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Short Communication

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Sexual dimorphism of *Canthophrys gongota* (Teleostei: Cobitidae) using landmark-based geometric morphometrics in the Atrai river of Bangladesh

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Abstract

Studies on sexual dimorphism of gongota Loach *Canthophrys gongota* (Local name: Pahari Gutum) was performed capturing them from the Atrai River of Dinajpur district in Bangladesh. Females had light blotches and patches with thick and rounded pectoral and pelvic fins while males having dark blotches and patches with thin and comparatively pointed paired fins. Body size, lengths of the anal fin and distances between the bases of pectoral, pelvic and caudal fins were significantly different ($5.62 < F < 11.65$, $P < 0.05$) between the sexes of *C. gongota*. The expansion factors of mean thin-plate grids and vectors also showed that the head region of males was statistically different from females, whereas abdomen and tail of the females were considerably broader than those of the males. Both PCA (principal component analysis) and DFA (discriminant function analysis) plots showed morphologically little overlapping of landmark points which discriminated the females from the males. These findings are the first records on the sexual dimorphism of this rare species that would be baseline in a future study.

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Introduction

Sexual dimorphism is the systematic variation in form and/or color between the sexes of animal taxa. Fishes display their sexual dimorphic traits in body size, shape, fins, and/or ornamentation (Echeverria, 1986). Morphological variations between sexes are not only found in primary sexual characters, but also in secondary sexual traits not directly associated with their spawning (Kitano *et al*., 2007). Some fishes exhibit temporary sexual characteristics (e.g. colors) but others show permanent (e.g., Clasper organ) structures (Saurabh *et al*., 2013). However, many fishes do not show any sexual disparities during their life cycle (Chu-Koo *et al.,* 2009). Sexual dimorphism of fishes can be determined by the surveillance of fish gonads by dissecting the individuals regardless of maturity and breeding season. In fishes, some morphological traits differ between sexes apparently playing vital roles in their spawning period (Plongsesthee *et al*, 2012). Although sexual behaviors are the most crucial facts in reproduction and autecology of fishes but no available information present on these aspects for loaches (Bohlen, 2008). The key sexual dimorphic traits are commonly used for identification and taxonomy of loach fishes but also have been applied to classify genera and subgenera (Nalbant, 1994).

In Bangladesh, a total of 265 species of freshwater fishes is recorded where at least 24 species of loaches belonging to 10 genera of three families (Rahman, 2005). The Atrai River is one of the major rivers in Dinajpur district of Bangladesh (Islam and Mia, 2016; Islam *et al.,* 2017; Islam *et al.,* 2018) enriched with five species of freshwater loaches (Chaki *et al.,* 2015). The gongota loach, *Canthophrys gongota* (Hamilton, 1822) under the family Cobitidae locally known as 'Pahari Gutum' are found in swamps, lakes and rivers of Mymensingh, Sylhet, Dinajpur and Rangpur districts of Bangladesh (Talwar and Jhingran, 1991).

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They show no external sexual dimorphism (Siddiqui *et al.*, 2007) and reported in the rivers close to foothills (Kottelat, 2012) from May to June (Saha and Saha, 2011). As some fishes primarily exhibit monomorphic traits but sexual dimorphic characters between sexes are anticipated because males and females show various reproductive roles for partner selection expressing their morphological variations (Casselman and Schulte-Hostedde, 2004). Besides, abdominal shape as a reproductive structure of mature fishes would be expected to change during spawning season (Hassell *et al.,* 2012). Geometric morphometrics is a graphical approach that provides more facilities to reveal, illustrate and quantify the structural variations of animal taxa using a more significant approach (Adams *et al.*, 2004; Slice, 2007). Now, it is widely applied to study the sexual dimorphism and ontogeny in fishes (Kitano *et al.,* 2012) including their intraspecific phenotypic differences. Even though sexual distinctions between males and females have been studied on some loaches (Bohlen, 2008; Plongsesthee *et al*., 2012) but without any earlier record. However, null hypothesis is that there is no significant variations between two sexes of this loach. Therefore, an attempt was made to assess the sexual dimorphism of *C. gongota* with a view to identify their key morphometric traits that will be convenient to separate females from the males.

