ORIGINAL ARTICLE

Differentiation of intraspecific phenotypic plasticity of elongate glassy perchlet, *Chanda nama*: Insights into landmark-based truss morphometric and meristic variations

Kamrun Naher Azad[1], Md. Sarower-E- Mahfuj[2], Towsif Iqbal[1], Khairun Naher Azad[3], Md. Arif Imtiaz Shafaq[1]

[1]Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Department of Fisheries and Marine Bioscience, Jashore University of Science and Technology, Jashore, Bangladesh
[3]Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh

ABSTRACT

Objective: Understanding intraspecific phenotypic plasticity is a prerequisite to fish stock identification and sustainable fisheries management. In this study, we assessed intraspecific phenotypic plasticity in terms of meristic and morphometric characters of wild populations of elongate glassy perchlet, *Chanda nama* from two different rivers, namely Madhumati River – Narail (MRN) and Tulshiganga River – Jaypurhat (TRJ), and an ox-bow lake, Jhapa Baor – Jashore (JBJ) in Bangladesh.
Materials and Methods: In this study, six meristics, 15 conventional morphometrics, and 23-truss-based morphometrics were subjected to a one-way analysis of variance (ANOVA), followed by the Tukey-HSD test. The mean values of three meristic counts and nine conventional and 12 truss-based morphometrics demonstrated significant differences in the ANOVA test. Furthermore, principal component analysis (PCA) and discrimination function analysis (DFA) were performed separately using conventional and truss-based morphometric data.
Results: In PCA analysis, four principal components were extracted and cumulatively demonstrated 51.848%. On the contrary, two discriminant functions (DF1, 63.5%; DF2, 36.5%) resulted from DFA analysis. In the bi-plot alignment from the discriminant space, all individuals were exceedingly separated among the three inhabitants. A dendrogram developed using conventional and truss morphometric characters confirmed that two clusters were formed among three populations. The TRJ population formed a distinct cluster, and the JBJ population formed a different cluster with a subcluster of MRN. In the discriminant function analysis, precise classification outcomes displayed 82% of individuals into their unique populations, whereas 66.9% of individuals were categorized as a cross-validated assemblage.
Conclusion: The baseline information resulting from the current study would be useful for environmental studies and further conservation of glassy perchlet populations in Bangladesh.

Introduction

Chanda (*C.*) *nama* is an essential freshwater and brackish water fish species under the family *Ambassidae*. It is mostly termed as a small indigenous species (SIS) in Bangladesh [1]. These species are naturally distributed in numerous freshwater habitats (i.e., rivers, lakes, haors, baors, canals, wetlands, and so on) of the South Asian countries [2]. Throughout the rainy season, these species are copiously found in flood plains and the adjacent paddy fields [3]. This fish is highly favored to the local fishermen due to its high market demand and the aquarium traders [4]. They mostly feed on small aquatic zooplankton, aquatic nematodes, and often minute scales of other fishes, i.e., lepidophagy [5]. In morphological perspectives, their body shape is bilaterally compressed. The dorsal and ventral characteristics of this species resemble convex shape. However, the natural populaces of this fish species are vehemently facing threats from several anthropogenic activities and frequently

hostile climate changes [6]. Moreover, indiscriminate illegal fishing gear, pollution from plastics and industry effluents, and irresponsible fishing activities pose a significant bottleneck to their sustainability in nature. Although this fish species is ostensibly declining in their habitats, this species is considered as least concerned both globally and nationally [7].

Knowledge of stock identification is essential in open water fishery management and sustainable uses of fish species for human welfare [8]. Inadequate information regarding fishery management may prime to abrupt modifications in phenotypical characteristics [9]. Nevertheless, suitable regulation of fish resources depends severely on the evidence concerning the ecology of population structure, life-history traits (i.e., larval development, age, growth, maturation, reproduction, and physiologies) [10]. However, to identify the suitable stocks in fisheries science, many outfits such as molecular techniques, tags, parasites infestation, and morphometric studies are used [11]. Besides, univariate and bivariate statistical analyses with a series of multivariate statistical analyses, including principal component analysis (PCA), discrimination function analysis (DFA), and classification analysis, are generally applied to differentiate fish stocks through intraspecific external morphometric variables [12]. Mahfuj et al. [13] recorded that identifying conventional morphometric studies was frequently analyzed by exploring distant phenotypical traits obtained through imaging systems.

Besides, landmark-based morphometric systems were introduced because of its accurate data assemblage and exact quantification for population discrimination [14]. In this regard, setting homologous landmarks are the best fit for describing common external phenotypical traits in a species [15]. Thus, homologous landmarks form a box-truss network by interconnecting to each point, representing a better shape across the whole body. Each distance from the box networks represents dominantly for stock discrimination studies [16]. The obtained interlandmark characteristics become pertinent for revising short duration of anthropogenic activities and biotic induced distinctions as well as stock identification [17], onto genital shifts in their life history [18], and other trait selection of conservation [19]. Consequently, elementary knowledge of phenotypical traits with evidence on population structures of *C. nama* is highly essential for identifying suitable stock from diverse water bodies.

Numerous research works have been carried out so far on *C. nama* species such as biodiversity status [20], trophic level status [21], feeding habits [22], and length and weight relationships [23], but a little research works have been conducted yet on the population differentiation using meristic, conventional, and landmark-based truss morphometrics in *C. nama*.

Moreover, the deltaic land of Bangladesh holds approximately 700 rivers and tributaries that run from north to south and eventually merge to the Bay of Bengal [24]. The Tulshiganga River flows from the Himalayan part of India. It then flows to Bangladesh through Dinajpur, Joypurhat, and Naogan districts, and this river finally merges with the Jamuna River system at Naogaon district [25]. Nowadays, the aquatic biodiversity of Tulshiganga River is facing a bottleneck due to several anthropogenic activities such as water pollution from industry, siltation, frequent climate changes, and so on [26]. Moreover, unrefined toxic waste from Joypurhat sugar mills creates a harsh environment for the aquatic ichthyofaunal diversity and deteriorates the water quality parameters of this river [27]. On the flip of the site, the Madhumati River, the principal distributary of the Padma River, is one of the lengthiest rivers (372-km) in Bangladesh. Currently, this river is vehemently facing high intrusion of saline water from the Bay of Bengal, resulting complete ecosystem shifted from freshwater to a brackish water system. The aquatic lives of this river are facing a significant threat due to the establishment of furnace oil-based power plant and dumping an inadequate amount of furnace oil as well as burnt mobil into the river [28]. Finally, Jhapa ox-bow lake, locally called Jhapa baor, is located at the Manirampur *Upazila* (sub-district) under the district of Jashore. Recently, two plastic-based floating bridges have been established on this ox-bow lake for transportation purposes and a recreation center for the tourists. As a result, the aquatic environment is getting worse day by day due to sound pollution, plastic pollution from the tourists, and chemical residues from agricultural activities [29]. Considering all these factors mentioned above, the Tulshiganga River – Joypurhat (TRJ), Madhumati River – Narail (MRN), and Jhapa Baor – Jashore (JBJ) in Bangladesh can be selected as sampling sites (Fig. 1). Hence, the present study has been conducted to examine the morphological variations among these three populations of *C. nama* using meristic counts, conventional morphometric, and truss-based morphometric characters. This is the first study of detecting the stock identification of *C. nama* from three different ecological niches through meristic characters, traditional morphometric, and truss-based morphometric characters.

Materials and Methods

Sample collection

A total of 153 samples of *C. nama* were collected from two diverse rivers and an ox-bow lake, namely, TRJ, MRN, and JBJ in Bangladesh using gill nets (mesh size: 16–17 mm) from July to December 2018 (Table 1). The fresh and undamaged samples were immediately removed from the gill nets on the specific sites as well as kept in an ice-box and then instantly brought into the Laboratory

of Aquaculture, under the Department of Aquaculture, Bangladesh Agricultural University, for examining their external phenotypes (i.e., counting of meristic characters and measuring of morphometric characters).

