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Genetic diversity of three generations tinfoil barb *Barbonymus schwanenfeldii* (Bleeker 1854) from West Kalimantan, Indonesia

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Abstract
The success of domestication programme and sustainability of aquaculture is highly dependent on the result of evaluation and understanding of the genetic diversity. This study is aimed to evaluate the genetic diversity of three generations, G-0, G-1, G-2 of tinfoil barb from West Kalimantan. Genetic analysis was conducted by RAPD using three primers (OPA 08, OPA 09 and OPC 02). The samples of caudal fin from each generation used 10 individuals. Formation of the population generation was done by individual selection method based superior growth. The results of intrapopulation diversity showed that the percentage polymorphism and heterozygosity of G-0 to G-2 has decreased by 30-40% with value 42.50% ; 0.163 (G-0), 27.50% ; 0.105 (G-1) and 15.00%; 0.071 (G-2). Based on the domesticated showed the genetic distance ranged from 0.26 to 0.36. The closest genetic distance was G-1 and G-2 (0.26). The domestication process that has been done can cause the reduction in genotype diversity of tinfoil barb.

Keywords: Barbonymus, barb, genetic, Kalimantan, Indonesia

Introduction
The success of domestication programme and aquaculture development depends on sustainable and quality management of genetic resources. Tinfoil barb *Barbonymus schwanenfeldii* (Bleeker 1854) is a native species of Southeast Asia [1,2]. In Indonesia was found in freshwaters of Sumatera, Kalimantan and has been reported in Java [3,4,5,6,7]. In West Kalimantan, tinfoil barb is high economic species (IDR.25.000-40.000,-/Kg) and potential as ornamental fish for small size.
Genetic diversity becomes a key parameter of *survival of fittest* population which ensure sustainability and ability to response passively natural selection and artificial [8,9]. Genetic diversity can be identified through a variety of morphological phenotype and genotype. Analysis of morphology on 3 generations of tinfoil barb from West Kalimantan has been done and the results was showed the highest similarity index (*sharing component*) of intrapopulation phenotype (76,7%) was in the G-1 and G-2 [6].
In support the success of tinfoil barb domestication from West Kalimantan, genetic analysis is also required based on genotype. Evaluation of genetic diversity has been done on domestication of mahseer [10]. In this study, genotype mapping was conducted using *Random Amplified Polymorphic DNA* (RAPD) which has capability to detect quickly one primer, without tracing the background of previous genome [11]. RAPD markers is ideal because of its high polymorphism[12]. In domestication and breeding studies, genetic variation analysis using RAPD has been done on lake kuromai rainbowfish *Melanotaenia parva* [13], orange clownfish *Amphiprion percula* [14], humpback grouper *Cromileptes altivelis* [15], common carp *Cyprinus carpio* [16] and mahseer [10]. The previous research reported that genetic variation was decrease or increase in each generation. In domestication, genetic variance influenced by increase the coefficient of inbreeding per generation and the amount random genetic drift [17]. This study was aimed to evaluate the genetic diversity of three generations of domesticated tinfoil barb from West Kalimantan.

2. Materials And Methods

2.1 Domestication

The founder generation (G-0) of tinfoil barb was captured from population consisting 50 individuals male and 100 individuals female sized 21,43 ± 1,21 cm and 432,25 ± 81,23 g. The first generation was formed by mating 25 pairs of G-0. After 14 months of grew out of G-1, 30 pairs of G-1 was spawned to produce the second generation (G-2).

2.2 Sample collection

The sample in this study was from three generation of domesticated tinfoil barb (G-0, G-1 and G-2), in the Central Institute for Fish Seed Production Anjungan, West Kalimantan. The Samples of caudal fin from each generation used 10 individuals. Approximately 1 cm of caudal fin was cut and preserved in a micro tube containing of alcohol 70% before the DNA extraction.

