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# Genetic Characterisation and Molecular Phylogeny of Mosquito *Aedes vittatus* Based on COI Gene from Bhawanipatna, Kalahandi, Odisha, India

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## 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

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Africa, Asia, Latin America, and Europe. 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.

**Objective:** The current study was designed for molecular characterisation 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 Indian 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 *Aedes* mosquitoes were surveyed weekly from the selected human dwelling, both inside and outside the house or premises of Bhawanipatna Municipal area from month June 2024 to 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 GenBank database using Basic Local Alignment Search Tool (BLAST) against the *Aedes vittatus* genomes in NCBI, GenBank. The complete sequences were deposited in GenBank with accession no. PQ477920.1.

**Conclusion:** *Aedes vittatus* vector might be introduced to Kalahandi, Odisha from neighbouring 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.

*Keywords: Aedes vittatus; molecular characterisation; Bhawanipatna; Odisha; COI.*

## 1. INTRODUCTION

According to Weaver et al. (2018) and Sudeep et al. (2017), *Aedes* (Fredwardsius) 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 *Aedes 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]. *Ae. vittatus* (Bigot, 1861) 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 *Ae. vittatus* has expanded its range across Africa, Asia, Latin America, and Europe [2,6,7], known for its preference for feeding on humans, *Ae. 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 characterisation of the *Ae. vittatus* mosquitoes collected from all parts of Bhawanipatna based on mitochondrial COI to provide a wider understanding of the phylogenetic relationships of *Ae. 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

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 and 83.17°E (Fig. 1), has a tropical wet and dry climate with annual average rainfall about 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.

