MORPHOLOGICAL AND MOLECULAR STUDIES ON THE MOSQUITO (DIPTERA: CULICIDAE) SPECIES BREEDING IN BRACKISH WATER HABITATS OF NORTH KERALA

Thesis submitted to the

UNIVERSITY OF CALICUT

For the award of the Degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

(Under the Faculty of Science)

By

SHAMNA. A. K

Under the Guidance and Supervision of

DR. SUMODAN.P. K

POST GRADUATE AND RESEARCH DEPARTMENT OF ZOOLOGY

GOVERNMENT COLLEGE MADAPPALLY

KERALA -INDIA

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PG & RESEARCH DEPARTMENT OF ZOOLOGY

GOVERNMENT COLLEGE MADAPPALLY

VADAKARA-

Dr. Sumodan P.K, MSC; Ph.D.

Research Guide in Zoology (Rtd.)

Government College, Madappally,

UNIVERSITY OF CALICUT

Phone :9846135324

Former Scientist at National Institute of Malaria Research

Former District Malaria Officer

Email: sumodanpk@gmail.com

CERTIFICATE

Certified that the thesis entitled "MORPHOLOGICAL AND MOLECULAR STUDIES ON THE MOSQUITO (DIPTERA: CULICIDAE) SPECIES BREEDING IN BRACKISH WATER HABITATS OF NORTH KERALA" submitted by Mrs. Shamna. A.K to the University of Calicut for the award of degree of Doctor of Philosophy in Zoology, is a bona fide record of research work done by her in this department under my supervision and guidance and that no part thereof has been presented before for any other degree.

Mrs. Shamna A.K has successfully completed the preliminary qualifying examinations prescribed by the University of Calicut.

Place: Madappally **Dr. P.K. SUMODAN**

Date: **Supervisor & Guide**

CERTIFICATE

Certified that the thesis entitled "Morphological and molecular studies on the mosquito (Diptera: Culicidae) species breeding in brackish water habitats of north Kerala" submitted by Mrs. Shamna. A.K to the University of Calicut for the award of degree of Doctor of Philosophy in Zoology, is a bona fide record of research work done by her in this department under my co-guidance and that no part thereof has been presented before for any other degree.

Mrs. Shamna A.K has successfully completed the preliminary qualifying examinations prescribed by the University of Calicut.

Place: Madappally **Dr. Thejass P**

Date: **CO-Guide**

DECLARATION

I do hereby declare that this thesis entitled "Morphological and molecular studies on the mosquito(Diptera: Culicidae)species breeding in brackish water habitats of north Kerala" is the original research work carried out by me under the supervision and guidance of Dr.Sumodan P.K, Research Guide, Post Graduate and Research Department of Zoology, Government College, Madappally in partial fulfillment of the requirements for the Ph. D in Zoology and no part of this work has been presented by me for any other degree, diploma or other similar title of any other University

Place: Madappally **SHAMNA.A.K**

Date:

PG & RESEARCH DEPARTMENT OF ZOOLOGY

GOVERNMENT COLLEGE MADAPPALLY

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Certified that the thesis entitled " MORPHOLOGICAL AND MOLECULAR STUDIES ON THE MOSQUITO (DIPTERA: CULICIDAE) SPECIES BREEDING IN BRACKISH WATER HABITATS OF NORTH KERALA".submitted by Mrs. Shamna AK to the University of Calicut for the award of degree of Doctor of Philosophy in Zoology, has made the corrections. suggestions from the adjudicators have been incorporated.

Place: Madappally **Dr. P.K. SUMODAN**

Date: **Supervisor & Guide**

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Haseena K V whose unwavering love and encouragement have been my source of strength throughout the journey of completing this thesis. Her sacrifices, wisdom and endless support have been instrumental in shaping both my personal and academic endeavors. This achievement is as much hers as it is mine, and I am profoundly thankful for her constant belief in my abilities. Also, I would like to thank them for supporting me wholeheartedly and providing me with everything I ask for. I extend profound appreciation to my father Abootty AK for his unwavering support and guidance during the process of completing this thesis. His wisdom, encouragement, and sacrifices have been a constant source of inspiration, shaping not only my academic pursuits but also my character. This accomplishment is a testament to his enduring belief in my capabilities, and I am sincerely grateful for the invaluable role he has played in my journey.

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Dedicated this thesis with extreme affection and gratitude to my family, friends and to my teachers

MORPHOLOGICAL AND MOLECULAR STUDIES ON THE MOSQUITO (DIPTERA: CULICIDAE) SPECIES BREEDING IN BRACKISH WATER HABITATS OF NORTH KERALA

Shamna AK

Supervisor : Dr. Sumodan PK

Abstract

Key words*: An. stephensi, An. barbirostris, Aedes albopictus, Culex sitiens, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus, Cx. gelidus* and *Cx. vishnui, An. subpictus B* and *An. subpictus A*

Mosquitoes live in almost every conceivable environment where water occurs because the existence of water is a necessity for their larval development.Fresh, brackish, and saline waters are respectively defined as containing <0.5,0.5-30 and >30 ppt (Ramaswamy, 2014). Mosquito larvae are mostly restricted to freshwater environments. Still, all 3 major genera of medical importance (*Aedes, Anopheles* and *Culex*) include both freshwater and saltwater species (Coluzzi and Sabatini 1969, Bradley 1987, Jude et;2012).

In Kerala, there are nearly 30 brackish water perennial/temporary estuaries, roughly parallel to the Arabian Sea, covering an area of 2, 42,600 ha.

The impact of rising Sea levels on coastal ecosystems can indeed exacerbate health risks by altering the habitats of disease vectors. Understanding and mitigating these environmental changes are crucial for preventing the spread of mosquito-borne diseases in vulnerable regions (Surendran, 2011, Ramaswamy, 2012).

Moreover, Kerala not only holds a high biodiversity of mosquitoes but has nearly half of its population living and working in coastal areas and maintaining close contact with the sea, creating a conducive situation for epidemiological processes.

An ecotype represents specialized groups within a species, adapted to specific local environments through genetic variations.

Mosquito larvae were collected from coastal areas of 4 districts (Kasargod, Kannur, Kozhikode and Malappuram) during the period of 4 years (January 2018 to March 2022).

The identified species were *Anopheles subpictus* species complex, *An. stephensi, An. barbirostris, Aedes albopictus, Culex sitiens, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus, Cx. gelidus* and *Cx. vishnui. An. subpictus* species complex was resolved as *An. subpictus B* and *An. subpictus A.*

Salinity in the fields

Salinity of the fields were measured using salinometer to classify the habitats as brackish or fresh water. The table below shows the salinity of the sample water from which the specimens were collected.

Table 1: salinity in the field

Mosquito	*Salinity in the fields (ppt)*
An. subpictus B	*28-32*
An. subpictus A	*0.3-20*
An. barbirostris	*0.1-2*
An. stephensi	*0.1-0.7*
Ae. albopictus	*0.1-5*
Cx.quinquefasciatus	*0.2-30*
Cx.sitiens	*2-32*
Cx.bitaeniorhynchus	*0.2-5*
Cx.tritaeniorhynchus	*0.3-5*
Cx.gelidus	*0.5-10*
Cx.vishnui	*0.3-6*

Morphologically all specimens were identified up to species level. of these collected specimens Anopheles subpictus was a species complex and to resolve this complex, molecular analysis was done. Anopheles stephensi was an incriminated vector of malaria in the state, by considering its vector status molecular analysis of Anopheles stephensi were also done. The sequences were deposited in the GENBANK and got accession numbers for the sequences.

Mosquito species encountered in both fresh water and brackish water are

- *An. barbirostris*
- *An. stephensi*
- *An. subpictus A*
- *Aedes albopictus*
- *Cx. tritaeniorhynchus*
- *Cx. sitiens*
- *Cx.quinquefasciatus*
- *Cx. gelidus*
- *Cx. bitaeniorhynchus*
- *Cx.vishnui*

കൊതുകിനെക്കുറിച്ചുള്ള മോർഫോളജിക്കൽ, മോളിക്യുലാർ പഠനങ്ങൾ (ഡിപ്റ്റെറ: ക്യൂലിസിഡേ) പ്രജനനം നടത്തുന്ന ജീവിവർഗ്ഗങ്ങൾ വടക്കൻ കേരളത്തിന്റെ ഉപ്പുവെള്ള ആവാസ കേന്ദ്രങ്ങൾ

ഷംന എ.കെ

സൂപ്പർവൈസർ : ഡോ.സുമോദൻ പി.കെ

അമൂർത്തമായ

പ്രധാന വാക്കുകൾ: *അൻ. സ്റ്റീഫൻസി, എ. ബാർബിറോസ്ട്രിസ്, ഈഡിസ് അൽബോപിക്റ്റസ്, ക്യൂലെക്സ് സിറ്റിയൻസ്, സിഎക്സ്. quinquefasciatus, Cx. tritaeniorhynchus, Cx. ബിറ്റേനിയോർഹൈഞ്ചസ്, Cx. ഗെലിഡസ്ഒപ്പംCx. വിഷ്ണു, അൻ. ഉപചിത്രം ബിഒപ്പംഎ. ഉപചിത്രം എ*

വെള്ളത്തിന്റെ അസ്തിത്വം അവയുടെ ലാർവകളുടെ വികാസത്തിന് അനിവാര്യമായതിനാൽ ജലം ഉണ്ടാകുന്ന എല്ലാ സങ്കൽപ്പിക്കാവുന്ന ചുറ്റുപാടുകളിലും കൊതുകുകൾ വസിക്കുന്നു. ശുദ്ധജലം, ഉപ്പുവെള്ളം, ഉപ്പുവെള്ളം എന്നിവ യഥാക്രമം <0.5,0.5-30, >30 ppt എന്നിവ അടങ്ങിയിരിക്കുന്നതായി നിർവചിച്ചിരിക്കുന്നു (രാമസ്വാമി, 2014) . കൊതുക് ലാർവകൾ കൂടുതലും ശുദ്ധജല ചുറ്റുപാടുകളിൽ പരിമിതപ്പെടുത്തിയിരിക്കുന്നു. എന്നിരുന്നാലും, മെഡിക്കൽ പ്രാധാന്യമുള്ള 3 പ്രധാന ജനുസ്സുകളും (*ഈഡിസ്, അനോഫിലിസ്*ഒപ്പം*ക്യൂലെക്സ്*) ശുദ്ധജലവും ഉപ്പുവെള്ളവും രണ്ട് ഇനങ്ങളും ഉൾപ്പെടുന്നു (കൊലൂസിയും സബാറ്റിനിയും 1969, ബ്രാഡ്ലി 1987, ജൂഡ് എറ്റ്;2012).

കേരളത്തിൽ ഏകദേശം 2,42,600 ഹെക്ടർ വിസ്തൃതിയിൽ അറബിക്കടലിന് സമാന്തരമായി ഏകദേശം 30 ലവണജല വറ്റാത്ത/താത്കാലിക അഴിമുഖങ്ങളുണ്ട്.

തീരദേശ ആവാസവ്യവസ്ഥയിൽ സമുദ്രനിരപ്പ് ഉയരുന്നതിന്റെ ആഘാതം, രോഗവാഹകരുടെ ആവാസവ്യവസ്ഥയിൽ മാറ്റം വരുത്തുന്നതിലൂടെ ആരോഗ്യപരമായ അപകടസാധ്യതകൾ വർദ്ധിപ്പിക്കും. ഈ പാരിസ്ഥിതിക മാറ്റങ്ങൾ മനസിലാക്കുകയും ലഘൂകരിക്കുകയും ചെയ്യുന്നത് ദുർബല പ്രദേശങ്ങളിൽ കൊതുക് പരത്തുന്ന രോഗങ്ങൾ പടരുന്നത് തടയുന്നതിന് നിർണായകമാണ് (സുരേന്ദ്രൻ, 2011, രാമസ്വാമി, 2012).

കൂടാതെ, കേരളത്തിൽ കൊതുകുകളുടെ ഉയർന്ന ജൈവവൈവിധ്യം ഉണ്ടെന്ന് മാത്രമല്ല, ജനസംഖ്യയുടെ പകുതിയോളം തീരപ്രദേശങ്ങളിൽ താമസിക്കുകയും ജോലി ചെയ്യുകയും ചെയ്യുന്നു, കടലുമായി അടുത്ത ബന്ധം പുലർത്തുന്നു, ഇത് പകർച്ചവ്യാധി പ്രക്രിയകൾക്ക് അനുകൂലമായ സാഹചര്യം സൃഷ്ടിക്കുന്നു.

ഒരു ഇക്കോടൈപ്പ് ജനിതക വ്യതിയാനങ്ങളിലൂടെ പ്രത്യേക പ്രാദേശിക പരിതസ്ഥിതികളുമായി പൊരുത്തപ്പെടുന്ന ഒരു സ്പീഷിസിനുള്ളിലെ പ്രത്യേക ഗ്രൂപ്പുകളെ പ്രതിനിധീകരിക്കുന്നു.

4 ജില്ലകളിലെ (കാസർകോട്, കണ്ണൂർ, കോഴിക്കോട്, മലപ്പുറം) തീരപ്രദേശങ്ങളിൽ നിന്ന് (2018 ജനുവരി മുതൽ 2022 മാർച്ച് വരെ) 4 വർഷത്തിനിടെ കൊതുകു ലാർവകൾ ശേഖരിച്ചു.

ഇവയായിരുന്നു തിരിച്ചറിഞ്ഞ ഇനങ്ങൾ*അനോഫിലസ് ഉപചിത്രം*സ്പീഷീസ് കോംപ്ലക്സ്, *An. സ്റ്റീഫൻസി, എ. ബാർബിറോസ്ട്രിസ്, ഈഡിസ് അൽബോപിക്റ്റസ്, ക്യൂലെക്സ് സിറ്റിയൻസ്, സിഎക്സ്. quinquefasciatus, Cx. ട്രൈറ്റേനിയോർഹൈഞ്ചസ്, Cx. ബിറ്റേനിയോർഹൈഞ്ചസ്, Cx. ഗെലിഡസ്ഒപ്പംCx. വിഷ്ണു. എ. ഉപചിത്രം*സ്പീഷീസ് കോംപ്ലക്സ് ആയി പരിഹരിച്ചു*എ. ഉപചിത്രം ബി*ഒപ്പം*എ. ഉപചിത്രം എ.*

വയലുകളിൽ ലവണാംശം

ആവാസവ്യവസ്ഥയെ ഉപ്പുവെള്ളമോ ശുദ്ധജലമോ ആയി തരംതിരിക്കാൻ സലിനോമീറ്റർ ഉപയോഗിച്ച് വയലുകളുടെ ലവണാംശം അളന്നു. സാമ്പിളുകൾ ശേഖരിച്ച സാമ്പിൾ വെള്ളത്തിന്റെ ലവണാംശം ചുവടെയുള്ള പട്ടിക കാണിക്കുന്നു.

പട്ടിക 1: വയലിലെ ലവണാംശം

കൊതുക്	*വയലുകളിലെ ലവണാംശം (ppt)*
എ. ഉപചിത്രം ബി	*28-32*
എ. ഉപചിത്രം എ	*0.3-20*
എ. ബാർബിറോസ്ട്രിസ്	*0.1-2*
എ. സ്ട്രറെഫെൻസി	*0.1-0.7*
Ae. ആൽബോപിക്ടസ്	*0.1-5*
Cx.quinquefasciatus	*0.2-30*
Cx.sitiens	*2-32*
Cx.biteniorhynchus	*0.2-5*
Cx.tritaeniorhynchus	*0.3-5*
Cx.gelidus	*0.5-10*
Cx.വിഷ്ണുയി	*0.3-6*

രൂപശാസ്ത്രപരമായി എല്ലാ മാതൃകകളും സ്പീഷീസ് ലെവൽ വരെ തിരിച്ചറിഞ്ഞു. ഈ ശേഖരിച്ച മാതൃകകളിൽ അനോഫിലിസ് സബ്പിക്റ്റസ് ഒരു സ്പീഷീസ് കോംപ്ലക്സ് ആയിരുന്നു, ഈ സങ്കീർണ്ണത പരിഹരിക്കാൻ, തന്മാത്രാ വിശകലനം നടത്തി. അനോഫിലിസ് സ്ട്രീഫൻസി, സംസ്ഥാനത്ത് മലേറിയയുടെ ഒരു കുറ്റകരമായ വെക്റ്റർ ആയിരുന്നു, അതിന്റെ വെക്റ്റർ നില പരിഗണിച്ച് അനോഫിലിസ് സ്ട്രീഫൻസിയുടെ തന്മാത്രാ വിശകലനവും നടത്തി. സീക്വൻസുകൾ GENBANK-ൽ നിക്ഷേപിക്കുകയും സീക്വൻസുകൾക്കുള്ള പ്രവേശന നമ്പറുകൾ ലഭിക്കുകയും ചെയ്തു.

ശുദ്ധജലത്തിലും ഉപ്പുവെള്ളത്തിലും കണ്ടുമുട്ടുന്ന കൊതുകുകൾ

- *എ. ബാർബിറോസ്ട്രിസ്*
- *എ. സ്ട്രറെഫെൻസി*
- *എ. ഉപചിത്രം എ*
- *ഈഡിസ് അൽബോപിക്ടസ്*
- *Cx. tritaeniorhynchus*
- *Cx. സിറ്റിയൻസ്*
- *Cx.quinquefasciatus*
- *Cx. ഗെലിഡസ്*
- *Cx. ബിറ്റേനിയോർഹൈഞ്ചസ്*
- *Cx.വിഷ്ണുയി*

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Chapter 1:

INTRODUCTION

CHAPTER 1

1. INTRODUCTION

1.1 General Introduction of Mosquitoes

The word mosquito is Spanish and Portuguese, meaning little fly (Brown and Lesley, 1993). The term mosquito is apparently of North American origin and dates to 1583. Aristotle documented the lifecycle and metamorphic abilities of mosquitoes and referred to them as empis in his Historia Animaliumin 300 BC. Mosquitoes have evolved; they first appeared during the Jurassic period, about 210 million years ago (Edwards, 1932). It is fascinating how mosquitoes have persisted through millions of years, evolving and adapting. Nature has perfected them through the process of evolution so that they can survive under harsh conditions and in a diversity of environments.

The mosquitoes (Diptera: Culicidae) are at the centre of global entomological research due to their importance as vectors of a wide range of debilitating viral and parasitic diseases affecting both humans and animals. Sir Patrick Manson, Sir Ronald Ross and Sir Walter Reed discovered that many species of mosquitoes were pests or vectors of pathogens. Mosquitoes indeed pose a significant health threat, transmitting various pathogens that cause diseases like malaria, dengue, chikungunya, West Nile virus, Japanese Encephalitis, lymphatic filariasis etc. The mosquitoes transmit the pathogens such as viruses(arboviruses), filarial worms(helminths) and protozoa. The focus on species like *Anopheles, Culex* and *Aedes* reflects their role in spreading these infections, making them a global public health concern. WHO (1996) declared the mosquito "public enemy number one". More than half of the world's population lives at risk of becoming infected by mosquitoes. Transmission of viruses, filarial worms or malaria parasites varies greatly among the ~3500 recognised mosquito species. The latest world malaria report of WHO shows that in 2021, there were an estimated 247 million cases of malaria worldwide compared to 245 million cases in 2020. It has been said that *Anopheles gambiae* as the primary transmitter of malaria parasites to humans, is the most dangerous animal in the world. Certainly, malaria has killed more people than all the wars that ever took place. Even now despite drugs and mosquito control, malaria claims the lives of 619,000 in the year 2021 compared to 625,000 in 2020. More than 3.9 billion people in over 129 countries are at risk of contracting dengue, with an estimated 96 million symptomatic cases and an estimated 40,000 deaths every year. Indeed, mosquitoes extend beyond tropical regions, causing nuisances and occasionally transmitting pathogens in temperate latitudes as well. Their adaptability to diverse environments emphasizes the global

impact of these vectors on public health zones. Vigilance and control measures are essential to mitigate the risks associated with mosquitoes in various climate.

1.2 Habitats of mosquitoes

Due to their ability to adapt to a wide range of habitats, mosquitoes are extremely successful organisms. Mosquitoes live in almost every conceivable environment where water occurs because the existence of water is a necessity for their larval development. They can be found anywhere in the world, except in permanently frozen areas. Oviposition sites of mosquitoes include different types which may differ in the point of the quantity and quality of water and environmental characteristics (Machault et al. 2009). The breeding habitat plays a crucial role in mosquito population dynamics. Each species has a preferred breeding site for oviposition. The density and distribution of the mosquito larval stages are closely associated with that of adults (Floore, 2006).

Mosquitoes breed in a wide range of water bodies, including temporary and permanent, highly polluted as well as clean, large, or small, stagnant or flowing and even the smallest accumulations such as water-filled buckets, flower vases, old tyres, coconut shells, hoof prints, or leaf axils (Metcalf, 1932). Complete metamorphosis takes place within the water and the adults are winged and capable of extensive flight. Adult mosquitoes differ greatly in their bionomics. Eg: - in terms of the host-seeking, biting and dispersal behaviour and strategies for reproduction. Both the sexes of mosquitoes feed on plant sugar, but only the female ingests blood, which they use to develop eggs (Michael, 1978). Females of most species feed primarily on the blood of wild or domestic animals and only a few are dedicated human feeders. Most mosquito species are not involved in pathogen transmission to humans, but those that are make a huge impact on global health.

The varied feeding habits of mosquitoes include nectar, honeydew, fruit juices, exudatesand blood (Peach, 2019). While many species primarily feed on plant substances, others especially females, rely on blood for egg production (Harbach, 2007). The broad spectrum of hosts, from warm blooded vertebrates to cold blooded animals such as snakes, turtles, toads, frogs, and other insects, including nymphal cicadas, lepidopterous larvae and mantidsunderscores the versatility of mosquito species in seeking nourishment for their reproductive needs(Lehane, 2005).

The time of feeding activityandflight is usually quite species-specific. Some species are active at night(nocturnal) or twilight(crepuscular) whereas others are active during the daylight hours(diurnal) (Crans, 1989). Despite our current knowledge of mosquito biology, practically nothing is known about the specific bionomics of most species.

1.3 Biology of Mosquitoes

As true flies, mosquitoes are slender, long-legged, holometabolous insects that are easily recognised by their long proboscis and the presence of scales on most of the body.

Life cycle

Like all flies, the mosquito life cycle consists of an egg, larva, pupa, and adult. The first 3 stages are largely aquatic. The life cycle is completed in 5-14 days. The larval and adult stages of mosquito exist in two different environments, each stage being under the influence of its immediate surroundings. (The typical mosquito life cycle is shown in Figure 1

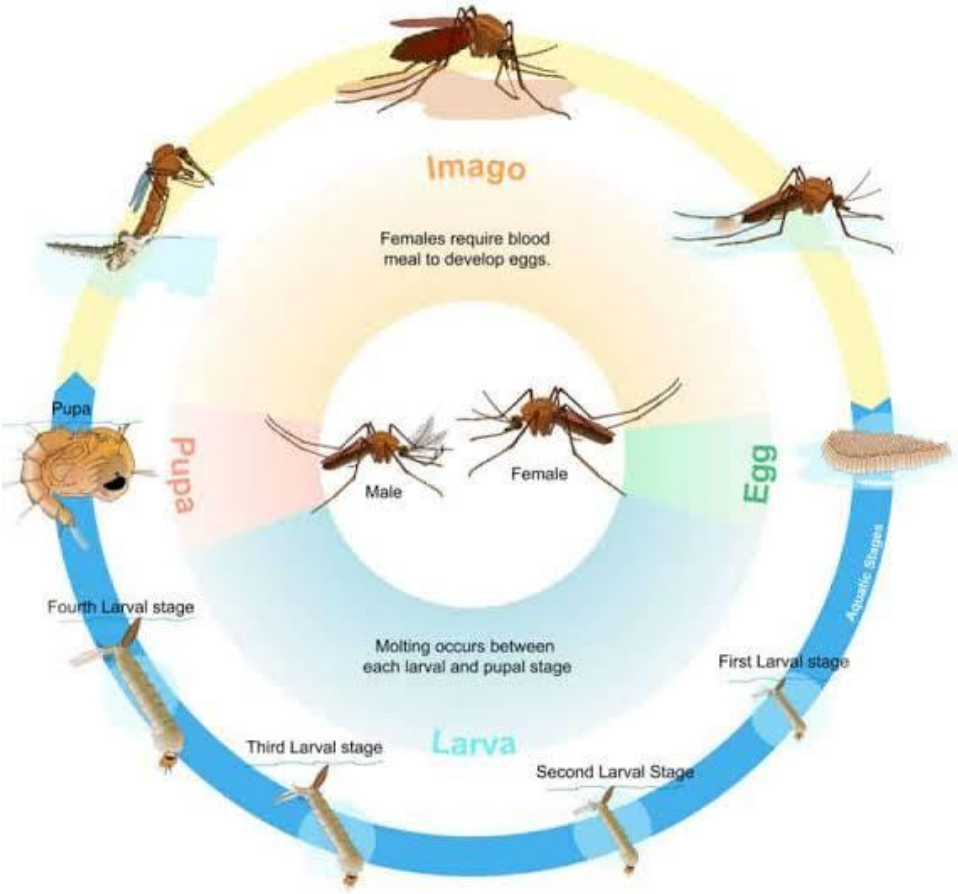


Fig. 1 Life cycle of Mosquito (http:// www. nemass mosquito.org/mosquitoes/pages/mosquito- life cycle)

Eggs

The eggs are laid on a surface of water and hatch into wrigglers, named due to their wriggling movement. In general, mosquito eggs fall into 3 distinct group

- Those that are laid singly on the water surface. Eggs of *Anopheline* mosquito. These are elongated and oval, usually pointed at one end, and are provided with a pair of lateral floats.
- Those that are glued together to form rafts, which float on the water. Eggs of *Culex, Culiseta, Coquillettidia*and *Ur00anotaenia* mosquito. This raft contains 200 or more eggs and remains afloat on the surface of the water until hatching occurs, usually within a few days.
- Those that are laid singly out of the water, on the soil. Eggs of *Orthopodomyia* and *Aedes*

Fig.2 Anopheles egg (C. Rexanne Connelly (2007))

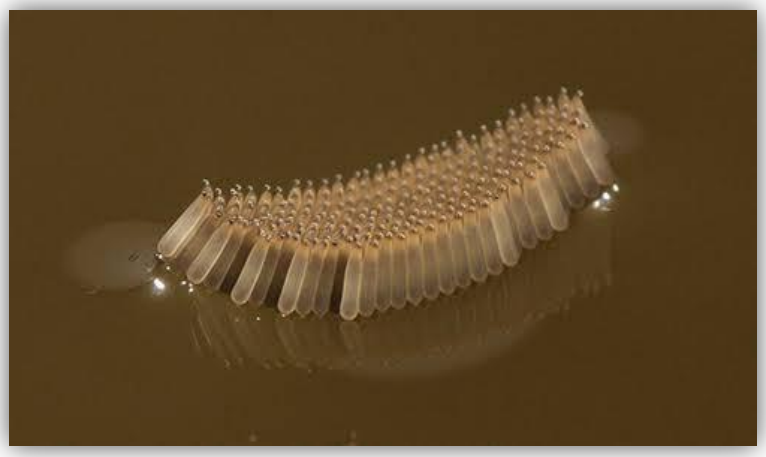


Fig. 3 Culex egg (Lawrence E. Reeves (2020))

Fig. 4 Aedes egg (Klivni 23)

Larvae

Theabsence of legs, the presence of a large distinct head bearing mouth brushes and antennae, a bulbous thorax that is wider than the head and abdomen, posterior anal papillae and either a pair of respiratory opening (subfamily Anophelinae) or an elongate siphon (subfamilyCulicinae) borne near the end of the abdomen are the characters which distinguishes mosquito larvae or wrigglers from other aquatic insects. The larvae thrust this tube above the water at intervals to breathe. Most of the larvae extract the microorganisms and suspended particulate matter from the water and feed on them with filamentous mouth brushes. Other species are obligatory or facultative predator mosquito species use their modified mouth brushes or grasping mandibles or maxillae to capture the immature stages of other mosquitoes and feed on them. When the food is scarce, some larvae resort to scavenging or cannibalism. The larvae of most mosquitoes came to water surface to obtain oxygen from the atmosphere. Four tracheal gills located on the last segment of the abdomen also supplies Oxygen.A specialised siphon is present in all species of *Mansonia* and *Coquillettidia* and some species of *Mimomyiato* obtain oxygen from the air vessels of aquatic plants. Respiration in *Aedomyia* species is by means of their enlarged antennae.Greatlyenlarged anal papillae that supplied with tracheae are present in some species and these species probably obtain dissolved oxygen from the water and seldom come to the surface. The larvae change into pupa by passing through 4 instars.

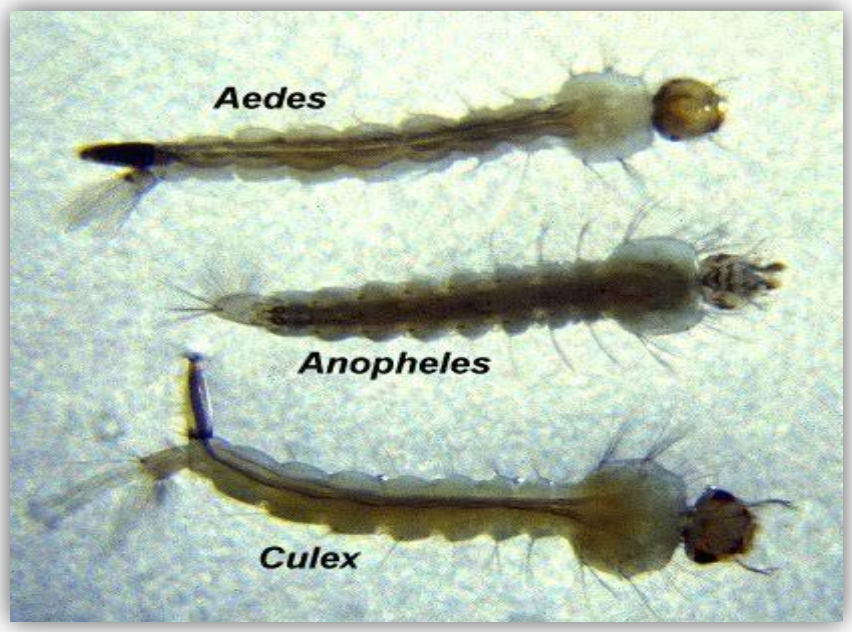


Fig. 5 Larvae of *Aedes, Anopheles* and *Culex*(Richard C Russell, 2000)

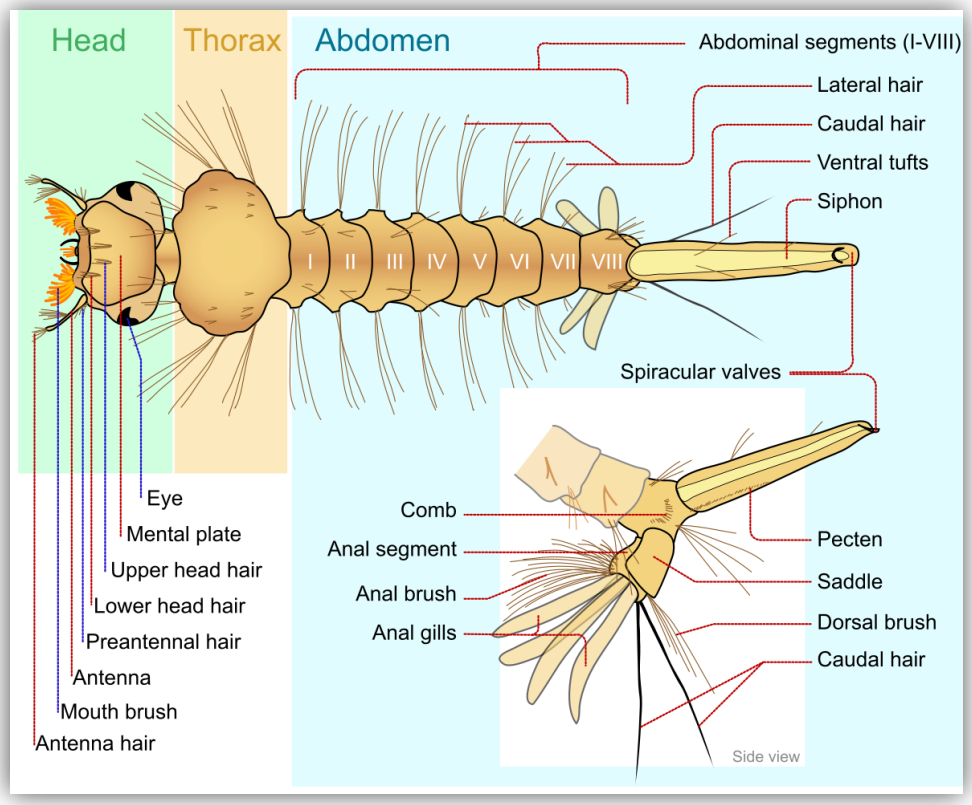


Fig. 6 Labelled diagram of a typical mosquito larvae (http://fmel.ufl.edu./key/images anatomy/larval/anat 2 John.JPG)

Pupae

Unlike most insects, pupae of mosquitoes are active and free swimmers and are called tumblers. Pupae respire through 2 trumpet-like tubes located on the thorax. Following a pupal period of short duration, usually 2 or 3 days, the outer skin of the cephalothorax of the pupa breaks at the weakest spot and through this opening, the adult emerges and after a moment flies away. Most mosquitoes have several generations each year.

The mosquitoes are attracted to hosts through moisture, lactic acid, carbon dioxide, body heat and movement (Hallem, 2004). The tube-like proboscis present in female adults can pierce the host skin and feed on blood. Blood feeding is essential for egg production because the blood contains protein and iron needed to produce eggs.

Fig. 7 Pupae of mosquito (Brook Jenson, 2022)

1.4 GENERAL MORPHOLOGY OF ADULT MOSQUITOES

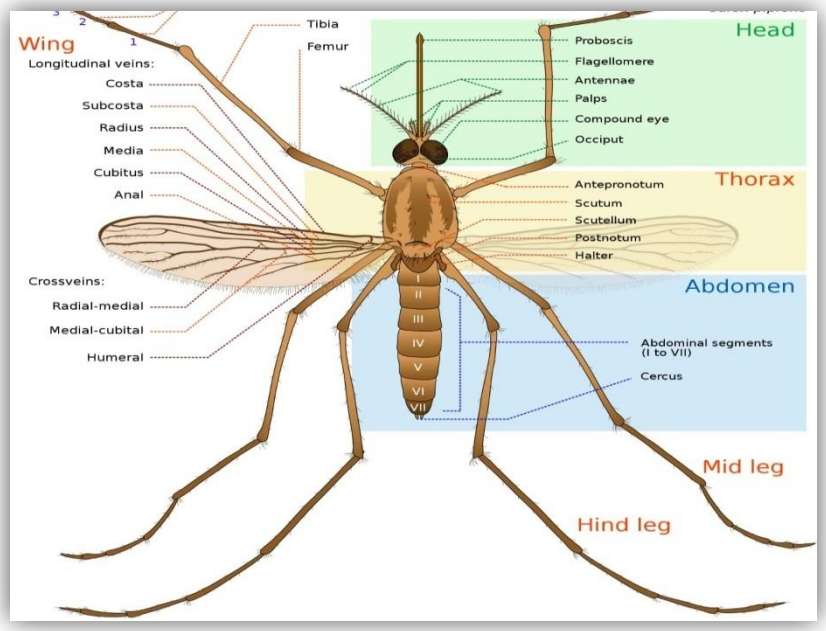


Fig. 8 Labelled diagram of a typical adult mosquito (Nicole Beaver, 2022)

Mosquitoes have a slender, delicate and long-legged body measuring 3-4 mm in length. The body is divisible into to head, thorax and abdomen (Fenemore, 1995).

Head

The head is globular and highly mobile on a slender neck. It bears a pair of compound eyes, antennae, and mouthparts (Herbert, 1956). The mouthparts are of piercing and sucking type. There is a pair of mandibles, a pair of maxillae, labium, labrum, epipharynx and hypopharynx.

Antennae:CO2 detection from a person's breath and movement of air is done by the long feather–like olfactory organs.

Eye: The movement is detected by two large compound eyes.

Palps: In between two antennae, there occurs palps.

Proboscis: Blood sucking organ in female mosquitoes, it pierces the skin and sucks out blood.

Thorax

The thorax consists of 3 segments; each segment bears a pair of legs. The legs are long, slender, and delicate. The mosquito has a single pair of wings attached to the mesothorax. The complete fringe of large scales around the wing margin and along each of the veins gives

the wing a peculiarly characteristic appearance. The mesothorax bears a pair of small drumsticks – shaped processes, called the halters or balancers (Little, 1963).

Halter: acts as a steering while flying, balancing organ

Wing: flying is done by a pair of wings.

Legs: like other insects,6 legs are present.

Femur: leg's first part.

Tibia: leg's 2nd part.

Tarsus: leg's last part, helps in mosquitoes to stand and walk on water.

Abdomen

The 10 segmented slender abdomen(Mani, 1973)serves as the stomach, reproductive system, and part of the respiratory system.

Genitalia: female release their eggs by means of their genitalia.

The abdomen terminates in two finger-like appendages, the cerci, which function in egg lying and copulation. In *Aedes* and *Psorophora* females, the cerci are visible, protruding from the tip of the abdomen while the cerci are retracted within the body and are not visibleIn many other genera (Burkett, 2013).

1.5 Vector status

A vector is any living agent that can transmit or spread disease. The vectors can be classified into two types; mechanical vectors and biological vectors. The mechanical vector passively transmits disease without biting whereas a biological vector transmits disease actively by biting (Garcia, 1999). Mosquitoes are biological vectors. Biological transmission is of 3 types.

1. Propagative-pathogenic organisms undergo no cyclic changes but multiply as in the case of yellow and dengue fever viruses within the vector.
2. Cyclo propagative- pathogenic organisms exhibit cyclic changes and multiply in the process as in malarial parasite
3. Cyclo-developmental- pathogenic organism undergoes only developmental changes but no multiplication as in filariasis.

1.6 Evolutionary History

It's fascinating how mosquitoes have been around for millions of years, adapting and evolving over time. Approximately 210 million years ago in the Jurassic period, mosquitoes came in to existence (Edwards, 1932). According to the University of Alaska, the oldest mosquito fossil is believed to be from 79 million years ago, though scientists believe mosquitoes have been in existence for 226 million years (Poinar et al, 2000). The oldest known mosquitoes are known from amber dating to the late Cretaceous. Molecular estimates suggest that the split between these two subfamilies occurred 197.5 million years ago, during the early Jurassic, but that major diversification did not take place until the Cretaceous (Lorenz et.al, 2021).

1.7 Systematics and Taxonomy

Absolutely, understanding the correct scientific name is fundamental in biological research. Systematics provides a framework for studying and organizing the diversity of organisms, helping researchers build upon cumulative knowledge about a particular species. The work of scientists like George Gaylord Simpson and Ernst Mayr has greatly contributed to shaping our understanding of the relationships between different species. Taxonomy is the theoretical study of classification including its bases, principles, procedures and rules(Simpson, 1961).The term classification should be used for the practical application of taxonomy to particular groups of organisms. In addition to classification, systematics also encompasses studies on ecology, evolution, and biodiversity (Narendran, 2006). In today's technology-orientated research world, it is important to realize the continuing value of systematics, the basic tenet of which is to combine diverse types of data to produce classifications that reflect the natural history of living organisms (Monis, 1999).

1.8 Taxonomic History of mosquitoes

Indeed, the history of mosquito taxonomy is fascinating. Linnaeus laid the foundation. Linnaeus, father of systematics, named the first genus *Culex* in the year 1735. He described 6 species belonging to this genus in the tenth edition of his book, the Systema Naturae in 1758. Subsequent researchers like Meigen (1818) described the genera *Aedes* and *Anopheles*. The link between mosquitoes and diseases like malaria and yellow fever further fueled the interest in the description and classification of mosquitoes. This intersection of taxonomy and public health has had a profound impact on our understanding of these disease vectors.In 1899, The

British Museum (Natural History) employed Fred V Theobald, starting the evolution of mosquito classification and taxonomy. Consequently, during the two decades following the publication of Theobald's Monograph of the Culicidae in 1910, significant changes were made toward a much more conservative system of classification. Particularly noteworthy were the efforts of F W Edwards in Europe and Harrison G Dyar in North America, whose work contributed most significantly to the acceptance of the broad group concepts (Edwards, 1932) that provided the framework on which the traditional classification (Stone et al., 1959, Belkin, 1962, Knight& Stone, 1977) of the twentieth century was built. The shifts in approach over those decades highlight the dynamic nature of scientific understanding.

1.9 Classification of Culicidae

Mosquito taxonomists use new methods of computerized and molecular analysis and comprehensive data sets to address the phylogeny and classification of mosquitoes. The family Culicidae, derived from *culex,* the Latin name for 'gnat' is a member of one of the main stocks of Nematocera, the infraorder Culicomorpha. It consists of two superfamilies (chironomoidea and Culicoidea) that include all the piercing/sucking nematocerans, both predators and blood-feeding biters. The superfamily Chironomoidea comprises the families Chironomidae and Thaumaleidae, which have non-piercing mouthparts, and Simuliidae and Ceratopogonidae, which pierce either vertebrates or invertebrates. The superfamily Culicoidea comprises the Dixidae, Corethrellidae, Chaoboridae, and Culicidae. Chaoboridae and Culicidae feed on vertebrate blood. Several of these families are superficially similar. However, among all the culicomorphs, the long proboscis of mosquitoes is distinctive. It is considered the most specialized of biting mouthparts among Nematocera and indicates a long and close association of mosquitoes with vertebrate animals.

Culicidae, the mosquito family, is comprised of 41 recognised genera incorporating about 3,500 species, many of which are vectors of disease pathogens. The largest number remaining to be discovered probably inhabits tropical rainforests, where faunas are more diverse but less well-surveyed than in temperate regions. Species that have been studied intensively often reveal that they consist of complexes of closely related species, indicating that many reproductively isolated and niche-specific forms remain to be identified or are undergoing speciation. The current Culicidae classification recognises three subfamilies: Anophelinae, Culicinae, and Toxorhynchitinae. Recent cladistic analysis of morphological and nucleotide-

sequence data supports the idea that the Anophelinae are only distantly related to the other subfamilies and that the Toxorhynchitinae do not merit subfamily status (Harbach and Kitching, 1998). Anophelinae eggs bear characteristic floats, their larvae lack air tubes, and adults have elongated palps in both sexes. Typical Culicinae and Toxorhynchitinae larvae have air tubes and adult females have short palps. Toxorhynchitines are all predaceous larvae. Are unusually large, and have a curved proboscis suited for feeding on only nectar.

The subfamily Culicinae includes about 1,500 species belonging to more than 20 genera. Two-thirds of the total described species of mosquitoes are scattered under the genera *Culex* and *Aedes*. Subfamily Anophelinae is further subdivided into three genera viz. *Anopheles, Chagasia,* and *Bironella*. Some members of the genus *Anopheles* are the vectors of human malaria throughout the world.*Chagasia* and *Bironella* are not reported from Indian region and are not involved in malaria transmission

1.10 Diversity and Distribution of Mosquitoes

Indeed, mosquitoes are widespread and divers, thriving in various climates globally. Their adaptability allows them to inhabit regions ranging from temperate to tropical, and surprisingly, even beyond the Arctic Circle. The multitude of mosquito species showcases the adaptability and resilience of this insect family. The mosquitoes are most diverse and least known in tropical forest environments. The greatest diversity of mosquito species is found in the neotropical region (31% of total known species) followed by the oriental (30%) and Afrotropical (22%). And Australasian (22%) regions. The Nearctic region (5%), including the United States and Canada, has the lowest species diversity (Rueda, 2008).

Along with the Neotropics India which is included in the oriental region is regarded as one of the richest biogeographic regions for mosquitoes in the world(Gaston & Hudson 1994). India is ranked fifth in terms of mosquito biodiversity after Brazil, Indonesia, Malaysia, and Thailand (Foley et al. 2007).

Documentation of mosquitoes in India shows that 404 species of mosquitoes (404/3541), which is >12% of all the world fauna, belonging to 50 genera and two subfamilies (12 tribes) are seen in India (Tyagi, 2015).

