Original Research Article

GENETIC CHARACTERISATION AND MOLECULAR PHYLOGENY OF *AEDES VITTATUS SPECIES* (DIPTERA: CULICIDAE) FROM BHAWANIPATNA, KALAHANDI, ODISHA, EASTERN INDIA BASED ON COI GENE

ABSTRACT

Introduction

Aedes vittatus is common throughout India and breeds in a variety of locations, including tree holes, cement tanks, rock pools, abandoned containers close to residential areas, and marsh pools. The invasive mosquito species *Aedes vittatus* has expanded its range across Africa, Asia, Latin America, and Europe. Mosquito species have been successfully identified by characterizing a portion of the cytochrome c oxidase subunit 1 (cox1) gene, particularly in light of the difficulty in differentiating mosquito larvae and the scarcity of qualified taxonomists. Hence, the current study was designed for molecular characterization of the *Aedes vittatus* mosquitoes collected from all parts of Bhawanipatna based on mitochondrial COI to provide a wider understanding of the phylogenetic relationships of *Aedes vittatus* mosquitoes that exist throughout India. The genetic relatedness between India mosquitoes and those reported from other parts of the world was also investigated.

Material and Methods

The collection of data was made from different locations of Bhawanipatna Municipal Corporation, the headquarter of the district of Kalahandi, Bhawanipatna is located at 19.9°N 83.17°E. Adult Collection: Adult *Aedes* mosquitoes were surveyed weekly from the selected human dwelling, both inside and outside the house or premises of Bhawanipatna Municipal area from last week of june-2024 to last week of October-2024. Adult mosquitoes were collected with the help of a manual aspirator tube and a torch light. DNA was isolated from the provided culture. Quality was evaluated on 1.8% Agarose Gel; a single band of high-molecular weight DNA has been observed. The consensus sequence was generated for each sample using BioEdit version 6.0.7 and were searched over the Gene Bank database using Basic Local Alignment Search Tool (BLAST) against the *Aedes vittatus* genomes in NCBI, Gene Bank. The complete sequences were deposited in Gene Bank with accession no. PQ477920.1

Discussion

The KSP03 isolate from Bhawanipatna, Kalahandi, matched the sequences that had previously been added to the NCBI Gene Bank from all around the world. *Aedes vittatus* was identified as our isolated strain, and it exhibited a high degree of similarity (99.85%) with accession number MK491498.1. However, when compared to *Aedes lineatopennis* (Thailand), *Aedes tarsalis* (Kenya), *Aedes centropunctatus* (USA), *Aedes cinereus* (Slovenia), *Aedes cumminsii* (Kenya), and *Aedes vexans* (Greece), both the Kerala03 and Kalahandi Odisha strains were similar to the Pakistan strain, *Aedes cogilli*.

Conclusion

Aedes vittatus vector might be introduced to Kalahandi, Odisha from neighboring states. The presence of this competent vector is most probably a risk of transmission of arboviruses such as dengue fever, yellow fever, West Nile virus, Zika virus, and chikungunya virus in this area.

Key Words: *Aedes vittatus* , Molecular Characterisation, Bhawanipatna, Odisha, COI

1. INTRODUCTION

According to Weaver et al. (2017) and Sudeep et al. (2017), Aedes mosquitoes are the main carriers of several mosquito-borne diseases (MBDs), such as dengue fever (DF), yellow fever (YF), chikungunya (CHIKV), and the Zika virus[1], [2]. In recent decades, the burden of these Aedes-Borne Diseases (ABDs) has increased dramatically on a global scale. This increase is partly explained by the mosquitoes' improved ability to transmit diseases due to their increasing tolerance to different pesticides and resilience to environmental stressors. Interestingly, there are notable variances in adaptive genetic variants across Aedes mosquito populations from various geographic areas. Because genetic analysis of local mosquito populations can provide important insights into their genetic composition, propensity for disease transmission, stability over time, and other pertinent aspects, effective control strategies are becoming more and more reliant on this method.Thus, genetic analysis of local mosquito populations is becoming more and more important for effective control measures because it can provide important information about the mosquitoes' genetic makeup, potential for disease transmission, stability over time, and other factors related to disease spread, like vector migration between regions [3].

