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MISLABELING OF JUVENILE *Tenualosa* spp. AS *gudusia chapra* IN THE FISH MARKETS AND RIVERS OF BANGLADESH

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ABSTRACT

Juveniles ilish, *Tenualosa* spp. Fowler, 1934 named Jhatka mislabeling is a documented problem that has significant effects on consumers' demand, resource limitation, and complex supply chain. When jhatka is sold as chapila, then its correct scientific/accepted name, jhatka fake permits unlawfully caught species entering the market. The result of fraudulent activity included consumers' losses, illegal harvesting, and more ilish resource degradation. These fraudulent activities must be defined. This paper examines the mislabeling of ilish at different stages, such as harvesting, market, and consumers level. Both morphomeristic and molecular analyses were used to identify the fishes. The samples were collected from different types of markets and rivers. Mislabeling at different stages of the supply chain, including illegal harvesting of jhatka has been identified. Circulating mislabeling records could also encourage law-enforcement agencies, fishermen, sellers, and consumers to more closely assess.

Keywords: DNA barcoding; ilish; supply chain

INTRODUCTION

Ilish *(Tenualosa* spp.) Fowler, 1934 is the national fish of Bangladesh, which is a delicious and commercially important fish in the different parts of Asia. Juvenile ilish named jhatka (up to 25 cm size locally named as Jatka) mislabeling is a problem in Bangladesh because it causes underreporting of species exploitation and has detrimental effects on conservation. Finally, Jhatka harvesting is one of the primary causes of ilish declination in Bangladesh. In Bangladesh, jhatka harvesting, catch, transportation, marketing, selling, and possessing have been banned from 1st November to 31st March every year (Protection and Conservation of Fish Act, 1950). Bangladesh government implements an ilisha fishery protection campaign for a certain period of every year in different months named "Operation Maa Ilish Rokksha" during peak breeding time of ilish and "Operation Jhatka" program for the peak season of Jhatka production to protect and increase the production of ilish fish in Bangladesh.

Furthermore, the Ministry of Fisheries and Livestock Act, as a leading agency, included with other different government organizations named local administration, NAVY, Air force, coast guard, police, Rapid Action Battalion (RAB), riverine police and Department of Fisheries (DoF) strongly involve implementing the campaign program every year. The objective of the campaign program is to awareness building of the ilish fisheries, the enforcement of laws and regulations, and the conservation of jhatka and mother ilish fish. Conversely, buyers and controllers impact the protection of ilish fish by monitoring. Selectively buying sustainably collected species of consumers affects the protection of fishes. Mislabeling has hindered this power and led to fish species' unexpected consumption. Here, we explore the consequences of such labeling incorrectness for best commercial and national/international popular fish ilish by DNA barcoding and morphologically.

Jhatka is available in rivers and coastal areas more or less year-round. Still, the period of highest abundance for harvesting is January to April, and sometimes it may extend up to May. In general, Jhatka looks like chapila (the adult Indian river shad) *Gudusia*

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chapra (Hamilton, 1822). Therefore, it is not easy to implement the Fisheries Act at the market for law-enforcement agencies. Conversely, fishermen, as well as people in business, mixed Jatka with chapila and sardines. Jatka is mostly mislabeled with closely related species like Chapila or Sardines, which have almost the same morphological structures. Because of the high demand for ilish fish, it is mostly mislabeled by morphologically alike Chapila fish in different fish markets.

The Chandpur District fisheries office said that despite a two-month ban in Chandpur, the fisherman continues to catch jatka in the Padma and Meghna rivers. In March 2020, the mobile court held sessions for jatka fishing. The authority conducted 223 drives and confiscated 6,919 Kg of Jatka. At the local market, a kilogram of jatka sells for tk 120. 12 to 14 thousand MT of Jatka was harvested from Bangladesh in 2017-2018 (interview with Principal Scientific Officer, Bangladesh Fisheries Research Institute (BFRI), Chandpur station). When law enforcement agencies are confronted with fishers, often they showed undesirables disagreement with them, puzzling that the fish are chapila. As a result, it creates vast community misperceptions and clashes as they repeatedly fail to differentiate between Jatka and Chapila. However, ilish (*Tenualosa* spp.) is the most vital marketable fish in Bangladesh, and a Geographical Indication (GI) Registration Certificate has been achieved for our national fish ilish. However, its production is obstructed for several reasons, such as overfishing of ilish and mother ilish, harvesting Jatka, water pollution, siltation of rivers, etc. If the Bangladesh government is able to stop or reduce Jatka harvesting from rivers, then it will enhance the country overall ilish production.