2.3 RAPD (*Random Amplified Polymorphic DNA*)

DNA extraction

DNA extraction was conducted by *Phenol-Chloroform* methods [18]. 5-10 mg pieces of fin were inserted into microtube 1.5 ml, which contained 500 µl TNES Urea solution and 10 µl proteinase K solution then was homogenized with vortex and incubated at 37 ºC for 24 hours until the tissue was destroyed. Then added 1.000 µl *Phenol Chloroform* and vortex were homogenized and centrifuged at 10.000 rpm for 10 minutes. The supernatant formed was transferred into a new microtube and added 1.000 µl of *ethanol* 90 % and 10 µl of sodium acetate. Afterwards a centrifuge at a speed of 10.000 rpm for 10 minutes. After that, the DNA deposit was seperated from the solution and dried at room temperature. Furthermore, the DNA pellet was dissolved in 100 µl *Tris - EDTA* (TE) *buffer* and stored at 4 ºC.

DNA amplication by FCR

DNA amplication was performed using PCR with three primers (OPA 8, OPA 9, and OPC 2) [5]. The composition of the material was used 1 µl DNA, 0.5 µl primer, 1 unit (10 µl) *dry taq* Promega, and *distilled water* (8.5 µl) with total volume of 20 µl. PCR program was used, including 94ºC pre-denaturation for 5 minutes, denaturasi of temperature 94ºC for 1 minutes, *annealing* of temperature 36ºC for 1 minutes, elongation of temperature 72ºC for 2,5 minutes, final elongation of 72ºC for 7 minutes and stabilization process of 4ºC for 3 minutes. The PCR process was done 45 cycles. The PCR result was then electrophoresed using 2% agarose gel in *Tris-Boric-EDTA* (TBE) *buffer*. The results were observed with a UV illuminator and documented with a Polaroid camera.

2.4 Statistical Analysis

Levels of intrapopulation genetic diversity were analyzed using Tingkat *Tools for Population Genetic Analysis* (TFPGA) programe refers to [19], whereas interpopulation relationships were analyzed based on genetic distance with UPGMA (*Unweight Pair Group Methods Arithmetic*) programe and presented in the form of dendrogram.

3.1 Results and Discussions

3.1 Genetic diversity of three generation of tinfoil barb

Amplification of DNA using three primer (OPA 08, OPA 09 dan OPC 02) on three generation of tinfoil barb from West Kalimantan was on figures 1, 2 and 3.

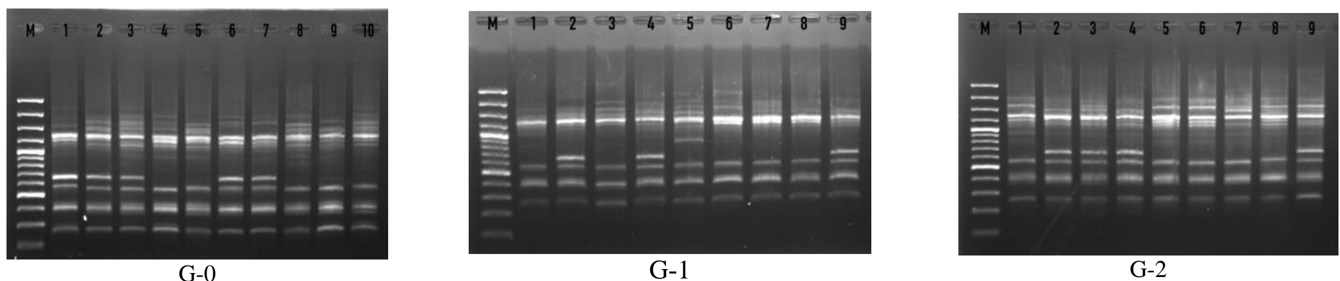


Fig 1: DNA amplification three generation of tinfoil barb (G-0, G-1, and G-2) from West Kalimantan with PCR-RAPD using OPA 08 primers (Description: number (1-10) = fish samples, M = marker (GeneRuler 100 bp Plus DNA Ladder, Thermo scientific).