Other Aedes species may also aid in the spread of arboviruses because of their similar vector requirements, in addition to the well-known Aedes aegypti and Ae. albopictus. Particularly concerning are species like Aedes *japonicus*, Aedes *vexans*, and Aedes vittatus, which have shown vector competence for a number of arboviruses, including the dengue, chikungunya, and Zika viruses[4]Aedes *vittatus* is common throughout India and breeds in a variety of locations, including tree holes, cement tanks, rock pools, abandoned containers close to residential areas, and marsh pools [5].

The invasive mosquito species Aedes (Fredwardsius) *vittatus* (Bigot, 1861) has expanded its range across Africa, Asia, Latin America, and Europe [2], [6], [7], known for its preference for feeding on humans, *Aedes vittatus is* a highly anthropophilic mosquito that thrives in environments close to human residences (peridomestic) as well as in forested areas (sylvatic) [8]. Mosquito species have been successfully identified by characterising a portion of the cytochrome c oxidase subunit 1 (cox1) gene, particularly in light of the difficulty in differentiating mosquito larvae and the scarcity of qualified taxonomists[9], [10], [11]. However, this approach relies on prior genetic data for each species [12]. This presents a notable limitation for *Aedes* mosquitoes, as genetic data is lacking for most Indian species within this genus, despite their role in transmitting pathogens [13], [14].

Hence, the current study was designed for molecular characterization of the *Aedes vittatus* mosquitoes collected from all parts of Bhawanipatna based on mitochondrial COI to provide a wider understanding of the phylogenetic relationships of *Aedes vittatus* mosquitoes that exist throughout India. The genetic relatedness between India mosquitoes and those reported from other parts of the world was also investigated.

2. MATERIALS AND METHODS

2.1 STUDY LOCATION

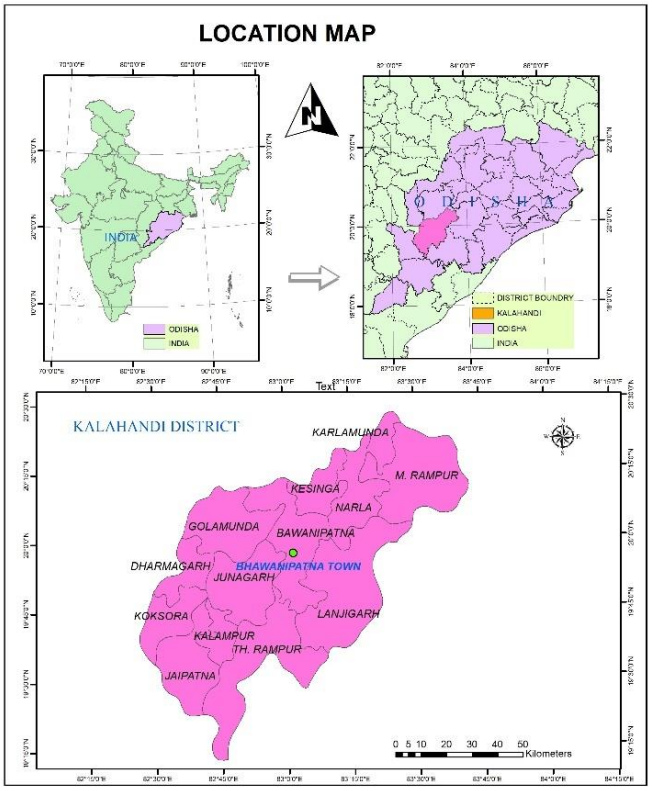

Figure 1: Showing study area at Bhawanipatna in Kalahandi district of Odisha, Eastern India

The collection of data was made from different locations of Bhawanipatna Municipal Corporation, the headquarter of the district of Kalahandi, Bhawanipatna is located at 19.9°N 83.17°E, has a tropical wet and dry climate, and the annual average rainfall is1300mm. The municipality has a population of 69,045 of which 35,506 are males while 33,539 are females residing in around 16,500 houses as per a report released by census India 2011.

