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Molecular comparison of *Clarias batrachus* (Linnaeus, 1758) found in India with the species reported from Bangladesh

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Abstract

In the present study molecular comparison of *Clarias batrachus* from Meerut, India was done with the same species reported from Bangladesh using 16S rRNA. Since, they are morphologically similar, the conservedness in sequences has been studied using ExpaRNA (online software). Besides this, the study is also supported by motif prediction using MEME online software which can be considered as a promising tool for fish species identification. This molecular comparison reveals intraspecific genetic variation in 16S rRNA sequences of *C.batrachus* from two geographically different locations due to phylogeographic factors.

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Introduction

Catfish dominates the domestic aquaculture industry all over the world. Air-breathing catfish from the family Clariidae is composed of 15 genera and 93 species, distributed in freshwaters of Africa, Asia Minor and Southeast Asia (Teugels *et al.* 2001; Teugels and Adriaens 2003). They are considered as highly prized fish because of their unique suprabranchial organs (Teugels and Adriaens 2003), which enable them to respirate atmospheric oxygen (Greenwood 1961; Graham 1997). Their air-breathing capacity in various hypoxic environments places these fishes as promising species with great economic potential in aquaculture.

Traditional methods of fish species identification was based on external morphological characters. Classical identification can sometimes be difficult in case of species showing morphological intraspecific variations. The development in molecular approaches considerably helped the identification of fish species (Kon *et al., 2007).* In present study to observe intraspecific variation, molecular comparison has been made between *C.batrachus* from Meerut, India and same species reported from Bangladesh using 16S ribosomal RNA marker. Mitochondrial genes such as 16S rRNA are common fish species identification markers that have been widely utilized in fisheries control (Greig *et al.*, 2005), and species delineation (Lemer *et al.*, 2007). Similar to other mitochondrial genes, in animal mitochondrial DNA, this mitochondrial gene has numerous nucleotide substitutions thus can be used for analysis of interspecies and intraspecific sequence variations in the mitochondrial genome.

C.batrachus of India and Bangladesh are same species so they should have conserved regions (motifs) in the sequence, number of motifs, their frequency and position must be similar. During the study, an attempt has been made to compare two similar species using molecular 16S rRNA sequences. Therefore, secondary structures for each sequence have also been generated separately using ExpaRNA software to compare the conservedness. The conserved sequences are found in all species but at distinct positions. Probable reasons for this genetic variation in the sequences of *C.batrachus* from India and Bangladesh are discussed in detail.

Materials and methods

Sampling site

Live fishes for the present investigation were collected from Ganga River at the site of Hastinapur, Meerut, India and were also purchased from the local fish markets.

Fish identification

The identification of fish was made with the help of classical works of Day (1878). Immediately after capture, fish were kept in an aquarium and acclimatized.

Molecular study

For molecular biological studies blood was taken from the caudal vein by heparanised syringe without sacrificing the fish. DNA was extracted from Geneaid Blood and Tissue kit following manufacturer's instructions. Methods of amplification and sequencing of fish DNA samples were followed from Dubey *et al* (2014). For this study, 16S region of mitochondrial gene and available primers were used for this region. The PCR products were visualized on 1.2 % agarose gels and the most intense products were selected for sequencing and sequenced bidirectionally using an ABI 3730 capillary sequencer using same primers which were used for amplification reaction following manufacturer's instructions.

Molecular analysis

Sequence products were subjected to BLAST (Basic Local Alignment Search Tool) for homology search. DNA sequences were aligned using Clustal W (Thompson *et al.*, 2003). The sequence of query species was compared with retrieved sequences (sequence of *C.batrachus*, Bangladesh: KF997532) to infer genetical distinction between them. The motifs and their regular expressions were predicted with the

help of online available software MEME (Timothy *et al.*, 1994). Conservedness in loops of RNA secondary structures was predicted with help of ExpaRNA (Smith *et al.*, 2010). Sequence alignement showing concensus region was done by LocARNA (Sebastian *et al.*, 2012).

