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Genetic Diversity Population of Beloso (*Glossogobius aureus*) in Lake Tempe, Sulawesi, Indonesia

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Abstract: Beloso fish (*Glossogobius aureus*) is a native species in Lake Tempe. There has been a decline in production and Beloso size, so that preservation should be done. The objective of this research was to determine the genetic diversity of various populations of Beloso fish. The research was conducted in Lake Tampe. Organ samples are meat. Beloso genetic diversity was measured with a set of extraction and purification of genomic tools, mt-DNA amplification and electrophoresis. Results of this study found that mt-DNA PCR product read at length 600 bp gene. The length of DNA fragments of beloso fish were sequenced with primer F and primer R is the range between 456 - 607 bp and 565 - 604 bp, respectively. Genetic distance of individual Beloso (*Glossogobius aureus*) in Lake Tempe is around 0.0012 - 0.0170. The genetic diversity of Beloso was very low leading to the lower income of the communities around Lake Tempe with a lower population and diversity of Beloso fish.

Key words: *Glossogobius aureus* · Purification · Genome · Mitochondrial DNA · Amplification · Electrophoresis

INTRODUCTION

Beloso (*Glossogobius giurus*) is one of species fish with high economic value and favored by people. Beloso has special flavor and distinctive with a thick flesh and few bones, it can be utilized in the form of fresh or dried. The fish is locally known as "Bungo". In the past, the fish has a high social value for the surrounding community, because only nobles and community leaders can consume it. Beloso fish has become one of the excellent fish, in addition to population and size and also because the price is high [1-3].

Beloso fish initially lives in estuaries [4-5] and then migrate to the Lake Tempe periodically through the river mouth of Pallime Cenranae [6-8]. One thing interesting is that the Beloso fish (*Glossogobius aureus*) is a native species [8-11] at Lake Tempe [1, 12-15], but it is not getting much attention both by researchers and local

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governments which led to minimal or no efforts towards its conservation. This can be seen by the lack of research especially on the aspects of genetic diversity for germplasm conservation resources and lack of effort from local government either in the form of research and protection of populations and their habitats. Therefore, the genetic diversity and population of the fish in Lake Tempe and the surrounding waters are declining in production with significant changes in species composition [16-19]. One of the fish species of high economic value that has significantly decreased in production is the Beloso (*Glossogobius aureus*). Its current species composition is only in the ratio of 1:47% to all fish in Lake Tempe with an average size of only about 120 - 200 mm [1, 20, 21]. This indicates that there is a possibility of reduction of genetic diversity of Beloso in this area [22, 23]. As there is very minimal hatching efforts, the decline in genetic variation can be caused by low or no possibility of gene exchange (gene flow) to other populations because the Beloso fish do not migrate too far as compared to the milkfish [12, 24, 25].

Studies on Beloso fish are still limited especially on the aspects of morphology and reproduction [13-15, 26-29] in the rivers of Sulawesi which form its main source of germplasm [16,18,19]. Therefore, efforts towards conserving the population of Beloso fish are critical. In order to realize the germplasm conservation of Beloso, monitoring and evaluation of its genetic variation has to be taken. Some research shows the importance of genetic variation in fish/shrimp that exist in nature and must be maintained when it will be used for seeding in hatchery [30]. Genetic variation can be evaluated in two ways, namely by allelic diversity and heterozygosity. Several methods can be used to estimate the level of genetic variation using molecular markers [31-33], including the mitochondrial DNA [34-37]. The objective of this study was therefore conducted to determine and identify the genetic variations of some populations of Beloso (*Glossogobius aureus*) at Lake Tempe and surrounding areas.

MATERIALS AND METHODS

Location of Study: The study was located in Lake Tempe and its surrounding waters, namely in the middle of the lake and in the surrounding rivers of Bila, Walanae and Cenranae. Samples were first analyzed in the Biotechnology Laboratory and DNA sequencing was performed in the First BASE Laboratories and Gemini Singapore Science Park in collaboration with PT. Genetics Science in Jakarta, Indonesia.

Methodology: Fish samples were taken from the sampling sites in the form of live or dead specimens (fresh or frozen). Beloso organ sample used in this study was its meat. Upon reaching the laboratory, the samples were preserved and stored at room temperature. Direct application of mitochondrial DNA techniques can be performed using samples of the fresh, frozen or stored in alcohol [38, 39]. Genetic diversity was measured by a set of extraction and purification of genomic tools, mt-DNA amplification and electrophoresis.

