Study of Landmark-based morphological variations of family Cyprinidae from the River Indus Punjab, Pakistan

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Abstract

Stock identification is the key to maintain fisheries conservation and management. The current study used morphometric, meristic and truss box analysis, body shape and color for identification of ten commercially important Cyprinids species *Catla catla*, *Labeo calbasu*, *Labeo gonius*, *Labeo boga*, *Labeo rohita*, *Cyprinus carpio*, *Cirrhinus mrigala*, *Cirrhinus reba*, *Salmostoma phulo* and *Systomus sarana* from Mianwali, Kallur Kot and Dera Ghazi. The inventory regarding stock identification was investigated using a total of 24 morphometric, 5 meristic and 30 truss measurements for each individual. To assess variations among the stock structure, univariate and multivariate analysis by using principle component analysis and cluster analysis were performed. In this study 24 morphometric parameters were analysed in which 16 characters in *C. catla*, 9 charaters in *L. calbasu*, 3 charaters in *L. gonius*, 6 charaters in *L. boga*, 8 charaters in *L. rohita*, 11 charaters in *C. carpio*, 9 charaters in *C. mrigala*, and 7 charaters in *S. sarana* were found significantly ($p<0.05$) variable among three different populations. Among three populations, non-significant difference was observed in *C. reba* and *S. phullo*. PC analysis of meristic parameters showed two principal components (PC1, 58.1% and PC2, 21.7%) together explained total variance of 79.8%. DFR, AFR and CFR showed a significant loading in PC1 and PC2 are responsible for species differentiation. Thirty truss box measurements were used in PC analysis revealed a total variability (84.5 %) between various variables. All truss measurements showed significantly ($p\leq 0.05$) strong correlation with each other. A dendrogram based on hierarchical cluster was obtained from the average measurement analysis of truss – based morphological characters showed twenty major clusters to elucidate the relationships among fish species and their environment. These differentiations are expected because of geographical isolation, environmental impact, genetic variations and due to different ancestral origin. There is no study on the taxonomic characterization of studied fishes from these regions of River Indus Punjab, Pakistan. This study established a novel and critical baseline for the taxonomic and phylogenetic characterization of commercially important Cyprinid species, highlighting significant morphological and genetic variations which is critical for effective fisheries conservation and management.

Keywords: Cyprinidae, Morphometric counts, Meristic counts, Truss-network analysis, Multivariate analysis (PCA), Conservation, River Indus

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Introduction

Aquatic diversity is a very essential phenomenon that gives us the idea to understand the life inside the water. Therefore, the study of ichthyodiversity is the first step to understanding the aquatic ecosystem in any targeted area (Faryal *et al*., 2015).

Freshwater fish diversity is considered the most diverse and characterizes a warm-water fish fauna. More than 35,000 fish species are present globally that contribute essentially to the prevailing vertebrates (Ude *et al*., 2020). In Pakistan, natural freshwater resources are existing in the form of natural lakes, dams, streams, and rivers which have great value and potential for aquaculture and fisheries practices. Most importantly, freshwater fish fauna is considered the most valuable and important food source across the globe (Khan *et al*., 2021). In Pakistan, a total of 531 fish species have been recognized, of which 298 are marine and 233 are freshwater fish species (Ghouri *et al*., 2020).

Economically 78 out of 233 freshwater fish species are more important. River Indus is one of the longest river systems in Pakistan. It starts from Tibetan Plateau, the district of Lake Manasarovar towards the Gilgit-Baltistan and Hindukush ranges flowing through the Ladakh region of Jammu and Kashmir. More than 180 freshwater fishes are located in the River Indus (Sheikh *et al*., 2017; Khan *et al*., 2021). Among the total fish diversity in Pakistan, 86 species of which 8 exotic and 78 indigenous have been recognized as "species of special importance" based on endemism, economic significance, IUCN status, and rarity (Rafique and Khan, 2012). Therefore, a significant extent of literature is existing about the fish diversity from various portions of Pakistan. Fish species that established the major riverine fish population belong to the family Cyprinidae. The Cyprinidae family is the major fish family in the freshwater ecosystem, with around 371 genera having 3038 species (Eschmeyer and Fong, 2016; Kaur *et al*., 2021). Cyprinidae family belongs to the order Cypriniforms, usually spread all over Asia, but the maximum species abundance is described in Southeast Asia (Alam *et al*., 2021). They are jawed fishes characterized as toothless and stomachless. Many species have food and marketable significance as cultivated fishes, famous ornamental fishes, game fishes, and ideal organisms for genomic improvement research (Balai *et al*., 2017; Alam *et al*., 2021).

Fish species of the genus Labeo belong to the family Cyprinidae. Labeo fishes only occur in South East Asia and Africa. After the Barbiinae, Labeo lineage is the second most vital group of the family Cyprinidae. In Asia, it represents almost 19.6% of cyprinid species structure (Sarma *et al*., 2017). Genus Labeo is the most dominating group of the family Cyprinidae. Out of 105 fish species of the Labeo genus around the world, 36 fish species of the genus Labeo are represented through the South and Southeast Asia and 69 occur in Africa (Sudha *et al*., 2015).

The most commercially important species of the Labeo genus are *Labeo rohita, Labeo gonius, Labeo calbasu, Labeo boga* and *Catla catla.* Increasing the importance of food, nutritional values, extraction oil, medicinal value, and ornamental purpose this species is widely used in different areas of Pakistan (Latif *et al.*, 2016; Anup and Biplab, 2021).

Having the highest market demand and accounting for a significant amount of freshwater fish output, it serves as the main commercial and cultivable fish species as well. Biomedical research, pest control, and a connection to the outdoors are just a few of the advantages that these freshwater fish give to human health and well-being (Lynch *et al*., 2016; Balai *et al*., 2017). *Cyprinus carip, Cirrhinus mrigala*, *Cirrhinus reba*, *Salmostoma phulo* and *Systomus sarana* are also regarded as economically and commercially high valued fishes in pakistan. Population of these freshwater fishes is declining in the rivers of Pakistan (Latif *et al*., 2016; Ethin *et al*., 2019).

The ecosystem of the River Indus has also been strongly disturbed by human involvement. Human overexploiting activities cause the loss of habitat and degradation of the freshwater ecosystem. Because of this many freshwater fish species have become endangered (Sheikh *et al*., 2017). Almost 20% of freshwater fish species have been declared either endangered or extinct. Examination of freshwater fish diversity from different parts of the River Indus indicates that they are in serious decline and need instant protection (Bajzik *et al*., 2012).

Therefore, improved conservational approaches and management plans will support and prevents the loss of ichthyodiversity. In order to avoid unfair competition and ensure proper labeling, some other techniques for the management and verification of commercial fishery products are required. Precise identification of species is an important component for management and conservation purposes. The identification of species of any animals is one of the major and difficult tasks for taxonomists (Ward *et al*., 2009). Identification on the basis of morphological character is a common traditional method based on visible features using various morphological keys (Karim *et al*., 2016; Iyiola *et al*., 2018; Naeem *et al*., 2020).

In systematic ichthyology investigations, morphometric features are one of the most essential keys. This information can be useful to examine and graphically show differences in shape (Mojekwu and Anumudu, 2015). It is also useful for assessing ontogenetic trait growth variability and population variance (Batubara *et al*., 2018). Morphometric measurement has been used to detect unknown hybrids, species and also changes in population of aquatic organisms (Park *et al*., 2013). Morphometry, according to Talwar and Jhingran, is the external measurement of an organism's bodily components, whereas meristic features are countable characters (Tripathy, 2020).

Meristic and morphometric features are widely used to distinguish between different fish species and populations. This approach, however, is ancient enough to distinguish species taxonomically, pure fish stocks and isolate different morphotypes (Parvej *et al*., 2014; Priyanka *et al*., 2018). Traditional approaches have been updated with sophisticated technologies from time to time. Image analysis, principal components analysis, multivariate analysis, and truss network analysis are examples of innovations that have been made to

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improve information and knowledge (Tripathy, 2020).

Truss values created with landmark points, as well as morphometric and meristic features measurements, are important tools that may be applied for stock identification, explaining population dynamics, and differentiating morphologically similar species from other species (Parvej *et al*., 2014). It is a network of vertical, horizontal, and diagonal distances between points chosen to split the body into units depending on local morphological features. This approach has benefits over traditional morphometric character sets, which generally comprise length, width, and depth data (Tripathy, 2020).

According to Dwivedi and Dubey, it is more useful than traditional morphometrics techniques since it gathers more data and employs a more effective strategy for describing the shape. It also helps in the extraction of morphometric variations in and between species (Dwivedi and Dubey, 2013). Several studies (Parvej *et al*., 2014; Mojekwu and Anumudu, 2015; Tripathy, 2020) highlighted the validity of the truss system of morphometric features, which ensures systematic form coverage and comprehensively and redundantly records landmark patterns (Gul *et al*., 2019).

Detailed study related to biometric characters till date has not been conducted on the Cyprinidae family from different regions of River Indus, Punjab, Pakistan. Data available on morphological variation of these species in natural populations is very limited and restricted to a particular area. To close the gap, present study was carried out with the goal of studying the characteristics of the economic importance of fish in this family. As a result, the objective of this study was to determine the morphometric, meristic, and truss box analyses, as well as their relationship within and between 10 species of the Cyprinidae family. This research will provide more useful information for conservation techniques and management strategies in the field of fisheries in the three different locations of the River Indus, Punjab, Pakistan.

Materials and methods

Sampling sites

The current study involved sampling from the River Indus of Punjab, Pakistan during October 2020 to February, 2022. In order to compare fish diversity, three locations of the River Indus were selected. Live fish samples were randomly collected from three different regions Mianwali (upstream), kallur kot (midstream) and Dera Ghazi Ghaat (downstream) of riverine system. At each location, basic information including latitude and longitude location were recorded. Identification of samples was done by using morphological parameters and truss network analysis (Fig. 1).

Collection of fish samples

The study involved ten freshwater fish species of total 90 fish specimens of the family Cyprinidae which includes *Labeo rohita, Labeo gonius, Labeo boga, Labeo calbasu, Catla catla, Cyprinus carpio, Cirrhinus mrigala, Cirrhinus reba, Salmostoma phullo,* and *Systomus sarana* were randomly collected from week to week from October, 2020 to Feburary, 2021 with the help of local fisherman. Fresh and

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undamaged specimens were preserved in an ice box and transported to the fisheries laboratory at Government College University Faisalabad, Pakistan, to examine the external phenotype (measuring and counting morphometric and meristic parameters). A biometric study was conducted on 10 species including 24 morphometric, 5 meristic counts and 12 landmarks determining thirty distances on fish body. Detailed description is given in the following section. After morphological measurements, specimens were preserved in 70% ethanol for further research and analysis (Table 1).

