MOLECULAR AND MORPHOLOGICAL IDENTIFICATION OF NGA MYIN YINN, *SILONIA SILONDIA* (HAMILTON, 1822) IN TWANTAY CANAL

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Abstract

The Silond Catfish (*Silonia silondia*), locally known as Nga Myin Yinn, is an economically significant freshwater species in Myanmar facing threats from overexploitation and habitat degradation. Genomic sequencing, alongside morphological analysis, is essential to support accurate identification and conservation efforts for this species. A total of 50 fingerling Silond catfish were collected from the Twantay Canal (a connection to the Ayeyarwady River) was obtained in May 2023. Species identification was confirmed using morphological characteristics, the FishBase application, and molecular analysis through cytochrome c oxidase I (coxI) gene sequencing. Two primer sets—Fish F2/R2 and LCO 1490 / HCO 2198—were used to amplify the *coxI* region of mitochondrial DNA successfully, yielding 525 bp sequences with the BigDye terminator sequencing method. Analysis of sequence data in the NCBI database showed a high homology (>93% sequence identity) between *S. silondia* populations from Myanmar, India and Bangladesh. Phylogenetic analysis using neighbor-joining (NJ) trees placed *S. silondia* from Myanmar in the same clade as *S. silondia* populations from Bangladesh, indicating strong genetic similarity across regions.

Keywords: *Silonia silondia*, Twantay canal, and Ayeyarwady River, Phylogenetic tree

Introduction

The Silond Catfish (*Silonia silondia*), locally known as Nga Myin Yinn in Myanmar, is one of the most valuable freshwater fish in the country, with market prices reaching up to 30,000 kyats/kg—making it more expensive than beef or pork. This species inhabits the Ayeyarwady River and its associated canals. *Silonia silondia* (Hamilton, 1822) belongs to the order Siluriformes (Nelson *et al*., 2016) and is distributed throughout South and Southeast Asia, including Bangladesh, India, Myanmar, Nepal, and Pakistan (Talwar and Jhingran, 1991; Gupta, 2015). It serves as a crucial target species for local fisheries, providing significant income and food security to communities in Myanmar.

Traditional fishing methods, such as the Nga Myin Yinn Tan long-line technique, are still widely practiced, particularly in the Katha, Mandalay, and Twantay townships. Despite its commercial importance, the population of *Silonia silondia* has been declining across its native range due to overfishing and habitat degradation, with local fishermen reporting decreasing catch rates. Although *S. silondia* is currently classified as "Least Concern" by the IUCN (2017), it is considered near-threatened in Pakistan (CAMB) and India (NBFRG) (Lakra *et al*., 2010) and has been listed as endangered in Bangladesh (IUCN, 2000). In Myanmar, reports from fishermen also indicate a marked decline in this species' abundance.

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To ensure the sustainability of *Silonia silondia*, targeted conservation measures and sustainable fishing practices are critical. Conservation strategies should include establishing protected areas, enforcing fishing quotas, and implementing habitat restoration efforts. Additionally, developing artificial propagation techniques could support sustainable production of this species. Unlike other freshwater fish already cultured in Myanmar, no artificial breeding methods have been established for *Silonia silondia*. The Department of Fisheries (DoF) has initiated preliminary studies to assess the feasibility of artificial propagation, focusing on fish behavior, nutritional requirements, and growth rates at the Twantay Township and Ta Loat Hla Fisheries Station in Maubin Township (Aye Aye Min, personal communication).

However, scientific research on *Silonia silondia* in Myanmar remains limited. Understanding the population structure, migration patterns, and species diversity requires reliable identification through both morphological and molecular methods, especially for the early life stages. Fingerlings of *S. silondia* are occasionally caught in the Twantay Canal, Yangon Region; however, they closely resemble fingerlings of the *Pangasius* species, making identification challenging. Therefore, in the present study, fingerling Silond catfish were collected from natural populations, and their species identity was confirmed through both morphological and molecular analyses.

