J. Ecophysiol. Occup. Hlth. 3 & 4 (2013) 57-65

Genetic Diversity in Four Species of Coldwater Fishes (Family Cyprinidae) using Random Amplified Polymorphic DNA Markers and Implication for their Conservation

Himani Pandey, B.K. Singh, S. Ali[1], G.K. Sivaraman and A. Barat*[1]

Department of Zoology, Kumaun University Nainital 263001, Uttarakhand, India
[1]Directorate of Coldwater Fisheries Research, Indian Council of Agricultural Research
Bhimtal-263136, Nainital, Uttarakhand, India

Abstract : *Information on the genetic profile of cultivable fish species is essential for studying molecular systematic and optimizing fisheries management and fish farming. In the Kumaun and Garhwal regions of Uttarakhand Himalayas, coldwater fish species (Cyprinidae) is widespread and endemic in the natural water bodies. The usefulness of random amplified polymorphic DNA (RAPD) was examined as a potential tool to test the genetic relationship among four predominant fish species, viz., Tor putitora, Schizothorax richardsonii, Garra gotyla and Barilius bendelisis (Cyprinidae: Pisces). The samples were collected from two different geographical region of Kumaun and Garhwal of Uttarakhand. Species specific markers were observed using 10 mer random primers through RAPD-PCR assay. The statistical analysis of RAPD was performed with the software POPGENE version 1.31. The phylogenetic trees based on RAPD-PCR, by using 15 random primers, showed a consistent result of forming two separate monophyletic groups consisting of T. putitora with S. richardsonii and Garra gotyla with B. bendelisis. The present investigation concluded that these methods could be a valuable tool for studying molecular systematic and establishing the taxonomic position among cyprinids coldwater fish species of India.*

Keywords: *S. richardsonii*, *T. putitora*, *B. bendelisis*, *G. gotyla*, RAPD-PCR, Genetic diversity, Phylogenetic relationship.

Introduction

Coldwater fishery is one of the emerging contributors to inland fish production of India through aquaculture and capture fisheries. The eastern Himalayas have a greater diversity of coldwater fish than the western Himalayas. 218 fish species are listed for the whole Himalayas. Subsistence and commercial fishes exploit the large fish, such as the cyprinids *Labeo dero, Tor putitora, Tot tor, Schizothorax richardsonii, Barilius bendelisis, as well as Garra gotyla and Crossocheilus diplochilus* (Sehgal, 1987; Tripathi, 2005). The taxonomic position of these four coldwater fish species vary according to different sources and no comparative studies on genetic variability and phylogenetic relationship are studied. Even though these species are classified under the family Cyprinidae, at subfamily level the classification is ambiguous. Various authors have classified them under different subfamilies leading to improper taxonomy of the species (Berg, 1940; Munro, 1982; Kapoor *et al*., 2002).

RAPD analysis has been described as a simple and easy method to detect polymorphisms based on the amplification of DNA segment with single primers of arbitrary nucleotide sequence (Williams *et al*., 1990; Welsh and McClelland, 1990). The application of RAPD technique has greatly increased the ability to understand the genetic diversity within and between the species. RAPD analysis has been used to evaluate genetic diversity for species, subspecies and population/stock identification in Guppy (Foo *et al.,* 1995), Tilapia (Bardakci *et al.,* 2004), Brown trout and Atlatic salmon (Elo *et al.,* 1997), Largemouth bass (Williams *et al.*, 1998), Ictalurid catfishes (Liu and Dunham,

*E-mail : abarat58@hotmail.com

1998), Common carp (Bartfai *et al.*, 2003). Naish *et al.* (1995) found the technique useful in detecting diversity within and between strains of *Oreochromis niloticus*. RAPD markers is good molecular marker to understand the genetic diversity within and between the species at the molecular level in pelagic fish populations (Kuusipalo, 1999), in aquarium fishes (Koh *et al*., 1999), in cultured catfish (Yoon and Kim, 2001), in cyprinidae fish (Callejas and Ochando, 2002), in Indian major carps (Barman *et al*., 2003), in six *Labeo* species (Das *et al*., 2005), in mosquito fish population (Grapputo *et al*., 2006) and in *Salminus brasiliensis* (Lopes *et al*., 2007). Some studies were found out on these coldwater fish species for assessing the genetic diversity and phylogeny (Sivaraman *et al*., 2010) also.

