PHYLOGENETIC RELATIONSHIP BETWEEN FOUR SPECIES USING DIVERGENT DOMAIN D9 AND D11 IN FAMILY: SILURIDAE (PISCES)

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Abstract

Molecular characterization and assessment of genetic variation in 28S region especially divergent domain D9 and D11 ribosomal DNA in fresh water catfish O*mpok pabda, O. pabo, O. bimaculatus* and *Wallago attu* was carried out. The nucleotide sequences and GC% of divergent domains of 28S ranged from 419-427 bp and 61% in D9 region and 686-688 bp and 55% in D11 region. In D9 region, *O. pabda* and *O. bimaculatus* were 100% similar, whereas other species were found 99% similar. In D11 region, *O. pabda* with *O. pabo* and *O. bimaculatus* were 100% similar and other species were 99% similar. After sequence analysis, *Ompok* species exhibited more variability from *W. attu*. DNA sequence data play an essential role in the reconstruction of evolutionary relationships among the organisms, resulting in insights in genetic affinities that may confirm or conflict with traditional taxonomy because of attractive properties, the rDNA is popular for examine phylogenetic relationships and for studying genetic variability and divergence within and between species.

Key words- *Ompok* species, rDNA, Base pair (bp), GC%.

INTRODUCTION

Studies of ribosomal RNA (rRNA) genes have gained prominence in a broad range of animals and plants, especially for identification of evolutionary relationships and the characterization of genome structure. The moderately repetitive nuclear ribosomal DNA (rDNA) is one of the most extensively sequenced markers, which consists of 18S, ITS 1, 5.8S, ITS 2 and domains of 28S. The nuclear rDNA copies within a genome can be highly homogenous because of concerted evolution of intra and inter chromosomal loci. DNA sequence of the ITS1 and ITS2 of the rRNA transcription unit have proven useful in resolving phylogenetic relationships for closely related taxa due to their relatively rapid evolution rate[1].

In higher eukaryotes, rRNA genes are organized as two distinct multigene families comprised of tandem arrayed repeats composed of hundreds to thousands copies. One class is represented by the 45S rDNA which consists of a transcriptional unit that code for 18S, 5.8S and 28S rRNAs, separated by ITSs and surrounded by intergenic spacers (IGS). The other class codes for 5S rRNA gene that consists of a highly conserved coding sequence of 120 base pairs (bp), which is separated from each transcriptional unit by a NTS. Each repeat of 28S rRNA gene is organized into several highly conserved cores interrupted by divergent domains, also called 'D domains. The divergent domains evolve rapidly with substitution rates that are at least two orders of magnitude higher than those of core regions. These domains show a high rate of insertion and deletion events. Variations in the more rapidly evolving divergent domains make them suitable for phylogenetic comparisons among closely related species. Therefore, in the present study, we report divergent domain D9 and D11 of 28S rDNA sequences and based on these sequences phylogenetic relationships among four species were investigated.

Due to attractive properties of fast evolving and repetitive nature, the rDNA is widely utilized for examining phylogenetic relationships and for studying genetic variability and divergence among the species.

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Table 1: Primer sequences of divergent domain D9 and D11

Serial no.	Code	Primer sequences
9	D9 F	5'-CGGCGGGAGTAACTATGACTCTCTTAAGGT-3'
10	D9 R	5'- CCGCCCCAGCCAAACTCCCCA-3'
11	D11 F	5'- TGAAATACCACTACTCTTATCGTT-3'
12	D11 R	5'- GGATTCTGACTTAGAGGCGTTCAG-3'

Fishes of genus *Ompok*, belonging to order Siluriformes and family Siluridae, have been reported in various water bodies of India. Freshwater catfishes *O. bimaculatus* (Indian butter catfish), *O. pabda* (Pabdah catfish), *O. pabo* (Pahboh) are among the highly priced and preferable fishes due to their high quality flesh and taste. These fishes have a wide geographical distribution covering Indus plain and adjoining hill area the of UP, Bihar, West Bengal, Kerala, Karnataka, North Eastern States of India, Bangladesh, Myanmar, Afghanistan, and Pakistan. *O. pabda* and *O. pabo* naturally occurs in muddy rivers, streams, ponds and lakes. The species supported a strong fishery in North Bihar and West Bengal during the early 1970s, but in the early 1980s sharp falls in catches were observed, indicating swift declines in those areas. Consequently, *O. pabda* and *O. pabo* has been listed as threatened fish species in India due to its decrease in abundance and restricted distribution. The *O. bimaculatus* generally inhabit in the similar habitat like *O. pabda.* This particular catfish has declared of threatened status in the Western Ghats in India and is documented as being found in both freshwater and brackish environments. *W. attu* is found in large rivers and lakes and can reach 2.4 m (8 feet) length. This fish is found from Bangladesh, Pakistan to Vietnam and Indonesia, and is also reported from Afghanistan. *Wallago* has been listed in low risk near threatened species.

The purpose of this study was to amplify, sequence and align the region of 28S (D9 and D11) regions in species of *O. pabda*, *O. bimaculatus*, *O. pabo* and *W. attu* for characterization of these sequences and phylogenetic relationship among these species. The results will provide useful information in species identification and genetic diversity assessment.

MATERIALS AND METHODS

Specimen's collection

The specimens of *O. bimaculatus*, *O. pabda*, *and O. pabo* were collected from river Ganga (West Bengal) near Farakka and *W. attu* was collected from river Gomti, Lucknow (Uttar Pradesh), India with the help of local fishermen. The specimens of all the species were in juvenile stage and the sex was unidentifiable by visual examination. For DNA isolation, blood samples were collected from fish specimens.

