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Molecular Phylogenetics and Population Structure of *Aedes lineatopennis* (Ludlow): Implications for Vector Potential and Public Health Risk

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Abstract

Introduction

The mosquito Aedes lineatopennis belongs to the subgenus Neomelaniconion and functions as a transmission agent for both the West Nile virus and Rift Valley fever virus. It also responsible for spreading of Japanese encephalitis virus and canine heartworm. The medical significance of this species exists alongside a lack of available documentation about it. This study analyzed the genetic variation and evolutionary links by calculation of different indices within Aedes lineatopennis population for the first time. Further its vectorial capacity was examined through the analysis of the mitochondrial COI gene. The findings will provide important data regarding the species' distribution throughout evolutionary development and ecological adaptations while uncovering possible public health concerns.

Results

Our study detected 21 unique haplotypes alongside a haplotype diversity rate of 0.9754 and a variance reading of 0.00023. The COI gene sequences in Aedes lineatopennis displayed 256 segregating sites yet demonstrated low nucleotide diversity with a value of 0.10724. The analysis showed significant results for Harpending's raggedness index (R2 = 0.0152) but the p-value exceeded 0.05. The D+ (1.88172) F+ (0.85790) and F (1.326) values from Fu and Li's results were positive despite lacking statistical significance. The Tajima's D statistic showed 1.53395 while Strobeck S registered at 0.487. The average evolutionary substitution rates at each site were estimated as 0.31, 0.60, 0.88, 1.22, and 1.99. Thymine/uracil formed 39.98% of the nucleotide composition while adenine followed at 29.53%, cytosine was 15.55%, and guanine made up 14.95%.

Conclusion

The genetic diversity within the Aedes lineatopennis population reveals recent population expansion alongside possible selective pressures which could eventually lead to its establishment among other Aedes species. The species could become a higher risk to human health and present more challenges for its control. At this moment we must develop prevention strategies to stop its growth and establishment during initial developmental phases.

Keywords: *Aedes Lineatopennis*, Molecular Phylogenetics, Population Genetics, Public Health

1. Introduction

Zoonotic diseases are caused by several biological agents, including parasites, viruses, and bacteria, which pass from nonhuman animals to humans Vector-borne diseases (VBDs) are particularly significant, as their transmission is closely linked to environmental factors including rainfall, humidity, and temperature. These diseases – malaria, dengue, and West Nile fever (transmitted by mosquitoes);

leishmaniasis (carried by sand flies); and Lyme disease (transmitted by ticks) represent major vector-borne diseases [1].

Mosquitoes are among the most climate-sensitive vectors. The *Aedes* genus encompasses species that display substantial adaptive capabilities largely because of their genetic diversity. The World Health Organisation (2024) reports that vector-borne diseases (VBDs) constitute approximately 17% of all infectious diseases worldwide, resulting in more than 700,000 fatalities per year. Multiple *Aedes* species serve as vectors for various arboviral diseases. The *Aedes aegypti* mosquito acts as a vector for the Zika virus, Rift Valley fever (RVF), West Nile virus (WNV), La Crosse encephalitis as well as yellow fever according to. The mosquito species *Aedes aegypti* and *Aedes albopictus* transmit dengue and chikungunya viruses according to, whereas *Aedes vittatus* carries Japanese encephalitis, West Nile virus, Chandipura virus, and Chittoor virus as stated by. Research into the medicinal importance of *Aedes lineatopennis* (Ludlow) remains inadequate. Despite having limited and outdated documentation *Ae. lineatopennis* has been identified as a potential vector for multiple diseases. Previous studies have identified *Ae. lineatopennis* as a vector in WNV transmission in Pakistan and RVF virus dispersal in Kenya, as well as Japanese encephalitis virus (JEV) spread in Malaysia and canine heartworm distribution in Thailand. While there are no documented instances verifying its involvement as a vector in India, the possibility of its reappearance due to increasing vectorial capacity must not be disregarded [2-8].

