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PHYLOGENETIC ANALYSIS *PANGASIONODON HYPOPHTHALMUS, PANGASIUS DJAMBAL, PANGASIONODON GIGAS, PANGASIUS SANITWONGSEI AND HYBRID P. HYPHOPHTHALMUS* X *P. DJAMBAL*

Analisis Filogenetik *Pangasionodon hypophthalmus, Pangasius djambal, Pangasionodon gigas, Pangasius sanitwongsei* dan Hibrid *P. hyphophthalmus* X *P. djambal*

Ibnu Dwi Buwono*, Yeni Mulyani and Afa Soraya

Fisheries Study Program, Faculty of Fisheries and Marine Sciences,

Universitas Padjadjaran 45363 Jatinangor, Indonesia

*Corresponding author: ibnu.dwi.buwono@unpad.ac.id

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ABSTRACT

This study aims to identify the types of striped catfish that have genetic variations and genetic relationships among the test striped catfish. Samples of sutchi catfish (*P. hypophthalmus*) and hybrid *P. hyphophthalmus* X *P. djambal* were obtained from Cijengkol and Subang, West Java. *P. djambal* from Cirata, *P. gigas* and *P. sanitwongsei* from ornamental fish shops in Jakarta. The source of genomic DNA was obtained from small pieces of the combined five caudal fins of each test striped catfish sample. Based on the results of amplification using OPA-2 and OPA-3 primers, genetic variations interpreted with the highest number of polymorphic fragments were found in *P. sanitwongsei* (3 fragments), *P. gigas* (2 fragments), hybrid *P. hyphophthalmus* X *P. djambal* (2 fragments) and *P. djambal* (1 fragment), respectively. The results of the NTSYS (Numerical Taxonomy and Multivariate Analysis System) program analysis of the OPA-2 and OPA-3 primer amplicons show that the genetic relationship between *P. hyphophthalmus*, *P. djambal*, and the hybrid of *P. hyphophthalmus* X *P. djambal* and *P. sanitwongsei* is relatively close (similarity index 71.30%) based on OPA-2 and 55.9% (based on OPA-3). Otherwise, *P. gigas* is relatively distant (similarity index 44%) based on the OPA-2 primer and 50% (based on the OPA-3 primer). The OPA-2 and OPA-3 primers can be used to detect polymorphisms of striped catfish species.

Keywords: Genetic Diversity, Polymorphism, Primer, Stripped Catfish,

ABSTRAK

Penelitian ini bertujuan mengidentifikasi jenis ikan patin yang memiliki variasi genetik dan hubungan kekerabatan genetik diantara patin uji. Sampel ikan patin siam (*P. hypophthalmus*) dan hibrid *P. hyphophthalmus* X *P. djambal* diperoleh dari Cijengkol dan Subang Jawa Barat. *P. djambal* dari Cirata, *P. gigas* dan *P. sanitwongsei* dari toko ikan hias Jakarta. Sumber DNA genomik diperoleh dari potongan kecil gabungan lima sirip ekor masing-masing sampel patin uji. Berdasarkan hasil amplifikasi menggunakan primer OPA-2 dan OPA-3, variasi genetik yang

diinterprestasikan dengan jumlah fragmen polimorfik terbanyak berturut-turut terdapat pada sampel *P. sanitwongsei* (3 fragmen), *P. gigas* (2 fragmen), hibrid *P. hyphophthalmus* **X** *P. bedado* (2 fragmen) dan *P. djambal* (1 fragmen). Hasil analisis program NTSYS (*Numerical Taxonomy and Multivariate Analysis System*) dari amplikon primer OPA-2 dan OPA-3, menunjukkan hubungan kekerabatan genetik *P. hyphophthalmus*, *P. djambal*, hibrid *P. hyphophthalmus* **X** *P. djambal* dan *P. sanitwongsei* tergolong dekat (indeks kesamaan 71,30%) berdasar OPA-2 dan 55,9% (berdasar OPA-3). Sebaliknya pada *P. gigas* tergolong jauh (indeks kesamaan 44 %) berdasar primer OPA-2 dan 50 % (berdasar primer OPA-3). Primer OPA-2 dan OPA-3 dapat digunakan untuk deteksi polimorfisme jenis ikan patin.

Kata kunci: Keragaman Genetik, Ikan Patin, Polimorfisme, Primer

INTRODUCTION

P. hypophthalmus as a cultivated fish is a freshwater fish commodity with economic value in meeting the community's food needs with high egg fecundity that can reach 135,000 eggs/kg (Sah *et al.,* 2018). This catfish originates from Thailand and was first introduced in Indonesia in 1972. Another type of catfish, namely *P. djambal*, has the potential to become an export commodity, successfully spawned by artificial mating in 1996 and introduced in 2000 (Cacot, 1999; Cacot *et al.,* 2002). Several types of catfish can be used as ornamental fish, including *P. sanitwongsei* and *P. gigas*, which have been known in Indonesia. As seed technology develops, cross-breeding with similarity of genus and species through hybridization program was carried out between *P. hypophthalmus* and *P. djambal* to produce hybrid catfish known as 'patin pasupati' (*Pangasius* sp.) by the Research Center for Breeding and Cultivation Technology of Freshwater Fisheries (LRPTBAT) Sukamandi, Subang, West Java. The flesh of this hybrid catfish is white and is an important criterion to fulfill the export quota of white-fleshed catfish (Khairuman & Amri, 2010).

