Genetic structure of mitochondrial cytochrome oxidase subunit I gene of the mosquito, *Armigeres subalbatus*

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Abstract

Molecular systematics encompasses a series of approaches in which phylogenetic relationships are inferred using information from macromolecules of the organisms under study. The experimental organism *Armigeres subalbatus* is a vector suspected of transmitting *Wuchereria bancrofti*. Sequencing was done for cytochrome oxidase subunit I (COI) gene of *Armigeres subalbatus*, to evaluate its relationship between the different species of mosquitoes and to generate a database for molecular barcoding of *A. subalbatus*. The mitochondrial cytochrome oxidase subunit I (COI) gene fragment of *Armigeres subalbatus yielded* a single product of length 522 bp and the sequence was deposited in GeneBank. The *A. subalbatus* of Kerala is showing 100% similarity to that obtained from Pakistan indicating the absence of geographical variation. The molecular barcode generated for *A. subalbatus* in the present study can be used for its accurate taxonomic identification.

Key words – *cytochrome oxidase subunit II, molecular phylogeny, Armigeres subalbatus, Kerala*

INTRODUCTION

Molecular systematics uses genetic markers to make inferences about population, process and phylogeny to establish a substantial comparative database for specific genes or proteins. Studies of molecular evolution use these data to evaluate rates, process and constraints on molecular change through time. Insect molecular systematics has complemented and enhanced value of morphological as well as ecological data contributing to evolutionary biology in process. The CO I gene is one of the most important protein coding genes of mt DNA and has been utilized in the studies of molecular evolution and classification of species.

The extreme diversity of insects and their economical, epidemiological and agricultural importance have made this group a major target of barcoding. The molecular taxonomic method has been

useful in the identification of vectors, that carrying pathogenic organisms, including the experimental organism *Armigeres subalbatus*. *Armigeres* adults are morphologically similar to species of other Aedine genera in the Oriental and Australasian Regions but they are generally larger and usually have the proboscis slightly curved downwards and flattened laterally. They are natural vectors of Japanese encephalitis. Also these species are found to be an excellent vector of filarial worm *Breinlia sergenti* and *Brugia pahangi*. Several species of *Armigeres* are suspected of transmitting *Wuchereria bancrofti.* Insect molecular systematics has complemented and enhanced the value of morphological and ecological data, making substantial contributions to evolutionary biology in the process.

Emerging infectious diseases represent a challenge for global economies and public health. About one fourth of the last pandemics have been originated by the spread of vector-borne pathogens. In this sense, the advent of modern molecular techniques has enhanced our capabilities to understand vector-host interactions and disease ecology. However, host identification protocols have poorly profited of international DNA barcoding initiatives and/or have focused exclusively on a limited array of vector species. Therefore, ascertaining the potential afforded by DNA barcoding tools in other vector-host systems of human and veterinary importance would represent a major advance in tracking pathogen life cycles and hosts (Alcaide *et al.*, 2009). This study show the applicability of a novel and efficient molecular method for the identification of the vertebrate host's DNA contained in the midgut of blood-feeding arthropods. A eukaryote-universal forward primer was designed and a vertebrate-specific reverse primer to selectively amplify 758 base pairs (bp) of the vertebrate mitochondrial Cytochrome c Oxidase Subunit I (COI) gene.

Close interactions between insects and plants have played a major role in the evolution of both these diverse groups of organisms. Studying these interactions, however, can be difficult because many insects, especially parasites, impinge most strongly on plants during larval stages when they are morphologically difficult to identify, and many belong to diverse groups for which most species remain undescribed. DNA barcoding was used to identify nondescript lepidopteran larvae that regularly parasitize flower buds of the coastal dune endemic *Camissoniopsis cheiranthifolia* (Onagraceae) (Emery *et al.*, 2009). Cytochrome oxidase I (COI) sequencing was used for the study of geographical variation of host–parasitoid interactions (Santos *et al.*, 2011).

Understanding of host–parasitoid associations is critical to the successful outcome of their utilization in biological control projects. However, identification of these parasitoids is often difficult because of their small size and generally similar morphological features, and hence, studies on the host–parasitoid associations.

