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Landmark-Based Morphometric and Meristic Variations of the Endangered Carp, Kalibaus *Labeo calbasu*, from Stocks of Two Isolated Rivers, the Jamuna and Halda, and a Hatchery

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Mostafa A. R. Hossain, Md. Nahiduzzaman, Debasish Saha, Mst. U. Habiba Khanam, and Md. S. Alam (2010) Landmark-based morphometric and meristic variations of the endangered carp, kalibaus *Labeo calbasu*, from stocks of two isolated rivers, the Jamuna and Halda, and a hatchery. *Zoological Studies* **49**(4): 556-563. Landmark-based morphometrics were examined to evaluate the population status of the endangered carp, kalibaus *Labeo calbasu*, collected from 2 isolated rivers (the Jamuna and Halda) and a hatchery. Morphometric characters along with truss network measurements and meristic counts were applied. Significant differences were observed in four (maximum body height, pre-orbital length, peduncle length, and maxillary barbel length) of 12 morphometric measurements, two (pectoral fin rays and scales above the lateral line) of 9 meristic counts, and four (8 to 9, 3 to 10, 2 to 10, and 1 to 11) of 22 truss network measurements among the stocks. For morphometric and landmark measurements, the 1st discriminant function (DF) accounted for 75.5% and the 2nd DF accounted for 24.5% of the among-group variability, and together they explained 100% of the total among-group variability. For the morphometric and truss network measurements, plotting DFs revealed high isolation of the stocks. The dendrogram based on morphometric and truss distance data placed the Jamuna and hatchery in 1 cluster and the Halda in another cluster, and the distance between the Halda and hatchery populations was the highest. http://zoolstud.sinica.edu.tw/Journals/49.9/556.pdf

Key words: Landmark, Morphometrics, Meristics, Fish stock, *Labeo calbasu*.

Bangladesh is a country of deltaic plains dominated by 3 major river systems: the Padma, Jamuna, and Meghna. Another important river is the Halda, which is geographically isolated from the 3 major river systems and is famous as a natural breeding ground of Indian major carp (ICM). The country has a rich diversity of various fish species and is ranked 3rd in inland fisheries in Asia after China and India with approximately 300 fresh- and brackish-water fish species (Hussain and Mazid 2001). Fish and fisheries play important roles in the economy of Bangladesh, and the country produces about 2.4 × 10^6 metric tons of fish per year. About 78% of the total production comes from inland resources, of which 42% is from culture and 36% from captured fisheries (DoF 2007).

Like other IMCs, kalibaus (*Labeo calbasu*, Hamilton 1822) is a teleost fish species distributed in Bangladesh, India, Pakistan, Myanmar, Thailand, and also South China (Day 1878). Once the species was abundant in all natural water bodies: rivers, beels (relatively large water bodies with static water in the Ganga-Brahmaputra floodplains of Bangladesh), haors (wetlands in the northeastern part of Bangladesh which physically are bowl- or saucer-shaped shallow depressions), baors (oxbow lakes, found mostly in the moribund delta as in northeastern Bangladesh), and lakes

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of Bangladesh. During the early 1980s, kalibaus was of great commercial importance like 3 other ICMs (Rohu *Labeo rohita*, Catla *Gibelion catla*, and Mrigal *Cirrhinus mrigala*), but fish farmers lost interest due to the unavailability of seeds from either natural or artificial sources. Gradually the abundance of the species seriously decreased in nature due to overfishing, habitat degradation, aquatic pollution, and several other anthropological reasons. Presently, kalibaus is regarded as endangered (IUCN-Bangladesh 2000) and in need of immediate measures to protect and conserve it.

Information on the biology and population structure of any species is a prerequisite for developing management and conservation strategies (Turan et al. 2006) and maybe applicable for studying short-term and environmentally induced variations. Morphometric differences among stocks of a species are recognized as important for evaluating the population structure and as a basis for identifying stocks (Ihssen et al. 1981, Templeman 1983, Smith and Jamieson 1986, Turan 2004, Turan et al. 2004b, Vishalakshi and Singh 2008, Randall and Pyle 2008). Morphometric and meristic characters of fish are the measurable or countable characters common to all fishes. Landmarks refer to some arbitrarily selected points on a fish's body, and with the help of these points, the individual fish shape can be analyzed. In other words, a landmark is a point of correspondence on an object that matches between and within populations (Barlow 1961, Swain and Foote 1999). Truss network systems constructed with the help of landmark points are powerful tools for stock identification. A sufficient degree of isolation may result in notable morphological, meristic, and shape differentiation among stocks of a species which may be recognizable as a basis for identifying the stocks. The characteristics may be more applicable for studying short-term, environmentally induced disparities, and the findings can be effectively used for improved fisheries management (Ihssen et al. 1981, Templeman 1983, Smith and Jamieson 1986, Turan 2004, Turan et al. 2004a b).