Ae. lineatopennis is classified under the subgenus Neomelaniconion and shows morphological features similar to *Ae. mcintoshi* causes frequent misidentification. The insect can be distinguished by its narrow vertex with erect forked scales beyond the occiput, a male maxillary palpus which surpasses the proboscis in size and a scutellum featuring uniformly narrow scales. Recent molecular techniques have shown that sequencing the mitochondrial cytochrome c oxidase subunit I (COI) gene serves as an accurate tool for identifying mosquito species at the species level [9-12].

Ae. lineatopennis is found mainly in Africa and its range continues into Southeast Asia and Australia. Recent studies have documented the presence of the species in India according to reports from the Indian subcontinent. Researchers believed Odisha's climate favored the existence of *Ae. lineatopennis* species. It thrives in Odisha owing to climatic similarities with regions where *Ae. mcintoshi* dwells. The study by Goud et al. (2023) mentioned *Ae. lineatopennis* yet lacks any verified records. But the authors have achieved the first molecular verified confirmation and identification of *Ae. lineatopennis* in this study. The study fills an important gap in understanding *Ae. lineatopennis* distribution by providing its first confirmed detection in Odisha. A thorough study of genetic structures has occurred for several medically relevant *Aedes* species such as *Ae. albopictus* yet no comparable research exists regarding the genetic diversity of *Ae. lineatopennis*. The genetic diversity studies of *Ae. albopictus*, and *Ae. Aegypti* have been investigated on global scale. The same study on *Ae. vittatus* has been done by Díez-Fernández et al. (2019). Despite *Ae. lineatopennis* bites both humans and animals aggressively and transmits various diseases yet we have no data about its worldwide distribution or genetic variation. A previous version of this article has been available as a preprint at research square. This study investigated *Ae. lineatopennis* genetic diversity and evolutionary relationships and evaluated its potential vector capacity through mitochondrial COI gene sequencing. The results will provide important information about the species' geographic distribution along with its evolutionary development and ecological adaptations while revealing potential public health issues. The insights derived from this research will play a crucial role in developing future strategies for vector surveillance and disease management [13-20].

2. Materials and Methods

2.1. Study Area

The study's fieldwork took place at the Manikya Vihar Campus which is located in Bhawanipatna within the Kalahandi District of southern Odisha, eastern India (**Figure 1**). Researchers gathered data from multiple locations throughout the Maa Manikeshwari University Campus. The town of Bhawanipatna is located at approximately 19.9°N latitude and 83.17°E longitude. This region experiences a tropical wet and dry climate known as savanna which receives about 1300 mm of rainfall each year. The geographic location of this district extends from 19.3°N to 21.5°N latitude and from 82.20°E to 83.47°E longitude.