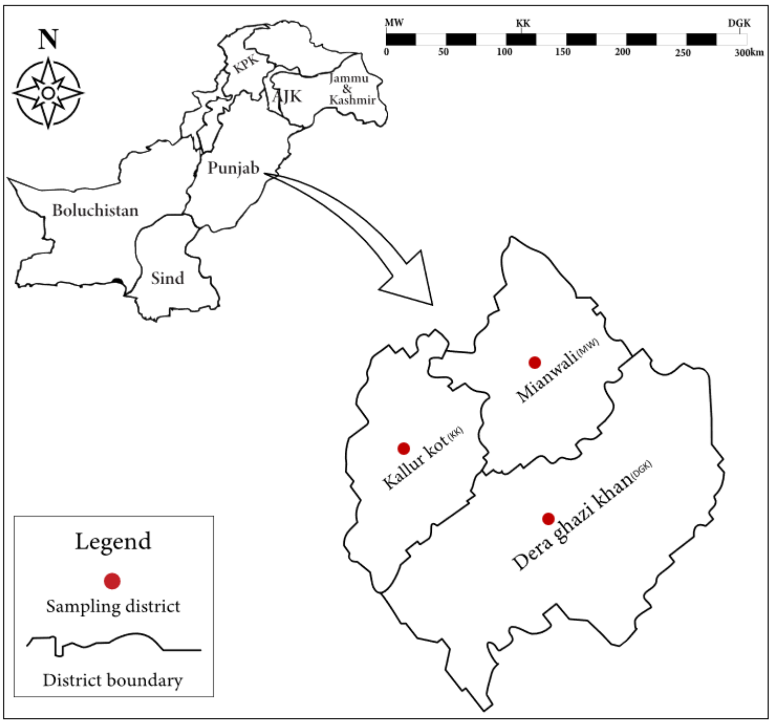

Figure 1: Map of the River Indus viewing three different sampling areas of fishes. 1. Mianwali; 2. Kallur kot and 3. Dera Ghazi Ghaat.

Table 1: List of freshwater fishes of family cyprinidae with their local name recorded from three different sites of River Indus Punjab, Pakistan.

Family	Scientific name	Local name	References
Cyprinidae	*Catla catla*	Thaila	(Muhammad *et al*., 2016)
	Labeo calbasu	Calbans, Dahi	(Abro *et al*., 2023)
	Labeo gonius	Sereha	(Muhammad *et al*., 2018)
	Labeo boga	Bhangan	(Latif *et al*., 2016)
	Labeo rohita	Rahu	(Muhammad *et al*., 2018)
	Cyprinus carpio	Gulfam, common carp	(Muhammad *et al*., 2018)
	Cirrihinus mrigala	Mori	(Abro *et al*., 2023; Muhammad *et al*., 2018)
	Cirrihinus reba	Suhni, Reba Machali	(Abro *et al*., 2023)
	Salmostoma phulo	Finescaled Razorbelly Minnow, Fulchela	(Abro *et al*., 2023)
	Systomus sarana	Olive barb	(Abro *et al*., 2023)

Species identification

The specimens of fish species were identified by invasive photographic techniques. Identification by images was focused on different body color patterns and fins. The digital camera was used to obtain the more accurate images of selected species. Photographs of all fish species were taken perpendicular only when all types of fish fins were fully extended. Twenty-nine measurements of body features (in mm) were taken using a digital caliper.

Measurement of Morphometric, Meristic and Truss Network Analysis

Morphometric counts

Each fish sample was removed from the ice box and washed with running tap water for measuring morphometric, meristic and truss box readings. The total 24 morphometric measurements viz, Total Length, Standard Length, Fork Length, Body Depth, Head Length, Head Depth, Eye Diameter, Pre-Orbital Length, Post-Orbital Length, Snout Length, Inter-Orbital, Upper Jaw Length, Lower Jaw Length, Pre-Dorsal Length, Post-Dorsal Length, Pre-Pectoral Length, Pre-Pelvic Length, Pre-Anal Length, Height of Dorsal Fin, Height of Anal Fin, Length of Dorsal-Fin Base, Length of Anal Fin Base, Caudal Peduncle Length and Caudal Peduncle Depth were measured in millimeter (Table 2, Fig. 2). These morphometric readings were measured with the help of a divider, common scale, measuring board, and vernier caliper to follow the method (Balai *et al*., 2017). The Total Length, Standard Length, and Fork Length were measured in centimeters and then converted into millimeters. Morphological identification followed studies by Samad *et al*., 2020; Mohammed, 2019 and Biswa, 2018.

Table 2: Description of morphometric and meristic measurements used in the study.

Morphometric Traits	Acronyms	Descriptions
Total Length	TL	The measurement from the tip of snout to the posterior edge of caudal fin
Standard Length	SL	Distance from snout to end of the vertebral column
Fork Length	FL	Distance from tip of snout to the point of bifurcation of caudal fin
Body Depth	BD	The Maximum vertical distance between ventral and dorsal edges of the body
Head Depth	HD	Vertical measurement just posterior to the eye orbits
Head Length	HL	Distance from the edge of the snout to the posterior edge of the operculum
Snout Length	SnL	The measurement from snout to anterior bony eye margin
Inter-Orbital	IO	Distance between the eye orbits
Eye Diameter	ED	Maximum length between anterior and posterior eye margins
Pre-Orbital Length	PrOL	Distance from the anterior part of the body to the front margin of the orbit
Post-Orbital Length	PsOL	Length from the posterior edge of orbit to end of operculum
Upper Jaw Length	UJL	Distance from anterior most part of premaxillary to the posterior edge of maxilla
Lower Jaw Length	LJL	Measurement between two endpoints along the lower jaw margin
Pre-Dorsal Length	PrDL	Distance from mouth to the origin of the first dorsal fin
Post-Dorsal Length	PsDL	Distance between the dorsal fin origin and caudal fin base
Pre-Anal Length	PrAL	Total length from mouth to the origin of anal fin rays
Pre-Pectoral Length	PrPecL	Area from the snout to the base of the first pectoral fin rays
Pre-Pelvic Length	PrPevL	Length from snout to the base of pelvic fin rays

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Table 2 (continued):

Morphometric Traits	Acronyms	Descriptions
Height of Dorsal Fin	HDF	Height from the base of the dorsal fin to the tip of the longest fin
Height of Anal Fin	HAF	Start from the base of the anal fin to the tip of the longest anal fin rays
Length of Dorsal Fin Base	LDFB	Length between the anterior and posterior edge of the dorsal fin along the fin base
Length of Anal Fin Base	LAFB	Area between anterior and posterior insertion of the anal fin
Caudal Peduncle Length	CPL	Horizontal length between the posterior edge of caudal fin
Caudal Peduncle Depth	CPD	Vertical depth of the caudal peduncle
Meristic Characters		
Dorsal Fin Rays	DFR	Total number of rays in the dorsal fin
Anal Fin Rays	AFR	Maximum number of the rays present on anal fin
Caudal Fin Rays	CFR	No. of maximum caudal fin rays
Pelvic Fin Rays	PevFR	Total rays on pelvic fin
Pectoral Fin Rays	PecFR	Total number of the rays of pectoral fin

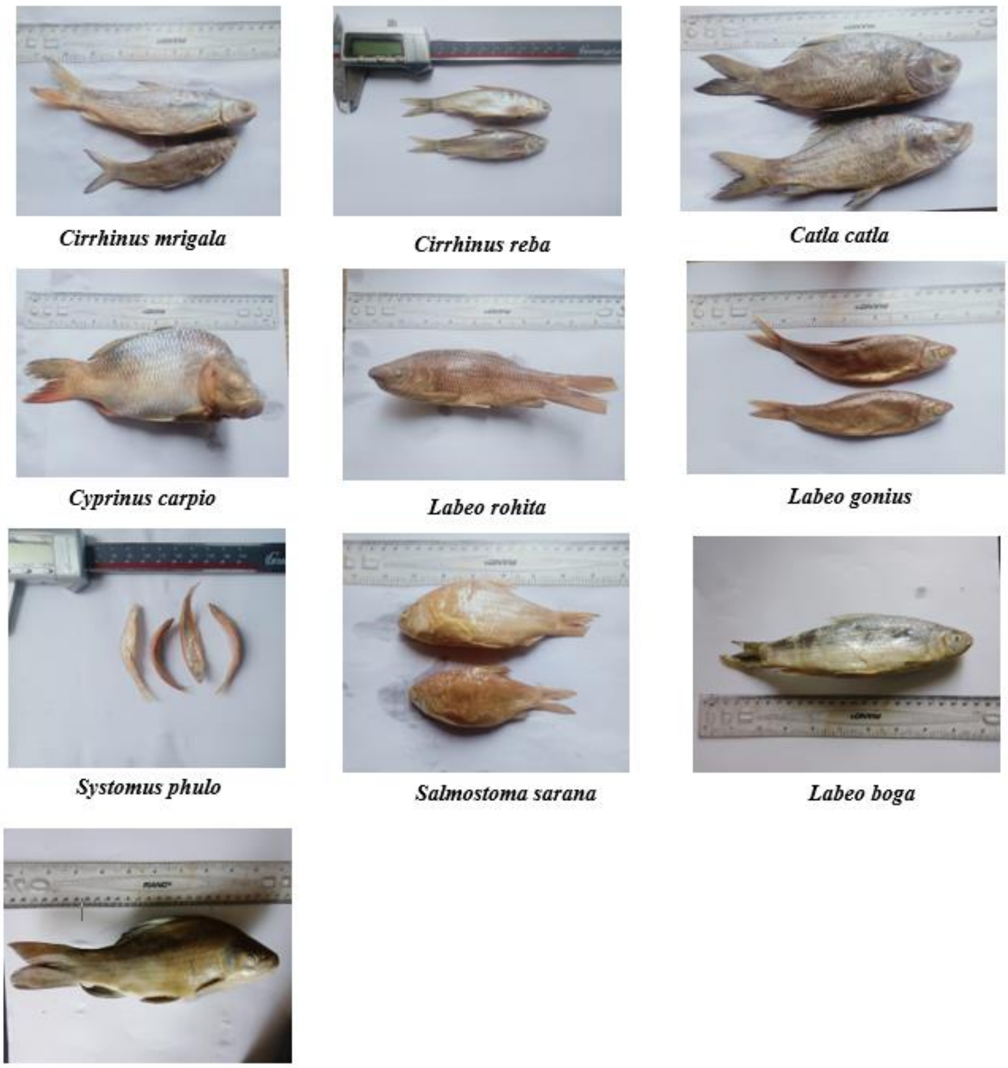

Figure 2: Different freshwater fish species of the family cyprinidae sampled from the River Indus.

