# DNA BARCODING OF SILURIFORM FISHES OF THE MEGHNA RIVER ESTRUARY

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# *CERTIFICATE*

*This is to certify that thesis entitled,* **"DNA BARCODING OF SILURIFORM FISHES OF THE MEGHNA RIVER ESTRUARY."** *submitted to the* **FACULTY OF AGRICULTURE**, *Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* **MASTER OF SCIENCE (MS) IN BIOTECHNOLOGY**, *embodies the result of a piece of bona fide research work carried out by* **NIAZ MORSHED MOLLIK**, *Registration No.* **19-10306** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.*

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**Place: Dhaka, Bangladesh**

Dedicated to

My Beloved Parents

& Sibling

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SAU, Dhaka **The Author**

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# DNA BARCODING OF SILURIFORM FISHES OF THE MEGHNA RIVER ESTRUARY

## ABSTRACT

The Meghna river estuary is the largest estuarine ecosystem in Bangladesh and the fish assemblage in this estuary is highly diverse. Estuaries are also very important as breeding and nursery grounds for a wide variety of fishes. Fish samples were collected from the different zone of the Meghna estuary around the year July 2020 to May 2021. In the present study, a total of 9 species were identified from the 7 seven genera and two families (Bagridae and Schilbeidae) under the order Siluriformes based on DNA barcoding approach along with morphology. The species were *M. bleekeri, M. gulio, M. vittatus, R. rita, S. aor* from the Bagridae family *and A. coila, C. garua, E. vacha, S. silondia* from the *Schilbeidae* family. The barcode sequences obtained from the present study showed high identity (> 99%) with the reference sequences deposited in the GenBank database. The assessment of species identities with previously known sequences and closely related species in NCBI BLAST generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. Sequences of the present study were clustered in a single clade with the sequences of conspecific species from GenBank which validate the genetic identification of the studied species. Genetic distances among the sequences of the present study and its compared sequences from GenBank ranged from 0.00 (0.2%) to 0.003 (0.3%) which also validates the genetic identification of this species. Fish diversity in the Meghna has long been declining due to the number of anthropogenic and natural factors. This study provides information on the species diversity of Siluriformes that will help in the management and conservation of the fish fauna of the Meghna river system.

# CHAPTER I

# INTRODUCTION

## 1.1. Background of the Study

Bangladesh has vast and rich fisheries resources. Bangladesh has three largest and powerful rivers— The Padma, The Meghna, and The Jamuna—feed one of the largest and busiest deltas in the world, with the largest flooded wetland on Earth and Asia's third-highest concentration of aquatic species after China and India, it is regarded as one of the most fisheries-friendly locations in the world. Within its borders and in the maritime territorial and economic zones, Bangladesh has enormous fishing potential, providing excellent opportunities for pisciculture. The country's fish diversity is greatly influenced by the floodplain, river systems, adequate rainfall, and temperature. Bangladesh, fortunate in having potential water resources, is one of the world's leading fish-producing countries with a total production of 45.03 lakh MT in FY 2019-20 (DoF, 2020). Fisheries sector contributes 3.52 percent to the national GDP and more than one-fourth (26.37%) to the total agricultural GDP (DoF, 2020).

Approximate over 700 species of marine fish species occur in Bangladesh (Habib and Islam 2020) a total of 260 freshwaters fish species in the Bangladesh (IUCN, 2000; Rahman, 2005). The diversified fisheries resources of the country are divided into two groups as Inland and Marine fisheries. The inland fishery is further divided into two subsectors: the inland capture fishery and inland culture fishery. The inland capture fisheries include exploit open water area of rivers and their tributaries, estuaries, the Sundarbans mangrove forest area, permanent wetlands beels and seasonal floodplains. The inland capture fisheries include production from closed water bodies such as ponds and ditches, ox-bow lakes, baors and coastal and inland shrimp and fish farm. The marine fisheries comprise industrial and trawl fisheries and small scales artisanal fisheries by coastal fisher communities (DoF, 2020).

According to the Sea Around Us Project, over 1,200 estuaries (including some lagoon systems and fjords) presents in over 120 countries and territories (Alder, 2003). These water bodies (of which over 95% have shape files) were selected such that the estuaries of all the world major rivers were included, as well as the small estuaries of countries without major rivers. In Bangladesh, there are about 20 estuaries throughout the coastal zone of Bangladesh as well as some complex estuarine ecosystems in natural and planted mangrove forest dominated areas, but relatively little is known (about the fisheries diversity and factors controlling their distribution and abundance (Shafi and Quddus, 1982; Islam, 2005; Ahammad, 2004).

The Meghna river is one of the most productive rivers of Bangladesh. The Meghna is a transboundary river shared by India and Bangladesh. The total area drained by the Meghna River Basin is 82,000 $km^2$, of which 47,000 square km (57% of the total area) is located in India and 35,000 square km (43% of the total area) is in Bangladesh. Specially, the Meghna River estuary is the largest estuarine ecosystem of Bangladesh, which supports the large diversity of flora and fauna. The Meghna Estuary is the easternmost sector of the Ganges delta. The estuary is formed inside Bangladesh by the joining of the Surma and Kushiyara rivers originating from the hilly region of eastern India.

Estuaries play a vital role in the life history development of many marine and brackish water coastal species, and some live out their entire life cycle within the estuarine environment. The majesty of estuaries is well understood in many parts of the world as breeding and nursery grounds for a wide variety of fishes. Estuaries are the meeting place of freshwater from rivers and saltwater from sea and, as such as, are dynamic environments characterized by large fluctuations in environmental conditions (James *et al*., 2007). Fisheries population in the estuary is very much dynamic in both the temporal and spatial spectrum. Intra-annual atmospheric changes, and short-term differences can also affect the interactions between the distribution and abundance of these communities (Noakes, 1992; Helfman, 1993; Axenrot et al., 2004). Estuaries are area of physical and biological ecotone between land, freshwaters, and the sea (Chowdhury et al., 2009).

The order Siluriformes (catfish) is one of the largest orders of teleosts containing more than 7293 valid species belonging to the 39 families, representing more than 12% of all teleosts

and over 6.3% of all vertebrates (Frick et al, 2022). Bangladesh has 76 species of catfish from 14 families (Habib and Islam 2020). Most catfish have a cylindrical body with a flattened ventral to allow for benthic feeding (Bruton, 1996). Catfish are named because of their whisker-like barbels located on the nose, each side of the mouth, and on the chin. Most catfish possess leading spines in their dorsal and pectoral fins. Catfish are scaleless, a characteristic of catfishes distinguishing them from most other teleost fish. However, some catfish, such as plecos, possess bony dermal plates covering their skin (Arce et al., 2013; Armbruster, 2004; Ferraris and Vari, 2012).

Catfish are highly diverse and distributed worldwide. They are commonly found in inland or coastal waters of all continents, including Antartica where fossils are found (Grande and Eastman, 1986). Catfish are most abundantly distributed in the tropics of South America, Africa and Asia (Lundberg and Friel, 2003). Due to their worldwide distribution and diversity, catfish are interesting models to ecologists and evolutionary biologists, and are important for biogeographical studies (Sullivan et al., 2006).

Catfish are quite hardy such that they are more adaptable for artificial spawning, handling, and culture. They possess all the characteristics necessary for aquaculture including relatively high fecundity, ability for artificial spawning, adaptability to earthen ponds for culture, high tolerance to low dissolved oxygen, relatively high resistance against infectious diseases, and relatively high feed conversion efficiency. It is such characteristics that make catfish one of the most popular groups of fish for aquaculture. In the world, a few major species are widely used for aquaculture, including channel catfish, blue catfish, walking catfish, shark catfish, Thai catfish, and African catfish. Catfish are of considerable economic importance for aquaculture and recreational fisheries. Its global importance is increasing as several countries in Asia, such as China, Vietnam, and Bangladesh are now heavily involved in catfish aquaculture (Liu, 2008).

The morphology of fishes historically has been the primary source of information for taxonomic and evolutionary studies. Morphology is a branch of life science dealing with the study of gross structure of an organism or taxon and its component parts. This includes aspects of the outward appearance (shape, structure, color, pattern, size), i.e. external

morphology as well as the form and structure of the internal parts like bones and organs, i.e. internal morphology (or anatomy). Morphological studies have been especially successful in defining species and in organizing these species into genera. Morphological keys are often effective only for a particular life stage or gender.

DNA barcoding is a tool that utilizes a short length of DNA of particular organism to identify its species (Herbert *et al.*, 2003). The main purpose of DNA barcoding is to identify an unknown organism and refer it to its corresponding species (Kress *et al.*, 2005). "DNA barcode" is a unique pattern of DNA sequence, which used to identify the living thing, small, damaged or industrially processed materials. DNA barcoding is a powerful marker to detect genetic uniqueness of individuals, population or species, can correct field misidentification of species more exact, and expands the technical expertise of taxonomist. DNA barcode differs from molecular phylogeny in which the main purpose is not only to determine the patterns of relationship but also to identify an unknown sample in terms of a pre-existing classification. DNA barcoding is an increasingly fashionable and novel concept that has generated in enhancing biodiversity field. However, this technique should be used in conjunction with others methods for effective conservation efforts. The application of DNA barcoding in fish identification has become popular in recent years.
Hebert *et al*. (2003) have demonstrated that the COI region is appropriate for discriminating between closely related species across diverse animal phyla and this technique has used for marine and freshwater fishes (Ward *et al.,* 2005). DNA barcoding using CO1 (Cytochrome c oxidase subunit I) is more authentic and helpful to create genetic color variations among species. Mitochondrial COI gene sequence is suitable because its sequence is conserved among conspecifics and the mutation rate of it's often fast enough to distinguish closely related species. DNA barcoding is a molecular tool uses standard genetic primers, traditionally the 600 to 800 segments of the mitochondrial gene cytochrome c oxidase I, to classify species.

DNA barcoding (using the mitochondrial COI gene) is a powerful molecular and taxonomic method in the identification of fish species. DNA barcoding of fishes of many countries has been done including Canada, Taiwan, and Indonesia (Hubert *et al*., 2015; Knebelsberger *et al.*, 2015). A particular region of the mitochondrial COI gene is PCR-

amplified and then sequenced and analyzed; resulting 4 different colors of bars that used to indicate A, T, G and C. Then following respective bars, each representing a DNA base, appear like a grocery barcode, hence the name of DNA barcoding.

There are several benefits of fish barcoding:

- Fish species can be identified easily by taxonomists
- Interspecies can be classified
- Previously unidentified fish species can be identified
- Proper identification of species that cannot be classified by traditional taxonomy.
- It provides permanent tags unchanged during taxonomic revision.

Although some studies were conducted to identify the fish species of Meghna river estuary (Hossain et al. 2012, Pramanik et al. 2017) those are based on the morphology. This is the first study where DNA Barcoding methods were applied to identify the species identification of the Meghna Estuary.

## 1.2. Objectives

- To identify the fish species of Siluriformes of the Meghna river estuary through DNA barcoding
- To study the phylogenetic relationship of identified species with the reference data.
- To study the genetic diversity of Siluriformes fish species of the Meghna river estuary.

# CHAPTER II
# REVIEW OF LITERATURE

**Pramanik et al. (2017)** conducted a study between January 2016 and December 2016 with a view to assessing the biodiversity of fishes in the Meghna River and their conservation status both in Bangladesh and global aspects. A total of 107 fish species belonging to 13 orders and 36 families were documented. Perciformes was found to be the most dominant order consisting 32% of the total fish population. Cyprinidae was found to be the richest family (16%). Twenty common groups were recorded in the studied areas. Estuary River was found to be the biggest habitat for the maximum number of fishes (43%). Twenty-one threatened fish species (20%) were recorded from the Meghna river in which 11 species (10.28%) were found as Vulnerable (VU), 8 species (7.48%) as Endangered (EN) and 2 species (2%) as Critically Endangered (CR).

**Hossain et al. (2012)** conducted a study to assess the fish diversity status with relation to major hydrological and meteorological parameters in both spatio-temporal scales in the Meghna Estuary. Findings showed that the Meghna river estuary is the habitat of 53 fish species and *Oxyurichthys microlepis, Hemiarius sona Arius thalassinus, Batrachocephalus mino* and *Arius caelatus* are the major contributory species (>6%) for both spatio-temporal scales. Water temperature and rainfall were found as major influential factors for species distribution.

**Mia *et al*. (2015)** mention that, a total of 20 species of fishes were identified in the catches of different nets in the Meghna river. The highest numbers (20) of species were recorded in the catches of ber jal while the lowest numbers (3) were recorded in case of moiya jal. Different species of fish fauna were caught by the fishers in the Meghna river including carps, barbs, minnows, catfish, gobies, perch, murrels, eels, small prawn and miscellaneous species. Most of them are found all the year round except carps, perch and murrells. Maximum catches are obtained during the month of July to December. The highest catch 500g and 86.11% was recorded whereas the lowest was 8g and 1.39% during the study period. The highest percentage of respondent (45%) caught fish of 3.1-4.0 kg/person with

maximum duration of 6-7h of fishing. Decline in fish catch (100%) was the greatest problem to the fishers followed by lack of capital for purchase of fishing gear and net. The status of fisheries at the Meghna river is closely related to the livelihood of fishermen. Steps to be taken at government and nongovernment level to support their livelihood.

**Bhuyan et al. (2016)** mention that a total of 69 fish species were identified during the study under 23 orders and 28 families. Among 69 fish species; 26 were found belong to Cyprinidae family followed by Bagaridae (5), Schilbeidae (4), Channidae (4), Ambassidae (2), Belontiidae (3), Siluridae (2), Notopteridae (2), Mastacembelidae (2) and others (19). During the study period, 7 species were found critically endangered, 15 species were endangered and 12 species were vulnerable while 26 species were not found in threatened position.

