STUDIES ON *AEDES CHRYSOLINEATUS* (DIPTERA: CULICIDAE) IN NORTH KERALA WITH A FOCUS ON ITS GEOGRAPHICAL DISTRIBUTION, HABITAT DIVERSITY AND CO-BREEDING WITH OTHER SPECIES WITH SPECIAL EMPHASIS ON THE VECTOR SPECIES *AEDES ALBOPICTUS*

Thesis Submitted to
the University of Calicut for the
Award of the Degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

(Under the Faculty of Science)

Submitted by

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I also certify that the corrections/suggestions recommended by adjudicators have been incorporated into the thesis.

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Co-guide

DECLARATION

I do hereby declare that this thesis entitled "**STUDIES ON *AEDES CHRYSOLINEATUS* (DIPTERA: CULICIDAE) IN NORTH KERALA WITH A FOCUS ON ITS GEOGRAPHICAL DISTRIBUTION, HABITAT DIVERSITY AND CO-BREEDING WITH OTHER SPECIES WITH SPECIAL EMPHASIS ON THE VECTOR SPECIES *AEDES ALBOPICTUS"*** submitted to the University of Calicut in partial fulfillment for the Doctoral degree in Zoology is a bonafide research work done by me under the supervision and guidance of **Dr. P. K. SUMODAN** Associate professor (Rtd), and **Dr. THEJASS. P (*Co-guide*)** Associate Professor, Department of Zoology, Govt. College Madappally and no part of the thesis has been presented by me for the award of any other degree, diploma or similar title. The contents of the thesis are undergone plagiarism check using 'iThenticate' software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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Dedicated to

The World's best Parents

&

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Whom I love and respect like God.....

ABSTRACT

The study investigates the geographical distribution and habitat diversity of *Aedes chrysolineatus*, and co-breeding with other species, especially with *Aedes albopictu*s in North Kerala districts, specifically in Kasaragod, Kannur, Wayanad, Kozhikode, and Malappuram. The research involved collecting mosquitoes from various habitats including, tree holes, latex collecting cups, areca leaf sheaths, rock holes, domestic containers, and plastic containers etc. A total of 4505 positive breeding habitats were identified all over the study areas. 8.6% of the breeding sites with *Aedes chrysolineatus* positivity were observed along with 34 species belonging to five genera. The species was most widespread in all panchayats of Wayanad district, followed by Kannur (5.6%), Kasaragod (5.2%), Kozhikode and Malappuram (4.2%).

The study found that *Aedes chrysolineatus* breeds most effectively in Latex collecting cups (20.05%), followed by areca leaf sheath (18.7%), tree holes (15.4%), plastic containers (10.5%), and plastic sheets/covers (10.2%). Wayanad district had the highest number of breeding habitats for *Aedes chrysolineatus* (40.5%), followed by Kozhikode (1.13%), Malappuram (0.83%), Kannur (0.73%), and Kasaragod (0.56%). The species' distribution varied within the 20-1200m elevation range, predominantly high in the high altitude (90.1%), low in upland (0.57%), and no distribution was found within (0-20 m) range of elevation. Month-wise, the density was the highest in July and lowest in May. The species' year-wise distribution was highest in 2019, followed by 2020, 2018, 2021, and 2017.

The study found that *Aedes chrysolineatus* was the dominant species in 85.06% of breeding habitats with *Aedes albopictus*. *Aedes chrysolineatus* was more competent in transforming resources into biomass, demonstrating a significant difference in resource utilization. Laboratory studies showed that *Aedes chrysolineatus* had a higher resource utilization capacity than *Aedes albopictus*, even at low (0.95 mg/l, 28°C) food concentrations. On the other hand, *Aedes albopictus* emerged faster and weighed more only at high (2.83 mg/l, 28°C) food concentrations. The study confirmed the competitiveness of *Aedes chrysolineatus* by assessing the relative crowding co-efficient (RCC), which was above 1.0 in low and medium quantities of food. *Aedes albopictus* plays a very important role in the epidemiology of *Aedes*-borne diseases in the state. This study revealed the

competitiveness of *Aedes chrysolinestus* over *Ae. albopictus* and its potential candidate for suppressing the density of *Ae. albopictus.*

Keywords: Aedes chrysolinestus, Habitat Diversity, co-breeding, *Aedes albopictus, Competetion study.*

സംഗ്രഹം

വടക്കൻ കേരള ജില്ലകളിലെ പ്രത്യേകിച്ച് കാസർഗോഡ്, കണ്ണൂർ, വയനാട്, കോഴിക്കോട്, മലപ്പുറം എന്നിവിടങ്ങളിലെ ഈഡിസ് ക്രൈസോലിനിയേറ്റസിന്റെ ഭൂമിശാസ്ത്രപരമായ വിതരണവും ആവാസ വൈവിധ്യവും മറ്റ് സ്പീഷീസുകളുമായുള്ള, പ്രത്യേകിച്ച് ഈഡിസ് ആൽബോപിക്റ്റസുമായി സഹ-പ്രജനനവും പഠനം അന്വേഷിക്കുന്നു. മരപ്പൊത്തുകൾ, റബർ പാൽ ശേഖരിക്കുന്ന പാത്രങ്ങൾ , കവുങ്ങിൻ പാള, പാറക്കുഴികൾ, ഗാർഹിക പാത്രങ്ങൾ, പ്ലാസ്റ്റിക് പാത്രങ്ങൾ തുടങ്ങി വിവിധ ആവാസവ്യവസ്ഥകളിൽ നിന്ന് കൊതുകുകളെ ശേഖരിക്കുകയുണ്ടായി. പഠന മേഖലകളിലുടനീളം മൊത്തം 4505 കൊതുകുകൾ പ്രജനനം ചെയ്ത ആവാസവ്യവസ്ഥകൾ കണ്ടെത്തി. ഈഡിസ് ക്രൈസോലിനിയേറ്റസ് കണ്ടെത്തിയ പ്രജനന കേന്ദ്രങ്ങളിൽ 8.6 ശതമാനത്തിൽ നിന്ന്, അഞ്ച് ജനസ്സിൽപ്പെട്ട 34 കൊതുകിനങ്ങളെ ശേഖരിച്ചു. വയനാട് ജില്ലയിലെ എല്ലാ പഞ്ചായത്തുകളിലും ഈ ഇനം വ്യാപകമായിരുന്നു, തുടർന്ന് കണ്ണൂർ (5.6%), കാസർഗോഡ് (5.2%), കോഴിക്കോട്, മലപ്പുറം (4.2%) ജില്ലകളിലും.

റബർ പാൽ ശേഖരിക്കുന്ന പാത്രങ്ങളിൽ (20.05%) ഈഡിസ് ക്രൈസോലിനിയേറ്റസ് ഏറ്റവും ഫലപ്രദമായി പ്രജനനം നടത്തുന്നതായി പഠനം കണ്ടെത്തി, തുടർന്ന് കവുങ്ങിൻ പാള, (18.7%) മരപ്പൊത്തുകൾ (15.4%) പ്ലാസ്റ്റിക് പാത്രങ്ങൾ (10.5%), പ്ലാസ്റ്റിക് ഷീറ്റുകൾ/കവറുകൾ (10.2%) തുടങ്ങിയവയും. വയനാട് ജില്ലയിലാണ് ഏറ്റവും കൂടുതൽ ഈഡിസ് ക്രൈസോലിനിയേറ്റസ് (40.5%)കണ്ടെത്തിയത്. അതിന് ശേഷം കോഴിക്കോട് (1.13%), മലപ്പുറം (0.83%), കണ്ണൂർ (0.73%), കാസർഗോഡ് (0.56%) ജില്ലകളും. 20-1200 മീറ്റർ ഉയരപരിധിക്കുള്ളിൽ ഈ ഇനത്തിൻറെ വിതരണം വ്യത്യാസപ്പെട്ടിരിക്കുന്നതായി കണ്ടെത്തി. മാസം തിരിച്ചുള്ള സാന്ദ്രത പരിശോധിച്ചപ്പോൾ ജൂലൈയിൽ ഏറ്റവും ഉയർന്നതും മെയ് മാസത്തിൽ ഏറ്റവും താഴ്ന്നതുമായിരുന്നു. വർഷം തിരിച്ചുള്ള ഈ ഇനത്തിൻറെ വിതരണം 2019 ലാണ് ഏറ്റവും ഉയർന്നത്. തുടർന്ന് 2020, 2018, 2021, 2017ലും രേഖപ്പെടുത്തി.

ഈഡിസ് ആൽബോപിക്കസിന്റെ കൂടെയുള്ള 85.06% പ്രജനന ആവാസവ്യവസ്ഥയിൽ ഈഡിസ് ക്രൈസോലിനിയേറ്റസ് പ്രബലമായ ഇനമാണെന്ന് പഠനം കണ്ടെത്തി. വിഭവങ്ങളെ ജൈവവസ്തുക്കളായി പരിവർത്തനം ചെയ്യുന്നതിൽ ഈഡിസ് ക്രൈസോലിനിയേറ്റസ് കൂടുതൽ കഴിവുള്ളതാണെന്നും ഇത് വിഭവ ഉപയോഗത്തിൽ കാര്യമായ വ്യത്യാസം പ്രകടമാക്കുന്നുവെന്നും പഠനം കണ്ടെത്തി. കുറഞ്ഞ (0.95 mg/l, 28°C) ഭക്ഷണ സാന്ദ്രതയിൽ പോലും ഈഡിസ് ആൽബോപിക്കസിനേക്കാൾ ഉയർന്ന വിഭവ വിനിയോഗ ശേഷി ഈഡിസ് ക്രൈസോലിനിയേറ്റസിന് ഉണ്ടെന്ന് ലബോറട്ടറി പഠനങ്ങൾ തെളിയിച്ചു. ഈഡിസ് ആൽബോപിക്കസ് വേഗത്തിൽ വിരിഞ്ഞു വരികയും, ഉയർന്ന (2.83 mg/l, 28°C) ഭക്ഷണ സാന്ദ്രതയിൽ മാത്രം ഭാരം കൂടുകയും ചെയ്തു. കുറഞ്ഞതും ഇടത്തരവുമായ അളവിൽ 1.0 ന് മുകളിലുള്ള ആപേക്ഷിക ക്രൗഡിംഗ് കോ-എഫിഷ്യന്റ് (ആർസിസി) വിലയിരുത്തിക്കൊണ്ട് ഈഡിസ് ക്രൈസോലിനിയേറ്റസിന്റെ മത്സരശേഷി പഠനം സ്ഥിരീകരിച്ചു. സംസ്ഥാനത്തെ ഈഡിസ് പരത്തുന്ന രോഗങ്ങളുടെ സംക്രമിക രോഗശാസ്ത്രത്തിൽ വളരെ പ്രധാനപ്പെട്ട ഒരു ഇനമാണ് ഈഡിസ് ആൽബോപിക്കസ്. ഈഡിസ് ആൽബോപിക്കസിന്റെ സാന്ദ്രത നിയന്ത്രിക്കുന്നതിന് ഉപയോഗിക്കാവുന്ന ഒരു കൊതുക് സ്പീഷീസാണ് ഈഡിസ് ക്രൈസോലിനിയേറ്റസ് എന്ന കണ്ടെത്തലാണ് ഈ പഠനത്തിന്റെ മൗലികമായ സംഭാവന.

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LIST OF ABBREVIATIONS OR SYMBOLS

cm	*Centimeter*
mm	*Millimeter*
m	*Meter*
No.	Number
°C	Degree centigrade
g/l	Gram per liter
pH	Potential of Hydrogen
+	Positive
-	Negative
%	Percent
<	Less than
>	Greater than
=	Equals
*	Asterisk
,	Comma
-	Hyphen
●	Dot or full stop
(	Bullet
)	Open parenthesis
	Close parenthesis
Ae.	*Aedes*
Cx.	*Culex*
Ar.	*Armigeres*
Hz.	*Heizmania*
Tx.	*Toxorhynchites*
Mn.	*Mansonia*

WHO	World Health Organization
JE	Japanese Encephalitis
RA	Relative Abundance
C	Distribution
CHIKV	Chikungunya Virus
C	Cranium or head capsule
CL	Clavicle
PT	Pecten
4-X	Ventral brush
T	Thorax
7-T	7^{th} Thoracic hair
6-M	6^{th} Meta thoracic
I	1
II	2
III	3
IV	4
V	5
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CHAPTER I
INTRODUCTION

Mosquitoes, which belong to the insect order Diptera, and family Culicidae, are as ancient as the dinosaurs. Though the oldest fossil mosquito, *Burmaculex antiquus*, obtained from a Burmese amber, is estimated to be 90-100 million years old, it is argued that the family Culicidae originated approximately 187 million years ago (Borkent, 1993; Borkent & Grimaldi, 2004). Hence, it can be safely assumed that the interaction between Man and Mosquitoes started with the birth of the first *Homo sapiens*. This interaction has been so violent that from the available data it has been extrapolated that half of the human population ever lived on earth died due to one or the other mosquito-borne diseases (Winegard, 2019).

1.1 *Mosquito-borne Diseases in the World*

Mosquito-borne diseases cause significant mortality and morbidity in the world. From the point of view of mortality caused, the leading mosquito-borne diseases are Malaria, Dengue, Yellow fever, Chikungunya, Zika, Japanese Encephalitis (JE), and West Nile Virus. Besides these, there are several other arboviral diseases and also the debilitating Lymphatic Filariasis. According to World Malaria Report 2022, there were 247 million malaria cases and 619000 deaths in the world in 2021(WHO, 2022). Besides Malaria, Dengue is also a major disease burden with an estimated 2.5 billion people at risk of this disease. Approximately 500000 people suffer from severe dengue every year with 2.5% mortality. Yellow Fever causes 200000 cases and 30000 deaths annually. JE is responsible for 50000 cases and 10000 deaths (WHO, 2014). It is predicted that by 2050, half of the world's population will be at risk of various arboviral diseases (Kraemer *et al.*, 2019). These alarming statistics make mosquitoes a subject of various kinds of investigations with the hope of toning down their menacing effects.

1.2 *Mosquito diversity*

The family Culicidae has two subfamilies viz., Anophelinae and Culicinae. While Anophelinae has three genera, Culicinae has 38. Under these 41 genera, approximately 3570 species have been described so far. Among them, the important disease vectors belong to the genera *Anopheles* (Malaria), *Aedes* (Dengue, Yellow Fever, Chikungunya, and Zika), *Culex* (Lymphatic Filariasis, Japanese Encephalitis, and West Nile Virus), and *Mansonia* (Lymphatic Filariasis) (Wilkerson *et al.*,2021).

1.3 *Mosquito and diseases in Kerala*

Being in the tropical region of the world, Kerala provides ideal conditions for the survival and proliferation of mosquitoes throughout the year. Studies on mosquitoes in the state were initiated by British workers at the beginning of the 20th century. The pioneering studies on mosquitoes in Kerala can be attributed to (Theobald, 1901) and (Giles, 1901). The former described three species from Kollam, viz., *Ficalbia minima*, *Mansonia annulifera* and *Mansonia uniformis*. The latter described *Cx. bitaeniorhynchus* and *Cx. tritaeniorhynchus* from Travancore (exact locality not mentioned). The mosquito fauna of the state is amply represented in the two volumes on the mosquito fauna of British India compiled by (Christophers, 1933) and (Barraud, 1934). Christopher reported 16 Anopheline species and Barraud 41 species under Culicinae and Toxorhynchitinae. (Iyengar, 1938) reported 72 species of mosquitoes from the state while studying the epidemiology of Filariasis in Travancore. In the post-independence period, (Tewari & Hiriyan, 1992) described two new species of *Aedes* from the state, viz., *Ae. agastyai* and *Ae. rubenae*. Hiriyan *et al.*,(2003) surveyed a Japanese encephalitis endemic area in Kerala and reported 21 species of mosquitoes. Subsequently, Arunachalam *et al*., (2004) reported 18 species of mosquitoes during a study to determine the vectors of Japanese encephalitis. Rajavel *et al.*, (2006) reported 17 species of mosquitoes from the Mangrove forests of Kannur in North Kerala. In 2009, Tyagi *et al.*, described a new species viz., *Anopheles pseudosundaicus* from Kollam (Tyagi *et al.*, 2009). Sumodan, (2012) reported the breeding of 12 species of mosquitoes in latex collecting containers in the rubber plantations of Kerala. As

many as 135 species of mosquitoes under 17 genera have been recorded from Kerala (Sumodan, 2014).

Kerala has a very long history of mosquito-borne diseases. The state had been haunted by malaria in its highlands and lymphatic filariasis in the coastal belt from prehistoric times. The prevalence of sickle cell anemia among the tribal population of Wayanad and Attappadi is solid proof of the antiquity of Malaria in the state (Feroze & Aravindan, 2001; Kaur *et al.,* 1997). In the pre-independent era, Malaria was a major disease burden causing significant rates of morbidity and mortality in the state (Covell and Singh, 1939). The disease was almost eradicated by the massive application of DDT under the WHO-UNICEF cosponsored pilot project during 1949-51 (Mara, 1949-51). However, during the post-eradication era, sporadic outbreaks of the disease have been reported from various parts of the state. Sumodan, (2002) reported the prevalence of indigenous and imported cases of Malaria from the Wayanad district and convincingly argued the importance of imported cases. Documentary evidence for the presence of Lymphatic Filariasis in Kerala goes back to 1709 when Clarke called elephantiasis legs in Cochin Malabar legs (Raghavan, 1957). Currently, the state is endemic to bancroftian and brugian forms of lymphatic filariasis and ranks second in India in terms of endemicity. 15.7% of the total cases are reported from the state (Agrawal & Sashindran, 2006). The first outbreak of dengue in Kerala was reported from Kottayam district in 1997 with 14 cases and 4 deaths, which was followed by a bigger outbreak with 67 cases and 13 deaths in 1998 in the same district. However, antibodies of dengue viruses were detected from human sera collected from various districts in the state as early as 1973 (Banerjee & Desai, 1973). In 2001, epidemic dengue resurged mainly in Kottayam, Idukki, and Ernakulam districts reporting 70 cases, followed by 219 cases in 2002 with some deaths. The year 2003 witnessed the spread of dengue throughout the state with 3546 confirmed cases and 68 deaths (Tyagi *et al.,* 2006). Since then, the state has been experiencing dengue outbreaks annually with varying degrees of severity. Das *et al.,* (2004) detected dengue virus in *Aedes albopictus* from specimens collected near Calicut Airport in Kerala, thus confirming the importance of the species in dengue transmission in the state. In 1996, there was an outbreak of

Japanese encephalitis in Kottayam and Alappuzha districts (John, 2006). Currently, the southern districts of Thiruvananthapuram, Kollam, Alappuzha, Kottayam, Ernakulam, and Thrissur are endemic to this disease. There have been a few Japanese encephalitis cases in North Kerala in recent years. Kerala state had the first outbreak of Chikungunya during June-July 2006 along the coastal areas of Alleppey, Quilon, and Thiruvananthapuram districts and again during May-August 2007 in Pathanamthitta, Kottayam, and Idukki districts (Kannan *et al.*, 2009; Manju & Sushamabai, 2009). Kumar *et al.*, (2008) reported the A226V mutation in the glycoprotein envelope 1 (E1) gene of the virus among isolates collected from the three worst-affected districts of the state during this outbreak. This mutation had already been suggested to be directly responsible for a significant increase in CHIKV infectivity in *Aedes albopictus*. Since then, the disease has been spreading its tentacles throughout the state. The first report of the West Nile Virus outbreak was in 2011 with 33 cases Anukumar *et al.*, (2014). Finally, Zika appeared in the state in 2021 as an outbreak in the capital city of Thiruvananthapuram (Lekshmi *et al.*, 2021).

1.4 *Aedes albopictus in Kerala*

With the prevalence of Dengue, Chikungunya, and Zika, *Aedes* species can be considered as the most important vector mosquitoes in the state. The state has three potential dengue vectors viz., *Aedes aegypti*, *Aedes albopictus*, and *Aedes vittatus*. The first two species are known vectors of Dengue, Chikungunya, and Zika. Among them, *Aedes albopictus* is distributed throughout the state, whereas *Aedes aegypti* is an urban species. *Aedes albopictus* has been incriminated as the vectors of Dengue and Chikungunya in Kerala. However, none of these viruses have been isolated from *Aedes aegypti* so far (Dhanda *et al.*, 1997; Niyas *et al.*, 2010; Thenmozhi *et al.*, 2007). This renders *Aedes albopictus* as a very important species in the epidemiology of *Aedes*-borne diseases in the state.

1.5 *Co-breeding of Aedes albopictus with Aedes chrysolineatus*

In a study conducted in the rubber plantations of Kozhikode, Kannur, and Wayanad districts of North Kerala, *Aedes albopictus* was found breeding along with

Aedes chrysolineatus, a non-vector species, in rainwater-filled latex cups. In the Wayanad district, both species were found breeding almost in the same number of cups. However, *Aedes chrysolineatus* larval density was much higher than that of *Aedes albopictus.* Co-breeding of these two species was observed in 277 cups. It was interesting to note that 74% of the adults which emerged from the cups in which they bred together, were composed of *Aedes chrysolineatus,* which could be due to its upper hand in the competition for resources (Sumodan, 2010). *Aedes chrysolineatus* was described as *Howardina chrysolineata* by Theobald in 1907 from Pundaluoya, Sri Lanka. The distinguishing character of the species is the golden yellow lines on the scutum. Scutum is deep brown or black, marked with sharply defined narrow lines of golden scales. There is a median line extending from the front back to scutellum, forking in front of antescutellar bare space, a pair of sub median lines which nearly meet a pair of lines curving from sides, and continued to lateral lobes of scutellum; another line of golden scales from wing-root, continued forwards a short distance. Mid-lobe of scutellum with narrow golden scales in the centre, flat dark scales on each side, lateral lobes with narrower dark scales. The species has been recorded from India (Kerala, Karnataka, Tamil Nadu, Eastern Himalayas), Bangladesh, Cambodia, Indonesia, Malaysia, Nepal, Sri Lanka, Thailand, and Vietnam (Barraud, 1934). Its vector status is unknown. Some species of the group were collected in human bait collection. However, its biting preference is yet to be understood (Knight, 1968).

As its popular name (Asian tiger) indicates, *Aedes albopictus* was originally an Asian species. However, through the used tyre trade, it has spread to the rest of the world and is currently a cosmopolitan species, recorded from as many as 132 countries. On the other hand, the distribution of *Aedes chrysolineatus* is limited to Asian countries. Currently, it is distributed in India, Sri Lanka, Japan, Malaya, Thailand, Indochina, Sumatra, and Java.

1.6 *The emergence of a hypothesis*

There have been several reports on the effect of co-breeding of mosquito species on the production, sex ratio, body size, and many other attributes of

participating species (Kweka *et al.,* 2012). The effects of density and species ratio on larval growth and mortality were documented in the laboratory studies of *Ae. aegypti* and *Ae. albopictus*. Furthermore, the same study recorded the competitive superiority of *Ae. aegypti* over *Ae. albopictus*. It was observed that larval mortality was higher in *Ae. albopictus*, when reared together (Moore & Fisher, 1969). In another field study, the competitive advantage of *Ae. albopictus* over *Ae. japonicus* was shown (Armistead *et al.,* 2008). The interaction between *Ae. albopictus* and *Ae. polynesiensis* revealed that *Ae. albopictus* develops faster than the opposite species irrespective of larval density, food supply, and competitive interaction and exhibits competitive superiority over *Ae. polynesiensis*, the vector of *Wuchereria bancrofti* (Lowrie, 1973). In an extreme case, it was reported from Australia that the introduction of the native species *Aedes notoscriptus* resulted in the elimination of the imported species *Aedes aegypti* (Russell, 1986). Considering these encouraging reports, it was hypothesized that co-breeding of *Aedes chrysolineatus* could have a negative effect on the productivity of *Aedes albopictus* in such habitats and could be used as a control strategy in the future. To support this hypothesis extensive field and laboratory investigations are required, which could be done in two stages. The first stage consists of collecting baseline data on the distribution and habitat diversity of *Aedes chrysolineatus*, the extent of co-breeding with *Aedes albopictus*, and its possible effect on the productivity of *Aedes albopictus.* The second stage consists of the study of the biting behaviour of *Aedes chrysolineatus*, its vector status, and laboratory and field trials on the efficacy of the introduction of *Aedes chrysolineatus* on the production of *Aedes albopictus*. In the present study, it has been decided to carry out the first stage of the investigation in five North Kerala districts, viz., Malappuram, Kozhikode, Wayanad, and Kasaragod with the following objectives.

OBJECTIVES

1. To study the geographical distribution and habitat diversity of *Aedes chrysolineatus* in North Kerala (Kasaragod, Kannur, Kozhikode, Wayanad and Malappuram) districts.

2. To investigate the co-breeding status of *Aedes chrysolineatus* with other mosquito species.

3. To investigate the possible effects of *Aedes chrysolineatus* breeding on the breeding of vector species *Aedes albopictus*.

The explanation of the findings of the present study has also carried out in four Chapters as follows:

Chapter IV **:** Morphology and Taxonomy of *Aedes chrysolineatus*

Chapter V **:** Geographical distribution and Habitat diversity of *Aedes chrysolineatus*

Chapter VI **:** Co-breeding of *Aedes chrysolineatus* with other mosquito species

Chapter VII **:** Effects of *Aedes chrysolineatus* breeding on the breeding of *Aedes albopictus*

CHAPTER II
REVIEW OF LITERATURE

At the 19th century, interest in mosquitoes grew due to their potential hazards. Grassi, (1899) initiated the taxonomy of Indian *Anopheles*, Gilles, (1899) investigated Indian Culicidae, and Theobald,(1901) published "The Monograph of the World Culicidae" a family that included all mosquitoes known to him in 149 genera.

A 1911 monograph by James & Liston significantly contributed to the study of Indian Anopheles Edward's significant contributions to the classification system were evident in a series of papers published between 1911 and 1932. Edwards (1923) from India identified five new species of the genus *Finlaya*, Barraud, (1923a) described five new species of *Stegomiya* and two new species of *Culex* from Assam. Barraud (1924) also described four new species of subgenus *Finlaya* from the western Himalayas and one new species of subgenus *Lophoceratomya* from the same region. Barraud (1931) described one species of subgenus *Stegomyia* from Bihar and eight new species of Indian Culicine mosquitoes, described eight new species of the subgenus *Aedes* and two new species of the genus *Stegomyia* from India.

