MORPHOLOGICAL AND MOLECULAR SYSTEMATICS OF *ANOPHELES* MOSQUITOES (DIPTERA: CULICIDAE: ANOPHELINAE) OF NORTH KERALA

Thesis Submitted to
the University of Calicut for the
Award of the Degree of
DOCTOR OF PHILOSOPHY IN ZOOLOGY
(Under the Faculty of Science)

Submitted by

VIPINYA C.

Under the Guidance & Supervision of

Dr. P.K. SUMODAN

PG & RESEARCH DEPARTMENT OF ZOOLOGY
GOVERNMENT COLLEGE MADAPPALLY
VADAKARA, CALICUT, KERALA,
INDIA – 673102
(Affiliated to University of Calicut)

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Dr. P.K. Sumodan, MSC; Ph.D.
Associate Professor (Rtd.) &
Research Supervisor,
Government College, Madappally,
University of Calicut
Phone: 9846135324
Email: sumodanpk@gmail.com

CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL AND MOLECULAR SYSTEMATICS OF *ANOPHELES* MOSQUITOES (DIPTERA: CULICIDAE: ANOPHELINAE) OF NORTH KERALA"** submitted to University of Calicut for the award of the degree of Doctor of Philosophy in Zoology is an authentic record of research work done by **Ms. Vipinya. C,** Department of Zoology, Government College Madappally, under my supervision and guidance and that no part thereof has been presented before for any other degree and also it is checked for plagiarism with iThenticate software from Calicut University.

Place: Madappally
Date:

Dr. P.K. SUMODAN
Supervisor & Guide

Dr. P.K. Sumodan, MSC; Ph.D.
Associate Professor (Rtd.) &
Research Supervisor,
Government College, Madappally,
University of Calicut
Phone: 9846135324
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I also certify that the corrections/suggestions recommended by adjudicators have been incorporated into the thesis.

Place: Madappally
Date:

Dr. P.K. SUMODAN
Supervisor & Guide

Dr. P. Thejass, MSC; Ph.D.
Associate Professor
Research Co-guide
Government College, Madappally,
University of Calicut
Phone: 9947361321
Email: thejassp@gmail.com

CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL AND MOLECULAR SYSTEMATICS OF *ANOPHELES* MOSQUITOES (DIPTERA: CULICIDAE: ANOPHELINAE) OF NORTH KERALA"** submitted to University of Calicut, for the award of the degree of **Doctor of Philosophy in Zoology** is a record of original and independent research work carried out **Ms. Vipinya. C,** Department of Zoology, Govt. College Madappally, under my guidance and supervision. The Thesis has not formed the basis for the award of any other Degree/Diploma of this or any other University.

Place: Madappally
Date:

Dr. THEJASS. P
Co-guide

DECLARATION

I do hereby declare that this thesis entitled **"Morphological and Molecular Systematics of *Anopheles* mosquitoes (Diptera: Culicidae: Anophelinae) of North Kerala"** is original research work carried out by me under the supervision and guidance of Dr. P. K. Sumodan, Research guide, postgraduate and research department of zoology, Government college Madappally in partial fulfillment of the requirement for the Ph.D. The content of the thesis has undergone plagiarism check, using iThenticate software at C. H. M. K Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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Date:

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Dedicated to,

My Beloved Teachers

My family

&

The most precious part of My life 'Aaru'

ABSTRACT

Mosquitoes belong to the family Culicidae, which is further subdivided into subfamilies Anophilinae and Culicinae. As the vectors of malaria, species of Anophelinae are considered as the most important of all mosquitoes. The subfamily Anophelinae is comprised of three genera: *Anopheles* Meigen, which has a nearly worldwide distribution, *Bironella* Theobald which is found in the Australasian region, and the Neotropical *Chagasia* Cruz. Nearly 51 species of *Anopheles* have been recorded from India. *An. culicifacies, An. fluviatilis, An. minimus, An. philippinensis, An. stephensi* and *An. sundaicus,* are the primary vectors of malaria in India. Besides, *An.subpictus* B has been reported to play an important role in malaria transmission in different parts of the county. Several vector species have been reported to exist as species complexes in various parts of the world including India. Hence, their specific identification is very important in planning malaria control strategies. Molecular techniques have been increasingly employed for this purpose in recent years.

The present study dealt with morphological and molecular analysis of *Anopheles* mosquitoes present in different localities of five North Kerala districts. 27 *Anopheles* species were recorded from Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram Districts. Out of these 10 species belonged to the subgenus *Anopheles,* and 17 species to the subgenus *Cellia.* Of the 27 species, two species, *An. sundaicus* and *An. subpictus* B are new records from Kerala. Besides, there are several new records from individual districts. From Kasaragod district, *An. fluviatilis,* and *An. theobaldi* were reported for the first time. From Kannur district seven species of *Anopheles* were reported for the first time viz., *An. crawfordi, An. nigerrimus, An. nitidus, An. sinensis, An. theobaldi, An. dirus,* and *An. sudaicus. Anopheles subpictus* B, the most potent malaria vector was reported from Kozhikode district for the first time. The present study also did the redescription of 27 species that had been collected. Molecular analysis of four malarial vector species, *Anopheles culicifacies, Anopheles dirus, Anopheles stephensi,* and *Anopheles subpictus* was conducted in the present study. *An. culicificies* species complex was resolved. The species prevalent in the study area was confirmed as *Anopheles culicifacies* B. This was the first time such a study was done in Kerala. The study also revealed the presence of *Anopheles dirus* D in the study area. The molecular analysis of the *subpictus* complex revealed the presence of *Anopheles subpictus* B, which is a piece of new information for the entire state.

സംഗ്രഹം

അനഫലിനേ, കൃലിസിനേ എന്നീ ഉപകുടുംബങ്ങളായി വീണ്ടും വിഭജിച്ചിരിക്കുന്ന കൃലിസിഡേ കുടുംബത്തിൽ പെടുന്ന പ്രാണികളാണ് കൊതുകുകൾ. മലേറിയയുടെ വാഹകർ എന്ന നിലയിൽ, എല്ലാ കൊതുകുകളിലും ഏറ്റവും പ്രധാനപ്പെട്ട ഇനമായി അനഫലിനേയെ കണക്കാക്കുന്നു. അനഫലിനേ എന്ന ഉപകുടുംബത്തിൽ മൂന്ന് ജനുസ്സുകൾ ഉൾപ്പെടുന്നു. ലോകമെമ്പാടും വിതരണം ചെയപ്പെട്ട അനഫലീസ് മെയ്ജൻ, ഓസ്ട്രേലിയൻ മേഖലയിൽ കാണപ്പെടുന്ന ബിറോണെല്ല തിയോബാൾഡ്, നിയോട്രോപ്പിക്കൽ മേഖലയിലുള്ള ചഗേഷ്യ ക്രൂസ് എന്നിവയാണ് ഈ ജനുസ്സുകൾ. ഏകദേശം 51 ഇനം അനഫലീസുകൾ ഇന്ത്യയിൽ നിന്ന് രേഖപ്പെടുത്തിയിട്ടുണ്ട്. അനഫലീസ് കൃലിസിഫസീസ്. , അന. ഫ്ലൂവിയറ്റിലിസ്, അന. മിനിമസ്, അന. ഫിലിപ്പിനെൻസിസ്, അന. സ്റ്റീഫെൻസി, അന. സൺഡൈക്കസ് എന്നിവ ഇന്ത്യയിലെ മലേറിയയുടെ പ്രാഥമിക വാഹകർ ആണ്. കൂടാതെ, രാജ്യത്തിന്റെ വിവിധ ഭാഗങ്ങളിൽ മലേറിയ പകരുന്നതിൽ അന. സബ്പിക്കസ് ബി ഒരു പ്രധാന പങ്ക് വഹിക്കുന്നതായി റിപ്പോർട്ട് ചെയ്യപ്പെട്ടിട്ടുണ്ട്. ഇന്ത്യയുൾപ്പെടെ ലോകത്തിന്റെ വിവിധ ഭാഗങ്ങളിൽ നിരവധി രോഗവാഹക സ്പീഷീസുകൾ സ്പീഷീസ് കോംപ്ലക്സുകളായി നിലനിൽക്കുന്നതായി റിപ്പോർട്ടുണ്ട്. അതിനാൽ, മലേറിയ നിയന്ത്രണ തന്ത്രങ്ങൾ ആസൂത്രണം ചെയ്യുന്നതിൽ അവയുടെ പ്രത്യേക തിരിച്ചറിയൽ വളരെ പ്രധാനമാണ്. സമീപ വർഷങ്ങളിൽ ഈ ആവശ്യത്തിനായി മോളികൃലർ ടെക്നിക്കുകൾ കൂടുതലായി ഉപയോഗിക്കപ്പെടുന്നുണ്ട്. വടക്കൻ കേരളത്തിലെ അഞ്ച് ജില്ലകളിലെ വിവിധ പ്രദേശങ്ങളിൽ കാണപ്പെടുന്ന അനഫലീസ് കൊതുകുകളുടെ രൂപശാസ്ത്രപരവും തന്മാത്രാ വിശകലനവുമാണ് ഇപ്പോഴത്തെ പഠനം കൈകാര്യം ചെയ്തത്. കാസർഗോഡ്, കണ്ണൂർ, കോഴിക്കോട്, വയനാട്, മലപ്പുറം ജില്ലകളിൽ 27 അനഫലീസ് സ്പീഷീസുകൾ രേഖപ്പെടുത്തിയിട്ടുണ്ട്. ഇതിൽ 10 സ്പീഷീസുകൾ അനഫലീസ് ഉപവിഭാഗത്തിലും 17 സ്പീഷീസുകൾ സെല്ലിയ ഉപവിഭാഗത്തിലുമാണ്. 27 ഇനങ്ങളിൽ, രണ്ട് ഇനങ്ങൾ, അന. സൺഡൈക്കസും അന. സബ്പിക്കസ് ബി യും കേരളത്തിൽ നിന്നുള്ള പുതിയ റെക്കോർഡുകളാണ്. കൂടാതെ, ഓരോ ജില്ലയിൽ നിന്നും നിരവധി പുതിയ റെക്കോർഡുകളുണ്ട്. കാസർഗോഡ് ജില്ലയിൽ നിന്ന്, അന. ഫ്ലൂവാറ്റിലിസ്, അന. തിയോബാൾഡി എന്നിവ ആദ്യമായി റിപ്പോർട്ട് ചെയ്യപ്പെട്ടു. കണ്ണൂർ ജില്ലയിൽ നിന്ന് ആദ്യമായി ഏഴ് ഇനം അനോഫിലീസുകൾ റിപ്പോർട്ട് ചെയ്യപ്പെട്ടു: അന. ക്രോഫോർഡി, അന. നൈജറിമസ്, അന. നൈറ്റിഡസ്, അന. സൈനെൻസിസ്, അന. തിയോബാൾഡി, അന. ഡൈറസ്, അന. സൺഡൈക്കസ്. പ്രധാനപ്പെട്ട ഒരു മലേറിയ വാഹകനായ അനഫലിസ് സബ്പിക്കസ് ബി ആദ്യമായി കോഴിക്കോട് ജില്ലയിൽ നിന്ന് റിപ്പോർട്ട് ചെയ്യപ്പെട്ടു. ശേഖരിച്ച 27 ഇനങ്ങളുടെ പുനർരൂപകൽപ്പനയും ഇപ്പോഴത്തെ പഠനം നടത്തി. നാല് മലേറിയ വെക്ടർ സ്പീഷീസുകളായ അനഫലീസ് കൃലിസിസ്, അനോഫീൽസ് ൈഡറസ്, അനോഫീൽസ് സ്റ്റെഫെൻസി, അനോഫീൽസ് സബ്പിക്കസ് എന്നിവയുടെ തന്മാത്രാ വിശകലനം ഈ പഠനത്തിൽ നടത്തി. ആൻ. കൃലിസിഫിസ് സ്പീഷീസ് കോംപ്ലക്സ് പരിഹരിച്ചു. പഠന മേഖലയിൽ പ്രചാരത്തിലുള്ള ഇനം അനോഫിലിസ് കൃലിസിഫേസീസ്, 'ബി' സ്പീഷീസ് ആണെന്ന് സ്ഥിരീകരിച്ചു. കേരളത്തിൽ ആദ്യമായാണ് ഇത്തരമൊരു പഠനം നടക്കുന്നത്. പഠന മേഖലയിൽ അന. ഡൈറസ് 'ഡി' യുടെ സാന്നിധ്യവും പഠനം വെളിപ്പെടുത്തി. അന.സബ്പിക്കസ് കോംപ്ലക്സിന്റെ തന്മാത്രാ വിശകലനം അന. സബ്പിക്കസ് ബിയുടെ സാന്നിധ്യം വെളിപ്പെടുത്തി. ഈ വിവരങ്ങൾ കേരളത്തെ സംബന്ധിച്ച് പുതിയവയാണ്.

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INTRODUCTION

Mosquitoes are an ancient group of insects belonging to the order Diptera, suborder Nematocera, and family Culicidae and are widely distributed around the World (Tandina., *et al*, 2018). Approximately 210 million years ago, mosquitoes evolved in the Jurassic period. The oldest fossil of a mosquito discovered so far is from Northern Burmese amber which is estimated to have lived during the Cretaceous period about 90-100 million years ago (Borkent and Grimaldi, 2004). The word "MOSQUITO" is from Spanish or Portuguese, meaning "little fly". These groups of insects are highly adaptable to diverse types of environmental conditions and is a monophyletic taxon (Wood & Borkent, 1989; Miller *et al*., 1997). Mosquitoes are prevalent throughout tropical and temperate regions of the world but completely absent in Iceland, a sub-polar island that is situated 6,000 m above sea level (Lane and Crosskey, 1993). The mosquito family Culicidae has two subfamilies, viz., Culicinae and Anophelinae. It contains around 3726 species (http://mosquito-taxonomic-inventory.info/). Their immature forms (eggs, larvae, and pupae) live in highly diverse habitats in terms of quality of water (potable water, organically polluted water, brackish water, etc.) and size of habitats (hoof prints, tree holes, paddy fields, wells, ponds, lakes, etc.) (Rattanarithikul *et al*., 2005; Harbach & Howard, 2007). Mosquitoes have attracted the attention of taxonomists and biologists all over the world due to their role in the transmission of various diseases like Malaria, Dengue, Yellow Fever, Lymphatic Filariasis, Japanese Encephalitis, Chikungunya, West Nile Virus, and Zika among many others (Kettle, 1995).

1.1 Mosquitoes in India

India is one of the world's 12 mega-diverse nations and has a significant number of all living things including mosquitoes. After Brazil, Indonesia, Malaysia, and Thailand, India is placed fifth in terms of mosquito diversity (Foley *et al*.,

2007). Serious efforts to study mosquito fauna in India began only after the discovery of the role of mosquitoes in the transmission of Malaria by Ronald Ross in 1897. Till then only two mosquito species were documented from the entire country (Giles, 1901). Since 1901 hundreds of mosquito species have been added to the mosquito fauna of India. In the pre-independent era, the major contributors were Giles (1901), Theobald (1901 and 1910), Edwards (1922), Christophers (1933), and Barraud (1934). In the post-independent era, the major pan-Indian faunal studies are attributed to those of Rao (1984), Nagpal and Sharma (1995). According to the latest catalog of Indian Mosquitoes, there are 404 mosquito species in the country (Tyagi *et al.*, 2015).

1.2 Mosquito: Taxonomy and Classification

Mosquito taxonomic research studies began with Linnaeus, who named the first genus *Culex* in 1735 and also did the description of 6 species belonging to this genus and recorded in his book Systema Nature. The genus *Aedes* and *Anopheles* were described by Meigen in 1818. The subfamily *Culicinae* consists of 38 genera and *Anophelinae* has three genera. The Culicinae is again segregated into 11 tribes. Aedeomylini (*Aedeomyia*), Aedini (*Aedes, Armigeres, Eretmapodites, Haemagogus, Heizmannia, Opifex, Psorophora, Psorophora, Udaya, verrallina, Zeugnomyia*), Culicini (*Culex, Deinocerites, Galindomyia, Lutzia*), Culicetini (*Culiceta*), Ficalbiini (*Ficalbia, Mimomyia*), Hodgesiini (*Hodgesia*), Mansoniini (*Coquillettidia and Mansonia*), Orthopodomyiini (*Orthopodomyia*), Sabethini (*Isostomyia, Johnbelkinia, Kimia, Limatus, Malaya, Maorigoeldia, Onirion, Runchomyia, Sabethes, Shannoniana, Topomyia, Trichoprosopon, Tripteroides, Wyeomyia*) Toxorhynchitinii (*Toxorhynchites*) and Uranotaeniini (*Uranoteania*) (Wilkerson *et al.,* 2021).

1.3 Subfamily *Anophelinae*

The subfamily *Anophelinae* comprises 485 officially recognized species, along with several nameless individuals from unofficially classified species complexes (WRBU, 2020). The subfamily *Anophelinae* consists of three genera viz., *Anopheles* Meigen, *Bironella* Theobald, and *Chagasia* Cruz (Wilkerson *et al*., 2021) Although an exhaustive phylogeny of these mosquitoes is far from complete, anopheline mosquito evolution has been the focus of conjecture and research for

decades (Harbach, 2015). The genera are commonly known as "Anophelines". The genus *Bironella* Theobald contains eight species that are exclusively native to the Australian region. The wing veins of Cu1 and M, which are wavy at their distal parts, and the palmate thoracic setae 1-M and 3-T in the larvae make them easily distinguishable from *Anopheles* (Becker *et al*., 2010). *Bironella* has three subgenera, *Bironella*, *Brugella*, and *Neobironella* (Harbach & Kitching, 2005). The genus *Chagasia* Cruz is located exclusively in the Neotropical region. It comprises only four species. The genus is a homologous group and it is not subdivided yet. (Becker *et al*., 2010).

Most of the Anopheline species belong to the genus *Anopheles*; which is again subdivided into seven subgenera: *Anopheles* Meigen*, Baimaia* Harbach, Rattanarithikul and Harrison*, Cellia* Theobald, *Kertezia* Theobald, *Lophopodomia* Antunes*, Nyssorhynchus* Blanchard, and *Stethomyia* Theobald. The primary defining characteristics of the subgenera are the number and distribution of specialized setae on the male genital monocoxites (Christophers, 1915). *Anopheles, cellia, and Nyssorhynchus*, the three largest subgenera again divided into informal taxonomic divisions (Christophers, 1915; Reid 1968; Harbach and Kitching, 2005). Subgenus *Anopheles* is divided into two sections and *Nyssorhynchus* into three sections (Reid & Knight, 1961; Faran, 1980; Harbach, 2004).

The genus *Anopheles* comprises around 420 species worldwide. The subgenus *Anopheles* is distributed all around the world's 12 zones with a maximum in the Malaysian zone with 56 species and a minimum of 11 species in the Afrotropical zone. 115 species of subgenus *cellia* which is recorded in Afrotropical zones is its highest number and the lowest distribution in South American zones with only one species. It is also distributed in the Australian and Oriental regions. The subgenus *Kerteszia* is reported only in the American zones: 11 species in South America, 5 species in Central America, and only one in North America. The *Lophopodomia* subgenus has been reported from South America(11sps) and Central America (1sps) (Nagpal & Sharma, 1995).

The phylogenetic study on the subfamily *Anophelinae* remains unresolved and the first study was conducted by Sallum *et al* in 2000 based on morphological characteristics. In their study, they concluded that, due to the inclusion of the genus

Bironella the genus *Anopheles* is paraphyletic. The other subgenera *Kertezia, Nyssorhynchus, Cellia, Lophopodomia Stethom*ia along with the genus *Bironella* are monophyletic. *Anopheles* was demonstrated to be a monophyletic group by Colluci and Sallum using 111 morphological features, 36 species, and five outgroup data, demonstrating that *Bironella* was a sister lineage (Collucci & Sallum, 2007).

1.4 External Morphology of *Anopheles* Mosquitoes (Fig.1)

Mosquitoes are slender, delicate, small, and long-legged insects with a pair of wings, and the whole body covered with hairs and scales and measured about 3-4 mm in length. The body is divided mainly into three regions- Head, Thorax, and Abdomen. The head is prognathous and globular. It is highly mobile and interacts with the thorax through a small neck. The head bears a pair of compound eyes, and 14-15 segmented antennae, which is plumose in males and pilose in females and mouthparts. Mouthparts are piercing and sucking type with a pair of mandibles, a pair of maxillae, labium, labrum, and hypopharynx. Proboscis is long, slender, black or brown coloured, and modified for piercing and sucking. Scales and setae cover the head, thorax, and abdomen; the coverage varies depending on the genus. The scales often cover the legs, wing veins, and wing edges. Scales cover the mosquito's integument widely. Scales are essentially flattened setae that contain colour and frequently have striated surfaces that provide optical effects, giving some physicochemical coordination. Although the colouration of scales can range from white to nearly black, while describing a species it is typically referred to as pale or dark (Becker *et al*., 2020).

Head

The sensory part of the mosquito body is the head. The two compound eyes are dominant in the roughly spherical head and are excellent visual organs even in low-light conditions. Chemosensory and mechanosensory antennae that develop between eyes are used for both functions. Antennae consists of three regions. The long, segmented, whip-like part of the antenna is known as flagellum. Each segment of the flagellum is known as flagellomere and is covered wholly by sensory setae. The basal portion of the flagellum is swollen or bulbous and is known as the pedicel; which plays a major role in the reception of vibration from sensory setae of the

flagellum. The forward projecting region of the head that gives paired maxillary palps and the proboscis is covered by the clypeus, which is located underneath the antennae. Maxillary palps simply referred to as palps, are shorter in females than males in the case of most mosquitoes. The head capsule is made up of several sclerites that have been combined to form the exterior of the head. Compound eyes take up a significant amount of the skull. The frons and vertex are parts of the head located below and above the frontal sutures, respectively. The occiput and post-occiput which resemble a ring are placed at the back of the head, between the vertex and cervix. The proboscis is nearly straight and cylindrical and articulated with the gnathocephalon and contains piercing mouthparts.

Thorax

The dorsal and lateral surfaces of the thorax are coated in scales that might be shiny or matte, white, brown, black, or nearly any other color. A pair of wings (on the mesothorax), a pair of halteres (metathoracic origin), three pairs of legs (one pair on each thoracic segment), and two respiratory spiracles (on each side) are all present on the thorax. Mosquitoes' wings are long and slender with wing veins that are covered with scales. Costa, subcostal, radius, media, cubitus, and anal vein are the longitudinal veins of wings with branches. Vein M1(anterior branch of media one) and vein 2(posterior branch) are the numerals designating the branches of these veins. Crossveins are short, generally transverse, and lie between two longitudinal veins. Both the dorsal and ventral sides of the veins are covered in scales, and the apex and posterior margin of the wing have a fringe of scales on them. The size and form of the scales on various wing sections vary greatly. Although many species have some pale scales mixed throughout or in patches, the majority of them have dark scales. Wing spots, which are distinct patches of pale and black scales found in most *Anopheles* species (http://mosquito-taxonomic-inventory.info/). The short, knobby halteres help to keep the balance during flight. Scutum which is known as the major dorsal portion of the thorax, the Scutellum is located posterior to the Scutum, while the mesopostnotum is located posterior to the scutellum.

Adult mosquito consists of three pairs of legs, foreleg, midleg, and hind leg (longest leg) respectively. The leg consists of five main segments. As the base

segment, the coxa is followed by the trochanter, femur, tibia, and tarsus. The subunits of the tarsus are known as tarsomere. It ends in a claw at the apical tarsomere.

Abdomen

There are ten segments in the abdomen, but only the first seven or eight are visible. In *Anophelinae*, the abdomen is nearly, or completely, absent of scales. Each segment is covered by dorsal and ventral plates, tergites, and sternites respectively. These tergites and sternites are interconnected by membranous exoskeleton. Cerci are finger-like appendages that function in egg-laying and copulation that terminates the abdomen (Burkett-Cadena, 2013). I-VII abdominal segments are similar in size and shape, VIII th segment is often smaller than the seven segments that came before it, shaped like a pentagon, and bearing the respiratory siphon and comb scales. *Anopheles* species lack siphon and breathe through a flattened spiracular mechanism on segment VII. Segment IX is a reduced structure and segment X or anal segment bears anal papillae, saddle, and ventral brush.

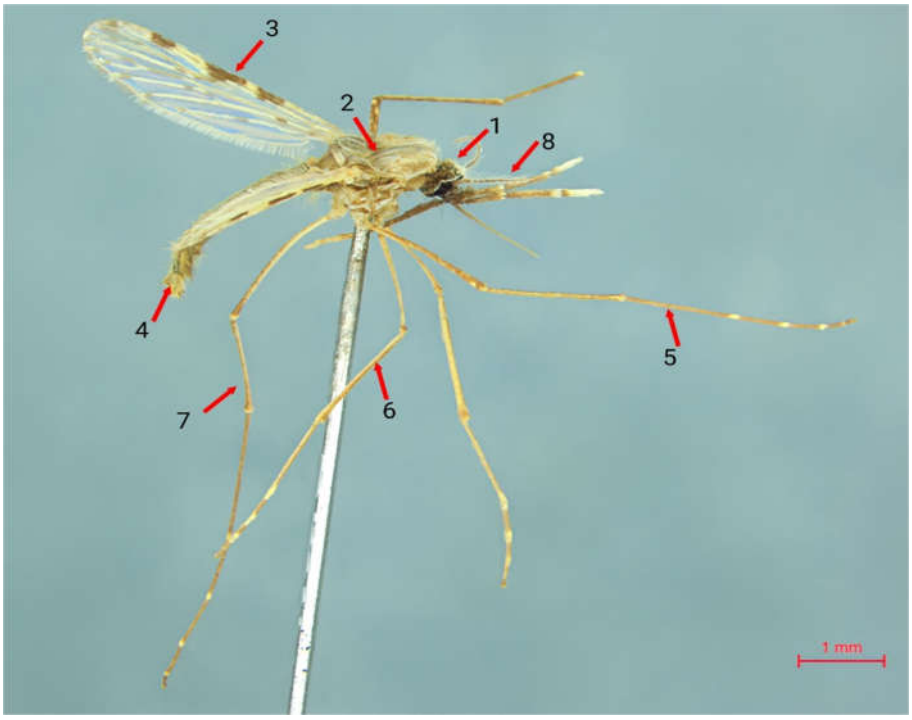


Fig. 1.1 Female Adult *Anopheles* Mosquito (1-Head, 2-Thorax, 3-Wing, 4-Abdomen, 5- Fore leg, 6- Mid leg, 7- Hind leg)

1.5 Life cycle (Fig 1.2)

The biology and life history of mosquitoes were studied in the 1890s (Dam, 1890). The life cycle of mosquitoes consists of four distinct and separate stages, Egg, Larva, Pupa, and Adult. Each stage is easily distinguished from each other by their distinctive appearance and it is known as complete metamorphosis.

Egg: There are several methods by which female mosquitoes lay their eggs. While selecting a location females consider temperature, salinity, and oxygen content. *Anopheles* eggs are easily identified by their canoe-like shape and the pair of floats on either side and laid singly on the water surface. An egg raft is roughly ¼ inch long and 1/8 wide, it resembles a soot particle floating on the water. There could be water in pools, Puddles, creeks, ditches, catch basins, horse troughs, barrels, ponds, or marshy places. There could be 50-100 eggs on each raft (https://www.swmosquito.org/mosquito-biology). Many other genera including *Aedes* lay their eggs singly on damp soil. Egg hatching happens when it is flooded with water. At about 30^0 C, eggs hatch into larvae in about 2-3 days. While in temperate zones (16^0C), about 7-14 days.

Larva: The egg hatches into larvae from 7 to 14 days or longer and it depends on water temperature. They commonly known as 'wrigglers', tend to congregate in regions where most protected from predators. *Anopheles*, lack respiratory siphons and lie parallel to the water surface to obtain oxygen through breathing openings along their abdomen. With the help of their specialized mouthparts, the larvae filter food from the water by feeding on bacteria, algae, plankton, fungi, and other microorganisms. Larvae undergo four molting events during their growth phase, with each molt resulting in increased size. The larvae stop feeding towards the end of the fourth instar and change into pupa after molting into its fourth instar.

Pupa: It is sensitive to light, shadows, and other disturbances and still needs to breathe air at the water's surface even though it is not feeding at this stage. Pupae are also known as "tumblers" since they are known to roll or tumble to get to deeper water. After a period of one and a half to four days, the pupa's skin breaks along the back, enabling the adult to emerge and rest on the water surface.

Adult: When the pupal case splits open, the adult mosquito emerges to the water surface and rests until its body hardens and dries.

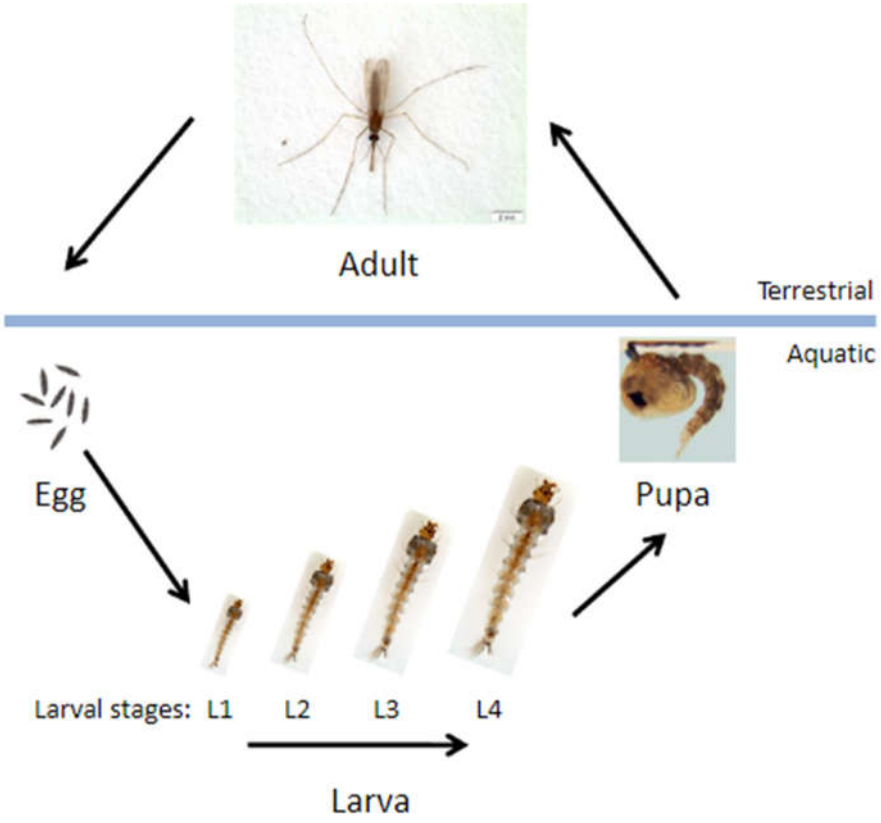


Fig.1.2 Life cycle of *Anopheles* Mosquitoes (William and Pinto, 2012)

1.6 Molecular Taxonomy of *Anopheles*

Through the determination of morphological characteristics utilized in dichotomous keys, classical taxonomy has allowed us to learn about mosquito species, resulting in a clustering that may not be similar in their distribution in ecosystems (Cova *et al*., 1974). Morphological identification needs skilled taxonomists, and the process itself takes a lot of time, especially when used by less experienced researchers. In addition to this, crucial morphological characteristics like scales and bristles are harmed due to inappropriate specimen care. So inadequate identification is frequently experienced (Abigail *et al*., 2014). Identification is also challenging using only taxonomic keys since members of species complexes exhibit comparable morphological characteristics. Due to the lack

of development of morphological characters in early larval stages, the majority of taxonomic keys are only applicable to adult female mosquitoes and larvae in their fourth instar (Jinbo *et al*., 2011). A supplementary approach to determining the evolutionary relationships between species that have been challenging to ascertain by morphological characteristics, sexual dimorphism, and developmental stages is molecular systematics (Shah *et al*., 2015). In 1993, DNA barcoding was developed as a method to standardize the use of molecular markers for species identification and taxonomic allocation through phylogenetic inference based on genetic diversity (Arnot *et al*., 1993, Andrew *et al*., 2021, Herbert, *et al*., 2003). This approach is based on the idea that each species has its genetic diversity (Herbert, *et al*., 2003). The nuclear internal transcribed spacer (ITS), cytochrome b oxidase, 12S rRNA, and nicotinamide adenine dinucleotide dehydrogenase were chosen as target genes in early DNA barcoding research. In recent years, the mitochondrial cytochrome c oxidase subunit 1(CO1) gene has grown popularity due to the simplicity of using a set of universal primers to amplify the gene and its capacity to provide a higher level of sequence variation between species than within species (Merget *et al*., 2012, Sevilla, *et al*., 2007, Shen *et al*., 2007, Vences *et al*., 2005 and Chu *et al*., 2006). Through the use of molecular taxonomy, one can identify mosquitoes down to the species or subspecies level, comprehend genetic diversity, and make inferences about evolution and phylogenetic relationships. DNA-based methodologies for the identification of mosquito species (Manonmani *et al*., 2001; Sing *et al*., 2004; Kang & Sim, 2013), molecular phylogeny (Shepard *et al*., 2006), and genetic diversity (Pfeiler *et al.,* 2013) have gained momentum in recent years as it is faster to perform and more reliable. Mosquitoes have the most barcodes of any group of barcoded insects likely as a result of their significance as carriers of numerous deadly human diseases (Ondrejicka *et al*., 2014). The Mosquito Barcoding Initiative Database is contained within the International Barcode of Life (IBOL), it consists of sequence data from 1182 species and about 37,000 specimens from all over the world (Laurito *et al*., 2022).

The mosquito species coming under the subfamily *Anophelinae* are exclusive vectors of human Malaria (Coetzee *et al*., 2013; Van Birtel *et al*., 2001).

Identification within the Genus *Anopheles* is challenging because of the prevalence of biotypes, ecotypes, and cytotypes which are difficult to differentiate based on morphological characteristics alone. Because of single species exhibits heterogenicity across a vast geographic range, the phenomenon of species complex further complicates identification (Anyanwu *et al*., 1997). Extensive barcoding research on *Anopheles* species was conducted in India. Some selected work from Northeast India as follows, Molecular evidence of *Anopheles nivipus* (Bhattacharya *et al*., 2010), spatial distribution, and molecular characterization of *Anopheles nivipus* and *An.philippinensis* (Sarma, 2012), same on *Anopheles maculatus* group (Singh *et al*., 2012), species identification and molecular characterization of the *Anopheles dirus* (Prakash *et al*., 2006), and the most recent research came from Meghalaya, a state in Northeastern India where *Anopheles dirus* has a dominant role in Malaria transmission and researchers identified the sibling species of this complex (Singh *et al*.,2023). From the Southern part of India, *Anopheles* phylogenetic studies are infrequent, especially from Kerala. Hence, molecular taxonomy in the present study was focused mainly on the identification of major malaria vectors prevalent in North Kerala.

1.7 Systematic Studies of *Anopheles* in India

During the pre-independence period, several publications dealt with either *Anophelinae* or *Culicinae* (Christophers, 1933; Barraud, 1934). Soon after Independence, much literature was published that exclusively dealt with *Anopheles* mosquitoes (Puri, 1949). But in several ways, lack of clarity on species synonymization, and various dark points in taxonomic identification persists in India. So, India needs updated taxonomic information on mosquito fauna, especially *Anopheles*. The last new species of *Anopheles* described was *Anopheles psedosundaicus* from Kerala (Tyagi *et al*., 2009). Mosquito studies in Kerala were pioneered by the British Scientists Christopher (1933) and Barraud (1934). Christopher reported 16 Anopheline species in his book “Fauna of British India” and Barraud reported 41 species under *Culicinae* and *Toxorhynchitinae* (Iyengar, 1938).

1.8 *Anopheles*: Major species complexes in India

In India, multiple *Anopheles* species are known to transmit Malaria, and the disease epidemiology is complicated because of the wide range of ecological and contextual factors (Avikar, *et al*., 2012; Singh & Mishra *et al*., 2009). There are 58 species in the Indian anopheline fauna, nine of which are malaria vectors. *An. fluviatilis, An. stephensi, An. culicifacies, An. mininus, An. dirus, and An. sundaicus* are the primary Malaria vectors. Except *An. stephensi* all of these are species complexes (http://www.mrcindia.org). Species complexes of *An. annularis* and *An. (philippinensis) nivipus* are among the vectors of secondary importance.

Anopheles vector species	Sibling species
Anopheles culicifacies	A, B, C, D, E
Anopheles fluviatilis	S, T, U, V
Anopheles dirus	D, E
Anopheles minimus	A, C, E
Anopheles sundaicus	A, D, E
Anopheles annularis	A & B
Anopheles subpictus	A, B, C, D
Anopheles philippinensis- nivipus	

1.9 Malaria

With 1.5 to 2.7 million deaths each year, malaria is still one of the world's most serious health issues. Globally in 2022, there were an estimated 249 million Malaria cases in 85 malaria endemic countries and areas (WHO, 2023). Malaria is the most significant parasitic illness in the world even though comprehensive control and elimination methods have been deployed in international and national malaria control programs (Al-Awadhi *et al*., 2021). Epidemiologically there are regional variations in the frequency of malaria cases, the cause of the disease, how severe it is, the prevalence of antibiotic resistance, and the fatality rate. According to the World Health Organization (WHO) most current annual global malaria report, there were an estimated 229 million cases of malaria in 87 countries with high malaria prevalence in 2019, a decrease of 9 million cases from 2000.

According to the 2022 World Malaria Report, there were 89 nations and 247 million malaria cases and 619000 malaria-related deaths in the world in 2021. WHO statistics show that 213 million or 93% of all malaria cases in 2018 occurred in the WHO's African region 3.4% of cases occurred in the Eastern Mediterranean region. Three nations India (58%), Indonesia (30%), and Myanmar (10%) are the top three contributors (WHO,2019). The burden of malaria in India is 3% of the total. India had a 49% decrease in reported cases and a 50.5% decrease in deaths from malaria compared to 2017, despite having the greatest malaria load in the SEA region (WMR, 2020).

Ronald Ross first identified the malaria parasite in a mosquito in 1897. The investigation on malaria transmission has been sparked by this significant finding reported in Secunderabad, India. Scientists have focused on cataloging the contributions of *Anopheles* mosquitoes to the spread of malaria. The entire cycle of malaria parasite development in humans and female *Anopheles* mosquitoes was discovered in 1898-1899 by Italian scientists Amico Bignami, Giuseppe Bastieanelli, and Battista Grassi. In 1900, Patrick Mansion and his associates proved that female Anopheles mosquitoes are the only vectors of Malaria.

Malaria in humans is caused by five Plasmodium species viz., *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi*. A total of 41 *Anopheles* mosquitoes can actively disperse plasmodium species, which causes human malaria (Foaster *et al*., 2017). Most malaria infections in Africa are caused by *P. falciparum*, which causes cerebral malaria and the majority of malaria-related mortality worldwide. Tropical, subtropical, and temperate zones all have a wider spread of *Plasmodium vivax*. However, the increasing prevalence of *Plasmodium vivax* infections, particularly in the Indian subcontinent poses unique diagnostic and therapeutic challenges (Dayanandha *et al*., 2018). The global distribution of *Plasmodium malariae* is uneven. *Plasmodium ovale* primarily prevalent in tropical regions, is the fourth and rare species of the malarial parasite. However, this species exists in some of the most

isolated tribal areas of Thailand and India (Orissa state) (Jumbulingam *et al*.,1989; Baired *et al*., 1990).

The malaria vectors incriminated from India include *An. culicifacies, An. fluviatilis, An.stephensi, An.philippinensis, An. dirus, An. annularis, An. sundaicus, An.aconitus, An. maculatus, An. tessellatus and An.subpictus* (Rao,1981). Among these vectors *An. culicifacies and An. stephensi* are the primary vectors of rural and urban Malaria in India respectively. The disease is distributed throughout the world in such a way that local climate, circumstances, terrain, and the socioeconomic position of the population all directly influence the parasite and vector species that are common there.

1.10 Malaria: Kerala Scenario

In the past, malaria was a major public health concern in Kerala. In prehistoric times Kerala had been haunted by Malaria among tribal people living in the hills and forests (DHS Kerala, 1964). Malaria originated in steep areas and progressively moved to foothills and surrounding areas where it caused sporadic outbreaks and deaths. Evidence of the long history of Malaria in the state comes from the high incidence of sickle cell anemia among the tribal people of Wayanad and Attapadi (Feroze & Aravindan, 2001; Kaur *et al*., 1997). Malaria was a serious illness burden in the state before independence, contributing significantly to the rates of morbidity and mortality (Covell & Singh, 1939).

In 1965, Kerala eliminated malaria through the National Malaria Control Programme, 1953 and the Malaria Eradication Programme launched in 1958. But around 1975, malaria cases were reintroduced (DHS Kerala,1978). Sumodan (2002) provided data on the frequency of both imported and indigenous cases of malaria in the Wayanad District and made a strong case for the significance of imported cases. During the past ten years, Kerala's malaria epidemiology has changed. The state's most malarious hill regions are now virtually devoid of the illness, but urban areas along the coast still have the disease at moderate rates. This shift may be due to the emergence of *Anopheles stephensi* in the coastal belt and the disappearance of *An. fluviatilis* from hills and foothills. This notorious vector or the urban malaria vector

An. stephensi contributed to the malaria outbreak in Valiyathura at Thiruvananthapuram in 1996. This was followed by another outbreak in Kasaragod in 1998 (DHS Kerala, 1997, 1998, 1999). (Table 1.1). At present, the rate of malaria infections in the state has increased due to the spread of *Anopheles stephensi* along the entire coastal region and in certain inland places (NVBDCP, 2020).

Table 1. Malaria situation in Kerala from 2010-2023 (Source: Directorate of Health Services, Kerala)

Year	Cases	Death
2010	2299	7
2011	1993	2
2012	2036	3
2013	1634	0
2014	1751	6
2015	1549	4
2016	1540	3
2017	1194	2
2018	908	0
2019	656	1
2020	268	1
2021	309	1
2022	439	0
2023	551	7

The knowledge of the vector species involved, their habitat preference, and geographical distributions are critical in planning and implementing control strategies against Malaria in the State. Hence it has become inevitable to have an in-depth understanding of the complete profile of *Anopheles.* Fairly extensive work has been done on the taxonomy of *Anopheles* in India. However, the status of *Anopheles* systematics is rudimentary and scattered in Kerala. No comprehensive surveys and

documentation of the genus have been undertaken in the state so far. The present study explored the complete taxonomic information on the *Anopheles* fauna of North Kerala districts. Besides, the resolution of vector species complexes based on molecular analyses was also attempted.

Objectves of study

1. To survey identify and describe *Anopheles* fauna of five North Kerala District (Kasargod, Kannur, Wayanad, Kozhikode & Malappuram) and to study the habitat preferences of various species.

2. Preparation of Dichotomous key up to species level.

3. Molecular taxonomy of malaria vectors.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Taxonomic Studies of *Anopheles* Mosquitoes

2.1.1 Global level

Mosquitoes have been the subject of comprehensive study worldwide ever since it was discovered that they could spread human diseases. The discovery that mosquitoes transmit Malaria and Yellow fever drastically changed this field and initiated the classification and description of mosquitoes. Mosquitoes belong to the Family Culicidae of the Order Diptera of Class Insecta (Knight & Stone, 1977; Ward, 1984, 1992; Harbach, 1994). The genus *Anopheles* was founded by Meigen in 1818.

Stephen (1828), described a new species of *Anopheles*, *An. plumbeus,* which was collected from North Ireland. Giles (1900), described two new species of *Anopheles* from West Africa. Theobald (1900) made an important contribution and added another new species (*Anopheles paludis*) to *Anopheles* diversity from Sierra Leone. Theobald (1900a) prepared a report on the collection of mosquitoes worldwide. Genus *Anopheles* was represented by twenty-two species of which ten were new, and constructed an extended monograph of the family Culicidae with a large number of species (3000 specimens). Theobald (1902) classified the Subfamily *Anophelinae* based on scales on the thorax, abdomen, and wings of fifty Known *Anopheles* species. Coquillett (1903) published a research article and added one more species of *Anopheles* (*Anopheles barberi*) from Plummers Island, Maryland. Favre (1903) published a book in which the author attempted to study Malaria in Russia. McCracken (1904) published an article and described *Anopheles* from California along with a description of a new species named *Anopheles franciscanus*. This species was found in small numbers occasionally in company with *Anopheles maculipennis*. Carter (1910) released an article with the description of a new anopheline from South Africa, described from two females that closely resembled *Pyretophorus sergentii* but differed in the wing spotting and leg banding and the

species was named *Pyretophorus transvaalensis*. Alcock (1910) published an article, that included 100 species from Genus *Anopheles* distributed in all the zoogeographical regions. Edwards (1911) described a new *Anopheles* from West Africa and published an article in the Bulletin of Entomological Research in which he described *Anopheles flavicosta* which closely resembled *An.pitchfordi* and *An.anstenii.* The second new species was *An.watsoni* which closely allied to *An.maculipalpis.* Alcock (1912) described a new species from the Malay Peninsula and the species was *Anopheles wellingtonianus.* The larvae were collected from the drinking water reservoir at Larut Hills. This species closely resembled *Anopheles barbirostris.* Alcock (1913) prepared a synopsis of *Anopheles* mosquitoes of Africa and the Oriental region. The work focused on identifying the *Anopheles* by distribution on scales on the body. Banks (1914) released a journal article, related to the emergence of a new malaria vector in Calamba and the species *Myzomyia febrifera* which very much resembled *Myzomyia funesta Giles* and *M. rossii*. Walker and Barber described this species. Christopher (1915) explained the male genitalia of Anopheles. Carter (1920), described the male genital armature of *Anopheles maculipennis* Meigen, *Anopheles bifurcates* Linnaeus, and *Anopheles plumbeus* Stephens in detail. Christophers (1922), published an article in the Indian Journal of Medical Research that described a new species of *Anopheles* in East Africa and he named the species *Anopheles kingi*, H H Kingi collected the specimen. This species closely resembled *Anopheles natalensis* in palpal ornamentation, characteristic speckling of the legs, and scaling and marking of the wings. Christophers (1923), published an article that explained a new record of *Anopheles* species from South America and the species was *Anopheles amazonicus*. This species closely resembled *Myzorhynchus* group. Root (1924) published an article and explained the male genitalia of American mosquitoes. Christophers (1926), described a new species from North China. The species was collected by W S Patton from Shan-tung, North China and it was described by Christophers, named the species as *An.pattoni*. It was very similar to *An.karwari* reported from India.