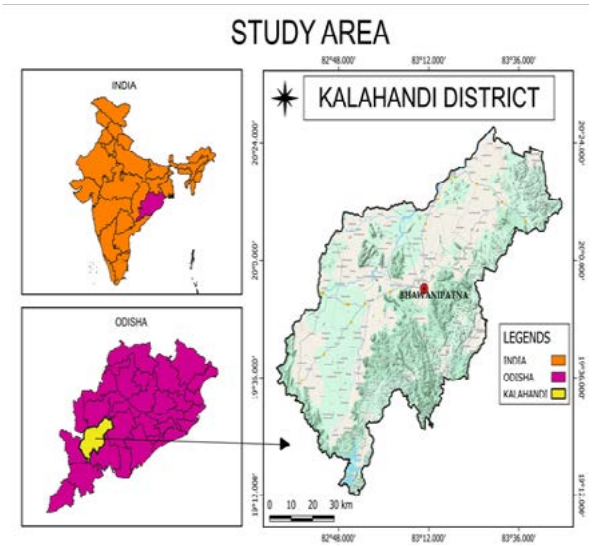

Figure 1: Showing Study Area

2.2. Mosquito Collection

Throughout the monsoon season from June to October 2024, authors gathered adult mosquito specimens bi-monthly from various locations on the Maa Manikeshwari University campus in Bhawanipatna. Collection was made by an aspirator tube and torchlight to target resting sites both inside buildings and outside areas. Researchers transferred captured mosquitoes into test tubes which contained between 3 to 5 insects each. The date, time, and exact collection site were recorded on every tube. The laboratory procedures involved anaesthetizing specimens and analyzing them with a binocular stereo zoom microscope to identify mosquito by morphological characteristics. The outdoor collections was made with suction tubes from open drains along with empty drums, discarded tyres, coconut shells and earthen pots combined with cement tanks and university grounds close to stagnant water bodies and swamps and other breeding areas for two-hour sessions during each collection.

2.3. DNA Extraction and PCR

The extraction of genomic DNA from each mosquito sample involved the use of the PureLink™ Genomic DNA Mini Kit. The authors adapted the conventional in vitro genomic extraction protocol with specific modifications to the PureLink™ Genomic DNA Mini Kit (Catalogue No. K1820-00) to achieve better DNA yield and purity. A standard volume of 100 µL of DNA extraction buffer was used to process each material which was then left to incubate at ambient temperature for 20–25 minutes to achieve tissue lysis. The sample was transferred to a vial filled with beads containing XPLOREGEN™ gDNA Extraction Buffer 1 before being vigorously vortexed at full speed for 10 minutes to ensure thorough homogenisation. Afterwards, 300 µL of XPLOREGEN™ gDNA Extraction Buffer 2 was added to the mixture and vortexed it for 7 more minutes. The homogenate underwent centrifugation at 10,000 rpm for 3 minutes while at room temperature in a sterile 2 mL microcentrifuge tube. After that, carefully 800 µL of the clear supernatant liquid was transferred into a new tube. The supernatant received 200 µL of XPLOREGEN™ gDNA Extraction Buffer 3 before being vortexed briefly for 5 seconds. The sample underwent an additional 2-minute centrifugation step at 10,000 rpm. The supernatant underwent DNA purification with XPLOREGEN™ Buffers 4, 5, and 6 through the manufacturer's recommended column-based DNA cleanup protocol. Following the final wash the spin column was centrifuged at 10,000 rpm for 5 minutes to remove excess wash buffer. Then 50 µL of XPLOREGEN™ Elution Buffer (Elution 1) was applied to the column membrane's center to elute the genomic DNA. The purified DNA transferred to a new microcentrifuge tube and stored at −20°C for later molecular studies. The spin columns were discarded following laboratory waste disposal guidelines.

2.4. Genomic Analysis

The Polymerase Chain Reaction (PCR) process took place in a reaction mixture with a total volume of 25 µL. The reaction mixture contained 10 µL of 10× Taq polymerase buffer with 3.3 mM $MgCl_2$ and 2.5 mM dNTPs as well as 1 µL of Taq DNA polymerase at 3 U/µL concentration together with 2 µL each of forward and reverse primers specific for the mitochondrial cytochrome c oxidase subunit I (COI) gene.

The following primer sequences that were used to amplify the COI gene:

- **Forward: GGTCAACAAATCATAAAGATATTGG**
- **Reverse: TAAACTTCAGGGTGACCAAAAAATCA**

Thermal cycling was performed according to the following protocol: The thermal cycling protocol began with a 3-minute denaturation at 94°C followed by 30 cycles which included 1-minute denaturation at 94°C and annealing at 50°C for 1 minute each with 2-minute extension at 72°C. During PCR, the final extension process at 72°C continued for 7 minutes to guarantee complete amplification of target DNA segments. The PCR amplification was checked by running their products on a 1.2% agarose gel containing ethidium bromide and subsequently examining them under UV light transillumination (**Figure 2**).

