

## Phylogenetic Analysis of *Cyclocheilichthys apogon* and *Cyclocheilichthys armatus* (Fish: Cyprinidae) from West Sumatra

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### ARTICLE INFO

#### Article history:

Received June 25, 2022

Received in revised form March 15, 2023

Accepted April 20, 2023

#### KEYWORDS:

Catua fish,  
Cytochrome b,  
Cytochrome oxidase-I,  
Genetic diversity,  
Monophyletic,  
Turiak fish

### ABSTRACT

*Cyclocheilichthys apogon* (Beardless Barb fish) and *Cyclocheilichthys armatus* (Hihgfin Barb fish) in West Sumatra is known by the local name Turiak and Catua fish, respectively, are economically important species. Reducing populations and limited biological information about these fishes make their genetic diversity and phylogenetic relationships crucial. The genetic variation was investigated by analyzing the sequencing of a part of mitochondrial DNA Cytochrome b (Cyt b) and Cytochrome oxidase-I (COI) genes of 23 individuals collected from West Sumatra populations. Phylogenetic analysis revealed the monophyletic lineage of the *Cyclocheilichthys* group and supported *Anematischthys* as the synonym of *Cyclocheilichthys*. *C. armatus* was closely related to *C. janthochir* and separated from *C. apogon* with sequences divergence were 12.7-14.0% (Cyt b) and 9.4%-10.0% (COI). *C. apogon* and *C. armatus* have low haplotype diversity and genetic diversity, respectively. Genetic diversity within and among populations with sequences divergence was 0.0-0.8% (Cyt b) and 0.0%-0.7% (COI), respectively. The low-level diversity may signify a possibility of gene flow within and among populations. The current data suggests all populations as a single stock unit. Present genetic information is important to establishing effective population management, conservation, and breeding strategies for Turiak and Catua fish.

## 1. Introduction

*Cyclocheilichthys* (Bleeker 1859a) is a Southeast Asia endemic fish widely distributed throughout Indonesia's lakes and rivers (Kottelat *et al.* 1993; Kottelat 2013). *Cyclocheilichthys* members are distinguished based on the rows' of pores on the snout, (Kottelat *et al.* 1993; Rainboth 1996). Some species currently belonging to *Cyclocheilichthys* were assigned earlier into different genera, such as *Barbus apogon* (Valenciennes 1842), *B. enoplos* (Bleeker 1850), *Puntius repasson* (Bleeker 1853), and *Anematischthys apogon* (Bleeker 1860). Furthermore, Bleeker (1859b) used *Cyclocheilichthys* and *Anematischthys* in the list species of Banka as *Cyclocheilichthys* (*Anematischthys*) *apogon*. However, Bleeker (1860) assigned *Anematischthys* as a subgenus and prioritized *Cyclocheilichthys* as a genus. Meanwhile, other authors (Kottelat 1999, 2013; Roberts 1989)

considered them as one genus (*Cyclocheilichthys*). Currently, *Cyclocheilichthys* consists of seven species (Kottelat 2013), whereas previously, there were eight species (*Cyclocheilichthys enoplos* transferred to the new genus as *Cyclocheilos*) (Rainboth 1996).

Among *Cyclocheilichthys*, five species are distributed in Indonesia, i.e., in Sumatra, Java, and Kalimantan (Kottelat *et al.* 1993; Kottelat 2013). In the previous study, Roesma (2011) found two species, *C. apogon* (Valenciennes, in Cuvier and Valenciennes 1842) and *C. armatus* (Valenciennes, in Cuvier and Valenciennes 1842) in West Sumatra rivers and lakes. The local names of these fish were Turiak and Catua fish, respectively, which are economically important as food and ornamental fish (Kottelat *et al.* 1993; Rainboth 1996). In the IUCN Red List (Lumbantobing and Vidthayanon 2020; Thinh *et al.* 2020), *C. apogon* and *C. armatus* status are noted as Least Concern (LC) with their wide distribution and still abundant. However, the fishers have recently found it hard to find *C. apogon* and *C. armatus* in West Sumatra rivers and lakes. In addition, human activities (overfishing), pollution, and habitat degradation may influence fish

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survival, thus reducing the number of populations (Sumaila *et al.* 2011). Moreover, they have limited biological information due to their lack of studies among them (Kottelat *et al.* 1993; Rachmatika *et al.* 2006; Suhendra *et al.* 2017).

Several *C. apogon* studies have been conducted only on the aspect of food composition (Hedianto *et al.* 2010), food and feeding habits (Hamid *et al.* 2015), genetic variation (Kenthao *et al.* 2016), reproductive biology (Suhendra *et al.* 2017), and morphological variation of *C. apogon* from Rivers in Thailand (Kenthao and Jearranaiprepame 2018). Meanwhile, the study about *C. armatus* was only conducted in the cytogenetic analysis (Chaiyasan *et al.* 2018). Therefore, there was no available genetic information on *C. apogon* and *C. armatus* in Sumatra. The information on genetic variation, population structure, and geographical distribution was required to determine effective management and conservation plan (Ward 2000; Tsukagoshi *et al.* 2015).

