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Original Article

Molecular insights into the genetic and haplotype diversity among four populations of *Catla catla* from Madhya Pradesh revealed through mtDNA *cyto b* gene sequences

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ABSTRACT

In the present investigation, the genetic structure of four populations of *Catla catla*, sequences of mitochondrial gene, *cytochrome b* (*cyto b*) from four populations were sequenced and analyzed. The sequences of mitochondrial regions revealed high haplotype diversity and low nucleotide diversity. The lowest 249 polymorphic sites and 0.00 parsimony informative sites were detected in populations of Fish Federation Pond (CCFFB) whereas highest 330 polymorphic sites and 56 parsimony informative sites were detected in populations of Narmada River (CCNRH) in the *cyto b* gene sequences in *Catla catla* populations. The twelve different haplotypes were detected among the four populations studied, lowest population specific haplotype as 2.00 was observed in Fish Federation Pond (CCFFB) and highest was in Population of Narmada River and Tighra reservoir. Sequencing of *cyto b* gene revealed 12 number of haplotypes (*h*) with haplotype (gene) diversity (*Hd*) 0.8736 and nucleotide diversity (π) 0.6474. These data clearly indicated that, feral/wild population showing highest values of polymorphisms, parsimony, haplotype diversity showing good, healthy habitat is lotic water (Narmada River) and lentic water body (Tighra reservoir). The results also concluded that the partial *cyto b* is polymorphic and can be a potential marker to determine ecological habitat based genetic differentiation among the populations.

1. Introduction

The genetic variations and gene flows within population is extremely useful for gathering information on individual's identity, breeding patterns, degree of relatedness and disturbance of genetic pool among them [1]. The genetic variation of a species is distributed both within populations, expressed as differences between individuals, and between populations, expressed as differences in the presence and frequency of alleles.

The development of highly variable molecular DNA marker and statistical methodologies for interpreting genetic data has provided the opportunity to gain an intricate understanding of population characteristics such as dispersal and genetic structure, which is important for the successful management of threatened species both in the wild and in captivity [2–5]. Globally, declines of freshwater animals have been much greater than losses of animals in terrestrial systems, and freshwater fishes are among the world's most endangered vertebrates [6]. Most of the fish used for human consumption is obtained through exploitation of wild populations. India is endowed with rich fish genetic biodiversity i.e., 2,200 fish species and ranks 9th in term of freshwater mega biodiversity [7].

Catla catla is an important species in fisheries and aquaculture [8] which is naturally found in the Indo-Gangetic river system [9]. *Catla catla* is declining mainly due to overfishing and habitat alterations [10]. The *cyto b* region of mt-DNA is one of the preferred mitochondrial genes for studying intraspecific genetic diversity in fishes [11–13]. The detailed comparative studies on natural population, cultured population and population reared by federation is not available, so, the present investigation is aimed to assess the potential of mt-DNA *cyto b* gene based comparative studies for wild and cultured populations collected from four different ecotypes.

2. Materials and methods

Lotic habitat, lentic habitat, man-made Pond (Fish Federation Pond) and Culture Pond were chosen to give a good representation

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of ecological and habitat variations (Table 1, Fig. 1) with respect to finding out of genetic traits among *Catla catla* populations. In this study, tried to analyze whether this species showing ecological based genetic variability which performed by mitochondrial DNA sequence (MTCYTOB gene) variations performed by a universal primers as forward: L14841 and reverse: H15149 (Table 2).

Catla catla populations were caught through commercial catches from above mentioned four sites i.e. Narmada River (n = 04), Tighra Reservoir (n = 04), Fish Federation Pond (n = 07 and Khatik Fish Farm (n = 05). Incisions (not more than 5–6 mm deep) were made and skin flap was removed. Small white muscle pieces were cut using surgical blade or small fine scissors. The muscles were kept on the aluminium foil labelled with fish number held over ice and the aluminium foil was folded as shown below and it was kept on ice temporarily and finally stored at –80 °C in the laboratory till further use for analysis. In the present investigation, molecular research methodologies were adopted to delineate the gene flow and hereditary traits among the *catla* populations.

2.1. Extraction of genomic DNA from tissues samples

Total genomic DNA was extracted as protocol provided by [14,15] with using some modifications. One hundred mg tissue sample was taken in pre-chilled eppendorf tube (1.5 ml capacity) and grinded tissue with the help of micro pestle within the tube. During grinding, added 0.5 ml of digestion buffer (100 mM Tris–HCl with pH 8.0, 10 mM EDTA with 8.0, 1.4 M NaCl, 1% SDS and 0.2% β-Mercaptoethanol) in tubes and added remaining 0.5 ml after grinding. Incubated samples at 50 °C for 30–60 min on dry bath with occasional shaking and then centrifuged at 5000 rpm for 10 min at room temperature. Collected supernatant in a fresh eppendorf tube and added equal volume of phenol:chloroform:iso amyl-alcohol (25:24:1) to the samples. Centrifuged again at 10,000 rpm for 10 min at 4 °C and transferred top aqueous layer to new tube. Added half volume of 7.5 M ammonium acetate and 2 volumes of 100% chilled ethanol. Tues kept in deep freezer for 1–2 h and centrifuged at 10,000 rpm for 10 min at 4 °C. Added 1 ml of 70% ethanol in the tubes for washing and centrifuged for 10 min at 10,000 rpm at 4 °C. Discarded upper aqueous layer and dried the pellet for 1–2 h at room temperature. Added 50 μl Tris–EDTA buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.6) and stayed for 2 h to dissolved the pellets.

