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Research Article

MOLECULAR PHYLOGENETIC STUDY OF *PERIPLANETA FULIGINOSA* FROM LAKSHADWEEP ISLANDS, INDIA USING CYTOCHROME OXIDASE SUBUNIT GENE SEQUENCE

Akhilesh, V. P.[1], Femida, M. P.[2] and Sebastian, C. D.[3]*
[1]Research Scholar, Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India
[2]Student, Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India
[3]Assistant Professor, Molecular Biology Laboratory, Department of Zoology, University of Calicut, Kerala, India
*Corresponding Author Email: drcdsebastian@gmail.com

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ABSTRACT

Cockroaches are insects of the order Blattoidea, sometimes also called Blattaria. Cockroaches live in a wide range of environments around the world, having broad, flattened bodies and relatively small heads. They are generalized insects, with few special adaptations and may be among the most primitive living neopteran insects. The smoky brown cockroach (*Periplaneta fuliginosa*) is a larger species of winged cockroach, which prefer warmer climates. Though closely related to American cockroach (*Periplaneta americana*), the smoky brown cockroach is readily distinguishable by its uniformly dark brown – mahogany coloration with a shiny thorax. No molecular barcoding data is available for this species that can be used for its precise identification. In this study, we have PCR amplified and sequenced cytochrome oxidase subunit I (COI) gene of *Periplaneta fuliginosa* collected from Lakshadweep Islands for molecular level identification and constructed phylogenetic tree for recognizing its evolutionary relationship. The amplified partial sequence of COI gene yielded a single product of 622 bp long fragment encoding 207 amino acids. The resultant COI gene sequence deposited in NCBI GenBank (Accession No. KM 985649) database can be used as molecular barcode of this species.

Keywords: Molecular systematics, *Periplaneta fuliginosa*, mitochondrial DNA, COI gene sequences.

INTRODUCTION

Insects are the major group of organisms in animal kingdom and are the most diverse and innumerable group. In such a case it is difficult to go for finding and studying characters of every individual and its relation with the other. *Periplaneta fuliginosa,* commonly known as the smoky brown cockroach, is a large species of winged cockroach. Although closely related to the American cockroach (*Periplaneta americana),* the smoky brown cockroach is readily distinguishable by its uniformly dark brown-mahogany coloration. The smoky brown cockroach life cycle requires about 320 days from egg to adult. The smoky brown cockroach is very common in Japan, as well as the Southern United States and tropical climates; notably, it can be found in Florida, Louisiana, Mississippi, Texas and other moist Gulf Costal States and along the Southern Mississippi River. The smoky brown cockroach prefers warmer climates and is not cold tolerant. The experimental organism, *P. fuliginosa* used for the present study was collected from Lakshadweep islands, India.

Molecular systematic uses genetic markers to make inferences about population process and phylogeny and in doing so creates substantial comparative database for specific genes or proteins[1]. There is a fundamental synergy between studies of molecular systematic and molecular evolution. Relevance of barcoding in insect studies was investigated by Bravo *et al* [2] using DNA barcodes to confirm the presence of a new invasive cockroach pest in New York city[3]. Here used DNA barcoding and morphological identification to confirm that this newly invasive pest species was indeed *Periplaneta japonica*. Species identification is unequivocally a valid use DNA barcoding, DNA sequences are used as markers prior established species in species identification DNA barcoding has also been used in well studied groups such as Lepidoptera[4]. DNA sequence based identification technique has revealed the morphological and ecological traits of many species during larval stages[5]. Barcoding is now used to understand to diversity of caterpillar fauna in various areas of the world[6].

Mitochondrial DNA has been one of the most widely used molecular markers for phylogenetic studies in animals because of its simple genome structure[7]. Among insects, the maximum number of mitochondrial genome has been characterized in order Diptera[8]. The most commonly sequenced regions in insect systematic are mitochondrial DNA and nuclear DNA. Mitochondrial DNA provides a powerful tool for studying relationships within species. Insect Mitochondrial genome is a double stranded circular genome which range from 14,503 bp to 19,571 bp in size. The sequence divergences at COI regularly enable the discrimination of closely allied species. The COI gene is generally effective as a barcode sequence, delivering more than 95% species level resolution[9]. DNA barcoding aims at identification of organisms by accessing their degree of DNA sequences similarity to a set of reference taxa[10].

In the present study, the sequencing at mitochondrial COI gene of *Periplaneta fuliginosa* has been done which can be used as its barcode for proper taxonomic identification.

MATERIALS AND METHODS

The experimental organism *Periplaneta fuliginosa* collected from Lakshadweep Islands (India) and it is commonly known as the smoky brown cockroach. It is morphologicaly identified by experts and preserved in 70% alcohol. It is although closely related to the American cockroach (*Periplaneta americana).*

DNA Extraction

DNA extraction was made from one of the thoracic legs of the experimental insect, *Periplaneta fuliginosa*. The tissue was homogenized and genomic DNA in the homogenate was isolated using M-N NucleoSpin Tissue Kit.