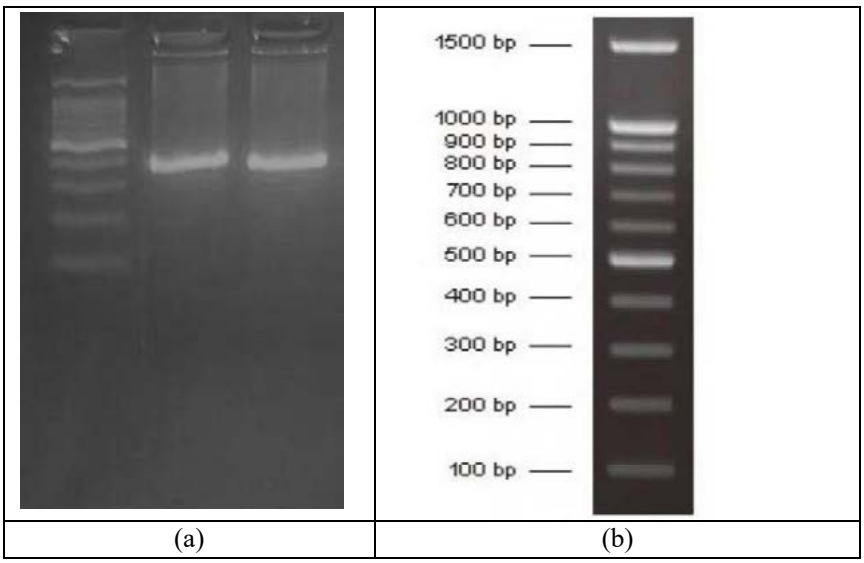

Figure 2: Agarose Gel (1.2%) Showing Single 700 BP Of Mitochondrial Cytochrome C Oxidase Subunit I Amplicon (A) Gel Image, (B) Marker Image

The BigDye™ Terminator v3.1 Cycle Sequencing Kit enabled bidirectional sequencing of the amplified products through the ABI 3130 Genetic Analyser. Sequencing reactions employed primers LCO1490 and Chelicerate Reverse 2. The raw sequencing data underwent processing and alignment through Clustal Wbefore adjustment with Bio Edit to prepare for phylogenetic analysis [19-21].

2.5. Data Analysis

The DnaSP software (22) was used to evaluate genetic diversity and molecular variation. The investigation identified haplotypes and evaluated key parameters such as synonymous versus non-synonymous substitution counts in addition to haplotype diversity (Hd), nucleotide diversity (π), and the average pairwise nucleotide differences between sequences.

The phylogenetic relationships first reconstructed through MEGA6 software received modifications via BioEdit software. This investigation assessed haplotypes' evolutionary relationships through fundamental statistical methods including Tajima's D neutrality test and phylogenetic tree creation via the neighbor-joining algorithm. The later analyses followed methodologies described in contemporary population genetics studies by. The obtained sequences were deposited in GenBank at the National Centre for Biotechnology Information (NCBI) to enable public access and academic reference [22,23].

2.6. Population Expansion

The DnaSP software by 22 evaluated demographic history and neutral evolution patterns through various neutrality tests statistics like Tajima's D, Fu and Li's D+ and F+, and R^2. The number of segregating sites was used to calculate Tajima's D which helped identify deviations from neutrality potentially caused by selection or population expansion. The Fu's Fs statistic was computed to assess demographic stability and potential population growth events following Ramos-Onsins and established methods.

3. Results

3.1. Morphological Characterization

The adult female mosquito that was caught showed certain morphological characteristics, like golden curved scales adorned the head while brown scales stood out behind the eyes. The occiput contained prominent patches of upright golden scales which were mixed with fine setae. The wings had an approximate length of 5 mm. The costal vein displayed a uniform dark scale covering while the subcostal area stayed mostly scale-free and showed a characteristic cream coloration. Dark scales primarily covered the legs while a pale longitudinal stripe appeared on the posterior parts of the femur, tibia, and the first tarsal segment. Additionally, the abdomen bore dense brown scaling. **Figure 3** provides a visual representation of these morphological traits.

