INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.vmsindia.org

Analysis of Genetic Divergence and Molecular Phylogenetic Relationship in Teleosts

D. M. Agase[1], S. B. Zade[2], A.M. Nagwnshi[2], R. R. Pimpalkar[2], M.R.Uke[3] and T. Srinivasu[3]

[1]D. D. Bhoyar Arts and Science College Mouda, Dist. Nagpur (MS) India
[2]Department of Zoology, R.T.M. Nagpur University, Nagpur (MS) India
[3]Department of Molecular Biology & Genetic Engineering, R.T.M. Nagpur University, Nagpur (MS) India
E-mail: sbt.durgesh@gmail.com

Abstract
Molecular phylogenetics is the fundamental aspect of biology. The similarity of molecular sequences in living organism strongly suggests that species descendent from a common ancestor. Molecular phylogenetics uses the sequence of molecules (nucleotides and amino acids) and how the change over time to infer these evolutionary relationship. The present study was undertaken the use of in silico approach and RAPD assay to generate species specific phylogeny and evaluate the relationship among different group of fresh water and aquarium fishes. In the present study, nine fresh water fishes and three ornamental fishes selected which belongs to the four different orders namely, Cypriniformes, Siluriformes, Perciformes and Cypridontiformes. These phylogenetic trees constructed from UPGMA method. RAPD analysis shows the great variation among different groups of fishes for the marker OPA6 and OPA7. In the present study, 51 RAPD bands were detected for primer OPA7 (GAAACGGGTG) while the lowest numbers of RAPD bands were scored for primer OPA9 (GGGTAACGCC). From this study OPA7 primer is found to be useful for analysis of genetic divergence and molecular phylogenetic relationship in Teleosts.
Keywords: Phylogenetics, Genetic Divergence, RAPD, In silico , UPGMA

1. INTRODUCTION

Fishes are an extremely diverse group of vertebrates. They exhibit remarkable level of diversity and it is important to humans from economical, ecological and cultural points of view. Due to some environmental effect various types of variation are found in fishes which affect their morphology, ecology, ethology and genetics. These genetic variations in a species enhance the capability of organism to adapt to changing environment and are necessary for survival of the species. In conjunction with other evolutionary forces like selection and genetic drift, genetic variation arises between individuals leading to differentiation at the level of population, species and higher order taxonomic groups (Volf.,2005).

In fishery science, genetic characterization is important for any successful fish breeding program as well as gene banks for conserving genetic resources. Information on genetic characterization within hatchery stocks indicates the level of success in their management. Many molecular techniques are now available, which allow Taxonomist and evolutionary biologists to determine the genetic architecture of a wide variety of closely related individuals (Goswami *et al,* 2016; Abdul and Muneer,2014).

The present study is undertaken with two major objectives. First one consisting of RAPD assay to generate species-specific RAPD profiles to infer the phylogeny. The second one consisting of an *In-silico* approach to reconstruct the phylogeny and evaluate the relationship between nine fresh water fishes and three ornamental fishes of four different orders.

2. MATERIAL AND METHOD

2.1 RAPD PROFILING

2.1.1 Taxon sampling and specimens

Three ornamental fishes were selected which belongs to four different orders namely, Cypriniformes, Siluriformes, Perciformes and Cypridontiformes. The nine freshwater fishes comprises *Catla catla* H., *Labeo rohita* H., *Cirrhinus mrigala* H., *Channa puncatas* B, *Clarias gariepinus* B., *Clarias batrachus* L., *Wallago attu* B., *Oreochromis mossambicus* P. and *Cyprinus carpio* L. collected from Ambazari lake of Nagpur. The three ornamental fishes comprise *Carassius auratus* L., *Poecilia reticulate* P. and *Danio rerio* H. which are collected from aquarium shop. Six fish specimen from each species were collected for genomic diversity analysis. Tissues were collected from fishes fin for isolation of DNA from tissue.

