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Mitochondrial DNA variation in natural populations of endangered Indian Feather-Back Fish, *Chitala chitala*

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Abstract Genetic variation at mitochondrial cytochrome *b* (cyt *b*) and D-loop region reveals the evidence of population sub-structuring in Indian populations of highly endangered primitive feather-back fish *Chitala chitala*. Samples collected through commercial catches from eight riverine populations from different geographical locations of India were analyzed for cyt *b* region (307 bp) and D-loop region (636–716 bp). The sequences of the both the mitochondrial regions revealed high haplotype diversity and low nucleotide diversity. The patterns of genetic diversity, haplotypes networks clearly indicated two distinct mitochondrial lineages and mismatch distribution strongly suggest a historical influence on the genetic structure of *C. chitala* populations. The baseline information on genetic variation and the evidence of population sub-structuring generated from this study would be useful for planning effective strategies for conservation and rehabilitation of this highly endangered species.

Keywords *Chitala chitala* · Genetic variation · Mitochondrial DNA · Cytochrome *b* · D-loop region

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Introduction

Estimates of genetic variation within and between populations can provide useful information on the level of interaction between local populations and permit assessment of the contribution of a meta-population structure to regional persistence of a species (reviewed in [1]). From conservation perspective, two types of independent units in a population of a species are suggested [2]. These are management units (MUs), representing populations that are demographically independent, and evolutionary significant units (ESUs), which represent historically isolated sets of populations that are on independent evolutionary trajectories, Molecular markers are an important tool for identifying population units that merit separate management and high priority for conservation.

Chitala chitala (Hamilton Buchanan), commonly known as Indian feather-back, (order Osteoglossiformes, family Notopteridae) is widely distributed in freshwater bodies of Indian subcontinent including India, except peninsular India [3], Bangladesh, Myanmar, Nepal and Pakistan [4]. Due to over exploitation both as ornamental and food fish, wild population of *C. chitala* has declined in its abundance and is presently listed under endangered (EN) category [5, 6]. The species is of considerable significance, not only for conservation but as a potential candidate species for aquaculture. This species fetches high market value, both as food fish [7] as well as for ornamental trade [8]. The research on artificial propagation of the species for in situ conservation is being pursued actively [6] and data on genetic variations can provide crucial input to plan the effective strategies for conservation and rehabilitation of natural populations of this species. In *C. chitala*, genetic variation in natural population was described using allozymes and nuclear markers, RAPD and microsatellites [9]. There is as yet no published work on

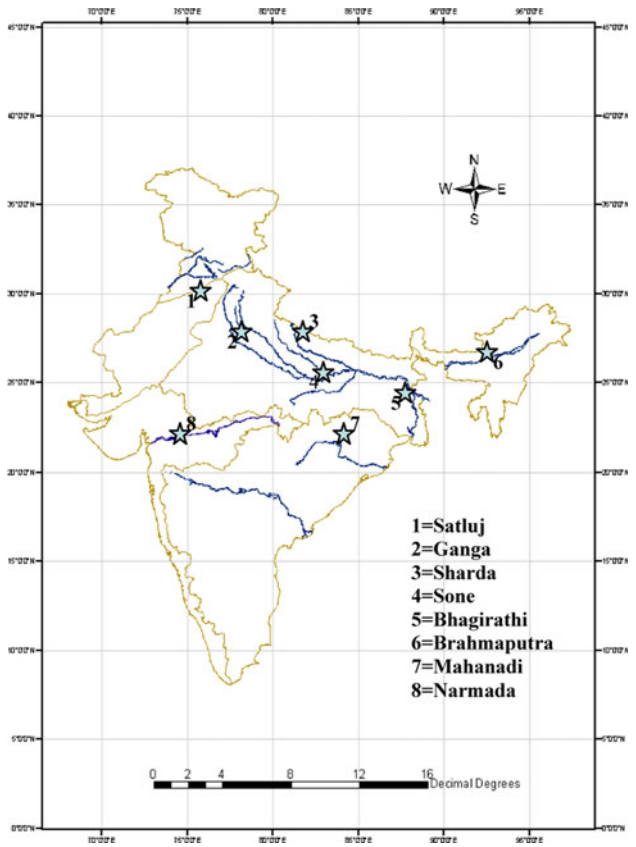

Fig. 1 Collection sites (*star*) of natural populations of *Chitala chitala* during present study

population structure analysis of *C. chitala* using any region on mitochondrial genome, however, data on various mitochondrial genes for evaluation of phylogenetic implications and molecular identification for few other fish species are available [10–12].

The aim of the present study was to use variation in mtDNA regions in *C. chitala*, to identify genetic units for conservation and also to investigate the effect of population reduction and fragmentation on the distribution of genetic variation.

Materials and methods

Specimens

Chitala chitala specimens were obtained through commercial riverine catches from eight rivers located in different geographical areas in India i.e. Satluj, Brahmaputra, Sharda, Ganga, Bhagirathi, Sone, Narmada and Mahanadi (Fig. 1, Table 1). The riverine locations were chosen to cover geographically distant populations of *C. chitala*. River Satluj is a part of Indus river system, whereas Brahmaputra and Ghagra belongs to Ganga basin [13]. River Narmada, Sone and Mahanadi originated from Amarkantak mountain ranges in Central India. Blood from individual fish was collected through puncture of caudal vein at fishing site and fixed in 95% ethanol in 1:5 ratio and stored at 4°C till use. The samples ($n = 46$) were obtained during May 2000 to May 2004.

