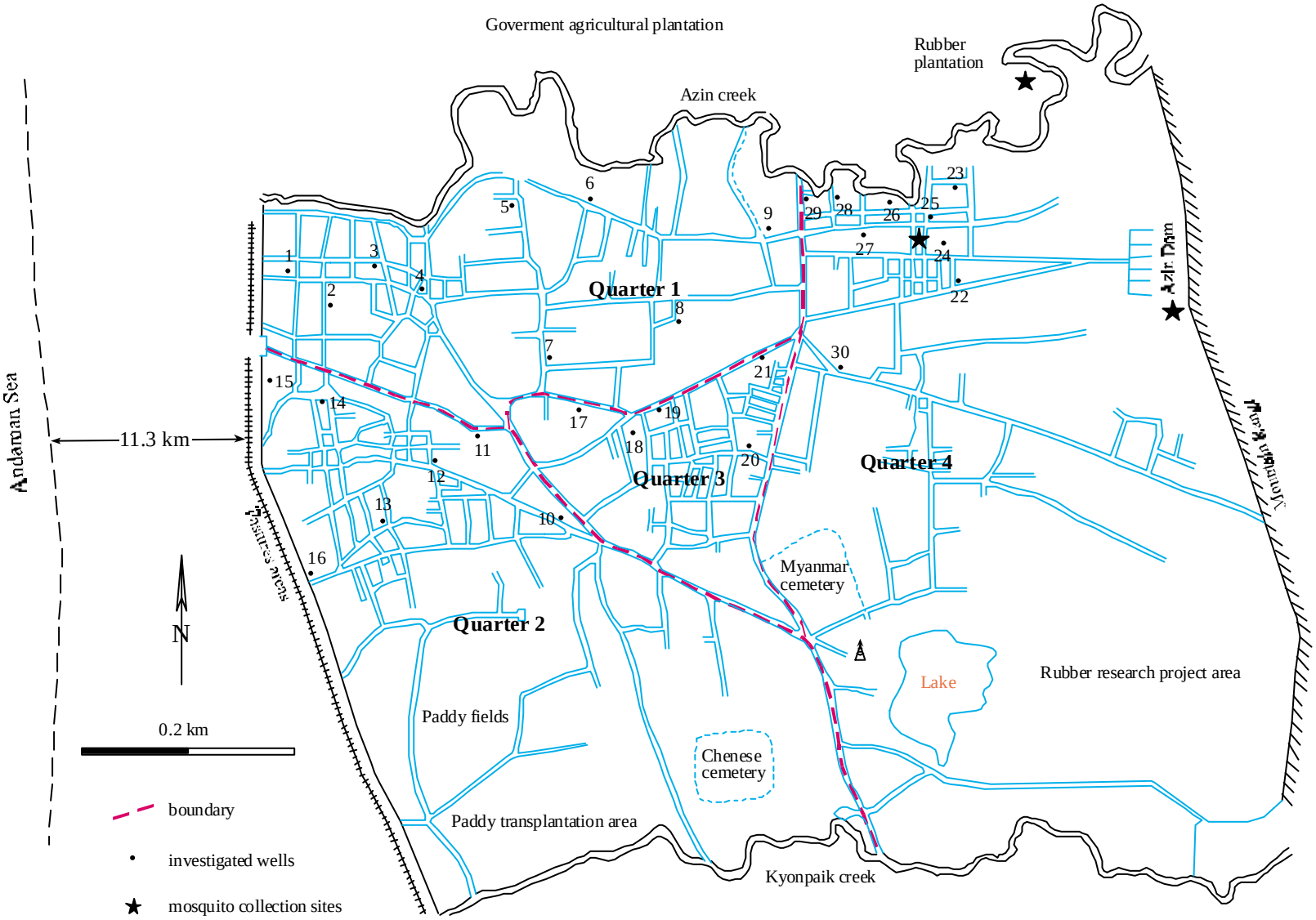


Fig. 3.2. Map of Mudon Town showing study sites.

3.2. Material and methods

3.2.1. Selection of 30 wells: Wells were selected randomly from all four Quarters. The criteria were as follows: (i) From each Quarter a lamp-post was chosen (each Quarter has between 14 to 18 lamp-posts and each lamp-post serves between 65 to 300 households). (ii) From each chosen lamp-post, three shady sites were selected randomly. (iii) From each shady site, within a radius of 135 m, 2-3 wells were chosen that were not in direct contact with sunlight during the afternoon. A total of 30 wells were selected, nine of which were in Quarter 1, seven in Quarter 2, five in Quarter 3 and nine in Quarter 4.

3.2.2. Larval surveys: (i) Larvae were sampled at mosquito breeding sites (domestic wells) on a weekly basis and appropriate data were recorded. Larval sampling was carried out by using a well net (Fig 2.79 and Photo 2.1). The net consisted of a conical shaped, white cloth dipper with a diameter of 35 cm and length of 46 cm. It was held at an angle by four strings and controlled by a long string or rope. The net was introduced into the well with the lower side of the net just under the surface of the water and its opening at an angle of about 45°. The net was moved slowly around the side of the well twice, then quickly withdrawn and inverted into a bowl of water. The larvae and pupae were collected in vials for species identification. Ten dips per well, per week were taken and the mean number of larvae and pupae per dip were determined.

(ii) Although the domestic wells were considered to be the major breeding habits during the rainy season, all stagnent water pockets, coconut shells, discarded tins, utensils, bamboo stumps, collecting bowls for the gum from rubber trees (Photo 2.2) and other containers were examined for larval breeding from domestic, rubber plantation and forest areas Methods for collecting larvae are described (in detail) in section 2.3.2.1.

(iii) As soon as possible, the species identification was made and all the specimens were stored in liquid nitrogen (-196 °C) or covered with dry ice for protein electrophoresis.

3.2.3. Adult collections: Three fixed catching stations (Fig. 3.2) were chosen in domestic area (DA), rubber plantation area (RPA) and forested area (FA). Indoor and outdoor biting catches were conducted in these three different ecological areas. All catchings were made in fixed stations throughout the study in the following way: (i) Human-bait hand catches (indoor and outdoor) with glass tubes and sucking tubes (aspirators) from 18:00 to 06:00 of the next day (Photo 2.3).

(ii) Using cattle-bait, big bednet catches were also made with sucking tubes (aspirators) from 18:00 to 06:00 of the next day (Photo 2.4). Methods for collecting adult mosquitoes are described in section 2.3.2.2.

(iii) As soon as possible, species identification was made and all the specimens were stored in liquid nitrogen (-196 °C) or covered with dry ice for protein electrophoresis.

3.2.4. Preparation of electrode buffer: Electrophoresis buffer systems were adapted to each enzyme to support the migration of the enzyme in an electrical field to the anode, distinguishable variants of each enzyme could be detected and distinct bands were built up. Five hundred ml of electrophoresis buffer were prepared, based on the standard procedures from Harris and Hopkinson (1976) and Shaw and Prasad (1970). Table 3.1 shows the different buffer systems, adapted to the enzyme and species specific requirements, that were prepared in the experiment with their corresponding recipes.

Table 3.1. Electrophoresis buffer systems and abbreviations of enzymes

No.	Electrode buffer	Gel buffer	Enzyme system	Author
1	0.05 M Tris/0.05 M NaH_2PO_4, pH 8.3	EP 1:10, pH 8.3	MPI, GPI	Harris & Hopkinson
2	0.1 M Tris/0.1 M Maleic acid/0.01 M $MgCl_2$/0.01 M EDTA, pH 8.3	EP 1:10, pH 8.3	PGM, HBDH	Harris & Hopkinson
3	0.155 M Tris/0.05 M Citric acid, pH 7.0	EP 1:10, pH 7.0	GOT, IDH, AK, HK, G3PDH, Aldox, MDH_{NAD}	Shaw & Prasad

3.2.5. Gel preparation: Gels used the appropriate gel buffer for the protein system. Nine ml of the prepared electrode buffer (Table 3.1) and 0.9 g agarose were filled up to 90 ml with distilled water in a stoppered Buckner vacuum flask. This was heated over a magnetic stirrer with continuous swirling until a vigorously boiling, clear, viscous solution was obtained (4-5 min). The gel solution was then poured in a sideways motion over a 21x12x2 mm sized horizontal glass gel plate. This was: (i) Cleaned with 70% ethanol; (ii) labelled with the name of the buffer system prepared; (iii) the date when the experiment was performed; (iv) the name of the mosquito samples that were tested in the experiment; and (v) a single line was drawn (4 cm) from the edge of the glass plate to serve as the point of origin for sample application. The gel was allowed to set at room temperature for about 10 minutes for

polymerization, then it was held at 4 °C until electrophoresis, in a moist plastic box. A ready prepared gel could be stored for one day at 4 °C.

3.2.6. Sample preparation: (i) One ml buffer solution (for samples) of 10 mM Tris/citric acid, pH 7.5 including 10 mM ß-mercaptoethanole, an antioxidant that protects the cystin groups of the enzymes, was prepared.
(ii) Twenty-two 1.5 ml plastic reaction (grinding) tubes were prepared with corresponding labels from 1 until 22.
(iii) Each of these vials (tubes) was filled with 50 µl of the buffer solution.
(iv) The mosquito samples were removed from liquid nitrogen (-196 °C) and each single specimen was transferred in each of these 22 vials.
(v) Tissue extracts were produced by homogenization for 15 seconds by using a high-speed ultrasound sonicator instrument (Sonopuls). The mixture was kept ice-cold (0 °C) during and after the homogenisation process.
(vi) Just prior to use, homogenates were centrifuged for one minute at 14,000 g in a table centrifuge (Eppendorf), to separate extracted soluble proteins (supernatant) from cellular debris.
(vii) The supernatant was then applied to the gel. Before adding the homogenate samples to the gel, a longitudinal paper strip was applied for two minutes. This was to remove excess moisture, which enabled the samples to penetrate the gel.
(viii) A slot guide of plexiglass with corresponding numbers from 1 to 22 was placed at the line of origin prior to electrophoresis. It was properly lined up at the middle in all the slots.
(ix) By the use of a micropipette, 3 to 5 µl of the homogenate samples from each of the 22 vials was fractionated and placed in the sample slots on the prepared gel for electrophoresis. After diffusion of the samples into the gel (2 min) the electrophoresis process could be started.

3.2.7. Preparation of equipment: Before sample preparation, every effort was made to have all the required solutions and equipment ready, as many proteins are unstable after destruction of the cellular environment (homogenization). The electrophoresis apparatus consisted of two buffer tanks (electrode chambers) and each buffer tank contained a platinium electrode, a buffered electrolyte solution (electrode buffer 250 ml each) and a high voltage electrical power source. The gel served as a bridge between the electrodes. The electrical current set up a charged field in the gel in which the protein molecules migrated and separated depending

on: (i) The number of positive versus negatively charged amino acid present in the protein; (ii) the size and configuration of the molecule with respect to the pore size of the gel matrix; (iii) the ionic and steric forces created by the buffer system (ion flow) and its specific pH; and (iv) other factors.

3.2.8. Electrophoresis: The electrophoresis tanks of a Pharmacia Multiphor II electrophoresis chamber, were filled with 250 ml (each of the tanks) electrode buffer. The gel on the supporting glass plate was placed on the cooling plate (cooling stage). Two pieces of wet cotton were folded and placed in each of the tanks and also covered about 2 cm of the gel at both edges serving as buffer bridge. Then the tank cover was closed and the power supply was turned on. To remove catalyst ions with a potential for enzyme inhibition a pre-run (program 1) was performed for 10 minutes at 200 volts. Program 2 was performed at 500 volts (sometimes 450 volts, depending on buffer strength) and cooled down to 4 °C. The electric current served to promote a charged field in the gel in which the protein molecules migrated and separated, depending on their net charge, caused by the number of positive versus negatively charged amino acids present in the protein.

3.2.9. Visual evidence of proteins: Proteins may be identified by unspecfic staining with silver-salt or coomassy-brillant blue. Much more specific and sensitive enzymes are shown by substrate-specific reaction. The standard procedure in the preparation of the stain followed Harris and Hopkinson (1976) and Murphy et al. (1996). After electrophoresis of the protein samples in the gel, the power was turned off and the gel was removed from the apparatus. The individual proteins were selectively stained and most of these stains provided a specific substrate and co-factors for the enzyme, allowing it to catalyze the particular reaction involved. A dye then reveals the formation of bands in the gel. The stain, substrate and any necessary co-factors are added, thoroughly mixed and poured immediately over the gel surface to give an even coverage of the gel. It may then be covered with clear plastic box to prevent desiccation and placed in darkness in an incubator at 37 °C until the bands have stained sufficiently for measuring. The results were usually observed within one hour but occasionally up to six hours were needed. Photograph were taken of these results and the migration of different isozymes were also recorded and drawn to be used in the interpretation of data. Table 3.2 shows the different types of stains that were prepared with their corresponding recipes.

Table 3.2. The different enzyme systems with their corresponding stain recipes (ingredients)

(a)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Mannosephosphate isomerase	MPI	5.3.1.8	0.2 M Tris/HCl, pH 7.5	50 ml
			Mannose-6-phosphate (Ba-salt)	10 mg
			1 M $MgCl_2$	0.2 ml
			Glucosephosphate isomerase	2 µl
			Glucose-6-phosphate dehydrogenase	0.15 mg (80 U)
			NADP	5 mg
			MTT	5 mg
			PMS	5 mg
			Agar	300 mg

(b)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Glucosephosphate isomerase	GPI	5.3.1.9	0.2 M Tris/HCl, pH 8.0	50 ml
			Fructose-6-phosphate (Ba-salt)	20 mg
			1 M $MgCl_2$	0.4 ml
			Glucose-6-phosphate dehydrogenase	0.1 mg (45 U)
			NADP	5 mg
			MTT	5 mg
			PMS	5 mg
			Agar	300 mg

(c)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Glutamat-oxalacetat-transaminase	GOT	2.6.1.1	0.1 M Tris/HCl, pH 8.0	50 ml
			L-Aspartic acid	100 mg
			2-Oxoglutarate	55 mg (pH 8)
			Pyridoxalephosphate	30 mg
			Fast Blue BB	125 mg

(d)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Hexokinase	HK	2.7.1.1	0.5 M Tris/HCl, pH 7.1	50 ml
			1 M $MgCl_2$	50 µl
			Glucose	40 mg
			ATP	10 mg
			Glucose-6-phosphate dehydrogenase	0.15 mg (80 U)
			NADP	5 mg
			MTT	8 mg
			PMS	2 mg
			Agar	300 mg

(e)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Adenylatekinase	AK	2.7.4.3	0.05 M Tris/HCl, pH 7.1	50 ml
			1 M $MgCl_2$	9 mg
			Glucose	40 mg
			ADP	8 mg
			Hexokinase	0.08 µl (160 U)
			Glucose-6-phosphate dehydrogenase	0.15 mg (80 U)
			NADP	5 mg
			MTT	8 mg
			PMS	2 mg
			Agar	300 mg

(f)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Phosphoglucomutase	PGM	2.7.5.1	0.5 M Tris/HCl, pH 8.0	50 ml
			Glucose-1-phosphate	40 mg
			Glucose-1, 6-diphosphate	< 1 mg
			1 M $MgCl_2$	0.5 ml
			Glucose-6-phosphate dehydrogenase	0.15 mg (80 U)
			MTT	5 mg
			PMS	5 mg
			Agar	300 mg

(g)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Glycerol-3-phosphate-dehydrogenase	G3PDH	1.1.1.8	0.2 M Tris/HCl, pH 8.7	50 ml
			1 M $MgCl_2$	0.1 ml
			a-ß-Glycerophosphate	15 mg
			NAD	20 mg
			MTT	5 mg
			PMS	5 mg
			Agar	300 mg

(h)

Enzyme	Abbreviation	E.C. No.	Stain Composition	Quantity
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30	0.3 M Phosphate buffer, pH 7.4	50 ml
			1 M $MgCl_2$	20 µl
			NaCl	250 mg
			DL-Hydroxybutyate	500 mg
			Gluconic acid	2 ml
			NAD	40 mg
			MTT	10 mg
			PMS	1 mg
			Agar	300 mg

(i)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Malate-dehydrogenase	MDH_{NAD}	1.1.1.37	0.1 M Tris/HCl, pH 8.0	50 ml
			L-Malic acid	350 mg, pH 8
			NAD	10 mg
			MTT	7.5 mg
			PMS	5 mg
			Agar	300 mg

(j)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Isocitrate-dehydrogenase	IDH	1.1.1.42	0.5 M Tris/HCl, pH 8.0	50 ml
			Isocitric acid	30 mg
			1 M $MgCl_2$	3 ml
			MTT	5 mg
			NADP	5 mg
			PMS	5 mg
			Agar	300 mg

(k)

Enzyme	Abbreviation	E.C. No.	Stain composition	Quantity
Aldehyde-oxidase	Aldox	1.2.3.2	0.1 M Tris/HCl, pH 7.5	50 ml
			Acetaldehyde	0.2 ml
			MTT	10 mg
			PMS	5 mg
			Agar	300 mg

Each enzyme was used in several electrophoretic runs for *An. maculipennis*, *An. stephensi* and three *An. dirus* populations.

3.2.10. Statistical analysis of the protein banding pattern (analysis of allozyme data): The different data from 11 enzymes analyzed by gel electrophoresis were recorded. The results in gels of the appearance of enzyme bands with different electrophoretic mobilities in different samples suggest that these electrophoretic variants of enzymes are encoded by different alleles. The appearance of a single band implies a gene locus, where gene products are electrophoretically indistinguishable, in other words no allelic variation is detectable. These loci are considered to be homozygous. The appearance of more than one band implies a heterozygote (which depends on the quaternary structure of the protein) or a homozygote at more than one locus. After the representation of the alleles, a hyphenated number (if enzymes of the same function are encoded by more than one locus) is assigned in order of electrophoretic mobility (1 being less anodal to 2). Allelic variants of enzymes are assigned by a subscript number depending on their relative electrophoretic mobility while the mobility

of the most common allozymes in *An. dirus* is expanded to the value of 100 mm. The relative mobility of each other allozyme is expanded by the same factor (e.g. PGM-100 and PGM-117, where PGM-100 is the most common allozyme in the reference population). The allozyme patterns in the gels (zymogram) obtained after electrophoresis depend on the structure of the respective enzyme (Evans, 1987).

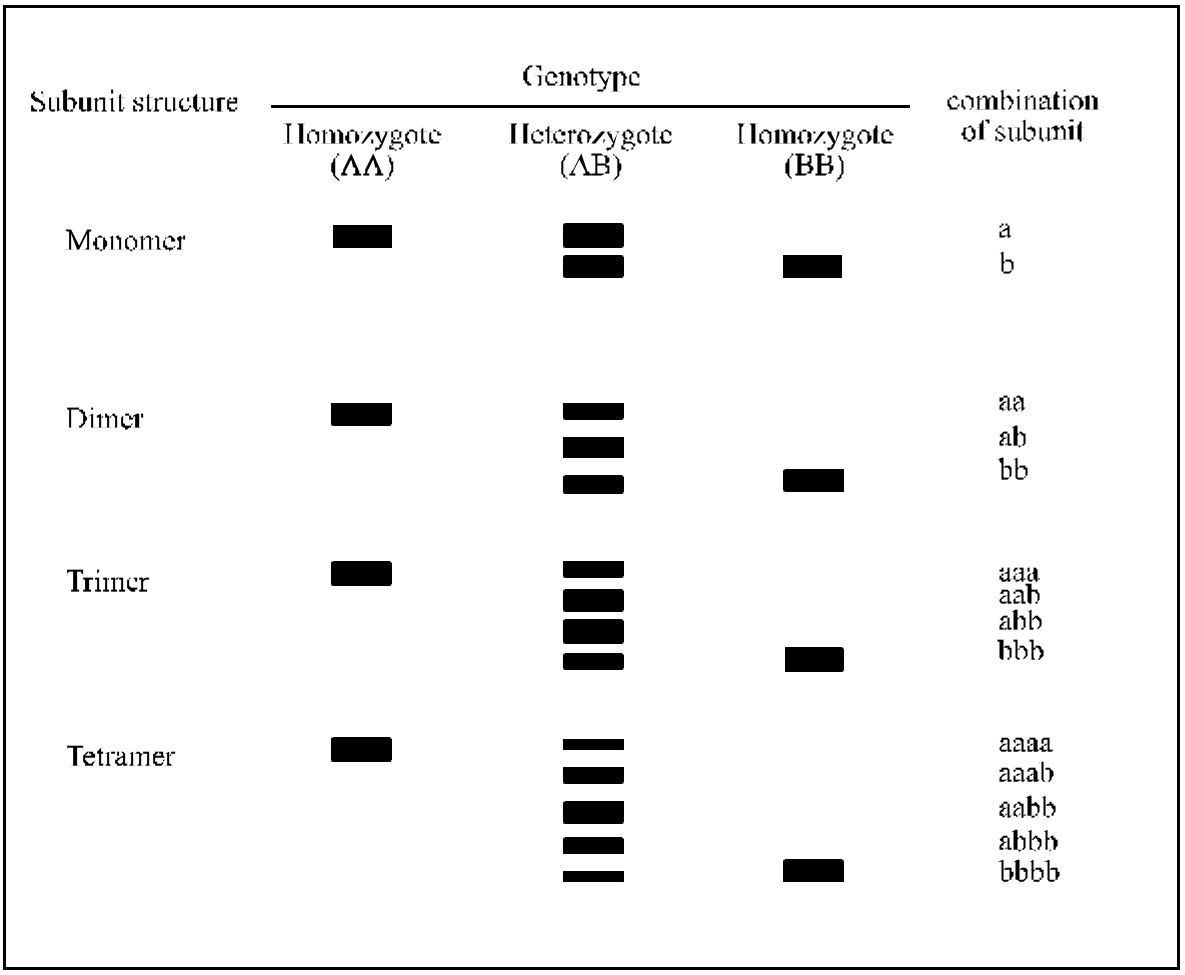


Fig. 3.3. Diagram of characteristic isozyme patterns expected in homozygotes in the case of enzymes with monomeric, dimeric, trimeric and tetrameric quarternary structure. The capital letters A and B respectively, represent the allelic background of the subunits of an enzyme, the small letters a and b represent the resulting subunits, which can be combined to multimeric proteins.

Figure 3.3 shows the diagram of isozyme patterns expected in homozygotes and heterozygotes for enzymes of common subunit composition. A monomeric enzyme consisting of one polypeptide chain causes a single band after electrophoresis when the underlying alleles of a individuals are identical. Then this gene locus is classified as homozygous. In the case of heterozygosity, two different polypeptide chains are produced caused by two different

responsible alleles. The resulting allozymes can be separated by electrophoresis into two distinct bands, representing a heterozygous gene locus. For example: PGM, HK.

In enzymes which consist of two polypeptides (i.e., of dimeric structure, such as IDH and MDH), the heterozygote is represented by three bands with the central one usually being the strongest due to the combination of both variants in one protein molecule. Nevertheless, monomorphic enzymes or homozygous gene loci are always represented by one band except of posttranscriptional or posttranslational (nongenetic) modifications. The characteristic electrophoresis patterns of heterozygotes shown diagrammatically are: In monomers there are two components (bands), with dimers three components, with trimers four components, with tetramers five components and so on. In each case the two outer components of the pattern represent the homomaric forms and the components with intermediate electrophoresis mobilities, the heteromeric forms.