2.1.2 Genomic DNA extraction

DNA was prepared from fin tissue described by Hillis & Moritz with some modifications (Hillis & Moritz,1990). Approximately 50 mg of the caudal fin tissue was cut into small pieces and suspended in 500 µl STE (0.1 M NaCl, 0.05 M Tris and 0.01 M EDTA, pH 8). After adding 30 µl SDS (10 per cent) and 30 µl proteinase K (10 mg/ml 1), the mixture was incubated at 50°C for 30 min. DNA was purified by successive extraction with phenol:chloroform:

isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1), respectively. DNA was precipitated with ice-cold absolute ethanol and washed with 70% ethanol. The pellet was dried and resuspended in 150 µl TE (10 mM Tris-HC1, 1 mM $Na_2EDTA.H20$, pH 7.2) and incubate the tube at 4°C until DNA has complete dissolved.

2.1.3 PCR primers

Five commercially available decamer primers (OPA-6, OPA-7, OPA-8, OPA-9 and OPA-14) from Operon technologies (Alameda, CA, USA) were used in the present study for RAPD-PCR amplification.

Table1: Sequence and operon codes of the random primers used to study variation in 12 Fish species

Primers Codes	Sequence (5' to 3')
OP A 06	GGTCCCTGAC
OP A 07	GAAACGGGTG
OP A 08	GTGACGTAGG
OP A 09	GGGTAACGCC
OP A 14	TCTGTGCTGG

2.1.4 PCR amplification

PCR reaction was carried out in a 25 µl reaction volume containing DNA template, 10X Taq polymerase buffer, and 200 µM of each dNTPs (Hi-Media, India). A negative control, without template DNA was included in each round of reactions. DNA amplification was performed in Eppendorf mastercycler. PCR thermal was performed in a 30 cycles. Each cycle consisted of 94° C denaturing for 30 sec, 40° C annealing 30 sec and 72° C extensions for 30 sec. The thermal cycles were started with an initial denaturizing of 95° C for 5 minutes and final 72° C extensions for 10 minutes for polishing the ends (making smooth) of PCR products.

2.1.5 Agarose gel electrophoresis

The resulting PCR products were resolved (10µl product mixed with 2 µl (6X) gel loading dye) on 1% agarose gel using submarine gel electrophoresis for 1 hour in 1X TAE buffer (Tris-HCL, Acetic acid, EDTA; pH 8.0). Subsequently, gels were stained with Ethidium bromide (10mg/ml) and photographed on an ultraviolet (UV) transilluminator using a gel documentation system (Bio Rad Gel Doc). A known DNA size marker was run with every gel (100bp ladder from Hi Media, India).

2.2 *IN SILICO* approch

Molecular phylogenetics is a fundamental aspect of bioinformatics. The similarity of molecular sequences in living organisms strongly suggests that species descended from a common ancestor. Similarities and divergence among related molecular sequences revealed by sequence alignment visualized in the form of Phylogenetic trees. Multiple Sequence Alignment methods were used to predict homology and estimate a phylogeny (Pearson,2013).

