Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

Check for updates

ROOTS PRESS

Research Article

Phenotypic plasticity and genetic stock identification of *Sperata seenghala* (Sykes, 1839), the giant river-catfish, at Indus River, Sindh, Pakistan

Sumera Noonari[1,2], Wazir Ali Baloch[1], Huan Gao[3], Anila Naz Soomro[1], Abdul Mueed Ganghro[1,2], Ghulam Rasool Qambrani[1,2]

[1]*Department of Fisheries and Aquatic Sciences, University of Sindh, Jamshoro, Sindh, Pakistan.*

[2]*Livestock and Fisheries Department, Government of Sindh, Hyderabad, Sindh, Pakistan.*

[3]*Huan Gao, School of Marine Science and Fisheries, Jiangsu Ocean University, China.*

ABSTRACT

Traditional fish identification is based on morphological characteristics, whereas DNA-based identification has recently been considered a more reliable alternative method. The catfish are a significant group of freshwater fishes. *Sperata seenghala* is among the largest freshwater catfish of the subcontinent, occurring in the Indus River. A morphometric study concluded that the Indus River system *Sperata* is closely related to *S. seenghala* with some physical differences. However, in the literature on Pakistan's fish diversity, only *S. seenghala* is documented. Research on *Speratra* in the Jamshoro stretch of the Indus River revealed it as *S. seenghala*. This ambiguity in the nomenclature of *S. seenghala* needs genetic identification to resolve this issue. Thus, the present study evaluated the phenotypic plasticity and genetic stock identification of the *S. seenghala* at the Indus River, Sindh, Pakistan.

There were 151 fish specimens collected from the commercial fish catch at the Indus River Jamshoro, from August 2020 to July 2022. The fish were measured for total length (TL) (cm) and body weight (g) on the spot and then brought to the laboratory for morphological identification and genetic study. The PCR (polymerase chain reaction) was used to amplify the mitochondrial COI gene using a primer pair for Fish F1 and Fish R1 primers. The sequencing, genetic variation, and phylogenetic relationships were established using DNAMAN and MEGA version 7 software. The study concluded that morphologically and genetically *Sperata* species occurring in the Indus River is *S. seenghala*, and there was no evidence of phenotypic plasticity or genetic difference. The present study aims to identify genetically *S. seenghala* of the Indus River in Pakistan.

Keywords: Catfish; Sperata seenghala; morphology; genetic study; Indus River.

OPEN ACCESS

Correspondence
Wazir Ali Baloch
wazir.baloch@usindh.edu.pk

Article History
Received: April 1, 2025
Accepted: April 20, 2025
Published: April 30, 2025

INTRODUCTION

The catfish are a crucial group of freshwater fishes. *Sperata seenghala* is a giant river catfish of the Bagridae family. It is known as Guizza, Guizzaayer, Auri, Ari, Pogal, Singhara and Seenghala. It is among the largest freshwater catfish; widely distributed in rivers, lakes, floodplains, inundated swamps, field ditches, canals, and other freshwater areas of Afghanistan, Pakistan, India, Bangladesh, Nepal, and Myanmar (Talwar and Jhingran, 1991; Mawa et al., 2022). It was documented as *Aorichthys aor* (Hamilton, 1822) and *Aorichthys Seenghala* (Sykes, 1841). Sykes (1839) described this species as *Platystoma seenghala*. Later on, the name of this species has several replacements (Bagrus, Aorichthys, Mystus) and at present, this fish is placed under the genus *Sperata* (Ferraris and Runge, 1999; Acharya et al., 2019). In Pakistan, Ahmad (1943) argued that the only species of the Singhari in the fish market of Lahore was *Mystus aor*. Mirza (1990) pointed out that only one species in Pakistan was Aorichthys aor. It was later concluded that the Indus River

specimens were somehow different from *Aorichthys aor* and *Aorichthys seenghala* and described them as a new subspecies *Aorichthys aor sarwari* (Mirza et al.,1992). Later on, the same species was documented as *Sperata sarwari* (Mirza, 2003). The Indus seenghala is more closely related to *Sperata seenghala* (Ferraris and Runge, 1999). In some studies, the *Sperata* species has been referred to as *Sperata sarwari* (Shakir, 2008; Shakir et al., 2008; Nawaz et al., 1994). In a morphometric study on Seenghala, Shakir (2008) found that the fish in the Indus River system are closely related to *S. seenghala*, though there are some physical differences between them. He emphasized the importance of genetic identification in clarifying this relationship. Literature on fish diversity in Pakistan, such as the work by Rifique and Huda (2012), has documented the species as *S. seenghala*. Similarly, a study conducted by Jatoi et al. (2013) in the Indus River at Jamshoro confirmed the presence of *S. seenghala*.

