MORPHOLOGICAL CHARACTERISATION OF CEPHALIC SENSILLA WITH RESPECT TO MAMMALIAN HOST PREFERENCES IN MOSQUITOES FROM SELECTED AREAS OF WAYANAD DISTRICT, 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

By

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Under the supervision of

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CERTIFICATE

This is to certify that the thesis entitled "**MORPHOLOGICAL CHARACTERISATION OF CEPHALIC SENSILLA WITH RESPECT TO MAMMALIAN HOST PREFERENCES IN MOSQUITOES FROM SELECTED AREAS OF WAYANAD DISTRICT, KERALA**" submitted to the University of Calicut for the award of the degreeof Doctor of Philosophy in Zoology under the Faculty of Science, is the record of the original work done by **Ms. MAIBY THANKACHAN** in the Molecular Biology Laboratory, Departmentof Zoology under my supervision and guidance, and that it has not formed the basis for the award of any degree / diploma or other similar title to any candidate of any University.

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This is certify that all corrections/suggestions recommended by the adjudicators in the Ph.D. thesis of **Ms. MAIBY THANKACHAN** entitled **"MORPHOLOGICAL CHARACTERISATION OF CEPHALIC SENSILLA WITH RESPECT TO MAMMALIAN HOST PREFERENCES IN MOSQUITOES FROM SELECTED AREAS OF WAYANAD DISTRICT, KERALA"** have been duly incorporated and the contents in the thesis and the soft copy are one and the same.

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DECLARATION

I hereby declare that the work presented in the thesis entitled "**MORPHOLOGICAL CHARACTERISATION OF CEPHALIC SENSILLA WITH RESPECT TO MAMMALIAN HOST PREFERENCES IN MOSQUITOES FROM SELECTED AREAS OF WAYANAD DISTRICT, KERALA**" is based on the original work done by me under the guidance of Dr. Sebastian C. D., Professor, Division of Molecular Biology, Department of Zoology, University of Calicut and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are 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.

Calicut University
14. 02. 2024

Maiby Thankachan

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ABSTRACT

Insects are the most diversified group in the history of life. Among the various insect groups, mosquitoes deserve special mention with significant ecological and public health importance. Mosquito-borne diseases are among the world's leading causes of illness and death. According to the World Health Organization, more than 300 million clinical cases of mosquito-borne illnesses report each year. In order to understand the vector dynamics and its disease transmission potentiality, it is indispensable to study certain fitness components related to the vector globally. Therefore, understanding the mosquito diversity and exploiting the proximate mechanisms of host location in mosquitoes will help to reduce their interaction with human hosts and the management of the transmission of infectious diseases.

The variety of mosquito species, particularly those that serve as vectors, is extremely high in Kerala. Here, the main mosquito-borne disease vectors that are abundant throughout the state are widely dispersed. Therefore, comprehensive information of the prevalence, distribution and biology of mosquitoes in the state would be useful for managing both present disease outbreaks and potential future outbreaks of diseases that are not now common in the state. The study area selected for the analysis was Mananthavady Taluk of Wayanad district, Kerala which is rich with different types of plantations, forest and urban and rural areas. The present work can be considered as a pioneer study from this area.

During the study period (2019-2022), a total of 80 species of mosquitoes belonging to 12 genera in 6 tribes and 2 subfamilies were collected and identified based on taxonomic keys. The 29 mosquito species among the collected specimens are mosquito vectors belonging to five genera namely *Aedes, Culex, Anopheles, Mansonia* and *Armigeres*. 21 species of mosquitoes and two genera namely *Malaya* and *Lutzia* are new reports to Kerala. The taxonomy of mosquitoes has been enhanced by two new records (*Uranotaenia* sp.) from the region.

35 mosquito species coming under 7 genera were barcoded using marker genes. The mitochondrial cytochrome oxidase I (CO I) gene sequences obtained were deposited in NCBI GenBank for worldwide accession with respective accession numbers

Molecular Phylogeny of Subfamilies Anophelinae and Culicinae were discussed with the construction of Maximum likelihood tree. 44 species (29 vector species and 15 non vector species) of mosquitoes from five genera namely Aedes, *Culex, Anopheles, Armigeres* and *Mansonia* were chosen for blood meal analysis, and their host preferences were discussed. Considering that the sensillae are very important for mosquitoes for the host selection, morphological studies based on the sensillae of antennae, maxillary palps and proboscis of 19 blood feeding female mosquitoes and one non blood feeding female mosquitoe *(Mal.genurostris*) were conducted along with their host preferences. The findings of the study are highly significant for the future references and for designing effective prevention and control strategies to mitigate the public health impact of mosquito-borne diseases.

സംഗ്രഹം

ലോകത്തിലെ തന്നെ ഏറ്റവും വൈവിധ്യമാർന്ന ജീവിവർഗമാണ് ഷഡ്പദങ്ങൾ. ഷഡ്പദങ്ങളുടെ വൈവിധ്യതയിൽ പാരിസ്ഥിതികമായും പൊതുജനാരോഗ്യപര മായും ഏറെ പ്രാധാന്യമുള്ള വർഗമാണ് കൊതുകുകൾ. കൊതുകു പരത്തുന്ന രോഗങ്ങളാണ് ലോകത്തിൽ ഏറ്റവും കൂടുതൽ മരണങ്ങൾക്ക് കാരണമാകുന്നത്. ലോകാരോഗ്യ സംഘടനയുടെ കണക്കനുസരിച്ച്, ഓരോ വർഷവും 300 ദശലക്ഷത്തിലധികം കൊതുകുജന്യ രോഗങ്ങൾ റിപ്പോർട്ട് ചെയ്യപ്പെടുന്നു.

രോഗവാഹകരുടെ ഗതിവിജ്ഞാനീയം മനസ്സിലാക്കുവാനും രോഗവ്യാപന സാധ്യത മനസ്സിലാക്കുവാനും, രോഗവാഹകരായ ഷഡ്പദങ്ങളുടെ ചില സ്ഥിതിവിവര ഘടകങ്ങൾ പഠിക്കേണ്ടത് അനിവാര്യമാണ്. അതായത് കൊതുകുകളുടെ വൈവിധ്യം മനസ്സിലാക്കുന്നതു വഴിയും കൊതുകുകളുടെ ആതിഥേയജീവികളുമായുള്ള സമീപന രീതികൾ മനസ്സിലാക്കുന്നത് വഴിയും മനുഷ്യ ആതിഥേയരുമായുള്ള അവയുടെ ഇടപഴകൽ കുറയ്ക്കുവാനും പകർച്ചവ്യാധികൾ പകരുന്നത് നിയന്ത്രിക്കുവാനും സാധിക്കും.

കൊതുകുകളുടെ വൈവിധ്യം, പ്രത്യേകിച്ച് രോഗവാഹികളായി പ്രവർത്തിക്കുന്നവ, കേരളത്തിൽ വളരെ കൂടുതലാണ്. അതിനാൽ, സംസ്ഥാനത്തെ കൊതുകുകളുടെ വ്യാപനം, ജീവശാസ്ത്രം എന്നിവയെക്കുറിച്ചുള്ള സമഗ്രമായ വിവരങ്ങൾ ശേഖരിക്കുന്ന തിലൂടെ, സംസ്ഥാനത്ത് ഇപ്പോഴുള്ള രോഗബാധകളും ഭാവിയിൽ പടർന്നുപിടിക്കാൻ സാധ്യതയുള്ള രോഗങ്ങളും ഒരു പരിധി വരെ നിയന്ത്രിക്കാൻ സാധിക്കും.

വിവിധതരം തോട്ടങ്ങൾ, വനം, നഗര-ഗ്രാമ പ്രദേശങ്ങൾ എന്നിവയാൽ സമ്പന്നമായ കേരളത്തിലെ വയനാട് ജില്ലയിലെ മാനന്തവാടി താലൂക്കാണ് വിശകലനത്തിനായി തിരഞ്ഞെടുത്ത പഠനമേഖല. പ്രസ്തുത മേഖലയിൽ ഈ രീതിയിൽ നടത്തപെടുന്ന ആദ്യത്തെ പഠനം കൂടിയാണ് ഇത്.

2019 മുതൽ 2022 വരെയുള്ള പഠന കാലയളവിൽ, 6 ഗോത്രങ്ങളിലെ 2 ഉപകുടുംബങ്ങളിലുമായി 12 ജനുസ്സുകളിൽ മൊത്തം 80 ഇനം കൊതുകുകളെ ടാക്സോണമിക് കീകളുടെ അടിസ്ഥാനത്തിൽ ശേഖരിക്കുകയും തിരിച്ചറിയുകയും ചെയ്തു. ശേഖരിച്ചവയിൽ 29 ഇനം കൊതുകുകൾ, ഈഡിസ്, കുലക്സ്, അനോഫിലിസ്, മൻസോണിയ, ആർമിഗെറസ് എന്നീ അഞ്ച് ജനുസ്സുകളിൽ പെട്ട

രോഗവാഹകരായ കൊതുകുകൾ ആയിരുന്നു. മലയ, ലുറ്റ്സിയ എന്നീ രണ്ട് ഇനങ്ങളും 15 മറ്റ ഇനങ്ങളും കേരളത്തിൽ നിന്നുള്ള പുതിയ റിപ്പോർട്ടുകളാണ്. പ്രദേശത്ത് നിന്ന് രണ്ട് പുതിയ റെക്കോർഡുകൾ (യുറനോട്ടേനിയ സ്പീഷീസ്.) കൂടി കണ്ടെത്തപ്പെട്ടു.

7 ജനുസ്സുകളിൽ പെടുന്ന 35 ഇനം കൊതുകുകളുടെ തന്മാത്രാതല വർഗ്ഗീകരണവും ഈ പഠനത്തിലൂടെ സാധ്യമായി. ഇവയുടെ മൈറ്റോകോൺഡ്രിയൽ സൈറ്റോക്രോം ഓക്സിഡേസ് സബ്യൂണിറ്റ് I (COI) ജീൻ ശ്രേണികൾ തന്മാത്രാതല പഠനങ്ങളുടെ ആഗോള ഡാറ്റാബേസ് ആയ എൻ. സി. ബി. ഐ - ജിൻബാങ്കിൽ സമർപ്പിച്ചു.

ഉപകുടുംബങ്ങളായ അനോഫെലിനേ, കുലിസിനേ എന്നിവയുടെ തന്മാത്രാതല വംശജനിതക വിശ്ലേഷണവും (Molecular Phylogeny) പഠനവിധേയമാക്കി. ഇതിനായി 'മാക്സിമം ലൈക്‌ലിഹുഡ്' എന്ന സാംഖ്യിക അനുമാന രീതി (statistical analysis) അവലംബിക്കുകയുണ്ടായി.

ഈഡിസ്, കൃലക്സ്, അനോഫിലിസ്, ആർമിഗെറസ്, മാൻസോണിയ എന്നീ അഞ്ച് ജനുസ്സുകളിൽ നിന്നുള്ള 44 ഇനം കൊതുകുകളെ അവയുടെ രക്ത-ഭക്ഷണ വിശകലനത്തിനായി തിരഞ്ഞെടുത്തു. അവയിൽ തന്നെ 29 ഇനം രോഗവാഹകരും 15 ഇനം രോഗവാഹകർ അല്ലാത്തവയും ആയിരുന്നു. ഓരോ ഇനവും ഏത് ആതിഥേയജീവിയോടാണ് കൂടുതൽ ഇടപഴകുന്നത് എന്നതനുസരിച്ചു ആതിഥേയ-മുൻഗണനാ ക്രമവും വിശകലനത്തിൽ ഉൾപ്പെടുത്തി.

ആതിഥേയ തിരഞ്ഞെടുപ്പിന് കൊതുകുകൾക്ക് സെൻസില വളരെ പ്രധാനമാണ് എന്നതിനാൽ, 19 രക്തം ഭക്ഷിക്കുന്ന പെൺകൊതുകുകളുടെയും രക്തം ഭക്ഷണമാക്കാത്ത ഒരു പെൺകൊതുകിന്റെയും (*Mal.genurostris*) ആന്റിനയുടെ സെൻസില, മാക്സില്ലറി പാൽപ്പ്, പ്രോബോസ്സിസ് എന്നിവയുടെ രൂപശാസ്ത്ര പഠനങ്ങളും ഈ ഗവേഷണത്തിലൂടെ സാധ്യമായി.

പഠനത്തിന്റെ കണ്ടെത്തലുകൾ കൊതുകുജന്യരോഗങ്ങളെ കുറിച്ചുള്ള ഭാവി പഠനങ്ങൾക്കും കൊതുക് പരത്തുന്ന രോഗങ്ങളുടെ ആഘാതം ലഘൂകരിക്കുന്നതിനും ഫലപ്രദമായ പ്രതിരോധ നിയന്ത്രണ തന്ത്രങ്ങൾ രൂപപ്പെടുത്തുന്നതിനും വളരെ സഹായകമാവുന്നതാണ്.

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ABBREVIATIONS

An	-	*Anopheles*
Cx	-	*Culex*
Ae	-	*Aedes*
Man	-	*Mansonia*
Lt	-	*Lutzia*
Hz	-	*Heizmannia*
Ver	-	*Verrallina*
Tp	-	*Tripteroides*
Ar	-	*Armigeres*
Ur	-	*Uranotaenia*
Or	-	*Orthopodomyia*
Mal	-	*Malaya*
RC	-	Resting Collection
ML	-	Man landing
AIC	-	Akaike Information Criterion
BI	-	Bayesian Inference
BIC	-	Bayesian information criterion
BLAST	-	Basic Local Alignment Search Tool
BS	-	Bootstrap
CO1	-	Cytochrome Oxidase I
Cyt.B	-	Cytochrome B
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylene diamine tetra acetic acid
dNTPs	-	Deoxynucleotide triphosphates
INSDC	-	International Nucleotide Sequence Database
	-	Collaboration
K2P	-	Kimura 2 Parameter
MEGA X	-	Molecular Evolutionary Genetics Analysis-X
ML	-	Maximum Likelihood
NCBI	-	National Centre for Biotechnology Information
PCR	-	Polymerase Chain Reaction
ºC	-	Degree Celsius

ºE	-	Degree East
ºN	-	Degree North
RNA	-	ribosomal ribonucleic acid
sp.	-	species
SCh1	-	Long Sensilla Chaetica
SCh2	-	Short Sensilla Chaetica
Tr1	-	Long sharp trichoidea
Tr2	-	Short sharp trichoidea
Tr3	-	Long blunt trichoidea
Tr4	-	Short blunt trichoidea
SBa	-	Sensilla Basiconica
SCoS	-	Small Sensilla Coeloconica
SCoL	-	Large Sensilla Coeloconica
SAm	-	Sensilla Ampullacea
SCp	-	Sensilla Capitate peg
SCm	-	Sensilla Companiformia
SST	-	Sensilla Sharp Trichoidea
SBT	-	Sensilla Blunt Trichoidea
STr	-	Sensilla Trichoidea

CHAPTER 1
INTRODUCTION

Insects are the most diversified group in the history of life over 400 million years. The ecological importance, diversity and longevity of insects make them the most successful group of species in the history of the Earth. More than any other eukaryotic animal, insects have been a major factor in the spread of pandemic diseases. Insect-borne diseases have claimed the lives of millions of people throughout history.

Mosquitoes belongs to the family Culicidae, constitute a diverse group of insects with over 3700 described species. Despite their diminutive size, these blood feeding insects hold considerable ecological importance, participating in various ecosystems as both pollinators and prey for other organisms. However their notoriety predominantly stems from their role as vectors for numerous infectious diseases like malaria, Dengue fever, Chikungunia, Yellow fever, Filariasis, Japanese encephalitis and so on.

The term ‘Culicies’ was first used by Roman author Plinius Secundus (AD23-79). The term "mosquito" has Spanish or Portuguese origins. Previously, the term for a mosquito was a ‘gnat’ or ‘culex.’ ‘A Handbook of the Gnats or Mosquitoes’ written by Giles in 1900, contains the word mosquito. Mosquitoes were not given a great deal of thought until Ross made his discovery of the involvement of mosquitoes in the malaria cycle in 1990. *Burmaculex antiquus*, a fossil mosquito, was the oldest mosquito ever discovered. It was characterised based on a single adult female that was only partially preserved in Burnese amber (Talib, 2018).

1.1. Mosquitoes in general

Mosquitoes are a large family of insects prevalent throughout the world's temperate and tropical regions, as well as outside of the Arctic Circle (Harbach, 2007). They fall under the categories of Class Insecta, Order Diptera, Suborder Nematocera and Family Culicidae. Three pairs of legs, head, thorax and abdomen

distinguishes insects from other Eukaryotes. Due to the modification of hind wings into club-shaped halteres, Diptera may easily be distinguished from all other insects. Nematocera is a suborder that has a delicate body, long, filamentous antennae and long, flexible maxillary palps. Mosquitoes are members of the family Culicidae. Head, thorax and abdomen are the three distinct parts of a mosquito's body.

The presence of a conspicuous forwardly extending proboscis, as well as the abundance of scales on the thorax, legs, abdomen, wing veins and as a fringe of scales along the posterior margin of the wings, distinguishes mosquitoes from other flies.

Mosquitoes are holometabolous insects that undergo complete metamorphosis with four distinct stages: egg, larva, pupa and adult. They breed in a variety of habitats which ranges from ponds to artificial containers. The larvae, often referred to as 'wrigglers' feed and develop in water before transforming into pupae. Various mosquito genera have distinct breeding preferences (Jebanesan, 2013; Selvan et al., 2015b). Density-dependent patterns and seasonal climate variations have a significant impact on mosquito abundance. Changes in climate may accelerate (or retard) the development, availability of breeding sites and food resources of certain species. (Franklin and Whelan, 2009).

The adults are winged, capable of prolonged flight and capable of a variety of activities on land. Only female mosquitoes consume blood, a crucial component for the development of their eggs. They locate hosts by detecting carbon dioxide, body heat and certain chemicals emitted by animals. Both sexes consume plant sugar, males on the other hand do not feed blood but primarily feed on nectar. Blood-feeding is either discretionary or completely absent in some species of mosquitoes and eggs are produced autogenously in them.

1.2. Taxonomy of mosquitoes

According to the Mosquito Taxonomic Inventory, currently, there are 3,724 extant species, divided into 113 genera and two subfamilies. The Subfamily Anophelinae comprises three genera while the subfamily Culicinae has 110 genera

divided into 11 tribes. Along with the Neotropics, the oriental region, which includes India, is regarded as one of the world's richest biogeographic regions for mosquitoes (Gaston and Hudson, 1994). After Brazil, Indonesia, Malaysia, and Thailand, India is placed fifth in terms of mosquito biodiversity (Foley et al., 2007). 410 species have previously been reported in India (WHO, 2014). These include *Anopheles stephensi*, an important vector of malaria, *Aedes aegypti*, a vector of dengue, and *Culex quinquefasciatus*, a vector of bancroftian filariasis, *Cx. tritaeniorhyncus* an important vector of Japanese Encephalitis, besides many other species involved in the transmission of arboviruses (Selvan et al., 2015a). Even in Kerala, mosquito-borne illnesses constitute a significant threat to public health. A total of 130 species representing 16 genera have been reported from the various study reports in Kerala (Sumodan, 2014; Aneesh et al., 2014;Thankachan and Gopinath, 2017; Balasubramanian et al., 2021).

1.3. DNA barcoding

DNA barcoding is an advanced taxonomic identification technique that utilizes the mitochondrial genome for species discrimination due to its lack of introns and infrequent recombination. The technique offers several advantages over the traditional morphological identification method, including clear differentiation between closely related species, identification of invasive species, recognition of cryptic species and prompt differentiation of mosquito species from a small tissue sample at any developmental stage. The method employed a number of genes, the mitochondrial cytochrome c oxidase subunit 1 (COI) gene being the most prevalent due to its precise inter species distinction (Herbert et al., 2003a).

Even though dengue and other vector-borne diseases are prevalent in majority of the states in India, only a small number of studies have examined the molecular epidemiology and evolutionary history of dengue vectors, indicating a lack of information for comparative analysis (Naddaf et al., 2012; Vadivalagan et al., 2016; Daude et al., 2017). Geographical distribution and climatic conditions are expected to have an impact on the mosquito population's capacity as a vector in the area. Additionally, as accurate identification is a crucial component of an effective

vector management programme, it is important to identify a species before linking it to a specific illness when it co-circulates with other sibling species. In several investigations, DNA barcoding has been used to distinguish between physically identical *Aedes* species that are otherwise challenging to recognize when the exterior traits are washed away.

Mosquitoes continue to be the main source of annoyance and the transmission of fatal illnesses to people. Vector-borne disease surveillance and control will be accurate with proper mosquito vector identification and categorization. The traditional morphology-based identification techniques take time and are frequently insufficient to identify species-level differences. Consequently, it is still important to use a multidisciplinary strategy that incorporates morphological and molecular methods.

1.4. Vectoral status of mosquitoes

Any organism (vertebrate or invertebrate) that functions as a carrier of an infectious agent between organisms of distinct species (Kuno and Chang, 2005) is one of the most inclusive definitions of a vector.

Arthropods are dangerous vectors of lethal illness that can spread diseases throughout the world's expanding human and animal populations as epidemics or pandemics. More than 150 species of arthropod vectors under the Class Insecta are known to be significant threats to human health and the majority of these species are largely restricted to the genera *Anopheles, Culex, and Aedes* . These organisms are known to be involved in the transmission of numerous vector borne diseases that cause significant morbidity and mortality in humans more than any other class of organisms (Mehlhorn et al., 2012; Severson and Behura, 2012; Taraphdar et al., 2012; Benelli, 2015).

Mosquito-borne diseases are prevalent in more than 100 countries, infecting 300-500 million people annually and resulting in approximately one million fatalities. Each year, mosquito-borne diseases affect more than 40 million individuals in India. Diseases transmitted by mosquitoes include Malaria, Dengue,

West Nile Virus, Chikungunya, Yellow Fever, Filariasis, Japanese Encephalitis, Saint Louis Encephalitis, Western Equine Encephalitis, Eastern Equine Encephalitis, Ross River Fever, Barmah Forest Fever, Venezuelan Equine Encephalitis, La Cross Encephalitis, Rift Valley Fever and Zika Fever. Among them, Malaria, Dengue fever, yellow fever, Chikungunya, Japanese Encephalitis, Filariasis, West Nile virus and Zika fever are the major mosquito borne diseases that spread in India.

Mosquito borne diseases are a major public health problem in Kerala, a southern state of India. Seven mosquito borne diseases viz., Malaria, Lymphatic Filariasis, Dengue, Chikungunya, Japanese Encephalitis, West Nile Virus and Zika Fever are prevalent in the state. The first outbreak of Filariasis, Malaria, Japanese encephalitis, Dengue, Chikungunya, West Nile virus and Zika fever occurred in Kerala during the years 1709, 1897, 1996, 1997, 2006, 2014 and 2021 respectively (Sumodan, 2019).

1.5. Blood feeding and Host preferences of mosquitoes

Blood-feeding patterns of mosquitoes influence the transmission and persistence of arboviruses (Daniel et al., 2019). Knowledge of their blood-feeding habits can shed light on disease dynamics and aid in the management of parasites that pose a threat to endemic wildlife.

For most animals, protein is a limiting factor for growth and reproduction. The adults of many mosquito species need such a nutritional boost as they emerge from their aquatic immature stage with a protein deficit. Pupae do not feed and the earlier filter feeding behaviour of mosquito larvae is often inadequate to accumulate sufficient protein to provide a reserve that can persist through metamorphosis to adulthood.Consequently adult female lack the protein needed to synthesize yolk and develops eggs. A few mosquito species are efficient at turning larval food into protein reserve. These species termed autogenous, can usually develop egg without blood.

According to Clements (1992) and Lehane (2005), the majority of adult female anautogenous mosquitoes feed by ingesting one vertebrate blood meal per

ovarian cycle and eating plant carbohydrates. For vitellogenesis, blood is used as a source of nutrients, and sugar acts as the substrate for the synthesis of glycogen and lipid. Some mosquito species are generalists and express opportunistic feeding behaviour while others are specialists feeding on selected species. This selective behaviour has great influence on disease transmission. The association between host biting rate and mosquito-borne pathogen reproduction rate raises the risk of spreading diseases (Takken et al., 1998; Macdonald, 1957; Dye, 1992). According to Gillies (1964) and Ulloa et al. (2004), mosquito's preference for hosts may be inherited, but it may also be influenced by ecological factors such host availability, host abundance, vector abundance, habitat, and climate (Thieman et al., 2011; Simpson et al., 2012).

Additionally, when hosts are few or limited, disease vectors may move to other habitats and alter their feeding habits to accommodate a wider variety of hosts. This change in disease vector's feeding habits may have significant effects on the dynamics of the disease and its transmission, particularly in new habitats. Hence understanding the dynamics of illness depends on knowledge of their spectrum of hosts and feeding preferences.

1.6. Olfaction and host preferences

Mosquitoes detect host emanations and olfactory cues by activating sensory neurons located in sensilla that resemble hairs. These olfactory sensilla can be found on the antennae, ovipositor, proboscis, maxillary palps, and tarsi (Hill et al., 2002). According to Mclver (1982), host-seeking behavior of mosquitoes enable them to spread a variety of diseases to people, is significantly influenced by their sensilla and sensory processes. According to Bowen (1991), the process of host discovery entails a guided flight towards the probable host with the use of chemical and visual cues. Bosch et al. (2000), clearly suggest that the perception of attractants such carbon dioxide, ammonia, and lactic acid forms the basis for the chemical identification of the host.

The sensory receptors, or sensilla, come in a variety of shapes and sizes, and they are positioned in specific areas on the maxillary palp, antennae, proboscis, etc.

The aporous sensilla would function as mechanoreceptors while porous sensilla are olfactory and contact chemoreceptors (Seenivasagan et al., 2009). Each sensilla is trained to detect a specific stimulus, such as variations in temperature and humidity, physical sensations, and odours of any type. A key element in the female mosquito's capacity to transmit disease is their behavioural reactions to locate their host. (Raza et al., 2021).

Variety of mosquito species, particularly those that serve as vectors, are extremely high in Kerala. The main mosquito-borne disease vectors that are abundant throughout the state are widely dispersed. Therefore, comprehensive information of the prevalence, distribution, and biology of mosquitoes in the state would be useful for managing both present disease outbreaks and potential future outbreaks that are not now common in the state. The study area selected for the analysis was Mananthavady Taluk of Wayanad district, Kerala which is rich with different types of plantations, forest and urban and rural areas. The present work can be considered as a pioneer study from this area. The number of mosquito species collected, abundance of mosquito vectors prevailed in the area are very important for the consideration of the control of mosquito vectors and thus the prevention of spreading of vector borne disease. Intensifying research on the frequency of mosquito vector–host contact will increase the probability of developing more effective disease prevention tools and strategies, strengthening the capacity for risk assessment and revealing insufficiently investigated fundamental and applied details of mosquito ecology and the role of mosquitoes in pathogen transmission. Considering that sensillae are very important for mosquitoes for the host selection, morphological studies based on the sensillae of antennae, maxillary palps and proboscis were carried out and the findings of the study are highly significant for the future references and control measures.

OBJECTIVES OF THE STUDY

1. To compliment the classification of mosquitoes using classical systematics based on field collections from Mananthavady Taluk, Wayanad.

2. To enrich the database of Culicines collected from selected habitats in Mananthavady Taluk with partial COI gene sequence .

3. To evaluate the phylogenetic relationship between the different species of mosquitoes under study.

4. To assess the mammalian host preference patterns among different species of female mosquitoes from the study area.

5. To analyse the morphological variations on the cephalic sensilla of the field samples of female mosquitoes.

CHAPTER 2
REVIEW OF LITERATURE

The field of medical entomology was born with Patrick Manson's groundbreaking discovery that mosquitoes may spread human filariasis in 1877. This was the first concrete proof, that the arachnids were involved in the transmission of illnesses to humans. The link between mosquitoes and the transmission of dengue (1903), yellow fever (1900), and malaria (1898) was established quickly following the discovery of mosquitoes as vectors of human filariasis. Mosquitoes have drawn the interest of entomologists and health professionals all around the world since it was discovered that they serve as human disease vectors.

2.1. Taxonomic status of mosquitoes

The studies on mosquitoes begun in 18th century with the publication of Systema Naturae by Linnaeus (1758). He was the first to give mosquitoes a scientific name, creating the Genus Culex to contain what he deemed mosquitoes. Fabricius in 1805 provided a list of the mosquito species in his revision of the Diptera. Meigen described A*nopheles* and *Aedes* in 1818 in addition to *Culex* by Linnaeus and he also described thirteen additional species.

The first collection of articles on the taxonomy of Indian *Anopheles* begun with the work of Grassi (1899) work and included works by Giles (1901), Theobald (1901, 1910), Liston (1901), James (1902), Cogill (1903), and many more. A significant contribution towards Indian *Anopheles* was made in the early 20th century by James and Liston (1911), who published a monograph that served primarily as a manual for species identification. Puri (1931) conducted extensive research on the Indian *Anopheles* larvae and wrote a thorough monograph on the subject. Edwards (1932) produced a thorough study in 'Genera Insectorum' on the phylogeny and classification of the Culicidae. The publication of Christophers' Fauna of British

India (1933) on the Tribe Anophelini was the most important revolutionary work in the early 20th century.

Anopheles received a lot of attention, whereas very less studies were done in Culicine mosquitoes. However, Baurraud (1934) explored the culicines of British India. This investigation uncovered so many culicine mosquitoes that Barraud publishing a series of papers in the Indian Journal of Medical Research under the title 'Revision of Culicine Mosquitoes of India' starting in 1923 and continuing until the release of his book 'Fauna of British India' in 1934.

The year 1934 marked the end of a period of intense taxonomic study on the Culicidae family, which made mosquitoes as one of the most well-known insect groups in the region. At this point, information on the mosquito population included 245 Culicine and 43 *Anopheles* species. Taxonomic investigation on mosquitoes moved quite slowly after 1934. Between 1934 and 1960, the number of new species discovered was incredibly low (Qutubuddin, 1960). The National Society of India for malaria and other mosquito-borne illnesses published an outstanding book in 1961 titled 'The Vectors of Malaria in India.' This article discussed ecology in connection to illness and the management of a few malaria vectors in India. 31 genera and 2401 species of mosquitoes were included in the global catalogues by Stone et al. (1959), and 34 genera and 2960 species by Knight and Stone (1977). Knowledge of a number of widespread Oriental species has been improved as a result of a review of the subgenus Culex in the Oriental region (Sirivanakarn, 1976). 42 species of the subgenus *Culex* were recognized in this revision, of which 5 were new and 37 were re validated and re characterized. The revision of the subgenus Stegomyia of *Aedes* (Huang, 1979) provided a guide for the identification of 37 species that are found in the oriental area and explains certain taxonomic issues. A balanced and comprehensive account of the Indian anophelines was presented in a monograph by Rao (1984). He has integrated all significant taxonomic advancements since the publication of Christopher's book in (1933).

Belkin's contribution to the classification of Culicidae, however, was made much earlier when he published Mosquitoes of the South Pacific (Belkin, 1962).

This work is considered the best example of a modern taxonomic study of an entire mosquito fauna because it incorporates all three levels of taxonomy: alpha, beta, and gamma.

The current classification status of Family Culicidae is that, the family includes 3724 extant species classified in two subfamilies and113 genera. The subfamilies include Anophelinae and Culicinae. The subfamily Anophelinae has 3 genera, 11 subgenera and 524 species. Whereas Subfamily Culicinae has 3200 species in110 genera segregated into 11 tribes. Aedinii is the largest tribe of mosquitoes with 1296 species.

Leaving aside the proposals of Reinert et al. (2004, 2006, 2008, and 2009), who divided the tribe Aedini into 82 genera instead of 10, there have been surprisingly few changes in the recognition of mosquito genera since Edwards (1932), despite the fact that the number of formally recognized species has more than doubled from 1400 to 3618. Reinert et al. elevated numerous subgenera and species groups of the tribe Aedini to generic level on the grounds that most phylogenetic systematists do not accept paraphyletic or polyphyletic classifications.

Currently the tribe Aedini has two types of classification viz, traditional classification (Knight and Stone, 1977) and phylogenetic classification (Reinert et al., 2009). According to traditional classification, the tribe is classified in 10 genera. The genera under this tribe are, *Aedes* (940 sp*), Armigeres* (58 sp), *Eretmapodites* (49 sp), *Haemagogus* (28 sp), *Heizmannia* (40 sp), *Opifex* (2sp), *Psorophora* (49 sp), *Udaya* (3 sps), *Verrallina* (95 sps) and *Zeugnomyia* (4 sp). respectively. Phylogenetic classification of the tribe consists of 82 genera (Reinert et al., 2009) (Mosquito Taxonomic Inventory). The current study follows the traditional classification of mosquitoes as there are still controversies regarding the phylogenetic classification.

2.2. Diversity of mosquitoes

The first to attempt to correlate the published descriptions on mosquitoes world wide was done by Giles, in his "Handbook of the Gnats or Mosquitoes" published in 1900 . He added about seventeen new species, most of which were native to India (Talib, 2018). With the publication of his five volumes of monograph between 1901 and 1910. Theobald opened up the study of mosquitoes to scientists worldwide. Brunetti (1907) compiled an annotated catalogue to provide a systematic list of the mosquitoes documented in the Oriental Region, including works from James (1899) to Banks (1906). Contemporary with this compendium is Leicester's (1908) monograph on the Culicidae of Malaya.

Edwards' works from 1911 to 1932 published the Genera Insectorum, a conservative system of classification for Culicidae, which reduced the number of genera from 149 to 30. He also placed all the vectors of human malaria in the genus Anopheles, rather than in many genera recognized by Theobald. Culicine mosquitoes of the Oriental region, particularly that of the British India, were investigated extensively by Barraud who brought out "A Revision of the Culicine Mosquitoes of India" in 26 parts through publications spanning from 1923 to 1929. He collated his work, which included the treatment of Culicidae by Edwards (1932) and Senior-White (1923), and published it in 1934 in his classic volume "Fauna of British India, including Ceylon and Burma."

South Asia Mosquito Project was parallel to Mosquitoes of the Middle America Project. Beginning in 1961, a major study of the mosquito fauna of Thailand was undertaken by the United States Army Medical Component Southeast Asia Treaty Organization (SEATO). A revision of the mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Island) and Korea was done by Bram (1967), Delfinado (1967,), Reinert (1970), Sirivanakarn (1972), and Huang (1972). In addition, the mosquito fauna of the Philippines has been extensively documented by Basio in a monograph (1971).

Reuben et al. (1993) investigated the subgroup *vishnui* of the subgenus *Culex*, which contains crucial vectors of Japanese encephalitis (JE). Harrison (1980) has

investigated the significant minimus group of the Myzomyia series of the subgenus *Cellia*, as well as the distribution and binomics in Southeast Asia. Chen et al. (2003) conducted molecular and morphological research in southern China on the Minimus group and determined its taxonomic, distributional, and vectoral status. Sallum et al. (2005) conducted a taxonomic revision of the leucosphyrus group, which is one of the most significant groups of malaria vectors in Southeast Asia. Rattanarithikul and Green (1986) studied the *An. maculatus* group and developed a key to adult females of the group, whereas Walton et al. (2007) studied genetic diversity and used the ITS2 region of rDNA for molecular identification of the species. Both Trung et al. (2004) and Manguin et al. (2008) have written about the bionomics and distribution of Southeast Asian malaria vectors.

During the early part of 21st century various studies of mosquitoes had been undertaken in different parts of the world *viz.* South Western Nigeria (Adeleke et al., 2010; Olayemi et al., 2014), Malaysia (Brant, 2011), Iran (Nikookar et al., 2010; Hanafi-Bojd et al., 2012; Jaberhashemi et al., 2022), Eastern Spain (Bernues-Baneres et al., 2013), Kenya (Lutomiah et al., 2013), Mexico (Bond et al., 2014), Indonesia (Anwar et al., 2015), Bhutan (Somboon et al., 2020), Netherland (Deblauwe et al., 2021), UK (Vaux et al., 2021) and Brazil (Silva-do-nascimento et al.,et al., 2021).

2.2.1. Distribution of mosquitoes – National status

In India, G. M. Giles was the first to publish his Compendium of the Gnats or Mosquitoes in 1900, to which he contributed approximately seventeen new species, the majority of which originated in India. In India, mosquito taxonomy remained stagnant for a long period. Malaria, which took a heavy toll on human life and cost India a great deal in terms of annual revenue, was given top priority among insect-borne diseases; consequently, work on mosquitoes of the genus *Anopheles* was initiated. The Malaria Survey of India (later renamed the Malaria Institute of India) began functioning as a full-fledged research institute first in Kassauli and then in New Delhi. Prior to Christophers' (1933) monograph, James and Liston's (1904) volume on the Anophelines of India served as the primary reference for identifying

species of *Anopheles*. The two monographs by Christopher on anophelines and Barraud on culicines, of the Indian subcontinent, published in 1933 and 1934, respectively, were the result of all the taxonomic research conducted in the subcontinent by the authors and their predecessors. James and Liston's (1904, 1911) volumes on Anophelines of India; Brunetti's (1907-1920) Annotated Catalogue of Culicidae and Critical Review of the Genera in Culicidae; and Puri (1931) Larvae of Anopheline Mosquitoes with a detailed description of those of the Indian Species are notable among the earlier works on Culicidae. The masterpieces of Gill (1917, 1920), Senior-White (1923), and Covell (1927) are also noteworthy. Christopher worked on the Anophelines of India from 191l to 1931, and published a number of articles, and described a great number of species.

India ranks fifth after Brazil, Indonesia, Malaysia, and Thailand in terms of mosquito biodiversity (Foley et al., 2007). Since the 1980s, faunistic surveys conducted in various regions of India have uncovered numerous species that are novel additions to the fauna of India's mosquitoes, despite their smaller numbers. A region-by-region survey of mosquitoes was the primary focus of the research team. Although the survey was conducted between October 1968 and September 1974, Hussainy (1981) documented the distribution of 14 Culicine species in the Bastar district of Madhya Pradesh. Important mosquito surveys, collections, and descriptions conducted in India since 1980 include: Nagpal and Sharma (1987); Rajput and Kulkarni (1990, 1991a,), Bhattacharya et al. (2000, 2002, 2003, 2004a, 2004b), Khan et al. (1998), Rajavel and Natarajan (2006, 2011) Rajavel et al. (1998, 2004, 2005), Rajput and Sing (1987a, 1987b), Reuben et al. (1993), Dutta et al. (2003) Tyagi et al. (1991) Sagandeep et al. (1994) and Jagbir and Kaur (1999). According to the taxonomic classification (Bhattacharya et al., 2014), the Indian mosquito fauna consists of 393 species (61 anophelines and 332 culicines) in 49 genera, and 41 subgenera.

During the second half of the 20th century and the beginning of the 21st century individual researchers contributed much to the field of mosquito taxonomy in various parts of India. 61 species of mosquitoes from 8 different genera –

Anopheles, Aedes, Armigeres, Coquillettidia, Culex, Mansonia, and *Toxorhynchites* – were discovered during a thorough study done in the eastern part of India by Nagpal and Sharma (1987), in western Himalaya (Rao et al., 1973), northeastern India (Dutta et al., 2003; Kaur and Kirti, 2003), Eastern and Western coasts (Rajavel et al., 2005), Gujarat and the Thar Desert region in northwestern Rajasthan (Tyagi, 1984, Sharma et al., 2021), southern India (Western Ghats and Eastern Ghats as well as Andaman and Nicobar Islands) (Reuben et al., 1993;Tyagi 2010, Muniratnam et al., 2014), Telangana (Suhasini and Sammaiah, 2014) and Tamil Nadu (Kumar et al., 2011; Amala and Anuradha, 2012; Karthikairaj et al., 2013; Varshini and Kanagappan, 2015) are few among them.

2.2.2. Distribution of mosquitoes – Regional status

Researchers from Kerala during the period as early as the 1900s focused on the studies on mosquitoes because of its correlation with the incidents of Brugian filariasis and malaria. James (1902), Giles (1902), Theobald (1901, 1910), James and Liston (1911), Cruickshank and Wright (1914), Brunetti (1920), and Covell (1927, 1931) provided early references to the presence and distribution of mosquitoes in Kerala. A notable contribution was given by Iyengar (1938) in this regard. His studies on epidemiology on filariasis in Travancore brought out many many species of mosquitoes to different genera . Covell and Singh (1939) made a survey in the mountainous and thickly forested regions of the Wayanad in 1938-39 and reported 19 species of *Anopheles*. Mathew (1939), Iyengar (1938), Nair and Roy (1958), Nair (1962), Daniel et al. (1986), Sabesan et al. (1991), and Mariappan et al. (1992, 1996) were some of the great contributors of mosquito diversity studies in 20th century.

During the 21st century many researchers came forward with their contribution in the field of mosquitoes. Hiriyan et al. (2003) reported 26 species of mosquitoes in 6 genera in Kuttanad region of Kerala. Sharma et al. (2004a), Kalra and Prasittisuk (2004), Sumodan (2003, 2014, 2019) were great contributors on the study and distribution of *Ae. albopictus.* According to Kalra and Prasittisuk (2004), this species is considered as the major Dengue vector species of Kerala. Rajavel et al.

(2006) identified 17 species of mosquitoes from 7 genera from the mangroves of Kannur. 27 species belonging to 7 genera were recorded from Thrissur by Sabu and Subramanian (2007). By compiling the study reports of Hiriyan et al. (2003), Rajavel et al. (2006) Thenmozhi et al. (2007) 50 different species of mosquitoes had been reported from various districts of Kerala. Jomon and Sudharmini (2009), Jomon et al., (2010), Balasubramanian and Nikhil, (2013), Asha and Aneesh (2014), Aneesh et al. (2014),Thankachan and Gopinath. (2017) and Seema et al. (2021) are a some of the recent contributors on the diversity studies on mosquito species in Kerala.

Very few studies on mosquitoes have been conducted in Wayanad District. In 2012, Sumodan conducted a study on the species diversity of mosquitoes breeding in rubber plantations and reported 12 species in 6 genera. Aneesh et al. (2014) surveyed mosquito species in Kuruva islands of Wayanad and recorded 18 species. In 2017, Thankachan and Gopinath conducted studies on the diversity of mosquitoes in the plantations of Wayanad district and reported 17 species in 6 genera. 7 species in 5 genera were reported by Shanasree and Sumodan (2019) in their studies on the diversity of tree hole breeding mosquitoes.

The different study reports from Kerala have all been combined, and it is observed that 130 species from 16 genera viz, *Anopheles* (31 sp), *Aedes* (31 sp), *Culex* (30 sp), *Mansonia* (4 sp), *Armigeres* (4 sp), *Heizmannia* (4 sp), *Uranotaenia* (11 sp), *orthopodomyia* (2 sp), *Mimomyia* (3 sp), *Verrallina* (4sp), and single species each from *Haemagogus*, *Topomyia, Ficalbia, Tripteroides, Coquilletidia* and *Toxorhynchitis* respectively, have been reported so far (Sumodan, 2014; Aneesh et al., 2014; Thankachan and Gopinath, 2017; Balasubramanian et al., 2021).

2.3. Molecular taxonomy and phylogenetic studies on mosquitoes

Medical entomology, where molecular methods to species diagnosis are frequently of significant assistance in the identification of all life stages, from eggs to adults, places a special emphasis on the capacity of DNA barcodes to identify species reliably, quickly, and economically.

Studies on Phylogenetic relationship among the mosquitoes initiated with study of Mallampalli (1995) as he worked on the phylogenetic relationship among the genus Culex in US. Later, studies were carried out by Miller et al., (1996) and Harbach and Kitching (1998). Like any other study, phylogenetic analysis was extensively explored in the 21st century by several scientists, including Navarro et al. (2000) and Sallum et al., (2002).

In 2002 Tautz and Arctander argued that DNA sequences serve as the primary foundation for biological identification, and Paul Hebert later postulated that sequencing the COI gene may facilitate DNA barcoding that would assist such classification. It has been demonstrated by Hebert et al. (2003a, b) that morphologically known animal species may be distinguished by the study of short, standardized genomic sections (DNA barcodes). The mitochondrial gene cytochrome c oxidase subunit 1 (CO1) is specifically mentioned as a potential standard target gene for a bio identification system.

So far, this methodology had been used to testify the identification of widely varied animals as Lepidoptera (Class: Insecta) (Hebert et al., 2003a, b, 2004a; Hajibabaei et al., 2006), birds (Class: Aves) (Hebert et al., 2004b), and tachinid parasitoids successfully. Currently, this technique is used to barcode several animal species, including fish and primates. (CBOL, 2005; Lorenz et al., 2005). Several genetic approaches have been applied to the identification of mosquito species, including protein electrophoresis (Green et al., 1992; Foley et al., 1995; Sukowati et al., 1999; Van Bortel et al., 1999), hybridization assays (Crampton and Hill, 1997; Cooper et al., 2002) and polymerase chain reaction (PCR)-based sequence analysis.

The 21st century marked the expansion of molecular research of the Culicidae in different parts of the world. The works of Chen et al. (2003), Wilkerson et al. (2003) in China, Cywinska et al. (2006) in Canada were first among them. Many countries adopted the identification of mosquitoes through DNA barcode like, China (Wang et al., 2012), Pakisthan (Ashfaq et al., 2014), Singapore (Chan et al., 2014), Antiokia (Hoyos et al., 2015), Beilgium (Versteirt et al., 2015, Smitz et al., 2021), Austria (Batovska et al., 2016), Sri Lanka (Weeraratne et al., 2018), Iran

(Doosti et al., 2018) UK (Hernández-Triana et al., 2019), Mexico (Adeniran et al., 2021), Brazil (Silva-do-Nascimento et al., 2021), Saudi Arabia (Noureldin et al., 2022), and Cambodia (Zhang et al., 2022) .

Molecular studies of Culicidae in India began with the work of Kshirsagar et al. (1997). He amplified mitochondrial 16S rRNA gene fragment from the cell line. Shouche and Patole (2000) analyzed a 450bp hyper variable region of the mitochondrial 16S rRNA gene in three major genera of mosquitoes, *Aedes, Anopheles* and *Culex.* Molecular and phylogenetic analysis studies in ITS region, 16S rDNA, 18S rDNA and 28S rDNA markers have been done by various researchers like Manonmani et al. (2001), Goswami et al. (2005), Prakash et al. (2006), Alam et al. (2006), Singh et al. (2010), Alam et al. (2006), Raghavendra et al. (2009), Dhananjeyan et al. (2010), Kohli et al. (2011a), Zomuanpuii et al. (2013) and Das et al. (2012).

Many scientists selected the mitochondrial genome for this approach, owing to its advantages such as maternal lineage, lack of recombination, lack of "indels," and higher mutation rates (Saccone et al., 1999). Among the mitochondrial genes, cytochrome c oxidase subunit 1 (COI) and II (COII) are reported to be the most conserved gene in the amino acid sequences and hence has distinct advantage for taxonomic studies (Knowlton and Weigt, 1998).

Goswami et al. (2005) developed PCR-RFLP of mitochondrial COII for the identification of members of *Anopheles culicifacies* complex. Kumar et al. (2007, 2013) studied DNA barcodes for several species of mosquitoes belonging to 15 genera, prevalent in India, which included major vector species. Bora et al. (2009) described variations in the three mitochondrial DNA markers viz., COI, COII and cytochrome b among *An. sundaicus* populations from Andaman and Nicobar Islands.

Sharma and Choudhry (2010) conducted sequence characterization of rDNA, ITS1, ITS2 and COII gene as potential molecular markers for studying genetic relatedness and phylogenetic kinship among six important species of genus *Anopheles*. Sharma et al. (2013) studied the phylogenetic relation among the four different mosquito species: *An. stephensi, Aedes aegypti, Ae. albopictus* and *Culex*

quinquefasciatus by using PCR-RFLP of COI gene. Many researchers like Daravath et al. (2013), Manonmani et al. (2013), Bindu and Sebastian (2014), Daravath et al., (2014), Murgan et al. (2015), Singh and Vashist (2017), Soni (2018) in Kerala have conducted studies on the DNA barcoding and molecular phylogeny analysis of mosquitoes in various parts of India. Recent studies in molecular phylogeny of mosquitoes in India was done by Panda and Barik (2022) in Odisha.

2.4. Vectoral status of mosquitoes

Diseases transmitted by vectors account for more than 17 percent of all infectious diseases and cause more than 700,000 fatalities annually. They might be caused by parasites, bacteria, or viruses (WHO, 2020). Fewer than 150 species of the arthropod class Insecta are public health-relevant vectors, and the majority are confined to the genera *Anopheles*, *Aedes*, and *Culex*, which are implicated in the transmission of a wide range of vector-borne diseases associated with significant morbidity and mortality among humans, more than any other group of organisms (Mehlhorn et al., 2012; Severson and Behura, 2012; Taraphdar et al., 2012; Benelli, 2015).

The most prevalent virus transmitted by Aedes mosquitoes is dengue. More than 3,9 billion persons in more than 129 countries are at risk of contracting dengue, with an estimated 96 million symptomatic cases and 40,000 fatalities annually. Chikungunya, Zika fever, yellow fever, West Nile fever, and Japanese encephalitis are additional vector-borne viral diseases (all transmitted by mosquitoes) (WHO, 2020).

According to the most recent World malaria report, in 2021 there were 247 million cases of malaria, compared to 245 million in 2020. The estimated number of malaria fatalities in 2021 decreased to 619 000 from 625 000 in 2020. During the two high years of the pandemic (2020 and 2021), COVID-related disruptions caused approximately 13 million additional malaria cases and 63 thousand additional malaria fatalities. The African Region of the World Health Organization continues to bear a disproportionate share of the global malaria burden. In 2021, approximately 95% of all malaria cases and 96% of fatalities occurred in the region.

About 80% of all malaria fatalities in the Region occurred in children under 5 years of age (WHO, 2020).

Chikungunya virus (CHIKV) was first identified in 1952 in the United Republic of Tanzania, and subsequently in other African and Asian nations (Staples et al. 2009). First urban epidemics were documented in Thailand in 1967 and India in the 1970s. Since 2004, CHIKV epidemics have become more frequent and pervasive, in part due to viral adaptations that facilitate the virus's transmission by *Aedes albopictus* mosquitoes. Over 110 countries in Asia, Africa, Europe, and the Americas have identified CHIKV.

Zika virus is a mosquito-borne virus that was first identified in a Rhesus macaque monkey in Uganda in 1947, followed by evidence of infection and disease in humans in other African countries during the 1950s. In Africa and Asia, sporadic human infections were detected between the 1960s and 1980s. Zika virus disease outbreaks have been reported since 2007 in Africa, the Americas, Asia, and the Pacific. In 2019, the first local cases of Zika virus disease transmitted by mosquitoes were documented in Europe, and in 2021, Zika virus outbreak activity was detected in India. Currently, 89 countries and territories have confirmed Zika virus infections transmitted by mosquitoes; however, global surveillance remains limited.

In 1871, Japan reported the first case of Japanese encephalitis virus disease (JE). JEV is the leading cause of viral encephalitis in many Asian nations, accounting for an estimated 68 000 clinical cases per year. 24 countries in WHO's South-East Asia and Western Pacific regions have endemic JEV transmission, putting more than 3 billion individuals at risk for infection. 863 million people in 47 countries are still at risk for lymphatic filariasis and require preventative chemotherapy to halt the spread of this parasitic infection. As of 2018, 51 million persons were infected, a 74% decline since the beginning of the WHO's Global Programme to Eliminate Lymphatic Filariasis in 2000 (WHO, 2020).

According to Bhattacharya et al.2014 and Kumar et al. (2017), in India, 32 mosquito species have been identified as vectors of numerous human pathogens. Six mosquito-borne diseases have been documented: Malaria, Japanese encephalitis,

Dengue, Chikungunya, West Nile, and Filariasis. Only *Anopheles* species belonging to the subgenus Cellia transmit malaria. Thirteen species of *Anopheles* have been identified as malaria vectors. Dengue and Chikungunya are transmitted exclusively by the two *Aedes* species. Japanese encephalitis is transmitted by sixteen different species, West Nile by two, and filariasis by four respectively (Talib, 2018).

Kerala has an extensive history of mosquito borne diseases. According to Sumodan (2014), the state had been haunted by malaria in its highlands and lymphatic filariasis in the coastal belt from pre-historic times. The prevalence of sickle cell anemia among the tribals of Wayanad and Attappadi is a conclusive evidence for the antiquity of malaria in the state (Kaur et al., 1997; Feroze and Aravindan, 2001). Documentary evidence for the presence of Lymphatic Filariasis in Kerala goes back to 1709 when Clarke called elephantiasis-legs in Cochin as Malabar legs (Raghavan, 1957). Currently, bancroftian and brugian forms of lymphatic filariasis are endemic in the state, which rates second in India in terms of endemicity. 15.7% of the total cases are reported form the state (Agarwal and Sashindran, 2006).

The first Dengue outbreak was reported from the Kottayam district of Kerala in 1997 with 14 cases and 4 deaths, which was followed by 67 cases and thirteen deaths in the same district in 1998. In 2003, there were 3,546, confirmed cases of dengue throughout the state and 68 fatalities (Tyagi and Dash 2006). Since then, the state has been experiencing yearly dengue outbreaks with varying degrees of severity. In 1996, there was an outbreak of Japanese encephalitis in Kottayam and Alappuzha districts (John, 2006).

In Kerala, the first pandemic of Chikungunya occurred in June-July 2006 in the coastal districts of Alleppey, Quilon, and Trivandrum, and then again in May-August 2007 in Pathanamthitta, Kollam, and Idukki (Kannan et al., 2009). Hiriyan et al. (2003) surveyed an endemic area for Japanese encephalitis in Kerala and identified 21 species of mosquitoes. In a subsequent study to determine the vectors of Japanese encephalitis, Arunachalam et al. (2004) identified 18 mosquito species. According to Sumodan (2014) *Anopheles culicifacies, An. stephensi, An. fluviatilis,*

An. dirus and *An. minimus* are primary vectors of malaria. Besides, *An. philippinensis, An. annularis* and *An. varuna* are secondary vectors. *Aedes agypti* and *Ae. albopictus* are established vectors of Dengue and Chikungunya. *Cx. quinquefasciatus* is the vector of bancroftian filariasis; *Cx. tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. gelidus* and *Cx. bitaeniorhynchus* are vectors of Japanese encephalitis. Three species of *Mansonia,* namely *Man.indiana*, *Man.annulifera* and *Man.uniformis* are vectors of Brugian filariasis.

2.5. Blood meal analysis of mosquitoes

Haematophagy is shared by females of most mosquito species (Harbach 2007), they utilize the energy from blood digestion mostly for egg development and maturation, enhancing their reproductive effectiveness (Phasomkusolsi et al., 2015). Blood meals might consist of blood from several host taxa, such fishes (Toma et al., 2014], amphibians, reptiles, birds, and mammals (Forattini et al., 1987). A species-specific host preference is an intrinsic trait having a genetic foundation, but it is modified by elements that affect patterns of host search and selection, such as host features and environmental circumstances (Takken and Verhulst, 2017).

The basic serological tool used in arthropod blood-meal identification was the precipitin test. Other methods like fluorescent antibody technique (Gentry et al., 1967), hemoglobin crystallization (Washino and Else, 1972) and passive hemagglutination inhibition (PHI) (Weitz, 1956; Kirsch and Murray, 1969; Tempelis and Rodrick, 1972) were also used for the blood meal analysis. The PHI technique has been adopted to identify blood feedings of mosquitoes (Tempelis and Rodrick, 1972). The precipitin test continues to be the fundamental serological instrument, despite the development of various alternative techniques for blood-meal identification. It needs the direct reaction of the blood meal, suspended in a diluent, with an antiserum. It is relatively simple to set up in the lab, regardless of the variation used.

Four precipitin methods have been used: (a) Ring test (Weitz, 1960), (b) Capillary tube test (Tempelis and Lofy, 1963), (c) Agar gel diffusion method (Crans, 1964), and (d) Microplate method (Tesh et al., 1971). The ring test was the first

method used and continues to be extensively applied (Boreham, 1972). The gel diffusion method has been assessed or used in at least 5 laboratories (Chamberlain and Sudia, 1967; Herndon and Ringle, 1967; Crans, 1969; Eliason, 1971; Sullivan et al., 1971). Crans (1969) reported that he had used it to test over 10,000 field-collected engorged mosquitoes.

Eliason (1971) has described a gel surface precipitation method. In this method, a little quantity (0.1ml) of antiserum is 'charged' onto an agar-coated slide. Drop-wise (0.001-0.003ml) additions of the suspended blood meals to the covered surface were made. Blood meals that react with the antiserum produced a precipitate. Tesh et al. (1971) described a fourth modification of precipitin test. It came up as a result of technical issues with the capillary tube precipitin test that were discovered during research on the host preferences of Panamanian phlebotomine sand flies. In this technique, little quantities of blood meal and antibody are allowed to be reacted in microplates. It is claimed that compared to the capillary precipitin test, this approach was simpler to interpret and produced more reliable results. Its main flaw is that, compared to the capillary tube approach, it requires more suspended blood meal (Tesh et al., 1971), which may be crucial when studying tiny arthropods that only consume a little amount of blood.

Another test for the identification of arthropod bloodmeals was the enzyme-linked immunosorbent assay (Tempelis, 1975; Washino and Tempelis, 1983). The identification of numerous arthropod bloodmeal sources is frequently restricted to order or family, despite the fact that these approaches have provided and continue to give useful, informative data. Increased precision in bloodmeal identification to the species or individual host level has been made possible by novel uses of polymerase chain reaction (PCR)-based methods and a growing body of vertebrate DNA sequence information available to the general public. The blood feeding behavior of numerous arthropods is being reviewed using modern molecular techniques due to the increased specificity attained using DNA-based methods.

DNA sequencing is the simplest and most precise approach to identify bloodmeals; but, the expense of sequencing can make this method unworkable for

the high-throughput processing of many samples. However, this method is best used when researching zoophilic arthropods that may feed on a variety of domestic and wild animal species, or when the arthropod's host range is entirely unknown (Kent et al., 2014).

When encountering only a few potential blood hosts, or when only broad taxonomic classification is desired, group-specific primers can be used to identify bloodmeals. Kent and Norris (2005) developed a multiplexed PCR assay to differentiate among five common vertebrate hosts of Anopheles mosquitoes in African villages. This assay uses a conserved reverse primer with animal-specific forward primers to amplify DNA fragments of differential size from each host that can be identified directly from agarose gel electrophoresis. PCR–RFLP diagnostics based on the mitochondrial cytochrome b (*cyt b*) have been developed and successfully used to identify the bloodmeals of ticks (Kirstein and Gray, 1996), mosquitoes (Ngo and Kramer, 2003; Oshagi et al., 2006) and tsetse flies (Steuber et al., 2005) to the genus or species level. A variation on RFLP is the terminal RFLP (T-RFLP). Meece et al. (2005) developed this technique for identifying the bloodmeals of mosquitoes. A real time TaqMan PCR assays (qPCR) based on a 358-bp section of *cyt b* was developed to identify mosquito bloodmeals originating from native Australian mammals. A multiplexed real-time PCR assay has also recently been developed to identify eight common blood hosts of fleas.

Heteroduplex analysis has identified bloodmeals to the species level for tsetse flies (Simo et al., 2008) and mosquitoes (Apperson et al., 2002; Lee et al., 2002; Richards et al., 2006), this technique is difficult to master and achieve reproducible results. Real-time qPCR (Sales et al., 2015; Kevin et al., 2020), Vossen et al., 2009), Droplet digital PCR (Rice et al., 2019 Microarray and Microsphere (Thiemann et al., 2011, 2012; Grubaugh et al., 2013), Mass spectrometry (Greenwalt et al., 2013), Stable isotope analysis (Rasgone 2008; Njabo et al., 2013) are some of the modern techniques used for the blood meal identification of arthropods (Borland and Kading 2021).

The earliest investigators to establish host preferences of mosquitoes by serological testing were King and Bull (1923). Their research confirmed the connection of *A. quadrimaculatus* to humans and emphasized the importance of this connection given that *A. quadrimaculatus* serves as the primary malaria vector in the southern United States. Since this trailblazing work, a great deal of other host selection research on malaria vectors has been carried out globally.

2.5.1. Anophiline mosquitoes

Numerous research on the host-feeding habits of several *Anopheles* mosquito species have been published. The most extensive work, which was extended over a period of 10 years (1955-1964) and worldwide in scope, was a coordinated study by the World Health Organization and the Lister Institute, England (Bruce-Chwatt et al., 1966). This study provided information from about 1,24,000 anopheline mosquitoes representing 92 species or species complexes. As the primary vector of malaria in much of Africa and recently implicated in filarial transmission (White 1971a), the *Anopheles gambiae* complex has been the subject of intensive biological study. Its principal hosts have been shown to be man and bovines (Hamon et al., 1964: White, 1971b). It is a complex of at least 5 sibling species (Davidson, 1964). The 2 species of greatest concern are *An. gambiae* A and B. During the course of many of the biological studies on these species, many attempts have been made to measure the relative vectorial activities of species A and B (White et al., 1972, White and Rosen, 1973). Numerous other investigations into this species also have already been done (Gillies, 1964; Joshi et al., 1973; Service, 1970; Haridi, 1972).

In 20th century, blood meal analyses of various *Anopheles* mosquito species were conducted worldwide. *An. subpictus subpictus* and *An. annularis* in India (Shalaby, 1969a, b). *An. sinensis* in Japan (Takahashi et al., 1971), *An. albimanus, An. pseudopunctipennis* and *An. punctimacula* in South America (Bruce-Chwatt et al., 1966), *An. freeborni, An. franciscanus* and *An. punctipennis* in the western states of Washington and California (Reeves and Hammon 1944a, 1962b, Hammon et al., 1945). *An. crucians* In Florida, (Edman, 1971) were few among them.

A small percentage of feedings by many Anopheles species were shown to be on birds (Bruce-Chwatt et al., 1966), usually less than 1% of the total Feedings. Since many *Anopheles* mosquitoes are malaria vectors, numerous scientists have contributed their findings to the analysis of blood meals from *Anopheles* mosquitoes in the twenty-first century. Anthropophilic nature *An. gambiae* and other *Anopheles* species were reported in Kenya (Joseph et al., 2003). USA (Marinotti et al., 2005) and in Brazil (Zimmerman et al., 2006).

The blood meal analysis studies in Rajasthan (Swami and Srivastava 2012) showed that that *An. subpictus* had a preference towards cattle blood, *An. culicifacies* and *An. stephensi* preferred human blood, while, *An. annularis* was noted to feed only on bovine blood. High anthropophilic nature of Malaria vectors *An. baimai*, *An. minimus* and *An. annularis* was observed in hilly areas of Bangladesh (Bashar et al., 2012). *Anopheles* mosquitoes collected in New Guinea were found with blood of humans, dogs, pigs and unexpected hosts like mice, bats, and marsupials (Logue et al., 2016). In 2017 experiment done in UK revealed *An. atroparvus* fed largely on rabbits and *An. messeae* on Cattle (Hernández-triana et al., 2017). In Iran feeding on birds were reported in *An. cruzii* (Shahhosseini et al., 2018).

