Morphological variations between native and Vietnam-originated striped snakeheads (*Channa striata*) in Bangladesh

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Abstract. The freshwater striped snakehead, *Channa striata*, known as "shol" in Bangladesh was introduced from Vietnam for commercial aquaculture that lacks morphological background. Therefore, the present study sought to differentiate various *C. striata* populations morphologically using one hundred eighty-six samples, consisting of nine native and two Vietnam-originated populations in Bangladesh. Seventeen morphometric and six meristic characters were studied, and the analysis highlighted that native and Vietnamese snakeheads were significantly different considering all morphological traits examined except eye diameter. The discrimination analysis illustrated that pelvic fin length (PVFL), pectoral fin length (PCFL), mouth gape (MG), and body width (BW) were the central characters caused to differentiate between native and Vietnamese populations. However, morphological findings compared with the previous findings reveal that Vietnam-originated snakeheads in Bangladesh do not correspond to wild morphotypes in Vietnam and that need to maintain carefully to avoid possible new morphotypes through genetic introgression and different culture condition practiced in Bangladesh.
Keywords: *Channa striata*, Morphological variation, Snakehead, Vietnam

Introduction

The striped snakehead (*Channa striata*) is a commercially important freshwater fish that belongs to the family Channidae of the order Anabantiformes. The members of Channidae (Nelson 1994), commonly known as snakeheads, comprise a vast proportion of total fish caught in Bangladesh (Ahmed *et al.* 2018). At least 40 species belong to the genus *Channa,* mainly occurring in southern Asia (Froese and Pauly 2022). Among them, *C. striata* has the highest level of within-species divergence (Adamson *et al.* 2010, Nguyen *et al.* 2016). In Bangladesh, there are four major species of snakeheads, *viz. C. striata*, *C. marulius*, *C. gachua* and *C. punctata* are also distributed in other Asian countries like India, Pakistan, Thailand, Vietnam, Malaysia, Laos, and China. However, *C. striata,* locally known as "shol"*,* is the most popular and commercially important snakehead species because of its exquisite taste, high market price, and medicinal properties enriched with high antioxidants and essential albumin (Roy *et al.* 2020, Hidayati *et al.* 2017, Mustafa *et al.* 2012).

The *C. striata* originated from Vietnam was introduced to Bangladesh in 2014 via Innovative Agro Aid Pvt. Ltd. for small-scale breeding and aquaculture, and the phenotypic status of that strain is still unclear (Nguyen *et al.* 2016). The popularity of this strain as an aquaculture species grew rapidly, and commercial farming also gradually increased throughout the country. It has a big chance to escape from the culture to the wild, which may affect the ecosystem (So 2009). Therefore, maintaining the morphological record of both wild and Vietnam-originated strains is essential for distinguishing and comparing them with each other.

Morphological analysis is considered a simple, cost-effective, and most common tool to identify and characterize fish stocks (Cadrin and Silva 2005, Siddik *et al.* 2016) and distinguish between fish populations (Cheng *et al.* 2005, Siddik *et al.* 2015). Nguyen *et al.* (2016) and Song *et al.* (2013) reported morphological characterization of *C. striata* in Vietnam and Malaysia. In Bangladesh, few reports on genetic characterization of *Channa* species using the mtDNA cytochrome c oxidase 1 (COI) gene (Ahmed *et al*. 2018) and genetic variation assessment of Bangladeshi and Vietnamese C. *striata* using PCR-RFLP analysis of the CO1 gene were also reported (Alam *et al*. 2021). Besides, no information is available regarding morphological differences between native Bangladeshi and Vietnam-originated snakeheads in Bangladesh. Interestingly, in the Mekong Delta of Vietnam, three distinct phenotypes of *C. striata* have been observed in the wild and aquaculture conditions (Nguyen *et al.* 2016). Integrating morphological analyses would be more powerful to differentiate the native and Vietnam-originated *C. striata*. From this perspective and to obtain fundamental knowledge for brood stock development and conservation, we collected *C. striata* across Bangladesh, including introduced Vietnamese populations, and performed morphological analyses to differentiate them.

