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Investigating the Population Genetic Structure of the Endangered Great Snakehead (*Channa marulius*) in Open Waterbodies of Bangladesh Using Mitochondrial DNA Markers

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ABSTRACT

The great snakehead, *Channa marulius*, is distributed across its natural distribution range of Southern and Southeast Asian countries. This valuable fish individual is endangered in Bangladesh, and its availability becomes limited only during winter which needs to be conserved, and no information has been recorded regarding the populational variations. In the current study, mitochondrial cytochrome b (Cytb) and cytochrome oxidase subunit 1 (CO1) genes were amplified and sequenced collecting samples from seven geographically distinct low-land ecosystems in Bangladesh. The amplification size was 742 (Cytb) and 558 (CO1) bp, and 10 and 11 haplotypes were found, respectively. Populations from Barishal, Sylhet, Mymensingh and Chattogram showed private haplotypes for both genes. The Cytb gene had the highest haplotype diversity (0.80) in the Mymensingh population, while the CO1 gene had the highest haplotype diversity (0.75) in the Rajshahi population. The Fst value showed the highest between Dhaka vs. Sylhet (0.912) in the case of the Cytb gene; whereas, the highest Fst value was recorded in Rajshahi vs. Sylhet, and Barishal vs. Sylhet (0.67) for CO1 gene, with a significance level of $P \leq 0.05$ for both genes. The phylogenetic tree constructed by both Cytb and CO1 genes produced three groups: Bangladeshi, shared with Indians, and shared with Pakistani strains. Finally, mtDNA genes identify a close but diversified relationship with the South Asian countries *C. marulius* species. Besides, several diversified groups were identified among Bangladeshi stripped snakehead populations that need to be carefully conserved for further extinction may happen.

INTRODUCTION

Among the freshwater fishes, Perciformes contribute to a large number of fishes in Bangladesh. One of the important families of the order Perciformes is Channidae, which consists of two genera, *Channa* and *Parachanna*. The members of the family Channidae commonly known as the snakeheads are native to Asia. Snakehead fishes are important

candidates for aquaculture in Bangladesh. There are five known species of *Channa* in Bangladesh, viz. *C. punctata* (Bloch, 1793) (spotted snakehead), *C. striata* (Bloch, 1793) (striped snakehead), , (great snakehead), *C. gachua* (Hamilton, 1822) and *C. barca* **(Rahman, 2005; IUCN, 2015)**. *C. marulius* (Hamilton, 1822) is widely distributed in Bangladesh, India, Pakistan, Nepal, Sri Lanka, Myanmar, Thailand and China. This species is known as 'Gajar Mach' in Bangladesh **(Talwar & Jhingran, 1991)**. It is highly valued and regarded for its flavor, high nutritional value, curative and medicinal properties, and it is occasionally suggested as a diet during the recovery period. Great snakehead (*C. marulius*) prefers sluggish or standing water in rivers, canals, lakes, and swamps that are characterized by submerged aquatic vegetation. It may also occupy areas of flooded forests and deeper riverine pools **(Courtenay & Williams, 2004)**. In our country, it generally prefers to inhabit the beel, canal and river bottom regions, and most often they are harvested during the dry season. *C. marulius* is reported to be the largest in the family of Channidae, reaching a length of 120- 122cm **(Talwar & Jhingran, 1992)**. However, their natural population has been steadily declining in recent years. Genetically unsound techniques that result in increasing inbreeding and genetic drift and the irreversible loss of fish species are making the issue worse. A thorough understanding of the genetic variability and genome organization of economically important fishes on the Indian subcontinent is required to develop effective strategies for the conservation of fish genetic resources in light of this serious scenario.

In our country, the culture of exotic species of *C. striata* is in practice. Recent analyses from **Kanon *et al*. (2022)** investigated the molecular differentiation between native and Vietnam-originated striped snakeheads (*C. striata*) in Bangladesh using the mitochondrial Cytb gene. **Alam *et al*. (2022)** also worked on native and Vietnamese *C. striata* to assess the genetic diversity, detection of strain-specific single nucleotide polymorphisms, and identification by PCR-RFLP analysis of the mitochondrial CO1 gene fragment. Although the fish species is endangered in its native habitat, *C. marulius* is another significant species from the genus *Channa*. Yet, there has been no definite published molecular research on this species. Thus, this circumstance necessitates a thorough investigation on this species' population status and conservation strategy.

