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# Genetic diversity of common carp *Cyprinus carpio* in the base population of a selective breeding programme in India

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**Abstract**
A selective breeding program for developing a suitable strain of *Cyprinus carpio* (Linnaeus, 1758) for inland saline aquaculture in India is in progress. At ICAR-CIFE, various geographical populations of common carp of India, viz. Madhya Pradesh (MP), Haryana (HR), Tripura (TR), Andhra Pradesh (AP), Manipur (MN), and Maharashtra (MH) formed the base population for selective breeding. The present study assesses the genetic diversity of these stocks using truss morphometry and mitochondrial DNA D loop marker analysis. The images of 600 fish were captured and digitized. The landmarks were identified, and an image network was constructed for truss analysis using tpsDig2 and PAST software. The data was subjected to scale transformation and factor analysis using SAS for Academics. The top 3 factors could explain 85.40% of the total variation. The results indicate stock-wise and sex-wise groupings. The mitochondrial DNA (D-loop) sequence analysis was conducted on 169 samples using MEGA6 software. The overall average haplotype and nucleotide diversity of the population were 0.08129 and 0.01134, respectively. Among stocks, the MP stock had a maximum of four haplotypes. The AMOVA results reveal that the stock AP is unique, and the other stocks form a single grouping. The information generated from the present study delineates genetic diversity among stocks and will aid in designing breeding plans.

**Keywords** Factor analysis · Haplotype diversity · AMOVA · Morphometry · D loop

## 1 Introduction

*Cyprinus carpio* (Linnaeus, 1758), belonging to the Family-Cyprinidae, Class-Osteichthyes, Order- Cypriniformes, is one of the world's most widely distributed and economically significant freshwater fish. In 2020, worldwide common carp production reached 4236.3 thousand tonnes and ranked fourth in finfish production, contributing 8.6% of total aquaculture production [1]. The fish has been introduced to most continents across fifty-nine countries. The common carp is an extensively translocated species around the world [2–4] including India, where it was introduced in 1959 for aquaculture purposes [5]. Due to its suitability for aquaculture, like eurythermal nature, faster growth, sturdy nature, market demand, etc., it has become a vital candidate species for freshwater aquaculture in the country [6]. It is one of the four fish species commonly farmed in India, singly or combined with the Indian Major carps (IMCs).

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There are three wide varieties of common carp; viz. *Cyprinus carpio communis* (scale carp), *Cyprinus carpio nudus* (leather carp), and *Cyprinus carpio specularis* (mirror carp) [7] cultured in India. The German strain of Mirror carp was first introduced in 1939 from Ceylon. The species was stocked in the Ooty Lake and established well in Nilgiri waters. In 1946, the German strain was introduced in Bhowali hatchery (Uttarakhand) for stocking in the Kumaon lakes. The Bangkok strain of common carp (*Cyprinus carpio communis*) is widely cultured in plains, while mirror carp (*Cyprinus carpio specularis*) is cultured in upland waters of hill states [8]. Further, the Amur strain of common carp was introduced in India [9] is widely propagated. The ICAR-DCFR imported Champa 1 and 2 cold varieties that was propagated in hilly areas of North India [6]. India's present common carp stocks are the intermixes of these few introductions, and majorly, it belongs to scale carp variety. The negative selection, coupled with inbreeding and early-age mating, has deteriorated the productivity of common carp in farms. It is essential to have selectively bred strains of common carp to sustain the common carp industry in India.

Groundwater salinization is a significant challenge globally but it also provides an opportunity to grow freshwater saline-tolerant and euryhaline species [10–12]. The common carp tolerates groundwater salinity up to 12ppt and can withstand severe winter months in North India, however mortality is reported at 15ppt [13, 14]. ICAR-CIFE has initiated a selective breeding program to develop a faster-growing, low saline-tolerant common carp strain for inland saline aquaculture. The various geographical populations of common carp of India viz., Madhya Pradesh (MP), Haryana (HR), Tripura (TR), Andhra Pradesh (AP), Manipur (MN), and Maharashtra (MH) formed the base population in the ongoing selective breeding program.

Geometric Morphometric (GM) is a robust method for studying and interpreting the shape compared to traditional methods of morphometry [15–19]. The GM is effective for solving evolutionary paradigms, individual genera, species, and populations identification, stocks, morphs, and even individuals' discrimination, reported in various fish studies [20, 21]. The mitochondrial D-loop is an exceptionally suitable marker for inter and intra-stock genetic diversity analyses due to its maternal mode of inheritance, a high evolutionary rate, and no recombination [22–24]. The D loop is used to decipher genetic structure [25], genetic differentiation [26], species validation [27], phylogeny [28] etc. The goal of the present study is to evaluate the genetic diversity in the base population using tools of morphometry and mitochondrial D loop marker. Assessing stocks' genetic diversity is imperative to delineate the germplasm genetic architecture, which will be a starting point for the long-term selective breeding program. The outcome will aid in designing appropriate mating plans in the selective breeding program.

## 2 Materials and methods

### 2.1 Sample collection

A total of 600 fish samples (100 from each population) representing various geographical locations viz., Madhya Pradesh, Manipur, Andhra Pradesh, Haryana, Maharashtra, and Tripura were used for the Truss morphometry analysis, and 169 sequences generated after proper quality screening represented the mitochondrial D loop study (Table 1 and Fig. 1). The samples were collected in February 2021 and the fish then had a pond age of 195 days and an actual age of 375 days approximately. The sampled population was cultured in inland saline groundwater-sourced ponds at a stocking density of 5000/ha. They were raised at two different salinities viz., 2–4 ppt and 6–8 ppt. The water depth in ponds was maintained between 1.2 and 1.5 m. They were fed ad libitum with commercially available carp feed twice daily (Crude protein 28% and 4% fat). The temperature range of water during the culture period was 10–32 °C.

### 2.2 Digitization of sample

To digitize the samples, the fish were anesthetized using clove oil and placed over a flat surface with a scale bar adjacent to it. Photographs of each fish were captured using a Nikon D90 with AF-S DX 18-105 mm (f/3.5–5.6G ED VR Lens) in JPEG format with a fixed resolution of 4288 X 2848 pixels. The images were captured and labelled with details, viz., pond number, name of the stock, and sample number for fish identification.

**Table 1** Details of stocks collected from different geographical locations

| States | Location | GPS |
|---|---|---|
| Madhya Pradesh (MP) | Govt. Fish Farm & Hatchery, Bhopal | 23°13′01.8″N 77°22′53.1″E |
| | Aadhya fisheries, Powarkheda | |
| | Vikash fisheries, Powarkheda | |
| Tripura (TR) | Govt. fish farm, Bishalgarh | 23°39′07.2″N 91°18′03.1″E |
| | Amulya Das Fish Hatchery, Agartala | |
| | Chakma fish farm, Dhalai | |
| Maharashtra(MH) | Goregaon Fish Farm and Hatchery (Pvt.) | 19°09′41.2″N 72°52′20.6″E |
| | Mass spawning of brooders collected from Nashik, Nagpur and Raigad | |
| Haryana (HR) | CIFE-Rohtak centre | 28°51′42.5″N 76°28′29.1″E |
| | Mass spawning of brooders collected from Rohtak, Hisar, Bhiwani and Sonipat | |
| Andhra Pradesh (AP) | Mass spawning of brooders collected from different locations of the state | 16°57′21.2″N 82°00′24.9″E |
| Manipur (MN) | Imoinu Fish Farm, Thoubal | 24°38′33.1″N 93°53′45.3″E |
| | Tomba & Sons Fish Farm, Hiyangthang | |
| | Eengaal Aqua, Lamphel | |
| | Baru Fish Farm, Wangjing | |

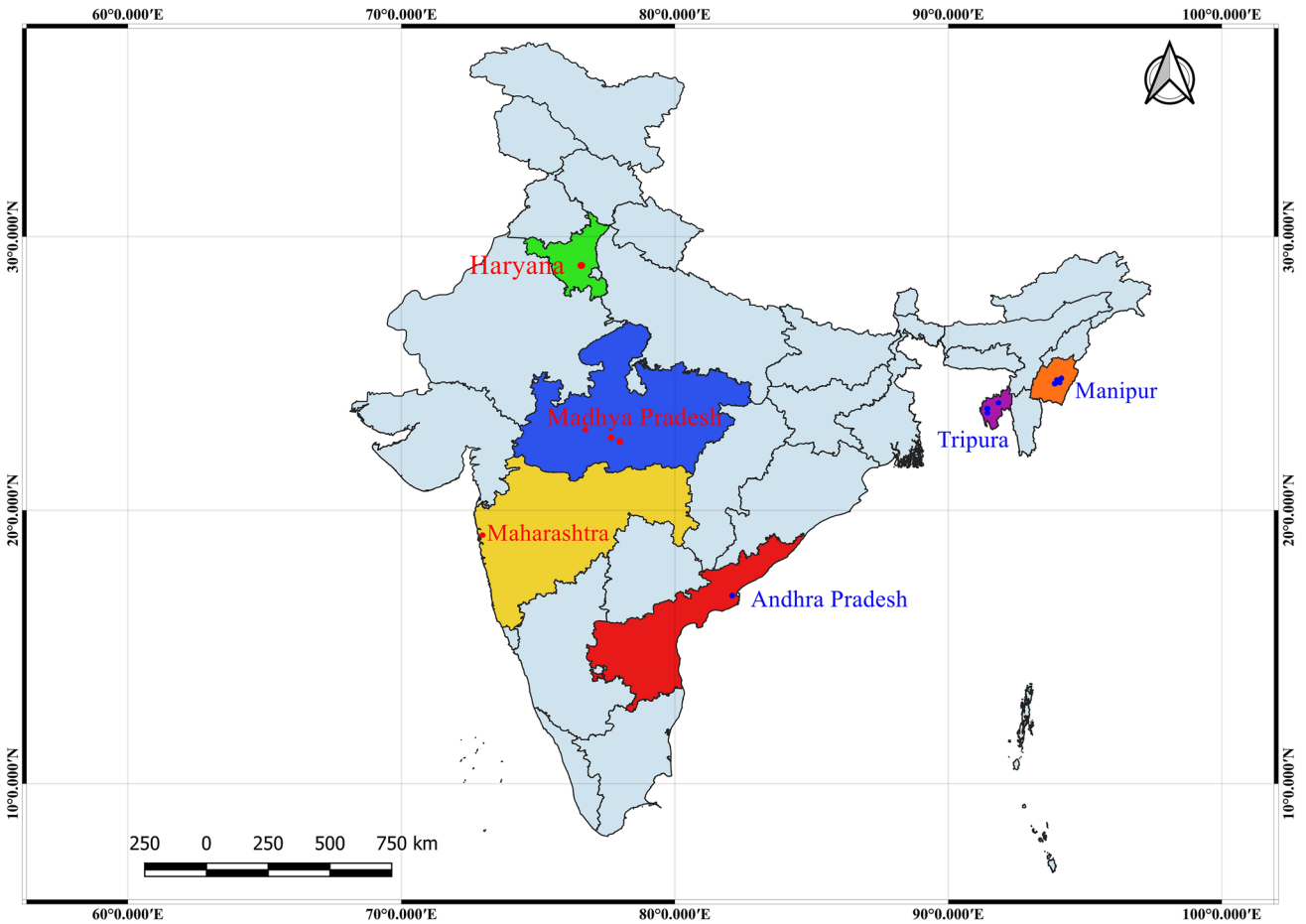


**Fig. 1** Stock Collection form Various Geographical Locations of India

### 2.3 Data retrieval

A linear combination of two software, tpsDig2 V2.1 and Paleontological Statistics (PAST), was used to extract truss distances from the digital images. The 15 landmarks were interconnected to form 37 truss points, thus forming a truss network (Fig. 2 and Table 2).

### 2.4 Statistical analysis

The statistical analysis was performed using SAS for Academics. The descriptive statistics, viz. minimum value and maximum value, mean, standard error, and coefficient of variance were estimated using the PROC MEANS procedure. The data was tested for normality by PROC UNIVARIATE, and box plots were generated using the PROC SGPLOT procedure. A linear model was fitted to estimate the least squares means and effects of stock, sex, and stock-by-sex interaction on various morphometric traits invoking PROC GLM procedure.

