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Comparative and evolutionary analysis of mitochondrial genes in Indian major carps

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ABSTRACT

Direct sequencing of mitochondrial DNA regions such as cytochrome *b*, ATPase 6/8 and control region was performed to study comparative and evolutionary status of the three mitochondrial genes in *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*. DNA sequence alignment among species using specific software revealed comparative rates of divergence with considerably faster and more heterogeneous substitution rate for control region as compared to cytochrome *b* and ATPase 6/8. Despite the relatively high variability of control region, the overall levels of sequence divergence were low in coding regions. Two protein coding genes and the control region with varying degree of sequence divergence established two distinct groups which are genetically distant from each other exhibiting identical phylogenetic structure in IMCs. Closest relationship was between *Labeo rohita* and *Catla catla* indicating that they might have diverged from a common ancestral stock in genealogical lineage whereas *Cirrhinus mrigala* showed greater divergence with all the three DNA regions studied. Findings of this study will help to understand evolution of mitochondrial DNA genes in carps and facilitate future investigations on phylogeographic structure of Indian carps.

1. Introduction

Indian major carps, *Labeo rohita* (*L. rohita*), *Catla catla* (*C. catla*) and *Cirrhinus mrigala* (*C. mrigala*) form the mainstay of freshwater aquaculture in the Indian subcontinent. Intensification of carp culture in recent years is attributed to their high commercial value and fast growth rate as well as the popularity. Together they contribute about 87% of inland aquaculture production in India (FAO, 2009). Genetic information on cultivable fish and shellfish species is useful for identification of stocks, stock enhancement, breeding programs, management for sustainable yields and preservation of genetic diversity (Dinesh et al., 1993; Garcia and Benzie, 1995; Tassanakajon et al., 1997). Techniques using mtDNA have been widely employed for aquaculture and fisheries related genetic studies because this marker has several useful characteristics including rapid rate of mutation making it effective for detecting recent population isolation (Ward and Grewe, 1994) and for establishing genealogical relationships among populations within species

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Table 1
Partial mtDNA genes of Indian major carps with Genbank Accession numbers.

Species	Genes	Accession No.
Labeo rohita	Cyt *b*	JN224867–JN224869
		JN253604–JN253629
	ATPase6/8	JN859621–JN859649
	CR	JN315802–JN315818
Catla catla	Cyt *b*	JN859708–JN859736
	ATPase6/8	JN859650–JN859677
	CR	JN859678–JN859707
Cirrhinus mrigala	Cyt *b*	JN859796–JN859825
	ATPase6/8	JN859737–JN859767
	CR	JN859768–JN859795

(Avise, 2000). From a population genetics perspective, mtDNA has been extensively used as a marker for evolution, genetic diversity studies and identification of species (Curole and Kocher, 1999). Mitochondrial genes have proven effective for elucidating phylogenetic and taxonomic relationships in many freshwater fish groups (Briolay et al., 1998; Na-Nakorn et al., 2006) and for investigating intraspecific variation and even for establishing species boundaries (Nguyen et al., 2008). Knowledge on evolutionary and biogeographical history of freshwater fish including carps is limited. In the present investigation, we studied comparative evolution of three mtDNA genes, control region (CR), cytochrome *b* (cyt *b*) and ATPase 6/8 in Indian major carps. Results of this study would be useful for comparative evolution of mtDNA in carps.

2. Materials and methods

2.1. Sample collection

A total of about 90 samples from three species (*C. catla*, *L. rohita* and *C. mrigala*) belonging to family Cyprinidae were collected during 2009–10 from the river Mahanadi (Latitude 20.27°N and Longitude 85.52°E). Morphological identification of species was done based on Talwar and Jhingran (1991).

2.2. DNA isolation

Fin clipping was done from each individual fish, preserved in 95% ethanol and stored at −20 °C until DNA extraction. Total DNA was isolated from fin tissue by proteinase K digestion followed by standard phenol and chloroform extraction (Sambrook et al., 1989). The DNA samples were then resuspended in 1× TE buffer. The concentration and purity of isolated DNA was estimated at wavelength 260/280 nm using a UV spectrophotometer.

2.3. Amplification and sequencing

The partial cyt *b* and CR genes were PCR amplified in a 25 μl reaction volume with 1X PCR buffer (Banglore genei), 0.25 mM of dNTP mix, 10 pmol of each primer, 0.25U of Taq polymerase and 50 ng/μl genomic DNA using a thermal cycler (ABI). The primer pairs used for PCR were L14841 (5′AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA 3′) and H15149 (5′AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA 3′) for cyt *b* gene (Kocher et al., 1989) and CR1 (5′ ACCCCTGGCTCCCAAAGC 3′) and CR2 (5′ GTTTCGGGGTTTGACAAGGATA 3′) for CR gene (Guo et al., 2003), respectively. The PCR temperature profile used was 1 cycle of initial denaturation at 94 °C for 4 min followed by 34 cycles (denaturation: 94 °C for 30 s, annealing: 50 °C

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Cyt b
[                    111 111 111 111 111 111 222 222 222 222]
[          112 247 889 001 134 455 566 677 888 001 122 244 455]
[       16 051 728 179 284 704 703 628 947 039 890 958 903 928]
#CM1502 TC CCT CTA TTC TCT CTT CTG CCC CCT AAG ATT TTG CGA TAC
#CM1509 .. ... ... ... ... ... ... ... ... G.. ... ... ... ...
#CM1514 A. ... .C. ... ... ... .C. ... ... ... ... ... ... ...
#CM1521 .. ... ... ... ... ... ... ... ... ... ..A ... ... ...
#CC1501 C. T.C TCT ..A .T. T.C TC. .T. .T. .CA ..A CAA TAG .CT
#CC1502 C. T.. TCT ..A .T. T.C TC. .T. .T. .CA ..A CAA TAG CCT
#LR2511 CT .TC TCT CCA CTC ... TCA T.T T.C ... CTA CAA TA. CCT
#LR2513 CT .TC TCT CCA CTC ... TCA T.T T.C ... CTA CAA TA. CCT
#LR2515 CT .TC TCT CCA CTC ... TCA T.T ..C ... CT. CAA TA. CCT
#LR2505 CT .TC TCT CCA CTC .C. TCA T.T T.C ... CT. CAA TA. CCT
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Fig. 1. Alignment of partial DNA sequences of cyt *b* gene. (Only variable sites are reported).