EUROPEAN ACADEMIC RESEARCH
Vol. V, Issue 2/ May 2017

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Genetic Variation among Ompok bimaculatus, Clupisoma sinensis, Labeo rohita and Puntius sophore Fishes from Jaikwadi and Vishnupuri Dams, from Godavari River in System Using Mitochondrial Cytochrome b Gene

RABEEHA MANKHI JEBUR
Basrah Southern Technical University
College of Health & Medical Technology, Basra, Iraq

Abstract:

Four species of fishes (Ompok bimaculatus, Clupisoma sinensis, Labeo rohita and Puntius sophore) were collected from two different location or population (Jaikwadi and Vishnupuri Dams) of Godavari river in system , twenty samples of each species were passed through several steps include (DNA isolation , design of specific primes for cytochrome b gene amplification, Quantification of DNA, PCR technical where cyt b gene amplified for Ompok bimaculatus & Clupisoma sinensis in this study was 1066 bp long and 1252 bp long was for (Labeo rohita and Puntius sophore) and sequencing process of cyt b gene for each species of fishes finally analysis of sequences to reveal genetic diversity among these species. The results showed after sequencing process for Ompok bimaculatus & Clupisoma sinensis species ,14 samples were analysed for Clupisoma sinensis at Vishnupuri dam for nucleotide composition and 16 samples for Ompok bimaculatus at Jaikwadi respectively, A (29.4%), T (U)(27.7%), G (14.5%), C (28.5%) and A (29.0%), T(U) (26.5%), G(14.4%), C(30.1%). nucleotide diversity, Pi(t) was 0.09309 between Nanded and Paithan populations . Two haplotypes of Ompok bimaculatus were formed while one haplotype of Clupisoma sinensis was formed, This indicates represent low genetic variation of Ompok bimaculatus. where as UPGMA cluster analysis showed sequence divergence only in Ompok

bimaculatus and no divergence in Clupisoma sinensis. Unfortunately, rest of two species (Labeo rohita and Puntius sophore) were failed to get their cyt b gene sequenes due to some technical reasons with trying of sequencing process.

Key words: Cytochrome b gene, genetic diversity, haplotypes, nucleotide composition, UPGMA & Pi(t) .

1. INTRODUCTION

Genetic variation describes naturally occurring genetic differences among individuals of the same species. This variation permits flexibility and survival of a population in the face of changing environmental circumstances. When a population interbreeds, nonrandom mating can sometimes occur because one organism chooses to mate with another based on certain traits. In this case, individuals in the population make specific behavioral choices, and these choices shape the genetic combinations that appear in successive generations [1]. The physical distribution of individuals affect a population with broad distribution, rarely it has the same genetic makeup over its entire range [1]. The individuals in a population living at one end of the range may live at a higher altitude and encounter different climatic conditions than others living at the opposite end at a lower altitude. At this more extreme boundary, the relative allele frequency may differ dramatically from those at the opposite boundary. If the individuals at either end of the range reconnect and continue mating, the resulting genetic intermixing can contribute to more genetic variation overall. However, if the range becomes wide enough that interbreeding between opposite ends becomes less and less likely, and the different forces acting at either end become more and more pronounced, and the individuals at each end of the population

range may eventually become genetically distinct from one another. Distribution is one way that genetic variation can be preserved in large populations over wide physical ranges, as different forces will shift relative allele frequencies in different ways at either end. In species composed of large numbers of migratory individuals, extrinsic barriers to migration imposed by landscape features are particularly important in determining population genetic structure.

