



## 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 characters in *L. calbasu*, 3 characters in *L. gonius*, 6 characters in *L. boga*, 8 characters in *L. rohita*, 11 characters in *C. carpio*, 9 characters in *C. mrigala*, and 7 characters 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 *Systemus 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

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*

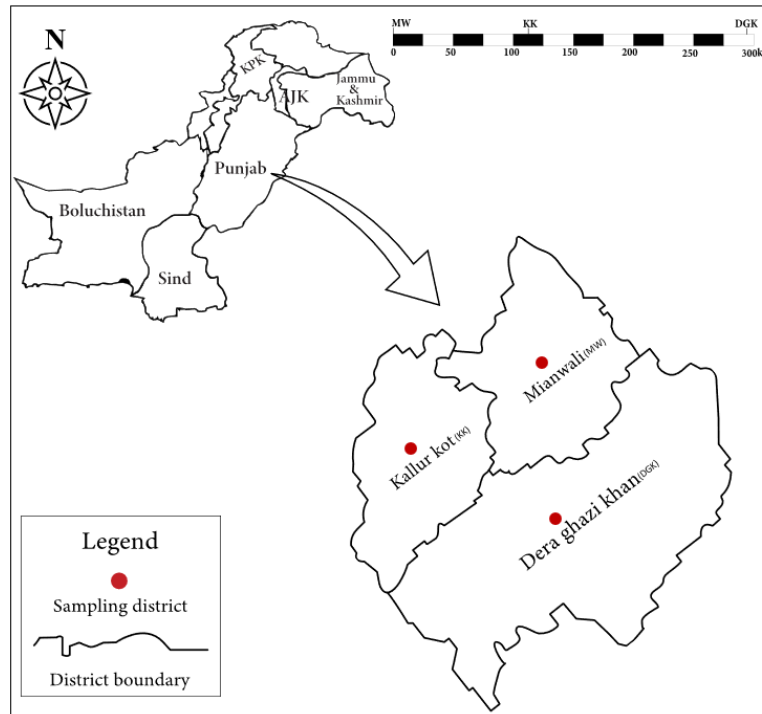
The current study involved sampling from the River Indus of Punjab, Pakistan during October 2020 to February, 2022. In order to compare fish diversity, three locations of the River Indus were selected. Live fish samples were randomly collected from three different regions Mianwali (upstream), kallur 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 February, 2021 with the help of local fisherman. Fresh and

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 | <i>Catla catla</i>        | Thaila                                 | (Muhammad <i>et al.</i> , 2016)                            |
|            | <i>Labeo calbasu</i>      | Calbans, Dahi                          | (Abro <i>et al.</i> , 2023)                                |
|            | <i>Labeo gonius</i>       | Sereha                                 | (Muhammad <i>et al.</i> , 2018)                            |
|            | <i>Labeo boga</i>         | Bhangan                                | (Latif <i>et al.</i> , 2016)                               |
|            | <i>Labeo rohita</i>       | Rahu                                   | (Muhammad <i>et al.</i> , 2018)                            |
|            | <i>Cyprinus carpio</i>    | Gulfam, common carp                    | (Muhammad <i>et al.</i> , 2018)                            |
|            | <i>Cirrihinus mrigala</i> | Mori                                   | (Abro <i>et al.</i> , 2023; Muhammad <i>et al.</i> , 2018) |
|            | <i>Cirrihinus reba</i>    | Suhni, Reba Machali                    | (Abro <i>et al.</i> , 2023)                                |
|            | <i>Salmostoma phulo</i>   | Finescaled Razorbelly Minnow, Fulchela | (Abro <i>et al.</i> , 2023)                                |
|            | <i>Systemus sarana</i>    | Olive barb                             | (Abro <i>et al.</i> , 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

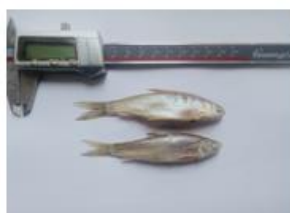
Length, Standard Length, Fork Length, Body Depth, Head Length, Head Depth, Eye Diameter, Pre-Orbital Length, Post-Orbital Length, Snout Length, Inter-Orbital, Upper Jaw Length, Lower Jaw Length, Pre-Dorsal Length, Post-Dorsal Length, Pre-Pectoral Length, Pre-Pelvic Length, Pre-Anal Length, Height of 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.