Meristic counts and morphometric measurements

First, each sample was removed from the ice-box and washed with fresh running tap water for meristic counts, measuring conventional morphometric and truss-based morphometric characters. Second, six meristic characters, namely, number of dorsal spine rays (DSR), number of dorsal soft fin rays (DSFR), number of caudal fin rays (CFR), number of anal fin rays (AFR), number of pelvic fin rays (PVFR), and number of pectoral-fin rays (PCFR) of each sample, were counted by using needles [10]. Next,

Figure 1. Map showing three different Bangladeshi freshwaters from where *Chanda nama* was collected.

each sample was placed on a coded white paper with a scale for taking the digital images by using the Cybershot DSC-W300 digital camera (Sony, China). The digital images were finally stored in the computer system, and the images resembled as a whole part of the body shape for measuring the conventional morphometric characters as well as the truss-based morphometric characters. After that, 16 conventional morphometric characters were measured, and 13 landmark points were fixed, and finally, the specific distances were measured from different points to points by using tpsDigV.2 1.1 software [14] (Figs. 2 and 3). Finally, all conventional and truss-based morphometric characters were recorded in a Microsoft Office Excel spreadsheet file. The same person collected all measurements for avoiding any type of error.

Data analysis

Before conducting in-depth data analysis, all conventional and truss-based morphometrics data were exposed to common descriptive inquiry to check their normality. In this case, no outliers were detected, and it was assumed that all data formed a normal distribution. Moreover, before running further analysis, the size effects of all conventional and truss-based morphometrics data were eliminated, described by Elliott et al. [30]. The allometric formula was used to remove the size effect from the dataset:

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

where M: Original measurement, M_{adj}: Size adjusted measurement, L_o: Total length of fish, and L_s: Overall mean of total length 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 groups.

The mean values of meristic characters, transformed conventional morphometric characters, and transformed truss-based morphometric characters were compared among populaces via one-way analysis of variance (ANOVA) followed by *post hoc* (Tukey-HSD) test. The size-adjusted data were also exposed to PCA and DFA with original and cross-validated classification analysis. However, by using ANOVA tests followed by Tukey-HSD *post hoc* tests, the exact causes of population-wise differences in morphological studies cannot be explained. Hence, multivariate analyses, i.e., PCA and discriminant

Table 1. Sources, sample size, total length, and date of collection of *C. nama* from the collection sites.

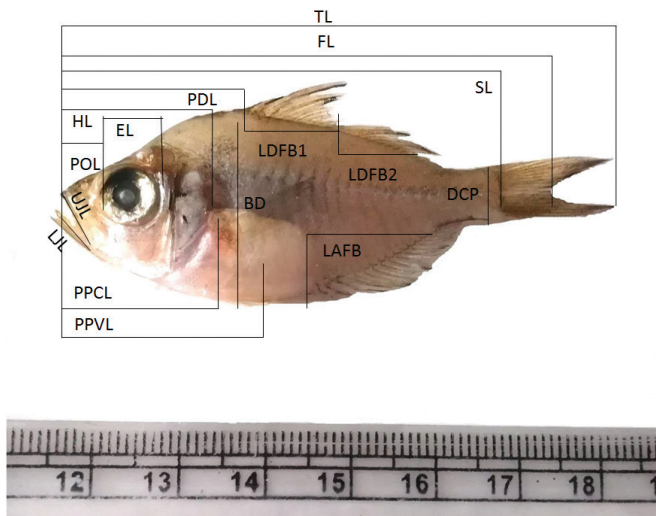| Sampling sites	Sample size (*n*)	Total length (Mean ± SD)	Geographical locations	Collection date
JBJ	61	5.66 ± 0.72	23.1778° N, 89.1801° E	15-07-2018
Tulshiganga River, Joypurhat	31	5.81 ± 0.53	25.0968° N, 89.0227° E	05-09-2018
Madhumati River, Narail	41	5.21 ± 0.81	23.1163° N, 89.5840° E	06-12-2018

Figure 2. Sixteen conventional morphometric characters were used for analysis in *Chanda nama.* The descriptions of landmarks are as follows, 1: TL (total length) – Distance from the tip of snout to the longest caudal fin ray, 2: SL – distance from the tip of snout to the end of the vertebral column, 3: FL – distance from the tip of the snout to the end of the fork of the caudal fin, 4: EL – distance from pre-orbital length to post-orbital length, 5: HL – distance from tip of snout to the posterior end of the opercular membrane, 6: BD – maximum depth between the dorsal and ventral portion, 7: UJL – straight line measurement between the snout tip and posterior edge of maxilla, 8: LJL – Straight line measurement between the snout tip and posterior edge of mandible, 9: POL – straight line measurement from front of snout to the fleshy anterior edge of the orbit, 10: PDL – straight line measurement from front of snout to the origin of the first ray of the first dorsal fin, 11: PPCL – straight line measurement from front of snout to origin of the pectoral fin, 12: PPVL – straight line measurement from front of snout to origin of pelvic fin, 13: LDFB1 – distance from base of dorsal fin ray to base of last dorsal ray, 14: LDFB2 – distance from first fin ray to last fin ray of second dorsal fin, 15: LAFB – distance from base of first anal fin ray to base of last anal fin ray, and 16: DCP – tapered region behind the dorsal and anal fins where the caudal fin attaches to the body.

function analyses, were performed by using conventional and truss morphometric characters combined to detect the best population as well as the actual sorts of morphometric features among the three populations. To address this issue, a major bottleneck was faced by inadequate samples regarding multivariate analyses during fish morphological studies. To conduct such types of multivariate analyses (PCA and DFA), a proportion of sample size (N) and the number of characters (P) must be considered as 3–3.5 [31]. However, insufficient sample size (N) may fail to analyze adequate covariances and morphological variations, which may ultimately interpret incorrect conclusions regarding the morphological studies in fishes [32].
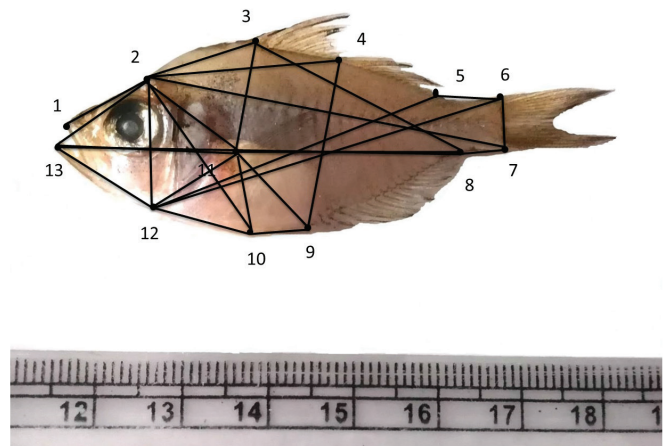

Figure 3. The position of landmarks for assembling the truss network on the fish body is illustrated as a closed circle, and truss-based morphometrics is indicated as interconnected measurements. The descriptions of landmarks are as follows: 1: Anterior tip of the upper snout, 2: Forehead (end of the frontal bone), 3: Origin of the first dorsal fin, 4: Endpoint of the first dorsal fin, 5: Endpoint of the second dorsal fin, 6: Dorsal origin of the caudal fin, 7: Ventral origin of the caudal fin, 8: Endpoint of the anal fin, 9: Origin of the anal fin, 10: Origin of the pelvic fin, 11: Origin of the pectoral fin, 12: Endpoint of the operculum at ventral side, and 13: Anterior tip of the lower snout.

Before conducting the PCA analysis, first, data were validated by using Bartlett's test of sphericity and the Kaiser–Meyer–Olkin (KMO) test. In general, the range of KMO value is 0–1 statistically. In PCA analysis, it was necessary to determine the number of factors for an accurate description of the variances and maximum contributions to differentiate the populations. In this context, the scree test is an established method, where the eigenvalues are considered more remarkable than a fixed unity to lessen the number of PCA analysis [33]. Hence, a scree test was performed. A Euclidean dendrogram [unweighted pair group method with arithmetic mean (UPGMA)] was drawn using transformed conventional and truss-based morphometric characters. The entire statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21 (SPSS, Chicago, IL).

Results

The minimum and maximum values of each meristic count ranged from 4 to 8 for DSR, 11 to 18 for DSFR, 15 to 25 for CFR, 14 to 28 for AFR, 5 to 9 for PVFR, and 4 to 8 for PCFR among three populations examined with corresponding descriptive statistical parameters (i.e., mean and standard deviation) (Table 2). The results obtained from the ANOVA test revealed that there were highly phenotypic divergences in different meristic characters among the three populations.

