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GENETIC RELATIONSHIP OF THE TRANSGENIC AND NON-TRANSGENIC MUTIARA CATFISH WITH THEIR HYBRIDS SANGKURIANG CATFISH

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Abstract. *The Transgenic "Mutiara" catfish is a fast growth fish containing Clarias gariepinus Growth Hormone (CgGH). Crossing between male transgenic Mutiara catfish and the non-transgenic one produced expecting F1 and F2 hybrids carrying superiority inheritance from the transgenic parent. This study analyzed genetic relationship using RAPD among the parent, hybrids and "Sangkuriang" catfish as additional material. Genomic DNA extraction and purification from catfish caudal fin tissue sample was performed using Wizard® Genomic DNA Purification kit (Promega). The selected primers used for the amplicons in the PCR process were OPA-03 and OPA-16. In the analysis phase, DNA fragments in the form of monomorphic and polymorphic fragments were then processed using the NTSYS-pc-2.02 (Numerical Taxonomy and Multivariate Analysis System) program and produced a phenogram. The results showed that only OPA-03 (5'-AGTCAGCCAC-3') primer enabled to determine DNA polymorphism among catfish samples. The closest genetic similarity index originates from the female broodstock of Non-Transgenic Mutiara catfish (P) (93%), while the furthest originates from the male broodstock of Mutiara Transgenic catfish (P) (51%) with F1 dan F2.*

Keywords: *genetic relationship, Mutiara catfish, polymorphism, Sangkuriang catfish, transgenic*

Citation
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INTRODUCTION

Freshwater aquaculture activities in Indonesia especially catfish farming are promising enterprise because their cultured is relatively easy and local market consumption demand is still high (Muhammad & Andriyanto, 2013). Short maintenance age (3-4 months) for the needs of "pecel lele" (Indonesian deep fried catfish) makes catfish a profitable business. This business opportunity encourages fish farmers to apply catfish culture intensively, with the use of high stocking densities and routine artificial feeding. This condition causes the demand for high catfish seeds and encourages the fish farmer to increase the frequency of broodstock spawning to fill the continued long-term demand.

Frequent use of the same broodstock in spawning by fish farmers (breeder) on freshwater aquaculture activities might cause the degradation of fish phenotype due to a high

rate of inbreeding. It leads to a decrease in superior character of the broodstock to offspring (Yousefian & Nejati, 2008; Chattopadhyay, 2017). Genetic improvement efforts that can be done is selective breeding (Iswanto et al., 2015). To date, catfish selective breeding programs have been carried out by the Fish Breeding Research Center (BPPI) Sukamandi which produces “Mutiara” catfish and has been officially disseminated to the public based on Decree of the Minister of Maritime Affairs and Fisheries Republic of Indonesia No.77/KEPMEN-KP/2015 (BPPI, 2014).

Genetic quality improvement of catfish can also be performed using hybridization and transgenesis technology. On hybridization, superior features within a few generations are not always inherited due to the effect of temporary dominance in the hybrid fish (Liu, 2007). While on transgenesis fish, the stability of inheritance from excellent character (growth) is displayed on the F1 and F2 offspring, showing growth gene construct integrated into the fish’s genome (Alimuddin et al., 2003; Rasal et al., 2016). Transgenesis application in Mutiara catfish using CgGH (*Clarias gariepinus* Growth Hormone), gene transfer (Buwono et al., 2016), and PhGH (*Pangasius hypophthalmus* Growth Hormone) gene transfer in African catfish (Dewi et al., 2013) were able to increase the catfish size two times larger than normal catfish.

Strain descent transgenic Mutiara catfish has been proved to have CgGH, while the genetic relationship among transgenic and non-transgenic Mutiara catfish broodstock (P) with first (F1) and second (F2) offspring hybrids of transgenic and non-transgenic Mutiara catfish need to be examined to determine stabilization of a decrease in the superior properties. Analysis of RAPD (Random Amplified Polymorphic DNA) were represented by monomorphic and polymorphic fragments that illustrate the genetic relationship of broodstock to each offspring (F = filial) (Dunham, 2004; El-Hawary et al., 2018). In this study, genetic analysis was carried out on the transgenic and non-transgenic Mutiara catfish broodstock (P) with F1, F2 offspring hybrids and Sangkuriang catfish as an effort to prevent inbreeding in the breeding program.