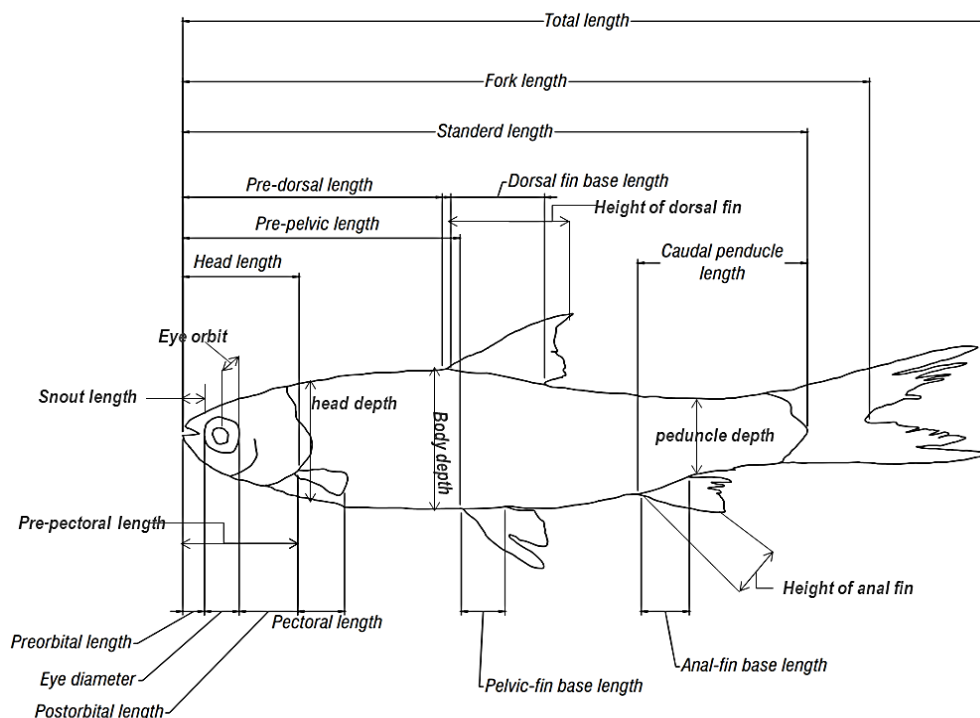
**Table 2: Description of morphometric and meristic measurements used in the study.**

| 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                                  |

**Table 2 (continued):**

| <b>Morphometric Traits</b> | <b>Acronyms</b> | <b>Descriptions</b>                                                                 |
|----------------------------|-----------------|-------------------------------------------------------------------------------------|
| 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                                               |
| <b>Meristic Characters</b> |                 |                                                                                     |
| 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                                            |

*Cirrhinus mrigala**Cirrhinus reba**Catla catla**Cyprinus carpio**Labeo rohita**Labeo gonius**Systomus phulo**Salmostoma sarana**Labeo boga**Labeo calbasu***Figure 2: Different freshwater fish species of the family cyprinidae sampled from the River Indus.**