This species is considered a highly valued edible fish due to its delicious taste and minimal intramuscular bones, making it a favorite among consumers (Saini, 2008; Yadav et al., 2017).

The mitochondrial DNA gene cytochrome oxidase subunit I (COI) functions as the most reliable bio-identification tool, identifying nearly all animal and plant species (Hebert et al., 2003; Hossain et al., 2023). The application of Kimura two-parameter (K2P) genetic distance in barcoding analysis (Kimura 1980) highlights that variations in barcodes reflect differences at the species, genus, family, and higher taxonomic levels. These methods have proven highly effective in identifying fish groups. The COI gene can distinguish between two or more genera through phylogenetic analysis (Lakra et al., 2007; Hajibabaei et al., 2007; Ward et al., 2005). Genetic diversity is crucial for a species' survival, adaptation, disease resistance, reproductive capacity, and resilience in changing environments, thereby playing a significant role in overall biodiversity (Surjya et al., 2022). This research focused on identifying catfish *S. seenghala* from the Indus River, Pakistan, through a combination of morphological analysis and DNA barcoding.

MATERIALS AND METHODS

The phenotypic study was undertaken at the Laboratory of Fisheries Science, University of Sindh, Jamshoro, Pakistan, while a genetic study was undertaken at the School of Marine Science and Fisheries, Jiangsu Ocean University, China.

Experimental Material and Treatments

Fish sampling

All 151 fish specimens were collected from the fish landing center near Jamshoro (commercial fish shops) from August 2020 to July 2022. The fish were measured for length-weight, and brought to the laboratory for morphological studies.

Phenotypic study

Each specimen was measured (cm) and weighed (g). A measuring board was used for fish measurement, while body weight was taken using an electric balance (OHAUS PRECISION Advanced MODEL GT400). Physical parameters were observed for phenotypic plasticity.

For the genetic study, fish samples from the liver and the muscles were taken and preserved in 70 % alcohol in centrifuge tubes and labeled. Fish specimens were identified using the conventional taxonomic key (Froese and Pauly, 2011; Jayaram, 1999; Menon, 1999; and Talwar and Jhingran, 1991, considering the morphometric characteristics.

DNA Extraction

The EZUP Colum animal genome DNA extraction kit (by Sango Biotech) was used to extract the DNA of the fish. About 400-600 mg of liver tissue was transferred to a 1.5ml centrifuge tube and ACL 180µl buffer was added and placed onto a thermo shaker for cracking the tissues at 56 °C for one hour. The hydrolyzed tissues were added with 200 µl of buffer CL in each centrifuge tube to separate impurities. The samples were shaken in the vortex for 10 seconds. About 200 µl of absolute ethyl alcohol was added to all samples, shaken well for 10 seconds and then the samples containing residues were transferred into the absorption column and rested for two minutes. The absorption columns were centrifuged for 60 seconds at 10,000 rpm and the supernatant was collected in a new absorption column. About 500 µl of CW1 solution was added to the absorption columns for 30 seconds at 10,000 rpm, and then the residues were discarded. In the same absorption columns, about 500 µl CW2 was added and centrifuged for 30 seconds at 10,000 rpm and the residues were discarded. The absorption columns were centrifuged again at 12,000 rpm for 2 minutes and transferred into new centrifuge tubes and 70 µl CE buffer solution was added. The new centrifuge tubes were centrifuged for 2 minutes at 12,000 rpm. Right after the centrifuge, the solution was transferred to new centrifuge tubes. The DNA was diluted in the solution and saved at -20 °C.

Measurement of DNA concentration

A UV-Vis Spectrophotometer Q 5000 was used to measure DNA concentration. The instrument was calibrated with

distilled water, using a 1.0 µl pipette. About 1.0 µl of DNA solution from each pipette was placed on the instrument, and the result was measured at 260 nm wavelength with the help of the software Q5000 VS 4.0.

PCR amplification

The COI gene barcoding was done successfully, using the fish primers (Ward et al. 2005) as shown in Table 2. The PCR reaction was 50 µl with the composition of (enzyme) 2X Taq master mix 25 µl, DNA concentration 1.4 µl, Primer F 2.0µl, Primer R 2.0µl, and DDH_2O 19.6µl (Table 3). The conditions for the PCR thermal cycler were set as follows: initial denaturation for 5 minutes at 95 °C, further with 30 cycles of denaturation for 30 seconds at 95 °C, annealing for 40 seconds at 61 °C, extension for 30 seconds at 72 °C and final extension at 72 °C for 7 minutes.