In recent years advanced techniques are widely used for blood meal analysis like MALDI-TOF MS in France (Diarra et al., 2019) and Africa (Niare et al., 2016) and Probe based qPCR in New Huinea (Keven et al., 2020).

2.5.2. Culicine mosquitoes

Studies on Culicine mosquitoes have focused more on the temperate climates of the New World in contrast to studies of *Anopheles* mosquitoes, which were focused primarily in tropical and subtropical areas of the Old World. The first study on host feeding was done on *Culex tarsalis*, (Reeves and Hammon, 1944; Hammon et al., 1945; Tempelis et al., 1967; Tempelis and Washino, 1967; Hayes et al., 1973). The *Cx. pipiens* complex is a significant group of mosquitoes whose eating habits have been well analyzed (Tempelis et al., 1970; Kokernot et al., 1969b; Liu et al., 1959; Lu et al., 1959).

Anthropophilic index for *Cx. quinquefasciatus* was studied in different parts of the world (Colless, 1959b; de Meillon et al., 1967). In East Africa 95% of *Cx. quinquefasciatus* fed on man or other primates (Heisch et al., 1959). In India, *Cx. quenquefasciatus* had shown preference for human blood over that of other mammals (Azmi et al., 2015). *Cx. quinquefasciatus* have a wide host preference range in Grenada, which is consistent with other studies (Garcia-Rejon et al., 2010; Stenn et al., 2018).

Cx. tritaeniorhynchus is a mosquito that is very significant in the Far East because it is a significant vector of Japanese B encephalitis. Numerous studies have been conducted on this mosquito, in Okinawa (Pennington and Phelps, 1968; Bendell, 1970), Malaya and Japan (Hurlbut, 1964; Macdonald et al., 1967; Takahashi et al., 1971; Christopher and Reuben, 1971) in India and China (Liu et al., 1959), Africa (Colless, 1959a; Snow and Boreham, 1973). Host preferences of *Cx. vishnui, Cx. pseudovishnui* and *Cx. bitaeniorhynchus* vectors of Japanese Encephalitis was done by Christopher and Reuben (1971) in India.

The first blood-meal analysis of *Ae. aegypti*, obtained from Kenya, were performed by Teesdale (1955). Studies shows that *Ae. aegypti* generally fed on man (Heisch et al., 1959) and reptiles (Nellis and Everard 1983; Ponlawat and Harrington, 2005; Jansen et al., 2009; Sivan et al., 2015; Khaklang and Kittayapong, 2014). Stenn et al. (2018) also conducted studies on the host preferences of *Ae. aegypti*.

Another *Aedes* mosquito of medical importance is *Ae. albopictus.* It feeds on mammals, and small percentage in birds (Tempelis et al., 1970). Anthropophilic nature of these mosquitoes were studied in Kolkata by Tandon and Ray (2000). Host preferences of *Ae. vexans* was done by Takahashi et al. (1971) and Reeves and Rudnik (1951). Studies on different species of Aedes like *Ae. melanimon, Ae. sierrensis, Ae. sticticus, Ae. dorsalis, Ae. nigromaculus,* and *Ae. cinereus* were done (Reeves and Hammon, 1962a; Edman and Downe, 1964; Tempelis and Washino, 1967; Tempelis et al., 1967; Hayes et al., 1973). Mammals, primarily cattle, were the main hosts in all of these studies. *Ae. taeniorhynchus* and *Ae. sollicitans,* fed predominantly on rabbits. *Ae. infirmatus* and *Ae. atlanticus* fed equally well on both

rabbits and armadillos. Another species, *Ae. fulvus pallens,* fed principally on armadillos (Edman, 1971). A relatively large collection of blood-engorged *Ae. canadensis* was made in the Pocomoke Swamp of Maryland (LeDuc et al., 1972). The main hosts for these mosquitoes were deer (47%), but the interesting aspect of this study was the fact that over 16% of these mosquitoes fed on reptiles *Ae. annulipes, Ae. cantaiis, Ae. caspius, Ae. cinereus; Ae. detritus, Ae. dorsalis, Ae. flavescens,* and *Ae. geniculatus,* were fed principally on large domestic mammals, primarily bovids (Service, 1969, 1971a).

The majority of *Aedes* species choose mammals as hosts. Under natural circumstances, they will occasionally attack birds, and a few species will feed on cold-blooded vertebrates (Taylor et al., 1971; LeDuc et al., 1972). 21st century is marked with various works of host preference patterns of mosquitoes in different parts of the world with various advanced technologies. Studies were done in Colorado (Lee et al., 2002), Florida (de Benedictis et al., 2003), USA (Molaei et al., 2007, Faraji et al., 2014), Africa (Delatte et al., 2010), Spain (Muñoz et al., 2011), Europe (Ventim et al., 2012), Kenya (Lutomiah et al., 2013), Israel (Valinsky et al., 2014), Andaman (Sivan et al., 2015), Thailand (Pengsakul et al., 2017), Switzerland (Schonenberger et al , 2015), UK (Hernández-triana et al., 2017), Iran (Shahhossein et al., 2018), Australia (Stephenson et al., 2018), Brazil (Santos et al., 2019), Greneda (Daniel et al., 2019) and Ethiopia (Guta et al., 2021).

Blood host feeding preferences of certain JE vectors were assessed in Kerala (Samuel et al., 2008), and the result indicated preferences for cattle, humans, and pigs. Numerous more genera of the family Culicinae have had their host ranges identified. These mosquito species were mainly discovered in the Western Hemisphere and are often regarded as pests of humans and domesticated animals.

2.6. Morphology of olfactory sensilla in mosquitoes

In mosquitoes and other insects, olfactory perception begins in their peripheral olfactory organs. Adult mosquitoes have olfactory receptor expression in their antennae, maxillary palps, proboscis, tarsi and ovipositor (Pitts et al., 2006, 2011; Athrey et al., 2017; Saveer et al., 2018, Melo et al., 2004; Bohbot et al., 2007;

Sparks and Dickens 2014; Matthews et al., 2016; Lombardo et al., 2017; Yamany et al., 2023; Zhou et al., 2014; Xia and Zwiebel, 2006; Leal et al., 2013).

The general morphology of the maxillary palps varies between species and sexes. Male *Anopheles* have long, club-shaped maxillary palps, whereas females have slightly shorter, cylindrical palps. Male and female Sabethes have palps that are small and similar in length. In *Aedes, Culex, Toxorhynchites*, and *Psorophora*, the males have maxillary palps that curve upwards, while females have maxillary palps that are straight and much shorter than the proboscis. Each mosquito maxillary palp comprises of five segments (Mclver, 1982).

Maxillary palps contain chemosensory sensilla and mechanosensory filaments, and are thus olfactory and mechanosensory organs, similar to antennae (McIver, 1971; McIver 1972). In contrast to antennae, the mechano sensitivity is achieved at the level of individual sensilla rather than the entire organ. The morphology of the proboscis is the most intriguing of the three olfactory organs and is associated with its function in blood intake. The typical mosquito proboscis contains six organs (a pair of maxillae with tooth-like structures, a pair of mandibles, a needle-like labrum, and a hypopharynx) enclosed in a labium that terminates in a labellum (Choo et al., 2015). All six organs are roughly the same length as the labium in females of blood-feeding species, and they penetrate the epidermis during blood-feeding. Males of these species have variable maxillae and mandibles (Wahid et al., 2003), and stylet innervation in *Aedes aegypti* is markedly sexually dimorphic (Jove et al., 2020). Males of blood-eating species cannot penetrate epidermis and do not consume blood. Males and females of the non-blood feeding *Malaya* and *Topomyia* species have lost their mandibles and maxillae entirely (Wahid et al., 2003). Compared to mosquitoes that depend on blood, the proboscises of certain non-bloodsucking species have strikingly different morphologies. For example, *Malaya* mosquitoes have a peculiar proboscis, enlarged at its distal end (Rattanarithikul et al., 2007). These mosquitoes feed on bamboo fluid obtained from Crematogaster ants via trophallaxis (Miyagi, 1981). *Toxorhynchites* mosquitoes do

not feed on blood and have a lengthy, curved proboscis, presumably to facilitate the nectar feeding on plants.

The primary functions of antennae are chemoreception and mechanoreception. Although elaborations into plumose, lamellate, or pectinate forms have arisen numerous times in different insect lineages, the general shape of most insect antennae is elongate and cylindrical. An insect antenna consists of three parts: the scape, the pedicel, and the flagellum. The first segment of the antenna, the scape, is affixed to the head by a rim of extensible inter segmental cuticle. The movements of an antenna are controlled in part by one or two pairs of muscles that attach to the head and the scape, respectively. The scape is connected to the next segment of the antenna, the pedicel, which contains the Johnston's organ, by an additional pair of muscles. The Johnston's organ of a male mosquito contains approximately 16,000 mechanosensory neurons, an extremely high number for such an organ. The antennae respond to changes in particle velocity by swaying back and forth at the pedicel, with the organ's first resonance mirroring the flight tone of the female mosquito, allowing it to perform its primary function, mate detection (Roth, 1948). Together, these two groups of muscles can manipulate an antenna in virtually any direction. The flagellum is the segment of the antenna with the most diverse morphology among insects. Each antenna of both male and female mosquitoes is composed of 13 evident flagellar segments. In females, the flagellar segments are roughly of equal length and exhibit a diversity of sensilla types. In most male mosquitoes, the basal 12 segments of the antennal shaft have whorls of robust fibrillae whose length decreases distally. The final two flagellar segments of the male possess the same diversity of sensilla types found along the length of the female's flagellum (Mclver, 1982). The primary function of antennae is to assess of the environment's physical and chemical characteristics. The innervated chemosensory and mechanosensory organs that are arranged on the antennae are used for detection. Typically, a single antenna contains multiple varieties of sensory organs with distinct properties.

Tarsi has five varieties of sensory sensilla: sensilla chaetica, grooved pegs, sensilla campaniform, sensilla basiconica, and sensilla coeloconica. The tarsal joints have two broad and serrated sensilla. On the tarsomeres, near the claws, and on the joints, there are two varieties of sensilla chaetica that differ considerably in morphology and morphometric parameters. On the ovipositor, two groups of four apical sensilla and short, spiny sub apical sensilla are sporadically distributed. The female external genitalia have two varieties of sensilla chaetica, which are arranged in rows and substantially smaller than their male counterparts. (Yamany et al., 2023).

The male antenna is briefly described by Johnston (1855), who first described the organ that bears his name. Child (1894) provides a more exact description of Johnston's organ in Culex. Johnston's organ in male and female *Cx. pipiens* is described by Eggeks (1924). According to Roth and Willis (1952), the female antenna of the *Ae. aegypti* could serve as a hygroreceptor. Detailed illustrations of the internal organization of the male antenna in three different mosquito species were provided by Risler (1953, 1955). Rahm (1957) discusses research on the olfactory function of antenna and other appendages in *Ae. aegypti*. In his work on *Ae. aegypti*, Christophers (1960) explains some of the setae and provides a description of the antenna. In 1963, Steward and Atwood conducted research on the sensory organs on mosquito antennae.

The morphological organization antennae in mosquitoes have been effectively retained. The 13 flagella that make up each antenna are covered with sensilla, which are sensory organs responsive to mechanical, thermal, hygro, and chemical stimuli (McIver, 1971). The sensilla found in culicine mosquitoes can be categorized as either olfactory or non-olfactory (Boo and McIver, 1976; Pitts and Zwiebel, 2006). Antennal sensilla have previously been described for several mosquito species (Coluzzi, 1964; Hill et al., 2009; Ismail, 1964; McIver, 1982; Sutcliffe, 1994).

The morphological bases for these senses in mosquitoes are summarized by Mclver (1970, 1971, 1972,1982) and Allen et al. (1987). According to Mclver (1982), in *Ae. aegypti*, 5 types of olfactory sensilla occur on the antennae (large and

small sensilla coeloconica, sensilla ampullaceae, grooved pegs, sensilla trichoidea) and one occurs on the palps (capitate pegs). In addition, large sensilla coelconica occur only on anopheline mosquitoes. Various scientists have given their contribution to the studies of various types of sensilla in mosquitoes; Capitate pegs (Mclver, 1970, 1971, Omer and Gillies, l97l; Sutcliffe et al., 1987), Grooved pegs (Mclver, 1974; Zacharuk, 1985), Large sensilla coeloconica (Ismail, 1962; Boo and Mclver, 1976; Mclver, 1982), Small sensilla coeloconica ((Mclver, 1973; Boo and Mclver, 1976; Mclver, 1982; Davis and Sokolove, 1975), Sensilla ampullaceae (Boo and Mclver 1976, Mclver and Siemicki 1979), Sensilla trichoidea (Omer and Gillies, l97l; Mclver, 1982). Pitts and Zwiebel (2006) examined the antennal sensilla of two female Anopheline sister species with different host ranges and discovered no physical changes between those sibling species in host preference. Sharon et al. (2009) described the antennal trichoid sensilla of the female Southern House Mosquito, *Cx. quinquefasciatus*.

The antennal sensilla of *Ae. albopictus* have undergone morphological examination by Seenivasagan (2009). Dhanalakshmi et al. (2018) have carried out a SEM analysis on the distribution of sensory structures on the antennae of female *Culex* mosquitoes. Ibrahim et al. (2018) examined the morphological characterisation and distribution of antennal sensilla in irradiated female *Cx. pipiens* mosquitoes (Diptera: Culicidae). A survey of chemoreceptive responses on different mosquito antennae by Yang et al. (2021) and studies on the sensory sensilla of tarsi and external genitalia of *Ae. albopictus* by Yamany et al., 2023 are the recent work done in this area. Though some works related to the mosquito antennae and host preferences were done in two sibling species of *Anopheles*, no other works were done correlating the host preferences and the morphology of the chemosensory organs of mosquito antennae.

CHAPTER 3
GENERAL METHODOLOGY

3.1. Study area

Wayanad, an elevated plateau in the Western Ghats located between 11°58°N – 11°30°N and 75°45°E – 76°28°E, was established on 01 November, 1980 as the 12th District of Kerala. The terms '*Vayal*' (swamps) and '*Nadu*' (place) are two local words that were combined to form the name 'WAYANAD.' It is an extension of the Deccan plateau to the west, bordered by Coorg and Mysore in the north and east; Nilgiri in the south; and Malappuram and Kozhikode in the south west (Fig. 3.1). Wayanad was previously catogorised into three regions: South Wayanad, North Wayanad, and South-east Wayanad (Nilgiri Wayanad). Wayanad's southeast section was given to Tamil Nadu, leaving the other two areas to make up the district that is the present Wayanad, which has an area of 2,130 square kilometers. The plateau is 700m above mean sea level (MSL) on average, but many of its summits are higher than 1500 meters. The only river, River Kabani, rises in the Western Ghats and flows east. Compared to the plains, the plateau has a very different climate.

A total of 885.92 sq. km. is covered by forests. The high altitude of the Wayanad district produces the ideal soil for the production of spices and perennial crops. Despite being seen as backward, this district produces crops like pepper, cardamom, coffee, tea, spices, and other condiments, making it possibly one of the State's top earners of foreign exchange. Paddy is another significant crop grown in the area and occupies roughly 19,308 hectares. The lowlands in this region offer the ideal soil for paddy farming, whilst the higher regions of the districts are the areas where tea, coffee, and spices are grown. In Wayanad, homestead farming of annuals and perennials is highly common. The main crops produced in these tiny holdings are vegetables, fruits trees like mango and jack fruit, papaya, pepper, coconut, and arecanut.

Wayanad, has a total human population of 817,420 as per Census 2011. The region experiences a tropical, humid environment with 3,000mm of annual precipitation on average. Mist is frequent from November to January, and after a few showers in April and May, the south-east monsoon contributes 75% of the yearly precipitation from June to August. This district consists of 3 Taluks viz, Vythiri, Mananthavady and Sulthan's Bathery with 49 revenue villages. Mananthavady is the only revenue division in the district. The present study was conducted in different locations of Mananthavady Taluk of Wayanad district (Table 3.1). Varieties of plantations along with favourable climatic conditions with intermittent rain, humidity and temperature ranges favour the multiplication of mosquitoes and thus an elevation of chances for vector borne diseases.

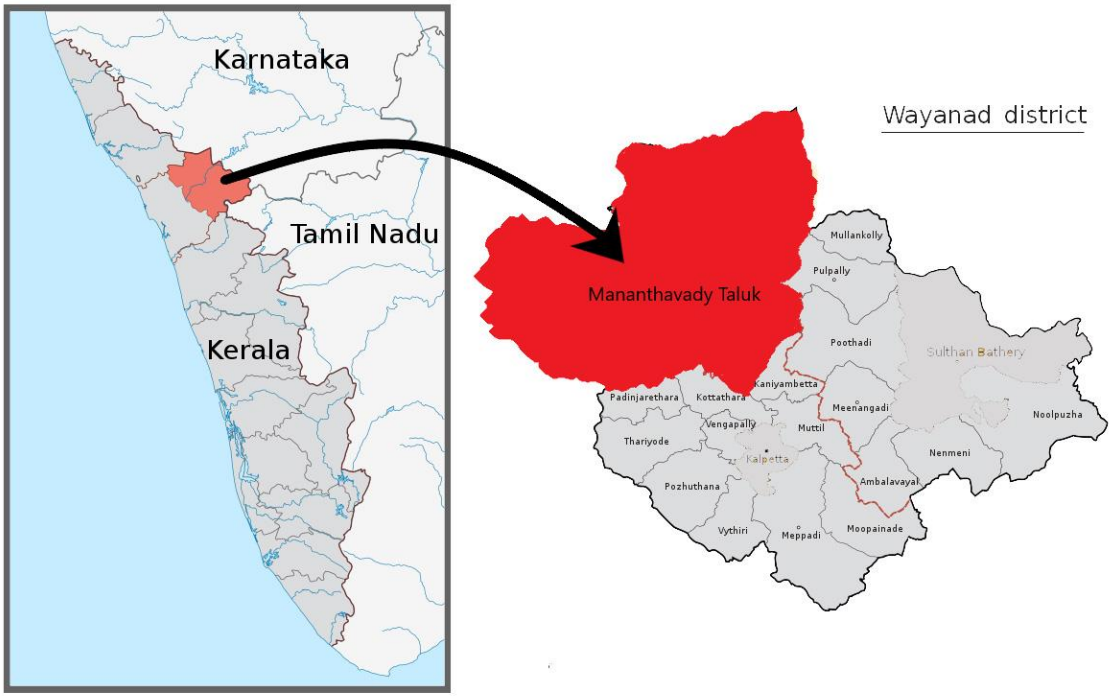

Figure 3.1: The study area selected for sampling during the present study

Table 3.1: Representative sampling sites in Mananthavady Taluk, Wayanad during the present study

Sl. No.	***Collection site***	***Geographic coordinates***
1	Mananthavady Town	11.8014° N, 76.0044° E
2	Kaniyaram	11.8192° N, 75.9908° E
3	Kuttimoola	11.8327° N,75.9913° E
4	Kallodi	11.7673° N,75.9623° E
5	Thettamala	11.7464° N, 75.9333° E
6	Makkiyad	11.7490319° N,75.90277°E
7	Kanjirangad	11.7575° N,75.9057° E
8	Paleri	11.7547° N,75.8781°E
9	Korom	11.7438° N,75.8810°E
10	Nadakkal	11.7414° N, 75.9937° E

3.2. Sample collection

Present study was conducted from October, 2019 to December, 2022. Collection has been done from each site every month during the period. Mosquitoes were collected randomly from different places of Wayanad district, Kerala, using the following techniques.

3.2.1. Immature collection

3.2.1.1. Dipping: Immature larvae were collected by dipping using ladle from different ground pools and sources like tanks, large containers and so on. The ladle was immersed in the breeding places at an angle of 45°. Four to five dips were taken from each site with an interval of 2-3 minutes between each dip. The water was examined carefully and the larvae that reached the surface were collected using a pipette and transferred to the labeled containers, filled half with water collected from the breeding place. The containers were then tied with net, plugged with cotton, and kept aside for adult emergence.

3.2.1.2. Pipetting: Larvae in small containers, coconut shells, bamboo holes, tree holes and leaf axils were collected by pipetting (Fig. 3.2). Collected larvae were transferred to the labeled containers and kept for emergence.

3.2.2. Adult collection

3.2.2.1. Light traps: Light traps were kept at different houses in Mananthavady area (Fig. 3.3). Time selected was from evening 6pm to morning 6am when the mosquitoes could be prominently spotted. The specimens collected were transferred to labeled containers, dried and preserved in 1,4-Dichlorobenzene for further studies.

3.2.2.2. Aspirator: Resting collection and man landing collection were done using Aspirator (Fig. 3.4). The mosquitoes in their biting and resting position were aspirated into the instrument and plugged with cotton at its opening. The mosquitoes were then transferred to labeled containers, dried, and preserved in 1,4-Dichlorobenzene for taxonomic studies.

3.2.2.3. Sweep net: The flying mosquitoes were trapped by sweeping net manually over the mosquitoes (Fig. 3.5). The collected ones were aspirated into labeled specimen jars and preserved in 1,4-Dichlorobenzene for identification. Sweep net collection method was commonly adopted in different plantations like coffee, pepper, banana, cashew, rubber, areca palm and mixed plantations.

3.3. Specimen preservation and mounting

3.3.1. Tube mounting: Adults collected in the field were transported to the laboratory and killed using ethyl acetate. Each adult was mounted on a Minuten Pin under a stereo microscope. The pin was inserted along the lateral side of the mosquito keeping the proboscis towards left. After pinning the other end of the pin was inserted in to the cork using a forceps. Care was taken in the arrangements of wings and legs to make all characters visible for the study. Cork was inserted into a glass vial and preserved using 1,4-Dichlorobenzene to safeguard the specimen from fungus and from other insects (Fig. 3.6).

3.3.2. Slide mounting: For the correct identification of the species, associated larvae of some of the adults as well as the genitalia of some of the species were slide mounted. Hoyer's medium is considered to the best medium for mosquito material by Belkin (1962). It is the modified of Berlese's medium, prepared by dissolving 30g of clear gum arabic in 50ml of distilled water. After allowed to stand for overnight, filtered through four- or five-fold muslin cloth and then add 200g of

chloral hydrate ($C_2H_3Cl_3O_2$) by stirring thoroughly followed by the addition of 20ml Glycerine.

3.3.3. Whole larva mounting: The specimens were kept in the clearing reagent (Gater's fluid: 80g of chloral hydrate in 20ml of 30% acetic acid) for one day after pinning one or two places in the sides of thorax and abdomen. The specimens were then transferred to the slides with the clearing reagent and an incision was made carefully in the VII segment using dissection needle without damaging the hairs and were mounted in Hoyer's medium (Fig. 3.7).

3.3.4. Male genitalia mounting: For the species determination of certain mosquitoes, structure of male genitalia was observed (Fig. 3.8). Therefore, slide mounting of male genitalia was made using Hoyer's medium. The procedure followed was: (i) adults were relaxed in a moist test tube for at least 2 hours individually, (ii) the tip of the abdomen was cut at about the middle of segment VII with the pair of scissors under the stereoscopic microscope, (iii) the genitalia was transferred to the diluted soap solution for 12 hours, (iv) the specimen was washed in distilled water and transferred to clearing reagent for 12 hours. (v) the specimen was transferred to a slide in a drop of clearing agent and the segment VII was torn off gently with a needle to separate the sternite and tergite, and (vi) the genitalia were mounted in Hoyer's medium with the ventral side on the top.

Figure 3.2: Pippetting the larvvae from the tree hole

Figure 3.3: light trap for adult collection

Figure 3.4: Using Aspirator for adult collection

Figure 3.5: Sweep net collection

Figure 3.6: Tube mounting the adult

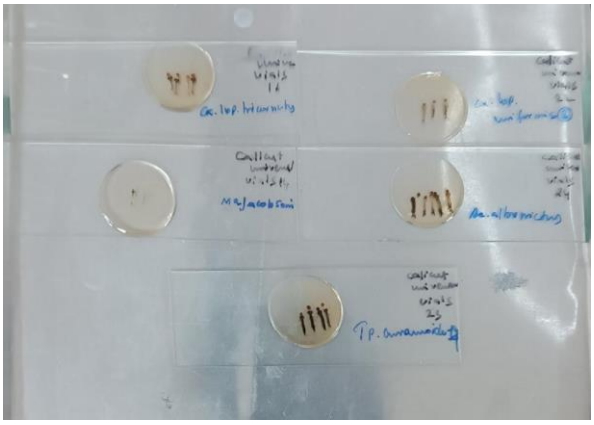

Figure 3.7: Whole larva mounting

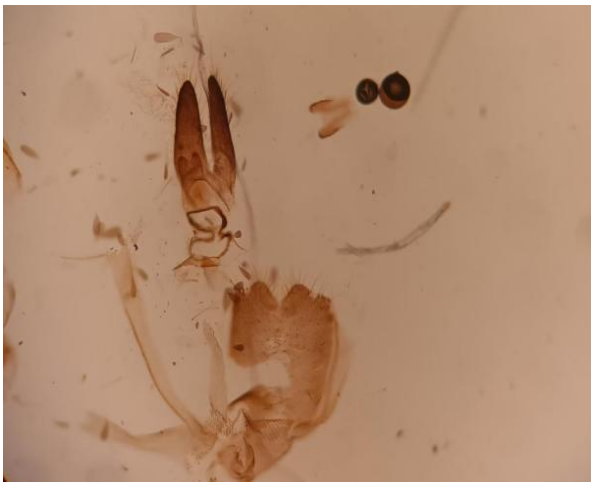

Figure 3.8: Genitalia mounting

3.4. Morphological identification of mosquitoes

Taxonomic identification of adults and larvae were carried out under microscopes – Carl Zeiss Stemi 2000 (Stereomicroscope) and Olympus CH-2 (Compound microscope). All the collected mosquitoes were preserved using 1,4-Dichlorobenzene. They were identified to the species level using taxonomic keys of Christophers (1933), Barraud (1934), Reinert (1973), Reid (1968), Sirivanakaran (1972, 1976, 1977), Harbach (2017) and confirmed with the help of experts from ICMR – Vector Control Research Centre, Pondicherry, India.

3.5. Molecular identification of mosquitoes

Complete genomic DNA from the individual mosquitoes were extracted using DNA extraction kit (Macherey-Nagel Inc.) in accordance with the manufacturer's instructions and the mitochondrial cytochrome oxidase subunit I (MT-CO1) marker gene sequence was amplified by polymerase chain reaction (PCR) using the appropriate primers (Table 3.2). A 50µl reaction volume containing 5µl of template DNA, 5µl of 10X reaction buffer (100mM Tris pH 9.0, 500mM KCl, 15mM $MgCl_2$, 0.1% Gelatin), 1µl of 10mM dNTPs, 1µl of each primer, 0.5µl Taq polymerase (2.5 units), and nuclease-free water was used for the PCR reaction. PCR conditions for CUL primers (Kumar et al., 2007) were as follows: an initial denaturation of 5min (95°C) was followed by five cycles of 94°C for 40sec (denaturation), 45°C for 1min (annealing), and 72°C for 1min (extension) and 35 cycles of 94°C for 40sec (denaturation), 51°C for 1min (annealing), 72°C for 1min (extension), and a final extension at 72°C for 10min.

The PCR thermal profile for LCO-HCO primers (Folmer et al., 1994) was as follows: initial heating to 95^0 C for 5min after which the cycle begins by DNA denaturation, allowed at 95^0 C for 10sec, followed by annealing of primer allowed at 52^0 C for 1min after which extension is allowed at 72^0 C for 45sec. The number of cycles is set at 35 followed by a final extension at 72^0 C for 7min.

Table 3.2: List of primer sequences used for the PCR amplification of marker gene regions in the present study

Primer Name	***Target Region***	***Direction***	***Base Sequence (5' → 3')***
CUL F	MT-CO1	Forward	GGATTTGGAAATTGATTAGTTCCTT
CUL R	MT-CO1	Reverse	AAAAATTTTAATTCCAGTTGGAACAGC
LCO 1490	MT-CO1	Forward	GGTCAACAAATCATAAAGATATTGG
HCO 2198	MT-CO1	Reverse	GCGAACAAGTACCGTGAGGG

A 1% agarose gel was used (Fig. 3.9) to test the integrity of the amplified fragments before performing sequencing. The sequencing of the amplified DNA fragment from both forward and reverse ends were performed using the Sanger's dideoxy chain termination sequencing method (Sanger and Coulson, 1975) at Agrigenome Laboratories Ltd., Cochin with ABI 3730XL automated sequencer. There is a higher probability of obtaining longer sequences by sequencing from both ends while using forward and reverse primers than by only using a primer in one way. From electropherograms, sequences were extracted using the Finch TV (Treves, 2010) application. In order to prevent sequencing errors, forward and reverse sequences of CO1 gene segments from each specimen were repeatedly aligned using the ClustalW (Thompson et al., 1994) programme integrated with BioEdit software. The 5'-3' contig sequence that was created using both the forward and reverse sequences were then submitted in FASTA format to the public database of the INSDC (International Nucleotide Sequence Database Collaboration) of NCBI GenBank, where it was assigned an accession number for all the sequences submitted. The Basic Local Alignment Search Tool (BLAST) was used to determine how similar the sequences were to other sequences in the NCBI database (Altschul et al., 1990). The BLAST search yielded similarities with mosquito sequences that are already in the database.

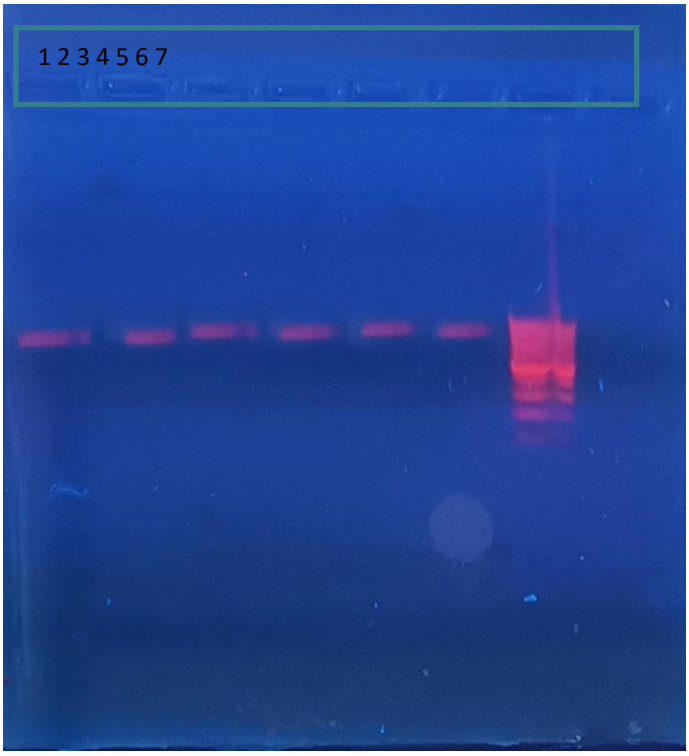

Figure 3.9. 1% Agarose gel showing (a) Lanes 1- 6-amplified CO1 region (~700bp), (b)lane 7 1kb DNA Ladder.

3.6. Molecular phylogenetic analyses

The Molecular Evolutionary Genetics Analysis Version X (MEGA X) software (Kumar et al., 2018) was used for phylogenetic analysis in order to analyze maximum likelihood (ML). A minimal Akaike information criterion (AIC) value (Posada and Buckley, 2004) and Bayesian Information Criterion (BIC) value were used to select the best-fit nucleotide substitution model among the 24 models offered in MEGA X (Kumar et al., 2018). The bootstrap values ran for 1000 iterations were used to estimate the reliability of the Maximum Likelihood phylogenetic tree. Kimura's 2-parameter model was used to create a phylogenetic tree and generate the intraspecific and interspecific genetic diversity (Kimura, 1980). MEGA X software was used to calculate percentage nucleotide distances. The findings were displayed as corresponding trees and tables. Nodes with ML bootstrap (BS) values greater than 90 were considered to be highly supported, those with BS values between 70 and 90 were considered to be moderately supported, and those with BS values between 50 and 69 were considered to be weakly supported. BS values under 50 were regarded as being unsubstantiated.

3.7. Blood meal analysis

Serological techniques based on the precipitin reaction have been widely used for the identification of unknown bloods. For blood meal analysis, blood fed mosquitoes were collected from cattle sheds, piggery, and human dwellings from different selected area (Fig. 3.10). Resting collection was done from 6pm to 6am. Collected mosquitoes were identified based on taxonomic keys (Christophers, 1933; Barraud, 1934) and the blood from the abdomen were spotted on the Whatman No.1 filter paper using the blunt end of the needle (Fig. 3.11). Each sample was properly labelled on the filter paper. After drying filter papers were stored at 4℃.

Anti bovine, anti pig and anti human antiserums were used for the analysis. Antigen preparation (source of blood meal) was done by cutting the mosquito blood meal spot on the filter pater into small pieces and soaked in 50µl of phosphate buffered saline (PBS) in a sterile 1.5 microcentrifuge and incubated at room temperature for overnight. Following day 10µl of antiserum and 10µl of antigen were loaded in 1% agarose gel according to the template (Fig. 3.12) prepared based on the number of samples and antiserum. Gel tray was stored in closed chamber and kept overnight and precipitin bands were formed on the following day (Fig. 3.13).

Figure 3.10: Resting collection using Aspirator.

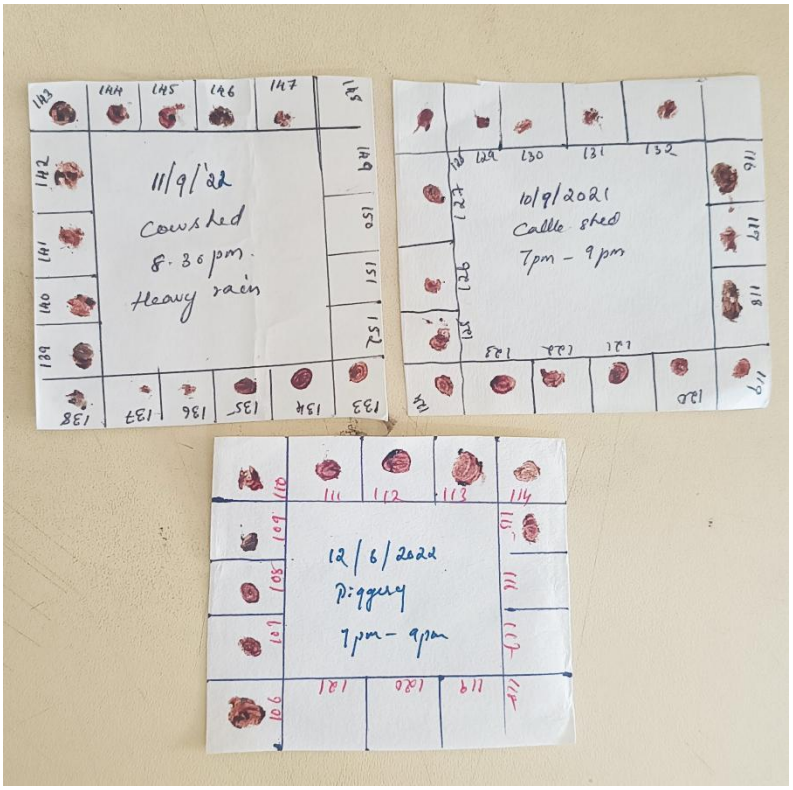

Figure 3.11: Whatman filter paper with blood samples

	1	2	3	4	5	6	7	8	9
1	H	S1	B	S2	H	S3	B	S4	H
2	S5	P	S6	P	S7	P	S8	P	S9
3	B	S10	H	S11	B	S12	H	S13	B
4	S14	P	S15	P	S16	P	S17	P	S18
5	H	S19	B	S20	H	S21	B	S22	H
6	S23	P	S24	P	S25	P	S26	P	S27
7	B	S28	H	S29	B	S30	H	S31	B

Figure 3.12: No. of antiserum tested: 3, Human, bovine and Pig antiserums

H = Human antiserum, B = Bovine antiserum, P= Pig antiserum

S = Sample (Source of mosquito blood meal)

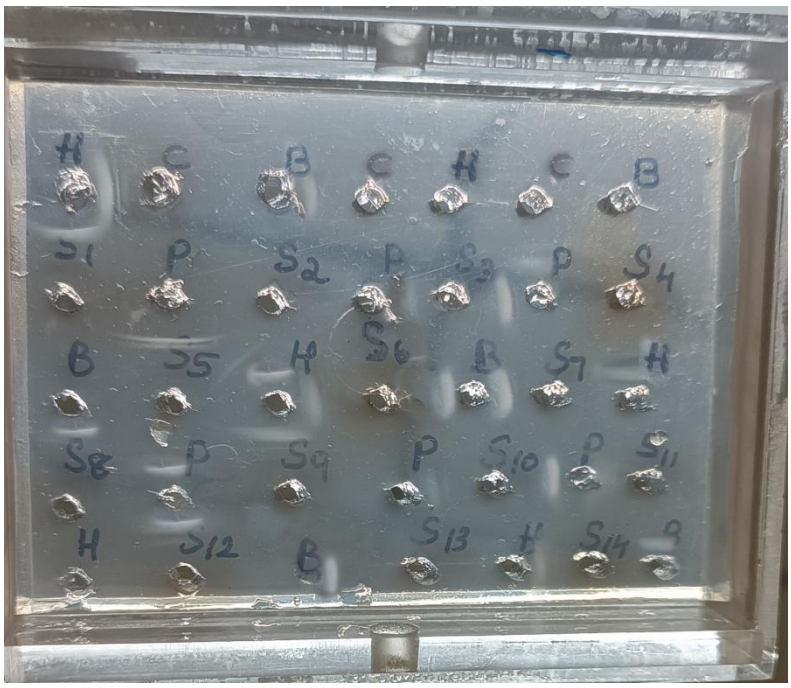

Figure 3.13: Agarose gel with precipitin bands

3.8. Analysis of cephalic olfactory structures

Antennae, maxillary palps, and proboscis of female mosquitoes were hand dissected from anesthetized specimens. For scanning electron microscopic analysis, the dry samples were mounted on a 1cm diameter stub with conductive carbon tape and stored at 30°C overnight. The sample was sputtered with gold palladium metal sheet for 50sec in a Quorum SC 7620 sputter coater. The imaging of olfactory structures was carried out using Zeiss Gemini Field Emission Scanning Electron Microscope 300.

CHAPTER 4
MORPHOTAXONOMY OF MOSQUITOES

4.1. Specimen collection data

Family Culicidae belongs to the Order Diptera, a large and abundant group of mosquitoes that occur throughout temperate and tropical regions of the world, well beyond the Arctic Circle. The family includes 3724 extant species classified in two subfamilies and 113 genera. The Subfamily Anophelinae comprises of three genera and Subfamily Culicinae has 110 genera segregated into 11 tribes. According to the traditional classification the family and subfamily Culicinae include 41 and 38 genera respectively. (Mosquito Taxonomic Inventory).

During the present study, a total of 80 species of mosquitoes belonging to 12 genera in 6 tribes and 2 subfamilies were collected and identified based on taxonomic keys (Christophers, 1933; Barraud, 1934; Reinert, 1973; Sirivanakaran, 1972, 1976, 1977; Harbach, 2017) and confirmed with the help of experts from ICMR – Vector Control Research Centre, Pondicherry, India (Table 4.1). 19 species were recorded from Sub family Anophelinae. In Subfamily Culicinae, most number of mosquito species were recorded from the Tribe Culicini (25), followed by Tribes Aedini (20),Uranotaeniini (9), Mansoniini and Sabethinii (3 each), and Orthopodomyiini (1). Single species each from genera *Verrallina, Tripteroides, Lutzia, Orthopodomyia,* and *Heizmannia* were recorded .

Table 4.1: The list of species collected from Mananthavady Taluk during the present study

Sl. No.	Taxa	Status
	ORDER: DIPTERA **FAMILY: CULICIDAE** **SUBFAMILY: CULICINAE** Meigen, 1818 **Tribe 1:Aedeomyiini** Theobald, 1901 **Genus*: Aedes*** Meigen, 1818 **Subgenus: Aedimorphus** Theobald, 1903	
1	*Aedes*(*Aedimorphus*) *vexans* Meigen, 1830	Reported from Kerala Sumodan (2014)
	Subgenus: Stegomyia Theobald, 1901	
2	*Aedes*(*Stegomyia*)*aegypti* Linnaeus, 1762	Reported from KeralaBarraud (1934)
3	*Aedes*(*Stegomyia*)*albopictus* Skuse, 1894	Reportedfrom KeralaBarraud (1934)

4	*Aedes(Stegomyia) subalbopictus*Barraud, 1931	Reported from Kerala Sumodan (2012)
5	*Aedes(Stegomyia)novalbopictus* Barraud, 1931	Reported from Kerala Thankachan et al., (2017)
6	*Aedes(Stegomyia) pseudalbopictus* Borel, 1928	Reported from Kerala Thankachan et al.,(2017)
	Subgenus: *Phagomyia* Theobald, 1905	
7	*Aedes (Phagomyia) cogilli* Edwards, 1922	Reported from Kerala Sumodan (2012)
	Subgenus: *Christophersiomyia* Barraud, 1923	
8	*Aedes(Christophersiomyia) thomsoni* Theobald, 1905	**First Report from Kerala**
	Subgenus: *Alloeomyia* Reinert, Harbach & Kitching, 2008	
9	*Aedes(Alloeomyia) pseudotaeniatus* Giles, 1901	Reported from Kerala Aneesh et al., (2014)
	Subgenus: *Hulecoeteomyia* Theobald, 1904	
10	*Aedes (Hulecoeteomyia) harveyi* Barraud, 1923	Reported from Kerala Sumodan (2012)
11	*Aedes (Hulecoeteomyia) chrysolineatus* Theobald, 1907	Reported from KeralaBarraud (1934)
	Subgenus: *Neomelaniconion* Newstead, 1907	
12	***Aedes (Neomelaniconion) lineatopenne* Ludlow, 1905**	**First Report from Kerala**
	Subgenus:*Paraedes* Edwards, 1934	
13	*Aedes (Paraedes) menoni*Mattingly, 1958	Reported from KeralaMattingly (1958)
14	*Aedes (Paraedes) barraudi* Edwards, 1934	Reported from KeralaTewari & Hiriyan (1994)
	Subgenus: *Downsiomyia* Vargas, 1950	
15	*Aedes (Downsiomyia) niveus* Ludlow, 1903	Reported from Kerala Aneesh et al., (2014)
	Subgenus*: Fredwardsius* Reinert, 2000	
16	*Aedes (Fredwardsius) vittatus* Bigot, 1861	Reported from KeralaBarraud (1934)
	Genus*: Armigeres* Theobald, 1901 **Subgenus: *Armigeres*** Theobald, 1901	
17	*Armigeres* (*Armigeres*) *subalbatus* Coquillett, 1898	Reported from Kerala Barraud (1934)
18	*Armigeres (Armigeres) aureolineatus* Leicester, 1908	Reported from Kerala Barraud (1934)
	Genus: *Heizmannia* Ludlow, 1905 **Subgenus: *Heizmannia*** Ludlow, 1905	
19	*Heizmannia (Heizmannia) chandi* Edwards, 1922	Reported from KeralaEdwards (1922)
	Genus*: Verrallina* Theobald, 1903 **Subgenus*: Neomacleaya*** Theobald, 1907	
20	***Verrallina (Neomacleaya) indica* Theobald, 1907**	**First Report from Kerala**

	Tribe 2: Culicini Meigen, 1818 **Genus*: Culex*** Linnaeus, 1758 **Subgenus*: Culex*** Linnaeus, 1758	
21	*Culex (Culex) gelidus* Theobald, 1901	Reported from KeralaBarraud (1934)
22	*Culex (Culex) fuscocephala* Theobald, 1907	Reported from KeralaArunachalam et al (2004)
23	*Culex (Culex) tritaeniorhynchus* Giles, 1901	Reported from Kerala Giles (1901)
24	*Culex (Culex) mimulus* Edwards, 1915	Reported from KeralaBarraud (1934)
25	*Culex (Culex) quinquefasciatus* Say, 1823	Reported from Kerala Barraud (1934)
26	*Culex (Culex) pseudovishnui* Colless, 1957	Reported from Kerala Rajavel et al (2006)
27	*Culex (Culex) vishnui* Theobald, 1901	Reported from Kerala Barraud (1934)
28	***Culex (Culex) barraudi* Edwards, 1922**	**First report from Kerala**
29	***Culex (Culex) hutchinsoni* Barraud, 1924**	**First report from Kerala**
30	***Culex (Culex) murrelli* Lien, 1968**	**First report from Kerala**
31	*Culex (Culex) whitmorei* Giles, 1904	Reported from KeralaBarraud (1934)
	Subgenus: *Oculeomyia* Theobald, 1907	
32	*Culex* (Oculeomyia) *bitaeniorhynchus* Giles, 1901	Reported from Kerala Giles (1901)
33	*Culex (*Oculeomyia) *sinensis* Theobald, 190	Reported from Kerala Balasubramanian & Nikhil, 2013
34	*Culex (*Oculeomyia) *infula*Theobald, 1901	Reported from Kerala Arunachalam et al (2004)
	Subgenus: *Culiciomyia* Theobald, 1907	
35	***Culex (Culiciomyia) nigropunctatus* Edwards, 1926**	**First Report from Kerala**
36	*Culex (Culiciomyia) pallidothorax* Theobald, 1905	Reported from Kerala Balasubramanian & Nikhil, 2013
	Subgenus*: Eumelanomyia*Theobald, 1909	
37	*Culex (Eumelanomyia) malayi* Leicester, 1908	Reported from KeralaBalasubramanian & Nikhil, 2013
38	***Culex (Eumelanomyia) foliatus* Brug, 1932**	**First report from Kerala**
39	*Culex (Eumelanomyia) brevipalpis* Giles, 1902	Reported from KeralaMariappan et al (1996)
	Subgenus: *Lophoceraomyia* Theobald, 1905	
40	*Culex (Lophoceraomyia) uniformis* Theobald, 1905	Reported from KeralaSumodan (2012)
41	***Culex (Lophoceraomyia) bicornutus* Theobald, 1910**	**First Report from Kerala**
42	***Culex (Lophoceraomyia) wilfredi* Colless, 1965**	**First report from Kerala**

43	***Culex (Lophoceraomyia) minor* Leicester, 1908**	**First report from Kerala**
44	*Culex (Lophoceraomyia) cinctellus* Edwards, 1922	Reported from KeralaBarraud (1934)
	Genus*: Lutzia* Theobald, 1903 **Subgenus: *Metalutzia*** Tanaka, 2003	
45	***Lutzia (Metalutzia)*** *halifaxii* **Theobald, 1903**	**First report from Kerala**
	Tribe 3:Mansoniini Belkin, 1962 **Genus: *Mansonia*** Blanchard, 1901 **Subgenus: Mansonioides** Theobald, 1907	
46	*Mansonia* (*Mansonioides*) *indiana* Edwards, 1930	Reported from Kerala Iyengar (1932)
47	*Mansonia* (*Mansonioides*) *uniformis* Theobald, 1901	Reported from Kerala Theobald (1901)
48	*Mansonia* (*Mansonioides*) *annulifera* Theobald, 1901	Reported from KeralaTheobald (1901)
	Tribe 4: Uranotaeniini Lahille, 1904 **Genus: *Uranotaenia*** Lynch Arribalzaga, 1891 **Subgenus: *Pseudoficalbia*** Theobald, 1912	
49	***Uranotaenia (Pseudoficalbia) obscura* Edwards, 1915**	**First Report from Kerala**
50	***Uranotaenia (Pseudoficalbia) nivipleura* Leicester, 1908**	**First report from Kerala**
51	*Uranotaenia (Pseudoficalbia) pseudostricklandi* Natarajan, Rajavel & Jambulingam, 2018	Reported from Kerala Natarajan et al., (2018)
	Subgenus: *Uranotaenia* *L*ynch Arribalzaga, 1891	
52	***Uranotaenia(Uranotaenia)* rutherfordi Edwards, 1922**	**First report from Kerala**
53	*Uranotaenia (Uranotaenia) testacea* Theobald, 1905	Reported from KeralaMariappan et al (1997
54	***Uranotaenia (Uranotaenia) macfarlanei* Edwards, 1914**	**First report from Kerala**
55	***Uranotaenia (Uranotaenia) campestris* Leicester, 1908**	**First Report from Kerala**
56	***Uranotaenia (Uranotaenia)* sp. 1**	**New recport**
57	***Uranotaenia (Uranotaenia)* sp. 2**	**New report**
	Tribe 5: *Sabethini* Blanchard, 1905 **Genus: *Malaya*** Leicester, 1908	
58	***Malaya genurostris* Leicester, 1908**	**First report from Kerala**
59	***Malaya jacobsoni* Edwards, 1930**	**First report from Kerala**
	Genus: *Tripteroides* Giles, 1904 **Subgenus: Rachionotomyia** Theobald, 1905	
60	***Tripteroides (Rachionotomyia)*** *aranoides* **Theobald, 1901**	**First Report from Kerala**
	Tribe 6: Orthopodomyiini Belkin, Heinemann & Page, 1970 **Genus: *Orthopodomyia*** Theobald, 1904	

61	*Orthopodomyia anopheloides* Giles, 1903	Reported from KeralaBarraud (1934)
	SUBFAMILY:ANOPHELINAE Grassi, 1900 **Genus: *Anopheles***Meigen, 1818 **Subgenus: Anopheles** Meigen, 1818	
62	*Anopheles (Anopheles) barbirostris* van der Wulp, 1884	Reported from Kerala (Christophers (1933)
63	*Anopheles (Anopheles) insulaeflorum* Swellengrebel & Swellengrebel de Graaf, 1920	Reported from Kerala(Nagpal and Sharma (1995)
64	*Anopheles (Anopheles) peditaeniatus* Leicester, 1908	Reported from Kerala Arunachalam et al (2004)
65	*Anopheles* (*Anopheles*) *aitkenii* James, 1903	Reported from Kerala (Nagpal and Sharma (1995)
66	*Anopheles (Anopheles) nigerrimus* Giles, 1900	Reported from Kerala(Nagpal and Sharma (1995)
67	*Anopheles (Anopheles) culiciformis* Cogill, 1903	Reported from Kerala (Christophers (1933)
68	***Anopheles (Anopheles) crawfordi* Reid, 1953**	**First Report from Kerala**
	Subgenus: Cellia Theobald, 1902	
69	*Anopheles (Cellia) elegans* James, 1903	Reported from Kerala (Nagpal and Sharma (1995)
70	*Anopheles (Cellia) vagus* Donitz, 1902	Reported from Kerala(Christophers (1933)
71	*Anopheles (Cellia) jamesii* Theobald, 1901	Reported from Kerala(Christophers (1933)
72	*Anopheles (Cellia) tessellatus* Theobald, 1901	Reported from Kerala (Christophers (1933)
73	*Anopheles (Cellia) subpictus* Grassi, 1899	Reported from Kerala (Christophers (1933)
74	*Anopheles (Cellia) splendidus* Koidzumi, 1920	Reported from Kerala (Christophers (1933)
75	*Anopheles (Cellia) karwari* James, 1903	Reported from Kerala (Christophers (1933)
76	*Anopheles (Cellia) stephensi* Liston, 1901	Reported from Kerala(Christophers (1933)
77	*Anopheles (Cellia) jeyporiensis* James, 1902	Reported from Kerala(Christophers (1933)
78	*Anopheles (Cellia)fluviatilis* James, 1902	Reported from Kerala (Christophers (1933)
79	*Anopheles (Cellia) theobaldi* Giles,1901	Reported from Kerala
80	*Anopheles (Cellia) culicifacies* Giles,1901	Reported from Kerala(Christophers (1933)

Twenty nine mosquito species among the collected specimens were disease vectors belonging to five genera: *Aedes*,*Culex Anopheles*, *Mansonia* and *Armigeres* (Table 4.2)..This include *Ae.aegypti* (a major vector of Dengue) , *An.stephensi* (a major Vector of Malaria), *Cx.tritaeniorhyncus* (a major vector of Japanese Encephalitis) and so on.

Table 4.2: Vectoral status of mosquito species from the study area

Sl. No.	Genus	Species	Vectoral status to diseases
1.	*Culex*	*tritaeniorhyncus*	Japanese Encephalitis, West Nile (Pushparaj et al., 2018)
2.	*Culex*	*vishnui*	JapaneseEncephalitis,Filariasis (Pushparaj et al., 2018)
3.	*Culex*	*pseudovishnui*	Japanese Encephalitis (Sarika et al., 2012)
4.	*Culex*	*gelidus*	Japanese Encephalitis, Filariasis (Sarika et al., 2012)
5.	*Culex*	*infula*	Japanese Encephalitis (Ranjana et al.,2021)
6.	*Culex*	*bitaeniorhyncus*	Japanese Encephalitis, Filariasis (Thenmozhi et al., 2013)
7.	*Culex*	*fuscocephala*	Japanese Encephalitis (Pushparaj et al., 2018)
8.	*Culex*	*quinquefasciatus*	Japanese Encephalitis, Filariasis, West Nile (Thenmozhi et al., 2013)
9.	*Culex*	*whitemorei*	Japanese Encephalitis (Sarika et al., 2012)
10.	*Anopheles*	*stephensi*	Malaria, Filariasis (Saeung et al ., 2013)
11.	*Anopheles*	*vagus*	Malaria, (Alam et al., 2017)
12.	*Anopheles*	*subpictus*	Japanese Encephalitis (Sarika et al., 2012)
13.	*Anopheles*	*elegans*	Malaria (Subbarao et al., 2019)
14.	*Anopheles*	*fluvialitis*	Malaria (Subbarao et al., 2019)
15.	*Anopheles*	*nigerrimus*	Malaria, Filariasis (Saeung et al ., 2013)
16.	*Anopheles*	*barbirostris*	Japanese Encephalitis, Filariasis, (Claudia et al., 2009)
17.	*Anopheles*	*peditaeniatus*	Japanese Encephalitis, Filariasis (Sarika et al., 2012), (Saeung et al ., 2013)
18.	*Anopheles*	*jeyporienis*	Malaria (Dash et al., 2007)
19.	*Anopheles*	*crawfordi*	Filariasis (Saeung et al ., 2013)
20.	*Anopheles*	*culicifacies*	Malaria (Dash et al., 2007)
21.	*Aedes*	*albopictus*	Dengue, Chikungunya, Zika, (Carrington et al., 2014)
22.	*Aedes*	*vexans*	West Nile, Rift Valley (Birnberg et al., 2019)
23.	*Aedes*	*aegypti*	Dengue, Chikungunya, Yellow fever, (Carrington et al., 2014)
24.	*Aedes*	*vittatus*	Dengue, Chikungunya (Sudeep , 2017)
25.	*Aedes*	*niveus*	Filariasis, (Das et al., 2004)
26.	*Mansonia*	*uniformis*	Japanese Encephalitis, Filariasis (Sarika et al., 2012)
27.	*Mansonia*	*indiana*	Japanese Encephalitis, Filariasis (Pratiwi et al., 2021)
28.	*Mansonia*	*annulifera*	Japanese Encephalitis, Filariasis (Pratiwi et al., 2021)
29.	*Armigeres*	*sabalbatus*	Japanese Encephalitis, (Liu et al., 2013, Aneesh et al., 2014)

The taxonomic keys were prepared for the species collected and identified from the study area during the present study. Adult keys to the Genus, Subgenus and species level are provided below. Descriptions and images of the species collected from Mananthavady Taluk, Wayanad is given (Fig. 4.1).

4.2 Taxonomic key to the genus, subgenus and species collected from Mananthavady Taluk, Wayanad

1. Palpus about equal to the length of proboscis; scutellum evenly rounded; abdomen with sterna and terga largely or wholly devoid of scales..... (Genus: ***Anopheles***) 55

– Palpus is shorter than proboscis; scutellum tri-lobed; abdomen with scales 2

2. Vein 6 ending well beyond level of fork of vein 5; margin of squama with fringed 3

– Vein 6 short, ending before or at about level of fork of vein 5 ---- (Genera: ***Malaya, Uranotaenia***) 47

3. Pulvilli present 4

– Pulvilli absent or rudimentary 5

4. Four or more lower mesepimeral bristles present; very narrow pale apical bands ..... ***Lutzia halifaxi***

– None, one or two lower mesepimeral bristles present ---- (Genus: ***Culex***) 29

5. Post-spiracular bristles absent (Genera: ***Tripteriodes, Orthopodomyia,*** **Heizmannia**) -..... 6

– Post-spiracular bristles present ---- (Genera: ***Mansonia, Armigeres, Aedes)*** --- 8

6. Spiracular bristles present; femora unspotted; margin of mesonotum not white; scales around eye margin with not brilliant blue reflection .... ***Tripteroides aranoides***

– Spiracular bristles absent ..... - 7

7. First segment of fore tarsi longer than last four segment together; postnotum without setae; three white spots on the costa on apical ½ of wing; segment 4 of hind tarsi with black subapical ring, segment 5 white .... ***Orthopodomyia anopheloides***

– First segment of fore tarsi not so; postnotum with setae; antepronotum with silvery white scale; outstanding plume scales vein 2.1and 2.2 line . ... ***- Heizmannia chandi***

8. Wings scales usually broad many with asymmetrical; brown to yellowish in colour ..... (Genus: ***Mansonia***) 9

– Wings scales not broad and not asymmetrical 11

9. Mesonotum with distinct 4-6 white spot; yellowish colour; broad scales in mid lobe of scutellum ..... ***Mansonia annulifera***

– Mesonotum without distinct white spot; brown to dark brown colour 10

10. Mesonotum with a pair of sub lateral greenish stripe; post-pronotum with broad scales ..... ***Mansonia uniformis***

– Mesonotum dark brown with indistinct spots of scales; post-pronotum with narrow scales ..... ***Mansonia indiana***

11. Proboscis is stout with somewhat laterally compressed on downwards towards tip .....(Genus: ***Armigeres***)..... 12

– Proboscis slender and straight ..... (Genus: ***Aedes***) 13

12. Mesonotum with a pair of well-defined sub median gold lines: lateral white marking on abdominal tergites produce on to dorsum ....***Armigeres aureolineatus***

– Mesonotum and abdominal tergites not so ..... ***Armigeres subalbatus***

13. Segment VIII narrow and completely retractile; cerci long, narrow and projecting from segment VII 14

– Segment VIII broad and completely retractile; cerci short and broad 17

14. Mesonotum with conspicuous lateral yellow scaling; abdomen with pale yellow basal band ..... ***Neomelaniconion lineatopenne***

– Mesonotum and abdomen scaling not so 15

15. Hind tarsi marked with distinct basal pale bands; acrostichal setae present; pale scales on anterior surface of mid-femur evenly sprinkled; both claws of hind tarsi simple ..... ***Aedimorphus vexans***

– Hind tarsi dark; acrostichal setae absent; scutum with golden scale on acrostichal area ..... (Subgenus: ***Paraedes***) 16

16. Broad scale on antepronotum ..... ***Paraedes barraudi***

– Narrow scale on antepronotum ..... ***Paraedes menoni***

17. Hind tarsi completely dark 18

– Hind tarsi pale rings 19

18. Broad pale patch on anterior scutum; scutellar scales broad ..... ***Finlaya niveus***

– Pale patch on anterior scutum absent; scutellar scale narrow (female genitalia examined to differentiate species) ..... ***Verrallina indica***

19. Proboscis with a white ring; absence of white spots on scutum; hind tibia with a white ring on basal half .....***Christophersiomyia thomsoni***

– Proboscis dark 20

20. Scutum with 4-6 prominent white spots; all tibia with white ring .... ***Fredwarsisus vittatus***

– Scutum with a patch of white scales or with pale or yellow line of narrow scales 21

21. Hind tarsal segments with both apical and basal white rings.....22

– Hind tarsal with basal white rings only 23

22. Mesonotum with white patch in front; post-pronotum with only small patch of white scales on posterior border .....***Finlaya cogilli***

– Mesonotum with pale lines; femora with white longitudinal line for whole length .....***Finlaya pseudotaeniatus***

23. Dark brown species: mesonotum marked with narrow lines of golden scales.....24

– Black species with snow white marking . ..... (Subgenus: ***Stegomyia***) 25

24. Proboscis pale on underside; mid femur dark on anterior surface ....***Finlaya harveyi***

– Proboscis pale scaling both on upper and underside .....***Finlaya chrysolineatus***

25. Mesonotum with a pair of lateral curved white lines; two dots of white scales on clypeus ..... ***Stegomyia aegypti***

– Mesonotum with narrow white line; clypeus without white scales 26

26. Wing root with broad white scales .....***Stegomyia albopictus***

– Wing root with narrow white/yellowish scales 27

27. Wing root with yellowish scales; fore &mid femora with some pale scales scattered on anterior surface .....***Stegomyia novalbopictus***

– Wing root with white scales 28

28. Pre-scutellar space with patch of broad dark scales on each side; post-spiracular area with scales .....***Stegomyia pseudoalbopictus***

– Pre-scutullar space and postspicular area without scales .... ***Stegomyia subalbopictus***

29. Pleuron with distinct scale patches on at least upper and lower mesokatepisternum and anterior mesepimeron ..... (Subgenus: ***Culex***) 30

– Pleuron without distinct scale patches 41

30. Abdominal terga II-VI with apical bands and/or apical lateral pale patches .. 31

– Abdominal terga II-VI with basal pale bands only 33

31. Wing with dark scales on all veins; anterior surface of fore and mid femora moderately to strongly speckled with numerous pale scales; prosternum with a small patch of pale scales; clypeus with any pale scales .....***Culex sinensis***

– Wing with a mix of dark and pale scales 32

32. Abdominal terga II-VII with broad apical bands ..... ***Culex bitaeniorhynchus***

– Abdominal terga II-VI largely dark or with narrow apical bands ...***Culex infula***

33. Proboscis dark 34

– Proboscis with pale band on middle 35
34. Abdominal terga without pale bands; pleuron with striking pattern of dark and pale stripes ..... ***Culex fuscophala***

– Abdominal terga with pale bands; pleuron without pattern of dark and pale stripes ..... ***Culex quinquefasciatus***
35. Wing with pale spot on at least two areas of costa and 1 area of other veins (larvae for species confirmation) ..... ….....***Cx. mimulus, Cx. murrelli*** and ***Cx. hutchinsoni***

– Wing without distinct pale spots 36
36. Anterior 0.7 of scutum covered with pure white scales 37

– Anterior 0.7 of scutum covered with yellow, golden, or dark scales 38
37. Anterior surface of fore and mid femora without speckling of pale scales; pale band of proboscis narrow .....***Culex gelidus***

– Anterior surface of fore and mid femora speckled with pale scales; pale band of proboscis broad .....***Culex whitmorei***
38. Mid-femur with longitudinal pale stripe on anterior surface; pale scales on postspiracular area ..... ***Culex barraudi***

– Mid-femur without longitudinal pale stripe; post-spiracular without pale scales . ….....39
39. Anterior surface of fore and mid femora speckled with pale scales; anterior surface of hind femur with pale stripe not contrasting with dark scaled area .. ...… ….....***Culex vishnui***

– Anterior surface of fore and mid femora entirely dark; anterior surface of hind femur not with pale stripe 40
40. Proboscis often with accessory pale patches on ventral surface; hind femur pale with apical dark ring .....***Culex tritaeniorhynchus***

– Proboscis with accessory pale patches; anterior surface of hind femur with pale stripe contrasting with dark scaled area ..... ***Culex pseudovishnui***
41. Acrostichal setae well developed on scutum ..... (Subgenus: ***Eumelanomyia***) 42

– Acrostichal setae not developed except anterior end ..... (Subgenera: ***Culiciomyia*** and ***Lophoceraomyia***) 44
42. Lower mesepimeral setae absent ..... ***Eumelanomyia brevipalpis***

– One or more lower mesepimeral setae present 43
43. Decumbent scales on anterior dorsal margin of vertex broad ..... …..... ***Eumelanomyia malayi***

– Decumbent scales on anterior dorsal margin of vertex entirely narrow ….....… ….....***Eumelanomyia foliates***

44. Scaling on scutum sparse, rough in appearance; wings scales usually scandy, especially on vein 6 .....(Subgenus: ***Lophoceraomyia***) 45

– Scaling on scutum very dense, smooth in appearance; wing scales dense .....(Subgenus: ***Culiciomyia)*** 46

45. Abdominal terga with basal pale bands ..... ***Lophoceraomyia cinctellus***

– Abdominal terga with basal pale bands ..... (Females difficult to separate, used male antenatal and genitalia characters to confirm, identified only with larva) .....***L. bicornutus, L. uniformis, L. minor*** and ***L. wilfredi***

46. Integument of the pleuron with a prominent, isolated, very dark brown to black spot present on the upper mesepimeron . ..... ***Culiciomyia nigropunctatus***

– Integument of the pleuron with brown pattern stretching from the prespicular areas across the pre-alar area and terminating at the upper mesepimeron .... ***Culiciomyia pallidothorax***

47. Tip of proboscis swollen, upturned, and hairy .....(Genus: ***Malaya***) 48

– Tip of proboscis not so .....(Genus: ***Uranotaenia)*** 49

48. Clypeus yellowish white; a line of silvery scales between eyes; antepronotum and front of vertex with silvery scales .....***Malaya genurostris***

– Clypeus dark; no silvery scales between eyes; antepronotum and front of vertex with blue or bluish-violet scales ..... ***Malaya jacobsoni***

49. Hind tarsi with white markings 50

– Hind tarsi entirely dark 51

50. Wing scales dark ..... ***Uranotaenia testacea***

– Wing with a large patch of white scales ..... ***Uranotaenia rutherfordi***

51. Some white, blue, creamy flat broad scales along lateral margin of mesonotum in front of wing root 52

– No pale or blue flat scales on lateral margin of mesonotum in front of wing root 53

52. Dorsum of mesonotum covered with a mixture of pale brown, ochreous and dark brown scales . .....***Uranotaenia macferlanei***

– Dorsum of mesonotum covered with deep brown scales ....***Uranotaenia compestris***

53. Abdomen banded; pleurae pale yellowish with conspicuous brownish-black markings; a conspicuous median patch of grey-white scales on the mesepimeron ..... ***Uranotaenia pseudostrickilandi***

– Abdomen non-banded; pleural integument uniform in colour 54

54. Scutellar integument dark reddish, narrowly pale yellowish on lateral margin; antepronotum without scales ..... ***Uranotaenia nivipleura***

– Scutellar integument light or pale brown; pleuron without yellowish scales; antepronotum with greyish or bronzy scales ..... ***Uranotaenia obscura***

55. Wings dark 56

– Wings with pale marking.....58
56. Head scales very narrow, rod-like; palpi equal to the proboscis 57

– Head scales broad; palpi shorter than proboscis ..... ***Anopheles culiciformis***
57. Pre-scutellar space with setae ..... ***Anopheles insulaeflorum***

– Pre-scutellar space without setae .....***Anopheles aitkeni***
58. Less than four dark areas on costa, involving both the costa and vein 1 59

– At least four dark areas on costa, involving both the costa and vein 1 62
59. Palpi without distinct pale marking; a tuft of scales on ventral side of 7th abdominal segment .....***Anopheles barbirostris***

– Palpi with pale marking; no scale tufts on ventral side of 7th abdominal segment 60
60. Pale bands on hind tarsi narrow, apical only, 4th segment without basal pale band; apical fringe spot extending from end of vein 1 or 2.1 at least as far as 3; wing pattern sharp, the dark marks short and well defend; tip vein 1 pale ....***Anopheles crawfordi***

– Pale bands on hind tarsi broad, third band crossing the joint on the base of 4th segment; wing pattern darker; basal dark mark on 5 long 61
61. Costa usually with a few scattered pale scales in basal half; remigium without a line of white scales along the front .....***Anopheles nigerrimus***

– Costa without pale scales in basal half; remigium with a line of white scales along the front .....***Anopheles peditaeniatus***
62. Tip of hind tarsus not white 63

– Tip of hind tarsus white 68
63. Femora and tibiae speckled; palpi with two broad pale band intervening with a narrow dark band ..... ***Anopheles stephensi***

– Femora and tibiae not speckled; palpi with subapical pale band narrow, intervening dark areas varies 64
64. Basal third of costa uninterruptedly dark; palpi with subapical dark band broader than apical pale band 65

– Basal third of costa with a pale interruption; palpi with subapical dark band equal or shorter than apical pale band 67
65. Fore tarsomeres with white bands .....***Anopheles jeyporiensis***

– Fore tarsomeres without white bands 66
66. Inner costa uninterrupted; fringe spots on all veins but 6. ...***Anopheles fluviatilis***

– Inner costa interrupted; fringe spots at one or two veins only ------ ***Anopheles culicifacies***

67. Palpi with apical pale band equal to subapical dark band ---------- ***Anopheles subpictus***

– Palpi with subapical dark band equal to subapical band; apical pale band broader .....***Anopheles vagus***

68. Femora and tibiae unspeckled; tarsi with only one segment white; commonly with broad white bands above this ..... ***Anopheles karwari***

– Femora and tibiae speckled ..... - 69

69. Palpi with four bands; vein 6 with more than three dark areas 70

– Palpi with three bands; vein 6 with three or less dark areas.....71

70. Tibio-tarsal joint of hind leg with broad and conspicuous white band ....***Anopheles elegans***

– Tibio-tarsal joint of hind leg without broad and conspicuous white band .. .. ***Anopheles tessellatus***

71. Palpi with apical pale band more or less equal to subapical dark band and unspeckled .....***Anopheles jamesi***

– Palpi with a narrow dark band between two broad pale apical bands and conspicuous speckled 72

72. Hind tarsomeres 5,4 and 3 completely pale.....***Anopheles splendidus***

– Hind tarsomere 5 and part of 4 only pale .....***Anopheles theobaldi***

4.3. Description of mosquito species recorded from Mananthavady Taluk (Wayanad) during the present study

4.3.1. SUBFAMILY: CULICINAE

Culicinae is the largest subfamily of mosquitoes that includes 3,115 species in 110 genera and two groups of incertae sedis (uncertain placement), such as ‘Aedes’ and ‘Ochlerotatus’ sensu auctorum (according to the original and subsequent authors of description). The species in this subfamily are known as ‘culicines,’ however species in the tribe Aedini are often called ‘aedines’ and species in the tribe Sabethini are called ‘sabethines.’

