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Evaluation of genetic diversity in natural populations of *Channa marulius* (Hamilton, 1822) through RAPD markers in major rivers of Punjab, Pakistan

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ABSTRACT

Channa marulius is a valuable food fish, known as "Sol". In Pakistan, *C. marulius* plays an important role to meeting the protein requirement of people in their daily diet. Due to overfishing, destruction of habitat, and water pollution, the population of *C. marulius* is declining rapidly. Therefore, for the conservation of *C. marulius*, we evaluated its genetic diversity by collecting specimens from five major rivers (Chenab, Indus, Ravi, Jhelum, and Sutlej) of Punjab, Pakistan using random amplified polymorphic DNA (RAPD) molecular markers. 75 specimens of *C. marulius* were collected from five rivers and evaluated its genetic diversity by using five RAPD DNA markers OPA-02, OPA-05, OPA-07, OPA-11, and OPA-16. Results showed 106 polymorphic bands out of a total of 154 bands and detected five primers associated with genetic diversity. The highest genetic diversity was observed in River Jhelum with 28/35 (80%) polymorphic bands. In others, it is recorded as 26 (78.78%) in Ravi, 20 (74.07%) in Sutlej, 24 (71.44%) in Indus and lowest in River Chenab with 08 (28.58%) polymorphic bands. Nei's genetic diversity (h) observed in Jhelum (0.782) and Chenab (0.323) reflect high genetic diversity in Jhelum and lowest genetic diversity in Chenab. UPGMA dendrogram showed that the population of *C. marulius* in Sutlej and Ravi has close genetic connectivity and genetic similarity and similarly the population of *C. marulius* in Indus and Chenab also has close genetic connectivity and genetic similarity. The present study reports that the lowest genetic diversity in the Chenab River is a matter of serious concern for fisheries resource managers to take immediate steps for *C. marulius* conservation.

Keywords: Genetic diversity, RAPD markers, Natural populations, *Channa marulius*, Fisheries, Pakistan.

1. INTRODUCTION

Evaluation of genetic diversity laid the foundation of fishery management and conservation (Gu et al., 2021; Zhang et al., 2023). The ability of a species to adapt to the changing environmental conditions strongly supports the genetic diversity it possesses (Naeem et al., 2011). It is well known that morphometry is the simplest and most straightforward method for identifying species (Naeem et al., 2010; Naeem et al., 2012). It can characterize morphological variation and

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examine any potential relationships between variation and genetic differentiation (Mayanglambam et al., 2024; Yousaf et al., 2011). But for the effective management of fishery resources evaluation of population differentiation and genetic diversity are the key requirements (Ukenye et al., 2019; Ismat et al., 2013).

To study population structure and genetic diversity in different fish species Randomly Amplified Polymorphic DNA (RAPD) markers are widely used (Chauhan and Rajiv, 2010; Chen et al., 2020). DNA markers are universally used to evaluate the genetic diversity of freshwater fishes. Randomly Amplified Polymorphic DNA (RAPD) markers significantly used for the analysis of genetic diversity (Ahmad and Naeem, 2023).

There are two genera in the Family Channidae: Channa and Parachanna (Nelson, 1984). Due to the consumer preferences, these fish often collected from natural freshwater bodies in Pakistan and sold alive (Mirza, 1982; Rahman, 2005; Naeem et al., 2010). *C. marulius* (Hamilton, 1822) and *C. punctatus* (Bloch, 1793) among the snakeheads are commercially important fish and used for ornamental trade (Courtenay and Williams, 2004). Snakehead and murrel of genus Channa tropical freshwater fishes widely used for human consumption (Hossain et al., 2008; Khan and Naeem, 2024) and additionally in pharmaceutical and medicinal purposes (Michelle et al., 2004; Kousar et al., 2021).

In developing countries, fish is considered as one of the best sources of protein (Naeem et al., 2016). The nutritional value of the whole body is often used as an index of fish quality (Pervaiz et al., 2012). *C. marulius* species has fast growth rate and reach up to a length of 120–122 cm (Talwar and Jhingran, 1992; Naeem et al., 2011). Riverine water bodies of Pakistan have significant ichthyodiversity (Urooj et al., 2011). Due to decreasing flows, extended dry periods and overexploitation have led to the decrease in fisheries resources (Ashraf et al., 2011). Studies about genetic differentiation of *C. marulius* populations in Pakistan are still at basic level (Ahmad and Naeem, 2022). There is an urgent need to understand the changes in genetic diversity of the fish populations in river system of Pakistan. Therefore, it is necessary to understand the genetic diversity of the *C. marulius* fish populations among Pakistan Rivers (Khan and Naeem, 2024).

The paper evaluates the genetic diversity in natural populations of *Channa marulius* (Hamilton, 1822), family Channidae from five rivers of Pakistan, Sutlej, Ravi, Jhelum, Indus, and Chenab.