In recent years, different molecular techniques, using nuclear and mitochondrial DNA (mtDNA), have provided new information concerning the genetic variability of wild and cultivated populations of several fish species (Was, A. and R. Wenne, 2002). Mitochondrial DNA is maternally inherited without genetic recombination. The evolutionary rate as well as the genetic differentiation of mtDNA among populations is thought to be approximately 5-10 times higher than that exhibited by nuclear genes (Birky C.W., T. Maruyana and P. Fuerst, 1983). Mitochondrial DNA represents a significant marker system for use in population and phylogenetic studies. An extensive review of the advantages of mt DNA as a tool for population genetic analysis has been provided (Avise, J.C., 1991). Among many mitochondrial genes, cytochrome b has been used successfully to identify genetic variation in many fish species (McVeigh H. P *et.al* 1991). Cytochrome b tends to show intra specific variation mainly in 3rd position of codon which can be used to identify stocks. Variation in mtDNA Cytochrome b gene has been used for poplation studies in cyprinidae fishes (Fayazi J *et.al* 2006). The genetic diversity of Ompok bimaculatus was previously studied by Malakar A K *et. al* in 2012. The aim of present study was to present an assessment of the genetic variability due to effect of extrinsic barriers. Three species (*Ompok bimaculatus, Labeo rohita* and *Puntius sophore*) were selected from two populations of Jaikwadi and Vishnupuri dams which are some of the largest irrigation projects on

Godavari river in the Indian state of Maharashtra. n order to understand the effect of extrinsic barriers on genetic variation and population structure on fishes, three species were selected from The hierarchical analysis of genetic variance revealed that the differentiation was relative high. The geographic pattern of differentiation was accounted by analysis of molecular variance within population and among population. The understanding of genetic differentiation of O. Bimaculatus, L. rohita and P. sophore populations will play a key role in conservation and management of the fish species. Yet, the identification of such barriers and the prediction of their impacts in shaping intraspecific genetic diversity remain a major challenge in population biology (Slatkin 1985; Sork et al. 1999; Wiegand et al. 1999). Studies dealing with the effect of landscape in shaping population structure have largly focused on the consequences of habitat fragmentation caused by recent anthropogenic disturbance (e.g., Aldrich et al. 1998; Gibbs 1998; Van Dongen et al. 1998). Although obviously relevant, such studies generally lack a historical perspective on the influence of landscape structure on temporal dynamics of genetic connectivity among natural populations (Fahrig and Merriam 1994; McCauley 1995; McCauley et al. 1995). Consequently, there is a need for detailed empirical studies that simultaneously quantify the effects of individual landscape features at various geographic scales (Shaw et al. 1994; Kudoh and Whigham 1997; Keyghobadi et al. 1999; Sork et al. 1999). Yet, the identification of such barriers and the prediction of their impacts in shaping intraspecific genetic diversity remain a major challenge in population biology (Slatkin 1985; Sork et al. 1999; Wiegand et al. 1999). Studies dealing with the effect of landscape in shaping population structure have largely focused on the consequences of habitat fragmentation caused by recent anthropogenic disturbance (e.g., Aldrich et al. 1998; Gibbs 1998; Van Dongen et al. 1998). Although obviously relevant, such

studies generally lack a historical perspective on the influence of landscape structure on temporal dynamics of genetic connectivity among natural populations (Fahrig and Merriam 1994; McCauley 1995; McCauley et al. 1995). Consequently, there is a need for detailed empirical studies that simultaneously quantify the effects of individual landscape features at various geographic scales (Shaw et al. 1994; Kudoh and Whigham 1997; Keyghobadi et al. 1999; Sork et al. 1999).

2. MATERIAL AND METHODS

The Godavari River is the second largest river in India originating in the Western Ghats Trimbakeshwar, in the Nashik flowing eastwardly across the Deccan Plateau through the states of Maharashtra with a catchment area of 312,812 km2 and a long-term average annual surface flow of 110 km3. The Jayakwadi dam near Paithan is one of the largest earthen dam in India (height 41.30 m and catchment area 21,750 km^2). This dam was built to address the problem of drought in Marathwada region and problem of flood along the bank of river. While Vishnupuri dam near Nanded is one of the largest lift irrigation projects in Asia.(Fig.1)

