Research Article

Study on Ovitrap Surveillance and Molecular Phylogeny of Dengue Vectors (*Aedes aegypti* and *Aedes albopictus*) based on Cytochrome C Oxidase Subunit I (COI) Genes from Different Habitat Types of North Eastern, India

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ABSTRACT

Background: Larval surveillance plays crucial role in monitoring dengue vector surveillance. Conventional vector indices are failed in monitoring vector abundance and risk of transmission. ovitrap surveillance and molecular phylogeny study was conducted. **Materials and Methods:** Ovitrap surveillance was carried using different ovitrap indices which correlate with environmental parameters. Phylogeny study was carried out using mtCOI genes as morphological characterization of mosquitoes lacks accuracy to characterized closely related species. **Results:** Findings revealed that *Aedes albopictus* was dominant over *Aedes aegypti* in ovitrap placement locations. And temperature, rainfall and habitat types serve as major factors contributing to variations in ovitrap indices. Molecular phylogeny revealed that the isolates form distinct grouped into separate clusters which indicate polymorphisms in gene among the isolates and signify sequence diversity of mt COI genes in *Aedes* mosquito species differs geographically which further differentiate a population and brings new identity. **Conclusion:** The study helps in measuring the population status of dengue vector abundance, forecasting dengue outbreaks and served as a powerful indicator to adopt different control measures.

Keywords: *Aedes albopictus*, *Aedes aegypti*, Population, Polymorphisms.

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INTRODUCTION

The dengue fever cause by *Aedes* vectors both *A. aegypti* and *A. albopictus* was continuously spreading in tropical and subtropical region of the globe, causing illness and fatality.[1] In India, most of the districts and cities are endemic to dengue infection.[2] It was considered as major public health problem in India because of its high morbidity and mortality. Urbanization, population movement and failures of public health measures are the major cause of increasing dengue positive cases.[3-7] Vector surveillance was the only option to determine the vector abundance, vector habitats, and spatiotemporal risk factors with respect to dengue transmission.[8] Most of the countries, vector surveillance is heavily rely on larval surveillance.[9] In India, the house index (the percentage of houses positive with larvae or pupae), container index (the percentage of positive water filled containers infested with larvae or pupae) and Breteau index (the number of positive containers for *Aedes* vector per 100 houses inspected) are the main indices which have been used as indicators for risk of dengue transmission.[10-12] But sometimes *Aedes* indices prove to be incapable of determining vector abundance and transmission risk as, *Aedes* mosquito vectorial capacity varies by region, and the diagnostic techniques are inadequate and also the situation is made more

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difficult by the broad size and regional differences in socioeconomic condition.[9,13] For better understand of population dynamics and the distribution of dengue vectors for prevention and control and to replace long-term vector surveillance was the ovitrap surveillance method. In ovitrap surveillance, Ovitrap is simple and efficient technology and helps in tracking dengue vectors.[9] Ovitrap based surveillance was employed in many Asian countries like Hong Kong, Singapore, and Australia in routine dengue surveillance.[4,14-16] This technique helps to find both young mosquitoes and the eggs laid by gravid female mosquitoes.[17,18]

In this study, indices based on ovitrap surveillance, namely the positive Ovitrap Index (OI; total no. of positive ovitrap/ total no. of ovitrap placed), Trap Positivity Index (TPI; Total no. of positive egg sheets/ total no. of egg sheets installed in each ovitraps) were calculated to correlate. And molecular phylogeny was studied to obtain the evolutionary relatedness among the dengue vectors of study sites with the global isolates. The aim of the study to develop effective vector surveillance strategies to improve the data collection efficiency and to determine dengue risk zones in a short period of time to provide effective vector control measures.