3.2.10.1. Allele and genotype frequencies: The band patterns that appear in gels represent phenotypes with a linear genetic background. Allozymic variation reflects a part of the genetic variation at the DNA-level. Diploid organisms such as the mosquito have two copies of each gene locus (except for the few genes of the sex chromosomes in males). The products of both gene copies can be identical (monomorphic) or can differ in the amino acid sequence. The underlying variants of the same gene locus are called alleles.

The genotype describes the combination of identical or different alleles in one specimen. The genotypic frequencies in a population depend on the allele frequencies in the population (Hardy-Weinberg-equilibrium). The degree of heterozygosity reflects the frequency of combination of different alleles and provides a measure of the genetic variability at a distinct locus or over all gene loci considered, in the population. This is calculated by adding up the numbers of each heterozygous at a given locus and the total number for all genotypes combined (i.e., the sample total).

Beside the genotype frequencies, the frequencies of each allele at each locus is calculated. The relative allele frequencies are obtained by counting the number of times each variant was found and dividing this by the total number of distinguishable alleles in the sample. The frequency of an allele calculated in this way is the frequency of individuals homozygous for that allele, plus half the frequency of heterozygotes for that allele. Each allele frequency will be calculated separately for each population and when totalled the sum should be equal to 1.0. This is completed using the formula below (Evans, 1987).

$$p_A = \frac{N_{AA} + \frac{1}{2} N_{AB}}{N_{total}}; \qquad p_B = \frac{\frac{1}{2} N_{AB} + N_{BB}}{N_{total}}, \qquad (1)$$

where A, B are allele frequencies and N is the sample size.

3.2.10.2. Variance and confidence intervals of the allele frequencies: Allele frequencies are obtained from the randomly chosen samples out of the whole population. They accurately reflect those frequencies in the population as a whole, particularly if the samples are large enough in number. In this case, it is of great importance to calculate the variance of each allele frequency as a measure of the accuracy of the estimate for the real population`s state. The formula for doing this, together with an algebraic representation of the above method for estimating allele frequencies (Evans, 1987) is:

$$Var\,(p_A) = \frac{p_A\,(1 - p_A)}{2\,N_{total}}, \qquad (2)$$

where Var (p_A) is the variance of allele frequency p_A of allele A and N_{total} is the size of the mosquito sample. The formula for estimating confidence intervals {VI }, Weir (1990) is:

$$VI(p_A) = p_A \pm 2\,v\,Var\,(p_A). \qquad (3)$$

3.2.11. Testing for fit to Hardy-Weinberg expectations: Most polymorphic enzymes where the sample size has allowed a statistically meaningful test are in the Hardy-Weinberg Equilibrium (HWE). The HWE was established by Hardy and Weinberg in 1908. It states that in a randomly mating population, the expected distribution of genotypes is determined by the random combination of alleles, and this results in an equlibium being set up among genotypic frequencies at any given locus that remains constant from one generation to the other.

The genotypic frequencies equilibrium are given by the square of the allele frequencies, so that with two alleles A and B with frequencies *p* and *q*, the frequencies of the three possible genotypes are:

$$(p + q)^2 = p^2 + 2pq + q^2. \qquad (4)$$

In the case of three or more alleles, a polynomial expansion is used {i.e., *n* alleles with frequencies *p*1, *p*2…*p*n yield genotype frequencies (*p*1, *p*2…*p*n)^2}. From the observed allele frequencies, the expected frequency of each genotype is obtained, and when multiplied by the number of individuals in the population sample, gives the expected Hardy Weinberg Equilibrium distribution.

Expected genotype frequencies can be calculated in several ways. One way is to test heterozygosities rather than genotype frequencies; i.e., to pool all homozygotes and all heterozygotes at each locus regardless of allele and test them against predictions of the HWE rule about the expected pooled respective numbers (Dobzhansky and Epling, 1944; Pamilo and Varvio-Aho, 1984). The test statistic is:

$$X^2 = \frac{\left(\left| 2N \sum_{i<j}^{n} p_i p_j - \sum_{i<j} O_{ij} \right| - c\right)^2}{2N \sum_{i<j}^{n} p_i p_j} + \frac{\left(\left| N \sum_{i=1}^{n} p_i^2 - \sum_{i=1} O_{ii} \right| - c\right)^2}{N \sum_{i=1}^{n} p_i^2}, \qquad (5)$$

where N is the number of individuals sampled, p_i and p_j are estimated frequencies of alleles i and j $(i<j)$, n is the number of alleles at the locus, O_{ij} the number of heterozygotes of alleles i and j, and O_{ii} is the number of homozygotes of allele i. There is always one degree of freedom in this test, and c is the correction factor for continuity. The value of c is usually 0.5 but Emigh (1980) suggested a value of 0.25 is preferable for HWE testing.

For testing individual genotype frequencies one could calculate the expected numbers from the HWE formula as Np_i^2 for homozygotes and as $2Np_i p_j$ for heterozygotes. However, for small sample sizes (<100 individuals, according to Spiess, 1989) a special problem arises. Levene (1949), Haldane (1954) and Smith (1970) pointed out that a finite sample from a population at HWE overrepresents the number of homozygotes; they used a correction (usually referred to as “Levene`s correction”) for the asymptotic nature of gene frequencies. The corrected expected values are:

for heterozygotes:

$$E_{ij} = \frac{4 N^2 p_i p_j}{2N - 1}; \qquad (6)$$

for homozygotes:

$$E_{ij} = \frac{N p_i (2 N p_i - 1)}{2 N - 1}. \qquad (7)$$

Emigh (1980) found that the use of these expectations in goodness-of-fit X^2 tests produces results that are, by-and-large, compatible with exact tests. No matter how individual expected genotype frequencies are estimated, the goodness-of-fit X^2 statistic is:

$$X^2 = \sum_{i<j}^{n} \frac{(|O_{ij} - E_{ij}| - c)^2}{E_{ij}} + \sum_{i=1}^{n} \frac{(|O_{ii} - E_{ii}| - c)^2}{E_{ij}}. \qquad (8)$$

Where c is the correction factor, which could be 0.5 or 0.25 in the case of two alleles, and should be 0 for more than two alleles. Because one degree of freedom is lost for each estimated allele frequency, the degrees of freedom (df) for this test are not equal to the number of genotype classes minus one (Dobzhansky and Levene, 1948; Crisp et al., 1978) i.e.,

$$df = \frac{n(n-1)}{2}. \qquad (9)$$

A problem that invariably arises in goodness-of-fit testing with the sample sizes usually employed in electrophoretic surveys, is that rare alleles lead to small expected genotype frequencies. Because expected values appear in the denominator, the X^2 test can

produce artificially inflated singnificance values. When Levene`s correction of expected values is used, a X^2 test will be impossible whenever $p_i = 1/(2N)$, i.e., whenever a single allele of a particular kind has been observed, because the expected number of homozygotes for this allele will be zero. Above this limiting value, there is uncertainty as to when it is necessary to seek special remedies (Horn, 1977). The Hardy-Weinberg test statistic for two alleles were comforted with exact critical values presented by Vithayasai (1973). Roscoe and Byars (1971) suggested that it is sufficient to have a sample size that exceeds twice the number of genotype classes when the significance level a= 0.05 and four times the number of genotype classes when a= 0.01.

3.2.12. Polymorphism and heterozygosity: Two measures of the degree of genetic variation in a population are the amount of heterozygosity and the proportion of loci that are polymorphic in the population.

A genetic polymorphism is the occurrence of two or more alleles at one locus, each with appreciable frequency in the same population. A locus is considered polymorphic when the frequency of the most common allele is less than 0.99 (or alternatively less than 0.95). To estimate the proportion of polymorphic loci ($P_{\varnothing}$) for a population where a number of loci have been examined, the number of polymorphic loci (L_{poly}) is counted and then divided by the total number of loci (L) examined. The accuracy of this estimate depends upon the number of loci examined and on the number of individuals. The formula for proportion of polymorphic loci, $P_{\varnothing}$, (Evans, 1987) is:

$$P_{Æ} = \frac{L_{poly}}{L}. \qquad (10)$$

The degree of heterozygosity can be calculated in a number of ways, depending on whether variation of one locus within individuals or populations is examined. The values may be also be estimated as observed frequencies of heterozygotes, expected frequencies (assuming Hardy Weinberg Equilibria) or from allele frequency data. It is important to distinguish between these different heterozygosity estimates because they yield differences in the degree of variance associated with each parameter.

The degree of heterozygosity at one specific gene locus in a population estimates the probability of two alleles from the same locus, taken at random from the population, being different.

The mean observed heterozygosity ($H_{\varnothing}$) in a sampled population is usually calculated for a number of loci. This involves counting the number of individuals heterozygous at a particular locus and dividing by the total number of individuals examined. This is then repeated for other loci and a mean estimate obtained by averaging the values over all loci. Alternatively, the number of heterozygotes across all loci [the degree of heterozygosity at the locus (H_L)] may be calculated for each individual, summed for all individuals, and then divided by total number of individuals to give a mean heterozygosity estimate for the population as before.

$$H_L = \frac{N_{AB}}{N_{total}} . \qquad (11)$$

If the population is not in Hardy Weinberg Equllibrium, the observed heterozygosity, may not reflect well the amount of genetic variation in the population. This can be overcome by calculating the expected heterozygosity (H_{exp}) from the allele frequencies, using the formula:

$$H_{exp} - \sum_{i}^{m} = 1 p_i^2 , \qquad (12)$$

where p_i is the frequency of the *i*-th allele at a locus, with *n* alleles (Nei, 1975), and averaging values over all loci. The expected heterozygosity may then be compared with the observed values and chi-square tested.

3.2.13. Measures of genetic identity and genetic distance: Relative allelic frequencies are used to calculate Nei`s (1972) genetic similarity (I_L), genetic identity ($I_{\varnothing}$) and genetic distance (D) according to Mueller and Ayala (1982) corrected for sample size (Nei, 1978). The genetic similarity (I) per locus between two populations is measured by:

$$I_L = \frac{? X_i Y_i}{v ? X_i^2 ? Y_i^2} , \qquad (13)$$

where x_i , y_i represent the products $x_1 y_1$, $x_2 y_2$, …… etc.

A commonly algorithum to calculate the mean genetic identity (**I**) between populations was proposed by Nei, (1972, 1973):

$$I = \frac{J_{xy}}{\sqrt{J_x J_y}}, \qquad (14)$$

where $J_x = S_i S_j X^2_{ij} /r$, $J_y = S_i S_j y^2_{ij} /r$, in which x_{ij} and y_{ij} are the frequencies of the i-th locus in populations X and Y respectively and r is the number of loci studied.

The genetic distance (D), quantifies the genetic differentiation between populations using relative allele frequencies as differentiating characters. It was measured by using Nei`s, (1973) methods:

$$D = - \log_e I \qquad (15)$$

3.2.1.4. The construction of dendrogram

Phylogenetic tree or dendrogram construction can be computed with the PHYLIP phylogency inference package, version 3.6 (alpha 3) by Joseph Felsenstein (Seattle, U.S.A.), 2002, using the Kitsch program. This program carries out the Fitch-Margoliash and Least Squares methods, plus a variety of others of the same family, with the assumption that all tip species are contemporaneous, and that there is an evolutionary clock. The branches of the tree cannot be of arbitrary length, but are constrained so that the total length from the root of the tree to any species is the same. The Kitsch program has an option to make trees by the minimum evolution distance matrix method. This method minimises an objective function, the sum of squares, not only setting the levels of the clusters to do so, but rearranging the hierarchy of clusters to try to find alternative clusterings that give a lower overall sum of squares.

These statistical methods were applied to data and experimental work and the results are detailed in the following section.

3.3. Results

The data presented below provide indications of protein electrophoretic variability in three populations of *An. dirus* and the outgroup taxa. The genetic variability, and differentiation are described and analyzed. As outgroup taxa the tropical species *An. stephensi* and *An. maculipennis* from temperate regions were used.

Each of the enzyme systems show a unique formation of bands in the gel. Photographs were taken of each run of enzyme electrophoresis for documentation processes. The electrophoresis conditions were adjusted between pH 7.0 and 8.5 so that the proteins of interest usually migrated from the origin (4 cm from cathode) to the top (anode), only few variants migrated to the cathode. The interpretation of the zymograms leads to the quantification of allele frequencies, genotype frequencies as well as the variances in comparison between the different populations.

a. Mannosephosphate isomerase (MPI) E.C. 5.3.1.8

Buffer system: number 1, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 31 mA, incubation time for staining at 37°C: 20 min.

Three electrophoretic variants depending on only one MPI-gene locus were observed in three species (five populations) that were tested (Fig. 3.4). These were MPI* 100, 102 and 107.

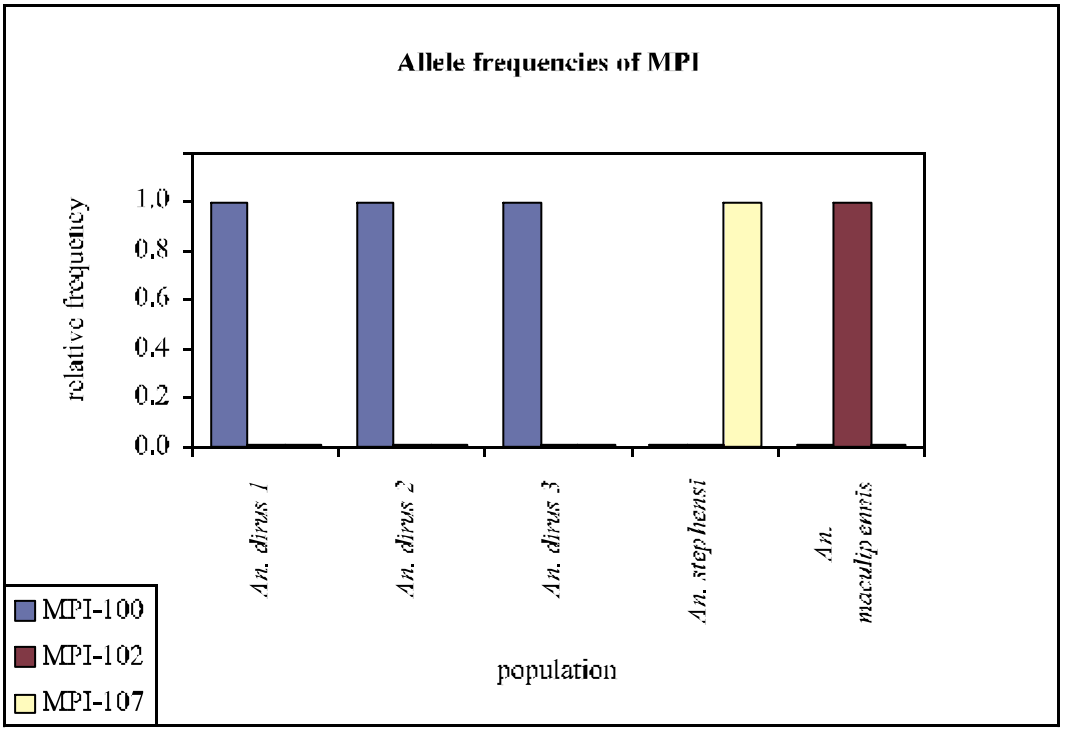
Table 3.3. Observed absolute (Abs) and relative (Rel) abundance of ***MPI*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy Weinberg-Equilibrium using allele frequencies

Locus	***MPI***									**Total**
Genotype	***100/100***			***102/102***			***107/107***			
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	12	1.0000	1.0000	0	0	0	0	0	0	12
An. dirus 2	12	1.0000	1.0000	0	0	0	0	0	0	12
An. dirus 3	16	1.0000	1.0000	0	0	0	0	0	0	16
An. stephensi	0	0	0	0	0	0	8	1.0000	1.0000	8
An. maculipennis	0	0	0	9	1.0000	1.0000	0	0	0	9

Allele MPI*100 was observed in *An. dirus* 1 (forested area), *An. dirus* 2 (rubber plantation area) and *An. dirus* 3 (domestic area), with a frequency of 1.0000 respectively. However this allele was absent in *An. stephensi* and *An. maculipennis* populations. Allel MPI*102 was observed in *An. maculipennis* with a frequency of 1.0000. This allele was not observed in *An. dirus* populations and *An. stephensi*. A species specific allele (MPI*109) was observed in *An. stephensi* with a frequency of 1.0000 (Table 3.3, 3.4 and Graph 3.1).

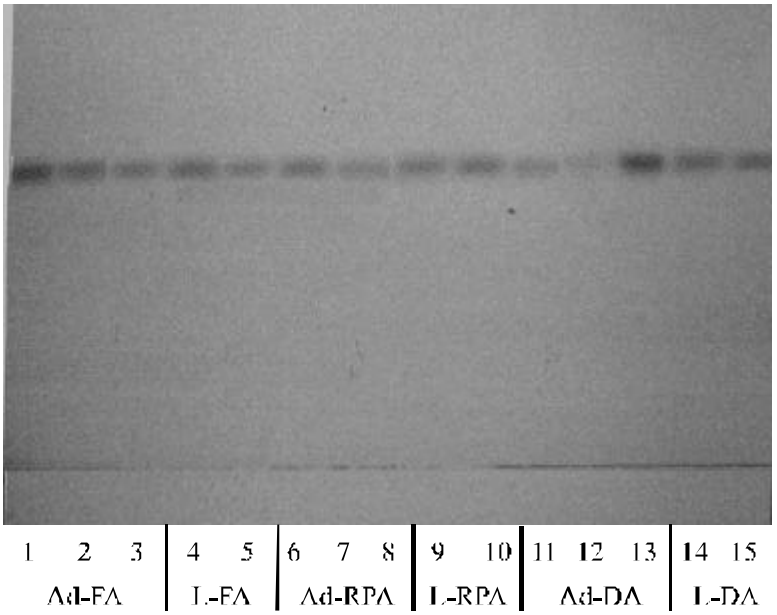
Table 3.4. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus *MPI* in five *Anopheles* populations

Allele	***MPI-100***				***MPI-102***				***MPI-107***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	24	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 2	24	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 3	32	1.0000	0	0	0	0	0	0	0	0	0	0
An. stephensi	0	0	0	0	0	0	0	0	16	1.0000	0	0
An. maculipennis	0	0	0	0	18	1.0000	0	0	0	0	0	0

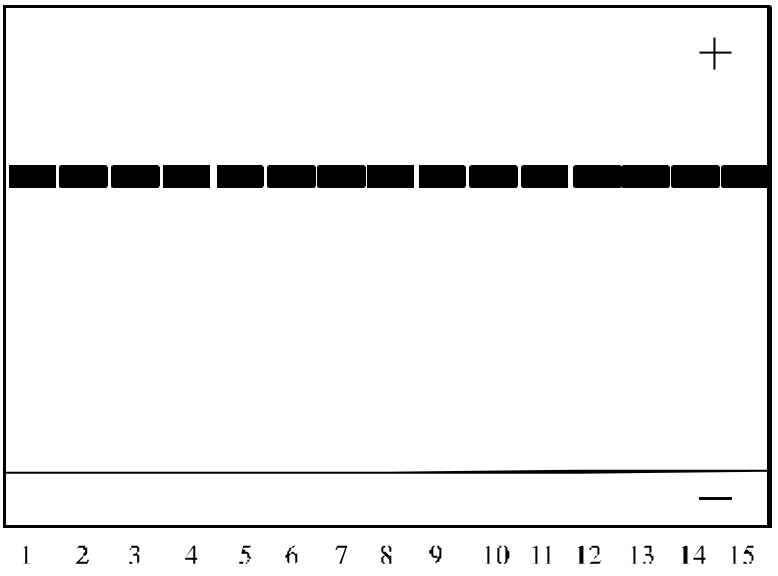


Graph 3.1. The graph shows the allele frequencies of MPI* in five populations

A.



B.



C.

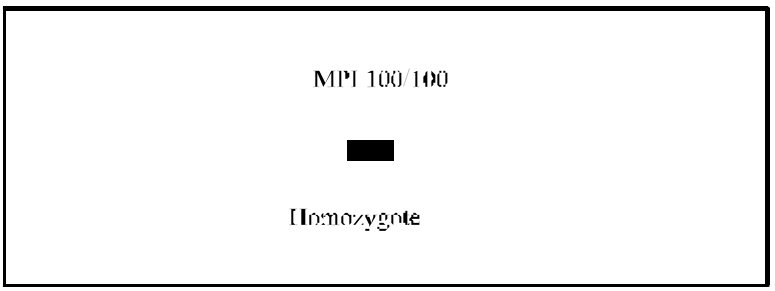


Fig. 3.4. (A) Photograph showing the electrophoretic mobilities of MPI in three *An. dirus* populations. (B) Diagrammatic representation of MPI enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

b. Glucosephosphat isomerase (GPI) E.C. 5.3.1.9

Buffer system: number 1, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 49 mA, incubation time for staining at 37°C: 15 min.

Glucosephosphat-isomerase (GPI) activity was detected, but separation into electrophoretic variants (bands) was not satisfactory. Thus, GPI-allozymes were not taken into account.

c. Glutamat oxalacetat transaminase (GOT) E.C. 2.6.1.1

Buffer system: number 3, gel temperature: 4°C *running time*: 66 min., *voltage (limited):* 450 V, *ampere:* 77 mA, *incubation time for staining at 37°C: 2 hours.*

Similar to the GPI-enzyme, Glutamat oxalacetat transaminase (GOT) activity could be observed but a separation into clear bands was not possible.

d. Hexokinase (HK) E.C. 2.7.1.1

Buffer system: number 3, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 55 mA, incubation time for staining at 37°C: 1 hour.

In *Anopheles* mosquitoes two co-dominant gene-loci, HK-1* and HK-2* were detected (Fig. 3.5). In HK-1 three alleles were observed, HK-1*94, 97 and 100. The HK-1*100 allele was exclusively present in *An. dirus* populations (*An. dirus* 1, *An. dirus* 2 and *An. dirus* 3) and showed a frequency of 1.0000. This allele was observed in both *An. stephensi* and *An. maculipennis* populations. Allele HK-1*94 was found only in the *An. stephensi* population and HK-1*97 only in *An. maculipennis* with frequencies of 1.0000 respectively. These HK-1*94 and HK-1*97 alleles were not observed in three populations of *An. dirus* (Table 3.5, 3.6 and Graph 3.2).

Also at the gene locus HK-2*, three alleles were found. These were HK-2*100, 104 and 109. HK-2*100 was the commonest allele in three populations of *An. dirus* with a frequency of 1.0000. HK-2* 104 was present only in *An. stephensi* population and HK-2*109 was observed only in *An. maculipennis*, both with relative frequencies of 1.0000. HK-2*104 and HK-2*109 were not present in three populations of *An. dirus* (Table 3.7, 3.8 and Graph 3.3).