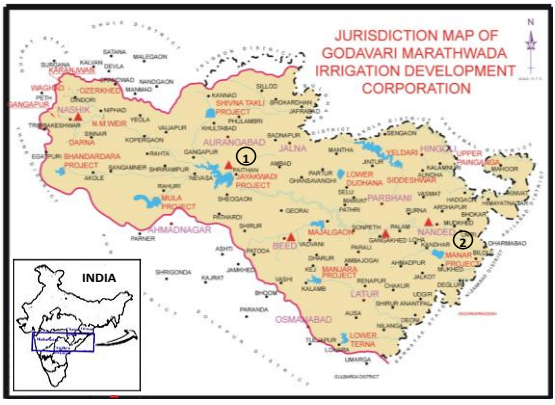


Figure 1: Location of sampling stations on Godavari river in Marathwada Juridisdiction. (Map was adopted from Irrigation development corporation Marashtra, India)

The four fish species were collected from two sampling stations (table 1) and fin clip tissue samples having approximate size of 1-4 sq. cm were collected from caudal fin or left side of Pectoral or pelvic fin without disturbing their morphology. Then this tissue samples were preserved in 100% ethanol in 1.5ml microtubes with appropriate code as tagged on specimens. The Fish specimens were preserved in 70% ethanol.

Srno.	Sampling station	Impounds	Jaikwadi dam	Vishnupuri dam
1	*Ompok bimaculatus*	Godavari river	20	0
2	*Clupisoma sinensis*	Godavari river	0	20
3	*Labeo rohita*	Godavari river	20	20
4	*Puntius sophore*	Godavari river	20	20

Table 1: List of specimens collected from two sampling stations of Godavari river (MS).

2.1. Sample Processing

The tissue samples were then sub sampled and processed further for genomic DNA extraction and tissue stock was preserved at -20^{0}c. The Specimens were washed and permanently preserved in glass jars after morphological investigations in fresh 70% ethanol at room temperature. The genomic DNA was extracted by using C-TAB method / Promega wizard genomic DNA isolation kit . The isolated DNA was further investigated for quantified by using spectrophotometer for 20 samples for each species and for quality check by agarose gel electrophoresis for all species of fishes and resolved on 1% agarose gels are shown in figures.3 (a,b,c ,d).

2.2. DNA Extraction

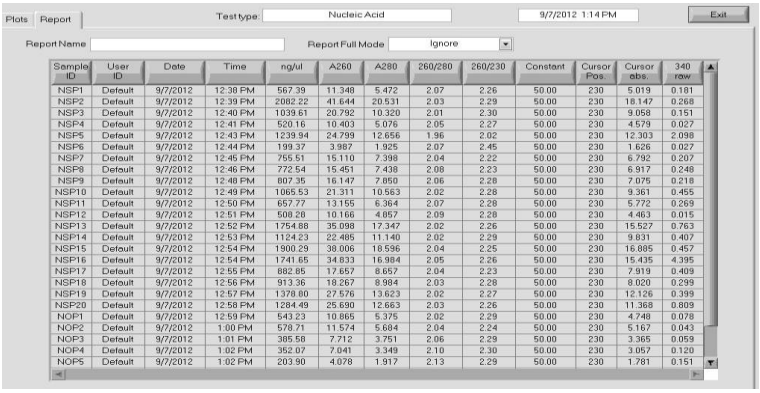
DNA was extracted from ethanol- preserved fin clips using CTAB method (Innis et al.1989). was performed as below

