Molecular phylogenetic of silver barb *barbonymus gonionotus* (bleeker, 1849) (cypriniformes: cyprinidae) in Java, Indonesia

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Abstract. *Barbonymus gonionotus* is a species of cyprinid native to river drainages in southeastern Asia, which occurs in the Cambodia, Vietnam, Thailand, Laos and Indonesia. This study aims to identify the molecular phylogenetic of *Barbonymus gonionotus* in Java, Indonesia based on the Cytochrome C Oxidase Subunit I (COI) gene. Specimen from the Opak river had 656 base pairs with the Identity values 100% to *Barbonymus gonionotus*. The genetic distance of *Barbonymus gonionotus* in the Java was 0.000-0.005. Phylogenetic tree of *Barbonymus gonionotus* in the Java is provided.

1. Introduction

The South-east Asian cyprinid *Barbonymus gonionotus* (Bleeker, 1849) [1,2,3], is a freshwater fish that also known as *Puntius gonionotus* and *Barbodes gonionotus*, native to Cambodia [4], Thailand [5], Laos [6], Vietnam [7], and Indonesia [8]. In Indonesia, this species found in Sumatra and Java [9], furthermore *B. gonionotus* also found in Sulawesi [10] as introduced species.

In Java, *Barbonymus gonionotus* ranged widely in the fresh waters across the Mainland which include West Java, Central Java, and East Java [11]. The presence of *B. gonionotus* in Java has been recorded [12], but the molecular phylogenetic *B. gonionotus* in Java has not been studied yet. This topic would increase knowledge of molecular identification [13] and phylogenetic [14]. This study was using
the DNA Barcoding method [15] with the specific gene in the mitochondrial (mtDNA) genome [16], cytochrome c oxidase subunit I (COI) gene.

2. Materials and methods

2.1 Sample collection and study sites

We collected The twenty-five live specimens with a total length between 14 cm and 31 cm using cast-nets on 15 Agustus 2021 in the Opak River, Sleman Regency, Yogyakarta (7°53'14"S;110°23'21"E) (Figure 1).

![Figure 1](image1.png)

Figure 1. The red square marks the location of Opak River in Java

Twenty (20) specimens were kept as livestock at the Fish Reproduction Laboratory, Brawijaya University, Malang Indonesia, and were transported in polyethylene bags with oxygen [17], four (4) specimens preserved in 10% formalin solution to keep as preserved specimens [18] and deposited at the Zoology Laboratory, Generasi Biologi Indonesia Fondation, Gresik, Indonesia. One (1) specimen preserved in 96% alcohol solution [19,20] for molecular identification (Figure 2).

![Figure 2](image2.png)

Figure 2. Specimens of *B. gonionotus* was captured on 15 August 2021 in the Opak River, Sleman Regency, Yogyakarta
Twelve (12) sample sequences COI gene were downloaded from GenBank, eight (8) *Barbonymus gonionotus* (KU692338.1; KU692335.1; KU692342.1; KU692333.1; KU692329.1; KU692337.1; KU692336.1) [12], one (1) *Barbonymus balleroides* (KU692329.1) [12], one (1) *Barbonymus schwanenfeldii* (KT001008.1), two (2) *Barbonymus altus* (JX066755.1; JQ346154.1) [21,22], and one (1) sample as outgroup *Catostomus commersonii* (HQ557394.1) [23].

2.2 DNA extraction, isolation and amplification
Genomic DNA extraction of the specimen was using the KIT method: Genomic DNeasy Blood & Qiagen Tissue Kit. Amplification (PCR) of cytochrome C oxidase subunit I (COI) gene was carried out with the AmpliTaq RedTM (Applied Biosystems) and the jgLCO1490 and jgHCO2198 primers [24]. PCR reaction and thermocycling profile with initial denaturation at 94 °C for 3 minutes, 35 cycles of 94 °C for 30 seconds, annealing at 50 °C for 30 seconds and an extension stage at 72 °C for 2 min [25]. PCR product visualized using 1% agarose gel stained [26] Biotium® gel red stain. The PCR product that successfully amplified was sent to DNA Sequencing facility and sequenced using Sanger sequencing methods.

2.3 Data analysis
Sequencing editing and alignment was conducted using the Clustal W method in MEGA6 software [27] and identified in NCBI (National Center for Biotechnology Information) Genbank online (www.ncbi.nlm.gov), with BLASTn (Basic Local Alignment Search Tool-nucleotide) method as a Identification Engine. The phylogenetic tree was constructed using Neighbor-Joining method [28] with a bootstrap test of 10000 replications [29] and the evolutionary distance was calculated using the Kimura 2-parameter method [30].