The phylogenetic study is one of the studies that provide basic data and information to determine the appropriate step for management and conservation (Barik *et al.* 2018; Eagderi *et al.* 2017; Kenthao *et al.* 2016; Pasco-viel *et al.* 2012; Ryman 1991; Ward 2000; Roesma *et al.* 2018, 2019, 2020, 2022; Zareian and Esmaeili 2017). Mitochondrial DNA (mtDNA) has been used for phylogenetic studies due to maternal inheritance, no recombination, fast evolutionary rate relatively, and high nucleotide substitution rate (Behera *et al.* 2017; Brown *et al.* 1979; Chakrabarty 2010; Domingues *et al.* 2007; Timm *et al.* 2008; Morrison *et al.* 2009). Two genes of mtDNA (Cytochrome b and Cytochrome oxidase-I) having the conserved region and high mutation rate, therefore widely used to identify genetic diversity, differentiate species, phylogenetic study, and DNA barcoding for various taxa in animals (Hebert *et al.* 2003; Chen *et al.* 2018; Jennings *et al.* 2019; Kenthao *et al.* 2016; Paine *et al.* 2007; Vandewoestijne *et al.* 2004; Ward *et al.* 2005; Young *et al.* 2013; Zhang and Hewtl 1997).

Combining two genes is more reliable for detecting species's phylogenetic relationship and genetic variation. Further research using two mtDNA genes (Cyt b and COI) is needed. Although *C. apogon* and *C. armatus* have wide distribution, the limited biological information, especially genetics, and the population decline will impact their existence. The availability of genetic data is needed as primary data

for effective population management, conservation plans, and breeding strategies. Therefore, using two mtDNA genes, the present study investigated the genetic diversity and phylogenetic relationship of *C. apogon* and *C. armatus* in West Sumatra.

## 2. Materials and Methods

### 2.1. Sample Collection

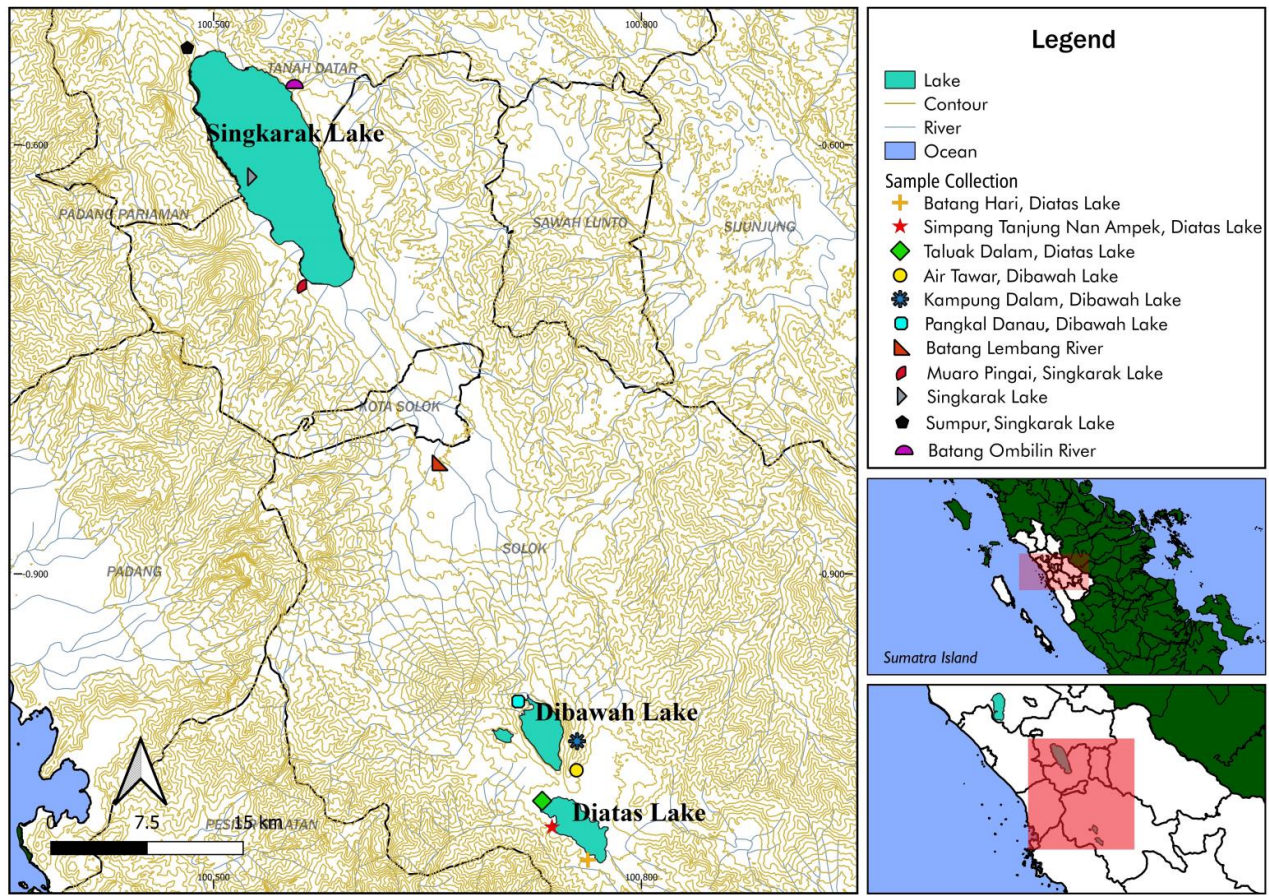
Samples were caught with gill nets and electrofishing in the three lake populations in West Sumatra (Singkarak Lake, Diatas Lake, and Dibawah Lake). The three lake populations are divided into 11 sub-populations among them; Singkarak Lake population consists of five sub-populations (Sumpur, Muara/Muaro Pingai, Batang Lembang, Batang Ombilin, and Singkarak Lake); Diatas Lake population consists of three sub-populations (Batang Hari, Simpang Tanjung Nan Ampek, and Teluk/Taluak Dalam), and Dibawah Lake population consist of three sub-populations (Air Tawar, Kampung Dalam, and Pangkal/Kapalo Danau) (Figure 1). The sample collection has obtained a legal permit from Biology Department at Andalas University. 23 individuals from 11 sub-populations were used for phylogenetic analysis consisting of 20 individuals of *C. apogon* and three individuals of *C. armatus* (Table 1).