Mitochondrial DNA (mtDNA) has been widely used as a genetic marker in phylogenetic studies of animals since it evolves considerably more frequently than nuclear DNA **(Brown *et al*., 1979)**. In fact, groups that have only recently been separated from one another differ due to the fast-paced sequence alterations in mtDNA. Cytb and CO1 genes are considered one of the widely used markers in the studies of population genetics and evolution since they are the most conservative protein-coding genes found in the mitochondrial genomes of animals. The mtDNA Cytb region is generally more variable **(Meyer *et al*., 1994)**. The mitochondrial Cytb gene is used for the phylogenetic study of closely related taxa and the evaluation of intraspecific genetic differentiation. Due to the following crucial features, CO1 is a bio-identical marker with good categorization power for practically all animal phyla; It is simple to isolate and has a lot of copies **(Cywinska *et al*., 2006)**, Insertion and deletion are unusual, free of recombination **(Daravath *et al*., 2013)**. In addition, it appears to have sufficient sequence uniqueness to distinguish closely related species because there are few variations within the species **(Hebert *et al*., 2003; Hebert *et al*., 2004)** and can be retrieved from both tiny and deteriorated samples **(Waugh, 2007)**. Sometimes, a clear conclusion can't be drawn

if one gene is used. Rather, if we use two genes, more strong information can be found. Therefore, we have used two genes (CO1 and Cytb) for the present study. This kind of dual gene utilization was done by **Habib *et al.* (2012)**, who successfully determined variations at interpopulation as well as intrapopulation levels in wild *C. marulius* from the Indian water by using ATPase 8 and ATPase 6 mtDNA sequences. According to the recent study of the genus *Channa* in India by **Imran *et al.* (2021)**, a tendency toward genomic homogeneity in *C. marulius*, which is evident from the intra-specific variation, suggests that the species is more susceptible to environmental pressures. Their findings imply that immediate action is required to protect *C. marulius*, including the implementation of particular conservation measures and appropriate breeding programs to increase their genome variability, which may make these species resilient to environmental whims.

Channa marulius is declining in the wild day by day, and now it is in an endangered condition; this is the main hindrance to conducting samples for any research work since it is very difficult to find a vast number of samples, and very little molecular research found on this species in our country. Given thiese data, this species was chosen to study its genetic variability, phylogenetic pattern, and genetic relatedness within and among various populations so that it can assume or evolve some conservative measures along with gaining knowledge about their present status. In this study, identification, genetic diversity, and phylogeny pattern of *C. marulius* species of family Channidae, belonging to the seven divisions of Bangladesh were approached using mtDNA sequence of Cytb and CO1 region, which might be helpful for effective management and conservation strategies of *C. marulius*. Two genes were used to observe the genetic variation pattern among the populations and determine if they are potential markers for studying variation both at an interpopulation level as well as an intrapopulation level. Genetic structure was assessed by evaluating the pattern of variation in mtDNA sequences within this selected geographic range, and this genetic study provides the much-needed details on these fish's genetic make-ups, which will enable us to develop an all-encompassing plan for successful conservation by propagating them in their native habitat along with referring the absolute population to be selected for brood fish and to take an attempt for establishing a sanctuary for the weak population.

MATERIALS AND METHODS

1. Sample collection and isolation of genomic DNA

Fish samples were collected from the natural habitat of seven divisions in Bangladesh. As this fish is already in endangered condition, and thus it was hard to collect a large number of samples from nature. However, detailed information about the samples used in this study is presented in Table (1). The fish samples were brought to the laboratory in a fresh condition in an icebox. DNA was extracted using Gene JET genomic DNA purification kit (Thermo Scientific), following the manufacturer's protocol with slight modifications. Fin tissues were used for DNA extraction.

2. PCR amplification

Two primer sets (Table 2) were used to amplify two genes (Cytb, CO1) of the mitochondrial DNA of *C. marulius*. Consequently, PCR amplification of the mtDNA Cytb gene was performed using GLUDG-L_Modified_Forward and CB3-

H_Modified_Reverse, and the CO1 gene was amplified using CO1_Fish_F1 and CO1_Fish_R1(Table 2). The fragment size of PCR amplification for Cytb was about 897bp, and for CO1 it was about 774bp.

Table 1. List of samples used in the present study

Source	Location	Sample code	Sample number
Shatla Beel, Barishal	22°90′31.41″ N/ 90°06 ′ 77.58″ E	B	7
Boalia Beel, Kustia, Khulna	23°96′ 04.23″ N/ 88°86′ 33.45″ E	K	5
Gazna Beel, Rajshashi	23°63′04.96″ N/ 89°00′ 41.02″ E	R	9
Chatla Beel, Sylhet	24°63′ 29.42″ N/ 92°08′ 87.63″ E	S	8
Megha Beel, Netrokona, Mymensingh	24°87′ 35.30″ N/ 90°77′ 04.97″ E	M	13
Chinadi Beel, Narsingdi, Dhaka	24°06′ 28.78″ N/90°66′ 91.63″ E	D	15
Ghugrar Beel, Comilla, Chattogram	23°48′ 64.70″ N/91°00′ 49.49″ E	C	10
Total samples			67