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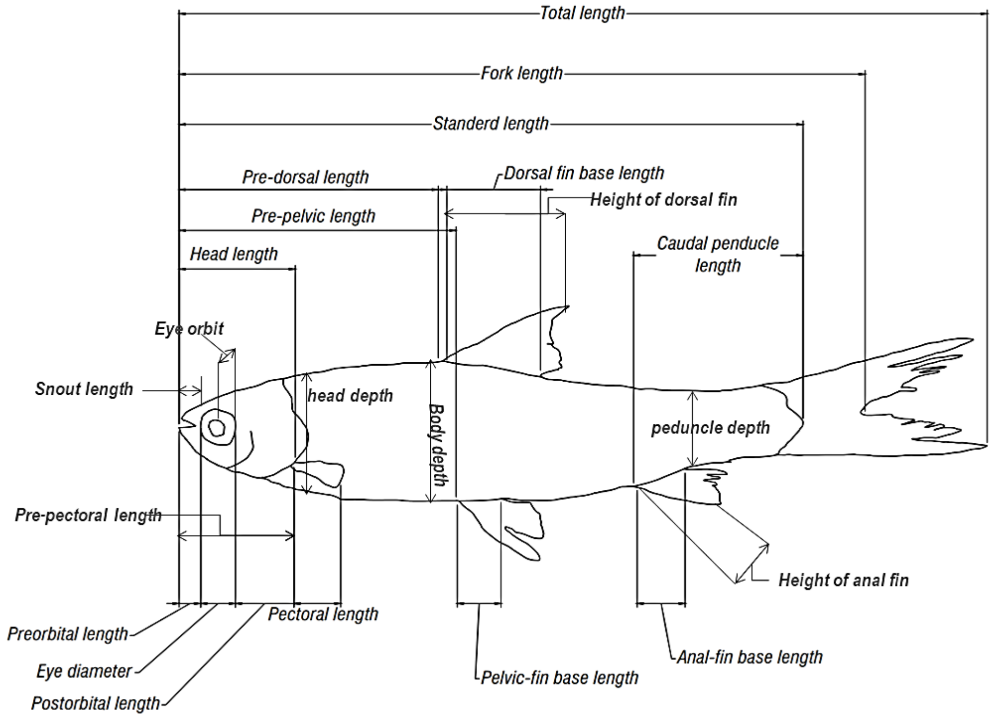

Figure 3: Photograph showing morphometric and meristic measurements of fish of family cyprinidae Total Length (TL), Standard Length (SL), Fork Length (FL), Body Depth (BD), Head Length (HL), Head Depth (HD), Eye Diameter (ED), Pre-Orbital Length (PrOL), Post-Orbital Length (PsOL), Snout Length (SnL), Inter-Orbital (IO), Upper Jaw Length (UJL), Lower Jaw Length (LJL), Pre-Dorsal Length (PrDL), Post-Dorsal Length (PsDL), Pre-Pectoral Length (PrPecL), Pre-Pelvic Length (PrPevL), Pre-Anal Length (PrAL), Height of Dorsal Fin (HDF), Height of Anal Fin (HAF), Length of Dorsal Fin Base (LDFB), Length of Anal Fin Base (LAFB), Caudal Peduncle Depth (CPD) and Caudal Peduncle Length (CPL).

Meristic measurements

Five meristic characters, dorsal fin ray, anal-fin ray, caudal fin ray, pectoral fin ray, and pelvic fin ray were calculated for this study. These fin rays were counted with the help of needles, and magnifying glass and principal rays were counted as separate rays (Parvej *et al*., 2014). Meristic counts for each fish sample (Table 2 and Fig. 3) followed studies by Kaur, 2021 and Kumari *et al*., 2020.

Truss Network Analysis

The truss network described the body shape, depth, width, and morphometric characteristics of fish species. Landmarks distances of species were measured to construct a network on the body of fish. Twelve landmarks that determined thirty distances on fish bodies were marked and measured (Fig. 4a, b). Each box was obtained from the distances on the graph-paper which were measured using Vernier calipers.

Statistical analysis

Data visualization and the statistical analysis were implemented by using R statistical software (R studio core team, 2021) by R integrated development environment in R studio team, 2021. Data were subjected to an analysis of variance (ANOVA) and the means values were compared by using tukey pairwise test at

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($p \leq 0.05$) difference between and among sites and species. Morphological characters (morphometric and meristic counts) and truss analysis was measured by using multivariate analysis (PCA by ggbiplot), correlation matrix (ggbiplot2) and heatmaps were plotted by customized code (pheatmap) and hierarchical cluster plot by using R statistical software.

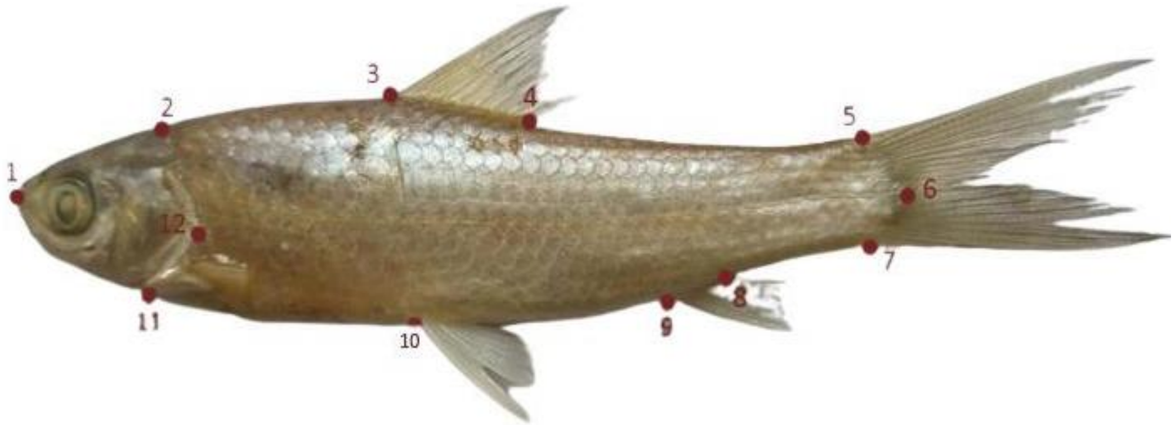

Figure 4a: Image of fish showing the twelve selected anatomical landmarks in *Crrihinus reba.* Landmarks refers to, 1. Anterior tip of snout; 2. Most posterior aspect of neurocranium; 3. Origin of the dorsal fin; 4. Insertion of the dorsal fin; 5. Dorsal side of the caudal peduncle; 6. End of the lateral line; 7. Ventral side of the caudal peduncle; 8. Insertion of the anal fin; 9. Origin of the anal fin; 10. Origin of pelvic fin; 11. Ventral junction of the operculum 12. End of operculum (Hossain *et al.,*, 2010).

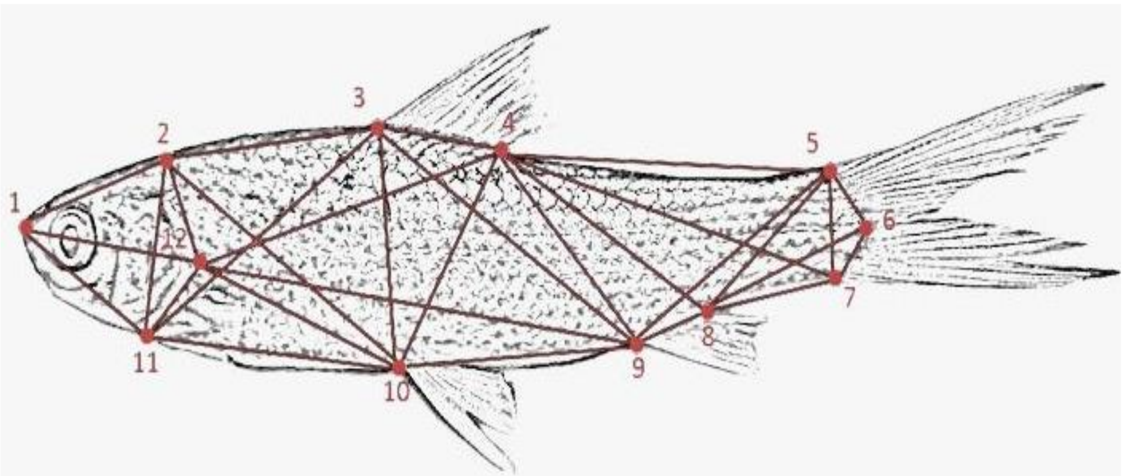

Figure 4b: Schematic image depicting 12 anatomical landmarks creating 30 truss networks on the fish body shown as a close circles and associated truss box to infer morphological differences among fish populations and closed circles indicated as interconnected measurements are as given, T1 (1-2), T2 (1-11), T3(1-12), T4(2-3), T5(2-10), T6(2-11), T7(2-12), T8(3-4), T9(3-11), T10(4-5), T11(4-7), T12(3-9), T13(3-10), T14(4-8), T15(4-9), T16(4-10), T17(4-12), T18(5-6), T19(5-7), T20(5-8), T21(5-9), T22(6-7), T23(6-8), T24(7-8), T25(8-9), T26(9-10), T27(9-12), T28(10-11), T29(10-12), T30(11-12).

Results

Body color and shape

During the survey of fish fauna belonging to family cyprinidae from the River Indus at Mianwali, Kallur kot and Dera Ghazi Ghaat, 90 fishes were reported which belong to single family cyrinidae and 6 genera (Table 1). Genera were Catla,

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Labeo, Cyprinus, Cirrihinus, Salmostoma and Systomus. *Cyprinus carpio* was greenish brown to reddish brown with slightly golden shade. In *Labeo rohita*, back was black and sides became brownish when preserved in formalin. Fins were dark gray and iris rims was red. The edge of each scale has a dark brown tinge.

In *Catla catla*, dorsal side (back) was dirty greenish and ventral side was silvery brown. Fins color was black with light base color. *Labeo boga* has a narrow mouth with no lateral lobe and has two minute maxillary barbels. Body color was dark gray to orange and fins with reddish tinge. The body color of *Labeo calbasu* was blackish with lighter on ventral side. It has four pairs of barbels with very minute gill rakers. The mouth was narrow with depressed snout and has thick lips. In *Labeo gonius*, body color was reddish golden to orange with convex dorsal profile than abdomen. The mouth is narrow with short barbels. Fins color was reddish brown and pectrol fin was as long as head.

The dorsal side of *Cirrhinus mrigala* was dark gray to slight greenish and ventral side was silvery. In large specimen, fin color was light orange. It has convex dorsal profile than abdomen. In *Cirrhinus reba*, body color was orange gray dorsally and silvery on ventral side. The fin color was grayish on top with slight orange base. Body was elongate and head depth was greater than head length. Small number of *Systomus sarana* was found in these three locations. The body color was brown to greenish and became dark orange due to the effect of formalin. Head depth was also greater than head length. *Salmostoma phulo* was dark orange on both dorsal and ventral side. Mid body color near the lateral line was silvey gray. Fin color was brown.

Morphometric measurements

To study morphological variations, fish samples were collected from three different sites Mianwali, Kallur kot and Dera Ghazi Ghaat of the River Indus Punjab, Pakistan. The mean and standard error of morphometric measurements are presented in Table 3. In *Catla catla* Maximum mean value of total length (TL), standard length (SL), fork length (FL), body depth (BD), head length (HL), and head depth (HD), post-orbital length (PsOL), inter orbital (IO), pre-dorsal length (PrDL), post-dorsal length (PsDL), pre-pectoral length (PrPecL), pre-pelvic length (PrPevL), pre-anal length (PrAL), height of dorsal fin (HDF), height of anal fin (HAF) and length of dorsal fin base (LDFB) were observed in MW population than other sites KK, DGG. These traits in MW population showed significance difference with the same traits in KK, DGG population. Non-significance difference in eye diameter (ED), pre-orbital length (PrOL), snout length (SnL), upper jaw length (UJL), lower jaw length (LJL), length of anal fin base (LAFB), caudal peduncle depth (CPD), and Caudal Peduncle Length (CPL) were observed among all three sites in *Catla catla*. Biometric analysis of *C.catla* from MW, KK and DGG population average total length, standard length and forked length were recorded 137.8±1.96 (94.97±214.67mm), 107.8±2.53 (72.27±166.67 mm) and 118.63±1.95 (77.97±186.33mm), respectively.

In *Labeo calbasu* significance difference was observed in TL parameter between all three sampling sites. Highest mean values of SL, FL, PsDL were observed in fish present in MW location.

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Table 3: Morphometric and meristic characters (Mean±SE) of different fish species of labeo genus collected from diverse ecozones of Punjab, Pakistan.