This study was done to sequence cytochrome oxidase subunit I (COI) gene of *Armigeres subalbatus*, to evaluate its relationship between the different species of mosquitoes and to generate a database for molecular barcoding of *A. subalbatus.*

METHODOLOGY

The genomic DNA was extracted from one of the thoracic legs of the experimental insect, *Armigeres subalbatus* mosquito, a vector of filarial parasite *Wuchereria bancrofti*, using GeNei Ultrapure Mammalian Genomic DNA Prep Kit (GeNei, Bangalore).

Sequencing of genomic DNA

2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the forward primer with DNA sequence 5'-GGTCAACAAATCATAAAGATATTG G -3' and reverse primer with DNA sequence 5'-TAAACTTCAGGGTGACCAAAAAAT CA -3'. The PCR reaction mixture consisted of 2 nanogram of genomic DNA (1 µl), 0.5 µl each forward and reverse primers at a concentration of 5 µM, 0.5 µl of dNTPs (2.5 mM), 2.5 µl 10X reaction buffer, 0.5 µl Taq polymerase (5 U/µl) and 19.5 µl H_2O. The PCR profile consisted of an initial denaturation step of 5 min at 95°C, followed by 30 cycles of 10s at 95°C, 30s at 55°C and 45s at 72°C and ending with a final phase of 72°C for 3 min. PCR product was column purified using Mo Bio UltraClean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California). The purified PCR product was sequenced at SciGenom Labs Private Ltd, Cochin. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis.

Phylogenetic Analysis

The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme (Altschul *et al*., 1990) of NCBI (www.ncbi.nlm.nih.gov/). The phylogenetic tree was plotted in neighbor joining method using MEGA 5 software (Tamura *et al*., 2011).

RESULT

The mitochondrial cytochrome oxidase subunit I (COI) gene fragment of *Armigeres subalbatus* yielded a single product of length 522 bp (GenBank Accession: KM 096999) by means of PCR. The *A. subalbatus* of Kerala is showing 100% similarity to that obtained from Pakisthan (KF 406789, KF 406783). Japan and Pakisthan, all of them belonging to the same family Culicidae. This indicates the absence of evolutionary forces among them even though they are geographically separated. The *A. subalbatus* of Kerala is 87% and 86% similar with *Aedes lineatopennis* of Japan (AB 738145) and Thailand (HQ398909) respectively. *A. subalbatus* of Kerala is about 86% similar to culex species of

The molecular barcode generated for *A. subalbatus* in the present study can be

used for its accurate taxonomic identification and also to distinguish between the vector and non-vector based diagnostic methods.

>*Armigeres subalbatus* isolate CUAS 01 mitochondrial cytochrome oxidase subunit I (COI) partial nucleotide sequence, 522 bases

TAGTGGGAACTTCTTTAAGTATTTTAATTCGAACAGAATTAAATCACCCT
GGAGTATTTATTGGAAATGATCAAATTTATAATGTAATTGTAACAGCTCA
TGCTTTTATTATAATTTTTTTTATAGTTATACCAATTATAATTGGAGGAT
TTGGAAATTGATTAGTACCCCTTATACTTGGAGCTCCAGATATAGCCTTC
CCTCGAATAAATAATATAAGTTTTTGAATATTACCCCCTTCATTAACTCT
ACTAATTTCAAGTTCTTTAGTAGAAACAGGAGCTGGAACTGGATGAACCG
TTTATCCTCCTTTATCTTCTGGAACTGCCCATGCTGGAGCTTCTGTTGAT
TTAGCTATTTTCTCTCTTCATTTAGCAGGTATTTCTTCTATTTTGGGAGC
AGTAAATTTTATTACAACTGTAATTAATATACGATCATCAGGGATTACTC
TTGATCGATTACCCTTATTTGTTTGATCTGTTGTTATTACAGCTATTTTA
CTTCTTCTTTCTTTACCAGTTT

Table.1 The evolutionary divergence between *A. subalbatus* (Kerala) with other species

