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ANIMAL GENETICS

ATPase 8/6 Gene Based Genetic Diversity Assessment of Snakehead Murrel, *Channa striata* (Perciformes, Channidae)[1]

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Abstract—The mitochondrial DNA (mtDNA) ATPase 8/6 genes have been used in phylogenetic as well as in phylogeographic studies along with other mtDNA markers. In this study, ATPase gene sequences were used to assess the genetic structuring and phylogeographic patterns in *Channa striata*. Out of 884 nucleotide positions generated in ATPase 8/6 genes, 76 were polymorphic. The study suggested 23 unique haplotypes from
1 67 individuals of nine populations collected from different riverine systems of India. The ATPase 8/6 sequence revealed highest haplotype as well as nucleotide diversities in Imphal River population and lowest diversities in Tapti River population. The pattern of genetic diversity and haplotype network indicated distinct mitochondrial lineages for Chaliyar population, whereas mismatch distribution strongly suggested a population expansion in mid pleistocene epoch (0.4 Mya) with distinct genetic structuring in *C. striata*. The baseline information on genetic variation and the population sub-structuring would facilitate conservation and management of this important snakehead murrel.

Keywords: ATPase 8/6, *C. striata*, genetic variation, mtDNA polymorphism, phylogeography.

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INTRODUCTION

Channa striata (Bloch, 1793), commonly known as striped snakehead murrel, is a tropical freshwater food fish in the Asia-Pacific region [1] and has also been used for medicinal and pharmaceutical purposes [2]. It is a carnivorous, air-breathing species found across the North part of Asia, southern China, Indochina and the Sunda Islands in ponds, swamps, rivers, small streams, canals, drains, reservoirs, rice fields, mining pools and lakes. It is abundant in nature and may survive for a longer time without water. Culture of snakehead is important in certain countries like India, Pakistan, Tibet, Nepal, Bhutan, Bangladesh and Sri Lanka [3]. In this region, the aquaculture potential of the species has not been fully exploited despite its high market price and tolerance to adverse climatic conditions including air breathing capability [1].

Being an important food-fish, indiscriminate harvesting has resulted in decline in the stocks of the species [4]. An earlier study of this species has focused on reproductive biology [5], breeding [6], medicinal properties [2–7], biochemical composition [8], dietary intake [9], ecology [10] and morphological characters [11]. In *C. striata*, genetic variation in natural populations of Malaysia was described using mitochondrial COI and nuclear microsatellites [12, 13] markers. For conservation and management of this species, information on relevant population genetics is essential, specifically through assessment of its genetic diversity and structuring for potential brood-stock identification. As information on population structure of this species in Indian region is not available, so this study was undertaken to assess the genetic diversity of the natural populations for utilization in management of the species in culture systems.

The mitochondrial ATPase subunits 8/6 were sequenced to examine the genetic diversity and structure of *C. striata* stocks within India and particularly to infer the mechanisms or forces most likely to have been involved in distribution of snakehead populations; thus, providing genetic information for broodstock management and species conservation in nature.

MATERIALS AND METHODS

Sample Collection

A total of 67 individuals, representing nine populations of *C. striata* from India, were sampled during the years 2010 to 2013. Details of sample collection, i.e. river, sample size, sampling locations with lati-

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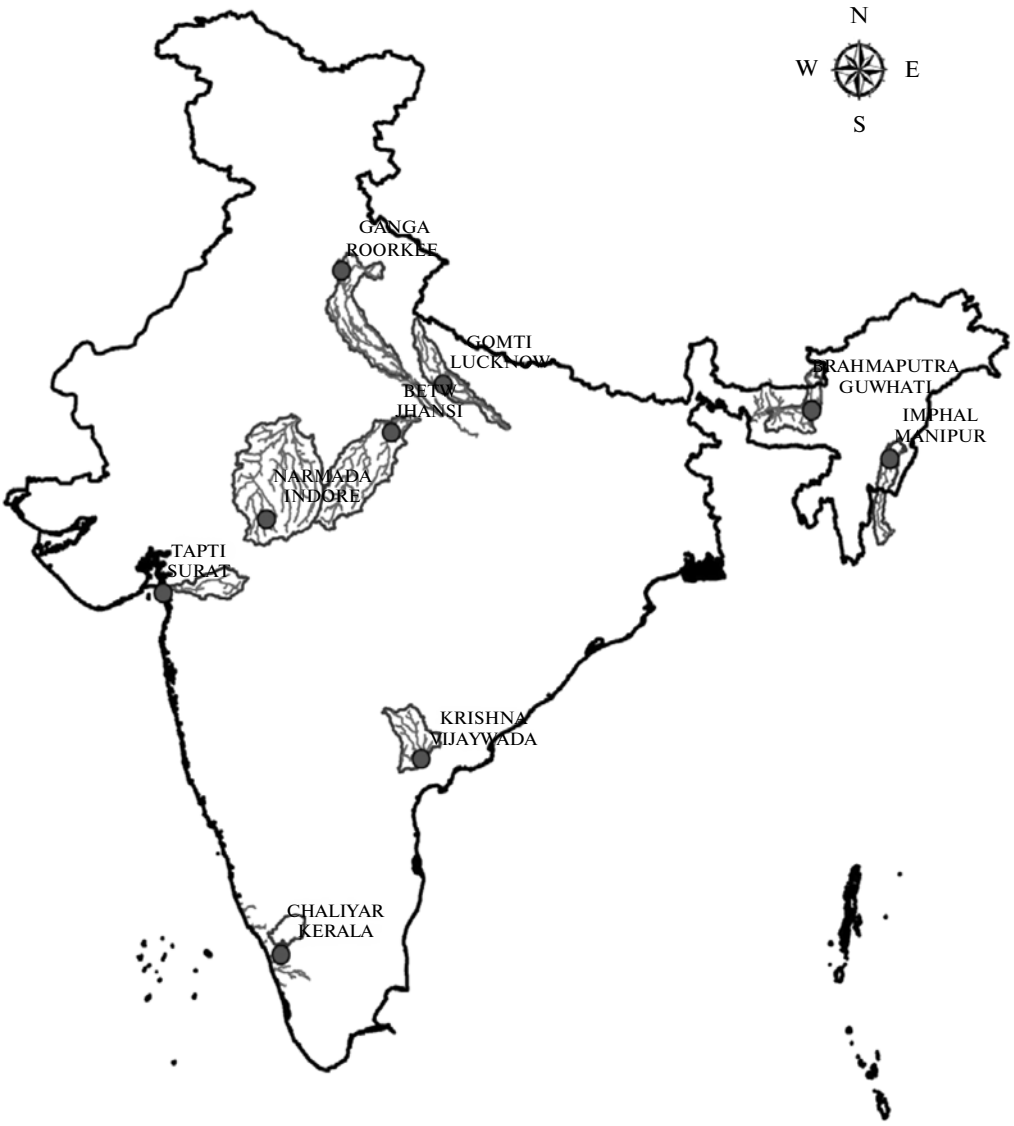


