

Molecular Phylogeny Analysis Of Southern House Mosquito, *Culex quinquefasciatus* (Diptera: Culicidae) Derived From Mitochondrial DNA Sequences

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Abstract

Southern house mosquito, Culex quinquefasciatus vectors of important diseases, such as filariasis, Japanese encephalitis, dengue fever and avian malaria. Here we report the partial DNA sequence of cytochrome oxidase subunit II (COII) of *C. quinquefasciatus* and its phylogenetic status. In Partial COII DNA sequence of *C. quinquefasciatus* has no intraspecies divergence with *C. quinquefasciatus* of USA, Japan and Malaysia COII gene (GenBank Accession Number: JQ716608, JQ716607), but Partial COII DNA sequence of *C. quinquefasciatus* is 5% difference to that of *C. bitaeniorhynchus*, *C. nigropunctatus* COII gene (GenBank Accession Number: HQ398952 and HQ398950) and 10% to that of *Aedes taeniorhynchus* and *Anopheles hyrcanus* CO II gene sequences (GenBank Accession Number: FM992197 and HQ197484). Phylogenetically the nearest relative of *C. quinquefasciatus* are *C. pipiens*

Key words- cytochrome oxidase subunit II, *Culex quinquefasciatus*, phylogeny

Introduction

Southern house mosquito, *C. quinquefasciatus* is an important vector of periodic filariasis in some parts of the world (Belkin, 1968), and is known to carry and transmit *Wuchereria bancrofti* to some degree of efficacy in many regions of the globe. *C. quinquefasciatus* is able to transmit Ross River Virus, Alfuy, Almpiwar, Corriparta, Dengue, Sindbis, Japanese Encephalitis Virus (Reuben *et al.*, 1994). *C. quinquefasciatus* is also a major vector of bird pox and the avian malaria-causing protozoa (Derraik, 2004). It is a strong-winged domestic species seen all over India in and around human dwellings. Rapid urbanization and industrialization without adequate drainage facilities are responsible for its increased dispersal. In the present study, the sequencing of mitochondrial CO II gene of *C.*

quinquefasciatus (earlier known as *C. fatigans*), the Southern house mosquito, has been done which can be used as its barcode for proper taxonomic identification.

C. quinquefasciatus is a medium sized brown mosquito that exists throughout the tropics and the lower latitudes of temperate regions. Adults vary from 3.96 to 4.25 mm in length (Lima *et al.*, 2003). The mosquito is brown with the proboscis, thorax, wings, and tarsi darker than the rest of the body. The head is light brown with the lightest portion in the center. The antennae and the proboscis are about the same length, but in some cases the antennae are slightly shorter than the proboscis. The flagellum has thirteen segments that have few to no scales (Sirivanakarn *et al.*, 1978). The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. The bands barely touch the basolateral spots taking on a half-moon shape.

C. quinquefasciatus is a sub-tropical species usually found within the latitudes 36° N and 36° S. However, in the U.S. between 36° N and 39° N there is a broad hybrid zone where *C. quinquefasciatus* freely mates with *C. pipiens* Linnaeus, which is usually not found south of 39° N. Mating between these two members of the *C. pipiens* complex produce viable offspring within the hybrid zone. The extent of the hybridization is extensive and the members are sometimes considered to be subspecies, *C. pipiens quinquefasciatus* and *C. pipiens pipiens* respectively. *C. quinquefasciatus* is found in North America, South America, Australia, Asia, Africa, Middle East, and New Zealand. In U.S, this species ranges from Virginia across the southern plains to southern California, and from as far north as southern Iowa, south to Texas and Florida. There are reports of *C. quinquefasciatus* as far north as Indiana in the U.S. The species has also been collected in Hawaii. In Florida, *C. quinquefasciatus* is found in all 67 counties.