Only limited information is available on kalibaus morphometry (Rao and Rao 1972, Gupta and Jhingran 1973, Chatterji et al. 1980, Rahman 2005), and there have been few attempts to evaluate the population structure using different methods based on phenotypical and genetic aspects. The present study deals with the population structure of kalibaus from a phenotypical point of view to determine the morphometrics among the stocks.

MATERIALS AND METHODS

Collection of samples

During Oct. - Dec. 2007, 129 kalibaus (*L. calbasu*) were collected live in oxygenated polythene bags from 3 sources: 2 rivers, the Jamuna River in Bogra District and the Halda River in Chittagong, and a hatchery in Mymensingh (Fig. 1). The fish were 11.3-36.1 cm in total length (TL) and 18-475 g in weight. The sample size, mean TL, and weight are presented in table 1.

Twelve morphometric characters and body weights of the fish were measured to an accuracy of 0.05 mm and 1.0 g, respectively (Table 2). In total, 9 meristic characters (dorsal, pectoral, pelvic, anal, and caudal fin rays, lateral line scales, scales above and below the lateral line, and vertebra counts) were analyzed. Eleven landmarks delineating 22 distances were measured on the body (Fig. 2). Each landmark was obtained by placing a fish on graph paper, and then the landmarks were detected with colored pointers. Finally, distances on the graph paper were measured using vernier calipers.

Statistical analyses

Prior to the analysis, size effects from the dataset were eliminated. Variations were attributed to body shape differences, and not to the relative sizes of the fish. In the present study, there were significant linear correlations among all measured characters and the TL of the fish. Therefore, it was necessary to remove size-dependent variations from all of the characters. An allometric formula given by Elliott et al. (1995) with slight modification was used to remove the size effect from the dataset:

$$M_{adj} = M\,(L_s / L_o)^{b};$$

where M is the original measurement, M_{adj} is the size-adjusted measurement, L_o is the TL of the fish, and L_s is the overall mean of the TL for all fish from all samples. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log L_o, using all fish in all groups. The efficiency of the size-adjustment transformations was assessed by testing the significance of the correlation between a transformed variable and the TL. The sex of specimens was determined macroscopically, and there were no significant differences of tested variables between the sexes within the same stock. Meristic characters were compared using the non-parametric Kruskal-Wallis test. A univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. In addition, size-adjusted data were standardized and submitted to a discriminant function (DF) analysis (DFA). A dendrogram of the populations based on the morphometric and landmark distance data was drawn using the unweighted pair group method analysis (UPGMA). All statistical analyses