Fig. 1. Sampling locations of *C. striata*.

tude/longitude and time of collection are shown in Fig. 1 and Table 1. These populations, classified into nine categories (i.e. Betwa, Brahmaputra, Chaliyar, Ganga, Gomti, Imphal, Krishna, Narmada and Tapti Rivers), were located in different geographical areas and rivers in India. These riverine locations were selected so as to cover geographically distant populations. Gomti and Betwa (Vindhya Range) belongs to Ganges River basin in Northern India, Narmada (Amarkantak Hill) and Tapti (Satpura Range) in Central India. Brahmaputra River and Imphal River are major rivers in North East, part of India. Chaliyar and Krishna Rivers originated from Western Ghat in southern India, but flowing west-ward and east-ward, respectively. Fish muscle tissue samples were collected, fixed in 95% ethanol and stored at 4°C for further use. Voucher specimens were preserved in formalin.

DNA Isolation, Amplification and Sequencing

Genomic DNA was isolated from ethanol preserved muscle tissue using standard phenol-chloroform method. The DNA pellet was dissolved in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and the DNA quality as well as concentration was determined on 0.7% agarose gel and with Nanodrop 2000 (Thermo Scientific, USA). The genomic DNA was diluted to 50 ng/μL and stored at 4°C. ATPase 8/6 regions of mitochondrial DNA (mtDNA) was amplified in 50 μL reaction volumes. The PCR reaction mix contained 10× buffer (with 15 mM $MgCl_2$), 10 mM dNTPs mix. 10 pmole of each Primer (ATP.2L8331 and COIII.2H 9236), 2.5 U *Taq* DNA polymerase and 200 ng genomic DNA. Amplifications were performed in thermal cycler (Eppendorf AG 22331 Hamburg, Germany) with initial denaturation at 94°C for 3 min,

Table 1. Sampling locations of *C. striata* used in the present study

River	*N*	Sampling locations (latitude and longitude)	Sampling time
Gomti	9	Lucknow, Uttar Pradesh (26°52′ N; 80°55′ E)	March, 2010
Narmada	8	Indore, Madhya Pradesh (23°10′ N; 75°45′ E)	November, 2012
Betwa	10	Jhansi, Uttar Pradesh (25°33′ N; 79°24′ E)	November, 2012
Tapti	4	Surat, Gujarat (21°14′ N; 72°24′ E)	November, 2012
Chaliyar	8	Nilambur, Kerala (11°15′ N; 76°11′ E)	February, 2013
Krishna	9	Vijaywada, Andhra Pradesh (16°36′ N; 80°17′ E)	February, 2013
Ganga	10	Roorkee, Uttarakhand (29°51′ N; 77°18′ E)	March, 2013
Brahmaputra	6	Guwahati, Assam (26°10′ N; 91°41′ E)	March, 2013
Imphal	3	Imphal, Manipur (24°50′ N; 93°58′ E)	March, 2013
Total	67	–	–

Note: *N* = number of individuals.

followed by 33 cycles of denaturation at 94°C for 45 s, annealing at 54°C temperature for 30 s and elongation at 72°C for 1 min, with a final elongation at 72°C for 10 min. The amplified PCR products were checked on 1% agarose gel and sequenced from both side by the dideoxynucleotide chain-termination method [14] using ABI sequencer 3730. The quality of sequencing results was checked by viewing the electropherograms in Finch TV (Geospiza, Inc., Seattle, WA 98119) software.

Sequence Alignment

The total 67 sequences were aligned using Clustal W [15] in MEGA 5.05 software [16]. The mismatches were referred against the electropherograms and the sequences submitted to GenBank (Table 2). All parameters were used as default with gap opening penalty 15, gap extension penalty 6.66 and multiple alignment parameters set as gap opening penalty of 15, gap extension 6.66, DNA weight matrix IUB and Transition weight 0.5.

Genetic Diversity Analysis

Intra-population diversity was analyzed by haplotype diversity and nucleotide diversity. The overall genetic differentiation between each sampled population was tested using pairwise *F*-statistics. For deriving the correlation between geographical distribution and genetic structure, genetic differentiation of all the nine populations was calculated in the framework of a predefined geographical locations and analysis of molecular variance (AMOVA) based on pairwise squared Euclidean distances matrix amongst populations and between individuals [17]. All populations analyses were performed using Arlequin version 3.5 [18]. The measurement of contribution of diversity (CSR), differentiation (CDR) and allelic richness (CTR) of each population were computed by Contrib (version 1.02) software [19].

Phylogeographic Analysis

Sequence data were analyzed through neighbor-joining (NJ) and maximum-likelihood (ML) methods with bootstraps of 1,000 replicates. All phylogenetic analyses were carried out using MEGA (version 5.05) software. The UPGMA cladogram was computed on the basis of pairwise genetic relatedness (i.e. F_{st} scores). To find out whether ancestral haplotypes are extinct, dataset was analyzed using phylogeographical techniques based on haplotype networks using NETWORK (version 4612) software package [20]. The *p*-distance was computed using between group mean distances option in MEGA to find the proportions of nucleotides at particular positions are different.

Test of population patterns, isolation by distance (IBD) and relationships among geographical distance of the rivers and genetic distance among stocks was done using Mantel test [21, 22] in XLSTAT-2010 software with 10,000 permutations. Pairwise F_{st} value were genetic distance matrix between populations, while geographical distances (in kilometer) between populations were sampling site of the river.

