Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i7.7228

MORPHOMETRIC AND POPULATION GENETIC ANALYSIS OF ENDAGERED CATFISH *RITA RITA* FROM RIVER INDUS AND CHENAB PUNJAB, PAKISTAN

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ABSTRACT
The common name of *Rita rita* fish is Rita that is a commercially important catfish, valued for its good taste and rich protein content. However, there is a lack of sufficient local data on its molecular identification and phylogenetic analysis. *R. rita* specimens were sampled from four distinct geographical sites in Punjab province, along the Indus and Chenab rivers in Pakistan. Morphometric measurements and molecular techniques were employed to assess intraspecific variations among populations of *R. rita*. Mitochondrial DNA was extracted from *R. rita* tissue, and the COI gene was utilized for molecular identification. Principal Component Analysis (PCA) was used to estimate variation levels. Morphometric data clearly segregated *R. rita* from different locations into two distinct categories, accounting for 99.7% of cumulative variability. Non-significance ($P < 0.05$) among *R. rita* indicated a unique control haplotype localized within the sub-population. Intraspecific distances ranged from 0.000 to 0.068 across the four populations, while sequences retrieved from the NCBI database spanned 0.000 to 0.059, with sequence diversity ranging from 0.000 to 0.0071 in this intraspecific comparison. A cladogram was constructed to visualize the phylogenetic relationships among *R. rita* from different geographical locations. The phylogenetic analysis of *R. rita* populations in this study provides valuable insights into genetic variations within the species, essential for devising effective conservation strategies in Pakistan's fisheries management programs. The study identified significant intraspecific genetic variations in *R. rita* across different locations in Punjab, Pakistan, using morphometric and molecular techniques, providing crucial insights for effective conservation strategies.

Keywords: *Rita rita*, Morphometric parameters, COI gene, Phylogeny, Principal component analysis

INTRODUCTION
The rapidly increasing global aquaculture industry is a major source of high-quality food products, animal protein, job opportunities, health status, and economic benefits (Boyd et al., 2022; Manzoor et al., 2023a). Aquaculture contributions to aquatic products reached 122.6 million metric tons, valued at 281.5 billion US dollars, with an annual growth rate of 6.7% (Emeish et al., 2023; Prakoso et al., 2023). The current worldwide per capita fish consumption is 20.5 kg (Kazmi et al., 2023; Mahmood

et al., 2024; Ullah et al., 2023a). Fish is a cost-effective and second-ranked protein source (Manzoor et al., 2023b; Ullah et al., 2023b), accounting for 60% of protein intake (Mansoor et al., 2023; Saad et al., 2023). Approximately 225 Bagrid catfish species across 19 genera belong to the family Bagridae, which is widely distributed in Africa and Asia (Mitra et al., 2017). *R. rita*, a member of the Bagridae family within the Siluriformes order, is commonly known as catfish. *R. rita* is primarily a riverine fish species and is found in rivers and streams across South Asia, Bangladesh, Afghanistan, Pakistan, India, Nepal, and Myanmar (Khondker et al., 2023; Froese and Pauly, 2007). This species inhabits freshwater environments, as well as estuaries and coastal waters (Yashpal et al., 2006; Khondker et al., 2023). Notably, it is esteemed for its taste, nutritional value, and medicinal properties. *R. rita* muscles are protein-rich (17.2-19.5%), low in fat (1.01-2.70%), abundant in minerals (0.89-1.07%), and a source of vitamin A, contributing to its high market demand and price (Khondker et al., 2023; Bengtsson et al., 2012).

The identification of fish species was done by measuring different body characteristics (Hubert et al., 2015; Lashari et al., 2004). In Indonesia, the Bagridae family comprises 60 freshwater fish species found exclusively in Java, Sumatra, and Kalimantan, with some species also present in Thailand and India (Supiwong et al., 2013; Punhal et al., 2018). Despite its significance, *R. rita* remains reliant on wild sources for reproduction, as attempts at domestication have been unsuccessful. Historical data indicate that human-induced factors, such as overfishing, habitat degradation, pollution, and other anthropogenic influences, have significantly reduced its native population (Parveen and Faisal, 2002; IUCN, 2015). Presently, the conservation status of *R. rita* varies: it is endangered in Bangladesh, near threatened in India, and least concern globally (IUCN, 2015; Gupta, 2015).

It's imperative to conserve and manage the genetic diversity and structure of *R. rita*, considering the potential effects of population decline on genetic health. Small, fragmented populations face risks such as loss of genetic diversity, harmful allele fixation, inbreeding, and reduced fitness (Frankham et al., 2009), which can hinder their ability to adapt to ecological changes and heighten the risk of extinction (Frankham, 2005). *R. rita* holds commercial importance in catfish farming and fishing, particularly in the rivers of South Asia, where it is appreciated for its taste and high protein content. Recently, it has even gained recognition as an ornamental fish. However, its status as a critically endangered species in Bangladesh highlights the decline due to overexploitation and ecosystem changes (Jafri et al., 1998; ICUN, 2003).