Sequencing of genomic DNA

About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the forward primer, 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer, 5'- TAAACTTCAGGGTGACCAAAAAATCA -3'. The PCR reaction mixture consisted of 2 ng of genomic DNA (1µl), 0.5µl each forward and reverse primer with at a concentration of 5 µM, 0.5 µl $dNTP_S$ (2.5mM), 2.5 µl 10X reaction buffer, 0.5 µl Taq polymerase(5U/ 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. The PCR products were resolved on a 2% TAE- agarose gel, for confirmation of the target gene amplification. The PCR product was column purified and was sequenced using Sanger's method[11].

Phylogenetic Analysis

The obtained sequence was checked for its quality by examining chromatograms and the forward and reverse sequence were assembled using Clustal W and the consensus was taken for the analysis. The final sequence was searched for its similarity using BLAST of NCBI (www.ncbi.nlm.nih.gov/). The phylogenetic tree was plotted using neighbor joining method using by MEGA6 software[12].

RESULTS

The PCR product of the mitochondrial cytochrome oxidase subunit I (CO I) gene *Periplaneta fuliginosa* yielded a single product of 622 bp. The sequence is found to be novel and the same has been deposited in the NCBI GenBank (Accession No. KM 985649). The PCR amplified COI gene of *Periplaneta fuliginosa* yielded the chromatogram shown in the Figure 1. The phylogenetic tree plotted using neighbor joining method in Rectangle and Radial forms exhibited in Figure 2.

