Asian Journal of Biochemistry, Genetics and Molecular Biology

8(1): 1-8, 2021; Article no.AJBGMB.68016
ISSN: 2582-3698

Determination of Genetic Diversity of Four Guppy Strains Using the Random Amplified Polymorphic DNA-PCR

Riva Hafidah[1*], Ayi Yustiati[1], Yuniar Mulyani[1] and Ibnu Bangkit Bioshina Suryadi[1]

[1]Fisheries Study Program, Fishery and Marine Sciences Faculty, Padjadjaran University, Jln. Raya Jatinangor KM 21, Sumedang 45363, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. Author RH designed the study wrote the first draft of the manuscript managed the literature searches and managed the analyses of the study. Author AY designed the study and wrote the protocol. Authors AY, YM, and IBBS managed to check and revise the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2021/v8i130183
Editor(s):
(1) Dr. Gul Ozcan, University of Istanbul, Turkey.
Reviewers:
(1) Laura Borelli Thomaz Carreira, Universidade Estadual Paulista (UNESP), Brazil.
(2) Amin Golpour Dehsari, Institute of Hydrobiology, Czech Academy of Science, Czechia.
(3) Leonardo Luiz Calado, Federal University of Rio Grande do Norte, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68016

Original Research Article

Received 01 March 2021
Accepted 11 May 2021
Published 18 May 2021

ABSTRACT

This research aims to determine genetic diversity of four strains guppy, respectively are japan blue double sword (JBD), japan blue tiger double sword (JBTD), blue moscow (BM), and panda guppy (PG) with RAPD-PCR method. The obtained genetic diversity data is used as guide reference for hybridization between four strains. The research was conducted in September 2020 to April 2021 with explorative methods and in qualitative and quantitative descriptive analysis. The research was carried out in biotechnology Laboratory, Fishery and Marine Sciences Faculty and Central Laboratory, Padjadjaran University, Indonesia. Strains of JBD, JBTD, BM obtained from Cilengkrang Subdistrict, Bandung and PG strain obtained from Parung market, Bogor. Primary OPA-03 (AGTCAGCCAC) is used for standard parameters to interpret genetic diversity among four strains of guppy. Based on results, amplification with OPA-03 primary visualize 25 bands that include five polymorphic bands and 20 monomorphic bands. The phylogenetic tree result show that

**Corresponding author: E-mail: rivahafidah@gmail.com;*

there are two relationship groups. The first group are JBD, JBTD, and BM with similarity index in the range of 80-89% The first group consist two sub groups of relationship. The first sub group are JBD and JBTD with similarity index of 89%. The second sub group is BM with similarity index of 80%. The second group is PG with similarity index of 65.5%.

Keywords: Genetic diversity; guppy; RAPD-PCR; phylogenetic tree.

1. INTRODUCTION

Guppy *(Poecilia reticulata*) is freshwater ornamental fish with high economic value, both domestically and abroad. Guppy can be kept in the aquarium and can also be used as contest fish. Guppy fish production in 2015-2018 in Indonesia increased by 82.5% [1]. The increase occurred due to market demand.

The appeal of guppy are color, pattern, and shape of the tail. According to [2] there are 14 variety of patterns that snakeskin, tuxedo, cobra, leopard tail, grass tail, lace tail, and mosaic tail, and has a variety of colors that are variations of single color, bicolor, and multicolor.

The high demand of new variety of colors, patterns, and shapes of the tail make guppy breeders continue to make cross efforts to get new guppy variations. Continuous cross-crossing experiments will increase the risk of inbreeding. Therefore, Hybridization can produce superior phenotype properties and reduce the risk of inbreeding.

Hybridization is cross between two different individuals to obtain superior offspring [3]. According to [4] hybridization is a cross breeding between two species of fish whose diversity is close, but has different properties and characteristics, so that it will produce offspring with the desired traits. According to [5] the key to success in hybridization activities is that both fish have the same number of chromosomes and have the same biological and reproductive properties.

The selection of a parent for hybridization can be seen from its genetic diversity. Genetic diversity data can be used as reference data for selecting parent to hybridization. This hybridization will produce superior progeny. Strains of Japan blue double sword (JBD), Japan blue tiger double sword (JBTD), blue Moscow (BM), and panda guppy (PG) are not yet known genetic diversity data.

Determine genetic diversity of fish can be known through Polymerase Chain Reaction (PCR) technique with Random Amplified of Polymorphic DNA (RAPD) method. According to [6], PCR with RAPD method can detect monomorphic fragments of DNA and can be interpreted through phylogenetic tree. The advantages of RAPD method are to detect nucleotide sequences with only one primary, have high polymorphism, and it can be used without knowing the background of the previous genome [7]. RAPD can be used to analyze genetic variations that can be used for reproductive programs in the field of aquaculture [8].