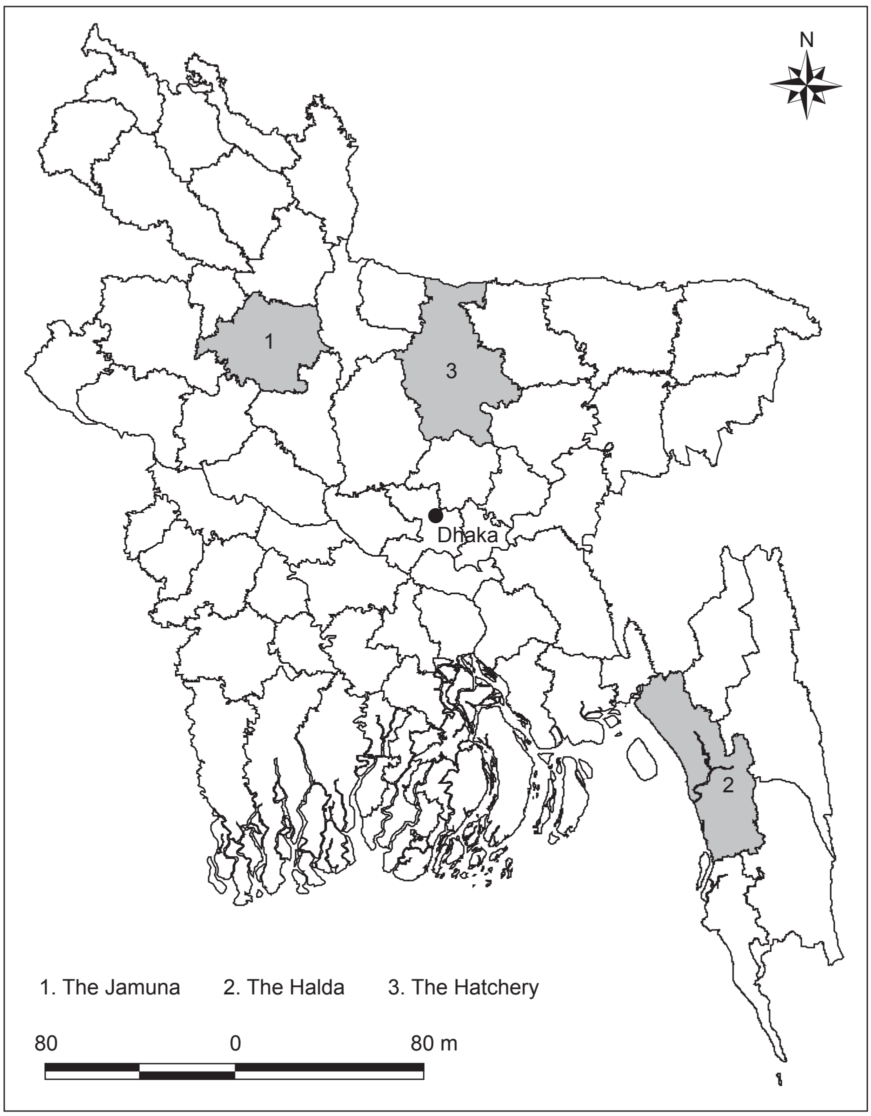

Fig. 1. Map of Bangladesh showing sampling sites of *Labeo calbasu*.

Table 1. Collection of *Labeo calbasu* samples from different stocks

Source of fish samples	Status of the fish stocks	Sample size	Mean total length (cm) ± S.E.	Mean weight (g) ± S.E.
Jamuna River in Bogra District	Wild	47	31.25 ± 0.65	293.63 ± 23.66
Halda River at Chittagong	Wild	29	24.56 ± 0.09	159.76 ± 0.86
Hatchery at Mymensingh	Hatchery	53	16.11 ± 0.6	45.33 ± 3.40

were done using SPSS vers. 11.5 (SPSS, Chicago, IL, USA).

RESULTS

Meristic counts

Meristic counts of all samples ranged 16-18 (median (m_e), 17) for dorsal fin rays, 7 or 8 (m_e, 8) for anal fin rays, 26 or 27 (m_e, 27) for caudal fin rays, 17-19 (m_e, 18) for pectoral fin rays, 9 for pelvic fin rays, 41-43 (m_e, 42) for lateral line scales, 7.5-8.5 (m_e, 7.5) for scales above the lateral line, 6.5-7.5 (m_e, 6.5) for scales below the lateral line, and 32 or 33 (m_e, 33) for the number of vertebrae. Meristic counts were compared among the 3 populations (Jamuna, Halda, and hatchery populations). Mean numbers of anal, caudal, and pelvic fin rays, and scales below the lateral line, and number of vertebrae did not differ among fish from these stocks (Kruskal-Wallis test, $p > 0.05$), but difference occurred in other characters ($d.f. = 2$, pectoral fin rays: H = 6.75, $p < 0.05$ and scales above the lateral line: H = 6.61, $p < 0.05$).

Morphometric and landmark distances

The efficiency of the allometric formula in removing the size effect from the data was justified by using correlations between the TL and adjusted characters. Among the 11 transformed morphometric and 22 truss measurements, 2 measurements (standard length and post-orbital length) were found to be significantly correlated ($p < 0.05$). Therefore, the 2 measurements were not considered for further calculation. None of the truss measurements or remaining 9 morphometric

