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MOLECULAR IDENTIFICATION OF RIBBON FISH (*EUPLEUROGRAMMUS SP.*) USING PARTIAL SEQUENCE OF MITOCHONDRIAL COI GENE

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Abstract: Dry fish is considered as a delicacy in the menu of many people of Bangladesh. The most economically important marine and freshwater dried fishes are ribbon fish, bombay duck, olive barb, pomfret, shrimp, etc. In this study, fresh ribbon fish sample was collected from Cox's Bazar and morphologically identified as ribbon fish. The collected fish was confirmed through a molecular technique using mitochondrial COI gene (cytochrome c oxidase subunit I) to avoid morphological ambiguity, which is first reported data from Bangladesh. Partial sequence of this COI gene was amplified using a genus specific universal primer set. The target nucleotide sequence of COI gene was determined successfully with 616 bp length and the ribbon fish was identified as *Eupleurogrammus* sp. Within the amplified region GC and AT content were 45.5% and 54.5%, respectively. 16.88% interspecific polymorphism was observed when compared with other species under genus *Eupleurogrammus* collected from Gen Bank database. This sequence will be used as molecular bar code for authentic identification of *Eupleurogrammus* sp. at genus level.

Key words: Molecular Identification, Mitochondrial COI gene, Ribbon fish.

INTRODUCTION

Bangladesh is a land full of fisheries resources including vast marine life. The primary protein source in Bangladeshi diet is fish. It contributes about 60% of total animal protein. BBS (2017) reported that in Bangladesh per head fish consumption reaches up to 62.58 g which is higher than their daily protein demand (60 g). Fisheries sector plays a very important role in the national economy and considered as the second most important sectors to contribute in the field of export earnings of Bangladesh (Samsuzzaman *et al.* 2014). This sector contributes around 25.72% to the agricultural Gross Domestic Product (GDP) and 3.50% to the GDP of the country (FRSS 2019). The ribbon fish is under the family Trichiuridae, belong to the order Perciformes, and has a very

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common appearance in the dry fish industry of Bangladesh (Firawati *et al.* 2016, Paul *et al.* 2018). It is a common to abundant species and can be found in oceans throughout the world of tropical and temperate regions. They are mainly benthopelagic, but may appear at the surface during the night (Kim *et al.* 1998). They are commercially very important in the north east part of Bay of Bengal. Bangladesh has reported genus *Trichiurus, Lepturacanthus* and *Eupleurogrammus* from the family Trichiuridae (Rahman *et al.* 2009), while only species of the genus *Eupleurogrammus* has been reported from Bangladesh. It also has very few molecular data on NCBI. Morphology can be affected by environmental factors and some other reasons in the developmental stages and these factors can cause difficulties in the identification. In that context, it is neceassary to proper idenditification of this economically important fish for Bangladesh. Due to sometimes morphological identification contradictory with releated fish, therefore, molecular analysis is very important to identify the genus accurately. Mitochondrial DNA is usually used for molecular identification of organisms. The COI (cytochrome c oxidase subunit I) region of mtDNA is one of the very suitable regions for the identification of fish, ttherefore, in this study, a fragment of COI gene was amplified and sequenced confirm this valuable fish as *Eupleurogrammus* at genus level. This information can be used for further research to conserve this genus and enrich the marine biodiversity of Bangladesh.

MATERIAL AND METHODS

Sample collection, morphological analysis of the voucher specimen: A total of 4 samples of ribbon fish (*Eupleurogrammus* sp.) were collected from Cox's Bazar (Fishery Ghats, Fish Market). From the collected fishes only one sample was considered for further study and was washed for taking nice photograph. The collected specimen was brought to the Laboratory of Genetics and Molecular Biology, Department of Zoology, University of Dhaka for further experiment. The sample was then morphologically identified up to genus level by conventional method (Rahman *et al.,* 2009). Different body parts of the collected fish were measured (Table 1) and preserved in 70% ethanol. The preserved samples were stored in refrigerator at 4°C.

DNA extraction, PCR amplification and sequencing: Genomic DNA was extracted from 25 g muscle tissue of the observed fish species using 'Monarch Genomic DNA Extraction Kit' by Biolab and the mentioned extraction protocol was used for DNA extraction. Standard PCR was performed using a set of genus specific universal primer for CO1 gene amplification and were analyzed by electrophoresis in a 0.8 % agarose gel. The forward and reverse primer sequences were considered as 5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′ and 5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′ respectively (Ahmed *et al.,* 2021). PCR

amplification was sequenced using Sanger dideoxy sequencing method.