2. MATERIALS AND METHODS

This research was conducted in September 2020 to April 2021 at Biotechnology Laboratory, Fishery and Marine Sciences Faculty, Padjadjaran University for DNA isolation, DNA amplification, and electrophoresis results of DNA isolation and amplification. DNA purity calculation at the Central Laboratory, Padjadjaran University. The research method used explorative methods without experimental design with analyzed descriptively qualitatively and quantitatively. The samples used were guppy caudal fins from four different strains. Determination of genetic diversity used Polymerase Chain Reaction (PCR) with Random Amplified Polymorphic DNA (RAPD) method and used two universal primers namely OPA-02 and OPA-03. Only one primary was chosen for further analysis, the primary that show more bands. The DNA test resultswas analyzedby using the NTSYS program.

2.1 Procedures

Research procedures include:

2.1.1 DNA isolation

This research used Genomic DNA Purification Kit (Promega). 10 mg of caudal fin was added into microtube 1.5 µ l and mashed until smooth, added 300 µl Nuclei lysis solution, homogenized with vortex 10 seconds, and incubated at 65^{o}C for 30 minutes. Added 1.5 µl RNAse, flipped 2-5 times, incubated at 37^{o}C for 30 minutes, and cooled for 5 minutes at room temperature. Added 100 mL precipitation solution protein,

homogenized with vortex for 10 seconds, and centrifuged at 13,000 rpm for 4 minutes. Supernatant was transferred to new microtube, added 300 µl isopropanol and centrifuged at 13,000 rpm for 1 minute. Supernatant was discarded, then added 300 µl ethanol, and centrifuged at 13,000 rpm for 1 minute. Ethanol was discarded and pellet dried for 15 minutes. Added 50 µl Rehydration solution and incubated at 65^{o}C for 60 minutes. The tube stored at -20^{o}C.

2.1.2 Calculation of DNA purity

DNA purity quantification is a quantitative test of DNA. The test used the Multimode Reader Infinite 200 PRO NanoQuant spectrophotometer tool at wavelengths 260 and 280 nm. Added 2 µl DNA isolation result and 2 µl nuclease free water solution into the cuvette, then the absorbance value on the tool was set with wavelengths of 260 nm and 280 nm. The results recorded and calculated.

2.1.3 DNA amplification

DNA amplification procedure was carried out by added 12.5 µl GoTaq Green Master Mix (Promega), 2 µl DNA genome, 1.3 µl primer, and 9.2 µl nuclease free water into microtube 0.2 µl and homogenized with vortex 10 seconds.

Microtube inserted into the PCR machine with the program: pre - denaturation (94^{0}C, 2 minutes, 1 cycle), denaturation (94^{0}C, 1 minute, 45 cycle), annealing (36^{0}C, 1 minute, 45 cycle), extension (72^{0}C, 2 minutes, 45 cycles), final extension (72^{0}C, 10 minutes, 1 cycle), hold (4^{0}C, 3 minutes, 1 cycle) [9].

2.1.4 Electrophoresis

DNA isolation and amplification results are qualitatively tested through electrophoresis with agarose gel. Electrophoresis procedure was to make agarose concentration 1% by added 0.8 gr agarose powder and 80 ml TAE 1x solution into the erlenmeyer, then put in the microwave for 3 minutes, added 0.8 μl gel red then homogenized. Cooled the solution and poured into agarose mold that had been equipped with a comb, waited until frozen. TAE 1x buffer is poured with sub-marine technique.

For first test sample was filled with 2 µl DNA Ladder 1kb and 2 µl loading dye. The next test was filled with 4 µl DNA template and 2 µl loading dye. Electrophoresis tank was given electricity at 75V for 45 minutes (for DNA isolation results) and 90 minutes (for DNA amplification results).

2.2 Data Analysis

Data analysis was conducted qualitatively and quantitatively. Quantitative data was obtained from the calculation of DNA purity. Qualitative data was obtained from the bands visualized and not visualized on gel agarose, then qualitative data was created in the form of a binary matrix with Microsoft Excel 1997-2003 and processed using NTSYS program to bring up the phylogenetic tree.

3. RESULTS AND DISCUSSION

3.1 DNA Isolation Result

DNA isolation results show that the bands produced on each strain is thick and without smear (Fig. 1). JBD strain shows the most obvious band without smear, BM strain shows the thinnest band among other strains, while JBTD and PG show thick band. DNA isolation conducted in this research is successfully purifies DNA. Smear indicates that isolated DNA contains material other than DNA or DNA degradation occurs [10,9].