Hence, this study aims to assess the genetic diversity and population structure of the endangered catfish *R. rita* in the Indus and Chenab rivers. This analysis will contribute to a broader understanding of the species' conservation needs and strategies.

MATERIALS AND METHODS

Samples collection and transportation

A total of 200 samples of *R. rita* were collected from four different geographical locations within the Indus riverine system of Pakistan: the River Indus (Taunsa Barrage and Chashma Barrage) and the River Chenab (Trimmu Barrage and Head Panjnad). These locations are situated in different geographic areas of Punjab, Pakistan, as shown in Fig. 1. The collected samples were transported in an ice box to the Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, for further analysis.

Morphometric analysis

The morphometric parameters such as fish body weight, fork length, total length, head length, paired pectoral fin length, paired pelvic fin length, and caudal fin length were recorded for each individual sample collected from different sites (Rasool et al., 2013).

DNA extraction

DNA was extracted using the phenol-chloroform protocol (Sambrook and Russell, 2001). The DNA was diluted to a final concentration of 100 ng/mL. The COI gene from *R. rita* fish samples was amplified using a combination of forward and reverse primers; LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3'HCO 1298 5'- TAAACTTCAGGGTGACCAAAA AATCA-3' (Pramono et al., 2019).

PCR amplification

A PCR reaction with a total volume of 25 µl was prepared, containing 5 µl of genomic DNA, 2 µl of forward and reverse primers each, 12 µl of DreamTaq Green PCR Master Mix (2X) (Catalog number: K1081, ThermoFisher Scientific, USA), and 4 µl of deionized water. The COI barcode region of 724 bp was amplified in a thermal cycler (Bio-Rad, USA, Model 1861096) under the following thermal conditions: The PCR program consisted of 35 cycles of pre-denaturation incubation at 95°C for 3 minutes, denaturation at 98°C for 10 seconds, annealing at 50°C for 30 seconds, and extension at 68°C for 1 minute; followed by holding the mixture at 40°C (Pramono et al. 2019).

Gel-electrophoresis and sequencing

The 1% agarose gel (Invitrogen), stained with 5 µl of ethidium bromide, was visualized using a Gel Documentation System (Bio-Rad, USA). The COI gene was detected and subsequently sequenced using the Sanger sequencing method (BGI Hong Kong Co. Ltd., China).

Morphometric and molecular data analysis

Regression analysis was used to estimate the length-weight relationship, while analysis of variance (ANOVA) was performed to compare four populations from sampling sites based on morphometric parameters using XLSTAT version 21.1.1 (Rasool et al., 2013). Alignment of the sequenced data was performed with sequences from NCBI using ClustalW (Thompson et al., 1997). The sequences were submitted to NCBI GenBank for accession numbers. Furthermore, the extent of sequence variation between species was calculated by averaging pairwise comparisons, and the number of haplotypes was tested using DnaSp 5.10 (Librado and Rozas, 2009). Based on the Kimura 2-parameter (K2P) model, neighbor-joining (NJ) trees were constructed using MEGA 11 (Kimura, 1980; Tamura et al., 2013).

RESULTS

Morphometric parameters

The results from the Principal Component Analysis (PCA) clearly illustrated a relationship between the increasing number of factors or components and the corresponding decrease in eigenvalues. This trend was most pronounced at the second factor, as depicted in Table 1. Transformation analysis highlighted that the first two factors (F1 and F2) significantly contributed to the variance, as indicated by their bold values in Table 1. Applying the Kaiser criterion, which was based on eigenvalues exceeding one, the PCA for the morphometric analysis of *R. rita* populations showed that despite limited distinctiveness among the four populations, 99.7% of the fish were correctly classified into their respective species clusters.

The first factor (F1), comprising parameters such as fish body weight, total length, dorsal fin length, caudal fin length, anal fin length, average length of paired pectoral fins, and average length of paired pelvic fins, collectively explains 94.6% of the total variability. The second distinct factor (F2), primarily represented by the fork length of the fish body, contributes 5.1% to the cumulative variability, as detailed in Table 1. Positive correlations observed between fish body weight and fork length, total length, head length, anal fin length, and caudal fin length are shown in Table 2.

The graphs derived from PCA-processed data illustrated relationships among variables (morphometric parameters), observations (samples from four sites), and a bi-plot that represents both. These visualizations assessed the similarities among individuals from diverse populations across four

geographical locations. Through PCA transformation, the graph based on F1 and F2 factors showed that these factors collectively explain 99.7% of the variability, with F1 contributing 94.6% and F2 contributing 5.1%. In this variable plot, all populations exhibited minor differences within the range of 0 to +1.0 (Fig. 2).

The graphs derived from PCA-processed data illustrated relationships among variables (morphometric parameters), observations (samples from four sites), and a bi-plot that represents both. These visualizations assessed the similarities among individuals from diverse populations across four geographical locations. Through PCA transformation, the graph based on F1 and F2 factors showed that these factors collectively explained 99.7% of the variability, with F1 contributing 94.6% and F2 contributing 5.1%. In this variable plot, all populations exhibited minor differences within the range of 0 to +1.0 (Fig. 2).