Materials and Methods

Sample collection: Native and Vietnam-originated striped snakeheads (*C. striata)* were collected from natural habitats and hatcheries. Native 136 samples were collected from natural sources of 9 districts of Bangladesh *viz.* Rangamati, Patuakhlai, Dinajpur, Natore, Mymensingh, Netrokona, Kishoreganj, Sylhet, and Gazipur, that largely covered all the divisions of Bangladesh and the Vietnam-originated 50 samples were collected from two local hatcheries- Reliance Aqua Farms and Biswas Agro Fisheries, located in Trishal, Mymensingh (Table I).

Table I. List of the sample of *C. striata* used in the study

Population	Source	location	Sample code	Sample number
Native	Kaptai Lake, Rangamati	22°35'34"N 92°12'49"E	R	24
	Jamla beel, Patuakhali	22°26'35"N 90°23'12"E	P	20
	Halti beel, Natore	24°30'49"N 88°59'30"E	N	20
	Ashura beel, Dinajpur	25°26'3"N 89°4'2"E	D	22
	Tanguar haor, Sylhet	25°09'01"N 91°03'37"E	S	15
	Chamra Bondor, Kishoreganj	24°28'49"N 90°57'27"E	K	10
	Kangsa River, Mohonganj, Netrokona	25°0'50"N 90°32'40"E	Ne	10
	Bayhe beel, Mymensingh	24°42'21"N 90°19'37"E	M	5
	Balu river, Gazipur	23°58'27"N 90°27'49"E	G	10
Vietnam	Biswas Agro Fisheries, Trishal, Mymensingh	24°39'0"N 90°21'13"E	BV	25
	Reliance Aqua Farms, Trishal, Mymensingh	24°39'2"N 90°24'7"E	RV	25
	Total sample			186

Morphometric and meristic data were measured following Rahman (1989) and; Doherty and McCarthy (2004) with some modifications (Fig. 1).
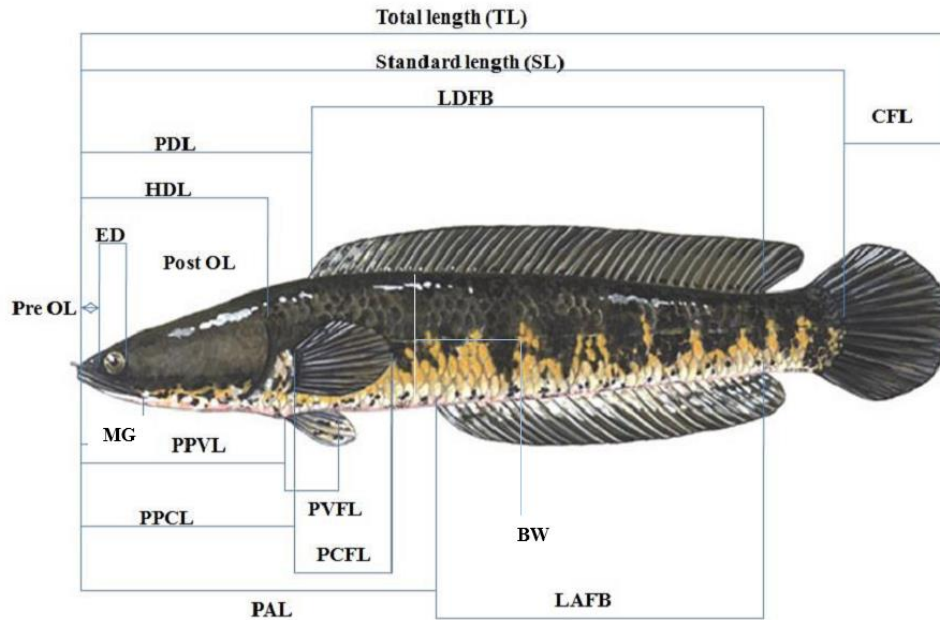
Fig. 1. The morphometric and meristic characteristics of *C. striata* followed in the present study.TL = Total length, SL = Standard length, PVFL = Pelvic fin Length, PCFL = Pectoral fin length, CFL = Caudal fin length, PDL= Pre dorsal length, PAL = Pre anal length, PPCL = Pre-pectoral length, PPVL = Pre pelvic fin length, LDFB = Length of dorsal fin base, LAFB = Length of anal fin base, MG = Mouth gape, BW = Body width, ED = Eye diameter, HDL = Head length, Pre OL = Pre orbital length, Post OL = Post orbital length.