Morphometric characters	*Catla catla*			*Labeo calbasu*			*Labeo gonius*		
	MW	KK	DGG	MW	KK	DGG	MW	KK	DGG
TL	214.67±3.18a	103.74±1.96b	94.97±0.73b	223.67±7.31a	182±3.61b	160.67±1.76c	171.67±1.20a	162.33±2.73a	154.33±1.45b
SL	166.67±3.84a	84.37±3.47b	72.27±0.29b	173.67±7.69a	139±2.65b	131.98±0.99b	135±1.53a	127±2.08a	119.67±1.45b
FL	186.33±3.53a	91.58±1.89b	77.97±0.42b	195.33±7.06a	157.67±3.28b	145.51±1.37b	149.67±1.76a	139±2.65b	130.67±1.20b
BD	54.57±2.49a	20.27±0.41b	20.24±0.44b	58.66±3.48a	45.05±0.88a	46.44±0.39a	40.43±1.60a	35.51±0.89a	36.83±0.16a
HL	51.71±1.14a	28.7±1.03b	26.21±0.22b	41.13±1.57a	35.01±0.77a	29.70±1.06a	29.13±0.73a	27.02±0.33a	27.23±0.12a
HD	46.83±0.49a	20.62±0.49b	18.45±0.16b	26.55±0.46a	27.08±0.47a	25.60±0.21a	23.72±3.34a	19.18±2.12a	23.27±0.13a
ED	8.17±0.26a	5.23±0.16a	4.93±0.05a	7.72±0.32a	8.03±0.10a	6.64±0.22a	6.92±0.53a	6.34±0.67a	7.42±0.11a
PrOL	20.06±0.95a	8.62±0.27a	8.79±0.02a	17.68±0.07a	16.31±0.48a	14.3±0.23a	9.25±0.42a	8.15±1.06a	10.11±0.11a
PsOL	27.92±0.11a	13.43±0.23b	12.64±0.22b	17.21±1.68a	13.63±0.65a	12.05±0.14a	12.23±1.39a	10.16±0.46a	10.4±0.10a
SnL	23.01±0.39a	11.06±0.50a	9.96±0.07a	20.52±0.50a	19.60±0.25a	17.12±0.48a	13.02±0.59a	11.61±0.78a	13.08±0.08a
IO	29.28±0.36aA	10.81±0.24b	10.09±0.08b	24.31±0.37a	20.09±1.29a	17.85±0.17a	14.52±0.65a	13.72±0.29a	14.16±0.07a
UJL	15.79±0.39aA	8.42±0.08a	8.23±0.10a	7.13±0.10a	6.57±0.16a	5.45±0.28a	4.23±0.04a	3.39±0.19a	3.08±0.06a
LJL	15.18±0.39aA	7.98±0.05a	7.76±0.05a	6.33±0.14a	5.33±0.22a	3.71±0.28a	3.66±0.06a	2.57±0.06a	2.41±0.05a
PrDL	80.14±1.36a	36.06±0.57b	34.99±0.50b	84.92±1.00a	73.98±1.84a	66.02±1.58b	61.51±1.32a	55.72±1.59a	51.53±0.54a
PsDL	42.77±0.45a	19.12±0.25b	18.35±0.15b	52.60±0.42a	36.69±3.83b	34.12±0.37b	43.28±2.25a	42.14±1.05a	40.10±0.16a
PrPecL	53.92±0.70a	26.00±1.04b	23.39±0.18b	42.89±0.78a	35.38±1.33a	28.60±1.29b	30.42±1.15a	28.13±0.88a	29.56±0.22a
PrPevL	91.47±1.51a	38.7±1.38b	35.31±0.13b	83.18±1.74a	76.54±1.08a	64.3±2.05b	68.26±0.14a	65.43±0.16a	64.93±0.14a
PrAL	136.62±1.21a	58.16±0.91b	55.74±0.14b	124.22±2.23a	111.03±1.65a	96.28±1.40b	105.46±0.40a	97.70±2.36a	94.52±0.26a
HDF	43.03±1.45a	19.98±0.24b	19.28±0.11b	48.76±3.64a	43.28±0.24b	38.18±1.75a	27.12±0.58a	23.62±1.02a	23.09±0.23a
HAF	34.20±1.34a	15.28±1.52b	10.71±0.17b	39.30±1.76aA	34.8±0.45a	30.27±1.04a	20.66±1.28a	19.05±0.65a	18.88±0.13a
LDFB	48.88±1.15a	19.03±0.55b	18.06±0.05b	46.04±0.65a	38.31±0.75a	35.5±0.58a	27.39±0.47a	27.88±0.56a	28.56±0.24a
LAFB	14.24±0.27a	5.36±0.27a	4.83±0.10a	17.68±0.18a	14.05±0.87a	12.71±0.23a	10.19±0.79a	8.09±0.54a	9.05±0.09a
CPD	23.21±0.29a	9.29±0.34a	8.38±0.10a	26.25±0.51a	20.97±1.23a	18.43±0.24a	15.89±0.69a	14.05±0.83a	15.43±0.13a
CPL	21.7±0.93a	9.60±0.19a	9.82±0.06a	25.05±0.60a	23.34±0.22a	21.02±0.76a	20.33±0.21a	14.53±0.87a	15.25±0.23a
				Meristic traits					
DFR	16±0.58a	14±0.58a	15±0.58a	15.58±0.58a	15.33±0.33a	15.33±0.88a	15±0.58a	15±0.58a	15.33±0.33a
AFR	7.33±0.33a	6.33±0.33a	6.33±0.33a	7±0.33a	7±0.33a	7±0.33a	6.33±0.33a	7±0a	7±0a
CFR	25±2.52a	20±0.58a	20.33±0.88a	24±2.08a	21.33±0.67a	21.67±0.33a	21.67±1.45a	22.67±0.33a	23±0.58a
PecFR	14.67±0.33a	15.67±0.33a	15.33±0.33a	18.33±0.33a	18.33±0.33a	18.33±0.33a	13.33±1.67a	13.33±0.33a	14±0.58a
PevFR	9±0aA	8.67±0.33a	8.33±0.33a	9.33±0.33a	9.33±0.33a	9.33±0.33a	9±0.58a	9±0a	9.33±0.33a

Table 3 (continued):

Morphometric characters	*Labeo boga*			*Labeo rohita*		
	MW	KK	DGG	MW	KK	DGG
TL	221.67±4.41c	278.33±1.2a	242.67±1.45b	285.67±7.45aA	254.33±3.84b	226±3.46c
SL	169.67±2.6c	212.67±6.36a	188.33±1.2b	219.93±7.63aA	192.06±4.28b	179±1.73b
FL	187.67±1.86c	238.67±7.36a	207.67±1.45b	250.73±10.02aA	225.83±3.7b	203±4.04c
BD	47.97±1.38a	51.28±0.79a	50.07±0.02a	68.34±2.12aA	60.80±0.92a	56.62±1.67a
HL	37.72±1.62a	40.59±6.52a	42.43±0.22a	62.43±2.69aA	56.21±1.5a	49.4±0.97a
HD	29.05±1.67a	26.85±1.85a	34.22±0.25a	50.05±1.5aA	44.13±0.72a	37.91±1.62a
ED	15.45±1.14a	7.13±0.02b	7.41±0.17b	32.36±2.55aA	28.87±0.58a	26.33±0.41a
PrOL	13.75±1.07a	17.55±0.51a	16.16±0.07a	24.30±0.46aA	21.98±0.34a	19.63±0.76a
PsOL	16.26±0.81a	23.88±0.14a	20.24±0.34a	33.11±1.24aA	27.08±0.9a	22.61±0.64a
SnL	17.41±1.25a	20.71±0.13a	19.98±0.05a	28.89±0.98aA	26.14±0.14a	23.40±0.58a
IO	16.14±0.36a	17.65±0.19a	17.34±0.06a	8.83±0.04a	8.48±0.11a	8.31±0.04a
UJL	9.03±0.12a	12.11±0.6a	9.52±0.26a	10.72±0.63a	9.68±0.07a	7.47±0.52a
LJL	8.30±0.09a	7.81±0.35a	8.79±0.06a	8.56±0.46a	7.26±0.3a	5.63±0.24a
PrDL	75.09±2.14b	91.02±0.31a	82.66±0.19a	115.07±1.84aA	101.83±0.4b	91.21±4.4b
PsDL	64.88±1.6a	74.63±0.71aA	71.45±0.13a	72.67±1.56a	65.13±0.64a	56.94±1.65b
PrPecL	39.88±1.04a	44.10±1.16a	42.32±0.2a	62.04±1.56aA	54.73±0.77a	47.30±1.92b
PrPevL	83.92±4.08a	97.54±0.09a	93.69±0.28a	117.95±2.2aA	103.17±1.00b	91.35±3.03b
PrAL	134.91±3.79b	158.02±1.53a	146.94±0.11a	184.36±1.07aA	147.15±0.77b	132.89±3.64b
HDF	44.22±0.51a	51.21±0.09a	45.90±0.11a	51.89±1.46aA	46.49±0.52a	39.77±1.25a
HAF	31.69±0.19a	39.26±0.6aA	32.99±0.12a	29.82±0.88a	27.89±0.65a	27.83±0.09a
LDFB	30.43±0.9a	35.79±0.34a	34.24±0.23a	50.06±1.4aA	44.57±0.67a	40.70±0.87a
LAFB	17.87±0.7a	14.92±0.62a	18.52±0.27aA	18.50±0.59aA	15.83±0.25a	12.62±0.32a

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Table 3 (continued):

Morphometric characters	***Labeo boga***			***Labeo rohita***		
	MW	**KK**	**DGG**	**MW**	**KK**	**DGG**
CPD	20.93±1.14a	24.98±0.02a	24.78±0.28a	30.87±1.12aA	27.65±0.41a	24.17±0.66a
CPL	23.57±0.47a	32.72±0.3aA	25.49±0.16a	27.52±0.73a	23.83±0.2a	20.83±0.56a
			Meristic traits			
DFR	11±0a	10.67±0.33a	10.33±0.33a	13±0.58a	13±0.33a	13±0.33a
AFR	8.33±0.33a	6.33±0.33a	6.33±0.33a	7.67±0.88a	8±0a	8.67±0.33a
CFR	22.67±0.88a	23±0a	23.33±0.33a	24±1a	23.33±0.33a	22.67±0.33a
PecFR	13.33±0.33a	13.67±0.33a	13.67±0.33a	15.67±1.67a	17.67±0.67a	13.67±0.33a
PevFR	9±0a	9.33±0.33a	9±0a	8±0.58a	7.67±0.33a	8±0.58a

Means bearing, different small letters (a, b, c) indicate significant ($p \leq 0.05$) differences between collection sites of five different fish species. Capital letters (A, B, C) indicate significant ($p \leq 0.05$) difference among species. Means in the same rows sharing the same superscript letters are not significantly different. Means with different superscripts letter are significantly different for each morphometric and meristic variable.

There was no significance difference in mean percentage of BD, HL, HD, ED, PrOL, PsOL, SnL, IO, UJL, LJL, HAF, LDFB, LAFB, CPD, and CPL were observed among all three locations MW, KK and DGG. Average total length, standard length and forked length from three sites were recorded 1.88.8±4.22 (160.67±223.67mm), 148.21±3.78 (131.98±173.67 mm) and 166.17±3.90 (145.51±195.33mm), respectively.