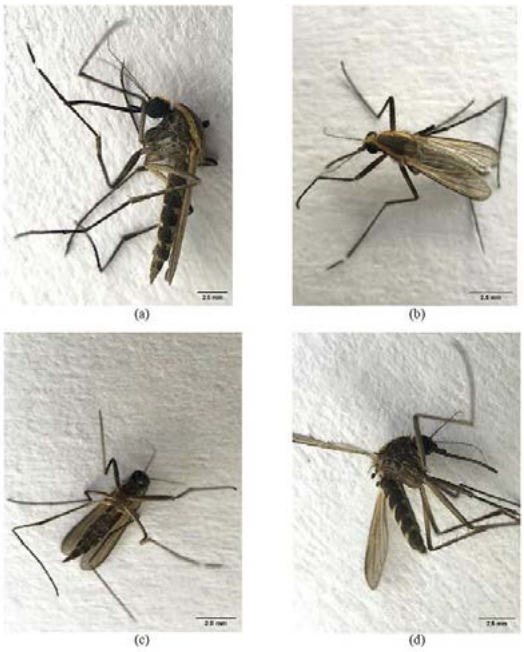

Figure 3: Different Views of Female *Aedes lineatopennis*: (A) Right Lateral, (B) Dorsal, (C) Ventral, and (D) Left Lateral

3.2 Species Confirmation Through Molecular Identification

Sl. No.	Organism Name	Accession No.	% Match
1	Aedes lineatopennis isolate M22-4 cytochrome c oxidase subunit I (COX1) gene.	OK413076.1	99.55%
2	Aedes lineatopennis mitochondrial COI gene for cytochrome oxidase subunit 1.	AB73814S.1	99.54%
3	Aedes lineatopennis isolate M14-1 cytochrome c oxidase subunit I (COX1) gene.	OK413075.1	99.39%
4	Aedes lineatopennis isolate M17-1 cytochrome c oxidase subunit I (COX1) gene.	OK413074.1	99.39%
5	Aedes lineatopennis isolate M13-2 cytochrome c oxidase subunit I (cox1) gene.	OK413077.1	99.09%
6	Aedes lineatopennis mitochondrial COI gene for cytochrome oxidase subunit 1, partial cds, isolate: 351YON2005	AB738144.1	98.92%
7	Aedes lineatopennis isolate 35-3 cytochrome oxidase subunit I (COI) gene.	KT358468.1	98.92%
8	Aedes lineatopennis clone Ae.lin3 cytochrome oxidase subunit I (COI) gene.	MF179164.1	98.92%
9	Aedes lineatopennis voucher TH150-1 cytochrome oxidase subunit I (COI) gene.	HQ398909.1	98.77%
10	Aedes lineatopennis isolate 35-1 cytochrome oxidase subunit I (COI) gene.	KT358466.1	98.61%

Table 1: blast result of our isolated *ae. lineatopennis* COI Sequences with Other COI Sequences Of *AE. Lineatopennis* Avaibalbe In Genbank

Through initial morphological assessment and identifiable diagnostic features, it was confirmed that the specimen belonged to *Ae. lineatopennis*. Sequence analysis provided molecular confirmation for the specimen identification. The resulting DNA sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) algorithm (24). DNA sequence analysis demonstrated a 99.5% similarity with the *Ae. lineatopennis* isolate M22-4 (GenBank accession no. OK413076.1) (**Table 1**). This information, thereby corroborating the morphological identification as identified earlier.

3.3. Molecular Diversity and Population Genetics Analysis

The COI gene sequence retrieved after PCR amplification and sequencing was modified before being placed in the GenBank database under accession number PQ788195 (**Figure 2**). One newly generated sequence and 28 reference sequences from GenBank were used in the molecular analysis to identify 21 distinct haplotypes. The calculated haplotype diversity of 0.9754 along with low variance measurement of 0.00023 demonstrated significant genetic variation among the sequences presented in **Table 2**. Analysis found an average of 41.825 pairwise nucleotide differences between sequences, while all 917 examined nucleotide positions remained synonymous without any detected nonsynonymous mutations. The sequences exhibited low nucleotide diversity with a value of π = 0.10724 and revealed 256 segregating sites.