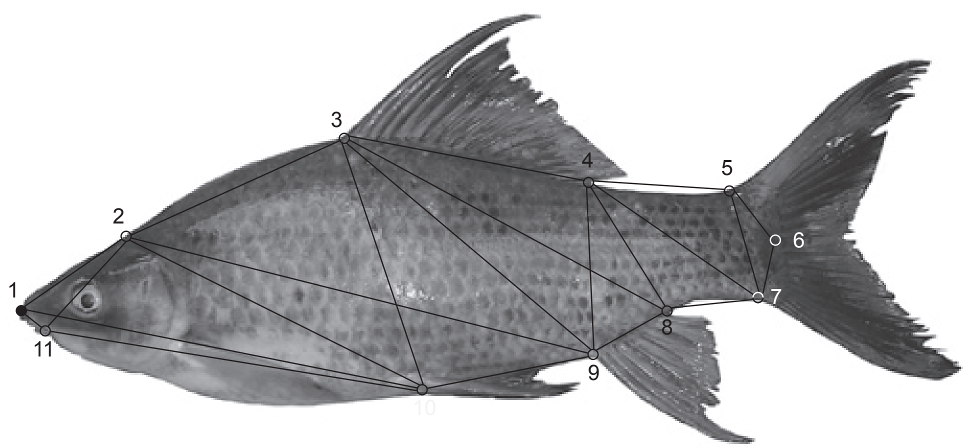

Fig. 2. Locations of 11 landmarks used for the shape analysis of *Labeo calbasu*.

Table 2. Morphometric characters used for analysis of *Labeo calbasu* stock variations

Character	Description
Total length (TL)	Distance from the tip of the snout to the longest caudal fin ray
Standard length (SL)	Distance from the tip of the snout to the end of the vertebral column
Fork length (FL)	Distance from the tip of the snout to the middle part of the fork of the tail
Head length (HL)	Distance from the tip of the snout to the posterior margin of the opercula
Maximum body height (MBH)	Vertical distance from the anterior part of the 1st dorsal fin and ventral part of the body
Eye length (EL)	Diameter of the eye
Pre-orbital length (PrOL)	Distance from the tip of the snout to the anterior margin of the eye
Post-orbital length (POL)	Distance from the posterior margin of the eye to the end of the operculum
Peduncle length (PL)	Length of the peduncle
Pre-dorsal length (PrDL)	Distance from the snout tip to the anterior base of the dorsal fin
Maxillary barbel length (MxBL)	Length of the maxillary barbel
Mandibular barbel length (MnBL)	Length of the mandibular barbel

measurements showed a significant correlation with TL. Univariate statistics (ANOVA) showed that 4 (maximum body height (MBH), pre-orbital length (PrOL), peduncle length (PL), and maxillary barbel length (MxBL)) of 9 morphometric and 4 (8 to 9, 3 to 10, 2 to 10, and 1 to 11) of the 22 truss measurements significantly differed to varying degrees ($p < 0.05$ or < 0.001) among samples (Table 3).

The DFA produced 2 DFs (the 1st and 2nd DFs) for both morphometric and landmark measurements. For morphometric and landmark measurements, the 1st DF accounted for 75.5% and the 2nd DF accounted for 24.5% of among-group variability, and together they explained 100% of the total among-group variability.

Table 3. Univariate statistics (ANOVA) testing differences among samples from 9 morphometric and 22 truss measurements

Character	Wilks' lambda	F	Significance
FL	0.98	0.60	0.53
HL	0.94	1.85	0.17
MBH	0.84	5.74	0.005*
EL	0.96	1.26	0.29
PrOL	0.82	6.93	0.002*
PL	0.32	67.43	0***
PrDL	0.92	2.85	0.065
MxBL	0.85	5.59	0.006*
MnBL	0.97	1.07	0.35
1 to 2	0.99	0.06	0.945
2 to 3	0.96	1.42	0.249
3 to 4	0.98	0.75	0.476
4 to 5	0.96	1.24	0.296
5 to 6	0.98	0.80	0.455
6 to 7	0.98	0.62	0.543
5 to 7	0.99	0.20	0.816
7 to 8	0.96	1.33	0.273
4 to 7	0.99	0.13	0.877
4 to 8	0.99	0.13	0.882
4 to 9	0.99	0.08	0.924
8 to 9	0.91	3.25	0.045*
2 to 9	0.98	0.81	0.451
3 to 8	0.99	0.47	0.625
3 to 9	0.95	1.59	0.212
9 to 10	1	0.01	0.996
3 to 10	0.69	14.28	0***
2 to 10	0.835	6.57	0.003**
1 to 10	0.98	0.66	0.521
10 to 11	0.98	0.60	0.551
2 to 11	0.99	0.49	0.617
1 to 11	0.90	3.39	0.04**

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Characters are defined in table 2.

