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Ayi Yustiati
Lecturer of Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia

Hana Junita Simangunsong
Lecturer of Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia

Ujang Subhan
Lecturer of Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia

Dian Yuni Pratiwi
Lecturer of Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia

Corresponding Author:
Dian Yuni Pratiwi
Lecturer of Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia

Genetic relationship analysis of hybrid green catfish (*Hemibagrus nemurus*) with striped catfish (*Pangasianodon hypophthalmus*) by random amplified polymorphic Dna (Rapd) method

Ayi Yustiati, Hana Junita Simangunsong, Ujang Subhan and Dian Yuni Pratiwi

Abstract
This research conducted to determine the genetic variation on male green catfish (*Hemibagrus nemurus)*, female striped catfish (*Pangasianodon hypophthalmus)* and genetic relationship of hybrid to each of parent. The research method was an explorative experiment. This research was conducted using striped female striped catfish, male green catfish, and the hybrid of striped female catfish and male green catfish. Genetic variation analysis was performed using the RAPD-PCR technique using primary OPA-2 and OPA-3 methods. However, the result of DNA amplification with OPA-3 was better than OPA-2. Therefore, further analysis was carried out using data from OPA-3. The genetic relationship was analyzed based on the similarity index which calculated by *Numerical Taxonomy and Multivariate Analysis System* (NTSYS) program. The result showed that the similarity index between hybrid and female striped catfish was 82%, while the similarity index between female striped catfish and male green catfish was 31%. Thereby, the inheritance of hybrid comes more from female striped catfish.

Keywords: Green catfish, female striped catfish, hybrid, genetic relationship

1. Introduction
In 2030, the human population is estimated to reach 8.2 billion. So that, there will be an increase in demand for nutritious food sources. Global food production with good quality from land and water must increase to meet human needs. Fisheries is one of the sectors that is important in providing foods and healthy diet for the human population. Globally, fish provide 16.6% of protein from all animal protein. Because of that, the demands and production for fish in the world tend to increase. Fish supply is expected to increase from 154 million tons in 2011 to 186 million tons in 2030 [1].

Not only in the world, now days, fish demand and production in Indonesia shows the increasing trend year by year [1]. Fish consumption in Indonesia is increasing from 32.25 kg/capita/year in 2011 to 47.34 kg/capita/year in 2017 [2]. The ministry of maritime affairs and fisheries reported that the value of Fisheries Gross Domestic Product which is increased in recent years. The value of fisheries Gross Domestic Product in 2019 reached 62.24 trillion. This value was 6.24% greater than the value of Gross Domestic Product in the year 2018. This increasing Gross Domestic Product value was supported by the increasing number of aquaculture production which reached 8.2 million tons in 2019. While in 2018, it was 6.7 million tons [3].

Fisheries is also a sector that contributes to the Indonesian economy because a lot of fisheries products are exported. Indonesian fishery products had also been exported to 157 countries [3]. The value of fisheries product was US$ 4.2 billion dollars in 2012. The sector also opens employment opportunities of around 6.4 million direct jobs and fulfills the needs of 54.8% of protein for the domestic population [4]. Because of The fisheries products' demands are high and continue to grow every year, so it's must be balanced with the availability of prime and excellent fingerlings.

One type of fish that is widely cultivated and has high economic value in Indonesia is the striped catfish (*Pangasianodon hypophthalmus*). This fish was introduced from Thailand to

Indonesia in 1972. Striped catfish have an elongated body shape, are slightly flattened and do not have scales. Striped catfish have a relatively small head with a wide mouth. Body length can reach 120 cm. The body of the striped catfish is silver white with a bluish back.

Striped catfish (*P. hypophthalmus*) has been established by the Ministry of Maritime Affairs and Fisheries (KKP) as a developed fishery commodity for the acceleration of industrialization programs. This fish is also widely cultivated in various Asian countries such as Bangladesh, India, Malaysia, Laos, and Indonesia [5]. This is because striped catfish has several advantages, such as faster growth compared with local catfish, high market demand, large size, high enough protein [6], low production cost, high stress tolerance, and disease resistance. This fish is able to survive in very bad conditions [7].

