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COI Gene Sequencing for Identification of a Freshwater Fish from Maharashtra

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ABSTRACT

Identification of fishes can become problematic as depending upon the habitat and life stage, fishes can show diversity in colour, shape and size. Sequence variability in the cytochrome oxidase subunit I better known as COI region is considered as one of the most preferred marker for identifying unknown animal species. DNA was extracted from a freshwater fish and its COI region was amplified using specific primers and sequenced. This sequence of the amplified COI region was analyzed using the BLAST tool and compared with the available database to identify the fish based on the sequence similarity.

KEYWORDS Fish, PCR, BLAST, COI, gene sequencing, BLAST

INTRODUCTION

DNA barcoding involves the use of a short, agreed-upon region of the genome for sequence comparison for identifying unknown biological material to species level. In animals, locus for choice is the cytochrome oxidase subunit I (COI) region of mitochondria (Hebert ***et al***., 2003).

The usefulness of COI sequencing as a reliable means of identification of fish species has been reported by many researchers (Hubert ***et al***., 2008; Mabragana ***et al***., 2011; Zhang and Hanner, 2012, Kim ***et al***., 2012). However, its use for identification of local fishes in Maharashtra is still in preliminary stages. In view of this, the present study was undertaken.

MATERIALS AND METHODS

Collection of samples

A commonly available, local fish was collected from the Mula river near Pune, Maharashtra. After collection, it was identified as a fish belonging to the family Cyprinidae based on its morphological characteristics. The collected fish samples (total ten) were immediately immersed in 70% ethanol for preservation in separate sterile containers. After that, the samples were brought to the laboratory in Mumbai for further analysis following the methodology by Gomes ***et al***., (1999).

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. Fish F1 and Fish R1 were used for this purpose. PCR amplification was carried out by using Biometra thermal cycler (T-Personal 48). The PCR amplification cycle consisted of a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 55°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C. The reagents used were procured from GeNei.

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 sample was approximately 450 bp.

Sequencing of PCR product

The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). The sample was sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product, Fish F1- 5′ (TCAACCAACCACAAAGACATTGGCAC3′) sequencing primer was used.

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 fish from Mula River

Genomic DNA extracted from the fish collected from the Mula River, Pune when targeted to amplify its COI region by PCR produced a sequence of 469 bp as shown in the Fig.1.

> FISH FROM MULA RIVER<PUNE>MAHARASHTRA
ATGACCAAATTTATAACGTTATTGTTACTGCCCACGCTTTTGTAATAATCTTCTTT
ATAGTTATACCTATCCTAATCGGGGGATTTGGAAACTGACTCGTACCCTTAATAA
TCGGAGCCCCCGACATGGCATTTCCACGGATAAATAATATAAGCTTCTGACTTCT
ACCCCCGTCATTCCTCCTACTATTAGCCTCCTCTGGGGTTGAGGCTGGGGCCG
GAACAGGATGAACAGTTTATCCACCCCTTGCAGGCAACCTAGCCCATACAGGAG
CATCAGTAGATTTAACAATTTTTTCATTACACCTAGCAGGTGTATCATCAATTCTA
GGGGCTATTAATTTTATTACCACAACAATTAATATGAAACCCCCAGCTATTTCCC
AATATCAAACACCTCTATTTGTGTGGTCTGTACTTGTAACTGCCGTACTACTCCT
TTTGTCACTACCAGTATTAGCTGCCGGTAT

Figure 1: COI gene sequence for the unknown fish

Results for BLAST Analysis

The amplified COI gene sequence of the fish sample collected from the Mula River was compared with the existing sequences in the nucleotide database library and by this analysis, it was identified as ***Garra mullya*** as it showed 100% sequence similarity with the already deposited COI gene sequences of ***Garra mullya*** (Table 1).

Description	Identity	Accession
Garra mullya isolate Fish3 cytochrome oxidase subunit I (COI) gene	100%	KJ588170.1
Garra sp. 1 KKL-2013 voucher GSP-150 (COI) gene	99%	KF550083.1

Garra sp. 1 KKL-2013 voucher GSP-112 (COI) gene	98%	KF550070.1
Garra mullya voucher NF436 (COI) gene	98%	JX983294.1

Table 1: BLAST results for the Fish sample

So using the amplified COI gene sequences, the unknown fish sample collected from the Mula River was identified as ***Garra mullya***. It is a fish endemic to peninsular India and has been reported from Andhra Pradesh, Chattisgarh, Goa, Gujarat, Jharkand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and West Bengal states. It is classified into Kingdom: Animalia; Phylum: Chordata; Class: Actinopterygii; Order: Cypriniformes and Family: Cyprinidae. So based on the morphological characteristics, even though family was identified, the actual identification was made easier with the aid of gene sequence of COI.

Garra mullya has been previously reported from the state of Maharashtra, especially Pune region by many researchers (Tonapi and Mulherkar, 1963; Wagh and Ghate, 2003; Chandanshive ***et al***., 2007). So the results in the present study confirm the presence of this fish species in the region using a molecular marker.

In the present study, primers for targeting the COI region were selected as suggested by Lakra ***et al***., (2011) who employed these primers to establish the DNA barcodes for Indian marine fishes. As promising results were observed in the present investigation even for the freshwater fish, these primers could be used for analyzing more fish from the local region which remain unexplored for molecular analyses.

CONCLUSION

We can therefore conclude that amplified COI gene sequence can be used for barcoding animals such as fishes to study and identify them in a quicker, accurate and cost effective way.

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