**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

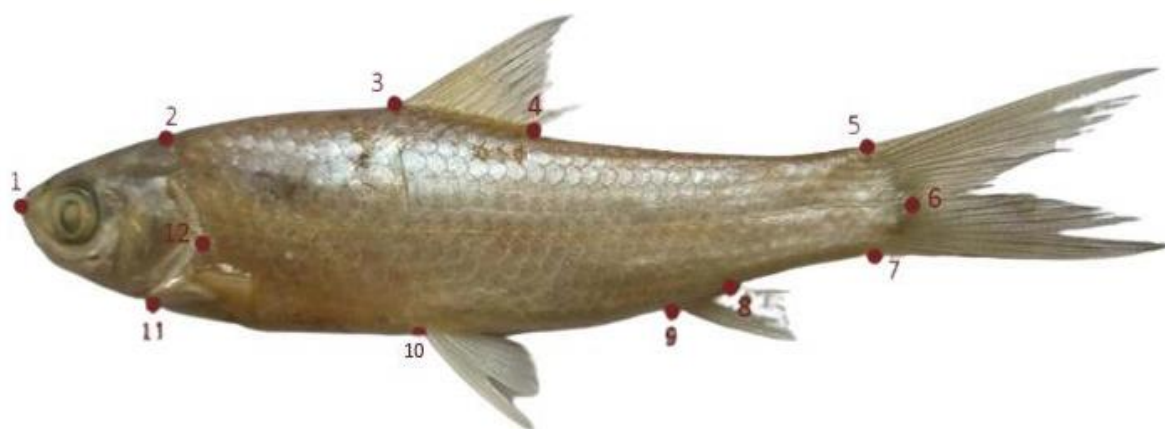
construct a network on the body of fish. Twelve landmarks that determined thirty distances on fish bodies were marked and measured (Fig. 4a, b). Each box was obtained from the distances on the graph-paper which were measured using Vernier calipers.

### Statistical analysis

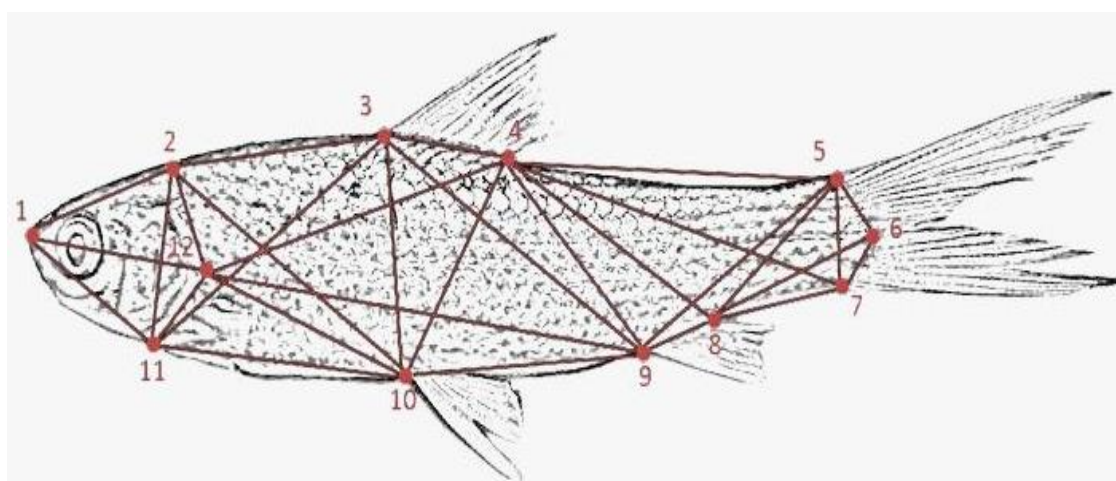
Data visualization and the statistical analysis were implemented by using R statistical software (R studio core team, 2021) by R integrated development environment in R studio team, 2021. Data were subjected to an analysis of variance (ANOVA) and the means values were compared by using tukey pairwise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

( $p \leq 0.05$ ) 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,

Labeo, Cyprinus, Cirrihinus, Salmostoma and Systemus. *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 pectoral 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 *Systemus 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 silvery 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  $137.8 \pm 1.96$  ( $94.97 \pm 214.67$ mm),  $107.8 \pm 2.53$  ( $72.27 \pm 166.67$  mm) and  $118.63 \pm 1.95$  ( $77.97 \pm 186.33$ mm), 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.

