ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(4): 268-272

www.fisheriesjournal.com
Received: 03-05-2016
Accepted: 04-06-2016

Buddhadev Sarmah
Directorate of Extension Education, Assam Agricultural University, Jorhat-785013, Assam, India.

Bhaswati Sarmah
Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat-785013, Assam, India.

Correspondence
Bhaswati Sarmah
Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat-785013, Assam, India.

mtDNA cytochrome-b gene assisted genetic diversity study of *Catla catla*

Buddhadev Sarmah and Bhaswati Sarmah

Abstract
Catla catla is an important carp species found in the river systems of India and its neighbouring countries with a vast scope for commercialization. In order to determine the genetic variation in Catla population collected from different parts of the country (India), the mtDNA cytochrome-b gene was chosen as molecular marker which could provide useful insights into genetic diversity, origin and divergence of the population. In this investigation, 6 haplotypes with 13 polymorphic sites were detected, out of which all 13 were polymorphic singleton sites with no parsimony informative site. The average nucleotide composition of all Catla haplotype sequences was 30.75% Adenine, 27.45% Thymine, 13.41% Guanine and 28.39% Cytosine. The Nucleotide diversity of the haplotypes is 0.008627 ± 0.005855 and the mean number of pair wise differences is 3.666667 ± 2.155097. Clustering of the samples collected from the same river systems into different groups suggests the variability present in the environment

Keywords: *Catla catla*, mtDNA, genetic variation, haplotypes, diversity

1. Introduction
Catla catla, an Indian Major Carp, belonging to the family Cyprinidae [1] enjoy a prime position in the Indian aquaculture scenario due to their fast growing nature and taste. It is one of the major aquaculture species of India, Bangladesh, Myanmar (Burma) and Pakistan [2] and is generally esteemed for eating purposes next to *Labeo rohita*. This species, like any other domesticated fish species, is also found in rivers, lakes, tanks and ponds, and is often predisposed to variation [3]. Catla (common name) is native to the rivers of north India, plains of Indus river of Pakistan, rivers of Bangladesh, Nepal and Myanmar. As reported by Talwar and Jhingran [4], Catla fish breeds in the flowing water of riverine ecosystem. The popularity of Catla increased in carp polyculture systems among the fish farmers of Indian peninsula *i.e.,* India, Bangladesh, Myanmar, Laos, Pakistan and Thailand, due to some specific reasons, such as, its growth rate is very high in comparison to other carps, feeding habit is also different, as they are surface feeder and above all the consumer's preference.
The detection of genetic variation at the species, population and within population level is of great importance for sustainable aquaculture practices [5]. Variation at the population level can provide an idea about different genetic classes, the genetic diversity among them and their evolutionary relationship with wild relatives. The information on the identity of individuals, breeding pattern, degree of relatedness and distribution of genetic variation among them could easily be meet by knowing the genetic variability within the population [6]. The variability of qualitative and quantitative characteristics in Catla are enriched and its genetic diversity increased as a consequence of its adaptability in both wild and culture conditions [7].
The evolution of one species to another can be seen though the changes in Mitochondrial DNA (mtDNA) of closely related species. The mtDNA is often used as a genetic marker system, either alone or in combination with nuclear markers such as microsatellites, for assessing the population structure, gene flow and phylogeny [8]. The cytochrome b region of mtDNA is one of the preferred mitochondrial genes for studying intraspecific genetic variability in fish [9] including many cyprinids [10, 11]. Information on molecular markers in Catla is limited to the identification of microsatellite loci and divergence from other Indian major carps using RAPD markers [12]. There is no documented information based on intraspecific variation analysing mitochondrial genes.
Keeping these views in mind, a study was attempted to determine the genetic variations in Catla as inferred from mitochondrial cyt-b gene from a population collected from different parts of India. This study will also work as a survey to understand the changes of the population as well as provide a proper knowledge of the genetic make-up and variability of fish stocks which will, in turn, help us in the management, conservation of endangered species and improvement of stocks of cultivable species.

