MOLECULAR GENETIC VARIATION IN *ANABAS TESTUDINEUS* (BLOCH, 1792) FROM SOME RIVERS OF MYANMAR

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Abstract

A total of thirteen fish samples of *Anabas testudineus* were collected from four different geographical sites, Maubin, Sittwe, Dawei and Kalay of Myanmar, to examine the morphology and molecular genetics during the study period of December 2019 to August 2020. In the present study, the 15 morphometric variable characteristics were recorded for all *Anabas* specimens. The largest total length of 17cm was observed for the species of Maubin and the smallest of 8.5cm for that of Sittwe. The dorsal fin had 16 to 18 strong spines and 8 to 10 soft rays inserted over or slightly in advance of the pectoral fins; the anal fin was with 8 to 11 spines and 9 to 11 soft rays. The body weight was the highest, 82.10g, in Maubin and the lowest is 11.26g in Sittwe. Extraction of high-quality and quantity DNA was conducted, that was a fundamental requirement for genetic research. The highest quality of DNA from fish samples (value of A260/A280 was 2.02) from Sittwe, and the lowest quality (value of A260/A280 was 1.74) from Maubin were obtained. The mitochondrial cytochrome oxidase subunit I (COI) genes were amplified using PCR reaction. A product ~700 bp length was obtained. After the sequencing, a total 4 haplotypes with 2 variable sites, haplotype diversity of 0.67 and nucleotide diversity of 0.32%, were noted for CO1 gene (615 bp, n=4) dataset of the studied species. Genetic distance ranged from 0.0016 to 0.0032% among four samples of *Anabas testudineus* for CO1 gene. In Neighbor-joinging (NJ) and Maximum likelihood (ML) phylogenetic tree analyses, all four samples of native Myanmar *Anabas testudineus* clustered into a strong single cluster for all datasets. Inferred ancestral sequences tree was constructed using CO1: AS, AD, AM1 and AK among sites treated as being uniform G nucleotide.

Keywords *Anabas testudineus,* CO1 gene, haplotype, nucleotide diversity, genetic distance, phylogenetic tree.

Introduction

Perciformes is the largest and most diverse order of teleosts in the world, containing about 41 % of all bony fish comprising greater than 10,000 species and about 160 families (Nelson, 2006). The family Anabantidae belonged to Perciformes, with thirty-four species in it. There are two identified species in the genus *Anabas*: *Anabas cobojius* (Hamilton, 1822) and *Anabas testudineus* (Bloch, 1792 Froese and Pauly, 2012). The climbing perch, *A. testudineus* (Bloch, 1792) is an associate of this family, being considerable in several components of Asia: Bangladesh, China, India, Malaysia, Myanmar, Pakistan, Philippines, Sri Lanka, and Thailand (Rahman, 2005). This is a very hardy fish and plays a significant role in fisheries and aquaculture practices (Froese and Pauly, 2014). Therefore, it will bear extremely adverse water conditions such as low oxygen, polluted water, and so on (Pethiyagoda, 1991) through an accessory air respiration organ referred to as the labyrinth organ (Rahman, 1989).

In Myanmar, the climbing perch is known by the local name of Nga bye ma and well known as a delicacy for its great taste. This species is considered as a valuable item of diet for sick and convalescents (Saha, 1971).

The application of DNA barcoding in the form of sequence data of cytochrome c oxidase subunit I mitochondrial gene (mtDNA-COI) has been appreciably used for taxonomy study and organism identification (Yudhistira and Arisuryanti, 2019). It is now considered highly desirable to include sequences from mitochondrial COI gene to identify freshwater fish species accurately

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(Dahruddin *et al*., 2017). In fish, DNA barcodes were advanced as a rapid and correct device for species identity through the use of universal primers (Ivanova *et al.*, 2007).

Generally, all the freshwater fish were identified based on their morphology. Nevertheless, the morphological identification, sometimes, wasn't generally inaccurate and incorrect e.g. climbing perch and gourami (RIPED, 2008). Molecular identity the usage of the COI gene as a DNA barcoding marker is required to investigate the correct species name of the freshwater fish COI gene as a DNA barcoding marker has many advantages such as it can be used for small amount samples of the life stages, and differentiation between similar phenotypes of fish (Dudu *et al*., 2016).

Traditional morphology-based taxonomic procedures are time-consuming and now no constantly sufficient for identity to the species level, and therefore a multidisciplinary technique to taxonomy that consists of morphological, molecular, and distributional data is vital (Krzywinski and Besansky, 2003). Hebert *et al.* (2003a, b) have proven that the analysis of short, standardized genomic regions (DNA barcodes) can discriminate morphologically recognized animal species. In fact, it has already been established that only those COI sequences that meet these strict standards might be precise as DNA barcodes by the National Center for Biotechnology Information's GenBank (NCBI, GenBank; www.ncbi.nlm.nih. gov/Genbank), the European Molecular Biology Laboratory (EMBL; www.embl.org), and the DNA Data Bank of Japan (DDBJ; www.ddbj.nig.ac.jp).