Sl. No.	Genetic variability indices	Value	Remark
1.	Number of sequences	29	Available in the World (Total)
2.	Number of sites (L)	390	_
3.	Number of segregating Sites/ polymorphic (S)	256	_
4.	Proportion of segregating sites (S/L×100)	65.6%	Indicates moderate to high genetic diversity in the population
5.	Nucleotide diversity (π)	0.10724	
6.	Pairwise nucleotide differences (K_a)	41.82	
7.	Theta (per site) from eta (Θ_s)	0.107244	
8.	Theta (per sequence) from eta (Θ_g)	41.825123	
9.	Numberof haplotypes (H)	21	
10.	Haploid diversity (H_d)	0.975369	
11.	Variance of haploid diversity (Vhd)	0.00023	Indicates the same haplotype or exhibits minimal genetic differences or homogeneous population

Table 2: Genetic Variability Indices For The *AE. Lineatopennis* Samples from Whole World

Sl. No.	Population expansion indices	Value	Remark
1.	Fu and Li's D test (D+)	1.88172	neutral processes (not significant)
2.	Fu and Li's F test (F+)	0.85790	neutral evolutionary processes (not significant)
3.	Fu's Fs statistic (Fs)	1.326	no recent population expansion (not significant)
4.	Strobeck's statistic (S)	0.487	neutral processes (not significant)
5.	Tajima's D (D)	1.53395	rich diversity of mutations (not significant)
6.	Harpending's raggedness statistic (R2)	0.0152	has undergone a recent population expansion (statistically significant)

Table 3: Population Expansion Indices for the *Ae. lineatopennis* Samples from Whole World

The analysis of nucleotide pairs showed one site with polymorphism. The Harpending's raggedness index value was 0.0152 for demographic expansion pattern analysis despite being positive its statistical significance remained below the threshold (P > 0.05). The Fu and Li's D+ test showed 1.88172 as a positive result and F+ displayed 0.85790 but none achieved statistical significance according to **Table 3** with Fu's Fs scoring 1.326. The Strobeck's S value reached 0.487 and Tajima's D value reached 1.53395 but neither revealed any statistically significant departure from neutrality according to the P value (P > 0.10). The researchers used the Tamura-Nei model along with gamma distribution to evaluate substitution patterns. The model identified five distinct classes to handle variable evolutionary substitution rates across sites which averaged 0.31, 0.60, 0.88, 1.22, and 1.99 substitutions per site. The nucleotide composition of the dataset displayed A = 29.53%, T = 39.98%, C = 15.55%, and G = 14.95% which indicates an AT bias characteristic of mitochondrial genes.

4. Discussion

Recently, out of several VBDs, *Aedes* Borne Diseases (ABDs) have increased significantly because of climate changes. The most common and medically important *Aedes* mosquitoes, *Ae. aegypti*, *Ae. albopictus*, and *Ae. vittatus*, are widespread in tropical, subtropical, and certain temperate regions of the world. The most neglected species of *Ae. lineatopennis*, was originally indigenous to Africa, but it has since spread

to Asia, Australia, and India. However, morphological identification of this species is challenging due to its similarities with other *Aedes* species. But the golden body colour may be the most important and easiest to identify this mosquito. Researchers use the mitochondrial cytochrome oxidase I (COI) gene to confirm species levels, conduct population studies, and comprehend their evolutionary relationships. In this study COI gene sequences were used to look at the global genetic variety of *Ae. lineatopennis*. The confirmation of the presence of this species in Odisha, India, is the first recorded collecting of *Ae. lineatopennis*. This finding contributes to our understanding of the species' distribution. Thus, the presence of this species in Odisha indicated that it may be present in other parts of the country where similar types of habitats are present, like extreme environmental conditions. It is caught in the rainy season at locations near human dwellings, shaded areas, swamps, and wet forest environments, indicating that it may breed in stagnant water with more organic content (**Figure 4**). Additionally, it mphasises that there must be ongoing surveillance and research to monitor the potential implications for public health and vector control strategies.