**Begum et al.** (2019) conducted to be known the water qualities and richness of fisheries resources in the Meghna river at Narsingdi region. The result of the study showed that the temperature, EC, TDS, DO, BOD, pH and hardness of the Meghna river water were ranged from 19.1 to 19.6ºC, 525 to 714 µS/cm, 113 to 197.67 mg/l, 1.23 to 2.9 mg/l, 7.72 to 7.91 and 94 to 126 mg/l, respectively during the study period. The obtained results assured the desired quality of water in terms of above parameters except DO. The study identified a total of nine fish species under five orders and six families. Here Foli (*Notopterus notopterus*) considered as vulnerable accordingly IUCN (2000). It was also found that water quality degradation (33.75%) negatively influenced the abundance of fish species. Proper management and monitoring along with implementation of existing laws and regulations for industrial discharge of waste should be carried out to maintain the water quality of the Meghna river so that the river can remain as a healthy ecosystem and habitat for freshwater fishes.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1. Collection of Samples

Fish samples were collected by hands, lining, traps, and from the landing center around the Meghna Estuary (Fig. 1). After collection, samples were transferred to the Laboratories for morphological identification. After morphological study, samples were preserved in 95% ethanol for subsequent molecular work in Aquatic Bioresource laboratory (ABR Lab) at Sher-e-Bangla Agricultural University (SAU), Dhaka.

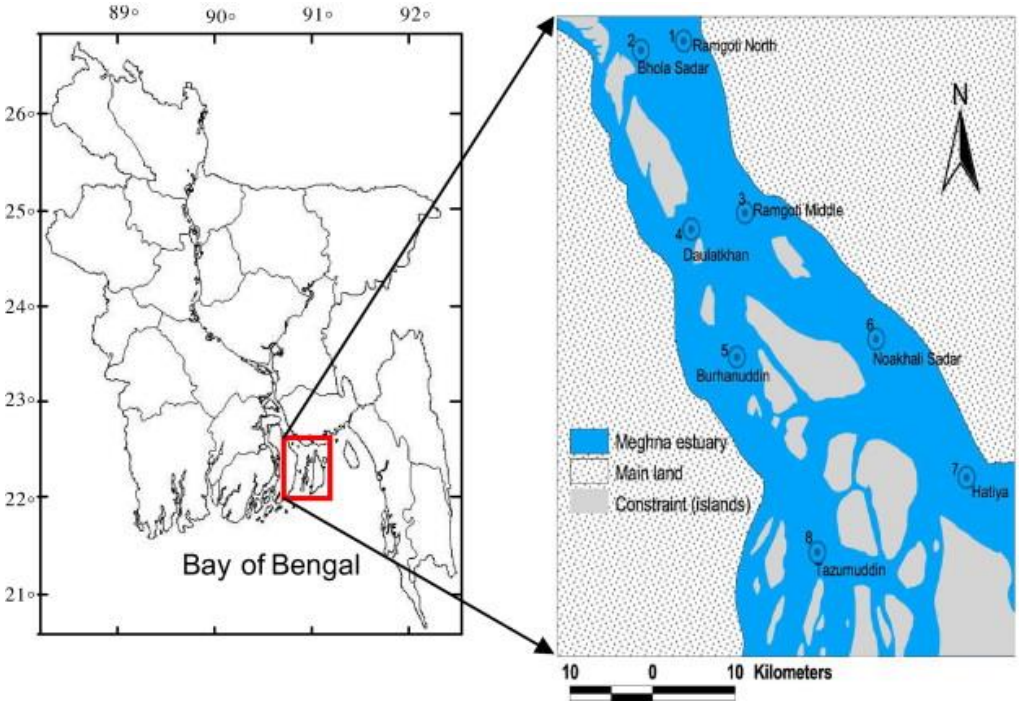



**Figure 1:** Sampling location in the Meghna Estuary, Bangladesh

## 3.2. Study Period

This study was conducted from July 2020 to May 2021.

## 3.3. Study Framework

The whole study was divided into several parts i.e. literature review, sample collection, morphological study, molecular study, sequence analysis and thesis writing. The detail study framework has been given in the Table 1.

**Table 1:** Framework of the study

| **Works** | **2020-2021** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | March | April | May | June |
| **Literature review** | | | | | | | | | | | | |
| **Sample collection** | | | | | | | | | | | | |
| **Molecular study** | | | | | | | | | | | | |
| **Sequence analysis** | | | | | | | | | | | | |
| **Thesis writing** | | | | | | | | | | | | |

## 3.4. Sampling Frequency

Sampling was done monthly basis. A total of 5 sampling has been done from July 2020 to May 2021.

**Table 2:** Sampling frequency.

| **Sampling Number** | **Date** |
|---|---|
| First | 25 July, 2020 |
| Second | 15 October, 2020 |
| Third | 16 December, 2020 |
| Fourth | 26 January, 2021 |
| Fifth | 17 March, 2021 |

## 3.5. Materials

Following instruments, kits and chemicals were used in this present study-

## 3.5.1. Experimental Instruments

Instruments used in the present study are-

**Small Instruments:**

- Scale
- Chopping board
- Aluminum foil
- Fish pots
- Tag paper
- Pipettes
- Trays
- Dissecting box
- 250ml conical flask
- Comb
- Gel tank
- Gel chamber
- Gel documentation chamber (INGENIUS$^3$)
- Purification column
- Commercial Sequencer
- Sprayer
- Ice
- Qubit 3.0 fluorometer
- Spin Column CB3

**Heavy Equipment:**

- Electric balance
- Refrigerator's
- Spin down machine
- Vortex machine
- Centrifuge machine

- Computer
- Micro-oven
- Polymerase chain reaction (PCR) machine or Thermal cycler
- Incubator or heat block

**Plastic ware/Glassware:**

- 1.5 ml micro-centrifuge tube
- 2 ml micro-centrifuge tube
- Assay tubes
- 0.2 ml Polymerase chain reaction (PCR) tubes

## 3.5.2. Chemical materials

TIANamp Marine Animals DNA Kit (Company-TIANGEN®) containing

- Buffer GA
- Proteinase k
- Buffer GB
- Ethanol (96 − 100%)
- Buffer GD
- Buffer PW
- Buffer TE

- Forward and Reverse primers
- Qubit® buffer
- Qubit® reagent
- Master mix containing –
- *Taq* DNA polymerase
- Nuclease free water
- Distilled water
- dNTP mix
- EZ - Vision ® In-Gel Solution
- 1% Agarose gel
- 0.5X TAE buffer
- DNA Ladder

- 0.5X Tris – Borate – EDTA buffer (TBE buffer)
- QIAquick PCR purification kit containing -
    - Buffer PB
    - Buffer PE
    - Elusion buffer

## 3.6. Molecular Study

DNA barcoding was used to identify and resolve the taxonomic ambiguities of the fish species. After morphological studies, Cytochromc oxidase/I (COI) of mitochondrial DNA (mtDNA) was used as for DNA barcoding of the fish species. All the molecular study has been carried out in Aquatic Biodiversity Research Lab, Dept. of Fisheries Biology and Genetics, Sher- e- Bangla Agricultural University, Dhaka-1207, Bangladesh.

## 3.7. Collection of Tissue Sample

For genomic DNA extraction about 0.03g fresh tissue samples were collected from muscle just below the dorsal fin or from the caudal fin or fin tissue (especially caudal fin) of each specimen using a sterile scalpel, scissor, and forceps.

## 3.8. DNA Barcoding Protocol

DNA Barcoding includes the following successive stages.

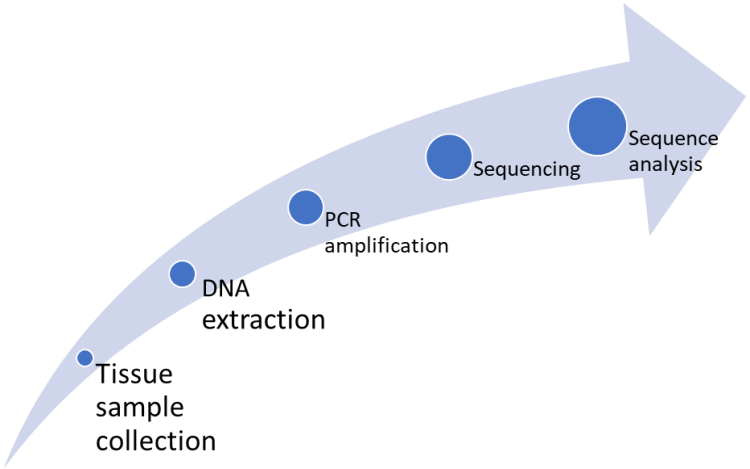



**Figure 2:** Outline of the present study.

## 3.9. Genomic DNA Extraction

Genomic DNA was extracted from collected tissue sample by using TIANamp Marine Animals DNA Kit from TIANGEN® (Fig. 3). The gDNA extraction protocol of TIANamp Marine Animals DNA Kit has been shown below:

1. Preparation of samples: Up to 0.03g muscle tissue was cut into small pieces and then the pieces of tissue were placed in a 1.5 ml micro-centrifuge tube. 200 $\mu l$ Buffer GA was added into the sample containing tube and the tube was vortex for 15 s.
2. Then 20 $\mu l$ Proteinase k (20 mg/ml) was added into the tube and the mixture was mixed thoroughly by vortex. Brief centrifugation (spin down) of the tube was done to remove drops from the inside of the lid. Then the tube was incubated at 56˚C in a heat block until the tissue was completely lysed. Brief centrifugation (spin down) of the tube was done to remove drops from the inside of the lid.
3. Subsequently then 200 $\mu l$ Buffer GB was added into the tube and the mixture was mixed thoroughly by vortex. The tube was incubated at 70˚C for 10 min to yield a homogeneous solution. Brief centrifugation (spin down) of the tube was done to remove drops from the inside of the lid.
4. Then 200 $\mu l$ ethanol ($96 - 100\%$) was added into the tube and the mixture was mixed thoroughly by vortex for 15 s. A white precipitate might form by addition of ethanol. Brief centrifugation (spin down) of the tube was done to remove drops from the inside of the lid.
5. Then the mixture was pipetted from step 4 into the Spin Column CB3 (in a 2 ml collection tube) and the Spin column was centrifuged at 12,000 rpm (~ 13,400 × $g$) for 30s. Flow–through was discarded and the spin column was placed into the collection tube.
6. After ward 500 $\mu l$ Buffer GD was added into the Spin Column CB3, and the Spin column was centrifuged at 12,000 rpm (~ 13,400 × $g$) for 30 s. The flow-through was discarded and the spin column was placed into the collection tube.
7. Then, 600 $\mu l$ Buffer PW had been added) was added into the Spin Column CB3, and the Spin column was centrifuged at 12,000 rpm (~ 13,400 × $g$) for 30 s. The

flow-through was discarded and the spin column was placed into the collection tube.

8. Then, repetition of step 7 was done.
9. Then, the collection tube containing the spin column was centrifuged at 12,000 rpm (~ 13,400 × $g$) for 2 min to dry the membrane completely.
10. Finally, the Spin Column CB3 was placed in a new clean 1.5 ml micro-centrifuge tube, and 50 - 200 $\mu l$ Buffer TE was pipetted directly to the center of the membrane. The tube was incubated at room temperature (15 – 25˚C) for 2-5 min, and then centrifuged for 2 min at 12,000 rpm (~ 13,400 × $g$). Then the Spin column CB3 was removed from 1.5 ml micro-centrifuge tube. Finally, the extracted DNA contained in 1.5 ml micro-centrifuge tube was stored at 4 $^0$C.

Adding Buffer GA

Incubating at 70˚C for 10 min

Centrifuging at 12,000 rpm

Centrifuging running

Sample mixing by vortex

Brief Centrifugation

**Figure 3**: Pictorial views of Genomic DNA Extraction

## 3.10. PCR Amplification of Mitochondrial COI Gene Region

Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a DNA sequence. PCR was performed in a 25 $\mu l$ reaction mixture in small reaction tubes (0.2 ml) in a Thermal cycler (2720 Thermal Cycler, Applied Biosystems). The components of the reaction mixture for mitochondrial (mtDNA) COI have been shown in the Table 3.

**Table 3:** Components of PCR reaction for mtDNA COI region.

| **Components** | **Amount Per sample** |
|---|---|
| Green buffer | 2.5$\mu l$ |
| Forward primer | 1.0$\mu l$ |
| Reverse primer | 1.0$\mu l$ |
| Template DNA | 3.0$\mu l$ |
| Nuclease free water | 16.5$\mu l$ |
| *Taq* DNA polymerase | 0.5$\mu l$ |
| dNTP mix | 0.5$\mu l$ |
| **Total** | **25.0 $\mu l$** |

Each primer is prepared by mixing 90 $\mu l$ of deionized water with 10 $\mu l$ of each primer. *Taq* DNA polymerase was added just before the start of the reaction. Then the PCR tubes were transferred to PCR thermal cycler for the amplification of the reaction. The information on the primer sets used for amplifying the DNA barcode region of the mitochondrial COI gene of fish has been given in the Table 4.

**Table 4:** The Primer information was used for amplification of the mtDNA COI barcode gene region.

| **Name of the primer** | **Primer sequence (5' – 3')** | **Tm (°C)** | **Direction** | **Reference** |
|---|---|---|---|---|
| FishF1 | TCAACCAACCACAAAGACATTGGCAC | 63.7 | Forward | Ward et al. 2005 |
| FishR1 | TAGACTTCTGGGTGGCCAAAGAATCA | 62.8 | Reverse | |

The mtDNA COI region is amplified around the 650 bp region of the COI gene. Thermal cycling conditions include an initial denaturation temperature of $95^0$C for 2 minutes and subsequently, $94^0$C for 40 seconds for denaturation, $54^0$C cycles for 40 seconds for annealing, 72°C for 1 minute for an extension for 35 cycle followed by 72°C for 10 minutes for the final extension.

