Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (October–December 2012) 82(4):497–501
DOI 10.1007/s40011-012-0061-x

RESEARCH ARTICLE

Assessment of ATPase 8 and ATPase 6 mtDNA Sequences in Genetic Diversity Studies of *Channa marulius* (Channidae: Perciformes)

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Received: 22 February 2012 / Revised: 26 June 2012 / Accepted: 18 July 2012 / Published online: 22 August 2012

Abstract *Channa marulius* is a commercially important fish of South East Asia. The present study evaluates the potential of complete ATPase 6/8 region of mitochondrial DNA as a marker region to determine the phylogeography of *C. marulius* from Indian rivers. Analysis of 842 bp of ATPase 6/8 region from 3 unlinked river basins; Mahanadi, Teesta and Yamuna generated 7 haplotypes with 8 variable sites and a haplotype diversity of 0.876. Out of the total variation, 22.05 % was due to variation within the population and 77.95 % due to variation among populations. Population structuring was revealed by high and significant Fst value of 0.77. The results revealed that 842 bp of ATPase 6/8 region could be a promising marker for determining variations at interpopulation as well as intrapopulation levels in wild *C. marulius*. These results would facilitate conservation and management of this important species.

Keywords ATPase 8 · ATPase 6 · *Channa marulius* · Genetic diversity

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Introduction

Channa marulius is a commercially important, freshwater fish native of India, Bangladesh, China, Thailand, Cambodia, Sri Lanka and Pakistan. In India, it is reported from the rivers of Indo-Gangetic plains and also in many parts of peninsular India. *C. marulius* is an esteemed table delicacy and is considered a potential aquaculture species too. Due to its great reduction in wild, *C. marulius* is categorised as lower risk- near threatened (LRnt), [1]. Therefore, development of stock structure data is a critical input for conservation and management of this important fishery resource.

Molecular genetic markers have become valuable tools in population genetics, conservation biology and evolutionary studies [2]. These markers have been used to estimate effective population size [3], historical bottlenecks [4], and sex specific gene flow [5]. However, selection of appropriate markers that is useful to determine genetic variation is prerequisite for any population genetic study [6]. Mitochondrial DNA is widely used to determine the variation at interspecific and intraspecific levels [7, 8]. Since mtDNA substitution rates are homogenous across lineages, the date of divergence can also be estimated based on genetic distance data [9]. ATPase8 and ATPase6 genes of mtDNA are generally variable in vertebrates [10]. These genes have been consistently found to have high evolutionary rate (1.3 % per million years) in fishes [11]. ATPase 8 and ATPase 6 regions have been successfully analysed for both phylogeny as well as phylogeography in several fish species, [12–14]. An analysis of the variation of ATPase 8 and ATPase 6 DNA in different Cyprinids has proved that these genes are useful genetic markers to monitor the variations in progeny of crosses [15]. A recent study by Yan et al. [16] also suggests that ATPase