Morphometric data were collected by measuring various parts of the body's length and width and the descriptive statistics of the measurement are given in Table II. Meristic characteristics were analyzed by studying lateral lines count, scale count, and fin count (6 counts), and morphometric data were measured (17 measures) and the data is given in Table III.

Table II. Descriptive statistics of the morphometric characters of native and Vietnamese populations of *C. striata*

Characteristics	Native		Vietnamese	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
TL	29.8 ± 0.42	0.16	15.41 ± 0.24	0.11
SL	25.61 ± 0.37	0.17	13.09 ± 0.21	0.11
PVFL	2.88 ± 0.05	0.19	1.63 ± 0.03	0.14
PCFL	3.92 ± 0.07	0.20	2.34 ± 0.04	0.12
CFL	4.20 ± 0.07	0.21	2.37 ± 0.04	0.11
PDL	8.83 ± 0.13	0.17	4.59 ± 0.08	0.12
PAL	13.52 ± 0.24	0.20	7.10 ± 0.12	0.12
PPCL	8.22 ± 0.11	0.16	4.44 ± 0.07	0.12

PPVL	8.79 ± 0.13	0.17	4.61 ± 0.08	0.12
LDFB	15.18 ± 0.23	0.18	7.56 ± 0.13	0.12
LAFB	9.25 ± 0.16	0.20	4.81 ± 0.08	0.12
MG	3.39 ± 0.05	0.17	1.62 ± 0.03	0.14
BW	3.73 ± 0.09	0.29	2.20 ± 0.04	0.13
ED	0.69 ± 0.02	0.29	0.66 ± 0.01	0.10
HDL	8 ± 0.11	0.16	4.23 ± 0.08	0.14
Pre OL	1.56 ± 0.03	0.23	0.80 ± 0.02	0.17
Post OL	5.57 ± 0.09	0.19	2.94 ± 0.06	0.14

Table III. Meristic characteristics of Native and Vietnamese *C. striata*

Characteristics	Range		Rahman (2005)
	Native	Vietnamese	
DFR	41-45	37-42	22-46
AFR	24-28	25-28	24-27
CFR	12-16	13-16	Absent
PVFR	6-7	6	6
PCFR	10-18	13-16	15-17
LLS	46-62	54-58	54-60

DFR = Dorsal fin ray, AFR = Anal fin ray, CFR= Caudal fin ray, PVFR = Pelvic fin ray, PCFR = Pectoral fin ray, LLS = Lateral line scale.

Morphometric analysis: Data on the 17 morphometric measurements of native and Vietnam-originated individuals were analyzed using univariate and multivariate techniques. The multivariate analysis of variance (MANOVA) was conducted to test the difference of the mean of all morphological traits of native and Vietnamese populations jointly. The principal components are effective tools to find the sources of the maximal amount of variance of the variables by aiding in data reduction. This technique was employed to identify the sources of variation due to morphological characters of native and Vietnamese populations separately. The cumulative proportion of variance is considered the benchmark in determining the number of factors to extract used in this study. Finally, discriminant analyses were used to classify the group membership. All the analysis was performed by statistical package R, and a 5% level of significance threshold was considered for all tests.