Table 2. List of the primers used in the present study

Primer	Sequence	Size	Reference
CO1_Fish_F1	5′-TCAACCAACCACAAAGACATTGGCAC-3′,	26bp	**Ward *et al.* (2005)**
CO1_Fish_R1	5′-TAGACTTCTGGGTGGCCAAAG-AATCA-3'	26bp	**Ward *et al.* (2005)**
GLUDG-L_Modified_Forward	5’-TGA CTT GAA RAA CCA YCG TTG-3’	21bp	This study
CB3-H_Modified_Reverse	5’-GGC AAA GAG AAA RTA TCA TTC-3’	21bp	This study

*R=G or A; Y= T or C

The final volume of the PCR mixture was 50µL, and the mixture contained 5µL template DNA, 2µL forward and 2µL reverse primer, 25µL PCR master mixture (Thermo Scientific), and 16µL double distilled water. Before conducting the actual amplification reactions, PCR conditions were improved by adjusting several parameters. In a thermal cycler, 40 cycles of denaturation at 94°C for 1 minute, annealing at 49°C for 1 minute 30 seconds, and extension at 72°C for 1 minute were used to conduct PCR. One cycle at 94°C for two minutes began the cycle, which was followed by a 10-minute cycle at 72°C

and a holding period at 4°C. The PCR results were electrophoresed (Mupid-2plus, Advance) in 0.9% agarose gel at 100 volts for 30 minutes to determine the size of the amplified DNA. The migration distance in the gel was then compared to DNA fragments of known molecular size (1 kb Gene ruler). Before using a UV transilluminator (Gel Doc^{TM}EZ Imager) to see the gel, it was stained with ethidium bromide for around 45 minutes and then washed in water.

3. PCR product purification and sequencing

PCR products were purified by using the molecular biology purification kit (Thermo Scientific) according to the manufacturer's protocol. The purified PCR product of each sample was stored and used for sequencing analysis. Sequencing reactions were carried out using the big dye terminator sequencing kit (v3.1; Applied Biosystems, USA). Cycle sequencing was carried out for 30 cycles with the following temperature profile: 94°C for 30s; 50°C for 30s, and 72°C for 1min and 30s, preceded by 3min at 94°C and followed by 8min at 72°C after cycling completion, and the sample was cooled to 4°C. Sequencing analysis was conducted on a capillary electrophoresis DNA analyzer (ABI Prism 3130xl Genetic Analyzer; Applied Biosystems, USA). In the procedure, bidirectional sequencing was carried out, and the sequencing data were curated and converted into FASTA format, and the size of the final useable sequence was determined as 742 bp for the Cytb gene, and 558 bp for the CO1 gene.

4. Molecular analysis

Using MEGA (version 11)'s implementation of CLUSTAL W (version 1.6), the mtDNA Cytb and CO1 gene sequences were aligned and modified before being manually adjusted. Sequence ambiguities were corrected by analyzing the chromatogram with Chromas software (2.1.1) and using the BLAST search to confirm the identity of the sequence by comparing it to other sequences that were present in the NCBI database (http://www.ncbi.nih.gov). The sequence determined in this study was deposited in the online GenBank databases under Accession No. LC705703 - LC705712 for Cytb gene and Accession No. LC705713- LC705723 for CO1 gene. Using sequences, MEGA software estimated the phylogenetic tree to show the link between populations. DnaSP6 calculated the fixation index, nucleotide diversity and other genetic distance indices. Using MEGA's (Version 11) MCL method, some phylogenetic analysis, Tajima's neutrality test, and evolutionary distances were calculated. Arlequin (Version 3.5) was used to perform AMOVA (analysis of molecular variance) analysis **(Excoffier *et al.*, 2005)**.

RESULTS

1. Haplotypes and sequence divergence

Any changes of base pair in any site of sequences were considered as a haplotype. Thus, 10 haplotypes were identified from the mtDNA Cytb gene and named H1, H2, H3, H4, H5, H6, H7, H8, H9 and H10. A private group of haplotypes was observed among Barishal (H1, H3), Sylhet (H5), Mymensingh (H6, H7, H8) and Chattogram (H10) populations. The rest of the haplotypes (H2, H4, and H9) were shared by the other populations (Fig. 1).

