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ANALYSIS OF GENETIC DIVERSITY OF OSPHRONEMIDAE FAMILY FISHES USING THE RAPD-PCR METHOD

Analisis Keragaman Genetik Ikan Famili Osphronemidae dengan Metode RAPD-PCR

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ABSTRACT

Currently research on genetic diversity analysis is needed and is important information to maintain its survival. Osphronemidae family fish are fish that are often found in various regions. Generally, fish from this family are ornamental fish which are characterized by having a variety of colors. This requires supporting data to determine the level of relationship between fish in the Osphronemidae family. Therefore, research was carried out on the genetic diversity of fish in the Osphronemidae family using the RAPD-PCR method. The research was carried out using goldfish (MK), *Blue gourami* (SP), gourami (GM), dumbo ear betta (DE), crowntail betta (ST), wild betta (WB), and halfmoon betta (HM). The stages carried out are sample preparation, isolation, PCR, data analysis. The results showed that the fourth highest level of kinship was between SP3 (*Blue gourami*), GM1 (gourami), WB19 (wild betta), DE3 (dumbo ear betta), ST10 (crowntail betta), and HM3 (halfmoon betta), namely 0.18 or 18%. Meanwhile, the sample included in the outgroup is MK1 (goldfish) has no relationship with other samples.

Keyword : Betta, Genetic Diversity, Osphronemidae, RAPD-PCR

ABSTRAK

Saat ini penelitian mengenai analisis keragaman genetik diperlukan dan menjadi informasi penting untuk menjaga kelangsungan hidupnya. Ikan famili Osphronemidae merupakan ikan yang banyak ditemukan di berbagai daerah. Umumnya ikan dari famili ini merupakan ikan hias yang bercirikan mempunyai warna yang beranekaragam. Hal ini diperlukan data pendukung untuk mengetahui tingkat kekerabatan antara ikan famili Osphronemidae. Oleh karena itu dilaksanakan penelitian mengenai keragaman genetik ikan famili Osphronemidae den gan metode RAPD-PCR. Penelitian dilaksanakan dengan menggunakan ikan mas koki (MK), ikan sepat biru (SP), ikan gurame (GM), ikan cupang dumbo ear (DE), ikan cupang serit (ST), ikan cupang wild betta (WB), dan cupang halfmoon (HM). Tahapan yang dilakukan adalah persiapan sampel, isolasi, PCR, analisis data. Hasil diperoleh bahwa tingkat kekerabatan tertinggi keempat antara SP3 (ikan sepat biru biru), GM1 (ikan gurame), WB19 (ikan cupang

wild betta), DE3 (ikan cupang dumbo ear), ST10 (ikan cupang serit), dan HM3 (ikan cupang halfmoon), yakni sebesar 0,18 atau 18%. Sedangkan sampel yang termasuk outgroup yaitu MK1 (ikan mas koki) tidak memiliki kekerabatan dengan sampel lainnya.

Kata Kunci : Cupang, Keragaman Genetik, Osphronemidae, RAPD-PCR

INTRODUCTION

Osphronemidae is one type groups of fish that have labyrinth as a means of breathing. The habitat Lots found in swamps and the Mula river. The spread found in Asia, starting from India , India to China. This group of fish makes nest by the way make the foam then used as a nesting place (Veronica *et al*., 2017) . Fish belonging to the family Osphronemidae that is various types of betta fish (*Betta* sp.) (Ajai *et al.,* 2020), Blue Sepat Fish (*Trichogaster pectoralis*) (Pujiyani & Rukayah, 2019), Gourami Fish (*Osphronemus gourami*) (Mahyuddin, 2009). Several types of betta fish include the Dumbo Ear, Halfmoon, Crown Tail, Wild Betta. Fish from family Osphronemidae It is a food fish and can also be used as an ornamental fish (Susanto, 2014). Decorative fish is many types of fish maintained Because beauty color and body shape (Fadzilah *et al*., 2023) .

