



## Stock identification of the Reba carp, *Cirrhinus reba*: Insight into conventional and truss network-based morphometrics

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### ABSTRACT

The floodplains and ox-bow lake located in the south-western region of Bangladesh is currently facing a remarkable decrease in the number of their wild fish stock. Identification of fish stock along with wild and near-threatened fish conservation management is highly crucial. Samples of wild *Cirrhinus reba* were gathered from Bergobindapur Boar, Jhapa Boar, and Bornir Beel. The stock status of wild *C. reba* was assessed utilizing conventional and truss network-based morphometric examinations. Discriminant function analyses (DFA), univariate analyses were used. Univariate statistics (ANOVA) showed six out of eight conventional morphometric characters and eleven out of the twenty-two truss-based morphometric characters impressively exhibited significant differences among specimens. Two discriminant functions (1st and 2nd DFs) for both truss and morphometric measurements produced by the (DFA). In DFA, stocks are isolated with a lower extent of overlapping in both truss and morphometric measurements. This breakthrough proposed that there was partial isolation among the three stocks. Due to various hereditary origins, morphological variation among the stocks was noticed. These morphological differences are crucial in making the right decisions for proper management and conservation, as well as mass seed production, to ensure their long-term viability.

### INTRODUCTION

In open water fishery management and sustainable uses of fish species for human welfare, stock identification is critical (Kalhor *et al.*, 2015). Insufficient knowledge regarding fishery management could lead to sudden alterations in phenotypic characters (morphological characters) (Turan *et al.*, 2004). Numerous methods have effectively been established for stock identification of a fish species. Among all the methods regarding stock identification, morphological analysis is a state-of-art strategy with many inclinations concerning quick and supportive in regards to phenotypic varieties of fishes

(Keivany and Mohsen, 2017). Along these, meristic and morphometric characters are much of the time utilized techniques in fisheries research for stock distinguishing framework as well as stock separation of a fish species (Cadrin, 2000). Revealing of morphological changes within fish populaces in its ecological assortment may bring up the event of stock organization (Agüero and Rodriguez, 2004). Furthermore, morphometric characters play an important role in fisheries research, as they are used to examine population ontogeny and morphological patterns across different geographical areas (Mahfuj *et al.*, 2017). Ecological components are primarily responsible for intraspecific morphological variation (Tălu, 2012). Similarly, synergistic impacts of allelic recombination and environmental effects on different developments would create shape change among stocks (Garrod and Horwood, 1984). To consider the weaknesses of regular morphometric methodologies, 'the truss network' study has been continuously used in fisheries research with various examinations techniques, for example, univariate, bivariate and multivariate measurements (Cadrin, 2000; Hossain *et al.*, 2010; Gain *et al.*, 2017; Mahfuj *et al.*, 2017). In this regard, image processing procedures are well-known for obtaining external phenotypical features in traditional and truss-based morphometrics studies (Cavalcanti *et al.*, 1999; Turan, 2004; Mahfuj *et al.*, 2019a). Due to its ease of comprehension, depiction, and appropriate quantification of body shape, a modified box-truss network based on interconnected landmarks has recently gained much scientific attention for achieving appropriate phenotypic variation and stock identification (Rohlf, 1990). Analogous landmarks are shared different forms among biological samples (Bookstein, 1990, Turan *et al.*, 2004) that are commonly used as streamlined on a target entity, implying at the intra and inter stock standard (Swain *et al.*, 1999).