Results

The descriptive statistics and the coefficient of variation (CV %) of both native and Vietnamese 17 morphometric measures are presented in Table II. The low CV of each measurement indicated less variation among the intra class population and each sample was phenotypically homogeneous. The box whisker plot of the morphometric characters provided visual evidence of differences between native and Vietnamese populations of *C. striata* as well as the ranges of morphological measurements of Vietnamese *C. striata* were larger than those of natives (Fig. 2).
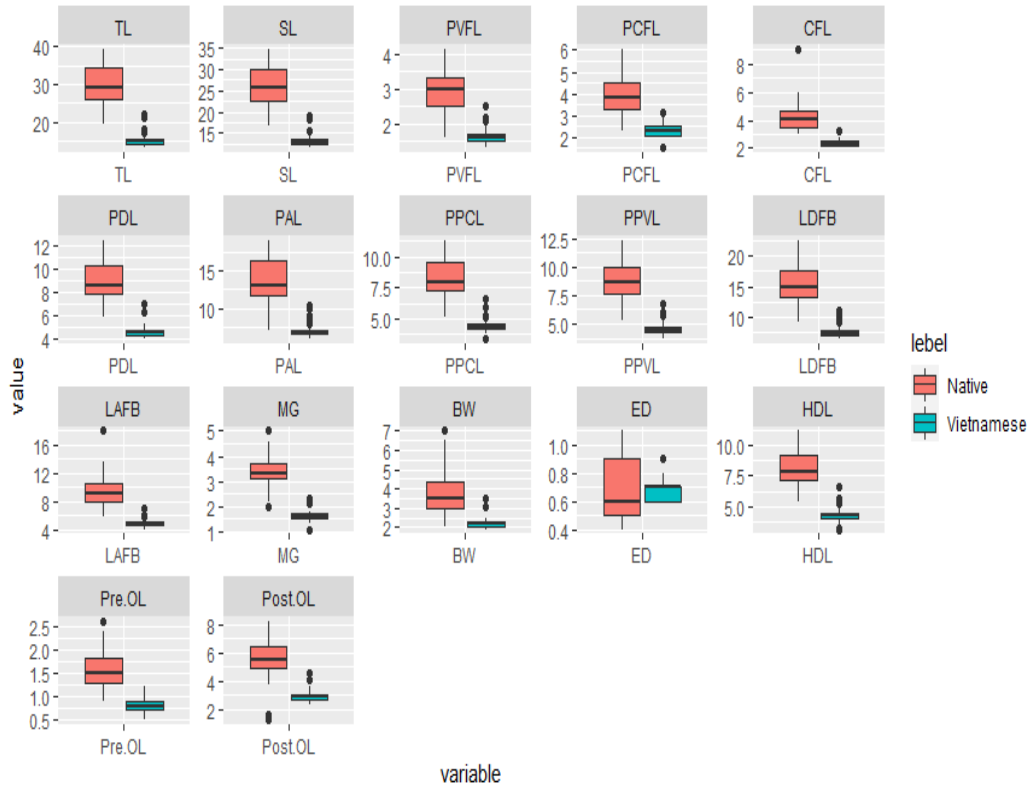
Fig. 2. Boxplot of the morphometric characters of native and Vietnamese populations of *C. striata*.

The outliers in the native populations provided massive variation in the data. The MANOVA concluded, that the mean difference of all 17 morphological characters between native and Vietnamese was statistically significant at a 5% level of significance except for ED. Therefore, it could be concluded that the variation across the two strains originated from other 16 measurements. The samples were collected from different sources, and the variation due to sources was examined by principal component analysis separately for two groups. The principal component, along with eigenvalues and proportion of variations, was explained in Table IV.

Table IV. Explained variation associated with loadings

Characteristics	Native			Vietnamese		
	PC1	PC2	PC3	PC1	PC2	PC3
TL	0.26	-0.25	0.12	**0.27**	**-0.69**	0.11
SL	0.24	-0.30	0.18	**0.27**	-0.04	0.15
PVFL	0.24	0.23	0.04	0.24	-0.07	-0.08
PCFL	0.26	0.14	-0.08	0.21	-0.24	-0.05
CFL	0.22	0.21	-0.11	0.25	-0.11	-0.16
PDL	0.26	-0.10	0.17	0.26	-0.04	0.08
PAL	0.24	-0.17	-0.21	0.26	0.01	0.02
PPCL	**0.27**	-0.10	0.003	0.26	-0.02	0.01
PPVL	**0.27**	-0.09	0.05	**0.27**	-0.01	0.02
LDFB	0.26	-0.18	0.09	0.26	-0.05	0.26
LAFB	0.25	-0.05	0.36	0.25	-0.07	0.24
MG	0.26	0.08	0.08	0.21	0.29	**-0.46**
BW	0.25	0.15	-0.02	0.24	-0.22	0.09
ED	0.14	**0.70**	0.03	0.12	0.68	0.43
HDL	0.26	-0.1	-0.06	0.24	-0.002	0.02
Pre OL	0.23	0.30	**0.42**	0.18	0.54	-0.42
Post OL	0.21	-0.11	**-0.83**	0.26	-0.05	0.04
Eigen value	13.45	1.36	0.5	13.03	1.07	0.73
Proportion of Variance	0.80	0.08	0.03	0.77	0.06	0.04