Manalang (1930) described the morphology and classification of the Philippine variety of *Anopheles minimus* Theobald in detail. Evans (1931) described

a new subspecies of *Anopheles funestus* Giles from Southern Rodentia. A new variety of *Anopheles maculipennis* from southern Europe was reported by Hackett and Lewis (1935). A new species of *Anopheles*, *Anopheles habibi* was described by Mulligan and Puri (1936) in Quetta, Baluchistan. Urbino (1936), analyzed eggs of some Philippine *Anopheles* species and prepared an identification key based on the structure of the eggs. King (1936) described a new *Anopheles* from the Phillipines and the species was named *Anopheles cristatus*. Komp (1936), described a species from Panama, based on specimens collected from hollow trees and stream banks from an altitude of about 6500 feet, and named the species *Anopheles chiriquiensis*. Chang (1937), studied the maxillary teeth in the *Anopheles* and *Hyrcanus var. sinensis* Wiedemann in the Shanghai region. Crawford (1938), studied the structure of pupae and described the pupal stages of 17 species of *Anopheles* in detail. Hurlburt (1938), described the eggs of *Anopheles walkeri*. They also studied the overwintering of the eggs of *An. walkeri* under experimental conditions. *Anopheles sogdianus*, a new species from Tadjakstan, was described by Keshishian (1938). Aitken (1939) explained the *Anopheles macculipennis* complex in Western Africa and gave details on a new species in this complex *Anopheles maculipennis freeborni*. Bates (1940) explained the taxonomic status of the *Anopheles maculipennis* complex.

King and Bradley (1941), carried out a study on *Anopheles* species in the Nearctic region. They explained the general morphology and classified the *Anopheles* species in detail with taxonomic keys based on the characters they studied. Belkin and Ralph (1944) reported and described a new species from the Solomon Islands named *Anopheles linguae.* From there they collected four distinct forms of Anophelines (two forms of *An.punctulatus* Donitz and another was a new one. Causey and Deane (1944) identified and prepared illustrated keys to the eggs of thirty Brazilian Anophelines with several new species collected from the Amazon Valley in the northeastern and eastern coastal regions. They collected eggs of more than 28000 female anophelines and reared them into adults. During this study, they also described three new species, *Anopheles (nyssorhynchus) sawyeri, Anopheles (Nyssorhynchus) galvaoi, and Chagasia rozeboomi.* The eggs of nine species were

described for the first time. The key of thirty species were prepared based on floats, frills, and pattern of exochorion present. Lee (1944), described a new species named *Anopheles powelli* species collected from northern Australia and the species resembled *Culex*. In Australia, this species resembled *An.stigmaticus* Skuse and *An.aitkenii* James. Woodhill and Lee (1944), explained a subspecies of *Anopheles amictus*, the species was originally described by Edwards. Belkin (1945) identified a new species of *Anopheles nataliae* from the Guddal Canal in New Georgia. Belkin and Knight (1945) reported and described the taxonomic status and biology of *An. punctulatus* and *An. punctulatus moluccensis* in the Solomon Islands and New hebrids. They made an intensive collection of immatures and rearing was also done. Causey *et al.,* (1946) they prepared an identification key based on male genetalic characters of 34 species of Anophelinae from the Northeast and Amazon region of Brazil.

Ried (1953) studied on *Anopheles hyrcanus* group and discussed its classification, distribution, evolution, biology, and relation to disease in detail from Southeast Asia. Aitken (1953), published an article that dealt with *Anopheles* fauna of Sardinia. During their study period, they identified seven species of *Anopheles* Viz., *Anopheles labranchiae labranchiae* Falleroni, *Anopheles melanoon melanoon* Hackett, *An. clavingert*, *An.algeriensis* Theobald, *An. marteri* Senvet and Prunelle, *An.plumbeus* Stephens, *An. Hispaniola*. They also explained a brief description of the various life cycle stages of each mosquito. Pringle (1954) published a key to the adult Anopheline mosquitoes of Iraq and its surrounding regions. Hara (1957) studied female terminalia of 12 species of genus *Anopheles* belonging to the subgenus *Anopheles* and Myzomyia in detail and also provided keys for these mosquitoes. Ohmori (1957) explained four species of sinensis group ie, *Anopheles yatsus- hiroensis, Anopheles sinensis* Weidemann, *Anopheles sineroides* and *Anopheles lesteri* from Japan. Chang and Huang (1954 and 1955), explained anopheline diversity in Taiwan. Dobrotworsky (1957) made notes on Australian mosquitoes with the description of *Anopheles skuse* and also a new species description (*An. papuensis*). Buttiker and Bales (1959), carried out a study on *Anopheles minimus* group and discovered a new species and described it, and named

it *Anopheles pampanae* from Cambodia. Shahgudian (1960) published a key in the Anophelines mosquitoes from Iran. Services (1962) published a key to the West African Anophelini. A key to the adult female and larval Anopheline mosquitoes of the Philippines was published by Baisas and Dowell (1965). Scanlon *et al.,* (1968), released an annotated checklist of the fauna of *Anopheles* from Thailand. Reid (1968) developed a key to the Borneo and Malayan Anopheline mosquitoes. Cagampang-Romas and Darsie (1970) released a key to the *Anopheles* mosquito population on the island of the Philippines.

A Turkish *Anopheles maculipennis* complex was described by Postiglione *et al.,* (1970). Harrison (1972) published an article that dealt with the anopheles Hyrcanus complex of Southeast Asia. This study examined about a thousand specimens and interpreted Ried's works in 1953, 1963, and 1968. The new interpretation based on analysis of characters from all life stages and explained the affinities within the southeast Asian *Anopheles hyrcanus* complex. Harrison (1973) published an article and described a new species of the barbirostris species complex from Sri Lanka named *Anopheles reidi.* He compared the specimen morphologically with other members of the barbirostris complex and members of the Bancrofti species complex. Harrison & Scanlon (1974), described a new species in the aitkenii complex named *Anopheles pilinotum* and this was a redefined species of *An. insulaeflorum*. Xu Jin-Jiang and Feng (1975) studied the hyrcanus group in China from 1962 to 1965. They discussed in detail on life stages, bionomics, and distribution of the hyrcanus group of China and the group consists of nine closely related species. Of these nine two were new species- *Anopheles lesteri* antropophagus (subspecies) and *Anopheles kiangsuensis*. Meillon & Eeden (1976) they reported and described a new species from South Africa- *Anopheles (cellia) deaconi.* The species closely related to *Anopheles listeri* a very common species in that area. Darsie & Cagampang Ramos (1977) studied *Anopheles ludlowae* cabrerai a new sub-species described by the authors itself in 1969. This study explained the *Anopheles ludlowae* cabrerai was a new synonym of the subspecies *Anopheles ludlowae* with morphological variation and without reproductive isolation. Kanda & Oguma (1978) published an article discussing with description of a new species

named *Anopheles engarensis* in Japan. This species has been separated from *Anopheles sinensis*. Python and Harrison (1979) reported and described a new species from Thailand under the leucosphyrus group a major vector of Malaria named *Anopheles dirus*. Faran (1981), published an article in mosquito systematics, they synonymized the species *Anopheles noroestenensis* with *Anopheles evansi* based on a morphological comparison of male genitalia of these species and also described the male genitalia of *An. evansi.*

Dong (1984) described a new species *Anopheles kunmingenesis* from China and the species closely related to *Anopheles sinensis* from Hyrcanus group. White (1985) described the new species *Anopheles bwambe* from the Ugandan Semliki Valley. Rattanarthikul and Green (1987) formally recognize the species of *Anopheles maculatus* group occurring in Thailand and they also included the description of two new species. Tewari *et al*., (1987) reported 31 Anopheline species from hill ranges of Western Ghats. Bhatt *et al*., (1987) published an article, they studied the outdoor resting habitat of mosquitoes in the Kheda districts of Gujarat using artificial pit shelters and revealed that mosquitoes prefer to rest in pit shelters and these can be used for ecological and behavioral studies. Raiput & Singh (1987) reported *Anopheles minimus* Theobald, from Manipur for the first time it is an important vector of malaria in the Northeast Region. *Anopheles paltdnieni*, a new species from the Sultanate of Oman, was described by Shidrawi and Gillies (1987). Peyton & Ramalingam (1988), described a new species from peninsular Malaysia named *Anopheles nemophilous*. Barr and Guptavanij (1988), described a new species of *Anopheles maculipennis* complex and named the species as *Anopheles hermi*. Mostly they identified the species using the key of Belkin and McDonald and also certain means of identification by Chromosome analysis. In 1990, Wilkerson and Strickman released a key to the female *Anopheles* mosquitoes found in Mexico and Central America. Wilkerson (1991) described a new species in the Arribalzagia series from Peru and named the species as *Anopheles calderoni*. From Iran, *Anopheles culicifacies* were reported by Zaim and Javaherian (1991). Click (1992), published a key to the female *Anopheles* mosquitoes of Egypt and Southwest Asia. A catalog of the mosquitoes of the world was published by Ward (1992). Coetzee

(1995), described a new species *Anopheles crypticus* from South Africa, and the species was distinguished from *Anopheles coustani*. Earlier this species was known as *Anopheles coustani* species B. Differences between *An.crypticus* and *An. coustani* showed on the chromosomal map. Nguen *et al*., (2000) described *Anopheles (Anopheles) nimpe* as a new species in the coastal area of Southern Vietnam. Sallum *et al*., (2000), worked on subgenus Kerteszia, redescribed, and compared *Anopheles laneanu* Correa with other species belonging to the subgenus Kerteszia. Samboon *et al*., (2000) compared the species *Anopheles flavirostris, Anopheles minimus,* and a new sibling species- species E based on SEM studies of cibarial armature collected from different regions. Sallum *et al*., (2002) by using a scanning electron microscope characterize the ultrastructure of eggs of *Anopheles galvaoi* Cansey and *Anopheles evansae* Brethes. Reuda *et al*., (2003) gave a comprehensive morphological explanation for the species *Anopheles pseudopunctipennis* in Central and South America. Rubio-Paris *et al*., (2003) published a key for species *Anopheles marajoara* from subgenus *Nyssorhynchus.* Vythilian *et al.,* (2003) carried out a study on the prevalence of *Anopheles* in three malaria-endemic villeges of Sekong province, Lao PDR. Trung *et al*., (2004) analyzed malarial vectors in different geographical areas of Southeast Asia. Jaichapor *et al*., (2005) conducted a study on a morphological variation on the wing of *Anopheles minimus* A from Thailand. Sedaghat & Harbach (2005), based on Iranian manuals, reports, and information published prepared a checklist of *Anopheles* species. Sallum *et al*., (2005) described six new species from *Anopheles leucosphyrus* group from an oriental region. Sallum *et al*., (2005) revised 20 species from subgenus *cellia* group *Leucosphyrus* and series *Neomyzomia.* In this piece of work, the authors also included description of male and female adults, genitalia of males, pupa, and fourth instar larvae.

Azari-Hamidian *et al*., (2006) reported and studied in details of *Anopheles peditaneatus* from Iran for the first time. In the same year, another study from Kenya by Joseph *et al.* explained the immature stages of *Anopheles arabiensis* and other mosquitoes from the rice ecosystem. Mutuku *et al*., (2006), a study conducted *on Anopheles gambiae* complex from rural villages of western Kenya and explained the pupal habitat productivity of this species. Mwangangi *et al*., (2006) conducted a

comparative study on rice growth cycle and immature stages of *Anopheles arabiensis* patton. Qui *et al*., (2006) studied on olfactory receptor gene in antennal neurons of *Anopheles gambiae* complex and revealed that these genes are expressed after the first blood meal. Helena and Wilkerson (2007) described *Anopheles albitarsis* complex from Colombia. Walton *et al*., (2007) conducted molecular studies on *Anopheles annularis* and included 5 currently recognized species in South Asia. Brochero *et al*., (2007) reported a new malaria vector from Colombia and it was a new species belonging to the *Anopheles albitarsis* complex. Okech *et al*., (2007) studied the effect of soil substrates of larval breeding habitats on larval development time, pupation rates, and vector competence of *Anopheles gambiae* to *Plasmodium falciparum*. Rueda *et al*., (2007) conducted a study on the distribution pattern of the *Anopheles Hyrcanus* group in China and examined the distribution and vector status of *Anopheles sinensis*, with the help of a scanning electron microscope they also conducted a detailed study on parts of the eggs of six species belongs to Hyrcanus group. Ramos *et al*., (2008) a morphological study on the species *Anopheles nuneztovari* Gabaldon and also studied their intraspecific variation in wings of three. Blanford *et al*., (2009) conducted an experimental study regarding the thermal behavior of *Anopheles stephensi with* locust biopesticides and fungal biopesticides and concluded that no evidence of the thermal behavior of *An.stephensi* with fungal pesticides used for malaria control. Stops *et al*., (2009) conducted a bionomics study of *Anopheles* mosquitoes from two ecologically distinct places in West Java, Indonesia. The study concluded that lots of variations occurred in the studied specimens. Djadia *et al*., (2009) discovered a new member of the Hyrcanus group from Iran and it was the first record from Iran. Motoki *et al*. (2009) analysed the *Anopheles albitarsis* complex. Malkawa *et al.,* (2009) first recorded *Anopheles balabacensis* from Western Sumbawa Island, Indonesia. Kim *et al*., (2009) conducted a study on the larvae of *Anopheles lindasayi* Japonicus. Oyewole *et al*., (2009) investigated the physical and chemical properties of breeding habitats of *Anopheles* and prepared entomological indices from Korea. Wanji *et al*., (2009) examined the breeding sites of *Anopheles* Mount Cameroon region regarding their distribution, environmental characterization, etc. Basseri *et al*., (2010)

investigated the seasonal abundance and host feeding patterns of vectors of Malaria in endemic areas of Iran and concluded that after feeding on human beings vectors tended to rest in animal shelters.

Gonzalez *et al*., (2010) explained the species *Anopheles calderoni* Wilkerson from Colombia for the first time and confirmed by molecular analysis. Rueda *et al.*, (2010) examined the potential vector of Malaria in Korea the *Anopheles belenrae.* Rueda *et al.,* (2010a) Seasonal occurrence, larval habitats, and distribution of potential vector of malaria in the Republic of Korea. Juri *et al*., (2011) recorded *Anopheles neomaculipalpus* Curry an important vector of Malaria from Northwestern Argentina for the first time. Imbahale *et al*., (2011) investigated larval habitats of *Anopheles*. *Anopheles gambiae* important Malaria vector at that time and breeds both in temporary and permanent habitats. Ndoen *et al*., (2011) examined the activity pattern of the Anopheline mosquito from dusk to dawn, types of resting places selected in coastal as well as hilly areas, and nature host seeking in Anophelines. Sinka *et al*., (2011) published a review of Dominant vector species in the Asia-Pacific region and presented a distribution map of 19 *Anopheles* vector species along with a bionomics summary of each species/species complex created using Boosted Regression Tree (BRT). Chouaia et al. (2012) detailed investigation of delayed larval development in *Anopheles stephensi* Liston. Kweka *et al*., (2012) studied the co-breeding of *Anopheles gambiae* and *Culex quinquifaciatus* and concluded that the co-existence between two species in semi-natural conditions affects the body size of *Anopheles gambiae.* Tisgratog *et al*., (2012) investigated the blood-feeding nature, pattern, and host preferences of *Anopheles minimus* from Western Thailand and assumed that *Anopheles minumus* mostly spent outdoors rather than indoors and humans were blood-feeding hosts. Gunuthilaka *et al*., (2012) investigated the blood-sucking nature of Anopheline mosquitoes of Sri Lanka and also studied malaria-transmitting species among Anophelines. Coetzee *et al*., (2013), based on molecular and bionomical evidence named and described two species of *Anopheles gambiae* complex. Dabire *et al*., (2013) studied the swarming behavior of *Anopheles arabiensis* in Bobo-Dioulassoba City West Africa. Dadzie *et al*., (2013) from Ghana, investigated the species composition of *Anopheles funestus*

Giles and their role in Malaria transmission. Hii & Rueda *et al*., (2013) reported *Anopheles dirus* Harrison and Peyton in Vietnam for the first time vector of *plasmodium knowlesi* and also studied vector behavior, ecology, and degree of contact between humans of Anophelines. Ngo *et al*., (2013) described *Anopheles dangi* a new species of the Hyrcanus group a close relative of *Anopheles crawfordi* Reid, based on the humeral pale spot on the costa of the wing they differentiate the new species from the existing one. Danabalan *et al*., (2014) investigated the host preference nature of Anopheles *maculipennis*. Faulde *et al*., (2014) recorded *Anopheles stephensi* for the first time from Djibouti, Horn of Africa.

Rueda *et al*., (2014) a preliminary study on the distribution of *Anopheles hyrcanus* in Kyushu Island from Japan. Saeung *et al*., (2014) constructed an ultrastructural key for *Anopheles hyrcanus* group using images from a scanning electron microscope from Thailand. Kabirul Bashar & Nobuko Tuno (2014), conducted studies on the seasonal abundance of *Anopheles* mosquitoes and their association with meteorological factors and malaria incidence in Bangladesh. A total of 2,443 female anophelines, representing 22 species were captured. Every female *Anopheles* was tested for *P. falciparum*, *P.vivax* 210, and *P.vivax* 247 CSP, of which 10 species were found positive.The CSP-positive species were *An. annularis, An. baimaii, An. barbirostris, An. jeyporiensis, An. karwari, An. minimus s.l., An. philippinensis, An. umbrosus, An. vagus, An. wilmori, and An. philippinensis* were the dominant species present almost throughout the year with highest peaks in March and smallest peaks in September but *An. baimaii* and *An. willmori* were found during monsoon (July -September) only. Lag rainfall and relative humidity were the most significant variables influencing *An. baimaii, An. willmori, An. vagus, and An. subpictus* density in the Kumari area. The abundance of these four species positively related to malaria cases.

LMO Martin *et al.,* (2018) *Anopheles* mosquitoes were collected in four different landscapes of Cruzeiro do Sul□Acre, the current area with the highest malaria transmission in Brazil and performed adult mosquito collections every three months over two years and associated vector occurrence with local abiotic factors. A

total of 1,754 *Anopheles* belonging to nine species were collected, but only four of them (*An. albitarsiss*.l . Lynch□Arribalzaga, *An. braziliensis* Chagas, *An. peryassui* Dyar and Knab, and *An. triannulatus* Neiva and Pinto) represented 77.1% of the total. Vector density and diversity were uneven across field sites and collection periods. Higher *Anopheles* abundance (54.8%) and richness were observed in a deforested palm tree area (IFC), with *An. braziliensis* the most frequent mosquito (40.5%). Only 7.3% of mosquitoes were collected in the SAB village, but 66.4% of them were *An. darlingi* and *An. oswaldoi*, species often regarded as primary and secondary vectors of malaria in the Amazon region. Michel N *et al*., (2022) published an article systematic review on the diversity and distribution of *Anopheles* species in Gabon and revealed that 41 *Anopheles* species were present in Gabon and prominent were gambiae complex species, Nili complexes, Funestus complexes.

2.1.2 National level

The Anopheline faunal studies in India started at the beginning of the twentieth century. Giles (1901b) described four new species belonging to the genus *Anopheles* from India. Bently (1902) investigated and described one new species of genus *Anopheles* from Tezpur district, Assam. James and Liston (1904) studied and described Indian anopheline fauna and published a monograph of the Anopheles mosquitoes of India. Theobald discovered four new genera, and described twenty new Anopheline species of genus *Anopheles* and also identified one new variety from India. Christophers (1911) published an article in the Journal of Paludism he described a new species of *Anopheles* from India (*Neocellia fowleri*) and stated that this species has a resemblance with *N. fuliginous*. Christophers (1913, 1915, 1916, 1916a, 1923, and 1924) contributed to the Indian Anopheline taxonomy by releasing a series of research papers. James & Liston (1911) wrote a book on Indian Anophelines and described thirty *Anopheles* species from Simla. In addition to the above work of James and Liston, Brahmachari. Bahadur (1912), studied the Anophelines diversity in Calcutta over one year and reported mainly three *Anopheles* species *Myzomia ludlowii*, *Myzomia culicifacies,* and new species

Myzomia brahmachari. Prasad (1918) published an article, that described a new species *Anopheles annandelei* the specimen collected from Sureil in the Darjeeling District and species closely related to *Anopheles ascitica*. Iyengar (1922) prepared a description larvae of *Anopheles annanddalei* and differentiated its peculiarities. Christophers (1924), described some *Anopheles* mosquitoes seen in the Himalayan region and also in the peninsular and explained *An.gigas* and *An.lindesayi* both are restricted to high altitudes and found the whole length of the Himalayan chain. The *An.gigas* found in south India resembles *An.sinensis* in the North region. Christophers (1924a), again released an article related to Indian anophelines. In addition to the discussion related to *An.gigas* and *An.lindesayi* again added three varieties of *Anopheles*. Covell (1927) made a distribution study of Indian anophelines and compared the species with Ceylon. Strickland & Chowdhury (1927) studied and described a new species of *Anopheles* from Bengal.

Sinton & Covell (1927) investigated the morphological features of the buccal cavity of 52 species of *Anopheles* except *Chagasia*. Puri (1929) investigated and described a new species of *Anopheles* from South India breeds in tree holes. Christophers (1931) published a synoptic table of *Anopheles* in India. Covell (1931) conducted a detailed study on *Anopheles* in India. Puri (1931) studied the external morphology of larvae, and mounting techniques, of *Anopheles* mosquitoes in detail. Puri's (1928, 1929, 1930, 1937, 1948, and 1948a) contribution to the taxonomy of Anophelines of India was remarkable. Among this most significant one was the synoptic table for the identification of Indian Anophelines. Christophers (1933) made a major contribution and published the key family *Culicinae* and tribe *Anophelini.* In the same year, the book "Fauna of British India" was an outstanding contribution of Christophers to the Taxonomy of mosquitoes in India. This comprehensive taxonomic publication described 42 Anopheles in India detailed information on each species was depicted in this book. Also provided information regarding breeding habitats, distribution, and bionomics of each species. Rao et al. (1938) based on the egg size of *Anopheles stephensi*, made a comparative study of variants of this species; *Anopheles stephensi* type and *Anopheles stephensi*

mysorensis. Rao (1952) investigated and recorded 24 Anopheline species in the district of Shimoga and Hassan of Mysore state.

Bhombore *et al*., (1954) study from the Western hill rock Mysore state and investigated the seasonal prevalence of Anophelines and listed 23 species from there. Rao (1981) a major contribution to anophelines with, a detailed study on type locality, name, taxonomy, distribution, and prevalence of *Anopheles* found in neighboring other countries of India. Reuben & Suguna (1983) described the morphological differences between sibling species of *Anopheles subpictus* Grassi from India and concluded that species A resembles *An. subpictus* from India and species B resembles *An. subpictus* from Malaysia. Nagpal (1983,1983a, and 1987) published a series of papers on morphological variations in Anopheline species collected from Assam, the Northeastern region of Kutch, and the Andaman Islands. Sucharid & Choochote (1983) conducted a morphometric analysis of male genitalia and clasper movements during the induced copulation of two species *Anopheles balabacencis* and *Anopheles dirus.* Malhotra *et al*., (1987) surveyed the Tenga valley of Arunachal Pradesh and reported 12 species of Anopheles with a high density of *Anopheles vagus* and *Anopheles maculatus* and also published the systemic list of 45 species of *Anopheles* from the Northeast region in 1998. Rajput & Sing (1987a) carried out studies in the Northeastern region, from Manipur they reported *Anopheles minimus* Theobald for the first time.

Tewari *et al*., (1987) studied the diversity of genus *Anopheles* in hill ranges of the Western Ghats and reported 31 species. Das *et al*., (1990) accoutered pictorial key to the Indian Anophelines. Nagpal (1990) carried out studies of 216 specimens of *Anopheles stephensi* Liston regarding the morphological variations in their wing and palpi collected from Gujarat. Sixteen species of *Anopheles* were reported by Pal & Dutta (1992) from three districts in Arunachal Pradesh. Chakarborty *et al*., (1998) conducted studies on the seasonal prevalence of larvae of *Anopheles stephensi* in an Urban Garden in Central Calcutta and found that *type* and *mysorensis*. Malhotra *et al*., (2000) studied the surface morphology of eggs of *Anopheles stephensi* with the help of SEM. Sharma and Hamzakoya (2001) investigated the geographical

distribution of *Anopheles stephensi* and *Aedes aegypti* in the Arabian Sea Island of Lakshadweep. Chaudhari and Gupta (2003) carried out SEM studies on the principal malaria vector *Anopheles culicifacies*. In the next year, conducted same type of studies in another principal vector *Anopheles stephensi.* Harbach (2004) published an article related to the classification of the genus *Anopheles* and its phylogenetic relationship and made a comparison between subgenera included in the genus *Anopheles*. Kirti and Kaur (2004) studied different types of (8) morphological wing variation and palpi variation in the natural population of *Anopheles pulcherrimus* from Northwest India. Kirti and Kaur (2004a) studied the wing and palpi variation of *Anopheles subpictus* from Northwest India. Pramanik *et al*., (2006) conducted a survey of Anophelines in Urban and rural areas of West Bengal. Singh *et al*., (2006) conducted studies on malaria vectors; *Anopheles fluviatilis* species (s) and *Anopheles minimus* species (C). Kalitha (2007) investigated the seasonal prevalence of *Anopheles* in Sonapur districts of Assam. Kamal and Mahantha (2007) studied the distribution and prevalence of *Anopheles* in Darrang districts of Assam. One new species belonging to the genus *Anopheles* was described by Sathe and Tingare (2007) from India. Banerjee *et al.,* (2008) using artificial neural networks, classified and identified *Anopheles* mosquitoes. Bhatt *et al.,* (2008) investigated the prevalence of *Anopheles culicifacies* of the Kheda district, Gujarat, and also studied its role in Malaria transmission.

Devi & Jauhari (2008) studied the distribution of *Anopheles* in seven districts of Uttrakhand. Suman *et al*., (2008) carried out studies on *Anopheles* mosquito larvae regarding the morphological sexual dimorphism and to identify and differentiate male and female larvae they used spots present at the third and fourth abdominal segments of larvae. Barik *et al*., (2009) studied the bionomics of sibling species of *Anopheles culicifacies* (A, B, C, D, and E). Pradya *et al*., (2009) investigated the cibarial armature of *Anopheles dirus* complex with the help of SEM. Sedaghat *et al*., (2009) carried out studies on *Anopheles persiensis* about the morphology of adults, eggs, larvae, and pupae. Sathe & Jagtap (2010a, 2010b) investigated the diseases carried by mosquitoes as well as the biodiversity of Anopheline mosquitoes in the Western Ghats. Amala *et al*., (2011) carried out a

diversity study of mosquitoes (genus *Anopheles, Aedes, and Culex*) in the Dindigul district of Tamil Nadu. Kirti *et al*., (2011) investigated and studied morphological variation in the wing and palpi of *Anopheles stephensi.* Sahu *et al*., (2011) conducted a study on seasonal prevalence & resting *Anopheles minimus* Theobald *and An. fluviatilis* James (Diptera: Culicidae) in east Central India. Kirti *et al.,* (2012) studied the peculiarities of the eggs of *Anopheles peditaneatus* from Punjab. In the same year, Kirti *et al* studied wing and palpi variation in *Anopheles pulcherrimus*.

Based on morphological variation, Kirti *et al*., (2013) recorded six types of *Anopheles splendidus* and *Anopheles willmori* from Northwest India. Kumari *et al*., (2013) conducted a study on the prevalence of sibling species of *Anopheles subpictus* in Odisha and studied its role in malaria transmission in four districts. Also reported *Anopheles subpictus* B from Odisha for the first time. Singh *et al*., (2013) published a review paper on the Status of *Anopheles annularis* in India and bionomics and its role in Malaria transmission were discussed in this article. Singh *et al*., (2014) investigated bionomics and the role of *Anopheles subpictus* in the Indian scenario. Kumar *et al*., (2016) investigated Anopheline fauna in Goa from May 2013 to April 2015 and also carried out DNA analysis of mosquito DNA for detecting plasmodium infection. Dev & Manguin (2016) studied on dominant vector species *Anopheles minimus* prevalent in Northeastern states and East central state of Odisha and gave an in-depth study on its systematic position, bionomical characteristics, and distribution in India. Gayan Dharmasiri *et al*., (2017) a study to know the vector mosquito fauna concerning the diversity of selected areas of Satturthaluk, four villages of Virudhunagar district, Tamilnadu. Twelve species belonging to four genera were recorded (2017) carried out a series of entomological investigations in Mannar Districts and reported *Anopheles stephensi* for the first time. The species were found to be co-breeding with *An. subpictus, An.varuna,* and *An.culicifacies.* Sharmah *et al*., (2019) conducted an entomological survey to detect the presence of *Anopheles baimaii* from Tripura State Northeast of India. Subbarao *et al.,* (2019) published a review article, that discussed the need for the study to generate data that fill the gaps in existing knowledge in vector biology and bionomics to eliminate Malaria in India. Vidhya *et al.,* (2019) conducted an

entomologic and parasitologic survey of Anophelines in the Selected islands of Andaman and Nicobar Islands and identified their role in the transmission of *Plasmodium kwolesi.*

Sharma *et al*., (2020) conducted a comparative study *Anopheles fluviatilis sensu lato* and its close relative species of *Anopheles minimus* complex and studied variation in palpal ornamentation of *Anopheles fluviatilis* species T and U and their taxonomic consequence. The study concluded that palpal ornamentation may not be a reliable characteristic for the identification of *Anopheles minimus* and *Anopheles fluviatilis*. Kareemi *et al*., (2021) carried out a study of tribal areas of Chhattisgarh state and investigated seasonal and ecotype-wise distribution, breeding habitats, sibling species composition, insecticide susceptibility, and role in the transmission of the local vector population and concluded that *An.culicifacies* was the dominant species and played a major role in disease transmission. Bhari *et al*., (2022) conducted a systematic longitudinal study to know about the bionomics of *Anopheles culicifacies* in the Kheda and Panchamahal Districts of Gujarat and concluded that this species found to be endophilic, zoophagic behavior and resistant to alpha-cypermethrin. They suggested that frequent evaluation of vector control measures should needed in these areas.

Khan *et al*., (2023) carried out a study on the bionomics of primary vectors *Anopheles minimus* and *Anopheles baimaii* in the Tripura and Meghalaya North East states of India. *An.baimaii* which prefers to be bred in natural and manmade depressions near the forested areas.

2.1.3 Kerala

Many mosquito species from several genera have been reported from various parts of Keala. During the year before independence, British researchers conducted the majority of the taxonomic investigations on the state's mosquito population (Sumodan, 2014). Early references on the distribution and prevalence of mosquitoes in Kerala were provided by Theobald (1901, 1902, 1905, 1910), James and Liston (1911), Giles (1902), and James (1902). Covell and Harbhagwan (1939) surveyed the mountains and forested areas of Wayanad district and reported 19 species of

Anopheles. Mathew (1939) reported malarial vector species that were once found in Travancore state. Mariappan et al. (1992, 1996, 1997) carried out a study in Kochi and surrounding areas and reported 7 species of *Anopheles*. They reported 35 species of mosquitoes belonging to several genera: 3 species of *Aedes*, 1 *Armigeres* species, 14 species of *Culex*, and 4 species of *Uranotaenia*. Rajavel *et al*., (2006) conducted studies in the Mangrove Forest of Kannur and reported 17 species belonging to 7 genera. Sandeep (2008) published an article about the control, Eradication, and Resurgence of Malaria during the Past 50 years. Balasubramanian & Nikhil (2013) conducted mosquitoes diversity study from the Alappuzha and Kottayam Districts of Kerala. They recorded a total of 44 species belonging to 11 genera from Alappuzha district and 21 species belonging to 9 genera were recorded from Kottayam district. Species belonging to the genus *Anopheles* include *An.barbiristris, An.jamesii, An. subpictus, An. peditaneatus and An. vagus*. Aneesh *et al*., (2014) conducted a survey of Mosquitoes from Kurava Island of Wayanad district and their vector status was analyzed. They identified around 18 mosquito species belonging to 5 genera *Anopheles, Aedes, Culex, Mansonia,* and *Armigeres. An. peditaniatus, An. pallidus, An. subpictus, An. vagus* were Anopheles identified in this study.

Sajith *et al.,* (2016) studied the diversity assessment of mosquitos about the occurrence of vector-borne diseases in the Thalassery Municipal area of Malabar Coast Kerala. They found that the species diversity of adult mosquitos was higher during post-monsoon season (2 genera,5 species) than during premonsoon season (1 genera,2 species). However, a reversal in species diversity has been noticed with mosquito larvae with higher species diversity during pre-monsoon season (4 genera,8 species) than post-monsoon season (4 genera,6 species). In both seasons' *culex quinquefaciatus* and *Anopheles stephensi* were the most predominant species in terms of relative abundance and distribution. Sajith *et al.,* (2016) studied habitat characterization and diversity assessment of mosquitos from selected coastal districts of Kerala India and described that mosquitos stand at the top spreading many devastating diseases.

Maiby Thankachan and Arya Gopinath (2017) surveyed the diversity of mosquito species in the plantation area of Manathavady municipal area for six months from February to July 2016 larvae and adults of the mosquitoes were collected and identified by running appropriate keys. A total of seventeen mosquito species belong to six genera. Namely, *Anopheles, Culex, Aedes, Armigeres, Uranotaenia,* and *Triteroider* were identified. Aedes (10), culex (3), Anopheles (1), Uranotaenius (1), Tripteroides (1), and Armigeres (1) respectively. Radhakrishnan A (2019) conducted a diversity study of mosquito species in the Eranakulam Districts of Kerala and recorded 26 species from 6 genera. *Anopheles* was the most dominant among them, 11 species were recorded.

2.2 Phylogenetic Studies of *Anopheles*

2.2.1 Global level

Chen *et al*., (2002) conducted molecular and morphological studies on the *Anopheles minimus* group of mosquitoes in Southern China, molecular characterization using SSCPs and D3 region of the 28S rDNA and COII locus. Species A and C were found to be sympatric and molecular study states that species A and C are morphological variants of species A. Nadaaf *et al*., (2003) conducted a study to determine the composition and distribution of members of fluviatilis complex in the Republic of Iran using PCR assay of ITS2 regions and reported *Anopheles fluviatilis* species Y (Similar to species S in India) for the first time from Iran. Foster *et al*., (2013) studied *Anopheles (Nyssorhynchus*) from Brazil. Undergo morphological as well as molecular aspects of species identification. In their point of view, the CO I gene and other single genes were poor at confirming species; but, when three genes were combined, they performed significantly better. Surendran *et al.,* (2013) conducted molecular studies of *Anopheles culicifacies* found in Sri Lanka, and this work aimed to investigate the variability in COII and other loci in *Anopheles culicifacies* from Sri Lanka. Foley *et al*., (2014) conducted a study on the distribution, evolution, and vectorial capacity of *Anopheles albitarsis* group. Harbach and Kitching (2015) revisited the phylogenetic relationship of anophelines using state-of-the-art software and presented a refined interpretation of relationships

and set data including new species. Tainchum *et al.,* (2015) *Anopheles* species diversity and distribution of the malaria vectors of Thailand. In Thailand, seven *Anopheles* species within three species assemblages have been incriminated as important malaria vectors. Comprehensive maps showing the countrywide geographical distribution of primary and secondary malaria vector species have not yet been developed; the maps that do exist are typically restricted to specific areas or are out of date. In addition, with the advent of molecular-based species-identification tools, the geographical locations of various sibling species have been more clearly defined in the country. Weeraratne *et al*., (2017) conducted molecular characterization of Anophelines mosquitoes from eight geographical locations in Sri Lanka, and identified 15 species of Anophelines using combined effort of both morphological and molecular (COI and ITS 2) techniques.

Surendran *et al*., (2018) studied the genotype and biotype of invasive *Anopheles stephensi* in Mannar Islands of Sri Lanka and provided more details on molecular aspects of *An. stephensi* and detected type-form (Indian urban vector) vector for the first time, concluded that all the DNA samples matched with existing sequences of *An.stephensi* found in the Middle East and India. Baskin *et al*., (2020) conducted a molecular study on eighteen species of *Anopheles* Islands of Sulawesi, Indonesia. Namgay *et al*., (2020) studied the species *Anopheles lindesayi* collected from various parts of Bhutan and compared it with same species reported from Thailand, Japan, South Korea, China, and India morphologically and molecularly. Sallum *et al*., (2020) conducted morphological studies on Genus *Anopheles* found in South America and constructed an identification key. Tyagi *et al*., (2021) published a review to evaluate the utility of complete mitochondrial genome in the phylogenetic classification of species of *Anopheles*. They concluded that whole mitochondrial genomes are more reliable than those based on single or partial mitochondrial genes in determining the taxonomic and phylogenetic status of these species. Akeju *et al.,* (2022) investigated molecular identification and variation in the wing among the females of *Anopheles gambiae* complex and *Anopheles funestus* group found in five different locations in Akure North Local Government Area, Nigeria. The third main dark spot in the costa region of *An.gambiae* complex only variation among species

in this complex. *Anopheles leesoni* was the only species identified from the funestus group from the study area. Jeon *et a*l., (2023) investigated *Anopheles* species from the Republic of Korea and performed phylogenetic analysis using CO I and ITS 2 and TH genes for comparison of species studied and concluded that ITS2 and TH were more useful for resolving the phylogenetic correlation of the species. Ochomo *et al*., (2023) carried out surveillance of both adult and larval stages of *Anopheles stephensi* in two districts of Northern Kenya. Multiple methods of identification were carried out including morphological key, ITS2 and COI PCR, and Sanger and next-generation sequencing.

2.2.2 National level

Even though the diversity of mosquito species in India is among the highest, little is known about the molecular taxonomy of the malaria vectors found here (Dixit *et al*., 2010). Singh *et al.,* (2006) studied the con-specificity of two nominal species *Anopheles fluviatilis* S and *Anopheles minimus* C by analyzing the DNA sequences of ITS2 and D2-D3 domains of 28S rDNA. The phylogenetic analysis of this study revealed that *An.fluviatilis* S distantly related to *An.minimus* C as compared to other members of fluviatilis complex. Raghavendra *et al.,* (2009) carried out phylogenetic studies in the *Anopheles culicifacies* complex (2 groups A/D and B/D/E), they examined sequence variations among members of the complex within the variable D2 domain of the 28S rDNA subunit. Mosquitoes were collected from different areas of India and a PCR assay was conducted. The study revealed that, even though the five sibling species evolved distinct bionomic characteristics, group 1 developed pre-mating barriers and group 2 had not yet developed post-mating barriers still speciation ongoing. Swain *et al*., (2010) carried out molecular identification and phylogeny of Myzomyia and Neocellia series of *Anopheles* subgenus *Cellia* were estimated using D3 sequences. Dixit *et al*., (2010) conducted a multilocus molecular phylogenetic study of Indian Anophelines and *Anopheles gambiae* at each individual genetic region using NJ, ML, MP, and Beysian approaches and calculated divergence of Indian malarial vector species and added a new temporal dimension to the knowledge of evolution of mosquitoes. This

information became useful in the case of the evolution of subgenus *cellia*. Sarma *et al*., (2012) conducted a molecular characterization and phylogenetic study of *Anopheles nivipes* and *Anopheles philippinensis* in Norteast India. Das *et al.,* (2013) conducted studies on *Anopheles culicifacies* complex in malaria-endemic regions of Odisha. This study revealed that *An.culicifacies* E was detected for the first time and confirmed by using molecular phylogenetics and plays a significant role in malaria transmission in Odisha. Paul *et al.,* (2015) conducted a systematic survey of *Anopheles* mosquitoes in Morga, West Bengal, and studied the seasonal abundance, morphological variation, and molecular characterization of *Anopheles subpictus* and *Anopheles vagus*. The study revealed that *An.subpictus* and *An.vagus* were closely related in their GC content and number of repeated sequences and restriction sites. Singh & Vashist (2017) carried out studies on the genus *Anopheles* collected from different localities of Punjab and generated a molecular database of these specimens using the mitochondrial gene COII and studied intra and interspecific relationships between the collected specimens. This study identified four *Anopheles* species morphologically as well as molecularly: *Anopheles annularis*, *Anopheles pulcherrimus, Anopheles peditaenaitus,* and *Anopheles stephensi* and concluded that the COII gene was found to be quite useful for identifying and distinguishing species belonging to genus *Anopheles.*

Sindhania *et al*., (2020) studied the molecular forms of *Anopheles subpictus* and *Anopheles sundaicus* in the Indian Subcontinent, collected specimens from the mainland, Andaman and Nicobar Islands, and Sri Lanka, conducted DNA sequencing of ITS- 2 and CO-II. Phylogenetic analysis revealed that these two species belong to two clades: i) Subpictus clade with *An. subpictus* A and ii) Sundaicus clade with members of Sundaicus complex and *An. subpictus* B and C. Singh *et al*., (2023) studied *Anopheles* species composition and genetic diversity in Meghalaya state of India. They identified 19 *Anopheles* species from two districts of Meghalaya and phylogenetic studies were conducted based on COI sequencing. Two new species *An.xuii* and *An.dissidens* reported from the study area for the first time. Sindhania *et al*., (2023) studied the differential insecticide- susceptibility of two molecular forms of *Anopheles subpictus* complex (A & B) against DDT and

Pyrethroids and the occurrence of knockdown resistance (*Kdr*) mutations in these forms. This study reported that, contrasting differences in insecticide resistance among these molecular forms against DDT and pyrethroids and form B phylogenetically closer to the Sundaicus complex. Compared to Form B, Form A showed a significantly higher level of insecticide resistance. Rajkonwar *et al.,* (2024) carried out a comprehensive entomological survey in the Alipurduar District of West Bengal and reported *An.pseudowillmori* and *An.sawadwongporni* for the first time. By using morphological and molecular techniques they reported significant vectors of malaria *An.annularis* and *An. culicifacies* from the study area.

2.2.3 Kerala

Tyagi *et al*., (2009) reported and described a new species belonging to the Pyretophorus series, *Anopheles pseudosundaicus* collected from the Coastal areas of the Alappuzha and Kollam districts of Kerala. They described and distinguished it from *An.subpictus* and *An.sundaicus* performed a phylogenetic study of CO I sequences and concluded that phylogenetically this species is distant from *An.sundaicus* and closer to *Anopheles subpictus*. Thankachan *et al.,* (2023) molecular as well as phylogenetic study of vector mosquitoes from Mananthavady Taluk, Wayanad district. They investigated around 17 mosquito species belonging to 5 genera including *Anopheles*.

The assessment of the review of the literature showed that, with a large amount of literature available on the morphology of Anophelines of India, there remains much to know regarding the systematics, distribution, and phylogenetics of *Anopheles* mosquitoes in India as well as Kerala. On account of this, the study was undertaken to know the complete information on *Anopheles* diversity in North Kerala and the distribution of malarial vector species morphologically and molecularly.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study site

Kerala is a geographically unique state in India, nestled between the Arabian Sea in the west and the Western Ghats to the East. Its distinct topography includes many hills, high mountains, valleys, deep forests, lakes, beaches, and backwaters. The Western Ghats are a range of hills and mountains that rise gradually from Kerala's rainy and sweltering coastal plain. Palakkad Gap, a 30-40 Km broad geological feature, is one of Kerala's unique features (Subramanyam & Nayar, 1974; Joshy *et al*., 2018). This geographical barrier appears to split Kerala into two regions: North Kerala, which is located north of the Palakkad Gap, and South Kerala, the region situated to the south of the Palakkad Gap (Subramanyam & Nayar, 1974). The present study has been conducted in North Kerala districts except Palakkad. North Kerala lies between latitudes 12.7848^0N and 10.7502^0N and longitudes 74.8675^0E and 76.7016^0E. The study area has five districts, Malappuram, Kozhikode, Wayanad, Kannur, and Kasaragod (Fig.1).

Kasaragod

Kasaragod is the Northernmost district of Kerala with a total area of 1989 Ha. It lies between $12^0$5' N and $74^0$9' E. It has an average elevation of 62 feet (19 meters). The district boundaries are, the Arabian Sea to the west, the Dhakshina Kannada District to the north, Kodagu District to the southeast, and Kannur District to the south.

Kannur

Kannur district is located between latitudes $11^0$40' and $12^0$48' North and longitudes $74^0$52' and $76^0$56' East. The district is bordered by the Western Ghats in the east (Coorg District, Karnataka), Kozhikode and Wayanad districts to the South,

the Arabian Sea, in the west, and Kasaragod district in the North. The length of the coastal line is 82 km or 13.9% of Kerala's total coastline.

