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Morphological and mitochondrial cytochrome oxidase-I gene-based characterisation of *Culex quinquefasciatus* from central Kerala#

S. Anupriya[1], Bindu Lakshmanan[1]*, R. Radhika[1], Asha Rajagopal[1], K. Syamala[1], M.K. Sahala Fathima[1], Gowri S. Nair[1] and Vrinda K. Menon[2]

[1]Department of Veterinary Parasitology, [2]Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala - 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India

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Abstract

Culex quinquefasciatus *is a principal vector of* filarial and viral infections *and a key contributor to lymphatic filariosis transmission in Kerala, India. Accurate identification of mosquito vectors is crucial for surveillance and control and, morphological differentiation can be challenging among closely related species. This study characterised Culex quinquefasciatus from Thrissur and Ernakulam districts of Kerala using morphological and molecular approaches. A total of 6,421 mosquitoes were studies, with 271 specimens identified* as *Cx. quinquefasciatus and 42 as members of the Cx. vishnui group. Morphological features of Cx. quinquefasciatus included brown to golden-brown bodies, short palpi, unbanded proboscis and tarsi, and narrow pale abdominal bands. Molecular characterisation involved amplification of a 665 bp fragment of the mitochondrial COI gene with PCR yielding specific amplicons and sequencing confirming 100 per cent identity with published Cx. quinquefasciatus sequences. Phylogenetic analysis clustered the Kerala isolate with Indian and UK sequences, distinct from other Culex species, validating species identification and supporting its use in vector surveillance and control programs*.

Key words: *Cx. quinquefasciatus, prevalence, Kerala, phylogeny*

Mosquitoes are among the most important arthropod vectors of human and animal diseases, transmitting a wide range of pathogens including protozoa, nematodes, and viruses (Madhav *et al*., 2024). Belonging to the family *Culicidae*, they comprise of more than 3,600 species worldwide, with only a fraction recognized as vectors of medical and veterinary significance (Soghigian *et al*., 2023). Their ability to acquire and transmit pathogens during blood feeding has made them a central concern in public health, particularly in tropical and subtropical regions where environmental conditions favour their proliferation (WHO, 2024). Among the globally important mosquito genera, *Culex* (*Cx.)* stands out in South and Southeast Asia for its prominent role in transmitting parasitic and viral diseases affecting both humans and animals, including lymphatic filariosis, Japanese encephalitis, and West Nile fever (WHO, 2024).

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**Corresponding author: bindul@kvasu.ac.in, Ph. 9447486722*

In Kerala, *Cx. quinquefasciatus* and members of the *Cx. vishnui* subgroup are widely distributed, thriving in close association with human settlements and agricultural landscapes. *Cx. quinquefasciatus* serves as the principal vector of *Wuchereria bancrofti*, thereby sustaining lymphatic filariasis transmission in endemic foci of the state. Meanwhile, *Cx. tritaeniorhynchus*, a predominant rice field breeder and a key member of the *Cx. vishnui* subgroup, functions as an efficient vector of Japanese encephalitis in southern India (Mathiarasan *et al*., 2025). The ecology of Kerala, characterised by heavy rainfall, extensive paddy fields, backwaters, and peri-urban habitats, provides ideal breeding opportunities for *Culex* mosquitoes. Factors such as rapid urbanisation, poor sanitation, and climate variability further contribute to mosquito proliferation and increase in human vector contact, thereby amplifying the risk of spillover infections. Although chemical, biological, and environmental control strategies have been implemented, their overall effectiveness remains limited due to insecticide resistance, operational challenges, and the remarkable ecological adaptability of *Culex* species (Rashmi *et al*., 2025).

