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DNA polymorphism and relationships among the three different riverine populations of spotted snakehead (*Channa punctata*)

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DNA polymorphism refers the presence of different alleles of a gene because of the changes in the DNA sequences and the total number of genetic characteristics are determinant for the genetic makeup of a species. Genetic diversity refers to the diversity of genes within a species passed from one generation to next (Verma 2017). Each species of fish possesses a collection of genes of unique features. The presence of the different variety of genes in an individual or entire population of an organism determines the ability to tolerate the stress given by the environmental factors. Genetic diversity is an essential characteristic of any population of an organism for the fitness of individuals and also survival of the whole population related to adaptation to the changing environmental conditions and stress. Hence the loss of genetic diversity in any species will decrease its capability for an adaptation and increases the chance of extinction (Isagi *et al.* 2020).

Molecular markers have played an important role in understanding the basis of polymorphism within a species, and population differentiation. Random amplified polymorphic DNA (RAPD) is the simplest and most popular PCR (polymerase chain reaction) based technique which is easy to use, fast and low cost. RAPD has been used to find out the genetic variation in genus *Synodontis* (Abu-Almaaty and Ebied 2018), genus *Scarus* (Mohammed 2018), *Coptodon zillii* and *Oreochromis aureus* (Al-Khafaji *et al.* 2019), *Amblyceps mangois* (Mukhopadhyay and Bhattacharjee 2019), Carangidae family (Al-Faisal *et al.* 2019), *Monopterus cuchia* (Abedin *et al.* 2020), *Clarias batrachus* (Miah *et al.* 2020), guppy fish (Nuradha *et al.* 2021), and *Clarias gariepinus* (Adesola *et al.* 2020, Normala 2021). The study on genetic diversity of *Channa punctata* (Bloch 1793) was carried out by some workers such as Nagarajan *et al.* (2006), Masih and Masih (2010), Bhat *et al.* (2014), Kashyap *et al.* (2016) but still information on comparative aspect of DNA polymorphism in different populations of the *C. punctata* is scanty. Therefore, keeping the paucity of information in mind the present study was carried out to analyze genetic DNA polymorphism in *C. punctata* collected from three different rivers of northern India.

A total of 90 samples of *C. punctata* (30 from each river) were collected from three different rivers namely Gomti at Lucknow (26°51′30″ N 80°56′14″ E), Ganga at Kanpur (26°37′08″ N 80°16′26″ E) and Ken at Banda (25°46′N 80°31′E) of Ganges basin of northern India by using cast and drag nets with the help of local fishermen. These sampling sites are geographically and distantly distinct from each other and characterized by different environmental conditions. Genomic DNA was isolated from the blood of the fish using methods as described by Ruzzante *et al.* (1996) for RAPD-PCR. Random primers of Operon 'B' series (USA) were used for RAPD. 20 primers were screened for amplification in PCR. RAPD bands were scored for the presence (1) and absence (0) of bands. The raw data was used to determine Nei's (1978) unbiased genetic identity (I), genetic distance (D), percentage of polymorphism and a dendogram was constructed using unweighted pair group method with arithmetic mean (UPGMA) using software POPGENE 1.32.

The 5 primers out of 20 RAPD primers gave reproducible and clear resolution in banding patterns in screening of primers. Other 15 primers either did not amplify the products at all or produced inconsistent results so were excluded from the further study. These five primers showed high GC content of 60–70% (Table 1). The details of the images of the RAPD amplified bands by five primers are given in Fig. 1. A total of 54 loci were generated from 5 RAPD primers from the genomic DNA of the fish of three rivers in which monomorphic and polymorphic loci were 3(5.56%) and 51(94.44%) respectively indicating higher hetrozygocity as compared to homozygocity. Unique bands or population specific bands were not found in the analysis. Maximum number of loci were 13 generated by primer OPB18 which showed maximum polymorphism while primer OPB11 generated minimum loci indicating minimum polymorphism. Ganga population showed highest genetic