Kozhikode

Kozhikode district is situated on the southwest coast of India and lies between 11°15' and 11 25°North and 75° 46' and 75.77' East. The district is bordered by the Arabian Sea in the west, Kannur district in the north, Wayanad district in the east, and Malappuram district in the south. Geographically, the district is divided into three distinct areas: the lateritic midland, sandy areas, and the rocky highlands formed by the hilly portions of the Western Ghats. The lateritic midlands make up 1343.50 sq km and the sandy coastal belt is 362.85 sq km of the overall area of 2344 Sq km and also 637.65 sq km of rocky hills.

Wayanad

Wayanad is a majestic plateau that is situated between Latitude 11 27'and 15 58' North and longitude 75 47' and 70 27' East. The district's borders are as follows: to the east of Mysore and Nilgiris, to the north of Coorg district, to the south by Malappuram district, and to the west of Kozhikode district. It is located between 700 and 2100 meters above Sea level. The district's terrain varies with meadows, valleys, and hilly regions. In addition, the climate varies greatly based on altitude and topography.

Malappuram

Malappuram is bounded to the northwest by the Kozhikode district, to the northeast by the Wayanad district, to the east by Nigiri hills, to the south by the Palakkad district, to the southeast by Thrissur district, and the west by the Arabian Sea. The total geographic area is around 3,554 km. It is the third-largest district in the state. The geographical coordinates of the districts are 10^0N- 12^0N latitude and 75^0E- 77^0E longitude. The district is divided into three regions, lowland coastal area,

midland, and mountainous highland region, bounded on the east by the Western Ghats.

3.2 Climate

Kerala typically has a humid equatorial tropical climate (Chacko & Renuka, 2002). The Western Ghats have a significant impact on the weather. On the windward side of the area, the heavy rainfall is caused by the forced ascent of monsoon currents. Rainfall intensity is influenced by many factors, including slope steepness, current direction, and strength and altitude. Kerala receives an average of 3000 mm of rain annually. The lowlands typically experience temperatures between 28^0 and 32^0 C, whereas the highlands have temperatures of roughly 20^0 C. Rainfall offers opportunities and breeding grounds for mosquitoes to multiply across the environment. An increase in rainfall results in additional possible breeding sites, which ultimately results in an increased number of mosquito hatching (Kakarla *et al*., 2020). North Kerala is an excellent place for taxonomic studies especially mosquitoes because of its great biodiversity, which is mostly attributed to its tropical climate, seasonal rainfall, and incorporation of portions of the Western Ghats hotspot region.

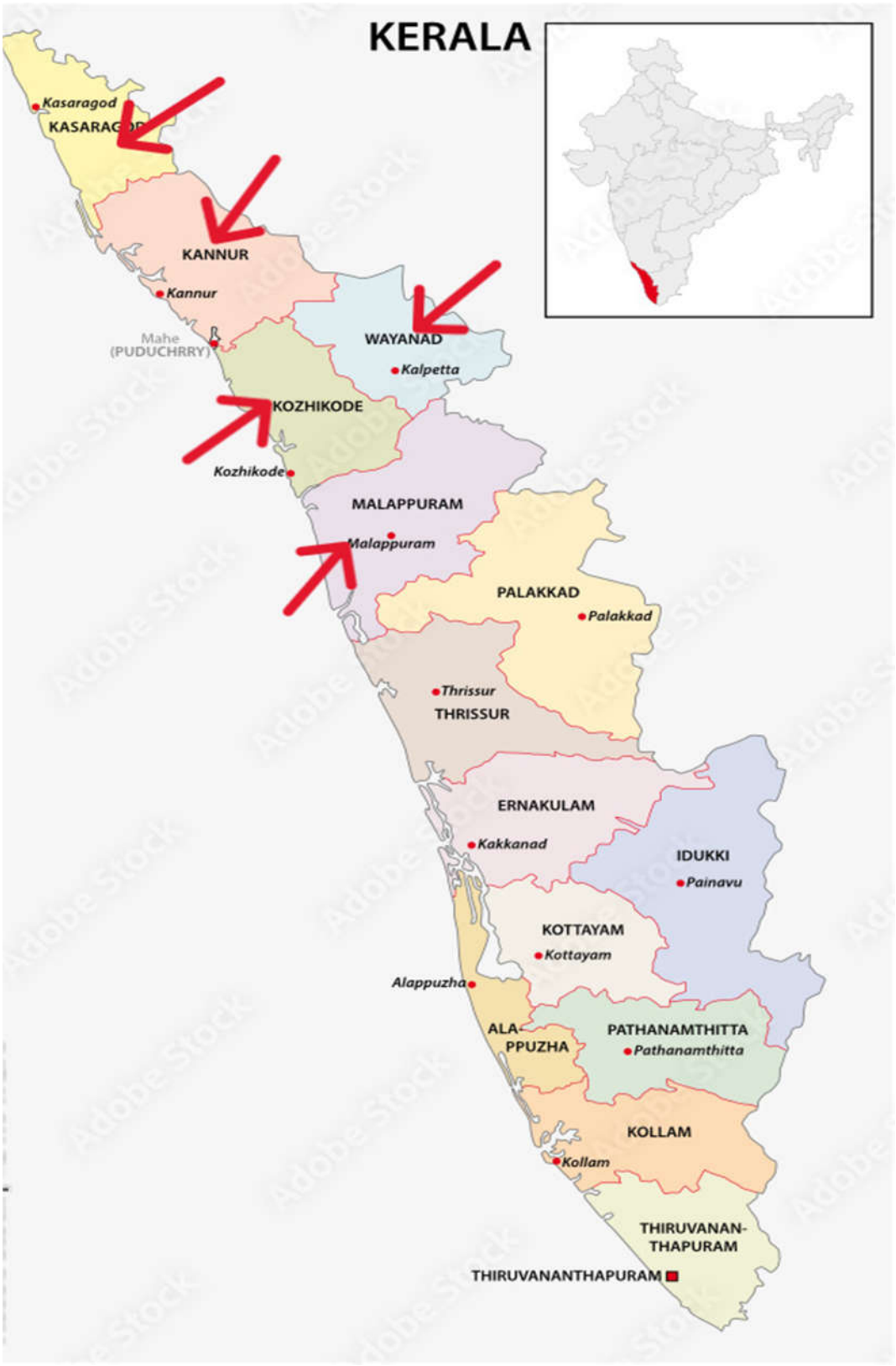


Fig. 3.1: Study area

3.3 Survey, identification, and description of the *Anopheles* fauna and their habitats

Background

Choosing the right mosquito collection techniques is essential to assess how vector control measures affect entomological results. There are four primary types of mosquito sampling techniques: techniques for collecting flying adults, resting adults, larvae, and eggs. In this work to do sampling, two strategies were adopted; cross-sectional design and longitudinal survey. At randomly chosen or risk-based research sites, over a brief period, cross-sectional surveys collect data to evaluate the presence or absence of vectorial capacity of the target species. Longitudinal survey involves repeated sampling of the same sites.

Materials used for collection or field study

- Dipper
- Test tubes
- Specimen tubes
- Suction tube
- Fine mesh insect net
- Ladle (circular mouth spoon)
- White plastic containers
- Droppers
- Labels
- Pipettes
- Ethanol
- Nylon cloth
- Rubber band
- Brush
- Salino meter

- UV- based Light trap
- Smartphone

3.3.1: Mosquito Survey:

For surveying *Anopheles* species, two methodologies were adopted, viz., collection of immature stages (larvae and pupae) and collection of adults.

3.3.1.1: Collection of immature stages:

Random sampling methods were adopted for the study. The larval and pupal collection mainly depends on the size and type of their breeding sites. The collection was done from 2019 to 2023 in five districts of North Kerala (Kasaragod, Kannur, Kozhikode, Wayanad, and Malapparum). Dippers of 300 ml capacity and white bowls were used for larger water bodies such as ditches, ponds, rivers, streams, drains, pools, puddles, tree holes, river margins, rice fields, cement tanks, and artificial containers (fig: 3.3a). Evaluation of habitats was done following Service (1993). Shallow skim and partial submersion dipping methods were used for sampling *Anopheles* immature stages. In the shallow skim method, the dipper's leading edge was submerged 2.5 cm below the water's surface, at a 45^0 angle. Along the water's surface, the dippers were drawn. The dipper was buried along the emergent vegetation at an angle of 45^0. In the case of partial submersion method dipper was not moved horizontally. The larvae collected from different breeding places were kept in different wide-mouthed bottles with suitable labeling (Date of collection, Type of habitat, salinity). The mouths of these bottles were covered with nylon cloth, and transported to the laboratory.

The bottles were allowed to stand in a warm place, immature stages were allowed to emerge and they were collected and stored in clean glass vials. Emerged adults were photographed using a Leica stereo zoom microscope and then identified using standard taxonomic keys (Das *et al*., 1990, Christophers 1933, Nagpal *et al*., 1995). To confirm the correctness of the identification of species, visited the mosquito museum of ICMR Vector Control Research Centre, Pondicherry (ICMR-VCRC).

Fig. 3.3a: Dipper used for larval collection

Mosquito Larval Collection in different Habitats

3.3.1.2: Collection of Adult Mosquitoes:

A. Hand collection:

Adult mosquitoes from indoors (houses and cattle sheds) were collected either in the early mornings (4.00 am to 5.00 am) or evenings (7.00 pm to 11.00 pm) using aspirators and transferred to the test tubes. The tubes were labeled with essential information such as location, date and time of collection, type of structure, etc., and brought to the laboratory for identification (Fig. 3.3b).

Fig.3.3b Collection of adult mosquitoes using Aspirator

B. By light traps:

Adult mosquitoes were collected by using the UV-based insect PRO Active Gravid cum Light Trap (AGLT) (Fig. 3.3c) in the areas of mosquito activity. These traps help to capture full and half-gravid mosquitoes. The trap could be placed safely inside a house or animal dwelling place for the collection of anophelines. The trap runs on a 4.5 to 12v blowing unit that blows attracted mosquitoes into the cage containers.

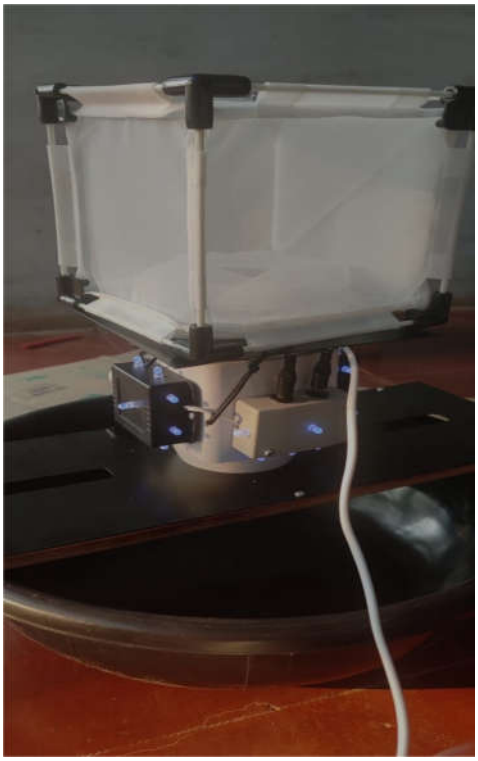


Fig. 3.3c Collection of adult mosquito using Insect PRO Active Light trap

3.3.2: Mounting of Adult Mosquitoes: Killing, Pinning, Preservation and Labelling.

In making the collection of mosquitoes, they must be killed, dried, and preserved in bottles. After the emergence of adults, the killing/anesthetizing was carried out with the help of a cotton piece soaked in diethyl ether placed inside a glass jar. After killing, a few mosquitoes of both sexes were selected from the jar as representatives of a particular species and were pinned through the ventral side of the thorax using minute No.20 pins (Special entomological pin). Before pinning the mosquito one side of the pin was fixed on the cork of a specimen vial. (Fig. 3.3d, 3.3e). Para dichloro benzene (1, 4 para dichloro benzene) was used to preserve the adults. It is a chlorinated aromatic hydrocarbon compound used as a fumigant insecticide and repellent. Pinned specimens were kept in glass bottles filled with para dichloro benzene closed with a bark cork (Fig. 3.3f). The specimens in glass bottles were labeled with information on name, date of collection, and specimen number. A field diary was maintained for recording further details of each specimen collected. The collected adult mosquitoes were photographed under a Leica stereo-zoom microscope (Fig.3.3g)

Fig. 3.3d Entomological pin

Fig.3.3e Pinning of the mosquito

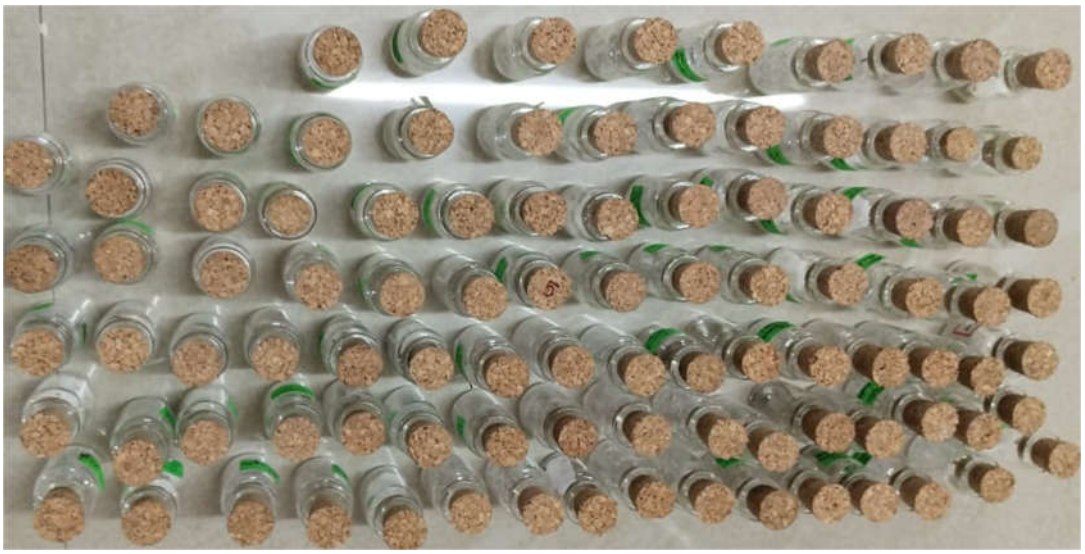


Fig. 3.3f Preservation of Mosquitoes

Fig.3.3g Viewing specimen under Leica stereo zoom Microscope

3.3.3: Identification:

Characters used in adult identification

Head: Head scales are single-type, erect, fan-shaped, truncated, and usually have 12-15 striations. These scales are black at the sides and back of the head and pale at the front of the vertex.

Antennae: Consist of torus and flagellar segments, 15 segmented, in female antennae torus are smaller and larger in males. First flagellar segment scales are dark in females. Scales commonly present on first and second flagellar segments.

Clypeus: Usually bare area but tufts of scales present in some species.

Palpi: The first segment is usually small. Ornamentation, mainly in the form of pale scales, forming bands, present on 2-3 and 3-4 segments.

Proboscis: Labium is uniformly dark, in a few species apical half is golden or light.

Thorax: Prothoracic lobes with erect scales, clusters of propleural hairs present, absent, or reduced depending on species. Mesonotum bare and shiny.

Wing: With longitudinal veins 1-6, 1(R1), 3(R4+5), and 6 (A) branched; 2(RS), 4 (M), and 5(Cu) unbranched, costa (thick anterior border of the wing), subcostal (parallel to costa). Wing ornamentation consists of alternate dark and white areas. Several species are without wing ornamentation.

Legs: Three pairs of legs, fore, mid, and hind legs originating from the thorax. Each leg has a coxa, trochanter, long femur, slender tibia, and five segmented tarsus. Ornamentation of the leg depends on the presence or absence of pale and dark bands present in different parts of the leg. The legs are covered with scales except coxa.

Abdomen: Composed of 10 segments, eight segments are present externally, 8^{th,} and 9th segments are modified as genital organs. The presence or absence of scaling, hair, coxites, or cerci on the abdomen is a useful feature in identification.

Morphology of *Anopheles*- Adult female

Fig a: 1-Head, 2-Thorax, 3-Wing, 4- Abdomen, 5- Fore leg, 6- Mid leg, 7- Hind leg.

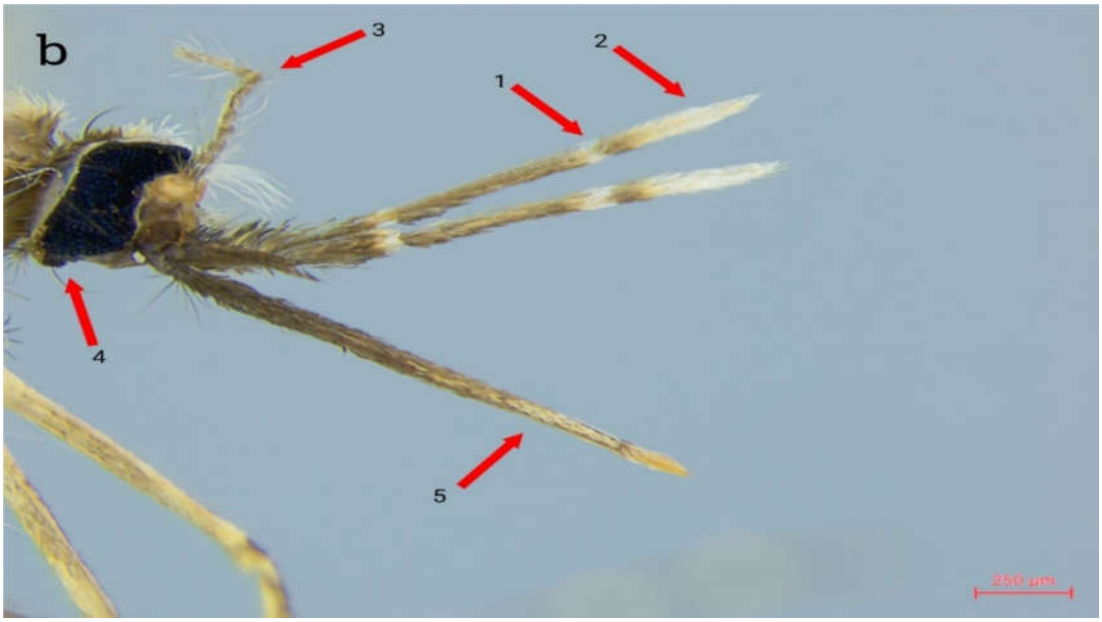


Fig b: 1- Sub apical pale band, 2- Apical pale band, 3- Antenna, 4-Eye, 5- Proboscis

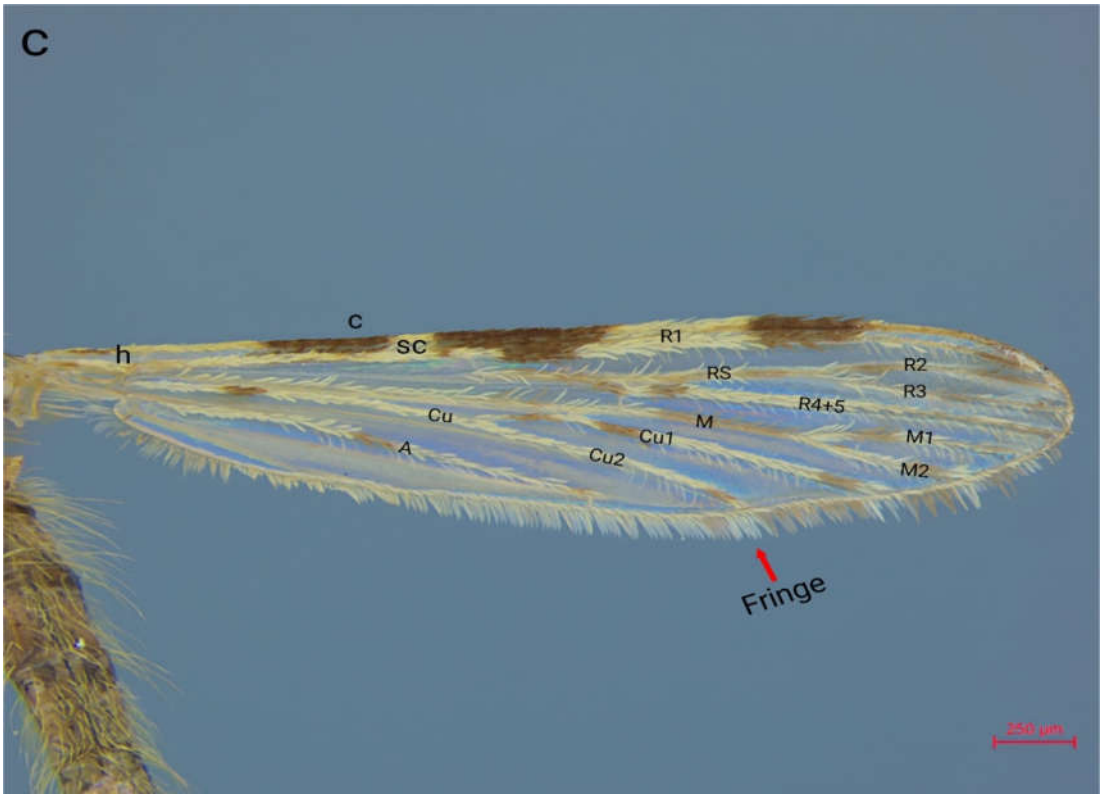


Fig c: Wing**; h**-Humeral, **C**- Costa, **SC**- Subcostal, **R1**(Radius-1)-Vein 1, **RS** (Radial sector) Vein 2**, R2**- Anterior branch of vein 1, **R3**- Posterior branch of vein 2, **R4+5**- Vein 3, **M** (Median)- Vein 4, **M1**- Anterior branch of vein 4, **M2**- Posterior branch of Vein 4, **Cu** (cubitus)- Vein 5, **Cu1**- Anterior branch of vein 5, **Cu2**- Posterior branch of vein 5, **A** (anal)- Vein 6

Fig d: Speckling on hind leg of *Anopheles* mosquito

Fig e: Hind leg; **Fe-III**- Hind leg femur, **Ti-III**- Hind leg tibia, **Ta-III1 to Ta-III5**- Hind leg tarsus

3.3.4. Preparation of Dichotomous key up to species level

The *Anopheles* fauna of Kerala presently consists of 36 species (Sumodan, 2024). Based on existing literature, 23 species were identified and redescribed from North Kerala. A dichotomous key was prepared using the morphological characters of all species encountered during the present study and the species reported earlier.

Terminology used for the description of female *Anopheles* Mosquitoes

C -Costa
Sc -Subcosta
R - Radius
M -Media
Cu -Cubitus
A -Anal vein
h -Humeral cross vein
re -Remigium
rm -Radiomedial cross vein
m cu -Mediocubial cross vein
scr -Subcoastal radial vein
Ant -Antenna
Ap -Antepronotum
CE -Compound eye
Clp -Clypeus
Fl -Flagellum
Mplp -Maxillary palp
M1 -Media-one
M2 -Media-two
P -Proboscis
PA -Post spiracular area
Pe -Pedicel
S -Sternum
SA -Subspiracular area
R1 -Radius-one
R2 -Radius-two
R3 -Radius-three
Wf -Wing fringe
R2+3 -Radius-two-plus-three
R4+5 -Radius-four-plus-five
$Ta\text{-}III_1$-$Ta\text{-}III_5$ -Hindtarsomere 1-5
Te -tergum
Ti-III -Hindtibia
V -Vertex

Abbreviations Used

BNMH	British National Museum of History
NM	National Museum
US	United States

RUM	Rome University Museum
ZMH	Zoological Museum of Humboldt
USNM	United States National Museum

3.4 Molecular taxonomy of Malaria vectors

DNA Barcoding using universal primers of COI

Genomic DNA Isolation

Soon after the specimens were collected, the DNA was extracted before they were dried. Until DNA extraction, the obtained specimens were stored in a freezer at -20^{0}C. The genomic DNA of mosquitoes was isolated from the tissues using a HiPurA genomic isolation kit from HIMEDIA following the manufacturer's instructions. Because the mosquito was a tiny insect genomic DNA was isolated from its entire body. 20 µl of proteinase k (10 mg/ µl) was added to the tissue homogenates to lyse them using lysis buffer. Silicate columns were used to purify the DNA extracted from lysed cells. TE buffer with a PH of 8 was used to elute the pure DNA from the column (Sambrook *et al*., 1989). The eluted DNA served as the template for the PCR amplification of the COI gene.

PCR Amplification of COI gene

After extraction of DNA for the amplification of mitochondrial cytochrome oxidase subunit I (COI) forward and reverse primers were used (`5'-GGTCAACAAATCATAAAGATATTGG-3'F` and `5'TAAACTTCAGGGTGACCAAAAAATCA-3'R`) (Folmer *et al*., 1994). An automated thermal cycler (Sure Cycler 8800, Agilent Technologies USA) was used to do the PCR amplification. The PCR reaction mixture was composed of 25 µl of the 2x premix of Emarald AMP GT PCR master mix, 2 µl each of the forward and reverse primers (5 µM), 1 µl template DNA, and 20 µl of sterile dH2O to make the total volume of 50 µl. The PCR temperature profile was as follows: initial denaturation at 94^0 C for up to 5 minutes followed by 30 cycles of denaturation at 94^0 C for 10 seconds, annealing at 56^0 C for 45 seconds, and elongation at 72^0 C for 45 seconds, concluded with a final extension of 72^0 C for 3 minutes. The reaction mixture was stored at 4^0 C.

PCR Product Analysis- Agarose Gel Electrophoresis

The PCR products were resolved using agarose gel electrophoresis, by loading 5 µl from the 50 µl of the PCR product into the wells of 1% ethidium bromide-stained agarose gel (Sambrook *et al*., 1989). A gel document system (Bio-Rad EZ gel documentation system) was used to visualize the gel following electrophoresis. StrataPrep PCR purification kit (Agilent Technologies, USA) was used to purify the amplified PCR product by the manufacturer's instructions.

DNA Sequencing

The purified PCR products were sequenced from both ends at Agrigenom Labs. Pvt. Ltd. Kakkanad, Kochi using Sangr's sequencing technique (Sanger *et al*., 1977). Using Sequencer 5.3 (Gene Codes Corporation, Ann Arbour, Michigan, USA), the forward and reverse sequences were assembled, and the consensus sequence was produced. The NCBI's BLASTn tool was used to search for sequence similarities in the consensus sequences that were produced (Altschul *et al*., 1990). Similar sequences were selected, and the most similar sequences were used to determine the phylogenetic relationship between them.

Phylogenetic Analysis

The evolutionary history was inferred using the Neighbor-Joining method (Saitou *et al*., 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 667 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.,* 2021).

CHAPTER 4
RESULTS

4.1 *Anopheles* fauna of North Kerala districts

Mosquito surveys in Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram districts yielded 27 Anopheline species. Of these, 10 species belonged to the subgenus *Anopheles* (*Anopheles aitkenii, Anopheles culiciformis, Anopheles barbirostris, Anopheles umbrosus, Anopheles crawfordi, Anopheles peditaneatus, Anopheles gigas, Anopheles nigerrimus, Anopheles nitidus,* and *Anopheles sinensis*) and 17 species to the subgenus *Cellia* (*Anopheles culicifacies, Anopheles jeyporiensis Anopheles fluviatilis, Anopheles dthali, Anopheles subpictus A, Anopheles subpictus B, Anopheles vagus, Anopheles sundaicus, Anopheles stephensi, Anopheles theobaldi, Anopheles karwari Anopheles jamesii, Anopheles splendidus, Anopheles tesellatus, Anopheles kochi, Anopheles dirus,* and *Anopheles elegans*). The species detected the districts from which they were recorded and the breeding habitats they preferred are given Table 4.1 and Table 4.2.

Table 4.1: *Anopheles* species diversity of *Anopheles* in North Kerala

Sl No.	Species	Subgenus	Habitats	Districts
1	*Anopheles aitkenii*	*Anopheles*	Stream shaded with trees	Wayanad
2	*Anopheles umbrosus*	*Anopheles*	Arecanut garden	Wayanad
3	*Anopheles culiciformis*	*Anopheles*	Small ditches near pond	Wayanad
4	*Anopheles barbirostris*	*Anopheles*	Pools with aquatic vegetation, rice fields, tanks, slow-running streams	Kannur, Kozhikode, Wayanad, Malappuram
5	*Anopheles crawfordi*	*Anopheles*	Marshes, ground pools, ditches	Kannur, Wayanad
6	*Anopheles*	*Anopheles*	Rice fields, marshes, ditches, ponds, shallow	Kannur

	peditaneatus		wells	
7	*Anopheles gigas*	*Anopheles*	Rice fields	Wayanad
8	*Anopheles nigerrimus*	*Anopheles*	Rice fields with vegetation, lake, grassy pools, shady ponds, slow-moving waters	Wayanad, Kannur, Malappuram
9	*Anopheles nitidus*	*Anopheles*	Rock pools, rice fields, ditches, seepages	Wayanad, Kannur, Malappuram
10	*Anopheles sinensis*	*Anopheles*	Slow running stream with aquatic vegetation	Wayanad, Malappuram
11	*Anopheles culicifacies*	*Cellia*	River bed pools, rock pools, river margins	Kozhikode, Wayanad
12	*Anopheles jeyporiensis*	*Cellia*	Rice fields, slow running stream with aquatic vegetation	Wayanad, Malappuram
13	*Anopheles fluviatilis*	*Cellia*	Slow moving streams with vegetation	Kasaragod, Wayanad
14	*Anopheles dthali*	*Cellia*	Streams covered by pebbles, rice fields, seepages	Wayanad
15	*Anopheles subpictus A*	*Cellia*	Wells, marshy mangrove fields, lake margins, cement cisterns	Kozhikode, Kannur, Kasaragod
16	*Anopheles subpictus B*	*Cellia*	Marshy mangrove fields	Kozhikode
17	*Anopheles vagus*	*Cellia*	Rice fields, wells, ground pools, shaded part of ponds, lakes	Kozhikode, Kannur, Wayanad, Malappuram
18	*Anopheles sundaicus*	*Cellia*	Fresh water ditches, small puddles	Kannur
19	*Anopheles stephensi*	*Cellia*	Wells, overhead tanks, cement tanks, ground level water tanks	Kozhikode, Kannur, Kasaragod, Malappuram
20	*Anopheles theobaldi*	*Cellia*	Ponds, rice fields, river bed pools, ditches	Kozhikode, Kannur, Kasaragod, Malappuram, Wayanad
21	*Anopheles karwari*	*Cellia*	Clear shaded streams, pools, seepages, drains	Kozhikode, Kannur, Kasaragod, Malappuram, Wayanad

22	*Anopheles jamesii*	*Cellia*	Growing rice fields with aquatic vegetation, rain water pools, ponds, river bed pools	Kozhikode, Kannur, Kasaragod, Malappuram, Wayanad
23	*Anopheles splendidus*	*Cellia*	River bed with algal vegetation, pond with aquatic vegetation	Wayanad
24	*Anopheles tessellatus*	*Cellia*	Wells, rice fields, stagnant water under shade	Wayanad, Kozhikode
25	*Anopheles kochi*	*cellia*	Stagnant waterbodies	Wayanad
26	*Anopheles dirus*	*Cellia*	Rocky streams, rain water collections, borrow pits, stagnant water in forest	Kozhikode, Kannur, Wayanad
27	*Anopheles elegans*	*Cellia*	Arecanut gardens, rock pools in forested areas	Kozhikode, Malappuram

4.2. Habitat preferences of *Anopheles* species

The present study identified around 27 *Anopheles* species from north Kerala that can be detected in various habitats (Table 4.2). They preferred to breed in various habitats such as freshwater bodies (clean, stagnant, slow-running streams or rivers), vegetated areas, rainwater accumulations, flooded areas, irrigation canals, and artificial containers.

Table 4.2 Habitat preferences of collected *Anopheles* species

Species	**Different types of Habitats surveyed**												
	RF	**RP**	**WL**	**FSA**	**RS**	**SWB**	**TH**	**DP**	**AB**	**CT**	**OHT**	**ML**	**MH**
Anopheles aitkenii	-	-	-	-	+	+	-	-	-	-	-	-	-
Anopheles umbrosus	-	-	-	-	+	+	-	-	-	-	-	-	-
Anopheles culiciformis	-	-	-	+	+	+	-	-	-	-	-	-	-
Anopheles barbirostris	+	+	+	+	-	+	-	+	-	+	-	+	+
Anopheles crawfordi	+	-	-	+	-	-	-	+	-	-	-	-	-

Anopheles peditaneatus	+	-	-	+	+	+	-	-	-	-	-	-	-
Anopheles gigas	+	+	-	+	+	+	-	-	-	-	-	-	-
Anopheles nigerrimus	+	+	-	+	+	+	-	-	+	+	+	-	-
Anopheles nitidus	+	+	-	+	+	+	-	-	-	-	+	+	+
Anopheles sinensis	-	-	-	-	-	+	-	-	-	-	-	-	-
Anopheles culicifacies	-	+	-	+	+	-	-	-	+	+	+	-	-
Anopheles jeyporiensis	+	-	-	+	-	+	-	-	-	-	-	-	-
Anopheles fluviatilis	-	-	-	+	+	-	-	-	-	-	-	-	-
Anopheles dthali	+	-	-	-	-	-	-	-	-	-	-	-	-
Anopheles subpictus	+	+	+	-	-	+	+	+	+	+	+	+	+
Anopheles vagus	+	-	+	-	-	+	+	+	+	+	+	-	-
Anopheles sundaicus	+	-	-	-	-	-	-	-	-	-	-	+	+
Anopheles stephensi	-	+	+	-	-	+	+	+	+	+	+	+	+
Anopheles theobaldi	+	+	-	+	+	+	+	+	-	+	-	-	+
Anopheles karwari	+	+	-	+	+	+	+	+	+	+	+	+	-
Anopheles jamesii	+	+	-	+	+	+	+	+	+	_	_	_	_
Anopheles splendidus	-	+	+	-	-	-	-	-	-	-	-	-	-
Anopheles tessellatus	+	-	+	-	-	+	-	-	-	+	+	+	+
Anopheles kochi	-	-	-	-	-	-	+	+	-	-	-	-	-
Anopheles dirus	+	+	-	+	+	+	-	-	-	-	-	-	-
Anopheles elegans	+	-	-	+	+	+	-	-	-	-	-	-	-

(RF- rice fields, RP- rock pools, WL- wells, FSA- freshwater stream with aquatic vegetation, RS- running streams, SWB- stagnant water bodies, TH- tree holes, DP- ditches and puddles, AB- abandoned boats, GP- ground pools, CT- cement tanks, OHT- overhead tanks, ML- marshy lands, MH- mangrove habitats).

4.3 Re- description of collected *Anopheles* mosquitoes

4.3.1 Systematic position

CLASS: INSECTA

ORDER: DIPTERA

FAMILY: CULICIDAE

Subfamily: Anophelinae Grassi, 1900

Genus 1: *Anopheles* Meigen, 1818

Subgenus*: Anopheles* Meigen,

aitkenii group, Ried & Knight, 1961

- *An.aitkenii* James, 1903
- *An.insulaeflorum,* 1920

asciaticus group, Reid, 1968

- *An.annandalei* Prashad, 1918

barbirostris group, Reid & Knight, 1961

- *An.barbirostris* Van der Wulp, 1884

culiciformis group, Reid & Knight, 1961

- *An.culiciformis* Cogil, 1903
- *An.sintoni* Puri, 1929

hyrcanus group, Reid,1953

- *An.crawfordi* Reid, 1953
- *An.peditaneatus* Leicester, 1908

- *An.nigerrimus* Giles, 1900
- *An.nitidus* Harrison, Scalon & Reid, 1973
- *An.sinensis* Wiedemann

lindesayi group, Reid & Knight, 1961

- *An.gigas* Giles, 1908

Subgenus: *Cellia* Theobald

Myzomyia series

- *An.dthali* Patton, 1905
- *An.majidi* Young & Majid, 1928

funestus Group, Garros et al., 2005b

- *An.jeyporiensis* James, 1902
- *An.varuna* Iyengar, 1924
- *An.aconitus* Donitz, 1902
- *An.culicifacies* Giles, 1901
- *An.fluviatilis* James, 1902
- *An.minimus* Theobald, 1901

Neocellia series

- *An.karwari* James, 1903
- *An.moghulensis* Christophers, 1924
- *An.stephensi* Liston, 1901
- *An.theobaldi* Giles, 1901

annularis Group, Reid,1968

- *An.annularis* Van der Wulp, 1884
- *An.pallidus* Theobald, 1901
- *An.philippinensis* Ludlow1902
- *An.nivipus* Theobald, 1903

jamesii Group, Rattanarithikul & Green, 1987

- *An.jamesii* Theobald, 1901
- *An.splendidus* Koidzumi, 1920

Neomyzomia series

kochi group, Rattanarithikul et al., 2006b

- *An.kochi* Donitz, 1901

lecosphyrus Group, Ried, 1949

- *An.dirus* Python & Harrison, , 1979
- *An.elegans* (James), 1903

tesellatus Group, Rattanarithikul et al., 2006b

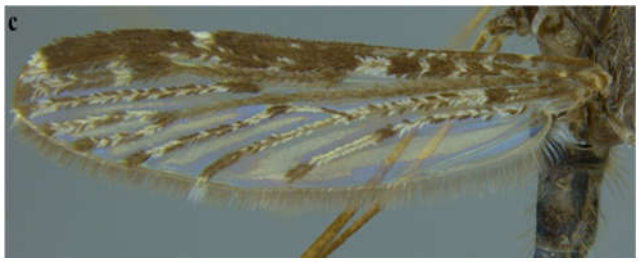
- *An.tesellatus* Theobald, 1901

pyretophorus Series, Edwards, 1932a

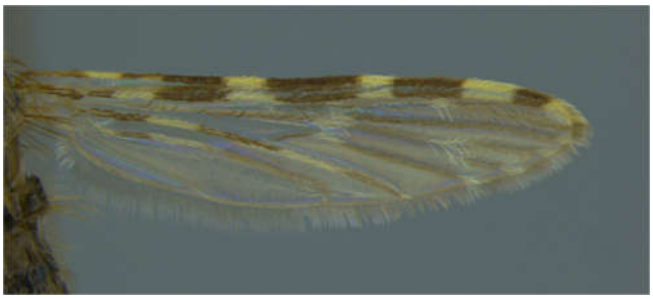
- *An.vagus* Donitz, 1902
- *An.subpictus* Grassi, 1899
- *An.sundaicus* Rodenwaldt, 1925

4.3.2 Key to the subgenera

1. Wing completely dark or if pale areas present, dark areas on costa involving both costa and vein 1 are less than four in number; parabasal spines of male two; pharyngeal armature absent .....subgenus *Anopheles*



2. Wing always with pale and dark markings; dark areas on costa also involving vein 1 four or more in number; parabasal spines of male four or five in a group .... subgenus *Cellia*



Subgenus *Anopheles,* Meigen 1818

The present study reported 10 species belong to this subgenus and detailed redescriptions are given below:

1. *Anopheles aitkenii* James, 1903

Stethomyia fragilis Theobald, 1903: 257. (BMNH, London). Type: Described from 2♂♂. Type locality: Kuala Lumpur (Synonymized by Stanton, Jou. Lon. Sch. T. Med. 1912: 4).

Stethomyia pallida Ludlow, 1905: 129. (NM, US) Type: described from single ♀, Camp Stotsenberg, Pampamga (synonymized by Alcock, Jou. Lon. Sch. T. Med. 1913:159).

Anopheles treacherii Leicester, 1908: 19. (BMNH, London) Type: ♀&♂. Fed. Malay states. (Synonymized by James and Stanton 1912: 515).

Anopheles aitkenii James, 1903: 23. (BMNH, London) Type: Type locality: Goa, Karwar, India.

Material examined: Plesiotype: 1♀, Kerala: Thonichal, Wayanad district. Coll. C. Vipinya, 07.iii.2019.

Other material examined: Kerala: 2♀& 2♂, Thonichal, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 2 ♂, Tharuvana, Wayanad district. Coll. St. Mable Rose, 12. Iv. 2019; 3♀ & 5♂, Mananthavady, Wayanad district. Coll. C. vipinya, 12. Iv.2019.

Diagnosis: Female (Plate 1, Fig.1, Map-1) Small to moderate-sized, brown-coloured, 3.1 mm long.

Head: Covered with linear scales sparsely scattered; narrow vertical area; vertical hairs pale coloured; prominent frontal area; narrow ocular scales; compound eyes rounded and black; proboscis brown with black tip, 2.34 mm long.

Antennae*:* With few pale hairs and devoid of scales; 1.53 mm long, yellowish brown, last two segments swollen, hairy, and pilose; palpi 2.37 mm long, as long as the proboscis, thin, delicate, straight, slender, uniform thickness, without pale bands; the tip of palpi apical segment short and swollen with scales.

Thorax*:* 1.48 mm long, short, rounded, usually dark coloured; mesonotum dull brown or pale coloured, entirely devoid of scales.

Wings: 3.67 mm long, 1.52 mm wide, wing entirely dark, unspotted with marked wing scaling; scales on wing border extended up to base; costa with three dark scaled spots, large middle dark spot; vein 1(R1) dark, vein 2 (RS) dark, vein 3(R4+5) dark, vein 4 (M) dark, vein 5(Cu) dark, vein 6 (anal) dark; fringe spot absent on all the veins.

Legs: 5.68 mm long, coxa 0.4 mm long, thin; femur 1.56 mm long; femur, tibia, and tarsus uniformly dark; tibia, 1.37 mm long, darkish, not speckled; tarsus five segmented, fore-leg tarsomeres without broad white bands.

Abdomen: 1.64 mm long, dark with dark hairs; even on cerci scales absent.

Male: Characters are same as in females, smaller than females; 2.51 mm long, slender, with plumose antenna, uniformly dark; palpi unbanded and dark; dorsal portion of abdomen including coxites devoid of scales.

Bionomics: Breeding habitats include, small pools, marshes, channels, rivers whose flow is not continuous, rock pools, streams heavily shaded with plants, drains in tea gardens, and wells.

Distribution: In India: recorded from Sikkim, Meghalaya, Manipur, Assam, Bihar, Maharashtra, Uttar Pradesh, Kerala, Tamil Nadu, Odisha, Punjab Andra Pradesh and West Bengal also from Andamans in different locations, not recorded from Central Provinces and in this study, reported this species from Wayanad from stream shaded with trees.

Remarks: *An.aitkenii* belong to the subgenus *Anopheles*. Its main identification features are the following,

1. Palpi, wing, and legs are completely dark.
2. Fringe spots are absent in all the veins.

This species runs close to *An.insulaeflorum*, *An.pinjaurensis*, and *An.bengalensis* and forms the *aitkenii* group. Since it is impossible to resolve these species' specific identities based on morphological characters alone, the specimens identified as *An.aitkenii* in this study could be a complex of the above species.

2. ***Anopheles umbrosus*** **(Theobald), 1903**

Material examined: Plesiotype: Kerala: 1♀, Kannavum, Kannur district. Coll. C. Vipinya, 12. v.2021.

Other material examined: Kerala: 3♀ & 5♂, Mananthavady, Wayanad 13♀& 6♂, Makkiyad, Wayanad district. Coll. C. Vipinya, 14.iii.2021; 13♀ & 8♂, Kavumandam, Wayanad district. Coll. C. Vipinya, 18. v.2021; 12♀ & 8♂, Kannavum, Kannur district. Coll. C. Vipinya, 12. v.2021; 14♀ & 5♂, Koorara,

Kannur district. Coll. C. Vipinya, 02.i.2020; 2♀ & 5♂, Vilangad, Kozhikode Coll. C. Vipinya, 2.xii.2021.

Diagnosis: Female (Plate-2, Figs. 2a-e, Map-1) Large-sized, black-coloured 3.54 mm long.

Head: Covered with broad, short black type scales; narrow vertical area with pale vertical spot, vertical hairs pale coloured (fig.2b); prominent frontal tuft with white hairs; narrow elongated ocular scales; compound eyes rounded and black; proboscis brown with black tip 2.58 mm long covered with black scales.

Antennae: With small dark and pale scales present on first and second filamental segment; 1.58 mm long, yellowish brown, 15 segmented, last two segment swollen hairy, and pilose (Fig.3b); palpi 2.57 mm long, as long as the proboscis, erect, thin, delicate, straight, slender, uniform thickness covered with long broad scales, without pale bands (fig.3b).

Thorax: 1.55 mm long, usually dark coloured; mesonotum black, median area not pale as lateral area, covered with narrow hair-like scales; lateral area brown or pale coloured; pre-scutellar space with short fine setae extending to scutellum; scutellum with large and smaller hairs; pleura darkish with horizontal pale area without scales.

Wings (Fig.2c): 3.85 mm long, 1.59 mm wide; without broad apical fringe area, apex entirely dark; wing veins covered with proximal area of costa with scattered pale scales, vein with two white spots; vein 1(R1) with two pale spot, vein 2 (RS) entirely dark scaled two white scaled patches on distal branch, Vein 3 (R4+5) dark scales covered, interrupted by few white scales, vein 4 (M) dark scaled on main branch, on its branches both dark and white scales present, vein 5(Cu) dark and white scales, vein 6 (anal) with two distinct pale spots one on middle and other on distal end; wing fringe major part is dark also with pale spot on the apex of wing.