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

| Morphometric characters | <i>Catla catla</i> |              |             | <i>Labeo calbasu</i> |              |              | <i>Labeo gonius</i> |              |              |
|-------------------------|--------------------|--------------|-------------|----------------------|--------------|--------------|---------------------|--------------|--------------|
|                         | 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±1.39a        | 11.06±0.50a  | 9.96±0.07a  | 20.52±1.60a          | 19.60±0.25a  | 17.12±0.48a  | 13.02±0.59a         | 11.61±0.78a  | 13.08±0.08a  |
| IO                      | 29.28±0.36a        | 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±0.39a        | 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±0.39a        | 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±1.76a          | 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  |
| <b>Meristic traits</b>  |                    |              |             |                      |              |              |                     |              |              |
| 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 | <i>Labeo boga</i> |              |              | <i>Labeo rohita</i> |              |              |
|-------------------------|-------------------|--------------|--------------|---------------------|--------------|--------------|
|                         | 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  |

**Table 3 (continued):**

| Morphometric characters | <i>Labeo boga</i> |             |             | <i>Labeo rohita</i> |             |             |
|-------------------------|-------------------|-------------|-------------|---------------------|-------------|-------------|
|                         | 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  $188.8 \pm 4.22$  ( $160.67 \pm 223.67$ mm),  $148.21 \pm 3.78$  ( $131.98 \pm 173.67$  mm) and  $166.17 \pm 3.90$  ( $145.51 \pm 195.33$ mm), 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  $162.78 \pm 1.79$  ( $154.33 \pm 171.67$ mm),  $127.22 \pm 1.68$  ( $119.67 \pm 135$ mm) and  $139.78 \pm 1.87$  ( $130.67 \pm 149.67$ mm), 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  $247.56 \pm 2.35$  ( $221.67 \pm 278.33$ mm),  $190.22 \pm 3.39$  ( $169.67 \pm 212.67$ mm),  $211.33 \pm 3.56$  ( $187.67 \pm 238.67$ mm),  $255.33 \pm 4.92$  ( $226 \pm 285.67$ mm),  $197 \pm 4.55$  ( $179 \pm 219.93$ mm) and  $226.52 \pm 5.92$  ( $203 \pm 250.73$ mm), 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

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 *Systemus 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, Cirrihinus, Salmostoma and Systemus genus) collected from diverse ecozones of Punjab, Pakistan.**

| Morphometric characters | <i>Cyprinus carpio</i> |              |              | <i>Cirrihinus mrigala</i> |               |              | <i>Cirrihinus reba</i> |              |              |
|-------------------------|------------------------|--------------|--------------|---------------------------|---------------|--------------|------------------------|--------------|--------------|
|                         | 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         |

**Table 4 (continued):**

| Morphometric characters | <i>Salmostoma phulo</i> |             |             | <i>Systomus sarana</i> |              |              |
|-------------------------|-------------------------|-------------|-------------|------------------------|--------------|--------------|
|                         | 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  |
| <b>Meristic traits</b>  |                         |             |             |                        |              |              |
| 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.*

*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. rohita* 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)