MATERIALS AND METHODS

Two-years-old transgenic and non-transgenic Mutiara catfish broodstock (P), one-year-old F1 offspring, one-month-old F2 offspring and one-year-old Sangkuriang catfish were used as research samples. Fish genomic DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega). The genomic DNA extraction was carried out using catfish caudal fin tissue, hence DNA can be obtained without injure or kill the fish. The presence and quality of genomic DNA were determined by doing DNA quantitative testing with the spectrophotometer through calculating the ratio of Å260 / Å280 nm wherein the UV light wavelength of 260 nm is absorbed by the double-helix DNA, while long-wave UV light at 280 nm is absorbed by the proteins or phenols contaminant, thus the measurement of the purity in the genomic DNA is known (Fatchiyah et al., 2011).

The process of amplification using two kinds of arbitrary size 10 nucleotide primers with 10 base pairs manufactured by Operon Technology (Alameda, California), namely OPA-03 and OPA-16 (Table 1). Two primers selected from the reference from several studies, OPA-03 primer used by Danish et al. (2012) in *Clarias batracus*; Diani (2013) in local, Dumbo and Sangkuriang catfish; and Buwono et al. (2018) in F1 offspring hybrids of transgenic and non-transgenic Mutiara catfish and Sangkuriang catfish, while OPA-16

primer used by Beheary et al. (2015) in *Clarias gariepinus* and Buwono et al. (2018) in F1 offspring hybrids of transgenic and non-transgenic Mutiara catfish and Sangkuriang catfish. Both primers (OPA-03 and OPA-16) considered as a selected primer that has a complementary sequence with the transgenic and non-transgenic Mutiara catfish broodstock (P) with F1, F2 offspring hybrids and Sangkuriang catfish sequence.

Table 1. RAPD Primer List With Sequence Bases

Primer	Sequences
OPA-03	AGT CAG CCAC
OPA-16	AGC CAG CGAA

Exploratory method and qualitative descriptive analysis were used in this study. PCR were performed in 25 µl reaction volume containing GoTaq fast 2G ® master mix 12.5 µl, Nuclease-Free Water (NFW) 9.5 µl, 1.0 µl RAPD Primer (OPA-03 and OPA-16), and template DNA 2.0 µl. The DNA qualitative test performed by agarose gel electrophoresis (Williams et al., 1990). The electrophoresis was carried out at 60 volts for 35 min using 1% agarose gel and TBE buffer. The gel was removed and stained with 5 µl EtBr (Ethidium Bromide) solution for 25 minutes and rinsed with 100 ml distilled water for 8 minutes. Reaction mixtures were amplified in Sensoquest Labcycler, programmed for 45 cycles with a final extension step at 72°C for 5 minutes. Each cycle consists of denaturation at 94 °C for 1 minunte, annealing at 36°C for 1 minute and extension at 72°C for 2 minutes. PCR reaction product was separated by electrophoresis in 1% agarose using TBE buffer for 85 min at 55 volts.

The results of DNA amplification using RAPD primers were interpreted with the NTSYSpc-2.02 (Numerical Taxonomy and Multivariate Analysis System) program to determine genetic relationships among samples. This program will display genetic similarities. The genetic distance was calculated using a method developed by Nei & Li (1979) that the GD (genetic distance) = 1 - GS (genetic similarity).

RESULTS AND DISCUSSION

Genomic DNA Extraction

The degree of purity of DNA has a ratio value of 1.8 to 2.0 (Barbas et al., 2007). If the value of the ratio is less than 1.8 then there is still protein or phenol contamination in the solution (Tenriulo et al., 2001), while the greater ratio more than 2.0 was indicated DNA contamination of RNA (Khosravinia et al, 2007). According to the results (Table 2), all samples were in range ratio expressing the purity of tested DNA. Furthermore the genomic DNA tested by electrophoresis and results test shown in Figure 1.

The electrophoresis genome results showed smear visualization. Therefore, DNA quality measurement indicated eligible and qualified to be samples templates for DNA amplification.