According to Hubert *et al*., (2008) sequencing the fish cytochrome oxidase subunit 1 (COI) gene is an efficient DNA barcoding technique for identifying freshwater fish species and creating a phylogenetic tree. It can aid in our understanding of the evolutionary history of morphological and ecological traits in marine invertebrates and other organisms (Arrigoni *et al.*, 2014).

Hence, in the present study, an attempt was made to analyze the genetic diversity of native Myanmar *Anabas testudineus* (Nga bye ma) based on morphology and COI sequence data to investigate the genetic variation between populations.

Materials and Methods

Study areas

The present study areas for sample collection were located in Ayeyarwady Region, Tanintharyi Region, Rakhine State, and Sagaing Region (Fig.1). Molecular genetics laboratory works were carried out at the Department of Zoology, Dagon University.

Study period

The study was carried out from December 2019 to August 2020.

Specimen collection

A total of 13 fish specimens of native climbing perch *Anabas testudineus* (Bloch, 1792) were randomly collected from local fishermen at four geographical sites; Maubin, Ayeyarwady Region (n=10), Dawei, Tanintharyi Region (n=1), Sittwe, Rakhine State (n=1), and Kalay, Sagaing Region (n=1). The sample codes were named as Maubin (AM), Dawei (AD), Sittwe (AS) and Kalay (AK).

Morphometric examination

Initially, the external characteristics including color, body shape, head, and tail of collected fish individuals were examined according to the methods of Talwar and

Jhingran (1991) and Barman *et al*. (2014). Then, the morphometric measurements such as body weight, total length, standard length, and 12 other parameters including dorsal fins, anal fins, pectoral fins, and so on were taken. After the examination, the fins (dorsal, anal, and pectoral) were clipped and immediately preserved in the 1.5 ml microcentrifuge tube containing 70% Analytical grade ethanol and kept in a freezer (-20°C) until the DNA Extraction.

Genetic analysis

Genomic DNA extraction

For the genomic DNA extraction from the stored fin tissues, approximately ≤25 mg of the stored fin tissues were taken and put into 1.5 ml microcentrifuge tube. Then, genomic DNA was extracted following the protocol provided by the PureLink Genomic Kit (Invitrogen).

Quantification of extracted genomic DNA

The quality and quantity of extracted genomic DNA were measured by using NanoDrop^{TM} spectrophotometer. The amount and purity of extracted DNA (1µl) were measured and observed for A260/280 ratio and A260/230.

PCR amplification using COI gene primers

The amplification of DNA was carried out in thermal-cycler (Simpli Amp PCR System). The COI gene approximately ~700 bp fragment length located in the mitochondrial genome of the climbing perch was successfully amplified using the universal fish primer named Fish F1 and Fish R1 (Ward *et al*., 2005). The sequences of the primers are: Fish forward F1 (5´TCA ACC AAC CAC AAA GAC ATT GGC AC 3´) and Fish reversed R1 (5´TAG ACT TCT GGG TGG CCA AAG AAT CA 3´). Each 25 µL PCR reaction comprises as: 2 µL of extracted DNA template, 12.5 µL of Gold Taq® G2 Master mix (Ref. M743A, Promega, Madison, WI USA), 1 µL (0.01 mM) of forward primer, 1 µL (0.01 mM) of reverse primer, and 8.5 µl of nuclease free water (Ref. P119A, Promega, Madison, WI USA). The PCR thermal cycling conditions involved an initial denaturation at 94°C for 3 min, denaturation at 94°C for 45 s, annealing temperature of 50°C for 45 s and elongation temperature of 72°C for 1 min for 30 cycles, and concluded with a final elongation step at 72°C for 7 min followed by a hold at 4°C.

Agarose gel electrophoresis

The amplified PCR products were checked in 1% agarose gel at 135 VDC for 25 mins. Gel was then stained using ethidium bromide (EtBr) solution. PCR bands were visualized under the UV transilluminator and photographed. The 100 bp ladder (Thermo Fisher Scientific) was used to estimate PCR band size in bp (base pairs).

Purification of PCR product

The successful PCR products were purified by using Exosap Kit (Thermo Fisher Scientific BallicsUAB). Firstly, 5 µl of PCR product was mixed with 2 µl of Exosap. And then, thermal cycling was done at 37ºC for 15 min and 80 ºC for 15 min.