4.3.1.1. Tribe: Aedinii Neveu-Lemaire, 1902

The traditional classification of tribe Aedini (Knight and Stone, 1977) included ten genera, the largest of which was *Aedes*. A series of studies that started

with the restoration of Verrallina and Ayurakitia to generic status (Reinert, 1999, 2000a, respectively) followed by the seminal study of Reinert (2000b), who conducted a thorough and systematic analysis of more than 65% of the species of the genus *Aedes* that were recognized at the time, resulted in radical changes to the traditional classification of the genus *Aedes*. Many of the Aedini tribe's subgenera and species groupings were elevated to generic status by Reinert et al. It should be noted that many of the taxa recognized as subgenera in the traditional classification of genus *Aedes* were originally described as genera (Mosquito Taxonomic Inventory). Present study follows the traditional classification and the new classification is given in the Annexure. Out of the ten genera of the tribe Aedini, species from three genera were collected during the current study.

4.3.1.1.1. Genus: *Aedes* Meigen, 1818

4.3.1.1.1.1. Subgenus: Aedimorphus Theobald, 1903

Aedes (Aedimorphus) vexans Meigen, 1830

Materials examined: 2♀, 1♂ India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 21-xii-2019, Resting collection. Samples collected: 2♀ and 1♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : A species of average size with minimal ornamentation and basally ringed hind tarsals. Numerous black upright scales on the nape and vertex, and narrow yellowish scales on the vertex that are laterally bordered by dark scales. The integument of the mesonotum is almost black, covered with golden-brown narrow scales. Narrow and pale scutellar scales. Several large, creamy, or white scale patches on the pleurae. Wings are with dark scales . Femora brown with white scale specks. Tibia appeared speckled when viewed from the front but was primarily pale when viewed from the back. Absence of a base-visible pale ring on the hind tibia. Tarsi is dark brown with slender, pale basal rings (Barraud, 1934; Yiau-Min Huang,1972).

4.3.1.1.2. Subgenus: Stegomyia Theobald, 1901

Aedes (Stegomyia)aegypti Linnaeus, 1762

Materials examined: 2♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N,75.90277°E) 12-xi -2021, Larval collection. Samples collected: 2♀. Habitat: Fallen areca palm leaves. Coll: Maiby Thankachan.

Diagnosis: A medium sized dark species with contrasting silvery white ornamentation on the head, scutum, legs and abdomen. It has a distinct white sutal marking which forms the typical 'lyre shaped' pattern of the species. The clypeus haslateral white scales and the pedicel has large patches of white scales at the sides. The vertex has a median line of broad white scales. Scutum is either black or brown with two sub median-longitudinal white stripes and white lyre-shaped patterns. Occiput is the only part of the vertex with upright forked scales. Broadly white-scaled paratergite. Two white scale patches separated on a mesepimeron. All femora have white knee spots. The fore and mid femur with narrow and longitudinal white stripes on the anterior surface. All tibiae are dark anteriorly (Barraud, 1934; Huang, 1972).

Aedes (Stegomyia) albopictus Skuse, 1894

Materials examined: 2♀, 2♂ India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Makkiyad (11.7490319 ° N,75.90277° E), Kuttimoola (11.8327° N,75.9913°) 29-x-2019, 10-x-2020, 20-vi-2021, 23-viii-2021, 15-iv-2022, Man landing, Larval collection. Samples collected: 1115♀and 178♂. Habitat: Mixed plantation, plastic containers, tree hole, rubber latex collecting cups. Coll: Maiby Thankachan.

Diagnosis : A slender silvery-white median line extending nearly the entire length of the mesonotum. The white scale patches on clypeus are absent. All lobes of the scutellar scale are flat and white as snow. In front of the wing-root on the mesonotum's border, but not continuing over the wing-root, there is a line of flat, silvery scales. On the pleurae, there are uneven patches of white scales. White median-longitudinal band on the scutum. Mesepimeron with white scale patches that

are not separated and create white patches in a V shape. Broad white rings are seen on all segments of the hind tarsi. Abdomen with bands. Anterior part of the mid-femur without a white stripe running through it (Barraud, 1934; Yiau-Min Huang, 1972).

Aedes (Stegomyia) subalbopictus Barraud, 1931

Materials examined: 2♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 10-ii-2020, Man landing. Samples collected: 2♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis **:** It is similar to *Ae.albopictus* In terms of ornamentation on the head, thorax, and legs, but can be differentiated by a completely black dorsum of the abdomen without any indication of basal bands; however, it does have small basal, lateral, silvery-white patches on the tergites. The palps and proboscis are both about the same length. The tip of the typical style is long and pointed. Scutum with a pronounced median stripe that forks at the start of the pre-scutellar space and narrows in front of the pre-scutellar bare space. Just before the level of the wing root, there is a patch of narrow, curved white scales on the lateral border, and there are a few more narrow, pale scales there. Mid and fore femora are dark. large white band anteriorly on the hind femur (Barraud, 1934; Yiau-Min Huang, 1972).

Aedes (Stegomyia) novalbopictus Barraud, 1931

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 30-v-2020, Man landing. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis **:** Similar to *Ae. Albopictus* and differes in the scales at the wing root. There are a few narrow, curved pale yellowish scales on the lateral border, and also a few more over the wing root. Neither the post-spiracular nor the sub-spiracular regions have scales. Dark scales on the anterior fore and mid-femora, with occasional lighter scales. White scales on the first portion of the abdomen. Tergites II–VI has side spots (Barraud, 1934; Yiau-Min Huang, 1972).

Aedes (Stegomyia) pseudalbopictus Borel, 1928

Materials examined: 2♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 05-i-2021, Man landing. Samples collected: 2♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The structure of the male terminalia distinguishes it from *Ae. albopictus*. Having black scales, the male proboscis is longer than the palpi by more than half the length of the terminal segment. Scutum has a pronounced median white line and slender black scales. On either side of the pre-scutellar area, there is a patch of wide, black scales. Before the level of the wing root, there is a patch of thin, curving white scales on the lateral border, and there are a few more scales over the wing root. White scales in the post-spiracular region and pale scales in the sub-spiracular region. Dark anteriorly and pale posteriorly define the fore and mid-femur. Segment I of the abdominal wall has white scales on the lateral side. Each Terga II–Vi has a lateral white speck and a basal white band (Barraud, 1934; Yiau-Min Huang, 1972).

4.3.1.1.1.3. Subgenus: Phagomyia Theobald, 1905

Aedes (Phagomyia) cogilli **Edwards, 1922**

Materials examined: 2♀, 1♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N,75.90277° E), Kuttimoola (11.8327° N, 75.9913°E) 27-x-2020, Man landing and Larval collection. Samples collected: 2♀and 1♂. Habitat: Forest, Rubber latex collecting cups. Coll: Maiby Thankachan.

Diagnosis : Black and white species of average size. The edges of the eyes have a thin border of white scales. A little area of flat, white scales on the lower posterior border is present on the post-pronotum. Prealar white patches are smaller; the white patch on the front of the mesonotum is a bit larger, rounder, and more silky. White flat scales cover the middle lobe of the scutellum. The lateral lobes have flat black scales. There are no prominent scale tufts on the venter of the abdomen (Barraud, (1934).

4.3.1.1.1.4. Subgenus: Christophersiomyia Barraud, 1923

Aedes (Christophersiomyia) thomsoni Theobald, 1905

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N,75.90277° E) 11-xi-2020, Larval collection. Samples collected: 1♀. Habitat: Cashew tree hole. Coll: Maiby Thankachan.

Diagnosis : Palpi is dark scaled. Torus has a patch of broad white scales on median surface. Thorax is dark brown covered with white scales throughout. Silvery white and light brown scaling, as well as two dots that resemble brown eyes, adorn the mesonotum. Variable amounts of pale brown scaling can be seen on the posterior mesonotum. Scutellum lobes are covered in flat, white scales. Proboscis has a white ring around it. White ring on basal half of the hind tibia. Coxa has white scales except fore and mid coxa with a few dark scales. All tarsi are dark scaled on dorsal surface but white on ventral surface. Sternite II and III are largely white scaled (Barraud, 1934; Abererombie, 1977).

4.3.1.1.1.5. Subgenus: Alloeomyia Reinert, Harbach & Kitching, 2008

Aedes (Alloeomyia) pseudotaeniatus Giles, 1901

Materials examined: 2♀, 2♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E) 18-vi-2020, Man Landing and Larval collection. Samples collected: 44♀ and 20♂. Habitat: Mixed plantation, rain water collected in plastic sheets. Coll: Maiby Thankachan.

Diagnosis : A median pale line runs from nape to front of vertex. An irregular patch of broad white scales are seen on the pleura, and a line of pale lanceolate scales on the antepronotum. A thin band of white scales are seen along the costal margin close to the base of the wings. Legs are either black or dark brown. Femora and tibia of the front and middle legs have long, thin pale longitudinal stripes on both sides. The dorsum of the abdomen is black, with thin, pale basal stripes on II – VI (Barraud, 1934).

4.3.1.1.1.6. Subgenus: Hulecoeteomyia Theobald, 1904

Aedes (Hulecoeteomyia) chrysolineatus Theobald, 1907

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), Kuttimoola (11.8327° N, 75.9913°E) 24-vi-2020, Man landing and Larval collection. Samples collected: 65 ♀ and 20 ♂. Habitat: Mixed plantation, rain water collected in plastic sheets, rubber latex collecting cups, forest. Coll: Maiby Thankachan.

Diagnosis : Palpi are brownish-black with a white tip. A median line running from the front to the back of the scutellum, forking in front of the ante-scutellar bare space, a pair of sub-median lines that nearly meet a pair of lines curving from the sides that continues to lateral lobes of the scutellum are all characteristics of the mesonotum, which is a deep brown or black colour. Another line of golden scales from the wing root continues forwards a short distance. From near the base, the proboscis is pale beneath, for more than half the length. The ventral margin of the fore femur is light anteriorly. Femur has a pale midline. The entire posterior length of the fore and mid femora is pale. Abdomen is nearly black with broad basal lateral silvery specks and yellowish basal streaks. Sternites have basal white bands (Barraud, 1934).

Aedes (Hulecoeteomyia) harveyi Barraud, 1923

Materials examined: 2♀, India: Kerala, Wayanad, Makkiyad (11.7490319 ° N,75.90277° E) 10-xi-2019, Man landing. Samples collected: 2♀. Habitat: Mixed plantation, forest. Coll: Maiby Thankachan.

Diagnosis : The species closely resembles *Ae.Chrysolineatus*. Torus with dark scaling mesally; Palpus is white tipped. Only the centre of the proboscis is noticeably pale underneath; the edges and top surface are black. Anterior pronotum is with broad white scales, posterior pronotum with narrow whitish or yellowish scales. A small patch of broad white scales is located ventro-posteriorly. Median scutal line is forked posteriorly. Sub medial line is broken at the level of scutal angle. Few or no pale scales on the anterior surface of the fore and middle femora. The tip

of the hind femur and the base of the hind tibia are less extensively white beneath. Although tergites exhibit significant lateral basal silvery patches, the dorsum of the abdomen is completely dark and devoid of any pale bands (Barraud, 1934).

4.3.1.1.1.7. Subgenus: Neomelaniconion Newstead, 1907

Aedes (Neomelaniconion) lineatopenne Ludlow, 1905

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 27-x -2019, Man landing. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Dark brown species . Mesonotum's sides are yellow. Dark brown, un-banded legs are present. Scales on vein 1 and the stem of vein 5 in wings are light yellow. Pale yellow basal stripes on the abdomen (Barraud, 1934).

4.3.1.1.1.8. Subgenus: Paraedes Edwards, 1934

***Aedes (Paraedes) menoni* Mattingly, 1958**

Materials examined: 8♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 4-i-2021, Man landing, Samples collected: 8♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Palpi are longer than the proboscis. Vertex with narrow and curved golden- white scales. Scutellar integument has dark golden-brown scales. Post pronotum with several narrow curved reddish-brown scales on dorsal area. Pro pleuron with a patch of broad silvery white scales and several brown setae. Paratergite is bare. Post-spiracular area has 3,4 golden brown setae. Mesepimeron contains broad silvery white scales. Coxa I-III have golden brown setae. Femur I-II with anterior brown scales. Dorsal and ventral veins of the wing with brown scales (Barraud, 1934).

Aedes (Paraedes) barraudi Edwards, 1934

Materials examined: 2♀, India: Kerala, Wayanad, Paleri(11.7547° N, 75.8781° E), 03-xii-2020. Man landing. Samples collected: 2♀. Habitat: Cashew plantation. Coll: Maiby Thankachan.

Diagnosis : Palpi are loner than the proboscis. Clypeus is dark brown. Vertex covered with broad dark brown scales. Occiput with narrow white scales and a few erect golden white and brown scales. Scutal integument is reddish brown. Scutum covered with reddish brown scales. Post pronotum with a few narrow curved reddish-brown scales. Post spiracular area with a few broad white scales and 3,4 golden setae. Mesepisternum with upper and lower posterior patches of broad white scales. Coxa I-III have several golden or brown setae. Trochanters I- III are with white scales. Femora I and II with anterior brown scales. Abdominal terga II to VI dark brown scaled with latero-basal white scaled patch. Terga and sterna with numerous short golden setae (Barraud, 1934).

4.3.1.1.1.9. Subgenus: Downsiomyia Vargas, 1950

Aedes (Downsiomyia) niveus Ludlow, 1903

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N,75.90277° E) 15-ix-2021, Larval collection. Samples collected: 5♀ and 1♂. Habitat: Cashew tree hole. Coll: Maiby Thankachan.

Diagnosis : Mesonotum has a large, snow-white patch in the front that is separated into lateral portions. Black scales cover the scutella. Except for a thin band of light scales at the base of the costa, the wings are not spotted or speckled. Anteriorly, the fore femur is black. Hind femur has at least a basal two-thirds of whiteness. The tarsi and tibiae are entirely brownish-black. The abdomen is dark (Barraud, 1934).

4.3.1.1.1.10. Subgenus: Fredwardsius Reinert, 2000

Aedes (Fredwardsius) vittatus Bigot, 1861

Materials examined: 6♀, 4♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Makkiyad (11.7490319° N, 75.90277° E), 29-xi-2019, 12-viii-2022, Man landing. Samples collected: 4♀ and 1♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The proboscis is as long as the fore femur. Its median part has a band of whitish scales. The palps have a few white scales in the middle and the apex is broadly pale scaled. The clypeus has lateral patches of white scales. The mesonotum has three pairs of prominent silvery whitish spots. The anterior two pairs are larger than the posterior pair. All three lobes of scutellum have broad white scales. The mesepisternum has upper and lower scale patches. Femora and tibia with preapical white rings and medial white rings respectively. The hind tibia has a distinct median white ring. The tarsi of the fore and mid legs have narrow white basal rings at the tarsomere. The abdominal terga are predominantly covered with black scales (Barraud, 1934).

4.3.1.1.2. Genus: *Armigeres* Theobald, 1901

4.3.1.1.2.1. Subgenus: Armigeres Theobald, 1901

Armigeres (Armigeres) aureolineatus Leicester, 1908

Materials examined: 8♀, 4♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E) 16-vi-2020, Larval collection. Samples collected: 8♀ and 4♂. Habitat: fallen areca palm leaves. Coll: Maiby Thankachan.

Diagnosis : Mesonotum with a curving line of similar scales across each wing root and two distinct sub median golden lines. On the dorsum, the abdominal tergites created lateral white marks. Sternite III–VI with small, black bands at the apex. Tarsi and tibiae are both dark brown Without any light bands (Barraud, 1934).

Armigeres* (*Armigeres*) *subalbatus Coquillett, 1898

Materials examined: 2♀, 2♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Makkiyad (11.7490319° N, 75.90277° E), Kuttimoola (11.8327° N, 75.9913° E) 24-xi-2019, 12-vi-2020, 22-viii-2021, Man landing, Resting collection, and Larval collection. Samples collected: 218♀ and 140♂. Habitat: Mixed plantation, thermocol sheet, plastic sheets, rubber latex collecting water, fallen areca palm leaves. Coll: Maiby Thankachan.

Diagnosis **:** Mesepimeron's posterior border lacks setae. Complete line of pale scales is present along the lateral border of the scutum. A broad white band runs from the post-ventral surface of the hind femur almost to the apex. There are no apical yellowish spots on abdominal terga II to VI. Broad, dark stripes are found at the apex of abdominal sterna III–V, measuring 0.50, 0.33, and 0.25 l, respectively (Barraud, 1934).

4.3.1.1.3. Genus: *Heizmannia* Ludlow,1905

4.3.1.1.3.1. Subgenus: Heizmannia Ludlow, 1905

Heizmannia (Heizmannia) chandi Edwards, 1922

Materials examined: 5♀, 1♂, India: Kerala, Makkiyad (11.7490319° N, 75.90277° E) 10-viii-2020, Larval collection. Samples collected: 5♀, 1♂. Habitat: Forest. Coll: Maiby Thankachan.

Diagnosis **:** On the middle lobe of the scutellum, there is a little patch of silvery scales. Antepronotum is covered in scales that are silvery-white. Bright green or bluish-green lustre can be found on mesonotal scales. Outstanding plume-scales on veins 2.1 and 2.2 are linear. Dorsal edge of the hind femur is black From the base to the knee joint. A large dense tuft of relatively short, twisted hairs on Coxite near the base. style with a protrusion that resembles an elbow near the base (Barraud, 1934).

4.3.1.1.4. Genus: *Verrallina* Theobald, 1903

4.3.1.1.4.1. Subgenus: *Neomacleaya* Theobald, 1907

Verrallina (Neomacleaya) indica Theobald, 1907

Materials examined: 6♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 24-xi-2019, 12-iii-2020, Man landing, sweep net collection. Samples collected: 18♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The vertex's decumbent scales are wide. Scutum is with dorso-central setae. Acrostichal setae range in number from few to many. The scales of the scuta and scutella are all thin and curved. Post-pronotum may be bare or covered entirely with curved, thin scales, with the exception of the meso-postnotum, which is often bare. Post-spiracular setae are present, whereas the pre-spiracular region is barren. Alula have marginal scales that are fairly wide. Tarsi I-III are dark scaled.

Female genitalia – Tergum VIII is short, wider than the length, index is about 0.35-0.57 and nearly entirely covered with broad scales. Sternum VIII has numerous thin setae on apical lobes, largely covered with broad scales. Post-genital lobe is relatively narrow, with deep median caudal indentation. Upper vaginal lip with median posterior area. Cauda of spermathecal eminence is developed into large vertical shield; lower vaginal sclerite is large, not contiguous with lower vaginal lip which is usually spiculate. Insula is well-defined, small, with a few minute tuberculi. Spermathecal eminence is complex, with numerous well-developed spicules (Mosquito Taxonomic Inventory).

4.3.1.2. Tribe: Culicini Meigen, 1818

The tribe consists of 817 species organized into four genera. Culex is the largest genus by a wide margin, with 789 species distributed across 27 subgenera. In comparison, there are 18 species of Deinocerites, one species of Galindomyia, and nine species of Lutzia. Multiple species of the subgenera Culex and Melanoconion play a role in the transmission of agents that cause human diseases, including filariasis and arboviral encephalitis (Mosquito Taxonomic Inventory).

4.3.1.2.1. Genus: *Culex* Linnaeus, 1758.

4.3.1.2.1.1. Subgenus: Culex Linnaeus, 1758

Culex (Culex) gelidus Theobald, 1901

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Nadakkal (11.7414° N, 75.9937° E), 04-ix-2019, Larval collection, Resting collection. Samples collected: 95♀ and 15♂. Habitat: Mixed plantation, Banana field. Coll: Maiby Thankachan.

Diagnosis : The anterior surface of fore and mid-femora are without speckling. The scutellum and the pre-scutellar region behind the wing base have black scales. The proboscis' pale band is short and does not extend as far as the black area's base. The anterior 0.7 of the scutum is covered in pure white scales. There are no pale band speckles on the wings. Terga II–VI of abdomen have basal pale stripes (Barraud, (1934).

Culex (Culex) fuscocephalus Theobald, 1907

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Nadakkal (11.7414° N, 75.9937° E), 2-x-2020, Larval collection. Samples collected: 11♀ and 4♂. Habitat: Mixed plantation, Banana field. Coll: Maiby Thankachan.

Diagnosis : The main distinguishing features of this species are its unbanded abdomen, dull brown color as well as the presence of two horizontal black bands on the pleurae that are split by a white-scaled border. The vertex is covered with a pattern of brown and white upright scales and narrow scales, with a patch of large white scales on each side. Underneath, the proboscis is light brown in colour. Brown describes the palpi. The legs have a deep brown colour. The dorsal edge of the hind femur is mostly pale, with brown scales extending toward the apex. A more or less distinct pale stripe can be seen on the outside of the hind tibia.Tarsi are brown. Dusky brown hairs cover the posterior borders of the tergites on the dorsal side of the abdomen, giving it a banded look (Barraud, 1934).

Culex (Culex) tritaeniorhynchus Giles, 1901

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 4-xi-2021, Resting collection. Samples collected: 15♀ and 7♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Dark brown and erect scales on the vertex. Palpi is dark and the proboscis with narrow median pale ring and some pale scales scattering on the lateral and ventral surface. On the anterior surface of the scutum, all the scales are dark brown. The distal end of the hind femur is marked by a distinct, narrow dark ring. The fore and mid-femora are completely dark. Basal pale bands on abdominal terga II to VI. There is no pale area on the wing tip. There are no lower epimeral setae (Barraud, 1934).

Culex (Culex) mimulus Edwards, 1915

Materials examined: 1♀. India: Kerala, Wayanad, Nadakkal (11.7414° N, 75.9937° E), 06-xii-2019, Larval collection. Samples collected: 1♀. Habitat: Banana field. Coll: Maiby Thankachan.

Diagnosis : Palpi is completely dark. A few pale scales are present on the segment 4 of the apex. Probosis is with very distinct median pale ring which occupies 0.2 times of total length. Mesonotal integument is reddish brown to black. Antepronotum and post-pronotum with narrow, yellow, or golden scales. Anterior surface of the fore and mid femur is dark. Tarsomere I-IV of all legs are with broad basal and narrow apical pale bands. Wing vein C is without pale scale, streak, or spot. Abdominal terga II- VII with basal pale bands (Sirivanakaran, 1976).

Culex (Culex) quinquefasciatus Say, 1823

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Nadakkal (11.7414° N, 75.9937° E), 14-v-2021, Man landing and Resting collection. Samples collected: 10♀. Habitat: Mixed plantation, Banana field. Coll: Maiby Thankachan.

Diagnosis : There are no pale bands on tarsi and proboscis. There are one or two lower mesepimeral setae. Pronotum and mesonotum are of with similar colouration. There are no black or light stripes on Pleuron. There is no pale scale patch in the post-spiracular region. Scales on the wing veins are dark and dense. Abdominal tergum I with dark median scale patch. Terga II- VII with evenly broad basal pale bands and basolateral pale spots (Barraud, 1934; Sirivanakaran, 1976).

Culex (Culex) pseudovishnui Colless, 1957

Materials examined: 1♀, 1♂ India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 04-xi-2021, Resting collection, Samples collected: 15♀ and 2♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : On the vertex, erect scales are typically black laterally and pale in the centre. The majority of the scutum has pale beige, yellow, or golden scales. Proboscis is banded without any pale patch on the side. A light stripe on the front surface of the hind femur stands out against the black sections well. Pale scales completely encircle the pre-scutellar region (Barraud, 1934).

Culex (Culex) vishnui Theobald, 1901

Materials examined: 3♀, 2♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 24-i-2020, Resting collection. Samples collected: 15♀ and 8♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The ventral surface of the proboscis is banded without any additional pale patches or stripes. Vertex scales were upright and dark and pale in colour. The hind femur's front surface features a pale stripe that blends in with the surrounding area of dark scales and contains one to several scattered pale scales. The integument of the scutum is light brown. At least on the apico-dorsal region, the anterior surface of the fore and mid femora is speckled with numerous whitish scales. Scales of various shades of beige, yellow, golden, or dark cover the anterior surface of the scutum. Tergae II–VI of abdomen have basal pale stripes (Barraud, 1934; Sirivanakaran, 1976).

Culex (Culex) barraudi Edwards, 1922

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Nadakkal (11.7414° N, 75.9937° E), 05-vi-2020, Larval collection. Samples collected: 2♀. Habitat: Mixed plantation, plastic containers, Banana field. Coll: Maiby Thankachan.

Diagnosis : Brownish or blackish scales are present on the head. Tip of the palpi is pale and the proboscis is with median pale ring. Mesonotum and pleural integument are dark brown. Anterior surface of the fore femur is completely dark. Anterior surface of the hind femur is with longitudinal pale scales. Wing scales are dark narrow and dense. Abdominal terga II-VII with basal pale bands that are narrower on the posterior segments (Sirivanakaran, 1976).

Culex (Culex) hutchinsoni Barraud, 1924

Materials examined: 2♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Nadakkal (11.7414° N, 75.9937° E). 05-vi -2020, Larval collection. Samples collected: 2♀. Habitat: Mixed plantation, banana field. Coll: Maiby Thankachan.

Diagnosis : Yellowish brown or golden scales are seen on the vertex. Whitish scales on the scutellar and pre-scutellar space. Pronotal integument is dark brown; antepronotum with narrow golden brown and post-pronotum with dark brown scales. Anterior surface of the fore and mid femora are dark scaled. Anterior surface of the hind femur has a pale longitudinal stripe. All tibiae and tarsi are entirely dark. Abdominal tergum I with median patch of dark scale. Tergum II is with basal median pale streaks and tergum VIII is largely pale yellowish (Sirivanakaran, 1976).

Culex (Culex) murrelli Lien, 1968

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 05-vi-2020, Larval collection. Samples collected: 1♀. Habitat: Mixed plantation, plastic containers. Coll: Maiby Thankachan.

Diagnosis : Palpi is completely dark with a few pale scales at the segment 4 of the apex. Proboscis is with a very distinct median pale ring which occupies 0.2 times of total length. Mesonotal integument is reddish brown to black. Antepronotum and post-pronotum with narrow, yellow, or golden scales. Anterior surface of the fore and mid femur is dark. Tarsomere I-IV of all legs with broad basal and narrow apical pale bands. Base of the wing vein C is without any pale scale streak or line on posterior surface. First costal pale spot at the middle of vein C restricted to C, Sc and fR1 (Sirivanakaran, 1976).

Culex (Culex) whitmorei Giles, 1904

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N,75.90277°E), 12-v -2022, Resting collection. Samples collected: 2♀ and 6. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Dark brown proboscis with a broad median band of white scales. Proboscis is with several white scales at the tip. Scutum with a pattern of silver white scales which extends through the pre-scutellar space and on to the mid-lobe of the scutum. Dorsal wing scales are brown. Anterior surface of the hind femur with a variable scattering of pale scales medially. Predominantly dark hind tibia with a median stripe of pale scales. Hind tarsus dark with moderately broad basal white bands on tarsomere I-V. Abdominal terga is dark scaled with variable pale basal bands on segments II-VII. Basal bands is expanded medially but not reaching the lateral margins. Sternum is predominantly pale (Barraud, 1934; Sirivanakaran, 1976).

4.3.1.2.1.2. Subgenus: Oculeomyia Theobald, 1907

Culex (Oculeomyia) bitaeniorhynchus Giles, 1901.

Materials examined: 4♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 24-xi-2020, Resting collection, man landing. Samples collected: 8♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Speckled wings, banded proboscis, apical yellow bands on the abdominal tergites serve as indicators of this species. Black palpi with light scales at the tip. Black proboscis with a wide, creamy median stripe and light scaling toward

the tip. The wing has broad, pale yellow and black scales mixed together that give it a speckled appearance. On the femora, tibiae, and first segments of the tarsus, the legs are brownish-black and heavily covered in whitish scales. Dark tarsi with basal and narrow, light-colored rings. Generally black or with a scattering of pale scales on the abdominal tergites, with somewhat broad apical yellow stripes (Barraud, 1934; Sirivanakaran, 1976).

Culex* (*Oculeomyia*) *sinensis Theobald, 1903

Materials examined: 1♀, India: Kerala, Wayanad, Nadakkal (11.7414° N, 75.9937° E), 30-xii-2019, Larval collection. Samples collected: 1♀. Habitat: Banana field. Coll: Maiby Thankachan.

Diagnosis : It is identical to *Cx. bitaeniorhynchus* but can be differentiated by its wings with completely dark scales. Proboscis is dark brown with a broad median pale band. Pale ochreous or greyish white scales cover the mesonotum from the front all the way back almost to the level of the wing roots. Dark brown legs are with lighter speckling on the foreleg femur and tibia. Hind tibia with a mixture of dark and pale scales. Narrow, pale rings is seen near the base of the tarsi. Brownish-black abdominal tergites have fine ochreous apical streaks (Barraud, 1934; Sirivanakaran, 1976).

Culex (Oculeomyia) infula Theobald, 1901

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 12-ix-2019, Man landing. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Pale rings encircle the proboscis and tarsi. In addition to having narrow apical bands, apico-lateral yellowish patches, and median basal pale bands or spots, the abdominal terga II to IV are often dark in colour. Terga V-VII have narrow bands at the apex and base. The wings and legs have light to medium speckling. There is no definite pale area on the wing tips (Barraud, 1934; Sirivanakaran, 1976).

4.3.1.2.1.3. Subgenus: Culiciomyia Theobald, 1907

Culex (Culiciomyia) nigropunctatus Edwards, 1926

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N,75.90277° E), 12-v-2021, Larval collection. Samples collected: 25♀ and 6♂. Habitat: Fallen areca palm leaves. Coll: Maiby Thankachan.

Diagnosis : The palpi and the proboscis are uniformly dark scaled. Sides of the head have a patch of flat, white scales. Darker upright scales and slender, ochreous scales cover the vertex. Pleurae is a light brown colour with two less prominent dark spots, one posterior to the post-pronotum and one in the centre of the sternopleura, as well as a very distinct velvety black mark on the upper portion of the mesepimeron. The hind femur is pale except dorsally, and the legs are brown anteriorly. Brown abdominal tergites have thin, ochreous-coloured basal bands. Sterna is uniformly pale (Barraud, 1934).

Culex (Culiciomyia) pallidothorax Theobald, 1905

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 12-viii-2020, Larval collection. Samples collected: 25♀ and 8♂. Habitat: Mixed plantation, plastic containers. Coll: Maiby Thankachan.

Diagnosis : The palpi and proboscis are uniformly dark scaled. The tiny scales on the head are brown rather than white and are darker. Instead of being deep dark brown, the scales that cover the mesonotum are dark fawn-brown. On the sternopleura, there is a dark spot, and the top section of the pleura has a dark stripe. The anterior fork-cell of the wing is about 2/4 times the length of the stem. Legs are dark brown, but white scales are present on the ventral margin of the hind femur. Dorsally, the hind femur lacks a prominent pale knee patch. The posterior borders of pale basal stripes on the abdomen are either straight or slightly rounded (Barraud, 1934).

4.3.1.2.1.4. Subgenus: Eumelanomyia Theobald, 1909

Culex (Eumelanomyia) malayi Leicester, 1908

Materials examined: 1♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 30-xii-2019, Light trap. Samples collected: 1♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Small dark species. Proboscis with 4 dark labial basal setae, the longest ones are as long as palpus. Scutum, scutellum and pronotum are brown. Head is covered with small light-coloured flat scales. Antennae, palpi and proboscis are brownish black. Palpi is about 1/6th the length of the proboscis. Mesonotum is covered with brown narrow scales. Pleura is dark on upper half and greenish below. Wings are dark scaled. Legs are deep brown. Fore and mid femur lighter posteriorly. Hind femur is pale. Abdomen is brownish black dorsally (Sirivanakaran, 1972).

Culex (Eumelanomyia) foliatus Brug, 1932

Materials examined: 1♀, India: Kerala, Wayanad, Paleri (11.7547° N, 75.8781° E), 06-v-2021, 14-x-2021, Larval collection. Samples collected: 1♀. Habitat: Banana field. Coll: Maiby Thankachan.

Diagnosis : Very small species without prominent ornamentation or colour patterns. Vertex with dark brown scales but some lighter scales on the posterior line. Scutum and scutellum are dark brown but with faintly lighter stripes on dorso-central area. Pleuron is uniformly dark brown. Except for the pale scaled anterior surface of hind femur, all legs are dark scaled. Abdominal terga and sterna are completely dark (Sirivanakaran, 1972).

Culex (Eumelanomyia) brevipalpis Giles, 1902

Materials examined: 12♀, 4♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 24-xi-2019, Man landing, Larval collection. Samples collected: 3♀ and 4♂. Habitat: Mixed plantation, Plastic container. Coll: Maiby Thankachan.

Diagnosis : Small, dark species distinguished by a unique palpi shape. On the vertex and nape, there are narrow pale scales and erect yellow scales. Palpi is dark brown. With some form of externally erect hairs and a few strong bristly hairs at the tip, the lengthy segment is bent inward. The penultimate portion is curved and fairly long. The final segment is small and has strong, fine bristles near the tip (Sirivanakaran, 1972).

4.3.1.2.1.5. Subgenus: Lophoceratomyia Theobald, 1905

Culex (Lophoceraomyia) uniformis Theobald, 1905

Materials examined: 1♀, India: Kerala, Wayanad, Kuttimoola (11.8327° N, 75.9913° E), 4-vi-2020, Larval collection. Samples collected: 4♀. Habitat: Rubber latex collecting cup. Coll: Maiby Thankachan.

Diagnosis : The last two segments of the palp are clearly hairy, and longer than the proboscis by more than the length of the apical segment. Segment 6 of the antenna has a tuft of scales, some of which are large and very long. The tip of the longest extends past the matted and twisted tufts on segments 7-9 and the matted tuft on segment 9. On segment 10, there are no such scales. Narrow scales that are brownish in color cover the mesonotum. The venter is paler and the abdominal tergites are brownish-black in colour (Barraud, 1934; Sirivanakaran, 1977).

Culex (Lophoceraomyia) bicornutus Theobald, 1910.

Materials examined: 1♀, 1♂ India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Kuttimoola (11.8327° N, 75.9913° E), 4-vi-2020, Larval collection. Samples collected: 15♀ and 7♂. Habitat: Mixed plantation, plastic containers, rubber latex collecting cups. Coll: Maiby Thankachan.

Diagnosis : Species is exceedingly similar *Cx. minor*. The palpi is dark scaled with 0.2 times length of the proboscis. The last two segments of the palpi have a few hairs and are just slightly longer than the proboscis. A tuft of thin, uniformly sized scales that resembles hairs are present on segment 6 of the antenna. The antennal torus lacks any prominence. Further up the dorsal edge of coxite, there was a row of

seven recurved hairs. Mesonotum is brown or black scaled. Upper surface of the post-pronotum is without any setae or scales. Anterior surface of the hind femur with moderately broad pale stripe from base to apex. Abdominal terga are black scaled sterna are pale yellowish or white (Sirivanakaran, 1977).

Culex (Lophoceraomyia) wilfredi Colless, 1965

Materials Examined :1♂ India: Kerala, Wayanad, Paleri (11.7547° N, 75.8781° E), 02-i-2020, Larval collection. Samples collected: 1♂. Habitat: Banana Field. Coll: Maiby Thankachan.

Diagnosis **:** Scales on the vertex are narrow, linear, and fine. Proboscis is with 4 labial basal setae, 2 lateral ones about 0.5 of palpal length. Scales on the mesonotum and that of pleuron are reddish to black. Anterior surface of the hind femur with a broad longitudinal whitish stripe extending from base to apex. Wing scales on the R2 and R3 are narrow. Abdominal terga are dark scaled and sterna is pale yellow scaled (Sirivanakaran, 1977).

Culex (Lophoceraomyia) minor Leicester, 1908

Materials examined: 6♀, 2♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 23-x-2020, Larval collection. Samples collected: 6♀ and 2♂. Habitat: Mixed plantation, Plastic container. Coll: Maiby Thankachan.

Diagnosis **:** The palpi is dark scaled and longer than the proboscis. The last two segments of the palpi have few hairs and are just slightly longer than the proboscis. A tuft of thin, uniformly sized scales that resembles hairs are present on segment 6 of the antenna. The antennal torus lacks any prominence. Further up the dorsal edge of coxite, there was a row of seven recurved hairs. Mesonotum is brown or black scaled. Upper surface of the post-pronotum is without any setae or scales. Anterior surface of the hind femur with moderately broad pale stripe from base to apex. Abdominal terga are black scaled, sterna are pale yellowish or white (Barraud, 1934; Sirivanakaran, 1977).

Culex (Lophoceraomyia) cinctellus Edwards, 1922

Materials examined: 1♂, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 30-xii-2019, Light trap. Samples collected: 1♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The antennal structure and banded abdomen help to identify the species. On the vertex and nape of the head, there are numerous, erect, slender dark brown scales. Antennae are without prominence on the torus. Segment 6 has larger scales, and segments 7-9 have matted and twisted segments. Segment 10 has four extremely long bristles, several relatively short, thick hairs, and segment 11, which is brownish-black with well-defined basal transverse ochreous bands (Barraud, 1934; Sirivanakaran, 1977)

4.3.1.2.2. Genus: *Lutzia* Theobald, 1903

4.3.1.2.2.1. Subgenus: Metalutzia **Tanaka, 2003**

Lutzia (Metalutzia) halifaxii Theobald, 1903

Materials Examined :1♀ India: Kerala, Wayanad, Paleri (11.7547° N,75.8781°E), 02-i-2020, Larval collection. Samples collected: 1♂. Habitat: Slow running water in banana plantation field. Coll: Maiby Thankachan.

Diagnosis : The adult is very similar to *Lutzia vorax*. It is a darker species. The mesonotum is covered with brownish - black scales, with paler scales forming a very distinct pattern. The dorsum of the abdomen is entirely brownish- black. There are some narrow pale ochreous or white apical bands on the abdomen. Anterior surface of the hind femur is with numerous dark scales on the basal 1/2; apical 1/2 is without a distinct line of pale scaling to the tip (Barraud, 1934).

4.3.1.3. Tribe: Mansoniini Belkin, 1962

The two genera Coquillettidia (58 species) and Mansonia (25 species) comprise the 82 species that make up the tribe Mansoniini. The larval and, to a lesser extent, pupal stages of Mansoniini species are most distinctively characterised.

The adults are readily misconstrued for Culex or Aedine mosquitoes due to their heterogeneous appearance. Several species of the tribe have been discovered to be naturally infected with arboviruses and microfilariae and have been shown to transmit them under experimental conditions. Some species are invasive and vicious biters (Mosquito Taxonomic Inventory).

4.3.1.3.1. Genus: *Mansonia* Blanchard, 1901

4.3.1.3.1.1. Subgenus: Mansonioides Theobald, 1907

Mansonia* (*Mansonioides*) *indiana Edwards, 1930

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 10-vii-2020, Resting collection. Samples collected: 15♀ and 7♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis **:** Extremely comparable to *Mansonia uniformis*. Scaling is uniformly dark brown on the mesonotum. On tergite VIII, the chitinous hooks are widely separated, bent, and lack a clear separation between them and the median teeth. Legs and wings have specks on them (Barraud, 1934).

Mansonia* (*Mansonioides*) *uniformis Theobald, 1901

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 10-vii-2020, Resting collection. Samples collected: 11♀ and 2♂. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis **:** The mesonotum is covered with light brown and greenish scales that form two broad sublateral stripes that run from the front to over the wing roots. Broad, asymmetrical yellowish and dark brown scales are scattered throughout the wings. On the outside of the hind femur, there are four or five oblique pale marks. Dorsum of the abdomen is dark brown with lateral white and yellowish patches (Barraud, 1934).

Mansonia* (*Mansonioides*) *annulifera Theobald, 1901

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 17-ii-2022, Man landing. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Mesonotum with fine golden or yellow scales. Near the front edge, there are 2 distinct round white spots, a pair of spots behind them, and three less prominent markings at or near the level of the wing roots. Broad, asymmetrical scales that are both yellowish and dark brown are spotted all over the wings. The legs are yellowish and have several white rings on them. Brown scales cover the abdomen's dorsum (Barraud, 1934).

4.3.1.4. Tribe: Uranotaeniini Lahille, 1904

The tribe Uranotaeniini consist of 272 species of a single genus, Uranotaenia. It is commonly regarded as one of the most primordial Culicinae groups. There is no medical or economic importance associated with the species of this tribe. The species belonging to the tribe Uranotaeniini are small mosquitoes with the following characteristics: Maxillary palps very short, consisting of a single palpomere; wing membrane with incredibly minute and numerous microtrichia that are not visible at lower magnification; vein scales typically all broad and small, truncate or rounded at the apex (Mosquito Taxonomic Inventory).

4.3.1.4.1. Genus: Uranotaenia Lynch Arribalzaga, 1891

4.3.1.4.1.1. Subgenus: Pseudoficalbia Theobald, 1912

Uranotaenia (Pseudoficalbia) obscura Edwards, 1915.

Materials examined: 1♀, India: Kerala, Wayanad, Kuttimoola (11.8327° N, 75.9913° E), 15-v-2020, Larval collection. Samples collected: 1♀. Habitat: Rubber latex collecting cup. Coll: Maiby Thankachan.

Diagnosis : A small, dark brown species. Dark brown flat scales and a fair number of erect scales cover the vertex and nape. The proboscis, clypeus, palpi, and

antennae are all dark brown. Scales on the wings are black. Legs are dark brown, with pale brown femora underneath. The first segment of the hind tarsus is clearly longer than the tibia. The venter and dorsum of the abdomen are both dark brown and unmarked (Barraud, 1934).

Uranotaenia (Pseudoficalbia) nivipleura Leicester, 1908

Materials examined: 5♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E) 16-ix-2021, Larval collection. Samples collected: 5♀. Habitat: Fallen areca palm leaves. Coll: Maiby Thankachan.

Diagnosis : The entire hind tarsi are black. The abdomen is non banded with a dark brown dorsum and pale brown venter. Small pale scales that extend almost continuously from the wing root to the front or lateral edges of the mesonotum. The long, curving bristles of the mesonotum are noticeable. All the pleurae are pale (Barraud, 1934).

Uranotaenia (Pseudoficalbia)pseudostricklandi Natarajan, Rajavel & Jambulingam, 2018

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 15-v-2022, Resting collection. Samples collected: 1♀. Habitat: Cattle shed in a mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The species resemble *U. stricklandi*. The main differences between both are: Mesepimeron is dark without a median patch of greyish -white translucent scales. Lower half of metapleuron is dark. Thoracic pleura is pale yellow with conspicuous brownish -black markings. Mesonotum is dark brown with scales of same colour. Abdominal terga is predominantly dark brownish black scaled with distinct, narrow, uniform basal ochreous bands on terga III-VI (Natarajan, Rajavel & Jambulingam, 2017).

4.3.1.4.1.2. Subgenus: Uranotaenia Lynch Arribalzaga, 1891

Uranotaenia (Uranotaenia) rutherfordi Edwards, 1922

Materials examined: 1♀, India: Kerala, Wayanad, Paleri (11.7547° N, 75.8781° E) 21-v-2021, Larval collection. Samples collected: 1♀. Habitat: Slow running water in banana field, Coll: Maiby Thankachan.

Diagnosis : Completely dark proboscis and three white patches on the hind tibia. No white knee patches on the femora. Costa is completely dark. Segments 1-3 of the hind tarsi have white rings at the base and apex (Barraud, 1934).

Uranotaenia (Uranotaenia) testacea Theobald, 1905

Materials examined: 1♂, India: Kerala, Wayanad, Paleri (11.7547° N,75.8781°E), 06-v-2021, Larval collection. Samples collected: 1♂. Habitat: Banana field water. Coll: Maiby Thankachan.

Diagnosis : A medium-sized, dark species with blue scales only on the head and pleurae, not above the wing root. The top of the head is covered in flat, vivid blue or bluish-silver scales. Its postnotum is a pale brown color. A line of flat, bluish-silver scales extends from the head, over the pleurae, to the anterior margin of the mesepimeron. The apical 2/3rd of segment 3 and segments 4 and 5 of the hind tarsi are creamy white, and segment 1 of the hind tarsi is slightly longer than the tibia. The tibio-tarsal joint occasionally has a pale spot underneath it. The dorsum of the abdomen is darker while the ventral portion is paler (Barraud, 1934).

Uranotaenia (Uranotaenia) macfarlanei Edwards, 1914

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E) 30-v-2020, Larval collection. Samples collected: 1♀. Habitat: Forest. Coll: Maiby Thankachan.

Diagnosis : Distinct from other species by absence of blue tint in thoracic scales and abdominal patterns. White scales with a thin border that widens at the edges go along the eyes. A mixture of dark and light brown small scales covers the

mesonotum's deep brown integument in a rather dense manner. Except for a row of light-coloured scales running down the basal 1/4th of vein I, the wings are darkly scaled. The tips of the femur and tibia are slightly pale (Barraud, 1934).

Uranotaenia (Uranotaenia) campestris Leicester, 1908

Materials examined: 1♀, India: Kerala, Wayanad, Paleri (11.7547° N, 75.8781° E) 21-v-2021, Larval collection. Samples collected: 1♀. Habitat: Banana field water. Coll: Maiby Thankachan.

Diagnosis : Characterized by abdominal tergites with an apical band. On the borders of the eyes, a narrow band of bluish-white scales is visible. The proboscis, clypeus, palpi, and antennae are all dark brown. From the wing root to the post-pronotum, a broad band of bluish-white scales extends along the lateral margin. Wings have dark scales, with the exception of a line of light scales running down vein 1 from base to almost level with base of 2 (Barraud, 1934).

4.3.1.5. Tribe: *Sabethini* Blanchard, 1905

The tribe Sabethini includes 439 currently recognized species that comprise 14 genera: Isostomyia (4), Johnbelkinia (3), Kimia (5), Limatus (9), Malaya (12), Maorigoeldia (1), Onirion(7), Runchomyia (8), Sabethes (42), Shannoniana (3), Topomyia (68), Trichoprosopon (14), Tripteroides (123) and Wyeomyia (140). The tribe's species are referred to as 'sabethines.' Sabethines, as a whole, are not significant as disease vectors. Sabethine larvae are almost exclusively restricted to water contained in deceased and living plant matter, such as leaf axils, bromeliads, bamboo, tree-hole etc (Mosquito taxonomic Inventory).

4.3.1.5.1. Genus: *Malaya* Leicester, 1908

Malaya genurostris Leicester, 1908

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), Paleri (11.7547° N, 75.8781° E), 15-vi-2020, 18-viii-2021, Larval collection. Samples collected: 12♀. Habitat: Banana leaf axils. Coll: Maiby Thankachan.

Diagnosis : The species may be distinguished from *Mal. jacobsoni* by its yellowish-white clypeus, line of silvery scales between the eyes, wider silvery patch laterally on tergite IV, and silvery white scales on the front of the vertex. The proboscis is somewhat longer than the dark-brown antennae. The mesonotum's integument is dark brown, and from the front border to around the middle of the mesonotum, there is a clearly defined median line of flat bluish-violet scales with a metallic shine. Pleuron and postnotum are both dark brown. Wing is darkly scaled, and vein 6's termination is often a bit closer to the tip of the wing than the level of vein 5's fork and vein 2's base. Legs are dark brown, with whiter undersides on the femora. In some positions, all legs have a golden shine. Tibia's first segment of the hind tarsus is somewhat longer. Abdominal tergites are brownish-black, with huge silvery patches on I, II, IV, and VI, with IV having the largest of these patches (Barraud, 1934).

Malaya jacobsoni Edwards, 1930

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 15-vi-2020, Larval collection. Samples collected: 2♀. Habitat: Banana leaf axils. Coll: Maiby Thankachan.

Diagnosis : Adults resemble *Mal. genurostris* but have the following differences from them: Clypeus is dark. No scaling line can be observed, and the clypeus and tori both have mild pruinescence. Scales on the front of the vertex and on the antepronotum between the eyes are blue or bluish-violet, not silvery-white with a bluish shine. Mesonotum and pleurae have black integument. Both sexes have a large, completely black apical region of the probosis. The silvery band on the lateral side of abdominal tergite IV is smaller (Barraud, 1934).

4.3.1.5.2. Genus: *Tripteroides* Giles, 1904

4.3.1.5.2.1. Subgenus: Rachionotomyia Theobald, 1905

Tripteroides (Rachionotomyia) aranoides Theobald, 1901

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 15-x-2022, Larval collection. Samples collected: 2♀. Habitat: Cut bamboo stems. Coll: Maiby Thankachan.

Diagnosis : The majority of the head is covered with broad, flat, dark brown scales. The eye borders have a thin blue scale border. Palpi and proboscis are both dark brown in color, perhaps with a bronzy shine. Palpi is one-sixth of proboscis' length. White scales partially cover the clypeus. Broad, dark-grayish-brown scales cover the mesonotum; there are no dorso-central bristles. Flat, grayish-dark scutellar scales. On the antepronotum and post-pronotum, white scales may be observed. White scales cover Pleuron's surface. The integument is brown or yellowish. Non-banded, dark brown legs are present. The femora's undersides are lighter. The dorsum of the abdomen is brownish-black with a deep blue sheen (Barraud, 1934).

4.3.1.6. Tribe: Orthopodomyiini Belkin, Heinemann & Page, 1970

Tribe Orthopodomyiini consists of 36 species of Orthopodomyia. The species and species groups differ primarily in their thoracic and wing ornamentation. The ornamentation of the proboscis, maxillary palps, legs, and abdomen are all highly variable. Tribe's species are not medically significant (Mosquito Taxonomic Inventory).

4.3.1.6.1. Genus: *Orthopodomyia* Theobald, 1904

Orthopodomyia anopheloides Giles, 1903

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 02-i-2021, Larval collection. Samples collected: 1♀. Habitat: Cashew tree hole. Coll: Maiby Thankachan.

Diagnosis : The dorsal surface of the head densely covers with large, pale upright and forked scales. The front and eye margins are covered in a few narrow yellow scales, and the sides are heavily covered in black and numerous flat white scales. On the antenna's torus and first flagellar segment, there are a few white scales. More than half the length of the proboscis is made up of the palpi, which is black or dark brown with white markings in the middle and at the tip. Scales on the wings are both dark and light. Femora are dark brown with scales that are light and golden in colour. Anteriorly, the fore and mid femora are springled with light scales. The tips of all tibiae are white. Abdomen is dark brown. Tergite II–VII have thin white streaks at the base (Barraud, 1934).

4.3.2. SUBFAMILY: ANOPHELINAE

The subfamily Anophelinae consists of 501 recognised species. There are numerous genetic species of sibling species complexes that awaits official names. Three genera comprise the subfamily, namely, Anopheles, Bironella, and Chagasia. Anophelines are the common name for mosquitoes pertaining to these genera. Adult anophelines are readily identifiable based on their physical characteristics. Most species stand with their bodies at an angle of 45° to the surface, and their wing veins are covered with dark and light patches of scales. Some species' wing veins are completely covered in brown scales. Except for Bironella, the maxillary palpi of both sexes are roughly the same length as the proboscis. Occasionally, female palpi have semi-erect scales that give them an unkempt aspect. In Anopheles and Bironella, the scutellum is evenly spherical, whereas it is trilobed in Chagasia. The abdominal sterna and terga typically lack scales completely or nearly (Mosquito Taxonomic Inventory).

4.3.2.1. Genus: *Anopheles* Meigen, 1818

4.3.2.1.1. Subgenus: Anopheles Meigen, 1818

Anopheles (Anopheles) barbirostris Van der Wulp, 1884

Materials examined: 3♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Makkiyad (11.7490319° N, 75.90277° E), Nadakkal (11.7414° N,

75.9937° E), 11-vi-2020, Man landing, Larval collection. Samples collected: 3♀ and 1♂. Habitat: Mixed plantation, Forest, Banana field. Coll: Maiby Thankachan.

Diagnosis : A large, black anopheline. The occiput has relatively short, wide, and tightly spaced scales. Palpi is totally dark. Clypeus with no lateral scale tufts. Scutellum with big chaetae and a few tiny whitish hairs. Veins 1 and 3 have two milky white patches, and the region of the costa and subcosta is under four. Internal and exterior white lines on the hind femur. Tibiae have a distinct mark that is pale at the very base and at the points. A pale ring is found at the base of the femora. White scales cover the ventral surface of the abdomen. On the ventral side of the seventh abdominal segment, there is a tuft of hairs (Christophers, 1933).

Anopheles (Anopheles) insulaeflorum Swellengrebel & Swellengrebel de Graaf, 1920.

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 04-vii-2020, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The adult is quite similar to *An. aitkeni*. The thoracic pre-scutellar area is occupied by setae. Palpi are modest in size, entirely black, and extend to the proboscis. Costa and Subcosta entirely are dark. At the femur and tibia's extremities, there are no pale rings. Larvae in their fourth instar have certain peculiar traits that set them apart from *An. aitkeni*. The lateral hairs on IIIsegment are finer and have fewer branches than those on I and II, whereas the palmate hairs on the thorax and I–VII are very well developed. Abdominal palmate hair filaments have relatively little differentiation (Christophers, 1933).

Anopheles (Anopheles) peditaeniatus Leicester, 1908

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 12-iv-2020, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Less than 4 percent of the costa and subcosta are pale, including vein 1.Palpi is four-banded. Pale skin covers the palpi's tip. The inner costa does not have any pale scales, and vein 5 does not have any fringe spots. There are two faint bands on the tarsomeres of the hind legs (Christophers, 1933).

Anopheles (Anopheles) aitkeni James, 1903

Materials examined: 4♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277° E), 06-v-2022, Man landing. Samples collected: 4♀. Habitat: Forest. Coll: Maiby Thankachan.

Diagnosis : A small to moderately sized, delicate brown anopheline. Abruptly becomes smaller towards the eyes, creating a little sulcus-like area. The terminal two segments of the palpi are somewhat enlarged, giving them a characteristic club-like appearance. The palpi are equal to the proboscis, entirely dark, and scales are modest in size. Pleura is devoid of scales. There are no setae in the thorax's pre-scutellar area. Costa and Subcosta are dark. At the femur and tibia's ends, there are no pale rings. The dorsal lobe of the harpago typically has three spines; larvae with ic bifurcate around 1/4th of the way from the base (Christophers, 1933).

Anopheles (Anopheles) nigerrimus Giles, 1900.

Materials examined: 2♀, India: Kerala, Wayanad, Paleri (11.7547° N,75.8781°E), 06-v-2021, Larval collection. Samples collected: 2♀. Habitat: Banana field. Coll: Maiby Thankachan.

Diagnosis : Dark and big anopheline. Scutellum covered with thick, black hairs. Darkened pleurae are identified by thin, horizontal lines. Antennae with several broad white scales, often on the first five or more flagellar segments, and a few tiny pale scales on the torso. Pale tip and four bands on the palpi. Vein 1 is included in the pale region on the costa and subcosta, which is smaller than 4. On wing vein 5, the basal dark mark is large—0.4 times the length of the Cu stem. The amount of marking on tarsi varies greatly; typically, segments 1-3 on the fore and mid legs and

segments 1-4 on the hind legs have pale apical bands of modest length (Christophers, 1933).

Anopheles (Anopheles) culiciformis Cogill, 1903

Materials examined: 4♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E) 14-v-2021, Resting collection. Samples collected: 4♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : A black anopheline with a head that is only lightly decorated. The vertex abruptly narrows at the eyes to form a groove resembling a sulcus. The palpi are smaller than the proboscis and have broad, totally dark scales. Antennae having several big, black scales on the first segments of their flagella. The colour of the mesonotum is either dark brown or blackish, and it is uniformly coloured with indistinct lines. Pale markings are absent on the wing. The legs are evenly dark Without knee spots or tarsal bands. Even on cerci, the belly lacks scales and has dark hairs (Christophers, 1933).