Bioinformatics analysis: COI gene sequence of experimental fish and other existing sequence of closely related fish genus were collected from GenBank database (NCBI). Local alignment was done with the help of serial cloner software. MEGA 7 software was used for multiple sequence alignment which was followed by polymorphic sites analysis and for construction of phylogenetic tree. Sequence alignment and pairwise distance calculation was used to conduct two or more biological sequences of similar length.

RESULTS AND DISCUSSION

Morphological identification of the voucher specimen: Body of the voucher specimen was elongated, eel-like with large mouth and dermal process on tip of both jaws and the colour was steely blue with metallic reflections. Lower jaw was measured longer than upper one containing fang like teeth. Minute spinules were found buried in skin in anal fin area. The fins have an anterior spine and posterior soft rays. Meristics and Morphometric characteristics of the experimental fish were studied (Fig. 1) and different body parts and fin rays have been shown in tables 1 and 2. All these morphological data indicate the fish to be under genus *Eupleurogrammus* of family Trichiuridae under the order Perciformes (Rahman *et al.,* 2009).

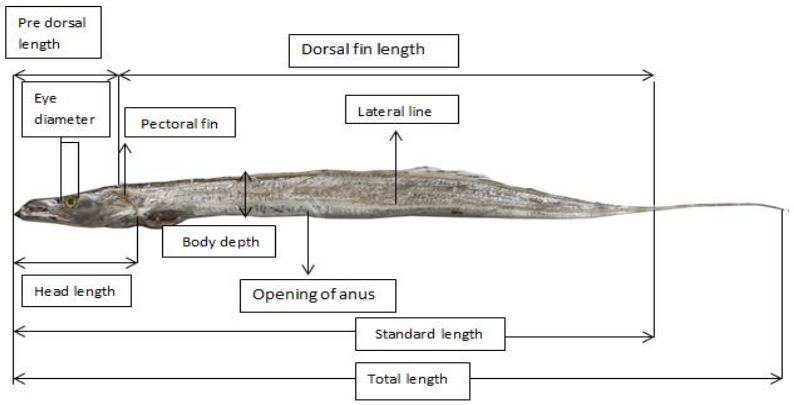

Fig. 1: Measurement of different parts of ribbon fish.

Molecular confirmation of experimental fish using mitochondrial CO1 gene: According to the above morphological characteristics, the experimental sample was identified as genus *Eupleurogrammus*.

Table 1. Meristic characteristics of the sample specimen using Principal Component Analysis (PCA)

Morphometric Characters:	Measurements
Total Length	67cm
Standard Length	55.5 Cm
Body Depth	5cm
Tail Length	13cm
Trunk Length	49cm
Pre-Orbital Length	3.9cm
Post-Orbital Length	4cm
Eye-Diameter	1.5cm
Dorsal Fin Base Length	49cm
Pectoral Fin Base	2.1cm

Table 2. Fin formula of sample specimen

Fin Formula	Numbers
Dorsal Fin Number	1
Dorsal Fin Spine	3
Dorsal Fin Rays	135-140
Pectoral Fin Number	1
Pectoral Fin Ray	17

Checking Extracted DNA: Based on agaroge gel electrophoresis, obtained band was very clear and prominent which indicates the quality of extracted DNA was good enough for amplification through PCR method for sequencing (Fig. 2a).

PCR amplification of targeted region: Partial region of COI was amplified through PCR technique. For amplifying mitochondrial COI gene of both vertebrate and invertebrate, genus specific universal primers were used successfully (Ahmed *et al.,* 2021). Agarose Gel Electrophoresis bands for the PCR products have been shown in fig. 2b and the amplified region was around 650bp long.

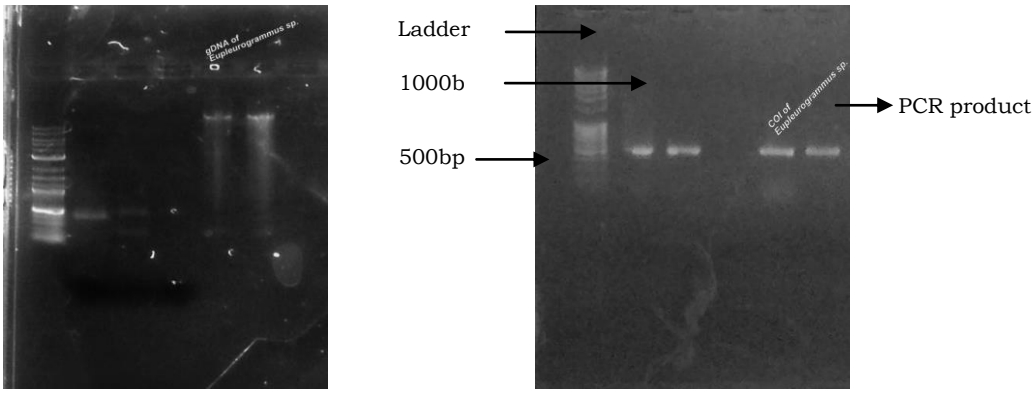

Fig. 2. Banding pattern of (a) extracted gDNA and (b) PCR product of COI gene of *Eupleurogrammus* sp. Lane 1 denotes DNA ladder of 1 kb.