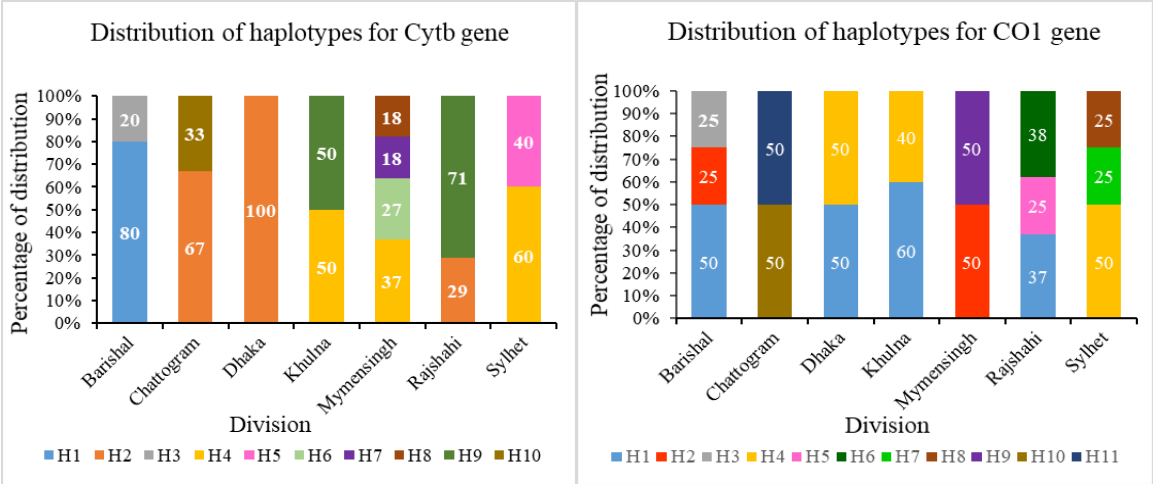

Fig. 1. Distribution of haplotypes (in percentage) for populations of *C. marulius* in seven sampling locations

The nucleotide frequencies are 25.65% (A), 26.42% (T), 33.73% (C) and 14.20% (G). The transition/transversion rate ratios are k1 = 8.672 (purines) and k2 = 6.364 (pyrimidines). The overall transition/transversion bias is R = 3.684, where R = [A×G×k1+ T×C×k2]/ [(A+G)×(T+C)]. 7 parsimony informative sites, 119 singleton sites, and 126 variable sites were found **(Tamura, 2021)**.

A total of 11 haplotypes were identified from the mtDNA CO1 gene, and the haplotypes were named as H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, and H11. A private group of haplotypes was observed among Barishal (H3), Rajshahi (H5, H6), Sylhet (H7, H8), Mymensingh (H9) and Chattogram (H10, H11) populations. The rest of the haplotypes (H1, H2, and H4) were shared by the other populations (Fig. 1). The nucleotide frequencies are 22.97% (A), 27.66% (T), 31.26% (C), and 18.10% (G). The transition/transversion rate ratios are k1 = 6.05 (purines) and k2 = 2.075 (pyrimidines). The overall transition/transversion bias is R = 1.781, where R = [A×G×k1 + T×C×k2]/ [(A+G) × (T+C)], and there were 5 parsimony informative sites, 17 singleton sites, 22 variable sites **(Tamura, 2021)**.

Tajima's D calculated based on native populations in Bangladesh was -1.939347 for the Cytb gene, and -1.446678 for the CO1 gene. According to Tajima 1989, a negative Tajima's D indicates an overabundance of low frequency polymorphisms compared to expectations, which denotes population size expansion (e.g., after a bottleneck or a selective sweep) **(Tajima, 1989; Nei, 2000)**. **Mostafa *et al.* (2009)** and **Galib *et al.* (2013)** studied *C. marulius* population distribution in its natural habitat along with the prediction of future degradation and low abundance; their data recorded justify the present endangered condition of *C. marulius* in open waterbodies of Bangladesh.

2. Genetic variation within the populations

For the Cytb mtDNA gene, the highest haplotype diversity (0.80) was found in the Mymensingh population and the lowest haplotype diversity (0.00) was found in the Dhaka population. The moderate to highest haplotype diversity was found in Khulna and Sylhet populations. The highest nucleotide diversity (0.088) was found in the Chattogram

population, while the lowest nucleotide diversity (0.00) was found in the Dhaka population. The overall nucleotide diversity and the number of segregating sites were 0.014 and 136, respectively (Table 3).

Table 3. Haplotype and nucleotide diversity of *C. marulius* found in the present study

Population	No. of Haplotypes		Haplotype diversity		Nucleotide diversity (pi)	
	Cytb	CO1	Cytb	CO1	Cytb	CO1
Barishal	2	3	0.400	0.714	0.001	0.002
Khulna	2	2	0.667	0.600	0.003	0.003
Rajshahi	2	3	0.476	0.750	0.001	0.003
Sylhet	2	3	0.600	0.714	0.001	0.002
Mymensingh	4	2	0.800	0.667	0.004	0.002
Dhaka	1	2	0.000	0.667	0.000	0.004
Chattogram	2	2	0.533	0.667	0.089	0.014

For the mtDNA CO1 gene, the highest and lowest haplotype diversity was found in the Rajshahi (0.75) and Khulna (0.60) populations, respectively. It indicates that the Rajshahi population is genetically more diverse than other populations. The moderate to the highest haplotype diversity was found in Sylhet and Barishal populations. In the case of nucleotide diversity, the highest value (0.014) was found in the Chattogram population, and the lowest value (0.002) was found in the Barishal, Sylhet and Mymensingh populations. The overall nucleotide diversity and the number of segregating sites were 0.004 and 29, respectively (Table 3).