*Cirrhinus reba*, also known as bhagna bata, tatkini, or raik, is an economically significant and suitable native minor carp species found in the Indian subcontinent like Bangladesh and India (Rahman, 2005; Talwar and Jhingran, 1991). The majority of the rivers, small creeks, floodplains and lakes of Bangladesh are the natural habitat for *C. reba* (Akhteruzzaman *et al.*, 1998; Hussain and Mazid, 2001). Because of its excellent taste and oily flesh attributes likewise as having a reasonable proportion of minerals, protein, and unsaturated fat it is significantly standard fish to purchasers (Afroz and Begum, 2014; Rahman, 2005). As an omnivore and column feeder fish species *C. reba* can grow up to a length of 40–50 cm and within 1+ year they can achieve the sexual development (Akhteruzzaman *et al.*, 1998; Roos *et al.*, 2003; Rahman, 2005). Normally, spawning period starts in April and remain until August and fertility of female *C. reba* is somewhere in the range of 200,000 and 250,000 egg kg/body weight (Hussain and Mazid, 2001). In spite of the fact that nursing of fry and induced breeding are conducted by certain scientists and for commercial purposes production of this species (Akhteruzzaman *et al.*, 1998; Rahman *et al.*, 2009; Sarkar *et al.*, 2004; Mohanta *et al.*, 2008). In Indian subcontinent during last twenty years due to overfishing stocks of

this species from natural reservoirs have dramatically deteriorated (Sarkar *et al.*, 2004) and inappropriate fishing practices prompting habitat loss for spawning and siltation (Ahmad *et al.*, 2013; Sarkar *et al.*, 2004). Presently, in Bangladesh *C. reba* is named in the vulnerable species list (IUCN, 2000). In this regard, distinguishing the wild stocks of *C. reba* for the improvement of proper incubation center stocks through impressive breeding innovation is crucial, and overall production of seed and aquaculture sustainability indeed a significant breakthrough (Sarkar *et al.*, 2004). However, there is little data accessible on the stocks of *C. reba* populaces possessing natural reservoir of Bangladesh because of the shortage of research regarding stock identification except Ethin *et al.* (2019) and/or proper governmental action plans. The current investigation was hence completed to recognize the stock design of *C. reba* based on morphometric characters and truss network estimations in three freshwaters; Bergobindapur *Baor*, Bornir *Beel*, and Jhapa *Baor* of Southwestern region of Bangladesh.

## MATERIALS AND METHODS

### Sampling

An aggregate of 125 specimens of *Cirrhinus reba* were collected from three distinctive freshwater sources *viz.* Bergobindapur *Baor*, Jashore, (BBJ); Bornir *Beel*, Gopalganj (BBG) and Jhapa *Baor*, Jashore (JBJ) (Table 1) during August, 2019 to November, 2019. The samples were brought in live condition utilizing oxygenated polythene bags. For laboratory works samples were then carried to the laboratory of Fish physiology laboratory under the department of Fisheries and Marine Bioscience in Jashore University of Science and Technology (JUST), Bangladesh.

**Table 1:** Details of Sampling of *Cirrhinus reba* collected from three distinct regions of Bangladesh

Sl no.	Stocks name and location	Abbreviations	Locations	Number of specimens
1	Bergobindapur <i>Baor</i> , Jashore	BBJ	23.11°N, 89.38°E	58
2	Bornir <i>Beel</i> , Gopalganj	BBG	23.16°N, 89.21°E	46
3	Jhapa <i>Baor</i> , Jashore	JBJ	23.16°N, 89.21°E	21

### Imaging of samples

First and foremost, tested fresh samples were primary scrubbed in normal tap water flow system, dragging and set on a flat board with obscure white paper respectively as a preliminary procedure. To make the origin and insertion focuses noticeable, the fin rays were raised and put on the platform. Every single individual was marked with a particular code for legitimate recognition. To collect the computerized pictures a Cyber-

shot digital camera (Model-DSC-W730, Sony) was utilized that gave a total structure of body format (Cadrin and Friedland, 1999).

### Morphometric and truss network data measurement

Eight conventional morphometric characters were chosen and measured from left to right directions across the fish body by utilizing programming software tpsDig2v2.1 (Rohlf, 2006) (Table 2) and Fig. (1.a).