Sanger sequencing was performed unidirectional for the fish species.

Analysis of the Data

The sequences were analyzed and aligned using BioEdit version 7.0.5.3. The identity match was performed using NCBI. The Neighbor-Joining (NJ) method, as proposed by Saitou and Nei (1987), was employed to generate trees. The K2P model was used for this purpose, using MEGA version X software.

RESULTS AND DISCUSSION

The body of *S. seenghala* is elongated, narrow from side to side, and notably deep from back to belly. Its snout is wide and flattened. Barbels extend backward, reaching beyond the pelvic fins or even as far as the anal fins. It has a dorsal fin containing a single spine with a few rays, while another is an adipose fin. The dorsal spine is relatively soft, smooth on the anterior side, serrated on the posterior side, and approximately equal in length to the head (excluding the snout). The adipose fin is fairly large, with a basal length that equals or exceeds that of the rayed dorsal, and the intermediate distance is similar in length.

The pectoral fin reaches more than halfway to the pelvic fin; its spine is stiffer than that of the dorsal fin and about half the length of the head, with external roughness and internal denticulations. The pelvic fin, positioned abdominally, starts behind the last ray of the dorsal fin and extends over two-thirds of the distance to the anal fin. The caudal fin is deeply forked, with the upper lobe longer and curving downward at the tip. The fish color is grayish on its back and silvery on the sides and belly, having a clear dark spot on the adipose fin.

Length-Weight Parameters

The relationship between body weight and body length is an important parameter, being used to determine the mathematical relationship in fisheries research. The length-weight regression parameters are given in Table 1. In the male population of *S. seenghala*, the length-weight coefficient of determination value, r^2 = 0.967, indicates a close relationship between the two parameters. The value of intercept a is 0.0032 while the confidence limit is 0.0030 - 0.0033. The body coefficient *b* value is 3.08 while the confidence limit is 2.853-3.1513.

The coefficient of determination r^2 in the female fish populations was r^2 = 0.956, showing a close relationship between length and weight. The value of a in the female population is 0.0026, and the confidence limit is 0.00013- 0.0074. Whereas the b value is 3.17, and the confidence limit is 3.015-3.327.

In the mixed population, the coefficient of determination of the two parameters is r^2 = 0.969, showing a close relationship between the two parameters. The value of intercept a is 0.0024 and the confidence limit is 0.0012- 0.0026, whereas the b value is 3.15 and the confidence limit is 3.019-3.37.

Length-Weight Relationship Equations

The equations of the relationship between length and weight were calculated separately for male, female, and mixed populations of *S. seenghala*, which are as follows.

Log W= 0.0032+3.08 log L (Male)
Log W= 0.0026+3.17 log L (Female)
Log W= 0.0024+3.15 log L (Mixed)

Isometric growth was evident in male populations, whereas female populations and mixed populations showed a slight positive allometric growth. It is widely known that the regression b value represents the body shape, and it is closely related to the weight, which is influenced by ecological factors such as temperature, food supply, spawning conditions, and other factors, such as sex, age, fishing time, and area and fishing vessels (Mirza and Javed, 1992). The positive allometric growth in females is attributed to high voracious feeding of female fish before and after the breeding season during the gonad development season, which makes them heavier than that of male *S. seenghala*.

Sexual Dimorphism

In fish populations, sexual dimorphism between males and females is not apparent. The vent-sex study in catfishes is,

Table1. Length-weight relationship parameters of *Sperata seenghala* from Indus River, district Jamshoro.

		Total Length (cm)		Total Weight (g)		Regression analyses				
Sex	*n*	Min	Max	Min	Max	*a*	CL	*b*	CL	r^2
Male	68	46	92	557	5120	0.0032	0.0026 - 0.1099	3.08	2.834-3.171	0.967
Female	83	49	94	581	5430	0.0026	0.00231- 0.00289	3.17	2.913-3.231	0.956
Mixed	151	48	94	557	5430	0.0024	0.00219- 0.00254	3.15	2.964-3.259	0.969

however, an alternate option for sex determination. In the case of *S. seenghala* one can identify the fish sex with a careful examination of the vent, where males possess a prominent papilla that is not seen in females. The sex difference based on genital papilla was also suggested by those who observed a soft, elongated structure in male fish (genital papilla) that was broad at the base and gradually tapering towards the end. The tip of the papilla was beyond the base of the first anal fin while in the female only the genital pore was present (Figure 1).