The purified PCR product was done before sequencing. For this reaction, the same primer set (Forward and Reversed) was separately used for each purified PCR product of the sample. For the control, pGEM -3zf (+) as the template sample and -20M13 as control primer were used. Total reaction volume was 20 µl and 30 cycle sequencing. Each 20 µl of sequencing components were prepared as follows: 4 µl of Big Dye Mix, 2 µl of 5X buffer, 11 µl of Nuclease free water, 1 µl of primer and 2 µl of 1st purification product. The running conditions for the sequencing were as follows: hold duration at 96 ºC for 2min, denaturation at 96 ºC for 30 sec, annealing temperature of 55 ºC for 15 sec, and extension temperature of 60 ºC for 4min followed by a hold at 4 ºC.

For the preparation of 2nd purification using Centri-sep (Thermo Fisher U.S.A), the cap of the tube was opened and added 800 µl nuclease-free water. After that the bottom cap of the tube was removed and placed into a new 2 µl collection tube and checked to drain and incubated 30 min at room temperature. The reaction vessels can be successfully precipitated and become visible and measure at least 200-250 µl removed by pipetting. Totally the removed solvent had to retain 500 µl. Finally, the collection tube was discarded and placed it in the new sterile 1.5 ml microcentrifuge tube.

Next step, 20 µl of cycle sequencing product was slowly added at the center of the centri-sep by pipetting. After that, the tube was centrifuged for 2 min at 1800 rpm and it would be resuspended in a 1.5 ml sterile tube and obtained a second purification product. It was kept into a laminar flow and inverted it for overnight. If the dry sample was added 20 µl Hidi formamide (Thermo Fisher Scientific) into the tube and mixed well by vortex to obtain a homogeneous solution and spring down by mini centrifuge. Eventually, this tube was incubated in 95 ºC for 2 min and immediately transferred into the -20 ºC and left for 2 min. This prepared sample can be ready to use in the 96 well plate by pipetting.

Sequencing in ABI 3500 Genetic Analyzer

DNA sequencing was done in ABI 3500 Genetic Analyzer auto sequencer (Applied Biosystems). The obtained sequence data were downloaded from ABI 3500 Computer onto CD discs and transferred to the laptop computer. And then, the sequence was edited by using MEGA-X software. After alignment, the sequencing data from the chromatogram was converted into STARDANT format and identified the sequence using BLAST at the nucleotide database of the National Center for Biotechnology Information (NCBI) to determine the best match homology.

Genetic analysis

Haplotype number, haplotype diversity, nucleotide diversity, polymorphic sites, genetic distances, and phylogenetic analyses of the aligned sequences were conducted by using MEGA X software, BioEdit V.7, and DNA Sequence Polymorphism V6.12.03.

The reference sequences for *Anabas* species were obtained from GenBank/ EMBL database.

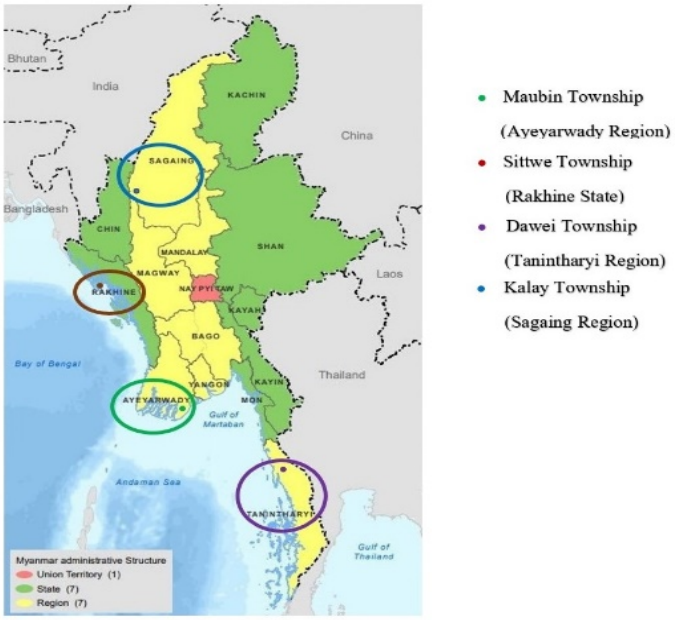


Figure 1 Location map of fish sampling sites in Myanmar

Results

External appearance of studied *Anabas testudineus* species

External characteristics including color, body shape, head and tail of fish individuals were observed in the present study. Some remarkable change in color for *A. testudineus* was observed among the collected specimens from different study sites. The color of the dorsal surface is dark to pale greenish, very pale below, back dusky to olive found in Maubin and Dawei. Ventrally longitudinal stripes were observed on the head of all examined *Anabas* species. The iris is golden reddish color in the fish from Maubin.