DNA extraction and sequencing

Genomic DNA was extracted from ethanol fixed blood samples, using a modified phenol–chloroform method [14], re-suspend in TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) and concentration was determined through serial dilutions on 0.7% agarose gel in 0.5× TAE (1× = 40 mM Tris Acetate, 1 mM EDTA). Individuals were amplified for cytochrome *b* (cyt *b*) (L14841 and H15149; [15]) and D-loop (L15998-PRO and H strand Primer CSBDH; [16]) region of mitochondrial DNA. Amplification was carried out in a 25 µl reaction mixture, which included 1× PCR buffer (10 mM Tris–HCl, pH 9.0), 50 mM KCl, 0.01% gelatin, 0.2 mM of each dNTPs, 1.5 mM of $MgCl_2$, 20 pmol of each primer), 1.5 U Taq DNA polymerase, and 100 ng of template DNA. Polymerase chain reaction was performed using MJ Research thermal cycler (PTC-200) with initial denaturation at 94°C for 5 min followed by 25 cycles of 94°C for

Table 1 Collection of samples of *Chitala chitala* from natural population used in the study

River	*N*	Location/position	Year of sampling
Satluj	8	Hari Ke Pattan, Punjab (31°09′N; 74°56′E)	May, 2000
Ganga	4	Bijnour, U. P. (29°19′N; 78°04′E)	May, 2002
Sharda	9	Sharda Barrage, U. P. (28°21′N; 88°11′E)	June, 2001
Sone	4	Rewa, M. P. (24°31′N; 81°17′E)	November, 2002
Bhagirathi	5	Farrakka, West Bengal (24°05′N; 88°06′E)	July, 2003
Brahmaputra	7	Kalang, Assam (26°16′N; 91°46′E)	January, 2001
Mahanadi	5	Sambalpur, Orissa (20°27′N; 85°52′E)	May, 2004
Narmada	5	Bhopal, M. P. (23°14′N; 77°23′E)	April, 2003
Total set	47		

N number of samples studied

30 s and at 55°C annealing temperature for 30 s, elongation at 72°C for 1 min, with a final elongation at 72°C for 4 min and finally soak at 4°C. The PCR amplified products were checked through 2% Agarose gel electrophoresis and were purified using QIAquick PCR purification kit (QIAGEN, Crawley, West Sussex, UK) and sequenced bidirectionally on MEGABACE 500 at NBFGR, Lucknow following manufacturers' recommendations.

Sequence alignment

Both mitochondrial regions amplified were sequenced in both directions to check the validity of the sequence data. All sequences were aligned using clustal W [17]. Alignments were checked manually and repeated using different values for parameters. For cyt *b* all parameters were as default with pair-wise parameters set at gap opening penalty 10, gap extension penalty 0.1 and multiple alignment parameters set at gap opening penalty of 10, gap extension 0.2. D-loop alignment parameters were as default except for pairwise and multiple alignment parameters that were both set at gap opening penalty 10 and gap extension penalty 5.

Population genetic analysis

Five different geographical groups were defined a priori river systems-Indus (River Satluj), Ganges (Rivers Ganga, Sharda, Sone and Bhagirathi), Brahmaputra (River Brahmaputra), Narmada (River Brahmaputra) and Mahanadi (River Mahanadi) river systems. Intra-population diversity was analyzed by estimating gene diversity (h), considering the probability that two randomly chosen haplotypes are different [18], and nucleotide diversity (π), based on the probability that two randomly chosen homologous nucleotides are different [18, 19].

The overall genetic differentiation between each sample population was tested using pair-wise F-statistics [20]. This approach does not allow the specific testing of hypotheses that relate geographical proximity of populations to genetic population structure. To achieve this, genetic differentiation was tested in the framework of a predefined geographical structure of the samples using analysis of molecular variance (AMOVA) based on pair-wise squared- Euclidean distances between haplotypes [21]. All population analyses were performed using Arlequin version 3.11 [22].

Phylogeographical analysis

Phylogenetic analysis was carried out on both cyt *b* and D-loop sequences along with outgroup taxa in order to ascertain how populations were related to each other and whether there was any evidence of historical dispersal and colonization between regions (i.e. whether one population was derived from another). Sequence data were subsequently analyzed using distance (neighbour joining) and maximum-parsimony methods. The sampling error of neighbour-joining (NJ) and maximum parsimony (MP) trees was analyzed using bootstraps of 1000 replicates where possible followed by the construction of majority-rule trees. For maximum-parsimony analysis, only 100 bootstraps were possible for D-loop sequences, because of limitations in computational power. All phylogenetic analyses were carried out using MEGA. As because phylogenetic analysis makes assumptions that are invalid at the population level (i.e. ancestral haplotypes are extinct), data were also analyzed using phylogeographical techniques based on haplotype networks as implemented by the software package TCS version 1.13 [23].

Neutrality and demographic history

Demographic history was investigated by analyzing mismatch distributions of pair-wise differences between all individuals of each population using Software package Arlequin version 3.11 [22]. This kind of analysis can discriminate whether a population has undergone a rapid population expansion (possibly after a bottleneck) or has remained stable over time. The mismatch distribution will appear unimodal (like a Poisson curve) if accumulation of new mutations is greater than the loss of variation through genetic drift, and multimodal if the generation of new mutations is offset by random genetic drift [24]. Arlequin was also used to test for departures from mutation-drift equilibrium with Tajima's D-test [25]. The time of possible population expansions (t) was calculated through the relationship $\tau = 2ut$ [24], where τ is the mode of the mismatch distribution, u is the mutation rate of the sequence considering that $u = 2\mu k$ (μ is the mutation rate per nucleotide and k is the number of nucleotides). A mutation rate of 2% per nucleotide per million years (Myr) was used for cyt *b* as the mean rate for vertebrate mtDNA [26]. The D-loop region evolves faster than this rate in fish and a mean value of 3.6% per Myr was selected as the mean mutation rate [27].