1. Dry the alcohol dipped samples, transfer it to micro centrifuge tube.

2. Add 60c prewarmed CTAB (600ul +3ul of Beta-mercapto ethanol +10ul of 20%SDS).
3. After crushing add 3ul of proteinase-k and incubated over night.
4. Vortex each tube for 5 min vigorously.
5. Keep it over night incubation and vortex after interval of 2-3 hours if possible after incubation cooled samples to room temperature, and centrifuge at 14.000 rpm for 10 minutes.
6. Take supernatant and discard the pellet.
7. Transfer 400ul of sample into fresh tube.
8. Add equal volume of phenol: chloroform: isomyl alcohol (25:24:1) and mixed it for 5 minutes (vortex).
9. After mixing, centrifuge at 14.000 rpm for 10 min .
10. Then take supernatant to fresh tube and discard the pellet.
11. Add chloroform: isomyl alcohol(24:1),600ul in each tube ,and mix it(vortex).
12. Centrifuge at 12.000rpm (10 min).
13. Take supernatant in fresh tube and discard the debris.
14. Add chilled isopropanol (400ul) and mixed slowly until white flakes appear(usually does not appear).
15. Then keep it at(- 35 or -50 c) in deep freeze (1 hour).
16. Brought the samples at room temperature.
17. Centrifuge at 10.000 rpm(10 min).
18. Decant the supernatant and add 70% chilled from refrigerator ethanol(400ul)+ ammonium acetate (100ul) already liquid. to the pellet for washing.(mild vortexing).
19. Centrifuge at 10.000 rpm(10 min).
20. Decant the supernatant carefully, and add 400ul of absolute alcohol and centrifuge at 10.000 rpm for (10 min).
21. Decant the supernatant and dry the pellet at room temp.dry it for 2-3 hours.
22. Dissolve in TE Buffer.(overnight).

2.3. Quantification of DNA

The DNA isolated is quantified by using Nanodrop (260/280 ratio). The contamination of sample with RNA or Phenolic compounds is detected and samples are processed further for PCR reaction. (Fig. 2).


Plots | Report — Test type: Nucleic Acid — 9/7/2012 1:14 PM — Exit

Report Name — Report Full Mode: Ignore

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos	Cursor abs	340 raw
NSP1	Default	9/7/2012	12:38 PM	567.39	11.348	5.472	2.07	2.26	50.00	230	5.019	0.181
NSP2	Default	9/7/2012	12:39 PM	2082.22	41.644	20.531	2.03	2.29	50.00	230	18.147	0.268
NSP3	Default	9/7/2012	12:40 PM	1039.61	20.792	10.320	2.01	2.30	50.00	230	9.058	0.151
NSP4	Default	9/7/2012	12:41 PM	520.16	10.403	5.076	2.05	2.27	50.00	230	4.579	0.027
NSP5	Default	9/7/2012	12:43 PM	1239.94	24.799	12.656	1.96	2.02	50.00	230	12.303	2.098
NSP6	Default	9/7/2012	12:44 PM	199.37	3.987	1.925	2.07	2.45	50.00	230	1.626	0.027
NSP7	Default	9/7/2012	12:45 PM	755.51	15.110	7.398	2.04	2.22	50.00	230	6.792	0.207
NSP8	Default	9/7/2012	12:46 PM	772.54	15.451	7.438	2.08	2.23	50.00	230	6.917	0.248
NSP9	Default	9/7/2012	12:48 PM	807.35	16.147	7.850	2.06	2.28	50.00	230	7.075	0.218
NSP10	Default	9/7/2012	12:49 PM	1065.53	21.311	10.563	2.02	2.28	50.00	230	9.361	0.455
NSP11	Default	9/7/2012	12:50 PM	657.77	13.155	6.364	2.07	2.28	50.00	230	5.772	0.269
NSP12	Default	9/7/2012	12:51 PM	508.28	10.166	4.857	2.09	2.28	50.00	230	4.463	0.015
NSP13	Default	9/7/2012	12:52 PM	1754.88	35.098	17.347	2.02	2.26	50.00	230	15.527	0.763
NSP14	Default	9/7/2012	12:53 PM	1124.23	22.485	11.140	2.02	2.29	50.00	230	9.831	0.407
NSP15	Default	9/7/2012	12:54 PM	1900.29	38.006	18.596	2.04	2.25	50.00	230	16.885	0.457
NSP16	Default	9/7/2012	12:54 PM	1741.65	34.833	16.984	2.05	2.26	50.00	230	15.435	4.395
NSP17	Default	9/7/2012	12:55 PM	882.85	17.657	8.657	2.04	2.23	50.00	230	7.919	0.409
NSP18	Default	9/7/2012	12:56 PM	913.36	18.267	8.984	2.03	2.28	50.00	230	8.020	0.299
NSP19	Default	9/7/2012	12:57 PM	1378.80	27.576	13.623	2.02	2.27	50.00	230	12.126	0.399
NSP20	Default	9/7/2012	12:58 PM	1284.49	25.690	12.663	2.03	2.26	50.00	230	11.368	0.809
NOP1	Default	9/7/2012	12:59 PM	543.23	10.865	5.375	2.02	2.29	50.00	230	4.748	0.078
NOP2	Default	9/7/2012	1:00 PM	578.71	11.574	5.684	2.04	2.24	50.00	230	5.167	0.043
NOP3	Default	9/7/2012	1:01 PM	385.56	7.712	3.751	2.06	2.29	50.00	230	3.365	0.059
NOP4	Default	9/7/2012	1:02 PM	352.07	7.041	3.349	2.10	2.30	50.00	230	3.057	0.120
NOP5	Default	9/7/2012	1:02 PM	203.90	4.078	1.917	2.13	2.29	50.00	230	1.781	0.151