Figure 4: collection sites of *Ae. lineatopennis*, Near to Human Drawelling Sheaded, Swamp and Wet Forest Area.

In this study 21 unique haplotypes were identified, indicating significant genetic diversity in *Ae. lineatopennis*. As well as it showed significant haplotype diversity (Hd = 0.975369) and high value of nucleotide diversity (π = 0.10724) indicating a notable mutation rate in the COI gene of *Ae. lineatopennis*. The region-specific haplotypes are developed by ecological spread and geographic isolation. Gene flow between genetically diverse groups, presumably influenced by human activities (insecticide application) or certain climatic conditions, has contributed to this diversity. So, these factors may have amplified genetic variation by promoting adaptive genotypes. Here we can compare the indigenous populations with nonnative. *Ae. albopictus* populations indigenous to East Asia. It shows more superior genetic diversity compared to those in imported areas, presumably owing to a more extensive evolutionary history in their native habitat . Similar type of result also reported on *Ae. aegypti* from Hainan Island and the Leizhou Peninsula indicates significant diversity, likely affected by anthropogenic spread . In Asia and North America have frequent use of different insecticides (DDT, organochlorines, organophosphates, pyrethroids, and carbamates. The creation and imposition of selection pressures result in the promotion of adaptive genetic changes *Ae. aegypti* displays minimal genetic diversity worldwide because of consistent ecological conditions across its habitats the considerable diversity seen in *Ae. lineatopennis* may indicate that they originated from common ancestral populations but adapted to different environmental niches [23-28]

Here we got two contrasting values of two metrics. The value of the haplotype count was high, whereas the variance of haplotype diversity (Vhd = 0.00023) was small. It indicated a relative homogeneity among these populations. This uniformity may indicate a recent shared ancestry or a historical bottleneck event that reduced genetic variation, which has permitted a limited number of haplotypes to prevail. Such patterns usually arise when small founding populations establish themselves in new ecological niches. The movement of genes across global boundaries through international commerce and travel can diminish regional genetic distinctions[29].

The COI gene shows extensive polymorphism and diversity as demonstrated by metrics such as numerous segregating sites along with pairwise nucleotide variations (Ka) and theta values per site and sequence (Θs and Θg). These tendencies are likely attributable to ecological stressors, accelerated reproduction, widespread global dispersion, and historical demographic changes. *Ae. lineatopennis* demonstrates mitochondrial gene diversity levels surpassing 0.1 (Θs), in contrast to the typical range of 0.001 to 0.02 per site observed in mammals, indicating significant environmental adaptability across continents [30,31].

The several selection tests (Fu and Li's D, Tajima's D, and the S statistic) provided positive value. But, the results were statistically non-significant ($p > 0.05$). This showed that neutral evolutionary mechanisms such as genetic drift may be at play; whereas Fu's Fs value suggested resistance to population increase. Further, the Harpending's R^2

value was low, supporting the idea of a recent population expansion [32].

A phylogenetic tree derived from 29 worldwide *Ae. lineatopennis* sequences exhibited several prominent clades (**Figure 5**). The *Ae. lineatopennis* samples from Australia formed a singular cluster. Whereas same sampale from China and the UK were categorised into three separate branches each. Indian samples exhibited genetic affinity to specimens from Thailand and China, indicating a potential introduction into India from these areas. The presence of similar genetic profiles in different areas supports the idea that genes are constantly flowing between them. However, there was no clear proof was found linking genetic differences to changes in their habitats [26-33].

