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Research Article
Landmark-based Truss Morphometrics Delineate the Stock Structure of *Lepidocephalichthys guntea*

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Abstract

Background and Objective: Despite the status of *Lepidocephalichthys guntea* stocks are regarding as a least concern species in Bangladeshi freshwaters but indiscriminate fishing and human made anthropogenic activities will be led more vulnerable if such kinds of activities are frequently practiced in future. The present study was aimed to describe the stock structure of peppered loach, *L. guntea* on the bases of landmark-based truss morphometric analysis. **Materials and Methods:** Wild fish samples were collected from 2 rivers viz. Bhairab river, Jashore (BRJ) (n = 21); Nabaganga river, Jhenaidah (NRJ) (n = 49) and 2 natural water bodies called as beel viz. Chalan beel, Pabna (CBP) (n = 45) and Dhakuria beel, Jashore (DBJ) (n = 20) in Bangladesh. Then the samples were used to capture in digital images and subsequently 16 general morphometrics and 23 truss distances were measured by using tpsDig2v2.1 software. Then the morphometrics data and truss measurements were subjected to univariate statistics of variance (ANOVA) and discriminant function analysis (DFA) by using SPSS 21 software version. **Results:** In univariate statistics conferred that all measurements including general morphometrics and truss distances were significantly different among samples in varying degrees and in DFA showed that the first discriminant function (DF) accounted for 56.9%, the second DF accounted for 29.1% and the third DF accounted for 14%, respectively among group variability. Plotting DFs revealed that the stocks were clearly separated from each other in the discriminant space with virtually overlapping in varying degrees. Two main clusters were formed among four stocks based on the rescaled distance cluster combine. The NRJ formed a single cluster and the CBP and DBJ aggregately formed another two separate clusters. **Conclusion:** These results are suggested that the presence of different stocks of *L. guntea* from 4 freshwaters. The baseline information of the study will be helped in further *ex-situ* conservation, breeding as well as protect them from extinction.

Key words: Morphometric, *Lepidocephalichthys guntea*, truss network, stock identification, Bangladesh

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recognition and identification of different stocks of a fish species is a rudimentary requisite for its conservation, management and exploitation[1]. There are several ways to distinguish a fish stock, the landmark-based and geometric morphometric methods is a relatively modern method with many advantages including being cost effective, fast and useful[2] and has been successfully used in many studies[3-6]. Therefore, morphometric characters play a vital role in fisheries research, as it is used for comparing life history and morphological trends of stocks across habitats[7,8]. Besides, morphometric characters are more resistant and exhibits as unchanged condition through generation to generation but when the environmental stressors and other factors like inbreeding, hybridization and bottleneck affects are extremely affected in a stock, then morphometrics traits exhibit a significant disparity[9-11]. Morphological variation within species level is mainly triggered by environmental factors[12]. Numerous approaches, for instance morphological features, traditional tags, parasites as usual tags, otolith chemistry and molecular genetics have been extensively used for the purpose of stock discrimination among which morphometric characters are one of the most often employed techniques. Although, traditional morphometric methods have tremendous restrictions but recently a system using morphometric dimensions entitled 'the truss system' has been extensively practised for purposes of stock identification or discrimination[13]. The truss methodology is involving of measurements across body distances joining two or more morphological landmarks from a consecutive series of interrelated lines. Such powerful techniques can identify changes in organizations of a fish that are not easily detected through traditional forms of measurements or by the naked eye. Researchers might be benefited from this application by using landmark-based truss morphometric because this procedure is currently a state-of-art tool in vertebrate morphogenesis research[14].

Peppered loach, *Lepidocephalichthys guntea*[15] is categorically common freshwater fish species available in aquatic environment ranges in south east Asian countries[16,17]. This fish species is available in swamps, streams, floodplains and beels throughout Bangladesh and commonly occurs in the streams of northern region of Bangladesh[18]. Generally, it is a potamodromous species and feeds on small insect larvae and bottom detritus[19]. Albeit, *L. guntea* is the most plentiful species amongst other species of the genus *Lepidocephalichthys* and deliberated as least concerned species[20] but indiscriminate fishing pressure and other human made activities such as pollution, urbanization, overfishing will ultimately lead the status of this species make more vulnerable for their living. Above this considering fact, in the present study, landmark-based truss morphometric study was used in *L. guntea* stock. Therefore, the present research was examined to assess their stock structure inhabiting four ecological sources from Bangladeshi freshwater for its sustainable conservation and management.