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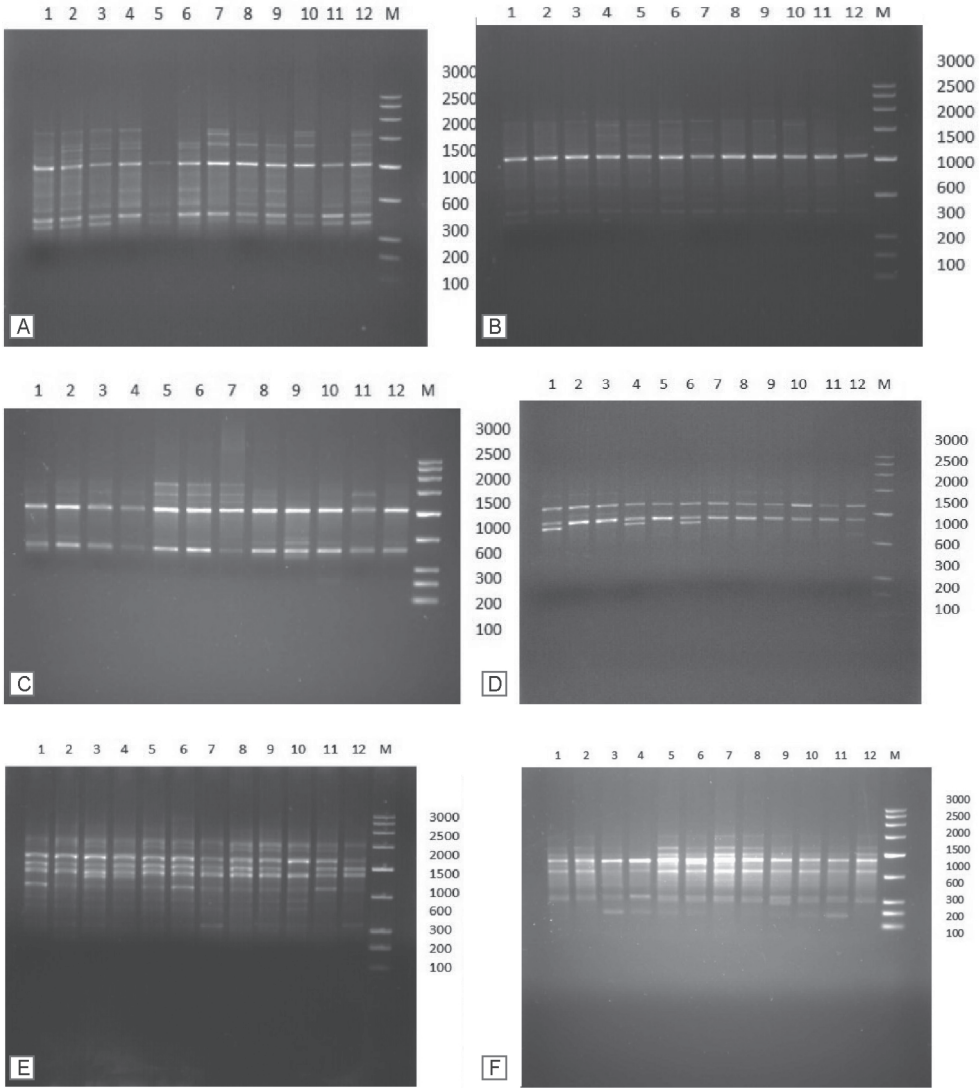

Fig. 1. RAPD band patterns generated by primer OPB-4 in the *C. punctata* of the river Gomti (A), Ganga (B), Ken (C) and primer OPB-18 in the *C. punctata* of the river Gomti (D), Ganga (E) and Ken (F).

polymorphism with 33 (61.11%) polymorphic loci out of 54 loci, whereas 55.56% and 46.30% polymorphism were recorded in Ken and Gomti populations of *C. punctata* respectively. The total mean genetic diversity of all the three populations of the fish was 0.3082 with the maximum value of 0.2274 recorded in the fish of the river Ganga which was followed by 0.2023 and 0.1463 in the population of the fish collected from Ken and Gomti respectively. Ganga and Ken populations showed maximum genetic identity (Table 2) and the dendogram constructed using UPGMA also depicted that the Ganga and Ken populations are more similar to each other rather than to the population of the fish of river Gomti (Fig. 2).

Genetic diversity is the base for other high levels of biodiversity at population and species level (Coker 2017) and has emerged as one of the potent issues in conservation

Table 1. Sequence, G+C content, number of total loci and number of polymorphic loci produced by the five RAPD primers in the three populations of *C. punctata*

Primer	Primer sequence	G+C (%)	Number of loci amplified	Poly-morphic total
OPB-4	5′-GGACTGGAGT-3′	60	11	9
OPB-6	5′-TGCTCTGCCC-3′	70	11	11
OPB-11	5′-GTAGACCCGT-3′	70	8	8
OPB-12	5′-CCTTGACGCA-3′	60	11	11
OPB-18	5′-CCACAGCAGT-3′	60	13	12
Combined			54	51

Table 2. Nei's (1978) unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) between the three populations of *C. punctata*