In meristic parameters, three characters, namely, DSR ($p <$ 0.001), AFR ($p <$ 0.001), and PCFR ($p <$ 0.001) parameters, demonstrated significant disparities while three remaining characters, namely, DSFR ($p >$ 0.05), CFR ($p >$ 0.05), and PVFR ($p >$ 0.05) showed no significant differences among the three populations. The MRN population showed significant differences from JBJ and TRJ populations for DSR character. In addition, the MRN population demonstrated a significant deviation from the JBJ and TRJ populations for AFR character. Moreover, the JBJ population exposed significant differences from TRJ and MRN populations for PCFR characters.

On the flip of site, nine conventional morphometric characters showed significant differences ($p <$ 0.05 and $p <$ 0.001) out of 15 conventional morphometric characters (Table 3). The JBJ population demonstrated a highly significant difference from the MRN population, whereas the TRJ population demonstrated intermediate between the two populations for the character of eye length (EL) ($p <$ 0.001). In addition, MRN population showed a significant disparity from the JBJ and TRJ populations for the character of head length (HL) ($p <$ 0.001). In addition, the TRJ population exposed a significant difference from

Table 2. Comparison of the mean with standard deviation (SD) of meristic characters of *C. nama* in three populations.

Characters (meristic)	JBJ (*n* = 61)		TRJ (*n* = 31)		MRN (*n* = 41)		ANOVA test	
	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	*f*	*p*-value
DSR	5–8	6.68 ± 0.84[a]	5–8	6.58 ± 0.72[a]	4–7	5.31 ± 0.61[b]	44.913	0.000***
DSFR	12–16	12.92 ± 1.58	11–18	13.64 ± 1.70	11–17	13.12 ± 1.41	2.231	0.112
CFR	17–25	19.03 ± 2.41	16–24	19.20 ± 1.88	15–24	19.58 ± 2.03	0.785	0.458
AFR	15–28	18.00 ± 2.18[b]	14–22	17.80 ± 1.93[b]	16–24	19.76 ± 1.92[a]	11.283	0.000***
PVFR	5–9	5.90 ± 1.01	5–7	5.74 ± 0.63	5–7	5.56 ± 0.63	2.077	0.129
PCFR	5–8	6.18 ± 1.08 [a]	5–7	5.61 ± 0.66[b]	4–7	5.48 ± 0.63[b]	8.880	0.000***

Min = Minimum; Max = Maximum; SD = Standard deviation; f = The ratio of between-group variability and within-group variability; P = Significance level; ANOVA = Analysis of variance (one-way).
Means with different superscripts letters are significantly different for each meristic variable.
* $p <$ 0.05, ** $p <$ 0.01, and ***$p <$ 0.001. The acronyms of meristic and population names were described in materials and methods section.

Table 3. Comparison of the mean with standard deviation (SD) of conventional morphometric characters of *C.nama* in three populations.

Morphometric characters	JBJ		TRJ		MRN		ANOVA test	
	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	*f*	*p*-value
SL	3.17–5.84	4.44 ± 0.11	3.73–5.44	4.47 ± 0.21	3.06–5.74	4.43 ± 0.14	0.839	0.434
FL	0.25–0.78	0.51 ± 0.10	0.38–0.79	0.50 ± 0.08	0.34–0.88	0.53 ± 0.10	0.686	0.505
EL	0.31–0.65	0.49 ± 0.06 [a]	0.33–0.63	0.45 ± 0.07 [ab]	0.27–0.65	0.43 ± 0.06 [b]	9.984	0.000***
HL	1.08–2.04	1.51 ± 0.13 [a]	1.14–1.18	1.47 ± 0.13 [a]	0.87–1.57	1.33 ± 0.12 [b]	24.385	0.000***
BD	1.48–2.27	1.62 ± 0.24 [b]	1.39–2.09	1.75 ± 0.11 [a]	1.11–2.12	1.62 ± 0.22 [b]	3.889	0.023*
UJL	0.14–0.46	0.28 ± 0.06	0.21–0.42	0.26 ± 0.04	0.17–0.43	0.26 ± 0.05	1.836	0.164
LJL	0.19–0.55	0.33 ± 0.07	0.25–0.57	0.35 ± 0.05	0.21–0.52	0.33 ± 0.06	1.041	0.356
POL	0.28–0.73	0.49 ± 0.08 [c]	0.43–0.83	0.55 ± 0.08 [b]	0.26–0.57	0.42 ± 0.07 [a]	22.829	0.000***
PDL	1.37–2.42	1.88 ± 0.13 [b]	1.71–2.71	1.97 ± 0.14 [a]	1.21–2.17	1.81 ± 0.15 [b]	9.909	0.000***
PPCL	1.06–2.17	1.66 ± 0.13 [b]	1.47–2.10	1.73 ± 0.11 [a]	1.07–1.89	1.56 ± 0.14 [a]	13.927	0.000***
PPVL	0.89–1.27	1.57 ± 0.16 [b]	1.41–2.25	1.75 ± 0.14 [a]	1.11–1.75	1.45 ± 0.16 [c]	29.399	0.000***
LDFB1	0.51–1.73	0.94 ± 0.22	0.65–1.44	0.93 ± 0.12	0.51–1.21	0.93 ± 0.11	2.109	0.125
LDFB2	0.67–1.97	1.11 ± 0.16 [b]	1.00–1.82	1.28 ± 0.14 [a]	0.59–1.43	1.08 ± 0.17 [b]	14.735	0.000***
LAFB	1.13–2.34	1.55 ± 0.19	1.07–2.14	1.57 ± 0.16	1.16–2.17	1.64 ± 0.17	2.960	0.055
DCP	0.28–0.73	0.52 ± 0.06 [c]	0.33–0.65	0.47 ± 0.06 [b]	0.35–0.73	0.55 ± 0.06 [a]	14.497	0.000***

Min = Minimum; Max = Maximum; SD = Standard deviation; f = The ratio of between-group variability and within-group variability; P = Significance level; ANOVA = Analysis of variance (One-way).
Means with different superscripts letters are significantly different for each morphometric variable.
* $p <$ 0.05, ** $p <$ 0.01, and *** $p <$ 0.001. The acronyms of morphometric and population names were described in the materials and methods section.

the JBJ and MRN populations for the characters of body depth (BD) ($p < 0.05$), predorsal length (PDL) ($p < 0.001$), and length of second dorsal fin base (LDFB2) ($p < 0.001$), respectively. Moreover, the MRN, JBJ, and TRJ populations showed significant differences among each other for the characters of preorbital length (POL) ($p < 0.001$), depth of caudal peduncle (DCP) ($p < 0.001$), and pre-pelvic length (PPVL) ($p < 0.001$), respectively. Furthermore, the prepectoral length (PPCL) ($p < 0.001$) character showed a significant disparity in the JBJ population compared to the TRJ and MRN populations. On the contrary, the following characters, namely, standard length (SL) ($p > 0.05$), fork length (FL) ($p > 0.05$), upper jaw length (UJL) ($p > 0.05$), lower Jaw Length (LJL) ($p > 0.05$), length of first dorsal fin base (LDFB1) ($p > 0.05$), and length of anal fin base (LAFB) ($p > 0.05$), did not confer any significant differences among the three populations.

However, in truss morphometric characters, 11 characters were found significantly different ($p < 0.05$ and $p < 0.001$) out of 23 characters among the three populations (Table 4). The TRJ population unveiled significant difference from the MRN population, whereas the JBJ population formed as intermediate between the TRJ and MRN populations for the characters of 1–2 ($p < 0.05$), 11–12 ($p < 0.05$), 2–9 ($p < 0.05$), 2–11 ($p < 0.05$), 2–12 ($p < 0.05$), and 4–9 ($p < 0.05$). Besides, the JBJ population exhibited a significant difference from TRJ population, but the MRN population did not show significant differences from the JBJ and TRJ populations for the character of 7–8 ($p < 0.05$). Similarly, the JBJ population showed a significant difference than the MRN population, whereas the TRJ population showed intermediate between the populations of JBJ and MRN for the character of 3–8 ($p < 0.05$). In addition, TRJ population formed a significant differences with the JBJ and MRN populations for the

Table 4. Comparison of the mean with standard deviation (SD) of truss-based morphometric characters of *C. nama* in three populations.

