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COMPARATIVE MORPHO-MERISTIC VARIATIONS OF ASIAN STINGING CATFISH (*HETEROPNEUSTES FOSSILIS*) COLLECTED FROM NATURAL AND HATCHERY SOURCES IN BANGLADESH

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ABSTRACT: The present study sought to differentiate various sources of *Heteropneustes fossilis* samples morphologically using two hundred and twenty-five samples from six natural and three hatchery sources in Bangladesh. Eighteen morphometric and five meristic characters were studied, and the analysis highlighted that natural stocks were significantly different from hatchery stocks. The multivariate analysis showed a significant difference ($p<0.05$) in morphometric features between Asian stinging catfish from natural and hatchery origins such as pectoral fin length (PCFL), pre-anal fin length (PAL), pre-pectoral fin length (PPCL), length of dorsal fin base (LDFB), body width (BW), eye diameter (ED), postorbital (Post OL) as well as meristic characters i.e. caudal fin ray (CFR). Significant differences in morphometric and meristic characteristics were also found using the nonparametric Kruskal-Wallis (H) test. Natural and hatchery sources of *H. fossilis* strongly differ in standard length (SL) and post-orbital length (Post OL), as identified by principal component analysis. Additionally, the discriminate analysis identified total length (TL), pre-anal fin length (PAL), pre-pectoral fin length (PPCL), and body width (BW) as distinguishing traits. According to hierarchical cluster analysis, there was a minimum divergence between the natural and hatchery sources, and it partially separated the two sources. However, hatchery-originated stinging catfish must be utilized carefully in Bangladesh's diverse cultural contexts; the current result may help determine if new morphotypes develop from genetic introgression and other causes.

Key words: Asian Stinging Catfish, Morphological variation, Nature and Hatchery populations, Morphotypes

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INTRODUCTION

The stinging catfish (*H. fossilis*) belongs to the family Heteropneustidae of the order Siluriformes, distributed in freshwater habitats of Bangladesh, India, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam. The members of the Siluriformes order are called catfish. The species can be distinguished by their elongated and compressed bodies, small mouths with fleshy lips, short dorsal fins, pectoral fins with serrated spine, and ribbon-like longer anal fins (Hossain *et al.*, 2013). The stinging catfishes are often inhabited in ponds, ditches, canals, flooded rice fields, swamps, marshes, waterlogged areas, and rivers of Bangladesh (Hossain *et al.*, 2013). There are up to six species of *Heteropneustes- H. fossilis* (Bloch, 1794) (Stinging catfish), *H. fuscus* (Plamoottil, 2022), *H. kemratensis* (Fowler, 1937), *H. longipectoralis* (Devi & Raghunathan, 1999), *H. nani* (Hossain et al., 2013), *H. microps* (Gunther, 1864). Among them, *H. fossilis,* and *H. nani* are found in Bangladesh (Hossain et al., 2013). *H. fossilis*, locally known as 'Shing,' is the most popular and commercially essential catfish species due to its high market price and nutritional value, i.e., low-fat content and source of high amounts of iron and calcium (Hossain *et al.*, 2013). However, *H. fossilis* is reported from Bangladesh (Samad *et al.*, 2010; Hossain, 2011) with an observation of the high level of genetic variability within and between the populations (Nasren *et al.*, 2009).

Morphometric and meristic data for stinging catfish (*H. fossilis*) are crucial for several important reasons: species identification, population studies, age and growth analysis, health and condition assessment, ecological studies, taxonomy and phylogenetics, and conservation. There are morphometric differences between nature and hatchery sources *H. fossilis*. During a disaster, there is a high risk of hatchery-sourced fish escaping into nature, threatening natural fish's ecological and genetic integrity. Therefore, maintaining the morphological data of both nature and hatchery strains is crucial for distinguishing them.

Morphological analysis is a simple, cost-effective, and most common tool to identify and characterize fish stocks (Siddik *et al.*, 2016) and distinguish between fish populations (Siddik *et al.*, 2015). Rahman *et al.* (2019) reported morphometric and meristic characterization of *H. fossilis* in Bangladesh. Few reports on the genetic characterization of *Heteropneustes spp*. using microsatellite DNA markers (Sultana *et al.*, 2015); genetic variation and differentiation in *H. fossilis* populations assessed by heterologous microsatellite DNA markers (Nasren *et al.*, 2009); and genetic variation of *H. fossilis* obtained by RAPD analysis (Islam *et al.*, 2011) have been published in Bangladesh. There is no information about morphological differences between nature and hatchery stinging catfish in Bangladesh. Morphological analyses would be more potent in differentiating the nature and hatchery sources of *H. fossilis*. From this

perspective, to obtain rudimentary knowledge for species identification, broodstock development, seed development, and conservation, we have collected *H. fossilis* from Bangladesh and performed morphological analyses to differentiate nature and hatchery sources of stinging catfish.

MATERIAL AND METHODS

Sample Collection: Natural and hatchery-reared stinging catfish (*H. fossilis*) were gathered from various districts of Bangladesh. 150 samples were obtained from natural sources in six districts of Bangladesh, namely Cumilla, Gazipur, Bagerhat, Jamalpur, Pabna, and Sylhet, while 75 samples were collected from hatchery sources in three districts of Bangladesh, namely Rangamati, Jashore, and Mymensingh (Table 1); that primarily covered most of the divisions of Bangladesh. A total of 225 samples were used for morphometric and meristic analysis. The samples were measured, followed by (Rahman *et al.*, 2019) with some modifications (Figure 2).