Materials and methods

Study area

A total of 128 individuals (64 males and 64 females) of *C. gongota* were collected directly from fishermen of the Atrai River (25.924° N, 88.724° E) in Dinajpur district located in the northwest region of Bangladesh (Fig. 1). Fishermen caught fishes by using fine meshed seine net (15×3.5 m^2, mesh size 4 mm). Fishes were immediately (within 1.5-2.0 h) transported with ice box and carried to the laboratory of the Department of Fisheries Biology and Genetics under Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Identification and measurement of specimens

At first, external form and/or ornamentation of the collected specimens were examined to find out any traits as reliable indicators in their body followed by Plongsesthee *et al.* (2012) with some modifications. Body weight (W_B) of each individual was measured with a digital balance (HD-602ND, MEGA, Japan) to the nearest 0.1 g for male and female, respectively. Eight lengths of fins (cm) and their distances between bases (cm) were also taken with a vernier caliper to the nearest 0.1 cm for each fish specimen to determine any

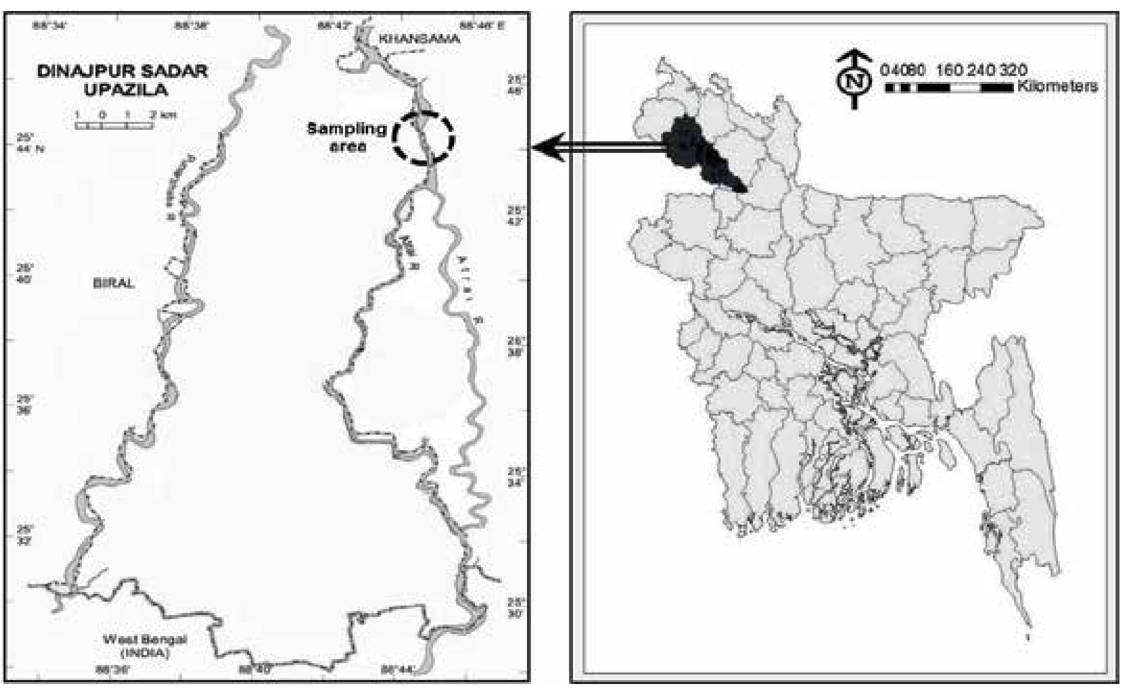

Fig. 1. Sampling area (black circle: 25.924° N, 88.724° E) in the Atrai River basin of Dinajpur district of Bangladesh

dissimilarity between the sexes of *C. gongota* (Table I; Fig. 2 and 3). Finally, fish samples were dissected to collect their gonads for precision of prediction by visual observation of sexes. After gonad collection, sexes were finally detected by observing their testes and ovary (Esmaeili *et al.*, 2017). Before dissection, photographs of the specimen were taken in order to grasp their main sexual dimorphic characters using geometric morphometric analysis. Individuals that had damaged fins (e.g. dorsal and caudal fins) not considered for imaging and analysis.