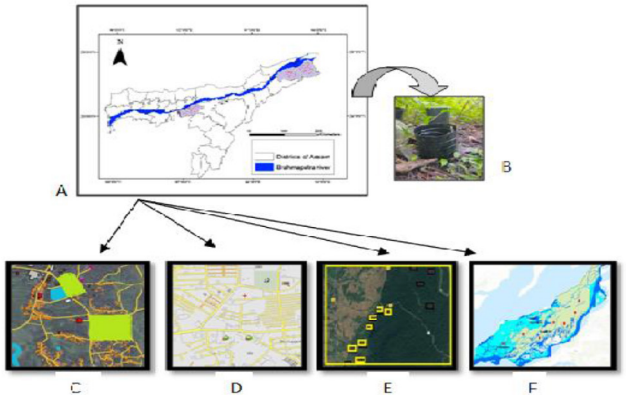

Plate 1: Google earth map showing dengue vector ovitrap surveillance in different selected locations of both Dibrugarh (27.47° N to 94.91° E) and Tinsukia (27.48° N to 95.36° E) districts of upper Brahmaputra valley of Assam, India. (A) Represent study areas in map of Assam, (B) solid black ovitraps installed at households, (c) Tea-estates areas, (D) industrial areas, (E) Forest areas, (F) riverside areas respectively.

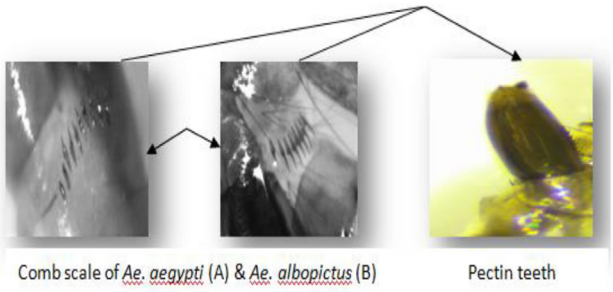

Figure 1: *Aedes* mosquito's larval identification of Microscopic image (10×) of larvae showing differences in morphological structure of comb scales on the eighth segment of the abdomen and the shape of the pectin teeth on the siphon.

MATERIALS AND METHODS

Study areas and ovitap surveillance

Ovitrap survey was conducted in housing areas of four different habitat types of Dibrugarh (27.4705° N, 94.9125° E) and Tinsukia (27.4886° N, 95.3558° E) district of upper Brahmaputra valley of Assam on a fourth nightly basis for a period of one year (2019-2020) and to obtained a comparative view along with this habitat types, the ovitrap survey was also conducted in four different dengue endemic zones of Assam, Guwahati which falls under lower Brahmaputra valley of Assam. India. Mostly the ovitrap is a black plastic container of varying sizes. The inner walls of ovitraps were lined with Whitman filter paper of size 190 mm. Two-thirds of the ovitrap filled with water and filter paper was dipped in that water which act as a female *Aedes* oviposition site. In each household of all the study sites, the ovitraps were placed in three different consecutive heights (3ft, 6ft and 12ft) in a shady place near the houses. Thus, a total of 240 ovitraps were installed in all the study sites. Each ovitrap was marked with a serial number, date and study sites. Global Positioning System coordinates (Garmin eTrex 10×) was use to record the coordinates of study sites (Plate 1).

Mosquito collection and rearing

From the ovitraps, all the immature both larvae, and pupae and eggs sheets were collected two times in an interval of 15 days per month from all the study sites and brought to the entomology laboratory. All immature collected from the ovitrap were counted, identified and reared for adult emergence for species identification. Two Vector indices were used in this study, namely, OI and TRP of all the study sites were determined.

Morphological identification

The larvae collected from ovitraps placed at different sampling sites were identified using morphological characteristics such as pectin teeth and comb scale, and the adults reared from larvae were identified using standard keys (Figure 1).[19]

Molecular Identification

Adult specimens after morphological identification were identified as *A. aegypti* and *A. albopictus* by observing morphological characteristics. After that individual adults from different sampling areas were selected for molecular characterization to study the molecular variations.

Genomic and mitochondrial DNA extraction

Whole DNA was extracted from individual adult reared mosquito by DNeasy® Blood and Tissue Kit (QIAGEN, USA) according to standard protocol. Isolated DNA was eluted with supplied elution buffer and send to the Heredity Biosciences Labs Pvt. Ltd., Odisha, India for gene sequence analysis. Statistical analyses were done using SPSS version 22.

RESULTS

During the survey period from 2019–2020, the study areas received an average temperature of 23.7°C (max: 24.8°C and min: 22.8°C). Monthly average precipitation of 167.76 mm, with February and December was recorded to be the driest month (0.2 mm) and June, July and August, the wettest months (483.2 mm) (Figure 2A).