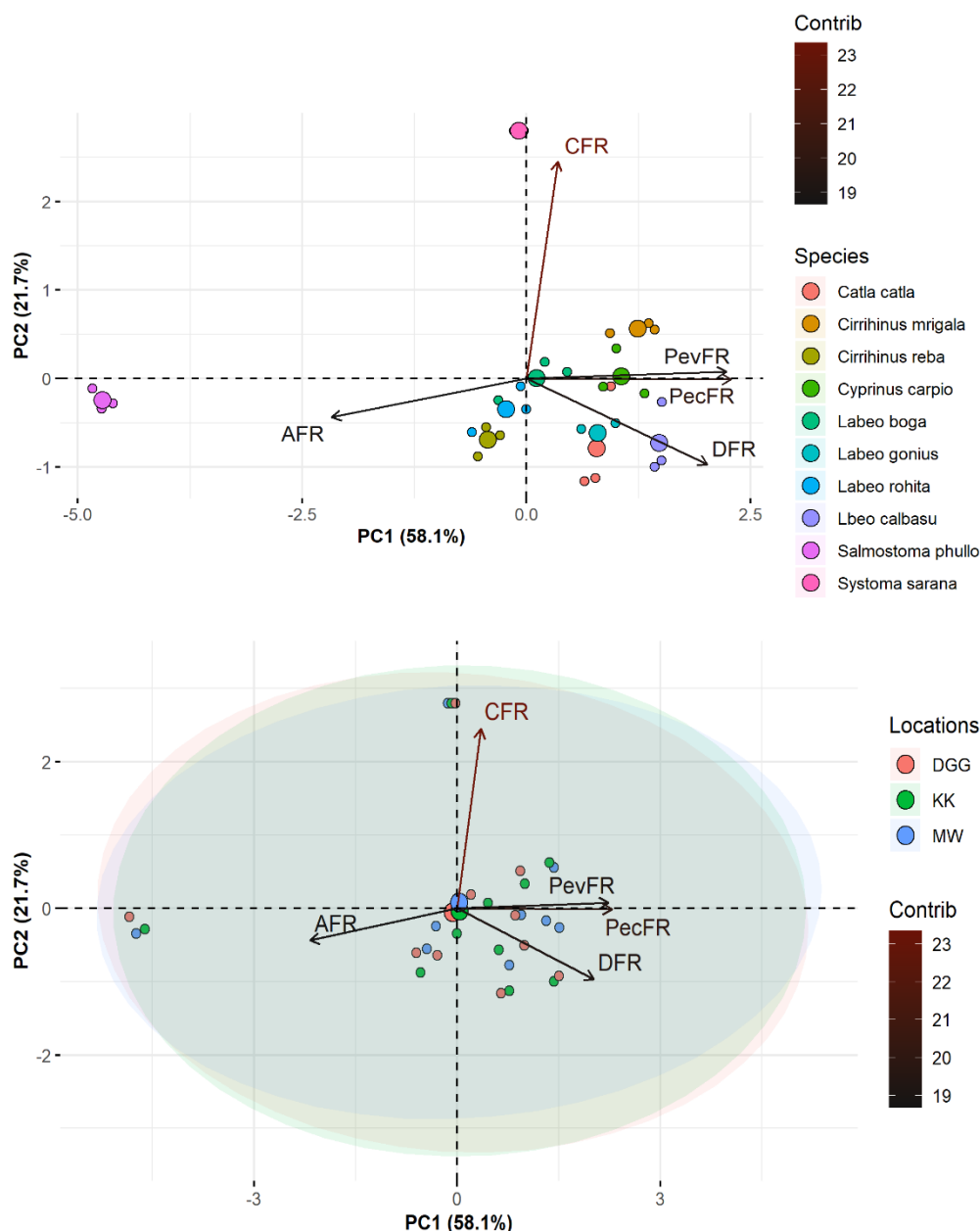
Principal component analysis of meristic characters demonstrated a significant variability between species collected from different sites of Indus River Punjab, Pakistan. Two principal components (PC1, 58.1% and PC2, 21.7%) with a total variance of 79.8% were selected. The PCAs showed a cumulative variability (79.8 %) for fish species. The *Systema 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 ( $p \leq 0.05$ ) with higher positive eigenvalues. However, species *Cirrihinuis 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)