MATERIALS AND METHODS

Fish sampling: A total of 135 individuals of *L. guntea* were collected from four different fresh water sources viz. Nabaganga River (NRJ); Bhairab River (BRJ); Chalan beel (CBP); and Dhakuria beel (DBJ) (Fig. 1, Table 1) during August, 2017 to October, 2017. The samples were transported using polythene bags. The fish specimens were 5.09-9.28 cm in Total Length (TL).

Imaging of fish samples: Firstly, sampled specimens were cleansed in running water and positioned on a flat sheet with opaque white paper as a background. Then fin rays were erected on the platform to make the original shape of each fish and easily visible the insertion points. Each sample was marked with an exact code for proper documentation. A Cyber-shot DSC-W730 digital camera (Sony, China) was used to capture the digital images, which delivered a complete archive of body shape and permitted a replication of the measurements when necessary[21].

Measurements of morphometric and truss data: A total of 16 morphometric characters were measured from left to right side across the fish body using software platform tpsDig2v2.1[22] (Table 2).

For truss measurement the truss networks were constructed by interconnecting 10 landmarks point which form a total of 23 truss measurements (Fig. 2). The general morphometrics and truss distances from digital images of specimens were conducted using software platform[22] tpsDig2v2.1. All measurements were subsequently transferred to Microsoft Excel spread sheet 2007 version and SPSS 21 version software for further analysis[23].

Statistical analysis: An allometric formula was used to remove the size effect from the dataset[24]:

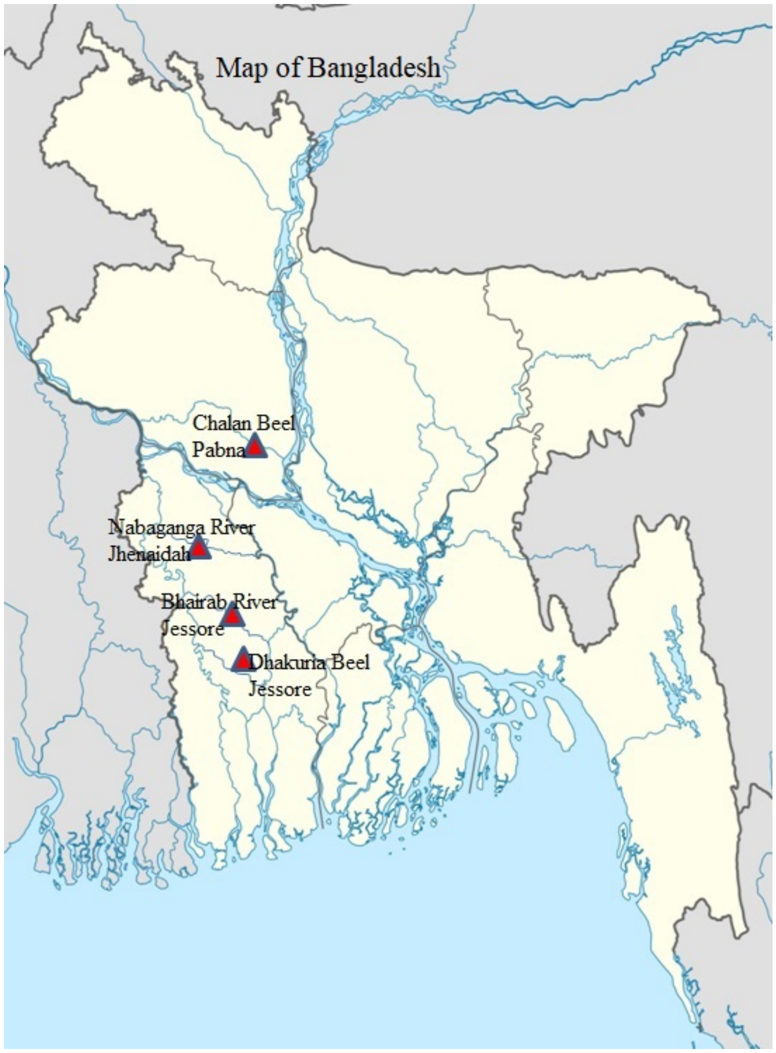

Fig. 1: Map of Bangladesh showing collection sites of *Lepidocephalichthys guntea* from four freshwater sources