Results

Sequence variation

Cyt b region

The 5′ end of the cyt *b* mtDNA region (307 bp) was amplified from a total of 46 individuals (accession no. GQ476686-731) collected from eight different riverine locations. The alignment of the sequences revealed eight

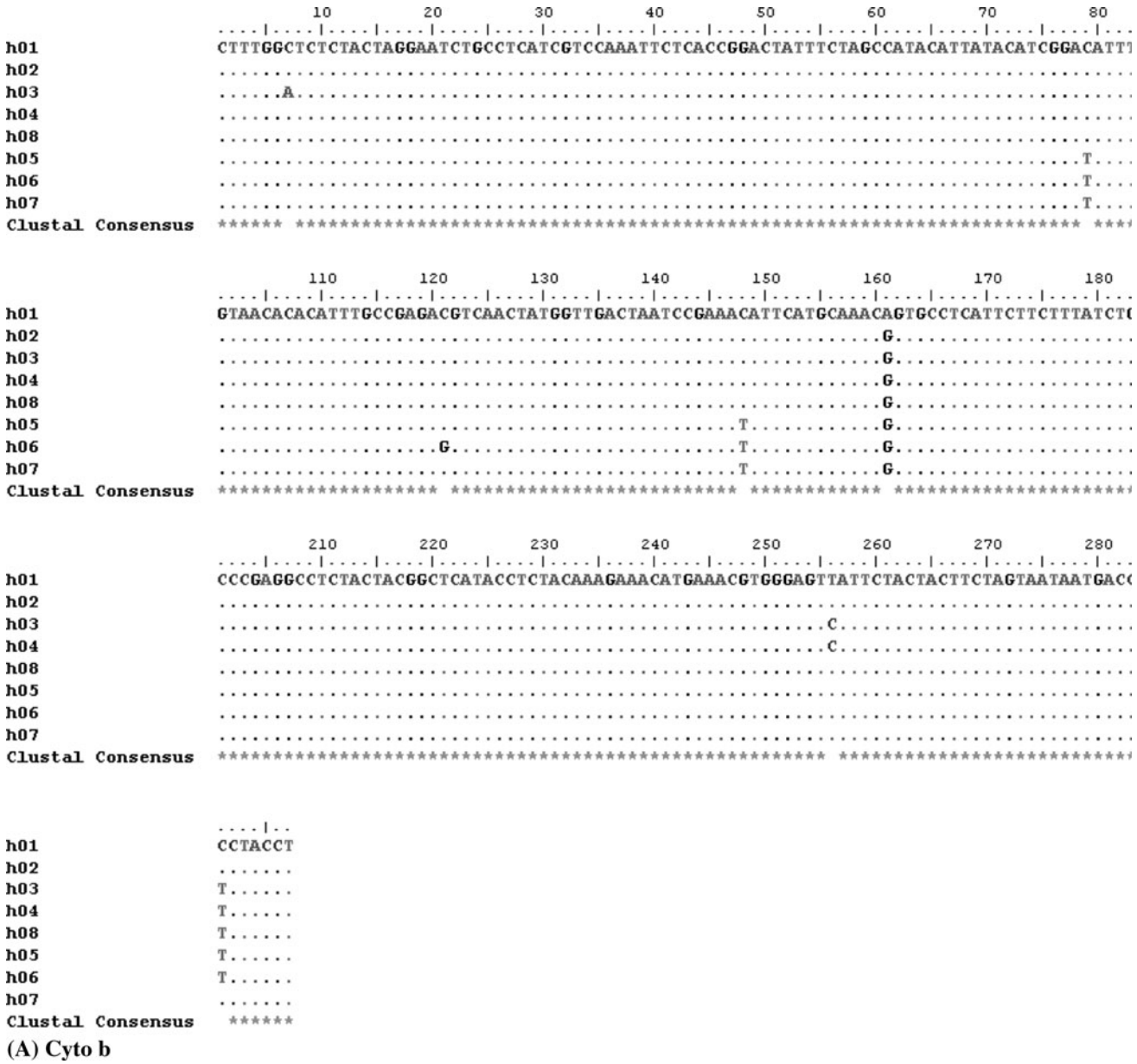

Fig. 2 Haplotypes of cyt *b* and D-loop regions in *Chitala chitala* natural populations in present study

different haplotypes defined by seven divergent nucleotide sites. For the 307 bases, 300 were constant, 4 variables with parsimony informative and 3 singletons.

The variable sites that exist at third position of respective codons were considered silent. Most nucleotide variation resulted from transitions followed by transversions with an expected ti/tv ratio of 18.0. The nucleotide frequencies were T = 0.303, C = 0.280, A = 0.261 and G = 0.156. The two most common haplotypes h2 and h5 (Fig. 2a) were found in individuals from all the rivers, except river Satluj and Mahanadi respectively (Table S1 in Supplementary material). Other six haplotypes were observed to be exclusive to one of the rivers such as h1 (Narmada); h3 and h4 (Mahanadi); h6 (Brahmaputra); h7 (Ganga) and h8 (Bhagirathi). The samples from river Satluj had only one haplotype i.e. h5.