Table 3.5. Observed absolute (Abs) and relative (Rel) abundance of ***HK-1*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-Equilibrium using allele frequencies

Locus	***HK-1***									
Genotype	***94/94***			***97/97***			***100/100***			**Total**
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	0	0	0	15	1.0000	1.0000	15
An. dirus 2	0	0	0	0	0	0	14	1.0000	1.0000	14
An. dirus 3	0	0	0	0	0	0	17	1.0000	1.0000	17
An. stephensi	8	1.0000	1.0000	0	0	0	0	0	0	8
An. maculipennis	0	0	0	12	1.0000	1.0000	0	0	0	12

Table 3.6. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the Locus ***HK-1*** in five *Anopheles* populations

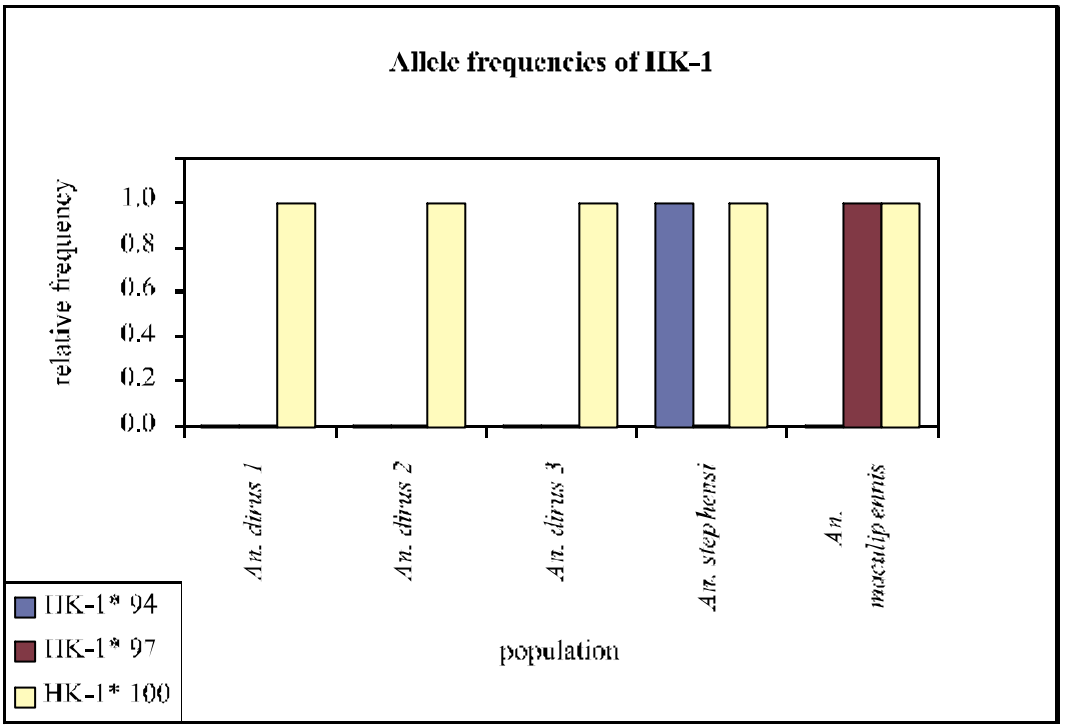
Allele	***HK-1* 94***				***HK-1* 97***				***HK-1* 100***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0	0	0	0	0	30	1.0000	0	0
An. dirus 2	0	0	0	0	0	0	0	0	28	1.0000	0	0
An. dirus 3	0	0	0	0	0	0	0	0	34	1.0000	0	0
An. stephensi	16	1.0000	0	0	0	0	0	0	0	0	0	0
An. maculipennis	0	0	0	0	24	1.0000	0	0	0	0	0	0

Table 3.7. Observed absolute (Abs) and relative (Rel) abundance of ***HK-2*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-Equilibrium using allele frequencies

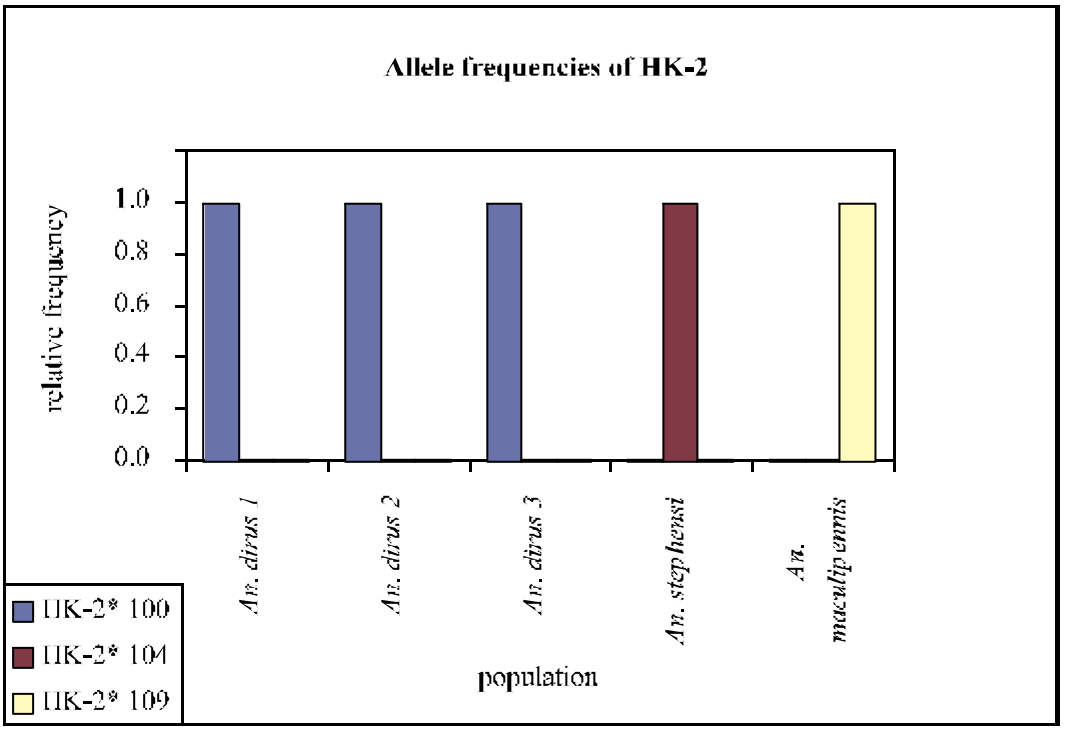
Locus	***HK-2***									
Genotype	***100/100***			***104/104***			***109/109***			**Total**
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	10	1.0000	1.0000	0	0	0	0	0	0	10
An. dirus 2	10	1.0000	1.0000	0	0	0	0	0	0	10
An. dirus 3	12	1.0000	1.0000	0	0	0	0	0	0	12
An. stephensi	0	0	0	8	1.0000	1.0000	0	0	0	8
An. maculipennis	0	0	0	0	0	0	6	1.0000	1.0000	6

Table 3.8. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***HK-2*** in five *Anopheles* populations

Allele	***HK-2* 100***				***HK-2* 104***				***HK-2* 109***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	20	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 2	20	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 3	24	1.0000	0	0	0	0	0	0	0	0	0	0
An. stephensi	0	0	0	0	16	1.0000	0	0	0	0	0	0
An. maculipennis	0	0	0	0	0	0	0	0	12	1.0000	0	0



Graph 3.2. The graph shows the allele frequencies of HK-1* in five populations



Graph 3.3. The graph shows the allele frequencies of HK-2* in five populations

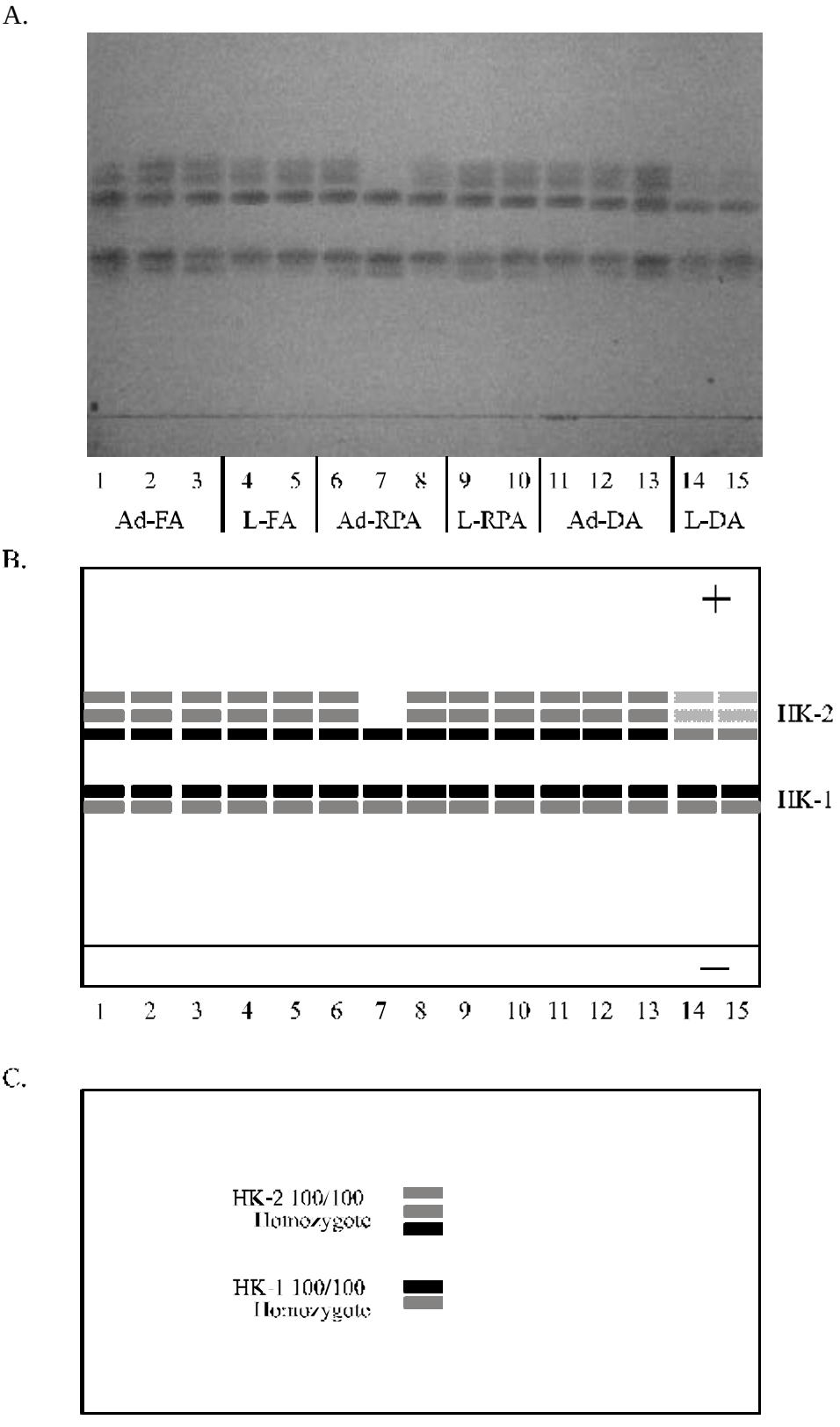


Fig. 3.5. (A) Photograph showing the electrophoretic mobilities of HK in three *An. dirus* populations. (B) Diagrammatic representation of HK enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA= Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

e. Adenylatkinase (AK) **E.C. 2.7.4.3**

Buffer system: number 3, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 55 mA, incubation time for staining at 37°C: 15 min.

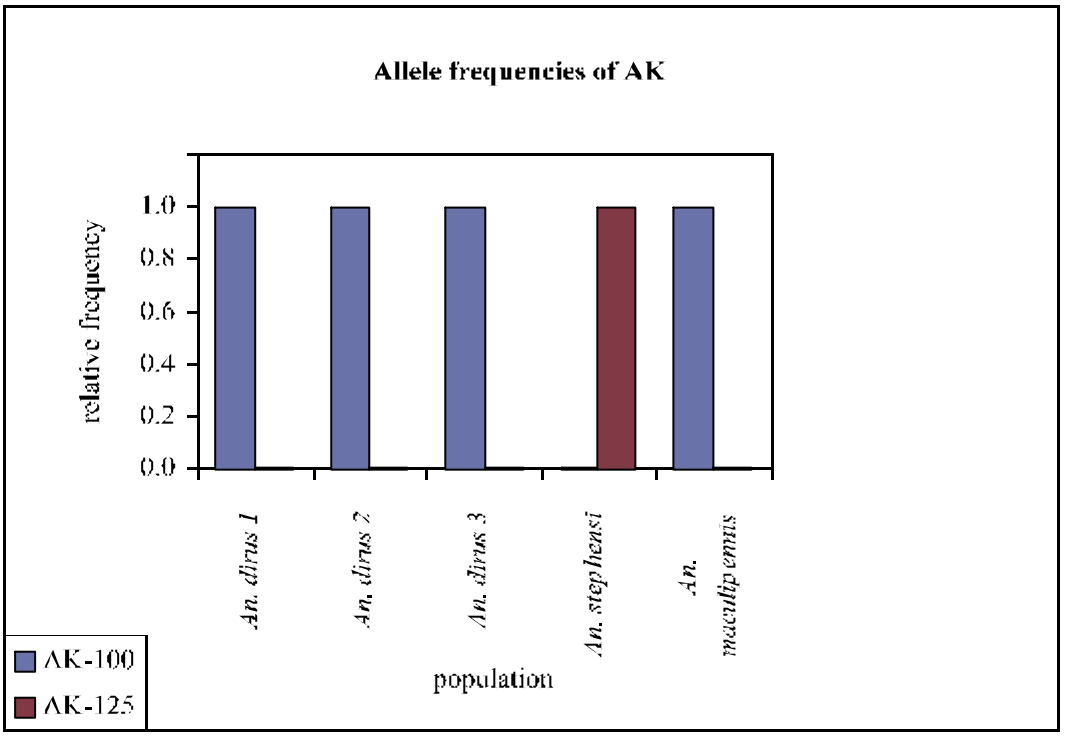
Table 3.9. Observed absolute (Abs) and relative (Rel) abundance of ***AK*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-Equilibrium using allele frequencies

Locus	**AK**						**Total**
Genotype	**100/100**			**125/125**			
Population	Abs.	Rel.	Ex-h	Abs.	Rel.	Ex-h	
An. dirus 1	11	1.0000	1.0000	0	0	0	11
An. dirus 2	5	1.0000	1.0000	0	0	0	5
An. dirus 3	5	1.0000	1.0000	0	0	0	5
An. stephensi	0	0	0	4	1.0000	1.0000	4
An. maculipennis	8	1.0000	1.0000	0	0	0	8

Table 3.10. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***AK*** in five *Anopheles* populations

Allele	***AK-100***				***AK-125***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	10	1.0000	0	0	0	0	0	0
An. dirus 2	10	1.0000	0	0	0	0	0	0
An. dirus 3	22	1.0000	0	0	0	0	0	0
An. stephensi	0	0	0	0	8	1.0000	0	0
An. maculipennis	16	1.0000	0	0	0	0	0	0

The two allozymic variants observed in AK were designated as alleles AK*100 and AK*125 (Fig. 3.6). Allele AK*100 was observed in three populations of *An. dirus* (*An. dirus* 1, *An. dirus* 2 and *An. dirus* 3) and *An. maculipennis* with a frequency of 1.0000. This allele was absent in *An. stephensi.* Instead AK*125 was only observed in *An. stephensi* population exclusively with a frequency of 1.0000 (see Table 3.9, 3.10 and Graph 3.4).



Graph 3.4. The graph shows the allele frequencies of AK* in five populations

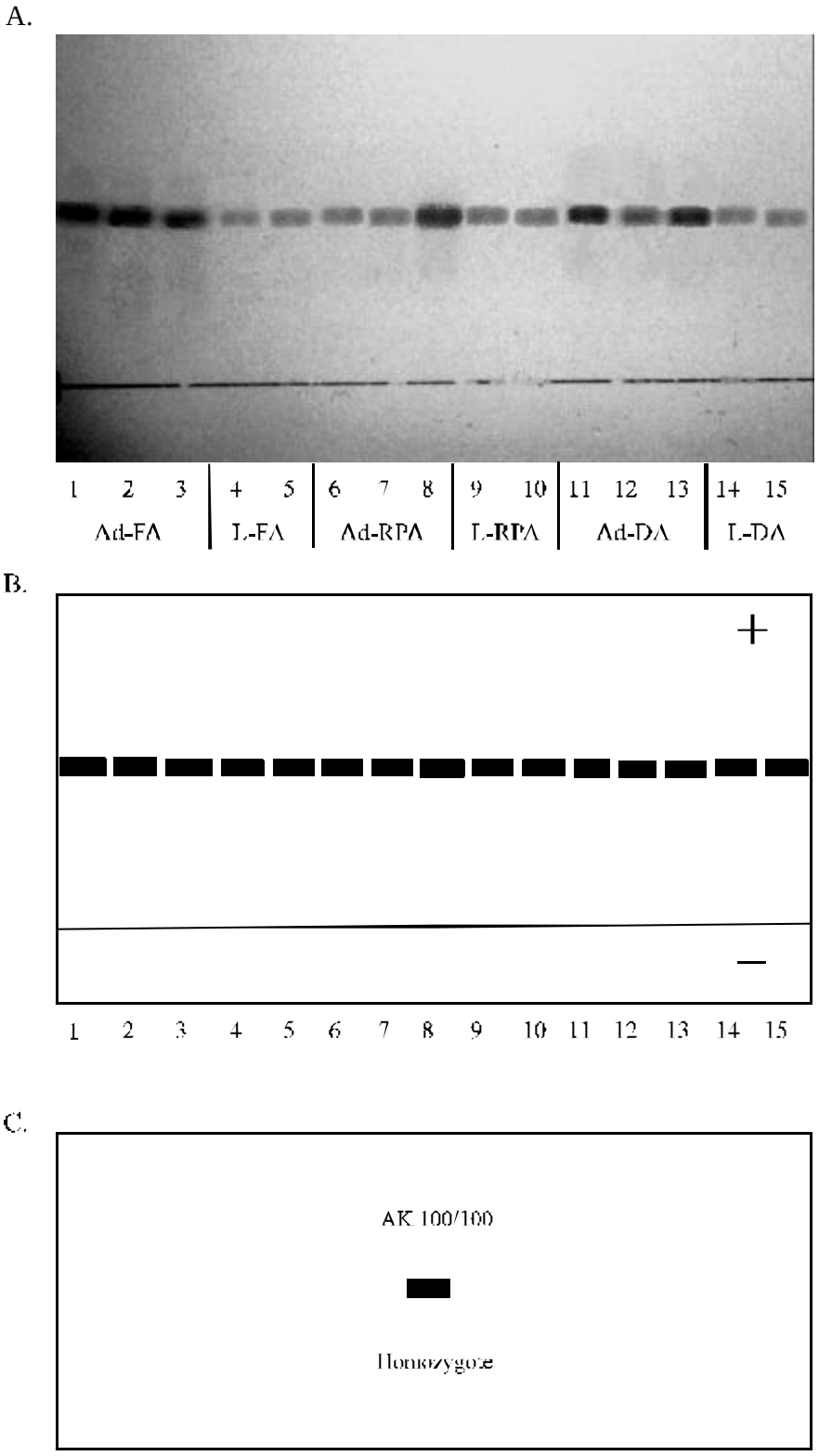


Fig. 3.6. (A) Photograph showing the electrophoretic mobilities of AK in three *An. dirus* populations. (B) Diagrammatic representation of AK enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA= Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

f. Phosphoglucomutase (PGM) E.C. 2.7.5.1

Buffer system: number 2, gel temperature: 4°C running time: 66 min., voltage (limited): 450 V, ampere: 87 mA, incubation time for staining at 37°C: 10 min.

Seven electrophoretic variants depending on only one PGM-gene locus were observed in the three *Anopheles* species (five populations). These were PGM*50, PGM*74, PGM*75, PGM*89, PGM*100, PGM*104 and PGM*117 (Fig. 3.7).

In the *An. maculipennis* population, three different alleles, PGM*50, PGM*75 and PGM*89 were observed. These alleles showed frequencies of 0.0312, 0.7813 and 0.1875 respectively. These alleles were absent in three populations of *An. dirus* (*An. dirus* 1, *An. dirus* 2 and *An. dirus* 3) and *An. stephensi.*

In the *An. stephensi* population, two different alleles were observed. These were PGM*74 and PGM*104 with frequencies of 0.1667 and 0.8333 respectively. However these alleles were not present in *An. dirus* and *An. maculipennis.*

PGM*117 was observed in *An. dirus* 1 (forested) and *An. dirus* 3 (domestic) populations. It showed frequencies of 0.0294 and 0.1191 respectively. However this allele was absent in *An. dirus* 2 (rubber plantation), *An. maculipennis* and *An. stephensi* populations. The most common allele in three populations of *An. dirus* was PGM*100 with relative frequencies of 0.9705, 1.0000 and 0.8809 respectively. This allele PGM*100 was not observed in *An. maculipennis* and *An. stephensi* populations (see Table 3.11, 3.12 and Graph 3.5).

