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SHORT COMMUNICATION

Genetic characterization of *Bagarius* species using cytochrome *c* oxidase I and cytochrome *b* genes

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Abstract

In this study, we first inferred the genetic variability of two *Bagarius bagarius* populations collected from Ganges and Brahmaputra rivers of India using two mtDNA markers. Sequence analysis of *COI* gene did not show significant differences between two populations whereas cytochrome *b* gene showed significant differences between two populations. Followed by, genetic relationship of *B. bagarius* and *B. yarrielli* was analyzed using *COI* and cytochrome *b* gene and the results showed a higher level genetic variation between two species. The present study provides support for the suitability of *COI* and cytochrome *b* genes for the identification of *B. bagarius* and *B. yarrielli*.

Keywords

B. bagarius and *B. yarrielli*, *COI* gene, cytochrome *b* gene

History

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Introduction

Bagarius is one of the widely distributed fish genus in South Asian countries, which includes four species namely, *B. bagarius*, *B. rutilus*, *B. suchus*, and *B. yarrelli.* Among the four species, *B. bagarius* has got wide distribution in Indian river systems (Roberts, 1983). It was described from the Ganges, known locally by several names ''Gorua'', ''Baghar'', and ''Bagari''. It is an important edible fish and fetches a high prize in the local markets due to its less spines and taste. The natural stocks of this species have declined drastically for the last two decades, which has seriously affected the livelihoods of local fishing communities (Allan et al., 2005). Subsequently, it has been categorized as a threatened species as per IUCN (Menon, 1989). Genetic variability analysis within and between populations can provide genetic relationship of the local populations, which is essential information for the effective fishery resource management. Among the molecular markers, cytochrome *c* oxidase I (COI) and cytochrome *b* genes are the widely used mitochondrial genes for studying intra and inter species genetic variability in fish (Habib et al., 2011; Luhariya et al., 2012; Mandal et al., 2012). In spite of its economic importance and conservation status, the genetic variability analysis on *Bagarius* species has not been conducted yet. In this study, the mtDNA *COI* and cytochrome *b* genes were used to examine the population structure of *B. bagarius* and genetic variability between *B. bagarius* and *B. yarrelli*.

Methods

A total of 26 *B. bagarius* samples were collected from Ganges river at Uttar Pradesh and Bihar (13 nos.) and Brahmaputra river at Assam and Arunachal Pradesh (13 nos.). Genomic DNA was isolated from fin clips using Invitrogen's ''Pure Link Genomic DNA Mini Kit'' following the manufactures instructions. The *COI* and cytochrome *b* genes were amplified with standard PCR conditions using the universal primers FishF1 and FishR1 (Ward et al., 2005) and L14724 and H15915 (Xiao et al., 2001), respectively. Sequencing was performed using Big Dye Terminator on ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). In addition to that, the genetic variability between *B. bagarius* and *B. yarrielli* was analyzed using *COI* and cytochrome *b* gene sequences. The *COI* and cytochrome *b* gene sequences of *B. yarrielli* were retrieved from GenBank (Table 1). Sequences were edited and aligned using CLUSTAL X and MEGA software (Kumar et al., 2004). Haplotype diversity, AMOVA, and pair wise differences between populations (F_{ST}) were calculated using ARLEQUIN (Schneider et al., 2002). The phylogenetic network was drawn using the software NETWORK (Schneider et al., 2002).

Results

Genetic diversity between *B. bagarius* populations

COI gene

The aligned 644 bp sequences of COI gene revealed seven unique haplotypes resulting from 10 polymorphic sites on 24 fish samples (Figure 1A). The Ganges and Brahmaputra populations showed four haplotypes each with seven and three polymorphic sites, respectively. The haplotype diversity was found to be 0.5256 ± 0.1527 and 0.4909 ± 0.1754, respectively. Among the seven haplotypes, Hap3 was found in both the populations with high frequency (17 on 24) whereas others are unique to respective populations (Hap1, Hap2, and Hap4 observed in Ganges population; Hap5, Hap6, and Hap7 observed in Brahmaputra population). The AMOVA analysis showed 99.12% variation

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within population whereas 0.88% was between populations. The pairwise F_{ST} indicated less variation (0.0088) between two populations.