2.2 SAMPLE COLLECTION

Adult Collection: Adult Aedes mosquitoes were surveyed weakly from the selected human dwelling, both inside and outside the house or premises of Bhawanipatna Municipal area from last week of june-2024 to last week of October-2024. Adult mosquitoes were collected with the help of a manual aspirator tube and a torch light[15]. Immediately after collection, the mosquitoes were transferred into test tubes at the rate of 3-4 mosquitoes per tube. The date, place, and time of collection were marked on each test tube. The mosquitoes were anesthetized and identified under a binocular stereo zoom microscope in the laboratory-based on the standard morphological keys[16]. Indoor collections were made during the morning hour between 6 am and 10 am. Larval Collection: Weekly Larval collections were made at random from indoor (earthen pot, cement tank, plastic container, flower pot, and plastic bucket etc.) and outdoor (cement tank, tree hole, coconut shell, metal drum, plant pot, plastic container, and discarded tire etc.) breeding sites. The location (indoor or outdoor), date and time, type of habitat, and the number of larvae collected were recorded. The immature stages were collected with the help of a glass dropper and transferred to the laboratory in plastic containers, for development into mature stage and identification of mosquito at the species level.

2.3 MORPHOLOGICAL IDENTIFICATION

(a) (b)

Figure 2: (a) *Aedes vittatus* (Male) and (b)*Aedes vittatus* (Female)

The larvae collected from different sampling sites were identified using morphological characteristics such as comb scale and pecten teeth, and the adults reared from larvae were identified using standard keys[16].The important diagnostic characteristics to confirm the occurrence of *Ae. vittatus* specimens from the sampling location. The mosquito specimens were identified as Ae. *vittatus* and differentiated from other found Aedes mosquitoes by the following morphological characteristics. The presence of narrow dark scales and three pairs of small round white spots distributed along the dorsocentral area of the scutum (Figure 2). The identified Aedes specimens with tibiae dark, each with a subbasal white spot and a white band at about basal 0.33 on fore- and midand at about 0.50 on hind-tibia (Figure2). In addition, the mosquito specimens have a distinct white band on the proboscis [17].

2.3 PCR AMPLIFICATION OF COI PARTIAL DNA SEQUENCE AND SEQUENCING

2.3.1 Isolation

For DNA Isolation,Hi-PurA Insect DNA Purification Kit Catalog No. MB569-20PR from Hi-Media was used.

2.3.2 Agarose gel electrophoresis

The amplified DNA was separated by electrophoresis in 0.8% agarose gel run in 1× TAE buffer at 50V for 30 to 45 minutes till DNA fragments are migrated well. The gel was photographed on gel documentation system.

2.3.3 PCR Amplification

Isolated DNA was amplified with mitochondrial cytochrome c oxidase subunit I (COI) genes Specific Primer (**LCO 1490 & HCO 2148**) using Veriti® 96 well Thermal Cycler. A single discrete PCR amplicon band of **~700 bp** was observed (**Figure 3**). The PCR amplicon was bead purified and further subjected to Sanger Sequencing.

2.3.4 Sequencing.

Bi-directional DNA sequencing reaction of PCR amplicon was carried out with **LCO 1490- TCC GTA GGT GAA CCT GC GG& HCO 2148- TCC TCC GCT TAT TGA TAT GC** primers using BDT v3.1 Cycle sequencing kit on ABI 3500Dx Genetic Analyzer.