Neutrality and Demographic History

Arlequin computer software version 3.5 [18] was used for pairwise mismatch distribution analyses and selective neutrality tests. Raggedness index was included in mismatch analysis to determine goodness of fit for unimodal distribution [23]. Two tests, *viz.* Tajima's *D*-test [24] and Fu's *Fs*-test [25], were applied under the infinite site model to test the selective neutrality of the DNA sequences without recombination (Fig. 2). The significance of *D*-statistic is tested by generating random samples under the hypothesis of

Table 2. Number of haplotypes in nine distinctly located rivers from India

Rivers → Sample size → Haplotype↓	Ganga 10	Gomti 9	Betwa 10	Narmada 8	Tapti 4	Krishna 9	Chaliyar 8	Brahmaputra 6	Imphal 3	GenBank Acc. No.
h_1	8	0	0	0	0	0	0	0	0	KJ538595
h_2	1	0	0	0	4	0	0	0	0	KJ538626
h_3	1	0	0	0	0	0	0	0	0	KJ538591
h_4	0	1	1	0	0	0	0	0	0	KJ538608
h_5	0	2	0	0	0	0	0	0	0	KJ538598
h_6	0	6	9	6	0	0	0	0	0	KJ538620
h_7	0	0	0	2	0	0	0	0	0	KJ538622
h_8	0	0	0	0	0	3	0	0	0	KJ538631
h_9	0	0	0	0	0	4	0	0	0	KJ538635
h_{10}	0	0	0	0	0	1	0	0	0	KJ538632
h_{11}	0	0	0	0	0	1	0	0	0	KJ538633
h_{12}	0	0	0	0	0	0	1	0	0	KJ538636
h_{13}	0	0	0	0	0	0	1	0	0	KJ538637
h_{14}	0	0	0	0	0	0	2	0	0	KJ538639
h_{15}	0	0	0	0	0	0	1	0	0	KJ538640
h_{16}	0	0	0	0	0	0	2	0	0	KJ538642
h_{17}	0	0	0	0	0	0	1	0	0	KJ538643
h_{18}	0	0	0	0	0	0	0	2	0	KJ538645
h_{19}	0	0	0	0	0	0	0	3	0	KJ538644
h_{20}	0	0	0	0	0	0	0	1	0	KJ538646
h_{21}	0	0	0	0	0	0	0	0	1	KJ865050
h_{22}	0	0	0	0	0	0	0	0	1	KJ865051
h_{23}	0	0	0	0	0	0	0	0	1	KJ865052

selective neutrality and populations equilibrate, using a coalescent simulation algorithm [26].

Mismatch frequency distribution of the stocks' haplotypes were calculated on the basis of three parameter, i.e. θ_0 (value before population growth), θ_1 (value after the population expansion) and τ (expressed in units of mutational time since expansion). These frequency distributions were used to investigate the historic demographic expansion of the populations in million years ago [Mya, 27–29]. The parameters of the demographic expansion (θ_0, θ_1 and τ) were computed on the basis of generalized non-linear least-square approach and confidence intervals of the parameter were computed with bootstrap of 1,000 replicates [30].

The result has been expressed in mutational time scale, $\tau = 2ut$, where u is the mutation rate for whole haplotype, t (generation time in years) and τ measures it in units of $1/2u$ generations. The $u = 2\mu k$, where μ is the mutation rate per nucleotide and k is the number of nucleotides in the sequence of examined stocks. The value of t was calculated as a time after population expansion using 1.3% nucleotide substitution rate per million years of ATPase gene as mean rate reported earlier for vertebrate mtDNA [31].

RESULTS

Sequence Variation

A total of 67 sequences of the mitochondrial ATPase 8/6 regions from nine different riverine locations, submitted in GenBank database (Acc. no. KJ538586–KJ538652), were analyzed for studying genetic variation in natural populations of *C. striata*. In the sequenced data, ATPase 8 gene ranged from 1–168 bp and ATPase 6 from 159–884 bp with an overlapping region of 10 bp (159–168 bp). Out of the 884 sites in the dataset, 808 were constant, 76 variable with 64 parsimony informative and 12 singleton sites. These two

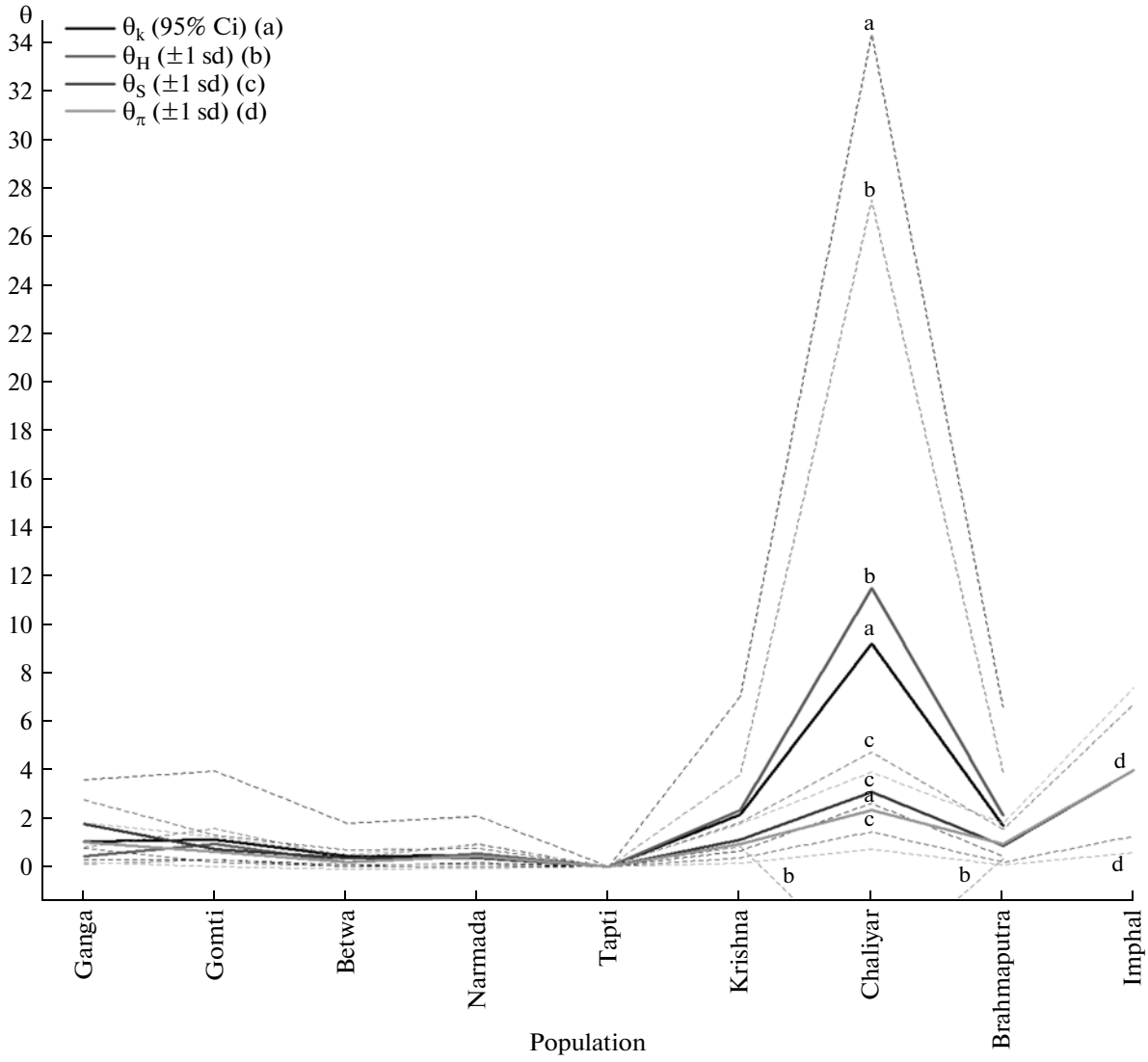


Fig. 2. Graph showing molecular diversity indices based on Tajma's *D* neutrality test. θ_H = observed similarity, θ_s = observed segregating sites, θ_k = observed number of alleles, θ_π = observed mean no of pairwise differences.

regions were analyzed collectively for phylogeographic analyses of *C. striata*. The nucleotide variations resulted from transitions (ts) and transversions (tv) with an expected ts/tv ratio of 1.311. The average nucleotide composition was: T = 28.3%, C = 32.8%, A = 27.2% and G = 11.7%.