2. Materials and methods

2.1 Sample collection

In the present investigation, Catla samples were collected from natural ecosystem (such as rivers and wetlands), culture system (ponds and fisheries) as well as market places from different locations of India (Figure 1). Three fish samples (replicates) were collected from all the collection sites totaling 156 fish samples from 52 different locations of India (Figure 1, Supplementary 1).

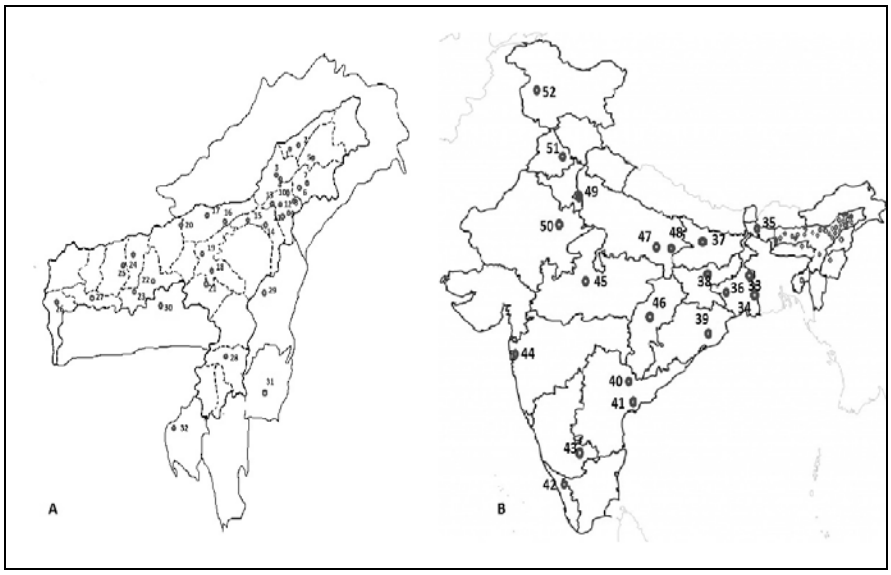

Fig 1: Map of sample collection sites of *Catla catla* in the North-East region **(A)** and overall India **(B)**.

2.2 DNA extraction, PCR amplification and DNA sequencing

DNA extraction of the fish samples was performed in two replications using the QIAGEN-DNeasy Blood and Tissue kit following the procedure of Krista *et al*. [13], which was followed by PCR amplification in a Mastercycler and the mtDNA sequences containing the cyt-b gene were isolated [14]. Amplification of a part of mtDNA was performed in 15µl reaction mixture followed by purification of the amplified products by adding 1 µl of Exo-SAP (Shrimp Alkaline Phosphatase) per 10 µl reaction. After this the purified PCR products were kept at -20°C for further sequencing. The PCR products were sequenced using both the amplifying primers (L14724 and H15149) [15] by the ABI Prism Big Dye Terminator Cycle Sequencing kit according to the manufacturer's instructions. The purified products were then analyzed with ABI Prism 3100 DNA Analyzer (Applied Biosystems).

2.3 Analysis of DNA sequences

The Chromas software was used to check the validity and errors of the sequences obtained. The cyt-b sequences obtained had both forward and reverse sequences which were imported to MEGA version 5.0 [16] and then compared and visually refined. The sequences were allowed to align from the forward and reverse sections of DNA in MEGA. The alignment was also done in BioEdit [17]. The sequenced data were analyzed with the help of BLAST (www.ncbi.nlm.nih.gov.) for homology search. Neighbor-joining and Maximum likelihood Phylogenetic trees were drawn from the haplotypes obtained from the samples resolving the relationships among closely related haplotypes (NC002709 was used as an out group).