The truss measurements were log-transformed, and the outliers were deleted. The correlation coefficient 'r' was estimated by invoking the PROC CORR procedure. The allometric approach removed the size-dependent variation [29]. The transformation removed the effects of body length successfully.

$$M_{trans} = \log M - \beta(\log SL - \log SL_{mean}) \quad (1)$$

where $M_{trans}$ is the final transformed measurement, log $M$ is the natural log transform of the original measurement, $\beta$ is the within-group slope regressions of the log $M$ vs log SL, SL is the standard length of the fish, and $SL_{mean}$ is the stock-wise mean of the standard length.

The FACTOR analysis was performed on 39 truss measurements using the PROC FACTOR procedure of SAS. The factors were extracted using the Maximum likelihood method. The factors were retained for the rotation procedure based on meaningful biological groupings. The only retained factors were subjected to a rotation procedure using the Varimax (orthogonal) rotation and scratching procedure [30]. Further, to determine the classification and error rate, the discriminant analysis was conducted. The number of observations and percent classified stock-wise and sex-wise were done using the generalized squared distance function of the PROC DISCRIM procedure of SAS.

### 2.5 Genomic DNA extraction

About 169 fin clip specimens representing the six geographical populations of common carp were collected and preserved in absolute alcohol and further subjected to genomic DNA isolation using the standard phenol–chloroform method [31]. The integrity of the extracted DNA was evaluated by 0.8% agarose gel electrophoresis.

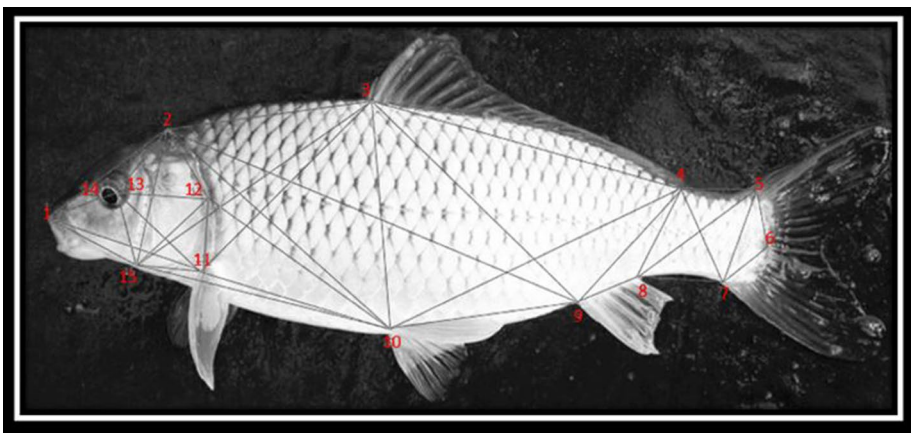


**Fig. 2** Landmark points on *Cyprinus carpio* used for Truss Morphometry

**Table 2** Truss distances obtained from the selected 15 anatomical landmarks

| Truss Landmarks | Traits | Definition |
|---|---|---|
| 1 to 2 | SOL | Anterior tip of the snout on upper jaw to Nape above insertion of opercle |
| 1 to 11 | SPTL | Anterior tip of the snout on upper jaw to Origin of pectoral fin |
| 1 to 14 | SPEL | Anterior tip of the snout on upper jaw to Posterior orbit of eye |
| 1 to 15 | SVOL | Anterior tip of the snout on upper jaw to Ventral insertion of the opercle |
| 2 to 3 | ODL | Nape above insertion of opercle to Origin of the dorsal fin |
| 2 to 9 | OAW | Nape above insertion of opercle to Origin of anal fin |
| 2 to 10 | OPW | Nape above insertion of opercle to Origin of pelvic fin |
| 2 to 12 | OOEW | Nape above insertion of opercle to Opercle end |
| 2 to 13 | OAEW | Nape above insertion of opercle to Anterior orbit of eye |
| 2 to 15 | OVOW | Nape above insertion of opercle to Ventral insertion of the opercle |
| 3 to 4 | DEDL | Origin of the dorsal fin to End of dorsal fin base |
| 3 to 9 | DAW | Origin of the dorsal fin to Origin of anal fin |
| 3 to 10 | DPW | Origin of the dorsal fin to Origin of pelvic fin |
| 3 to 11 | DPTW | Origin of the dorsal fin to Origin of pectoral fin |
| 3 to 15 | DVOW | Origin of the dorsal fin to Ventral insertion of the opercle |
| 4 to 5 | EDCL | End of dorsal fin base to Dorsal origin of caudal fin |
| 4 to 7 | EDVCW | End of dorsal fin base to Ventral origin of caudal fin |
| 4 to 8 | EDEAW | End of dorsal fin base to End of anal fin base |
| 4 to 9 | EDAW | End of dorsal fin base to Origin of anal fin |
| 4 to 10 | EDPW | End of dorsal fin base to Origin of pelvic fin |
| 5 to 6 | DCVCW | Dorsal origin of caudal fin to Posterior end of vertebral column |
| 5 to 7 | DCVCPW | Dorsal origin of caudal fin to Ventral origin of caudal fin |
| 5 to 8 | DCEAW | Dorsal origin of caudal fin to End of anal fin base |
| 6 to 7 | VCVCL | Posterior end of vertebral column to Ventral origin of caudal fin |
| 7 to 8 | VCEAL | Ventral origin of caudal fin to End of anal fin base |
| 8 to 9 | EAAL | End of anal fin base to Origin of anal fin |
| 9 to 10 | APL | Origin of anal fin to Origin of pelvic fin |
| 10 to 11 | PPTL | Origin of pelvic fin to Origin of pectoral fin |
| 10 to 12 | POEW | Origin of pelvic fin to Opercle end |
| 10 to 15 | PVOL | Origin of pelvic fin to Ventral insertion of the opercle |
| 11 to 12 | PTOEW | Origin of pectoral fin to Opercle end |
| 11 to 13 | PTAEW | Origin of pectoral fin to Anterior orbit of eye |
| 11 to 15 | PTVOL | Origin of pectoral fin to Ventral insertion of the opercle |
| 12 to 13 | OAEL | Opercle end to Anterior orbit of eye |
| 12 to 15 | OEVOW | Opercle end to Ventral insertion of the opercle |
| 13 to 14 | AEPEL | Anterior orbit of eye to Posterior orbit of eye |
| 13 to15 | AEVOW | Anterior orbit of eye to Ventral insertion of the opercle |

## 2.6 PCR amplification of Dloop and sequencing

The primer combination of 5′AACTCTCACCCCTGGCTACCAAAG3′ (forward) and 5′CTAGGACTCATCTTAGCATCTTCA GTG3′ (reverse) were employed to amplify the desired D loop fragment of 1 Kb using 50 µL reaction volume that consisted of 200 ng template DNA, 10 pmol of each primer, 200 µM of each dNTP, one units of Taq DNA polymerase and 10 × Taq buffer with 1.5 mM $MgCl_2$. The reaction mixture was added to a heated lid thermocycler in 0.2 mL PCR tubes (BioRad, USA). The PCR program consisted of initial denaturation at 95 °C for 3 min and 35 cycles of denaturation at 95 °C for 30 s, annealing temperatures at 59 °C for 30 s, extension for 1.2 min at 72 °C and final extension was set at 72 °C for 8 min. The PCR-amplified products were purified with a gel extraction kit (Qiagen, Germany), and the purified products were sequenced using the Sanger sequencing method.

The alignment of amplified mitochondrial D loop sequences and the sequence composition was done using Clustal Omega [32] and MEGA X [33], respectively. The DnaSP v5 [34] was used to estimate the Haplotype (Hd) and nucleotide (p) diversity values. The Arlequin V3 [35] was used to estimate the genetic differentiation between each population ($F_{ST}$). The genetic variability among and within the population was determined by the Analysis of Molecular variance (AMOVA) technique [36]. The Network version 5.0 (Fluxus-engineering.com, [37]) was used to construct a haplotype network following the median-joining method using. The DnaSp v5 was used to estimate Tajima's D value to assess the genetic equilibrium of populations [38, 39]. The phylogenetics analysis was performed using MEGA X. A phylogenetic tree was constructed using Neighbour Joining method [33].

## 3 Results

### 3.1 Descriptive statistics

All the measured fish were sexually mature and exhibited secondary sexual characteristics viz roughness in lateral body, ooze of milt in males, and softening and rounding as well as reddening of protrusion of anal papilla and vent in females. About 39 morphometric measurements (truss distances) were retrieved from 600 fish belonging to six geographical stocks. The data represented 15 landmarks (39 traits) of common carp comprising three major regions: head, body curvature, and caudal region. The descriptive statistics for all the truss distances, standard length (SL), and body weight (BW) is provided in Table 3. The overall average body weight (BW) and standard length (SL) of fish were 163.68 ± 1.89 g and 17.07 ± 0.07 cm, respectively. The body weight showed the highest CV of 28.16 compared to the standard length. The traits PTVOL, EDCL, VCEAL, EAAL, AEVOW, and BW exhibited the highest CV of 38.43, 30.07, 22.82, 23.45, 21.28, and 28.16 respectively, compared to the other traits. The lowest CV was observed for DVOW, DAW, DPTW, and SOL traits.

### 3.2 Least squares means

The least squares mean and standard errors (stock and sex-wise) for all the morphometric measurements are provided in (Tables 4, 5, 6, 7, 8, 9, 10). The female fish had a significantly higher body weight of 170.80 g ± 2.25 compared to male fish. There was no significant difference in standard length between the sexes. Out of the 39 traits examined and analyzed, the traits viz SL, OAW, DAW, DPTW, ODL, DVOW, DPW, and PTOEW significantly differed between the stocks. The traits OAW (Nape above insertion of opercle to Origin of anal fin), DAW (Origin of the dorsal fin to Origin of anal fin), DPTW (Origin of the dorsal fin to Origin of pectoral fin), ODL (Nape above insertion of opercle to Origin of the dorsal fin), and DVOW (Origin of the dorsal fin to Ventral insertion of the opercle) were found to be significantly higher in Haryana stock compared to all the other stocks with a mean value of, 11.57 ± 0.06, 7.96 ± 0.04, 6.58 ± 0.03, 4.98 ± 0.03, 7.74 ± 0.04 cm respectively.

The trait SL (Standard length) in Tripura stock was found to be significantly higher compared to all the other stocks, with a mean value of 17.59 ± 0.143 cm, while the trait DPW (Origin of the dorsal fin to Origin of pelvic fin) was found to be significantly lower in Tripura stock when compared with the rest of the stock with a mean value of 6.20 ± 0.03 cm. Sex-wise, significant differences were observed between male and female fish. Out of the 39 traits, the traits viz DAW, DCVCW, DCVCPW, VCVCL, EDAW and EAAL were found to be significantly different between the sex, and males exhibited the highest value compared to female fish.

The effect of various factors on the morphometric distances is provided in Tables 11, 12, 13, 14. The effect of SL, BW, Stock, Sex, and stock-sex interaction was estimated. The effect of Standard length was significant on the traits viz ODL and EAAL. The body weight significantly affected all the traits except trait EDCL. The stock had a significant effect on all the traits except the trait EDCL. The sex had a significant effect on trait EAAL.

