

## Original Research Article

# GENETIC CHARACTERISATION AND MOLECULAR PHYLOGENY OF MOSQUITO *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 Characterization, 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-A. vexans*; and *Aedes-A. 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-A. (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-A. 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 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

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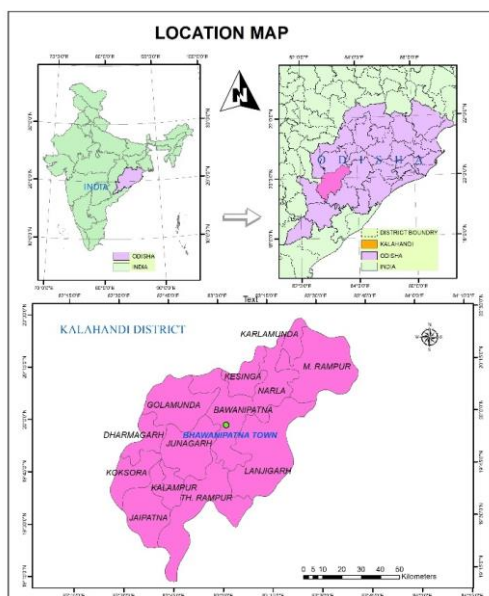
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qualified taxonomists [9], [10], [11]. However, this approach relies on prior genetic data for each species [12]. This presents a notable limitation for *Aedes* mosquitoes, as genetic data is lacking for most Indian species within this genus, despite 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-A. 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 is 1300mm. 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.

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## 2.2 SAMPLE COLLECTION

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[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

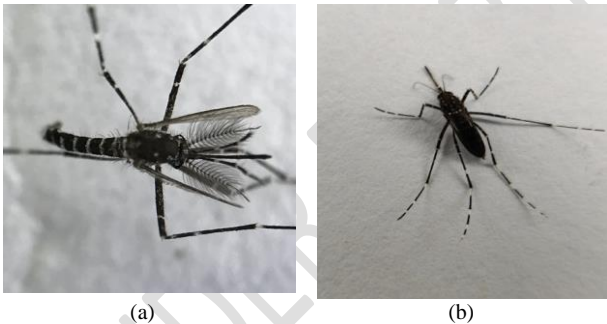


Figure 2: (a) *Aedes vittatus* (Male) and (b) *Aedes vittatus* (Female)

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 dorso-central area of the scutum (Figure 2). The identified *Aedes* specimens with dark tibiae, each with a sub-basal white spot and a white band at about basal 0.33 on fore- and mid-tibia at about 0.50 on hind-tibia (Figure 2). In addition, the mosquito specimens have a distinct white band on the proboscis [17].

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## 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.

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### 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 until DNA fragments are migrated well. The gel was photographed on gel documentation system.

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### 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.

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**Figure 3:** ~~1.8%~~ Agarose gel (1.8%) 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 *A. edes 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 *A. edes 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+~~n~~Non\_coding. 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.