Table 3.11. Observed absolute (Abs) and relative (Rel) abundance of ***PGM*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-Equilibrium using allele frequencies

Locus	***PGM***								
Genotype	***89/89***			***100/100***			***100/117***		
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.
An. dirus 1	0	0	0	16	0.9411	0.9418	1	0.0588	0.0571
An. dirus 2	0	0	0	17	1.0000	1.0000	0	0	0
An. dirus 3	0	0	0	16	0.7619	0.7760	5	0.2380	0.2097
An. stephensi	0	0	0	0	0	0	0	0	0
An. maculipennis	0	0.0000	0.0352	0	0	0	0	0	0

continuation

Locus	***PGM***						
Genotype	***104/104***			***117/117***			**Total**
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	0	0	0	17
An. dirus 2	0	0	0	0	0	0	17
An. dirus 3	0	0	0	0	0	0.0142	21
An. stephensi	4	0.6666	0.6944	0	0	0	6
An. maculipennis	0	0	0	0	0	0	16

Table 3.12. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***PGM*** in five *Anopheles* populations

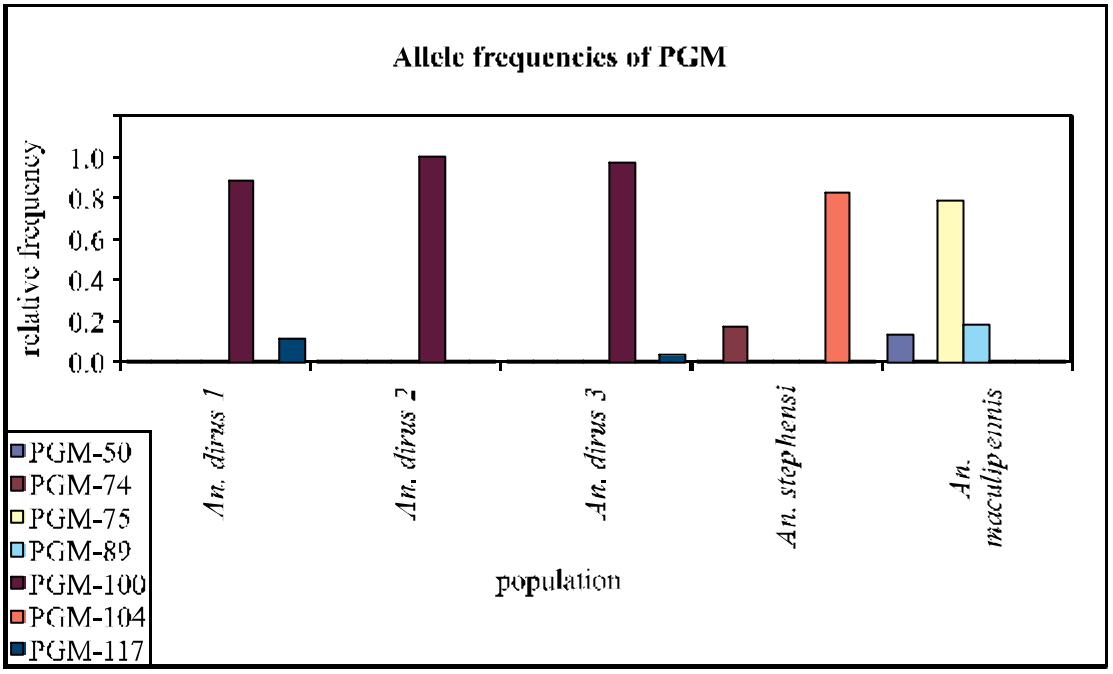
Allele	***PGM-50***				***PGM-74***				***PGM-75***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0	0	0	0	0	0	0	0	0
An. dirus 2	0	0	0	0	0	0	0	0	0	0	0	0
An. dirus 3	0	0	0	0	0	0	0	0	0	0	0	0
An. stephensi	0	0	0	0	2	0.1667	0.2151	0.0116	0	0	0	0
An. maculipennis	1	0.0312	0.06	0.0009	0	0	0	0	25	0.7813	0.1456	0.0053

continuation

Allele	***PGM-89***				***PGM-100***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0	33	0.9705	0.0566	0.0008
An. dirus 2	0	0	0	0	34	1.0000	0	0
An. dirus 3	0	0	0	0	37	0.8809	0.1	0.0025
An. stephensi	0	0	0	0	0	0	0	0
An. maculipennis	6	0.1875	0.1386	0.0048	0	0	0	0

continuation

Allele	***PGM-104***				***PGM-117***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0	1	0.0294	0.0566	0.0008
An. dirus 2	0	0	0	0	0	0	0	0
An. dirus 3	0	0	0	0	5	0.1191	0.1000	0.0025
An. stephensi	10	0.8333	0.2154	0.0116	0	0	0	0
An. maculipennis	0	0	0	0	0	0	0	0



Graph 3.5. The graph shows the allele frequencies of PGM* in five populations

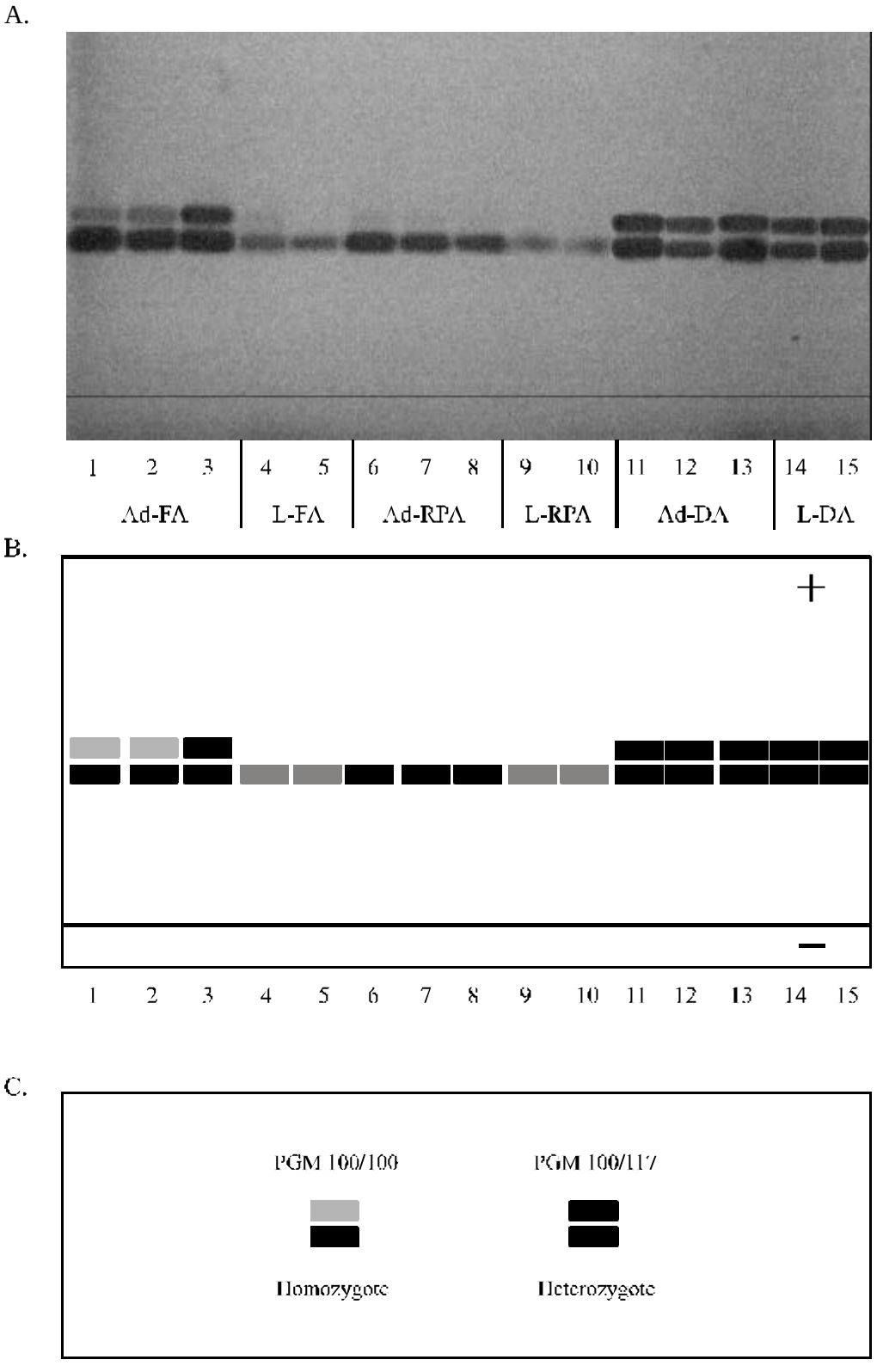


Fig. 3.7. (A) A photo which shows the electrophoretic mobilities of PGM in three *An. dirus* populations. (B) Diagrammatic representation of PGM enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

g. Glycerin-3-phosphate dehydrogenase (G3PDH) E.C. 1.1.1.8

Buffer system: number 3, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 51 mA, incubation time for staining at 37°C: 15 min.

Four electrophoretic variants were observed in G3PDH representing four different alleles in the three *Anopheles* species. These were G3PDH*100, G3PDH*117, G3PDH*130 and G3PDH*136 (Fig. 3.8). In three different populations of *An. dirus,* only one G3PDH*100 was observed with a frequency of 1.0000, although this allele was also present both in *An. maculipennis* and *An. stephensi* populations. G3PDH*117 and G3PDH*136 were observed only in *An. maculipennis* with a frequencies of 0.2500 respectively. These alleles were not present in the other populations. G3PDH*130 was observed only in *An. stephensi* with a frequency of 0.3333 (see Table 3.13, 3.14 and Graph 3.6).

Table 3.13. Observed absolute (Abs) and relative (Rel) abundance of ***G3PDH*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-Equilibrium using allele frequencies

Locus	***G3PDH***								
Genotype	***100/100***			***117/117***			***117/136***		
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.
An. dirus 1	15	1.0000	1.0000	0	0	0	0	0	0
An. dirus 2	15	1.0000	1.0000	0	0	0	0	0	0
An. dirus 3	15	1.0000	1.0000	0	0	0	0	0	0
An. stephensi	4	0.6666	0.4444	0	0	0	0	0	0
An. maculipennis	2	0.5000	0.2500	0	0	0.0625	2	0.5000	0.1250

continuation

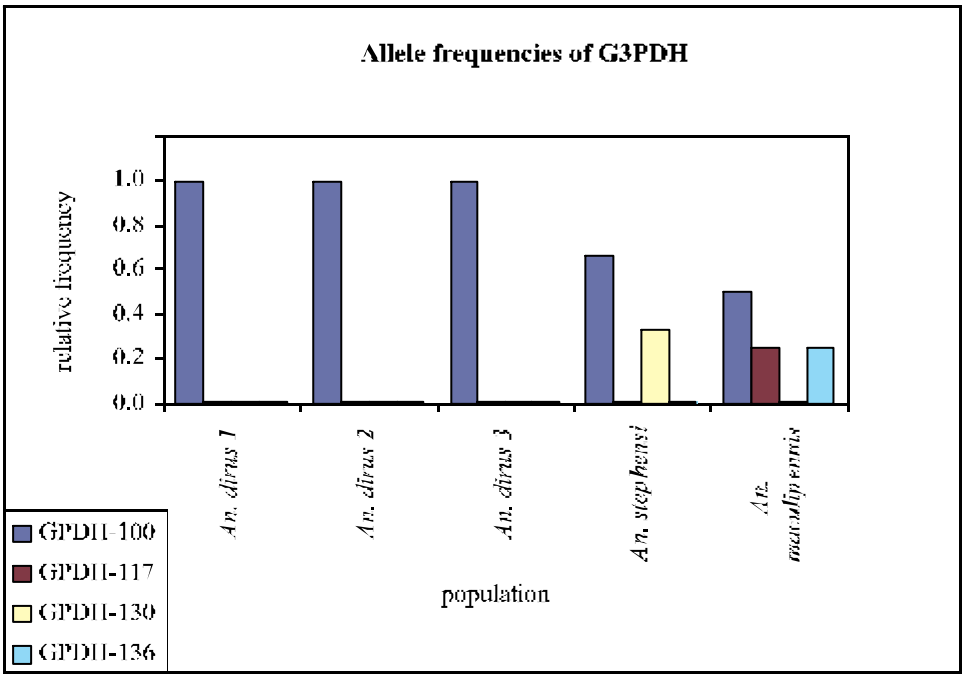
Locus	***G3PDH***						**Total**
Genotype	***130/130***			***136/136***			
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	0	0	0	15
An. dirus 2	0	0	0	0	0	0	15
An. dirus 3	0	0	0	0	0	0	15
An. stephensi	2	0.3333	0.1110	0	0	0	6
An. maculipennis	0	0	0	0	0	0.0625	4

Table 3.14. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***G3PDH*** in five *Anopheles* populations

Allele	***G3PDH-100***				***G3PDH-117***				***G3PDH-130***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	30	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 2	30	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 3	30	1.0000	0	0	0	0	0	0	0	0	0	0
An. stephensi	8	0.6667	0.2722	0.0185	0	0	0	0	4	0.3333	0.2722	0.0185
An. maculipennis	4	0.5000	0.3538	0.0313	2	0.2500	0.3062	0.0234	0	0	0	0

continuation

Allele	***G3PDH-136***			
Population	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0
An. dirus 2	0	0	0	0
An. dirus 3	0	0	0	0
An. stephensi	0	0	0	0
An. maculipennis	2	0.2500	0.0306	0.0234



Graph 3.6. The graph shows the allele frequencies of G3PDH* in five populations

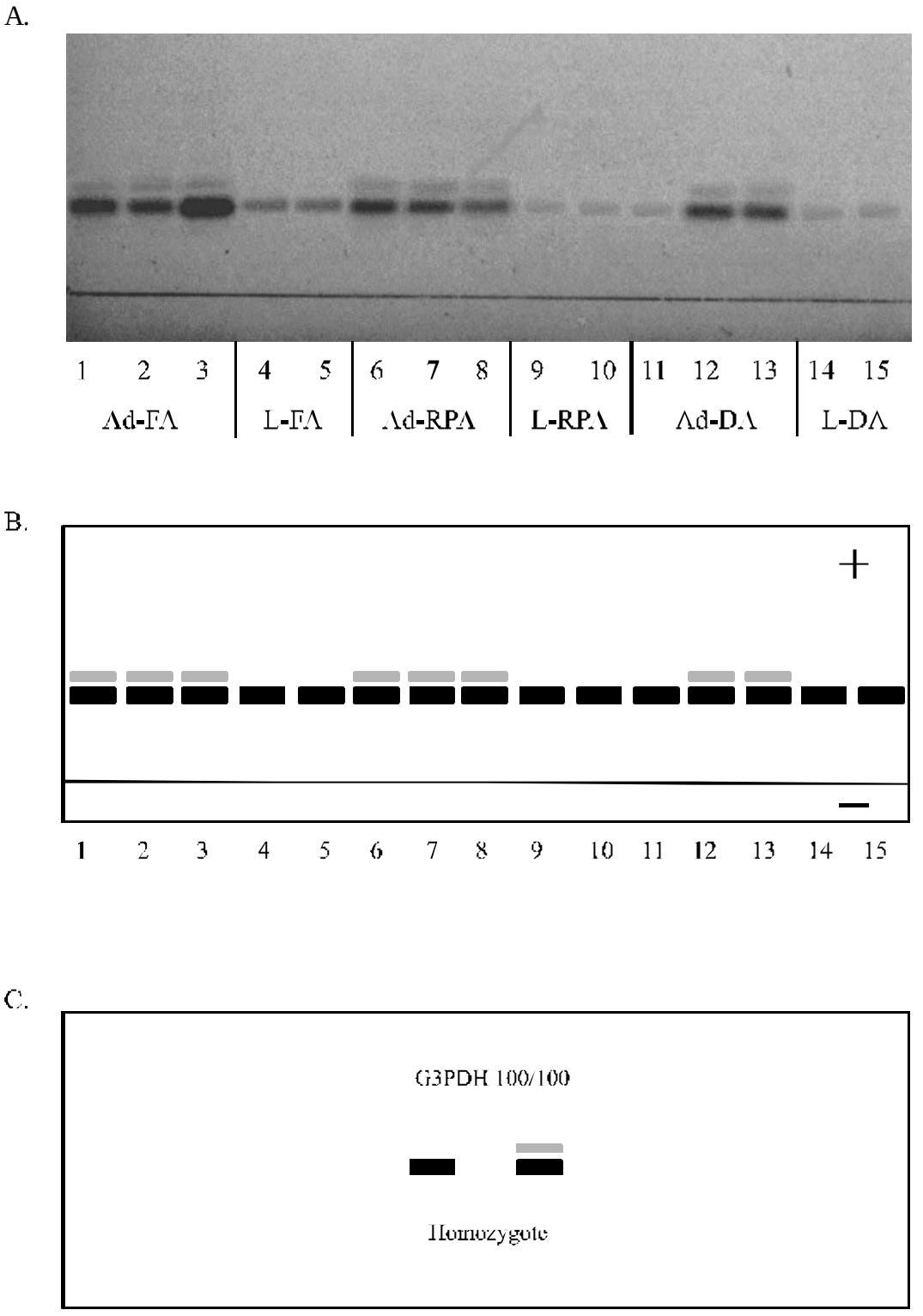


Fig. 3.8. (A) Photograph showing the electrophoretic mobilities of G3PDH in three *An. dirus* populations. (B) Diagrammatic representation of G3PDH enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

h. Hydroxybutyrate dehydrogenase (HBDH) E.C. 1.1.1.30

Buffer system: number 2, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 77 mA, incubation time for staning at 37°C: 15 min.

In HBDH four allozymic variants were observed. They were designated as alleles HBDH*100, HBDH*108, HBDH*150 and HBDH*174 (Fig. 3.9). Allele HBDH*100 was found only in the *An. dirus* (*An. dirus* 1, *An. dirus* 2 and *An. dirus* 3) populations with frequency of 1.0000. This allele was not present in the other populations. HBDH*108 and HBDH*174 were observed only in *An. maculipennis* with frequencies of 0.8333 and 0.1667 respectively. HBDH*150 was observed only in *An. stephensi* population (private allele, genetic marker) with a frequency of 1.0000. (Table 3.15, 3.16 and Graph 3.7).

Table 3.15. Observed absolute (Abs) and relative (Rel) abundance of ***HBDH*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-equilibrium using allele frequencies

Locus	***HBDH***								
Genotype	***100/100***			***108/108***			***108/174***		
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.
An. dirus 1	10	1.0000	1.0000	0	0	0	0	0	0
An. dirus 2	10	1.0000	1.0000	0	0	0	0	0	0
An. dirus 3	14	1.0000	1.0000	0	0	0	0	0	0
An. stephensi	0	0	0	0	0	0	0	0	0
An. maculipennis	0	0	0	4	0.6666	0.6944	2	0.3333	0.2778

continuation

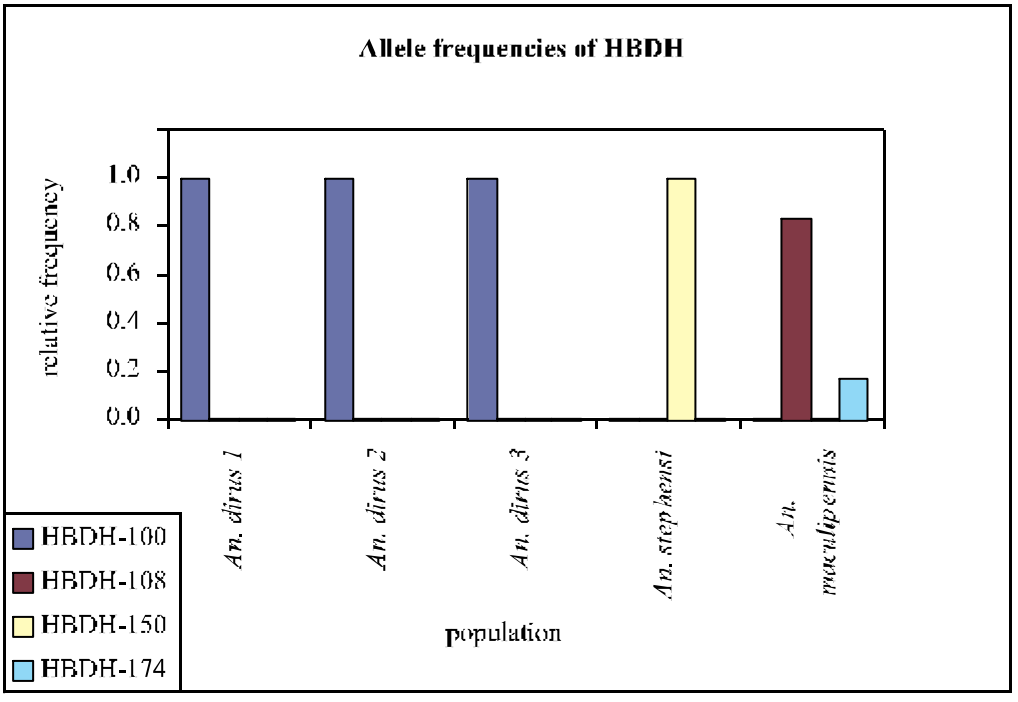
Locus	***HBDH***						**Total**
Genotype	***150/150***			***174/174***			
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	0	0	0	10
An. dirus 2	0	0	0	0	0	0	10
An. dirus 3	0	0	0	0	0	0	14
An. stephensi	6	1.0000	1.0000	0	0	0	6
An. maculipennis	0	0	0	0	0	0.0277	6

Table 3.16. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***HBDH*** in five *Anopheles* populations

Allele	***HBDH-100***				***HBDH-108***				***HBDH-150***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	20	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 2	20	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 3	28	1.0000	0	0	0	0	0	0	0	0	0	0
An. stephensi	0	0	0	0	0	0	0	0	12	1.0000	0	0
An. maculipennis	0	0	0	0	10	0.8333	0.2154	0.0116	0	0	0	0

continuation

Allele	***HBDH-174***			
Population	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0
An. dirus 2	0	0	0	0
An. dirus 3	0	0	0	0
An. stephensi	0	0	0	0
An. maculipennis	2	0.1667	0.2152	0.0115



Graph 3.7. The graph shows the allele frequencies of HBDH* in five populations

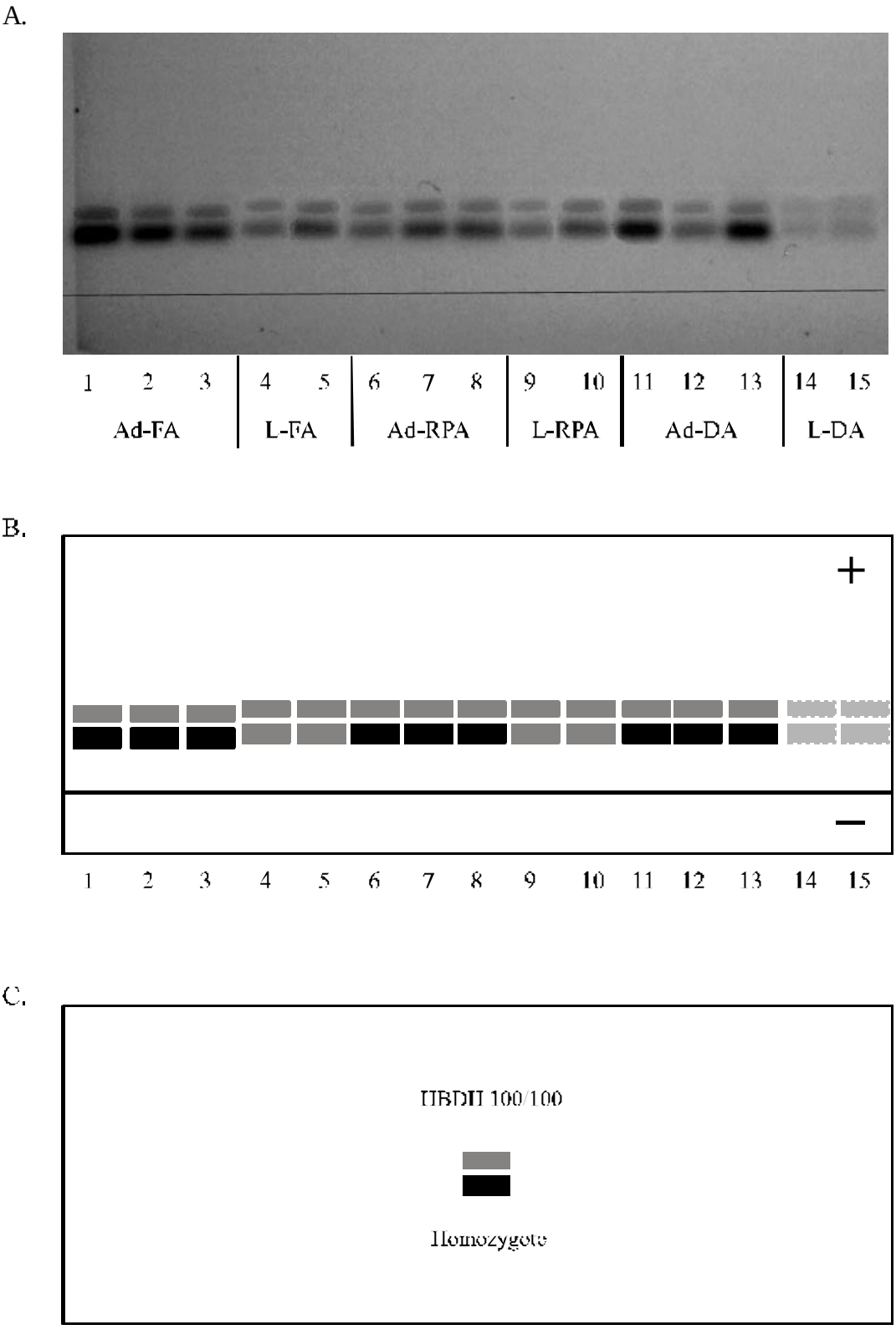


Fig. 3.9. (A) Photograph showing the electrophoretic mobilities of HBDH* in three *An. dirus* populations. (B) Diagrammatic representation of HBDH* enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

i. Malat dehydrogenase (MDH_{NAD}) **E.C. 1.1.1.37**

Buffer system: number3, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 77 mA, incubation time for staning at 37°C: 15 min.