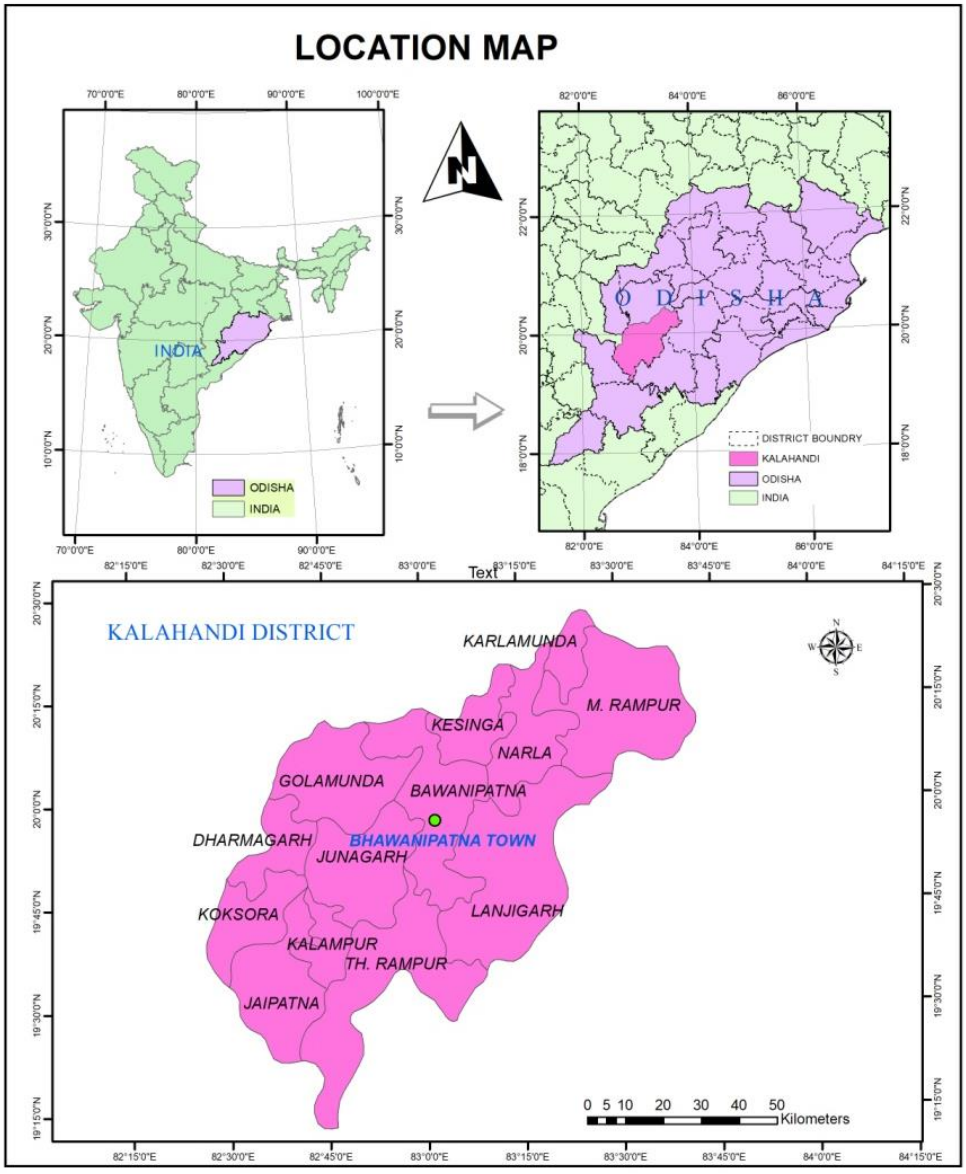



**Fig. 1. Map showing study area at Bhawanipatna in Kalahandi district of Odisha**

## 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 month June 2024 to 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].During the study hour, 286 *Aedes* mosquitoes in total were collected, of which 198 were female and 88 were male. However, 126 *Ae. albopictus*, 45 *Ae. aegypti*, and 27 *Ae. vittatus* were found among the 198 female *Aedes*. Similarly, of the 88 male *Aedes*, 19 were *Ae. vittatus*, 50 were *Ae. albopictus*, and 29 were *Ae. aegypti*.

**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, 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.

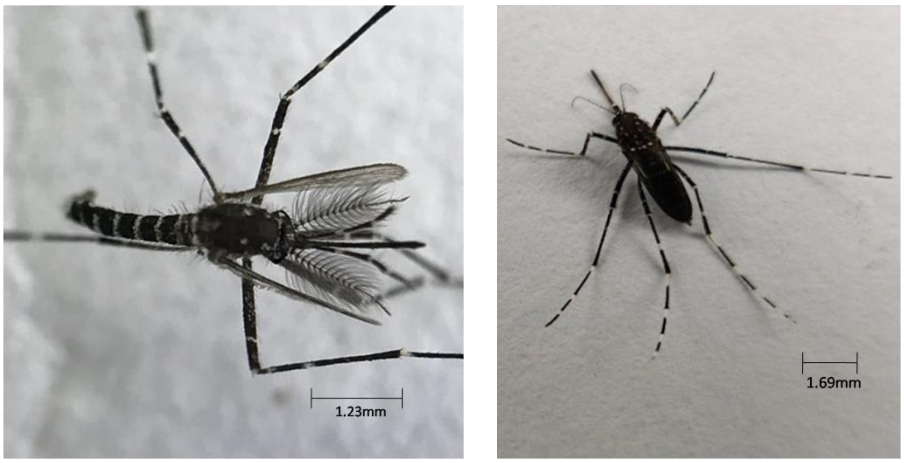
## 2.3 Morphological Identification

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 (Fig. 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- and at about 0.50 on hind-tibia (Fig. 2). In addition, the mosquito specimens have a distinct white band on the proboscis [17].

## 2.4 PCR Amplification of COI Partial DNA Sequence and Sequencing

### 2.4.1 Isolation

We used Hi-PurA Insect DNA Purification Kit Catalog No. MB569-20PR from Hi-Media for the DNA Isolation from *Aedes vittatus*.