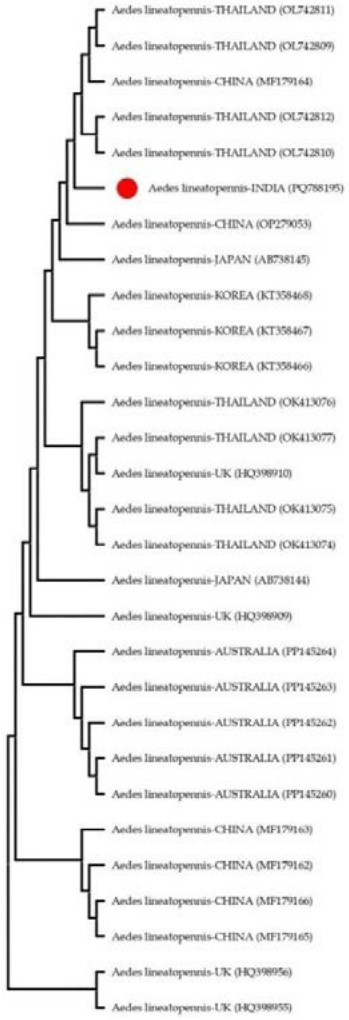

Figure 5: Molecular Phylogenetic Analysis of *Ae. lineatopennis* By Maximum Likelihood Method of The Whole World Sample Data, Red Dot Marked Indicates Specimen from This Research

The application of the Discrete Gamma Distribution model (+G) provided valuable insight into the heterogeneity of evolutionary rates of the mitochondrial cytochrome oxidase I (mtCOI) gene. This variation could cause certain nucleotide positions to evolve at differing speeds. The gamma distribution was divided into five unique rate categories during this research. It represented a spectrum of substitution rates per site: 0.31, 0.60, 0.88, 1.22, and 1.99. Genes showing the lowest evolutionary change rate (0.31 substitutions per site) appear to be experiencing significant purifying selection whereas those evolving at the maximum rate (1.99 substitutions per site) seem to exhibit fewer selective constraints or positive selection which could enable adaptive evolution under environmental or host-pathogen pressures. The nucleotide composition of the mtCOI gene in *Ae. lineatopennis* showed a notable bias: adenine (A) comprises 29.53%, thymine/uracil (T/U) 39.98%, cytosine (C) 15.55%, and guanine (G) 14.95%. This AT-rich profile, with a combined A+T/U content of 69.51%, is characteristic of mitochondrial genomes and may reflect AT-biased mutation patterns or specific selective pressures. These findings suggesting that the mtCOI gene exhibits a mosaic of evolutionary pressures. Where some regions being highly conserved due to essential functional roles, while others evolve more rapidly, because of adaptation to facilitate. The presence of both slow- and fast-evolving sites highlights the complexity of mitochondrial gene evolution in *Ae. lineatopennis*. It underscores the gene's utility in revealing evolutionary patterns and mutational processes within mosquito populations.

5. Conclusion

The *Ae. lineatopennis* population has significant genetic diversity, indicating recent population expansion and possible selective pressures; in the future, it may be more established with other *Aedes* species. Together, they may have more nuances for human health and be difficult to control. So this is the right time to plan to inhibit its expansion and establishment. Because there was some proof of the vectorial potential of WNV, RVF, JEV, and canine heartworm in the past, it should not be ignored further. This study shows a genetically heterogeneous *Ae. lineatopennis* population influenced by demographic shifts and evolutionary pressures on the mt COI gene.

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List of Abbreviations

COI : cytochrome c oxidase i
MT: mitochondrial
AE.: *aedes*
HD: haplotype diversity
BLAST: basic local alignment search tool
VHD : variance of haploid diversity

Declaration

Erhics Approval and Consent to Participate

"Not applicable"

Animal Ethics

'Not applicable'

Consent for Publication

'Not applicable'

Availability of Data and Material

'Not applicable'

Competing Interests

"The authors declare that they have no competing interests"

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