Table 3.17. Observed absolute (Abs) and relative (Rel) abundance of ***MDH_{NAD}*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-equilibrium using allele frequencies

Locus	***MDH_{NAD}***								
Genotype	***85/85***			***90/90***			***100/100***		
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.
An. dirus 1	0	0	0	0	0	0	8	1.0000	1.0000
An. dirus 2	0	0	0	0	0	0	8	1.0000	1.0000
An. dirus 3	0	0	0	0	0	0	9	0.8181	0.8262
An. stephensi	0	0	0	4	1.0000	1.0000	0	0	0
An. maculipennis	12	1.0000	1.0000	0	0	0	0	0	0

continuation

Locus	***MDH_{NAD}***						**Total**
Genotype	***100/115***			***115/115***			
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	0	0	0	8
An. dirus 2	0	0	0	0	0	0	8
An. dirus 3	2	0.1818	0.1653	0	0	0.0083	11
An. stephensi	0	0	0	0	0	0	4
An. maculipennis	0	0	0	0	0	0	12

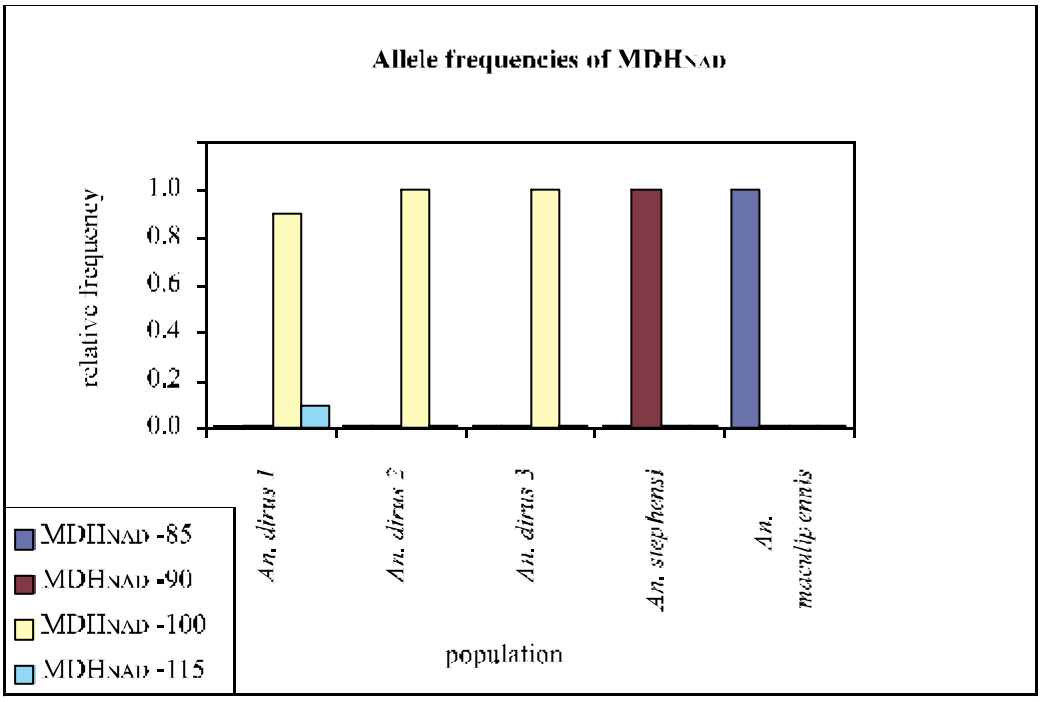
Table 3.18. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus MDH_{NAD} in five *Anopheles* populations

Allele	$MDH_{NAD\ -85}$				$MDH_{NAD\ -90}$				$MDH_{NAD\ -100}$			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0	0	0	0	0	16	1.0000	0	0
An. dirus 2	0	0	0	0	0	0	0	0	16	1.0000	0	0
An. dirus 3	0	0	0	0	0	0	0	0	20	0.9091	0.1233	0.0038
An. stephensi	0	0	0	0	8	1.0000	0	0	0	0	0	0
An. maculipennis	24	1.0000	0	0	0	0	0	0	0	0	0	0

continuation

Allele	$MDH_{NAD\ -115}$			
Population	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0
An. dirus 2	0	0	0	0
An. dirus 3	2	0.0909	0.1233	0.0038
An. stephensi	0	0	0	0
An. maculipennis	0	0	0	0

In MDH_{NAD}, four alleles were observed in the samples. These were MDH_{NAD}*85, MDH_{NAD}*90, MDH_{NAD}*100 and MDH_{NAD}*115 (Fig. 3.10). Allele MDH_{NAD}*85 appeared only in the sample of *An. maculipennis* population with a frequency of 1.0000. It was missing in populations of *An. dirus* 1, *An. dirus* 2, *An. dirus* 3 and *An. stephensi.* In *An. stephensi* population, MDH_{NAD}*90 was observed but was not present in other populations. This allele showed relative frequencies of 1.0000. The most common allele in three different *An. dirus* populations, MDH_{NAD}*100 was observed in *An. dirus* 1, *An. dirus* 2 and *An. dirus* 3 with frequencies of 1.0000, 1.0000 and 0.9091 respectively. However, in a population of *An. dirus* 3 (from domestic area) allele MDH_{NAD}*115 was also observed and this allele showed a frequency of 0.0909. Alleles MDH_{NAD}*100 and MDH_{NAD}*115 were not observed in *An. maculipennis* and *An. stephensi* populations (Table 3.17, 3.18 and Graph 3.8).



Graph 3.8. The graph shows the allele frequencies of MDH_{NAD} in five populations

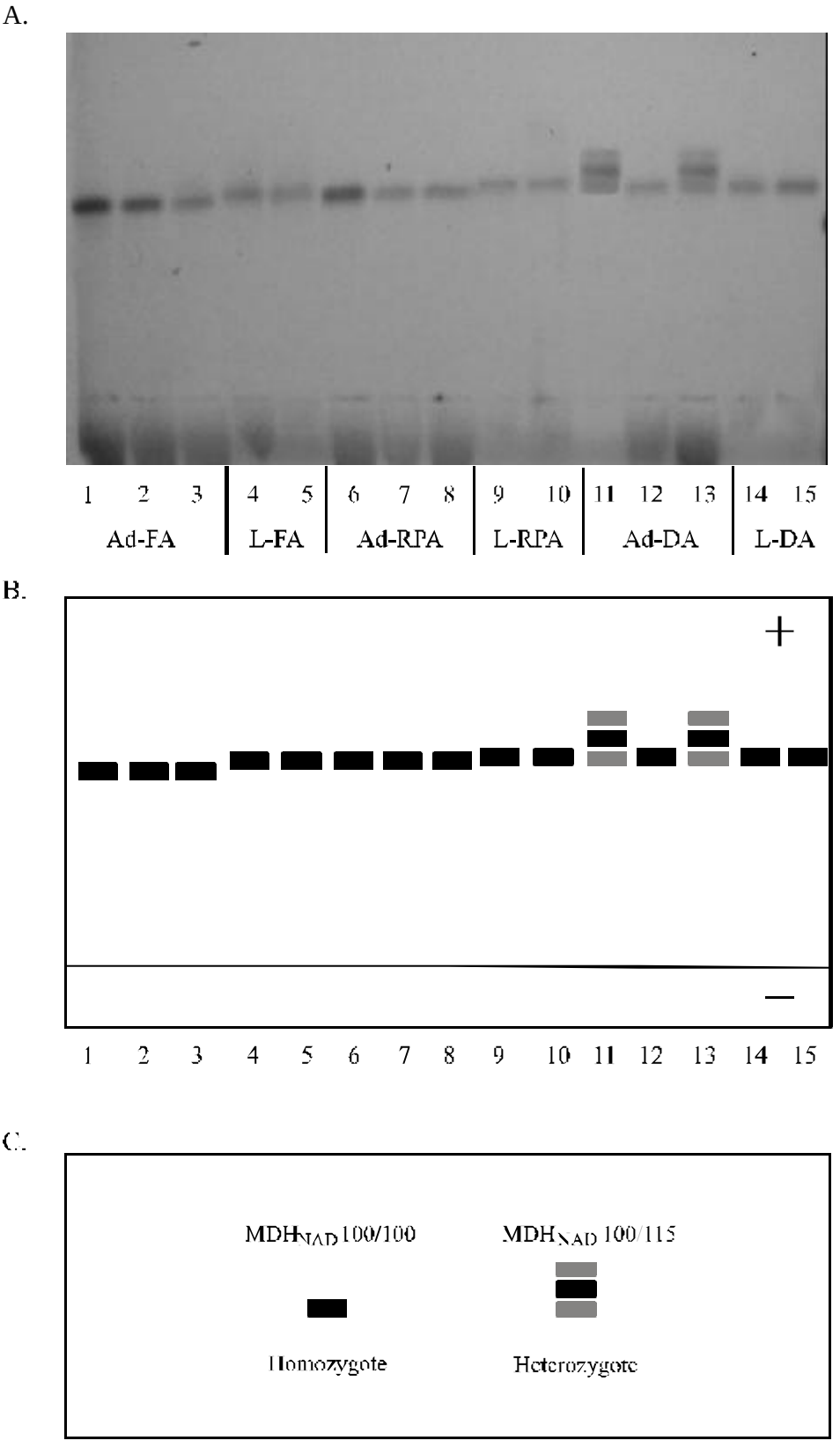


Fig. 3.10. (A) Photograph showing the electrophoretic mobilities of MDH_{NAD} in three *An. dirus* populations. (B) Diagrammatic representation of MDH_{NAD} enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

j. Isocitric dehydrogenase (IDH) E.C. 1.1.1.42

Buffer system: number 3, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 40 mA, incubation time for staning at 37°C: 1 hour.

Isocitrat dehydrogenase (IDH) enzyme is visualized in *Anopheles* mosquitoes by two gene-loci, IDH-1* and IDH-2*.

In the samples that were tested, IDH-1* could not be observed with good (consistent) results and the bands were also not clear in the photographs. IDH-1* appeared in *An. maculipennis* and *An. stephensi* populations but enzyme activity was too weak in the samples of the three different *An. dirus* populations.

Table 3.19. Observed absolute (Abs) and relative (Rel) abundance of ***IDH-2*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-equilibrium using allele frequencies

Locus	***IDH-2***								
Genotype	***157/157***			***157/183***			***160/160***		
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.
An. dirus 1	0	0	0	0	0	0	0	0	0
An. dirus 2	0	0	0	0	0	0	0	0	0
An. dirus 3	0	0	0	0	0	0	0	0	0
An. stephensi	4	0.6666	0.6943	2	0.3333	0.2778	0	0	0
An. maculipennis	0	0	0	0	0	0	0	0	0.0017

continuation

Locus	***IDH-2***			**Total**
Genotype	***183/183***			
Population	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	12
An. dirus 2	0	0	0	12
An. dirus 3	0	0	0	14
An. stephensi	0	0	0.0278	6
An. maculipennis	0	0	0	12

Table 3.20. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***IDH-2***in five *Anopheles* populations

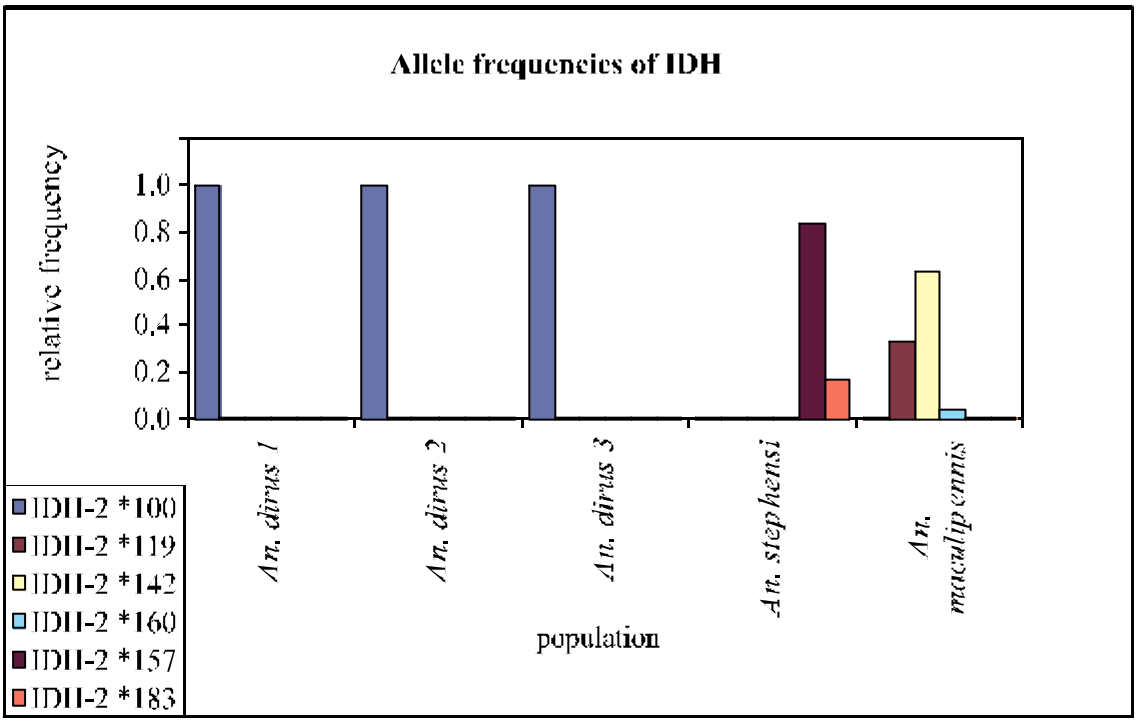
Allele	***IDH-2 *100***				***IDH-2 *119***				***IDH-2 *142***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	24	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 2	24	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 3	28	1.0000	0	0	0	0	0	0	0	0	0	0
An. stephensi	0	0	0	0	0	0	0	0	0	0	0	0
An. maculipennis	0	0	0	0	8	0.3333	0.1929	0.0093	15	0.6250	0.1980	0.0098

continuation

Allele	***IDH-2 *157***				***IDH-2 *160***				***IDH-2 *183***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	0	0	0	0	0	0	0	0	0	0	0	0
An. dirus 2	0	0	0	0	0	0	0	0	0	0	0	0
An. dirus 3	0	0	0	0	0	0	0	0	0	0	0	0
An. stephensi	10	0.8333	0.2154	0.0116	0	0	0	0	2	0.1667	0.2154	0.0116
An. maculipennis	0	0	0	0	1	0.0417	0.08	0.0016	0	0	0	0

At the gene locus IDH-2*, six allozymic variants representing six alleles were detected. These were IDH-2*100, IDH-2*119, IDH-2*142, IDH-2*157, IDH-2*160 and IDH-2*183 (Fig. 3.11). IDH-2*100 was present only in three different *An. dirus* (*An. dirus* 1, *An. dirus* 2 and *An. dirus* 3) populations. It was absent in the other populations. This allele showed a frequency of 1.0000 represented by monomorphism banding patterns.

IDH-2*157, and IDH-2*183 were observed only in *An. stephensi* with a frequencies of 0.8333 and 0.1667 respectively. These alleles were not present in other populations. In *An. maculipennis* population, three different alleles were observed. These were IDH-2*119, IDH-2*142, IDH-2*160 with a frequencies of 0.3333, 0.6250 and 0.0417 respectively. However these alleles were not present in three populations of *An. dirus* and *An stephensi* populations (Table 3.19, 3.20 and Graph 3.9).



Graph 3.9. The graph shows the allele frequencies of IDH-2* in five populations

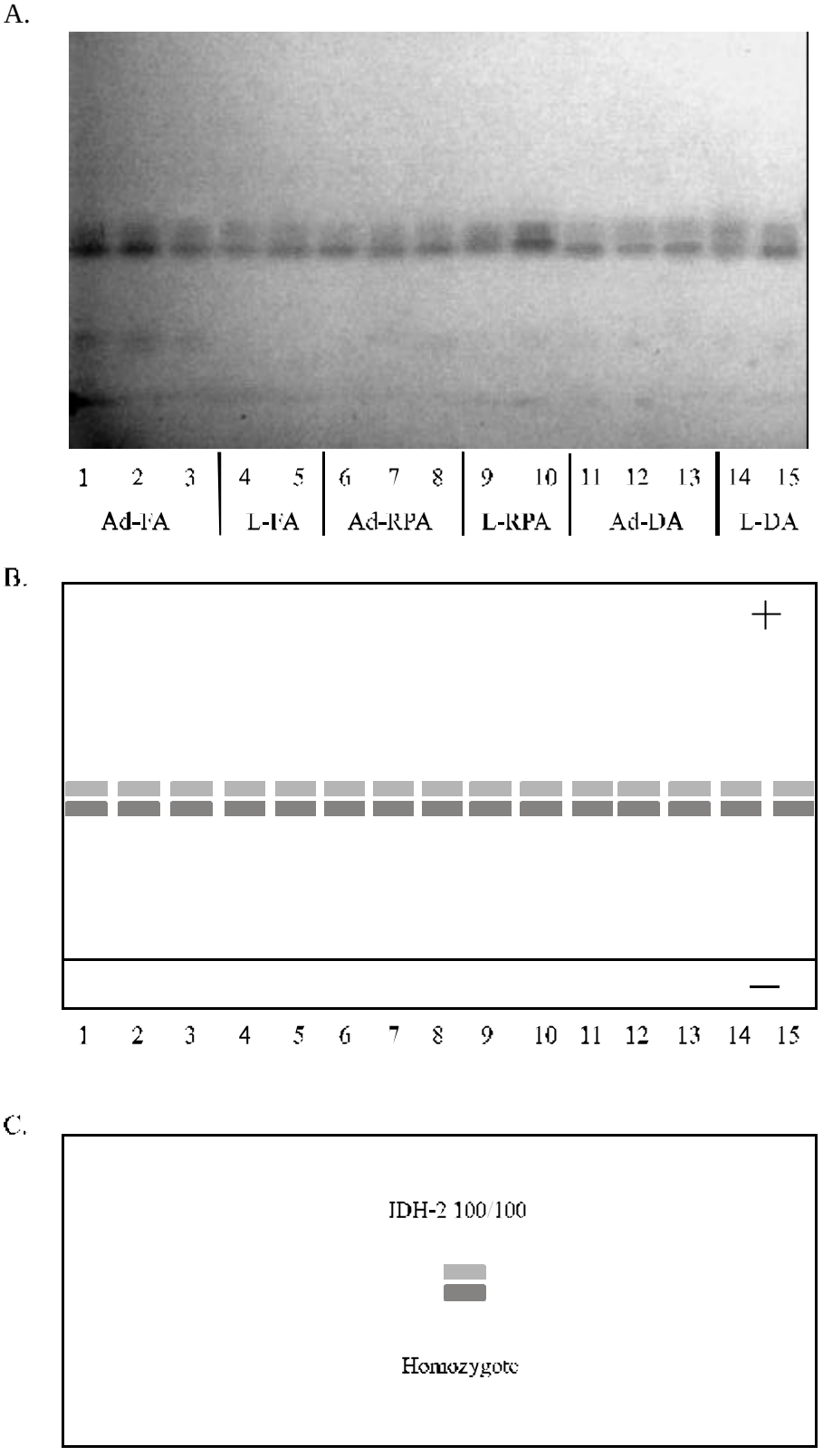


Fig. 3.11. (A) Photograph showing the electrophoretic mobilities of IDH-2 in three *An. dirus* populations. (B) Diagrammatic representation of IDH-2 enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

k. Aldehyde oxidase (Aldox) E.C. 1.2.3.2

Buffer system: number 3, gel temperature: 4°C running time: 60 min., voltage (limited): 500 V, ampere: 77 mA, incubation time for staning at 37°C: 15 min.

Table 3.21. Observed absolute (Abs) and relative (Rel) abundance of ***Aldox*** genotypes compared with expected (Exp) genotype frequencies calculated by following the Hardy-Weinberg-equilibrium using allele frequencies

Locus	***Aldox***								
Genotype	***100/100***			***100/150***			***110/150***		
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.
An. dirus 1	10	1.0000	1.0000	0	0	0	0	0	0
An. dirus 2	10	1.0000	1.0000	0	0	0	0	0	0
An. dirus 3	9	0.8181	0.8263	2	0.1818	0.1653	0	0	0
An. stephensi	2	1.0000	1.0000	0	0	0	0	0	0
An. maculipennis	0	0	0	0	0	0	1	0.2000	0.1800

continuation

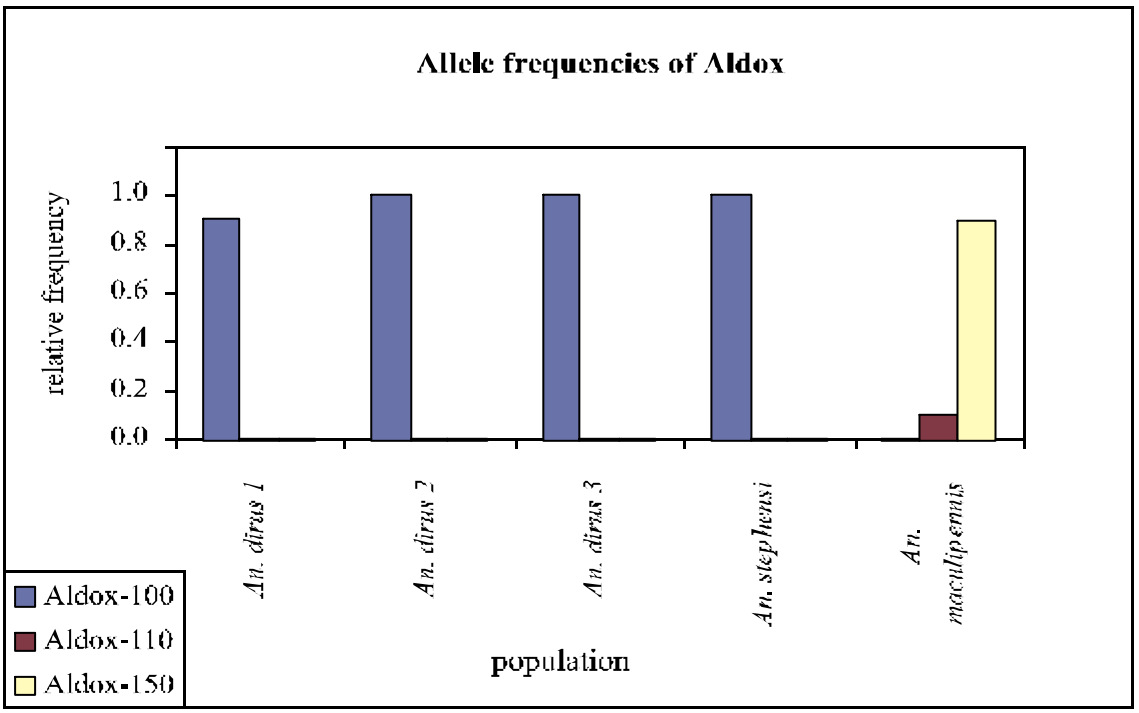
Locus	***Aldox***						**Total**
Genotype	***150/150***			***110/110***			
Population	Abs.	Rel.	Exp.	Abs.	Rel.	Exp.	
An. dirus 1	0	0	0	0	0	0	10
An. dirus 2	0	0	0	0	0	0	10
An. dirus 3	0	0	0	0	0	0	11
An. stephensi	0	0	0	0	0	0	2
An. maculipennis	4	0.8000	0.8100	0	0	0.0100	5

At the Aldox-gene-locus three alleles were observed, Aldox*100, Aldox*110 and Aldox*150 (Fig. 3.12). The most common Aldox*-allele of the *Anopheles* populations, Aldox*100 was detected in three different *An. dirus* (*An. dirus* 1, *An. dirus* 2 and *An. dirus* 3) populations and *An. stephensi* population with frequencies of 1.0000, 1.0000, 0.9091 and 1.0000 respectively. However, in the population of *An. dirus* 3 (from domestic area) allele Aldox*150 was also observed with a frequency of 0.0909. Allele Aldox*100 was not present

in the *An. maculipennis* population. However in the *An. maculipennis*, two different alleles were observed namely Aldox*110 and Aldox*150 with frequencies of 0.1000 and 0.9000 respectively. These alleles were not present in three populations of *An. dirus* and *An stephensi* population (Table 3.21, 3.22 and Graph 3.10).