**(a)** **(b)**

**Fig. 2. (a) *Aedes vittatus* (Male) and (b) *Aedes vittatus* (Female)**

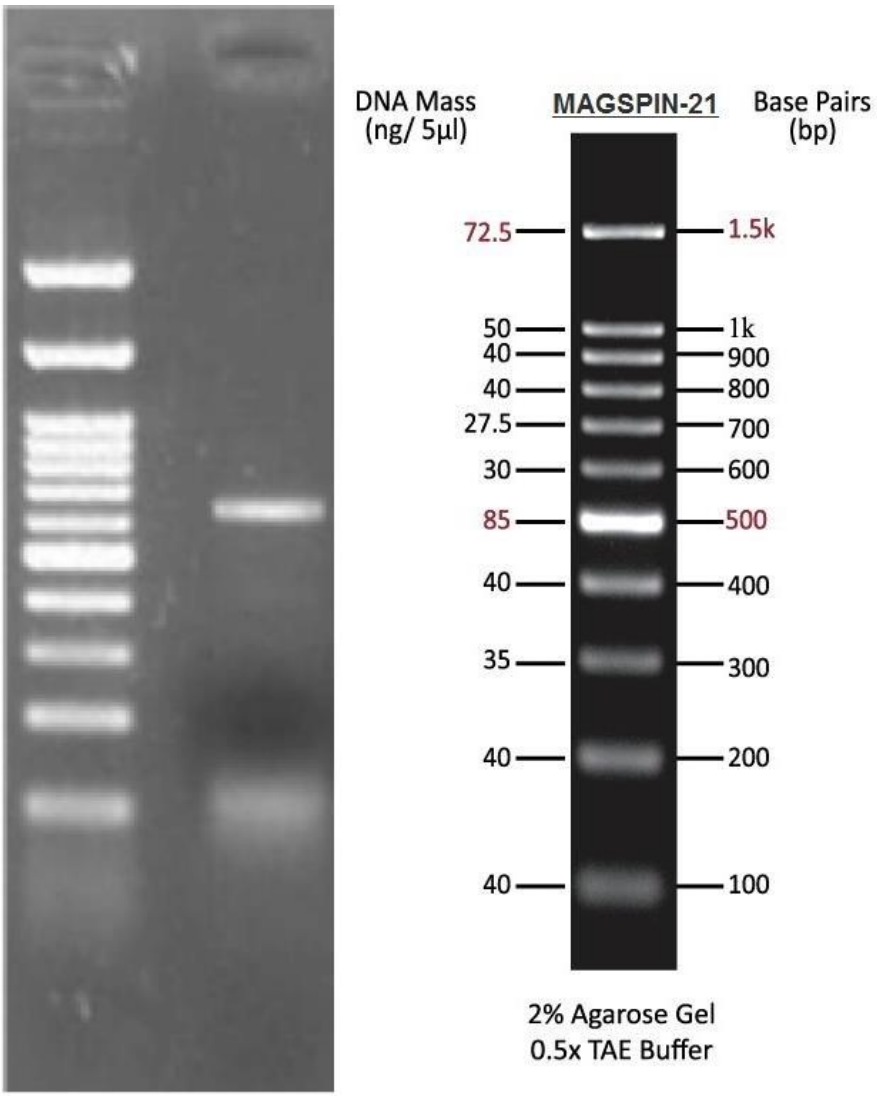



**Fig. 3. Agarose gel (1.8%) showing single 700 bp of mitochondrial cytochrome c oxidase subunit I amplicon**

### 2.4.2 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 were migrated well. The gel was photographed on gel documentation system by usingiBright™ CL750 Imaging System model.

### 2.4.3 PCR amplification

The 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 (Fig. 3). The PCR amplicon was bead purified and further subjected to Sanger Sequencing.

### 2.4.4 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.

## 2.5 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 mtCOI sequence of *Ae. vittatus (*our isolated strain KSP02) were compared with the whole world samples of gene COI of *Aedes* species found from other countries (Fig. 4); further it also compared with the COI gene sequence of Indian *Ae. Vittatus* (Fig. 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 were 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. RESULTS

The KSP02 isolate from Bhawanipatna, Kalahandi, matched the sequences that had previously been added to the NCBI GenBank (Fig. 4) from all around the world. *Ae. 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 KSP02-identified strain from Bhawanipatna, Kalahandi (Fig. 4). Additionally, the COI sequences of *Ae. vittatus* from India that were already in the NCBI GenBank agree with our isolated strain KSP02 from Bhawanipatna. The KSP02 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 KSP02 was the least similar (99.03%) to Kolkata strain (accession number PQ483326.1). However, the Indian *Ae. 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 KSP02 separated strain.