These constraints highlight the necessity for sustained surveillance and integrated management approaches to reduce the growing burden of *Culex*-borne diseases in Kerala. A critical component of such surveillance is the accurate identification of mosquito species, which is essential for understanding their role in disease transmission and for designing targeted control measures. However, traditional morphological identification has inherent limitations, particularly when dealing with closely related or cryptic species, as overlapping characters, subtle morphological differences, or specimen damage during collection can hinder accurate classification (Thankachan *et al*., 2023). This issue is especially relevant in species complexes and subgroups such as the *Culex vishnui* subgroup (*Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, and *Cx. vishnui*), where minor structural differences mask significant variation in vectorial capacity (Saiwichai *et al*., 2023). Molecular characterisation offers a reliable solution to these challenges. DNA-based approaches, particularly PCR amplification of mitochondrial genes such as *cytochrome oxidase I* (*COI*), enable precise identification and differentiation of sibling and cryptic species (Thankachan *et al*., 2021). Beyond species recognition, molecular tools are valuable for confirming vector incrimination, monitoring genetic diversity and population dispersal, detecting insecticide resistance mutations, and elucidating evolutionary relationships (Madhav *et al*., 2024). In Kerala, where multiple *Culex* species coexist across diverse ecological settings, molecular characterisation provides a critical framework for strengthening vector surveillance and implementing evidence-based, sustainable control strategies (Thankachan *et al*., 2023). In this background a study was conducted to identify the prevalence and to characterise the *Culex* spp. in central Kerala.

Materials and methods

Mosquitoes were collected from multiple locations across Thrissur and Ernakulam districts between July 2024 and August 2025. Collection was performed using a mechanical aspirator, and specimens were morphologically identified to species level under a stereozoom microscope using standard taxonomic keys (Gyawali *et al*., 2025). For molecular analyses, mosquito genomic DNA was extracted using the salting-out method.

Molecular characterisation of *Cx. quinquefasciatus* was performed to confirm species identity and assess phylogenetic relationships among local mosquito populations. A 665 bp fragment of the mitochondrial *cytochrome oxidase I* (*COI*) gene was amplified using primers (Forward: 5'-ATT GGA TTA TTA GGA TTT ATT G-3'; Reverse: 5'-GCA GGA GGA AGA GTA TGA TAT C-3') previously described by Daravath *et al*. (2015). The PCR amplification was carried out in 20 µL reactions containing 10 µL of 2X EmeraldAmp® GT PCR Master Mix, 1 µL of each primer (25 pmol/µL), 3 µL of template DNA extracted from individual mosquitoes, and 5 µL of nuclease-free water. Thermal cycling was performed in a gradient-capable thermal cycler and included an initial denaturation at 95°C for 3 min, followed by 34 cycles consisting of denaturation at 95°C for 1 min, primer annealing at 52–58°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 5 min to ensure complete amplification of the target region. Amplification was verified by electrophoresis on a 1.5% agarose gel containing ethidium bromide and visualized under UV illumination. The purified amplicons were then subjected to bidirectional Sanger sequencing at Gene Spec Biosciences, Kochi, to ensure accurate sequence determination. Raw sequences were quality-checked, trimmed and aligned using EMBOSS software, and the resulting consensus sequences were compared against the National Centre for Biotechnology Information (NCBI) GenBank database using the BLASTn algorithm to verify species identity.

Phylogenetic analysis was subsequently conducted to explore the genetic relationships between *Cx. quinquefasciatus* and other mosquito species. Multiple sequence alignment was performed using ClustalW and phylogenetic trees were constructed in MEGA v12 using the Neighbor-Joining method. Bootstrap analysis with 1,000 replicates was applied to assess the robustness of tree topology and nodal support, providing insight into the evolutionary relationships and genetic divergence among the studied mosquito species.

Results and discussion

A total of 6,421 mosquitoes were collected from households of Thrissur and Ernakulam districts. Among these, 271 specimens were identified as *Cx. quinquefasciatus* and 42 as members of the *Cx. vishnui*

group, based on distinctive morphological characteristics observed under a stereozoom microscope.