Further development of catfish cultivation among farmers has resulted in a tendency for genetic variation in fish to decrease compared to their parents. Crossbreeding of catfish of the same species, which is common in community hatcheries, causes the genetic traits inherited from the parents to degrade. Indications of decreased genetic quality in fish are characterized by traits such as slow growth and decreased immunity, which are thought to be related to inbreeding among the spawned catfish parents (Vu *et al.,* 2020). Information on genetic variation can be obtained through several methods, one of which is the genotypic diversity approach, namely through DNA polymorphism analysis. Fish genetic diversity, indicated by the level of polymorphism, has important value in fish cultivation programs. Increasing genetic diversity facilitates improving the genetic quality of fish. Polymorphism analysis using Random Amplified Polymorphism DNA is used to examine the genetic relationship of fish as a way to determine the potential for superior parents to be crossed (Tamanna *et al.,* 2012). This polymorphic DNA marker has been used in genetic variation analysis research in three catfish populations, *Mystus vittatus* (Bloch) from the Chalan, Mohanganj and Kangsha rivers in Bangladesh. The RAPD test is relatively simple, fast, and inexpensive, and is the method of choice for observing genetic diversity, genetic distance and phylogenetic tree construction as well as kinship relationships that occur between fish species or strains (Avise, 2012). Gene mapping and genetic variation in channel catfish (*Ictalurus punctatus*) can be done using the RAPD technique (Liu *et al.,* 1999), as well as the genetic distance between discus fish (*Symphysodon* spp.) obtained from the wild and fish from cultivation (Koh *et al.,* 1999). Because genetic variation and distance can affect the improvement of the genetic quality of catfish, it is necessary to identify genetic variation in the test catfish through diversity and kinship analysis as an effort to breed catfish to prevent genetic decline due to inbreeding.

RESEARCH METHODS

Test Fish

Test samples of *P. hypophthalmus* were taken from the Cijengkol Freshwater Aquaculture Development Center, West Java, *P. djambal* from a catfish farmer in Cirata, West Java, and a hybrid of *P. hyphophthalmus* (*P. djambal*) from Subang, West Java, and *P. sanitwongsei* and *P. gigas* from an ornamental fish shop in Jakarta, Indonesia. From each location of origin of the catfish, 5 individuals were taken as test samples. A general description of the morphology of the five catfish species is described below.

Sutchi catfish **(***P. hypophthalmus***)**

P. hypophthalmus (Figure 1) is a common and widely cultivated catfish species in Indonesia (Mahyuddin, 2010). Catfish began to be spawned in Indonesia in 1980, and in the 1990s, catfish cultivation began to develop rapidly in West Java, Lampung, South Sumatra, and Kalimantan.

Figure 1. *P. hypophthalmus*

The Siamese catfish's body shape resembles that of the local catfish (*P. djambal*), but with a more substantial build. Its entire body is predominantly silvery white with red fins. Its head is relatively small, with its mouth located at the lower end of the head, a characteristic of the catfish family. Two pairs of short whiskers, which function as feelers, are located directly at the corners of its mouth.

P. djambal Bleeker

This catfish is a type of freshwater fish native to Indonesia and was domesticated by the Sukamandi Freshwater Fisheries Breeding and Cultivation Technology Research Center in 1997. The geographical distribution of the jambal catfish (Figure 2) is quite extensive, spanning almost all of Indonesia. *P. djambal* has white, fibrous flesh, making it a suitable export commodity.

The dorsal fin has hard rays that develop into large, serrated barbs at the rear. There are six or seven soft rays on the dorsal fin. The dorsal fin has a hump (a small adipose fin). The caudal fin is V-shaped (does not form a fork). The anal fin consists of 30-33 soft rays, while the pelvic fins have six soft rays. The pectoral fins have 12-13 soft rays and one hard ray that develops into a barb (Roberts, 1999). The head is flatter and longer than that of the Siamese catfish or the pasupati catfish. The color pattern of the fins is black, and the body color is silvery black with larger eyes than the Siamese catfish.

Figure 2. *P. djambal*

Hibrid *P. hyphophthalmus* **X** *P. djambal*

The hybrid *P. hyphophthalmus* **X** *P. djambal* is known as 'patin pasupati' derived from the abbreviation "patin super harapan pertiwi" (Figure 3) is a new type of patin fish native to Indonesia. This hybrid patin is produced from a cross between the Siamese patin and the Jambal patin produced by the Sukamandi Freshwater Fish Breeding Research Center, Subang, West Java, established by the Decree of the Minister of Maritime Affairs and Fisheries Number KEP.25/MEN/2006.

Figure 3. Pasupati (hibrid *P. hyphophthalmus* **X** *P. djambal*)

The morphological characteristics of the three catfish species mentioned above are quite similar, and all three have barbs on their dorsal and pectoral fins. The eyes of the Pasupati catfish are slightly larger than those of the Siamese catfish. The nostrils are relatively enlarged, and the subterminal mouth is relatively small and widens laterally. The distance between the tip of the snout and the edge of the eye is shorter than that of the Jambal catfish. The caudal fin forms a fork and is symmetrical, similar to the tail fin of the Siamese catfish (Gustiano & Kristanto, 2007).