Based on the Ministry of Maritime Affairs and Fisheries data, Striped catfish production in Indonesia increased from year to year about 391,151 tons in 2018.This value was 22.2 percent higher than the production of catfish in 2017. In 2018, catfish for the first time was exported to the kingdom of Saudi Arabia to be given to pilgrims from Indonesia. These reasons make catfish widely cultivated in Indonesia. However, one of the weaknesses of Striped catfish is yellowish-colored flesh.

Another type of popular fish, green catfish (*Hemibagrus nemurus*) is also an aquatic commodity that has prospects for cultivation. The advantage of green catfish is that it has white, thick and contains high protein. This fish can reach sizes from 750-1000 g/individual. Because of that, *H. nemurus* have a high price from 60.000 to 75.000 IDR/kg [8]. If this fish has been processed into smoked fish, the selling price can reach Rp.150.000 – 250.000/Kg [9].

The activity of crossing between one species could decrease genetic quality in offspring. One effort to prevent the decline of genes is to hybridize between different types of fish, but have a close relationship such as striped catfish and green catfish. Therefore, to improve fish quality, hybridization needs to be done. The hybrid is expected to has rapid growth such as striped catfish with white, thick and thornless meat such as green catfish. Thus, hybrid from striped catfish and green catfish can have a higher selling price.

The hybrid can have different genetic variation with the two parents. The method that can be used to determine genetic variation is the Random Amplified Polymorphic DNA or commonly referred to as RAPD-PCR [10]. The RAPD technique is useful and sensitive in differentiating various fish genera and species [11]. Detected polymorphic DNA fragments can interpret the genetic relationship of hybrid offspring with male parent and female parent [12].

This research was aimed to determine the genetic variation on male green catfish (*H. nemurus)*, female striped catfish (*P. hypophthalmus)* and genetic relationship between hybrid to each of parent.

2. Materials and Methods

The research activities were carried out at the Ciparanje Laboratory Pool, Unpad and Biotechnology Laboratory of FPIK UNPAD. The test samples were female striped catfish, male green catfish, and larvae of hybrid between female striped catfish and green catfish. Larvae of hybrid was 1 week old. These research activities were carried out through several stages such as DNA isolation, DNA quantitative by spectrophotometer, electrophoresis, and Random Amplified Polymorphic DNA (RAPD) analysis.

2.1 DNA Isolation

DNA isolation was carried out to separate chromosomal DNA or genomic DNA from other cell components. Ten mg caudal fins from male green catfish (*H. nemurus)*, female striped catfish (*P. hypophthalmus)*, and larvae of the hybrid were isolated using the Wizard Genomic DNA Purification Kit (Promega). After isolation, qualitative analysis was performed using electrophoresis in 1% agarose gel at 75 volts for 40 minutes. The size of the ladder was 1 kb. DNA isolation products were immersed in ethidium bromide (EtBr) for 30 and then visualized in UV light and photographed using a digital camera.

2.2 DNA Quantification

DNA quantification was carried out by using spectrophotometry. Double band DNA can absorb UV light at λ 260 nm, while protein or phenol contaminants will absorb light at λ 280 nm. DNA purity can be measured by calculating the absorbance value of λ 260 nm divided by the absorbance value of λ 280 nm (A260/A280). Good-quality DNA for molecular analysis will have an A_{260}/A_{280} ratio of 1.8–2.0 [13]. The Formula for calculating double-stranded DNA concentrations according to Button *et al*. [13].