3. Results and discussion

3.1 Sequences DNA
DNA-Barcoding of specimen from the Opak river are successfully sequenced with base-pair length of 656 bp by using the jgLCO1490 and jgHCO2198 primers [24] (Table 1). Hebert *et al.*, (2003) [15] said that fragments along 658 bp COI genes can be used as a basis for differentiating between animals.

| Table 1. Sequencing result of B. gonionotus from the Opak River, Sleman Regency, Yogyakarta |
|-----------------------------------------------|
| DNA Barcoding                                    |
| GGATAGTGGGAACCCTTAAGCCTTCATATTCTGAGCTGAACTTAGTCACACCCGGGTCACTTCTAGGGACGA |
| TCAAAATTTATTAACTGAAATTATGCTGATCTGTTACCCCTCTACTACTACCCCAGATATGCCATTTCCCACTTAAACACCA |
| TAAAGCTCTGTTACTGCGAGATATTTATCACTACTGCTAGCTTTCCGTTTGAAGGCGTGGCCTTCAGGAA |
| AGGTTGACAGTATATCTACCCCTACCTGCGAGGAACCTTGCCACCGGGAGGAGCTAGTACGATACCAATTTTC |
| TCACTTCACTTACTGCACTGACTGACTGTTATCACTGCTAGGCGTGGAGAAGCGGTTGCGGGAC |
| CCCAGGCGCTTATCGTAAATCAAAACATTTAGTTTGTGTGTATCGTACTGTGAAACCTGCCGCTACTCCTCCTCTCTC |
| GTCACTACCTGTTCGGCCGGGATTCAATGACAGTGCTACTCACCAACGCAATCCTTCCTTGAAGGATGGAAGGACCCG |

3.2 Species Identification
Specimen from the Opak river was compared to the GenBank NCBI (National Center for Biotechnology Information) and identified as *Barbonymus gonionotus* with a Query cover value 100%, Identity values 100% and E-value 0.0. According to the Hebert *et al.*, (2003) [15], species with 99-100% similarity
level are identical. The results of this study indicate that the Cytochrome C oxidase subunit I (COI) gene has a high level of accuracy for species identification, especially for fish identification.

**Table 2. Species Identification and Similarity**

| Specimen          | Similarity GenBank | Species identified | Accession Number (GenBank) | Phylum    | Class       | Family    | Family       | Genus       |
|-------------------|--------------------|--------------------|---------------------------|-----------|-------------|-----------|-------------|-------------|
| Barbonymus gonionotus | 100%               | Barbonymus gonionotus | KU69233 7                   | Chordata  | Actinopterygi | Cyprinidae | Barbonymus   | Barbonymus   |
| Barbonymus gonionotus | 99%                | Barbonymus gonionotus | KU69233 6                   | Chordata  | Actinopterygi | Cyprinidae | Barbonymus   | Barbonymus   |

3.3 Phylogenetic tree
Phylogenetic tree result from Neighbor-Joining method [28] showed that all the specimens from three populations (West Java, Central Java and East Java) are in one clade (Figure 3), there was no specific pattern of population difference between locations and population, therefore was considered as a mixing population.

![Phylogenetic tree](image)

**Figure 3. Phylogenetic tree of B. gonionotus in Java based on COI Gene**

3.4 Genetic distance
For the genetic distance in intra-population in the Java had diversity about 0.000 and 0.005 (Table 3). Where 0.000 is the closest distance that happened to the all East java population, two species of West java and between a species from Opak River with a species from West Java. 0.000 distance estimation value indicates that of 1000 base pairs, none of them have different base pairs. The highest genetic distance occurs in the population of West Java and East Java which is 0.005. Meanwhile, the research conducted by Fahmi et al., (2018) [31] on Tiger Fish from the Musi River in South Sumatra and the Kapuas River in West Kalimantan, there are also no specific differences between locations and populations, intra-population diversity is only 0.002 and 0.003 and is still in one clade.
Table 3. Genetic distance of *B. gonionotus*

|   | 1    | 2   | 3  | 4   | 5  | 6  | 7  | 8   |
|---|------|-----|----|-----|----|----|----|-----|
| 1 | Central Java |   |    |     |    |    |    |     |
| 2 | KU692338.1 East Java | 0.003 | | | | | | |
| 3 | KU692335.1 East Java | 0.003 | 0.000 | | | | | |
| 4 | KU692342.1 East Java | 0.003 | 0.000 | 0.000 | | | | |
| 5 | KU692333.1 East Java | 0.003 | 0.000 | 0.000 | 0.000 | | | |
| 6 | KU692339.1 West Java | 0.002 | 0.005 | 0.005 | 0.005 | 0.005 | | |
| 7 | KU692337.1 West Java | 0.000 | 0.003 | 0.003 | 0.003 | 0.003 | 0.002 | |
| 8 | KU692336.1 West Java | 0.002 | 0.005 | 0.005 | 0.005 | 0.005 | 0.000 | 0.002 |

The closest of *B. gonionotus* in Java could be caused by rivers in the Java Island was being connected to East Sunda River at last glacial era then being cut off and isolated due to rising sea levels [32, 33, 34]. The gene flow occurs over centuries, inherited genetics to the inheritor. For a native freshwater fish, molecular studies are important contributions for understanding species diversity [35, 36, 37].

4. Conclusion

DNA-Barcoding of specimen from the Opak river are successfully sequenced with base-pair length of 656 bp by using the jgLCO1490 and jgHCO2198 primers. Specimen was compared to the GenBank NCBI (National Center for Biotechnology Information) and identified as *Barbonymus gonionotus* with a Query cover value 100%, Identity values 100% and E-value 0.0. Phylogenetic tree result from Neighbor-Joining method showed that all the specimens from three populations (West Java, Central Java and East Java) are in one clade with the genetic distance about 0.000 and 0.005. Species from Opak River the closest to West Java about 0.000 distance estimation value that means none of them have different base pairs.

5. References

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