2. MATERIALS AND METHODS

2.1. Sample collection site

75 specimens (15 from each river) of *Channa marulius* were collected from River Sutlej, River Ravi, River Jhelum, River Indus, and River Chenab of Punjab, Pakistan (Fig 1).

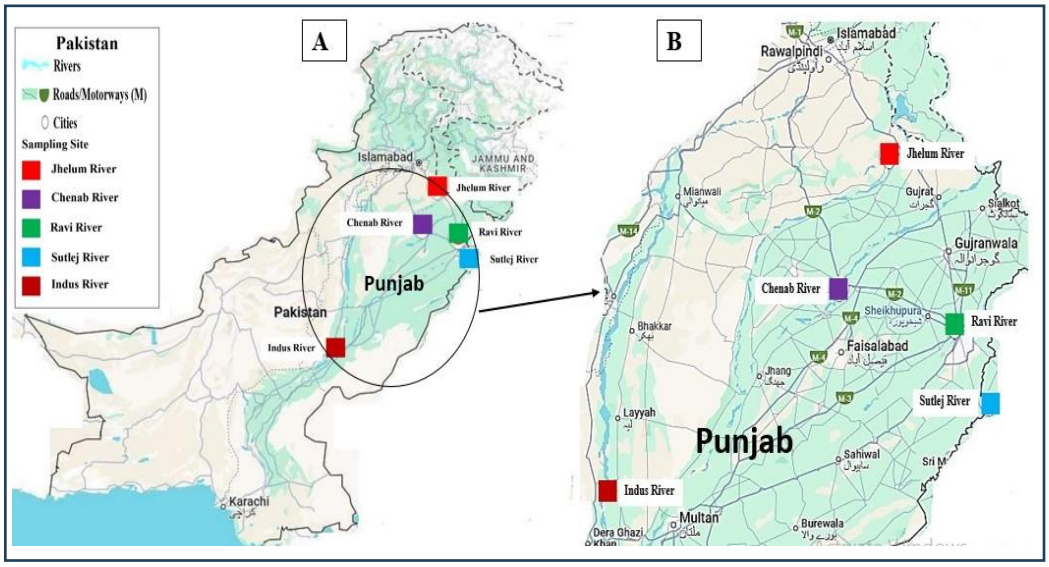

Figure 1. Study area-sampling sites of Channa marulius, A. Pakistan, and B. the study area.

Sample collection details, such as a river, sample size, and sampling locations with latitude/longitude provided in table 1. MS222 was used to euthanize fish. In concentrated MS222 (250 mg/L) solution, fish specimens were immersed and kept in solution for 10 min. After exposing towards MS222 solution fish stop breathing, lose consciousness followed by cessation of gills movement. After that the fish was kept in the freezer which makes it double sure the fish is dead and can be used for further studies (Ahmad and Naeem, 2023). Collected specimens were stored in ice bags and transported to fisheries research laboratory Institute of Zoology, Bahauddin Zakariya University Multan Pakistan. After that, fish muscle tissue excised for DNA extraction.

Table 1. Sample collection localities, coordinates (longitude/latitude) and collection date Channa marulius.

River	***Coordinates (Longitude/Latitude)***	***Sample collection Time***
Sutlej	69° 50’ 55.5"E, 29° 21’ 42.1"N	October 2020
Ravi	71° 49’ 42.5"E, 29° 11’ 12.2"N	October 2020
Jhelum	69° 30’ 14.4"E, 31° 39’47"N	October 2020

Indus	69° 49’ 56.1"E, 29° 31’45.2"N	October 2020
Chenab	72° 28’ 50.3"E, 31° 39’21.3"N	October 2020

2.2. Extraction of DNA and PCR amplification

DNA of 75 specimens of *Channa marulius* isolated with phenol-chloroform method using muscle tissue (Chowdhury et al., 2016). Five primers OPA-02, OPA-05, OPA-07, OPA-11 and OPA-16 were used for the PCR amplicons of Randomly Amplified Polymorphic DNA (Bhat et al., 2014) for screening *C. marulius* samples (Table 2).

Table 2. The primer code, sequence of primers, percentage of RAPD polymorphic and monomorphic loci detected among five river populations of C. marulius.