Fish samples were morphologically identified as *C. apogon* and *C. armatus* by Roesma (2011) based on the identification book (Kottelat *et al.* 1993; Rainboth 1996). The tissue samples were collected for genetic study and preserved in ethanol absolute. Individuals' samples were soaked in 10% formalin for several weeks. After that, the samples were washed using running water and stored in a box containing 70% ethanol.

### 2.2. DNA Extraction, Polymerase Chain Reaction (PCR), and DNA Sequencing

DNA from tissue samples was isolated using the protocol KIT Invitrogen (Thermo Fisher Scientific, AS). DNA amplification for Cyt b gene using primer pairs based on Roesma (2011) (5' CGATTCTTYGCNTTCCAYTTCYT 3' forward and 5' CCTCCRATCTTCCG ATTACAAGAC 3' reverse). DNA amplification for COI gene using primer pairs (FISH F1 and FISH R1) based on Ward *et al.* (2005) (5' TCAACCAACCACAAAGACATTGGCAC 3' forward and 5' TAGACTTCTGGGTGGCCAAAGAATCA3' reverse). The solution composition for the Cyt b and COI

### Map of sampling locations in the three lakes from West Sumatra



Map generated using QGIS v 3.24

Figure 1. Sampling locations of *Cyclocheilichthys apogon* and *Cyclocheilichthys armatus* in the three lake populations (11 sub-populations) from West Sumatra (source: administration map of West Sumatra and generated using QGIS v 3.24)

Table 1. Species list, number of samples (n) using Cytochrome b (Cyt b) and Cytochrome Oxidase I (COI) genes analysis, and sampling locations (11 sub-populations) of *Cyclocheilichthys apogon* and *Cyclocheilichthys armatus* in West Sumatra

Species	n		Sampling locations (sub-populations)
	Cyt b	COI	
<i>Cyclocheilichthys apogon</i>	2	2	Teluk/Taluak Dalam, Djabat Lake
<i>Cyclocheilichthys apogon</i>	1	2	Simpang Tanjung Nan Ampek, Djabat Lake
<i>Cyclocheilichthys apogon</i>	1	2	Batang Hari, Djabat Lake
<i>Cyclocheilichthys apogon</i>	4	2	Air Tawar, Dibawah Lake
<i>Cyclocheilichthys apogon</i>	2	2	Kampung Dalam, Dibawah Lake
<i>Cyclocheilichthys apogon</i>	1	2	Pangkal/Kapalo Danau, Dibawah Lake
<i>Cyclocheilichthys apogon</i>	3	3	Batang Lembang River
<i>Cyclocheilichthys apogon</i>	3	3	Muara/Muaro Pingai, Singkarak Lake
<i>Cyclocheilichthys apogon</i>	3	2	Sumpur, Singkarak Lake
<i>Cyclocheilichthys armatus</i>	1	-	Singkarak Lake
<i>Cyclocheilichthys armatus</i>	2	2	Batang Ombilin River



amplification follows the protocol from the previous studies (Roesma 2011; Roesma *et al.* 2018, 2019, 2020, 2022). The temperature setting of amplification cycles follows the protocol by Roesma (2011) for Cyt b and the protocol by Ward *et al.* (2005) for COI. DNA amplification products were checked by electrophoresis on 2% agarose gels. The successful products were sent for sequencing to MacroGen Inc. DNA Sequencing Laboratory (South Korea).

### 2.3. Data Analysis

The DNA STAR software is used to contig and checks the raw DNA sequences (Burland 2000). The contig sequence's similarity was checked with the Genbank sequences (Table 2) using the online website (Basic Local Alignment Search Tool). The Clustal X software was used for arranged sequences (Thompson *et al.* 1997) and then checked using the BIOEDIT software (Hall 1999). The amino acids of sequences were checked using the DNA to protein translation website (<http://insilico.ehu.es/translate>). The value of molecular diversity (polymorphism sites, nucleotide diversity, and haplotype diversity) was calculated with the DNA SP software (Rozas *et al.* 2003). Genetic distances and phylogenetic trees (represented by Maximum Likelihood (ML) tree) were computed and constructed using the Molecular Evolutionary Genetics Analysis (MEGA) 6.0 software with the Kimura two-parameter (K2P) model (Tamura *et al.* 2013).