3. Genetic variation between populations

Genetic variation between populations of *C. marulius* can be estimated by Fst values. In the present study, summarized genetic differentiation patterns between and within populations pairwise Fst, Gst, and Da values are presented in Table (4).

Table 4. Pairwise Fst value of *C. marulius* in the present study

Gene sequence	Fst Average (Max-Min)	Gst Average (Max-Min)	Da Average (Max-Min)
Cytb	0.440 (0.912-0.115)	0.251 (0.540-0.050)	0.005(0.014-0.0004)
CO1	0.305 (0.675-0.118)	0.124 (0.224-(-0.115))	0.003(0.010-(0.0007))

*For Fst value the differences are significant at P ≤5% level, for both genes P ≤ 0.0500

In the case of the Cytb mtDNA gene, the highest Fst value was found between Dhaka vs. Sylhet (0.912) indicating higher genetic differentiation of those populations. The lowest Fst value was found between Khulna vs. Mymensingh (0.115). The pairwise Fst value ranged from 0.115 to 0.912, with an average of 0.440, indicating that gene flow is

going on among the populations. For the CO1 mtDNA gene, the highest Fst value (0.67) was observed in Rajshahi vs. Sylhet, and Barishal vs. Sylhet indicating higher genetic differentiation of those populations. However, the lowest pairwise Fst value (0.118) was observed in Barishal vs. Khulna populations, and the average Fst value is 0.305. The highest Da value was found between Sylhet vs. Chattogram for Cytb and CO1 gene, and the average value are 0.005 and 0.003, respectively. The results of AMOVA attributed 24.10% and 43.71% of the variance to among populations for the Cytb gene and CO1 gene, respectively. Within-population variance for the CO1 gene and Cytb gene, respectively, was 56.28% and 75.69% (Table 5).

Table 5. Analysis of molecular variance (AMOVA) for populations of *C. marulius* in the present study

Source of variation	**Sum of squares**		**Variance components**		**Percentage variation (%)**	
	Cytb gene	CO1 gene	Cytb gene	CO1 gene	Cytb gene	CO1 Gene
Among populations	93.672	33.281	1.802	0.787	24.104	43.714
Within populations	187.203	34.475	5.673	1.014	75.896	56.286
Total	**280.875**	**67.756**	**7.475**	**1.801**		

*****AMOVA analysis was done at P=0.00 significance level

4. Phylogenetic analysis

The phylogenetic tree was constructed by the mtDNA Cytb and CO1 gene sequences obtained from the present study and data acquired from the gene bank after a homology search. Fig. (2) displays the phylogenetic tree and the corresponding relationships produced by the mtDNA Cytb and CO1 gene analysis.

The NJ tree (A) was constructed by Cytb mtDNA gene using present and online data, and haplotypes were separated into 4 groups with Indian *C. marulius* groups. Haplotypes (H3, H4, H7) and (H8, H9) produced distinct and separate groups in two clades. Whereas, haplotypes (H2, H5, H6, and H10) produced separate clades with distinct Indian groups; haplotype H1 produced another clade with the Pakistani group that was treated as an outgroup during tree formation.

A phylogenetic tree (B) was constructed by the mtDNA CO1 gene sequences including GenBank data. Some haplotypes (H7, H8, H4) created one cluster with the Indian and Bangladeshi groups, and some haplotypes (H3, H10, H11) created a cluster with the Indian group. H1 and H6 showed a close relationship with the Bangladeshi group separately. Whereas, H2 and H9 showed a close relationship with the Indian group separately. It was found that H5 was closer to the Pakistani group.