Table 1. Descriptive statistics and one-way ANOVA (P = 0.05) of body size and different fin's lengths with their base distances (cm) between sexes of *C. gongota*

General observation	Male						F-value	P-value
	Min	Max	Mean±SE	Min	Max	Mean±SE		
W_B	2.96	12.71	6.03±0.26	2.97	18.16	7.78±0.40	8.68	0.004
L_S	6.20	10.65	7.92±0.11	6.20	11.55	8.50±0.15	7.04	0.009
L_{PcF}	0.90	1.50	1.20±0.02	0.79	1.70	1.22±0.02	0.26	0.608
D_{PcFB}	0.40	0.75	0.55±0.01	0.40	0.80	0.61±0.01	11.65	0.001
L_{PvF}	0.95	1.50	1.18±0.02	0.65	1.60	1.21±0.02	0.54	0.464
D_{PvFB}	0.28	0.55	0.37±0.01	0.25	0.74	0.42±0.01	7.85	0.006
L_{DF}	0.55	1.20	0.84±0.02	0.09	1.50	0.91±0.02	0.99	0.321
L_{AF}	0.30	0.85	0.52±0.01	0.35	1.00	0.57±0.01	5.62	0.019
L_{CF}	1.20	1.80	1.52±0.02	0.90	2.02	1.49±0.02	1.14	0.287
D_{CFB}	0.45	0.80	0.57±0.01	0.40	0.97	0.61±0.01	5.68	0.019

WB, Body weight; LS, standard length; LPcF, length of pectoral fin; DPcFB, distance between bases of pectoral fin; LPvF , length of pelvic fin; DPvFB, distance between bases of pelvic fin; LDF, length of dorsal fin; LAF, length of anal fin; LCF, length of caudal fin; DCFB, distance between bases of caudal fin (length of caudal peduncle)

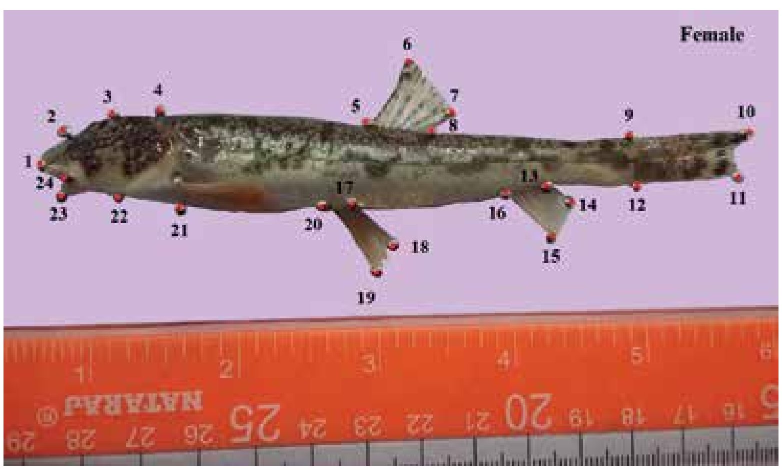

Fig. 2. a) Length and distances of unpaired fins of Canthophrys gongota: 5 to 8 (LDF), length of dorsal fin; 13 to 16 (LAF), length of anal fin; 9 to 10 (LCF), length of caudal fin; 9 to 12 (DCFB), distance between caudal fin bases. b) Location of 24 landmark points of this loach: 1) Tip of snout; 2) Middle of eyes; 3) Posterior of head; 4) Projection of operculum on dorsal profile; 5) Anterior insertion of dorsal fin; 6) Tip of posterior portion of dorsal fin; 7) Tip of anterior portion of dorsal fin; 8) Posterior insertion of dorsal fin; 9) Upper margin of caudal peduncle; 10) Tip of upper portion of caudal fin; 11) Tip of lower portion of caudal fin; 12) Lower margin of caudal peduncle; 13) Posterior insertion of anal fin; 14) Tip of posterior portion of anal fin; 15) Tip of anterior portion of anal fin; 16) Anterior insertion of anal fin; 17) Posterior insertion of pelvic fin; 18) Tip of posterior portion of pelvic fin; 19) Tip of anterior portion of pelvic fin; 20) Anterior insertion of pelvic fin; 21) Superior insertion of pectoral fin; 22) Insertion of operculum on ventral profile; 23) Posterior edge of lower jaw bone; and 24) Center of upper and lower jaw