Genetic diversity and population dynamic of *R. rita*

In this study, a total of four sequences were acquired and subsequently submitted to the GenBank database, where they had been assigned unique accession numbers ON1920187, ON920188, ON920189, and ON920190. Additionally, the current study identified five distinct haplotypes (h) based on the COI gene of *R. rita*. The overall haplotype diversity (Hd) was calculated at 0.59048, with an average of 11.79048 nucleotide differences (Kt), and a recorded nucleotide diversity (PiT) of 0.02005. An exceptional control haplotype was found within a specific sub-population of *R. rita*, despite the insignificance of the documented P value. The computed Kimura 2-parameter (K2P) genetic distance values, based on COI sequences, are presented in Table 4. These computations were executed using the MEGA X software. The Transition/Transversion bias (R) was estimated at 1.02.

The substitution pattern and rates were assessed using the Kimura 2-parameter model. The nucleotide frequencies were documented as follows: A = 26.63%, T/U = 30.05%, C = 25.99%, and G = 16.87%. Intra-species comparisons within *R. rita* from four distinct geographic locations exhibited a range of distances from 0.000 to 0.068. In contrast, when compared with sequences from the NCBI database, the distances ranged from 0.000 to 0.059. The pairwise sequence divergence findings are presented in Table 4. The intra-species sequence diversity demonstrated variability, with the highest diversity recorded as 0.071 and the lowest as 0.000. Using the Kimura 2-parameter (K2P) model, a Neighbor-Joining (NJ) tree was constructed based on both the gene sequences of *R. rita* from the present study and additional sequences retrieved from the database. The resulting NJ tree is depicted in Figure 5.

DISCUSSION

The results of the current study are consistent with those of Rabbani et al. (2024), who used principal component analysis to reveal that the first two factors (F1 and F2) significantly contribute to the variability. Applying the Kaiser criterion, which is based on eigenvalues greater than one, the PCA conducted for morphometric character analysis indicated that although the four populations of *R. rita* did not display high distinctiveness, 97.6% of the fish were correctly classified into their respective species clusters.

Factor one (F1) encompassed parameters such as fish body weight, total length, dorsal fin length, caudal fin length, anal fin length, the average length of paired pectoral fins, and the average length of paired pelvic fins, accounting for 94.6% of the cumulative variability. The second distinct group, represented by the fork length of the fish body and denoted as factor two (F2), contributed 3% to the cumulative variability.

Moreover, our findings are consistent with the study conducted by Kashyap et al. (2016) on the morphometric variation of C. punctatus. This research identified significant differences in multivariate morphometric analysis among sub-populations of the freshwater murrel, *C. punctatus*, inhabiting diverse habitats characterized by varying environmental conditions. The study revealed linear correlations between various body measurements and the overall length of the fish across these habitats. Among the seventeen morphometric parameters and five meristic parameters analyzed using

univariate (one-way ANOVA) analysis, fourteen and three parameters, respectively, showed statistically significant differences. These significant parameters were subsequently subjected to multivariate analysis, including principal component analysis and discriminant function analysis.

In the principal component analysis, the first component explained 57% of the total variance, while the second and third components contributed 13% and 6%, respectively, cumulatively explaining 76% of the total variance across the two sub-populations. Kamran et al. (2020) and Zhou et al. (2022) documented morphological distinctions among five Channa species, with their principal component analysis revealing that the first five principal components collectively accounted for 78.93% of the variance.

Moreover, our findings are consistent with the study conducted by Kashyap et al. (2016), on the morphometric variation of *C. punctatus*. This research identified significant differences in multivariate morphometric analysis among sub-populations of the freshwater murrel, *C. punctatus*, inhabiting diverse habitats with varying environmental conditions. The study revealed linear correlations between various body measurements and the overall length of the fish across these habitats. Among the seventeen morphometric parameters and five meristic parameters analyzed using univariate (one-way ANOVA) analysis, fourteen and three parameters, respectively, showed statistically significant differences. These significant parameters were subsequently subjected to multivariate analysis, including principal component and discriminant function analyses.

These results aligned with an earlier investigation (Zhu et al., 2013) that utilized partial cytochrome c oxidase subunit I (COI) to identify Channa species. Importantly, the genetic distance (K2P) between different species exceeded that within species. Among the species examined, the smallest genetic distance between species was observed between *C. argus* and *C. maculata* (0.091), while the largest was between *C. argus* and *C. striata* (0.219). Clear distinctions in genetic barcodes were evident between interspecific and intraspecific distances for each species.

In a similar way, Zou et al. (2020) utilized DNA barcoding and phylogenetic analysis to study catfish. Their findings revealed intraspecific genetic distances among 14 species ranging from 0 to 0.0368, with an average of 0.0063. Notably, the widest intraspecific genetic gap was observed within Pseudobagrus eupogon. Interspecific genetic distances varied from 0.0069 to 0.2088, averaging 0.1170 across distinct Bagridae species. The most significant interspecific genetic divergence occurred between Mystus wyckioides and Pelteobagrus intermedius (0.2088).