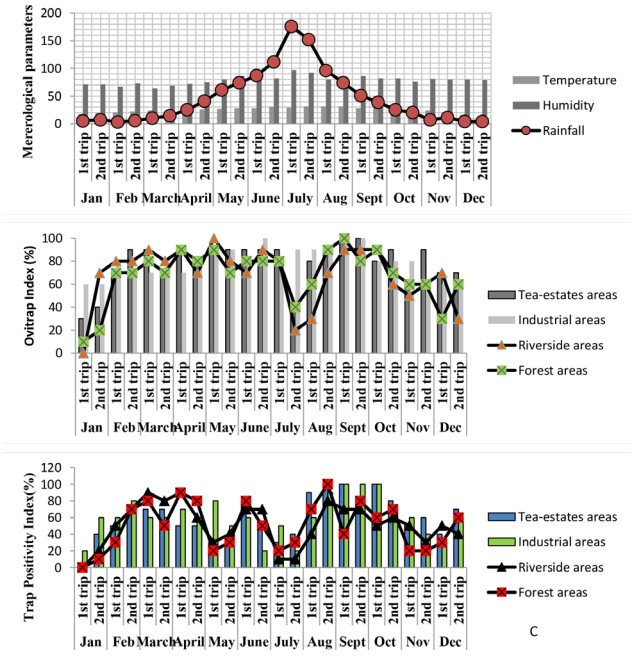

Figure 2: *Aedes* vector indices of ovitraps installed at different habitat types in Dibrugarh and Tinsukia district of Upper Brahmaputra valley of Assam, Northeast India with respect to meteorological parameters (A) month wise temperature, rainfall and humidity, (B) Ovitrap Index (%), (C) Trap positivity index (%).

Dengue vector abundance

A total of 65,283 *Aedes* spp. eggs were collected throughout the collection period from all the habitat types. And a total of 81,084 immature mosquitoes were collected from the ovitraps. Mostly two dengue vectors were collected from the ovitraps, namely *A. aegypti* and *A. albopictus*, from all the study sites. *A. albopictus* predominantly found in all the habitat type and maximum in tea-estates areas (18707) and industrial areas (19552) likewise the abundance of *A. aegypti* was also maximum in industrial areas (4133) followed by tea-estates (3352) followed by riverside areas (*A. aegypti*-3,029, and *A. albopictus*-17,253) and forest areas (*A. aegypti*-2,175 and *A. albopictus*- 12,820) (Table 1). The study on seasonal variation of ovitrap index of different study sites revealed that in winter season, the highest positive ovitrap index was recorded from industrial areas with an OI of (65±1.41) followed by tea-estates, flood plain riverside and forest areas with an OI of 61.66±3.09, 55±3.35 and 43.33±1.85. In pre-monsoon season the highest OI was recorded from tea-estates followed by floodplain riverside with an OI of 85±3.41, industrial, and forest areas with an OI of 85±3.41 and 85±1.28. Both industrial and forest areas found to have the same OI with an OI index of 80±3.65. In monsoon season, again, similar trend was found as winter season, the highest OI was found in industrial areas followed by tea-estates, forest and floodplain riverside areas with an OI of 88.33±3.07, 80±2.16, 71.66±1.49 and 60±1.54. In post-monsoon seasons, the highest OI was found in tea-estates areas followed by industrial, forest and flood-plain riverside areas with an OI of 86.66±2.14, 83.33±1.14, 76.66±2.66 and 73.33±1.60. Seasonal variation of ovitrap index of all the study areas showed significant difference. From the study of TPI, it was found that in winter season, the highest TPI was found in industrial areas with TPI of 51.66±2.09 followed by tea-estates, flood-plain riverside and forest areas with TPI of 45±1.56, 38.33±1.13 and 33.33±1.15. In pre-monsoon season, floodplain riverside areas found to have the highest TPI followed by tea-estates, forest

Table 1: Abundance of dengue vectors (*A. aegypti* and *A. albopictus*) that collected from each ovitrap placements in different habitat types.

Ovitrap placement habitats	Percentage of species abundance	
	A. aegypti	*A. albopictus*
Tea-estates areas	3352 (15.37%)	19552(90.55%)
Industrial areas	4133(17.44%)	18707(85.78%)
Floodplain areas	3092(15.19%)	12,820 (85.49%)
Forest areas	2175(14.50%)	17253(84.80%)

Table 2: Correlation between meteorological parameters and abundance of dengue vectors collected from ovitraps placements from different habitats.