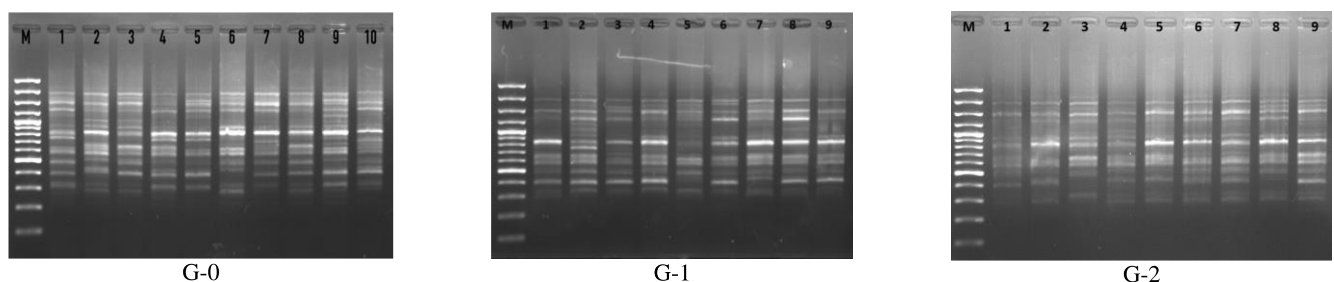
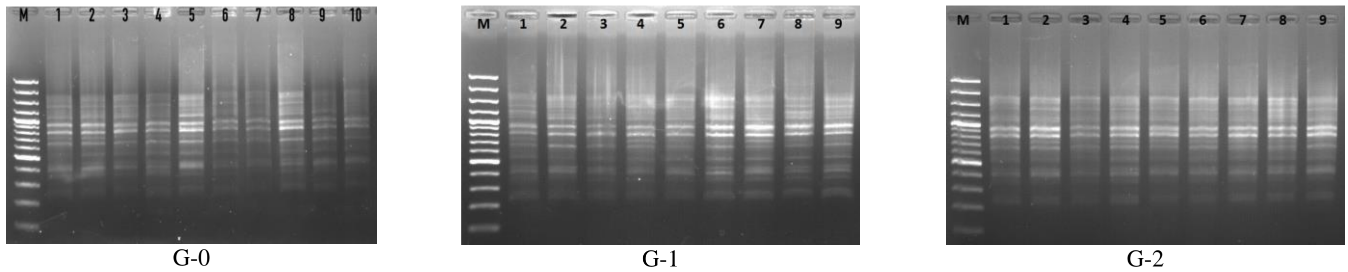


Fig 2: DNA amplification three generation of tinfoil barb (G-0, G-1, and G-2) from West Kalimantan with PCR-RAPD using OPA 09 primers (Description: number (1-10) = fish samples, M = marker (GeneRuler 100 bp Plus DNA Ladder, Thermo scientific).



G-0 G-1 G-2

Fig 3: DNA amplification three generation of tinfoil barb (G-0, G-1, and G-2) from West Kalimantan with PCR-RAPD using OPC 02 primers (Description: number (1-10) = fish samples, M = marker (GeneRuler 100 bp Plus DNA Ladder, Thermo scientific).

The number and size of amplified fragment is presented on Table 1. Diversity of RAPD profile showed that the size of amplified DNA fragment of 3 generations of tinfoil barb were same, ranged from 230-2400 bp. The number of fragments produced varied from 7 to 18 for G-0, 8 to19 for G-1, and10 to 19 for G-2.

Table 1: The number of fragments and size of DNA with PCR-RAPD using OPA 08, OPA 09 and OPC 02 primers of three generations of tinfoil barb from West Kalimantan.

Parameters	*population*		
	G-0	**G-1**	**G-2**
OPA 08 Number of fragment Size of fragment	 10-14 275-2000	 8-9 275-2000	 10-13 275-2000
OPA 09 Number of fragment Size of fragment	 15-18 290-2400	 13-19 290-2400	 14-19 290-2400
OPC 02 Number of fragment Size of fragment	 7-16 230-1800	 14-17 230-1800	 15-17 230-1800
Total Number of fragment Size of fragment	 7-18 230-2400	 8-19 230-2400	 10-19 230-2400

The percentage of polymorphism and heterozygosity were presented in Table 2.