C. quinquefasciatus females fly during the night to nutrient-rich standing water where they will lay their eggs. They will oviposit in waters ranging from waste water areas to bird baths, old tires, or any container that holds water. If the water evaporates before the eggs hatch or the larvae complete their life cycle, they die. Common to the *Culex* genus, the eggs of *C. quinquefasciatus* are laid in oval rafts loosely cemented together with 100 or more eggs in a raft which will normally hatch 24 to 30 hours after being oviposited.

The larvae feed on biotic material in the water and require between five to eight days completing their development at 30°C. The larvae progress through four larval instars, and towards the end of the fourth instar they stop eating and molt to the pupal stage. Following 36 hours at 27°C the adults emerge from the pupal stage. The time of development under natural conditions for all stages is variable and dependant on temperature. The larval head is short and stout becoming darker toward the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight

segments, the siphon, and the saddle. Each segment has a unique setae pattern. The siphon is on the dorsal side of the abdomen. In *C. quinquefasciatus* the siphon has four times longer and wide with multiple setae tufts. The saddle is barrel shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end. Similar to other mosquito species, *C. quinquefasciatus* pupae are comma shaped and consist of a fused head and thorax (cephalothorax and an abdomen). The cephalothorax colour varies with habitat and darkens on the posterior side. The trumpet, which is used for breathing, is a tube that widens and becomes lighter in color as it extends away from the body. The abdomen has eight segments. The first four segments are the darkest, and the color lightens towards the posterior. The paddle, at the apex of the abdomen, is translucent and robust with two small setae on the posterior end.

Both males and females take sugar meals from plants. Following mating, the female seeks a blood meal. *C. quinquefasciatus* are opportunistic feeders, feeding on mammals or birds throughout the night. Males survive only on sugar meals, while the female will take multiple blood meals. After a female mosquito digests the blood meal and the eggs develop, she finds a suitable place to lay her eggs, and the cycle begins again. A single female can lay up to five rafts of eggs in a lifetime. The number of eggs per raft varies with climatic conditions. This night time-active, opportunistic blood feeder is a vector of many of pathogens, several of which affect humans. Throughout much of the southern U.S., *C. quinquefasciatus* is the primary vector of St. Louis encephalitis virus (SLEv). It also transmits West Nile virus (WNV) (Shroyer, 2004).

Materials and methods

Sample collection and DNA extraction

The *C. quinquefasciatus* were collected from the Calicut university campus. Extraction of mitochondrial DNA was made from one of the thoracic legs of the experimental insect, *C. quinquefasciatus*. The genomic DNA was isolated using Genei Ultrapure Mammalian Genomic DNA Pre Kit (Bangalore, Genei) as per the Manufacturer's instruction.

PCR amplification and DNA Sequencing

About 2 nanogram of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit II (CO II) gene using the forward primer with DNA sequence 5'-ACCTTAAAAGCTATCGGTCATCAA -3' and reverse primer with DNA sequence 5'-GATTAGCACCACAAATTTCTGAAC -3'. The PCR reaction mixture consisted of 2 nanogram of genomic DNA, 1 µl each forward and reverse primer with at a concentration of 10 µM, 2.5 µl 10dNTP_s (2 mM), 2.5 µl 10X reaction buffer, 0.20 µl Taq polymerase (5U/ µl) and 12.8 µl H₂O. The PCR profile consisted of an initial denaturation step of 5 min at 95°C, followed by 30 cycles of

10s at 95°C, 10s at 55°C and 1 min at 72°C and ending with a final phase of 72°C for 3 min. The PCR products were resolved on a 1% TAE- agarose gel, stained with EtBr and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding coil fragment, the remaining portion of the PCR product was column purified using Mo Bio UltraClean PCR Clean-up Kit (Mo Bio Laboratories Inc., California) as per the manufacturer's instructions. The purified PCR product was sequenced from both ends using the forward and reverse primers used for the PCR using the Sanger's sequencing method at SciGenom Laboratory Private Ltd., Cochin.