Culex mosquitoes were medium-sized, measuring 4–10 mm in length, with brownish-grey to dull brown bodies and wings (W) covered with scales along the veins and margins (Tyagi *et al*., 2015). Females possessed short maxillary palps, shorter than the proboscis, whereas males exhibited long, plumose antennae (A) and palps equal to or exceeding the proboscis (P) length, reflecting their roles in host-seeking and mate detection. The slender bodies and long, delicate legs facilitated resting in sheltered habitats, and females typically had blunt-tipped abdomens with retracted cerci, characteristic of oviposition behaviour. These mosquitoes usually held their bodies parallel to resting surfaces, a posture that may enhance camouflage and reduce predation, demonstrating adaptations to peri-domestic and polluted water habitats (Gyawali *et al*., 2025). Among the collected specimens, *Cx. quinquefasciatus* (Southern House Mosquito) was the most abundant and readily distinguishable species. It was brown to golden-brown, with short palpi about one-fourth the length of the straight, uniformly dark proboscis. Both

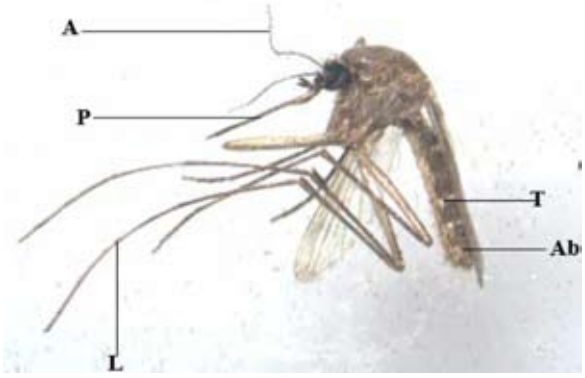

Fig. 1. *Cx. quinquefasciatus*- Female

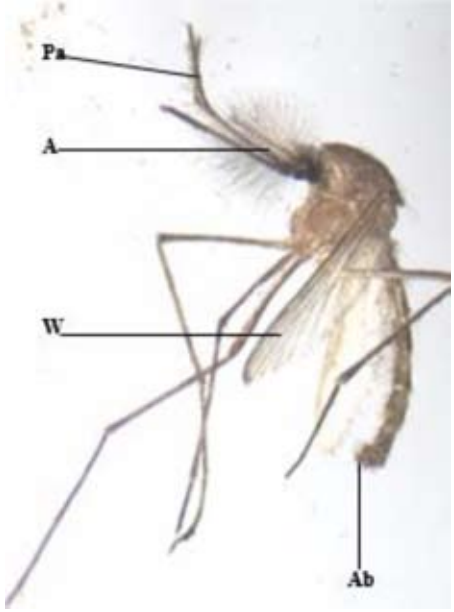

Fig. 2. *Cx. quinquefasciatus*- Male

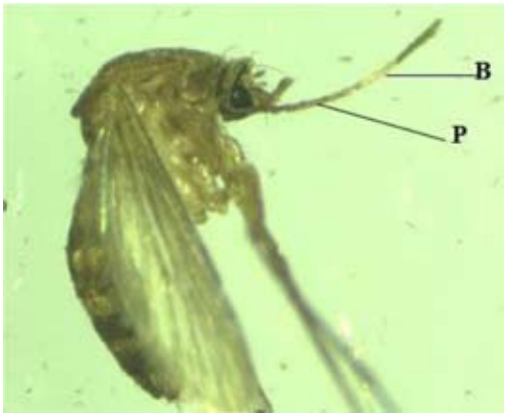

Fig. 3. *Cx. vishnui*-Proboscis

Fig. 4. *Cx. vishnui*-Male

the proboscis and tarsi were unbanded, wings lacked white spots, and the abdomen displayed narrow pale bands (Ab) on the tergites, ending in a blunt tip. The thorax was plain, and body scales were broad with intermixed black and pale scales, while legs (L) generally lacked white banding (Daravath *et al*., 2015). In contrast, members of the *Cx. vishnui* group differed from *Cx. quinquefasciatus* in several key morphological features, exhibiting generally darker brown bodies with variable pale scale patterns on the head, legs, and abdomen, as well as a distinct pale band (B) encircling the proboscis (P) and alternating pale and dark tarsal segments (L). The representative pictures are depicted in figures 1-4.