Digitalization of images and landmarks

During photography, each specimen placed in the center of a dissecting pad (32 × 23 cm^2) with a left side of its body facing. A total of 64 males and 64 females were taken into consideration for their photographs (4000 × 3000 pixels, 180 dpi) using a digital camera (Canon PowerShot SX510 HS) where fins of each fish were spread out and pinned in place to offer a distinct view of insertion points into their body. For measuring, a ruler was placed against the lower edge of dissecting pad. Twenty-four landmarks (equal to 24 x and 24 y coordinates) were selected to analyze body shape differences between the sexes using landmark-based geometric morphometrics. Pins inserted on each specimen at all landmarks before taking photographs to make sure the accuracy of landmark locations during digitization. Multiple photographs of an individual were taken to check the acquisition of a quality image. After photography, images reviewed to select the best image for each specimen. Landmark clarity, lighting and angle were examined when selecting a photograph. Once chosen, photograph file was renamed with specimen identity number to classify them accurately as their sex (Santos and Quilang, 2012; Reiss and Grothues, 2015). All pooled images were processed for digitization (Fig. 2) to decide sexual dimorphism where landmarks were digitized using tpsDig2 software (Rohlf, 2006). After opening the images through this software, the ruler was set for measuring distance and then mouse point was set on two landmark points to get correct and reliable measurements.

Statistical analysis

More or less mature fishes were used to lessen the effects of intrapopulation shape variations because of allometric growth pattern (Dorado *et al*., 2012). Specimen data put in rows and two-dimensional landmarks (x and y coordinates) along each row for general procrustes alignment (GPA) by rotating and translating to major axis for linear space (Rohlf and Slice, 1990). The resulting procustes distances were used to calculate in Multivariate Analysis of Variance (MANOVA) to observe whether the differences in morphological traits between sexes of *C. gongota* are significant or not. The analysis of similarity (ANOSIM) is a hypothetical test that was used to evaluate the phenotypic variations between sexes of this loach. The value of 'R' was calculated as $4 (r_b - r_w)/N(N-1)$, where 'r_b, is the average rank of all distances between groups, 'r_w' mean rank of all distances within groups and 'N' is the total number of sample (Clarke, 1993). The thin-plate spline algorithm was used to calculate the mean body shape between sexes of *C. gongota* (Bookstein, 1991). Discriminant Function Analysis (DFA) was also used to evaluate the total amount of difference between males and females. Principal Component Analysis (PCA) scatter diagram was also used to visualize the degree of differences between the sexes of this species.