**Table 2:** Sixteen morphometric characters were used for the inquiry of *Cirrhinus reba* stock variations

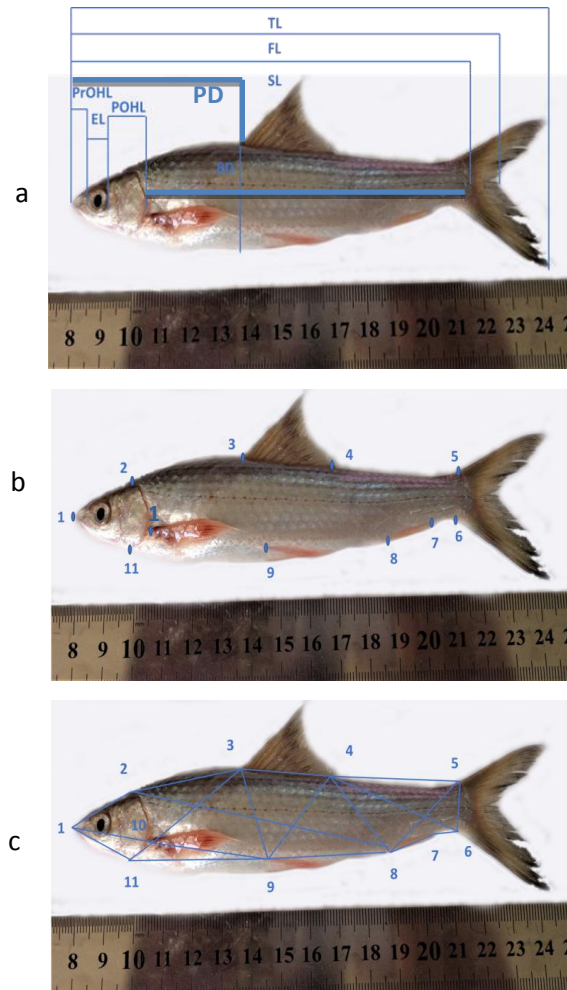
Characters and acronyms	Description
Total length (TL)	Straight line distance from the anterior tip of the upper jaw to the longest caudal fin ray
Fork length (FL)	Straight line distance from the anterior tip of the upper jaw to the endpoint of the fork of caudal fin rays
Standard length (SL)	Straight line distance from the anterior tip of the upper jaw to the end of the vertebral column
Pre-dorsal length (PDL)	Straight line distance from the front of the upper lip to the origin of the first ray of the first dorsal fin
Post orbital head length (POHL)	Straight line distance from the posterior margin of the eye to the endpoint of the operculum
Maximum body depth (BD)	Vertical maximum depth measured from the base of the first dorsal fin ray to the lowest point of bellyflap
Pre-orbital head length (PrOHL)	The anterior front of the upper lip to the fleshy anterior edge of the orbit
Eye length (EL)	Horizontal distance from fleshy anterior point to fleshy posterior part of the eye

For truss network estimation this network system was built by interconnecting 11 landmark points which possesses 22 truss networks Fig. (1. b-c). The derivations of morphometric and truss distances from the computerized images of samples were directed utilizing programming platform tpsDig2v2.1 (Rohlf, 2006).

### Statistical analyses

The entire truss-based and conventional morphometric data were firstly checked normal distribution for further analysis. Among all deliberate characters significant linear correlations were noticed with their TL. Therefore, to eliminate size-dependent variations from all of the specimens is highly crucial. According to Elliott *et al.* (1995) an allometric formula was utilized with modest alteration was made to eliminate the size from the dataset:  $M_{adj} = M (L_s/L_o)^b$ ; in this formula,  $M$  = the initial measurement,  $M_{adj}$  = the size-adjusted estimation,  $L_s$  = overall mean of the TL for all species of fish from all specimens and  $L_o$  = TL of the fish. Considering each character parameter  $b$  was determined from the observed data on the point of the slope of the regression of  $\log M$  on  $\log L_o$ . Univariate analysis of variance was applied to test the meaning of morphological

differences. Likewise, standardized size-adjusted data were submitted to a discriminant function (DF) analysis (DFA). Using the unweighted pair group method analysis (UPGMA), a dendrogram of the stocks based on the morphometric and landmark distance data was drawn. SPSS 21 (SPSS, Chicago, IL, USA) was used to analysis all the statistical data.