The aim of present study was to characterize the endemic fish species of coldwater bodies *Schizothorax richardsonii, Tor putitora, Garra gotyla* and *Barilius bendelisis* from Uttarakhand Himalayas (Kumaun and Garhwal region) on the basis of intraspecific and interspecific variations and to provide a phylogenetic hypothesis for the coldwater species.

Materials and Methods

Fish sampling

A total number of 60 individuals of four fishes (*Schizothorax richardsonii, Tor putitora, Garra gotyla and Barilius bendelisis*) were collected from two different geographically isolated location of Uttarakhand (Kumaun and Garhwal region) (Fig. 1, Table 1). Caudal fin sample of each fish were cut and placed in 2 ml vials containing 75% ethanol and voucher fish specimens immediately fixed in 5% formalin, the fin samples were kept in at -20^{0} C until DNA extraction.

Extraction of Genomic DNA

Total genomic DNA was isolated from 50 mg fin tissue samples preserved in absolute ethanol using proteinase K and phenol chloroform method (Sambrook *et al*., 1989). Isolated genomic DNA was precipitated with 2- 2.5 volume of chilled ethanol. The DNA pellets was washed twice with 70 % ethanol, air dried and resuspended in 1X TE (10 mM Tris-HCL, pH 8.0 and 1 mM ethylene diaminetetraacetic acid disodium salt) buffer and kept at -20^{0} C till further use. Contaminating RNA was removed by digested with RNAase A (60 min at 37^{0}). The quality of DNA was checked by 0.8% agarose gel electrophoresis and the concentration of the DNA was estimated in UV-VIS spectrophotometer (Thermo Scientific, England) at 260 nm and 280 nm absorbance. The concentration of the DNA was 50 µg/µl.

RAPD Primers and *PCR Amplifications*

Altogether 60 (10 mer) random primers of OPA, OPX and OPY series from Operon technology were screened. Of these, 15 primers (Table 2) those produced the strongest amplification with reproducible polymorphic bands were selected for this study. All the primers have ~60% G+C content and have shown good amplification in Cyprinid fish species. RAPD-PCR amplifications was performed in a total volume of 30 µl containing 50 ng of genomic DNA, 100 pM of random primer, 200 µM of each dNTP, 3 µL 10 X PCR buffer, and 1 U of Taq DNA polymerase. Amplification was carried out in a programmed DNA thermal cycler (Eppendorf) which consisted of initial denaturation at 94^{o} C for 5 min, followed by 35 cycles consisting of denaturation at 94^{o}C for 1 min, primer annealing at 36^{o}C for 1 min, primer extension at 72^{o}C for 1 min, and final extension at 72^{o}C for 5 min. One negative control reaction containing distilled water instead of template DNA was used to check any contamination. For each sample, 3 µl of PCR product was electrophoresed on 1.2% agarose gel followed by ethidium bromide staining, and visualized under UV illumination in the Gel-Doc system (Alpha Imager 3400, Alpha Innotech Corporation, USA). Molecular weights were determined using 1 kb and 100 bp DNA