Phylogenetic Analysis

The forward and reverse sequences were assembled by using ClustalW (<http://www.ebi.ac.uk/Tools/msa/clustalw2>) after removing the forward and reverse primer sequences and the consensus was taken for the analysis. The final sequence was searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/). The phylogenetic tree was plotted using neighbor joining method using by MEGA5 software (Tamura *et al.*, 2011).

Results and Discussion

The PCR amplification of partial mitochondrial cytochrome oxidase subunit II (CO II) gene fragment of *C. quinquefasciatus* yielded a single product of 509 bp. The comparison of sequence obtained with that of BOLD Animal Identification System revealed that the *C. quinquefasciatus* is 100% similar to that of *C. quinquefasciatus* of USA, Japan and Malaysia COII gene (GenBank Accession Number: JQ716608, JQ716607), 95% similar to that of *C. bitaeniorhynchus*, *C. nigropunctatus* COII gene (GenBank Accession Number: HQ398952 and HQ398950) and 90% similarity to that of *A. taeniorhynchus* and *A. hyrcanus* CO II gene sequences (GenBank Accession Number: FM992197 and HQ197484).

>*Culex quinquefasciatus* isolate mitochondrial cytochrome oxidase subunit II (CO II) partial nucleotide sequence, 509 bases

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TTTTATAAATTTAGAATTTGATTCATATATAATCCAACAAATGAATTAGATTTAAATGGATTCCGATTATT  
AGATGTTGATAATCGAATTATTTTACCATTAATAATCAAATTCGAATTTTAGTAACTGCTACTGATGTTCC  
TCACTCATGAACAGTTCCTTCTTTAGGAGTAAAATTGATGCTACTCCAGGCCGATTAAATCAAACAACTAATT  
TTCTAATTAATCAATCTGGTCTTTTTTTTTGGGCAATGTTTCNGAAATNTGNGGNGCTAATCTAATCINNCT  
NGAGTAGCATCAATTTTTACTCCTAAAGAAGGAACTGTTTCATGAGTGAAGAACATCAGTAGCAGTTACT  
AAAATTCGAATTTGATTATTTAATGGTAAAATAATTCGATTATCAACATCTAATAATCGGAATCCATTTAA  
ATCTAATTCATTTGTTGGAATTATATATGAATCAAATTTCTAAATTTATAAAATTAGAATATTCATAACTTCA  
GTATCATTGATG
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Figure 1: The DNA sequence of the mitochondrial cytochrome oxidase subunit II (CO II) gene of *Culex quinquefasciatus*.