Nucleotide sequences of COI gene: Nucleotide sequence was obtained by Sanger dideoxy sequencing method from Macrogen Inc., Seoul, Korea and 616 bp of nucleotide sequence was determined. The obtained sequence of COI gene was reported for the first time from Bangladesh and submitted to NCBI GenBank database with the accession number OP292642.1 and are shown in figure 3.

TTGGTGCATGAGCTGGGATAGTGGGCACAGCATTAAGCCTGCTTATCCGAGCAGAACTGAGCCAGCCA
GGCTCCCTCCTAGGTGACGATCAGATTTATAATGTGATTGTCACAGCACATGCTTTCGTTATAATTTTCTT
TATGGTCATACCAATCATAATTGGAGGTTTTGGAAACTGGCTCGTCCCACTTATAATTGGAGCCCCTGAC
ATAGCCTTCCCACGAATAAACAACATAAGCTTCTGGCTACTACCACCCTCTTTTGTCCTCCTTTTAGCCTC
TTCTGGTGTTGAAGCAGGAGCCGGCACTGGTTGAACAGTTTACCCGCCTCTAGCTAGCAATCTTGCCCA
CGCAGGCGCATCCGTAGATCTAACCATTTTCTCCCTCCATCTAGCAGGAATCTCTTCAATCCTGGGCGCT
ATTAACTTTATTACAACAATTATAAATATAAAACCTACAGCTATTACACAATTTCAAACCCCCCTCTTTGTAT
GATCTGTGCTAATTACAGCGGTTCTCCTGCTCCTATCCCTGCCCGTTCTTGCAGCCGGGATTACCATACT
TTTAACAGATCGAAACCTCAACACAACATTTTTTGACCCTTCAGGAGGAGGAGA

Fig.3. Partial nucleotide sequence of COI gene of *Euplurogrammus* sp. containing 616 bp.

Table 3. Nucleotide base pair study of the sample specimen and other related fishes

Name (Accession number)	Total number of				Percentage of GC (GC%)
	Adenin (A)	Cytosine (C)	Guanine (G)	Thiamine (T)	
Trichiurus lepturus nanhaiensis (KJ202212.1)	173	216	126	193	48.3%
Lepturacanthus savala (KY371669.1)	156	184	116	178	47.3%
Eupleurogrammus sp. (LC269213.1)	175	184	117	200	44.5%
Sample specimen (OP292642.1)	155	167	113	181`	45.5%
Eupleurogrammus sp. (MH235643.1)	166	178	113	194	44.7%
Eupleurogrammus sp. (LC269220.1)	170	182	118	202	44.6%

Analysis of nucleotide base pair from the obtained sequence: The sequence contained nucleotide bases Adenine, Thymine, Guanine, and Cytosine around 25.16%, 27.11%, 18.34% and 29.38% respectively. The A-T content was 9% higher than the G-C content. The AT/GC ratio was found to be 1.19. This finding is similar with that of other related fishes shown in table 3 (Ahmed *et al.,* 2021). Ahmed *et al.,* (2021), reported that the mitochondrial cytochrome c oxidase subunit I (COI) gene could be used as a marker for molecular characterization of marine and coastal fishes where the mean length of the sequences was above 600 base pairs which is almost similar with the present study. In Elasmobranchii (Sharks and rays), the nucleotide sequence analysis of COI gene indicated the average nucleotide frequencies among the four are to be A: 25.90%, T: 32.70%, G: 16.20% and C: 25.20% and in Osteichthyes (bony

fish), average nucleotide frequencies are to be A: 24.20%, T: 28.60%, G: 19.40% and C: 27.80%, which are almost similar to the present study (Ahmed *et al.,* 2021).