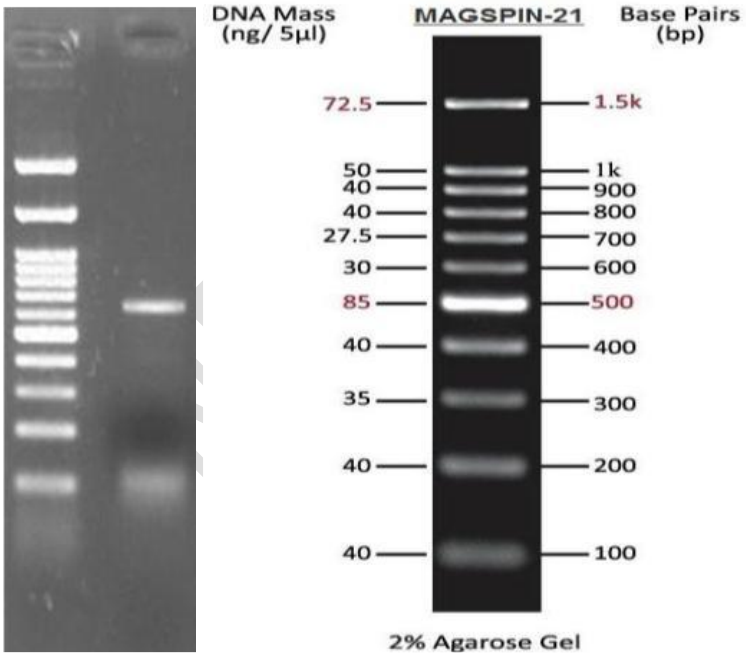

Figure 3: 1.8% Agarose gel showing single 700 bp of mitochondrial cytochrome c oxidase subunit I amplicon.

2.4 DATA ANALYSIS

The consensus sequence was generated for each sample using BioEdit version 6.0.7 and were searched over the GenBank database using Basic Local Alignment Search Tool (BLAST)against the *Ae. vittatus* genomes in GenBank (NCBI WEB SITE). The complete sequences were deposited in GenBank with accession no. PQ477920.1. The mt COI sequence of *Aedes vittatus (*our isolated strain KSP03) were compared with the whole world samples of gene COI of *Aedes* species found from other countries; further it also compared with the COI gene sequence of Indian *Aedes vittatus* (Figure 5) using Multiple Sequence Alignment (MSA) based on the sequences available in NCBI GenBank..

The evolutionary history was inferred using the Neighbor-Joining method [18]. Phylogenetic trees were built using maximum likelihood method (with 1000 bootstraps) with Kimura a cluster containing >50% bootstrap support, was considered significant. The evolutionary distances were computed using the Maximum Composite Likelihood method [19] and are in the units of the number of base substitutions per site. This analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X [20].

3. RESULT

The KSP03 isolate from Bhawanipatna, Kalahandi, matched the sequences that had previously been added to the NCBI Gene Bank (Figure) from all around the world. *Aedes vittatus* was identified as our isolated strain, and it exhibited a high degree of similarity (99.85%) with accession number MK491498.1, followed by 99.70%, 99.54%, 99.39%, and so on with other strain.