DNA Extraction: The method used in the extraction and purification of genomic DNA was based on a procedure using the Amersham-Pharmacial Prep™ Cell and Tissue Genomic Isolation Kit. The extracted organs are their meat. Genomic DNA extraction or purification was done by using the modified instructions [38, 40]. The results obtained give extraction results can be amplified further. DNA was extracted in this method is the total DNA/ genomic DNA and a whole section of existing cells in extract tissue.

DNA Extraction Procedure Using Phenol-Chloroform: As much as ± 50 mg - 100 mg of Beloso fish was preserved with a solution of 250 ml Buffer TNES Urea (6 M urea, 10 mM Tris-HCl, 125 mM NaCl, 10 mM EDTA, 1% SDS) in an Eppendorf 1.5 ml tube (stored at room temperature until DNA extraction) [38]. Before the DNA was extracted, the meat that has been preserved was added with 500 lysis buffer (0.5 M NaCl, 0.001 M EDTA), 1% SDS, 0.8% Triton X-100 and 0.1 MTris-HCl pH 9.0), 40 and 20 10% SDS-Proteinase K (20 mg/ml). After, it was incubated at 55°C for 1-3 hours and then added with another 12.5 RNAase (20 mg/mL) before it was finally stored at room temperature for 15-30 minutes.

Phenol was then added to Chloroform and Isoamyl alcohol at a ratio of PCIA 25: 24: 1. It was gently shaken to make it homogeneous and later allowed to stand at room temperature for 10 minutes and then centrifuged at 13,000 rpm for 8 minutes. The upper layer was taken and transferred to new Eppendorf tubes. The addition of PCIA was repeated once again with the same ratio as before. After, one part by volume solution of Chloroform: Isoamyl alcohol in the ratio (CIA 24: 1) was added, then centrifuged at 13,000 rpm for another 4 minutes.

The upper layer was again taken and then transferred to new tubes with two volume section of the cold ethanol solution added and inverted gently until it becomes homogeneous. It was further centrifuged at 6,000 rpm for 30 minutes after which the liquid was discarded. DNA pellets were washed with 1 ml 70% ethanol and centrifuged again at 6,000 rpm for 15 minutes. The final centrifugation the DNA pellet was dried and aired at room temperature, then added with 100ìl distilled water or TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) and finally further stored at - 20°C for further processing.

The presence of genomic DNA can be detected by electrophoresis of DNA which 3 ml was extracted and added to 1 ml loading buffer on 1% agarose gel inserted into the electrophoresis wells. Further electrophoresis was done with a voltage current automatically programmed. DNA electrophoresis process was stopped when there was a change from negative to the positive pole to three-quarters of the length of the gel. The presence of DNA in the gel can be viewed using ultraviolet illuminator. About 1% agarose gel was made by mixing of 0.5 g agarose with 50 ml solution of tris borate EDTA (TBE 1%). Colored solution was heated until it became clear, then poured into a mold and comb/comb attached to form a well/well. Gel that had been frozen can be directly used for electrophoresis or stored/stock by soaking in a solution of 1% TBE.

Amplification of Mitochondrial DNA: Amplification sequence mitochondrial DNA was done using a forward primer F (5'-CGC CTG TTT AAC AAA AAC AT - 3 '), total length = 20 and its reverse using mt-DNA primer R (5'-CCG GTC TGA ACT CAG ATC ATG T -3 '), with a total length = 22. Amplification performed using PCR method with composition: forward primer 2 ml, 2 ml reversor, 3 mL DNA and 18 ml water, with a total volume of 25 ml were mixed in 1 unit taq dry. Samples were loaded into PCR machine with a cycle at a temperature of 55°C pre-denaturated performed for 15 minutes and 94°C for 2 minutes, 40 cycles consisting of denaturation at a temperature of 94°C for 15 seconds, annealing 60 °C for 30 seconds and elongated to 68 °C for another minute. Final elongation was performed for a cycle at 68 °C for another 5 minutes. The last process was to stabilize at a temperature of up to 4 °C. The presence of PCR was checked on a 2% agarose gel electrophoresis and subsequently observed in the UV (ultraviolet) illuminator.