Result and discussion

Motif predictions

The motif prediction shows conserved regions (motifs) in each sequences of species, *C.batrachus* and for this, nucleotide sequences of 16S rRNA gene of *C.batrachus* (from India, Bangladesh) have been used for the motif identification with the help of online available software, MEME. The results of MEME showed three different kinds of motifs in 16S rRNA gene sequences of *C.batrachus* (**Fig.1**). Minimum motif width is of 39 bases and maximum motif width of 50 bases. Base length of motif one is 41 bases and motif two of 39 bases. However, motif three includes 41 bases. Motif one is denoted by red color, two by green and three as blue color (**Fig.2**). In sequence of India and Bangladesh Motif one was repeated 4 times, motif two 3 times and motif three 2 times. The position of motifs in Indian sequence is clearly distinct from Bangladesh. The order of motif was found same in sequences of India and Bangladesh but different in position (Table. 1).The shifting pattern is clearly seen in combined block diagram of motifs (Fig.2). This change may be because of changes in climatic conditions.

Table 1. Position of different motifs showing shifting and disposition.

Accession no.	Position of Motif 1 (base pairs)	Position of Motif 2 (base pairs)	Position of Motif 3 (base pairs)
KM494492	79-119	26-64	122-171
(India)	181-221	273-311	223-272
	355-395	491-529	
	444-484		
KF997532	92-132	39-77	135-184
(Bangladesh)	194-234	286-324	236-285
	368-408	504-542	
	457-497		

Secondary structure predictions

Percentage of Guanosine (G) and Cytosine (C) was calculated using GC calculator (http://www.genomicsplace.com/gc_calc.html). It was found 47.2% in species, *C.batrachus* (accession no. KM494492) from India, 46.4% in same species (KF997532) from Bangladesh. Thus, a structured RNA with higher GC content is likely to have more stable secondary structure.

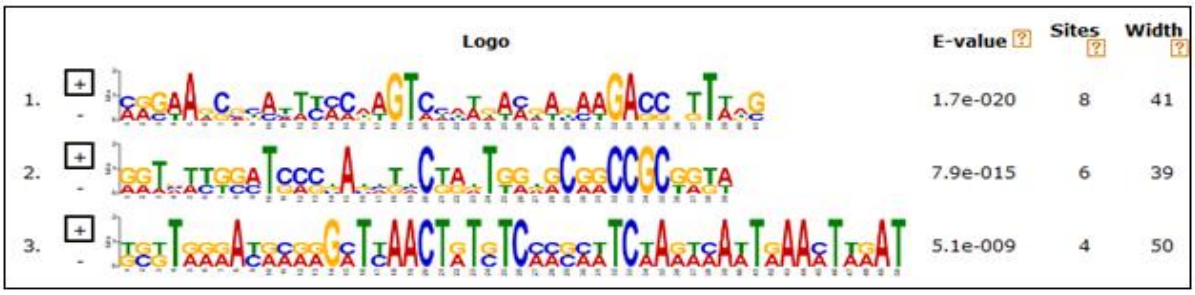

Fig. 1. Motif one, two and three.

Study of the RNA secondary structure further confirmed the conserved regions throughout sequence along various types of loops (hairpin, interior, multi, bulge and exterior loop) constructed by non-matched bases of sequences. Secondary structures (Fig. 3) for *C.batrachus* reported from India and Bangladesh were generated using ExpaRNA software that shows topological differences. Besides

the topology pattern, these structures infer the consensus sequences using seven color code patterns (violet, blue, light green, yellow, red, pink and sky blue). These color coded bases are exactly similar in a particular loop but at different position in sequences. In both RNA secondary structures generated, Color coded conserved regions in secondary structure further confirms that all have similar conserved regions in their genetic material but at different positions. It is noted that same genetic material of both the same species have some variations, incorporated in its position, to adapt to different environmental conditions.