## 3. Results

### 3.1. Sequence Data

#### 3.1.1. Cyt b Gene

A total of 39 sequences were analyzed where are, 16 of them obtained from GenBank, NCBI. BLAST analysis showed that the *Cyclocheilichthys* in West Sumatra were significantly similar (99-98%) with the GenBank sequence database. A total of 577 bp from 650 bp the partial region of the Cyt b gene, was used for the analysis. Out of 577 bp were analyzed, 257 bp (44.54%) as variable sites, where 204 bp (79.38%) of variable sites as parsimony sites, and 53 bp (20.62%) as singleton sites. The nucleotide base's average compositions were Guanine-deficient (12.30%), while the other nucleotides were Adenine: 32.00%, Cytosine: 29.20%, and Thymine: 26.50%. The sum of Adenine + thymine is 58.50% higher than Cytosine + Guanine. The Cyt b region analysis showed 310 mutations for all sequences, with a bias ratio of transitions to transversions was 2.551.

A total of 21 haplotypes were identified for all sequences. Seven haplotypes were observed for *Cyclocheilichthys* in West Sumatra and 14 for GenBank sequences. The mean haplotype diversity (Hd) and nucleotide diversity (Pi) for all Cyt b sequences were 0.870 and 0.110, respectively. While the haplotype diversity (Hd) among *Cyclocheilichthys* in West Sumatra of 0.628, and the nucleotide diversity (Pi) of 0.0277. Hap 01 was the dominant haplotype for

Table 2. List of species taxonomy, location, and GenBank accession numbers of samples

Family	Genus	Species	Location		Genbank accession numbers			
			Cyt b	COI	Cyt b	COI		
Cyprinidae	<i>Cyclocheilichthys</i>	<i>Cyclocheilichthys apogon</i>	-	Malaysia	-	JF781182.1		
			-	-	-	JF781184.1		
		<i>Cyclocheilichthys enoplos</i>	Laos	Laos	JQ346139.1	JQ346162.1		
			Cambodia	-	JX066771.1	-		
		<i>Cyclocheilichthys janthochir</i>	-	-	HM536808.1	HM536907.1		
			-	-	EU241418.1	JF915567.1		
		<i>Cyclocheilichthys heteronema</i>	Indonesia	-	KP712208.1	-		
			<i>Anematichthys</i>	<i>Anematichthys apogon</i>	Laos	Laos	JQ346138.1	JQ346161.1
		<i>Chosmochilus</i>		<i>Chosmochilus harmandi</i>	Laos	Laos	JQ346140.1	JQ346163.1
		<i>Chosmochilus cardinalis</i>		-	-	HQ235761.1	-	
		<i>Capoeta</i>	<i>Capoeta damascina</i>	Iran	Turkey	JF798326.1	KJ553216.1	
			<i>Capoeta caelestis</i>	-	Turkey	KY065261.1	KJ553030.1	
		<i>Acrossocheilus</i>	<i>Acrossocheilus paradoxus</i>	Taiwan	-	HM142579.1	MF122061.1	
			<i>Acrossocheilus cinctus</i>	China	China	JX066772.1	KJ994646.1	
<i>Leuciscus</i>	<i>Leuciscus idus</i>	-	German	HM560099.1	KM286734.1			
	<i>Leuciscus leuciscus</i>	Spain	Spain	AY509823.1	HM560272.1			
Botiidae	<i>Botia</i>	<i>Botia rostrata</i>	-	China	AY887788.1	KM610433.1		
Danioninae	<i>Danio</i>	<i>Danio choprai</i>	-	-	EF452740.1	EF452879.1		

*C. apogon* in West Sumatra and shared by *C. apogon* in nine different populations. Sequence divergences between *C. apogon* populations of 0.0-0.8%, sequence divergences between *C. armatus* populations of 0.0-0.4%, and sequence divergences between *C. apogon* and *C. armatus* species were 12.7-14.0%. At the same time, sequence divergences between outgroup (species name must be in italic *Botia rostrata* and *Danio choprai*) and ingroup ranged from 18.9 to 33.9%.

### 3.1.2. COI Gene

A total of 37 COI sequences were analyzed where are 15 of them obtained from GenBank, NCBI. BLAST analysis showed that the *Cyclocheilichthys* in West Sumatra were significantly similar (99-98%) with the GenBank sequence database. A total of 567 bp was used for the analysis, among 720 bp of the partial COI gene. Out of 567 bp were analyzed, 200 bp (35.27%) as variable sites, where 164 bp (82.00%) of them as parsimony sites, and 36 bp (18.00%) as singleton sites. The nucleotide base's average compositions were Guanine-deficient (16.30%), while the other nucleotides were Adenine: 27.60%, Cytosine: 27.20%, and Thymine: 28.90%. The sum of Adenine + Thymine is 56.50% higher than Cytosine + Guanine. The COI region analysis showed 270 mutations for all sequences, with a bias ratio of transitions to transversions was 4.02.