Primer code	***Sequences of primer***	***Total number of bands***	***Polymorphic band***		***Monomorphic band***	
			Total	***Percentage (%)***	***Total***	***Percentage (%)***
OPB-02	TGATCCCTGG	28	08	28.58	20	71.42
OPB-06	TGCTCTGCCC	31	24	77.41	07	22.59
OPC-11	AAAGCTGCGG	35	28	80.0	07	20.0
OPC-13	AAGCCTCGTC	33	26	78.78	07	21.22
OPC-15	GACGGATCAG	27	20	74.07	07	25.93
Total		**154**	**106**	**68.84**	**48**	**31.16**

PCR 25ml final product was prepared for each sample. The conditions of PCR amplification are set as; initial denaturation at 94°C at 5 minutes followed by 29 cycles at 94°C for 1 minute, annealing at 54°C for 1 minute, extension 72°C for 2 minutes and final extension 72°C for 10 minutes. The PCR amplicon products checked on 1.5% agarose gel and visualized under UV light. Genetic polymorphisms were detected on gel having PCR products stained with ethidium bromide. Amplicon bands of each gel photographed with Gel Documentation System (PHOTONYX DARKROOM NTGB/1003).

3. RESULTS

The results of *C. marulius* genetic diversity in five rivers showed 154 total RAPD loci with 106 polymorphic loci and 48 monomorphic loci using five RAPD primers (Table 2). OPB-02 marker produced a total of 28 amplicons (08 polymorphic and 20 monomorphic), OPB-06 marker 31 (24 polymorphic and 7 monomorphic), OPC-11 marker 35 (28 polymorphic and 7 monomorphic), OPC-13 marker 33 (26 polymorphic and 7 monomorphic), and OPC-15 marker 27 (20

polymorphic and 7 monomorphic). Percentage of RAPD polymorphic and monomorphic loci of five markers provided in table 2.

High genetic diversity is observed in *C. marulius* population in Jhelum as 28 (80%) and RAPD polymorphic loci detected includes 4 locus of OPB-02, 5 of OPB-06, 2 of OPC-11, 8 of OPC-13, and 9 of OPC-15. While low genetic diversity is observed in Chenab as 08 (28.58%) and RAPD polymorphic loci detected includes 0 locus of OPB-02, 01 of OPB-06, 02 of OPC-11, 04 of OPC-13, and 01 of OPC-15 (Table 3). The genetic diversity in Sutlej is 20 (74.07%) and RAPD polymorphic loci detected includes 01 locus of OPB-02, 04 of OPB-06, 04 of OPC-11, 08 of OPC-13, and 03 of OPC-15. In the case of Ravi, the genetic diversity is 26 (78.78%) and RAPD polymorphic loci detected includes 03 locus of OPB-02, 05 of OPB-06, 05 of OPC-11, 09 of OPC-13, and 04 of OPC-15. The genetic diversity observed in Indus is 24 (71.44%) and RAPD polymorphic loci detected includes 03 locus of OPB-02, 04 of OPB-06, 02 of OPC-11, 07 of OPC-13, and 08 of OPC-15 (Table 3).

In Chenab River the RAPD monomorphic loci detected is 20 (71.42%); in Indus, 7 (22.58%), in Jhelum, 7 (20.0%), in Ravi, 7 (21.21%) and in Sutlej, 7 (25.92%). River-wise monomorphic loci were detected for the River Chenab population (04 of loci of OPB-02, 05 loci of OPB-06, 05 loci of OPC-11, 0 loci of OPC-13, 06 loci of OPC-15), River Indus population (3 loci of OPB-02, 02 loci of OPB-06, 02 loci of OPC-11, 0 loci of OPC-13, 0 loci of OPC-15), River Jhelum population (3 loci of OPB-02, 02 loci of OPB-06, 02 loci of OPC-11, 0 loci of OPC-13, 0 loci of OPC-15), River Ravi population (4 loci of OPB-02, 01 loci of OPB-06, 01 loci of OPC-11, 0 loci of OPC-13, 01 loci of OPC-15), and River Sutlej population (3 loci of OPB-02, 01 loci of OPB-06, 01 loci of OPC-11, 0 loci of OPC-13, 02 loci of OPC-15) (Table 3 and Table 4).

Results of DNA fingerprints of PCR amplified product revealed genetic polymorphism in the band range of 100 base pairs to 800 base pairs among five riverine populations in Punjab, Pakistan (Figs 2A-2E). Interestingly, varied size range bands were observed, i.e. OPB-02 primer band range from 400 to 800 bp (Fig 2A); OPB-06 primer band range from 200 to 700 bp (Fig 2B); OPC-11 primer band range from 300 to 700 bp (Fig 2C); OPC-13 primer band range from 200 to 700 bp (Fig 2D); OPC-15 primer band range from 100 to 800 base pairs (Fig 2E).

Table 3. River wise RAPD genetic polymorphic, monomorphic and total bands detected in C. marulius of five riverine populations.