Male-Female Ratio

In natural populations, female fish are normally more populous while males are fewer in number. A similar pattern was evident in the case of *S. seenghala*, where the male-female ratio was determined to be 1.0:1.22.

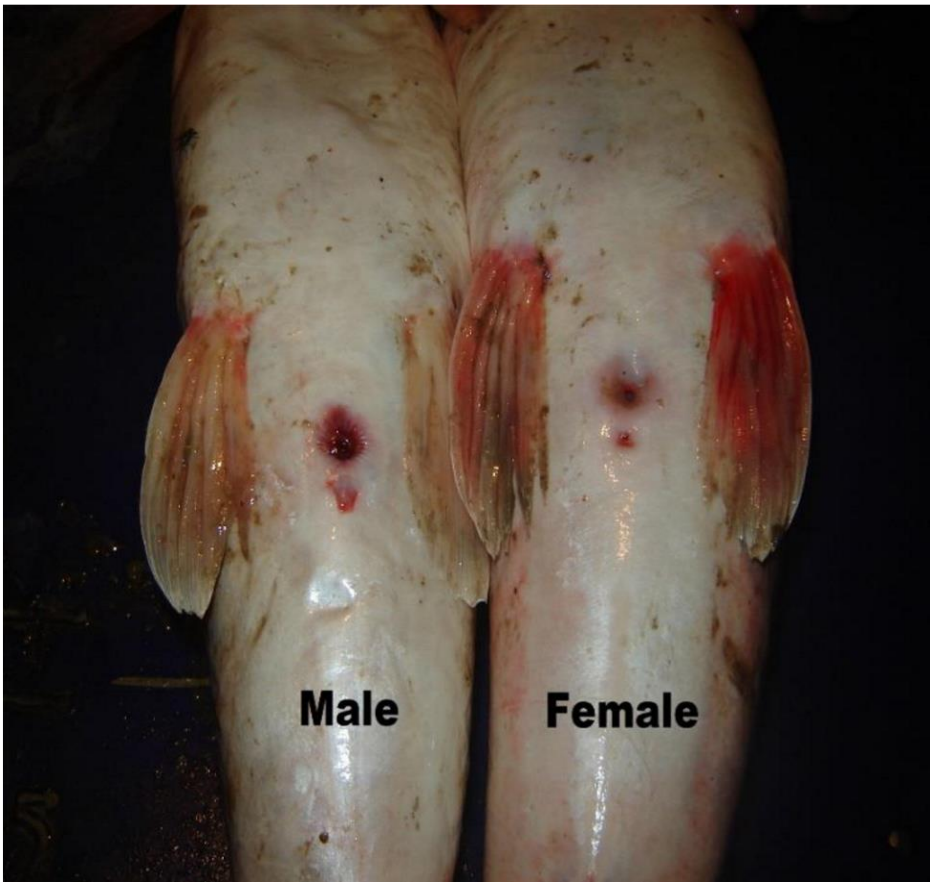

Figure 1. Dimorphism based on the vent-sex in male and female *Sperata seenghala*.

DNA-Based Identification

Genetic diversity

About 650 bp COI gene fragments were amplified by PCR using primer FISH F1 - C-3' and FISH R1 - A-3' (Table 2). This research focused on identifying catfish *S. seenghala* from the Indus River, Pakistan, through a combination of morphological analysis and DNA barcoding. The morphological examination of the specimens raised questions

regarding discrepancies between observed characteristics and documented descriptions. In some instances, distinguishing species using morphological keys proved challenging. The DNA barcoding approach helped address certain identification challenges and clarified the true species in the region. The PCR amplified DNA fragments corresponding to the COI gene were visualized by 1% agarose gel electrophoresis.

The analysis of nucleotide base frequencies, as presented in Table 3, revealed the overall observed mean values: thymine (T) at 28.4%, cytosine (C) at 20.4%, adenine (A) at 31.2%, and guanine G) at 19.9%. This examination of the base composition in COI sequences highlighted a notably high average adenine (A) content, contrasted by a relatively low guanine (G) content. Furthermore, the AT content was significantly higher than the GC content. Sequences with high counts of Adenine tend to have lower counts of Cytosine and Guanine, suggesting a compensatory relation between these nucleotides. The sequencing provides insights into the genetic diversity and evolutionary adaptation of *S. seenghala*. It also suggests that environmental adaptation as an ecological factor can influence nucleotide composition. Further, two different branches were found from the clade, as out-group sequences belonging to *Bagarius*. The neighbor phylogenetic tree of *S. seenghala* COI sequences was prepared. A single clade of *S. seenghala* was identified using the K2P model, while the out-group sequences performed *Bagarius bagarius* and *Bagarius Yarelli* species (Figure 2).