Data Analysis: To find similar genes produced, the nucleotide sequencing results aligned (alignment) with the existing fish sequences in Gene Bank using the program Basic Local alignment Search Toll (BLAST-N for nucleotide sequences). Beloso gene sequence was analyzed by using the program GENETYX version 7 to obtain the similarity of Beloso sequence.

RESULTS

Extraction of Beloso Genomes: Results of extract of Beloso genome populations (*Glossogobius aureus*) were measured by generuller "Low Range DNA Ladder" with a standard molecular size 100 bp (Figure 1).

Amplification (PCR) Mitochondrial DNA: The length of the Beloso fish gene that was successfully amplified (PCR) mt-DNA in this study was 600 bp (Figure 2).

The mitochondrial DNA PCR products were then sequenced to confirm the results of PCR amplification of mitochondrial DNA of the Beloso gene. Sequencing results are then read by the scanner sequence software.

Sequencing of PCR Amplification of Beloso Mitochondrial DNA (mt-DNA): Sequencing results from PCR amplification of mitochondrial DNA for Beloso fish was successfully done using sequencing software that can be read by a scanner. PCR analysis of mitochondrial DNA sequencing was carried back and forth by using mitochondrial DNA Primer F (*) and mitochondrial DNA primer R (#). The long sequences/fragments of DNA sequencing results are shown in Table 1. It can be seen from Table 1 that the length of DNA fragments of Beloso fish that live in Lake Tempe and surrounding waters which were sequenced with primer F is the range between 456 - 607bp and 565 - 604bp sequenced with primer R.

Nucleotide Sequence Beloso Sequencing: The results of PCR on Beloso mt-DNA sequencing can be seen below. The sequence is in order according to the table and sequential juxtaposed between the two primers used.

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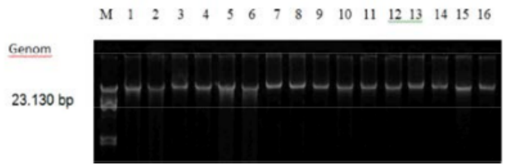
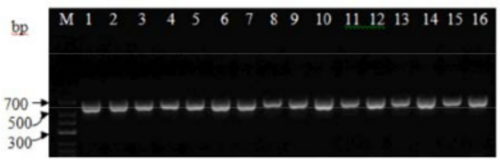
GACAAATGTTTTAACGGCCGCGGTATTTTGACCGT
GCGAAGGTAGCGTAATCACTTGTCTTTTAAATAG
AGACCTGTATGAACGGCATAACGAGGGCTGAGCTG
TCTCCTCCCTCCAGTCAATGAAATTGATCTATCCG
TGCAGAAGCGGACATGAAAACATAAGACGAGAA
GACCCTATGGAGCTTAAGACACAAGGACAGCCCA
CGTAAAACCCCCTAAATTTTAATTAGCGAAAACG
ATGTGAACCTCTGTCCTATCTCTTTGGTTGGGGCGA
CCGCGGGGAAACAAAAAACCCCCACGTGGAACGG
ACATCCTCCCGAAACAAGACCCACAAGTCGAACA
AGCAGAAATTCTGACCAAAAAGATCCGGCAACGC
CGATCAACGGACCCAGTTACCCTAGGGATAACAG
CGCAATCCCCTTTTAGAGCCCATATCGACAAGGGG



Note: M = H Ind Marker III; 1-4 = Genome IB Cenranae origin; 5-8 = IB Genomic origin When River; 9-12 = IB Genomic origin Tempe; 13-16 = IB Genomic origin

Fig. 1: Results extracted genome of Beloso fish populations (Glossogobius aureus), with a standard size of 100 bp DNA molecule Ledder (M)



Note: M = Marker Low Range; 1-4 = IB mtDNA PCR product origin Cenrana; 5-8 = IB mtDNA PCR product origin When the river; 9-12 = IB mtDNA PCR products Tempe origin; 13-16 = IB mtDNA PCR product origin Walanae

Fig. 2: The results of amplification (PCR) mt-DNA Beloso

Table 1: The DNA fragment length sequences of Beloso Fish, using mt-DNA Primer F (*) and R (#)