Figure 2: DNA quantification *for Puntius sophore, Clupisoma sinensis Ompok Pabda and Labeo rohita* using Nanodrop (260/280 ratio).

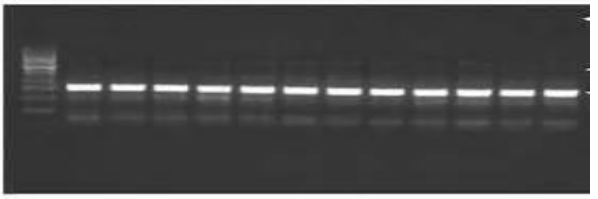


Fig. (3a): Genomic DNA bands extracted from Ompok bimaculatus on 1% agarose gel.

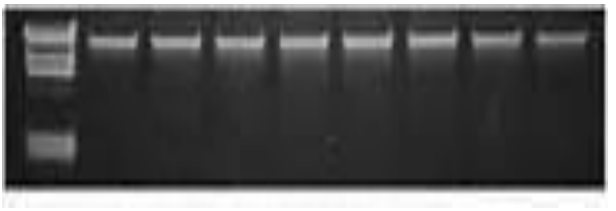


Fig. (3b): Genomic DNA bands extracted from Labeo rohita **on 1% agarose gel**.

Fig.(3c): Genomic DNA bands extracted from Clupisoma sinensis **on 1% agarose gel**.

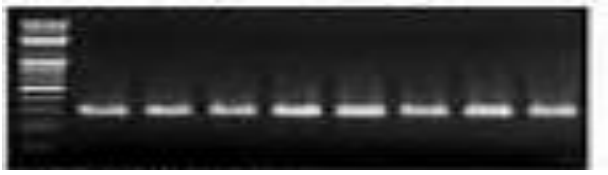


Fig. (3d): Genomic DNA bands extracted from Puntius sophore **on 1% agarose gel**.

In PCR, primers are used to determine the DNA fragment to be amplified by the PCR. The selection of oligonucleotide primers is often critical process for success of PCR. The selection and construction of primer sets for cytochrome b gene was done using software Primer 3 and Oligoclac which provided a small number of primer sets. The specific primer was chosen by trying all possible permutations of its placement, length, and relation to the other primers that meet conditions specified. Thus the primers having good resolution capacity gained high success rate in barcode recovery. The primers designed were mentioned in table below (table 2).

Species Name	Primer	Source	Sequence (5' - 3')
Ompok bimaculatus	OB_f1	NCBI- AY286130.1	AAACATAAATTTCCCTACATGC
	OB_r1	NCBI-AY286130.1	ATCATGCCAATAGAGCCTTA
Clupisoma sinensis	OB_f1	NCBI- AY286130.1	AAACATAAATTTCCCTACATGC
	OB_r1	NCBI-AY286130.1	ATCATGCCAATAGAGCCTTA
Labeo rohita	L14841	Kocher *et.al* 1989	AACAAGCTTCAATCCAACATCTCA
	H15630	Nazia *et.al* 2010	TTAATTTAGAATCCTAGCTTT
Puntius sophore	PS_f1	NCBI- EU241461.1	CATTGGCGTAGTCCTCTTAC
	PS_r1	NCBI- EU241461.1	TTTCTACATGTCCGGCTAGT

Table 2: List of primers designed for PCR and Sequencing of Cytochrome b gene.