Table 2. Primer sequences used for PCR amplification and sequencing through CO1 identification gene.

Primer	Sequence (5'→3')	TM (℃)
F1	tca acc aac cac aaa gac att ggc ac	60.2
R1	tag act tct ggg tgg cca aag aat ca	59.8

TM=Melting temperature.

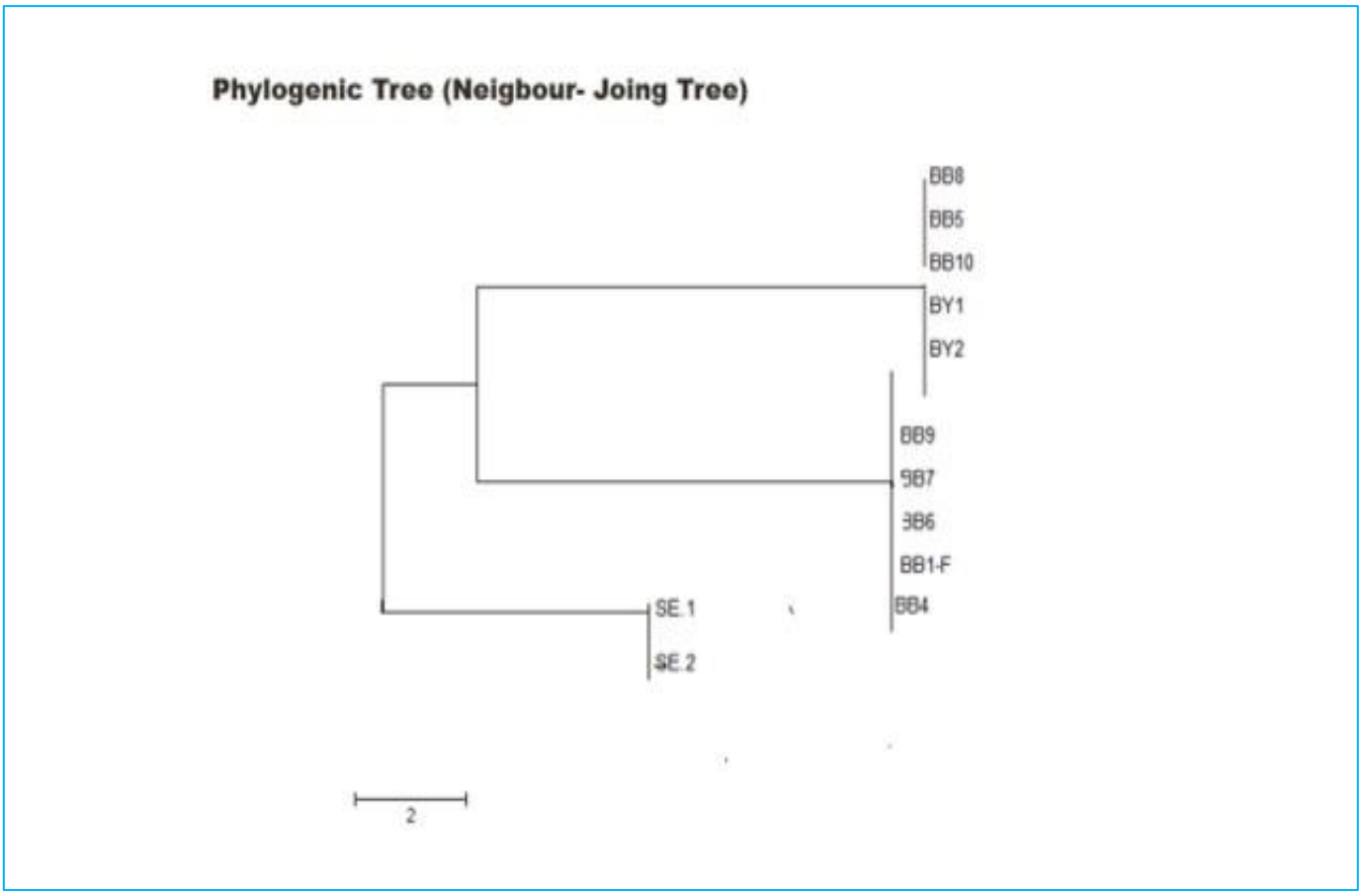

Figure 2. Phylogenetic tree of *S. Seenghala* fish that shows a single clade.

