

DNA Barcode_Snakeskin gouramy

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DNA barcoding of snakeskin gourami *Trichogaster pectoralis* and blue gourami *Trichogaster trichopterus* based on cytochrome c oxidase subunit I (COI) gene

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Abstract. Snakeskin gourami *Trichogaster pectoralis* and blue gourami *Trichogaster trichopterus* are the species from the genus of *Trichogaster*. There are three species of the genus *Trichogaster* in Indonesia. This research aimed to identify the sequence of cytochrome c oxidase subunit I (COI) gene of mtDNA, and construct the phylogenetic trees among species. The stage of research consisted of DNA isolation, DNA amplification using PCR (Polymerase Chain Reaction) and sequencing COI gene in mtDNA region. Sequencing of the COI gene produced 604 bp for snakeskin gourami and 628bp for blue gourami. The result of BLASTn (Basic Local Alignment Search Tool-nucleotide) analysis showed that snakeskin gourami from Kelekar River had 100% similarity of snakeskin from Indonesia and Philippines and farthest (88%) with snakeskin gourami from France. Blue gourami from Indralaya had 100% similarity with three spot gourami from Australia, South Africa and India and farthest (88%) with snakeskin gourami from Japan. The phylogenetic tree indicated that snakeskin and blue gourami were clustered in separate branches, where snakeskin gourami had 100% similarity to the same species from Indonesia and the Philippines, and blue gourami had 100% identity to the same species from Japan.

1. Introduction

These Indonesia has very high natural resources and biodiversity. One of the resources is the inland waters (freshwater). There are 353 species of fish in western Indonesia [1]. Some of the potential fish to be cultivated are snakeskin gourami (*Trichogaster pectoralis*), blue gourami (*Trichogaster trichopterus*), stripped snakehead (*Channa striata*), and Anabas testudineus [2,3]. "Sepat siam" or siamese gourami or snakeskin gourami were introduced from Thailand to Indonesia in 1934 as aquaculture fish in small ponds and farmland. This species inhabit swamps; a medium bodies with total length reaches 20 - 25 cm, compressed body shape, with slightly tapered mouth. The color of dorsal, tail, pectoral and anal fins are dark. Its anal fin is nearly the length of the body and the pelvic fins are long and thread-like [1]. Snakeskin gourami is classified as plankton feeders [4]. Meanwhile, blue gourami is an ornamental fish that live in fresh water, have small bodies. The blue gourami has two spots along each side of its body in line with the eye, considered the third spot [5], native to standing or slow-moving freshwater in Southeastern Asia, starting from Yunnan (China) through mainland southeast to Java, Borneo and Sumatra [6].



DNA barcoding is a species characterization and identification technique using DNA sequences. The cytochrome c oxidase subunit I (COI) gene is a protein coding in mitochondrial DNA that has been widely used as a tool for identifying animal species. For the animal group, the standard barcode is a 658 base pairs (basepair/bp) fragment. This sequence is widely used as a Barcode of Life to identify the kinship of fish species [3,7,8], catfish [9], the tilapia [10], sharks [11], tropical eels [12], Asian redbtail catfish [13], Rasbora group [14], and several species of fish in Lake Laut Tawar [15]. Presently, more than 5000 species of fish have been successfully identified using DNA barcodes [16], this proves that DNA barcoding techniques play an important role as taxonomic tools and genetically reveal different and separate species quickly and accurately.

DNA Barcode has an important role in revealing the genetics of a species, finding out the genetic status of the breeding process in fish. Iskandariah [17] conducted a study on genetic diversity of snakeskin gourami for aquaculture fish breeding programs, meanwhile Wibowo [18] has successfully barcoded Siamese gourami on the peatlands of the Musi Banyuasin waters in South Sumatra, but there was no DNA barcode yet for blue gourami. Therefore, the objective of current study was to investigate DNA barcodes in snakeskin gourami originating from the Kelekar River and blue gourami, and also to determine the phylogenetic structure and kinship level in comparison to other species recorded in the Genbank.

4 2. Materials and Methods

2.1. Biological materials

Snakeskin gourami were captured in the wild with the help of local fisherman from Kelekar River (Ogan Ilir Regency), while blue gourami were taken from ornamental fish sellers in Indralaya. Five individuals of each species were taken alive and put into an oxygenated plastic bag. Fish morphology (body color, body shape, head shape, fin shape) as well as morphometrics and meristics were analyzed in laboratory. Approximately 3 cm of a segment of the caudal fin was snipped and preserved in 99% ethanol (1:10 w:v), then stored in individual Eppendorf tubes at -20°C until required.