**Fig. 1.** Illustrated images of *Cirrhinus reba*. (a) Marking of the 8 conventional morphometric characters of along the fish body of *C. reba*. Morphometric features depicted in table 2. (b) Position of 11 landmarks delineated as close circle. Landmarks specify to (1) anterior tip of snout at upper jaw, (2) extreme posterior form of neurocranium, (3) initial point of dorsal fin, (4) last point of dorsal fin, (5) upper marginal point of dorsal skin and caudal fin, (6) lower marginal point of dorsal skin and caudal fin, (7) last point of anal fin, (8) start point anal fin, (9) start point of pelvic fin and (10) start point of pectoral fin, (11) last point of operculum. (c) The straight lines are constructed as truss-networks amidst the circles covering the whole external surface of *C. reba*.

## RESULTS

The allometric formula's intensity was justified and standardized by the correlation of TL with the remaining adjusted characters; as a result, the TL was later curtailed in further study. The correlation results indicated that all of the morphometric and truss measurements had a strong correlation with TL after the allometric transformation, indicating that the size effect had been eliminated from the equation. Univariate statistics (ANOVA) showed that seven, (FL, SL, TrL, PrOHL, EL, POHL, and BD) of eight conventional morphometrics and 6 (3-4, 5-6, 9-10, 1-11, 1-10 and 2-10) of the 22 truss measurements varied extensively (Table 3).

For both morphometric and truss measurements two DFs (1st and 2nd DFs) were produced by the discriminant function analysis (DFA). The first DF represented 92.4%, the second DF represented 7.6% among-group variability for truss and morphometric measurements, they clarified 100% of the total amid group variability (Table 4). With DFs and discriminant factors, pooled within-group correlations uncovered that, among the eight conventional morphometric estimations, SL, POHL and TrL overwhelmingly added to the first DF, the five estimations FL, PDL, PrOHL and BD added to the second DF (Table 4). Between all 22 truss measurements, four measurements (1-10, 2-10, 5-6 and 3-10) strongly contributed to the 1st DF, 18 truss measurements (3-9,10-11, 9-10,7-8, 3-4, 2-3, 9-11, 4-8, 6-7, 1-11, 1-2, 4-5, 4-6, 8-9, 2-8, 4-9, 5-8 and 2-9) added to the 2nd DF (Table 4).

Plotting all discriminant function analyses (DFA) showed a distinctive covering among the populaces for both morphometric and truss measurements. In the discriminant space the stocks are partially separated amid respective stock in Fig. (2) with lower extent of covering to a shifting degree among three stocks in both morphometric and truss measurements. Such innovation provided a suggestion that there was partial separation among three stocks like JBJ, BBG and BBJ. A Euclidian dendrogram depended on morphometric and truss measurement for three stocks of JBJ, BBJ and BBG. In Euclidian dendrogram 2 distinct clusters were framed; first group was shaped by stock of BBJ and was completely isolated, and second one was framed by cluster of BBG. Furthermore, JBJ stock shaped as a sub-cluster with BBG in Fig. (3).

By discriminant function analysis, a fair classification of individuals into their original stock differed by 100% in three stocks, and 100% of individual group cases could be categorized in their correct a priori classification (Table 5). Individuals from the BBJ, BBG, and JBJ stocks did not entirely contribute 100% to the initial grouping, and the individuals were partially mixed in with other stocks. However, 93.2% of individuals were correctly categorized in the original classification. This result showed that the individuals were clearly separated. In the cases of BBJ, BBG, and JBJ, morphometric measurements correctly categorized 97%, 88%, and 88% of the initial grouped cases,

respectively (Table 5). Using cross-validation study, maximum intermingling rates were observed in JBJ (60%), BBG (80%) and BBJ (91%) with 82.1% of average percentage levels observed in all stocks.