Consequently, the fishers livelihood with the Ilisha fishery will improve and consumers will get fish at a reduced price. Securing livelihoods of 11 percent of the total population of Bangladesh by involved in this sector on a full and part-time basis. National fish ilish as a single species has been making the highest contribution (around 12 percent) to the country's total fish production. Ilish production is 5.17 lakh MT in FY 2017-18 (DoF, 2018). Therefore, the current study's key objectives are to differentiate those fish species and protect ilish fish.

DNA barcode is an important tool for identifying any species, based on comparing the DNA barcode of the specimen to the DNA barcode of known species. DNA barcoding is a method of species identification using a short section of DNA relative to the entire genome and they can be found rationally, rapidly, and economically. The standard barcode region (648 nucleotide base pairs long) for higher animals is developing by the cytochrome C oxidase subunit 1 mitochondrial region (COI) method. DNA barcoding to identify fish has been used in several studies (Ward *et al.,* 2005; Smriti *et al.,* 2017). Furthermore, using morphometric & meristic characters and mitochondrial DNA sequence methods (John, 2009) were applying to resolve the taxonomic ambiguity of Punti fish, *Sahyadria denisonii* (Day, 1865) and *S. chalakkudiensis* (Menon, Rema Devi & Thobias, 1999).

Separately based on few morphometric and meristic characters of these two species has already been done (Day, 1880; Pillay *et al.,* 1957; Chondar, 1976; Jayaram, 1981; Whitehead, 1985; Rahman, 1989; Najero *et al.,* 2008; Smriti *et al.,* 2017). In the present study, both morphometric and molecular analysis was used to identify the fishes not only for differentiation but also for conservation purposes and will ultimately build awareness among the people from being fraudulence of buying mislabeled fishes and governmental staffs. Fish fake ultimately cheats consumers who fall victim to a bait and hurts honest fishermen and fish businesses.

MATERIALS AND METHODS

Sampling schedule and sites

Fresh twenty fish samples as chapila were collected from different habitats and markets are shown in Figure 1 and Table 1. Sample fishes were collected from the main river named the Meghna River (CCF) which were confiscated from fishermen by Coast Guard. Fishermen have argued with coast guard patrol peoples that it is chapila, not jatka fish. Tertiary river named Buriganga (BLF) direct from fisherman while they were catching fishes, three wholesale fish markets (where trade among fishermen and fish merchants and fish retailer) like Jatrabari (JNF), Kawran bazar (MF), Suarighat (SEF) bazar from aratdar and three retailer fish markets (where consumers direct buy fishes) like Rampura (RSF), Khilgoan-taltola (TCF), Hatirpul (EF) bazar from retailer at early morning in an icebox with sufficient ice. The specimens were preserved in a cool box and transferred at -20 ! deep freeze in the Fisheries Laboratory, Department of Zoology, Jagannath University, Dhaka until further study. All specimens were kept in the museum of the Zoology Department, Jagannath University as voucher specimens until completing the study.