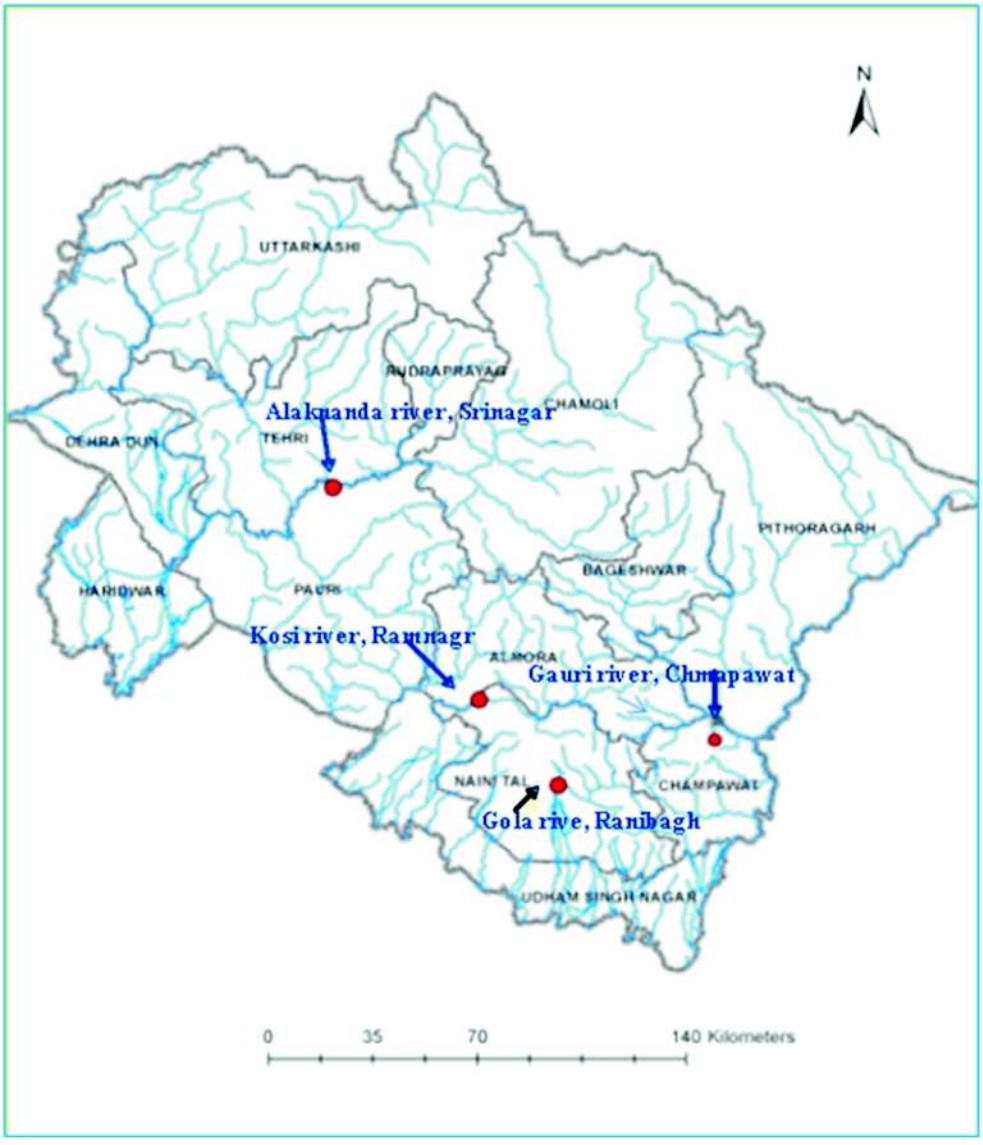

Fig. 1 Map showing the different sampling sites in the Uttarakhand region.

Table 1 Species, drainages, collection sites of specimens used in the study.

Species	No. of Specimen	Collection site of Kumaun	Collection site Garhwal
S richardsonii	60	Gauri river Champawat	Alaknanda River Srinagar
T. putitora	60	Kosi River, Ramnagar	Alaknanda River Srinagar
G. gotyla	60	Gola River, Ranibagh	Alaknanda River Srinagar
B. bendelisis	60	Gola River, Ranibagh	Alaknanda River Srinagar

markers (Fermentas, Canada), which was run parallel to the amplified product in gel or by the help of a computer program.

Sequence analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. RAPD calculations were carried out using the phylogenetic relationship analysis software, POPGENE 1.31 (Yeh *et al*., 1999). The UPGMA dendrogram of species was constructed based on Nei's (1972). Genetic differentiation (G_{st}) was calculated by using formula: Genetic dif (G_{st}) = 1-H_s/H_t, where H_s is sample gene diversity and H_t is total gene diversity. Gene flow was indirectly estimated among the species by using the formula: Nm = 0.5 (1 – G_{st})/G_{st} (McDermott and McDonald, 1993). Shannon's diversity index was calculated to provide a relative estimate of the degree of genetic variation within each species using POPGENE 1.31 (Yeh, 1999).

