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**Wila Karlina**

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra

**Dewi Imelda Roesma**

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra

**Djong Hon Tjong**

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra

## Phylogenetic study of *Puntius cf. binotatus* fish from Gunung Tujuh Lake in Sumatera Based on Cytochrome b Gene

Wila Karlina, Dewi Imelda Roesma, Djong Hon Tjong

### Abstract

Research on the study of phylogenetic *Puntius cf. binotatus* from Gunung Tujuh Lake based on cytochrome b gene. The study aimed to determine the phylogenetic relationships between *Puntius cf. binotatus* from Gunung Tujuh Lake and *Puntius binotatus* from other populations in West Sumatra. DNA sequences were alignment using Clustal X version 1.8 and phylogenetic tree was analyzed using MEGA 6.0. The alignment showed the number of partial of the cytochrome b gene was 530 base pair. The results of the study showed that the genetic divergences between them were 6.6%-11.2%. Therefore, it was possible to place the *Puntius cf. binotatus* as new species with its two unique haplotypes and from the tree, we concluded that there are at least four subspecies or species from our samples.

**Keywords:** phylogenetic, *Puntius cf. binotatus*, cytochrome b gene, genetic divergences

### 1. Introduction

Gunung Tujuh Lake is located in Jambi Province (Sumatera), at the altitude of 1,950 meters above the sea level. This lake has approximately 12 km<sup>2</sup> wide area and maximum of 40 meters depth. It is surrounded by volcanic mountains which has seven peaks and recorded as the highest lake in Southeast Asia [1]. The lake watering came from surrounding water and the outlet is a waterfall with about 75 meters in high. It flows into the East Coast of Sumatra Island through Batang Hari River, Jambi. This waterfall is a very potential as a barrier that does not allow fish migration into the lake. The waterfall can limit the spread of the fish [2]. There were two kind of fish in Gunung Tujuh Lake, one of them was *Puntius cf. binotatus* [3]. The population of *Puntius cf. binotatus* in Gunung Tujuh Lake is considered as a population that isolated from other freshwaters. The phylogenetic relationship with other populations in West Sumatra remains unknown. It is necessary to determine the phylogenetic relationships of *Puntius cf. binotatus* using cytochrome b gene mitochondrial DNA

### 2. Material and Method

In this research, samples were obtained from 14 population, consists of one population in Gunung Tujuh Lake, eight populations in other locations in West Sumatra (Gumanti River, Batang Lembang, Diatas Lake, Muaro Pingai River, Batang Tarok, Batang Kuranji, Asam River Maninjau ang Maninjau Lake) (Figure 1) [4], and five from GenBank (*Barbodes banksi*, *Barbodes aurotaeniatus*, *Barbodes binotatus*, *Barbodes everetti* and *Botia modesta* as outgroup). Total genomic DNA were extracted from about 15-20 mg of tissue according Protocol Kit INVITRO GEN PureLink™ Genomic DNA Mini Kit. Amplifications were carried out in thermal cycler (SensoQuest). Optimization of PCR was done using 25 µl reaction volumes containing 2 µl DNA (15-20 mg), 12 µl Go Taq Green (Promega), 1 µl primer forward, 1 µl primer reverse and 9 µl DDion. Primer were used 5' CGA TTC TTY GCN TTC CAY TTC YT 3' (forward) and 5' CCT CCR ATC TTC CGA TTA CAA GAC 3' (reverse) [4].

Amplifications were done with the PCR process: preliminary denaturation 95 °C for 4 minutes followed by 36 cycles of 94 °C for 1 minute for denaturation, 53 °C for 1 minute for annealing and 72 °C for 2 minutes for elongation. This was followed by a final extension period at 72 °C for 7 minutes before the sample were cooled to 4°C. Each PCR product was run on 2% agarose gel and were used for sequencing. The best PCR product were sequenced in *MacroGen USA DNA Sequencing Laboratory*.