### 3.3 Factor analysis

The factor analysis (overall) revealed that the first three factors explained 85.40% of the total morphometric variation, with eigen values of 64.14, 13.11 and 8.89, respectively (Table 15). The variables OAW, DAW, DPW, DPTW, EDPW, DCVCW, DCVCPW, VCVCL, OAEW, DEDL, and OAEL had the highest loading on factor-1(Table 15). The variables SVOL, AEVOW, PTVOL, OEVOW, and AEPEL were highest loaded on the second factor, and the variables EDCL, EDVCW, and APL highest loaded on the third factor occurred with variables (Table 15). The three factors are concentrated in the middle part of the body (Fig. 3), the head region (Fig. 4), and the caudal region (Fig. 5). This relationship is as expected as the trait variables

**Table 3** Descriptive statistics of truss measurements-overall

| Traits | N | Min | Max | Mean ± SE | CV |
|---|---|---|---|---|---|
| SL | 594 | 9.94 | 21.79 | 17.07 ± 0.07 | 9.79 |
| BW | 594 | 15.50 | 312.00 | 163.68 ± 1.89 | 28.16 |
| SOL | 594 | 2.73 | 5.56 | 4.30 ± 0.02 | 10.59 |
| SPTL | 594 | 2.74 | 5.89 | 4.26 ± 0.02 | 11.81 |
| SPEL | 594 | 0.81 | 2.62 | 1.40 ± 0.01 | 17.14 |
| SVOL | 594 | 2.17 | 14.14 | 3.41 ± 0.03 | 18.25 |
| ODL | 594 | 2.64 | 6.41 | 4.63 ± 0.02 | 12.45 |
| OAW | 594 | 6.07 | 14.75 | 10.89 ± 0.05 | 10.71 |
| OPW | 594 | 3.40 | 9.98 | 7.61 ± 0.03 | 10.71 |
| OOEW | 594 | 1.10 | 2.61 | 1.90 ± 0.01 | 12.26 |
| OAEW | 594 | 1.50 | 3.16 | 2.25 ± 0.01 | 12.25 |
| OVOW | 594 | 2.47 | 11.51 | 4.11 ± 0.02 | 12.67 |
| DEDL | 594 | 2.74 | 10.03 | 6.85 ± 0.04 | 13.76 |
| DAW | 594 | 4.33 | 10.38 | 7.46 ± 0.03 | 10.72 |
| DPW | 594 | 3.40 | 8.72 | 6.56 ± 0.03 | 10.99 |
| DPTW | 594 | 3.42 | 8.43 | 6.20 ± 0.03 | 10.73 |
| DVOW | 594 | 4.06 | 9.70 | 7.21 ± 0.03 | 10.45 |
| EDCL | 594 | 0.84 | 6.94 | 2.05 ± 0.03 | 30.07 |
| EDVCW | 594 | 1.86 | 7.61 | 3.42 ± 0.02 | 16.59 |
| EDEAW | 594 | 1.51 | 5.62 | 2.81 ± 0.02 | 14.77 |
| EDAW | 594 | 2.09 | 7.86 | 3.94 ± 0.02 | 12.35 |
| EDPW | 594 | 4.19 | 11.34 | 8.17 ± 0.04 | 11.34 |
| DCVCW | 594 | 0.76 | 2.23 | 1.46 ± 0.01 | 13.84 |
| DCVCPW | 594 | 1.18 | 3.83 | 2.67 ± 0.01 | 11.99 |
| DCEAW | 594 | 1.95 | 4.58 | 3.28 ± 0.02 | 13.72 |
| VCVCL | 594 | 0.76 | 2.30 | 1.51 ± 0.01 | 14.34 |
| VCEAL | 594 | 0.80 | 3.13 | 1.87 ± 0.02 | 22.82 |
| EAAL | 594 | 0.76 | 5.34 | 1.65 ± 0.02 | 23.45 |
| APL | 594 | 2.67 | 7.55 | 5.27 ± 0.03 | 12.41 |
| PPTL | 594 | 1.81 | 7.13 | 5.13 ± 0.03 | 12.50 |
| POEW | 594 | 2.28 | 8.15 | 5.94 ± 0.03 | 11.96 |
| PVOL | 594 | 2.92 | 11.00 | 6.08 ± 0.03 | 12.07 |
| PTOEW | 594 | 1.18 | 2.95 | 1.93 ± 0.01 | 14.45 |
| PTAEW | 594 | 1.62 | 3.86 | 2.77 ± 0.01 | 11.72 |
| PTVOL | 594 | 0.58 | 10.77 | 1.17 ± 0.02 | 38.43 |
| OAEL | 594 | 0.82 | 2.92 | 2.09 ± 0.01 | 11.92 |
| OEVOW | 594 | 1.52 | 12.35 | 2.62 ± 0.02 | 19.64 |
| AEPEL | 594 | 0.52 | 2.35 | 0.88 ± 0.01 | 14.78 |
| AEVOW | 594 | 1.31 | 13.18 | 2.51 ± 0.02 | 21.28 |

loading on the first factor concern the middle portion of fish, and these traits grow proportionately with each other. The relationships are represented further using bivariate plots, and the plots of factor-1, factor-2, and factor-3 revealed the separation of stocks (Figs. 6 and 7).

The first three factors explained 85.86% of the total morphometric variation for the male sex with eigen-values of 72.83, 24.19, and 9.43, respectively (Table 16). The variables ODL, OAW, DEDL, DAW, DPW, DPTW, DCVCW, DCVCPW, and VCVCL had the highest loading on factor-1 (Table 16). The variables SVOL, OVOW, PTVOL, OEVOW, AEPEL, and AEVOW are loaded on the second factor, and the variables EDCL, EDVCW, and APL loaded on the third factor (Table 16). The bivariate plots are further provided for depicting the separation of stocks. The bivariate plots between AP and TR, AP and HR, and AP and MP provided in Fig. 8, confirm the separation of AP stock from the rest of the other stocks. The first three factors for the female sex together explained 87.41% of the total morphometric variation with eigenvalues of 62.09, 17.38, and

**Table 4** Least squares means and standard errors of Body weight and Standard length

| Source | N = 599 | Body weight (g) | Standard length (cm) |
|---|---|---|---|
| Stock | AP | $160.72^{abc} \pm 4.10$ | $17.090^{ab} \pm 0.143$ |
| | HR | $145.42^{c} \pm 4.09$ | $17.48^{a} \pm 0.145$ |
| | MH | $183.14^{a} \pm 4.10$ | $16.55^{b} \pm 0.144$ |
| | MN | $171.67^{ab} \pm 4.11$ | $16.90^{ab} \pm 0.144$ |
| | MP | $176.33^{a} \pm 4.10$ | $17.01^{ab} \pm 0.144$ |
| | TR | $152.44^{bc} \pm 4.11$ | $17.59^{a} \pm 0.143$ |
| Sex | Female | $170.80^{a} \pm 2.25$ | $17.049^{a} \pm 0.079$ |
| | Male | $159.10^{b} \pm 2.50$ | $17.16^{a} \pm 0.088$ |

Means within each group of sources of variation viz stocks (AP, HR, MH, MN, MP and TR) and sex (Female and male) in the same column having different superscripts vary significantly from each other within the group ($p < 0.0001$). 'N' is number of observations

**Table 5** Least squares means and standard errors of various truss measurements

| Source | N = 599 | OAW | DAW | DPW | DPTW | EDPW | DCVCW | DCVCPW | VCVCL |
|---|---|---|---|---|---|---|---|---|---|
| Stock | AP | $10.68^{bc} \pm 0.06$ | $7.41^{bc} \pm 0.04$ | $6.49^{c} \pm 0.03$ | $6.13^{bcd} \pm 0.03$ | $8.25^{ab} \pm 0.05$ | $1.38^{c} \pm 0.01$ | $2.65^{b} \pm 0.02$ | $1.61^{ab} \pm 0.14$ |
| | HR | $11.57^{a} \pm 0.06$ | $7.96^{a} \pm 0.04$ | $6.85^{a} \pm 0.03$ | $6.58^{a} \pm 0.03$ | $8.48^{a} \pm 0.05$ | $1.53^{a} \pm 0.01$ | $2.79^{a} \pm 0.02$ | $1.63^{a} \pm 0.15$ |
| | MH | $10.57^{c} \pm 0.06$ | $7.23^{c} \pm 0.04$ | $6.59^{bc} \pm 0.03$ | $5.96^{d} \pm 0.03$ | $8.05^{bc} \pm 0.05$ | $1.43^{bc} \pm 0.01$ | $2.63^{b} \pm 0.02$ | $1.49^{cd} \pm 0.15$ |
| | MN | $10.90^{bc} \pm 0.06$ | $7.46^{bc} \pm 0.04$ | $6.62^{bc} \pm 0.03$ | $6.23^{bc} \pm 0.03$ | $8.09^{bc} \pm 0.05$ | $1.46^{ab} \pm 0.01$ | $2.61^{b} \pm 0.02$ | $1.41^{e} \pm 0.14$ |
| | MP | $10.98^{b} \pm 0.06$ | $7.57^{b} \pm 0.04$ | $6.72^{ab} \pm 0.03$ | $6.30^{b} \pm 0.03$ | $8.44^{a} \pm 0.05$ | $1.55^{a} \pm 0.01$ | $2.77^{a} \pm 0.02$ | $1.54^{bc} \pm 0.15$ |
| | TR | $10.77^{bc} \pm 0.06$ | $7.25b^{c} \pm 0.04$ | $6.20^{d} \pm 0.03$ | $6.09^{cd} \pm 0.03$ | $7.83^{c} \pm 0.05$ | $1.43^{bc} \pm 0.01$ | $2.58^{b} \pm 0.02$ | $1.42^{de} \pm 0.15$ |
| Sex | Female | $10.90^{a} \pm 0.03$ | $7.41^{a} \pm 0.02$ | $6.56^{a} \pm 0.01$ | $6.19^{a} \pm 0.01$ | $8.16^{a} \pm 0.02$ | $1.44^{a} \pm 0.008$ | $2.63^{a} \pm 0.01$ | $1.48^{a} \pm 0.008$ |
| | Male | $10.92^{a} \pm 0.03$ | $7.55^{b} \pm 0.02$ | $6.60^{a} \pm 0.02$ | $6.24^{a} \pm 0.02$ | $8.22^{a} \pm 0.03$ | $1.49^{b} \pm 0.008$ | $2.71^{b} \pm 0.01$ | $1.55^{b} \pm 0.009$ |

Means within each group of sources of variation viz stocks (AP, HR, MH, MN, MP and TR) and sex (Female and male) in the same column having different superscripts vary significantly from each other within the group ($p < 0.0001$). 'N' is number of observations

**Table 6** Least squares means and standard errors of various truss measurements

| Source | N = 599 | OAEW | DEDL | OAEL | SOL | SPTL | SPEL | SVOL | ODL |
|---|---|---|---|---|---|---|---|---|---|
| Stock | AP | $2.21^{b} \pm 0.02$ | $6.83^{abc} \pm 0.07$ | $1.98^{c} \pm 0.01$ | $4.21^{b} \pm 0.03$ | $4.01^{b} \pm 0.03$ | $1.42^{a} \pm 0.01$ | $3.27^{a} \pm 0.05$ | $4.52^{cd} \pm 0.03$ |
| | HR | $2.36^{a} \pm 0.02$ | $7.20^{a} \pm 0.07$ | $2.10^{ab} \pm 0.01$ | $4.43^{a} \pm 0.03$ | $4.37^{a} \pm 0.03$ | $1.36^{a} \pm 0.01$ | $3.43^{a} \pm 0.05$ | $4.98^{a} \pm 0.03$ |
| | MH | $2.15^{b} \pm 0.02$ | $6.51^{c} \pm 0.07$ | $2.07^{bc} \pm 0.01$ | $4.30^{ab} \pm 0.03$ | $4.26^{a} \pm 0.03$ | $1.45^{a} \pm 0.01$ | $3.36^{a} \pm 0.05$ | $4.33^{d} \pm 0.03$ |
| | MN | $2.24^{ab} \pm 0.02$ | $7.00^{ab} \pm 0.07$ | $2.18^{a} \pm 0.01$ | $4.19^{b} \pm 0.03$ | $4.23^{ab} \pm 0.03$ | $1.34^{a} \pm 0.01$ | $3.34^{a} \pm 0.05$ | $4.74^{b} \pm 0.03$ |
| | MP | $2.25^{ab} \pm 0.02$ | $6.88^{abc} \pm 0.07$ | $2.14^{ab} \pm 0.01$ | $4.41^{a} \pm 0.03$ | $4.33^{a} \pm 0.03$ | $1.44^{a} \pm 0.01$ | $3.51^{a} \pm 0.05$ | $4.61^{bc} \pm 0.03$ |
| | TR | $2.28^{ab} \pm 0.02$ | $6.79^{bc} \pm 0.07$ | $2.10^{ab} \pm 0.01$ | $4.26^{ab} \pm 0.03$ | $4.36^{a} \pm 0.03$ | $1.40^{a} \pm 0.01$ | $3.58^{a} \pm 0.05$ | $4.62^{bc} \pm 0.03$ |
| Sex | Female | $2.24^{a} \pm 0.01$ | $6.79^{a} \pm 0.03$ | $2.09^{a} \pm 0.01$ | $4.32^{a} \pm 0.01$ | $4.29^{a} \pm 0.02$ | $1.42^{a} \pm 0.01$ | $3.43^{a} \pm 0.03$ | $4.64^{a} \pm 0.02$ |
| | Male | $2.25^{a} \pm 0.01$ | $6.95^{a} \pm 0.04$ | $2.10^{a} \pm 0.01$ | $4.28^{a} \pm 0.02$ | $4.23^{a} \pm 0.02$ | $1.39^{a} \pm 0.01$ | $3.40^{a} \pm 0.03$ | $4.63^{a} \pm 0.02$ |

Means within each group of sources of variation viz stocks (AP, HR, MH, MN, MP and TR) and sex (Female and male) in the same column having different superscripts vary significantly from each other within the group ($p < 0.0001$). 'N' is number of observations

15.91, respectively (Table 17). The variables OAW, DEDL, EDPW, EAAL, AEPEL, DCVCW, DCVCPW, and VCVCL had the highest loading on factor-1 (Table 17). The variables SPTL, SPEL, SVOL, and PTVOL are loaded on the second factor, and the variables EDCL, EDVCW, and EDEAW are loaded on the third factor (Table 17).