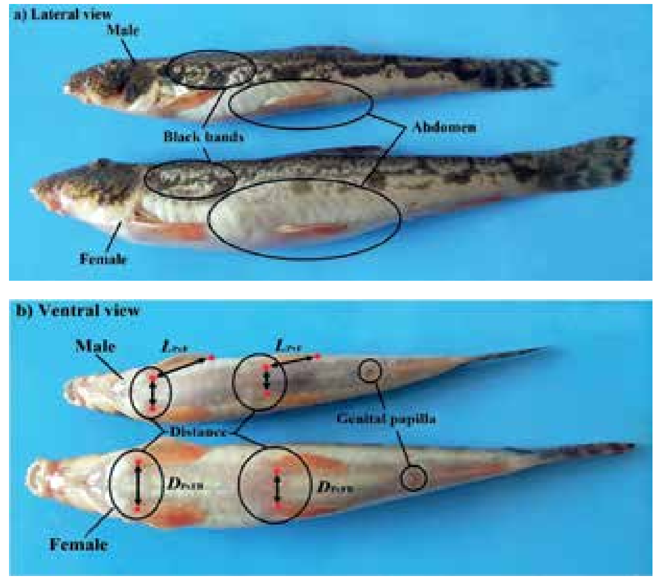

Fig. 3. a) Body coloration and abdomen between sexes of C. gongota (Standard length 10.65 and 11.55 cm; body weight 12.71 and 18.16 g for male and female, respectively. b) Length of paired fins and their distances between bases (LPcF, length of pectoral fin; LPvF, length of pelvic fin; DPcFB, distance between pectoral fin bases; DPvFB, distance between pectoral fin bases)

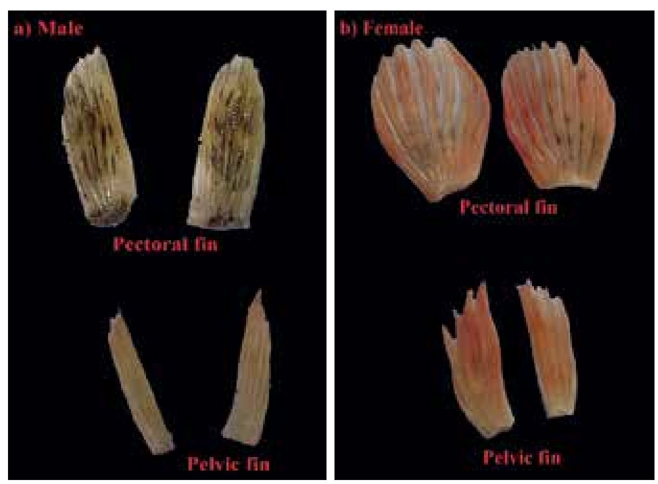

Fig. 4. Sexual dimorphism in the paired fins between sexes of *C. gongota*

Results and discussion

Sexual dimorphism through general observation

In this study, it was observed that females had a larger body (standard length), broader abdomen, comparatively thick and rounded paired fins (pectoral and pelvic) having relatively greater distances between bases of two fins than males (Fig. 3 and 4). In contrast, males had thin and slightly pointed pectoral and pelvic fins compared to the females. Besides, females also had reddish orange (during spawning period) to cream (in pre and post-spawning time) colored ovaries with the rough surface while males carried whitish colored smooth testes. Moreover, body size (standard length and body weight) and distances between bases of paired (pectoral and pelvic) and unpaired (anal and caudal) fins were significantly varied (F > 5.62, P < 0.01) while no statistical divergences (F < 1.14, P > 0.05) were observed in the lengths of dorsal, pectoral, pelvic and caudal fins between sexes of *C. gongota* (Table I).

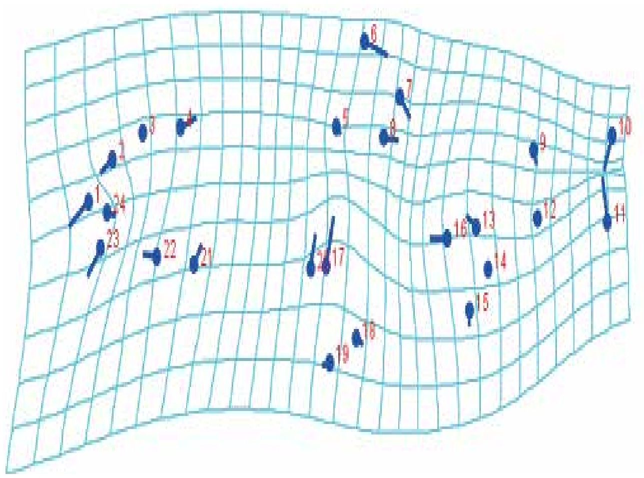

Fig. 5. Relative splines and vectors showing shape variation along the discriminators