Chao Praya giant catfish (P. sanitwongsei)

P. sanitwongsei (Figure 4) is generally known in Indonesia as an ornamental fish due to its shark-like morphology, often called the iridescent freshwater shark. This fish has a distinctive, upright, elongated dorsal fin like a shark. *P. sanitwongsei* has a distinct body shape, unlike the Pangasius genus in general, with its elongated body and long fins (Gustiano & Pouyaud, 2006).

Figure 4. *P. sanitwongsei*

Giant Mekong catfish (*P. gigas*)

P. gigas (Figure 5) is a species of giant catfish native to the Mekong River in Thailand, reaching lengths of up to 3 meters (Hogan, 2004). The eyes are located below the upper mouth and are similar in size to those of the Siamese catfish. The caudal fin is V-shaped, almost identical to the tail of the Siamese catfish. The head is shaped somewhat like that of the Siamese catfish.

Figure 5. *P. gigas*

Genomic DNA Isolation

A small piece of the tail fin of the test catfish was used as a source of genomic DNA (DNA template) to copy polymorphic fragments using RAPD primers. Subsequent DNA isolation followed the Wizard® genomic purification kit (Promega) protocol. The quantity and quality of the sample DNA were measured using a UV-Vis spectrophotometer to determine the concentration and purity of the DNA. The ratio between the sample DNA at A260 nm and A280 nm was considered pure if it ranged between 1.8 and 2.0 (Barbas *et al.,* 2001).

Primer RAPD-PCR

The primers used for the polymorphism analysis of the test catfish (Table 1) refer to the complementary genetic variation research of Pangasius bocourti which produces polymorphic fragments, namely OPA-2, OPA-3, OPA-7, and OPA-9 (Liu *et al.,* 1999; Champasri *et al.,* 2008).

Table 1. OPA primary sequences

Primer	*Nucleotide Base Sequence*
OPA-2	5'-TGCCGAGCTG-3'
OPA-3	5'-AGTCAGCCAC-3'
OPA-7	5'-GAAACGGGTG-3'
OPA-9	5'-GGGTAACGCC-3'

Amplifikasi DNA

RAPD-PCR reaction mixture formulation for genomic DNA amplification of catfish test using primers presented in Table 1 in a total volume of 25 µL Each sample was reacted with go taq® green master mix 2X (Promega) 12.5 µl, RAPD primer 1.25 µl, 2 µl sample DNA as template and 9.25 µl Nuclease Free Water. DNA amplification program settings for PCR cycles on test samples are presented in Table 2.

Table 2. RAPD-PCR Program

Stage	*Process*	*Temperature (ºC)*	*Time (minutes)*	*Cycle*
1	Pre-denaturation	94	2	1 x
2	Denaturation	94	1	45 x
3	Annealing	36	1	
4	Extension	72	2	
5	Final extension	72	10	1 x
6	Hold	4	10	

Elektroforesis

The PCR products were then separated using 1.4% agarose gel electrophoresis method (60 amperes, 50 volts, 90 minutes) and the DNA bands were documented using a UV transilluminator (λ-280 nm).

Analisis Polimorfisme

Genetic diversity analysis of the test catfish samples was conducted by inputting the presence or absence of amplified DNA fragments and translating them into numerical data. The presence of DNA amplicons was translated as one (1), and those that were not present were translated as zero (0). DNA fragments amplified in one catfish sample and not in another were interpreted as polymorphic fragments, indicating genetic variation in the organism (Liu & Cordes, 2004).

The genetic relationships of the test catfish DNA samples were determined by calculating a similarity index based on the numerical amplicon data. The similarity index calculation used the NTSYS program for kinship analysis among the test catfish. The genetic distance between the test samples was analyzed using the Unweighted Pair Group Method with Arithmetic Averages program in the NTSYS software. The resulting data was a phylogenetic tree presented in the form of a phenogram (Alam *et al.,* 2006).

RESULT

Genomic DNA

The presence of genomic DNA in the test catfish can be demonstrated by 1% agarose gel electrophoresis (Figure 6). The quality of genomic DNA from the test catfish fin samples (wells 1-6 in Figure 6) was relatively high, as indicated by the thickness of the DNA bands in each test catfish sample, and was suitable for use as a template for RAPD primers.