Amplification of Cytochrome b gene was attempted using species specific primers (Table 2) for PCR reactions using Kappa biosystems kit in 96-well plates. The reaction master mix consisted of 9.6 µl 10% trehalose, 7 µl H_2O, 2.5 µl 10X PCR buffer 'B', 0.8 µl $MgCl_2$, 2 µl 2.5mM DNTP, 1 µl 10mM forward primer, 1 µl 10mM reverse primer and 0.1 µl taq polymerase (5 units). The PCR reaction profile was comprised of an initial step of 2 min at 95^0C and 35 cycles of 30 sec at 94^0C denaturation , 30 sec at (52-49)^{0}C annealing rang for each species, and 30 sec at 72^0C extension stage, with a final extension at 72^0C for 10 min. Amplicons were visualized on 1.2% agarose Gel , were mentioned in figures ,4(a,b,c,d) below:

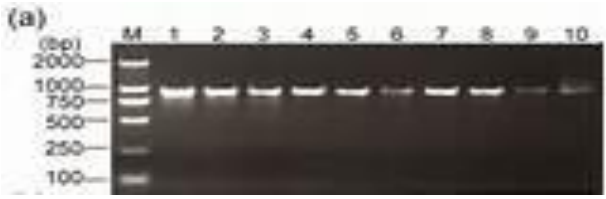


Fig.(4a): Gel image of Cytochrome b gene of Ompok bimaculatus on 1.2% agarose Gel.

Fig.(4b): Gel image of Cytochrome b gene of Labeo rohita on 1.2% agarose Gel.

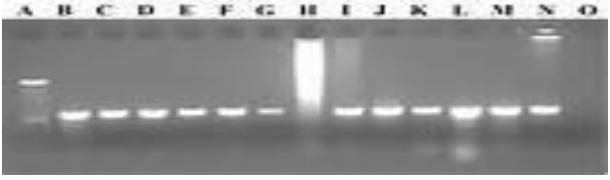


Fig.(4c): Gel image of Cytochrome b gene of Clupisoma sinensis on 1.2% agarose Gel.

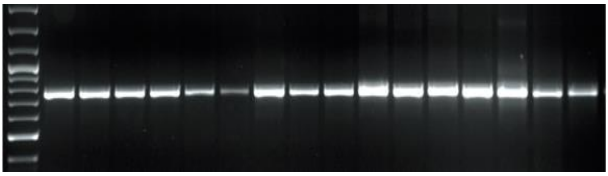


Fig.(4d) : Gel image of Cytochrome b gene of Puntius sophore on 1.2% agarose Gel.

These PCR products were Processed for cleanup to remove un incorporated nucleotide and residual primers. Sequencing reactions used PCR forward and reverse primers using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems,Inc.) because it provides robust sequencing chemistry. It generally produces long reads of 750 base pairs, even on GC-rich templates. The reaction profile was comprised of an initial step of 3 min a 96^0C and 35 cycles of 30 sec at 96^0C, 15 sec at 55^0C,and 4 min at 60^0C. The Cycle sequencing is followed by sequencing cleanup by ethanol precipitation method which is then followed by dissolving template in hidi formamide. These samples were processed for sequencing in ABI 3130 Genetic analyzer capillary sequencer. This barcode product is bi directionally sequenced to aid full length barcode sequence by avoiding problems in signal deterioration that often occur near the end of a read.

2.4. Sequence analysis

There are varieties of DNA analysis software packages used widely.