Similar findings were reported in a study conducted by Kamran et al. (2020), which focused on identifying and analyzing Channa species from Pakistan's riverine system using the COI gene as a DNA barcoding marker. According to their results using the K2P model, intraspecific divergence across the three species ranged from 0.00 to 0.023, while interspecific divergence varied from 0.182 to 0.195. The most notable genetic distance was observed between *C. striata* and *C. marulius*, whereas the smallest distance occurred between *C. punctata* and *C. marulius*. The Neighbor Joining (NJ) tree, constructed based on the K2P model for twenty-seven specimens within the Channidae family, revealed three distinct clades, confirming the presence of distinct species. These findings provide additional support for a consistent phylogenetic relationship among the species investigated.

Similar findings were observed in a study by Rabbani et al. (2024) on the morphometric and molecular characterization of *Channa marulius* from the riverine system of Punjab, Pakistan. Their research revealed that intra-species genetic distances for *C. marulius* across four different geographic locations ranged from 0.000 to 0.001. When compared with sequences from the NCBI database, intra-species genetic distances ranged from 0.000 to 0.003. The diversity of intra-species sequences varied from 0.006 to 0.000. Additionally, the study identified five haplotypes (h) based on the COI gene of *C. marulius*. Overall, haplotype diversity (Hd) was calculated as 0.42647, with an average number of nucleotide differences (Kt) of 1.01471, and nucleotide diversity (PiT) recorded at 0.00182.

CONCLUSION

The study identified significant intraspecific genetic variations in *R. rita* across different locations in Punjab, Pakistan, using morphometric and molecular techniques, providing crucial insights for effective conservation strategies.

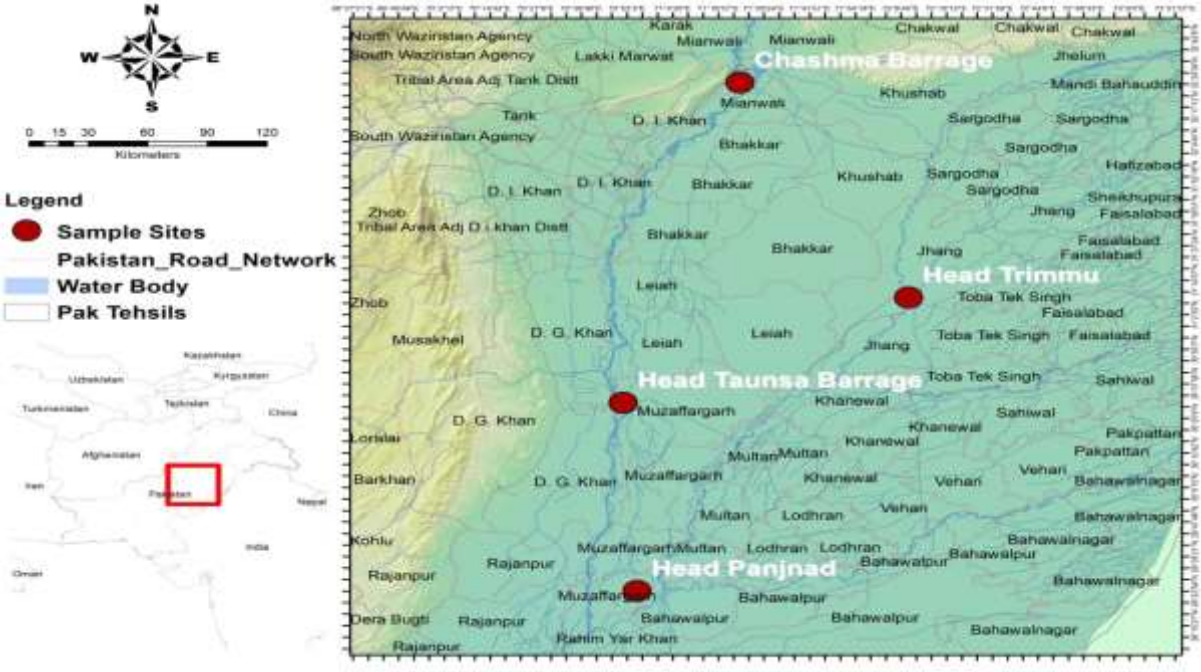

Fig 1. Sampling locations and geographical coordinates of sampled sites

Table 1. Eigen values for morphometric parameters of *Rita rita*

Eigenvalue	7.5695	0.4073	0.0138	0.0056	0.0023	0.0013	0.0002	0
Proportion	0.946	0.051	0.002	0.001	0	0	0	0
Cumulative	0.946	0.997	0.999	1	1	1	1	1