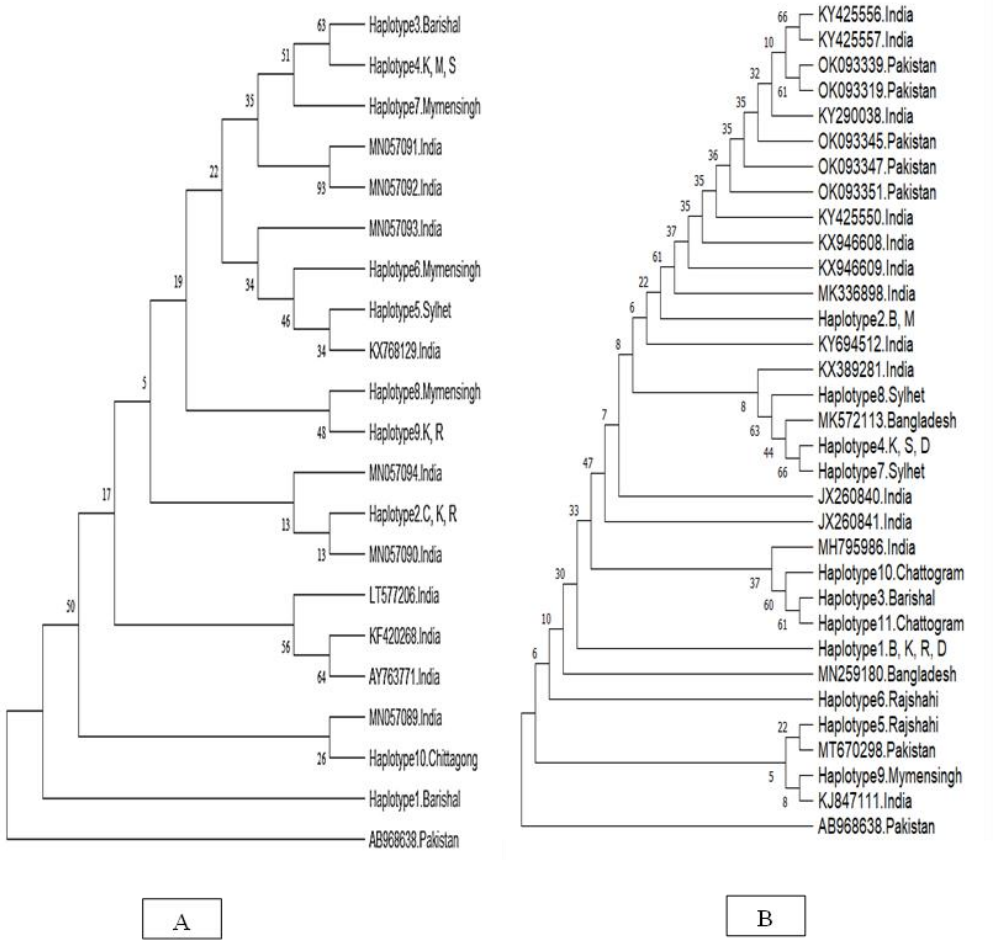

Fig. 2. Phylogenetic relationships constructed by mtDNA Cytochrome b region (Cytb) (A) and Cytochrome oxidase subunit 1 (CO1) (B) of different populations of *C. marulius.* Here B= Barishal, C= Chattogram, D= Dhaka, K= Khulna, M= Mymensingh, R= Rajshahi, S= Sylhet.

DISCUSSION

Taking immediate action and implementing conservation strategies is of utmost importance because *C. marulius* has been recently added to the Red List of Bangladesh by the International Union for Conservation of Nature (IUCN). Understanding a species' population genetic structure is essential for creating management and conservation plans for both naturally occurring fish populations and fish species that are endangered. Detailed molecular work with this species is not yet done in our country though some molecular work is done with the *Channa* genus they do not convey species specific information on genetic diversity and population status. The goal of the current work on the analysis of the Cytb and CO1 genes was to ascertain the genetic diversity and phylogenetic relationships among the populations of the species *C. marulius* and it will be the 1st molecular report on genetic diversity analysis of this endangered fish in Bangladesh.

In this study, we analyzed 742 bp fragments of the Cytb gene and 558 bp fragments of the CO1 gene in seven populations of *C. marulius.* The overall transition to transversion ratio (3.684) of the Cytb mtDNA gene in the present study is higher than that of the 307 bp Cytb gene sequence of *C. marulius* in India, where the transition to transversion ratio was 3.1 reported by **Habib *et al.* (2011)**. Genetic analysis of nucleotide sequences of Cytb in *C. marulius* was A + T rich (52.07%), which is near to the values (53.65% and 57.4%) observed in the studies of **Habib *et al.* (2011)** and **Imran *et al.* (2021)** in India, respectively. On the other hand, the overall transition to transversion ratio is 1.781 for the CO1 gene in our study which is lower than the transition/transversion ratio of up to 2.30 for the CO1 gene of *Channa argus* in a study conducted by **Zhou *et al.* (2019)** in China. The A + T content (50.63%) of CO1 in *C. marulius* is lower than those found by **Jamaluddin *et al.* (2011)** and **Alam *et al.* (2022)**. However, the G+C content (49.37%) obtained in the present study is almost similar to that reported in the work of **Lakra *et al.* (2010)**.