Table 2: Percentage of polymorphism and heterozigosity of three generations of tinfoil barb from West Kalimantan using OPA 08, OPA 09 and OPC 02 primers.

Genetic diversity	**Tinfoil barb**		
	G-0	**G-1**	**G-2**
Polymorphism (%)	42,50	27,50	15,00
Heterozigosity	0,163	0,105	0,071

Based on the analysis of genetic diversity, the percentage of polymorphism and heterozygosity was decreased from G-0 to G-2. Heterozygosity obtained in G-2 was decreased by 35% from G-0 and 32% of G-1, while the percentage of polymorphism in the G-2 was decrease 35% from G-0 and 45% from G-1.

Differences polymorphism DNA bands generated depends on annealing sites and can be used to provide an overview of the level of genetic diversity of a population [20]. Impairment percentage polymorphism value of second generation (G-2) in tonfoil barb from West Kalimantan is closely related to the decrease of heterozygosity, this may be due to population isolation and genetic drift [17]. The decline in the percentage of polymorphism and heterozygosity values often occur due to inbreeding and natural migration limitations [21;22]. In addition, the selection intensity level can be calculated a large role in determining decline of polymorphism and heterozygosity [23]. The decline in the genetic diversity e.g. heterozygosity and polymorphism, may occur due to the loss of alleles during adaptation process.

Pairwise comparison test FST (Table 3) showed significant different in genetic diversity between G-1 and G-0 (P<0,05). FST test results indicated that the G-0 of tinfoil barb was indeed unexplored.

Table 3: Pairwise comparison FST Percentage of three generations of tinfoil barb from West Kalimantan.

Population	**G-0**	**G-1**	**G-2**
G-0	******		
G-1	0,0085*	******	
G-2	0,4007	0,9997	******

Description: significantly different (P<0.05).

The analysis of genetic diversity interpopulation, genetic distance of each generation (Table 4) showed that the furthest genetic distance occurred between G-0 and G-1 (0,362). The genetic relationship among different generation was presented on figure 4.

Table 4: Genetic distance of three generations of tinfoil barb from West Kalimantan.

Population	**G-0**	**G-1**	**G-2**
G-0	******		
G-1	0,3615	******	
G-2	0,2983	0,2656	******

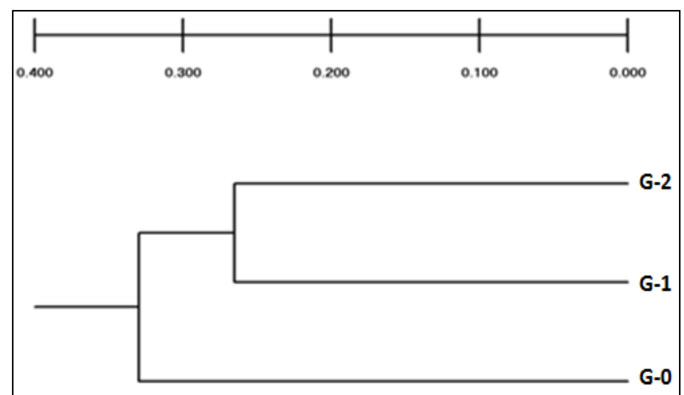


Fig 4: Genetic relationship among three generations of tinfoil barb from domesticated programe based on OPA 08, OPA 09 and OPC 02 diversity.

Genetic diversity is a key parameter of population fitness that ensures its sustainability and the ability to respond passively to natural or artificial selection [9]. Differences in genetic diversity may increase the genetic distance among populations and is generally used as a consideration in the selection and crossbreeding. In general, low intrapopulation

genetic diversity will result in negative traits including slow growth, reproduction and low adaptation rates [24].

4. Conclusion

The genetic diversity of tinfoil barb from West Kalimantan showed that the percentage of polymorphism and heterozygosity value decreases with every generation, with the closest genetic distance among G-1 and G-2 (0.266). The result of genotype data from generation to generation enabled to used as baseline information to support the stability of broodstock quality and seed of tinfoil barb.

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