Table 2. Catfish Genome DNA Purity Test Ratio Value

No.	Sample	Abs260	Abs280	Ratio	Consentration (ng/µl)
1	(P) Male Transgenic Mutiara Catfish (♂MTG)	0.112	0.061	1.836	280
2	(P) Female Non Transgenic Mutiara Catfish (♀ MNT)	0.163	0.086	1.895	407.5
3	(P) Male Non Transgenic Mutiara Catfish (♂MNT)	0.177	0.097	1.824	442.5
4	(F1) MTG >< MNT Male Catfish	0.258	0.143	1.804	645
5	(F1) MNT >< MNT Female Catfish	0.129	0.07	1.842	322.5
6	(F2) MTG_MNT >< MNT_MNT Catfish	0.13	0.068	1.911	325
7	Sangkuriang Catfish (control)	0.216	0.111	1.945	540

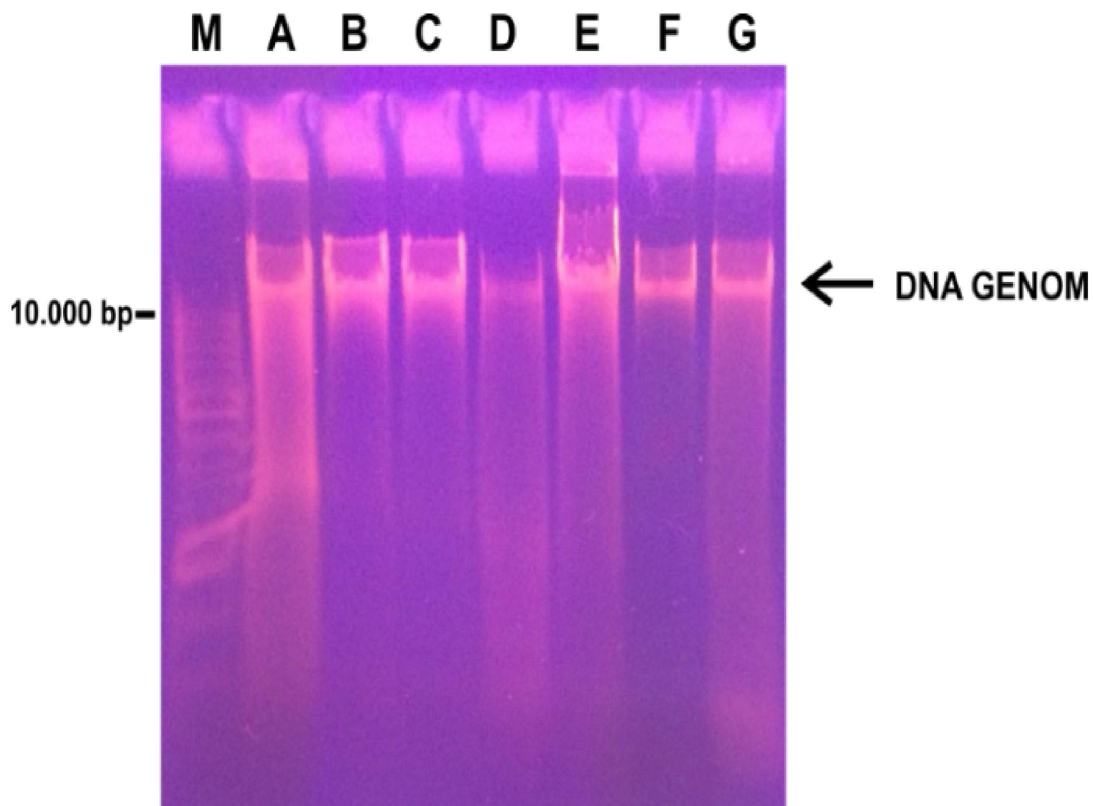

Figure 1. Catfish Genomic DNA Electrophoresis Results
A = (P) Male Transgenic Mutiara Catfish (♂MTG); B = (P) Female Non Transgenic Mutiara Catfish (♀ MNT); C = (P) Male Non Transgenic Mutiara Catfish (♂MNT); D = (F1) MTG >< MNT Male Catfish; E = (F1) MNT >< MNT Female Catfish; F = (F2) MTG_MNT >< MNT_MNT Catfish; G = Sangkuriang Catfish (control)

Polymorphisms and Heterozygosity

Electropherogram results from two PCR primers showed OPA-03 primer was the most complementary primer sequences with catfish samples sequences than OPA-16 primer (Figure 2). Annealing temperature precision is factor that affect the success of primer complementary with a DNA sequence in the PCR amplification process thus DNA fragments appear (Zulfahmi, 2013). In OPA-16, primer all samples were copied but only produced one line of monomorphic fragments, which indicates all samples closely related. While a number of polymorphic fragments detected by OPA-03, this provides a basis to determine genetic variations among samples. Genetic variations (polymorphisms) will serve as a reference point in the management of sustainable aquaculture (Danish et al., 2012).