The summarized results of discriminant analysis are presented in the following Table 5. Firstly, the analysis was conducted considering all 17 measurements. The four most critical morphometric traits, namely PVFL, PCFL, MG, and BW, were selected according to significant loadings compared to other characteristics. Therefore, these four characteristics contributed to separating the native and Vietnamese; the discrimination function correctly classified around 98.9% of the total sample. All the Vietnamese samples were correctly classified, while two native samples were misclassified, and the between-group variance was 31.64. The cross-validation technique was applied by partitioning the whole data; 90% of the data was used for modeling purposes, and the rest was used for validation. According to the cross-validation technique, the discrimination rule successfully classified about 95% of the sample into respective groups in the prediction phase. Furthermore, in the validation phase, the rest, 10% of the total sample, resulted that one native being misclassified; therefore, PVFL, PCFL, MG, and BW were sufficient to recognize and determine the two populations' structures. The following Fig. 3 also provides evidence that the discriminant function could separate the sample into distinct groups.
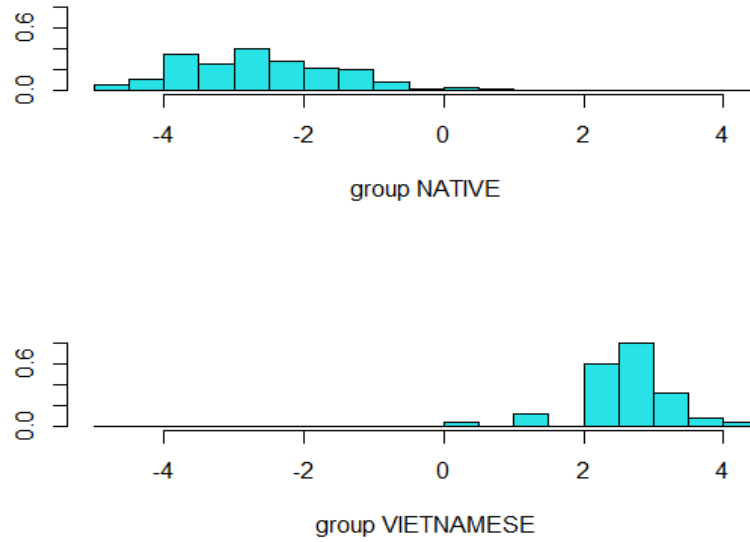
Fig. 3. Discriminate function separated native and Vietnamese populations into distinct groups.

Table V. Summary of discriminate analysis

Characteristics	LD1
TL	-0.17
SL	0.05
PVFL	**-0.80**
PCFL	**0.74**
CFL	-0.33
PDL	-0.36
PAL	0.02
PPCL	0.31
PPVL	-0.13
LDFB	-0.08
LAFB	0.16
MG	**-2.37**
BW	**1.54**
ED	0.26
HDL	0.07
Pre OL	0.02
Post OL	-0.30
Misclassification error	0.011

The variation was also found in the case of 6 meristic counts between native and Vietnamese samples. The Vietnamese had the maximum number of dorsal fin rays than the natives, and around 64% of Vietnamese had 56 dorsal fin rays, and 81% of natives had 43-45 dorsal fin rays. A total of 80% of Vietnamese had 25-26 anal fin rays, while 92% of natives had a total of 25-27 anal fin rays. Therefore, since the anal fin ray was wider than in Vietnamese, more variation was observed in natives compared to Vietnamese due to the anal fin ray. A total of 84% of Vietnamese had 15 caudal fin rays. On the contrary, 90% of natives had 13-14 caudal fin rays. The number of pelvic fin rays of Vietnamese was six, as most natives (90%) had six pelvic fin rays. Around 94% of Vietnamese and 85% of natives had 15-16 pectoral fin rays, 64% of Vietnamese had 56 lateral line scales whereas 70% of natives had 58-60 lateral line scales.