Population Variation

Hierarchical AMOVA analysis of ATPase 8/6 sequences explained 93.87 and 6.12%) of variations among and within populations, respectively (Table 3). The haplotype diversity in natural populations (Table 4) ranged from 0.0000 (Tapti) to 1.0000 (Imphal), similarly the nucleotide diversity varied from 0.000000 (Tapti) to 0.004525 (Imphal). The significant contribution by means of total genetic diversity and total genetic differentiation of sampled rivers explained as a total allelic richness presented in Fig. 3.

Phylogeographical Relationship

The AMOVA indicated that a high proportion of the total variance was attributed to among population differences with a significant value ($p < 0.001$) and confirm population structure in this species. Estimates of genetic relatedness (F_{st} statistics) as well as *p*-distance scores of nine populations are given in Table 5. The Chaliyar population showed low level of genetic relatedness from all other populations and significant genetic differentiation was not detected between populations of Ganga with Narmada as well as Gomti with Betwa and Narmada.

Table 3. Hierarchical AMOVA of ATPase 8/6 sequence of *C. striata* samples (Fixation index and *p*-value with variance components were estimated using ARLEQUIN (ver. 3.5) at 1,000 random permutation of data matrix)

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Fixation index	*p*-value
Among populations	8	431.882	7.28319 Va	93.87107	F_{st}: 0.93871	<0.001
Within populations	58	27.581	0.47553 Vb	6.12893		
Total	66	459.463	7.75872	100.00		

Table 4. ATPase 8/6 haplotype (h) and nucleotide (π) diversities in 9 populations of *C. striata*

River	Haplotype diversity (h)	Nucleotide diversity (π)
Ganga	0.3778 ± 0.1813	0.001131 ± 0.000937
Gomti	0.5556 ± 0.1653	0.000691 ± 0.000680
Betwa	0.2000 ± 0.1541	0.000226 ± 0.000344
Narmada	0.4286 ± 0.1687	0.000485 ± 0.000552
Tapti	0.0000 ± 0.0000	0.000000 ± 0.000000
Krishna	0.7500 ± 0.1121	0.001068 ± 0.000912
Chaliyar	0.9286 ± 0.0844	0.002626 ± 0.001824
Brahmaputra	0.7333 ± 0.1552	0.001056 ± 0.000964
Imphal	1.0000 ± 0.2722	0.004525 ± 0.003845

Phylogenetic Analysis and Population Differentiation

Analyses of haplotypes produced same phylogenetic tree using NJ and ML with slight difference in bootstrap values. The alignment of the sequences resulted in 23 haplotypes (Fig. 4, Table 2). The haplotype networks created for ATPase 8/6 region sequences are presented in Fig. 5,*a*. The most common haplotype, h6, were found in 21 individuals belonging to three rivers (six of Gomti, six of Narmada and nine of Betwa). The population of Ganga was shared with haplotypes h1, h2 and h3. Haplotype h4 was found in two individuals (one of Narmada and one of Gomti). Haplotypes h5 and h7 were found in two individuals each of Gomti and Narmada populations, respectively. Haplotypes h8–h11 were found in Krishna population only. Haplotypes h12–h17 were observed only in Chaliyar population. Similarly, haplotypes h18-h20 and h2l–h23 were found in Brahmaputra and Imphal river populations only. The samples from river Tapti had only one haplotype, i.e. h2.

With observed haplotypes, two distinct clades were observed using ATPase 8/6 markers; however, no specific clade contained haplotypes from single geographical region. The overall F_{st} was 0.93871. The F_{st}

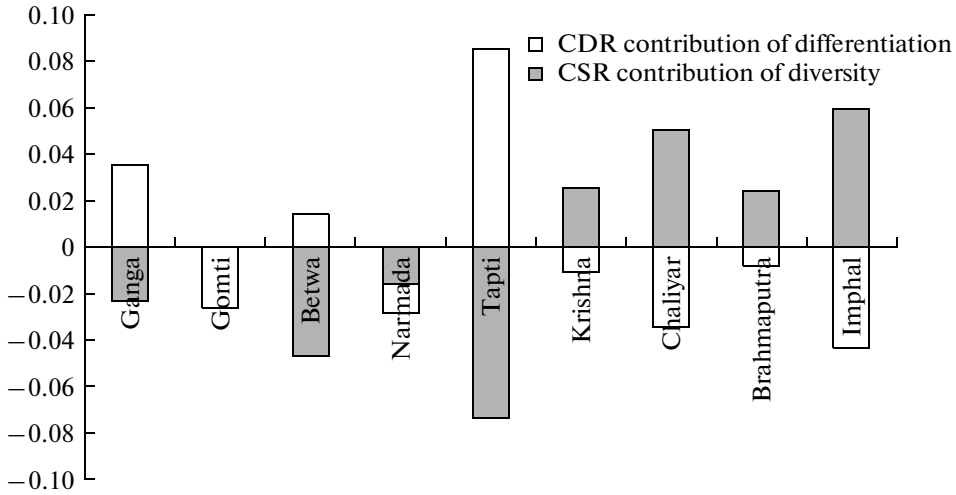


Fig. 3. Population-wise contribution of diversity (CSR) and contribution of differentiation (CDR) of *C. striata*, depicted by allelic richness.