All-encompassing work on the classification and systematics of the Culicidae was published by Edwards (1932) in Genera Insectorum. Edwards established the family Culicidae, which consisted of 1400 species belonging to 39 genera. Early in the 20th century Christophers (1933) published Monographs on the *Anophelines* of British India. Enough details about the names and systematics of the species, their breeding grounds, adult bionomics, distribution, and relationships to diseases were included in this volume. Christopher recognized and described four subgenera and then knew 43 species of mosquitoes under the genus *Anopheles*.

Splendid work on the *Culicines* of British India was done by (Barraud, 1934). During that period, Anopheles was prioritized above Culicinae mosquitoes.

'Revision of *Culicine* mosquitoes of India' was delivered in the Indian Journal of Medical Research. Barraud (1923b) recognized 16 genera and described 245 species of mosquitoes under the subfamily Culicinae. The genus *Aedes* contains 110 species and 12 genera. As an exhaustive monograph on Indian Culicinae, Barraud's "Fauna of British India" remained relevant. Both the publications of Christophers, (1933) and Barraud, (1934) marked a landmark in the history of mosquito studies in the subcontinent. An era of vigorous taxonomic research on the Culicidae came to an end in 1934, making mosquitoes one of the most well-known insect groups in the region. Since 1934, not many taxonomic studies of Indian *Culicidae* have been advanced. Most of the studies have mainly focused on *Anopheles* mosquitoes due to their high impact in Malaria in the country.

The family Culicidae encompasses three subfamilies *Anophelinae*, *Culicinae*, and *Toxorhychitinae* (Service, 2012). Representatives of *Anophelinae* and Culicidae are medically significant. The medically significant genera *Culex, Aedes, Mansonia, Anopheles, Haemagogus, and Sabethus* are coming under this. *Aedes* is the majort tribe of mosquitoes with 1256 species of ten genera, most significant according to community health concerns belongs to the tribe *Aedini*, the largest tribe of *Culicidae* with 1240 recorded species. Genus *Aedes* is subdivided into several subgenera comprised of over 900 species (Belkin, 1962). Various species of *Aedes* transmit arbovirus that have caused magnitudes of outbreaks across the globe. *Stegomyia* is the most significant subgenus in the medical angle followed by subgenus *Finlaya*. Barraud, (1923b) described new species added more information on the genera *Stegomyia* Theobald and *Finlaya* Theobald. Edward (1932) identified the Chrysolineata group as an oriental, Australasian group with distinctive features such as basal white bands on the hind tarsi, unlined femora, and scutum with longitudinal white or golden scales (Knight, 1947).

Knight, (1968) published first paper in a series of revisionary paper on *Aedes (Finlaya)* mosquitoes of Southeast Asia, categorization based on several structural and biological characters. Knight and Marks (1952) put forward the classification of group D into eight subgroups of which three were in Southeast Asia, specifically

Subgroup I, Chrysolineatus; Subgroup II, Aureostiatus; and Subgroup IV, Togoi. Subgroup I is distributed across oriental region and present in Palaearctic region. The Subgroup I, presently include *chrysolineatus (Theobald), formosensis Yamada, harveyi (Barraud), japonicus japonicus (Theobald), japonicus shintienensis Tsai* and *Lien, jugraensis (Leicester), koreicus (Edwards), nigrorhynchus Brug, rizali (Banks), saxicola Edwards,* and *sherki* Knight. Except *Koreicus,* rest of the species are appear in Southeast Asia. Larvae of *Chrysolineatus* Subgroups are mainly found in Container habitats; rock holes, tree holes, leaf axils and occasionally found in artificial habitat types, with some species being taken in individual catches. *Finlaya Kochi* (Theobald) was explained based on morphology, female and male genitalia, pupae and fourth instar larvae (Reinert & Harbach, 2005). Based on a comparative morphological analysis of the female genitalia, *Finlaya* was divided into seven species assemblages, one of which is the *Chrysolineatus* Assemblage (Reinert, 2002). The *Chrysolineatus* Assemblage differs from other assemblages by the presence of the following characters of the female genitalia: characteristic round shape and absence of scales on cercus; tergum IX comprised of 2 moderately pigmented lateral plates separated by lighter pigmented area; posterior margin of sternum VIII with minute to small median emargination, with numerous short, slightly curved setae (Natarajan *et al.,* 2016). Reinert *et al.,* (2008) assigned the *Chrysolineatus* subgroup to the genus *Hulecoeteomyia*, which was later downgraded to the status of the subgenus (Wilkerson, 2015).

2.1 *Geographical Distribution and Habitat diversity studies*

Mosquitos are found worldwide, primarily in tropical and temperate regions, with diverse species found in various breeding sources, except in high salt water concentrations (Rueda, 2008). Records of Carpenter & Lacasse, (1954), explained various collection methods of mosquitos and preparation for study, keys to genera and species, description of larvae, male and female, and distribution and bionomics status of each species. Provided insight into Global Mosquito Biogeography from Country Species Records (Foley *et al.,* 2007). Review on natural habitats of *Ae. aegypti* in the Caribbean, recorded twelve natural habitats: tree holes, leaf axils,

bromeliads, rock holes, bamboo internodes, papaya stumps, coconut shells, calabashes, ground pools, crab holes, conch shells, and coral rock holes. *Ae. aegypti* mainly used the habitat of calabash fruit and tree holes each with 32.1% and 25.8 % respectively were documented (Chadee *et al.,* 1998). The survey of (Kumar *et al.,* 2020), recorded various mosquito species habitats such as cesspits, cesspools, drainage, septic tank, natural or artificial containers, ponds, rice field, rice pots etc. of which cesspits, cesspools, drainage, septic tank and rice field were identified as the significant breeding sites of mosquitos. The diversity was recorded found high from August to December and January to May.

Physicochemical elements of breeding habitat as the significant factor which influence the breeding, and distribution of mosquito species. The larval habitat diversity studies of (Amini *et al.,* 2020) recorded twenty two natural breeding grounds. The distribution of mosquito species potentially affected by diverse environmental influences as the physicochemical factors in the larval habitats, interspecific association and climate (Okogun *et al.,* 2003). Mosquito micro habitat survey of (Amusan & Ogbogu, 2020) found that drainage channels represents the greatest larval habitat with abundance were found in the dry season. Amerasinghe *et al.,* (2001) conducted larval survey in the irrigation tanks, recorded 36.9% of *Aedes*, 35.2% of *Culex*, and 27.9% of *Anopheles* species.

In the studies of (Mbanzulu *et al.,* 2022) It was found that physiochemical parameters in the breeding habitats influence growth and breeding of *Aedes* larvae, and water temperature, dissolved oxygen, turbidity and salinity in breeding waters of *Aedes aegypti* and *Aedes albopictus* nearly parallel. *Aedes* species with turbidity, dissolved oxygen and salinity in larval habitat was recorded 19.15, 1, and 0.115 respectively whereas in *Culex* with 55, 0.8, and 0.29 respectively.

The distribution and occurrence of *Aedes* species was found affected by dissolved oxygen and salinity, and the species proliferation was indirectly supported by temperature, pH, and turbidity. Breeding habitat of *Ae. aegypti* and *Ae. albopictus* were short term containers. *Ae. albopictus* species were found in various habitats such as ponds, margins of streams, tree holes and dried up wells, whereas *Ae.*

aegypti habitats were found in rain water trapped fallen leaves. *Ae. albopictus* displayed better distribution and abundance than *Ae. aegypti* (Ranasinghe *et al.*, 2020). In *Aedes albopictus* the presence and profusion of larvae in breeding sites was determined by the pH and type of habitat, whereas in *Ae. aegypti* the profusion of the species was connected with pH and salinity, while the larval presence were correlated with habitat type and dissolved oxygen (Medeiros-Sousa *et al.,* 2020). No significant changes were discovered in the choice of pH, turbidity, alkalinity, TDS, etc., and the selection of breeding environment was connected to the presence of temperature and chloride particles in diverse species (Amini *et al.,* 2020).

Findings of (Pemola & Jauhari, 2007) shows that greater number of species were found in rock holes and streams with 18 number each and trailed by 16 species harboring seepage pools, whereas smallest number of species were identified from shallow pits. According to the availability of sunlit and quadrats, profusion of larvae in the mesocosms changes were documented by (Roy *et al.*, 2019), in the studies of distribution of larvae in the rice fields ecosystem. Larval association was found greater in habitat with floating vegetation, denoting that habitat heterogeneity have an effect on the distribution of larvae in the accessible habitats. Suganthi *et al.* (2014) found optimal mosquito breeding in outdoor containers filled with rain fall and water, including grinding stones, mud pots, cement cisterns, rock holes, tree holes, and metal vessels. A meaningful connection was found between larval profusion in the breeding habitat with volume of water filled in containers, in contrast no meaningful connection was found between altitude of the site and larval species diversity as stated by (Singh *et al.,* 2019). The dominant breeder found in the artificial container was *Ochlerotatus japonicas,* which occupied greater number in discarded tanks.

Bond *et al.*, (2005) found that, fish in breeding habitats increase larval development period, decreasing resource utilization and producing small adults, while algal cover doesn't affect resource utilization but affects adult wing length. Environmental factors significantly influence mosquito density, with temperature and relative humidity affecting maturation time and species survival (Selvan *et al.*,

2020). The availability of resting sites and breeding sites promotes the distribution of adult mosquitoes (Selven *et al.,* 2015). The global distribution of *Ae. aegypti* and *Ae. albopictus* is influenced by climate fluctuations, with favorable monthly rainfall and temperature ranges of 50-200mm and 10-30°C respectively (Laporta *et al.,* 2023). Mosquito faunal studies of (Attaullah *et al.,* 2023) identified specimens from the habitats of freshwater bodies, animal sheds, rice fields, indoors, drains, and sewage water, showing significant differences in the diversity of mosquitoes. The studies of Lubna *et al.,* (2023) documented species diversity patterns in various container breeding habitats in Peshawar, including permanent, temporary, and natural environments. Human activities in China have led to the growth of mosquito populations, as evidenced by mapping of 339 mosquito species and 35 arboviruses (Wang *et al.,* 2022). This study recorded that *Cx. triteaniorhynchus* species harbors the most arboviruses.

The *Aedes* mosquito species are found in various habitats around human dwellings in Yaoundé, as per documented evidence of Djiappi-Tchamen *et al.,* (2022) in their studies. The study found that urban and peri-urban areas have higher *Ae. albopictus* diversity, with most breeding sites being discarded tires, while rural areas have a dominant *Ae. aegypti* diversity. *Aedes aegypti*, and *Ae. albopictus* mosquito density was induced mainly by artificial and natural breeding habitats, environmental, and climatic factors (Palaniyandi *et al.,* 2020). The altitudinal distribution of 34 species from five genera, including *Aedes, Anopheles, Culex, Armigeres,* and *Uranoteania,* was observed at elevations ranging from 300-2000m (Devi & Jauhari, 2004).

Muja-Bajraktari *et al.,* (2019) recorded the significant distribution and variations in mosquito species between the two central plains of Kosovo. *et al.,* (2018) found that environmental conditions significantly influence the spatial distribution of exportable insects in South Korea. The study by Ferede *et al.,* (2018) found that discarded tires are the most preferred breeding habitat for *Aedes* mosquito species in residential areas of Ethiopia. The abundance of mosquitos in their preferred breeding microhabitats in refuse damps was surveyed (Ikpeama *et al.,*

2017). According to their findings the breeding of mosquitos being favored by blocked gutters, empty cans and ground pools in the study site. The report from the urban and rural areas of Gwalior of Madhya Pradesh by (Priyalika & Gupta, 2017), identified nine species of mosquitoes belonging to 4 genera, viz., *Aedes, Culex, Anopheles,* and *Armigeres*. *Culex quinquefasciatus* was found in the highest number in urban areas, followed by *Anopheles stephensi* in the lowest number in urban areas and *Armigeres subalbatus* in rural areas. The mosquito fauna was evaluated based on habitat characteristics in two coastal districts of Kerala on a seasonal basis (Sajith *et al.,* 2016), found that in the pre-monsoon and post-monsoon seasons with 50% and 83% respectively of the total breeding habitats were represented by sewerage. Fatima *et al.,* (2016) explored the spatial distribution of *Ae. aegypti* in Dengue endemic regions of Pakistan. Selven *et al.,* (2015) identified 12 species of mosquitoes belonging to three genera from tree holes in Puducherry Union territory. Makesh kumar & Jebanesan, (2014) identified 11 species of mosquitoes from tree holes in Kolli hills in the Eastern Ghats and also reported 25 new species from Pondicherry. Bhat & Krishnamoorthy (2014), documented the *Aedes* species distribution in Tirunelveli a study has found that the most popular types of storage containers used by the inhabitants of the area were plastic drums, cement tanks, aluminum and plastic containers, which act as the primary breeding source in the area. Mosquito species biodiversity in phytotelmata from the Western Ghats was studied by (Munirathinam *et al.,* 2014), who recorded 10 *Anopheles* and 114 *Culicinae* in 11 habitats. The most dominant habitat was tree holes followed by bamboo stumps, log holes, leaf axils etc.

Vijayakumar *et al.,* (2014) noticed that distribution of *Ae. albopictus* is high in peri-domestic areas, and the most suitable habitat was discarded tires. Mosquito faunal studies (Balasubramanian & Nikhil, 2013), shows that paddy fields, mud pools, fallow fields and artificial containers act as suitable habitats for immature in the Alappuzha district, and Kottayam district in which tree-holes, coconut shells, artificial container and leaf axils as the prime habitats. Dash, (2011) studied mosquito fauna inhabiting shoreline habitats of the Orissa and documented that 55 species of mosquitoes belong to 12 genera. Larval habitat survey of Aditya *et*

al.,(2006), reported number of mosquito species differs in relation to the month and habitat types and found that the most suitable breeding sources were temporary pools followed by cemented water tanks. Harding *et al.,* (2007) found that mosquito larvae in the Kingdom of Tonga have a nearly parallel number of breeding habitats in both rural and urban areas. Rajavel *et al.,* (2006) recorded seventeen species of mosquitoes belonging to seven genera from the Mangrove Forest of Kannur, the most common breeding habitats found were tree holes and swamp pools.

The faunal studies in the parts of Garhwal found that species richness was greater in the region of dense forest riverine areas (Pemola & Jauhari, 2005) and species diversity was found higher in lower altitudinal range than higher (Pemola & Jauhari, 2004). And reported the species abundant habitat were rock holes and streams while the minimum number of species was recovered from seepage pits. In the survey of Chareonviriyaphap *et al.,* (2003) to assess the larval breeding grounds and ascertain the abundance of larvae during the dry season in all five geographical regions of Thailand, recorded plastic containers and broken cans were found to be the most important breeding sites for *Ae. albopictus* during the dry season, whereas *Ae. aegypti* are more likely to be bred in water jars.

2.2 *Co-breeding of mosquitoes*

Organisms are interdependent and mutually supporting, forming ecological communities. These communities consist of populations of species that interact directly and indirectly, varying based on environmental conditions and evolutionary context. In an ecosystem, interactions are interspecific; occur between the species while intraspecific; occur within the species (Lang *et al*., 2013). Numerous species of mosquitoes associated in same breeding habitats, the major habitats were tree holes, discarded tires and containers; *Ae. aegypti, Ae. dendrophilus, Ae. furcife*r, and *Ae. africanus* were found in same habitats in the rural areas, whereas *Ae. aegypti* correlated with *Ae. vittatus* in suburban areas (Zahouli *et al.*, 2017). Co-habitation of five species in thirty-one instances was found in the rubber plantations (Sumodan, 2012). Species breeding in the same containers often leads to competitive interactions and sometimes coexistence or displacements of one of the species. The

effects of density and species ratio on larval growth and mortality were documented in the laboratory studies of *Ae. aegypti* and *Ae. albopictus* (Moore & Fisher, 1969). Co-breeding mosquito species in the man-made containers was recorded in Mississippi (Goddard *et al.,* 2017). Moreover, it was identified that the co-occurrences of various mosquito species, such as *Cx. quinquefasciatus* with *Cx. salinarius* and *An. quadrimaculatus* in artificial containers, *An. punctipennis* was co-habited with *Cx. restuans*, and *Cx. coronator* co-habited with *Tx. r. septentrionalis* in May.

Observations of (Chumsri *et al.,* 2020) indicates that mixed breeding of species in which number breeding habitats were higher in the dry season and the coexistence of *Aedes albopictus, Aedes aegypti* and *Culex* larvae were observed. Coexistence mechanisms explained by (Laporta & Mureb Sallum, 2014) in microhabitat, habitat and landscape scales indicates that coexistence works synchronously at those three levels of scales. And recorded co-habitation of *An. Bellator*, and *Cx. imitator.* Co- habitation of strong competitors teams *An. bellator*, *An. cruzii,* and *An. scapularis* , *An. serratus* not observed at habitat and micro habitat levels, in contrast cohabitation were found at landscape level. Enhanced food source and density of larvae were related with reductions in the maturation time (Yoshioka *et al.,* 2012). Egg laying site selection is influenced by larval habitat density and food concentration. Few outdoor breeding sites show co-breeding of *Ae. albopictus* and *Cx. quinquefasciatus*, while *Ae. albopictus* and *Ae. aegypti* are not observed as stated by (Saleeza *et al.,* 2013).

Competition between species has obtained more important concern among biotic interactions, chiefly in the areas of invasion biology, where superior invasive species competitively displace the established species (Holway, 1999; Juliano *et al.,* 2004; Petren *et al.,* 1993). Environmental factors frequently influence how native and invading species behave in terms of competition (Daehler, 2003). The interspecific larval competition is proposed as an fascinating mechanism to illustrate the invasion success of *Aedes aegypti* in Asia (Lounibos, 2007), based on the superiority in laboratory studies of *Ae. aegypti* in competition with the native

species *Ae. albopictus* (Moore & Fisher, 1969), *Ae. aegypti* larvae reliably succeeded over *Ae. albopictus* in the presence of nutritious artificial food (Black *et al.,* 1988). This competitive result was inconsistent with the population declines of *Ae. aegypti* by *Ae. albopictus* in the South Eastern USA (Hobbs *et al.,* 1991; O'Meara *et al.,* 1995). Co-existence of *Ae. aegypti* and *Ae. albopictus* were found at ovitrap to, micro habitat and habitat scales in Argentina (Faraone *et al.,* 2021).

Laboratory experiments revealed that *Ae. albopictus* would eliminate cage occupants of *Aedes polynesiensis* (Gubler, 1970). Leaf litter is a basal resource in competition experiments (Barrera, 1996; Juliano, 1998b). Irregular competition between *Ae. albopictus* and *Cx. quinquefasciatus* indicates that under resource paucity conditions competition between two species occurs. *Ae. aegypti* act as active feeder and food biomass conversion is very fast (Santana-Martínez *et al.,* 2017). According to laboratory tests, *Ae. albopictus* survival and maturation period are correlated with the densities of the same species, but *Cx. pipiens*, whose densities are influenced by both *Ae. albopictus* and the same species, exhibits a competitive advantage over *Ae. albopictus.* (Costanzo, *et al.,* 2005a). Differences in aerial surroundings may determine the influence of interspecific larval competition among sites. Therefore, the outcome of competitive declines may be influenced by living and non-living factors through effects on the egg to adult stages (Lounibos, 2007). Competitive ability of *Ae. albopictus* was affected by environmental differences among regions, which influenced its invasion success and impact (Leisnham *et al.,* 2009). Interspecific competition among *Cx. pipiens* and *Ae. albopictus* is normal in temperate climates and boosts higher mosquito numbers produced by higher temperatures (Marini *et al.,* 2017). The competitive exclusion and coexistence sites experiment reveals that the competitive gains of *Ae. albopictus* larvae may lower by higher egg mortality in drier, hotter environments (Juliano *et al.,* 2002). Thus, co-habitation and elimination may be similar in aqueous environments (Juliano *et al.,* 2004). *Aedes albopictus* was more successful in habitats where with food paucity, and the temperature was 25±2°C. By comparison, *Ae. albopictus* is less successful at 20 degrees Celsius (Carrieri *et al.,* 2003). Temperature, larval diet, and number may all play a role in controlling the survival and maturation rate of *Ae. aegypti* (Couret

et al., 2014). And their co-operative interactions are essential in the growth rate variations for the egg to adult emergence. In the reviews of the interspecific interactions among mosquitoes (Juliano, 2009), it has been postulated that non-native and native species of the same genus might compete extremely due to their competition for resources than between less closely related species; this explains why most invasive species are from exotic genera (Darwin, 1859; Elton, 1958). The level of intraspecific competition had an essential effect on adult survival under minimal humidity conditions for *Ae. aegypti* but not for *Ae. albopictus* (Reiskind & Lounibos, 2009).

Competitive shifts and coexistence work among immature habitats (Juliano, 1998b) and may act concurrently in the same system. Reiskind & Lounibos, (2009) recorded the impact of larval competition on adult maturation time in the mosquitoes *Ae. aegypti*. And recognized that larval competition was common in container-inhabiting mosquitoes and stated that larval competition could lead to increasing or decreasing effects on adult body size, survivorship, growth, and mosquito body size reveals the availability of resources in the larval habitats (Juliano *et al.,* 2002; Lounibos *et al.,* 2002). Prior field laboratory experiments have revealed that habitats used by container breeding mosquito species can significantly impact their potential to attain and survive as adults (Daugherty *et al.,* 2000; Fish & Carpenter, 1982). Competition is common in resource-paucity conditions, *Ae. albopictus* was more efficient when competing under restricted or poor-quality resources (Carrieri *et al.,* 2003). Mosquito-rearing studies show that higher larval numbers lead to declined larval survival (Tun-Lin *et al.,* 2000). Intraspecific competition during the immature stages is one of the main reasons behind adult fitness (Bradshaw & Holzapfel, 1986). Seasonal variations in the environment alters competitive ability and equalize the effects (O'Neal & Juliano, 2013).

Quantity of resource will be crucial for larvae in all instars, and growth inhibitors produced by later instars may have their greatest effects on earlier instars (Ikeshoji & Mulla, 1970). The paucity of resources in the breeding grounds leads to a prolonged time for larval growth, resulting in a smaller size at metamorphosis

(Arnaldo, 2006). Adult body size, survival, productivity, mating success, and flight capacity are all reduced (Hard & Bradshaw, 1993; Steinwascher, 1982). Wing length specify an exact indicator of fecundity in adults (Armbruster & Hutchinson, 2002). Several studies have exploited this relationship to investigate mosquito ecology and behavior (Alto *et al.,* 2005, 2008; Reiskind & Lounibos, 2009). The growth of larvae to adulthood and their existence to adulthood, body size, and longevity are largely interconnected to their larval density within the breeding habitats (Alto *et al.,* 2008b; Griswold, *et al.,* 2005; Bevins, 2008).

Parker *et al.,*(2018) recorded that the negative effect of interspecific competition was high in small and medium containers. In reverse, the negative effects of intraspecific competition were higher in large containers, also found that *Ae. aegypti* may be better in utilizing resources in small, medium-sized containers. The container size can mediate the outcomes of competition for *Ae. albopictus* and *Ae. aegypti*. Often, the interspecific competition in the larval environment was irregular, resulting in the complete elimination of the inferior competitor (Chesson, 2000; Costanzo, *et al.,* 2005b; Lawton & Hassell, 1981). Interspecific competition influences the structure of populations of container-breeding *Aedes albopictus* and *Aedes aegypti* mosquitoes (Barrera, 1996).

The variations in the number of *Ae. aegypti* adults was related to interspecific competition in their larval stages (O'Meara *et al.,* 1995). *Aedes albopictus* larvae were consistently superior to *Ae. aegypti* in terms of growth and survivorship; and analysis of population growth factors revealed that interspecific larval competition was the appropriate description on the declines of *Ae. aegypti* populations in containers with leaf litter substrates (Juliano, 1998a). Although interspecific larval competition is an important reduction mechanism, the predictability based on the outcomes of laboratory experiments has proven exclusive. Probable mechanism to explain the co-existence of *Ae. albopictus* and *Oc. triseriatus* was differential breeding ground selection (Lounibos *et al.,* 2001). Studies reveals that *Ae. albopictus* has superior resource-utilizing abilities compared to its competitors, and its larvae spend more time on feeding activities than *Ae.*

aegypti larvae (Carrieri *et al.,* 2003; Marini *et al.,* 2017; Yee *et al.,* 2004). Larva of *Ae. albopictus* exhibits dominant competitive abilities, affecting interspecific competition and negatively impacting *Oc. triseriatus* survival, as indicated by the relative crowding coefficient index (Bevins, 2007).

