Fish Identification based on Partial Fragments of The Mitochondrial COI Subunit I Gene

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Abstract. The mtDNA sequences revealed that several of the fish studied were *Hampala macrolepidota* and *Barbonymus gonionotus*. The objective of this research was to learn the pattern of COI gene in mtDNA and establish a phylogenetic tree. Basic Local Alignment Search Tool-nucleotide (BLASTn) confirmed that *Barbonymus gonionotus* from the Ranau Lake, South Sumatera has 100% matching ranges to the species from Memberamo River (Indonesia), India, Bangladesh, Thailand (Mae Khlong), Indo-Myanmar, and Malaysia_1. The lowest closeness (98.76%) is related to species from Thailand (Lower Ing). The Blast investigation appears us that the level of familiarity was very high, it is coming to 98-100% in *Barbonymus gonionotus*. *Hampala macrolepidota* had 100% matching ranges to the species from Indonesia (SouthaSumatera_1) and Vietnam. They had 99.05%-99.84% closeness from Malaysia_1,2&3, Indonesia (South Sumatera_2&3, Java and Bali_1,2&3).

1 Introduction

Based on water volume, Ranau Lake is the second-largest the largest lake in Indonesia [15]. Some species that can be found in Ranau Lake are *Hampala macrolepidota* and *Barbonymus gonionotus*. Both belong to the family of cyprinidae. Although identifying the species by morphology has several limitations [13], molecular identification techniques have an advantage in rapid and accurate assessment [17]. COI-based on molecular method can identify specimens precisely, reliably, accessible, and at low cost [11]. DNA identification provides greater precision in species or lineage identification, including amplification and sequencing short mitochondrial COI genes [12]. *Barbonymus gonionotus* is very suitable as an ornamental fish. The population of this type of fish has experienced a decline in the last ten years due to logging, overfishing, and fishing practices that are not environmentally friendly by fishermen in the waters of Nagan [1]. Hampala macrolepidota has a black bar between the dorsal fin and pelvic fin in adult stages, which becomes less distinct in enormous specimens [5]. The study aims to identify several types of fishes using DNA sequences in the Ranau Lake and to obtain a more definite identification based on information on the nucleotide sequences of *Barbonymus gonionotus* and *Hampala macrolepidota*.

2 Materials And Methods

2.1. Biological materials

Barbonymus gonionotus and *Hampala macrolepidota* were collected using net and assistance from local fishermen. Three individuals of each type were obtained and stored in approximately 0.5 cm2 of solute 99% ethanol, placed in a test tube of 1.5 ml, and then kept in the refrigerator (18°C).

2.1.1 DNA extraction

Genomic DNA was obtained by extracting each fish tissue (approximately 2.5 mm^2). If a polymerase chain reaction (PCR) is to be used in extracting, the DNA sample should be kept in a freezer (18°C).

2.1.2 DNA amplification

The DNA of *Barbonymus gonionotus* and *Hampala macrolepidota* was extracted and amplified using the following pairs of common primer COI-Fish-R and Fish-COI-F as follows: Fish COIF (5'ACT TCA AAC TTC CAY AAA GAY ATY GG-3`) and COIFishR (5` TAG ACT TCT GGG TGG CCR AAR AAY CA-3`) [7]. PCR carries out this DNA amplification. Genomic DNA extraction resulted in a COI gene fragment of 540 bp. Use Bioline's taq ReadyMix to perform PCR with a final volume of 50 µl. Each reaction contains 1 µl FishF2 primer, 1 µl FishR2 M primer, 21 µl nuclease-free water, 25 µl my taq ReadyMix and 2 µl DNA template. The stage of pre-denaturation at 95°C for 1 minute, the second stage consists of 35 cycles, where each process is denatured at 95°C for 15 seconds, and direct bonding (annealing) to 55 Celsius degrees for 15 seconds, 72 Celsius degrees temperature extension for 30 seconds, the last stage of the form is 72 Celsius degrees final extension 3 minutes, and at a specific temperature 6°C 6 minutes. In addition, the PCR products were run on a 120v, 250A electrophoresis instrument for 5 minutes, and GelDoc was used for visualization. Use the 100 bp marker [16] to observe PCR results of DNA size. The amplified DNA fragments were separated from an agarose gel (1%) and stained with ethidium bromide.

2.2. Data analysis

The length of a DNA sequence of *Barbonymus goninotus* is 707 bp, and for *Hampala macrolepidota* is 680 bp. The results of nucleotide sequencing were manually edited using chromatogram-based Bioedit software [2]. In the next step, the MEGA 6.0 program (Molecular Evolutionary Genetics Analysis) [6] was used to align and modify the nucleotide sequence using Clustal W [4]. To determine DNA sequence similarity, the DNA sequence was transferred to BLASTn for nucleotide recovery. Data were provided by the National Center for Biotechnology Information (GenBank NCBI) used the neighbor-joining (NJ) method to align all the sequences of the phylogenetic tree.

3 Results

BLASTn evaluation confirmed that *Barbonymus gonionotus* from Lake Ranau in South Sumatra showed a 100% similarity level to the identical species from Memberamo River (Indonesia), India, Bangladesh, Thailand (Mae Khlong), Indo-Myanmar, Malaysia_1. The lowest similarity stage (98.76%) was represented by the same species in Thailand (Lower Ing). The stage of similarity acquired from BLASTn analysis was very high, attaining 98-100 % in *Barbonymus gonionotus* (Table 1). *Hampala macrolepidota* is 100% similar to sane

species in Indonesia (South Sumatera_1) and Vietnam. Their similarities ranged from 99.05% to 99.8 % in Malaysia_1, 2 and 3, Indonesia (South Sumatra_2 and 3, Java and Bali_1, 2 and 3) (Table 2).