Metrological parameters	Ranges	Correlation coefficients				*P*-value			
		F.A	R.A	I.A	T.A	R.A	F.A	I.A	T.A
$Temp_{max-min}$	37°C - 8°C	0.91	0.56	0.85	0.91	0.01	0.01	0.01	0.01
$Rainfall_{max-min}$	484mm-14mm	0.73	-0.19	0.76	0.81	0.05	0.01	0.01	0.01
$Relative\ humidity_{max-min}$	90%- 60%	0.25	0.47	0.25	0.29	0.27	0.5	0.21	0.18

Table 3: Linear regression analysis of vector abundance with three different levels of elevations.

Dengue risk zone in upper Brahmaputra valley of Assam	Three different elevations	Standard error	Degree of freedom	Sig.
Forest areas	3ft	33.27	4	0.03
Riverside areas	6ft	37.93	9	0.00
Industrial areas	12ft	31.46	9	0.04
Tea-estates areas				
Dengue endemic zones in lower Brahmaputra valley of Assam	**Three different elevations**			
Sonapur	3ft	33.27	4	0.03
Dispur	6ft	37.93	9	0.04
Gandhibasti	12ft	31.46	9	0.04
6th mile		33.27	4	0.03

areas and industrial with TPI of 65±1.56, 63.33±3.21, 58.33±1.94 and 50 ±3.30. In case of monsoon season, tea-estates areas found to have the highest TPI of 65±1.76 followed forest, industrial and floodplain riverside areas with TPI of 58.33±2.49, 50±1.94 and 46.66±1.82. In post-monsoon season, the highest TPI was recorded from both tea-estates and industrial areas having same TPI of (78.33±2.33) followed floodplain riverside and forest areas with TPI of 55±3.19 and 48.33±1.46 (Figure 2c). From the ovitrap surveillance, it was found that, industrial areas showed the highest percentage of immature counts of *A. aegypti*, (17.44%) followed by tea-estates, (15.37%), flood-plain riverside, (15.19%) and forest areas, (14.50%). For *A. albopictus*, tea-esates areas showed the highest percentage of *A. albopictus* abundance, (90.55%) followed by industrial areas, (85.78%), flood-plain riverside areas, (85.49%) and forest areas, (84.80%) (Figure 2)

Based on correlation co-efficient between three major meteorological parameters temperature/rainfall/ humidity (both min./max.) and dengue vector abundance, all the study sites were positively correlate with the all the three parameters except the riverside areas which was negatively correlate with the rainfall (-0.19). With temperature tea-estates areas (0.91), industrial areas (0.85), riverside areas (0.56) and forest areas (0.91). With rainfall, tea-estates areas, industrial areas, riverside areas and forest areas shows (0.81, 0.76, -0.19 and 0.73) similarly with humidity, tea-estates areas, industrial areas, riverside areas and forest areas shows (0.29, 0.25, 0.47 and 0.25) respectively (Table 2).

Again, while considering the effect of ovitraps with three different levels of elevations, 3ft, 6ft and 12ft above ground level in all the four habitat types of upper Brahmaputra valley of Assam along with four dengue prone zones of capital city of Assam, India that comes under lower Brahmaputra valley of Assam, to get a comparative view of dengue vector abundance. The 3ft height above the ground level showed highest percentage of positive ovitraps followed by 6ft and 12ft. Among all the study sites Sonapur recorded maximum percentage of positive ovitraps that were installed in all three levels of elevations, in 3ft it was (80%), in 6^{ft} it was (73.68%) and in 12ft height it was (60%) as compared to other study sites and the study sites, 6^{th} mile shows lowest percentage of positive ovitraps per ovitrap installed in all the three level of elevations i.e., 28.57%, 14.28% and 0%. From the liner regression analysis of vector abundance with three different levels of elevations, all the study sites show significant relation with the three different level of heights $p<0.05$.(Table 3).

The study of water quality parameters was of great importance in determination of *Aedes* vector abundance. From the analysis of three major water quality parameters i.e., pH, TDS and DO, it was found that the *Aedes* vector abundance was highly correlated with the high pH and

Table 4: Average vector abundance (*A. aegypti* and *A. albopictus*) with relation to water quality parameters of Study sites.