Primer code	***River Chenab***			***River Indus***			***River Jhelum***			***River Ravi***			***River Sutlej***		
	Polymorphic band	***Monomorphic band***	***RAPD total band***	***Polymorphic band***	***Monomorphic band***	***RAPD total band***	***Polymorphic band***	***Monomorphic band***	***RAPD total band***	***Polymorphic band***	***Monomorphic band***	***RAPD total band***	***Polymorphic band***	***Monomorphic band***	***RAPD band***
OPB-02	0	04	04	03	03	06	04	03	07	03	04	07	01	03	04
OPB-06	01	05	06	04	02	06	05	02	07	05	01	06	04	01	05
OPC-11	02	05	07	02	02	04	02	02	04	05	01	06	04	01	05
OPC-13	04	0	04	07	0	07	08	0	08	09	0	09	08	0	08
OPC-15	01	06	07	08	0	08	09	0	09	04	01	05	03	02	05
Total	**08**	**20**	**28**	**24**	**07**	**31**	**28**	**07**	**35**	**26**	**07**	**33**	**20**	**07**	**27**

Table 4. River wise percentage (%age) of RAPD polymorphic and monomorphic loci among five riverine populations of C. marulius.

Primer code	***River Chenab***			***River Indus***			***River Jhelum***			***River Ravi***			***River Sutlej***		
	Polymorphic band %	***Monomorphic band %***	***RAPD total band %***	***Polymorphic band %***	***Monomorphic band %***	***RAPD total band %***	***Polymorphic band %***	***Monomorphic band %***	***RAPD total band %***	***Polymorphic band %***	***Monomorphic band %***	***RAPD total band %***	***Polymorphic band %***	***Monomorphic band %***	***RAPD band %***
OPB-02	0	100	100	50	50	100	57.14	42.85	100	42.85	57.14	100	25	75	100
OPB-06	16.66	83.34	100	66.66	33.34	100	71.42	28.58	100	83.33	16.67	100	80	20	100
OPC-11	28.57	71.42	100	50	50	100	50	50	100	83.33	16.67	100	80	20	100
OPC-13	100	0	100	100	0	100	100	0	100	100	0	100	100	0	100
OPC-15	14.28	85.71	100	100	0	100	100	0	100	80	20	100	60	40	100
Total	**28.58**	**71.42**	**100**	**71.44**	**22.58**	**100**	**80**	**20**	**100**	**78.78**	**21.21**	100	**74.07**	**25.92**	100

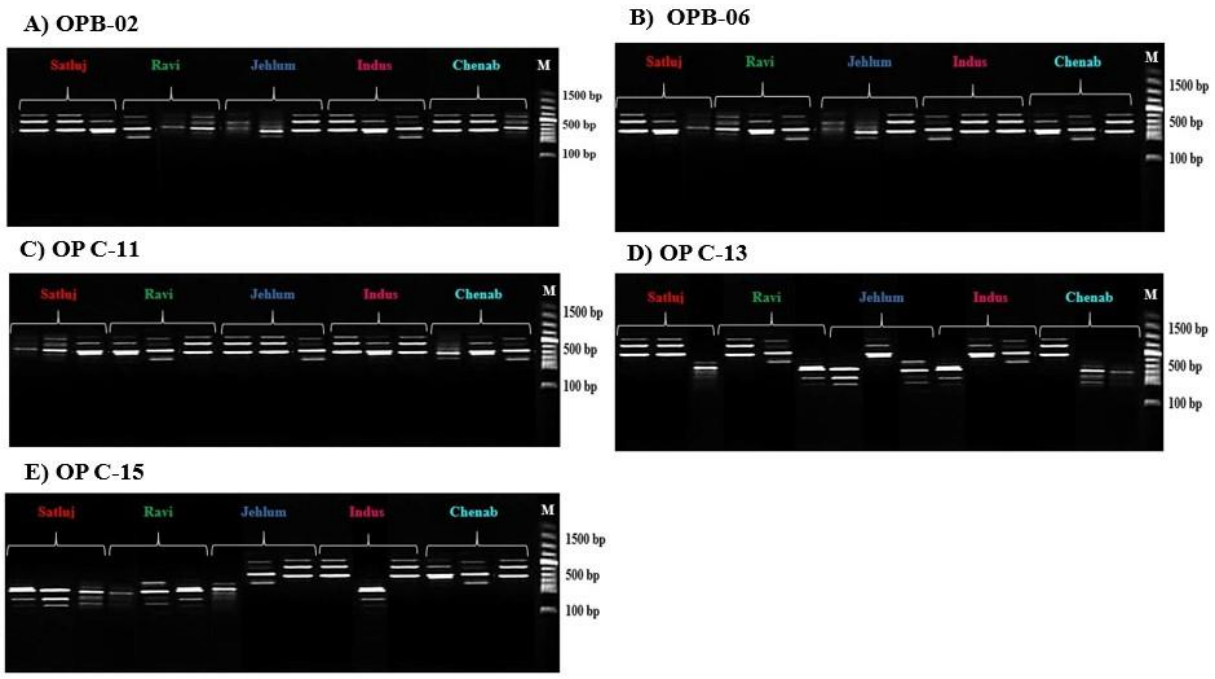

Figure 2. DNA fingerprints of PCR amplified product (A) OPB-02 primer band range 400 to 800 bp; (B) OPB-06 primer band range 200 to 700 bp (C); OPC-11 primer band range 300 to 700 bp; (D) OPC-13 primer band range 200 to 700 bp; (E). OPC-15 primer band range 100-800 base pairs.