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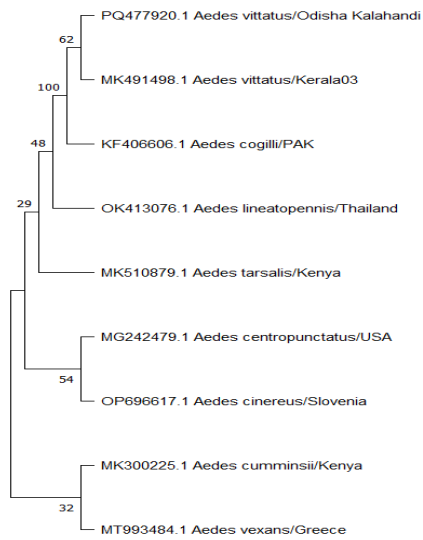
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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus cytochrome oxidase subunit 1 gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1247	1247	100%	0.0	99.85%	679	<a href="#">MK491498.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher NP2_16S cytochrome c oxidase subunit 1 (COX1) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1223	1223	99%	0.0	99.11%	676	<a href="#">OL348176.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher NP2_15S cytochrome c oxidase subunit 1 (COX1) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1221	1221	99%	0.0	99.26%	679	<a href="#">OL348175.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher NP2_14S cytochrome c oxidase subunit 1 (COX1) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1210	1210	97%	0.0	99.70%	664	<a href="#">OL348174.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher SLIM21 cytochrome c oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1208	1208	96%	0.0	99.85%	657	<a href="#">MH330197.1</a>
<input checked="" type="checkbox"/> <a href="#">Phagomyia cogilli voucher NIBGE.MOS-00829 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1205	1205	97%	0.0	99.70%	658	<a href="#">KF406606.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher WRBU-1943-99 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1205	1205	97%	0.0	99.70%	658	<a href="#">MT519729.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01591 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1205	1205	97%	0.0	99.70%	658	<a href="#">KF406613.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher WRBU-1943-75 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1205	1205	97%	0.0	99.70%	658	<a href="#">MT519730.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-00829 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1199	1199	97%	0.0	99.54%	658	<a href="#">KF406608.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01583 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1199	1199	97%	0.0	99.54%	658	<a href="#">KF406619.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher NP2_14M cytochrome c oxidase subunit 1 (COX1) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1197	1197	96%	0.0	99.54%	657	<a href="#">OL331077.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01586 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1195	1195	97%	0.0	99.39%	658	<a href="#">KF406584.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.DIP-00375 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1195	1195	97%	0.0	99.39%	658	<a href="#">KF406618.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01755 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1194	1194	97%	0.0	99.39%	658	<a href="#">KF406595.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.DIP-00374 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1194	1194	97%	0.0	99.39%	658	<a href="#">KF406620.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01778 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1194	1194	97%	0.0	99.39%	658	<a href="#">KF406581.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01588 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1194	1194	97%	0.0	99.39%	658	<a href="#">KF406621.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes cogilli voucher NIBGE.MOS-01779 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochondrial</a>	<a href="#">Phagomyia cogilli</a>	1194	1194	97%	0.0	99.39%	658	<a href="#">KF406585.1</a>
<input checked="" type="checkbox"/> <a href="#">Aedes vittatus voucher NP2_16M cytochrome c oxidase subunit 1 (COX1) gene, partial cds: mitochondrial</a>	<a href="#">Aedes vittatus</a>	1192	1192	96%	0.0	99.39%	657	<a href="#">OL331079.1</a>

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								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> <a href="#">MK491498.1 /Kerala03</a>		1245	1245	99%	0.0	99.85%	678	Query_5737388
<input checked="" type="checkbox"/> <a href="#">OL851671.1 /Tamilnadu02</a>		1175	1175	95%	0.0	99.38%	650	Query_5737384
<input checked="" type="checkbox"/> <a href="#">QP317577.1 /Tamilnadu</a>		1175	1175	95%	0.0	99.38%	650	Query_5737383
<input checked="" type="checkbox"/> <a href="#">MZ828135.1 /Tamilnadu03</a>		1090	1090	88%	0.0	99.60%	599	Query_5737385
<input checked="" type="checkbox"/> <a href="#">MT858330.1 /Kerala02</a>		968	968	77%	0.0	99.81%	527	Query_5737387
<input checked="" type="checkbox"/> <a href="#">QR879749.1 /Odisha</a>		929	929	74%	0.0	99.80%	506	Query_5737378
<input checked="" type="checkbox"/> <a href="#">PQ483327.1 /Kolkata02</a>		880	880	71%	0.0	99.38%	732	Query_5737379
<input checked="" type="checkbox"/> <a href="#">MW931755.1 /Kerala01</a>		863	863	70%	0.0	99.37%	724	Query_5737386
<input checked="" type="checkbox"/> <a href="#">PQ483324.1 /Kolkata04</a>		802	802	65%	0.0	99.32%	694	Query_5737382
<input checked="" type="checkbox"/> <a href="#">PQ483326.1 /Kolkata</a>		785	785	63%	0.0	99.77%	678	Query_5737380
<input checked="" type="checkbox"/> <a href="#">PQ483325.1 /Kolkata03</a>		675	675	55%	0.0	99.20%	626	Query_5737381
<input checked="" type="checkbox"/> <a href="#">MK243685.1 /Tamilnadu04</a>		649	649	52%	0.0	99.44%	357	Query_5737389
<input checked="" type="checkbox"/> <a href="#">KR872404.1 /Coimbatore</a>		597	597	71%	7e-174	89.03%	502	Query_5737391
<input checked="" type="checkbox"/> <a href="#">AY834246.1 /Pondicherry</a>		597	597	71%	7e-174	89.03%	512	Query_5737390