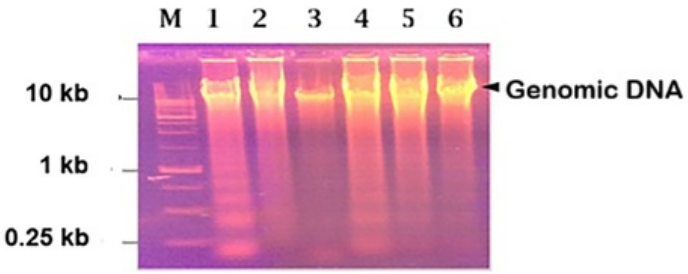

Figure 6. Genomic DNA of the test catfish, each gel well contains a combination of five small pieces of each test catfish caudal fin.
M = DNA Ladder Marker 1 kb; 1= *P. hypophthalmus* (Cijengkol); 2= hibrid *P. hyphophthalmus* **X** *P. djambal* Sukamandi); 3= *P. hypophthalmus* (Subang); 4= *P. djambal* (Cirata); 5= *P. gigas*; 6= *P. sanitwongsei*

Tabel 3. Konsentrasi DNA genomik patin

Sample	DNA Concentration (ng/µl)	Absorbance Ratio 260nm/280nm
P. hypophthalmus (Cijengkol)	24,80	1,811
Hibrid *P. hyphophthalmus* **X** *P. djambal* (Sukamandi)	25,95	1,597
P. hypophthalmus (Subang)	23,60	1,823
P. djambal (Cirata)	25,83	1,849
P. gigas	28,03	1,783
P. sanitwongsei	25,93	1,866

Produk PCR Dengan Primer OPA-2 Dan OPA-3

The results of the amplification of catfish DNA from the test samples with RAPD primers provided varying sizes and numbers of polymorphic fragments based on the OPA-2 primer (Figure 7A) and the OPA-3 primer (Figure 7B).

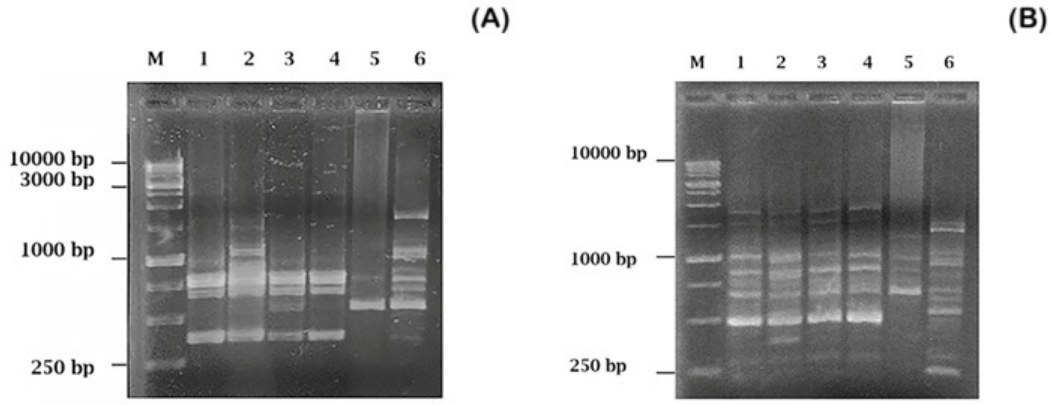

Figure 7. PCR products with primer OPA-2 (A) and primer OPA-3 (B)
M= Marker DNA Ladder 1 kb; 1= *P. hypophthalmus* (Cijengkol); 2= hibrid *P. hyphophthalmus* **X** *P. djambal* Sukamandi); 3= *P. hypophthalmus* (Subang); 4= *P. djambal* (Cirata); 5= *P. gigas*; 6= *P. sanitwongsei*

PCR products with primers OPA-7 and OPA-9

Especially for primers OPA-7 and OPA-9, not all catfish DNA samples could be amplified by both primers (Figure 8A, 8B).

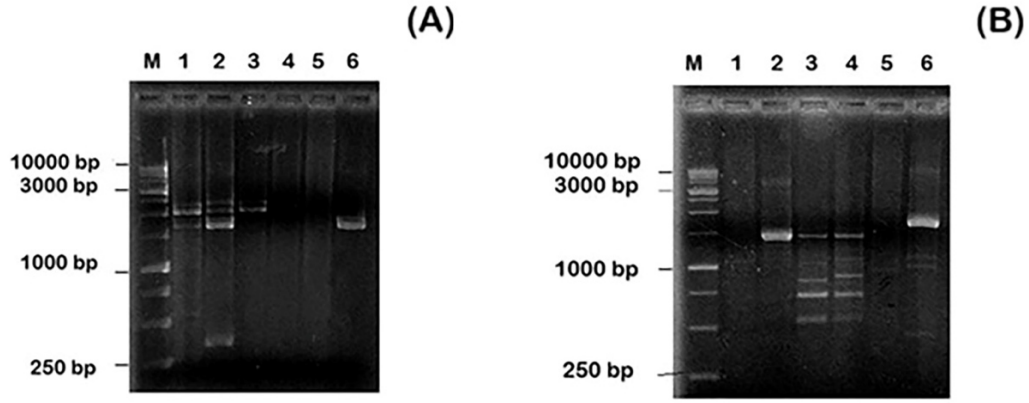

Figure 8. PCR product of primer OPA-07 (A) and PCR product of primer OPA-09 (B)
M = Marker DNA Ladder 1 kb; 1= *P. hypophthalmus* (Cijengkol); 2= hibrid *P. hyphophthalmus* **X** *P. djambal* Sukamandi); 3= *P. hypophthalmus* (Subang); 4= *P. djambal* (Cirata); 5= *P. gigas*; 6= *P. sanitwongsei*

Polymorphic Fragments

DNA molecular markers are essentially specific DNA segments that can indicate different polymorphic fragments in each individual within a species (Phadphon *et al.,* 2019). Polymorphic fragments are DNA bands that appear at a certain size, but are not found in other samples. Table 4 presents the polymorphic fragment profiles generated by both OPA-2 and OPA-3 primers to determine genetic variation in each tested catfish related to polymorphism in the fish.