Discussion

The native and Vietnam-originated *C. striata* were significantly different according to their morphometric characteristics and meristic counts. Spatial variation was also determined among natives since the population was collected from several natural sources (Hossain *et al.* 2008). Though the Vietnamese population was collected from the cultivated farm, less variation in morphometric measurements was observed. Therefore, principal component analysis was conducted separately for native and Vietnamese *C. striata* to determine the morphometric characteristics that cause the maximum variation among groups due to different origins. Li *et al.* (2016) modeled that morphometric classification between the sexes of *C. striata* species, nine of the 10 morphometric measurements revealed no significant difference but TL (Total length) performed significantly greater values for males than for females among 272 sex-identified individuals (108 males and 164 females) in Subtropical Island.

According to principal component analysis (Table III), the intra-class variation of native and Vietnam-originated populations, and three principal components were extracted separately for native and Vietnamese populations. Around 90% of the total variation among the native groups can be explained by these principal components, whereas three principal components capture a total 83% variation of the Vietnamese population. It was reasonable since the Vietnamese were cultivated artificially. Their feeding habit and other management factors were similar. The first principal component (PC1) obtained from native explained 80% of the total variation of 17 measurements. However, the second and third principal component's importance is sometimes constructive (Delling *et al.* 2000) to explain the variation. The PPCL and PPVL had the maximum character loadings in PC1, ED had the maximum loadings in PC2, and Post OL had in PC3. These variables, different length measurements such as Pre-pectoral length, Pre pelvic fin length, Eye diameter, and Post orbital length, are factors that may consider the sources of variation among the native group. On the other hand, TL, SL, and PPVL had the highest loading in PC1 while TL and MG had maximum loadings in PC2 and PC3, respectively. Hence, the Total length, Standard length, Pre pelvic fin length, and Mouth gape were the influential factors that cause the differences among the Vietnamese. Therefore, PCA is considered a valuable tool to find the morphological characteristics of several species that are regarded as significant sources of variation among populations (Arechavala-Lopez *et al.* 2012) as well as between populations (Specziar *et al.* 2009, Turan *et al.* 2005, Alp and Kara 2007, Yakubu and Okunsebor 2011, Nguyen *et al.* 2016).

Discriminant analyses are popularly applied in identifying key factors that separate the fish population into distinct groups (Maric *et al.* 2004, Bektas and Belduz 2009, Yakubu and Okunsebor 2011). Turan *et al.* (2005) and Pollar *et al.* (2007) proposed a discriminant function that successfully identified 78% of the total population of *Clarias gariepinus*, and 95.6% of *Tor tambroides*, respectively. The cross-validation method was carried out in a later case and found a

48

93.1% of success rate. In this research, the discriminant analysis provided a clear idea regarding classifying factors of native and Vietnamese of *C. striata*. The classification rule was successfully allocated around 99% of *C. striata* into their particular groups. Furthermore, the cross-validation approach was used to validate this discriminant analysis, correctly classifying 95% of the population into their respective groups. The Pelvic fin Length (PVFL), Pectoral fin length (PCFL), Mouth gape (MG), and Body width (BW) were identified as classifying factors that could separate native and Vietnamese populations of *C. striata*. Therefore, these discriminating variables were statistically adequate to determine the population morphology of fish species and could be helpful for farmers and consumers in identifying native and Vietnamese populations of *C. striata*.

Different fin ray ranges have distinguished native and Vietnamese *C. striata* communities in the case of meristic characteristics. We have reported caudal fin ray count (CFR) for the first time for native and Vietnamese populations. We also compared meristic data with the published book by Rahman (2005) (Table III). Besides, Ahmed *et al.* (2018) compared the morphology of four species of *Channa* (*C. punctata, C. striata, C. marulius, C. gachua*) with a limited sample and the fin count of *C. striata* by them provide only a single value. On the other hand, we used an extensive sampling of native and exotic *C. striata* to generate meristic characteristic ranges, likewise to Rahman (2005). However, our fin count range also agreed with Ahmed *et al.* (2018).