D-loop region

Nucleotide sequences from D-loop regions were generated from 37 *C. chitala* individuals (accession no. GQ868713-749), and the length of sequences varied from 636 to 716 bp. The length difference was caused by variable numbers of tandem repeat units of 49 bp each (data

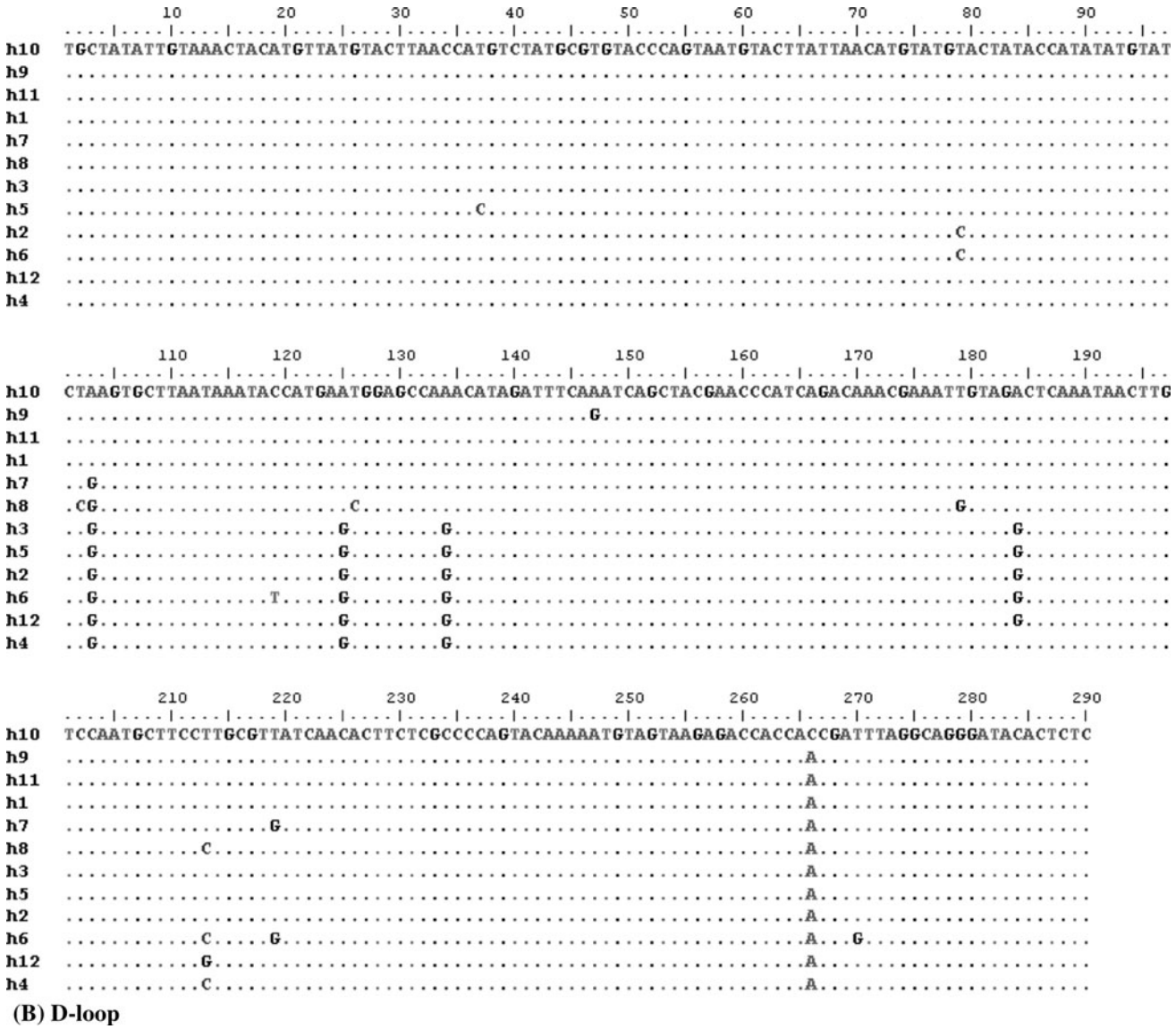

Fig. 2 continued

presented elsewhere). The tandem repeat regions from D-loop sequences were removed and the remaining 291 bp sequences were used for subsequent population analysis. There were 18 variable positions with 9 parsimony informative sites, which defined 37 sequences into 12 haplotypes. Most nucleotide variation resulted from transitions followed by transversions with an expected ti/tv ratio of 4.0. The nucleotide frequencies were T = 0.284, C = 0.223, A = 0.336 and G = 0.157. The most common haplotype h1 (Fig. 2b) was found in individuals from all groups, except group I. Haplotypes h2, h5 and h6 were observed only in Satluj samples, h7 and h8 in Brahmaputra, h10 in Sharda while h12 in Sone only. The samples from Narmada were observed to have only one haplotype h1 (Table S1 in Supplementary material).