Table 4. Polymorphic fragments generated by primer OPA-2

Fragment size	*P. hypophthalmus* Cijengkol	*'patin pasupati'*	*P. hypophthalmus* Subang	*P. djambal* Cirata	*P. gigas*	*P. sanitwongsei*
1731 bp						---*
1650 bp						---*
1500 bp		---*				
1300 bp						---*
1100 bp		---				---
1000 bp	---	---	---	---		---
950 bp	---	---	---	---		---
850 bp	---	---	---	---	---	---
800 bp	---	---	---	---	---	---
750 bp	---	---	---	---		---
700 bp				---	---	---
650 bp				---*		
500 bp		---*				
400 bp	---	---	---	---		---

--- = monomorphic fragments
---* = polymorphic fragments

Meanwhile, the polymorphic fragment profiles generated by the OPA-3 primers are presented in Table 5.

Table 5. Amplification of OPA-3 primer polymorphic fragments

Fragment size	*P. hypophthalmus* Cijengkol	*'patin pasupati'*	*P. hypophthalmus* Subang	*P. djambal* Cirata	*P. gigas*	*P. sanitwongsei*
1861 bp	---	---	---	---		
1610 bp						---*
1500 bp						---*
1450 bp					---*	
1300 bp				---*		
1000 bp	---	---	---	---	---	---
975 bp	---	---	---	---	---	
850 bp						
900 bp					---*	
850 bp	---	---	---	---	---	
750 bp	---	---	---	---	---	
700 bp	---	---	---			---
650 bp	---	---	---	---		
600 bp	---	---	---	---		
500 bp	---	---	---	---		
350 bp		---*				
300 bp	---	---	---	---		
250bp						---*

--- = monomorphic fragments
---* = polymorphic fragments

Genetic Relationship of Catfish

The genetic relationships obtained from the results of NTSYS data processing based on the OPA-2 primer are grouped into 5, namely *P. hypophthalmus* relatives with *P. djambal*, *P. djambal* relatives,' patin pasupati' relatives, *P. sanitwongsei* relatives and *P. gigas* relatives (Figure 9).

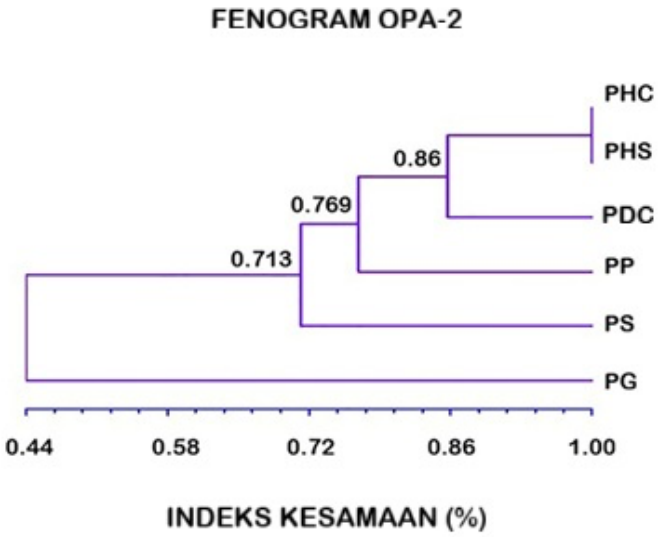

Figure 9. Phenogram of catfish with OPA-2 primer
PHC: *P. hypophthalmus* Cijengkol; PHS: *P. hypophthalmus* Subang;
PDC: *P. djambal* Cirata; PP: 'patin pasupati '; PS: *P. sanitwongsei*;
PG: *P. gigas*

The interpretation of the inner kinship relationship shown by the primary phenogram of OPA-3 (Figure 10) is slightly different from the primary phenogram of OPA-2.

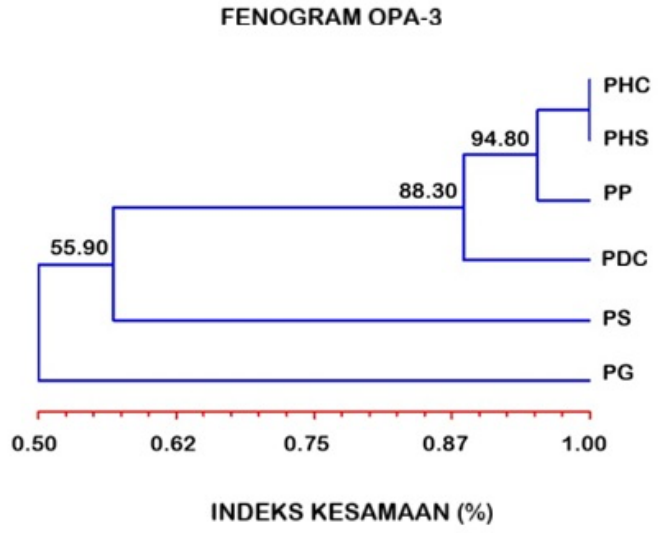

Figure 10. Phenogram of catfish with OPA-3 primer
PHC: *P. hypophthalmus* Cijengkol; PHS: *P. hypophthalmus* Subang;
PDC: *P. djambal* Cirata; PP: 'patin pasupati '; PS: *P. sanitwongsei*;
PG: *P. gigas*