118 species of mosquitoes under 15 genera are reported from Kerala. The genera reported from the state so far are *Anopheles, Aedes, Culex, Mansonia, Armigeres, Heizmannia, Uranotaenia, Orthopodomyia, Haemogogus, Topomyia, Ficalbia, Myomyia, Tripteroides, Verralina*and*Toxorhynchites* (Sumodan, 2014).

1.11 Morphological and Molecular Identification of Mosquitoes

The intricate evolutionary trends of life are understood by the description and study of biodiversity. Toward this goal, the Linnaean classification of animals and plants made an important step. For more than two centuries we followed the systematics based on morphological characteristics. In addition to morphological techniques, several approaches to identify mosquitoes have been used, including cytogenetics (polytene chromosomes with discernible banding patterns), electrophoresis (allozymes); and other molecular methods involving DNA, particularly microsatellites, markers, randomly amplified polymorphic DNA, and PCR and amplification of rDNA (Walton et al.,1999). Omics technologies play a crucial role in dissecting the intricate variations within mosquito populations, providing deeper insights into their evolutionary biology.

Vector control is one of the most successful strategies for the suppression of mosquito-borne diseases. As mosquitoes are vectors of many human diseases, accurate identification is essential in implementing vector control programmes. Two major approaches used in species identification are morphological characters and DNA barcoding. Most of the epidemiologically important mosquito species exist in the form of complexes of two or more cryptic species with similar overlapping or confusing morphotaxonomic characters making their identification difficult. These complexes were resolved by a multi-disciplinary approach including analysis of polytene chromosomes, amino acids, and enzyme profiles. As a result, a variety of molecular markers were developed to establish the exact taxonomic and phylogenetic status of species and their populations (Kaur,2010). Unambiguous species identification is done by simple and reliable molecular systematics (Manguin et al. 2008). Different target genes such as internal transcribed spacer 2(ITS 2), Cytochrome oxidase I (COI) and II(COII) have been used for species identification in many *Anopheles* complexes (Yajun et al. 2006). Compared to traditional morphology-based taxonomy, the modern system of taxonomy-DNA barcoding produces results with very high precision and accuracy within a short period (Thilini, 2017).It’s fascinating how mitochondrial genes, especially the COI gene, with their abundance, lack of introns, limited recombination exposure and haploid inheritance serve as superior markers. The COI gene’s variations make it a reliable tool for DNA barcoding and species identification in mosquitoes, while its conserved domains and variable regions contribute to evolutionary studies.

1.12 Mosquito-Borne Diseases

MOSQUITO	DISEASE CAUSED	TYPE OF PATHOGEN
Aedes	Chikungunya	Virus
Aedes	Dengue fever	Virus
Aedes	Lymphatic filariasis	Parasite
Aedes	Rift valley fever	Virus
Aedes	Yellow fever	Virus
Aedes	Zika	Virus
Anopheles	Malaria	Parasite
Anopheles	Lymphatic filariasis	Parasite
Culex	Japanese encephalitis	Virus
Culex	Lymphatic filariasis	Parasite
Culex	West Nile Virus	Virus

Table 1: Mosquito borne diseases(Source: WHO 2020)

1.13 Control of mosquitoes

The intricate relationship between mosquito vectors and the transmission of diseases like malaria and lymphatic filariasis has persisted despite a century of awareness in the medical community. Control efforts have faced challenges, including the rapid emergence of insecticide-resistant mosquitoes and drug resistant parasites. Beyond these biological factors, environmental, economic, sociological, political and demographic elements also contribute significantly to the re-emergence of mosquito borne diseases in tropical and subtropical regions, particularly affecting less privileged populations. Traditional mosquito control methods, once centered on environmental modification and pesticide use, face limitations due to concerns about environmental and human health, as well as the development of pesticide-resistant mosquitoes. In response, molecular biology and genomics are now driving research towards innovative disease control strategies. Depending on the context, source reduction, biocontrol, larviciding, or adulticiding may be employed using techniques like habitat modification, pesticides, biological control agents, and trapping to manage mosquito populations effectively.

1.14 Brackish water habitats

Fresh, brackish, and saline waters are respectively defined as containing <0.5,0.5-30 and >30 ppt (Ramaswamy, 2014). Mosquito larvae are mostly restricted to freshwater environments. Still, all 3 major genera of medical importance (*Aedes*, *Anopheles* and *Culex*) include both freshwater and saltwater species (Coluzzi and Sabatini 1969, Bradley 1987, Jude et;2012).

Brackish water is any water that exhibits salinity intermediate between seawater and fresh water. It is formed along coastlines by mixing wherever seawater is diluted by fresh water to form water of intermediate salinity. Three main types of brackish water are recognised.

1.Estuaries: the regions through which rivers discharge to the sea.

2. Coastal lagoons: bodies of coastal water that are separated from an adjacent sea by barriers of sand or shingle but that nevertheless receive a significant input of seawater.

3. Inland seas such as the Baltic that have a very limited exchange with the ocean into which copious freshwater discharges.

Other less important categories of brackish water include small high-level rock pools on rocky shores and saltpans on salt marshes and numerous manmade brackish habitats such as those drainage ditches that have faulty sluices, boating lakes fed by pumped seawater, scrapes in low-lying coastal bird reserves.

1.15 Brackish water habitats of Kerala

Coastal brackish water wetlands are among the most common and productive habitats that play an important ecological role in the interface between marine and terrestrial environments. Because of their location, these wetlands are spatially and temporally dynamic and highly productive ecosystems, with a wide variety of critical habitats and species including vector mosquitoes and significant biting pests. The backwater system along the southwestern coast of India, bordering Kerala, is a vital inland water resource, constituting about 68% of Kerala's inland water. Its unique blend of freshwater and marine characteristics creates a rich ecosystem, fostering diverse fauna and flora. The estuarine environment supports economically significant marine and freshwater organisms, with the backwaters of Kerala spanning the entire coastal length of the state. In Kerala, there are nearly 30 brackish water perennial/temporary estuaries, roughly parallel to the Arabian Sea, covering an area of 2, 42,600 ha.

1.16 Climate change and habitat adaptation

The interconnectedness of modern transport systems, coupled with global factors like economic growth and climate change, indeed poses challenges in managing the spread of diseases and invasive species. Addressing these issues requires international cooperation and proactive measures to mitigate potential threats to public health and ecosystems (Manguin and Boete, 2010).

Global increases in temperatures and urbanisation are impacting the epidemiology of mosquito-borne diseases. Urbanization processes create suitable habitats for vector mosquitoes in which there are a reduced number of predators, and human hosts are widely available (Wilke, 2019). The impact of rising Sea levels on coastal ecosystems can indeed exacerbate health risks by altering the habitats of disease vectors. Understanding and mitigating these environmental changes are crucial for preventing the spread of mosquito-borne diseases in vulnerable regions (Surendran, 2011, Ramaswamy, 2012). Over the coming century global climate change remains one of the biggest environmental threats to human welfare. It has been reported that global warming changes the distribution, intensity of transmission and seasonality of malaria in sub-Saharan Africa. Global climate change remains one of the biggest environmental threats to human welfare over the coming century. Malaria is considered one of the major vector-bornediseases most sensitive to changing environmental conditions (Martens, 1998, Rogers, 2000).

Recent reports show that differences in salinity tolerance may also underlie habitat segregation between the closely related siblings of mosquito complexes. Therefore, salinity tolerance of mosquito larvae constitutes a major physiological factor that characterizes the ecological niche of these species' complex and may be pivotal to adaptive radiation and speciation that have occurred or are still undergoing in those species' complexes. Alternation between dilution by rain and, salinity increase by evaporation or flooding of coastal marshes occurs in the breeding sites of brackish water mosquitoes. In addition, anthropological factors can also influence the number of salts in breeding sites by modifying coastal areas and polluting urban breeding sites. Favourable conditions for brackish water larvae also include organic pollution in the form of putrefying masses of vegetation. As a result, the larvae of these mosquitoes respond to this changing condition by adapting at varying levels of salinity (Kengne 2019). The over-exploitation of groundwater from aquifers for agricultural, domestic, and industrial use has led to increasing salinisation of groundwater in many parts of the world.

Among the potential effects of climate change on human health, the impact of vector-borne diseases has attracted increasing attention in recent years. Kerala is a suitable place to carry out studies on mosquito-borne diseases. Since warm temperatures and rainfall in the area favour the development and survival of mosquitoes and humans are heavily exposed to mosquito biting. Moreover, Kerala not only holds a high biodiversity of mosquitoes but has nearly half of its population living and working in coastal areas and maintaining close contact with the sea, creating a conducive situation for epidemiological processes. The changes in the temporal and spatial pattern of climate variables due to climate change will affect the biology and ecology of vectors and consequently increase the risk of vector-borne disease transmission.

For the last four decades, Kerala state has undergone numerous ecological changes in the form of water extraction and changes in water courses and construction of irrigation canals including habitat modification for the development of agriculture which has resulted in the vast expansion of water bodies that support mosquito breeding (Jomon, 2009).

1.17 Ecotypes

An ecotype represents specialized groups within a species, adapted to specific local environments through genetic variations. This adaptation helps them thrive in particular ecological niches. The implication is that those individuals who were best adapted to the prevailing conditions left the most offspring. Moreover, those more successful individuals carry genes that are partly responsible for their success in that environment. Thus, the adaptations of these ecotypes are based on the interactions of their own species' sets of genes with their environment. It is possible that some of the observed variation in ecotypes results from the ability of individuals which have some range of response as the environment changes. An ecotype is a phenotype that is permanently adapted to the new habitat. Therefore, it is a genotypically adapted phenotype. Ecotypes showcase phenotypic variations within a species, but these differences are typically not significant enough to merit classification as a subspecies. As a result, ecotypes don’t hold a specific taxonomic rank. They are capable of interbreeding with other geographically adjacent ecotypes without loss of fertility or vigour.

1.18 Objectives

1)To identify and document the mosquito species breeding in brackish water habitats in North Kerala (Kasargod, Kannur, Kozhikode, and Malappuram districts) using morphological and molecular techniques.

2)To study the Salinity tolerance of mosquitoes breeding in brackish water.
3)To compare the salinity tolerance of mosquitoes breeding in brackish water with the same species breeding in freshwater in order to investigate the possibility of ecotypes.
4)To prepare pictorial key to the species of mosquitoes breeding in brackish water habitats of North Kerala.

1.19 Scope of the study

Most of the faunistic studies of mosquitoes provide information on the distribution of mosquito species in different regions or states. The species diversity of mosquito vectors plays a crucial role inthe transmission of diseases. Mosquito species that breed in salt marshes along the Arabic coast of Kerala can be a considerable nuisance to humans and are likely to vary in their importance in transmitting a range of arboviruses and parasites.

The projected climate change scenario estimates that the atmospheric temperature across Kerala will rise by 2°C by the year 2050(source: Department of Environment and Climate Change, govt. of Kerala). also, it is estimated that if the sea level rises by one meter, 169 km² of the coastal region would be inundated.

To comprehend the epidemiology of different diseases, an inventory of mosquito biodiversity is essential. Such systematic inquiries on the geographic distribution of insect vectors will help to evaluate the transmission risk of vector-borne diseases in a better way. Assessment of habitat characteristics of mosquitoes in the area helps in the proper understanding of the growth conditions they prefer and aids in the process of area-wise eradication measures.

Creating a species inventory is fundamental for documenting mosquito biodiversity in epidemic and endemic areas. Simplifying and expediting the identification process, especially through straightforward and rapid methodologies like DNA barcoding, is crucial for effective taxonomical study and subsequent management strategies.The application of DNA barcoding in identifying mosquito species, especially when

morphological characteristics are insufficient, is a promising approach. This molecular technique proves valuable, particularly in swiftly identifying major vectors, aiding efficient management during disease outbreaks.

Indeed, identifying and locating mosquito breeding habitats is crucial for health authorities to implement targeted vector control strategies. Mosquitoes display preference in habitat selection, and periodic screening of larval habitats becomes mandatory for the precise application of larvicides against specific vectors, enhancing the effectiveness of control measures.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Global level

The history of mosquito taxonomy, starting with Linnaeus in 1735.He is considered to be the father of systematics and named the first genus *Culex* in the year 1735. He described 6 species belonging to this genus in the tenth edition of his book, the Systema Naturae in 1758. Meigen's (1818) descriptions of the genera Aedes and Anopheles marked a pivotal moment in mosquito taxonomy. The subsequent revelation in the early 19th century that mosquitoes transmit malaria and yellow fever sparked widespread interest, leading to increased efforts in the description and classification of these insects. The employment of Fred V Theobald by the British Museum (Natural History) in 1899 led to the introduction of numerous new generic names for classifying mosquito species. However, over time, it became evident that Theobald's classification system was deemed impractical and lacked a natural basis. Following Theobald's Monograph of the Culicidae in 1910, substantial changes occurred in mosquito taxonomy over two decades. F.W Edwards in Europe and Harrison G Dyar in North America played crucial roles in advocating for a more conservative classification system. Edward's work in 1932, emphasizing broad group concepts, laid the foundation for the traditional classification of the twentieth century by researchers such as Stone et al. (1959), Belkin (1962), and Knight& Stone (1977).

Christopher (1933), Edwards (1941), Stone (1961, 1963, 1967, 1970), Taylor (1967), Reid (1968), Smith (1969), Rajavel etal., (2004, 2005), Tyagi et., (2015) were the eminent scientists worked on mosquito taxonomy from different parts of the world.

James and Liston (1911), Barraud (1934), Stone et al., (1959), Christopher (1960, Reid and Knight (1961), Knight and Stone (1977), Nair and Mathew (1993), Nagpal and Sharma (1987, 1995), Sathe (2006) etc., have been worked on the taxonomy of Indian mosquitoes.

'Mosquitoes of the world' is an extremely useful resource for entomologists and it provides a good starting point for newcomers to the field. Mosquitoes of the World by Wilkerson RC, Linton YM, Strickman D is the most comprehensive reference work on mosquitoes ever made. part 1 consists of ten chapters on a wide range of topics such as evolution, life cycle, development, feeding, mating etc. Mosquitoes of the World is a composite guide to the taxonomy and systematics of mosquitoes. The second part of volume 1 comprises diagnostic morphological characters, systematics, distributions, bionomics, associated pathogens and

exemplar DNA sequences for each of the 41 genera and 128 globally important mosquito species. Volume 2 is the first printed catalogue of all described mosquito species (including fossils and synonyms) in over 40 years, and follows Knight and Stone's seminal work published in 1977.During the intervening four decades, the number of validated species and subspecies has increased from 3,133 to 3,700: an increase of 16%. Alan Clements 'three-volumeBiology of Mosquitoes together with Mike Services' classic "Mosquito Ecology: Field SamplingMethods would be a good start for anyone venturing into the world of mosquitoes.

Salinity is described as the mass in grams of dissolved inorganic matter in 1 kg of water (Stumm and Morgan, 1996). It is hence asserted as parts per thousand. Seawater has a notably uniform salinity between 33 and 37 ppt. water salinity is an attribute of the total concentration of soluble inorganic ions (Canedo-Arguelles et al.2013) and is largely attributed to sodium and chloride (Canedo-Arguelles et al.2016).

Coastal brackish water is among the most common and productive mosquito larval habitats on earth. These habitats are also important for public health worldwide as immature habitats for disease vector mosquitoes (Leisnham and Mohapatra, 2011; Van Schie et al., 2009; Medlock et al.,2005; James et al., 2012 and Becker et al., 2003).

According to Tennessen (1993), habitats having shallow water bodies with higher nutrient and salinity levels have higher rates of mosquito survival due to abundant food sources and low predator populations.

Biologists have long been fascinated by the capacity of mosquito larvae to survive in saline environments that are much too toxic for any aquatic vertebrate.The effect of salinity on the development of mosquitoes and the range of tolerance of salinity has been investigated by several workers since 1914.

Ochlerotatus (Aedes)sollicitans,Ochlerotatuscantator, Aedes aegypti, Culex pipiens, and *Culex salinarius* were tested in the USA for the effects of the pure salts found in the sea water-calcium chloride, magnesium sulphate, potassium chloride, magnesium sulphate, potassium chloride and sodium chloride. The effects of seawater of various degrees of concentration were also studied. *Culex pipiens* hatched in salt water were unable to withstand the toxic action of salt. *Aedes sollicitans* and *Aedes cantator* were more susceptible to a high concentration of sea water. It was found that even a 40% solution of seawater was not strong enough to kill the larvae of Aedes sollicitans. Field observations show that in large shallow

salt pools, mosquito larvae may gradually become acclimatized as the salinity increases owing to evaporation (Chidester F E, 1916).

Studies were carried out in Puerto Rico to find out the relation of mosquitoes to the salt content of their breeding places. Some of the mosquitoes were found in the salinity ranges of 0-950 parts chlorine per 100,000. They include *Anopheles crucians*, *Anopheles albimanus*, and *Anopheles grabhami* and were confined to brackish water (Tulloch GS, 1937).

To confirm the previous work by Wanson and Nicolay that *Culex fatigans (Culex quinquifasciatus)* in the Belgian Congo develop normally in water containing 30 g chloride per litre, some laboratory studies were carried out. The results show that first or fourth instar larvae did not develop normally in concentrations greater than 10 gm salts per litre. When the salinity of the water gradually raised from 9-11-16 gm, a few adults were obtained from water containing 14 gm. Pupae were not affected by salinity and developed into adults in water containing 105 gm salts per litre (Woodhill AR, 1938).

Beadle (1939) was one of the first to investigate the physiology and focused on *Aedes detritus*, a species that resides in coastal marshes in northern Europe. He was the first to demonstrate that the larvae found in saline waters possess specific osmoregulatory mechanisms not found in freshwater forms. Much work has been done with marine, brackish, and freshwater animals to discover the nature of the mechanism by which the composition and concentration of the body fluids can be maintained differently from those of the external medium. He demonstrated that the organ in the posterior portion of the insect is responsible for the osmoregulation.

Breeding of *Aedes sollicitans* was investigated in Illinois and Indiana in areas that were intermittently flooded with salt water. The salinity was found to be 6.8-8.8 ppt. The larvae showed a surprising tolerance for acid water (pH2.7). Aedes dorsalis was also present in areas flooded with salt water from oil wells (H.L. Fellton, 1944).

The mosquitoes *Aedes taeniorhynchus* and *Aedes sollicitans* lay eggs in saline soil which are likely to be flooded by either tide or rain water. These two mosquitoes were restricted to coastal areas with a few exceptions (Maurice W Provost, 1951).

Bates (1959) suggested that the difference in salinity tolerance may be useful in determining the different subspecies of *An. maculipennis* complex in Europe. Marchal (1959) found variation in the anopheline populations of a pond in which salinity varied greatly during the

rainy season, even though the fluctuations in salinity showed no direct influence on larval morphology. The eggs of *Culiseta* incidence were collected from water with salinity in the range of 7-7.5 ppt.

Anopheles sundaicus breeds in 13-18 ppt either natural or manmade habitats, in Java, Indonesia. The natural swamps are due to the silting up of the river mouths, causing brackish water lagoons. Manmade sites are due to the clearing of mangrove swamps and the making of fish ponds or salt evaporation pools (Van F Ronnefeldt, 1959).

Rearing of the salt water, coastal breeder *Anopheles litoralis* King (a suspected secondary malaria vector in the Philippines) is completed in the saline water. Saline water was used for rearing the immature stages and for the oviposition medium (Richard F Darsie et. al, 1970).

Malaria transmission by *Anopheles litoralis* King, a saltwater breeder was found in the malaria survey carried out by Benjamin D Cabrera, in 1970 in Pangutaran, Sulu, Republic of Philippines.

In contrast with the relative stability of the sea, the waters of estuaries and brackish water environments are characterized by sudden and often extensive changes in salinity and temperature. The organisms that live in these physiologically demanding environments are expected to exhibit physiological responses to fluctuating parameters. The brackish water environments have played a bottleneck role in the emergence of animal life from its original home, the sea. Relatively few species are characteristic of brackish waters and the majority of them are euryhaline (Kinne, 1971).

Anal papillae from fourth instar larvae of the saltwater mosquito *Aedes campestris* were studied in both hyperosmotic and hypoosmotic external media. It is suggested that in both conditions anal papillae are actively engaged in ion transport and the changes in transport rates may be due to minor structural modifications such as permeability changes or activation of enzyme systems already present in the cell membranes (J Meredith et.al, 1973).

Ramsay (1950) estimated that the rectum was the site of ion concentration and the anus is responsible for the excretion of hyperosmotic urine. Similar results were obtained in the species *Opifex fuscus*, a species endemic to New Zealand (Nicolson and Leader, 1974).

An important feature found in the saline water forms of *Aedes* and *Opifex* is a two-part rectum, in contrast to freshwater forms that have a rectum with only one segment. The

additional segment found in saltwater breeders acts as a salt gland (Bradley and Philips (1975, 1977).

Anopheles sundaicus and *Anopheles subpictus* breed mostly in the brackish water fishponds which exist in clusters along the entire coastline. Low salinity of 5-10 ppt and extensive surface coverage with green algae favour high larval densities of *Anopheles sundaicus*, while *Anopheles subpictus* thrives in highly saline water. Although they cohabit in the same pond, *Anopheles subpictus* larvae always appeared in far greater numbers than *Anopheles sundaicus* (RT Collins et. al, 1979).

Studies in Japan show that the larvae of the marine mosquito *Aedes togoi* tolerate environmental salinities ranging from freshwater to 300% seawater. The physiology of larvae especially the rectum is involved in hyperosmotic urine production and the anal papillae appear to be the extra renal organ (Koshu Asakura, 1980).

The correlation of density of adults of *Anopheles merus* with rainfall and salinity at their breeding sites in Kenya was studied and peak density was found with salinities of 30-50% sea water in the breeding sites. Laboratory experiments showed that larvae could complete development in 0-100% seawater, with optimum development at 0, 40 and 60% seawater. Density was found to decrease with increasing rainfall (F.W Mosha et. al, 1982).

Aedes mosquitoes are known for their adaptability to a wide range of osmotic concentrations ranging from distilled water to several times the concentration of seawater during their larval development (TJ Bradley et. al, 1984).

Larvae of *Culisetainornata* in California can complete development and can survive in dilutions of seawater. These species can regulate both sodium and chloride ion concentrations in the haemolymph over the full range of salinities tested (Margaret Garrett, 1984). They propose that mosquitoes exhibiting this osmoregulatory pattern should be described as brackish water species.

It's interesting to note the specific salinity ranges for the mosquito species found in the brackish pond on the Kenya Coast. It shows the following ranges. *Aedes albocephalus* (salinity range 21-30 ppt), *Culex sitiens* (31-40), *Culex tritaneorhynchus* (41-50), *Culex thalassius* (41-50) and *Anopheles gambiae* complex (13-54) (L. M. N. Rogo et. al, 1985). The absence of larvae beyond certain salinity thresholds indicates the critical role of salinity in

their breeding conditions. No larvae were present when salinity was found above 54 through evaporation or fell below 13 after rain.

Larvae studied in the genera *Culex* and *Culiseta* show that a sufficient pattern of osmoregulatory strategy was present in these species (TJ Bradley, 1987).

Culex tarsalis larvae were capable of surviving and developing in dilutions of seawater ranging from 0 mosmol |-1 to 700 mosmol|-1. In every concentration of seawater tested, the larvae regulate the levels of Na, K, Mg, Ca, and Cl in the haemolymph. An increase in haemolymph osmotic concentration observed in media above 400 mosmol |-1 is due to the accumulation of proline, serine, and trehalose (Margaret A Garrett, 1987).

The varying tolerance levels to seawater among different Afrotropical Anopheline species such as *Anopheles tenebrosus*, *An. mousinhoi*, *An. pharoensis* and *An. quadriannulatus* as observed by Maureen Coetzee in 1988, highlight the diverse adaptations within this group. *An. merus* showed significantly better survival in 25% seawater.

Tropical coastal areas are generally overgrown by mangroves. Mangroves are distributed in tropical and subtropical regions in the intertidal saline zone (K. Kathiresan et.al, 2001). The mangrove habitat is rich in detritus surface and high in organic soil content which feeds mosquito larvae (S.A Ritchie et.al, 1991). Factors that contribute to mangrove growth include salinity, DO, P, sand, mud, temperature, pH, Organic matter, and N (E.D Hastuti et.al, 2012).

The survival of immature stages of *Culex sitiens* was found in 6-8 ppt salinity in Brisbane, Australia (P. Mottram, 1994). They also found that *Aedes vigilax* larvae can survive up to 25 ppt salinity. In Australia, *Culex sitiens* occurs along the brackish coastal wetlands.

Ross River virus was isolated from brackish water breeding *Aedes funereus* and *Culex sitiens*, and salt marsh breeding *Aedes vigilax* from Brisbane, Australia (Scott A Ritchie, 1994). This study implicates brackish and saline mosquitoes as important suburban vectors of the Ross River virus and indicates the need for refocusing mosquito control priorities.

Saline tolerance of two species -i.e., *Aedes nigromaculis* and *Aedes melanimon* were examined by performing survival tests in water ranging from 10-100% seawater (Wesley B Grueber, 1994). *Aedes nigromaculis* showed increased mortality above 30% seawater, while *Aedes melanimon*showed high rates of survival in all salinities tested.

Studies related to salinity experiments in Oman encountered mosquitoes in a tank having salinity in the range of 25% seawater. Salinity tolerance estimations were also done. *Culex Sinaiticus* survived in 50% seawater. *Culex sitiens* were most successful in 66% seawater, and survival was significantly reduced in fresh water. *Culex quinquifasciatus* survived in 28% seawater. Some *Anopheles stephensi* were able to tolerate 50% seawater (Derek Roberts, 1996).

A study related to the physical and chemical characteristics of breeding places of mosquitoes in Kuwait estimated that the breeding places have salinity in the range of 0.2 to 30%. The species encountered were *Culex pipiens*, *Culex quinquifasciatus*, *Culex tritaneorhynchus*, *Culisetalongiareolata*, *Culisetainornata*, *Anopheles stephensi*, and *Aedes caspius*. As regards chemical factors, larvae of all mosquito species in Kuwait state are mostly alkalinophiles (AM Salit, 1996).

Brackish pools, with salinities as high as 35 ppt, were used by *An. farauti* and *An. hilli* in the larval sites surveyed in Northern Australia (R. D Cooper, 1996). *Anopheles farauti* and *An. hilli* have adapted to saline conditions (Russell 1979, Sweeny, 1987) In this survey it was found that brackish water sites were occasionally utilized by *An. annulipes, An. amictus, An. novaguinensis, An. meraukensis*and *An. bancroftii.*

Culex sitiens was collected in salinities between 10 and 115%, with peak abundance in 30% sea water in Oman (DM Roberts, 1997). *Cx.tritaeniorhynchus* had a peak abundance in 30% seawater.

Mosquitoes breeding in coastal saline habitats of Australia such as *Aedes alternans*, *Aedes camptorhynchus, Aedes vigilax*and *Culex sitiens* may tolerate salinity to a greater extent. *Aedes vigilax* is the most important coastal vector of Ross River and Barmah Forest viruses (Russell,1998).

Larvae of *Aedes togoi* are abundant in the supralittoral rock pools in Hong Kong with salinity ranging from 0% (after rainfall) to three times seawater strength- 100% (in summer). Larvae on the shore can tolerate higher salinities than recorded in the laboratory which may be due to behavioural mechanisms that allow *Aedes to go* to live in such an osmotically stressful environment (Gray A Williams, 2000).

Aedes togoi adult emergence was investigated in the brackish water of rock pools near a coastal area in Pusan, South Korea (Dong-Kyu Lee, 2001).

An investigation of salinity tolerance of *Culex quinquifasciatus* and *Culex tarsalis* shows that these two mosquito larvae were capable of tolerating salinity using an unknown mechanism (ML Patrick et. al, 2000, 2001).

One of the most common and productive mosquito larval habitats on earth was coastal brackish water. As larval habitats for disease vector mosquitoes, these coastal habitats are also important for public health worldwide (Becker et.al.,2003, Medlock et.al., 2005, Van Schie et.al.,2009, Mohapatra, 2011, James et.al., 2011).

The studies on the oriental *Anopheles sundaicus* shows that the capacity of *Anopheles sundaicus* to develop in seawater, various concentration of brackish water, and freshwater represent a threat to coastal and island populations of humans in Southeast Asia. However, its presence is restricted along the coast. *Anopheles pseudopunctipennis* also can develop in a range of habitats from freshwater to seawater (Isabelle Dusfour, 2004).

Bradley (1994) reported that only 5% of all extant species within the family Culicidae are capable of surviving in salt water. Salt resistance has evolved at least 5 times independently in the Culicidae. Mosquito larvae breed in various habitats, with most species being found in freshwater (Sanchez-Ribas etal;2015). Anyhow, some larvae breed in brackish water with varying levels of salinity (Chirebvu and Chimbari 2015). Saltwater tolerance is likely a derived trait and freshwater restriction is an ancestral condition in Culicidae, (Bradley 2008, Albers, and Bradley, 2011). Some mosquito larvae can tolerate certain levels of salinity and finally survive to adulthood, fly away, and transmit diseases (Patrick et al;2001). On the other hand, recent studies have shown that vector mosquitoes are adapting to exceptional environments for breeding, with exalted levels of salinity (Waniwa, 2011).

Salinity tolerance is a significant factor that controls the ecology of disease vector mosquitoes by deciding their choice of larval habitats and ultimately governs their ecological and geographical distribution (Ramaswamy and Surendran 2011). 10 different genera ofmosquitoes show salt water tolerance and thus appear to have developed gradually in independent mosquito lineages (O'Meara, 1976,Bradley 2008,Albers and Bradley 2011).The mosquito larvae which is tolerant to salt water can develop normally in both freshwater and saline habitats, but selection of saltwatermay be adaptive because of high nutrient levels, reduced competition and predation in brackish or saline sites, particularly those sites related to rapid changes to salinity (Bradley 2008).

Several mosquito species with salinity-tolerant larvae are vectors of human arboviral and parasitic diseases in many parts of the world. Eg: - *Ae. togoi* Theobald found in coastal marshes and *Ae. Taeniorhynchus* Weidmann in splash pools, whose osmoregulatory mechanisms have been well studied. *Ae. aegypti* and *Ae. albopictus* can oviposit and undergo pre-imaginal development in fresh and brackish water under field conditions, but that oviposition diminishes with increasing salinity of water.

The studies in Haroonabad, Pakistan related to mosquito species breeding in wastewater estimated 4 species of *Anopheles*, and two species of *Culex*. The identified *Anopheles* are *Anopheles subpictus*, *Anopheles stephensi*, *Anopheles culicifacies*and *Anopheles pulcherrimus.* The two species of *Culex* are *Culex quinquifasciatus* and Culex tritaneorhynchus. Almost similar results were obtained from Faisalabad, Pakistan. *Anopheles subpictus*and *Anopheles stephensi*were the dominant *Anopheles* species in wastewater-irrigated sites, with *Anopheles culicifacies* recorded in low numbers. Among the *Culex* species, *Culex tritaneorhynchus* was most frequently recorded and *Culex quinquifasciatus* was the second most abundant species encountered in wastewater-irrigated areas (Muhammad Mukhtar et. al, 2003, 2006).

Verrallinafunerea, a vector of the Ross River virus was found to be a brackish waterbreeder with salinity in the range of 0-35 ppt in southeast Queen Island, Australia. It is a brackish water mosquito species found most commonly in Indonesia, Papua New Guinea, and the northeastern coastal regions of Australia (Jason AL Jeffrey, 2005, 2006).

A larval survey in relation to environmental factors in Indonesia revealed that *Anopheles aconitus*and *Anopheles barbirostris* were associated with paddies with relatively shallow water depths, higher water temperatures, higher acidity, and salinity concentrations (Criag A stoops et. al, 2007).

Among *Anopheles* species in Southeast Asia, *Anopheles sundaicus* is a principal malaria vector along coastal areas of the mainland and islands. Its reported coastal distribution ranges from northern India to southern Vietnam and the islands of Nicobar, Andaman, Borneo, Java, Sumatra, and Sulawesi (Isabelle Dusfour et. al, 2007).

Studies in Sucre state, Venezuela for the control of malarial vector *Anopheles aquasalis* show that the vector can be found in a variety of habitats such as lakes, canals, marshes, mangrove swamps, or flooded fields that vary greatly in salinities i.e., 0.4-38.4 ppt. Laboratory

experiments have confirmed that this species is physiologically adapted to salinities of between 10 and 20 ppt (Osborn et. al, 2007).

Salt tolerance in the two closely related isomorphic species. ie. An. farauti species No.7 and *An. farauti* Laveran s.s appears to be a shared derived character within the Farauti complex. The seawater tolerance test was previously thought to be a diagnostic for the saltwater tolerant *An. farauti Laveran s.s. An. farauti* No.7 also shares this character thereby making their identification difficult ((D.H Foley et.al, 2008).

The species *Aedes melanimon* provides an interesting situation. The larvae have a two-part rectum and can survive in full-strength seawater. In nature, however, the larvae are found in freshwater habitats in flooded pastures in Southern California. That is the physiology and ecology of the species do not always map precisely on each other. Another species *Aedes nigromaculis*, with one rectum, is a sister species to *Aedes sollicitans*- a saline water species, the closest relative to *Aedes taeniorhynchus* another saline water species. This species cluster provides the strongest evidence that reversion to the freshwater condition has occurred in this clade (T J Bradley, 2008).

The impact of dryland salinity and waterlogging in inland southwestern Australia, as discussed by Andrew J et. al, in 2008, is notable for its connection to the succession of salinity tolerant mosquito species *Aedes camptorhynchus*.This ecological shift contributes to an elevated risk of Ross River Virus transmission in the region.

The diversity and seasonality of the mosquito community studies in the northern Adelaide region of South Australia over 7 years reveals that salt marsh mosquitoes *Aedes camptorhynchus* and *Aedes vigilax* were most abundant in the coastal areas. These two salt marsh mosquitoes cause extreme levels of nuisance biting (Craig R Williams et. al, 2009).

Aedes natronius, Culex tenagius and *Culex nakuruensis* were found to breed in salinities ranging from 0-8% NaCl in the region of Lake Manyara, a saline lake in Tanzania. It is considered that these species were able to breed in both fresh and saltwater (A.R. Njogu, 2009).

The most likely vectors of arboviruses and sources of mosquito nuisance in Spencer Gulf Coast of South Australia have been identified as salt marsh breeding species *Aedes camptorhynchus* and *Aedes vigilax* (Samantha R Williams et. al, 2009).

Competitively inferior halotolerant mosquito species *Aedes alboannulatus* and *Aedes camptorhynchus* are released into the ecosystem when salinity increases. This study also suggests that salinity in the Western Australia Wheatbelt may facilitate a greater abundance of halotolerant mosquitoes *Aedes alboannulatus* and *Aedes camptorhynchus* – a vector of Ross River Virus (Scott Carver et. al, 2010).

Larval abundance with topography in Indonesia shows that the major malaria vectors namely *Anopheles sundaicus, Anopheles subpictus, Anopheles maculatus,* and *Anopheles annularis* were associated with coastal areas. Non-vector species Anopheles vagus was also encountered in coastal areas (Ermi Ndoen et. al, 2010).

The larval survey conducted in the eastern province of Sri Lanka after the 2004 Asian tsunami, as reported by Jude in 2010, revealed the presence of *Anopheles culicifacies* E, a significant vector for falciparum and vivax malaria in Sri Lanka. Notably, this species demonstrated an ability to breed in brackish water habitats. Additionally, Anopheles subpictus species B, known for breeding in saltwater and considered a secondary malaria vector in the country, was identified at the same site.

The larval survey carried out in Thailand shows that *Anopheles epiroticus* (*Anopheles sundaicus A)* can bred in fresh, brackish, and saltwater habitats with salinity ranging from 0.5 to 119.4 g/l. It is a vector of malaria. It was the dominant species encountered there (Suchada Sumruayphol et. al, 2010).

Studies in Brazil indicate that the larvae of several mosquito species are tolerant to salinity, including some species of the genus Aedes. Observations related to *Ae. aegypti* have indicated that the species occurs in concentrations of up to 16 ppt (Marylene de Brito Arduino). Studies in Sri Lanka reported that, *Ae. albopictus* and *Ae. aegypti* can oviposit and undergo breeding in fresh and brackish water under field conditions, but that oviposition diminishes with increasing salinity of water (Surendran). In Brunei, Darussalam larval stages of *Ae. albopictus* were found in brackish water collections up to 8 ppt salinity (Fakhriedzwan). The adaptability of *Anopheles culicifacies* as observed in Oman and Sri Lanka, showcases its ability to breed in concrete reservoir tanks with brackish water, even though it generally thrives in freshwater. The tolerance of *An. culicifacies* E to salinity changes in its breeding grounds, influenced by monsoonal rain, adds an interesting dimension to its ecological dynamics. Additionally, the broader context of various anopheline species complexes such as *An. melas, An. merus* in the *An. gambiae* complex, *An. farauti* No.1 in the

farauti complex, and the *An. sundaicus* complex in Asia, highlights the diverse salinity tolerance within different mosquito species associated with malaria transmission in coastal areas worldwide (Jude et al., 2010).

A survey carried out in eastern Saudi Arabia encountered 5 species namely *Aedes caspius*, *Anopheles multicolor*, *Culex perexiguus*, *Culex pipiens* and *Culex pusillus*. The first four are major vectors. All these are encountered from breeding habitats with a salinity range of 0.3-6.4% of salinity (Ashraf M Ahmed et. al, 2011).

The physiological and morphological parameters associated with salinity tolerance were examined in the genus of *Ochlerotatus*. Saline tolerance was assayed for 11 species, six of them were found to be physiologically restricted to freshwater habitats and the remaining five could successfully osmoregulate in both freshwater and saline water, including seawater. The 6 obligatory freshwater forms had only one rectal segment and the euryhaline osmoregulators had two rectal segments (Albers, 2011).

Breeding of *Culex sitiens* and *Anopheles sundaicus* has been detected in brackish water habitats in Phang Nga province, Thailand (Samrerng et. al, 2011). The salinity was found in the range of 2.2-27.8 ppt. Their study revealed that some freshwater sites had changed into brackish water sites after the tsunami hit. *Culex sitiens* was found to be the most dominant species.

The studies related to climate change and mosquito-borne diseases in Thailand show that increased density of *Culex sitiens*, an established vector of arboviruses and *Anopheles sundaicus*was observed in an area of Thailand affected by the Tsunami. Large-scale shrimp farming in the Mekong Delta of Vietnam locally increased the density of *An.sundaicus*. Higher densities of Aedes camptorhynchus have been associated with increasing salinization of freshwater bodies due to intensive agriculture in western Australia. A reduction in the habitat of a brackish water vector has been associated with a decrease in malaria in Western Europe. *An. atroparvus* of *An.maculipennis* complex was primarily responsible for transmitting malaria until the early 1900s in marshland areas of England. The draining of coastal marshes reduced the breeding sites for *An.atroparvus*.Greater availability of brackish water bodies can lead to freshwater breeding mosquitoes adapting to breed in them. E g: - *An. stephensi* and *An. culicifacies*(typical freshwater breeders) were found breeding in brackish water bodies immediately after the tsunami of 2004 in India and five years later in eastern Sri Lanka (Ramasamy et. al, 2011).

Recent studies in coastal areas of Northern Sri Lanka by Jude, 2012 reported the breeding of several mosquitoes including vectors in collections of brackish and saline water habitats. The encountered larvae include *Aedes aegypti, Aedes albopictus, Anopheles subpictus, Anopheles barbirostris, Anopheles varuna, Anopheles culicifacies, Culex sitiens, Lutziafuscanus* within the range of salinities 2 to 68 ppt.

Studies in Thailand show that *Culex sitiens* was the dominant species found in almost every stagnant, brackish water site. A study was undertaken in the tsunami-hit areas in Phang Nga province, Thailand (SamrerngPrummongkol et. al, 2012).

The studies in Australia reveal that *Culex quinquifasciatus* can breed in brackish water and it was known to breed in brackish to saline water (Richard Russell, 2012).

In a study to implicate the performance and competitive interactions of *Aedes aegypti* and *Ae. albopictus* in different salt concentrations in North Carolina, USA it is found that *Aedes aegypti* survived in the highest salt conditions. This tolerance may allow it to use coastal containers effectively. But the ultimate effects of salt on the co-existence of these species or the exclusion of either species remain unknown (Yee et.al, 2013).

The research on the *Anopheles funestus* group to find out the effects of different salt concentrations on survival rates of larvae shows that the two members of the *Anopheles funestus* group i.e., *An. funestus* and *An. rivulorum* shows different responses toward different saline concentrations. *An. funestus* shows negative trends in hatch rate and survival rate with increasing salt concentrations. *An. rivulorum* can tolerate salt concentration (Koekemoer et. al, 2013).

Diversity studies in accordance with the physicochemical parameters in Egypt reveal the presence of 5 Culicines and 2 Anopheline mosquito species in saline breeding habitats such as released water, sewage water tanks and drainage canals. The five culicine are *Culex pipiens, Culex pusillus, Culex perexiguus, Culex theleri*and *Ochlerotatuscaspius.* The two Anophelines are *Anopheles multicolor* and *Anopheles pharoensis*. The salinity of the breeding habitats was found to be in the range of 198.4 to 698.1 mg/L (Iman, 2013).

Brackish water with salinity of 2-15 ppt in discarded plastic and glass containers, abandoned fishing boats and unused wells in coastal peri-urban environments were found to contain *Aedes aegypti* and *Aedes albopictus* larvae in Sri Lanka. This finding raises the possibility of the adaptation of dengue and chikungunya vectors to brackish water (Ramasamy et. al, 2015).

80% of global malaria morbidity and mortality is due to *Anopheles gambiae* complex. *An. merus* is a member of *An. gambiae* complex. An. merus is known to breed in salty water. Laboratory studies showed that the larvae can survive up to 25% salinity. *An. merus* had a positive correlation with a salinity of (26.4%), suggesting this species can tolerate the presence of salinity in its habitat (Pamela Kipyab et. al, 2015).

The vectors which are previously thought to be exclusive freshwater breeders can undergo pre-imaginal developments in brackish water habitats. Eg: - Recent evidence shows that *Aedes albopictus*, *Aedes aegypti* and *Anopheles culicifacies*were brackish water breeders (Ramasamy et. al, 2015).

MOSQUITO	DISEASE CAUSED	TYPE OF PATHOGEN
Aedes	Chikungunya	Virus
Aedes	Dengue fever	Virus
Aedes	Lymphatic filariasis	Parasite
Aedes	Rift valley fever	Virus
Aedes	Yellow fever	Virus
Aedes	Zika	Virus
Anopheles	Malaria	Parasite
Anopheles	Lymphatic filariasis	Parasite
Culex	Japanese encephalitis	Virus
Culex	Lymphatic filariasis	Parasite
Culex	West Nile Virus	Virus

Table 1: Mosquito borne diseases(Source: WHO 2020)

A study conducted in Negombo estuary (a partially enclosed coastal body of brackish water), revealed that *Aedes albopictus* and *Aedes aegypti* can oviposit in brackish water in field and laboratory conditions. Negombo, being a fishing village and having a high population density, the study identifies the potential risk of dengue transmission in the area (Madhushika et. al, 2016)

To find the effect of salt on *Anopheles gambiae* under laboratory conditions, a test was conducted. No larval mortality occurred between 0.03 to 13.25 ppt salinity. The level of water salinity may indicate the presence or absence of *An. gambiae* mosquito larvae and this information can be used for disease control purposes (Lukwa, et. al, 2017).

Culex quinquifasciatus, *Culex sitiens*, *Culex gelidus*, *Anopheles epiroticus* and *Aedes aegypti* were found breeding in brackish water in coastal habitats of Samut Songkhram Province, Thailand (Chaiphongpachara, 2017).

A laboratory study in France to determine the osmotic responses of 5 larvae after 24 h exposure to varying salinities, it is shown that Aedes albopictus was the most tolerant species followed by *Anopheles coluzzii, Aedes aegypti, Culex quinquifasciatus*and In *Anopheles In gambiae* in In decreasing order. *Culex pipiens* was the least tolerant species. (Pierre Kengne, 2019).

Recent studies in Mauritania, a West African country reveal that first record of *An. multicolor* in the country and they found that *An. multicolor* and *An. arabiensis* co-exist at the larval stage in the same breeding place and can develop in highly saline water. In the laboratory testing, it is found that both larvae can survive 28.9% salinity (Muhamed Aly et. al, 2020).