Materials and Methods

Specimen collection

A total of 50 fish samples were collected from the Twantay Canal, Yangon region (16.74833°N, and 96.08643°E) using a net fence (Pike-Bawoon), with permission from the Department of Fisheries and the license owner (Plate 1 A and B) in May 2024. All samples were transported in oxygen-filled plastic bags with water (Plate 1 C) to the Laboratory of Aquatic Animal Diseases at the University of Yangon. In the laboratory, the fish were temporarily placed in cement tanks with aeration. These tanks were disinfected with 20% NaOH for 48 hours prior to introducing the fish.

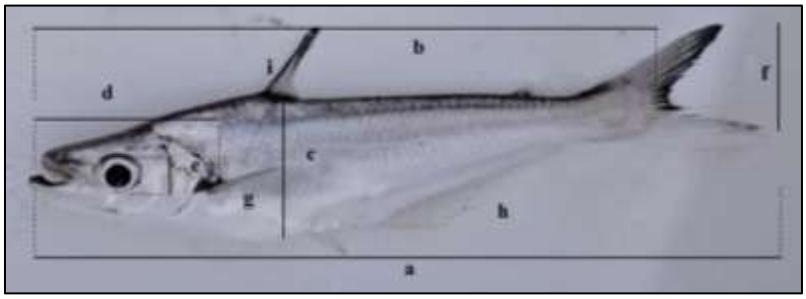
A. Department of fisheries station located at Twantay Canal B. Sample collection C. Transferred of fish using oxygen filled plastic bags

Plate 1 Sample collection at Twantay Canal

Morphological Identification of Fish Species

For the morphological identification of fish species, a total of 3 individuals were collected for morphometric measurements. Detailed measurements included total length, weight, standard length, body depth, head length, eye length, pectoral fin length, dorsal fin height, and the number of fin rays. Additionally, the number of scales along the lateral line, the number of gill rakers on

each arch, the number of teeth in the upper and lower jaws, and the structure of the adipose fin were recorded in Plate 2. Photographic records were taken while the fish were in fresh condition. Measurements were obtained from the sampled fish to assist in species identification, utilizing the identification keys provided by Day (1878), Lagler (1977), Talwar and Jhingran (1991) and Ferraris Jr. (1997).



Measuring of fingerling *Silonia silondia*

a. Total length, b. Standard length, c. Body depth, d. Head length, e. Eye length, f. Caudal fins, g. Pectoral fins, h. Dorsal fins and i. Pelvic fins

Plate 2 Morphometric measurements of fish

Molecular Identification of Fish Species

The muscle near the caudal fins was collected for DNA extraction for species identification of fish. A total of six individuals including three individuals conducted for morphology identification were collected for muscle samples. The samples were named Sample SS1, SS2, SS3, SS4, SS5 and SS6. Among them, three fish samples (SS1, SS2 and SS3) were prepared for the cytochrome c oxidase (*coxI*) region of mitochondrial DNA (mtDNA) amplification using two pairs of primers: forward primer F1 and reverse primer R1, and forward primer F2 and reverse primer R2, as some freshwater fish do not amplify well with the F1 and R1 primers (Ward *et. al.*, 2005).

In addition, other three fish samples (SS4, SS5 and SS6) were amplified for the *coxI* region of mtDNA using forward primer LCO 1490 and reverse primer HCO 2198 (Folmer *et al.*, 1994). Primers were selected based on the availability of the primer bank in the department. The amplified of the *coxI* using different primers sets are shown in Fig. (1).