Results and Discussion

In the present study 15 primers generated a total number of 150 fragments, with the approximate size ranging from 250 to 3000 bp. Siraj *et al.* (2007) used a maximum of 10 -15 random primers and observed more numbers of amplified fragments (226-449) with 79% and 100% polymorphism of size range from 100bp-1500bp respectively. The number of polymorphic loci was 96 for *S. richardsonii*, 108 for *B. bendelisis*, 116 for *G. gotyla* and 117 for *T. putitora*. The percentage of polymorphic loci (P) was 64.00% for *S. richardsonii*, 72.00% for *B. bendelisis*, 77.33% for *G. gotyla* and 78.00% for *T. putitora*. The overall number of polymorphic loci for Garhwal species was 150 and overall percentage of polymorphic loci was 100%. In accordance with our study Das *et al.* (2005) observed the varied range of 42.6%, 31.7%, 19.2%, 16.8% and 14.3% polymorphic loci in different carp species. RAPD loci showed the highest level of within population genetic diversities, including allele frequencies and ne estimates. However, Nei's genetic diversity was found to be higher in *G. gotyla* (0.3303) and lower genetic diversity was found in *S. richardsonii* (0.2812) for Garhwal region. The Shannon's index ranged from 0.4025 for *S. richardsonii* to 0.4765 *B. bendelisis*. Overall Shannon's diversity was 0.6028 for all four species from Garhwal. Total genetic diversity (Ht) was 0.4164, and the genetic diversity within populations (Hs) was 0.3137**.** Similarly, Bardakci *et al.* (2004) and Grapputo *et al.* (2006) were also observed the genetic diversity index ranging from 0.1022-0.1643(0.122), 0.0579and 0.1563 among different fish population respectively. The coefficient of genetic differentiation (Gst) varied among 150 loci typed from 0.0023 to 0.8384, and the overall average Gst value was 0.2466 with a gene flow (Nm) = 1.5276. Nei's (1972) genetic distance between population, computed from combined data for all fifteen primers was 0.1915 for *G. gotyla* to *S. richardsonii* 0.1982 (Table 3).

The frequency of polymorphism was not the same among the four wild species in this study. The number of polymorphic loci was 101 for *S. richardsonii*, 104 for *B. bendelisis*, 93 for *G. gotyla* and 117 for *T. putitora*. The percentage of polymorphic loci (P) was 67.33% for *S. richardsonii*, 69.33% for *B. bendelisis*, 62.00% for *G. gotyla* and 78.00% for *T. putitora*. The overall number of polymorphic loci for Kumaun species was 149 and overall percentage of polymorphic loci was 99.33%. Higher genetic diversity was found in the *T. putitora* (0.3217) and lower genetic diversity was found for the *G. gotyla* (0.2600)**.** The overall genetic diversity within Kumaun species was 0.3860. The Shannon's index ranged from 0.3766 for *G. gotyla* to 0.4683 for *T. putitora***.** Overall Shannon's diversity was 0.5675 for all four species from Kumaun region. Total genetic diversity (Ht) was 0.3860, and the genetic diversity within populations (Hs) was 0.2894**.** The coefficient of genetic differentiation (Gst) varied among 150 loci typed from 0.0000 to 0.9031, and the overall average Gst value was

Table 2 Primers and primer sequences used for the detection of polymorphism in coldwater fishes.

	Oligos used as RAPD PCR			
Sl. No.	Primer Name	Sequence (5'to 3')	GC content (%)	Length (mer)
1	OPA1	CAGGCCCTTC	70%	10
2	OPA4	AATCGGGCTG	60%	10
3	OPA7	GAAACGGGTG	60%	10
4	OPA10	GTGATCGCAG	60%	10
5	OPA20	GTTGCGATCC	60%	10
6	OPX1	CTGGGCACGA	70%	10
7	OPX4	CCGCTACCGA	70%	10
8	OPX5	CCTTTCCCTC	60%	10
9	OPX7	GAGCGAGGCT	70%	10
10	OPX8	CAGGGGTGGA	70%	10
11	OPY2	CATCGCCGCA	70%	10
12	OPY6	AAGGCTCACC	60%	10
13	OPY7	AGAGCCGTCA	60%	10
14	OPY10	CAAACGTGGG	60%	10
15	OPY20	AGCCGTGGAA	60%	10

Table 3 Nei's unbiased measures of Genetic identity and genetic distance between fish populations from Garhwal region.