Recent studies in Sri Lanka reveals the distribution of different Anopheline larvae in a variety of habitats with different range of salinity. *Anopheles stephensi* was found to breed in salinity1.9-3.4 ppt, *Anopheles subpictus* (0.9-3.4 ppt), *Anopheles culicifacies* (0.9-3.4 ppt), and *Anopheles varuna* (0.5-1.4). the ability of these species to breed in brackish water habitats poses a threat to disease transmission in coastal areas (Surendran et. al, 2020).

Studies in Indonesia related to *Anopheles sundaicus* show that immature stages are typically associated with coastal, sunlit brackish water habitats and often with floating algal mats. The salinity of coastal habitats typically ranges between1.2-1.8% (seawater ~3.5%) (Din Syafruddin et.al, 2020)

Two species of *Ochlerotatus*- i.e., *Ochlerotatus dorsalis* and *Ochlerotatuscaspius* larvae were encountered from drainage water, where the salinity was 100% in Qatar. Saline water was the preferable site for females to lay their eggs in these two species (Fathima AA, et. al, 2020).

A detailed study was conducted on the aquaporin expression of *Aedes aegypti* larvae developed in fresh water and brackish water in Canada recently. The results show that aquaporin expression in the osmoregulatory organs is mostly consistent between larvae that are developing in fresh water and brackish water. This suggests that aquaporin may not have major roles in adapting to long-term survival in brackish water. *Aedes aegypti* larvae are osmo-regulators maintaining their hemolymph osmolarity in a range of ˜250 to 300 mOsmolI-1. Excess water should be eliminated and ions should be conserved in fresh water.

But in brackish water, they must reduce the accumulation of salts. (Lidiya Misyura et. al, 2020).

Climatic (Temperature, rainfall, humidity) changes due to global warming can expand the geographical range of vector mosquitoes, extend the disease transmission season, shorten the gonotrophic cycle and reduce the time taken for ingested virus to develop to infectivity in mosquitoes, thereby increasing the propagation rates of arboviral diseases by mosquitoes. Rising sea levels can therefore increase the abundance of salinity-tolerant mosquito vectors and lead to the adaptation of normally freshwater vectors to brackish water, thereby additionally enhancing transmission of mosquito-borne diseases in coastal areas. The predicted increase in the worldwide population density of coastal areas from 87 persons per km^2 in the year 2000 to 134 persons per km^2 in 2050 is also likely to exacerbate the situation by increasing human vector contact.

The species that had the highest occurrence in brackish water habitats with different salinities were *Aedes albopictus, Culex iridescens,* and *Culex dolosus* in the study done in Brazil. The study indicates that although *Aedes albopictus* preferentially inhabits mediums with lower salinity, it has a high tolerance to high salt concentration (Laura et. al, 2021).

Recent studies in Indonesia (Arini Ratnasari et.al, 2021) suggest that on a laboratory scale, *Aedes aegypti* can survive, tolerate salinity, and put eggs of up to 15 ppt salinity. The salinity tolerance test showed that 4^{th} instar larvae are highly adapted to the saline environment. Larvae from coastal areas are better able to tolerate salinity up to 13 ppt while inland larvae can only tolerate salinity up to 10 ppt.

Recent water quality assessment of mosquito breeding sites in Egypt estimates that there was a significant positive correlation between the density of *Culex pipiens* and *Culex perexiguus* and salinity (Alaa Nagy et. al, 2021).

Culex sitiens represented over 60% of all collected mosquitoes in Koh Kong Mangrove forests, Cambodia. *Aedes albopictus* and *Culex vishnui* were collected during both the dry and rainy seasons. *Culex quinquifasciatusand Aedes aegypti* were also detected in the mangroves (Pierre-Olivier Maquart, 2022).

Mosquito species develop different strategies to adapt to high levels of salinity, and tolerance to this factor varies between species. Salinity and conductivity can be considered predictor variables for the occurrence of mosquito larvae, as an increase in the values of these

parameters results in decreased species diversity, in turn increasing the abundance of salinity-tolerant species. Several mosquito species vary greatly in their tolerance to salinity and about 5% of mosquito species survive in very saline conditions.

Some *Culex* species are known to inhabit fresh water, while others preferentially inhabit saline water. Among *Culex, Culex tarsalis* has a high salinity tolerance. *Culex quinquifasciatus* has also shown high survival rates in saline water as this species is adapted to highly polluted aquatic habitats, and pollution increases salt concentrations in natural habitats. *Culex dolosus*and *Culex iridescens* are other species that seem to have developed mechanisms to tolerate high salinities and are found in mediums with different salinities and different types of aquatic habitats.

2.2 National level (INDIA)

Mosquito faunistic studies in the Indian subcontinent started in 1900 when Giles entitled his book A Handbook of the Gnats or Mosquitoes and himself added some seventeen new species. However, it was Theobald who in his gigantic task of grappling with the Culicidae of the World, in the five volumes of his monograph published over the years 1901-1910 opened the study of mosquitoesto workers all over the world. Later, two monographs by Christophers on Anophelines and Barraud on Culicines marked as a landmark in the history of mosquito studies in the Indian subcontinent were published in 1933 and 1934 respectively. The publications were the result of all the taxonomic studies in the subcontinent made by the earlier workers and the authors themselves. Noteworthy among the earlier works are James and Liston's (1904) volume on Anophelines of India; the Annotated Catalogue of Culicidae and the critical Review of the genera in Culicidae by Brunetti (1907-1920), and Larvae of Anopheline Mosquitoes by Puri (1931). After their monumental work, not many comprehensive biosystematics studies of Indian Culicidae have been undertaken and the year 1934 marks the end of an era of very active taxonomic research on Culicidae by which mosquitoes became, one of the best-known groups of insects in the area. During the period a total of 288 mosquito species (Anopheline -43, Culicines-245) were described from the Indian subcontinent. Due to a fervent nationwide anti-malaria campaign between 1950 and 1980, hardly any attention was given to the non-anopheline mosquitoes, and the biomedical significance of culicine mosquitoes was almost completely overshadowed in terms of their taxonomic biodiversity, many of the culicine mosquitoes playing key roles in transmitting several deadly and debilitating diseases. However, a faunistic study regarding culicines

(Anopheline also) was carried out by Rao and his colleagues from 1967 to 1969 in the Himalayan region and was published in subsequent years (Rao et al. 1973, Bhat and Kulkarni 1983, and Rao 1984). The recent review of the published studies showed that the Indian mosquito fauna comprises 393 species (Anophelines-61, Culicines-332) in 49 genera and 41 subgenera with the latest taxonomic situation (Bhattacharyya et al.2014).

Habitat characterization of anopheline larvae of the Dutch East Indies was described. *Anopheles umbrous is* always found in brackish or salt water in mangrove swamps. *Anopheles sinensis* also found in brackish water. *Anopheles maculatus* occurs down to the seashore, even in somewhat brackish water. *Anopheles ludlowi* breeds in brackish water (NH Sweliengrebel et. al, 1919).

Neogi SK's study in 1936 on salt lakes of Calcutta proved insights into the correlation between the breeding of *Anopheles sundaicus* environmental factors. The high, positive, and significant coefficient of correlation observed between breeding and salinity suggests that the salinity of the lakes is conductive to the development of Anopheles sundaicus larvae. Neogi further postulates a potential link between the salinity of breeding places and the endemicity of malaria in suburban areas of Calcutta. Notably, Anopheles sundaicus was found to be typically breed in brackish water but exhibited adaptability to water with varying degrees of salinity.

Sibling species B of *Anopheles subpictus* B has been incriminated as a vector in some coastal areas of south India (Russel and Jacob, 1939, Russel and Rao, 1940, Panicker et al., 1981).

A series of observations on the physico-chemical factors related to the breeding of *Anopheles sundaicus* in Calcutta shows a positive correlation between breeding and salinity (Sen, 1957).

Anopheles subpictus acts as a vector in West Bengal, India in the absence of any other recognized primary vector in the area. Species B breeds in brackish water (Soumendranath Chatterjee et. al, 2000).

The salinity of the larval habitat of *Verrallinalugubris* (Barraud) was found to be 11-13 ppt in Pichavaram mangrove forests and Maravakadu mangrove forest in the eastern coast of south India (A.R Rajavel et. al, 2005). Associated mosquito species *were Culex sitiens, Ochlerotatusportonovoensis,* and *Anopheles subpictus*Grassi.

A larval survey was carried out after the tsunami waves of Dec 26, 2004, in south India by Vector Control Research Centre, Pondicherry revealing the breeding of malaria vectors in

brackish water. *An. culicifacies* breeds in 2.3- 7.1 ppt salinity. *An. stephensi* breeds in 0.08-0.6 ppt salinity. From their findings, it is seen that the tsunami-hit areas are highly receptive to malaria, with the presence of two major malaria vectors- i.e., *An. stephensi* and *An. culicifacies*.

The study conducted at Andaman and Nicobar Islands by Krishnamoorthy K et al., reported that salinity ranging from 3- 42.50 ppt was found to support profuse breeding of *An. sundaicus* and *An. subpictus*.

Anopheles sundaicus is an important malaria vector breed in freshwater as well as in brackish water in Andaman and Nicobar Islands (Mohammad Tauqeer Alam et. al, 2006).

The salinity of the mosquito larval habitats in the mangroves of India was found to range from 100 to 37,500 mg/litre. Larvae of *An. subpictus, Cx. Sitiens, Cx. Tritaeniorhynchus* occurs in water having high salinity (AR Rajvel et al.,2008).

Anopheles subpictus has been found to play an important role in malaria transmission as a secondary vector in certain parts of Odisha and coastal areas of south India. It breeds in a variety of fresh as well as saline water habitats (Raj Kumar Singh et. al, 2014).

Among the total of 23 Anopheline species *Anopheles sundaicus* is the incriminated vector in Andaman and Nicobar Islands. These vector species breed both in fresh and brackish water. Filamentous floating algae and aquatic plants appear to be crucial for the development of the larvae (IP Sunish et. al, 2015).

Anopheles subpictus is found to be a fast-establishing vector of malaria in coastal urban cities and the sibling species B has been incriminated in the coastal region breeding in brackish water bodies (Ashwani Kumar et. al, 2020).

2.3 State level (KERALA)

Studies on the diversity of mosquitoes in Kerala started at the beginning of the twentieth century as a consequence of the path-breaking discovery of mosquitoes as vectors of malaria by Ronald Ross in 1897. The pioneering studies on mosquitoes in Kerala can be attributed to Theobald (1901) and Giles (1901). In 1901 Theobald described 3 new species and Giles 2 species. The bulk of the taxonomic studies on mosquitoes of the state were done by British workers in the pre-independent period.

Brackish water mosquitoes in mangrove forests of marshy areas are very big problems in Vypeen Island, Kochi. The major encountered species from adult collections were *Culex sitiens, Culex quinquifasciatus, Aedes aegypti, Aedes albopictus* and *Armigeressubalbatus*(T Mariappan et. al, 1996).

A larval survey carried out in Lakshadweep Islands, encountered 5 species of mosquitoes namely, *Anopheles stephensi, Anopheles varuna, Culex quinquifasciatus, Aedes albopictus* and *Aedes aegypti (*SK Sharma et. al, 2001).

Mosquito species that breed in salt marshes along the Arabic coast of Kerala can be a considerable nuisance to humans and are likely to vary in their importance in transmitting a range of arboviruses including Japanese Encephalitis Virus (JEV) and West Nile Virus (WNV). Mosquito species such as *Cx.gelidus, Cx.tritaneorhynchus, Cx. sitiens*show broad salinity tolerance and their abundance is positively associated with increasing salinity(Balasubramanian,2019).

CHAPTER 3

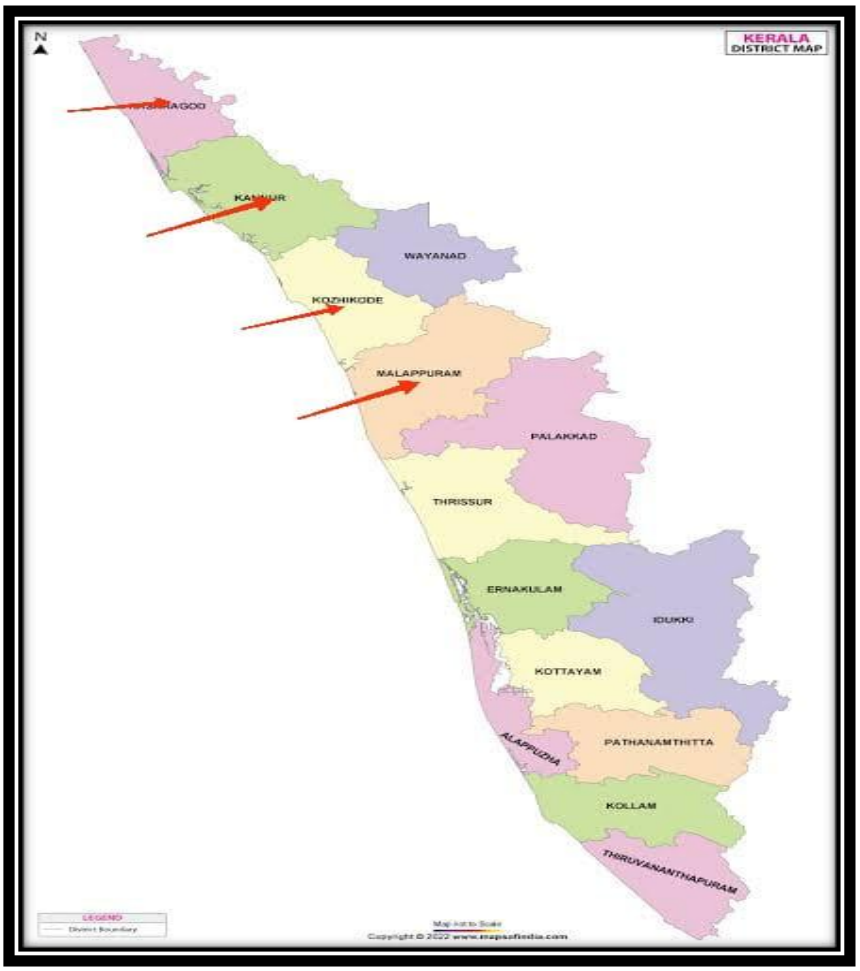
Materials and Methods

1.To identify and document the mosquito species breeding in brackish water habitats in North Kerala (Kasargod, Kannur, Kozhikode, and Malappuram districts) using morphological and molecular techniques.

3.1 STUDY AREA

It is fascinating to learn about Kerala's geographical diversity, from its lowland coastal areas with beaches and backwaters to the midland valleys and highland region with steep hills and forests. The hydrological dynamics of its numerous rivers add another layer to the state's unique characteristics. Kerala, with a total area of 38,863 sq. km (urban 3365 sq. km and rural 35,498 sq. km), is one of the country's smaller states. Shaped as a high vertical slope with an average breadth of 50 km, it is endowed with diversified climatic richness, with a pinch of hydrological concern as 41 of its 44 rivers originating in the western ghat empty into the Arabian Sea in less than 48 hours after a rain. The state's lowland region, which accounts for 10% of the total area, runs along the coastline and embodies beaches, swamps, and lagoons, besides backwaters, paddy fields and coconut plantations. Kerala's midland (42% of the total land mass) is primarily made up of valleys with undulating small hills and meandering passages. The highland region (48% of the total state's area), with steep hills, is rife with forests and small streams. Absolutely, Kerala's distinctive physiographic setting contributes to its rich ecological diversity. The combination of the narrow coastal strip, the western ghats, and the interconnected lagoons and estuaries fosters a unique environment that influences both the landscape and the high population density in the region (860 persons/km²).Covering an area of 38,864 km², Kerala is situated between latitudes 8"18' and 12"48'N and longitudes 71"53' and 77"24'E. notably, the coastal wetlands, constituting 937.3 km², play a significant role in the state's unique physiographic setting (Nair and Sankar 2002). Covering approximately 590 km, the coastal line extends over nine districts with the northern limit at Manjeswaram (Kasargod) and the southern limit at Pozhiyar (Thiruvananthapuram). The coastal region exhibits asymmetrical topography featuring undulating subdued hills and steep slopes. Altitude ranges from below mean sea level (MSL) to 2694 m above MSL, as reported by Jagtap et al.,2004. Kerala, boasting 44 rivers and an extensive network of estuaries and backwaters experiencing tidal action, originally had 700 km² of mangroves along its coast (Ramachandran et al.,1986). However, the current extent has significantly reduced to 9 km² (FSI, 2019). The remaining mangrove patches that still are

dispersed across ten coastal districts of the state, with the northern zone was higher (11.91 km² out of 17.82 km²). North Kerala refers to Kasargod, Kannur, Kozhikode, Malappuram and Wayanad. Among these the first four districts are coastal. The study area is the coastal area of North Kerala. Kerala a state in India has about 217 wetland areas constituting one-fifth of its total area, out of which 93 are coastal wetlands of area of 93730.5 ha. Brackish water collections in unused wells, discarded artificial containers, abandoned boats, brackish water bodies like lagoons, estuaries, coastal marshes, and tidal pools, as well as ponds, lakes and wells near urbanized coasts were continuously monitored for larval breeding.



Map 1 showing North Kerala

Kasargod

Kasargod is the northernmost district of Kerala and is also known as Saptha Bhasha Sangama Bhoomi. It is located at 12.5°N 75.0°E. It has an average elevation of 19 meters (62 feet). The district is bounded by Dhakshina Kannada District to the north, Western Ghats to the

northeast, Kodagu District to the southeast, Kannur District to the south and Arabian Sea to the west. Kasargod district has the maximum number of rivers in Kerala-12.

Map 2 shows the Kasargod district

Kannur

Kannur situated at 11°52’29.3” N 75°22’29.1” E on Kerala’s west coast, spanning an area of 2,996 km². Kannur has 7.55 sq km of mangroves, i.e., around 45% of Kerala’s total

mangrove forest cover. Kannur has an elevation of 1.02 meters or 3.3 feet along the coast of the Laccadive Sea, with a sandy coastal area. Kannur is located north of Kozhikode, south of Kasargod and Mangalore, west of the western ghat regions of Kodagu and Wayanad and east of the Laccadive Sea.

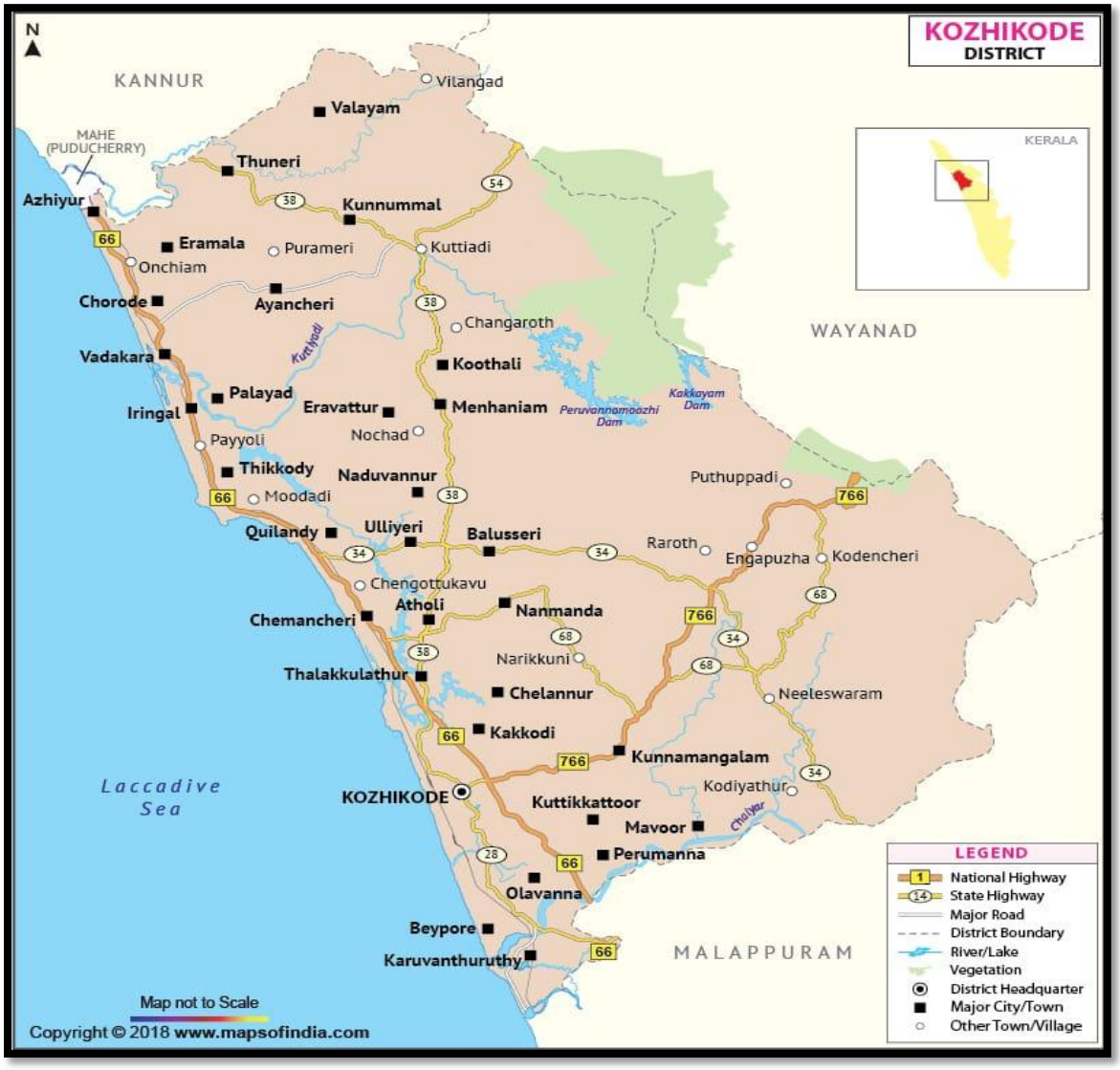
Map 3 shows Kannur district

Kozhikode

Kozhikode district is in the northern part of Kerala state, India between 11°15’ and 11 25°North and 75° 46’ and 75.77’ East. The district is bordered on the North by Kannur, south by Malappuram and east by Wayanad districts of Kerala state with the Laccadive Sea on its western boundary. The district extends to an area of 2344 km² with a total population of about 1.4 million people. There is an extended coastal line running from Kadalundi in the south to the boundary of Mahe Union territory in the north, which extends to more than 80 km.

Kozhikode district in north Kerala has diverse habitats endowed with parts of the western ghats viz., laterite hills in the midlands, wetlands, streams, rivers, estuaries in the coastal area and a long stretch of sea shore. Physiographically, the district is divisible into 3 zones, namely the lowland, midland and the highlands. The lowland coastal zone which spans approximately 362.85 km², is about 15.55 per cent of the total land cover of the district. The midland portion is almost 55% of the total area of the district. The highland portion of the district constitutes about 27% of the total land.

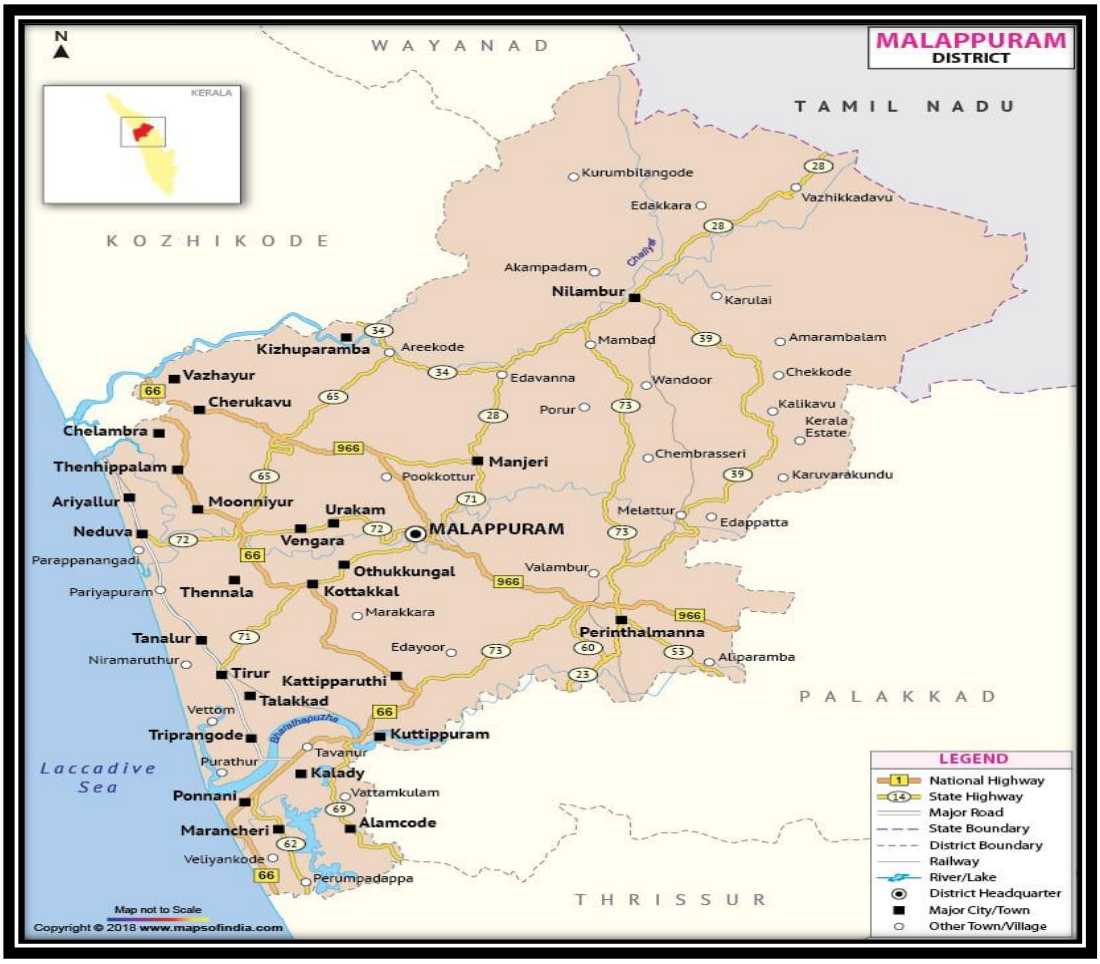
In the river mouth of the coastal zone, very characteristic formations of mangroves flourished in the past. Relicts of such formations can be seen now at Kadalundi, Feroke, and Beypore, and according to a very rough estimate of 1992, the district has 20 ha of undisturbed and 120 ha of degraded mangroves much scattered in distribution.



Map 4 shows Kozhikode district

Malappuram

Malappuram is located between 75° and 77° East longitude and 10° and 12° North latitude. It covers about 3548 km² area. Malappuram is bordered by Palakkad and Thrissur districts in the south and Kozhikode in the north with the western and eastern boundaries delimited by the Laccadive Sea and parts of Tamil Nadu state respectively. There is about 70 km long coastal line in the district, which extends from Kadalundi in the north to Palappetty in the south with a fishing harbour at Ponnani. The lowlands are situated within 8 m above MSL, extending along the coastal belt to a length of about 70 km from Vallikkunnu to Perumpadappu. In the lowlands of the district, the major formation of biodiversity is seen in the backwaters and associated marshes, ponds, streams and waterlogged fields, sea coasts and adjoining landscapes up to an altitude of about 8 m from the sea level. Mangroves are seen along the mouths of rivers joining the sea and along the banks of backwaters, especially at Beypore, chaliyar, Kadalundi etc. Such areas are limited to only about two hectares in the whole of the district, even though potential areas of the vegetation type extend to more than 10 hectares.



Map 5 shows the Malappuram district

Area with special interest

1. **Kadalundi**

The Kadalundi estuary (11°7’28”-11°8’01” N and 75°49’36” - 75°50’20” E),

Situated at the mouth of the Kadalundi River in Kerala, India, is characterized by two channels enclosing a small island. With mangroves scattered along its borders and mudflats providing foraging grounds, it serves as a habitat for numerous wintering and resident water birds, particularly waders. Designated as India’s first community reserve in 2007, the KadalundiVallikkunnu community reserve span 1.5km² and is jointly owned by the villages of Kadalundi and Vallikkunnu.

Map 6 showing Kadalundi

2. Kaipad rice fields -Ezhome

Kaipad is a saline-prone naturally organic rice production tract of north Kerala, India falling in Kozhikode, Kannur and Kasargod districts in 4100 hectares. A major part of the area is in the Kannur district extending mainly into four panchayats namely Ezhome, Pattuvam, Cherukunnu and Kannapuram. The Kaipad system of rice cultivation is an integrated organic farming system in which rice cultivation and aquaculture go together in coastal brackish water marshes that are rich in organic matter. The network of backwaters and estuaries serves as an inlet of sea water and causes salinity in the area. This ecosystem is rich in biodiversity concerning flora and fauna. Mangroves which are seen on the fringes of back waters and estuaries are characteristic features of Kaipad tracts. As the Kaipad tract is coastal to the river which merges into to sea, there will be floods during monsoon and salinity during the summer season. Kaipad ecosystem consists of marshy swamps, ponds and paddy fields which help in controlling sedimentation, flood and pollution. The river water is usually saline except during monsoon. The Kaipad tract is located approximately 11.25°N 75.77°E/12.5°N 75.0°E. Kaipad rice tract of Kannur district mainly lies on the bank of Valapattanam and Kuppam in the panchayats Ezhome, Pattuvam, Kannapuram, Cherukunnu, Pazhathi, Chelora, Narath, Kolacheri, Chirakkal, Munderi, Elayavoor, Kuttiyattoor and Mayyil. In Kannur districts, the approximate area of the Kaipad tract is 3400 hectors.

Map 7 showing Ezhome- Kaipad rice fields

3. Kavvayi river basin

Kavvayi River basin is located between 12° 05’ to 12° 15’ North latitude and 75° 05’ to 75° 20’ East longitude. It spread over an area of 164.76 km² and spreads over nine local administrative bodies in the districts of Kannur and Kasargod. The Kavvayi River emerges from the Cheemeni laterite hills at an elevation of 119 m above MSL having a length of 31 km and directly flows into the Kavvayi backwater. The river basin is a topographically complex, biodiversity-rich, fragmented and densely populated cultural landscape. Even though the Kavvayi River is prominent among the 14 rivers originating in midland in Kerala there is no reserved forest patch in the river basin (Alex, 2018).

Map 8 shows Kavvayi Island and the beach

4. Harbours in North Kerala

Kasargod	Kasargod
	Cheruvathoor
	Manjeswaram
Kannur	Thalai
	Maplabay
	Azheekkal
Kozhikode	Beypore
	Puthiyappa
	Koyilandi
	Vellayil
	Chombal
Malappuram	Ponnani
	Thanur

Table 2 Harbours of north Kerala

3.2 Collection

Indeed, various methods are employed to monitor mosquito abundance and assess the risk of vector- borne diseases. Adult collection methods such as traps or human landing collections, help gauge adult mosquito populations, while pupal and larval collections target earlier life stages. Ovitraps designated to attract egg-lying mosquitoes, provide insights into breeding sites. These monitoring efforts play a crucial role in formulating effective strategies to control and prevent the spread of diseases carried by mosquitoes. The collection was done strictly in brackish water habitats (habitats with salinity in the range of 0.5-30 ppt. To collect the brackish water breeders only larval collection was possible. Adult collection was not preferable because freshwater breeders may also be encountered during adult collection.

Larval collection

Larval collection is of key importance as all mosquitoes rely on water bodies for their development. Besides, larval sampling is of particular interest to mosquito species whose females are not or are only rarely attracted to the commonly used trap systems.

Depending on the size and design of the breeding sites, mosquito larvae can be collected by netting, dipping or sucking. Larger water bodies can be sampled by classic dippers or plastic trays, or by fine-meshed (≤ 0.5 mm) aquatic nets (aquarium water nets) and sieves. Smaller water bodies can be checked for the presence of larvae by dipping with a ladle or by aspirating water with a tube or a pipette. Collected water can be inspected better for the presence of juveniles when decanted in a white plastic tray/bowl.

Mosquito larvae were collected from coastal areas of 4 districts (Kasargod, Kannur, Kozhikode and Malappuram) during the period of 4 years (January 2018 to March 2022). All artificial and natural containers holding water with salinity in the range of 0.5-30 ppt, both indoor and outdoor were visually inspected for the presence of mosquito larvae. Each type of container positive with mosquito larvae and pupae was classified as a breeding site. Larvae were collected using an 8 cm diameter and 240 ml capacity dipper.

Fig. 9 Dipper used for larval collection

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Fig 1-8COLLECTION DONE IN HARBOURS OF NORTH KERALA

Fig 9- 35 COLLECTION DONE IN MARSHY LANDS AND MANGROVE AREAS 9

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Check list of Materials used for collection

- Smartphone or field data reporting system, Salinometer, Dipper, White plastic tray, Fine mesh aquatic net, Fine mesh sieve, Kitchen ladle, Tubes, Pipettes, Vial, Ethanol, Droppers, Labels, Pencil, Field magnifying glass, Glass wares

Standard dipping methods

Dipping method	Targeted mosquito genera	Method details	Notes
Shallow skim	Anopheles	The dipper is submerged at approximately 45°with the leading edge, about 2.5 cm below the water surface, and is filled at the end of the stroke while being drawn along the water surface.	When the dipper enters the water, this method proves effective for Anopheles larvae, which linger at the water surface longer than other mosquito larvae. A recommended sampling techniques involves submerged macrophytes with leaves positioned just below the water surface.
Complete submersion	Aedes, ochlerotatus, culex, culiseta	In floodwater habitats, the dipper is rapidly submerged in open water. It is then brought up to the water surface, utilizing larvae reactions to the disturbance caused by submerging the dipper	Primarily employed for rapidly responsive and visible mosquito larvae, this method is also suitable for sampling larvae near vegetation. The dipper is brought to the water's surface while in contact the emergent vegetations.

Partial submersion	Anopheles, culex, culiseta	Submerging at approximately 45°along emergent vegetation, water swiftly flows into the dipper. The dipper remains stationary horizontally but can be moved vertically to scrape along the edge of the emergent vegetation.	This method is effective for sampling in robust emergent vegetation. The water flow creates suction into the dipper, and scraping also gathers small insect predators and herbivores associated with mosquito larvae on or near vegetation.
Flow in	Aedes, ochlerotatus, culex	Applied in shallow water where depth is less than height of the dipper's ladle, this technique involves pushing the dipper's bottom into the substrate, allowing water, larvae, and debris to flow into the dipper	Effective in shallow habitats, root masses, and other environments shallower than the dipper's profile, this method proves to be successful.
Scraping	Coquillettidia	To dislodge attached larvae, the dipper is scraped against the underside of floating vegetation using a typically vigorous back-and-forth motion.	This technique is employed to sample larvae residing under and typically attached to floating vegetation or the roots of floating plants. The vigorous back-and - forth motion, performed with the dipper completely submerged, is most effective when using dippers with a screened bottom.

Simple ladle	Culex	A swift flip of the wrist is employed to completely submerge the dipper just below the water surface, akin to the motion of taking a drink of water.	This method is not preferred, especially when the sample is not taken adjacent to a mosquito microhabitat. However, it can be suitable in hypereutrophic situations where larvae abundance often reaches around 1000/dip.
Background	Aedes, ochlerotatus	The dipper is utilized to create a light background, enhancing visibility of darker-colored immature mosquitoes. Once located, mosquitoes are swiftly collected by pulling the dipper through the water surface.	This technique is primarily employed for identifying mosquitoes inhabiting woodland ponds and pools.

Fig.10 salinometer used to determine the salinity of the sample

3.3 Rearing of adults

The collected specimens were brought to the research laboratory of the government college Madappally. Larvae were reared in plastic bowls of 300 ml capacity. Each bowl was fed 100mg per day of a 2:1 mixture of finely ground fish pellets: Baker's yeast for the first 3 days of post-emergence, and 135mg per day thereafter. For all experiments involving exposure to saline water, solutions of commercial NaCl were prepared with distilled water. (Bradley J. White et., al.). The female mosquitoes were fed on rat's blood and made to lay eggs in a rearing cage. All colonies were maintained at a research laboratory of Government College Madappally.

3.4 Killing and pinning of adult mosquitoes

After emerging, the killing of adult mosquitoes was carried out with the help of cotton swabs soaked in diethyl ether placed inside a glass jar. Representative specimens of each mosquito species were pinned through the thorax. Special entomological pins known as minutens (No.20) were passed through the mosquito thorax so that a quarter of the pin was above the specimen. Pins were inserted into the mosquito so that it is at right angles to the transverse and longitudinal axes of the mosquito's body. These pins do not become corroded by mosquito body fluids.

Fig.11 Pinning of Mosquito

3.5 Preservation of adult mosquitoes

For preserving the adults, 1, 4-dichloro benzene (para dichloro benzene) was used. Para dichloro benzene is a chlorinated aromatic hydrocarbon compound used as a fumigant insecticide and repellant. Pinned specimens were kept in a glass bottle impregnated with para dichloro benzene with a bark cork.

Fig.12Preservationofmosquito3.6Labelling of the specimens

The specimens in the glass bottle were labelled with the information of specimen number, name, habitat, and date of collection. Further details were recorded in the field dairy.

3.7 Identification

3.7.1 Morphological Identification

Larvae

Morphological features used in identifying a larva

- Head – the head is round or slightly oblong and slightly flattened.
- Thorax – appears distinct from the head, separated by a very narrow neck.
- Abdomen – segmented section behind the thorax. The abdomen has 10 segments, but not all are distinct. In Aedes and Culex, the ninth segment is not distinct; in Anopheles the tenth.

- Anal papillae – the four white protrusions on the anal segment which perform osmotic regulation of the organism.
- Hairs -the number, position, and arrangement of hairs on the larva can be diagnostic.
- Siphon – is an air tube on the 8th abdominal segment. All genera – except one have a siphon.
- Pecten – pecten is a row of closely set teeth or spines on each side of the siphon.
- Comb scales – a line or patch of scales found on the 8th abdominal segment in most genera.
- Saddle – a dark thickened band on the anal segment. It can ring the segment, be in two pieces, or appear as a saddle.
- Abdominal hairs – the placement and number of hairs on the abdomen can be diagnostic. Setae, brushes, tufts, and hairs.
- Setae -another word for insect hairs

- Brush – a clump of tufts
- Anal brush -anal brush is used like a rudder when the larva is swimming.
- Tuft – more than one hair growing together.

Anopheles larvae

If the larvae are lying flat on the surface, they are from the genus *Anopheles.* This is the only genus of mosquito that lies flat on the surface. All others are suspended from the surface at an angle. *Anopheles* does not have a siphon. Instead, it lays parallel to the surface and breathes through openings on its 8th abdominal segment(spiracles).

Aedes larvae

The comb scales on the eighth segment of the abdomen, the shape of the pecten teeth on the siphon, and the number of setae at the ventral brush distinguish *Ae. albopictus* larvae. The comb scales lack lateral denticles while the pecten teeth exhibit three well-defined pointed denticles (Cheong,1986, WHO,1995 as cited by Sivanathan,2006). *Ae. albopictus* larvae's ventral brush(4-X) features only 4 pairs setae (Harrison &Rattanarithikul,1973; Rueda,2004). In pupae of Aedes the absence of a tracheiod portion in the trumpet meatus/respiratory

trumpet serves as a distinguishing characteristic from other mosquito genera. *Ae. albopictus* pupae display a midrib of paddle reaching apex with visible setae along the margin.

Culex larvae

Culex larvae were more or less like *Aedes* larvae. But the siphon of *Culex* larvae was longer and had a lighter colour, their body was also hairy compared to *Aedes.*

Morphological features used in the identification keys of adults

Head, Proboscis, Antenna, Maxillary palpomere 4, Clypeus, Vertex, Occiput, Scutum, Alula, Scutellum, Paratergite, Prespiracular area, Mesepimeron, Foreleg, Wing, Vein Cu, Vein 1A, Femur, Abdominal terga, Midleg, Hindleg.

Differentiating male and female mosquitoes

- Antennae in males are bushier/hairier than females.
- Males have pincer-like claspers (sexual organs) at the end of the abdomen.
- Palps on either side of the proboscis are long and generally of similar length to the proboscis in males.

Some female mosquitoes (Anopheles species) also have long palps but the antennae are not as heavily "feathered"

The emerged adults were identified by pictorial keys prepared by Bina Pani Das (for the genus Anopheles). For the emerged culex identification, an "Illustrated keys to species of Culex (Culex) associated with Japanese Encephalitis in southeast Asia" was used (Reuben R, Tewari S C, Hiriyan J, Akiyama J 1994). For the emerged aedes identification, "pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission (LM Reuda, 2004) was used.

3.7.2Molecular resolution of species complexes

Genomic DNA isolation

Until genomic isolation, the specimens were stored in the laboratory at -20^{o} C. The Genomic DNA of mosquitoes was extracted using a HiPurA genomic DNA isolation kit from HIMEDIA following manufactures manufacturer-suggested protocol. The mosquito's genomic DNA was isolated from the whole body as the insect was small. The tissue homogenates were lysed by using a lysis buffer along with the action of 20 µl proteinase k

(10mg/ µl). The DNA from lysed cells were purified by using silicate columns. The elution of the purified DNA from the column is by using TE buffer with P^H 8 [Sambrook et al, 1989]. The eluted DNA was used as a template for PCR amplification of the COI gene and ITS 2 sequences.

PCR Amplification of COI gene

The COI gene was amplified by using the FOM F (5'-GGTCAACAAATCATAAAGATATTGG-3') and FOMR (5'–TAAACTTCAGGGTGACCAAAAAATCA- 3') primers (Folmer *et al.* 1994). The PCR amplification was done using an automated Thermal cycler (Sure Cycler 8800, Agilent Technologies USA). The PCR reaction mixture consisted of 25 µl of 2x premix of Emarald AMP GT PCR master mix, 2 µl Forward Primer (5 µM), 2 µl reverse Primer (5 µM), 1µl template DNA and then made up to 50µl by adding 20 µl of sterile dH_2O. The temperature profile of PCR consisted of an initial denaturation at 94^0 C for up to 5 minutes followed by 30 cycles of denaturation at 94^0 C for 10 seconds, annealing at 56^0 C for 45 seconds and elongation at 72^0 C for 45 seconds. It concluded with a final extension at 72^0 C for 3 minutes. The reaction mixture was stored at 4^0 C.

PCR Amplification of ITS 2 regions

The ITS-2 region between 5.8S rDNA and 28S rDNA genes was amplified using common forward primer SubF (5' - ACTGCAGGACACATGAACACCG- 3') and species-specific reverse primers SubA (5'- GCTTGTTGTCGAACCGTGCGAT - 3') and SubB (5'-ATCCGGTTGATACAGGACGCAC-3'). The PCR reaction mixture consisted of 25 µl of 2x premix of Emarald AMP GT PCR master mix, 2 µl Forward Primer (5 µM), 2 µl reverse Primer (5 µM), 1 µl template DNA and then made up to 50µl by adding 20 µl of sterile dH_2O. The PCR conditions for both PCRs were an initial denaturation at 95°C for 5 minutes followed by 30 cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 45 seconds followed by a final extension at 72°C for 7 minutes.

The amplification was checked by loading 5 µl from the 50 µl of PCR product into the wells of 1% ethidium bromide-stained agarose gel [Sambrook et al, 1989] and the remaining 45 µl PCR product was retained for sequencing. After electrophoresis, the gel was visualized in a gel document system (Bio-Rad EZ gel documentation system). The amplified PCR product was purified by using a StrataPrep PCR purification kit (Agilent Technologies, USA) as per the manufacturer's instruction.

DNA sequencing

The purified PCR products were sequenced at AgriGenom Labs Pvt. Ltd, Kakkanad, Kochi using Sanger's sequencing technique [Sanger et al, 1977]. From the both ends the sequencing were done.