A total of 18 haplotypes were identified with four haplotypes for *Cyclocheilichthys* in West Sumatra and 14 haplotypes for GenBank sequences. The mean haplotype diversity (Hd) and nucleotide diversity (Pi) for all COI sequences were 0.742 and 0.080, respectively. While the haplotype diversity (Hd) among *Cyclocheilichthys* in West Sumatra of 0.260 and nucleotide diversity (Pi) of 0.014. Hap 01 was the dominant haplotype of *C. apogon* from West Sumatra and was shared by *C. apogon* in nine different populations. Sequence divergences between *C. apogon* populations of 0.0-0.2%, sequence divergences between *C. armatus* populations were 0.7%, and sequence divergences between *C. apogon* and *C. armatus* species were 9.4%-10.0%. While sequence divergences between outgroup (*Botia rostrata* and *Danio choprai*) and ingroup ranged from 20.3 to 27.0%.

### 3.1.3. Phylogenetic Relationships

The phylogenetic trees are constructed using an ML tree (Figure 2 and 3) with a 1,000 bootstrap. The bootstrap values showed the reliability level of a phylogenetic tree. The value at each branching indicates the percentage of bootstrap support. The bootstrap value above 50 indicates a confidence level in the medium to high range, and the bootstrap value below 50 indicates the low range. Based on the analysis of two genes, the phylogenetic trees showed that *Cyclocheilichthys* formed a monophyletic lineage (first cluster).

The phylogenetic trees (Cyt b and COI) have the same lineages with two main clusters. The first cluster of the trees is the *Cyclocheilichthys* groups, which consists of three sublineages; *C. apogon* and *A. apogon* (first sublineage), *C. heteronema* (second sublineage), *C. janthochir* and *C. armatus* (third sublineage) for Cyt b gene and two sublineages; *C. apogon*, *A. apogon* (first sublineage), and *C. janthochir* and *C. armatus* (second sublineage) for the COI gene. *C. apogon* and *C. armatus* in West Sumatra species separated into different sublineages with sequence divergences were 12.7-14.0% (Cyt b) and 9.4-10.0% (COI). Therefore, the first cluster's sublineage is occupied by *C. apogon* and *A. apogon*, supported by the highly bootstrap value. The sequence divergence between *C. apogon* and *A. apogon* was 12.9-13.2% (Cyt b) and 8.0-8.5% (COI). While *C. apogon* in West Sumatra populations (first sublineage) have low sequence divergences were 0.0-0.8% (Cyt b) and 0.0-0.2% (COI), respectively. These values suggested *C. apogon* populations have low genetic diversity.

The second sublineage in the Cyt b tree has only one member, namely *C. heteronema*. The sequence divergence between the second sublineage with other sublineages in the first cluster was 14.0-18.8%. At the same time, the second sublineage in the COI tree consists of *C. armatus* and *C. janthochir*. These species have a high sequence divergence of 9.2-10.3% to the first sublineage (different species). However, *C. armatus* and *C. janthochir* are different species and have low sequence divergences (4.3-5.1%). *C. armatus* in West Sumatra populations have low sequence divergences of 0.0-0.4% (Cyt b) and 0.7% (COI), respectively. These values suggested that *C. armatus*