Sl. No.	Species names	Percentage of divergence
1.	KM 096999 Armigeres subalbatus	
2.	KF406789 Armigeres subalbatus	0%
3.	KF406783 Armigeres subalbatus	0%
4.	KF406762 Armigeres subalbatus	0%
5.	KF406753 Armigeres subalbatus	0%
6.	KC970285 Armigeres subalbatus	0%
7.	KJ410334 Armigeres subalbatus	0%
8.	KF406788 Armigeres subalbatus	0%
9.	KF406787 Armigeres subalbatus	0%
10.	KF406784 Armigeres subalbatus	0%
11.	KF406776 Armigeres subalbatus	0%
12.	AY729986 Armigeres subalbatus	0%
13.	AB690838 Armigeres subalbatus	2%

14.	AB738145 Aedes lineatopennis	13%
15.	AB690849 Culex tritaeniorhynchus	13%
16.	AB738144 Aedes lineatopennis	14%
17.	HQ398909 Aedes lineatopennis	14%
18.	AB738141 Culex tritaeniorhynchus	14%
19.	AB690855 Culex tritaeniorhynchus	14%
20.	KF407884 Culex tritaeniorhynchus	14%
21.	AB738248 Culex tritaeniorhynchus	14%
22.	AB738247 Culex tritaeniorhynchus	14%
23.	KF407908 Culex tritaeniorhynchus	14%
24.	KF407907 Culex tritaeniorhynchus	14%
25.	KF407897 Culex tritaeniorhynchus	14%
26.	KF407888 Culex tritaeniorhynchus	14%
27.	KF407886 Culex tritaeniorhynchus	14%
28.	KF407885 Culex tritaeniorhynchus	14%
29.	KF407853 Culex tritaeniorhynchus	14%
30.	KF407847 Culex tritaeniorhynchus	14%