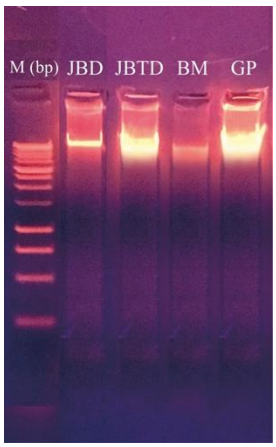

Fig. 1. Electrophoresis of DNA isolation result
Note: bp = base pair; M = Marker DNA Ladder 1 Kb; JBD = Japan Blue Double Sword; JBTD = Japan Blue Tiger Double Sword; BM = Blue Moscow; PG = Panda Guppy

This research used Genomic DNA Purification Kit (Promega) isolation kit. Genomic DNA Purification Kit (Promega) has been tested to show good purity results [11,12]. Isolated fish samples that have been preservation first, the composition of the preservation solution is by mixing alcohol 95% and glycerin at a ratio of 4:1

[13]. Caudal fins are an easy part to destroy, easy to get DNA samples [9]. DNA isolation is an early stage for molecularly identifying fish species, the success of DNA isolation based on the quality and quantity of DNA purity produced.

DNA purity calculation result of Japan blue double sword (JBD), Japan blue tiger double sword (JBTD), blue Moscow (BM), panda guppy (PG) have purity values of 1.93; 1.92; 1.93; 1.93 (Table 1). The purity results are classified as pure, because it is in the range of 1.8-2.0. Purity values in the range of 1.8-2.0 indicate that the DNA is pure [14]. According to [9], purity values above 2.0 indicate that there is RNA contamination, whereas if the purity value is below 1.8 indicates that there is protein contamination.

The principle of DNA concentration calculation is based on ultra violet (UV) irradiation of the spectrophotometer absorbed by nucleotides and proteins in the solution, the more UV rays are absorbed then the high DNA concentration [14,12].

3.2 DNA Amplification

OPA-03 primer showed more bands than OPA-02. Then, DNA amplification result by using OPA-03 primary analyzed further. The number of DNA bands that appear from four strains using the primary OPA - 03 is 25 bands (Fig. 2), with the number of 5 polymorphic bands and 20 monomorphic bands (Table 2).

The DNA fragment size results ranged from 224.02-5070.55 bp, this suggests that the results obtained are normal. According to [15], the normal base pair (bp) found in fish ranges from 200-1500 bp, while the base pair commonly found in fish is around 300-1500 bp.

Japan blue double sword (JBD) show 19 bands consisting of 18 monomorphic bands and 1 polymorphic band, Japan blue tiger double sword (JBTD) show 17 monomorphic bands, blue Moscow (BM) show 17 bands consisting of 16 monomorphic bands and 1 polymorphic band, and panda guppy (PG) show 17 bands consisting of 14 monomorphic bands and 3 polymorphic bands. Polymorphic band is a DNA band that appears at a certain size and is found in only one sample, while monomorphic band is the band that appears on all samples [16].

The panda guppy (PG) strain have the highest number of three polymorphic bands at the size of 4290.24, 2433.95, and 510,52 base pairs. JBD and BM strains have each one polymorphic band located at the size of 670,57 and 5070.55 base pairs. The large number of polymorphic bands in fish strains indicates that there are genetic variations.

Polymorphic of JBD strain is thought to give rise to the shape of double sword caudal fin. Polymorphic of BM strain is thought to give rise to a full variation of dark blue color. Polymorphic of GP strain is caused by the origin of GP are different from the other three strains and GP strain is cross-results.PG strains have the largest genetic variation from other strains. According to the results of research [9], polymorphic band shows the existence of genetic variations between strains of nirwanalumajang and nirwanawanayasa.

JBD and JBTD strains have 16 monomorphic bands. According to [17], monomorphic nucleotide sequences will express phenotype similarities, phenotypes can be known from morphological, anatomical, and physiological. When viewed in terms of phenotypes, these two fish have similarities such as the shape of the tail double sword and the color of the yellow and light blue body.