Multivariate approaches were used to examine data from 18 morphometric and 5 meristic assessments of nature and hatchery individuals. The multivariate analysis of variance (MANOVA) was used to compare the mean of all morphological features in natural and hatchery populations. Separate analyses were performed for morphometric and meristic characters. The analysis used the raw meristic data since meristic characteristics were unaffected by fish size.

However, to avoid possible biases produced by size effects on the morphometric variables, all morphometric characters were standardized by the formula,

$ACi = \log OCi - [\beta \times (\log TLi - \log MTL)]$ (Claytor & MacCrimmon, 1987) where,

ACi is the adjusted logarithmic character measurements of the ith specimen (i=1,2, 3..);

OCi is the unadjusted character measurement of the ith specimen (i=1,2, 3..);

β is the common within-group regression coefficient of that character against total length after the logarithmic transformation of both variables;

TLi is the total length of the ith specimen (i=1,2, 3..), and MTL is the overall mean total length.

The allometric formula's effectiveness in eliminating the size effect from the data was supported by the correlation between total length and adjusted characters. Thus, total length was the first parameter to be removed and was not altered. All other parameters were then standardized using this parameter as the standard by a methodology similar to that used by Mollah *et al.* (2012) for *Glossogobius giuris*.

Table 1: List of the samples of *H. fossilis* used in the present study

Population	Source	Coordinates	Sample No.
Nature	Kodaliar Beel, Bagerhat	22°53'45.44"N 89°48'5.94"E	25
	Gomoti River, Cumilla	23°32'3.48"N 90°42'20.96"E	25
	Belai Beel, Gazipur	24° 2'3.33"N 90°30'10.15"E	25
	Pachkani Beel, Jamalpur	24°56'0.52"N 89°55'40.53"E	25
	Moktar Beel, Pabna	24° 3'30.38"N 89°30'3.01"E	25
	Borobagha Beel, Kushiara River, Sylhet	24°39'12.26"N 91°49'36.02"E	25
Hatchery	Provita Fish Hatchery, Mymensingh	24°45'24.08"N 90°24'33.77"E	25
	National Fish Hatchery, Jashore	23°14'1.11"N 89° 7'48.39"E	25
	Kaptai Lake, Rangamati	22°42'47.09"N 92°16'0.19"E	25
Total Sample			225

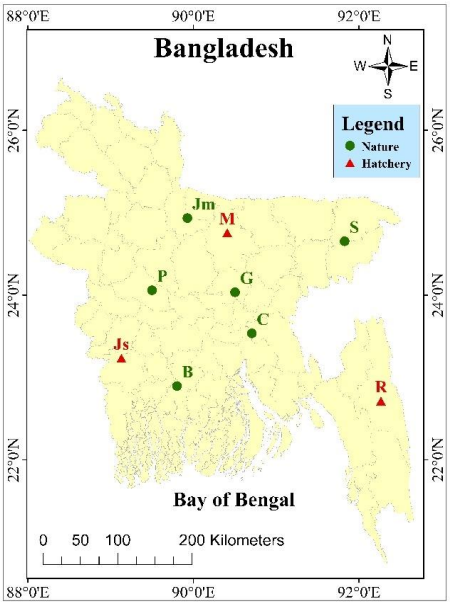

Fig. 1. The map illustrates the sampling locations used in the present study. B: Bagerhat, C: Cumilla, G: Gazipur, Jm: Jamalpur, Js: Jashore, M: Mymensingh, P: Pabna, R: Rangamati, S: Sylhet.

The Kruskal-Wallis (H) test was used to compare the group means. To illustrate the two groups' clustering pattern, two dendrograms were created using the ward linkage approach. IBM SPSS, version 26.0. IBM Corp (Armonk,

NY, USA) was used for the MANOVA test, the Kruskal–Wallis (H) test, and the construction of the dendrograms. The principal component analysis (PCA) was used to differentiate the sources of the highest variation owing to physical characteristics in nature and hatchery populations. The discriminant analysis