Population variability

Parameters of genetic variation in *C. chitala* for cyt *b* and D-loop region are given in Table 2. In cyt *b* region, haplotype diversity (*h*), within the geographical populations was high ranging from 0.0 (Satluj) to 0.733 (Brahmaputra). Nucleotide diversity (π) was generally low ranging from 0.0 (Satluj) to 0.007 (Brahmaputra). In D-loop region also,

Table 2 Intra-population nucleotide (π) and haplotype (h) diversities for *Chitala chitala* for cyt *b* and D-loop region

River	Haplotype diversity (h)	Nucleotide diversity (π)
Cyt *b*		
Satluj	0.0000 ± 0.0000	0.0000 ± 0.0000
Ganga	0.6667 ± 0.2041	0.0043 ± 0.0040
Sharda	0.2222 ± 0.162	0.0022 ± 0.0021
Sone	0.6667 ± 0.2041	0.0065 ± 0.0055
Bhagirathi	0.7000 ± 0.2184	0.0045 ± 0.0039
Brahmaputra	0.7333 ± 0.1552	0.0069 ± 0.0052
Mahanadi	0.7000 ± 0.2184	0.0052 ± 0.0043
Narmada	0.4000 ± 0.2373	0.0013 ± 0.0017
D-loop		
Satluj	0.7500 ± 0.1391	0.0076 ± 0.0054
Ganga	0.8333 ± 0.2224	0.0208 ± 0.0150
Sharda	0.6944 ± 0.1470	0.0291 ± 0.0169
Sone	0.8333 ± 0.2224	0.0253 ± 0.0179
Brahmaputra	0.9524 ± 0.0955	0.0181 ± 0.0114
Narmada	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 3 Hierarchical analysis of molecular variance (AMOVA) analysis of *Chitala chitala* for cyt *b* and D-loop regions of mtDNA

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P* values
Cyt *b*						
Among groups	4	4.120	0.359	38.72	FCT:0.387	<0.05
Among populations within groups	3	2.124	0.033	3.56	FSC:0.058	<0.005
Within populations	37	19.800	0.535	57.73	FST:0.422	<0.001
D-loop						
Among groups	3	3.492	0.121	27.67	FCT:0.277	NS
Among populations within groups	2	0.369	−0.032	−7.19	FSC:−0.099	NS
Within populations	31	10.760	0.347	79.52	FST:0.205	<0.001

Table 4 Pairwise F_{st} and *P* values (Kimura-2P distance) for different *Chitala chitala* population for cyt *b* (below diagonal) and D-loop (above diagonal) sequences

	Satl	Gang	Shar	Sone	Bhag	Brah	Maha	Narm
Satl	–	0.5902**	0.6697**	0.3282*	–	0.5445*	–	0.7924**
Gang	0.7611**	–	−0.1094	−0.0512	–	−0.1142	–	0.0606
Shar	0.8591**	0.1590	–	0.1496	–	0.0714	–	−0.0517
Sone	0.4815	0.0000	0.2062	–	–	0.0148	–	0.3585
Bhag	0.7342**	0.0226	0.0000	0.0000	–	–	–	–
Brah	0.3926*	0.0000	0.2017	0.0000	0.00000	–	–	0.1245
Maha	0.7936**	0.2637	0.1377	0.1503	0.00000	0.1716	–	–
Narm	0.9499**	0.3114	0.0000	0.3357	0.10122	0.2953	0.1678	–

Satl Satluj, *Gang* Ganga, *Shar* Sharda, *Sone* Sone, *Bhag* Bhagirathi, *Brah* Brahmaputra, *Maha* Mahanadi, *Narm* Narmada

* Significant at $P < 0.05$; ** Significant at $P < 0.001$

haplotype diversity (h), within the geographical populations was high ranging from 0.0 (Narmada) to 0.952 (Brahmaputra) and nucleotide diversity (π) generally low from 0.0 (Narmada) to 0.029 (Sharda).

Phylogeographical relationships of populations

The analysis of molecular variance (Table 3) on samples from five groups (Indus, Ganges, Brahmaputra, Mahanadi

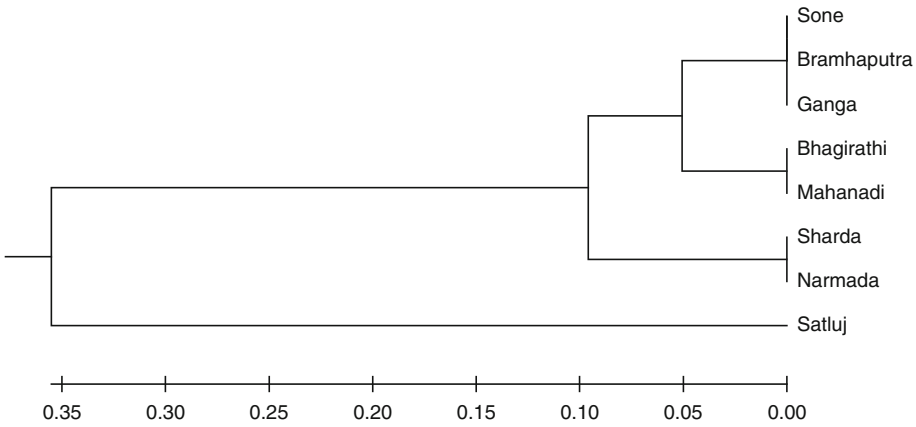

Fig. 3 UPGMA tree depiction for genetic relatedness among wild populations of *Chitala chitala* on the basis of cyt *b* region of mitochondrial DNA