## 3.11. Gel Electrophoresis and Documentation of PCR Products

PCR products were examined by 1% agarose gel electrophoresis with a standard size marker (DNA ladder) (Fig. 4). The procedure for 50ml of 1% agarose gel formation and the process of gel electrophoresis and documentation of PCR products has been given below:

1. About 0.5g of agarose was weighted and mixed it with add 50ml TAE Buffer in a 250ml conical flask.
2. For dissolving the agarose was heated in a micro-oven for about 40 s and then a swirl was given to it. Then it was left to cool on the bench for 5 minutes until the temperature is down to about 60 ºC.
3. Then 5 $\mu l$ EZ-Vision ® In-Gel Solution was added and then swirled to mix. DNA bands will emit a whitish-blue fluorescence against a dark background using a standard trans illuminator (254 or 302 nm).
5. Then the mixture was poured on gel chamber. After inserting the comb, the gel mixture was kept up for 30 minutes for solidification. Then the gel was ready for gel electrophoresis.
6. Then the solidified gel was poured slowly into the gel tank and pushed any bubbles away to the side using a disposable tip.
7. After that 0.5X *TAE* buffer was poured into the gel tank to submerge the gel at 2 – 5mm depth. This is running buffer.
8. Then a volume of 5 µl PCR product from each sample and a ladder (5 µl) (1 Kb DNA Ladder) were placed into the comb – like chambers in the gel. Gel electrophoresis was performed in a 0.5X Tris – Acetate– EDTA (TAE) buffer for 20 minutes at 100 volts.

10. After electrophoresis, the gel was placed in the documentation chamber (INGENIUS[3]) and the flow UV – ray was kept on watching the band in the connected computer by using GeneSys software.


**Figure 4:** Gel electrophoresis and documentation

## 3.12. Purification of PCR Products

Purification of PCR products was conducted by QIAquick PCR purification kit. Steps of Purification of PCR products by QIAquick PCR purification kit have been given below-

- One volume of PCR products (18 µl) was mixed with 5 volumes (18 x 5 = 90 µl) of PB buffer.
- The mixture was transferred to the purification kit (column) after pipetting.
- The mixture was centrifuged for 1 minute.
- Then 750 µl PE buffer was added to the mixture in the column for the purpose of washing. Centrifugation was done again for 1 minute.
- Vacuum was applied and after discarding flow – through the column was placed back in the same tube. Again, centrifugation of the column was done for 1 minute.
- After that each column was placed in a clean 1.5 ml micro – centrifuge tube.
- To elute DNA, 30 µl EB (elution buffer) was added to the center of the QIAquick membrane and it was kept for 2 minutes and then centrifugation of the column was done for 1 minute.
- Finally, the column was discarded, and the liquid was transferred to another 2 ml micro – centrifuged tube. It was stored in $-20^0$C for estimating the concentration of purified DNA.

## 3.13. DNA Sequencing

After measuring concentration, the amplified PCR products were sent to a foreign institute for sequencing via a commercial DNA sequencing company. The DNA were sequenced in both directions by using Commercial Sequencer through a native company "Biotech Concern". The sequencing results were received by Email.

## 3.14. Sequence Editing

The received 5 Bagridae and 4 Schilbeidae DNA sequences were edited using the Software Geneious 9.1.5 with the help of Chromas Lit.

## 3.15. Identification of the Samples by BLAST Search

The mtDNA COI barcode region sequences of 5 Bagridae and 4 Schilbeidae samples of different fishes were identified by NCBI Blast search. Beside this, the morphological characters provided in the section was also used to verify the identification of the fish designated by NCBI.

## 3.16. Genetic Distance and Genetic Diversity

Genetic diversity of the fish and the mean genetic distance among the studied samples, and the genetic distance between the studied fish and the same species of other countries particularly of Asia were evaluated using Kimura two-parameter model in MEGA7 (Kimura, 1980).

## 3.17. Construction of Phylogenetic Tree

A phylogenetic tree was constructed using the sequences of different species obtained from the present study with Gene Bank sequence of the same species from NCBI from the other regions to understand the genetic difference. Nucleic acid sequences were aligned using ClustalW version 1.6 with the Alignment Explorer of the MEGA version 7 (Kumar et al., 2016). Among the 24 different nucleotide substitution models best model have been selected for constructing phylogenetic tree. For phylogenetic analyses, Neighbour-Joining (NJ) tree (Saitou and Nei, 1987) was constructed by MEGA7. The validity of the tree was examined by the bootstrap method (Felsenstein, 1985).

## 3.18. Statistical analysis

The statistical analyses were carried out using the statistical software package MEGA version 7.0. The pairwise sequence divergence among species was calculated according to Kimura two-parameter model in MEGA (Kimura, 1980). Tajima's test of neutrality has been done to observe the nucleotide diversity (Tajima, 1989). The probability of strict neutrality or positive selection that have been occurred was tested through Fisher exact test of neutrality (Zhang *et al.*, 1997).

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1. Identification of the Species

A total of 9 species were identified from the 7 seven genera, two families (Bagridae and Schilbeidae) under the order Siluriformes based on morphology and DNA barcoding approach. The species were *M. bleekeri, M. gulio, M. vittatus, R. rita, S. aor* from Bagridae and *A. coila, C. garua, E. vacha, S. silondia* from Schilbeidae family. List of identified species and given the Table 5.

**Table 5:** List of identified Bagridae and Schilbeidae from the Meghna Estuary, Bangladesh in the present study.

| **Family** | **Species Name** | **English Name** | **Local Name** | **Lab Code** | **IUCN Status** | **NCBI Blast search result** |
|---|---|---|---|---|---|---|
| Bagridae | *Mystus bleekeri* | Day's mystus | Tengra, Golsha-tengra | F2110ME-35 | LC | 99% |
| | *Mystus gulio* | Long whiskers catfish | Nuna-tengra | F1908ME-23, F1908ME-24 F1908ME-25 | NE | 99% |
| | *Mystus vittatus* | Striped dwarf catfish | Tengra | F2110ME-31 | LC | 99% |
| | *Rita rita* | *Rita* | *Rita* | F1812ME38 | NE | 99% |
| | *Sperata aor* | Long-whiskered catfish | Ayre | F1812ME-29 | LC | 99% |
| Schilbeidae | *Ailia coila* | Gangetic ailia | Kajuli | F1812ME-14 | LC | 99% |
| | *Clupisoma garua* | Garua bachcha | Ghaira | F1812ME-43 | LC | 99% |
| | *Eutropiichthys vacha* | Batchwa vacha | Bacha | F2110ME-52 | LC | 99% |
| | *Silonia silondia* | Silond catfish | Shillong | F1812ME-44 | LC | 99% |

## 4.2. Identification of the Species of *Mystus bleekeri*

**Species Name:** *Mystus bleekeri* (Bloch, 1794)

**English name:** Day's mystus

**Local Name:** Tengra, Golsha- tengra

**IUCN status:** Least concern (LC).

**Figure 5:** Lateral view of *M. bleekeri*

### 4.2.1. Identifying Characteristics

Body elongated and compressed. Head depressed. mouth terminal. It has four pairs barbel. Its maxillary barbels long up to anal fin sometimes larger than anal fin. Dorsal spine smooth. Adipose fin present. Caudal fin forked. The least height of caudal peduncle about 2 times its height. Dorsal side brownish, lighter at below. On above and below lateral line there is two longitudinal bands. A dark shoulders spot behind the head. Fins are greyish-white at the edges side dark (Fig. 5). Meristic characteristics of *M. bleekeri* in this present study and its comparison with other studies are given in the Table 6.

**Table 6:** Meristic characteristics of *M. bleekeri* in this present study and its comparison with other studies.

| Characteristics | Present study | (Rahman, 2005) |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/9 | I/9 |
| Pelvic fin | 6 | 6 |
| Anal fin | 9 | 9 |

## 4.2.2. Habit and Habitat

Freshwater; rivers, canals, khals, beels and other freshwater bodies in Bangladesh (Rahman, 2005). Inhibits lakes, tanks, rivers (Talwar and Jhingran, 1991).

## 4.2.3. Genetic Description of *M. bleekeri*

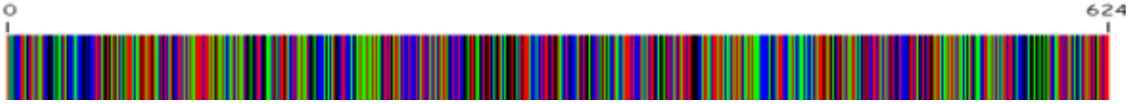
A total of 624 nucleotide base pairs (Fig. 7) was obtained from the alignments of a COI gene sequence (Fig. 9) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the sequences of *M. bleekeri* deposited in the GenBank database from Bangladesh (MK572335) and India (KT896741, KX266834 and KP939357). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 8) generated 99% identities indicating the effectiveness of COI sequences to provide species-level resolution. *M. bleekeri* sequences of the present study were clustered in a single clade with the sequences from Bangladesh (MK572335) and India (KT896741, KX266834, and KP939357) which validate the genetic identification of this species. Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh ranged from 0.002 (0.2%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table **7**). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 29.6%, C= 28.0%, A= 26.3%, G=16.1%.


**Figure 6:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *M. bleekeri*.The species *S. silondia* was used as an out-group. Bootstrap support of >92% are shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 7:** Pairwise genetic distance among the six *M. bleekeri* individuals used to construct the phylogenetic tree.

| Sl No | Scientific Name | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | *Mystus_bleekeri*_F2110ME-35 | | | | |
| 2 | *Mystus_bleekeri*_KX266834.1_India | 0.002 | | | |
| 3 | *Mystus_bleekeri*_KP939357.1_India | 0.002 | 0.003 | | |
| 4 | *Mystus_bleekeri*_MK572335.1_Bangladesh | 0.002 | 0.003 | 0.003 | |
| 5 | *Mystus_bleekeri*_KT896741.1_India | 0.000 | 0.002 | 0.002 | 0.002 |
| **Overall=0.002%** | | | | | |

**DNA Barcode:** *Mystus bleekeri*




TACAGCCCTTAGCTTACTAATCCGGGCAGAACTAGCCCAACCCGGCGCCCTTCTAGGTGATGATCAGATTTACAAT
GTTATTGTAACTGCTCATGCCTTCATCATAATTTTCTTTATAGTAATACCAATCATAATCGGAGGCTTCGGAAACTG
ACTTGTACCTCTAATAATCGGAGCCCCAGACATGGCCTTCCCACGAATAAATAATATAAGCTTCTGATTACTCCCTC
CCTCATTCCTACTACTATTAGCCTCCTCCGGAGTTGAAGCTGGTGCAGGTACAGGGTGGACTGTTTACCCACCTCT
TGCTGGTAACCTCGCCCATGCTGGAGCCTCAGTAGACCTAACCATTTTCTCCCTACATCTTGCAGGAGTCTCATCT
ATTCTTGGGGCCATTAACTTTATTACAACTATTATCAATATAAAACCCCCAGCCATTTCCCAATACCAAACACCACT
ATTTGTATGAGCTGTACTAATTACAGCTGTCCTTCTACTACTGTCGCTCCCCGTTCTAGCTGCTGGTATTACAATAT
TACTAACAGATCGAAACCTTAATACTACATTCTTCGACCCGGCAGGAGGAGGAGATCCAATTCTCTATCAGCACTT
ATTCTGATTCTTT

**Figure 7:** Illustrate of DNA Barcode of *M. bleekeri*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mystus bleekeri isolate bf83 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1142 | 1142 | 98% | 0.0 | 100.00% | 624 | MK359935.1 |
| Mystus bleekeri isolate UC-MR37 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 98% | 0.0 | 100.00% | 655 | MN096212.1 |
| Mystus bleekeri from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 98% | 0.0 | 100.00% | 655 | MK572334.1 |
| Mystus bleekeri isolate ZSI_MRF8 cytochrome oxidase subunit I gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 98% | 0.0 | 100.00% | 645 | MK029805.1 |
| Mystus bleekeri voucher NBFGRMU 8028R cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 98% | 0.0 | 100.00% | 655 | KT896741.1 |
| Mystus bleekeri voucher MB-2001 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 100% | 0.0 | 99.52% | 707 | KJ936764.1 |
| Mystus bleekeri voucher MB-1001 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 98% | 0.0 | 100.00% | 655 | KJ936689.1 |
| Mystus bleekeri voucher SRC-ANU-13A cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus bleekeri | 1138 | 1138 | 99% | 0.0 | 99.84% | 660 | KP939357.1 |

**Figure 8:** NCBI Blast search result of *M. bleekeri*

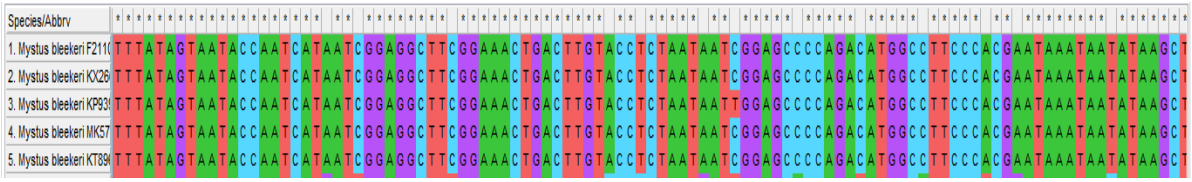



**Figure 9:** Alignment sequence of *M. bleekeri*

# 4.3. Identification of the Species of *Mystus* gulio

**Species Name:** *Mystus gulio (*Hamilton, 1822)

**English name:** Long whiskers catfish

**Local name:** Nuna-tengra

**IUCN status:** Least concern (LC).

**Figure 10:** Lateral view of *M. gulio*

## 4.3.1. Identifying Characteristics

Head depressed. Body elongated and compressed. Its upper surface rough and granulated. Barbels four pairs. Maxillary barbels extend to end of pelvic fins. Mouth terminal. Dorsal spine strong and serrated. Adipose fin small and caudal fin forked and caudal peduncle equal at height. Body color bluish-brown on head and back. Brown on the back. Dull white below. Mandibular barbells are partly black and partly white (Fig. 10). Meristic characteristics of *M. gulio* in this present study and its comparison with other studies are given in Table 8.