Haplotypes were represented graphically using NETWORK 4.6.0.0 software [18] for visualization of the relationship among the haplotypes. Parsimony network of all the haplotypes were constructed in the TCS version 1.21 [19]. Genetic distances among different mtDNA sequences were calculated by Kimura 2 parameter method and a matrix was made to calculate pair wise genetic distance within the population using MEGA5.0 [16]. DnaSP 5.0 [20] was used to define the haplotype diversity and polymorphic sites from the aligned data sets. The Nucleotide diversity of the haplotypes, mean number of pairwise differences and the molecular diversity indexes were calculated with ARLEQUIN version 3.0 [21].

3. Results and Discussion

Sequencing of 425 bp of cytochrome b gene fragments of Catla from 156 samples of India resulted in six different haplotypes (CAJOR5, CWBNA2, CADHE2, CPNBC1, CRJJA, CJKDA1) (Supplementary 1&2). The Neighbor-joining phylogenetic tree (Figure 2 (A)) and Maximum likelihood tree (Figure 2 (B)) was also constructed from the haplotype sequences which exhibited that two haplotypes, CWBNA2 and CADHE2, cluster separately from the rest with moderate bootstrap value. The neighbor joining tree shows that the haplotypes do not have any significant bootstrap values. A common haplotype (CAJOR5) was shared among the samples collected from all over India which is perhaps the introduced stock or the dominant stock population. In this dendogram, a haplotype of Catla collected from Noihati of West Bengal province (CWBNA2) has clustered separately indicating more genetic variation than the other haplotypes. NC002079 was used as an out-group for construction of the Neighbor-joining and Maximum likelihood tree. Clustering of the samples from the same river systems into different groups suggest the variability of the environmental conditions. The geographical distance as well as isolation of the closed water bodies has also resulted in the samples having different morphological characters. Introduced populations (from river systems into lakes) do not show phenotypic differentiation among populations as there has been not enough time to generate these [22].

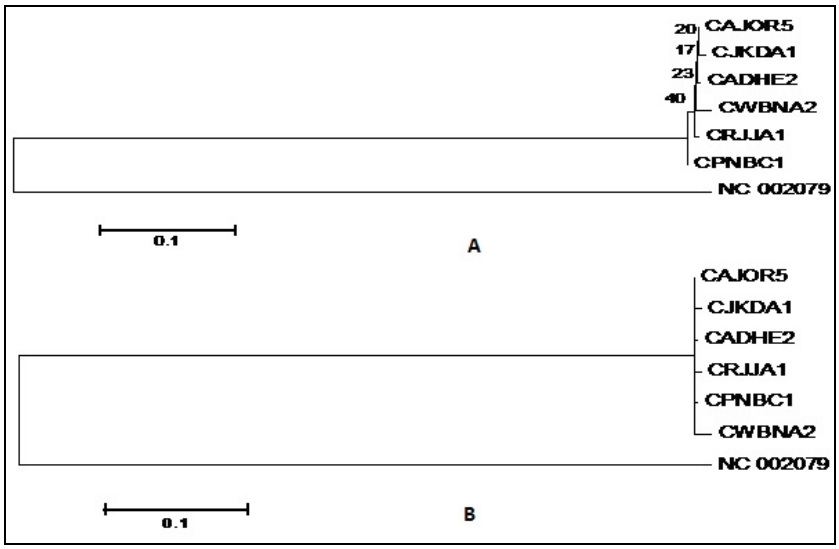

Fig 2: Neighbor-Joining tree (Fig A) and Maximum likelihood tree (Fig B) displaying relationships among the 6 haplotypes obtained from 156 Catla samples of India. NC002079 is used as an out-group. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

Out of 13 polymorphic sites, all were detected as singleton sites and there is no parsimony informative site (Table 1). The haplotypes CADHE2, CPNBC1, CRJJA1, CJKDA1 and CWBNA2 differ by one, one, two, two and five substitutions respectively from CAJOR5. This natural genetic variation may have occurred due to different environmental condition faced in the process of evolution [23]. The Nucleotide diversity of the haplotypes is 0.008627 ± 0.005855 and the mean number of pairwise differences is 3.666667 ± 2.155097. The molecular diversity indexes resulted in a total of 13 substitutions, of which 7 are transitions, 6 are transversions, and no indels.