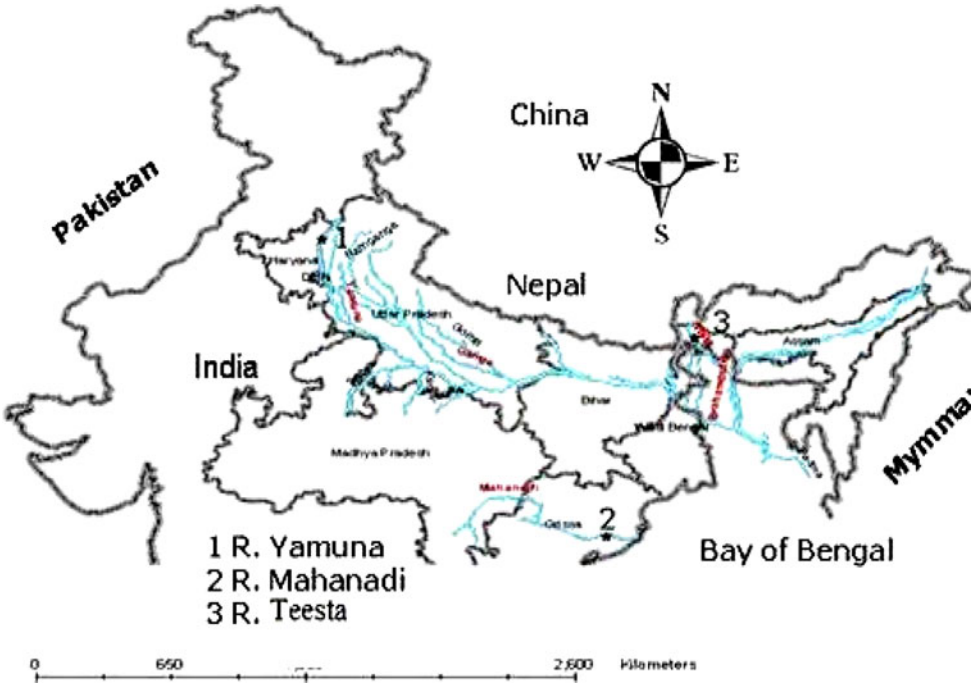

Fig. 1 Locations of sampling station (*Asterisk*) across different rover basins for population structure study of *C. marulius*

6/8 genes are valuable genetic markers to track genealogies and variations in progenies of the hybrids.

The research on application of mtDNA markers on Murrels (Family: Channidae), has been primarily limited to determine phylogeny using Cytochrome b region [17], NADH dehydrogenase subunit 1 and 2 of mitochondrial DNA [18]. The present study analyses complete sequences of mitochondrial ATPase 8 and ATPase 6 regions in *C. marulius* collected from 3 distant major rivers namely, Mahanadi, Teesta and Yamuna, to determine genetic variation and evaluate their potential in determining genetic differentiation in natural populations.

Material and Methods

Sample Collection

Five specimens of *C. marulius* were collected through commercial catches from Teesta, Mahanadi and Yamuna rivers, belonging to different basins (Fig. 1). The blood was extracted through caudal puncture and fixed in 95 % ethanol in the ratio 1:5. Teesta river is a tributary of Brahmaputra river which is a part of Ganga river system. Samples were collected from Teesta Barrage (26°45′16.83″N; 88°36′02.83″E) in north West Bengal. River Yamuna is a tributary of Ganga river system and the samples for this river were collected from Yamuna Nagar (29°58′47.01″N; 76°54′47.40″E). River Mahanadi is a separate river originating from central plateau in India and drains into Bay of Bengal. The samples from this river were collected from Cuttack (21°58′N; 86°07′E) in Orissa.

DNA Preparation

Total Genomic DNA from 5 fishes collected from each site was extracted from blood using Phenol–Chloroform Method of Ruzzante et al. [19]. DNA was amplified using Universal Primers for mtDNA ATPase8 and ATPase6 region; ATP8.2 L8331and CO111.2H9236 [20]. PCR conditions were: 94 °C, 5 min,: 30 cycles of 94 °C for 30 s; 55 °C for 1 min; 72 °C for 1 min 30 s and final extension for 10 min at 72 °C.

DNA Sequencing

Double stranded PCR product was purified using elution method from Low melting Agarose gel. The purified PCR products were sequenced.

Analysis of DNA Sequences

DNA sequences were aligned using ClustalW and analysed for determining parameters of population genetic variation. MEGA 4.1 [21] was used to estimate parameters of genetic variation. Sequence composition and molecular diversity indices, genetic differentiation and Fst values were calculated using Arlequin 3.11 [22] and haplotype diversity was estimated using DnaSP 4.5 [23].

Table 1 Different haplotypes and their consensus sequences detected in 3 populations of *Channa marulius*

Position	212 A	302 A	316 G	392 T	500 T	515 G	800 A	804 G	Haplotype occurrence in river (n)	Gen Bank ID
Haplotype1	.	.	.	.	.	.	.	.	Mahanadi; n = 3	GQ-415682
Haplotype2	.	.	A	.	.	.	.	.	Mahanadi; n = 2	GQ-415684
Haplotype3	.	.	.	C	.	.	.	.	Teesta; n = 4	GQ-894728
Haplotype4	.	.	.	.	C	A	.	.	Teesta; n = 1	GQ-894729
Haplotype5	.	.	.	.	.	A	.	.	Yamuna; n = 2	GQ-894732
Haplotype6	C	T	.	C	.	.	.	.	Yamuna; n = 2	GQ-894733
Haplotype7	C	T	.	C	.	.	G	C	Yamuna; n = 1	GQ-894736

The dots refer to the identical positions to reference consensus sequence

Table 2 Pair wise Fst values between 3 different populations of *Channa marulius* from Indian sub continent