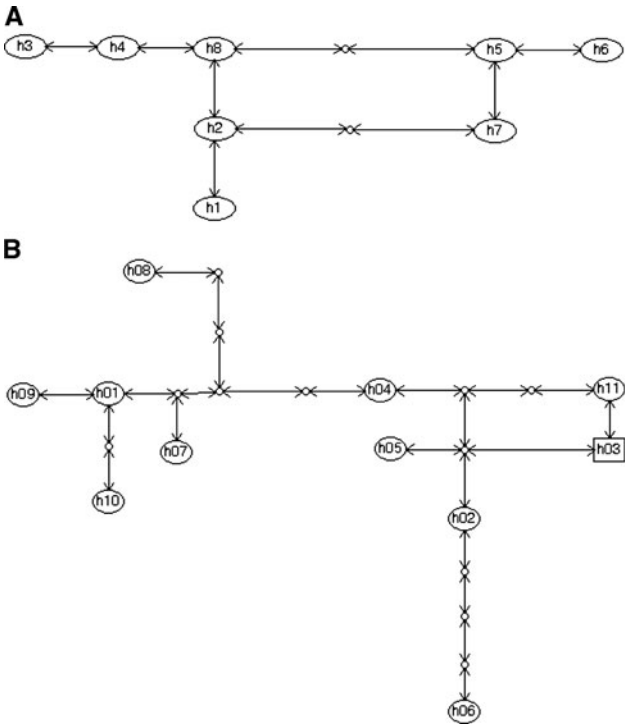

Fig. 4 Minimum spanning network analysis of haplotypes of mitochondrial DNA regions of natural populations of *Chitala chitala* (**a** cyt *b*, **b** D-loop)

and Narmada) for cyt *b* and four groups (Indus, Ganges, Brahmaputra and Narmada) for D-loop region indicated that a high proportion of the total variance was attributed to differences among populations with a significant value ($P < 0.0002$) for both the regions cyt *b* D-loop region, indicating there is geographical structure between groups.

Estimates of genetic differentiation between all eight populations for cyt *b* and D-loop region, using *F*-statistics, are given in Table 4, with respective *P* values. The samples from Satluj showed high levels of genetic differentiation from all the other populations, except with Sone for cyt *b*. Significant genetic differentiation was not detected between populations of Ganges, Mahanadi and Narmada. Similar pattern was observed from D-loop sequences, where Satluj samples were highly differentiated from other populations under study. The genetic relationship among populations is depicted in Fig. 3 on the basis of cyt *b* region as UPGMA tree on the basis of F_{st} values. Similar pattern was observed for D-loop region.

Phylogenetic analysis

The haplotype network derived from cyt b region sequences is presented in Fig. 4a. The one of the most common haplotypes h2, represents individuals from the Ganges, Mahanadi and Narmada. The Satluj samples were solely represented by second most common haplotype h5, while it was present in Brahmaputra, Sharda, Sone and Bhagirathi and totally absent in Mahanadi and Narmada. In the D-loop region sequences haplotype network (Fig. 4b), one of the most common haplotypes h1, represents individuals from the Ganges, and Narmada. Second most common haplotype h2 represented by the Satluj samples, only.

With both the markers, two distinct clades among haplotypes were observed, however no specific clade contain haplotypes from single geographical region is consistent with the non-significant 'among regions' variance component from (FCT) AMOVA (Table 3). Nevertheless, significant pair-wise F_{st} values were observed between Satluj and rest of populations. This suggests strong genetic differentiation between these two regions.

Demographic history and neutrality

Pair-wise mismatch distribution and results of Tajima's D-tests performed for cyt *b* and D-loop region on each population are given in Fig. 5. The parameters of the model of sudden expansion [24] and goodness of fit test to the model are given as Table S2 in Supplementary material.