Concentration (µg/ml) = A_{260} reading – A_{320} reading) × dilution factor × 50µg/ml

Dilution factor = 50 times

2.3 DNA Amplification

The DNA was then amplified using the OPA-2 Primer (5'TGCCGAGCTG-3') and OPA-3 Primer (5'AGTCAGCCAC-3 '). Amplification was carried out using the PCR method with the composition of the ingredients: GoTaq® green master mix as much as 12.5 µl, primer as much as 1.25 µl, Template DNA 2 µl, and Nucleus Free Water 9.25 µl. Furthermore, all ingredients were included in a thermocycler with a cycle of 45 cycles. One cycle of initial denaturation at 94ºC for 2 minutes, 45 subsequent cycles consisting of denaturation at 94ºC for 1 minute, 36ºC annealing for 1 minute and elongation of 72ºC for 2 minutes. Final elongation at 72ºC for 10 minutes. The PCR results were seen through electrophoresis in 1% agarose gel at 75 volts for 70 minutes. DNA amplification products were immersed in ethidium bromide (EtBr) for 30 and then visualized in UV light and photographed using a digital camera.

2.4 Genetic Relationship Analysis

The genetic relationship can be known by calculating the similarity index based on amplified numeric data bands. The genetic relationship was analyzed based on the similarity index calculated through the Numerical Taxonomy and Multivariate Analysis System (NTSYS) program.

Results and Discussions

3.1 DNA Isolation and DNA Quantification

Isolation of genomic DNA is the process of separating DNA molecules from other molecules in the cell nucleus. DNA isolation is a process to obtain pure DNA that can be used for examination and diagnosis purposes in an organism [14]. The Quality and quantity from DNA isolation of male green catfish (*H. nemurus)*, female striped catfish (*P. hypophthalmus)* and hybrid can be known by *electrophoresis and* spectrophotometry. Qualitative analysis by

electrophoresis showed that all DNA samples have been successfully isolated and produce a single fragment with a molecular weight above 10,000 bp.

Based on the result of electrophoresis on figure 1, there was thick smear in the female striped catfish and a thin smear in the male green catfish. In the hybrid sample, the result of DNA isolation was clearly and there was no smear. Smears indicated the presence of contaminants such as protein or RNA in DNA isolation. The DNA purity of the three samples was 1.550-1.728. It indicated the presence of protein contamination. Sambrook and Russel [15] said that good-quality DNA for molecular analysis will have an A260/A280 ratio of 1.8-2.0. Ghatak *et al.* [16] said that a ratio below 1.8 indicates protein contaminants. While the ratio above 2 indicates RNA contamination. These contaminants can inhibit the amplification process (PCR) [17]. Therefore, DNA samples were purified. Purification of DNA isolates from RNA contaminants can be done by adding RNAse. Purification of DNA isolates from proteins can be done by adding phenol-chloroform [18].

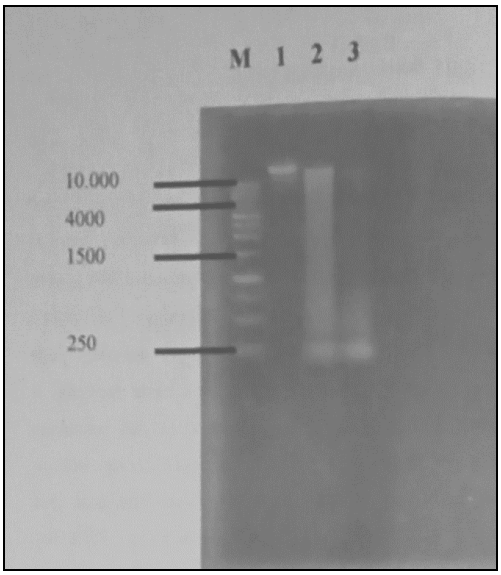

Fig 1: DNA Isolation Figure Information: M: Marker 1 kb; 1: Hybrid 2: Striped Catfish (*P. hypophthalmus*) 3: Green Catfish (*H. Nemurus*)

The spectrophotometry results showed that the pssurity value of each sample varied between 1.550-1.728 (Table 1). The DNA purity in female striped catfish (*P. hypophthalmus)* was 1,728. While in male green catfish (*H. nemurus)* was 1,550. In the hybrid was 1,680.