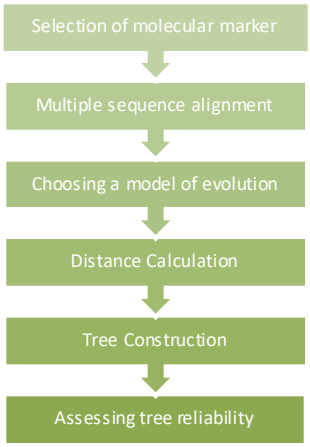

Figure 1: Steps in Phylogenetic Analysis

2.2. 1 Selection of molecular marker for *in silico* approch

The choice of molecular markers is an important because it can make a major difference in obtaining a correct tree. A single-copy gene may be more useful than multiple-copy gene. The substitution rate should be optimum so as to provide enough informative sites. Too many base variations among the taxa, is not preferable which may not reflect the true ancestry. So far, these molecular studies of divergence have drawn on DNA or amino acid sequence data for highly conserved genes, particularly the structural ribosomal genes 18S/16S/5S/28S, the nuclear protein-coding gene elongation factor-1a (EF-1α) and the slowly evolving mitochondrial gene cytochrome c oxidase I (COI), histone H3, U2 snRNA and many more genes which are widely distributed (Anand Patwardhan *et al,* 2014). In the present study we have analyzed and compiled 10 datasets of marker genes that are shared by different fish models. Ten datasets of marker genes were 18S rRNA, Beta-actin, Cytochrome-b, Cytochrome oxidase, RAG 1, GSTT, GAPDH, HSP70, TLR1 and TLR3. Marker genes sequences were retrieved from National Center for Biotechnology Information (NCBI) through a

sequence retrieval system (Patwardhan *et al.*, 2014).

2.2.2 Phylogenetic tree reconstruction by UPGMA method

The simplest distance based clustering method is UPGMA, which builds a tree by a sequential clustering. Given a distance matrix, it starts by grouping two taxa with the smallest pairwise distance in the distance matrix. A node is placed at the midpoint or half distance between them. It then creates a reduced matrix by treating the new cluster as a single taxon. The distances between this new composite taxon and all remaining taxa are calculated to create a reduced matrix. The same grouping process is repeated and another newly reduced matrix is created. The iteration continues until all taxa are placed on the tree. The last taxon added is considered the out group producing a rooted tree. The basic assumption of the UPGMA method is that all taxa evolve at a constant rate and that they are equally distant from the root, implying that a molecular clock is in effect (Graur*et al.*,2000).

3. RESULTS

3.1 RAPD PROFILING

We have estimated 5 different decamer primers of OPA6, OPA7, OPA8, OPA9 and OPA14 (Operon technologies Inc., Alameda, CA, USA) having 60-70% GC content. The number of amplified bands those detected varied on the basis of types of primers, species and individuals. A series of discrete bands were obtained after amplification of DNA samples of some fish species with three primers (Table No 2). The different primers produced different banding patterns (Figure 2-4). For the comparison of these patterns, a set of distinct, well separated bands were selected, avoiding the weak and unresolved bands to find out consistent banding pattern among the individuals within specific populations of the fish. Only two primers (OPA6, OPA7 and OPA9) produced potential markers.

The data of inter species specific analytical study indicates that a total of 74 DNA markers (considering all bands) were detected in all group of fish species, out of which, 38 bands were polymorphic, can be considered as useful RAPD markers (Table No 2). The remaining 36 bands were monomorphic suggesting that all three fish species have a common ancestor and are genetically closer to each other. The largest number of RAPD bands were detected for primer OPA7 (51 bands, Figure 3) while the lowest number was scored for primer OPA9 (4 bands, Figure 4). The molecular weight of scorable bands generated by these primers ranged from 200 to 2000 bp. Highest molecular weight range was exhibited for OPA7 (App. 1300bp) while it was lowest for OPA9 (App. 300bp). Number of polymorphic bands produced per primer ranged from one to five with an average of 3.06 bands per primer. Seven scorable bands were obtained by these primers having molecular weight more than 1000 bp and 29 scorable bands were having molecular weight of more than 500 bp (52.73 %). Large numbers of family and species specific bands were scored within RAPD profiles produced by these primers. Out of 38 polymorphic bands, 15 bands were counted as species specific. 12 species specific markers were scored for *Labio rohita* while 9 markers each for *Catla catla* and *C. mrigala* species scored for *Labio rohita* while 9 markers each for *Catla catla* and *C. mrigala* species.

Table 2 : No. of bands observed in different primers, OPA 6, OPA 7 and OPA 9.

