Volume-5, Issue-9, September- 2016 • ISSN No 2277 - 8160 IF : 3.62 | IC Value 70.36

Original Research Paper Zoology

Identification of the Freshwater fish *Bangana ariza* Using COI Gene Sequencing

*** V. N. Magare**

Principal, Dept. of Zoology, Kirti M. Doongursee College of Arts, Science and Commerce, Dadar (W), Mumbai -400028,
* Corresponding author

ABSTRACT

Bangana ariza is a common freshwater fish in India and is also used for aquaculture. Owing to the diversity in body colour and the stripe pattern, its identification could lead to an error. Sequence diversity in the cytochrome oxidase subunit 1 (COI) is routinely used for identifying and classifying animals such as fish. An attempt was made in the present study to investigate the efficacy of COI gene in DNA barcoding to identify the samples of Bangana ariza collected from two different water bodies in Maharashtra. Sequence of the amplified COI region when analyzed by BLAST tool was able to accurately identify the fish samples based on their sequence similarities.

KEYWORDS : COI, phylogeny, Maharashtra, freshwater fish, India, DNA barcoding

INTRODUCTION

Accurate species identification is perquisite not only for scientific studies but also commercial and aquacultural point of view. Fish show remarkable diversity in their shapes, sizes and colours. Therefore, identification done solely on the basis of morphological characteristics could be misleading. In view of this, identification techniques based on the sequence diversity of the DNA have been developed and have been found to be more accurate and reliable.

DNA barcoding is one such a novel technique designed to provide rapid, accurate, and automatable species identifications by using short, standardized gene regions as internal species tags (Hebert and Gregory, 2005). For many animal taxa, sequence divergences within the 5'region of the mitochondrial cytochrome oxidase subunit I (COI) gene are generally much greater between species than within species. This in turn suggests that the approach is extensively applicable among phylogenetically distant animal groups. Many studies have shown that intraspecific variation of COI barcodes is generally pretty small and clearly discriminable from interspecific variation (Zhang and Hanner, 2012).

Bangana ariza better known by the name Reba is an important member of rey-finned freshwater fish belonging to the family cyprinidae. Found throughout the Indian sub-continent, this fish is considered to be very suitable for aquaculture owing to its prolific breeding capacity.

Identification of this can be tricky as it is found in various colour and also the thin stripes on its body can also be confusing. In view of this, an attempt was made to identify this fish by analyzing the sequence of the amplified COI region of the mitochondria of its genomic DNA

MATERIALS AND METHODS

Collection of samples

Ten samples of *Bangana ariza* were collected from two different water bodies viz. from the Krishna river, Satara district of Maharashtra and from a local aquaculture farm near Wai, Satara. After identification based on their morphological characteristics, the collected fish samples 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 samples was approximately 500-600 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 the sample was centrifuged at 10,000 rpm for 5 min. It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product Fish F1- 5' (TCAACCAACCACAAAGACATTGGCAC 3') sequencing primer was used.

Analysis using BLAST

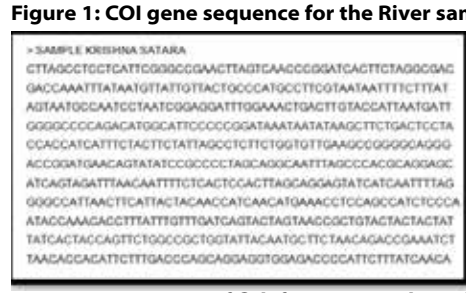
DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Centre for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION

COI gene sequence of fish from Krishna River

Genomic DNA extracted from the fish *Bangana ariza* collected from the Krishna River when targeted to amplify its COI region by PCR produced a sequence of 602 bp as shown in the Fig.1.

Figure 1: COI gene sequence for the River sample



COI gene sequence of fish from aquaculture farm

Genomic DNA extracted from the fish collected from the fish farm

when targeted to amplify its COI region produced a PCR product of 523 bp which was sequenced and shown in the Fig.2.

Figure 2: COI gene sequence for fish farm sample

Analysis using BLAST

The amplified COI gene sequence of the fish sample collected from the Krishna river was compared with the existing sequences in the nucleotide database library and by this analysis, it was confirmed to be *Bangana ariza* as it showed 100% sequence similarity with the already deposited COI gene sequences of *Bangana ariza* (Table 1). Similarly, BLAST analysis for COI gene sequence of the sample from fish farm also confirmed it as *Bangana ariza* (Table 2).

Description	Identity	Accession
Bangana ariza isolate Fish4 cytochrome oxidase subunit I (COI) gene	100%	KJ588169.1
Bangana ariza isolate Fish1 (COI) gene	100%	KJ588172.1
Bangana ariza isolate Fish2 (COI) gene	100%	KJ588171.1
Cirrhinus reba voucher ZMUD:048 (COI) gene	90%	KX455893.1

Table 1: BLAST results for the River sample

Description	Identity	Accession
Bangana ariza isolate Fish4 cytochrome oxidase subunit I (COI) gene	100%	KJ588169.1
Bangana ariza isolate Fish1 c (COI) gene	100%	KJ588172.1
Bangana ariza isolate Fish2 (COI) gene	100%	KJ588171.1
Labiobarbus fasciatus voucher BIF0684 (COI) gene	90%	KU692576.1

Table 2: BLAST results for fish farm sample

So using the amplified COI gene sequences, samples of the fish *Bangana ariza* collected from the two different habitats (natural and artificial) were confirmed as *Bangana ariza*. It is important to mention that morphologically both the fish samples did show variations in their body colour and stripe patterns but the gene sequence of COI was able to identify them accurately at the molecular level.

In the present study, primers for targeting the COI region were selected as suggested by Lakra *et al*., (2011) who employed these primers for DNA barcoding Indian marine fishes. As promising results were observed in the present investigation even for 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 identifying morphologically dissimilar fish accurately.

REFERENCES:

[1] Gomes, C., Oxenford, H. A. and Dales, R. G. B. (1999), "Mitochondrial DNA D-Loop Variation and Implications for Stock Structure of the Four-Wing Flyingfish, *Hirundichthys Affinis* in the Central Western Atlantic." 64 (3), 485-500.

[2] Hebert, P. D. N., and Gregory T. R. (2005), "The promise of DNA barcoding for Taxonomy." Syst. Biol., 54(5), 852-859.

[3] Lakra, W.S., Verma, M.S., Goswami, M., Lal, K. K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K. V., Ward, R.D. and Hebert, P. (2011), "DNA barcoding Indian marine fishes." Molecular Ecology Resources, 11, 60-71

[4] Zhang, J. and Hanner, R. (2012), "Molecular Approach to the Identification of Fish in the South China Sea." PLoS ONE, 7(2), e30621.