Table 5. Pairwise F_{st} (below diagonal) and p-distances (above diagonal) values among *C. striata* populations

Rivers	Ganga	Gomti	Betwa	Narmada	Tapti	Krishna	Chaliyar	Brahmaputra	Imphal
Ganga	–	0.00275	0.00248	0.00265	0.00418	0.00607	0.06450	0.00403	0.00720
Gomti	0.66540*	–	0.00046	0.00065	0.00263	0.00452	0.06267	0.00226	0.00527
Betwa	0.72700*	0.01983	–	0.00039	0.00237	0.00426	0.06247	0.00199	0.00501
Narmada	0.68447	0.10581	0.11454*	–	0.00254	0.00443	0.06264	0.00216	0.00518
Tapti	0.80626*	0.81705*	0.92979*	0.87011*	–	0.00188	0.06235	0.00188	0.00641
Krishna	0.81912*	0.80607*	0.85358*	0.82142*	0.62059*	–	0.06348	0.00377	0.00678
Chaliyar	0.97371*	0.97582*	0.98055*	0.97641*	0.97212*	0.97315*	–	0.06198	0.06537
Brahmaputra	0.72715*	0.62770*	0.72658*	0.66158*	0.66481*	0.71856*	0.96980*	–	0.00603
Imphal	0.73366*	0.68301*	0.75962*	0.69483*	0.70754*	0.71583*	0.95556*	0.63634*	–

* $p < 0.05$.

values of the populations were significantly different from each other, except Gomti with Narmada and Betwa as well as Ganga with Narmada for ATPase 8/6 which explained the genetic relatedness amongst the river. The UPGMA tree, created on the basis of pairwise all F_{st} values, divided the undertaken populations in to 5 clades (Fig. 5,*b*; Table 5).

Isolation by Distance (IBD)

The IBD patterns were supported when all the subject populations compared using Mantel test, i.e. the genetic distance among the populations were increasing with the increase of geographical distance (Fig. 6, Table 6). Observed IBD between Gomti, Betwa with Narmada ($r = 0.847$, $p > 0.500$) and Brahmaputra, Imphal with Tapti, Krishna ($r = 0.195$, $p > 0.542$) were non-significant. Other observed IBDs, like Gomti, Betwa, Narmada with Brahmaputra, Imphal ($r = 0.729$, $p < 0.031$), Gomti, Betwa, Narmada, Brahmaputra, Imphal with Ganga ($r = 0.569$, $p < 0.026$), Gomti, Betwa, Narmada with Tapti, Krishna ($r = 0.662$, $p < 0.047$) were significantly different. The overall population IBD ($r = 0.465$, $p < 0.0001$) was also significantly different. The clear positive correlations were also observed between geographical distances and pairwise genetic relatedness (F_{st})) among the overall population.

Demographic History and Neutrality

Pairwise mismatch distribution and results of Tajima's *D*-test and Fu's *Fs*-test performed for ATPase 8/6 region on each population are given in Fig. 7. The parameters of the sudden expansion [28] and goodness of fit test to the model are given in Table 7. All histograms presented unimodal curves characteristics suggesting the populations have passed through a recent demographic expansion. Populations from Betwa, Chaliyar, Gomti and Krishna exhibited moderate to high negative values for both Tajima's *D*-statistic and Fu's *Fs*-test. Ganga population showed high negative

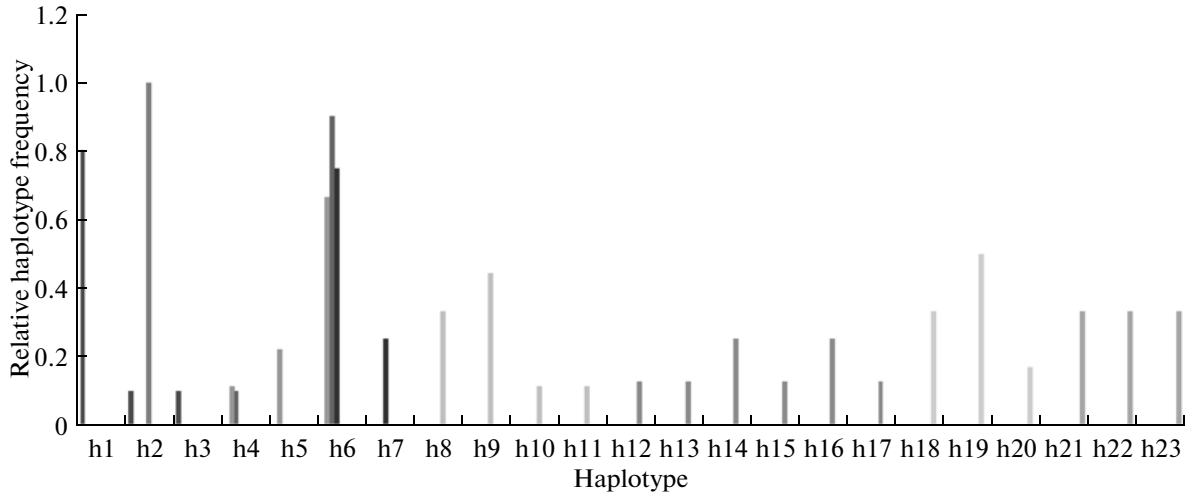


Fig. 4. Graph represents relative frequency of different haplotypes (h1–h23) among rivers: Ganga (h1–h3), Betwa (h4, h6), Tapti (h2), Chailyar (h12–h17), Imphal (h21–h23), Gomti (h4–h6), Narmada (h6, h7), Krishna (h8–h11), Brahmaputra (h18–h20).