The forward and reverse sequences were assembled and the consensus sequence was generated using Sequencher 5.3 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The consensus sequence obtained was searched for its sequence similarities using the BLASTn programme of NCBI [Altschul et al, 1990]. Similar sequences were selected from the nucleotide database of GenBank and the phylogenetic relationships were identified from the most similar sequences (Tables 3 and 4). The homologous sequences retrieved from GenBank were used for the construction of a phylogenetic tree using the Maximum likelihood method in the MEGA XI application

Table 3 for phylogenetic tree construction

S.I. No.	GenBank accession	Species	Location
1	KX632059	*Anopheles subpictus*	India: Panaji, Goa
2	KP165072	*Anopheles subpictus*	Sri Lanka
3	KX632060	*Anopheles subpictus*	India: Panaji, Goa
4	KX632062	*Anopheles subpictus*	India: Panaji, Goa
5	KX632063	*Anopheles subpictus*	India: Panaji, Goa
6	MW078486	*Anopheles subpictus*	India: Delhi
7	KJ437453	*Anopheles subpictus*	Sri Lanka
8	KJ437450	*Anopheles subpictus*	Sri Lanka
9	KC191826*	*Anopheles subpictus*	Sri Lanka: Kallady
10	GQ870334*	*Anopheles subpictus*	Myanmar: Rakhine, Sittwe
11	AF406615	*Anopheles subpictus*	Sri Lanka
12	AY049004	*Anopheles subpictus*	Sri Lanka: Coastal area
13	AF406616	*Anopheles subpictus*	Sri Lanka
14	MT068434	*Anopheles subpictus*	Thailand: Phetchaburi, Tha Yang
15	MT068425	*Anopheles subpictus*	Thailand: Chiang Mai, Mueang Chiang Mai
16	GQ870330	*Anopheles subpictus*	Viet Nam: Ninh Binh, Kim Son District

17	GQ870329	*Anopheles subpictus*	Cambodia: Kampot, Kampot Province, Kampong Bay
18	AB731656	*Anopheles subpictus*	Viet Nam: Hai Phong, Do Son
19	GQ870333	*Anopheles subpictus*	Thailand: Phang Nga
20	GQ870328	*Anopheles subpictus*	Indonesia: Lesser Sunda Islands, Flores
21	GQ870327	*Anopheles subpictus*	Indonesia: Lesser Sunda Islands, Flores
22	GQ870326	*Anopheles subpictus*	Cambodia: Kampot, Kampot Province, Kampong Bay
23	MW078489	*Anopheles epiroticus*	Sun I Nicobar
24	MW078488		Sun I Myanmar
25	MF599123	*Anopheles sundaicus*	India: Andaman and Nicobar Islands
26	JN675920		Indonesia
27	GQ480823	*Anopheles vagus*	East Timor
28	AF469855	*Anopheles epiroticus*	Thailand
29	AF369560	*Anopheles sundaicus*	Malaysia: Lundu
30	GQ870332	*Anopheles indefinitus*	Philippines: Luzon, Laguna Bay, Morong Bataan, Minanga
31	KY000690	*Anopheles vagus*	Sri Lanka
32	KP165078	*Anopheles subpictus*	Sri Lanka
33	KJ437451	*Anopheles subpictus*	Sri Lanka
34	KC191825*	*Anopheles subpictus*	Sri Lanka, Suthumalai
35	GQ870337*	*Anopheles subpictus*	Sri Lanka, Monaragala

Table 4 for phylogenetic tree construction

1	HQ609066	*Anopheles subpictus*	Myanmar, Rakhine Sittwe
2	HQ609030	*Anopheles subpictus*	Myanmar, Rakhine Sittwe
3	KJ461784	*Anopheles subpictus*	Sri Lanka
4	HQ609056*	*Anopheles subpictus*	Myanmar
5	HQ609053*	*Anopheles subpictus*	Myanmar
6	HQ609042*	*Anopheles subpictus*	Myanmar
7	HQ609044*	*Anopheles subpictus*	Myanmar
8	HQ609047*	*Anopheles subpictus*	Myanmar

9	HQ609034*	*Anopheles subpictus*	Myanmar
10	HQ609055*	*Anopheles subpictus*	Myanmar
11	HQ609052*	*Anopheles subpictus*	Myanmar
12	MT066053	*Anopheles subpictus*	Thailand, ChiangMai, San PaTong
13	MT066048	*Anopheles subpictus*	Thailand, Chiang Mai, Maeon
14	MT066056	Anopheles subpictus	Thailand, Phetchaburi, Tha Yang
15	MT066046	*Anopheles subpictus*	Thailand, Chiang Mai, Mueang Chiang Mai
16	MT066047	*Anopheles subpictus*	Thailand, Chiang Mai, Maeon
17	MT066052	*Anopheles subpictus*	Thailand, Chiang Mai, Hang Dong
18	MT066051	*Anopheles subpictus*	Thailand, Chiang Mai, Doi Saket
19	MT669951	*Anopheles sundaicus*	Malaysia, Sabah,Kudat,Paradason
20	MH924526	*Anopheles darlingi*	Colombia
21	KJ492682	*Anopheles albitarsis*	Brazil, Maranhao, Balsas
22	KJ492753	*Anopheles deaneorum*	Brazil, MatoGrosso do sul, Aquidauana, pousada
23	DQ076223	*Anopheles marijuana*	New York
24	DQ267688*	*Anopheles subpictus*	India, Tamilnadu
25	FM994156*	*Anopheles subpictus*	India
26	FM992376	*Anopheles subpictus*	India, Orissa, Bantala, Angul district
27	DQ310146*	*Anopheles subpictus*	India, Pondicherry, Moorthikuppam
28	FM992375*	*Anopheles subpictus*	India, Orissa

2) To study the Salinity tolerance of mosquitoes breeding in brackish water.

Salinity is the measure of the number of grams of salts per kilogram of seawater, which is expressed in parts per thousand. Parts per thousand can be defined as how many parts, or grams, of the salt there are per thousand parts or kilogram (1000 g) of seawater. It is represented by the symbol ‰ (Duncan Seraphin et.al, 1995).

Salinity in ppt = grams of dissolved salts/1000 grams of seawater

The average salinity of seawater is about 35‰. Sea water generally ranges from 33‰-38‰.

3.8 Preparation of NaCl

The salinity of the field was monitored using a salinometer. For obtaining salinity tolerance estimation of collected specimens, larvae should be treated with saline water. For this NaCl solution was prepared using the following formula

A one percent solution is defined as 1 gram of solute per 100 millilitres final volume

for example, 1g of NaCl, made up to 100 ml with distilled water, is a 1%NaCl solution. Likewise, 35‰ is equal to 3.5% i.e., 35/1000=3.5/100=3.5%

A one ppt solution of NaCl is prepared by dissolving 1g NaCl in 1000 ml water.

By using this conversion formula salinities of different concentrations were prepared using Instant Ocean Sea salt.

3.9Salinity tolerance estimation

While collecting larvae and pupae salinity of the water in the habitats was recorded using a salinometer. Following hatching 20 larvae from each colony were promptly counted, transferred to plastic trays with brackish water from their natural habitats. Further, larvae were exposed to higher as well as lower salt concentrations in the laboratory. Third instar larvae were exposed to 0, 5, 10, 15, 20, 25, 30 and 35 ppt initially. Once a tolerable salinity was obtained, they were further exposed to still narrower ranges of salinity. Higher concentrations were prepared in distilled water using instant ocean sea salt. The experiment was done in duplicate. A control was maintained in the water sample collected from the field. Survival of the larvae were visually scored. Confirmation of death was achieved through stimulation using a glass rod.

(Bradley J. White et al;2013)

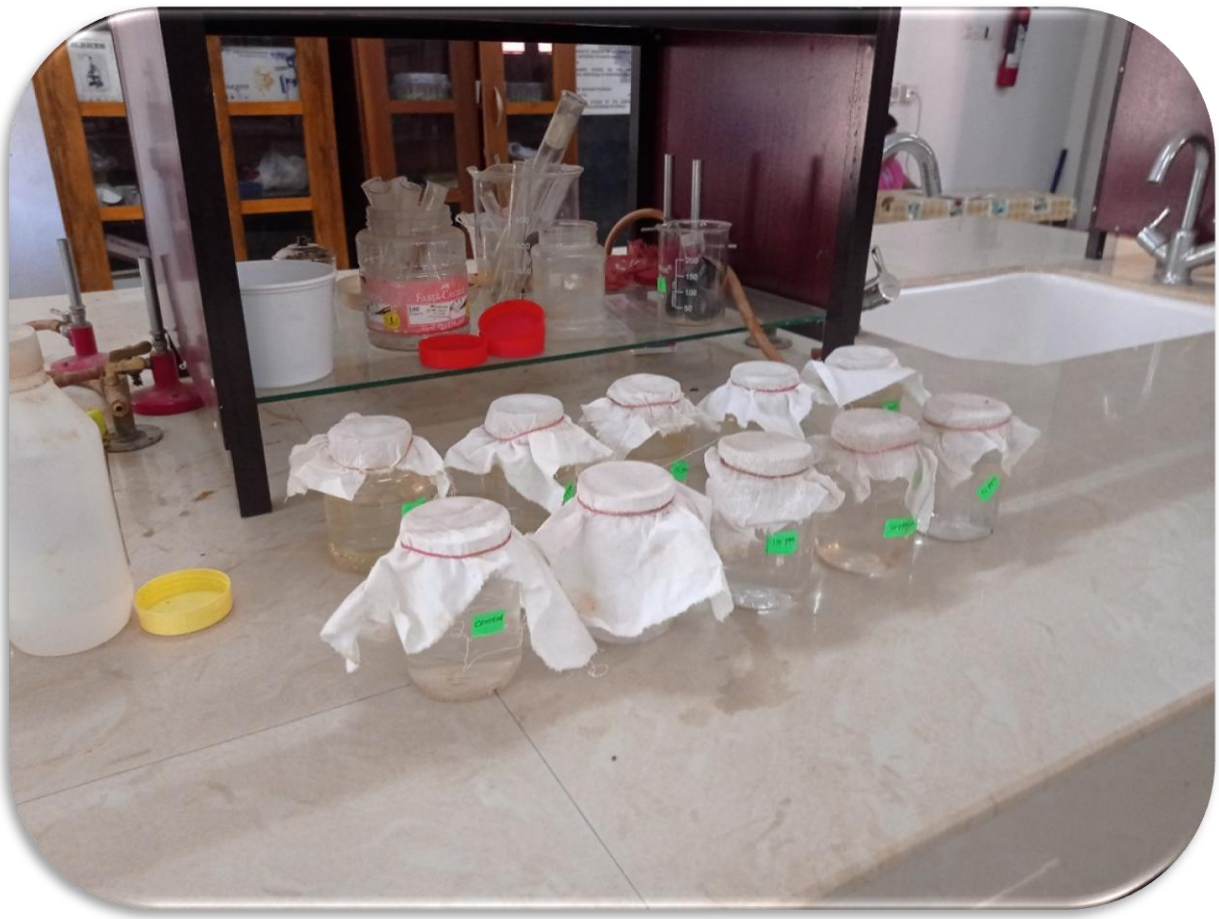


Fig. 13 Salinity tolerance estimation in the laboratory

3)To compare the salinity tolerance of mosquitoes breeding in brackish water with the same species breeding in freshwater to investigate the possibility of ecotypes.

Those mosquito species that breed in brackish water, as well as freshwater, were compared for their salinity tolerance using the procedure discussed below.

Salinity tolerance estimation

While collecting larvae and pupae salinity of the water in the habitats was recorded using a salinometer. Following hatching 20 larvae from each colony were promptly counted, transferred to plastic trays with brackish water or fresh water from their natural habitats. Further, larvae were exposed to higher as well as lower salt concentrations in the laboratory. Third instar larvae were exposed to 0, 5, 10, 15, 20, 25, 30 and 35 ppt initially. Once a tolerable salinity was obtained, they were further exposed to still narrower ranges of salinity. Higher concentrations were prepared in distilled water using instant ocean sea salt. The

experiment was done in duplicate. A control was maintained in the water sample collected from the field. Survival of the larvae were visually scored. Confirmation of death was achieved through stimulation using a glass rod.

(Bradley J. White et al;2013)

4)To prepare pictorial key to the species of mosquitoes breeding in brackish water habitats of North Kerala.

Taxonomic characters of the mosquito species recorded in the study were photographed by using Leica model MSY266, type DMC 2900 microscope. Using these high-definition pictures, a pictorial key for their identification was constructed.

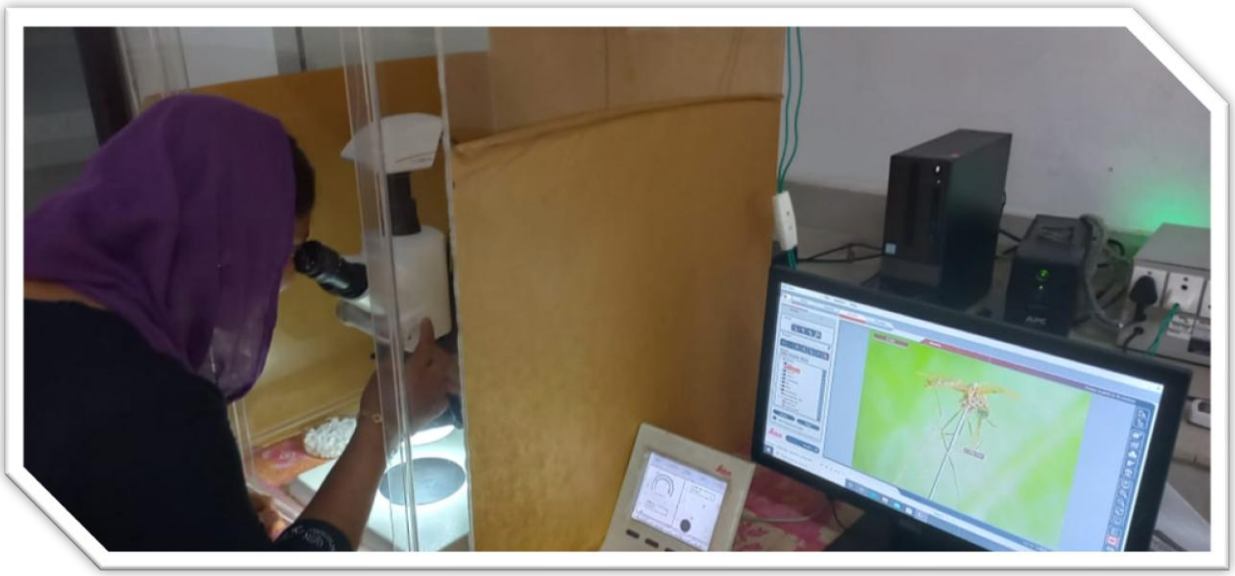


Fig. 14 Pictorial key preparation using a Leica microscope

CHAPTER 4

RESULTS

4.1 Documentation of mosquitoes

Documentation of the mosquito species breeding in brackish water habitats in North Kerala (Kasargod, Kannur, Kozhikode, and Malappuram districts) using morphological and molecular techniques.

Brackish water is defined as having salinity in the range of 0.5-30 ppt salinity. Different types of habitats were found having brackish water with salinity ranging between 0.5-30 ppt salinity viz., unused wells, ornamental ponds, unmaintained water bodies, ditches, discarded artificial containers, abandoned boats, brackish water bodies like lagoons, estuaries, coastal marshes, and tidal pools, as well as ponds, lakes, wells near urbanized coasts and rice fields with brackish water (Kaipad rice fields). To check the possibility of ecotypes, continuous monitoring was also done in freshwater habitats with a salinity of 0.1 -0.5 ppt. So, the whole collection was done in habitats with salinity ranging from 0.1-30 ppt.

The present study revealed that several mosquito species in coastal areas of north Kerala can undergo larval development in brackish water habitats. A total of 159,602 mosquito larvae and pupae were collected along the coastal brackish water bodies with salinity levels up to 2-32 ppt (parts per thousand). The collection was done during 2018 January – 2022 May in which 11 species and three genera were identified. The identified genera were *Aedes, Anopheles* and *Culex.* Of these collected mosquitoes *Culex* was the predominant genera with 6 species followed by *Anopheles* with 4 species. *Aedes* was the least collected species with only one species. The identified species were *Anopheles subpictus*species complex, *An. stephensi, An. barbirostris, Aedes albopictus, Culex sitiens, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus, Cx. gelidus*and *Cx. vishnui. An. subpictus*species complex was resolved as *An. subpictus B* and *An. subpictus A.*

4.2. District wise distribution of mosquitoes

The detailed district-wise distribution of the collected species is given below.

1. *An. subpictus* B

An. subpictus B was collected from Azhiyoor, Vadakara, Kadalundi and Beypore of Kozhikode district and Vallikkunnu and Ponnani of Malappuram district.

2. *An. subpictus* A

An. subpictus A was collected from Azhiyur, Vadakara, Koyilandi, and Chengottkavu of Kozhikode district and Vallikkunnu, Vettom and Purathur of Malappuram district.

3. An. barbirostris

An. barbirostris was collected from

Chorode, Vadakara, Koyilandi, Payyoli, Eramala, Azhiyur, Beypore, Feroke, Ayancheri, Chengottkavu, Kozhikode - Kozhikode district.

Ramanthala, Cheruthazham, Madayi, Ezhome, cherukunnu, Mattool, Azhikode, Valapattanam, Chirakkal – Kannur district.

Vazhayur, Cherukavu, Pallikkal, Peruvanur, Vallikkunnu, Moonniyur, Tanalur, Vettom, Ponnani, Veliyamkode, Alamkode- Malappuram district.3. Aedes albopictus

4. An. stephensi

*An. stephensi*was collected from abandoned boats of Chombala harbours, Koyilandi Harbour, Puthiyappa, and Vellayil harbours. It was found to be very low in density and only a few larvae were collected from brackish water habitats. It was only collected from Kozhikode district.

5. Ae. albopictus

Ae. albopictus was collected from
Azhiyur, Eramala, Vadakara, Payyoli, Thikkodi, Moodady, Koyilandi, Chengottkavu, Thalakulathur, Elathoor, Beypore-Kozhikode.

Peringome, Cheruthazham, Kannapuram, Madayi, Ezhome, Pattuvam, Cherukunnu, Mattool, Kalliassery, Valapattanam, Chirakkal, Muzhappilangad, Dharmadam- Kannur

Vazhakkad, Pulikkal, Chelembra, Vallikkunnu, Vengara, Ozhur, Tanur, Vettom, Purathur, Ponnani, Veliyamkode, Perumpadappu- Malappuram

6.Cx. sitiens

Cx. sitiens was collected from Azhiyur, Chorode, Vadakara, Ayancheri, Payyoli, Thikkodi, Moodady, Koyilandi, Chengottkavu, Chelannur, Elathoor, Kakkodi, Cheruvannur, Beypore, feroke, Kadalundi -Kozhikode.

Cherupuzha, Peringome, Karivellur, Payyannur, Ramanthala, Madayi, Ezhome, pattuvam, Mattool, Azhikode, Kalliassery, Pappinissery, Valapattanam, Chirakkal, Kannur, Muzhappilangad, Dharmadam, Eranholi, Thalassery, Mahe, Chokli.

Vazhayur, Cherukavu, Vallikkunnu, Pulikkal, Moonniyur, Peruvallur, Parappangadi, Thirungadi, Nannambra, Tanur, Tirur, Vettom, Purathur, Ponnani, Maranchery, Alamkode, Perumpadappu – Malappuram.

Mangalappady, Kumbla, Kasargod, Chemnad, Uduma, Pallikkara, Ajanoor, Kanjangad, Neeleswaram, Madikkai, Cheruvathur, Karivellur, Pilicode – Kasargod.

7.*Cx. quinquefasciatus*

*Cx. quinquefasciatus*was collected from

Kadalundi, Feroke, Beypore, Olavanna, Cheruvannur, Kozhikode, Kakkodi, Thalakulathur, Chemanchery, Chengottkavu, Koyilandi, Moodady, Naduvannur, Arikkulam, Keezhariyur, Thurayur, Thikkodi, Payyoli, Vadakara, Eramala, Ayancheri, Azhiyur- Kozhikode

Payyannur, Eramamkuttoor, Kadannappalli, Cheruthazham, Ezhome, Madayi, Cherukunnu, Pattuvam, Kannapuram, Mattool, Azhikode, Valapattanam, Chirakkal, Narath, Chembilode, Muzhappilangad, Dharmadam, Thalassery, Mahe -Kannur

Chelembra, Thenhipalam, Pallikkal, Vallikkunnu, Moonniyur, Parappanangadi, Thennala, Edarikode, Ponmukulum, Talakkad, Vettom, Purathur, Triprangode, Thirunavaya, Maranchery, Nannamukku – Malappuram

Mangalappady, Kumbla, Madhur, Mograputhur, Uduma, Pallikkara, Ajanoor, Pullur-Periya, Madikkai, Neeleswaram, Cheruvathur, Cheemeni -Kasargod

8.Cx. tritaeniorhynchus

Cx. tritaeniorhynchus was collected from

Azhiyoor, Vadakara, Payyoli, Moodady, Arikkulam, Koyilandi, Chengottkavu, Chemanchery, Thalakulathur, Elathoor, Kakkodi, Peruvayal, Beypore – Kozhikode

Ezhome, Mattool, Thaliparamba, Azhikode, Chirakkal, Muzhappilangad, Dharmadam, Thalassery – Kannur

Vallikkunnu, Pallikkal, Peruvallur, Moonniyur, Ozhur, Ponmundam, Cheriyamundam, Tirur, Veliyamkode, Ponnani – Malappuram

Chengala, Muliyar, Uduma, Ajanoor, Kanhangad, Neeleswaram, Karivelloor, Valiyaparamba, Pilicode –Kasargod

9.Cx. bitaeniorhynchus

Azhiyoor, Vadakara, Villiappally, Thurayur, Thikkodi, Chengottkavu, Chemanchery, Beypore, Kadalundi Kozhikode

Kunhimangalam, Ramanthala, Madayi, Ezhome, Mattool, Narath, Thaliparamba, Dharmadam, Thalassery – Kannur

Vazhakkad, Vazhayur, Pulikkal, Cherukavu, Pallikkal, vallikkunnu, Nannambra, Ozhur, Tanalur, Tavanur, Vattamkulam - Malappuram

Kumbla, Chemnad, Periya, Ajanoor, Madikkai, Kinanoor, Cheemeni, Thrikkaripur -Kasargod

10.Cx. gelidus

Azhiyoor, Edacheri, Chorode, Thikkodi, Koyilandi, Chengottkavu, Chemanchery, Elathoor, Cheruvannur, Beypore, Feroke, Kadalundi – Kozhikode

Ezhome, pattuvam, Chirakkal, Kannur, Muzhappilangad, Dharmadam, Eranholi, Thalassery, Mahe – Kannur

Vallikkunnu, Peruvallur, Pallikkal, Tanur, Tirur, Vettom, Thalakkad, Triprangode, Purathur, Vattamkulam, Veliyamkode, Alamkode- Malappuram

Meenja, Puthige, Kumbla, Chemnod, Uduma, Pallikkara, Kanhangad, Cheruvathur, Valiyaparamba- Kasargod

11.Cx. vishnui

Azhiyur, Vadakara, Chorode, Payyoli, Thikkodi, Koyilandi, Chengottkavu, Beypore, Feroke, Kadalundi - Kozhikode

Ezhome, pattuvam, Madayi, Kunhimangalam, Chirakkal, Muzhappilangad, Dharmadam Kannur

Vallikkunnu, Parappangadi, Tanur, Purathur, Ponnani, Veliyamkode- Malappuram

Kumbla, Kasargod, Uduma, Kanhangad, Cheruvathur, Karivellur, Valiyaparamba – Kasargod

In addition to these localities, almost all species were also collected from harbours of north Kerala i.e., Puthiyappa, Vellayil, Koyilandi, Chombala, Thalayi

Within the above enlisted species Anopheles stephensi was only encountered from slightly brackish water and it is only collected during the year 2021. So, it was excluded from the year wise analysis. Though it was less in number, only the salinity tolerance estimation was done.

4.3. Habitats of collected mosquitoes

Table 5 showing the habitats: eleven species were collected from the following habitats

Species	Types of habitats positive for larval breeding with salinity in ppt in brackets																		
	AB	DC	BD	RP	WLC	WLF	TH	TY	STR	GP	OHT	CP	WD	ME	ML	RF	CS	KRF	SWB	BP
An. subpictus B														+(20-30)	+(28-32)				+(20-30)	
An. subpictus A			+(16)		+(1)	+(.3)								+(20)	+(18)				+(15)	
An. barbirostris	+(.3)	+(1)		+(.1)	+(.7)	+(.1)				+(.1)	+(.1)		+(.4)					+(2)	+(1)	
An. stephensi	+(.2-.7)	+(.2)			+(.4)	+(.1)				+(.1)	+(.3)									
Ae. albopictus	+(.2-.7)	+(.1-2)	+(4)				+(.2)	+(.1-.3)		+(.3-.5)	+(.1-.3)	+(.2-.5)	+(.1-.3)				+(.3)		+(2)	+(5)
Cx.tritaeniorhynchus		+(.4)	+(4)									+(.3)				+(.5)		+(3)		+(5)
Cx.sitiens		+(2)											+(5)	+(30)	+(28-30)				+(30)	+(10)
Cx.quinquefasciatus	+(.4)	+(2)								+(.2)		+(.4)		+(30)	+(28-30)				+(30)	+(20)
Cx.bitaeniorhynchus	+(.3)	+(.3)							+(.2)		+(.2)		+(.2)					+(5)	+(3)	+(2)
Cx.gelidus	+(.7)	+(3)												+(10)	+(8)		+(.5)	+(5)	+(5)	+(7)
Cx. vishnui	+(.3)	+(2)													+(6)	+(.5)		+(5)	+(5)	+(6)

+ = present, AB= Abandoned boats, DC= Discarded containers, BD= Brackish ditches, RP= Rock pools, WLC= well in coastal areas, WLF= Well in fresh water areas, TH= Tree holes, GP= Ground pools, TY= Tyres, STR- Streams, OHT= Overhead tank, CP= Cement pots, WD= Water drums, MR= Mangrove ecosystem, ML= Marshy land, RF= Rice fields, CS= Coconut shells, KRF= Kaipad rice fields, SWB= Stagnant water bodies, BP= Brackish pits.

On an overall assessment, it has been noticed that the larval density was greater in habitats like mangrove ecosystems, marshy lands, stagnant water bodies, discarded containers, Kaipad rice fields and rice fields. In terms of larvae, Culex larvae show maximum density when compared to Aedes and Anopheles.

Of the surveyed habitats *An. subpictus* B was collected from only brackish water habitats. It was collected from mangrove ecosystems having salinity in the range of 20-30 ppt, marshy land having salinity in the range of 28-32 ppt, and stagnant water bodies in coastal areas having salinity in the range of 20-30 ppt.

An. subpictus A larvae were collected from brackish water ditches with a salinity of 16 ppt, Wells in coastal areas with salinity1 ppt, wells in freshwater areas having 0.3 ppt salinity, mangrove ecosystems with having salinity of 20 ppt, marshy land with salinity 18 ppt, and stagnant water bodies having salinity 15 ppt.

Larvae of *An. barbirostris* were collected from abandoned boats with 0.3 ppt, discarded containers and rock pools with a salinity 1 ppt, wells in coastal areas having salinity 0.7 ppt, wells in freshwater areas with 0.1ppt, ground pools with 0.1 ppt salinity, overhead tank with 0.1 ppt salinity, water drum having salinity 0.4 ppt, Kaipad rice field with 2 ppt salinity and stagnant water bodies having salinity 1 ppt.

An. stephensi larvae were collected from abandoned boats with a salinity of 0.2-0.7 ppt, discarded containers with salinity of 0.2 ppt, wells in coastal areas with a salinity 0.4 ppt, wells in freshwater habitats having salinity of 0.1 ppt, ground pools with 0.1 salinity and overhead tank with salinity 0.3 ppt.

Ae. albopictus larvae were collected from abandoned boats with salinity 0.2-0.7 ppt, discarded containers of 0.1-2 ppt, brackish water ditches of 4 ppt, tree holes with 0.2 ppt salinity, tyres having salinity in the range of 0.1-0.3 ppt, ground pools having salinity in the range of 0.3-0.5 ppt, overhead tank with 0.1-0.3 ppt salinity, cement pots of 0.2-0.5 ppt

salinity, water drums with 0.1-0.3 ppt salinity, coconut shells having 0.3 ppt salinity, stagnant water bodies with salinity 2 ppt and brackish pits of salinity 5 ppt.

Larval population of *Cx. tritaeniorhynchus* were observed in discarded containers having a salinity of 0.4 ppt, brackish water ditches of 4 ppt salinity, cement pots having a salinity 0.3 ppt, rice fields with a salinity 0.5 ppt, Kaipad rice fields with 3 ppt salinity, and brackish pits with 5 ppt salinity.

Larval collections of *Cx. sitiens* has been recorded mainly from brackish water habitats like discarded containers of salinity of 2 ppt, water drums with 5 ppt salinity, mangrove ecosystems with 30 ppt, marshy land with 28-30 ppt, stagnant water bodies having salinity of 30 ppt, and brackish pits with 10 ppt.

Larval populations of *Cx. quinquefasciatus* were recorded from discarded containers of salinity of 2 ppt, mangrove ecosystems with salinity of 30 ppt, marshy lands having salinity in the range of 28-30 ppt, stagnant water bodies of 30 ppt salinity, brackish water pits of 20 ppt salinity, cement pots of 0.4 ppt salinity, and ground pools of 0.2 ppt salinity.

Cx. bitaeniorhynchus larvae were abundant in abandoned boats of 0.3 ppt, discarded containers with salinity of 0.3 ppt, overhead tank with 0.2 ppt, water drum with 0.2 ppt, Kaipad rice fields with 5 ppt salinity, stagnant water bodies with 3 ppt salinity, brackish pits of salinity 2 ppt and stream margins of 0.2 ppt salinity.

Larvae of *Cx. gelidus* prefer both fresh and brackish water equally. Larvae were collected from abandoned boats with salinity 0.7 ppt, discarded containers of salinity 3 ppt, mangrove ecosystem with salinity 10 ppt, marshy land with 8 ppt, rice fields with 0.5 ppt, Kaipad rice fields with 5 ppt salinity, coconut shells with 0.5 ppt, stagnant water bodies with 5 ppt salinity and brackish pits with 7 ppt salinity.

The larvae of *Cx. vishnui* has been recorded from abandoned boats with 0.3 ppt, discarded containers having salinity 2 ppt, marshy lands with salinity 6 ppt, rice fields of 0.5 ppt salinity, Kaipad rice fields with 5 ppt salinity, stagnant water bodies having salinity 5 ppt and brackish pits with 6 ppt salinity.

The larval density was studied in detail and it was found that larvae of culex observed in all aquatic habitats and their high density was found in almost every habitat. Maximum immature stages recorded in mangrove ecosystems and marshy land. Culex species were the

most abundant and found over, all habitats most frequently in rice fields, Kaipad rice fields etc.

4.4. Density of collected mosquitoes

Table 6: Density of *Anopheles* larvae in different habitat types in north Kerala

Sl. No	Habitat type(n)	Total no. of dips	Total no. of larvae	No. of larvae per dip	Total no. of pupae	No. of pupae per dip
1.	Mangrove ecosystem (60)	1500	3038	2.02	235	0.15
2	Marshy land (48)	1200	2370	1.975	200	0.16
3	Stagnant water bodies (72)	1800	1632	0.90	230	0.12
4	Brackish ditches (56)	1400	1890	1.35	277	0.19
5	Wells in coastal areas (134)	3350	150	0.04	41	0.01
6	Wells in fresh water areas (137)	3425	137	0.04	36	0.01
7	Discarded containers (258)	6450	1983	0.30	268	0.04
8	Rock pools (45)	1125	1786	1.58	256	0.22
9	Ground pools (37)	925	1746	1.887	234	0.25
10	Overhead tanks (29)	725	3428	4.72	229	0.31
11	Water drum (34)	850	1960	2.30	218	0.25
12	Abandoned boats (175)	4375	2148	0.49	197	0.04
13	Kaipad rice fields (24)	600	1756	2.92	239	0.39
14	Total	27725	24024	0.86	2660	0.09

The larval density of *Anopheles* was found to be very less when compared to *Culex* and *Aedes*. Among the surveyed habitats the maximum density was found in overhead tank. With 725 dips in 29 overhead tank, 3428 larvae were collected with density 4.72. The minimum

density was found in wells in coastal and fresh water areas. With 3350 dips 134 wells in coastal areas only 150 larvae were collected with density 0.04 and with 3425 dips in 137 wells in fresh water areas only 137 larvae were collected with density 0.04. The maximum pupal density was found in Kaipad rice fields. With 600 dips in 24 Kaipad rice fields 239 pupae were collected with density 0.39. The least density was found in wells in coastal areas and wells in fresh water areas i.e., 0.01 density each.

Table 7: Density of *Aedes* larvae in different habitat types in north Kerala

Sl.no	Habitat type (n)	Total no. of dips	Total no. of larvae	No. of larvae per dip	Total no. of pupae	No. of pupae per dip
1	Abandoned boats (225)	4500	1890	0.42	200	0.04
2	Discarded containers (558)	1000	1750	1.7	185	0.18
3	Brackish ditches (32)	640	898	1.4	198	0.30
4	Tree holes (134)	2680	1364	0.5	144	0.05
5	Tyres (56)	1120	828	0.7	163	0.14
6	Ground pools (49)	980	1008	1.02	172	0.17
7	Overhead tank (90)	1800	1158	0.64	159	0.08
8	Cement pots (106)	2120	1024	0.48	134	0.06
9	Water drums (75)	1500	1037	0.69	107	0.07
10	Coconut shells (289)	1445	758	0.52	162	0.11
11	Stagnant water bodies (32)	640	218	0.34	93	0.14
12	Brackish pits (24)	480	164	0.34	84	0.17
13	Total	10160	12097	1.19	1801	0.17

The density study of Aedes larvae shows that maximum larval density was found in discarded containers, where 558 discarded containers were sampled with 1000 dips ,1750 larvae were collected and density was found to be 1.7. the pupal density also shows the same trend, discarded containers show maximum density. The minimum larval density was found in stagnant water bodies and brackish pits. The larval density was found to be 0.34. maximum pupal density was found in brackish ditches i.e., 0.30. the minimum larval density was found in abandoned boats i.e., 0.04,

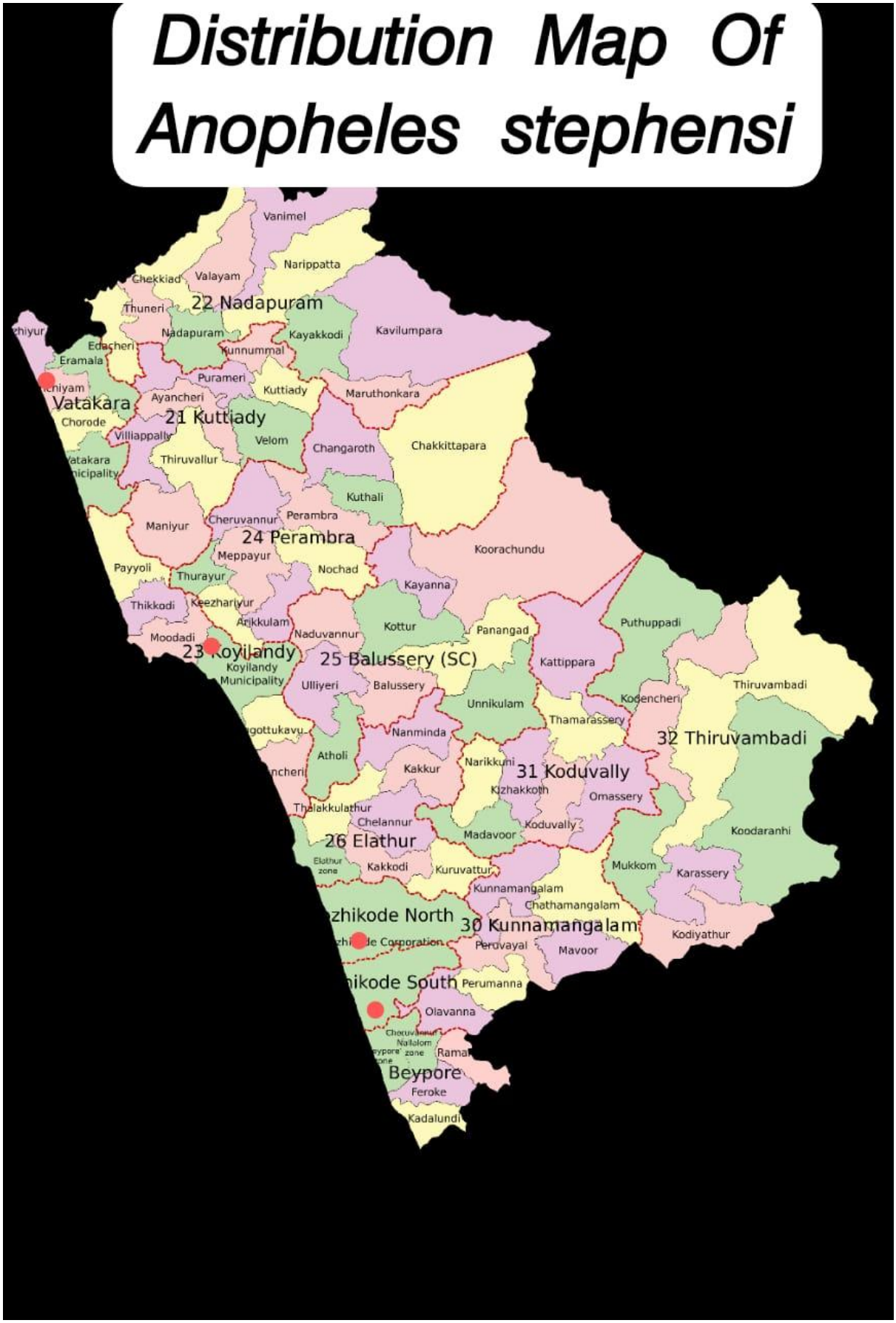
Table8:Density of *Culex* larvae in different habitat types in north Kerala

Sl. No	Habitat types (n)	Total no. of dips	Total no. of larvae	No. of larvae per dip	Total no. of pupae	No. of pupae per dip
1	Rice fields (35)	350	6487	18.53	1017	2.90
2	Kaipad rice fields (12)	180	8439	46.88	969	5.38
3	Water drums (21)	210	7957	37.89	1114	5.30
4	Cement pots (32)	320	8079	25.24	948	2.96
5	Brackish pits (23)	230	7375	32.06	799	3.47
6	Discarded containers (134)	200	6846	34.23	868	4.34
7	Brackish ditches (28)	280	7938	28.35	1124	4.01
8	Mangrove ecosystems (19)	200	9400	47	784	3.92
9	Marshy lands (16)	200	9900	49.5	1350	6.75
10	Stagnant water bodies (26)	260	7734	29.74	807	3.01
11	Ground pools (16)	160	4249	26.55	899	5.61
12	Abandoned boats (175)	600	8734	14.55	967	1.61
13	Stream margins (29)	290	5017	17.3	571	1.96
14	Coconut shells (197)	394	7865	19.96	783	1.98
15	Total	3874	106020	27.36	13000	3.35

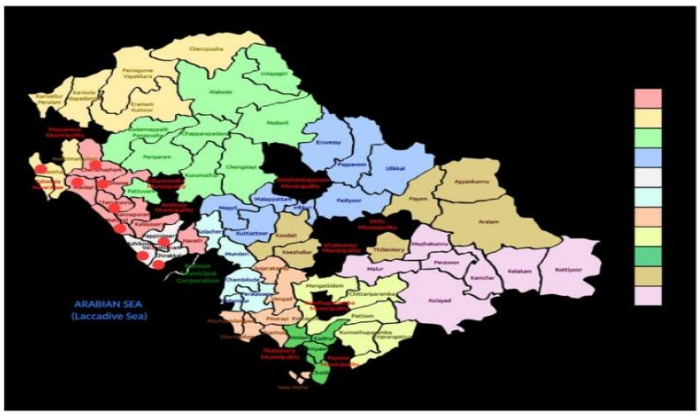
Larval density was very higher in culex larvae when compared to Aedes and Anopheles larvae. Maximum larval density was found in marshy lands. With 200 dips in 16 marshy lands, the density was found to be 49.5. in addition to marshy lands, Kaipad rice fields and mangrove ecosystem were also shown 40 above densities. The least density was found in stream margins, 5017larvae got from 29 stream margins with 290 dips and having density 17.3. The maximum pupal density was found in marshy lands, with 200 dips in 16 marshy lands, the density was found to be 6.75. Like larval density, least pupal density was found in stream margins i.e.,1.98.