Moreover, in nature Osphoronemidae fish group have role Ecological importance for nature and humans (Ilham *et al*., 2021) . Based on research results (Diniya et al., 2014) explain that besides eat animals Small fish in the water, this fish is also a grazer fish that eats remains organisms that stick between aquatic plants such as weed (Diniya *et al*., 2014). Blue catfish blue plays an important role in controlling weeds and phytoplankton to prevent this from happening flowering, so ecosystem surrounding still stable. Apart from eating aquatic plants and phytoplankton , blue catfish blue also eats flick mosquito *Anopheles* sp. reason malaria in humans (Asmiani *et al*., 2013).

The availability of genetic information in the Osphronemidae family is important information in an effort to maintain its survival. One method that can be used to assess genetic variation between populations is to use *Random Amplified Polymorphic DNA* (RAPD-PCR). Genetic markers using the RAPD-PCR method use a single primer with a short oligonucleotide of 10 base pairs as a primer that will bind to the complement part. This method can detect DNA polymorphisms which are used as genetic markers to determine kinship relationships (Anggereini, 2008) .

The advantage of the RAPD-PCR method is that the single primer used can be amplified randomly (Hayuningtyas & Kadarini, 2016) , and the acquisition of molecular data is relatively fast and cheap (Hayuningtyas & Kadarini, 2016) . The RAPD-PCR method can quickly produce molecular markers to describe genetic diversity compared to the *amplified fragment length polymorphism* (AFLP) and *random fragment length polymorphism* (RFLP) methods (Randriani & Tresniawati, 2012) . Molecular markers using the RAPD-PCR method have been widely used to detect genetic diversity in several ornamental fish populations, one of which is guppy fish (*Poecilia reticulata*) (Ekasanti *et al*., 2023).

Based on this, it is necessary to carry out research to examine it diversity of fish families Osphronemidae in a area that becomes its habitat. Remember importance benefit economics and ecology of fish families Osphronemidae in nature, then data and information related to diversity are needed family fish life Osphronemidae as an attempt management source Power the genetics of the fish to maintain continuity his life.

RESEARCH METHODS

There were seven species of test fish with 10 fish each used in this study , namely goldfish (MK), blue catfish (SP), gourami fish (GM), betta fish *dumbo ear* (DE), betta fish serit (ST), betta fish *wild betta* (WB), and betta *halfmoon* (HM). The stages carried out are

samples of fish fins that will be observed taken , cut use scissors surgery on his fins . The sample is immersed in the solution preservative (4 alcohol : glycerol 1). The carp's tail fin is taken 10 mg and put into a 1.5 mL tube . Samples were crushed use chopsticks until fine . Added 300 ul Solution Nuclei Lysed and homogenized use vortex for 10 seconds Then incubated / heated use water bath at 65 ^{o}C for 30 minutes. Sample added Solution RNAse as much as 1.5 ul and turned 2-5 times with the aim of mixing evenly, after that it was incubated again in a water bath at 37 ^{o}C for 30 minutes. After incubation , the samples were left at room temperature for 5 minutes. Added 100 ul Solution Protein precipitation and homogenization use vortex for 10 seconds , then in cold ice for 5 minutes and centrifuged for 4 minutes with a centrifuge speed of 13,000 rpm. Supernatant transferred into a new tube filled with 300 ul isopranol , then homogenized and centrifuged at 13,000 for 1 minute. Supernatant discarded and 300 ul 7% ethanol was added and centrifuged again at 13,000 rpm for one minute. Ethanol discarded and pelleted dried in air for 15 minutes. 50 ul Solution Rehydrate added to the containing tube pellets and then incubated in a water bath at 65 ^{o}C for 1 hour. The resulting solution of the DNA isolation process is a solution containing a DNA sample that is ready to be used as a DNA template in the tube process furthermore that is amplification and electrophoresis . Furthermore tube stored at -20 ^{o}C.