	B. bendelisis (g)	*G. gotyla* (g)	*S. richardsonii* (g)	*T. putitora* (g)
B. bendelisis (g)	-----	0.8257	0.7692	0.8109
G. gotyla (g)	0.1915	-----	0.7729	0.8202
S. richardsonii (g)	0.2624	0.2576	-----	0.8520
T. putitora (g)	0.2095	0.1982	0.1601	-----

Table 4 Nei's unbiased measures of Genetic identity and genetic distance between fish populations from Kumaun region.

	B. bendelisis (k)	*G. gotyla* (k)	*S. richardsonii* (k)	*T. putitora* (k)
B. bendelisis (k)	-----	0.8580	0.8058	0.8062
G. gotyla (k)	0.1531	-----	0.8109	0.8109
S. richardsonii (k)	0.2159	0.2097	-----	0.8638
T. putitora (k)	0.2154	0.2096	0.1464	-----

Table 5 Genetic identity and genetic distance between and within fish populations from Kumaun and Garhwal regions.

	B. b(g)	*B. b*(k)	*G. g*(g)	*G. g*(k)	*S. r* (g)	*S. r* (k)	*T. p* (g)	*T. p* (k)
B. b(g)	-----	0.8754	0.8257	0.8158	0.7692	0.7884	0.8109	0.7802
B. b(k)	0.1331	-----	0.8536	0.8580	0.7941	0.8058	0.8314	0.8062
G. g(g)	0.1915	0.1583	-----	0.8524	0.7729	0.8046	0.8202	0.8115
G. g(k)	0.2036	0.1531	0.1597	-----	0.8138	0.8109	0.8091	0.8109
S. r (g)	0.2624	0.2305	0.2576	0.2061	-----	0.8860	0.8520	0.8070
S. r (k)	0.2377	0.2159	0.2175	0.2097	0.1210	-----	0.8754	0.8638
T. p (g)	0.2095	0.1846	0.1982	0.2119	0.1601	0.1331	-----	0.9087
T. p (k)	0.2482	0.2154	0.2088	0.2096	0.2145	0.1464	0.0958	-----

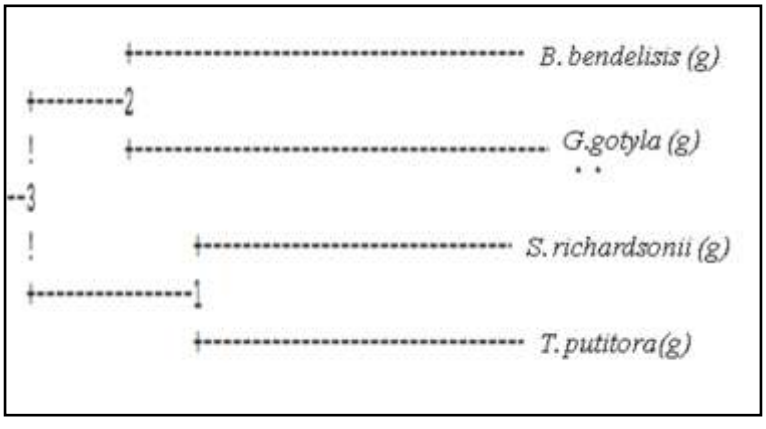

Fig. 2 UPGMA dendrogram showing the phylogenetic relationship from Kumaun region using the RAPD markers.