Sequences from all across the world that had previously been deposited in the NCBI GenBank matched the KSP03-identified strain from Bhawanipatna, Kalahandi (Figure 4). Our isolated strain, *Aedes vittatus*, shared a high degree of identification (99.85%) with accession number MK491498.1. 99.70%, 99.54%, 99.39%, and so forth came next. Additionally, the COI sequences of *Aedes vittatus* from India that were already in the NCBI GenBank agree with our isolated strain KSP03 from Bhawanipatna. The KSP03 isolate was most similar to the Kerala03 strain (accession number MK491498.1) (99.85%), followed by the Kerala02 strain (accession number MT858330.1) (99.81%), the Kolkata strain (accession number PQ483326.1) (99.77%), and so on. However, the isolated strain KSP03 was the least similar (99.03%) to Kolkata strain (accession number PQ483326.1). However, the Indian *Aedes vittatus* isolates from Coimbatore and Pondicherry, with accession number KR872404.1 and AY834246.1, respectively, shared the least amount of similarity (99.03%) with the KSP03 separated strain.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aedes vittatus cytochrome oxidase subunit I gene, partial cds; mitochondrial	Aedes vittatus	1247	1247	100%	0.0	99.85%	679	MK491498.1
Aedes vittatus voucher NP2_16S cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Aedes vittatus	1223	1223	99%	0.0	99.11%	676	OL348176.1
Aedes vittatus voucher NP2_15S cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Aedes vittatus	1221	1221	99%	0.0	99.26%	679	OL348175.1
Aedes vittatus voucher NP2_14S cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Aedes vittatus	1210	1210	97%	0.0	99.70%	664	OL348174.1
Aedes vittatus voucher SL/M21 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Aedes vittatus	1208	1208	96%	0.0	99.85%	657	MH330197.1
Aedes cogilli voucher NIBGE MOS-00828 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1205	1205	97%	0.0	99.70%	658	KF406606.1
Aedes vittatus voucher WRBU-1943-99 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Aedes vittatus	1205	1205	97%	0.0	99.70%	658	MT519729.1
Aedes cogilli voucher NIBGE MOS-01591 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1205	1205	97%	0.0	99.70%	658	KF406613.1
Aedes vittatus voucher WRBU-1943-75 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Aedes vittatus	1205	1205	97%	0.0	99.70%	658	MT519730.1
Aedes cogilli voucher NIBGE MOS-00829 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1199	1199	97%	0.0	99.54%	658	KF406580.1
Aedes cogilli voucher NIBGE MOS-01583 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1199	1199	97%	0.0	99.54%	658	KF406619.1
Aedes vittatus voucher NP2_14M cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Aedes vittatus	1197	1197	96%	0.0	99.54%	657	OL331077.1
Aedes cogilli voucher NIBGE MOS-01586 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1195	1195	97%	0.0	99.39%	658	KF406584.1
Aedes cogilli voucher NIBGE DIP-00375 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1195	1195	97%	0.0	99.39%	658	KF406618.1
Aedes cogilli voucher NIBGE MOS-01755 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1194	1194	97%	0.0	99.39%	658	KF406595.1
Aedes cogilli voucher NIBGE DIP-00374 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1194	1194	97%	0.0	99.39%	658	KF406620.1
Aedes cogilli voucher NIBGE MOS-01778 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1194	1194	97%	0.0	99.39%	658	KF406581.1
Aedes cogilli voucher NIBGE MOS-01588 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1194	1194	97%	0.0	99.39%	658	KF406621.1
Aedes cogilli voucher NIBGE MOS-01779 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Phagomyia cogilli	1194	1194	97%	0.0	99.39%	658	KF406585.1
Aedes vittatus voucher NP2_16M cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Aedes vittatus	1192	1192	96%	0.0	99.39%	657	OL331079.1

Figure 4: Sequence from the whole world producing significant alignments

Descriptions | Graphic Summary | Alignments

Sequences producing significant alignments Download Select columns Show 100

select all *14 sequences selected* Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
MK491498.1 /Kerla03		1245	1245	99%	0.0	99.85%	678	Query_5737388
OL851671.1 /Tamilnadu02		1175	1175	95%	0.0	99.38%	650	Query_5737384
OP317577.1 /Tamilnadu		1175	1175	95%	0.0	99.38%	650	Query_5737383
MZ828135.1 /Tamilnadu03		1090	1090	88%	0.0	99.50%	599	Query_5737385
MT858330.1 /Kerala02		968	968	77%	0.0	99.81%	527	Query_5737387
OR879749.1 /Odisha		929	929	74%	0.0	99.80%	506	Query_5737378
PQ483327.1 /Kolkata02		880	880	71%	0.0	99.38%	732	Query_5737379
MW931755.1 /Kerala01		863	863	70%	0.0	99.37%	724	Query_5737386
PQ483324.1 /Kolkata04		802	802	65%	0.0	99.32%	694	Query_5737382
PQ483326.1 /Kolkata		785	785	63%	0.0	99.77%	678	Query_5737380
PQ483325.1 /Kolkata03		675	675	55%	0.0	99.20%	626	Query_5737381
MK243685.1 /Tamilnadu04		649	649	52%	0.0	99.44%	357	Query_5737389
KR872404.1 /Coimbatore		597	597	71%	7e-174	89.03%	502	Query_5737391
AY834246.1 /Pondicherry		597	597	71%	7e-174	89.03%	512	Query_5737390