	JBJ		TRJ		MRN		ANOVA test	
	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	*f*	*p*-value
1–2	0.73–1.41	1.09 ± 0.15 [ab]	0.87–1.54	1.12 ± 0.15 [a]	0.41–1.46	1.02 ± 0.15 [b]	4.095	0.019*
2–3	0.57–1.36	0.93 ± 0.18	0.51–1.43	0.92 ± 0.17	0.52–1.17	0.92 ± 0.14	0.967	0.383
5–6	0.25–1.33	0.71 ± 0.19	0.35–1.03	0.63 ± 0.14	0.36–1.24	0.71 ± 0.16	2.210	0.114
7–8	0.37–1.32	0.67 ± 0.16 [a]	0.38–0.91	0.58 ± 0.12 [b]	0.40–1.06	0.64 ± 0.10 [ab]	3.684	0.028*
9–10	0.52–1.32	0.88 ± 0.14	0.50–1.26	0.90 ± 0.15	0.42–1.21	0.76 ± 0.12	2.879	0.060
10–11	0.44–1.18	0.69 ± 0.12	0.39–0.98	0.67 ± 0.13	0.36–0.97	0.68 ± 0.14	0.169	0.845
11–12	0.38–1.58	0.68 ± 0.16 [ab]	0.62–1.17	0.74 ± 0.11 [a]	0.32–0.87	0.65 ± 0.12 [b]	3.928	0.022*
11–13	1.07–2.30	1.58 ± 0.22 [b]	1.33–2.20	1.71 ± 0.16 [a]	0.82–2.22	1.57 ± 0.17 [b]	5.189	0.007**
2–14	1.18–3.80	1.76 ± 0.36	1.57–2.58	1.77 ± 0.35	0.93–2.35	1.76 ± 0.19	0.011	0.989
2–7	2.58–4.82	3.52 ± 0.34	2.82–4.69	3.59 ± 0.34	1.99–4.96	3.55 ± 0.34	0.353	0.704
2–9	1.22–3.02	2.17 ± 0.32 [ab]	1.93–3.12	2.30 ± 0.21 [a]	1.01–2.93	2.06 ± 0.24 [b]	6.538	0.002**
2–11	0.56–2.83	1.07 ± 0.32 [ab]	0.90–2.02	1.18 ± 0.22 [a]	0.38–1.89	0.99 ± 0.22 [b]	3.872	0.023*
2—12	0.90–1.85	1.30 ± 0.17 [ab]	1.03–1.85	1.37 ± 0.15 [a]	0.52–1.55	1.22 ± 0.17 [b]	6.558	0.002**
2–13	0.95–2.09	1.37 ± 0.20 [a]	1.12–1.91	1.41 ± 0.17 [a]	0.60–1.68	1.23 ± 0.17 [b]	9.476	0.000***
3–8	1.60–3.33	2.36 ± 0.28 [a]	2.12–3.04	2.47 ± 0.21 [ab]	1.07–3.24	2.29 ± 0.24 [b]	4.119	0.018*
3–10	1.16–2.41	1.82 ± 0.26 [b]	1.54–2.55	1.75 ± 0.20 [a]	0.76–2.31	1.75 ± 0.33 [b]	7.816	0.001***
4–9	1.04–2.16	1.71 ± 0.24 [ab]	1.09–2.36	1.82 ± 0.21 [a]	0.77–2.18	1.64 ± 0.25 [b]	5.016	0.008**
5–12	2.02–4.65	2.94 ± 0.38	2.06–3.75	3.03 ± 0.27	1.47–3.76	2.90 ± 0.30	1.265	0.286
6–12	2.43–4.75	3.51 ± 0.32	3.20–4.38	3.63 ± 0.27	1.89–4.77	3.55 ± 0.29	1.510	0.225
7–11	1.52–3.95	2.93 ± 0.35	2.36–3.74	2.89 ± 0.29	1.56–4.12	2.91 ± 0.37	0.132	0.876
8–13	2.15–5.33	3.85 ± 0.51	3.23–4.93	4.02 ± 0.35	1.92–5.59	3.84 ± 0.37	1.900	0.154
9–11	0.86–1.72	1.24 ± 0.18	0.86–2.71	1.32 ± 0.37	0.70–1.73	1.18 ± 0.15	2.625	0.076
10–13	1.18–2.78	1.91 ± 0.24	1.51–2.51	2.00 ± 0.23	1.05–3.12	1.95 ± 0.29	1.283	0.281

Min = Minimum; Max = Maximum; SD = Standard deviation; f = The ratio of between-group variability and within-group variability; P = Significance level; ANOVA = Analysis of variance (One-way).
Means with different superscripts letters are significantly different for each truss-based morphometric variable.
*$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. The acronyms of truss-based morphometric characters and population names were described in the materials and methods section.

characters of 11–13 ($p < 0.05$) and 3–10 ($p < 0.05$). Similarly, the MRN population showed a significant disparity from the JBJ and TRJ populations for the character of 2–13 ($p < 0.001$). Alternatively, the remaining 12 characters, namely, 2–3 ($p > 0.05$), 5–6 ($p > 0.05$), 9–10 ($p > 0.05$), 10–11 ($p > 0.05$), 2–14 ($p > 0.05$), 2–7 ($p > 0.05$), 5–12 ($p > 0.05$), 6–12 ($p > 0.05$), 7–11 ($p > 0.05$), 8–13 ($p > 0.05$), 9–11 ($p > 0.05$), and 10–13 ($p > 0.05$), did not confer any significant variations among the three populations from the ANOVA test.

However, in the present study, we used 133 (N) fish samples from three locations and measured 38 (P) conventional morphometric characters and truss-based morphometric characters combinedly. Thus, we analyzed the ratio of 3.5 (N:P) for conventional and truss-based morphometric characters before conducting further analysis of PCA and DFA to evaluate the same characters to discriminate against the populations. The KMO value in the current study was obtained at 0.817. Moreover, a significant result ($p < 0.05$) was also revealed from Bartlett's test of sphericity. In the scree test, 38 eigenvalues were observed. Among the 38 eigenvalues, only the first four factors were considered, where 51.848% cumulative variances were recorded with eigenvalues greater than 2, whereas the remaining factors were curtailed (Fig. 4).

The proportion loadings of the first four factors from principal components (PC1, PC2, PC3, and PC4, respectively) defined 27.681%, 12.210%, 6.577%, and 5.379% of the variance, respectively (Table 5). To maintain the constructive explanation, we fixed the factor loadings those were equal to 0.50 and beyond. Thus, the most important loadings on PC1 were HL, PDL, PPCL, PPVL, 1–2, 2–3, 11–12, 11–13, 2–7, 2–9, 2–11, 2–12, 2–13, 3–8, 3–10, 4–9, 5–12, 6–12, 8–13, and 10–13 (Table 6).

In the subsequent DFA, two discriminant functions (DF) were observed, where DF1 showed 63.5% variance and discriminant function (DF2) showed 36.5% variance (Table 7). The conventional and truss-based morphometric characters with significant loadings in first DF1 were PPVL, POL, PPCL, DCP, LDFB2, PDL, 2–13, 3–10, 2–12, 2–9, 4–9, 1–2, 3–8, 11–13, 2–11, 11–12, 9–11, 9–10, 5–6, 8–13, 5–12, 2–3, SL, FL, and 2–4, aggregately filling 63.5% of the total variance. Those mentioned above, 25 conventional and truss-based morphometric characters, characterized the entire external part of the fish body. On the other hand, the second DF2 clarified 36.5% of the total variance, where HL, EL, 7–8, UJL, LDFB1, BD, LAFB, 10–13, 6–12, LJL, 2–7, 7–11, and 10–11 characters were significantly contributed to the head region and body region.

In the bi-plot orientation from the discriminant space, all individuals were highly separated among the three populations (Fig. 5). Based on the discriminant function analysis, accurate classification results displayed 82% of individuals into their unique populations, whereas 66.9% of individuals were classified as a cross-validated group (Table 8). The original and cross-validated ratios of appropriately

Table 5. Fraction-based loadings (i.e., eigenvalues, percentage of variance, and percentage of cumulative variance) of principal components in *C. nama*.