## 4. DISCUSSION

As of 1st November 2024, the NCBI GenBank contained 102 COI gene sequences of *Ae. vittatus,* including the KSP02 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 *Ae*. *lineatopennis* (Thailand), *Ae. tarsalis* (Kenya), *Ae. centropunctatus* (USA), *Ae. cinereus* (Slovenia), *Ae.* cumminsii (Kenya), and *Ae. vexans* (Greece), both the Kerala03 and Kalahandi Odisha strains were similar to the Pakistan strain, *Ae. cogilli* locations are marked in Figs. 4, 6 & 8.

There has no continuous evidence of transmission by *Ae. vittatus*, largely due to the lack of molecular characterisation of this species. However, based on the available data, it is likely that *Ae. vittatus* has evolved in distinct ecotypes, leading to different evolutionary pathways. This variation could increase the species' vectorial capacity in various regions worldwide. According to certain reports, *Ae. vittatus* transmitted the Zika virus in Africa, America, and Asia [21–23] and the Dengue virus in Africa, Asia, and Europe [24,25]. Additionally, there was evidence that *Ae. vittatus* was responsible for the Yellow fever virus in Africa and South America [26,27] and Chikungunya in Africa, America, Asia, and Europe [26]. In Africa, Asia, and Europe, these mosquitoes were also the cause of Encephalities [28]. In the Indian subcontinent, these mosquitoes can carry Dengue, Chikungunya, Yellow fever, or Encephalities at any time. Globalisation, urbanisation, and climate change significantly modify the dynamics of disease transmission and enhance the involvement of neglected species, such as *Ae. vittatus*, in disease propagation [29]. It is predicted that, regardless of its native range, climate change will encourage incursions into colder subtropical regions in countries throughout Asia, Europe, and North America. *Ae. vittatus* is found in rural, urban, and peri-urban locations throughout tropical and subtropical regions. It is found at heights from sea level up to 2,500 meters above sea level, thriving in temperatures ranging from 15 to 30°C [30]. The species is able to adapt to a wide range of habitats, including forests,

grasslands, lakes, and deserts. Furthermore, this mosquito species may expand to new regions of the Americas, Europe, and Oceania [30]. Despite arbovirus outbreaks in other parts of the world, *Ae. vittatus* has not yet been connected to any cases in India.