Population	River Gomti	River Ganga	River Ken
River Gomti		0.7682	0.7748
River Ganga	0.2637		0.8378
River Ken	0.2551	0.1769	

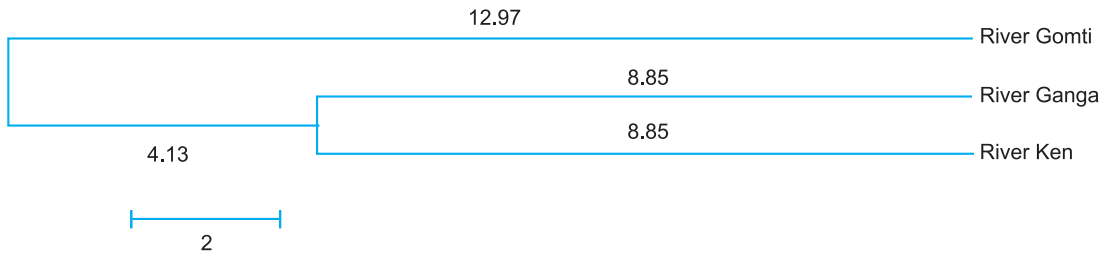

Fig. 2. Dendogram showing relationships between the populations of *C. punctata* of three different rivers.

biology for the sustainability of populations within a species. RAPD markers have been reported to have a wide range of applications in population genetics, genetic characterization and breeding programmes (Mahboob 2019, Mukhopadhyay and Bhattacharjee 2019, Adesola 2020). Mahboob *et al.* (2019) calculated genetic polymorphism in tilapia (*Oreochromis niloticus*) by using RAPD markers for the purpose of evolving strategies to conserve their diversity. The polymorphic DNA bands detected in the present study can be used as genetic markers to select the breeders from the desired population under the selective breeding programme.

In the present study, 94.44% DNA polymorphism were recorded in the three populations of *C. punctata* which indicated a large amount of genetic variation. DNA polymorphism of 46.30, 55.56, and 61.11% were recorded in Gomti, Ken and Ganga respectively indicating that the *C. punctata* of river Ganga has highest genetic diversity while that of Gomti has lowest. This diversity may be due to different environmental conditions of the habitats because the presence of genetic variability among populations is considered to be essential for their ability to survive and successfully respond to the environmental changes (Manel *et al.* 2020). Genetic variation allows adaptation to the changing environmental conditions of the habitat (Megbowon 2019). Maximum value of genetic identity was recorded between the populations of *C. punctata* collected from Ganga and Ken while maximum genetic distance was recorded between Gomti and Ganga populations of the fish. River Ken travels a distance of about 427 km before merging, whereas river Gomti extends to 960 km from its origin and then finally merges into river Ganga.

Genetic identity, genetic distance and dendogram indicated that the fish populations of the river Ganga and Ken are closer as compared to that of Gomti. Genetic difference increases as geographical isolation increases has been revealed in the present study, showing significant correlation between genetic similarity and geographical distance.

SUMMARY

The current study was aimed to investigate the DNA polymorphism of *C. punctata* from three different rivers of northern region of India. For this, *C. punctata* of river Ganga and its tributaries (river Gomti and river Ken) was carried out using RAPD-PCR (Random amplified polymorphic DNA-polymerase chain reaction). A total of 54 loci were amplified by five selected primers out of 20 having GC content of 60% to 70%. The fish population of river Ganga showed highest genetic polymorphism with 33 (61.11%) polymorphic loci out of 54 loci whereas a total of 55.56% and 46.30% DNA polymorphism were recorded in the fish populations of Ken and Gomti respectively. Maximum genetic identity and minimum genetic distance was recorded between the fish populations of Ganga and Ken showing that these two populations are more similar to each other than to Gomti population. Dendogram based on genetic distance using UPGMA also confirmed that Ganga and Ken population are closer to each other.

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REFERENCES

Abedin M M, Eshik M M E, Punom N J, Begum M K and Rahman M S. 2020. Genetic characterization of Swamp Eel of Bangladesh through DNA barcoding and RAPD techniques. *Journal of the Asiatic Society of Bangladesh, Science* **46**(2): 117–31.

Abu-Almaaty A H and Ebied A B M. 2018. Genetic characterization of four fish species of genus *Synodontis* using RAPD Marker. *Indian Journal of Geo Marine Sciences* **47**(12): 2395–2406.

Adesola O O F, Awodiran M O, Olaleye V F and Awopetu J I. 2020. Genetic characterization based on RAPD-PCR in cultured strains of *Clarias gariepinus* (Siluriformes: Clariidae). *Genetics of Aquatic Organisms* **4**(2): 81–88.