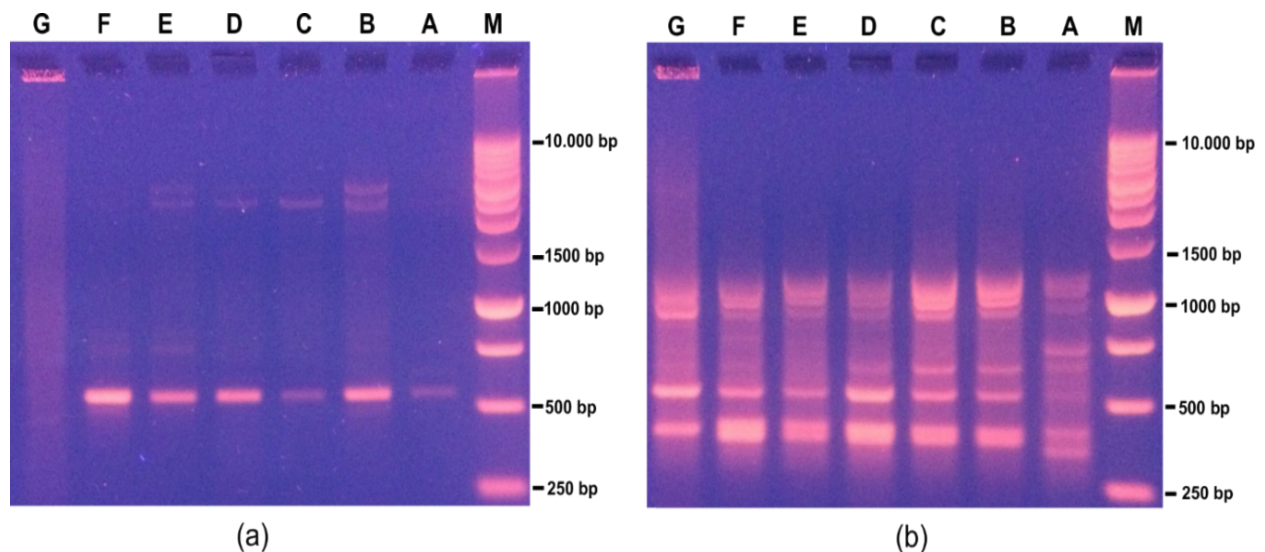

Figure 2. a) DNA Amplification Result using OPA-16 Primer; b) DNA Amplification Result using OPA-03 Primer

Monomorphic and polymorphic fragments sample using OPA-03 spread to the seven samples (Table 3). The monomorphic fragment is a DNA fragment found in all of the samples at the same size, while polymorphic fragments detected at a specific size and do not recover in other samples. The OPA - 03 primers produced 55 DNA fragments at 342 - 1249 bp, which 6 of them were polymorphic. Highly reproducible fragment in size range of 200-150 bp generally found in fish (Liu et al., 1999). Danish et al. (2012) also reported OPA-03 primers produced 23 fragments appeared at 240-1360 bp on *Clarias bathracus* DNA amplification. The presence of polymorphic fragments exhibited a genetic variation among samples that plays an essential role in improving the fish fitness (survival) so it can adapt to the changes in environmental conditions (Dunham, 2004).

The RAPD-PCR fragment results indicated a particular gene linkage as well as a distinct phenotype expression (FAO, 2001; Liu & Cordes, 2004) that expressed in the Male Transgenic Mutiara Catfish (P) and (F2) offspring hybrids Catfish with 5 and 1 polymorphic fragment respectively (Table 3). RAPD as dominant trait produced homozygotes as much as heterozygotes allele while the heterozygotes generated the polymorphic alleles (Liu, 2007). Buwono et al. (2016) reported transgenic Mutiara catfish has success-

Jurnal Biodjati 4(2):204-213, November 2019

JURNAL BIODJATI

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fully inserted by CgGH that constructed from two GH gene derived from the fish own body (endogenous) and GH from outside the body (exogenous) thus effects of transgenic catfish revealed polymorphisms.

The polymorphisms found in the transgenic Mutiara catfish (samples A and F) more than non-transgenic Mutiara catfish (samples B, C, E and G) that tend to produce monomorphic fragments. Buwono et al. (2018) reported non-transgenic Mutiara catfish and Sangkuriang catfish have a lack of diversity (monomorphism), while in this study (Table 3) the monomorphic fragments were generated in all samples on 396 bp, 523 bp and 1035 bp (indicated by parallel fragments). The presence of monomorphic fragments on all samples implies a high level of similarity among samples (Dunham, 2004). Four African catfish strains such as Dumbo, Sangkuriang, Egypt and Paiton catfish were crossed and became the former base population of Mutiara catfish (BPPI, 2014). It explained that the seven samples of this study are descendants of African catfish thus have a kinship relationship.