Pooled within-group correlations between discriminant variables and DFs revealed that among the 9 morphometric measurements, 7 measurements of PL, PrOL, MBH, MxBL pre-dorsal length (PrDL), head length (HL), and fork length (FL) dominantly contributed to the 1st DF, while the remaining 2 (eye length (EL) and mandibular barbel length (MnBL)) contributed to the 2nd DF (Table 4). Among the 22 truss measurements, 10 measurements (10 to 11, 3 to 8, 2 to 9, 4 to 8, 2 to 3, 4 to 5, 7 to 8, 6 to 7, 5 to 7, and 1 to 2) dominantly contributed to the 1st DF, and the remaining 12 contributed to the 2nd DF (Table 4).

A dendrogram based on morphometric and landmark distance data was constructed for the Jamuna, Halda, and hatchery populations. The distances of squared Euclidean dissimilarity values were nearest between the Jamuna and hatchery populations. The Halda population was isolated from other 2 populations (Fig. 3).

DISCUSSION

In the present study, meristic counts of all samples ranged 16-18 rays for the dorsal fin, 17-19 rays for the pectoral fin, 9 rays for the pelvic fin, 7 or 8 rays for the anal fin, and 26 or 27 rays for the caudal fin. Lateral line scales, scales above the lateral line, scales below the lateral line, number of vertebrae ranged 41-43, 7.5-8.5, 6.5-7.5, and 32 or 33, respectively. These results are similar to those reported by Rahman (2005) for kalibaus. The mean numbers of pectoral fin rays and scales above the lateral line significantly differed ($p < 0.05$) among the 3 stocks. Nakamura (2003) found differences in meristic counts in Japanese charr (*Salvelinus leucomaenis*) among the river systems (Naka and Tone rivers, central Japan) and among the tributaries of the Naka River (Ashinagasawa, Akasawa, Ushirosawa and Moto-okashirasawa streams). In the present study, highly significant morphological variations were found among the Halda, Jamuna, and hatchery kalibaus populations. The phenotypic discreteness suggests a direct relationship between the extent of phenotypic divergence and geographic separation, which indicates that geographic separation is a limiting factor to migration among stocks. Turan et al. (2004a) also found similar results for *Liza abu* (Heckel 1843) populations from the Orontes, Euphrates, and Tigris Rivers in Turkey.

Morphometric differences among stocks are expected, because they are geographically separated and may have originated from different ancestors. Therefore, it is not unlikely that obvious environmental variations exist in these 3 habitats (the Halda and Jamuna Rivers, and the hatchery).

Fish are very sensitive to environmental changes and quickly adapt themselves by changing necessary morphometrics. It is well-known that morphological characters can show high plasticity in response to differences in environmental conditions, such as food abundance and temperature (Allendorf and Phelps 1988, Swain et al. 1991, Wimberger 1992). In general, fish demonstrate greater variances in morphological traits both within and between populations than any other vertebrates, and are more susceptible to environmentally induced morphological variations (Allendorf et al. 1987, Wimberger 1992).

The phenotypic plasticity of fish is very high. They adapt quickly by modifying their physiology and behavior to environmental changes. These modifications ultimately change their morphology (Stearns 1983). In a small country like Bangladesh, there are probably very small environmental changes from place to place. In spite of this, the Halda River possesses a unique environment that differs from other rivers of Bangladesh. However, due to small environmental differences, the resulting morphological differences in fish may be so small that they might be impossible to discern with gross morphomeristic characters. Therefore, truss network measurements were employed in this experiment. Truss network systems are a powerful tool for identifying stocks of fish species (Turan et al. 2004b). An unbiased network of morphometric measurements over a 2 dimensional outline of a fish removes the need to find the types of characters and optimal number of characters for stock separation, and provides information over the entire fish form (Turan et al. 2004b). The truss network system can effectively be used to distinguish between the hatchery and wild stocks. In this case, more-significant differences were expected because of the 2 completely different habitats i.e., one is an open-water habitat and the other is closed water. Environmentally induced phenotypic variations; however, may

Table 4. Pooled within-group correlations between discriminating variables and discriminant functions (DFs; variables ordered by size of correlation within function, *denotes the largest correlation between each variable and DFs)