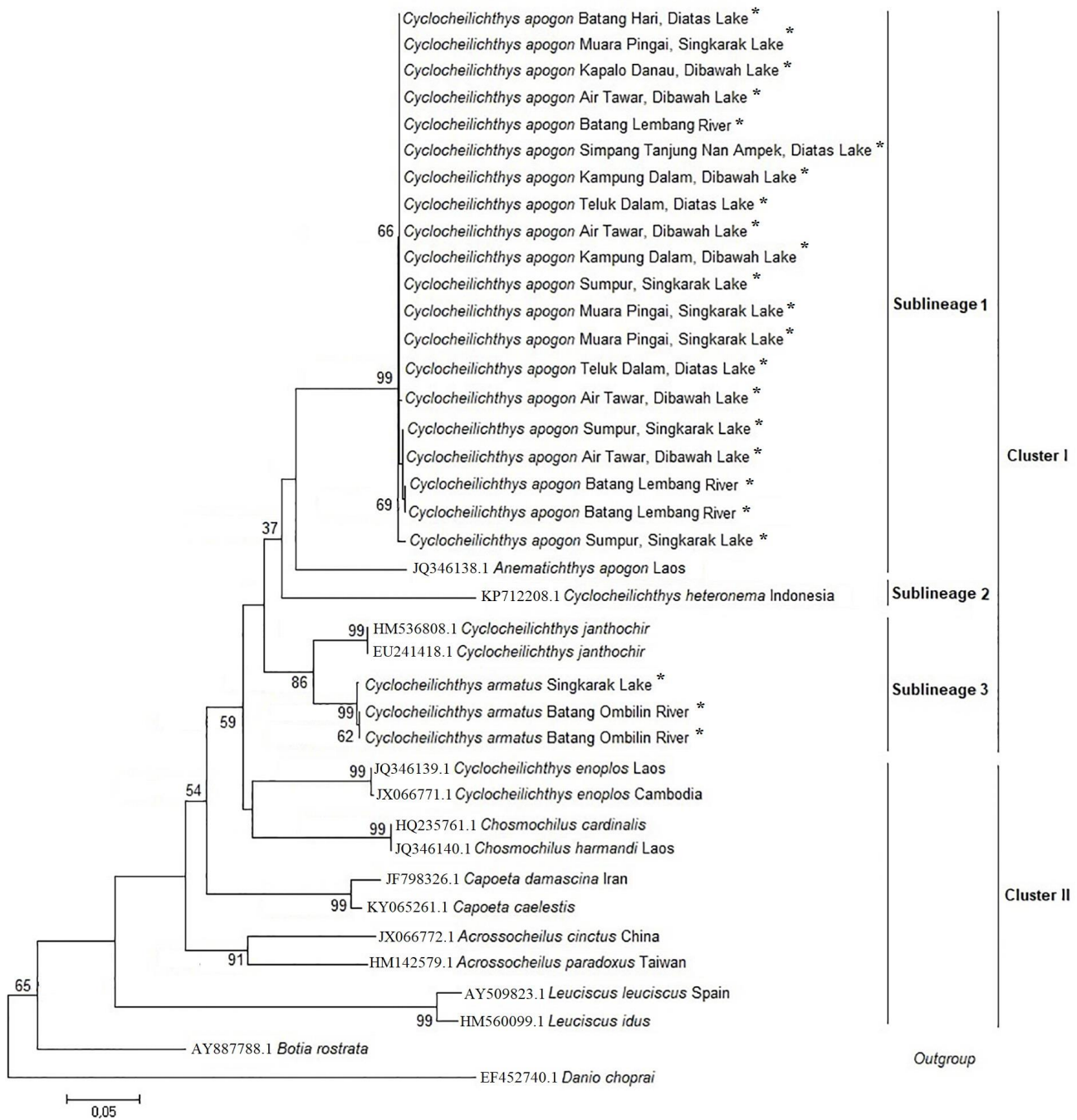


Figure 2. The Maximum Likelihood (ML) phylogenetic tree of the partial gene of the Cyt b gene with bootstrapping of 1,000 replicates. The number at each branching indicates the percentage of bootstrap support. The asterisk (\*) showed the *Cyclocheilichthys apogon* and *Cyclocheilichthys armatus* sequence from West Sumatra