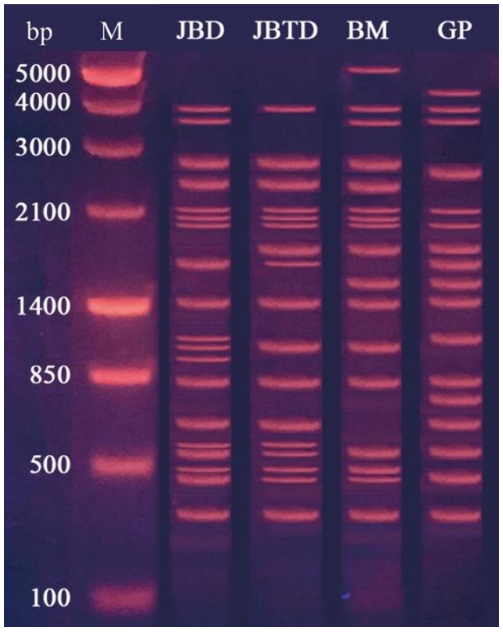

Fig. 2. Electrophoresis of DNA Amplification Result

Note: bp = base pair; M = Marker DNA Ladder 1 Kb; JBD = Japan Blue Double Sword; JBTD = Japan Blue Tiger Double Sword; BM = Blue Moscow; PG = Panda Guppy

3.3 Genetic Relationship Analysis

Phylogenetic tree describes a relationship between fish species or strains. The phylogenetic tree is a method to detect relationship of species and the degree of evolution. The results of phylogenetic tree can be seen in Fig. 3.

Based on the results of the phylogenetic tree obtained, there are two relationship groups. The first group are JBD, JBTD, and BM which have a similarity index in the range of 80-89%. The first group consist two sub groups of relationship. The first sub group are JBD and JBTD with similarity index of 89%. The second sub group is BM with similarity index of 80%. The second group is PG which have similarity index of 65,5%.

The first sub group are JBD and JBTD. The similarities between JBD and JBTD has the shape of a double sword caudal fin, yellow color from head to abdomen, light blue color on the abdomen to the tail. The difference between both fish is that JBTD has tiger pattern, while JBD does not have tiger pattern. According to [18], tiger pattern shows the dotted pattern in the fins. The dotted pattern is effected by aggregation of melanophores (black color cell).

The Japan blue strain originated in the Kanagawa River, Japan, which was discovered around the late 80s. It was first introduced in Japanese Fish Magazine in 1994. Japan blue has a metallic light blue color on the head to abdomen, solid color fins, dot patterns, mosaics, red or variations of other colors. The location of the japan blue gene is in the Y-chromosome, depending on male gender [19]. The double sword tail shape is on X-linked and Y-linked [19]. Tiger pattern in JBTD is a recessive gene, this gene is also known as Europian Gold, IFGA Bronze or Asian Tiger [20], According to [19] tiger is commonly known in Asia.

The second sub group is BM with similarity index of 80%. BM has the shape of caudal triangle tail fin, and dark blue color from the head to the caudal fins. Moscow strain originated from Russia, Moscow characteristic has a solid color on the head. The Moscow gene is found in Y^{mw}, only the male guppy has the Moscow gene [19].

The second group is PG with similarity index of 65.5%. PG color on the head to the abdomen is white, while from the abdomen to tail is dark blue, the color of the fins caudal are dark blue and clear white, the color of the dorsal fin is dark blue. PG strain is the result of a cross between female pink guppy and male Moscow [19], This is thought to be a factor GP has a low similarity index because GP is a strain of cross-results.

PG fish body is smaller than other strains. PG fish obtained from Parung market, Bogor. While the other strains cultivated in Cilengkrangsubdistric, Bandung. The interaction between genotypes and the environment will give rise to phenotypes or visible properties [7]. According to [5] the interaction between the environment and genotypes occurs because the response of some alleles to phenotype expression that appears will different in different environments. According to [21] phenotype character of guppy is the result of adaptation to the environment with different abiotic factors or environmental adaptation with different biotic factors such as predators or parasites.

The results of determine genetic diversity of four guppy strains using the RAPD method with primary OPA-03 successfully analyzed the relationship of four guppy strains. it related with research by [16] on the analysis of green snake skin revolutions and black variations in guppy fish using RAPD markers, the results suggested that the brood and F1 have mendelian inheritance patterns. Furthermore, [22] research of eight guppy fish used for genetic analysis using RAPD techniques with 9 primers showed the highest genetic differences between king and flaminggo (0.604), while the genetic equation of all guppy fish samples was (minumun 0.670 and maximum 0.948).