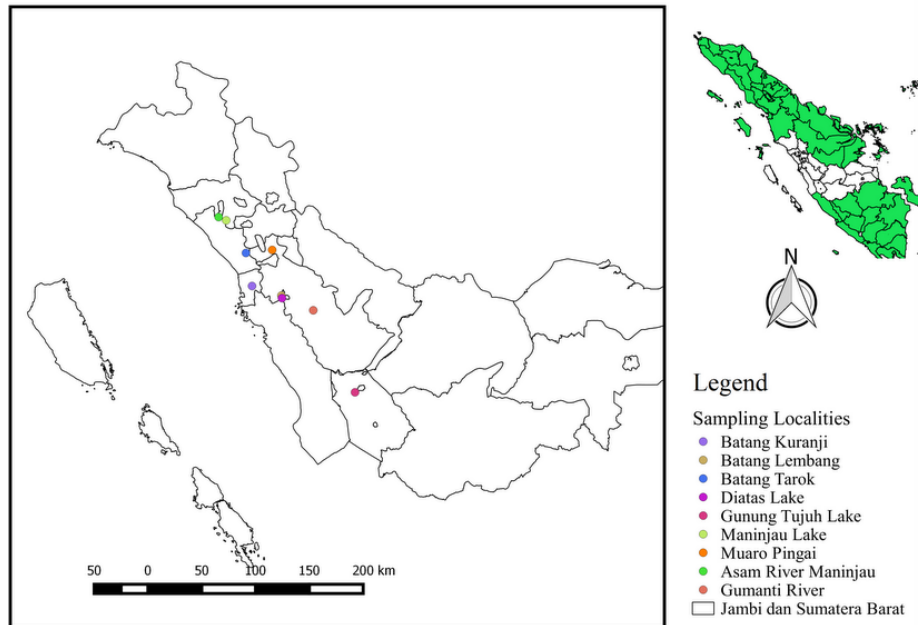
### Correspondence

**Dewi Imelda Roesma**

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra

DNA sequences forward and reverse form of *Puntius cf. binotatus* were contig using DNA STAR program [5]. DNA sequences of *Puntius cf. binotatus* from Gunung Tujuh Lake were compared with observing the similarity DNA sequences in GenBank NCBI (National Center of Biotechnology Information) <http://blast-ncbi.nlm.nih.gov/Blast> and some populations of *Puntius binotatus* that have been researched [4]. All DNA sequences were aligned using Clustal X version 1.8

[6]. Alignments were edited by Bioedit programing [7]. Translate DNA used program online DNA to Protein Translation [8]. The number of polymorphic sites were determined using the software program DNA Sequence Polymorphism 5.10. [9]. The analysis of phylogenetic tree based on DNA sequences cytochrome b gene mitochondrial DNA used the software program MEGA (Molecular Evolutionary Genetics Analysis) 6.0 [10].