In *Labeo gonius* increased values of TL and SL were observed between MW and KK population while maximum FL was found in MW population. Out of 24 morphological characters, 21 characters showed a non-significant difference ($p \leq$ 0.05) among the population of *L. gonius* of the Mianwali (MW), Kallur kot (KK) and Dera ghazi ghat (DGG). Average TL, SL and FL were recorded 162.78±1.79 (154.33±171.67mm), 127.22±1.68 (119.67±135mm) and 139.78±1.87 (130.67±149.67mm), respectively. Significance differences in TL, SL and FL were observed among all three populations of *Labeo boga*. The maximum PrDL and PrAL was found in Kallur kot, and Dera ghazi ghat population but ED was in Mianwali population and remaining characters had a statistically non-significant ($p \leq$ 0.05) among population. In *L. rohita*, highest mean values of SL, PrDL, PrPevL and PrAL were found in MW population while PsDL and PrPecL was found maximum in MW and KK population. Significance difference in TL and FL was found in all three populations of *Rahu*. Similarly in *L. boga* and *L. rohita* average TL, SL and FL were measured 247.56±2.35 (221.67±278.33mm), 190.22±3.39 (169.67±212.67mm), 211.33±3.56 (187.67±238.67mm), 255.33±4.92 (226±285.67mm), 197±4.55 (179±219.93mm) and 226.52±5.92 (203±250.73mm), respectively (Table 3).

Significance differences in TL, SL, FL and PrAL were observed between all three populations of *Cyprinus carpio.* Moreover, the KK population showed significant differences from MW and DGG populations for BD, HL, PrDL, PrPecL, PrPevL, HDF and LDFB characters. In *C. mrigala*, the DGG population demonstrated a highly significant difference in SL, FL from the MW and KK population while a significance difference in TL and PsDL was found between all three populations. The

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KK population showed significant differences from MW and DGG populations for BD, PrDL, PrPecL, PrPevL and PrAL characters. All morphometric parameters showed no significant differences among the three populations Of *Cirrihinus reba* and *Salmostoma phulo*. Three characters, namely, TL (p<0.05), SL (p<0.05), and FL (p<0.05) parameters, demonstrated significant disparities among three population of *Systomus sarana*. In addition, MW population revealed a significant deviation from KK and DGG populations for character PrDL and PrAL while KK population demonstrated significance from MW and DGG population for BD and PrPevL parameters (Table 4).

Table 4: Morphometric and meristic characters of different fish species (Cyprinus, Cirrhinus, Salmostoma and Systomus genus) collected from diverse ecozones of Punjab, Pakistan.

Morphometric characters	*Cyprinus carpio*			*Cirrihinus mrigala*			*Cirrihinus reba*		
	MW	**KK**	**DGG**	**MW**	**KK**	**DGG**	**MW**	**KK**	**DGG**
TL	295.12±13.2aA	198.51±6.34c	240.57±1.45b	263.38±5.81a	243.93±2.14b	183.85±5.91c	107.50±0.56a	105.89±0.22a	102.52±1.02a
SL	235.67±12.65aA	161.13±7.06c	182.35±0.84b	219.23±4.57a	205.43±10.61a	140.37±5.28b	85.85±0.69a	83.41±0.61a	81.80±0.20a
FL	273.86±13.03aA	172.56±5.68c	220.21±1.33b	237.25±4.37a	222.03±10.42a	150.27±5.10b	93.59±0.36a	91.60±0.89a	89.39±0.31a
BD	78.61± 4.65aA	52.45±0.56b	65.37±0.77a	53.50±0.95a	50.70±0.17a	38.11±2.37b	20.08±0.10a	20.01±0.68a	19.29±0.37a
HL	63.36± 2.51aA	43.42±1.49b	54.54±0.64a	47.94±0.21a	44.63±0.43a	34.55±0.76a	19.35±0.33a	18.44±0.27a	17.32±0.35a
HD	45.07±1.34aA	39.35±0.65a	39.78±0.74a	22.66±0.41a	20.88±0.15a	17.84±0.73a	11.88±0.08a	11.52±0.17a	10.78±0.18a
ED	22.73±1.04aA	17.36±0.69a	18.58±0.29a	22.29±0.22a	20.65±0.24a	14.84±1.04a	5±0.22a	5.18±0.07a	5.12±0.06a
PrOL	24.66±1.33aA	16.84±0.98a	19.81±0.35a	14.94±0.25a	14.02±0.11a	10.6±0.64a	6.64±0.05a	6.53±0.19a	5.69±0.10a
PsOL	28.48±1.85aA	21.23±1.25a	23.43±0.40a	22.75±0.61a	21.32±0.12a	15.58±0.71a	8.55±0.14a	7.97±0.13a	7.54±0.06a
SnL	27.81±1.67aA	20.13±0.88a	22.70±0.33a	18.22±0.39a	16.93±0.11a	13.00±0.78a	8.87±0.06a	8.48±0.20a	8.27±0.02a
IO	9.96±0.47a	8.87±0.06a	9.25±0.07a	9.55±0.39a	8.44±0.12a	8.24±0.11a	8.63±0.03a	8.77±0.06a	8.44±0.11a
UJL	14.39±0.54aA	11.09±0.95a	13.5±0.04a	11.04±0.56a	9.72±0.07a	9.22±0.22a	4.18±0.05a	4.26±0.29a	3.76±0.18a
LJL	12.75±0.56aA	9.40±0.64a	11.55±0.05a	10.47±0.57a	8.22±0.06a	7.25±0.23a	3.73±0.03a	3.73±0.19a	3.53±0.11a
PrDL	120.34±5.57aA	85.06±4.83b	105.60±1.13a	94.99±1.01a	91.84±0.18a	73.63±1.50b	38.21±0.42a	37.1±0.30a	36.37±0.07a
PsDL	30.49±1.23a	31.19±0.35a	29.44±0.72a	94.84±0.88aA	73.71±6.79b	43.84±1.33c	30.85±0.45a	29.24±0.45a	29.57±0.52a
PrPecL	62.48±2.56aA	42.67±2.63b	54.47±0.39a	49.97±1.04a	46.58±0.30a	32.36±1.71b	20.01±0.09a	19.71±0.06a	18.75±0.17a
PrPevL	112.06±4.06a	82.20±4.27b	102.38±0.76a	117.08±0.84aA	108.58±1.41a	75.84±1.40b	41.05±0.10a	40.98±0.45a	39.24±0.38a
PrAL	176.56±7.73aA	126.60±5.58c	157.61±0.81b	169.07±0.77a	155.32±3.11a	111.62±3.36b	62.16±1.70a	61.80±0.91a	61.04±0.15a
HDF	46.00±2.12aA	30.72±3.82b	39.49±0.50a	46.52±0.68aA	42.45±0.74a	32.88±1.38a	18.18±0.01a	19.41±0.71a	19.52±0.40a
HAF	38.05±2.08aA	27.73±2.69a	32.39±0.42a	38.47±0.93aA	36.53±0.05a	26.27±1.08a	12.39±0.04a	12.88±0.22a	13.27±0.06a
LDFB	88.99±3.12aA	68.81±6.21b	80.80±0.50a	41.29±0.73a	38.35±0.50a	30.79±1.15a	11.49±0.21a	11.27±0.10a	10.77±0.12a
LAFB	23.73±0.98aA	17.71±1.01a	19.46±0.43a	17.11±0.19a	16.51±0.26a	11.5±0.28a	6.13±0.03a	6.19±0.06a	6.25±0.05a
CPD	33.62±1.99aA	24.45±1.89a	27.32±0.61a	23.37±0.74a	21.37±0.33a	16.43±1.06a	9.29±0.05a	9.16±0.22a	8.94±0.07a
CPL	27.60±1.24aA	21.74±2.08a	24.03±0.49a	24.37±0.85a	22.80±0.10a	15.35±1.23a	12.14±0.17a	11.56±0.68a	10.58±0.05a
					Meristic traits				
DFR	16.67±0.33a	15.67±0.33a	15.67±0.33a	13.33±0.33a	13.67±0.33a	13.67±0.33a	9±0aA	9±0a	9±0a
AFR	7.67±0.33a	5.67±0.67a	6.67±0.88a	7.67±0.33a	7.33±0.33a	7.33±0.33a	6.33±0.33a	7±0a	6.33±0.33a
CFR	25±1a	26±1a	24.67±1.20a	26.33±0.33a	26.67±0.88a	26.33±0.88a	20±0aA	19±0.58a	19.67±0.33a
PecFR	14.33±0.33a	15±0a	14.67±0.33a	17.67±0.33a	17.67±0.33a	17.67±0.33a	11.33±0.33a	12±1a	12.33±0.67a
PevFR	9.67±0.33a	8.33±0.33a	8.67±0.33a	9.67±0.33a	9.33±0.33a	8.67±0.33a	9±0aA	9±0a	9±0a

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Table 4 (continued):

Morphometric characters	*Salmostoma phulo*			*Systomus sarana*		
	MW	KK	DGG	MW	KK	DGG
TL	61.62±0.71a	58.83±0.27a	57.24±0.35a	157.90±6.01a	116.21±2.37c	135.32±2.04b
SL	52.38±0.38a	48.42±0.88a	48.28±0.39a	124.94±6.05a	90.09±1.68c	106.18±2b
FL	56.78±0.41a	54.18±0.25a	52.76±1.09a	143.41±5.36a	100.89±2.16c	116.55±1.59b
BD	11.58±0.29a	10.18±0.52a	10.72±0.14a	43.76±1.28a	23.65±1.41b	36.17±1.08a
HL	9.94±0.23a	9.21±0.31a	9.23±0.51a	31.28±1.42a	20.59±1.46a	25.84±1.29a
HD	4.58±0.18a	4.18±0.23a	4.52±0.09a	19.71±0.64a	12.77±1.07a	15.05±1.14a
ED	2.34±0.13a	1.56±0.24a	1.85±0.17a	8.9±0.25a	5.39±0.25a	5.53±0.31a
PrOL	2.22±0.12a	1.40±0.41a	1.65±0.30a	8.56±0.13a	6.25±0.22a	6.72±0.50a
PsOL	3.78±0.16a	3.04±0.26a	3.28±0.27a	14.82±1.20a	8.86±0.56a	10.78±0.49a
SnL	2.87±0.15a	2.57±0.04a	2.58±0.14a	13.18±0.79a	8.67±0.33a	9.75±0.60a
IO	1.49±0.15a	1.43±0.05a	1.46±0.03a	16.06±0.62aA	10.02±0.63a	14.07±0.66a
UJL	1.89±0.11a	1.6±0.13a	1.84±0.10a	8.74±0.12a	5.17±0.81a	7.91±0.07a
LJL	2.04±0.12a	1.95±0.05a	2.04±0.02a	7.97±0.16a	4.84±0.80a	6.91±0.28a
PrDL	31.78±0.21a	31.21±0.50a	31.12±0.38a	65.91±4.17a	40.79±1.40b	50.92±1.26b
PsDL	11.21±0.12a	10.84±0.12a	10.48±0.57a	42.24±0.90a	35.89±1.55a	39.6±0.60a
PrPecL	11.88±0.19a	10.04±0.27a	10.86±0.95a	32.30±1.38a	21.59±0.97a	27.28±0.49a
PrPevL	23.67±0.20a	21.79±0.71a	22.87±0.35a	59.71±1.16a	43.37±1.15b	55.25±0.57a
PrAL	31.76±0.34a	30.57±0.25a	31.42±0.36a	92.27±3.36a	63.01±2.26b	78.83±1.14b
HDF	5.07±0.10a	4.60±0.19a	4.89±0.08a	25.75±0.85a	21.52±0.28a	22.81±0.55a
HAF	6.72±0.28a	4.94±0.27a	5.81±0.53a	19.41±0.35a	14.34±0.88a	16.53±0.45a
LDFB	3.71±0.18a	3.5±0.02a	3.55±0.07a	19.07±0.99a	12.11±0.68a	15.74±0.45a
LAFB	6.72±0.10a	5.53±0.67a	6.09±0.25a	11.70±0.69a	8.94±0.33a	9.49±0.32a
CPD	3.78±0.19a	2.72±0.08a	3.16±0.27a	16.51±0.73a	9.12±0.60a	13.98±0.12a
CPL	6.87±0.17a	6.09±0.17a	6.27±0.59a	18.26±1.07a	12.52±0.35a	14.93±0.21a
Meristic traits						
DFR	6.67±0.33a	6.67±0.33a	5.67±0.33a	8.33±0.33a	8.33±0.33a	8.33±0.33a
AFR	13±0a	12.33±0.33a	12.67±0.33a	6.33±0.33a	6.33±0.33a	6.33±0.33a
CFR	22.33±0.33a	22.33±0.33a	22.67±0.33a	32.33±0.67a	32.33±0.67a	32.33±0.33a
PecFR	7±0a	6.67±0.33a	6.67±0.33a	13.33±0.88a	13.67±0.33a	14±0.58a
PevFR	6.67±0.33a	6.67±0.33a	6.67±0.33a	8.67±0.33a	8.67±0.33a	8.67±0.33a

Means bearing, different small letters (a, b, c) indicate significant ($p \leq 0.05$) differences between collection sites of five different fish species. Capital letters (A, B, C) indicate significant ($p \leq 0.05$) difference among species. Means in the same rows sharing the same superscript letters are not significantly different. Means with different superscripts letter are significantly different for each morphometric and meristic variable.