Component reagents for PCR are made use 0.2 ml microtube. A total of 12.5 µl solution MyTaq Master Mix is taken use micropipette and inserted into the microtube. *Nuclease Free Water* (NFW) added to the microtube as much as 9.5 µl then homogenized. Primer OP G - 1 6 (AGCGTCCTCC) added to the microtube as much as 1 µl. template DNA 2 µl was put into a microtube Then homogenized. The stages of the PCR program in this research were initial denaturation at 94 ^{o}C for 2 minutes, 35 cycles consisting of denaturation at 94 ^{o}C for 1 minute, annealing at 36 ^{o}C for 1 minute, extension at 72 ^{o}C for 2 minutes and final extension at 4 ^{o}C for 7 minutes.

Next, the sample results are checked by performing electrophoresis. Electrophoresis created using powder agarose weighed as much as 1 g. TAE was measured as much as 100 ml. Put the Scott bottle in the microwave for 1 minute. Scott's bottle was removed from the microwave and opened. Added 10µl gel red Then shaken until homogeneous. Pour in the mixture powder agarose, TAE and gel red, leave until congested. Analysis of electrophoresis data DNA amplification is processed using CorelDraw X7 software so that it can be analyzed size DNA arrangement specific. The final stage is carried out that is process data using application NTSYSpc 2.02, then the dendrogram graphic results are obtained which can then be analyzed.

RESULTS

Genomic DNA isolation applies procedures and methods according to the Wizard Genomic DNA Purification Kit, also known as the Promega Kit. The body part of the fish sampled was the tail of the fish. The tail is the part of the fish's body that moves most actively in the water and requires energy to regenerate muscle cells. This part of the tail is used as a sample to obtain a part that can represent the cells in the body of the fish which are used as test samples in good condition. Apart from that, the tail part that is sampled can also be taken directly without having to kill the fish.