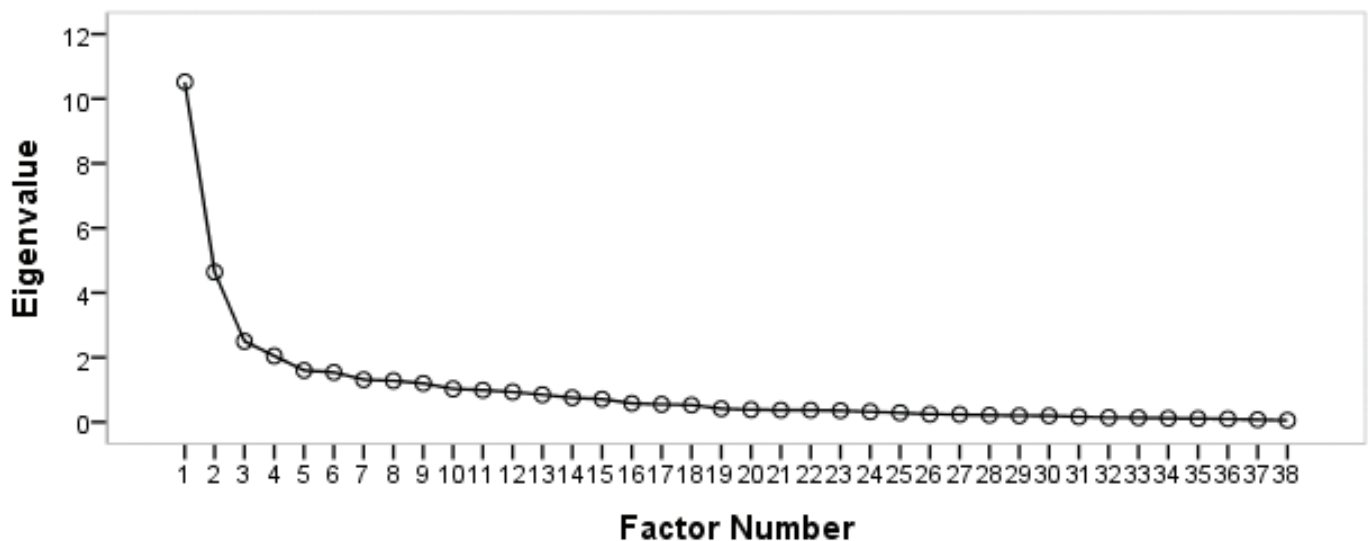| Component	Eigenvalues	% of Variance	Cumulative %
PC 1	10.519	27.681	27.681
PC 2	4.640	12.210	39.891
PC 3	2.499	6.577	46.469
PC 4	2.044	5.379	51.848

Figure 4. Scree plot of PCA derived from conventional and truss morphometric characters in *Chanda nama*. A total of 38 eigenvalues were observed and corresponded to 100% of cumulative variances.

Table 6. Component loadings of first four principal components (PC) of conventional morphometric and truss morphometric characters in *C. nama*.

Characters	PC 1	PC 2	PC 3	PC 4
SL	0.170	0.259	0.624	−0.309
FL	0.074	−0.056	−0.429	0.256
EL	0.315	−0.417	−0.003	−0.117
HL	0.540	−0.454	0.241	−0.135
BD	0.474	−0.443	−0.214	−0.390
UJL	0.104	−0.210	0.285	0.714
LJL	0.087	−0.088	0.267	0.764
POL	0.455	−0.405	0.293	0.019
PDL	0.500	−0.473	0.380	−0.157
PPCL	0.569	−0.496	0.232	−0.181
PPVL	0.505	−0.591	0.217	−0.238
LDFB1	0.296	−0.064	−0.260	0.101
LDFB2	0.150	−0.248	0.171	0.156
LAFB	−0.014	0.048	−0.372	−0.284
DCP	−0.175	0.196	0.100	−0.154
1–2	0.628	−0.269	0.205	0.141
2–3	0.541	0.351	−0.087	−0.186
5–6	−0.073	0.507	0.428	−0.279
7–8	−0.050	0.390	0.299	−0.076
9–10	0.306	0.113	0.255	−0.331
10–11	0.467	−0.038	0.125	0.065
11–12	0.595	−0.224	−0.239	−0.050
11–13	0.836	0.085	−0.137	0.031
2–14	0.439	0.142	−0.455	−0.017
2–7	0.540	0.711	0.065	0.043
2–9	0.769	0.183	0.042	0.057
2–11	0.663	0.050	−0.236	−0.002
2–12	0.800	−0.193	−0.094	0.117
2–13	0.636	−0.338	0.217	0.179
3–8	0.711	0.355	−0.130	0.006
3–10	0.841	−0.179	−0.189	−0.034
4–9	0.779	−0.143	−0.308	−0.052
5–12	0.733	0.369	−0.151	0.075
6–12	0.662	0.599	0.076	0.025
7–11	0.377	0.640	0.134	0.071
8–13	0.670	0.437	0.038	0.053
9–11	0.417	0.380	0.308	0.006
10–13	0.703	0.210	0.001	0.172

Character descriptions were given in the materials and methods section.

Table 7. Discriminant function scores of *C. nama* obtained using conventional morphometric characters and truss morphometric characters.

Characters	DF1 (63.5%)	DF2 (36.5%)
PPVL	0.508*	−0.079
POL	0.450*	0.042
PPCL	0.351*	0.047
DCP	−0.333*	0.026
LDFB2	0.332*	−0.193
PDL	0.296*	−0.037
2–13	0.276*	0.120
3–10	0.252*	−0.102
2–12	0.242*	0.006
2–9	0.241*	−0.016
4–9	0.209*	−0.042
1–2	0.188*	0.042
3–8	0.188*	−0.048
11–13	0.185*	−0.144
2–11	0.185*	−0.023
11–12	0.182*	−0.058
9–11	0.152*	−0.027
9–10	0.145*	0.091
5–6	−0.115*	0.106
8–13	0.108*	−0.096
5–12	0.103*	−0.034
2–13	0.084*	−0.053
SL	0.083*	−0.032
FL	−0.077*	−0.021
2–4	0.009*	0.004
HL	0.347	0.411*
EL	0.139	0.348*
7–8	−0.101	0.199*
UJL	0.022	0.166*
LDFB1	0.059	−0.163*
BD	0.147	−0.151*
LAFB	−0.120	−0144*
10–13	0.043	−0.129*
6–12	0.065	−0.127*
LJL	0.073	−0.084*
2–7	0.023	−0.067*
7–11	−0.012	0.043*
10–11	−0.022	0.042*

Character descriptions were given in the materials and methods section.

classified individuals were demonstrated as a maximum of 87.8% and 75.6%, respectively, for MRN, followed by 80.6% and 64.5%, respectively, for TRJ and 78.7% and 62.3% for JBJ. A UPGMA dendrogram was developed based on

Euclidean distances between the population centroids and mainly displayed two main clusters, where the TRJ population formed a distinct cluster. The JBJ population developed a different cluster with a sub-cluster of MRN (Fig. 6).
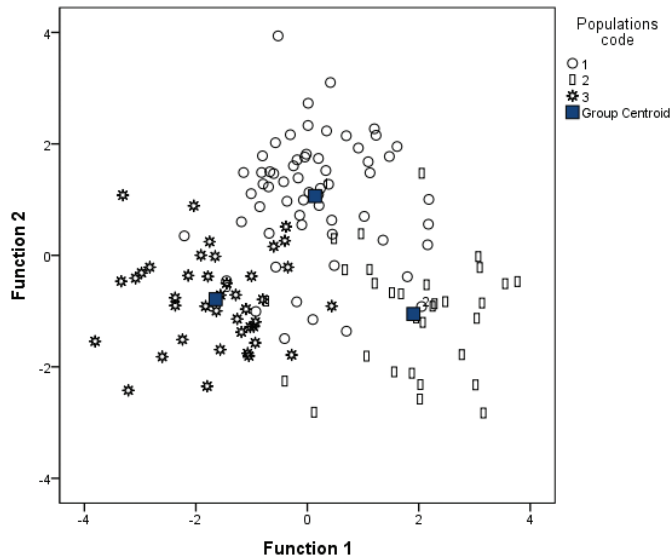
Figure 5. Bi-plot orientation was obtained from DFA by using conventional and truss morphometric variables for *Chanda nama*. Populations code represents: 1: Jhapa Baor - Jashore (JBJ); 2: Tulshiganga River Joypurhat (TRJ); and 3: Madhumati River Narail (MRN) in Bangladesh.