DISCUSSION

Genomic DNA quality

The results of measuring the genomic DNA concentration of the test catfish using a UV-vis spectrophotometer ranged from 23.60–28.03 ng/µl (Table 3). This DNA concentration meets the requirements for use as a template in PCR work, between 10–30 ng/µl (Asif *et al.,* 2021). The genomic quality of the test catfish samples had a fairly high purity value with an absorbance (A) ratio of 260 nm/280 nm of around 1.6–1.9 (Barbas *et al.,* 2001).

Amplification Products

Based on the results of Figure 7A, the OPA-2 primers were able to amplify DNA fragments from each tested catfish sample, indicating that the primers are suitable for detecting the genetic diversity of catfish. The variation in DNA fragment amplicons in hybrid catfish (a cross between *P. hyphophthalmus* and *P. djambal*) and *P. sanitwongsei* was greater than in either *P. hyphophthalmus* or *P. djambal*. In addition to detecting genetic variation in hybrid catfish, the primers also detected variation in DNA fragments in *Pangasionodon gigas* and *P. sanitwongsei*. These results indicate that the OPA-02 primers are a molecular marker for the presence of genotypic variation in hybrid catfish, distinct from the phenotypes of the Siamese and Jambal catfish, their parents. Similar results were also shown for polymorphisms in Clarias batrachus (Danish *et al.,* 2012) and Channa marulius (Khan & Naeem, 2025) populations, indicating that the OPA-2 primer can be used as a genetic marker for superior broodstock selection in fish genetic improvement efforts. RAPD is associated with dominant traits and is inherited according to Mendelian methods, indicating that polymorphic amplification products aid in characterizing individuals within a population. The high frequency and uniform distribution in most eukaryotic genomes (including fish), as well as the high degree of variation, encourage its use in superior broodstock selection and genetic kinship (Muneer *et al*., 2012). Randomly amplified polymorphic DNA is a common molecular marker used for genetic analysis, population genealogy, and species taxonomy, including fish (Gjedrem, 2012). Several researchers have demonstrated the usefulness of the RAPD-PCR method in assessing genetic trait differences between fish populations located at distant locations (Bardakci & Skibinski, 1994).

Besides the OPA-2 primer, genetic diversity in the test catfish samples was also detected by the OPA-3 primer (Figure 7B) indicating that the OPA-3 primer can be used as a genetic marker for polymorphism analysis of the test catfish strains. The OPA-3 primer (Figure 7B) produced more polymorphic fragments than the OPA-2 primer, indicating differences in the number and position of DNA fragments in each test catfish sample. The polymorphic fragment profile of the *P. hyphophthalmus* ✗ *P. djambal* hybrid differed from *P. hyphophthalmus* and *P. djambal*, as well as from *P. gigas* and *P. sanitwongsei*. Variations and close genetic distances among all test catfish samples (*P. hypophthalmus* Cijengkol, *P. hyphophthalmus* ✗ *P. djambal* hybrid, *P. hypophthalmus* Subang, *P. djambal* Cirata, *P. gigas, P. sanitwongsei*) could be detected by the OPA-3 primer, indicating that this primer is suitable for polymorphism analysis and genetic potential of the parent. Genetic variation indicated by the presence of polymorphic fragments amplified by primers OPA-2 and OPA-3 (Figure 7A, 7B), indicates that both primers are suitable for species identification and phylogenetic analysis of catfish (Pouyaud *et al.,* 2004; Tamanna *et al.,* 2012; Vu *et al.,* 2020). Primer OPA-2 is more sensitive than OPA-3 in detecting the presence of polymorphic fragments in the hybrid *P. hyphophthalmus* ✗ *P. djambal* as a hybrid fish. Conversely, primer OPA-3 is sensitive in detecting the number of polymorphic fragments in *P. djambal, P. gigas* and *P. sanitwongsei* compared to OPA-2 (Figure 7A and 7B). Similar results were also shown in polymorphism analysis in *Pangasius pangasius* populations,

that primers OPA-2 and OPA-3 produced varying numbers of polymorphic fragments (Hassan & Naeem, 2023). This indication explains that genetic variations interpreted by polymorphic fragments can be detected by suitable RAPD primers, such as primers OPA-2 and OPA-3 in the five tested catfish samples (Liu & Cordes, 2004).