The present study was focused on the genetic identification and analysis of the catfish species *Sperata seenghala* from the Indus River, Pakistan. A combination of morphological examination and DNA barcoding technique was used.

Many researchers emphasize the challenges of species identification based on morphological characteristics only and suggest the utility of molecular tools, such as DNA barcoding, in resolving taxonomic ambiguities (Hebert et al., 2003). The EZUP Colum animal genome DNA extraction kit (by Sango Biotech) was used to extract DNA from fish liver tissue. High-quality DNA suitable for molecular analyses was obtained. A protocol involving tissue lysis, clearing impurities, and column purification effectively isolated pure DNA (Sambrook and Russell, 2001). The DNA concentration measurement was performed using a UV-Vis spectrophotometer at 260 nm, confirming the presence of DNA, with the A260/A280 ratio indicating the purity of the extracted DNA. This step is critical for ensuring the reliability of subsequent PCR amplification and sequencing (Wilfinger et al., 1997).
The effective intensification of the cytochrome C Oxidase subunit I (COI) quality, a standard marker for DNA barcoding, underscores the viability of the preliminaries and PCR conditions utilized (Table 3).

Table 3. Components of the mixture for polymerase chain reaction (PCR).

Component	Quantity (µL)
2X taq master mix	25
Primer -F	2
Primer-R	2
DNA	1.4
DDH_20	19.6

Table 4. Composition and percentage of bases of *S. seenghala*.

SEQUENCE NAME	COMPOSITION					PERCENTAGE				
	A	C	G	T	OTHERS	A	C	G	T	OTHERS
SE1	94	83	91	103	0.0	25.3	22.4	24.5	27.8	0.0
SE2	113	78	70	109	0.0	30.5	21.1	18.9	29.5	0.0
SE3	119	56	62	127	0.0	32.7	15.4	17.0	34.9	0.0
SE4	119	54.5	70	127	0.0	31.8	15.2	17.0	34.9	0.0
SE5	78	81.9	68	90	0.0	24.6	25.6	21.5	28.4	0.0
SE6	111	81.9	68	90	0.0	14.6	25.6	21.5	28.4	0.0
SE7	119	54	68.1	90	0.0	14.6	25.06	22.0	27.2	0.0
SE8	117	49	67	90	0.0	14.6	22.89	20.1	27.9	0.0
SE9	113	49	63.8	103	0.0	24.9	22.5	20.85	27.8	0.0
SE10	111	82.4	68.4	94	0.0	14.9	24.8	20.9	28.6	0.0
SE11	116	55.0	79.8	122	0.0	31.4	24.0	19.8	31.5	0.0
SE12	99.8	80.2	74.8	90	0.0	14.8	25.4	22.6	27.6	0.0
SE13	111	84.9	78.2	99.1	0.0	14.5	24.7	20.4	27.2	0.0
SE14	119	82.4	72.0	108	0.0	20.9	25.8	21.7	27.8	0.0

SE15	110	78.9	69.8	98.9	0.0	14.6	21.8	22.1	29.9	0.0
SE16	117	77.4	77.0	95.8	0.0	14.8	21.5	20.5	26.0	0.0
SE 17	111	81.6	68.5	90.8	0.0	30.5	24.9	22.9	29.2	0.0
SE18	118	85.2	71.8	105	0.0	25.3	22.5	24.9	32.8	0.0
SE19	119	78.9	65.9	99.0	0.0	14.0	21.8	25	29.0	0.0
SE20	111	82.9	68.3	92.8	0.0	23.5	22.1	22.1	28.5	0.0

CONCLUSION

This study conclusively identifies the *Sperata* fish populations of the Indus River in Sindh, Pakistan, as *Sperata seenghala* through integrated morphological and genetic analyses. Our findings not only advance the understanding of this species' genetic diversity and evolutionary biology but also demonstrate the critical role of DNA barcoding in resolving taxonomic ambiguities and informing conservation strategies. By establishing a reliable genetic baseline for *S. seenghala*, this work provides a foundation for future research on population dynamics, habitat management, and biodiversity preservation in the Indus River ecosystem.