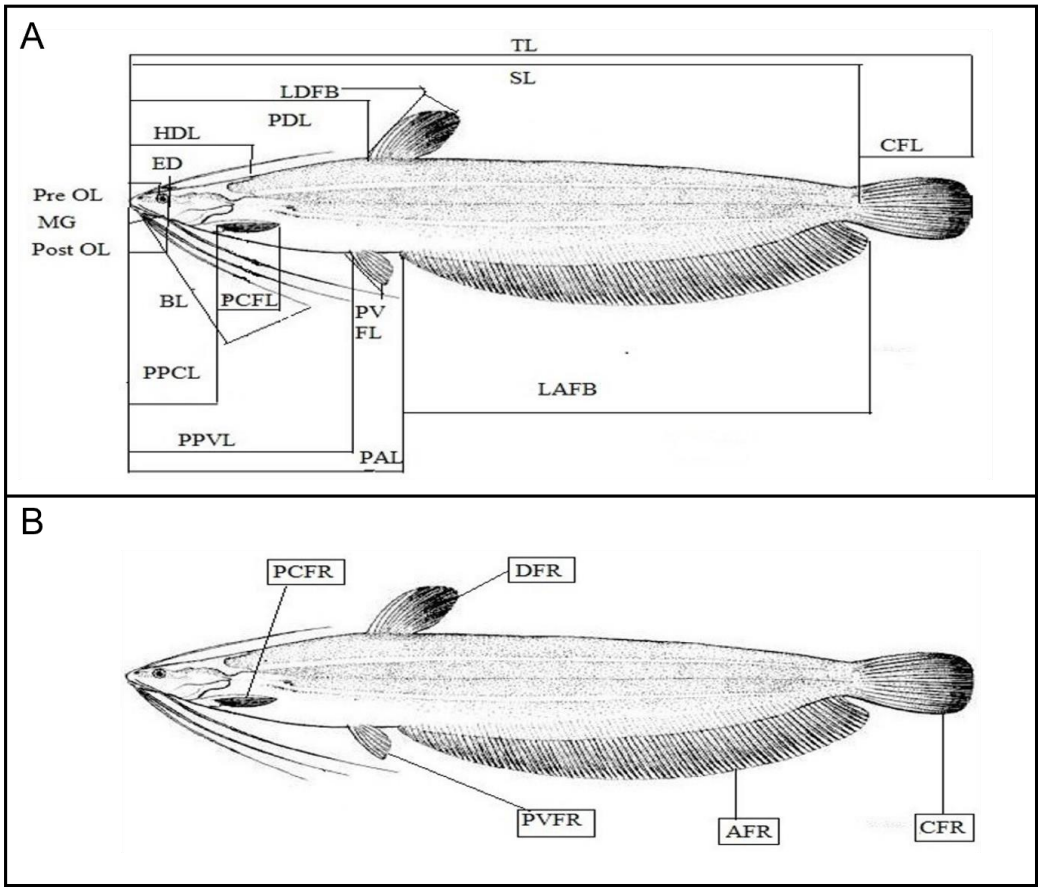

Fig. 2. The morphometric (A) and meristic (B) characteristics of *H. fossilis* followed in the present study. SL = Standard length, TL = Total length, PVFL = Pelvic fin Length, PCFL = Pectoral fin length, CFL = Caudal fin length, PDL= Pre dorsal fin length, PAL = Pre anal fin length, PPCL = Pre-pectoral fin length, PPVL = Pre pelvic fin length, LDFB = Length of dorsal fin base, LAFB = Length of anal fin base, BL= Barbel length, MG = Mouth gape, BW = Body width, ED = Eye diameter, HDL = Head length, Pre OL = Pre orbital length, Post OL = Post orbital length, DFR= Dorsal Fin Ray, PCFR=Pectoral Fin Ray, PVFR= Pelvic Fin Ray, AER= Anal Fin Ray, CFR= Caudal Fin Ray.

Data analysis

determined which variables discriminate between the two groups, considering all 18 measurements. For the box plot, principal component analysis (PCA), and discriminant analysis, the R tool (version 4.3.1) was utilized. All tests were conducted with a significance threshold of 5%.

RESULTS AND DISCUSSION

Morphometric data were acquired by measuring various sections of the body's length and width (18 measurements). Table 2 shows the descriptive statistics and the coefficient of variation (CV %) of the 18 morphometric measures from nature and hatcheries. The low CV of each measurement indicated less variation among the intra-class population, and each sample was phenotypically homogeneous. Meristic traits were studied by counting fin rays (5 counts), and the results are shown in Table 3.

Table 2: Descriptive statistics of the morphometric characters of Nature and Hatchery populations of *H. fossilis*

Characteristics	Nature		Hatchery	
	Mean ± SEM	Coefficient of variation (%)	Mean ± SEM	Coefficient of variation (%)
SL	14.81±1.05	17.34	15.03±1.04	11.95
TL	16.37±1.14	17.12	16.60±1.08	11.23
PVFL	1.17±0.09	18.35	1.16±0.07	9.87
PCFL	1.46±0.12	20.29	1.56±0.07	9.57
CFL	1.56±0.10	16.41	1.58±0.04	3.92
PDL	4.59±0.32	17.19	4.64±0.31	11.53
PAL	5.71±0.38	16.45	5.91±0.48	14.1
PPCL	2.19±0.16	18.01	2.04±0.09	7.43
PPVL	4.67±0.33	17.07	4.85±0.38	13.58
LDFB	1.26±0.09	17.96	1.36±0.04	4.57
LAFB	8.95±0.65	17.89	9.03±0.62	11.95
BL	4.43±0.47	25.76	4.22±0.52	21.38
MG	1.0±0.08	20.2	0.98±0.05	8.33
BW	1.94±0.17	20.87	2.03±0.16	13.59
ED	0.38±0.01	7.3	0.33±0.01	3.64
HDL	2.54±0.15	14.17	2.55±0.14	9.55
Pre OL	0.63±0.04	16.36	0.66±0.02	6.1
Post OL	1.0±0.06	15.27	0.95±0.02	3.31

*Mean value in cm

The morphometric features and meristic counts of the natural and hatchery strains obtained from various places demonstrated a considerable ($p<0.05$) variance in size. Due to their collection from different natural sources and cultivation on farms in Bangladesh, spatial heterogeneity was also noted

independently in the natural and hatchery populations. The mean TL and SL of the natural population were determined to be 16.37 cm and 14.81 cm, respectively, while the mean TL and SL of the hatchery population were 16.60 cm and 15.03 cm. Like these two, the two populations differed in every other morphometric parameter.