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

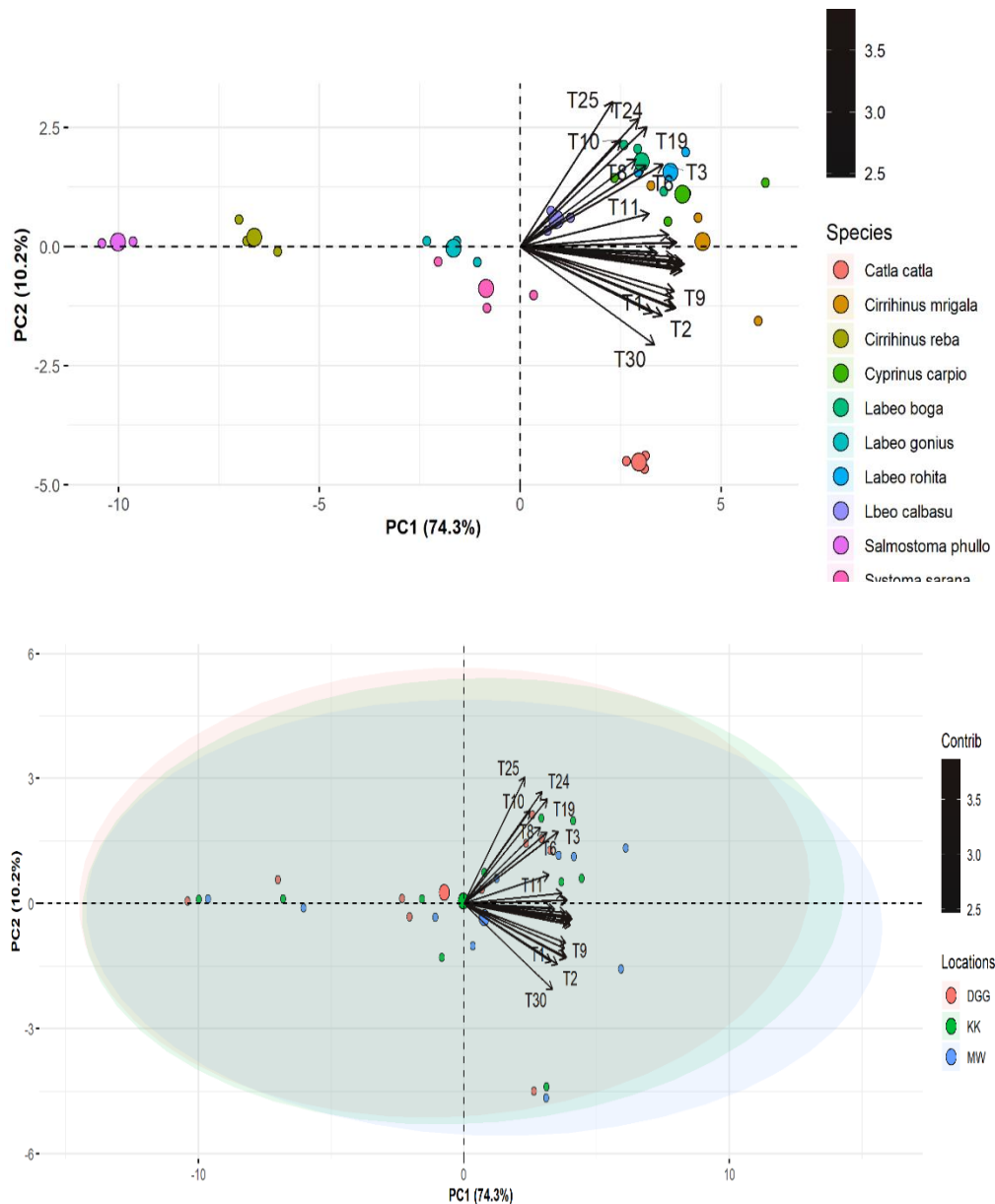
The meristic and truss box characters of different fishes had been shown a significantly ( $p \leq 0.05$ ) strong correlation with each other. The meristic traits such as PecFR and PevFR were positively and

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

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. phullo* and *S. sarana* of

fish assemblages as shown by dendrogram in Figure 9. The dendrogram 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.