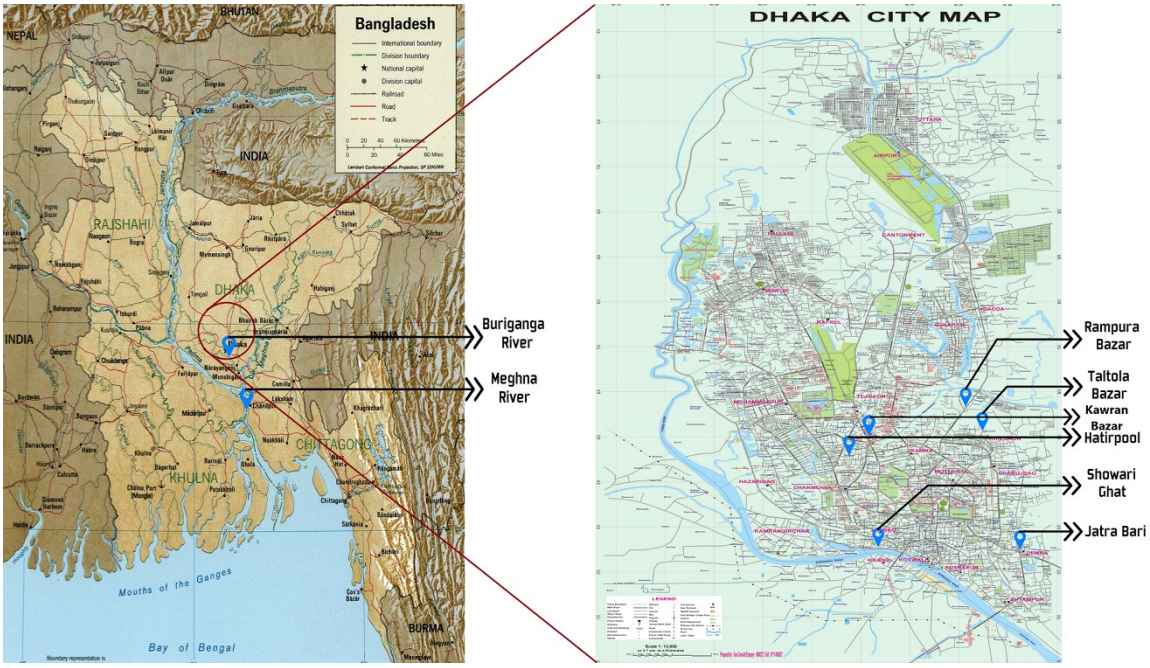

Figure 1. Sampling sites in the two rivers (left) and six markets (right).

Table 1. Sampling schedule, habitat, site and sampling name with number, code and sampling picture.

Sl no	Sampling date	Types of sampling sites	Name of sampling site	Sample name	Number of fish individual	Voucher specimen code	Sampling picture
01.	02.02.2015	Main River	Meghna River (Chandpur)	Chapila	03	CCF	
02.	23.02.2015	Tertiary River	Buriganga River	Chapila	03	BLF	
03.	18.02.2015	Whole sale Fish Market	Jatrabari Bazar	Chapila	01	JNF	
04.	19.02.2015		Kawran Bazar	Chapila	02	MF	
05.	18.02.2015		Suarighat Bazar	Chapila	03	SEF	
06.	18.02.2015	Retailer Market	Rampura Bazar	Chapila	02	RSF	
07.	13.04.2015		KhilgoanTaltola Bazar	Chapila	03	TCF	
08.	11.03.2015		Hatirpul Bazar	Chapila	03	EF	

Taxonomic procedure

Morphomeristics

The fish length was measured in centimeter to the nearest 0.01, and weight was measured in gram. Morphometrics and meristics methods were similar to those described by Allen and Talbot (1985). A total of eight meristic and 20 morphometrics characters were considered, and some descriptive characters such as body and fin coloration were observed. The morphomeristics study was carried out in the Fisheries Laboratory, Department of Zoology, Jagannath University, Dhaka, Bangladesh.

Molecular approaches

The molecular experiment was carried out in the Zoology Section, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. For the molecular study, 8 fish individuals were selected, including a single individual from each of the two rivers (CCF and BLF), five wholesale fish markets (JNF, MF, TCF, RSF, and SEF), and one retailer fish market (EF).