Studied interspecific larval competition of *Ae. albopictus* and *Ae. japonicus*, and found that *Ae. albopictus* eliminate *Ae. japonicus* competitively (Armistead *et al.,* 2008). *Aedes aegypti*, a long-standing resident and invasive species in the United States, was the dominant artificial container species in Eastern regions prior to *Ae. albopictus'* arrival (Lounibos *et al.,* 2002). As *Ae. albopictus* spread, however, *Ae. aegypti* population reductions were extremely rapid. Major declines of *Ae. aegypti* has been seen in most areas where *Ae. albopictus* invaded, the replacement has not been complete everywhere (Hornby *et al.,* 1994). *Aedes triseriatus (say),* and *Ae. japonicas japonicus* (Theobald) is the other container species invaded by *Ae. albopictus* in the United States (Fader, 2016). The Eastern tree holes mosquito is the dominant tree holes species in the Eastern United States, native to be affected by *Ae. albopictus* (Lounibos *et al.,* 2001). Under food paucity, *Ae. triseriatus* is an inferior resource competitor to *Ae. albopictus* (Livdahl & Willey, 1991; Teng & Apperson, 2000). *Aedes triseriatus* would eliminated from tire habitats but not tree holes due to different tree holes resources (Livdahl & Willey, 1991). Studies support this prediction as *Ae. triseriatus* seem less affected by *Ae. albopictus* invasion in tree holes than artificial sites by Florida, *Ae. japonicus* likely invaded North America in a shipment of used tires. They are arriving in the late 1990s from Japan, *Ae. sirrensis* (Ludlow) was the dominant tree hole mosquito in the Western United States and progressively interacts with the exceeding *Ae. albopictus* population in California (Kesavaraju *et al.,* 2014; Washburn & Hartmann, 1992). Under limited resource conditions, two studies have tested the competition between *Ae. sierrensis* and *Ae. albopictus*, which is a superior competitor (Fader, 2016). Coexistence of *Cx. pipiens (L)* with *Ae. albopictus* was primarily observed in artificial containers in the Northern United States and Southern Canada. In many of the competition experiments *Cx. pipiens* was found to be an inferior competitor to *Ae. albopictus*, has a sufficiently low overlap with *Ae. albopictus* to avoid

competitive displacements (Carrieri *et al.,* 2003; Costanzo, *et al.,* 2005; Murrell & Juliano, 2012). *Ae. albopictus* is a superior resource competitor to the Southern house mosquito, *Cx. quinquefasciatus* (Say) (Allgood & Yee, 2014; Daniels *et al.,* 2016). *Aedes albopictus,* an invasive Asian mosquito, became prevalent in the mid-1980s in large areas of the United States. (Black *et al.,* 1988), Europe, Africa, and South America during the last two decades (Lounibos *et al.,* 2002) since it invaded most of the South-Eastern United states (O'Meara *et al.,* 1995). In the Southern U S, the spread of *Ae. albopictus* coincided with declines in the range and abundance of the resident *Ae. aegypti* in artificial containers reviewed by (Juliano *et al.,* 2004), *albopictus* is superior to many resident container mosquitoes as a competitor (Aliabadi & Juliano, 2002; Daugherty *et al.,* 2000; Yee *et al.,* 2004).

The critical determinant of every competition is greater efficiency in acquiring a limited resource, an advantage (Yee *et al.,* 2004). Competitive advantage varies according to environmental factors such as habitat drying (Costanzo, *et al.,* 2005), resource type (Barrera, 1996), container type (Livdahl & Willey, 1991). *Aedes albopictus* larvae competitively eliminate *Ae. aegypti* in inadequate container habitats of suburban and rural areas (Britch *et al.,* 2008). Multiple factors like detritus type, nutrient levels, and water temperature within container habitats can lower competitive exclusion, leading to the co-breeding observed at some locations (Farjana *et al.,* 2012; Murrell & Juliano, 2008).

Competitive interactions of invasive *Ae. albopictus* with resident *Ae. aegypti* results in the coexistence or exclusion of the latter species (Leisnham *et al.,* 2009). The invasion success of many species into occupied niches outcomes the vital role of local competition (Duyck *et al.,* 2006; Yasuda *et al.,* 2004). The spread of *Ae. albopictus* has been associated with a decline or local elimination of a closely related species, *Ae. aegypti* (O'Meara *et al.,* 1995). *Ae. albopictus* invaded the Americas in the 16th century from its origin in tropical Africa (Lounibos, 2002). In fields (Juliano, 1998b), and laboratories (Barrera, 1996; Murrell & Juliano, 2008). Competitive experiments have shown that *Ae. albopictus* was a potential competitor for resources with *Ae. aegypti* in containers. These experiments have typically

involved the effect of competition on the immature stages, ignoring effects that may be expressed in resulting adults (Costanzo, *et al.*, 2005). The Asian tiger mosquito has a highly competitive ability and ecological plasticity (Bargielowski *et al.*, 2015). Generally, species can adapt quickly and deal with various conditions; in some countries, *Ae. albopictus* even started to displace *Ae. aegypti* or exploit other species ,*Cx. pipiens* habitats (Paupy *et al.*, 2009).

In the field, larvae of both species coexist in the same container, but in the laboratory experiments, *Oc. triseriatus* has a longer development time than *Ae. albopictus* could be a better competitor (Teng & Apperson, 2000).The presence of *Oc. triseriatus* in mixed conditions did not significantly affect *Ae. albopictus* survival, signifying that *Oc. triseriatus* had no positive or negative effect on *Ae. albopictus* metamorphic success (Bevins, 2007).

Literature on *Aedes chrysolineatus* was few, on account of this study was undertaken to know the habitat diversity and co-breeding status of *Aedes chrysolineatus*, especially with *Ae. albopictus* in North Kerala.

CHAPTER III
MATERIALS AND METHODS

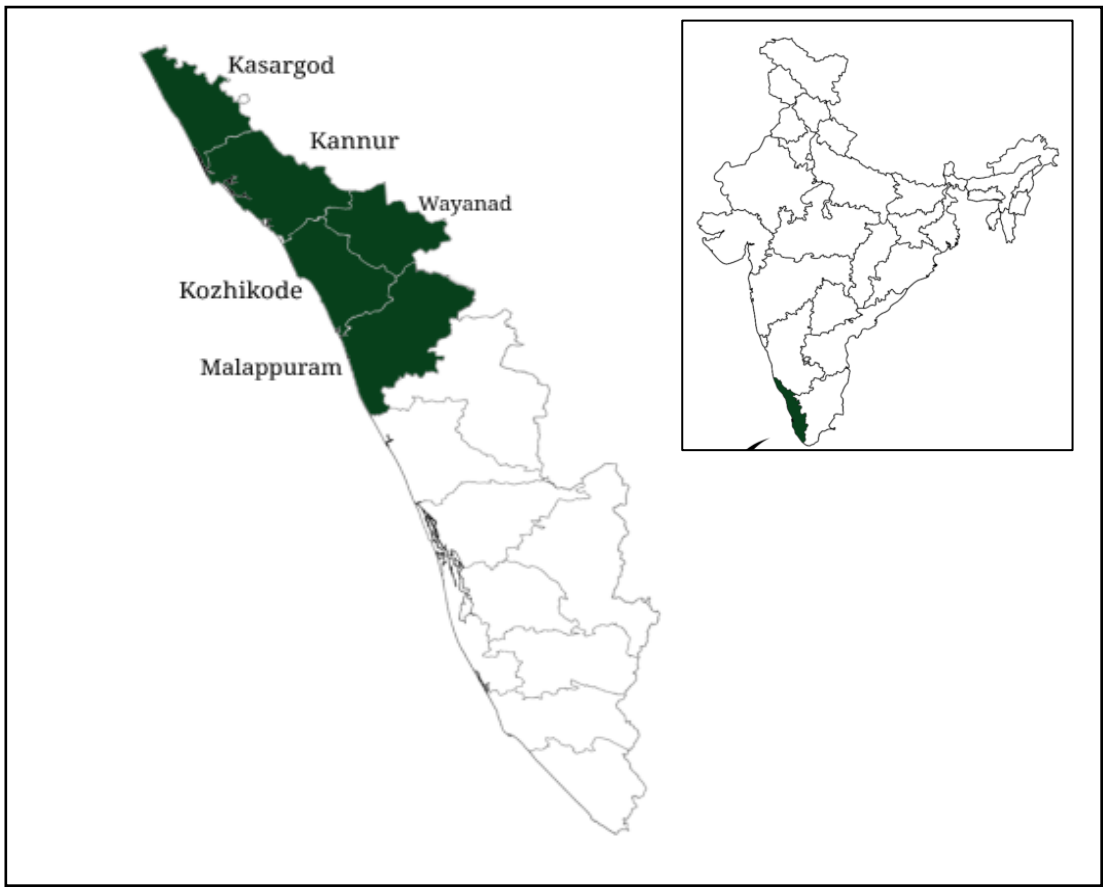
3.1 Study Designs

The study was carried out by sample survey from January 2017 to December 2021. Five Northern Kerala districts viz., Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram were selected for the collection of mosquitos. Surveys were conducted in all seasons.

3.2. Study area (Map.3.1)

Kasaragod, Kannur, Kozhikode, Wayanad, and the Malappuram districts are located towards the Northern side of Kerala state. These five districts are diverse and unique in various aspects, as detailed below.

Map (3.1): Study Area



a. Physiography in the study area (Map.3.2)

Kasaragod: Kasaragod, the northernmost district of Kerala has a total geographical area of 1989.00 Ha. It lies between 12 5’ N and 75 0’E. The average elevation of the district is19 meters (62 feet). Kannur district outlines the district to the south, the Arabian Sea to the west and Mangalore, Karnataka to the north and the Western Ghat to the east.

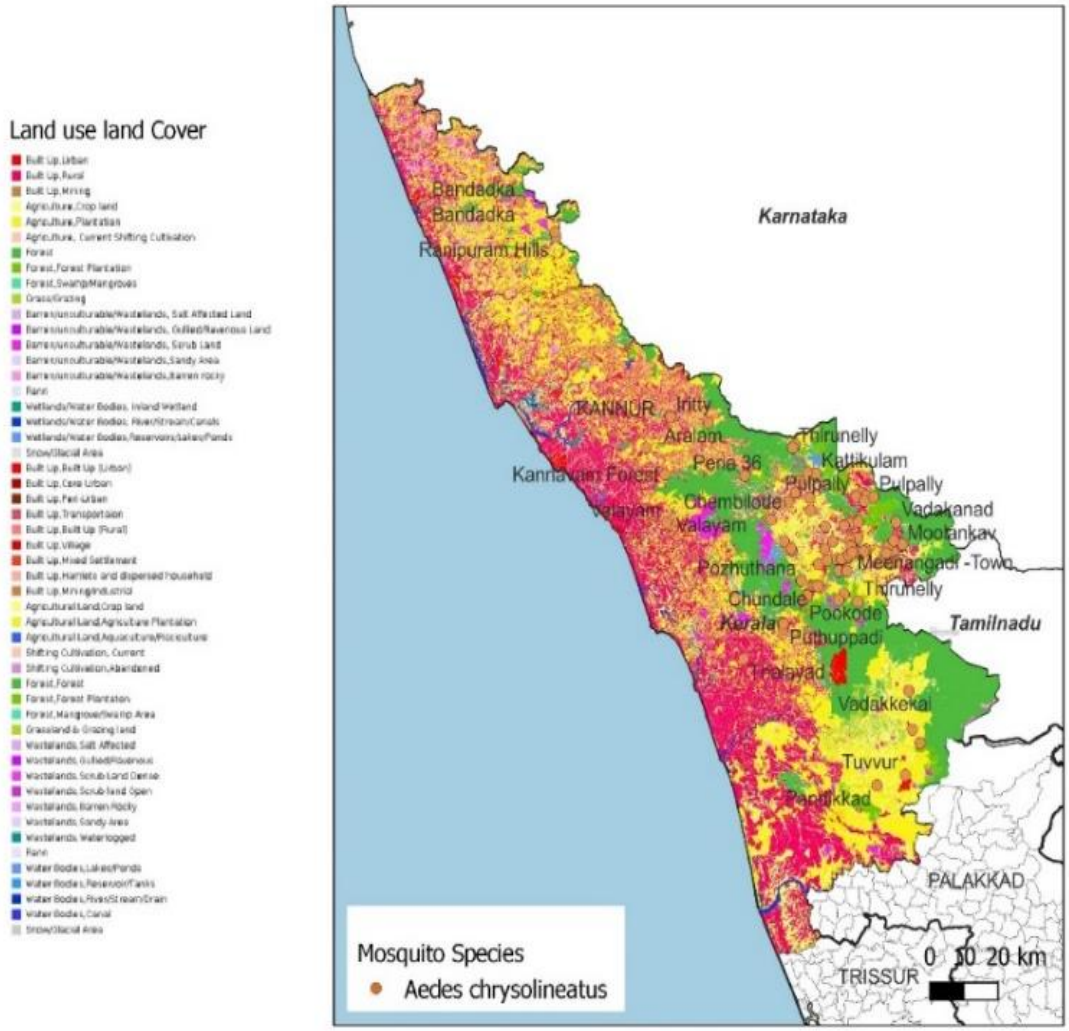
Kannur: Total area of 2961Km^2 and lies between 11 8’N and 75 3’E. The district's borders are as follows: Wayanad district to the east, Mahe (Pondicherry state) to the south-west, Kozhikode district to the south, and Kasaragod district to the north. It is situated along the Laccadive Sea Coast at a height of 1.02 meters, or 3.3 feet, with a sandy coastline region. Coastal line length is 82 km which is 13.9% of the total coastal line of Kerala.

Wayanad: Wayanad is a rural district and a hill station in Kerala with an area of about 2130 sq. km and lies between Latitude 11 27’ & 15 58’ and longitude 75 47’& 70 27’. The district is located in the north-east part of Kerala and the edge of the Deccan Plateau in the south. The district is outlined to the east by the districts of Mysore and Nilgiris in Karnataka and Tamil Nadu, to the north by the Coorg district in Karnataka, to the south by the Malappuram district, and to the west by the districts of Kozhikode and Kannur in Kerala. Its main feature is the Western Ghat mountain range with an altitude between 700-2100 meters, with an elevated ridges diffused with dump forest, intricate jungles, and deepest valleys.

Kozhikode: The district spans 2345 square kilometers and is located between longitudes 75 30' and 76 8’'E and latitudes 11 08' and 11 50'. The district is surrounded by Kannur, Wayanad, Malappuram, and the Arabian Sea on the north, east, south, and west respectively. Its average altitude is 127 meters.

Malappuram: Malappuram district has an area of 1372sq. m. and lies between 75 to 77 east longitude and 10 to 12 of north longitude and has an elevation of 223 feet. It is the third largest district in the state surrounded by Nilgiri Hills in the east and in the west Arabian Sea, and Wayanad and Kozhikode districts in the north, and Thrissur and Palakkad districts in the south. It has an altitude range between 115 meters to 2954 meters.

Map (3.2): Physiography in the Study Area



b. Demography in the study area

As the report of 2011 survey, Kasaragod district was home to 13.07 lakh people and about 90.09 percent of literacy rates were recorded. There were 657 people per square kilometer. Total number of households was 273410.

As per 2011 survey, Kannur district was home to 25.23 lakh inhabitants with a 84.7 percent of literacy rate. Density of the population per square kilometer is 852. The total households in Kannur district is 554298. In the survey report of 2011, Kozhikode district was home to 30.86 Lakh people, and 95.08 percent of average literacy. And density of the population per square kilometer was 1316. Total

households is 697710. In accordance with the 2011 survey, Wayanad and Malappuram districts had a population of 8.17 Lakhs and 41.13 Lakhs with an average literacy of 89.03% and 93.57% respectively. And peoples density per square kilometer was 384 and 1157. The total number of households was 190894 and 793999 respectively.

c. Climate

The climate condition of all districts can be divided into four seasons, the summer season (March to May), the southwest monsoon (June to September), the post-monsoon season (October and November), and the winter season (December to February). The Kasaragod district features, tropical and subtropical climate. And receives 3350mm rainfall annually, June to August experiences the highest rainfall of the total. The climate of Kannur district is tropical. The district annually gets rainfall of 2410 millimeter, the highest precipitation falls in June and the driest month is February and average annual temperature is 26.4°C. Kozhikode district has a tropical monsoon climate, normally experiencing an average of 3266 mm rainfall annually. Wayanad district enjoys healthy climate. The district experience mean rainfall of 2322 mm. High rainfall areas in the districts are Lakkidi, Vythiri, and Meppadi, the annual rainfall of these three regions falls into 3000-4000 mm. High-altitude regions experience severe cold.

d. Agriculture

Agriculture forms one of the main incomes of all districts. Agriculture forms the major source of income for the populaces of Kasaragod district. Heterogeneity in cultivation is the main feature of agriculture. Three natural divisions are composed of 3 types of soil; Laterite in the highland region, a red ferruginous loam mixed with sand and clay in the midland region, and the coastal strip are sandy. Forest and hilly areas comprise timber, teak, rubber, cashew, and ginger. In the areas of coast constituted with, vegetables, paddy, areca nut, cashew, coconut, tobacco, and tapioca, etc. In the plateau areas, cashew trees are cultivated, and areca nut, pepper, and cocoa are grown in some patches. One of the major backbones of the Kannur district is agriculture. Paddy, cashew, tapioca, areca nut, and rubber are the main

cultivation crops. In Kozhikode the major crops cultivated are coconut, paddy, banana, tubers, spices, and tree crops. Agriculture forms the major economy of the Wayanad district, characterized by the cultivation of plantation crops and spices. The major cultivations are coffee, tea (31,792), pepper, cardamom (38,348 ha), and rubber (63,015ha). Coffee forms 33.7 percent of the total cropped area (66,999 ha), 78 percent of the Kerala's coffee area. In Malappuram district, 2.09 lakh hectares of land are available for agriculture and it is characterized by the cultivation of paddy, coconut, tapioca, areca nut, cashew nut, banana, rubber, ginger, pulses, and pepper.

e. Terrains

Kasaragod, Kannur, and Kozhikode districts comprise high land, midland, and low land, the stretch of sandy beaches with 362.85 square kilometer, the rocky mountains (637.65 sq. km) in the hilly sides of the Western Ghats, and midland (1343.50 sq. km), with laterite. The four taluks are distributed across these three regions. Wayanad district is located at an altitude of 700-1200 meters from the sea level. The district comprises hilly areas, valleys, and meadows. The highest peak ranges from 1500 m to 2100 m height. Malappuram district comprises three natural divisions; lowland, midland, and highland.

Collection methods

3.3 Collection of Immature mosquitoes

Immature stages (Larvae and Pupae) of mosquito were collected using a different methods depending upon the habitat type. The habitats sampled include containers, stagnant pools, discarded tyres, rock pools, tree holes, domestic runoff etc. where mosquito breeds. Habitat evaluation methods described by (Service, 1993) were selected in collecting the larvae from different habitats. Larvae and pupae were sampled utilizing dippers from their habitats (fig. 3.3a) in ground pools, suction tubes (fig 3.3 b), and pipettes (fig 3.3c) in tree holes. Small containers were fully emptied into the collection bottles (fig 3.3d) (Service, 1993; WHO, 1975). Sample from each habitat was maintained separately in suitably labeled (Date of collection, type of habitat, and study area) containers (fig 3.3e), and then transported to the laboratory.

Fig 3.3 (a-d): Collection Methods and materials : (a) Dippers, (b) Sample collection using suction tubes, (c) Pipette, (d) Latex cup emptying to bottles

Immature stages were allowed to emerge and were collected and stored in vials (fig 3.3f) and all the collected mosquitos were photographed under stereo zoom microscope (fig 3.3g) in a magnified form and then identified using the standard keys and nomenclature (Barraud, 1934). In cases of doubt confirmation was done with assistance of Mosquito museum of ICMR- Vector Control Research Centre, Pondicherry.

Fig. 3.3 (e-h): (e) Samples bottle with larvae, (f) Adult mosquito stored in vials, (g) Stereo zoom microscope, (h) Mounted larval slides

3.31 Morphology of larvae and pupae

a. Preservation and mounting techniques for immature: (Reagents used; 70% Alcohol, *Canada Balsm, *Ethyl Cellosolve).

Field-collected larvae were killed in warm water and stored in a small vial containing 75-80% ethyl alcohol and transferred the specimens from alcohol to cellosolve for 15 minutes. The specimens were removed from cellosolve and placed the middle of the slide with the dorsal side up (these steps are common for larvae and pupae). A small amount of Canada balsam was dropped on the larval or pupal specimen. Mounting of larvae was carried out by placing head pointing down, and organised the head, thorax, and abdomen in a normal manner, then split abdominal segments between VI and VII. Placed the terminal segments with siphon to the left. More Canada balsam was added to the specimen and checked the position of setae and larval, then carefully covered specimen with a 22 mm rectangular cover glass (fig 3.3h).

For pupae cephalothorax was separated from the metanotum and abdomen and mounted the specimen pointing down, placed the metanotum and abdomen dorsal side up then turned the cephalothorax left side up and placed it below the metanotum. More Canada balsam was added to the specimen, and checked the correctness of position of the specimen. The specimen was covered with a 15 mm round cover glass. The slides were dried (larval and pupal) in an oven at 45 to 55℃ for one-two weeks (Rattanarithikul, 1982). Diagrams of *Ae. chrysolineatus* larvae and pupae taken from descriptions of (Knight, 1968).

3. 4 Adult collections of mosquitoes

Adult mosquitoes were collected by using insect PRO Active gravid cum Light Trap (ALGT) (fig.3.4a). Resting adult mosquitos were collected by using a manually prepared aspirator (fig.3.4b), and emerging adults from larvae or pupae were collected.

Fig 3.4 Collection tools (a-c): (a) insect PRO Active Gravid cum Light Trap (AGLT), (b) Manualy prepared Aspirator, (c) Entomological pins, (d) Pinned specimens

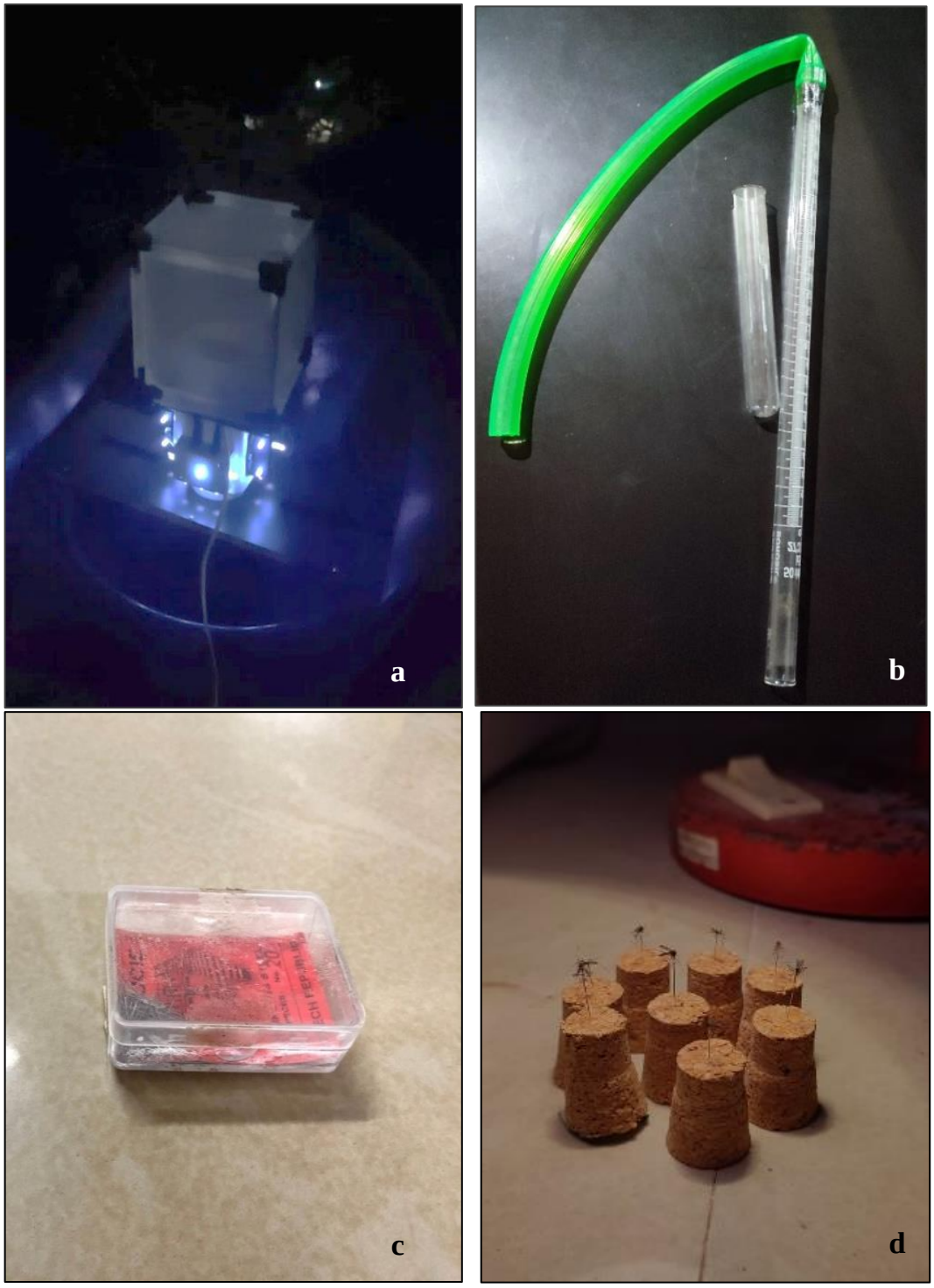


Fig 3.4 (e) Pinned specimen photographs

3. 41 Morphology of adult

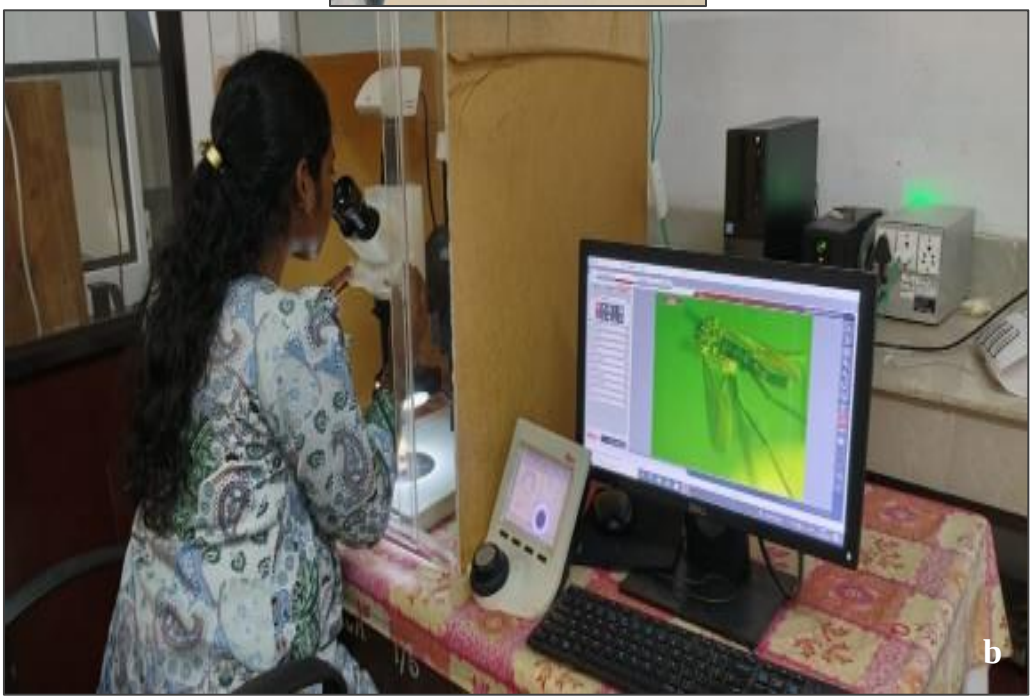
a. Pinning and labeling of Adult Specimens

After adult emergence, adults were held for at least 24 hours before killing. Cotton piece soaked in diethyl ether was used for killing, placed in a glass jar. Adult mosquitoes were mounted by pins (fig.3.4c) in the mesothoracic area and placed in corks of glass vials (fig. 3.4d). The collection details, including districts, locality, elevation range, seasons, and breeding habitat, were noted.