Table 3: Meristic characteristics of Nature and Hatchery populations of *H. fossilis*

Characteristics	Range	
	Nature	Hatchery
DFR	5-7	5-8
AFR	54-75	55-73
CFR	11-19	13-18
PVFR	5-7	5-7
PCFR	5-8	5-8

Regarding meristic counts, Hatchery sources had the highest observed number of dorsal fin rays (8), which was not recorded in natural sources. Only 1.33% of hatchery samples exhibited this maximum number (8 DFR). Additionally, 65.33% of hatchery samples and 81.33% of natural samples had 6 dorsal fin rays. Meanwhile, the nature group had the highest anal fin ray count of 75, but this was found in only 0.44% of the nature samples. The most common anal fin ray count for both nature and hatchery sources is 67. In the nature group, 11.33% of fish have this count, while in the hatchery group, 10.67% have it. In the nature group, the most common caudal fin ray count is 14, found in 31.33% of fish, while in the hatchery group, the most common count is 16, found in 33.33%. The most common pelvic fin ray count is 6 in both nature and hatchery groups, with 80% of fish in nature and 74.67% of fish in the hatchery having this count. The most common pectoral fin ray count for both nature and hatchery is 6, with 68.67% of fish in nature and 61.33% of fish in the hatchery having this count. Additionally, the hatchery population had the highest percentage (12.44%) of the maximum pectoral fin ray count (8).

Similar to this study, Shafi and Quddus (2001) reported 7 dorsal fin rays, 7 pectoral fin rays, 6 pelvic fin rays, 60-79 anal fin rays, and 19 caudal fin rays. In another study, Rahman *et al.* (2019) compared the morphology of *H. fossilis* with a limited sample from the same source and the fin count of *H. fossilis.* They started with 7 dorsal fins, 7-8 pectoral fin rays, 6 pelvic fin rays, 64-69 anal fin rays, and 16-18 caudal fin rays. In this study, extensive

sampling of *H. fossilis* from multiple sources of nature and hatchery was used to generate meristic characteristic ranges

Box Plot Analysis: The box whisker plot of the morphometric features also demonstrated differences between natural and hatchery populations of H. fossilis. The morphological measurement ranges of *H. fossilis* in nature were greater than those of hatcheries (Fig. 3). The median values for several characteristics, including PVFL, PPCL, LDFB, and ED, are typically higher in the nature group. The hatchery group frequently has higher median values for attributes like TL, SL, PCFL, PDL, PAL, PPVL, and HDL. The nature group tends to have more outliers and comprehensive ranges for several characteristics, suggesting more significant variability. Hatchery groups typically exhibit less variability due to their more compact ranges. These boxplots visually compare the distributions of different factors across the nature and hatchery groups by displaying the differences in central tendency (medians) and variability (range and outliers).

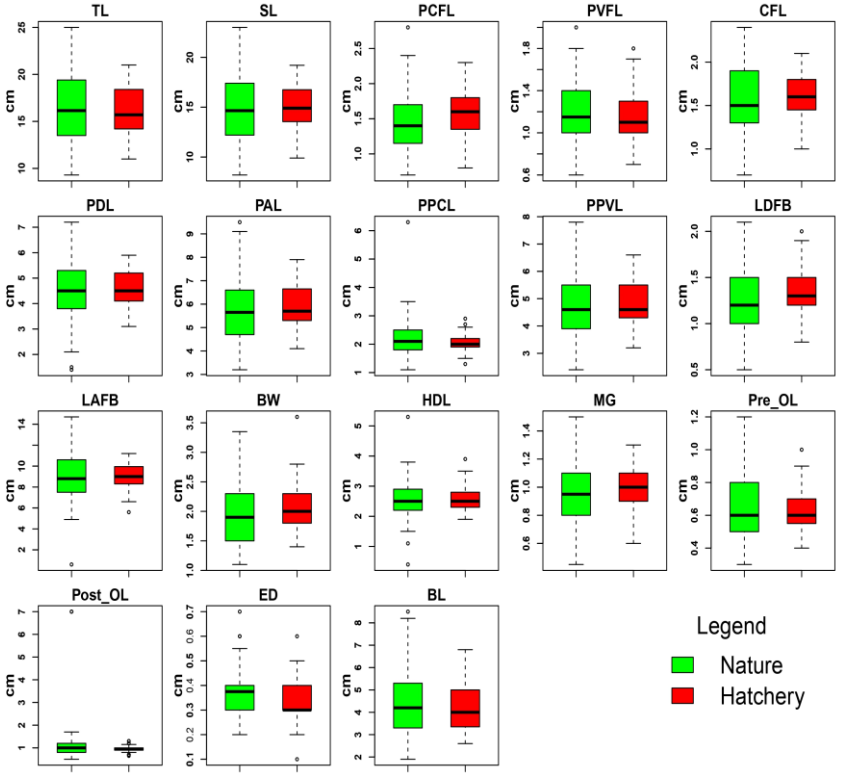

Fig. 3. Boxplot of the morphometric characters of nature and hatchery populations of *H. fossilis*.

The MANOVA indicated that the mean difference between natural and hatchery *H. fossilis* was statistically significant at the 5% significance level. As a

result, it is possible to deduce that all of the measures caused the variation between the two sources.

Multivariate analysis: In morphometric analysis, multivariate analysis of variance (MANOVA) revealed significant variation (Wilk's Lambda = 0.717, $p<0.05$) in the case of PCFL, PAL, PPCL, LDFB, BW, ED, Post OL between nature and hatchery populations. In meristic analysis, multivariate analysis of variance (MANOVA) revealed significant variation (Wilk's Lambda = 0.943, $p<0.05$) in the case of CFR between Nature and Hatchery populations of *H. fossilis* (Table 4). Hossen *et al.* (2017) reported significant variation ($p<0.01$) between SL, BD, ED, DFL, and AFL, but no significant difference in the lowest body depth, PCFL, PVFL, and meristic analysis; considerable variation was found ($p<0.01$) in case of DFS, DFR, PVFR, and AFS of Indigenous and Thai *A. testudineus*,

Table 4. Morphometric and meristic characters showing significant and insignificant differences between Nature and Hatchery populations of *H. fossilis*

Characters	F*	P value	Characters	F*	P value
SL	0	0.997	MG	1.54	0.22
PVFL	0.184	0.67	BW	7.24	0.01*
PCFL	6.74	0.01*	ED	10.89	0*
CFL	0.398	0.53	HDL	0	0.96
PDL	0.21	0.65	Pre OL	0.06	0.812
PAL	4.82	0.03*	Post OL	4.36	0.04*
PPCL	20.24	0*	DFR	1.03	0.312
PPVL	1.13	0.29	AFR	0.53	0.47
LDFB	7.1	0.01*	CFR	9.51	0.00*
LAFB	0.17	0.68	PVFR	2	0.16
BL	1.26	0.26	PCFR	0.9	0.33

* Values of the parameter differ significantly ($p<0.05$)
*F value of Multivariate ANOVA test.