For each sample, about 20-100 mg of tissue was collected from the selected part (below dorsal fin) of fish with a sterile scalpel. Genomic DNA was extracted by using DNA extraction kit (Promega, USA) and phenol-chloroform method (Sambrook *et al.,* 1989) using Lysis buffer, proteinase K, Phenol: Chloroform: Isoamyl alcohol (25:24:1), Chloroform: Isoamyl alcohol (24:1), Absolute ethanol, 70% ethanol, and TE buffer. The extracted DNA was measured by two methods- Gel electrophoresis and UV-Spectrophotometry (Nanodrop spectrophotometer ND-2000, Thermo Scientific, USA). To amplify the target DNA segment (COI gene), PCR master mix with template DNA and specific primer for fish species was run in PCR thermal cycler following the cycle, initialization step consists of heating the reaction to a temperature of 94°C-96°C is held for 1-9 min. The denaturation step is the first regular cycling event and consists of heating the reaction to 94°C-98°C for 22-30 seconds. In the annealing, step temperature is lowered 50°-65°C for 20-40 seconds allowing annealing of the primers to the single-stranded DNA template. The extension step commonly used a temperature at 72°C to synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template 5'-3' direction. The final elongation step is occasionally performed at a temperature of 70-74°C for 5-15 mins for fully extended and the final hold step at 4-15°C for an indefinite time may be employed for the short-term storage of the reaction. After that, the PCR product was checked by running agarose gel electrophoresis and banding pattern was used for qualitative and quantitative analysis. Approximately 655 bp were amplified from the COI in mitochondrial DNA using different combinations of two newly designed primers (Ward *et al.,* 2005) Fish F1: TCAACCAACCACAAAGACATTGGCAC and Fish R1: TAGACTTCTGGGTGGCCAAAGAATCA (primer 1), Fish F2: TCGACTAATCATAAAGATATCGGCAC and Fish R2: ACTTCAGGGTGACCGAAGAATCAGAA (primer 2). Following PCR amplification, the COI PCR product was cleaned up by PCR purification Kit. For samples showing clean, discrete PCR products proceeded directly to sequencing. The purified products were labeled using the Bio Dye Terminator v.3.1 cycle sequencing kit (Sanger sequencer, Model: 3031 Genetic Analyzer) in a 39 total reaction mixture of 10 ìl containing 4.94 ìl of nanopure water,1.94 ìl of 5x Big Dye buffer, 2 ìl MI3F or MI3R,0.12 ìl of Big Dye terminator and 1 ìlExo SAP product. In this study, the sequencing has been done from First BASE Laboratories SdnBhd No. 7-1 to 73, Jalan SP 2/7, Taman Serdang Perdana, Seksyen 2, 43300 Seri Kembangan, Selangor, Malaysia. PCR amplification and sequencing of the barcode segment (COI gene) of target fish species were followed by the analysis of the sequence based on Bioinformatics tools - Chromas Lite and Geneious R8. For sequence analysis, Chromas Lite was used to viewing the chromatogram figure and the sequence data were transferred to FASTA format. All sequences were proofread and assembled using the software SeqMan (DNAStar, USA). All sequences were blasted within the nucleotide database for the authentication of the morphological identification at the National Centre for Biotechnology Information databases (NCBI) to determine the highest homology and thus to identify the species. The software MEGA 6.0 (Tamura *et al.,* 2013) was used to form the Neighbor-joining (NJ) tree based on the Kimura 2 parameter model (K2P) and 1000 bootstrap replications.

Supply chain

There are different types of customers as well as sellers in the wholesaler and retailer market. These customers can receive fish directly from fishermen or from other vendors/suppliers. Consumers receive fish from sellers in the retails market.

RESULTS AND DISCUSSION

Results

Fraud labeling was investigated as a role of wholesaler and retailer fish markets within the capitals

and rivers where the number of samples was collected. However, In the present study, 20 fish samples from 6 local different types of fish markets and 2 river sites (SEF, JNF, RSF, TCF, MF, EF, CCF, and BLF) were collected and showed consistency in both morphological and molecular investigation.

Morphological identification

The morphometric and meristic characteristics of these fishes are given in Table 2. According to morphological and meristic analysis, collected fishes from CCF, JNF, RSF, BLF, and EF sites were matched to *Tenualosa ilisha* (Hamilton, 1822), SEF site matched to *Tenualosa toli* (Valenciennes, 1847), and TCF and MF sites matched to *Gudusia chapra*.