Sexual size dimorphism using geometric morphology

During an experiment, a significant difference (P < 0.01) was observed between sexes of *C. gongota* based on body shape variations using MANOVA test (Wilk's lambda = 0.18, P < 0.01) representing sexual dimorphism. The ANOSIM compared the mean of ranked dissimilarities between groups to mean of ranked dissimilarities within groups exhibiting variations (R = 0.61, P < 0.01) between males and females (Table III). This deformation grid depicted the shape transformation associated with first canonical variate (CV_1) axis where eigen value, % of variance and Mahalanobis distances between group were 1.77, 99.22% and 2.64, respectively discriminating the sexes. The deformation grid associated with CV_1 illustrated that the most effective discriminators were 1, 2, 4, 23 (head region); 6, 7 (dorsal fin); 10, 11 (caudal fin); 21, 22 (bases of pectoral fin); 17, 20 (bases of pelvic fin) and 13, 16 (anal fin bases) between the sexes of this loach (Fig. 5). Besides, higher values of expansion factor were found in the head region for males (> 1.17) and in abdomen mainly in front of pelvic fin bases for females (> 0.58).

Using principal components analysis (PCA), the variance of PCA_1 and PCA_2 were 79.68% and 8.23%, respectively. The points that cluster together had similar shapes but far removed showed different shapes (Fig. 6). A discriminant function analysis (DFA) was used to judge distinctions between males and females of *C. gongota* where the group was correctly classified as 97.66% (F = 7.01, P < 0.01). The low overlap and high spread of DFA values explained higher and significant variations along mean values of DFA. The first six relative warps scores (RWs) of Pahari Gutum were RW_1 (36.22%), RW_2 (24.42 %), RW_3 (9.38%), RW_4 (6.87%), RW_5 (3.54%) and RW_6 (2.41%) accounted to 82.85% of total variance and eigen value of these scores were 0.077, 0.052, 0.0201, 0.015, 0.008 and 0.005, respectively. The percentage of RW_1 was larger showing higher differences compared to other RW scores. The first two relative warps accounted for 60.64% of total variance in 24 landmark points. Positioning in the plot relative to other individuals indicated the degree of similarity where individuals with positive amplitude on the first relative warp were similar in body shape and vice-versa.

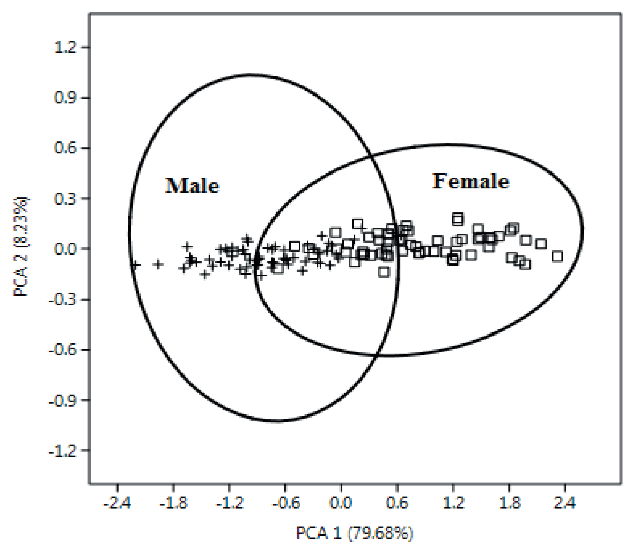

Fig. 6. PCA scatter plot showing the distribution of male and female of *C. gongota* based on body shape (+ male; ❑ female)