Morphometric measurements of studied *Anabas testudineus* species

According to the data obtained, four *Anabas* species are similar in eye diameter (0.8cm). Dorsal spine rays (17), dorsal soft rays (8), and anal soft rays (8) were recorded at the same number. The variation of total length (17cm) and body weight (82.10g) was highest in the fish of Maubin *Anabas* whereas in the smallest of total length (8.5cm) and body weight (11.26g) in the fish of Sittwe. All studied species have dorsal fin (16-18) strong spines and the inserted soft rays (8-10) over or slightly in advance of pectoral fins; anal fin was (8-11) spines and soft rays (9-11) (Plate 1).

Concentration and purity of extracted genomic DNA

The different total concentrations of genomic DNA were observed as Maubin (516.3 ng/µl), Sittwe (1248.5 ng/µl), Dawei (212.9 ng/µl), and Kalay (1353.3 ng/µl). The results of calculated (A260/280nm) ratio by the measurement of the NanoDrop spectrophotometer showed the values of Maubin (1.74), Sittwe (2.02), Dawei (1.80), and Kalay (2.00) indicating a high yield of genomic DNA quality. Similarly, absorbance at 260/230 nm ratio had acceptable contamination showing the value of 1.78, 2.36, 1.95, and 2.16 respectively. It indicated continuing PCR amplification process (Table 1).

PCR amplification using CO1 (Cytochrome oxidase subunit 1) marker gene

All extracted DNA samples were successfully amplified using CO1 primers by PCR. The product was visualized under UV light with 1% agarose gel after electrophoresis checking. The length of mtDNA fragments was ~700 bp by comparing the length of 100 bp Ladder (Plate 2). All PCR products were used in the next step of DNA sequencing.

Genetic Diversity

After the sequencing on ABI 3500 Genetic Analyzer, four CO1 gene sequences (615 bp) were obtained.

Haplotype and nucleotide diversity

Out of four individuals analyzed two different haplotypes were yielded from two variable sites. Haplotype 2 was found in fish individuals from Sittwe and Dawei. The transitional mutation points from C to T were found at the point of 6 bp and 594 bp of the sequence. The haplotype diversity (HD) was 0.67. The nucleotide diversity (π, %) was 0.32% (Table 2).

Genetic distances

Table 3 showed the pairwise genetic distance and evolutionary divergence among the species collected from different study sites.

Phylogenetic relationship

The phylogenetic relationships of *Anabas* species were investigated based on the CO1 gene (615 bp) using the three phylogenetic reconstruction methods.

The obtained four sequence datasets were analyzed by aligning with a total of 16 reference sequences obtained from GenBank. There were six sequences from India, four sequences from Indonesia, one sequence from Korea and Vietnam, and four sequences from Bangladesh (Table 4).

The pairwise genetic distance and evolutionary divergence of the studied sequence with the reference dataset were shown in (Table 5) and sequence alignment stated.

The nucleotide BLAST analysis result and identification of all twenty data of COI mitochondrial gene sequence of the fishes from different regions were obtained. The sequences of the COI mitochondrial gene of sample AK, AM 1, AD, and AS have a similarity of 90-93% if compared to *A. testudineus* recorded in the GenBank database by applying BLAST analysis and identification. The specimen had a similarity score between 92-95% with the species which had been recorded at GenBank. Again, *A. cobojius* as an outgroup database had a similarity of 76-95%.

Multiple phylogenetic analyses showed nearly identical topologies. All examined species belonged to one clade.

Neighbor-joining (NJ) tree analysis and maximum likelihood (ML) tree analysis

This was strongly supported by the neighbor-joining phylogenetic tree, which divided the haplotypes into four discrete allopatric clusters, corresponding to the individual drainage system. However, as noted in the nucleotide divergence values, the differentiation was correlated with geographical distance. The examined haplotypes were located in one cluster with a high supporting bootstrap value.

All other references from GenBank, consisting *Anabas* species formed distinct species-specific clades in the NJ tree. Outgroup is *A. cobojius* references from India and Bangladesh (Fig. 1).

Also, the examined haplotype was located within the same clade in maximum likelihood (ML) tree analysis (Fig. 2).

Inferred ancestral sequences tree

The tree showed a set of possible nucleotides (states) at each ancestral node based on their inferred likelihood at sites A, T, C, and G. For each node only the most probable sites are shown in Fig 3. The initial tree was inferred using the method, AS, AD, AM1, and AK among sites were treated as being uniform G nucleotide. T nucleotide among sites in India, A nucleotide sites in Indonesia, and C nucleotide among sites in Bangladesh.

Molecular genetic characteristics among Myanmar *Anabas testudineus*

Table 6 shows a summary of data analysis of CO1 615bp (n=4) sequence analysis. Out of four sequences, two haplotypes were noted (two in AS and AD with two variable sites: the other identical in AM1 and AK) with a total number of mutations (n=2). In four sequences of study Myanmar *Anabas* species, the nucleotide diversity (π, *%)* was 0.32% and haplotype diversity (HD) was 0.67.