Table 1: Sampling details of *Lepidocephalichthys guntea* from four freshwater sources in Bangladesh

Sample No.	Stocks	Abbreviations	Locations	No. of specimens	Mean SL (cm) (SD)
1	Nabaganga River, Jhenaidah	NRJ	23.11°N 89.38°E	49	5.29±0.79
2	Bhairab River, Jashore	BRJ	23.16°N 89.21°E	21	6.43±0.67
3	Chalan beel, Pabna	CBP	24.15°N 89.44°E	45	6.36±0.66
4	Dhakuria beel, Jashore	DBJ	23.16°N 89.21°E	20	6.70±0.75

$$M_{adj} = M\left(\frac{L_s}{L_o}\right)^b$$

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. A univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. Additionally, size-adjusted data were standardized and submitted to a discriminant function (DF) analysis (DFA). A dendrogram of the stocks based on the data was drawn using the

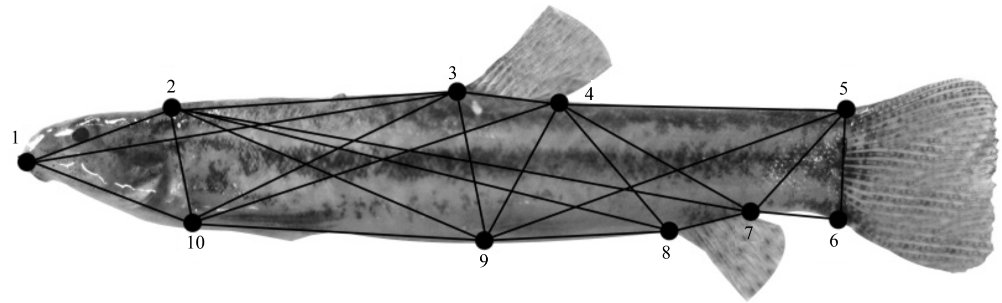

Fig. 2: Location of the 10 landmarks for constructing the truss network on fish body illustrated as close circle and morphometric distance measures between the circles as lines

Landmarks refer to 1: Anterior tip of snout at upper jaw, 2: Most posterior aspect of neurocranium, 3: Origin of dorsal fin, 4: Insertion of dorsal fin, 5: Anterior attachment of dorsal membrane from caudal fin, 6: Anterior attachment of ventral membrane from caudal fin, 7: Insertion of anal fin, 8: Origin of anal fin, 9: Insertion of pelvic fin, 10: Insertion of pectoral fin

Table 2: Description of 16 morphometric characters of *Lepidocephalichthys guntea*

Characters	Description
Total length (TL)	Distance from the tip of the upper jaw to the longest caudal fin ray
Standard length (SL)	Distance from the tip of the upper jaw to the end of the vertebral column
Pre-dorsal length (PDL)	Front of the upper lip to the origin of the first ray of the first dorsal fin
Post orbital head length (POL)	Distance from the posterior margin of the eye to the end of the operculum
Pre-pectoral length (PPCL)	Front of the lower lip to the origin of the pectoral fin
Pre-pelvic length (PPVL)	Front of the upper lip to the origin of the pelvic fin
Length of the dorsal fin base (LDFB)	From base of dorsal fin ray to base of last dorsal ray
Length of soft dorsal fin ray (LDSFR)	From base to tip of the soft dorsal ray
Length of anal fin base (LAFB)	From base of first anal fin ray to base of last anal ray
Caudal fin length (CFL)	From tail base to tip of the caudal fin
Length of soft anal ray (LSAR)	From base to tip of the soft anal ray
Body depth (BD)	Maximum depth measured from the base of the first dorsal fin ray
Pre-orbital length (PreOL)	The front of the upper lip to the fleshy anterior edge of the orbit
Eye diameter (ED)	The greatest crystal like diameter of the orbit
Head length (HL)	From the front of the lower lip to the posterior end of the opercular membrane
Inter orbital (IO)	Distance between dorsal side of both eyes

unweighted pair group method analysis. All statistical analysis were done using SPSS 21 (SPSS, Chicago, IL, USA).