Distribution Map of *Anopheles subpicus B*

Distribution Map of *Anopheles subpicus A*

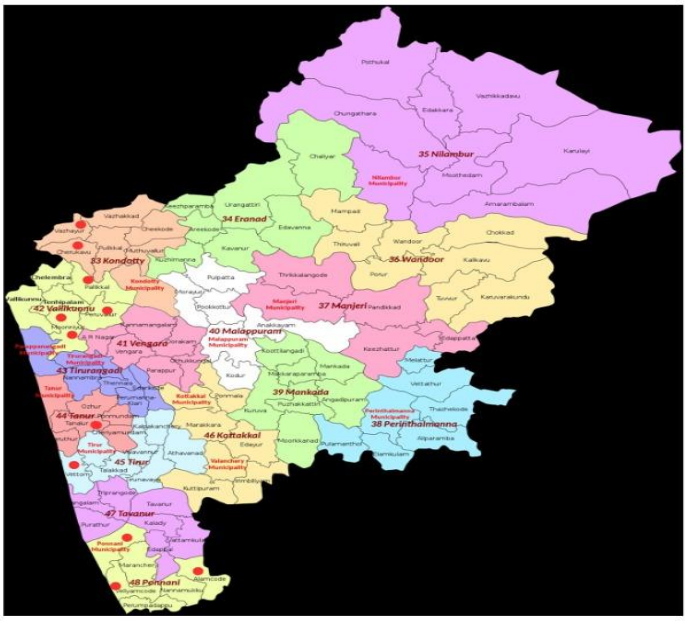

Distribution Map Of
Anopheles stephensi
Vanimel
Narippatta
Chekkiad
Valayam
22 Nadapuram
Thuneri
Nadapuram
Kayakkodi
Kavilumpara
Edacheri
Kunnummal
Eramala
Purameri
Kuttiady
Maruthonkara
Ayancheri
Vatakara
21 Kuttiady
Chorode
Villiappally
Velom
Changaroth
Chakkittapara
Thiruvallur
Kuthali
Cheruvannur
Perambra
Maniyur
24 Perambra
Koorachundu
Meppayur
Payyoli
Thurayur
Nochad
Kayanna
Thikkodi
Keezhariyur
Arikkulam
Kottur
Puthuppadi
Moodadi
Naduvannur
Panangad
23 Koyilandy
25 Balussery (SC)
Kattippara
Koyilandy
Municipality
Ulliyeri
Balussery
Thiruvambadi
Unnikulam
Kodencheri
Thamarassery
Nanminda
32 Thiruvambadi
Atholi
Kakkur
Narikkuni
31 Koduvally
Kizhakkoth
Omassery
Thalakkulathur
Chelannur
Koduvally
Madavoor
Koodaranhi
26 Elathur
Elathur zone
Kakkodi
Kuruvattur
Mukkom
Karassery
Kunnamangalam
Chathamangalam
zhikode North
30 Kunnamangalam
Kodiyathur
Peruvayal
Mavoor
hikode South
Perumanna
Olavanna
Nallalom zone
Beypore
Feroke
Kadalundi

Distribution Map of *Anopheles barbirostris*



■ Kannur

■ Kozhikode



■ Malappuram

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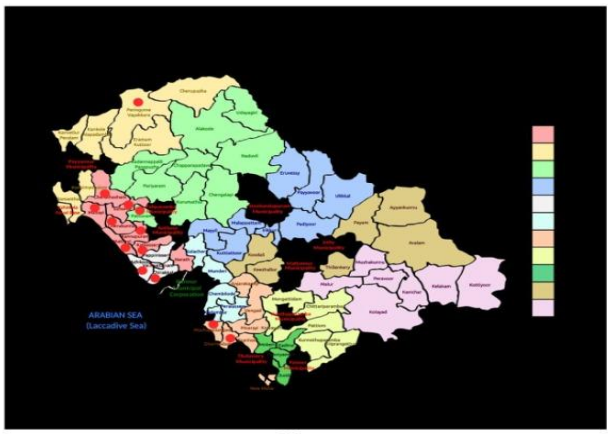
Distribution Map of *Aedes albopictus*



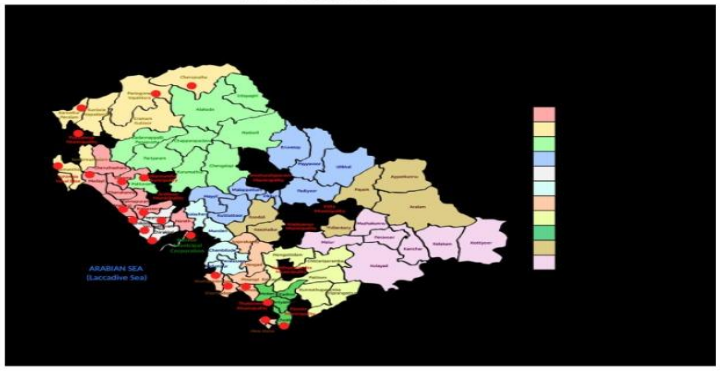
■ Kannur

■ Kozhikode

■ Malappuram

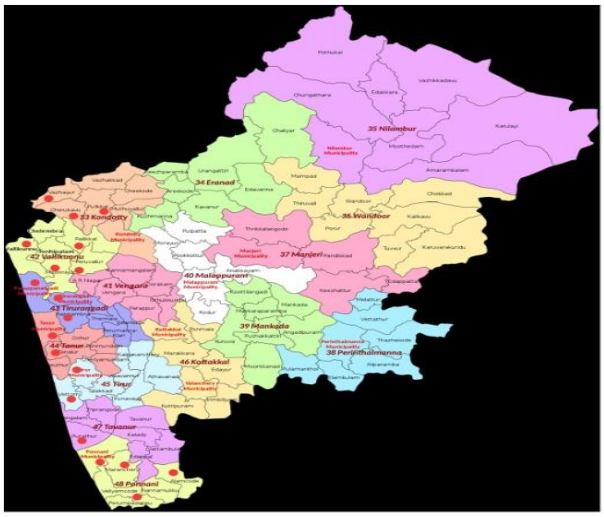
Distribution Map of *Culex sitiens*

■ Kannur



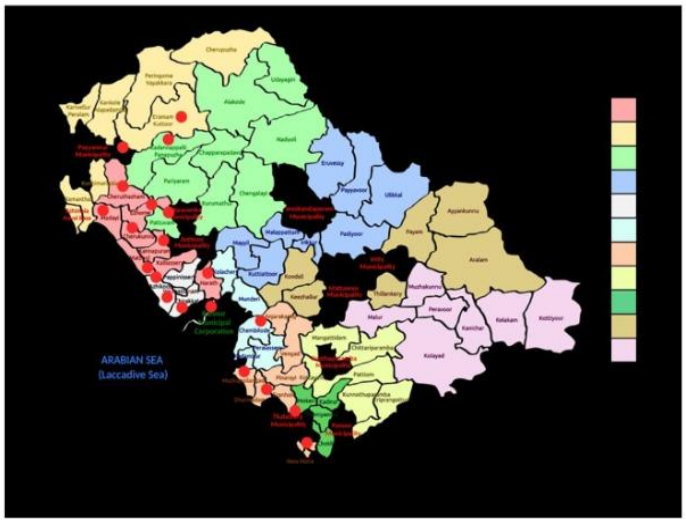
■ Kozhikode

■ Malappuram



Distribution Map of *Culex quinquefasciatus*

■ **Kannur**

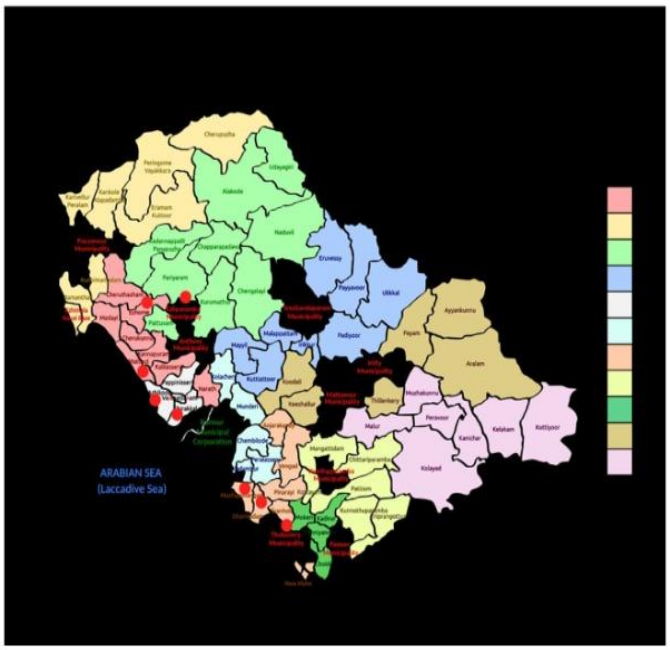


■ **Kozhikode**

■ **Malappuram**

Distribution Map of *Culex tritaeniorhynchus*

■ **Kannur**

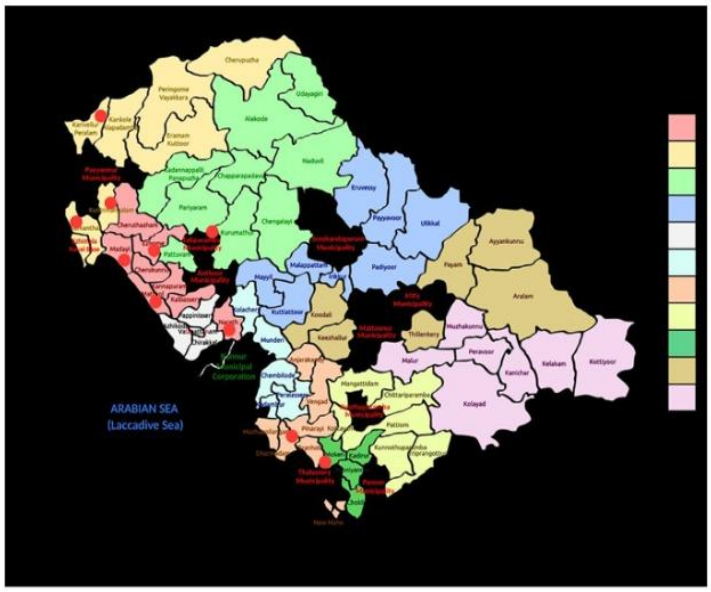


■ **Kozhikode**

■ **Malappuram**

Distribution Map of *Culex bitaeniorhynchus*

■ **Kannur**

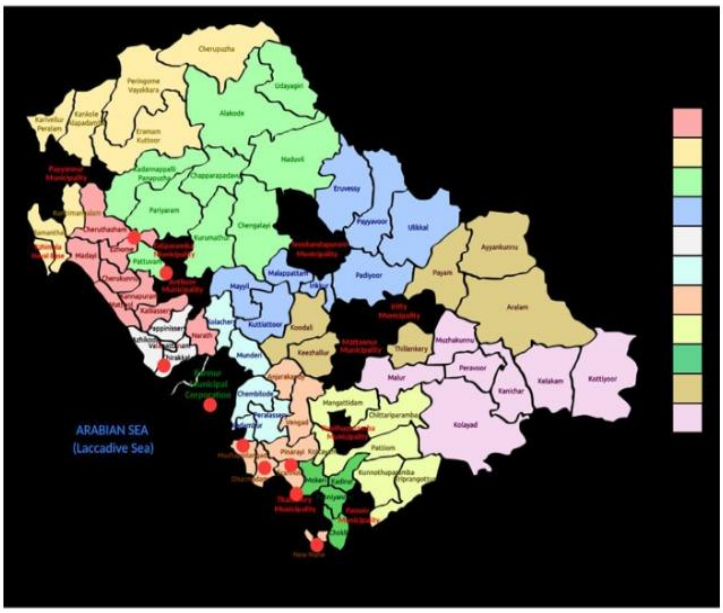


■ **Kozhikode**

■ **Malappuram**

Distribution Map of *Culex gelidus*

■ **Kannur**

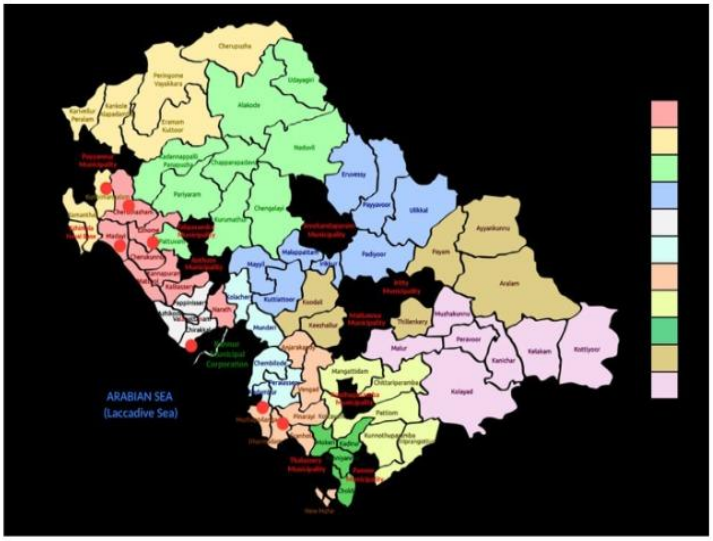


■ **Kozhikode**

■ **Malappuram**

Distribution Map of *Culex vishnui*

■ Kannur



■ Kozhikode

■ Malappuram

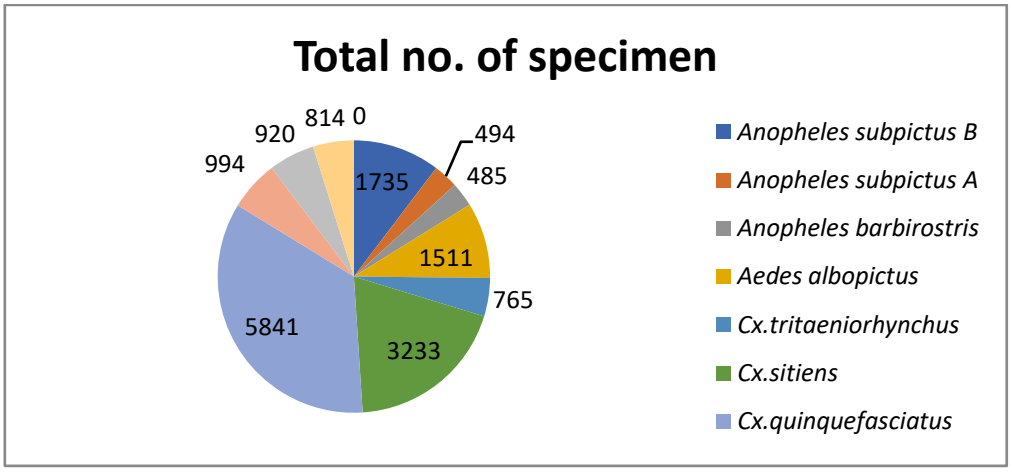
4.6 Salinity in the fields

Salinity of the fields were measured using salinometer to classify the habitats as brackish or fresh water. The table below shows the salinity of the sample water from which the specimens were collected.

Table 9: salinity in the field

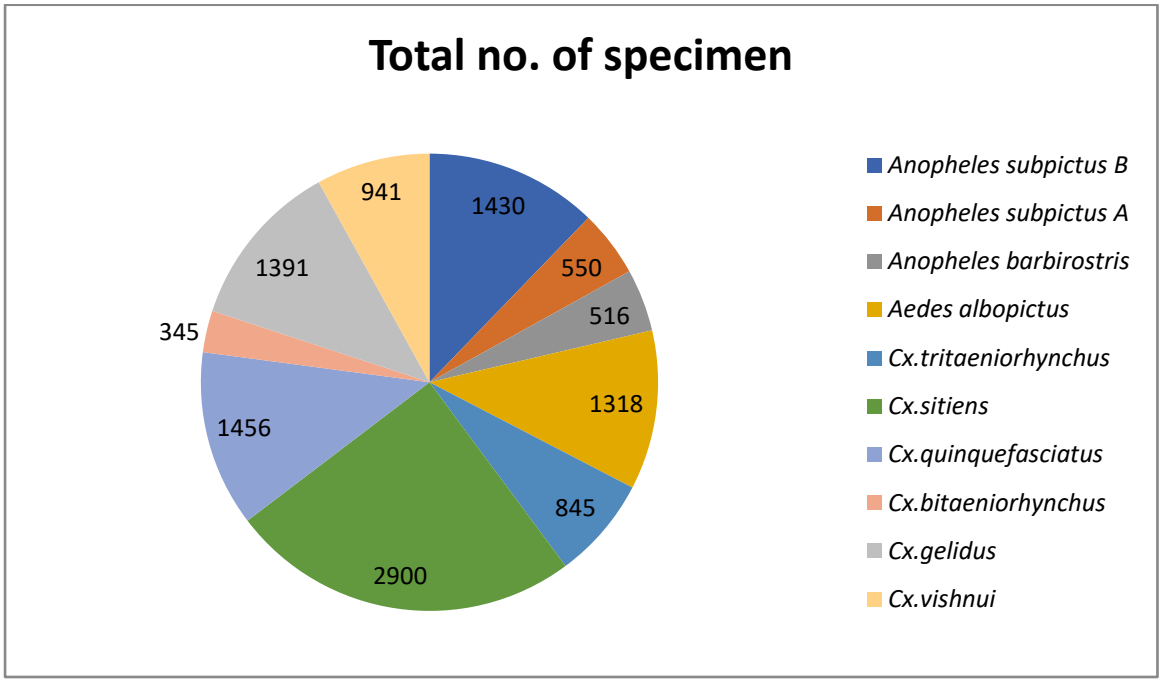
Mosquito	Salinity in the fields (ppt)
An. subpictus B	28-32
An. subpictus A	0.3-20
An. barbirostris	0.1-2
An. stephensi	0.1-0.7
Ae. albopictus	0.1-5
Cx.quinquefasciatus	0.2-30
Cx.sitiens	2-32
Cx.bitaeniorhynchus	0.2-5
Cx.tritaeniorhynchus	0.3-5
Cx.gelidus	0.5-10
Cx.vishnui	0.3-6

4.7 Year wise analysis of collection



Pie diagram showing Species Encountered in 2018

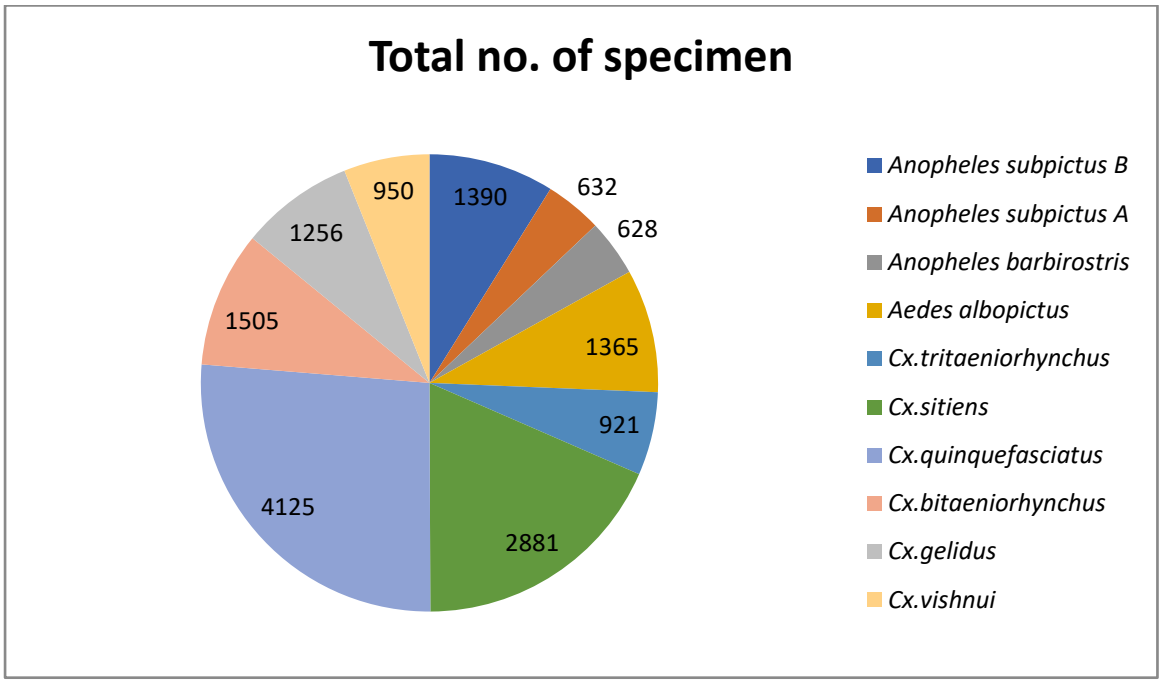
A total of 16792 of 3rd, 4th instar larvae and pupae were collected in 2018. Altogether 10 species under 3 genera were identified. Among the collected species *Culex quinquefasciatus* were the dominant species collected (34.78%). *Cx. sitiens* (19.25%), *An. subpictus* B (10.33%), *Ae. albopictus* (8.99%), *Cx. bitaeniorhynchus* (5.91%), *Cx. gelidus* (5.47%), *Cx. vishnui* (4.84%), *Cx. tritaeniorhynchus* (4.55%), *An. subpictus* A (2.94%), *An. barbirostris* (2.88%) were also collected. An. barbirostris were the least collected species. It was observed during the study that mangrove marshes and ditches in mangrove areas were the most preferred habitat for breeding by brackish water mosquitoes.



Pie diagram showing Species Encountered in 2019

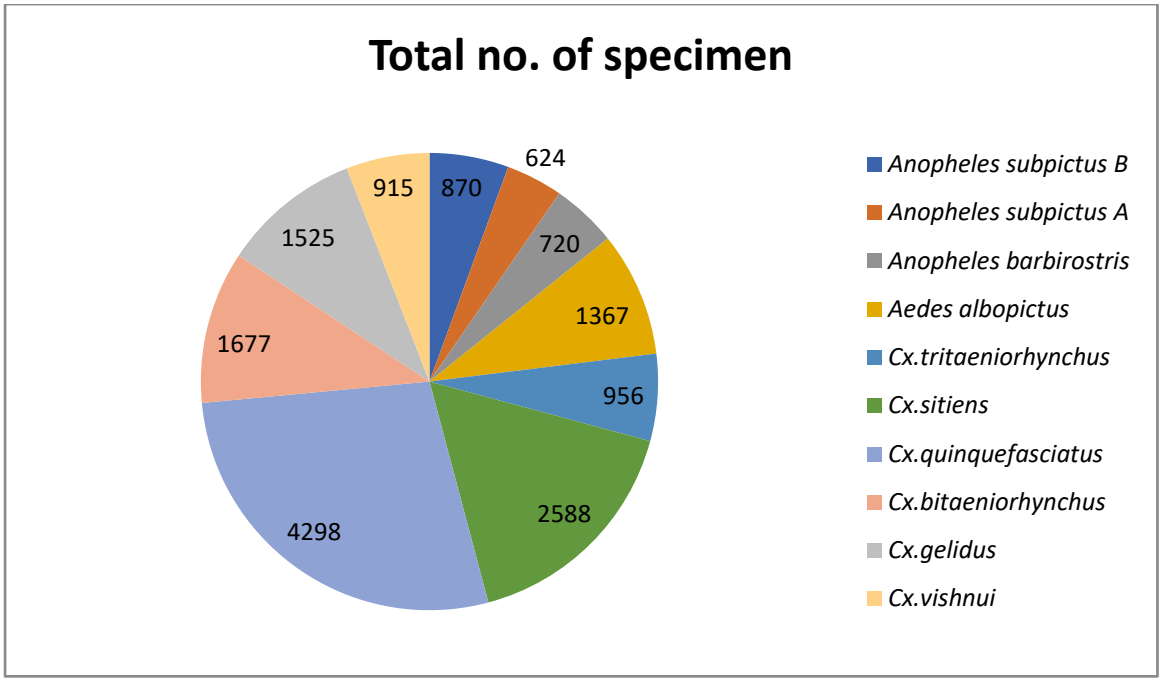
A total of 15770 3rd, 4th instar larvae and pupae were collected during the year 2019. Mosquitoes belongs to 10 species under 3 genera were morphologically identified. Among these *Culex quinquefasciatus* were the dominant species collected (28.81%). *Anopheles barbirostris* was the least collected species (3.27%). *Culex sitiens*(18.38%), *An. subpictus B* (9.06%),*Cx. gelidus*(8.82%),*Cx. bitaeniorhynchus*(8.46%), *Ae. albopictus* (8.35%),*Cx. vishnui*(5.96%),*Cx. tritaeniorhynchus (5.35%) and An. subpictus*(3.48%)were also collected during this period. It was observed during the study that stagnant water bodies in mangrove area and ditches in mangroves were the most preferred habitat for breeding of brackish water

breeders. Discarded containers in coastal areas and rice fields in coastal areas were found to be the second preferred habitats of mosquito breeding in brackish water.



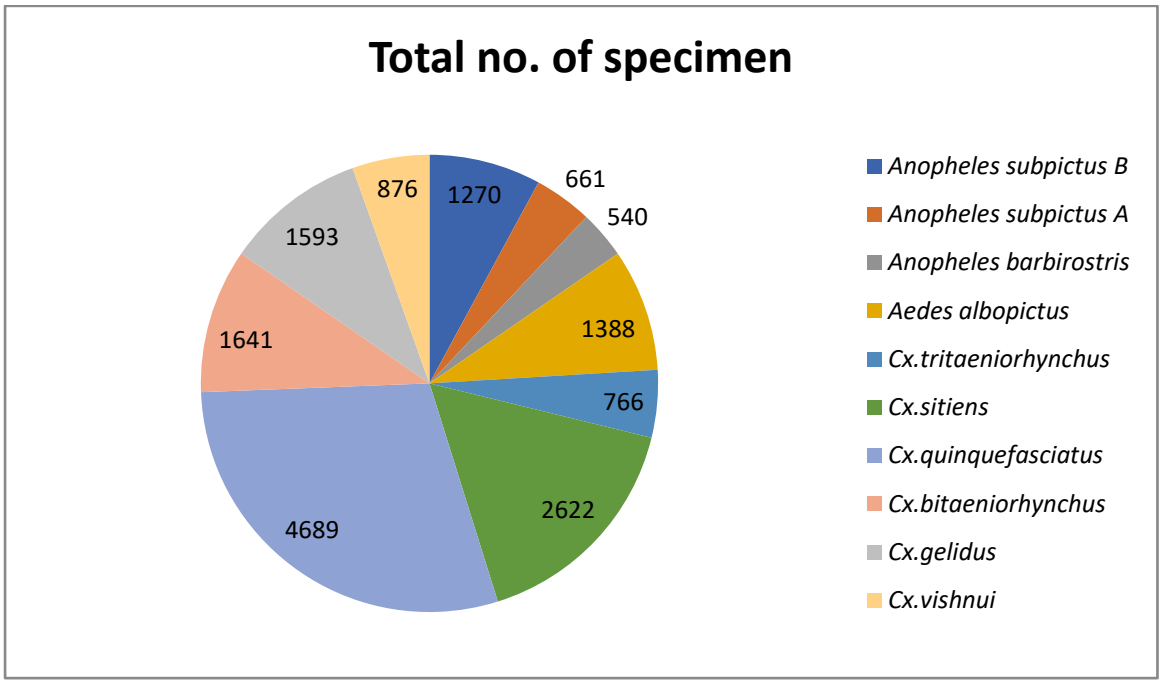
Pie diagram showing Species Encountered in 2020

During 2020 a total of 15653 mosquitoes were collected. Ten species of mosquitoes under 3 genera were morphologically identified. *Culex quinquefasciatus* was the dominant one among the collected species (26.35%). *Cx.sitiens* was the second dominant species collected during 2020(18.40%). *An. barbirostris* was the least collected species (4.01%). *Cx. bitaeniorhynchus* (9.61%), *An. subpictus* B (8.88%), *Ae. albopictus* (8.72%), *Cx. gelidus* (8.02%), *Cx. vishnui* (6.06%) and *An. subpictus* A (4.03%) were also collected. The preferred habitats of mosquitoes were found to be coastal marshes and stagnant water bodies in mangroves.



Pie diagram showing Species Encountered in 2021

In 2021 a total of 15540 mosquitoes were collected. Ten species of mosquitoes under 3 genera were morphologically identified. *Culex quinquefasciatus* was the dominant species collected (27.69%). *Anopheles subpictus* A was the least collected species (4.01%). *Cx.sitiens*, *Cx. bitaeniorhynchus*, *Cx. gelidus* (9.81%), *Ae.albopictus*(8.79%),*Cx.tritaeniorhynchus*(6.15%), *An.subpictus* B(5.59%), and *An.barbirostris*(4.63%) were also collected during 2021. Kaipad rice fields in Ezhome were found to be the most preferred habitat of brackish water breeding mosquito.



Pie diagram showing Species Encountered in 2022

A total of 16046 mosquitoes were collected during 2022. Ten species under 3 genera were morphologically identified. *Culex quinquefasciatus* was the dominant species collected (29.22%) and *Anopheles barbirostris* was the least collected species (3.36%). *Cx.sitiens* (16.34%), *Cx. bitaeniorhynchus* (10.22%), *Cx. gelidus* (9.92%), *Ae. albopictus* (8.65%), *An. subpictus* B (7.91%), *Cx. vishnui* (5.45%), *Cx. tritaeniorhynchus*(4.77%) and *An. subpictus* A (4.12%) were also collected.

4.8 Distribution of species in different season

Collection was done in pre monsoon, monsoon, and post monsoon periods.

The table below shows the year wise distribution of collected species in pre monsoon, monsoon, and post monsoon periods.

Table 10: Seasonwise distribution

Species name	Year	Premonsoon	Monsoon	Post monsoon	Total
An. subpictus B	2018	1774	540	1156	3470
	2019	1400	480	980	2860
	2020	1560	580	640	2780
	2021	1000	280	460	1740
	2022	1340	780	420	2540
An. subpictus A	2018	408	260	320	988
	2019	500	360	240	1100
	2020	624	280	360	1264
	2021	556	274	418	1248
	2022	596	330	396	1322
An. stephensi	2018	0	0	0	0
	2019	0	0	0	0
	2020	0	0	0	0
	2021	46	0	23	69
	2022	0	0	0	0

An. barbirostris	2018	400	200	370	970
	2019	492	246	294	1032
	2020	530	334	392	1256
	2021	600	380	460	1440
	2022	578	216	286	1080
Aedes albopictus	2018	1400	700	922	3022
	2019	1394	1068	708	2636
	2020	1156	774	800	2730
	2021	1358	596	780	2734
	2022	1196	680	900	2776
Cx.sitiens	2018	3000	1400	2066	6466
	2019	2468	1358	1974	5800
	2020	2690	1314	1758	5762
	2021	2552	1134	1490	5176
	2022	2376	1312	1556	5244
Cx.bitaeniorhynchus	2018	1200	388	400	1988
	2019	1474	506	690	2670
	2020	1390	708	912	3010
	2021	1572	690	1092	3354
	2022	1374	862	1046	3282
Cx.tritaeniorhynchus	2018	700	340	490	1530
	2019	756	426	508	1690
	2020	796	468	578	1842
	2021	804	508	600	1912
	2022	616	400	516	1532
Cx.quinquefasciatus	2018	4800	2882	4000	11400
	2019	3758	2418	2912	8800
	2020	3534	2092	2684	8000
	2021	3810	2006	2780	8200
	2022	3580	2490	3308	9000
Cx.gelidus	2018	1000	240	600	1840
	2019	1380	588	814	2782

	2020	1226	502	784	2512
	2021	1440	620	990	3050
	2022	1382	774	1030	3186
Cx.vishnui	2018	800	228	600	1628
	2019	868	356	658	1882
	2020	906	300	694	1900
	2021	782	438	710	1830
	2022	756	416	580	1752

4.9 Brief description of collected species

1. An. subpictus B

An. subpictus complex comprise of four reproductively distinct species, named as A, B, C and D (Goutham Chandra 2010). Reuben & Suguna1983 stated morphological differences in eggs, larvae, pupae and adults between sibling species A, B, C, D. The morphological differences include the ridge number on egg floats, egg frill, larval and pupal setae, adult female palpi (Suguna etal.1994). *Anopheles subpictus* B breed in Brackish water (Chandra G, etal 2010). Species A, C, D occur in freshwater habitats of Inland areas, while the salt water species B occurs sympatrically with the others in coastal villages (S. G. Suguna etal. 1994). The brackish water form, sibling species B of *An. subpictus* has been incriminated as a vector in some coastal areas of South India (Russell & Jacob 1939, Russell & Rao 1940, Panicker etal, 1981).

Morphologically identified *Anopheles subpictus* species complex were resolved using molecular methods and was shown to be *Anopheles subpictus* B. It was found to be breed in brackish water. Identifying features of *Anopheles subpictus B* is enlisted below.

Female palpi with three pale band, subapical dark band equal or nearly equal to the apical pale band

Thorax: Antepronotal scales absent; upper proepisternal setae present.

Legs: Fore tarsi with broad pale bands.

Hind tarsomere 5 not white.

Femur and tibia not speckled

Wings with 4 or more dark spots on costa [1, 2, 3, 4], which also involve vein 1.

Abdomen: Segments VII and VIII and cercus with at least a few scales.

2. Anopheles subpictus A

Morphologically identified *Anopheles subpictus* complex were resolved using molecular methods. It was found to be *Anopheles subpictus* A.

3.Anopheles stephensi

Very attractive species with ornate, heavily spotted upper legs. Maxillary palpus with pale bands. Erect head scales broad, white on vertex and dark brown laterally and posteriorly.

Thorax: scutum with obvious pale scales in addition to setae. Scutal fossa with scattered pale scales.

Wing: vein 1A with 3 dark spots, wing with scattered pale spots present on nearly all veins.

Abdomen: V-VIII-S usually with pale scales, II-VII-Te without dark scale tufts.

4. Anopheles barbirostris

Proboscis entirely dark scaled, Palpi without any pale markings and with dark numerous and erect scales; pedicel with dorsal and lateral scales; clypeus without dark scales at sides.

Thorax: Antepronotal scales present; pleuron with white scale patches.

Abdomen: sterna with median patches of pale scales and rows of pale scales on lateral margins; Female with prominent tuft of dark scales on ventral side of abdominal segment VII.

Legs: Fe III mostly dark, without preapical pale band; Ta II 1-5 not banded.

Wing: with 3 large dark spots on costa(C) and veins R-R1inner quarter of costa with scattered pale scales, usually with fringe spot at vein 5.2.

Wing with narrow lower apical fringe spot opposite to vein 3only.

Inner quarter of costa mainly dark, sometimes with a few scattered pale scales

5.Aedes albopictus

Commonly known as Asian tiger mosquito. It is one of the best-known mosquitoes in the world.

Head: Proboscis entirely dark scaled; palpus with white scales at apex; pedicel with scales on lateral surfaces.

Thorax: Scutum with median longitudinal stripe; antealar area with patch of broad pale scales; mesepimeron with lower scales; paratergite with scales; postpronotal scales present; postspiracular scales absent; proepisternal scales present; scutal angles without pale scales; subspiracular area with broad white scales.

Legs: Silvery or white scale patches on legs; Ta I-III 1-5 with only basal bands.

Abdomen: Tergal scales basal, often not connected with lateral pale scales; I -Te without median patch of white scales.

6. Culex sitiens

Culex sitiens is primarily dark brown, with a large median white band on the proboscis, and pale stripes across the abdominal segments and leg joints, giving almost wasp- like appearance.

Head: proboscis with broad median pale area; vertex all brown scaled.

Thorax: Scutum with dark brown scales and small pale scale patches on margins and on Prescutellar area; Acrostichal setae present. Pleuron with distinct patches of scales; lower mesepimeral setae absent.

Wing: Dark scaled; vein R2+3 shorter than vein R2.

Legs: Fe I. II speckled anteriorly; Ta I-III with pale bands at articulations.

7. Culex quinquefasciatus

Head: Proboscis without distinct median pale band.

Thorax: Postspiracular scales absent; prealar scales absent; lower mesepimeron with one seta; C-I with a few dark scales; scutal integuments yellowish or pale brown.

Legs: Ta I-III entirely dark. Anterior surface of Fe-I, Fe-II, and Ti I-III without median longitudinal pale stripes.

Wings: Wings entirely dark scaled; vein R2+3 short, <0.25 length of cell R2.

Abdomen: Terga bands with pale yellowish basal bands; abdominal sterna not banded.

8. Culex bitaeniorhynchus

Head: Proboscis with median pale band and two dorsolateral pale spots at labellum.

Thorax: Acrostichal setae present; lower mesepimeral setae absent.

Abdomen: With intermixed pale and dark scales.

Leg: Fe I-III and Ti I-III without rows of pale spots; Ta I-III with basal pale bands.

9. Culex tritaeniorhynchus

Head: Proboscis with broad median pale band; vertex with erect brownish scales.

Thorax: Scutum with unicolorous dark scales; Acrostichal setae present; pleuron with distinct scale patches; postspiracular scales absent; mesepimeral setae absent; lower mesokatepisternal scales present.

Legs: Fe I, II dark anteriorly; Ta I-III with pale bands.

Wing: Dark scaled; vein R2+3 shorter than R2.

Abdomen: Terga only with basal pale bands.

10. Culex gelidus

Head: Proboscis with broad median pale band; vertex with erect white scales.

Thorax: Distinct double row of Acrostichal setae present; scutum white-scaled anterior to Prescutellar area; pleuron with distinct scale patches; lower mesepimeral setae absent.

Legs: Ta I-III with pale bands at articulations; Fe II, III without pale speckling on anterior surfaces.

Abdomen: Terga with broad basal pale bands.

11. Culex vishnui

Head: Proboscis with distinct median band not extending basally onto ventral surface; vertex pale brown-scaled;

Thorax: Acrostichal setae present; pleuron with distinct scale patches; postspiracular scales absent; lower mesepimeron setae absent; terga with only basal bands.

Wing: Vein R2+3 shorter than vein R2.
Legs: Ta I-III pale banded, Fe I, II without anterior speckling Fe III with apical dark band.

4.10 Molecular resolution of collected species complex

Morphologically all specimens were identified up to species level. of these collected specimens *Anopheles subpictus*was a species complex and to resolve this complex, molecular analysis was done. *Anopheles stephensi*was an incriminated vector of malaria in the state, by considering its vector status molecular analysis of *Anopheles stephensi* were also done. The sequences were deposited in the GENBANK and got accession numbers for the sequences.

The phylogenetic tree of *Anopheles subpictus* B using COI and ITS 2 were given below.

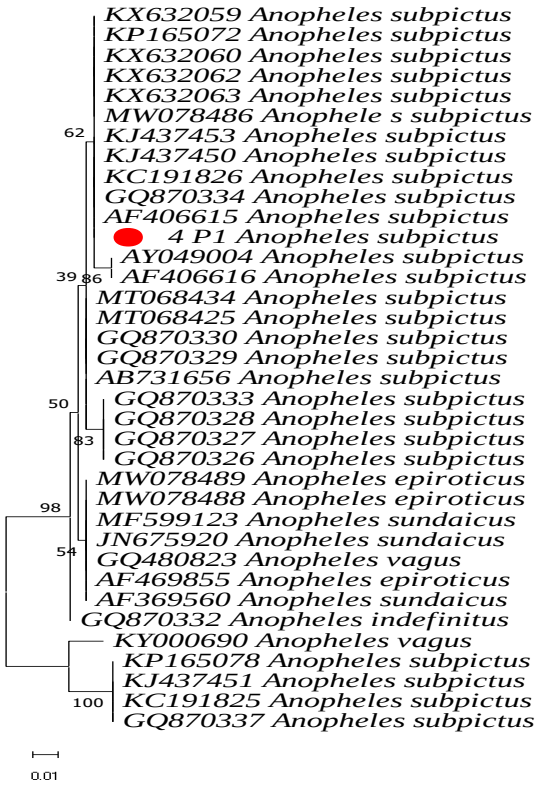


Fig 15: Evolutionary analysis of *Anopheles subpictus* based on ITS2 sequences using Maximum Likelihood method

PHYLOGENETIC ANALYSIS

Phylogenetic analysis based on ITS 2 sequences

>OL604470.1 Anopheles subpictus isolate P1 internal transcribed spacer 2, partial sequence

TTTCACCCGACCGATGCACACATCCTTGAGTGCCTACTAGGTACTTCGATTTTCCTATAA
TTAGACTACAGACGGGCGCCACTAATGGGCTGACGGGTTATCCGTCGTCTGGCGTGCGAC
TGTGCAGCATGGCGTGCTCGGGTCTCGGCGTGGACCCTTGGGCGCTGAAAGTGGATACTC
TGTTTGAGCGGCACCTTTGCGTGTGCTCTCCTAAGTGTCGACGTATGGTGAGGGTAGTGT
CAAGCCGCACGGTGCGACAACACAAGCGTACTGTCGAGTTTGGTGCAATCGGATGCCTAC
TACCATGGGCGGTGCCGGCGTGCATTCAACACTCGACGTGTGCGTCCTG

Fig 16: Consensus sequence of ITS2 region of *Anopheles subpictus* (OL604470)

The 349 bp long nucleotide sequence of ITS2 region was deposited in GenBank nucleotide database with accession number OL604470. The evolutionary history of *An. subpictus* was inferred by using the Maximum Likelihood method and Jukes-Cantor model [Jukes and Cantor, 1969]. The morphologically identified *An. subpictus* species B was sequenced for the rDNA ITS 2 regions. The resulting 294 bp sequences together with GenBank entries for *An. subpictus* from India, Thailand, Sri Lanka and Myanmar and sequences of *An. vagus, An. epiroticus, An. sundaicus*and *An. Indefinitus*were used in phylogenetic tree construction. The tree with the highest log likelihood (-567.73) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Jukes-Cantor model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 36 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 294 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [Tamura et al., 2021]. The resulting tree with samples names and corresponding GenBank accession numbers is given in figure 2. The phylogenetic tree reveals 2 distinct clades. Species B clade and species A clade. Morphologically identified sample 4P1 *An. subpictus* belongs to species B clade and from herein we consider individuals in this clade belongs to species B. The separate clade is referred to as species A clade. The ITS 2 gene tree indicates that species B closely related to *An. Sundaicus, An. vagus,* and *An. epiroticus* and species A form a distinct clade.

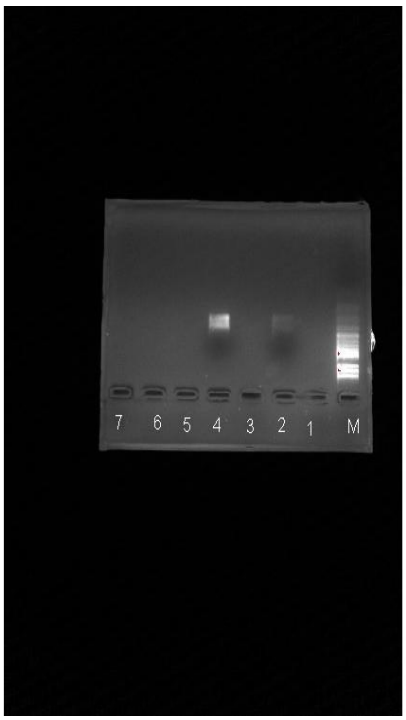


Figure 17:Gel image of ITS 2 sequence of *An. subpictus*B. M : 100 bp marker, 2&4 species B

Fig: Evolutionary analysis of *Anopheles subpictus* based on COI sequences using Maximum Likelihood method

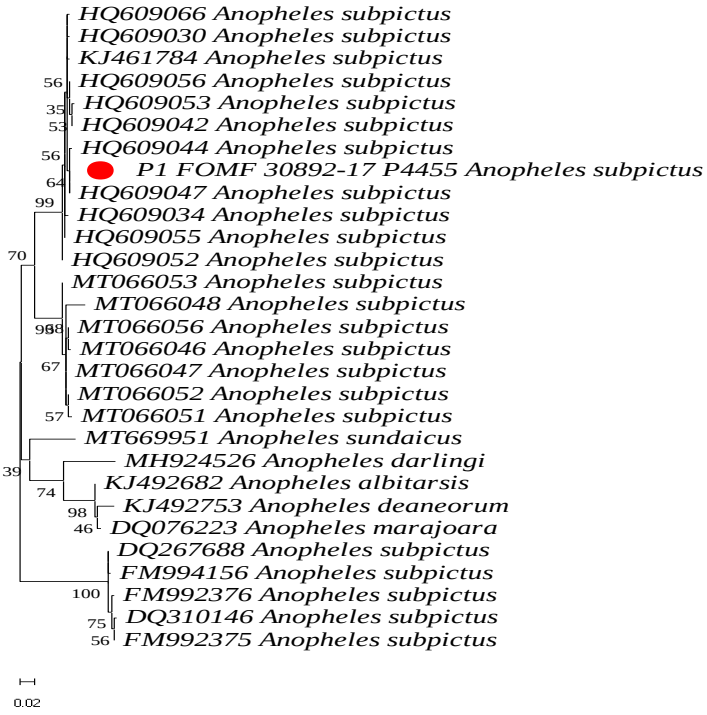


Fig18: Evolutionary analysis of *Anopheles subpictus* based on COI sequences using Maximum Likelihood method

PHYLOGENETIC ANALYSIS BY COI SEQUENCES

>OL587934.1 Anopheles subpictus voucher P1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

TGGATAGTGGGTACTTCTTTAAGAATTTTAATTCGAGCTGAATTAGGTCATCCAGGAGCT

TTTATTGGTGATGATCAAATTTATAATGTAATTGTTACTGCTCATGCATTTATTATAATT

TTCTTTATAGTAATACCAATTATAATTGGTGGATTTGGAAACTGATTAGTGCCTCTTATA

CTAGGAGCGCCTGATATAGCATTCCCTCGAATAAACAATATAAGATTTTGAATATTACCT

CCTTCTTTAACACTTTTAATTTCTAGTAGTATAGTGGAAAATGGGGCGGGTACAGGATGA

ACTGTTTACCCTCCGCTATCTTCTGGGATTGCACATGCAGGGGCATCAGTTGATTTAGCA

ATTTTTTCTCTACATTTAGCTGGTATTTCTTCAATTTTAGGAGCAGTAAATTTTATTACT

ACAGTAATTAATATACGATCTCCAGGAATTACTTTAGACCGAATACCATTGTTTGTATGA

TCAGTAGTAATTACAGCAATTTTATTATTGTTATCACTGCCAGTATTAGCTGGAGCTATT

ACTATATTATTAACTGATCGAAATTTAAATACCTCTTTCTTTGACCCAGCTGGAGGAGGA

GATCCTATTTTATATCAACATTTATTTTGATTTTTTGGTCACCCTG

Fig. 19 Consensus sequence of COX 1 of *Anopheles Subpictus* P1 (OL587934)

The 646bp long nucleotide sequence of mitochondrial COI region obtained from morphologically identified species Bwas deposited in GenBank nucleotide database with accession number OL587934**.**It was aligned along with GenBank entries for *An. subpictus*from India, Sri Lanka, Myanmar, and Thailand *An. sundaicus* from Malaysia, *An. darlingi* from Colombia, *An. albitarsis* from Brazil, *An. deaneorum* from Brazil and *An. marajoara* from New York. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model [Tamura, 1992]. The tree with the highest log likelihood (-1302.58) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2397)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 408 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [Tamura et. al., 2021]. In the COI gene tree, two distinct clades obtained. Species B clade and species A clade. In Concordance with the ITS 2 gene tree, the COI tree suggested that species A form a separate clade, species B closely related to *Anopheles sundaicus*.