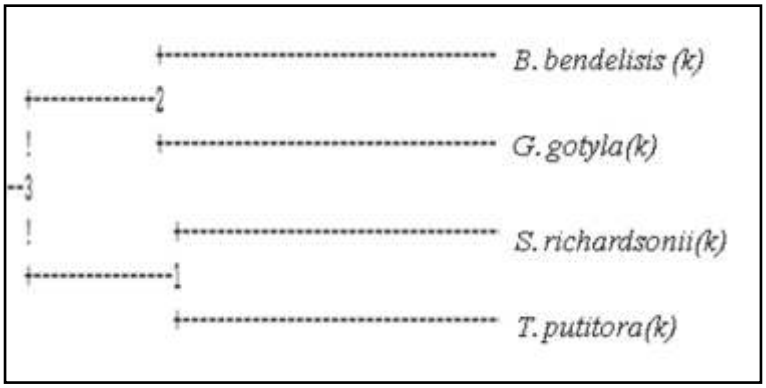

Fig. 3 UPGMA dendrogram showing the phylogenetic relationship from Garhwal region using the RAPD markers.

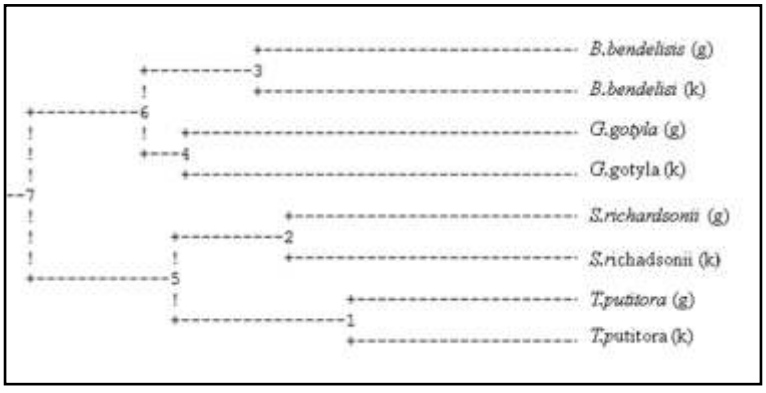

Fig. 4 UPMGA dendrogram showing the phylogenetic relationship from both (Kumaun and Garhwal) regions using the RAPD markers.

0.2502 with a gene flow (Nm) = 1.4985. This result suggests that the gene flow in the Kumaun populations seems to be slow and indicative of little migration among population. Genetic distance between populations, computed from combined data for all fifteen primers was 0.1531 for *G. gotyla* to 0.2159 for *S. richardsonii* (Table 4).

The overall genetic diversity within and between Garhwal and Kumaun species was 0.4129. Overall Shannon's diversity was 0.6005 for all four species from Kumaun and Garhwal region. The genetic diversity within populations (Hs) was 0.3016. The coefficient of genetic differentiation (Gst) varied among 150 loci typed from 0.0337 to 0.8576, and the overall Gst value was 0.2696 with a gene flow (Nm) = 1.3548 for both region. Genetic distance within and between populations computed from combined data for all fifteen primers (Table 5). This result suggests that the gene flow in the Kumaun populations seems to be slow and indicative of little migration among population. Garhwal region is having higher proportion of polymorphism as compared to Kumaun region.

RAPD markers have been used successfully to determine species- specific traits in fishes and for constructing phylogenetic relation among species and sub-species (Callejas and Ochando, 1998). In the present investigation phylogenetic analysis by RAPD showed two clusters for Kumaun and Garhwal region, first cluster consist of the *S. richardsonii* and *T. putitora*, the second cluster consisting *G. gotyla* and *B. bendelisis* (Fig. 4). Genetic distance data showed that *S. richardsonii* and *T. putitora* are closely related to each other and *G. gotyla* and *B. bendelisis* are closely related to each other