Table 2
Primers used for PCR amplification of mitochondrial *cyto b* gene in the present studies on *C. catla* populations.

Primer Name	Sequence (5′ → 3′)
L14841	AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA
H15149	AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTCA

2.2. Quantification of extracted genomic DNA and integrity checking

The yield of extracted DNA from fish tissues in ng/μl was measured using a UV Spectrophotometer (ND-1000) at 260 nm and 280 nm wavelength. The purity of DNA was determined by calculating the ratio of absorbance at 260–280 nm. The ratio of absorption at 260 nm v/s 280 nm should 1.8 is commonly used to assess the purity of DNA with respect to protein contamination, since protein (in particular, the aromatic amino acid) tends to absorb at 280 nm. The DNA sample is considered as pure when the 260 to 280 ratio comes near 1.8. But the DNA sample having ratio 1.5–2.0

Table 1
Sample collection sites along with coordinates for estimation intraspecific genetic variance among *C. catla* species.

S. No.	Sample Code	Sampling Site	Geographical Coordinates	Samples Size (n)
1.	CCNRH	Narmada River, Hoshangabad	22.75°N 77.72°	04
2.	CCTRG	Tighra Reservoir, Gwalior	26°13′17.11″N 78°00′6.52″E	04
3.	CCFFB	Fish Federation Pond, Bhopal	23.2084°N, 77.3790°E	07
4.	CCKFB	Khatik Fish Farm, Bhopal	23.2437°N, 77.4731°E	05

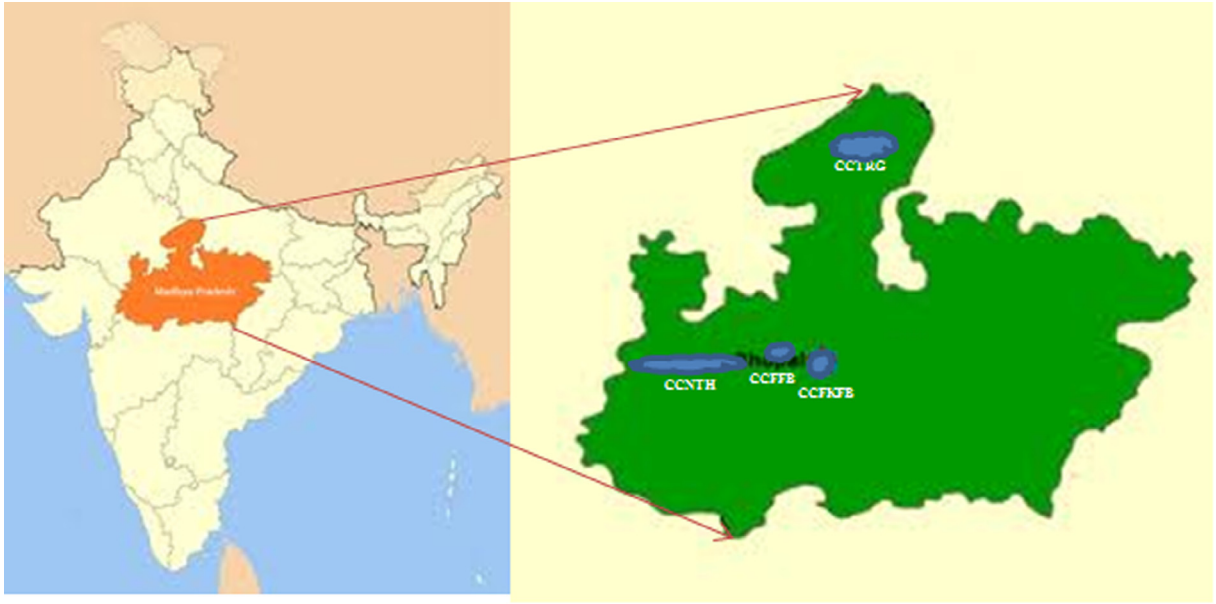

Fig. 1. Map showing samples collection sites i.e. Narmada River (CCNRH), Tighra Reservoir (CCTRG), Fish Federation Pond (CCFFB), Khatik Fish Farm (CCKFB).

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