## 4.3.2. Habit and Habitat

Firstly, it's a brackish water fish that enters and lives in freshwater fish also enters tidal rivers of Bangladesh and in the Bay of Bengal (Rahman, 2005). Inhibits estuaries and tidal rivers and lakes; ascends freshwater and often enters sea (Talwar and Jhingran, 1991). Found in canals, beels, haors, oxbow lakes, rivers, and estuaries (Shafi and Quddus, 2001). Available in the Meghna river and the Sundarbans of Bangladesh (Rahman, 2005).

**Table 8:** Meristic characteristics of *M. gulio* in this present study and its comparison with another study.

| Characteristics | Present study | Rahman, 2005 |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/9 | I/9 |
| Pelvic fin | 6 | 6 |
| Anal fin | 15 | 15 |

## 4.3.3. Genetic Description of *M. gulio*

A total of 641 nucleotide base pairs (Fig. 12) were obtained from the alignments of three COI gene sequences (Fig. 14) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the three sequences of *M. gulio* deposited in the GenBank database from Bangladesh (KX455905, MK572347 and MN083111). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 13) generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *M. gulio* sequences of the present study were clustered in a single clade with the three sequences from Bangladesh submitted in the GenBank (KX455905, MK572347 and MN083111) which validate the genetic identification of this species**.** Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh ranged from 0.00 (0.0%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table 9). The total number of nucleotide (A, T, G, C) composition among the sequences of the present study have been observed. The average nucleotide composition was T= 31.2%, C= 25.6%, A= 24.5%, G=18.7%.

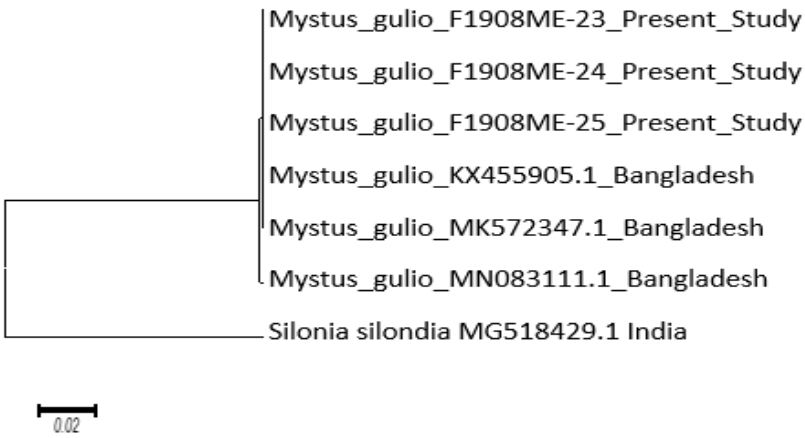
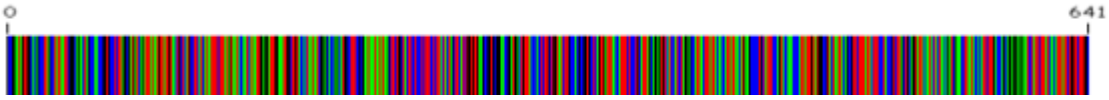



**Figure 11:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *M. gulio*.The species *S. silondia* was used as an out-group. Bootstrap support of >90% are shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 9:** Pairwise genetic distance among the six *M. gulio* individuals used to construct the phylogenetic tree.

| **SI No.** | **Scientific Name** | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 1 | *Mystus_gulio*_F1908ME-23_Present_Study | | | | | |
| 2 | *Mystus_gulio*_F1908ME-24_Present_Study | 0.000 | | | | |
| 3 | *Mystus_gulio*_F1908ME-25_Present_Study | 0.000 | 0.000 | | | |
| 4 | *Mystus_gulio*_KX455905.1_Bangladesh | 0.000 | 0.000 | 0.000 | | |
| 5 | *Mystus_gulio*_MK572347.1_Bangladesh | 0.000 | 0.000 | 0.000 | 0.000 | |
| 6 | *Mystus_gulio*_MN083111.1_Bangladesh | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 |
| **Overall=0.001%** | | | | | | |

**DNA Barcode:** *Mystus gulio*




CGGTGCTTGAGCCGGATAGTTGGCACAGCCCTTAGCCTATTAATTCGGGCAGAACTAGCCC
AACCCGGTGCCCTCTTAGGCGATGATCAGATTTATAATGTTATTGTAACTGCTCATGCCTTC
ATCATAATTTTCTTTATAGTAATACCAATCATAATTGGAGGATTTGGAAATTGACTTGTACC
ACTAATAATCGGAGCACCAGACATGGCCTTTCCGCGAATAAATAATATAAGCTTCTGACTA
CTTCCTCCCTCTTTCCTCTTACTACTAGCTTCATCTGGTGTTGAAGCCGGTGCGGGAACCGG
ATGAACTGTTTACCCACCTCTTGCTGGTAATCTTGCCCACGCCGGTGCCTCAGTTGATTTAA
CCATTTTCTCCCTGCATTTAGCAGGAGTGTCATCCATCCTAGGAGCCATTAACTTTATTACA
ACTATTATTAACATGAAGCCCCCTGCTATTTCCCAGTACCAAACCCCATTATTTGTATGAGC
CGTACTAATTACAGCCGTTCTTTTATTACTTTCCCTACCCGTCTTAGCTGCAGGTATTACAAT
ACTACTAACAGATCGAAACCTTAATACCACATTCTTTGACCCAGCAGGAGGAGGAGATCCT
ATTCTTTATCAACATTTATTCTGATTCTTTGCC

**Figure 12:** Illustrate of DNA Barcode of *M. gulio*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mystus gulio voucher ZMUD:120 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus gulio | 1194 | 1194 | 99% | 0.0 | 99.85% | 696 | KX455898.1 |
| Mystus gulio voucher FBRC_ZSI_DNA1002_F4481 cytochrome c oxidase subunit I (COX1) gene, partial cds;... | Mystus gulio | 1194 | 1194 | 99% | 0.0 | 99.85% | 678 | OM274001.1 |
| Mystus gulio voucher ZMUD:120.2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus gulio | 1188 | 1188 | 99% | 0.0 | 99.69% | 696 | KX455905.1 |
| Mystus gulio from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus gulio | 1175 | 1175 | 98% | 0.0 | 99.84% | 655 | MK572349.1 |
| Mystus gulio from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus gulio | 1171 | 1171 | 98% | 0.0 | 99.69% | 655 | MK572346.1 |
| Mystus gulio from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus gulio | 1170 | 1170 | 98% | 0.0 | 99.69% | 655 | MK572348.1 |
| Mystus gulio from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus gulio | 1168 | 1168 | 97% | 0.0 | 99.69% | 655 | MK572350.1 |
| Mystus gulio from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Mystus gulio | 1164 | 1164 | 98% | 0.0 | 99.53% | 655 | MK572345.1 |

**Figure 13:** NCBI Blast search result of *M. gulio*

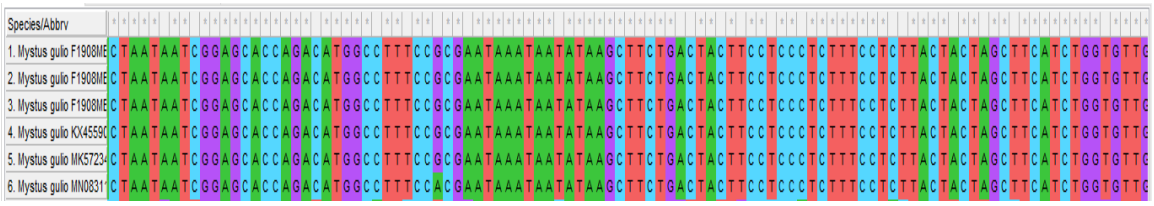



**Figure 14:** Alignment sequence of *M. gulio*

# 4.4. Identification of the Species of *Mystus* vittatus

**Species Name:** *Mystus vittatus* (Bloch, 1794)

**English name:** Striped dwarf catfish

**Local Name:** Tengra

**IUCN status:** Least concern (LC).

**Figure 15**: lateral view of *M. vittatus*

## 4.4.1. Identified Characteristics

Body elongated and slightly compressed. 4 pairs of barbels, maxillary barbels extending beyond the pelvic fins, often to the end of the anal fin. Dorsal spine is weak, finely serrated on its inner edge. Adipose fin small inserted much behind rayed dorsal fin but anterior to the anal fin. Lateral line present and straight. Color varies with age; generally delicate gray-silvery to shining golden, with about 5 pale blue or dark brown to deep black longitudinal on the side. A narrow dusky spot often presents on the shoulder. The fins are glass, with dark tips. Meristic characteristics of *M. vittatus* in this present study and its comparison with other studies are given in Table 10.

**Table 10:** Meristic characteristics of *M. vittatus* in this present study and its comparison with other studies.

| Characteristics | Present study | Rahman, 2005 |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/8 | I/8 |
| Pelvic fin | 6 | 6 |
| Anal fin | 9 | 9 |
| Caudal Fin | 17 | 17 |

## 4.4.2. Habit and Habitat

Found in freshwater bodies; flooded canals, beels, paddy and jute fields, streams, haors, oxbow lakes and rivers in swarms during the rainy season (Bhuiyan, 1964; Shafi and Quddus, 2001). Inhibits standing and flowing water bodies, even in the tidal zone (Talwar and Jhingran, 1991). Recorded from Chalan Beel (Galib *et al.*, 2009a).

## 4.4.3. Genetic Description of *M. vittatus*

A total of 616 nucleotide base pairs (Fig. 17) were obtained from the alignments of an COI gene sequences (Fig. 19) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query

sequence showed high identity (> 99%) with the three sequences of *M. vittatus* deposited in the GenBank database from India (KZ959638, JN228949) and Bangladesh (KT364780). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 18) generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *M. vittatus* sequences of the present study were clustered in a single clade with the two sequences from India (KZ959638, JN228949) and one sequence from Bangladesh (KT364780) submitted in the GenBank which validates the genetic identification of this species (Fig. 16). Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh ranged from 0.002 (0.2%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table 11). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 30.8%, C= 27.9%, A= 23.7%, G=17.6%.

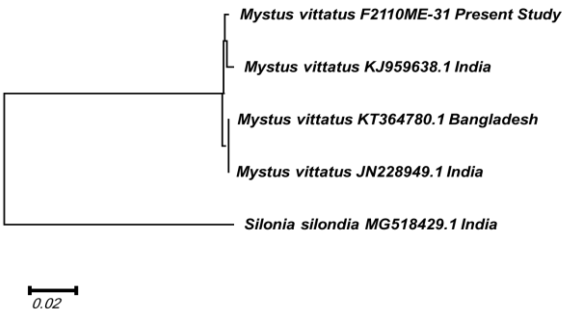
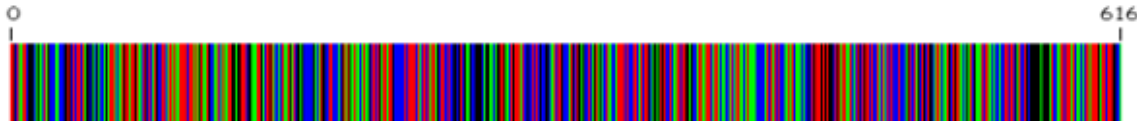



**Figure 16:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *M. vittatus*. The species *S. silondia* was used as an out-group. Bootstrap support of >92% are shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 11:** Pairwise genetic distance among the four *M. vittatus* individuals used to construct the phylogenetic tree.

| **SI No** | **Scientific Name** | 1 | 2 | 3 |
|---|---|---|---|---|
| 1 | *Mystus_vittatus*_F2110ME-31 | | | |
| 2 | *Mystus_vittatus*_KT364780.1_Bangladesh | 0.002 | | |
| 3 | *Mystus_vittatus*_KJ959638.1_India | 0.003 | 0.005 | |
| 4 | *Mystus_vittatus*_JN228949.1_India | 0.002 | 0.000 | 0.005 |
| **Overall=0.003%** | | | | |

**DNA Barcode:** *Mystus vittatus*




TTGCTAATTCGGGCAGAACTAGCCCAACCCGGTGCTCTTCTAGGCGACGACCAGATTTACAATGTTATTGTAACTG
CTCATGCCTTCATTATAATTTTCTTTATAGTAATGCCAATCATAATTGGAGGTTTCGGAAATTGACTTGTACCACTA
ATGATCGGAGCCCCCGACATGGCCTTCCCACGTATGAATAACATAAGCTTCTGATTATTGCCCCCCTCTTTTCTACT
TCTATTAGCTTCCTCCGGCGTCGAAGCCGGGGGCAGGAACAGGTTGAACTGTTTATCCGCCTCTCGCCGGCAATCT
TGCCCATGCTGGAGCCTCAGTAGACCTAACCATTTTCTCCCTTCATCTTGCAGGAGTGTCCTCAATTCTTGGAGCC
ATTAATTTCATTACAACCATCATTAACATAAAACCCCCATCTATCTCCCAATATCAAACACCGCTGTTTGTGTGGGC
TGTATTAATCACTGCTGTTCTCCTACTACTCTCCCTCCCAGTCCTAGCTGCTGGCATTACAATGCTGTTAACAGATC
GAAATCTTAACACCACCTTCTTCGATCCTGCGGGGGGAGGGGATCCAATTCTTTATCAACACTTATTTTGATTCTTT
GGCCA