Out of the total 6427 mosquitoes collected, *Cx. quinquefasciatus* comprised 4.22 per cent (n=271) and *Cx*. *vishnui* accounted for 0.65 per cent (n=42). An entomological survey conducted by Sabu and Subramanian (2007) in Thrissur, Kerala, between January and December 2002, identified *Cx. tritaeniorhynchus* as the most common species in cattle sheds, whereas *Cx. quinquefasciatus* was most frequently observed in human dwellings. The collection points in the present study were also households and aligns to these observations. Korgaonkar *et al.* (2008) also observed that the dominant species was *Cx. quinquefasciatus*, accounting for 47.8 per cent of all female mosquitoes in Panaji, Goa. A comprehensive entomological survey by Singh *et al.* (2023) documented the presence of 393 mosquito species in India, with *Anophelinae* contributing 61 species and *Culicinae* accounting for 332 species across 11 tribes and

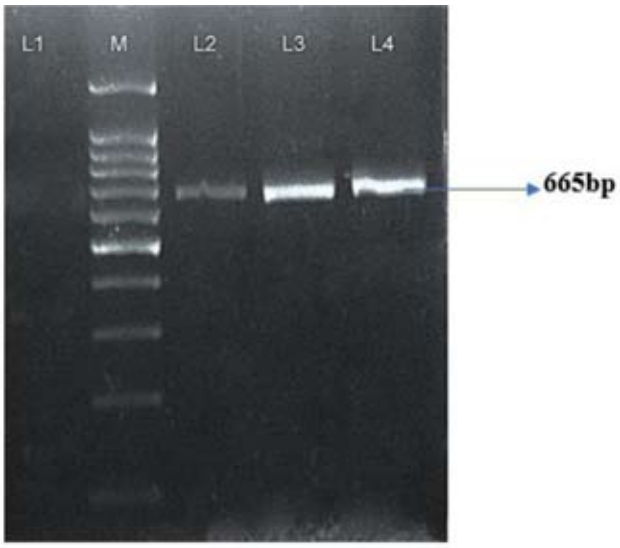

Fig. 5. Amplicons of *COI* gene of *Cx. quinquefasciatus.* L1: NTC, M:100 bp ladder, L2-L4: positive

48 genera. *Culex quinquefasciatus* was the most abundant in Coimbatore (Tamil Nadu) while *Cx. tritaeniorhynchus* was abundant in Bellary (Karnataka). Arumugam *et al.* (2024) investigated mosquito prevalence in urban areas of Udupi and Dakshina Kannada districts, Karnataka and reported that *Cx. quinquefasciatus* was the predominant species, comprising 77.9 per cent of all specimens collected.

Molecular characterisation of *Cx. quinquefasciatus* was performed to confirm species identity and provide a reliable genetic reference for vector surveillance. Patsoula *et al.* (2006) utilised the internal transcribed spacer 2 (ITS2) region of ribosomal DNA and the mitochondrial cytochrome oxidase I (*COI*) gene for precise mosquito species differentiation. They documented that though *COI* PCR products were similar in size, sequencing revealed sufficient interspecific variation to support phylogenetic differentiation. A 665 bp fragment of the *COI* gene was amplified using species-specific primers (Daravath *et al*., 2015), with gradient PCR conducted using DNA from *Cx. quinquefasciatus* as a positive control. The assay produced strong and specific amplicons across all tested annealing temperatures (53–56°C), with no cross-amplification observed in non-target mosquito species or negative controls, demonstrating the high specificity of the protocol. Based on band intensity, an annealing temperature of 55°C was selected for amplifying the remaining DNA samples (Fig. 5). The PCR products were sequenced using the Sanger dideoxy chain termination method, and BLASTn analysis revealed 100 per cent identity with existing *Cx. quinquefasciatus* sequences in GenBank. The sequence was submitted under accession number PV802545, providing a confirmed molecular reference for the Kerala isolate. Phylogenetic analysis using the Neighbor-Joining method in MEGA12, with 1000 bootstrap replicates, included *Cx. quinquefasciatus* sequences from India (Hyderabad, Kerala), Brazil, Colombia, UK, and Australia, alongside related taxa (*Cx. pipiens, Cx. tritaeniorhynchus, Cx. vishnui,* and *Cx. pseudovishnui*), with *Haemaphysalis bispinosa* as a distant outgroup (Fig. 6). The Kerala isolate