Cytochrome b gene

Sequence data for the 913 bp regions of 25 fish resulted in four polymorphic sites, which defined seven unique haplotypes (Figure 1B). The Ganges population showed five haplotypes with three polymorphic sites where as the Brahmaputra population showed two haplotypes with two polymorphic sites. The haplotype diversity was found to be of 0.8030 ± 0.0903 and 0.1538 ± 0.1261, respectively. Among the seven haplotypes, Hap1–Hap5 were found in Ganges population whereas Hap6 and Hap7 were found in Brahmaputra population. At nucleotide position 657, a ''T'' was prominent in Brahmaputra population instead ''C'', which unambiguously separated both the populations. The AMOVA analysis showed 59.23% variation within population whereas 40.77% was between populations. In contrast to *COI* gene, the pairwise F_{ST} showed high differences (0.5923) between populations.

Table 1. GenBank accession numbers for the mitochondrial COI and cytochrome *b* gene sequences used in this study.

S. no	Species	GenBank accession number	Gene
1.	*B. bagarius*	KP829955–KP829978	*COI*
		JN815268	
		JN697602	
		JN697601	
		EU417763	
		EU417762	
		FJ459434	
2.	*B. bagarius*	KP829930–KP829954	Cytochrome *b*
3.	*B. yarrelli*	HM156377–HM156380	*COI*
		JQ026260	
		EU490855	
4.	*B. yarrelli*	HQ322524	Cytochrome *b*
		HQ322525	
		AF416897	
		DQ119406	
		EU490904	
		JQ026260	

Genetic diversity between *B. bagarius* and *B. yarrelli*

COI gene

The sequences were truncated to 581 bp length for which sequences were available for *B. yarrelli*. Sequences data for the 581 bp regions of 36 fish revealed eight unique haplotypes resulting from 78 polymorphic sites. *B. bagarius* showed five haplotypes with five polymorphic sites whereas the *B. yarrelli* showed three haplotypes with 49 polymorphic sites. Among the eight haplotypes, Hap3 was found to be recurrent in *B. bagarius* (25 on 30). *B. yarrelli* with moderate sample size found to be divergent with high number of polymorphic sites and high haplotype diversity (0.6010 ± 0.2152) as compared with *B. bagarius* (0.3080 ± 0.1075). The AMOVA analysis showed 93.66% variation between species whereas 6.34% was within species. The pairwise F_{ST} values showed high level variation (0.9366) between *B. bagarius* and *B. yarrelli*.