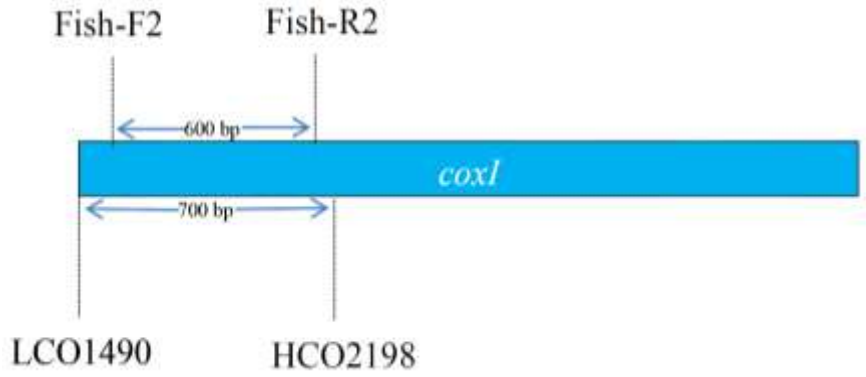


Figure. 1 The amplified regions of the *coxI* using two different primers sets

Genomic DNA Extraction of Fish Species

Fish muscle tissues were preserved in 70% ethanol in tissue sample bottle with labelled. The QIAGEN DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA) was used for DNA extraction. Briefly, approximately 5 mg of tissue was digested in an extraction buffer solution (100 mM Tris, pH 8.0; 100 mM EDTA, pH 8.0; 100 mM NaCl) with Proteinase K (20 mg/ml) at 72°C for 4 to 24 hours. Remaining proteins and polysaccharides were removed using a DNeasy Mini spin column. The precipitated DNA was washed with 100% ethanol, resuspended in buffer AE, and stored in a freezer until utilization. The concentration of nucleic acid fish samples was determined by using NanoDrop one 2000 Spectrophotometer.

PCR Amplification and Gel Electrophosis of Fish Species

For PCR, 5 µl of extracted DNA was combined with 15 µl of water, 2.5 µl of each 25 µM primer, and 25 µl of AMPIGENE® HS Taq DNA Polymerase. The PCR profile for the Fish F1/Fish R1 and Fish F2/Fish R2 primers included an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 1 minute, finishing with a final extension at 72°C for 10 minutes. For the LCO 1490/HCO 2198 primers, the PCR profile started with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 40°C for 1 minute, and extension at 72°C for 30 seconds, concluding with a final extension at 72°C for 7 minutes. The primer sequence and amplify regions were described in Table. (1).

The PCR products were separated and visualized using electrophoresis on a 2.0% agarose gel stained with GR Green loading dye (Wiz Bio Solution, Republic of Korea). A 100 bp DNA marker (Wizbio Solution Co. Ltd., Korea) was included for size comparison. Gel bands were visualized with a blue light transilluminator (GeneDireX, Inc.).

Table 1 Primers for the PCR amplification of fish species

Primers	Primer Sequences	Expected amplicon size	Reference
Fish-F1	5’TCAACCAACCACAAAGACATTGGCAC3’	600	Ward *et al.*, 2005
Fish-R1	5’TAGACTTCTGGGTGGCCAAAGAATCA3’		
Fish-F2	5’TCGACTAATCATAAAGATATCGGCAC3’	655	
Fish-R2	5’ACTTCAGGGTGACCGAAGAATCAGAA3’		
LCO1490	5’GGTCAACAAATCATAAAGATATTGG3’	700	Folmer *et al*., 1994
HCO2198	5’TAAACTTCAGGGTGACCAAAAAATCA3’		

Gel Purification and DNA Sequencing of Fish Species

Distant DNA bands were excised using a blade and transferred to 1.5 ml tubes (Plate 3). DNA extraction and purification from the bands were performed using the FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co., Ltd.), following the manufacturer's instructions. The purified amplicons were then submitted to Macrogen, Inc. (Geumchun-gu, Seoul, Korea) for DNA sequencing. Single-pass sequencing was conducted on the template using forward primers. The sequencing reactions were carried out with the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) and the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), adhering to the manufacturer's protocols.