Table 3. Polymorphic and monomorphic fragments Catfish from OPA-03 Primer Test

Distance Fragments of the wells (bp)	A	B	C	D	E	F	G
1249	--*						
1173	--*						
1101	--	--		--	--		
1035	--^	--^	--^	--^	--^	--^	--^
986	--		--			--	
926		--	--	--	--	--	--
784						--*	
743	--*						
636	--	--	--	--		--	--
580	--*						
523	--^	--^	--^	--^	--^	--^	--^
416				--	--	--	
396	--^	--^	--^	--^	--^	--^	--^
377		--	--	--	--	--	--
342	--*						

--^ = monomorphic fragment; --* = polymorphic fragment

A = (P) Male Transgenic Mutiara Catfish (♂MTG); B = (P) Female Non Transgenic Mutiara Catfish (♀ MNT); C = (P) Male Non Transgenic Mutiara Catfish (♂MNT); D = (F1) MTG >< MNT Male Catfish; E = (F1) MNT >< MNT Female Catfish; F = (F2) MTG_MNT >< MNT_MNT Catfish; G = Sangkuriang Catfish (control)

Genetic Relationship Analysis

The results of the RAPD analysis using the OPA-03 of the seven isolates genomic DNA (Figure 3) divided into two main clusters to produce the dendrogram of genetic relationship (Mahaputro et al., 2012; Adolfsson, et al., 2018).

Cluster I consisting of male transgenic Mutiara catfish (P) (sample A) that has 51% genetic similarity with other 6 samples. Based on the Nei & Li (1979) method, genetic distance (=GD) value estimated by genetic similarity relatively high 49%. This excessive value indicating high-level genetic variation due to sample A is the broodstock that not descended from reproduction but emanates from Mutiara catfish transferred growth hormone (GH) gene of Dumbo catfish. This maybe the reason for genetic variations (polymorphisms) compared with non-transgenic Mutiara catfish and the descendants.

Cluster II involving sample B, sample C, sample D (offspring of sample A and B), sample E (offspring of sample B and C), sample F (offspring of sample D and E), and samples G divided into three subcluster. Subcluster I involves Sangkuriang catfish (sample G) with female non-transgenic Mutiara catfish (P) (sample B), non-transgenic Mutiara catfish (P) (sample C), F1 hybrid offspring of male transgenic Mutiara catfish (P) with female non-transgenic Mutiara catfish broodstock (sample D), F1 hybrid offspring of male non-transgenic Mutiara catfish (P) with female non-transgenic Mutiara catfish broodstock (sample E), and F2 hybrid offspring of F1 transgenic and non-transgenic Mutiara catfish (sample F). Subcluster II involves 2 samples of fish (sample C and F), while subcluster III involves 3 samples of fish (sample B, D and E).

Genetic distance among samples in subcluster I (24%) relatively lower compared to cluster I (similarity 76%). The fact that Sangkuriang catfish which was one of the founders of Mutiara catfish history formation is a hybrid backcross result, made the samples (F1 and F2 offspring hybrids of Mutiara catfish) separated apart genetically as revealed by genetic distance analysis. Liu (2007) reported that superior features are not always inherited in hybrid offspring strains or not permanent. Moreover, subcluster II and subcluster III exhibited GS of 78% while the GD apart from each other by 22%, indicating low-level genetic variation compared with subcluster I. This implies more homogenity among five genetic fish samples (sample B, D, E, C and F). However, subcluster II that consists of sample C and sample F, has 87% GS with 13% GD which affirms the genetic distance between these fish higher when compared with subcluster III. One polymorphic fragment that appears on sample F (Table 3) indicates that genetic variation detected, contribution of alleles from the male transgenic Mutiara catfish (P) estimated as a cause of the polymorphism. Buwono et al. (2018) reported polymorphic fragments appeared on the F1 hybrid offspring transgenic and non-transgenic Mutiara catfish.

