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Sequence Analysis of Coi Gene to Identify Insects Collected from Goa, India

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ABSTRACT

DNA barcoding is a rapid, accurate, cost effective and easy to perform technique of species identification. Sequence variability in the cytochrome oxidase subunit I better known as COI region is considered as one of the most preferred marker for establishing a species specific DNA barcode. An attempt was made in the current study to check the usefulness of the COI gene sequence to identify some insects collected from the forest of Goa state. Sequence of the amplified COI region when analyzed by BLAST tool was able to identify the insect samples based on their sequence similarities.

KEYWORDS DNA barcoding, PCR, BLAST, Taxonomy, Entomology

INTRODUCTION

DNA sequences have been used for species identification since a long time. Recently it has been shown that in many groups, including insects, interspecific variation in DNA sequences of some genes is much higher than intraspecific. This understanding led to the development of DNA barcoding which involves the use of a short, agreed-upon region of the genome for sequence comparison for identifying unknown biological material to species level. Mitochondrial genome continues to remain the primary choice for barcoding in animals. In this genome, locus for choice is the cytochrome oxidase subunit I (COI) region (Hebert ***et al***., 2003).

Even though the usefulness of COI as a reliable means of species identification has been reported by many researchers in a number of animals (Ratnasingham and Hebert, 2013; Pentinsaari ***et al***., 2014; Stork ***et al***., 2015), there is not much data available on its use in India for identification of insects. In view of this, an attempt was made to identify some insects using the sequence of the amplified COI region of the mitochondria which were collected from the forest of Goa.

MATERIALS AND METHODS

Collection of samples

Sample collection was carried out in the forest of Goa state. Two morphologically different looking insects were collected 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 650-750 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 747 bp which was sequenced and shown in the Fig.1.

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>INSGOA1
CCTTAAATTTTTGGAGCATGATCAGGAATAGTAGGTACTTCATTAAGAAT
GCTTATTCGAGCAGAATTAGGACGACCTGGAACATTCATTGGTGATGATC
AAATTTATAATGTTGTTGTTACAGCCCACGCTTTTATTATAATTTTTTTCAT
AGTTATACCTATTTTAATTGGGGGATTCGGAAATTGACTTGTCCCTCTTAT
ATTAGGAGCACCAGATATGGCATTTCCTCGAATAAATAATATAAGTTTTTG
ACTTCTTCCCCCTTCTTTAACTCTTCTTCTTTCTAGTTCATTTGTAGAAAAT
GGAGCAGGAACTGGTTGAACTGTTTATCCTCCACTTTCTGCAGCAATTGC
TCATAGTGGGGCTTCTGTAGATTTAGCTATTTTTTCTTTACATTTAGCTGG
AGTATCATCAATTTTAGGTTCAGTAAATTTTATTACTACAGTTATTAATATA
CGAGCTAATGGAATTACTCTGGATCGAATACCTCTTTTCGTTTGATCAGT
AGTAATTACTACTGTTCTCCTATTGCTTTCTTTACCTGTATTAGCAGGAGC
TATTACTATACTTCTAACTGATCGAAATTTAAATACATCTTTTTTTGATCCT
GCAGGAGGTGGAGACCCTATTCTTTATCAACATTTATTTTGATTTTTTGGT
CACCCTGAAGTTAAAATTAATTTTGGGGGGAGGATTGAAAAGGAGAAATA
GCTAAATAGTCGTGAGACATACTCGTGCAGACTG
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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 669 bp which was sequenced and shown in the Fig.2.

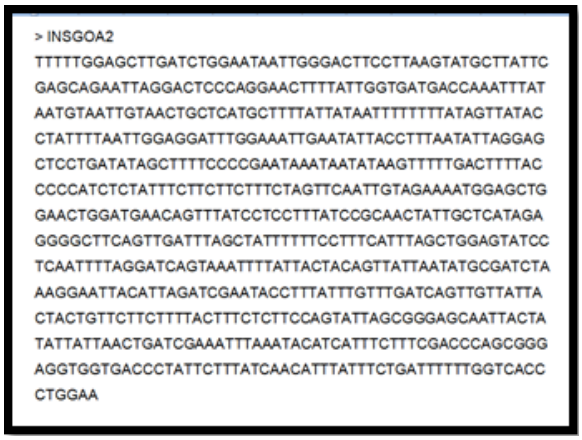

Figure 2: Partial COI gene sequence for Insect 2

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 ***Chironomus circumdatus*** as it showed 99% sequence similarity to that of isolate MN7 having an accession number JQ287749.1, with the isolate LL1-2 with the accession number KJ530965.1 and with the voucher specimen NIBGE MOS-00108 having an accession number KJ768129.1

The amplified COI gene sequence of the Insect No. 2 when compared with the existing sequences in the nucleotide database library, was identified to be ***Kiefferulus barbatitarsis*** as it showed 93% sequence similarity to that of voucher specimen of NIGLAS TA18, TA19 and TA23 having the accession numbers KP902779.1, KP902780.1 and KP902782.1 respectively.

So using the amplified COI gene sequences, two insect samples collected from the forest of Goa were identified as ***Kiefferulus barbatitarsis*** 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 vindicating that sequence diversity in COI region can be used for species 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.

COI barcoding sequences can be used to discover cryptic species: closely related and similar morphologically, and, for this reason, overlooked by traditional morphology-based approaches. DNA barcodes can also be used to link different life stages, e.g. larvae, pupae and adults (Virgilio ***et al***., 2010). This is particularly useful in situations where multiple species co-occur, or larvae are difficult to rear. Additionally, barcoding the samples from different populations allow reconstruction of the Pleistocene history of species distribution and, eventually, the process of geographical speciation (Hebert ***et al***., 2016). Considering this, more studies are being carried out to extend the use of this marker to create species specific barcodes.

CONCLUSION

We can therefore conclude that amplified COI gene sequence can be used for barcoding animals such as insects to study and identify them in a quicker, cheaper and accurate manner.

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