Table 1: The Purity Value of DNA Isolation From Striped Catfish, Green Catfish, and Hybrid

No	Sampel	Abs260nm	Abs280nm	Ratio
1	Female Striped Catfish	0.159	0.092	1.728
2	Male Green Catfish	0.062	0.040	1.550
3	Hybrid	0.084	0.050	1.680

3.2 DNA Amplification

The RAPD technique was useful and sensitive in differentiating various fish genera and species [11]. This technique was widely used in identifying genetic diversity at the intraspecies [19] and interspecies levels [20]. RAPD markers were analyzed according to the bands on RAPD gels. There are two kind of band will be produced by RAPD. There are polymorphic bands and monomorphic bands. Polymorphic band is band of a certain size that appear only in one sample and not found in the other samples. Meanwhile, the monomorphic bands is a band of a certain size that appears in all samples. The number of polymorphic bands present in a sample indicates genetic variation.

The results of sample amplification using two primers (OPA 02 and OPA 03) showed that OPA 03 produce more DNA fragments than OPA 02. The results of DNA Amplification Products were shown in Table 2 (OPA 02) and Table 3 (OPA 03). Therefore, the genetic relationship analysis of the sample in this study used primer OPA 03. Selecting a primer for RAPD was important [21]. Each primer has its attachment site, consequently the DNA bands produced by each primer become different, both in terms of the number of base pairs and the number of DNA fragments [22]. In this research, OPA 03 was used for the genetic relationship analysis because the result of DNA amplification with OPA 03 was better than OPA 02. OPA 03 produced more DNA fragments than OPA 02. The more number of DNA fragments produced by OPA 03 indicated that the base sequence OPA 03 primers were more compatible with the genomes of the samples.

The highest polymorphic percentages with OPA 02 were obtained in male green catfish. The number of polymorphic fragments in female striped catfish was 1 band or 5.56%, in male green catfish there were 10 polymorphic bands (58.82%) and there were no polymorphic bands in hybrid. The number of monomorphic bands in male green catfish, female striped catfish, hybrid was 10 (41.18%), 17 (94.44), and 18 (100%) respectively.

The highest polymorphic percentages with OPA 03 were also obtained in male green catfish. The number of polymorphic fragments in hybrid was 3 bands or 11.54%, in male green catfish there were 4 polymorphic bands (30.77%) and there were no polymorphic bands in female striped catfish. This indicates that the genetic of the male green catfish is more variable.

The existence of this polymorphic in hybrid sample indicated genetic variation between hybrid and parents. Zalapa *et al.* [23] said that hybridization is one way to increase genetic diversity. The hybridization will allow gene modification in offspring. Hybrid will express a combination of characteristics that lie between the two parents [24]. This result also indicates that male green catfish has higher genetic diversity than female striped catfish and hybrid. Genetic diversity can affect the organism ability to respond to the natural and artificial selection [25]. High diversity populations can also indicate a high capacity to adapt to stressful environments, productivity and population persistence than low diversity populations [26].

Using OPA 03, there were 26 monomorphic bands in the hybrid sample. There were 20 monomorphic bands in female striped catfish. All of the bands from the female striped catfish also appeared on the same size in hybrid. There were only 9 monomorphic bands in male green catfish. The existence of monomorphic nucleotide sequences might be able to express phenotypic similarities between hybrid, female catfish, and male green catfish. The monomorphic nucleotide sequence might express phenotypic similarities in the population [27]. The possibility of this same phenotype can be known in terms of morphological, anatomical, and physiological. Based on the result of the existence of monomorphic bands, the hybrid might be able to express more phenotypic similarities with the female striped catfish than male green catfish.