Study sites	*A. aegypti*	*A. albopictus*	pH	TDS	DO
Tea-estates areas	172.21±3.82	814.67±0.98	7.28±0.07	150.14±2.67	4.02±0.61
Industrial areas	268.16±1.11	779.46±0.99	7.14±0.09	82.67±3.19	4.12±0.84
Floodplain areas	115.13±1.98	718.88±3.56	7.11±0.11	60.81±1.14	4.80±0.43
Forest areas	38.17±2.85	534.17±2.48	7.08±0.14	114.90±3.25	4.20±0.41

Table 5: Significant test analysis of Vector abundance with that of water quality parameters.

	Parameters	N	DF	F
1.	Ph with *A. aegypti* of all the study sites	24	3	16.63
2.	Ph with *A. albopictus* of all the study sites	24	3	16.63
3.	TDS with *A. aegypti* of all the study sites	24	3	25.27
4.	TDS with *A. albopictus* of all the study sites	24	3	8.47
5.	DO with *A. aegypti* of all the study sites	24	3	25.27
6.	DO with *A. albopictus* of all the study sites	24	3	12.98

*The mean difference is significant at 0.05 levels

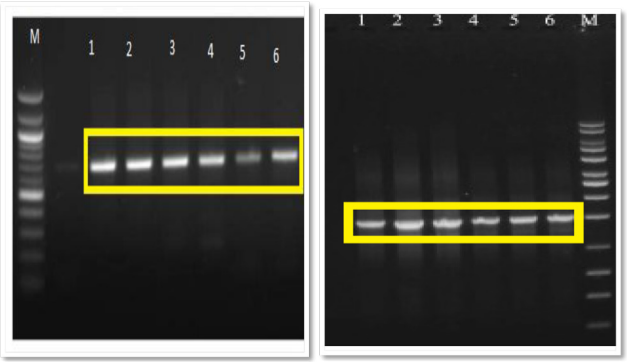

Figure 3: Represents the amplification of mitochondrial CO1 gene using the specific primers, each lane shows the amplified product of both *A. aegypti* (A) and *A. albopictus* (B) of sampling sites. Approximately 735 base pair with marker.

TDS with low dissolve oxygen in study sites. Among the study sites, the highest vector abundance was showed by tea-estates areas i.e. 172.21±3.82 for *A. aegypti* and 814.67±0.98 for *A. albopictus*, which correlated with the highest pH of 7.28±0.07 and TDS of 150.14±2.67 with low DO of 4.02±0.61 as compared to other study areas followed by industrial with average vector abundance 268.16±1.11 for *A. aegypti* and 779.46±0.99 for *A. albopictus* of which correlate with pH- 7.14±0.09, TDS- 82.67±3.19 and DO-4.12±0.84, floodplain riverside with average vector abundance of 115.13±1.98 for *A. aegypti* and 718.88±3.56 for *A. albopictus* of which correlate with pH- 7.11±0.11, TDS- 60.81±1.14 and DO-4.80±0.43 and forest areas with average vector abundance of 38.17±2.85 for *A. aegypti* and 534.17±2.48 for *A. albopictus* which correlate with pH-7.08±0.14, TDS- 114.90±3.25 and DO-4.20±0.41 respectively. From study it was found that the water quality ranges of both industrial areas and tea-estates provides more suitable breeding ground for dengue vectors as the vector abundance is found to be maximum in these two sampling sites (Figure 4), (Table 4). All the water quality parameters recorded to be statistically significant at ≤0.05 significant level with that of vector abundance (Table 5).

The amplification of the partial mt COI portion of genomic DNA was done by using forward primers of 5- GGATTGGAAATTGATAGTTCCTT and reverse primer of 3 AAAATTTAATTCCATTGAACAGC primers by using standard PCR protocol and yielded band of approximately size 700bp fragments for both *A. aegypti* and *A. albopictus*.[20] Further sequencing of both amplicons confirmed that all analyzed individuals belonged to *A. aegypti* and *A. albopictus*. Both the vectors displayed different amplicon size for mt COI gene (Figure 4).