Legs (fig.2d): 5. 64 mm long, coxa 0.4 mm long, thin; femur 1.72 mm long, femur with pale band at extreme base; tibia in fore, mid and hind legs pale at base and tip; tibio-tarsal joints and tarsal joints pale banded; tibia,1.37 mm long, darkish, not speckled; tarsus five segmented, fore-leg tarsomeres without broad white bands.

Abdomen (fig.2e): 1.68 mm long, usually dark purple with many hairs; narrow pale scale on VIII segment, segment VII with a black tuft of hairs; cerci with hairs only.

Male: Characters are same as in females, smaller than females, 3.71 mm long, slender, with plumose antenna with dark scales, uniformly dark; palpi unbanded and dark; dorsal portion abdomen without tuft of black scales segment VII; coxites with numerous scales.

Bionomics: Rice fields, ponds and ground pools with aquatic vegetation, slow running streams, shaded parts of rivers, shallow wells, plastic as well as cement tanks, stagnant water pools with aquatic vegetation.

Distribution: India: recorded throughout the country. Kerala: reported from Alappuzha (Radhakrishnan A, 2019). The present study reported this species from Wayanad in the Arecanut garden.

Remarks: *An. umbrosus* is a species complex consisting of eleven species. This species is considered a vector of malaria in Indonesia. The main identification characteristics *An. umbrosus* of are the following;

1. palpi completely dark
2. Wing: inner costa with pale interruptions
3. Hind leg tarsomere with broad white bands
4. Fringe spot present at vein 1 and vein 3

3. *Anopheles culiciformis* Cogill, 1903

Anopheles culiciformis Cogill, 1903: 333. (BMNH, London) Type: unknown. Type locality: Karwar, Bombay.

Material examined: Plesiotype: Kerala, 1♀, 4th mile, Wayanad district. Coll. C. Vipinya, 03.iii.2019.

Other material examined: Kerala: 1♀ & 2♂, 4th mile, Wayanad district. Coll. C. Vipinya, 03.iii.2019. 2♀ & 2♂, Korome, Wayanad district. Coll. C. Vipinya, 12. Iv.2021.

Diagnosis: Female (Plate 3, Fig.3, Map-1) Small to moderate-sized, dark coloured 3.23 mm long.

Head: Covered with narrow, brown, upright scales sparsely scattered; narrow vertical area, vertical area without pale spot; no prominent frontal tuft of hairs; narrow ocular scales; compound eyes rounded and black; proboscis brown with black tip 2.43 mm long.

Antennae: With few pale hairs and numerous dark scales on first flagellar segment; 1.58 mm long, yellowish brown, 15 segmented, last two segments swollen hairy, and pilose; palpi 2.38 mm long, a little shorter than proboscis, moderate thickness, uniform distribution of scales shaggy effect, delicate, straight, without pale bands.

Thorax: 1.43 mm long, short, rounded, usually dark coloured; mesonotum dull brown or blackish, indistinct line present, entirely devoid of scales; scutellum dark with long brown scales, pleura dark grey coloured.

Wings: 3.69 mm long,1.54 mm wide, wing entirely dark, usually unspotted covered with spindle-shaped scales; vein 1(R1) dark, vein 2 (RS) dark, vein 3(R4+5) dark, vein 4 (M) dark, vein 5(Cu) dark, vein 6 (anal) dark; fringe spot absent on all the veins.

Legs: 5.7 mm long, coxa 0.4 mm long, thin; femur 1.59 mm long; femur, tibia, and tarsus uniformly dark in colour; tibia, 1.39 mm long, darkish, not speckled; tarsus five segmented, entirely brown covered with scales.

Abdomen: 1.66 mm long, usually dark light or brown covered with dark hairs; even on cerci scales absent.

Male; Characters are same as in females, smaller than females,2.92 mm long, slender, with plumose antenna, uniformly dark; palpi unbanded and dark; dorsal portion abdomen including coxites devoid of scales.

Bionomics: Forest species mainly breed in tree holes and holes on cut bamboo.

Distribution: In India: Uttar Pradesh, West Bengal, Andra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Goa, and Kerala. In Kerala: Kozhikode (Puduppadi). In this study the specimens were collected from Wayanad district.

Remarks: *An.culiciformis* belongs to the *Culiciformis* group, and its main identification characteristics are the following,

1. Wing and legs completely dark
2. Palpi completely dark and slightly shorter than proboscis.

This species differs from *An.aitkenii* and *An.insulaeflorum* by thicker palpi and normal head scales and differ from *An.sintoni* only by larval and male genital characters. It is a species complex consisting of five species; *An.alongensis, An.kyodawensis, An.sintonoides, An.sintoni,* and *An.culiciformis.*

4. *Anopheles barbirostris* Van der Wulp, 1884

Anopheles vanus Walker, 1860:91. (BNMH, London) Type: ♂ described, Type locality: Makessar, Celebes.

Anopheles martni Laveran, 1902: 907 Type: Unknown, Type locality: West of Pursat, Cambodia (Synonym by Edwards., Gen.insect. 1932).

Anopheles barbirostris Van der Wulp, 1884: 248. (SMNH, Leyden) Type locality: Mount Ardjoeno, Java.

Material examined: Plesiotype: Kerala: 1♀, Koorachund, Kozhikode district. Coll. C. Vipinya,13. x.2019.

Other material examined: Kerala: 21♀ & 8♂, Koorachund, Kozhikode district. Coll. C. vipinya, 13. x.2019; 13♀ & 6♂, Thirunelli, Wayanad district. Coll. C. Vipinya, 08.ii.2020; 22♀ & 2♂, Tharuvana, Wayanad district. Coll. C. Vipinya, 13. v.2020; 3♀ & 5♂, Mananthavady, Wayanad 13♀& 6♂, Makkiyad, Wayanad district. Coll. C. Vipinya, 14.iii.2021; 13♀ & 8♂, Kavumandam, Wayanad district. Coll. C. Vipinya, 18. v.2021; 12♀ & 8♂, Kannavum, Kannur district. Coll. C. Vipinya, 12. v.2021; 3♀ & 4♂, Pakkayil, Kozhikode district. Coll.C. Vipinya 22.viii.2021; 6♀ &

4♂, Thottilppalam, Kozhikode Coll. C. Vipinya, 23.vi.2021; 14♀ & 5♂, Koorara, Kannur district. Coll. C. Vipinya, 02.i.2020; 2♀ & 5♂, Vilangad, Kozhikode Coll. C. Vipinya, 2.xii.2021. 11♀ & 6♂, Nilabmur, Malappuram district. Coll. C. Vipinya, 19.ii.2022; 2♀ & 2♂, Kalikavu, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 4♀ & 12♂, Kakkadampoyil, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 13♀ & 6♂, Nedumkayam Malappuram district. Coll. C. Vipinya, 19.iii.2022;

Diagnosis: Female (Plate-4, Figs. 4a-e, Map-1) Large-sized, black-coloured 3.74 mm long.

Head: Covered with broad, short black type scales; narrow vertical area with pale vertical spot, vertical hairs pale coloured (fig.4b); prominent frontal tuft with white hairs; narrow elongated ocular scales; compound eyes rounded and black; proboscis brown with black tip 2.58 mm long covered with black scales.

Antennae: With small dark and pale scales present on first and second filamental segment; 1.58 mm long, yellowish brown, 15 segmented, last two segment swollen hairy, and pilose (Fig.4b); palpi 2.57 mm long, as long as the proboscis, erect, thin, delicate, straight, slender, uniform thickness covered with long broad scales, without pale bands (fig.4b).

Thorax: 1.55 mm long, usually dark coloured; mesonotum black, median area not pale as lateral area, covered with narrow hair-like scales; lateral area brown or pale coloured; pre-scutellar space with short fine setae extending to scutellum; scutellum with large and smaller hairs; pleura darkish with horizontal pale area without scales.

Wings (Fig.4c): 3.85 mm long, 1.5 mm wide; as in *Anopheles nigerrimus* without broad apical fringe area, apex entirely dark; wing veins covered with proximal area of costa with scattered pale scales, vein with two white spots; vein 1(R1) with two pale spot, vein 2 (RS) entirely dark scaled two white scaled patches on distal branch, Vein 3 (R4+5) dark scales covered, vein 4 (M) dark scaled on main branch, on its branches both dark and white scales present, vein 5(Cu) dark and white scales, vein 6 (anal) with two distinct pale spots one on middle and other on distal end; wing fringe major part is dark also with pale spot on the apex of wing.

Legs (fig.4d): 5. 64 mm long, coxa 0.4 mm long, thin; femur 1.72 mm long, femur with pale band at extreme base; tibia in fore, mid and hind legs pale at base and tip; tibio-tarsal joints and tarsal joints pale banded; tibia,1.37 mm long, darkish, not speckled; tarsus five segmented, fore-leg tarsomeres without broad white bands.

Abdomen (fig.4e): 1.68 mm long, usually dark purple with many hairs; narrow pale scale on VIII segment, segment VII with a black tuft of hairs; cerci with hairs only.

Male: Characters are same as in females, smaller than females, 3.71 mm long, slender, with plumose antenna with dark scales, uniformly dark; palpi unbanded and dark; dorsal portion abdomen without tuft of black scales segment VII; coxites with numerous scales.

Bionomics: Rice fields, ponds and ground pools with aquatic vegetation, slow running streams, shaded parts of rivers, shallow wells, plastic as well as cement tanks, stagnant water pools with aquatic vegetation.

Distribution: India: recorded throughout the country. Kerala: reported from Alappuzha (Radhakrishnan A, 2019).

Remarks: *An.barbirostris* is a member of the *barbirostris* sub-group and it is a species complex consisting of eleven species. This species is considered as a vector of malaria and filaria in Southeast Asia. The main identification characteristics of *An.barbirostris* are the following;

1. Palpi completely dark

2.Wing: inner costa with pale interruptions

3. Hind leg tarsomere with broad white bands

5. *Anopheles crawfordi* Reid, 1953

Anopheles crawfordi Reid, 1953: 43 (BMNH, London). Type: Type locality: Kuala Lumpur.

Material examined: Plesiotype: Kerala: 1♀, Tharuvana, Wayanad district. Coll. C. vipinya, 12. Iv.2019.

Other material examined: Kerala: 21♀ & 12♂, Tharuvana, Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 3♀ & 5♂, Mananthavady, Wayanad district. Coll. C. Vipinya, 113. Iv.2019; 2♀& 2♂, Kannavam Kannur district. Coll. C. Vipinya, 10.v.2022; 24♀ & 20♂, Thaliparamba Kannur district. Coll. C. Vipinya, 10.iv.2023.

Diagnosis: Female (Plate 5, Figs. 5a-e, Map-1) Small to moderate-sized, brown coloured 2.87 mm long.

Head(fig.5b): Covered with sparsely scattered linear scales; narrow vertical area, vertical hairs pale coloured; prominent frontal area; narrow ocular scales; compound eyes rounded and black; proboscis brown with black tip 2.33 mm long.

Antennae (fig.5b): With few pale hairs and devoid of scales; 1.52 mm long, yellowish brown, 15 segmented, last two segments swollen, hairy and pilose; palpi 2.41 mm long, as long as the proboscis, thin, delicate, straight, slender, uniform thickness, pale scales present on segment 2 with 4 small pale bands present: the tip of palpi apical segment short and swollen with scales.

Thorax (fig.5d): 1.45 mm long, short, rounded, usually dark coloured; mesonotum dull brown or pale coloured, entirely devoid of scales; scutum greyish with well-developed dark lines.

Wings (fig.5c): 3.66 mm long,1.53 mm wide, wing entirely dark, costa dark with subcostal pale spots, unspotted with marked wing scaling, scales on wing border extended up to base; costa with three dark scaled spots, large middle dark spot; vein 1(R1) dark without pale scales on preapical dark area pale scales on tip, vein 2 (RS) largely pale, vein 3(R4+5)small dark spot on proximal and distal area, vein 4 (M) majority pale, scattered pale area present, vein 5(Cu) proximal part pale several pale scales, vein 6 (anal) dark; fringe spot absent on all the veins. broad apical pale fringe spot present.

Leg (fig.5e): 5.65 mm long, coxa 0.4 mm long, thin, femur 1.53 mm long, femur, tibia, and tarsus uniformly dark in colour, tibia, 1.37 mm long, darkish, not speckled; tarsus five segmented, fore-leg: tibia without pale spot, tarsomere Ta-I1-3 with broad

white bands, mid-leg: Ta-II1-4 with broad apical pale band, hind leg: as same as in mid-leg tarsomere.

Abdomen: 1.63 mm long, usually dark light with dark hairs; even on cerci scales absent.

Male: Characters are same as in females, smaller than females, 2.51 mm long, slender, with plumose antenna, uniformly dark; palpi segment 2-3 with pale scales; dorsal portion abdomen including coxites devoid of scales.

Bionomics: This species particularly breeds in rice fields, marshes, swamps and ground pools

Distribution: India: recorded from Assam, Manipur, Kerala, and Tripura. Kerala: Rajavel *et al*., (2011), reported this species from Wayanad (Edakkal, Kalpetta). In this work, the species were collected from Wayanad and Kannur districts.

Remarks: The present study detected its presence from Kannur district for the first time. The characteristic identification features of *An.crawfordi* are the following;

1. Palpi with four pale bands.
2. Fore, mid and hind tarsomere with broad pale bands.
3. Tip of Vein 1 pale scaled.

6. *Anopheles peditaeniatus* Leicester, 1908

Myzorhynchus peditaeniatus Leicester, 1908: 31. (BMNH, London) Type: described from 2♂♂. Type locality: Kuala Lumpur. Synonymized by Stanton, Jou. Lon. Sch. T. Med. 1912: 4.

Myzorhynchus sinenesis var. peditaeniatus James & Stanton, 1912:61. (NM, US) Type: described single ♀, Camp Stotsenberg, Pampamga synonymized by Alcock, Jou. Lon. Sch. T. Med. 1913:159.

Anopheles peditaeniatus Leicester, 1908: 18. (BNMH, London) Type: syntypes, Type locality: Malaya, Malaysia.

Materials examined: Plesiotype: Kerala:1♀, Kaniyambetta, Wayanad district. Coll. C. Vipinya, 12. Iv.2019.

Other material examined: Kerala: 13♀ & 11♂, Kaniyambetta, Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 2♀ & 4♂, Meppadi, Wayanad district. Coll. C. Vipinya,13. Iv.2019; 2♀ & 7♂, ezhome Kannur district. Coll. C. Vipinya, 30.vii.2022; 12♀ & 3♂, Kelakam Kannur district. Coll. C. Vipinya, 30.vii.2022; 2♀ & 8♂, Thaliparamba Kannur district. Coll. C. Vipinya, 10.iv.2023; 2♀ & 4♂, Mayyil Kannur district. Coll. C. Vipinya, 10.iv.2023.

Diagnosis: Female (Plate 6, Figs. 6a-d, Map-2) Moderate-sized, brow coloured 2.89 mm long.

Head (fig.6b): Covered with narrow, linear scales sparsely scattered; narrow vertical area, vertical hairs pale coloured; prominent frontal area with tuft of hairs; narrow ocular scales; compound eyes rounded and black; proboscis brown with black tip 2.41 mm long.

Antennae (fig.6b): With few pale hairs and devoid of scales; 1.53 mm long, yellowish brown, 15 segmented, last two segments swollen hairy, and pilose; palpi 2.42 mm long, as long as the proboscis, thin, delicate, straight, slender, uniform thickness, pale scales present on segment 2 with 4 distinct pale bands present; the tip of palpi apical segment short and swollen with scales.

Thorax: 1.47 mm long, short, rounded, usually dark coloured; mesonotum dull brown or pale coloured, entirely devoid of scales; scutum greyish with well-developed median dark lines.

Wings (fig.6c): 3.67 mm long,1.54 mm wide, wing pale and dark in appearance; costa dark with subcostal pale spots and preapical pale spots, numerous scattered pale spots present; vein 1(R1) with numerous pale scales dark without pale scales on preapical dark area pale scales on tip, vein 2 (RS) largely pale, vein 3(R4+5)small dark spot on proximal and distal area, vein 4 (M) majority pale area scattered pale area present, vein 5(Cu) proximal part pale several pale scales, vein 6 (anal) dark; fringe spot absent on all the veins; broad apical pale fringe spot present.

Legs (fig.6d): 5.65 mm long, coxa 0.4 mm long, thin, femur 1.53 mm long, femur, tibia, and tarsus uniformly dark in colour; tibia, 1.37 mm long, darkish, not

speckled; tarsus five segmented, fore-leg: tibia without pale spot, tarsomere Ta-I_3 with broad white bands. mid leg: Ta-II_4 with broad apical pale band, hind leg: as same as in mid-leg tarsomere.

Abdomen (fig.6d): 1.63 mm long, usually dark light with dark hairs; even on Cerci scales absent.

Male: Characters are same as in females, smaller than females,2.51 mm long, slender, with plumose antenna, uniformly dark; palpi segment 2-3 with pale scales, dorsal portion abdomen including coxites devoid of scales.

Bionomics: This species mainly breeds in rice fields, and also prefers to breed in habitats like ditches and marshes, ponds, temporary pools, margins of streams and rivers with aquatic vegetation, shallow wells, and animal footprints.

Distribution: India: recorded from Goa, Tamil Nadu, and Kerala. Kerala: Arunachalam *et al*., 2004 reported from Alappuzha, and Wayanad, Kannur (present study).

Remarks: This is the first report from North Kerala. Characteristic features are:

1. Palpi with 4 small pale bands
2. Inner side of Costa completely dark

7. *Anopheles gigas* Giles, 1901

Material examined: Plesiotype: Kerala: 1♀, Vythiri, Wayanad district. Coll. C. Vipinya, 07.iii.2019.

Other material examined: Kerala: 2♀ & 7♂, Vythiri, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 2♀ & 8 ♂, Tharuvana, Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 3♀ & 5♂, Mananthavady, Wayanad district. Coll. C. Vipinya, 12. Iv.2019.

Diagnosis: Female (Plate 7, Figs. 7a-d, Map-2) large-sized, light-coloured 3.86 mm long.

Head (fig.7b): Covered with normal type scales; narrow vertical area, vertical hairs pale coloured; prominent frontal area; narrow elongated ocular scales; compound eyes rounded and black; proboscis brown with black tip 2.54 mm long.

Antennae (fig.7b): With dark and pale scales present on the first and second filamental segment; 1.59 mm long, yellowish brown, 15 segmented, last two segments swollen hairy, and pilose; palpi 2.55 mm long, as long as the proboscis, erect, thin, delicate, straight, slender, uniform thickness, without pale bands, few pale scales at joints: the tip of palpi apical segment short and swollen with scales.

Thorax: 1.53 mm long, usually dark coloured; mesonotum median area pale coloured, entirely devoid of scales, lateral area brown or pale coloured; pre-scutellar space with short fine setae extending to scutellum; scutellum with large and smaller hairs; pleura darkish with horizontal pale area without scales.

Wings (fig.7c): 3.78 mm long,1.55 mm wide, wing dark with absence of pale regions in costa and vein 1, usually unspotted with marked wing scaling, scales on wing border extended up to the base, costa with three dark scaled spots, large middle dark spot; vein 1(R1) dark, vein 2 (RS) dark, vein 3(R4+5) dark, vein 4 (M) dark, vein 5(Cu) dark, vein 6 (anal) with pale spot-on outer half; fringe spot absent on all the veins.

Legs (fig.7d): 5.64 mm long, coxa 0.4 mm long, thin; femur 1.72 mm long; femur with pale band at extreme base; tibia of fore, mid and hind legs pale at base and tip; tibiotarsal joints and tarsal joints pale banded, tibia,1.37 mm long, darkish, not speckled; tarsus five segmented, fore-leg tarsomeres without broad white bands.

Abdomen; 1.68 mm long, usually pale with pale hairs; scales absent even on Cerci.

Male: Characters are same as in females, smaller than females,2.51 mm long, slender, with plumose antenna, uniformly dark; palpi unbanded and dark; dorsal portion abdomen including coxites devoid of scales.

Bionomics: Breeding places include, edges of ponds with aquatic vegetation, marshy places with grass, small pools and hill streams, rice fields, seepage pools, forest pools, rock holes, rainwater collections, and spring bed pools.

Distribution: India: Arunachal Pradesh, Assam, Delhi, Haryana, Himachal Pradesh, Karnataka, Kashmir, Manipur, Meghalaya, Mizoram, Nagaland, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal, and Kerala. Kerala: Radhakrishnan (2019) reported from Ernakulam (Central Kerala), Wayanad (present study).

Remarks; It is reported from North Kerala (Wayanad) for the first time. The characteristic identification features of *An.gigas* are the following;

1. Palpi are completely dark but small bands are present at the joints.
2. Basal dark area of vein 5 (CU) 1/6/ of the stem
3. Inner costa interrupted
4. Vein 6 with a pale spot on the outer half.

8. *Anopheles nigerrimus* Giles, 1900

Anopheles sinensis subsps. *indiensis* Theobald, 1901:145. (BNMH, London) Type: ♀ described, Type locality: Madras, Central Province, India. (Synonymized Christophers Ind.Med.Res.Mem. 1924:29).

Anopheles pursati Laveran, 1902: 907. Type: ♀described, Type locality: West of Pursat, Cambodia (Synonym of An. Hyrcanus by Edwards., Gen.insect. 1932).

Anopheles bentleyi Bentley, 1902: 15. Type: ♀described, Type locality: Tezpur, Assam (Synonym by Christophers Ind.Med.Res.Mem. 1924:29).

Myzorhynchus minutus Theobald, 1903: 91. (BNMH, London) Type: ♀described, Type locality: Lahore, Punjab, India (Synonym by James & Liston Anopheles Mosquitoes of India 1911: 120).

Myzorhynchus argyropus Swelleng, 1914: 334. Type: nill, Type locality: Java (Synonym by Barraud and Christophers Rec. Mal. Surv. 1931:153).

Anopheles nigerrimus Giles, 1900: 374. (BMNH, London) Type: Type locality: Calcutta, India.

Materials examined: Plesiotype: Kerala: 1♀, Vythiri, Wayanad district. Coll. C. Vipinya, 07. iii.2019.

Other material examined: Kerala: 6♀ & 1♂, Vythiri, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 1♀ & 3♂, Makkiyad, Wayanad district. Coll. C. Vipinya, 12.

Iv.2019; 2♀ & 7♂, Mananthavady Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 1♀ & 3♂, Kelakam Kannur district. Coll. C. Vipinya, 30.vii.2022; 2♀ & 5♂, Thaliparamba Kannur district. Coll. C. Vipinya, 10.iv.2023; 5♀ & 1♂, Kakkadampoyil, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 6♀ & 2♂, Nedumkayam, Malappuram district. Coll. C. Vipinya, 19.iii.2022.

Diagnosis: Female (Plate 8, Figs.8a-d, Map-2) Medium to large-sized, dark brown-coloured mosquitoes, 3.45mm long.

Head (fig.8b): Covered with normal black and white upright and pale scales in the front area with dark scales on behind; well-marked pale-coloured vertical area; ocular area well developed with scales arranged in a single line in the anterior region; prominent frontal tuft with white scales; compound eyes rounded and black; proboscis cylindrical and covered with black scales, tip yellowish white 2.86 mm long.

Antennae: With few minute scales on the torus and white scales on 1-5 flagellar segment; 1.46 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.45 mm long, as long as the proboscis, moderately thick, slender, scales present throughout its length, four pale bands: white apical band other bands are narrow at 2-3, 3-4, and 4-5.

Thorax: 1.35 mm long, covered with dense black scales; mesonotum black, median area lighter than lateral area, tuft of white scales present on lateral area; scutellum dark with dark hairs present; pleura dark.

Wings (fig.8c): 3.54 mm long,1.45 mm wide, pale coloured and spotted with dark marks; wing veins covered with broad spindle-shaped white and black scales those in *Anopheles barbirostris*; costa majority black, with three-minute white spots in outer third, one spot present junction of outer and middle third of costa, second one at distal end, third large spot also at distal end. vein 1(R1) with two white spots covered with light and dark scales, vein 2 (RS) covered with both dark and light scales vein 1 s light spot extended to the bifurcation of second vein, vein 3 (R4+5) mostly pale areas except basal and distal portion, vein 4 (M) with light and dark

scales except its proximal and distal extremity, vein 5(Cu) black at the origin rest of its part light coloured, vein 6 (anal) mostly pale coloured with two dark scaled spots; wing fringe with dark scales except at the apex.

Legs (fig.8d): 5.72 mm long, coxa 0.5 mm long, broad; femur 1.56 mm long; femur, tibia, and tarsus covered with dark and light scales, light scales present at all joints; tibia, 1.45 mm long, usually pale, not speckled; tarsus five segmented, fore, mid, and leg tarsomere (Ta-I, II) with broad white bands present, hind leg (Ta-III) tarsomere with apical white bands.

Abdomen: 1.58 mm long, mainly dark in colour, covered with light and dark hairs, devoid of scales, scales present on Te-VIII at its posterior border; cerci with hairs only.

Male: Characters are the same as in females, smaller than females, 2.89mm long, slender, with plumose antennae, and uniformly dark; antennae without scales, major portion of the palpi pale coloured; abdominal segment without scales; coxites with narrow dark and pale scales.

Bionomics: mainly breeds in rice fields with well-grown aquatic vegetation, but also breeds in shady edges of ponds with aquatic vegetation, tanks, drains, shaded lakes, borrow pits, and pools

Distribution: India except Himachal Pradesh, *An.nigerrimus* recorded throughout the country. Also reported from Andaman and Lakshadweep Islands. Kerala: Balasubramanian *et al*., (2013) reported from Alappuzha, and Kottayam: Radhakrishnan (2018) reported from Ernakulam. Present study- Wayanad, Kannur, and Malappuram.

Remarks: New record from the study area, the presence of this species in Kannur, Wayanad, and Malappuram was detected for the first time. Major identification characteristics of *An.nigerrmus* are the following;

1. Palpi with four pale bands
2. Tarsomere with broad pale bands

3. Basal dark area of vein 5 (CU) is ½ of the stem

9. *Anopheles nitidus*, Harrison, Scanlon & Reid, 1973

Anopheles sinensis subsps. *indiensis* Theobald, 1901:145. (BNMH, London) Type: ♀ described, Type locality: Madras, Central Province, India. (Synonymized Christophers Ind.Med.Res.Mem. 1924:29)

Material examined: Plesiotype: Kerala: 1♀, Ambalavayal, Wayanad district. Coll. C. Vipinya, 07.ii.2020.

Other material examined: Kerala: 4♀ & 5♂, Ambalavayal, Wayanad district. Coll. C. Vipinya, 07.ii.2020; 2♀ & 6 ♂, Korome, Wayanad district. Coll. C. Vipinya, 16. v.2021; 3♀ & 4♂, Mananthavady, Wayanad district, Coll. C. Vipinya, 24.vi.2022; 1♀& 3♂, Kelakam Kannur district. Coll. C. Vipinya, 30.vii.2022; 5♀ & 1♂, Kakkadampoyil, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 6♀ & 2♂, Nedumkayam Malappuram district. Coll. C. Vipinya, 19.iii.2022; 3♀ & 1♂, Anakkayam, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 2♀ & 4♂, Kootilangadi Malappuram district. Coll. C. Vipinya, 19.iii.2022; 2♀ & 5♂, Thaliparamba Kannur district. Coll. C. Vipinya, 10.iv.2023; 1♀& 4♂, Irikkur Kannur district. Coll. C. Vipinya, 1.v.2023.

Diagnosis: Female (Plate 9, Fig.9a-e, Map-) Medium to large-sized, dark brown-coloured mosquitoes, 3.45mm long.

Head (fig.9b): Covered with normal black and white upright and pale scales in the front area with dark scales on behind; well-marked pale-coloured vertical area; ocular area well developed with scales arranged in a single line in the anterior region; prominent frontal tuft with white scales; compound eyes rounded and black; proboscis cylindrical and covered with black scales, tip yellowish white 2.86 mm long.

Antennae (fig.9b): With few minute scales on the torus and white scales on 1-5 flagellar segment; 1.46 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.484 mm long, as long as the proboscis, moderately thick, slender, scales

present throughout its length, four pale bands: broad apical band other bands are narrow at 2-3, 3-4, and 4-5.

Thorax: 1.35 mm long, covered with dense black scales; mesonotum black, median area lighter than lateral area; tuft of white scales presents on lateral area; scutellum dark with dark hairs present; pleura dark.

Wings (fig.9c): 3.54 mm long,1.45 mm wide, pale coloured and spotted with dark marks, wing veins covered with broad spindle-shaped white and black scales those in *Anopheles barbirostris*; costa majority black, with three-minute white spots in outer third, one spot present junction of outer and middle third of costa, second one at distal end, third large spot also at distal end. vein 1(R1) with two white spots covered with light and dark scales, vein 2 (RS) covered with both dark and light scales vein 1's light spot extended to bifurcation of second vein, vein 3(R4+5) mostly pale areas except basal and distal portion, vein 4 (M) with light and dark scales except its proximal and distal extremity, vein 5(Cu) black at the origin rest of its part light coloured, vein 6 (anal) mostly pale coloured with two dark scaled spots; wing fringe with dark scales except at the apex.

Legs (fig.9d): 5.72 mm long, coxa 0.5 mm long, broad, femur 1.56 mm long, femur, tibia, and tarsus covered with dark and light scales, light scales present at all joints, tibia, 1.45 mm long, usually pale, not speckled, tarsus five segmented, fore, mid, and leg tarsomere (Ta-I, II) with broad white bands present, hind leg (Ta-III) tarsomere with apical white bands.

Abdomen(fig.9e): 1.58 mm long, mainly dark in colour, covered with light and dark hairs, devoid of scales, scales present on Te-VIII at its posterior border; cerci with hairs only.

Male: characters are same as in females, smaller than females, 2.89mm long, slender, with plumose antennae, uniformly dark; antennae without scales; major portion of the palpi pale coloured, abdominal segment without scales; coxites with narrow dark and pale scales.

Bionomics: commonly breeds in swamps, rice fields, burrowpits, and rock pools.

Distribution: India: recorded from Assam, Manipur and Kerala. In the present study the species was recorded from Kannur, Wayanad, and Malappuram for the first time.

Remarks: Its characteristic features for identification are following;

1. Dark area present on the basal pat of wing vein 5
2. 4 pale banded palpi

***10. Anopheles sinensis* Wiedermann, 1828**

Material examined: Plesiotype: Kerala: 1♀, Nedumkayam Malappuram district. Coll. C. Vipinya, 19.iii.2022.

Other material examined: Kerala: 2♀ & 6 ♂, Korome, Wayanad district. Coll. C. Vipinya, 16. v.2021; 3♀ & 4♂, Mananthavady, Wayanad district, Coll. C. Vipinya, 24.vi.2022; 1♀& 3♂, Kelakam Kannur district. Coll. C. Vipinya, 30.vii.2022; 5♀ & 1♂, Kakkadampoyil, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 6♀ & 2♂, Nedumkayam Malappuram district. Coll. C. Vipinya, 19.iii.2022; 3♀ & 1♂, Anakkayam, Malappuram district. Coll. C. Vipinya, 20.ii.2022;

Diagnosis: Female (Plate 10, Fig.10a-d, Map-3) Medium to large-sized, dark-coloured mosquitoes, 3.4mm long.

Head (fig.10b): Covered with normal black and white upright and pale scales in the front area with dark scales on behind; well-marked vertical area; ocular area well developed with scales arranged in a single line in the anterior region; prominent frontal tuft with white scales; compound eyes rounded and black; proboscis cylindrical and covered with black scales, tip yellowish white 2.8 mm long.

Antennae (fig.10b): With few minute scales on the torus and white scales on 1-5 flagellar segment; 1.46 mm long, light coloured, hairy, and pilose; palpi 2.84 mm long, as long as the proboscis, moderately thick, slender, scales present throughout its length, four pale bands: broad apical band other bands are narrow at 2-3, 3-4, and 4-5.

Thorax: 1.37 mm long, covered with dense black scales; mesonotum black, median area lighter than lateral area; tuft of white scales presents on lateral area; scutellum dark with dark hairs present; pleura dark.

Wings (fig.10c): 3.52 mm long,1.42 mm wide, pale coloured and spotted with dark marks, wing veins covered with broad spindle-shaped white and black scales; costa majority black, with three-minute white spots in outer third, one spot present junction of outer and middle third of costa, second one at distal end, third large spot also at distal end. vein 1(R1) with two white spots covered with light and dark scales, vein 2 (RS) covered with both dark and light scales vein 1's light spot extended to bifurcation of second vein, vein 3(R4+5) mostly pale areas except basal and distal portion, vein 4 (M) with light and dark scales except its proximal and distal extremity, vein 5(Cu) black at the origin rest of its part light coloured, vein 6 (anal) mostly pale coloured with two dark scaled spots; wing fringe with dark scales except at the apex.

Legs (fig.10d): 5.7 mm long, coxa 0.5 mm long, broad, femur 1.6 mm long, femur, tibia, and tarsus covered with dark and light scales, light scales present at all joints, tibia, 1.45 mm long, usually pale, not speckled, tarsus five segmented, fore, mid, and leg tarsomere (Ta-I, II) with broad white bands present, hind leg (Ta-III) tarsomere with apical white bands.

Abdomen: 1.58 mm long, mainly dark in colour, covered with light and dark hairs, devoid of scales, scales present on Te-VIII at its posterior border; cerci with hairs only.

Male: Characters are same as in females, smaller than females, 2.89mm long, slender, with plumose antennae, uniformly dark; antennae without scales; major portion of the palpi pale coloured, abdominal segment without scales; coxites with narrow dark and pale scales.

Bionomics: Commonly breeds in swamps, rice fields, burrowpits, and rock pools.

Distribution: India: recorded from Assam, Manipur, Meghalaya, Mizoram, Odisha, Punjab, Tamil Nadu and Kerala (Alappuzha). Kerala: This species was reported from

Alappuzha. This species was reported from Wayanad, Malappuram and Kannur districts for the first time in this study.

Remarks: Its characteristic features for identification are the following;

1. 4 pale banded palpi
2. Vein 5 with fringe spot
3. Costa (inner) completely dark

Subgenus *Cellia* Theobald

Subgenus *Cellia* is the largest subgenus of the subfamily *Anophelinae.* It has been divided into six series- *Cellia, Myzomia, Neocellia, Neomyzomia, Paramyzomia*, and *Pyretophorus* (Grjebine, 1966; Gillies and de Million, 1968; Ried, 1968). The present study reported 17 species belong to this subgenus.

Series *myzomyia*

11. *Anopheles culicifacies* Giles, 1901

Anopheles listonii Giles, 1901: 197. (BMNH, London) Type: single ♀, Ellichpur, Berars, India. (synonymized with *An. culicifacies*, Theobald 1901: 41)

Anopheles indica Theobald,1901: 183 (BMNH, London) type: single ♀, Madras, India. (synonymized with Theobald 1902: 377)

Myzomia culicifacies var. punjabensis, James and Liston,1911: 72(BMNH, London) type: ♀, Punjab, India. (synonymized with pigment anomaly by Christophers 1916: 463).

Anopheles culicifacies Giles, 1901: 196. (BNMH, London) Type locality: Hoshangabad, India.

Material examined: Plesiotype: Kerala: 1♀, Thottilppalam, Kozhikode district, Coll.C. Vipinya, 03. xii.2019.

Other materials examined: Kerala: 4♀ & 5♂, Thottilppalam, Kozhikode district, Coll.C. Vipinya, 03. xii.2019; 3♀ & 4♂, Kannavam, Kannur district, Coll.C. Vipinya.22.iii.2020; 3♀ & 2♂ Mananthavady, Wayanad district, Coll. Mable Rose.12.vi.2020.

Diagnosis: Female (Plate 11, Figs.11a-d, Map-2) small to medium-sized mosquitoes, 3.8 mm long.

Head (fig.11b): Blackish brown with narrow scale type; vertex darkish not flattened; scanty ocular scales with ocular space 0.22 mm long; compound eyes rounded and black; proboscis blackish or brownish 2.12 mm long.

Antennae: Without scales few hairs present on the first segment, 1.9 mm long, yellowish brown, 15segmented, hairy, and pilose; palpi 2.1 mm long, as long as proboscis, slender, apical pale band nearly equal to the subapical dark band.

Thorax: 1.4 mm long, brown or blackish; centre of scutum without scales, light-coloured ante pronotal scales absent.

Wings (fig.11c): 3.34 mm long,0.61 mm width; base of costa with interruption just external to the cross vein, scales with yellow colour present on veins, vein 3 (R4+5) mainly dark, vein R1 without pale interruption, fringe spot present on 4.2 and 5.1 only, cubitus bifurcated.

Legs (fig.11d): 6.4 mm long, coaxa 0.21 mm long, broad; femur 1.59 mm long, cylindrical, slightly yellow with scales, without splecking; tibia, 1.83 mm long, yellowish, not speckled; tarsus five segmented, tarsomeres without bands.

Abdomen: 1.68 mm long, devoid of scales, reddish brown.

Male: Smaller than females, 3.12 mm long, slender, with a plumose antenna.

Bionomics: Prefers to breed in rice fields, rock pools with aquatic vegetation, river margins and river bed pools, rainwater collections, wells, pools, and brackish water pools.

Distribution: In India: recorded throughout the country except Andaman and Nicobar Islands. Kerala: Iyengar,(1938) reported from Travancore, and Mathew, (1939) reported from Travancore. In this study, the species were reported from Kozhikode (Rockpool with vegetation) and Wayanad districts

Remarks: *An.culicifacies* is one of the important primary Malaria vectors in India and it is a species complex consisting of five sibling species designated from A to E. Species B is most predominant and present throughout the country. Its main identification features are following;

1. Apical pale band nearly equal to pre-apical dark band.
2. Tarsomere without bands
3. Vein 3 (R4+5) mainly dark
4. Inner costa interrupted

12. *Anopheles jeyporiensis* James, 1902

Material examined: Plesiotype: Kerala: 1♀, Mananthavady, Wayanad district Coll. St. Mable Rose.15. v.2020.

Other material examined: Kerala: 1♀ & 2♂, Kannavam, Kannur district Coll.C. Vipinya. 22.iv.2020; 1♀& 1♂, Mananthavady, Wayanad district Coll. C. Vipinya.15. v.2020.

Diagnosis: Female (Plate 12, Figs.12a-e, Map-3) small-sized mosquitoes 2.5 mm long.

Head (fig.12b): Normal type scales with well-marked vertical spot; well-developed frontal tuft; large vertical area; vertex darkish not flattened; ocular scales arranged in many rows; compound eyes rounded and black; proboscis blackish or brownish 2.02 mm long.

Antennae: Without scales, few narrow hairs present on the first segment; 1.8 mm long, yellowish brown, hairy, and pilose; palpi 2 mm long, as long as proboscis, slender, three pale bands: at the apex, junction between second and third, last band between third and fourth, apical pale band nearly or equal to the pre-apical dark band.

Thorax: 1.3 mm long, yellowish and white hair-like scales arranged as one median, two longitudinal lateral lines; mesonotum with narrow white scales.

Wings(fig.12d): 3.3 mm long,0.39 mm wide, long spindle-shaped scales cover the wings; costa with four yellowish-white interruptions, larger one at the proximal part and the smallest one at the distal part, vein 1(R1); four pale scaled areas corresponding to coastal pale bands, vein 2(RS); three pale bands, vein 3 (R4+5) mainly pale, vein 4 (M); mostly dark scaled, vein 5 (Cu) mostly pale scales at its bifurcation it is dark scaled, vein 6 (anal) most part dark scales but two small pale areas; except for vein 6 fringe spot present on all other veins.

Legs (fig.12e): 6.2 mm long, coxa 0.11 mm long, broad; femur 0.99 mm long; femur, tibia, and tarsus uniformly dark in colour scales; narrow band present at the joints, without scales and speckling; tibia1.54 mm long, yellowish, not speckled; tarsus five segmented, tarsomeres without bands.

Abdomen: 1.52 mm long, devoid of scales, mainly dark, covered with yellowish white hairs.

Male: smaller than females,2.5 mm long, slender, with a plumose antenna.

Bionomics: Breeds in pools, streams slow-moving water with aquatic vegetation, seepage channels of Dams, irrigation channels, wells, margins of lakes, borrow pits, tanks, rice fields,

Distribution: In India, it is recorded throughout the country. Kerala: Iyengar (1934) reported it from Travancore. In this study, it was reported from Kannur, Wayanad, and Malappuram.

Remarks: It is considered a secondary vector of Malaria in China Its characteristics features are following;

1. Apical pale band nearly equal to pre-apical dark band
2. Tarsomere with small narrow band bands
3. Fringe spot present on all veins

***13. Anopheles fluviatilis* James, 1902**

Anopheles listonii Liston, 1901: 361 (BMNH, London). Type: two ♀♀ labelled, Ellichpur, India. Synonymized by Edwards., 1932.

Myzomia leptomeres Theobald, 1903: 38 (BMNH, London). Type: single ♀ described, Lahore, India. Synonymised by Christophers., 1924.

Anopheles fluviatilis James, 1902: 106. Type locality: Nagpur and Jeypore Hill Tracts, India.

Material examined: Plesiotype: Kerala: 1♀, Ranipuram, Kasaragod district, Coll. C. Vipinya, 12. vii.2020.

Other material examined: Kerala: 2♀ & 3♂, Ranipuram, Kasaragod district, Coll.C. Vipinya, 12. vii.2020; 1♀ & 2♂, Kannavam, Kannur district Coll.C. Vipinya. 22.iv.2020; 1♀& 1♂, Mananthavady, Wayanad district Coll. St. Mable Rose.15. v.2020.

Diagnosis: Female (Plate 13, Figs.13a-e, Map-3) small-sized mosquitoes, very dark in colour 2.9 mm long.

Head (fig.13b): Normal type of upright scales with a frontal tuft of hair; large vertical area; vertex darkish not flattened; ocular scales arranged in many rows; compound eyes rounded and black; proboscis yellowish-white tip and blackish or brownish 2.02 mm long.

Antennae: Without scales, few narrow hairs present on the first segment; 1.8 mm long, yellowish brown, 15segmented, hairy, and pilose; palpi 2 mm long, as long as proboscis, slender, three pale bands: at the apex, junction between second and third, last band between third and fourth, apical pale band nearly or equal to the pre-apical dark band.

Thorax: 1.3 mm long, yellowish and white hair-like scales arranged as one median, two longitudinal lateral lines; dark-coloured mesothorax; median tuft scales present on ante pronotum.

Wings(fig.13d): 3.23 mm long,0.39 mm wide, long spindle-shaped scales cover the wings; costa with four yellowish-white interruptions, larger one at the proximal part

and the smallest one at the distal part, vein 1(R1); four pale scaled areas corresponding to coastal pale bands, vein 2(RS); three pale bands, vein 3 (R4+5) mainly pale, vein 4 (M); mostly dark scaled, vein 5 (Cu) mostly pale scales at its bifurcation it is dark scaled, vein 6 (anal) most part dark scales but two small pale areas; except for vein 6 fringe spot present on all other veins.

Legs (fig.13e): 6.2 mm long, coxa 0.11 mm long, broad; femur 0.99 mm long; femur, tibia, and tarsus uniformly dark in colour scales; narrow band present at the joints, without scales and speckling; tibia1.54 mm long, yellowish, not speckled; tarsus five segmented, tarsomeres without bands.

Abdomen: 1.52 mm long, devoid of scales, mainly dark, covered with yellowish-white hairs.

Male: Smaller than females,2.5 mm long, slender, with a plumose antenna.

Bionomics: Breeds in pools, streams slow-moving water with aquatic vegetation, seepage channels of Dams, irrigation channels, wells, margins of lakes, borrow pits, tanks, rice fields,

Distribution: In India: recorded throughout the country. Kerala: Covell,(1927) reported from Wayanad, Mathew, (1939) reported from Travancore. Covell & Harbhagwan, (1939) reported from Wayanad, and Balasubramanian, (2013) reported from Alappuzha. We reported this species from Wayanad and Kasaragod.

Remarks: This study reported the species from Kasaragod for the first time. *An.fluviatilis* is a species complex of three sibling species (Known as S, T, and U). species S common in India and Nepal and it is an efficient malaria vector in forested areas in several states. Its characteristics features are following;

1. Apical pale band nearly equal to pre-apical dark band
2. Tarsomere without bands

14. *Anopheles dthali* Patton, 1905

Anopheles rhodensiensis Christophers and Khazan Chand, 1905 (unknown), Type locality: D'thala, Yemen.

Material examined: Plesiotype: Kerala: 2♀ & 3♂, Kanjirangad, Wayanad district. Coll. C. Vipinya, 10.xi.2020.

Diagnosis: Female (Plate 14, Figs.14, Map-3) Small-sized, pale-coloured mosquitoes, 2.10 mm long. Look like *An.culicifacies* in appearance.

Head: Narrow rod-like scales with light yellowish; narrow vertical area; vertical hairs forming a single row on the eighter side; narrow ocular scales arranged in a single row; frontal tuft absent compound eyes rounded and black; proboscis yellowish-white tip and blackish or brownish 2.3 mm long.

Antennae: Without scales; 1.61 mm long, yellowish brown, 15segmented, hairy, and pilose; palpi 2.1 mm long, as long as the proboscis, thin, slender, uniform thickness, two pale bands: the tip of palpi dark (apical band with pale hairs no definite band), the first pale band in between second and third segment, the second pale band in between third and fourth segment.

Thorax: 1.45 mm long; mesonotum yellowish and white or bluish transparent appearance without hairs and scales; pleura without scales.