### 3.4 Discriminant analysis

The traits with high loadings on the first factor, second factor, and third factor in the factor analysis of both stock-wise and sex-wise were subjected to discriminant analysis. With a total of 9 traits viz DAW, DPW, DPTW, EDPW,

**Table 7** Least squares means and standard errors of various truss measurements

| Source | N = 599 | OPW | OOEW | OAEW | OVOW | DVOW | EDCL | EDVCW |
|---|---|---|---|---|---|---|---|---|
| Stock | AP | $7.36^{c} \pm 0.04$ | $1.98^{a} \pm 0.18$ | $2.21^{b} \pm 0.02$ | $4.17^{abc} \pm 0.04$ | $7.22^{b} \pm 0.03$ | $2.16^{a} \pm 0.05$ | $3.46^{a} \pm 0.05$ |
| | HR | $7.87^{a} \pm 0.04$ | $1.96^{a} \pm 0.18$ | $2.36^{a} \pm 0.02$ | $4.29^{a} \pm 0.04$ | $7.74^{a} \pm 0.04$ | $2.12^{a} \pm 0.06$ | $3.61^{a} \pm 0.05$ |
| | MH | $7.65^{ab} \pm 0.04$ | $1.82^{b} \pm 0.18$ | $2.15^{b} \pm 0.02$ | $4.02^{bc} \pm 0.04$ | $6.95^{c} \pm 0.04$ | $1.93^{a} \pm 0.06$ | $3.33^{a} \pm 0.05$ |
| | MN | $7.67^{ab} \pm 0.04$ | $1.89^{ab} \pm 0.18$ | $2.24^{ab} \pm 0.02$ | $3.95^{c} \pm 0.04$ | $7.17^{bc} \pm 0.03$ | $1.99^{a} \pm 0.06$ | $3.40^{a} \pm 0.05$ |
| | MP | $7.67^{ab} \pm 0.04$ | $1.83^{b} \pm 0.18$ | $2.25^{ab} \pm 0.02$ | $4.22^{ab} \pm 0.04$ | $7.30^{b} \pm 0.04$ | $1.90^{a} \pm 0.06$ | $3.38^{a} \pm 0.05$ |
| | TR | $7.51^{bc} \pm 0.04$ | $1.91^{ab} \pm 0.18$ | $2.28^{ab} \pm 0.02$ | $4.04^{bc} \pm 0.04$ | $6.98^{c} \pm 0.04$ | $2.15^{a} \pm 0.06$ | $3.37^{a} \pm 0.05$ |
| Sex | Female | $7.66^{a} \pm 0.02$ | $1.89^{a} \pm 0.01$ | $2.24^{a} \pm 0.01$ | $4.10^{a} \pm 0.01$ | $7.20^{a} \pm 0.02$ | $2.10^{a} \pm 0.03$ | $3.44^{a} \pm 0.02$ |
| | Male | $7.59^{a} \pm 0.02$ | $1.90^{a} \pm 0.01$ | $2.25^{a} \pm 0.01$ | $4.13^{a} \pm 0.01$ | $7.25^{a} \pm 0.02$ | $1.99^{a} \pm 0.03$ | $3.41^{a} \pm 0.03$ |

Means within each group of sources of variation viz stocks (AP, HR, MH, MN, MP and TR) and sex (Female and male) in the same column having different superscripts vary significantly from each other within the group ($p < 0.0001$). 'N' is number of observations

**Table 8** Least squares means and standard errors of various truss measurements

| Source | N = 599 | EDEAW | EDAW | DCEAW | VCEAL | EAAL | APL | PPTL | POEW |
|---|---|---|---|---|---|---|---|---|---|
| Stock | AP | $2.83^{a} \pm 0.03$ | $4.03^{ab} \pm 0.03$ | $3.34^{a} \pm 0.03$ | $1.91^{a} \pm 0.03$ | $1.77^{a} \pm 0.03$ | $5.28^{bc} \pm 0.04$ | $4.96^{c} \pm 0.03$ | $5.67^{c} \pm 0.03$ |
| | HR | $2.92^{a} \pm 0.03$ | $4.10^{a} \pm 0.03$ | $3.33^{a} \pm 0.03$ | $1.88^{ab} \pm 0.04$ | $1.81^{a} \pm 0.03$ | $5.57^{a} \pm 0.04$ | $5.29^{a} \pm 0.04$ | $6.18^{a} \pm 0.04$ |
| | MH | $2.73^{a} \pm 0.03$ | $3.78^{c} \pm 0.03$ | $3.11^{b} \pm 0.03$ | $1.67^{b} \pm 0.04$ | $1.52^{b} \pm 0.03$ | $5.27^{bc} \pm 0.04$ | $5.19^{ab} \pm 0.03$ | $6.01^{ab} \pm 0.04$ |
| | MN | $2.74^{a} \pm 0.03$ | $3.88^{bc} \pm 0.03$ | $3.13^{b} \pm 0.03$ | $1.83^{ab} \pm 0.03$ | $1.67^{ab} \pm 0.03$ | $5.13^{cd} \pm 0.04$ | $5.156^{abc} \pm 0.03$ | $5.97^{ab} \pm 0.03$ |
| | MP | $2.88^{a} \pm 0.03$ | $4.09^{a} \pm 0.03$ | $3.39^{a} \pm 0.03$ | $1.87^{ab} \pm 0.04$ | $1.68^{ab} \pm 0.03$ | $5.44^{ab} \pm 0.04$ | $5.11^{abc} \pm 0.03$ | $6.03^{ab} \pm 0.03$ |
| | TR | $2.76^{a} \pm 0.03$ | $3.82^{c} \pm 0.03$ | $3.38^{a} \pm 0.03$ | $2.01^{a} \pm 0.04$ | $1.51^{b} \pm 0.03$ | $5.002^{d} \pm 0.04$ | $5.07^{bc} \pm 0.03$ | $5.84^{bc} \pm 0.03$ |
| Sex | Female | $2.80^{a} \pm 0.01$ | $3.89^{a} \pm 0.01$ | $3.27^{a} \pm 0.01$ | $1.90^{a} \pm 0.02$ | $1.59^{a} \pm 0.01$ | $5.30^{a} \pm 0.02$ | $5.16^{a} \pm 0.02$ | $6.001^{a} \pm 0.02$ |
| | Male | $2.82^{a} \pm 0.01$ | $4.01^{b} \pm 0.02$ | $3.29^{a} \pm 0.02$ | $1.83^{a} \pm 0.02$ | $1.73^{b} \pm 0.02$ | $5.26^{a} \pm 0.02$ | $5.10^{a} \pm 0.02$ | $5.90^{a} \pm 0.02$ |

Means within each group of sources of variation viz stocks (AP, HR, MH, MN, MP and TR) and sex (Female and male) in the same column having different superscripts vary significantly from each other within the group ($p < 0.0001$). 'N' is number of observations

**Table 9** Least squares means and standard errors of various truss measurements

| Source | N = 599 | PVOL | PTOEW | PTAEW | PTVOL | OEVOW | AEPEL | AEVOW |
|---|---|---|---|---|---|---|---|---|
| Stock | AP | $6.013^{b} \pm 0.04$ | $1.73^{c} \pm 0.01$ | $2.64^{c} \pm 0.02$ | $1.31^{a} \pm 0.04$ | $2.61^{a} \pm 0.04$ | $0.88^{bc} \pm 0.01$ | $2.55^{a} \pm 0.04$ |
| | HR | $6.33^{a} \pm 0.04$ | $1.94^{b} \pm 0.01$ | $2.81^{ab} \pm 0.02$ | $1.30^{a} \pm 0.04$ | $2.71^{a} \pm 0.04$ | $0.95^{a} \pm 0.01$ | $2.53^{a} \pm 0.05$ |
| | MH | $6.15^{ab} \pm 0.04$ | $1.97^{ab} \pm 0.01$ | $2.74^{bc} \pm 0.02$ | $1.16^{ab} \pm 0.04$ | $2.64^{a} \pm 0.04$ | $0.91^{ab} \pm 0.01$ | $2.46^{a} \pm 0.05$ |
| | MN | $6.09^{ab} \pm 0.04$ | $1.92^{b} \pm 0.01$ | $2.78^{ab} \pm 0.02$ | $1.11^{ab} \pm 0.04$ | $2.52^{a} \pm 0.04$ | $0.82^{c} \pm 0.01$ | $2.39^{a} \pm 0.05$ |
| | MP | $6.004^{b} \pm 0.04$ | $2.08^{a} \pm 0.01$ | $2.88^{a} \pm 0.02$ | $1.13^{ab} \pm 0.04$ | $2.75^{a} \pm 0.04$ | $0.88^{bc} \pm 0.01$ | $2.66^{a} \pm 0.04$ |
| | TR | $5.94^{b} \pm 0.04$ | $1.92^{b} \pm 0.01$ | $2.76^{ab} \pm 0.02$ | $1.038^{a} \pm 0.04$ | $2.48^{a} \pm 0.04$ | $0.83^{c} \pm 0.01$ | $2.45^{a} \pm 0.04$ |
| Sex | Female | $6.09^{a} \pm 0.02$ | $1.92^{a} \pm 0.01$ | $2.79^{a} \pm 0.01$ | $1.15^{a} \pm 0.02$ | $2.59^{a} \pm 0.02$ | $0.87^{a} \pm 0.006$ | $2.51^{a} \pm 0.02$ |
| | Male | $6.08^{a} \pm 0.02$ | $1.93^{a} \pm 0.01$ | $2.75^{a} \pm 0.01$ | $1.20^{a} \pm 0.02$ | $2.65^{a} \pm 0.02$ | $0.88^{a} \pm 0.007$ | $2.51^{a} \pm 0.03$ |

Means within each group of sources of variation viz stocks (AP, HR, MH, MN, MP and TR) and sex (Female and male) in the same column having different superscripts vary significantly from each other within the group ($p < 0.0001$). 'N' is number of observations

DCVCPW, SVOL, EDCL, AEVOW, and OEVOW, classification of stocks was achieved for AP, HR, MH, MN, MP, and TR at a classification rate of 95%, 59%, 67%, 59%, 69%, and 71% respectively. Discriminant analysis was performed for the overall stock points towards misclassification rate of 5%,41%, 33%, 40%, 31%, and 29% for AP, HR, MH, MN, MP, and TR, respectively (Table 18). The misclassification rate for sex is provided in Table 19.