The received rDNA sequences of mt COI gene were probe for identification using BLAST, NCBI so to aligned the sequences of isolates of present study to the similar isolated obtained from already submitted databases in NCBI for construction of phylogenetic tree to determined genetic diversity and evolutionary relationship among the isolates from different habitat types. Phylogenic tree was constructed in two different forms; one was constructed in-between the isolates obtained in present work collected from different sampling sites and one was in between isolates study in present work with that of similar isolates submitted in gene bank database in NCBI. Based on mt COI gene sequences of *A. aegypti*, maximum likelihood tree shows that out of six isolates, two isolates were distinctly separated into two clusters one is Code 5, Guwahati and another one is Code 6, ICMR strains collected from Dibrugarh, Assam) and other four isolated were sub-clustered into two clusters one between Code 1, Industrial areas and Code2, Tea-estates areas and second was in between Code 3, Riverside areas and Code 4, Forest areas and out of six isolates Code 1 and 2 and Code 3 and 4 shares 80% and 96% similarity with

one another and shows close proximity with each other whereas Code 5 shares 61% similarity with Code 1 and Code 2 and Code 6 shares 68% similarity with all the four isolates collected from different habitat types for *A. aegypti*.

In *A. albopictus*, similar pattern was observed, out of six isolates, Code 1' and Code 3'were distinctly separated forming separate cluster. Whereas (Code 2'and 5') and (Code 4' and 6') sub-clustered into two individual cluster. Among the six isolates both (Code 2' and 5') shared 90% and (Code 4' and 6') shared 76% similarity. In second phylogenetic tree analysis between isolates of present study and similar isolates obtained from BLAST result revealed that the isolates of *A. aegypti* shows similar alignment with isolates from two different states and countries i.e., from India (Assam and Maharashtra) and Srilanka, Kenya and Africa respectively. Out of six isolates of *A. aegypti*, four isolates show close proximity with the isolates from Assam obtained from genebank database, NCBI with Accession no. KX227735.1, KX227743.1, KX227739.1, whereas isolates of *A. albopictus* aligned with the isolates from Assam, India, China and Nepal and all the isolates shares maximum similarities with the isolates from China expect Code6' i.e., DRL, Tezpur which shows close proximity with isolates from Assam, India. Molecular phylogenetic analysis also revealed that *A. aegypti* collected from study sites has its origin in African countries while *A. albopictus* has in Asian countries (Figure 4).

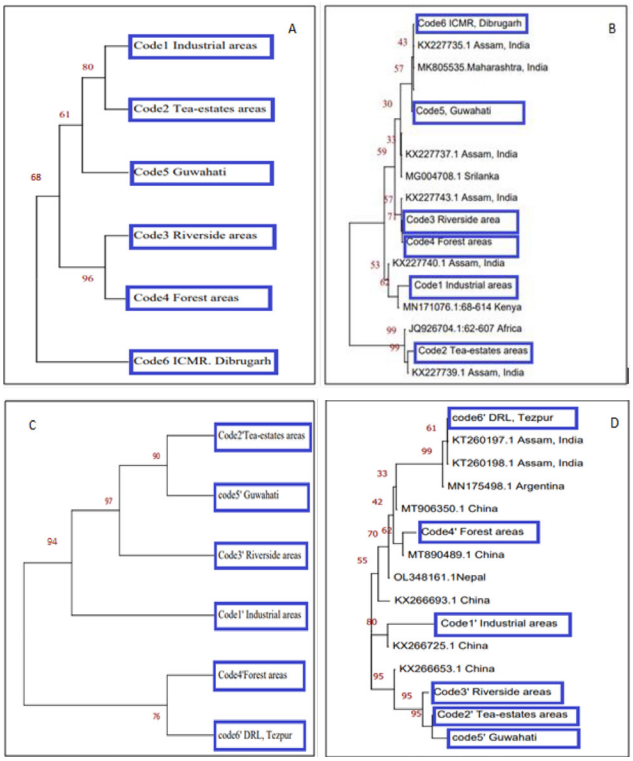

Figure 4: Construction of molecular phylogeny for mitochondrial COI gene of both *A. aegypti* (a and b) and *A. albopictus* (c and d). The evolutionary history was inferred by using the maximum likelihood method based on the Tamura 3-parameter model. The bootstrap consensus tree inferred from 1000 replicates is used to represent the evolutionary history of the taxa analyzed.