Kruskal-Wallis (H) test of the morphometric data showed that nature and hatchery populations of *H. fossilis* are significantly different from each other ($p<0.05$) concerning PCFL, PAL, PPCL, LDFB, BW, ED, Post OL (Table 5). Kruskal-Wallis (H) test of the meristic data of the two strains showed that Nature and Hatchery populations of *H. fossilis* are significantly different from each other ($p<0.05$) concerning CFR. However, a Kruskal-Wallis (H) test of

morphometric characters between Indigenous and Thai *A. testudineus* revealed a significant difference (p<0.01) concerning SL, HL, BD, ED, LDF, PCVL, and AFL

Table 5. Kruskal-Wallis (H) test for comparison of morphometric and meristic characters between Nature and Hatchery populations of *H. fossilis*

Characters	Mean rank		*H*	df	*p-value*
	Nature	Hatchery			
SL	111.35	116.29	0.29	1	0.59
PVFL	114.97	109.07	0.41	1	0.52
PCFL	103.65	131.69	9.28	1	0.00*
CFL	111.96	115.08	0.12	1	0.74
PDL	114.44	110.12	0.22	1	0.64
PAL	104.67	129.67	7.38	1	0.01*
PPCL	125.85	87.29	17.54	1	0.00*
PPVL	109.38	120.24	1.39	1	0.24
LDFB	103.89	131.23	8.82	1	0.00*
LAFB	118.8	101.4	3.57	1	0.06
BL	116.41	106.19	1.23	1	0.27
MG	110.89	117.23	0.47	1	0.49
BW	104.38	130.24	7.89	1	0.01*
ED	122.43	94.15	9.44	1	0.00*
HDL	116.01	106.99	0.96	1	0.33
Pre OL	114.63	109.75	0.28	1	0.6
Post OL	120.82	97.36	6.49	1	0.01*
DFR	110.78	117.43	0.94	1	0.33
AFR	110.23	118.53	0.82	1	0.37
CFR	102.84	133.32	11.53	1	0.00*
PVFR	109.85	119.3	2.03	1	0.15
PCFR	115.49	108.03	0.94	1	0.33

but no significant difference (p> 0.01) in PVFL and meristic characters, a significant difference (p<0.01) between native and Thai *A. testudineus* based on DFS, DFR, PVFR, and AFS but no significant difference (p>0.01) in PCFR, AFR, CFR (Hossen *et al.*, 2017).

Principal Component Analysis: The variation in the unadjusted data of the morphometric parameters related to the source was evaluated individually for the two populations using principal component analysis. The morphometric

characters used to cluster the populations displayed a high admixture of clusters presented with a 95% confidence ellipse (Figure 4). Regarding nature plot (A), the groups overlap, particularly around the center, indicating similarities in the data. However, Sylhet has a more dispersed distribution, indicating higher variability within this group. Bagerhat and Chandpur samples also exhibited broader variability, overlapping slightly with Gazipur. The tight clustering of populations such as Jamalpur and Pabna suggests homogeneity within these regions. In hatchery plot (B), the groups have more distinct separations than the nature plot, indicating more apparent differences. There is some overlap between Mymensingh and Rangamati, but Jashore shows a distinct cluster. This differentiation could be due to selective breeding, different management practices, or varying environmental conditions in the hatcheries. The observed overlap among the hatchery groups indicates that, while some differentiation exists, there is still considerable genetic or phenotypic similarity within the hatchery populations. Table 6 explains the principal component, the eigenvalues, and the proportion of variance.

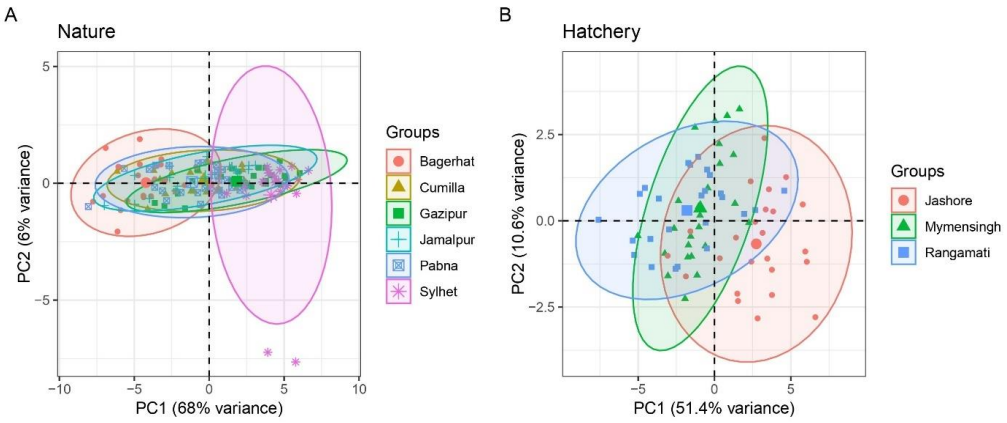

Fig. 4. Principle component analysis (PCA) plot based on morphometric characters of the nine nature and hatchery populations of *H. fossilis*.