Figure 5: Sequence from India producing significant alignments



**Figure 6: Molecular phylogenetic analysis of *A. vittatus* by Maximum Likelihood method of World.**

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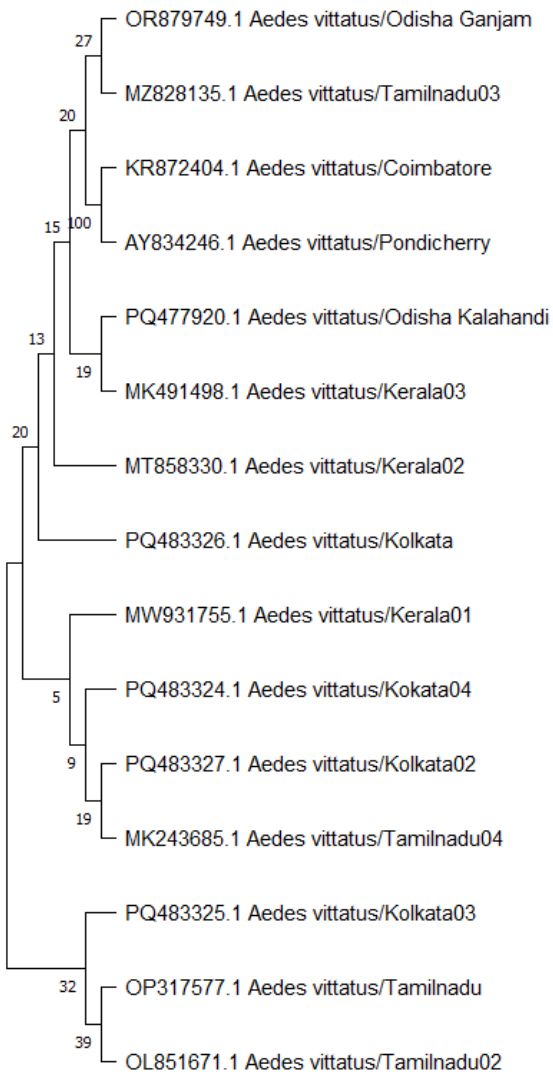


Figure 7: Molecular Phylogenetic analysis of *A. vittatus* by Maximum Likelihood method of India.