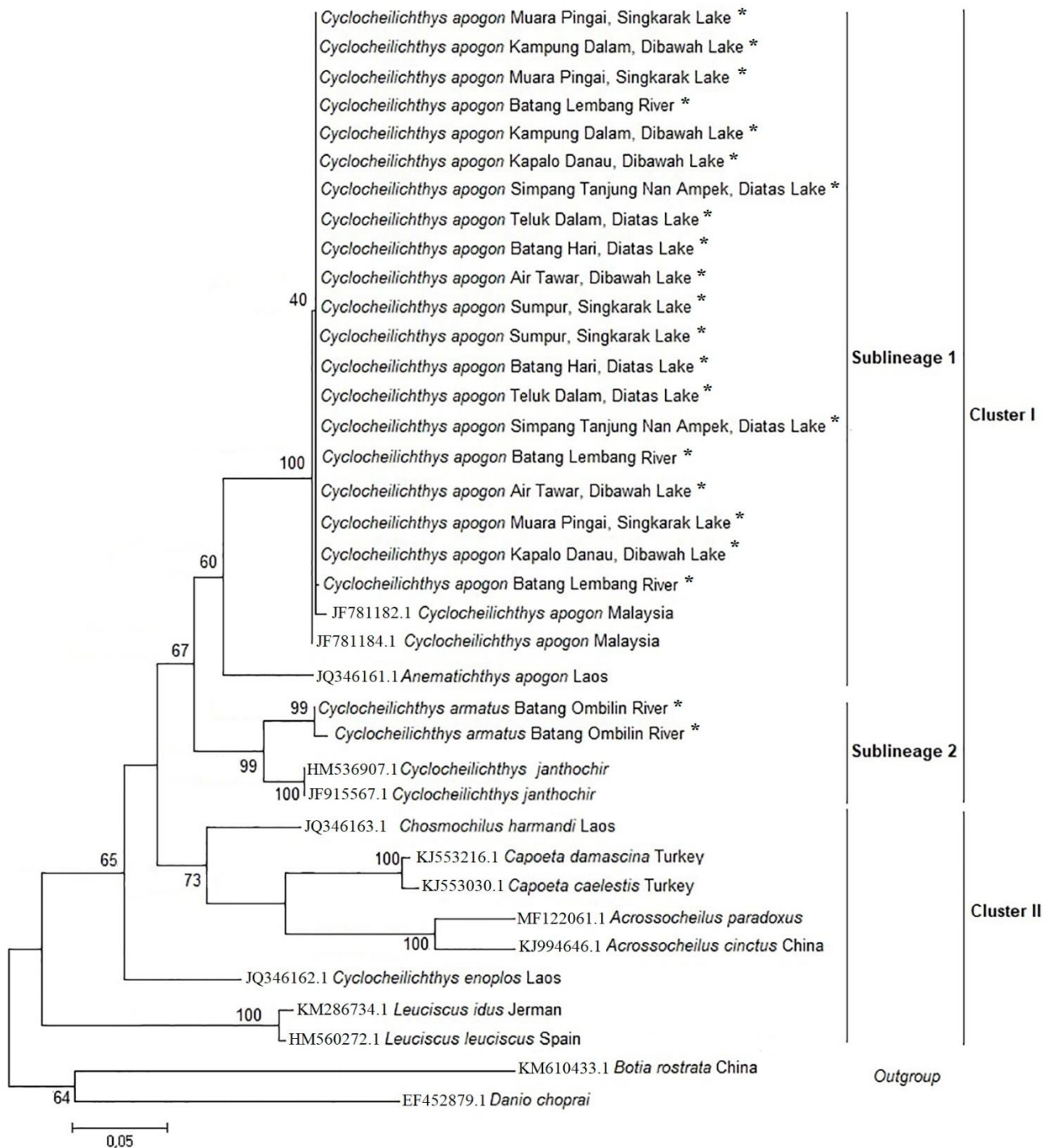


Figure 3. The Maximum Likelihood (ML) phylogenetic tree of the partial gene of the COI gene with bootstrapping of 1,000 replicates. The number at each branching indicates the percentage of bootstrap support. The asterisk (\*) showed the *Cyclocheilichthys apogon* and *Cyclocheilichthys armatus* sequence from West Sumatra



populations have low genetic diversity. The third sublineage of the Cyt b tree consists of *C. armatus* and *C. janthochir*, with 12.7-16.4% sequence divergences from the other sublineage in the first cluster. *C. armatus* and *C. janthochir* (Cyt b gene) also have low sequence divergences (6.6%) as different species, similar to the result obtained in the COI result. The COI tree only has two sublineages due to the lack of other *Cyclocheilichthys* COI sequences in Genbank, NCBI.

The second cluster of the genus was considered a synonym to *Cyclocheilichthys* (*Cosmochilus*, *Accrossocheilus*, *Capoeta*, and *Leuciscus*). The first and second clusters were separated with high sequence divergences of 14.5-27.8% (Cyt b) and 11.1-19.4% (COI). The high sequence divergence supports the separation between *Cyclocheilichthys* and the members of the second cluster into the different genera. *C. enoplos* was present in the second cluster and separated from *Cyclocheilichthys* groups (first cluster). *C. enoplos* was closely related to the other genera (*Cosmochilus*) in the Cyt b tree. At the same time, *C. enoplos* formed the separate sublineage from other *Cyclocheilichthys* in the COI tree. *C. enoplos* separated from the *Cyclocheilichthys* group with the sequence divergences was 11.7-17.8% (Cyt b) and 11.1-13.7% (COI).

#### 4. Discussion

The partial sequence (Cyt b and COI gene) of *C. apogon* and *C. armatus* in West Sumatra had been successfully amplified, and the sequence similarities 99% with the GenBank database. The nucleotide base's average compositions were almost similar to the previous *C. apogon* study by Kenthao *et al.* (2016). AT ratio was higher than the GC ratio, consistent with earlier studies for Cyprinidae (Johns and Avise 1998; Kenthao *et al.* 2016). The AT ratio higher than GC is characteristic of mtDNA's genome in vertebrates (Hubert *et al.* 2008). Guanine composition is lower than other nucleotide bases. Strange and Maiden (2009) reported that guanine composition is approximately 10% between other nucleotides, lower in the third codon than in the first and second codons. Our results provided insight into the *Cyclocheilichthys* group's phylogenetic relationship and genetic variation, especially the *C. apogon* and *C. armatus*. Phylogenetic analysis showed *Cyclocheilichthys* form a monophyletic group and *Cosmochilus* as a sister

group. In the previous study by Pasco-viel *et al.* (2012), *Cyclocheilichthys* form a non-monophyletic group, whereas *C. enoplos* are grouped with *Puntioplites* and *Cosmochilus* groups. Therefore, Pasco-viel *et al.* (2012) considered *C. enoplos* as *Cyclocheilichthys* species and proposed *C. armatus*, *C. apogon*, and *C. repasson* as *Anematichthys* species. However, Kottelat (2013) reported that *C. armatus*, *C. apogon*, and *C. repasson* as valid species of *Cyclocheilichthys* and considered *Anematichthys* synonym name, and changed *Cyclocheilichthys enoplos* to the new genus (*Cyclocheilos enoplos*). Consequently, Pasco-Viel *et al.* (2013) corrected the previous report and agreed with Kottelat's opinion. In this study, *C. enoplos* separated from the *Cyclocheilichthys* group's with high sequence divergences (difference at the genera level). Therefore, our results supported the alteration of *C. enoplos* into new genera as *Cyclocheilos enoplos* by Kottelat (2013).

*A. apogon* and *C. apogon* clustered together in the first cluster with sequences divergence was 12.9-13.2% (Cyt b) and 8.0-8.5% (COI). The value of sequence divergences reported by Field (Kartavtsev 2011, 2013) places *C. apogon* and *A. apogon* at the same species level. The taxonomy revision by Kottelat (2013) supported the result of this study and stated that *A. apogon* is a synonym of *C. apogon* (the same species).

*C. apogon* and *C. armatus* have been collected from three lake populations consisting of 11 sub-populations in West Sumatra. The phylogenetic tree showed all populations in West Sumatra grouped in the same cluster with low sequence divergences. *C. apogon* and *C. armatus* in West Sumatra species separated into different sublineages with the sequence divergences were 12.7-14.0% (Cyt b) and 9.4-10.0% (COI). Based on (Kartavtsev 2011, 2013), this value supported *C. apogon* and *C. armatus* as different species in the same genus.