Sr. No.	Fish Species	No. of bands with primers		
		OPA 6	OPA7	OPA9
1	*Catla catla* (10 bands)	04	06	00
2	*Cirrihinus mrigala*(07 bands)	03	04	00
3	*Labeo rohita*(05 bands)	01	04	00
4	*Channa punctatus* (02bands)	00	02	00
5	*Clarias batrachus*(08 bands)	00	07	01
6	*Clarias gariepinus*(07 bands)	00	05	02
7	*Wallago attu*(10 bands)	04	05	01
8	*Oreochromis mossambicus* (03bands)	00	03	00
9	*Cyprinus carpio*(04 bands)	01	03	00
10	*Carassius auratus*(09 bands)	02	06	01
11	*Poicelia reticulata*(04 bands)	01	03	00
12	*Danio rerio*(05 bands)	02	03	00

RAPD RESULTS

1. Primer OPA 6

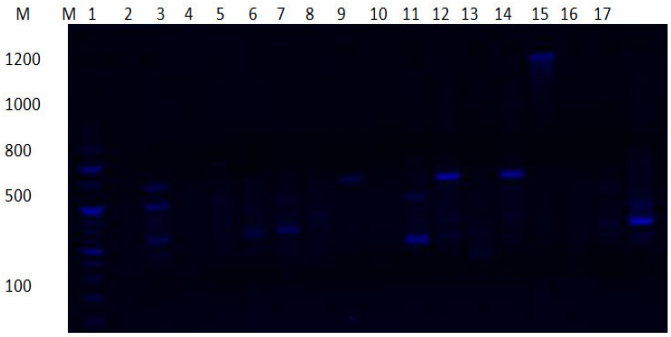

Figure 2: RAPD banding pattern amplified by OPA-6 Primer (GGTCCCTGAC), Lane M- 100 bp DNA ladder, Lane 1- *C.gariepinus*, Lane 2- *W.attu,* Lane 3 & 15-*C.batrachus*, Lane 4- *C.punctatus,* Lane 5 & 6- *C.mrigala,* Lane 7- *L.rohita,* Lane 8 & 14- *C.carpio,* Lane 9- *O.mossambicus*, Lane 10- *C.auratus,* Lane 11 & 13- *D.rerio,* Lane 12- *P.reticulata,* Lane 16 & 17- *C.catla.*

2. Primer OPA 7:

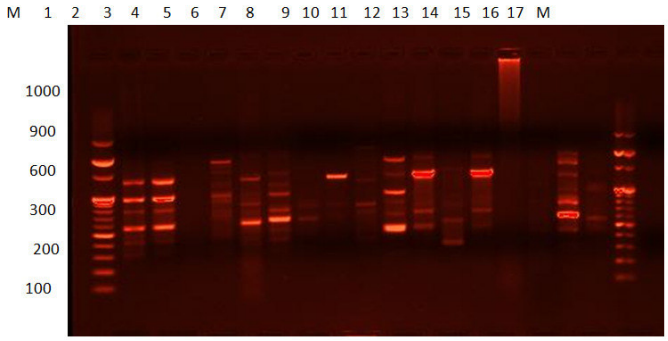

Figure 3: RAPD banding pattern amplified by OPA- 7 Primer (GAAACGGGTG), Lane M - 100 bp DNA ladder, Lane 1&2- *W. attu,* Lane 3 & 7 -*C.punctatus*, Lane 4- *C.gariepinus*, Lane 5- *C.mrigala,* Lane 6- *C.batrachus*, Lane 8 & 15- *C.carpio,* Lane 9 & 14- *O.mossambicus*, Lane 10- *C.catla,* Lane 11& 13- *L.rohita,* Lane 12- *D.rerio,* Lane 16-*C.auratus,* and Lane 17- *P.reticulata.*