| 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 |

**Fig. 4. Sequences 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 |

**Fig. 5. Sequences from India producing significant alignments**

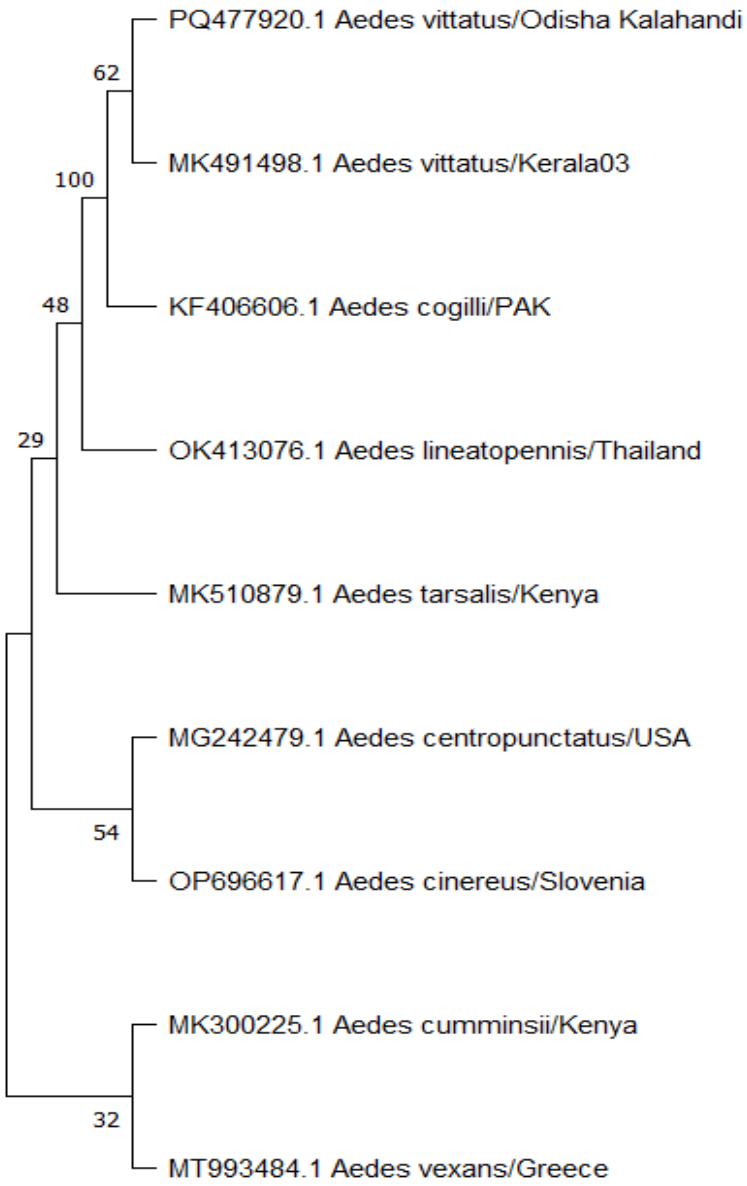



**Fig. 6. Molecular phylogenetic analysis of *Ae. vittatus* by Maximum Likelihood method of the World**

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 *Ae. 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 *Ae. vittatus* strains identified from Ganjam, Odisha were far from our isolated strain KSP02, which was highly similar to the *Ae. vittatus* of Kerala03 strain found in a single clade in India (Fig. 7). Our strain was also more similar to the Kerala03 strain than Kerala02 and Kerala01 strains because they belonged to separate clades. Furthermore, because it belonged to various clades, our isolated strain of *Ae. vittatus* KSP02 was close to the Kolkata strain (PQ483326.1) of *Ae. vittatus* but also far from the Kolkata4 and Kolkata2 strains. It is surprising that the *Ae. vittatus* strain from Kolkata03 was farther distant than the other strains from Kolkata 01, 02, and 04 strains in terms of distance (Table 1).