3. 5 Identification of immature species and adults

Mounted larval and pupal specimens were photographed under phase contrast microscope (fig.3.5a). Collected adult mosquitos were photographed under stereo zoom microscope (fig.3.5b), in a magnified form and then identified using the standard keys and nomenclature (Barraud, 1934).

Fig 3.5 (a-b): Viewing specimens under Microscopes: a) Phase contrast Microscope, **b)** Stereo Zoom Microscope



3.6 Taxonomy

Taxonomical details of *Aedes chrysolineatus* have been gathered by surveying published literature.

3.7 Phylogenetic Analysis

The mitochondrial COXI sequence isolated from an *Aedes chrysolineatus* (SH1) collected from Wayanad was aligned with similar sequences of different *Aedes* mosquitoes retrieved from the NCBI GenBank database. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). An optimal tree was constructed and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Tamura *et al.,* 2004). This analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 942 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.,* 2021). Other sequences related to this Analysis were taken from NCBI.

*(*SH 1, name given to Ae. chrysolineatus specimens for identification)*

3. 8 Laboratory study

Experiments were carried out following Carrieri *et al.,* (2003). Field collected larvae of *Ae. chrysolineatus* and *Ae. albopictus* larvae were placed in a rearing cage at 28℃, 75% relative humidity (RH), and a light period of 12:12 L:D. Field-collected larvae were separated and placed in 300 ml of de-chlorinated water. Three food doses (dog biscuits) were used: 0.95mg/larvae, 1.9 mg/larvae, and 2.83 mg/larvae at 28, 22℃. In addition, the influence of air temperature was studied by comparing larval competition at 22℃ and 28℃ using the intermediate food dose of 1.9 mg/larvae. The dosage that was previously identified as the most suitable for

demonstrating competition between two species. The dry body weight, rate of adult production rate corresponding to each temperature and food dose were evaluated to establish the competition between the following ratios of *Ae. chrysolineatus* and *Ae. albopictus* larvae (1:0, 3:1, 1:1, 1:3, 0:1), 10 larvae were used as one unit in all the ratios (e.g.: 1:3 means 10 *Ae. chrysolineatus* : 30 *Ae. albopictus*). During each of the 6 days of larval development the food was supplied in doses proportional to age, i.e.; 10% on the first and second day, 15% on the third, 21% on the 4th day and 22% on the 5th and 6th day. Pupae were collected and placed in separate containers. Adults who emerged were counted and placed at a temperature of -20℃ and then dried, and weighed (SCALETEC Analytical balance, SAB-224 CL) (Fig. 3.7).

Fig 3.7: SCALETEC Analytical Balance (SAB-224 CL)

3. 9 Data analysis

3.91 Geographical distribution studies: In order to study the distribution pattern of *Aedes chrysolineatus*, mosquito breeding habitats were surveyed in all Panchayats of the districts under study. Collection details were recorded, and tabularized and percentages were calculate using Microsoft Excel.

3.92 Altitudinal distribution: While doing habitat surveys altitudes were recorded for each locality. These altitudes ranges were tabularized as mid land, mid upland, and high ranges. These data were plotted in the QGIS software for obtaining altitudinal distribution maps.

3.93 Seasonal fluctuation: Month-wise fluctuation of the density of *Ae.chrysolineatus* was assessed using emergence data. For this purpose, number of adult mosquitoes emerging each month was recorded separately and analyzed.

3.94 Map preparation

Sample collection data with location, latitude, longitude and elevation were maintained, and sorted out in Microsoft Excel 2013. Maps were created in QGIS software 3.34, using the geo co-ordinates of the collection site.

3.95 Relative abundance and distribution pattern of *Aedes chrysolineatus*

Relative abundance and distribution pattern were calculated using the following equations (Ali *et al.*, 2013; Attaullah *et al.*, 2023; Rydzanicz and Lonc (2003) & Sengil *et al.*, 2011).

Relative abundance (RA) = $\frac{I}{L} \times 100$ where I 'is the number of collected specimens of each species, and 'L' is the total no of collections. Mosquito species were classified according the rates of relative abundance as follows,

i) Satellite; RA < 1%,

ii) Sub-dominant; RA < 5%

iii) Dominant species; RA > 5%

$$Distribution\ (C) = \frac{n}{N} \times 100$$

Where, 'n' is the number of sites in which species were found and 'N' is the total number of sites surveyed. In supporting the rate of (C), distribution level of the species as follows,

i) C =0-20% (sporadic),

ii) C =20.1-40% (infrequent)

iii) C =40.1-60% (moderate)

iv) C=60.1-80% (frequent)

v) C =80.1-100% (constant).

(* Note: Distribution level of *Ae. chrysolineatus* only mentioned in results)

3. 96 Co-breeding and competition studies:

a). The analysis of competitive advantage was evaluated using one way analysis of variance (ANOVA) by comparing the number of adult mosquitoes emerged between the co-breeding of *Ae. chrysolineatus* and *Ae. albopictus*. For this analysis, data were sorted out, and the significance was determined at $P \leq 0.05$, these analyses were performed using Microsoft Excel version 2013.

If, $P \leq 0.05$, then the result is significant.

b). Competition study of the species were studied by data recorded, sorted and analyzed in Microsoft Excel. Competitive advantages of the two species were confirmed using the measure, Relative crowding Co-efficient (RCC) analyzed by using following modified equations of Novak *et al.*, (1993) and Oberg *et al.,* (1996), as follows:

$$\frac{X_{1:1}/Y_{1:1}}{X_{1:0}/Y_{0:1}},$$

$X_{i:j}(Y_{i:j})$ = Mean biomass of the species X or Y; the ratio $X:Y$ is $i:j$

$$\frac{\frac{1}{3}\left[\frac{1}{3}\left(\frac{X_{3:1}}{Y_{3:1}}\right)+\left(\frac{X_{1:1}}{Y_{1:1}}\right)+3\left(\frac{X_{1:3}}{Y_{1:3}}\right)\right]}{\left(\frac{X_{1:0}}{Y_{0:1}}\right)}$$

Values of RCC indicates as follows:

i). RCC = 1, two species are equal competitors

ii). RCC > 1, Species X is a superior competitor to the species Y

iii). RCC < 1, Species Y prevails

CHAPTER IV

MORPHOLOGY AND TAXONOMY OF *AEDES CHRYSOLINEATUS*

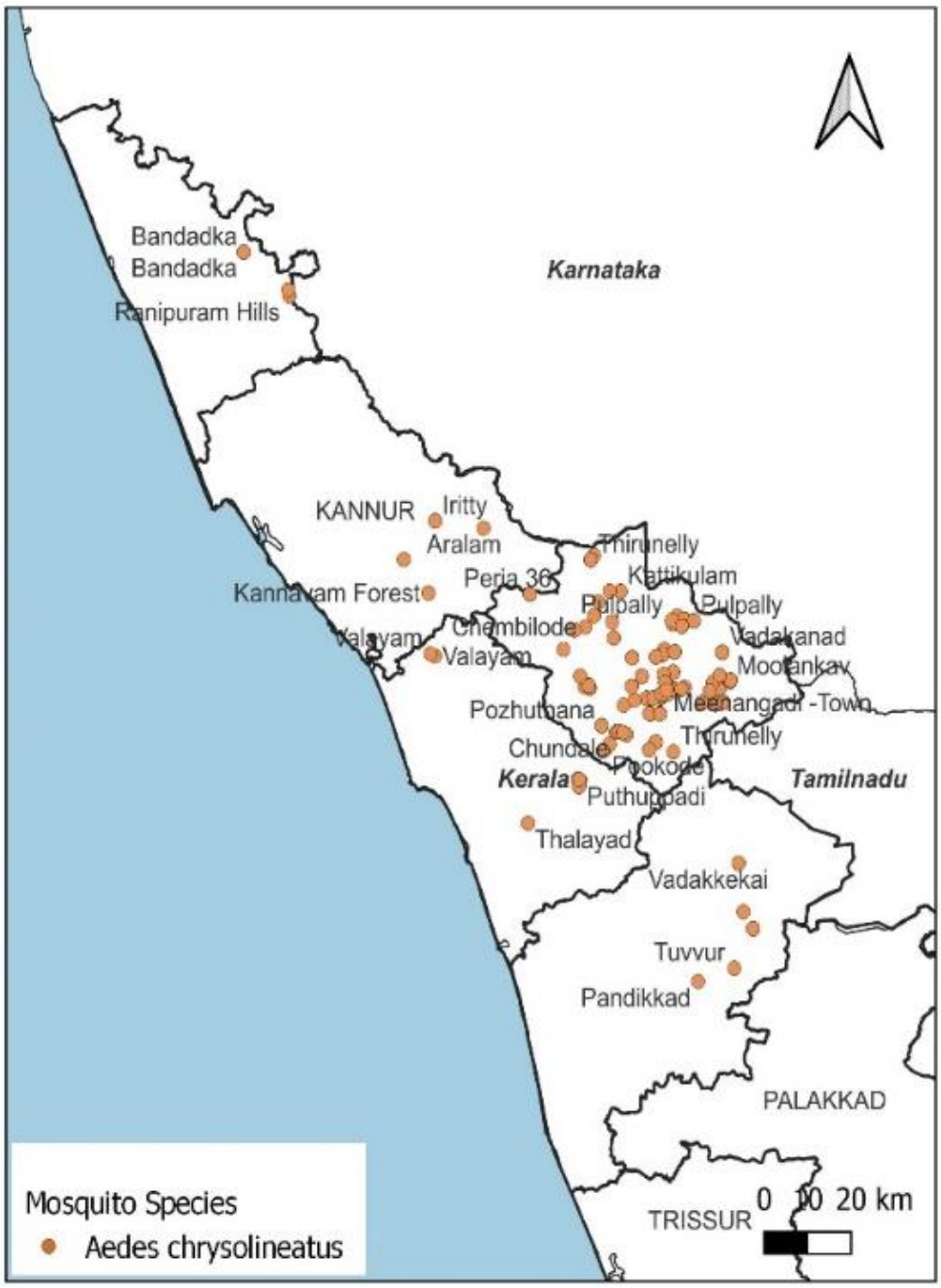
4.1. Introduction

Aedes chrysolineatus was described by Theobald in 1907 as *Howardina chrysolineatus* based on a female specimen collected from Pundaluoya in Sri Lanka. The meaning of the specific name is gold-lined, referring to the golden yellow lines on its scutum. This characteristic earned it the informal name Gold-banded Sri Lankan Pointy Mosquito. After the original description, Barraud (1934) described the adult male, female, and larva under the name *Aedes chrysolineatus* (Barraud, 1934; Wilkerson *et al.,* 2021). In this chapter detailed morphological characteristics of the adults, larva, and pupa are described, with a note on its taxonomy.

4.2. Materials and Methods

Larval and adult Samples were collected between January 2017 to December 2021 in North Kerala districts (Kasaragod, Kannur, Wayanad, Kozhikode, and Malappuram) (Map.4.1). Various collection methods, slide preparation (larvae and pupae), pinning, labeling, identification of *Aedes chrysolineatus* adult specimens, and methods of phylogenetic analysis were mentioned in Chapter III (section 3.3-3.7).

Map (4.1): Collection sites



4.3. Results

4. 31 *Morphology of Aedes chrysolineatus*

a. Adult Female (fig. 4.3: a, b) ***Head***: brownish, pale behind and above the eyes. Eyes are separated above antennae, with inter-ocular space featuring whitish setae and white curved patches, blackish or creamy white scales on the midline of occiput, and erect scales. The vertex had a narrow, pale median band of scales, which later widened into a broad, scanty patch. A line of scales along the median half of the eye margin, which are alike; with dark scales between the ocular pale line and a posterior patch of narrow pale scales. Vertex with broad pale scales laterally, and a small anterior patch of dark sales medially in this area. Forked upright scales are plenteous dorsally from eye margin to nape.

Proboscis (fig. 4.3: c, d)**:** 1.9-2.1 mm in length and is about the same length as the fore femur. Posteriorly with broad pale scaling from base to apex. *Palpus* is 1/4 to 1/5th the length of proboscis, palpomeres dark with pale scaled at the apex. *Antenna* brown with hairy internodes, 4/5th to the length of the proboscis. 2nd flagellomere, somewhat curved and hairy with white scales at the tip.

Thorax: (fig.4.3: d, e) 1.0-1.1 mm in length. Scutum integument brown covered with narrow curved yellowish pale scales. The pale scales are arranged in full-length narrow longitudinal traces as follows. Scutum with median longitudinal pale line forked posteriorly at pre-scutellar area. A distinct median line in acrostichal setae. Sub median lines tend to be broken at the scutal angle. The anterior cease of the posterior component of the line is regularly curved alongside the scutal angle. A slightly curved line over the wing base, a small patch of long narrow curved pale scales just before wing base. Scutellum: lateral lobe with dark sparse scales, mid lobe with pale scales broadened flat lying dark scales laterally. Pleurae deep brown with a patch of broad white scales. Proepisternal, post spiracular, prealar (between the lobes), upper sternopleural, lower posterior sternopleural, and upper half more of the mesepimeral with white scales. Para tergite and sub spiracular space devoid of scales.

Wings: *Wings* 3.82-3.86 mm, usually a short line of white scaling on the outer or underside of the costa at the base otherwise completely dark-scaled, Halters brownish colored.

Legs (fig. 4.3: f, g)**:** basal pale bands on the mid and hind pairs. Coxae pale, fore, and mid coxae additionally with dark scales ventrally and fore coxa usually with pale scales anterior-ventrally to the dark scales. Fore femur 2.1-2.2 mm. The apex of the femora and tibiae are pale. The posterior surface of the fore-femur is a broad dorsal white scale near the base to the apex (may be interrupted or diminished subapically). The anterior surface of the mid femur with the basal half was largely white, this white continued along the apical half as a ventral band. Hind femur anteriorly with broad pale or white area from near base to the region of middle connecting ventrally with a similar area on the posterior surface, dorsal surface dark along whole length whitish area beneath the apex, this extending apico -dorsally on to both anterior and posterior surfaces. Tibia each with a narrow apical white band. *Mid and hind femur*: Lateral portion of the hind femur with white area mesally. Tarsus I, II, and III with white bands; fore and mid tarsi with distinct white bands; mid tarsus with 3 white bands, IV& V of the mid tarsus not. Hind tarsus with one or more white bands at the base of the segments I-III.

Abdomen: Abdomen 2.5-2.7 mm. Integument and thoracic pleura brownish. Abdominal terga with dark scaled, sternite with a white band in all segments likewise terga. The abdominal spiracle is dark colored (fig. 4.3 h).

Male (fig.4.3: i, j): In general, similar to female except for sexual character**.** ***Head:*** *Proboscis*: 1.2-1.3 mm in length; pale yellow color at the apex and narrow white band medially, pale beneath from near the base to just beyond the middle and also at the apex, except these area upper surface is dark.

Palpus: 0.8- 1mm long with four palpomeres; I& III somewhat equal in length and the 2nd one is large. Whitish ring in between segments I and II pale hairs between the segments, and the apex is slightly swollen with few hairy processes. Pale scaling at the joints of II to V.

Legs: Ventral border of forefemur pale along anteriorly mid femur dark anteriorly or with longitudinal median pale scaling hind femur white beneath at tip. Wing approximately 2.8 -3.0 mm in length.

Abdomen (fig.4.3 k): Dorso-basal pale scaling of terga extremely variable. Tergal lobes IX each with 15-35 setae.

b. *Pupa*. (Fig.4.4 a, b) Cephalothorax: Hair 1-C at least two times as long as hair 2-C; 10-C to the median vertical longitudinal plane of 11-C; 11-C much elongated and single.

Abdomen (Fig.4.5*)*: Hair of the segments, I-II is mostly well developed, with multiple branches; hair 2 of segment I and 3-I guessed d; hair 2-VI well towards the median vertical longitudinal plane of hair l-VI; Hair 3 of segment, I-III highly elongated and single; hair 5, segments IV-VII remarkably elongate, single; hair 6-VII almost alike to hair 9, with 3- 4 simple, spiny branches which may be forked; hair 9, segments I-VI minute; 9-VII alike to six but typically larger and with more branches; 9-VIII larger than on VII with 3-15 simple or pointed branches which are normally forked; Paddle hair, 1-P oblong, nearly equal to 5-VII in development; paddle margins that are typically minutely and sparsely fringed on the basal half, with moderate sub marginal spiculation apically.

c. *Larva* (fig.4.6a): *Head* (4.6b, 4.7a): 1-1.05 mm in length somewhat round shape, brownish color, head seta 4C small variously(1-6) branched 6C single or with 2 equal branches, seta 4C closer to 6C than to 5-C, 6-C with 4-6 branches, basal maxillary hair with 3-4 branches, 7C alike to 6C and 14-C with 2-3 branches. Antenna long with spiculation, Hair, 1-A with 2- 4 branches. Hairs 5C antenna is short and narrow without an articulated apical segment. Thorax (4.7a) without four sets of stout dorsal spines, thoracic setae stellate. Metathoracic hair 7-T with elongated tapered branches and development into hair 6-M.

Abdomen: abdominal segment VIII (4.7 b) devoid of chitinized plate. Abdominal hair I-X not branched, infrequently divided sub basally. Hair 1-VIII with 1-6 branches, 2-VIII& 4-VIII, single, 3-VIII, with 6-10 branches, and 5-VIII with 4-8

branches. Respiratory siphon 2.2-2.61 mm in length, with single sub ventral setae, siphonal tuft not stout (fig. 4.6 c); tips usually reaching or slightly exceeding siphon apex. Air tube not over for time as long as basal width, air tube without an apical row of stout spines. Pecten teeth extend to near the apex of the air tube (fig.4.6 c). Pecten with 13-21 teeth; between siphonal tuft and apex of the air tube, two- three pecten teeth present. Comb scales arranged in a triangular patch (fig.4.6 (d, e) and 4.7b) or three rows with 30-61 comb scales usually pitchfork-shaped. Individual comb scales tapered distinctly to a stout central spine; denticles are conspicuous and elongate. Saddle, 0.36 mm in length. Ventral brush (4-X) with 4 or more pairs of seta, seems 10-14 tufts (4.7 b).

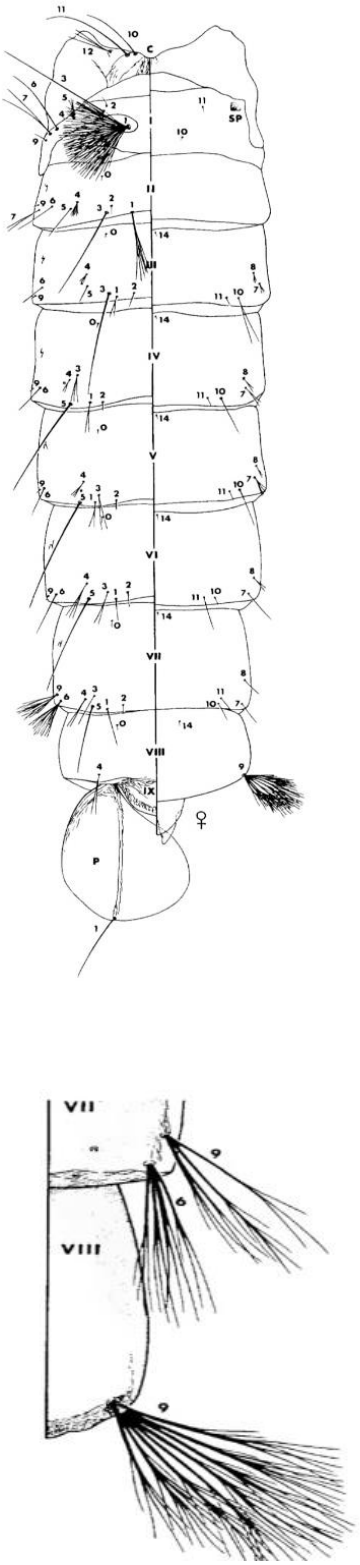
Adult *Aedes chrysolineatus* Fig 4.3 (a-k), Female Fig 4.3 (a-d): **(**a) whole specimen female, (b) Dorso-lateral view of female, (c) Dorsal view of proboscis, palpi female, (d) Lateral view of the female scutum

***Ae. chrysolineatus* Female, Fig 4.3 (e-h):** (e) Dorsal view of the scutum Female, (f) lateral view of hind leg female (g) lateral view of the foreleg female, (h) dorsal view of abdomen female

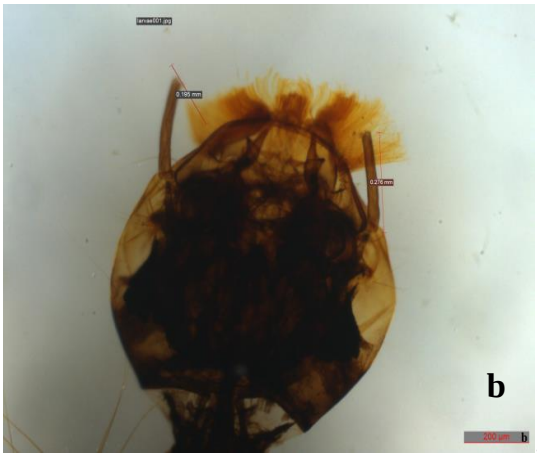
***Ae. chrysolineatus* Male, Fig 4.3 (i-k):** (i) dorsal view of the scutum male, (j) whole specimen male, (k) Lateral view of abdomen male

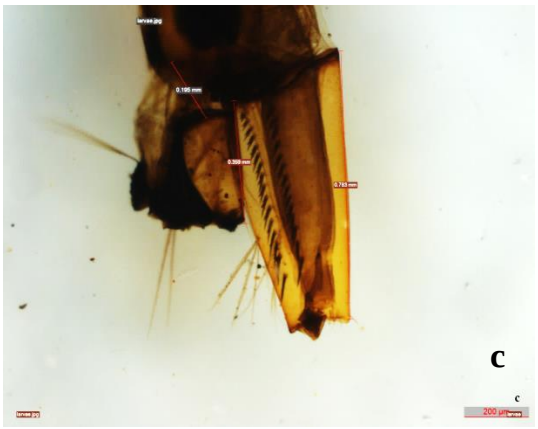
Pupa *Aedes chrysolineatus.* Fig. 4.4 (a-b): (a) whole pupae, (b) paddle with genital lobe

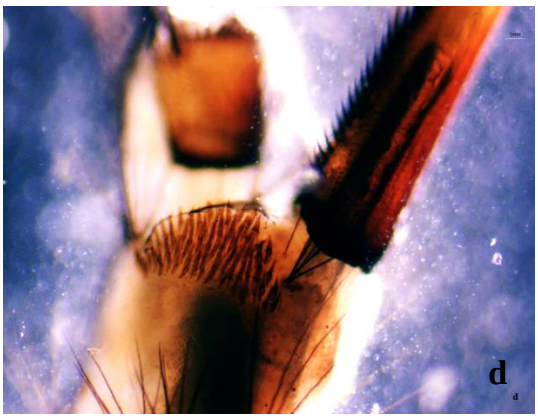
Fig. 4.5: **Diagrams pupae**



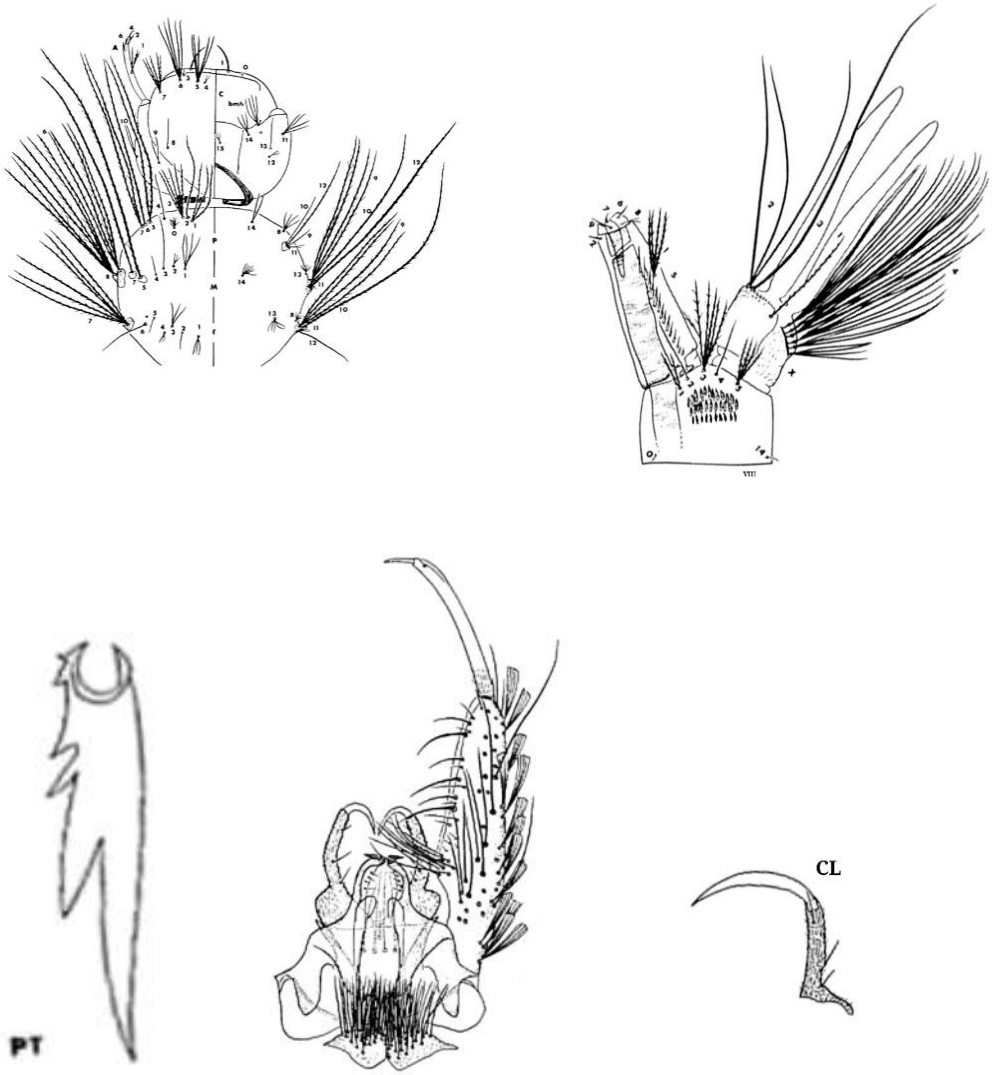
Larvae ***Aedes chrysolineatus*: Fig. 4.6 (a-e):** (a) whole larvae, (b) Head, (c) siphon tube with pecten teeth, (d- e) comb scales.