Isolation of genomic DNA and PCR amplification

The genomic DNA was extracted from the whole blood using the standard phenol-chloroform–isoamylalcohol [2]. The amplification of divergent

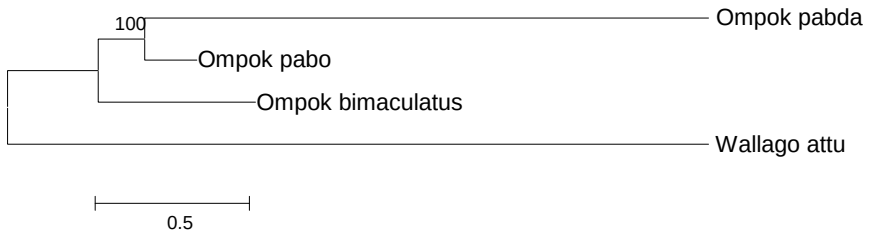

Fig.1. Phylogenetic tree between four species

domains of 28S especially D9 and D11 region in subject species were carried out in a BIO-RAD thermal cycler. The primers for 28S were taken from Zardoya [3]. A standard PCR reaction was performed using: 10 pmoles of each forward (F) and reverse (R) primers (Table 1), 10 mM dNTP mix, 1U Taq DNA Polymerase (Chromous), 10X *Taq* buffer and 50-90 ng of genomic DNA in a final reaction volume of 50 µl. The PCR cycling conditions were: initial denaturation at 94 ^{o}C for 4 min; followed by 35 cycles of denaturation at 94^{o}C for 30 sec; primer annealing at 55 ^{o}C for 35 sec; primer extension at 72 ^{o}C for 45 sec; with post cycling extension at 72 ^{o}C for 10 min. Amplified products were run on 1.5% agarose gel stained with ethidium bromide. The PCR products were custom sequenced and the sequences were submitted to NCBI database.

Sequence analysis and phylogenetic tree construction

The sequences were analyzed using ClustalW multiple sequence alignment program. The phylogeny was inferred using the Neighbor-Joining method[4] in MEGA4 program [5].The evolutionary distances were computed using the Maximum Composite Likelihood method [6]and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

RESULT

The nucleotide sequences and GC% of divergent domains of 28S ranged from 419-427 bp and 61% in D9 region (NCBI Accession No: GU385701- *O. pabda,* GU385703- *O. pabo*, GU385730- *O. bimaculatus*, GU385702- *W. attu*) and 686-688 bp and 55% in D11 region (Accession no: GU385707, GU385705, GU385706, GU385704). In D9 region, *O. pabda* and *O. bimaculatus* were 100% similar, whereas other species were found 99% similar. In D11 region, *O. pabda* with *O. pabo* and *O. bimaculatus* were 100% similar and other species were 99% similar.

Phylogenetic analysis

The optimal tree with the sum of branch length was 0.52205557 in 28S rDNAs. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein 1985). On the basis of phylogenetic tree, the *Ompok* species were closely related to each other in comparison to *W. attu.* It was found that the genetic distance between *O. pabda* and *W. attu* was highest, followed by *O. bimaculatus* and *W. attu* in the pair wise comparison. Since the genetic identity between *O. pabda* and *O. pabo* was highest, it can be said that these two species were most closely related as compared to the other species combinations (Fig. 1).

DISCUSSION

This is the first report on nucleotide composition of 28S rDNAs (D9 and D11) and phylogenetic relationship among the undertaken silurid species. The G+C contents were higher than A+T contents in all the species. The sequence divergence has shown inter-specific variations, which were comparatively high because of the presence of transition, transversion and indels (insertions/ deletions). The 28S rRNA genes in fishes are slightly shorter than mammals[7] while working with mouse, suggested that major variations in 28S rRNA gene size during the evolution have been restricted to a unique set of a few sites within a largely conserved secondary core structure. The divergent domains, responsible for the large increase in size of the molecule, from prokaryotes to higher eukaryotes, represent half the mouse 28S rRNA length. In the present study, major differences occurred between the sequences lengths of D9 and D11. The sequence length of D9 region is shorter than D11 region. Phylogenetic hypotheses of the evolutionary relationships among species provide frameworks for comparative research on mechanisms of diversification and speciation. These phylogenies are also valuable resources for people concerned with conservation in that they provide a relatively objective means of quantifying evolutionary distinctiveness and resolving taxonomic ambiguities involving rare taxa. Routine application of genetic methods in fish conservation and management is still relatively scarce and awaits the availability of simpler, accurate and easily implemented methods. Divergent domains are widely and routinely used in analysis of species relationships by using a phylogenetic structure method in various organisms [8-10]. It was successfully applied in analysis of phylogenetic relationship among the siluridae species and the conclusions from phylogenetic tree were well in agreement with those from analysis based on morphological systematics and other PCR based molecular techniques. Our study demonstrated that D9 and D11 domain provide strong phylogenetic relationship between *Ompok* species belong to the same genus.

The present study reaffirms that the ribosomal genes possesses the general trend of variability between the species as well as the conserved ness in same family. The species displaying the conserved ness

belong to the same geographical locations with diverse ecological conditions [11].

ACKNOWLEDGEMENT

The authors are thankful to the Director, National Bureau Fish Genetic Resources, Lucknow for providing facilities to carry out this piece of research work. They are also thankful to Head, Department of Zoology, University of Lucknow for granting permission to the first author to work at NBFGR.

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