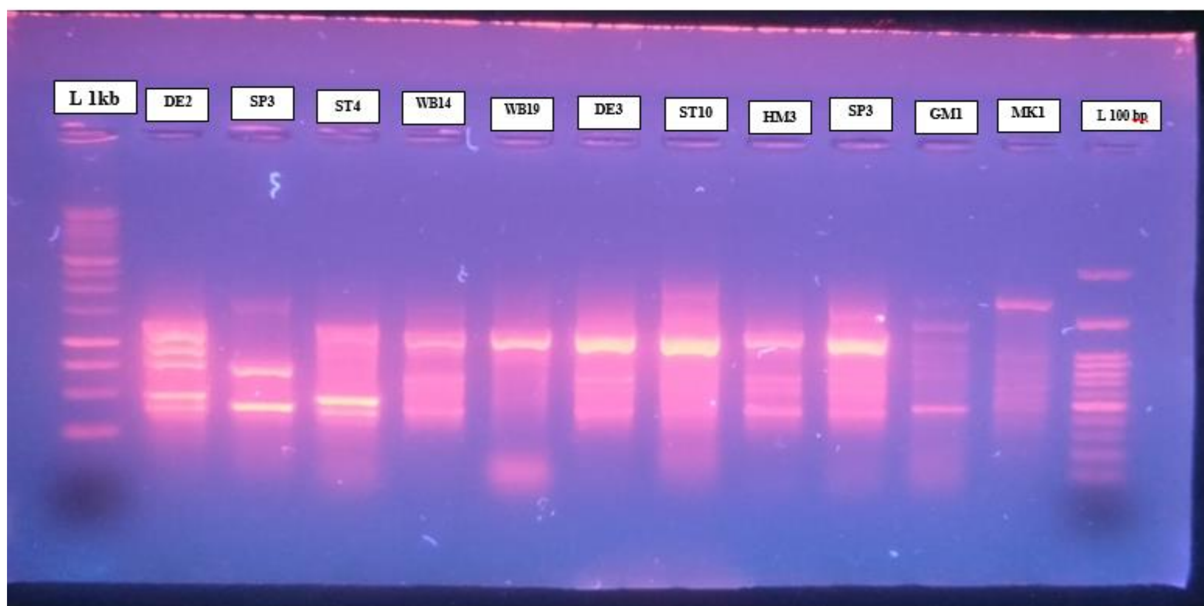

Figure 1. PCR Electrophoresis Results with OPG-16 Primer

The results of the amplification process are in Figure 1 which shows the presence of band fragments of DNA produced by the OPG-16 primer. These fragments are produced in various ways due to differences in the nucleotide sequence at the primer attachment site. Reinforced tape depends largely on the quantity, quality and suitability of the site and primer used. These primer attachment sites are distributed randomly throughout the genome, and polymorphisms in these areas will result in different amplifications.

Table 1 . Amount Tape DNA Sample Test

Fragment Distance (mm)	Mark Base Pair	Amount Tape	Number of Polymorphism Bands	Number of Monomorphism Bands
47	3000	1	1	0
49	3000	1	1	0
52	3000	1	1	0
55	1500	1	1	0
58	1500	4	0	4
62	900	1	1	0
63	900	4	0	4
67	800	2	0	2
69	700	3	0	3
71	600	2	0	2
76	500	1	1	0
79	400	2	0	2
81	400	5	0	5
86	300	1	1	0
Total		**29**	**7**	**22**

Grouping of DNA bands based on size showed that there were polymorphic DNA bands and monomorphic DNA bands (Table 1). Based on this grouping, it was found that most of the DNA fragments in the test samples produced using these primers were monomorphic, and only a small portion of the test samples produced polymorphic DNA fragments. Monomorphic

is the nature of the DNA fragments found in each sample, while polymorphic is the nature of the fragments

The OPG-16 primer produces the majority of DNA fragments in the test samples produced by this primer which are monomorphic, and only a small portion of the test samples produce polymorphic DNA fragments. The following are the results of RAPD-PCR data processing which are presented in the form of a phenogram or phylogeny tree resulting from data processing using the NTSYS application.

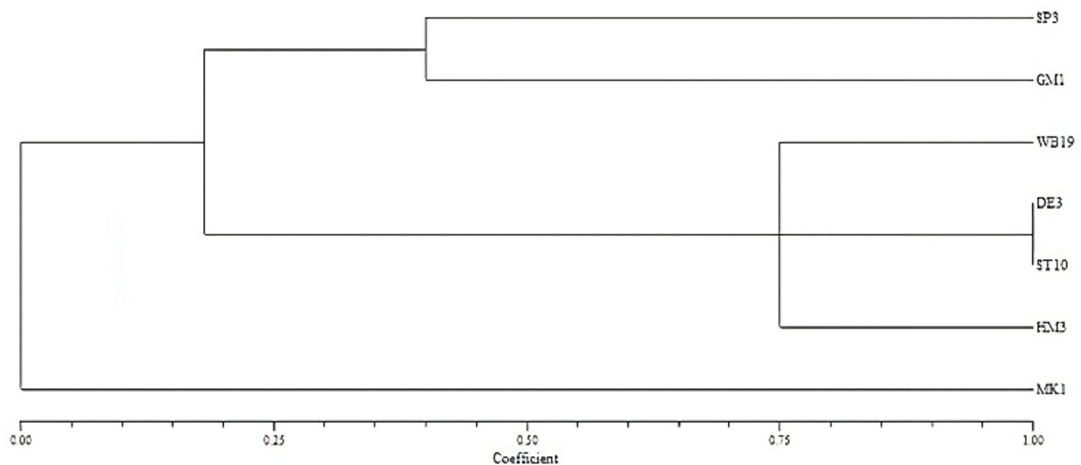

Figure 2. Phylogeny Tree Result of Analysis with NTSYS

Based on the phylogeny tree, it was found that samples DE3 (dumbo ear betta fish) and ST10 (serit betta fish) had the highest level of relationship, namely 1.00 or 100%. This shows that the level of relationship between these two test samples is very close. The second highest level of kinship is between samples WB19 (wild betta fish), DE3 (dumbo ear betta fish), ST10 (serit betta fish), and HM3 (halfmoon betta fish), namely 0.75 or 75%. Furthermore, there is the third highest level of kinship, namely 0.4 or 40% between SP3 (blue sepat fish) and GM1 (gourami fish).