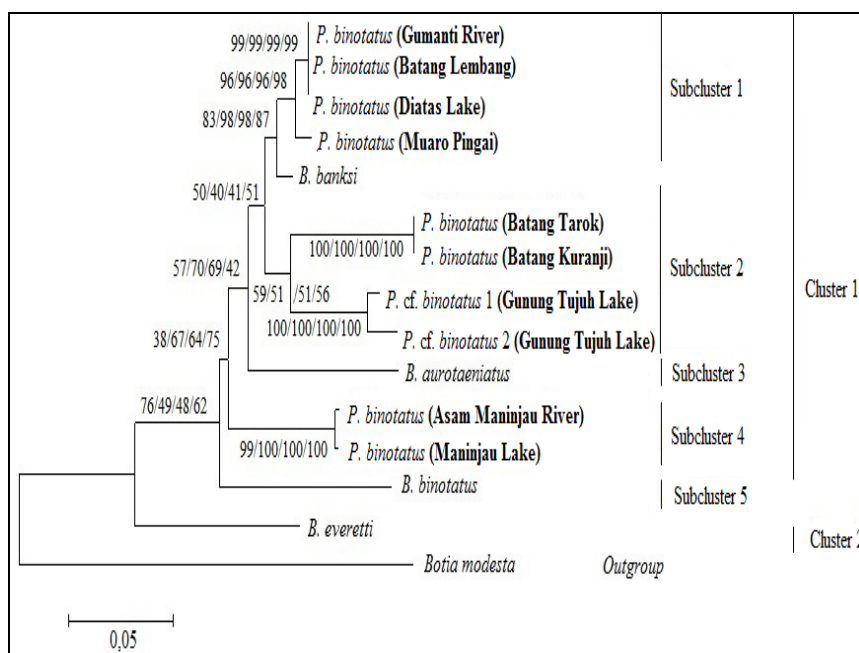


**Fig 1:** Sampling localities of *Puntius cf. binotatus* in Gunung Tujuh Lake and *Puntius binotatus* eight populations in other locations in West Sumatra

**3. Results and Discussion**

The 530 bp part of the cytochrome b gene mitochondrial DNA was analyzed. The composition of nucleotide bases of the cytochrome b gene mitochondrial DNA were 32.4% A (adenine), 24.9% T (Thymine), 30.4% C (Cytosine) and 12.3% G (Guanine). The composition of nucleotide bases were similar to those previously reported for fish cytochrome b sequences [11, 12, 13].

From the 530 bp was obtained, 364 bp were categorized as conserved site, 166 pb as variable site, 96 bp as parsimony site and 70 bp as a singleton site. There were 12 haplotypes from 14 populations which has been analyzed. Thus it can be asserted that the genus *Puntius* on the samples tested (eight populations in West Sumatra and Gunung Tujuh Lake) has an inter-population variation with the presence of 7 haplotypes.



**Fig 2:** The phylogenetic tree of genus *Puntius* based on cytochrome b gene with bootstrapping 1000 (ML/NJ/ME/MP), in brackets indicate the sampling location

The Figure 2 showed the relationship of the genus *Puntius* based on cytochrome b genes. There were two main clusters. The first cluster consists of *P. binotatus*, *Puntius* cf. *binotatus*, *B. banksi* and *B. aurotaeniatus*. The second cluster consists of *B. everetti*. The first cluster consists of five subcluster. Members of the first subcluster were *P. binotatus* from Batang Lembang, Diatas Lake, Gumanti River, Muaro Pingai River and *B. banksi* from Asia. Members of the second subcluster were *P. binotatus* from Batang Tarok and Batang Kuranji and *Puntius* cf. *binotatus* from Gunung Tujuh Lake. Member of the third subcluster was *B. aurotaeniatus* from Laos. Members of the fourth subcluster were *P. binotatus* from Maninjau Lake and Asam River Maninjau. Member of the fifth subcluster was *B. binotatus* from Asia. The division of five subcluster supported by bootstrap values 76/49/48/62 (ML/NJ/ME/MP). Based on the data, genus *Puntius* from our samples (*Puntius* cf. *binotatus* and *P. binotatus*) consist of at least four subspecies or species. According to Kottelat [14], *Puntius* is synonymous name of *Barbodes*.

The genetic divergences of *Puntius* cf. *binotatus* and *P. binotatus* (subcluster 1) were 6.6%-8.1%, *Puntius* cf. *binotatus* and *P. binotatus* (subcluster 2) were 9.1%-9.8%, and *Puntius* cf. *binotatus* and *P. binotatus* (subcluster 4) were 10.8%-11.2%. Therefore, genetic divergences between them were 6.6%-11.2%. This value indicates that the *Puntius* cf. *binotatus* is in a different group with *P. binotatus* from other subcluster and can be asserted as a new species. Based on cytochrome b gene of mitochondrial DNA in vertebrates, the sequence divergence on subspecies, semi-species, and sibling species level was about  $5.10 \pm 0.91\%$ , and the level of different species in a single genus was about  $10.31 \pm 0.93\%$  [15].

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