Figure 5: Sequence from India producing significant alignments

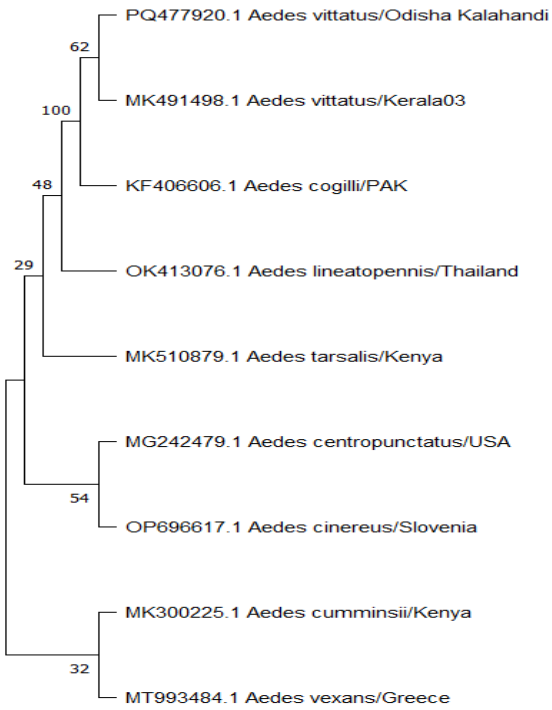

Figure 6: Molecular Phylogenetic analysis by Maximum Likelihood method of World.

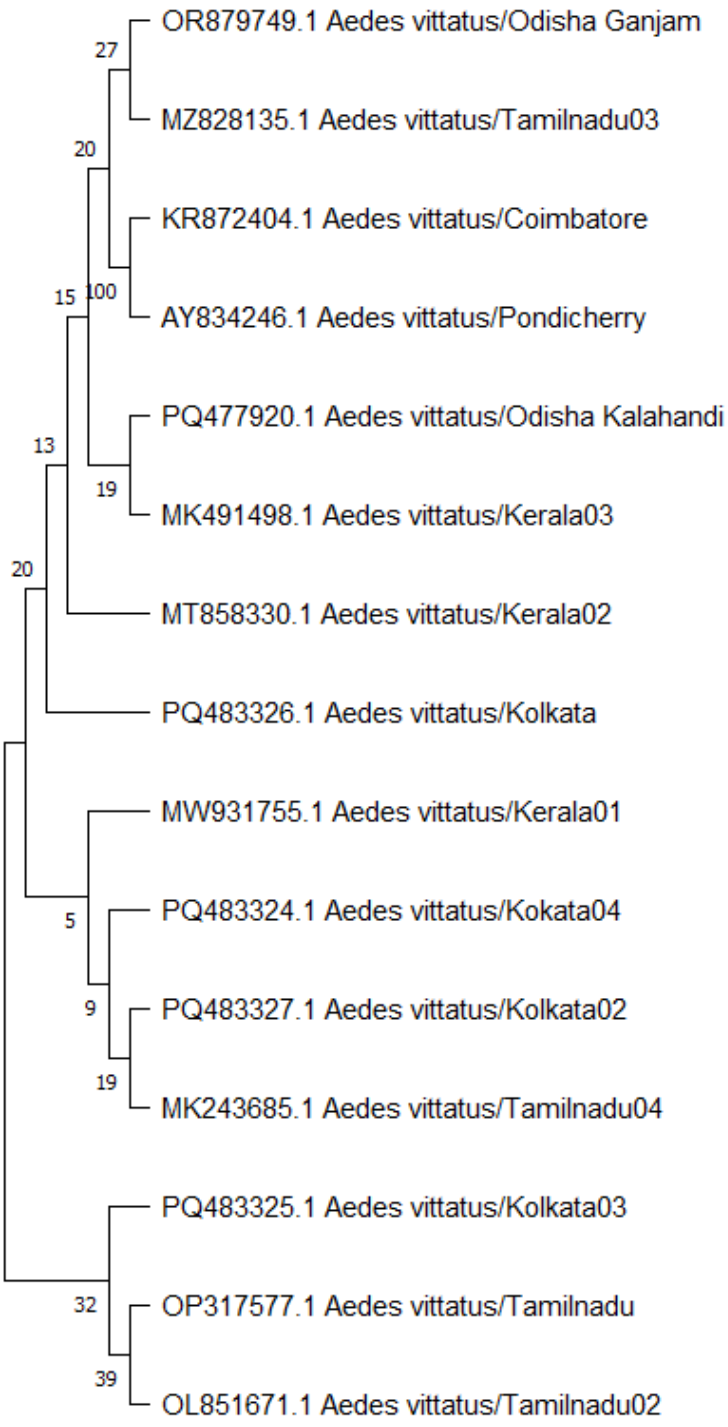

Figure 7: Molecular Phylogenetic analysis by Maximum Likelihood method of India.