Diagrams larvae fig. 4.7(a-e): (a) Head, Thorax, (b) Segments VIII-X, (c) Pecten teeth, (d) Gonostylus, (e) Clavicle

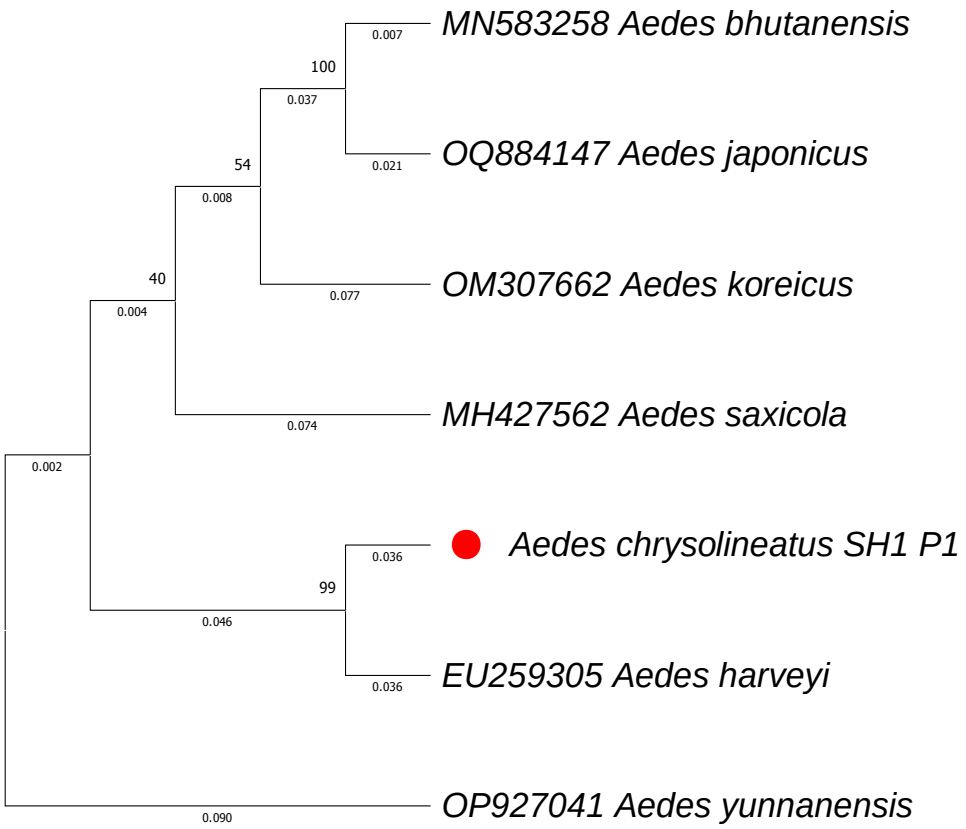


4. 32 *Taxonomy*

As mentioned earlier *Aedes chrysolineatus* was described by Theobald in 1907 as *Howardina chrysolineatus*. Earlier, it was included under the subgenus *Finlaya* and subgroup *Chrysolineatus* (Knight, 1968). Based on a comparative, morphological analysis of the female genitalia, *Finlaya* was divided into seven species assemblages, one of which is the *Chrysolineatus* Assemblage (Reinert, 2002). The *Chrysolineatus* Assemblage differs from other assemblages by the presence of the following characters of the female genitalia: characteristic round shape and absence of scales on cercus; tergum IX comprised of 2 moderately pigmented lateral plates separated by lighter pigmented area; posterior margin of sternum VIII with minute to small median emargination, with numerous short, slightly curved setae (Natarajan *et al*., 2016). Reinert *et al*., (2008) assigned the *Chrysolineatus* subgroup to the genus *Hulecoeteomyia*, which was later downgraded to the status of the subgenus (Wilkerson, 2015).

Phylogenetic Analysis: Phylogenetic analysis has shown that *Aedes chrysolineatus* is more associated with *Aedes harveyi* (EU259305). They were found in the same clade and are monophyletic. *Ae. chrysolineatus* is paraphyletic with *Ae. saxicola*, *Ae. koreicus*, *Ae. japonicus* and *Ae. bhutanensis* were found more distant from it. *Ae. yunnanensis* is distant forming an outgroup in the tree (Fig.4.8).

Phylogenetic tree (Fig. 4.8): Neighbor-Joining showing the phylogenetic relationship of different *Aedes* mosquitoes based on their mitochondrial COXI gene sequences



CHAPTER V

GEOGRAPHICAL DISTRIBUTION AND HABITAT DIVERSITY OF *AEDES CHRYSOLINEATUS*

5.1 Introduction

Mosquito species vary in geographical distribution and habitat diversity. The geographical distribution is influenced by various physical and climatological factors. The habitats of mosquitoes are exceptionally varied from small to large containers and also vary from species to species depending upon environmental and climatic conditions. Some species choose habitats with vegetation, some breed in open, bright pools, and also in leaf axils of some plants, arboreal cavities, and artificial containers (Carpenter & Lacasse, 1954; Manzoor *et al.*, 2013). Mosquito distribution also depends on the geographical and climatological peculiarities.

Each mosquito species has its breeding preference. The immature forms of mosquitoes were collected from the sides of the slow-running stream, crevices in rock and tree holes, and from a grinding stone, ground pools, blocked gutters, and empty cans (Amala & Anuradha, 2012; Ikpeama *et al.*, 2017). Immature forms of many *Anopheles* species were collected in the shady areas of the stream and grassy margins of the slow-running streams (Amala & Anuradha, 2012). *Culex quinquefasciatus* prefer to breed in drainages, polluted water in ditches, and water accumulated in a variety of containers: ceramic vessels, plastic vessels, metal vessels, tucker boxes, and plastic water barrels (Ikpeama *et al.*, 2017; Prechaporn *et al.*, 2007).

Many of the *Aedes* species are container breeders, breeding in natural habitats such as tree holes, rock pools, bamboo stumps, leaf axils and coconut shells, artificial containers and tyres (Bonizzoni *et al.*, 2013), in some instances, cesspits and sewage systems were also exploited (Pramanik *et al.*, 2007). The *Aedes* genus of mosquitos reproduces in phytotelmata at considerably lesser elevation and with a reduced water capacity compared to the *Culex* genus (Adebote *et al.*, 2008). Larval

forms of *Ae. aegypti* and *Ae. albopictus* were identified in internodes of bamboo (Müller *et al.,* 2022). Breeding adaptability of *Ae. chrysolineatus* was found in latex collecting cups, tree holes, areca leaf sheaths, and domestic containers (Shanasree & Sumodan, 2019a, 2019b; Sumodan, 2012). Larvae of *Ae. chrysolineatus,* along with other species such as *Ae. albopictus, Ae. greeni, Ae. vittatus, Ae. aegypti, Ar. aureolineatus, Ar. subalbatus, Culex mimuloides, Cx. quinquefasciatus, Hz. greeni and Tx. spendens* were obtained from coconut shells, discarded containers, dirty water pools, and *Ae. vexans* were found in paddy fields and dirty water pools (Balasubramanian & Nikhil, 2013). Breeding of *Ae. albopictus* has also been reported in rock holes and *Ae. aegypti* in grinding stones (Amala & Anuradha, 2012). *Aedes aegypti* breeds in various freshwater containers: drums, buckets, tyres, and pots (Ngugi *et al.,* 2017). There are only very few studies on the habitat diversity and distribution of *Aedes chrysolineatus* in Kerala. In the present study a detailed survey to map the distribution and also to elucidate the habitat diversity of this species was carried out in five North Kerala districts viz., Kasaragod, Kannur, Kozhikode, Wayanad, and Malappuram from 2017 to 2021.

5.2 Materials and Methods

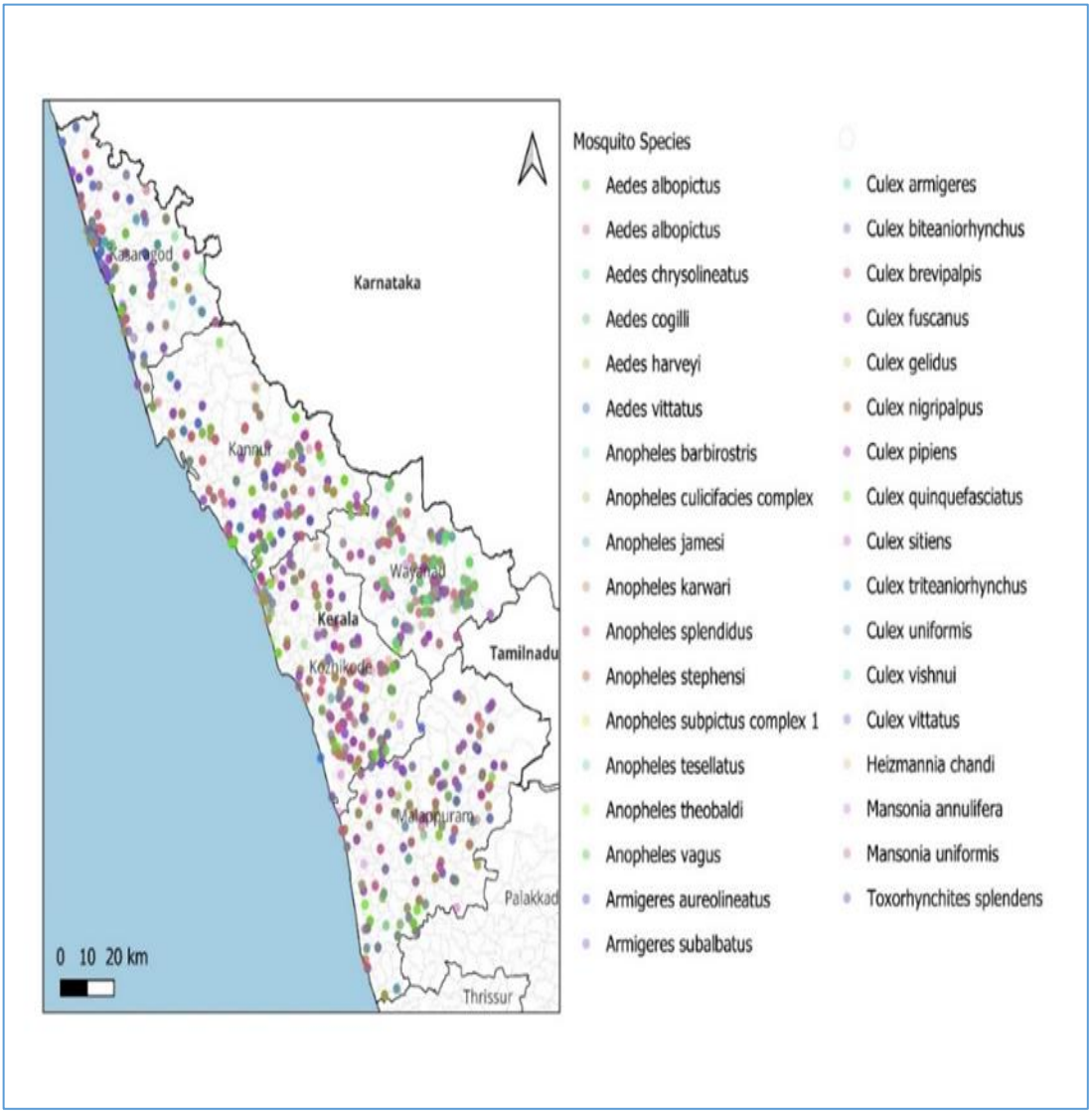
Study area, survey of *Ae. chrysolineatus* immature were done as methods described in Chapter III, section 3.3, and data analysis: geographical distribution of the *Ae. chrysolineatus,* altitudinal distribution, seasonal fluctuation in the species density and map preparations were also described in the same chapter, sections (3.91, 3.92, 3.93, and 3.94).

5.3 Results

5.31 District wise distribution of Aedes chrysolineatus in North Kerala

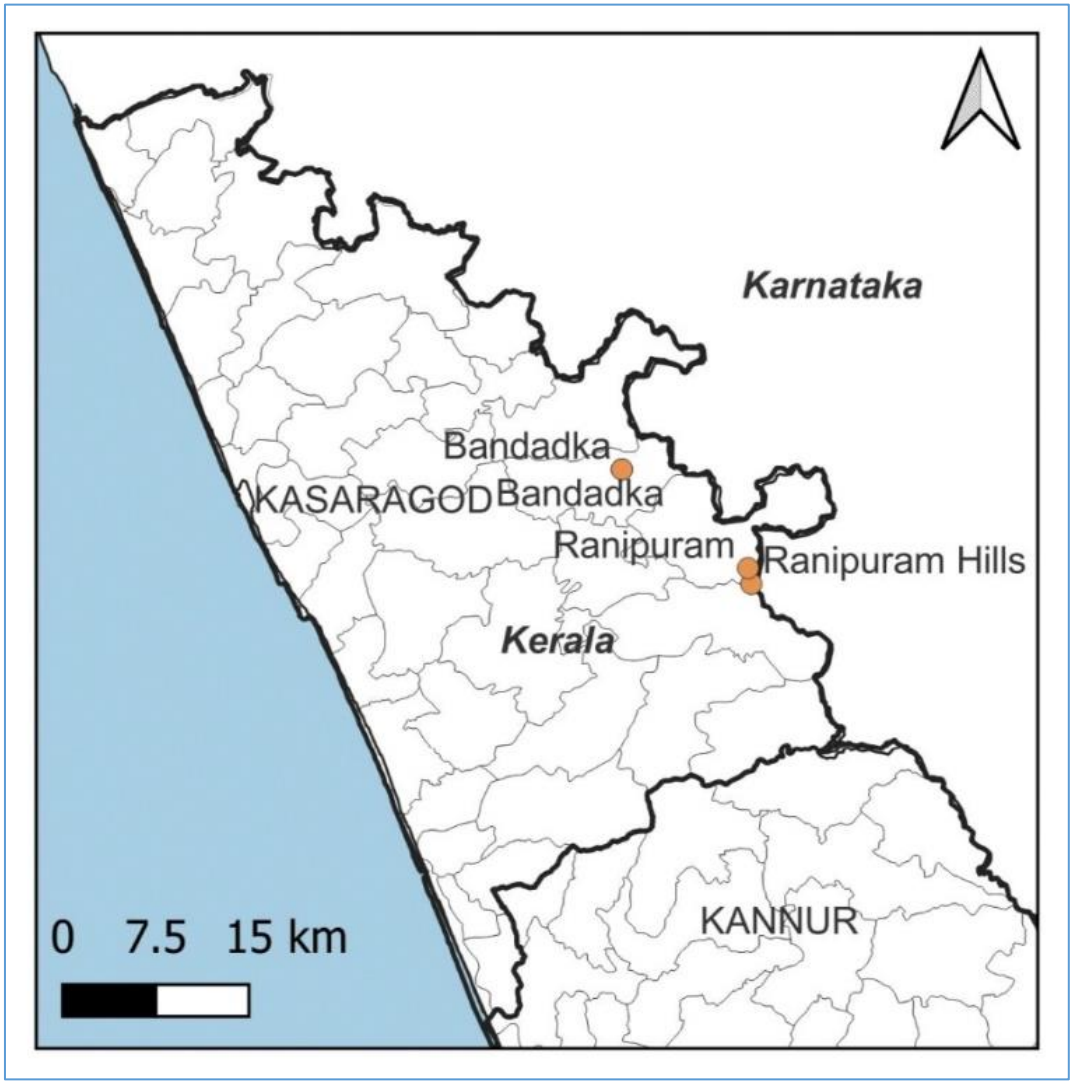
Map 5.1 shows the localities where the surveys for the breeding of *Ae. chrysolineatus* were carried out. A total of 8418 localities were surveyed. Besides, *Ae. chrysolineatus* 34 other species were also obtained from the surveys. Separate color codes are given the species. The details of district wise distribution of *Ae. chrysolineatus* are given separately in the following sections.

Map (5.1): Survey sites in North Kerala and the mosquito species emerged



Kasaragod district: Kasaragod district has a total of 38 panchayats. Mosquito surveys were carried out in all panchayats. Out of 38 panchayats, breeding of *Ae. chrysolineatus* was observed in two panchayats only (5.2%)(Table.5.1). These two Panchayats were Panathady and Kuttikol (Map. 5.2).

Map (5.2): Distribution of *Ae. chrysolineatus* in Kasaragod district

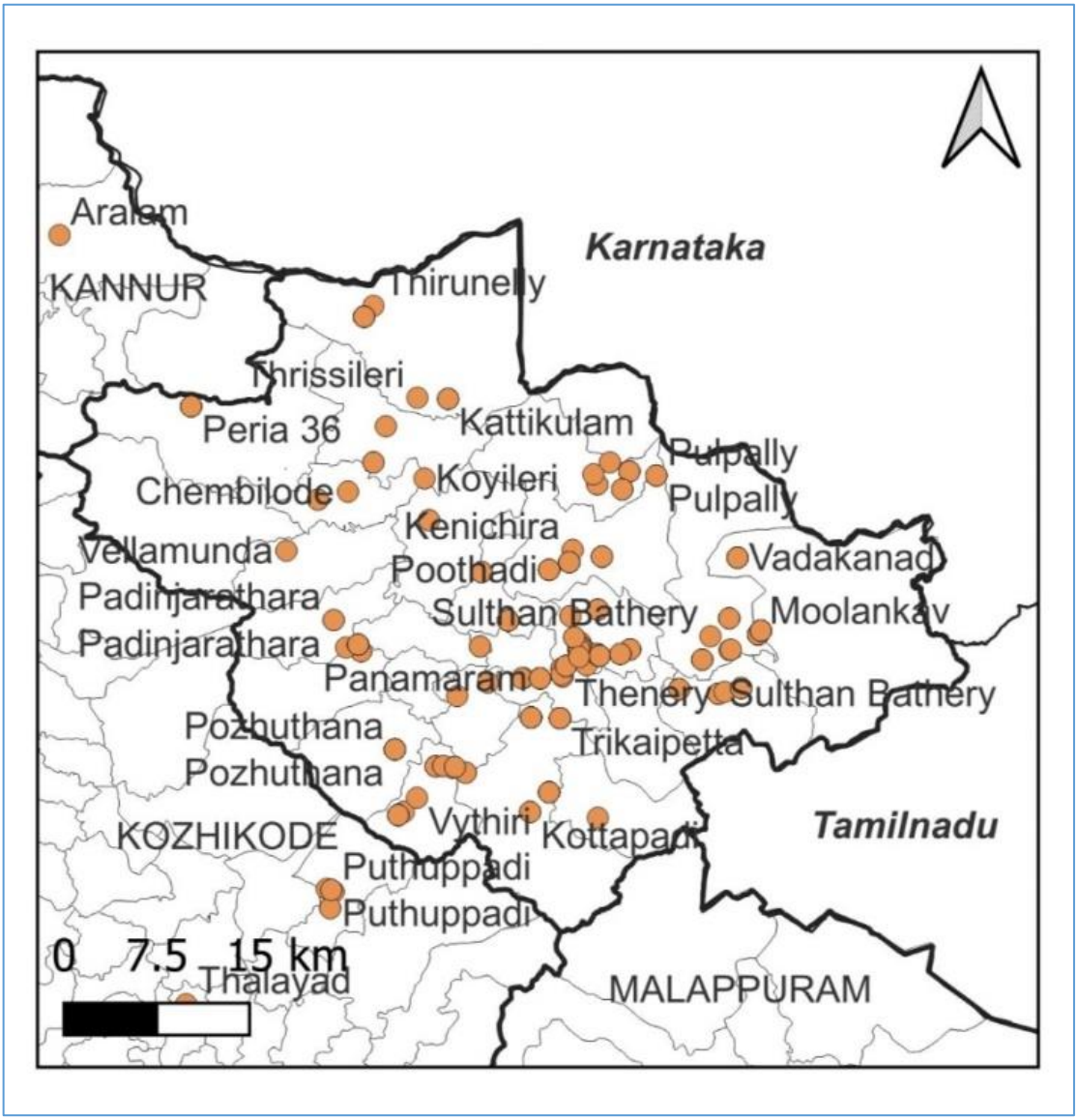


Kannur district: Kannur district has a total of 71 panchayats. Mosquito surveys were carried out in all panchayats (Map.5.3). Out of 71 Panchayats, breeding of *Ae. chrysolineatus* was observed in four Panchayats (5.6%) (Table.5.1). The four panchayats were Aralam, Maloor, Chittariparamba, and Kolayad.

Map (5.3): Distribution of *Ae. chrysolineatus* in Kannur district

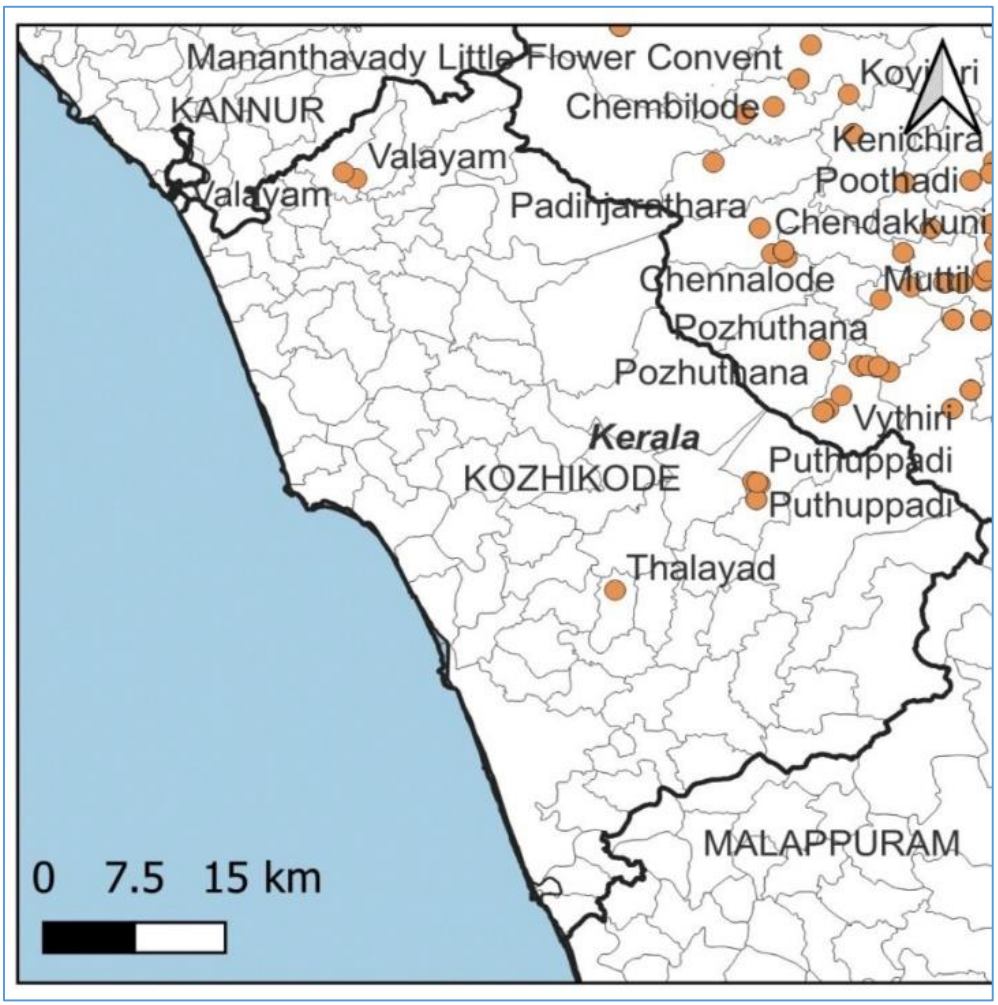
Wayanad district: Wayanad district has a total of 23 panchayats. Mosquito surveys were carried out in all panchayats. Out of 23 Panchayats, breeding of *Ae. chrysolineatus* was observed in all Panchayats (100%)(Table.5.1). The panchayats were, Ambalavayal, Edavaka, Kaniyambetta, Kottathara, Meenangadi, Meppadi, Mullankolly, Muppainadu, Muttil, Nenmeni, Noolpuzha, Padinharathara, Panamaram, Poothadi, Pozhuthana, Pulppalli, Thariode, Thavinhal, Thirunelly, Thodernadu, Vellamunda, Vengapally, and Vythiri (Map 5.4).

Map (5.4): Distribution of *Ae. chrysolineatus* in Wayanad district



Kozhikode district: Kozhikode district has a total of 70 panchayats. Mosquito surveys were carried out in all panchayats. Out of 70 Panchayats, breeding of *Ae. chrysolineatus* was observed in three Panchayats (4.2%)(Table.5.1). The three panchayats were Puthuppady, Koorachund, and Valayam respectively (Map 5.5).