1) DNA Star (Lasergene) provides another platform for sequence analysis and **primer design**, DNA map drawing etc.
2) Codon code Aligner is a leading software program for DNA sequence analysis. Widely used in evolution research and biogeography, it offers many features including ability to compare contigs with **ClustalW** and

muscle, trace sharpening, and base calling with PHRED, as well as standard features like assembly, trace and contig editing, end clipping, and mutation detection.

3) Mega5 is Molecular Evolutionary Genetics Analysis software that uses maximum likelihood (ML) analyses for inferring **evolutionary trees**, =selecting best-fit **substitution models** (nucleotide or amino acid), inferring ancestral states and sequences (along with probabilities), and estimating evolutionary rates site-by-site. The MEGA user interface has now been enhanced to be activity driven to make it easier for the use of both beginners and experienced scientists.
4) Arlequin is a software for **population genetic data analysis** with quite a large set of methods and stastical tests, in order to extract information on genetic and demographic features of a collection of population samples.

3. RESULTS

Cytochrome b gene amplified for *Ompok bimaculatus* & *Clupisoma sinensis* in this study was 1066 bp long. The *Ompok bimaculatus* & *Clupisoma sinensis* was sequenced for cytochrome b analysis. Unfortunately, rest of two species *Labeo rohita* and *Puntius sophore* were in process of sequencing as they were failed due to some technical reasons .

The average nucleotide percentage among 40 samples of two population of was studied. At Vishnupuri dam for *Clupisoma sinensis* 14 samples were analysed for nucleotide composition showing A (29.4%), T (U) (27.7%), G (14.5%), C (28.5%) and at Jaikwadi for *Ompok bimaculatus* 16 samples were studied showing nucleotide composition A (29.0%), T(U) (26.5%), G(14.4%), C(30.1%). Cyt b gene revealed 100 variable

sites and 100 parsimonic informative sites for 1066 bp long region. The nucleotide diversity, Pi(t) was 0.09309 between Nanded and Paithan populations. A total of three distinct cyt b mt DNA haplotypes were identified in two populations of Ompok pabda (Table 3). that were indicated to DNA polymorphism observed among two species(Table 4). .

Haplotype	Vishnupuri dam	Jaikwadi dam
Hap 1	4	0
Hap 2	10	0
Hap 3	0	16

Table 3: Number of haplotypes detected in two different populations of Ompok bimaculatus at Jaikwadi dam and Clupisoma sinensis Vishnupuri dam.

	Jaikwadi dam (Clupisoma sinensis)	Vishnupuri dam (Ompok bimaculatus)
Number of Haplotypes	1	2
Haplotype diversity	0	0.44
Variance of Haplotype diversity	0	0.01254
Nucleotide diversity	0	0.00159

Table 4: DNA polymorphism observed among two species.

The genetic variation of *Clupisoma sinensis* was not observed within species from Jaikwadi dam while in *Ompok bimaculatus* genetic diversity was observed in means of haplotype diversity and nucleotide diversity.

UPGMA Dendrogram based on cyt b gene sequences shows that, two different clusters of Ompok bimaculatus are formed (Figures(5 and 6). Mean genetic distance between populations observed is 0.01823 thus showing very less genetic variation among individuals of same species of Nanded dam.

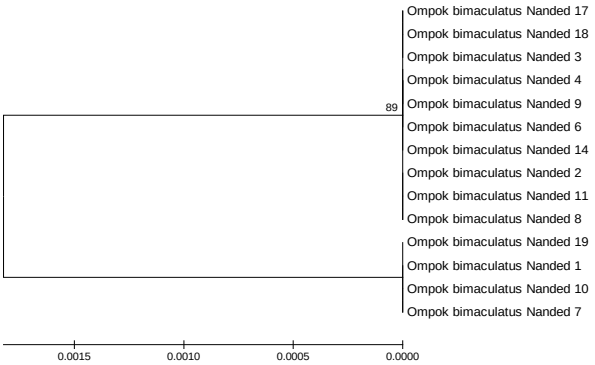


Figure 5: UPGMA Dendrogram, based on the nucleotide divergence, showing the relationship between the Vishnupuri populations of Ompok bimaculutus.