5 2.2. DNA extraction

The total DNA genome was extracted using the Genomic DNA Extraction Kit (GeneAid) following the method in the manual. In general, DNA extraction consists of 5 stages: cell lysis, RNase treatment, DNA precipitation, washing and dissolution of DNA. The RNase incubation phase was carried out to reduce RNA contamination. Each fin sample (about 2 mm²) was used in DNA isolation. DNA samples were then stored in a freezer (-20°C) until required for PCR (polymerase chain reaction).

2.3. DNA amplification

The DNA amplification was conducted using Polymerase Chain Reaction (PCR) method. DNA genome obtained from extraction (five individuals per species) were used to obtain the COI gene fragment (655 bp) from mitochondrial DNA with primer pairs of FishF2-5' TCGACTAATCATAAAGATATCGGCAC 3' and FishR2-5' ACTTCAGGGTGACCGAAGAATCAGAA 3' (17). PCR was performed in 40 µl final volumes using KAPA HiFi HotStart ReadyMix from Kapa Biosystem. Each reaction contained 1.6 µl 10 µM FishF2 primer, 1.6 µl 10 µM FishR2 primer, 14.8 µl nuclease-free water, 20 µl 2x KAPA HiFi HotStart ReadyMix and 2 µl DNA template.

DNA amplification was carried out by stages: initiation cycle at 95 °C for 1 minute, denaturation at 95 °C for 15 seconds, annealing at 57 °C for 15 seconds, Extension or elongation at 72 °C for 10 seconds in 28 cycles and the extension at 72 °C for 4 minutes. Furthermore, the PCR product was run through 1% agarose gel electrophoresis with 75V for 35 minutes and visualized using GelDoc. The DNA size of the PCR results was observed using a 1 Kb marker.

2.4. Sequencing of COI gene

DNA samples of snakeskin and blue gourami which were successfully amplified using PCR were then electrophoresized along with 1 Kb markers to recognize the target area of the COI gene product. PCR products of known size then sequenced at DNA sequencing service at 1st Base Singapore through the services of the Genetica Science Institute in Jakarta.

2.5. Data Analysis

COI sequences of two species that have been saved in the form of fasta format are then aligned using MEGA 6.0 software for trimming process. The lengths of the aligned sequences were 604 bp for snakeskin gourami, 628 bp for Blue gourami with no gaps within sequences. They were then blasted to BLAST for nucleotide, which is used to determine the homology of a DNA sequence with data provided in Genbank NCBI (National Center for Biotechnology Information). Furthermore, all sequences are aligned for phylogenetic trees using the Neighbor Joining (NJ) method.

3. Results and Discussion

3.1 The sequence Similarity and Genetic Tree

BLAST analysis showed Snakeskin gourami (*Trichopodus pectoralis*) from the Kelekar River Inderalaya had 100% similarity levels to the same species from Java and Bali (Indonesia). The lowest level of similarity (88%) was denoted to the same species from France. Meanwhile, Blue gourami (*Trichopodus trichopterus*) denoted the highest level of similarity (100%) to the same species from South Africa, Australia and India. The lowest level of similarity (88%) for Blue gourami was indicated to Siamese gourami originating from Japan. The level of similarity obtained from the BLAST analysis was quite high, reaching 88-100% in Snakeskin gourami. This indicated that identifying species using DNA barcoding is very promising. The COI is effectively used as an identification tool because intraspecific variation is low, but has high interspecific variation values especially in adjacent taxa [19].

Table 1. The BLASTn of COI nucleotide of Siamese gourami.

No	Species	Identity (%)	Accession	Origin
1	<i>Trichopodus pectoralis</i>	100	KU692927	Indonesia (Java and Bali)
2	<i>Trichogaster pectoralis</i>	100	Q682728	Philippines
3	<i>Trichopodus pectoralis</i>	99	LC190090	Japan (Kyoto)
4	<i>Trichopodus pectoralis</i>	99	KX817198	Malaysia (Terengganu)
5	<i>Trichogaster pectoralis</i>	99	KM213050	Indonesia (South Sumatera)
6	<i>Trichopodus pectoralis</i>	98	KX817208	Malaysia (Terengganu2)
7	<i>Trichopodus pectoralis</i>	97	KX817204	Malaysia (Terengganu3)
8	<i>Trichopodus pectoralis</i>	96	KX817205	Malaysia (Terengganu4)
9	<i>Trichogaster trichopterus</i>	90	JQ661373	Thailand (Phayao)
10	<i>Trichogaster trichopterus</i>	90	JN896635	Thailand (Muang)
11	<i>Trichogaster trichopterus</i>	89	KM213048	Indonesia (SUMSEL2)
12	<i>Trichogaster trichopterus</i>	89	KC789556	Philippines (Nueva Ecija)
13	<i>Trichopodus trichopterus</i>	89	KU569064	South Africa (Grahamstown)
14	<i>Trichopodus trichopterus</i>	89	KJ669650	Australia (Canberra)
15	<i>Trichogaster trichopterus</i>	88	JQ667586	India (Maharashtra)
16	<i>Trichopodus trichopterus</i>	88	KU692928	France (Herault2)