Wings: 3.45 mm long,1.30 mm wide, wing entirely devoid of pale interruptions including apex and fringe areas, vein 1(R1) to vein 6 (anal) completely dark; some pale interruptions present on cross vein areas.

Legs: 5.45 mm long, coxa 0.14 mm long, broad; femur 1.12 mm long; femur, tibia, and tarsus uniformly dark in colour; tibia, 1.34 mm long, darkish, not speckled; tarsus five segmented, tarsomeres without bands.

Abdomen: 1.62 mm long, devoid of scales, alternate dark and white patches, covered with whitish hairs.

Male: Characters are the same as in females, smaller than females,1.51 mm long, slender, with plumose antenna, uniformly dark.

Bionomics: Rice fields, streams and river margins with vegetation, and water seepages.

Distribution: In India: recorded extreme northwest, Jammu and Kashmir, Maharashtra, Punjab, and Kerala. In this study, the species was reported from Wayanad.

Remarks: This species reported from the study area for the first time and its main identification features are the following;

1. Tip of palpi dark
2. Tarsomere without bands
3. Vein 1 and vein 6 completely dark

Series *Pseudomyzomia*

***Anopheles subpictus* Grassi, 1899**

Anopheles rosii Giles, 1899: 101 (RUM, Rome). Type: ♂ and ♀ type specimens. India: Calcutta. (synonymized by Edwards 1920 (129) Bulletin of Entomological Research).

Aldrichia error Theobald, 1903: 353 (BMNH, London). Type: one ♀, India, Calcutta.

Anopheles subpictus Grassi, 1899: 100 (RUM, Rome) Type locality: India.

Material examined: Plesiotype: Kerala: 1♀, Kadalundi, Kozhikode district. Coll. C. vipinya, 26.i.2019.

Other materials examined: Kerala: 15♀ & 25♂, Kadalundi, Kozhikode district. Coll. C. Vipinya, 26.i.2019; 12♀ & 8♂, Kavilumpara, Kozhikode district. Coll. C. Vipinya, 11. Iv.2019; 3♀ & 5♂, Ayancheri, Kozhikode district, Coll. C. Vipinya, 10.iii.2020; 12♀ & 8♂, Kuttiady, Kozhikode district. Coll. C. Vipinya, 11. v.2020; 3♀ & 5♂, Koodaranji, Kozhikode district, Coll. C. vipinya, 6.vii.2020; 5♀& 2♂,

Chengottkavu, Kozhikode district. Coll. C. Vipinya. 11.x. 20205♀ & 2♂, Koothali, Kozhikode district. Coll. C. Vipinya, 21.ii.2021; 2♀ & 1♂, Kunjippally, Kozhikode district, Coll. C. Vipinya, 12.iii.2022; 5♀& 2♂, Ajanur, Kasaragod district. Coll. C. Vipinya, 28.iv.2022; 5♀& 2♂, Adhur, Kasaragod district, Coll. C. Vipinya, 14.x.2022.

Diagnosis: Female (Plate 15, Figs.15a-d, Map-3) Medium-sized, light brown-coloured mosquitoes, 3.21 mm long.

15. Anopheles subpictus B

Head (fig.15b): Covered with narrow black and white scales; narrow vertical area, vertical hairs pale coloured; prominent frontal tuft; narrow ocular scales; compound eyes rounded and black; proboscis brown with yellow tip 2.4 mm long.

Antennae: With few pale hairs and scales; 1.54 mm long, yellowish brown, 15segmented, hairy, and pilose; palpi 2.41 mm long, as long as the proboscis, thin, slender, uniform thickness, three pale bands: the tip of palpi pale and broad, remaining bands are narrow, the first pale band in between fourth and third segment, others pale band in between third and second segments; apical pale band nearly equal to pre-apical dark band.

Thorax: 1.5 mm long, yellowish and golden hairs and hair-like scales arranged in indistinct longitudinal lines; mesonotum pale, pale golden hairs present on median area.

Wings (fig.15d): 3.67 mm long,1.51 mm wide; wing with pale interruptions including apex and fringe areas, more than four pale coloured areas; costa with three dark scaled spots, large middle dark spot; vein 1(R1) with five dark spots, four of these similar and fifth lies close to the inner end of the vein, vein 2 (RS) with two dark spots; vein 3(R4+5) with three similar dark spots; vein 4 (M) before it's bifurcation two dark spots; vein 5 (Cu) one spot before bifurcation, anterior branch with three and posterior with one dark spot; vein 6 (anal) with two dark spots; fringe spot on all the veins.

Legs: 5.64 mm long, coxa 0.4 mm long, broad; femur 1.5 mm long, femur, tibia, and tarsus uniformly dark in colour; tibia, 1.34 mm long, darkish, not speckled; tarsus five segmented, fore-leg tarsomeres with broad white bands.

Abdomen (fig.15d): 1.76 mm long, light golden hairs, alternate dark and white patches, covered with whitish hairs; cerci with dark scales; posterior portion of segments VII and VIII with pale scales.

Male: Characters are the same as in females, smaller than females,2.51 mm long, slender, with plumose antenna, uniformly dark; major portion of palpi pale coloured, dorsal portion abdominal segment VIII with broad pale scales instead of hairs.

Bionomics: Breeds in a variety of habitats. Flowing or stagnant water with or without vegetation, shaded places, borrow pits, channels, wells, ponds, cement tanks, brackish water bodies, rice fields, lake margins, tree holes, and ground pools.

Distribution: Kerala: Alappuzha (Balasubramanian and Nikhil, 2013), Kannur (Rajavel *et al*., 2006), Ernakulam (Radhakrishnan, 2019).

Remarks: *Anopheles subpictus* is a species consisting of two sibling species, *An. subpictus* A and B. Species B is the potential vector of malaria in India. Both species are morphologically similar and they differ only in their molecular characteristics. Its characteristic features are the following;

1. Apical pale band and nearly equal to the pre-apical dark band
2. Foreleg tarsomere with broad bands
3. Fringe spot present on all veins

16. Anopheles subpictus A

In the present study *An. subpictus A* is collected from water held in marshy mangrove area in Vatakara, Kozhikode District. Morphologically this species showed close similarity with *Anopheles subpictus* B. But the molecular resolution of COI gene showed the difference that they were 2 separate species (Details were attached in the molecular part).

17. ***Anopheles vagus*** **Donitz, 1902**

Anopheles indefinita, Theobald 1907: 47 (ZMH, France).

Anopheles immaculatus James, 1903: 35 (BMNH, London). Type: described single ♀, India, Goa synonymized by Edwards 1921.

Myzomyia flava, Geneesk 1917: 807 (BMNH, London). Type: 3♀♀& 3♂♂. Java, Soerabaia. Synonimyzed by Edwards and Swellengrebel in 1917.

Anopheles vagus Donitz, 1902: 15. (ZMHU, France) Type locality: Fort de Kock, Java.

Material examined: Plesiotype: Kerala: 1♀, Thirunelly, Wayanad district. Coll. C. Vipinya, 07.iii.2019;

Other material examined: Kerala: 16♀ & 11♂, Thirunelly, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 20♀ & 12♂, Kavumannam, Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 4♀ & 1♂, Kavilumpara, Kozhikode district, Coll. C. Vipinya.12. v. 2019; 4♀ & 1♂, Ayancheri, Kozhikode district, Coll. C. Vipinya. 13.x.2019; 2♀ & 5♂, Changaroth, Kozhikode district, Coll. C. Vipinya.12. v. 2019; 3♀ & 5♂, Chundale, Wayanad district. Coll. C. Vipinya. 12.vi.2020; 3♀ & 5♂, Nadavayal, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 8♀ & 9♂, Thodernad, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 4♀ & 7♂, Pulpally, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 5♀ & 4♂, Kalnad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 7♀ & 4♂, Uduma, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 2♀ & 1♂, Kodakkad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 2♀ & 2♂, Adoor, Kasaragod district, Coll. C. Vipinya. 19.xii.2020; 2♀ & 7♂, Bellur, Kasaragod district, Coll. C. Vipinya. 19.xii.2020; 2♀ & 4♂, Madhur, Kasaragod district, Coll. C. Vipinya. 19.xii.2020; 4♀ & 7♂, Kalikavu, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 4♀ & 7♂, Kottakkal, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 5♀ & 6♂, Vengara, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 5♀ & 6♂, Tirur, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 5♀ & 4♂, Tavanur, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 4♀ & 7♂, Kuttippuram, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 4♀ & 3♂, Balussery, Kozhikode district, Coll. C. Vipinya. 23.xii.2021; 4♀ & 2♂, Kakkur, Kozhikode district, Coll. C. Vipinya. 23.xii.2021; 5♀ & 6♂, Thiruvambady,

Kozhikode district, Coll. C. Vipinya. 23.xii.2021; 3♀ & 8♂, Areekode, Malappuram district, Coll. C. Vipinya. 2.vii.2023; 4♀ & 7♂, Mambad, Malappuram district, Coll. 7♀ &11♂, Eramala, Kozhikode district, Coll. C. Vipinya. 12.vi.2021; 2♀ & 5♂, Perinthalmanna, Malappuram district, Coll. C. Vipinya. 2.vii.2023;

Diagnosis: Female (Plate 16, Figs.16a-e, Map-3) Medium-sized, close resemblance with *An.subpictus*, 3.26 mm long.

Head (fig.16b): Covered with narrow black and white scales; narrow vertical area, vertical hairs pale coloured; prominent frontal tuft; narrow ocular scales; compound eyes rounded and black; proboscis brown with yellow tip 2.4 mm long.

Antennae: With few pale hairs and scales; 1.55 mm long, yellowish brown, 15 segmented, hairy, and pilose; palpi 2.41 mm long, as long as the proboscis, thin, slender, uniform thickness, three pale bands: the tip of palpi pale and broad, remaining bands are narrow, the first pale band in between fourth and third segment, others pale band in between third and second segments; pre-apical dark band 1/4th or 1/5th length of apical pale band.

Thorax (fig.16d): 1.54 mm long, yellowish and golden hairs, hair-like scales arranged in indistinct longitudinal lines; mesonotum pale, pale golden hairs present on median area.

Wings (fig.16c): 3.64 mm long,1.5 mm wide, wing with pale interruptions including apex and fringe areas, more than four pale coloured areas; costa with three dark scaled spots, large middle dark spot; vein 1(R1) with five dark spots, four of these similar and fifth lies close to inner end of vein, vein 2 (RS) with two dark spots, vein 3(R4+5) with three similar dark spots. vein 4 (M) before its bifurcation two dark spots. vein 5 (Cu) one spot before bifurcation, anterior branch with three and posterior with one dark spot. vein 6 (anal) with two dark spots; fringe spots on all the veins.

Legs (fig.16e): 5.64 mm long, coxa 0.4 mm long, broad, femur 1.5 mm long, femur, tibia, and tarsus uniformly dark in colour, tibia, 1.34 mm long, darkish, not speckled, tarsus five segmented, fore-leg tarsomeres with broad white bands.

Abdomen: 1.76 mm long, light golden hairs, alternately dark and white patches, covered with whitish hairs. Cerci with dark scales; posterior portion of segments VII and VIII with pale scales.

Male: Characters are same as in females, smaller than females,2.51 mm long, slender, with plumose antenna, uniformly dark; major portion of palpi pale coloured; dorsal portion abdominal segment VIII with broad pale scales instead of hairs.

Bionomics: Breeding habitats were similar to *An.subpictus.* breeds in variety of habitats. In stagnant or flowing water bodies, pools with aquatic vegetation.

Distribution: India: found extensively throughout the country except Himachal Pradesh and Punjab. Kerala: Alappuzha (Balasubramanian and Nikhil, (2013)), Ernakulam (Radhakrishnan, (2019)), and Wayanad (Aneesh, (2014)), in this study reported from all five districts.

Remarks: *Anopheles vagus* is not a vector of Malaria in India, but it has been identified as a potential vector of Malaria, Filariasis and Japanese encephalitis in Asia. Its characteristic features are the following;

1. Broad white band present on foreleg tarsomere.
2. All the wing veins with fringe spots.
3. The length of the apical band is twice that of the pre-apical dark band.

18. *Anopheles sundaicus* Rodenwaldt, 1925

Myzomyia ludlowi var. *flavescens,* 1921: 47 (RUM, Rome). Type: unknown. Java: Soerabaia. (synonymized by Christophers 1932 (60) Ind. Med. Res. Mem).

Anopheles sundaicus Rodenwaldt, 1925: 173. (SBC) Type: neotype, Type locality: Sunda Islands.

Materials examined: Plesiotype 1♀, Kerala: Ezhome, Kannur district. Coll. C. vipinya, 07.iii.2022

Other material examined: 5♀ & 2♂, Ezhome, Kannur district. Coll. C. vipinya, 07.iii.2022; 2♀ & 2♂, Thaliparamba, Kannur district. Coll. C. Vipinya, 12. Iv.2022;

3♀ & 1♂, Chirakkal, Kannur district. Coll. C. Vipinya, 04.iii.2023; 1♀ & 2♂, Mayyil, Kannur district. Coll. C. Vipinya, 04.iii.2023.

Diagnosis: Female (Plate 17, Figs.17a-e, Map-3) Medium-sized, dark-coloured mosquitoes, 3.01 mm long.

Head (fig.17b): Covered with black and white scales; narrow vertical area, vertical hairs pale coloured; prominent frontal tuft; narrow ocular scales; compound eyes rounded and black; proboscis entirely dark coloured 2.45 mm long.

Antennae: With few pale hairs and scales; 1.5 mm long, yellowish brown, 15segmented, hairy, and pilose; palpi 2.456 mm long, as long as the proboscis, thin, slender, uniform thickness, three pale bands: subapical dark band greater than or equal to apical pale band and subapical pale band much narrower than apical pale band, the first pale band in between fourth and third segment, others pale band in between third and second segments.

Thorax (fig.17d): 1.53 mm long, lateral area comparatively dark compared to median area; mesonotum dark; ante-pronotal scales absent.

Wings (fig.17e): 3.69 mm long,1.45 mm wide, wing with pale interruptions including apex and fringe areas, more than four pale coloured areas; costa with three dark scaled spots, large middle dark spot; vein 1(R1) with four dark spots, vein 2 (RS) with two dark spots; vein 3(R4+5) with three similar dark spots; vein 4 (M) before it's bifurcation two dark spots, anterior branch two spots and in posterior branch three spots; vein 5(Cu) one spot before bifurcation, anterior branch with three and posterior with one dark spot; vein 6 (anal) with two dark spots; fringe spot on all the veins.

Legs (fig.17e): 5.7 mm long, coxa 0.5 mm long, broad; femur 1.5 mm long; femur, tibia, and tarsus uniformly dark in colour; tibia, 1.32 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomeres with broad white bands.

Abdomen: 1.76 mm long, light golden hairs; alternate dark and white patches, covered with whitish hairs; cerci with dark scales; posterior portion of segments VII and VIII with pale scales.

Male: Characters are same as in females, smaller than females, 2.51 mm long, slender, with plumose antenna, uniformly dark; major portion of the palpi pale coloured, dorsal portion abdominal segment VIII with broad pale scales instead of hairs.

Bionomics: Mainly breeds in brackish water also a freshwater breeder, major breeding places are the following; saltwater lagoons, wells, pools in coastal areas, pits and swamps filled with brackish water.

Distribution: In India: at earlier time, this species was reported from, Andra Pradesh, Odisha, Tamil Nadu, and West Bengal. Now widely prevalent in the Andaman and Nicobar Islands. Kerala: Kannur.

Remarks: This is for the first time *An. sundaicus is* reported from Kerala. It is the principal vector of malaria in Andaman -Nicobar Islands. *An .sundaicus* is a complex of three isomorphic species (species A, B, and C). Its characteristics features are the following;

1. Apical pale band nearly equal to pre-apical dark band
2. Legs with speckling
3. Fore leg tarsomere with broad white bands.

Series Neocellia

19. *Anopheles stephensi* Liston, 1901

Anopheles metaboles Theobald, 1902: 374 (BNMH, London). Type: 5 described, Lahore, Punjab, India. (synonymized by James and Liston 1911 (113) Anop. Mosq. Ind.).

Neocellia intermedia Rothwell, 1907: 34 (BNMH, London). Type: described from 3 and 1, Deesa, Gujarat, India (synonymized by James and Liston).

Anopheles folquei de Mello, 1918: 63 (IM, Culcutta). Type: ♀, Pragana, Gujarat, India (Synonymized by Christophers Ind. Med. Res.).

Anopheles stephensi Liston, 1901: 441 (BMNH, London) Type locality: Ellichpur, India.

Materials examined: Plesiotype: Kerala: 1♀, Muzhappilangad, Kannur district. Coll. C. vipinya, 15.iv.2021.

Other material examined: Kerala: 4♀ &1♂, Valayam, Kozhikode district, Coll. C. Vipinya. 13.x.2019; 3♀ & 2♂, Payyoli, Kozhikode district, Coll. C. Vipinya. 10.xi.2020; 13♀ & 8♂, Koyilandy, Kozhikode district, Coll. C. Vipinya. 10.xi.2020; 1♀ & 1♂, Kalnad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020.; 1♂, Uppala, Kasaragod district, Coll. C. Vipinya. 19.xii.2020. 1♀ & 1♂, Cheruvathur, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 1♀ & 1♂, Uduma, Kasaragod district, Coll. C. Vipinya. 18.xii.20203♀ & 7♂, Chengottkavu, Kozhikode district, Coll. C. Vipinya. 10.xi.2020; 5♀ & 5♂, Olavanna, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 5♀ & 4♂, Mukkam, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 1♀ & 1♂, Koduvally, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 4♀ & 8♂, Payyannur, Kannur district. Coll. C. vipinya, 15.iv.2021; 6♀ & 4♂, Pariyaram, Kannur district. Coll. C. vipinya, 15.iv.2021; 2♀ & 3♂, Kannur, Kannur district. Coll. C. vipinya, 15.iv.2021; 7♀ & 2♂, Ayancheri, Kozhikode district, Coll. C. Vipinya. 15.iii.2021; 8♀ & 3♂, Edakkad, Kannur district. Coll. C. vipinya, 15.iv.2021; 5♀ & 7♂, Muzhappilangad, Kannur district. Coll. C. vipinya, 15.iv.2021; 5♀ & 2♂, Karivallur, Kannur district. Coll. C. vipinya, 07.iii.2022; 1♀ & 3♂, Changaroth, Kozhikode district, Coll. C. Vipinya. 15.vi.2022; 4♀ & 7♂, Koorachund, Kozhikode district, Coll. C. Vipinya. 15.vi.2022; 1♀ & 2♂, Koothali, Kozhikode district, Coll. C. Vipinya. 15.vi.2022; 3♀ & 5♂, Mahe, Kannur district. Coll. C. vipinya, 5.vii.2023; 1♀ & 3♂, Madayi, Kannur district. Coll. C. vipinya, 23. viii.2023. 2♀ & 4♂, Mayyil, Kannur district. Coll. C. vipinya, 23. viii.2023; 1♀ & 1♂, Kuruvattoor, Kozhikode district, Coll. C. Vipinya. 11.v.2023;

Diagnosis: Female (Plate 18, Figs.18a-d, Map-4) Medium-sized, pale-coloured mosquitoes, 3.01 mm long.

Head (fig.18b): Covered with brown upright forked normal and white scales; narrow pale coloured vertical area; prominent frontal tuft; narrow ocular scales forming; compound eyes rounded and black; proboscis cylindrical and yellowish tip 2.64 mm long; vertex covered with broad white scales and dark scales on laterally and posteriorly.

Antennae: With few pale hairs and scales; 1.5 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.456 mm long, as long as the proboscis, thin, slender, uniform thickness, three pale bands and speckled: apical and subapical pale band are equal about 0.34 mm and third band is narrow.

Thorax: 1.34 mm long, lateral area comparatively dark compared to median area; ante-pronotum and pronotum with white scales; scutum with pale scales and dark bristles; lateral side of thorax is dark.

Wings (fig. 18c): 3.51 mm long,1.43 mm wide, wing with pale interruptions including apex and fringe areas and covered with broad spindle-shaped scales; more than four pale coloured areas; costa with seven dark scaled spots, large middle dark spot; vein 1(R1) with four dark spots; vein 2 (RS) with two dark spots, area of bifurcation dark scaled; vein 3(R4+5) with two similar dark spots; vein 4 (M) two dark spots; the anterior branch two spots and in posterior branch also two spots; vein 5(Cu) single spot before bifurcation, anterior branch with three and posterior with one dark spot; vein 6 (anal) with three dark spots; fringe spot on all the veins.

Legs: 5.75 mm long, coxa 0.52 mm long, broad; femur 1.63 mm long; femur, tibia, and tarsus uniformly dark in colour; tibia, 1.32 mm long, darkish, speckled; tarsus five segmented, fore-leg yellowish white band present on second and third tarsal segments.

***Abdomen*:** 1.64 mm long, covered with numerous setae and whitish hairs; abdominal segments with II-VII terga without dark scale tufts; cerci with dark scales.

Male: Characters are same as in females, smaller than females, 2.55 mm long, slender, with plumose antenna; uniformly dark; major portion of the palpi pale

coloured; dorsal portion abdominal segment VIII with broad pale scales instead of hairs.

Bionomics: Commonly known as urban malaria mosquitoes, in urban areas it predominantly breeds in wells, overhead and ground-level water tanks, cisterns, tanks, coolers, roof gutters and other artificial containers and is also reported from brackish water habitats.

Distribution: India: recorded from all states of India in different locations and also recorded from Andamans and Lakshadweep Islands. Kerala: Kozhikode, Kasaragod, Malappurum

Remarks: Primary vector of Malaria in India, exists in three ecological variants ie, *type, intermediate, and mysorensis* forms. *Type* form is the efficient vector of Malaria in urban areas. Its characteristics identification features are the following:

1. Apical and subapical pale bands equal.
2. Palpi and legs with speckling
3. Fore leg tarsomere without broad white bands.

20. *Anopheles theobaldi* Giles, 1901

Anopheles theobaldi 1901: 198 (BNMH, London). Type: ♀ described, Ellichpur, Amaroti Dist. India.

Materials examined: Plesiotype: Kerala: 1♀, Tuneri, Kozhikode district, Coll. C. Vipinya. 23.xii.2021.

Other materials examined: Kerala: 16♀& 11♂, Thirunelly, Wayanad district. Coll. C. vipinya, 07.iii.2019; 8♀ & 9♂, Thodernad, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 4♀ & 7♂, Pulpally, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 4♀ & 2♂, Kakkur, Kozhikode district, Coll. C. Vipinya. 23. xii.2021; 2♀ & 2♂, Perambra, Kozhikode district, Coll. C. Vipinya. 25. ix.2021; 4♀ & 1♂, Maruthomkara, Kozhikode district, Coll. C. Vipinya. 13. vi.2021; 1♀, Kayakkodi, Kozhikode district, Coll. C. Vipinya. 23. xii.2021; 2♀ & 2♂, Thikkodi, Kozhikode

district, Coll. C. Vipinya. 25. ix.2021; 1♀ & 2♂, Vatakara, Kozhikode district, Coll. C. Vipinya. 28. x.2022. 1♀ & 1♂, Kayanna, Kozhikode district, Coll. C. Vipinya. 23. x.2022; 1♀ & 1♂, Nochad, Kozhikode district, Coll. C. Vipinya. 23. x.2022; 2♀ & 6♂, Chakkittappara, Kozhikode district, Coll. C. Vipinya. 17.v.2022; 3♀ & 6♂, Puthuppadi, Kozhikode district, Coll. C. Vipinya. 12.iii.2023; 1♀ & 4♂, Kodencheri, Kozhikode district, Coll. C. Vipinya. 12. iii.2023; 2♀ & 1♂, Thalakkulathur, Kozhikode district, Coll. C. Vipinya. 12. iii.2023; 2♀ & 1♂, Kuruvattor, Kozhikode district, Coll. C. Vipinya. 12. iii.2023; 2♀ & 1♂, Koduvally, Kozhikode district, Coll. C. Vipinya. 12. iii.2023; 2♀ & 1♂, Mukkam, Kozhikode district, Coll. C. Vipinya. 12. iii.2023; 1♀ & 2♂, Mayyil, Kannur district. Coll. C. vipinya, 04.iii.2023.

Diagnosis: Female (Plate 19, Figs.19a-e, Map-4) Moderate-sized, pale-coloured mosquitoes, 2.93 mm long.

Head (Fig.19b): Covered with narrow normal black and pale scales; narrow pale coloured vertical area; prominent frontal tuft; narrow ocular scales forming single front line; compound eyes rounded and black; proboscis cylindrical and back with yellowish tip 2.43 mm long.

Antennae: With few hairs and minute scales; 1.5 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.45 mm long, as long as the proboscis, moderately thick, slender, three pale bands and speckled: apical and subapical pale bands equal and separated by apical dark band.

Thorax: 1.4 mm long, dorsal surface covered with white scales lateral area comparatively dark compared to median area; median area covered with pale white scales.

Wings: 3.45 mm long,1.32 mm wide, pale coloured and spotted with dark marks, covered with broad spindle-shaped scales; costa with six dark scaled spots, large middle dark spot; two small dark spots situated at the inner end of wing, vein 1(R1) with four dark spots, usually pale area at its base; vein 2 (RS) with two dark spots, most of the part white Area of bifurcation dark scaled; vein 3(R4+5) with three

similar dark spots; vein 4 (M) two long dark spots on the main trunk; vein 5(Cu) single spot before bifurcation, anterior branch with three and posterior with one dark spot; vein 6 (anal) with three dark spots; fringe spot on all the veins.

Legs (Fig.19c); 5.7 mm long, coxa 0.5 mm long, broad; femur 1.61 mm long; femur, tibia, and tarsus covered with dark and light scales; tibia, 1.3 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomere (Ta-I_1) yellowish white band present on second (Ta-I_2) and third (Ta-I_3) tarsal segments, hind leg tarsomere (Ta-III 4and 5) completely white and Ta-III_3 partially white.

Abdomen (Fig.19d): 1.62 mm long, covered with numerous setae and hair scales on dorsal area; abdominal segments with II-VII terga without dark scale tufts; cerci with dark scales.

Male: Characters are the same as in females, smaller than females, 2.65 mm long, slender, with plumose antenna, uniformly dark; major portion of the palpi pale coloured, dorsal portion abdominal segment VIII with broad pale scales instead of hairs.

Bionomics: Predominantly breeds in rice fields, ponds, tanks, and river bed pools with bright light areas.

Distribution; India: recorded from all states of India in different locations. Also recorded from Andamans and Lakshadweep. The present study detected its presence in all the North Kerala districts.

Remarks: This species of *Anopheles* is not a vector of Malaria Indian region. Its characteristics features are following;

1. Equal-sized apical and sub-apical pale bands are separated by narrow preapical dark band.
2. Hind tarsomere with speckling, foreleg tarsomere with broad white bands.

21. *Anopheles karwari* James, 1903

Anopheles nigrans Stanton 1912:7 (BNMH, London). Type locality: Karwar, Bombay, India. (Synonymized by Christophers Ind. Journ. Med. Res. 1916: 469).

Anopheles karwari James, 1903: 102. (BMNH, London) Type locality: Karwar, India.

Materials examined: Plesiotype: Kerala: 1♀, Vatakara, Kozhikode district. Coll.C. Vipinya 21.vi.2019.

Other material examined: Kerala: 1♀ & 1♂, Vatakara, Kozhikode district. Coll.C. Vipinya 21.vi.2019; 6♀& 1♂, Vythiri, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 1♀ & 3♂, Kayanna, Kozhikode district. Coll.C. Vipinya 2.v.2019; 16♀& 11♂, Thirunelly, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 1♀ & 1♂, Vatakara, Kozhikode district. Coll.C. Vipinya 21.vi.2019;.1♀ & 1♂, Orkatteri, Kozhikode district. Coll.C. Vipinya 21.vi.2019; 1♀ & 1♂, Iringal, Kozhikode district. Coll.C. Vipinya 21.vi.2019; 15♀& 25♂, Kadalundi, Kozhikode district. Coll. C. vipinya, 26.i.2019; 11♀ & 1♂, Padannakkad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 1♀ & 1♂, Poochakkad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 5♀ & 6♂, Tirur, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 3♀ & 5♂, Nadavayal, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 4♀ & 7♂, Pulpally, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 3♀ & 5♂, Thonikadavu, Kozhikode district, Coll. C. Vipinya, 6.vii.2020; 12♀ & 9♂, Vilangad, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 2♀& 4♂, Kottakkal, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 1♀ & 5♂, Olavanna, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 5♀ & 4♂, Mukkam, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 1♀& 2♂, Kayakkodi, Kozhikode district, Coll. C. Vipinya. 23. xii.2021; 2♀ & 3♂, Kodencheri, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 19♀ & 12♂, Thonikkadavu, Kozhikode district. Coll.C. Vipinya 13.vii.2022; 2♀ & 2♂, Perambra, Kozhikode district, Coll. C. Vipinya. 25. ix.2021; 1♀ & 1♂, Kayanna, Kozhikode district, Coll. C. Vipinya. 23. x.2022; 6♀& 2♂, Nedumkayam Malappuram district. Coll. C. Vipinya, 19.iii.2022; 1♀& 1♂, Nilambur, Malappuram district. Coll. C. Vipinya, 120.iii.2022; 1♀& 1♂, Ponnani, Malappuram district.

Coll. C. Vipinya, 120.iii.2022; 2♀& 4♂, Mayyil Kannur district. Coll. C. Vipinya, 10.iv.2023.

Diagnosis: Female (Plate 20, Figs.120a-d, Map-5) Medium-sized, pale-coloured mosquitoes, 2.86mm long.

Head (fig.20b): Covered with normal white upright and pale scales in front area dark scales on behind; well-marked pale coloured vertical area; ocular area with scales arranged in single line in anterior region; prominent frontal tuft; compound eyes rounded and black; proboscis cylindrical and back with yellowish tip 2.33 mm long.

Antennae: With few hairs and whitish or silvery scales; 1.4 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.37 mm long, as long as the proboscis, moderately thick, slender, four pale bands: apical and subapical pale bands equal and broad, other bands narrow.

Thorax (fig.20d): 1.37 mm long, covered with snowy white spindle-shaped scales and hairs on dorsal side lateral area comparatively dark devoid of scales; scutellum with some scales as in thorax; median area covered with pale white scales.

Wings: 3.42 mm long,1.25 mm wide, pale coloured and spotted with dark marks, wing veins covered with broad spindle-shaped white and black scales; costa with large four dark scaled spots; vein 1(R1) with four dark spots exactly those in costa except middle area, vein 2 (RS) with single dark spots before bifurcation, two spots present on each branch, vein 3(R4+5) mostly pale areas except basal and distal portion, vein 4 (M) three dark spots on the main trunk, two spots present on each branch, vein 5(Cu) single spot before bifurcation, anterior branch with three and posterior with one dark spot, vein 6 (anal) with two dark spots; fringe spot on all the veins.

Legs (fig.20e): 5.64 mm long, coxa 0.5 mm long, broad; femur 1.59 mm long; femur, tibia, and tarsus covered with dark and light scales; tibia, 1.33 mm long, darkish, not speckled; tarsus five segmented, fore-leg tarsomere (Ta-I1) yellowish

white band present on second (Ta-I2) and third (Ta-I3) tarsal segments, hind leg Ta-III 1 and 2 tarsomere with apical white bands, Ta-III 5 completely white.

Abdomen: 1.57 mm long; covered with numerous setae and light yellowish hairs and golden scales on segments VII- VIII (Te-VII-VIII); abdominal segments with II-VI terga without dark scale tufts; cerci with light scales above, dark below.

Male: Characters are the same as in females, smaller than females; 2.81mm long, slender, with plumose antenna, uniformly dark; major portion of the palpi pale coloured; dorsal portion abdominal segment VIII with broad golden scales instead of hairs.

Bionomics: important breeding habitats are the following; sunny or shaded parts of rivers, pools, streams, ponds, wells, seepages, tanks, and springs, with or without green aquatic vegetation.

Distribution; In India: Rajasthan, Uttar Pradesh, Madhya Pradesh, Bihar, West Bengal, Odisha, Assam, Meghalaya, Tripura, Arunachal Pradesh, Maharashtra, Andra Pradesh, Goa, Karnataka, Kerala, and Tamil Nadu. In Kerala: Ernakulam (Radhakrishnan, 2019). The present study detected its presence in all five North Kerala districts for the first time.

Remarks: This species is not regarded as a vector of Malaria. Its important features are following;

1. Ta-III completely white
2. Palpi with four bands

22. *Anopheles Jamesii* Theobald, 1901

Materials examined: Plesiotype: Kerala:1 ♀, Orkatteri, Kozhikode district. Coll.C. Vipinya 21.vi.2019.

Other material examined: Kerala: 16♀& 11♂, Thirunelly, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 3♀ & 2♂, Orkatteri, Kozhikode district. Coll.C. Vipinya 21.vi.2019; 1♀ & 1♂, Iringal, Kozhikode district. Coll.C. Vipinya 21.vi.2019; 1♀ &

1♂, Vatakara, Kozhikode district. Coll.C. Vipinya 21.vi.2019; 15♀& 25♂, Kadalundi, Kozhikode district. Coll. C. vipinya, 26.i.2019; 1♀ & 3♂, Kayanna, Kozhikode district. Coll.C. Vipinya 2.v.2019; 6♀& 1♂, Vythiri, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 4♀ & 7♂, Pulpally, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 11♀ & 1♂, Padannakkad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 1♀ & 1♂, Poochakkad, Kasaragod district, Coll. C. Vipinya. 18.xii.2020; 3♀ & 5♂, Thonikadavu, Kozhikode district, Coll. C. Vipinya, 6.vii.2020; 1♀ & 5♂, Thonikadavu, Kozhikode district, Coll. C. Vipinya, 6.vii.2020; 3♀ & 5♂, Nadavayal, Wayanad district. Coll. C. Vipinya. 20.ix.2020; 12♀ & 9♂, Vilangad, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 5♀ & 6♂, Tirur, Malappuram district, Coll. C. Vipinya. 20.vi.2021; 2♀ &2♂, Perambra, Kozhikode district, Coll. C. Vipinya. 25. ix.2021 1♀& 2♂, Kayakkodi, Kozhikode district, Coll. C. Vipinya. 23. xii.2021; 1♀ & 1♂, Kottakkal, Malappuram district, Coll. C. Vipinya. 20.vi.2021; ♀ & 5♂, Olavanna, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 1♀& 2♂, Kayakkodi, Kozhikode district, Coll. C. Vipinya. 23. xii.2021; 5♀ &4♂, Mukkam, Kozhikode district, Coll. C. Vipinya. 19.x.2021; 2♀ & 3♂, Kodencheri, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 36♀& 2♂, Nedumkayam Malappuram district. Coll. C. vipinya, 19.iii.2022; 1♀& 1♂, Nilambur, Malappuram district. Coll. C. Vipinya, 120.iii.2022; 1♀& 1♂, Ponnani, Malappuram district. Coll. C. Vipinya, 120.iii.2022; 1♀ & 1♂, Kayanna, Kozhikode district, Coll. C. Vipinya. 23. x.2022; 19♀ & 12♂, Thonikkadavu, Kozhikode district. Coll.C. Vipinya 13.vii.2022; 6♀& 2♂, Nedumkayam Malappuram district. Coll. C. Vipinya, 19.iii.2022; 1♀& 1♂, Nilambur, Malappuram district. Coll. C. Vipinya, 120.iii.2022; 1♀& 1♂, Ponnani, Malappuram district. Coll. C. Vipinya, 120.iii.2022; 2♀& 4♂, Mayyil Kannur district. Coll. C. Vipinya, 10.iv.2023.

Diagnosis: Female (Plate 21, Figs.21a-e, Map-5) Small-medium sized, pale-coloured mosquitoes, 2.61mm long.

Head (fig.21b): Covered with normal white upright and pale scales; well-marked pale coloured vertical area; ocular area with scales arranged in single line in anterior

region; prominent frontal tuft; compound eyes rounded and black; proboscis cylindrical and back with white scales at tip 2.32 mm long.

Antennae: With few hairs and whitish scales in first flagellar segment; 1.38 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.34 mm long, as long as the proboscis, moderately thick, slender, three pale bands: broad apical pale bands equal to subapical dark band, other two bands narrow at 2-3 and 3-4.

Thorax: 1.29 mm long, meso-thorax covered with white spindle-shaped scales and hairs on dorsal side lateral area comparatively dark less covered with scales and hairs; median area covered with pale white scales.

Wings(fig.21c): 3.34mm long,1.22 mm wide, pale coloured and spotted with dark marks; wing veins covered with broad spindle-shaped white and black scales; costa with four white scaled spots; vein 1(R1) with four white spots exactly those in costa except proximal area, addition of two-minute white spot beneath central black spots, vein 2 (RS) mostly with white scales, single black spots present on anterior branch , two in posterior branch, vein 3(R4+5) mostly pale areas except basal and distal portion, vein 4 (M) mostly white scaled, two dark spots on main trunk, two spots present on each branches, vein 5(Cu) single spot before bifurcation, anterior branch with two and posterior with one dark spot, vein 6 (anal) with three dark spots; fringe spot on all the veins.

Legs (fig.21e): 5.58 mm long, coxa 0.5 mm long, broad; femur 1.58 mm long; femur, tibia, and tarsus covered with light scales; tibia, 1.35 mm long, darkish, speckled; tarsus five segmented, hind leg Ta-III 1 and 2 tarsomere with apical white bands, Ta-III 3,4 and 5 completely white.

Abdomen (fig.21d): 1.52 mm long; covered with numerous setae and light yellowish hairs and golden scales and narrow golden hairs on all segments; abdominal segments with II-VI terga without dark scale tufts; cerci with light scales above, dark below.

Male: Characters are same as in females, smaller than females, 2.63mm long, slender, with plumose antenna, uniformly dark; major portion of the palpi pale

coloured; dorsal portion abdominal segment VIII with broad golden scales instead of hairs.

Bionomics: Prefers to breed in growing rice with vegetation, ponds, rivers, rainwater pools, slowly flowing streams with aquatic vegetation, wells with aquatic vegetation, and marshy fields.

Distribution; In India: recorded from Kerala, Tamil Nadu, Karnataka, Goa, Andra Pradesh, Maharashtra, Odisha, Gujarat, Madhya Pradesh, Rajasthan, West Bengal, Uttar Pradesh, Bihar, Assam, Meghalaya, Arunachal Pradesh, Tripura, Punjab, and Haryana. In Kerala: Kannur (Rajavel et al., 2006), Alappuzha (Arunachalam et al., 2004; Balasubramanian and Nikhil, 2013), Ernakulam (Radhakrishnan, 2019). According to the present study, the species was recorded from Kozhikode, Kasaragod, Wayanad, Malappuram and Kannur.

Remarks: The presence of this species in North Kerala was a first report. It is a non-malaria vector and one of the most abundant *Anopheles* species in India. Its identifying features are following;

1. Fore, mid and hind legs with speckling
2. Distal part of abdominal segment with golden hairs.
3. Ta-III 3,4 and 5 completely white.

23. *Anopheles splendidus* Koidzumi, 1920

Nysorhynchus maculipalpis Theobald, 1903:99 (BNMH, London). Type: ♀ described, Type locality: Nagpur, Central Province, India. (Synonymized by Edwards Gen. Insect. 1932)

Anopheles maculipalpis James & Liston, 1904: 95. Type locality: India (Synonymized by Christophers Christophers., Ind. Med. Res. Mem 1924: 66)

Materials examined: Plesiotype: Kerala: 1♀, Korome, Wayanad district. Coll. C. vipinya, 07.iii.2019.

Other material examined: Kerala: 6♀& 1♂, Korome, Wayanad district. Coll. C. Vipinya, 07.iii.2019; 2♀ & 12♂, Kanjiragad, Wayanad district. Coll. C. Vipinya, 12.

Iv.2019; 3♀ & 5♂, Vellamunda, Wayanad district. Coll. C. Vipinya. 12.vi.2020; 3♀ & 5♂, Mananthavady, Wayanad district. Coll. C. Vipinya. 20.ix.2020.

Diagnosis: Female (Plate 22, Figs.22a-e, Map-5) Medium to large-sized, pale-coloured mosquitoes, 2.92mm long.

Head (Fig.22b): Covered with normal white upright and pale scales in front area dark scales on behind; well-marked pale coloured vertical area; ocular area with scales arranged in single line in anterior region; prominent frontal tuft; compound eyes rounded and black; proboscis cylindrical and back including tip 2.42 mm long.

Antennae (Fig.22b): With few minute scales on torus and first flagellar segment; 1.43 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.44 mm long, as long as the proboscis, moderately thick, slender, broad apical pale bands with speckling, three pale bands: apical and subapical pale bands equal and broad, other bands narrow.

Thorax: 1.35 mm long, covered with snowy white scales and hairs on dorsal side lateral area comparatively dark due the presence of dark hairs, devoid of scales.

Wings (Fig.22b): 3.42 mm long,1.25 mm wide, pale coloured and spotted with dark marks; wing veins covered with broad spindle-shaped white and black scales; costa with three distinct white spots, vein 1(R1) with three dark spots exactly those in Costa except middle area, vein 2 (RS) with white scales at its bifurcation, one spot present on each branch, vein 3(R4+5) mostly pale areas except basal and distal portion, vein 4 (M) mainly dark except its proximal extremity, single dark spots present on each branch, vein 5(Cu) most part light coloured, two dark in main branch, anterior branch with three and posterior with two dark spot. vein 6 (anal) mostly pale coloured with three dark spots; wing fringe with light scales.

Legs (Fig.22e): 5.7 mm long, coxa 0.5 mm long, broad; femur 1.56 mm long; femur, tibia, and tarsus covered with dark and light scales; tibia, 1.35 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomere (Ta-I_1) with white bands present on second (Ta-I_2) and third (Ta-I_3) tarsal segments, hind leg Ta-III 1 and 2 tarsomere with apical white bands, Ta-III 3-5 completely white.

Abdomen (Fig.22d): 1.54 mm long, mainly dark in colour, covered with light and dark hairs; abdominal segments with II-VI terga without dark scale tufts; cerci with dense black scales.

Male: Characters are same as in females, smaller than females, 2.87mm long, slender, with plumose antenna, uniformly dark; major portion of the palpi pale coloured; dorsal portion abdominal segment mainly dark narrow pale scales present; coxites with narrow dark and pale scales.

Bionomics: Predominantly breeds in forest pools, river beds with aquatic vegetation, ditches, rock holes, tree holes, streams, and pools with aquatic vegetation.

Distribution; In India: except Tripura, Mizoram, and Nagaland, recorded from all other states of India in different locations. In Kerala: Malappuram and Trissur (Sajith *et al*., 2016). Present study- Wayanad

Remarks: The present study recorded the presence of *Anopheles splendidus* from Wayanad for the first time. No role in Malaria transmission. Its major features are the following;

1. Legs and palpi with speckling.
2. Equal apical and sub apical band.
3. Ta-III 3-5 atleast completely white.

24. *Anopheles tessellatus* Theobald, 1901

Anopheles formosae Hatori, 1901:275 (BNMH, London). Type: single ♀ described, Type locality: Formosa. (Synonymized by Yamada Ind. Journ. Med. Res. 1925: 483).

Anopheles deceptor Donitz, 1902:60 (BNMH, London). Type: single ♀ described, Type locality: Sumatra. (Synonymized by Stanton Bull. Ent. Res. 1913: 129).

Myzomyia thorntonii Lodlow, 1904:69 (USNM, Washington). Type: 3♀♀ described, Type locality: Oras Samar and Cottabato. (Synonymized by Edwards Bull. Ent. Res. 1913: 221).

Dactylomyia ceylonica Newsted & Cartor, 1910:377. Type: single♀ described, Type locality: Trichomalee, Ceylon. (Synonymized by Stanton Bull. loc. cit. 1913).

Anopheles kinoshitai Koidzumi, 1917:133. Type: single♀ described, Type locality: Trichomalee, Ceylon. (Synonymized by Stanton Bull. loc. cit. 1913).

Material examined: Plesiotype: Kerala: 1♀, Kanjiragad, Wayanad district. Coll. C. Vipinya, 12. Iv.2019.

Other materials examined: Kerala: 2♀ & 2♂, Kanjiragad, Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 6♀& 1♂, Vythiri, Wayanad district. Coll. C. vipinya, 07.iii.2019; 5♀ & 6♂, Manathavady, Wayanad district. Coll. C. vipinya, 12. Iv.2019; 7♀ & 11♂, Chombala, Kozhikode district, Coll. C. Vipinya. 12.vi.2021; 2♀ & 2♂, Chorode, Kozhikode district, Coll. C. Vipinya. 12.vi.2021; 3♀ & 4♂, pakkayil, Kozhikode district. Coll.C. Vipinya 22. viii. 2021;

Diagnosis: Female (Plate 23, Figs.23a-e, Map-6) Medium-sized, pale-coloured mosquitoes, 2.85mm long.

Head: Covered with normal white upright and pale scales in front area dark scales on behind; well-marked pale coloured vertical spot; vertex broad, ocular area with scales arranged in single line in anterior region; sparse frontal tuft; compound eyes rounded and black; proboscis cylindrical and black half of the portion yellowish 2.35 mm long.

Antennae (fig.23b): With few hairs and whitish or silvery scales on first flagellar segment (fs); 1.34 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.36 mm long, as long as the proboscis, moderately thick, slender, three broad pale bands: pale bands are separated by short dark band in between them.