**Figure 17:** Illustrate of DNA Barcode of *M. vittatus*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mystus vittatus voucher DUZM122 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus vittatus | 1129 | 1129 | 99% | 0.0 | 99.84% | 679 | KT364780.1 |
| Mystus vittatus voucher FBRC_ZSI_DNA818_F3798 cytochrome c oxidase subunit I (COX1) gene, partial cds;... | Mystus vittatus | 1129 | 1129 | 100% | 0.0 | 99.68% | 640 | MW485071.1 |
| Mystus tengara isolate G97 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Mystus tengara | 1125 | 1125 | 99% | 0.0 | 99.67% | 636 | MT928147.1 |
| Mystus tengara isolate G30 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Mystus tengara | 1125 | 1125 | 99% | 0.0 | 99.67% | 636 | MT928145.1 |
| Mystus vittatus isolate SGMCAC-MOF3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus vittatus | 1123 | 1123 | 99% | 0.0 | 99.67% | 621 | KJ959638.1 |
| Mystus tengara isolate K23 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Mystus tengara | 1120 | 1120 | 99% | 0.0 | 99.51% | 636 | MT928144.1 |
| Mystus tengara isolate 11413 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus tengara | 1118 | 1118 | 100% | 0.0 | 99.35% | 633 | MN083145.1 |
| Mystus vittatus isolate bf74 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus vittatus | 1118 | 1118 | 99% | 0.0 | 99.67% | 630 | MK359926.1 |
| Mystus vittatus isolate bf37 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Mystus vittatus | 1118 | 1118 | 99% | 0.0 | 99.67% | 618 | MK359890.1 |

**Figure 18:** NCBI Blast search result of *M. vittatus*


**Figure 19:** Alignment sequence of *M. vittatus*

# 4.5. Identification of the Species of *Rita rita*

**Species Name:** *Rita rita (*Hamilton, 1822)

**English name**: Rita

**Local name:** Rita

**IUCN status:** Least concern (LC).

**Figure 20:** Lateral view of *R. rita*

## 4.5.1. Identifying Characteristics

Body elongated with depressed head. Nostril wide apart. Mouth transverse with 3 pairs of barbels. Straight lateral line with the strong dorsal spine. Pectoral spine shorter than dorsal spine. Caudal forked. Body color green or greenish above and flanks, sometimes brownish/blackish; dull white below (Fig. 20). Meristic characteristics of this present study and its comparison with other studies are given in the Table 12.

**Table 12:** Meristic characteristics of *R. rita* in this present study and its comparison with other study.

| Characteristics | Present study | Rahman, 2005 |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/10 | I/10 |
| Pelvic fin | 8 | 8 |
| Anal fin | 10 | 10 |
| Caudal Fin | 22 | 22 |

## 4.5.2. Habit and Habitat

Freshwater and tidal waters (Talwar and Jhingran, 1991). In rivers and estuaries of Bangladesh; also recorded from the Meghna river and Chalan Beel (Rahman, 2005).

## 4.5.3. Genetic Description of *Rita rita*

A total of 648 nucleotide base pairs (Fig. 22) were obtained from the alignments of a COI gene sequence (Fig. 24) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the three sequences of *R. rita* deposited in the GenBank database from India (MH047229 and MH047230) and Bangladesh (KT762374 and OK103946). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 23) generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *R. rita* sequences of the present study were clustered in a single clade with the two sequences from India (MH047229 and MH047230) and two sequences from Bangladesh (KT762374 and OK103946) submitted in the GenBank which validates the genetic identification of this

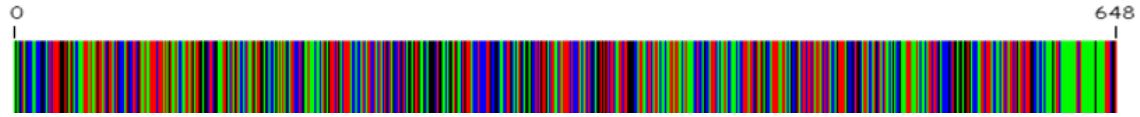
species (Fig. 21). Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are 0.00 (0.0%) which also validates the genetic identification of this species (Table 13). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 30.6%, C= 25.8%, A= 27.5%, G=16.1%.


**Figure 21:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *R. rita*. The species *S. silondia* was used as an out-group. Bootstrap support of >92% is shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 13:** Pairwise genetic distance among the six *R. rita* individuals was used to construct the phylogenetic tree.

| **SI No** | **Scientific Name** | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | *Rita_rita*_F1812ME38_Present_Study | | | | |
| 2 | *Rita_rita*_KT762374.1_Bangladesh | 0.000 | | | |
| 3 | *Rita_rita*_OK103946.1_Bangladesh | 0.000 | 0.000 | | |
| 4 | *Rita_rita*_MH047230.1_India | 0.000 | 0.000 | 0.000 | |
| 5 | *Rita_rita*_MH047229.1_India | 0.000 | 0.000 | 0.000 | 0.000 |
| **Overall=0.000%** | | | | | |

**DNA Barcode:** *Rita rita*




AGAGCTAGCCCAACCCGGCACTCTTTTGGGTGATGACCAAATTTATAATGTTATCGTTACTGCCCATGCCTTCGTA
ATAATTTTCTTTATAGTAATACCAATTATGATTGGAGGCTTCGGAAATTGACTAGTACCACTAATGATTGGAGCAC
CAGATATAGCATTCCCTCGAATAAACAACATGAGTTTCTGACTTTTACCACCATCATTCCTACTACTGCTAGCCTCG
TCAGGAGTTGAAGCAGGGGCTGGAACAGGATGAACTGTTTACCCTCCCCTTGCCGGCAACCTTGCACATGCAGG
AGCCTCTGTTGACTTAACTATTTTCTCCCTACATCTCGCAGGTGTGTCTTCAATTCTTGGGGCCATTAACTTCATTAC
AACAATTATTAACATAAAACCTCCAGCCATTTCACAATACCAAACACCTTTATTCGTATGAGCCATTTTAATTACAG
CCGTACTTCTTCTATTATCTCTACCAGTTTTAGCCGCTGGCATTACCATGCTACTAACAGACCGAAATTTAAATACA
ACATTCTTCGACCCCGCTGGAGGAGGTGACCCAATTCTTTACCAACATCTATTCTGATTCTTTGGCCACCACAAAA
GATTCAAAAAAAAATCTAAAAAAAAGAAAAATTTCGGT

**Figure 22:** Illustrate of DNA Barcode of *R. rita*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rita rita voucher DUZM124.1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Rita rita | 1118 | 1118 | 93% | 0.0 | 100.00% | 685 | KT762374.1 |
| Rita rita voucher F1812ME-38 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Rita rita | 1110 | 1110 | 92% | 0.0 | 100.00% | 643 | OK103946.1 |
| Rita rita voucher DUZM124 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Rita rita | 1109 | 1109 | 92% | 0.0 | 100.00% | 653 | KT364781.1 |
| Rita rita voucher COE-AS-353 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Rita rita | 1107 | 1107 | 92% | 0.0 | 99.83% | 639 | MH165300.1 |
| Rita rita voucher Pak-RR2 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Rita rita | 1107 | 1107 | 92% | 0.0 | 99.83% | 639 | ON920188.1 |
| Rita rita voucher RR1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Rita rita | 1105 | 1105 | 92% | 0.0 | 99.83% | 636 | MT812028.1 |
| Rita rita isolate Rth_04 cytochrome oxidase subunit I gene, partial cds; mitochondrial | Rita rita | 1103 | 1103 | 91% | 0.0 | 100.00% | 629 | MH047230.1 |
| Rita rita isolate Rtg_02 cytochrome oxidase subunit I gene, partial cds; mitochondrial | Rita rita | 1103 | 1103 | 91% | 0.0 | 100.00% | 631 | MH047229.1 |
| Rita rita voucher DOF104 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Rita rita | 1099 | 1099 | 91% | 0.0 | 100.00% | 622 | JX105469.1 |

**Figure 23:** NCBI Blast search result of *R. rita*

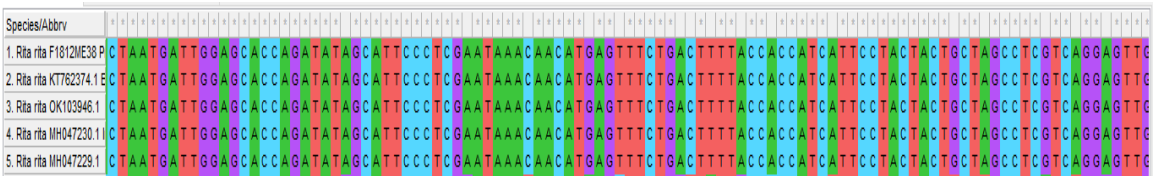



**Figure 24:** Alignment sequence of *R. rita*

# 4.6. Identification of the Species of *Sperata* aor

**Species Name:** *Sperata aor (*Hamilton,1822)

**English name:** Long-whiskered catfish

**Local name:** Ayre

**IUCN status:** Least concern (LC).

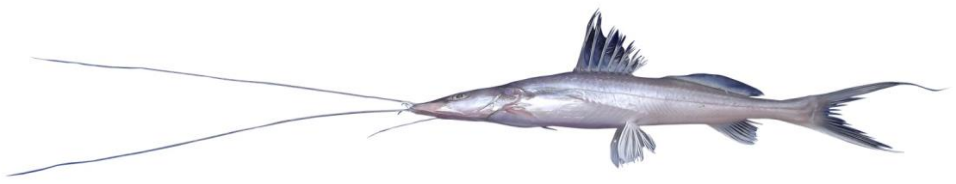


**Figure 25:** Lateral view of *S. aor*

## 4.6.1. Identifying Characteristics

Body elongated, head depressed and mouth sub-terminal. Eyes are transversely oval situated on the dorsal portion of the head. Nostril 2 pairs. 4 pairs of barbels, maxillary pair reaches to the base of the caudal, the dorsal. Dorsal and pectoral fins contain a strong spine and dorsal spine finely serrated on its posterior edge. Adipose fin well developed and originated near caudal fin. Caudal forked and upper lobe slightly longer than lower. Lateral line present and complete (Fig. 25)**.** Meristic characteristics of this present study and its comparison with other studies are given in Table 14.

**Table 14:** Meristic characteristics of *S. aor* in this present study and its comparison with other study.

| Characteristics | Present study | Talwar and Jhingran, 1991 |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/10 | I10 |
| Pelvic fin | I/5 | I/5 |
| Anal fin | 12 | 12 |
| Caudal Fin | 17 | 17 |

## 4.6.2. Habit and Habitat

Bottom living fish. Commonly found in freshwater and brackish water (Bhuiyan, 1964). Some common habitats are rivers, khals, canals, beels, ponds, lakes, ditches, inundated fields, reservoirs etc (IUCN Bangladesh, 2000).

## 4.6.3. Genetic Description of *Sperata aor*

A total of 634 nucleotide base pairs (Fig. 27) were obtained from the alignments of one COI gene sequence (Fig. 29) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the three sequences of *S. aor* deposited in the GenBank database from India (KY290057, MH047237 and MH047238) and one sequence

from Bangladesh (KT762381). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 28) generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *S. aor* sequences of the present study were clustered in a single clade with the three sequences from Bangladesh from India (KY290057, MH047237 and MH047238) and one sequences from Bangladesh (KT762381) submitted in the GenBank which validates the genetic identification of this species (Fig. 26)**.** Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are 0.00 (0.0%) which also validates the genetic identification of this species (Table 15). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 39.4%, C= 26.4%, A= 27.2% and G=17.0%.