The fourth highest level of kinship between SP3 (blue sepat fish), GM1 (gourami fish), WB19 (wild betta betta fish), DE3 (dumbo ear betta fish), ST10 (serit betta fish), and HM3 (halfmoon betta fish), namely of 0.18 or 18%. This shows that the level of relationship between these species is increasingly distant. There is an outgroup sample, namely MK1 (goldfish) which is not related to the other samples. This is appropriate if seen from the taxonomy of the fish samples. Blue catfish, gourami, wild betta bettas, dumbo ear bettas, serit bettas, and halfmoon bettas come from the same family, namely Osphronemidae, while goldfish come from the Cyprinidae family.

DISCUSSION

The RAPD-PCR method is a method for identifying a large number of DNA polymorphisms in the genome which are used as genetic *markers* and determining the relationship of organisms quickly and efficiently. With this type of polymorphism, RAPD-PCR is very suitable for the study of genetic diversity, kinship relationships, genetic maps, and DNA fingerprinting. The RAPD-PCR method uses short oligonucleotides (usually 10 bp) as primers that will bind to the complement sites. This method relies on using a single primer (synthetic oligonucleotide) to start PCR (Anggereini, 2008).

Analysis is carried out in 3 stages, DNA amplification, electrophoresis and

polymorphism analysis (Utomo *et al*., 2021). The initial stage of DNA amplification was carried out using the RAPD-PCR method with the primer, namely OPG-16. DNA polymorphism data is in the form of images of DNA bands which are then analyzed, DNA bands are detected, and DNA bands are grouped. Visualization of the electrophoresis results under ultraviolet light will provide an overview of the DNA bands and patterns of DNA fragments resulting from restriction enzyme digestion. The length of the DNA bands and fragment patterns are printed and adjusted to the 100 bp DNA ladder marker.

Monomorphic fragments are a picture of DNA bands that appear at a certain size, and in other samples DNA bands at the same size are found (Mahrus *et al*., 2022). The low polymorphism obtained could be due to the gene strain of the test sample having low nucleotide diversity. If the nucleotide diversity value is less than 1%, it indicates that the molecular marker has low nucleotide diversity (Kochzius *et al*., 2010).

Low polymorphism indicates that polymorphic DNA bands are not found specifically in certain size groups in certain test samples. It was found that there were differences in band length measurements between PCR results from different species, this is a common phenomenon even though the amplification of these fragments uses the same primer pair (Mahrus *et al*., 2022). This can happen, because each test sample species has specific sequences in its genome that differ from one species to another, resulting in different sizes when amplified from different species.

A phylogenetic tree is a form of description of the genealogy of living things, both animals and plants, with branches resembling a tree (Lubis, 2014). Phylogenetics is a method used to analyze the phylogenetic relationship of a living creature. In principle, molecular phylogenetic approaches can be used to differentiate and identify specimens that are difficult to identify (Dharmayanti, 2011).

CONCLUSION

The fourth highest level of kinship between SP3 (blue sepat fish), GM1 (gourami fish), WB19 (wild betta betta fish), DE3 (dumbo ear betta fish), ST10 (serit betta fish), and HM3 (halfmoon betta fish), namely of 0.18 or 18%. Meanwhile, the sample belonging to the outgroup, namely MK1 (goldfish), has no relationship with the other samples.

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