Character	First DF	Second DF
PL	0.267*	0.001
PrOL	-0.086*	-0.006
10 to 11	-0.080*	-0.076
MBH	-0.076*	-0.031
MxBL	-0.074*	0.042
3 to 8	-0.065*	-0.019
2 to 9	-0.057*	-0.010
PrDL	-0.055*	0.013
4 to 8	-0.049*	0.010
HL	-0.044*	-0.016
2 to 3	-0.037*	-0.021
4 to 5	0.036*	0.009
7 to 8	0.036*	-0.024
FL	-0.025*	0.012
6 to 7	-0.025*	-0.014
5 to 7	-0.015*	-0.002
1 to 2	-0.008*	0.003
8 to 9	-0.067	-0.159*
2 to 11	-0.020	-0.098*
4 to 7	0.074	0.079*
2 to 10	0.064	0.076*
9 to 10	0.053	-0.073*
EL	0.011	0.070*
MnBL	-0.007	0.067*
3 to 10	-0.003	-0.062*
1 to 11	0.047	0.050*
1 to 10	-0.032	-0.043*
3 to 9	-0.031	-0.042*
3 to 4	-0.024	-0.031*
5 to 6	-0.025	-0.030*
4 to 9	-0.011	0.012*

Characters are defined in table 2.

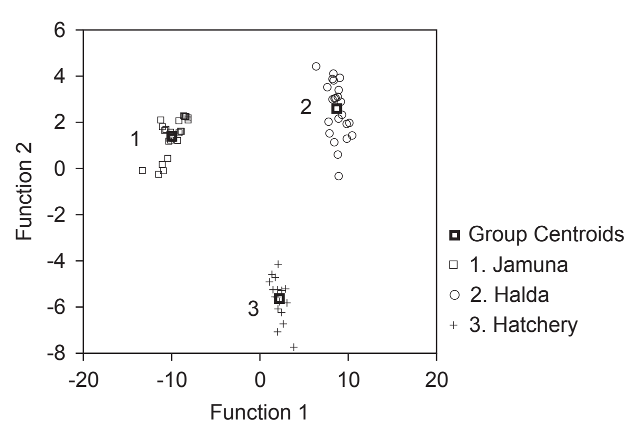

Fig. 3. Sample centroids of the discriminant function scores based on morphometric and truss measurements.

have advantages in the stock structure analysis of exploited species, especially when the time is insufficient for significant genetic differentiation to accumulate among populations. Genetic markers might not be sufficient to detect existing genetic variation among populations, and also only a small proportion of DNA is analyzed by genetic markers.

Relationships among the 3 stocks differed according to whether the 1st or 2nd DF was considered (Fig. 3). Considering 1st DF, the hatchery stock displayed intermediate characteristics between the Jamuna and Halda stocks. As the hatchery owners use broodfish collected from both the Jamuna and Halda Rivers, 2 major natural sources of kalibaus in the country, the hatchery stocks showed some nearness to both stocks. Based on the 2nd DF, the Jamuna and Halda stocks, however, broadly overlapped, while the hatchery stock clearly differed. The 1st DF accounted for much more (75.5%) of the among-group variability than did the 2nd DF (24.5%). It is obvious that the 2nd DF explains much less of the variance than does the 1st DEF. The 2nd DF is; therefore, much less informative in explaining differences among the stocks.

The dendrogram employed in this study resulted in 2 clusters: the Jamuna and hatchery stocks in one and the Halda stock in another (Fig. 4). The difference between the hatchery and wild stocks may have been due to environmental as well as genetic variations.

Plotting DFs revealed high isolation in morphometrics among the stocks. The results of the study are useful as baseline information of *L. calbasu* populations for further studies. In both aquaculture and open-water management, it is essential to select genetically superior stocks along with better features. More research especially genetic studies and investigations of the impacts of environmental factors is needed for conservation and mass seed production of selected stocks to pave the way to saving this endangered species from extinction.

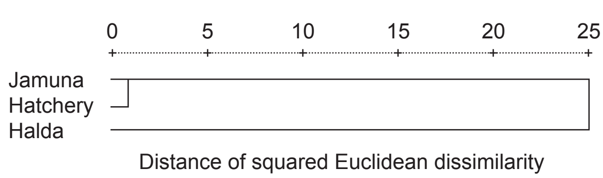

Fig. 4. Dendrogram based on morphometric characters and landmark distances of the Jamuna, Halda and hatchery populations.

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