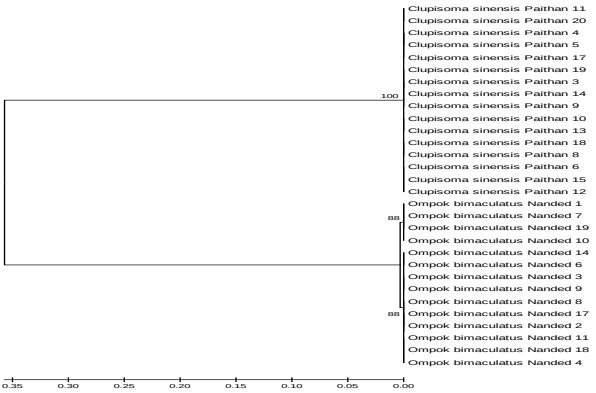


Figure 6: UPGMA Dendrogram, based on the nucleotide divergence, showing the relationship between the Jaikwadi population Paithan and Vishnupuri population Nanded.

4. DISCUSSION

The present study reveals genetic diversity of *Ompok bimaculatus* from Jaikwadi dam and *Clupisoma sinensis* from Vishnupuri dam of Aurangabad, Maharashtra. Two haplotypes of Ompok bimaculatus and one haplotype of Clupisoma sinensis were formed. The diversity indices represent low genetic

variation of Ompok bimaculatus. While UPGMA cluster analysis showed sequence divergence only in Ompok bimaculatus and no divergence in Clupisoma sinensis.

The *Labeo rohita* and *Puntius sophore* species are still to be sequenced and analysed for their diversity indices. Thus current work determines low diversity of Ompok bimaculatus and no diversity of Clupisoma sinensis among the population of Jaikwadi and Vishnupuri dam.

REFERENCES

1) Aldrich, P. R., J. L. Hamrick, P. Charvarriaga, and G. Kochert.1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree Symphonia globulifera. Mol. Ecol. 7:933–944
2) Fahrig, L., and G. Merriam. 1994. Conservation of fragmented populations. Cons. Biol. 8:50–59.
3) Shaw, P. W., G. R. Carvalho, A. E. Magurran, and B. H. Sheghers.1991. Population differentiation in Trinidadian guppies: patterns and problems. J. Fish Biol. 39:203–209
4) Was, A. and R. Wenne, 2002. Genetic differentiation in hatchery and wild sea trout (Salmo trutta) in the Southern Baltic at microsatellite loci. Aquaculture., 204: 493-506.
5) Birky C.W., T. Maruyana and P. Fuerst, 1983. An approach to population and evolution genetic theory for genes in mitochondria and chloroplasts and some results. Genetics., 103: 513-527.
6) Avise, J.C., 1991. Ten unorthodox perspectives on evolution prompted by comparative population

genetics findings on mitochondrial DNA. Annu. Rev. Genet., 25: 45-69.

7) McVeigh H. P., S. E. Bartlett and W. S. Davidson, 1991. Polymerase chain reaction/direct sequence analysis of the cytochrome b gene in Salmo salar. Aquaculture., 95: 225–233.
8) Fayazi J., M. Moradi, G. Rahimi, R. Ashtyani and H. Galledari, 2006. Genetic differentiation and phylogenetic relationships among Barbus xanthopterus (Cyprinidae) populations in south west of Iran using mitochondrial DNA markers. Pak J. Biol. Sci., 9(12): 2249-2254.
9) Seema Sah, Ashoktaru Barat, Veena Pande, Jyoti Sati and Chirag Goel, 2011. Population Structure of Indian Hill Trout(Barilius bendelisis) Inferred from Variation in Mitochondrial Dna Sequences Advances in Biological Research 5 (2): 93-98.
10) http://www.nature.com/scitable/topicpage/the-genetic-variation-in-a-population-is-6526354