Table 1: Polymorphic sites within 6 haplotype sequences of Catla. Dots denote the identical nucleotides

Individual	Nucleotide positions										
	170	171	218	267	387	399	401	410	419	423	424
Cajor5	A	T	A	A	T	A	G	C	C	C	C
Cadhe2	•	•	•	•	•	•	A	•	•	•	•
Cpnbc1	•	•	•	•	•	•	•	•	•	•	A
Crjja1	T	A	•	•	•	•	•	•	•	•	•
Cjkda1	•	•	•	•	•	T	•	•	•	A	•
Cwbna2	•	•	T	G	C	•	•	T	T	•	•

The Nucleotide compositions of the 6 haplotypes are shown in the Figure 3. The average nucleotide composition for all the haplotype sequences was 30.75% Adenine, 27.45% Thymine, 13.41% Guanine and 28.39% Cytosine. CADHE2 and CPNBC1 exhibited the highest Adenine composition of 31.1% while CWBNA2 showed the highest Thymine concentration (27.8%). A high concentration of Cytosine (28.5%) was displayed by CAJOR5, CADHE2 and CRJJA1 while CWBNA2 recorded the highest Guanine concentration (13.6%).

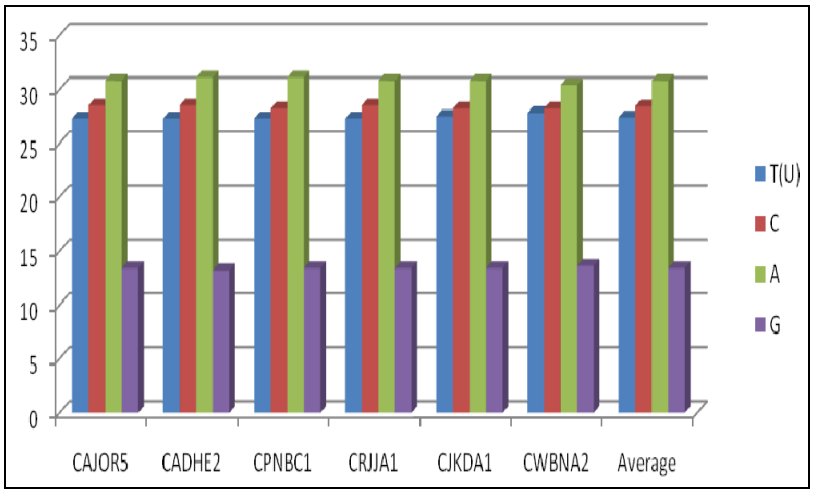

Fig 3: Nucleotide composition of the 6 haplotypes of Catla

Pair-wise genetic distance values among haplotypes calculated in MEGA have showed that the genetic distance between CAJOR5 and CADHE2 is 0.002, CAJOR5 and CWBNA2 is 0.012 and CADHE2 and CWBNA2 is 0.014. The genetic distance was found to be 0.007 between CPNBC1 and CJKDA1 as well as CPNBC1 and CRJJA1 (Table 2). The Haplotype 6 (CWBNA2) found to have more distance from the remaining haplotypes.

Table 2: Pair-wise genetic distance values among 6 haplotypes of Catla

	1	2	3	4	5
(CAJOR5)					
(CADHE2)	0.002				
(CPNBC1)	0.002	0.005			
(CRJJA1)	0.005	0.007	0.007		
(CJKDA1)	0.005	0.007	0.007	0.009	
(CWBNA2)	0.012	0.014	0.017	0.017	0.017

The Median joining network and Parsimony network of all the six haplotypes based on cytochrome b sequences data of Catla were constructed from the all the six haplotypes of India (Figure 4 & Figure 5).