3. Primer OPA 9:

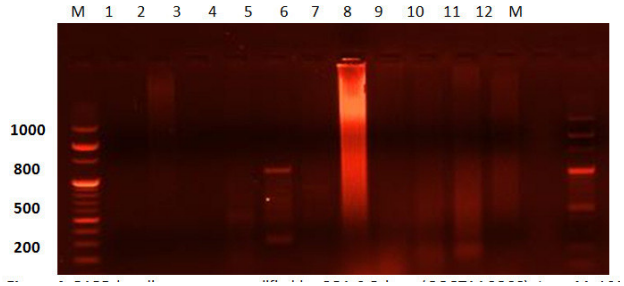

Figure 4: RAPD banding pattern amplified by OPA-9 Primer (GGGTAACGCC), Lane M- 100 bp DNA ladder, Lane 1- *C.catla,* Lane 2- *C.mrigala,* Lane 3- *L.rohita,* Lane 4- *C.punctatus,* Lane 5- *C.gariepinus,* Lane 6- *C.batrachus,* Lane 7- *C.carpio,* Lane 8- *O.mossambicus*, Lane 9- *W.attu,* Lane 10

3.2 Phylogenetic tree

We have analyzed and compiled 10 datasets of marker genes that are shared by different teleost fish models. Marker gene sequences were retrieved from Gene bank. Multiple sequence alignment of retrieved marker gene sequences were performed with the help of Clustal Omega. Overall distance matrix (Table no 3) and phylogenetic tree (Figure 5) of 12 teleost fish created by **UPGMA** method are as follow:

	Cc	Cm	Lr	Cp	Cb	Cg	Wa	Om	Cyc	Ca	Pr	Dr
Cc	0											
Cm	0.107	0										
Lr	0.164	0.332	0									
Cp	0.478	0.415	0.532	0								
Cb	0.464	0.388	0.301	0.533	0							
Cg	0.451	0.422	0.338	0.686	0.266	0						
Wa	0.177	0.181	0.186	0.416	0.123	0.276	0					
Om	0.468	0.433	0.441	0.584	0.432	0.357	0.362	0				
Cyc	0.222	0.348	0.291	0.552	0.331	0.286	0.159	0.427	0			
Ca	0.321	0.314	0.183	0.566	0.249	0.423	0.198	0.423	0.377	0		
Pr	0.325	0.353	0.46	0.343	0.311	0.511	0.296	0.443	0.476	0.452	0	0.456
Dr	0.199	0.323	0.236	0.62	0.479	0.39	0.286	0.516	0.329	0.327	0.436	0

Abbreviations: *Cc- Catla catla, Cm- Cirrhinus mrigala, Lr- Labeo rohita, Cp- Channa punctatus, Cb- Clarias batrachus, Cg- Clarias gariepinus, Wa- Wallago attu, Om-Oreochromis mossambicus, Cyc- Cyprinus carpio, Ca- Carasius auratus, Dr- Danio rerio, Pr- Poecilia reticulate*

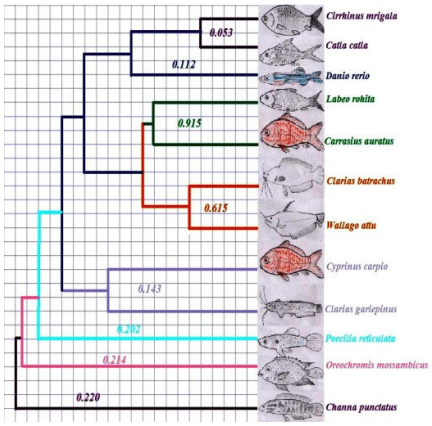

Figure 5: UPGMA Phylogenetic tree depicting the evolutionary relationships among four orders namely,*Cypriniformes*, *Siluriformes*, *Perciformes* and *Cypridontiformes* based on distance Matrix.