Thorax: 1.38 mm long, covered with few scales and hairs on dorsal side; lateral area comparatively pale with narrow white scales; mesonotum with broad markings; scutellum with some scales as in thorax; median area covered with pale white scales; pleura dark.

Wings (Fig.23c): 3.38 mm long,1.15 mm wide, pale coloured and spotted with dark marks; wing veins covered with broad spindle-shaped white and black scales; costa without basal pale spot, vein 1(R1) with 1-4 pale interruptions exactly those in costa except middle area, pre sector dark mark basally extended up to the humeral dark mark of costa, vein 2 (RS) with three dark spots before bifurcation, three spots present on each branch, vein 3(R4+5) mostly pale areas except basal and distal portion, seven small dark scaled area present, vein 4 (M) three large dark spots on the main trunk, three spots present on each branch, vein 5(Cu) four dark spot before bifurcation, anterior branch with five and posterior with four dark spots, vein 6 (anal) with five dark spots; fringe spot on all the veins.

Legs (Fig.23d): 5.54 mm long, coxa 0.5 mm long, broad; femur 1.55 mm long; femur, tibia, and tarsus covered with light scales; tibia, 1.35 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomere (Ta-I5) yellowish-white scales present on the tip, other tarsal segments with white scales and form bands, mid-leg tarsomeres are same as in foreleg, hind leg, femur and tibia speckled with white scales, Ta-III5 tip has few white scales.

Abdomen: 1.57 mm long, covered with numerous setae and light yellowish hairs, Te-VI with narrow light brown scales, Te-VII with light or dark brown scales, Te-VIII covered with golden scale and golden scales on segment VII- VIII (Te-VII-VIII). Cerci without scales above, dark below.

Male: Characters are same as in females, smaller than females, 2.86mm long, slender, with plumose antenna one or two black scales on first flagellar segment, uniformly dark; major portion of the palpi pale coloured with marginal hairs; dorsal portion abdominal segment VIII with broad golden scales instead of hairs.

Bionomics: Breeds in rice fields, wells, sunlight or shaded parts of dirty stagnant waters, slow running streams.

Distribution; In India: except Punjab, Haryana, Himachal Pradesh, Arunachal Pradesh, and Mizoram, recorded from all other states of India in different locations.

Also recorded from Andaman Islands. Kerela (present study)- Wayanad and Kozhikode.

Remarks: It is not a potent vector but acts as a suspected vector of malaria in India. Its features are following;

1. Palpi with four bands
2. Speckled legs
3. ½ of proboscis yellow coloured

***25. Anopheles kochi* Doenitz, 1901**

Anopheles ocellatus Theobald, 1901:174 (BNMH, London). Type: single ♀ described, Type locality: Taiping, Perak. (Synonymized by Theobald Ind. Journ. Med. Res. 1925: 483).

Cellia flava Ludlow, 1908:32 (USNM, Washington). Type: 4♀ described, Type locality: Camp Wilhelm, Tayubar. (Synonymized by Edwards,Bull. Ent. Res. 1913: 222).

Christophersia halli James, 1904:33 (IM, Calcutta). Type: 3♀♀ described, Type locality: Sylhet, Assam, India. (Synonymized by James and Stanton Congress FEATM. 1914: 515).

Material examined: Plesiotype: Kerala: 1♀, Kanjiragad, Wayanad district. Coll. C. Vipinya, 12. Iv.2019.

Other materials examined: Kerala: 2♀ & 2♂, Kanjiragad, Wayanad district. Coll. C. Vipinya, 12. Iv.2019; 6♀& 1♂, Vythiri, Wayanad district. Coll. C. vipinya, 07.iii.2019; 5♀ & 6♂, Manathavady, Wayanad district. Coll. C. vipinya, 12. Iv.2019; 7♀ & 11♂, Engapuzha, Kozhikode district, Coll. C. Vipinya. 12.vi.2021; 2♀ & 2♂, Olavanna, Kozhikode district, Coll. C. Vipinya. 12.vi.2021; 3♀ & 4♂, Aralum Kannur district. Coll.C. Vipinya 22. viii. 2021;

Diagnosis: Female (Plate 24, Figs.24a-e, Map-6) Medium-sized, pale-coloured mosquitoes, 2.73mm long.

Head: Covered with normal scales, front area dark scales; well-marked pale coloured vertical spot; vertex broad, ocular area with scales arranged in single line in

anterior region; sparse frontal tuft; compound eyes rounded and black; proboscis cylindrical and black half of the portion yellowish 2.35 mm long.

Antennae (fig.24b): With few hairs and whitish or silvery scales on first flagellar segment (fs); 1.4 mm long, light coloured, hairy, and pilose; palpi 2.3 mm long, as long as the proboscis, moderately thick, slender, three broad pale bands: pale bands are separated by short dark band in between them.

Thorax: 1.3 mm long, covered with few scales and hairs on dorsal side; lateral area comparatively pale with narrow white scales; mesonotum with broad markings; scutellum with some scales as in thorax; median area covered with pale white scales; pleura dark.

Wings (Fig.24c): 3.38 mm long,1.15 mm wide, pale coloured and spotted with dark marks; wing veins covered with broad spindle-shaped white and black scales; costa without basal pale spot, vein 1(R1) with 1-4 pale interruptions exactly those in costa except middle area, pre sector dark mark basally extended up to the humeral dark mark of costa, vein 2 (RS) with three dark spots before bifurcation, three spots present on each branch, vein 3(R4+5) mostly pale areas except basal and distal portion, seven small dark scaled area present, vein 4 (M) three large dark spots on the main trunk, three spots present on each branch, vein 5(Cu) four dark spot before bifurcation, anterior branch with five and posterior with four dark spots, vein 6 (anal) with five dark spots; fringe spot on all the veins.

Legs (Fig.24d): 5.54 mm long, coxa 0.5 mm long, broad; femur 1.55 mm long; femur, tibia, and tarsus covered with light scales; tibia, 1.35 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomere (Ta-I5) yellowish-white scales present on the tip, other tarsal segments with white scales and form bands, mid-leg tarsomeres are same as in foreleg, hind leg, femur and tibia speckled with white scales, Ta-III5 tip has few white scales.

Abdomen: 1.57 mm long, covered with numerous setae and light yellowish hairs, Te-VI with narrow light brown scales, Te-VII with light or dark brown scales, Te-

VIII covered with golden scale and golden scales on segment VII- VIII (Te-VII-VIII). Cerci without scales above, dark below.

Male: Characters are same as in females, smaller than females, 2.86mm long, slender, with plumose antenna one or two black scales on first flagellar segment, uniformly dark; major portion of the palpi pale coloured with marginal hairs; dorsal portion abdominal segment VIII with broad golden scales instead of hairs.

Bionomics: Breeds in rice fields, wells, sunlight or shaded parts of dirty stagnant waters, slow running streams.

Distribution; In India: except Punjab, Haryana, Himachal Pradesh, Arunachal Pradesh, and Mizoram, recorded from all other states of India in different locations. Also recorded from Andaman Islands. Present study- Wayanad and Malappuram.

Remarks: It is not a potent vector but acts as a suspected vector of malaria in India. Its features are following;

1. Palpi with four bands
2. Speckled legs
3. ½ of proboscis yellow coloured

26. *Anopheles dirus* Python & Harrison, 1979

Anopheles leucosphyrus balabacensis Colless, 1957: 137. Type: single♀ described, Type locality: Trichomalee, Ceylon. (Synonymized by Stanton Bull. loc. cit. 1913).

Anopheles dirus Python & Harrison, 1979: 40 (USNM, Washington) Type locality: Ban Bu Phram, Thailand.

Material examined: Plesiotype: 1♀, Vilangad, Kozhikode district. Coll.C. Vipinya 21.vi.2021

Other materials examined: 1♀ & 2♂, Kannavam, Kannur district Coll.C. Vipinya. 22.iv.2020; 12♀ & 9♂, Vilangad, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 7♀ & 9♂, Olavanna, Kozhikode district. Coll.C. Vipinya 02.v.2021; 3♀ & 7♂,

Kuttiady, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 13♀ & 6♂, Pasukkadavu, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 4♀ & 7♂, Nilambur, Malappuram district, Coll. C. Vipinya. 20. vi.2021.

Diagnosis: Female (Plate 25, Figs.25a-e, Map-6) Medium-sized, pale-coloured mosquitoes, 2.95mm long.

Head (Fig. 25b): Covered with narrow normal white upright and pale scales in front area dark scales on behind; well-marked pale coloured vertical spot; vertex broad, ocular area with scales arranged in single line in anterior region; sparse frontal tuft; compound eyes rounded and black; proboscis cylindrical and black half of the portion yellowish 2.4 mm long.

Antennae: With few hairs and whitish or silvery scales on first flagellar segment (fs); 1.34 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.41 mm long, as long as the proboscis, moderately thick, slender, four broad pale bands: pale bands are separated by short dark band in between them.

Thorax: 1.38 mm long, covered with few scales and hairs on dorsal side; lateral area comparatively pale with narrow white scales; mesonotum with broad markings; scutellum with some scales as in thorax; median area covered with pale white scales; pleura dark.

Wings (Fig. 25c): 3.38 mm long,1.15 mm wide, pale coloured and spotted with dark marks, wing veins covered with broad spindle-shaped white and black scales; white spots costa with large four dark scaled spots and three small dots, vein 1(R1) with 1-4 pale spots exactly those in costa except middle area, vein 2 (RS) with three dark spots before bifurcation, three spots present on each branches, vein 3(R4+5) mostly pale areas except basal and distal portion, seven small dark scaled area present, vein 4 (M) three large dark spots on main trunk, three spots present on each branches, vein 5(Cu) four dark spot before bifurcation, anterior branch with five and posterior with four dark spot, vein 6 (anal) with five dark spots; fringe spot absent on vein 5 and 6 but present in between these two.

Legs (Fig. 25d): 5.5 mm long, coxa 0.5 mm long, broad; femur 1.5 mm long; femur, tibia, and tarsus covered with light scales; tibia, 1.35 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomere (Ta-I_5) yellowish white scales present on tip other tarsal segment with white scales and form bands, mid leg tarsomeres are same as in foreleg, hind leg, femur and tibia speckled with white scales; broad white spot present at the junction between femur and tibia Ta-III5 tip has few white scales.

***Abdomen*:** 1.57 mm long, covered with numerous setae and light yellowish hairs; Te-VI few narrow light brown scales, Te-VII with light narrow scales, Te-VIII extensively covered with golden scale and setae. Cerci without scales above, dark below.

Male: Characters are same as in females, smaller than females, 2.86mm long, slender, with plumose antenna 1 or two black scales on first flagellar segment, uniformly dark; major portion of the palpi pale coloured with marginal hairs; dorsal portion abdominal segment VIII with broad golden scales instead of hairs.

Bionomics: Breeds largely in forested areas, and its habitats are following; borrow pits, rainwater collections, small, shallow, shaded parts of pools, puddles, gem pits, animal footprints, wheel ruts, and hollow logs. Most abundant during the rainy season.

Distribution: In India: Recorded from Arunachal Pradesh, Assam, Meghalaya, Mizoram, Tripura, West Bengal, Tamil Nadu, Kerala, and Karnataka. In Kerala: Wayanad (Nagpal and Sharma, 1995), Kottayam (Balasubramanian and Nikhil, 2013), present study- Kozhikode and Kannur and Malappuram.

Remarks: The present study reported this species from Kozhikode, Kannur and Malappuram for the first time. *Anopheles dirus is* a complex consisting of seven sibling species*, An.dirus* (*An.dirus* A), *An.cracens* (*An.dirus* B), *An.scanloni* (*An.dirus* C), *An.baimaii* (*An.dirus* D), *An.elegans* (*An.dirus* E), *An.nemophilous* (An.dirus F) and *An.takasagoensis. An.elegans* has now been renamed as *An.mirans* and is not part of the complex.

27. *Anopheles elegans* James, 1903

Myzomyia elegans James 1903:56(BNMH, London) Type: single♀ described, Type locality: India, Karnataka, Karwar.

Anopheles balabacensis of Reid, 1970: 56

Anopheles dirus species E of Tewari et al., 1987: 25

Anopheles leucosphyrus of Christophers 1933: 177

Materials examined: Plesiotype: 1♀, Kerala: Thottilppalam, Kozhikode district, Coll.C. Vipinya, 03. xii.2019

Other materials examined: 3♀& 4♂, Thottilppalam, Kozhikode district, Coll.C. Vipinya, 03. xii.2019.1♀ & 3♂, Vilangad, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 3♀ & 6♂, Pasukkadavu, Kozhikode district. Coll.C. Vipinya 21.vi.2021; 1♀ & 1♂, Kakkadampoyil, Malappuram district. Coll. C. Vipinya, 20.ii.2022; 4♀ & 7♂, Vayalada, Kozhikode district. Coll.C. Vipinya 22. viii. 2023.

Diagnosis: Female (Plate 26, Figs.26a-e, Map-6) Medium-sized, pale-coloured mosquitoes, 2.95mm long.

Head (Figs.26b): Covered with white upright and pale scales in front area with dark scales on behind; well-marked pale-coloured vertical spot; vertex broad, ocular area with scales arranged in single line in anterior region; sparse frontal tuft; compound eyes rounded and black; proboscis cylindrical and black half of the portion yellowish 2.4 mm long.

Antennae: With few hairs and whitish or silvery scales on the first flagellar segment (fs); 1.34 mm long, light coloured, 15segmented, hairy, and pilose; palpi 2.41 mm long, as long as the proboscis and longer than fore femur, moderately thick, slender, four broad pale bands: pale bands are separated by short dark band in between them.

Thorax: 1.38 mm long, covered with few scales and hairs on dorsal side, lateral area comparatively pale with narrow white scales; mesonotum with broad markings;

scutellum with some scales as in thorax; median area covered with pale white scales; pleura dark.

Wings (Figs.26c): 3.38 mm long,1.15 mm wide, pale coloured and spotted with dark marks; wing veins covered with broad spindle-shaped white and cream coloured scales; white spots costa with large four dark scaled spots and three small dots; vein 1(R1) with 1-4 pale spots exactly those in costa except middle area, vein 2 (RS) with three dark spots before bifurcation, three spots present on each branch, vein 3(R4+5) mostly pale areas except basal and distal portion, seven small dark scaled area present, vein 4 (M) three large dark spots on the main trunk, three spots present on each branch, vein 5(Cu) four dark spot before bifurcation, anterior branch with five and posterior with four dark spots, vein 6 (anal) with five dark spots, fringe spot absent on vein 5 and 6 but present in between these two.

Legs (Figs.26d): 5.5 mm long, coxa 0.5 mm long, broad; femur 1.5 mm long; femur, tibia, and tarsus covered with light scales; tibia, 1.35 mm long, darkish, speckled; tarsus five segmented, fore-leg tarsomere (Ta-I_5) yellowish-white scales present on tip other tarsal segments with white scales and form bands, mid leg tarsomeres are same as in foreleg, hind leg, femur and tibia speckled with white scales, broad white spot present at the junction between femur and tibia Ta-III_5 tip has few white scales and apical pale band of hind tibia with longitudinal dark strip on ventral side.

***Abdomen*:** 1.57 mm long, covered with numerous setae and light yellowish hairs; Te-VI few narrow light brown scales, Te-VII with light narrow scales, Te-VIII extensively covered with golden scale and setae, cerci without scales above, dark below.

***Male*:** Characters are same as in females, smaller than females, 2.86mm long, slender, with plumose antenna one or two black scales on first flagellar segment, uniformly dark; major portion of the palpi pale coloured with marginal hairs; dorsal portion abdominal segment VIII with broad golden scales instead of hairs.

***Bionomics*:** Predominantly breeds in borrow pits, rainwater collections, small, shallow, shaded parts of pools, puddles, gem pits, animal footprints, wheel ruts, tree holes, rock pools, ground pools, stagnant waters, and hollow logs.

Distribution: In India: recorded from Southwest states of India; Andra Pradesh, Karnataka, Kerala, Maharashtra, and Tamil Nadu. Kerala: Wayanad (Nagpal and Sharma, (1995); Thankachan M *et al*., (2019)). In this study, this species was recorded from Kozhikode and Malappuram.

Remarks: *An.elegans* have been incriminated as the natural vector of simian malaria in southwest India and Sri Lanka, but there is no evidence of *An.elegans* being involved in human malaria transmission. In Kerala, this species was reported only from the Wayanad district. However, the present work reported it from Kozhikode and Malappuram for the first time.

List of *Anopheles* mosquitoes encountered during the present study

1. *Anopheles aitkenii* James, 1903
2. *Anopheles umbrosus*
3. *Anopheles culiciformis* Cogill, 1903
4. *Anopheles barbirostris* Van der Wulp, 1884
5. *Anopheles crawfordi* Reid, 1953
6. *Anopheles peditaeniatus* Leicester, 1908
7. *Anopheles gigas* Giles, 1901
8. *Anopheles nigerrimus* Giles, 1900
9. *Anopheles nitidus*, Harrison, Scanlon & Reid, 1973
10. *Anopheles sinensis* (Weidemann), 1828
11. *Anopheles culicifacies* Giles, 1901

12. *Anopheles jeyporiensis* James, 1902

13. *Anopheles fluviatilis* James, 1902

14. *Anopheles dthali* Patton, 1905

15. *Anopheles subpictus A & B* Grassi, 1899

16. *Anopheles subpictus B*

17. *Anopheles vagus* Donitz, 1902

18. *Anopheles sundaicus* Rodenwaldt, 1925

19. *Anopheles stephensi* Liston, 1901

20. *Anopheles theobaldi* Giles, 1901

21. *Anopheles karwari* James, 1903

22. *Anopheles Jamesii* Theobald, 1901

23. *Anopheles splendidus* Koidzumi, 1920

24. Anopheles kochi Doenitz, 1901

25. *Anopheles tessellatus* Theobald, 1901

26. *Anopheles dirus* Python & Harrison, 1979

27. *Anopheles elegans* James, 1903

***Anopheles* species recorded from Kerala by other authors**

1. *Anopheles aconitus* Doenitz, 1902

Medium-sized mosquito; palpi with three pale bands, apical and subapical pale bands are equal and separated by pre apical dark band; three pairs of legs are completely dark without any white patches; wing costa with less than four pale areas; abdomen and cerci without any scales.

2. *Anopheles annadalei* Prashad, 1918

Medium-sized mosquito; palpi without a distinct pale band but small bands are present at joints; fore, mid, and hind legs completely dark, a prominent tuft of white and dark scales present at hind femur; the majority of the wing without any pale interruptions.

3. *Anopheles annularis* Van der Wulp, 1884

Medium-sized mosquito; white scales present on frontal tuft; palpi with 3 pale bands; wing costa mainly dark with less than 3 pale interruptions; femur and tibia completely dark in all three pair of legs, tarsomere III3-III5 completely white.

4. *Anopheles insulaeflorum* (Swell. & Swell.), 1919

Dark-coloured mosquito; palpi completely dark without any pale interruptions; wing completely dark; legs completely dark.

5. *Anopheles interruptus* Puri, 1929

Small-sized mosquito; palpi completely dark but small bands present at each joints; majority of wing veins are dark coloured, inner costa interrupted and subcostal pale spot present; legs completely dark, apex of hind femur with prominent tuft of white and dark scales.

6. *Anopheles leucosphyrus* Donitz, 1901

Small to medium-sized mosquito; head with normal type of scales; palpi without pale interruptions; wing completely dark; fore, mid, and hind leg completely dark.

7. *Anopheles lindesayi* Giles, 1900

Dark-coloured, medium-sized mosquito; head scales are brown-coloured; palpi completely dark without pale bands; inner costa completely dark; hind femur with pale band.

8. ***Anopheles maculatus*** **Theobald, 1901**

Medium-sized mosquito; head with pale coloured scales; palpi with three pale bands, length of apical and subapical pale band equal and separated by pre apical dark band; speckled legs, hind tarsomere with broad white bands; most of wings are pale coloured, costa with more than three pale interruptions.

9. ***Anopheles majidi*** **Young &Majid, 1928**

Small-sized mosquito; palpi with three pale bands, apical and sub apical pale band equal and separated by preapical dark band; wing with pale interruptions on costa, fringe spot present on vein 5 only; foreleg tarsomere with broad white bands, hindleg tarsomere 5 completely white, tarsomere 4 and 5 partially white.

10. ***Anopheles minimus*** **Theobald, 1901**

Medium-sized mosquito; palpi with three pale bands, apical and subapical pale bands equal and separated by preapical dark band; wing with pale interruptions, inner costa interrupted; legs without pale bands.

11. ***Anopheles moghulensis*** **Christophers, 1924**

Medium-sized mosquito; palpi with three pale bands, apical pale band nearly equal to pre apical dark band; tarsomere with broad white bands; fringe spot present on all vein except vein 6.

12. ***Anopheles pallidus*** **Theobald, 1901**

Medium-sized mosquito; wing with pale interruptions; palpi with pale bands, apical pale band nearly equal to preapical dark band; hindleg tarsomere 3, 4, and 5 completely white; fringe spot present on all veins.

13. ***Anopheles philippinensis*** **Ludlow, 1902**

Large-sized mosquito; palpi with three pale bands, apical and subapical pale band nearly equal separated by pre apical dark band; foreleg tarsomere with broad white bands, hindleg tarsomere 3, 4, and 5 completely white.

14. *Anopheles psuedosundaicus* Tyagi *et al*., 2009

Dark-coloured, medium-sized mosquito; palpi with three pale bands, apical pale band shorter than pre apical dark band; wing with pale interruptions, fringe spot absent on vein1; abdominal segment with pale scale patches.

15. *Anopheles pseudowillmori* Theobald, 1910

Medium-sized brown-coloured mosquito; palpi with three pale bands apical and subapical pale bands equal and separated by preapical dark band; legs with speckling, foreleg tarsomere with broad white bands; hindleg tarsomere 5 completely white.

16. *Anopheles varuna* Iyengar, 1921

Medium-sized, dark-coloured mosquito; palpi with three pale bands, apical and subapical pale band equal and separated by preapical dark band, tip of proboscis yellow in colour; wing with pale interruptions, fringe spot absent on vein 6; tarsomere without pale bands.

17. *Anopheles sintoni* Puri,1929

Small-sized mosquito; palpi completely dark and slightly shorter than proboscis; wing completely dark; legs completely dark.

4.4 Dichotomous key to the identification of Female *Anopheles* mosquito species in Kerala

Key to the species of Subgenus *Anopheles*

1. Wing entirely dark without pale areas **2**

Wing with contrasting dark and pale areas **5**

2(1). Head scales long and narrow (aitkenii group) **3**

Head scales broad and normal type (culiciformis group) **4**

3(2). Pre-scutellar space with fine setae extending up to scutellum: Larva; palmate hair present on abdominal segment I-VII; inner clypeal hair absent ..... ***Anopheles insulaeflorum***

Pre-scutellar space without setae; above the scutellum is bare area: Larva; inner clypeal hair 1-2 branches at ¼ th of base ..... ***Anopheles aitkenii***

4(2). Larva: one or two branches present on frontal hair; abdominal segment III-VI with long lateral hairs; prothorax- its ventral surface hair not spine-like..... ***. culiciformis***

Larva: feathered and short frontal hair; abdominal segment III-VI with long lateral hair with branches; prothorax- ventral surface hair spine like ..... ***An. sintoni***

5(1). Anterior margin of wing with less than four dark areas present on costa and radius R (vein1) (Subgenus *Anopheles*) **6**

Anterior margin of wing with four or more dark areas on costa and radius R (Vein 1) ..... **(Subgenus *Cellia*)**

6(5). Tuft of white scales present at the tip of hind femur visible to naked eye **7**

Hind femur without such scales..... **8**

7(6). Sub costal pale spot present on costa; small pale band present at the joint of leg ..... ***Anopheles interruptus***

Costa without sub costal pale spot; small pale band present at the joint of legs ..... ***Anopheles annandalei***

8(6). Inner part of costa mainly dark; single apical pale spot present on costa; broad white band present on hind femur ..... ***Anopheles lindesayi***

Costa with pale interruptions; hind femur without such band..... **9**

9(8). Costa with three or more pale spots at its inner quarter including pre-sector pale spot ..... ***Anopheles gigas***

Costa mainly dark; few scattered pale scales present **10**

10(9). Palpi with three or more pale bands **11**

Palpi without bands **15**

11(10). Scattered pale scales present on Costa **12**

Costa entirely dark **13**

12(11). Costa with scattered pale scales; hind leg tarsomere (Ta-III) with pale bands; Wing vein 5 with short basal dark spot .....***Anopheles nitidus***

Costa with pale scales; hind leg tarsomere (Ta-III) with pale bands; wing vein 5 with long basal dark spot .....***Anopheles nigerrimus***

13(11). Hind tarsomere with apical pale band at 4th and 5th tarsomere; humeral cross vein with dark scales **14**

Hind tarsomere with narrow bands; humeral cross vein without dark scales... ....***Anopheles peditaeneatus***

14(13). Fringe spot present on vein 5 (Cu2); wing with large apical pale spot; apex of vein 1 dark scaled .....***Anopheles sinensis***

Fringe spot absent on vein 5 (Cu2); apex of vein 1 pale scaled .....

Anopheles crawfordi

15(10). Ventral side of abdominal segment VII with tuft of dark scales; costa with scattered pale scales; fringe spot at vein 5 .....***Anopheles barbirostris***

Ventral side of abdomen without prominent tuft of dark scales; costa without pale scales; no fringe spot .....***Anopheles umbrosus***

2.1.2 Key to the species of sub genus *Cellia*

1. Anterior margin of wing with four or more dark areas on costa and radius R (Vein 1) (Subgenus Cellia) **2**

Anterior margin of wing with less than four dark areas presents on costa and radius R (Vein 1) ..... **Subgenus Anopheles (In part)**

2(1). Femora and tibia speckled **3**

Femora and tibia not speckled **12**

3(2). Tip of hind tarsomere 5 (Ta-III_5) not white **4**

Tip of hind tarsomere 5 (Ta-III_5) white **9**

4(3). Maxillary palpi (Mplp) with three pale bands **5**

Maxillary palpi (Mplp) with four pale bands **6**

5(4). Maxillary palpi speckled, broad apical and subapical pale band of palpi equal and separated by dark band; abdominal terga covered with pale scales (II-VIII) ..... ***Anopheles stephensi***

Maxillary palpi not speckled; apical and subapical pale band un equal .... ***Anopheles sundaicus***

6(4). Tibio tarsal joint of hind leg with broad pale band..... **7**

Tibio tarsal joint of hind leg without broad pale band **8**

7(6). Length of proboscis longer than 1 1/4th of the length of fore femora; a longitudinal dark mark present on ventral part of hind tibia .... ***Anopheles elegans***

Length of proboscis equal or shorter than 1 1/4th of the length of fore femora; ventral part of hind tibia without longitudinal dark mark..... .... ***Anopheles dirus***

8(6). Apex of proboscis is pale; three broad and one narrow pale band present on palpi; abdominal sternites with prominent tuft of dark scales..... ..... ***Anopheles kochi***

Apex of proboscis is pale; three broad and one narrow pale band present on palpi; abdominal sternites without prominent tuft of dark scales..... ..... ***Anopheles tessellatus***

9(3). Hind tarsomere Ta-III_3 not completely white, Ta-III_4 and Ta-III_5 completely white... ***Anopheles theobaldi***

Hind tarsomere Ta-III_3, Ta-III_4 and Ta-III_5 entirely white **10**

10(9). Broad subapical pale band which is equal to apical pale band; palpus segment with 2nd and 3rd with speckling.. ..... ***Anopheles splendidus***

Sub apical pale band of not equal to apical; palpi without speckling **11**

11(10). Abdominal terga (VI-VII) with golden scales inner and outer part of costa mainly pale ..... ***Anopheles jamesii***

Abdominal terga (VI-VII) without golden scales (dark colour), costa mainly dark . ..... ***Anopheles pseudojamesii***

12(2). Hind tarsomere with broad pale bands; Ta-III_5 (Hind tarsomere 5) entirely white. **13**

Hind tarsomere without broad pale band; Ta-III5 (Hind tarsomere 5) not white entirely **17**

13(12). Tarsomere 5 (Ta-III5) and part of 4 (Ta-III4) white **14**

Tarsomere 5 (Ta-III5), 4(Ta-III4), and 3 (Ta-III3) entirely white **15**

14(13*).* Mplp with three pale bands ..... ***Anopheles majidi***

Mplp with four pale bands .....***Anopheles karwari***

15(13). Dorsal side of abdomen with narrow scales; vein 5 (Cu) mainly dark, dark spot present at bifurcation point .....***Anopheles annularis***

Dorsum of abdomen with narrow scales; vein 5 (Cu) white; no dark spot at the point of bifurcation **16**

16(15). Abdomen with few or no pale scales except tip of dorsum and ventrum; apex of hind tarsomere 1 with narrow pale band . .....***Anopheles philippinensis***

Abdomen with scattered pale scales on most of the segment ventrally and on five or six segment dorsally; apex of tarsomere 1 without pale band…….... ... .***Anopheles pallidus***

17(12). Fore leg tarsomere with broad white bands; mid and hind leg tarsomere usually narrow banded **18**

Fore leg tarsomere without banded or narrow band present **19**

18(17). Female palpi with apical pale three times or less than the length of pre apical pale band ..... ***Anopheles vagus***

Female palpi with apical pale band equal or nearly equal to sub apical dark band.. .....***Anopheles subpictus* complex**

19(17). Wing: female mosquito with pale spotting on and vein 1 only…..

.... *Anopheles dthali*

Wing: pale spots present on all veins **20**

20(19). Female palpi apical area pale banded; wing: vein R4+5(vein 3) mainly dark . . ..***culicifacies complex***

Female palpi with apical area dark coloured; Wing: vein R4+5 (vein 3) mostly pale **21**

21(20). Palp: both apical and subapical pale band broader than the subapical dark band (Minimus group). **22**

Palpi: subapical dark band much broader than subapical pale band. **24**

22(21). Wing: vein 6 (anal) with two dark spots on proximal or distal part; vein 2.1 with pale spot ..... ***Anopheles aconitus***

Wing: vein 6 with one large spot on apical half; vein 2.1 pale areas in base and apex **23**

23(22). Wing: basal third of costa with one pale interruption; fore leg tarsomere with minute apical pale band ..... ***Anopheles minimus***

Wing: basal third costa entirely dark; fore leg tarsomere entirely dark .... ***Anopheles varuna***

24(21). One or more pale interruptions on basal third of costa; vein 6 (A) mainly dark; hind leg tarsomere with pale interruptions ..... ***Anopheles jeyporiensis***

Proximal part without pale interruptions **25**

25(24). Hind tarsomere Ta-III_{1-4} with narrow pale bands; vein 6(A) with three dark areas ..... ***Anopheles moghulensis***

Hind leg tarsomere dark without pale bands; vein 6 (A) with 2 dark area .... ***Anopheles fluviatilis***

4.5 Molecular taxonomy of Malaria vectors

Malarial vectors are included in complexes or groups of species that have comparable morphological characteristics making it difficult or impossible to distinguish between them. Recent developments in molecular taxonomy made it possible to identify species precisely using simple and reliable methods (Manguin *et al*., 2008). In India, based on morphological and cytological characteristics and a few molecular features, the taxonomic status of malarial vectors has been documented. However, multilocus DNA sequences are still rarely used in phylogenetic analysis of Anopheles mosquitoes (Dixit *et al*., 2010). Using cytochrome oxidase c subunit, I (COI) may clarify the ambiguity arising from incorrect taxonomic identification of mosquito vector complexes (Anoop Kumar *et al*., 2019). In Kerala, only a few research articles correlating molecular studies on mosquito species. still, there are no molecular studies on the resolution of malarial vector species complex from Kerala.

From North Kerala (Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram) we collected 27 species of *Anopheles* mosquitoes belonging to the subgenus *Anopheles* and *Cellia*. Of these 27 species, five species were the primary vector of Malaria, they are *Anopheles stephensi, Anopheles culicifacies, Anopheles dirus, Anopheles fluviatilis, Anopheles subpictus* B. Except for *An.stephensi,* all other species are complex forms. We conducted a molecular analysis of the above complex species and also another *species An. elegans,* details are given below:

Sequencing of DNA, Molecular identification and Phylogenetic analysis were done with these five species collected from different localities of North Kerala. Morphologically these species were identified using taxonomic keys. Final nucleotide sequences were submitted to NCBI Gen Bank and got accession numbers for each sequence. Table 4.5 shows the data from the collected specimen and the Accession number obtained from NCBI Gen Bank.

Table 4.5 List of COI gene sequences of *Anopheles* species of North Kerala with NCBI Accession Number

Sl. No.	Species Name	Voucher No.	Accession No. (COI)	Locality of Collection	Length of Sequence
1	*An. culicifacies*	ACC2	PP469556	Thottilppalam, Kozhikode	630bp
2	*An. dirus*	AD3	PP469557	Vilangad, Kozhikode	609bp
3	*An. stephensi*	VPY5	PP469559	Ponnani, Malappuram	612bp
4	*An. subpictus*	VPY2	PP469560	Kadalundi, Kozhikode	622bp

4.5.1 *Anopheles culicifacies* Giles, 1901

GenBank Accession Number: PP469556

4.5.1.1 Taxonomic Classification

Order: Diptera

Suborder: Nematocera

Family: Culicinae

Subfamily: Anophelinae

Genus: *Anopheles*

Subgenus: *Cellia*

Series: Myzomyia

Group: Funestus

Subgroup: *Culicifacies*

4.5.1.2 Analysis

After extraction of DNA for the amplification of mitochondrial cytochrome oxidase subunit I (COI) forward and reverse primers (Former et al., 1994) were used (`5'-GGTCAACAAATCATAAAGATATTGG-3'F` and `5'TAAACTTCAGGGTGACCAAAAAATCA-3'R`). *Anopheles culicifacies* were collected from the rock pool region of Thottilppalam (11.6776^{0}N, 75.7793^{0}E), Kozhikode District, North Kerala. PCR amplification product consists of around 630 base pairs sequences of COI gene fragments. This sequence was deposited in NCBI GenBank and got an Accession Number PP469556. The following are the figures for this species' DNA sequence, phylogenetic tree, and chromatogram.

```
>ACC.2, 630bp
TTATTTTCGGTGCTTGAGCTGGGATAGTAGGAACTTCATTAAGAATTCTTATTCGAGCTG
AATTGGGTCACCCAGGAGCTTTTATTGGAGATGATCAAATTTATAACGTAATCGTAACTG
CTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGGAGGATTTGGAA
ATTGACTTGTTCCATTAATATTAGGAGCACCAGATATAGCTTTCCCTCGAATAAATAATA
TAAGATTTTGAATACTTCCTCCTTCTTTAACACTTCTTATTTCTAGAAGTATAGTAGAAA
ATGGAGCAGGAACTGGATGAACTGTATACCCTCCATTATCTTCTGGAATTGCTCATGCAG
GGGCTTCAGTAGATTTAGCTATTTTTTCTTTACATTTAGCAGGAATTTCTTCAATTTTAG
GGGCAGTAAATTTTATTACTACTGTTATTAATATACGATCTCCGGGAATTACATTAGATC
GAATACCATTATTTGTTTGATCTGTAGTAATTACTGCTATTTTATTATTATTATCATTAC
CCGTATTAGCTGGAGCTATTACAATATTATTAACTGATCGAAATTTAAATACTTCATTCT
TTGATCCAGCAGGAGGAGGAGATCCAATTT
```

Fig. 4.5.1a: Nucleotide sequences of COI gene of *Anopheles culicifacies*

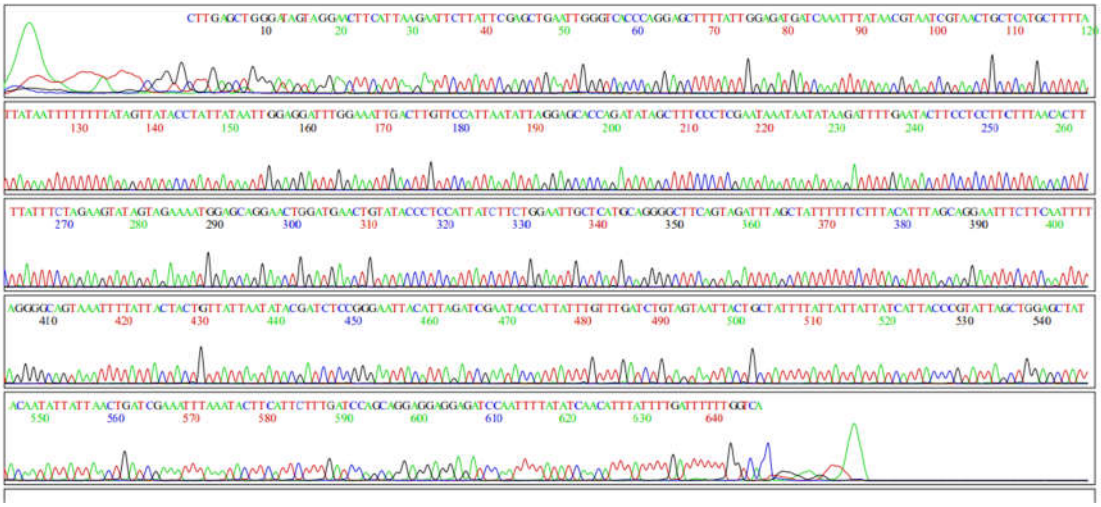


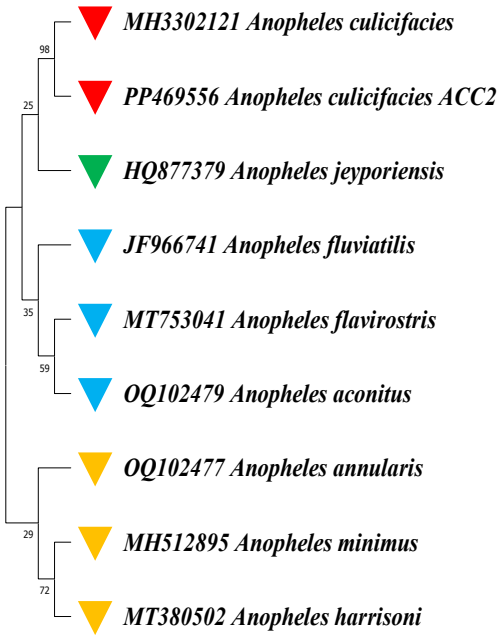
Fig 4.5.1b The sequencing chromatogram of forward DNA of *Anopheles culicifacies*

Table 4.5.1: BLAST hit table shows Percentage identity between *Anopheles culicifacies* and its closely related species

SI. No.	Species	Accession No.	Location	%Identity
1	*Anopheles culicifacies*	MH330212	Sri Lanka: Pasyala	99.53
2	*Anopheles flavirostris*	MT753041	Indonesia: Sulawesi	95.27
3	*Anopheles aconitus*	OQ102479	Indonesia: Yogyakarta	94.89
4	*Anopheles minimus*	MH512895	India: Patiala	94.58
5	*Anopheles jeyporiensis*	HQ877379	Viet Nam: Binh Dinh	93.49
6	*Anopheles fluviatilis*	JF966741	Iran: Tehran	93.45
7	*Anopheles harrisoni*	MT380502	Viet Nam	93.04
8	*Anopheles annularis*	OQ102477	Indonesia: Yogyakarta	91.95

The *Anopheles culicifacies* sequences used in the present study (ACC2) showed 99.53% sequence similarity with *An.culicifacies* from Sri Lanka (MH330212). At the same time, the similarity was 94.58% with *Anopheles* species (MH512895) from India. *An. flavirostris* (MT753041) from Indonesia showed 95.27% similarity.

The sequences with top similarities were identified from the BLAST and the sequences were used with ACC2 for phylogenetic analysis using MEGA 11, the Neighbor-joining trees were represented below in Fig. 4.5.1b.



▼ species collected from Kozhikode district

▼, ▼ closely related anopheles species (paraphyletic and polyphyletic)

▼ unrelated species

Fig. 4.5.1c: Phylogenetic tree of *Anopheles culicifacies* constructed by Neighbor-Joining method

The variations and similarities were reflected in the NJ- tree constructed using sequences obtained from the database. *An. culicifacies* (ACC2) used in the present study were found to share a single clade (monophyletic) with *An. culicifacies* (MH3302121▼) from Sri Lanka with 99.53% sequence similarity. *An.jeyporiensis* (HQ877379▼) found in the next clade showed a paraphyletic relationship with ACC2 with sequence similarity of 93.49%. *An.fluviatilis* (JF966741▼), *An.flavirostris* (MT753041▼), and *An.aconitus* (OQ102477▼) showed a polyphyletic relationship with ACC2 sequence with a sequence similarity of 93.25%, 95.27%, and 94.89% respectively. *Anopheles annularis* (OQ102477, Indonesia), *Anopheles minimus* (MH512895, India), *Anopheles harrisoni* (MT380502, Viet Nam) of this cladogram were found unrelated species.

By analysing the nucleotide sequences or BLAST analysis of 630 bp of *An. culicifacies*, showed that phylogenetic relationship with other *Anopheles* species found in different geographic locations. *An. culicifacies* (PP469556) shows 99.53 % identity *with An. culicifacies* found in Sri Lanka: Pasyala (MH330212), 95.27 % identity with *An. flavirostris* in Indonesia (MT753041), 91.95 % identity with *An. annularis* found in Indonesia: Yogyakarta.

4.5.1.3 Discussion

Anopheles culicifacies small-sized mosquito, distributed in Afghanistan, Bangladesh, Cambodia, India, Iran, Myanmar, Nepal Pakistan, Thailand, Viet Nam, Laos, and Sri Lanka. It is an important vector of Malaria and exists as a complex of five morphologically indistinguishable species A, B, C, D, and E with varied geographical distributional patterns and occur in all mainland zones. Species A and B are sympatric in North and South India, with species A predominant in the north and species B in the south (Sharma and Dev, 2015) The species preferred to breed in seepage, unused wells, irrigation channels, field channels, rock pools with vegetation, etc. The most suitable breeding places are small rocky pools and perennial streams with clear water and the least suitable places are highly turbid, brackish water rich with planktons and blue-green algae (Sharma and Dev, 2015). All members of the complex were identified by Geeta Goswami et al., 2000 using allele-specific polymerase chain reaction. Among these, the use of diagnostic fixed readable inversions in the ovarian nurse cell's polytene chromosome helps to identify sibling species and it is the only method available to differentiate the four sibling species (Sharma and Dev, 2015). In the present study, this species was collected from Kozhikode a North Kerala district, phylogenetically this species shares a common ancestry with *Anopheles culicifacies* from Sri Lanka (MH330212). Here, these two species were represented as sibling species, and using polytene chromosome analysis they confirmed that it was species B. So, without any doubt, the isolated sequence is *An. culicifacies* found in Kozhikode Districts of North Kerala belong to *Anopheles culicifacies* B the poor vector of malaria in India. In

Kerala, poor studies were carried out regarding the molecular aspect of *Anopheles culicifacies.* So, the study was a novel molecular work from Kerala.

4.5.2 *Anopheles dirus* Peyton & Harrison, 1979

Gen Bank Accession Number: PP459557

4.5.2.1 Taxonomic Classification

Order : Diptera

Suborder : Nematocera

Family : Culicinae

Subfamily : Anophelinae

Genus : *Anopheles*

Subgenus : *Cellia*

Series : Neomyzomyia

Group : Leucosphyrus

Subgroup : Lecosphyrus

4.5.2.2 Analysis

After extraction of DNA for the amplification of mitochondrial cytochrome oxidase subunit I (COI) forward and reverse primers (Folmer et al., 1994) were used (`5'-GGTCAACAAATCATAAAGATATTGG-3'F` and `5'TAAACTTCAGGGTGACCAAAAAATCA-3'R`). *Anopheles dirus* was collected from the rock pool region of Vilangad, Kozhikode District, North Kerala. PCR amplification product consists of around 609 base pairs sequences of COI gene fragments. This sequence was deposited in NCBI GenBank and got an Accession Number PP469557. Following are the figures of, the DNA sequence (Fig.4.5.2a), Phylogenetic tree (Fig. 4.5.2c), and chromatogram (4.5.2b) of this species.