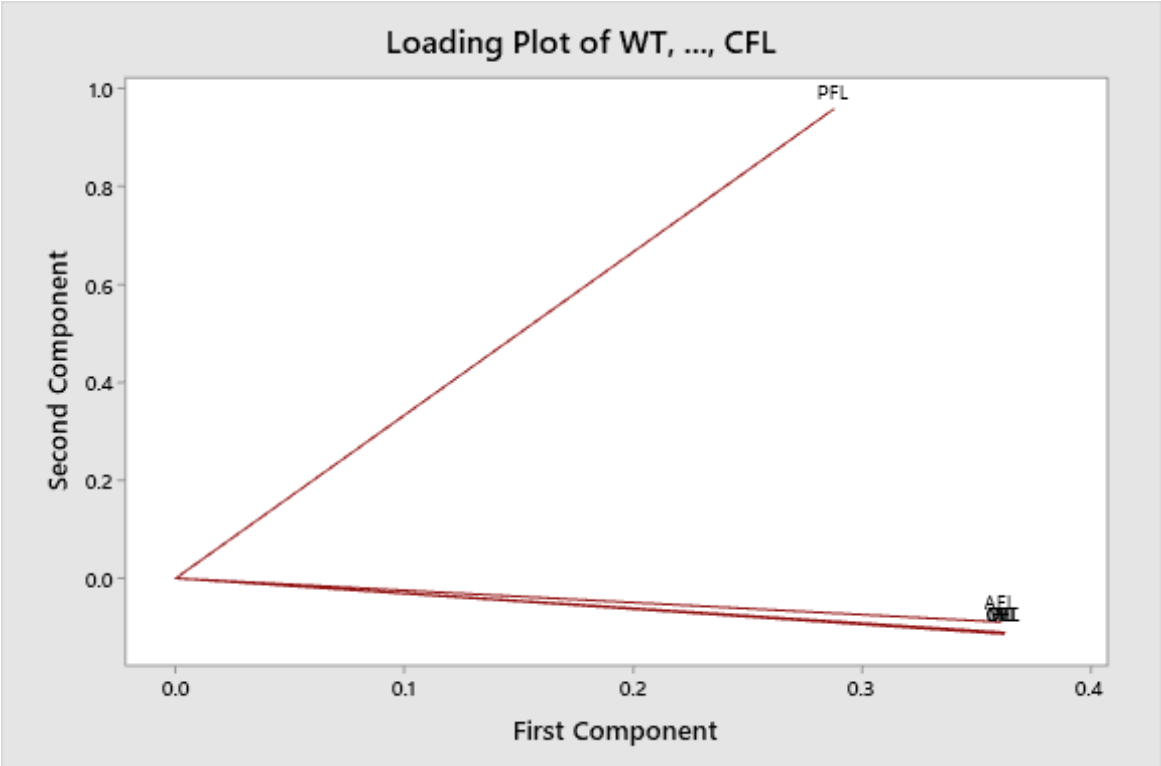

Fig 2. Morphometric variable plot

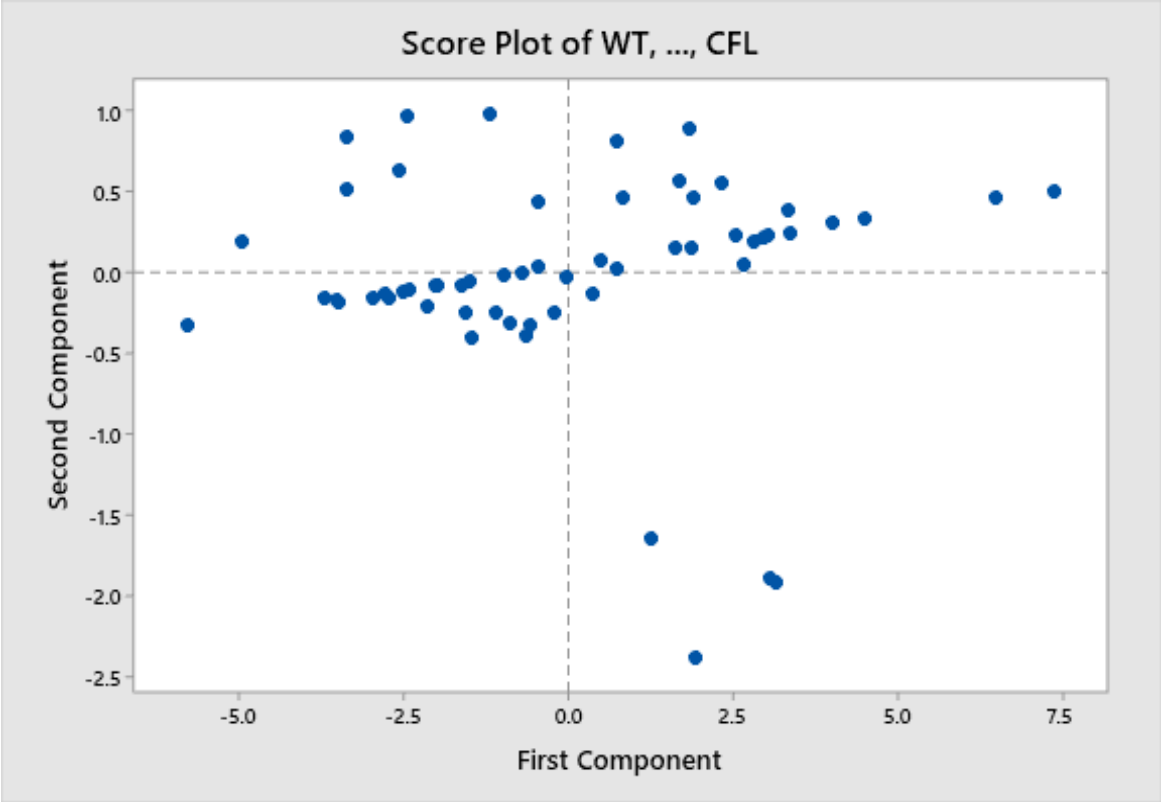

Fig 3. Samples observations Score-Plot

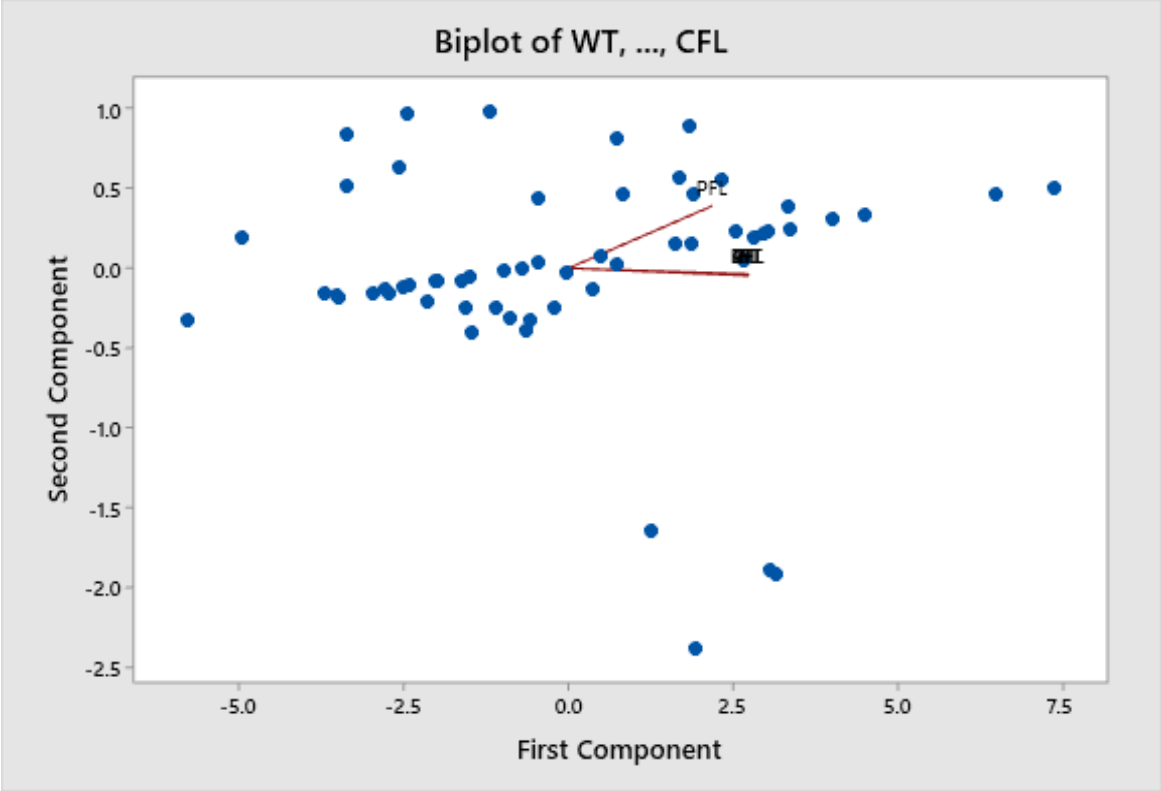

Fig 4. Variables and observations Bi-Plot

Fig 5. NJ Tree constructed for *R. rita* of different Sites

Table 2. Correlation Matrix of *R. rita*

Variables	WT	FL	TL	HL	DFL	PFL	AFL	CFL
WT								
FL	0.998							
TL	1.000	0.998						
HL	0.999	0.958	0.998					
DFL	1.000	0.997	1.000	0.998				
PFL	0.746	0.745	0.746	0.745	0.745			
AFL	0.992	0.988	0.993	0.992	0.993	0.751		
CFL	0.998	0.995	0.998	0.996	0.998	0.745	0.990	

Table 3. P distance of *R. rita* Sequences

	ON920187	ON920188	ON920189	ON920190	MT670302	MT670304	MH165300	MK480372	MK572552	MK572551	MH087043	MH087033	MH165300	KT762374	KT364781
ON920187															
ON920188	0.057														
ON920189	0.068	0.017													
ON920190	0.066	0.014	0.000												
MT670302	0.066	0.014	0.000	0.000											
MT670304	0.068	0.017	0.000	0.000	0.000										
MH165300	0.057	0.000	0.017	0.014	0.014	0.017									
MK480372	0.000	0.057	0.068	0.066	0.066	0.068	0.057								
MK572552	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057							
MK572551	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057	0.000						
MH087043	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057	0.000	0.000					
MH087033	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057	0.000	0.000	0.000				
MH165300	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057	0.000	0.000	0.000	0.000			
KT762374	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057	0.000	0.000	0.000	0.000	0.000		
KT364781	0.057	0.000	0.013	0.013	0.013	0.013	0.000	0.057	0.000	0.000	0.000	0.000	0.000	0.000	