Table 3.22. Absolute (Abs) and relative (Rel) allele frequencies as well as variances (V) and the confidence intervals (VI) of the locus ***Aldox*** in five *Anopheles* populations

Allele	***Aldox-100***				***Aldox-110***				***Aldox-150***			
Population	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.	Abs.	Rel.	V(+ -)	Var.
An. dirus 1	20	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 2	20	1.0000	0	0	0	0	0	0	0	0	0	0
An. dirus 3	20	0.9091	0.1233	0.0038	0	0	0	0	2	0.0909	0.1233	0.0038
An. stephensi	4	1.0000	0	0	0	0	0	0	0	0	0	0
An. maculipennis	0	0	0	0	1	0.1000	0.1898	0.009	9	0.9	0.1898	0.009



Graph 3.10. The graph shows the allele frequencies of Aldox* in five populations

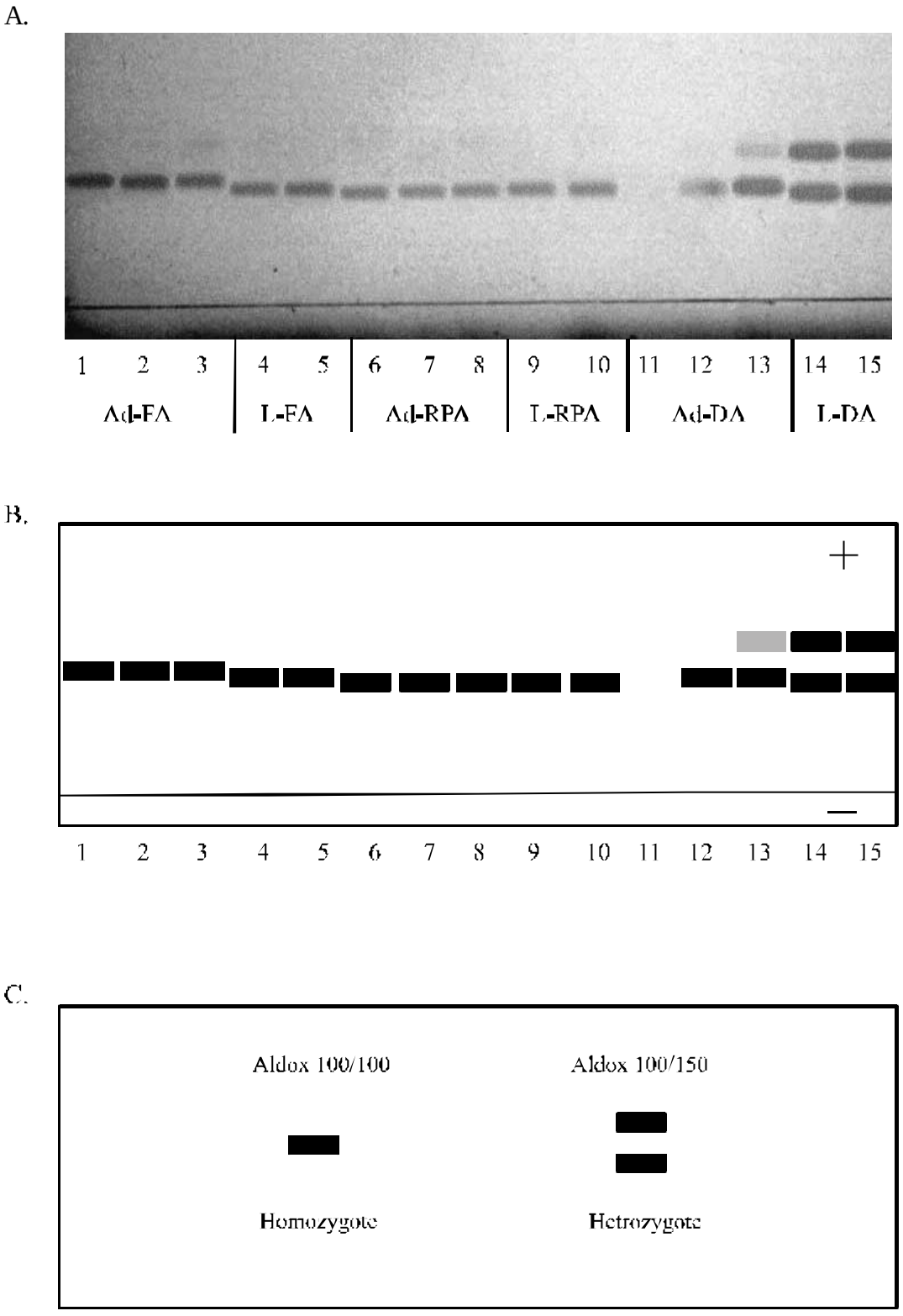


Fig. 3.12. (A) Photograph showing the electrophoretic mobilities of Aldox in three *An. dirus* populations. (B) Diagrammatic representation of Aldox enzyme banding patterns. (C) Isozyme patterns. (Ad-FA=Adult Forested Area, L-FA=Larva Forested Area, Ad-RPA=Adult Rubber Plantation Area, L-RPA=Larva Rubber Plantation Area, Ad-DA=Adult Domestic Area, L-DA=Larva Domestic Area)

3.3.1. Statistical analyses

Tables 3.23, 3.24, 3.25, 3.26 and 3.27 show polymorphism (p) and the degree of heterozygosity (H_L) at the locus estimates for five populations. Tables 3.28, 3.29, 3.30 and 3.31 show comformity to expectations of the Hardy-Weinberg rule with x^2 (chi square)values for population 1 (*An. dirus* from forested area), population 3 (*An. dirus* from domestic area), population 4 (*An. stephensi* from Indonesia) and population 5 (*An. maculipennis* from Germany).

The values of Nei`s standard genetic idenity (I) and genetic distance (D) between five populations are presented in Table 3.32. Polylogenetic dendrograms revealed by the PHYLIP phylogency inference package program based on the Kitsch method is presented in Fig. 3.13.

Table 3.23. Polymorphism (p) and the degree of heterozygosity (H_L) at the locus estimates for population 1 (*An. dirus* from forested area)

Enzyme locus	Allele	Frequencies	Polymorphism (p) or monomorphism (m)	Degree of heterozygosity at the locus (H_L)
MPI	100	1.0000	m	0
HK-1	100	1.0000	m	0
HK-2	100	1.0000	m	0
AK	100	1.0000	m	0
PGM	100	0.9705	p	0.0588
	117	0.0294		
G3PDH	100	1.0000	m	0
HBDH	100	1.0000	m	0
MDH_{NAD}	100	1.0000	m	0
IDH-2	100	1.0000	m	0
Aldox	100	1.0000	m	0
			$P_{\varnothing}$= 1/10=0.1	$H_{\varnothing}$= 0.0005

$P_{\varnothing}$= average number of polymorphism, $H_{\varnothing}$= average number of heterozygosity

Table 3.24. Polymorphism (p) and the degree of heterozygosity at the locus (H_L) estimates for population 2 (*An. dirus* from rubber plantation area)

Enzyme locus	Allele	Frequencies	Polymorphism (p) or monomorphism (m)	Degree of heterozygosity at the locus (H_L)
MPI	100	1.0000	m	0
HK-1	100	1.0000	m	0
HK-2	100	1.0000	m	0
AK	100	1.0000	m	0
PGM	100	1.0000	m	0
G3PDH	100	1.0000	m	0
HBDH	100	1.0000	m	0
MDH_{NAD}	100	1.0000	m	0
IDH-2	100	1.0000	m	0
Aldox	100	1.0000	m	0
			$P_{\varnothing}$= 0/10=0	$H_{\varnothing}$= 0

$P_{\varnothing}$= average number of polymorphism, $H_{\varnothing}$= average number of heterozygosity

Table 3.25. Polymorphism (p) and the degree of heterozygosity at the locus (H_L) estimates for population 3 (*An. dirus* from domestic area)

Enzyme locus	Allele	Frequencies	Polymorphism (p) or monomorphism (m)	Degree of heterozygosity at the locus (H_L)
MPI	100	1.0000	m	0
HK-1	100	1.0000	m	0
HK-2	100	1.0000	m	0
AK	100	1.0000	m	0
PGM	100	0.8809	p	0.2381
	117	0.1191		
G3PDH	100	1.0000	m	0
HBDH	100	1.0000	m	0
MDH_{NAD}	100	0.9090	p	0.1818
	115	0.0909		
IDH-2	100	1.0000	m	0
Aldox	100	0.9090		
	150	0.0909	p	0.1818
			$P_{\varnothing}$= 3/10=0.3	$H_{\varnothing}$= 0.0602

$P_{\varnothing}$= average number of polymorphism, $H_{\varnothing}$= average number of heterozygosity

Table 3.26. Polymorphism (p) and the degree of heterozygosity at the locus (H_L) estimates for population 4 (*An. stephensi* from Indonesia)

Enzyme locus	Allele	Frequencies	Polymorphism (p) or monomorphism (m)	Degree of heterozygosity at the locus (H_L)
MPI	107	1.0000	m	0
HK-1	94	1.0000	m	0
HK-2	104	1.0000	m	0
AK	125	1.0000	m	0
PGM	74	0.1666		
	104	0.8333	p	0.3333
G3PDH	100	0.6666	p	0
	130	0.3333		0
HBDH	150	1.0000	m	0
MDH_{NAD}	100	1.0000	m	0
IDH-2	157	0.8333		
	183	0.1667	p	0.3333
Aldox	100	1.0000	m	0
			$P_{\varnothing}$= 3/10=0.3	$H_{\varnothing}$= 0.0667

$P_{\varnothing}$= average number of polymorphism, $H_{\varnothing}$= average number of heterozygosity

Table 3.27. Polymorphism (p) and the degree of heterozygosity at the locus (H_L) estimates for population 5 (*An. maculipennis* from Germany)

Enzyme locus	Allele	Frequencies	Polymorphism (p) or monomorphism (m)	Degree of heterozygosity at the locus (H_L)
MPI	102	1.0000	m	0
HK-1	97	1.0000	m	0
HK-2	100	1.0000	m	0
AK	100	1.0000	m	0
PGM	50	0.0312	p	0.4375
	75	0.7813		
	89	0.1875		
G3PDH	117	0.2500	p	0.5000
	136	0.2500		
HBDH	108	0.8333	p	0.3333
	174	0.1667		
MDH_{NAD}	100	1.0000	m	0
IDH-2	119	0.3334	p	0.5833
	142	0.6250		
	160	0.0416		
Aldox	110	0.1000	p	0.2000
	150	0.9000		
			$P_\varnothing$= 5/10=0.5	$H_\varnothing$= 0.2054

$P_\varnothing$= average number of Polymorphism, $H_\varnothing$= average number of heterozygosity

Table 3.28. Comformity to expectations of the Hardy-Weinberg rule for population 1 (*An. dirus*, from forested area)

Locus	Genotype	Frequency		X^2
		Observed	Expected	
PGM	100/100	16	15.9970	
	100/117	1	0.9995	
	117/117	0	0.0001	0-3 Heterozygote

Table 3.29. Comformity to expectations of the Hardy-Weinberg rule for population 3 (*An. dirus*, from domestic area)

Locus	Genotype	Frequency		X^2
		Observed	Expected	
PGM	100/100	16	16.2419	
	100/117	5	4.5090	
	117/117	0	0.2437	2-6 Heterozygote
MDH_{NAD}	100/100	9	9.0457	
	100/115	2	1.9043	
	115/115	0	0.0476	1-3 Heterozygote
Aldox	100/100	9	9.0457	
	100/150	2	1.9043	
	150/150	0	0.0476	1-3 Heterozygote

Table 3.30. Comformity to expectations of the Hardy-Weinberg rule for population 4 (*An. stephensi* from Indonesia)

Locus	Genotype	Frequency		X^2
		Observed	Expected	
PGM	74/74	0	0.0908	
	74/104	2	1.8174	
	104/104	4	4.0905	1-3 Heterozygote

Table 3.31. Comformity to expectations of the Hardy-Weinberg rule for population 5 (*An. maculipennis* from Germany)

Locus	Genotype	Frequency		X^2
		Observed	Expected	
PGM	50/50	0	0.2165	
	75/75	9	9.6786	
	50/75	1	0.8052	
	75/89	6	4.8390	
	89/89	0	0.4838	
New frequency was built by *PGM* alleles 50 and 89.				
	50+89/50+89	0	0.7899	
	50+89/75	7	5.6442	
	75/75	9	9.6786	2-8 Heterozygote
G3PDH	117/117	0	0.1428	
	117/136	2	0.5714	
	136/136	0	0.1428	1-3 Heterozygote
HBDH	108/108	4	4.0905	
	108/174	2	1.8184	
	174/174	0	0.0909	1-3 Heterozygote
IDH-2	119/119	1	1.2171	
	119/142	6	5.2169	
	142/142	4	4.5652	
	142/160	1	0.6511	
	160/160	0	0.00003	
New frequency was built by *IDH-2* alleles 119 and 160.				
	119+160/119+160	1	1.5643	
	119+160/142	7	5.8680	
	142/142	4	4.5652	4-10 Heterozygote
Aldox	110/110	0	0	
	110/150	1	1	
	150/150	4	4	

Table 3.32. Genetic identity (I, above the diagonal) and genetic distance (D, below the diagonal) between 5 populations

Population	Population 1 *An. dirus* 1 (forested)	Population 2 *An. dirus* 2 (rubber plantation)	Population 3 *An. dirus* 3 (domestic)	Population 4 *An. stephensi (Indonesia)*	Population 5 *An. maculipennis (Germany)*
Population 1 *An. dirus* 1 (forested area)	*	0.9999	0.9978	0.1761	0.1674
Population 2 *An. dirus* 2 (rubber plantation)	0.0001	*	0.9972	0.1756	0.1670
Population 3 *An. dirus* 3 (domestic area)	0.0022	0.0028	*	0.1707	0.1810
Population 4 *An. stephensi (Indonesia)*	1.7362	1.7391	1.7674	*	0.0391
Population 5 *An. maculipennis (Germany)*	1.7869	1.7897	1.7088	3.2411	*

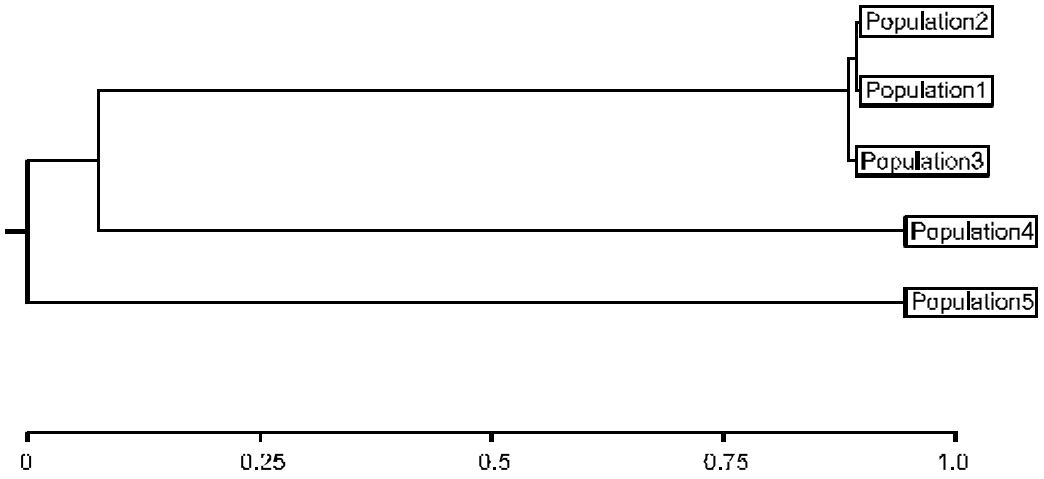


Fig. 3.13. Dendrogram based on Nei`s genetic distance data (Table 3.32) between five populations

3.4. Conclusion and discussion

In recent years, studies have been conducted on the cytogenetica and genetics of *Anopheles.* These studies have been productive and significant progress includes mapping of morphological mutants (Benedict et al., 1979), linkage group-chromosome correlation (Rabbani and Seawright, 1976), induction and characterization of chromosome aberrations (Rabbani and Kitzmiller, 1972, 1975), and synthesis of a genetic sexing system for the preferential elimination of females (Kaiser et al., 1978).

Although *An. dirus* is one species potentially hazardous to public health in Myanmar, there is little information on the population genetics of this species. The formal genetics of esterases in a laboratory strain of *An. dirus* had been reported by Moe Moe (1985) and Myat Myat Thu et al., (1986). However, there was only one biochemical genetic study of *An. dirus* by Htay Aung (1995) and no other malaria vectors in Myanmar had ever been studied.

In the present study, three population samples of *An. dirus* have been researched: *An. dirus* 1 (forest), *An. dirus* 2 (rubber plantation) and *An. dirus* 3 (from domestic location), around Mudon Township, Mon State, were analyzed in eleven gene enzyme systems comprising twelve presumptive loci. The twelve enzymatic loci included the following: (a) Mannosephosphate isomerase (MPI*) (b) glucosephosphate isomerase (GPI*), (c) glutamat-oxalacetat transaminase (GOT*), (d) hexokinase (HK-1*, -2*), (e) adenylatkinase (AK*), (f) phospho-glucomutase (PGM*), (g) glycerin-3-phosphate dehydrogenase (G3PDH*), (h) hydroxybutyrate dehydrogenase (HBDH*), (i) malat dehydrogenase (MDH_{NAD}*), (j) isocitrat dehydrogenase (IDH-2*) and (k) aldehyde oxidase (Aldox*).

The banding patterns of allozymes of each species that were tested in gel electrophoresis were recorded. Based on the data obtained from the electrophoretic migration of enzymes, the following values were computed in each population: relative frequencies of alleles and genotypes, confidence interval and the variances of allele frequencies, degree of heterozygosity and polymorphism and Nei`s genetic distance values between the species.

The results after electrophoretic analysis of the hexokinase (HK) enzyme, exhibited two zones of activity. These zones represented the enzyme loci HK-1* and HK-2*. In HK-1* and HK-2*, there was no polymorphism and thus no differentation between the three different populations of *An. dirus* (of the HK-locus) was found. In each case only one allele was observed and designated as allele HK*100 (HK-1*100 and HK-2*100 respectively). The HK-1* locus showed strong activity. The zymograms of the populations clearly showed that the zone-1- allozymes are controlled by one gene-locus (two loci) in HK-1 and interpreted as a

one-locus- two bands system. In addition to the alleles of HK-2, there were three bands commonly observed in zone 2.

Genetic studies on hexokinase of anopheline species are very limited. Steiner et al., (1979) described the polymorphism for several species from Brazil. They noted three distinct zones of activity for zymograms prepared from adult mosquitoes. The zone located closest to the anode was designated Hk-F, and their data indicated this zone was under the control of an autosomal locus with two co-dominant alleles. A middle zone (Hk-M) consisted of two bands in each homozygous mosquito. Thus Hk-M was interpreted as a one-locus- two bands system. In heterozygotes, three bands was observed. Their slowest, third zone (Hk-S) consisted of a one-gene; one-band type displaying faint hexokinases activity, and three bands observed at this locus indicated two codominant alleles. But this HK-activity-zone was not observed in *An. dirus* populations (probably due to the weak enzymatic activity).

My observations of hexokinases activity in *An. dirus* paralleled those described by Steiner et al., (1979); and Narang et al., (1981), with the important expectation that no "simultaneous heterozygosity" was found in all the *An. dirus* populations.

The enzymatic locus of phosphoglucomutase (PGM), showed biallelic polymorphism in *An.dirus* population, with two co-dominant alleles PGM*100 and PGM*117. Allele PGM*117 was found in *An. dirus* 1 (forest) and *An. dirus* 3 (domestic) respectively, but not in *An. dirus* 2 (rubber plantation) while PGM*100 (fixed allele) was present in all three populations of *An. dirus.*

Allozymes of PGM of *An. dirus* populations have a simple monofactorial inheritance. The quarternary structure of this enzyme results in a monomeric molecule and thus mosquito`s heterozygous at the PGM locus show two bands corresponding to the respective alleles. This one gene-one band situation, typical for anopheline species, holds true in 13 species of neotropical anopheline mosquitoes (Narang et al.,1981). The relatively high level of PGM polymorphism in this species is consistent with similar reports on other mosquito species. Among palearctic anophelines of the subgenus *Anopheles,* a total of six PGM alleles have been reported in sibling species of the *An. maculipennis* complex, with four alleles in *An. plumbeus,* three in the *An. claviger* group, and two alleles in a single sample of the Mediterranean species *An. algeriensis* (Bullini and Coluzzi, 1973) confirming the results of this study of PGM activity in *An. dirus.*

Adenylatkinase (AK) showed only a single allele designated as AK*100. Thus in AK*, there was no polymorphism and no detectable differentation between three different *An. dirus* populations.

The enzymatic loci of mannosephosphate isomerase (MPI) and glucosephosphate isomerase (GPI), showed only one allele in each *An. dirus* populations designated alleles MPI*100 and GPI*100 respectively.

Also the enzymatic locus of glycerin-3-phosphate dehydrogenase (G3PDH) there was no differentation between three *An. dirus* populations because only one allele was observed and designated as allele G3PDH*100. The hydroxybutyrate dehydrogenase (HBDH) enzyme showed the similar results as G3PDH with one fixed allele HBDH*100.

In malat dehydrogenase (MDH_{NAD}), two allozymes were observed, representing the allele MDH_{NAD}*100 and the rare one, MDH_{NAD}*115. MDH_{NAD}*115 was found only in *An. dirus* 3 (domestic), while MDH_{NAD}*100 was present in all three populations. Consequently, only in *An. dirus* 3, was heterozygosity observed at this locus.

The enzyme locus isocitrat dehydrogenase (IDH*) showed two zones of activity representing two presumptive IDH-loci, but only one of them exhibited a clear banding pattern. Only one allele IDH-2*100 was observed in *An. dirus* populations and thus the homozygous genotype IDH-2* 100/100 was present in all of the *An. dirus* populations.

In the electrophoresis of aldehyde oxidase (Aldox), two allozymes were observed, representing the alleles Aldox*100 and Aldox*150. There were two genotypes observed, namely; Aldox 100/100 and Aldox 100/150.

Three loci (PGM*, MDH_{NAD}* and Aldox*) were indicated polymorphic in *An. dirus* populations.