Sample	DNA Fragment Length (bp) Sequencing Results
IB 2	603* / 596#
IB 3	586* / 597#
IB 5	592* / 588#
IB 6	456* / 565#
IB 7	607* / 601#
IB 8	590* / 592#
IB 11	602* / 591#
IB 12	604* / 597#
IB13	599* / 598#
IB 15	593* / 593#
IB 16	599* / 604#

GTTTACGACCTCGATGTTGGATCAGGACATCCTAA
TGGTGCAGCCGCTATTAAGGGTTCGTTTGTTCAACG
ATTAAAGTCCTACATGATCTGAGTTCAGACCGGA
ACTTGAACAGAATNCA

>1st_BASE_719511_IB_2_mt___R:
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CACCATTAGGATGTCCTGATCCAACATCGAGGTCG
TAAACCCCCTTGTCGATATGGGCTCTAAAAGGGGA
TTGCGCTGTTATCCCTAGGGTAACTGGGTCCGTTGA
TCGGCGTTGCCGGATCTTTTTGGTCAGAATTTCTGC
TTGTTCGACTTGTGGGTCTTGTTTCGGGAGGATGTC
CGTTCCACGTGGGGGTTTTTTGTTTCCCCGCGGTCGC
CCCAACCAAAGAGATAGGACAGAGGTTCACATCG
TTTTCGCTAATTAAAATTTAGGGGGTTTTACGTGG
GCTGTCCTTGTGTCTTAAGCTCCATAGGGTCTTCTC
GTCTTATGTTTTCATGTCCGCTTCTGCACGGATAGA
TCAATTTCATTGACTGGAGGGAGGAGACAGCTCAG
CCCTCGTTATGCCGTTCATACAGGTCTCTATTTAA
AAGACAAGTGATTACGCTACCTTCGCACGGTCAA
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CTCCTCCCTCCAGTCAATGAAATTGATCTATCCGT
GCAGAAGCGGACATGAAAACATAAGACGAGAAG
ACCCTATGGAGCTTAAGACACAAGGACAGCCCAC
GTAAAACCCCCTAAATTTTAATTAGCGAAAACG
ATGTGAACCTCTGTCCTATCTCTTTGGTTGGGGCGA
CCGCGGGGAAACAAAAAACCCCCACGTGGAACGG
ACATCCTCCCGAAACAAGACCCACAAGTCGAACA
AGCAGAAATTCTGACCAAAAAGATCCGGCAACGC
CGATCAACGGACCCAGTTACCCTAGGGATAACAG
CGCAATCCCCTTTTAGAGCCCATATCGACAAGGGG
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AACCCCCTTGTCGATATGGGCTCTAAAAGGGGATT
GCGCTGTTATCCCTAGGGTAACTGGGTCCGTTGATC
GGCGTTGCCGGATCTTTTTGGTCAGAATTTCTGCTT
GTTCGACTTGTGGGTCTTGTTTCGGGAGGATGTCCG
TTCCACGTGGGGGTTTTTTGTTTCCCCGCGGTCGCCC
CAACCAAAGAGATAGGACAGAGGTTCACATCGTT
TTCGCTAATTAAAATTTAGGGGGTTTTACGTGGGC
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CTTATGTTTTCATGTCCGCTTCTGCACGGATAGATC
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GACAAGTGATTACGCTACCTTCGCACGGTCAAAA

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GCAGAAGCGGACATGAAAACATAAGACGAGAAG
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GACCCTATGGAGCTTAAGACACAAGGACAGCCCA
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GGAAAAAGA

From the list of nucleotide sequences obtained from sequencing results showed a trend existing diversity of Beloso fish in the waters of the lake and surrounding wrought.

Phylogenetic Beloso Fish: The phylogenetic tree species found that the Glossogobius *sp* gene was quite similar to the group fish of Beloso and distant from the *Gibisoma bosci*, *Enypnias seminudus* and *Enypnias acearas* (Figure 3.)

Fish Gene Sequence Similarity with Beloso: BLAST-N analysis of the genes for gene Beloso in Gen Bank fish shows many similarities with fish genes have been reported (Table 2). Table 2 listed some types of fish with similarity or likeness to 88% Beloso. BLAST-N analysis of the Beloso genes shows a very high sequence similarity (93% - 94%) with a fish gene *Glossogobius sp*. The alignment results give confidence that the Beloso fish genes in Lake Tempe and its surrounding are the same genes from the *Glossogobius sp*.