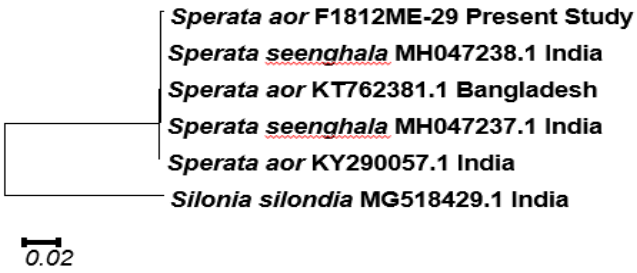
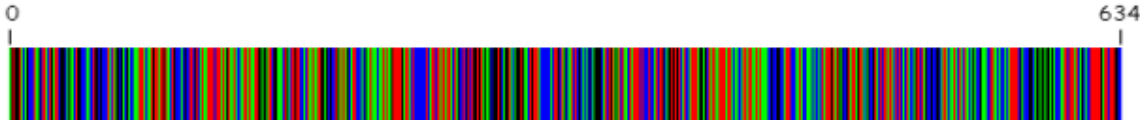



**Figure 26:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *S. aor*. The species *S. silondia* was used as an out-group. Bootstrap support of >92% is shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 15:** Pairwise genetic distance among the six *S. aor* individuals used to construct the phylogenetic tree.

| Sl No | Scientific Name | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | *Sperata_aor*_F1812ME-29_Present_Study | | | | |
| 2 | *Sperata_aor*_KT762381.1_Bangladesh | 0.002 | | | |
| 3 | *Sperata_ aor* _MH047238.1_India | 0.002 | 0.000 | | |
| 4 | *Sperata_ aor* _MH047237.1_India | 0.002 | 0.000 | 0.000 | |
| 5 | *Sperata_aor*_KY290057.1_India | 0.002 | 0.000 | 0.000 | 0.000 |
| **Overall=0.001%** | | | | | |

**DNA Barcode:** *Sperata aor*




AGTGGGTACAGCCCTAAGCTTACTGATTCGGGCAGAACTGGCCCAACCTGGCGCCCTACTAGGCGACGACCAAA
TTTACAATGTTATTGTAACTGCCCACGCCTTCGTCATAATTTTCTTTATAGTAATACCAATTATAATTGGAGGATTC
GGAAACTGACTTGTTCCACTAATAATTGGAGCGCCTGATATAGCATTCCCACGAATAAACAATATAAGTTTTTGAT
TACTCCCCCCCTCATTCTTATTATTACTAGCCTCGTCTGGTGTAGAAGCGGGTGCAGGTACAGGGTGAACTGTTTA
CCCCCCACTTGCTAGCAATCTCGCACACGCAGGGGCATCTGTAGATCTAACTATTTTCTCCCTCCATCTTGCAGGT
GTGTCATCCATTTTAGGAGCTATTAATTTTATTACAACCATTATTAATATAAAACCGCCGGCCATTTCCCAATACCA
AACACCACTATTTGTATGAGCCGTATTAGTTACAGCCGTACTCTTATTACTCTCACTACCAGTTCTAGCCGCCGGCA
TTACAATACTACTAACAGACCGAAACCTTAACACCACATTTTTCGACCCGGCAGGAGGAGGAGACCCAATTCTTTA
TCAGCACCTTTTTTGATTCTTTGGCC

**Figure 27:** Illustrate of DNA Barcode of *S. aor*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sperata aor voucher DUZM125 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Sperata aor | 1168 | 1168 | 100% | 0.0 | 99.84% | 685 | KT762381.1 |
| Sperata aor isolate aor-2 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Sperata aor | 1162 | 1162 | 99% | 0.0 | 99.84% | 632 | MT448048.1 |
| Sperata aor isolate aor-1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Sperata aor | 1162 | 1162 | 99% | 0.0 | 99.84% | 632 | MT448047.1 |
| Sperata seenghala isolate Msh_07 cytochrome oxidase subunit I gene, partial cds; mitochondrial | Sperata seenghala | 1160 | 1160 | 99% | 0.0 | 99.84% | 631 | MH047238.1 |
| Sperata seenghala isolate Msg_02 cytochrome oxidase subunit I gene, partial cds; mitochondrial | Sperata seenghala | 1160 | 1160 | 99% | 0.0 | 99.84% | 631 | MH047237.1 |

**Figure 28:** NCBI Blast search result of *S. aor*

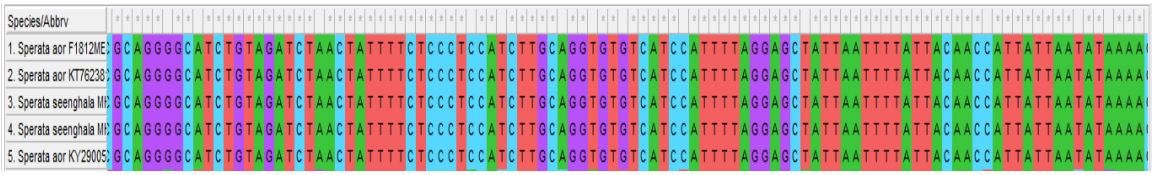



**Figure 29:** Alignment sequence of *S. aor*

# 4.7. Identification of the Species of *Ailia coila*

**Species Name:** *Ailia coila (*Hamilton, 1822)

**English name:** Gangetic ailia

**Local name:** Kajuli

**IUCN status:** Least Concern (LC).

**Figure 30.** Lateral view of *A. coila*

## 4.7.1. Identifying Characteristics

Body elongated, head depressed and mouth sub terminal. Eyes transversely oval situated on the dorsal portion of head. Nostril 2 pairs. 4 pairs of barbels, maxillary pair reaches to the base of caudal fin. Dorsal and pectoral fins contain a strong spine and dorsal spine finely serrated on its posterior edge. Adipose fin well developed and originated near caudal fin. Caudal forked and upper lobe slightly longer than lower. Lateral line present and complete (Fig. 30). Meristic characteristics of this present study and its comparison with other studies are given in the Table 16.

**Table 16:** Meristic characteristics of *A. coila* in this present study and its comparison with other study.

| Characteristics | Present study | Talwar and Jhingran, 1991 |
|---|---|---|
| First dorsal fin | - | - |
| Second dorsal fin | - | - |
| Pectoral fin | I/14 | I/14-16 |
| Pelvic fin | 6 | 6 |
| Anal fin | 67 | 58-75 |

## 4.7.2. Habit and Habitat

Bottom living fish. Commonly found in freshwater and brackish water. Some common habitats are rivers, khals, canals, beels, ponds, lakes, ditches, inundated fields, reservoirs etc. (IUCN Bangladesh, 2000;). Recorded from Chalan Beel (Galib *et al.*, 2009).

## 4.7.3. Genetic Description of *Ailia coila*

A total of 620 nucleotide base pairs (Fig. 32) were obtained from the alignments of two COI gene sequences (Fig. 34) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the two sequences of *A. coila* deposited in the GenBank database from India (KJ959640, MK577978) and one sequence from Bangladesh (KT364761). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 33) generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *A. coila* sequences

of the present study were clustered in a single clade with the two sequences from India (KJ959640, MK577978) and one sequence from Bangladesh (KT364761) submitted in the GenBank which validates the genetic identification of this species (Fig. 31). Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are 0.00 (0.0%) which also validates the genetic identification of this species (Table 17). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 31.4%, C= 24.4%, A= 28.0%, G=16.2%.

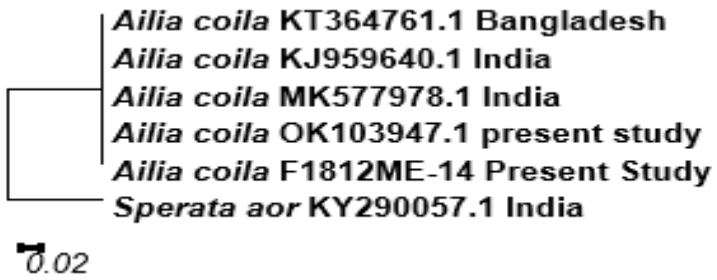
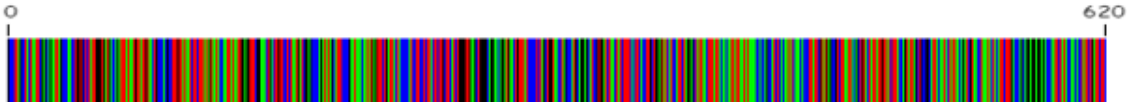



**Figure 31:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *A. coila*. The species *S. aor* was used as an out-group. Bootstrap support of >92% is shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 17:** Pairwise genetic distance among the six *A. coila* individuals used to construct the phylogenetic tree.

| **Sl No** | **Scientific Name** | 1 | 2 | 4 | 4 |
|---|---|---|---|---|---|
| 1 | *Ailia_coila*_OK103947.1_present_study | | | | |
| 2 | *Ailia_coila*_F1812ME-14 | 0.000 | | | |
| 3 | *Ailia_coila*_MK577978.1_India | 0.000 | 0.000 | | |
| 4 | *Ailia_coila*_KT364761.1_Bangladesh | 0.000 | 0.000 | 0.000 | |
| 5 | *Ailia_coila*_KJ959640.1_India | 0.000 | 0.000 | 0.000 | 0.000 |
| **Overall=0.000%** | | | | | |

**DNA Barcode:** *Ailia coila*




AGTTGGTACAGCCCTTAGCCTACTAATTCGAGCAGAATTAGCCCAACCTGGTGCTCTATTGGGTGATGACCAGAT
TTATAATGTTATTGTAACTGCCCACGCCTTTGTTATAATCTTCTTTATAGTAATACCAATTATAATTGGAGGTTTCG
GAAACTGACTTGTTCCTCTAATGATCGGAGCCCCAGATATAGCATTTCCCCGAATAAACAATATGAGTTTCTGATT
ACTACCCCCATCCTTCCTCTTACTTCTTGCTTCATCTGGGGTTGAAGCTGGGGCAGGAACAGGATGAACTGTTTAT
CCCCCTCTAGCTGGTAACCTAGCCCATGCAGGAGCATCCGTGGATCTAACTATCTTTTCACTTCACCTTGCTGGAG
TATCATCCATTCTAGGAGCTATTAACTTTATTACAACAATTATTAATATAAAACCACCAGCCATCTCACAATATCAA
ACACCCCTATTCGTATGAGCCGTCTTATTACAGCTGTACTATTATTGTTATCTTTACCAGTATTAGCTGCCGGCATT
ACAATACTATTAACAGATCGTAACCTAAATACTACATTCTTTGACCAGCAGGAGGAGGAGACCCAATTCTATACCA
ACACCTATTCTGATTCTTTGGCC

**Figure 32:** Illustrate of DNA Barcode of *A. coila*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ailia coila voucher F1812ME-15 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial | Ailia coila | 1160 | 1160 | 100% | 0.0 | 99.69% | 635 | OK103947.1 |
| Ailia coila voucher DUZM131 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Ailia coila | 1157 | 1157 | 99% | 0.0 | 99.68% | 679 | KT364761.1 |
| Ailia coila isolate SGMCAC-MOF6 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Ailia coila | 1153 | 1153 | 99% | 0.0 | 99.68% | 633 | KJ959640.1 |
| Ailia coila mitochondrion, complete genome | Ailia coila | 1151 | 1151 | 99% | 0.0 | 99.68% | 16497 | NC_046497.1 |
| Ailia coila mitochondrion, complete genome | Ailia coila | 1151 | 1151 | 99% | 0.0 | 99.68% | 16497 | MK348534.1 |
| Ailia coila cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Ailia coila | 1151 | 1151 | 99% | 0.0 | 99.68% | 648 | MK577978.1 |
| Ailia coila isolate bf100 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Ailia coila | 1140 | 1140 | 98% | 0.0 | 99.68% | 624 | MK359950.1 |
| Ailia coila isolate 11424 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Ailia coila | 1138 | 1138 | 98% | 0.0 | 99.52% | 641 | MN083152.1 |
| Ailia coila voucher CIFRI 8064S cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Ailia coila | 1136 | 1136 | 97% | 0.0 | 99.68% | 655 | MG518408.1 |

**Figure 33:** NCBI Blast search result of *A. coila*

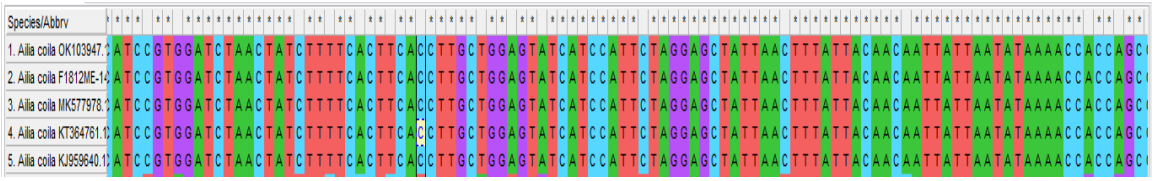



**Figure 34:** Alignment sequence of *A. coila*

# 4.8. Identification of the Species of *Clupisoma garua*

**Species Name:** *Clupisoma garua* (Hamilton, 1822)

**English Name:** Garua bachcha

**Local Name:** Ghaira

**IUCN status:** Least Concern (LC).

**Figure 35:** Lateral view of *C. garua*

## 4.8.1. Identifying Characteristics

Body elongated and laterally compressed. Eye with board adipose lids. Mouth moderate and sub-terminal. Upper jaw longer than lower jaw. Barbel present and 4 pairs. Wide gill opening. Dorsal spine slender and comparatively weak than pectoral. It also shorter than head and serrated posteriorly. No adipose fin in adult. Body dark on back and whitish or silvery at the sides and abdomen. Lateral line present but not so conspicuous (Fig. 35). Meristic characteristics of this present study and its comparison with other studies are given in the Table 18.

**Table 18:** Meristic characteristics of ***C. garua*** in this present study and its comparison with other study.

| Characteristics | Present study | Rahman, 1989 |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/11 | I/11 |
| Pelvic fin | 6 | 6 |
| Anal fin | III, 28-29 | III, 27-30 |

## 4.8.2. Habit and Habitat

Commonly found in freshwater bodies. Niche is bottom layer of water body. Recorded in Chalan Beel of Bangladesh (Galib *et al.*, 2009).

## 4.8.3. Genetic Description of *Clupisoma garua*

A total of 641 nucleotide base pairs (Fig. 37) were obtained from the alignments of two COI gene sequences (Fig. 39) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the three sequences of *C. garua* deposited in the GenBank database from India (MG518411, MG518412, and JN628921). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 38) generated 99% of identities indicating the effectiveness

of COI sequences to provide species-level resolution. *C. garua* sequences of the present study were clustered in a single clade with the three sequences from India (MG518411, MG518412, and JN628921) submitted in the GenBank which validates the genetic identification of this species (Fig. 36). Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are ranged from 0.00 (0.0%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table 19). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 30.3%, C= 25.4%, A= 27.0%, G=17.3%.