	Mahanadi	Teesta	Yamuna
Mahanadi	0		
Teesta	0.5	0	
Yamuna	0.83	0.8	0

Results

Out of a total of 842 bp of mitochondrial gene amplified, 168 bp fragment was of ATPase 8 and 684 bp of ATPase 6 and the two fragments were analyzed together to determine genetic variation. The stop codon for the ATPase 8 was from 166 to 168 bp position and start codon for ATPase 6 was from 159 to 161 bp. An overlapping region of 10 bp was also observed from position 159 to168 bp. The average frequencies of four nucleotides for all the 15 samples of *C. marulius* were A: 26.23 %; T: 26.21 %; G: 11.71 %; C: 35.74 %. Nucleotide sequences of ATPase 8 and 6 in *C. marulius* were A + T rich (52.44 %) and transition to transversion ratio was 1.373. Co-efficient of differentiation for all the 3 population samples was 0.660. Mean number of polymorphic loci was estimated to be: 2.00 ± 1.00 and mean nucleotide diversity (pi) was 0.843 ± 0.340. ATPase 8 and 6 sequence data generated total 7 haplotypes; 2 for Mahanadi, 3 for Yamuna and 2 for Teesta samples. Haplotype frequencies, occurrence in rivers and GenBank accession numbers are given in Table 1. Total 8 variable sites were identified and 6 of them were parsimony informative. Haplotype Diversity (Hd) was found to be 0.876 and variance of Hd was 0.0026 ± 0.052. Analysis of Molecular Variance (AMOVA) revealed that out of total variation, only 22.05 % was contributed due to variation within the population; however 77.95 % was attributed to differentiation among populations and population structuring revealed by high and significant F*st* value of 0.77. Pair wise F*st* values ranged from 0.062 to 0.127 and were significant for all the 3 population pairs ($p < 0.05$ Table 2). Population specific Fst values were approximately equal to 0.768 for all the 3 populations. Overall Mean *p* distance for all the 3 populations was 0.003 and was found to be significant ($p < 0.05$).

Discussion

The present study reveals that highly significant genetic differentiation exists in *C. marulius* inhabiting three river basins namely Mahanadi, Yamuna and Teesta, based on the analysis of 842 bp mtDNA ATPase 8 and ATPase 6 sequences. ATPase 8 and 6 regions of mtDNA genome are successfully amplified in *C. marulius* using the primer pair described by Sivasundar et al. [20]. The primer pair has been reported to be useful for detecting intraspecific variation in several species across orders like Atheriniformes [24], Characiformes [20], Clupeiformes [25], Synbranchiformes [26], Petromyzontiformes [27], Tetrodontiformes [28], Orectolobiformes [29], Salmoniformes [25, 30], Siluriformes [31], Cypriniformes [16, 32–34] and Perciformes [12–14].The order of most represented bases is C > A > T > G and the level of G observed in ATPase gene was in accordance with the consistent level in case of Cytochrome b gene in *C. marulius* and also other Snakeheads [17]. Nucleotide sequences of ATPase region in *C. marulius* were A + T rich (52.44 %), which are similar to many other fish species [35].

In spite of all the 3 populations exhibiting more than 1 haplotype, no shared haplotype was observed in the present study. AMOVA revealed low within population variation in *C. marulius* (22.05 %) but it was high variation among the population (77.95 %). These values are in agreement with Vrijenhoek [36] who reported 67.6 % variation among population and 32.4 %, within population in a non migratory fish. Hence the genetic divergence level between populations, observed in this study is higher than that reported for a non migratory fish. This observation can be

attributed to the fact that murrels are known as local migrants and their sole movement is for the purpose of feeding or for locating suitable breeding grounds or in search of new water to avoid stress conditions of existing ecosystem [37]. F*st* value also support the presence of significant genetic divergence between populations of Mahanadi, Teesta and Yamuna rivers, even with the limited sample size.

Conclusion

Thus, it is concluded that sequences of ATPase 8 and ATPase 6 regions of mtDNA are potential markers for studying variation both at interpopulation level as well as intrapopulation level. Moreover because of its high rate of evolution in ATPase gene, it is able to distinguish between haplotypes even in the small sample size, which can be of significant advantage to the species found in low abundance and belonging to threatened category. In the present study, ATPase 8 and 6 genes not only expressed intraspecific diversity but are also able to represent haplotypes in each population as well as variation in the progeny. The present findings clearly demonstrate the utility of this otherwise less studied region of mtDNA for population genetics, conservation and management of wild *C. marulius* fishery.

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