>AD.3 609bp
CCTGAGCAGGTATAGTAGGAACTTCTTTAAGAATCTTAATTCGAGCTGAATTAGGACATC
CAGGAGCTTTTATTGGAGATGATCAAATTTATAATGTTATTGTTACTGCTCACGCATTTA
TCATAATTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTTC
CTTTAATACTAGGAGCACCAGATATGGCATTTCCTCGAATAAATAATATAAGTTTTTGAA
TATTACCTCCTTCACTTACACTTTTAATTTCTAGAAGTATAGTAGAAAATGGAGCAGGTA
CAGGATGAACAGTTTATCCCCCTTTATCATCTGGGATTGCTCATGCCGGAGCTTCTGTTG
ATTTAGCAATTTTTTCTTTACATTTAGCAGGAATTTCTTCTATTTTAGGGGCAGTAAATT
TTATTACTACTGTAATTAATATGCGATCGCCAGGAATTACTTTAGATCGAATACCTTTAT
TTGTTTGATCAGTTGTAATTACTGCAATTTTATTACTTTTATCTTTGCCCGTATTAGCAG
GAGCAATTACTATACTATTAACGGACCGAAATTTAAATACTTCATTTTTTGACCCAGCTG
GTGGAGGAG

Fig. 4.5.2a: Nucleotide sequences of COI gene of *Anopheles dirus*

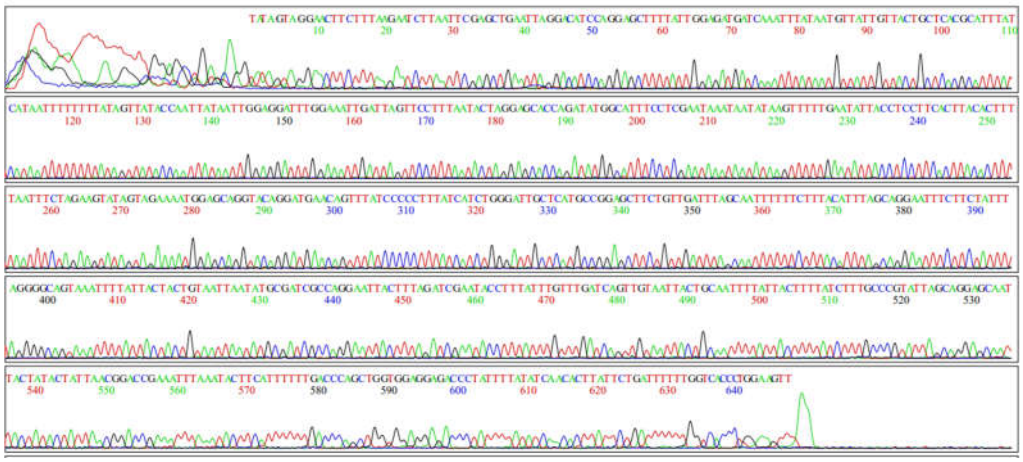


Fig 4.5.2b The sequencing chromatogram of forward sequences of *Anopheles dirus*

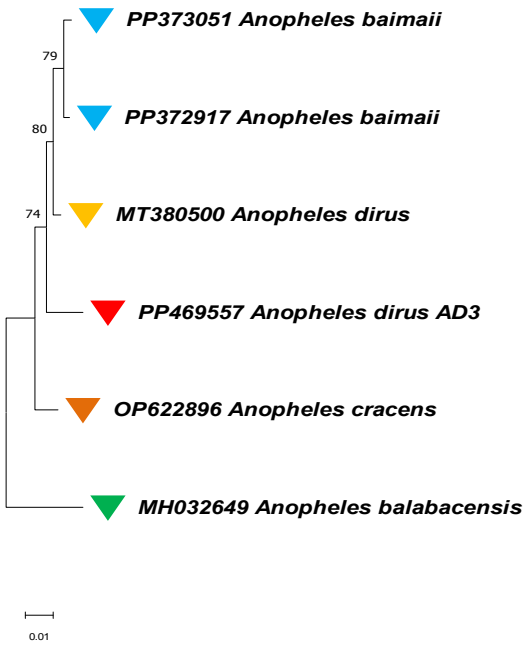
Table 4.5.2: BLAST hit table shows Percentage identity between *Anopheles dirus* and its closely related species

SI No.	Species	Accession No.	Location	%Identity
1	*Anopheles dirus*	MT380500	Viet Nam	98.23
2	*Anopheles baimaii*	PP373050	Myanmar	98.07
3	*Anopheles cracens*	OP622896	Malaysia	97.34
4	*Anopheles balabacensis*	MH032649	Malaysia	95
5	*Anopheles baimaii*	PP372917	Thailand	98.26

The specimen AD3 was identified as *Anopheles dirus* using the taxonomic key (Das *et al*., 1990). The BLAST analysis of 609 bp of mosquito *An.dirus* showed

a significant relationship with different *Anopheles* species. The genetic identity analysis depicts the divergence of different geographically isolated species of *Anopheles* with various related species. *An.dirus* isolated from a shaded rock pool region with aquatic vegetation near the forest area of Vilangad, Kozhikode District (GenBank Accession No. PP469557) showed 98.23% similarity with *An.dirus* (MT80500) from Viet Nam. While the similarity was 98.07 % with *Anopheles baimaii* (PP373050) from Myanmar. Another sequence analysis of *Anopheles baimaii* (PP372917) from Myanmar showed 98.26% similarity with AD3. *Anopheles cracens* (OP622896) from Malasia showed 97.34% similarity with AD3 and 95 % similarity with *Anopheles balabacensis* (MH032649) from Malasia.

The sequence with high similarities was sorted from the nucleotide sequence deposit from GenBank and used with the AD3 sequence finally phylogenetic analysis was done in MEGA 11 and a phylogenetic tree was derived (Neighbor-Joining method) and shown below (Fig. 4.2c).



▼ **species collected from Vilangad, Kozhikode District (AD3)**

▼, ▼, ▼ ▼ **closely related species**

Fig. 4.5.2c: Phylogenetic tree of *Anopheles dirus* constructed by Neighbor-Joining method.

4.5.2.3 Discussion

An. dirus is widely distributed in Taiwan, India, Nepal, Bhutan, Veit Nam, Lao, and Cambodia. In India, this species is widely distributed in Uttar Pradesh, Kerala, and Karnataka. The species complex comprises seven sibling species (Python, 1989). Species A, B, C, D, E, and F and sibling species were identified by cross-mating experiments, karyotypic studies, polytene chromosome banding patterns, DNA probes, and egg morphology (Phungam *et al*., 2017). Among these species, *An. dirus* D most prevalent in india (Sarmah et al., 2019). Species D in India is referred to as *An. baimaii* a close relative of *An. dirus*. It is widely abundant in northeastern states and the habitats follow small, temporary shaded pools of water in hilly regions. In the present study, this species was isolated from the shaded part of the rock pool area of Mahe River Vilangad, a fragment forest-covered area of Kozhikode district. AD3 showed 98.26% sequence similarity with *An.baimaii* (PP273917) from Thailand and 98.07 % similarity with An.baimaii (PP373050) from Myanmar in the DataBase of GenBank. So based on the above data AD3 sequence was isolated from Kozhikode District *An.dirus* D, which showed a polyphyletic relationship with PP273917 and PP373050.

4.5.3 *Anopheles stephensi* Liston, 1901

GenBank Accession Number: PP469559

4.5.3.1 Taxonomic Classification

Order	:	Diptera
Suborder	:	Nematocera
Family	:	Culicinae
Subfamily	:	Anophelinae
Genus	:	Anopheles
Subgenus	:	Cellia
Series	:	Neocellia

4.5.3.2. Analysis

After extraction of DNA for the amplification of mitochondrial cytochrome oxidase subunit I (COI) forward and reverse primers were used (5'-GGTCAACAAATCATAA AGATATTGG-3'F and 5'TAAACTTCAGGGTGACCAAAAAATCA-3'R). *Anopheles stephensi* were collected from the abandoned fishing boats in Malappuram District, North Kerala. PCR amplification product consists of around 641 base pairs sequences of COI gene fragments. This sequence was deposited in NCBI GenBank and got an Accession Number PP469559. Following are the figures of, the DNA sequence (Fig.4.5.3a), Phylogenetic tree (Fig. 4.5.3c), and chromatogram (4.5.3b) of this species.

>Anopheles_stephensi_VPY5
AGTAGGAACATCTTTAAGAATTCTTATTCGAGCTGAATTAGGACACCCAGGAGCATTTAT
TGGAGACGATCAAATTTATAATGTAATTGTAACTGCTCATGCTTTTATTATAATTTTCTT
TATAGTTATACCTATTATAATTGGGGGATTTGGAAATTGATTAGTTCCTTTAATATTAGG
AGCACCAGATATAGCATTTCCTCGAATAAATAATATAAGATTTTGAATATTACCCCCCTC
ATTAACTCTTTTAATTTCTAGAAGTATAGTAGAAAATGGAGCAGGAACAGGATGAACTGT
TTATCCGCCTTTATCGTCTGGAATTGCTCACGCTGGGGCTTCAGTAGATTTAGCAATTTT
TTCATTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCAGTTAATTTTATTACTACAGT
AATTAATATACGATCGCCAGGAATTACGTTAGACCGAATACCTTTATTCGTTTGATCTGT
TGTAATTACTGCTATTTTATTATTATTATCATTACCTGTATTAGCTGGAGCTATTACTAT
ATTACTTACAGACCGAAATTTAAATACATCTTTTTTCGACCCAGCTGGAGGAGGAGACCC
CATTTTATATCAACATTTATTTTGATTTTTTGGTCACCCTG

Fig. 4.5.3a: Nucleotide sequences of COI gene of *Anopheles stephensi*

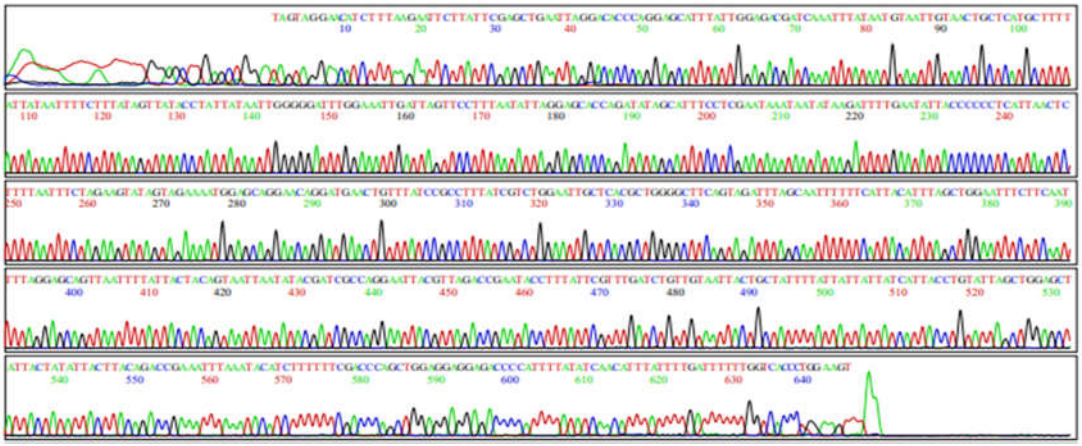


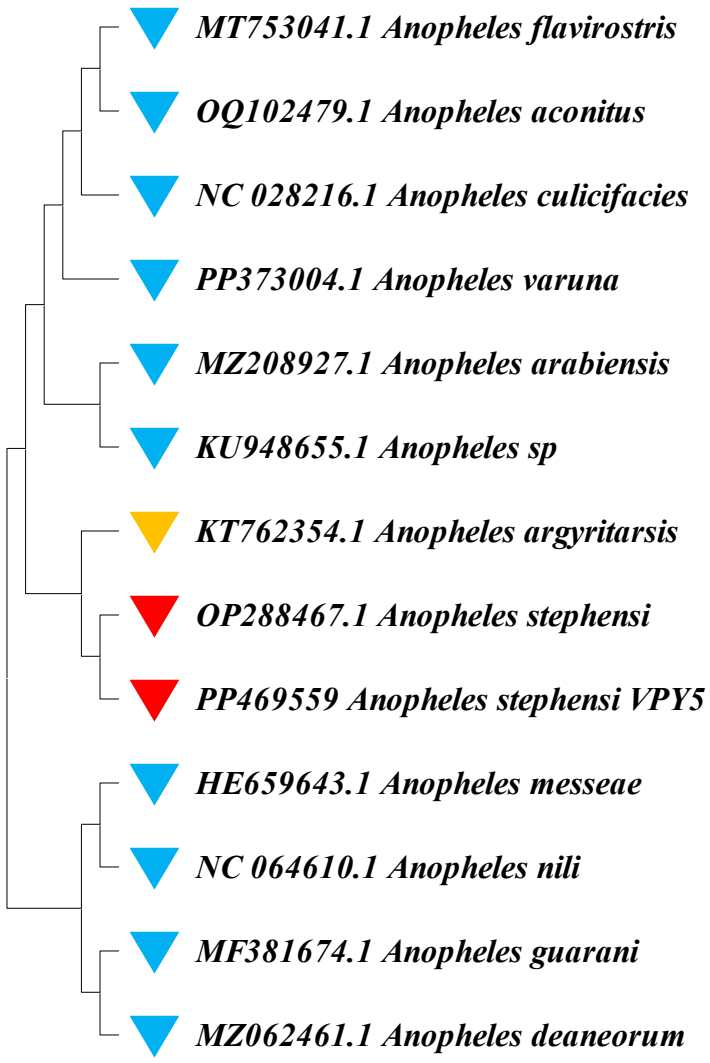
Fig. 4.5.3b The sequencing chromatogram of forward nucleotides of *Anopheles stephensi*

Table 4.5.3: BLAST hit table shows Percentage identity between *Anopheles stephensi* and its closely related species

Sl No.	Species	Accession number	Location	% Similarity
1	*Anopheles stephensi*	OP288467	Kerala	100
2	*Anopheles arabiensis*	MZ208927	Saudi Arabia	91.84
3	*Anopheles sp.*	KU948655	London	91.8
4	*Anopheles argyritarsis*	KT762354	Brazil	91.8
5	*Anopheles varuna*	PP373004	Thailand	91.69
6	*Anopheles flavirostris*	MT753041	Indonesia	91.68
7	*Anopheles messeae*	HE659643	Russia	91.56
8	*Anopheles guarani*	MF381674	Brazil	91.42
9	*Anopheles deaneorum*	MZ062461	USA	91.31
10	*Anopheles nilli*	NC_064610	USA	91.26
11	*Anopheles culicifacies*	NC_028216	USA	91.24
12	*Anopheles aconitus*	OQ102479	Indonesia	91.19

The voucher VPY5 was isolated from specimens collected from water holding in abandoned fishing boats in Ponnani, Malappuram district. Morphologically the species identified as *Anopheles stephensi* using Taxonomic key (Das *et al*., 1990). The BLAST analysis of 641 bp of specimen VPY5 showed significant similarity with anopheles species present in different geographical areas. *An.stephensi* VPY5 (PP469559) used in the present study showed 100% similarity with *An.stephensi* (OP288467) from Kerala. The percentage similarity of VPY5 with Other *Anopheles* species is shown in table 4.5.3.

The phylogenetic relationship of *An. stephensi* VPY5 was done using MEGA11 and revealed the phylogeny with the Neighbor-joining tree developed using sequences from GenBank Data Base (Fig. 4.5.3c)



▼ **Species collected from Ponnani**

▼▼ **Closely related species (paraphyletic and polyphyletic)**

Fig. 4.5.3c: Phylogenetic tree of *Anopheles stephensi* constructed by Neighbor-Joining method.

The evolutionary % similarity analysis of *An.stephensi* VPY5 (PP469559) between 12 species revealed that VPY5 shared a single clade (monophyletic) with *An.stephensi* (OP288467▼) from Kerala with 100 % sequence similarity. Parallel to this clade another species *An. argyritarsis* (KT62354▼) from Brazil with a sequence similarity of 91.8% (paraphyletic). which showed that phylogenetically *An. argyritarsis* closest relative of *An.stephensi.*

4.5.3.3 Discussion

Anopheles stephensi is a major Malaria vector in India commonly Known as Urban Malaria Vector. It is widely distributed in Afghanistan, Bangladesh, India, China, Myanmar, Iraq, Taiwan and Thailand. This species is native to the Indian subcontinent, but now expanding its geographic range to Ethiopia, Sudan, Horn of Africa, Somalia, Yemen, Kenya, Ghana, and Nigeria. *An.stephensi* VPY5 (PP469559) used in the present study showed 100% similarity with *An.stephensi* (OP288467) from Kerala.

4.5.4 *Anopheles subpictus* B Grassi, 1899

GenBank Accession number PP469560

4.5.4.1 Taxonomic Classification

Order : Diptera

Suborder : Nematocera

Family : Culicinae

Subfamily : Anophelinae

Genus : Anopheles

Subgenus : Cellia

Series : Pyretophorus

Complex : Subpictus

4.5.4.2 Analysis

After extraction of DNA for the amplification of mitochondrial cytochrome oxidase subunit I (COI) forward and reverse primers were used (5'-GGTCAACAAATCATAAAGATATTGG-3'F and 5'TAAACTTCAGGGTGACCAAAAAATCA-3'R). *Anopheles subpictus B* was collected from the marshy ditches and paddles of the Kadalundi River, Kozhikode District, North Kerala. PCR amplification product

consists of around 646 base pairs sequences of COI gene fragments. This sequence was deposited in NCBI GenBank and got an Accession Number (PP469560). Following are the figures of, the DNA sequence (Fig.4.5.4a), Phylogenetic tree (Fig. 4.5.4c), and chromatogram (4.5.4b) of this species.

>Anopheles_subpictus_VPY2
ATAGTGGGTACTTCTTTAAGAATTTTAATTCGAGCTGAATTAGGTCATCC
AGGAGCTTTTATTGGTGATGATCAAATTTATAATGTAATTGTTACTGCTC
ATGCATTTATTATAATTTTCTTTATAGTAATACCAATTATAATTGGTGGA
TTTGGAAACTGATTAGTGCCTCTTATACTAGGAGCGCCTGATATAGCATT
CCCTCGAATAAACAATATAAGATTTTGAATATTACCTCCTTCTTTAACAC
TTTTAATTTCTAGTAGTATAGTGGAAAATGGGGCGGGTACAGGATGAACT
GTTTACCCTCCGCTATCTTCTGGGATTGCACATGCAGGGGCATCAGTTGA
TTTAGCAATTTTTTCTCTACATTTAGCTGGTATTTCTTCAATTTTAGGAG
CAGTAAATTTTATTACTACAGTAATTAATATACGATCTCCAGGAATTACT
TTAGACCGAATACCATTGTTTGTATGATCAGTAGTAATTACAGCAATTTT
ATTATTGTTATCACTGCCAGTATTAGCTGGAGCTATTACTATATTATTAA
CTGATCGAAATTTAAATACCTCTTTCTTTGACCCAGCTGGAGGAGGAGAT
CCTATTTTATATCAACATTTATTTTGATTTTTTGGTCACC

Fig. 4.5.4a: Nucleotide sequences of COI gene of *Anopheles subpictus*

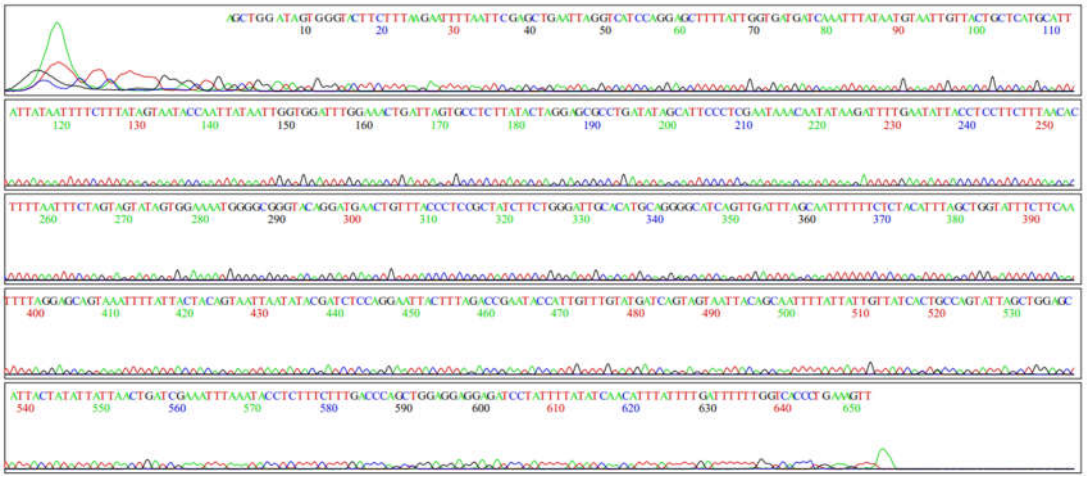


Fig. 4.5.4b The sequencing chromatogram of forward DNA of *Anopheles subpictus*

Table 4.5.4: BLAST hit table shows Percentage identity between *Anopheles subpictus* and its closely related species

Sl No.	Species	Accession Number	Location	% similarity
1	*Anopheles subpictusB VPY2*	PP469560	Kerala	100
2	*Anopheles subpictus*	OL587934	Kerala	100
3	*Anopheles epiroticus*	NC_028217	USA	91.25
4	*Anopheles sundaicus*	MT669951	Malaysia	91.25
5	*Anopheles marajoara*	DQ076223	USA	91.02
6	*Anopheles albitarsis*	KJ492698	USA	90.87
7	*Anopheles darlingi*	HM022406	Colombia	90.55
8	*Anopheles deaneorum*	MZ062461	USA	90.39
9	*Anopheles rangeli*	NC_037786	USA	90.31

4.5.4.3 Discussion

The 646 long nucleotide sequences of COI region isolated from morphologically confirmed species B collected from Kadalundi, Kozhikode district, were deposited in Gen Bank and got Accession number (PP469560). These nucleotide sequences were aligned along with Gen Bank entries of *Anopheles subpictus* from Kerala, *An.epiroticus* from USA, and *An.sundaicus* from Malaysia, *An.darlingi* from Colombia, *An.marajoara* from USA, *An.albitarsis* from USA and *An.rangeli* from USA. For the evolutionary analysis, 9 nucleotide sequences were used.

The phylogenetic study revealed that *Anopheles subpictus* (OL587934) from Kerala showed 100 % sequence similarity and shared a common clade in their neighbour-joining tree. This was identified in the subpictus B clade (Surendran, 2010). It showed that these two species originated from the same ancestor or in the same clade (monophyletic origin). *Anopheles epiroticus* (NC_028217) from the USA and *Anopheles sundaicus* (MT669951) from Malaysia showed paraphyletic relationship with the *Anopheles subpictus* (PP469560). *Anopheles albitarsis* (KJ492698) from the USA, *Anopheles darlingi* (HM022406) from Colombia, *Anopheles deaneorum* (MZ062461) and *Anopheles rangeli* (NC_037786) from the

USA showed polyphyletic relationship with the present nucleotide sequences of *Anopheles subpictus*. So without any doubt, the morphologically identified *An. subpictus* confirmed as *Anopheles subpictus* B.

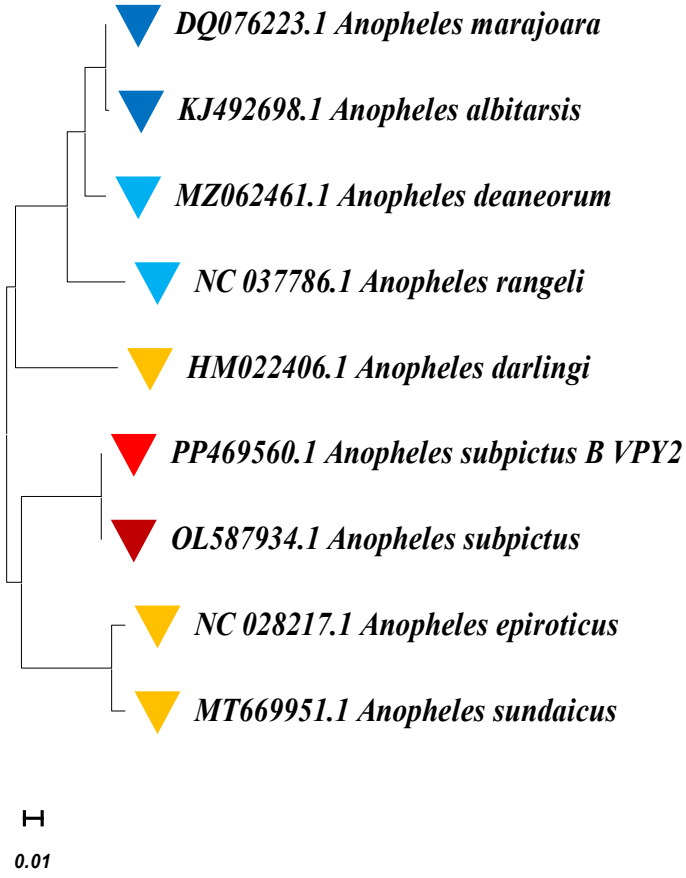


Fig. 4.5.4c: Phylogenetic tree of *Anopheles subpictus B* constructed by Neighbor-Joining method.

▼species collected from Kadalundi

▼▼▼closely related species

PLATE 1

Fig 1: Female *Anopheles aitkenii*

PLATE 2

Fig.2. (a-d), a-*Anopheles umbrosus* Female, b- palpi, c- wing, d- hind leg

PLATE 3

Fig 3: Female *Anopheles culiciformis*

PLATE 4

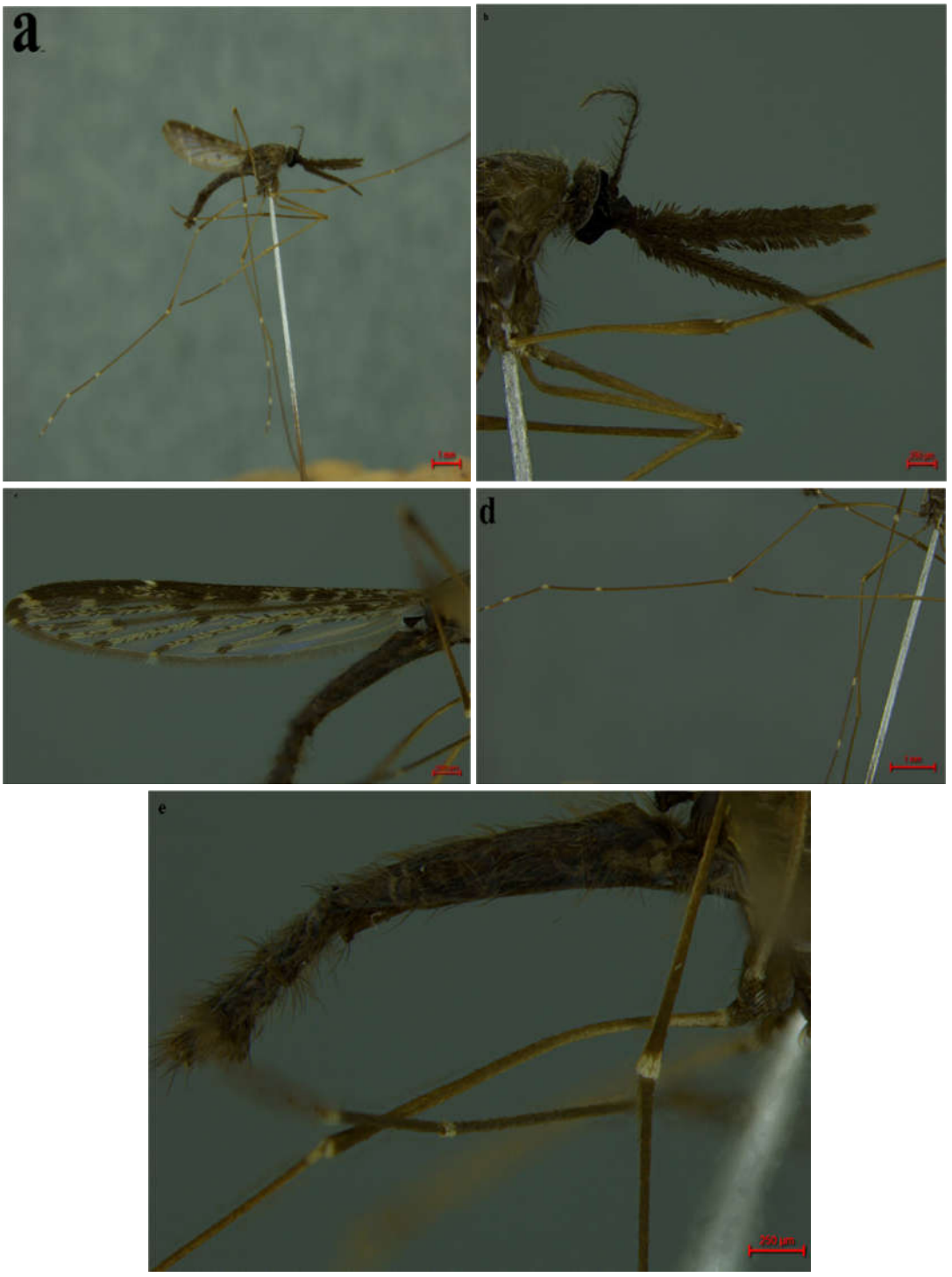


Fig. 4 (a-e); a- *An.barbirostris* female**, b-** Female palpi**, c-** Wing**, d-** Hind leg**, e-** Abdomen

PLATE 5

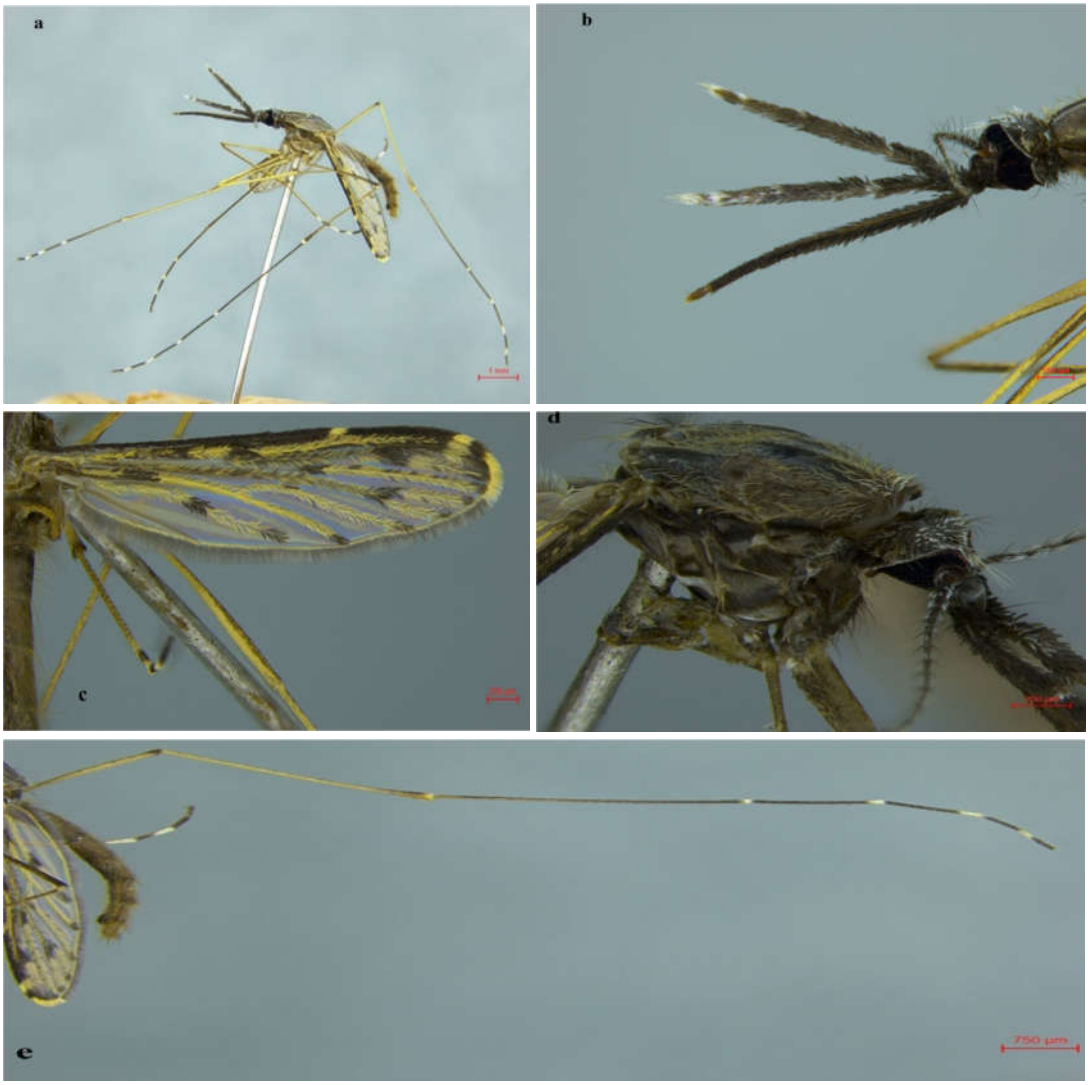


Fig.5(a-e); a- *An.crawfordi* female, **b**- Female palpi, **c**- Wing, **d**- Thorax, **e**- Hind leg

PLATE 6

Fig 6(a-d): a-*Anopheles peditaeniatus* female, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 7

Fig 7 (a-d): a- Female *Anopheles gigas*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 8

Fig 8 (a-d): a- Female *Anopheles nigerrimus*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 9

Fig 9 (a-e): a- Female *Anopheles nitidus*, **b**- Female palpi, **c**- Wing, **d**- Hind leg, e- Abdomen

PLATE 10

Fig 10 (a-d): a- Female *Anopheles sinensis*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 11

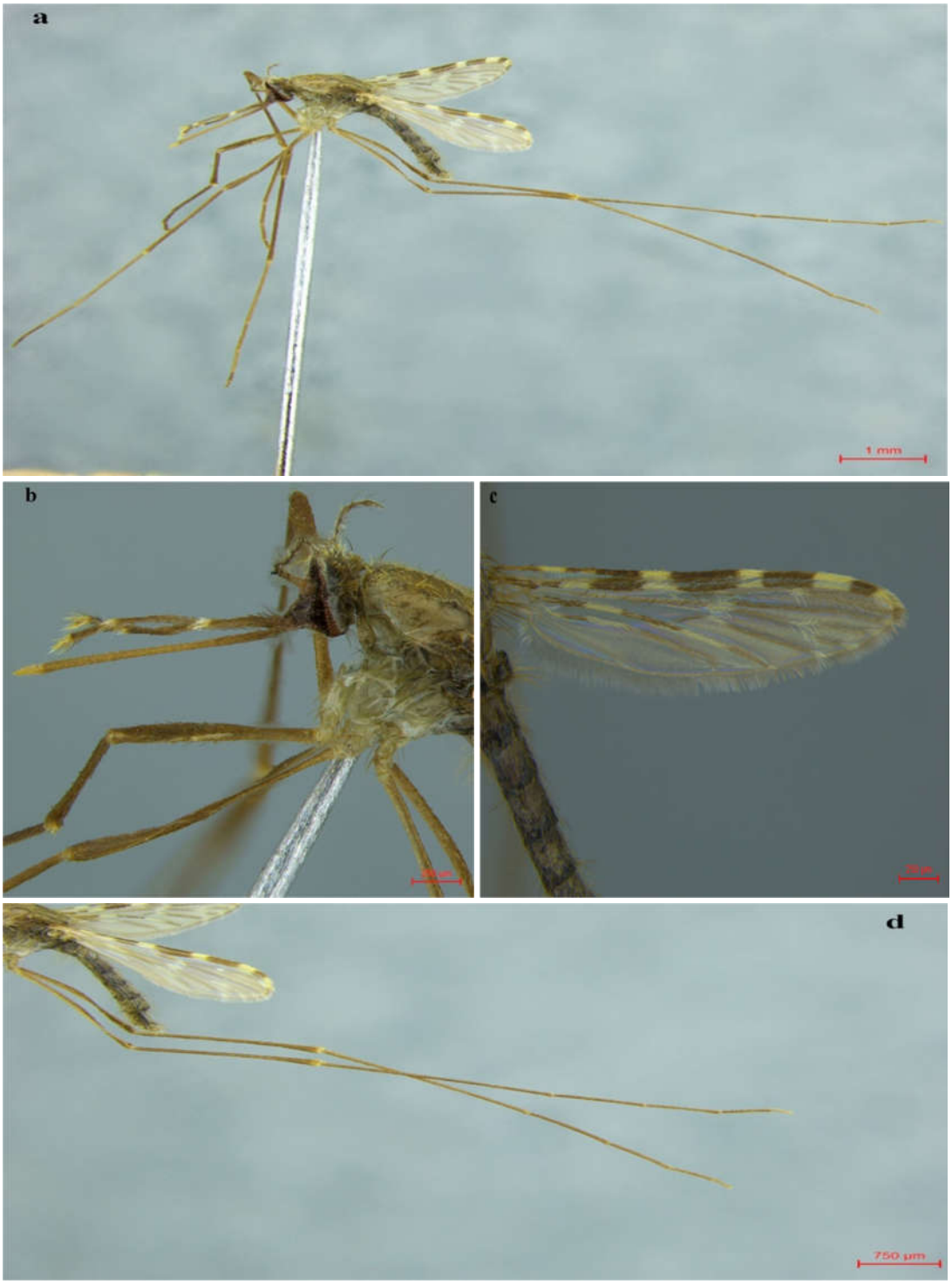


Fig 11(a-d): a- Female *Anopheles culicifacies*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 12

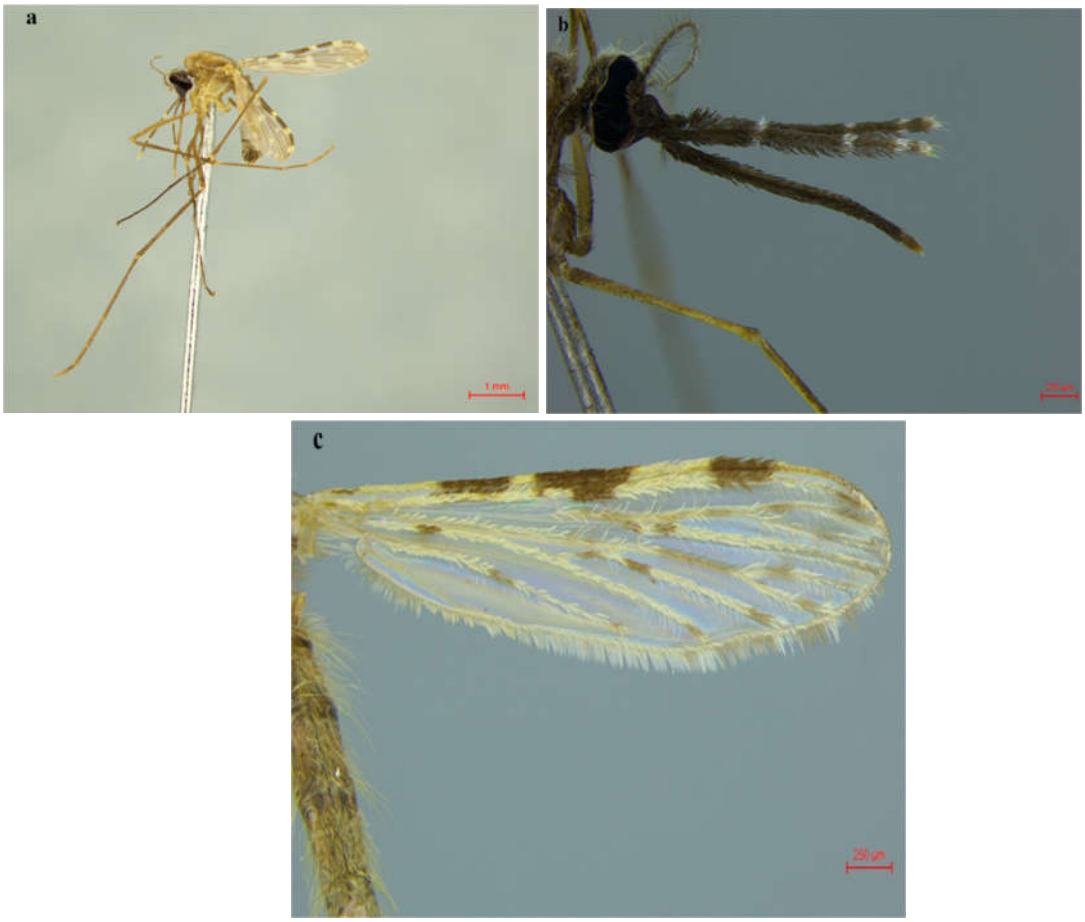


Fig. 12 (a-c) *Anopheles jeyporiensis* Female.a- Whole, b- Palpi, c- Wing

PLATE 13

Fig 13 (a-e): a- Female *Anopheles fluviatilis*, **b**- Female palpi, **c**- Thorax(lateral), **d**- Wing, e- Thoracic legs.