**Table 3.** Univariate statistics testing variance (ANOVA) between specimens from 8 conventional morphometrics and 22 truss measurements of *Cirrhinus reba* collected from three stocks in Bangladesh

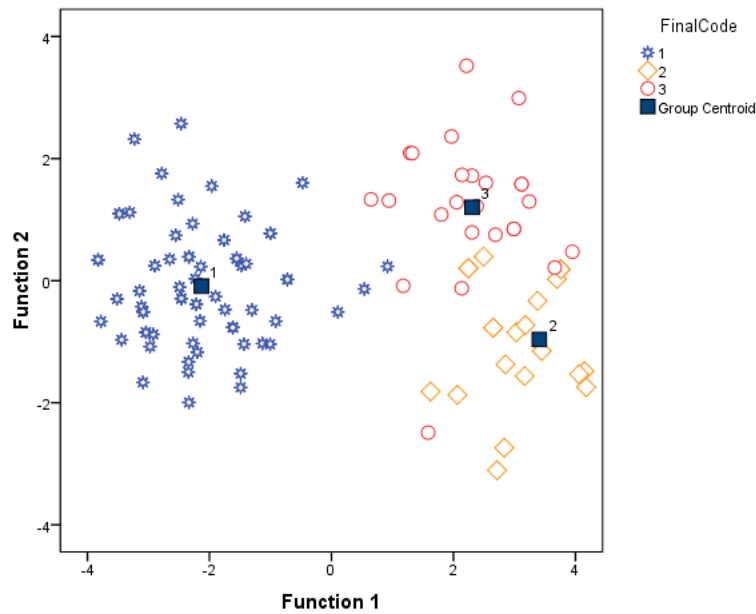
Characters	Wilks' Lambda	F	df1	df2	Significant
FL	0.942	3.522	2	114	0.033*
SL	0.378	93.787	2	114	0.000***
TrL	0.930	4.311	2	114	0.016*
PDL	0.956	2.650	2	114	0.075
PrOHL	0.895	6.699	2	114	0.002**
EL	0.944	3.397	2	114	0.037*
POHL	0.877	7.959	2	114	0.001**
BD	0.873	8.301	2	114	0.000***
1-2	0.991	0.493	2	114	0.612
2-3	0.964	2.133	2	114	0.123
3-4	0.945	3.298	2	114	0.041*
4-5	0.992	0.482	2	114	0.619
5-6	0.947	3.205	2	114	0.044*
6-7	0.979	1.251	2	114	0.290
7-8	0.952	2.877	2	114	0.060
8-9	0.993	0.399	2	114	0.672
9-10	0.910	5.643	2	114	0.005**
9-11	0.956	2.607	2	114	0.078
10-11	0.957	2.562	2	114	0.082
1-11	0.926	4.574	2	114	0.012**
1-10	0.885	7.409	2	114	0.001**
2-10	0.919	5.025	2	114	0.008**
3-10	0.960	2.395	2	114	0.096
3-9	0.959	2.415	2	114	0.094
4-8	0.977	1.337	2	114	0.267
4-9	0.992	0.433	2	114	0.649
5-8	0.997	0.187	2	114	0.830
4-6	0.987	0.763	2	114	0.468
2-9	1.000	0.000	2	114	1.000
2-8	0.980	1.148	2	114	0.321