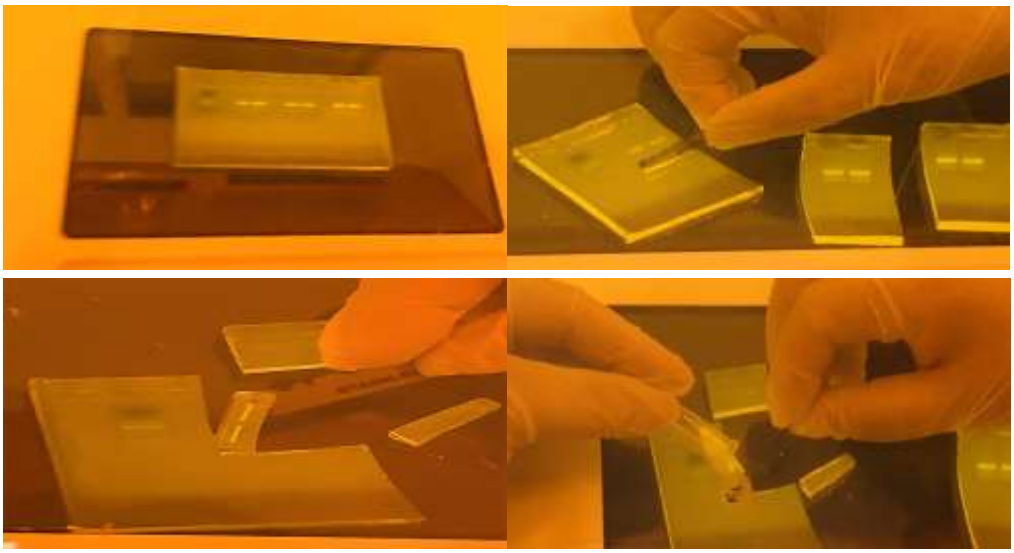


Plate 3 Cutting Distinct Bands of PCR Amplified Fish Samples for Gel purification

Sequence Alignment and Phylogenetic Analysis of Fish Species

Among the six samples five sequences were qualify for the alignment. The sequences from the five individual fish were aligned using MEGA 11 software. Manual editing was performed to remove mismatched sites and gaps. The ten most similar reference sequences, retrieved from the NCBI database, were compared to those obtained in this study. For a comprehensive analysis, the acquired sequences and reference sequences underwent multiple sequence alignment using the Muscle alignment tool in MEGA 11. The aligned sequences were then used to construct a phylogenetic tree, employing the Neighbor-Joining method with 1000 bootstrap replicates for validation.

Results

Morphological Description of *Silonia silondia*

A total of 50 individuals (13.86 ± 2.02 cm and 17.92 ± 10.09 g) were collected and their morphological identification was carried out. The species was confirmed as Silond catfish, *Silonia silondia* (Hamilton, 1822), through morphological characteristics using the FishBase software.

The fin formula of collected fish samples are is D1.I/7; D.0; P.I/11-13; V.6; A.40-46 (4/35-44); C.17.

D1. I/7

D1 refers to the first dorsal fin. **I** indicate the number of spines in that fin, which is 1.**7** refers to the number of soft rays in the first dorsal fin.

D. 0:

D refers to second dorsal fin was absent.

P. I/11-13:

P refers to the pectoral fins. **I** indicate 1 spine in the pectoral fin. **11-13** refers to the range of soft rays in the pectoral fin of collected samples.

V. 6:

V refers to the ventral (pelvic) fins. The number **6** indicates that there are 6 soft rays in the ventral fins selected fish.

A. 40-46 (4/35-44):

A refers to the anal fin. The range **40-46** indicates the number of soft rays in the anal fin of sampled fish. The notation **(4/35-44)** provides additional information that there are 4 spines in the anal fin, with a range of 35 to 44 soft rays.

C. 17:

C refers to the caudal fin. The number **17** indicates that there are 17 rays in the caudal fin.

According to the morphological identification, the fish is identified as *Silonia silondia*.

DNA Concentration of *Silonia silondia*

The extracted DNA concentrations of fish of fish samples was determined by using NanoDrop one 2000 Spectrophotometer. DNA concentration ranged from 32.8 to 329.6 ng/µl. The purity ratios (A260/A280) of the samples varied between 1.39 - 2.07 (Plate 4).