The OPA-7 primer can only amplify DNA fragments of *P. hypophthalmus* (Cijengkol), *P. hypophthalmus* ✗ *P. djambal* (Sukamandi), *P. hypophthalmus* (Subang) and *P. sanitwongsei* samples with a smaller number of fragments, while *P. djambal* (Cirata) and *P. gigas* were not amplified (Figure 8A). On the other hand, the OPA-9 primer only amplifies DNA fragments of *P. hypophthalmus* ✗ *P. djambal* (Sukamandi), *P. hypophthalmus* (Subang), *P. djambal* (Cirata) and *P. gigas* samples, while the DNA samples of *P. hypophthalmus* (Cijengkol) and *P. sanitwongsei* were not amplified (Figure 8B). These results indicate that both OPA-7 and OPA-9 primers are not suitable for analyzing genetic relationships among the six types of catfish, considering that the DNA fragments of *P. djambal* Cirata and *P. gigas* samples were not amplified by OPA-7 primers and *P. hypophthalmus* Cijengkol and *P. gigas* were not amplified by OPA-9 primers (Figures 8A, 8B).

Polymorphism Analysis

The number of polymorphic fragments in the pasupati catfish sample was 2 fragments, in *P. djambal* only 1 fragment and *P. sanitwongsei* 3 fragments (Table 4) reflecting genetic variations associated with morphological differences in the six tested catfish species. Dominant morphological differences, especially found in the shape of the head of *P. djambal* (Figure 2) which is different from other test samples, as well as the shape of the dorsal fin of *P. sanitwongsei* (Figure 4) and *P. gigas* (Figure 5) which is different from other samples. This indication is confirmed by the presence of polymorphic fragments in Table 4. Sensitive molecular markers will provide genetic variations related to dominant phenotypes for specific characteristics of a species that are useful in selective breeding programs and facilitate genetic selection of broodstock fish (Popoola *et al.,* 2014; Sultana *et al.,* 2010; Danish *et al.,* 2012; Phromthep *et al.,* 2025). The number of polymorphic fragments in fish genomic DNA is related to dominant phenotypes and facilitates the exploitation of advantageous quantitative phenotypes for selection purposes. Based on Table 4, the polymorphic fragments of *P. sanitwongsei* (3 fragments) and the pasupati catfish (2 fragments) exhibit relatively high genetic variation and allow for crossbreeding to improve specific phenotypes, given the higher number of polymorphic fragments compared to *P. hypophthalmus* and *P. djambal*. Hybrid fish resulting from crosses between *P. hypophthalmus* (♀) and *P. nasutus* (♂) and between *P. hypophthalmus* (♀) and *P. bocourti* (♂) exhibit heterosis based on analysis of morphometric traits related to hybrid fish polymorphism (Yusoff *et al.,* 2019; Gutiérrez-Barragán, 2025). This finding suggests that polymorphism analysis in patin fish has the potential to enhance heterosis in the selection of superior fish.

The polymorphic fragments produced by the OPA-3 primer, in contrast to the OPA-2 primer, were 2 fragments in the P. gigas sample, 1 fragment in the pasupati catfish, 1 fragment in the *P. djambal* and 3 fragments in the *P. sanitwongsei* (Table 5). Both the OPA-2 and OPA-3 primers were consistent in detecting the number of polymorphic fragments in *P. djambal* and *P. sanitwongsei*, respectively, of 1 and 3. Research conducted on three species of catfish (*P. hypophthalmus, P. larnaudii, P. sanitwongsei*) showed quite high genetic diversity among the 3 species in nature, indicating the presence of polymorphic fragments (Na-Nakorn *et al.,* 2009; Duong *et al.,* 2022). Genetic kinship analysis that has been carried out on *P. djambal* and *P. sanitwongsei* showed different clusters (Gustiano *et al.,* 2021). Similar genetic closeness studies have also shown between *P. sanitwongsei* and *P. djambal* at 50% (Pouyaud *et al.,* 2004).

This explanation is consistent with the results of the study of six types of catfish in Table 5, which shows that *P. djambal, P. gigas*, and *P. sanitwongsei* are different catfish groups. The presence of monomorphic fragments in the test catfish samples produced by both OPA-2 and OPA-3 primers indicates that the genetic relationship of the fish is relatively close, while polymorphic fragments indicate a relatively distant relationship.

Genetic Distance

P. hypophthalmus from Cijengkol and Subang are identical because they have a high similarity index (almost 100%), on the other hand, the genetic closeness of *P. djambal* with *P. hypophthalmus* Cijengkol and Subang is 86% which indicates that most of the morphological characteristics of the three patin are similar with slight differences in body color, eye size and caudal fin shape (Figures 1, 2). The DNA fragment amplified by the OPA-2 primer also shows a high similarity between *P. hypophthalmus Cijengkol,* Subang and *P. djambal* (Figure 2). Patin pasupati as a hybrid fish derived from *P. hypophthalmus* and *P. djambal,* inherits the genetic characteristics of both parents as parents, which is shown based on the DNA fragment amplified by the OPA-2 primer (Figure 7A) with a genetic closeness of 76.9% (Figure 9). Phenotypic variations (body color, eye size, caudal fin shape) that emerged in the Pasupati catfish, a cross between *P. hypophthalmus* and *P. djambal* (Table 4), resulted in a slight decrease in the genetic similarity index (Figure 9).