(Fig. 2 and 3). In this study that highest genetic distance were observed between *S. richardsonii* and *B. bendelisis for both region (Table 3 and 4)*. Brahmane *et al.* (2006) observed the lowest genetic distance (0.213) between the Allahabad and Lal Gola population and highest (0.394) in between the Allahabad and Bhadbhud population. In the present study analysis of RAPD of four Cyprinidae fishes of Kumaun and Garhwal region of Uttarakhand suggests clear relationship between *B. bendelisis* and *G. gotyla*, and between *S. richardsonii* and *T. putitora.* The present phylogenetic tree also showed some relation with the karyotypic phylogeny and chromosomal duplication process of these species. KhudaBukhsh *et al.* (1986) viewed a diploid-polyploid relationship among some members within family Cyprinidae. The chromosomes numbers of these Cyprinidae fishes were considered in phylogenetic analysis that provides a clear pattern of relationship between these four species. The Cyprinidae fishes *T. putitora* (2n=100) and *S. richardsonii* (2n=98) have a close relationship and cluster together while *B. bendelisis* (n=50) and *G. gotyla* (n-50) were cluster together and more divergent from *S. richardsonii* and *T. putitora.* Therefore, the role played by polyploidy in the evolution of fish karyotypes seems to be quite significant in the present study using RAPD. This study may be useful in determining evolutionary characteristics of these four species and compare the genetic diversity in different geographically isolated natural populations. This would be important for breeding programmes because maintaining genetic diversity within breeding populations is necessary to maximize hybrid vigour, because this species is not a migratory species and possibly suffers from inbreeding depression. The markers characterized in this study will be useful for linkage mapping and the location of quantitative trait loci. The cross species amplification of these markers in other Teleostei fishes in the present findings showed their abilities to study the genetic diversity analysis in those respective species and would be useful for full fledged genetic structure analysis.

Acknowledgement

The authors are thankful to Indian Council of Agricultural Research, for financial support. We also acknowledge the laboratory facility provided by Directorate of Coldwater Fisheries Research, Bhimtal to carry out this study.

References

Ambak, M.A., Bolong, A.A., Ismail, P. and MinhTam, B. (2006) Genetic variation of Snakehead fish (Channa striata) population using Random Amplified Polymorphic DNA. *Biotechnol.,* **5**, 104-110.

Baradakci, F. and Skibinski, D.O.F. (1994) Application of the RAPD technique in Tilapia fish species and subspecies identification. *Heredity,* **73**, 117-123.

Bartfai, R., Egedi, S., Yue, G.H., Kovacs, B., Urbanyi, B., Tamas, G., Horvath, L. and Orban, L. (2003) Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. *Aquacul.,* **219**, 157–167.

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. *Aquacul.,* **217**, 115–123.

Bardakci, F., Tatar, N. and Hrbek, T. (2004) Genetic relationships between Anatolian species and sub- species of *Aphanuis Narda*, 1827 (Pisces, Cyprinodontiformes) based on RAPD markers. *Biologia, Bratislava*, **595**, 559–566.

Brahmane, M.P., Das, M.K., Sinha, M.R., Sugunan, V.V., Mukherjee, A., Singh, S.N., Prakash, S., Maurye, P. and Hajra, A. (2006) Use of RAPD fingerprinting for Delineating population Hilsa shad *tenualosa ilisha* **(**Hamilton, 1822). *Genetic Mol. Res.,* **5,** 64–652.

Berg, L.S. (1940) Classification of fishes, both recent and fossil. *Trav. Inst. Zool. Acad. Sci. U.S.S.R*., vol. 5, No. 2, 517 pp.

Callejas, C. and Ochando, M.D. (2002) Phylogenetic relationships among Spanish *Barbus* Species (Pisces, Cyprinidae) shown by RAPD markers. *Heredity,* **89**, 36–43.

Das, P., Prasad, H., Meher, P.K., Barat, A. and Jana, R.K. (2005) Evaluation of genetic relationship among six Labeo species using Random amplified polymorphic DNA (RAPD). *Aquacult. Res.,* **36**, 564-569.

Elo, K., Ivanoff, S., Jukka, A., Vuorinen, J. and Piironen, J. (1997) Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. *Aquacul.,* **152**, 55-56.

Foo, C.L., Dinesh, K.R., Lim, T.M., Chan, W.K. and Phang, V.P.E. (1995) Inheritance of RAPD markers in the guppy fish, *Poecilia Reticulate. Zool. Sci.,* **12**, 535.

Grapputo, A., Bisazza, A. and Pilastro, A. (2006) Invasion success despite reduction of genetic diversity in the European population of eastern mosquito fish (*Gambusia holbrooki*). *Italian J. Zool.,* **73**, 67-73.

Kapoor, D., Dayal, R. and Ponniah, A.G. (2002) Fish Biodiversity of India. Director NBFGR, Canal Ring Road, Lucknow, India. Pp. 104-221.