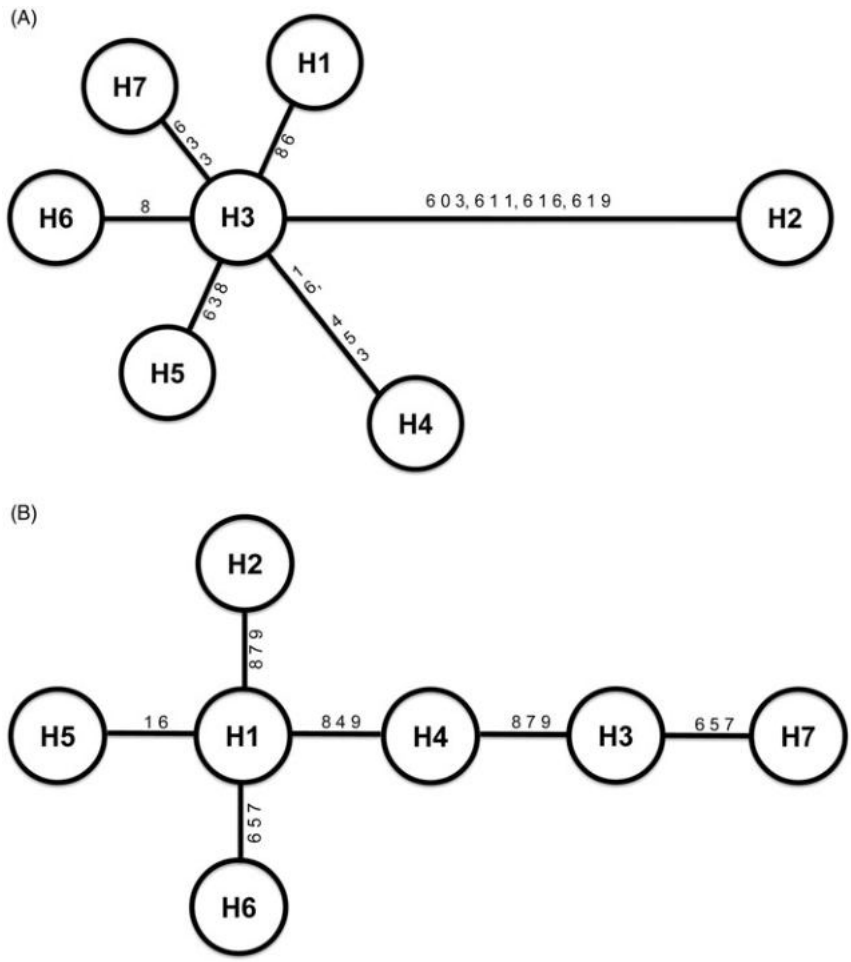

Figure 1. Haplotype network for *COI* (A) and cytochrome *b* (B) genes of *B. bagarius*.

Cytochrome b gene

The 913 bp length cytochrome *b* gene revealed 12 unique haplotypes resulting from 132 polymorphic sites on 31 fishes. The *B. bagarius* showed seven haplotypes with four polymorphic sites whereas the *B. yarrelli* showed five haplotypes with 92 polymorphic sites. Among the seven haplotypes, Hap6 was found to be more frequent in *B. bagarius* (12 on 30). *Bagarius yarrelli* with moderate sample size was found to be divergent with a high number of polymorphic sites and high haplotype diversities (0.9333 ± 0.1217) as compared with *B. bagarius* (0.7637 ± 0.0774). The AMOVA analysis showed 85.08% variation between two species whereas within species was 14.92%. The pairwise F_{ST} values showed high level variation (0.8508) between *B. bagarius* and *B. yarrelli*.

Discussion

In this study, the genetic structure of *B. bagarius* was studied using two mitochondrial DNA markers. A total of 14 different haplotypes were found in *Bagarius* populations for COI and cytochrome *b* genes. However, only a single haplotype (Hap3 of the COI) was shared between two populations, which confirmed that these two populations were evolved from a common ancestor assuming Hap3 was the founding haplotype. The rest of the haplotypes would have evolved after the fragmentation. Among the two markers, cytochrome *b* gene showed high genetic variation between populations (59%) whereas *COI* gene showed high variation within population (99%). The genetic variation obtained in this study for within and between populations was higher than that of reported previously for migratory fishes (Vrijenhoek, 1998). The pairwise F_{ST} value was significantly high (0.59) between two *B. bagarius* populations for cytochrome *b* gene. Such high inter-population diversity can be expected as these two populations were collected from two different rivers (Habib et al., 2011; Ward et al., 1994). A nucleotide ''T'' was prominent in Brahmaputra population instead ''C'' at nucleotide position 657 in Cytochrome *b* gene, which unambiguously distinguish both the populations further which can be used as population specific marker in conservation studies. It indicates that currently there is no gene flow between Ganges and Brahmaputra river populations. The absence of gene flow further suggests that genetically isolated *B. bagarius* stocks exist in the Indian region. It has been reported that populations growing in different habitats are likely to diverge genetically from one another due to adaptive radiation (Greenwood, 1991). Mandal et al. (2009) have also observed similar results in *C. chitala* populations of Ganges and Brahmaputra. Followed by, *COI* and cytochrome *b* gene sequences have been used to characterize *B. bagarius* and *B. yarrielli*, which clearly distinguished both the species and strongly support the classification based on the morphological traits. The interspecific variation was greater than intraspecific variation, which is in agreement with the results reported previously for *COI* and cytochrome *b* genes (Habib et al., 2011; Nadiatul et al., 2011).

In conclusion, the present study clearly shows that the mtDNA *COI* and cytochrome *b* are found to be potential markers for studying the variations both among the population and the species. This study has provided basic information on the genetic variability of *Bagarius* species, which can be used to develop strategies and management plan for the conservation of threatened *Bagarius* species.

Declaration of interest

The authors acknowledge the Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, New Delhi, for providing financial assistance (No. SR/FT/LS-40/2010). The authors declare that there are no competing interests. The authors alone are responsible for the content and writing of the paper.

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