The similarity index between *P. hypophthalmus* Cijengkol (PHC), *P. hypophthalmus* Subang (PHS), *P. djambal* Cirata (PDC), *Pasupati catfish* (PP), *P. sanitwongsei* (PS), and *P. gigas* (PG) was 86%, respectively, between PHC or PHS and PDC. Furthermore, between PDC, PHC or PHS and PP, it was 76.9%, and between PHC or PHS, PDC, PP, and PS, it was 71.3%, indicating a relatively close genetic relationship (Figure 9). PG is distantly related to the other five catfish species (PHC, PHS, PDC, PP, PS), as indicated by a similarity index value of 44% (Figure 9), indicating dissimilarity. This dissimilarity of the PG samples is related to the small number of monomorphic fragments detected by the OPA-2 primer (3, Table 4), reinforcing the genetic dissimilarity of PG. Hybridization results between *P. gigas* and *P. hypophthalmus* indicate a genetic relationship between *P. gigas* and the *P. gigas* hybrid, with *P. hypophthalmus* having a distant genetic distance from *P. gigas* (Phadphon *et al.,* 2019). This indication indicates that *P. gigas* represents a distinct catfish cluster from the other test samples, as confirmed in Figure 9. The more distantly related an organism is, the greater the likelihood of differences in the gene structure contained within its chromosomes (Baack & Rieseberg, 2007; Wei *et al.,* 2002).

Based on the OPA-3 phenogram, the genetic similarity index between PHC or PHS and PP was 94.80%, between PHC or PHS, PP and PDC was 88.30% and between PHC or PHS, PP, PDC and PS was 55.90%. Between PHC or PHS, PP, PDC, PS and PG was 50%, indicating that PG is a genetically different group of patin with the five types of patin tested (Figure 10). The consistency of genetic similarity between PS and PG was also shown by the OPA-2 primer (44%) and the OPA-3 primer, explaining that *P. sanitwongsei* and *P. gigas* are different groups of patin. These results also explain the kinship research between *P. sanitwongsei* and *P. gigas* based on the cytochrome b and 12S rRNA genes showing different catfish groups (similarity index 63%) (Karinthanyakit & Jondeung, 2012) originating from the Chao Phraya River (*P. sanitwongsei*) and Mekong (*P. gigas*). Other studies also show similar results that the genetic kinship between *P. sanitwongsei* (Pangasius group) and *P. gigas* (Pangasionodon group) is only 28% (Duong *et al.,* 2022). Meanwhile, the phylogenetic analysis between *P. hypophthalmus* and *P. gigas* based on the cytochrome b gene is 57% (Tran *et al.,* 2017), consistent with the results of the OPA-2 (44%) and OPA-3 (50%) phenograms (Figures 9, 10).

The genetic similarity index between *P. djambal* and *P. hypophthalmus* based on OPA-2 was 86%, and based on OPA-3 was 88.30%, indicating the consistency of both primers in determining the genetic relationship of the two types of catfish (Figures 9 and 10). However, another study based on cytochrome b gene analysis, the genetic closeness between *P. djambal* and *P. hypophthalmus* was 52% (Duong et al., 2022), indicating that *P. djambal* and *P. hypophthalmus* are different catfish groups (Gustiano *et al.,* 2021). Meanwhile, PP, which is a hybrid of *P. hypophthalmus* and *P. djambal*, showed a genetic closeness to PDC of 76.9% (based on OPA-2) and 88.30% (based on OPA-3). This difference is caused by RAPD primers only detecting genetic variations associated with dominant phenotypes, while not being able to detect co-dominant (heterozygous) phenotypes (Liu *et al.,* 2009; Huang *et al.,* 2005). However, RAPD can be used as an initial method to exploit dominant phenotypes associated with the potential superiority of a fish species. The *P. hypophthalmus* ✗ *P. djambal* hybrid still allows for selection for its potential superiority, considering the presence of polymorphic fragments detected by both OPA-2 and OPA-3 primers (Tables 4 and 5). The results of hybridization studies on catfish, showed the presence of polymorphic fragments tended to increase compared to both parents, indicating that RAPD molecular markers can be used to detect dominant phenotype variations (Huang *et al.,* 2005). The genetic diversity of *P. gigas* and *P. sanitwongsei* is high compared to other patin samples (Tables 4, 5) indicating its potential as a superior parent candidate. The existence of genetic variation in parent fish needs to be maintained in each generation to maintain potential superiority and avoid inbreeding in offspring (Liu *et al.,* 1999; Silverstein *et al.,* 2004).

CONCLUSION

P. hypophthalmus (Cijengkol and Subang) exhibited low genetic diversity, while high genetic diversity (the presence of polymorphic fragments) was found in *P. sanitwongsei, P. gigas*, the *P. hypopthalmus-P. djambal* hybrid, and *P. djambal*, respectively. The genetic relationship between *P. hypophthalmus*, the *P. hypopthalmus-P. djambal* hybrid, and *P. djambal* was relatively close (76.90% based on OPA-2 and 88.30% based on OPA-3). Genetic similarity with *P. sanitwongsei* was 55.90% (OPA-2) and 71.30% (OPA-3), and with *P. gigas* was 44% (OPA-2) and 50% (OPA-3). The OPA-2 and OPA-3 primers can be used for the analysis of catfish polymorphisms associated with dominant phenotypes.

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