The average haplotype diversity for the Cytb gene (0.496) in the current study is lower than the average haplotype diversity for the Cytb region of mitochondrial DNA (0.763) in the Indian *C. marulius* **(Habib *et al.*, 2011)**. However, the highest haplotype diversity (0.80) is similar to the findings of **Rahim *et al.* (2012)** in *C. striata* in Malaysia. On the other hand, the highest haplotype diversity (0.75) for the CO1 gene is almost similar to those of **Siti-Balkhis *et al.* (2011)** in Malaysia and **Alam *et al.* (2022)** in Bangladesh who worked on the CO1gene of *C. striata* species. But average haplotype diversity (0.68) is lower than the average haplotype diversity value (0.8018) of **Song *et al.* (2013)** who worked in Malaysia on *C. striata* specimens using the mtDNA CO1 gene. High haplotype diversity recommends large, stable population sizes, environmental variability and life-history features that allow for fast population expansion **(Liu *et al.*, 2008)**. The average nucleotide diversity for Cytb was 0.014049, whereas **Habib *et al.* (2011)** found a nucleotide diversity value of 0.0128 in the Indian *C. marulius* using the Cytb mtDNA gene. The highest nucleotide diversity for the CO1 mtDNA gene was 0.01434; whereas, **Barman *et al.* (2014)** found the highest nucleotide diversity (Pi) of *Channa* species 0.16678 in India by using the CO1 gene. Therefore, the haplotype and nucleotide diversity value from the present study seems to be similar to the previous studies.

From the analysis of the Cytb gene, we found that the Mymensingh population has a significant population size, with the highest haplotype diversity and is possibly connected to other populations as haplotype H4 that was shared by Khulna and Sylhet populations. On the other hand, the analysis of the CO1 gene showed that the Rajshahi population has a significant population size with the highest haplotype diversity and is possibly connected to other populations as haplotype H1 that was shared by Barishal, Khulna and Dhaka populations. The private haplotypes that are found in most of the populations might be due to the distinctive natural habitat of these populations. Whereas, the highest nucleotide diversity was found in the Chattogram population for both genes, which indicates that, this population is genetically more diversified than other populations. One of the key components of species conservation is genetic diversity **(Miller, 1997; Almeida *et al.*, 2003; Baisvar *et al.*, 2019)**. In addition, the population's heterogeneity is a crucial evolutionary indicator for determining the dynamics and survival of the populations **(Reed, 2009)**. Therefore, the findings from the analysis of

population diversity patterns will aid in our understanding of where to put our conservation plan and what type of conservation (*in-situ* or *ex-situ*) is appropriate for which location.

According to **Nei (1986)** and **Wright (1978)**, the Fst value ranging from 0 - 0.05, 0.05-0.15, 0.15-0.25, and >0.25 indicate little, moderate, considered, and more prominent levels of genetic differentiation, respectively. In the case of the Cytb mtDNA gene, the highest Fst value was found between Dhaka vs. Sylhet (0.911); whereas for the CO1 mtDNA gene, the highest Fst value was found between Rajshahi vs. Sylhet and Barishal vs. Sylhet (0.67), indicating the higher genetic distance of those populations. High genetic differentiation among these populations could happen for several reasons as already their abundance is very low in their natural habitat, populations are geographically isolated, populations could have evolved in isolation after fragmentation from common ancestors, and according to **Chondar (1999)**, snakeheads are local migrants traveling only for a short distance for the purpose of feeding or for locating suitable breeding grounds or in search of new water to avoid stress conditions of the existing ecosystem. The lowest Fst value was found between Khulna vs. Mymensingh (0.114) for the Cytb gene, and Barishal vs. Khulna (0.118) for the CO1 gene. There is some ancient river connection between Barishal and Khulna divisions which may result in a lower Fst value. However, the interspecies distance with CO1 ranged from 0.049 to 0.2460 in the Indian Channidae fish population **(Lakra *et al*., 2010)**, which is lower than the range detected in the present research. In the present study, we found some interesting results in the case of pairwise Fst value of the Cytb gene; in spite of having a long geographical distance of Mymensingh with Khulna, the Fst value was the lowest among them, indicating that there is some need for more clarification. **Hussain *et al*. (2019)** reported the same scenario as they also found less genetic differentiation between populations of *C. marulius* having large geographical distances among them. They explained it as human mediated translocation or event of any ancient connectivity, or simply it could be a drawback of using Cytb gene as a marker. The lack of genetic differentiation among adjacent populations is not so unexpected. But when distant populations show less genetic differentiation, then demographic and historical ecology explanations are the main factors for the justification of such less genetic differentiation and may include the ancient connectivity among populations **(Steven, 2004)**. More frequent sampling from that locations, biogeographic study, and analysis with multiple mtDNA genes can be a way to clarify the result.