AUTHOR'S CONTRIBUTION

All authors contributed to various aspects of the research and manuscript preparation, and they approved the final version of the manuscript for submission.

FUNDING

There was no funding available for the present research work.

AVAILABILITY OF DATA AND MATERIAL

Data presented in this study will be available on a fair request to the corresponding author.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable to this publication.

CONSENT FOR PUBLICATION

I would like to confirm that all authors listed in the manuscript have provided their consent for publication.

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

ACKNOWLEDGEMENT

We extend our sincere gratitude to Professor Dr. Gao Huan for his invaluable guidance, generous provision of laboratory facilities, and expert assistance with DNA sequencing. His contributions were instrumental to the success of this research.

REFERENCES

Acharya, A.P., Pavan-Kumar, A., Gireesh-Babu, P., Joshi, C.G., Chaudhari, A. & Krishna, G. (2019). Population genetics of Indian giant river-catfish, Sperata seenghala (Sykes, 1839) using microsatellite markers. Aquatic Living Resources, 32: 4. https://doi.org/10.1051/alr/2019002

Ahmed, N. (1943). Fauna of Lahore. 5 Fishes of Lahore. Bulletin of the Department of Zoology, Punjab University, Lahore, 1: 253–374.

Brown, W.M., George, M. & Wilson, A.C. (1979). Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America, 76(4): 1967–1971.

Chen, Y., Chen, X., Wang, H., Bao, Y., & Zhang, W. (2020). Translational selection dominates over mutational bias in shaping nucleotide composition in bacteria. Nature Communications, 11(1), 1–12. https://doi.org/10.1038/s41467-020-18064-w

Dayrat, B. (2005). Towards integrative taxonomy. Biological Journal of the Linnean Society, 85(3): 407–417.

Ferraris, C.J. Jr. & Runge, K.E. (1999). Revision of the South Asian bagrid catfish genus Sperata, four species of catfishes of the Punarbhaba River in India. Indian Journal of Fisheries, 58(4): 79–85.

Forsberg, K.J., Patel, S., Witt, E., Wang, B., Ellison, T.D., Dantas, G. & Andersson, D.I. (2017). Temperature drives evolutionary rates in prokaryotes. The ISME Journal, 11(8): 1879–1886. https://doi.org/10.1038/ismej.2017.33

Frank, A.C. & Lobry, J.R. (1999). Asymmetric substitution patterns: A review of possible underlying mutational or selective mechanisms. Gene, 238(1): 65–77.

Froese, R. & Pauly, D. (Eds.) (2011). FishBase. [version 10/2011]. http://www.fishbase.org

Hajibabaei, M., Singer, G.A., Hebert, P.D. & Hickey, D.A. (2007). DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics, 23(4): 167–172.

Hamilton, F. (1822). An account of the fishes found in the river Ganges and its tributaries. Edinburgh and London.

Hebert, P.D.N., Ratnasingham, S. & de Waard, J.R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B: Biological Sciences, 270(Suppl 1): S96–S99. http://doi.org/10.1098/rsbl.2003.0025

Hossain, M.Y., Uddin, M., Rahman, M.A., Haque, M.K., Kormoker, T., Samad, M.A., Tanjin, S., Rahman, M.A., Parvin, M.F., Sarmin, M.S. & Mawa, Z., 2023. Species identification, reproductive biology, and nutritional value of marine shellfish (Meretrix lyrata) in the Bay of Bengal. Marine Environmental Research, 192, p.106222.

IUCN. (2020). Global Standard for Nature-based Solutions. A user-friendly framework for the verification, design and scaling up of NbS. First edition.

Jayaram, K.C. (1999). Systematic account of Siluriformes fishes. In: The fresh water fishes of the Indian region. Narendra Publishing House, New Delhi, 220-318 pp.

Jatoi, S., Baloch, W.A., Soomro, A.N. & Gachal, G.S. (2013). Length-weight relationship of the Silurid catfish Sperata seenghala Sykes 1839 (Bagridae) from Indus River, Sindh, Pakistan. Sindh University Research Journal-SURJ (Science Series), 45(4): 661-664.