Map (5.5): Distribution of *Ae. chrysolineatus* in Kozhikode district



Malappuram district: Malappuram district has a total of 94 panchayats. Mosquito surveys were carried out in all panchayats. Out of 94 Panchayats, breeding of *Ae. chrysolineatus* was observed in four Panchayats (4.2%)(Table.5.1). The three panchayats respectively were Chokkadu, Pandikkad, Karulai, and Tuvvur (Map 5.6).

Map (5.6): Distribution of *Ae. chrysolineatus* in Malappuram district

Table.5.1: Distribution of *Ae.chrysolineatus* in North Kerala district panchayats

Sl. No	Districts	Total no. of Panchayats	Number positive for *Ae. chrysolineatus* in Panchayats
1	Kasaragod	38	2 (5.2%)
2	Kannur	71	4 (5.6%)
3	Wayanad	23	28 (100%)
4	Kozhikode	70	3(4.2%)
5	Malappuram	94	4(4.2%)

5.32 *Altitudinal distribution of Aedes chrysolineatus*

The study reveals the occurrence of *Ae. chrysolineatus* within the altitudinal range of 20-1200 m elevation. The distribution of *Ae. chrysolineatus* according to the altitude or elevation is shown in (Table. 5.2, Fig.5.1, and Map.5.7). *Ae. chrysolineatus* was widespread within the range of 600-1200m. The maximum of 90.1% of the species distribution was found within the high range (600-1200 m), followed by midland (20-100 m) with 8.01%, mid upland (100-300 m) with (1.26%), and (0.57%) distribution was found within the upland range of 300-600 m. The species was not observed within the lowland region of (0-20 m) range of elevation.

Map (5.7): Altitudinal distribution of *Ae. chrysolineatus* in North Kerala

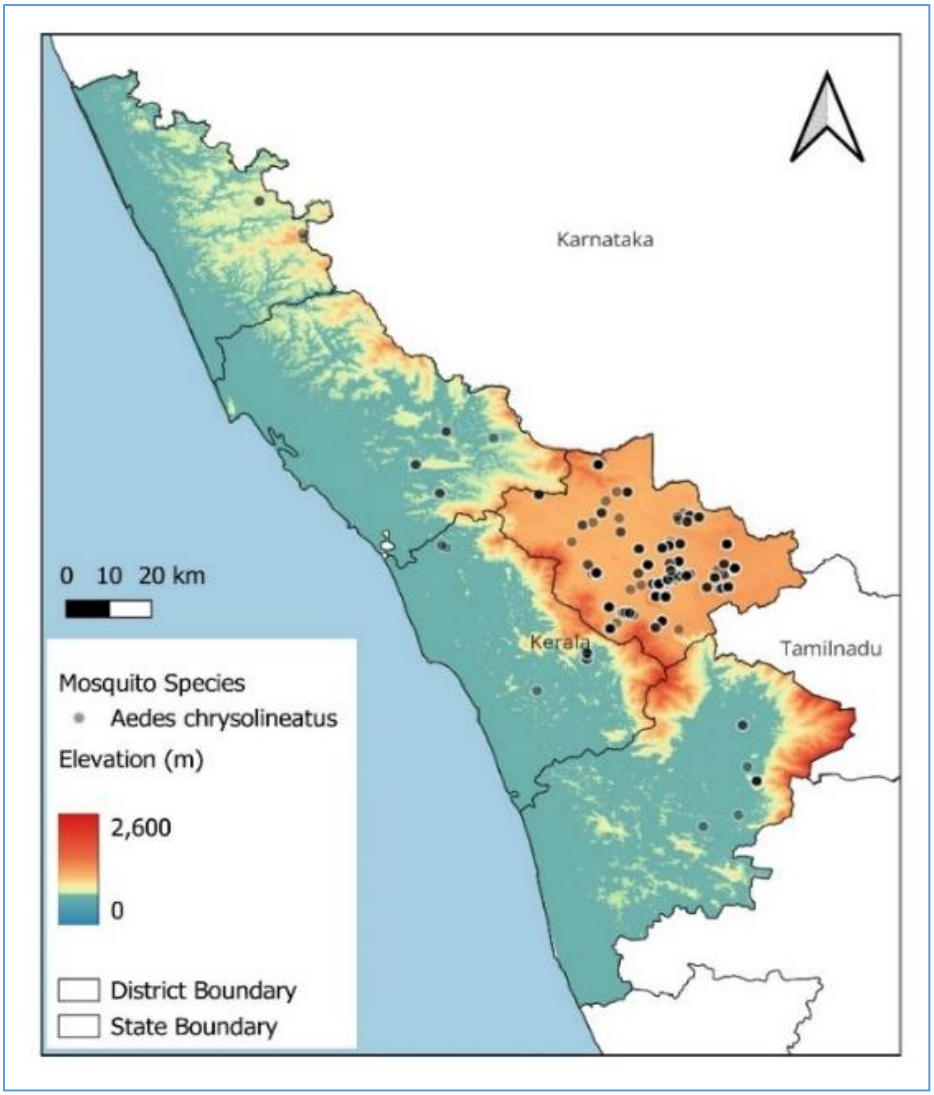
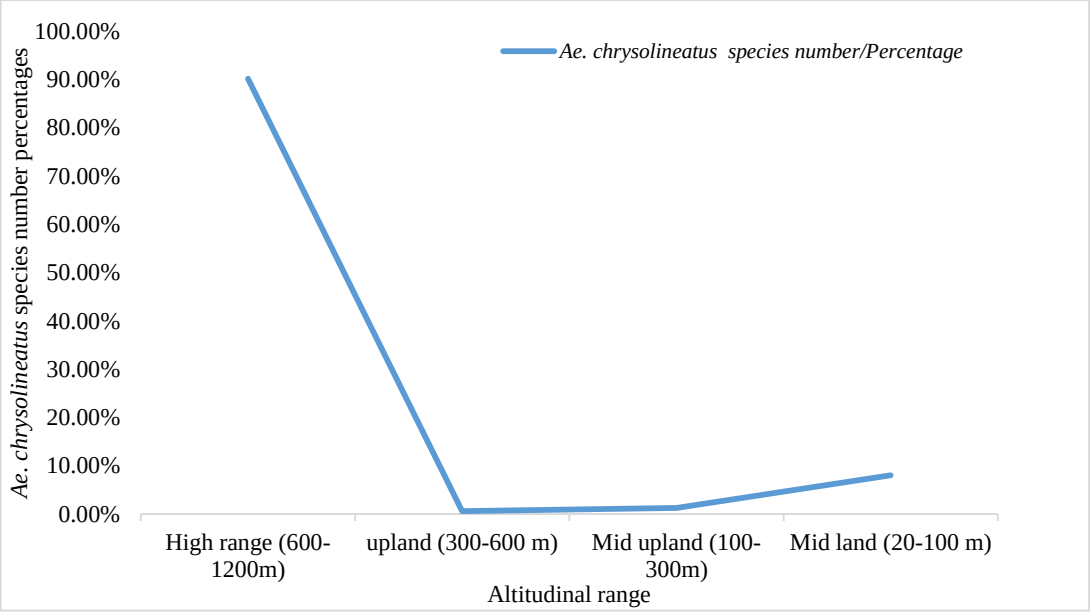


Table.5.2: Altitudinal distribution of *Ae. chrysolineatus*

Terrains	Altitudinal distribution of *Ae. chrysolineaus in* North Kerala					
	Wayanad	**Kozhikode**	**Malappuram**	**Kannur**	**Kasaragod**	**Total**
High range (600-1200m)	2359 (90.07%)	0	0	0	2 (0.07%)	**2361 (90.1%)**
Up land (20-100m)	0	0	0	0	15 (0.57%)	**15(0.57%)**

Mid upland (100-300m)	0	0	33 (1.52%)	0	0	**33 (1.26%)**
Mid land (20-100m)	0	90 (3.43%)	47 (1.79%)	65 (2.48%)	8 (0.30%)	**210 (8.01%)**
	2359 (90.07%)	**90 (3.43%)**	**80 (3.05%)**	**65 (2.48%)**	**25 (0.95%)**	**2619**

Fig.5.1: Distribution of *Ae. chrysolineatus* according to elevation ranges



5.33 Monthly variation in the Aedes chrysolineatus density

Ae. chrysolineatus showed monthly variation in density, which was reflected in the emergence of adults from the samples collected. Except in April, breeding of the species was observed in all eleven months. The maximum density was obtained in July (22.06%), followed by September (21.30%). The lowest density was in May (0.64%). The densities in other months were as follows: November (13.6%), February (9.62%), October (8.05%), August (6.52%), March (6.3%), December (6.1%), June (3.58%) and January (2.06%) respectively (Table 5.3, Fig.5.2).

The year-wise distribution of *Ae. chrysolineatus* shows that the highest number of species was observed in 2019, followed by the order 2020>2018>2021>2017 each constituted with the species numbers 47.15%, 17.60%, 13.70%, 13.60% and 7.78% respectively (Fig.5.3). The highest number of species were found in September 2019, constituting 5.5% of the total collection. In comparison, the lowest was found in January 2021, which represents 0.076%. In 2017, species number was found to be high in September and lowest in November, constituting 2.63% and 0.19%. In 2018, July represented the highest species number and the lowest was observed in November with 0.57%, whereas in 2019, the lowest number of species was found in August with 0.22%. In 2020 and 2021, the highest number of species were found in July, which is 8.74% and 8.43%, respectively. Moreover, the lowest percentages were in October, 2.9% and January, 0.076%, respectively.

Table.5.3: Monthly variation in *Ae. chrysolineatus* numbers

Month	Number of *Ae. chrysolineatus*	%
January	54	2.06%
February	252	9.62%
March	165	6.3%
May	17	0.64%
June	94	3.58%
July	578	22.06%
August	171	6.52%
September	558	21.3%
October	211	8.05%
November	358	13.6%
December	161	6.1%
Total (N)	**2619**	**100**

Fig. 5.2: Monthly distribution of *Ae. chrysolineatus*

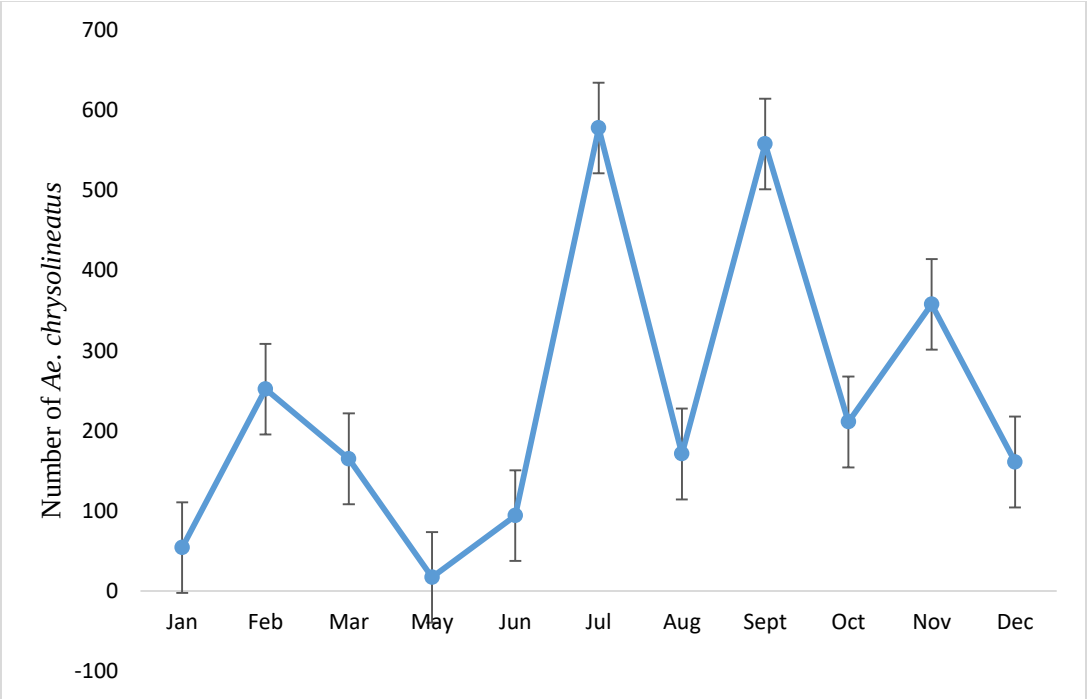
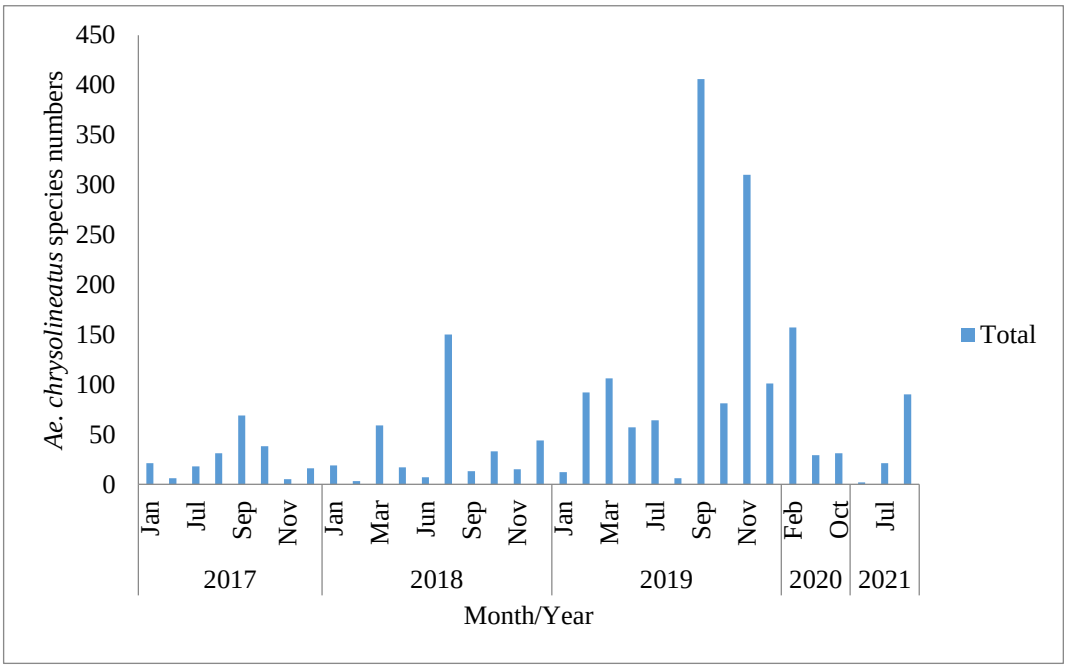


Fig. 5.3 Years wise density pattern of *Ae. chrysolineatus*



5. 34 *Habitat diversity of Aedes chrysolineatus in North Kerala*

A total of 4505 (53.5%) (Table.5.4) positive larval habitats were identified, of which 8.6% had *Ae. chrysolineatus* breeding. Overall, 23 habitats were recorded, of which seventeen (73.9%) habitats supported *Ae. chrysolineatus* breeding. The available habitats in the study area were, latex collecting cups (20.05%), areca leaf sheath (18.76%), tree holes (15.42%), plastic containers (10.53%) and plastic sheet/cover (10.28%), coconut shell (5.9%), fallen leaves (0.77%), Tank (3.59%), Tires (1.79%), metal containers (1.79%), boat (1.28%), flower pot (1.79%), Thermocol (1.54%), cattle shed (0.25%), footprint (0.25%), rock hole (0.25%) and others (5.6%) (Grinding stone, broken toys, disposable plates, glass bottle, Aluminum foil, polythene sheet, Rubber bucket etc). District- wise breeding habitat preferences of *Ae. chrysolineatus* is given in (Table. 5.5, Table 5.6, Fig. 5.4, and Fig. 5.5 a-z).

Table.5.4: Details of positive breeding habitats of five districts based on total habitats surveyed

Sl. No	Districts	Total no. of habitats surveyed	Number of positive habitats	Number of habitats positive for *Ae. chrysolineatus* larvae
1	Kasaragod	1232	713 (57.8%)	4 (0.56%)
2	Kannur	2026	954 (47.08%)	7 (0.73%)
3	Wayanad	1018	887 (87.1%)	359 (40.8%)
4	Kozhikode	1827	879 (48.1%)	10 (1.13%)
5	Malappuram	2315	1072 (46.3%)	9 (0.83%)
Total		8418	4505 (53.5%)	389 (8.6%)

Kasaragod district: A total of 1232 breeding habitats were surveyed in Kasaragod district. Out of the 1232 habitats, (57.8%) were positive for mosquito larval presence. Breeding of *Ae. chrysolineatus* was observed in four habitats (0.56%). There was only one habitat, viz., areca leaf sheaths, supported *Ae. chrysolineatus* breeding.

Kannur district: Of the total 2026 breeding habitats surveyed, (47.08%) were with mosquito larval presence, of which breeding of *Ae. chrysolineatus* was observed in seven habitats (0.73%). The habitats were areca leaf sheaths, represented by three in number (42.8%), tree holes, and drum each with two in number (28.5%) respectively.

Wayanad district: Total of 1018 mosquito breeding habitats surveyed in Wayanad district. Out of the 1018 habitats, (87.1%) were positive for mosquito larvae, of which breeding of *Ae. chrysolineatus* was observed in 359 habitats (40.5%). The species prefer to breed in both natural and artificial breeding habitats; the prime habitats noted were, areca leaf sheaths (18.10%), latex collecting cups (17.82%), tree holes (15.59%), plastic containers (11.42%), plastic sheets/cover (11.14%), coconut shell and others (6.12%) each. *Aedes chrysolineatus* (51.4%) was determined to be the dominating species in the areca leaf sheath (Shanasree & Sumodan, 2019a). Furthermore, the species least utilize habitats such as tanks

(3.34%), metal containers, flower pots and tires, each with (1.94%), and habitats such as boats (1.39%), Thermocol (1.67%), In the Wayanad district, Areca leaf sheaths, latex collecting cups and tree holes form the prime breeding habitat of the species, which together constitute 50% of the positive *Ae. chrysolineatus* containers.

Kozhikode district: Out of the 1827 habitat surveyed, (48.1%) were with mosquito larval presence, of which breeding of *Ae. chrysolineatus* was observed in 10 habitats (1.13%). The significant habitat in the study area were, latex collecting cups with six in number (60%), areca leaf sheath, coconut shell, fallen leaves, and tree holes each represented by one habitats each with (10%) respectively.

Malappuram district: As like other districts, 2315 habitats were surveyed, out of the 2315 habitats, (46.3%) were with larval presence, of which, breeding of *Ae. chrysolineatus* was observed in nine habitats (0.83%). The *Ae. chrysolineatus* positive habitats were, latex collecting cups, eight in number (88.8%), and tree holes represents one habitat (11.11%) respectively.

Table.5.5: Breeding habitat preferences of *Ae. chrysolineatus* in each district

Sl. no	*Ae. chrysolineatus* positive breeding habitats	No. of habitats in each Districts					Total
		Kasaragod	Kannur	Wayanad	Kozhikode	Malappuram	
1	Areca leaf sheath	4 (100%)	3 (42.8%)	65 (18.1%)	1 (10%)		73 (18.7%)
2	Latex collecting cups			64 (17.8%)	6 (60%)	8 (88.8%)	78 (20.05%
3	Tree holes		2 (28.5%)	56 (15.59%)	1 (10%)	1 (11.1%)	60 (15.4%)
4	Plastic container			41 (11.4%)			41 (10.5%)
5	Plastic sheet/cover			40 (11.14%)			40 (10.2%)
6	Coconut shell			22 (6.12%)	1 (10%)		23 (5.9%)
7	Tank/ Drum		2 (28.5%)	12 (3.34%)			14 (3.5%)
8	Flower pots			7 (1.94%)			7 (1.79%)
9	Tyre			7 (1.94%)			7 (1.79%)
10	Metal container			7 (1.94%)			7 (1.79%)
11	Thermocol			6 (1.64%)			6 (1.54%)

12	Boat			5 (1.39%)			5 (1.28%)
13	Fallen leaves			2 (0.55%)	1 (10%)		3 (0.77%)
14	Foot print			1 (0.27%)			1 (0.25%)
15	Rock hole			1 (0.27%)			1 (0.25%)
16	Cattle shed			1 (0.27%)			1 (0.25%)
17	Others			22 (6.12%)			22 (5.6%)
	Total	4 (0.56%)	7 (0.73%)	359 (40.5%)	10 (1.13%)	9 (0.83%)	**389**

**Others(Grinding stone, broken toys, disposable plates, glass bottle, Aluminum foil, polythene sheet, Rubber bucket)*

Table. 5.6: Different types of habitat supporting breeding of *Ae. chrysolineatus* in the study area

Types of habitat	Water holding positive habitats		*Ae. chrysolineatus* (+ve) habitats	
	N =4505	%	N= 389(8.6%)	%
Plastic container	663	14.7	41	10.53
Latex collecting cups	637	14.13	78	20.05
Areca leaf sheath	616	13.67	73	18.76
Coconut shell	472	10.47	23	5.91
Plastic sheet/cover	450	9.9	40	10.28
Drum/tank	438	9.72	14	3.59
Tree holes	268	5.94	60	15.42
Flower pot/mud pot	155	3.44	7	1.79
Metal container	137	3.04	7	1.79
Fallen leaves	128	2.84	3	0.77
Tire	103	2.28	7	1.79
Thermocol	55	1.22	6	1.54
Boat	48	1.06	5	1.28
Rock hole	19	0.42	1	0.25
River side/pond/pool	14	0.31	-	-
Cattle shed	9	0.19	1	0.25
Rice field	7	0.15	-	-
Concrete canal	5	0.11	-	-
Ditches/swamps	3	0.06	-	-
Foot print	3	0.06	1	0.25
Bamboo stumps	2	0.04	-	-
Marshy areas	3	0.0.6	-	-
Others	270	5.9	22	5.6

Fig. 5.4: Different types of habitat supporting breeding of *Ae. chrysolineatus*

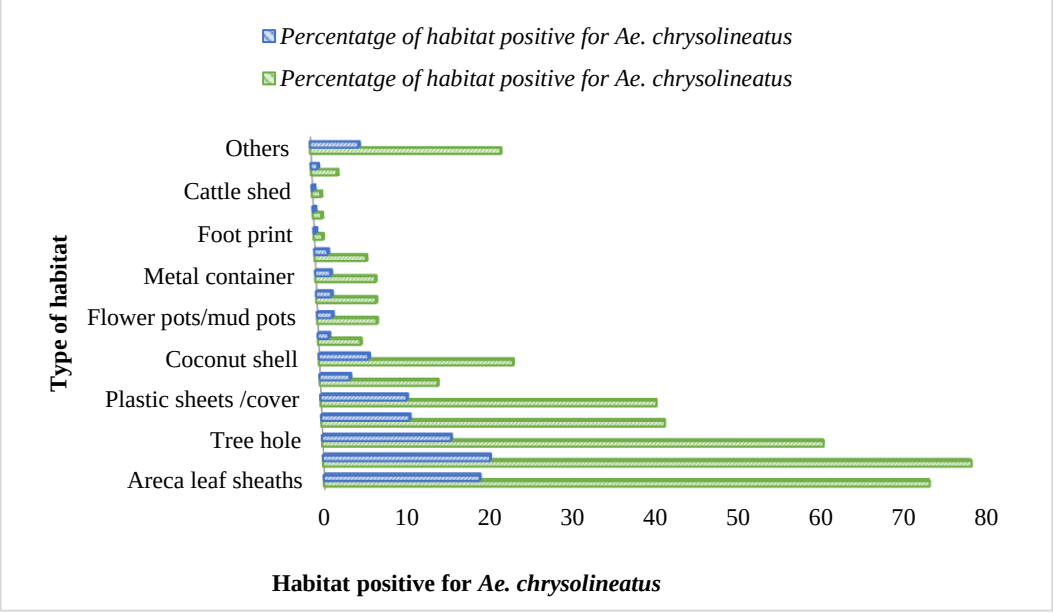


Fig. 5.5 (a-d): Habitat diversity photograph of *Ae. chrysolineatus* (a) latex collecting cups, (b) rock holes, (c) areca cut hole, (d) areca leaf sheaths

Fig. 5.5 (e-h): (e) steel container, (f) bucket, (g) rubber cut hole, (h) cement tank

Fig. 5.5 (i-l): (i) coconut cut hole, (j) plastic sheet holding water, (k) plastic mug, (l) grinding stone

Fig. 5.5 (m-p): (m) plastic cups, (n) aluminum container, (o) pots, (p) cooker

Fig. 5.5 (q-t): (q) steel cup, (r) coffee tree hole, (s) cashew tree hole, (t) tyre

Fig. 5.5 (u-x): (u) plastic cover, (v) plastic bottle, (w) bamboo cuts, (x) broken chair

Fig. 5.5 (y-z): (y) coconut shell, (z) tank

5.35 Habitat of other species

The habitat diversity of 34 species of mosquitos detected during the study period is shown in (Table.5.7). *Ae. albopictus, Ae. chrysolineatus, Ae. vittatus, Ar. subalbatus, Cx. quinquefasciatus, Cx. pipiens* were found in more than 15 habitats. A total of 23 habitats were recorded; the available habitats in the study area were tree holes, fallen leaves, coconut shells, latex collecting cups, plastic containers, plastic sheet/cover metal containers, rock holes, areca sheaths, tyre, boats, flower pots/mud pot, bamboo stumps, rice field, footprints, thermocol, cattle shed, drainage/canal, marshy areas, river/pond/pool, and ditches/swamps and others (broken toys, shoe holding water, pipe, disposable plate, grinding stone, glass bottle etc). A total of 4505 containers were identified as potential breeding sites. The prime habitats were the plastic containers with (14.71%), latex collecting cups with (14.13%), areca sheaths with (13.67%) and (10. 47%) of coconut shells, plastic sheets/ covers, (9.9%) of tree holes, and others with (5.9%) each, respectively.