3.1. RAPD Diversity and Genetic Differentiation

A total of 154 scorable bands were observed. Among them, polymorphic are 106 (68.84%) and monomorphic bands are 48 (31.16%). The pattern of genetic diversity observed in the five rivers is Jhelum> Ravi> Sutlej> Indus> Chenab (Table 3). Nei's genetic diversity (h) observed as 0.782 for Jhelum, 0.685 for Ravi, 0.672 for Sutlej, 0.611 for Indus and 0.323 for Chenab (Table 5).

Table 5. Analysis of Nei's genetic diversity and Nei's (1978) unbiased genetic distance C. marulius.

River	***Parameters of Nei's genetic diversity***		***Nei's (1978) Unbiased genetic distance***					
	Number of specimens	***Nei's genetic diversity (h)±SD***	***Population***	***Sutlej***	***Ravi***	***Jhelum***	***Indus***	***Chenab***
Chenab	15	**0.323**±0.0112	**Sutlej**	****	0.1120	0.2382	0.2292	0.4319
Indus	15	0.611±0.0143	**Ravi**	0.1120	****	0.2720	0.2520	0.3120
Jhelum	15	**0.782**±0.0213	**Jhelum**	0.2382	0.2720	****	0.4719	0.5119
Ravi	15	0.685±0.0243	**Indus**	0.2292	0.2520	0.4719	****	0.3119
Sutlej	15	0.672±0.0135	**Chenab**	0.4319	0.3120	0.5119	0.3119	****

3.2. Analysis of Genetic Distances and Genetic Connectivity Among Rivers

RAPD analysis data reported high genetic distance 0.5119 observed between Jhelum and Chenab whereas the lowest genetic distance observed 0.1120 between Ravi and Sutlej (Table 5). UPGMA dendrogram of genetic connectivity showed that Jhelum produced independent clade whereas two sub-clusters were also observed. Sutlej and Ravi found in one sub-cluster, Indus, and Chenab in another sub-cluster (Fig 3). UPGMA dendrogram revealed close genetic connectivity and genetic similarity among Sutlej and Ravi fish populations and Indus and Chenab riverine populations of *C. marulius*.

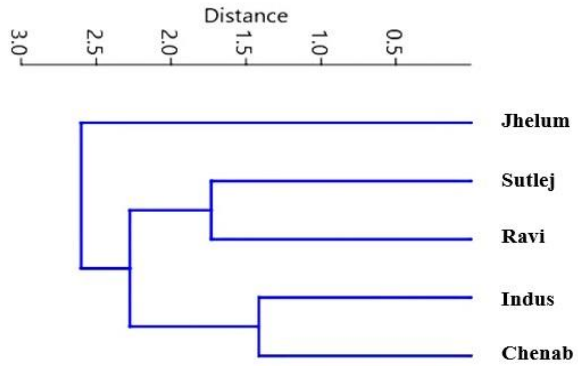

Figure 3. RAPD polymorphic UPGMA dendrogram showed genetic connectivity among five riverine populations.

4. DISCUSSION

The evaluation of genetic diversity is necessary for fisheries management and conservation (Jamsari et al., 2011; Xu et al., 2018; Gu et al., 2021; Zhang et al., 2023). In fishes, the genetic diversity correlates to their habitat (Excoffier et al., 2010; Ahmad and Naeem, 2023). The factors like mutations, genetic drift and habitat destruction may affect the genetic size of population, which effectively reduce genetic diversity (Ahmad and Naeem, 2023; Kebtieneh et al., 2024). The data of present study provides valuable information about genetic diversity of *C. marulius* in five major rivers of Pakistan.

Table 2 of present study reported 106 polymorphic loci among five riverine natural population of *C. marulius* as compared to Bhat et al. (2014), who reported 87 polymorphic loci in *Channa striatus*, Ola-Oladimeji et al. (2020) in *Clarias gariepinus* and Hassan and Naeem (2023) in *Pangasius pangasius*.

Table 3 of present study reported high genetic diversity 80% of *C. marulius* population in River Jhelum whereas, low genetic diversity 28.58% in River Chenab as Bhat et al. (2014), who

reported 87, high 92.31% and low 80.00% genetic diversity in *Channa striatus*, Ola-Oladimeji et al. (2020) in *Clarias gariepinus* and Hassan and Naeem (2023) in *Pangasius pangasius*.