RESULTS

In univariate statistics (ANOVA) showed that 15 morphometric and 23 truss measurements were significantly different among samples in varying degrees (p<0.001) (Table 3).

Discriminant function analysis produced three discriminant functions viz. DF1, DF2 and DF3 for both morphometric and truss measurements. The first DF accounted for 56.9%, the second DF accounted for 29.1% and the 3rd DF accounted for 14%, respectively among group variability, explaining 100% of total variability (Table 4). The individual of each stock was clearly separated in the discriminant space (Fig. 3) with virtually overlapping in varying degrees. This finding suggested that there was separation among stocks.

Pooled within-groups correlations between discriminant variables and DFs showed that among 15 morphometric measurements, 1 measurement dominantly contributed to first DF and 14 morphometric measurements are contributed to the second DF. In case of truss measurements, among the twenty three measurements, one measurement contributed to first DF and the remaining truss measurements contributed to the third DF (Table 4).

A dendrogram based on morphometric and truss measurements, two main clusters were formed among four stocks. The NRJ formed a single cluster and the CBP and DBJ aggregately formed another separate cluster and BRJ stock formed a sub-cluster with CBP (Fig. 4).

On the basis of morphometric and truss measurements 93.3, 90.5, 93.9 and 100.0% of original grouped cases were correctly classified of CBP, BRJ, NRJ and DBJ stocks, respectively and a total of 94.1% of original grouped cases correctly classified (Table 5).

Table 3: ANOVA results of 15 morphometric characters and 23 truss measurements of *Lepidocephalichthys guntea* from Nabaganga River, Bhairab River, Chalan beel and Dhakuria beel in Bangladeshi freshwaters

Characters	Wilks' Lambda	F-value	p-value
SL	0.66	22.44	0.000***
PDL	0.62	25.97	0.000***
POL	0.66	21.97	0.000***
PPCL	0.62	26.55	0.000***
PPVL	0.67	21.48	0.000***
LDFB	0.67	21.02	0.000***
LDSFR	0.87	6.43	0.000***
LAFB	0.64	23.56	0.000***
CFL	0.88	5.45	0.001***
LSAR	0.66	21.80	0.000***
BD	0.64	24.42	0.000***
PreOL	0.53	38.08	0.000***
ED	0.72	17.01	0.000***
HL	0.72	16.40	0.000***
IO	0.69	19.15	0.000***
1-2	0.57	32.53	0.000***
2-3	0.68	19.96	0.000***
3-4	0.71	17.45	0.000***
4-5	0.73	15.49	0.000***
5-6	0.62	26.08	0.000***
6-7	0.80	10.60	0.000***
7-8	0.69	19.56	0.000***
8-9	0.73	16.13	0.000***
9-10	0.66	21.92	0.000***
10-1	0.62	25.72	0.000***
10-2	0.67	21.51	0.000***
10-3	0.67	21.36	0.000***
3-9	0.65	22.82	0.000***
4-9	0.70	18.43	0.000***
4-8	0.68	19.79	0.000***
4-7	0.67	21.34	0.000***
5-7	0.70	18.56	0.000***
4-10	0.66	22.35	0.000***
2-9	0.66	21.70	0.000***
2-8	0.67	20.67	0.000***
2-7	0.67	20.99	0.000***
1-3	0.69	18.81	0.000***
5-9	0.71	17.70	0.000***

SL: Standard length, PDL: Pre-dorsal length, POL: Post orbital head length, PPCL: Pre-pectoral length, PPVL: Pre-pelvic length, LDFB: Length of the dorsal fin base, LDSFR: Length of soft dorsal fin ray, LAFB: Length of anal fin base, CFL: Caudal fin length, LSAR: Length of soft anal ray, BD: Body depth, PreOL: Pre-orbital length, ED: Eye diameter, IO: Inter orbital

Table 4: Pooled within-group correlation between discriminating variables and discriminant functions