**Table 4.** Pooled within-group correlations between discriminating variables and discriminant functions

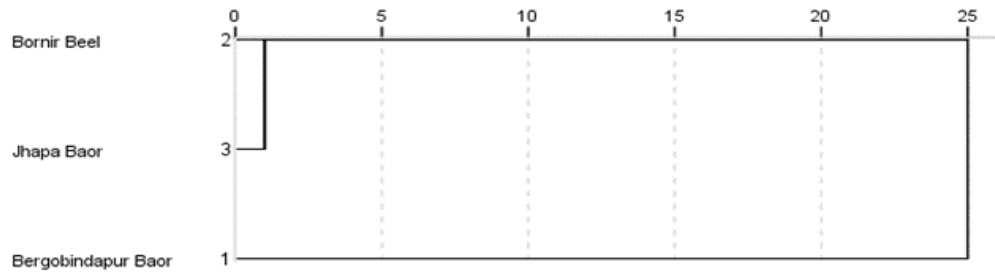
<b>Characteristics</b>	<b>DF1</b>	<b>DF2</b>
SL	-0.505*	0.177
POHL	-0.146*	-0.084
1-10	-0.140*	-0.105
2-10	-0.115*	0.085
TrL	-0.106*	-0.092
5-6	-0.094*	0.023
3-10	-0.079*	-0.069
FL	-0.018	0.337*
3-9	-0.005	-0.284*
10-11	0.028	0.276*
PDL	0.038	-0.267*
PrOHL	-0.115	0.253*
9-10	0.102	0.251*
7-8	0.055	0.244*
3-4	0.072	0.216*
2-3	-0.047	0.211*
9-11	0.060	0.210*
4-8	0.013	-0.206*
EL	-0.082	0.179*
6-7	-0.032	0.172*
BD	0.144	-0.159*
1-11	-0.104	0.146*
1-2	0.003	0.128*
4-5	-0.008	-0.124*
4-6	-0.032	0.115*
8-9	-0.012	-0.108*
2-8	0.051	0.081*
4-9	0.032	0.047*
5-8	0.020	0.035*
2-9	0.000	-0.002*

\*signifies the maximum correlation between each variable and DFs)





**Fig. 2.** Sample centroid of the discriminant function is scored based on general morphometric and truss measurements of *Cirrhinus reba* stocks from three freshwater reservoir of Bangladesh. Stocks are coded as: 1. Bergobindopur *Baor*, Jashore (BBJ); 2. Bornir *Beel*, (Gopalganj); 3. Jhapa *Baor*, Jashore (JBJ).



**Fig. 3.** Dendrogram based on morphometric and truss networks of *Cirrhinus reba* stocks from 3 distinct freshwaters in Bangladesh.

Table 5. Appropriate distributions of each individual of *Cirrhinus reba* collected from three diverse freshwater sources of Bangladesh; Bergobindopur *Baor*, Jashore (BBJ); Bornir *Beel*, (Gopalganj); Jhapa *Baor*, Jashore (JBJ)

	Stock Code	Predicted Group Membership			Total (%)
		BBJ	BBG	JBJ	
Original <sup>a</sup>	BBJ	65 (97%)	0	2 (3%)	67 (100%)
	BBG	0	22 (88%)	3 (12%)	25 (100%)
	JBJ	0	3 (12%)	22 (88%)	25 (100%)
Cross-validated <sup>b</sup>	BBJ	61 (91%)	0	6 (9%)	67 (100%)
	BBG	0	20 (80%)	5 (20%)	25 (100%)
	JBJ	2 (8%)	8 (32%)	15 (60%)	25 (100%)

<sup>a</sup> 93.2% of unique assembled cases properly classified

<sup>b</sup> 82.1% of cross-validated assembled cases appropriately categorized

## DISCUSSION

Results from this experiment shows that morphometric features of *Cirrhinus reba* shifted essentially within the considered stocks. So that these stocks went through few degree morphological shifts throughout the direction of the time because of topographical confinement or may be against various precursors. They may likewise be appearing from various ecological and variation in the condition of the habitats. After all, in morphological characteristics to ecological variations fish displays greater pliancy (**Wimberger, 1992**). The fluctuation of morphometric counts of *C. reba* have likewise been accounted for in the finding of **Rahman (2005)**. The previously mentioned specialist may have tested stocks that was encountering comparable ecological conditions or had a typical progenitor and consequently were not go through separation notwithstanding being secluded geologically. Notwithstanding morphometric characters, a truss network framework have been utilized by various researcher and has significant rule to recognize species, subspecies, races, and strains (**Siddik *et al.*, 2016; Ethin *et al.*, 2019; Gain *et al.*, 2017; Mahfuj *et al.*, 2017; Azad *et al.*, 2020; Mahfuj *et al.*, 2020a**). Through ecological changes such characters can be also adjusted during early life-history phases of fish species (**Cheverud, 1988; Reyment, 1985**). Phenotypic divergence among *C. reba* specimens were resulted from Size-adjusted morphometric and landmark data. The existence of three distinct morphologically differentiated stocks was revealed by the discriminant function analysis and dendrogram. From the other two stocks Bergobindapur Baor stock itself a highly distinct stock, and formed the first group due its geographical location. The Bornir *Beel* itself formed another group, whereas Jhapa Baor formed a sub-cluster with Bornir *Beel*. There are no possibilities of migration among the individuals of three stocks (i.e. Bergobindapur *Baor*, Jhapa *Baor* and the Bornir *Beel*)