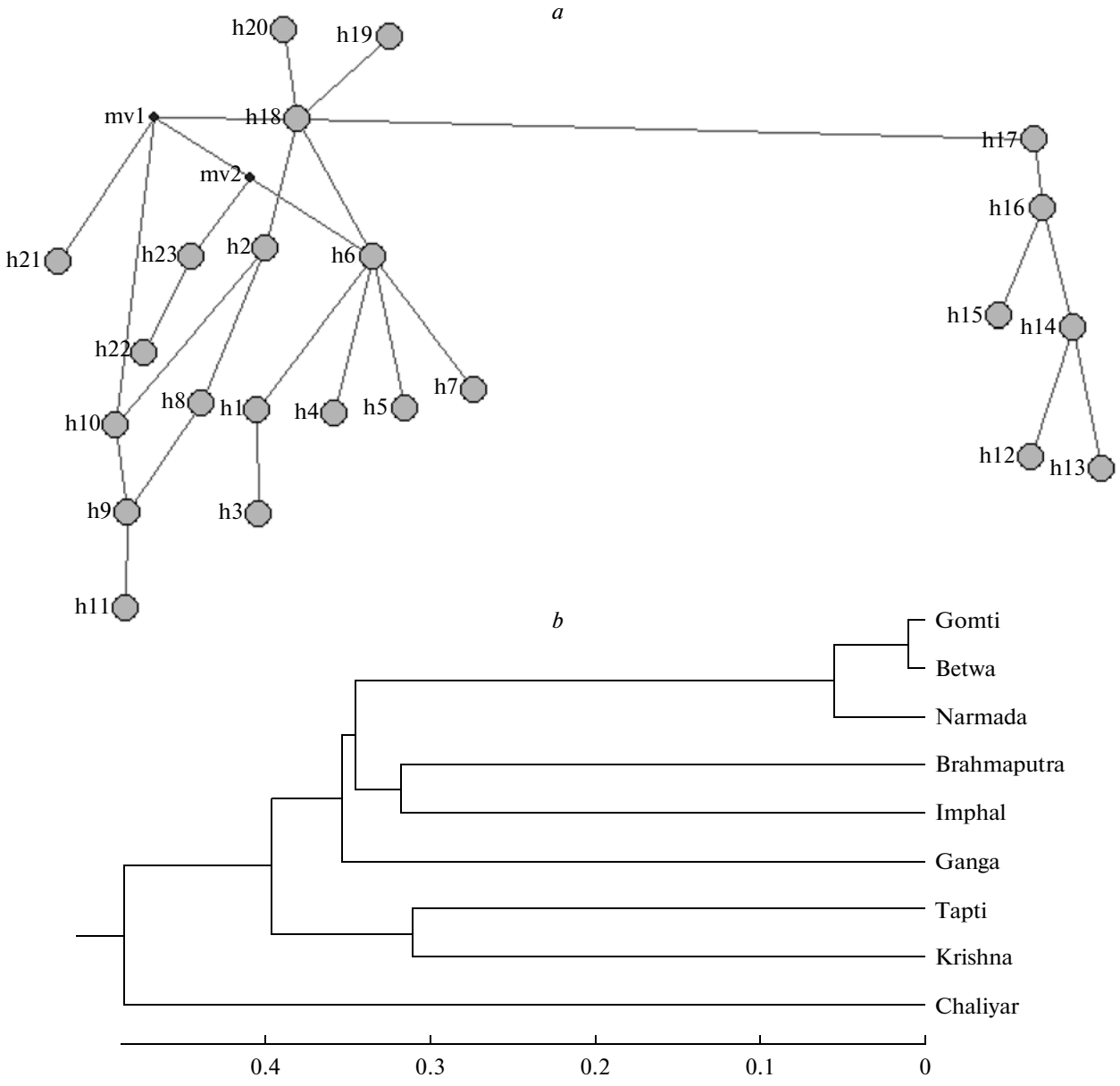


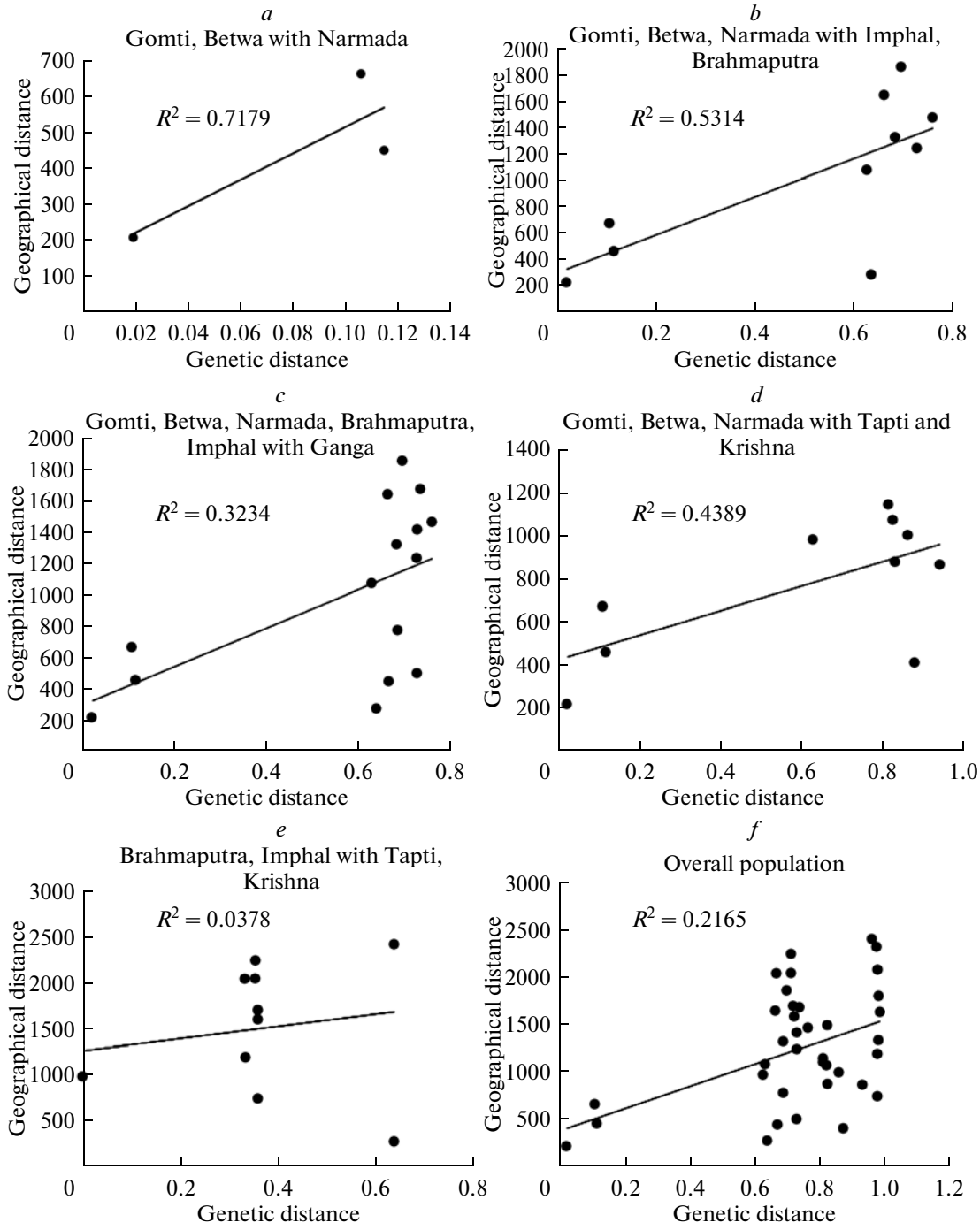
Fig. 5. Haplotype (h1–h23) (a) network of ATPase 8/6 gene of nine natural populations of *C. striata* across India and (b) pairwise F_{st} of ATPase 8/6 gene based UPGMA tree detecting genetic relatedness among natural populations of *C. striata*.