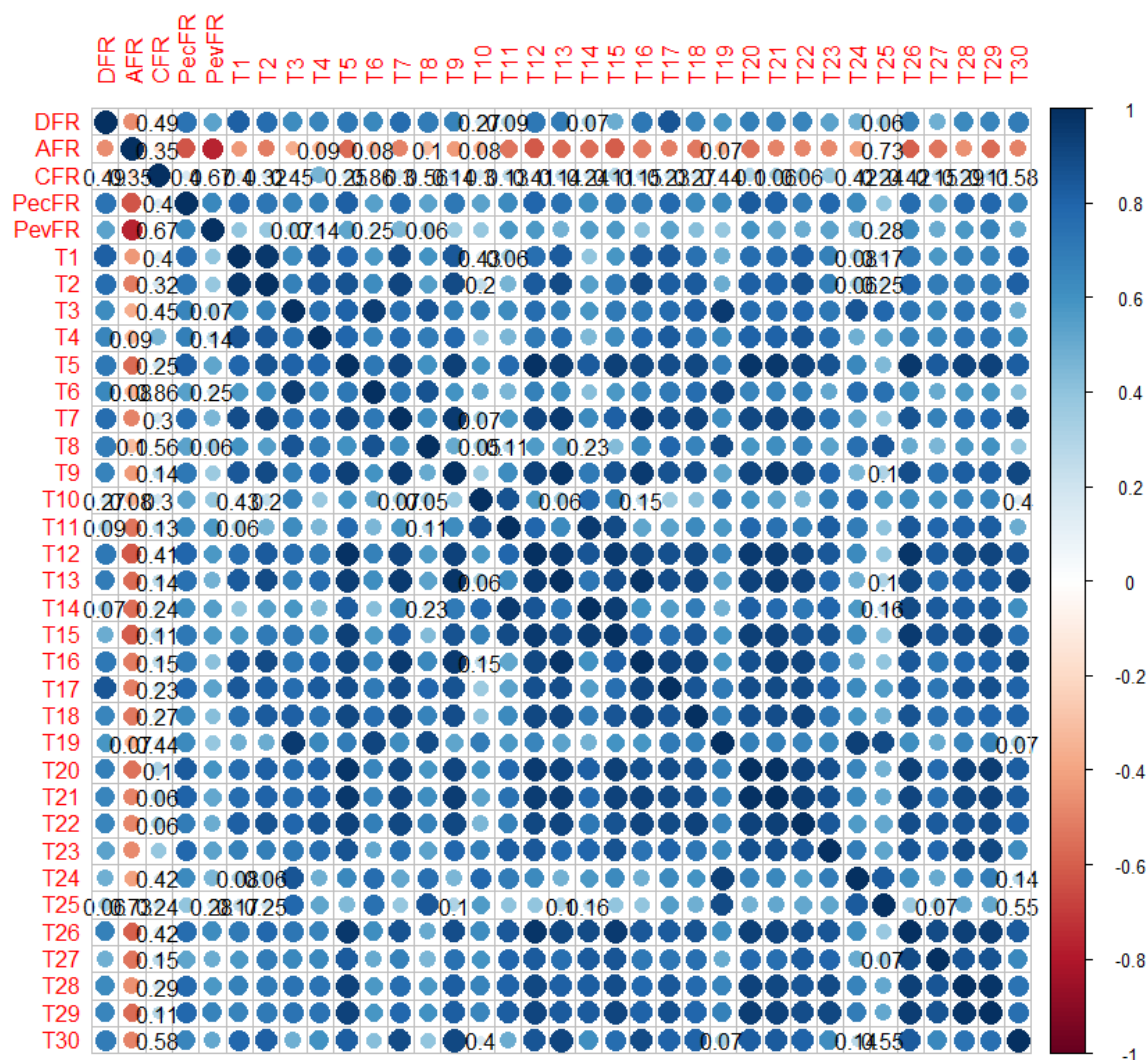
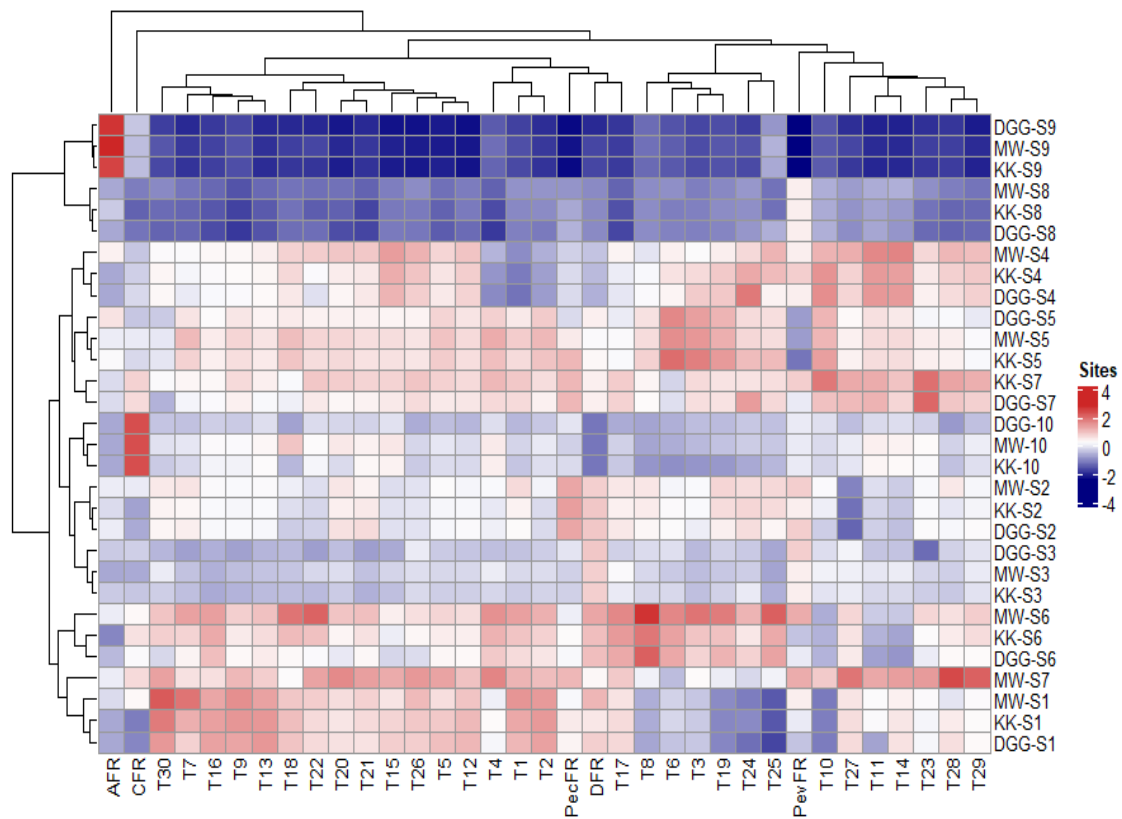
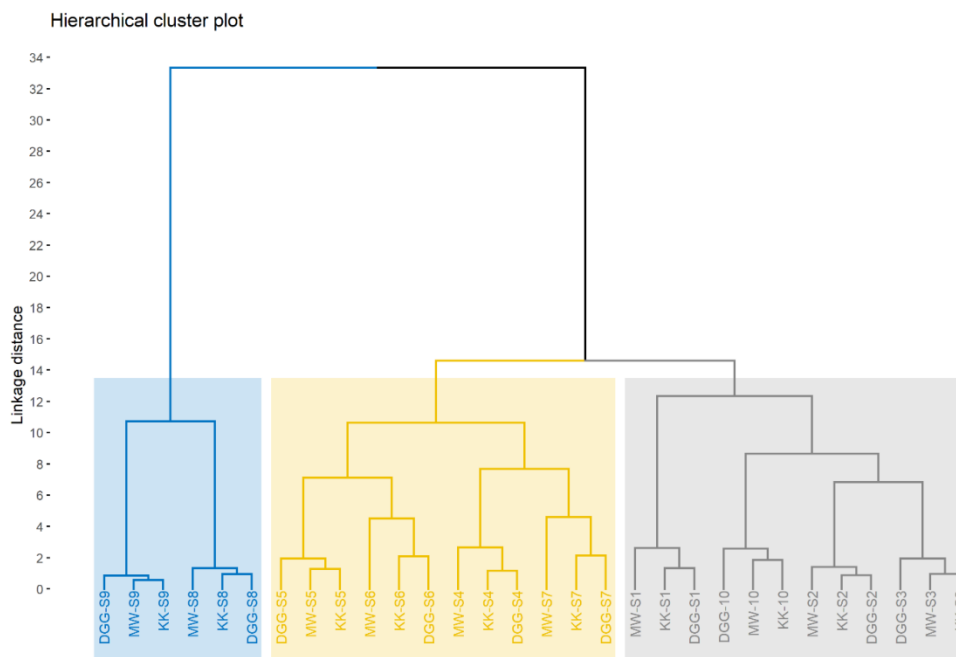


Figure 7: Pearson correlation matrix between truss box and meristic characters of different fish species.



**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)).**

## 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

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 February was warmer than the temperature in November and December. That's why more fish species were reported in the month of October and February 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

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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