In the *Cyclocheilichthys* lineages, the phylogenetic analysis tree showed *C. armatus* closer related to *C. janthochir* than to *C. apogon*. *C. armatus* and *C. janthochir* have lower genetic distance than *C. armatus* and *C. apogon*. Yang *et al.* (2012) also reported that *C. janthochir* is closer related to *C. armatus* in the phylogenetic tree.

Based on haplotype diversity and genetic distance, the phylogenetic trees show the relationship and genetic diversity between populations. *C. apogon* from three lake populations (nine sub-populations) has



five haplotypes for Cyt b and two haplotypes for COI analysis. *C. armatus* from Singkarak Lake populations (two sub-populations) has two haplotypes for Cyt b and one haplotype in one population for COI analysis. Genetic distance within and among *C. apogon* species was 0.0-0.8% (Cyt b and COI), and within and among *C. Armatus* species was 0.0-0.7% (Cyt b and COI). The low haplotype diversity and genetic distance showed the low genetic diversity of *C. apogon* and *C. armatus* species, respectively.

These conditions can be associated with geographical distances and the gene flow between populations. The results showed the gene flow consistent with the geographical distances from populations. Wang *et al.* (2000) reported that haplotype and nucleotide diversity was associated with abundance, dispersal, and geographical distance. Diatas Lake, Dibawah Lake, and Singkarak Lake are estimated to have been connected in the past, making it easier to mix populations genetically. Therefore, it's possible to result in the low genetic diversity of *Cyclocheilichthys* species in West Sumatra.

The same case was also reported on *Barbodes banksi* (Cyprinidae) from Diatas Lake, Dibawah Lake, and Singkarak Lake populations with low genetic diversity because those three lakes have been connected in the past (Roesma *et al.* 2018). Furthermore, there are two main rivers among several rivers as an inlet of Singkarak Lake; the Sumpur River (Northwest) and the Sumani River (Southeast). Dibawah Lake has an outlet namely the Batang Lembang River (Peranginangin *et al.* 2004; Siddiq *et al.* 2019). The situation allows Dibawah Lake and Singkarak Lake to be interconnected by the Batang Lembang River and Sumani River. Therefore, the fish populations are categorized as sympatric populations in the past. Singkarak Lake only has one outlet namely the Ombilin River (Peranginangin *et al.* 2004).

Diatas Lake and Dibawah Lake are known as the Twin Lakes and are not interconnected fields (Lehmusluoto and Machbub 1997). Based on the explanation of an active region, the Bukit Barisan mountains always experience tectonic-volcanic dynamics (Katili 1980). Roesma *et al.* (2018) reported the possibility of concatenating Diatas and Dibawah lakes in the past. This condition allows gene flow between lake populations, maintaining their genetic similarities. Therefore Diatas Lake, Dibawah Lake,

and Singkarak Lake populations can be categorized as sympatric. The three concatenated lakes flowed east of the Bukit Barisan mountain range. Wang *et al.* (2000) explained that geographic isolation becomes the barrier to migration between populations. It's become one factor that appears the genetic variations. However, some rare floods created migration opportunities across the drainage systems at lower elevations, which reduced the difference between populations.

There are too many factors that contribute to genetic variations. Franck *et al.* (1998) stated that the high nucleotide diversity occurred due to geographical isolation and high species abundance. Vice versa, low nucleotide diversity can happen due to the high gene flow between populations or bottleneck phenomenon. Hedrick (1999), Ma *et al.* (2010), Wang *et al.* (2014) described that demographic history, geographic separation, habitat differences, reproductive strategy, and human activities influence the genetic diversity among populations. Kenthao *et al.* (2016) also reported the low genetic diversity of *C. apogon* in Northeastern Thailand. It's possible due to the lack of isolation by geographical distance and gene flow between populations. Zheng and Yang (2017) revealed that *Acrossocheilus yunnanensis* (Cyprinidae) populations originating from the same drainage or adjacent regions showed low haplotype and nucleotide diversity due to the genetic mixing of populations. Khan *et al.* (2017) also reported that factors like hybridization, incomplete lineage sorting, and homoplasy might contribute to the lack of variation in *Schizothoracine* species.

This study provides the information that *C. apogon* and *C. armatus* in the wildlife, represented by three lake populations in West Sumatra have low genetic diversity. These results suggest all populations as a single stock unit. Waples *et al.* (2008) stated that the availability of genetic diversity data of fish species becomes important because it is needed as a recommendation for resource recovery and fishery management. This data provides information for determine the population to be conserved and the population for cultivation. Wang *et al.* (2014) described the various anthropogenic pressures, overfishing, and habitat degradation as negative effects on species' sustainability thus, more attention should be paid to species conservation. Populations with high haplotype diversity can be kept as variation sources and carry out conservation efforts

without overfishing. At the same time, populations with low genetic variation (same haplotype) can be used as a stock unit for cultivation. The future study by expanding the location study to determine the existence of fish with a non-invasive method can be applied to keep the declining population. This study provides the first barcode and genetic information of *C. apogon* and *C. armatus* in West Sumatra, which is useful as reference sequences for Sumatra populations.

### Acknowledgements

The author thanks for the work permit to the Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University. This paper was written reported based on data from research conducted in Genetic and Biomolecular Laboratory. We also want to express our gratitude to students who helped us in the field and laboratory work in the Genetic and Biomolecular Laboratory.

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