Table 1 Concentration and purity of extracted genomic DNA from *A. testudineus*

Sr. No.	Specimen code	Amount ng/µL	A260/A280	A260/A230
1.	AM 1	516.3	1.74	1.78
2.	AS	1248.5	2.02	2.36
3.	AD	212.9	1.80	1.95
4.	AK	1353.3	2.00	2.16

Table 2 Polymorphic sites, haplotype diversity, and nucleotide diversity

No. of Sequences (n)	No. of haplotype	No. of polymorphic sites	Haplotype diversity (HD)	Nucleotide diversity (π, %)
4	2	2	0.67	0.32

Table 3 Net nucleotide diversities between the haplotypes as a pairwise distance measured based on 615 bp of CO1 gene

Lineage	AK	AM 1	AS	AD
AK	-			
AM 1	0.0016	-		
AS	0.0032	0.0032	-	
AD	0.0032	0.0032	0.0000	-

Table 4 Haplotypes and accession numbers of fish CO1 mtDNA sequences

Haplotype	GenBank Accession No.	Location
AM 1 AK AS AD		This study
Anabas testudineus, India (2019)	MK213550.1	GenBank
Anabas testudineus, India (2019)	MK213553.1	
Anabas testudineus, Indonesia (2020)	MN640070.1	
Anabas testudineus, Indonesia (2020)	MN640071.1	
Anabas testudineus, Indonesia (2020)	MN640072.1	
Anabas testudineus, Indonesia (2020)	KU692243.1	
Anabas testudineus, India (2019)	JX983214.1	
Anabas testudineus, India (2019)	MK213554.1	
Anabas testudineus, Korea (2019)	MK359929.1	
Anabas testudineus, Bangladesh (2019)	MG552721.1	
Anabas testudineus, Bangladesh (2019)	MN083164.1	
Anabas testudineus Vietnam (2019)	MH721200.1	
Anabas cobojius India (2019)	MK213553.1	Outgroup GenBank
Anabas cobojius India (2014)	KC774636.1	
Anabas cobojius Bangladesh (2019)	MK572025.1	
Anabas cobojius Bangladesh (2019)	MK572024.1	

Dataset: 20 sequences of CO1 mtDNA

Table 5 Estimates of evolutionary divergence between studied sequences and references from GenBank

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AK_(Kalay)																				
AM_1_(Maubin)	0.0016																			
AS_(Sittwe)	0.0032	0.0032																		
AD (Dawei)	0.0032	0.0032	0.0000																	
A.testudineus_MK213550.1-India	0.0000	0.0016	0.0032	0.0032																
A.testudineus_MK213553.1-India	5.1291	5.2133	5.1858	5.1858	5.1291															
A.testudineus_MK213554.1-India	5.0565	5.1389	5.1100	5.1100	5.0565	0.0031														
A.testudineus_MN640070.1-Indonesia	5.8462	5.8462	5.9582	5.9582	5.8462	6.9555	7.1047													
A.testudineus_MN640071.1-Indonesia	7.3007	7.1484	7.2856	7.2856	7.3007	5.9100	6.0015	6.3644												
A.testudineus_MN640072.1-Indonesia	6.3631	6.3631	6.3871	6.3871	6.3631	6.5165	6.5165	8.0287	6.8593											
A.testudineus_KU692243.1-Indonesia	10.5546	10.4321	10.3902	10.3902	10.5546	11.1892	11.2749	6.5597	8.1281	6.3094										
A.testudineus_JX983214.1-India	10.7766	10.6623	10.6321	10.6321	10.7766	11.1795	11.2647	6.4342	8.0095	6.3650	0.0785									
A.cobojius_MK213553.1-India	5.1291	5.2133	5.1858	5.1858	5.1291	0.0000	0.0031	6.9555	5.9100	6.5165	11.1892	11.1795								
A.cobojius_MK572025.1-Bangladesh	10.7766	10.6623	10.6321	10.6321	10.7766	11.1795	11.2647	6.4596	8.0095	6.3464	0.0768	0.0015	11.1795							
A.cobojius_MK572024.1-Bangladesh	10.7766	10.6623	10.6321	10.6321	10.7766	11.1795	11.2647	6.4596	8.0095	6.3464	0.0768	0.0015	11.1795	0.0000						
A.cobojius_KC774636.1-India	5.1181	5.2019	5.1974	5.1974	5.1181	4.7117	4.6409	5.4906	6.1860	5.2928	11.4381	11.5990	4.7117	11.5990	11.5990					
A.testudineus_MG552721.1-Bangladesh	10.4147	10.4147	10.4910	10.4910	10.4147	11.8105	11.8856	4.3640	11.1522	7.3899	8.1293	8.0265	11.8105	8.0017	8.0017	11.2445				
A.testudineus_MK359929.1-Korea	8.0786	8.0786	8.0786	8.0786	8.0786	8.0322	7.9273	7.8321	8.5152	11.3792	6.2799	6.3089	8.0322	6.2848	6.2848	10.9055	4.5419			
A.testudineus_MN083164.1-Bangladesh	9.2751	9.2751	11.3476	11.3476	9.2751	8.2493	8.1749	8.2635	11.7704	11.3964	6.6303	6.7165	8.2493	6.6914	6.6914	7.9281	7.9456	4.8519		
A.testudineus_MH721200.1-Vietnam	5.0983	5.0983	5.0039	5.0039	5.0983	5.4063	5.3094	4.7327	6.5584	7.1619	10.7367	10.8228	5.4063	10.7969	10.7969	4.7531	8.1605	8.0850	11.0477	