H. fossilis morphometric characteristics and meristic counts from nature and hatchery differed significantly. Because the population was gathered from various natural sources, spatial variance among locals was also determined (Hossain *et al.*, 2008). Even though the hatchery population was drawn from a cultivated farm, morphometric parameters had less diversity. As a result, principal component analysis was performed independently on *H. fossilis* from nature and *H. fossilis* from hatcheries to find the morphometric traits that produce the most difference among groups due to different origins. The intra-class variance of nature and hatchery-derived populations was recovered separately using principal component analysis (Table 6), and three principal

Table 6. Explained variation associated with loadings

Characteristics	Nature			Hatchery		
	PC1	PC2	PC3	PC1	PC2	PC3
TL	-0.28	-0.01	-0.04	-0.28	-0.14	-0.17
SL	-0.28	-0.01	-0.06	-0.32	0.01	-0.12
PVFL	-0.24	-0.01	0.35	-0.05	0.51	0.28
PCFL	-0.24	-0.02	0.31	-0.23	0.29	-0.10
CFL	-0.24	-0.04	0.04	-0.18	0.30	0.01
PDL	-0.26	0.00	-0.09	-0.32	-0.12	-0.03
PAL	-0.27	0.03	-0.15	-0.32	-0.06	-0.13
PPCL	-0.24	0.07	-0.24	-0.11	0.22	-0.42
PPVL	-0.27	0.04	-0.16	-0.32	-0.07	-0.09
LDFB	-0.19	-0.02	0.60	-0.14	0.47	0.06
LAFB	-0.26	-0.05	0.02	-0.31	-0.01	-0.14
MG	-0.21	0.09	-0.46	-0.24	-0.12	0.00
BW	-0.27	-0.02	0.04	-0.28	-0.20	0.03
ED	-0.16	-0.37	-0.08	-0.10	0.25	0.37
HDL	-0.23	-0.18	0.14	-0.28	0.06	0.08
Pre OL	-0.23	0.28	-0.19	-0.17	-0.32	0.40
Post OL	-0.03	0.85	0.17	-0.19	-0.12	0.57
BL	-0.22	0.02	0.05	-0.09	0.07	0.15
Eigen Values	12.15	1.08	0.98	8.61	1.95	1.66
Proportion of variance	0.68	0.06	0.05	0.48	0.11	0.09

components were extracted separately for nature and hatchery populations. These principal components explain approximately 80% of the total variation among nature groups, whereas three principal components explain 68% of the variation in the hatchery population. It was reasonable because the hatchery sources' stinging catfish were artificially cultivated, and their feeding habits and other management aspects were identical (Jannat et al., 2022). in the case of nature groups, environmental variation and fluctuations in naturally open habitats created more plasticity in morphology (Duong *et al.*, 2019). The native first principal component (PC1) accounted for 68% of the total variation of 18 measurements. However, the importance of the second and third principal components is sometimes constructive (Delling et al., 2000) in explaining the variation. In nature, the TL and SL had the highest character loadings in PC1, Post OL had the highest loadings in PC2, and LDFB had the highest loadings in

PC3. These variables, including length measurements like total length, standard length, postorbital length, and dorsal fin base length, may be considered sources of diversity among the nature group. In the hatchery, PDL, PAL, and PPVL had the most significant loadings in PC1, but PVFL and Post OL had the most loadings in PC2 and PC3, respectively. As a result, the pre-dorsal length, pre-anal length, pre-pelvic fin length, pelvic fin length, and post-orbital were the most critical factors that caused the hatchery population differences. Jannat *et al.* (2022) found the sources of variation between native and Vietnamese *C. striata* may be considered by examining various length measurements, including pre-pectoral length, pre-pelvic fin length, eye diameter, and post-orbital length. Mouth gape, pre-pelvic fin length, total length, and standard length were the key variables influencing the variations amongst Vietnamese people. As a result, PCA is recognized as a good technique for determining the physical traits of numerous species that are considered substantial sources of variation within and between populations (Yakubu and Okunsebor, 2011; Nguyen *et al.*, 2016).

This study discovered significant differences in the morphological traits of natural and hatchery sources of *H. fossilis.* Morphometric and meristic traits have long been recognized as critical tools for determining the distinctive characteristics of a species. (Musikasinthorn, 2000). The current PCA results show that two characteristics (SL and Post OL) are responsible for the difference between natural and hatchery *H. fossilis* in Bangladesh. It is possible, however, that separate phenotypic differences in *H. fossilis* from two independent origins resulted in cryptic morphotypes. The commercially cultured hatchery *H. fossilis* shared these characteristics, which might be attributed to identical management practices and feeding habits. These parameters are occasionally influenced by the fish's food and eating habits, and such carnivorous fish (Narejo *et al.*, 2016) of native *H. fossilis* retrieved from nature may impact that cause. Commercially cultivated *H. fossilis*, on the other hand, are entirely dependent on a pelleted diet, which can lead to differences in these characteristics in hatchery populations. Finally, the present *H. fossilis* morphometric and meristic separation criteria can be used to differentiate between native and hatchery strains of *H. fossilis* observed in Bangladesh.