DISCUSSION

Owing to human behavior, vector-borne diseases increased manifold worldwide mostly in tropical and sub-tropical countries. One of the important measures for the control of vector-borne diseases lies on the accurate and realistic collection of data through proper monitoring of vector species in disease-prone areas or the areas, which have an equal chance of disease outbreak in coming years. This helps in increases the rate of success of vector control program. This could be achieved by conducting different vector surveillance methods according to place. Based on ovitrap surveillance it was found that immature stages of *A. albopictus* were dominant than *A. aegypti*. Which indicated that *A. albopictus* shared the same habitat with *A. aegypti* as their oviposition sites (Table 1). The finding revealed that *A. albopictus* have the flexibility of choosing oviposition sites for laying eggs in both natural and artificial containers in urbanized habitat.[21] For this reason a mixed infestation was observed in all the four habitat types except in forest areas. Additionally, *A. albopictus* is an opportunistic biter which prefers to take human blood over animal blood.[22] While its feeding habits vary depending on the region or habitat type from where the mosquito population originates. In this study only the outdoor ovitrap surveillance was preferred to examine in four different habitats. As from earlier study in different countries confirmed that, outdoor ovitrap surveillance contain the highest density of mosquito eggs than indoor ovitraps. This was also proved from our previous study on container surveillance of dengue vectors in and around the housing areas.[23] Similarly in traditional larval indices surveillance at four villages in Central Java and found significant outside infestation in flower pots (26 percent) and discarded tyres (53 percent).[24] Higher than 10% ovitrap index indicate a possible risk of dengue outbreak which was recorded in present study. The effect of three important meteorological parameters, temperature, humidity and rainfall against ovitraps revealed that both temperature and rainfall was a crucial factor in anticipating rises in dengue vector populations.[25] As rainfall creates favorable habitats for oviposition and larval development by filling natural and manufactured water-holding

containers which results in a high adult population both outside and inside of homes along with suitable temperature that exist throughout the year and clearly indicated by both OI and TPI result. Both temperature and precipitation showed the highest significant correlation with ovitrap indices in present study (Table 2). similarly precipitation increases the number of eggs counts of *Aedes* spp. in Brazil.[25] Accordingly a research conducted on Italy and found that rainfall was significantly correlated with female abundance.[26] Similar trend was observed in Yogyakarta, Indonesia by BG-Sentinel trap and found that larval count of *Aedes* spp. were significantly correlated with rainfall.[27] In various Asian nations, a correlation between rainfall and temperature and the occurrence of dengue has been documented.[28,29] This finding might be useful for motivating the public health authority to adapt vector control measures to clean up the water holding containers in dengue risk zones to decrease the breeding habitats. From the study of vertical dispersion of *Aedes* spp., it was observed that the correlation between positive ovitrap and larval collection with that of height was less. Due to this reason, a lesser *Aedes* populations was found at heights of high-rise apartments (Table 3). And this study was also supported by similar study carried in different countries like Malaysia, Singapore and many other countries worldwide and also from the present study it was observed that *A. aegypti* can take flight much higher that *A. albopictus*.[14] This was probably the first study conducted in our region. Therefore, it is strongly advised to do larger-scale studies to observe at the vertical dispersal of *Aedes* mosquitoes. Which help to provide clear view of transmission of dengue near future as there was greater risk of maximum dispersal of dengue vector to high rise apartment from ground level because of insecticide pressure on breeding surface at ground level and also the human and animal too act as carriers of this vector.[30] Study on water quality parameters with the ovitrap in different study sites was conducted as most mosquitoes choose their breeding locations based on their desired traits that may allow them to survive and for the dynamics of their population.[31,32] A number of variables that are physical and chemical that can impact the locations for those who can both attract and deter insect's mosquitoes. A certain site situation may be favored by most species, though others might not. A study has revealed that different types of plants and various chemical characteristics of the larval environment in per domestic areas are associated content of physical and chemical traits that could the growth and survival of larvae.[33] The present study strongly indicated that *Aedes* spp. mostly suitable to breed in water pH slightly alkaline and TDS in-between 60 to 200 ranges. These ranges were mostly favored by both industrial and tea-estates areas as maximum positive ovitraps along with higher dengue vector abundance were found in these two study sites. From the water quality parameters, it was found that *A. albopictus* survive and developed in a variety of habitat such as fresh water, brackish water or any water (clear, turbid or polluted), that were used for drinking or washing purposes as compared to *A. aegypti*. And this was proved by present study as the *A. albopictus* vector abundance was maximum then *A. aegypti*. Due to this reason *A. albopictus* has the greater plasticity of breeding habitat and infested in *A. aegypti* breeding sites and this was strongly suggested by many countries where similar work has been done.[34] This study was first reported in our areas, where water quality parameters were study in relation with the ovitraps. DNA-based identification techniques like rDNA region and mt gene COI could overcome the restricted ability of morphological identification methods to distinguish between sibling and closely related species of mosquitoes. Thus, during vector monitoring for arboviral outbreaks like dengue, this approach can be utilized to identify both adult and aquatic stages of the *Aedes* vectors. Results demonstrated a high degree of genetic congruence based on mitochondrial COI gene homology, which is 90% percent between the *Aedes* spp. (Figure 4). Similar finding was observed in previous study in Assam, India.[35] According to the phylogenetic study, both mosquito species used in this investigation are classified separately with rare exceptions and some Indian counterparts different from other regions of the world. Global map was created based on the available barcode sequences, shows the geographical distribution of the two vectors. As, the rise in dengue cases in both Dibrugarh and Tinsukia districts of Assam raises important questioning regarding the origin and establishment of this species in these areas. Beside the taxonomic identification, proper molecular identification of dengue vectors was of great importance in recent times. The challenge of finding and eliminating these mosquitoes from the infested area drove the desire to determine where this population originated in order to better understand its ecology and a general assessment of how long it has been there. These details help in evaluating the control measures and surveillance requirements. Further habitat wise molecular analysis of *A. albopictus* and *A. aegypti* based on PCR was displayed for the first time in Assam. From the present study it was cleared that the developing countries like India, dengue already has a significant impact and poses a high