Anopheles (Anopheles) crawfordi Reid, 1953

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 16-xi-2019, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Adult resembles *An. sinensis*. Palpi with four pale bands. Scattered pale scales are absent between the bands. Mesonotum with well-marked eye spots. Wing pattern is sharp and bright. Humeral cross vein bare or with only one or two dark scales, vein 1 without scattered pale scales in the preapical dark mark but extreme tip is pale. Coxa is with few pale sales. Pale bands at the hind tarsi are narrow (Reid, 1953).

4.3.2.1.2. Subgenus: Cellia Theobald, 1902

Anopheles (Cellia) elegans James, 1903

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), Makkiyad (11.7490319° N, 75.90277° E), 18-ii-2021, Resting

collection. Samples collected: 5♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Palpi is with four white bands. Wing scales are pale coloured. Legs are speckled. Femur, tibiae and tarsomeres 1 are dark scaled. Fore tarsomeres 2, 3 and 4 with broad, basal, and apical bands of white scales. Hind tarsomere 2 and 3 are with short apical pale bands, 4 with basal and apical pale bands and 5 with pale scales at the apex. Abdominal terga Vi without scales. Abdominal terga VII with few narrow cream-coloured scales and tergum VIII with long cream coloured to golden yellow scales. Sternum VII with patch of dark scale and sternum VIII with patch of white scales (Sallum et al., 2005).

Anopheles (Cellia) vagus Donitz, 1902

Materials examined: 1♀, India: Kerala, Wayanad, Makkiyad (11.7490319° N, 75.90277°E), 17-iv-2020, Larval collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The adult resembles *An. subpictus* very well, although it differs in the following areas: On female palpi, the subapical dark band is substantially less and only 1/4 to 1/5 times as long as the apical pale area. In the direction of the proboscis extremity, there is a light tache. Typically, the subapical black patch on the costa is small (Christophers, 1933).

Anopheles (Cellia) jamesi Theobald, 1901

Materials examined: 7♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 18-i-2022, Resting collection. Samples collected: 7♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Pleura lacks scales and is dark. The hairs are spiracular. The pre-apical dark band and the apical pale band of the proboscis are roughly similar in size. Black and light portions in the apical half of the wing are almost equal, with vein 6 having three little dark patches and vein 5 being widely pale. Leg speckling is

present. The hind tarsomeres 5, 4, and 3 are totally pale. The inner costa is mostly black, while the region where wing vein 5 splits is pale (Christophers, 1933).

Anopheles (Cellia) tessellatus Theobald, 1901

Materials examined: 2♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 08-ii-2021, Larval collection. Samples collected: 2♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The head scales are with a wide, pale vertical patch. Thorax with apical dark scales. The pleurae have noticeable scales and are black. Palpi are with a second pale band at the tip of segment two, a patch of pale scales on segment three, and three broad white apical bands divided by two narrow black bands. The veins of the wings have more dark spots, and vein 6 has a fringe patch and border scales that almost reach the base of the wings. The legs have specks. The basal portion of the front femoral bone is significantly enlarged. Narrow bands are found on the tarsomeres of the hind legs (Christophers, 1933).

Anopheles (Cellia) subpictus Grassi 1899

Materials examined: 3♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 10-i-2021, Resting collection. Samples collected: 3♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The black region between the apical and preapical pale bands and the apical pale area of palpi are approximately equal in length. The mesonotum has thick scales. The labium is uniformly dark. Pleurae found with a few scales. Three tiny, black accessory dots can be seen at the base of the costa. Femur with base-positioned dark rings. The forelegs' tibiae are noticeably pale underneath, especially at the tips. The mid- and hind-leg tarsi have much narrower bands, primarily apical. Golden hairs and several slender, yellow scales on the abdomen. Scales on the cerci are black (Christophers, 1933).)

Anopheles (Cellia) splendidus Koidzumi, 1920

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 18-ii-2021, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : A well-defined white vertical area on the head. On vein 1, pale bands are commonly bridged with dark patches. The pale band on the proboscis is almost identical to the sub apical pale band. Segment 3 has two or more noticeable white spots, as well as speckling on the front and hind legs. 5 and 4 of the hind tarsomeres are entirely pale. It has a dark abdomen. Inconspicuous scales identify the venter of VII and the hind border of VIII (Christophers, 1933).

Anopheles (Cellia) karwari James, 1903

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 18-ii-2021, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : The palpi have four bands. The apical segment is not entirely white, with a dark band separating it towards the base. The following pale region covers a significant portion of segment 4, and the seeming additional band is the standard band in segments 3–4. Antepronotum of the thorax without scales. More than 3 percent of the costa and subcosta are pale, including vein 1. The legs are devoid of speckles. On the hind leg, tarsomeres 5, 4, and 3 are dark. The dorsum of VIII is black with light yellowish hairs and slender golden scales (Christophers, 1933).

Anopheles (Cellia) stephensi Liston, 1901

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 18-ii-2021, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Wide-scaled thorax. The dark area in between the broad pale bands at the apex and base of the palps is narrower than either band. More than four

pale patches on the costa and subcosta of antennae with little pale scales on the torus. The tibia and femur have specks. The interior surfaces of the hind femur and tibia are pale. Abdomen with tergites II to VIII having small scales that get wider as they get closer to the posterior (Christophers, 1933).

Anopheles (Cellia) jeyporiensis James, 1902

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 19-ii-2020, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Mesonotum has slender, white scales covering the median area. Scales are absent from the pleura. More than 3 percent of the costa and subcosta are pale. When compared to the posterior forked cell, the anterior forked cell is farther from the base of the costa. There are no specks on the legs. On the hind leg, tarsomeres 5, 4, and 3 are dark. Bands are seen on the middle and fore tarsomeres of the leg. Even on cerci, the abdomen is completely devoid of scales (Christophers, 1933).

Anopheles (Cellia) fluviatilis James, 1902

Materials examined: 1♀, 1♂, India: Kerala, Wayanad, Makkiyad (11.749031° N, 75.9027° E), 18-i-2022, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Small to medium sized species. Wing vein R4+5 with long pale area in the middle. Base of vein R is pale. Palpus with three pale bands. Preapical dark band is very broad, much wider than either of the two apical pale bands. Mesonotum is without scales, lightly covered with setae. Tarsi is entirely dark or with very minute pale bands (Christophers, 1933).

***Anopheles (Cellia) theobaldi* Giles,1901**

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 18-vi-2022, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Pale area on costa and subcosta including vein 1 is greater than 3. Femur and tibia of forlegs and hind legs are speckled. Three banded palpi. Apical pale band is nearly equal to the subapical pale band. Hind tarsomeres 5 and 4 are pale (Christophers, 1933).

***Anopheles (Cellia) culicifacies* Giles,1901**

Materials examined: 1♀, India: Kerala, Wayanad, Mananthavady (11.8014° N, 76.0044° E), 18-vi-2022, Resting collection. Samples collected: 1♀. Habitat: Mixed plantation. Coll: Maiby Thankachan.

Diagnosis : Small to medium sized species. Scales are of normal type. Vertical chaetae pale or darkish forming five hairs on either side, making an imperfect ventral tuft. Plapi with short terminal segment. Dark area between apical and subapical pale bands many times the length of apical pale area. Base of costa with interruption. Fringe spots at one or two veins only. Tarsi are unbanded. Coxae is devoid of scales. Abdomen is devoid of scales (Christophers, 1933).

4.4. Recognition of New Species:

Two new *Uranataenia* species larvae have been collected and identified to subgenus level. Some striking features in the body hairs and arrangements regarded these as different species. It needs pupal and adult association for the confirmation up to species level. The specimens are preserved in ICMR-Vector Control Research Centre, Pondicherry.

4.5. Discussion

The different study reports from Kerala have all been combined, and it is observed that 130 species from 16 genera viz, *Anopheles* (31 sp), *Aedes* (31

sp.),*Culex* (30 sp.), *Mansonia* (4 sp.), *Armigeres* (4 sp.), *Heizmannia* (4 sp.), *Uranotaenia* (11 sp.), *Orthopodomyia* (2 sp.), *Mimomyia* (3 sp.), *Verrallina* (4 sp.), and single species each from *Haemagogus*, *Topomyia*, *Ficalbia*, *Tripteroides*, *Coquilletidia* and *Toxorhynchitis* respectively, have been reported so far (Sumodan, 2014; Aneesh et al., 2014; Thankachan and Gopinath, 2017; Balasubramanian et al., 2021). The present study reported 80 mosquitoes from 12 genera; 10 genera which were previously reported in Kerala and 2 genera namely *Malaya* and *Lutzia* are new reports to Kerala. Out of 80 species collected, 29 species from 5 genera are vectors of various diseases like Dengue Fever, Chikungunya, Yellow Fever, Zika Fever, Japanese Encephalitis, Filariasis etc. Fifty one non medically important mosquito species from 7 Genera were also been reported. Most of the *Aedes* species were abundant during monsoon as it preferred clear stagnant waters for egg laying. Anopheles species were abundant during post monsoon period.

Abundance of mosquitoes were observed during monsoon seasons whereas diversity was more during pre-monsoon and post-monsoon seasons where intermittent rain favoured the multiplications of mosquitoes. This is in support with the result of Abdelrazec and Gumel (2017) who depicts a peak in mosquito abundance for temperature and rainfall values in the range 20−25°C. The climate of Wayanad is in concordance with this temperature.

Very few studies on mosquitoes of Wayanad district have been conducted. The area is plentiful with various types of plantations, forest, and a humid climate that is ideal for mosquito breeding. Fallen areca palm leaves, paddy fields, and slow-running waters in the banana cultivation field are all rich mosquito larval habitats. The collection of 80 species of mosquitoes belonging to 12 genera in the Mananthavady Taluk of Wayanad district demonstrates the area's abundance of mosquito species. 21 new reports from Kerala (*Ae. thomsoni, Ae. lineatopenne, Cx. barraudi, Cx. hutchinsoni, Cx. murreli, Cx. foliatus, Cx. wilfredi, Cx. minor, Cx.nigropunctatus, Cx.bicornutus, Tr.aranoides, Lt. halifaxi, Ur. nivipleura, Ur. rutherfordi, Ur. macfarlanei,Ur.obscura, Ur.campestris, An.crowfordi, Ver.indica,*

Mal. genurostris and *Mal. jacobsoni*), 29 vector species, (Table 4.2) and two new records (*Uranotaenia* sp) contribute significantly to the taxonomy of mosquitoes.

Of the 29 mosquito vectors collected, *Ae. albopictus, Ae. aegypti* and *Ae. vittatus* are the vectors of Dengue, Chikungunya, and Yellow fever. *Ae. albopictus* is also a Zika virus carrier. During the collection, *Ae. Albopictus* was the second most prevalent species after *Ar. sabalbatus*. The species abundance was more during monsoon season. Kalra and Prasittisuk (2004) and Jomon et al. (2009) also support this result as they indicate *Ae.albopictus* as the major species in prevalence and distribution in their studies conducted in different parts of Kerala. Japanese encephalitis (JE) vectors dominated all other vectors collected from the area with sixteen species namely, *Cx. vishnui, Cx. pseudovishnui, Cx. tritaeniorhyncus, Cx. gelidus, Cx. fuscocephala, Cx. bitaeniorhyncus, Cx. infula, Cx. quinquefasciatus, Cx. whitemorei, An. subpictus, An. barbirostris, An. peditaeniatus, Man. indiana, Man. uniformis, Man. annulifera* and *Ar.sabalbatus*. *Ar. subalbatus* is an incriminated vector of JE (Liu et al., 2013; Das et al., 1983; Aneesh et al., 2014) and this species prevailed every other species in the collection, regardless of season or location. The species abundance of *Ae. albopictus* and *Ar. sabalbatus* of the area is in support with the findings of Sumodan (2014), in his studies on the medically important vectors in Wayanad. All the vectors identified in India and abroad for lymphatic filariasis viz., *Cx quinquefasciatus, Cx. vishnui, Cx. bitaeniorhyncus, Cx. gelidus, , An. stephensi, An. nigerrimus, An. peditaeniatus, An. crawfordi, Man. indiana, Man. uniformis* and *Ae. niveus* were collected from the area. The malaria vectors found in this area are *An. stephensi, An. vagus, An. nigerrimus, An. fluvialitis, An. jeyporiensis, An.culicifacies* and *An. elegans.* Mosquito vectors were abundant in urban areas than rural areas which itself indicates the dangerous situations of vector borne diseases.

CHAPTER 5

MOLECULAR BARCODING OF MOSQUITOES

During the study period (2019—2022), mosquitoes classified under 12 genera were collected from Mananthavady Taluk, Wayanad, out of which 35 mosquito species coming under 7 genera were barcoded using marker genes. The single species representing the genera *Uranotaenia, Heizmannia, Orthopodomyia, Tripteroides* and *Lutzia* were not barcoded as only single specimen was collected from the study area and need to be kept as voucher species. The mitochondrial cytochrome oxidase I (CO I) gene sequences of these species were submitted to NCBI GenBank for worldwide accession with respective accession numbers (Table 5.1). All the sequences had accurate match with their own haplotypes in the NCBI with significant percentage identities. The accuracy of the identification was demonstrated by the fact that every DNA sequence in the current investigation shared 98 to 100% of similarities with the sequences in BLAST.

Table 5.1: List of mosquito species barcoded during the present study with their Voucher number, Accession number and list of representative barcode figures

Sl. No.	Species	Voucher Number	Accession Number	Figure Nos.
	Genus: Aedes			
1.	*Aedes aegypti*	CUMM175	OR130932.1	5.1.1(a) – (c)
2.	*Aedes albopictus*	CUMM6	MW542315	5.1.2(a) – (c)
3.	*Aedes subalbopictus*	CUMM54	MW931745	5.1.3(a) – (c)
4.	*Aedes vittatus*	CUMM62	MW931755	5.1.4(a) – (c)
5.	*Aedes pseudotaeniatus*	CUMM38	MW931741	5.1.5(a) – (c)
6.	*Aedes chrysolineatus*	CUMM28	MW931765	5.1.6(a) – (c)
7.	*Aedes niveus*	CUMM79	ON506043	5.1.7(a) – (c)
8.	*Aedes barraudi*	CUMM19	MW549045	5.1.8(a) – (c)
9.	*Aedes cogilli*	CUMM68	OP078702	5.1.9(a) – (c)
	Genus: Culex			
10.	*Culex gelidus*	CUMM9	MW542314	5.2.1(a) – (c)
11.	*Culex fuscocephala*	CUMM3	MW535377	5.2.2(a) – (c)
12.	*Culex tritaeniorhyncus*	CUMM65	MW922794	5.2.3(a) – (c)

13.	*Culex quinquefasciatus*	CUMM59	MW926770	5.2.4(a) – (c)
14.	*Culex pseudovishnui*	CUMM49	MW922745	5.2.5(a) – (c)
15.	*Culex vishnui*	CUMM39	MW549044	5.2.6(a) – (c)
16.	*Culex bitaeniorhyncus*	CUMM55	MW555571	5.2.7(a) – (c)
17.	*Culex infula*	CUMM63	MW922750	5.2.8(a) – (c)
18.	*Culex pallidothorax*	CUMM7	MW542320	5.2.9(a) – (c)
19.	*Culex uniformis*	CUMM90	OM368631	5.2.10(a) – (c)
20.	*Culex minor*	CUMM57	MW555438	5.2.11(a) – (c)
	Genus: Mansonia			
21.	*Mansonia indiana*	CUMM41	MW922742	5.3.1(a) – (c)
22.	*Mansonia uniformis*	CUMM11	MW542318	5.3.2(a) – (c)
	Genus: Armigeres			
23.	*Armigeres sabalbatus*	CUMM5	MW542319	5.4.1(a) – (c)
24.	*Armigeres aureolineatus*	CUMM29	OP093565	5.4.2(a) – (c)
	Genus: Malaya			
25.	*Malaya genurostris*	CUMM23	MW549050	5.5.1(a) – (c)
	Genus: Verralina			
26.	*Verralina indica*	CUMM8	OP107393	5.6.1(a) – (c)
	Genus: Anopheles			
27.	*Anopheles barbirostris*	CUMM13	MW922751	5.7.1(a) – (c)
28.	*Anopheles insuleiflorum*	CUMM135	OP028210	5.7.2(a) – (c)
29.	*Anopheles aitkeni*	CUMM106	OP024181	5.7.3(a) – (c)
30.	*Anopheles crawfordi*	CUMM64	OM368571	5.7.4(a) – (c)
31.	*Anopheles jamesi*	CUMM66	MW931754	5.7.5(a) – (c)
32.	*Anopheles splendidus*	CUMM94	OM368636	5.7.6(a) – (c)
33.	*Anopheles stephensi*	CUMM40	MW549046	5.7.7(a) – (c)
34.	*Anopheles karwari*	CUMM161	OQ286389	5.7.8(a) – (c)
35.	*Anopheles elegans*	CUMM160	OQ509988	5.7.9(a) – (c)

>seq1*Aedes (Stegomyia) aegypti* CUMM175 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

ACTTAGCCACCCTGGTATATTTATTGGGAATGACCAAATTTATAATGTAATTGTAA
CAGCTCATGCATTTATTATAATTTTCTTTATAGTAATACCAATTATAATTGGAGGA
TTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCCTGATATAGCCTTTCCTCG
AATAAATAATATAAGTTTTTGAATACTACCTCCTTCATTGACTCTTCTATTATCAA
GCTCAATAGTAGAAAATGGGGCAGGAACTGGGTGAACAGTTTATCCTCCTCTCTCT
TCAGGAACAGCTCATGCTGGAGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTT
AGCTGGAATTTCCTCAATTTTAGGGGCAGTAAATTTTATTACAACTGTGATTAATA
TACGATCGTCAGGAATTACTTTAGATCGACTACCTTTATTTGTTTGATCTGTAGTT
ATTACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTACTAT
GTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATCGGAGGAGGAG
ATCCTATTTTATACCAACACTTA

Figure 5.1.1.(a): The partial Mitochondrial COI gene sequence of *Aedes (Stegomyia) aegypti*

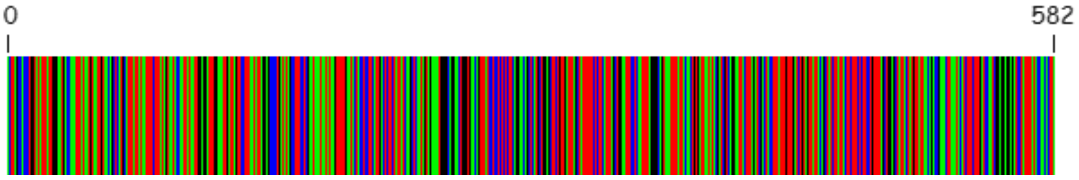

Figure 5.1.1.(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes (Stegomyia) aegypti*

>WIV81765.1cytochrome c oxidase subunit I Partial Amino acid sequences

LSHPGMFIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPR
MNNMSFWMLPPSLTLLLSSSMVENGAGTGWTVYPPLSSGTAHAGASVDLAIFSLHL
AGISSILGAVNFITTVINMRSSGITLDRLPLFVWSVVITAILLLLSLPVLAGAITM
LLTDRNLNTSFFDPIGGGDPILYQHL

Figure 5.1.1.(c): The translational product of the mitochondrial COI gene of *Aedes (Stegomyia) aegypti*

>seq1*Aedes (Stegomyia) albopictus* CUMM6 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TAAAGATATTGGAACATTATACTTTATTTTCGGTATTTGATCTGGAATAGTCGGAA
CTTCACTAAGAGTTTTAATTCGTATTGAACTTAGACATCCTGGTATATTTATTGGA
AATGATCAAATTTATAATGTAATTGTTACTGCTCATGCTTTTATTATAATTTTTTT
TATAGTAATACCTATCATAATTGGAGGATTTGGAAACTGACTAGTACCCTTAATAC
TAGGAGCCCCTGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGAATATTA
CCCCCCTCTTTAACACTGCTGCTTTCTAGTTCTATAGTAGAAAACGGAGCTGGAAC
AGGGTGAACGGTTTATCCTCCCCTTTCTTCTGGAACAGCTCATGCTGGGGCTTCAG
TTGATTTAGCAATTTTTTCTTTACATTTAGCGGGAATCTCATCTATTTTAGGAGCA
GTAAATTTTATTACAACTGTAATTAATATACGATCAGCTGGTATTACTCTTGATCG

ACTACCTTTATTTGTGTGATCAGTAGTAATTACAGCTATTTTATTACTTCTTTCTC
TACCCGTATTAGCCGGAGCTATTACTATATTATTAACAGACCGAAATTTAAATACA
TCTTTTTTTGATCCAATTGGAGGGGGAGACCCTATTTTATATCAACATTTATTTTG
ATTTTTTGG

Figure 5.1.2.(a): The partial Mitochondrial COI gene sequence of *Aedes (Stegomyia) albopictus*

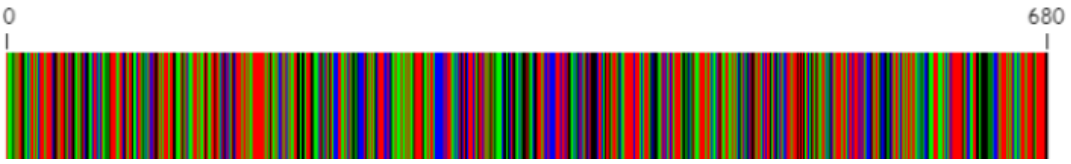

Figure 5.1.2.(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes (Stegomyia) albopictus*

>QQY98753.1 cytochrome c oxidase subunit I Partial Amino acid sequences

KDIGTLYFIFGIWSGMVGTSLSVLIRIELSHPGMFIGNDQIYNVIVTAHAFIMIFF
MVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGAGT
GWTVYPPLSSGTAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSAGITLDR
LPLFVWSVVITAILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPIYQHLFWF
FG

Figure 5.1.2.(c): The translational product of the mitochondrial COI gene of *Aedes (Stegomyia) albopictus*

>seq1*Aedes subalbopictus* CUMM54 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AAATTGATTAGTTCCTTTAATATTAGGGGCTCCTGATATAGCTTTCCCTCGAATAA
ATAATATAAGTTTTTGAATATTACCTCCTTCATTAACACTACTACTTTCTAGTTCT
ATAGTAGAAAATGGAGCTGGAACAGGTTGAACTGTTTATCCCCCTCTTTCTTCTGG
AACTGCTCATGCTGGGGCTTCAGTTGATTTAGCAATTTTTTCTTTACATTTAGCAG
GAATTTCTTCAATTTTAGGAGCAGTAAATTTTATTACTACTGTAATTAATATACGA
TCAGCAGGAATTACTCTTGACCGACTTCCTTTATTTGTTTGATCAGTAGTAATTAC
AGCTATTTTATTACTTCTTTCTTTACCTGTATTAGCAGGAGCTATTACTATACTAT
TAACAGATCGAAATTTAAATACATCATTCTTTGATCCAATTGGAGGAGGAGACCCA
ATTTTATATCAACATTTATTTTGATTCTTTGGACATCCTGAAGTTTACATTTTAAT
TCTTCCAGGATTTGGAATAATTTCTCATATTATTACACAAGAAAGAGGAAAAAAGG
AAACTTTTGGTACTTTAGGAATAATTTATGCTATATTAACAATTGGACTTCTAGGA
TTTATTGTTTGAGCTCACCATATATTTACAGTTGGAATAGATGTAG

Figure 5.1.3(a): The partial Mitochondrial COI gene sequence of *Aedes subalbopictus*

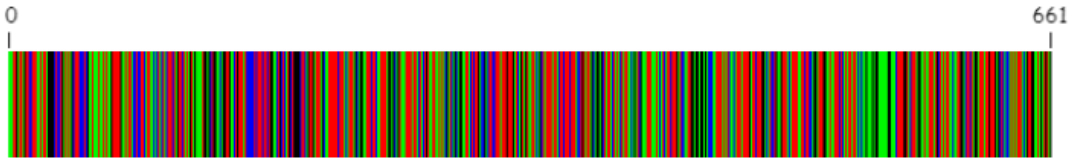

Figure 5.1.3(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes subalbopictus*

>QTW63365.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGAGTGWTVYPPLSSG
TAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSAGITLDRLPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLTIGLLGFIVWAHHMFTVGMDV

Figure 5.1.3(c): The translational product of the mitochondrial COI gene of *Aedes subalbopictus*

>seq1 *Aedes vittatus* CUMM62 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCTCGAATAAA
TAATATAAGTTTTTGAATATTACCTCCTTCATTAACACTACTACTTTCTAGTTCTA
TAGTAGAAAACGGAGCAGGAACAGGTTGAACAGTTTATCCTCCTCTATCTTCTGGG
ACTGCTCATGCTGGAGCATCAGTTGATTTAGCTATTTTTTCTCTTCATTTAGCAGG
GATTTCTTCAATTTTAGGAGCAGTAAATTTTATTACTACTGTAATTAATATACGAT
CAGCAGGAATTACTTTAGATCGTTTACCTTTATTTGTTTGATCTGTTGTAATTACA
GCTATTCTATTACTTTTATCATTACCAGTATTAGCAGGAGCTATTACTATATTATT
AACAGATCGAAATTTAAATACTTCATTCTTCGACCCAATTGGAGGAGGAGATCCTA
TTCTTTATCAACATTTATTTTGATTCTTTGGACATCCTGAAGTTTACATTTTAATT
CTTCCAGGATTTGGAATAATTTCTCATATTATTACTCAAGAAAGAGGAAAAAAGGA
AACATTTGGAACATTAGGAATAATTTATGCTATATTAACAATTGGTTTATTAGGAT
TTATTGTTTGAGCTCATCATATATTTACTGTAGGAATAGATGTAGATACACGAGCT
TACTTTACTTCTGCTACAATAATTATTGCTGTTCCAACTGGAATTAAAATTT

Figure 5.1.4.(a): The partial Mitochondrial COI gene sequence of *Aedes vittatus*

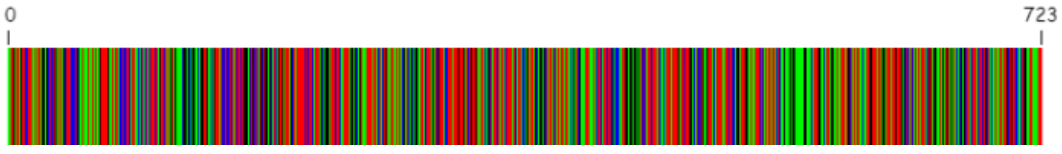

Figure 5.1.4.(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes vittatus*

>QTW63368.1cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGAGTGWTVYPPLSSG
TAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSAGITLDRLPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLTIGLLGFIVWAHHMFTVGMDVDTRA
YFTSATMIIAVPTGIKI

Figure 5.1.4.(c): The translational product of the mitochondrial COI gene of *Aedes vittatus*

>seq1 *Aedes pseudotaeniatus* CUMM38 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GAAATTGATTAGTTCCTTTAATATTAGGAGCCCCTGATATAGCATTTCCTCGGATA
AATAATATAAGTTTTTGAATACTACCTCCATCTTTAACCCTTCTTCTTTCTAGTAG
AATAGTTGAAAATGGATCAGGAACAGGTTGAACTGTTTACCCCCCTCTTTCATCGG
GGACTGCCCATGCAGGAGCTTCAGTAGACTTAACAATTTTTTCATTACATTTAGCT
GGTATTTCATCAATTTTAGGAGCTGTTAATTTTATTACAACTGTAATTAATATACG
ATCTGCTGGGATTACATTAGATCGATTACCTTTATTTGTTTGATCCGTTGTAATTA
CTGCAATTTTATTACTTTTATCTCTCCCTGTTTTAGCAGGAGCTATTACTATACTT
TTAACAGACCGAAACCTAAATACTTCATTTTTTGACCCAATTGGTGGGGGAGACCC
TATTTTATATCAACATTTATTTTGATTTTTTGGTCACCCAGAAGTTTATATTTTAA
TTTTACCAGGATTTGGAATAATTTCTCATATTATTACTCAAGAAAGAGGAAAAAAG
GAAACATTTGGAACATTAGGAATAATTTATGCTATATTAACTATTGGTTTATTAGG
ATTTATTGTATGAGCTCATCATATATTTACTGTAGGAATAGATGTAGATACACGAG
CTTACTTTACTTCAGCTACA

Figure 5.1.5.(a): The partial Mitochondrial COI gene sequence of *Aedes pseudotaeniatus*

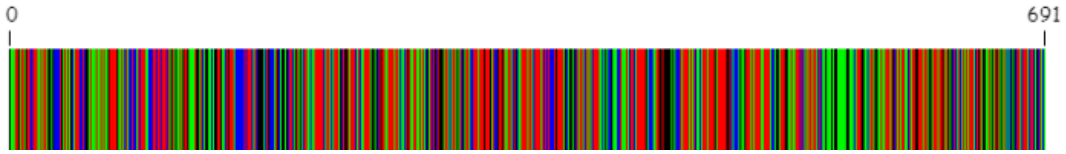

Figure 5.1.5.(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes pseudotaeniatus*

>QTW63364.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGSGTGWTVYPPLSSG
TAHAGASVDLTIFSLHLAGISSILGAVNFITTVINMRSAGITLDRLPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLTIGLLGFIVWAHHMFTVGMDVDTRA
YFTSAT

Figure 5.1.5.(c): The translational product of the mitochondrial COI gene of *Aedes pseudotaeniatus*

>seq1*Aedes chrysolineatus* CUMM28 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GAATATTTCCTCCATCATTAACACTATTAATTTCAGGTAGAATAGTAGAAAATGGA
GCTGGAACAGGATGAACAGTTTATCCTCCTTTATCATCTAGTACAGCTCATGCAGG
AGCATCTGTTGACTTAACAATTTTTTCTTTACATTTAGCCGGAGTTTCTTCAATTT
TAGGAGCAGTAAATTTTATTACTACTGTTATTAATATACGATCTTCAGGAATTACT
TTAGATCGAATACCTTTATTTGTTTGATCTGTTGTAATTACTGCAATTTTATTTCT
TCTTTCTCTTCCTGTTTTAGCTGGAGCTATTACTATACTTTTAACTGATCGTAATT
TAAATACTTCCTTCTTTGACCCTATAGGAGGAGGAGATCCTATTCTTTATCAACAT
TTATTCTGATTTTTTGGACATCCAGAAGTTTATATTTTAATTTTTCCTGGATTTGG
AATAATTTCTCATATTATTACACAAGAAAGAGGAAAAAAGGAAACATTTGGAACAT
TAGGAATAATTTATGCTATATTAGCAATTGGATTACTTGGATTTATTGTATGAGCC
CATCATATATTTACTGTAGGAATAGATGTAGAT

Figure 5.1.6.(a): The partial Mitochondrial COI gene sequence of *Aedes chrysolineatus*

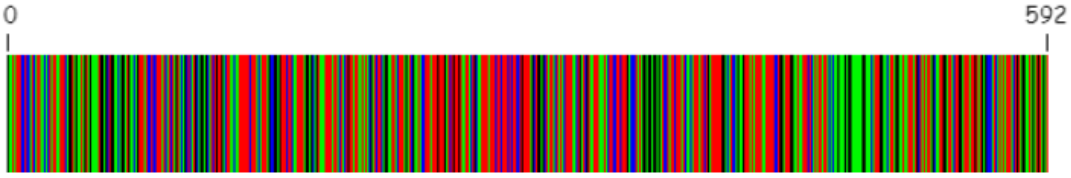

Figure 5.1.6.(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes chrysolineatus*

>QTW63369.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

MFPPSLTLLISGSMVENGAGTGWTVYPPLSSSTAHAGASVDLTIFSLHLAGVSSIL
GAVNFITTVINMRSSGITLDRMPLFVWSVVITAILFLLSLPVLAGAITMLLTDRNL
NTSFFDPMGGGDPILYQHLFWFFGHPEVYILIFPGFGMISHIITQESGKKETFGTL
GMIYAMLAIGLLGFIVWAHHMFTVGMDVD

Figure 5.1.6.(c): The translational product of the mitochondrial COI gene of *Aedes chrysolineatus*

>seq1 *Aedes niveus* CUMM79 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TTCTAGTAGTATAGTTGAAAATGGATCAGGAACAGGGTGAACTGTTTATCCTCCTC
TATCATCTGGAACTGCACATGCAGGAGCTTCAGTTGATTTAACAATTTTTTCTCTT
CATTTAGCCGGTATTTCTTCAATTTTAGGAGCAGTAAATTTTATTACTACTGTAAT
TAATATACGATCTTCAGGAATTACTGTGGATCGATTACCTTTATTTGTTTGATCTG
TTGTAATTACTGCTGTTTTATTACTTTTATCTTTACCTGTTTTAGCCGGAGCTATT
ACTATATTATTAACAGACCGAAATTTAAATACTTCATTCTTTGACCCAATTGGAGG
AGGAGACCCTATTTTATACCAACATTTATTTTGATTTTTTGGGCATCCTGAAGTTT
ATATTTTAATTTTACCAGGATTTGGTATAATTTCCCATATTATTACTCAAGAAAGA
GGTAAAAAGGAAACATTTGGAACTCTTGGGATAATTTATGCTATATTAACAATTGG
TTTATTAGGATT

Figure 5.1.7.(a): The partial Mitochondrial COI gene sequence of *Aedes niveus*

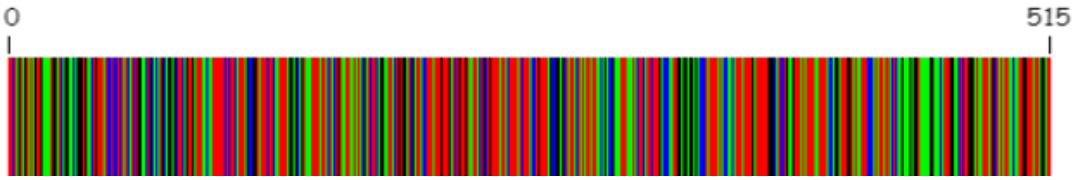

Figure 5.1.7(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes niveus*

>UQK90329.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

SSSMVENGSGTGWTVYPPLSSGTAHAGASVDLTIFSLHLAGISSILGAVNFITTVI
NMRSSGITVDRLPLFVWSVVITAVLLLLSLPVLAGAITMLLTDRNLNTSFFDPIGG
GDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFGTLGMIYAMLTIG
LLG

Figure 5.1.7(c): The translational product of the mitochondrial COI gene of *Aedes niveus*

>seq1 *Aedes barraudi* CUMM19 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AATTGATTAGTTCCTTTAATATTAGGAGCACCTGATATAGCTTTTCCTCGTATAAA
TAATATAAGTTTTTGACTCCTTCCTCCTTCATTAACTCTCCTCTTATCCAGCTCTA
TGGTAGAAAATGGAGCTGGGACAGGTTGAACTGTTTATCCTCCATTATCTTCAGGA
ACAGCCCATGCTGGAGGATCAGTTGATCTAGCTATTTTTTCCCTTCATTTAGCTGG
AATTTCTTCTATTTTAGGAGCTGTAAATTTTATTACAACTGTAATTAACATACGAT
CTGCCGGAATTACCCTTGACCGATTACCATTATTTGTTTGATCAGTTGTAATTACA
GCTATTTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGGGCTATTACAATACTATT
AACTGATCGAAATTTAAATACATCATTTTTTGACCCTATTGGTGGAGGAGACCCAA
TTTTGTATCAACATTTATTTTGATTTTTTGGGCATCCAGAAGTTTATATTTTAATT
TTACCCGGATTCGGAATAATTTCTCATATTATTACTCAAGAAAGAGGAAAAAAGGA
AACATTTGGAACATTAGGAATAATTTATGCTATACTTACAATTGGTTTATTGGGGT
TCATTGTTTGAGCCCATCATATATTTACAGTAGGAATAGATGTTGACACTCGAGCA
TACTTTACTTCTGCTACTATAATTATTGCTGTTCCAACTGGAATAAAAATTTT

Figure 5.1.8(a): The partial Mitochondrial COI gene sequence of *Aedes barraudi*

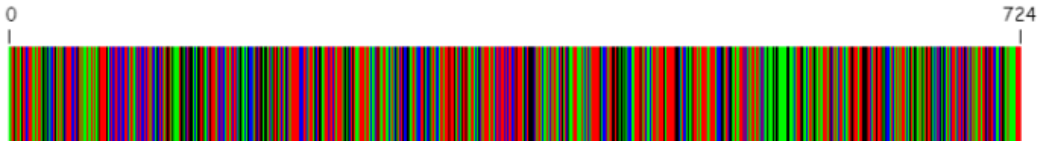

Figure 5.1.8(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes barraudi*

>URA20202.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLLSSSMVENGAGTGWTVYPPLSSG
TAHAGGSVDLAIFSLHLAGISSILGAVNFITTVINMRSAGITLDRLPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLTIGLLGFIVWAHHMFTVGMDVDTRA
YFTSATMIIAVPTGMKI

Figure 5.1.8(c): The translational product of the mitochondrial COI gene of *Aedes barraudi*

>seq1 *Aedes cogilli* CUMM68 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

CCTCGAATAAATAATATAAGTTTTTGAATATTACCCCCATCATTAACCCTTCTCCT
TTCCAGAAGTATAGTAGAAAATGGATCAGGAACTGGGTGAACCGTTTATCCTCCTC
TTTCATCAGGAGTAGCCCATGCTGGAGCTTCTGTAGATTTAACAATTTTTTCTCTT
CATTTAGCAGGAATTTCATCAATTTTAGGAGCAGTAAATTTTATTACTACTGTAAT
TAATATACGATCTTCCGGTATTACATTAGATCGTCTTCCTTTATTTGTTTGATCTG
TAGTAATTACAGCAATCTTATTACTTTTATCTTTACCTGTATTAGCAGGAGCAATT
ACTATATTATTAACAGATCGAAATTTAAATACTTCTTTCTTCGACCCAATCGGAGG
AGGAGACCCAATTTTATATCAACATTTATTTTGATTTTTTGGTCACCCAGAAGTTT
ATATTTTAATTCTACCCGGATTTGGAATAATTTCTCATATTATTACTCAAGAAAGA
GGAAAAAAGGAAACATTTGGAACATTAGGAATAATTTATGCAATATTAACAATTGG
ATTATTAGGATTCATTGTTTGAGCTCATCATATATTTACAGTAGGAATAGATGTAG
ATACACGAGCTTATTTTACTTCAGCTACAATAATCATTGCTGTTCCA

Figure 5.1.9(a): The partial Mitochondrial COI gene sequence of *Aedes cogilli*

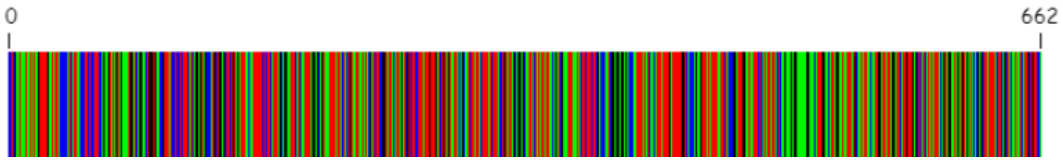

Figure 5.1.9(b): Molecular barcode of the partial mitochondrial COI gene of *Aedes cogilli*

>UUA62475.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

PRMNNMSFWMLPPSLTLLLSSSMVENGSGTGWTVYPPLSSGVAHAGASVDLTIFSL
HLAGISSILGAVNFITTVINMRSSGITLDRLPLFVWSVVITAILLLLSLPVLAGAI
TMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQES
GKKETFGTLGMIYAMLTIGLLGFIVWAHHMFTVGMDVDTRAYFTSATMIIAVP

Figure 5.1.9(c): The translational product of the mitochondrial COI gene of *Aedes cogilli*

>seq1 *Culex gelidus* CUMM9 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

CTTCATTAAGAATTCTAATTCGAGCAGAACTAAGTCAGCCTGGAGTATTTATTGGA
AATGATCAAATTTATAATGTTATTGTAACTGCTCACGCTTTTATTATAATTTTTTT
TATAGTTATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATAC
TAGGAGCTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGAATACTT
CCTCCTTCATTAACTTTACTACTTTCAAGTAGTTTAGTTGAAAATGGAGCTGGAAC
TGGATGAACAGTTTATCCCCCTCTTTCATCAGGTACAGCTCATGCTGGAGCTTCAG
TTGATTTAGCTATTTTTTCTTTACATTTAGCTGGGATTTCATCAATTTTAGGAGCA
GTAAATTTTATTACAACAGTAATTAATATACGATCTTCAGGAATTACACTTGATCG
AATACCTTTATTTGTTTGATCTGTAGTTATTACTGCTGTTTTATTACTCCTTTCAT
TACCCGTATTAGCTGGAGCTATTACAATATTATTAACTGATCGAAACCTAAATACT
TCATTTTTTGACCCTATTGGAGGAGGAGATCCTATTTTATACCAACATTTATTTTG
ATTTTTTGGTCACC

Figure 5.2.1(a): The partial Mitochondrial COI gene sequence of *Culex gelidus*

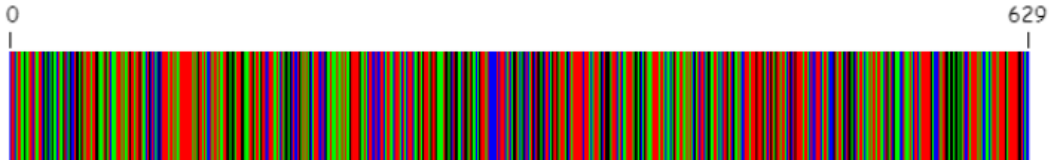

Figure 5.2.1(b): Molecular barcode of the partial mitochondrial COI gene of *Culex gelidus*

>QQY98752.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

SLSILIRAELSQPGVFIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSGTAHAGASV
DLAIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVITAVLLLLSL
PVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGH

Figure 5.2.1(c): The translational product of the mitochondrial COI gene of *Culex gelidus*

>seq1 *Culex fuscocephala* CUMM3 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TAAGTATTCTTATTCGAGCAGAATTAAGTCAACCCGGAGTTTTTATTGGAAATGAT
CAAATTTATAATGTAATTGTAACTGCTCATGCTTTTATTATAATTTTTTTTATAGT
TATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAG
CTCCAGATATAGCATTCCCTCGAATAAATAATATAAGTTTTTGAATACTACCACCT
TCTCTAACATTACTACTTTCAAGTAGTTTAGTAGAAAATGGAGCTGGAACTGGATG
AACAGTTTATCCCCCTCTTTCATCTGGGACAGCTCACGCCGGAGCATCAGTAGACT
TAGCTATTTTTTCTCTTCATTTAGCTGGGATTTCATCAATTTTAGGTGCTGTAAAT
TTTATTACAACAGTAATTAATATACGATCTTCAGGAATTACTTTAGATCGAATACC
ATTATTTGTTTGATCAGTAGTTATTACTGCTGTTTTACTTCTTTTATCTTTACCTG
TATTAGCCGGAGCTATTACTATATTATTAACAGATCGAAATTTAAATACTTCATTC
TTTGACCCAATTGGAGGAGGAGATCCAATTCTATATCAACATTTATTTTGATTTTT
TGGTCACCCTGA

Figure 5.2.2(a): The partial Mitochondrial COI gene sequence of *Culex fuscocephala*

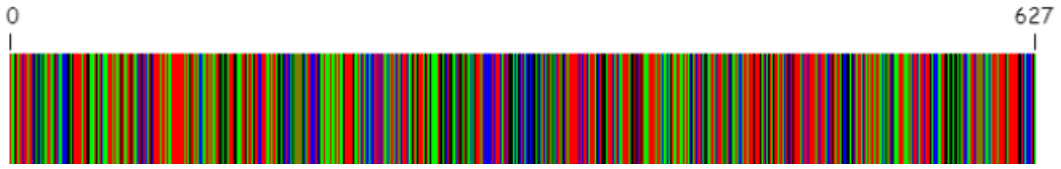

Figure 5.2.2(b): Molecular barcode of the partial mitochondrial COI gene of *Culex fuscocephala*

>QQY84135.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

SILIRAELSQPGVFIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGA
PDMAFPRMNNMSFWMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSGTAHAGASVDL
AIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVITAVLLLLSLPV
LAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHP

Figure 5.2.2(c): The translational product of the mitochondrial COI gene of *Culex fuscocephala*

>seq1 *Culex tritaeniorhyncus* CUMM65 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AAATTGATTAGTTCCTTTAATACTTGGAGCTCCTGATATGGCCTTTCCACGAATAA
ATAATATAAGTTTTTGAATACTACCTCCTTCATTAACTCTACTACTTTCAAGTAGT
TTAGTAGAAAATGGAGCTGGAACTGGATGAACAGTTTATCCACCCCTATCATCTGG
AACAGCACATGCTGGAGCTTCAGTTGATTTAGCTATTTTTTCTTTACATTTAGCTG
GGATTTCATCAATTTTAGGGGCAGTAAATTTTATTACAACAGTAATTAATATACGA
TCTTCAGGAATTACACTTGATCGAATACCTTTATTTGTTTGATCAGTAGTAATTAC
TGCTGTTTTATTACTTCTTTCACTACCAGTTTTAGCAGGAGCTATTACTATACTAT
TAACAGATCGAAATCTTAATACTTCATTCTTTGACCCAATTGGAGGAGGAGACCCA
ATTCTTTATCAACACTTATTCTGATTCTTTGGTCATCCAGAAGTATATATTTTAAT
TTTACCTGGATTTGGTATAATTTCTCATATTATTACTCAAGAAAGAGGAAAGAAGG
AAACATTTGGAACATTAGGAATAATTTATGCTATATTAGCTATTGGATTATTAGGG
TTTATTGTTTGAGCCCATCATATGTTTACAGTTGGAATAGATGTTGATACTCGAGC
TTACTTTACATCAGCTACAATAATTATTGCTGTTCCAA

Figure 5.2.3(a): The partial Mitochondrial COI gene sequence of *Culex tritaeniorhynchus*

Figure 5.2.3(b): Molecular barcode of the partial mitochondrial COI gene of *Culex tritaeniorhynchus*

>QTU76229.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSG
TAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVIT
AVLLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRA
YFTSATMIIAVP

Figure 5.2.3(c): The translational product of the mitochondrial COI gene of *Culex tritaeniorhynchus*

>seq1 *Culex quenquifasciatus* CUMM59 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TAAATAATATAAGTTTTTGAATACTACCTCCTTCATTGACACTACTACTTTCAAGT
AGTTTAGTAGAAAATGGGGCTGGGACTGGATGAACAGTGTATCCCCCTCTTTCATC
TGGAACAGCTCATGCTGGAGCTTCAGTAGACTTAGCTATTTTTTCTTTACATTTAG
CAGGAATTTCATCAATTTTAGGTGCAGTAAATTTTATTACAACAGTAATTAATATA
CGATCTTCAGGAATTACTCTTGATCGAATACCTTTATTTGTTTGATCAGTAGTAAT
TACTGCAGTTTTATTACTTCTTTCTTTACCTGTTTTAGCTGGTGCTATTACTATGT
TATTAACAGATCGAAATTTAAATACTTCATTCTTTGATCCAATTGGAGGAGGAGAT
CCAATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGAAGTTTATATTTT
AATTCTTCCAGGGTTTGGAATAATTTCTCATATTATTACTCAAGAAAGAGGAAAAA
AGGAAACATTTGGAACTTTAGGAATAATTTATGCTATATTAGCTATTGGTTTATTA
GGGTTTATTGTTTGAGCTCATCATATATTTACAGTTGGAATAGATGTTGATACACG
AGCTTATTTTACATCTGCTACAA

Figure 5.2.4(a): The partial Mitochondrial COI gene sequence of *Culex quinquefasciatus*

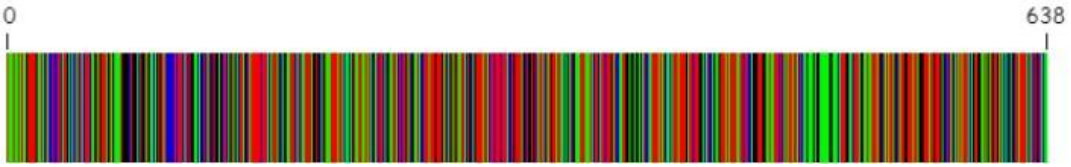

Figure 5.2.4(b): Molecular barcode of the partial mitochondrial COI gene of *Culex quinquefasciatus*

>QTV76169.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NNMSFWMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSGTAHAGASVDLAIFSLHLA
GISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVITAVLLLLSLPVLAGAITML
LTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKK
ETFGTLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRAYFTSAT

Figure 5.2.4(c): The translational product of the mitochondrial COI gene of *Culex quinquefasciatus*

>seq1 *Culex pseudovishnui* CUMM49 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AATTAAGTCAACCTGGTGTATTTATTGGAATTGATCAAATTTATAATGTTATTGTA
ACTGCTCACGCTTTTATTATAATTTTCTTTATAGTAATACCAATTATAATTGGTGG
ATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCTTTTCCAC
GAATAAATAATATAAGTTTTTGAATACTTCCTCCTTCATTAACTCTACTACTTTCA
AGTAGTTTAGTAGAAAATGGAGCTGGGACAGGATGAACAGTTTATCCTCCTTTATC
ATCTGGAACAGCACATGCAGGAGCTTCAGTTGATTTAGCTATTTTTTCTTTACACT
TAGCAGGGATTTCATCAATTTTAGGAGCAGTAAATTTTATTACTACAGTTATTAAT
ATACGATCATCAGGAATTACTCTTGATCGAATACCATTATTTGTATGATCAGTAGT
TATTACTGCTGTTTTATTACTTTTATCTTTACCAGTATTAGCTGGAGCTATTACTA
TATTATTAACTGATCGAAATTTAAATACTTCATTCTTTGATCCAATTGGAGGAGGA
GACCCTATTTTATATCAACATTTATTTTGATTCTTTGGACATCCAGAAGTTTACAT
TTTAATTTTACCAGGATTTGGTAAAATTTCTCATATTATTTCTCAAGAAAGAGGAA
AAAAGGAAACATTTGGAACATTAGGAATAATTTA

Figure 5.2.5(a): The partial Mitochondrial COI gene sequence of *Culex pseudovishnui*

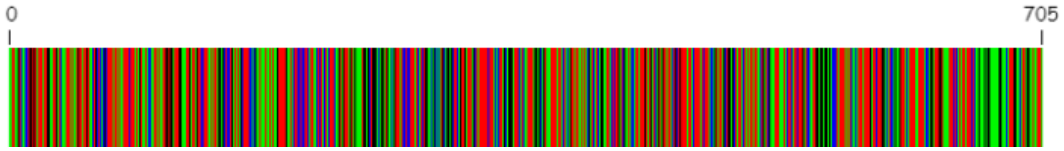

Figure 5.2.5(b): Molecular barcode of the partial mitochondrial COI gene of *Culex pseudovishnui*

>QTU76226.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

LSQPGVFIGIDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPR
MNNMSFWMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSGTAHAGASVDLAIFSLHL
AGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVITAVLLLLSLPVLAGAITM
LLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILILPGFGKISHIISQESGK
KETFGTLGMI

Figure 5.2.5(c): The translational product of the mitochondrial COI gene of *Culex pseudovishnui*

>seq1 *Culex vishnui* CUMM39 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

ACAAAATCATAAAGATATTGGAACATTATATTTTATTTTTGGGGCTTGAGCTGGAA
TAATTGGTACTTCTTTAAGTATTTTAATTCGTGCAGAATTAAGTCAACCCGGAGTA
TTTATTGGAAATGATCAAATTTATAATGTTATTGTAACTGCTCATGCTTTTATTAT
AATTTTCTTTATAGTAATACCTATTATAATTGGTGGATTTGGAAATTGATTAGTTC
CTTTAATGTTAGGAGCTCCTGATATAGCATTTCCACGAATAAATAATATAAGTTTT
TGAATACTTCCTCCTTCATTAACTTTACTACTTTCAAGTAGTTTAGTAGAAAATGG
AGCTGGGACAGGATGAACAGTTTATCCACCTTTATCATCTGGAACAGCCCACGCAG
GAGCTTCAGTTGATTTAGCTATTTTTTCTTTACATTTAGCAGGTATTTCATCAATT
TTAGGAGCAGTAAATTTTATTACTACAGTTATTAATATACGATCTTCAGGAATTAC
ACTTGATCGAATGCCATTATTTGTGTGATCAGTAGTTATTACTGCTGTTCTATTAC
TTTTATCTTTACCAGTATTAGCCGGAGCTATTACTATACTATTAACTGACCGAAAT
TTAAATACTTCATTCTTTGACCCAATTGGTGGAGGAGAC

Figure 5.2.6(a): The partial Mitochondrial COI gene sequence of *Culex vishnui*

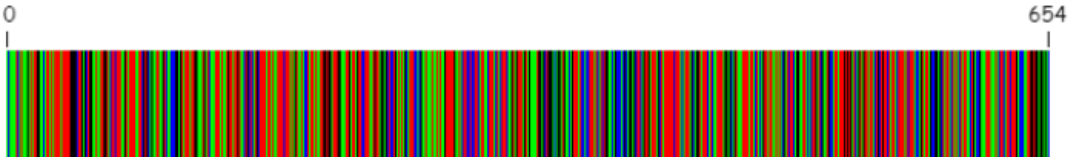

Figure 5.2.6(b): Molecular barcode of the partial mitochondrial COI gene of *Culex vishnui*

>QRA20201.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

QNHKDIGTLYFIFGAWAGMIGTSLSILIRAELSQPGVFIGNDQIYNVIVTAHAFIM
IFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSLVENG
AGTGWTVYPPLSSGTAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSSGIT
LDRMPLFVWSVVITAVLLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGD

Figure 5.2.6(c): The translational product of the mitochondrial COI gene of *Culex vishnui*

>seq1 *Culex bitaeniorhyncus* CUMM55 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCATTTCCTCGAATAA
ATAATATAAGTTTTTGAATACTACCTCCTTCATTAACTTTGCTACTTTCAAGTAGC
ATAGTTGAAAATGGAGCTGGAACTGGATGAACAGTTTACCCCCCACTTTCATCTGG
AACAGCCCATGCTGGAGCTTCAGTAGATTTAGCTATTTTTTCTCTTCATTTAGCTG
GAATTTCATCAATTTTAGGAGCTGTAAATTTTATTACAACAGTAATTAATATACGA
TCTTCAGGAATTACACTTGATCGAATACCTTTATTTGTATGATCAGTTGTAATTAC
TGCTATTTTATTACTTTTATCACTACCTGTCTTAGCTGGAGCTATTACTATATTAT
TAACAGATCGAAATTTAAATACTTCATTTTTTGATCCAATTGGAGGAGGAGATCCA
ATTTTATATCAACATTTATTTTGATTTTTTGGACATCCAGAAGTTTATATTTTAAT
TTTACCTGGATTTGGTATAATTTCTCATATTATTACTCAAGAAAGTGGTAAAAAGG
AAACATTTGGAACACTTGGAATAATTTATGCTATATTAGCTATTGGGTTATTAGGA
TTTATTGTTTGAGCTCATCATATATTTACAGTTGGAATAGATGTCGATACACGAGC
TTATTTTACTTCTGCTACAATAATTATTGCTGTTCCA

Figure 5.2.7 (a): The partial Mitochondrial COI gene sequence of *Culex bitaeniorhyncus*

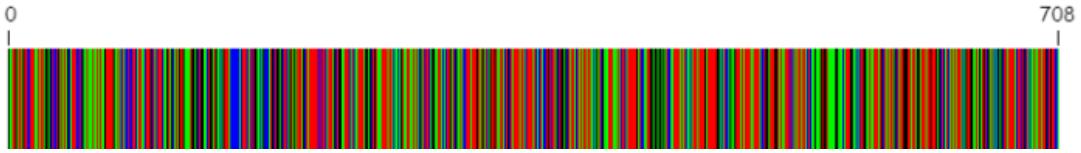

Figure 5.2.7 (b): Molecular barcode of the partial mitochondrial COI gene of *Culex bitaeniorhyncus*

>QRC50339.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGAGTGWTVYPPLSSG
TAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRA
YFTSATMIIAVP

Figure 5.2.7 (c): The translational product of the mitochondrial COI gene of *Culex bitaeniorhyncus*

>seq1 *Culex infula* CUMM63 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GAAAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCATTTCCTCGAAT
AAATAATATAAGTTTTTGAATACTACCTCCTTCATTAACTTTGTTACTTTCAAGTA
GCATAGTTGAAAATGGAGCTGGAACTGGATGAACAGTTTACCCCCCACTTTCATCT
GGAACAGCCCATGCCGGAGCTTCAGTAGATTTAGCTATTTTTTCTCTTCATTTAGC
TGGAATTTCATCAATTTTAGGAGCTGTAAATTTTATTACAACAGTAATTAATATAC
GATCTTCAGGAATTACACTTGATCGAATACCTTTATTTGTATGATCAGTAGTAATT
ACTGCTATTTTATTACTTTTATCATTACCTGTTTTAGCTGGAGCTATTACTATATT
ATTAACAGATCGAAATTTAAATACTTCATTTTTTGATCCTATTGGAGGAGGAGACC
CAATTTTATATCAACATTTATTTTGATTTTTTGGACATCCAGAAGTTTATATTTTA
ATTTTACCTGGATTTGGTATAATTTCTCATATTATTACTCAAGAAAGTGGTAAAAA
GGAAACATTTGGAACACTTGGAATAATTTATGCTATATTAGCTATTGGGTT

Figure 5.2.8(a): The partial Mitochondrial COI gene sequence of *Culex infula*

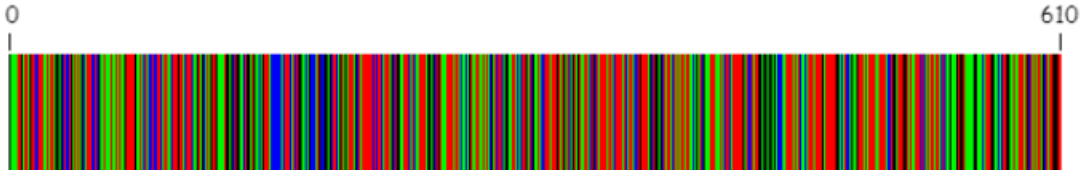

Figure 5.2.8(b): Molecular barcode of the partial mitochondrial COI gene of *Culex infula*

>QTU76227.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

ENWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGAGTGWTVYPPLSS
GTAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVI
TAILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYIL
ILPGFGMISHIITQESGKKETFGTLGMIYAMLAIG

Figure 5.2.8(c): The translational product of the mitochondrial COI gene of *Culex infula*

>seq1 *Culex pallidothorax* CUMM7 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TCTCTTAGTTTACTTATTCGAGCAGAATTAAGTCAACCTGGAGTATTTATTGGAAA
TGATCAAATTTATAATGTTATTGTAACTGCTCATGCTTTTATTATAATTTTTTTTA
TAGTAATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTCTTATATTA
GGAGCTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGAATACTTCC
CCCTTCTTTAACTTTACTACTTTCAAGTAGTATAGTAGAAAATGGAGCTGGGACAG
GATGAACAGTTTATCCACCTCTTTCTTCTGGAACTGCTCATGCAGGAGCTTCAGTT
GATTTAGCTATTTTTTCATTACATTTAGCTGGAATTTCATCTATTTTAGGAGCAGT
AAATTTTATTACAACAGTAATTAATATACGATCTTCAGGAATTACTCTTGATCGAA
TACCTTTATTTGTATGATCTGTAATTATTACTGCAGTATTATTACTTCTTTCTTTA
CCTGTATTAGCAGGAGCTATTACTATATTATTAACAGATCGAAATTTAAATACATC
ATTCTTTGACCCAATTGGAGGAGGAGATCCTATTCTATACCAACATTTATTTTGAT
TTTTTGG

Figure 5.2.9(a): The partial Mitochondrial COI gene sequence of *Culex pallidothorax*

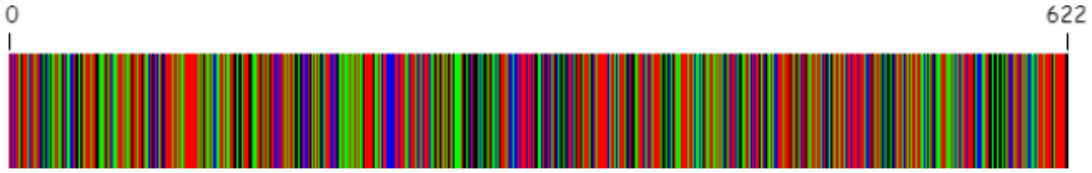

Figure 5.2.9(b): Molecular barcode of the partial mitochondrial COI gene of *Culex pallidothorax*

>QQY98758.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

SLSLLIRAELSQPGVFIGNDQIYNVIVTAHAFIMIFFMVMPIMIGGFGNWLVPLML
GAPDMAFPRMNNMSFWMLPPSLTLLLSSSMVENGAGTGWTVYPPLSSGTAHAGASV
DLAIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVIITAVLLLLSL
PVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFG

Figure 5.2.9(c): The translational product of the mitochondrial COI gene of *Culex pallidothorax*

>seq 1 *Culex uniformis* CUMM90 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

ATGAATACTTCCTCCTTCATTAACTTTACTCCTTTCCAGAAGTTTAGTAGAAAACG
GAGCTGGAACTGGATGAACAGTCTACCCTCCTTTATCTTCTGGAACTGCCCATGCT
GGAGCCTCTGTTGATTTAGCAATTTTTTCCCTTCATTTAGCAGGAATTTCTTCTAT
TTTAGGAGCAGTAAATTTTATTACTACAGTAATTAATATGCGATCTTCTGGGATTA
CTTTAGATCGAATACCTTTATTTGTATGATCAGTTGTTATTACAGCTATTTTATTA
CTTTTATCTCTTCCTGTTTTAGCAGGAGCTATTACTATATTATTAACAGATCGAAA
TTTAAATACATCATTTTTTGACCCAATCGGTGGAGGAGACCCAATTTTATATCAAC
ATTTATTTTGATTTTTTGGTCACCCAGAAGTTTATATTTTAATTTTACCTGGATTT
GGAATAATTTCACATATTATTACTCAAGAAAGAGGAAAAAAGGAAACTTTTGGTAC
TTTAGGAATAATTTATGCTATGTTAGCTATTGGTTTATTAGGATTTATTGTATGAG
CACATCATATATTTACAGTAGGTATAGATGTTGATACACGAGCTTATTTTACTTCA
GCTACTATAATTATTGCTGTTCCAACTGGAATTAAAATTTTT

Figure 5.2.10(a): The partial Mitochondrial COI gene sequence of *Culex uniformis*

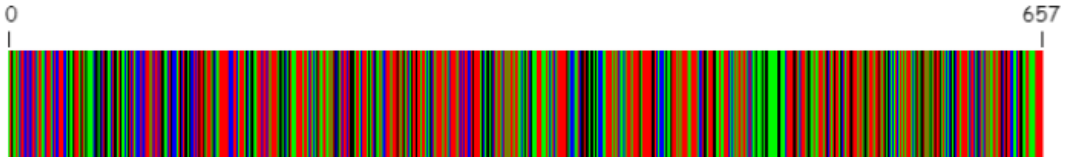

Figure 5.2.10(b)**:** Molecular barcode of the partial mitochondrial COI gene of *Culex uniformis*