Table 1. COI nucleotide of *Barbonymus gonionotus* based on BLASTn Method

No	Classification	Compatibility (%)	Accession number	Genesis
1	*Barbonymus gonionotus*	100	KJ936769.1	India
2	*Barbonymus gonionotus*	100	MK572052.1	Bangladesh
3	*Barbonymus gonionotus*	100	MK902689.1	Thailand (Mae Khlong)
4	*Barbonymus gonionotus*	100	MG736407.1	Indo-Myanmar
5	*Barbonymus gonionotus*	100	MK970395.1	IDN (Mamberamo)
6	*Barbonymus gonionotus*	100	KT001016.1	Malaysia_1
7	*Barbonymus gonionotus*	98.76	MK628326.1	Thailand (Lower Ing)
8	*Barbonymus gonionotus*	99.41	KT001015.1	Malaysia_2
9	*Barbonymus gonionotus*	100	MK970394.1	IDN (Memberamo_1)

Table 2. COI nucleotide of *Hampala macrolepidota* based on BLASTn Method

No	Classification	Compatibility (%)	Accession number	Genesis
1	*Hampala macrolepidota*	100	KM213069.1	IDN (South Sumatera_1)
2	*Hampala macrolepidota*	99,84	KM213080.1	IDN (South Sumatera_2)
3	*Hampala macrolepidota*	99,84	KU692540.1	IDN (Java and Bali_1)
4	*Hampala macrolepidota*	99,24	JF781170.1	Malaysia_1
5	*Hampala macrolepidota*	99,43	KU692541.1	IDN (Java and Bali_2)
6	*Hampala macrolepidota*	99,35	KT001059.1	Malaysia_2
7	*Hampala macrolepidota*	100	MK116339.1	Vietnam
8	*Hampala macrolepidota*	99,43	KU692543.1	IDN (Java and Bali_3)
9	*Hampala macrolepidota*	99,05	KT001032.1	Malaysia_3

4 Discussions

Barbonymus goninotus and *Hampala macrolepidota* are divided into different branches in the phylogenetic tree (Figure 1). By the use bootstrap of Neighbor-Joining method, the results of phylogenetic tree analysis show there were two main groups, particularly *Barbonymus gonionotus* and *Hampala macrolepidota*. *Hampala macrolepidota* from Indonesia have been clustered with the same species from Malaysia and Vietnam, with a baseline value of 74%. The starting value of *Barbonymus goninotus* and the same species from India, Bangladesh, Thailand, Indo-Myanmar, and Malaysia was 94%. This species, *Hampala macrolepidota*, was distributed at upstream Mekong River/lower reaches Lancang River [8], Malaysia [7], and Lake Ranau in Indonesia [14].

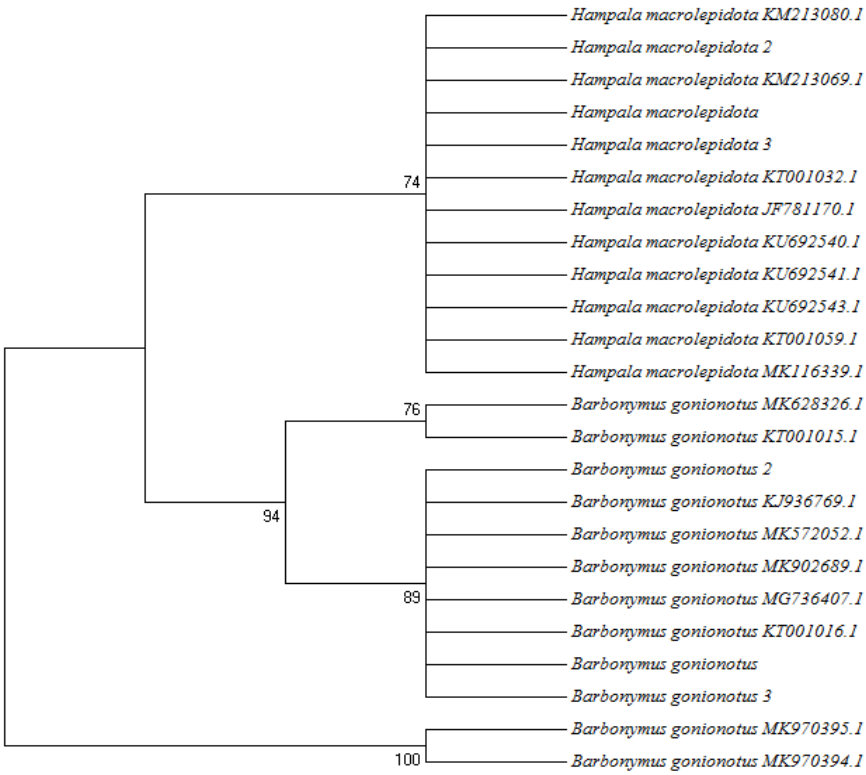

Fig. 1. Phylogenetic tree of *Barbonymus gonionotus* and *Hampala macrolepidota* by using the method of Neighbor-Joining (NJ) construction.

5 Conclusion

The length of the *Barbonymus goninotus* COI gene fragment is 707 bp, and the *Hampala macrolepidota* COI gene fragment length is 680 bp. *Barbonymus goninotus* is from Lake Ranau in South Sumatra and is 100% similar to the same species from the Memberamo River (Indonesia), India, Bangladesh, Thailand (Mae Khlong), Indo-Myanmar, and Malaysia_1. *Hampala macrolepidota* is 100% similar to the same species from Indonesia (South Sumatera_1) and Vietnam. Its similarities with Malaysia_1.2 and 3, Indonesia (South Sumatra_2 and 3, Java and Bali_1, 2, and 3) are 99.05%-99.84%.

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