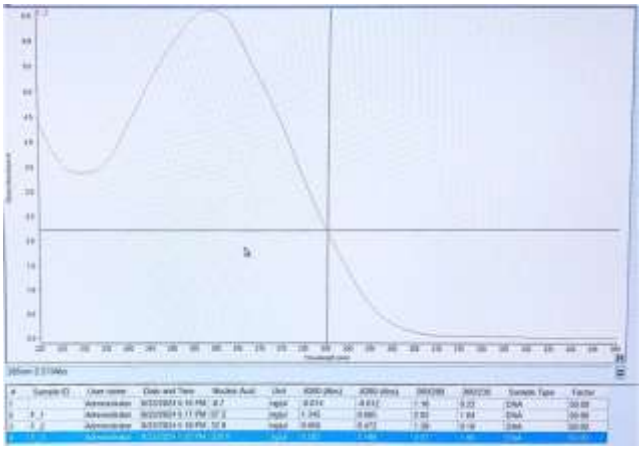
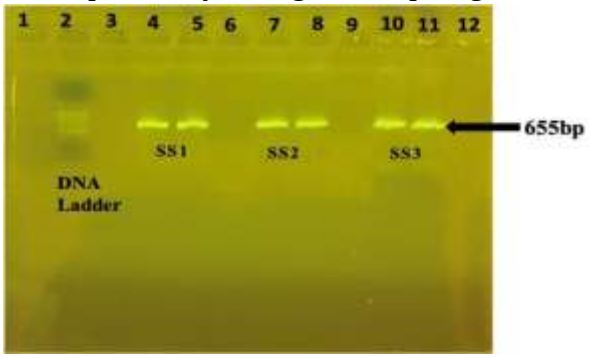


Plate 4 Graph of genomic DNA concentration of extracted from fish samples

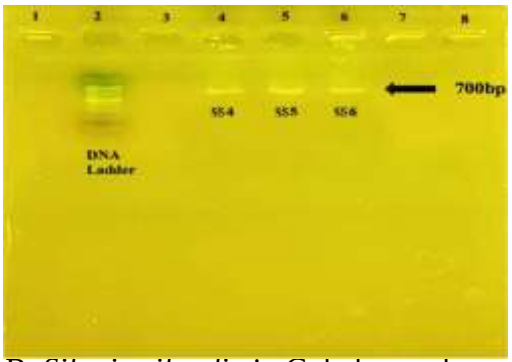
Amplification of *coxI* region of *Silonia silondia*

The *coxI* region of *Silonia silondia* samples was amplified using the FishF2/R2 primer set, with results shown in Plate 5 A. Lane 2 contains a 100 bp DNA ladder as a reference for fragment size. Lanes 4, 5, 7, 8, 10, and 11 display PCR results from three *Silonia silondia* samples (SS1, SS2 and SS3), showing successful amplification of a 655 bp fragment. In contrast, the FishF1/R1 primers could not amplified the fish samples.

For SS4, SS5, SS6, the *coxI* region of tissue samples was also successfully amplified with the LCO/HCO primers, yielding a 700 bp fragment as seen in Lanes 4, 5, and 6 (Plate 5 B).



A. *Silonia silondia* in Gel electrophoresis using Fish F_2 /R_2 primers



B. *Silonia silondia* in Gel electrophoresis using LCO1490/ HCO 2198 primers

Plate 5 PCR amplification of *Silonia silondia* using different primer pairs

Cytochrome c oxidase I (*coxI*) gene sequence analysis of *Silonia silondia* with the Fish-F2/ Fish-R2 primer pair

Among the three samples amplified with the Fish-F2/ Fish-R2 primers, two samples (SS1 and SS2) were successfully sequenced for their *coxI* regions. The third sample (SS3) showed contamination and noise, and thus was excluded from the data analysis.

The analysis indicated that the two *Silonia silondia* specimens form a slightly diverged clade with strong bootstrap support, reflecting a well-resolved evolutionary relationship with *Silonia silondia* and *Silonia childreni*. These species, collected from distinct geographic locations (Bangladesh and India, respectively), exhibited well-supported clades with bootstrap values of 94% and 93%, indicating considerable divergence from one another and from other groups in the tree. Notably, *Clupisoma bastari* from India clustered more closely with *Silonia silondia* than with other *Clupisoma* species (*C. prateri*, *C. garua*, and *C. sinense*), suggesting an unexpected phylogenetic relationship that warrants further investigation. The outgroup species, *Tenulosa ilisha* and *Monopterus javanensis*, formed a separate clade, which is characteristic of taxa outside the Siluriformes group, thereby contextualizing the relationships within the catfish lineage (Fig. 2, Table 2 and 3).