Al-Faisal A J, Mohamed A R M and Jaayid T A. 2019. DNA fingerprinting of the carangid fish species from the Iraqi marine waters by using RAPD technique. *International Journal of Fisheries and Aquatic Studies* **7**(4): 422–27.

Al-Khafaji, T Y, Ziyadi M S and Musad M K. 2019. DNA fingerprints of two tilapia fish species of euphrates river at governorate of al-muthanna using RAPD markers. *Engineering and Technology Journal* **37**(3C): 345–49.

Bhat A A, Haniffa M A, Milton M J, Paray B A, Divya P R and Gopalakrishnan A. 2014. Genetic variation of striped snakehead (*Channa striatus* Bloch, 1793) populations using random amplified polymorphic DNA (RAPD) markers. *International Journal of Biodiversity and Conservation* **6**: 363–72.

Coker O M. 2017. Importance of genetics in conservation of biodiversity. *Nigerian Journal of Wildlife Management* **1**(1):

11–18.

Isagi Y, Makino T, Hamabata T, Cao P L, Narita S, Komaki Y, Kurita K, Naiki A, Kameyama Y, Kondo T and Shibabayashi M. 2020. Significant loss of genetic diversity and accumulation of deleterious genetic variation in a critically endangered azalea species, *Rhododendron boninense*, growing on the Bonin Islands. *Plant Species Biology* **35**(3): 166–74.

Kashyap A, Awasthi M, Singh M and Serajuddin M. 2016. Genetic diversity of freshwater murrel *Channa punctatus* (Bloch, 1793). *Journal of Inland fisheries Society of India* **48**(2): 1–7.

Mahboob S, Al-Ghanim K A, Al-Misned F, Al-Balawi H A, Ashraf A and Al-Mulhim N M. 2019. Genetic diversity in tilapia populations in a freshwater reservoir assayed by randomly amplified polymorphic DNA markers. *Saudi Journal of Biological Sciences* **26**(2): 363–67.

Manel S, Guerin P E, Mouillot D, Blanchet S, Velez L, Albouy C and Pellissier L. 2020. Global determinants of freshwater and marine fish genetic diversity. *Nature Communications* **11**(1): 1–9.

Masih S A and Masih H. 2010. Assessment of genetic variability among fresh water murrels using random amplified polymorphic DNA markers. *Asian Journal of Animal Science* **5**: 10–13.

Megbowon I. 2019. Genetic evaluation of some tilapiine fishes using varying RAPD markers. *International Journal of Fisheries and Aquatic Studies* **7**(4): 275–79.

Miah M F, Rahman T, Shipa S A and Chakrabarty A. 2020. RAPD-based genotyping of walking catfish, *Clarias batrachus* in a population of Bangladesh. *Journal of Entomology and Zoology Studies* **8**(1): 885–88.

Mohammed E M. 2018. Genetic variation and phylogenetic relationship among four Parrotfishes (genus *Scarus*) in Hurghada, Red sea coast, Egypt based on RAPD markers. *Egyptian Academic Journal of Biological Sciences* **10**(1): 79–94.

Mukhopadhyay T and Bhattacharjee S. 2019. Genetic diversity and population structure analyses of threatened *Amblyceps mangois* from Sub-Himalayan West Bengal, India through RAPD and ISSR fingerprinting. *Croatian Journal of Fisheries* **77**(1): 33–50.

Nagarajan M, Haniffa M A, Gopalakrishnan A, Basheer V S and Muneer A. 2006. Genetic variability of *Channa punctatus* populations using randomly amplified polymorphic DNA. *Aquaculture Research* **37**: 1151–55.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**(3): 583–90.

Normala J, Okomoda V T, Mohd A A, Nur A A, Abol-Munafi A B and Md Sheriff S. 2021. Genetic Variation between Triploid and Diploid *Clarias gariepinus* (Burchell, 1822) Using RAPD Markers. *Veterinary Sciences* **8**(5): 75.

Nuradha I T, Yustiati A, Handaka A A and Bangkit I. 2021. Genetic relationship of four strains of Guppy (*Poecilia reticulata* Peters, 1859) Using RAPD-PCR Method. *Asian Journal of Biochemistry*, *Genetics and Molecular Biology* **7**(4): 15–24.

Ruzzante D E, Taggart C T, Cook D and Goddard S. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: Microsatellite DNA variation and antifreeze level. *Canadian Journal of Fisheries and Aquatic Sciences* **53**(3): 634–45.

Verma A K. 2017. Necessity of ecological balance for widespread biodiversity. *Indian Journal of Biology* **4**(2):158–60.