Plastic sheets/covers were noted as the significant breeding habitat for mosquitoes, harboring 18 species, followed by areca sheaths with 17 species, and 16 species each were found in the tree holes, latex collecting cups and plastic containers, coconut shell and other types of habitats each with 15 species, fallen leaves and boat with 13 and 12 species each, 11 species each were found in the tyre, tank and rock holes. Nine types of habitat harbor less than ten species (Table. 5.7).

Table. 5.7: Diversity of breeding habitats of various mosquito species in the study area

Immature Habitats / Species	Tree holes (coffee, jack, oak,)	Fallen leaves	Coconut shell	Bamboo stumps	Rice fields	Plastic containers	Plastic sheet/ cover	Tyre	Boat	Metal containers	Latex collecting cup	Areca leaf sheath	Rock holes	Footprints	Flower/ mud plots	Thermocol	Tank/ Drum	Drainage/ canal side	Marshy areas	River/ponds/pool	Ditches/swamps	Cattle shed	Others	Total
Ae. albopictus	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	19
Ae. chrysolineatus	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	17
Ae. harveyi	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Ae. vittatus	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	16
Ae. cogilli	+	+	+	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	7
Cx. quinquefasciatus	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	16
Cx. uniittatus	+	+	+	-	+	+	+	+	-	+	+	+	-	+	+	-	-	-	-	+	-	+	+	15
Cx. brevipalpis	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	14
Cx. vishnui	+	+	+	-	-	+	+	+	-	+	+	+	-	-	+	-	+	-	-	-	-	+	+	13
Cx. uniformis	-	+	-	+	-	+	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	8
Cx. fuscanus	-	+	+	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	8
Cx. vittatus	-		-	-	-	-		-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	4
Cx. nigripalpus	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2

Cx. sitiens	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	6
Cx. gelidus	+	+	-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	6
Cx. triteaniorhynchus	+	+	+	-	-	+	+	+	+	-	+	+	+	-	+		+	-	-	-	-	-	+	13
Cx. biteaniorhynchus	-		-	-	-	-		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Cx. armigeres	-		-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1
Ar. subalbatus	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	17
Ar. aureolineatus	+	+	+	-	-	+	+	+	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	11
Tx. splendens	+	+	+	-	-	-		-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	5
Hz. chandi	+	-	+	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	7
Mn. uniformis	-		-	-	-	-		-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1
Mn. annulifera	-		+	-	-	+		-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	3
An. barbirostris	-		-	-	-	-		-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	2
An. karwari	-		-	-	+	-		-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	3
An. stephensi	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	6
An. culicifacies	-		-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	1
An. theobaldi	-		-	-	+	-		-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	3
An. vagus	-		-	-	-	+		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	2
An. jamesi	-		-	-	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
An. subpictus complex	-		-	-	-	-		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
An. splendidus	-		-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
An. tessellatus	-		-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1
Total	**16**	**13**	**15**	**2**	**6**	**16**	**18**	**11**	**12**	**10**	**16**	**17**	**11**	**3**	**10**	**6**	**11**	**2**	**2**	**8**	**3**	**7**	15	

'+' Sign indicates the presence and – 'the absence

5.4 Discussion and Conclusion

Geographical distribution of *Aedes chrysolineatus*: Breeding of *Aedes chrysolineatus* was observed in all study districts. However, the extent of its prevalence was varied among the districts. The highest prevalence was observed in Wayanad district as all the 23 Panchayats had its presence. The prevalence of the species in all other four districts are almost similar emerging from 4.2% (Malappuram and Kozhikode) to 5.6% (Kannur). Kasaragod has a prevalence almost near to that of Kannur (5.2%) (Table.5.1). The reason why the prevalence is so high in Wayanad could be safely attributed to the high elevation of the district. Maximum distribution of the species was within an altitude range of 600-1200 meters (Table.5.2). The entire Wayanad district lies at an altitude range between 700-2100 meters. All other districts are in the valley between the Arabian Sea and the Western Ghats. Hence, *Aedes chrysolineatus* can be considered as a high altitude species. However, since they were also observed breeding at altitudes 20-100 meters in all the four districts, it may not be difficult for the species to adapt to low altitudes also. Hence, their invasion to the coastal areas is also a possibility.

Habitat diversity: Similar to the other *Aedes* species, *Aedes chrysolineatus* has also been found to be container breeder. An array of 17 types of habitats were found to be the major habitats along with several minor ones. Both natural and artificial habitats supported breeding. It is interesting to note that the top two habitats- latex collecting cups (20.05%) and areca leaf sheaths (18.76%) are anthropogenic habitats as they are related to rubber plantations and areca farms respectively. While the first one is an artificial habitat, the second one is a natural one. Another habitat of major significance is tree holes. Barraud, (1934), reported tree holes, bamboo, and rock pools as their natural habitats. It is likely that they had adapted to the new habitats generated by land use changes. Sumodan, (2003) reported the importance of rubber plantations as an important ecosystem for the proliferation of the dengue vector *Aedes albopictus* in latex collection cups. Since latex collection cups is a preferred habitat of *Aedes chrysolineatus* also, investigating the interactions of these two species in the same habitat could be of significant interest.

CHAPTER VI

CO-BREEDING OF *AEDES CHRYSOLINEATUS* WITH OTHER MOSQUITO SPECIES

6.1 Introduction

Multiple species of mosquitoes hatch in the same container and share limited space and resources and similar habitat requirements and coexistence happens (Arnaldo, 2006). Co-breeding of species in the same tree hole is a strong indication of the enrichment of nutrients in that habitat (Shanasree & Sumodan, 2019). Competition in co-breeding habitats for resources leads to either displacement or reduction in the production of some species. Niche partitioning in breeding habitats concurrently leads to stable coexistence of competing species (Gilbert *et al.*, 2008). In the present study, the co-existence of *Ae. chrysolineatus* with other species have been investigated with the objectives of understanding the diversity of co-existing species, its distribution pattern and also its abundance with respect to other species.

6.2 Methodology

Survey of the immature species of *Ae. chrysolineatus* and *Ae. albopictus* were done as per the methods described in Chapter III, section (3.3). The data were analyzed using analysis of variance (ANOVA) with Microsoft Excel (mentioned in Chapter III, section 3.96: a)

6.3 Results

6.31 *Co-breeding of Aedes chrysolineatus with other mosquito species*

Aedes chrysolineatus was found breeding in association with other species in 55.8% (n=217) of the breeding sites in which they were positive. In the remaining habitats (44.2%) the breeding was alone. Co-breeding was observed with 11 species viz., *Aedes albopictus, Ae. cogilli, Ae. vittatus, Armigeres subalbatus, Ar. aureolineatus, Culex biteaniorhynchus, Cx. brevipalpis, Cx. quinquefasciatus, Cx.*

univittatus, Cx. vishnui, and *Heizmannia chandi.* The co-breeding was in various combinations with 1- 4 species (Table.6.1).

Table 6.1: Co-breeding of *Ae. chrysolineatus* and other species in the same habitats

Sl. No	Species co-breeding with *Ae. chrysolineatus*	Number of habitat	Percentage %
1	*Ae. chrysolineatus, Ae. albopictus*	112	51.6%
2	*Ae. chrysolineatus, Cx. quinquefasciatus*	27	12.4%
3	*Ae. chrysolineatus, Ar. subalbatus*	17	7.83%
4	*Ae. chrysolineatus, Cx. brevipalpis*	7	3.2%
5	*Ae. chrysolineatus, Ar. aureolineatus*	7	3.2%
6	*Ae. chrysolineatus, Ae. albopictus, Ar. subalbatus*	*13*	1.38%
7	*Ae. chrysolineatus, Ae. albopictus, Cx. univittatus*	*2*	0.92%
8	*Ae. chrysolineatus, Cx. quinquefasciatus, Ae. albopictus*	19	8.7%
9	*Ae. chrysolineatus, Ae. albopictus, Cx. vishnui*	1	0.46%
10	*Ae. chrysolineatus ,Cx. brevipalpis, Heizmannia chandi*	1	0. 46%
11	*Ae. chrysolineatus, Cx. brevipalpis, Ae. albopictus*	1	0. 46%
12	*Ae. chrysolineatus, Hz.chandi, Ar. aureolineatus*	1	0. 46%
13	*Ae. chrysolineatus, Cx. quinquefasciatus, Ae. cogilli*	1	0.46%
14	*Ae. chrysolineatus, Ar. subalbatus, Cx. univittatus*	1	0.46%
15	*Ae. chrysolineatus, Ar. subalbatus, Ae. vittatus*	1	0.46%
16	*Ae. chrysolineatus Cx. quinquefasciatus, Ar. subalbatus, Ae. albopictus*	2	0.92%
17	*Ae. chrysolineatus, Ae. albopictus, Cx. brevipalpis, Cx. biteaniorhynchus*	1	0.46%
18	*Ae. chrysolineatus, Ae. albopictus, Cx. brevipalpis, Ar. subalbatus*	1	0.46%
19	*Ae. chrysolineatus, Ar. subalbatus, Ae. albopictus, Cx. brevipalpis, Hz. chandi*	2	0.92%
	Total	**217**	**100 %**

a. **Co-breeding with one species:** Five species viz., *Ae. albopictus, Ar. aureolineatus, Ar. subalbatus, Cx. brevipalpis,* and *Cx. quinquefasciatus* were found breeding with *Ae. chrysolineatus* in varying number of habitats. Such co-breeding was found in (78.3%) of breeding habitats. Percentage of co-breeding with *Ae. albopictus* was (51.6%), with *Cx. quinquefasciatus (*12.4%), with *Ar. subalbatus (*7.83%), with *Cx. brevipalpis(* 3.2%), and with *Ar. aureolineatus* (3.2%).

b. **Co-breeding with two species:** Co-breeding with two species occurred in 18.9% of the habitats in 10 different combinations of species. The percentage of habitats with different species were as follows: *Cx. quinquefasciatus,* and *Ae. albopictus*–8.7%; *Ae. albopictus,* and *Ar. subalbatus*-1.38%; *Ae. albopictus* and *Cx. univittatus*-0.92%; *Ae. albopictus,* and *Cx. vishnui*-0.46%; *Cx. brevipalpis,* and *Hz. chandi*- 0.46%; *Cx. brevipalpis,* and *Ae. albopictus*- 0.46%; *Hz. chandi,* and *Ar. aureolineatus*- *0.46%; Cx. quinquefasciatus,* and *Ae. cogilli*-0.46%*;* and *Ar. subalbatus,* and *Cx. univittatus*-0.46%, and *Ar. subalbatus,* and *Ae. vittatus*- 0.46%.

c. **Co-breeding with three species:** Co-breeding with three species occurred in 1.84% of the habitats in 3 different combinations of species. The percentage of habitats with different species were as follows: *Cx. quinquefasciatus, Ar. subalbatus,* and *Ae. albopictus*- 0.92%; *Ae. albopictus, Cx. brevipalpis,* and *Cx. biteaniorhynchus*- 0.46%; and *Ae. albopictus, Cx. brevipalpis,* and *Ar. subalbatus*- 0.46%.

d. **Co-breeding with four species:** Co-breeding with four species occurred in 0.92% of the habitats in a single combination of species. The species were *Ar. subalbatus, Ae. albopictus, Cx. brevipalpis,* and *Hz. chandi*.

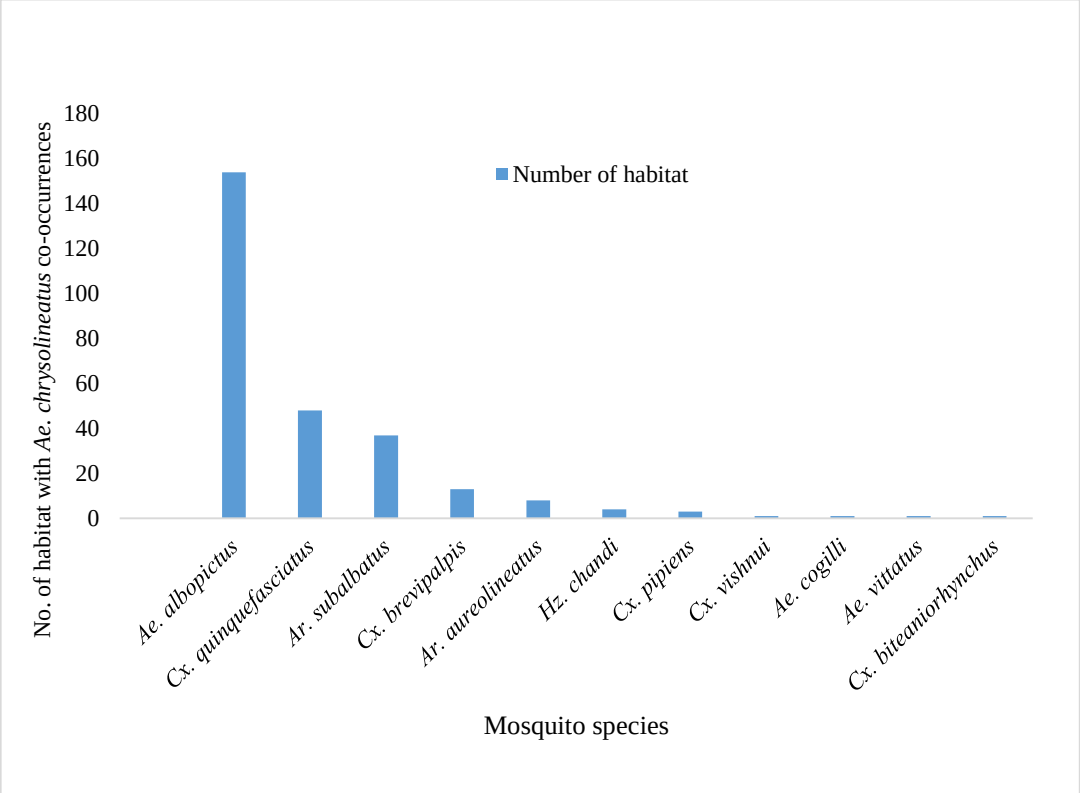
e. **Frequency of co-breeding** (Table. 6.2; Fig 6.1). *Ae. chrysolineatus* most frequently co-bred with *Ae. albopictus*. They were found co-existing in 71% of positive breeding sites. This was followed by *Cx. quinquefasciatus* (22.1%), *Ar. subalbatus* (17.05 %), *Cx. brevipalpis* (6%), and 3.6%, 1.84% and 1.4% sites respectively with *Ar. aureolineatus, Hz. chandi,* and *Cx.*

univittatus. Below one percent of co-breeding were found with *Ae. vittatus, Ae. cogilli, Cx. vishnui, Cx. biteaniorhynchus* each with (0.46%) respectively. 91.5% of habitats with *Ae. chrysolineatus* - *Ae. albopictus* combinations were observed in Wayanad district, followed by (4.57%) in Kozhikode district, and Malappuram district constituted (3.9%).

Table. 6.2 Frequency of co-breeding with other species

Sl. No	Species co-occurrence with *Ae. chrysolineatus*	Number of habitat	Percentage %
1	*Ae. chrysolineatus, Ae. albopictus*	154	71%
2	*Ae. chrysolineatus, Cx. quinquefasciatus*	48	22.1%
3	*Ae. chrysolineatus, Ar. subalbatus*	37	17.05%
4	*Ae. chrysolineatus, Cx. brevipalpis*	13	6%
5	*Ae. chrysolineatus, Ar. aureolineatus*	8	3.6%
6	*Ae. chrysolineatus, Hz. chandi*	*4*	1.84%
7	*Ae. chrysolineatus, Cx. univittatus*	*3*	1.4%
8	*Ae. chrysolineatus, Cx. vishnui*	1	0.46%
9	*Ae. chrysolineatus, Ae. cogilli*	1	0.46%
10	*Ae. chrysolineatus, Ae. vittatus*	1	0. 46%
11	*Ae. chrysolineatus, Cx. biteaniorhynchus*	1	0. 46%
	Total	271	100 %

Fig. 6.1: Co-breeding of *Ae. chrysolineatus* with other species in the same habitats



f. **Habitats with co-breeding:** Mixed breeding of 2 to 5 species including *Ae. chrysolineatus* were observed in different breeding habitats in North Kerala. Mixed breeding of five species (*Ae. chrysolineatus, Ar. subalbatus, Ae. albopictus, Cx. brevipalpis, Heizmannia chandi*) were observed (Table. 6.3) in different tree holes of (Therakam and Silver oak) Kattikulam Wayanad. Mixed breeding of four species were observed in four different habitats, areca leaf sheaths (Cheeramkunnu), mud pots (Purakkadi), boat (Pookode), and latex collecting cup (Manichira). Co-breeding of three species were found in 41 habitats, in which 37 breeding habitats were found in Wayanad, three in Kozhikode, and one in Kannur. The habitats were plastic containers (5), areca leaf sheaths (7), coconut shells (2), latex collecting cups (11), tree holes (4: coffee, mango, vaka, and teak), plastic sheets/covers (7), boat (1), flower pots (2), fallen leaves (1) and glass bottles (1) etc. Co-breeding of two species were found in 170 habitats, the habitats were latex collecting cups

(50), areca leaf sheaths (30), plastic containers (23), tree holes (areca cut holes, mango, coffee, rubber: 21), plastic sheets/cover (18), coconut shell (11), Tank (5), glass bottle (4), fallen leaves (3), boat and Thermocol with (2) respectively.

Table 6.3: Co-breeding pattern in various breeding habitats in North Kerala

Breeding Habitats	Number of Habitats		
	With five species	With four species	With three species
Tree holes	2		4
Areca nut leaf sheaths		1	7
Latex collection cups		1	11
Mud pots		1	2
Fallen leaves			1
Coconut shell			2
Plastic containers			5
Plastic sheet/covers			7
Glass bottle			1
Boat		1	1
Total	**2**	**4**	**41**

6. 32 *Relative abundance and distribution pattern of Aedes chrysolineatus*

As can be seen (Table 6.4), a total of Two thousand, six hundred and nineteen *Ae. chrysolineatus* adults emerged from the samples collected from the five North Kerala districts during the study period, constituting 6.65% of the total adults emerged. In Wayanad district, *Ae. chrysolineatus* is the dominant species (RA=10.42%) with constant distribution (C=92.2%), where as in Kannur (RA=1.35%, C=1.79%), Kozhikode (RA=2.27%, C=2.57%) and Malappuram (RA=1.68%, C=2.31%) district the species is subdominant with sporadic distribution. In Kasaragod district *Ae. chrysolineatus* is a satellite species (RA=0.78%) with sporadic distribution (C=1.02%). Wayanad district showed a high relative abundance (10.42%) of *Ae. chrysolineatus*, followed by Kozhikode, Malappuram, Kannur and Kasaragod districts. Moreover, below one per cent was recorded from Kasaragod (0.78%) districts. Distribution of *Ae. chrysolineatus* species was higher in the Wayanad district, with species inhabiting 359 (92.2%) breeding habitats, followed by Kozhikode district, comprises 10 (2.57%) breeding habitats, Malappuram with nine (2.31%) habitats, and Kannur with seven (1.7%). The least number of breeding habitats (four), were found in Kasaragod (1.02%) district.

Table 6. 4: Relative abundance and distribution of *Ae. chrysolineatus* in different habitats in North Kerala

Districts	**No. of adult mosquitoes emerged**	**No. of *Ae. chrysolineatus***	**No. of *Ae. chrysolineatus* +ve breeding habitats**	***RA**	**Status**	**C***	**Status**
Wayanad	22627	2359	359	10.42%	Dominant	92.2%	Constant
Kozhikode	3949	90	10	2.27%	Subdominant	2.57%	Sporadic
Malappuram	4760	80	9	1.68%	Subdominant	2.31%	Sporadic
Kannur	4790	65	7	1.35%	Subdominant	1.79%	Sporadic
Kasaragod	3203	25	4	0.78%	Satellite	1.02%	Sporadic
	N=39329	2619	N=389				

6.4 Discussion and Conclusion

Co-breeding of *Ae. chrysolineatus* with other mosquito species was assessed in all five districts with interesting outcomes. It is interesting to note that in the majority of habitats (55.8%) it was found breeding with 1 to 4 other species in various combinations. Two factors determine co-breeding of mosquito species. The first one is related to the breeding habitats. Physicochemical factors such as pH, salinity, turbidity, sunlight, air temperature, dissolved organic and inorganic matter, chlorine, magnesium, cadmium, and sulphur; degree of eutrophication, depth, height, water volume, and surface area, influence the growth and survival of mosquito larvae (Abdel-Hamid *et al.,* 2009; Adebote *et al.,* 2008; Yadav *et al.,* 2012). In this study, tree holes were found to support the maximum number of species (five), followed by areca nut leaf sheaths, mud pots, boat and latex collection cups (four).

The second factor is related to the species themselves. The species should be able to either co-operate by partitioning the niche, or compete with the other species for resources. One of the interesting findings was the combination of *Ae. albopictus* and *Ae.chrysolineatus*. Among the species which co-existed with *Ae.chrysolineatus*, *Ae. albopictus* was the one with the maximum frequency, with 71% having these species together. It supports our hypothesis of a possible competition between these two species, especially in Wayanad district.

Further, relative abundance, and distribution of *Ae. chrysolineatus* was found very high in Wayanad district as it turned out to be a dominant and constant species. This means, the species has a very strong foothold in the district. This is another clue suggesting its high competitiveness over other species. These findings are significantly encouraging to pursue further investigations in to its interactions with *Ae. albopictus.*

CHAPTER VII

EFFECTS OF *AEDES CHRYSOLINEATUS* BREEDING ON THE BREEDING OF *AEDES ALBOPICTUS*

7.1 Introduction

Breeding of multiple species of mosquitos in the same habitat leads to competition for resources until they attain pupal stages. These interactions may be interspecific or intraspecific. A superior competitor exclude the inferior competitor (Holway, 1999; Juliano *et al.,* 2004; Petren *et al.,* 1993). Potentiality of positive habitat and its requirements finalize the breeding, interactions and subsequent adult production (Banerjee *et al.,* 2015). *Ae. albopictus* is a vector of many deadly diseases. The invasion of this species in many countries resulted in in the displacement of many indigenous species of mosquitoes. Environmental conditions in the breeding habitats was the main reason behind competitiveness of indigenous species against invasive species (Daehler, 2003). Parker *et al.,* (2018) demonstrated that the negative effect of interspecific competition was high in small and medium containers. In contrast, the negative effects of intraspecific competition were greater in big containers. In this chapter, the effect of *Aedes chrysolineatus* breeding on *Aedes albopictus* is discussed in detail.

7.2 Methodology

7.21 Field study: Surveys for immature stages of mosquitoes were carried out in all study districts following the methods described in chapter III, section (3.3). Sample with mixed breeding of *Ae. chrysolineatus* and *Ae. albopictus* were segregated in the laboratory. Number of adult mosquitoes emerging from the samples were counted and recorded for further analysis. Analysis was done using ANOVA (3.96: a).

7.22 Laboratory study: Experiments were carried out for the assessment of adult production rate and dry body weight of *Ae. chrysolineatus* and *Ae. albopictus* under three different food doses and temperatures. Methods following Carrieri *et al.,* (2003), described in chapter III, section (3.8).

7.23 Data analysis: Relative crowding Co-efficient (RCC) analyzed by using following modified equations of Novak *et al.*, (1993), and Oberg *et al.,* (1996). Described in Chapter III, section (3.96: b)

7. 3 Results

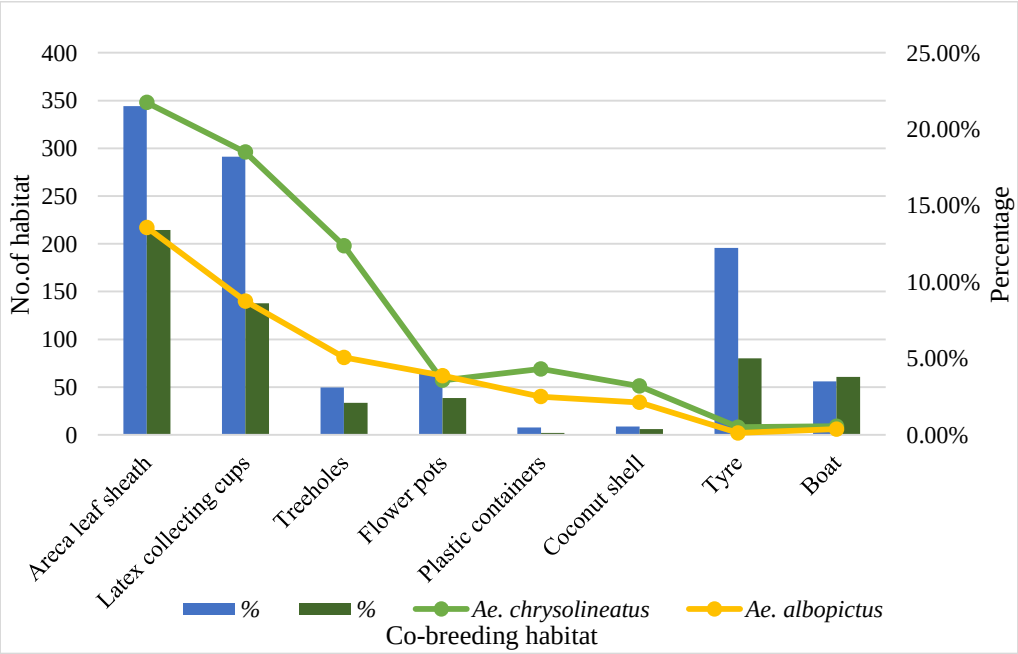
7. 31 *Co-breeding association of Ae. chrysolineatus and Ae. albopictus in the field-collected samples*

Co-breeding of *Ae. chrysolineatus* with *Ae. albopictus* were found in 71% of breeding habitats, where mixed breeding was encountered (See Chapter VI). Co-occurrences of *Ae. chrysolineatus* and *Ae. albopictus* was observed in eight types of breeding containers, of which areca leaf sheath and latex collecting cups were the most important among them, which constituted 31.8% and 29.2% , followed by tree holes with 12.23% respectively. In contrast, the least number of habitats with co-occurrences of the species were found in tyre and boat each with 0.64%. A total of 1618 adult mosquitoes belonging to *Ae. chrysolineatus* and *Ae. albopictus* emerged from the samples in which they co-existed. Of the total adults 34.9% emerged from areca leaf sheath followed by latex collecting cups (26.9%), and tree holes (17.2%). Other habitats contributed far less than these three habitats (Table.7.1, Fig 7.1). Emergence data showed that 64% species was *Ae. chrysolineatus*, whereas only 36% species was *Ae. albopictus.*

Table.7.1: Record of *Ae. chrysolineatus* and *Ae. albopictus* mixed breeding habitats

Co-breeding habitats	N	*Ae. chrysolineatus*	*Ae. albopictus*	Total species (%)
Areca leaf sheath	49	348	217	565(34.9%)
Latex collecting cups	45	296	140	436(26.9%)
Tree holes	25	198	81	279(17.9)%
Flower pots	15	57	62	119(7.3)%
Plastic containers	10	69	40	109(6.7%)
Coconut shell	8	51	34	85(5.2%)
Tyre	1	8	2	10(0.61)%
Boat	1	9	6	15(0.92)%
Total	**15 4**	**1036 (64%)**	**582(36%)**	**1618**

Fig.7.1: Record of *Ae. chrysolineatus* and *Ae. albopictus* mixed breeding habitats



Emergence data showed that in the case of mixed breeding with *Ae. albopictus*, *Ae. chrysolineatus* was the dominant species in 85.06% of breeding habitats, as more *Ae. chrysolineatus* emerged as adults ($F=70.01,\ P=2.1\times10^{-15}$), whereas in only 12.98% breeding sites, more *Ae. albopictus* emerged as adults (Table.7.2). This indicates that *Ae. chrysolineatus* is more competent in transforming resources into biomass, than *Ae. albopictus.* While sharing habitat with *Ae. chrysolineatus, Ae. albopictus* was very slow in transforming resources into biomass. Resource utilization of both species showed a significant difference, and the result is highly statistically significant, as *Ae.chrysolineatus* has a competitive advantage over *Ae. albopictus* in the field collected samples.