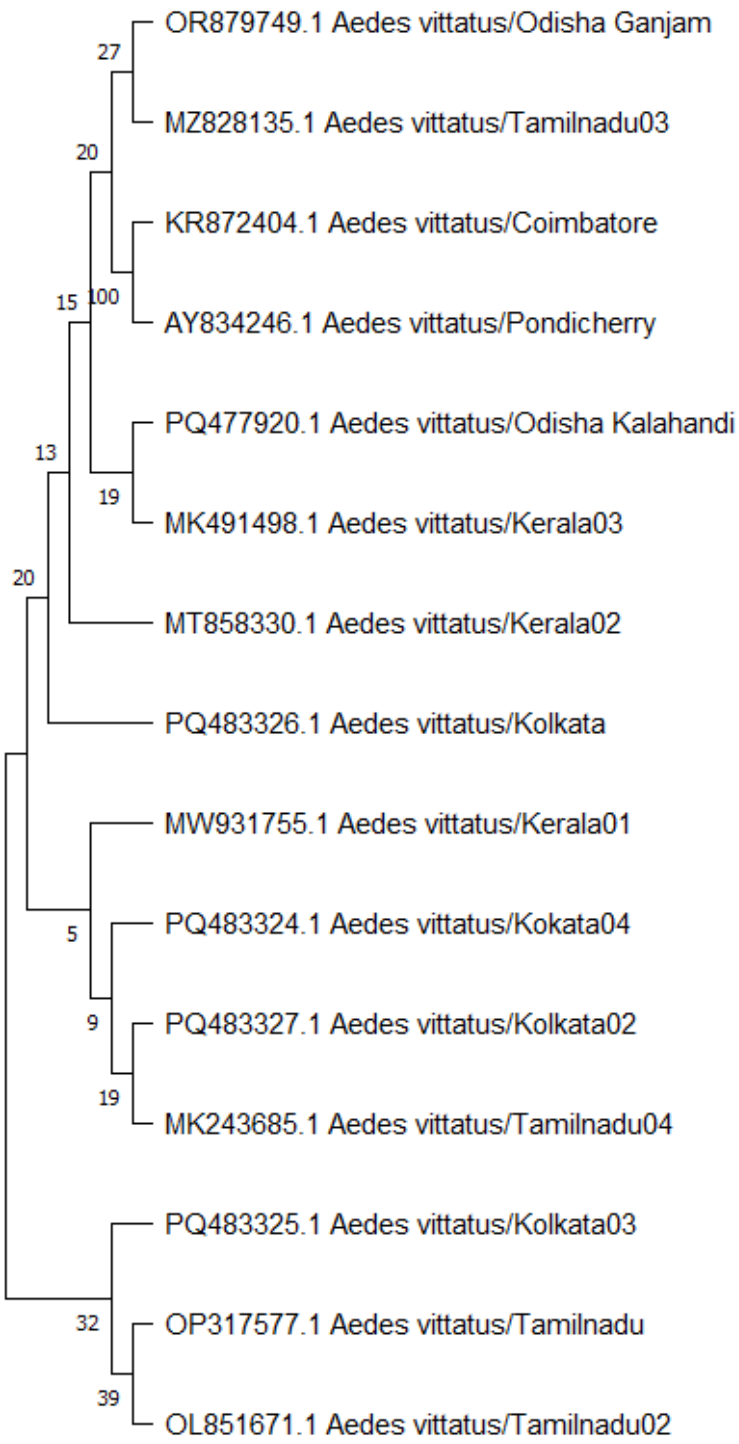



**Fig. 7. Molecular phylogenetic analysis of *Ae. vittatus* by Maximum Likelihood method of India**

**Table 1. Distance matrix of Indian COI gene sequences of *Aedes vittatus***

| 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 | 0.0082136 | | | | | | | | | | | | | | |
| PQ483326.1_*Aedes_vittatus*/Kolkata | 0.0023364 | 0.0059259 | | | | | | | | | | | | | |
| PQ483325.1_*Aedes_vittatus*/Kolkata03 | 0.0080000 | 0.0016077 | 0.0048000 | | | | | | | | | | | | |
| PQ483324.1_*Aedes_vittatus*/Kokata04 | 0.0067416 | 0.0028902 | 0.0058997 | 0.0031949 | | | | | | | | | | | |
| OR879749.1_Aedes_*vittatus*/Odisha_Ganjam | 0.0019763 | 0.0097087 | 0.0028329 | 0.0100000 | 0.0081081 | | | | | | | | | | |
| OP317577.1_*Aedes_vittatus*/Tamilnadu | 0.0061824 | 0.0043860 | 0.0075567 | 0.0000000 | 0.0024155 | 0.0079051 | | | | | | | | | |
| OL851671.1_*Aedes_vittatus*/Tamilnadu02 | 0.0061728 | 0.0043764 | 0.0075377 | 0.0000000 | 0.0024096 | 0.0079051 | 0.0000000 | | | | | | | | |
| MZ828135.1_*Aedes_vittatus*/Tamilnadu03 | 0.0050083 | 0.0110865 | 0.0051020 | 0.0117994 | 0.0097800 | 0.0039526 | 0.0100167 | 0.0100167 | | | | | | | |
| MW931755.1_*Aedes_vittatus*/Kerala01 | 0.0063025 | 0.0013831 | 0.0044379 | 0.0000000 | 0.0014430 | 0.0074813 | 0.0022472 | 0.0022422 | 0.0090909 | | | | | | |
| MT858330.1_*Aedes_vittatus*/Kerala02 | 0.0018975 | 0.0045249 | 0.0026110 | 0.0030303 | 0.0025000 | 0.0040241 | 0.0037951 | 0.0037951 | 0.0056926 | 0.0023202 | | | | | |
| MK491498.1_*Aedes_vittatus*/Kerala03 | 0.0014749 | 0.0102459 | 0.0046620 | 0.0106383 | 0.0089686 | 0.0019763 | 0.0046368 | 0.0046296 | 0.0050083 | 0.0083857 | 0.0018975 | | | | |
| MK243685.1_*Aedes_vittatus*/Tamilnadu04 | 0.0056022 | 0.0056022 | 0.0056022 | 0.0061538 | 0.0056022 | 0.0090361 | 0.0084034 | 0.0084034 | 0.0112045 | 0.0056022 | 0.0084034 | 0.0056022 | | | |
| KR872404.1_*Aedes_vittatus*/Coimbatore | 0.1157025 | 0.1170635 | 0.1272321 | 0.1265823 | 0.1225806 | 0.1198044 | 0.1125828 | 0.1123348 | 0.1160714 | 0.1149194 | 0.1138952 | 0.1175258 | 0.1316527 | | |
| AY834246.1_*Aedes_vittatus*/Pondicherry | 0.1157025 | 0.1167315 | 0.1266376 | 0.1259259 | 0.1221053 | 0.1198044 | 0.1125828 | 0.1123348 | 0.1160714 | 0.1146245 | 0.1138952 | 0.1175258 | 0.1316527 | 0.0000000 | |