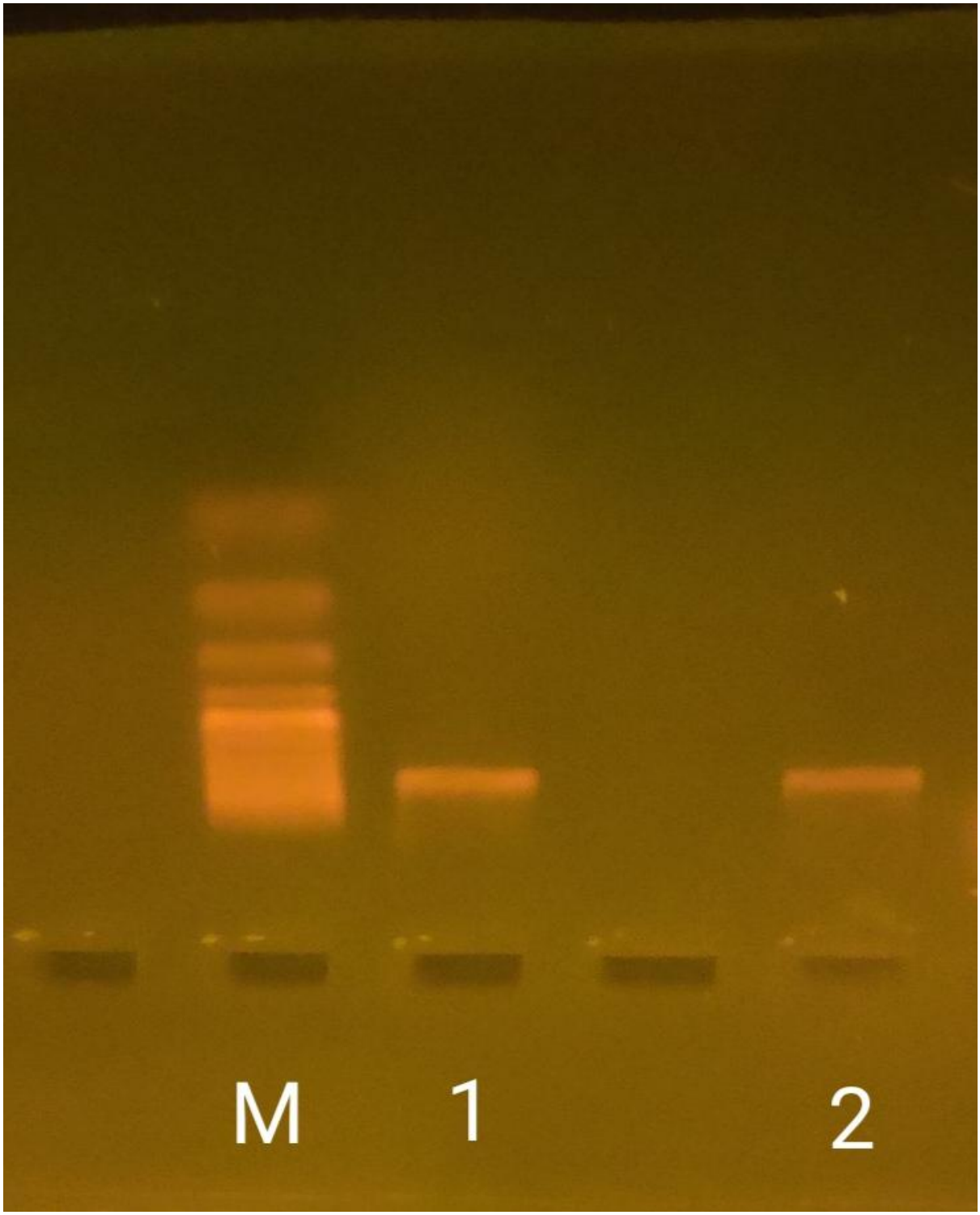


Figure 20: Gel image of COI sequence of An. subpictus B. M :100 bp marker, 1&2 species B

The phylogenetic analyses of the present study are consistent with the previous studies conducted for ITS 2 and COI, which separated the species in to two clades.

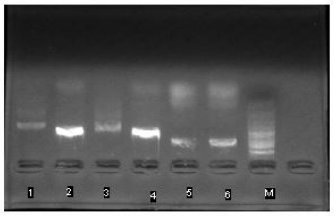
Both ITS 2 and COI sequences revealed two divergent clades indicating that the subpictus complex in Kerala corresponds to species B. Phylogenetic analysis showed that species A and species B do not form a monophyletic clade but instead share genetic similarity with *Anopheles vagus* and *Anopheles sundaicus* respectively. Species B of *An. subpictus* was found to be a close relative of *An. sundaicus* (Surendran, 2010) As a result Surendran et al. recognized them as sundaicus complex. The sample P1 included in the *subpictus* B clade

This study revealed the presence of one molecular form in sundaicus- subpictus complex in the Kerala state. Phylogenetic analysis using ITS 2 sequences revealed two distinct clades where *An. subpictus* B, *An. epiroticus*, *An. sundaicus*, *An. indefinitus*, *An. vagus* was found in one clade (ITS 2) and *An. subpictus* A form a separate clade. Earlier phylogenetic studies in India (Ankita Sindhaniya ,2020) also shows similar results. Phylogenetic analysis using COI sequences also reveals two distinct clades where *An. subpictus*B, *An. sundaicus*, *An. darlingi*, *An. albitarsis*, *An. deaneorum*, *An. marajoara* were found in one clade and molecular form of An. subpictus A form a distinct clade. Previous investigation also indicated the same by Surendran et.al in their work from Sri Lanka (Surendran et al., 2013).

The phylogenetic tree revealed *An. subpictus* species is not monophyletic, in the present tree they form two distinct clades. The clade representing subpictus A is distinct from other anopheles' species and claiming a separate lineage. The GenBank accessions KC191826* and GQ870334* are found in the same clade of the present isolate P1 and They were described as subpictus B by Surendran and others from Sri Lanka (Surendran et al., 2013). The results of molecular analysis confirming the morphological identification of the sample P1 as *An. subpictus* B. The GenBank accessions KC191826* and GQ870334*based on ITS2 region and HQ609056*, HQ609053*, HQ609042*, HQ609044*, HQ609047*, HQ609034*, HQ609055* and HQ609052* based on COI region were identified as subpictus B whereas KC191825* and GQ870337* base on ITS2 and DQ267688*, FM994156*, DQ310146* and FM992375* based on COI region were identified as subpictus A by Surendran et al in their work from Sri Lanka (Surendran et al., 2013).

Figure 21:Gel images of *An. subpictus* A and *An. subpictus* B

A 1.5% agarose gel image showing the amplification of the diagnostic fragments for species A and B of the subpictus complex in the ASPCR assay. M 100 bp marker, 1 control without DNA, 2 &4 Species B, 5&6 Species A.



>OP288467.1 Anopheles stephensi voucher VP4 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

AGTAGGAACATCTTTAAGAATTCTTATTCGAGCTGAATTAGGACACCCAGGAGCATTTATTGGAGACGAT

CAAATTTATAATGTAATTGTAACTGCTCATGCTTTTATTATAATTTTCTTTATAGTTATACCTATTATAA

TTGGGGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCACCAGATATAGCATTTCCTCGAATAAA

TAATATAAGATTTTGAATATTACCCCCCTCATTAACTCTTTTAATTTCTAGAAGTATAGTAGAAAATGGA

GCAGGAACAGGATGAACTGTTTATCCGCCTTTATCGTCTGGAATTGCTCACGCTGGGGCTTCAGTAGATT

TAGCAATTTTTTCATTACATTTAGCTGGAATTTCTTCAATTTTAGGAGCAGTTAATTTTATTACTACAGT

AATTAATATACGATCGCCAGGAATTACGTTAGACCGAATACCTTTATTCGTTTGATCTGTTGTAATTACT

GCTATTTTATTATTATTATCATTACCTGTATTAGCTGGAGCTATTACTATATTACTTACAGACCGAAATT

TAAATACATCTTTTTTCGACCCAGCTGGAGGAGGAGACCCCATTTTATATCAACATTTATTTTGATTTTT

TGGTCACCCTG

Fig. 22: Consensus sequence of COX 1 of *Anopheles stephensi* (OP288467)

4.11 Salinity tolerance estimation in the laboratory

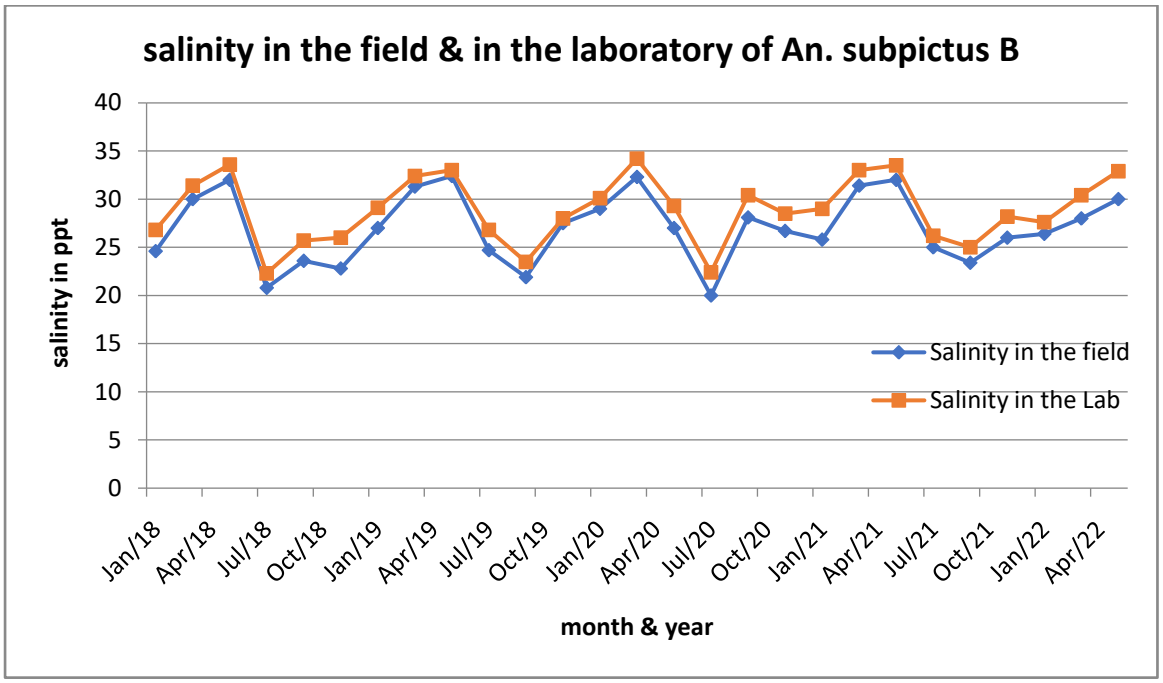
2)To study the Salinity tolerance of mosquitoes breeding in brackish water.

A series of concentrations of instant ocean sea salt ranging from distilled water to 50 ppt was employed, being kept at room temperature. The larvae grow rapidly in pure water and lower salt concentration, being all at the 4^{th} or final instar in 5 days. Growth is very slightly delayed in 5-10 ppt salinity. There is greater inhibition at 35ppt salinity. At above 35 ppt, there is often considerable mortality and the growth of the survivors is extremely slow, there being almost no growth, and larvae still in the 3^{rd} or 4^{th} instar at the end of 5 days. At above 40 ppt, the larvae fail to grow, and die in about 24 hours. These are all dead in 1 hour in 50 ppt, and in about half an hour at 55 ppt and over.

Table :11 Salinity of *An. subpictus* B in the field

Month & year of collection	Salinity of the water samples (ppt)
January 2018	24.6
March 2018	30
May 2018	32
July 2018	20.8
September 2018	23.6
November 2018	22.8
January 2019	27
March 2019	31.3
May 2019	32.4
July 2019	24.7
September 2019	21.9
November 2019	27.5
January 2020	29

March 2020	**32.3**
May 2020	**27**
July 2020	**20**
September 2020	**28.1**
November 2020	**26.7**
January 2021	**25.8**
March 2021	**31.4**
May 2021	**32**
July 2021	**25**
September 2021	**23.4**
November 2021	**26**
January 2022	**26.4**
March 2022	**28**
May 2022	**30**



The detailed table of salinity tolerance study in the laboratory of the collected brackish water breeders were given below.

Table 12. showing salinity tolerance estimation of *An. subpictus B*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	20	20	20
10	20	20	20	20
15	20	20	20	20
20	20	20	20	20
25	20	20	20	20
30	20	20	20	20
35	20	9	7	0
40	20	0	0	0

An. subpictus B was collected from brackish water habitats with salinity ranging from 28-30 ppt. The larvae of an. subpictus B employed for salinity tolerance estimation. The larvae were treated with a series of concentrations of instant sea salt ranging from distilled water to 50 ppt salinity. The larvae of *An. subpictus* B grow rapidly in about water having salinity up to 30 ppt. The growth of larvae very slightly delayed in 32 ppt salinity. There is greater inhibition of growth at 33 ppt salinity. At above 35 ppt salinity there is often considerable mortality and no survivors above this salinity. The larvae were again employed for salinity tolerance estimation in narrow range of salinity.

Table 13. Salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
36	20	0	0	0
37	20	0	0	0
38	20	0	0	0
39	20	0	0	0
40	20	0	0	0

A series of concentrations of instant ocean salt ranging from 36 to 40 ppt were employed, being kept at room temperature. There is a greater inhibition of growth at 35 ppt above. At above 35 ppt salinity only one larva remains after 24 hours, the larvae fail to grow, and all die in 48 hours. 35 ppt was the maximum tolerable salinity for *An. subpictus* B in the laboratory.

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	20	20	20
10	20	20	20	20
15	20	20	20	20
20	20	20	20	20
25	20	0	0	0
30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
50	20	0	0	0

Table 14 showing salinity tolerance estimation of *An. subpictus A*.

The larvae of *An. subpictus* A were collected from brackish water having salinity in the range 15-20 ppt. A series of concentrations of salinity ranging from distilled water to 50 ppt were employed at room temperature. The larvae grow rapidly up to 20 ppt and larvae fail to grow and all die in about 24 hours at 25 ppt salinity.

Table 15. Showing Salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
21	20	20	20	20
22	20	20	20	20
23	20	7	2	0
24	20	1	0	0
25	20	0	0	0
26	20	0	0	0
27	20	0	0	0
28	20	0	0	0
29	0	0	0	0

The same experiment was performed with narrow range of salinity ranging from 21-29 ppt salinity. There is greater inhibition of growth of larvae at 23 ppt salinity and there is often considerable mortality and the growth of the survivors is extremely slow and after 24 hours only 7 out of 20 larvae survived and after 48 hours only 2 of them remains. At 24 ppt all larvae except one fail to grow and die in 48 hours. So, 24 ppt was the maximum tolerable salinity of *An. subpictus* A.

Table 16. showing salinity tolerance estimation of *Aedes albopictus*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	20	20	20
10	20	20	20	20
15	20	8	5	0
20	20	0	0	0
25	20	0	0	0

30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Ae. albopictus were collected from brackish water habitat with salinity ranging from 2-5 ppt. A series of concentrations of sodium ocean sea salt ranging from 0-50 ppt was employed, being kept at room temperature. The larvae grow rapidly in pure water and up to 10 ppt salinity. Growth is slightly delayed in 15 ppt salinity and all larvae died in 20 ppt salinity. The salinity tolerance estimation was done in narrower range and the table was given below.

Table 17 showingSalinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
11	20	20	20	20
12	20	20	20	20
13	20	16	10	8
14	20	4	3	1
15	20	0	0	0
16	20	0	0	0
17	20	0	0	0
18	20	0	0	0
19	20	0	0	0

Instant ocean sea salt solutions of 11-19 ppt were employed under room temperature. The larvae grow rapidly up to 12 ppt salinity. Growth was delayed in 13 ppt and there was greater inhibition of growth in 14 ppt. At 14 ppt salinity, there was often considerable mortality and growth of the survivors were extremely slow and only 4 larvae out of 20 remained after 24 hours and only 3 larvae remained after 48 hours. Only one larva remained after 72 hours. So, the maximum tolerable salinity of Ae. albopictus was 14 ppt salinity.

Table 18. showing salinity tolerance estimation of *culex quinquifasciatus*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	20	20	20
10	20	20	20	20
15	20	20	20	20
20	20	20	20	20
25	20	20	20	20
30	20	20	20	20
35	20	5	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

*Culex quinquefasciatus*larvae were collected from brackish water habitats having salinity in the range of 28-30 ppt. Larvae of *Culex quinquefasciatus* were employed for salinity tolerance estimation at room temperature. A series of concentrations of instant ocean sea salt ranging from distilled water to 50 ppt salinity. The larvae grow rapidly up to 25 ppt salinity. There is greater inhibition of growth of larvae at 30 ppt salinity. There is often considerable mortality and the growth of survivors is extremely slow at 35 ppt. only 5 out of 20 remains alive after 24 hours at 35 ppt. The larvae fail to grow and die in 48 hours.

All larvae remain dead at above 35 ppt salinity.

Table 19 showing Salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
36	20	0	0	0
37	20	0	0	0
38	20	0	0	0
39	20	0	0	0

To know the tolerance estimation between 35-40 ppt salinity, the larvae were employed for salinity estimation in narrower range. It was shown that the maximum tolerable salinity for *Culex quinquefasciatus* was 35 ppt salinity. No larvae remain alive above 35 ppt salinity.

Table 20 Showing salinity tolerance estimation of *Culex sitiens*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	20	20	20
10	20	20	20	20
15	20	20	20	20
20	20	20	20	20
25	20	20	20	20
30	20	20	20	20
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Culex sitiens*were collected from brackish water habitats having salinity in the range of 28-30 ppt. When employed to salinity tolerance estimation for a series of concentration ranging from distilled water to 50 ppt salinity, it was found that the larvae were unaffected by a concentration of 25 ppt and below. Growth of the larvae was slightly delayed at 30 ppt salinity. There was a considerable mortality above 35 ppt and a very few survive. They were nearly all dead in 24 hours.

Table 21 showing salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
31	20	20	20	20
32	20	20	20	20
33	20	10	7	2
34	20	0	0	0
35	20	0	0	0
36	20	0	0	0
37	20	0	0	0
38	20	0	0	0
39	20	0	0	0

Salinity tolerance estimation in narrow range of salinity for a series of concentration ranging from 31-39 ppt shows that larvae were unaffected by a concentration of 32 ppt salinity. It was found that at 33 ppt salinity, the growth of the survivors was extremely slow and there was considerable mortality at this salinity. Only half of the larvae remain alive after 24 hours and 7 larvae out of 20 remain alive after48 hours. Only 2 of them remain alive after 72 hours. At 34 ppt salinity the larvae fail to grow and all die in about 24 hours. So, the maximum tolerable salinity for *Culex sitiens* was found to be 33 ppt salinity.

Table 22showing salinity tolerance estimation of *Anopheles barbirostrus*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	8	3	1
10	20	0	0	0
15	20	0	0	0
20	20	0	0	0
25	20	0	0	0

30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Anopheles barbirostris* were collected from brackish water habitats of salinity in the range of 1-2 ppt. A series of concentrations of instant ocean sea salt ranging from distilled water to 50 ppt salinity were employed under room temperature for obtaining salinity tolerance of *An. barbirostris* larvae. The larvae show less tolerance towards brackish water and it was found that growth of the larvae stopped at 10 ppt and all larvae become dead after 24 hours at this salinity.

Table 23 showing salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
5	20	7	5	0
6	20	0	0	0
7	20	0	0	0
8	20	0	0	0
9	20	0	0	0

In narrow range of salinity tolerance estimation of *An. barbirostris*, it was found that the larvae were very much affected by salinity concentration of 6 ppt and above. All larvae dead in about 24 hours in 6 ppt salinity. At 5 ppt salinity only 7 out of 20 larvae survive after 24 hours at 5 ppt salinity and only 5 of them survive after 48 hours at same salinity and all larvae dead after 72 hours. So, the maximum tolerable salinity of *An. barbirostris* was found to be 5 ppt.

Table 24 showing salinity tolerance estimation of *Culex bitaeniorhynchus*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	14	9	7
10	20	0	0	0
15	20	0	0	0
20	20	0	0	0
25	20	0	0	0
30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Culex bitaeniorhynchus* were collected from brackish water habitats of salinity 3-5 ppt. The larvae were employed for salinity tolerance estimation in the laboratory. A series of concentration of instant ocean sea salt ranging from distilled water to 50 ppt salinity. All experiment were done at room temperature. The larvae grow rapidly at distilled water and shows considerable mortality and the growth of the survivors was extremely slow. Only 14 out of 20 larvae remain alive at 5 ppt salinity after 24 hours and only 9 out of 20 larvae survived after 48 hours at same salinity. All larvae except 7 dead after 72 hours at 5 ppt. The larvae fail to grow and die in about 24 hours at 10 ppt.

Table 25 showing salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
6	20	5	2	1
7	20	3	1	0
8	20	1	0	0
9	20	0	0	0

In narrow range of salinity tolerance estimation, it was found that larval growth was inhibited greatly at 7 ppt salinity. Only 3 out of 20 larvae remain unaffected after 24 hours at 7 ppt and only one larva remains alive after 48 hours at 7 ppt salinity and no larvae survive after 72 hours at 7 ppt. At 8 ppt salinity one out of 20 larvae remain unaffected after 24 hours and all larvae dead after 48 hours. So, the maximum tolerable salinity of *Cx. bitaeniorhynchus* was found to be 8.

Table 26 showing salinity tolerance estimation of *Culex tritaeniorhynchus*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	12	7	6
10	20	0	0	0
15	20	0	0	0
20	20	0	0	0
25	20	0	0	0
30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Culex tritaeniorhynchus* were collected from brackish water habitats having salinity in the range of 4-5 ppt. Salinity tolerance estimation with a series of concentration of instant ocean sea salt at room temperature shows that growth was very slightly delayed at 5 ppt salinity. At 5 ppt salinity only 12 out of 20 larvae remain unaffected after 24 hours and only 7 out of 20 larvae survived after 48 hours at same salinity. After 72 hours only 6 larvae remain out of 20 at 5 ppt.

Table 27 showing salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
5	20	12	7	6
6	20	9	6	6
7	20	5	3	0
8	20	3	2	0
9	20	2	1	0

It was found that growth was delayed at 6 ppt salinity and considerable mortality was occurred at 7 ppt salinity. At 9 ppt salinity only 2 larvae were unaffected after 24 hours and one larva survived after 48 hours. All larvae became dead after 72 hours. So, the maximum tolerable limit of salinity was 9 ppt.

Table 28 showing salinity tolerance estimation of *Culex gelidus*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	20	20	20
10	20	20	20	20
15	20	18	12	5
20	20	0	0	0
25	20	0	0	0
30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Culex gelidus*were collected from brackish water habitats having salinity in the range of 7-10 ppt salinity. A series of concentrations of instant ocean sea salt solution ranging from distilled water to 50 ppt salinity were employed at room temperature. Larvae grow rapidly up to 10 ppt. at 15 ppt salinity growth is very slightly delayed 18 out of 20 larvae were unaffected after 24 hours. At 15 ppt salinity only 12 larvae remain alive after 48 hours and only 5 larvae survived after 72 hours.

Table 29 showing salinity tolerance estimation in narrower range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
16	20	14	9	3
17	20	10	7	2
18	20	10	6	2
19	20	8	4	1

The estimation in the narrow range shows that the maximum tolerable salinity of *Culex gelidus* larvae were 19 ppt salinity. Larvae were greatly affected at 19 ppt salinity. Only 8 larvae were unaffected after 24 hours at 19 ppt and only 4 larvae were unaffected after 48 hours. Only one larva was unaffected by 19 ppt salinity after 72 hours.

Table 30 showing salinity tolerance estimation of *Culex vishnui*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	16	10	9
10	20	0	0	0
15	20	0	0	0
20	20	0	0	0
25	20	0	0	0
30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Culex vishnui* were collected from brackish water habitats having salinity in the range of 4-7 ppt. Different concentrations of instant ocean sea salt starting from distilled water to 50 ppt salinity were employed for salinity tolerance estimation at room temperature. It was found that the larvae were unaffected by a concentration up to 5 ppt salinity. Growth was very slightly delayed at 5 ppt salinity and only 16 out of 20 larvae were survived after 24 hours, 10 out of 20 were remained after 48 hours, only 9 out of 20 larvae survived after 72 hours.

Table 31 showing salinity tolerance estimation in narrow range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
6	20	8	5	0
7	20	6	3	0
8	20	4	2	0
9	20	2	1	0

Salinity tolerance estimation in narrow range shows that there was a greater inhibition of growth at 6 ppt salinity and above. All larvae dead about 72 hours. The maximum tolerable salinity of *Cx. vishnui* was found to be 9 ppt. Only 2 out of 20 larvae unaffected at 9 ppt after 24 hours, and only one larva survives after 48 hours.

Table 32 showing salinity tolerance of *Anopheles stephensi*

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
0	20	20	20	20
5	20	0	0	0
10	20	0	0	0
15	20	0	0	0
20	20	0	0	0
25	20	0	0	0
30	20	0	0	0
35	20	0	0	0
40	20	0	0	0
45	20	0	0	0
50	20	0	0	0

Larvae of *Anopheles stephensi* were collected from slightly brackish water habitats having salinity in the range 0.7-1 ppt from abandoned boats of Chombala harbour. The number of larvae were also less when compared to other mosquito species. A series of concentrations of instant ocean sea salt ranging from distilled water to 50 ppt salinity were employed for salinity tolerance estimation. It was found that larvae fail to grow at 5 ppt salinity.

Table 33 showing salinity tolerance estimation in narrow range of salinity

Salinity (ppt)	No. of larvae	Survival after 24 h	Survival after 48 h	Survival after 72 h
1	20	8	5	0
2	20	6	3	0
3	20	0	0	0
4	20	0	0	0

In narrow range of salinity tolerance estimation, it was found that the larvae were greatly affected by 1 ppt salinity and above and the maximum tolerable salinity of *An. stephensi* was found to be 2 ppt. above 2 ppt salinity larvae fail to grow and all larvae dead after 24 hours.

4.12 Study of ecotypes

3)To compare the salinity tolerance of mosquitoes breeding in brackish water with the same species breeding in freshwater to investigate the possibility of ecotypes.

To enlist the ecotypes, collection was also done in fresh water habitats. Fresh water is having salinity in the range of 0.1-0.5 ppt. Habitats like ponds, streams, swamps, marshes, used wells, artificial containers, manmade ponds, natural ponds, drainage ditches, swamps , cement tanks, building under construction, discarded tyres, abandoned boats, domestic containers, mud pots, plastic buckets, plastic drums, coconut shells, leaf axils, folded plastic sheets, pools, puddles, ditches, borrow pits, rice fields, tree holes, wetlands were continuously surveyed and found positive for mosquito larvae.

Mosquito species encountered in both fresh water and brackish water are

- *An. barbirostris*
- *An. stephensi*
- *An. subpictus A*
- *Aedes albopictus*
- *Cx. tritaeniorhynchus*
- *Cx. sitiens*
- *Cx.quinquefasciatus*
- *Cx. gelidus*
- *Cx. bitaeniorhynchus*
- *Cx.vishnui*

Fresh, brackish, saline waters are respectively defined as containing <0.5,0.5-30 and >30 ppt (R. Ramaswamy 2014).

An. subpictus A was collected from freshwater having salinity in the range of 0.3 ppt. The larvae grow rapidly up to 0.5 ppt. *An. stephensi* was collected from fresh water having salinity in the range 0.2 ppt and the larvae survived in 0.5 ppt. An. barbirostris was collected from freshwater having salinity in the range of 0.1 ppt and found to grow rapidly up to 0.5 ppt salinity. Ae. Albopictus was collected from freshwater habitat with salinity in the range of 0.2 ppt and grow rapidly upto 0.5 ppt. *Cx. Tritaeniorhynchus* and *Cx. bitaeniorhynchus* were collected from 0.1 ppt salinity and found to grow rapidly up to 0.5 ppt salinity. *Cx. sitiens* and *Cx. quinquefaciatus* were also encountered in freshwater habitats having salinity in the range of 0.2 ppt. The 2 larvae were found to grow rapidly in 0.5 ppt salinity. *Cx. vishnui* larvae were also collected from freshwater habitats having salinity in the range of 0.2 ppt salinity and found to grow rapidly up to 0.5 ppt salinity.

An ecotype is a population or subspecies or race that is adapted to local environmental conditions. It describes a genetically distinct geographic variety, population, or race within a species which is genotypically adapted to specific environmental conditions. An ecotype is a variant in which the phenotypic differences are too few to warrant being classified as a subspecies. Therefore, ecotypes have no taxonomic rank. They are capable of interbreeding with other geographically adjacent ecotypes without loss of fertility or vigor.

Here, mosquito species were encountered from both brackish water and fresh water habitats. But morphologically there were no differences between these two groups.

4.13 Pictorial keys of the collected species

4)To prepare pictorial key to the species of mosquitoes breeding in brackish water habitats of North Kerala.

*An. subpictus*A and B

1.Wings entirely without pale markings ……. 2

Wings with pale markings……... 3

3(1). Wings with 3 or less than three dark spots on costa [1, 2, 3], which also in vein 1. (Subgenus Anopheles, in part) ……. 5

Wings with 4 or more dark spots on costa [1, 2, 3, 4], which also involve vein 1. (subgenus Cellia)…… 4

4(3). Femur and tibia speckled. (section A)……... 7

Femur and tibia not speckled. (section B)…… 6

6(4). Hind tarsomere 5 white…… 8

Hind tarsomere 5 not white…….. 9

9(6). Fore tarsi with broad pale bands…….. 10

Fore tarsi with narrow pale bands or unbanded…….. 11

10(9). Female palpi with subapical dark band equal or nearly equal to the apical pale band…… (subpictus species complex)

An. barbirostris

1.Wings entirely without pale markings ……. 2

Wings with pale markings…… 3

3(1). Wings with 3 or less than three dark spots on costa [1, 2, 3], which also in vein 1. (Subgenus Anopheles, in part) ……. 4

Wings with 4 or more dark spots on costa [1, 2, 3, 4], which also involve vein 1. (subgenus Cellia)……

4(3). Apex of hind femur with a prominent tuft of white scales precededby a tuft of black scales visible to the naked eye……. 5

(Asiaticus group)

Hind femur not so…… 6

6(4). Hind femur with a broad white band; costa with only apical spot……lindesayi

Hind femur without such a band……...7

7(6). Wing: inner quarter of costa with large pale spots, costa with three or more pale spots

[1, 2, 3, 4] including a presector pale spot(but in male: preapical pale spot usually extending on vein 2.1). very large species(wing length 5-6mm……gigas

Inner quarter of costa mainly dark, sometimes with a few scattered pale scales……8

8(7). Palpi with definite pale markings; clypeus of female with tuft of dark scales at sides……Hyrcanus group

Palpi without any pale markings; clypeus without dark scales at sides…….9

9(8). Female with prominent tuft of dark scales on ventral side of abdominal segment VII. Wing: inner quarter of costa with scattered pale scales, usually with fringe spot at vein 5.2 (barbirostrisgroup)…… 10

Female without such tuft. Wing: inner quarter of costa without pale scales, no fringe spot at vein 5.2 (umbrosusgroup)…….. 11

10(9). Wing with narrow lower apical fringe spot opposite to vein 3only; abdominal sternite II-VI usually with some pale scales.(barbirostris subgroup)…… barbirostris

Aedes albopictus

1. Head. Vertex with broad erect forked scales numerous, not restricted to occiput; proboscis with a white band…….2

Head. Vertex with broad erect forked scales not numerous, restricted to occiput; proboscis without a white band……3

2(1). Abdomen speckled dorsally……*Aedes (Diceromyia)furcifer*

Abdomen not speckled dorsally…….*Aedes (Diceromyia)taylori*

3(1). Leg. Femora with white knee spot; midfemora without 3 large white patches on anterior surface; hind tarsomere 5 entirely white……..... 4

Femora without white knee spot; midfemora with 3 large white patches on anterior surface; hind tarsomere 5 entirely dark……5

4(3). Thorax. Scutum black or brown with a pair of submedian-longitudinal white stripes, but without median longitudinal white stripe, or with white lyre-shaped markings;

mesepimeron with two well separated white scale patches. Leg. anterior portion of midfemur with a longitudinal white stripe. Head. clypeus with white scale patches…….....*Aedes (Stegomyia)aegypti*

Thorax. Scutum with a narrow median longitudinal white stripe; mesepimeron with white scale patches not separated, forming V – shaped white patch. leg. Anterior portion of midfemur without a longitudinal white stripe. Head. Clypeus without white scale patches…….*Aedes (Stegomyia)albopictus*

Culex quinquefasciatus

1. Proboscis and tarsi without pale rings; 1 or 2 lower mesepimeral setae present……..2

Proboscis and tarsi with pale rings; lower mesepimeral setae absent……....4

2(1) Abdominal terga without pale bands, occasionally a few indistinct bands on posterior segments; pleuron with striking pattern of dark and pale stripes…… *fuscocephala*

Abdominal terga with basal pale bands; pleuron without striking pattern of dark and pale stripes……3

3(2). Postspiracular area with pale scale patch…… *perexiguus*

Postspiracular area without pale scale patch……*quinquifasciatus*

Culex bitaeniorhynchus

1. Proboscis and tarsi without pale rings; 1 or 2 lower mesepimeral setae present……3

Proboscis and tarsi with pale rings; lower mesepimeral setae absent……..2

2(1). Wing with pale spots on at least 2 areas of costa and 1 area of other veins ……*mimeticus s*ub group

Wing without distinct pale spots……..4

4(2). Abdominal terga II-VIII largely yellowish or golden; pale yellow scales on apical portions of wing veins C, R1, R2, R3 and R4+5 forming apical pale area…….*epidesmus*

Abdominal terga II-VIII with dark and pale bands, or completely dark, wing tip without definite pale area……5

5(4). Wing heavily speckled with pale scales; abdominal terga II-VI with apical pale bands and/ or apicolateral pale patches and basal palebands……6

Wing without speckling of pale scales; abdominal terga II-VI with basal pale bands…..7

6(5). Abdominal terga II-VII with evenly broad apical pale bands and without apicolateral pale patches; femora, tibia, and wings heavily speckled with pale scales……..*bitaeniorhynchus*

Culex gelidus

1. Proboscis and tarsi without pale rings; 1 or 2 lower mesepimeral setae present……..2

Proboscis and tarsi with pale rings; lower mesepimeral setae absent……4

4(1). Wing with pale spots on at least 2 areas of costa and 1 area of other veins ……*mimeticus*sub group

Wing without distinct pale spots……..5

5(4). Abdominal terga II-VIII largely yellowish or golden; pale yellow scales on apical portions of wing veins C, R1, R2, R3 and R4+5 forming apical pale area……*epidesmus*

Abdominal terga II-VIII with dark and pale bands, or completely dark, wing tip without definite pale area……6

6(5). Wing heavily speckled with pale scales; abdominal terga II-VI with apical pale bands and/ or apicolateral pale patches and basal pale bands…….7

Wing without speckling of pale scales; abdominal terga II-VI with basal pale bands…..8

8(6). Anterior 0.7 of scutum covered with pure white scales……...9

Anterior 0.7 of scutum covered with beige, yellow, golden, or dark scales……..10

9(8). Anterior surface of fore and midfemorawithout speckling without speckling of pale scales; scales on prescutellar space, behind wing base, and on scutellum entirely dark; pale band on proboscis narrow, narrow, less than length of basal dark area……...*gelidus*

Culex sitiens

1. Proboscis and tarsi without pale rings; 1 or 2 lower mesepimeral setae present……..2

Proboscis and tarsi with pale rings; lower mesepimeral setae absent……4

4(1). Wing with pale spots on at least 2 areas of costa and 1 area of other veins ……mimeticus sub group

Wing without distinct pale spots……5

5(4). Abdominal terga II-VIII largely yellowish or golden; pale yellow scales on apical portions of wing veins C, R1, R2, R3 and R4+5 forming apical pale area……epidesmus

Abdominal terga II-VIII with dark and pale bands, or completely dark, wing tip without definite pale area……..6

6(5). Wing heavily speckled with pale scales; abdominal terga II-VI with apical pale bands and/ or apicolateral pale patches and basal pale bands…….7

Wing without speckling of pale scales; abdominal terga II-VI with basal pale bands…..8

8(6). Anterior 0.7 of scutum covered with pure white scales……..9

Anterior 0.7 of scutum covered with beige, yellow, golden, or dark scales……..10

10(8). Anterior surface of fore and midfemora speckled with several pale scales, at least on apicodorsal surface……...11

Anterior surface of fore and midfemora entirely dark……12

11(10). Wing scales mainly dark; scutal integument dark; speckling of pale scales on femora contrasting sharply with dark scaled area……...*sitiens, annulirostris*

Culex vishnui

1. Proboscis and tarsi without pale rings; 1 or 2 lower mesepimeral setae present…2

Proboscis and tarsi with pale rings; lower mesepimeral setae absent…….4

4(1). Wing with pale spots on at least 2 areas of costa and 1 area of other veins …….mimeticus sub group

Wing without distinct pale spots……..5

5(4). Abdominal terga II-VIII largely yellowish or golden; pale yellow scales on apical portions of wing veins C, R1, R2, R3 and R4+5 forming apical pale area……epidesmus

Abdominal terga II-VIII with dark and pale bands, or completely dark, wing tip without definite pale area…….6

6(5). Wing heavily speckled with pale scales; abdominal terga II-VI with apical pale bands and/ or apicolateral pale patches and basal pale bands…….7

Wing without speckling of pale scales; abdominal terga II-VI with basal pale bands…..8

8(6). Anterior 0.7 of scutum covered with pure white scales……..9

Anterior 0.7 of scutum covered with beige, yellow, golden, or dark scales……..10

10(8). Anterior surface of fore and midfemora speckled with several pale scales, at least on apicodorsal surface……...11

Anterior surface of fore and midfemora entirely dark……12

11(10). Wing scales mainly dark; scutal integument dark; speckling of pale scales on femora contrasting sharply with dark scaled area……*sitiens, annulirostris*

Wing with few to several scattered pale scales; scutal integument light brown, speckling of pale scales on femora not contrasting sharply with dark scaled are……..*vishnui*

Culex tritaeniorhynchus

1. Proboscis and tarsi without pale rings; 1 or 2 lower mesepimeral setae present…….2

Proboscis and tarsi with pale rings; lower mesepimeral setae absent……4

4(1). Wing with pale spots on at least 2 areas of costa and 1 area of other veins ……mimeticus sub group

Wing without distinct pale spots……..5

5(4). Abdominal terga II-VIII largely yellowish or golden; pale yellow scales on apical portions of wing veins C, R1, R2, R3 and R4+5 forming apical pale area……*epidesmus*

Abdominal terga II-VIII with dark and pale bands, or completely dark, wing tip without definite pale area……...6

6(5). Wing heavily speckled with pale scales; abdominal terga II-VI with apical pale bands and/ or apicolateral pale patches and basal pale bands……..7

Wing without speckling of pale scales; abdominal terga II-VI with basal pale bands…..8

8(6). Anterior 0.7 of scutum covered with pure white scales……..9

Anterior 0.7 of scutum covered with beige, yellow, golden, or dark scales……..10

10(8). Anterior surface of fore and midfemora speckled with several pale scales, at least on apicodorsal surface……..11

Anterior surface of fore and midfemora entirely dark……12

12(10). Erect scales on vertex and scales on anterior 0.7 of scutum entirely deep brown; proboscis often with accessory pale patches on ventral surface, proximal to median pale ring; hindfemur pale with distinct, narrow dark ring distally……...*tritaeniorhynchus*

Anopheles subpictus B - Proboscis

Anopheles subpictus B

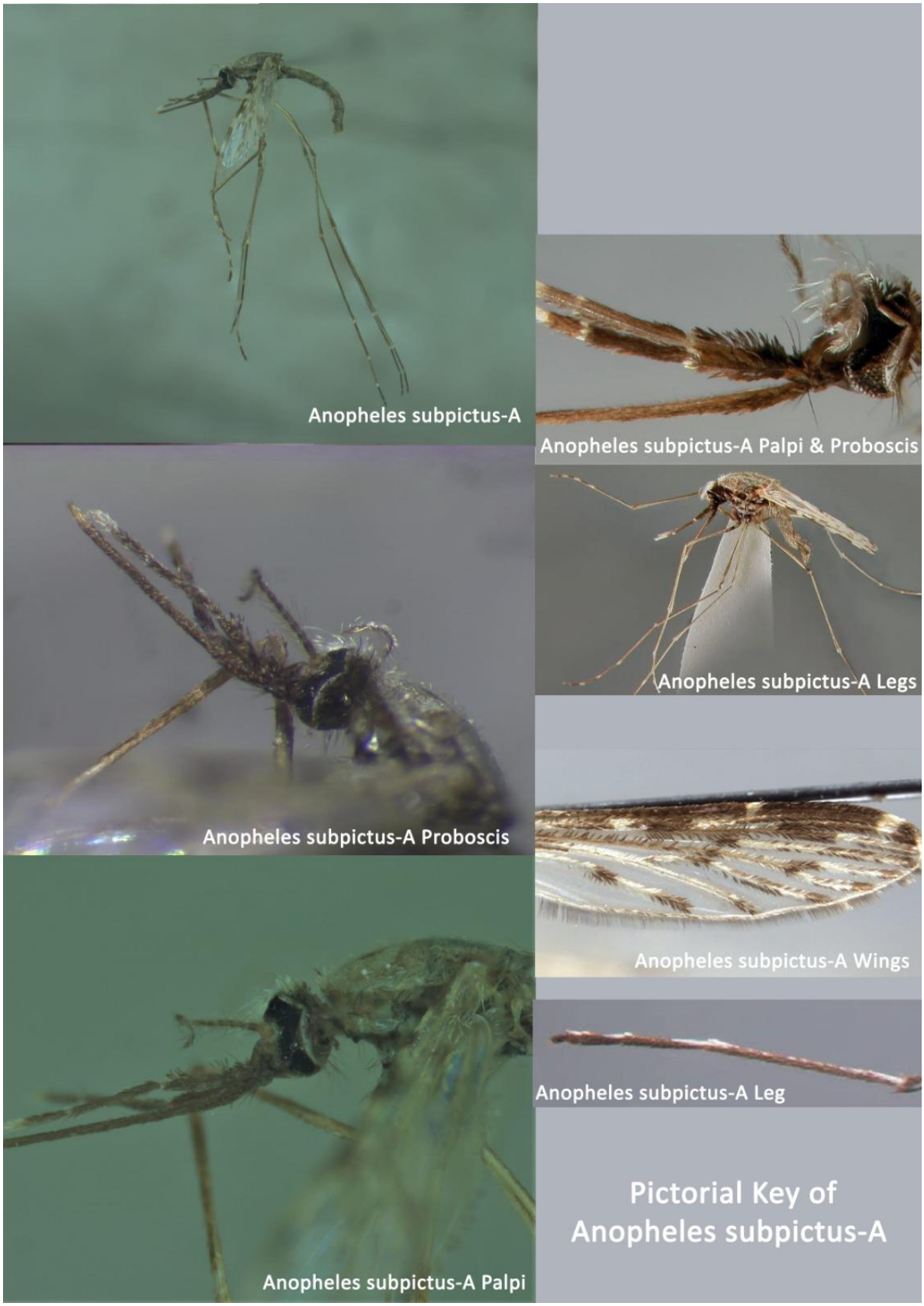
Anopheles subpictus B - Palp

Anopheles subpictus B - Legs

Anopheles subpictus B - Hind Tarsi

Anopheles subpictus B - Wing

Pictorial Key of Anopheles subpictus B


Anopheles subpictus-A
Anopheles subpictus-A Palpi & Proboscis
Anopheles subpictus-A Legs
Anopheles subpictus-A Proboscis
Anopheles subpictus-A Wings
Anopheles subpictus-A Leg
Anopheles subpictus-A Palpi
Pictorial Key of
Anopheles subpictus-A