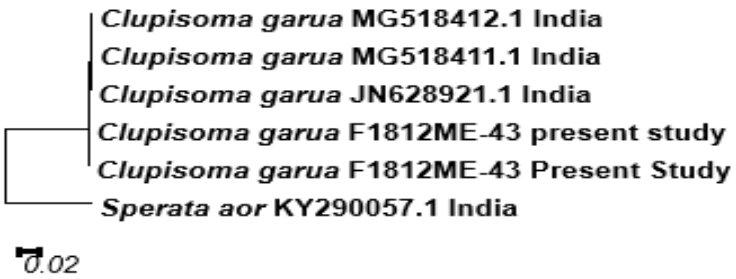



**Figure 36:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *C. garua*. The species *S. aor* was used as an out-group. Bootstrap support of >92% is shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 19:** Pairwise genetic distance among the *C. garua* individuals used to construct the phylogenetic tree.

| Sl No | Scientific Name | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | *Clupisoma_garua*_F1812ME-43_present_study | | | | |
| 2 | *Clupisoma_garua*_F1812ME-43 | 0.000 | | | |
| 3 | *Clupisoma_garua*_MG518412.1_India | 0.003 | 0.003 | | |
| 4 | *Clupisoma_garua*_MG518411.1_India | 0.003 | 0.003 | 0.000 | |
| 5 | *Clupisoma_garua*_JN628921.1_India | 0.002 | 0.002 | 0.002 | 0.002 |
| **Overall=0.002%** | | | | | |

**DNA Barcode:** *Clupisoma garua*


GGTGCCTGAGCCGGATAGTTGGCACAGCCCTTAGCCTACTAATTCGGGCAGAACTAGCCCAACCTGGTACTCTAC
TGGGCGATGACCAGATTTATAATGTTATTGTTACTGCCCATGCCTTCATCATAATTTTCTTTATAGTAATACCAATC
ATAATTGGAGGATTTGGAAATTGACTCGTTCCCCTAATGATTGGGGCACCAGACATGGCATTCCCTCGAATAAAT
AACATAAGCTTCTGATTACTACCCCCATCTTTCCTGCTACTTCTTGCCTCATCTGGAGTTGAAGCAGGAGCAGGAA
CAGGGTGAACTGTATACCCCCCTCTCGCTGGCAACCTGGCACATGCAGGAGCTTCTGTAGATTTAACTATCTTCTC
CCTCCACCTTGCTGGGGTTTCATCAATTTTAGGAGCAATTAATTTTATTACAACTATTATTAATATGAAACCCCCAG
CTATTTCACAGTATCAAACACCTCTATTTGTATGAGCCGTATTAATTACAGCCGTACTACTTCTGCTGTCTCTACCA
GTATTAGCCGCTGGGATTACAATACTACTAACAGATCGAAACCTAAATACCACATTCTTCGACCCGGCAGGGGGA
GGAGATCCAATTCTTTATCAACACCTTTTCTGATTCTTTGGCC

**Figure 37:** Illustrate of DNA Barcode of *C. garua*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Clupisoma garua from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1173 | 1173 | 98% | 0.0 | 99.84% | 655 | MK572133.1 |
| Clupisoma garua from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1168 | 1168 | 98% | 0.0 | 99.69% | 655 | MK572134.1 |
| Clupisoma garua from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1168 | 1168 | 98% | 0.0 | 99.69% | 655 | MK572131.1 |
| Clupisoma prateri voucher DUZM133.4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial | Clupisoma prateri | 1168 | 1168 | 98% | 0.0 | 99.69% | 655 | MG969517.1 |
| Clupisoma garua from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1166 | 1166 | 97% | 0.0 | 99.84% | 651 | MK572132.1 |
| Clupisoma garua from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1162 | 1162 | 97% | 0.0 | 99.69% | 652 | MK572130.1 |
| Clupisoma garua voucher CIFRI 8049K cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1162 | 1162 | 98% | 0.0 | 99.53% | 655 | MG518414.1 |
| Clupisoma garua voucher CIFRI 8049J cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1162 | 1162 | 98% | 0.0 | 99.53% | 655 | MG518413.1 |
| Clupisoma garua voucher CIFRI 8049I cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Clupisoma garua | 1162 | 1162 | 98% | 0.0 | 99.53% | 655 | MG518412.1 |

**Figure 38:** NCBI Blast search result of *C. garua*

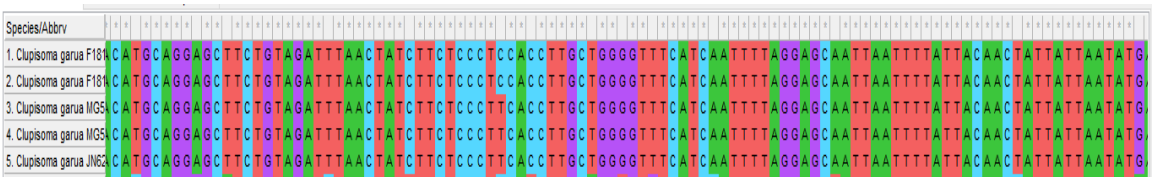



**Figure 39:** Alignment sequence of *C. garua*

# 4.9. Identification of the Species *Eutropiichthys vacha*

**Species Name:** *Eutropiichthys vacha (*Hamilton, 1822)

**English Name:** Batchwa vacha

**Local Name:** Bacha

**IUCN status:** Least Concern (LC).

**Figure 40:** Lateral view of *E. vacha*

## 4.9.1. Identifying Characteristics

Body elongated and laterally compressed. Dorsal and ventral profile almost equally convex. Upper jaw slightly longer than lower. 4 pairs of barbels. Dorsal spine serrated posteriorly and pectoral serrated internally. Adipose fin always present and caudal deeply forked. Pectoral and anal fins with reddish margin. Lateral line present and complete (Fig. 40). Meristic characteristics of this present study and its comparison with other studies are given in the Table 20.

**Table 20:** Meristic characteristics of ***E. vacha*** in this present study and its comparison with other study.

| Characteristics | Present study | Rahman, 2005 |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Second dorsal fin | - | - |
| Pectoral fin | I/13 | I/13-14 |
| Pelvic fin | 6 | 6 |
| Anal fin | III/47 | III/46-48 |

## 4.9.2. Habit and Habitat

Fresh and tidal waters; Surface feeder (Talwar and Jhingran, 1991; IUCN Bangladesh, 2000).

## 4.9.3. Genetic Description of *Eutropiichthys vacha*

A total of 618 nucleotide base pairs (Fig. 42) were obtained from the alignments of one COI gene sequences (Fig. 44) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the two sequences of *E. vacha* deposited in the GenBank database from India (MG518425 and MT812299) and three sequences from Bangladesh (KT364762 and KT165299). The assessment of species identities with previously known sequences and closely related species in NCBI BLAST (Fig. 43)

generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *E. vacha* sequences of the present study were clustered in a single clade with the two sequences from India (MG518425 and MT812299) and two sequences from Bangladesh (KT364762 and KT165299) submitted in the GenBank which validates the genetic identification of this species (Fig. 41). Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are ranged from 0.00 (0.0%) to 0.02 (0.2%) which also validates the genetic identification of this species (Table 21). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 28.8%, C= 27.2%, A= 26.1%, G=17.9%.

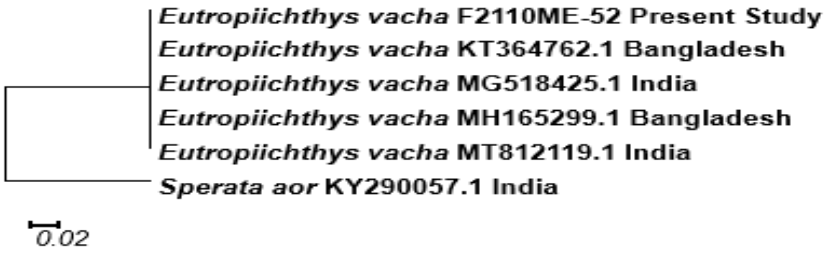
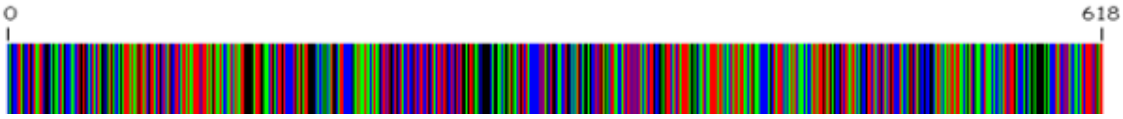



**Figure 41:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *E. vacha*. The species *S. aor* was used as an out-group. Bootstrap support of >92% is shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 21:** Pairwise genetic distance among the *E. vacha* individuals used to construct the phylogenetic tree.

| Sl No | Scientific Name | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 1 | *Eutropiichthys_vacha*_F2110ME-52_Present_Study | | | | |
| 2 | *Eutropiichthys_vacha*_KT364762.1_Bangladesh | 0.000 | | | |
| 3 | *Eutropiichthys_vacha*_MH165299.1_Bangladesh | 0.002 | 0.002 | | |
| 4 | *Eutropiichthys_vacha*_MT812119.1_India | 0.002 | 0.002 | 0.000 | |
| 5 | *Eutropiichthys_vacha*_MG518425.1_India | 0.000 | 0.000 | 0.002 | 0.002 |
| **Overall=0.001%** | | | | | |

**DNA Barcode:** *Eutropiichthys vacha*




CAGCCCTTAGCCTGCTAATTCGGGCAGAACTAGCCCAACCTGGTGCACTACTAGGCGATGACCAGATTTATAATG
TTATCGTTACTGCTCATGCCTTCATTATAATTTTCTTTATAGTAATACCAATCATAATTGGGGGATTTGGAAACTGA
CTTGTCCCCCTGATGATTGGGGCACCAGACATGGCATTCCCCCGAATAAATAACATAAGCTTCTGGCTGCTACCTC
CGTCCTTCCTGCTACTTCTTGCCTCGTCTGGAGTTGAAGCGGGGGCAGGAACAGGATGAACTGTCTACCCCCCTCT
AGCTGGCAACCTGGCACATGCAGGAGCCTCTGTAGACTTAACCATCTTCTCTCTACATCTTGCCGGAGTTTCATCT
ATTTTAGGAGCAATCAATTTTATTACAACTATTATTAACATGAAACCCCCAGCTATTTCACAATATCAAACACCATT
ATTTGTATGAGCCGTCTTAATTACAGCCGTTCTACTGCTACTGTCCCTACCAGTGCTAGCCGCCGGCATTACAATAT
TATTAACAGATCGAAACCTAAACACCACATTCTTTGACCCAGCAGGAGGGGGAGACCCTATCCTCTACCAACATCT
TTTCTGATT

**Figure 42:** Illustrate of DNA Barcode of *E. vacha*


**Figure 43:** NCBI Blast search result of *E. vacha*

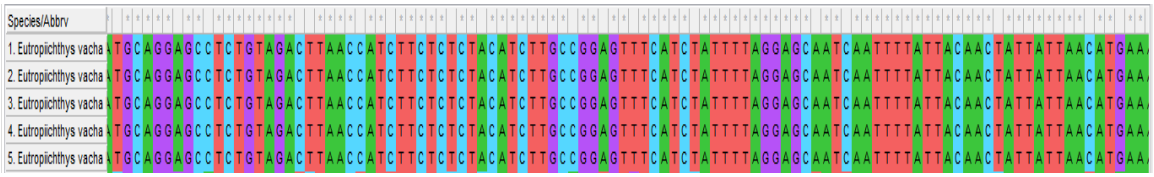



**Figure 44:** Alignment sequence of *E. vacha*

# 4.10. Identification of the Species *Silonia silondia*

**Species Name: S***ilonia silondia* (Hamilton,1822)

**English name:** Silond catfish

**Local name:** Shillong

**IUCN status:** Least Concern (LC).

**Figure 45:** Lateral view of *S. silondia*

## 4.10.1. Identifying Characteristics

Body elongated and deeply compressed. Mouth terminal and lower jaw little longer. Snout broad and rounded. Eyes with narrow adipose lids. Barbels 2 pairs. Dorsal spine comparatively weak than pectoral spine and both spines fin serrated posteriorly. The body color is yellowish-green on back, silvery purple on flanks and abdomen, golden tinge present on both sides of head. Caudal, anal and pelvic bases yellowish (Fig. 45). Meristic characteristics of this present study and its comparison with other studies are given in the Table 22.

**Table 22:** Meristic characteristics of *S. silondia* in this present study and its comparison with other study.

| Characteristics | Present study | Reference (Range) |
|---|---|---|
| First dorsal fin | I/7 | I/7 |
| Pectoral fin | I12 | I/11-13 |
| Pelvic fin | 6 | 6 |
| Anal fin | 45 | 40-46 |
| Caudal fin | 27 | 17-22 |

## 4.10.2. Habit and Habitat

Commonly found in estuaries and rivers (mainly fluviatile) but also be alive in tanks and large reservoirs (IUCN Bangladesh, 2000). Inhibits estuaries and rivers throughout Bangladesh (Rahman, 2005). Not found in closed waters (Shafi and Quddus, 2001).

## 4.10.3. Genetic Description of *Silonia silondia*

A total of 627 nucleotide base pairs (Fig. 47) were obtained from the alignments of one COI gene sequence (Fig. 49) after removing the ambiguous sequences near primer ends. For molecular identification, at first, checked the COI barcode sequence of collected fish samples from this study using the BLAST search tools provided in the GenBank. The query sequence showed high identity (> 99%) with the one sequences of *S. silondia* deposited in the GenBank database from India (MG518429) and three sequences from Bangladesh (OK103949 and MN259178). The assessment of species identities with previously known

sequences and closely related species in NCBI BLAST (Fig. 48) generated 99% of identities indicating the effectiveness of COI sequences to provide species-level resolution. *S. silondia* sequences of the present study were clustered in a single clade with one sequence from India (MG518429) and two sequences from Bangladesh (OK103949 and MN259178) submitted in the GenBank which validates the genetic identification of this species (Fig. 46) Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are ranged from 0.00 (0.0%) to 0.02 (0.2%) which also validates the genetic identification of this species (Table 23). The total number of nucleotide (A, T, G, C) compositions among the sequences of the present study have been observed. The average nucleotide composition was T= 29.9%, C= 26.5%, A= 26.2%, G=17.3%.