Table 2. The BLASTn of COI nucleotide of *Blue gourami*

No	Species	Identity (%)	Accession	Origin
1	<i>Trichopodus trichopterus</i>	100	KU569063	South Africa (Grahamstown)
2	<i>Trichopodus trichopterus</i>	100	KJ669650	Australia (Canberra)
3	<i>Trichogaster trichopterus</i>	100	JQ667580	India (Aurangabad)
4	<i>Trichogaster trichopterus</i>	99	KM213048	Indonesia (South Sumatera)
5	<i>Trichogaster trichopterus</i>	99	KC789557	Philippines (Nueva Ecija)
6	<i>Trichogaster trichopterus</i>	99	JQ667586	India (Maharashtra)
7	<i>Trichogaster trichopterus</i>	99	KU692940	Indonesia (Java and Bali)
8	<i>Trichogaster sp.</i>	99	JF752335	India (Kochi)
9	<i>Trichogaster trichopterus</i>	96	JQ661373	Thailand (Muang)
10	<i>Trichogaster trichopterus</i>	96	JN896636	Thailand (Phayao)
11	<i>Trichogaster pectoralis</i>	89	HQ682726	Philippines (Quezon City)
12	<i>Trichogaster pectoralis</i>	89	KX817201	Malaysia (Terengganu)
13	<i>Trichogaster pectoralis</i>	88	LC190090	Japan (Kyoto)

3.2 Phylogenetics

The phylogenetic tree showed that Snakeskin and Blue gourami were clustered in separate branches (Figure 2). Phylogenetic analysis combines molecular biology techniques with statistics to reconstruct phylogenetic relationships [20]. The phylogeny tree construction was carried out to describe the phylogenetic between species [21]. The results of the phylogenetic tree analysis using the Neighbor-Joining method with Bootstrap 1000x indicated that there were two main clusters, namely Siamese gourami (*Trichogaster pectoralis*) and Blue gourami (*Trichogaster trichopterus*). Siamese gourami originating from Indonesia were in the same cluster to the same species from Japan, and the Philippines with 80% bootstrap value. While blue gourami have a 100% bootstrap value with the same species originating from France, South Africa, Indonesia, India and Australia.

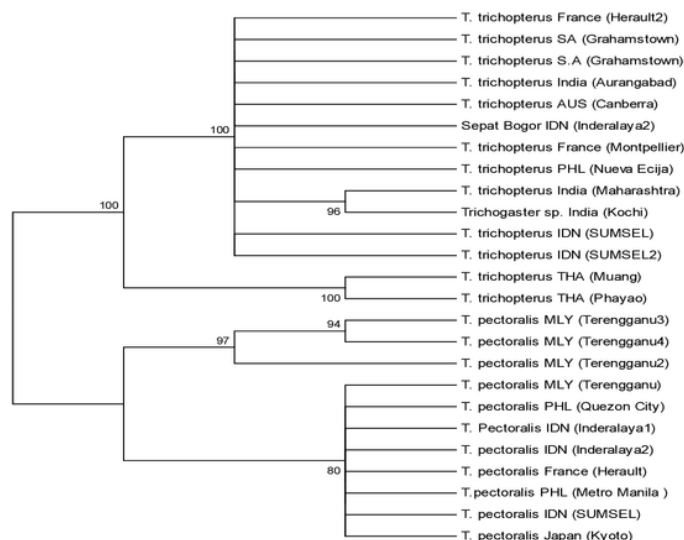


Figure 1. Phylogenetic tree of Snakeskin and Blue gourami constructed using *Neighbor-Joining* (NJ) method.

The bootstrap was calculated to evaluate branch stability [22]. The bootstrap value in the phylogenetic tree is stable if the bootstrap value is above 95% and it is unstable if the bootstrap value is below 70%. Phylogenetic analysis of a species can be carried out on morphological characters and genes through sequences of mitochondrial DNA. The use of sequences of mitochondrial DNA clarifies the relationship of species to evolutionary blurring due to variations in morphology [23]. Phylogenetic relationships also described the possibility of genetic mixture between populations [24], caused by some factors for instance genetic flow and human introduction activities.

4. Conclusion

The COI gene fragment length was 604bp for snakeskin gourami and 628bp for blue gourami. Snakeskin gourami (*Trichogaster pectoralis*) had 100% similarity to the same species from Indonesia and the Philippines, and had the farthest percentage of 88% with blue gourami (*Trichogaster trichopterus*) from France. Blue gourami had 100% identity to the same species from Japan.

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