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Identification of Some Local Insects Using Dna Barcoding

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ABSTRACT

Sequence diversity in the cytochrome oxidase subunit 1 (COI) is often used for DNA barcoding different animals in order to identify and classify them. There is dearth of information available on such studies done in India, especially in the Maharashtra state. So the present investigation was carried out to investigate the efficacy of COI gene in DNA barcoding to identify some local insects collected from a nearby forest. Sequence of the amplified COI region when analyzed by BLAST tool was able to identify the insect samples based on their sequence similarities.

KEYWORDS : Insects, classification, COI, phylogeny, Maharashtra

INTRODUCTION

DNA barcoding is a novel system designed to provide rapid, accurate, and automatable species identifications by using short, standardized gene regions as internal species tags (Hebert and Gregory, 2005).

In case of animal kingdom, the sequence diversity in cytochrome c oxidase subunit 1 (COI) is often analysed for establishing the barcode. It is suggested that the diversity in the amino acid sequences coded by the 5′ section of this mitochondrial gene is sufficient to reliably place species into higher taxonomic categories (from phyla to orders) (Hebert et al., 2003).

It is already known that DNA barcoding is reliable, easy to perform and cost effective molecular tool for identification of many animal species especially the metazoan taxa which includes insects (Virgilio et al., 2010)

In view of this, an attempt was made to identify some insects collected from the local forest of Maharashtra using the sequence of the amplified COI region of the mitochondrion.

MATERIALS AND METHODS

Collection of samples

Two different insects were collected from the local forest of Ratnagiri, Maharashtra state and immediately immersed in 90% ethanol for preservation in separate sterile containers. After that, the samples were brought to the laboratory in Mumbai for further analysis.

DNA extraction and quantification

DNA from the samples was extracted using Genelute Genomic DNA extraction kit (Sigma, G1N70-1KT) by following manufacturer's instructions and by treating with proteinase K and RNase A solutions. The extraction process was completed by adding the lysate to the GenElute Miniprep Binding Column and centrifugation. Concentration of DNA was determined using UV-1800 spectrophotometer.

Amplification of COI gene by using PCR

Polymerase chain reaction (PCR) was used to amplify the Cytochrome oxidase I (COI) region of mitochondria from the extracted genomic DNA. Two primers specific to mitochondrial COI sequence viz. LCO1490 and HCO2198 were used for this purpose. PCR amplification was carried out by using Biometra thermal cycler (T-Personal 48).

Agarose gel electrophoresis

Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The band size obtained for both the samples was approximately 600-700 bp.

Sequencing of PCR product

The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). 100 µl of PCR-A buffer was added to the 25 µl of reaction. The sample was mixed and transferred to column placed in 2 ml collection tube and centrifuged at 10,000 rpm for 1 min. The filtrate was discarded. 700 µl of W2 buffer was added to the column and centrifuged at 10,000rpm for 2 min. This step was repeated twice. The column was transferred to a new tube. 25 µl of Eluent was added into the column and incubated at room temperature for 2 min. Then centrifuged at 10,000 rpm for 5 min. It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained.

Analysis using BLAST

The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Centre for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION

COI gene sequence of insect 1

Genomic DNA extracted from the insect no. 1 when targeted to amplify its COI region produced a PCR product of 677 bp which was sequenced and shown in the Fig.1.

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> INS1
TTTATAATGTAATTGTAACTGCTCACGCTTTTATTATAATTTTTTTCATAGTTATGCCT
ATTTTAATTGGTGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCACCTGATA
TAGCATTTCCCCGAATAAATAATATAAGTTTTTGACTTTTACCCCCATCTCTTACTCT
TTTACTTTCAAGTTCAATTGTAGAAAATGGGGCTGGAACAGGATGAACAGTTTATCC
TCCCTTATCCGCAGCTATTGCACATAGTGGGGCTTCAGTTGATTTAGCAATTTTCTC
CCTTCATTTAGCAGGAGTATCTTCTATTTTAGGATCTGTAAATTTTATTACTACAGTT
ATTAATATGCGAGCTAATGGAATTACATTAGATCGAATACCTTTATTTGTTTGATCAG
TTGTTATTACTACTGTTCTTCTTTTACTTTCTCTTCCAGTATTAGCGGGAGCAATTAC
AATATTATTAACTGATCGAAATTTAAATACATCATTCTTTGACCCAGCGGGAGGTGG
TGACCCTATTCTTTATCAACATTTATTCTGATTTTTTGGTCACCCTGGAAGTTAAAAT
CCCACCCCCGGCCGCCAGGCCAGAGGCGGACCGCCAGAACCCCGAAGGCTAGC
TCGGCGGAAGAAGCAGAGTTAAAGGCGGTGGGCGGAAACACTGAGC
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Figure 1: Partial COI gene sequence for Insect 1

COI gene sequence of insect 2

Genomic DNA extracted from the insect no. 1 when targeted to amplify its COI region produced a PCR product of 582 bp which was sequenced and shown in the Fig.2.

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> INS2
TACAGCCCACGCTTTTATTATAATTTTTTTCATAGTTATACCTATTTTAATT
GGGGGATTCGGAAATTGACTTGTCCCTCTTATATTAGGAGCACCAGATAT
GGCATTTCCTCGAATAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTT
AACTCTTCTTCTTTCTAGTTCATTTGTAGAAAATGGAGCAGGAACTGGTT
GAACTGTTTATCCTCCACTTTCTGCAGCAATTGCTCATAGTGGGGCTTCT
GTAGATTTAGCTATTTTTTCTTTACATTTAGCTGGAGTATCATCAATTTTAG
GTTCAGTAAATTTTATTACTACAGTTATTAATATACGAGCTAATGGAATTA
CTCTGGATCGAATACCTCTTTTCGTTTGATCAGTAGTAATTACTACTGTTC
TCCTATTGCTTTCTTTACCTGTATTAGCAGGAGCTATTACTATACTTCTAA
CTGATCGAAATTTAAATACATCTTTTTTTGATCCTGCAGGAGGTGGAGAC
CCTATTCTTTATCAACATTTATTTTGATTTTTTGGTCACCCTGAAGTTAAAA
TTAATTTTGGGGGGAGGATTGA
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Figure 2: Partial COI gene sequence for Insect 2

Analysis using BLAST

The amplified COI gene sequence of Insect No. 1 was compared with the existing sequences in the nucleotide database library and by this analysis, it was identified to be *Nilodorum tainanus* as it showed 98% sequence similarity to that of isolate: NIESD0117 having an accession number AB838672.1.

The amplified COI gene sequence of Insect No. 2 when compared with the existing sequences in the nucleotide database library, was identified to be *Chironomus circumdatus* as it showed 99% sequence similarity to that of isolate MN7 having an accession number JQ287749.1 and with the COI gene sequence of voucher KMCC5 having an accession number of KT212974.1.

So using the amplified COI gene sequences, two insect samples collected from the local forest were identified as *Nilodorum tainanus* and *Chironomus circumdatus*. Both the insects are classified into Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta; Order: Diptera and Family: Chironomidae. It is important to mention that even though both the insects belonged to same family, gene sequence of COI was able to differentiate them and help in the correct identification.

In the present study, primers for targeting the COI region were selected as suggested by Kim *et al*., (2012) who employed these primers for DNA barcoding insects, fish and shellfish in Korea. As promising results were observed in the present investigation, these primers could be used for analyzing more insects and other animals from local region which remain unexplored for molecular analyses.

CONCLUSIONS

It can be concluded that amplified COI gene sequence can be used for barcoding animals especially insects to study and identify them in a quicker, cheaper and accurate manner.

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