Table 4 of present study percentage of polymorphic loci 68.84% and monomorphic 31.16% as Bhat et al. (2014), who reported 87, high 92.31% and low 80.00% genetic diversity in *Channa striatus*, Ola-Oladimeji et al. (2020) in *Clarias gariepinus* and Hassan and Naeem (2023) in *Pangasius pangasius*.

Table 5 Nei's genetic diversity (h) observed as Jhelum 0.782, Ravi 0.685, Sutlej 0.672, Indus 0.611 and Chenab 0.323 as Bhat et al. (2014) used Nei's method to report genetic diversity in *Channa striatus*, Ola-Oladimeji et al. (2020) in *Clarias gariepinus*, and Hassan and Naeem (2023) in *Pangasius pangasius*.

Table 5 of present study reported high UPGMA genetic distance 0.5119 between Jhelum and Chenab whereas the lowest genetic distance observed 0.1120 between Ravi and Sutlej as Bhat et al. (2014) used UPGMA method to report genetic distance in *Channa striatus*, Ola-Oladimeji et al. (2020) in *Clarias gariepinus* and Hassan and Naeem (2023) in *Pangasius pangasius*.

Figure 2 of present study showed varied size bands range 100-800 base pairs as compared to Bhat et al. (2014), who reported bands range 309-3029 base pairs, Ola-Oladimeji et al. (2020) in *Clarias gariepinus* and Hassan and Naeem (2023) in *Pangasius pangasius*.

Populations having low rate of genetic diversity may be at risk of species reduction. This may be due to mutations and genetic drift in the small population sizes (Gu et al., 2021; Zhang et al., 2023). In the present study, the observed pattern of genetic diversity among five populations of *C. marulius* seems to be consequence of gene flow between populations and phylogeographic events. These populations of *C. marulius* can be important for their adaptive significance and should be conserving on a priority basis.

5. CONCLUSION

Genetic diversity and population structure are vital factors for the management and conservation of fish species. Present study revealed the degree of genetic diversity of *C. marulius* in five rivers (Chenab, Indus, Jhelum, Ravi and Sutlej) of Pakistan. Results reported the high genetic diversity of *C. marulius* population in Jhelum River then Ravi, Sutlej and Indus whereas lowest genetic diversity of *C. marulius* in Chenab River. Consequently, proof of the lowest genetic diversity of

C. marulius in Chenab River is a matter of concern for fish resource managers and needs to take necessary action for its conservation to attain the sustainability.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

8. REFERENCE

Ahmad, W & Naeem, M. 2022. Genetic diversity in natural population of *Notopterus notopterus* evaluated through mitochondrial DNA marker ATPase 6/8 regions and cytochrome b gene. *Molecular Biology Reports*, **49(5)**: 3903–3910, DOI: 10.1007/s11033-022-07240-4.

Ahmad, W & Naeem, M. 2023. Analysis of efficiency of seven genomic DNA isolation techniques through applications of DNA barcode, PCR and Nanodrop from fins of *Notopterus notopterus* (PALLAS, 1769). *Malaysian Journal of Biochemistry & Molecular Biology*, **1(1)**: 77-87.

Ahmad, W & Naeem, M. 2023. Comparison of seven genomic DNA isolation techniques from internal organs, gills and muscle tissues of *Notopterus notopterus* (Notopteridae) using PCR amplification and Nanodrop. *International Journal of Biology and Chemistry*, **16(1)**: 4-16, DOI: 10.26577/ijbch.2023.v16.i1.01.

Ahmad, W & Naeem, M. 2023. Molecular Identification and Genetic Diversity of *Notopterus notopterus* (Pallas, 1769) Using Mitochondrial COI Gene and RAPD Markers in Riverine Water of Pakistan. *Punjab University Journal of Zoology*, **38(2)**: 181-190, DOI: 10.17582/journal.pujz/2023.38.2.181.190.

Ashraf, M., Zafar, A & Naeem, M. 2011. Comparative Studies on the Seasonal Variations in the Nutritional Values of Three Carnivorous Fish Species. *International Journal of Agriculture and Biology*, **13(5)**: 701-706.

Bhat, A. A., Haniffa, M. A., Milton, M. J., Paray, B. A., Divya, P. R & 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(5)**: 363-372, DOI: 10.5897/IJBC2013.0649.

Chauhan, T & Rajiv, K. 2010. Molecular markers and their applications in fisheries and aquaculture. *Advance in Bioscience and Biotechnology*, **1(4)**: 281–291, DOI: 10.4236/abb.2010.14037.

Chen, W., Li, C., Chen, F., Li, Y., Yang, J., Li, J & Li, X. 2020. Phylogeographic analyses of a migratory freshwater fish (*Megalobra materminalis*) reveal a shallow genetic structure and pronounced effects of sea-level changes. *Gene*, **737(1)**: 1478–1488, DOI: https://doi.org/10.1016/j.gene.2020.144478.