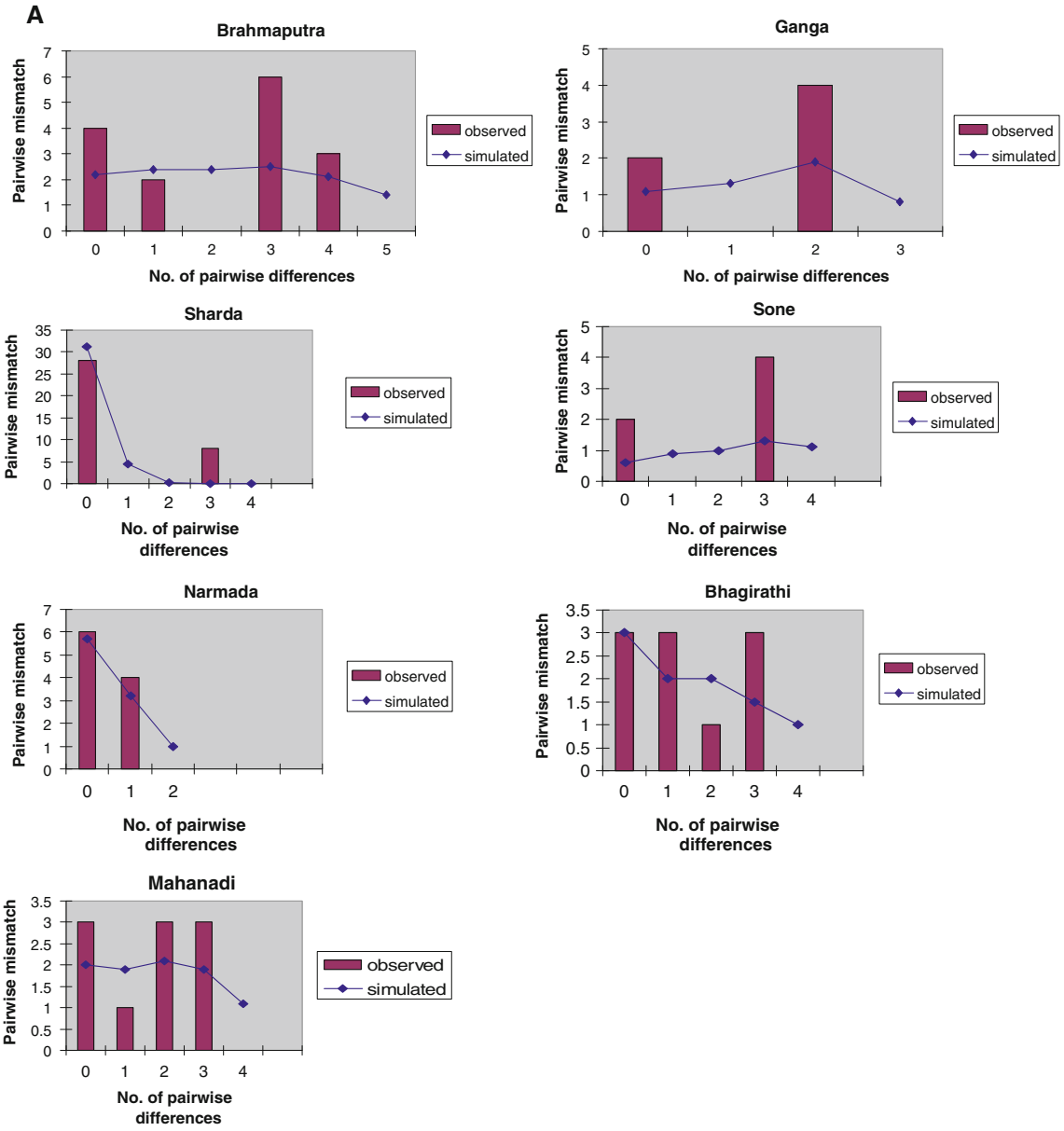

Fig. 5 Minimum spanning network analysis of haplotypes of mitochondrial DNA regions of natural populations of *Chitala chitala* (**a** cyt *b*, **b** D-loop)

For cyt *b*, the Satluj population could not be done for this analysis. All histogram presented multimodal curves, characteristics of population with constant size over time. Sharda, Narmada and Bhagirathi populations exhibited moderate to highly negative D-values, but only Sharda population was significant. This population presents a unimodal curve with significant negative D-value indicating a sudden expansion in population size. A time of expansion was estimated at approximately 2.07 Myr. For D-loop region, Satluj, Brahmaputra, Ganga and Sharda populations exhibited moderate to highly negative D-values, but none of the population was significant (Table S3 in Supplementary material).

Discussion

Variation in mtDNA cyt *b* sequences indicated population sub-structuring in wild population of Indian feather-back *C. chitala*, from different rivers. All the rivers appeared, over the course, as lateral rivers in Himalayas. Remote sensing and archaeological evidences suggest a mighty