Genetic distance values were characteristic of those observed among local conspecific populations of invertebrates (Ayala, 1975), indicating considerable gene flow even among geographically remote populations. Therefore, according to Barr`s criteria (1982), *An. dirus* populations around the Mudon area cannot be considered to be specifically distinct, but rather should be regarded as a collection of interbreeding populations. Interestingly, the isozyme profiles observed from this study resembled those of Italian populations of *Cx. pipiens* (Villani et al., 1986) and Egyptian populations of *Cx. pipiens* (Farid et al., 1991). The *An. dirus* populations were only slightly different in this study. This finding was in agreement with Hassan (1988).

In the present study, the average genetic distance values between any two of the three populations studied was less than 0.005. This value is similar to those of Yong et al., (1981) and Htay Aung (1995). Tabachnick and Powell (1979) also gave a similar report for the yellow fever mosquito *Aedes aegypti.* As in the case of *Aedes aegypti*, the genetic distance

(D) values between taxa are small when compared to similar values obtained in species of *Drosophila* and other organisms (Ayala, 1975).

An important application of multilocus electrophoretic techniques is their use in taxonomy and the evidence that they provide for phylogenetic interpretation. One reason why enzymatic characters are so useful in phylogenetic studies is that they generally reflect homologous conditions. For most loci which are now studied electrophoretically, common function generally implies common origin (Avise, 1974). Moreover, for most structural genes investigated by electrophoretic means, evolution by allelic substitution at the codon level is a slow process. The probability of back mutations or parallel mutations at a codon is negligibly small, unless evolutionary time is very large.

Another advantage is that it is possible to use many independent characters whose genetic bases are known. This permits the application of a phenetic taxonomic procedure to characters which have a phylogenetic value. Finally the degrees of genetic similarity or distance between species can be correlated to evolutionary time, and this property further improves phylogenetic analysis.

The method most commonly used to express synthetically the degrees of similarity in a complex of conspecific and heterospecific populations is the construction of bidimensional dendrograms (tree diagrams). There is a vast literature on methods of constructing such dendrograms (Sneath and Sokal, 1973) and on their relevance in phylogenetic analysis (Platnick, 1977).

To construct a dendrogram of this type, it is necessary to begin from a matrix of genetic distances as illustrated in Table 3.32. Phylogenetic relationships between the respective population pairs among the five gene pools were demonstrated by dendrograms using the Kitsch program (Felsenstein, 2002), presented in Fig.3.13. This dendrogram clustered the populations in two forms; three *An. dirus* population groups together in two groups. The first group (population 1, *An. dirus* from forested area and population 2, *An. dirus* from rubber plantation area) to be clustered are those with the smallest genetic distance. Populations of second group are developed from the first group and population 3 (*An. dirus* from domestic location). These two groups of *An. dirus* are then combined and taken to be a single group. The populations of third group entity are from another cluster for *An. dirus* combined group and population 4 (*An. stephensi* from Indonesia). New estimates of genetic distance between this combined group and other groups are then calculated (Nei, 1975).

The dendrogram can be used to extract two types of information: one, absolute, which indicates the actual distances among the various taxa on the basis of their positions and those

of the various nodes on the scale of genetic distance; the other, relative, which considers the position of a particular taxon in a given cluster. Some phylogenetic inference is derived mainly from the second type of information, which gives more precise indications of phyletic relationships within a certain group, provided that the dendrogram includes an exhaustive sample of the taxa existing within the group. It should be pointed out that a dendrogram cannot be stricly superimposed on a phyletic tree, in that cladograms reflect only degrees of relationships (e.g. closest relatives), while phylogenetic trees attempt to detect ancestor-descendant relationships by specifying either available or hypothetical taxa as ancestors.

When considering the congruence between electrophoretic and morphological data, it should be remembered that electrophoresis is a means of obtaining data and not a way to systematics. For this reason it is not quite correct to speak of the electrophoretic approach to systematics as opposed to the morphological. A fully reliable comparison should be made only by considering the two sets of data (i.e. allozymic and morphometric) in the same taxonomic group, analyzed by the same procedure.

The available evidence shows that agreement generally exists between electrophoretic data and morphological characters in establishing systematic relationships. In several instances, the arrangements based on gene-enzymes studied closely correspond to relationships previously recognized on morphological or karyological bases (Avise, 1974; Mickevich and Johnson, 1976).

The relationship between three populations of *An. dirus* is reinforced by similar morphology and complete genetic compatibility (Graham et al., 1972; Thompson et al., 1981). There were no significant differences between these three populations of *An. dirus* in morphological characters and electrophorectic patterns. The present biochemical findings of close affinity between three populations (from the forested, rubber plantation and domestic areas) concurs with similarity of morphological features observed in these three populations. It was learnt that *An. dirus* from the domestic, rubber plantation and forested areas, were closely related to each other and no indications for restricted gene flow were found.

This finding is in agreement with Htay Aung **(**1995). Because all three populations of *An. dirus* are so similar in morphology, the presence of diagnostic isoenzymes for at least laboratory colony stock is important for identification purposes (Htay Aung, 1995).

Also, the average degree of polymorphism was 0.1000 for *An. dirus* (population 1) from forested area, 0.3000 for *An. dirus* (population 3) from domestic area and the average number of heterozygosity was 0.0005 for population 1 and 0.0602 for population 3 (Table 3.23 and 3.25). In many cases of X^2 testings the observed frequency values were greater than

0.5 (Table 3.28 and 3.29), this may be due to sampling error or small sample sizes. Although the present populations are not identical in genetic constitution, the genetic variations observed are within the boundaries of geographical populations and of small populations. However, the values of genetic similarity (Nei I = 0.9999 for population 1 and 0.9978 for population 3) and genetic distance (D = 0.0001 for population 1 and 0.0022 for population 3) are in good accordance with those reported for geographic populations of various invertebrates. Thus it can be concluded that the sample of these three populations of Mudon area are conspecific populations of *An. dirus.*

4. General conclusion

4. General conclusion

Part I: The anopheline mosquitoes of Myanmar

Thirty-six species of anopheline mosquitoes recorded in Myanmar have been considered in detail with particular reference to the distribution, breeding sites, seasonal prevalence, adult behaviour and relation to malaria of each species and some general aspects of anopheline behaviour have been discussed.

Classification has been made of all recorded species with regard to their relation to malaria transmission and the distribution of malaria in Myanmar as follows:

1. Anopheline vectors of Myanmar

The decision as to whether or not a particular species is a vector of malaria was made by taking the following considerations into account.

(a) Dissection records: The evidence that can incriminate a species as a vector is the finding of sporozoites in the salivary glands on numerous occasions. The occasional finding of gut infection without corresponding salivary gland infection can not be considered as evidence that the species concerned is transmitting malaria.

(b) Feeding habits: If it can be shown, either by night collections or by an extensive series of preciption tests (WHO, 1975) that a particular species is feeding mainly on human blood rather than on animal blood, then that species must be considered as a potential vector.

(c) Distribution: Usually there is a correlation between the distribution of a suspected species and the distribution of malaria.

(d) Seasonal prevalence: A species that is transmitting malaria in any area must be abundant in that area at the time when most malaria cases are occurring.

(e) Evidence from other sources: In case of doubt it is sometimes helpful to compare the behaviour of a particular species with the evidence available from other countries especially in regard to feeding habits, seasonal incidence and dissection records.

Out of 36 species of anophelines distributed throughout the country, ten species have been found to be infected with malaria parasites based on the entomological and parasitological studies. The species can be classified according to their vector competence as follows:

(I) Primary vector: Responsible for regular, annual malaria transmission; (i) *Anopheles dirus* and (ii) *Anopheles minimus.*

(II) Secondary vector: Predominantly cattle-feeders which may, under abnormal conditions,

feed on man. Often abundant and capable, therefore, of transmitting malaria; (iii) *Anopheles aconitus* (iv) *Anopheles annularis* (v) *Anopheles culicifacies* (vi) *Anopheles sinensis* (vii) *Anopheles jeyporensis* (viii) *Anopheles maculatus* (ix) *Anopheles philippinensis* (x) *Anopheles sundaicus.*

(i) *Anopheles dirus:* *Anopheles dirus* is the major vector of malaria in Myanmar and is mostly associated with forested foothills, deep forest and domestic wells (Oo et al., 2002). This species can be found in certain regions breeding in water-fill bamboo stumps. There is a well-marked post-monsoon transmission season with a peak in October (Oo et al., 2003 a).

(ii) *Anopheles minimus**:*** *Anopheles minimus* is essentially a mosquito of the hilly regions, either low or rolling foothills or narrow river valleys in mountain ranges. When found in the plains, it is always associated with extensive irrigation systems. The topographical conditions where *Anopheles minimus* is recorded range from coastal plains through narrow river valleys to foothills and plateau. *Anopheles minimus* has not been recorded in locations over 915 m above sea level. It is the most dangerous vector responsible for hyperendemic and stable malaria in the foothill and sub-mountain regions (Fox, 1949; Khin Maung Kyi, 1970; Khin Maung Kyi and Win, 1976; Myo Paing et al., 1988 and 1989 b; VBDC report, 2000). In areas under the influence of this species, there is a well-marked pre-monsoon transmission season with a peak in May and June and also post-monsoon incidence, with a peak in November and December (Oo et al., 2003 b).

(iii) *Anopheles aconitus:* *Anopheles aconitus* is widely distributed and recorded in various types of localities especially in hilly tracts, foothill areas and also in the plains of central and southern Myanmar (Khin Maung Kyi, 1971; Myo Paing, 1990 a). This species is mainly a cattle-feeder and cannot be considered as a major vector of any importance in Myanmar. However, when cattle are not avalible, it is possible that this species fed predominantely on humans especially when it occurs in large numbers. It has to be considered as a secondary vector in some isolated localities (Oo et al., 2003 b).

(iv) *Anopheles annularis:* *Anopheles annularis* is a common anopheline mosquito in all areas of Myanmar. Though it is not important as a major vector in Myanmar as a whole, it has considerable local importance in the Rakhine coastal region, where it is responsible for severe epidemic outbreaks (Khin Maung Kyi, 1972; Myo Paing, 1990 a). The villages along

this coastal region are subjected to periodic cyclones. Following the cyclones, an outbreak of malaria epidemic usually occurrs in the devastated areas. Pools and ditches in and around the villages are filled with water and *Anopheles annularis* could be found to breed in profusion. There is a well-marked transmission season from October to March (Oo et al., 2003 b).

(v) *Anopheles culicifacies:* The distribution of *Anopheles culicifacies* is mainly confined to the plain and irrigated areas, especially in the central dry zone. It is observed to be a highly domestic and zoophilic mosquito in Myanmar. Although it is a plain species, it is found at moderate altitude like the Shan Plateau (Shan State) in the eastern part of Myanmar. Mosquitoes breed in fresh and clear water with vegetation but also in sandy pools and rock pools. Though it may not be a major vector of malaria in Myanmar, *Anopheles culicifacies* has a considerable secondary importance wherever present in hyperendemic foothill areas (Khin Maung Kyi, 1972; Myo Paing, 1990 b). In the plains of central Myanmar where it is the only vector species, it has been known to be responsible for outbreaks of sporadic epidemics (Oo et al., 2003 b).

(vi) *Anopheles sinensis:* This shows a fairly limited distribution in Myanmar. According to the observations, it might be concluded that the seasonal incidence of this species in Myanmar appears to be at the end of the monsoon months of August and September (Oo et al., 2003 b). *Anopheles sinensis* is mainly a cattle-feeder, but will attack man if cattle are not available. *Anopheles sinensis* is an important malaria vector only at the Myanmar-China border (Shan State) when it occurs in high densities (Khin Maung Kyi, 1971; VBDC report, 2000).

(vii) *Anopheles jeyporensis:* This mosquito can be found in hilly tracts, foothill areas and coastal plains (Oo et al., 2003 b). *Anopheles jeyporensis* has a very limited distribution. It is abundant only in two localities in Rakhine State (Myanmar-Bangladesh border area) and Sagaing Division (Kabaw valley area). It is a secondary vector on the Myanmar-Bangladesh border area and on the Myanmar-China border area (Shan State), when it occurs in great numbers (VBDC report, 2000). Elsewhere it is found in very small numbers.

(viii) *Anopheles maculatus:* *Anopheles maculatus* is primarly recorded from forested foothills, deep forest camps and in rocky mountainous areas about 1220 m above sea level. It could not be found in plain areas far away from the foothills. This species could be regarded

as a secondary vector especially in Tanintharyi Division, southern part of Myanmar (Myo Paing, 1990 c; VBDC report, 2000; Oo et al., 2003 b).

(ix) *Anopheles philippinensis:* *Anopheles philippinensis* has been recorded from all parts of the country and all types of terrain except Tanintharyi Division, southern Myanmar (Oo et al., 2003 b). It has been incriminated as a vector in valley regions (Bahmor and Kabaw valley, Sagaing Division) and is a local vector of minor importance along the Myanmar-Bangladesh border area. Elsewhere it does not appear to be an efficient vector (Fox, 1949; Khin Maung Kyi, 1972; VBDC report, 2000). There is a well-marked transmission season during monsoon periods (June to September).

(x) *Anopheles sundaicus:* This species is confined only to the coastal areas such as Rakhine State, Tanintharyi Division and lower reaches of the Ayeyarwady Division (delta) where the creeks are subject to tidal influence. *Anopheles sundaicus* is responsible for regular annual malaria transmission in certain areas where it occurs in great numbers. Irregular local outbreaks of malaria vary from year to year in some localities. The bionomics of *Anopheles sundaicus* in Rakhine are identical with those of this species elsewhere. Even though this species has not been found to be infected with *Plasmodium*, it is likely that in Myanmar, this species is involved as vector during local outbreaks of malaria in different parts of the coastal areas of the country especially in Rakhine State and Chaungtha area, Pathein Township, Ayeyarwady Division. The transmission season is post-monsoon period (Khin Maung Kyi, 1972; VBDC report, 2000; Oo et al., 2003 b).

Non vectors, either entirely cattle-feeders or too scarce generally to be of any importance (Oo et al., 2003 b) include**:** *Anopheles aitkenii, Anopheles argyropus, Anopheles barbirostris, Anopheles bengalensis, Anopheles fluviatilis, Anopheles gigas, Anopheles nitidus, Anopheles insulaeflorum, Anopheles jamesii, Anopheles karwari, Anopheles kochi, Anopheles kyondawensis, Anopheles lindesayi, Anopheles willmori, Anopheles majidi, Anopheles nigerrimus, Anopheles pallidus, Anopheles peditaeniatus, Anopheles pseudojamesii, Anopheles splendidus, Anopheles stephensi, Anopheles subpictus, Anopheles tessellatus, Anopheles theobaldi, Anopheles vagus* and *Anopheles varuna*.

It is realized that the above classification is provisional only, having been drawn up solely from the material that has been submitted in this paper. Further evidence may well

alter the status of some of the species. *Anopheles jeyporensis,* for instance, has yet to be studied in Myanmar under normal conditions. The exact role played by *Anopheles aconitus* in Myanmar has yet to be definitely proved, as much more research is required into the behaviour of the species listed as secondary vectors.

It is quite evident that are many gaps in our knowledge and that many problems of anopheline behaviour will require solution before any successful attempt can be made to tackle the major problem of malaria control in Myanmar.

2. The distribution of malaria in Myanmar

A brief description of the physical features of seven well-marked geomorphological regions has been given in section 1.1.2 with Fig. 1.2., and the distribution of malaria in these regions which will be discussed further below. In this section an attempt will be made to describe, in general terms only, the incidence of malaria in certain natural geomorphological regions of Myanmar (Fig. 4.1). It is emphasized that this is not a detailed account of the epidemiology of malaria in Myanmar, but merely a general statement inserted with a view to simplifying the later sections of this Thesis when an attempt will be made to correlate the distribution of certain anopheline species with the distribution of malaria.

The following material has been used in the compilation of this summary:

(a) Dissection records on malaria infection rate during the study period.

(b) Annual reports (unpublished), Vector Borne Disease Control Department (VBDC), 1982-2002.

(c) Malaria situation of Myanmar, VBDC, (1977-2002) (unpublished).

(d) Fox. D. G. R. (1949): Anopheline Mosquitoes in Burma. M.D. Thesis.

(e) Khin Maung Kyi. (1970 to 1976): Malaria vectors in Burma and The Anopheline mosquitoes of Burma.

Region 1- Rakhine Coastal Strip: The incidence rate is high throughout this region and the evidence suggests that regular annual transmission takes place. Fox (1949) postulated the following probable sequence of malaria transmission:

(i) Mountain area: definite pre-monsoon season (April-May) with possible monsoon transmission also in the jungle areas.

(ii) Hillock area: long pre-monsoon season (February-May) with possible post-monsoon season until the cold weather starts.

(iii) Coastal area: sporadic transmission at any season but there appears to be a definite

increase associated with the spring and autumn equinoctial tides. The intensity of malaria varies from year to year and isolated epidemics can occur.

Region 2- Western Hills: Little is known about conditions in the northern portion but as the scattered villages usually lie 915 m above sea level they are probably malaria free. Further south, malaria is hyperendemic in the valleys wherever perennial streams exit. In the Kabaw and Kale valleys (Fig. 2.67), for example, transmission occurs throughout the year with well-marked pre-monsoon, monsoon and post-monsoon peaks.

Region 3- Northern Hills: On the whole the main river valleys (Fig. 2.67) of the Ayeyarwady, Chindwin and the Mu rivers, are relatively healthy except where villages encroach upon the foothill regions, where severe malaria transmission occurs. The lesser valleys, between two watersheds such as the Hukaung valley (Fig. 2.74), and Pinlebu area (Fig. 2.67) with railroad north from Wuntho Town to Mogaung Town are all intensely malarial. The seasonal incidence is mainly post-monsoon (September to December).

Region 4- Dry Zone: Large portions of the Dry Zone are malaria free. In fact, in this region, the disease is associated only with two distinct types of locality.
(i) In the neighbourhood of the foothills bordering the plains, both in the east and the west.
(ii) In areas where an extensive irrigation system exits.

The malaria season usually extends from July to March with a peak in December or January. An unusual feature of the malaria incidence in this region is the sporadic occurrence of localised epidemics. The factors responsible for these epidemics are a little obscure; in some years they appear to have been associated with a failure of the monsoon while in others extensive flooding has been held responsible. Occasionally an extension of the irrigation system has been a precipitating factor.

Region 5- Southern Plain and Delta: There is a patchy distribution of malaria observed in this region. In the neighbourhood of the foothills of the Shan Plateau, the Bago Yoma mountain range (Fig. 2.64) and the Rakhine Yoma mountain range (Fig. 2.70), malaria is hyperendemic. Large areas of the lower Ayeyarwady valley (Fig. 2.65) and Sittang valley (Fig. 2.64 and 2.71) are malaria free while elsewhere in this Delta localized areas of endemic malaria are found.

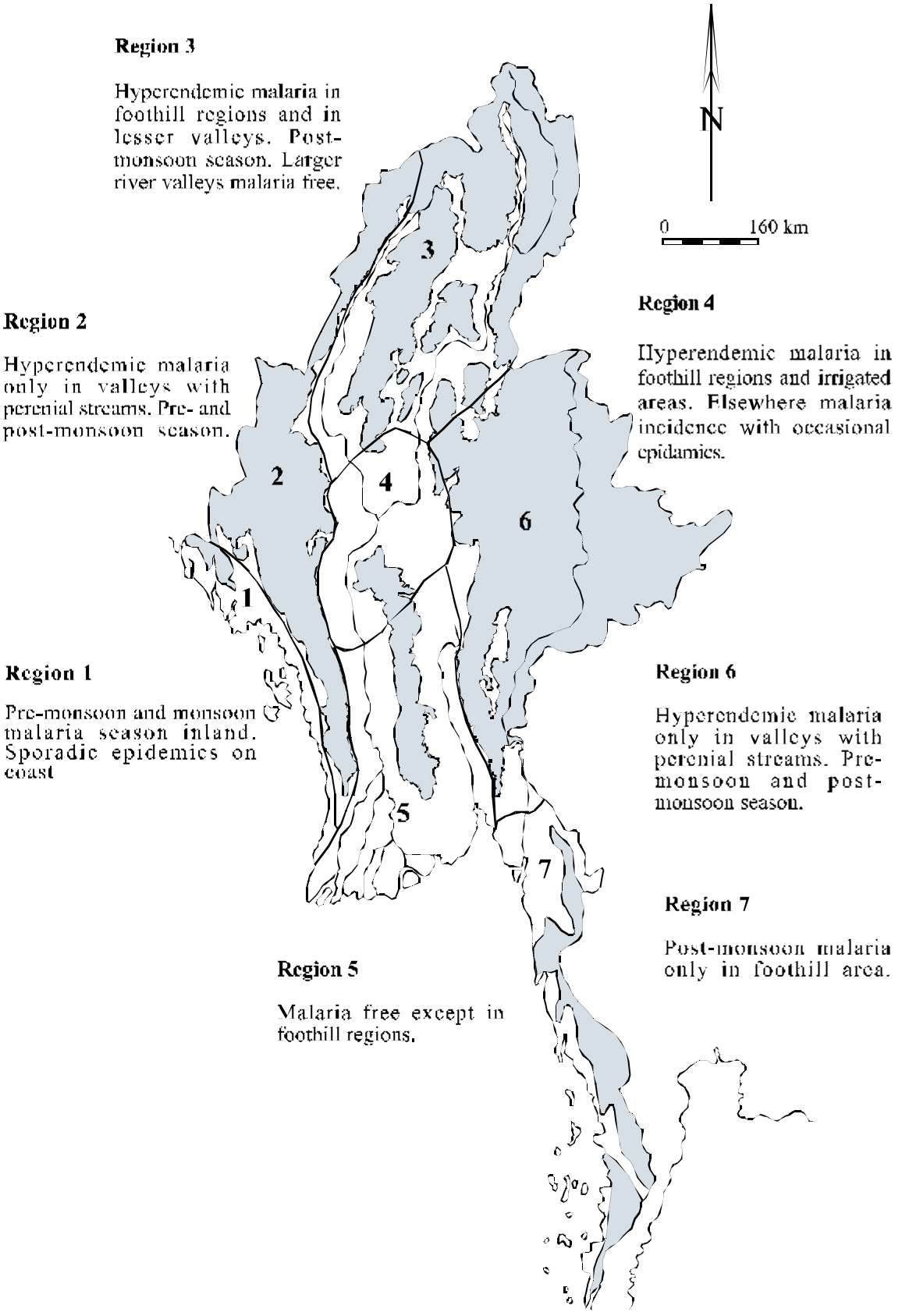


Fig. 4.1. Distribution of malaria in Myanmar (modified after Fox, 1949)
Shaded areas indicate heights over 300 meters

The seasonal incidence is not clearly defined but is probably mainly post-monsoon from September to December. In addition, in the Delta, there is some evidence of localized epidemics of malaria occurring at regular intervals, often associated with a failure of the monsoon.

Region 6- Shan Plateau: Malaria is hyperendemic throughout in association with perennial streams and terraced rice cultivation in the valleys. Locations from 1067 to 1830 m above sea level are generally malaria free. There is a well-marked pre-monsoon transmission season with a peak in May or June and more severe post-monsoon season with a peak in November or December.

Region 7- Tanintharyi Coastal Strip: Malaria in this region is confined to the foothill tracts of the Tanintharyi mountain range (see Fig. 2.69) and transmission is mainly post-monsoon.