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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	0.00 821 36														
PQ483326.1_Aedes_vittatus /Kolkata	0.00 233 64	0.00 592 59													
PQ483325.1_Aedes_vittatus /Kolkata03	0.00 800 00	0.00 160 77	0.00 480 00												
PQ483324.1_Aedes_vittatus /Kolkata04	0.00 674 16	0.00 289 02	0.00 589 97	0.00 319 49											
OR879749.1_Aedes_vittatus /Odisha_Ganjam	0.00 197 63	0.00 970 87	0.00 283 29	0.00 000 00	0.00 810 81										
OP317577.1_Aedes_vittatus /Tamilnadu	0.00 618 24	0.00 438 60	0.00 755 67	0.00 000 00	0.00 241 55	0.00 790 51									
OL851671.1_Aedes_vittatus /Tamilnadu02	0.00 617 28	0.00 437 64	0.00 753 77	0.00 000 00	0.00 240 96	0.00 790 51	0.00 000 00								
MZ828135.1_Aedes_vittatus /Tamilnadu03	0.00 500 83	0.01 108 65	0.00 510 20	0.01 179 94	0.00 978 00	0.00 395 26	0.01 001 67	0.01 001 67							
MW931755.1_Aedes_vittatus /Kerala01	0.00 630 25	0.00 138 31	0.00 443 79	0.00 000 00	0.00 144 30	0.00 748 13	0.00 224 72	0.00 224 22	0.00 909 09						
MT858330.1_Aedes_vittatus /Kerala02	0.00 189 75	0.00 452 49	0.00 261 10	0.00 303 03	0.00 250 00	0.00 402 41	0.00 379 51	0.00 379 51	0.00 569 26	0.00 232 02					
MK491498.1_Aedes_vittatus /Kerala03	0.00 147 49	0.01 024 59	0.00 466 20	0.01 063 83	0.00 896 86	0.00 197 63	0.00 463 68	0.00 462 96	0.00 500 83	0.00 838 57	0.00 189 75				
MK243685.1_Aedes_vittatus /Tamilnadu04	0.00 560 22	0.00 560 22	0.00 560 22	0.00 615 38	0.00 560 22	0.00 903 61	0.00 840 34	0.00 840 34	0.01 120 45	0.00 560 22	0.00 840 34	0.00 560 22			
KR872404.1_Aedes_vittatus /Coimbatore	0.11 570 25	0.11 706 35	0.12 723 21	0.12 658 23	0.12 258 06	0.11 980 44	0.11 258 28	0.11 233 48	0.11 607 14	0.11 491 94	0.11 389 52	0.11 752 58	0.13 165 27		
AY834246.1_Aedes_vittatus /Pondicherry	0.11 570 25	0.11 673 15	0.12 663 76	0.12 592 59	0.12 210 53	0.11 980 44	0.11 258 28	0.11 233 48	0.11 607 14	0.11 462 45	0.11 389 52	0.11 752 58	0.13 165 27	0.00 000 00	

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

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#### 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.

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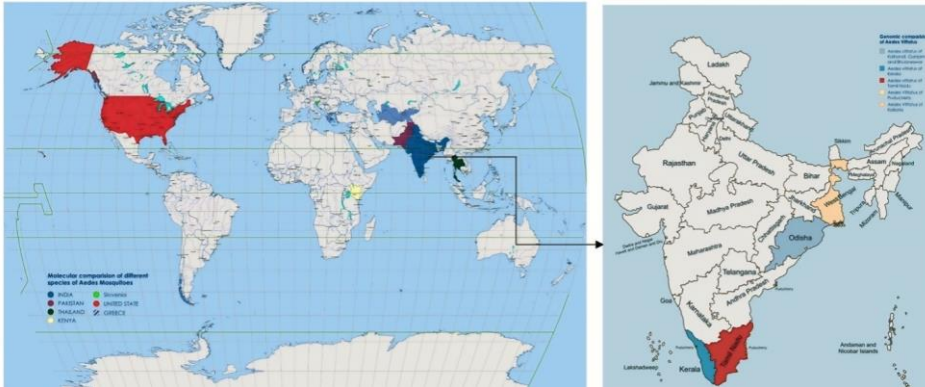
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**Figure 8:** *Aedes* species of different countries having maximum similarity with Kalahandi, Odisha isolated *Aedes vittatus* strain (This world map has no scale was made online, and the website link was <https://www.mapchart.net/india.html>)

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.

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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 Kerala01strains because they belonged to separate clades. Furthermore, because it belongs to various clades, our isolated strain of *Aedes vittatus* KSP03 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.

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## 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.

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

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