Pictorial Key of Anopheles barbirostris

Aedes albopictus Palpi & Proboscis

Aedes albopictus abdomen

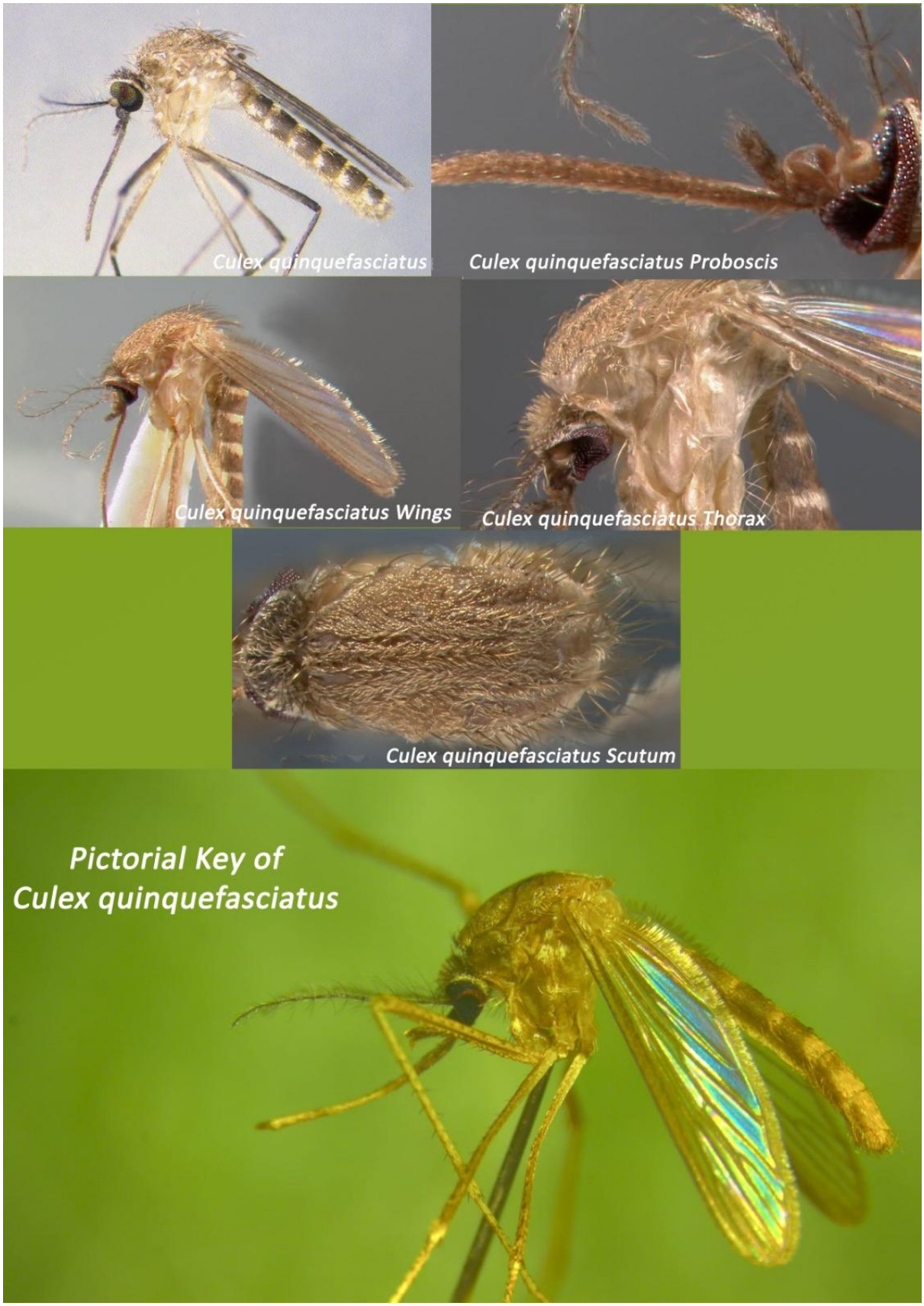
Aedes albopictus scutum

Aedes albopictus Wings & Legs

Aedes albopictus Leg

Aedes albopictus

Pictorial Key of
Aedes albopictus


Culex quinquefasciatus
Culex quinquefasciatus Proboscis
Culex quinquefasciatus Wings
Culex quinquefasciatus Thorax
Culex quinquefasciatus Scutum
Pictorial Key of
Culex quinquefasciatus

Culex sitiens

Culex sitiens Scutum

Culex sitiens **Palpi & Proboscis**

Culex sitiens Wing

Culex sitiens Leg

Pictorial Key of *Culex sitiens*

Pictorial Key of *Culex vishnui*

Culex vishnui

Culex vishnui Legs

Culex vishnui Thorax

Culex vishnui Proboscis

Culex vishnui Scutum

Culex vishnui Wings

Culex vishnui Abdomen

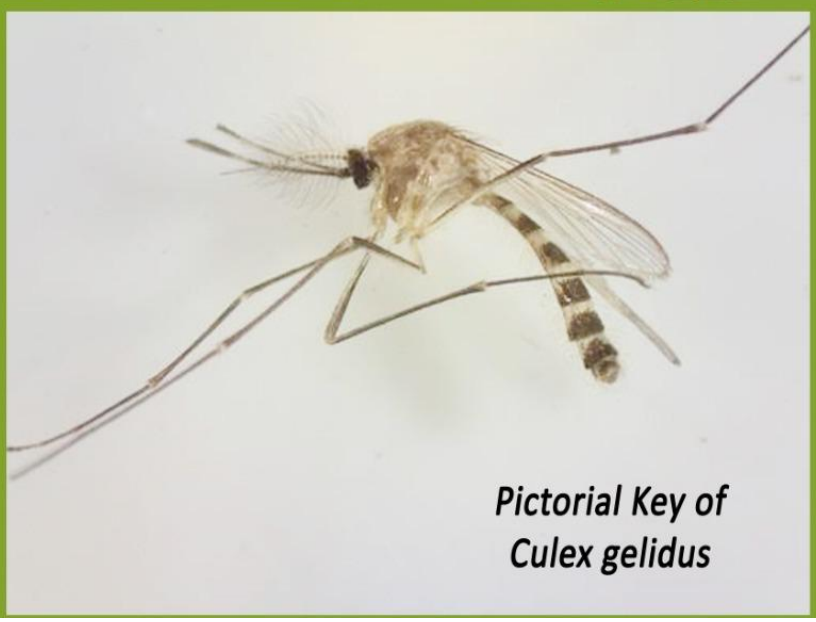

Culex gelidus


Culex gelidus Proboscis


Culex gelidus Scutum


Culex gelidus Abdomen


Culex gelidus Wing & legs


Pictorial Key of
Culex gelidus

Culex tritaeniorhynchus

Culex tritaeniorhynchus Proboscis

Culex tritaeniorhynchus Abdomen

Culex tritaeniorhynchus Thorax

Culex tritaeniorhynchus Scutum

Culex tritaeniorhynchus Wing

Pictorial Key of Culex tritaeniorhynchus

Culex bitaeniorhynchus

Culex bitaeniorhynchus Wings

Culex bitaeniorhynchus Scutum

Culex bitaeniorhynchus Abdomen

Culex bitaeniorhynchus Proboscis

Pictorial Key of
Culex bitaeniorhynchus

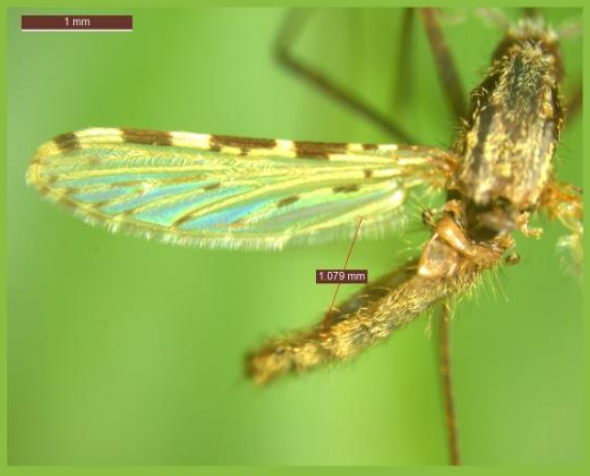
Pictorial key of *Anopheles stephensi*

***Anopheles stephensi* Leg**

***Anopheles stephensi* Palps**

***Anopheles stephensi* Thorax**

***Anopheles stephensi* Vertex**



***Anopheles stephensi* Wing**

CHAPTER 5

DISCUSSION

DISCUSSION

5.1 Vector status of mosquitoes breeding in brackish water

Mosquitoes are known to breed in various types of water, including fresh water, brackish water, and even some saline environments. Brackish water, which is a mixture of fresh water and salt water, typically found in coastal regions, estuaries, and manfrooves, can be a breeding ground for certain species of mosquitoes.

The results of this study imply that several mosquito species in coastal areas of north Kerala can undergo larval development in brackish water habitats. The mosquito species that breed in brackish water are adapted to specific salinity ranges. The role of coastal brackish water ecosystems in vector-borne disease transmission has not been well studied in Kerala. Coastal brackish water wetlands act as the interface between marine and terrestrial habitats and it was one of the most productive habitats (Bruland, 2008). Mosquitoes breeds in brackish water can spread Malaria, Dengue fever, and West Nile fever.

Among the collected 4 species of *Anopheles, Anopheles stephensi* and *Anopheles subpictus* were primary vectors of malaria. *Aedes albopictus*, the vector of dengue, chikungunya, yellow fever and zika was also encountered in brackish water habitats. Among the collected 6 species of culex, all of them were incriminated vectors. *Culex quinquefasciatus* was vector of bancroftian filariasis. The remaining five species i.e., *Culex sitiens*, *Culex gelidus, Culex tritaeniorhynchus*, *Culex bitaeniorhynchus* and *Culex vishnui* were the vectors of JE.

Although *An. stephensi* the major malaria vector in Kerala is recognised as a freshwater mosquito, it has been recently reported to undergo larval development in brackish water of up to 3.5 ppt in Sri Lanka (Surendran, 2019). The present finding shows that this is also the case in Kerala. In our study *An. stephensi* was found to undergo larval development in water with 0.2-0.7 ppt salinity.

An. subpictus B is a well-known saline-tolerant mosquito vector of malaria in Sri Lanka (Surendran, 2011) and India (Goutham, 2010). However, its role in disease transmission in Kerala has not been established. Malaria in Kerala is mainly transmitted by *An. stephensi*. The present study implies that the larvae of *An. subpictus* B can undergo development in brackish water habitats with salinity 28-32 ppt. This is the first study in Kerala that detects the ability of *An. subpictus* B to breed in brackish water.

CHARACTERS	*An. subpictus* A	*An. subpictus* B	*An. subpictus* C	*An. subpictus* D
Egg-ridges frill	35(31-36) opaque	18(16-20) transparent	27(25-29) Semi-transparent	22(21-24) Semi transparent
Larvae-seta 4M	2 branched	2 branched	3 branched	3 branched
Pupa- seta 7-1	simple	4-5 branched	2 branched	3 branched
Adult female palp	Apical pale band longer than sub apical dark band	Apical pale band shorter than sub apical dark band	Apical pale band equal to sub apical dark band	Apical pale band equal to sub apical dark band

Source: SG Suguna 1991

This high salinity tolerance was associated with malaria transmission in coastal areas of north Kerala.

Although Larval development of *Ae. albopictus* is known to exist in a freshwater environment for many years, few recent findings have revealed the possibility of *Aedes* breeding and immature stage development in brackish water conditions of up to 15 ppt salinity in Sri Lanka (Ramasamy, 2011) and Brunei Darussalam (Idris, 2013). In the present study, though the natural breeding of *Ae. albopictus* was found in brackish water with up to 4.5 ppt only, they could tolerate a salinity of up to 14 ppt in the laboratory, which is comparable to the Sri Lankan strain of the species. This is the first study demonstrating the adaptation of the predominantly freshwater breeding *Ae. albopictus* to brackish water in Kerala.

Culex sitiens is a well-known brackish water breeder and vector of Japanese encephalitis virus. The findings of this study recorded that the larvae of Cx.*sitiens* can undergo larval development in brackish water habitats with 2-32 ppt salinity.

The major vector of the Japanese encephalitis virus is *Cx. gelidus* and *Cx. tritaeniorhynchus* are known to exist in brackish water habitats up to salinity 7 ppt in Alappuzha district (Balasubramanian, 2021). The findings of this study recorded that *Cx. tritaeniorhynchus* was found to breed in 0.3-5 ppt salinity and *Cx. gelidus* was found to breed in 0.5-10 ppt salinity.

Cx. vishnui and *Cx. bitaeniorhynchus* are also vectors of the Japanese encephalitis virus. The findings of this study showed that *Cx. vishnui* can undergo preimaginal development in brackish water habitats with 0.3-6 ppt salinity and *Cx. bitaeniorhynchus* breeds in habitats with 0.2-5 ppt salinity.

Culex quinquefasciatus is a well-known brackish water breeder and vector of bancroftian filariasis. The present investigation implies that it can undergo larval development in 0.2- 30 ppt salinity.

Table 34: List of collected vectors during the study

Genera	Species	Vector status
Anopheles	*subpictus B*	Potential vector of Malaria
	stephensi	The primary vector of Malaria
Aedes	*albopictus*	Primary vector of Dengue, chikungunya, yellow fever, zika
Culex	*Sitiens*	Japanese encephalitis
	Quinquefasciatus	Bancroftian filariasis
	bitaeniorhynchus	Japanese encephalitis
	tritaeniorhynchus	Japanese encephalitis
	Vishnui	Japanese encephalitis
	gelidus	Japanese encephalitis

Kerala state has reported many outbreaks of malaria, dengue, chikungunya, Japanese encephalitis, etc in coastal areas recently.

5.2 Mosquito-borne diseases in Kerala

Diseases		2019	2020	2021	2022
Malaria	Cases	656	268	309	439
	Death	1	1	1	0
Dengue	Cases	4651	2722	3251	4468
	Death	14	22	27	58
Chikungunya	Cases	109	558	334	66
	Death	0	0	0	0

Japanese encephalitis virus	Cases	11	0	0	2
	Death	2	0	0	0
West Nile virus	Cases	11	0	1	3
	Death	2	0	0	1
Zika	Cases	0	0	90	15
	Death	0	0	0	0

Table 35: Mosquito-borne diseases in Kerala

Table 36-39 showing mosquito borne diseases in north Kerala

5.3. Mosquito-borne diseases in North Kerala

Malappuram					
Disease		2019	2020	2021	2022
Malaria	Cases	66	27	25	38
	Death	1	0	0	0
Dengue	Cases	361	60	169	267
	Death	0	1	2	4
Chikungunya	Cases	1	1	0	0
	Death	0	0	0	0
Japanese encephalitis	Cases	0	0	0	0
	Death	0	0	0	0
West Nile fever	Cases	4	0	1	0
	Death	2	0	0	0
Zika	Cases	0	0	0	0
	Death	0	0	0	0

Kozhikode					
Disease		2019	2020	2021	2022
Malaria	Cases	79	19	23	38
	Death	0	0	0	0
Dengue	Cases	405	69	209	44
	Death	2	2	6	2

Chikungunya	Cases	0	0	0	0
	Death	0	0	0	0
Japanese encephalitis	Cases	4	0	0	1
	Death	0	0	0	0
West Nile fever	Cases	5	0	0	0
	Death	0	0	0	0
Zika	Cases	0	0	1	0
	Death	0	0	0	0

Kannur					
Disease		2019	2020	2021	2022
Malaria	Cases	62	15	24	40
	Death	0	0	0	0
Dengue	Cases	212	268	116	110
	Death	0	5	2	0
Chikungunya	Cases	0	1	1	0
	Death	0	0	0	0
Japanese encephalitis	Cases	0	0	0	0
	Death	0	0	0	0
West Nile fever	Cases	1	0	0	0
	Death	0	0	0	0
Zika	Cases	0	0	0	0
	Death	0	0	0	0

Kasargod					
Disease		2019	2020	2021	2022
Malaria	Cases	70	31	27	17
	Death	0	0	0	0
Dengue	Cases	242	117	429	227
	Death	0	2	2	1

Chikungunya	Cases	0	0	7	0
	Death	0	0	0	0
Japanese encephalitis	Cases	1	0	0	0
	Death	0	0	0	0
West Nile fever	Cases	0	0	0	0
	Death	0	0	0	0
Zika	Cases	0	0	0	0
	Death	0	0	0	0

Rising sea levels can lead to the expansion of brackish and saline water bodies in coastal areas and it may also lead to an increase in the density of salinity-tolerant mosquito vectors and cause freshwater mosquito vectors to adapt to brackish water habitats (Ramasamy, 2012). Such developments can lead to an increase in the density of vectors relative to humans. The temporal and spatial pattern of climate variable changes due to climate change will affect the biology and ecology of vectors and thereby increase the risk of vector-borne disease transmission. In many recent studies, investigators have examined the relationship between climatic variation and the occurrence of vector-borne diseases especially mosquito-borne diseases like Malaria, Japanese encephalitis virus, West Nile virus, Dengue, Chikungunya virus etc. As vector-borne diseases are emerging in coastal regions, they are causing a significant health risk.So the surveillance and control of mosquito populations in brackish water areas are crucial.

CHAPTER 6

Conclusion

The present study reveals, for the first time in Kerala, the detection of *An. subpictus* B, the primary vector of malaria, breeds in brackish water. The coastal population of the subpictus complex have been particularly incriminated as malaria vectors in India (Panicker1981). *An. subpictus* B has been specifically implicated in transmitting malaria on the west coast of Sri Lanka (Abhayawardhana1996). In that regard, the precise identification of sibling species in *An. subpictus* species complex a pivotal role in malaria elimination. sporadic cases of malaria have been reported from the coastal areas of the state in recent times. Being a coastal state, the detection of this vector species is very important from the point of view of malaria epidemiology in Kerala. There is limited research work available on this species in India, especially in Kerala. it was thought prudent to review the bionomics and the role of *An. subpictus* in malaria transmission in coastal villages of Kerala. This necessitates a need for constant monitoring of brackish water breeding habitats of the state and initiating appropriate control measures wherever necessary, to prevent malaria outbreaks.

Mosquito species that breed in salt marshes along the coastal belt of Kerala are vectors of many arboviral and protozoan diseases. Over the past several decades, Kerala has experienced a population increase along the coastal area and a change in environmental conditions. This change may have direct or indirect effects on the disappearance of some species as well as the reappearance or introduction of new species. Kerala has reported many outbreaks of dengue, chikungunya, malaria, Japanese encephalitis, and West Nile Encephalitis in its coastal districts. As vector-borne diseases are emerging in coastal regions, they are causing a significant health risk.

Encephalitis virus outbreak was reported in coastal areas of many countries and it was found to be transmitted by saline-tolerant mosquitoes which aggressively bite both birds and mammals (Freitas, 2012). Coastal wetlands provide habitats for migrating and resident birds by acting as nurseries for juvenile fish and invertebrates including mosquitoes. The rise of brackish water habitats in coastal areas favours the emergence of saline-tolerant mosquitoes and it also leads to the adaptation of freshwater vectors to breed in brackish waters (Ramasamy, 2012). This often leads to changes in mosquito population dynamics and species composition

Formation of ecotype plays of pivotal role in evolution, as it shapes species diversity and has a role in range expansion, response to climate change and ecological speciation. Ecotype

formation also has public health significance. Control of mosquito-borne diseases may be complicated by local adaptations in the vector that diversify the ecological and environmental range and alter epidemiologically relevant behaviour.

The most striking ecological difference among mosquito taxa is the larval habitat. In a heterogeneous environment, ecotypic differentiation implies some form of resource partitioning.

CHAPTER 7

Recommendation

This study was conducted to investigate and document the mosquito species breeding in brackish water habitats of north Kerala. The present study reveals for the first time in Kerala, the ability of malaria vector *An. subpictus* B of subpictus complex and *An. stephensi* to breed in brackish water.

The present study has also brought the hitherto neglected brackish water habitats in the coastal areas of Kerala into the limelight in the context of the epidemiology of diseases transmitted by *Ae. albopictus*. This is the first study demonstrating the adaptation of the predominantly freshwater breeding Ae. albopictus to brackish water in Kerala.

The greater availability of brackish water habitats can lead to the adaptation of freshwater-breeding mosquitoes to brackish water breeding.

Demonstrations of the ability of mosquito vectors to tolerate brackish waters in the field and the laboratory, conditions indicate the importance. At present, for curing arboviral diseases the drugs that provide relief from the symptoms are only available. Vaccines are currently available for only yellow fever and JE. The existence of four serotypes of viruses hampers the development of a vaccine against dengue. Therefore, the observed transmission of mosquito-borne diseases in coastal areas and brackish water areas of Kerala can have serious consequences in the field of health.

Brackish water bodies are frequently neglected in vector control programs due to the belief that the vectors can breed only in fresh water. It is important to raise awareness among the health authorities and common people on the risks associated with brackish water breeders. It is very important in the context of rising sea levels due to global warming.

Since the number of brackish water habitats with vectors in the present study was comparatively less, it could be argued that the phenomenon of adaptation to brackish water is in its initial stage. However, as time elapses, the chances of the saline-tolerant population proliferating cannot be ruled out.

Dengue is a regular phenomenon in all districts of the state including Kannur, Kozhikode, Malappuram and Kasargod with a sizeable number of cases and even a few deaths every year. In the absence of an effective vaccine, dengue control strategies focus on the reduction and elimination of Aedes breeding in domestic and peri-domestic container habitats. Hence, the brackish water habitats are largely neglected. This could have serious consequences in the

dengue scenario of the state soon. Besides dengue, two other Aedes-borne diseases viz., Chikungunya and Zika, are also prevalent in the state. Considering all these factors, serious efforts are needed to monitor and design control strategies to tackle this new phenomenon. The study is the first of its kind in the state. Adaptation of *Ae. albopictus* to brackish water could have serious consequences in the dengue scenario of the state. Hence, immediate strategies to monitor and contain the proliferation of saline-tolerant populations of the species are recommended. To encounter the brackish water habitats in Kerala, an extended study in the whole state is also recommended.

CHAPTER 8

REFERENCES

AdulsakWijit, AtipornSaeung et.al (2013). DNA barcoding for the identification of eight species members of the Thai Hyrcanus group and investigation of their stenogamousbehaviour. C. R Biologies 336: 449-456.

A K Bradley, B M Greenwood, A M Greenwood, P Byass et al (1987). Mortality and morbidity from malaria among children in a rural area of the Gambia, West Africa. Transactions of the royal society of tropical medicine and hygiene. Vol 81. Issue 3. Pages 478- 486.

Alaa Nagy, Ahmed El- Zeiny, Manal Elshaier, Mohamed Sowilem, WedadAtwa(2021). Water quality assessment of mosquito breeding water localities in the Nile Valley of Giza Governorate, Journal of Environmental Sciences, Mansoura University, 50(1), 1-10.

Ammini PL, Srinivasan J, Ramani K, Beena MR, SeynudeenMB(2010).Marine Fisheries Information Service T&E Series 1-10.

AM Salit, SS Al-Tubiakh, SA El-Fiki, OH Enan, K Wildey (1996). Physical and chemical properties of different types of mosquito aquatic breeding places in Kuwait State, Proceedings of the Second International Conference on Urban pests2, 185-193.

Andre B.B. Wilke, Catherine Chase et al (2019). Urbanization creates diverse aquatic habitats for immature mosquitoes in urban areas. Nature Sci Rep 9,15335.

Andrew Jardine, MDA Lindsay, CA Johansen, Angus Cook, Philip Weinstein (2008). Impact of dryland salinity on population dynamics of vector mosquitoes (Diptera: Culicidae) of Ross River Virus in inland areas of southwestern Western Australia, Journal of Medical Entomology, 45(6), 1011-1022.

Anil Varughese, Chitra Purushothaman (2021). Climate change and public health in India: The 2018 Kerala floods, World medical &health policy.13(1):16-35.

Ankitha Sindhania, Manoj K Das et al (2021). Molecular forms of *Anopheles subpictus* and *Anopheles sundaicus* in the Indian subcontinent. Malaria Journal, 19:417

Armbruster, P., & Hutchinson, R. A. (2002). Pupal mass and wing length as indicators of fecundity in Aedes albopictus and Aedes geniculatus (diptera: Culicidae). Journal of Medical Entomology, 39(4), 699–704. https://doi.org/10.1603/0022-2585-39.4.699

Ariniratnasari, Arif Rahman Jabal, Syaharibulan, Irfan Idris, Nur Rahma et. al (2021). Salinity tolerance of larvae *Aedes aegypti* inland and coastal habitats in Pasangkayu, West Sulawesi, Indonesia. Biodiversitas. Vol.22. Number 3. Pages 1203-1210.

Arnaldo, M. (2006). Differences in performance of Aedes aegypti larvae raised at different densities in tires and ovitraps under field conditions in Argentina. Journal of Vector Ecology, 31(2), 1710(2006)31[371:dipoaa]2.0.co;2

A.R.Njogu and G. Kinoti (1971). Observations on the breeding sites of mosquitoes in Lake Manyara, a saline lake in the East African Rift Valley. Bulletin of Entomological Research, 60(3), 473-479.

AR Rajavel, R Natarajan, A Munirathinam and K Vaidyanathan (2005). The larval habitat of *Verrallinalugubris* and chaetotaxy of field-collected larvae. Journal of the American Mosquito Control Association. 21(1). 96-97.

A. R. Rajavel, A. Munirathinam, R. Natarajan and K. Vaidyanathan (2001). Species diversity of Mosquitoes (Diptera: Culicidae) in mangrove Ecosystem in South India. Entomon:26(3&4):271-277.

AR Rajavel and R Natarajan (2008). Mosquitoes of the mangrove forests of India: Part 7- An Overview. Journal of the American Mosquito Control Association,24(4):478-488.

Ashraf M Ahmed, Essam A Shaalan, Mourad A M Aboul Soud, Frederic Tripet and Abdulaziz A, Al-Khedhairy (2011). Mosquito vectors survey in the AL-Ahsaa district of eastern Saudi Arabia, Journal of Insect Science 11:176.

Ashwani Kumar, K Hari Krishnan Raju, Vas Dev (2020). An emerging vector of malaria in urban India. Vector biology and control an update for malaria Elimination Initiative in India, 105.

Ashwani Kumar, Rajeshwari Hosmani, et al (2016). *Anopheles subpictus* carries human malaria parasites in an urban area of Western India and may facilitate perennial malaria transmission. Malaria Journal 15: 124.

Balasubramanian R, Nadh V A, Sahina S (2021). Ecology of breeding habitats of mosquito population and screening for the virus of Japanese encephalitis and west Nile in the coastal area of Kerala, India. J Vector Borne Dis; 58:232-9

Balasubramanian R, Nadh V A, Sahina S, Sreelekha K P, Nikhil T L (2019). Effects of different salinity levels on larval growth and development of disease vectors of Culex species. Journal of Environmental Biology., 40,1115-1122.

Balasubramanian R, T. L. Nikhil (2015). Effects of rainfall and salinity increase on prevalence of vector mosquitoes in coastal areas of Alappuzha district, Kerala. Journal of environmental Biology:36(6):1325.

Banerjee, S., Mohan, S., Saha, N., Mohanty, S. P., Saha, G. K., & Aditya, G. (2015). Pupal productivity & nutrient reserves of Aedes mosquitoes breeding in sewage drains & other habitats of Kolkata, India: Implications for habitat expansion & vector management. Indian Journal of https://doi.org/10.4103/0971-5916.176632.

BarraudP.J.1934.Diptera Vol V.Family Culicidae. Tribes Megarhinini and Culicini. In: The Fauna of British India, including Ceylon and Burma.R.B.S. Sewell and P.W. Edwards(eds.) Taylor and Francis.London.

Bargielowski, I. E., Lounibos, L. P., Shin, D., Smartt, C. T., Carrasquilla, M. C., Henry, A., Navarro, J. C., Paupy, C., & Dennett, J. A. (2015). Widespread evidence for interspecific mating between Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in nature. Infection, Genetics and Evolution, 36, 456–461. https://doi.org/10.1016/j.meegid.2015.08.016.

Becker, N., D. Petric, M. Zgomba, C. Boase, C. Dahl, J. Lane and A. Kaiser (2003): Mosquitoes and their control. Kluwer Academic/Plenum Publishing, New York.

Benjamin D Cabrera, Oscar L Ramos, Ildefonso T Cruz (1970). Malaria transmission by *Anopheles litoralis* King, a saltwater breeder, in Pangutaran, Sulu, Republic of the Philippines. Southeast Asian Journal of Tropical Medicine and Public Health. 1(2). 193-204.

Belkin, J. . (1962). The Mosquitoes of the South Pacific (Diptera, Culicidae), Vol. 2. Cambridge University Press, https://www.cabdirect.org/cabdirect/abstract/19501100562 2(1), 412. Bevins, S. N.

(2007). Timing of resource input and larval competition between invasive and native container-inhabiting mosquitoes (Diptera: Culicidae). Journal of Vector Ecology, 32(2), 1710(2007)32[252:torial]2.0.co;2 252–262. https://doi.org/10.3376/1081

Bevins, S. N. (2008). Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). Biological Invasions, 10(7), 1109–1117. https://doi.org/10.1007/s10530-007-9188-8

Bhat, M. A., & Krishnamoorthy, K. (2014). Original Research Article Entomological investigation and distribution of Aedes mosquitoes in. International Journal of Current Microbiology and Applied Sciences, 3(10), 253–260.

Black, W. C., Rai, K. S., Turco, B. J., & Arroyo, D. C. (1989). Laboratory study of competition between United States strains of Aedes albopictus and Aedes aegypti (Diptera: Culicidae). Journal of Medical Entomology, 26(4), 260–271. https://doi.org/10.1093/jmedent/26.4.260

Bina Pani Das, R. Rajagopal, J. Akiyama. Pictorial key to the species of Indian Anopheline Mosquitoes.Zoology.2(3):131-162.

BK Tyagi, A Munirathinam, A Venkatesh (2015). A catalogue of Indian mosquitoes. International journal of mosquito research. 50(625): 50-97.

Bradley J. White, Frank H Collins, Nora J Besansky (2011). Evolution of *Anopheles gambiae* about humans and malaria. Annual review of ecology, evolution, and systematics 42, 111-132.

Bradley J. White, Peter N. Kundert, Nora J. Besanky (2013). Dose and developmental responses of *Anopheles merus* larvae to salinity. The Journal of Experimental Biology. 216(18):3433-3441.

Brown, Lesley (1993). The New Shorter Oxford English Dictionary on Historical Principles. Oxford [Eng.]: Clarendon. ISBN 978-0-19-861271-1.

Bruland GL (2008). Coastal wetlands: function and role in reducing the impact of land-based management. in A. Fares and A. I. AlKadi, editors. Coastal watershed management. WIT Press .Southhampton: UK.

Burkett -Cadena, N.D (2013). Mosquitoes of the southeastern United States. Tuscaloosa: The University of Alabama Press.202 pages.

Cameron SL (2014). Insect mitochondrial genomics: implications for evolution and phylogeny. Annu. Rev. Entomol. 59. 95-117.

Chaiphongpachara. T and Sumruayphol.S (2017). Species diversity and distribution of mosquito vectors in coastal habitats of Samut Songkhram province, Thailand. Tropical Biomedicine 34(3):524-532.

Chidester F.E (1916). The influence of salinity on the development of certain species of mosquito larvae and its bearing on the problem of the distribution of species, Bulletin. New Jersey Agricultural Experiment Station, No. 299 pp

Christophers, S R (1933). The fauna of British India including Ceylon and Burma. Diptera vol. IV. Family Culicidae. Tribe Anopheline. Taylor &Francis, London, UK.

Craig A Stoops, Yoyo R Gionar, PriyantoSismadi, Iqbal RF Elayazar, Michael J Bangs, SupratmanSukowati (2007). Environmental factors associated with spatial and temporal distribution of Anopheles (Diptera: Culicidae) larvae in Sukabumi, West Java, Indonesia. Journal of Medical Entomology, 44(4), 543-553.

Craig R Williams, Samantha R Williams, Jay Nicholson, Susan M Little, Julie Riordan, Stephen R Fricker, Michael J Kokkinn (2009). Diversity and seasonal succession of coastal mosquitoes (Diptera: Culicidae) in the northern Adelaide region of South Australia. Australian Journal of Entomology, 48(2), 107-112.

Cran, Wayne J (1989). Resting boxes as mosquito surveillance tools. Proceedings of the Eighty-Second Annual Meeting of the New Jersey Mosquito Control Association. Pp.53-57.

D.A Yee, E. Himel, M.H Reiskind, S.M Vamosi (2013). Implications of saline concentrations for the performance and competitive interactions of the mosquitoes *Aedes aegypti* and *Aedes albopictus*, Medical and veterinary entomology, 28(1), 60-69.

Derek Roberts (1996). Mosquitoes (Diptera: Culicidae) breeding in brackish water: female ovipositional preferences or larval survival, Journal of Medical Entomology 33(4), 525-530.

D.H. Foley and J.H. Bryan (2008). Shared salinity tolerance invalidates a test for the malaria vector *An. farauti*s on Guadalcanal, Solomon Islands, Medical and veterinary entomology, 14(4), 450-452.

Din Syafruddin, Yulia E Lestari, Dendi H Permana, Puji BS Asih et.al (2020). *Anopheles sundaicus* complex and the presence of *Anopheles epiroticus* in Indonesia. PloS Neglected Tropical Diseases, 14(7).

DM Roberts, RJ Irving- Bell (1997). Salinity and microhabitat preferences in mosquito larvae from southern Oman. Journal of Arid Environments 37(3), 497-504.

Dong-Kyu Lee (2001). Field evaluation of an insect growth regulator, pyriproxyfen, against *Aedes togoi* larvae in brackish water in South Korea. Journal of vector ecology 26, 39-42.

D P W Jayatunga, N Harischandra et al (2021). Compensatory base changes reveal sexual incompatibility among members of the *Anopheles subpictussensu lato* (Diptera: Culicidae) species complex in Sri Lanka. Life, 11,211.

Duncan Seraphin, K. Philippoff, J. Pottenger, F. M. Kaupp, L. Lurie, M. H. Lin D and Baumgartner E (1995). The fluid Earth and The Living Ocean.

E.D.Hastuti, S.Anggoro and R.Pribadi (2012). The effects of environmental factors on the dynamic growth pattern of mangrove *Avicennia marina*. J. Coast. Dev. Vol.16. No. 1. Page 57-61.

Ermi Ndoen, Clyde Wild, Pat Dale, Neil Sipe, Mike Dale (2010). Relationships between anopheline mosquitoes and topography in West Timor and Java, Indonesia. Malaria Journal, 242

Fathima Abdulla Alkhayat, Abu H Ahmad, Junaid Rahim et. al (2020). Characterization of mosquito larval habitats in Qatar, Saudi Journal of Biological Sciences, 27(1), 2358-2365.

Fenemore P G and Alka Prakash. 1995. Applied entomology, Wiley Eastern Limited New Age International, New Delhi.

Freitas FB, Novo MT, Esteves A, Almeida AP (2012). Species Composition and WNV Screening of Mosquitoes from Lagoons in a Wetland Area of the Algarve, Portugal. Front Physiol. 2:122

F.W Mosha and C.M Mutero (1982). The influence of salinity on larval development and population dynamics of *Anopheles merus* Donitz (Diptera: Culicidae), Bulletin of Entomological Research, 72(1): 119-128.

Feroze, M., & Aravindan, K. P. (2001). Sickle cell disease in Wayanad, Kerala: Gene frequencies and disease characteristics. In National Medical Journal of India (Vol. 14, Issue 5, pp. 267–270).

Fish, D., & Carpenter, S. R. (1982). Leaf litter and larval mosquito dynamics in tree-hole ecosystems‟ Durland. Ecology, 63(2), 283–288.

Foley, D. H., Rueda, L. M., & Wilkerson, R. (2007). Insight into Global Mosquito Biogeography from Country Species Records. J. Med. Enlomol, 44(4), 554–567. https://doi.org/10.1603/0022-2585(2007)44.

Garcia, Lynne S (1999). Classification of human parasites, vectors and similar organisms. Clinical Infectious diseases. 29(4): 734-736.

George F O'Meara (1976). Saltmarsh mosquitoes (Diptera: Culicidae). Marine insects, 303-333

Getachew et.al (2020). Anopheles larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. Malaria Journal. 19:65.

Githeko,A;Lindsay,S;Confalonieri,U;Patz,J (2000).Climate change and vector-borne diseases, a regional analysis.Bull.WHO 78Available online:http://www.who.int/bulletin/archives/78%289%291136.pdf(accessed on 2 september 2014).

Goutam Chandra, Indranil Bhattacharjee, Soumendranath Chatterjee (2010). A review on *Anopheles subpictus*Grassi - A biological vector. Acta Tropica115: 142-154.

Gray A Williams (2000). Osmoregulation in larvae of the mosquito *Aedes togoi* from supralittoral rock pools at Cape Daguilar, Hong Kong, The marine flora and fauna of Hong Kong and southern China, V 5, 277.

Gunasekaran K et. al (2005). Malaria receptivity in the tsunami hit coastal villages of southern India. Lancet Infect Dis 5(9):531-532.

Hallem E A, Nicole Fox A, Zwiebel LJ, Carlson JR. Olfaction: Mosquito receptor for human-sweat odorant. Nature. 427(6971):212-3.

H.L. Felton(1944). The breeding of the salt marsh mosquito in Midwestern states, Journal of Economic Entomology,37(2), 245-247.

Herbert H Ross: 1956. A Text Book of Entomology, John Wiley And Sons, Inc., USA

Idris FHJ, Usman A, Suredran SN, Ramasamy R (2013). Detection of *Aedes albopictus* pre-imaginal stages in brackish water habitats in Brunei Darussalam. J VectEcol :38:197-199.

Iman Mohamed Bahgat (2013). Impact of physical and chemical characteristics of breeding sites on mosquito larval abundance at Ismailia Governorate, Egypt, Journal of Egyptian Society of Parasitology, 43(2), 399-406.

IP Sunish, AN Shriram, Amitabh De, PaluruVijayachari (2015). Malaria in the Andaman and Nicobar Islands: Challenges and opportunities for elimination. Asian Pacific Journal of Tropical Disease, 5(10),837-840.

Isabelle Dusfour, Johan R Michaux, Ralph E Harbach, Sylvie Manguin (2007). Speciation and phylogeography of the Southeast Asian *Anopheles sundaicus* complex. Infection, Genetics and Evolution, 7(4): 484-493.

Isabelle Dusfour, Ralph E Harbach, Sylvie Manguin (2004). Bionomics and systematics of the oriental *Anopheles sundaicus* complex in relation to malaria transmission and vector control. American Journal of Tropical Medicine and Hygiene, American Society of Tropical Medicine and Hygiene, 71(4), 518-524.

James- Pirri, M.J., R.M. Erwin. D.J Prosser and J.D. Taylor (2012). Responses of salt marsh ecosystems to mosquito control management practices along the Atlantic coast (USA). Rest. Ecol.,19,395-404.

Jason AL Jeffery, Brian H Kay, Peter A Ryan (2005). Development time and survival of *Verrallinafunerea* (Theobald)(Diptera: Culicidae) immatures and other brackish water mosquito species in southeast Queen's Island, Australia. Australian Journal of Entomology 44(3). 226-232.

Jason AL Jeffery, Brian H Kay, Peter A Ryan (2006). Role of *Verrallinafunerea* (Diptera: Culicidae) in Transmission of Barmah Forest Virus and Ross River Virus in Coastal Areas of Eastern Australia. Journal of Medical Entomology 43(6),1239-1247.

Johansson,MA;Dominici,F,Glass,G.E(2009). Local and global effect of climate on dengue transmission in Puerto Rico. PLoSNegl.Trop.Dis. doi: 10.1371/jornal.pntd.0000382.

J Meredith, J E Philips (1973). Ultrastructure of the anal papillae of a saltwater mosquito larva, *Aedes campestris*, Journal of insect physiology, 19(6), 1157-1172.

Jomon KV, Sudharmini S, Thomas T Valampampil (2009). Aedes mosquitoes in arboviral epidemic-prone area of Kottayam district, Kerala, India. Acad Review; 171-178.

Juliano, S. A. (2009). Species Interactions Among Larval Mosquitoes: Context Dependence Across Habitat Gradients. Annu Rev https://doi.org/10.1146/annurev.ento.54.110807.090611 Entomol, 54, 37–56.

Juliano, S. A., Lounibos, L. P., & Meara, G. F. O. (2004). A field test for competitive effects of Aedes albopictus on Ae . aegypti in South Florida : differences between sites of coexistence and exclusion. https://doi.org/10.1007/s00442-004-1532-4 Oecologia, 139, 583–593.

Juliano, S. A., O‟Meara, G. F., Morrill, J. R., &Cutwa, M. M. (2002). Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia, 130(3), 458–469. https://doi.org/10.1007/s004420100811

Jukes T.H. and Cantor C.R. (**1969**). Evolution of protein molecules. In Munro HN, editor, *Mammalian Protein Metabolism*, pp. 21-132, Academic Press, New York.

Kadarkarai Murugan, ChithravelVadivalagan et al (2016). DNA barcoding and molecular evolution of mosquito vectors of medical and veterinary importance. ParasitolRes.115:107-121

Kaliannagoun Krishnamoorthy, Jambulingam P, Natarajan R, Shriram AN, Das PK, and Sehgal SC (2005). Altered environment and risk of malaria outbreak in South Andaman, Andaman and Nicobar Islands, India affected by the tsunami disaster. Malaria Journal. 4:32.

K Balaraman, S Sabesan, P Jambulingam, K Gunasekaran, PS Boopathi Doss (2005). Risk of the outbreak of vector-borne diseases in the tsunami hit areas of southern India, The Lancet of Infectious Diseases, 5(3), 128-129.

Kelebogile Waniwa (2011). Salinity tolerance of the aquatic stages of *Anopheles funestus* group. Thesis submitted for the Master of Science (Med) degree, faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa.

K. Kathiresan and B.L. Bingham (2001). Biology of mangroves and mangrove ecosystems. Advances in Marine Biology.

KKN Nair, George Mathew (2010). Biodiversity of Malappuram district Kerala state. KFRI extension report No. 33(6).

KKN Nair, George Mathew (2010). Biodiversity of Kozhikode district Kerala state. KFRI extension report No. 33(7).

KKWT Madhushika, PD Dayananda, HSD Fernando et. al (2016). High salinity tolerance of Aedes to breed in brackish waters around the Negombo Estuary. Proceedings of International Forestry and Environment Symposium 21.

KM Arif, PK Prasadan, S Babu (2011). Conservation significance of the Kadalundi- vallikkunnu community reserve. Current science. Vol.101(6). 717-718.

KN Panicker (1981). *Anopheles subpictus*, vector of malaria in coastal villages of southeast Asia. Current science 50, 694-695.

Kokila Sivabalakrishnan, GajapathyKanapathy et al (2020). Population genetic structure of *An. subpictus* species B using COII and Cytbmarkers.Journal of Jaffna Science Association. 2(1), 38-47.

Koshu Asakura (1980). The anal portion as a salt excreting organ in a seawater mosquito larva *Aedes togoi* Theobald, Journal of comparative physiology, 138, 59-65.

Krishnamoorthy K, Natarajan R, Jambulingam P et al (2005). Altered environment and risk of malaria outbreak in South Andaman, Andaman & Nicobar Islands, India affected by the tsunami disaster. Malaria journal 4:32.

Laura Cristina Multini, Rafael Oliveira- Christe, Antonio Ralph Medeiros- Sousa, et. al (2021). The influence of the pH and salinity of water in breeding sites on the occurrence and community composition of immature mosquitoes in the green belt of the city of Sao Paulo, Brazil, Insects, 12(9),797.

LC Beadle (1939). Regulation of the haemolymph in the saline water mosquito larva Aedes detritus Edw. Journal of Experimental Biology, 16(3), 346-362.

Lehane, M.J(2005). The Biology of Blood Sucking in Insects. Cambridge University Press. Pp 151.

Leisnham, P.T. and S.S Mohapatra (2011). Mosquitoes associated with ditch-plugged and control tidal salt marshes on the Delmarva Peninsula. Inter. J. Environ. Res. Pub. Hlth., 8, 3099-3113.

Leopoldo M Reuda (2004). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. Zootaxa. 1-877354-47-3.

Leopoldo M Reuda, Mustapha Debboun (2020). Mosquitoes, communities, and public health in Texas. Science Direct. 3-7.

Lidiya Misyura, Elia Grieco Guardian, Andrea Claire Durant, Andrew Donini (2020). A comparison of aquaporin expression in mosquito larvae (*Aedes aegypti*) that develop in hypo-osmotic fresh water and iso-osmotic brackish water. 15(8).

Little V A (1963). General and Applied Entomology, Harper and Row, Publishers, New York and Evanston.

L.L.Koekemoer, K. Waniwa, B.D. Brook, G. Nkosi, A. Mabuza, M. Coetzee (2013). Larval salinity tolerance of two members of the *Anopheles funestus* group. Medical and veterinary entomology., 28(2), 187-192.

L. M. N. Rogo, G. B. White and R. C. Odhiambo (1985). Salinity relationships of mosquitoes breeding in a brackish pond on the Kenya coast. International Journal of Tropical Insect Science. 6(1). 91-95.

Lorenz, Camila; Alves, Joao M.P; Foster, Peter G (2021). Phylogeny and temporal diversification of mosquitoes (Diptera: Culicidae) with an emphasis on the Neotropical fauna". Systematic Entomology. 46(4):798-811.

MA Albers, T J Bradley (2011). On the evolution of saline tolerance in the larvae of mosquitoes in the genus Ochlerotatus. Physiological and biochemical zoology 84(3), 258- 267.