Among all species *C. catla* (MW) has large average IO, UJL, and LJL but *L. boga* (KK) has maximum average PsDL, HAF and CPL than other species. Other 18 characters were found to be maximum in *L. rohita* in MW population (Table 3). Similarly *Systomus sarana* has maximum average IO and *Cirrihinus mrigala* has highest mean PsDL and PrPevL in MW population than other species. Remaining twenty one traits were found to be significant in *Cyprinus carpio* (MW) from other sites (Table 4). These results are important to compare among fish species of cyprinid. All the morphometric characters, their maximum, minimum mean and standard error are shown in Table 3 and 4. Biometric study revealed that sufficient numbers of mature fish species are available in these sites of the River Indus Punjab, Pakistan.

Meristic counts

Table 2 shows the five meristic traits i.e., dorsal fin ray (DFC), anal fin ray (AFR), caudal fin ray (CFR), pelvic fin ray (PevFR) and pectoral fin ray (PecFR) investigated in this study. There were no differences in DFC among *C. catla*, *L.*

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calbasu, *L. boga*, and *C. reba*, PecFR in *L. gonius*, *C. mrigala*, and *L. calbasu*, AFR in *C. mrigala* and *L. calbasu*, CFR in *C. mrigala*, *S. phulo*, and *S. sarana* and PevFR in *C. reba*, *L. calbasu*, *L. gonius* and *L. boga*. In *C. catla* DFR, CFR, PecFR, ranged from 14-16, 6-8, 20-25 and 14-15, respectively along with pelvic fin rays (PevFR) recorded 8-9 numbers in (Table 3). In *L. calbasu* caudal fin rays, Pectoral fin rays, Pelvic fin rays ranged from 21-24, 18 and 9.33.

The range of DFR in *L. calbasu* and *L. gonius* was same for all the populations in different locations *viz.* 15 while in *L. boga* and *L. rohit* it was ranged 10-11 and 13. In case of AFR it was 7 for *L. calbasu* and 7-8 for *L. rohita* but for *L. gonius* and *L. boga* it was 6-7 and 6-8. Counts of CFR showed slight variation among species ranging from 21-24 in overall. Similarly for PecFR the ranges were 18-19, 13-14, 13 and 13-17 for *L. calbasu*, *L. gonius*, *L. boga* and *L. rohita*, respectively.

In case of PevFR it was 7-8 for *L. rohita* but for *L. calbasu*, *L. gonius* and *L. boga* it was 9 for all the three populations (Table 3). The range of DFR and PevFR in *C. reba* was same for all three population 9 while in *C. carpio*, *C. mrigala*, *S. phulo* and *S. sarana* DFR ranged from 15-16, 13-14, 5-6 and 8-9. In the case of AFR it ranged from 6-7 for *C. reba* and *S. sarana* but it was 5-7, 7 and 12-13 for *C. carpio*, *C. mrigala* and *S. phulo*. In the case of PevFR it was 8-9 for *C. carpio*, *C. mrigala*, *S. sarana* but it was 6-7 for *S. phulo* (Table 4).

Multivariate analysis

Principal component analysis (PCAs)

Principal component analysis of meristic characters demonstrated a significant variability between species collected from different sites of Indus River Punjab, Pakistan. Two principal components (PC1, 58.1% and PC2, 21.7%) with a total variance of 79.8% were selected. The PCAs showed a cumulative variability (79.8 %) for fish species. The *Systoma sarana* showed a higher positive eigenvalue and plotted toward the PC2 upper axis. The specie *Labeo gonius* and *Catla catla* were strongly associated with each other and showed negative lower eigenvalues. The *Cirrihinuis reba* and *Labeo rohita* were closely related to each other and plotted toward the PC1. The locations for meristic characters had shown a close association between PevFR and PecFR, while a strong negative association was excelled between AFR and DFR (Fig. 5).

Image based truss-box analysis

Results obtained by using univariate analysis are not to be enough to distinguish fish stocks from three sites of Indus River. The effects of variables on PC were calculated to study which truss measurement segregate stocks most effectively. Truss box analysis data revealed a total variability (84.5%) between various variables. The species *Cyprinus carpio*, *Labeo rohita*, *Labeo boga* and *Labeo gonius* were plotted closely in PC2 significantly ($p \le 0.05$) with higher positive eigenvalues. However, species *Cirrihinus reba* and *Salmostoma phullo* had not shown any association with other species among three populations and were plotted near to central axis separately (Fig. 6a). The Truss box traits T25(8-9):T10(4-5), T24(7-8):T16(4-10), T16(4-10):T18(5-6)

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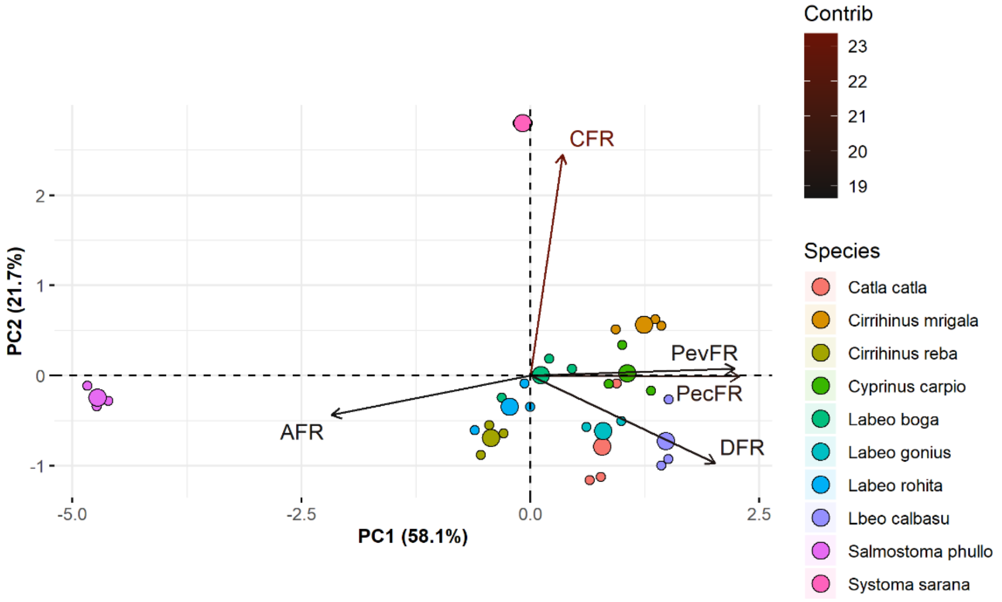
and T3(1-12):T19(5-7) were strongly influenced by each other with higher positive eigenvalues, while T9(3-11):T1(1-2) were negatively contributed to each other (Fig. 6b).



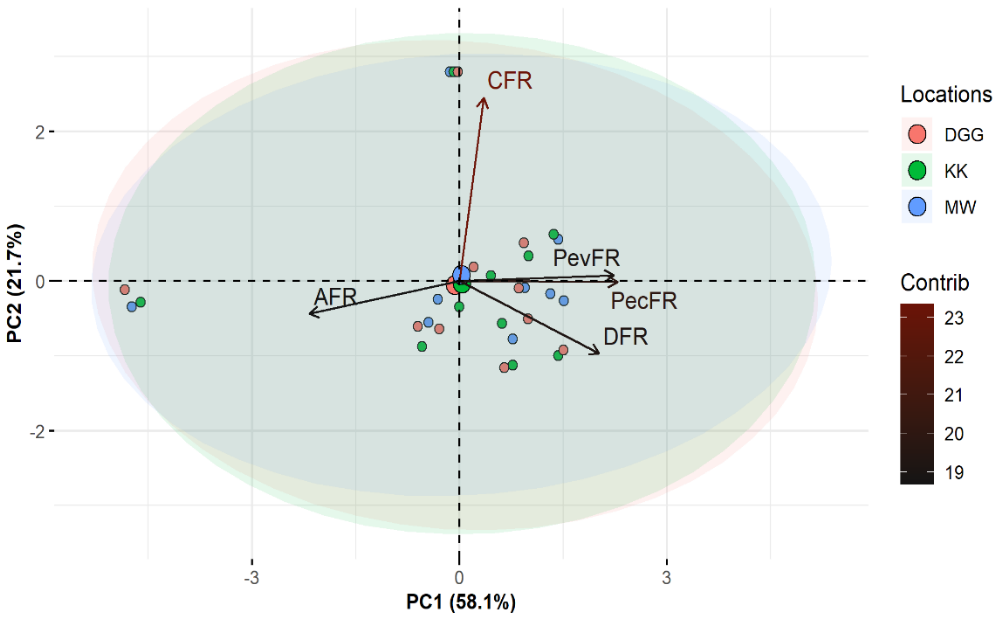


Figure 5: Principal component analysis (PCA) biplot of meristic characters for a) different species of fishes b) collected from different locations of Punjab, Pakistan.

Correlation matrix and clustered heatmap

The meristic and truss box characters of different fishes had been shown a significantly ($p \leq 0.05$) strong correlation with each other. The meristic traits such as PecFR and PevFR were positively and strongly correlated with all Truss box characters. However, the AFR showed a negative relation with all characters of Truss and meristic characters (Fig. 7).

The clustered heatmap was constructed to show the influence of various meristic

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and truss box characters. The AFR and CFR were tightly grouped and exhibited a strong influence on Truss analysis characters. All truss traits showed a negative influence with the DGG-S9, MW,-S9, KK-S9 and MW-S8. The MW-S6, KK-S6, and DGG-S6 were strongly and positively influenced by DFR, T7 (2-12), T8 (3-4), T6 (2-11), T3 (1-12), T19 (5-7) and T25 (8-9) (Fig. 8).