Discriminant analysis: Table 7 summarizes the findings of the discriminant analysis. The analysis revealed TL, PAL, PPCL, and BW as the most considerable loadings relative to other characteristics. As a result, these four traits helped distinguish between nature-stinging and hatchery-stinging catfish. 14 nature and 39 hatchery samples were misclassified, with a 78.37 between-group variation. The cross-validation technique was utilized by partitioning the entire

Table 7. Summary of discriminate analysis

Characteristics	LD1	Characteristics	LD1
TL	**2.826**	LAFB	-0.039
SL	-0.442	MG	0.019
PVFL	-0.013	BW	**-0.988**
PCFL	-0.698	ED	0.11
CFL	-0.275	HDL	0.135
PDL	0.322	Pre OL	-0.036
PAL	**-1.18**	Post OL	0.241
PPCL	**0.888**	BL	0.146
PPVL	-0.697	Misclassification error	0.235
LDFB	-0.129		

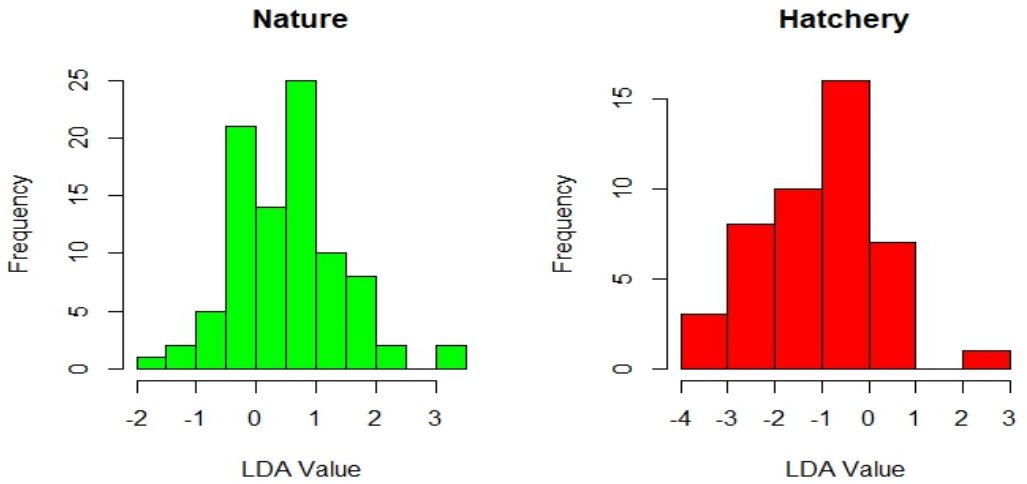

Fig. 5. Discriminate function separated nature and hatchery populations into distinct groups.

data set; 90% of the data was used for modeling and the remaining 10% for validation. According to the cross-validation technique, the discriminating Figure 5 demonstrates how the discriminant function can divide the sample into several groups. The discrimination function correctly classified around 76.44% of the total sample rule correctly classified approximately 90% of the sample into corresponding groups during the prediction phase. Furthermore, during the validation phase, 10% of the overall sample resulted in 2 nature and 4 hatchery samples being misclassified. As a result, TL, PAL, PPCL, and BW were sufficient to detect and differentiate the two populations' structures.

Discriminant analyses are commonly used to find significant characteristics that differentiate the fish population into discrete groups (Yakubu and Okunsebor, 2011). Around 77% of *H. fossilis* were successfully categorized into their respective groups. Furthermore, the cross-validation approach was used to validate this discriminant analysis, which properly assigned 90% of the population to their relevant categories. The total length (TL), pre-anal fin length (PAL), pre-pectoral fin length (PPCL), and body width (BW) of *H. fossilis* were discovered as distinguishing criteria that may separate between natural and hatchery populations. As a result, these distinguishing characteristics were statistically acceptable for determining the population morphology of fish species. They could aid farmers and consumers in identifying *H. fossilis* populations in nature and hatcheries.

Rahman *et al.* (2014) found for the morphometric data of *H. fossilis*, the discriminant function (DF) analysis yielded two DFs (the first and second DF). Concerning morphometric data, the first DF explained 83.9% of the variability among groups, while the second DF accounted for 16.1%, explaining 100% of the total among-group variability. Using the classification rule, Jannat et al. (2022) successfully classified roughly 99% of C. striata into their respective groupings. Turan *et al.* (2005) and Pollard et al. (2007) proposed a discriminant function that successfully identified 78% of the total population of Clarias gariepinus and 95.6% of Tor embroider, respectively. The cross-validation approach was used in a subsequent instance, and the success percentage was 93.1%.

Cluster analysis: The dendrogram based on the hierarchical cluster analysis using size-adjusted morphometric characters for *H. fossilis* of the two populations, which is collected from 9 districts, is shown in Figure 6. The dendrogram formed two main clusters, nature Gazipur, Jamalpur, Cumilla, Bagerhat *H. fossilis,* and hatchery *H. fossilis* collected from Rangamati in one cluster and the remaining 2 nature and 2 hatchery sources *H. fossilis* populations collected from the rest of the locations remained in another cluster. This indicates that these two strains were not separated regarding morphometric characters. The results obtained from hierarchical cluster analysis for meristic characters are presented as a dendrogram in Figure 7. The two strains did not cluster differently because nature Bagerhat, nature Jamalpur, hatchery Jashore, and Mymensingh are in one cluster, and the hatchery Rangamati population groups with the other three natural populations, namely Gazipur, Sylhet, and Cumilla. Therefore, the nature- and hatchery-originated populations could not be separated. Similar dendrograms were employed by Samaradivakara *et al.* (2012) to show the morphological divergence of four Sri Lankan tilapia populations. However, pond, haor, and river populations of *Glossogobius giuris*