**Table 10** Least squares means and standard error for Sex-wise significant traits

| Stock | Sex | DAW | DCVCW | DCVCPW |
|---|---|---|---|---|
| AP | Female | $7.22^{def} \pm 0.058$ | $1.33^{d} \pm 0.019$ | $2.54^{cd} \pm 0.026$ |
| AP | Male | $7.60^{abc} \pm 0.064$ | $1.43^{bcd} \pm 0.021$ | $2.76^{a} \pm 0.029$ |
| HR | Female | $7.93^{ab} \pm 0.057$ | $1.51^{abc} \pm 0.019$ | $2.81^{a} \pm 0.026$ |
| HR | Male | $7.99^{a} \pm 0.066$ | $1.54^{ab} \pm 0.022$ | $2.78^{a} \pm 0.030$ |
| MH | Female | $7.14^{ef} \pm 0.059$ | $1.41^{bcd} \pm 0.020$ | $2.57^{cd} \pm 0.027$ |
| MH | Male | $7.31^{cdef} \pm 0.064$ | $1.45^{abcd} \pm 0.021$ | $2.69^{abc} \pm 0.029$ |
| MN | Female | $7.42^{cdef} \pm 0.059$ | $1.43^{bcd} \pm 0.020$ | $2.58^{bcd} \pm 0.027$ |
| MN | Male | $7.51^{cde} \pm 0.063$ | $1.50^{abc} \pm 0.021$ | $2.64^{abcd} \pm 0.029$ |
| MP | Female | $7.60^{abc} \pm 0.057$ | $1.56^{a} \pm 0.019$ | $2.80^{a} \pm 0.026$ |
| MP | Male | $7.54^{cd} \pm 0.066$ | $1.53^{ab} \pm 0.022$ | $2.75^{ab} \pm 0.030$ |
| TR | Female | $7.12^{f} \pm 0.058$ | $1.40^{cd} \pm 0.019$ | $2.51^{d} \pm 0.026$ |
| TR | Male | $7.37^{cdef} \pm 0.066$ | $1.47^{abc} \pm 0.022$ | $2.65^{abcd} \pm 0.030$ |
| Stock | Sex | VCVCL | EDAW | EAAL |
| AP | Female | $1.53^{bc} \pm 0.019$ | $3.87^{bc} \pm 0.043$ | $1.66^{abc} \pm 0.046$ |
| AP | Male | $1.68^{a} \pm 0.022$ | $4.19^{a} \pm 0.048$ | $1.88^{a} \pm 0.051$ |
| HR | Female | $1.62^{ab} \pm 0.019$ | $4.09^{ab} \pm 0.043$ | $1.77^{a} \pm 0.046$ |
| HR | Male | $1.63^{ab} \pm 0.022$ | $4.12^{ab} \pm 0.048$ | $1.85^{a} \pm 0.053$ |
| MH | Female | $1.45^{cd} \pm 0.020$ | $3.68^{c} \pm 0.045$ | $1.44^{bc} \pm 0.047$ |
| MH | Male | $1.54^{bc} \pm 0.021$ | $3.88^{bc} \pm 0.048$ | $1.60^{abc} \pm 0.051$ |
| MN | Female | $1.39^{d} \pm 0.020$ | $3.83^{bc} \pm 0.044$ | $1.60^{abc} \pm 0.047$ |
| MN | Male | $1.42^{cd} \pm 0.021$ | $3.92^{abc} \pm 0.048$ | $1.73^{ab} \pm 0.051$ |
| MP | Female | $1.54^{bc} \pm 0.019$ | $4.11^{ab} \pm 0.032$ | $1.67^{abc} \pm 0.045$ |
| MP | Male | $1.54^{bc} \pm 0.022$ | $4.07^{ab} \pm 0.050$ | $1.69^{abc} \pm 0.053$ |
| TR | Female | $1.37^{d} \pm 0.019$ | $3.75^{c} \pm 0.043$ | $1.42^{c} \pm 0.046$ |
| TR | Male | $1.46^{cd} \pm 0.022$ | $3.88^{bc} \pm 0.049$ | $1.60^{abc} \pm 0.052$ |

The varying supercscripts indicates statistically significant Tukey groupings

**Table 11** ANOVA for different truss measurements

| Source | df | SOL | SPTL | SPEL | SVOL | ODL | OPW | OOEW |
|---|---|---|---|---|---|---|---|---|
| SL | 1 | $1.405^{ns}$ | $2.22^{ns}$ | $0.144^{ns}$ | $1.109^{ns}$ | 3.01* | $1.81^{ns}$ | $0.22^{ns}$ |
| BW | 1 | 31.26* | 27.35* | 3.162* | 15.95* | 53.11* | 168.4* | 6.855* |
| Stock | 5 | 0.996* | 1.73* | $0.167^{ns}$ | $1.306^{ns}$ | 4.47* | 2.91* | 0.388* |
| Sex | 1 | $0.351^{ns}$ | $0.48^{ns}$ | $0.101^{ns}$ | $0.122^{ns}$ | $0.003^{ns}$ | $0.71^{ns}$ | $0.02^{ns}$ |
| Stock*Sex | 5 | $0.053^{ns}$ | $0.37^{ns}$ | $0.093^{ns}$ | $0.77^{ns}$ | $0.23^{ns}$ | $0.12^{ns}$ | $0.09^{ns}$ |
| Error | 585 | 0.107 | 0.147 | 0.047 | 0.32 | 0.146 | 0.184 | 0.034 |
| $R^2$ (%) | | 52.18 | 45.59 | 23.49 | 19.40 | 58.48 | 74.43 | 39.37 |

*$p < 0.0001$, *ns* not significant ($p > 0.0001$)

* indicates the values are statistically significant at p values less than 0.0001

**Table 12** ANOVA for different truss measurements

| Source | df | OAEW | OVOW | DVOW | EDCL | EDVCW | EDEAW | EDAW |
|---|---|---|---|---|---|---|---|---|
| SL | 1 | $0.684^{ns}$ | $0.66^{ns}$ | 2.75* | $1.88^{ns}$ | 2.860* | $0.529^{ns}$ | $1.371^{ns}$ |
| BW | 1 | 9.035* | 35.48* | 140.71* | $3.342^{ns}$ | 18.53* | 23.387* | 43.53* |
| Stock | 5 | 0.437* | 1.67* | 7.752* | $1.16^{ns}$ | $0.932^{ns}$ | 0.580* | 1.945* |
| Sex | 1 | $0.004^{ns}$ | $0.07^{ns}$ | $0.49^{ns}$ | $1.74^{ns}$ | $0.126^{ns}$ | $0.124^{ns}$ | 2.20* |
| Stock*Sex | 5 | $0.107^{ns}$ | $0.54^{ns}$ | $0.59^{ns}$ | $0.37^{ns}$ | $0.363^{ns}$ | $0.149^{ns}$ | $0.394^{ns}$ |
| Error | 585 | 0.046 | 0.167 | 0.155 | 0.350 | 0.252 | 0.106 | 0.106 |
| $R^2$ (%) | | 42.40 | 41.79 | 74.89 | 9.61 | 24.38 | 41.21 | 58.57 |

*$p < 0.0001$, *ns* not significant ($p > 0.0001$)

* indicates the values are statistically significant at p values less than 0.0001

**Table 13** ANOVA for different truss measurements

| Source | df | DCEAW | VCEAL | EAAL | APL | PPTL | POEW |
|---|---|---|---|---|---|---|---|
| SL | 1 | 1.480* | 1.63$^{ns}$ | 3.148* | 1.353$^{ns}$ | 0.608$^{ns}$ | 1.305$^{ns}$ |
| BW | 1 | 27.7092* | 3.18* | 1.89* | 85.72* | 91.86* | 116.43* |
| Stock | 5 | 1.517* | 1.160* | 1.519* | 4.243* | 1.239* | 2.97* |
| Sex | 1 | 0.048$^{ns}$ | 0.771$^{ns}$ | 2.69* | 0.187$^{ns}$ | 0.422$^{ns}$ | 1.264$^{ns}$ |
| Stock*Sex | 5 | 0.196$^{ns}$ | 0.069$^{ns}$ | 0.12$^{ns}$ | 0.166$^{ns}$ | 0.16$^{ns}$ | 0.127$^{ns}$ |
| Error | 585 | 0.109 | 0.154 | 0.119 | 0.167 | 0.152 | 0.153 |
| $R^2$ (%) | | 48.85 | 16.80 | 22.01 | 63.98 | 65.01 | 71.79 |

*$p < 0.0001$, *ns* not significant ($p > 0.0001$)

* indicates the values are statistically significant at p values less than 0.0001

**Table 14** ANOVA for different truss measurements

| Source | df | PVOL | PTOEW | PTAEW | PTVOL | OEVOW | AEPEL | AEVOW |
|---|---|---|---|---|---|---|---|---|
| SL | 1 | 0.59$^{ns}$ | 0.150$^{ns}$ | 1.276* | 0.005* | 0.010* | 0.000$^{ns}$ | 0.379$^{ns}$ |
| BW | 1 | 120.22* | 9.211* | 16.96* | 2.67* | 14.60* | 0.639* | 13.85* |
| Stock | 5 | 1.95* | 1.230* | 0.608* | 1.187$^{ns}$ | 1.061$^{ns}$ | 0.234* | 0.862$^{ns}$ |
| Sex | 1 | 0.001$^{ns}$ | 0.014$^{ns}$ | 0.201$^{ns}$ | 0.286$^{ns}$ | 0.50$^{ns}$ | 0.011$^{ns}$ | 0.002$^{ns}$ |
| Stock*Sex | 5 | 0.47$^{ns}$ | 0.061$^{ns}$ | 0.037$^{ns}$ | 0.257$^{ns}$ | 0.493$^{ns}$ | 0.021$^{ns}$ | 0.359$^{ns}$ |
| Error | 585 | 0.21 | 0.037 | 0.043 | 0.189 | 0.217 | 0.013 | 0.239 |
| $R^2$ (%) | | 62.75 | 53.79 | 62.19 | 8.43 | 19.99 | 21.40 | 18.90 |

*$p < 0.0001$, *ns* not significant ($p > 0.0001$)

* indicates the values are statistically significant at p values less than 0.0001

**Table 15** Variable loadings for the truss data from rotated factor- Overall

| Variables | Truss distance | Factor-1 | Factor-2 | Factor-3 |
|---|---|---|---|---|
| OAW | 2–9 | 64* | 14 | 25 |
| DAW | 3–9 | 88* | 27 | 22 |
| DPW | 3–10 | 81* | 34 | 14 |
| DPTW | 3–11 | 86* | 26 | 20 |
| EDPW | 4–10 | 84* | 36 | 0 |
| DCVCW | 5–6 | 68* | 25 | 18 |
| DCVCPW | 5–7 | 81* | 29 | 11 |
| VCVCL | 6–7 | 71* | 22 | – 4 |
| VCEAL | 7–8 | 17 | 15 | 10 |
| SVOL | 1–15 | 19 | 80* | 15 |
| OOEW | 2–12 | 0 | 3 | – 13 |
| OAEW | 2–13 | 61* | 25 | 14 |
| DEDL | 3–4 | 73* | 13 | – 12 |
| EDCL | 4–5 | – 3 | 9 | 100* |
| EDVCW | 4–7 | 30 | 20 | 54* |
| AEVOW | 13–15 | 29 | 91* | 4 |
| EAAL | 8–9 | 36 | 20 | – 5 |
| APL | 9–10 | 18 | 15 | 71* |
| PTVOL | 11–15 | 21 | 51* | – 4 |
| OAEL | 12–13 | 57* | 21 | 27 |
| OEVOW | 12–15 | 36 | 78* | 9 |
| AEPEL | 13–14 | 27 | 49* | 6 |

* indicates the values are statistically significant at p values less than 0.0001