The lowest GD (7-11%) were found in subcluster III involving sample B, sample D (offspring of sample A and B), and sample E (offspring of sample B and C) while their GS values range from 89-93%. The genetic similarity approaching 100% showed a close genetic affinity (Muharam et al., 2012). It confirmed that F1 hybrid offspring of female non-transgenic Mutiara catfish (P) (sample B) crossed with male transgenic Mutiara catfish (P) (sample A) or male non-transgenic Mutiara catfish (P) (sample C) have predominance inheritance from female broodstock (sample B), thus genetic similarity between female broodstock and F1 is very close. Cheng et al. (2015) reported F1 hybrid offspring of

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Epinephelus fuscoguttatus (female) (P) >< *Epinephelus lanceolatus* (male) (P) had inherited the mitochondrial DNA (mtDNA) from maternal parent or closely related with *Epinephelus fuscoguttatus*. Furthermore, Permana et al. (2009) affirmed that the contribution of mtDNA can be inherited only from the mother (maternal inheritance).

The genetic similarity level described by genetic distance and similarity index of individual members of the population (Muharam et al., 2012; Arifin et al., 2017). The high-level of similarity index indicated low-level of genetic variation and generated to inbreeding among a samples (Sofro, 1994). Mating between non-transgenic Mutiara catfish broodstock in the same population should be avoided due to high closely related potential. The closest genetic similarity index of 93% originates from the female broodstock of non-transgenic Mutiara catfish (P) (sample B) and (F1) MTG >< MNT Male Catfish (sample D) had inbreeding potential. While the farthest genetic similarity index of 51% originates from the male broodstock of male transgenic Mutiara catfish (P) (sample A) with female F1 and F2. Offspring hybrids mating are recommended for producing catfish offspring with better genetic quality. In conclusion, male transgenic Mutiara catfish (P) are distinctive species based on the total number of polymorphic fragments thus increase genetic variation within population.

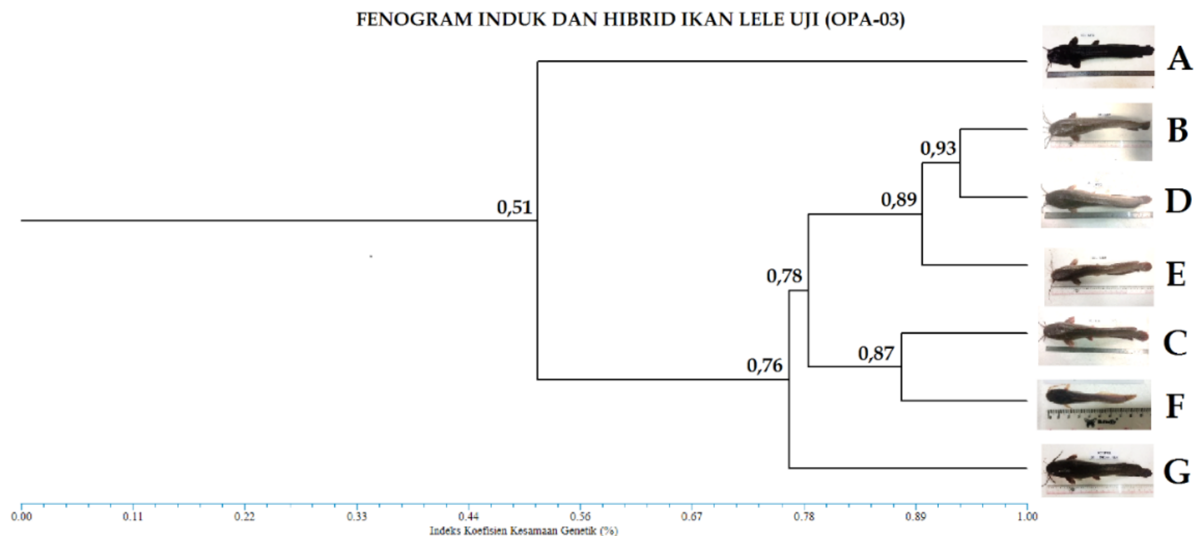

Figure 3. Fenogram Parent and Hybrid Catfish Test (OPA-03)

A = (P) Male Transgenic Mutiara Catfish (♂MTG); B = (P) Female Non Transgenic Mutiara Catfish (♀ MNT); C = (P) Male Non Transgenic Mutiara Catfish (♂MNT); D = (F1) MTG >< MNT Male Catfish; E = (F1) MNT >< MNT Female Catfish; F = (F2) MTG_MNT >< MNT_MNT Catfish; G = Sangkuriang Catfish (control)

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