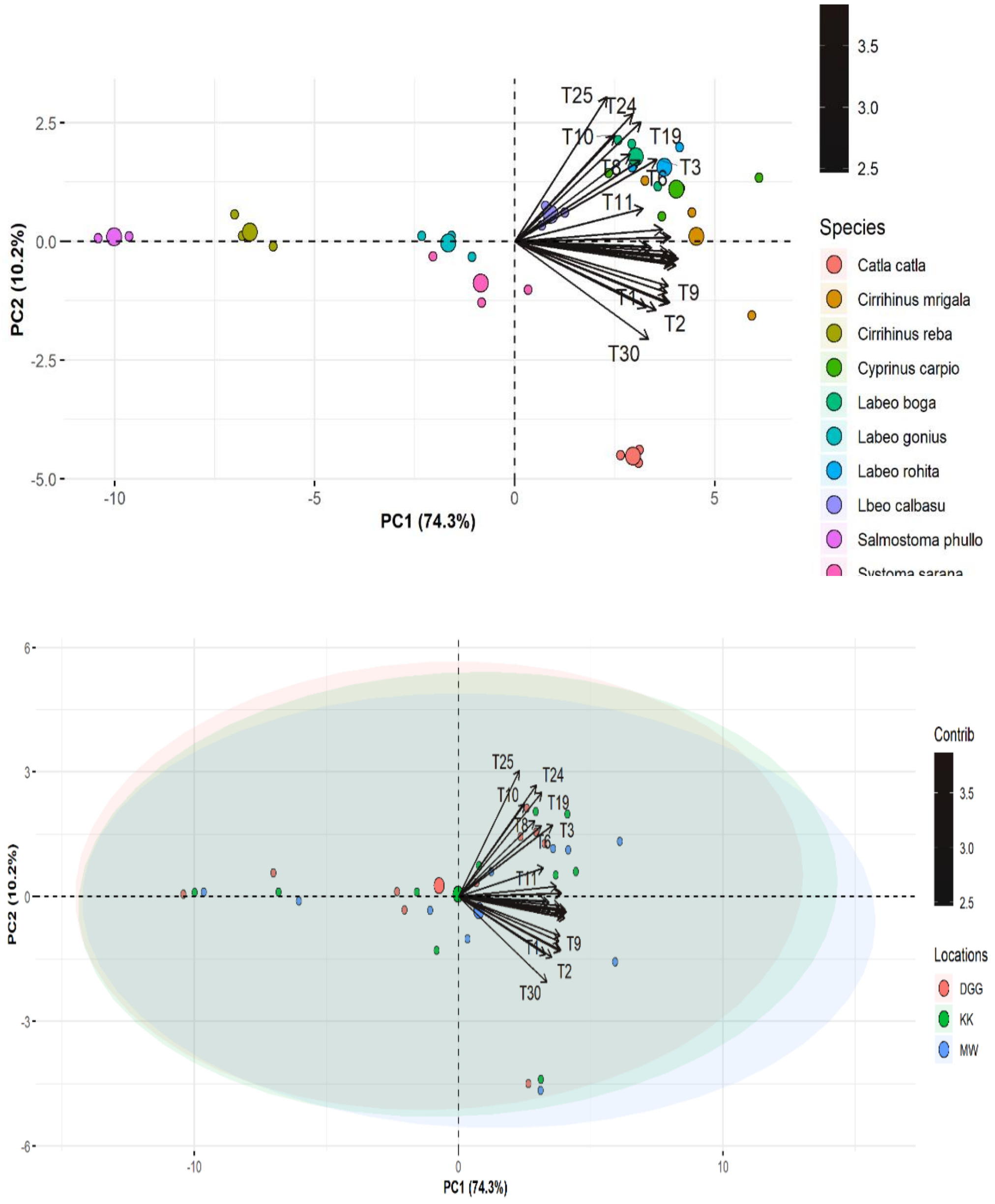

Figure 6: (a,b): Principal component analysis (PCA) biplot of Truss box traits for a) different species of fishes b) collected from different locations of Punjab, Pakistan.

Hierarchical cluster map

Hierarchical cluster analysis of the identified fish species demonstrated ten distinct groups *C. catla*, *L. calbasu*, *L. gonius*, *L. boga*, *L. rohita*, *C. carpio*, *C. mrigala*, *C. reba*, *S. phulo* and *S. sarana* of

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fish assemblages as shown by dendogram in Figure 9. The dendogram was obtained from the average measurement analysis of truss – based morphological characters with ten freshwater fish species taken, showed three major and twenty sub-clusters. Three Populations of S8 and S9 species form one cluster and S4, S5, S6, S7 form second cluster and S1, S2, S3, S10 form third cluster which shows close similarities with each other. Each fish members of assemblage illustrate closer similarities in ecological niche.

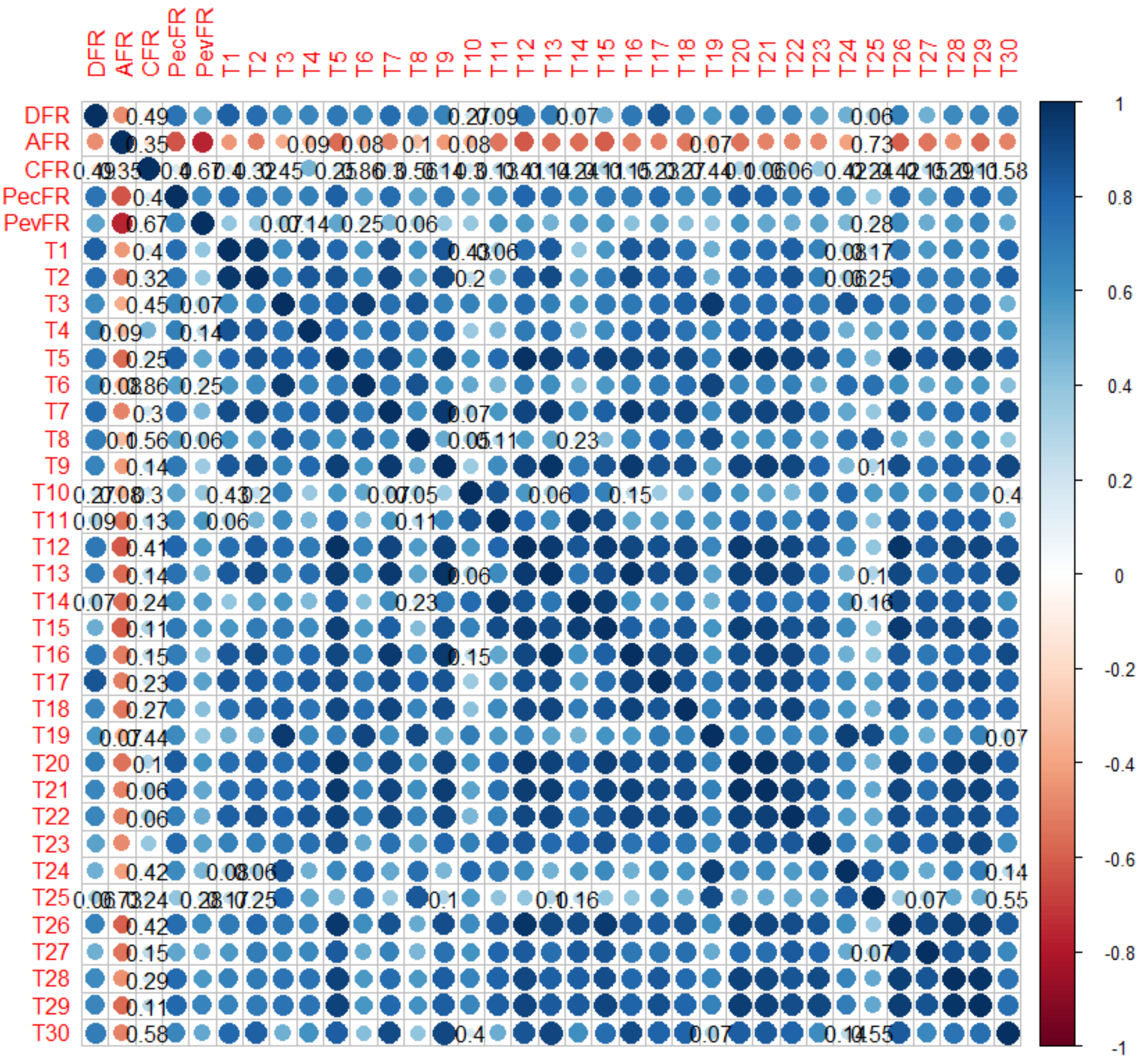

Figure 7: Pearson correlation matric between truss box and meristic characters of different fish species.

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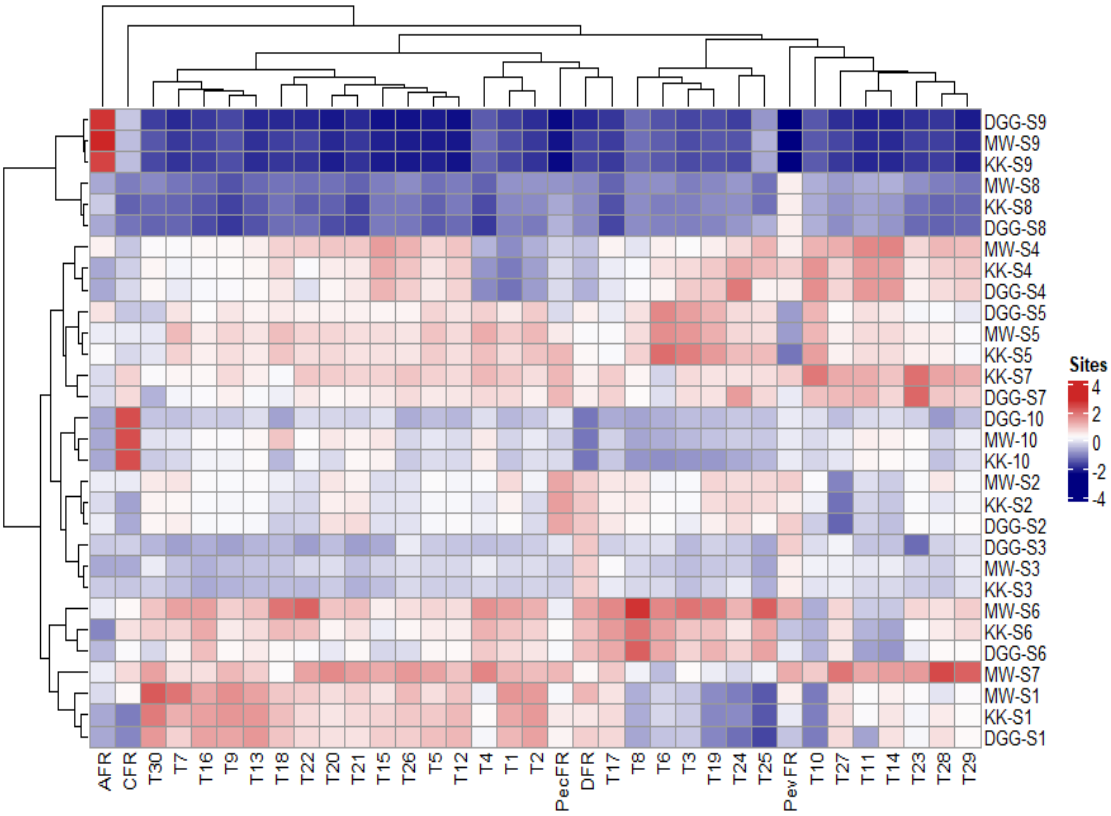

Figure 8: Clustered heatmap among morphological traits, and truss box measurements of fish species of cyprinidae from different locations, showing significant difference.