Madushika K.K.W.T, Dayananda P.D, Fernando H.S.D, Fernando A.L, De Silva H (2016). High salinity tolerance of Aedes to breed in brackish waters around the Negombo Estuary; vol.21(2016):21st International Forestry and environment symposium.

Mani M, S, (1973). General Entomology, Oxford and IBH Publishing Co., New Delhi.

Margaret Garrett and Timothy J. Bradley (1984). The pattern of osmotic regulation in larvae of the mosquito Culiseta inornate. J. exp. Biol. 113. 133-141.

Margaret A Garrett, Timothy J Bradley (1987). Extracellular accumulation of proline, serine and trehalose in the haemolymph of osmoconforming brackish water mosquitoes. Journal of Experimental Biology 129(1), 231-238.

Marjorie L Patrick, Timothy J Bradley (2000). The physiology of salinity tolerance in larvae of two species of Culex mosquitoes: the role of compatible solutes. Journal of Experimental Biology 203(4), 821-830.

Marylene de Brito Arduino, LF Mucci, LL Nunes Serpa, M de Moura Rodrigues (2015). Effect of salinity on the behaviour of *Aedes aegypti* populations from the coastal plateau of southeastern Brazil. Journal of vector-borne diseases 52(1), 79.

Maureen Coetzee and David Lusueur (1988). Effect of salinity on the larvae of some Afrotropical anopheline mosquitoes. Medical and Veterinary Entomology. 2(4), 385-390.

Maurice W Provost (1951). The occurrence of salt marsh mosquitoes in the interior of Florida, The Florida Entomologist 34(2), 48-53.

Mayagaya VS, Ntamatungiro AJ, Moore SJ, et al (2015). Evaluating preservation methods for identifying *Anopheles gambiae*s.s and *Anopheles arabiensis* complex mosquito's species using near infra-red spectroscopy. Parasites and Vectors 8, 60.

M Coluzzi, A Sebatini, V Petrarcha, M A Di Deco (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. Transactions of the royal society of tropical medicine and hygiene. Vol 73. Issue 5, pages 483- 497.

Medlock, J.M., K.R. Snow and S. Leach (2005). Potential transmission of West Nile virus in the British Isles: An ecological review of candidate mosquito bridge vectors. Medical Vete. Entomo., 19,2-21.

Metcalf, C.L. and W.P. Flint (1932). Fundamentals of Insect Life, McGraw-Hill Book Company, Inc., New York and London.

Michael D. Atkins (1978). Insects in Perspective, Macmillan Publishing Co., Inc., New York and London.

M L Patrick, R J Gonzalez, T J Bradley (2001). Sodium chloride regulation in freshwater and osmoconforming larvae of Culex mosquitoes. Journal of experimental biology 204(19), 3345-3354.

Mohammad Tauqeer Alam, Manoj K Das et al (2006). Molecular identification of *Anopheles* (Cellia) *sundaicus* from the Andaman and Nicobar Islands of India. Acta Tropica, 97(1): 10-18.

Mohamed Aly OuldLemrabott, Gilbert Le Goff, Pierre Kengne et. al (2020). First report of *Anopheles* (Cellia) *multicolour*during a study of tolerance to salinity of *Anopheles arabiensis* larvae in Nouakchott, Mauritania, Parasites and vectors, 13:522.

Muhammad Abdur Rahman, Muhammad MahbuburRahman and Md, Nazimuzzaman (2019). Impact of salinity on infectious disease outbreaks: Experiences from the Global Coastal Region. Springer Nature Switzerland AG.

Muhammad Mukthar, Jeroen Ensink, Wim Van der Hoek, Felix Prashantha Amerasinghe, FlemmingKonradsen (2006). Importance of waste stabilization ponds and wastewater irrigation in the generation of vector mosquitoes in Pakistan, 43(5):9996-1003.

Mukhtar M, Herrel N, Amerasinghe FP, Ensink J, van der Hoek W, Konradsen F (2003). Role of wastewater irrigation in mosquito breeding in south Punjab, Pakistan. Southeast Asian J Trop Med Public Health,34(1):72-80.

Nancy Mae Scherer (1977). The function of the anal papillae of saline-water mosquito larvae. University of British Columbia.

Neogi SK (1936). Study of the bionomics of *Anopheles sundaicus* (A. Ludlow) in the salt lakes of Calcutta. Records of the Malaria Survey of India, 6(1):31-47.

Neogi SK (1936). Relation of the salinity of the breeding places of *Anopheles sundaicus* with the endemicity of malaria in the suburban areas of Calcutta. Rec, Malar. Surv. India, 6(2):233-238.

NH Sweliengrebel, JMH Swellengrebel-de Graff (1919). Description of the Anopheline larvae of Netherlands India, so far as they are known till now. Mededelingen van den BurgerlijkenGaneeskundigenDienst in Nederlandsch- Indie pp48.

Nicolson SW and Leader JP (1974). The permeability to water of the cuticle of the larva of Opifex fuscus (Hutton) (Diptera: Culicidae). Journal of Experimental Biology, 60,593-604.

N. Lukwa, T. Mduluza, C. Nyoni, M. Zimba (2017). To what extent does salt (NaCl) affect *Anopheles gambiae* sensu lato mosquito larvae survival. Journal of Entomological and Acarological Research, 49(2).

N Pradeep Kumar, A R Rajavel et al (2007). DNA barcodes can distinguish species of Indian mosquitoes. Journal of Medical Entomology, 44(1):1-7.

Nwaefuna K, BagshawII, Gbogbo F, Osae M (2013). Oviposition and development of *Anopheles coluzzii* Coetzee and Wilkerson in salt water. Malar Res Treat, 7:9523962.

Osborn FR, Herrera MJ, Gomez CJ, Salazar (2007). A. Comparison of two commercial formulations of *Bacillus thuringiensis var. israelensis* for the control of *Anopheles aquasalis* (Diptera: Culicidae) at three salt concentrations. Mem. Inst. Oswaldo Cruz, 102(1).

Pamela C Kipyab, Battan M Khaemba, Joseph M Mwangangi and Charles M Mbogo (2015). The physicochemical and environmental factors affecting the distribution of *Anopheles merus* along the Kenyan coast. Parasites and vectors, 8:221.

Panicker KN, Geetha Bai M, Bheema Rao US, Viswam K, Suryanarayanamurthy U (1981). *Anopheles subpictus*, vector of malaria in coastal villages of south-east India. Current Science, 50, 694-695.

Paul F Russel, TC Rao, VP Jacob (1939). *Anopheles subpictus* Grassi 1899 and *An. vagus* Donitz 1902, found naturally infected with malaria plasmodia in southeastern India. Jour Mal Inst Ind 2, 95-100.

Paul F Russell, T Ramachandra Rao (1940). On habitat and association of species of anopheline larvae in southeastern madras. Journal of Malaria Institute of India 3(1).

Paul Reuter (2001). Climate change and mosquito-borne diseases. Environmental health perspectives 109 suppl 1,141-161.

Pavilupillai J Jude, Sangaralingam Dharshini, MuthuladchumyVinobaba, Sinnathamby N Surendran, Ranjan Ramasamy (2010). *Anopheles culicifacies* breeding in brackish waters in Sri Lanka and implications for malaria control. Malaria Journal 9:106.

Pavilupillai J Jude, T Tharmasegaram, GobikaSivasubramaniyam, Sinnathamby N Surendran, Ranjan Ramasamy (2012). Salinity tolerant larvae of mosquito vectors in the tropical coast of Jaffna, Sri Lanka and the effect of salinity on the toxicity of Bacillus thuringiensis to *Aedes aegypti* larvae. Parasites and vectors 5(1). 1-8.

Peach D A, Gries G (2019). Mosquito phytophagy- sources exploited, ecological function, and evolutionary transition to haematophagy. EntomologiaExperimentalis et Applicata. 168(2): 120-136.

Pierre Kengne, Guy Charmantier, Eva Blondeau-Bidet, Carlo costantini, Diego Ayala (2019). Tolerance of disease vector mosquitoes to brackish water and their osmoregulatory ability. Ecosphere 10(10), e02783.

Pierre- Olivier Maquart, CheaSokha, Sebastien Boyer (2022). Mosquito (Diptera: Culicidae) diversity and medical importance in Koh Kong mangrove forests, Cambodia. 16(3):121-129.

P Mottram, B.H.Kay and J.D Fanning (1994). Development and survival of *Culex sitiens* Wiedemann (Diptera: Culicidae) in Relation to Temperature and Salinity. J. Aust. ent. Soc.33:81-85.

Poinar, G.O; et al (2000). *Paleo culicisminutus*(Diptera: Culicidae) n. gen., n. sp., from Cretaceous Canadian amber with a summary of described fossil mosquitoes. Acta v GeologicaHispanica. 35:119-128.

PT Monis (1999). Invited review The importance of systematics in parasitological research, International journal for parasitology. Vol 29. Issue 3. 381-388.

P Sen (1957). Importance of Albuminoid Ammonia and salinity in the breeding of *Anopheles sundaicus* (Rodenw). Indian Journal of Public Health, 1(4), 252-3.

Rahees N, Kiran M, Vishal V (2014). Phytosociological analysis of mangrove forest at KadalundiVallikkunnu community reserve Kerala. International journal of science, environment and Technology. Vol.3. No. 6:2154-2159.

Rajesh Dhakane, Kiran Ahirrao et al (2021). DNA based identification and control of disease spreading mosquito species: A review. Journal of applied biology and biotechnology 9(1),124-134.

Raj Kumar Singh, Gaurav Kumar, Pradeep Kumar Mittal, Ramesh Chand Dhiman (2014). Bionomics and vector potential of *Anopheles subpictus* as a malaria vector in India: An overview. Int J Mosq Res 1(1), 29-37.

Ralph E Harbarch (2007). The Culicidae (Diptera): A review of Taxonomy, Classification and Phylogeny. Zootaxa 1668:591-638.

Ralph E Harbarch (2011). Classification within the cosmopolitan genus Culex (Diptera: Culicidae): the foundation for molecular systematics and phylogenetic research. Acta Trop. Oct-Nov.

Ramsay JA (1950). Osmotic regulation in mosquito larvae. Journal of Experimental Biology 27, 145-180.

Ramasamy R, Surendran SN (2012). Global climate change and its potential impact on disease transmission by salinity-tolerant mosquito vectors in coastal zones. FrontPhysiol; 3: 198.

Ranjan Ramasamy, Sinnathamby N Surendran (2011). Possible impact of rising sea levels on vector-borne infectious diseases. BMC Infectious Diseases. 11:18.

Ranjan Ramasamy, Sinnathamby N Surendran, Pavilupillai J Jude, Sangaralingam Dharshini (2011). Larval development of *Aedes aegypti* and *Aedes albopictus* in peri-urban brackish water and its implications for transmission of arboviral diseases. pLoSNegl trop Dis5(11), C1369.

Ranjan Ramasamy, Sinnathamby N Surendran, Pavilupillai J Jude, Sangaralingam Dharshini (2015). Adaptation of mosquito vectors to salinity and its impact on mosquito-borne disease transmission in the South and Southeast Asian tropics. Socio-ecological dimensions of infectious diseases in Southeast Asia, 107-122.

Ranjan Ramasamy, P J Jude, T Veluppillai, S N Surendran (2014). Biological differences between brackish and freshwater-derived *Aedes aegypti* from two locations in the Jaffna peninsula of Sri Lanka and the implications for arboviral disease transmission. PLoS ONE 9(8): e104977.

R.C Russell (1979). A study of the influence of some environmental factors on the development of *Anopheles annulipes* Walker and Anopheles amictushilli Woodhill and Lee (Diptera: Culicidae). Gen. Appl. Entomol. 11:42-45.

R.C Russell (1998). Mosquito-borne arboviruses in Australia: the current scene and implications of climate change for human health. Int. J. Parasitol.28:955-969.

R. D. Cooper, S.P. Francis, D. G. E. Waterson, R. G. Piper and A. W. Sweeney (1996). Distribution of Anopheline mosquitoes in Northern Australia. Journal of American Mosquito Control Association, 12(4): 656-663.

Reiter. P (2001). Climate change and mosquito-borne diseases. Environ.Hlth.Perspect: 109:141-161.

Richard C Russell (2012). A review of the status and significance of the species within the *Culex pipiens* group in Australia. Journal of the American Mosquito Control Association 28(4s), 24-27.

Richard F Darsie, Adela Cagampang- Ramos (1970). The colonization of *Anopheles litoralis* King in the Philippines. Journal of Medical Entomology, 7(5):518-521.

Robert D Cooper, Michael D Edstien, Stephen P Frances, Nigel W Beebe (2010). Malaria vectors of Timor-Leste. Malaria journal.9:40.

R. Reuben, S G Suguna (1983). Morphological differences between sibling species of the taxon *Anopheles subpictus* Grassi in India, with notes on relationships with known forms.

RT Collins, RK Jung, H Anoez, RH Sutrisno, Putut D (1979). A Study of the coastal malaria vectors, *Anopheles sundaicus* (Rodenwaldt) and *Anopheles subpictus* Grassi, in South Sulawesi, Sulawesi, Indonesia. WHO/MAL/79.913.

Russel PF and Jacob VP (1939). Epidemiology of malaria in Ennore Nellore coastal area, Madras Presidency, India. J. Mal. Inst. India, 2, 131-152.

Russel PF and Rao TR (1940). Natural malarial infections in South Indian Anophelines with special reference to *Anopheles culicifacies*. J. Mal. Inst. India, 3, 543-562.

Samantha R Williams, Michael J Kokkinn, Stephen R Fricker, Melanie J Schneemilch, Matthew J Humphrey, Criag R Williams (2009). Mosquitoes (Diptera: Culicidae) of the Spencer Gulf Coast of South Australia, Transactions of the Royal Society of South Australia, 133(1), 51-56.

SamrerngPrummongkol, ChotechuangPanasoponkul, ChamnarnApiwathnasorn, Usa Lek-Uthai (2012). Biology of *Culex sitiens*, a predominant mosquito in Phang Nga, Thailand after a tsunami. Journal of Insect Science 12(1).

S.A. Ritchie and E.S Johnson (1991). Distribution and sampling of *Aedes taeniorhynchus*(Diptera: Culicidae) eggs in a Florida mangrove forest. J. Med.Entomol.

Satarupa Paul, Amit Chattopadhyay et al (2013). Anopheline diversity: Morphological and molecular variation of *An. subpictus* in rural and urban areas of West Bengal. Journal of entomology and zoology studies.1(2):35-40.

Satarupa Paul, Amit Chattopadhyay et al (2015). Studies on seasonal abundance & molecular characterization of *Anopheles subpictus* and *Anopheles vagus* based on ITS2 sequence variability. International Journal of Mosquito Research.2(3):131-135.

Scott A Ritchie, Ian D. Fanning, Debbie A Phillips et. al (1997). Ross River Virus in Mosquitoes (Diptera: Culicidae) During the 1994 Epidemic around Brisbane, Australia. Journal of Medical Entomology. Vol.34. Issue 2. Pages 156-159.

Scott Carver, Helen Spafford, Andrew Storey and Philip Weinstein (2010). The role of predators, competitors, and secondary salinization in structuring Mosquito (Diptera: Culicidae) assemblages in ephemeral water bodies of the Wheatbelt of Western Australia, Environ. Entomol, 39(3): 798-810.

S G Suguna, K Gopalan Rathinam, AR Rajavel, VD Handa (1994). Morphological and chromosomal descriptions of new species in the *Anopheles subpictus* complex. Medical and veterinary entomology 8(1), 88-94.

Sinnathamby N Surendran, Devojit K Sarma, Pavilupillai J Jude, Petri Kemppainen, Nadarajah Kanthakumaran, KanapathyGajapathy, Lalanthika BS Peiris, Ranjan Ramasamy (2013). Molecular characterization and identification of members of *Anopheles subpictus* complex in Sri Lanka. Malaria Journal. 12:304.

Sinnathamby N Surendran, Kokila Sivabalakrishnan, ArthiyanSivasingham et.al (2019) anthropogenic factors driving recent range expansion of the malaria vector *Anopheles stephensi*. Front. Public Health Vol. 7.

Sinnathamby N Surendran, Pavilupillai J Jude, Ranjan Ramasamy (2011). variations in salinity tolerance of malaria vectors of the *Anopheles subpictus* complex in Sri Lanka and the implications for malaria transmission. Parasites and vectors. 4:117.

Sinnathamby N Surendran, Pavilupillai J Jude, Veluppillai Thabothiny, Selvarajah Raveendran (2012). Pre-imaginal development of *Aedes aegypti* in brackish and freshwater urban domestic wells in Sri Lanka. Journal of vector ecology 37(2),471-473.

Sinnathamby N Surendran et al (2010). Genetic evidence for malaria vectors of the *Anopheles sundaicus* complex in Sri Lanka with morphological characteristics attributed to *Anopheles subpictus* species B. Malaria Journal, 9:343.

Sinnathamby N Surendran, Tibutius TP Jayadas, AnnathuraiTharsan et. al (2020). Anopheline bionomics, insecticide resistance and transnational dispersion in the context of controlling a possible recurrence of malaria transmission in Jaffna city in northern Sri Lanka. Parasites and vectors, 13, 156.

SK Sharma and KK Hamzakoya (2001). Geographical spread of *Anopheles stephensi*, vector of urban malaria, and *Aedes aegypti*, vector of Dengue/DHF, in the Arabian Sea Islands of Lakshadweep, India. Dengue Bulletin, WHO, Vol 25.

Soumendranath Chatterjee and Goutham Chandra (2000). Role of *Anopheles subpictus* as a primary vector of malaria in an area in India. Jpn. J. Trop. Med. Hyg., 28(3): 177-181.

Sreelekshmi S, BijeeshKV, Bijoy S, Harikrishnan M (2021). Regional studies in marine science vol 41101573.

SuchadaSumruayphol, Chamnarn A piwathnasorn, NarumonKomalamisra, et. Al (2010). Bionomic status of *Anopheles epiroticus* Linton&Harbarch, a coastal malaria vector, in Rayong Province, Thailand, Southeast Asian J Trop Med Public Health, 41(3).

Sumodan PK (2014). Mosquitoes in Kerala: Diversity and public health implications. Conference paper. Conference: Insect diversity of Western Ghats- an approach towards modern conservation strategies. At Thaliparamba. Kerala.

Sweeney. A.W (1987). Larval salinity tolerances of the sibling species of *Anopheles farauti*. Journal of American Mosquito Control Association. 3:589-592.

Sylvie Manguin, Claire Garros, Isabelle Dusfour, RE Harbarch, M Coosemans (2008). Bionomics, taxonomy, and distribution of the major malaria vector taxa of Anopheles subgenus Cellia in Southeast Asia: an updated review. Infection, genetics and evolution 8(4), 489-503.

Tamura K., Stecher G., and Kumar S. (**2021**). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* https://doi.org/10.1093/molbev/msab120.3. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Taruna Kaura, Monika Sharma, Sudarshan Chaudhry, Asha Chaudhry (2010). Sequence polymorphism in spacer ITS2 of *Anopheles* (Cellia) *subpictus* Grassi (Diptera: Culicidae). Caryologia. 63:2, 124-133.

TC Narendran(2000). The importance of systematics. Resonance 5(6), 60-68.

Tennessen K.J (1993). Production and suppression of mosquitoes in constructed wetlands. In: Moshiri, G. A. (Ed.), Constructed wetlands for water quality improvement. Lewis Publishers Michigan, USA, 591-601 PP.

Thilini C Weeraratne, Sinnathamby N Surendran, et al (2018). Genetic diversity and population structure of malaria vector mosquitoes *Anopheles subpictus, Anopheles peditaeniatus* and *Anopheles vagus* in five districts of Sri Lanka. Malaria Journal 17:271.

Thilini C Weeraratne, Sinnathamby N Surendran, et al (2017). Molecular characterization of Anopheline (Diptera: Culicidae) mosquitoes from eight geographical locations of Sri Lanka. Malaria journal16:234

Thomas M. Clark, Benjamin J. Flis, Susanna K. Remold (2004). Difference in the effect of salinity on larval growth and development programs of a freshwater and a euryhaline mosquito species (Insecta: Diptera, Culicidae). Journal of Experimental Biology 207(13),2289-2295.

Thomas G Floore (2006). Mosquito larval control practices: past and present. Journal of the American Mosquito Control Association 22(3), 527-533.

T. J Bradley, K Strange, JE Phillips (1984). Osmotic and ionic regulation in saline-water mosquito larvae. Osmoregulation in Estuarine and Marine Animals, vol 9, 35-50.

T.J Bradley (1987). Physiology of osmoregulation in mosquitoes. Annual review of entomology 32(1), 439-462.

T J Bradley (1994). The role of physiological capacity, morphology, and phylogeny in determining habitat use in mosquitoes. Ecological morphology; integrative organismal biology, 303-318.

T J Bradley (2008). Saline water insects: ecology, physiology and evolution. Aquatic insects: challenges to populations. UK: CAB International, 20-35.

T Mariappan, N Arunachalam, CMR Reddy, S Sabesan and KN Panicker (1996). Brackish water mosquito problem of Vypeen Island, Cochin, Kerala. Southeast Asian J Trop Med Public Health. 27(1).

Tulloch G.S (1937). The brackish water mosquitoes of Puerto Rico, 21(4): 581-583.

Van F Ronnefeldt (1959). *Anopheles sundaicus* as a vector of Malaria in Middle Java. Zeitschrift fur Tropenmedizin und Parasitologie, 10(1), 38-47.

Van Schie, C., H. Spafford, S. Carver and P. Weinstein (2009). Salinity tolerance of *Aedes camptorhynchus (*Diptera: Culicidae) from two regions in southwestern Australia. Australian J. Entomo., 48,293-299.

Weaver,S.C, Riesen W.K (2010).Present and future arboviral threats. Antiviral Res.85:328

Wesley B Grueber and Timothy J Bradley (1994). The evolution of increased salinity tolerance in larvae of Aedes mosquitoes: a phylogenetic analysis. Physiological zoology 67(3), 566-579.

Woodhill A. R. (1938). Salinity tolerance and pH range of Culex fatigans Wied, with notes on the anal papillae of salt water mosquitoes, Proceedings of the Linnean Society of New South Wales, 63(3-4), 273-281.

World Health Organization 2019.Dengue guidelines for diagnosis, treatment, prevention and control.WHO/HTM/NTD/DEN/2019.

Yadav. P (2009). Factors affecting mosquito populations in created wetlands. Environmental Science Graduate Program of the Ohio State University. 1:1-43.

Yajun Ma, Shizhu Li, Jiannong Xu (2006). Molecular identification and phylogeny of the maculatus group of Anopheles mosquitoes (Diptera: Culicidae) based on nuclear and mitochondrial DNA sequences. Acta Tropica 99(2-3), 272-280.

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Detection of *Aedes albopictus* (Diptera: Culicidae) breeding in brackish water habitats in coastal Kerala, India and its implications for dengue scenario in the state

Shamna A K, Vipinya C, Sumodan P K*
Department of Zoology, Government College Madappally, Vadakara, Kerala, India

Abstract
There is a long and widely held view that *Ae. albopictus*, the vector of Dengue, Chikungunya, Zika and Yellow fever breed only in fresh water habitats. Larval source reduction efforts worldwide, therefore focus on freshwater habitats of this vector. However, *Ae. albopictus* was recently shown to undergo larval development in brackish water with salinity up to 15 ppt in various parts of the world. In the present study breeding of *Ae. albopictus* was detected in three types of breeding habitats with brackish water *viz*., shallow ponds, plastic containers and discarded bottles in the coastal areas of Kannur and Kozhikode districts of Kerala, India. The salinity of water in these habitats ranged from 2.6 to 4.5 ppt. When tested in laboratory, the highest salinity tolerance was found to be 12 ppt. Since dengue is the most important mosquito-borne disease in the state, adaptation of *Ae. albopictus* to coastal brackish water habitats could have major consequences for the dengue scenario in the state. Besides dengue, Chikungunya and Zika are also prevalent in the state. This necessitates a need for constant monitoring of brackish water breeding habitats of the state and initiate appropriate control measures wherever necessary, in order to prevent the diseases vectored by *Ae. albopictus*.

Keywords: aedes albopictus, brackish water, salinity, vector, climate change

Introduction
Dengue is a mosquito borne viral disease transmitted mainly by *Aedes aegypti* and *Ae. albopictus*. These mosquitoes are also vectors of chikungunya, yellow fever and zika viruses. Dengue is widespread throughout the tropics, with local variations in risk influenced by climate parameters, as well as social and environmental factors [1]. India reported 123106 cases till October 2021of which the contribution of Kerala was 3794 cases (Source: National Vector Borne Disease Control Programme). Brackish water habitats are a neglected source of dengue vectors on tropical beaches in the world [2] *Aedes albopictus* breed and undergo larval and pupal development in natural and artificial fresh water collections in the urban and peri urban environment. Although Larval development of *Ae. albopictus* is known to exist in fresh water environment for many years, few recent findings have revealed the possibility of *Aedes* breeding and immature stage development in brackish water conditions [3]. Water with<0.5 ppt, 0.5-30 ppt and >30 ppt salt are termed as fresh, brackish and saline respectively [4]. Approximately 5% of mosquito species are adapted to undergo larval development in brackish and saline waters [5]. Many countries, particularly in Southeast Asia, have extensive coastlines, high coast to land area ratios and, a large proportion of their populations living in coastal areas. Such brackish water habitats are potential sources of vectors that may contribute to the transmission of dengue and other arboviral diseases in coastal areas [6]. The state of Kerala in South India has a total coastline of 589.5 kilometers, which forms 10% of India's total coastline. The present study was originally planned to investigate mosquito breeding in brackish water habitats, especially disease vectors and also to estimate the salinity tolerance of the local populations of the species.

Materials and Methods
Study area
Surveys for detecting mosquito breeding in brackish water was conducted randomly in all types of habitats in the coastal areas of Kannur and Kozhikode districts of North Kerala from January to December 2021. Surveys were restricted within 2 KM from the coastline. Ten localities were selected from each district for the study *viz*., Kurichiyil, Chalil, Thalayi, Edakkad, Dharmadam, Muzhappilangad, Mattool, Ezhome, Puthiyangadi, Thayyil (Kannur district), Azhiyoor, Chombala, Kuriyadi, Vadakara, Koyilandy, Thikkodi, Ezhukudikkal, Puthiyappa, Vellayil, Chaliyam and Beypore (Kozhikode district).

Larval collection and measurement of salinity
Before collecting larvae, salinity of the water was measured using a portable salinometer. Water with salinity ranging between 0.5 and 30 ppt were considered as brackish [5]. Larvae from large habitats were collected using 8 cm diameter and 240 ml capacity dipper. From smaller containers larvae were picked up by droppers. Fourth

instar larvae were identified using published identification keys in the field. Early instar larvae were reared in the laboratory to fourth intsar larvae. Identities were further conferred after emergence using adult keys [7].

Salinity tolerance study in the laboratory

For obtaining larvae for salinity tolerance study, female *Ae. albopictus* mosquitoes were fed on rat's blood and made to lay eggs in a rearing cage. Larvae were reared in plastic bowls of 300 ml capacity. Each bowl was fed on 2:1 mixture of finely ground fish pellets: baker's yeast. Solutions of commercial NaCl were prepared with distilled water following published procedures [8]. These preparations were placed in 500 mL plastic containers (each concentration was placed separately). Initially, larvae were exposed to six concentrations *viz*., 0 ppt, 10 ppt, 20 ppt, 30 ppt, 40 ppt and 50 ppt. There were 4 replicates for each concentration. NaCl solutions were left for 1hour before introducing mosquito larvae. Salinity tolerance was estimated following published procedures [5]. 20 fourth instar larvae were placed in each test solution. After 24 hours of exposure, larval survival was scored by eye. Larval death was confirmed by repeated stimulation with a plastic pipette to test for a motile response. It was repeated in 48 and 72 hours respectively. Once a tolerable salinity was obtained, they were further exposed to still narrower ranges of salinity in 1 ppt increments.

Data on dengue

Data on dengue cases and deaths were obtained from Directorate of Health Services, Thiruvananthapuram

Results and Discussion

***Ae. albopictus* breeding in brackish water**

Seven types of habitats were found having brackish water with salinity ranging between 0.5 and 30 ppt *viz*., cement tanks, plastic tanks, wells, shallow ponds, ditches, discarded bottles and different types of plastic containers. Breeding of *Anopheles subpictus* and *Culex sitiens* was observed in habitats having salinity ranging between 2.0- 28.0 ppt. *Ae. albopictus* breeding was observed in four shallow ponds and nine plastic containers in Ezhome (Kannur district) and three discarded bottles in Azhiyur (Kozhikode district). The salinity of water in these habitats ranged between 2.6 to 4.5 ppt (Table 1).

Table 1: Brackish water habitats with *Aedes albopictus* breeding

Locality / District	Habitat (Number)	Immature stages	Salinity range (ppt)
Ezhome/ Kannur	Shallow ponds (4)	All larval instars and Pupae	2.6 to 3.2
Ezhome/ Kannur	Plastic containers (9)	Third and Fourth Larvae	3.0 to 3.5
Azhiyur/ Kozhikode	Discarded bottles (3)	All larval instars	4.5

***Salinity tolerance of larvae* in laboratory**

In the first experiment, with broad ranges of salinity (0-50 ppt in 10 ppt increment), 100 % survival of larvae of *Ae. albopictus* was recorded in the samples with up to 10 ppt ((Table-2). In the second experiment, with narrow ranges of salinity (10 to 19 ppt in 1 ppt increment), 100% survival was recorded up to 12 ppt (Table-3).

Table 2: Salinity tolerance of fourth instar *Ae. albopictus* larvae (0 to 50 ppt)

Salinity (ppt)	Number of larvae exposed	Number of larvae survived		
		24 hours	48 hours	72 hours
0	20	20	20	20
10	20	20	20	20
20	20	0	0	0
30	20	0	0	0
40	20	0	0	0
50	20	0	0	0

Table 3: Salinity tolerance of fourth instar *Ae. albopictus* larvae (10 to 20 ppt)

Salinity (ppt)	Number of larvae exposed	Number of larvae survived		
		24 hours	48 hours	72 hours
10	20	20	20	20
11	20	20	20	20
12	20	20	20	20
13	20	16	10	8
14	20	4	3	1
15	20	0	0	0
16	20	0	0	0
17	20	0	0	0
18	20	0	0	0
19	20	0	0	0

Dengue situation

Both Kannur and Kozhikode districts reported dengue cases consistently (Table 4). From 2017 to 2021 the number of cases in Kannur ranged from 116 to 629. During the same period Kozhikode district recorded 69 to 1354. Deaths due to dengue in Kannur district ranged from 0 to 5 and the same was 2 to 7 in Kozhikode district.

Table 4: Dengue cases in Kannur and Kozhikode districts from 2017 to 2021

Year	Dengue cases (death)	
	Kannur	Kozhikode
2017	629 (0)	1354 (7)
2018	338 (3)	247 (3)
2019	212 (0)	405 (2)
2020	268 (5)	69 (2)
2021	116 (2)	209 (6)

The present study has brought the hitherto neglected brackish water habitats in the coastal areas of Kerala into lime light in the context of the epidemiology of diseases transmitted by *Ae. albopictus*. This is the first study demonstrating the adaptation of the predominantly freshwater breeding *Ae. albopictus* to brackish water in Kerala. In Sri Lanka, brackish water with salinity of 2 to 15 ppt in discarded plastic and glass containers, abandoned fishing boats and unused wells in coastal peri-urban environment were found to contain *Ae. aegypti* and *Ae. albopictus* larvae. In Jaffna city of Sri Lanka, higher incidences of dengue were reported from areas in the vicinity of brackish water habitats [2]. In the present study, though natural breeding of *Ae. albopictus* was found in brackish water with up to 4.5 ppt only, they could tolerate a salinity up to 12 ppt in the laboratory, which is comparable to the Sri Lankan strain of the species. Since the number of brackish water habitats with *Ae. albopictus* in the present study was comparatively less, it could be argued that the phenomenon of adaptation to brackish water is in its initial stage. However, as time elapses, the chances of the saline tolerant population to proliferate cannot be ruled out. Dengue is a regular phenomenon in all districts of the state including Kannur and Kozhikode with sizeable number of cases and even a few deaths every year. In the absence of an effective vaccine, dengue control strategies focus on the reduction and elimination of *Aedes* breeding in domestic and peri-domestic container habitats. Hence, the brackish water habitats are largely neglected. This could have serious consequences in the dengue scenario of the state in the near future. Besides dengue, two other *Aedes*-borne diseases *viz*., Chikungunya and Zika, are also prevalent in the state. Considering all these factors, serious efforts are needed to monitor and design control strategies to tackle this new phenomenon.

Conclusion

Breeding of *Aedes albopictus*, a predominantly freshwater species, was detected in three types of breeding habitats with brackish water *viz*., shallow ponds, plastic containers and discarded containers in two localities in the coastal areas of Kannur and Kozhikode districts of Kerala. The salinity of these habitats ranged from 2.6 to 4.5 ppt. In the laboratory the fourth instar larvae showed a maximum salinity tolerance of 12 ppt. The study is the first of its kind in the state. Adaptation of *Ae. albopictus* to brackish water could have serious consequences in the dengue scenario of the state. Hence, immediate strategies to monitor and contain the proliferation of saline tolerant populations of the species is recommended.

References

1. World Health Organization. Dengue: Guidelines for diagnosis, treatment, prevention and control, WHO, Geneva, 2019.
2. Ramasamy R, Surendran SN, Jude PJ, Dharshini S, Vinobaba M. Larval development of *Aedes aegypti* and *Aedes albopictus* in peri-urban brackish water and its implications for transmission of arboviral diseases. pLoS Negl trop Dis,2011:5(11):1-10.
3. Weaver SC, Riesen WK. Present and future arboviral threats. Antiviral Res,2010:85(2):328-345.
4. Surendran SN, Jude PJ, Ramasamy R. Variations in salinity tolerance of malaria vectors of the *Anopheles subpictus* complex in Sri Lanka and the implications for malaria transmission. Parasites and vectors,2011:4(117):1-9.
5. White BJ, Kundert PN, Besanky NJ. Dose and developmental responses of *Anopheles merus* larvae to salinity. The Journal of experimental Biology,2013:216(18):3433-3441.
6. Surendran SN, Jude PJ, Thabothiny V, Raveendran S. Pre imaginal development of *Aedes aegypti* in brackish and freshwater urban domestic wells in Sri Lanka. Journal of Vector Ecology,2012:37(2):471-473.
7. Tyagi BK, Munirathinam A, Venkatesh A. A catalogue of Indian mosquitoes. International Journal of Mosquito Research,2015:2(2):50-97.
8. Lukwa N, Mduluza T, Nyoni C, Zimba M. To what extent does salt (NaCl) affect *Anopheles gambiae* sensu lato mosquito larvae survival. Journal of Entomological and Acarological Research,2017:49:(6)594:85-88.

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Distribution pattern of *Anopheles stephensi* (Diptera: Culicidae) in north Malabar region of Kerala and its potential role in malaria transmission

Vipinya C, Shamna A K, Sumodan P K*
Research, Department of Zoology, Government College, Madappally, Vadakara, Kerala, India

Abstract
Anopheles stephensi is an important vector of urban malaria in the Indian subcontinent. The present study was planned to investigate the prevalence and distribution of this notorious vector in North Malabar region of Kerala. Larval collections were done from various potential breeding habitats, viz., domestic containers, water collection in the buildings under construction, cement tanks, wells, seepage canals, boats, fresh water ponds, streams, swamp, marshes, artificial containers, overhead tanks and ground level water tanks. The study was conducted in five North Kerala districts viz., Kasaragod, Kannur, Kozhikode, Malappuram and Wayanad from 2019 to 2021. *Anopheles stephensi* was found breeding in 6 breeding habitats viz., buildings under construction, cement tanks, wells, ponds, boats and ground level water tanks. The positive habitats were distributed in urban, suburban and rural areas. For the first time the species was detected from a non-coastal rural area, indicating its invasion from urban centres. Considering the efficiency of *Anopheles stephensi* as a vector of Malaria, constant surveillance and preventive measures are recommended.

Keywords: *Anopheles stephensi*, habitat preference, primary vector, malaria control

Introduction
Geoclimatic peculiarities and other natural habitats in India form a mosquito-genic condition conducive for the transmission of vector borne diseases. Mosquitoes belong to the top order disease vectors, responsible for spreading several diseases such as Malaria, Dengue, Japanese Encephalitis, Chikungunya, Zika, West Nile Virus and Lymphatic Filariasis. Among these Malaria is one of the major public health problems in India [1]. As per the World Malaria Report 2021 there were an estimated 241 million malaria cases and 627 000 malaria deaths worldwide in 2020. This represents about 14 million more cases in 2020 compared to 2019, and 69 000 more deaths. Approximately two thirds of these additional deaths (47000) were linked to disruptions in the provision of malaria prevention, diagnosis and treatment during the covid pandemic [2]. In India National Vector Borne Disease Control Programme reported 186532 cases and 93 deaths in the same period. The contribution of Kerala was 268 cases and one death. *Anopheles stephensi* is an important vector of urban Malaria in the Indian subcontinent [3, 4, 5]. In Kerala, this species was first reported from Kochi in 1992. Subsequently, it was reported from Thiruvananthapuram, Kollam, Thrissur, Valancherry (Malappuram), Kasaragod and Thodupuzha (Idukki) [6]. However, there were no systematic study on the prevalence, habitat specificity and distribution of this important vector species in North Malabar. Hence, the present study was carried out in five Northern districts viz., Kasaragod, Kannur, Kozhikode, Malappuram and Wayanad from 2019 to 2021. `

Materials and Methods
Study area
North Malabar refers to the geographic area of southwest India covering mainly six districts of the state Kerala. These districts are enriched with major and minor water bodies which have strong influence on breeding of mosquitoes especially *Anopheles stephensi.* The study was conducted in five North Kerala districts viz., Kasaragod, Kannur, Kozhikode, Malappuram and Wayanad.

Sample collection and identification
Immature stages of *Anopheles stephensi* were collected from all possible breeding habitats using 300 ml bowls and dippers. The collected larvae and pupae were transported to the laboratory in plastic containers. For rearing, water samples containing larvae/pupae from each site were covered with nylon cloth to avoid the escape of adult mosquitoes. Emerging adults were collected and anesthetized using diethyl ether, and the newly emerged adults were identified using taxonomic keys [7].

Data on Malaria
Data on Malaria were obtained from the daily bulletins published by the Directorate of Health Services, Government of Kerala on their website.

Results and Discussion

Breeding of *Anopheles stephensi*

During the study period 12 types of potential breeding habitats were surveyed and 6 of them were found to have *An. stephensi* breeding. The positive habitats were curing water (water collection in buildings under construction), cement tanks, wells, abandoned boats in fishing harbours, ponds and sump tanks. The positive habitats were detected from four urban, one suburban and two rural areas (Table 1). Among the breeding habitats only ponds were natural habitats. All others were man-made artificial habits. Except for Ayancheri, all other localities from where An. stephensi was collected were coastal areas. Ayancheri was 12 KM away from the coastline. Breeding was encountered in Kannur, Kozhikode and Malappuram districts.

Table 1: Habitat preference of *An. Stephensi*

Breeding habitats surveyed	Localities – Districts	Urban/ suburban/ rural	Breeding status of *An. stephensi*	
			Positive	Negative
Domestic containers				Yes
Building under construction	Ayanchery-Kozhikode	Rural **	Yes	
Cement tanks	Pakkayil-Kozhikode	Urban *	Yes	
Wells	Pakkayil-Kozhikode Kunjippally-Kozhikode	Urban * Suburban *	Yes	
Seepage water				Yes
Canals				Yes
Boats (abandoned in coastal areas)	Chombala, Koyilandy, Puthiyappa, Beypure_-Kozhikode Ponnani- Malappuram	Urban *	Yes	
Ponds	Pattiad-Kozhikode	Urban *	Yes	
Artificial containers				Yes
Over head tanks				Yes
Sump tanks	Muzhappilangad Kannur	Rural *	Yes	
Fresh water streams				Yes

* Coastal ** Non-coastal

Malaria Cases

From January 2020 to December 2021 a total of 134 cases were reported from the five districts (Table 2). Of these 112 were *Plasmodium vivax* (*Pv*), 15were *Plasmodium falciparum* (*Pf)* and 7 were mixed. While 3 cases were indigenously acquired, the remaining 131 cases were imported cases from other states. The indigenous cases were reported from Kasaragod, Kozhikode and Wayanad districts.

Table 2: Malaria cases reported from January 2020- December 2021

Districts	No. of Malaria cases reported	Type of infection			Indigenous	Imported
		Pv	*Pf*	Mix		
Kasaragod	31	26	3	2	1	30
Kannur	24	20	2	2	0	24
Kozhikode	28	23	4	1	1	27
Wayanad	9	9			1	8
Malappuram	42	34	6	2	0	42
Total	134	112	15	7	3	131

Discussion

Malaria is a tropical endemic disease. In India six species of *Anopheles* act as primary vectors. Among them *An. stephensi* is the most important one. Recently, it has been recognized as an invasive species and has been reported from Sri Lanka and Africa which were free of this species [8, 9]. In Kerala, before the eradication of Malaria in 1965 *Anopheles fluviatilis* was the major malaria vector [10]. However, when malaria re-emerged in the state in 1996 and 1998 (Thiruvanathapuram and Kasaragod respectively), the vector was *Anopheles stephensi* [6]. Hence, *Anopheles stephensi* driven malaria is a recent phenomenon in the state. The present study indicated a diverse assemblage of breeding habitats in the study areas. Though *An. stephensi* is primarily an urban vector, its presence was detected from a rural area also. Besides, breeding was encountered in a non-coastal locality. This shows the spread of the vector from coastal urban centres to the surrounding areas. Though the number of indigenous Malaria reported from the study were very few, the number of imported malaria cases is a matter of concern as they can provide source of infection. Additionally, the invasion of the species to rural areas is also worrisome from the point of view of malaria epidemiology. The confluence of vectors and parasites could trigger

new outbreaks in such areas. Hence, it is necessary to plan strategies to monitor the prevalence of this species and its control.

Conclusion

An. Stephensi is a primary vector of Malaria in India and it has started invading countries outside its primary distribution. In Kerala Malaria due to *An. stephensi* is a relatively new phenomenon. The present study detected the breeding *An. stephensi* in six types of habitats. The most significant observation in the study was the detection of the species in a non-coastal rural area, indicating the invasion of the species from urban centres. Considering the high vectorial capacity and invasiveness of the species, active surveillance for the detection and elimination of this species is warranted in the state.

References

1. National Vector Borne Disease Control Programme. Government of India. http://www.nvbdcp.gov.in
2. World Health Organisation. World Malaria Report WHO, Geneva, 2021, 263.
3. Gakhar SK, Sharma R, Sharma A. Population genetic structure of malaria vector Anopheles stephensi Liston (Diptera: Culicidae). Indian J Exp Biol,2013:51(4):273-279.
4. Nagpal BN, Srivastava A, Kalra NL, Subbarao SK. Spiracular indices on *Anopheles stephensi*: a taxonomic tool to identify ecological variants. Journal of Medical Entomology,2003:40(6):747-749.
5. Sweet WC, Rao B. Races of Anopheles stephensi Liston, 1901. Ind Med Gaz,1937:72(11):665-674.
6. Raveendran S, Rakesh PS, Dev S, Prasannakumar P, Vijayakumar N. Investigation of an outbreak of malaria in a non- endemic coastal area, Kerala, Southern India. Int J Community Med Public Health,2016:3(12):3581-3583.
7. Das BP, Rajagopal R, Akiyamma J. Pictorial key to the species of Indian Anopheline mosquitoes. Journal of pure and applied Zoology,1990:2(3):131-162.
8. Gayan Dharmasiri AG, Perera AY, Harishchandra J, Herath H, Aravindan K, Jayasoorya HTR *et al*. First record of Anopheles stephensi in Sri Lanka: a potential challenge for prevention of malaria reintroduction. Malar J,2017:6(1):326.
9. Sinkaa ME, Pirononb S, Masseyc NC, Longbottoms J, Hemingway J, Moyes CL *et al*. A new malaria vector in Africa: Predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. PNAS,2020:117:(40):1-9.
10. Covell G, Singh H. Malaria in the Wayanad, South India. Journal of Malaria Institute of India,1939:2:341-376.