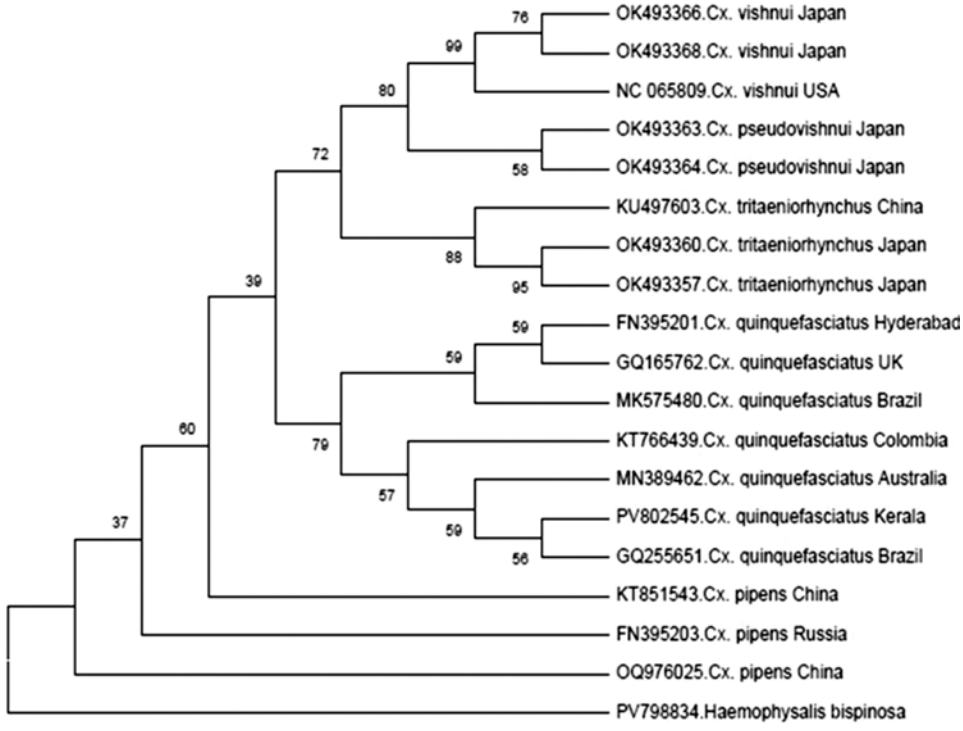

Fig. 6. Phylogenetic tree constructed using *COI* gene nucleotide sequences of *Cx. quinquefasciatus*

clustered closely with sequences from India and the UK, supported by 95 per cent bootstrap values, and was clearly separated from *Cx. pipiens* and *Cx. tritaeniorhynchus*, which formed distinct clades, while members of the *Cx. vishnui* group resolved as a separate clade with strong bootstrap support. These findings confirm the molecular identity of the Kerala isolate, demonstrate the discriminative power of the COI gene for interspecific differentiation, and indicate genetic homogeneity of *Cx. quinquefasciatus* populations across geographically distant regions, aligning with previous molecular studies (Daravath *et al*., 2015).

The use of the *COI* gene for DNA barcoding has become a standard tool in vector systematics due to its ability to delineate morphologically cryptic species, trace phylogeographic patterns, and monitor vector movement and evolution (Soghigian *et al.,* 2023). The molecular validation of species identity ensures precision in vector surveillance and control programs, which is critical for the success of ongoing filariosis elimination efforts under the National Vector Borne Disease Control Programme.

Conclusion

Morphological and molecular analyses confirmed the identity of *Cx. quinquefasciatus* from Kerala and distinguished it from closely related species. The integration of *COI*-based sequencing with traditional taxonomy strengthens vector surveillance efforts and supports targeted control of lymphatic filariosis.

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Conflict of interest

None

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