Molecular identification of sample fish

Blast results of COI gene sequences

Among 8 sequenced samples, 4 different species were identified after the blast in the NCBI reference database (Table 3). Among 7 samples amplified by primer-1, three samples were detected as *T. ilisha* (CCF, JNF, and BLF). Two samples were detected as *G. chapra* (MF and TCF). One was *T. toil* by molecular identification (SEF). Another one was also *T. ilisha* (EF) but less similarity within the species. RSF was not identified by primer-1 (Table 3). Among 7 samples amplified by primer-2, two samples were detected as *T. ilisha* (CCF and JNF) and one was contaminated (BLF) (Table 3). Two samples were detected as *G. chapra* (MF and TCF). One was *T. toil* by molecular identification (SEF). Only one sample was *Sardinella jussieu* (Lacepède, 1803) (RSF). EF was not identified by primer-2 but morphometrically identified as *T. ilisha* (Table 1, 2, 3).

Table 2. The morpho-meristic measurements of the collected fish sample from six markets and two rivers.

Morphomeristics Variables	CCF (*T. ilisha*)	SEF (*T. toli*)	JNF (*T. ilisha*)	RSF (*T. ilisha*)	BLF (*T. ilisha*)	TCF (*G. chapra*)	EF (*T. ilisha*)	MF (*G. chapra*)
Total length	20.4	18.5	17.5	15.1	8.8	10.3	11.2	14
Fork length	17	15.8	14.2	12.3	7.3	8.7	10.3	12.2
Standard length	16	13	12.1	10.3	5	5.1	8.5	11
Predorsal length	6.6	6.6	6.6	4	2.9	4	3.9	5.8
Head length	4.2	3.2	4.3	2	1.6	1.6	2.5	3.5
Preorbital length	0.9	1.3	0.9	0.9	0.4	0.3	0.6	0.7
Post orbital length	2.5	1.3	2.6	1.5	0.8	0.8	1.2	1.8
Eye diameter	0.8	1.1	0.7	0.6	0.3	0.5	0.5	o.8
Body depth	1.8	1.7	4.1	2.5	0.6	0.8	0.7	1.2
Dorsal fin base	2.1	1.8	2.4	1.2	1	1	1.3	1.6
Peduncle depth	0.7	0.4	0.6	1	0.2	0.3	0.2	0.5
Peduncle length	1.1	0.8	1	0.9	0.5	0.7	0.5	1.1
Length of upper jaw	1.9	1.8	1.9	0.8	0.9	0.7	1.4	1.3
Length of lower jaw	1.5	1.6	1.7	0.9	0.8	0.8	1.1	1.6
Jaw gape	1.3	0.7	1.3	0.3	0.7	1	1.3	1.6
Pectoral fin base	2.5	1.4	2.6	1.5	1	1.3	1.5	1.3
Pelvic fin base	1.5	0.7	1.6	1	0.7	0.8	1	1.2
Anal fin base	2.1	3.8	2.2	2	1	1	1.3	2.2
Length of caudal fin	1.5	1.3	1.5	1.8	0.7	0.6	1	1.2
Dorsal fin ray	19	17	19	19	17	13	19	15
Pectoral fin ray	15	14	16	14	12	12	16	12
Pelvic fin ray	8	8	8	7	8	8	8	7
Anal fin ray	24	21	23	18	21	24	23	24
Branchiostegal ray	5 pair	5 pair	5 pair	4 pair	5 pair	6 pair	5 pair	6 pair
Scutes	31	29	30	32	32	27	25	26

Table 3. Molecular identification of samples using DNA barcoding with conventional morphological identification.