Table 6 COI gene sequence with variable sites of fish *Anabas* species from different sites

n = 4; variable sites = 2; haplotype = 2
615 bp alignment dataset

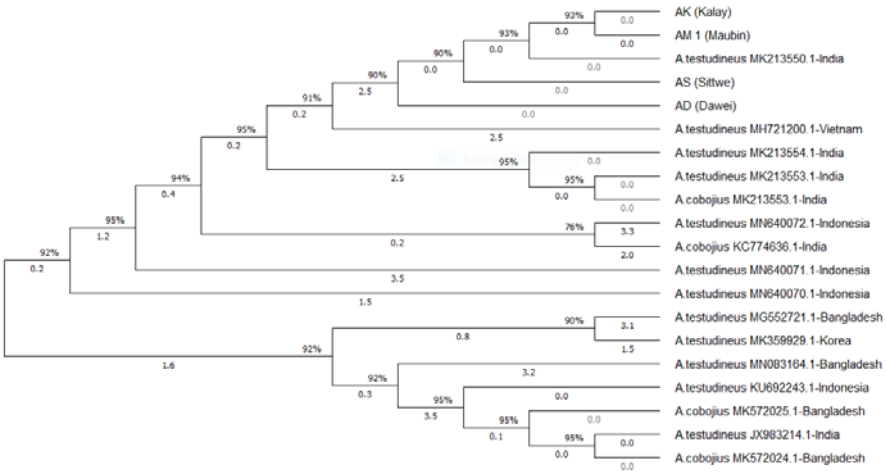


Figure 1 Neighbor joining phylogenetic tree constructed by using CO1 (615 bp) dataset of the present studied *Anabas testudineus* (n=4).

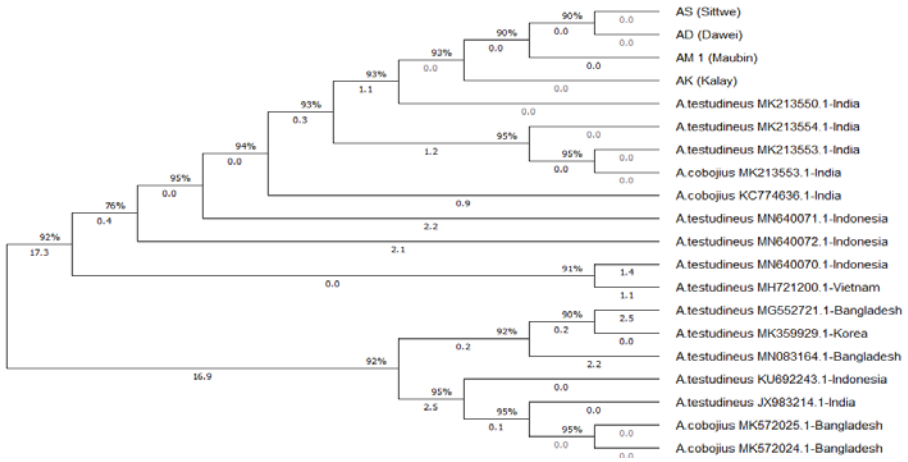


Figure 2 Maximum Likelihood phylogenetic tree constructed by using CO1 (615 bp) dataset of the studied *Anabas testudineus* (n=4).