PLATE 14

fig. 14. ***Anopheles dthati*** **Female**

PLATE 15

Fig 15 (a-d): a- Female *Anopheles subpictus*, **b**- Female palpi, **c**- Wing, **d**- Abdomen, e- Fore leg

PLATE 16

Fig 16 (a-e): a- Female *Anopheles vagus*, **b-** Female palpi, **c-** Wing, **d** Thorax(lateral), e- Hind leg

PLATE 17

Fig 17 (a-e): a-Female *Anopheles sundaicus*, **b**- Female palpi, **c**- Wing, **d**- Thorax(lateral) e- Hind leg

PLATE 18

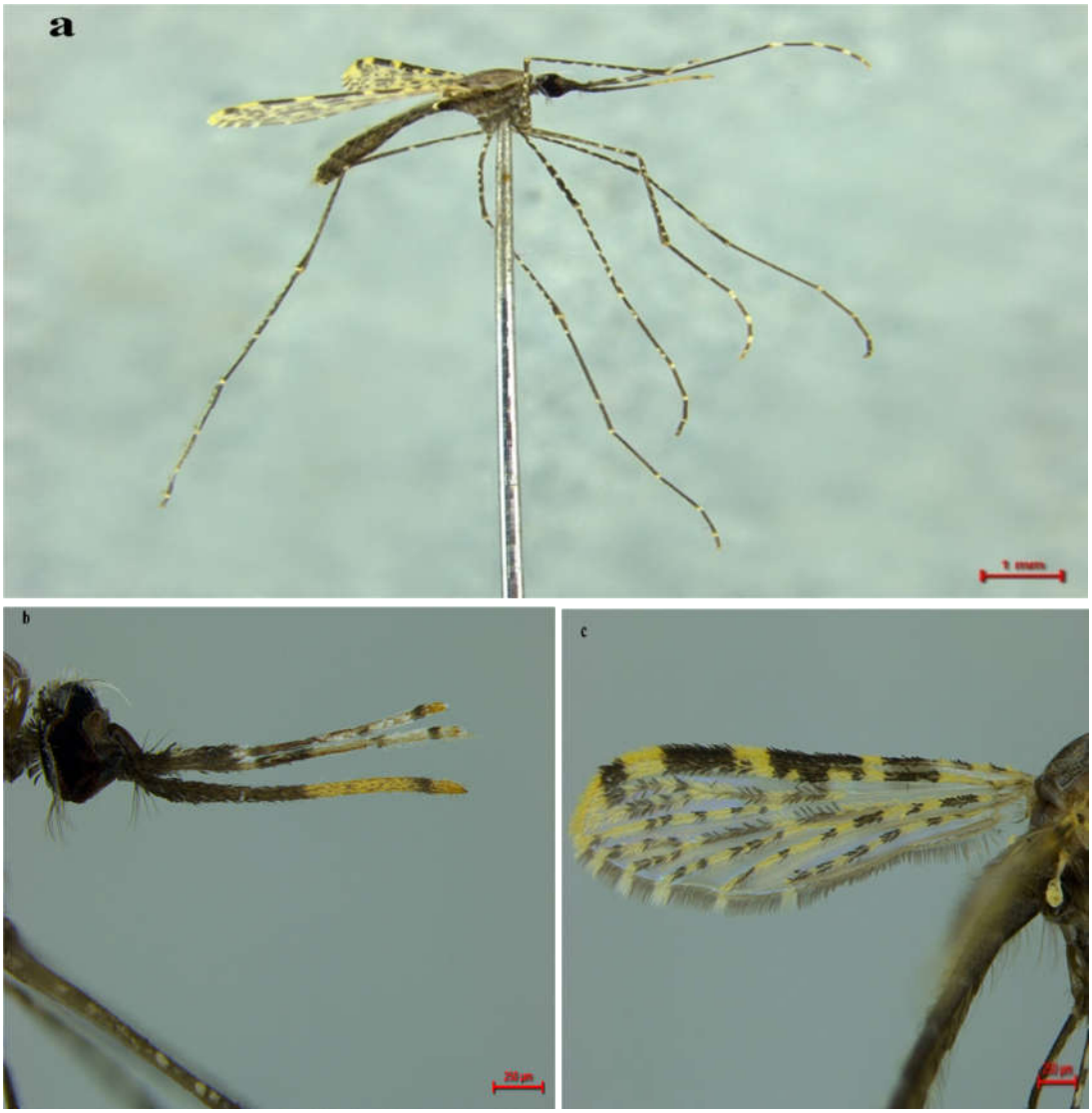


Fig 18 (a-e): a- Female *Anopheles stephensi*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 19

Fig 19 (a-e): a- Female *Anopheles theobaldi*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 20

Fig 20 (a-d): a-Female *Anopheles karwari*, **b**- Female palpi, **c**- Hind leg, **d**- Abdomen

PLATE 21

Fig 21 (a-e): a- Female *Anopheles jamesii*, **b**- Female palpi, **c**- Wing, **d**- Abdome **e**- Hind leg

PLATE 22

Fig 22 (a-e): a- Female *Anopheles splendidus*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

PLATE 23

Fig 23 (a-e) a- Female *An. tesellatus*, **b**- Female palpi, **c**-Wing, **d**- Hind leg

PLATE 24

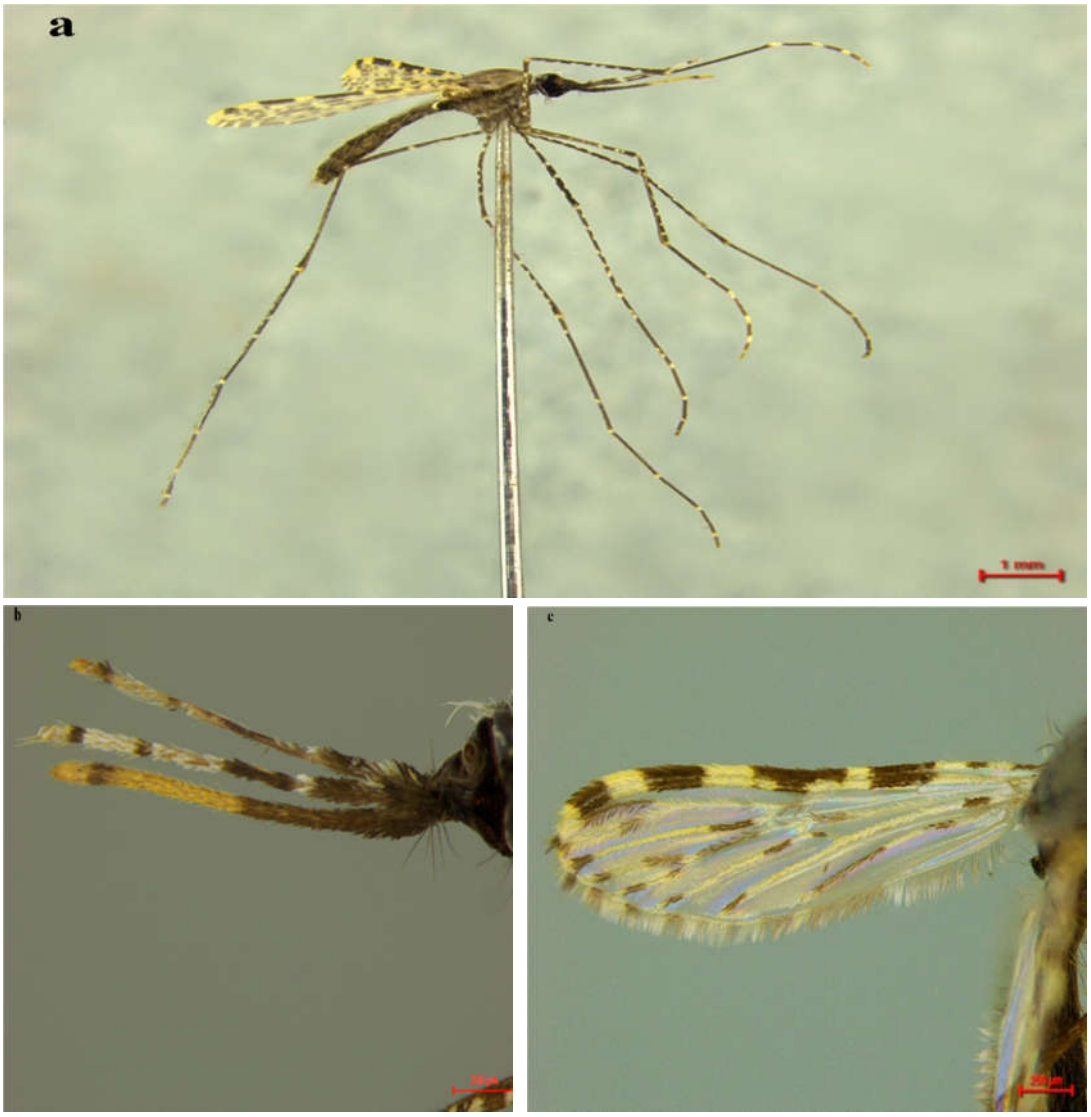


Fig. 24 (a-c). *Anopheles kochi*. a- female, b- Palpi, c- Wing

PLATE 25

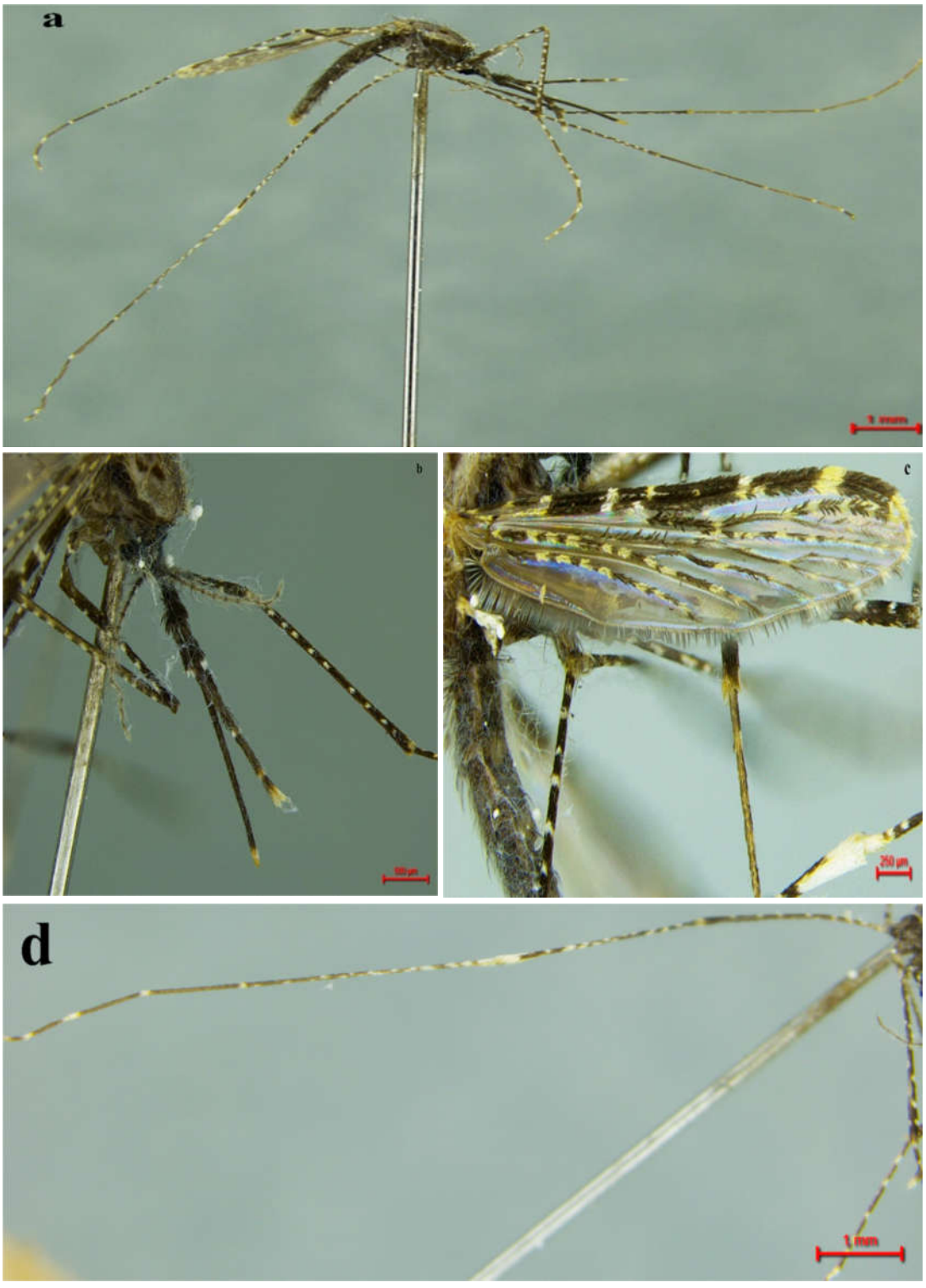


Fig 25 (a-e): a- Female *Anopheles dirus*, **b**- Female palpi, **c**- Wing, **d**- Hind leg

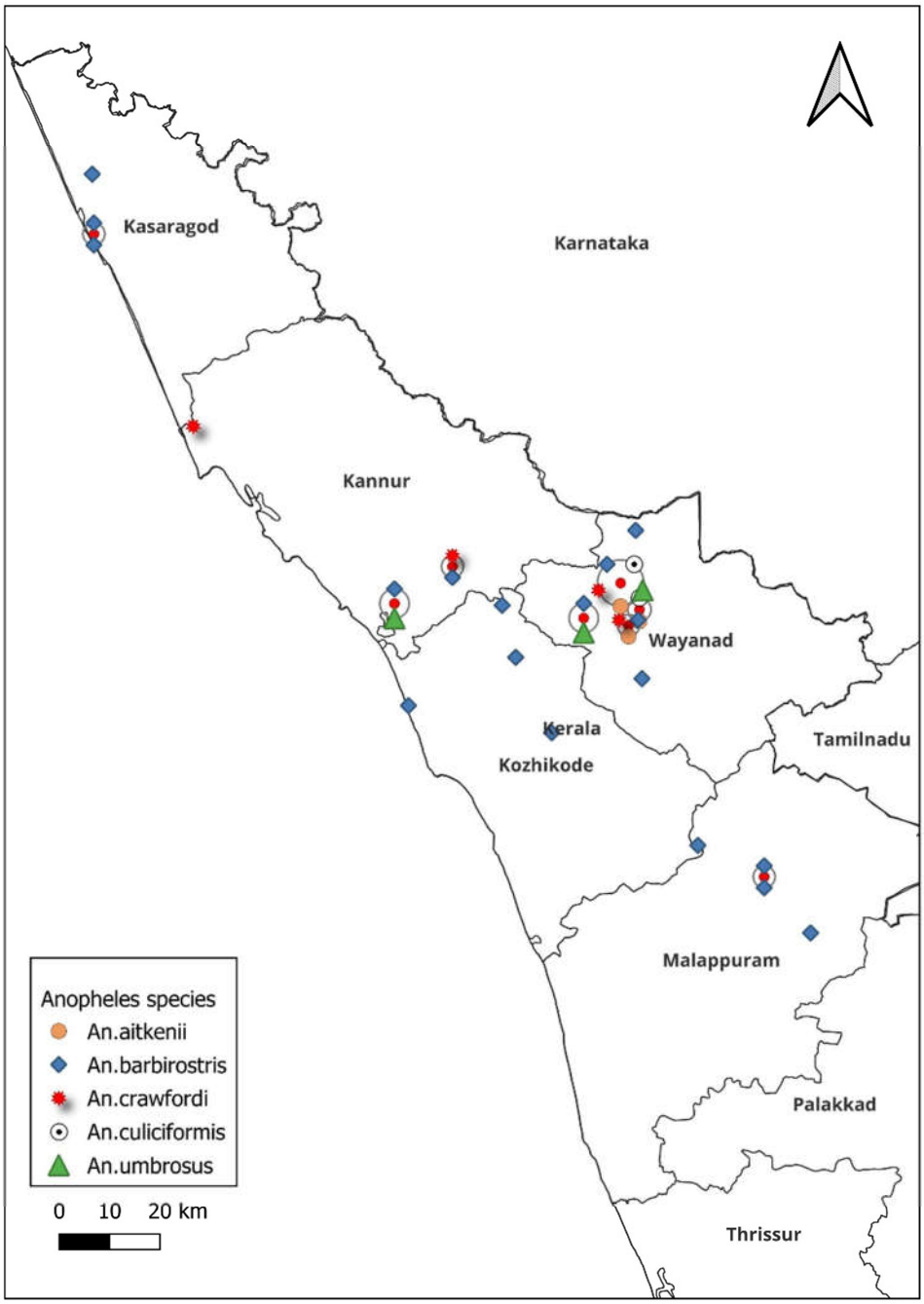
PLATE 26

Fig 26 (a-e): a-Female *An.elegans*, **b-** Female palpi, **c**-Wing, **d-** Hind leg

Distribution map of *Anopheles* in North Kerala

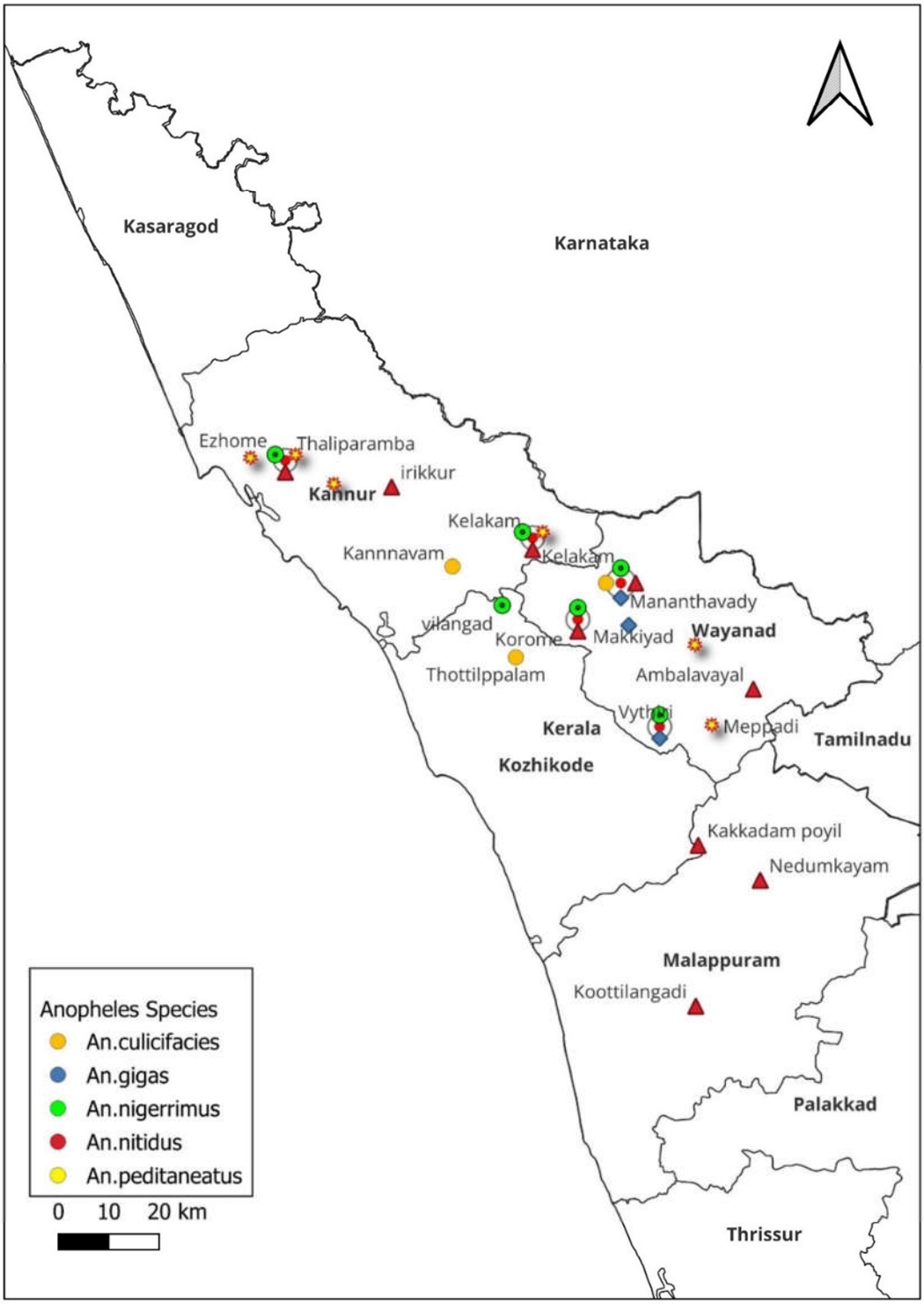
MAP 1

Distribution of Anopheles Mosquitoes in North Kerala- map 1



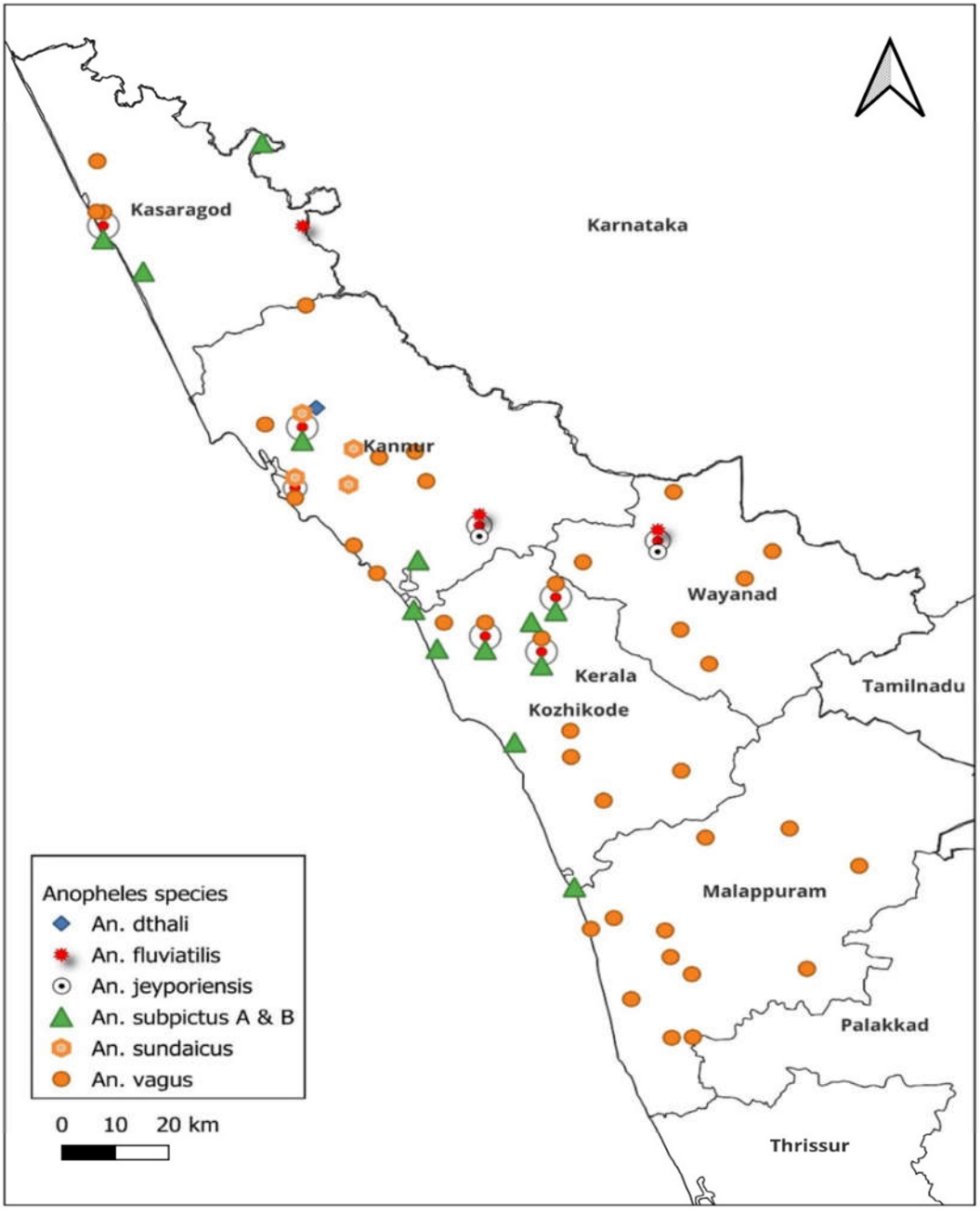
MAP 2

Distribution of Anopheles Mosquitoes in North Kerala - map 2



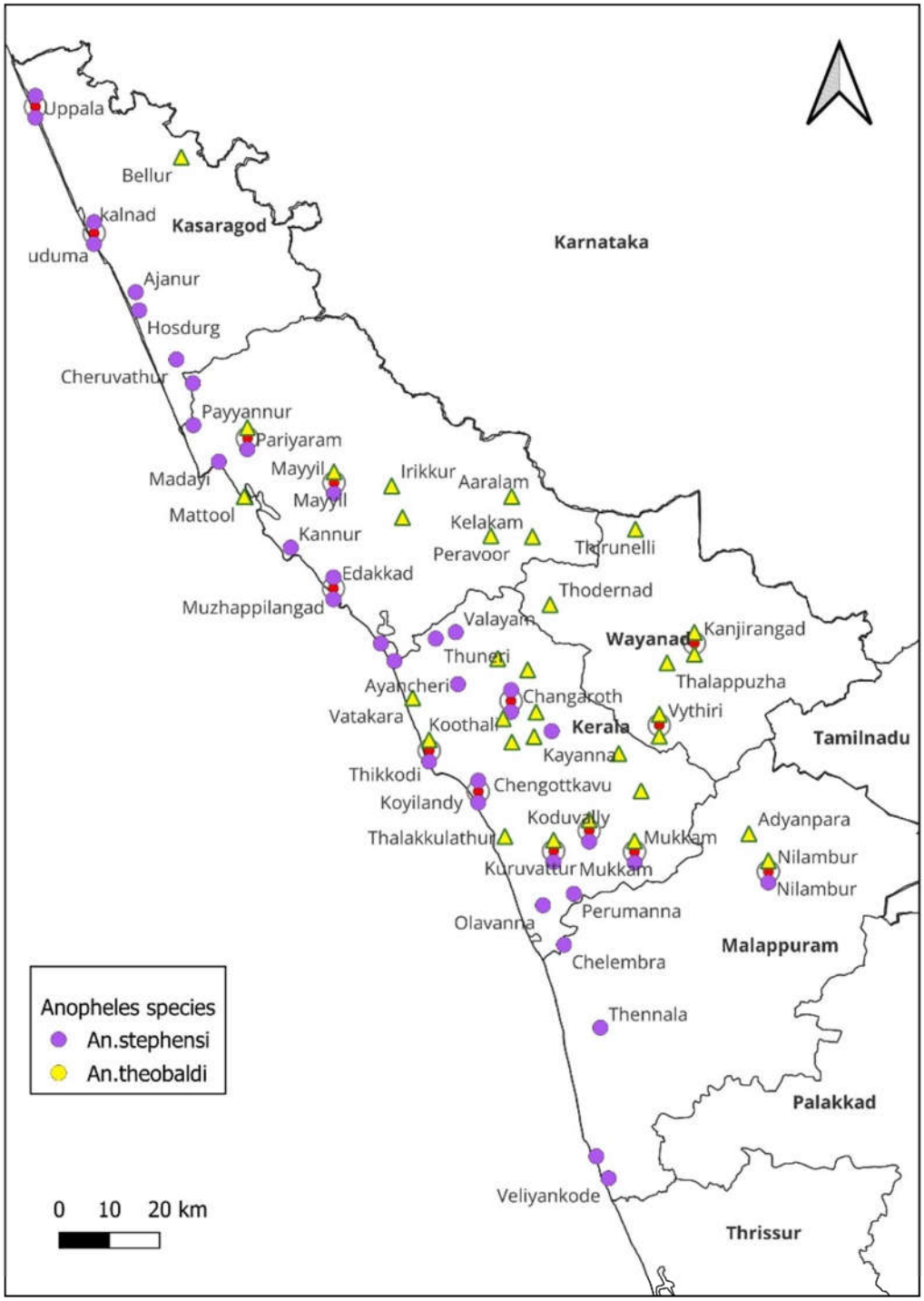
MAP 3

Distribution of Anopheles Mosquitoes in North Kerala- map 3



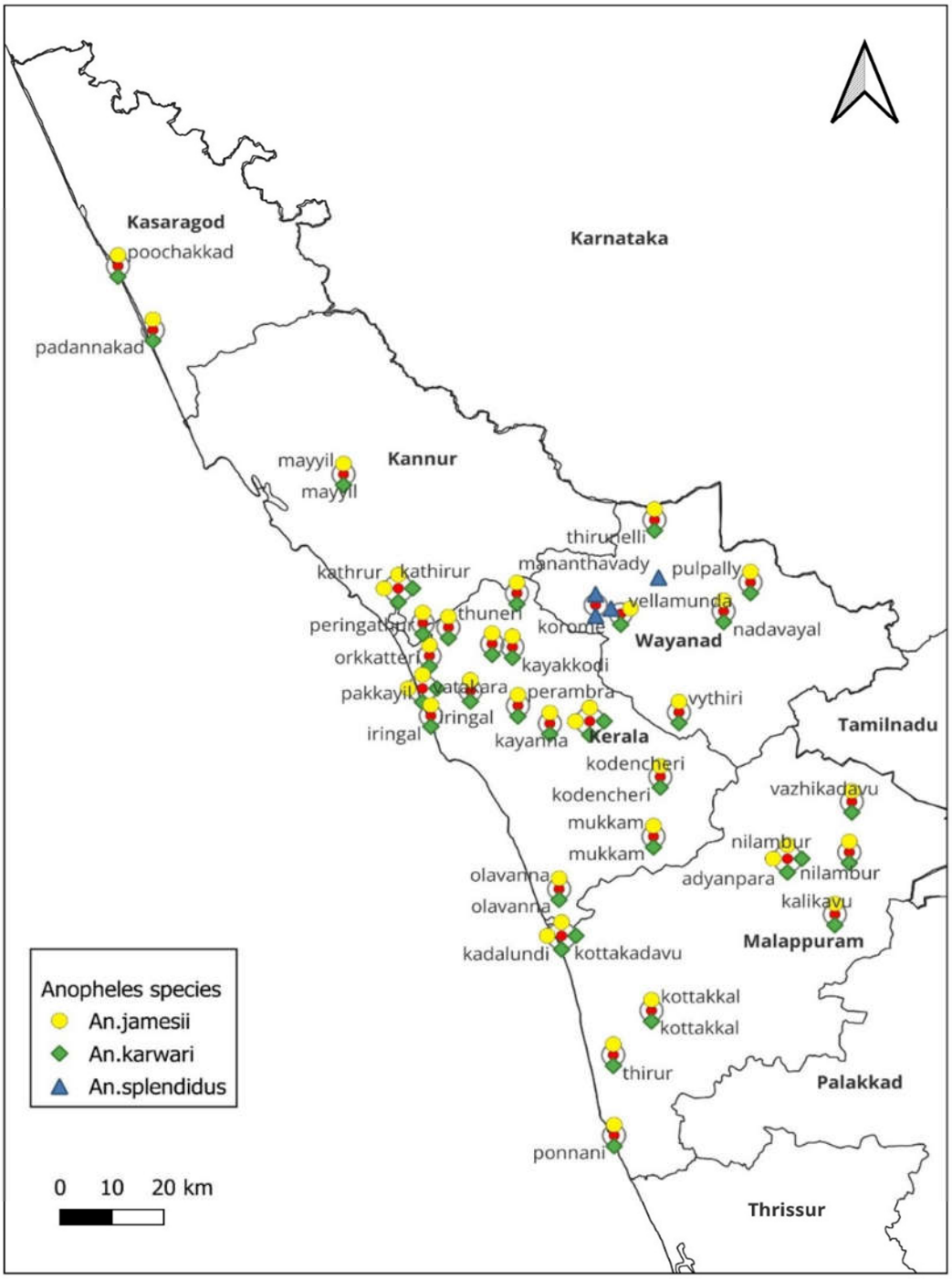
MAP 4

Distribution of Anopheles Mosquitoes in North Kerala - map 4



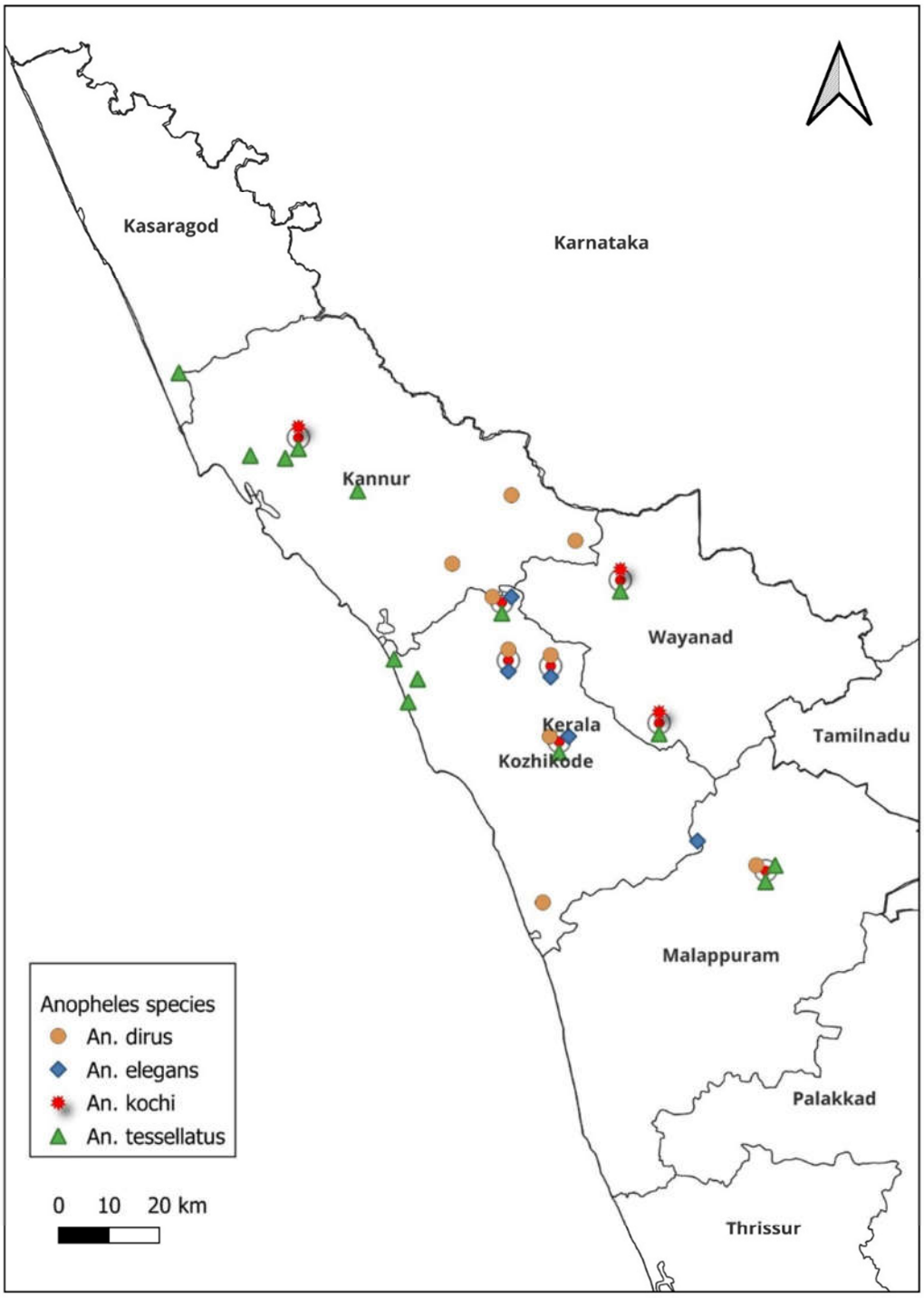
MAP 5

Distribution of Anopheles Mosquitoes in North Kerala- map 5



MAP 6

Distribution of Anopheles Mosquitoes in North Kerala- map 6



CHAPTER 5
DISCUSSION

Mosquitoes are an important group of insects responsible for the transmission of many medically important pathogens and parasites such as viruses, bacteria, nematodes, and protozoans which cause serious diseases like Malaria, Dengue, Yellow fever, Zika, West Nile Virus, and Lymphatic Filariasis (Kettle, 1995; Lehane, 1991; Dahmana and Mediannikov, 2020). The primary role that *Anopheles* mosquitoes play in the spread of human malaria is well-documented. Of the approximately 450 known species of *Anopheles*, about 40 are capable of transmitting human malaria parasites at high enough transmission rates to result in serious illness (MAP, 2019). Malaria in humans is caused by five Plasmodium species, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi*. The prominent vectors of malaria include *An. culicifacies, An. fluviatilis, An.stephensi, An.philippinensis, An. dirus, An. minimus, An. annularis, An. sundaicus, An.aconitus, An.maculatus, An. tessellatus and An.subpictus* (Rao,1981). Among these vectors the principal vectors in India are *An. culicifacies* (rural vector), *An. stephensi* (urban vector), *An. fluviatilis* (foothill vector), *An. dirus and An. minimus* (vectors in Northeastern India), and *An. sundaicus* (vector in Andaman and Nicobar Islands).

Many researchers have so far studied the mosquito fauna of Kerala. In 1901, Giles and Theobald, two taxonomists from England, independently began the first investigations on the mosquito fauna of Kerala (Sumodan, 2024). Currently, mosquito fauna of Kerala consists of 151 species under 18 genera. The present study dealt with the morphological and molecular identification of *Anopheles* mosquitoes distributed in different localities of five districts of North Kerala. Several studies have been conducted concerning the diversity of mosquitoes in various parts of Kerala. All these studies were limited to one or two districts or particular localities of the state only (Rajavel *et al*., studied mosquitoes in Kannur District, Balasubramanian and Nikhil, 2013 studied mosquitoes of Alappuzha and Kottayam,

Radhakrishnan, 2018 studied mosquitoes present in Ernakulam District). However, these studies were focused on mosquito fauna as a whole. The present study concentrated on the genus *Anopheles* of five districts of North Kerala viz., Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram. No comprehensive surveys and documentation of the genus have been undertaken in these areas in the past. This study also focused on the resolution of vector species complexes of malaria using molecular techniques.

5.1 Diversity of *Anopheles* fauna in North Kerala

Anopheles fauna of the state consists of around 39 species including primary (5) and secondary vectors (3) of malaria (Sumodan, 2024). In the present study, 27 species of the genus *Anopheles* belonging to the subgenera *Anopheles* and *Cellia* were recorded from the study districts mentioned above. Out of these 10 species belonged to the subgenus *Anopheles,* and 17 species to the subgenus *Cellia.* Of the 27 species, one species is a new record from Kerala. Besides, there were several new records from individual districts. From Kasaragod district, *An. fluviatilis,* and *An. theobaldi* were reported for the first time. From Kannur district six species of *Anopheles* were reported for the first time viz., *An. crawfordi, An. nigerrimus, An. nitidus, An. sinensis, An. theobaldi, An. dirus. Anopheles subpictus* B, the most potent malaria vector was reported from Kozhikode district for the first time. Compared to other districts the majority of new records was from Wayanad. This study recorderd *An. umbrosus, An. gigas, An. nigerrimus, An. nitidus, An. sinensis, An. dthali, An. theobaldi, An. splendidus* for the first time from Wayanad district. From Malappuram, the study reported *An. nigerrimus, An. nitidus, An. sinensis, An. theobaldi,*and *An. dirus* for the first time. The present study also did the redescription of 27 species that had been collected. This study also provided a dichotomous Key of the genus *Anopheles* of Kerala. Among the 27 species, some species such as, *An. fluviatilis, An. theobaldi, An. nigerrimus, An. nitidus, An. sinensis, An. splendidus* were already reported from different parts of Kerala (Mariappan *et al*., 1997; Arunachalam *et al*., 2004; Balasubramanian and Nikhil, 2013; Radhakrishnan, 2018). The present study detected six primary vectors of

Malaria from the study area: *An. sundaicus* (New report from Kerala), *An. dirus. An. culicifacies, An. fluviatilis, An.stephensi and An.subpictus* B (First record from Kerala).

The species *Anopheles sundaicus* Rodenwaldt, 1925 from subgenus *Cellia* was reported from the state for the first time. In India, this species acts as the principal vector of Malaria and it was last recorded in the Parganas district of West Bengal in 1974-75. Now the species is prevalent only in the Andaman and Nicobar Islands (Subbarao *et al*., 2019). The specimens were collected from Thaliparamba in Kannur district. Morphologically this species was identified as *An. sundaicus* using a taxonomic key (Das *et al.,*1990) but could not be confirmed molecularly due to paucity of fresh specimens. *Anopheles sundaicus* preferably breeds in coastal habitats, but we collected this species from freshwater habitats of salinity >1.00 ppt. This species is known for transmitting *Plasmodium vivax* and *Plasmodium falciparum.*

Anopheles subpictus Grassi was collected from different habitats in Kozhikode, Kannur, and Kasaragod districts and breeds in freshwater as well as brackish water. Four sibling species of *Anopheles subpictus* are A, B, C, and D. *An. subpictus* B is a vector of Malaria mostly in coastal areas of India and Sri Lanka (Panicker *et al*., 1981; Amerasinghe *et al*., 1992, Dahmana and Mediannikov, 2020). In our study, we detected the presence of larvae of *Anopheles subpictus* B in brackish water of salinity 28-32 ppt. This species primarily transmits *Plasmodium falciparum* and *Plasmodium vivax.* Its adaptability to various breeding sites makes it a persistent threat in endemic regions.

Anopheles fluviatilis James was collected from slow-running streams with aquatic vegetation in the hilly area of the Kasaragod district, Ranipuram. This study morphologically confirmed the species as *An. fluviatilis* but was not able to carry out the molecular studies due to a lack of fresh specimens. The presence of this species in Kerala underscores the need for targeted malaria control programs, particularly in rural and forested regions. Effective management of this vector is essential to reduce malaria transmission and improve public health outcomes.

Anopheles stephensi Liston, the primary vector of malaria in urban areas was collected from Kasaragod, Kannur, Kozhikode, and Malappuram districts. Unlike other malaria vectors that prefer rural settings, *Anopheles stephensi* breeds in urban areas, exploiting man-made water containers for larval development. The presence of this vector increases the risk of urban malaria outbreaks, necessitating enhanced surveillance and control measures.

Anopheles dirus Python & Harrison, another prominent vector of malaria, has also been identified in Kerala and is known for its efficiency in transmitting malaria, especially in forested and rural areas. The present study reported this species from shallow and shaded parts of streams in forested areas of Kozhikode and Kannur Districts. The presence of this species in North Kerala contributes to the risk of Malaria outbreaks, especially in areas close to forests.

Anopheles culicifacies Giles, is a significant malaria vector in Kerala, with diverse genetic populations. The present study reported this species from the Kozhikode and Wayanad districts.

Anopheles dthali belonging to subgenus *Cellia* reported from Kerala for the first time from Wayanad District. It is a secondary vector of malaria in southern parts of Iran but not a vector in India (Nagpal and Sharma, 1995).

Anopheles gigas species belonging to the subgenus *Anopheles* was also reported from the study area for the first time.

The following species reported earlier from the study districts could not be detected in the present study. This is probably due to the fact that, instead of adult collection the major method of collection resorted to in this study was larval collection. The reason for preferring larval collection was to document the larval habitats, which is essential for implementing non-chemical strategies of mosquito control.

1. *Anopheles aconitus* Doenitz, 1902
2. *Anopheles annadalei* Prashad, 1918

3. *Anopheles annularis* Van der Wulp, 1884
4. *Anopheles insulaeflorum* (Swell. & Swell.), 1919
5. *Anopheles interruptus* Puri, 1929
6. *Anopheles leucosphyrus* Donitz, 1901
7. *Anopheles lindesayi* Giles, 1900
8. *Anopheles maculatus* Theobald, 1901
9. *Anopheles majidi* Young &Majid, 1928
10. *Anopheles minimus* Theobald, 1901
11. *Anopheles pallidus* Theobald, 1901
12. *Anopheles philippinensis* Ludlow, 1902
13. *Anopheles psuedosundaicus* Tyagi *et al*., 2009
14. *Anopheles pseudowillmori* Theobald, 1910
15. *Anopheles varuna* Iyengar, 1921
16. *Anopheles sintoni* Puri,1929
17. *Anopheles moghulensis* Christophers, 1924

5.2 Malaria Situation in the study area

From 2019 to 2023, Kerala experienced a fluctuating number of malaria cases, with most infections being imported from other states, largely due to an influx of migrant workers. There was a moderate range (17-70) of Malaria attacks in Kasaragod district. An average range of 15-62 cases of malaria was reported in Kannur district during 2019- 2023 and this includes both indigenous and imported cases. In Wayanad, comparatively low incidence of malaria was reported from 2019 to 2023 and the range was 5-15 cases. Compared to other North Kerala districts Kozhikode and Malappuram districts reported high rate of Malaria incidence. Kozhikode reported 19- 79 cases and Malappuram 25-66 cases (Source: Directorate of Health Sciences, Kerala).

5.3 Phylogenetic study

Molecular analysis of four malarial vector species, *Anopheles culicifacies, Anopheles dirus, Anopheles stephensi,* and *Anopheles subpictus* was conducted in the present study. Through molecular analysis techniques, the *An. culicificies* species complex was resolved. The complex is a group of five species, viz., A, B, C, D, and E. The species prevalent in the study area was confirmed as *Anopheles culicifacies* B. This was the first time such a study was done in Kerala. *Anopheles dirus* belongs to the *An. dirus* complex, having seven species, viz., A, B, C, D, E, and F. In the present study, it was confirmed that the species prevalent in the study area is *Anopheles dirus* D. *Anopheles stephensi*, the urban vector of malaria in Kerala was found to have its presence in all districts in the study area except Wayanad. Molecular study was conducted on this species and the sequences was deposited in Gen Bank. *Anopheles subpictus* complex consists of 2 subspecies A and B. Morphologically both species are almost identical. By analyzing the sequence molecularly, this study confirmed the presence of two species of *Anopheles subpictus* species viz., A and B from Kerala. The presence of *Anopheles subpictus* B is a new information for the entire state. Since this is a vector species, this has an added significance in the Malaria epidemiology of the state.

5.4 Significance of the study

Besides documenting the Anopheline fauna from the study area, this study is also significant in relation to Public Health of the study area. As discussed above, Anophelines are the vectors of Malaria. Though the number of malaria cases being reported is low in the study districts, the presence of six primary vectors is a matter of worry. Every district was found to have at least one primary vector. In a 2002 study, the presence of *An. culicifacies* in very low density was found sufficient to transmit malaria from an imported malaria patient to a local resident in Wayanad (Sumodan, 2002). This is evidence for the importance of the presence of primary malaria vectors in a scenario of imported malaria cases. Imported malaria could trigger malaria outbreaks in non-malarious areas in the presence of vectors. This makes the faunal study of Anophelines significant in the state.

CHAPTER 6
SUMMARY AND CONCLUSION

The study was undertaken on the Morphological and Molecular systematics of Anopheles mosquitoes of North Kerala (Diptera: Culicidae: *Anophelinae*).

The investigation on the *Anopheles* fauna was carried out for a period of 5 years (2019-2023) in different areas of Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram districts and collected a total of 15,845 specimens belonging to the genus *Anopheles.* A total of 27 species from two subgenera, viz., *Anopheles* and *Cellia* were recorded from the study as listed below:

Anopheles aitkenii James, 1903

Anopheles umbrosus (Theobald), 1903

Anopheles culiciformis Cogill, 1903

Anopheles barbirostris Van der Wulp, 1884

Anopheles crawfordi Reid, 1953

Anopheles peditaeniatus Leicester, 1908

Anopheles gigas Giles, 1901

Anopheles nigerrimus Giles, 1900

Anopheles nitidus, Harrison, Scanlon & Reid, 1973

Anopheles sinensis (Weidemann), 1828

Anopheles culicifacies Giles, 1901

Anopheles jeyporiensis James, 1902

Anopheles fluviatilis James, 1902

Anopheles dthali Patton, 1905

Anopheles subpictus A & B Grassi, 1899

Anopheles subpictus B

Anopheles vagus Donitz, 1902

Anopheles sundaicus Rodenwaldt, 1925

Anopheles stephensi Liston, 1901

Anopheles theobaldi Giles, 1901

Anopheles karwari James, 1903

Anopheles Jamesii Theobald, 1901

Anopheles splendidus Koidzumi, 1920

Anopheles kochi Doenitz, 1901

Anopheles tessellatus Theobald, 1901

Anopheles dirus Python & Harrison, 1979

Anopheles elegans James, 1903

Among the collected species, 6 species act as primary vectors of Malaria *An. sundaicus* (New report from Kerala), *An. dirus. An. culicifacies, An. fluviatilis, An.stephensi and An.subpictus* B. The present study conducted molecular analyses of 4 vector species and resolved their molecular complexes.

1. *Anopheles subpictus*- resolved as *Anopheles subpictus* A and B
2. *Anopheles dirus*- resolves as species D
3. *Anopheles culicifacies*- resolved as Species B
4. *Anopheles stephensi*- urban malaria vector

Malaria, one of the most dreaded mosquito-borne diseases in the world has been staging a comeback in Kerala recently. This study provided in-depth information on the *Anopheles* fauna of North Kerala. Hence, this information will be helpful for the vector control programmes of the state and can manage current and future outbreaks of the disease in the state.

New state record of the present study

Anopheles sundaicus

Anopheles subpictus B

New District records of the present study

Kasaragod- *An. fluviatilis, An. theobaldi*

Kannur- *An. theobaldi, An. crawfordi, An. nigerrimus, An. nitidus, An. sinensis, An. Dirus, & An. sundaicus.*

Kozhikode- *Anopheles subpictus* B

Wayanad- *An. umbrosus, An. gigas, An. nigerrimus, An. nitidus, An. sinensis, An. dthali, An. theobaldi, An. splendidus*

Malappuram- *An. nigerrimus, An. nitidus, An. sinensis, An. theobaldi, An. dirus*

CHAPTER 7
RECOMMENDATIONS

The present study recorded and redescribed the 27 *Anopheles* species from five North Kerala districts (Kasaragod, Kannur, Wayanad, Kozhikode, and Malappuram). This study reported *Anopheles subpictus* B and *Anopheles sundaicus*, the primary vectors of Malaria from Kerala for the first time. Among the 27 species, six species were identified as primary vectors of Malaria. This study also revealed the molecular characterization of these vector species and sequences were deposited in Gen Bank.

The study of *Anopheles* mosquitoes in Kerala is crucial due to the significant role these mosquitoes play in malaria transmission. Accurately identifying and mapping malarial vector species is vital for malarial control programs. Studying the distribution of *Anopheles* species across different ecological zones in Kerala helps identify high-risk areas and deploy resources efficiently. In the present study molecular characterization of vector species was also performed. So, the present study is limited to North Kerala districts only. Similar studies in other districts of Kerala are recommended. Though the presence of *An. sundaicus* recorded from two localities in Kannur district, its molecular identity was not confirmed due to paucity of specimens. Hence, intensive collections from all over Kerala needs to be undertaken to study its distribution and also to reveal its molecular identity.

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