Part II: Special consideration of *Anopheles dirus*

The present study on *Anopheles dirus* includes adult biology, larva ecology, epidemiology (section 2.3.3.2) and genetics (Chapter 3. Biochemical studies on *Anopheles dirus* collected from Mudon).

Anopheles dirus is an important (one of the primary vectors) vector of malaria in Myanmar and the neighbouring countries. Malaria remains a serious public health problem in Mon State (VBDC report, 1998). In Mudon (Mon State), *Anopheles dirus* larvae were found in large numbers within domestic wells. These wells provided water for drinking, washing, bathing and cooking. During the rainy season, the larval and pupal density of *An.dirus* was usually high, whereas during the cool-dry season, their numbers were reduced.

Anopheles dirus, a forest breeder, is gradually becoming a peridomestic breeder and adapting itself favourably and rapidly to this new enviroment. The need was felt to study how this forest breeder *Anopheles dirus* is becoming a peridomestic breeder and adapting to the new environment so as to provide knowledge for further research and control needed in Mudon, Mon State (section 2.3.3.2).

The biology and ecology mainly concerned the development and the factors that influenced the seasonal abundance of the *Anopheles dirus* mosquitoes. The factors included are both biological and environmental. Among the biological factors, adult and larva densities were found to be positively correlated with rainfall. (Table 2.1.2., Table 2.2.18. and

Fig. 2.93). More *Anopheles dirus* mosquitoes were caught during the rainy season than other seasons (Tables 2.2.18 and 2.2.20). The water level is influenced by rainfall and shade. The heavier the rainfall, the higher the water level. The wells under tree canopy usually contain more water and provide better conditions for larval breeding. During the rainy season larval density was very high. Most of the wells retain more water (high water level). These wells had more debris (more organic food for mosquito larvae) and received more shade (because of greater cloudiness during most of the day), enabling more mosquitoes to survive and develop into healthy and vigorous adults (Kitthawee et al., 1990).

An. dirus is well known for breeding in shaded habitats in forested areas. Due to deforestation resulting in shortage of *An. dirus,* residential wells appear to have provided new breeding resources. The wells are usually shaded, densely vegetated and relatively cool due to the underground water supply and are therefore, similar to the natural breeding places. The close contact of one of the major malaria vectors with humans could be one of the reasons for the increasing number of malaria cases in this region (Oo et al., 2002).

Part II mainly concerns the genetic studies of the *Anopheles dirus* from three different localities (domestic, rubber plantation and forested areas) around Mudon area. Genetic studies of mosquito species have been retarded by the lack of efficient, accurate and dependable methods by which to identify individual gene loci and their allelic arrays. The application of electrophoretic techniques to identify variable (polymorphic) gene loci in mosquitoes seems currently the best way to resolve this problem (Steiner et al., 1979; Murphy et al., 1996).

In this study, field-collected specimens (both adults and larvae) were stored in liquid nitrogen (-196 °C) or covered with dry ice for protein electrophoresis. These specimens were used in horizontal ultrathin agarose electrophoresis for identification. A more detailed analysis was performed in the present study using 11 enzyme systems to determine the degree of genetic differentiation among these populations in comparision with *Anopheles maculipennis* from Germany and *Anopheles stephensi* from Indonesia.

The use of biochemical keys requires a knowledge not only of the appearances of enzyme genotypes, but also of their relative numbers in populations. In a randomly mating population, genotypes occur in characteristic numbers (expressed as frequencies, or percentages) which can be estimated by Hardy-Weinberg distribution of population genetics where the first step is to confirm the observed allele frequencies (Berlocher, 1980).

The study includes the assessment of the degree of polymorphism, heterogeneity status, genetic identity (**I**), genetic distance (**D**) and the significance of the findings were

discussed. The genetic identity values between these three populations of *An. dirus* ranged from 0.9978 to 0.9999 (Table 3.32) which is the generally accepted range for conspecific populations (Ayala et al., 1975). The high values of genetic similarity indices suggest that natural populations of *An. dirus* in Mudon area share an undifferentiated gene pool. The very low genetic distance D (between 0.0001 to 0.0022- Table 3.32) also indicates that these three populations of *Anopheles dirus* from Mudon area are parts of a metapopulation without measureable adaptations due to selective conditions in ecologically different breeding sites. The enzyme electrophoretic results do not indicate any barrier to gene flow between the *An. dirus* populations.

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APPENDICES

Appendix A

The main differences between *Anopheles kyondawensis* and *Anopheles insulaeflorum* are:

(a) In the latter species, palmate hairs on abdominal segment I are well developed whereas this is not so in *An. kyondawensis.*

(b) The frontal hairs are short and branched in the former species but are of normal length and branched in the latter.

Appendix B

Anopheles willmori

This was the only recognized variety of *maculatus* and differs from *An. maculatus* in having profuse scaling on tergites III-VII. A large proportion of individuals of *willmori* also show speckling on the palpi. The larvae of the two forms are, however, indistinguishable.

Appendix C

Table 1. Occurrence of anopheline fauna during the study period and their locations

N.B.
p.v. = primary vector
(+) = seldom
\+ = few
++ = common
¦ = abundant

1.Yangon Division (see section 2.3.1and Fig. 2.63)																																						
			p.v.		secondary vector								non vector																											
No. of study areas	Township	Sampling sites	*An. dirus*	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	Taikkyi	Yesitkan village	(+)	(+)	+	¦	+	++	(+)	¦	++				¦		(+)		++		¦	(+)	(+)			(+)		¦	(+)				+	+	++		¦	+
2	Taikkyi	Leinmawchan vil.	(+)	(+)	+	¦	+	++	(+)	¦	++				¦		(+)		++		¦	(+)	(+)			(+)		¦	(+)				+	+	++		¦	+
3	Hlegu	Yenetgyi village	(+)	(+)	+	++	+	++		¦	+				¦		(+)				¦							¦						(+)	+		¦	+
4	Hlegu	Paunggyi village	(+)		+	++	+	++	(+)		+				¦						¦												(+)			¦	(+)
5	Hlegu	Kyaukain village			+	++	+	++			+				¦						¦															¦	(+)
6	Insein	Strand road area			+	+		+		++					¦						¦							+		+						¦	(+)
7	N.Okkalapa	Ayeyar 15th street				+		+	(+)																											¦	(+)
8	Bahan	Ngadatkyi area				+	(+)	+																													
9	Mayangon	Thiri Avenue				+	(+)	+	(+)																													

2. Bago Division (see section 2.3.1. and Fig. 2.64)

			p.v.		secondary vector								non vector																																
No. of study areas	Township	Sampling sites	*An. dirus*	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
10	Okpo	Kaingdawsu village	++			+				++																												
11	Okpo	Yebya camp	++	+		+																																
12	Kyauktagar	Myochaung village				+																																
13	Kyauktagar	Htiphado village		++							++						(+)																					
14	Kyauktagar	Kyarkyaungthaik		++					(+)																													
		Phado village		++				++																				(+)										
15	Kyauktagar	Katsene camp																																				
16	Oktwin	Thabyewa village		++		+					++																											
17	Letpadan	Hsachaung village				+																										+						
18	Pyu	Khintan village			+	+	+	++		++					++						+													+		++	(+)
19	Thayawady	Hlelangu village									++																											
20	Kyauktagar	Ngokto village				+																																
		Gwegone village				+																																
21	Kyauktagar	Bwechin village				+					++																											
22	Taungoo	Taungoo Town			+	+	++		(+)						++		(+)		++		++								(+)			+	+	+	++			(+)
23	Yedashe	Sezongone village				+	+			++											++											+	+	+			++	
24	Bago	Bago Town			+	+	++		(+)						+		(+)				+													+	++		++	
25	Thayawady	Thayawady Town			+	+																													+		++	
26	Pyay	Shwekuyad			+	+																						++		++		(+)			+			
		Htanpauk village			+										++						+											+					++	
		Kyauklongyi			+	+	+		(+)	+	+				+		++		++		+		(+)						(+)			+	++				++	
27	Paukhaung	Gonemingone	+	+	+					++	++																							++	+			
		Taunglae village			+	++	+			++	++										++		(+)										++					
28	Shwedaung	Shwedaung Town			+	+	+				+				+		(+)				+											+		++	+		++	

3. Ayeyarwady Division (see section 2.3.1 and Fig. 2.65)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																												
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
29	Myaungmya	Magyipin village			++	++					+	++			++																					++	(+)
30	Chaungthar	Seikkyi village	(+)		+				(+)	+																						++				++	
31	Chaungthar	Chaungthar Town																													+	++					
32	Hinthada	Hinthada Town					(+)								+																		++			++	(+)
33	Pathein	Thabyuchaung		(+)	++	+		++		+																								+		++	
		Thalatkhwar village				+		++							+						+													+		++	
34	Ngaputaw	Kaditchaung village	(+)									++																										

4. Mandalay Division (see section 2.3.1 and Fig. 2.66)																																						
			p.v.		secondary vector								non vector																									
No. of study areas	Township	Sampling sites	*An. dirus*	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
35	Madaya	Sedawgyi	+	¦		++		++		¦	¦																								+				
36	Madaya	Botegone village		++	++	++	++	+	(+)	++	++																						+					
		Zeephyupin	++	++	++	++	++	+		++	++						(+)													+			+		+			+
37	Mandalay	North, Mandalay				¦	++								++		(+)						(+)					¦				+	++	+	+		++	+
38	Patheingyi	Shwesaryan		++			++										(+)																++					+
		Tawsu village			++		++																															
39	Amarapura	Sargalein village		++	++	++	++	+		++	¦																						¦					+
		Nyaungnibin			++	++		+			+																						¦					+
40	Singu	Nweyone village		++	+	++	++	+		++	+																						+					+
		Shwepyi village				++					+																						+					
41	Singaing	Sezone village			+	++	++	+	(+)	+	+																						+					+
		Badaquinn village		++																																		
42	Meiktila	Aungpintha			+		++				+																						+					
43	Kyaukse	Lunkyaw village		++	++	++	¦	+		+	+						++						(+)					¦		++			+	+	+		++	+
44	Kyaukpadaung	Le`gwa village		++	+	+	++	+		+	¦				¦													++					+					+
45	Nyaung-oo	Shwe-hlaing					++																															
46	Taungtha	Semikan village		++	+	+	++	+			++																						+					+
		Magyibin village				+	++																															
47	Thabeikkyin	Tagaung village			+	+		+			+																						+					+
		Taungtalone		++	+	+	++			+	+						(+)																					+
48	Mogok	Shwenyaungbin		++	+	+	+	++		¦							(+)																+					+
49	Mogok	Kabaing village		++	+	+			+	¦	+				+	(+)		(+)					(+)					++				(+)					++	+

continuation Mandalay Division

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																									
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
50	Pyinoolwin	Wetwun village		++	¦		+	++	(+)	++	¦				++		(+)				+							++	+			+	+	+			++	+
51	Pyinmana	Moeswe village					++																										+					+
		Aungthukha		++	+	+	++	++	(+)	++	++																						+					
52	Mandalay-Pyinoolwin	Foothill area		++	++	+	++	++		++	¦				¦		++				++		+					¦				+	++		¦		¦	+
53	Meiktila	Wundwin village				+	++			+					+																	(+)		+	+		++	+
54	Thazi	Indaingtha village	+	+	+	+	+	++	+	+	+															+							+		+			
55	Yamethin	Kinywa-indaing	(+)	+		+	++	+	+	+	+				+						+	(+)	(+)					+				+	+	+	+		++	+
56	Lewe	Chinsu village	+	+	+	+	+	+		+	+																						+		+			+
		Kantha village					+			+																												

5. Sagaing Division (see section 2.3.1 and Fig. 2.67)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																													
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
57	Mawlaik	Khamaya (368)		++	+	+	-	++	+	++	-		(+)		+	(+)			+			+	++			-		++	+			+	++	++	+		++	+
58	Kale	Kabaw valley	(+)	++	++	++	++	+	+	++	++				+	(+)					++		++					++	+				++	++	+		++	
59	Kale	Kale valley		++	++	+	+	+		+	++				+	(+)					++		++			++		++					++	++	+		++	+
60	Tamu	Tamu Town	+	+	+	+	+	+		+	+				+	+					+	+	-					+				+		+	+		++	+
		Boke-kann		+	+	+	+	+	+	+	+			(+)	+								++						+			+	+				++	
61	Sagaing	Myothit Quarter				+	+	+															+									+		+			++	
62	Monywa	Monywa Town				+	-			+	+				+																	+	-	++			++	+
63	Wuntho	Bonchaung village	+	+	+	+	++	+		+	+				+		++				+		++					+	+			+	+	+	+		++	+
		Pay-gone village			+	+				+	+																					+						+
64	Inntaw	Nabar village		+	+	+	+	+		+	++				+		++				++	+	+				(+)					+	+	+	+		++	+
65	Katha	Katha Town	(+)	+	+	+	-	++		+	-				+		++					+	-			-	(+)	+	+			+	+	-	+		++	+
66	Shwebo	Shwebo Town			+	++									++		-						++					+	+			+	++	++	+		++	+

N.B. only adult of *An. majidi* (column no.28) have been recorded.

6. Magwe Division (see section 2.3.1 and Fig. 2.68)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																															
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
67	Yenangyaung	Ywathitgyi village		(+)			++																					++					+					
68	Chauk	Mingyan village	(+)	(+)			++																										+					
69	Myaing	Lingadaw village					++																															
70	Saw	Yinkekaung		(+)		++	++				+						+																+	+			++	
71	Gawgaw	Khaungtone		(+)			++			+																							+	+			++	
72	pakoku	sinlan village				[illegible]											+																				++	
73	Seikpyu	Koedaung village					++																															
74	Taungdwingyi	Kamontaung	(+)	(+)	+	++	++	+		+	+				+						+											(+)	+	+			++	
		Thabyepin					++																															++	
75	Natmauk	Khinkaung					++																										+	+			++	
76	Myothit	Yeayetaing		(+)		++	++	+		+	+																						+	+			++	
77	Magwe	Tegyigone				[illegible]	++			+	+				+													++					++	[illegible]			++	
78	Mezali	Mezali Town					++				+																											
79	Yesagyo	Zitaw village				+	++	+		+	+																						+	+			++	
80	Mayde	Mogaung																															+					
81	Thayet	Ywathit village				++	++				+				+																		+				++	
82	Kama	Gyopin village				++	++	+		+					+																		+	+				
83	Sinbaungwe	Nyaungbintha					++																															
84	Mindon	Zeedaw village			+	++	++	+		+																								+			++	
85	Pwinbyu	Thanatwa		(+)	+	[illegible]	++	+		+	+				[illegible]												++					+				++	
86	Minbu	Minbu Town									+				++		+																+	++			++	
87	Sagu	Sagu Town					++																															
88	Salin	Linzin village			+		++	+			+				+		+												(+)			+	+			++	
89	Sedoktaya	Sedoktaya Town				++	++	+			+																						+	+			++	
90	Ngape	Padan village			+	++	++	+		+	+				+						+												+	+	+		++	

7. Tanintharyi Division (see section 2.3.1 and Fig. 2.69)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																																
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
91	Myeik	Shwedu	++																																										
92	Bokpyin	Hangadaing		++								++																						+										
93	Kawthaung	Namtun		++	++	++						++			+																							++						
94	Dawei	Sanchi village		++	++	++	+	+			+	++			+						+	(+)	+			+	(+)		+					+			++	+						
95	Myeik	Monsu village	++	++																		(+)																						

N.B. only adult of *An. majidi* (column no.28) have been recorded.

8. Rakhine State (see section 2.3.1 and Fig. 2.70)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																																	
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
96	Sittwe	Sittwe Town	(+)		–	–	+	+	+	+	+	–	(+)		+	(+)						+						++						+	+		++	+
97	Kyaukpyu	Kyaukpyu Town			–	–	+	+	+	++	+	–			+							+						+			+			+	+		–	+
98	Myebon	Myebon Town										++			+																			+				
99	Gwe	Gwe Town						+		+		++																										
100	Taungpyo	Bangladesh border			++	++		+	++		–	++			+																					++	
101	Taungpyo	Taungyo Town		++		–		+			++	++			+						+	+											+	+	+		++	
102	Maungdaw	10th miles	(+)	++	++	–	+	+	++	++	+				+						+	+											+	+	+		++	+
103	Minbya	Minbya Town					+	+							+								+									(+)	+	+	+		++	+
104	Myohaung	Shwegudaung			–							++																										+
105	Buthedaung	Kardi village		++	++	–		+		++	+	++			+																			+			++	
106	Ann	Ann Town																																				

N.B. only larvae of *An. pseudojamesi* (column no.32) have been recorded.

9. Mon State (see section 2.3.1 and Fig. 2.71)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																															
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
107	Mawlamyine	Taungwaing	++			+	+	++		+	+				+			(+)			++													+		+	
		Innwaing	++		+	+	+	++	(+)	+	++	++			+				+	+	++	(+)	(+)	(+)			(+)	+	(+)	+				++	+		+	+
108	Chaungzone	Tagukkana	++		+	+		+		+	+	+			+						++	(+)	(+)						(+)					+	+		+	+
109	Thanbyuzayat	Wetlay village	-									(+)									++	(+)						+							+			+	+
110	Kyaikmaraw	Kalinekaning	++			+		+		+	+				+						++					+								+			+	
111	Mawlamyine	Kawkalok	++		++																																	+	
112	Mudon	Quarter 4	-		++	+				+	+				+				+		++	(+)				+	(+)	+		+		(+)		++	+		+	+
113	Thaton	Thinhtow	++			+		+			+				+						++													-			+	
114	Belin	Botayza	++			+		+			+				+						++														+		+	
115	Kyaikto	Kinmonchaung	++			+		+			+										++																+	

N.B. (i) only larvae of *An. kyondawensis* (column no.25) have been recorded.
(ii) only adult of *An. majidi* (column no.28) have been recorded.

10. Shan State (see section 2.3.1 and Fig. 2.72)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																															
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
116	Momeik	Khalaya	+	+	+	+	+	+		+	++				+			(+)			+		+								+			+	+		++	
117	Hsipaw	Hsipaw Town	(+)	+	+	+	++	++		+	+				+		+						(+)					+						+	+		++	
118	Lashio	Mansan fall	(+)	+	+	+	++	++	+	+	+		(+)		+	(+)	+	(+)			+	(+)	(+)			++	(+)	+				(+)			+	+	(+)	++	+
119	Mabein	Mabein Town				+														(+)																			
120	Kyaukme	Quarter 4	(+)	+	+	+		++		+	+				+		+																				++	+
121	Loilem	Loilem Town				+																										(+)	+					
122	Kunlong	Kunlong Town		+	+	+		++		+					+																							
123	Chinshwehor	China border					+	+	++																													
124	Kengtung	Kengtung Town		+	+	+	+	+		+	++				+						+	(+)	+					+				(+)	+				++	+
125	Tachileik	Tachileik Town					+																														++	
126	Heho	Heho Town				+	+																															+
127	Hopone	Hopone Town		+	+	+	+	++		+					+		+																				++	+
128	Yutsawk	Yutsawk Town		+	+	+	+	+					(+)																									
129	Aungban	Aungban Town				+											+																					
130	Pindaya	Thayetgon		+	+	+			+	+	+				+								(+)														++	
131	Nyaungshwe	Nyaungshwe Town		+	+	+	+			+	+				+																						++	+
132	Ywangan	Myogyi village		+	+	+	+			+	+						+																					
133	Kalaw	Nanpandit village		+	+	+	+			+	+		(+)		+	(+)	+				+		(+)		(+)	++	(+)		+				+				++	+
134	Namsam	Namsam Town											(+)			(+)		(+)																				
135	Shwenyaung	Phetmun village		+	+	+	++		+	+	+				+													+						+			++	+
136	Sesai	Aungthapye Quarter		+	+	+		+	+	+	+				+																							
137	Indaw	Indaw Town		+	+	+		+		+	+						+				+															+	
138	Taunggyi	Yebu village		+	+	+	+	+	+	+	+				+						+		(+)									(+)	+		+		+	(+)

N.B. (i) only larvae of *An. insulaeflorum* (column no.21) have been recorded. (ii) only adult of *An. majidi* (column no.28) have been recorded.

11. Kayah State (see section 2.3.1 and Fig. 2.73)																																						
			p.v.		secondary vector								non vector																									
No. of study areas	Township	Sampling sites	*An. dirus*	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
139	Mawchi	Mawchi mines		+		+	+			+			(+)			(+)					+		(+)														+	
140	Demoesoe	Myenigone				+	+	+			+				(+)																			+			+	
141	Loikaw	Nwalasoe		+	+	+	+	+		+	+				(+)								(+)				(+)		+					+	+		+	(+)

N.B. only adult of *An. majidi* (column no.28) have been recorded.

12. Kachin State (see section 2.3.1 and Fig. 2.74)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																													
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nititdus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
142	Myitkyina	Mawphaung	(+)	++	+	+	+	+	+	+	++				+		(+)						(+)			+	(+)	++				+	+		+		++	++
143	Mohnyin	Aungthapye		+	+	++		+		+	+															+							+				++	
144	Kamaing	Lawa				++																																		
145	Tanaing	Minekune		+	+	++		+		+	+				+						+																		
146	Moegaung	Kyuntaw				+		+		+	+				+								(+)			+												++	++
147	Bhamo	Bhamo Town	(+)	+	+	+	+	+	+	+	++				+						+		(+)			+		++						++	+		++	++
148	Myothit	Myothit Town				+		+													+						(+)										++	

N.B. only adult of *An. majidi* (column no.28) have been recorded.

13. Chin State (see section 2.3.1 and Fig. 2.75)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																													
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
149	Falam	Panunchaung		+		+		+		+	+		(+)			(+)		(+)															+				+	
150	Paletwa	Htaungza		+		+		+		+	+										(+)	(+)							+								+	
151	Mindat	Mindat Town		+									(+)			(+)									(+)							(+)							
152	Kanpetlet	Syawlaung		+									(+)			(+)									(+)							(+)					+	

14. Kayin State (see section 2.3.1 and Fig. 2.76)

No. of study areas	Township	Sampling sites	p.v.		secondary vector								non vector																																
			An. dirus	*An. minimus*	*An. aconitus*	*An. annularis*	*An. culicifacies*	*An. sinensis*	*An. jeyporiensis*	*An. maculatus*	*An. philippinensis*	*An. sundaicus*	*An. aitkenii*	*An. argyropus*	*An. barbirostris*	*An. bengalensis*	*An. fluviatilis*	*An. gigas*	*An. nitidus*	*An. insulaeflorum*	*An. Jamesii*	*An. karwari*	*An. kochi*	*An. kyondawensis*	*An. lindesayi*	*An. willmori*	*An. majidi*	*An. nigerrimus*	*An. pallidus*	*An. peditaeniatus*	*An. pseudojamesi*	*An. splendidus*	*An. stephensi*	*An. subpictus*	*An. tesselatus*	*An. theobaldi*	*An. vagus*	*An. varuna*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
153	Thandaung	Thandaung Town					+			++	+		(+)			(+)		(+)	+													+	+				++	+
154	Kawkareik	Khamaya				+		+		++	+				+						++	(+)												+			++	
155	Kyrainseikkyi	Kyaikdon		+		+		+		++	+				+						++	(+)													+			++	+
156	Pyapon	Kamanoung						+							+						++		+					+	++			+		+	+			++	
157	Hpaan	Haungkhayaing	++	+	+	+		+		++	+				+						++	(+)	+										+	+	+	+		++	+