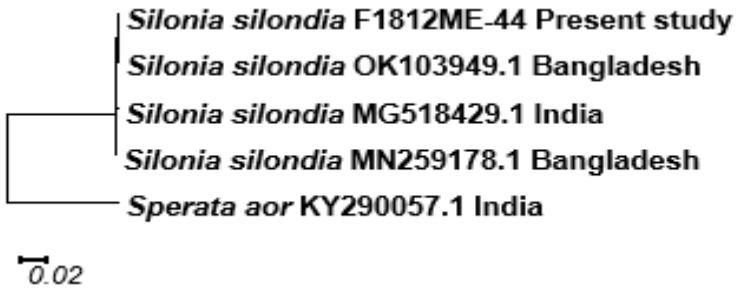
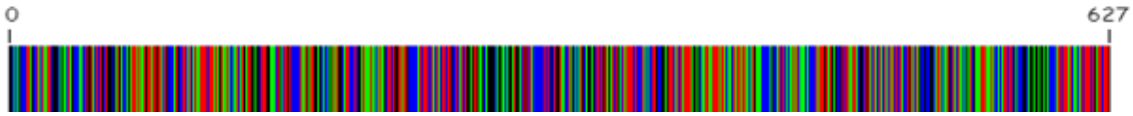



**Figure 46:** Neighbour-joining phylogenetic tree constructed based on mitochondrial COI barcode sequences of *S. silondia*. The species *S. aor* was used as an out-group. Bootstrap support of >92% is shown above branches. The scale bar indicates nucleotide substitutions per site.

**Table 23:** Pairwise genetic distance among the *S. silondia* individuals used to construct the phylogenetic tree.

| Sl No | Scientific Name | 1 | 2 | 3 |
|---|---|---|---|---|
| 1 | *Silonia_silondia*_F1812ME-44_Present_study | | | |
| 2 | *Silonia_silondia*_OK103949.1_Bangladesh | 0.000 | | |
| 3 | *Silonia_silondia*_MN259178.1_Bangladesh | 0.003 | 0.003 | |
| 4 | *Silonia_silondia*_MG518429.1_India | 0.002 | 0.002 | 0.002 |
| **Overall=0.002%** | | | | |

**DNA Barcode:** *Silonia silondia*




GGCACAGCCCTTAGCCTACTAATTCGGGCAGAACTAGCCCAACCTGGTACTCTGCTGGGCGATGACCAGATTTAT
AATGTTATTGTTACTGCCCATGCCTTCATCATAATTTTCTTTATAGTAATACCAATCATGATTGGAGGCTTTGGAAA
CTGACTTGTCCCCCTAATGATTGGAGCACCAGATATGGCATTCCCTCGGATAAATAACATAAGCTTCTGGTTATTG
CCCCCATCTTTCCTACTACTTCTTGCCTCATCTGGAGTTGAAGCAGGGGCAGGAACAGGATGGACTGTCTACCCCC
CTCTCGCTGGTAACCTAGCACATGCAGGAGCTTCTGTAGATCTGACTATTTTCTCCCTACACCTTGCCGGAGTTTCA
TCTATTTTAGGAGCAATTAATTTTATTACAACTATTATTAACATGAAACCCCCAGCCATCTCACAATATCAAACACC
CCTATTTGTATGAGCCGTCTTAATTACGGCTGTACTACTACTACTATCTCTACCTGTACTAGCCGCCGGCATTACAA
TGCTACTAACAGATCGAAATTTAAATACCACATTCTTCGACCCGGCAGGAGGAGGAGACCCCATTCTTTATCAACA
TCTTTTCTGATTCTTTG

**Figure 47:** Illustrate of DNA Barcode of *S. silondia*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Silonia silondia isolate E04 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Silonia silondia | 1146 | 1146 | 99% | 0.0 | 99.68% | 633 | MN259178.1 |
| Silonia silondia from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Silonia silondia | 1136 | 1136 | 98% | 0.0 | 99.84% | 655 | MK572596.1 |
| Silonia silondia from Bangladesh cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | Silonia silondia | 1136 | 1136 | 98% | 0.0 | 99.84% | 655 | MK572595.1 |
| Silonia silondia voucher CIFRI 8240E cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Silonia silondia | 1136 | 1136 | 98% | 0.0 | 99.84% | 655 | MG518430.1 |
| Silonia silondia voucher CIFRI 8240D cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Silonia silondia | 1136 | 1136 | 98% | 0.0 | 99.84% | 655 | MG518429.1 |
| Silonia silondia voucher CIFRI 8240B cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Silonia silondia | 1136 | 1136 | 98% | 0.0 | 99.84% | 655 | MG518427.1 |
| Silonia silondia voucher CIFRI 8240A cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Silonia silondia | 1136 | 1136 | 98% | 0.0 | 99.84% | 655 | MG518426.1 |
| Silonia silondia voucher CIFRI 8240C cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | Silonia silondia | 1131 | 1131 | 98% | 0.0 | 99.68% | 655 | MG518428.1 |

**Figure 48:** NCBI Blast search result of *S. silondia*

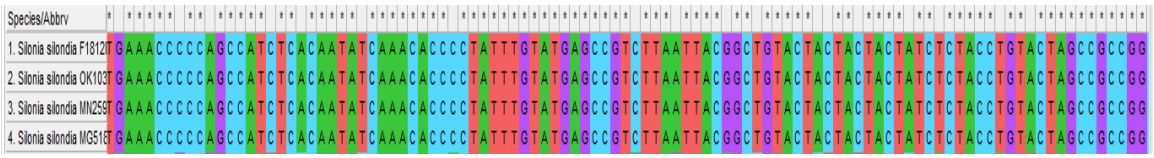



**Figure 49:** Alignment sequence of *S. silondia*

## 4.11 Overall genetic description of studied sequences

Combined morphological and molecular analyses confirmed a total of 9 fish species belonging to 2 families and 8 genera. A total of 10 COI sequences of these species were obtained in the study. The consensus length after editing all COI barcode sequences was larger than 600 bp, and no stop codons and insertions were observed in any of the sequences.

In the phylogenetic tree, COI barcode sequences of each species discriminated against all other species and clustered the similar species under the same nodes with significant bootstrap values 80–100% (Fig. 50 and table 24). The assessment of species identities with previously known sequences and closely related species in BLAST and BOLD databases

generated 98–100% identities indicating the effectiveness of COI sequences to provide species-level resolution. The genetic distances among the studied sequences ranged from 0.00% to 22%.

The COI sequences (10 sequences) obtained from 9 species comprised 10 haplotypes with 126 polymorphic sites. The nucleotide diversity was calculated as 0.16 ± 0.05 (mean ± SD) and the haplotype diversity was 0.95 ± 0.06 (mean ± SD) for the COI sequences obtained in the present study. Parsimony informative sites of two, three, and four variants were 52, 31, and 14.

The mean nucleotide base compositions were calculated as A= 26.3%, T= 30.1%, C= 26.3%, and G=17.3%. The base composition analysis for the COI sequence showed that the average T content was the highest and the average G content was the lowest; the GC content was 44.3%. The overall mean distance of the sequences was 17%.

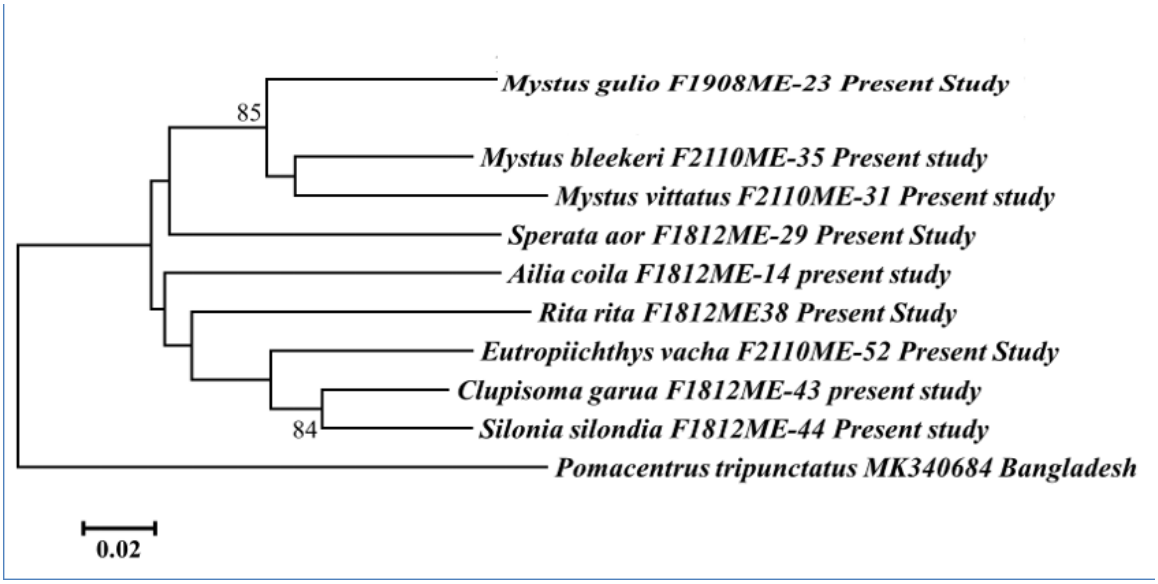



**Figure 50:** Neighbor-joining phylogenetic tree for COI gene sequences of collected fish species in the present study. Bootstrap support of >80% is shown above branches. Scale represents substitution rate per site. *Pomacentrus tripunctatus* (MK340684 Bangladesh) was used as outgroup.

**Table 24:** Genetic distance among all of the studied sequences.

| Sl No | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Ailia_coila*_F1812ME-14 | - | | | | | | | |
| 2 | *Clupisoma_garua*_F1812ME-43 | 0.16 | | | | | | | |
| 3 | *Eutropiichthys_vacha*_F2110ME-52 | 0.19 | 0.10 | | | | | | |
| 4 | *Mystus_bleekeri*_F2110ME-35 | 0.20 | 0.18 | 0.17 | | | | | |
| 5 | *Mystus_gulio*_F1908ME-23 | 0.20 | 0.18 | 0.18 | 0.13 | | | | |
| 6 | *Mystus_vittatus*_F2110ME-31 | 0.22 | 0.19 | 0.20 | 0.12 | 0.16 | | | |
| 7 | *Rita_rita*_F1812ME38 | 0.20 | 0.17 | 0.18 | 0.20 | 0.21 | 0.21 | | |
| 8 | *Silonia_silondia*_F1812ME-44 | 0.17 | 0.08 | 0.12 | 0.17 | 0.20 | 0.20 | 0.20 | |
| 9 | *Sperata_aor*_F1812ME-29 | 0.19 | 0.19 | 0.22 | 0.16 | 0.20 | 0.20 | 0.20 | 0.21 |

# CHAPTER V

# SUMMARY AND CONCLUSION

In the present study, we have identified 9 siluriformes species through morphology and DNA barcoding. The assessment of species identities with previously known sequences and closely related species in NCBI BLAST generated 99% identities indicating the effectiveness of COI sequences to provide species-level resolution.

Genetic distances among the sequences of *M. bleekeri* of the present study and its compared sequences from India and Bangladesh ranged from 0.002 (0.2%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table **7**). Genetic distances among the sequences of *M. gulio* of the present study and its compared sequences from India and Bangladesh ranged from 0.00 (0.0%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table 9). Genetic distances among the sequences of *M. vittatus* of the present study and its compared sequences from India and Bangladesh ranged from 0.002 (0.2%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table 11). Genetic distances among the sequences of the present study and its compared sequences from India and Bangladesh are 0.00 (0.0%) which also validates the genetic identification of this species (Table 13). Genetic distances among the sequences of *Sperata aor* the present study and its compared sequences from India and Bangladesh are 0.00 (0.0%) which also validates the genetic identification of this species (Table 15). Genetic distances among the sequences of *A. coila* of the present study and its compared sequences from India and Bangladesh are 0.00 (0.0%) which also validates the genetic identification of this species (Table 17). Genetic distances among the sequences of the *C. garua* of the present study and its compared sequences from India and Bangladesh are ranged from 0.00 (0.0%) to 0.003 (0.3%) which also validates the genetic identification of this species (Table 19). Genetic distances among the sequences of *E. vacha* of the present study and its compared sequences from India and Bangladesh are ranged from 0.00 (0.0%) to 0.02 (0.2%) which also validates the genetic identification of this species (Table 21). Genetic distances among the sequences of *S. silondia* of the present study and its compared

sequences from India and Bangladesh are ranged from 0.00 (0.0%) to 0.02 (0.2%) which also validates the genetic identification of this species (Table 23).

The present study suggested that siluriformes species were sometimes difficult to differentiate morphologically due to key features being quite similar, but their identification was confirmed by the molecular analysis results. Some species of siluriformes fishes of the present study had phenotypic variation in their external features in different stage of life cycle, which seems as different species. However, these individuals were found as same species by DNA Barcoding. That's why, combination of both morphological and genetic analyses are highly recommended for identification of siluriformes species. So, DNA barcode can be used for the rapid analysis for identification of siluriformes species. The present study can make a contribution in the fishery management and conservation of the species of Bagridae and Schilbeidae in Bangladesh.

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