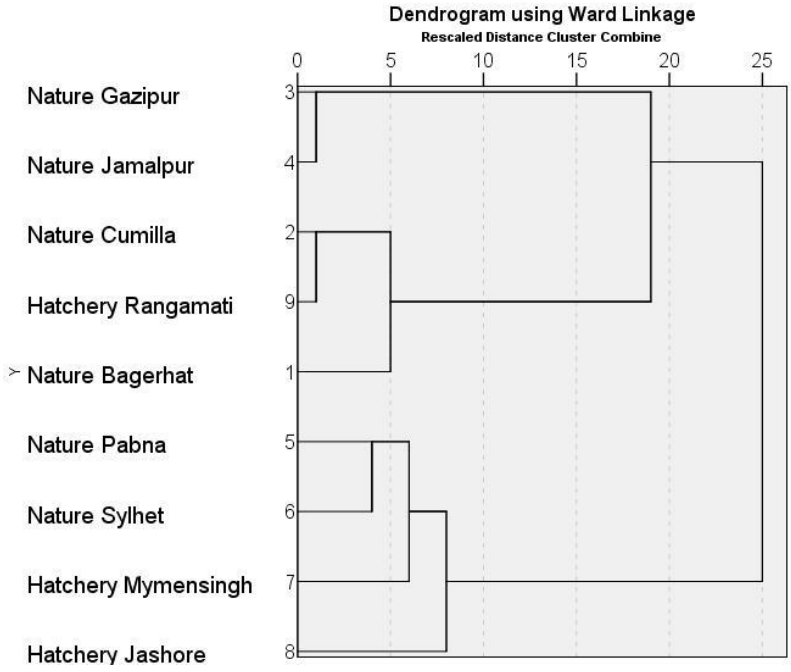

Fig. 6. Dendrogram obtained for morphometric characters of nature and hatchery populations of *H. fossilis*

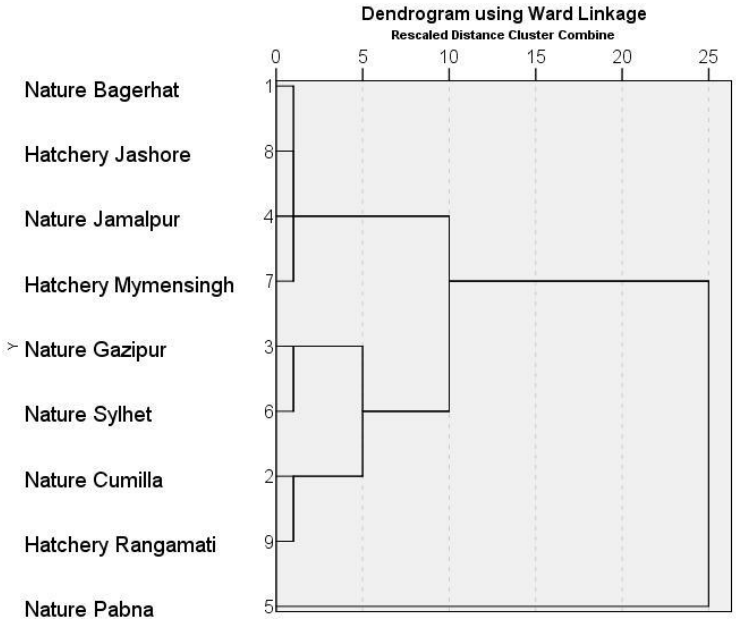

Fig. 7. Dendrogram obtained for meristic characters of nature and hatchery populations of *H. fossilis*.

collected from three different regions of Bangladesh were also shown to have a clustering pattern using a similar type of dendrogram by Mollah *et al.* (2012). The present study's findings were similar to those of the other two investigations. For individuals of every species, the taxonomic formula and morphological characteristics should fall within the same range (Alam *et al.*, 2014). Nonetheless, some diversity was found in the morphometric and meristic characteristics in the current investigation. Hossen et al. (2017) found that a dendrogram based on hierarchical cluster analysis of morphometric characters for *A. testudineus* successfully separated the two stocks from native and Thai origin. However, in this study, the results of the hierarchical cluster analysis using meristic characters did not group the two populations as seen in the dendrogram derived for the morphometric characters. It was, therefore, impossible to fully separate the two populations.

Examining the physical characteristics and meristic count differences between different strains of *H. fossilis* helps to understand the population variations and structure of the species. It can assist in developing management and breeding strategies targeted at different ecological populations. This study demonstrates that although *H. fossilis* populations that originated in nature and hatcheries may be identified using the morpho-meristic approach, a combination of molecular biology techniques is required for a more accurate and scientific evaluation of the different populations.

CONCLUSION

The present study found that *H. fossilis* from both natural and hatchery origins displayed differences in various morphological characteristics even though they are in the same taxonomic category. According to principal component analysis, the distinguishing morphological features- standard length (SL) and postorbital (post-OL)- can differentiate nature and hatchery *H. fossilis* in Bangladesh. In contrast, according to discriminant analysis, total length (TL), pre-anal fin length (PAL), pre-pectoral fin length (PPCL), and body width (BW) were the most distinguishing morphological characters to differentiate between the natural and hatchery populations. Meristic counts were constant except in caudal fin ray (CFR). Eventually, hatchery-originated *H. fossilis* populations might change due to diverse aquaculture practices, potentially resulting in genetic mixing and reduced natural adaptability. Finally, morphological identification of the two different sources can be utilized for a conservation plan to protect different morphotypes that may arise through introgression in the future.

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