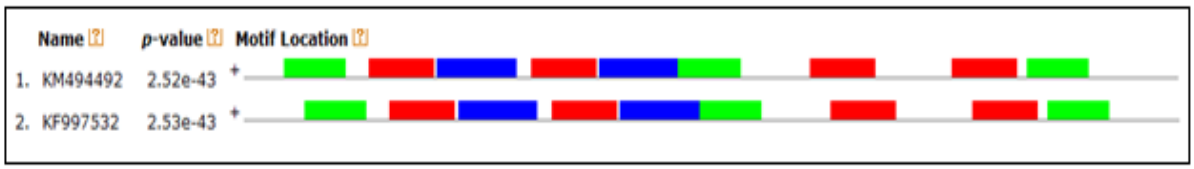

Fig. 2. Combined block diagram showing motifs in 16S rRNA sequences of *C.batrachus* of India and Bangladesh.

Sequences of 16S RNA gene of Indian and Bangladesh sequence of *C.batrachus* have been aligned using LocARNA, to predict the consensus region, showing color coded plots of alignment (primary results). Consensus sequences are deeply colored. The conservation profile for aligned sequences is given at the base line of each row of alignment in grey color (Fig. 4).

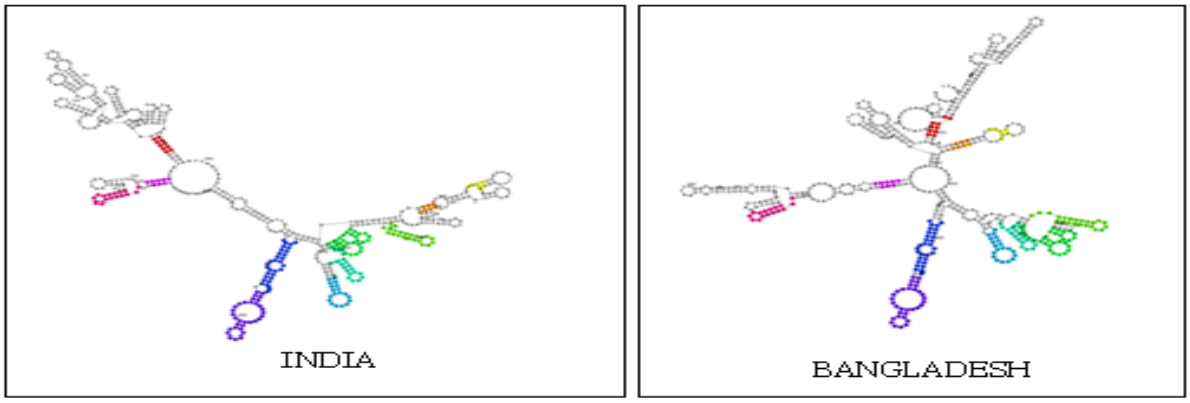

Fig. 3. Secondary structures of Indian and Bangladesh *C.batrachus,* showing conserved regions in different. Loops.

This molecular analysis reveals the genetic similarity between same species of *C. batrachus* from different regions with minor variations. Reasons for genetic variations are due to their geographical distribution or adaptation in different regions. At molecular level, this genetic comparison between Indian and Bangladesh species may provide further clues for understanding the evolution of the species. RNA secondary structure analysis along with motif prediction could be a valuable tool for species discrimination.

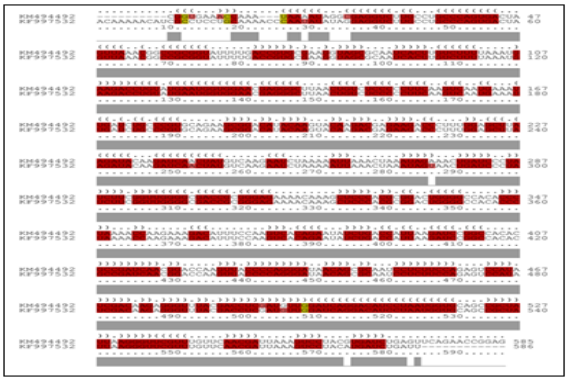

Fig. 4. Sequence alignement showing consensus regions.

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