Similar Fst value for both Cytb and CO1 gene was also observed among some populations as; Barishal vs. Sylhet, Barishal vs. Chattogram, Khulna vs. Sylhet, Khulna vs. Mymensingh, Khulna vs. Rajshahi, and Sylhet vs. Rajshahi, and their Fst value ranged from 0.11 to 0.82. While other *Channa* species such as *C. striata* of the Malaysian population attributed the highest inter-population genetic distance (0.01) in the CO1 region **(Siti-Balkhis *et al*., 2011)**, *C. argus* showed a maximum genetic distance of less than 0.010 in China using the CO1 region **(Zhou *et al*., 2019)** and *C. striata* of Bangladeshi population showed the Fst value ranging from low to high (0.0 to 0.75) within the native populations **(Kanon *et al*., 2022)**. That means genetic distance is variable according to the geographical distribution pattern and migration rate among the populations, and some populations still maintain isolation, and interbreeding may occur between the populations. It has been identified that the species *C. marulius* is likely to

exhibit such a high degree of genetic differentiation, which may have been influenced by the species' limited dispersal.

The lowest Da values of various population pairs indicated that they were less segregated; individual might openly interbreed, and might elucidate more migration. On the other hand, higher values indicated lower gene flow, lower allelic frequency, and lower interbreeding (e.g., Sylhet vs. Chattogram, Mymensingh vs. Chattogram populations for both genes). To determine the genetic variability within the population and among the population of *C. marulius*, AMOVA was performed. AMOVA analysis showed variations among populations of 24.10439% and 43.71403% for the Cytb gene and CO1 gene, respectively. However, according to **Vrijenhoek (1998)**, among-population variance for non-migratory fishes such as *C. striata* was 32.4%; the same result was reported in the study of **Alam *et al.* (2022)**. Within the population, variations were 75.89561%; and 56.28597% for the Cytb gene and CO1 gene, respectively. **Jamaluddin *et al.* (2011)** studied AMOVA between populations of *Channa striata* in Perak State and found variance among populations and within populations, with values of 62.10 & 33.06%, respectively. In this respect, **Hussain *et al.* (2019)** found that AMOVA among populations and within populations of *C. marulius* was 62.05 & 33.03%, respectively. Our findings of AMOVA analysis are higher within population variations than the previous studies, which can be attributed to the decreased breeding grounds, decreased population size, genetic drift and population bottleneck, which also indicate the endangered condition of this species in Bangladesh.

In the past, morphometric and meristic characteristics were the only tools used for inferring fish phylogenetic relationships and understanding speciation **(Musikasinthorn, 2000)**. However, it is difficult to differentiate between fishes, especially Channidae species because of the similarity in their external morphology **(Khan *et al*., 2013; Miyan *et al*., 2014)**. Therefore, the construction of phylogenetic trees based on only morphology is controversial due to the complex evolutionary changes in their morphological and physiological characters. A large variety of snakeheads is available in Bangladesh, and their identification based on morphological characteristics alone is highly ambiguous. There are many reported cases of mislabeling Channidae species all over the world **(Galal-Khallaf *et al*., 2014; Nagalakshmi *et al*., 2016)**. For the evolutionary and ecological processes, the phylogeny of species is playing an important role due to its fitness in ecological function **(Baisvar *et al*., 2019)**. The present study revealed the phylogenetic relationship of Bangladeshi haplotypes with neighboring country India's *C. marulius* species. Both Cytb and CO1 mtDNA genes showed a close relationship with neighboring country's *C. marulius* species. The present analysis showed almost similar results for both Cytb and CO1 genes and can be used for this type of molecular research. In the case of using a single mtDNA gene for molecular identification of this species, it would be better to use the CO1 gene since it exploited more uniform genetic and nucleotide diversity among the populations; in addition, this gene produced more haplotypes than the Cytb gene in the present study. In spite of having a small sample size, these results might be useful as a guideline for conservation studies in this region and for generating basic information about this species. The Cytb and CO1 fragment, taken together, will be a promising marker to identify the distribution and pattern of genetic diversity throughout the large native distribution of great snakehead fishes (*C. marulius*).

CONCLUSION

The present experiment was a study of mtDNA Cytb and CO1 gene, and it aimed to reveal genetic variability of *C. marulius* inhabiting different sites in Bangladesh with variable environmental conditions to give an insight into conservation pattern. Results showed moderate variations in the mitochondrial genome of *C. marulius* and the utility of molecular markers showing intraspecific variations as well as inter-population variations. Though a limited number of samples was used in the present study, the molecular analysis revealed the pairwise genetic distance between the populations from different locations with interesting findings. The phylogenetic tree constructed by combining data from the present study and online GenBank data showed similarity with the neighboring populations from India and Pakistan. It can be assumed that genetic variations were probably because of geographical distribution patterns, adaptation in different regions, genetic makeup and limited gene flow. It is confirmed that, at the molecular level, this genetic comparison between the populations of great snakehead fish from several parts of Bangladesh may provide further clues to comprehend the conservation pattern of the species in the near future.

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