>UJO74359.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

WMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSGTAHAGASVDLAIFSLHLAGISSI
LGAVNFITTVINMRSSGITLDRMPLFVWSVVITAILLLLSLPVLAGAITMLLTDRN
LNTSFFDPIGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFGT
LGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRAYFTSATMIIAVPTGIKIF

Figure 5.2.10(c): The translational product of the mitochondrial COI gene of *Culex uniformis*

>seq1 *Culex minor* CUMM57 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TTTTTGAATACTACCTCCTTCATTAACACTTCTACTTTCTAGTAGTTTAGTAGAAA
ATGGAGCTGGGACTGGATGAACAGTATACCCCCCACTTTCATCTGGTACTGCTCAT
GCTGGAGCATCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCTTC
TATTTTAGGAGCTGTAAATTTTATTACTACAGTAATTAATATACGATCTTCAGGAA
TTACTTTAGACCGAATACCTTTATTTGTATGATCTGTAGTTATTACAGCTATTTTA
TTACTTTTATCATTACCTGTTTTAGCCGGAGCTATTACTATATTATTAACAGATCG
AAATCTAAATACTTCCTTTTTCGATCCTATTGGAGGAGGAGATCCAATTTTATATC
AACACTTATTTTGATTTTTTGGACACCCAGAAGTATATATTTTAATTTTACCAGGA
TTTGGAATAATTTCTCATATTATTACCCAAGAAAGTGGTAAAAAGGAAACTTTTGG
AACTTTAGGAATAATTTATGCTATATTAGCTATTGGTTTATTAGGATTTATTGTAT
GAGCTCACCATATATTTACAGTTGGAATAGATGTAGATACACGTGCTTATTTTACC
TCAGCTACAATAATTATTGCTGTTCC

Figure 5.2.11(a): The partial Mitochondrial COI gene sequence of *Culex minor*

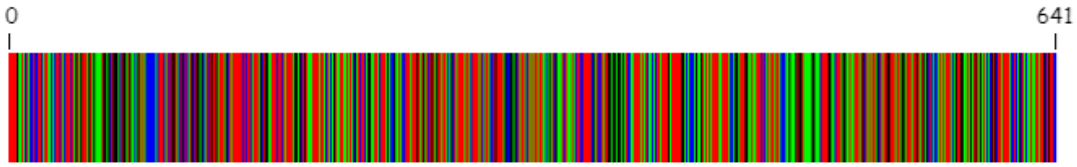

Figure 5.2.11(b): Molecular barcode of the partial mitochondrial COI gene of *Culex minor*

>QRC50337.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

FWMLPPSLTLLLSSSLVENGAGTGWTVYPPLSSGTAHAGASVDLAIFSLHLAGISS
ILGAVNFITTVINMRSSGITLDRMPLFVWSVVITAILLLLSLPVLAGAITMLLTDR
NLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFG
TLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRAYFTSATMIIAVP

Figure 5.2.11(c): The translational product of the mitochondrial COI gene of *Culex minor*

>seq1 *Mansonia indiana* CUMM41 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AATTGATTAGTTCCCCTTATATTAGGAGCCCCTGATATAGCATTTCCTCGAATAAA
TAATATAAGATTTTGACTTTTACCTCCATCATTAACATTATTAATTTCAGGAGGTA
TAGTAGAAAACGGGGCTGGTACAGGTTGAACTGTTTACCCCCCTCTATCTGCCAAC
ACTGCTCATACAGGAGCATCAGTTGATTTAACAATTTTTTCTCTCCACTTAGCCGG
AGTATCCTCAATTTTAGGTGCAGTAAATTTTATTACTACTGTAATTAATATACGAT
CCTCAGGAATTACATTAGATCGAATACCATTATTTGTTTGATCAGTTGTAATTACA
GCAATTTTATTACTCCTCTCCCTCCCTGTTTTAGCTGGAGCTATTACTATGCTTCT
AACTGATCGTAATTTAAATACATCATTCTTTGATCCAATAGGAGGAGGAGACCCTA
TTTTATATCAACATCTCTTTTGATTTTTTGGACACCCAGAAGTTTACATTTTAATT
CTACCCGGATTTGGTATAATTTCTCACATTATTACTCAAGAAAGTGGTAAAAAGGA
AACATTTGGAACATTAGGAATAATTTATGCTATATTAGCTATTGGATTATTAGGGT
TCATCGT

Figure 5.3.1(a): The partial Mitochondrial COI gene sequence of *Mansonia indiana*

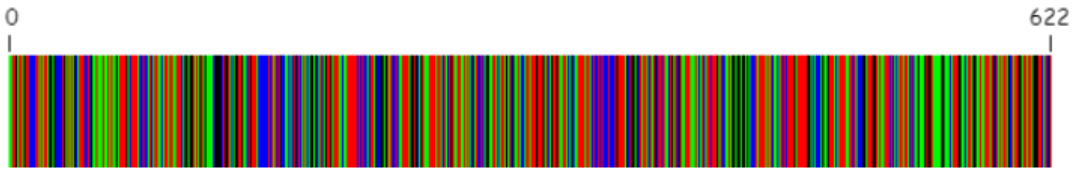

Figure 5.3.1(b): Molecular barcode of the partial mitochondrial COI gene of *Mansonia indiana*

>QTU76225.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISGGMVENGAGTGWTVYPPLSAN
TAHTGASVDLTIFSLHLAGVSSILGAVNFITTVINMRSSGITLDRMPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPMGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLAIGLLGFIV

Figure 5.3.1(c): The translational product of the mitochondrial COI gene of *Mansonia indiana*

>seq1 *Mansonia uniformis* CUMM11 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCATTTCCTCGAATAA
ATAATATAAGATTTTGACTTTTACCTCCATCATTAACATTATTAATTTCAGGAGGA
ATAGTAGAAAATGGGGCTGGAACTGGATGAACAGTTTATCCTCCTTTATCAGCTAA
TACAGCTCATACTGGAGCATCTGTTGACTTAACAATTTTTTCTTTACATTTAGCCG
GAGTTTCTTCAATTTTAGGAGCAGTAAATTTTATTACTACTGTTATTAATATACGA
TCTTCAGGAATTACTTTAGACCGAATACCTCTATTTGTATGATCTGTTGTAATTAC
AGCAATTTTATTACTTCTTTCCCTTCCTGTTTTAGCTGGAGCTATTACAATACTTT
TAACTGATCGTAATTTAAATACATCCTTCTTTGACCCTATAGGAGGAGGAGACCCT
ATTCTTTATCAACACTTATTCTGATTTTTTGGACATCCAGAAGTTTATATTTTAAT
TTTACCTGGATTTGGAATAATTTCTCATATTATTACACAAGAAAGAGGAAAAAAGG
AAACATTTGGAACATTAGGAATAATTTATGCAATATTAGCAATTGGATTATTAGGA
TTTATTGTATGAGCCCACCATATATTTACTGTAGGAATAGATGTTGATACTCGAGC
TTACTTTACATCTGCTACTATAATTATTGCTGTTCCAAA

Figure 5.3.2(a): The partial Mitochondrial COI gene sequence of *Mansonia uniformis*

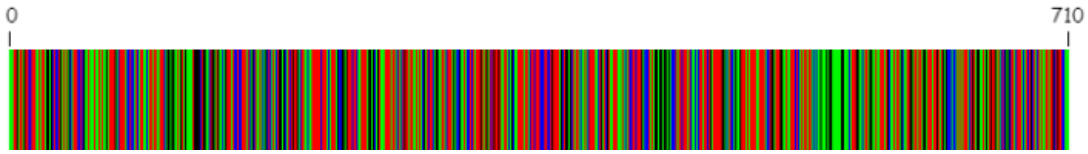

Figure 5.3.2(b): Molecular barcode of the partial mitochondrial COI gene of *Mansonia uniformis*

>QQY98757.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLLISGGMVENGAGTGWTVYPPLSAN
TAHTGASVDLTIFSLHLAGVSSILGAVNFITTVINMRSSGITLDRMPLFVWSVVIT
AILLLLSLPVLAGAITMLLTDRNLNTSFFDPMGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRA
YFTSATMIIAVP

Figure 5.3.2(c): The translational product of the mitochondrial COI gene of *Mansonia uniformis*

>seq1 *Armigeres sabalbatus* CUMM5 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

ATAAAGATATTGGAACTTTATATTTTATTTTTGGTGCTTGAGCTGGAATAGTGGGA
ACTTCTTTAAGTATTTTAATTCGAACAGAATTAAATCACCCTGGAGTATTTATTGG
AAATGATCAAATTTATAATGTAATTGTAACAGCTCATGCTTTTATTATAATTTTTT
TTATAGTTATACCAATTATAATTGGAGGATTTGGAAATTGATTAGTACCCCTTATA
CTTGGAGCTCCAGATATAGCCTTCCCTCGAATAAATAATATAAGTTTTTGAATATT
ACCCCCTTCATTAACTCTACTAATTTCAAGTTCTTTAGTAGAAACAGGAGCTGGAA
CTGGATGAACCGTTTATCCTCCTTTATCTTCTGGAACTGCCCATGCTGGAGCTTCT
GTTGATTTAGCTATTTTCTCTCTTCATTTAGCAGGTATTTCTTCTATTTTGGGAGC
AGTAAATTTTATTACAACTGTAATTAATATACGATCATCAGGGATTACTCTTGATC
GATTACCCTTATTTGTTTGATCTGTTGTTATTACAGCTATTTTACTTCTTCTTTCT
TTACCAGTTTTAGCAGGAGCTATTACTATACTATTAACTGATCGGAATTTAAATAC
CTCATTCTTTGACCCAATTGGAGGAGGAGATCCGATCTTATACCAACATTTATTTT
GATTTTTTGGTCACC

Figure 5.4.1(a): The partial Mitochondrial COI gene sequence of *Armigeres sabalbatus*

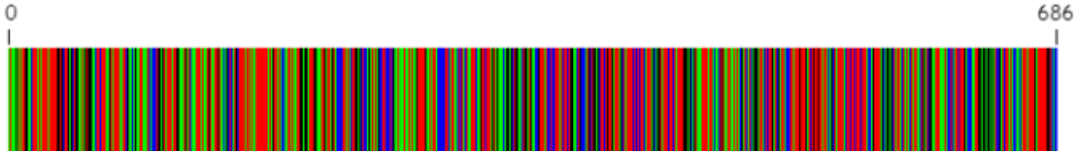

Figure 5.4.1(b): Molecular barcode of the partial mitochondrial COI gene of *Armigeres sabalbatus*

>QQY98756.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

KDIGTLYFIFGAWAGMVGTSLSILIRTELNHPGVFIGNDQIYNVIVTAHAFIMIFF
MVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLISSSLVETGAGT
GWTVYPPLSSGTAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSSGITLDR
LPLFVWSVVITAILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFW
FFGH

Figure 5.4.1(c): The translational product of the mitochondrial COI gene of *Armigeres sabalbatus*

>seq1 *Armigeres aureolineatus* CUMM29 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TTTCATCAATTTTAGGAGCAGTAAATTTTATTACTACAGTAATTAATATACGATCT
TCAGGAATTACTCTTGATCGATTACCTTTATTTGTCTGATCTGTTGTTATTACAGC
TATTTTACTTCTTCTTTCTTTACCTGTTTTAGCCGGAGCTATTACTATATTATTAA
CTGATCGAAATTTAAATACTTCATTTTTTGACCCAATTGGAGGAGGAGATCCTATT
TTATATCAACATTTATTCTGATTTTTTGGACACCCTGAAGTTTATATTTTAATTTT
ACCTGGATTTGGTATAATTTCTCATATTATTACTCAAGAAAGAGGAAAAAAGGAAA
CATTTG

Figure 5.4.2(a): The partial Mitochondrial COI gene sequence of *Armigeres aureolineatus*

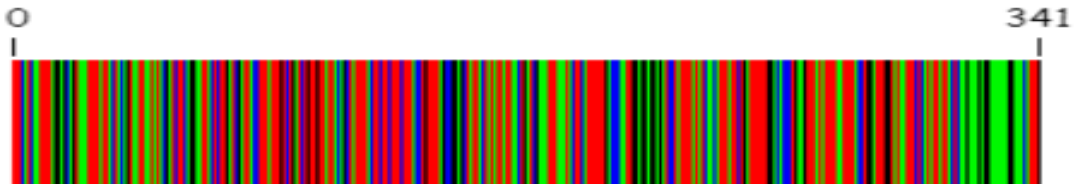

Figure 5.4.2(b): Molecular barcode of the partial mitochondrial COI gene of *Armigeres aureolineatus*

>UUB67889.1cytochrome c oxidase subunit I Partial Amino acid Sequences

SSILGAVNFITTVINMRSSGITLDRLPLFVWSVVITAILLLLSLPVLAGAITMLLT
DRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKET
F

Figure 5.4.2(c): The translational product of the mitochondrial COI gene of *Armigeres aureolineatus*

>seq1 *Malaya genurostris* CUMM23 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TTATAGTAGCAGAAGTAAAATAAGCTCGTGTATCTACATCTATACCTACTGTAAAT
ATATGATGTGCTCAAACAATAAAACCAAGTAATCCAATTGCTAATATAGCATAAAT
TATTCCTAAAGTACCAAAAGTTTCCTTTTTTCCTCTTTCTTGTGTAATAATATGAG
AAATTATACCAAATCCTGGAAGAATTAAAATATAAACTTCTGGATGTCCAAAAAAT
CAAAATAAATGTTGATAAAGAATAGGATCTCCTCCTCCAATAGGGTCAAAGAATGA
AGTATTAAAATTACGATCTGTTAATAATATAGTAATAGCTCCTGCTAATACAGGTA
AAGATAATAAAAGAAGTAAGGCAGTAATTACTACAGATCAAACAAATAATGGTATT
CGATCTAGAGTAATTCCAGAAGATCGTATATTAATTACTGTTGTAATAAAATTAAC
AGCTCCTAAAATAGAAGAAATTCCAGCTAAATGAAGAGAAAAAATAGATAAATCAA
CAGAAGCTCCTGCATGGGCATTATTAGAAGAGAGAGGTGGATAAACTGTTCATCCT
GTTCCAGCTCCATTTTCTACTATACTACCTGACAATAATAATATTAAAGAAGGTGG
AAGTATTCAAAAACTTATATTATTTATTCGAGGGAAAGCTATATCAGGTGCACCTA
ATATTAAAGGAACTAATCAATTCC

Figure 5.5.1(a): The partial Mitochondrial COI gene sequence of *Malaya genurostris*

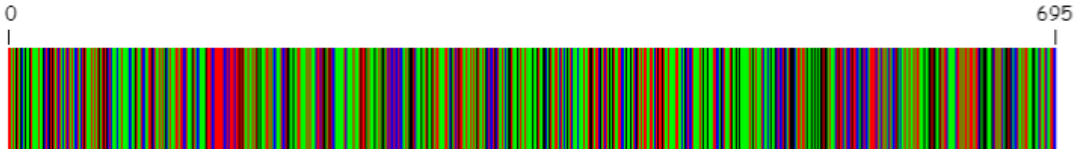

Figure 5.5.1(b): Molecular barcode of the partial mitochondrial COI gene of *Malaya genurostris*

>QRA20207.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

NWLVPLMLGAPDMAFPRMNNMSFWMLPPSLMLLLSGSMVENGAGTGWTVYPPLSSN
NAHAGASVDLSIFSLHLAGISSILGAVNFITTVINMRSSGITLDRMPLFVWSVVIT
ALLLLLSLPVLAGAITMLLTDRNFNTSFFDPIGGGDPILYQHLFWFFGHPEVYILI
LPGFGMISHIITQESGKKETFGTLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRA
YFTSATM

Figure 5.5.1(c): The translational product of the mitochondrial COI gene of *Malaya genurostris*

>seq1 *Verrallina indica* CUMM8 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GAGGATTCGGAAATTGATTAGTTCCTTTAATATTAGGAGCACCTGATATAGCTTTT
CCTCGTATAAATAATATAAGTTTTTGACTCCTTCCTCCTTCACTTACACTCTTCTT
ATCCAGTTCTATGGTAGAAAATGGAGCTGGTACAGGTTGAACTGTTTATCCTCCAT
TATCTGCAGGAACAGCTCATGCTGGAGGATCAGTTGATTTAGCTATTTTTTCTCTT
CATTTAGCTGGAATTTCTTCTATTTTAGGAGCTGTAAATTTTATTACAACTGTAAT
TAATATACGATCTGCTGGAATTACTCTTGATCGTCTTCCATTATTTGTTTGATCTG
TTGTTATTACAGCTATTTTACTTCTTCTTTCTCTTCCTGTATTAGCTGGAGCAATT
ACTATATTATTAACAGATCGAAATTTAAATACTTCATTCTTTGACCCAATTGGAGG
AGGTGATCCTATTTTATACCAACATTTATTCTGATTTTTTGGACATCCAGAAGTTT
ATATTTTAATTCTTCCAGGATTTGGTCTAATTTCTC

Figure 5.6.1(a): The partial mitochondrial COI gene sequence of *Verrallina indica*

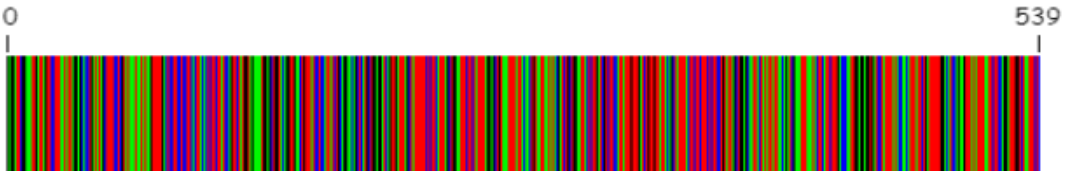

Figure 5.6.1(b): Molecular barcode of the partial mitochondrial COI gene of *Verrallina indica*

>UUC26816.1cytochrome c oxidase subunit I Partial Amino acid Sequences

GFGNWLVPLMLGAPDMAFPRMNNMSFWLLPPSLTLFLSSSMVENGAGTGWTVYPPL
SAGTAHAGGSVDLAIFSLHLAGISSILGAVNFITTVINMRSAGITLDRLPLFVWSV
VITAILLLLSLPVLAGAITMLLTDRNLNTSFFDPIGGGDPILYQHLFWFFGHPEVY
ILILPGFGLIS

Figure 5.6.1(c): The translational product of the mitochondrial COI gene of *Verrallina indica*

>seq1 *Anopheles barbirostris* CUMM13 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GGATTCGGAAATTGATTAGTTCCTTTAATATTAGGAGCTCCTGATATAGCTTTTCC
TCGAATAAATAATATAAGATTTTGAATATTACCTCTTTCTCTTACTTTATTAATTT
TTAGAAGTATAGTAGAAAATGGTGCCGGAACTGGATGAACTGTATATCCACCTTTA
TCTTCTGGAATTGCACATGCAGGAGCTTCTGTTGATTTAGCTATTTTTTCATTACA
TTTAGCAGGAATTTCTTCAATTTTAGGAGCAGTAAATTTTATTACTACTGTTATTA
ATATACGTTCACCAGGAATTACTTTAGATCGAATACCATTATTTGTCTGATCTGTA
GTTATTACAGCAATTCTTTTATTATTATCTTTACCAGTATTAGCAGGAGCAATTAC
TATATTATTAACTGATCGAAATTTAAATATCTCATTTTTTGACCCCGCAGGAGGAG
GAGATCCAATTTTATATCAACATTTATTTTGATTTTTCGGACATCCTGAAGTTTAT
ATTTTAATTTTACCAGGATTTGGAATAATTTCACATATTATTACTCAAGAAAGAGG
GAAAAAGGAAACTTTTGGTATTTTAGGTATAATATATGCAATATTAGCTATTGGTT
TATTAGGATTTATTGTATGAGCTCATTATATAT

Figure 5.7.1(a): The partial Mitochondrial COI gene sequence of *Anopheles barbirostris*

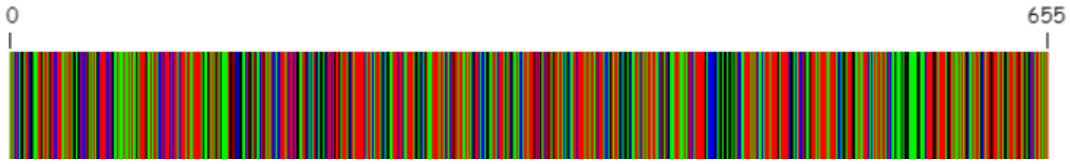

Figure 5.7.1(b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles barbirostris*

>QTU76228.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

GFGNWLVPLMLGAPDMAFPRMNNMSFWMLPLSLTLLIFSSMVENGAGTGWTVYPPL
SSGIAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSPGITLDRMPLFVWSV
VITAILLLLSLPVLAGAITMLLTDRNLNISFFDPAGGGDPILYQHLFWFFGHPEVY
ILILPGFGMISHIITQESGKKETFGILGMMYAMLAIGLLGFIVWAHYM

Figure 5.7.1(c): The translational product of the mitochondrial COI gene of *Anopheles barbirostris*

>seq1 *Anopheles insuleiflorum* CUMM135 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GATTTGGAAATTGATTAGTTCCTTTAATGCTAGGAGCACCTGATATAGCATTTCCA
CGAATAAATAATATAAGATTTTGAATACTACCCCCTTCACTTACTTTATTAATTAC
AAGTAGTATAGTAGAAAATGGAGCCGGTACTGGATGAACTGTTTATCCACCTCTTT
CTTCTGGTATTGCTCATGCAGGAGCTTCTGTAGATTTAGCAATTTTCTCTTTACAT
TTAGCTGGGATTTCTTCAATTTTAGGAGCTGTAAATTTTATTACAACTGTTATTAA
TATACGTTCACCTGGAATTACTCTTGATCGAATACCTTTATTTGTTTGATCTGTTG
TAATTACTGCTGTTTTATTATTATTATCTTTACCTGTACTAGCTGGAGCTATTACT
ATATTATTAACTGATCGAAATTTAAATACATCATTCTTTGATCCAGCTGGAGGAGG
AGACCCAATTCTATACCAACATTTATTTTGATTTTTTGGTCACCCAGAAGTATATA

TTTTAATTTTACCTGGATTTGGTATAATTTCTCATATTATTACTCAAGAAAGAGGA
AAAAAGGAAACTTTCGGAAATCTAGGAATAATTTATGCTATATTAGCAATTGGATT
ATTAGGATTTATTGTTTGAGCTCATCATATATTTACAGTTGGAATAGACGTAGATA
CTCGAGCCTACTTTACTTCTGCAAC

Figure 5.7.2(a): The partial Mitochondrial COI gene sequence of *Anopheles insulaeflorum*

Figure 5.7.2(b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles insulaeflorum*

>UTT74799.1cytochrome c oxidase subunit I Partial Amino acid Sequences

FGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLITSSMVENGAGTGWTVYPPLS
SGIAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSPGITLDRMPLFVWSVV
ITAVLLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
LILPGFGMISHIITQESGKKETFGNLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDT
RAYFTSAT

Figure 5.7.2(c): The translational product of the mitochondrial COI gene of *Anopheles insulaeflorum*

>seq1 *Anopheles aitkenii* CUMM106 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

ATTTTGAATACTACCCCCTTCACTTACTTTATTAATTACAAGTAGTATAGTAGAAA
ATGGAGCCGGTACTGGATGAACTGTTTATCCACCTCTTTCTTCTGGTATTGCTCAT
GCAGGAGCTTCTGTAGATTTAGCAATTTTCTCTTTACATTTAGCTGGGATTTCTTC
AATTTTAGGAGCTGTAAATTTTATTACAACTGTTATTAATATACGTTCACCTGGAA
TTACTCTTGATCGAATACCTTTATTTGTTTGATCTGTTGTAATTACTGCTGTTTTA
TTATTATTATCTTTACCTGTACTAGCTGGAGCTATTACTATATTATTAACTGATCG
AAATTTAAATACATCATTCTTTGATCCAGCTGGAGGAGGAGACCCAATTCTATACC
AACATTTATTTTGATTTTTTGGTCACCCAGAAGTATATATTTTAATTTTACCTGGA
TTTGGTATAATTTCTCATATTATTACTCAAGAAAGAGGAAAAAAGGAAACTTTCGG
AAATCTAGGAATAATTTATGCTATATTAGCAATTGGATTATTAGGATTTATTGTTT
GAGCTCATCATATATTTACAGTTGGAAT

Figure 5.7.3(a): The partial Mitochondrial COI gene sequence of *Anopheles aitkenii*

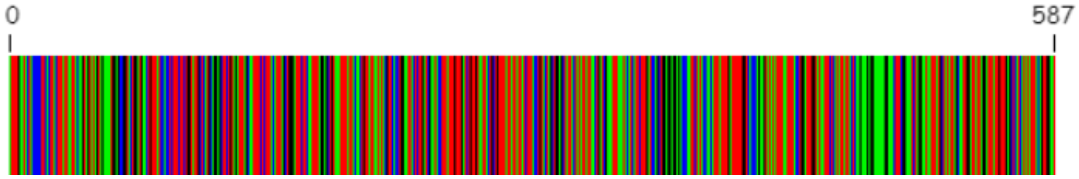

Figure 5.7.3(b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles aitkenii*

>UTS98182.1cytochrome c oxidase subunit I Partial Amino acid Sequences

FWMLPPSLTLLITSSMVENGAGTGWTVYPPLSSGIAHAGASVDLAIFSLHLAGISS
ILGAVNFITTVINMRSPGITLDRMPLFVWSVVITAVLLLLSLPVLAGAITMLLTDR
NLNTSFFDPAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFG
NLGMIYAMLAIGLLGFIVWAHHMFTVG

Figure 5.7.3(c): The translational product of the mitochondrial COI gene of *Anopheles aitkenii*

>seq1 *Anopheles crawfordi* CUMM64 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

AATATTACCTCCTTCCTTAACTTTACTAATTTCTAGAAGAATAGTAGAAAATGGAG
CAGGAACAGGGTGAACTGTTTATCCACCCCTTTCATCTGGAATTGCTCATGCTGGA
GCATCCGTAGATTTAGCTATTTTTTCATTACATTTAGCTGGAATTTCTTCAATTTT
AGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCCCCAGGAATTACAT
TAGATCGAATACCTTTATTTGTCTGATCCGTAGTAATTACAGCAGTATTATTATTA
TTATCTTTACCAGTATTAGCTGGAGCTATTACTATGCTTTTAACAGATCGAAACTT
AAATACTTCTTTCTTTGATCCAGCTGGAGGAGGAGACCCAATTTTATACCAACATT
TATTTTGATTCTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGA
ATAATTTCTCATATTATTACACAAGAAAGTGGTAAAAAGGAAACTTTCGGAAACTT
GGGAATAATTTATGCTATACTAGCAATTGGATTACTAGGATTTATTGTATGAGCCC
ATCATATATTTACAGTCGGAATAGACGTAGATACTCGAGCTTATTTCACTTCAGCA
ACTATAATTATTGCTGTTCCAACTGGAATAAAAATTTT

Figure 5.7.4 (a): The partial Mitochondrial COI gene sequence of *Anopheles crawfordi*

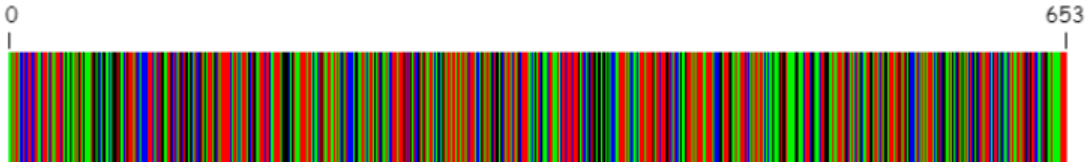

Figure 5.7.4 (b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles crawfordi*

>UJO74356.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

MLPPSLTLLISSSMVENGAGTGWTVYPPLSSGIAHAGASVDLAIFSLHLAGISSIL
GAVNFITTVINMRSPGITLDRMPLFVWSVVITAVLLLLSLPVLAGAITMLLTDRNL
NTSFFDPAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGKKETFGNL
GMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRAYFTSATMIIAVPTGMKI

Figure 5.7.4 (c): The translational product of the mitochondrial COI gene of *Anopheles crawfordi*

>seq1 *Anopheles jamesi* CUMM66 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GATTTGGAAATTGATTAGTTCCTTTAATGTTGGGAGCTCCAGATATAGCATTCCCA
CGAATAAATAATATAAGATTTTGAATACTTCCTCCCTCATTAACCCTTTTAATTTC
TAGAAGTATAGTAGAAAATGGGGCAGGAACAGGTTGAACTGTTTATCCTCCTCTTT
CTTCAGGAATTGCTCACGCAGGAGCTTCAGTAGATTTAGCTATTTTTTCTTTACAT
TTAGCGGGGATTTCATCAATTTTAGGTGCTGTAAATTTTATTACTACAGTTATTAA
TATACGATCGCCAGGAATTACATTAGATCGAATACCTTTATTTGTATGATCAGTAG
TAATTACTGCTATTTTATTATTATTATCATTGCCAGTATTAGCAGGAGCTATCACT
ATATTACTTACAGATCGTAATTTAAATACTTCTTTTTTCGATCCTGCGGGAGGAGG
AGATCCGATCTTATATCAACACTTATTTTGATTTTTTGGGCATCCAGAAGTTTACA
TTTTAATTTTACCTGGATTTGGGATAATTTCTCATATTATTACACAAGAAAGAGGT
AAAAAGGAAACATTTGGAAATTTAGGAATAATTTATGCTATATTAGCAATTGGATT
ATTAGGTTTTATTGTATGAGCTCATCATATATTTACAGTCGGAATAGATGTAGATA
CCCGAGCTTATTTTACTTCTGCTACAAT

Figure 5.7.5 (a): The partial Mitochondrial COI gene sequence of *Anopheles jamesi*

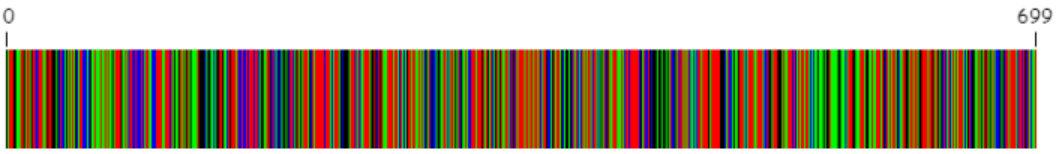

Figure 5.7.5 (b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles jamesi*

>QTW63367.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

FGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLISSSMVENGAGTGWTVYPPLS
SGIAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSPGITLRMPLFVWSVVI
TAILLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHPEVYIL
ILPGFGMISHIITQESGKKETFGNLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTR
AYFTSAT

Figure 5.7.5 (c): The translational product of the mitochondrial COI gene of *Anopheles jamesi*

>seq1 *Anopheles splendidus* CUMM94 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GAATAAATAATATAAGTTTTTGAATGCTTCCTCCCTCATTAACTTTACTTATTTCT
AGAAGTATAGTAGAAAATGGAGCAGGAACAGGATGAACTGTATACCCCCCTCTTTC
ATCAGGAATTGCTCATGCGGGAGCCTCAGTAGATTTAGCTATTTTTTCATTACATT
TAGCAGGAATTTCATCTATTTTAGGGGCAGTAAATTTTATTACTACAGTAATTAAT
ATACGATCACCAGGAATTACATTAGACCGAATACCTTTATTTGTTTGATCAGTTGT
AATTACTGCAATTTTATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATTACAA
TACTTCTTACAGATCGAAATTTAAATACATCTTTTTTCGATCCTGCTGGAGGAGGA
GATCCAATTCTATATCAACATTTATTCTGATTCTTTGGACACCCAGAAGTATATAT
TTTAATTTTACCAGGATTTGGTATAATTTCTCATATTATTACTCAAGAAAGTGGTA

AAAAGGAAACATTTGGAAACTTAGGAATAATTTATGCTATACTAGCAATTGGATTA
TTAGGATTTATTGTATGAGCACACCACATATTTACAGTTGGAATAGACGTTGACAC
ACGAGCTTATTTTACTTCTGCTACAATAATTATTGCTGTTCCAACT

Figure 5.7.6 (a): The partial Mitochondrial COI gene sequence of *Anopheles splendidus*

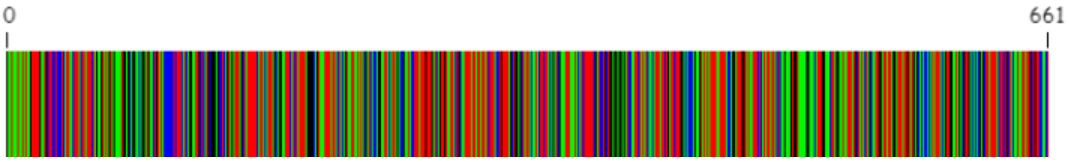

Figure 5.7.6 (b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles splendidus*

>UJO74361.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

MNNMSFWMLPPSLTLLISSSMVENGAGTGWTVYPPLSSGIAHAGASVDLAIFSLHL
AGISSILGAVNFITTVINMRSPGITLDRMPLFVWSVVITAILLLLSLPVLAGAITM
LLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHPEVYILILPGFGMISHIITQESGK
KETFGNLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRAYFTSATMIIAVPT

Figure 5.7.6 (c): The translational product of the mitochondrial COI gene of *Anopheles splendidus*

>seq1 Anopheles stephensi CUMM40 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GATTTGGGAATTGATTAGTTCCTTTAATATTAGGAGCACCAGATATAGCATTTCCT
CGAATAAATAATATAAGATTTTGAATATTACCCCCCTCATTAACTCTTTTAATTTC
TAGAAGTATAGTAGAAAATGGAGCAGGAACAGGATGAACTGTTTATCCGCCTTTAT
CGTCTGGAATTGCTCACGCTGGGGCTTCAGTAGATTTAGCAATTTTTTCATTACAT
TTAGCTGGAATTTCTTCAATTTTAGGAGCAGTTAATTTTATTACTACAGTAATTAA
TATACGATCGCCAGGAATTACGTTAGACCGAATACCTTTATTCGTTTGATCTGTTG
TAATTACTGCTATTTTATTATTATTATCATTACCTGTATTAGCTGGAGCTATTACT
ATATTACTTACAGACCGAAATTTAAATACATCTTTTTTCGACCCAGCTGGAGGAGG
AGACCCCATTTTATATCAACATTTATTTTGATTTTTTGGACACCCAGAAGTTTATA
TTTTAATTTTACCTGGATTTGGAATAATTTCACACATTATTACTCAAGAAAGAGGT
AAAAAGGAAACATTCGGAAATTTAGGAATAATTTATGCTATATTAGCAATTGGATT
ACTTGGATTTATCGTATGAGCCCACCATATGTTTACAGTAGGAATAGACGTAGATA
CTCGAGCTTATTTTACATCAGCTACAATAATTATTGCTGTTCCAACTGGAATTAAA
AATTT

Figure 5.7.7(a): The partial Mitochondrial COI gene sequence of *Anopheles stephensi*

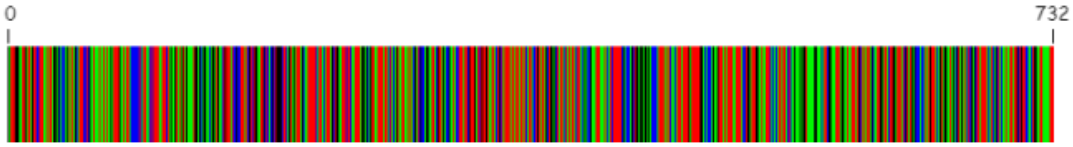

Figure 5.7.7(b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles stephensi*

>QRA20204.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

FGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLISSSMVENGAGTGWTVYPPLS
SGIAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSPGITLDRMPLFVWSVV
ITAILLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHPEVYI
LILPGFGMISHIITQESGKKETFGNLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDT
RAYFTSATMIIAVPTGIKN

Figure 5.7.7(c): The translational product of the mitochondrial COI gene of *Anopheles stephensi*

>seq1 Anopheles karwari CUMM161 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

TTCTTTAAGAATTCTAATTCGAGCTGAATTAGGTCATCCTGGAGCTTTTATTGGAG
ACGATCAAATTTATAATGTTATTGTAACCGCTCATGCTTTTATTATAATTTTTTTC
ATAGTTATGCCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATT
AGGAGCCCCAGATATAGCTTTTCCTCGAATAAATAATATAAGATTTTGAATATTAC
CTCCATCTCTTACACTCCTTATTTCTAGAAGTATAGTAGAAAACGGAGCCGGAACA
GGATGAACAGTTTACCCTCCTCTTTCTTCAGGAATTGCTCATGCAGGGGCTTCAGT
TGATTTAGCTATTTTTTCTTTACATTTAGCGGGGATTTCTTCTATTTTAGGAGCAG
TAAATTTTATTACTACAGTAATTAATATGCGATCCCCAGGAATTACTTTAGATCGA
ATACCATTATTTGTTTGATCTGTTGTAATTACAGCAATTTTATTACTATTATCATT
ACCAGTATTAGCTGGAGCTATTACAATACTACTTACTGATCGAAATTTAAATACAT
CATTTTTTGACCCTGCGGGAGGA

Figure 5.7.8(a): The partial Mitochondrial COI gene sequence of *Anopheles karwari*

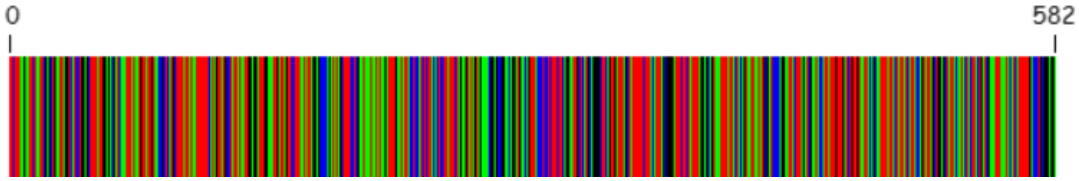

Figure 5.7.8(b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles karwari*

>WBV80334.1 cytochrome c oxidase subunit I Partial Amino acid Sequences

STNHKDIGTLYFIFGAWAGMVGTSLSILIRAELGHPGAFIGDDQIYNVIVTAHAFI
MIFFMVMPIMIGGFGNWLVPLMLGAPDMAFPRMNNMSFWMLPPSLTLLISSSMVEN
GAGTGWTVYPPLSSGIAHAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSPGI
TLDRMPLFVWSVVITAILLLLSLPVLAGAITMLLTDRNLNTSFFDPAGG

Figure 5.7.8(c): The translational product of the mitochondrial COI gene of *Anopheles karwari*

>seq1 Anopheles elegans CUMM160 cytochrome oxidase subunit 1 gene, partial cds, mitochondrial

GGTTAGTTCCTTTAATATTAGGAGCACCAGATATAGCATTTCCTCGAATAAACAAT
ATAAGTTTTTGAATACTACCTCCTGCCCTTACACTTTTAATTTCTAGTAGTATAGT
AGAAAATGGAGCAGGAACAGGTTGAACAGTTTATCCACCTCTATCTTCTGGAATTG
CACATGCGGGAGCCTCAGTTGATTTAGCAATTTTCTCTTTACATTTAGCAGGAATT
TCTTCTATTTTAGGAGCAGTAAATTTTATTACTACTGTAATTAATATACGATCTCC
TGGAATCACTTTAGATCGAATACCCTTATTTGTTTGATCTGTTGTAATTACTGCTA
TTTTATTACTTTTATCTTTACCAGTTTTAGCAGGAGCTATTACTATATTATTAACT
GATCGAAATTTAAATACTTCTTTTTTTGACCCTGCTGGAGGGGGAGACCCAATTTT
ATACCAACACTTATTTTGATTTTTTGGCCACCCAGAAGTTTATATTTTAATTTTAC
CTGGATTTGGTATAATTTCCCACATTATTACACAAGAAAGAGGAAAAAAGGAAACT
TTTGGTAATTTAGGAATAATTTACGCAATATTAGCAATTGGATTGTTAGGGTTCAT
TGTTTGAGCTCACCATATATTTACTGTTGGAATAGACGTAGATACACGAGCTTATT
TTACTTCTGCAACAATAATTATTGCTGTTCCAACTGG

Figure 5.7.9(a): The partial Mitochondrial COI gene sequence of *Anopheles elegans*

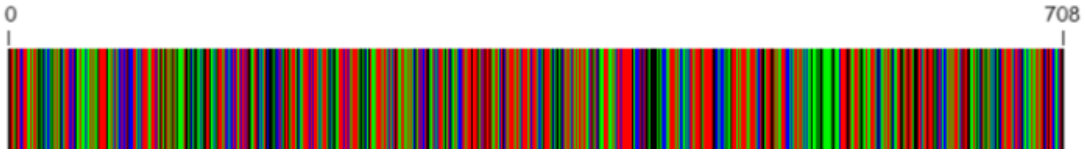

Figure 5.7.9(b): Molecular barcode of the partial mitochondrial COI gene of *Anopheles elegans*

>WDR70232.1cytochrome c oxidase subunit I Partial Amino acid Sequences

LVPLMLGAPDMAFPRMNNMSFWMLPPALTLLISSSMVENGAGTGWTVYPPLSSGIA
HAGASVDLAIFSLHLAGISSILGAVNFITTVINMRSPGITLDRMPLFVWSVVITAI
LLLLSLPVLAGAITMLLTDRNLNTSFFDPAGGGDPILYQHLFWFFGHPEVYILILP
GFGMISHIITQESGKKETFGNLGMIYAMLAIGLLGFIVWAHHMFTVGMDVDTRAYF
TSATMIIAVPTG

Figure 5.7.9(c): The translational product of the mitochondrial COI gene of *Anopheles elegans*

Discussion

Medical entomology, where molecular approaches are used for species diagnoses are of significant assistance in the identification of all life stages, from eggs to adults. It places a particular emphasis on the capacity of DNA barcodes in identifying species. The golden standard of barcode identification of species is the cytochrome c oxidase subunit I (COI) gene area of the mitochondrial genome (Hebert et al., 2003b) and has proved invaluable for distinguishing between mosquito species (Cywinska et al., 2006; Wang et al., 2012; Ashfaq et al., 2014). The sequences obtained in the present study did not show any indels and were properly aligned with the database sequences. The sequences lacked stop or nonsense codons, which are a hallmark of mitochondrial genes. Though Molecular sequencing of mosquitoes are being done worldwide, sequence reports from Kerala (a state widely renowned for its many mosquito-borne ailments) are very less. 20 of the 35 sequences submitted were significant mosquito vectors, which are known to carry a number of deadly diseases. The identification of mosquito species, especially the mosquito vectors, would benefit greatly from this work.

CHAPTER 6

MOLECULAR PHYLOGENY OF MOSQUITOES

Phylogenetic analysis provides insights into relationships at all levels of evolution. The phylogenetic tree is now available at all levels of taxonomic hierarchy for animals and plants, which play a pivotal role in comparative studies in diverse fields from ecology to molecular and comparative genetics (Solitis and Solitis, 2000).

6.1. Molecular Phylogeny of Subfamily Anophelinae

6.1.1. Subfamily Anophelinae

Subfamily Anophelinae includes 501 formally recognized species (Mosquito taxonomic inventory). *Anopheles* mosquitoes are one of the most studied members of the Culicidae family. The discovery of *Anopheles* as the exclusive vector for malaria transmission in humans has garnered much attention on study of this particular genus. Out of 17 species collected from the genera, 9 species representing two subgenera (Table 6.1.1) were selected for the phylogenetic analysis. The sequences of 13 species of Anopheles and an out group *Phlebotomus papatasi* were retrieved from NCBI Genbank (Table 6.1.2).

All the sequences were above 400 base pairs length. No stop codon or frame shifts were detected indicating the absence of pseudogenes (NUMTs). The sequences of all the species were already reported in NCBI and hence all our sequences were matching with the conspecific species of GenBank. This stipulate that COI sequences are useful in determining the species if the database is robust.

Table 6.1.1: List of COI sequences of Subfamily Anophelinae in Wayanad with voucher number and NCBI accession number

Sl. No.	Species Name	Voucher No.	Accession No.
1	*An. aitkenii*	CUMM106	OP024181.1
2	*An. splendidus*	CUMM94	OM368636.1
3	*An. stephensi*	CUMM40	MW549046.1
4	*An. insuleiflorum*	CUMM135	OP028210.1
5	*An. barbirostris*	CUMM13	MW922751.1
6	*An. jamesi*	CUMM66	MW931754.1
7	*An. crawfordi*	CUMM64	OM368571.1
8	*An. karwari*	CUMM161	OQ286389.1
9	*An.elegance*	CUMM160	OQ509988.1

Table 6.1.2: List of COI sequences of Subfamily Anophelinae retrieved from NCBI for phylogenetic analysis with Accession numbers

SI. No.	Species Name	Place of collection	Accession No.
1	*An. subpictus*	France	MT508474.1
2	*An. vagus*	France	MT434323.1
3	*An. tessellatus*	USA	MT257041.1
4	*An. nigerrimus*	Thailand	AB778798.1
5	*An. dirus*	France	MT434298.1
6	*An. peditaeniatus*	France	MT434316.1
7	*An. sinensis*	Syngapore	MW321920.1
8	*An. jeyporiensis*	Bangladesh	MK138573.1
9	*An. culicifacies*	Belgium	AF440397.1
10	*An. dthali*	Soudiarabia	KM068084.1
11	*An. varuna*	France	MT434331.1
12	*An. sundaicus*	England	AY789726.1
13	*Phlebotomus papatasi (Out Group)*	Serbia	KY848828.1

The maximum likelihood substitution matrix is shown in (Table 6.1.3).

The phylogenetic tree of Anophelinae based on COI sequences using Maximum likelihood (ML) method in MEGA X software are presented (Fig. 6.1.1).

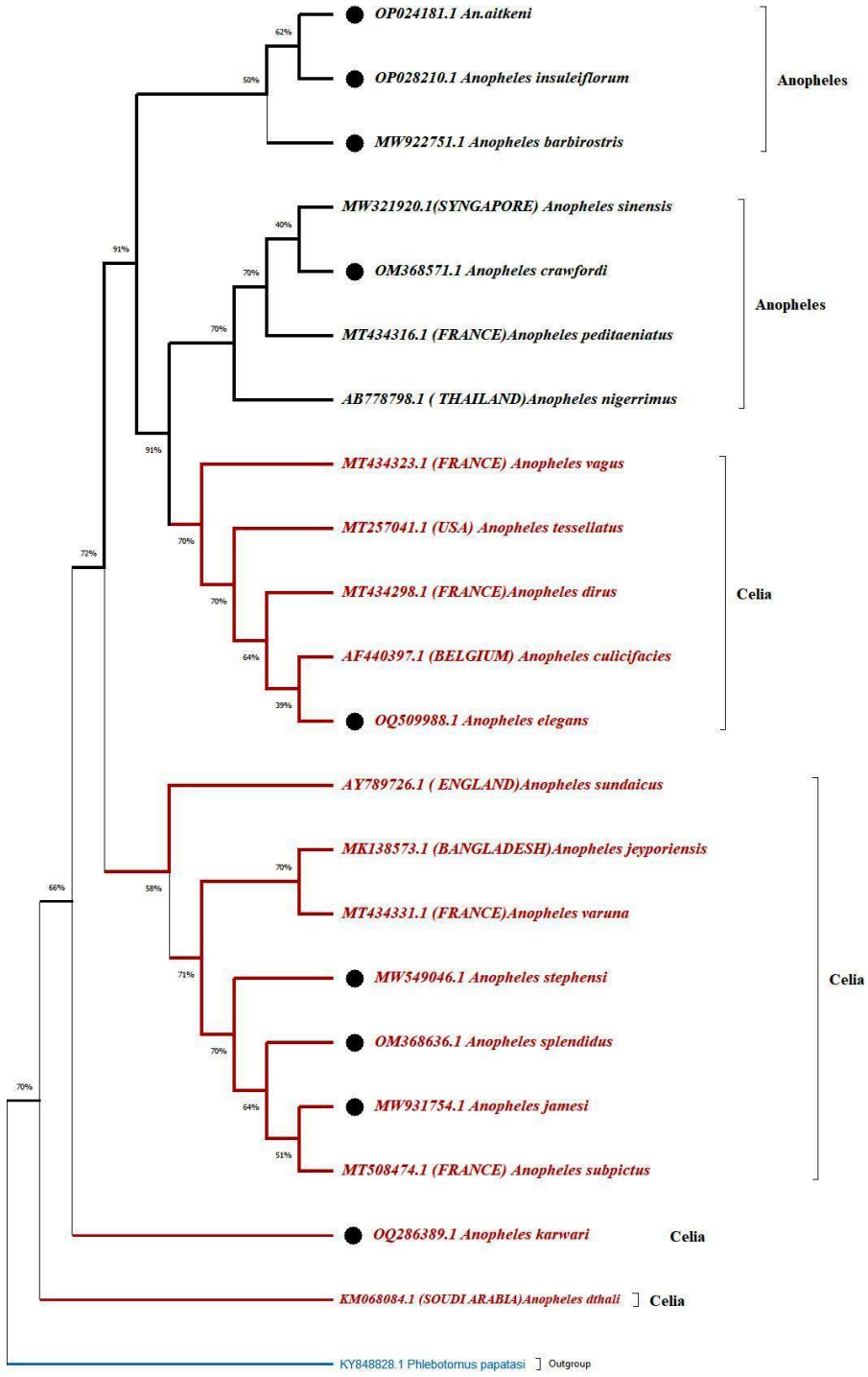

Figure 6.1.1: Phylogenetic tree of Subfamily Anophelinae based on COI gene sequences using Maximum likelihood method in MEGA X software. The species collected from the study area are bullet labelled.

Table 6.1.3: Maximum likelihood estimate of substitution matrix

	A	T/U	C	G
A	-	*7.05*	*7.05*	**10.90**
T/U	*7.05*		**10.90**	*7.05*
C	*7.05*	**10.90**		*7.05*
G	**10.90**	*7.05*	*7.05*	-

Rates of different transitional substitutions are shown in **bold** and those of transversion substitutions are shown in *italics*.

The nucleotide frequencies were A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -5898.353. the transition / transversion bias is 0.77.

Discussion

In phylogenetic tree, species of same subgenus were grouped together in same clade. Morphologically similar species shared the same clade in the tree. The species those shared sister clades were *An. aitkenii* and *An. insuleiflorum, An. sinensis* and *An. crawfordi, An. elegance* and *An. culicifacies, An. jeyporiensis* and *An. varuna, An. jamesi* and *An. subpictus.*

As in other insect groups (Sabir et al., 2019) codon structure of Cytochrome Oxidase I of Anophelinae collected were AT biased (Adenine 29.7%, Thymine 38.0%, Cytosine 15.9% and Guanine 16.4%). The mean diversity of the entire population and overall mean distance according to Kimura 2 parameter model was 0.18. The maximum pairwise distance was shown for *An. culicifacies* from all other species. No distances were shown between *An. aitkenii* and *An. insuleiflorum* as they were also very similar in morphology. The mean distance within the Subgenus *Anopheles* was 0.10 and Subgenus Celia was 0.21. The mean distance between Subgenus *Anopheles* and *Celia* was 0.170. A notable increase in K2P divergence was found across different taxonomic levels.

The best fit substitution model was GTR+G as it showed lowest BIC scores (Bayesian Information Criterion) and AICc (Akaike Information Criterion, corrected) values with maximum likelihood values. The analysis involved 22 sequences and coding positions were $1^{st}+2^{nd}+3^{rd}$.

6.2. Molecular Phylogeny of Subfamily Culicinae

The family Culicidae with many species of mosquitoes occurs thorough out the world, consists of 2 subfamilies viz., Culicinae and Anophilinae.

Culicinae is the largest subfamily of mosquitoes, containing 11 tribes. Species belonging to this subfamily are known as culicines. Molecular phylogenetic study of this subfamily collected from Mananthavady Taluk of Wayanad District, Kerala, resulted in 26 species belonging to 4 tribes and 6 genera (Table 6.2.1).

The CO1 sequences of all 26 species of 6 Genera are obtained with more than 400bp and are submitted to NCBI GenBank. The sequences of 10 species of subfamily Culicinae and an out group *Phlebotomus papatasi* were retrieved from NCBI GenBank for the phylogenetic analysis (Table 6.2.2). No stop codon or frame shifts were detected indicating the absence of pseudogenes (NUMTs). The sequences of all the species were already reported in Gen Bank and the sequences matched with the conspecific species of GenBank. This stipulate that COI sequences are useful in determining the species if the database is robust.

Table 6.2.1: List of COI sequences of Subfamily Culicinae in Wayanad with voucher number and NCBI accession number

Sl. No.	Species Name	Voucher No.	Accession No.
1.	*Ae. aegypti*	CUMM175	OR130932.1
2.	*Ae. albopictus*	CUMM6	MW542315.1
3.	*Ae. niveus*	CUMM79	ON506043.1
4.	*Ae. vittatus*	CUMM62	MW931755.1
5.	*Ae. paraedes barraudi*	CUMM19	MW549045.1
6.	*Ae. pseudotaeniatus*	CUMM38	MW931741.1
7.	*Ae. chrysolineatus*	CUMM28	MW931765.1
8.	*Ae. subalbopictus*	CUMM54	MW931745.1
9.	*Ae. cogilli*	CUMM68	OP078702.1
10.	*Cx. vishnui*	CUMM39	MW549044.1
11.	*Cx. gelidus*	CUMM9	MW542314.1

12.	*Cx. fuscocephala*	CUMM3	MW535377.1
13.	*Cx. quinquefasciatus*	CUMM59	MW926770.1
14.	*Cx. tritaeniorhynchus*	CUMM65	MW922794.1
15.	*Cx. infula*	CUMM63	MW922750.1
16.	*Cx. pseudovishnui*	CUMM49	MW922745.1
17.	*Cx. minor*	CUMM57	MW555438.1
18.	*Cx. bitaeniorhynchus*	CUMM55	MW555571.1
19.	*Cx. pallidothorax*	CUMM7	MW542320.1
20.	*Cx. uniformis*	CUMM90	OM368631.1
21.	*Mn. Indiana*	CUMM41	MW922742.1
22.	*Mn. uniformis*	CUMM11	MW542318.1
23.	*Ar. subalbatus*	CUMM5	MW542319.1
24.	*Ar.s aureolineatus*	CUMM29	OP093565.1
25.	*Mal. genurostris*	CUMM23	MW549050.1
26.	*Ver. indica*	CUMM8	OP107393.1

Table 6.2.2: List of COI sequences of Subfamily Culicinae retrieved from NCBI for phylogenetic analysis with Accession numbers

Sl. No.	Species Name	Place of collection	Accession No.
1	*Or. anopheloides*	Japan	LC054516.1
2	*Or. anopheloides*	India	AY917200.1
3	*Or. fascipes*	France	MF172346.1
4	*Ver. lugubris*	India	EU259290.1
5	*Ae.chrysolineatus*	India	EU259295.1
6	*Tr. aranoides*	India	AY917210.1
7	*Lt. halifaxii*	Thailand	MK271009.1
8	*Ur. nivipleura*	China	JQ728221.1
9	*Ur. obscura*	Singapore	MW321970.1
10	*Hz. chandi*	India	AY917208.1
11	*Phlebotomus papatasi* (Out Group)	Serbia	KY848828.1

The overall mean distance between the tribes is 0.18. The mean distances within the tribes and genus are shown in the Table 6.2.3. and 6.2.4.

Table 6.2.3: Intra tribe K2P divergence of Subfamily Culicinae

Sl. No.	Tribe	Distance within the tribe
1	Aedini	0.129191297
2	Culicini	0.097879093
3	Mansonini	0.10905285
4	Sabethini	0.796172401
5	Orthopodomyiini	0.100148236
6	Uranoteanini	0.11278077

Tribe Sabethini showed highest mean distances within the group. This tribe includes 2 species *Malaya genurostris* and *Trtipteroides aranoides*.

Table 6.2.4: The COI gene sequence divergences (K2P) within the Genus

SI. No	Genus	Distance within the Genus
1	*Aedes*	0.121019567
2	*Culex*	0.097425417
3	*Mansonia*	0.10905285
4	*Armigeres*	0.082084949
5	*Verrallina*	0.128672507
6	*Orthopomyia*	0.100148236
7	*Uranotaenia*	0.11278077

The phylogenetic tree of Culicinae based on COI sequences using Maximum likelihood (ML) method in MEGA X software are presented in (Figure 6.2.1.)

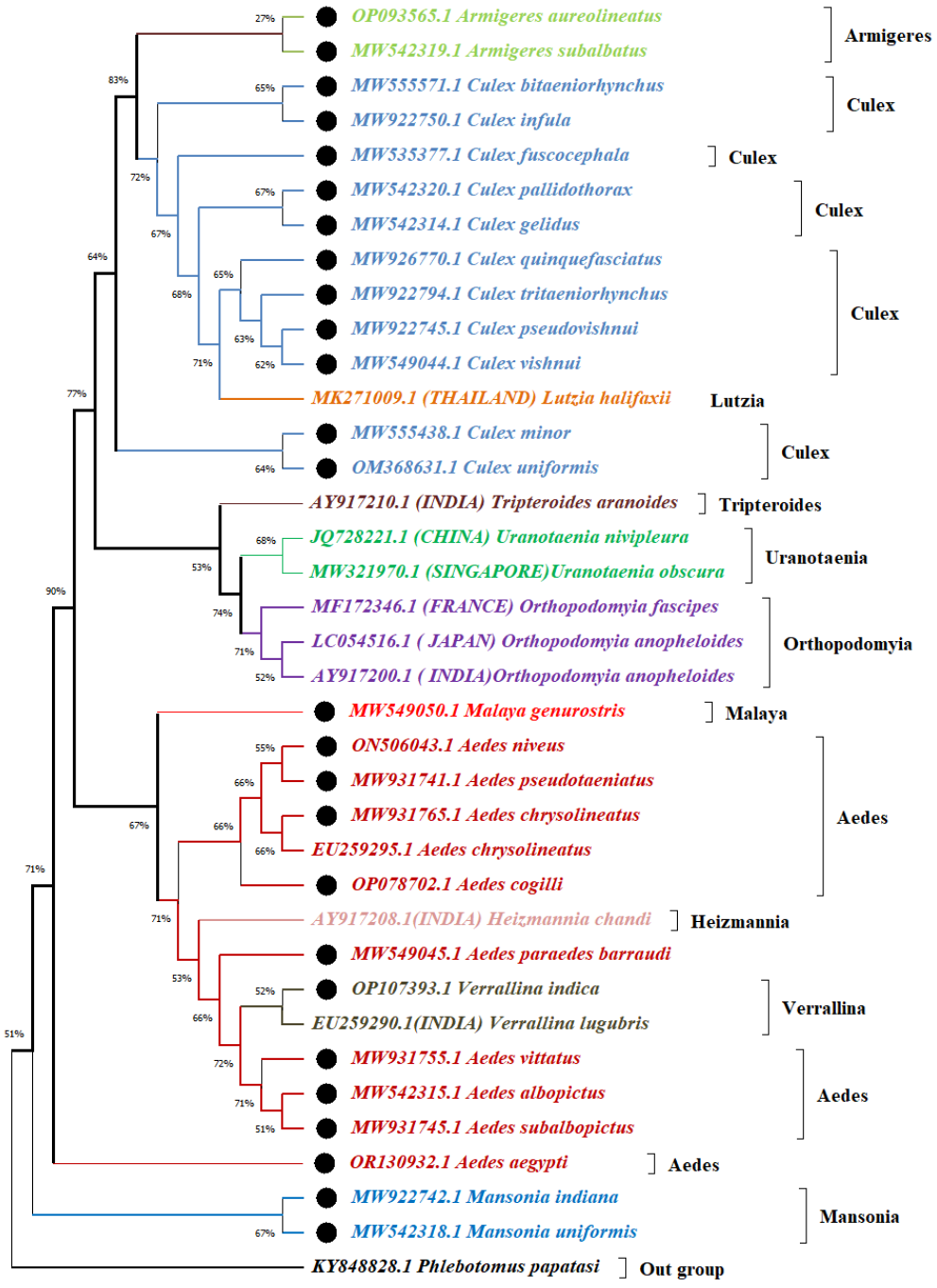

Figure 6.2.1: Phylogenetic tree of Subfamily Culicinae based on COI gene sequences using Maximum likelihood method in MEGA X software. The species collected from the study area are bullet labeled

Discussion

As in other insect groups (Sabir et al., 2019) codon structure of Cytochrome Oxidase I of Culicinae collected were AT biased (Adenine 29.7%, Thymine 38.7%, Cytosine 16.3% and Guanine 15.3%). *Mal. genurostris* belonging to the tribe Sabethini, showed greatest distance from all other species. Among the tribes, tribe Sabethini and among the genus, Genus Malaya showed more distance from all other groups. This was very evident in the tree as the species formed a separate clade. The lowest distance was shown between the tribes Aedinii and Culicinii. The species that shared sister clades were, *Cx vishnui* and *Cx.pseudovishnui* , C*x. infula* and *Cx. bitaeniorhyncus, Cx. gelidus* and *Cx. pallidothorax, Ar. sabalbatus* and *Ar. aureolineatus, Man. indiana* and *Man. uniformis*, *Ae. niveus* and *Ae. pseudotaeniatus, Ae. albopictus* and *Ae. subalbopictus,* and *Ver. indica* shared clade with *Ver. lugubris* . All the species shared sister clades also showed morphological similarity. The mean diversity of the entire population was 0.18. The estimated Transition/Transversion bias (R) was 0.91.

The coefficient of differentiation was 1183900534.11. The best fit substitution model was GTR+G as it showed lowest BIC (Bayesian Information Criterion) scores (15442.870) and AICc (Akaike Information Criterion, corrected) values (14819.187) with maximum likelihood values (-7331.314). This analysis involved 37 nucleotide sequences. Codon positions included were 1st+2nd+3rd. Rates of different transition substitutions were 11.89 and those of transversion substitutions were 6.55.

CHAPTER 7

MAMMALIAN HOST PREFERENCE PATTERNS AMONG THE FEMALE MOSQUITOES

The ability of insects to feed on blood is thought to have originated when plant-sucking insects accidently bit vertebrates and later acquired a digestive system that permitted the intake and utilization of the protein-rich resources for metabolic purposes (Waage, 1979). The tight relationship between chewing insects and vertebrates, in which the insects adapted to cues peculiar to vertebrates and occasionally nibbled on their skin, may have provided another avenue for evolutionary change (Lehane, 2005). An intense and closely related parallel parasitic evolution between the insect and the vertebrate host took place when blood became these insect's most important food source. The insect developed a reliance on host-specific cues during this process, enabling it to successfully recognize its host in a heterogeneous environment.

Both male and female adult mosquitoes may live solely off carbohydrates-rich foods like nectar or fruit juices (Foster et al., 1995). Plant sugars fuel physical activity by directly supplying energy for maintenance, enabling the replenishment of metabolic reserves, extending life, and extending lifespan (Barredo and Degennaro 2020). However, most species are also anautogenous, meaning that females must consume the blood of a vertebrate host in order to create eggs (Clements 1992).