4. DISCUSSION

The genetic structure of a population is not static. It is changeable, and the degree of the change depends on genetic as well as environmental interaction. RAPD PCR studies on *Channa punctatius* (Nagarajan *et al,* 2006) showed a significance correlation between genetic identity and geographical distance. M. Shanmughavalli *et al,* (2013) also reported the genetic variations among the species of *Poiecilia* by using RAPD. They showed that RAPD analysis is a rapid and convenient technique for estimating genetic variation between *P. latipinna* and *P. sphenops* and to generate useful genetic markers in molly fishes. RAPD fragments observed in the two individuals, showed a reasonable degree of genetic variation within and between the species. The population specific bands could not be discerned from the fragment patterns generated. The differentiation in various banding pattern have arisen in the present study may be due to drifting of allele frequencies at some loci. The present investigations shows the some degree of genomic polymorphism among the individuals of different fresh water and aquarium fish species which belongs to the four different orders of fisheries. Though RAPD analysis provides baseline information of the population genetics. Studies using microsatellite, allozyme and mitochondrial markers will further enhance our understanding of genetic variability among the different fish species which is economically and ecologically are more important.

4. CONCLUSIONS

Assessment of genetic diversity is important for studying molecular systematic, recognition of threatened and endangered species, optimizing fisheries management and fish farming. As Nagpur is one of the developing city in the Maharashtra, high levels of industrial pollution, construction of dams, hydropower plants, etc. affected the natural population of economically important fishes. These factors prevent the fish from migrating to their natural habitats and eliminate the opportunities for gene flow among the fish populations. These problems confused the fish farmers to identify the original fish stock and fish breeding. In order to

circumvent this problem, a need for testing species specificity and genetic variability by employing whole genome analysis in some Indian major carps from the lake in Nagpur. Therefore, the present study was undertaken the use of novel *in silico approach* and RAPD assay to generate species-specific phylogeny and evaluate the evolutionary relationship among different groups of fresh water and aquarium fishes.

In the present study, phylogenetic tree constructed from UPGMA method shows the splitting of taxa into two major clades, which is designated as Clade I and Clade II. Clade I contains *Cirrihina mrigala* H., *Catla catla* H., *Danio rerio* H., *Clarias batracus* L., *Wallago attu* B., *Labeo rohita* H., *Carassius auratus* L., *Cyprinus carpio* L., *Clarias gariepinus* B., *Poecilia reticulate* P. *and Oreochromis mosambicus* P. and clade II contains *Channa punctatus* B. This phylogenetic tree also shows the monophyletic origin *of Cirrihinus mrigala* H., *Catla catla* H., *Clarias batracus* L., *Wallago attu* B., *Labeo rohita* H., *Carassius auratus* L., *Cyprinus carpio* L., *Clarias gariepinus* B. and parphyletic origin of *Poecilia reticula* P. *Oreochromis mossambicus* P. and *channa punctatus* B. RAPD analysis shows the great variation among the different groups of fishes for the marker OPA 6 and OPA7. In the present study, the largest number of RAPD bands i.e. 51 bands were detected for primer OPA7 (GAAACGGGTG) while the lowest number i.e.5 bands was scored for primer OPA9 (GGGTAACGCC). This specific primer (OPA7) was found to be useful, resulting from different DNA polymorphism among individuals. Such a wide variation in the number of markers produced by these arbitrary primers attributed to the difference in the binding sites throughout the genome of the fish species under study. The diverse nature of DNA bands indicates the genetic distance between fish species but the presence of common bands indicates evolutionary relationship.

The present study concludes that information on the genetic structure of fish species is useful for identification of stocks, enhancement, management of breeding programs and preservation of genetic diversity. *In silico* study and RAPD markers are very useful methods for analysis of genetic divergence and molecular phylogenetic relationship fish species.

REFERENCES

Abdul, P.and Muneer, M.,(2014): Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies. Genetics Research International, 10.1155/2014/691759

Ali, B.A., Huang, T.H., Qin, D.N. and **Wang, X.M. (2004):** A review of Random Amplified Polymorphic DNA (RAPD) markers in fish. *Reviews in Fish Biology and Fisheries*, **14**: 443–453.

Bardakci, F. and **Skibinski, D.O.F. (1994):** Applications of the RAPD technique in tilapia fish: Species and subspecies identification, *Heredity.* **73**: 117-123.