danger for yellow fever. This emphasizes the necessity of a sizable global sequence collection to improve our comprehension of the diversity and dispersion of the mosquito species. Considering the rising incidence of Dengue in India, DNA bar-coding technique play an important role in understanding the mosquito speciation process in vector endemic zones

CONCLUSION

This study act as an efficacious tool for studying the habitat wise population dynamics status of *Aedes* mosquitoes in N.E. India. Helps in forecasting dengue endemic zones and outbreaks by serving as an early indicator to establish effective planning for necessary intervention strategies in environmental clean-up process as the study of both ovitrap surveillance. Molecular phylogeny provides proper information of biogeography, diversity, species abundance, moreover genetic structure of vector population among different habitat types of N.E. India.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

mt COI: Mitochondrial Cytochrome Oxidase I; **Labs:** Laboratory; **Pvt.:** Privatel; **Ltd.:** Limited; **OI:** Ovitrap index; **TPI:** Trap positivity index; **DNA:** Deoxyribonucleic acid; **SPSS:** Software platform offers advance statistical analysis; **pH:** Potential of hydrogen; **TDS:** Total dissolves solid; **DO:** Dissolve oxygen; **N.E.:** North east; **PCR:** Polymerize chain reaction; **BLAST:** Basic local alignment search tool; **NCBI:** National centre for biotechnology information; **Ft:** Feet; **Sig.:** Significant; **DRL:** Defence research laboratory; **ICMR:** Indian council of medical research; **Min:** Minimum; **Max:** Maximum.

SUMMARY

From the study of ovitrap surveillance, it was found that immature stages of *A. albopictus* were dominant over the *A. aegypti* in all the study sites. Mixed infestation of both the species in the same ovitrap was recorded. And among the different study sites, tea-estates areas account for the highest immature counts and 6th mile recorded the lowest. From the study of vector dispersal, 3ft height showed the highest immature counts followed by 6ft and 12ft above the ground level. And showed significant correlation with the vector count. The study of vector count with relation water quality parameters revealed that *Aedes* mosquito prefer to breed in high Ph and TDS and low DO. From the molecular phylogeny study, it was found that sequence of both *A. aegypti* and *A. albopictus* showed intra species divergences.

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