Table 8. Original and cross-validated classification of individuals of *C. nama* from three freshwaters, namely, Jhapa Baor - Jashore (JBJ), Tulshiganga River Joypurhat (TRJ); and Madhumati River Narail (MRN) in Bangladesh.

	Populations	Predicted group membership			Total
		JBJ	TRJ	MRN	
Original[a] (%)	JBJ	78.7	9.8	11.5	100.0
	TRJ	12.9	80.6	6.5	100.0
	MRN	9.8	2.4	87.8	100.0
Cross-validated[b] (%)	JBJ	62.3	21.3	16.4	100.0
	TRJ	25.8	64.5	9.7	100.0
	MRN	17.1	7.3	75.6	100.0

[a]82.0% of original grouped cases correctly classified.
[b]66.9% of cross-validated grouped cases correctly classified.

Discussion

Aquatic organisms like fishes often exhibit consistent phenotypical traits throughout their life cycle by numerous abiotic and biotic factors. Thus, phenotypical traits such as morphometric and meristic characters are frequently demonstrated as plastic in response to climatic action, anthropogenic activities, genetics, and epigenetics interactions within a species level. The present study focuses on the meristic and morphometric variations in natural SIS of *C. nama* estimated using meristic counts, conventional, and truss-based morphometrics.

The current study showed meristic counts of 4–8 for DSR, 11–18 for DSFR, 15–24 for CFR, 14–28 for AFR, 5–9 for PVFR, and 4–8 for PCFR. Moreover, in meristic characters, three parameters, namely, DSR, AFR, and PCFR, exhibited significant differences among six characters in ANOVA result followed by Tukey-HSD *post hoc* test. The present results fluctuate entirely with the findings of Hossain et al. [34], who recorded the meristic counts of *C. nama* species ranged from 8 for DSR, 21 to 22 for DSFR, 20 to 22 for CFR, 17 to18 for AFR, 6 for PVFR, and 8 to 0 for PCFR. This significant disparity in meristic traits might be caused by adjoined genetic sources and ecological dissimilarities initiated in diversified sampling sources [35,36]. Nonetheless, significant deviances in DSR, AFR, and PCFR might also be resulted by the consequence of environmental stimuli (current water speed), which formed during the period from embryonic development to late maturation stages [37]. Moreover, the alteration in the number of rays of pectoral fins and caudal fins may be due to the temperature in the respective ecological niches and variability of the abundance of natural foods [37,38]. Besides, abiotic factors such as vicissitudes of temperature, fluctuations of salinity, radiation and significant changes of dissolved oxygen, and degree of day time temperatures in water affect the meristic characters in fishes [39]. The meristic characters may be a practical means for ascertaining individuals from different populaces [40].
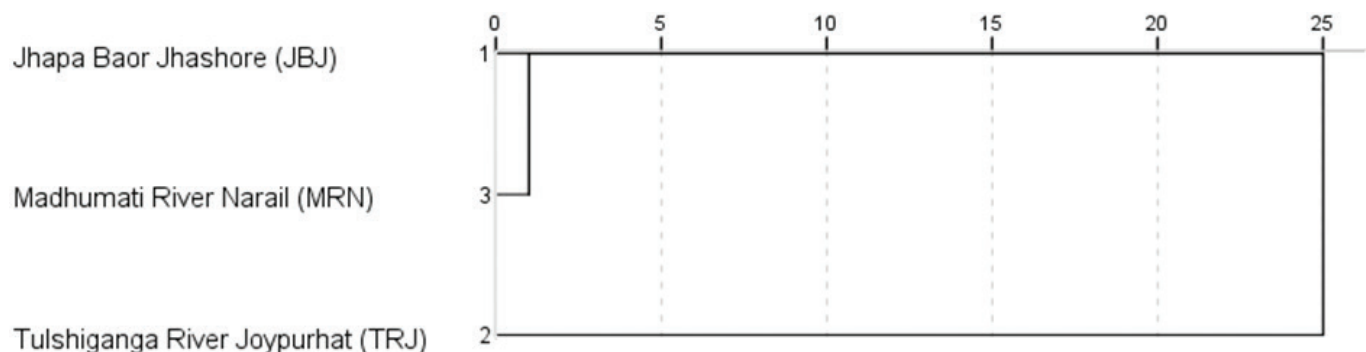

Figure 6. UPGMA dendrogram resulting from cluster analyses of conventional morphometric measurements and truss morphometric measurements for three different sources of *Chanda nama*.

In conventional morphometrics, nine characters showed significant variations out of 15 characters among the three populations. The significant characters are mainly categorized as three distinct regions (head and front region: EL, HL, POL, PDL, PPCL, and PPVL; body portion: BD and LDFB2; and caudal region: DCP) that are responsible for population differentiation in *C. nama*. Such a variation is highly parallel for freshwater species inhabiting in the same and different water bodies as described in *Channa punctatus* [41], *Labeo calbasu* [34], and *Xenentodon cancila* [42]. The significant characters of EL, HL, and BD are smaller in the JBJ population of *C. nama,* which could be attributed to the increase of turbidity mainly due to the creation of two floating bridges and other environmental factors. Turbid water influences in EL and HL development as well as responsible for population difference [43,44]. Moreover, environmental factors positively affect phenotypic variation in the head region; for instance, EL, HL, and POL. These types of phenotypic divergences may be caused by the availability of natural feed, dissolved oxygen, and light intensity [45,46].

In truss-based morphometrics, 11 characters (head region: 1–2, 11–12, 11–13, 2–11, 2–12, and 2–13; and body portion: 2–9, 3–8, 3–10, 4–9, and 2–9) were found to be significant out of 23 characters among three populations. Parallel results were obtained in *Sperata aor* [44], *Labeo ariza* [47], and *Labeo calbasu* [34]. These phenotypic alterations among three populations may be ensured due to their distinct environmental locations and the current environmental disparity of their three habitats or may be initiated from different ancestors and genetic variations.

However, in fish morphometric studies, the sample size is a matter for multivariate analyses (i.e., principal component analyses and discriminant function analysis) that always pose a question to differentiate fish population or stock. In earlier, researchers analyzed the ratio of the number of samples (N), and the number of morphometric characters (P) would be minimum at 3–3.5 [31]. Similarly, the ratio was 3.5 in the present study. Moreover, a lower value of *N* may mislead to capture the covariance or deviation of morphological characters and eventually interpret counterfeit conclusions concerning disparities among fish populations [32]. In this regard, AnvariFar et al. [48] observed that N:P ratios were 4.32 for Siah Mahi *C. gracilis* collected from the Tajan River. Moreover, Mir et al. [49] calculated the N:P proportion to be 14.03 for Indian Major Carp, *Labeo rohita* collected across the Ganga basin.

Multivariate analysis, such as PCA and discriminant function analysis, provides an advantageous system to differentiate dissimilar stocks within the same species [50]. Moreover, it is necessary to adjust the size-related parameters before conduct multivariate analysis (PCA and DFA) in the fish morphometric study to avoid the counterfeit results [48]. This consequence may be solely expected to phenotypical shape variations rather than allometric transformation among the individuals [51]. The KMO value of the study was 0.817. According to Kaiser [52], the KMO values can be ranked as different categories, for instance, moderate (0.5–0.7), good (0.7–0.8), and excellent (0.8–0.9). Consequently, the acquired result from KMO and Bartlett's tests recommended that the extracted data were proved as highly realistic from each sample for the factor analysis of conventional morphometrics and truss-based morphometric characters. It is noted that the factor loadings from four diverse factors were categorized as numerous levels, for instance, 0.30–0.39 dignified to be significant, 0.40–0.49 dignified to be more significant, and 0.50 or above dignified to be very significant. In the current study, we found the factor loadings equal to 0.50 and beyond, marked as very significant.