Table 2 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS1) and other species registered in NCBI database (The sequencing primer is Fish-F2/ R2)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	94.84	MK572595
Silonia childreni	India	93.98	MT654654
Clupisoma bastari	India	91.33	OP493140
Eutropiichthys vacha	India	90.89	KX946624
Proeutropiichthys taakree	India	90.74	OQ607735
Clupisoma sinense	China	90.45	JN020077
Clupisoma prateri	China	92.15	PP778663
Clupisoma garua	Pakistan	91.90	OP575582

Source: NCBI database.

Table 3 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS2) and other species registered in NCBI database (The sequencing primer is Fish-F2/R2)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	94.79	MK572595
Silonia childreni	India	93.92	MT654654
Clupisoma bastari	India	91.33	OP493140
Eutropiichthys vacha	India	90.79	KX946624
Proeutropiichthys taakree	India	90.68	OQ607735
Clupisoma sinense	China	90.51	JN020077
Clupisoma prateri	China	92.07	PP778663
Clupisoma garua	Pakistan	91.90	OP575582

Source: NCBI database.

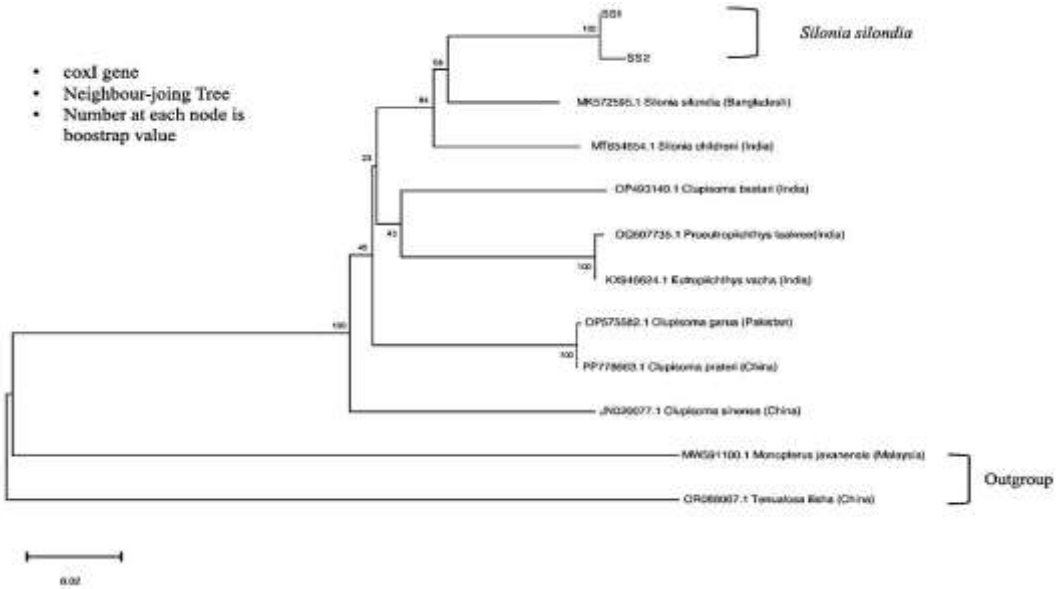


Figure. 2 Phylogenetic analysis of Species using Neighbor-Joining tree Based on *coxI* Gene Sequences amplified with Fish F2/R2 Primers

Cytochrome c oxidase I (*coxI*) gene sequence analysis of *Silonia silondia* with the LCO1490/ HCO 2198 primer pair

The resulting NJ tree indicated that the three *Silonia silondia* specimens formed a slightly diverged clade with strong bootstrap support, suggesting a well-defined evolutionary relationship with *Silonia silondia* and *Silonia childreni*. Specimens from geographically distinct regions—Bangladesh and India—exhibited well-supported clades with bootstrap values of 93% and 92%, respectively, highlighting significant divergence from each other and from other species in the analysis. Furthermore, species such as *Proeutropiichthys taakree* and *Eutropiichthys vacha* from the same region demonstrated a closer phylogenetic relationship with *Silonia silondia*, while the marine species *Tenulosa ilisha* and *Monopterus javanensis* clustered in a separate clade, reinforcing their role as outgroups in the phylogenetic analysis of these catfish species (Fig. 3, Table 4, 5 and 6).