Inter-specific sequence variation: When the sequence was searched through NCBI blast, it was found to be best matched with four sequences of COI gene of the genus *Eupleurogrammus*. Among them accession number-LC269220.1 showed 99.68% similarity with the sequence of the voucher specimen of present study. After that accession number- MH235643.1, LC269213.1, LC269209.1 were matched with 99.51% similarity. The obtained sequence of the voucher specimen was locally aligned in serial cloner with the matched sequence of MH235643.1. The polymorphic sites were shown in table 4.

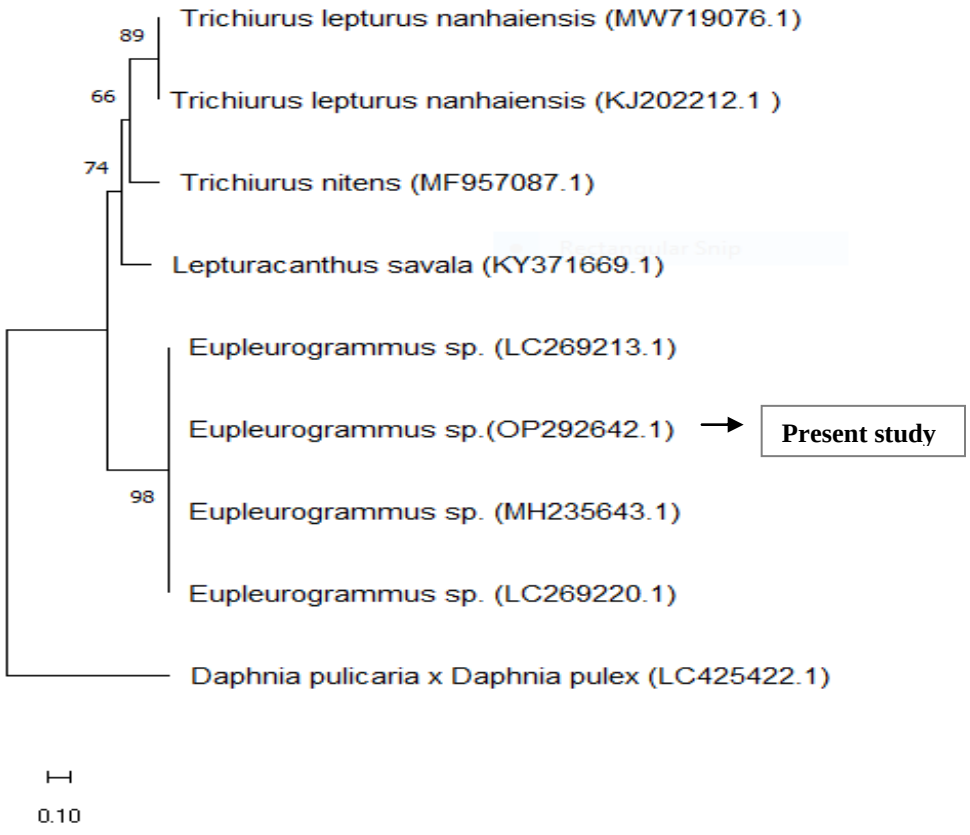

Fig. 4. Molecular phylogenetic tree using neighbor joining method based on partial nucleotide sequenced of COI gene of different ribbonfishes. Numbers of the tree representing the bootstrap value.

Phylogenetic relationship: A molecular phylogenetic tree was constructed based on partial nucleotide sequence of COI gene of ribbonfishes those were available in the NCBI database (Fig. 4). As Ribbon fish is a member of family Trichiuridae, a comparative study was performed among different fishes from this family using the neighbor joining method, with bootstrap value 100 where *Daphnia pulicaria x Daphnia pulex* was used as an out group. In the phylogenic tree all the four species of *Eupleurogrammus* form a monophyletic group with a bootstrap value of 98 which indicates strong relation among them and also confirms the sample specimen as genus *Eupleurogrammus*.

Table 4. Polymorphic Sites Analysis

Polymorphic Sites	Nucleotides showing polymorphic sites in *Eupleurogrammus* sp. (Present study)	Nucleotides showing polymorphic sites in *Eupleurogrammus* sp. Accession no: MH235643.1
17	G	A
455	T	C
512	G	A

CONCLUSION

Molecular techniques are very convenient for the taxonomic grouping of closely related animals including fish which are generally difficult to identify morphologically. It also creates opportunities to observe the continuous divergence of these animal groups over time. In this study, commercially important ribbon fish was identified genetically to the genus level where the COI gene sequence was considered and constructed phylogenic tree. This study would be the base information for further research to identify the genus *Eupleurogrammus* at species level. Further study should be considered based on COI, 16S rRNA, 12S rRNA, Cyt b genes from mitochondrial genome.

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