Sample ID	Morphological Identification	Molecular Identification (Primer-1)	Identity by NCBI blast result (%)	Molecular Identification (Primer-2)	Identity by NCBI blast result (%)
1. CCF	*T. ilisha*	*T. ilisha*	99%	*T. ilisha*	99%
2. SEF	*T. toli*	*T. toil*	99%	*T. toli*	99%
3. JNF	*T. ilisha*	*T. ilisha*	99%	*T. ilisha*	99%
4. RSF	*T. ilisha*	Not Identified	-	*S. jussieu*	91%
5. BLF	*T. ilisha*	*T. ilisha*	99%	Not Identified	-
6. TCF	*G. chapra*	*G. chapra*	100%	*G. chapra*	100%
7. EF	*T. ilisha*	*T. ilisha*	94 %	Not Identified	-
8. MF	*G. chapra*	*G. chapra*	100%	*G. chapra*	100%

Neighbor-Joining tree constructed using COI gene

Total 9 COI sequences among *T. ilisha* (JNF1, CCF1, BLF1 by primer 1 and CCF2 by primer 2), 4 for 2 individuals of *G. chapra* (MF1, TCF1 by primer 1 and MF2, TCF2 by primer 2) and one for a single individual of *T. toli* (SEF2 by primer 2) were selected as good sequences during the present study and submitted to GenBank with accession number (MW286124- MW286132). Three conspecies sequences were downloaded from GenBank and their accession number provided in the associated figure. Intraspecies genetic distances for *T. ilisha*, *T. toli,* and *G. chapra* ranged from 0.000 to 0.004 and interspecies distances ranged from 0.166 to 0.254, the threshold of species delimitation (0.035) distant exceeding (Ward *et al.,* 2005; Ward *et al.,* 2009) based on the metric of 10× the average intra-species genetic variation (Hebert *et al.,* 2004). The NJ tree based on COI gene sequences (Figure 2) revealed that the three different species *T. ilisha, T. toli,* and *G. chapra* formed monophyletic groups with reference sequences from NCBI of each.

Discussion

It is clear that fish fake continue to be a problem in Bangladesh, and our government needs to do more to tackle this once and for all. Fish fake ultimately cheats consumers who fall victim to a bait and hurts honest fishermen and fish businesses. It is critical to certify that all chapila sold in the fish market is honestly labelled. However, the present study was to differentiate Jatka from chapila to identify mislabeling, which has been randomly mixed with chapila mostly in the market and where fisherman catches such fish. Molecular analysis has been utilized for many years for fish species identification. Initially, allozyme differences were used (Avise, 1989), followed by mtDNA examination (Avise, 1994). DNA barcoding is becoming an increasingly popular method for identifying animal species (Hebert *et al.,* 2003; Costa and Carvalho, 2007). The differentiated four species of tuna (*Thunnus* spp. South, 1845) were identified by mtDNA sequencing (Bartlett and Davidson, 1991). The results of the present investigation clearly indicate that DNA barcoding is a dominant method and correctly detecting collected samples of different sources such as vender, fish markets, or rivers as different species instead of mislabeled chapila. Phylogenetic tree reconstruction methods such as NJ were used to justify the result of DNA barcode sequences. NJ tree was constructed for understanding the distance relationship among the sampling species. In the present study, *T. ilisha*, *G. chapra,* and *T. toli* had a considerable distance relationship. Therefore, this relationship confirmed the presence of different species mislabeled as one species. The samples, except for 5 individuals of *G. chapra* (TCF and MF) collected from different sites were mislabeled, with one species named Jatka (*T. ilisha*) being sold as chapila. We collected all the fish samples as a name of chapila but after morphological and DNA barcoding study we found 3 different species among them, most of the individuals were Jatka (ilish) which was mislabeled with chapila. So, a substantial amount of jatka are mislabeled for trading every day and it