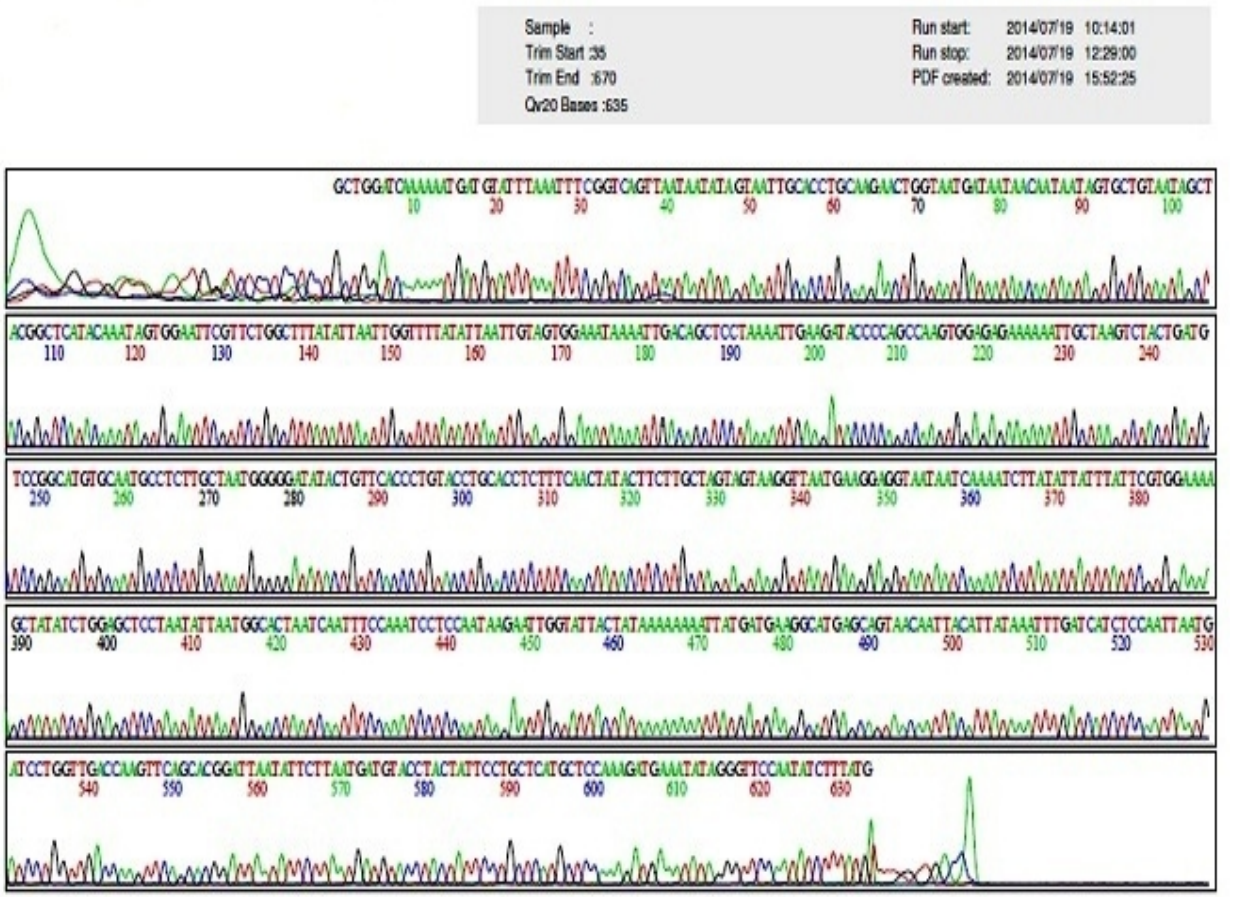

Figure 1: The chromatogram of PCR amplified COI gene of *Periplaneta fuliginosa*

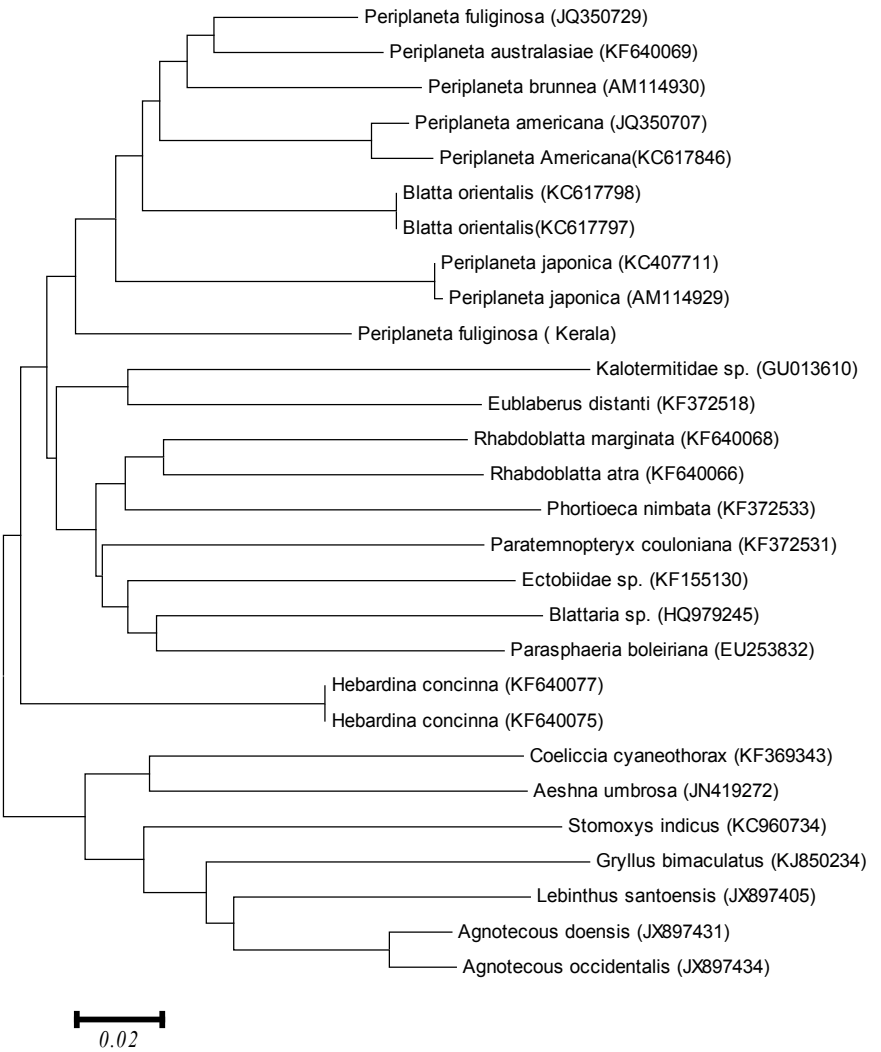

Figure 2: The phylogenetic tree plotted for *Periplaneta fuliginosa* was inferred using cytochrome oxidase subunit I (COI) gene partial sequence by Neighbor joining method.

DISCUSSION

Genetic diversity serves as a way for populations to adapt to changing environments. Genetic diversity is central to breeding success of most populations. Reduced genetic variation can greatly impair a population growth. The DNA sequences in an organism are maintained from generation to generation with very little change. Sequencing provides the order of individual nucleotides in DNA isolated from cells of animals or virtually any other source of genetic information. Cytochrome oxidase is one of a super family of proteins which act as the terminal enzymes of respiratory chains. There are two catalytic subunits, I and II. These are the most widely used gene for molecular barcoding and phylogeny analysis of organisms especially higher eukaryotes for its high level of sequence variation compared to other region of mitochondrial DNA. Molecular phylogenetic analysis using partial mitochondrial COI gene sequences were reported in a range of insect taxa like dipterans[13,14], lepidopterans[10,15], heteropterans[16] and hymenopterans[5,17].

Partial sequence of COI gene fragment of *Periplaneta fuliginosa* gene obtained was 89% similar to that of *Periplaneta fuliginosa* collected from South Korea (GenBank Accession No: JQ 350729), Japan (AB 126004) and to that of *P. australasiae* of China (KF 640069). Further it has 87% similar to COI sequence of *P. americana* (JQ 350707; KC617846) and 86% to that of *P. japonica* of Spain (KC 407711; AM 114929). The experimental organism collected from Lakshadweep is morphologically similar to that of *P. fuliginosa* as identified by Scientific Experts. But it shows 11-20% genetic divergence against different species of family because of their change in habitat or climatic factors. The COI barcode generated in the present study for *P. fuliginosa* is the first report from India.

The Neighbour joining tree with nucleotide sequence revealed that it is closer to *P. japonica, Blatta orientalis* and *P. americana*, with respect to mitochondrial COI gene sequences. The barcode generated for *P. fuliginosa* in the present study can be used for its accurate taxonomic identification.

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