Barman, H.K., Barat, A., Yadav, B.M., Banerjee, S., Meher, P.K., Reddy, P.V.G.K. and **Jana, R.K. (2003):** Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. *Aquaculture,* **217**: 115-123.

Bartfai, R., Egedi, S., Yue, G.H., Kovacs, B., Urbanyi, B., Tamas, G., Horvath, L. and **Orban, L. (2002):** Genetic analysis of two common carp brood stocks by RAPD and microsatellite markers. *Aquaculture,* **62220**: 1–11.

Bartish, I.V., Garkava, L.P., Rumpunen, K. and **Nybom, H. (2000):** Phylogenetic relationships and differentiation among and within populations of Chaenomeles Lindl. (*Rosaceae*) estimated with RAPDs and isozymes. *Theor. Appl. Genet.,* **101**: 554–561.

Bernardi, G. and **Talley, D. (2000):** Genetic evidence for limited dispersal in the coastal California killfish, *fundulus parvipinnis. Journal of Experimental Marine Biology and Ecology,* **255**: 187-199. 63

Brahman, M.P., Das, M.K., Singh, M.R., Sugunan, V.V., Mukharnmjee, S.N., Prakash, S., Maurye, P. and **Hajra, A. (2006):** Use of RAPD fingerprinting for the delineating populations of Hilsa shad *Tenualosailisha* (Hamilton, 1822) *Genet. Mol. Res.,* **5**: 643-652.

Chen, W.J., L. Lheknim, and **R. L. Mayden (2009):** Molecular systematics of the Cobitoidea (Teleostei: Cypriniformes) revisited and phylogenetic position of enigmatic loach Ellopostoma Vaillant 1902: Evidence from six nuclear genes. *Journal of Fish Biology,* **75**: 9 (December 2009).

Das, P., Barat, A., Meher, P.K., Ray, P.P. and **Majumdar, D. (2005a):** Isolation and characterization of polymorphic microsatellites in *Labeo rohita* and their cross-species amplification in related species. *Molecular Ecology Notes,* **5**: 231-233.

Das, P., Prasad, H., Meher, P.K., Barat, A. and **Jana, R.K. (2005b):** Evaluation of genetic relationship among six *Labeo* species using randomly amplified polymorphic DNA (RAPD). *Aquaculture Research,* **36**: 564-569. 6

Degani, G. (2004): Application of RAPD and *cytochrome b* sequences to the study of genetic

variations in F1 and F2 generations of two different strains of guppy (*Poecilia reticulata*) *Aquaculture Research*, **35**: 807- 815.

Dinesh, K.R., Lim, T.M., Chua, K.L., Chan, W.K. and **Phang, V.P. (1993):** RAPD analysis: an efficient method of DNA fingerprinting in fishes, *Zoology Sci.,* **10**: 849- 854.

Goswami, M., Mishra, A., Ninawe.,A.,Truda,V., and Lakra,W., (2016): Bio-banking: An Emerging Approach for Conservation of Fish Germplasm. Poult Fish Wildl Sci. Vol. 4 . 1000143.

Graur, D., and Li, W.-H. (2000): *Fundamentals of Molecular Evolution.* Sunderland, MA: Sinauer Associates.

Higgins, D.G. (2000): Amino acid-based phylogeny and alignment. *Adv. Protein Chem.* 54:99–135.

Koh, T.L., Khoo, G., Fan, L.Q. and **Phang, V.P.E. (1999):** Genetic diversity among wild forms and cultivated varieties of Discuc Symphysodon spp. as revealed by random amplified polymorphic DNA RAPD fingerprinting. *Aquaculture*, **173**: 485–497.

Lal, K.K., Chauhan, T., Mandal, A., Singh, R.K., Khulbe, L., Ponniah, A.G. and **Mohindra, V. (2004a):** Identification of microsatellite DNA markers for population structure analysis in Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882). *Journal of Applied Ichthyology,* **20**: 87-91.