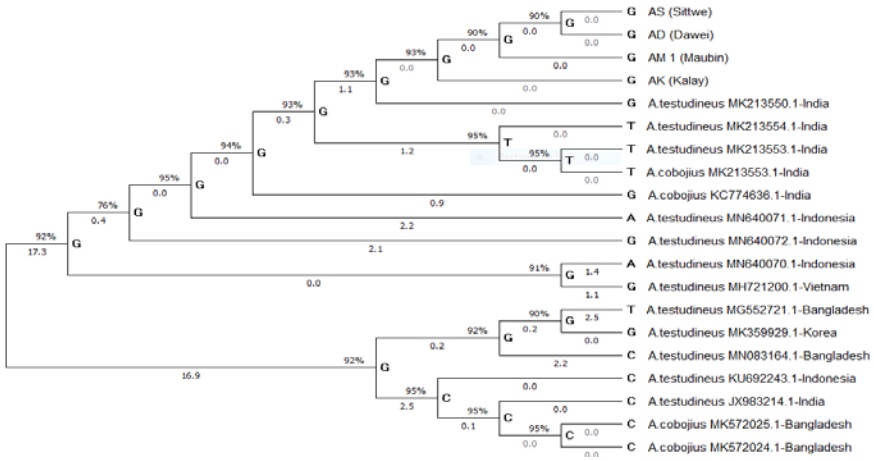
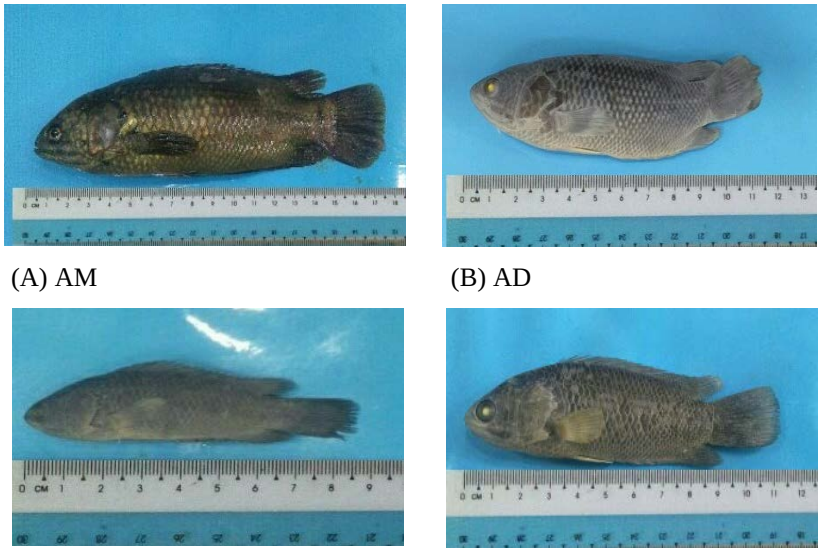


Figure 3 Inferred ancestral sequences tree constructed by using CO1 (615 bp) dataset of the studied *Anabas testudineus* (n=4).



(A) AM (B) AD

(C) AS (D) AK

Plate 1 Some studied fish *Anabas testudineus* from different collection sites
AM=Maubin, AS=Sittwe, AD= Dawei, AK= Kalay

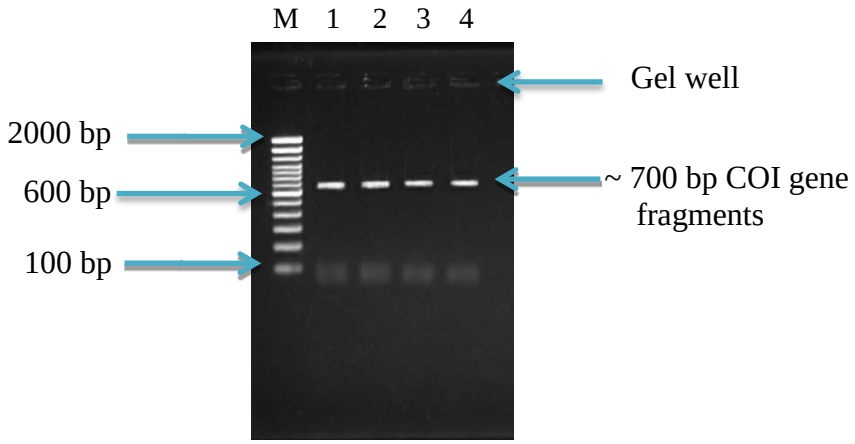


Plate 2 Gel Electrophoresis of PCR product of 1% agarose gel, Lanes 1: AM 1, 2: AS, 3: AD, 4: AK; Lanes M: CSL- MDNA 100 bp Makers; bp: base pair

Discussion

Anabas provides a significant contribution to the lake fisheries for as long as fifty years and as a native fish holds great ecological and economic significance with its exceptional export value and species abundance. *Anabas testudineus* (Bloch, 1792) is an economically critical freshwater species in Southeast Asia countries.

Traditionally, fish species identity has relied on morphological traits, such as body shape, number of scales or fin rays, and coloration patterns. Morphometric and meristic characters are utilized generally to distinguish fish stocks (Turan *et al*., 2004) and it has often been utilized in discrimination and classification studies by statistical techniques (Avsar, 1994). Variety studies based on morphological and molecular approaches can provide valuable information that helps in cataloguing the bioresources, their sustainable use and designing effective conservational techniques (Padmavathi and Gatreddi, 2017). In the present study, the fishes *Anabas* collected

from different geographical locations were separately distinguished and measured for total length (TL), body depth (BD), body weight (Gram) and so on. The result revealed that the variation of the highest length (total length) of the species ranged as 17cm in Maubin, 11.7cm in Kalay, 11.2 in Dawei and 8.5cm in Sittwe region. The body weight is highest in Maubin (82.10g) and lowest in Sittwe (11.26g).