Characters	DF1 (56.9%)	DF2 (29.1%)	DF3 (14%)
IO	0.367*	0.304	-0.326
1-3	0.354*	0.343	-0.267
PDL	0.052	0.769*	-0.064
1-2	0.104	0.767*	-0.133
BD	0.060	0.755*	-0.059
3-9	0.030	0.728*	-0.011
10-3	0.025	0.723*	-0.019
4-10	0.042	0.722*	-0.028
9-10	0.044	0.721*	-0.018
SL	0.082	0.715*	-0.009
2-8	0.072	0.715*	-0.047
4-9	0.058	0.714*	-0.021
PPCL	0.165	0.712*	-0.093
10-1	0.143	0.712*	-0.079
2-9	0.073	0.712*	-0.045
2-7	0.074	0.706*	-0.028
4-7	0.109	0.706*	0.002
2-3	0.053	0.702*	-0.008
PPVL	0.089	0.697*	-0.044
5-6	0.061	0.688*	0.021
4-8	0.062	0.687*	-0.044
8-9	0.065	0.685*	-0.059
10-2	0.097	0.683*	-0.098
5-9	0.092	0.666*	0.043
POL	0.129	0.657*	-0.101
ED	0.184	0.654*	-0.129
PreOL	0.436	0.650*	-0.138
LDFB	0.181	0.638*	-0.004
3-4	0.145	0.634*	-0.006
5-7	0.092	0.624*	0.140
4-5	0.112	0.622*	0.072
7-8	0.193	0.597*	0.150
LAFB	0.159	0.594*	0.078
LSAR	0.004	0.577*	0.156
CFL	-0.069	0.539*	0.049
HL	0.029	0.506*	-0.054
6-7	0.089	0.456*	0.254
LDSFR	-0.084	0.362*	-0.047

DFs: Variables ordered by size of correlation within function, *Largest correlation between each variable and DFs

DISCUSSION

In the present study, highly significant morphological variations were found in Univariate analysis (ANOVA) among four stocks of *L. guntea*. The geographic detachment is a constraining variable to movement among stocks and also found similar results for *Liza abu*[25] stocks from three rivers of Turkey and for *Macrognathus pancalus*[26] populations from four natural sources of Bangladesh. Normally, aquatic organisms expose phenotypic flexibility since they adjust hurriedly through adapting their functioning and behaviour to ecological vicissitudes[27,28]. Additional ecological effects could encompass heterochrony, fluctuations in the comparative timing of developmental events[29] such as shifts between growth and development[30]. Though, ecological stimuli on morphometric characters have not been deliberated in this work. Morphometric contrasts, however, among stocks are normal, since they are topographically isolated and may have originated from various predecessors. Therefore, it is doubtful that obvious environmental variations exist in four habitats in the present study. The plasticity of fish body is exceptionally sensitive to natural changes and rapidly

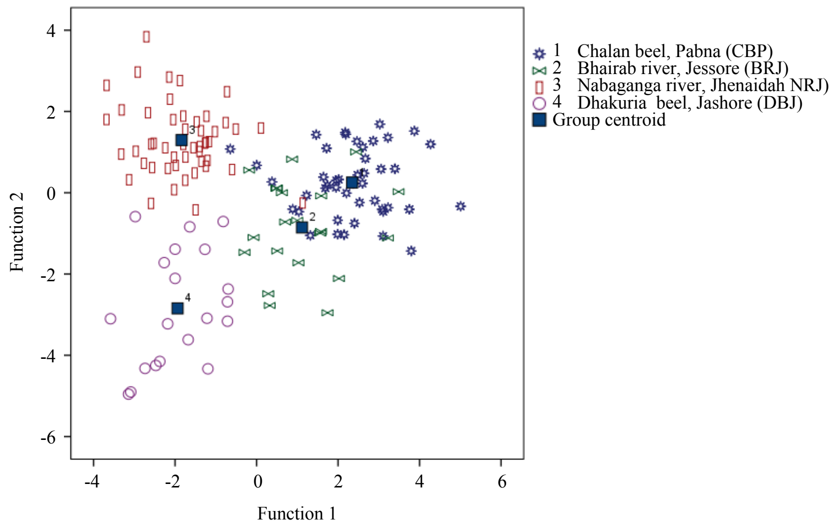

Fig. 3: Sample centroids of the discriminant function scores based on morphometric and truss measurements