In the present study, the classification results clearly supported higher phenotypical variations observed in *C. nama* among the three populations, namely Jhapa Baor (78.7%), Madhumati River (87.8%), and Tulshiganga River (80.6%), because the individuals might have limited chances of migration. Different results were observed in the population structure of *Ompok pabo* from Bergobindapur Baor (91.7%), Bhairab River (95.2%), and Kopotakho River (100%) by Mahfuj et al. [13]. In the same way, phenotypic differentiation was recorded in *Lepidocephalichthys guntea* from Chalan Beel (93.3%), Bhairab River (90.5%), Nabaganga River (93.9%), and Dhakuria floodplain (100%) [17]. The higher phenotypic divergences among the populations within different regions may be due to higher genetic effects such as gene flow (*mtDNA* and *cytochrome b*) over generations to generations. However, no previous work has been accomplished regarding the genetic variations of *C. nama* while studies reported on basic biology, morphology [53], length–length, and length–weight relationship [33]. The phenotypic variation of a fish may also be governed by genetic and environmental interactions [11]. Moreover, the correlation among the genetic variations and different morphological traits has been confirmed in natural fish populations [53], and similarly, both methods have been extensively used in population separation [54]. Nonetheless, the phenotypic disparity always replicates population distinction from many habitats using molecular levels [55]. Anthropogenic impacts can prime the heightening of pre-modified genetic disparity, reflecting higher inter-population genetic signatures [56].

Conclusion

The results of the current study through meristic counts, conventional morphometrics, and landmark-based truss morphometrics protocol revealed high levels of distinctions among the aforementioned three populations of *C. nama* in Bangladesh. The consequences of this research would address crucial information to resource augmentation, population delineation, and effective fishery management of this essential SIS. However, the application of other methods in population differentiation such as otolith shape and its micro-chemistry, life history analysis, tagging protocols, and molecular studies along with truss-based morphometrics are synergistically beneficial for addressing population differentiation. Therefore, more studies can be carried out by applying other established techniques to characterize individual stocks and conserve this wild SIS.

Acknowledgments

Conflicts of interest

The authors declared that they have no conflicts of interest related to this research.

Authors' contribution

KNA, MSM, and KNA executed the study design. KNA collected fish from different sources. KNA, MSM, TI, and MAIS participated in data collection. KNA and MSM performed all the tests. KNA, MSM, TI, and KNA drafted the manuscript; KNA, MSM, and KNA revised the manuscript. KNA and MSM critically checked the article and corrected the manuscript. All authors read and approved the latest version of this manuscript.

References

[1] Roos N, Islam MM, Thilsted SH. Small indigenous fish species in Bangladesh: contribution to vitamin A, calcium and iron intakes. J Nutr 2003;133(11):4021S–6S; https://doi.org/10.1093/jn/133.11.4021S

[2] Froese R, Pauly D. (Eds.). Fish base. World wide web electronic publication. 2018. Available via http://www.fishbase.org (Accessed 10 June 2018).

[3] Wahab MA. *Chanda nama*. In: Siddiqui KU, Islam MA, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA, Haque EU, Ahmed ZU, Begum ZNT, Hassan MA, Khondker M, Rahman MM. (eds.). Encyclopedia of flora and fauna of Bangladesh, Vol.23. Freshwater fishes. Asiatic Society of Bangladesh, Dhaka, Bangladesh, p 180, 2007.

[4] Dawes J. Complete encyclopedia of the freshwater aquarium. Firefly Books, Richmond Hill, ON, p 289, 2015.

[5] Ghrub AR, Winemiller KO. Ontogeny of scale feeding in the Asian glassfish, *Chandanama* (Ambassidae). Copeia 2004; 4:903–7; https://doi.org/10.1643/CE-02-095R1

[6] Hossain MY, Hossen MA, Yahya K, Islam MM, Islam MA, Ahmed KKU, et al. Threatened fishes of the world: *Ompok pabda* (Hamilton, 1822) Siluriformes: Siluridae). Croat J Fish 2015; 73:183–5; https://doi.org/10.14798/73.4.818

[7] Bangladesh, I. U. C. N. Red list of Bangladesh volume 5: Freshwater Fishes. In: Rahman MM (ed.). Chanda nama. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh, p 205, 2015.

[8] Begg GA, Friedland KD, Pearce JB. Stock identification and its role in stock assessment and fisheries management: an overview. Fish Res 1999; 43:1–8. https://doi.org/10.1016/S0165-7836(99)00062-4

[9] Turan C, Erguden D, Turan F, Gurlek M. Genetic and morphologic structure of Liza abu (Heckel, 1843) populations from the rivers Orontes, Euphrates and Tigris. Turk J Vet Anim Sci 2004; 28:729–34.

[10] Turan C, Ozturk B, Oral M, DuzgunesE. Morphometric and meristic variation between stocks of Bluefish (*Pomatomus saltatrix*) in the Black, Marmara, Aegean and northeastern Mediterranean Seas. Fish Res 2006; 79:139–47; http://doi.org/10.1016/j.fishres.2006.01.015

[11] Cadrin SX. Advances in m4t5orphometric identification of fishery stocks. Rev Fish Biol Fish 2000; 10: 91–112; https://doi.org/10.1023/A:1008939104413

[12] Cavalcanti MJ, Monteiro LR, Lopes P. Landmark-based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). Zool Stud 1999; 38:287–94.

[13] Mahfuj MS, Hossain MF, Jinia SS, Samad, MA. Meristic and morphometric variations of critically endangered butter catfish, Ompok pabo inhabiting three natural sources. Int J Biosci 2019; 14(4):518–27; https://doi.org/10.12692/ijb/14.4.518-527

[14] Rohlf FJ. Morphometrics. Annu Rev Ecol Syst. In: Rohlf FJ, 2004. TpsDig-Thin Plate Spline Digitise, Version 1.4 [Computer software] Stony Brook: Dept. Ecology and Evolution, State Univ, New York, NY, pp 299–316, 1990; https://doi.org/10.1146/annurev.es.21.110190.001503

[15] Swain DP, Foote CJ. Stocks and chameleons: the use of phenotypic variation in stock identification. Fish Res 1999; 43:113–28; https://doi.org/10.1016/S0165-7836(99)00069-7

[16] Strauss RE, Bookstein FL. The truss: body form reconstructions in morphometrics. Syst Biol 1982; 31:113–5; https://doi.org/10.1093/sysbio/31.2.113

[17] Mahfuj S, Said-ur-Rahman, Samad M. Landmark-based truss morphometrics delineate the stock structure of *Lepidocephalichthys guntea*. J Fish Aquat Sci 2019; 14:25–32; https://doi.org/10.3923/jfas.2019.25.32

[18] Hard J, Winans G, Richardson J. Phenotypic and genetic architecture of juvenile morphometry in Chinook salmon. J Hered 1999; 90:597–606; https://doi.org/10.1093/jhered/90.6.597

[19] Mahfuj MS, Khatun A, Boidya P, Samad MA. Meristic and morphometric variations of barred spiny eel *Macrognathus pancalus* populations from Bangladeshi freshwaters: an Insight Into Landmark-Based Truss Network System. Croat J Fish 2019; 77(1):7–18; https://doi.org/10.2478/cjf-2019-0002

[20] Galib SM, Naser SA, Mohsin ABM, Chaki N, Fahad MFH. Fish diversity of the River Choto Jamuna, Bangladesh: present status and conservation needs. Int J Biodivers Conserv 2013; 5(6):389–95.

[21] Halwart M, Gupta MV. (ed.). Culture of fish in ricefields. Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy, and the WorldFish Center, Penang, Malaysia, p 83, 2004.

[22] Grubh AR, Winemiller KO. Ontogeny of scale feeding in the Asian glassfish, *Chanda nama* (Ambassidae). Copeia 2004; 2004(4):903–7; https://doi.org/10.1643/CE-02-095R1

[23] Islam MR, Azom MG, Faridullah M, Mamun M. Length-weight relationship and condition factor of 13 fish species collected from the Atrai and Brahmaputra rivers, Bangladesh. J Bio Env Sci 2017; 10:123–33.

[24] Siddiqui KU, Islam MA, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA, et al. (eds.). Encyclopedia of flora and fauna of Bangladesh. Vol. 23. Freshwater Fishes. Asiatic Society of Bangladesh, Dhaka, Bangladesh, p 300, 2007.