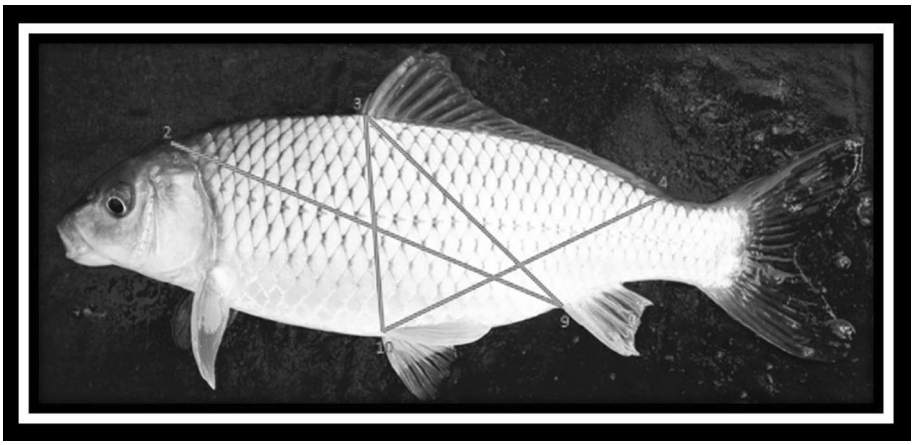


**Fig. 3** Landmark explained by Factor 1

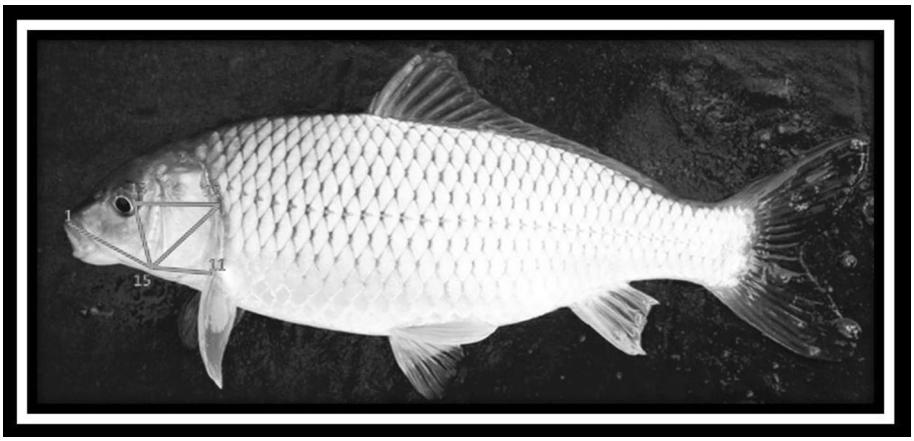


**Fig. 4** Landmark explained by Factor 2

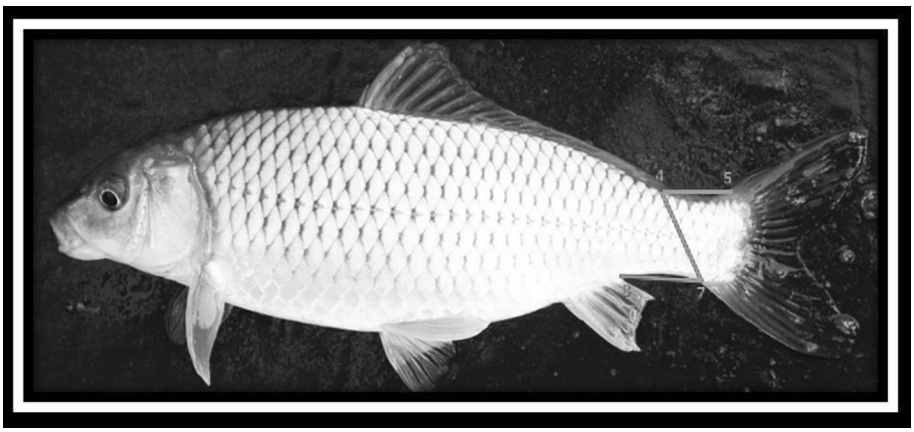


**Fig. 5** Landmark explained by Factor 3

### 3.5 Haplotype diversity, population structuring, and AMOVA

For better accuracy, complete sequences of mitochondrial D loop (~ 1 Kb) were amplified and sequenced in both orientations for all six stocks. The quality of the DNA sequences was verified based on phred score (Q > 30) of each base and was deposited to NCBI, GenBank (Table 20). Out of 169 individuals, 7 haplotypes were revealed by mitochondrial D loop region, among which AP, MP, MH, HR, MN, and TR stocks displayed 3, 4, 2, 1, 1, and 1 haplotypes, respectively. The haplotype frequency for the D loop region and the GenBank accession numbers are given in Table 20. Among all the six stocks, MP stock samples exhibited maximum haplotypes (Table 20). The average haplotype diversity of

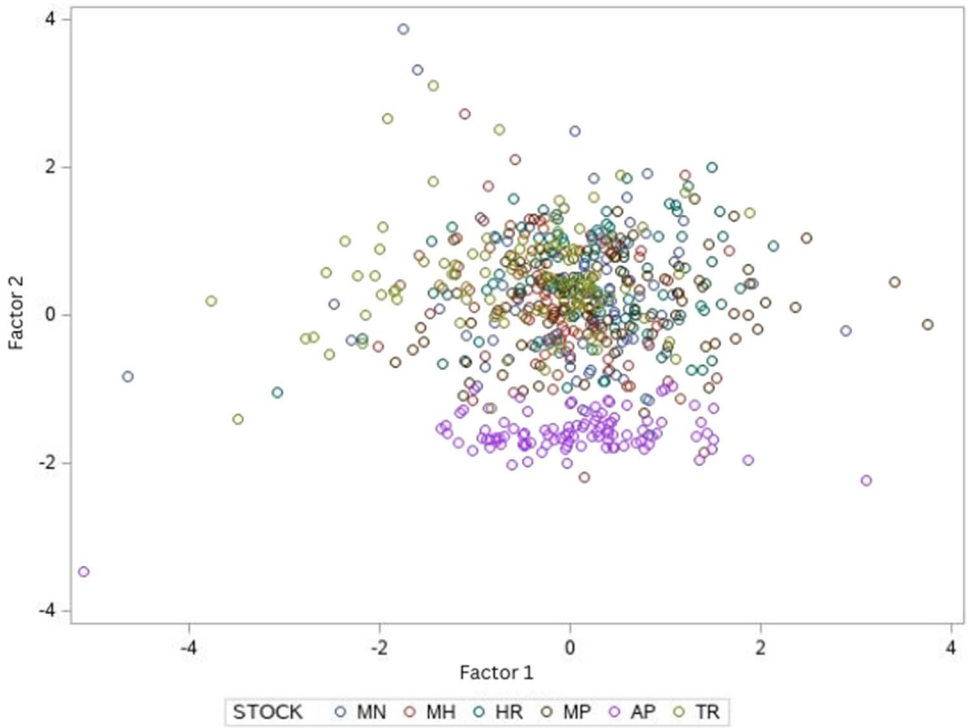


**Fig. 6** Bivariate plot for Factor 1 * Factor 2

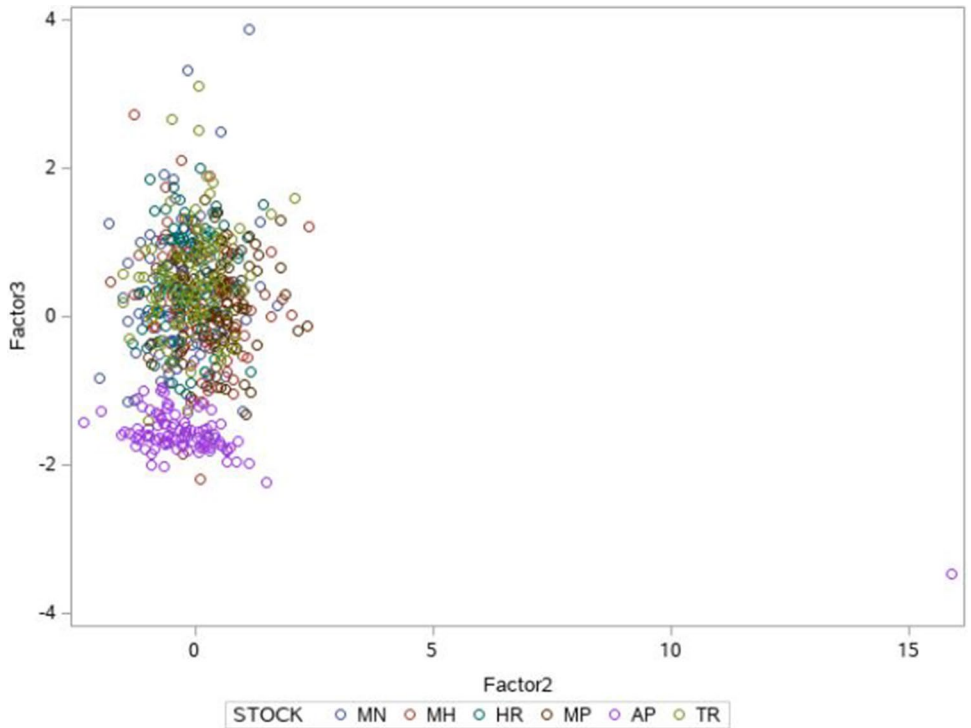


**Fig. 7** Bivariate plot for Factor 2 * Factor 3

all populations was 0.08129, and the average nucleotide diversity was 0.01134. Among the six stocks, the D loop region showed high haplotype diversity in AP stock (0.255) and moderate haplotype diversity in MP (0.157) and MH stock (0.066); however, no haplotype diversity was observed in the other three stocks (Table 21). The H1 haplotype was shared among all the six stocks. However, the nucleotide diversity was low for all the stocks. Population pairwise $F_{ST}$ values for both the DNA sequences ranged from 0.01 to 0.05 with a p value of $< 0.001$ (Table 22). The $F_{ST}$

**Table 16** Variable loadings for the truss data from rotated factor- Male

| Variables | Truss distance | Factor-1 | Factor-2 | Factor-3 |
|---|---|---|---|---|
| SVOL | 1–15 | 12 | 83* | 11 |
| ODL | 2–3 | 70* | 19 | 22 |
| OAW | 2–9 | 77* | 1 | 20 |
| OOEW | 2–12 | – 4 | 3 | – 14 |
| OVOW | 2–15 | 39 | 89* | – 2 |
| DEDL | 3–4 | 75* | 6 | – 6 |
| DAW | 3–9 | 91* | 23 | 19 |
| DPW | 3–10 | 77* | 32 | 14 |
| DPTW | 3–11 | 89* | 16 | 16 |
| EDCL | 4–5 | – 2 | 2 | 100* |
| EDVCW | 4–7 | 37 | 26 | 45* |
| DCVCW | 5–6 | 65* | 21 | 18 |
| DCVCPW | 5–7 | 75* | 28 | 5 |
| VCVCL | 6–7 | 62* | 29 | – 10 |
| VCEAL | 7–8 | 17 | 16 | 4 |
| EAAL | 8–9 | 22 | 30 | – 9 |
| APL | 9–10 | 14 | 4 | 73* |
| PTVOL | 11–15 | 8 | 72* | – 7 |
| OEVOW | 12–15 | 21 | 89* | 5 |
| AEPEL | 13–14 | 16 | 64* | 1 |
| AEVOW | 13–15 | 19 | 91* | 3 |

* indicates the values are statistically significant at p values less than 0.0001