Table 4. Kimura pairwise genetic distance

	ON920187	ON920188	ON920189	ON920190	MT670302	MT670304	MH165300	MK480372	MK572552	MK572551	MH087043	MH087033	MH165300	KT762374	KT364781_
ON920187															
ON920188	0.059														
ON920189	0.071	0.018													
ON920190	0.070	0.014	0.000												
MT670302	0.070	0.014	0.000	0.000											
MT670304	0.071	0.018	0.000	0.000	0.000										
MH165300	0.059	0.000	0.018	0.014	0.014	0.018									
MK480372	0.000	0.059	0.071	0.070	0.070	0.071	0.059								
MK572552	0.059	0.000	0.013	0.013	0.013	0.013	0.000	0.059							
MK572551	0.059	0.000	0.013	0.013	0.013	0.013	0.000	0.059	0.000						
MH087043	0.059	0.000	0.013	0.013	0.013	0.013	0.000	0.059	0.000	0.000					
MH087033	0.059	0.000	0.013	0.013	0.013	0.013	0.000	0.059	0.000	0.000	0.000				
MH165300	0.059	0.000	0.013	0.013	0.013	0.013	0.000	0.059	0.000	0.000	0.000	0.000			
KT762374	0.059	0.000	0.013	0.013	0.013	0.013	0.000	0.059	0.000	0.000	0.000	0.000	0.000		
KT364781	0.059	0.0000	0.013	0.013	0.013	0.013	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.000	

REFERENCES

1. Bengtsson L, Herschy RW, Fairbridge RW. Encyclopedia of lakes and reservoirs. Monographiae Biologicae 2012. 53:10-26.
2. Boyd CE, McNevin AA, Davis RP. The contribution of fisheries and aquaculture to the global protein supply. Food Secur 2022. 14(3):805-27.
3. Emeish WFA, Soliman SA, Ahmed AE, et al. Osmoregulatory genes expression analysis and chloride cells activity in common carp: insight from fish exposed to salinity. Pak Vet J 2023. 43(3): 515-522. http://dx.doi.org/10.29261/pakvetj/2023.069.
4. Frankham R, Ballou JD, Briscoe DA. Introduction to conservation genetics 2009. 2nd Edition. Cambridge University Press, Cambridge.
5. Frankham R. Stress and adaptation in conservation genetics. J Evol Biol 2005. 18(4):750-5.
6. Froese R, Pauly D. *Rita rita*. FishBase. June 2007 version.
7. Gupta S. *Rita rita* (Hamilton, 1822), a threatened fish of Indian Subcontinent. Int Res J Biolog Sci 2015. 4:70-73.
8. Hubert N, Kadarusman PD, Wibowo A, et al. DNA Barcoding Indonesian freshwater fishes: challenges and prospects. DNA Barcodes 2015. 3(1):144-69.
9. IUCN Bangladesh. Bangladesher Bipanno Prani. IUCN. The world conversation union 2003. 294
10. IUCN Bangladesh. Red List of Bangladesh Volume 5: Freshwater Fishes. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh 2015. 360 pp.
11. Jafri SI, Narejo NT, Hussain SM. Anomalous dorsal fin of a cat fish, *Rita rita*. Pak J Zool (Pakistan).1998. 30(2).
12. Kamran M, Yaqub A, Malkani N, et al. Identification and phylogenetic analysis of Channa species from riverine system of Pakistan using COI gene as a DNA barcoding marker. J Bioresour Manag 2020. 7(2):10.
13. Kashyap A, Awasthi M, Serajuddin M. Geographic morphometric variations of freshwater murrel, *Channa punctatus* from northern and eastern parts of India. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 2016. 86:367-73.
14. Kazmi SAH, Iqbal R, Al-Doaiss AA, et al. Azoxystrobin-induced oxidative stress in gills, hematological biomarkers and histopathological ailments in fresh water fish. Pak Vet J 2023. 43(2): 321-326. http://dx.doi.org/10.29261/pakvetj/2023.025.
15. Khondker M, Bhuiyan MA, Bashar MA. Towards an Appraisal of Biodiversity in Bangladesh. J Biodivers Conserv Bioresour Manag 2023. 9(1):79-100.
16. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980. 16: 111-120.
17. Lashari PK, Narejo NT, Mastoi AM et al. Some morphometric characters and their relationship in carp, *Cirrhinus reba* (Hamilton) from fishpond district Jacobabad, Sindh. Proc. Pak Congr Zool 2004. 24: 179-184.

18. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 2009. 25:1451-1452.
19. Mahmood S, Rasool F, Hafeez-urRehman M, Anjum KM. Molecular characterization of Aeromonas hydrophila detected in *Channa marulius* and *Sperata sarwari* sampled from rivers of Punjab in Pakistan. PLoS ONE 2024. 19(3): e0297979.
20. Mansoor A, Mehmood M, Hassan SMU, et al. Anti-bacterial effect of titanium-oxide nanoparticles and their application as alternative to antibiotics. Pak Vet J 2023. 43(2): 269-275. http://dx.doi.org/10.29261/pakvetj/2023.039.
21. Manzoor K, Rasool F, Khan N, Anjum KM, Parveen S. Prevalence and molecular detection of *Edwardsiella tarda* in cultured tilapia species of fish farms of Punjab in Pakistan and their postmortem examination. Pak Vet J 2023a. 43(2): 309-314.
22. Manzoor K, Rasool F, Khan N, Anjum KM, Parveen S. Resistance patterns of frequently applied antimicrobials and occurrence of antibiotic resistance genes in *Edwardsiella tarda* detected in edwardsiellosis-infected Tilapia species of fish farms of Punjab in Pakistan. J Microbiol Biotechnol 2023b. 33(5):668.
23. Mitra T, Ganguly S, Banerjee S, et al. Nutritional composition of different size groups of catfish *Rita rita* (Hamilton, 1822) from river Ganga. Ind J Fish 2017. 64:68-74.
24. Parveen S, Faisal IM. People versus power: The geopolitics of Kaptai Dam in Bangladesh. Int J Water Resour Develop 2002. 18(1):197-208.
25. Prakoso YA, Babazadeh D, Wijayanti AD. Potency of desert rose (*Adenium obesum* (Forssk.) Roem. & Schult.) flower extract against artificially induced furunculosis in oranda goldfish (*Carassius auratus auratus*). Pak Vet J 2023. 43(2): 339-344. http://dx.doi.org/10.29261/pakvetj/2023.024.
26. Pramono TB, Arfiati D, Widodo MS, Yanuhar U. Genetic characteristics of Senggaringan Fish (*Mystus singaringan*) from Klawing River, Brantas River and Thailand as the basis of conservation and domestication. Aquac Aquar Conserv Legis 2019. 12(4): 996-1004.
27. Punhal L, Laghari MY, Waryani B, et al. Genetic diversity and phylogenetic relationship of catfish Order Siluriformes inferred from mitochondrial gene sequence variation. Acta Scientific Agriculture 2018. 2(5):9-13.
28. Rabbani G, Rasool F, Fatima M, Majeed MBB. Morphometric and molecular characterization of *Channa marulius* from Riverine system of Punjab, Pakistan. Mol Biol Rep 2024. 51(1): 1-9.
29. Rasool F, Qureshi NA, Parveen S, et al. Morphometric parameters based study of the hatchery raised and natural populations of *Labeo rohita* Pakistan J Zool 2013. 903-907.
30. Saad N, El-Abasy MA, El-Khayat F, et al. Efficacy of chitosan nanoparticles as a natural antibacterial agent against pathogenic bacteria causing omphalitis in poultry. Pak Vet J 2023. 43(3): 573-578. http://dx.doi.org/10.29261/pakvetj/2023.065.
31. Sambrook J, Russell DW. Molecular Cloning: a Laboratory Manual, third ed. Cold Spring Harbor Laboratory Press, New York 2001.
32. Supiwong W, Liehr T, Cioffi MB, et al. Karyotype and cytogenetic mapping of 9 classes of repetitive DNAs in the genome of the naked catfish *Mystus bocourti* (Siluriformes, Bagridae). Mol Cytogen 2013. 6(1):51.
33. Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 2013. 30(12):2725-9.
34. Thompson JD, Gibson TJ, Plewniak F, et al. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research. 1997. 25(24):4876-82.
35. Ullah M, Rasool F, Khan N, Ali S, Sheikh AA. Antibiotic resistance and its gene profile in *Escherichia coli* isolated from diseased farm-raised carps in Punjab, Pakistan. Pak Vet J 2023a. 43(3): 470-476.

36. Ullah M, Rasool F, Khan N, Ali S, Sheikh AA. Molecular characterization and antimicrobial resistance among diarrheagenic and non-diarrheagenic *Escherichia coli* from healthy and diseased freshwater farmed fish. Paki J Agricul Sci 2023b. 60(4).
37. Yashpal M, Kumari U, Mittal S, et al. Surface architecture of the mouth cavity of a carnivorous fish *Rita rita* (Hamilton, 1822) (Siluriformes, Bagridae). Belgian J Zool 2006. 136(2):155.
38. Zhou A, Xie S, Sun Z, et al. Phylogenetic analysis of Channa genus based on morphological characters and X-ray imaging. Pak J Zool 2022. 54:693-700.
39. Zhu SR, Fu JJ, Wang Q, Li JL. Identification of Channa species using the partial cytochrome c oxidase subunit I (COI) gene as a DNA barcoding marker. Biochem Systemat Ecol 2013. 51:117-22.
40. Zou R, Liang C, Dai M, et al. DNA barcoding and phylogenetic analysis of bagrid catfish in China based on mitochondrial COI gene. Mitochondrial DNA Part A 2020. 31(2):73-80.