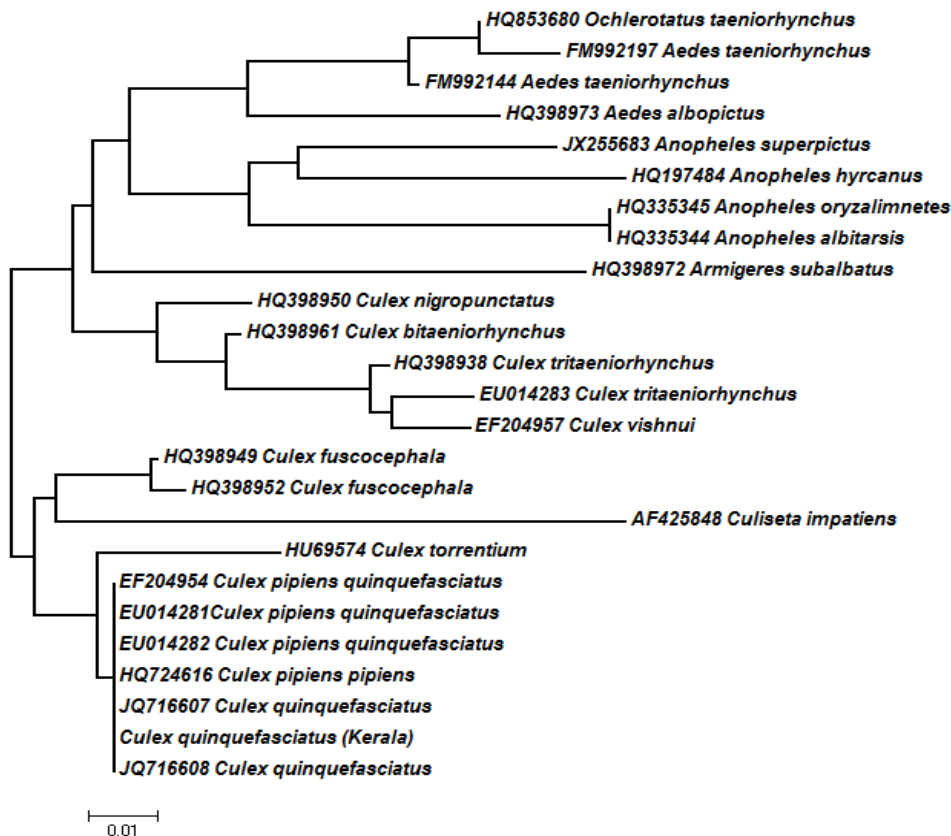


Figure 3: Phylogenetic status of *C. quinquefasciatus* compared with other *Culex* species .

Accession No. and Name of Species	Percentage of divergence
JQ716608 C. quinquefasciatus	0
JQ716607 C. quinquefasciatus	0
HQ724616 C. pipiens pipiens	0
EU014282 C. pipiens quinquefasciatus	0
EU014281C. pipiens quinquefasciatus	0
EF204954 C. pipiens quinquefasciatus	0
HQ398949 C. fuscocephala	3
HU69574 C. torrentium	3
HQ398952 C. fuscocephala	3
HQ398961 C. bitaeniorhynchus	5
HQ398938 C. tritaeniorhynchus	6
HQ398950 C. nigropunctatus	5
EU014283 Culex tritaeniorhynchus	8
EF204957 Culex vishnui	8
FM992144 Aedes taeniorhynchus	8
HQ398973 Aedes albopictus	9
HQ853680 Ochlerotatus taeniorhynchus	9
JX255683 Anopheles superpictus	10
HQ398972 Armigeres subalbatus	10
HQ335345 Anopheles oryzalimnetes	10
HQ335344 Anopheles albitarsis	10
HQ197484 Anopheles hyrcanus	10
AF425848 Culiseta impatiens	9
FM992197 Aedes taeniorhynchus	10

Figure5: Genetic divergence of COII sequence of *Culex quinquefasciatus* isolated from Kerala

The Neighbour joining tree with nucleotide sequences revealed that it is closer to *Culex* sp, *Aedes* sp and *Anopheles* sp, with respect to mitochondrial CO II gene sequences. The barcode generated for *C. quinquefasciatus* in the present study can be used for its accurate taxonomic identification.

Conclusions and Recommendations

The COII gene in the mitochondrial genome has been proved to be an excellent source of information for the set of closely related families belonging to the order Diptera. Variation in the nucleotide is fundamental property of all living organisms which can be used for their identification and phylogenetic status. The partial sequence of CO II gene of *C. quinquefasciatus* from Calicut University campus is 100% identical with that of *C. quinquefasciatus* isolated from USA, Japan and Malaysia. The COII sequence obtained in this study showed nucleotide variation of 5% to that of *C. bitaeniorhynchus*, *C. nigropunctatus* COII gene (GenBank Accession Number: HQ398952 and HQ398950) and 10% to that of *A. taeniorhynchus* and *A. hyrcanus* CO II gene sequences (GenBank Accession Number: FM992197 and HQ197484). Phylogenetically the nearest relative of *C. quinquefasciatus* are *C. pipiens* . The N-J tree with nucleotide sequences revealed that it is closer to *Culex* sp, *Aedes* sp, and *Anopheles* sp in their mitochondrial CO II gene sequences.

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