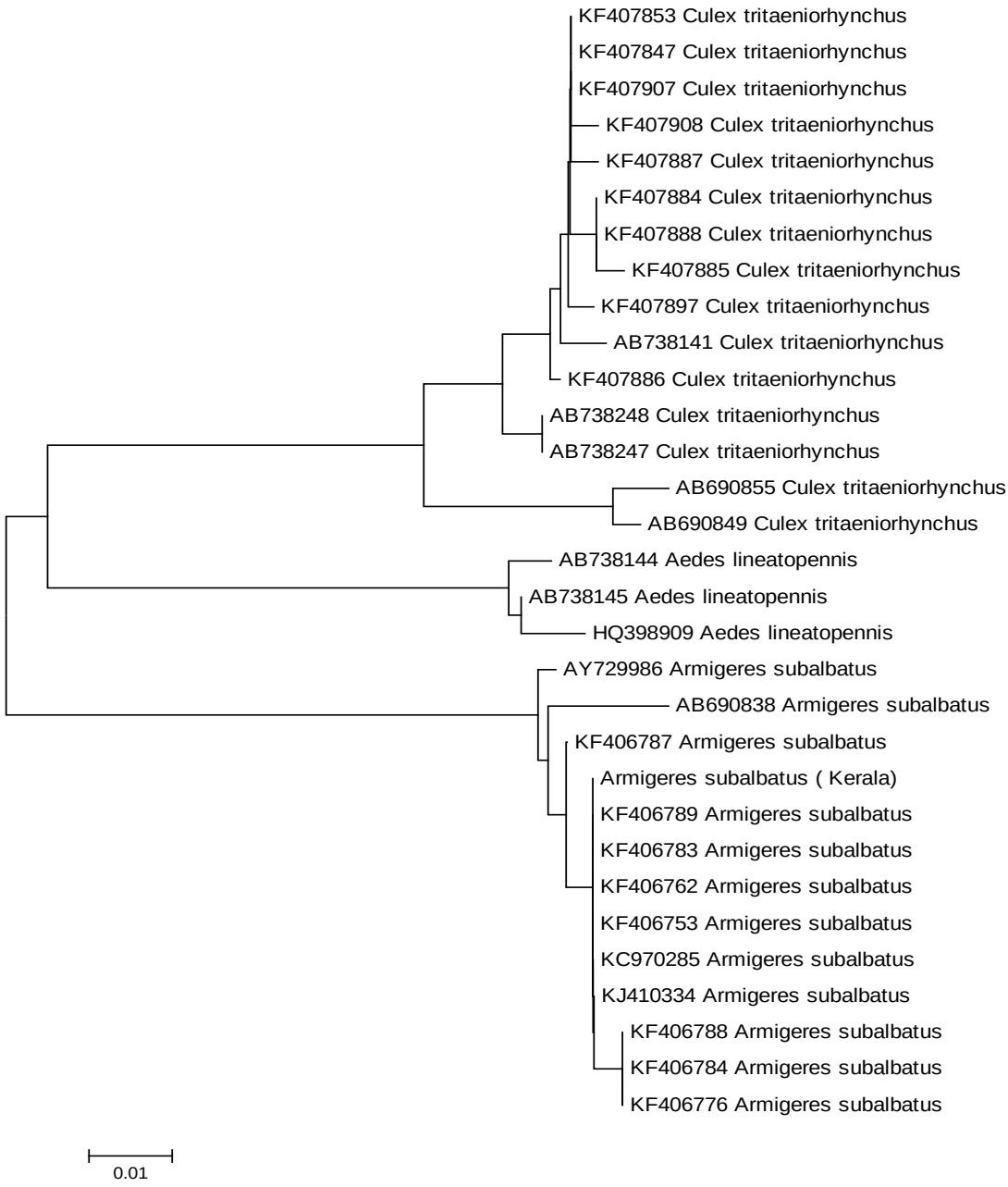

Fig.1 Evolutionary relationship of *A. subalbatus* with other species using neighbor joining method

DISCUSSION

Taxonomy is the study of identifying and classifying organisms based on their features. Traditional identification process is mainly by assessing morphological features which needs experts of that particular field. As the number of conventional taxonomists has decreased, molecular taxonomy is gaining importance today. A fragment of COI is considered as the standard DNA barcode region, which is very efficient in species identification. A 648-bp fragment has enough information and can be directly sequenced with a sequencer. Thus the barcoding can be a simple powerful tool for identifying and classifying different organisms. The mitochondrial cytochrome oxidase subunit I (COI) gene fragment of *Armigeres subalbatus* yielded a single product of length 522 bp and the sequence was deposited in GeneBank. Barcoding of 1684 mosquito specimens has been done by Asvfaq et al., 2014. Among them, 658bp length sequence of *A. subalbatus* (KF 406789) shows 100% similarity to the specimen isolated from Kerala (GenBank Accession: KM 096999). Along with a new species of tribe Aedini mosquito, *A. subalbatus* from Thailand was sequenced (Cook *et al.*, 2010). This yielded a product of 658bp (GenBank Accession: HQ 398908) which is 100% similar to *A. subalbatus of Kerala* which is 522bp length.

The *A. subalbatus* of Kerala (GenBank Accession: KM 096999) is showing 100% similarity to that obtained from Pakistan (KF 406789, KF 406783) indicating the absence of geographical variation. The A. subalbatus of Kerala has 87% and 86% similarity with *Aedes lineatopennis* of Japan (AB 738145) and Thailand (HQ 398909) respectively, and all belongs to the same family culicidae of order diptera.

CONCLUSION

Genetic diversity is central to the breeding success of most populations. Reduced genetic variation can greatly impair a population growth and can jeopardize the recovery of endangered species. The DNA sequences in organisms are maintained from generation to generation with very little change. Although such genetic stability is crucial for the survival of individuals, in the longer term the survival of organisms may depend on genetic variation, through which they can adapt to a changing environment. Thus an important property of the DNA in cells is its ability to undergo rearrangements that can vary the particular combination of genes present in any individual genome, as well as the timing and the level of expression of these genes.

In the present work, the experimental organism *A. subalbatus* from Kerala, which is a vector of filarial parasite, shows 100% similarity to that obtained from Pakisthan and 98% similarity to that from Japan. This indicates the absence of geographical variation among them. For future accurate taxonomic identification, the generated molecular barcode of *A. subalbatus* can be used.

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