[25] DoF. National fish week compendium. Department of Fisheries,Ministry of Fisheries and Livestock, Bangladesh, pp 144, 2015.

[26] Shammi M, Bhuiya GJA, Kamal AKI, Rahman MR, Rahman MM, Uddin MK. Investigation of salinity occurrences in Kumar-Madhumati River of Gopalganj District, Bangladesh. J Nat Sci Sus Tech 2012; 6(4):299–313.

[27] Dhaka Tribune, 2020. Sugar mill pollution turns Tulshiganga River jet black. Available via https://www.dhakatribune.com/ bangladesh/ nation/ 2020/01/27/ sugar-mill-pollution-turns- tulshiganga-river-jet-black (Accessed 22 June 2020).

[28] Dhaka Tribune, 2020. Gopalganj lifeline Madhumati under threat. Available via https://www.dhakatribune.com/ uncategorized/ 2014/09/10/ gopalganj-lifeline-madhumati- under-threat (Accessed 22 June 2020).

[29] Halim A, Sharmin S, Rahman H, Haque M, Rahman S, Islam S. Assessment of water quality parameters in baor environment, Bangladesh: a review. Int J Fish Aqua Stud 2018; 6(2):269–3.

[30] Elliott NG, Haskard K, Koslow JA. Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. J Fish Biol. 1995; 46(1):202–20; https://doi.org/10.1111/j.1095-8649.1995.tb05962.x

[31] Kocovsky PM, Adams JV, Bronte CR. The effect of sample size on the stability of principal component analysis of truss-based fish morphometrics. Trans Am Fish Soc 2008; 138:487–96; https://doi.org/10.1577/T08-091.1

[32] McGarigal K, Cushman S, Stafford S. Multivariate statistics for wildlife and ecology research. Springer Verlag, New York, NY, 2000; https://doi.org/10.1007/978-1-4612-1288-1

[33] Hossen M, Hossain M, Khatun D, Pramanik M, Uddin N, Parvin MF, et al. Morphometric and meristic traits of three ambassid fish species (*Chandanama*, *Parambassis lala* and *Parambassis ranga*). Indian J Geo Mar Sci 2020; 49(3):398–405.

[34] Hossain MA, Nahiduzzaman MD, Saha D, Khanam MUH, Alam MS. Landmark-based morphometric and meristic variations of the endangered carp, kalibausLabeocalbasu, from stocks of two isolated rivers, the Jamuna and Halda, and a hatchery. Zool Stud 2010; 49(4):556–63.

[35] Walsh MG, Bain MB, Squiers T, Waldman JR, Wirgin I. Morphological and genetic variation among shortnose sturgeon *Acipenser brevirostrum* from adjacent and distant rivers. Estuaries 2001; 24(1):41–8; https://doi.org/10.2307/1352811

[36] Saborido-Rey F, Nedreaas KH. 2000. Geographic variation of *Sebastesmentella* in the northeast arctic derived from a morphometric approach. ICES J Mar Sci 2000; 57(4):965–75; https://doi.org/10.1006/jmsc.2000.0703

[37] Lindsey CC. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J Fish Res Board Can 1975; 32:2453–69; https://doi.org/10.1139/f75-283

[38] Trabelsi M. Le complexe atherinaboyeri. Caractérisationbiométrique, biochimiqueetgénétique. Miseendedeuxnouvellesespècesd'athérinesdans le méditerranéen. Université de Tunis, Tunis, Tunisia, p 291, 2002.

[39] Kahilainen K, Østbye K. Morphological differentiation and resource polymorphism in three sympatric whitefish *Coregonus lavaretus* (L.) forms in a subarctic lake. J Fish Biol 2006; 68(1):63–79; https://doi.org/10.1111/j.0022-1112.2006.00876.x

[40] Carscadden JE, LeggeWC. Meristic differences in spawning populations of American shad, *Alosas apidissirna.* Evidence for homing to tributaries in the St. John River, New Brunswick. J Fish Res Board Can 1975; 32:653460; https://doi.org/10.1139/f75-084

[41] Khan MA, Miyan K, Khan S. Morphometric variation of snakehead fish, *Channa punctatus*, populations from three Indian rivers. J Appl Ichthyol 2013; 29(3):637–42; https://doi.org/10.1111/j.1439-0426.2012.02058.x

[42] Sarower-E-Mahfuj M, Rahman MM, Islam M, Samad MA, Paul AK, Adhikary RK. Landmark-based morphometric and meristic variations of freshwater garfish, Xenentodoncancila from four natural stocks of South-Western Bangladesh. J Adv Vet Anim Res 2019;6(1):117; https://doi.org/10.5455/javar.2019.f321

[43] Kotrschal K, Van Staaden MJ, Huber R. Fish brains: evolution and environmental relationships. Rev Fish Biol Fish 1998; 8(4):373–408; https://doi.org/10.1071/MF17306

[44] Khan MA, Nazir A. Stock delineation of the long-whiskered catfish, Sperataaor (Hamilton 1822), from River Ganga by using morphometrics. Mar Freshw Res 2019; 70(1):107–13.

[45] Langerhans RB, Chapman LJ, DeWitt TJ. Complex phenotype–environment associations revealed in an East African cyprinid. J Evol Biol 2007; 20(3):1171–81; https://doi.org/10.1111/j.1420-9101.2007.01282.x

[46] Chaiphongpachara T, Laojun S. Effectiveness of landmark-and semi-landmark-based geometric morphometric to identify four species of Culex mosquitoes in Thailand. J Adv Vet Anim Res 2019; 6(3):278–83; https://doi.org/10.5455/javar.2019.f345

[47] Ahammad AS, Ahmed MBU, Akhter S, Hossain MK. Landmark-based morphometric and meristic analysis in response to characterize the wild Bhagna, Labeoariza populations for its conservation. J Bangladesh Agric Univ 2018; 16(1):164–70; https://doi.org/10.3329/jbau.v16i1.36498

[48] AnvariFar H, Khyabani A, Farahmand H, Vatandoust S, AnvariFar H, Jahageerdar S. Detection of morphometric differentiation between isolated up and downstream populations of SiahMahi (*Capoeta capoeta gracilis*) (Pisces: Cyprinidae) in the Tajan River (Iran). Hydrobiologia 2011; 673:41–52; https://doi.org/10.1007/s10750-011-0748-7

[49] Mir JI, Sarkar UK, Dwivedi AK, Gusain OP, Jena JK. Stock structure analysis of *Labeorohita* (Hamilton, 1822) across the Ganga basin (India) using a truss network system. J Appl Ichthyol 2013; 29(5):1097–103; https://doi:10.1111/JAI.12141

[50] Karakousis Y, Triantaphyllidis C, Economidis PS. Morphological variability among seven populations of brown trout, *Salmon trutta* L., in Greece. J Fish Biol 1991; 38:807–17; https://doi.org/10.1111/j.1095-8649.1991.tb03620.x

[51] Tzeng TD. Morphological variation between populations of spotted mackerel (*Scomber australasicus*) off Taiwan. Fish Res 2004; 68:45–55; https://doi.org/10.1016/j.fishres.2004.02.011

[52] Kaiser HF. An index of factorial simplicity. Psychometrika 1974; 39:31–6; https://doi.org/10.1007/BF02291575

[53] Poulet N, Berrebi P, Crivelli AJ, Lek S, Argillier C. Genetic and morphometric variations in the pikeperch (*Sander lucioperca* L.) of a fragmented delta. Arch Hydrobiol 2004; 159:531–54; https://doi.org/10.1127/0003-9136/2004/0159-0531

[54] Agnese JF, Teugels GG, Galbusera P, Guyomard R, Volckaert F. Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *C. anguillaris* from Senegal. J Fish Biol 1997; 50:1143–57; https://doi.org/10.1111/j.1095-8649.1997.tb01643.x

[55] Ihssen PE, Evans DO, Christie WJ, Rechahn JA, DesJardine DL. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of Lake Whitefish (*Coregonus clupeaformis*) in the Great Lakes region. Can J Fish Aquat Sci 1981; 38:1790–807. https://doi.org/10.1139/f81-226

[56] Esguicero ALH, Arcifa SA. Fragmentation of a neotropical migratory fish population by a century old dam. Hydrobiologia 2010; 638:41–53; https://doi.org/10.1007/s10750-009-0008-2