Primary egg chambers, which include an oocyte, nurse cells, and enclosing follicle cells, helps in the development of mature eggs in the ovaries (Valzania et al., 2019). Until a bloodmeal is consumed, the development of primary egg chambers is halted (Roy et al., 2016). This initiates the vitellogenic phase of oogenesis. The processes controlling the vitellogenic phase are now well understood in *Ae. aegypti*, where intake of a bloodmeal triggers the release of two peptide hormones from the brain: ovary ecdysteroidogenic hormone (OEH) and insulin-like peptides (ILPs) (Roy et al., 2016). While ILP3 is necessary for triggering trypsin-like enzymes in the

midgut that digest the bloodmeal (Briegel, 2003; Isoe et al., 2009; Brackney et al., 2010), OEH powerfully stimulates follicular cells to create ecdysteroid hormones, most notably ecdysone (ECD) (Brown et al., 2008; Dhara et al., 2013). Mostly red blood cells (erythrocytes) in plasma make up the colloidal suspension of entire vertebrate blood. Additionally, the majority of the dry weight of whole blood is made up of proteins, with trace amounts of fat, carbohydrate, and other substances making up the remainder (de Smet, 1978). According to a number of studies, anautogenous mosquitoes lay eggs after ingesting blood from several vertebrates (Lyimo et al., 2012; Gordon, 1922), with clutch size correlated with blood volume ingested (McCann et al., 2009; Phasomkusolsil et al., 2015). the most crucial macro nutrient being protein (Sen, 1917, Lea and Delong 1958).

The mosquito penetrates the skin of the host with its needle-like mouthparts, or stylets, and inject saliva into the wound to prevent normal platelet aggregation and blood coagulation. According to Ribiero (1989), the substances in saliva shorten the time spent in probing and feeding. The saliva that the mosquito injects can cause the host to become infected when it is parasite-infested. Our perception of a mosquito bite is caused by the protein in saliva. As soon as the mosquito is disturbed or its stretch receptors alert it that its midgut is full of blood, it stops salivating and ingesting blood and takes off with its mouth parts still attached. After feeding, the female mosquitoes will rest until eggs are developed and blood is digested. Depending on its physiological condition, the female might wait to blood feed again until after the eggs are laid. Depending on the temperature, this time frame can extend anywhere from two to six days. (Gwadz, 1969). The mosquito flies away after finishing its meal since host stimuli no longer draw it in. Approximately 125 eggs mature inside the mosquito, and two to three days later, the mosquito lays its adult eggs near or on water. The female mosquito seeks out another host and ingests its blood after oviposition, which reactivates her host-seeking behaviour and starts a new reproductive cycle (Klowden, 1995). Identifying the host-feeding preferences of vector species reveals the vertebrate species susceptible to arthropod-borne pathogen transmission and the mechanism of disease spread in an environment (Alcaidae et al., 2009; Chaves et al., 2010; Brugman et al., 2015).

The present study was conducted over a period of 3 years from 2019 to 2022. Urban and rural regions of Mananthavady Taluk of Wayanad district of Kerala were selected to assess the mammalian host preferences by female mosquitoes. A total of 1,657 blood fed mosquitoes were collected from human dwellings, cattle sheds, and pig farms during the study period. Samples were collected using Resting collection (RC) and Man landing collection (ML) techniques (Mboera, 2005). Mosquitoes from five genera namely *Aedes, Culex, Anopheles, Armigeres* and *Mansonia* were chosen for the analysis, as these genera include vectors of medically important diseases (Table 7.1).

The feeding status of mosquitoes were visually categorized as unfed (UF), half-fed (HF), and fully-fed (F). The highest share of the samples comprises HF followed by F and UF. Majority of HF and F mosquito blood meals' hosts were identified successfully.

Table 7.1: Status of vector and non-vector mosquito genera collected from the study area

Sl. No.	Genus	Total No. of species collected	No. of vectors	No. of non-vectors
1	*Aedes*	10	5	5
2	*Culex*	14	10	4
3	*Anopheles*	15	11	5
4	*Mansonia*	3	3	-
5	*Armigeres*	1	1	-
	Total	**44**	**30**	**14**

Serological technique based on precipitin reaction was the method followed for the blood meal analysis. Precipitin bands formed in agarose gel of specific antigen (blood of the host) against antiserum was used for the analysis (Fig. 7.1). Result was interpreted by observing the position of the band near the antiserum.

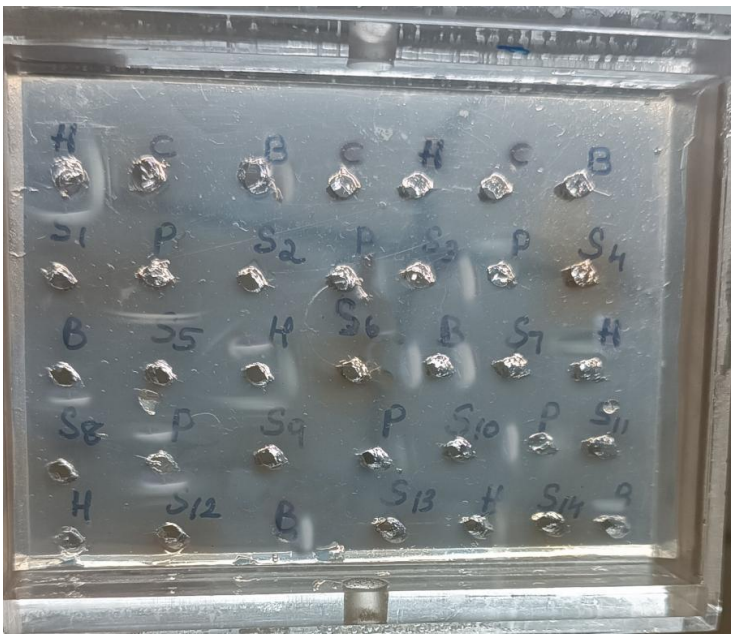

Figure 7.1: 1% Agarose gel with precipitin band indicating host preferences.

Blood meal analysis was performed on 44 species of mosquitoes coming under 5 genera, of which 30 species were identified as vectors of various diseases viz., Japanese encephalitis, filariasis, malaria, dengue fever, chikungunya, zika fever, yellow fever, and west Nile fever. and the remaining 14 were under non-vector species (Table 7.2). The genera A*nopheles* covers the highest number of blood-feeding mosquito vector species with eleven species each followed by *Culex* (10sp), *Aedes* (5sp.), *Manosia* (3sp.), and *Armigeres* (1spp.).

Ae. albopictus, Ae. vittatus, Ae. aegypti, Ae. niveus, and *Ae. vexans* were the vector species collected within the genus *Aedes*. Human blood was preferred by all species. *Ae. vexans* was detected for both human and bovine blood. There was a wide range in species abundance within the genera. *Ae. albopictus* was the most abundant species, while *Ae. aegypti, Ae. niveus,* and *Ae. vexans* were scarce in number.

The blood fed vector species reported from the study area under the genera *Culex* were *Cx. quinquefasciatus, Cx. vishnui, Cx. pseudovishnui, Cx. tritaeniorhyncus, Cx. bitaeniorhyncus, Cx. gelidus, Cx. whitemorei, Cx. fuscocephala, Cx. infula* and *Cx. pallidothorax. Cx. gelidus* was the most abundant

species collected during the study period with 236 blood fed species. *Cx. quinquefasciatus, Cx. vishnui, Cx. pseudovishnui, Cx. tritaeniorhyncus, Cx. bitaeniorhyncus* and *Cx. fuscopcephala* were less in number compared to *Cx. gelidus*. Species like *Cx. whitemorei, Cx. infula and Cx. pallidothorax* were the least collected ones. Except *Cx. quinquefasciatus, Cx. vishnui and Cx. pseudovishnui,* all other species of the genus *Culex* preferred cattle blood. These three species showed a preference for human as well as cattle blood. *Cx. quinquefasciatus* has a special preference for human blood. Both the genera *Aedes* and *Culex* showed great variation in the abundance of the species collected.

11 blood fed vector species were collected from the genera *Anopheles* namely, *An. barbirostris, An. peditaeniatus, An. stephensi, An. elegans , An. fluvialitis, An. nigerrimus, An. vagus, An. subpictus, An. jeyporiensis, An.culicifacies* and *An. crawfordi.* Genus *Anopheles* did not show much species abundance like *Culex* and *Aedes*. *An. barbirostris* and *An. subpictus* were more common species compared to other species. All the species of A*nopheles* collected, except *An. barbirostris* showed the diet of cattle blood. While a preference for both human and cattle blood could be observed in the case of *An. barbirostris*.

Among the three species collected from the genera *Mansonia, Man. annulifera* were least number collected, and was found to fed on human blood only. whereas the other two species, *Man. indiana* and *Man. uniformis* were abundant in numbers and were found to feed on all three hosts viz., human, cattle, and pig.

Table 7.2: List of mosquito vector species collected and their source of mammalian blood meal revealed in the present study

Sl. No	Blood-fed mosquito collected	Source of Blood meal			Collection method*
		Human	Cattle	Pig	
1.	*Ae. albopictus*	426	–	–	ML
2.	*Ae. vittatus*	33	–	–	ML
3.	*Ae. vexans*	4	3	–	RC
4.	*Ae. niveus*	2	–	–	ML
5.	*Ae. aegypti*	2	–	–	ML
6.	*Ar. sabalbatus*	274	410	56	ML, RC
7.	*Cx. quinquefasciatus*	32	1	–	RC
8.	*Cx. vishnui*	13	65	–	RC
9.	*Cx. pseudovishnui*	5	45	–	RC
10.	*Cx. tritaeniorhyncus*	–	74	–	RC
11.	*Cx. bitaeniorhyncus*	–	21	–	RC
12.	*Cx. whitemorei*	–	2	–	RC
13.	*Cx. gelidus*	–	236	–	RC
14.	*Cx. fuscocephala*	–	17	–	RC
15.	*Cx. pallidothorax*	–	4	–	RC
16.	*Cx. infula*	–	5	–	RC
17.	*An. barbirostris*	9	16	–	RC
18.	*An. peditaeniatus*	–	2	–	RC
19.	*An. stephensi*	–	3	–	RC
20.	*An. elegans*	–	8	–	RC
21.	*An. fluvialitis*	–	2	–	RC
22.	*An. nigerrimus*	–	1	–	RC
23.	*An. vagus*	–	6	–	RC
24.	*An. subpictus*	–	10	–	RC
25.	*An. jeyporiensis*	–	8	–	RC
26.	*An.culicifacies*	–	2	–	RC
27.	*An. crawfordi*	–	5	–	RC
28.	*Man. indiana*	14	38	14	RC
29.	*Man. uniformis*	11	45	11	RC
30.	*Man. annulifera*	3	–	–	ML

* RC – Resting collection, ML – Man landing collection

Ar. sabalbatus was the most common species collected during the study period with 740 individuals. It favoured each of the three hosts. Greater preference was given to cattle.The majority of Aedes species were collected using the Man landing

method, while the majority of Anopheles, Culex, and Mansonia species were caught using the resting collection method.

Five species of *Aedes,* five species of *Anopheles*, and four species of *Culex* have been identified as non-vector mosquitoes from the blood fed collected samples (Table 7.3). All species of *Aedes* (*Ae. harveyi, Ae. cogilli, Ae. novalbopictus, Ae. menoni and Ae. barraudi*) and *Culex* (*Cx. brevipalpis, Cx. bicornutus, Cx. nigropunctatus* and *Cx. uniformis*) showed positive results on human blood meal analysis. While blood meal anlysis of species from the genus *Anopheles* (*An. culiciformis, An. insuleiflorum, An. jamesi, An. karwari* and *An. splendidus*) presented positive results with blood source from cattle.

Table 7.3: List and Blood sources of non-vector mosquito species

Sl. No.	Blood-fed species collected	Source of Blood meal			Collection method*
		Human	Cattle	Pig	
1	*Ae. harveyi*	1	–	–	ML
2	*Ae. barraudi*	2	–	–	ML
3	*Ae. menoni*	3	–	–	ML
4	*Ae. novalbopictus*	2	–	–	ML
5	*Ae. cogilli*	4	–	–	ML
6	*Cx. brevipalpis*	2	–	–	ML
7	*Cx. uniformis*	2	–	–	ML
8	*Cx. bicornutus*	3	–	–	ML
9	*Cx. nigropunctatus*	1	–	–	RC
10	*An. culiciformis*	–	4	–	RC
11	*An. insuleiflorum*	–	1	–	RC
12	*An. jamesi*	–	54	–	RC
13	*An. karwari*	–	3	–	RC
14	*An. splendidus*	–	32	–	RC

* RC – Resting collection, ML – Man landing collection

Eleven vector species and five non-vector species were obtained from the Genus A*nopheles*, which accounted for 35% of the total mosquitoes collected. 10 Vector species and four non-vector species made up 33 percent of the genus *Culex.* 5 vector species and five non-vector species were obtained from the genus *Aedes*. It accounted for 23% of the whole collection. *Mansonia* accounted for 7% of the

overall collection with three vector species, while *Armigeres* was the least collected genus with a single species comprising 2% of the whole sample (Fig. 7.2). Two of the five genera namely *Armigeres* and *Mansonia*, have identical preferences for blood meals on all three hosts, human, cattle, and pig. Though *Anopheles* and *culex* showed an affinity for both cattle and human, a stronger preference could be observed towards cattle blood. Aedes stayed distinct because of its greater preference for human blood (Fig. 7.3).

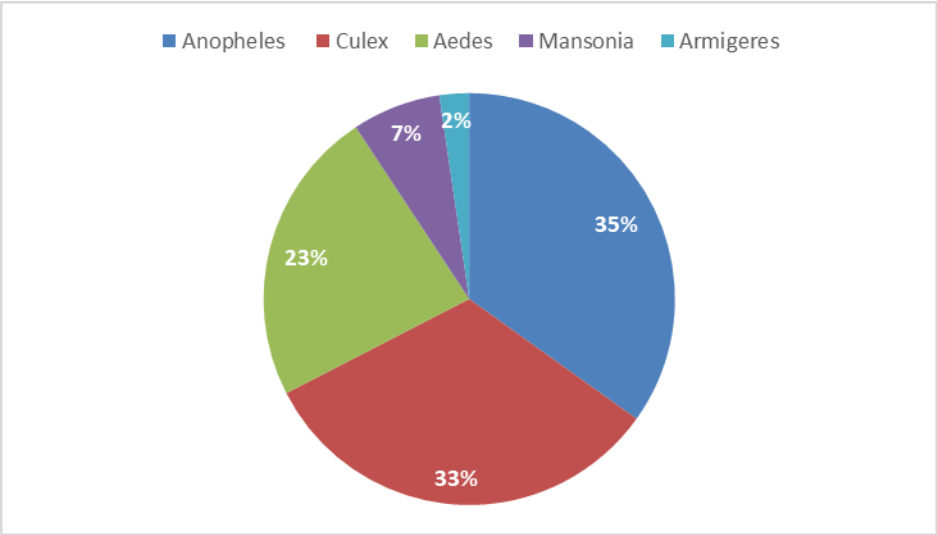

Figure 7.2: Pie chart showing the composition of blood fed mosquito species collected during the present study

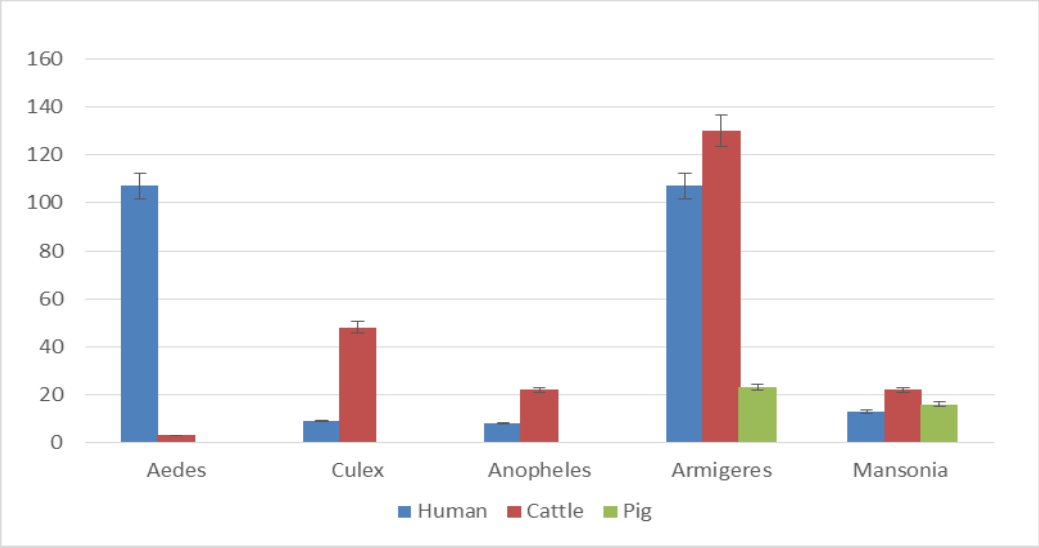

Figure 7.3: Clustered column showing the mammalian host preferences of mosquito vectors

DISCUSSION

A good number of mosquito species were found prevalent in human habitations and domestic animal sheds in the study area. Among the collected mosquitoes having vector status, *Cx.quinquefasciatus, Ae.aegypti* and *Ae albopictus* preferred human habitation than domestic animal sheds as their resting site. This result is in tune with the result of a similar study reported from West Bengal, where *Ae. albopictus* and *Cx. quinquefasciatus* found prevalent in human habitats (Azmi and Chatterjee 2015). This is a crucial point in respect to man-vector proximity, leading to disease transmission. The members of vector importance included under the genera *Anopheles* and *Culex* (except *Cx. quinquefasciatus*) preferred cattle shed for resting after blood meal. *Armigeres* and *Mansonia* mosquitoes were seen in all three habitats like human dwellings, cattle sheds, and piggeries. The cause of such a variation in resting sites might be linked to their blood feeding preferences.

Abundance of mosquitoes were observed during monsoon season whereas diversity was more during pre-monsoon season in which intermittent rain favoured the multiplications of mosquitoes. This is in support with the result of Abdelrazec and Gumel (2017) who depicted a peak in mosquito abundance for a temperature in the range 20−25°C and above average rainfall values. The climatic conditions of Wayanad does perfectly match with this study report.

Ar. sabalbatus, an incriminated vector of JE (Das et al., 1983; Aneesh et al., 2014), outnumbered all other species in resting as well as man landing collections regardless of the season. They were equally abundant during monsoon, pre-monsoon, and post-monsoon seasons. This species showed preferences toward all the three hosts selected for the present assessment.

In the present study, *Ae.albopictus*, a secondary vector for dengue fever, chikungunya, yellow fever, and zika fever, was the dominant mosquito species after *Ar. sabalbatus* in terms of their population. It was mostly collected through the man landing method, and this species dominated during the monsoon season. Although *Ae. aegypti* is the primary vector of these diseases, only few samples could be collected, indicating *Ae. albopictus* predominated the habitat during this study

period. Kalra and Prasittisuk (2004) and Jomon et al. (2009) also supported this result as they reported *Ae. albopictus* as the major species in prevalence and distribution from different parts of Kerala. Human blood was found in all specimens of this species upon dietary analysis. In addition to *Ae. albopictus, Ae. aegypti, Ae. vittatus, Man. annulifera,* and *Cx. quinquefasciatus* were the mosquitoes that preferred exclusively human blood. This suggests that these five mosquitoes were anthropophilic, whereas all other mosquitoes were zoophilic. While Janssen et al. (2015) supported the anthropophilic nature of *Cx. quinquefasciatus* with his studies among Mexico, Scott et al. (2000), Delatte et al. (2010), Heisch et al. (1959) agreed with the human preference of *Ae. albopictus* and *Ae. aegypti.*

The generalistic feeding behaviour of *Ae. albopictus* was asserted by some investigators like Savage et al. (1993) and Sivan et al (2015). The results from the present study substantially differed from their observations as all the *Ae. Albopictus* exhibited preference towards human blood. In-depth study of the feeding behaviour of this species is very crucial as it is an important vector and an abundant mosquito species of the area selected.

The zoophilic nature of *Cx. gelidus, Cx. tritaeniorhyncus,* and *Cx. infula* was confirmed through the studies from Gudallore and Thanjavoor as well as from Kerala (Samuel et al., 2008).

Some species were observed to ingest mixed blood meals. *Ar. sabalbatus, Cx. pseudovishnui,* and *Cx. vishnui* have been observed with human and bovine blood. *Ar. sabalbatus, Man. indiana,* and *Man. uniformis* were shown to have bovine and pig blood components. Double feeding nature of *Man. indiana* and *Man. uniformis* was in agreement with the studies of Samuel et al. (2008) who conducted studies in Kuttanad, Kerala. They observed the double feeding of human and cow and human and pig of both the species. The current study demonstrated the mixed nature of blood feeding from bovine and pig hosts by these mosquito species.

Multiple blood meals are important for mosquito fitness as well as for the reproduction. Second blood meal for some mosquitoes like *An. tessellatus* used for increased accumulation of metabolic reserves (Scott et al., 2012).

Research from several regions of the world revealed that mosquitoes engage in opportunistic feeding (Roy et al., 1991; Garcia-Rejon et al., 2010; Janssen et al., 2015; Stenn et al., 2018). Current study also in tune with the above findings showing mixed blood feeding behaviour of the mosquitoes. The final choice of their host by mosquitoes may depend on extrinsic or intrinsic factors, of which genetic variation is an important factor. Extrinsic factors such as availability of the host, its range of flight and resting habits, sleeping habits of the hosts, the odours given out by the host, the large exposed area of warm skin, the accessibility of the capillary blood vessels, the palatability of the blood (Kailash et al., 2012), body heat, body mass, gender, defensive behaviour, microorganisms present on the body, age, colour (Scott et al., 2012) etc. also play an important role in host selection.

Anautogenous female mosquitoes are unable to lay eggs without receiving blood from their vertebrate hosts (Clements, 1992). In contrast to other taxa, mammals have a larger plasma protein level than other species of vertebrates (de Smet, 1978). The three main plasma-derived proteins, serum albumin, fibrinogen, and globulins, differentially encouraged egg production in various mosquito species, such as *Ae. aegypti*, depending on the source of vertebrate hosts (Harrison et al., 2021).

Haemoglobin, the main blood cell protein, increased yolk deposition when taken from pigs but not from people, cows, or sheep. Different vertebrate serum albumins also have varying effects on egg development. According to Harrison et al. (2002), bovine serum albumin (BSA) induced ovarian ecdysteroidogenesis. Consequently, mosquitoes selectively choose mammals for their blood intake. Methods for controlling mosquitoes can be elucidated by studies on the vector's preferences for mammalian hosts. Compared to human residents, Cattle sheds have high mosquito population due to different defensive mechanisms of humans.

The genetic basis of mosquito's host selection is altered by the relative abundance of different host species. Both generalist and specialist prey on particular host species, which affects the spread of disease. Studies revealed that this species is exceptionally adapted to getting blood under a variety of situations, where the most

plentiful host species tends to be preferred, even when intrinsic preferences may prevail locally. This selective behaviour contrasts with that of specialist *An. gambiae*, which is anthropophilic and maintains this behaviour under various conditions, Occasional derivations of this behaviour are also documented, when human hosts are limited (Takken and Verhulst, 2013).

The relevance and the significance of the present study relies on the vectoral status of mosquitoes. The preferences of mosquitoes to specific hosts specially to human makes them to spread the deadly diseases faster. More number of mosquitoes with human blood were collected from the urban area, ie., Manathavady town, where the density of the population is richer than the rural area. The abundance and diversity of mosquito species were also observed to be higher in areas where both human and animal populations coexist. Mammals attract mosquitoes, specifically cattle and if they are in close proximity with human dwellings there is high risk and chances of spreading vector borne diseases. Some mosquitoes prefer multiple feeding as well, to improve the quality of the blood meal (Gillies, 1954, 1955). Though there are specific host preferences for some mosquitoes, majority are opportunistic feeders (Roy et al., 1991) and they feed on the availability of hosts. In urban areas where human populations are higher, there are high chances and risks of mosquito bites thus spreading of fatal vector borne diseases.

CHAPTER 8

DISTRIBUTION AND MORPHOLOGICAL VARIATIONS AMONG THE CEPHALIC SENSILLA OF MOSQUITOES

Olfactory driven behaviours enable mosquitoes to locate the hosts from which they obtain blood meals necessary for the production and maturation of eggs. In doing so, they spread disease to humans and animals (Bowen et al., 1995; Takken et al., 2013).

8.1. Cephalic structures in mosquitoes concerned with olfaction

The major cephalic appendages in mosquitoes housing olfactory sensilla are: one pair of antennae, one pair of maxillary palps and proboscis (Fig. 8.1a).

Scape, pedicel and 13 segmented flagellum forms the antennae of mosquitoes. Scape being the first segment connects to the second segment pedicel through a thin ring of chitinous tissue. A mass of neuronal components known as Johnston's organ is in the pedicel. The muscles that are placed at the base of scape and pedicel, helps in the movement of the antennae. Hair like sensillae that are located on the flagellomeres heps in the olfaction.

Males possess bushy antennae whereas females have plumose antennae (Fig. 8.1 a and 8.1b). The flagellar segments are of equal length in female and it consists of varieties of sensillae whereas it is unequal in male. Two terminal segments of male flagellae have sensilla like that of female and the rest of the basal segments are with whorls of fibrillae (MClver, 1982). There are five types of antennal sensillae in mosquitoes, namely, Sensilla Chaetica, Sensilla Trichoidea, Grooved Pegs, Sensilla Coeloconica and Sensilla Ampullacea. Besides olfaction, antennae also mediate sound detection in mosquitoes (Warren et al., 2010; Lapshin, 2012).

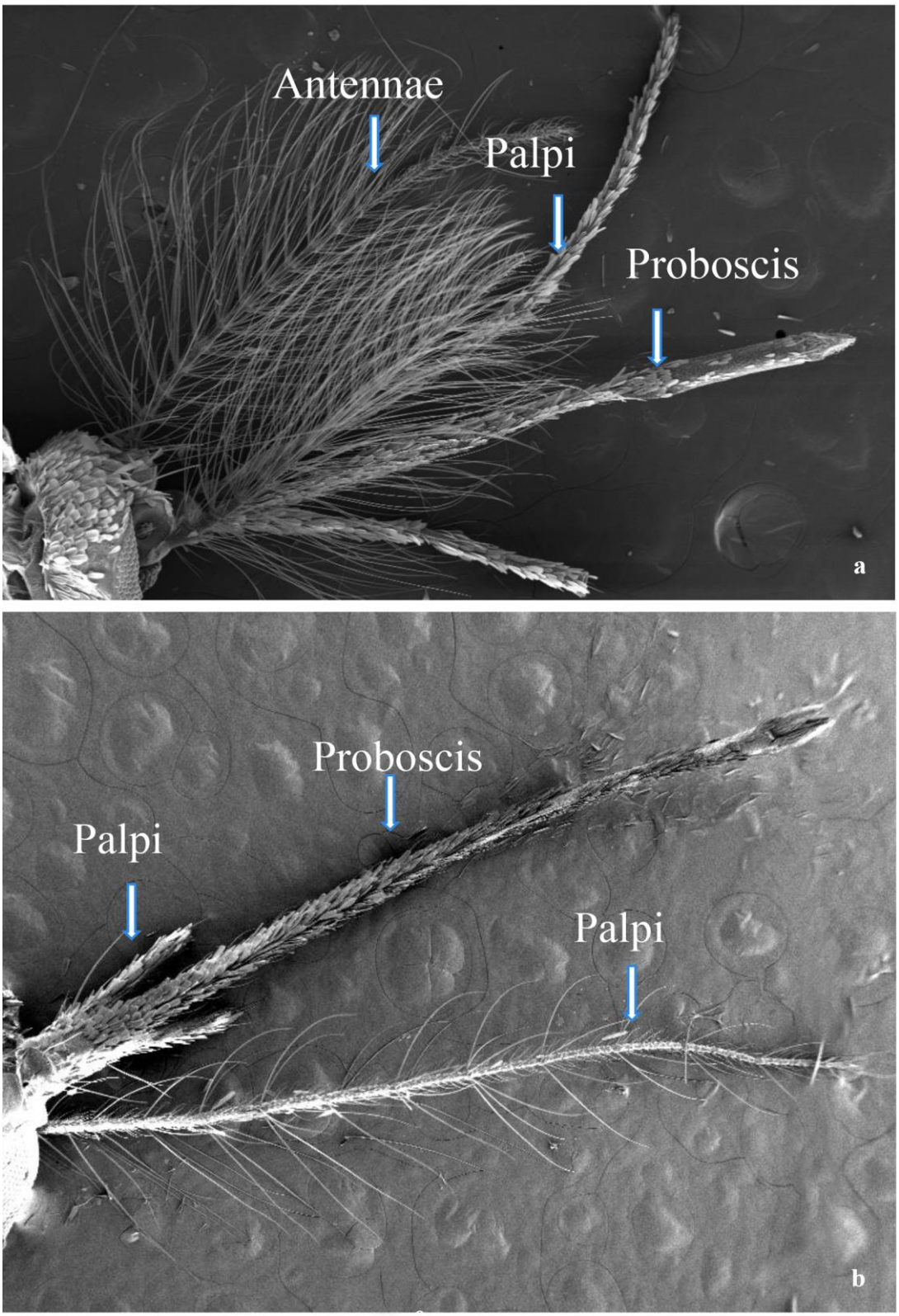

Figure. 8.1: a) Antennae , probosis ad Palpi of Male *Ae.albopictus* , b) Antennae , probosis ad Palpi of female *Ar.sabalbatus*.

The mosquito proboscis usually consists of six appendages (a pair of maxillae with teeth-like structures, a pair of mandibles, a needle-like labrum, and a hypopharynx) encased in a labium that ends in a labellum (Choo et al., 2015). In females of blood-feeding species, all the six appendages are about the same length as the labium and they pierce the host skin during blood-feeding. The length of maxillae and mandibles varies in males. Interestingly, both males and females of non-blood feeding genera (*Malaya* and *Topomyia*) are completely devoid of mandibles and maxillae (Wahid et al., 2003). Like antennae, proboscis also accommodates sensillae like Chaetica and Microtrichia, along with Squamiformia scales (Fig 8.2 a).

Maxillary palp of mosquitoes consists of five segments. In female Culicines the 5^{th} (most distal) segment is reduced to a knob, and in male Anophelines the 4^{th} and 5^{th} segments are expanded to a club shaped structure which forms the characteristic feature of the subfamily Anophelinae. Four types of sensillae were observed in the palpi of mosquitoes, namely, Microtrichia, Sensilla Chaetica, Sensilla Capitate peg and Companiform sensilla (Mclver and Charlton, 1970; Mclver, 1971; Mclver and Siemicki, 1975a) (Fig 8.2b).

8.2. Types of cephalic sensilla in female mosquitoes

Types of sensilla present on the cephalic organs of mosquito were: Sensilla Chaetica, Sensilla Coeloconica , Sensilla Trichoidea, Sensilla Basiconicaor Grooved Pegs, Sensilla Ampullaceae, Sensilla Capitate pegs, Sensilla Companiformia and Sensilla Squamiformia. Besides, there were small hair like structures called Microtrichia could also be detected (Table 8.1).

8.2.1. Sensilla Chaetica(SCh)

These sensilla are thick-walled and externally sturdy bristles that arise from a socket with fine serrations along the edge of grooves and have a sharp-pointed tip. There are two distinct varieties, long and short (SCh1 and SCh2), and they serve as mechano-sensilla (Fig. 8.4a). At the base of every flagellomere 2^{nd} to 13^{th}, long Chaetica sensilla are evenly distributed around the circumference of all lagellomeres in the antenna. These are also known as 'verticals' (Mclver, 1982).

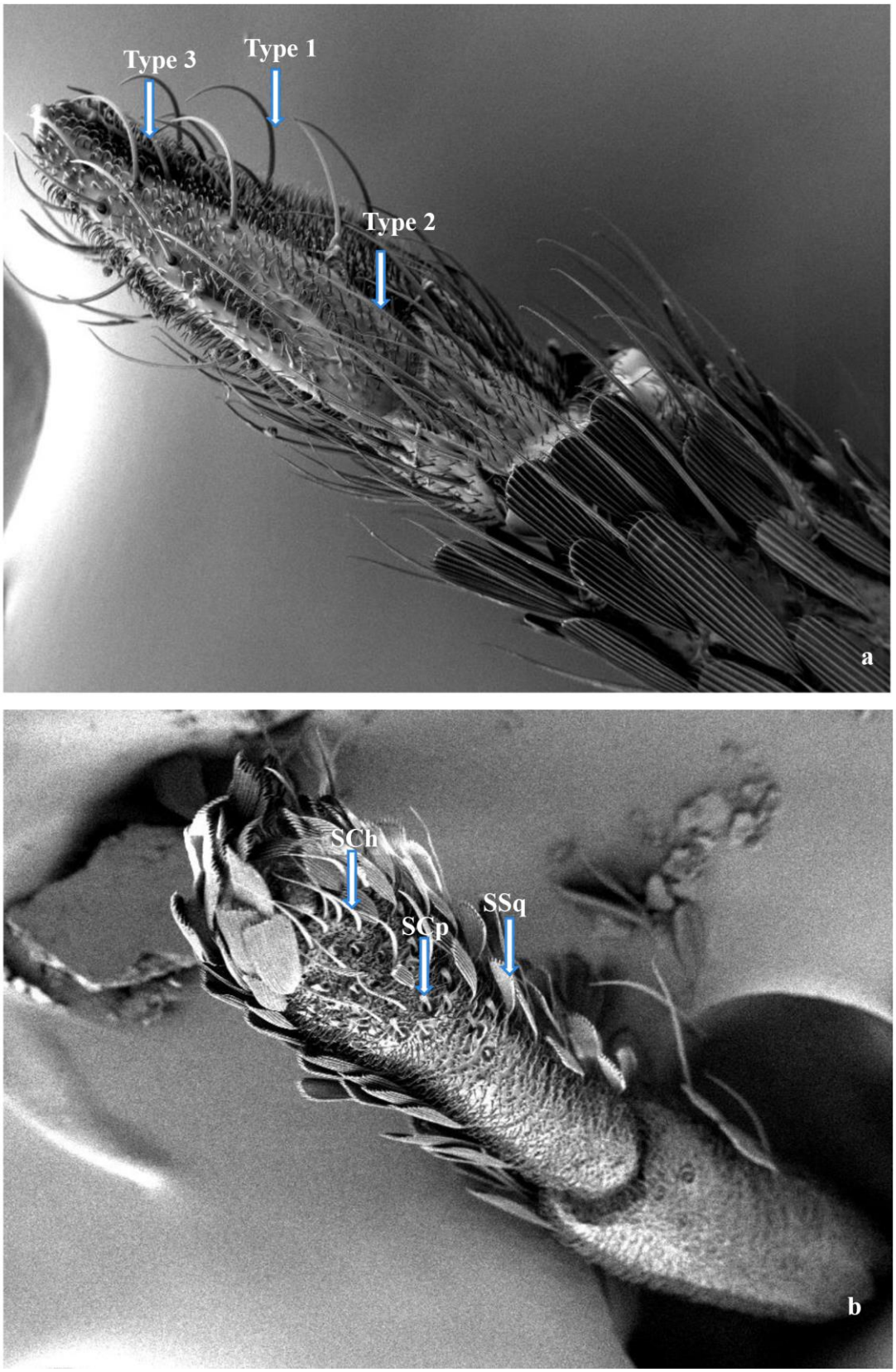

Figure.8.2: a) Proboscis of *Cx.quinquefasciatus*, b) Palpi of *Ae.vittatus*

8.2.2. Sensilla Coeloconica (SCo)

The Sensilla Coeloconica are of two types – large and small, on the basis of their shape and size.

Large Sensilla Coeloconica (SCoL), also known as 'sunken pegs' by Boo and Mclver (1976) and 'Sensilla Coeloconica ' by Ismail (1962), are exclusive to Anopheline mosquitoes. Female Anophelines, which typically have a few of these sensilla on each of the seven basal flagellomeres, have more than that of males of the same species, which have between 8 and 14 primarily on the sub terminal flagellomere (Mclver, 1982). Large Sensilla Coeloconica consist of short pegs embedded in the floor of a pit whose sides close partially over the peg point (Figs.8.3c, 8.4a, c, e & f). They are olfactory in function.

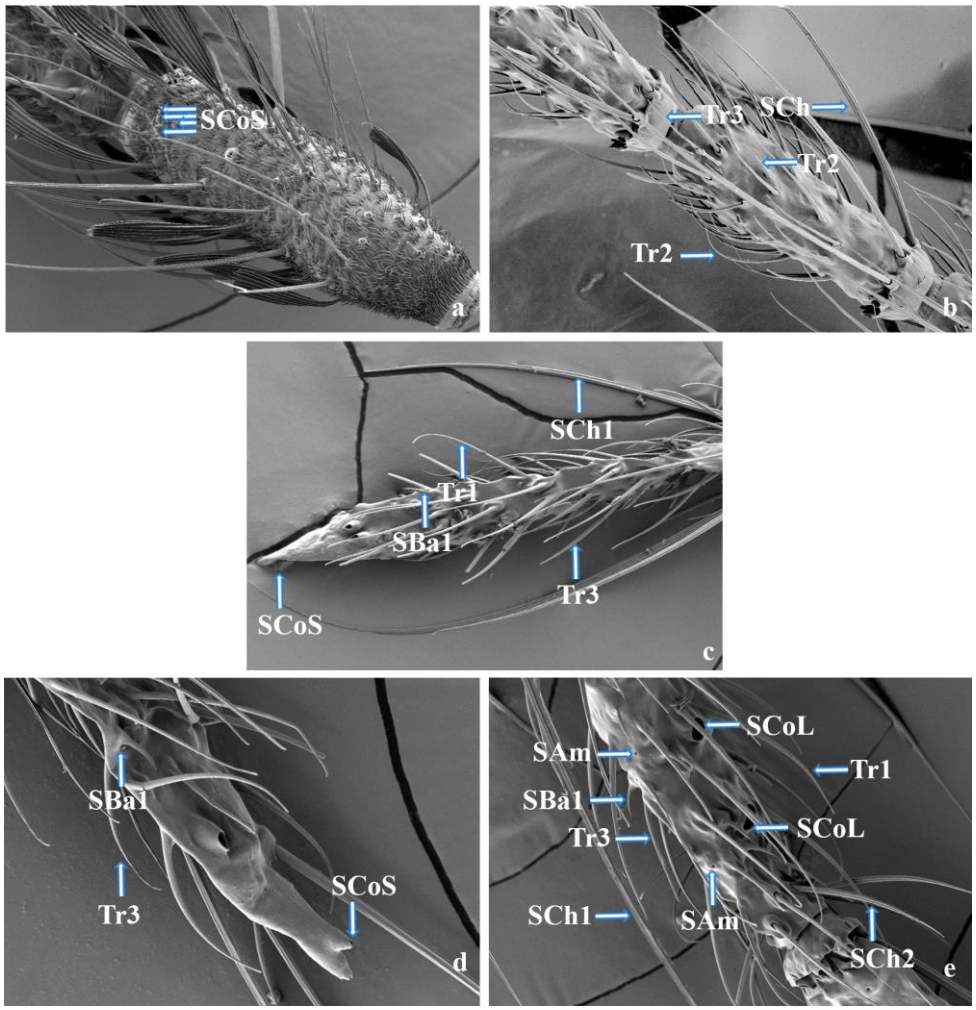

Figure 8.3: *An.barbirostris*-Antennae (a, b, c) *An.culiciformis*- Antennae (d,e)

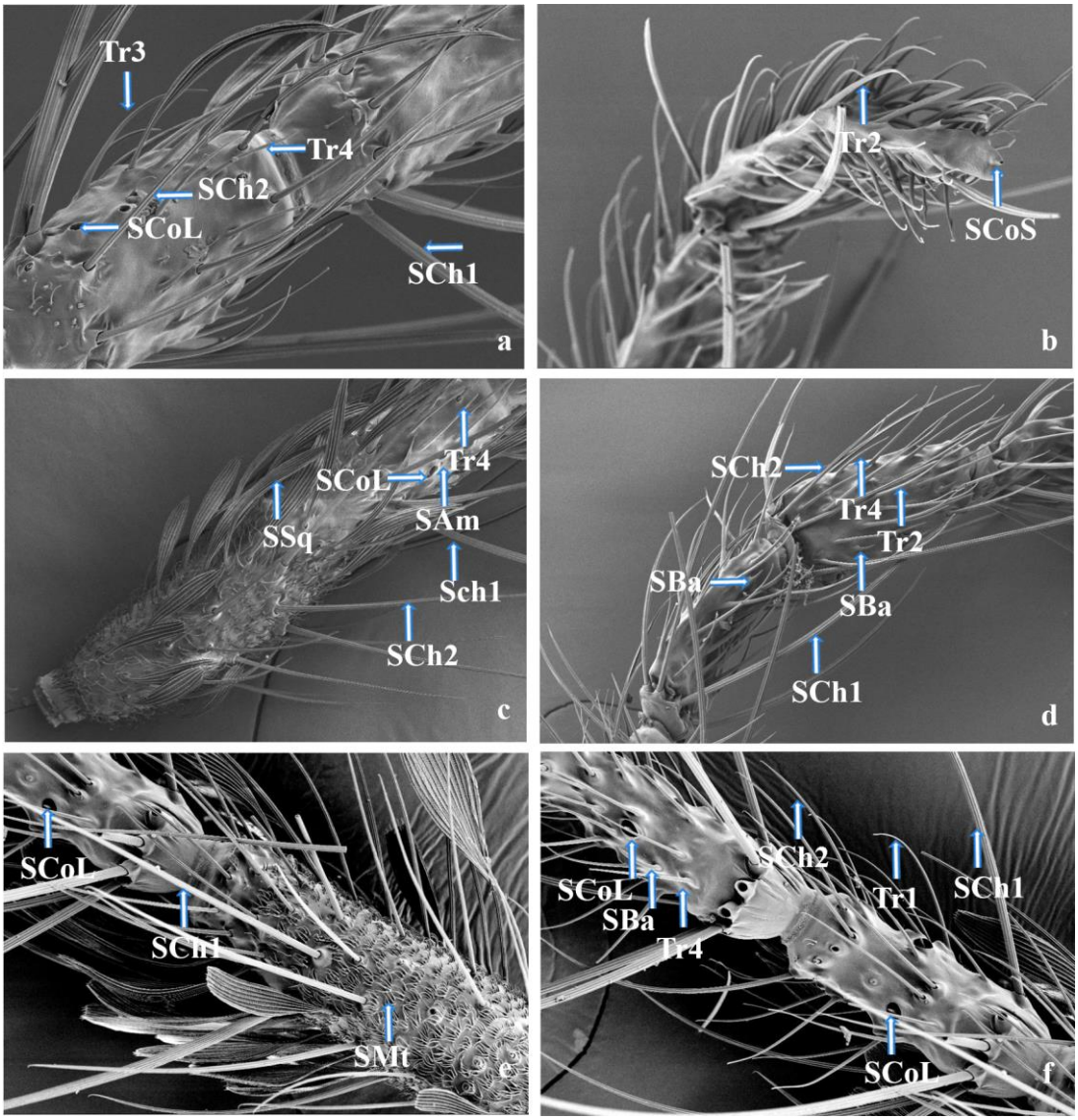

Figure 8.4: *An.stephensi*-Antennae (a,b,c) *An.nigerrimus*-Antennae (d,e)

Small Sensilla Coeloconica (SCoS), are produced in a cup-like depression of the antennal wall, these small, thick-walled sensilla are commonly known as pitted pegs. The pegs may or may not protrude through the circular apertures on the surface of the cuticle. Similar to Sensilla Basiconica, the surface of Sensilla Coeloconica is grooved along its length, though the channels are deeper. They have been provisionally categorized as hygro- and thermoreceptors (Hill et al., 2009). Sensilla Coeloconica has the peg positioned at the base of a pit with a small cuticular orifice, therefore, the peg is not visible. This type of sensillum was

discovered at the distal (13th) antennal flagellomere (Figs 8.3c, d; 8.4b; 8.5c, 8.6c, 8.8c & 8.9a).

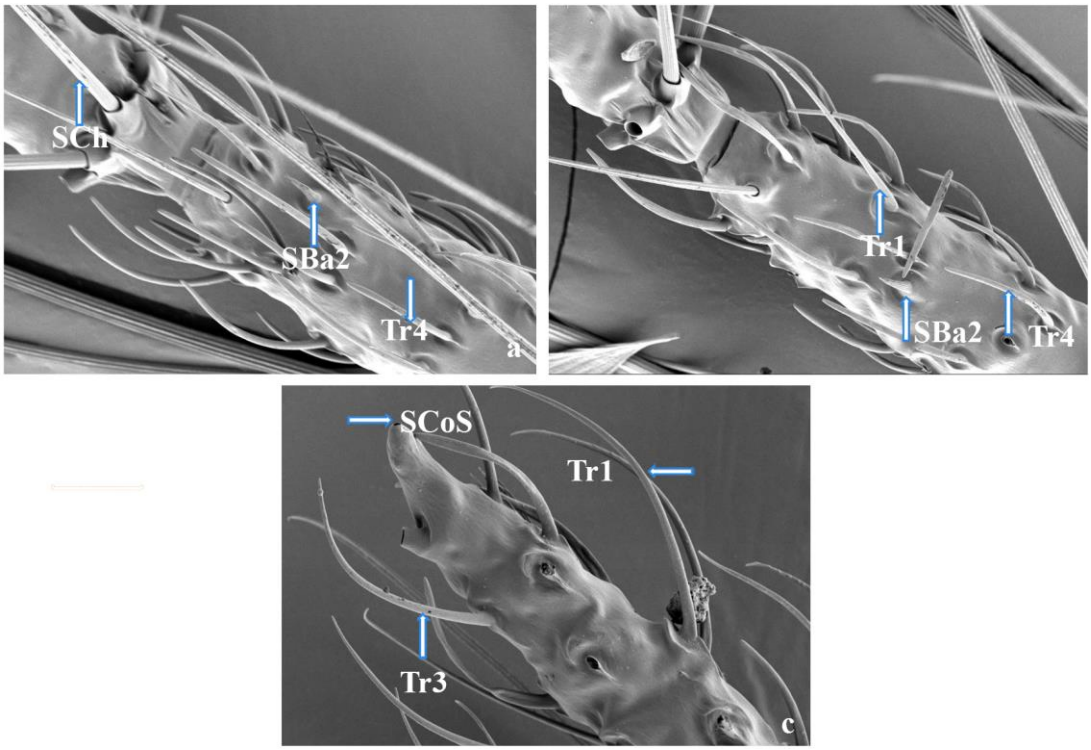

Figure 8.5: Antennae (Ae.aegypti)

8.2.3. Sensilla Trichoidea (STr)

Sensilla Trichoidea are sensory structures that have the appearance of hair but do not originate from a basal alveolus (Hill et al., 2009). Instead, STr function as olfactory organs. On flagellomeres 2nd to 13th, the majority of the sensilla that can be found are of this particular type. There are four distinct subtypes of Sensilla Trichoidea, which are denoted by the following designations: long sharp trichoidea (Tr1), short sharp trichoidea (Tr2), long blunt trichoidea (Tr3), and short blunt trichoidea (Tr4) (Figs. 8.4b, d; 8.5a,b, c.). The surface of long trichoidea is smooth and the number increases from the proximal end of flagellomeres to the distal end. On the flagellum, the number of short sharp trichoidea is significantly lower than the number of long sharp trichoidea. Sharp trichoidea are tapered to a point, whereas blunt trichoidea do not have this feature. In addition to this, the length of these sensilla appears to be more consistent than that of the sharp trichoidea. Blunt trichoidea are less in number compare to sharp trichoidea (Mclver, 1982).

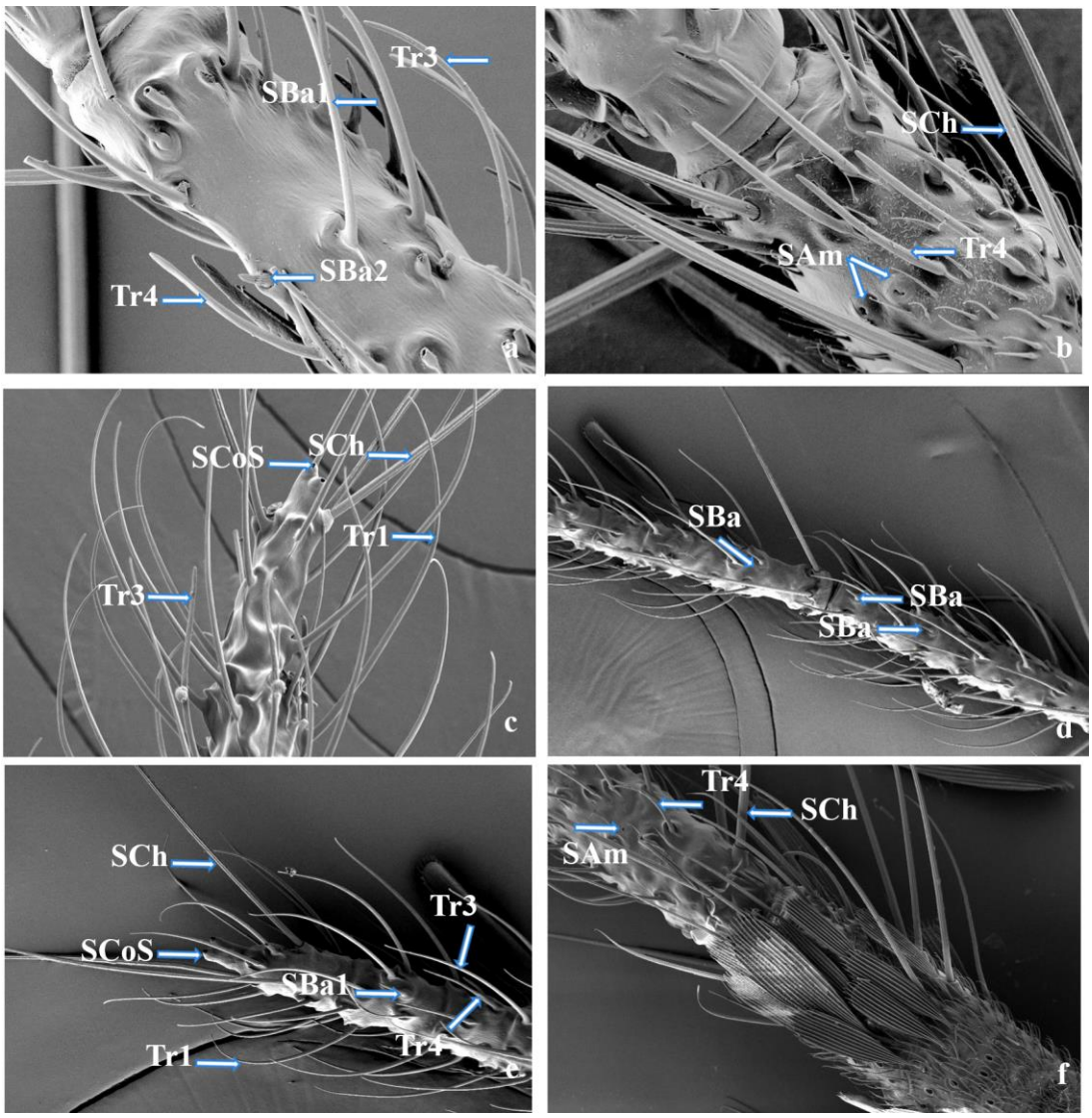

Figure 8.6: *Ae.albopictus* -Antennae (a,b,c) *Ae.vittatus* - Antennae (d,e,f)

8.2.4. Sensilla Basiconica or Grooved Peg (SBa)

They have the appearance of a short, peg-like structure, but their diameters vary. These pegs are separated into two kinds based on the length of their sensilla basiconica: long Sensilla Basiconica(SBa1) and short Sensilla Basiconica(SBa2) (Fig. 8.6a). SBa1 were found in greater numbers on the first basal segment of the flagellum, whereas SBa2 were distributed over the entire length of the flagellum. SBa act as a chemo-sensilla. Kellogg (1970) provided the physiological evidence that the grooved pegs of *Ae. aegypti* responded to the vapour of ammonia, acetone, and water (by excitation) and also responded to the vapour of acetic acid and anisole

(by inhibition). This proved that the grooved pegs have an olfactory function (Mclver, 1982).

8.2.5. Sensilla Ampullacea (SAm)

Its small peg-like organs are not readily visible as they are produced in pits with narrow or slit-like openings (Fig. 8.6b). This type of sensillum resides on the initial basal segment of flagellum. Sensilla Ampullacea has been suggested as thermo and hygroreceptors together with olfaction (Hill et al., 2009). Externally, the only evidence of a Sensillum Ampullacea is a small cuticular elevation with an elliptical orifice. The neck is a cuticle-lined tube projecting inward from the orifice and perpendicular to the long axis of the flagellum. The lumen of the neck progressively enlarges and opens into the flask-shaped chamber's side. A thick-walled pin protrudes upward from the chamber's floor (Mclver, 1982).

The remarkable morphological similarity between the Sensilla Ampullacea and the small Sensilla Coeloconica (SCoS) suggests that their functions are comparable. Therefore, it seems possible that the Sensilla Ampullacea, like the Sensilla Coeloconica , are sensitive to heat (MClever, 1982).

8.2.6. Sensilla Capitate pegs (SCp)

These sensilla are present on palpal segments 2, 3, and 4 in female Anophelines whereas on the 4^{th} segment in male Anophelines (Mclver and Siemicki, 1975). In the case of male and female Culicines, it is reported only on 4^{th} segment (Mclver and Charlton, 1970; Mclver, 197l). There are fewer than 20 per palp in female *Uranotaenia* sp. (Omer and Gillies, l97l) and more than 200 per palp in majority of *Culex* species. Palpal ablation studies suggested a CO_2 detection role for these sensilla in *Ae. aegypti* and *Cx. quinquefasciatus* (Bassler, 1958; Omer and Gillies, l97l). Sutcliffe et al. (1987) argued that the lamellate dendrite is the most likely CO_2 detector because all three biting groups (Simuliidae, Ceratopogonidae, and Culicidae) responded to CO_2 and the lamellate dendrite is the only dendrite shared by the capitate pegs of all three groups (Figs 8.15 b, 8.16b).

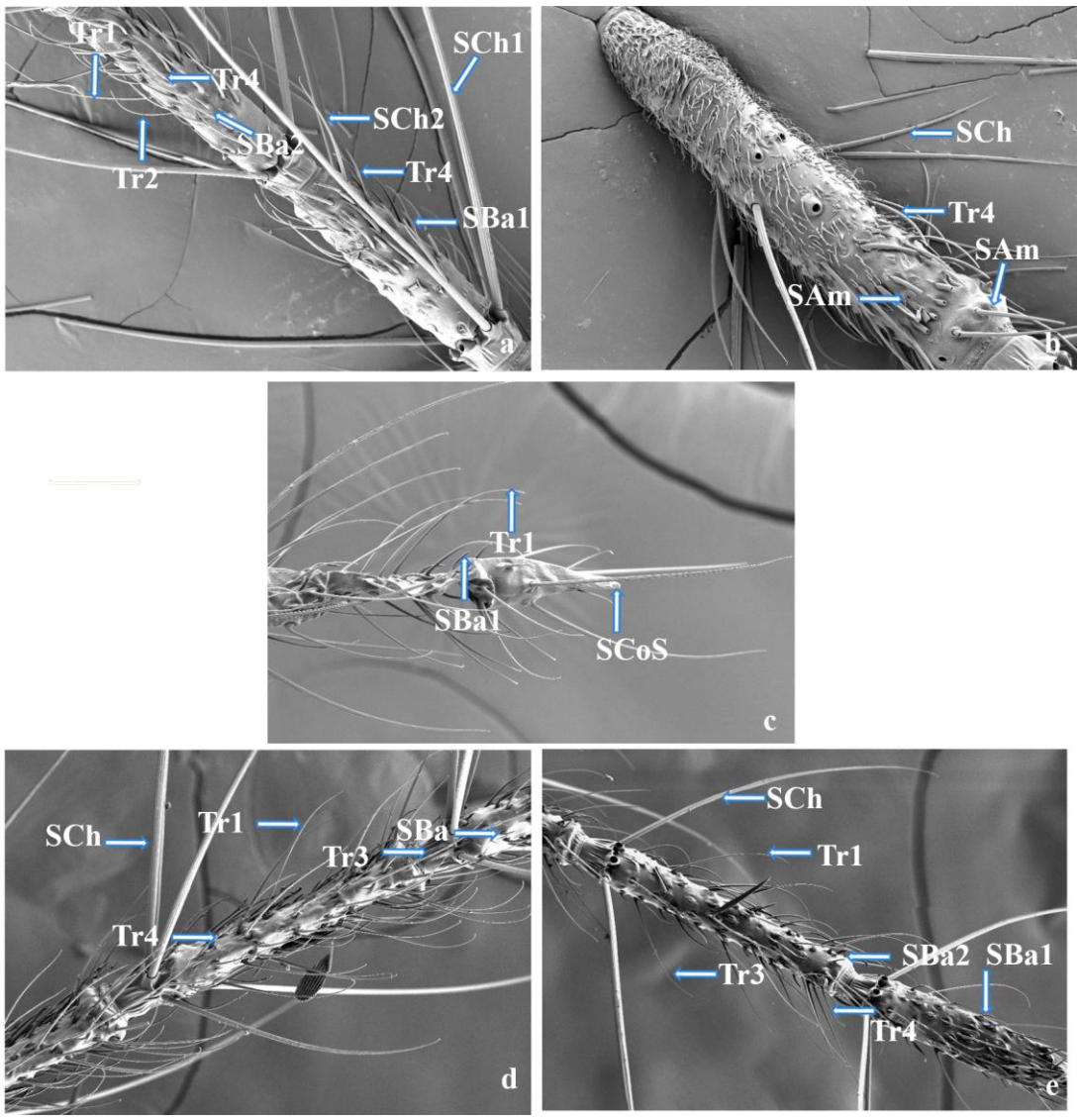

Figure 8.7: *Cx.gelidus*- Antennae (a,b,c) *Cx.bitaeniorhyncus*- Antennae (d,e)

8.2.7. Sensilla Companiformia (SCm)

On the palps of mosquitoes, Sensilla Companiformia was found close to the distal margin of 3rd segment. Each sensillum of a female *An. stephensi* consists of a dome-shaped cap that is hinged to a raised ring of cuticle that surrounds the sensillum. The cap is composed of three layers: an exocuticular layer on the exterior, a spongy layer in the middle, and a fibrous layer on the interior (Figs. 8.15c, 8.16c). According to Mclver and Siemicki (1975b), the rubbery protein resilin might be found in the middle layer of the cap, as well as possibly in the innermost layer. They

provide mechanical information. Sensilla Companiformia are proprioceptors, which respond to stress and strain exerted on the exoskeleton (Pringle, 1938a).

8.2.8. Sensilla Squamiformia (SSq)

They are typically present among the scales of flagellum in the form of slender scales (Fig. 8.13b). These sensilla may be considered to have a mechanical function, as they are able to detect stress in the cuticle caused by mechanical deformation (Fauchex, 1991).

8.2.9. Microtrichia (SMt)

These sensilla are non-innervated structures spread throughout the proboscis and palpi (Figs. 8.14d and 8.14e).

Among these, the sensilla solely concerned with olfaction are five in number, namely Sensilla Trichoidea, Sensilla Basiconica, Sensilla Ampullacea, Sensilla Coeloconica , and Sensilla Capitate pegs located on the maxillary palpi (Table 8.1).

Table 8.1: List of sensilla on the cephalic appendages of mosquitoes and their role in sensory perception

Sl. No.	Type of Sensilla	Sensory Perception
1	Trichoidea (STr)	Olfaction
2	Basiconica (SBa)	Olfaction (lactic acid, water vapours)
4	Ampullacea (SAm)	Olfaction, Hygroreception
4	Capitate peg (SCp)	Olfaction (CO_2)
5a	Large Coeloconica (SCoL)	Olfaction
5b	Small Coeloconica (SCoS)	Thermoreception
6	Chaetica (SCh)	Mechanoreception
7	Companiformia (SCm)	Mechanoreception
8	Squamiformia (SSq)	Mechanoreception
9	Microtrichia (SMt)	Mechanoreception

8.3. Genus specific variation of olfactory sensilla on the antenna of mosquitoes

A total of 19 species of blood feeding mosquitoes belonging to five genera viz., *Aedes, Culex, Anopheles, Mansonia, Armigeres* and one species of non-blood feeding mosquito belonging to the genus *Malaya* (*Mal. genurostris*) were taken for the structural analysis of olfactory sensilla through Scanning electron Microscopy (SEM) technique.

The first five genera of blood feeding mosquitoes are important vectors of human health diseases. The olfactory sensilla present on the antennae of these mosquitoes were given in Table 8.2.

Table 8.2: List of olfactory sensilla present on the antennae of different blood feeding mosquito species under five genera subjected to SEM analysis

Sl. No.	Sensilla	Anopheles	Aedes	Culex	Mansonia	Armigeres
1	STr1	+	+	+	+	+
2	STr2	+	+	+	+	+
3	STr3	+	+	+	+	+
4	STr4	+	+	+	+	+
5	SBa1	+	+	+	+	+
6	SBa2	+	+	+	+	+
7	SCoS	+	+	+	+	+
8	SCoL	+	-	-	-	-
9	SAm	+	+	+	+	+

The antennal olfactory sensilla commonly shared by all the members of the species under the five genera selected for the present study are: STr, SBa, SAm, SCoS.

The variations in the distribution of antennal sensilla on different flagellomeres of blood feeding mosquito species under the selected genera are given in Table 8.3.