D-statistic and positive *Fs* value. The population of Tapti did not show significant difference both for *D*-statistics and *Fs*-test. The population of Chaliyar showed higher and significant values ($p < 0.05$) for *Fs*-test. The time after recent expansion was estimated between 0.000 to 0.403 Mya for the Tapti populations. The significance of *D* values may be due to other (than selective effects) factors, like population expansion, bottleneck, or heterogeneity of mutation rates.

DISCUSSION

Nucleotide variations in ATPase 8/6 mtDNA sequences indicated sub-structuring in natural populations of Indian snakehead *C. striata* from different rivers amplified by using universal primer pair [32]. The ATPase 8/6 has been found to be highly polymorphic, thus, successfully used in this species as well as another species for intra-specific genetic diversity analyses. The ATPase 8/6 analyses showed nucleotide composition to be A+T (55.5%) rich, which are similar to many other fishes [33].

The analysis showed that haplotype h6 was shared between Gomti, Betwa Rivers in North India and also with Narmada River which flows parallel with Tapti River in Central India. The haplotypes of Narmada and Tapti rivers did not shared between them. Krishna and Chaliyar rivers flows in Central-Southern part of



1 **Fig. 6.** Pearson correlation (a–f) coefficients between geographic distance (km) and pairwise F_{st} values from nine riverine locations.

India, but Chaliyar River formed separate haplotypes lineage due to Indian Tectonic movement caused to change its course. On the other hand, Rivers Brahmaputra and Imphal, flowing in North-East part of India, did not shared haplotypes between them; even they are found in the same geographical region. Tapti River did not showed haplotype and nucleotide variation. *F*-statistics showed all the population significantly different, except population pair Ganga and Narmada, Gomti and Narmada and Gomti and Betwa that were non-significantly different. Chaliyar population showed higher significant Fst values across all over populations. Almost similar was obtained using *p*-distance scores. The haplotype networks showed that the Chaliyar River was located in different lineages with 52 nucleotide mutations from ancestor haplotype "h18"

Table 6. Isolation by distance (IBD) relationship obtained from genetic distance (F_{st}) and geographical distance (km) between nine riverine locations for *C. striata*. IBD was done among (UPGMA Cladogram) by removing clade at a time. The correlations between genetic (F_{st}) and geographical distances were computed using Mantel test at 10,000 permutations

Populations omitted	*r*	*p*-value
Gomti, Betwa with Narmada	0.847	0.500
Gomti, Betwa, Narmada with Brahmaputra	0.729*	0.031
Gomti, Betwa, Narmada, Brahmaputra, Imphal with Ganga	0.569*	0.026
Gomti, Betwa, Narmada with Tapti, Krishna	0.662*	0.047
Brahmaputra, Imphal with Tapti, Krishna	0.195	0.542
Overall populations	0.465*	0.000

Note: *r* = correlation coefficient; * indicates significant difference at $\alpha = 0.05$.

of Brahmaputra River. Phylogenetic analyses of haplotypes showed that all the mutation occurred at 3rd position of codon using Nj and ML clustering analyses with slight difference in its bootstrap values. The hierarchical AMOVA analysis showed very less (6.13%) within population variation and very high (93.87%) among population variation. It was earlier reported that the migratory fishes has 15 and 85%, whereas the non-migratory fish has 32.4 and 67.6% variations within and between the populations, respectively [34]. Same phenomenon represents the genetic structures of *C. striata* in natural populations, which are genetically different. One interesting finding was observed about Gomti river population where haplotype diversity did not showed contribution of total diversity with allelic richness and expansion of this population followed 0.050 Mya in late Pleistocene.

Chaliyar population was significantly different from other populations. This population did not shared with the samples of any other populations, hence, making another distinct cluster. These clustering resulted in two distinct lineages from haplotype network. This suggested that the two lineages could have evolved from genetically different ancestors. Even during present time, there is no effective gene flow between the Chaliyar population and the other river systems, such as Betwa, Brahmaputra, Ganges, Gomti, Imphal, Krishna and Narmada. The migration of fishes, initiated during the 60 Mya in Eocene and 10 Mya in Pleistocene, continued until taking apart of the Indo-Brahma River and formation of the separate Ganga and Brahmaputra river systems during the late Pleistocene.

It is quite likely that the ancestral stock may have the same gene pool with gene flow, and the changes in the course of rivers fragmented the populations. Presence of two clusters of haplotypes in the samples stud-

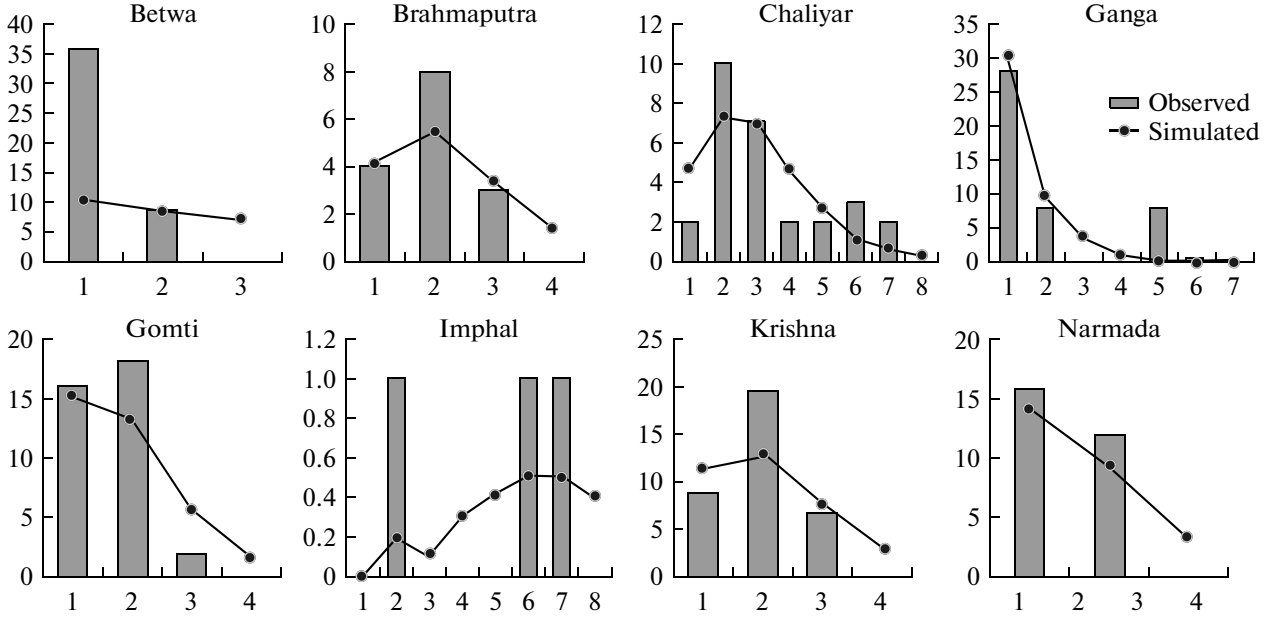


Fig. 7. The observed pairwise differences (bars) and the simulated mismatch distribution (line) under the sudden expansion model of ATPase 8/6 haplotype in *C. striata* populations, except Tapti.