Phylogenetic picture (Figure 4) showed that the genes of Beloso (*Glossogobius aureus*) is close to the *Glossogobius sp* and a bit far with the fish *Gibisoma bosci, Enypnias seminudus* and *Enypnias acearas*.

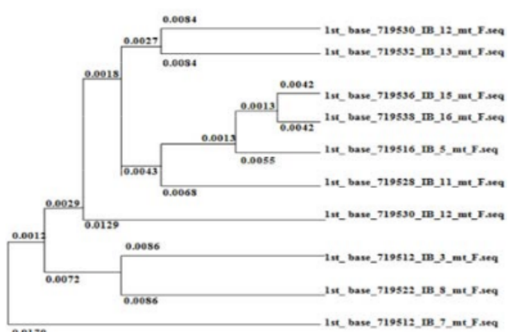

Fig. 3: Dendogram distance Beloso genetic individuals of Lake Tempe and surrounding Based Gene DNA sequences, 16S rRNA mt-DNA; UPGMA Analysis Method

Table 2: The gene sequence similarity with Beloso fish contained in Gene Bank

Description	Score maximum	Total score	Fulfillment query	Similarity max identity
Glossogobius sp DR-2004 haplotipe 1 16S ribosomal RNA gene partial sequence, mitochondrial	821	821	90%	94%
Glossogobius sp DR-2004 haplotipe 2 16S ribosomal RNA gene partial sequence, mitochondrial	815	815	90%	94%
Glossogobius sp DR-2004 Haplotipe 3 16S ribosomal RNA gene Partial sequence, mitochondrial	804	804	90%	93%
Siniperca knerii isolate DYG mitochondrion, complete genome	693	693	95%	89%
Siniperca scherzeri isolate BG mitochondrion, complete genome	689	689	95%	89%
Siniperca chuatsi mitochondrion, complete genome	689	689	95%	89%
Siniperca chuatsi 16S ribosomal RNA gene partial sequence, mitochondrial	688	688	95%	89%
Rhyacichthys guiberti isolate 15A 16S ribosomal RNA gene partial sequence, mitochondrial	688	688	95%	89%
Rhyacichthys guiberti isolate 15B 16S ribosomal RNA gene partial sequence, mitochondrial	688	688	95%	89%
Lutjanus rivulatus mitochondrion DNA, complete genome	678	678	95%	88%
Protogobius atiti isolate 36PK14 16S ribosomal RNA gene partial sequence, mitochondrial	676	676	95%	88%
Lutjanus kasmira mitochondrion, complete genome	673	673	95%	88%
Protogobius atiti isolate 35PK13 16S ribosomal RNA gene partial sequence, mitochondrial	669	669	95%	88%

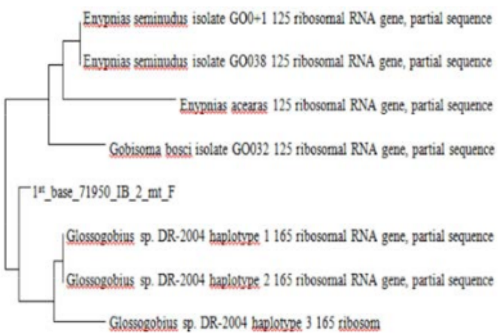

Fig. 4: Phylogenetic tree Beloso Fish Species of Lake Tempe and surrounding waters with outgroup Based on 16S rRNA Gene Sequences DNA mt-DNA.

CONCLUSION

It can be concluded that the genetic distance of Beloso (*Glossogobius aureus*) in Lake Tempe and surrounding waters based on DNA sequences by UPGMA analysis method is 0.0012 - 0.0170. It indicates that the genetic diversity of Beloso is already very low. Beloso fish test of sequence similarity indicates a very high similarity (93% - 94%) with gene *Glossogobius sp*. Genetic diversity of Beloso (*Glossogobius aurus*) is quite similar to the *Glossogobius sp* and different from the group *Gibisoma bosci*, *Enypnias seminudus* and *Enypnias acearas*. Further research should be conducted to compare the genetic characters of Beloso in Lake Tempe and the surrounding waters with existing Beloso fish habitat in other surrounding lakes located in Sulawesi, Indonesia.

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