Chowdhury, M. M., Rahman, A. S., Nahar, L., Rahman, M., Reza, A. H & Ahmed, M. S. 2016. Efficiency of different DNA extraction methods for fish tissues: a comparative analysis. *IOSR. Journal of Pharmacy and Biological Sciences*, **11(3):** 11–15, DOI: 10.9790/3008-1103041115.

Courtenay, W. R & Williams, J. D. 2004. Snakeheads (Pisces, Channidae) a biological synopsis and risk assessment. *USGS Circular,* **1251**: 1-143, DOI: 10.3133/cir1251.

Excoffier, L., Smouse, P. E & Quattro, J. M. 2010. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131(2)**: 991-1000, DOI: 10.1093/genetics/131.2.479.

Gu, S., Yi, M. R., He, X. B., Lin, P. S., Liu, W. H., Luo, Z. S., Lin, H. D & Yan, Y. R. 2021. Genetic diversity and population structure of cutlassfish (*Lepturacanthus savala*) along the coast of mainland China, as inferred by mitochondrial and microsatellite DNA markers. *Regional Studies in Marine Sciences*, **43(8)**: 101702-101781, DOI: 10.1016/j.rsma.2021.101702.

Hassan, S & Naeem, M. 2023. Assessment of genetic diversity by random amplified polymorphic DNA (RAPD) and ATPase 6/8 gene in *Pangasius pangasius*. *Journal of Animal and Plant Sciences*, **33(4)**: 911-919, DOI: 10.36899/JAPS.2023.4.0684.

Hossain, M. K, Latifa, G. A & Rahman, M. M. 2008. Observations on induced breeding of snakehead murrel, *Channa striatus* (Bloch, 1793). *International Journal of Sustain Crop Production,* **3(5)**: 65-68.

Ismat, N., Ashraf, M., Naeem, M & Rehman, M. H. 2013. Effect of different feed ingredients on growth and level of intestinal enzyme secretions in juvenile *Labeo rohita*, *Catla catla*,

Cirrhinus mrigala and *Hypophthalmicthys molitrix*. *International Journal of Aquaculture*, **3(1)**: 85-91, DOI: 10.5376/ija.2013.03.0016.

Jamsari, A. F., Pau, T. M & Sitiazizah, M. N. 2011. Genetic structure of the snakehead murrel, *Channa striata* (Channidae) based on the cytochrome c oxidase subunit I gene: influence of historical and geomorphological factors. *Genetics and Molecular Biology*, **34(1)**: 152-160, DOI: 10.1590/S1415-47572011000100026.

Kebtieneh, N., Alemayehu, K & Tilahun, G. 2024. Genetic Diversity and Population Structure of African Catfish (*Clarias gariepinus*) Species: Implications for Selection and Sustainable Genetic Improvement. A Review. *Journal of Aquaculture & Research Development*, **15(1)**: 828, DOI: 10.35248/2155-9546.24.14.828.

Khan, N. A & Naeem, M. 2024. Efficiency Evaluation of DNA Isolation Techniques in Fins of *Channa marulius* Using PCR Amplification and NanoDrop. *Momona Ethiopian Journal of Science*, **16(2)**: 331-346, DOI: 10.4314/mejs.v16i2.10.

Khan, N. A & Naeem, M. 2024. rDNA Barcode Base Molecular Identification and Evaluation of Genetic Connectivity of *Channa marulius* in Major Rivers of Pakistan. *Philippine Journal of Science*, **153(5)**: 1507-1522.

Kousar, S., Rehman, N., Javed, A., Hussain, A., Naeem, M., Masood, S., Ali, H. A., Manzoor, A., Khan, A. A., Akram, A., Iqbal, F., Zulfiqar, A., Jamshaid, M. B., Waqas, M., Waseem, A & Saeed, M. Q. 2021. Intensive Poultry Farming Practices Influence Antibiotic Resistance Profiles in *Pseudomonas aeruginosa* inhabiting Nearby Soils. *Infection and drug resistance*, **14(1)**: 4511-4516, DOI: 10.2147/IDR.S324055.

Mayanglambam, B. D., Kshetrimayum, P. D., Laishram, S. D & Asem, I. D. 2024. Genetic variability study of *Channa punctatus* (Bloch, 1793) using random amplified polymorphic DNA markers. *Journal of Experimental Zoology India*, **27(1)**: 1387-1390, DOI: 10.51470/jez.2024.27.1.1387.

Michelle, N. Y. T., Shanti, G & Loqman, M. Y. 2004. Effect of orally administered Channa striatus extract against experimentally induced osteoarthritis in rabbits. *International Journal of Applied Resource and Veterinary Medicine*, **2(3)**: 171-175.

Mirza, M. R. 1982. A Contribution to the Fishes of Lahore. Polymer Publications Urdu Bazaar, Lahore, Pakistan, 48p.