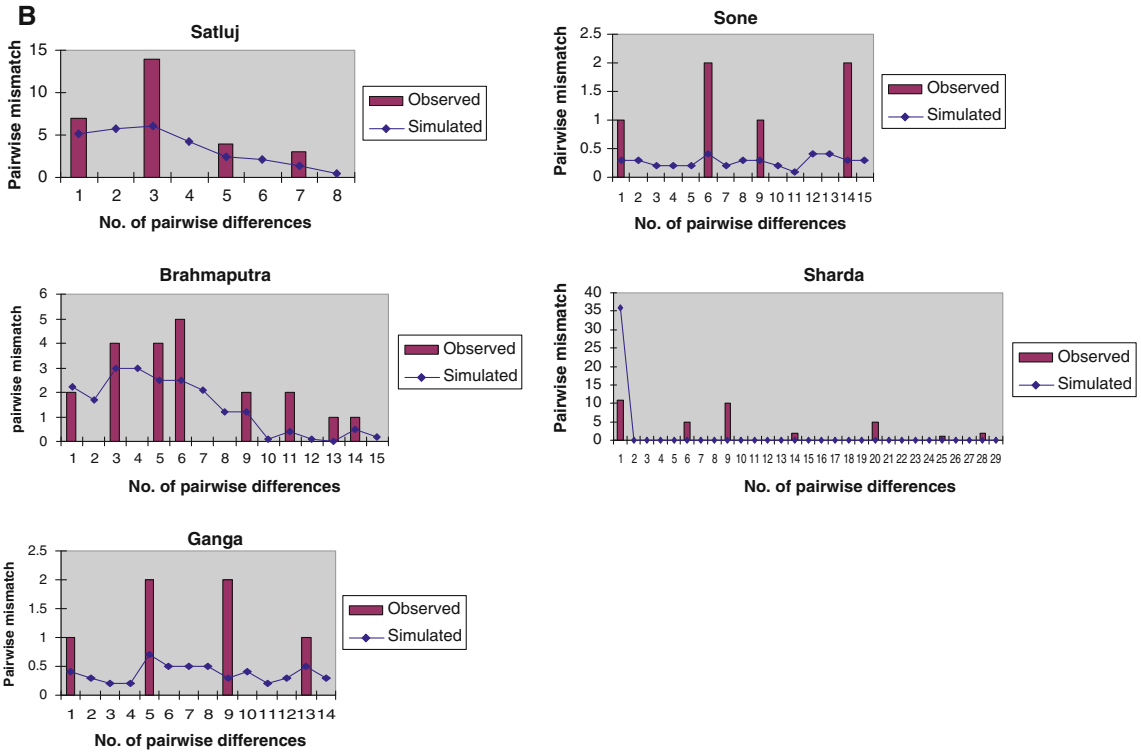

Fig. 5 continued

river Saraswati with Yamuna and Satluj rivers as its tributaries. Tectonic movement caused river Satluj to change its course and join the Indus system. On the other hand Yamuna changed to join Ganga [28]. Moderate to high genetic variation (h) and low to moderate nucleotide diversity (π) were found in all the populations studied, except for Satluj (for cyt *b*). *F*-statistics, AMOVA, haplotype network and phylogenetic analyses revealed genetic structure in wild *C. chitala* populations. Satluj population was significantly different from other populations except Sone population and consequently resolving into two distinct clusters for two distinct lineages. However, it could be noted that Satluj population shared at least some haplotypes with the samples from other populations. This suggested that two lineages could had evolved from common ancestor. During present time, there appears to be no effective gene flow between populations of the Indus and other river systems such as Ganges, Mahanadi, and Brahmaputra etc. However, the evidence of the existence of Indo-Brahma river that used to flow from present Assam to northwest to fall in present Arabian sea [28] could had been responsible for the distribution of common ancestral population and also the common haplotypes. The migration of fishes initiated during the Eocene (60 Myr ago) continued until dismemberment of the Indo-Brahma River and formation of the separate Indus, Ganga, and Brahmaputra river systems during the late Pleistocene. It is quite likely the ancestral stock could have been the same or a gene-pool with gene flow and change in the course of rivers fragmented the populations. Presence of two clusters of haplotypes in the samples studied in both cyt *b* and D-loop region also supported this view. However, exclusive occurrence of only one haplotype in Satluj could be possible, if at the time of fragmentation, the small founder population had either only that haplotype and alternate haplotypes in very small proportion that could not be found within the samples which were collected and analyzed. However, nuclear markers i.e. RAPD and microsatellites [9] had shown divergence within Ganges populations as well as in Brahmaputra where as Narmada were found to be differentiated from other populations. Our results demonstrated incongruence between mtDNA derived and nuclear based phylogenetic reconstructions, suggesting that sex-biased dispersal behaviors could have contributed to the observed disparities.

Satluj samples exhibited exclusive haplotype h5 for cyt *b* while had 4 haplotypes for D-loop region. Still likelihood of low founding population in Satluj is valid. The h3 is the haplotype of D-loop shared with populations from some rivers of Ganga Basin such as Sharda and Sone. Other 3 haplotypes h2, h5 and h6 are only observed in Satluj. This could be explained as the D-loop region is high evolving

region than the cyt *b*. The haplotypes h2, h5 and h6 are likely to had evolved after the fragmentation of Satluj population. These haplotypes might have evolved from a common ancestral haplotype or even could have been originated from h3.

Assuming the h3 (D-loop) haplotype could had been present in founding populations, it is interesting to note its presence only some of the populations of Ganga and associated rivers. Haplotypes h3 and h12 differ only by one base; the h12 could had replaced the presence of h3 in Brahmaputra and Ganga. All the rivers appeared, over the course, as lateral rivers in Himalayas. Remote sensing and archaeological evidences suggest a mighty river Saraswati with Yamuna and Satluj rivers as its tributaries. Tectonic movement caused river Satluj to change its course and join the Indus system. On the other hand Yamuna changed to join Ganga [28].

The Mahanadi river flowed westward as a part of the Narmada river started flowing southward through the Eastern Ghats during the Pleistocene. Therefore, low level of genetic differentiation between the various river samples and paleogeographic reconstructions indicate the possibility that the *C. chitala* from different river basins sampled here are likely to have evolved from a common ancestral gene pool. However, haplotypes h3 and h4 present in Mahanadi and h1 in Narmada had evolved much later.

The observed pattern of genetic variability in *C. chitala* can be attributed to a recent population expansion after a low effective population size caused by bottlenecks or founder events [29]. Such an explanation is also consistent with the dumble-shaped haplotype networks detected and mismatch distribution analysis further supports a population expansion, for populations Sharda. Such patterns of diversity, haplotype networks and mismatch distribution strongly suggest a historical influence on the genetic structure of *C. chitala* populations, as estimated by analysis of haplotype frequencies. Similar observation has also been made with allozyme and microsatellite markers used for genetic variation analysis for the same populations [9].

Mismatch distribution tests and significant Tajima's *D* values are indicative of population bottlenecks followed by expansion in Sharda population approx 2.07 Myr ago which indicates the scenario of a small and unstable population size. Though it may not be conclusive evidence, yet the results point out strong possibility that the *C. chitala* or their ancestors might have suffered reduction in size (bottleneck) in the historical past and thus losing the genetic variation. Another possible explanation for this lack of mtDNA diversity may be a selective sweep, since there is no recombination in the animal mitochondrial genomes [30]. Lack of genetic variation in *C. chitala* in the survey of thirty eight allozyme loci [31] agrees that the poor variation [32] is characteristic in *Chitala* genus. Lack of polymorphism could be the result of past bottleneck due to Pleistocene aridity in many species [33–36]. Fossil records of the family Osteoglossidae indicate these fishes to be between 38 and 55 Myr old. However, the present distribution of members of the Osteoglossidae family suggests that the group was present on Gondwana prior to Gondwana's fragmentation. Biogeographic evidence thus suggests a considerably greater age than the 55 Myr inferred from the fossil record [37].

Populations or species with reduced levels of genetic variation could be at risk of extinction due to both a limited adaptive potential to changing environment and the likely fixation of deleterious alleles as a consequence of genetic drift in the event of small effective population sizes. Hence, in view of the present results, the exploitation and rapid loss of *C. chitala* happening in wild raises concern and need mitigation through conservation plans based on genetic diversity data. Artificial propagation of this species has also been pursued successfully [4] which when considered with the prior knowledge on genetic composition of potential source populations can result in effective restoration programmes for conservation of wild populations [38].

This study generated basic information on the genetic variation parameters at two mtDNA regions in *C. chitala* and provided evidence of sub-structuring in this highly endangered primitive fish population in different rivers in India. The information could provide crucial input to develop rational programme for the conservation and management of declining population of *C. chitala* in nature.

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