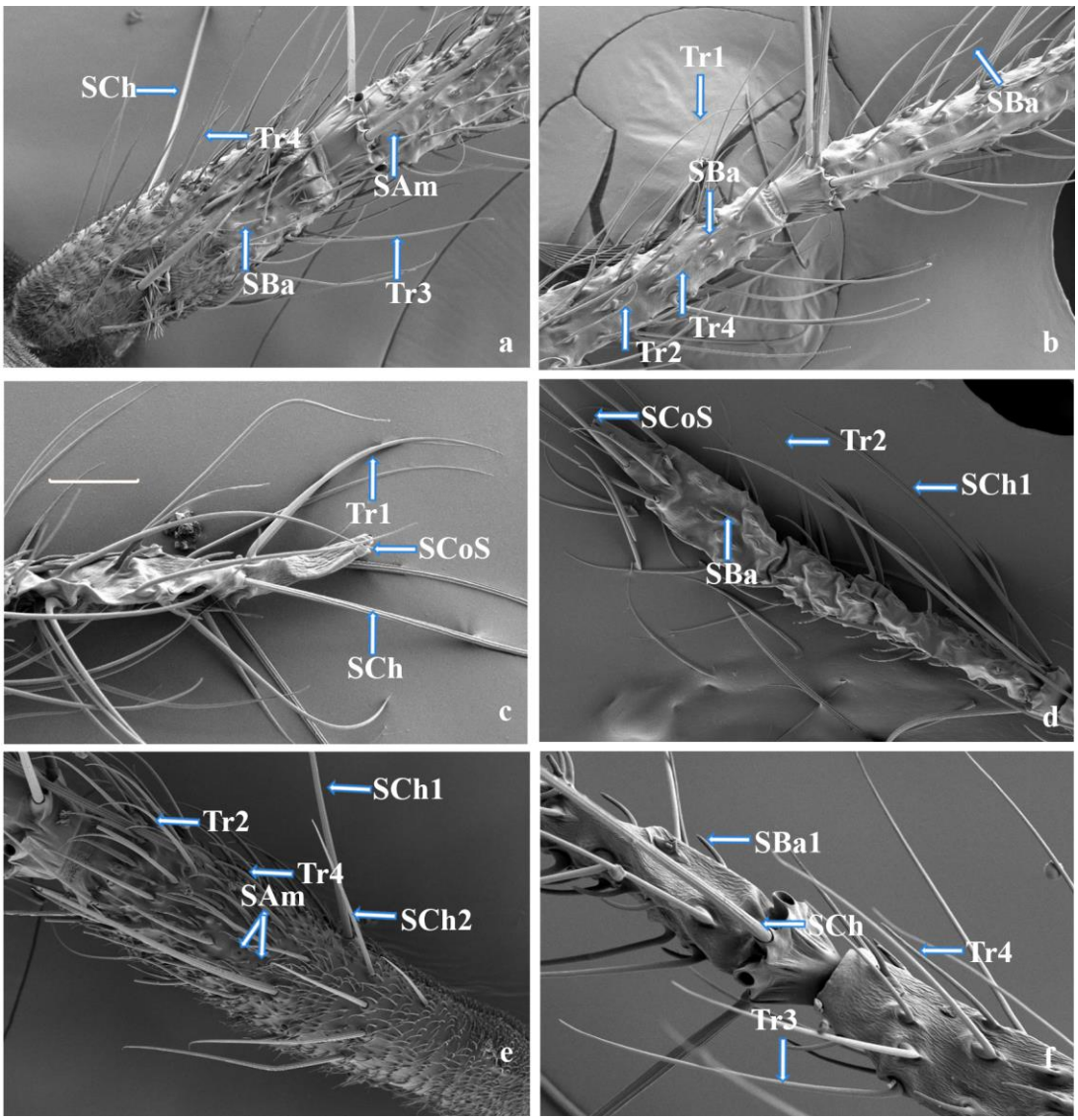

Figure 8.8: *Cx.pseudovishnui*- Antennae (a,b,c) *Cx.quinquefasciatus*-Antennae-(d,e,f)

Table 8.3: Variations in the distribution of antennal sensilla on different flagellomeres of blood feeding mosquito species under the selected genera

Genus	**1st and 2nd**	**3rd to 12th**	**13th**	**Most abundant**
Aedes	SSq, SCh1, SCh2, SBa, SST,SBT	SAm, SST,SBT,SBa	SCoS, SBT, SBa, SCh	SBa, SBT
Culex	SAm, SCh, STr1, SBa, SBT,	SAm, SST,SBT,SBa, SCh	SCoS, SBT, SST, SBa, SCh, SAm	SST
Anopheles	SSq, SCh1, SCh2, SCoS, SST, SCoL,	SAm, SCh, SCoL, SST,SBT, SBa, SAm	SCoS, SBT, SST, SBa. SCh	SCh, SST,
Mansonia	SAm, SST, SCh2, SCh1, SBT, SBa,	SAm,SST,SBT, SBa,	SCo, SST, SBa. SCh	SST
Armigeres	SSq, SCh1, SCh2	SAm,SST,SBT, SBa,	SCo, SBT, SST, SBa. SCh	SST

Sensilla Trichoidea was the most abundant type observed in this study and is regarded as the primary 'drivers' of various behaviours (Hill et al., 2002). Ismail (1964) also reported the occurrence of this type of sensillum in both Culicine and Anopheline mosquitoes. Even though it was found in all the flagellomeres, the 13th flagellomere displayed a greater number of Trichoidea in all genera (Figs. 8.3c, 8.4b, 8.6c,e; 8.7c, 8.11c,d and 8.12f). Among the four types of trichoidea, namely long and short sharp trichoidea (STr1 and STr2) and long and short blunt trichoidea (STr3 and STr4), the genus *Aedes* possess greater number of blunt trichoidea compared to the genera *Mansonia, Armigeres, Culex,* and *Anopheles* (Figs. 8.5a, 8.5b and 8.6b).

In comparison to other genera, *Aedes* and a few *Culex* species showed a higher number of SBa as they are more drawn towards human for blood feeding (Fig 8.6d and 8.8f). Based on the length, SBa are of two types viz., SBa1 (long) and SBa2 (short) and both types were found in all genera (Fig. 8.6a).

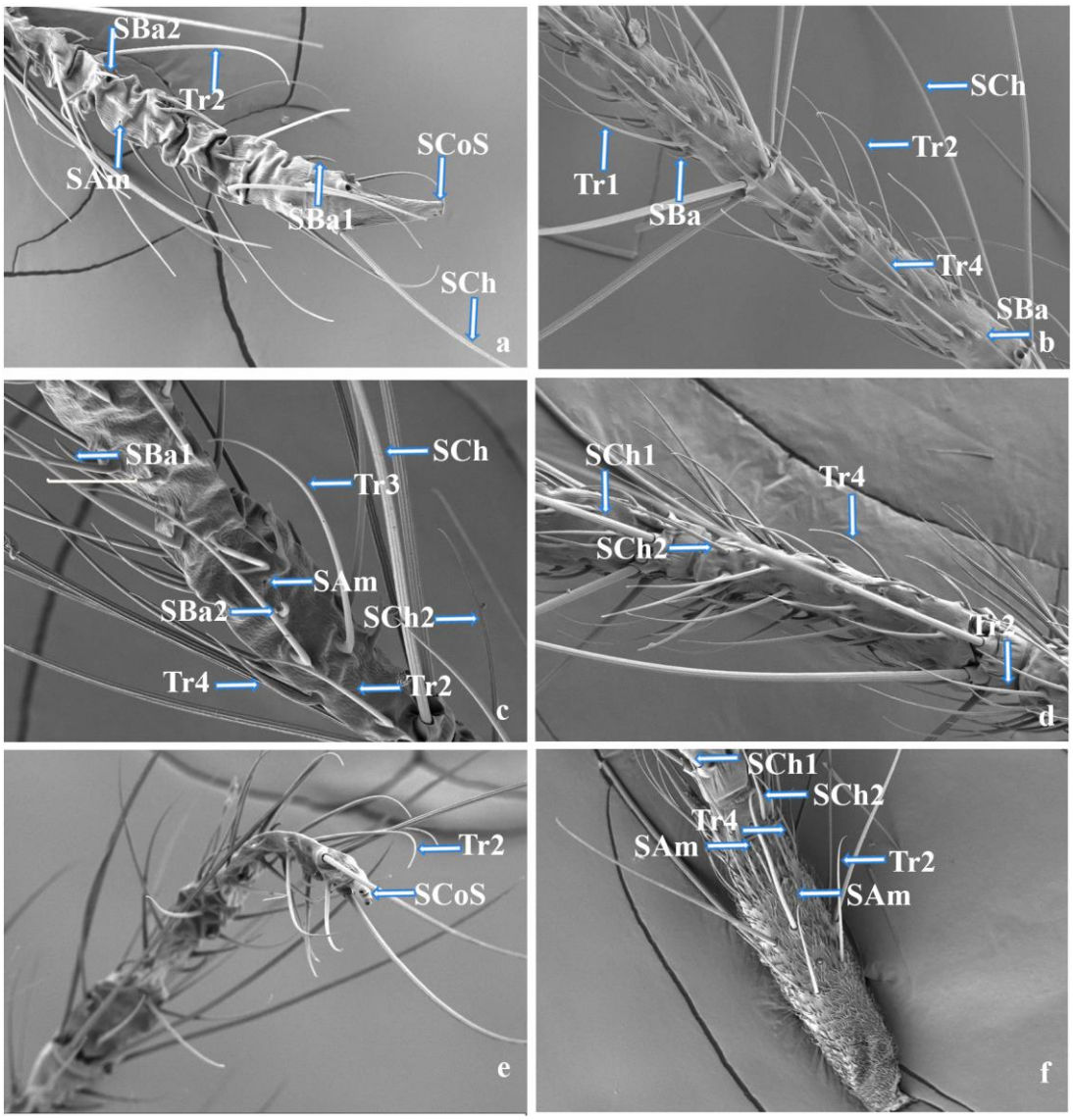

Figure 8.9: *Cx.tritaeniorhyncus*- Antennae (a,b,c) *Cx.fuscocephala*-Antennae-(d,e,f)

Sensilla Chaetica are long, rigid, hair-like structures set into a receptacle at the base and radially distributed in whorls along the flagellar margins. Regardless of difference in morphology or distribution, this type of sensilla was found in all sampled mosquito genera (Figs. 8.3 to 8.12). It has been reported that they are mechanoreceptive detectors (Hill et al., 2002). Greater number of Microtrichia were observed on the first flagellomere of all mosquitoes.

Sensilla Ampullacea, a thermo-hygro receptor sensilla with little olfactory role, was found in all genera. *Cx. quinquefasciatus* and *Ae. albopictus* have SAm

present very close to each other (Figs. 8.6b and 8.8e) while in all other species they are distally located (Figs. 8.3e, 8.6f, 8.9c, 8.9f, 8.10c, 8.10f, 8.11b, 8.11e, 8.12b and 8.12e).

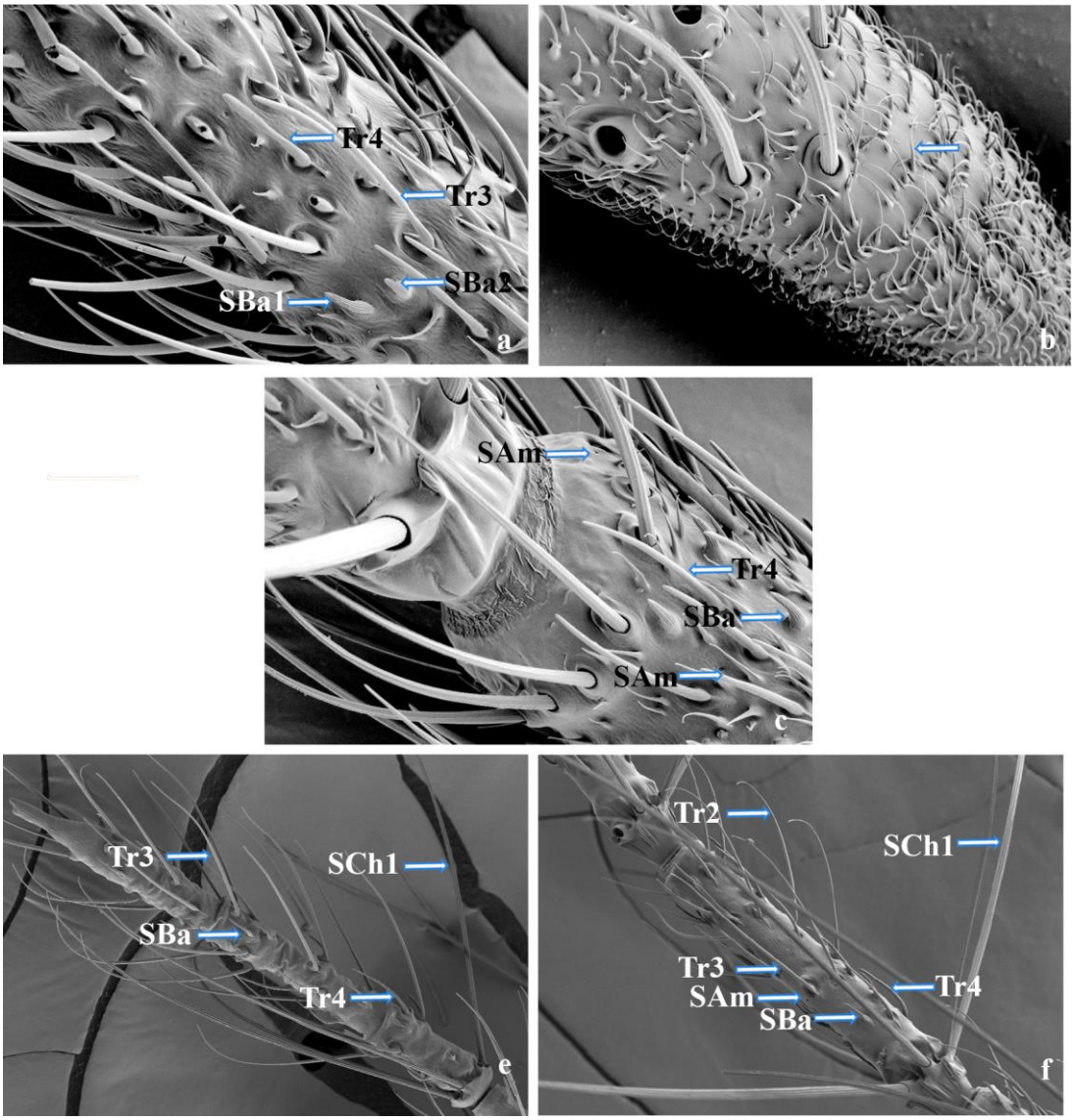

Figure.8.10: *Cx.vishnui*-Antennae (a,b,c) *Cx.whitemorei*- Antennae (d,e)

In all species studied under the five genera, small Sensilla Coeloconica (SCoS) was present only on the 13th flagellomere , except in the case of *An. barbirostris*, where the 1st flagellomere also possesses four small Sensilla Coeloconica in close proximity to one another (Fig. 8.3a). Large Sensilla

Coeloconica (SCoL) were found to be present in all species of A*nopheles* (Figs. 8.3e and 8.4 e & f) except for *An. barbirostris* (Figs. 8.3 a, b & c).

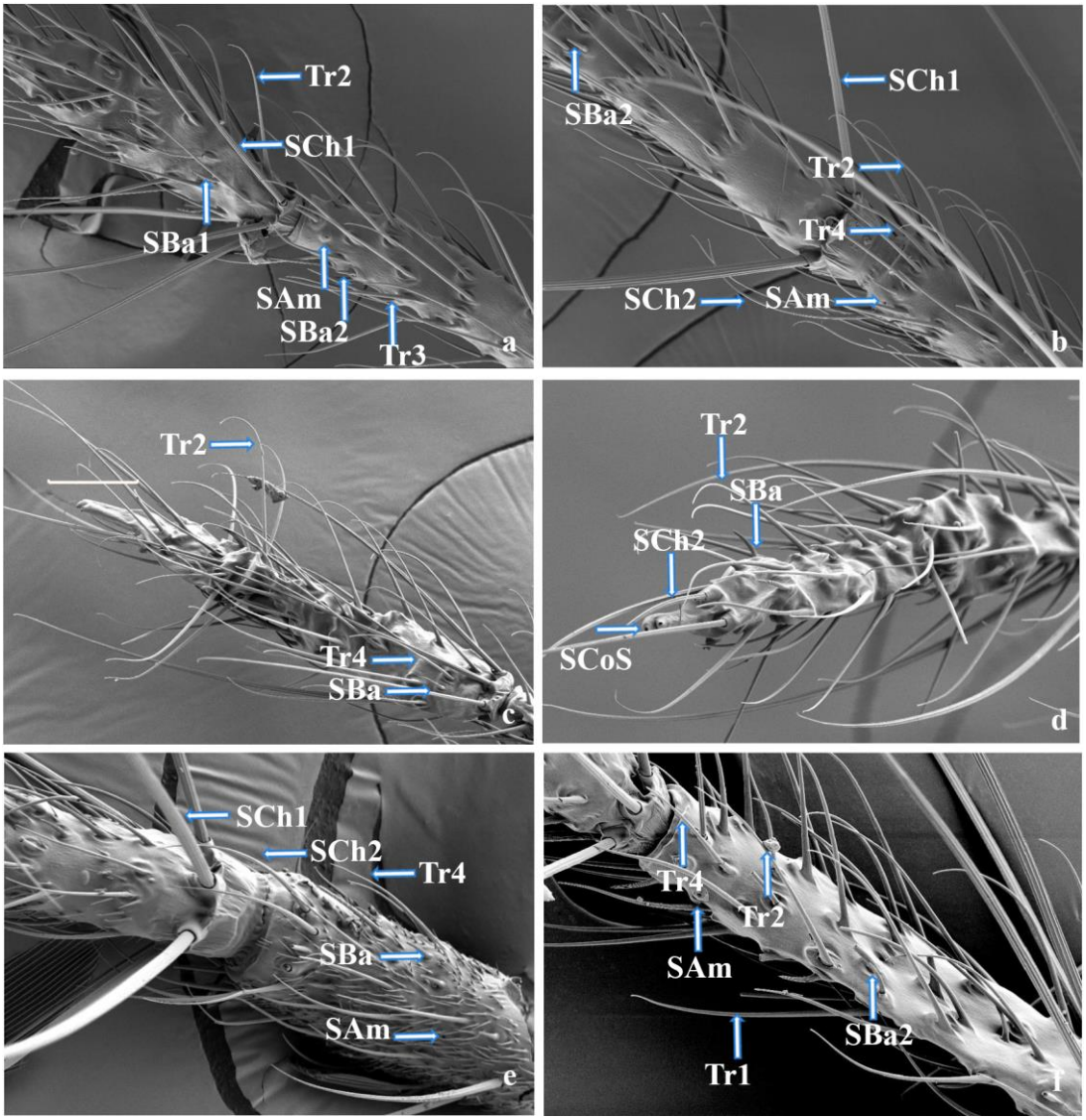

Figure 8.11: *Man.indiana* – Antennae (a,b,c) *Man.uniformis*-Antennae (d,e,f)

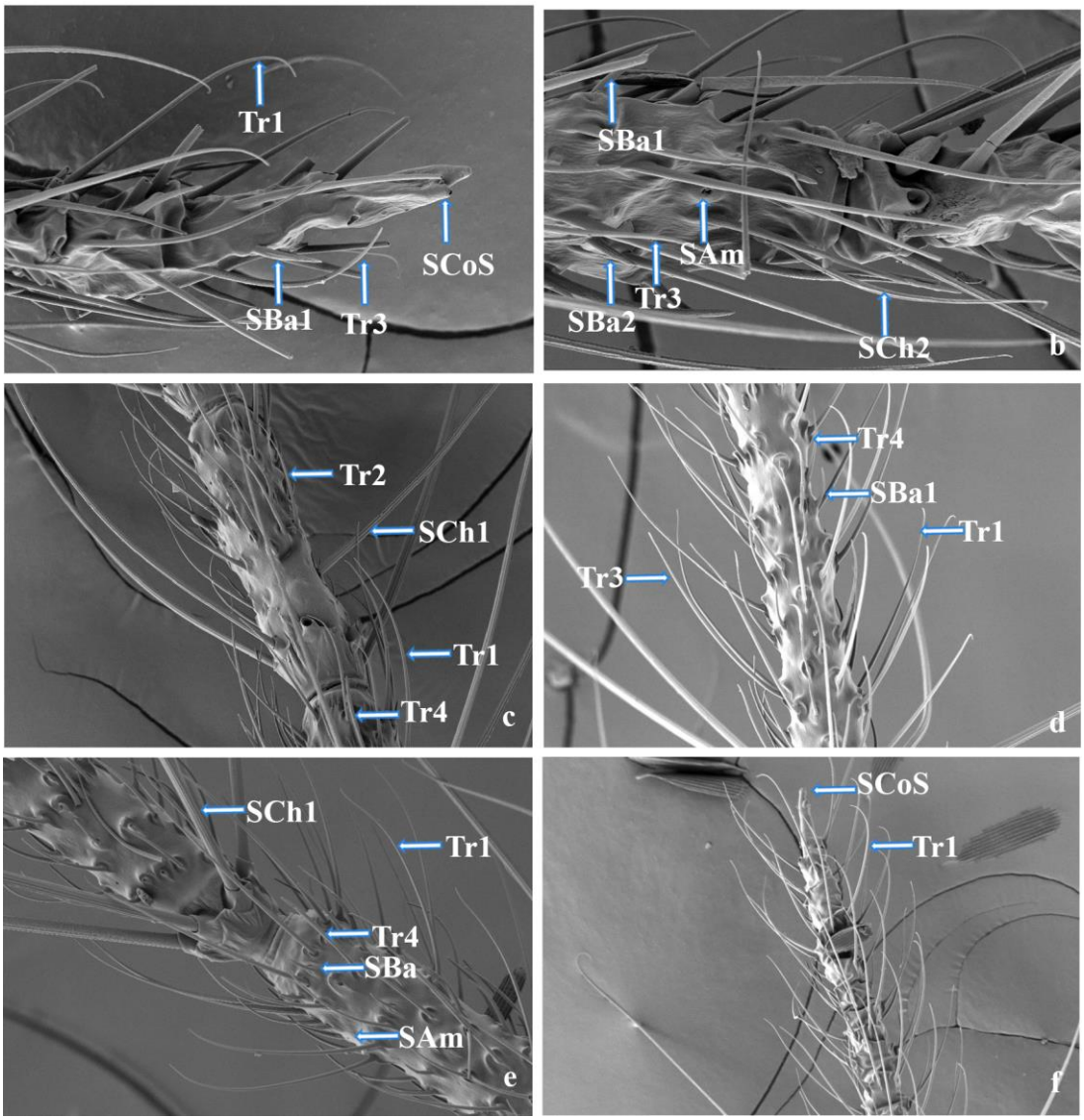

Figure 8.12: *Man.annulifera*- Antennae (a, b, c) *Ar.sabalbatus*-Ant (d, e, f)

8.4. Genus level variation of olfactory sensilla on the proboscis of mosquitoes

The proboscis of all blood feeding species under five genera exhibited pointed tip (Figs. 8.13 - 8.16). Sensilla Chaetica(SCh) was the predominant sensilla on the proboscis along with Squamiformia scales (SSq) and Microtrichia (SMt) Fig. 8.13c. Three types of chaetica, namely Type 1 (long and curved), Type 2 (long and straight) and Type 3 (short and curved) were also observed (Figs. 8.13a, c & d).

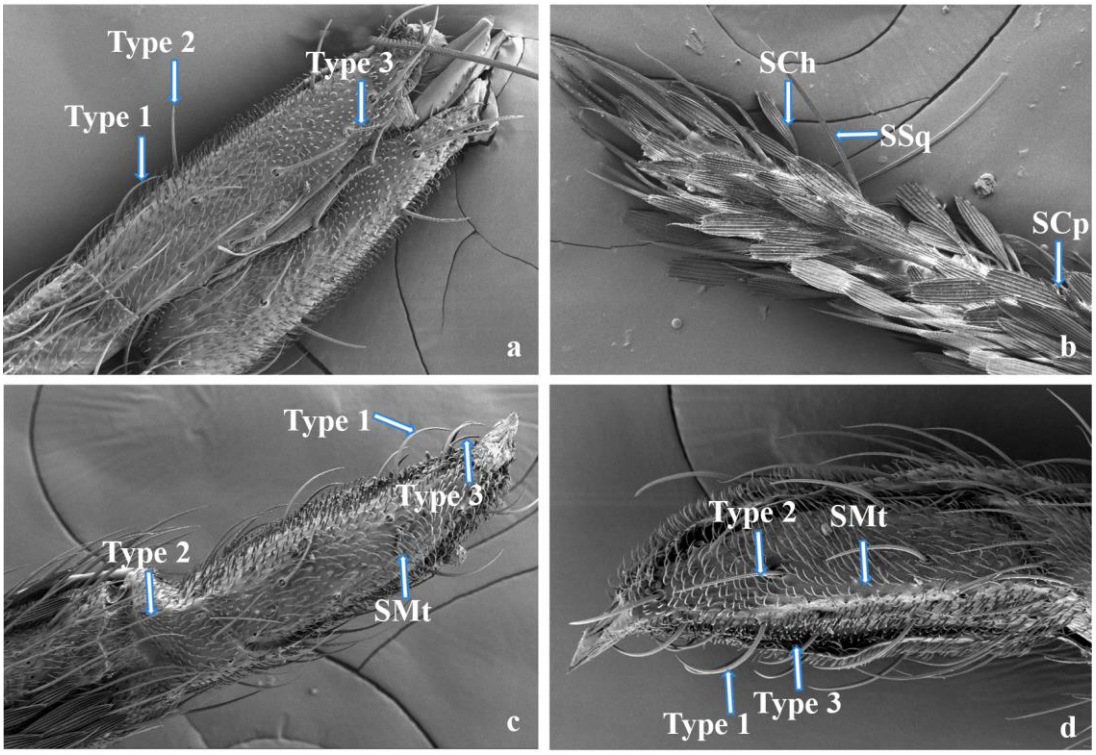

Figure 8.13: *An.stephensi*- a) Proboscis b) Palpi c) *An. barbirostris*-proboscis d) *An. culiciformis*- proboscis

Additionally, the length of the sensilla in the proboscis differed among genus. Compared to other genera, the number and size of SCh were very small in *Anopheles* (Figs. 8.13a, c). The length of Type 1 sensilla in this genus did not exceed the apex of the proboscis. In *An. stephensi* Type 1 sensilla are modest in size, and both Type 1 and Type 3 sensilla are few in numbers (Fig. 8.13a) and (Table 8.4).

Table 8.4: List of sensilla present on proboscis in different species of blood feeding mosquitoes subjected to analysis in the present study

Sl. No.	Sensilla	Aedes	Culex	Anopheles	Mansonia	Armigeres
1	Type 1 Chaetica	+	+	+	+	+
2	Type 2 Chaetica	+	+	+	+	+
3	Type 3 Chaetica	+	+	+	+	+
4	Squamiformia	+	+	+	+	+
5	Microtrichia	+	+	+	+	+

(+: Presence)

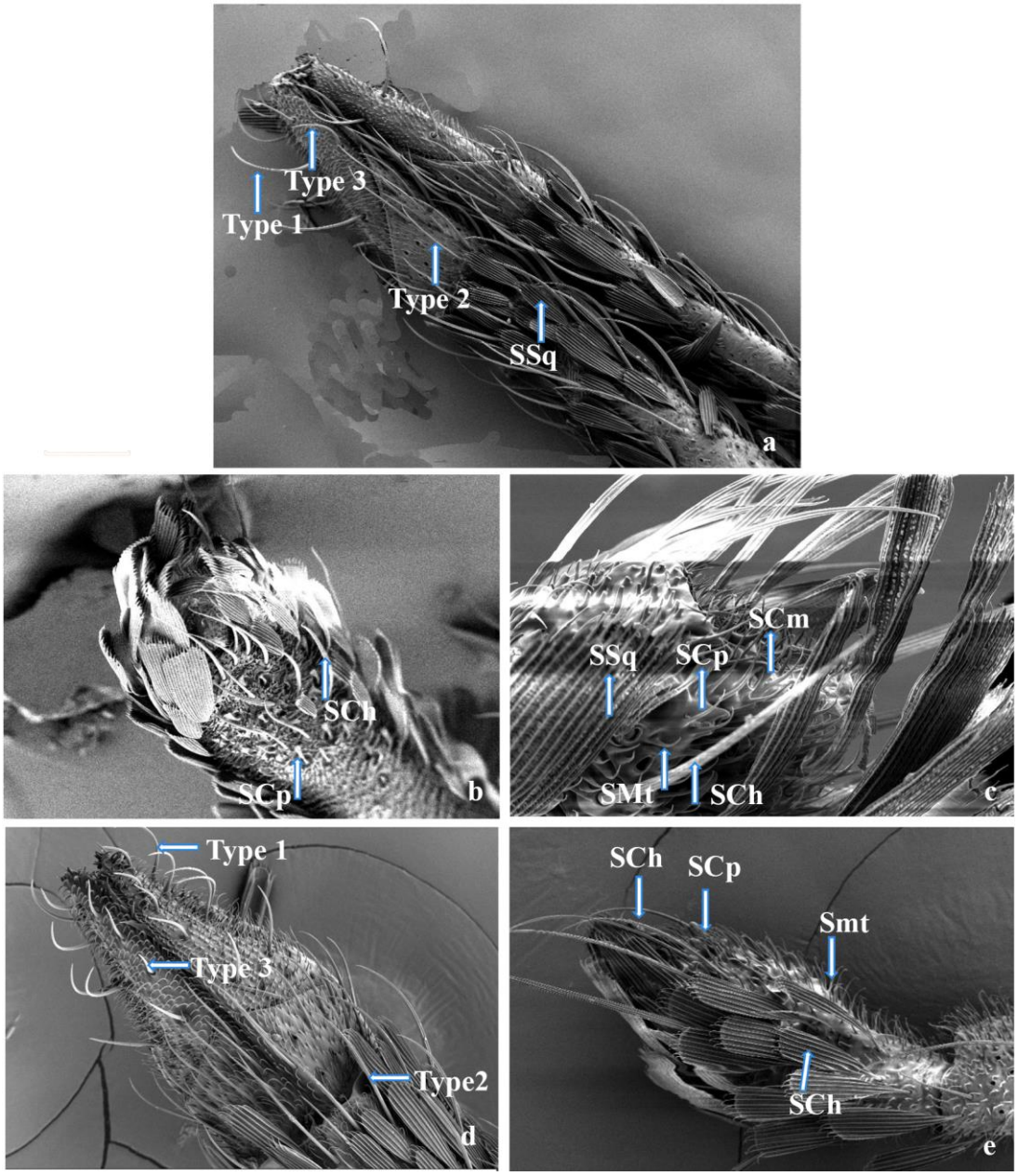

Figure 8.14: *Ae.vittatus*- a) Proboscis, b) palpi, c) Palpi *Ae.aegypti* d) Proboscis, e) palpi

Variation in shape of squamiformia scales could be observed in all species of the five genera. *Anopheles* was distinguished from all other genera by its slender, tapering Squamiformia (Fig. 8.13c). *Aedes* and *Culex* species have oval-shaped Squamiformia, while in *Culex* they were flatter than that of *Aedes* (Figs. 8.14 and 8.15). The genera *Armigeres* and *Mansonia* have flat squamiformia, and *Armigeres* has an ovoid distal end as well (Fig. 8.16).

8.5. Genus level variations of sensilla on the maxillary palp of mosquitoes

The general morphology of the maxillary palps varies with respect to species and sex. Male *Anopheles* have long, club-shaped maxillary palps, whereas females have slightly shorter, cylindrical palps. Males of *Aedes* and *Culex* have maxillary palps that curve upwards, whereas females have straight maxillary palps that are much shorter than the proboscis.

Maxillary palps possess chemosensory sensilla and mechanosensory filaments such as Sensilla Chaetica, Senilla Capitate pegs, Sensilla Campaniformia, and some non-innervated structures, like Microtrichia, making them olfactory and mechanosensory organs like antennae (McIver, 1971; McIver and Hudson, 1972) (Table 8.5).

Table 8.5: Distribution of sensilla on maxillary palp of of blood feeding mosquito species under the genera selected for the present study

Sl. No.	Sensilla	*Aedes*	*Culex*	*Anopheles*	*Mansonia*	*Armigeres*
1	Capitate peg	Few and small sized	Numerous	Very few	Very few and large sized	Few and finger shaped
2	Companiform	At the distal end	Proximal and distal end	Not observed	Not observed	3rd segment
3	Chaetica	At the proximal and distal end. Few	All segments. Numerous	All segments. Few	All segments, numerous	All segments. Numerous
4	Squamiformia	All segments and one side	All segments one side	All segments and in all sides	All segments, one side	All segments, one side
5	Microtrichia	Numerous and densely covered	Numerous and densely covered	Numerous and densely covered	Numerous and densely covered	Numerous and densely covered

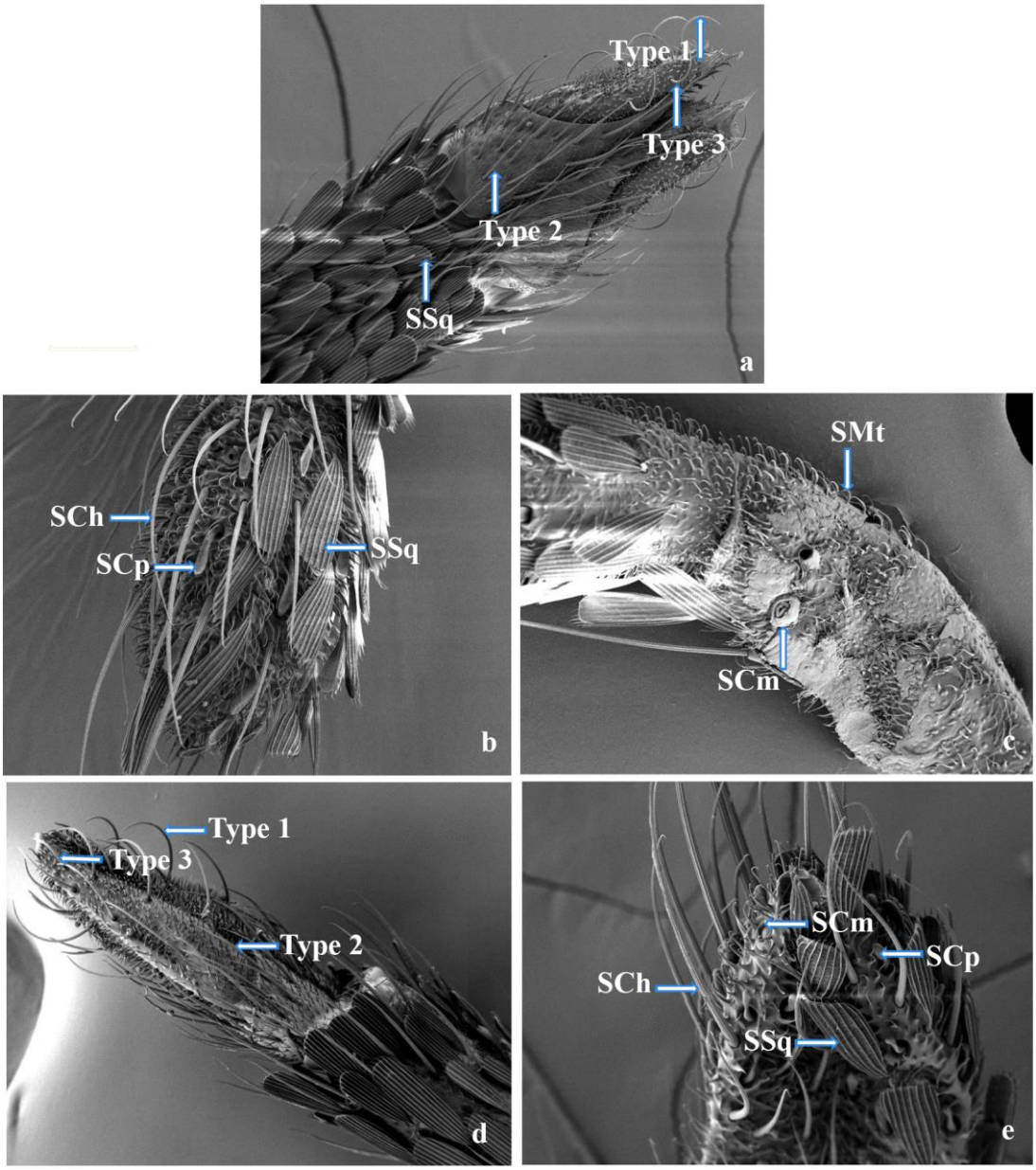

Figure 8.15: *Cx.tritaeniorhyncus* a) Proboscis, b) Palpi, c) palpi *Cx.quinquefasciatus*- d) Proboscis, e) Palpi

It is found that, in all species of blood feeding mosquitoes under study, the entire palps were densely covered by microtrichia (Figs. 8.14e, 8.15b,c; 8.16e). In all genera, except *Anopheles,* squamiformia (SSq) scales were found only on the dorsal and lateral sides (Figs. 8.14b, c; 8.15 b, e; 8.16 b & a) while in *Anopheles*, scales were found on all sides of palpi (Figs. 8.13b to 8.16). In the genera *Aedes* and *Anopheles,* Sensilla Chaetica (SCh) was found lesser in number (Figs. 8.14b,e & 8.14e), whereas chaetica is abundant in the other three genera viz., *Culex, Mansonia*

and *Armigeres* (Figs. 8.15b, e & 8.16b, c, e). In *Aedes*, SCh was only observed at the proximal and distal segments of the palpi while in other genera they were distributed on all palpal segments.

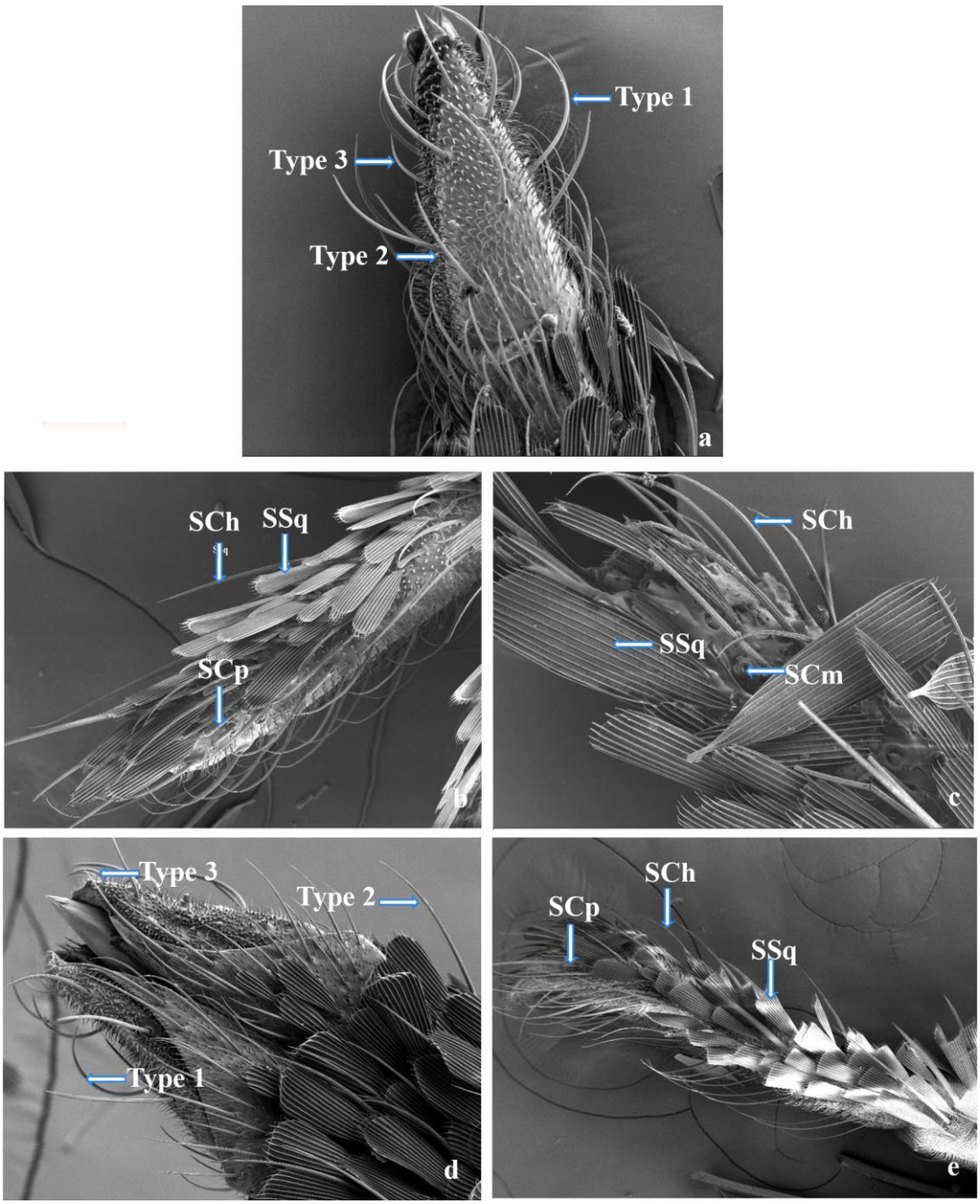

Figure 8.16: *Ar.sabalbatus* a) Proboscis, b) palpi, c) Palpi *Man.uniformis* d) proboscis, e) papli

Sensilla Companiformia (SCm) was not observed in *Anopheles* and *Mansonia* species in the present observation. Although it was present in all the other three genera, structural and positional differences were observed. It was seen distributed on the distal segment in *Ae. vittatus* (Fig. 8.14c) and *Cx. quinquefasciatus* (Fig. 8.15e), whereas on the proximal segment in *Cx. triteniorhyncus* (Fig. 8.15c).

In *Aedes*, *Culex*, *Armigeres*, and *Mansonia*, the capitate pegs were primarily found on the distal half of the ventral portion of 4th segment (Fig. 8.14b, c, e; to 8.15b, e; 8.16 b, e). This was consistent with the findings of Mclever (1972), on culicine. Capitate pegs (SCp) exhibited a unique structure in *Armigeres*, where it was a structure resembling a finger and was not located in a pit (Fig. 8.16b). In all the other genera studied, the SCp emerged from a pit, and its point was club-shaped (Figs. 8.13, 8.14 & 8.15). In *Mansonia*, only a small number of large-sized SCp were observed. In *Anopheles*, they were distributed among the SSq scales and were very few in numbers (Fig. 8.13b). Compared to all other genera, *Culex* had a greater number of Capitate pegs on distal segment (Figs. 8.15b and 8.15e).

8.6. Olfactory sensilla variation among blood feeding mosquitoes with host preference

Mosquitoes use five types of stimuli to locate hosts, namely CO_2, heat, body odour, water vapors, and visual indications with the help of Sensilla Capitate peg (SCp), Sensilla Coeloconica (SCo), Sensilla Ampullacea (SAm), Sensilla Trichoidea (STr), Sensilla Basiconica(SBa) and compound eyes respectively.

Among the five genera studied (*Aedes*, *Culex*, *Anopheles*, *Mansonia* and *Armigeres*), *Aedes* showed more preference to human blood than other hosts, while all other genera showed preference to all mammalian hosts under study viz., human, cattle, and pig. While comparing the sensilla of all the genera, it was noted that STr outnumbered all other sensilla in all species analysed. In *Aedes* species, more numbers of blunt trichoidea (STr3, STr4) were observed with varying length than smooth trichoidea (STr1, STr2). Whereas in all the other genera, smooth trichoidea (STr1, STr2) was abundant than blunt trichoidea (STr3, STr4). According to Hill et

al. (2002) majority of the sensilla are of this type and they are the principal drivers of various behaviours.

According to Steward et al. (1963), the number of STr and SBa varies from the proximal to the distal end of *Ae. aegypti*. At the proximal end, smooth trichoidea were scares, whereas their numbers increased at the distal end. Contrast is the case with blunt trichoidea, which was seen distributed greater at the proximal than the distal end. Like STr, SBa were more abundant at the distal end than at the proximal end, despite being extremely rare. Same pattern was observed in *Ae. albopictus* and *Ae. vittatus* (Fig. 8.6).

In the genus *Culex*, the arrangement and number of these three sensilla were identical. In *Armigeres* and *Mansonia*, there were greater blunt trichoidea (STr3, STr4) at the proximal end and fewer at the distal end, while there was a greater number of smooth trichoidea (STr1, STr2) at both the proximal and distal extremities. There was no evidence of gradual decrease or increase in number of STr in the mosquito species studied. Also, in the case of SBa, the distal end contains more numbers than the proximal end (Figs 8.11 to 8.12). Sensilla of the genus *Armigeres* have not been studied previously, making the present work as the pioneer study in this context. In the current study, *Armigeres* and *Mansonia* showed a general preference towards all the three mammalian hosts studied (humans, cattle, and pigs).

At the distal end of the Genus A*nopheles*, there were a greater number of smooth trichoidea (STr1, STr2) than at the proximal end. The length of smooth trichoidea, particularly STr1, is significantly shorter in the genus *Anopheles* compared to the other genera (Fig. 8.4). In *An. stephensi*, greater number of STr2 is present at the 13th segment (Fig. 8.4b). MClever (1982) elucidated the function of trichoidea, specifically that of STr1, as mediating the attractive odours emanating from human body. The presence of large numbers of smooth trichoidea at the distal end of the *Aedes* and *Culex* genera observed in the present investigation supports the above findings and explains its greater attraction towards human.

It was also found that grooved peg sensilla play an important role in host seeking behaviour in various mosquito species as it shows sensitivity to components of human perspiration, such as ammonia (Geier et al., 1999; Meijerink et al., 2001) and lactic acid (Davis et al., 1976). Gubler (2004) found that grooved pegs in *Ae. aegypti*, the principal vector of dengue, yellow fever, and other arboviruses around the community, had a high sensitivity to lactic acid. This finding highlights the significance of this type of sensilla in the processes of host seeking and disease transmission. It was discovered that the primary mosquito species responsible for human malaria, *An. gambiae*, has grooved pegs that are very similar to those of *Ae. aegypti* and other Culicines (Pitts and Zwiebel, 2006).

Grooved peg (SBa) was more abundant in the genus *Aedes* than in any other. All the three species of *Aedes* subjected to SEM analysis, *Ae. albopictus, Ae. aegypti*, and *Ae. vittatus*, possessed Sensilla Basiconica(SBa1 and SBa2), with SBa1 numerous than SBa2. *Cx. quinquefasciatus* preferred human over all other species within the genus *Culex*. And in this species, SBa1 was observed more frequently than SBa2. All mosquitoes with a preference for human blood exhibited the presence of SBa1. In the present study, *Ae. albopictus, Ae. aegypti, Ae. vittatus, Cx. quinquefasciatus, Cx. vishnui, Cx. pseudovishnui, Ar. sabalbatus, An. barbirostris, Man. indiana, Man. uniformis,* and *Man. annulera* were observed with preference towards human blood. All these species were found to possess SBa1 more prevalently than SBa2. This demonstrates the role of SBa1 in the human-seeking behaviour of all of these mosquito species. Variation in the length of grooved pegs in both sexes of *Culex* and *Aedes* were noted by MClever (1970, 1971). The longer forms of grooved pegs (SBa1) in these genera were found to be slender in shape. Seenivasagan et al. (2009) reported a significant variation in length and tip structure for grooved peg sensilla, indicating a possible difference in the odour molecule perception.

At the proximal end of the antenna some *Anopheles* species, specifically on the first and second flagellomeres, a greater number of elongated types of Sensilla Chaetica(SCh2) were observed (Fig. 8.4b). The mechanosensitive neurons in the

Sensilla Chaetica(SCh) may assist the female in orienting upwind towards air currents harbouring host-related stimuli. MClever (1982).

Genus level variation in the 13th flagellomere was also observed. It tapered into two terminal cones at its extremity. Small Sensilla Coeloconica (SCoS) originates from these cones. The structure, number, and pigmentation of these cones varied. The tip of antenna of *Ae. aegypti* (Fig. 8.5c) and *Ae. albopictus* (Fig. 8.6c) was not seen pigmented, whereas *Ae. vittatus* possess a pigmented circle at the apex (Fig. 8.6e). The cones were of two in number.

All species of the genus *Culex* studied – *Cx. quinquefasciatus* (Fig. 8.8d), *Cx. gelidus* (Fig. 8.7c), *Cx. pseudovishnui* (Fig. 8.8c), *Cx. tritaeniorhyncus* (Fig. 8.9a), and *Cx. fuscocephala* (Fig. 8.9e) –showed two unpigmented cones of equal length. In contrast, *Cx. whitemori* had one long cone and one short cone, both were unpigmented (Fig. 8.10d).

The genus *Armigeres* possessed three unpigmented cones. One was long and the other two were extremely short (Fig. 8.12f). The cones in the 13th flagellomere of all the three *Mansonia* species studied, namely *Man. indiana*, *Man. uniformis*, and *Man. annulifera*, were devoid of pigmentation. However, the size of the cones in *Man. annulifera* and *Man. uniformis* differs. *Man. indiana* has two cones of equal length (Fig. 8.11c) unlike the other. Anopheles exhibits variation in pigmentation and cone length. *An. barbirostris* and *An. stephensi* have unpigmented cones of which the *An. stephensi* had two cones of similar length (Fig. 8.4b), whereas *An. barbirostris* and *An. culiciformis* had cones of different lengths (Figs. 8.3c and 8.3d).

8.7. Male vz Female variation in Cephalic Sensilla

Two species of male mosquitoes namely *Ae.albopictus* and *An.stephensi* were subjected for SEM analysis. The two distal segments of Male antennae were observed with all the sensillae that were present in female except Sensilla Ampullacea. All the other segments were found with Sensilla Chaetica alone in whorls. Large Sensilla Coeloconica were observed in male antennae of *An.stephensi* similar to the female (Fig 8.17d).

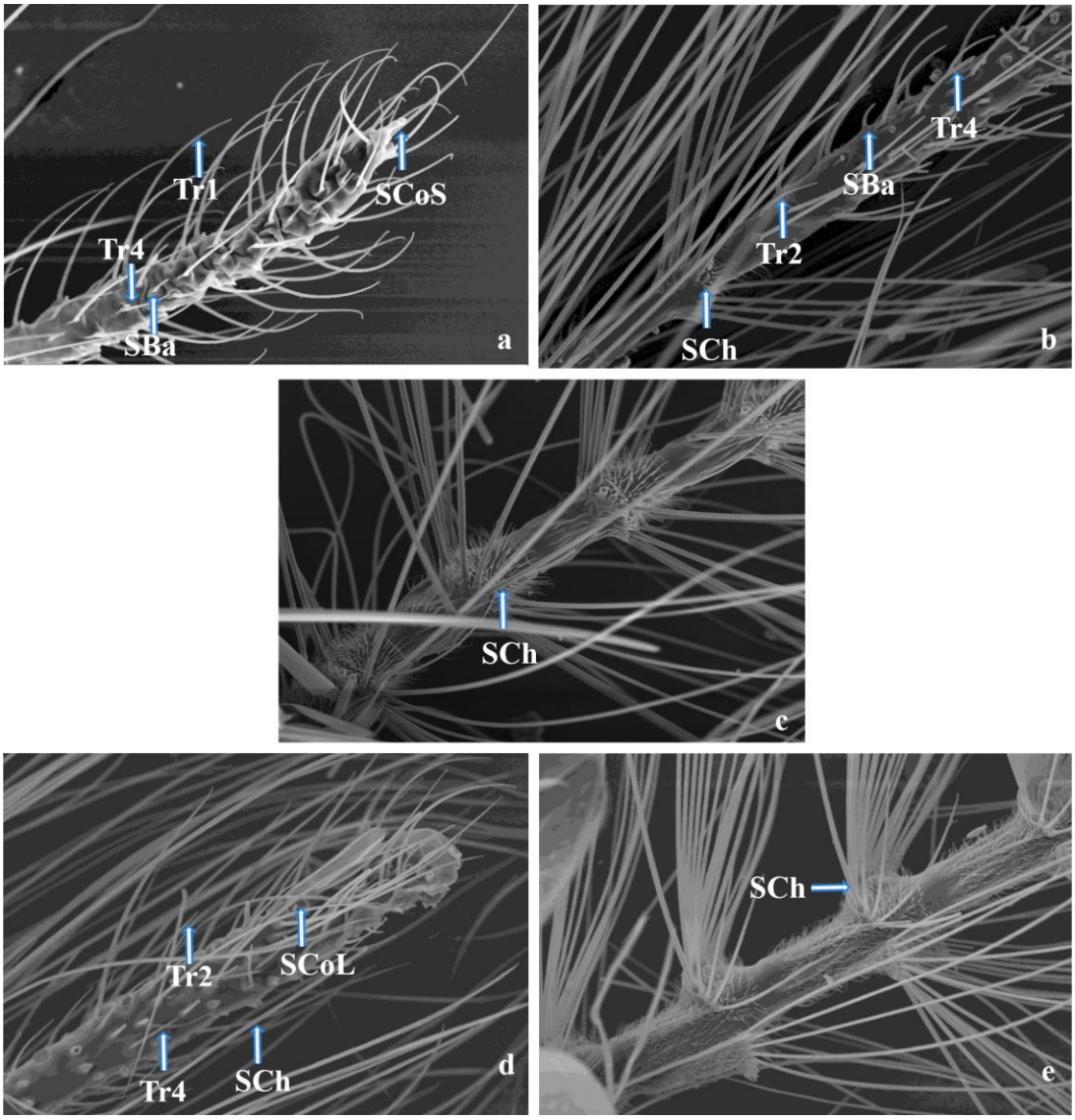

Figure 8.17: Antennae -*Ae.albopictus* male, (a,b,c) Antennae- *An.stephensi* male (d,e)

Numerous Microtrichea, Sensilla Squamiformia and both Type 2 and Type 3 Chaetica were observed in the proboscis of male mosquitoes similar to the female mosquitoes. Type 1 Chaetica were absent (Figs. 8.18 a,b)

The maxillary palps of male mosquitoes were found with Microtrichea, Sensilla Chaetica and Capitate peg. Sensilla Companiformia couldn't be observed in male mosquitoes which were analysed (Figs. 8.18c,d,e).

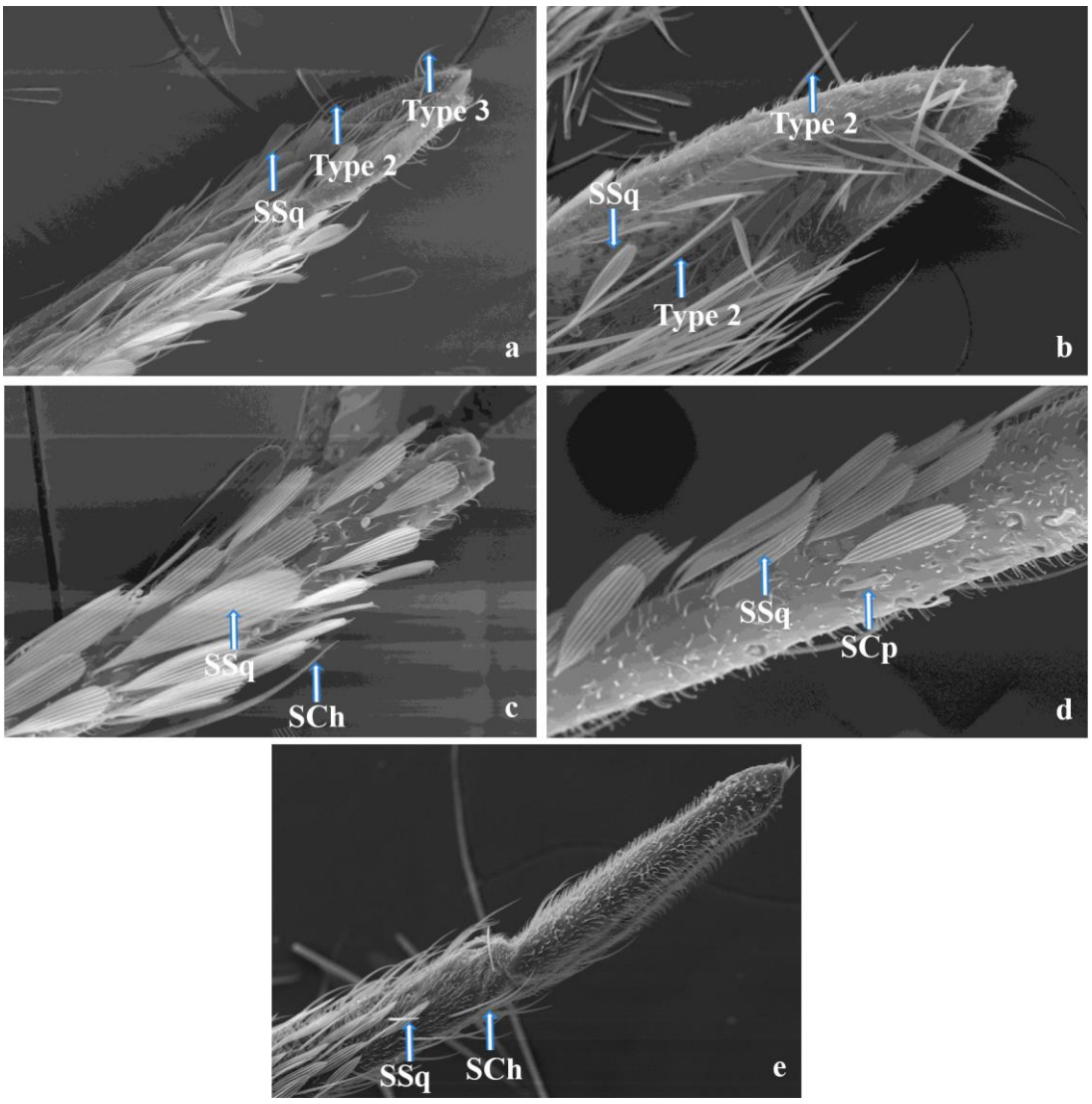

Figure. 8.18: a) Proboscis -*An.stephensi* male, b) Proboscis - *Ae.albopictus* male c) Palpi - *Ae.albopictus* male, d) Palpi - *Ae.albopictus* male, e) palpi - *An.stephensi* male.

8.8. Variation in the olfactory sensilla between blood-feeding and non-blood-feeding mosquitoes

One species of non-blood-feeding mosquito (*Malaya genurostris*) and 19 species of blood-feeding mosquitoes (described elsewhere) were subjected to SEM analysis in order to explain the possible differences in the presence and distribution of olfactory sensilla between the blood feeding and non-blood feeding mosquitoes.

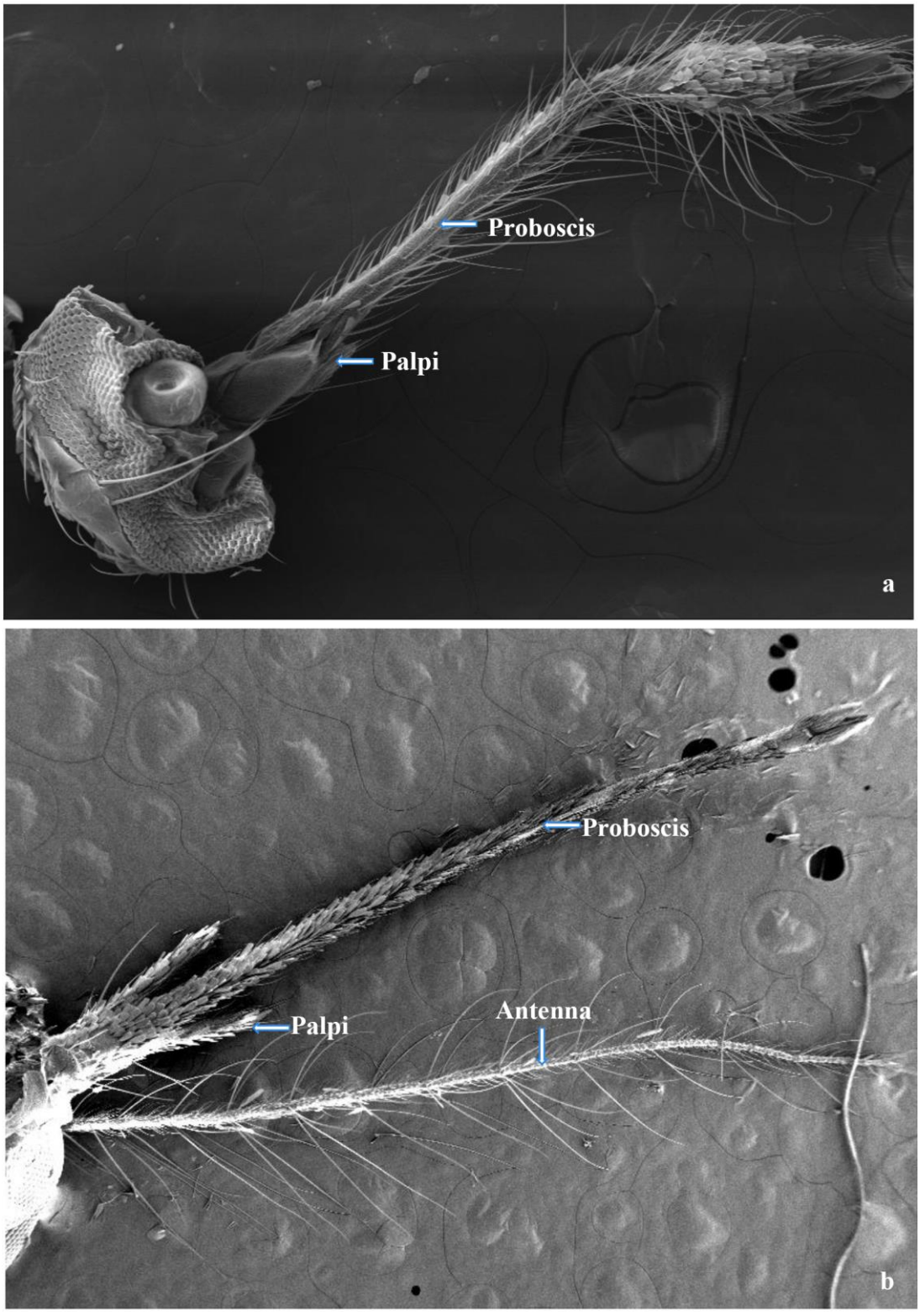

Figure 8.19: a) Proboscis and Palpi of *Mal.genurostris* b) Proboscis, Palpi and Antennae of *Ar.sabalbatus*

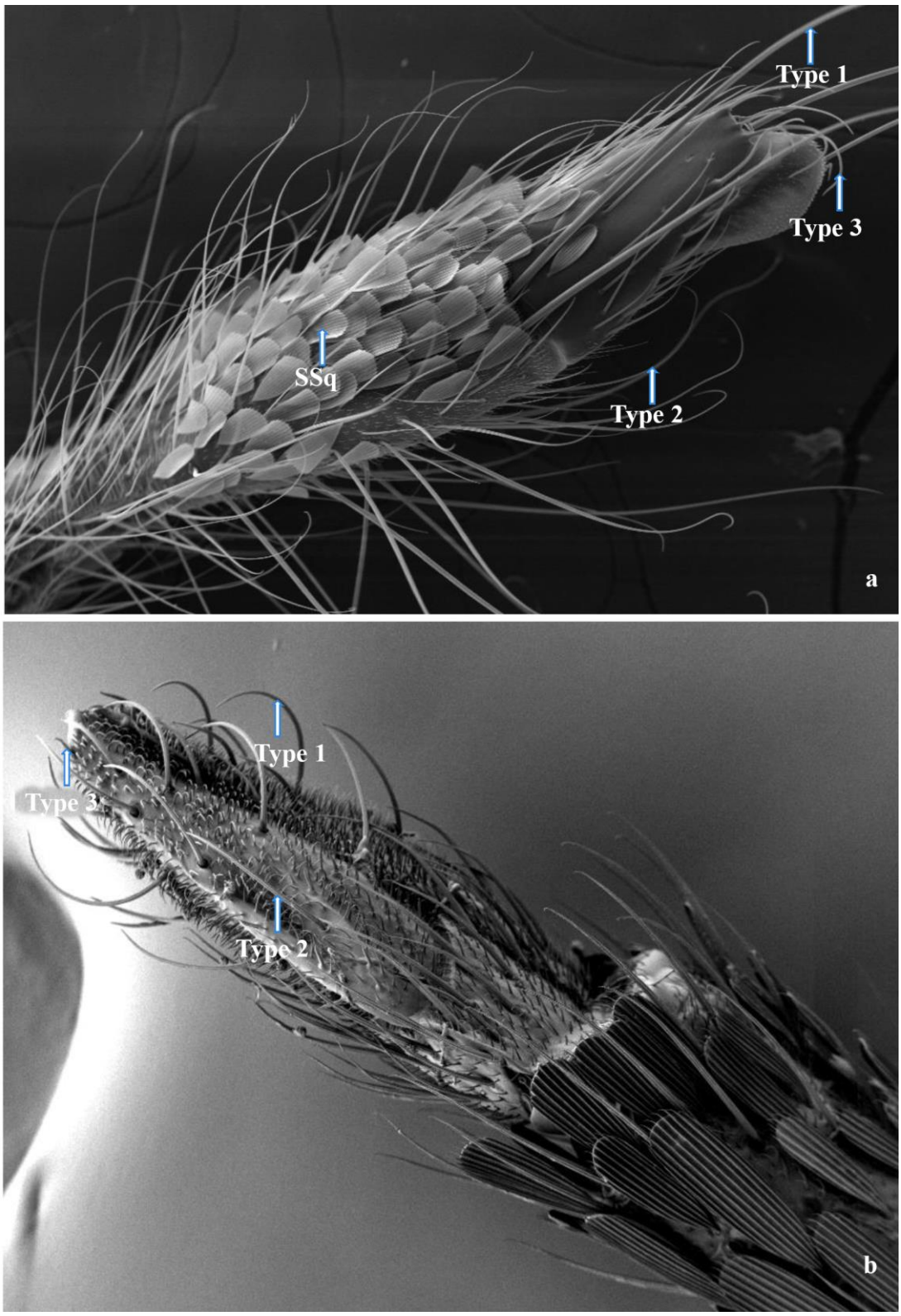

Figure 8.20: a) Proboscis (*Mal.genurostris*) b) Proboscis (*Cx.quinquefasciatus*)

The antennae of blood-feeding mosquitoes possessed all the five olfactory sensilla as explained earlier – Chaetica (SCh), Trichoidea (STr), Basiconica (SBa), Ampullacea (Sam), and Small Coeloconica (SCoS) – whereas in *Mal. genurostris* SAm and SCoS were not observed. Furthermore, *Mal. genurostris* had fewer number of SBa when compared to that of the other blood feeding species. *Mal. genurostris* lacked SCoS on its 13th flagellomere, whereas SCoS was invariably present on the 13th flagellomere of all other blood-feeding mosquitoes, regardless of genus level variation. Microtrichia, which was only seen on the proximal segment of the antennae of blood-feeding mosquitoes, thickly covered the whole flagellomeres in *Mal. genurostris*. *Mal. genurostris*, a mosquito that does not feed on blood, had a significantly low number of STr compared to blood-feeding mosquitoes (Fig. 8.21).