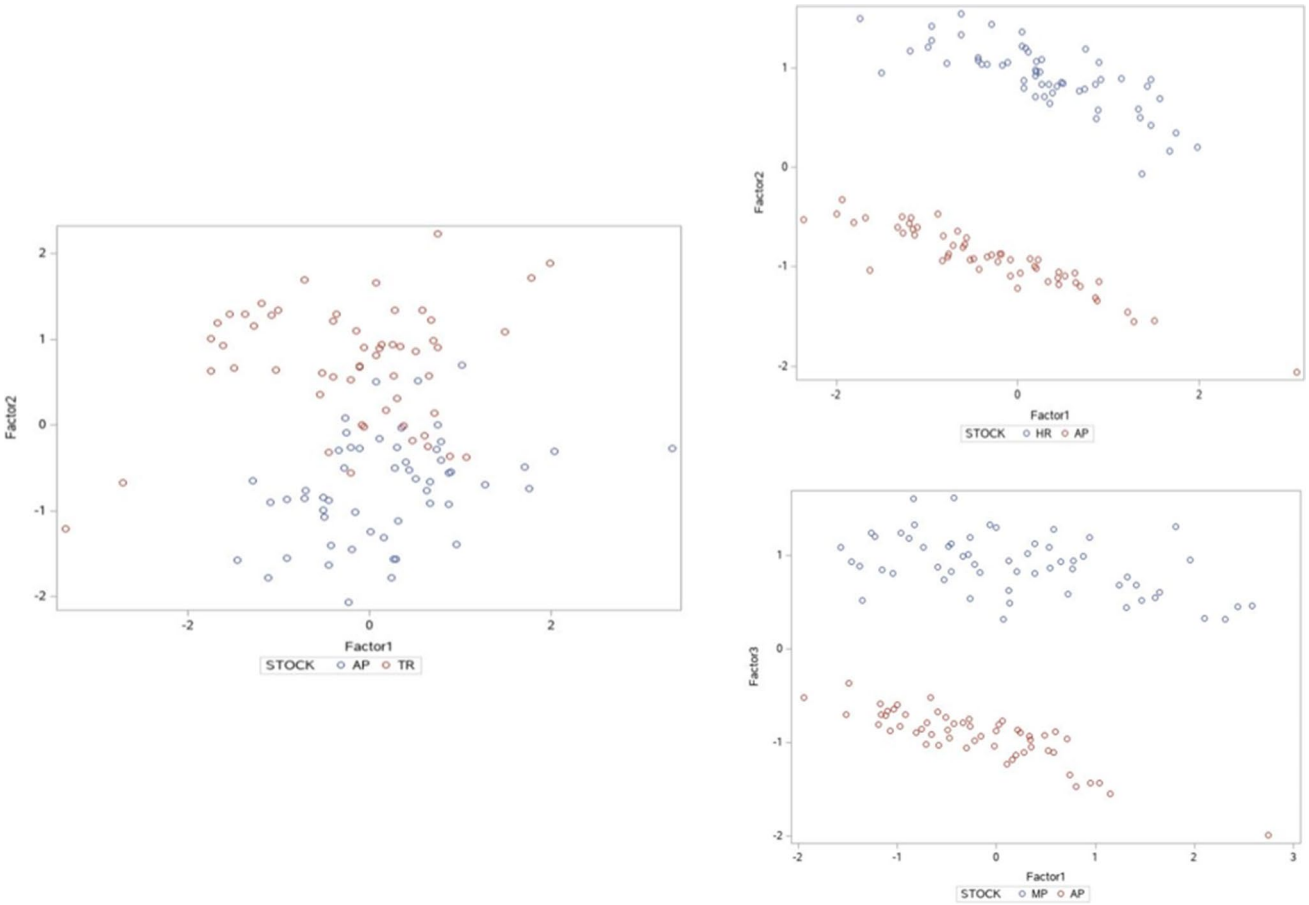


**Fig. 8** Bivariate plots for Factor 1 * Factor 2 (AP and other stocks)

**Table 17** Variable loadings for the truss data from rotated factor- Female

| Variables | Truss distance | Factor-1 | Factor-2 | Factor-3 |
|---|---|---|---|---|
| SPTL | 1–11 | 29 | 95* | 7 |
| SPEL | 1–14 | 9 | 75* | 6 |
| SVOL | 1–15 | 24 | 89* | 5 |
| OAW | 2–9 | 51* | 35 | 19 |
| OOEW | 2–12 | 1 | − 1 | 3 |
| DEDL | 3–4 | 71* | 29 | − 36 |
| EDCL | 4–5 | − 9 | 30 | 58* |
| EDVCW | 4–7 | 15 | 13 | 94* |
| EDEAW | 4–8 | 35 | 25 | 82* |
| EDPW | 4–10 | 85* | 29 | 4 |
| DCVCW | 5–6 | 74* | 25 | 16 |
| DCVCPW | 5–7 | 86* | 29 | 24 |
| VCVCL | 6–7 | 80* | 13 | 24 |
| VCEAL | 7–8 | 12 | 23 | 23 |
| EAAL | 8–9 | 47* | 2 | 16 |
| APL | 9–10 | 22 | 35 | 13 |
| PTVOL | 11–15 | 27 | 41* | 12 |
| AEPEL | 13–14 | 40* | 29 | 14 |

* indicates the values are statistically significant at p values less than 0.0001

**Table 18** Percentage of fish from each stock classified in the cross-validation of the Discriminant Analysis

| Stocks | AP | HR | MH | MN | MP | TR | Total |
|---|---|---|---|---|---|---|---|
| AP | 95 | 0 | 0 | 5 | 0 | 0 | 100 |
| | 95.00 | 0.00 | 0.00 | 5.00 | 0.00 | 0.00 | 100.00 |
| HR | 0 | 59 | 7 | 13 | 12 | 9 | 100 |
| | 0.00 | 59.00 | 7.00 | 13.00 | 12.00 | 9.00 | 100.00 |
| MH | 0 | 5 | 67 | 10 | 11 | 7 | 100 |
| | 0.00 | 5.00 | 67.00 | 10.00 | 11.00 | 7.00 | 100.00 |
| MN | 2 | 12 | 13 | 59 | 6 | 7 | 99 |
| | 2.02 | 12.12 | 13.13 | 59.60 | 6.06 | 7.07 | 100.00 |
| MP | 1 | 9 | 12 | 4 | 69 | 5 | 100 |
| | 1.00 | 9.00 | 12.00 | 4.00 | 69.00 | 5.00 | 100.00 |
| TR | 1 | 8 | 6 | 8 | 6 | 71 | 100 |
| | 1.00 | 8.00 | 6.00 | 8.00 | 6.00 | 71.00 | 100.00 |
| Total | 99 | 93 | 105 | 99 | 104 | 99 | 599 |
| | 16.53 | 15.53 | 17.53 | 16.53 | 17.36 | 16.53 | 100.00 |
| Priors | 0.16667 | 0.16667 | 0.16667 | 0.16667 | 0.16667 | 0.16667 | |
| Error count estimates for stock | | | | | | | |
| | AP | HR | MH | MN | MP | TR | Total |
| Rate | 0.0500 | 0.4100 | 0.3300 | 0.4040 | 0.3100 | 0.2900 | 0.2990 |
| Priors | 0.1667 | 0.1667 | 0.1667 | 0.1667 | 0.1667 | 0.1667 | |

results showed that the pair-wise $F_{ST}$ estimates were high between AP stock and HR, MN, and TR stocks, indicating high genetic differentiation between the Andhra stock and the later three stocks. The AMOVA is done in three other combinations, where AP is a distinct group. The population groups designed were (AP) (MH) (MN & TR & MP & HR), (AP) (MN & TR & MP & HR & MH), and (AP) (MP & HR & MH) (TR & MN). The AMOVA showed a higher proportion of total variance in the 3rd combination i.e., among groups of (AP) (MN & TR & MP & HR & MH) (Table 23). The DNA sequences showed significant negative Tajima's D values for four out of six stocks (Table 21).

**Table 19** Percentage of fish from each sex classified in the cross validation of the discriminant analysis

| Sex | Female | Male | Total |
|---|---|---|---|
| Female | 238 | 93 | 331 |
| | 71.90 | 28.10 | 100.00 |
| Male | 89 | 179 | 268 |
| | 33.21 | 66.79 | 100.00 |
| Total | 327 | 272 | 599 |
| | 54.59 | 45.41 | 100.00 |
| Priors | 0.5 | 0.5 | |
| Error count estimates for sex | | | |
| | Female | Male | Total |
| Rate | 0.2810 | 0.3321 | 0.3065 |
| Priors | 0.5000 | 0.5000 | |

**Table 20** Haplotype frequency for D –loop region and GenBank Accession numbers

| Haplotype | Maharashtra (MH) | Andhra Pradesh (AP) | Madhya Pradesh (MP) | Haryana (HR) | Tripura (TR) | Manipur (MN) | Accession numbers |
|---|---|---|---|---|---|---|---|
| H1 | 29 (0.17) | 19 (0.11) | 34 (0.20) | 30 (0.17) | 22 (0.13) | 28 (0.16) | OP271960- 2128 |
| H2 | | 1 (0.005) | | | | | OP271963 |
| H3 | | 2 (0.01) | | | | | OP271966, OP271968 |
| H4 | 1 (0.005) | | | | | | OP272025 |
| H5 | | | 1 (0.005) | | | | OP272088 |
| H6 | | | 1 (0.005) | | | | OP272101 |
| H7 | | | 1 (0.005) | | | | OP272106 |

**Table 21** Summary statistics for mtDNA haplogroups of Common carp

| Population | Andhra Pradesh | Madhya Pradesh | Maharashtra | Haryana | Manipur | Tripura | Overall |
|---|---|---|---|---|---|---|---|
| N | 22 | 37 | 30 | 30 | 28 | 22 | 169 |
| S | 3 | 171 | 2 | 0 | 0 | 0 | 172 |
| H | 3 | 4 | 2 | 1 | 1 | 1 | 7 |
| $H_d$ | 0.25541 | 0.15766 | 0.06667 | 0 | 0 | 0 | 0.08129 |
| $\pi_n$ | 0.00144 | 0.04970 | 0.00045 | 0 | 0 | 0 | 0.01134 |
| $D_T$ | − 0.769 | − 2.513*** | − 2.016* | − 2.482*** | − 1.838* | − 2.347** | − 2.879*** |
| Max K | 0.42857 | 14.75976 | 0.13333 | 0 | 0 | 0 | 3.36750 |

h: number of haplotypes; s: segregating sites; Hd: haplotype diversity; p: nucleotide diversity; DT: Tajima's D value; K: average number of nucleotide differences, Tajima's D significance $p < 0.001$ is marked by ***$p < 0.01$ marked by **$p < 0.05$ marked by *

**Table 22** Pairwise $F_{ST}$ (below diagonal) for D loop region of common carp from different geographical locations

| Population | Andhra Pradesh | Madhya Pradesh | Maharashtra | Haryana | Manipur | Tripura |
|---|---|---|---|---|---|---|
| Andhra Pradesh | – | – | – | – | – | – |
| Madhya Pradesh | 0.02065 | – | – | – | – | – |
| Maharashtra | 0.04418 | 0.01973 | – | – | – | – |
| Haryana | 0.05714 | 0.02131 | 0 | – | – | – |
| Manipur | 0.05714 | 0.02131 | 0 | 0 | – | – |
| Tripura | 0.05714 | 0.02131 | 0 | 0 | 0 | – |

**Table 23** AMOVA hierarchy design and analysis results of six common carp populations

| Source of variation | df | Sum of squares | Variance components | Percentage of variation | Fixation indices |
|---|---|---|---|---|---|
| Analysis1 (AP & MP & MH) (HR) (MN & TR) | | | | | |
| Among groups | 2 | 0.154 | 0.00027 Va | 0.66 | FSC: 0.02038 |
| Among populations within groups | 3 | 0.188 | 0.00083 Vb | 2.02 | FST: 0.0268 |
| Within populations | 163 | 6.4846 | 0.03979 Vc | 97.32 | FCT: 0.00655 |
| Total | 168 | 6.828 | 0.04089 | | |
| Analysis2 (AP) (MH) (MN & TR & MP & HR) | | | | | |
| Among groups | 2 | 0.231 | 0.00196 Va | 4.72 | FSC: – 0.00247 |
| Among populations within groups | 3 | 0.111 | – 0.00010 Vb | – 0.24 | FST: 0.0448 |
| Within populations | 163 | 6.486 | 0.03979 Vc | 95.52 | FCT: 0.04716 |
| Total | 168 | 6.828 | 0.04166 | | |
| Analysis3 (AP) (MN & TR & MP & HR & MH) | | | | | |
| Among groups | 1 | 0.215 | 0.00473 Va | 10.69 | FSC: – 0.00687 |
| Among populations within groups | 4 | 0.127 | – 0.00027 Vb | – 0.69 | FST: 0.10078 |
| Within populations | 163 | 6.486 | 0.03979 Vc | 89.92 | FCT: 0.10691 |
| Total | 168 | 6.828 | 0.04425 | | |
| Analysis4 (AP) (MP & HR & MH) (TR & MN) | | | | | |
| Among groups | 2 | 0.250 | 0.00194 Va | 4.68 | FSC: – 0.00768 |
| Among populations within groups | 3 | 0.092 | – 0.00030 Vb | – 0.73 | FST: 0.03949 |
| Within populations | 163 | 6.486 | 0.03979 Vc | 96.05 | FCT: 0.04681 |
| Total | 168 | 6.828 | 0.04143 | | |

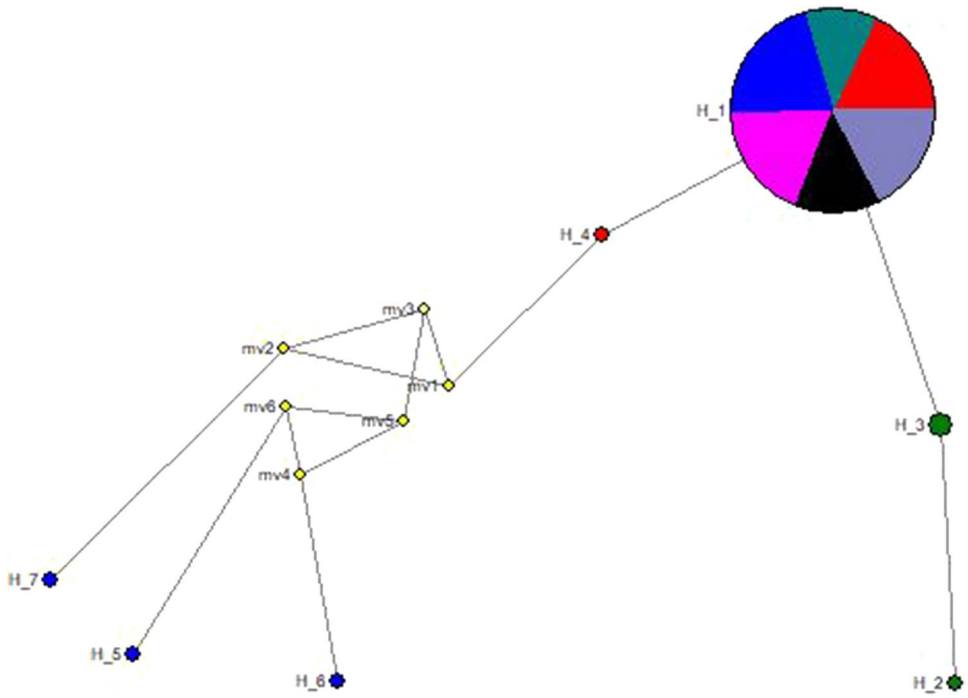


**Fig. 9** Haplotype network for common carp stocks based on mito D loop sequences

The haplotype network shows that the stocks of Maharashtra representing haplotype H4 and Madhya Pradesh (haplotype H5, H6, and H7) are joined by many median vectors and thus are closely related (Fig. 9). Gene flow between Maharashtra and Madhya Pradesh stocks is possible since most haplotypes are connected across the populations. Two closely related haplotypes (H2 and H3) were observed at the network's edge for Andhra Pradesh stock. The