These geographic variations indicate that the *C. reba* stocks in these three populations might not be are of the same ancestral origin. Moreover, the distances are varied from stocks to stocks. In some cases, it is very hard to clarify the reasons for morphological contrasts between populaces (**Cadrin, 2000**), yet it is accepted that these distinctions may be identified with genetical factors or be related with phenotypic versatility in light of various ecological variables in an area (**Murta, 2000**). Ecological variables might be one reason that clarifies the morphological differences identified in this examination. In morphological characters high plasticity can be observed in environmental factor like temperature and food availability (**Allendorf and Phelps, 1980; Wimberger, 1992**). Therefore, flowing water quality parameters among three habitats might be a crucial factor for the aforementioned stocks. Furthermore, ecological influences and rearing density simultaneously can modify the development of meristic characters and sometimes exposes natural aberrations in their development in varying degrees of aquatic organism (**Leary *et al.*, 1991**). Additionally, geographical variation is one of the major contributors of changes of meristic characters due to the fluctuations of

environmental circumstances (Asaduzzaman *et al.*, 2021; Gain *et al.*, 2017; Kashefi *et al.*, 2012; Mahfuj *et al.*, 2019a, b). Aggressive behavior (Mahfuj *et al.*, 2020b; Simon *et al.*, 2010) coupled with feeding habits might be an important factor of losing their fins and spines because of carnivorousness (Mishra *et al.*, 2013; Mahfuj *et al.*, 2021; Paul *et al.*, 2021) and omnivorousness (Sivakami, 1982) feeding pattern of *Lepidocephalichthys annandalei*. However, the phenotypic flexibility of fish is very high and they adjust rapidly by altering their body maintenance and conduct to natural vicissitudes due to the fluctuations of their habitats as well as their environments. These alterations, eventually, modify their morphology and physiology as well as swimming behavior (Mahfuj *et al.*, 2019a, b). In Bangladeshi water bodies there presumably little natural changes of water quality parameters from place to place. However, because of little natural contrasts, the subsequent morphological contrasts in fish might be small to the point that they may be difficult to recognize with net morphometric characters. Consequently, truss network estimations were utilized in this test. To distinguish contrasts between fish stocks, morphometric examination can be useful and are a fundamental apparatus for the segregation of fish stocks (Bailey, 1997; Palma and Andrade, 2002; Mahfuj *et al.*, 2019c). Additionally, morphometric estimations, joined with image analysis, address a technique for improving fish stock designs (Bailey, 1997; Mahfuj *et al.*, 2020a, b). Regardless, scientists have recognized the genuine phenomena of morphological and physiological modifications (for example genetic factors like epigenetic inheritance, characteristic determination) (Murta, 2000), and successful inbreeding and effective population size (He *et al.*, 2013). Besides, the stocks in this experiment may have begun from various predecessors. These clarifications are exceptionally coordinated with the past research achieved in *Labeo calbasu* (Hossain *et al.*, 2010), in *Labeo bata* (Mahfuj *et al.*, 2017), in *Labeo ariza* (Ahammad *et al.*, 2018), in *Cirrhinus cirrhosus* (Gain *et al.*, 2017), in *Glossogobius giuris* (Mollah *et al.*, 2013) and in *Ompok pabda* (Mahfuj *et al.*, 2019d).

## CONCLUSION

Because of various ancestral origin differences between morphological characteristics and the stock were seen. This experiment till now is the second one to describe the stock status of *Cirrhinus reba* in their wild freshwater habitat of Bangladesh. Further genetic investigations and the assessment of ecological effect on *C. reba* stocks in Bangladesh are proposed to help our discoveries.

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