Jukes, T.H. & Cantor, C.R. (1969). Evolution of Protein Molecules. In: Munro, H.N. (Ed.), Mammalian Protein Metabolism, Academic Press, New York, 21-132. http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7

Kimura, M. (1980). A Simple Method for Estimating Evolutionary Rate of Base Substitutions through Comparative Studies of Nucleotide Sequences. Journal of Molecular Evolution, 16: 111-120. https://doi.org/10.1007/BF01731581

Lakra, W.S., Goswami, M. & Mohindra, V. (2007). Molecular identification of five Indian sciaenids (Pisces: Perciformes, Sciaenidae) using RAPD markers. Hydrobiologia, 585: 359-363. doi:10.1007/s10750-006-0480-x

Lassalle, F., Périan, S., Bataillon, T., Nesme, X., Duret, L. & Daubin, V. (2015). GC-biased gene conversion in bacteria: A possible explanation for codon usage trends? PLoS Genetics, 11(3): e1005044. https://doi.org/10.1371/journal.pgen.1005044

Mawa, Z., Hossain, M., Hasan, M., Tanjin, S., Rahman, M.A., Ohtomi, J. & Islam, M.J. (2022). Life history traits of Mystus vittatus in the Ganges River, Bangladesh: recommendation for its sustainable management considering climate change. International Journal of Biometeorology, 66: 927-943. https://doi.org/10.1007/s00484-022-02249-7

Menon, A.G.K. (1999). Checklist - fresh water fishes of India. Records of the Zoological Survey of India, Miscellaneous Publication, Occasional Paper No. 175, 366 p.

Mirza, M.R. (1990). Freshwater fishes in Pakistan (in Urdu). Urdu Science Board, Lahore.

Mirza, M.R., Nawaz, H. & Javed, M.N. (1992). A note on the fishes of genus Aorichthys Wu with the description of a new subspecies. Pakistan Journal of Zoology, 24: 211-213.

Mirza, M.R. (2003). Checklist of freshwater fishes of Pakistan. Pakistan Journal of Zoology, Supplementary Series, 3: 1-30.

Nawaz, H., Sharif, M.H. & Mirza, M.R. (1994). Food of the singhari, Aorichthys aor sarwari, and observations on the biology of Mystus seenghala (Sykes) from the river Brahmaputra in Assam. Journal of Inland Fisheries Society of India, 21: 47-54.

Rafique, M. & Khan, N.U.H. (2012). Distribution and status of significant freshwater fishes of Pakistan. Records: Zoological Survey of Pakistan, 21: 90-95.

Saini, A., Dua, A. & Mohindra, V. (2008). Comparative morphometrics of two populations of giant river catfish (Mystus seenghala) from the Indus River system. Integrative Zoology, 3: 219-226.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4(4): 406-425.

Sambrook, J. & Russell, D.W. (2001). Detection of DNA in agarose gels. Molecular Cloning: A Laboratory Manual (3rd Ed.). Cold Spring Harbor Laboratory Press, New York: 5-14.

Sehgal, P. (1967). Food and feeding habits of Mystus seenghala Sykes. Research Bulletin of the Panjab University Science, 18: 149-155.
Seward, E.A. & Kelly, S. (2016). Energetic constraints drive nucleotide usage in prokaryotes. Proceedings of the Royal Society B, 283(1837): 20161456. https://doi.org/10.1098/rspb.2016.1456
Shakir, H.A. (2008). Meristic and morphometric study of Sperata sarwari from Mangla Lake, Pakistan. Punjab University Journal of Zoology, 23(1-2): 9-18.
Shakir, H.A., Mirza, M.R., Khan, A.M. & Abid, M. (2008). Weight length and condition factor relationship of Sperata sarwari (Singhari) from Mangla Lake, Pakistan. Journal of Animal and Plant Sciences, 18(4): 158-161.
Surjya, N.D., Abhijeet, S. & Anuj, T. (2022). Study on biometrics and genetic variability of giant river catfish Sperata seenghala from Harike Wetland - A Ramsar site. Journal of Experimental Zoology India, 25: 1123-1128. DocID: https://connectjournals.com/03895.2022.25.1123
Talwar, P.K. & Jhingran, A.G. (1991). Inland Fishes of India and Adjacent Countries, Vol. 1. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Bombay: 1027-1028.
Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462): 1847-1857.
Wei, Y., Silke, J. R., Xia, X., & Lin, W. (2019). Oxidative stress and mutational bias shape nucleotide composition in microbial genomes. Molecular Biology and Evolution, 36(5), 991–1003. https://doi.org/10.1093/molbev/msz036
Wilfinger, W.W., Mackey, K. & Chomczynski, P. (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques, 22(3): 474-481.
Yadav, A., Thakur, J.K. & Yadav, G. (2017). KIXBASE: A comprehensive web resource for identification and exploration of KIX domains. Scientific Reports, 7(1): 14924.