Table 4 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS4) and other species registered in NCBI database (The sequencing primer is LCO1490 /HCO 2198)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	93.98	MK572595
Silonia childreni	India	92.81	MT654654
Eutropiichthys vacha	India	91.05	KX946624
Proeutropiichthys taakree	India	91.27	OQ607735
Clupisoma sinense	China	90.61	JN020077
Clupisoma prateri	China	92.25	PP778663
Clupisoma garua	Pakistan	92.06	OP575582

Source: NCBI database.

Table 5 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS5) and other species registered in NCBI database (The sequencing primer is LCO1490 /HCO 2198)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	93.71	MK572595
Silonia childreni	India	92.72	MT654654
Eutropiichthys vacha	India	89.89	KX946624
Proeutropiichthys taakree	India	90.10	OQ607735
Clupisoma sinense	China	89.41	JN020077
Clupisoma prateri	China	90.95	PP778663
Clupisoma garua	Pakistan	90.95	OP575582

Source: NCBI database.

Table 6 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS6) and other species registered in NCBI database (The sequencing primer is LCO1490 /HCO 2198)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	93.99	MK572595
Silonia childreni	India	92.00	MT654654
Eutropiichthys vacha	India	91.04	KX946624
Proeutropiichthys taakree	India	91.26	OQ607735
Clupisoma sinense	China	90.60	JN020077
Clupisoma prateri	China	92.24	PP778663
Clupisoma garua	Pakistan	92.06	OP575582

Source: NCBI database.

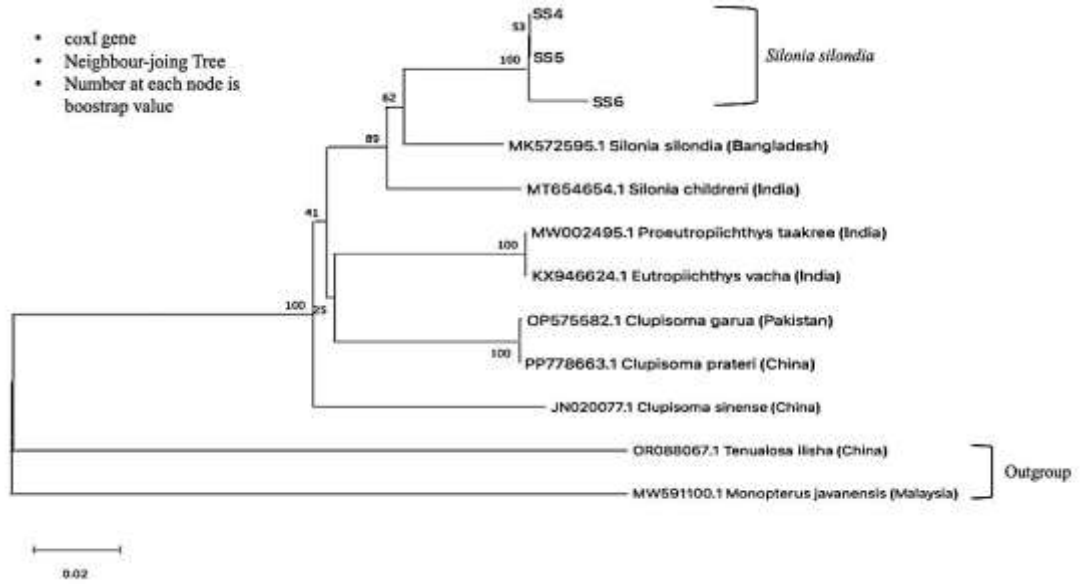


Figure. 3 Phylogenetic analysis of Species using Neighbor-Joining tree Based on *coxI* Gene m Sequences amplified with LCO1490 /HCO 2198 Primers

Discussion

In this study, two methods, morphological identification and DNA sequencing of the *coxI* region, were used to identify the species. The morphological analysis confirmed that the fish collected were *Silonia silondia*. Additionally, the phylogeny of *S. silondia* was analyzed using sequences from the Cytochrome c oxidase subunit 1 (*coxI*) gene. The *coxI* gene is often used for DNA barcoding, as it is crucial for cellular energy production and is highly conserved across species while still varying enough to differentiate them. Since 2003, studies have shown that the *coxI* gene is highly conserved within the same species but highly unique across different species (Ward *et al*., 2005).