Table 7. Summary of diversity, neutrality, and expansion time for *C. striata*, number of haplotype (h), segregating sites (*S*), Tajima's *D* and Fu's *Fs*, corresponding with *p*-values, time in number of generation (τ), time after expansion (*t*, in Mya) and mismatch distribution parameter estimates (θ_0, pre expansion population size; θ_1 post expansion population size), sum of squared deviation (SSD), Raggedness index (*r*)

Population	h	*S*	Tajima's *D*		Fu's *Fs*		Time after expansion event		Mismatch distribution		Goodness of fit test	
			D	*p*-value	*Fs*	*p*-value	τ	*t*	θ_0	θ_1	SSD	(*r*)
Ganga	3	5	−1.741	0.002*	0.477	0.588	3.000	0.192	0.000	0.531	0.0390	0.500
Gomti	3	2	−0.583	0.304	−0.532	0.141	0.787	0.050	0.000	99999	0.0275	0.203
Betwa	2	1	−1.111	0.175	−0.339	0.149	2.930	0.187	0.900	3.6000	0.3310	0.400
Narmada	2	1	0.333	0.775	0.536	0.353	0.623	0.039	0.000	99999	0.0101	0.204
Tapti	1	0	0.000	1.000	0.000	n.a.	n.a.	0.000#	0.000	0.0000	n.a.	n.a.
Krishna	4	3	−0.551	0.312	−1.156	0.064	1.203	0.077	0.000	99999	0.0408	0.261
Chaliyar	6	8	−1.197	0.033*	−2.237	0.044*	1.270	0.081	0.759	99999	0.0303	0.132
Brahmaputra	3	2	0.310	0.669	−0.304	0.218	1.186	0.076	0.000	99999	0.0309	0.222
Imphal	3	6	0.000	0.783	0.133	0.253	6.289	0.403	0.000	34.746	0.1863	0.444

#Note: Time of expansion (in Mya) of the Tapti population was not found due to single haplotype and less sample size.

* $p < 0.05$.

ied by ATPase 8/6 gene regions also supported this view.

However, exclusive occurrence of only one haplotype in Tapti could be possible, if at the time of fragmentation, the small founder population had either only one haplotype within the samples or population loss due to habitat destruction and overexploitation of *C. striata* species, especially in the western part of the headwater at Surat from where the samples were collected.

But less sample size ($n = 4$) of Tapti river is also supported the poor resolution of individuals variation. Another thing supporting the clustering is that the snakehead murrel exhibit parental care as their young ones move along with the mother in shoals, so they remained localize for long time in smaller size samples and have more possibility to be the progeny of limited number of parents or single parent. The Tapti and Narmada rivers started flowing Eastern to the Western Ghats during the Pleistocene. There was high level of genetic differentiation as well as diversity in the samples of Tapti. However, the paleogeographic reconstructions indicated the possibility that the *C. striata* from different river basins sampled are likely to have evolved from a common ancestral gene pool. However, haplotypes h2 were present in Tapti and h6 and h7 in Narmada population.

The observed genetic variability pattern in *C. striata* can be assigned to a recent population expansion after a low effective population size caused by bottlenecks or founder events [35]. Such an explanation is also consistent with the dumbbell-shaped haplotype networks detected and the mismatch distribution analysis further supports a population expansion for Chaliyar samples. Such patterns of diversity, haplotype networks and mismatch distribution strongly suggest a historical influence on the haplotypes frequencies and genetic distances (F_{st} values) of populations.

Mismatch distribution tests and significant Tajima's *D* and negative Fu's values indicated Chaliyar population expansion around 0.081 Mya, which showed a small and unstable population size. Expansion in Imphal, Ganga and Betwa populations happened around 0.403, 0.192 and 0.187 Mya (Pleistocene), respectively. Tapti did not show population expansion, either due to very small sample size or absence of haplotype diversity. Subsequently, fragmented population might be undergoing sudden expansion and formation of new haplotypes with new frequencies. The overall expansion time ranged from 0.031 to 0.403 Mya, when the population became more isolated to create different haplotypes and more acceptable as explained by IBD [22] for explaining two hypothesis [as suggested by 36]: (i) the river colonized by a single large heterogeneous lineage of fish that differentiated in several isolated groups in diverse areas, and (ii) river colonized by a multiple genetically different lineages of fish from different refugia that laterally homogenized in the river. In this study, the overall correlation is positive and significant and when compared cluster-wise according to UPGMA dendrogram, based on genetic relatedness of rivers, the only two clusters, viz. Ganga, Betwa with Narmada and Brahmaputra, Imphal with Tapti, Krishna, were non-significant between pairwise genetic distance (F_{st}) and geographical distance (km) of rivers. The sharing of common haplotypes, high within population variation and genetic relationship suggested that first two hypothesis are explained by population differentiation. Though, it may not be conclusive evidence, yet the results point out strong possibility of *C. striata* and their ancestors might have suffered reduction in size in the past and, thus, losing genetic variation. The Tapti population did not show intra-molecular diversity, while Chaliyar population showed maximum diversity.

Another possible explanation for the lack of mtDNA diversity within some locations may be due to selective sweep, since there is no recombination in the mitochondrial genomes [35]. Fossil records of the family Channidae indicated that these fishes to be 50 Mya old during early Eocene epoch. By 17 Mya during the early Miocene, Channidae had spread into Western and Central Eurasia. During the late Tortonian (8 Mya), they could be found throughout Africa and East Asia. The members of family Channidae were adapted to climate changes of high downfall with mean temperatures of 20°C. Thus, the snakehead migration to Europe and Asia corresponded to the development of the inter-tropical convergence zone, which has increased air humidity and step-up the East Asian monsoon [37]. The Pleistocene event may explain the most historical coalescence of a species whereby multiple installments of glaciations and de-glaciations occurred and with lowering and rising of sea water level around 2 Mya.

Populations of 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 harmful alleles as a result of genetic drift in the event of small effective population sizes. Hence, in the present study, the exploitation and rapid loss of *C. striata* happening in natural stocks is of concern and need moderation through genetic diversity data based conservation plans. Artificial propagation of this species, requiring prior knowledge on genetic composition of potential source populations, can result in effective restoration programs for conservation of natural populations [37].

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DECLARATIONS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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SPELL: 1. riverine
REPLY:1.correct