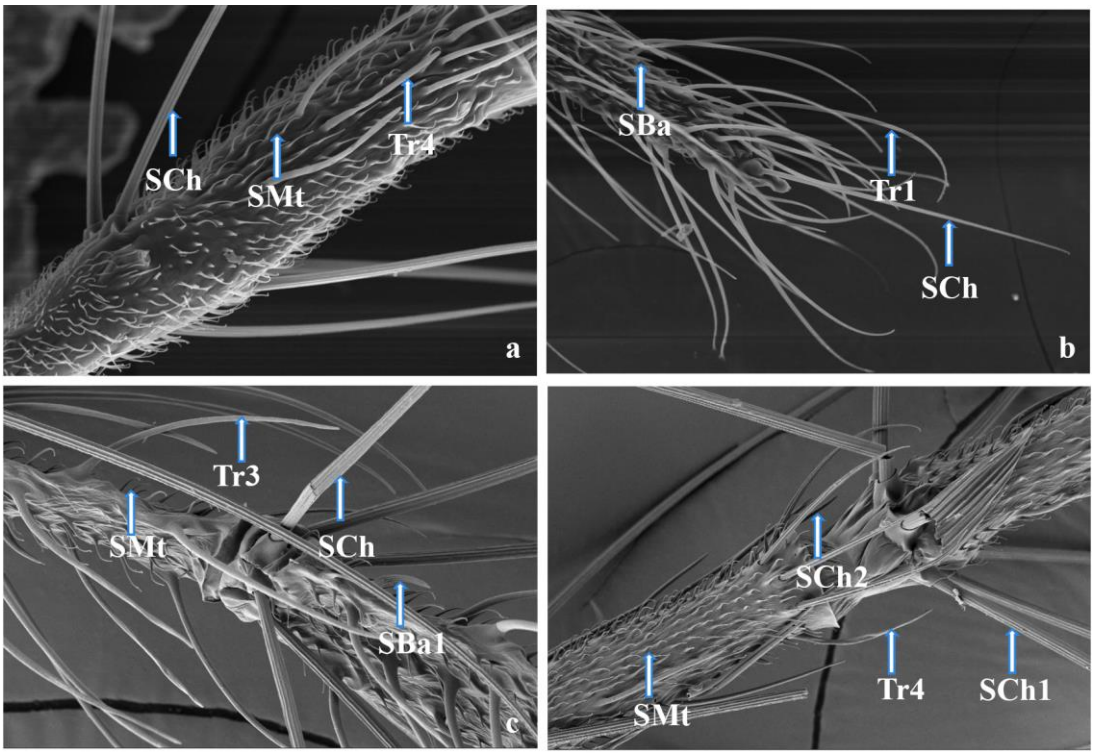

Figure 8.21: Antennae (*Mal.genurostris*)

Blood-feeding and non-blood-feeding mosquitoes exhibited substantially different structure for their proboscis (Fig. 8.19). The proboscis of *Mal. genurostris* had a blunt end, while it was pointed at the tip in the blood-feeding mosquito species. In comparison to *Mal. genurostris*, which had very few Squamiformia (SSq) present

on one side of the proboscis and on all sides at the swollen part of its tip, the SSq found densely covered the proboscis from base to tip invariably in blood-feeding mosquitoes (Figs. 8.19a and 8.20a). When present, the SSq were small sized in *Mal. genuristris* compared to other blood-feeding species (Fig. 8.20a). The difference in the morphological appearance of cephalic structures in blood-feeding and non-blood-feeding mosquitoes were given in Table 8.6.

Table 8.6: Comparison on the morphological appearance of cephalic structures in blood-feeding and non-blood-feeding mosquitoes.

Sl. No.	Structure	Blood feeding	Non-Blood feeding
1	Antennae	Feathery in male, slender in female	Slender in both male and female
2	Proboscis	More hairy and swollen at the tip	Less hairy and pointed at the tip
3	Palpi	Well developed with five segments	Very small, unsegmented, and attached with the proboscis

All three types of Sensilla Chaetica(Type1, Type2 and Type3) were present on the proboscis of both blood feeding and non-blood feeding mosquitoes. The entire proboscis was found to be densely covered with SCh from bottom to the tip in *Mal. genurostris* whereas in blood feeding mosquitoes SCh were very less in numbers (Fig. 8.20). In *Mal. genurostris*. four long Type 1 sensillae were present at the tip of the proboscis and this type of arrangement was not observed in any of the blood feeding mosquitoes under the present study (Fig. 8.22a). Sensilla Squamiformia (SSq) were the major and abundant mechanoreceptor sensilla in the blood feeding mosquitoes whereas in non-blood feeding mosquito, the SCh was found to act as the predominant mechanoreceptor. On the antennae and palpi of *Mal. genusrostris*, Microtrichia (SMt) were numerous, in contrast, sparsely distributed on the proboscis. The proboscis and palpi of blood-feeding mosquitoes had higher numbers of SMt, whereas on their antennae, it was seen distributed only on the proximal segment (Figs. 8.19 to 8.22).

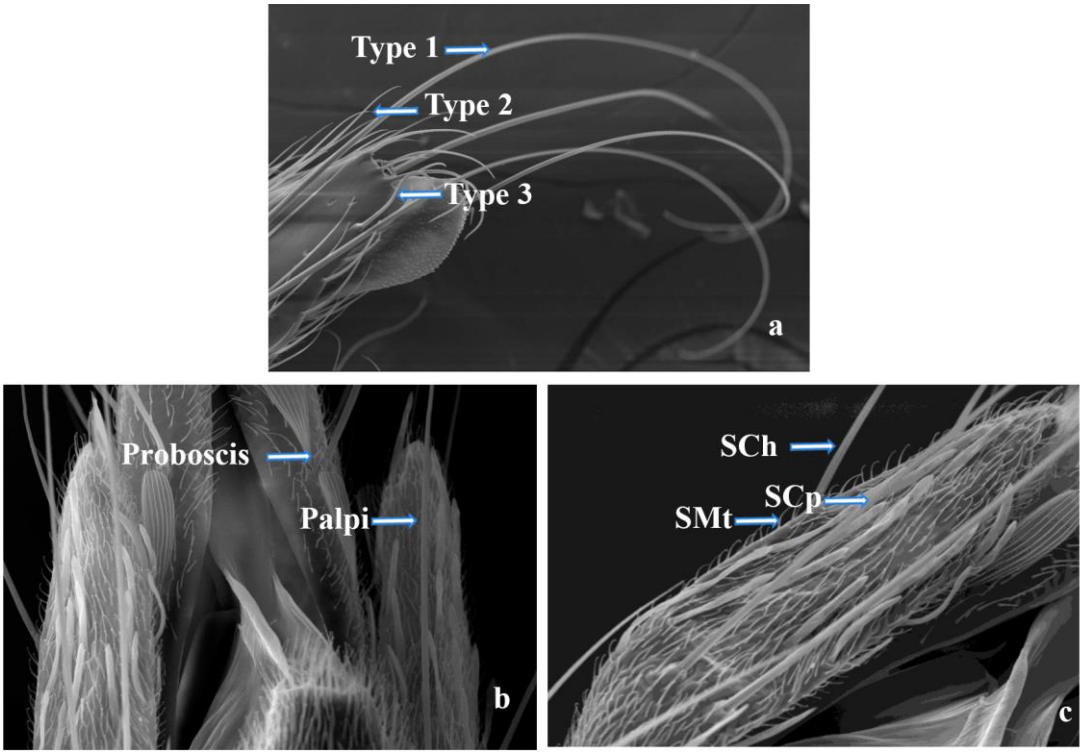

Figure 8.22: *Mal.genurostris* a) Proboscis, b) Palpi , c) Palpi

The palpi of blood feeding mosquitoes were seen well developed with five segments, whereas that of non-blood feeding mosquitoes were poorly developed, small, unsegmented, and was found attached with the proboscis (Fig. 8.19 and 8.22b). In both blood feeding and non-blood feeding mosquitoes palpi were completely covered with SMt and capitate peg (SCp). The shape of SCp was very unique in *Mal. genurostris* and it resembled the short blunt trichoidea (STr4) of the antennae (Fig. 8.22c). Club shaped SCp was observed generally in blood feeding mosquitoes. Sensilla Companiformia (SCm) were absent in *Mal. genurostris* but in all blood feeding mosquitoes it was present. Compared with the blood feeding mosquitoes SSq were very few in number in *Mal. genurostris.*

As SCo and SAm are responsible for thermoreception, these sensilla were completely absent in non-blood feeding mosquitoes where they feed only plant sap. SCp were plenty in numbers in non-blood feeding *Mal. genurostris*, as these sensilla are very much essential for CO_2 detection. Among the mechanoreceptors, SCh and

SMt are well developed and numerous than SSq and SCm in the non-blood feeding *Mal. genurostris*.

The difference in the distribution of various sensilla on the cephalic structures in blood-feeding and non-blood-feeding mosquitoes were given in Table 8.7.

Table 8.7: Comparison on the distribution of various sensilla on the cephalic structures in blood-feeding and non-blood-feeding mosquitoes.

Cephalic appendage	Type of Sensilla	Blood-feeding species	Non-blood-feeding species
Antenna	Trichoidea (STr)	Numerous	Very few
	Basiconica (SBa)	Numerous	Very few
	Ampullacea (SAm)	Numerous	Absent
	Coeloconica (SCo)	Present	Absent
	Chaetica (SCh)	Numerous	Numerous
	Squamiformia (SSq)	Numerous	Less in number
	Microtrichia (SMt)	Only on proximal flagellomere	Densely covers all flagellomeres
Proboscis	Chaetica (SCh)	Few, No Type 1 on the tip	Abundant, Four long Type 1 on the tip
	Squamiformia (SSq)	Large sized, densely covered from base to tip	Small, few on one side and swollen tip
	Microtrichia (SMt)	Greatly distributed	One or two in numbers
Maxillary palp	Capitate peg (SCp)	Poorly covered with, Club shaped	Completely covered with, Blunt type
	Microtrichia (SMt)	Greatly distributed	Greatly distributed
	Companiform (SCm)	Moderately present	Absent
	Squamiformia (SSq)	Numerous	Few in number

Conclusion

The genus *Aedes*, which showed more preferences towards human blood, has some peculiarities with the olfactory sensilla. It has more blunt trichoidea (STr3, STr4) than any other genera. As established, STr bear some relationship with blood feeding behaviour and the presence and abundance of STr in Aedes might have

specifically developed their elevated affinity towards human host. A pair of Sensilla Ampullacea (SAm) present in this genus was seen very closely located to each other. The palpi also remain distinct in having the Sensilla Chaetica(SCh) only at the proximal and distal end while all other genera have it in all segments.

The similarities in the sensilla of genera *Aedes* and *Culex* also were noteworthy. Presence of a greater number of Sensilla Basiconica(SBa) and large number of STr at the distal end was seen in both the genera. The Sensilla Squamiformia (SSq) of both the genera were oval in shape whereas in other genera it varied in shape. Presence of Sensilla Companiformia (SCm) at the distal end in both the genera also is remarkable. Both genera showed some similiarity in Phylogenetic tree as well as they shared the same clade.

The genus *Armigeres*, which has affinity for all three types of mammalian hosts observed, *viz* human, cattle and pig, has some peculiarities with their sensilla. The cones at 13th flagellomere were three in number compared to two in all other genera. The shape of capitate peg (SCp) on the maxillary palpi also remained distinct as it had finger shape and not located in sockets like other genera.

The members under family Anophelinae differed from Culicinae in the following characters of cephalic sensilla: (i) The large Sensilla Coeloconica (SCoL) was present only in Anophelinae, (ii) The length of Sensilla Chaetica(SCh) on proboscis was small and did not extend beyond the tip of the proboscis like that of Culicines, and (iii) The sensilla squamiformia (SSq) was present on all sides of the maxillary palpi.

There remains a lot of ambiguity about the connection between the olfactory sensilla of mosquitoes and their ability to locate hosts. There were some link observed between the occurrence and distribution of sensillae in mosquitoes and their host preference. This is likely because mosquitoes are opportunistic consumers. Blood is consumed in proportion to the number and frequency of hosts that are available in their habitat. The structural differences found in the antennae of mosquitoes were in tune with their taxonomic (genus and species) status as revealed during the present study. On the other hand, these distinctions might more accurately

represent phylogeny than behaviour or vectoral status. It was evident in the Phylogeny tree as non blood feeding *Mal.genurostris* formed a separate clade.

An. gambiae, a mosquito that feeds on humans and *An. quadriannulatus*, a mosquito that generally feeds on other mammals carry the same morphological type of sensilla on their antennae, and the densities of each type are effectively equal between the two species (Jason et al., 2006). Therefore, the absence of specialization at the gross morphological level of the antennae means that other factors are more likely to account for the olfactory-driven host choice difference between *An. gambiae* and *An. quadriannulatus*. The present study initiated a pilot investigation for future neurological, physiological, and genetic comparative studies that will hopefully shed light on any potential changes in olfactory perception that might exist among mosquitoes. It is clear from the analysis that, even though some morphological differences in sensilla could be observed with respect to difference in mammalian host, preferences of mosquitoes to hosts do not completely rely on morphological characters of their cephalic sensilla only. Instead, in the future, much more detailed analysis of the sensillae will have to be done in order to determine whether there is a definite correlation between the host preference and the distribution of sensilla.

CONCLUSION

- The present study (2019-2022) from Mananthavady Taluk of Wayanad, Kerala, reported 80 mosquitoes from 12 genera. This constitute 62% of the total mosquitoes reported so far from Kerala.
- 2 genera namely *Malaya* and *Lutzia* are new reports to Kerala.
- 29 medically important mosquito species from 5 genera and 50 non medically important mosquitoes species from 7 genera were reported.
- The current study contributes 21 new records from Kerala and 2 new records (*Uranotaenia* sp.) to the taxonomy of mosquitoes.
- 35 mosquito species coming under 7 genera were barcoded using marker genes and were submitted to NCBI for worldwide accession with respective accession numbers of which 20 species are vectors.
- Molecular phylogeny of subfamilies Anophelinae and Culicinae were discussed with the construction of Maximum likelihood tree.
- 44 species (29 vector species and 15 non vector species) of mosquitoes from 5 genera namely *Aedes, Culex, Anopheles, Armigeres* and *Mansonia* were chosen for blood meal analysis, and their host preferences were discussed.
- *Armigeres* and *Mansonia*, have identical preferences for blood meals on all three hosts viz., human, cattle, and pig.
- *Anopheles* and *Culex* showed an affinity for both cattle and human, a stronger preference could be observed towards cattle blood.
- *Aedes* stayed distinct because of its greater preference for human blood

- *Ae.albopictus, Ae.aegypti, Ae.vittatus, Man.annulifera,* and *Cx. quinquefasciatus* were the mosquitoes that preferred exclusively human blood in the current study.

- More number of mosquitoes with human blood were collected from the urban area, ie., Manathavady town, where the density of the population is rich than the rural area.

- More mosquitoes were observed both in abundance and diversity where human dwellings and animals coexist.

- The structural analysis of the cephalic sensilla of 19 blood feeding female mosquitoes and one non blood feeding female mosquitoe (*Mal.genurostris*) were discussed along with their host preferences.

- Mosquitoes, which has more preferences towards human blood, has more blunt trichoidea (Tr3, Tr4) than any species and pair of Sensilla Ampullacea (SAm) were seen very closely located to each other.

- The genus *Armigeres*, which has affinity for all three types of mammalian hosts observed, viz., human, cattle and pig, has some peculiarities with their sensilla. The cones at 13^{th} flagellomere were three in number which was two in number in all other genera. The shape of capitate peg (SCp) on the maxillary palpi also remain distinct as it has finger shape and not located in sockets like other genera.

- Great differences in the sensillae of antennae, proboscis and palpi of non blood feeding *Mal.genurostris* from all other blood feeding mosquitoes were observed.

RECOMMENDATIONS

- Mosquito preferences towards their hosts are not solely determined by the morphological features of their cephalic sensilla. Rather, to ascertain whether the distribution of sensilla and host preference are correlated, a far more thorough examination of the sensillae will need to be conducted in the future. Therefore, comparative research on the neurological, physiological, and genetic aspects of mosquito antenna sensilla are need to be conducted.

- Virological studies of the mosquito vectors of the study area have to be conducted.

- Taxonomic confirmation of the new species *Uranotaenia* sp. with the associated specimens from the study area as well as elaborate field search for the collection of more members are to be targeted.

Annexure

Classification of the Tribe Aedinii

Sl. No.	Phylogenetic Classification (Genus)	Traditional Classification	
		Genus	Subgenus
1.	*Aedes*	*Aedes*	Aedes
2.	*Abraedes*		Abraedes
3.	*Acartomyia*		Acartomyia
4.	*Aedimorphus*		Aedimorphus
5.	*Alanstonea*		Alanstonea
6.	*Albuginosus*		Albuginosus
7.	*Ayurakitia*		Ayurakitia
8.	*Aztecades*		Aztecades
9.	*Belkinius*		Belkinius
10.	*Bifidistylus*		Bifidistylus
11.	*Borichinda*		Borichinda
12.	*Bothaella*		Bothaella
13.	*Bruceharrisonius*		Bruceharrisonius
14.	*Cancraedes*		Cancraedes
15.	*Catageiomyia*		Catageiomyia
16.	*Catassomyia*		Catassomyia
17.	*Christophersiomyia*		Christophersiomyia
18.	*Collessius*		Collessius
19.	*Cornetius*		Cornetius
20.	*Dahliana*		Dahliana
21.	*Danielsia*		Danielsia
22.	*Dendroskusea*		Dendroskusea
23.	*Diceromyia*		Diceromyia
24.	*Downsiomyia*		Downsiomyia
25.	*Dobrotworskyius*		Dobrotworskyius
26.	*Edwardsaedes*		Edwardsaedes
27.	*Elpeytonius*		Elpeytonius
28.	*Finlaya*		Finlaya
29.	*Fredwardsius*		Fredwardsius
30.	*Georgecraigius*		Georgecraigius
31.	*Geoskusea*		Geoskusea
32.	*Gilesius*		Gilesius
33.	*Gymnometopa*		Gymnometopa

34.	*Halaedes*		Halaedes
35.	*Himalaius*		Himalaius
36.	*Hopkinsius*		Hopkinsius
37.	*Howardina*		Howardina
38.	*Huaedes*		Huaedes
39.	*Hulecoeteomyia*		Hulecoeteomyia
40.	*Indusius*		Indusius
41.	*Isoaedes*		Isoaedes
42.	*Jarnellius*		Jarnellius
43.	*jihlienius*		jihlienius
44.	*Kenknightia*		Kenknightia
45.	*kompia*		kompia
46.	*Leptosomatomyia*		Leptosomatomyia
47.	*Levua*		Levua
48.	*Lewnielsenius*		Lewnielsenius
49.	*Lorrainea*		Lorrainea
50.	*Luius*		Luius
51.	*macleaya*		macleaya
52.	*Molpemyia*		Molpemyia
53.	*mucidus*		mucidus
54.	*Neomelaniconion*		Neomelaniconion
55.	*Nyctomyia*		Nyctomyia
56.	*Ochlerotatus*		Ochlerotatus
57.	*Paraedes*		Paraedes
58.	*Patmarksia*		Patmarksia
59.	*Petermattinglysius*		Petermattinglysius
60.	*Phagomyia*		Phagomyia
61.	*Polyleptiomyia*		Polyleptiomyia
62.	*Pseudarmigeres*		Pseudarmigeres
63.	*Rampamyia*		Rampamyia
64.	*Rhinoskusea*		Rhinoskusea
65.	*Sallumia*		Sallumia
66.	*Scutomyia*		Scutomyia
67.	*Skusea*		Skusea
68.	*Stegomyia*		Stegomyia
69.	*Tewarius,*		Tewarius,
70.	*Tanakaius,*		Tanakaius,
71.	*Vansomerenis*		Vansomerenis
72.	*Zavortinkius*		Zavortinkius
73.	*Armigeres*	*Armigeres*	Armigeres

74.			Leicesteria
75.	*Eretmapodites*	*Eretmapodites*	Eretmapodites
76.	*Haemagogus*	*Haemagogus*	Haemagogus
77.			Conopostegus
78.	*Heizmannia*	*Heizmannia*	Heizmannia
79.			Mattinglya
80.	*Psorophora*	*Psorophora*	Psorophora
81.			Grabhamia
82.			Janthinosoma
83.	*Opifex*	*Opifex*	Opifex
84.			Nothoskusea
85.	*Udaya*	*Udaya*	Udaya
86.	*Verrallina*	*Verrallina*	Verrallina
87.			Harbachius
88.			Neomacleaya
89.	*Zeugnomyia*	*Zeugnomyia*	Zeugnomyia

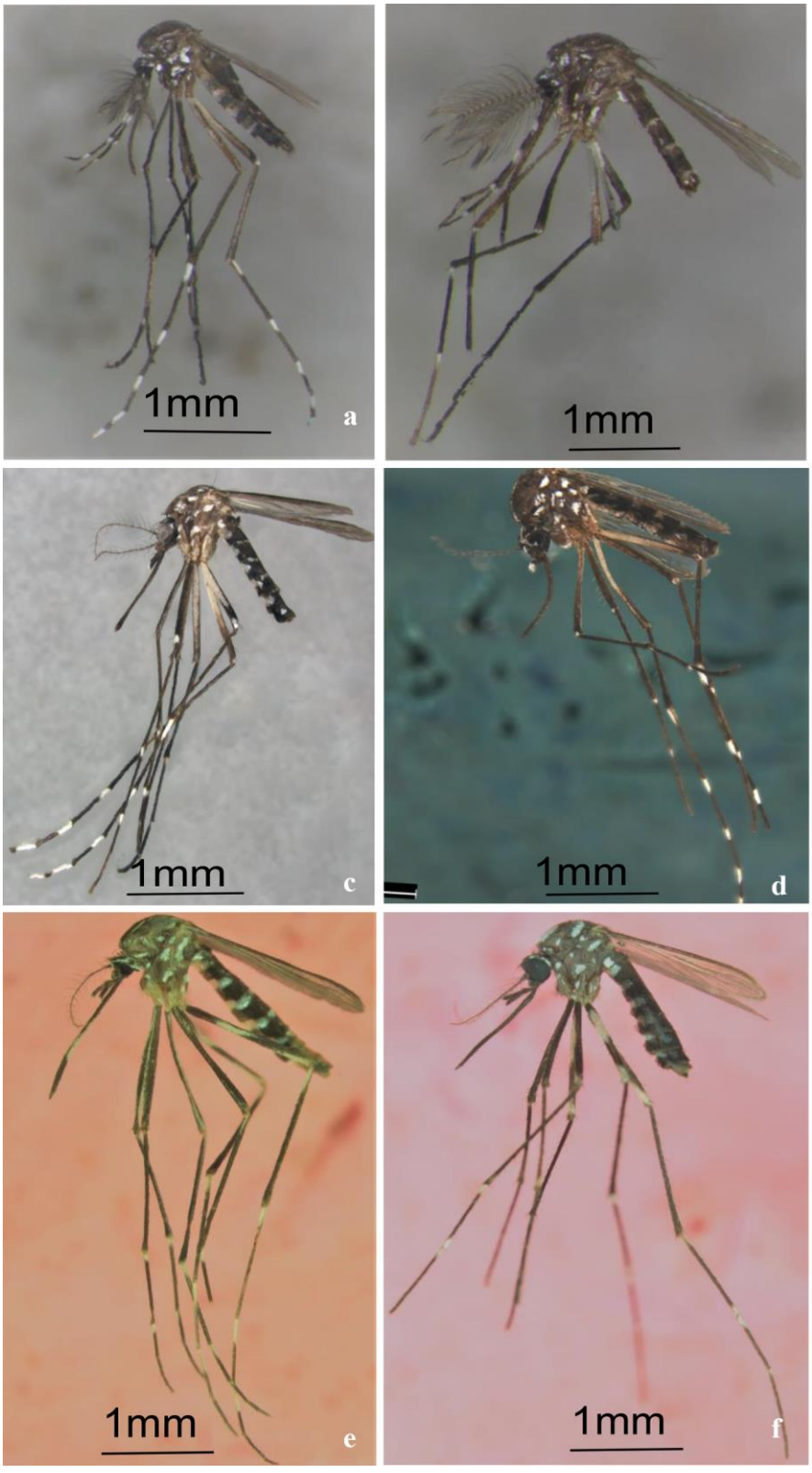

Figure: A1. a) *Ae.albopictus*, b) *Ae. psuedalbopictus*, c) *Ae.subalbopictus*, d) *Ae.novabopictus*, e) *Ae.chrysolineatus*, f) *Ae.cogilli*

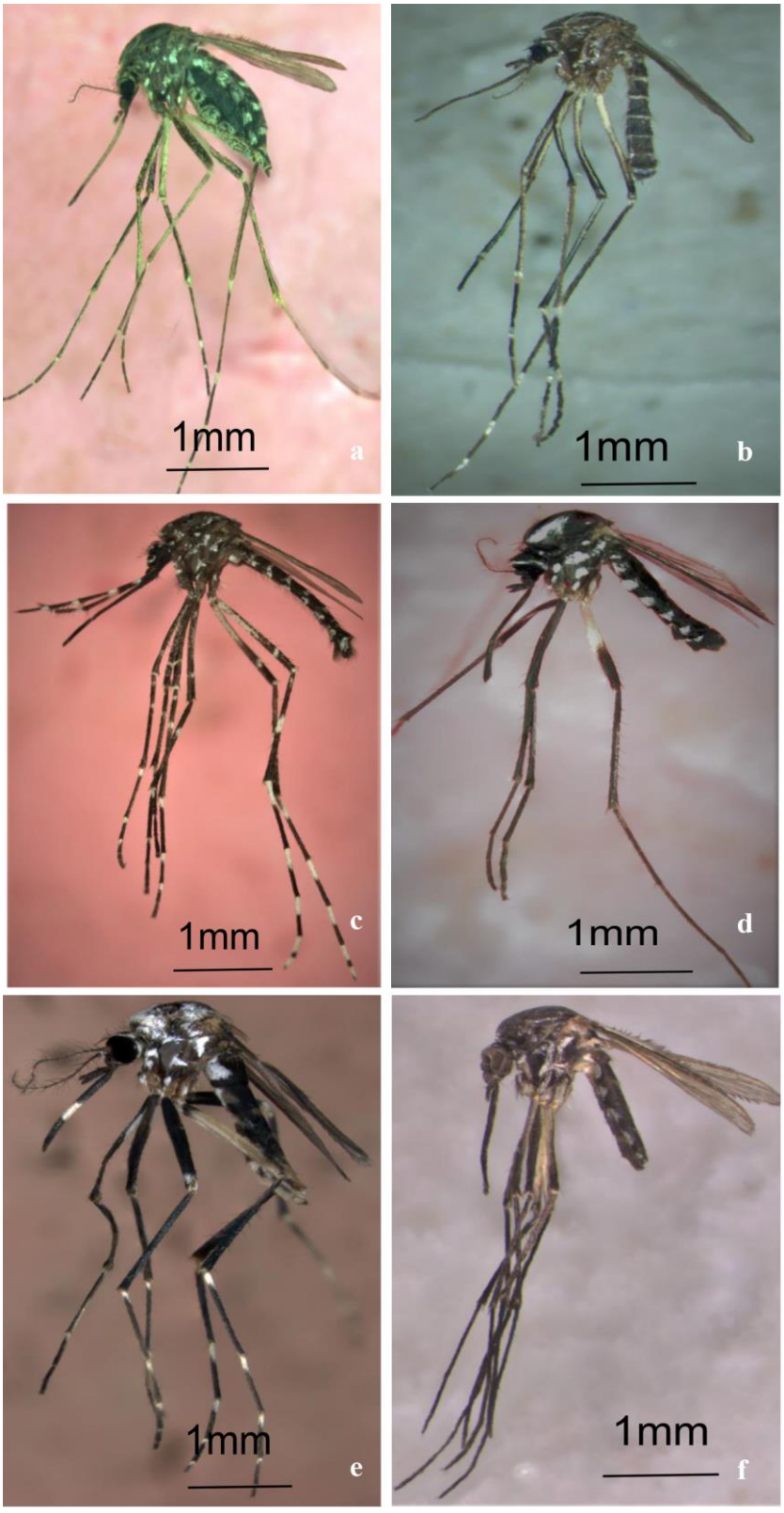

Figure A2. a) *Ae.vexans*, b) *Ae.pseudotaeniatus*, c) *Ae.vittatus*, d) *Ae.niveus*, e) *Ae.thomsoni*, f) *Ae.menoni*

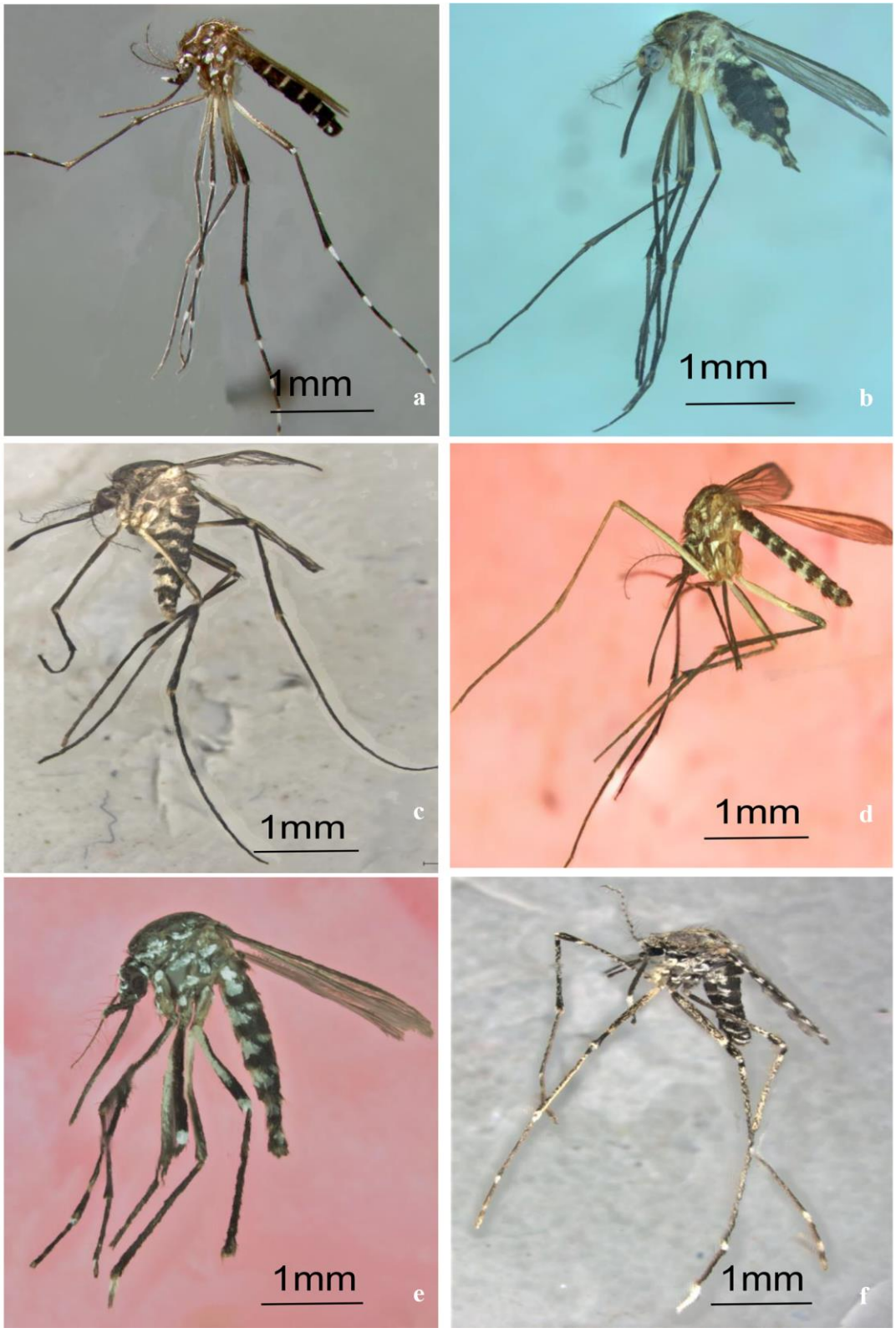

Figure A3. a) *Ae.aegypti*, b) *Ae. barraudi*, c) *Ar.sabalbatus*, d) *Ar.aureolineatus*, e) *Hz.chandi*, f) *Or.anopheloides*

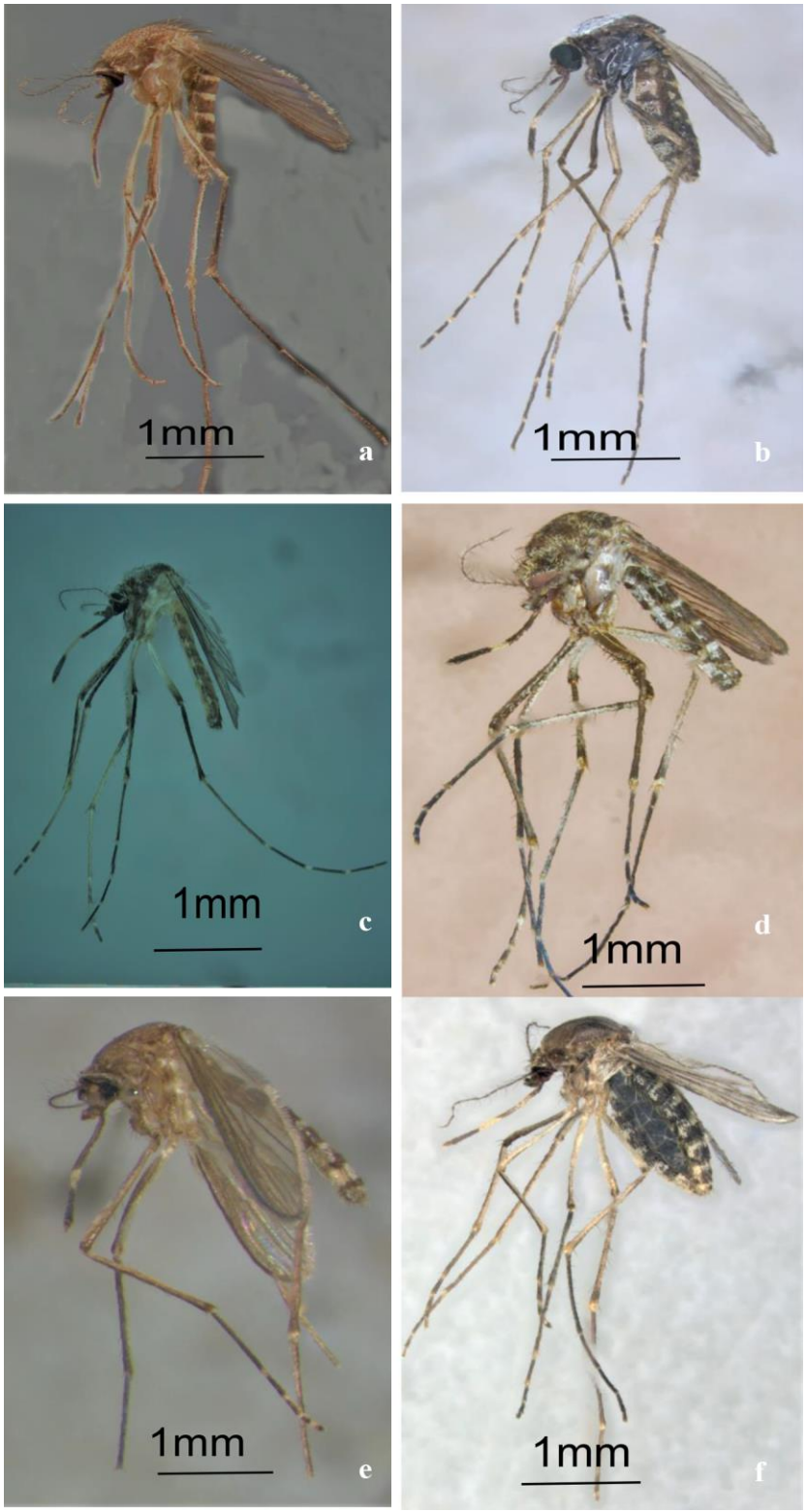

Figure A4. a) *Cx.quinquefasciatus*, b) *Cx.gelidus*, c) *Cx.whitemorei*, d) *Cx.vishnui*, e) *Cx.pseudovishnui*, f) *Cx.tritaeniorhyncus*

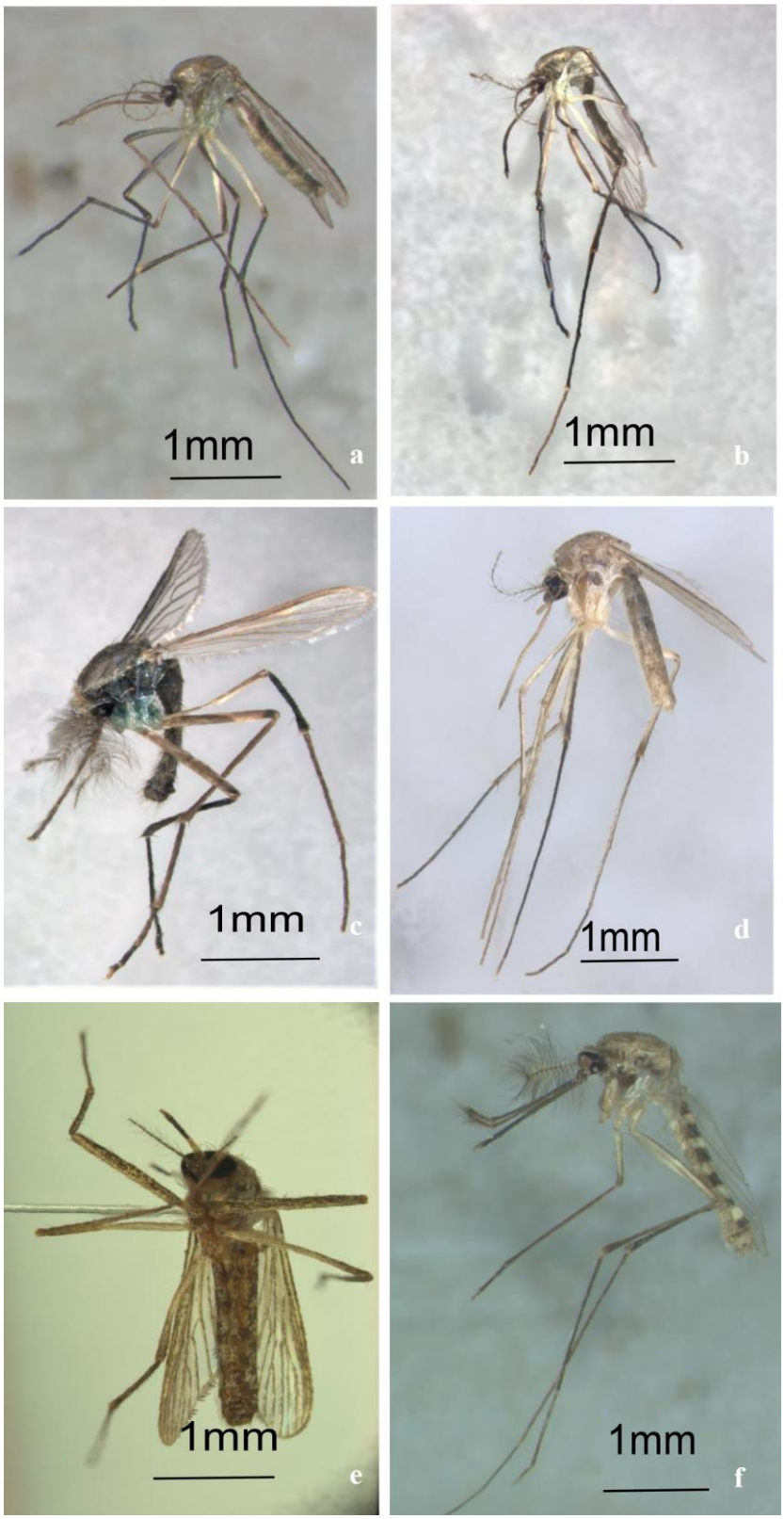

Figure A5. a) *Cx.bicornutus*, b) *Cx.brevipalpis*, c) *Cx.foliatus*, d) *Cx. fuscocephala*, e) *Cx.infula*, f) *Cx.pallidothorax*

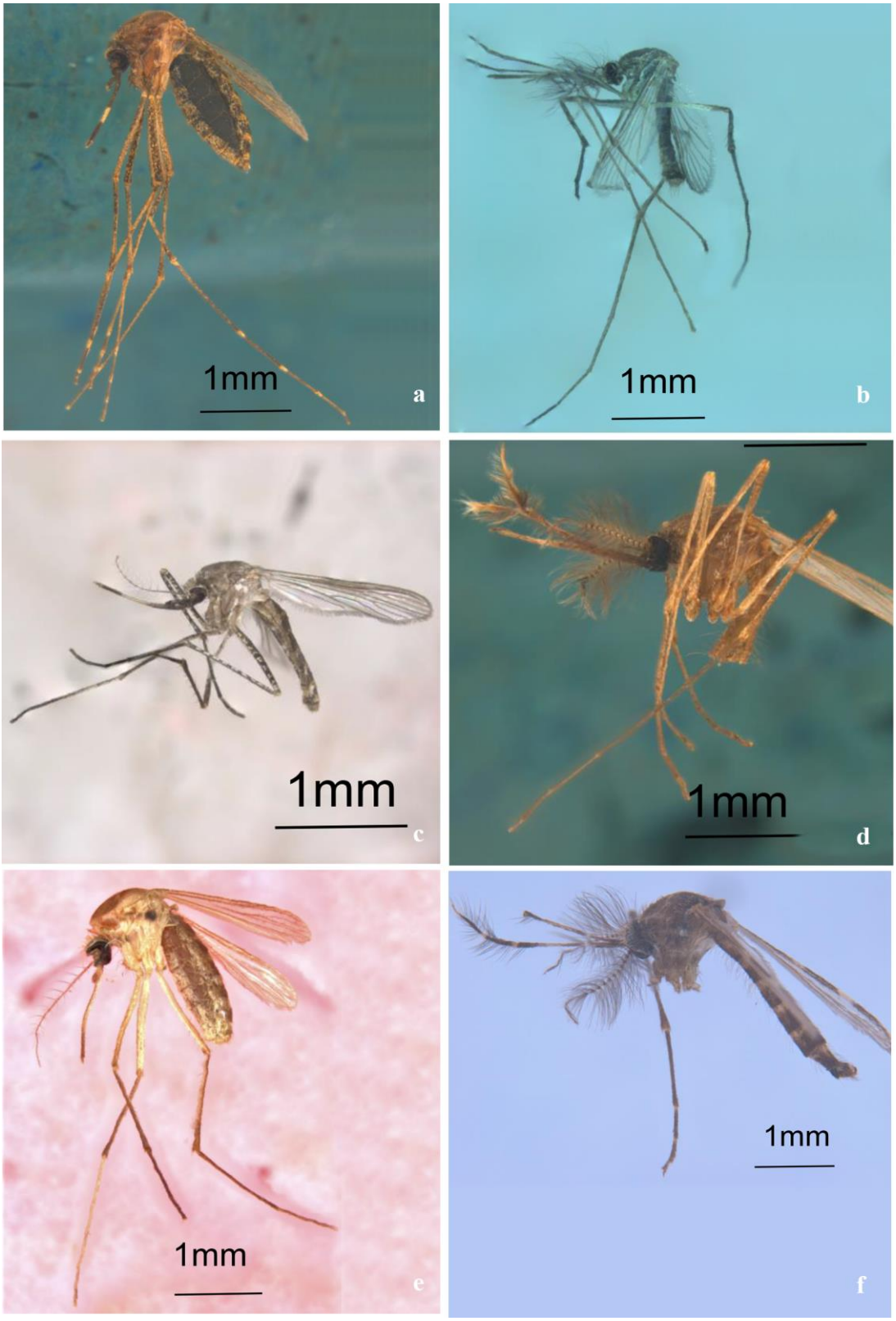

Figure A6. a) *Cx. bitaeniorhyncus*, b) *Cx. uniformis*, c) *Lt. halifaxii*, d) *Cx. sinensis*, e) *Cx. nigropunctatus*, f) *Cx. mimulus*

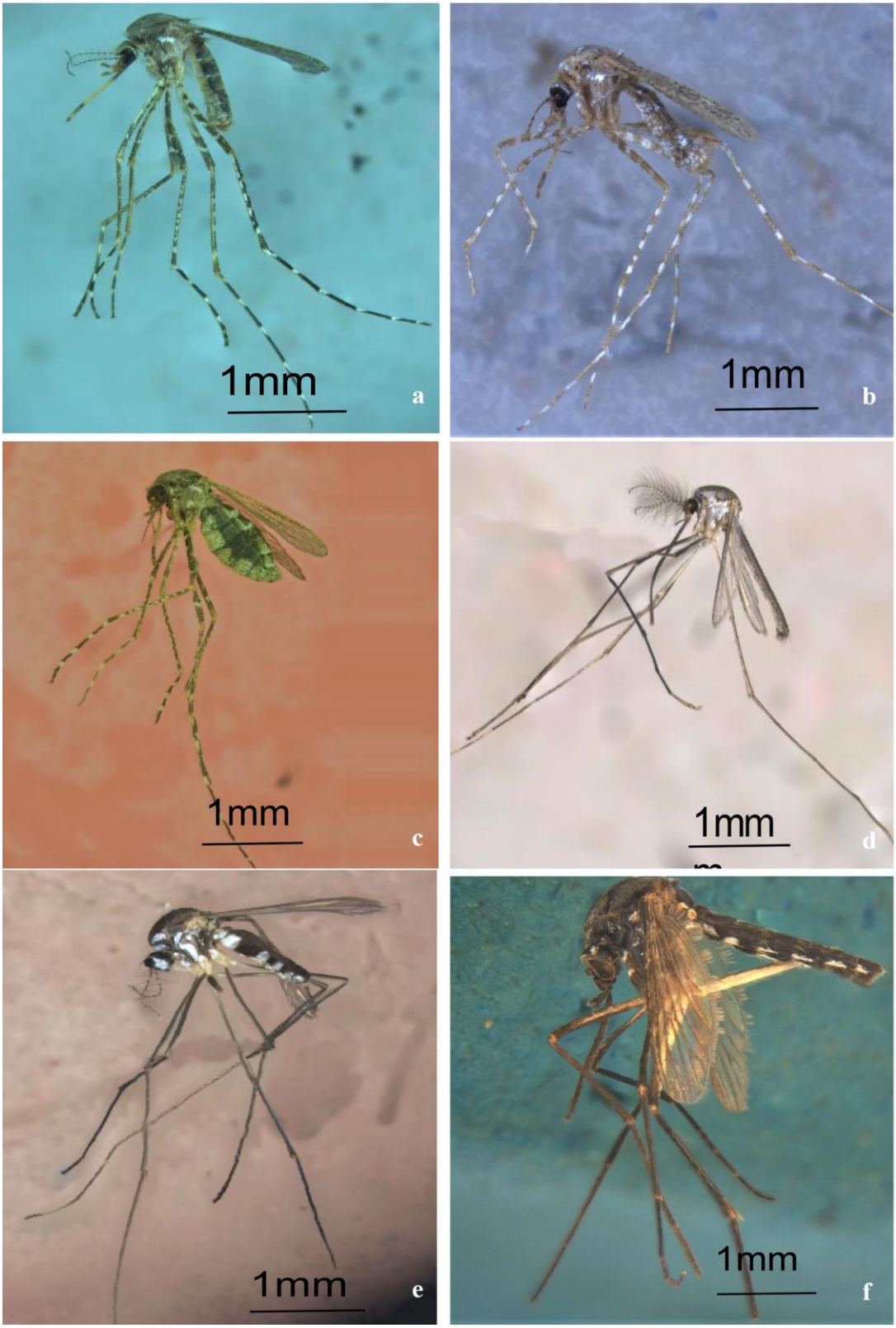

Figure A7. a) *Man. indiana*, b) *Man. annulifera*, c) *Man. uniformis*, d) *Tp. aranoides*, e) *Mal. genurostris*, f) *Ver. indica*

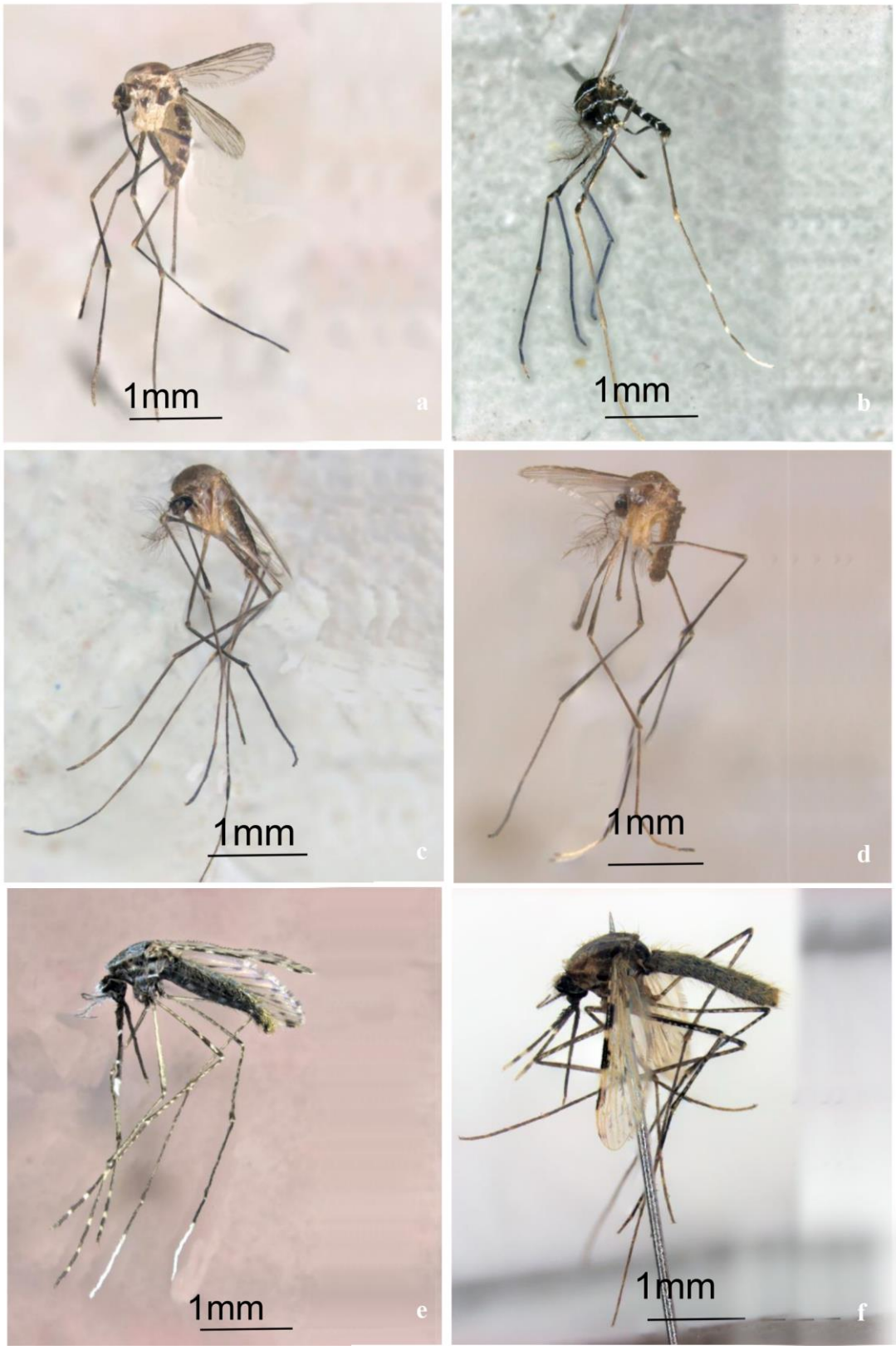

Figure A8. a) *Ur. pseudostricklandi*, b) *Ur. rutherfordi*, c) *Ur. nivipleura*, d) *Ur. testacea*, e) *An. jamesi*, f) *An. stephensi*

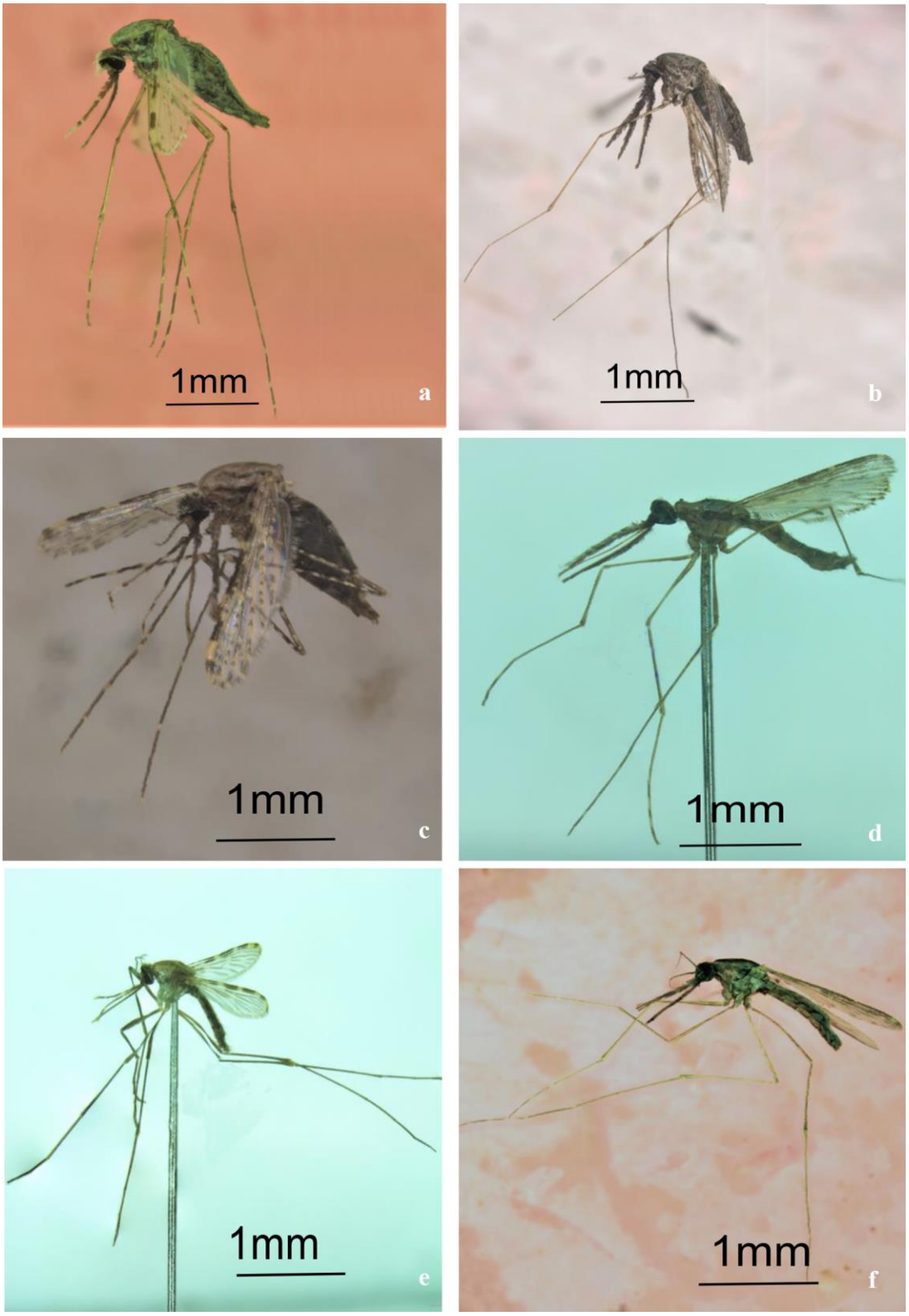

Figure A9. a) *An. vagus*, b) *An.nigerrimus*, c) *An.tessellatus*, d) *An.peditaeniatus*, e) *An. jeyporiensis*, f) *An. culiciformis*

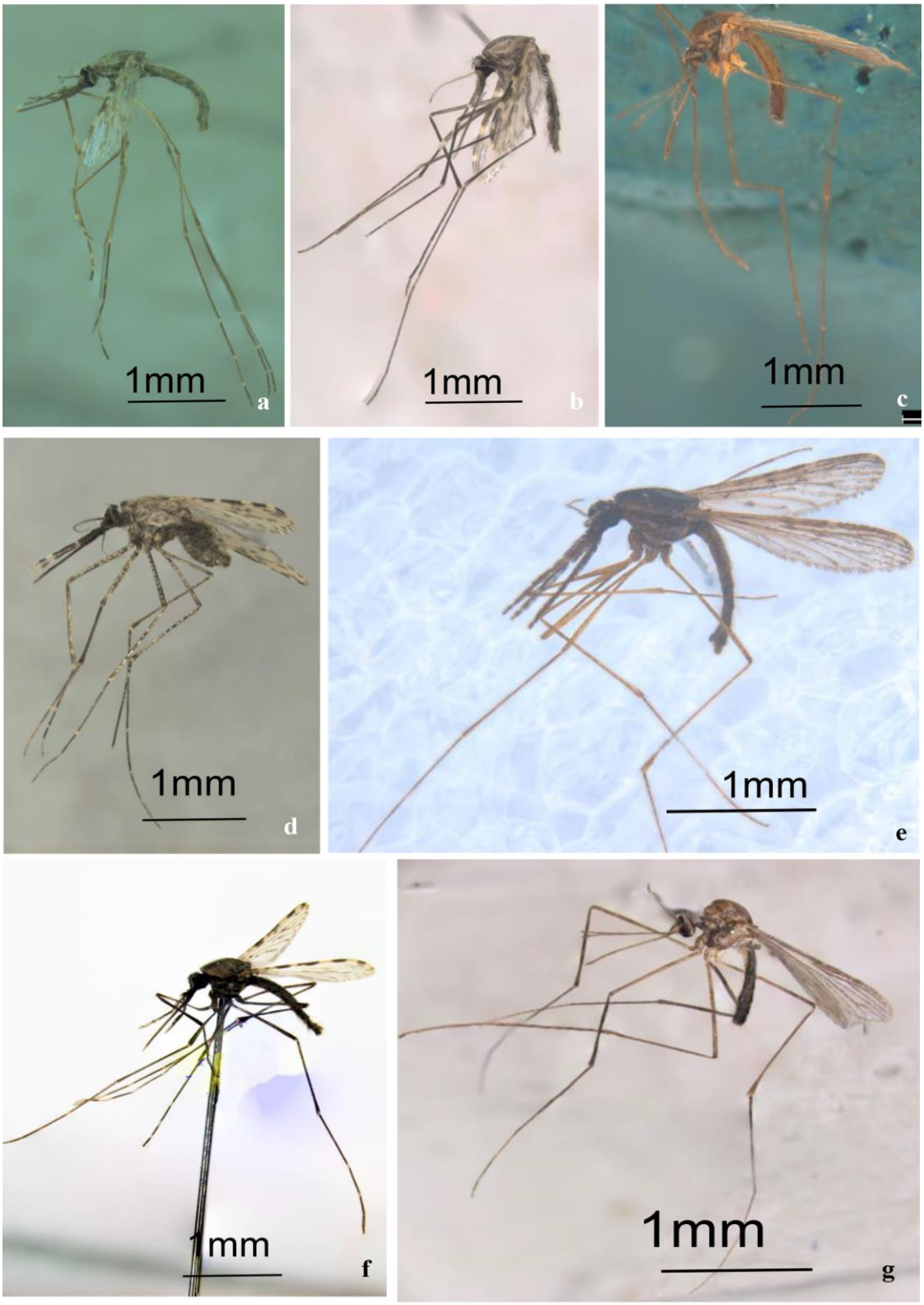

Figure A10. a) *An. subpictus*, b) *An. fluvialitis*, c) *An. insuleiflorum*, d) *An. elegans*, e) *An. barbirostris* , f) *An. aitkeni*, g) *An.karwari*.

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