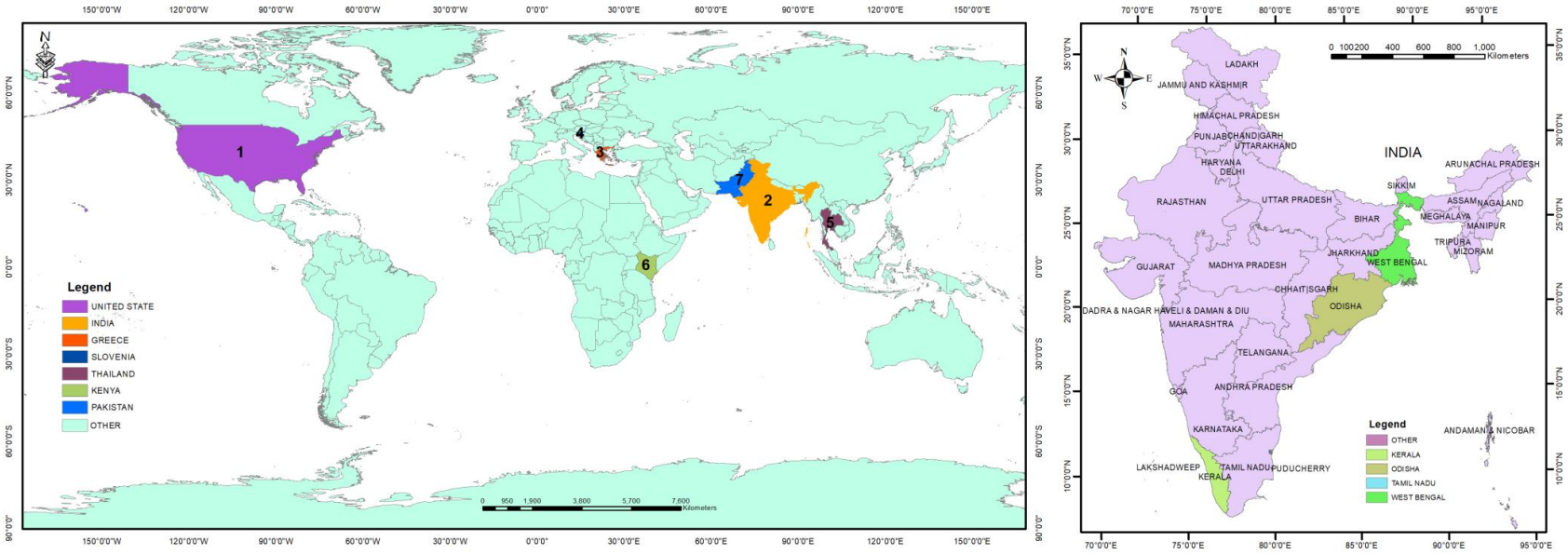



**Fig. 8. *Aedes* species of different countries having maximum similarity with Kalahandi, Odisha isolated *Aedes vittatus* strain (Map was created using ArcMap10.8.2 where the international boundary of the countries was not verified)**

## 5. CONCLUSION

*Ae. 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, *Ae. vittatus is* probably of special medical importance in addition to *Ae. aegypti* and *Ae. 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. The thorough study would improve our knowledge and supply crucial information to back up focused intervention tactics.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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