In the present study, *coxI* regions of *Silonia silondia* were sequenced using three primer sets. Of these, the Fish F1/R1 primer set did not successfully amplify the *S. silondia* samples. Liu *et al*. (2020) used Fish F1/R1 and Fish F2/R2 primers to amplify *COI* regions in 179 fish samples collected from Xinxiang and Zhengzhou in Henan Province, China. They reported that Fish F1/R1 amplified the *COI* gene in 63 (35.20%) samples, while F2/R2 amplified 132 (73.74%) samples. This finding suggests that certain fish species may not reliably amplify with the Fish F1/R1 primer, aligning with our result that Fish F1/R1 could not amplify *S. silondia*.

The LCO/HCO primer pair, a widely-used set in cytochrome c oxidase subunit I (*COI*) amplification studies, has varying annealing temperatures, ranging from 42 °C to 55 °C (Dahlgren *et al*., 2000; Kelly *et al*., 2007; Margam *et al*., 2010; Muller and Beheregaray, 2010). These primers, LCO1490 and HCO2198, are universal DNA primers applicable across vertebrates and various animals (Sharma and Konayashi, 2014). In this study, three samples were successfully amplified using this primer set.

The *coxI* sequence from this study was compared with available genetic data of *Silonia silondia*. However, data from Myanmar were absent. Toe Toe Soe *et al*. (2021) analyzed the genetic data of *S. silondia* in the Ayeyarwady River, Myanmar, using mitochondrial DNA markers *cytb* and *ATPase* genes, but they did not include the *COI* region. Consequently, the present *COI* sequence could not be compared to the sequences of *S. silondia* reported by Toe Toe Soe *et al*. (2021).

Additionally, Theint Sandar Saing (2018) studied molecular variation and phylogenetics of *Silonia silondia* using *COI* and *cytb* datasets. However, their *COI* data are not registered in the NCBI database, preventing direct comparison. Mandal *et al*. (2016) reported sequence analysis of *S. silondia* from four Indian rivers using *cytb* (1140 bp) and *ATPase 6/8* (842 bp) markers, but did not use the *COI* region. *S. silondia* is an economically important food fish, and analyzing molecular markers can reveal genetic differentiation within populations, as shown in Mandal *et al*. (2016). Using *COI* is advantageous because it is short enough for rapid sequencing and cost-efficiency while still distinguishing species-level variations (IUCN Bangladesh, 2014).

In this study, the *coxI* region of *Silonia silondia* was compared to sequences in the NCBI database. The closest match was with samples from Bangladesh, showing 94% similarity, indicating a high level of genetic homology. Ng and Tan (1999) discuss how freshwater fish distributions across historically connected river basins, particularly during periods of lower sea levels, facilitated gene flow between populations in Southeast Asia and the Indian subcontinent. This historical connectivity likely contributed to the observed genetic homology.

This study highlights the genetic variation of *Silonia silondia* in Myanmar relative to other countries. Such findings are essential for understanding genetic resources in freshwater species across historically connected landscapes.

Conclusion

This study successfully confirmed the species identity of Silonia silondia (Nga Myin Yinn) fingerlings from the Twantay Canal using both morphological and molecular coxI sequencing methods. Morphological and molecular analyses confirmed the species identity as *Silonia silondia*. Molecular identification was performed through cytochrome c oxidase I (*coxI*) gene sequencing using multiple primers. Phylogenetic analysis based on neighbor-joining (NJ) trees, constructed from *coxI* sequences with Fish F2/R2 and LCO1490 /HCO 2198 primers, included sequences from *Silonia silondia* specimens, respectively, along with reference sequences from GenBank. The coxI region of *S. silondia* collected in Myanmar is highly homology to *S. silondia* collected from Bangladesh rather than the India and China. These findings are essential for future conservation, genetic resource management, and the development of artificial propagation programs for this commercially vital but increasingly vulnerable fish species.

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