According to the present genetics study, the result was efficient in extracting genomic DNA from the dorsal fins of all fishes. Then Nanodrop spectrophotometer measurements indicated variation in concentration and purity of DNA extracts. The total concentrations ng/µl of the sample were found as AM 516.3, AS 1248.5, AD 212.9, and AK 1353.3. The accurate DNA extraction results at A260/280nm were calculated by visible spectrophotometer with the values AM 1.74, AS 2.02, AD 1.80, and AK 2.00 indicating high yield of genomic DNA quality. Also, the results of absorbance at 260/230 nm calculating with the values were recorded as AM 1.78, AS 2.36, AD 1.95, and AK 2.16. The results showed that the DNA of fish sample from Sittwe and Kalay were much better than other samples in DNA concentration.

As a promising alternative to the traditional species identity based on morphological characters, partial Cytochrome C oxidase subunit I (COI) sequences (DNA barcodes) had been recommended for standardized and routine species identification (Hebert *et al*., 2003b). The present work was concluded as the first attempt to assess the molecular genetic status and phylogenetics of the Myanmar Climbing perch *Anabas testudineus* (Bloch, 1792) (Nga bye ma) as a native species. By using COI barcoding and identification technique, the sequence was aligned on 615 bp length for comparison between different sequences. The sequence was checked for the mutation points before analysis. The COI data combined with the morphometric analysis enabled us to ascertain the fish species. Phylogenetic and molecular evolutionary analyses were conducted using the genetics software MEGA X (Molecular Evolutionary Genetics Analysis) (Tamura and Nei, 1993), by NJ (Neighbour-Joining) and Maximum Likelihood (ML) methods and Inferred ancestral sequences (Kumar, 2018) analyses.

The results revealed that the Nga bye ma specimens from four geographical sites (i.e Maubin, Sittwe, Dawei, and Kalay) clustered in a single clade with strong supporting confidence values in the Neighbor–joining (NJ) and Maximum likelihood (ML) phylogenetic tree analyses. This means that the Myanmar Nga bye ma (*Anabas testudineus*) is distinct species different from other related species referenced from GenBank such as *Anabas testudineus* and the same genus *Anabas cobojius.* Regarding haplotypes, CO1 analysis, it reveals only two haplotypes among the four Nga bye ma specimens from four geographical sites, with only two variable nucleotide sites found in only AS (Sittwe) and AD (Dawei) whereas AK (Kalay) and AM1 (Maubin) showing identical haplotype.

It indicated mitochondrial DNA lineage with two variable sites indicating genetic diversity among the populations of *Anabas testudineus* distributed in the coastal region, Ayeyarwady Delta, and Chindwin basin of Myanmar.

In the obtained data of the present study, evolutionary divergence composed of different CO1 haplotype sequences has no remarkable genetic distance ranged from 0.0016 to 0.0032%. If nucleotide G among sites AS, AD, AM1, and AK in native Nga bye ma in Myanmar is compared with the same G sites of India, Indonesia, Korea, and Vietnam, it showed similarity (90-95%) whereas nucleotide T sites of India and Bangladesh had (90-95%) and then outgrowth *A. cobojius* species of nucleotide C sites had (92-95%).

Conclusion

The results of the study provided inclusive records. Numerous studies had been devoted to investigate *A. testudineus* in Myanmar including morphological and molecular genetics analysis. The present study revealed remarkably low genetic variation of the fish *A. testudienus* from different regions. It was possible to produce the molecular database that assembles the molecular characteristics of the species distributed in Myanmar. These sequences can also be used in further studies to determine the genetic divergence of *Anabas* species distributed in different geographic locations. The present information assists the management of the fish population in the wild to prevent them from being extinct, and increase awareness of fishermen and local people.

Acknowledgments

I am greatly indebted to Dr Tin Maung Tun, Rector, and Dr Khin Chit Chit, Dr Cho Cho and Dr Thidar Aye, Pro-Rectors, University of Yangon for their constant encouragement. I am greatly indebted to Dr Moe Moe Dwe, Professor and Head (Retired), Zoology Department, Dagon University for his encouragement and valuable advice. Special thanks go to Dr Kay Lwin Tun, Professor and Head, Dr Sandar Win, Dr Aye Aye Khaing, and Dr Khin Wai Hlaing, Professors, Department of Zoology, University of Yangon for giving the opportunity to publish this research paper.

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