Mosquito Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PQ477920.1_Aedes_*vittatus* /Odisha_Kalahandi															
PQ483327.1_Aedes_*vittatus* /Kolkata02	0.00821 36														
PQ483326.1_Aedes_*vittatus* /Kolkata	0.00233 64	0.00592 59													
PQ483325.1_Aedes_*vittatus* /Kolkata03	0.00800 00	0.00160 77	0.00480 00												
PQ483324.1_Aedes_*vittatus* /Kokata04	0.00674 16	0.00289 02	0.00589 97	0.00319 49											
OR879749.1_Aedes_*vittatus* /Odisha_Ganjam	0.00197 63	0.00970 87	0.00283 29	0.01000 00	0.00810 81										
OP317577.1_Aedes_*vittatus* /Tamilnadu	0.00618 24	0.00438 60	0.00755 67	0.00000 00	0.00241 55	0.00790 51									
OL851671.1_Aedes_*vittatus* /Tamilnadu02	0.00617 28	0.00437 64	0.00753 77	0.00000 00	0.00240 96	0.00790 51	0.00000 00								
MZ828135.1_Aedes_*vittatus* /Tamilnadu03	0.00500 83	0.01108 65	0.00510 20	0.01179 94	0.00978 00	0.00395 26	0.01001 67	0.01001 67							
MW931755.1_Aedes_*vittatus* /Kerala01	0.00630 25	0.00138 31	0.00443 79	0.00000 00	0.00144 30	0.00748 13	0.00224 72	0.00224 22	0.00909 09						
MT858330.1_Aedes_*vittatus* /Kerala02	0.00189 75	0.00452 49	0.00261 10	0.00303 03	0.00250 00	0.00402 41	0.00379 51	0.00379 51	0.00569 26	0.00232 02					
MK491498.1_Aedes_*vittatus* /Kerala03	0.00147 49	0.01024 59	0.00466 20	0.01063 83	0.00896 86	0.00197 63	0.00463 68	0.00462 96	0.00500 83	0.00838 57	0.00189 75				
MK243685.1_Aedes_*vittatus* /Tamilnadu04	0.00560 22	0.00560 22	0.00560 22	0.00615 38	0.00560 22	0.00903 61	0.00840 34	0.00840 34	0.01120 45	0.00560 22	0.00840 34	0.00560 22			
KR872404.1_Aedes_*vittatus* /Coimbatore	0.11570 25	0.11706 35	0.12723 21	0.12658 23	0.12258 06	0.11980 44	0.11258 28	0.11233 48	0.11607 14	0.11491 94	0.11389 52	0.11752 58	0.13165 27		
AY834246.1_Aedes_*vittatus* /Pondicherry	0.11570 25	0.11673 15	0.12663 76	0.12592 59	0.12210 53	0.11980 44	0.11258 28	0.11233 48	0.11607 14	0.11462 45	0.11389 52	0.11752 58	0.13165 27	0.00000 00	

Table 1: Showing the distance matrix of Indian COI gene sequences of Aedes vittatus

4. DISCUSSION

As of 01-11-2024, the NCBI Gene Bank contained 102 COI gene sequences of *Aedes vittatus*, including the KSP03 isolated strain, out of these seven from Odisha and twenty-two from India. MK491498 and our isolated strain PQ477920 belonged to the same clade.However, when compared to Aedes *lineatopennis* (Thailand), Aedes *tarsalis* (Kenya), Aedes *centropunctatus* (USA), Aedes cinereus (Slovenia), Aedes cumminsii (Kenya), and Aedes *vexans* (Greece), both the Kerala03 and Kalahandi Odisha strains were similar to the Pakistan strain, Aedes cogilli locations are marked in figure 8.