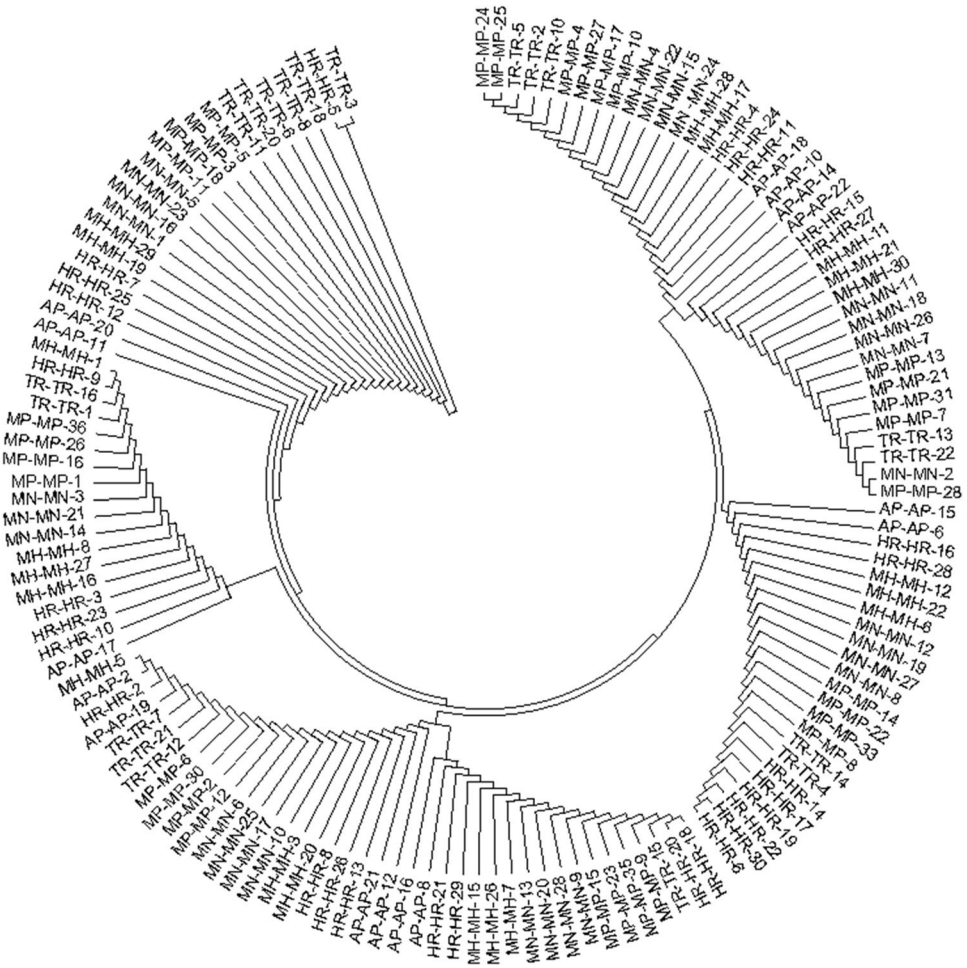


**Fig. 10** Phylogenetic tree for common carp stocks based on mito D loop sequences

Tripura and Andhra Pradesh stocks seem unique and distant from the rest of the population and, therefore, appear ancestral, conserving their haplotypes.

The phylogenetic tree is provided in Fig. 10. The samples from AP are distributed across the stocks. The AMOVA and phylogenetic tree construction using the NJ method relies on different principles. AMOVA examines the variance within and between groups based on genetic distances, while the NJ method constructs a tree based on pairwise genetic distances. The genetic distances used in both analyses may be capturing different aspects of the data, leading to discrepancies. The tree topology in present study does not reflect or be congruent with the AMOVA because the algorithms for both are different.

## 4 Discussion

In India, the common carp is widely cultured in monoculture and polyculture systems along with the major Indian carps. The present population of common carp in the country is an intermixer of only four/five introductions/imports. This population has been established from this limited founder number, and adverse selection coupled with inbreeding and breeding at younger ages has reduced the productivity of common carp. The lack of high-performing strains developed from selective breeding programs further threatens the common carp industry. In this regard, a selective breeding program for common carp exploiting its salinity and cold tolerance potential is initiated at ICAR-CIFE, India. The program aims to utilize fallow degraded soils and underground saline water for common carp culture. A base population for common carp was formed from various geographical populations of the country. Evaluating the standing genetic diversity at initiating a selective breeding program is imperative to minimize the inbreeding and associated risks of inbreeding depression. The present study assessed the genetic diversity utilizing truss morphometry and Mitochondrial D loop marker.

In the present study, the result obtained from the truss-based morphometrics indicated two major groupings among the six stocks viz., Andhra Pradesh-group1 and the rest of other stocks-group2. Such indications of stock structure arise from consideration of the first, second, and third factors. This analysis confirmed the variation evident in the middle part of the body, the head portion, and the caudal portion of the body. The factor analysis of common carp revealed meaningful loading on the central part of the body, the head, and the caudal portion. Similar studies in other fish species, too have delineated the stocks based on factor analysis. The factor analysis in *D. russelli* showed meaningful loading of the middle portion, the portion below the second dorsal fin, above the anal fin, and the caudal portion on the first and second factors, respectively. The factor analysis revealed the existence of two morphologically different stocks of *D. russelli* between the east and west coasts of India [40]. Similar studies wherein the population structure of *Barbodes carnaticus* species was delineated using conventional (based on body morphometrics and meristic) and image-based analysis (truss network system) methods [41]. They concluded that stock discrimination of this species was mainly due to geographic isolation, river ecology, and temperature variations. Similarly, the stock structure of *Chanos chanos* (Forsskål, 1775) in Indian waters was deciphered by truss network and otolith shape analysis [42].

In common carp, it is difficult to distinguish the sex until secondary sexual characters develop visually. The present study attempted to differentiate sex in common carp based on morphology and truss morphometry. The females in the present study were comparatively heavier than males in terms of body weight, whereas based on the standard length, there was no significant. The females were heavier because the GSI values of the female were high due to gonadal weight. The fish's weight was recorded during February, and it is an active breeding season for common carp in North India. Various studies support this finding, viz., the lowest and highest GSI obtained were 1.1 and 4 for males and 10 for females *C. Carpio* [43]. The higher GSI value was seen from February to April. The overall mean standard length was 17.07 ± 0.07 cm (female- 17.04 ± 0.07 cm and males- 17.16 ± 0.08 cm). Similar values for size at maturity of *C. carpio* are reported by various authors, viz., 17 cm for males and 21.5 cm for females [44], 15.8 cm and 22.5 cm for males and females [44], 27 cm and 28.3 cm for males and females [45, 46], 27 cm and 28.3 cm for males and females [45, 46]. A previous study on common carp reported that length is a good indicator of sex differentiation [47]. However, in our study, there was no significant difference in standard length between the sexes, and it can be concluded that the standard length was not a good indicator of sex differentiation for fish of the exact age. The growth of the common carp is sexually dimorphic, with the growth rate of females being at least 10% greater than that of males, especially after the juvenile stage [48, 49]. Similar results were obtained in the present study, wherein the body weight of the female sex was significantly higher than the males.

Out of the 39 traits in the present study, the traits viz; DAW, DCVCW, DCVCPW, VCVCL, EDAW, and EAAL were found to be significantly different between the sexes. The male sex of common carp of Andhra Pradesh and Tripura separates markedly from the male sex of other stocks. Similarly, the female sex of Andhra Pradesh separates distinctly from the rest of the females of different stocks. However, the female sex of Tripura stock exhibits decreased separation from females of the other stock. Truss morphometric method has been proven to be able to identify differences in the secondary sex of various fish species in which the dimorphisms are generally uncorrelated unclear such as in goldfish [50], gourami in pre-matured stadia [51], tilapia [52] and snakeskin gourami [53]. The factor analysis in the present study revealed significant loadings on body measurements that could delineate the sex in common carp. Factor 1 in male fish shows significant loadings in the dorsal region of the body (2 to 3 and 3 to 4), and body depth is the major contributor (points 3 to 10, 3 to 11, and 3 to 9). Factor 1 in female fish shows significant loadings in the ventral region of the body (9 to 10, 8 to 9, 2 to 9, and 4 to 10). The females of common carp were sexually mature when the observations were taken, and the loading points mentioned support this. Factor 2 in both male and female common carp exhibits significant loadings in the head region, suggesting the head region is the second most important contributor to the total variation. Similar studies revealed the sex determination of Kissing Gourami (*Helostoma temminckii* Cuvier, 1829) using the truss morphometrics method [54]. The results revealed that the truss morphometrics method could differentiate the male–female kissing gourami. A similar study was conducted on sexual dimorphism of Malaysian Mahseer, *Tor tambroides* brood stock by truss morphometry [55]. The morphometric characteristics included seven conventional and 21 Truss network system characteristics. The results delineated the sex determination/dimorphism of *T. Tambroides* broodstock, wild and hatchery-reared broodstock.

Mitochondrial markers have been widely used compared to nuclear markers to discriminate population and demography due to their high mutation rate, uniparental inheritance, and haploid nature [56]. In the present study, the results delineate genetic variation among geographical stocks. The average haplotype diversity of the entire population was 0.08129, and the average nucleotide diversity was estimated to be 0.01134. A similar study was conducted, where they assessed the genetic diversity in six stocks of common carp collected from Hungary, Indonesia, and Vietnam using the RAPD marker [57]. The intrapopulation similarity index was higher, but the interpopulation similarity was lower (20%). As

a result, two clusters were formed among common carp stocks, indicating the existence of considerable genetic variation among geographically isolated populations. However, our study reported high genetic diversity from the local stocks, though no stocks were imported from other countries. One reason that could be attributed to significant genetic variation within local stocks could be the marker of choice, "mtDNA D loop". The evolutionary rate of the mtDNA is about five to ten times faster than the nuclear genome because mutation accumulates slowly in nuclear genes [58]. The population pair-wise $F_{ST}$ values, AMOVA, and phylogenetic analyses in the present study indicated low genetic differentiation in *Cyprinus carpio* populations. Similar results were reported for the population genetic structure, demographic history, and migration patterns of the common carp from eight major drainages across China using mitochondrial COI and D loop sequences (1494 bp) from 241 individuals [59]. The AMOVA showed low population differentiation, with 11.60% molecular variance among river drainages, and the pair-wise $F_{ST}$ values between river drainages were moderate (0.0331–0.2617). The present study indicates population expansion for Madhya Pradesh, Maharashtra, Manipur, and Tripura stocks. Tajima's D value is sensitive to population fluctuation, and a significant negative value implies population expansion, while a positive Tajima's D value indicates population decline or over-dominant selection [38, 39, 60].

In conclusion, the truss analysis suggests variation among stocks, with Andhra Pradesh forming a distinct group and the rest others forming a different group. The truss analysis also revealed sexual dimorphism in common carp. Similar results were also observed in the mtDNA D-loop studies wherein Andhra Pradesh stocks form distinct groups with the rest of the population. The information generated in the present study will be helpful for the ongoing selective breeding program of common carp and the genetic management of this species in India.

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**Data availability** The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate** The study is approved by the Ethics Committee of ICAR-CIFE, Mumbai. The study was carried out in compliance with the Basel Declaration and ARRIVE guidelines. The authors confirm that the manuscript has been read and approved by named authors. The authors confirm that the order of authors listed in the manuscript has been approved by the named authors.

**Consent for publication** All the named authors agree to submit the paper for publication in Discover Animals.

**Competing interests** The authors declare no competing interests.



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