Khuda-Bukhsh, A.R., Chanda, T. and Barat, A. (1986) Karyomorphology and evolution in some Indian hillstearm fishes with particular references to polyploily in some species. In: IndoPacific Fishes (Eds. T. Uyeno; R. Arai; T. Taniuchi and K. Matsuura). *Ichthyol. Society of Japan, Tokyo, Japan*. pp.886-898.

Koh, T.L., Khoo, G., Fan, L.Q. and Phang, V.P.E. (1999) Genetic diversity among Wild forms and cultivated varieties of Discus (*Symphysodon spp*.) as revealed by Random amplified polymorphic DNA (RAPD) fingerprinting. *Aquacul.,* **173**, 485-497.

Kuusipalo, L. (1999) Genetic variation in the populations of Pelagic clupeids *Stolothrissa tanganicae* and *Limnothrissa miodon* and Nile perch (*Lates stappersii, L.mariae*) in lake Tanganyika.

Liu, Z.J. and Dunham, R.A. (1998) Genetic linkage and QTL mapping of Ictalurid catfish, *Ala. Agri. Exp. Stn. Bull.*, **321**, 1-19.

Lopes, C.M., Almeida Simoes, D.A.F., Orsi, M.L., Britto, S.G.D.C., Sirol, R.N. and Sodre, L.M.K. (2007) Fish passage ladders from Canoas complex – Paranapanema river: evaluation of genetic structure maintenance of *Salminus brasiliensis* (Teleostei: Characiformes). *Neotrop. Inchthyol.*, vol.**5**.

McDermott and McDonald (1993) *Ann. Rev. Phytopathol*., **31**, 353-373.

Munro, I.S.R. (1982) The Marine and Fresh Water Fishes of Ceylon. *Soni Reprints Agency,* Delhi, India. P. 349.

Naish, K.A., Warren, M., Baradaka, F., Skibinski, D.O.F., Carvalho, G.R. and Mair, G.C. (1995) Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). *Mol. Ecol.,* **4**, 271-274.

Nei, M. (1972) Genetic distance between populations. *Am. Naturalist*, **106**, 283- 292.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular cloning*: *A laboratory manual*, 2nd edn., Cold Spring Harbour Laboratory Press, New York.

Sehgal, K.L. (1987) Sport Fisheries in India. *ICAR Publication*, New Delhi, India. P.126.

Siraj, S.S., Esa, Y.B., Keong, B.P. and Daud, S.K., (2007) Genetic characterization of the two color-type of kelah. *Malays. Appl. Biol*., **36**, 23-29.

Sivaraman, G.K., Barat, A., Kapila, R., Nagappa, K. and Mahanta, P.C. (2010) Phylogenetic Analysis of Coldwater Fish Species (Cyprinids) of India using targeted mtDNA and RAPD-PCR markers. *IUP*., 43-53.

Tripathi, S.D. (2005) Aquaculture for food and Sportin the Hills, in Vass, K.K., Abidi, S.A.H. and Agarwal, V. P. (Eds.). *Proceedings of National Seminar on Aquatic Resource Management in Hills*.

Welsh and McClelland (1990) J. Welsh and M. McClelland, Fingerprinting genomes using PCR with arbitrary primers, *Nucleic Acids Res.,* 18 (1990), pp. 7213–7218.

Williams, J.G.K., Kubelik, A.R., Livak, J., Rafalski, J.A. and Tingey, S.V. (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.,* **18**, 6531-6535.

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 integrades. *Trans. Am. Fish. Soc.,* **127**, 825-832.

Yeh, F.C., Yang, R.C. and Boyle, T. (1999) POPGENE version 1.31, The Microsoft Window based freeware for Population Genetic Analysis, Edmanton, Alberta: University of Alberta, available in the internet at http://www.ualberta.ca/~fyeh/fyeh.

Yoon, J.M. and Kim, G.W. (2001) Random amplified polymorphic DNA-Polymerase Chain Reaction analysis of two different populations of cultured Korean Catfishes *Silurus asotus*. *Ind. Acad. Sci.,* **26**, 641-647.