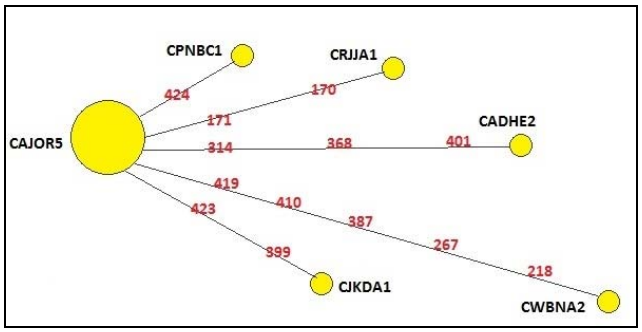

Fig 4: Median joining network of the five haplotypes of Catla obtained from 150 samples from different regions of India based on cytochrome b sequencs. Each circle indicates one haplotype and the length of the branch connecting two haplotypes is proportional to the number of mutations with their site.

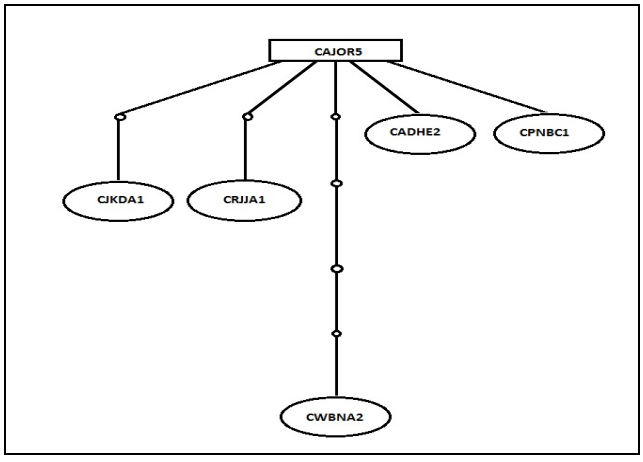

Fig 5: Parsimony network of all 6 haplotypes of Catla based on mtDNA sequence data obtained with TCS version 1.21 (Clement *et al*., 2000). Small circles in the network represent haplotypes not detected in the study.

These analyses reveal that in Indian geographical conditions, the Catla population has been undergoing mutation. Generally, individuals with greater genetic variability have higher growth rates, developmental stability, viability, fecundity, and resistance to environmental stress and diseases [22]. The findings also revealed that the genetic variation in Catla fish populations is more prominent in biodiversity hotspots zones in India. This variability of qualitative and quantitative characteristics in Catla population is enriched and its genetic diversity increased as a consequence of its adaptability in both wild and culture conditions [7]. These genetic variations could help the species in finding out the adaptation for the habitat niche utilization capability of that locality.

4. Conclusion

In the present study, on the genetic variation of Catla, it was found that the haplotypes CWBNA2 and CADHE2 clustered separately and shows more genetic variation than the other haplotypes in addition to displaying the maximum sequence divergence. A common haplotype (CAJOR5) was shared among the samples collected from all over India which is perhaps the introduced stock or the dominant stock population. Moreover, the haplotypes obtained from the same water system clustered separately indicating the variability present among them. We can also conclude that there is a great amount of genetic diversity in the Catla population which will help the species to adapt to the environmental changes occuring due to climate change senario. The species diversity identified will also be of tremendous importance for sustainable aquaculture practices. The species level genetic variation will help in identification of the taxonomic units and to determine the species individuality. Variation at the population level can provide an idea about different genetic classes, the genetic diversity among them and their evolutionary relationship with wild relatives.

5. Acknowledgements

The authors graciously acknowledge the help and guidance bestowed by Late Dr. Umesh C. Goswami, Professor, Department of Zoology, Gauhati University. The author also thanks Dr. U. Ramakrishnan and the staff of Lab 3 of NCBS-TIFR, Bangalore for providing the laboratory facility.

6. Conflict of Interest Statement

The authors declare that there is no conflict of interest.