This research revealed essential differences in the morphological characteristics of native and Vietnam-originated *C. striata*. Morphometric and meristic characteristics have long been regarded as critical instruments for determining a species' distinguishing characteristics (Musikasinthorn 2000). The present findings of PCA indicate four parameters (TL, SL, PVFL, and MG) were responsible for the difference between Bangladeshi wild and Vietnam-originated *C. striata* in Bangladesh. In contrast, Nguyen *et al.* (2016) found triangle (THS) and square head (SHS) parameters as the phenotypic difference of Vietnam *C. striata*. Nguyen *et al.* (2016) discussed three phenotypic variations (wild, triangle head, square head) of *C. striata* in Vietnam. It is complicated to compare Nguyen *et al.* (2016) data with the present findings due to the morphometric parameter used in both cases is not similar. However, it can be speculated that different phenotypic variations in *C. striata* of two countries resulted in cryptic morphotypes. The commercially cultured Vietnamese *C. striata* had similarities in this respect which may be causes of similar management systems and feeding habits. Sometimes mouth gape may depend on fishes' food and feeding habits, and such carnivorous fish of native *C. striata* collected from nature may impact that reason. Commercially cultured Vietnamese *C. striata*, on the other hand, are utterly reliant on pelleted feed, which can result in a more miniature mouth gape in Vietnamese populations. Finally, the current *C. striata* morphometric and meristic separation parameters may be effectively used to distinguish native and exotic strains of *C. striata* found in Bangladesh.

Our other molecular research showed that the Bangladeshi native *C. striata* produced a cluster with only Indian *C. striata*. On the other hand, the Vietnam-originated *C. striata* clustered with partial Indian, Vietnam, Thailand, Laos, and Malaysian samples of *C. striata*. At present morphological variations between Bangladeshi native and Vietnam-originated *C. striata* followed our molecular result (unpublished) with Alam *et al.* (2021). Thus it may be said that Bangladeshi native *C. striata* are closely related to Indian populations and maintain genetic differentiation. Besides, the distinct clade of *C. striata* may be due to the possible cryptic diversity of the snakehead species belonging to the same species within their distribution from South to East Asian countries (Adamson *et al.* 2010). Thus, the Vietnam-originated *C. striata* populations in Bangladesh belong to the East Asian group and still, it may be discussed that native *C. striata* populations maintain integrity, producing in the same clade. However, Tan *et al.* (2012) reported three distinct lineages of *C. striata* in Malaysia using the mtDNA ND5 gene. Phylogenetic reconstruction was also suggested by Wang and Yang (2011) for *C. striata*. Cryptic diversity of

the genus *Channa*, especially *C. gachua*, *C. marulius*, *C. punctata,* and *C. striata,* is also suggested by Serrao *et al.* (2014) after studying 36 putative species of the genus *Channa*. Though Ahmed *et al.* (2018) studied the Bangladeshi native *Channa* genus using the CO1 gene with a limited number of samples. Nguyen *et al.* (2016) noticed that the triangle head morphotype is closer to Malaysia and Indonesian *C. striata,* and the wild and square head morphotype is closer to the Indian sample. In the present study, the wild Bangladeshi type is closer to the Indian sample than the East Asian sample, and it can be said that Bangladeshi native *C. striata* is closer to the square and/or wild Vietnam morphotype. And Vietnam-originated *C. striata* in Bangladesh may be closer to the triangle head morphotype.

Conclusions

We discovered that native and Vietnamese *C. striata* differ in all morphological characters except eye diameter (ED). The four distinguishing morphological features such as pelvic fin length (PVFL), pectoral fin length (PCFL), body width (BW), and mouth gape (MG) can be used to differentiate native and Vietnam-originated *C. striata* in Bangladesh. Though it is difficult to identify exactly which morphotypes of *C. striata* were introduced from Vietnam in Bangladesh, and due to different aquaculture conditions, it may also be possible to find more diversified morphotypes in Bangladesh that still are within-species variation. As a consequence of the translocation, the Vietnam-originated *C. striata* in Bangladesh could put the Bangladeshi native populations at risk of genetic mixing with the Bangladeshi native population, leading to genetic attrition, reduced natural adaptability, and increased extinction risk. Thus, if any contamination has occurred due to hybridization, populations can be differentiated by the morphological differentiation found successfully in the present findings. However, it can be used to avoid genetic contamination and the smooth survival of native populations of *C. striata* in Bangladesh.

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