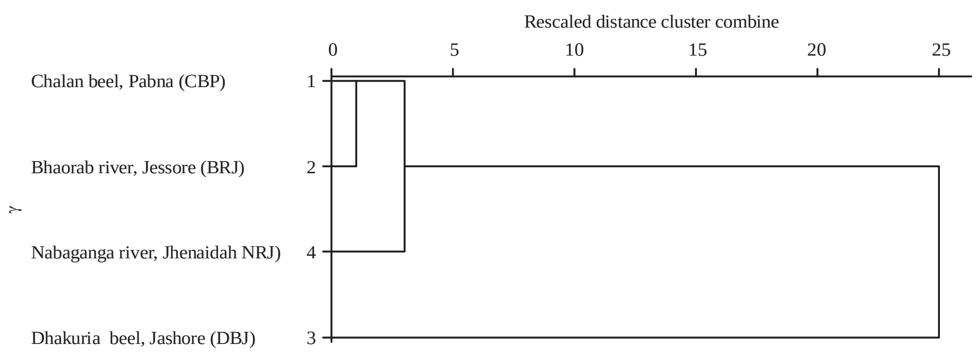

Fig. 4: Dendrogram based on morphometric and truss data of *Lepidocephalichthys guntea* populations collected from CBP, BRJ, NRJ and DBJ in Bangladesh

Table 5: Correct classifications of individuals (*Lepidocephalichthys guntea*) collected from four different freshwater sources viz. Chalan beel, Pabna (CBP); Bhairab river, Jashore (BRJ), Nabaganga river, Jhenaidah (NRJ) and Dhakuria beel, Jashore (DBJ)

		Predicted group membership				
Original	Stock name	CBP	BRJ	NRJ	DBJ	Total
Count	CBP	42	1	2	0	45
	BRJ	2	19	0	0	21
	NRJ	1	1	46	1	49
	DBJ	0	0	0	20	20
Percentage	CBP	93.3	2.2	4.4	0	100
	BRJ	9.5	90.5	0	0	100
	NRJ	2.0	2.0	93.9	2	100
	DBJ	0	0	0	100	100

adjusts by changing essential phenotypes. They immediately adjust hastily by altering their physiology and conduct to natural changes. These modifications finally change their morphology[31]. Morphological characters indicated high elasticity because of contrasts in natural conditions, for instance, food abundance and temperature[32-34]. In general, fish show more prominent changes in morphological characteristics both inside and between stocks than other vertebrates and are more defenceless to naturally incited morphological varieties[29-31].

Truss network systems are an effective manoeuvre for recognizing supplies of fish species[32]. A fair system of morphometric estimations over a two dimensional diagram of a fish evacuates the need to discover the sorts of characters and ideal number of characters for stock division and gives data over the whole fish shape[35,36]. For this situation, progressively critical contrasts were expected due to the four totally different habitats. The results of the investigation are helpful for *L. guntea* stocks. In open-water management, it is an elementary method to choose hereditarily superior stocks along with better features. More research particularly molecular researches are required for preservation and mass seed creation of the desired stocks to save this species from extinction. The present study manages a wide assortment of methods utilized for the morphological differentiation, influential factors in responsible of morphometric variation and overview the morphometric variation among fish stocks. It is apparent that the morphometric historic point attributes to distinguish phenotypic stocks is over extremely old, the improvement of truss system with the advances in expository strategies changed the investigation of morphometric variety which have expanded the energy of morphometric examination for stock identification. As a potential pointer of phenotypic stocks, investigation of morphometric milestones is a profitable device that supplements other stock identification strategies. All around, in the recent years the morphometric methods are boosting the utility of morphometric based research in fish stock identification to encourage the maintainable usage of fishery assets and biodiversity protection.

CONCLUSION

The morphometric diversity which was observed in this study will certainly give information on proper monitoring the status of the species in the south-western part of Bangladesh through appropriate management systems in future. The results of the research work will deliver valuable reference evidence of *L. guntea* stocks not only in south-western region of Bangladesh but also in entire geographic region of Bangladesh. Moreover, it is highly expected that this investigation will be useful and keeping in mind accomplish the knowledge in view of diversified morphometric procedures and to enhance stock identification.

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