Lal, K.K., Dhirendra Kumar., Srivastava, S.K., Mukherjee, A., Mohindra, V., Prakash, S., Sinha, M. and **Ponniah, A.G. (2004b):** Genetic variation in *Tenualosa ilisha* (Hamilton-buchnan) population in river Ganges. *Indian Journal of Fisheries,* **51**: 33-42. 66

Mohindra, V., Narain, L., Punia, P., Gopalakrishshnan, A., Mandal, A., Kapoor, D., Ponniah, A.G. and **Lal, K.K. (2005):** Microsatellite DNA markers for population – Genetic studies of *Labeo dyocheilus* (McClelland, 1839). *Journal of Applied Ichthyology,* **21**: 481-482.

Nei, M., and Kumar, S. (2000): *Molecular Evolution and Phylogenetics.* New York: Oxford Uni. Press.

Patwardhan,A.,Ray,S. and Roy.A.(2014): Molecular Markers in Phylogenetic Studies-A Review. Phylogen Evolution Biol 2014, 2:2

Pearson,W.R.(2013) An Introduction to Sequence Similarity ("Homology") Searching. Curr Protoc Bioinformaticsdoi: 10.1002/0471250953.bi0301s42.

Reddy, P.V.G.K. (2005): Genetic resources of Indian major carps. FAO Fisheries Technical Paper, **387**: FAO, *Daya Publishing House, New Delhi, India, pp.* 76.

Ryman, N., Utter, F. and **Laikre, L. (1995):** Protection of intra-specific biodiversity of exploited Fishes. *Rev. Fish. Biol. Fish.,* **5**: 417-446

Salemi, M., and Vandamme, A. (2003): *The Phylogenetics Handbook – A Practical Approach to DNA and Protein Phylogeny.* Cambridge, UK: Cambridge University Press.

Sambrook, J. and **D. Russell: Molecular cloning. (2001):** A laboratory manual **3rd** Edn. Cold Spring Harbor *Laboratory Press, New York.*

Seyoum, S. and **Kornfield, I. (1992):** Identification of the sub-species of *Oreochromis niloticus* (Pisces: *Cichlidae*) using restriction endonuclease analysis of mitochondrial DNA. *Aquaculture*, **102**: 29-42. 68

Shanmughavalli, M., Narmathanathiya, K., Arulvasu, C. and **Chandhirasekar, D. (2013):** Genetic variation between molly fishes *Poecilia latipinna* and *Poecilia sphenops* using RAPD assay. *Journal of Academia and Industrial Research (JAIR),* **2 (2)**: 83-88.

Shanmughavalli, M.,Narmathanathiya,K., Arulvasu,C., and Chandhirasekar,D. (2013): Genetic variation between molly fishes *Poecilia latipinna* and *Poecilia sphenops* using RAPD assay. Journal of Academia and Industrial Research (JAIR**)** 2,83-88.

Thornton, J. W., and DeSalle, R. (2000): Gene family evolution and homology: Genomics meets phylogenetics. *Annu. Rev. Genomics Hum. Genet.* 1:41–73.

Volff, J.N., (2005): Genome evolution and biodiversity in teleost fish Heredity, 94, 280–294.

Whelan, S., Lio, P., and Goldman, N.(2001): Molecular phylogenetics: State of the art methods for looking into the past. *Trends Genet.* 17:262–72.

Williams, D. J., Kazianis, S. and **Walter, R. B. (1998):** Use of random amplified polymorphic DNA (RAPD) for identification of largemouth bass subspecies and their intergrades. *Transactions of American Fisheries Society,* **127**: 825-832. 69

Yoon, J. M. and **Kim, G.W. (2001):** Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus. J. Biosci.,* **26**: 641–647.

Zhou, L., Wang, Y. and **Gui, J.F. (2000a):** Analysis of genetic heterogeneity among five gynogenetic clones of *silver crucian carp, Carassius auratus* gibelio *Bloch*, based on detection of RAPD molecular markers. *Cytogenet. Cell Genet.,* ***88*:** 133–139.

❖❖❖❖