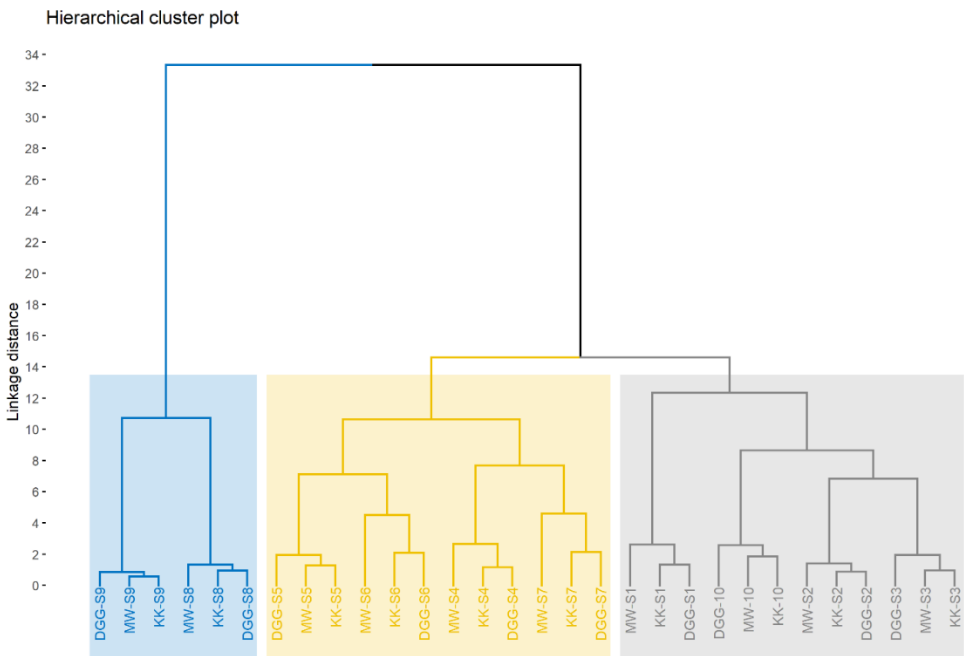

Figure 9: Hierarchical cluster map showing average linkage/grouping between sites and ten fishes of family Cyprinidae (*C. catla* (S1), *L. calbasu* (S2), *L. gonius* (S3), *L. boga* (S4), *L. rohita* (S5), *C. carpio* (S6), *C. mrigala* (S7), *C. reba* (S8), *S. phulo* (S9)and *S. sarana* (S10).

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Discussion

Total 90 fish species were recorded from the three populations of the River Indus Punjab, Pakistan and these belongs to family Cyprinidae. Fishes shows high degree of variations within and among fish population than other vertebrate and are more liable to morphological variations induced by local habitats (Wimberger, 1992; Biswas *et al*., 2018). Such variation particularly occurs due to separation of small portions of a population within habitat may cause notable genetic and phenotypic differentiation among populations within species (Turan *et al*., 2004). The abundance of fishes has been dropped due to alterations in the ecosystem of rivers and overharvesting which is caused by anthropogenic factors. Fish species that are specific and native to a particular region could improve both native species conservation and production (Rehman, 2015). The assessment of fish stock conformation is a useful tool for conserving and managing natural group's population. One of the most essential elements in concluding a valuable interpretation using a multivariate analysis is having a suitable sample size. During analysis to avoid inaccuracy, morphological character as well as truss network measurement along with principle analysis has been performed in this study (Nimalathasan, 2009).

In this study, meristic characters of all fishes were measured that showed some variability among fishes. These characters showed a little significant variation within sites of fishes. Recently a similar variability in meristic counts have been reported in *Alestes baremoze*, *Brycinus nurse*, *Alestes dentex*, and *Brycinus macrolepidotus* from River Nile at Kreima, *Labeo calbasu* from a hatchery and two isolated rivers, the Jamuna and the Halda and *Cirrhinus reba* (Hossain *et al*., 2010; Mohammed *et al*., 2019; Ethin *et al*., 2019). During early development, meristic characters are influenced noticeably by environmental factors especially by temperature. These traits may be size-dependent within and among species. Morphological characters are considered to be vital keys in ichthyology systematic studies. Morphological variations are rare within species but these variations are common in interspecies (Thangaraj *et al*., 2018).

Different morphometric characters were also measured for labeo genus fishes along with five other fish species *C. carpio*, *C. mrigala*, *C. reba*, *S. phulo* and *S. sarana.* Morphometric differences are expected within and among species because fishes can adapt quickly themselves by changing required morphometric due to environmental changes. When the average measurements of *Labeo rohita* from the three population was compared with other four species (Table 3), it was found that *L. rohita* (MW) has highest TL, SL, FL, BD, HL, HD, ED, PrOL, PsOL, SnL, PrDL, PrPecL, PrPevL, PrAL, HDF, LDFB, LAFB and CPD than other four species while *C. catla* (MW) has large average IO, UJL, and LJL but *L. boga* (KK) has maximum average PsDL, HAF and CPL than other species. On the other hand, *Cyprinus carpio* has highest average TL, SL, FL, BD, HL, HD, ED, PrOL, PsOL, SnL, UJL, LJL, PrDL, PrPecL, PrAL, HDF, HAF, LDFB, LAFB, CPD and CPL in MW population as compare to other four

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species in Table 4. Similarly *Systomus sarana* has maximum average IO and *Cirrihinus mrigala* has highest mean PsDL and PrPevL in MW population than other species.

It is quite difficult to explain the reasons of morphological variations among different fish population. It has been recommended that morphological traits of fish species are determined by genetics, environment and interaction between them (Poulet *et al*., 2004; Barman and Sharma, 2017). During the early development stages, environmental factors play a fundamental role when the phenotype of individual is more willing to environment impact are of certain importance (Pinheiro *et al*., 2005). Meanwhile, a fish shows higher plasticity in morphometric parameters to environmental fluctuations (Ethin *et al*., 2019). Some morphological characters showed overlapping between three populations of different fishes which may be due to similar environmental conditions and small geographic distances between these drainages. Several authors determined that water quality parameters and feeding behavior are also responsible for morphological variations in fishes (Keivany *et al*., 2016).

For the better understanding of differentiation of the studied fishes from MW, KK and DGG, hierarchical cluster analysis of truss-based morphological variations (morphometric, meristic counts, and truss-box measurements) were performed to investigate the relationship among different fish population. Cluster analysis showed that MW-S9 and KK-S9 fish population form one cluster whereas DGG-S9 forms another cluster. This result shows that MW-S9 has a greater morphological similarity with KK-S9. Similarly, the other cluster such as MW-S8 population was isolated from other 2 populations (KKS8, DGG-S8) and so on (Fig. 7). Dendrogram form three major cluster and 20 sub-cluster.

The basic aim of hierarchical cluster analysis is to represent the similarity and dissimilarity between sites and species based on the multiple variables associated with them that’s why similar fish species are depicted near from each other and dissimilar are positioned further apart from each other. In addition towards morphometric and meristic measurement, a landmark analysis is another essential criterion to detect and differentiate species, subspecies, strains and have been studied by many authors (Khan *et al*., 2013; Siddik *et al*., 2016; Barman *et al*., 2017; Biswas *et al*., 2018; Ethin *et al*., 2019).

These parameters can also be adjusted by environmental variations during early developmental stages of fish (Wimberger, 1992). The results obtained from the truss-box analysis revealed a significant phenotypic heterogeneity among fish populations. Truss box analysis data indicated a total variability (84.5%) between variables. Principle component analysis was carried out to describe the analysis of the results in more simple way. Correlation between component and variables called loading. This study showed two components with less than 1 eigenvalue. Mir *et al*. (2013) noted similar observation in *L. rohita* from the six drainage system of Ganga basin, where environmental circumstances were found to play a vital role in movement and spatial distribution. Hossain *et al*. (2010) applied PCA and DFA analysis in *L. calbasu* from three populations Jamuna River, Halda River and a hatchery to described the morphological variables among fishes due to the environmental variations and local fish migration.

The figures of PC1 and PC2 scores of each fish species indicated that, among three populations some were showing clearly distinct and others were overlapping. *C. carpio, L. rohita, L. boga* and *L. gonius* were plotted closely in PC2 with higher positive eigenvalues showed overlapping which may be due to similar environmental conditions and slight geographic distances between these drainages. However, species *Cirrihinus reba* and *Salmostoma phullo* revealed limited overlapping with other fishes possibly due to great distances or change environmental conditions. PCA ranked and selected five meristic and 30 truss-network measurements as reliable descriptive of ten fish species (Hossain *et al*., 2010) Stock identification using conventional and truss-based morphological parameters was studies in diversity of species. Ethin *et al*. (2019) applied multivariate discrimination analysis to four populations of *C. reba* from Padma River, Brahmaputra River, Jamuna River and Karatoya River and described landmark-based morphological discrimination due to fish migration and geographical distances.

Genetic differences due to natural selection, population movement, environmental variations and mutation resulting geographical position which may lead to variation in phenotypic characters between stocks. For a specific species, any characteristic can be assumed by its biotic aspects and location (Mahfuj *et al*., 2022). Kumar *et al.* (2010) elucidated some physiological changes that are primary drivers of phenomic differences in horse mackerel, which might due to available food and temperature. Many researchers investigated how conventional and truss-based morphometric traits can be used to differentiate the stocks population conformation studies using PC and CVA (Poulet *et al*., 2005).

Hence, there is also probability that the observed morphological differentiations are due to genetic variations and habitat condition changes among populations. These geographical variations indicate that fish stocks in these three populations might not be are the similar ancestral origin. To differentiate fish stocks, morphometric and meristic investigation can be helpful and are an essential tool for the segregation of fish stock (Palma and Andrade, 2002; Mahfuj *et al*., 2019c). Furthermore, morphometric assessments, joined with image analysis, report a technique for improving the fish stock identifications (Mahfuj *et al*., 2019a, Mahfuj *et al*., 2019b).

During study it was noted that temperature in October and Feburary was warmer than the temperature in November and December. That's why more fish species were reported in the month of October and Feburary than the month of November and December which confirmed more species are found in warmer condition as compared to colder. This study shows that River Indus at Mianwali, Kallur Kot and Dera Ghazi Ghaat mostly contain species in warmer temperature than cold water. The fish diversity at these study

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areas along with their local names are shown in Table 1.

As no study was present on these fishes, especially from these regions of River Indus system, morphometric and meristic traits would be useful in comparing the same and different species in different populations. The results obtained from the present study through landmark-based morphological variations of ten species belong to family cyprinidae from the three different population revealed significant differences among species. Though the River Indus is still conserving a good ichthyodiversity but at the same time human overexploiting activities, pollution, weed infestation and habitat loss would be the major threats as perceived in this study. The current study provided the data and basic information about the variations in fishes of cyprinidae using morphological characters and suggested that morphometric and meristic variations should be measured and considered in its biodiversity configuration.

It should also be used as preliminary step towards the fisheries conservation, management, commercial exploitation and stock improvement program. In order to have improved conservational and management plan and re-stocking methods, more readings are suggested in investigating other possible population structure will be illuminated using biochemical, environmental aspects and molecular techniques.

Novelty

In Pakistan, work has done on growth coefficient, length weight relationship and development of different fish fauna by using different methods. But no work is done on morphology of studied fishes in studied areas. Modern technologies (truss-network analysis) have occasionally been added to traditional tools which prior had never been used during the study of fish diversity in Pakistan.

Conclusion

The current study provided the baseline biological data that would be useful in facilitating the improvement of conserving and management strategies related to the fishery and conservation of studied fish species in designated regions of Indus River system. For better understanding, more research especially on molecular level is needed for conservation and to investigate the impacts of environmental factors.

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