7. References

1. Berg LS. Classification of fishes, both recent and fossil. Trav. Inst. Zool. Acad. Sci. USSR. 1940; 5(2):517.
2. Jhingran VG. Synopsis of Biological data on catla, *Catla catla* (Ham. 1822). FAO Fisheries Synopsis No. 32 Rev.1. 1968.
3. Beavan R. Handbook of the freshwater fishes of India. L. Reeve & Co., Covent Garden, London, 1877, 247.
4. Talwar PK, Jhingran AG. Inland Fisheries of India and adjacent countries. Oxford & IBH publishing co. PVT.LTD. New Delhi, Bombay, Calcutta. 1991; 1:541.
5. Das P, Prasad H, Meher PK, Barat A, Jana RK. Evaluation of genetic relationship among six *Labeo* species using randomly amplified polymorphic DNA (RAPD). Aquac. Res. 2005; 36(6):564-569.
6. Schierwater B, Streit B, Wagner GP, Desalle R. Molecular Ecology and Evolution: Approaches and Applications. Birkhauser Verlag, Basel, Switzerland, 1994, 495-508.
7. Bakos J, Gorda S. Genetic resources of common carp at the Fish Culture Research Institute, Food & Agriculture Org., Szarvas, Hungary 2001, 417.

8. Avise JC. Phylogeography- The History and Formation of Species. Harvard University Press, USA, 2000, 1-447.
9. Meyer A, Wilson AC. Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. J Mol. Evol. 1990; 31:359-364.
10. Li GY, Wang XZ, Zhao YH, Zhang J, Zhang CG, He SP. Speciation and phylogeography of *Opsariichthys bidens* (Pisces: Cypriniformes: Cyprinidae) in China: analysis of the cytochrome b gene of mtDNA from diverse populations. Zool Stud. 2009; 48:569-583.
11. Watanabe K, Kanagawa N, Kakioka R, Itai T, Mori S. Genetic diversity and conservation units in wild and captive populations of endangered freshwater fishes: a case of *Hemigrammocypris rasborella* in Shizuoka, Japan. Ichthyol Res. 2009; 56:411-416.
12. Rahman MM, Verdegem MCJ, Nagelkerke LAJ, Wahab MA, Milstein A, Verreth JAJ. Growth, production and food preference of rohu *Labeo rohita* (H.) in monoculture and in polyculture with common carp *Cyprinus carpio* (L.) under fed and non-fed ponds. Aquaculture. 2006; 257:359-372.
13. Krista LB, Michael DV, Cameron LA, Cynthia AP. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (*Centrocercus uropihasianus*). Conserv. Genet. 2005; 6:867-870.
14. Bartlett JMS, Stirling D. A Short History of the Polymerase Chain Reaction. PCR Protocols. Methods in Molecular Biology, 2003; 226(2nd ed.):3-6.
15. Irwin DM, Kocher TD, Wilson AC. Evolution of the cytochrome b gene of mammals. J Mol. Evol. 1991; 32:128-144.
16. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Bio. Evol. 2011; 28:2731-2739.
17. Hall T. BioEdit - Biological Sequence Alignment Editor. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html> 2005.
18. Bandelt RJ, Forster P, Rohl A. Median-joining networks for inferring intraspecific phylogenies. Mol. Bio. Evol. 1999; 16(1):37-48.
19. Clement M, Posada D, Crandall K. TCS: a computer program to estimate gene genealogies. Mol Ecol. 2000; 9(10):1657-1660.
20. Rozas J, Sanches JC, DelBarrio L, Messeguer L, Rozas R. DnaSP, DNA polymorphisms analyses by the coalescent and other methods. Bioinformatics. 2003; 19:2496-2497.
21. Excoffier L, Laval G, Schneider S. Arlequin 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online. 2005; 1:47-50.
22. Carvalho GR. Evolutionary aspects of fish distribution: genetic variability and adaptation. J Fish Biol. 1993; 43:53-73.
23. Singh AK, Pathak AK, Lakra WS. Invasion of an exotic fish-common carp, *Cyprinus carpio* L. (Actinopterygii: Cypriniformes: Cyprinidae) in the Ganga River, India and its impacts. Acta Ichthyol. Piscat. 2010; 40(1):11-19.