Table 2: The Results of DNA Amplification Products OPA 02

Sample	Amplified Fragments	Polymorphic Bands	Monomorphic Bands	% Polymorphic	% Monomorphic
Hybrid	18	0	18	0	100
Female Striped Catfish	18	1	17	5.56	94.44
Male Green Catfish	17	10	7	58.82	41.18

Table 3: The Results of DNA Amplification Products OPA 03

Sample	Amplified Fragments	Polymorphic Bands	Monomorphic Bands	% Polymorphic	% Monomorphic
Hybrid	26	3	23	11,54	88.46
Female Striped Catfish	20	0	20	0	100
Male Green Catfish	13	4	9	30.77	69.23

3.3 Genetic Relationship Analysis

The existence of monomorphic and polymorphic bands were then processed with the NTSYS program and UPGMA (Unweighted Pair Group Method with Arithmetic Mean Analysis). The results show that there were two groups. The first group was hybrid and female striped catfish with a similarity index value of 0.82. This coefficient value indicated that the two samples have 82% genetic similarity. In the second group consisted of male green catfish having a similarity index with group 1 of 0.31 or having 31% genetic similarity. These results indicated that the genetic of hybrid was similar and had a close relationship with female striped catfish.

4. Conclusion

It can be concluded that there was genetic variation between hybrid fish and their parents. Genetic of hybrid was more similar and had a closer relationship with female striped catfish than male green catfish.

5. References

1. Cruvinel WM, Junior DM, Araujo JAP, Catelan TTT, Silva de Siuza AW, Pereira de Silva N *et al*. Immune system – Part I Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. Brazilian Journal Rheumatology. 2010; 50(4):434-461.
2. Melki, Wike Ayu EP, Kurniati. Antibacterial Test of *Gracilaria* sp Extract. (Seaweed) Against *Escherichia coli* and *Staphylococcus aureus*. Ocean Sciences Study Program FMIPA Sriwijaya University, Palembang, 2011.
3. Dayuti S. Antibacterial activity of red algae (*Gracilaria verrucosa*) extract against *Escherichia coli* and *Salmonella typhimurium*. Aseanfen International Fisheries Symposium, 2017.
4. Pelczar MJ, Chan. Fundamentals of Microbiology. UI-Press, Jakarta, 2015.
5. Kobayashi M, Mssangi S, Batka M, Vannuccini S, Dey MM, Anderson JL. Fish to 2030: The Role and Opportunity for Aquaculture. Aquaculture Economics & Management. 2015; 19:282-300.
6. Firmansyah, Oktavilia S, Prayogi R, Abdulah R. Indonesian fish consumption: an analysis of dynamic panel regression model. IOP Conference Series: Earth and Environmental Science. 2019, 246.
7. Rimmer MA. Mariculture development in Indonesia: Prospects and Constraints. Indonesian Aquaculture Journal. 2010; 5(2).
8. Trana N, Rodriguez UP, Chana CY, Philips MJ, Mohana SV, Henrikssona PJG *et al*. Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the Asia Fish model. Marine Policy. 2017; 79:25-32.
9. Pamungkas W, Jusadi D, Zairin M, Setiawati M, Supriyono, Imron. Induction of ovarian rematuration in striped catfish (Pangasianodon hypophthalmus) using pregnant mare serum gonadotropin hormone in out-of spawning season. AACL Bioflux. 2019; 12(3).
10. Ali H, Rahmana MM, E-Jahan KM, Dhara GC. Production economics of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) farming under polyculture system in Bangladesh. Aquaculture. 2018; 491:381-390.
11. Chowdhury MA, Roy NC, Chowdhury A. Growth, yield and economic returns of striped catfish (*Pangasianodon hypophthalmus*) at different stocking densities under floodplain cage culture system. Egyptian Journal of Aquatic Research. 2020; 46:91-95.
12. Aryani N, Nuraini, Suharman I. Morphological characterization of baung fish (*Hemibagrus nemurus*) aquatic habitat on the different method based truss morfometrics. Journal of Fisheries and Aquaculture. 2013; 4(3):139-142.
13. Heltonika B, Karsih OR. Maintenance of green catfish (*Hemibagrus nemurus*) larvae with photoperiod technology. Berkala Perikanan Terubuk. 2017; 45(1):125-137.
14. Edward DD, Dearherage DE, Ernsting BR. Random Amplified Polymorphic DNA Analysis of Kinship Within Host-Associated Populations of The Symbiotic Water Mite *Unionicola folli* (Acari: Uniocolidae). Experimental & Applied Acarology. 2004; 34(1-2).
15. Asagbra MC, Adebayo AS, Ugwumba AAA, Anumudu CI. Genetic Characterization of Fin Fish Species From The Warri River at Ubeji, Niger Delta, Nigeria. African Journal of Biotechnology. 2014; 13(27).
16. Buwono ID, Lathifah AU, Subhan U. The genotypic diversity of Sangkuriang, Mutiara Transgenic and Non Transgenic Cat Fish on First Generation. Jurnal Biologi Indonesia. 2018; 14(1):133-141.
17. Barbas CF, Button DR, Scott JK, Silverman GJ. Quantitation of DNA dan RNA. Adapted from ”General Procedure” appendix 3. Cold Spring Harbor, NY, USA, 2001.
18. Chawla HS. Introduction to Plant Biotechnology. USA, Science Publishers. Inc, 2000.
19. Sambrook J, Russel. Moleculer Cloning: A Laboratory Manual. Cold Spring Harbour Laboratory Press, New York, USA, 2001.
20. Ghatak S, Muthukumaran RB, Nachimuthu SK. A Simple Method of Genomic DNA Extraction from Human Samples for PCR-RFLP Analysis. Journal of Biomolecular Techniques. 2013; 24:224-231.
21. Fatchiyah AEL, Widyarti, Rahayu S. Molecular Biology.