Body shape variations between sexes i.e. sexual dimorphism is the outputs of genetic changes mainly controlled by natural selection, choice of a couple and ecological selection (Blanckenhorn, 2005; Hassell *et al*., 2012) that were not considered in this study. This study reported that females of *C. gongota* were significantly larger in size than males which were consistent with the morphology of *Cobitis elongatoides* and *Sabanejewia balcanica* (Bohlen *et al.,* 2008), *C. calderoni* (Valladolid and Przybylski, 2008) and *Lepidocephalichthys* (Havird *et al.,* 2010). The reasons may be that female of loach may convert their energy for growth rather than reproduction until the second year whereas male uses more energy for breeding from their first-year life cycle (Eros, 2003). Studies regarding sexual dimorphism of this species have focused only on age and growth differences between males and females (Talwar and Jhingran, 1991). It was also observed that females had large and extended abdomen than those of males may be due to larger gonads demonstration which is similar to a loach *L. goalparensis* (Das *et al*, 2012). The males of *Schistura sexcauda* have a more slender abdomen than females (Plongsesthee *et al.,* 2012). Females of this gongota loach had a series of light brown blotches along sides and a less dark patch beneath the eyes compared to males which may possibly work as a functional gesture to entice females for mating or to counsel against males. Kottelat (2012) studied on over 60 loach species where all fishes carry small and extended body shape with different colored ornamentations, spots, and blotches. A sexually dimorphic black blotch present in pectoral fins of males *S. aurantiaca, S.* cf. *aurantiaca* and *S. sexcuada* while it is absent in females (Plongsesthee *et al.,* 2012). In common, males have statistically larger pectoral fins than females in most loaches such as *C. jadovaensis* (Mustafię *et al.,* 2008) and *S.* cf. *aurantiaca* (Plongsesthee *et al*., 2012). However, there were no significant variations in the lengths of pectoral, pelvic, caudal and dorsal fins between sexes of *C. gongota* which is similar to the morphology of *S. mahnerti* (Plongsesthee *et al*., 2012). But, a significantly larger anal fin was recorded in females than in males of this loach. Moreover, females of *C. gongota* also had significantly greater distances between the bases of pectoral, pelvic and caudal fins compared to males.

Landmark-based geometric morphometric is an important tool to examine the taxonomic and morphometric distinctions within and between species (AnvariFar *et al.,* 2011; Vilizzi and Kováč, 2014). There are no previous findings on sexual dimorphism of *C. gongota* (Pahari Gutum) or any other loaches (except three species of *Cobitis* reported by Mousavi-Sabet and Anvarifar, 2013) using truss-networks based geometric morphometric analysis. Therefore, it is not possible to compare the contemporaneous results with previous ones. However, the males and females of *C. gongota* showed sexual dimorphism based on their size independent morphometric traits which were similar to *Cobitis* sp. (Buj, *et al*., 2014) and to eight-barbel loach *Lefua* sp. (Aoyama, 2007). Using ANOSIM analysis, highly significant phenotypic variations also existed in the external morphology between sexes of *C. gongota* which may due to different roles played during their reproduction. These phenotypic changes may be due to genetical, environmental or their combinations (Pinheiro *et al.,* 2005). Thin plate analysis between males and females of gongota loach represented that variations were higher in the head and abdominal region compared to other body regions. Moreover, landmark plots presented low overlaps of shapes in both sexes of *C. gongota* where main dissimilarities mainly on the head and abdominal regions (an area between pectoral and pelvic fin bases). In *Mesopristes cancellatus*, differences were observed in the abdominal area and minimal variation on the tail between males and females (Barazona *et al*., 2015). The PCA scatter plot of *C. gongota* showed little overlapping of plots which implied that landmarks represented significant differences in body shapes of two sexes. In this study, PCA plots presented that both sexes of this fish population converge or overlapped at low level along the horizontal axis showing higher isolation between the sexes. Discriminant function analysis (DFA) could be a useful method to distinguish different species of the same genus or different stocks of the same species with respect to stock management programs (Karakousis *et al*., 1991). The overlap and spread of DFA represented 97.66% classification for *C. gongota* while 58.10%, 64.70% and 44.10 for *Cobitis* sp., *C. faridpaki* and *C. keyvani*, respectively (Mousavi-Sabet and Anvarifar, 2013). Besides, the individuals also grouped into 95.80% for *Salmo salar* (Solem and Berg, 2011) as different taxa.

However, variations between males and females of a fish species are important tools for fish stock assessment. The landmark-based geometric morphometric approach enabled us to quantify and visualize morphometric differences between sexes of *C. gongota.* This finding will be useful to identify sexes of *C. gongota* species where alteration patterns of sexes can be supportive to both in rearing and culturing with other developmental aspects of this species. Lastly, this landmark-based geometric morphometric study would be a baseline for future study on loaches within and among populations.

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