Table.7.2: Emergence data of mixed breeding of species of field collected samples.

Mosquito species	Count	Sum	Average	Variance
Ae. albopictus	154	582	3.779221	5.75486
Ae. chrysolineatus	154	1036	6.727273	13.36304

Source of Variation	SS	D f	MS	F	P-value	F crit
Between Groups	669.2078	1	669.2078	70.0085	2.12777E-15	3.872027
Within Groups	2925.039	306	9.558951			
Total	3594.247	307				

**P≤ 0.05, then the result is significant*

7. 32 *Larval competition for resources under laboratory conditions (Fig. 7 a-c)*

Data obtained from the laboratory experiments between *Ae. albopictus* and *Ae. chrysolineatus* revealed significant difference in the adult emergence rate of *Ae. albopictus* at three different food doses, 2.83mg/l, 1.9 mg/l, 0.95mg/l at 28°C and 1.9 mg/l at 22°C (Fig 7.2, 7.3 a-d). At food dose 0.95 mg/l at 28°C, the emergence rate of adult was only 11.07%. It increased to 92.2% in 2.83 mg/l. Beyond 80% of increase in adult emergence rate was found in low to high food dose change. No

significant difference was found in the adult production rate of *Ae. albopictus* at intermediate food doses, 1.9mg/l at 28°C and 22°C , which is 67.1% and 53.4% respectively. Where as in *Ae. chrysolineatus* at low food dose 0.95 mg/l at 28 °C, 70% of adult emergence was achieved. This increased to 91.25% at high dose of food, 2.83mg/l at 28°C (Table.7.3).

Fig. 7 Laboratory study (a-c): (a) *Ae. chrysolineatus* Larvae (left), (b) *Ae. albopictus* Larvae (right), (c) *Ae. chrysolineatus* and *Ae. albopictus* larvae in mixed treatments


c

No significant differences were observed in the adult emergence rate of *Ae. chrysolineatus* at three food doses 2.83 mg/l at 28°C, 1.9mg/l at 28°C and 22°C, which was 91.25%, 91% and 82.16% respectively. Maximum adult production rate was recorded in 2.83mg/l at 28°C and 1.9 mg/l at 28°C. Slight decrease in adult emergence frequency was observed in 1.9mg/l at 22℃ (82.16%) compared to 1.9 mg/l at 28°C (91%).

Table.7.3: Evaluation of some biological parameters of *Ae. chrysolineatus* and *Ae. albopictus* at three food doses

	Doses			
	2.83 mg/l 28℃	**1.9mg/l 28℃**	**0.95 mg/l 28℃**	**1.9 mg/l 22℃**
Adult emergence rate				
Ae. chrysolineatus	91.25±1.8	91± 1.90	70 ±5.4	82.16±2.2
Ae. albopictus	92.2±1.6	67.1±2.1	11.07±2.8	53.4±12.6
Mean adult weight (mg)				
Ae. chrysolineatus	0.90±0.026	0.81±0.021	0.64±0.028	0.76±0.014
Ae. albopictus	0.72±0.014	0.42±0.020	0.10±0.008	0.34±0.025

Fig.7.2 Record of adult biomass produced (*Ae. chrysolineatus* and *Ae. albopictus*) in relation to three different food doses.

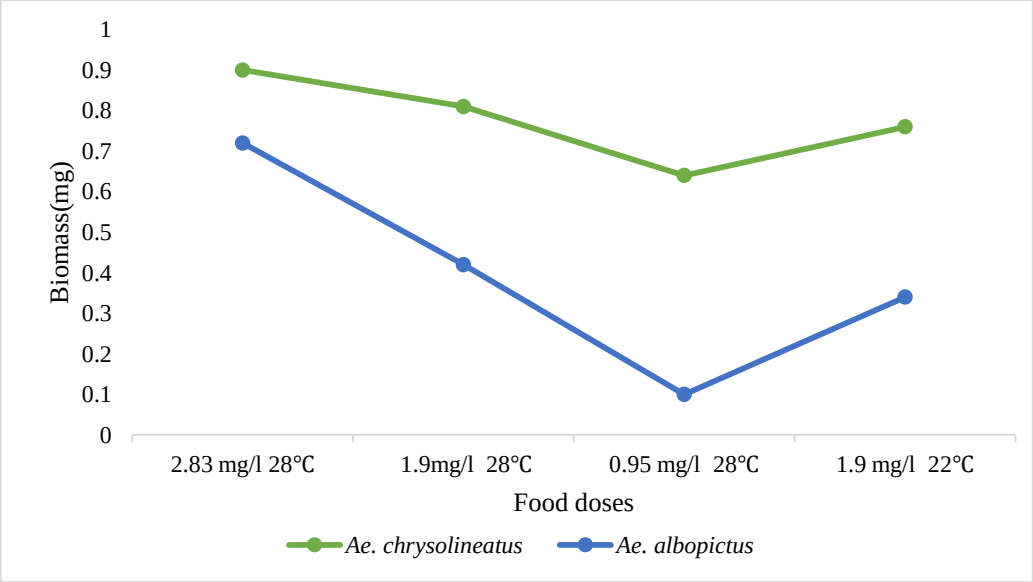


Fig. 7.3: Adult biomass produced based on the following competitive ratios: a) 2.83mg/l at 28 ℃; b) 1.9mg /l at 28 ℃; c) 0.95 mg/l 28 ℃; d) 1.9 mg/l at 22℃

Fig.7.3(a)

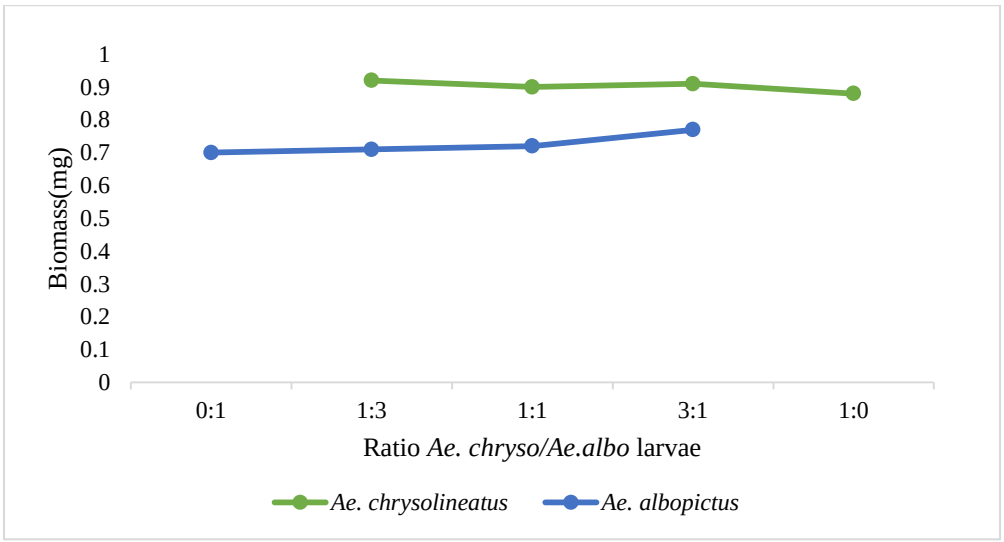


Fig.7.3 (b)

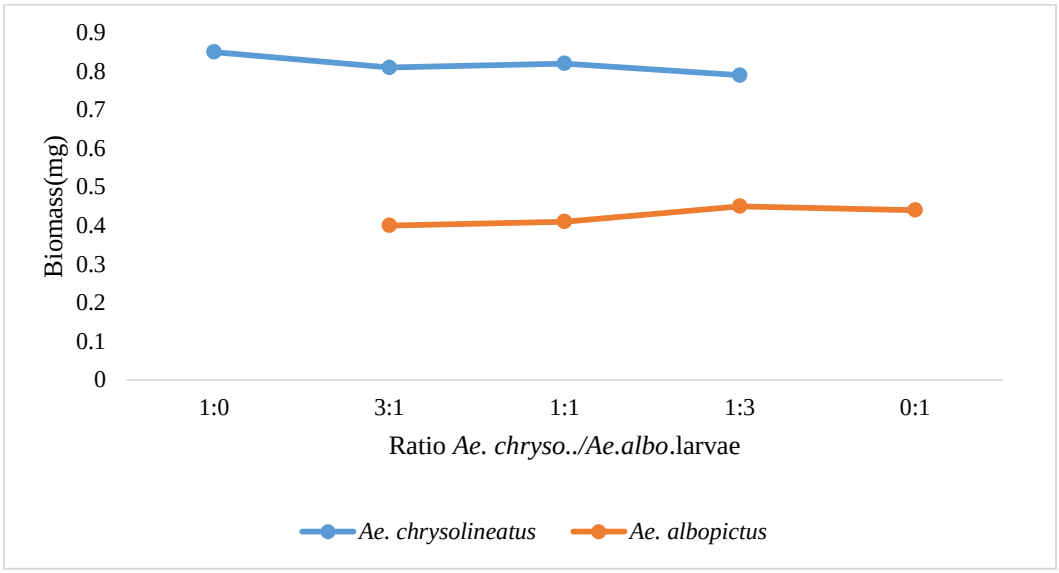


Fig.7.3(c)

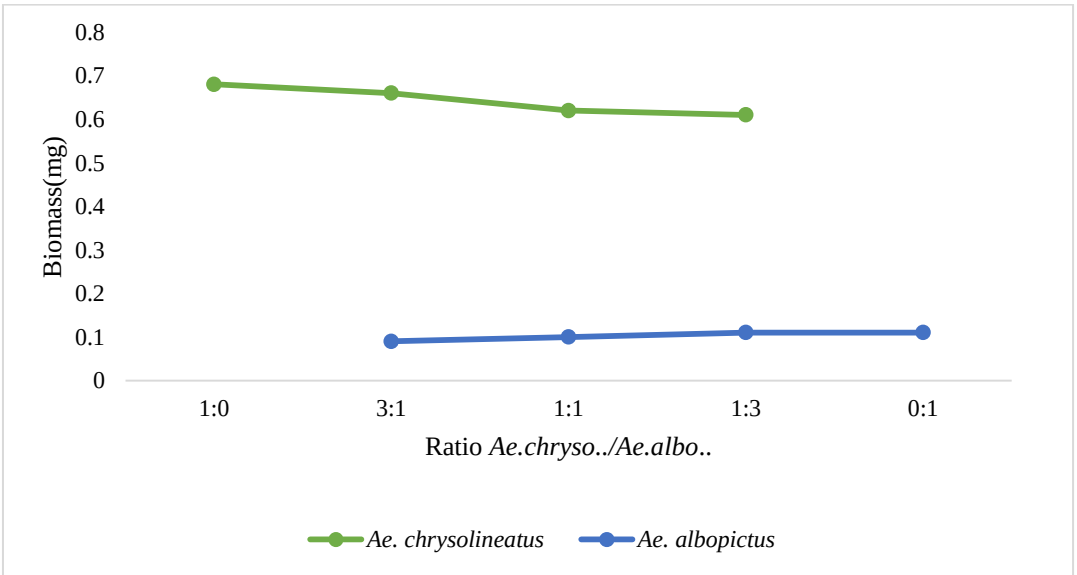
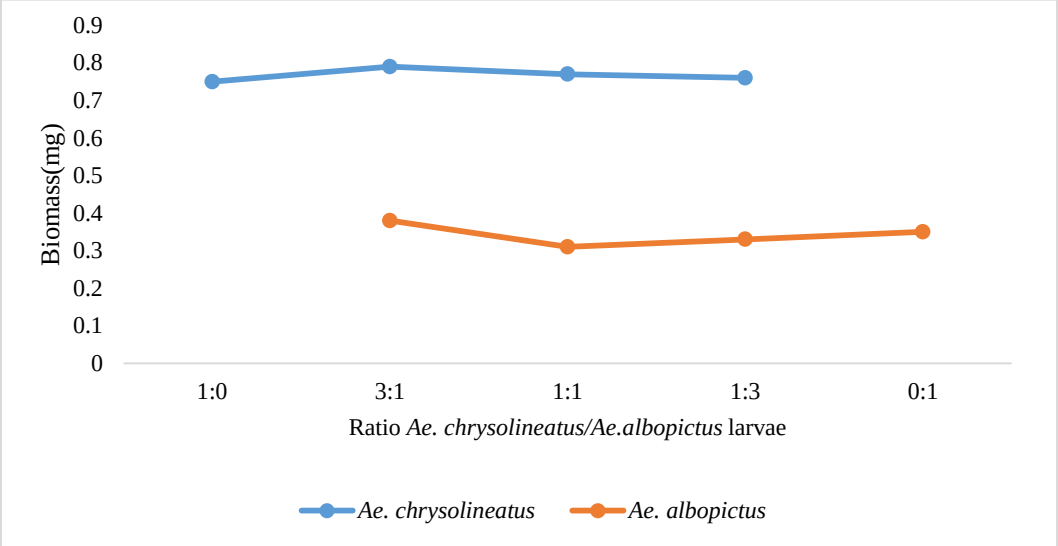


Fig.7.3(d)



With respect to adult weight, *Ae. chrysolineatus* showed a tendency to develop larger adult at the three food doses tested, 2.83 mg/l at 28°C, 1.9 mg/l at 28°C and 22°C , which were 0.90 mg, 0.81 mg and 0.76 mg respectively with 10% decrease in each food doses. Whereas slight decrease in the weight was found at dose 0.95 mg/l at 28°C which was 0.64mg (Table. 7.3). In contrast, major differences in the adult mean weight was observed at low food dose in *Ae. albopictus*, which was 0.10mg. At low food dose of 0.95 mg/l at 28°C, significant differences in the adult emergence rate of *Ae. chrysolineatus* vs *Ae. albopictus* was observed particularly at the ratios of 1:1 and 1:3 (Table.7.4). The development rate of *Ae. chrysolineatus* was 66% and 64%, whereas in the case of *Ae. albopictus* it was only 11% and 10.3% respectively at ratios 1:1 and 1:3. A significant difference in the adult mean weight was observed at these ratios. There was not found meaningful differences in the intermediate food dose at 22°C in both these species. Temperature was not found playing a particular role in mean adult weight changes in both the species. In contrast mean weight of both these species increased when there was sufficient availability of food sources, especially increase in the weight of *Ae. albopictus* .

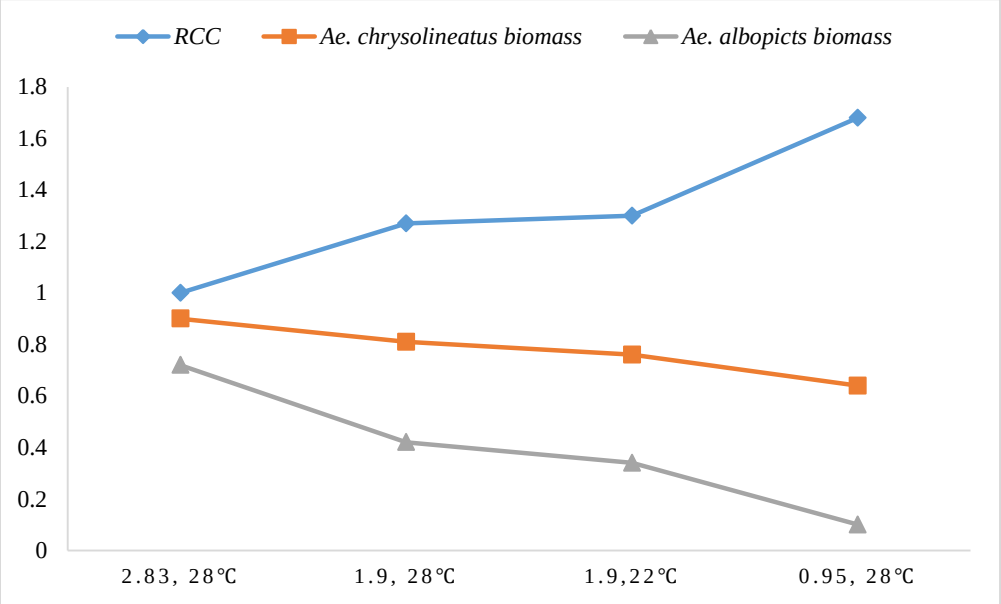
Table.7.4: Effect of competing on the adult weight (mg)

***Ae. chrysolineatus* / *Ae. albopictus* ratio**	***N***	***Ae. chrysolineatus* (adult emergence)**	***Ae. chrysolineatus* (adult wt)**	***Ae. albopictus* (adult emergence)**	***Ae. albopictus* (adult wt)**
2.83 mg/l 28℃					
1:0	4	88.5±0.18	0.88±0.014		
3:1	4	93±0.11	0.91±0.016	92.5± 0.02	0.77±0.026
1:1	4	90.5±0.10	0.9± 0.017	92.5± 0.024	0.72±0.023
1:3	4	93±0.11	0.92± 0.011	92± 0.03	0.71±0.07
0:1	4			92± 0.03	0.7±0.07
1.9mg/l 28℃					
1:0	4	91.5±0.75	0.85±0.021		
3:1	4	92±0.06	0.81±0.018	65±0.02	0.4±0.02
1:1	4	90.5±0.05	0.82±0.018	67.5±0.021	0.41±0.02
1:3	4	90±0.07	0.79±0.019	70.5±0.026	0.45±0.020
0:1	4			65.5±0.02	0.44±0.019
0.95 mg/l 28℃					
1:0	4	78±0.05	0.68±0.028		
3:1	4	72±0.042	0.66±0.025	15.5±2.8	0.09±0.008
1:1	4	66±0.04	0.62±0.026	11±2.4	0.1±0.01
1:3	4	64±0.041	0.61±0.027	10.3±2.7	0.11±0.02
0:1	4			7.5±2.6	0.11±0.02
1.9mg/l 22℃					
1:0	4	79.5±0.02	0.75±0.014		
3:1	4	85.65±0.021	0.79±0.016	33±12.6	0.38±0.025
1:1	4	81.5±0.03	0.77±0.013	54.5±12	0.31±0.024
1:3	4	82±0.022	0.79±0.016	58.6±11.1	0.33±0.026
0:1	4			67.5±1.2	0.35±0.025

***Relative crowding coefficient (RCC)*:** Relative crowding coefficient (RCC), indicated that in different food doses tested the competition between *Ae. chrysolineatus* and *Ae. albopictus*, favored *Ae. chrysolineatus.* RCC at 0.95 mg/l at 28°C =1.68, RCC at 1.9 mg/l at 22°C = 1.30, RCC at 1.9 mg/l at 28°C =1.27, RCC at 2.83 mg/l at 28°C = 1. At low food dose 0.95 mg/l at 28°C, RCC is 1.68, high level of competition occurs between the species, which favors *Ae. chrysolineatus.* Whereas in high food dose 2.83 mg/l at 28°C, RCC is 1, which implies that both species are equal competitors and there is no competitive advantage for both the

species. RCC was 1.30 and 1.27 at intermediate food doses at 22°C, 28°C which indicated the upper hand of *Ae. chrysolineatus* (Fig. 7.4).

Fig.7.4: RCC and biomass of *Ae. chrysolineatus* and *Ae. albopictus* in relation to different food doses



7.4 Discussion and Conclusion

When two mosquito species breed together in the same habitats, it is expected to have interactions between the two species. These interactions could be either competition or co-operation. Several studies in the past have shown competition between the species and in extreme instances total elimination of one of the species over a period of time (Moore and Fisher, 1969; Lowrie, 1973; Russel, 1986; Kweka *et al.*, 2012; Armistead *et al*., 2008). The present study was the first of its kind involving the co-breeding of *Ae. chrysolineatus* and *Ae. albopictus* and its effect on the production of the co-breeding species. Both field and laboratory assessments were carried out. The field study emphatically proved the significant competitiveness of *Ae. chrysolineatus* over *Ae. albopictus* as the 64 % of adults produced in the co-breeding habitats was *Ae. chrysolineatus*. Besides, from 85.06% of the co-breeding habitats *Ae. chrysolineatus* had the numerical superiority.

In the laboratory experiments to assess the competition between the two species under varying quantities of food and temperature interesting results were obtained. There was no competition in the case of high quantity of food but competitive advantage in favor of *Ae. chrysolineatus* was significant under low and medium quantity of food. However, temperature had no influence on species interaction.

The competitiveness was further confirmed by assessing Relative crowding co-efficient (RCC). At high quantity of food, RCC was 1.0 indicating lack of competition. However, RCCs were above 1.0 in the case of low and medium quantities of food.

Both field and laboratory studies indicates significant competition between *Ae. chrysolineatus* and *Ae. albopictus* with the former species having an edge over the latter. It is implied that under field conditions with limited food resources *Ae. chrysolineatus* could reduce the productivity *Ae. albopictus.* This qualifies *Ae. chrysolineatus* as a possible candidate for reducing the overall density of the vector species *Ae. albopictus.* In a state like Kerala with increasing trend of dengue, this could be another potential weapon in the arsenal against *Ae. albopictus* in rural areas where it is the dominant vector species.

CHAPTER VIII

SUMMARY

- During the present study breeding of *Aedes chrysolineatus* was observed in all study districts, with the highest prevalence in Wayanad district (100%) and the lowest in Malappuram and Kozhikode district (4.2%).
- Prevalence in the other two districts was 5.2% (Kasaragod) to 5.6% (Kannur).
- High prevalence in Wayanad could be due to high elevation, indicating potential adaptation to high altitudes.
- *Aedes chrysolineatus* is a container breeder, observed in 17 major habitats.
- Latex collecting cups and areca leaf sheaths are the top two anthropogenic habitats, related to rubber plantations and areca farms.
- Tree holes, bamboo, and rock pools are significant natural habitats.
- Rubber plantations are crucial for the proliferation of the dengue vector *Aedes albopictus* in latex collection cups. Investigating interactions between *Aedes chrysolineatus* and *Ae. albopictus* in the same habitat could be significant.
- Co-breeding of *Ae. chrysolineatus* with other Mosquito Species in Five Districts: In 55.8% of habitats *Ae. chrysolineatus* breeding was found breeding along with 1 to 4 other species.
- *Ae. albopictus* was the most frequent co-existing species with *Ae. chrysolineatus*, with 71% having them together.
- *Ae. chrysolineatus* was found to be a dominant and constant species in Wayanad district, indicating its high competitiveness over other species.

- Findings encourage further investigations into its interactions with *Ae. albopictus*.
- The study investigates the co-breeding of *Ae. chrysolineatus* and *Ae. albopictus* in the same habitats.
- Field studies show significant competitiveness between the two species, with *Ae. chrysolineatus* producing 64% of adults in co-breeding habitats.
- Laboratory experiments show no competition under high food quantities, but significant advantage for *Ae. chrysolineatus* under low and medium food quantities.
- Temperature does not influence species interaction.
- Relative crowding co-efficient (RCC) indicates no competition at high food quantities, but at low and medium food quantities.
- Both studies suggest *Ae. chrysolineatus* could reduce productivity of *Ae. albopictus* under limited food resources, potentially reducing its density in rural areas.

CHAPTER IX
RECOMMENDATIONS

The studies on *Aedes chrysolineatus* were like a journey into unchartered landscapes, without many models to follow. Considering the elaborateness of the topic this study was envisaged to be a long-term and stagewise study. Owing to time constraints only the first stage of the study could be carried out. The first stage consisted of collecting baseline data on the distribution and habitat diversity of *Aedes chrysolineatus*, the extent of co-breeding with *Aedes albopictus,* and its possible effect on the productivity of *Aedes albopictus.* The first stage could be considered as a theoretical foundation and the second stage a practical application of the theoretical principles arrived at. Hence, it is recommended to undertake the second stage of the study as follows:

1. Biting behaviour of *Ae. chrysolineatus.* The exact nature of the biting preference of this species is unknown. It should be confirmed that the release of this species in the field to inhibit *Ae. albopictus* population does not have any negative consequences on the people living in the trial area.

2. Vector status of *Ae. chrysolineatus.* It has to be ensured that this species does not transmit any human or animal diseases.

3. Laboratory and field trials on the efficacy of the introduction of *Aedes chrysolineatus* on the production of *Aedes albopictus*.

4. Assessment of the effect of the introduction of *Aedes chrysolineatus* on the diseases vectored by *Aedes albopictus*.

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