Basic Principle of Analysis. Erlangga, Jakarta, 2011.
22. Greco M, Sa'ez CA, Brown MT, Bitonti MB. A Simple and Effective Method for High Quality Co Extraction of Genomic DNA and Total RNA from Low Biomass *Ectocarpus siliculosus*, the Model Brown Alga. PLOS ONE, 2014; 9(5):1-13.
23. Pacheco ABF, Guth BEC, DFD Almeida, Ferreira LCS. Characterization of Enterotoxigenic *Escherichia coli* by Random Amplification of Polymorphic DNA. Research in Microbiology. 1996; 147:175-182.
24. Hadrys H, Balick M, Schierwater B. Applications of Random Amplified Polymorphic DNA (RAPD) in Molecular Ecology**.** Molecular Ecology. 1992; 1(1):55-63.
25. Mbwana J, Lin IB, Lyamuya E, Mhalu F, Lagergard T. Molecular Characterization of Haemophilus ducreyi Isolates from Different Geographical Locations. Journal of Clinical Microbiology. 2006; 44(1):132-137.
26. Ruwaida IP, Supriyado, Parjanto. Variability analysis of Sukun durian plant (*Durio zibethinus*) based on RAPD marker. Nusantara Bioscience. 2009; 1(2):84-91.
27. Zalapa JE, Brunet J, Guries RP. The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (ulmaceae). Evolutionary Applications. 2010; 3(2):157-168.
28. Stelkens RB, Brockhurst MA, Hurst GDD, Miller EI, Greig D. The effect of hybrid transgression on environmental tolerance inexperimental yeast crosses. Journal of Evolutionary Biology. 2014; 27:2507-2519.
29. Imron, Arifin OZ, Subagya. Characterization of Monomorphic Truss in Carp (Cyprinus carpio) Majalaya, Rajadanu, Wildan, and Sutisna Strains. Proceedings of the Conference on Fisheries Research Results, 2000.
30. Madduppa HH, Timm J, Kochzius M. Reduced Genetic Diversity in the Clown Anemonefish *Amphiprion ocellaris* in Exploited Reefs of Spermonde Archipelago, Indonesia. Frontier in Marine Science. 2018; 5(80).
31. Trijoko, Handayani NSN, Feranisa A. Morphological Characterization and Genetic Diversity of Macrobrachium rosebergii (De Man, 1879) crosses from Samas, Bone and Synthesis Populations. Jurnal Sain Veteriner. 2013; 31(20):227-242.