Naeem, M., Salam, A & Ishtiaq, A. 2011. Length–weight relationships of wild and farmed *Tor putitora* from Pakistan. *Journal of Applied Ichthyology*, **27(4)**: 1133-1134, DOI: 10.1111/j.1439-0426.2010.01613.

Naeem, M., Salam, A & Zuberi, A. 2016. Proximate composition of freshwater rainbow trout (*Oncorhynchus mykiss*) in relation to body size and condition factor from Pakistan. *Pakistan Journal of Agricultural Sciences,* **53(2)**: 497-502, DOI: 10.21162/PAKJAS/16.2653.

Naeem, M., Salam, A., Ishtiaq, A & Shafique, S. 2010. Length-weight and condition factor relationship of farmed hybrid (*Catla catla*♂ X *Labeo rohita*♀) from Multan, Pakistan. *Sindh University Research Journal (Science Series),* **42(2)**: 35-38.

Naeem, M., Salam, A., Tahir, S. S & Rauf, N. 2010. Assessment of the essential element and toxic heavy metals in hatchery reared *Oncorhynchus mykiss*. *International Journal of Agriculture and Biology,* **12(6)**: 935-938.

Naeem, M., Zuberi, A., Khan, N. A., Rasool, S. A., Ismail, H. F & Qamer, A. 2012. Some morphometric relationship traits of *Labeo bata* (Hamilton, 1822) from Head Panjnad, Pakistan. *African Journal of Biotechnology*, **11(88)**: 15465-15468, DOI: 10.5897/AJB11.3155.

Naeem, M., Zuberi, A., Salam, A., Ashraf, M., Elahi, N., Ali, M., Ishtiaq, A., Malik, T., Khan, M. J., Ayaz, M. M., Iqbal, M. J & Ahmad, B. 2011. Induced spawning, fecundity, fertilization rate and hatching rate of Grass carp (*Ctenopharyngodon idella*) by using a single intramuscular injection of ovaprim-C at a fish. *African Journal of Biotechnology*, **10(53)**: 11048-11048, DOI: 10.5897/AJB10.1481.

Nelson, J. S. 1984. Fishes of the World. New York John Wiley and Sons, 523p.

Ola-Oladimeji, F. A., Awodiran, M. O., Olaleye, V. F & 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, DOI: 10.4194/2459-1831-v4_2_03.

Pervaiz, K., Iqbal, Z. Mirza, M. R., Javed, M. N., Naeem, M & Ishtiaq, A. 2012. Length–weight, length–length relationships and feeding habits of wild Indus Mahseer, *Tor macrolepis*, from Attock, Pakistan. *Journal of Applied Ichthyology,* **28(1)**: 673-676, DOI: 10.1111/j.1439-0426.2012.01953.

Rahman, A. K. A. 2005. Freshwater Fishes of Bangladesh, 2nd Ed. The Zoological Society of Bangladesh, Department of Zoology, University of Dhaka, Dhaka-1000, 394p.

Talwar, P. K & Jhingran, A. G. 1992. Inland fishes of India and adjacent countries. A.A. *Alkema Rotterdam*, 1-1062.

Ukenye, E. A., Taiwo, I. A & Anyanwu, P. E. 2019. Morphological Genetic variation in *Tilapia guineensis* in West African coastal waters: A mini review. *Biotechnology Reports*, **24(1)**: e00362, DOI: 10.1016/j.btre.2019.e00362.

Urooj, N., Sahato, G. A., Lashari, K. H., Korai, A. L., Palh, Z. A & Naqvi, H. S. 2011. Ichthyodiversity of River Indus, at Jamshoro District, Sindh. *Sindh University Research Journal (Science Series)*, **43(1)**: 13-18.

Xu, S., Wang, Z., Zhang, Y., Gao, T., Zou, J & Zhang, X. 2018. Evidence for intra-mitochondrial variation in population genetic structure of *Platycephalus* sp.1 In the Northwestern Pacific. *Mitochondrial DNA Part A*, **30(2)**: 281–288, DOI: 10.1080/24701394.2018.1484119.

Yousaf, M., Salam, A & Naeem, M. 2011. Body composition of freshwater *Wallago attu* in relation to body size, condition factor and sex from southern Punjab, Pakistan. *African Journal of Biotechnology*, **10(20)**: 4265-4268, DOI: 10.5897/AJB10.1903.

Zhang, C., Chen, X., Yuan, L., Wu, Y., Ma, Y., Jie, W., Jiang, Y., Guo, J., Qiang, L., Han, C & Shu, H. 2023. Genetic diversity and population structure of Chinese Gizzard Shad *Clupanodon thrissain* South China based on morphological and molecular markers. *Global Ecology and Conservation*, **41**: e02367, DOI: 10.1016/j.gecco.2023.e02367.