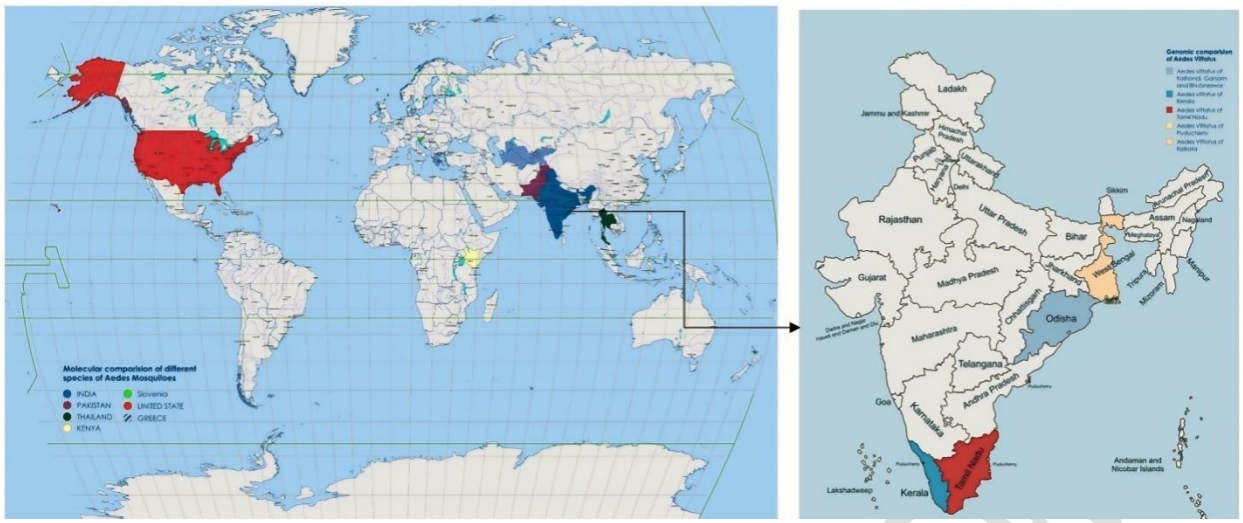

Figure 8: *Aedes* species of different countries having maxium similarity with kalahandi, Odisha isolated *Aedes vittatus* strain (This world map has no scale was made online, and the website link was https://www.mapchart.net/india.html)

There has been no continuous evidence of transmission by *Aedes vittatus*, largely due to the lack of molecular characterization of this species. However, based on the available data, it is likely that *Aedes vittatus* has evolved in distinct ecotypes, leading to different evolutionary pathways. This variation could increase the species' vectorial capacity in various regions worldwide. While there have been outbreaks of arboviruses in different parts of the world, no cases in India have been linked to *Aedes vittatus* to date. However, in the future, this species could emerge as an important vector in India due to the presence of diverse strains across different geographical areas. This makes it critical to monitor the genetic variation and transmission potential of *Aedes vittatus*. Morphological identification of *Aedes* species can often be confusing, so genetic identification using the COI gene should be adopted across India for effective surveillance of this vector.

Other *Aedes vittatus* strains identified from Ganjam, Odisha were far from our isolated strain KSP03, which was highly similar to the *Aedes vittatus* of Kerala03 strain found in a single clade in India. Our strain was also more similar to the Kerala03 strain than Kerala02 and Kerala01strainsbecause they belonged to separate clades. Furthermore, because it belongs to various clades, our isolated strain of *Aedes vittatus KSP*03 was close to the Kolkata strain (PQ483326.1) of *Aedes vittatus* but also far from the Kolkata4 and Kolkata2 strains. It is surprising that the *Aedes vittatus strain* from Kolkata 03 is farther distant than the other strains from Kolkata 01, 02, and 04 strains in terms of distance.

5.CONCLUSION

Aedes vittatus vector might be introduced to Kalahandi, Odisha from neighboring states. The presence of this competent vector is most probably a risk of transmission of arboviruses such as dengue fever, yellow fever, West Nile virus, Zika virus, and chikungunya virus in this area. Because of its high vector potential, *Aedes vittatus is* probably of special medical importance in addition to Aedes aegypti and Aedes albopictus. More entomological research is required to create efficient vector management strategies that can stop the spread of Ae. *vittatus* and associated arboviral diseases in Odisha.

Thorough study would improve our knowledge and supply crucial information to back up focused intervention tactics.

8. REFERENCES

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