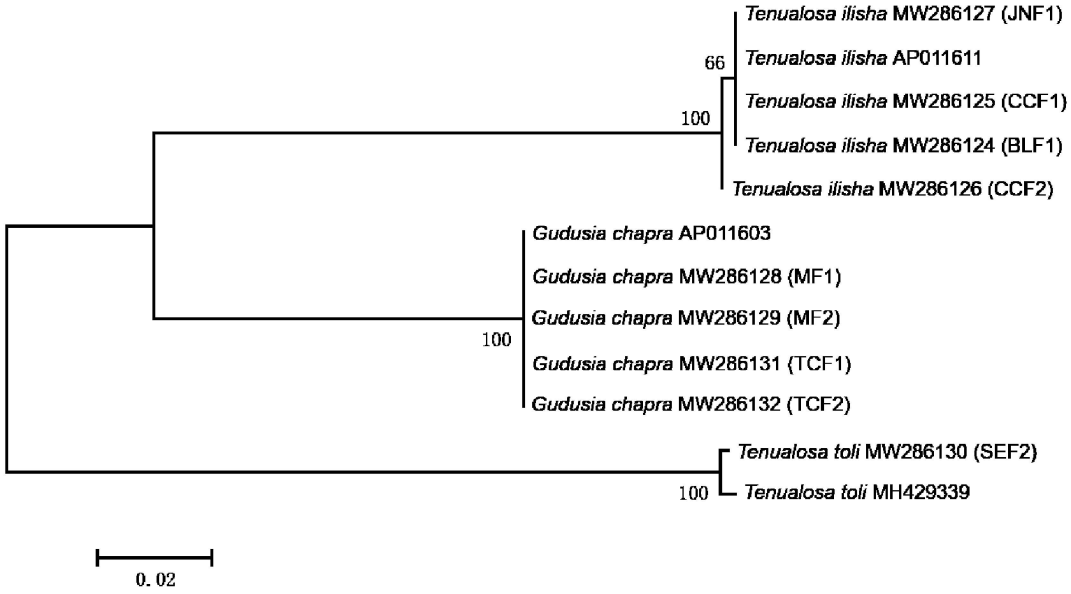

Figure 2. The neighbor-joining tree was constructed using the K2P model for 12 COI gene sequences of *T. ilisha*, *T. toli*, and *G. chapra*.

causes great loss to the economy of our country.

The most common substitute species for Jatka (*T. ilisha*) was common chapila in the wholesale and retailer market, tertiary and the main river has been reported. Furthermore, the vast majority of the customers/consumers could not distinguish jhatka from chapila. Our study measures mislabeling by fisherman and seller or consumers at a different fish market have been confirmed. Furthermore, the majority of exchanges recognized in our samples were, on average, fewer costly and apparently less wanted alternatives to jhatka. These results suggest a financial motivation because the alternative signifies lower-rated replacements.

During the "Jatka Operation", the Coast Guard seized the harvested jhatka, and the setting of the current jal (net) from the Meghna rivers indicates that undersized ilish fish is harvested. Yet, it violated the Protection and Conservation of Fish Act 1950. Furthermore, this result also indicates that the illegal setting of nets and the misreporting of the catch were confirmed in those habitats. This activity also indicates that a few fishermen still do not respect the Fishery Act 1950.

Ilish is transported through one or further transitional steps and later offers several chances for the legally and illegally sourced fish mixing, where the unlawful jhatka are basically legalized and later move into general trade as a lawful product. Considering the opportunity, jhatka mislabeling is significant for customers, fisheries administrators, and the ilish fish supply chain. In the present study, the result of the neighbor-joining tree clearly indicated the separation of the different roots of commonly called chapila/Jhatka in our local trade which badly impacts the future stock of our royal fish ilish. Selling and purchasing such fish species established severe financial fakes, and the consequences raised the unlawful dealing of our national fish from both economic and management topics of vision.

Circulating mislabeling records may motivate fishermen, sellers, and consumers to check that suppliers offer the right product. Therefore, controlling quality and identifying the species frequently traded in our country is vital.

CONCLUSION

Generally, we have purchased lots of Jhatka fish, such as so-called Chapila from different fish markets or vendors, which is wrong, as proved by our present investigation. We have also collected fish from the Meghna and the Buriganga rivers named chapila, but they are Ilish fish for both morphological and molecular identification. Mislabeling of jatka was confirmed at different stages of the supply chain. It greatly hamper to our economy and loyalty. Along with the government, we should take proper steps to save ilish.

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