Table 1. DNA purity

No	Sample	Abs 260	Abs 280	Value of DNA Purity
1	JBD	0,0702	0,036	1,93
2	JBTD	0,224	0,116	1,92
3	BM	0,12	0,062	1,93
4	PG	0,2046	0,106	1,93

Table 2. Polymorphic and Monomorphic DNA Bands

BP Position	JBD	JBTD	BM	PG
5070,55			--*	
4290,24				--*
3759,86	--	--	--	--
3413,09	--		--	--
2625,48	--	--	--	
2433,95				--*
2312,95	--	--	--	
1978,38	--	--	--	--
1876,73	--	--	--	
1716,78	--	--	--	--
1466,99		--	--	--
1289,48	--	--		--
1110,83			--	--
961,76	--	--	--	--
771,91	--			--
719,88	--	--	--	
670,57	--*			
592,88	--	--	--	--
510,52				--*
424,42	--	--		--
385,28	--	--		
345,47	--	--	--	--
314,10	--	--	--	
291,08	--	--	--	--
224,02	--	--	--	--

Note: -- = Monomorphic Band; -- = Polymorphic Band; JBD = Japan Blue Double Sword; JBTD = Japan Blue Tiger Double Sword; BM = Blue Moscow; PG = Panda Guppy*

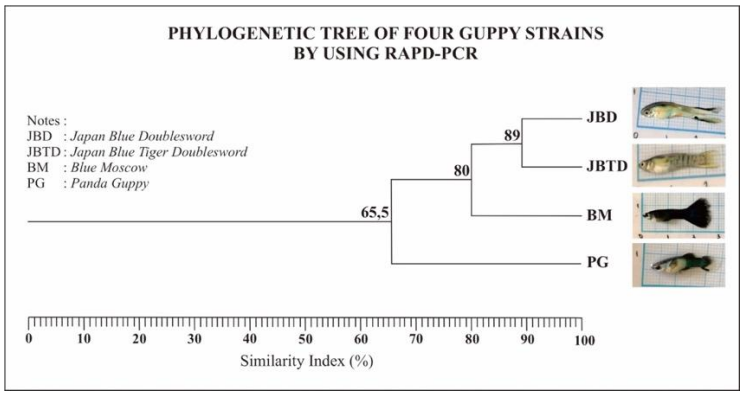

Fig. 3. Phylogenetic Tree of Four Guppy Strains with Primary OPA-03

Strains of JBD and JBTD have the highest similarity index, it means that these two fish have a lot of monomorphic bands. Thus, if these two fish are crossed then the success rate of the cross is high and it can increase the genetic variation of the parent. According to research conducted by [23], the cross between jambalpatin females and siampatin males that have low heterozygous successfully increases the proportion of heterozygous genes or increases the genetic variation of their offspring. According to [24], the value of genetic diversity is influenced by the proportion of heterozygous and homozygous genes, the higher proportion of homozygous genes it will have lower the genetic diversity, and the higher proportion of heterozygous genes, it will have higher genetic diversity.

Strains of JBD, BM, and GP have polymorphic band, it means the strains have high genetic variations. Thus, if between these three strains

are crossed, it will produce offspring with a new variation. According to [25], the presence of polymorphic band in fish will produce a new genetic structure in fish species.

4. CONCLUSION

Briefly, there are two relationship groups. The first group are japan blue double sword (JBD), japan blue tiger double sword (JBTD), and blue moscow (BM). The first group consist two sub groups of relationship. The first sub group are JBD and JBTD with similarity index of 89%. The second sub group is BM with similarity index of 80%. The second group is panda guppy (GP) with similarity index of 65.5%. Primary OPA-03 was able to show many bands in the genetic diversity analysis of guppy strains. GP showed 3 polymorphic bands which means GP strain have the higher genetic variations than others.

5. SUGGESTION

The suggestion from this research is to get a high success rate of cross breeding, you must to cross between japan blue double sword (JBD) and japan blue tiger double sword (JBTD). in other hand, if you want to produce strains with new variations, you must to cross between japan blue double sword (JBD), blue moscow(BM), and panda guppy (PG).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. DJPB1. Optimalisasi Potensi Budidaya Ikan Hias Nasional; 2019.
   Accessed 22 July 2020.
   Available:https://kkp.go.id/djpb/artikel/15803-optimalisasi-potensi-budidaya-ikan-hias-nasional.
2. Susanto H. Budidaya Ikan Guppy. Yogyakarta: Kanisius; 1990.
3. Arifin OZ, Hendri N, Muslim A, Yani dan, Yani A. HibridisasiIntraspesifik Antar Dua PopulasiIkan Gurami Galunggung (*Osphronemus goramy, Lacepede*, 1801). Jurnal Riset Akuakultur. 2017;12(1):315–323.
4. Arifin N, Muslim A, Hendri dan, Yani A. Karakteristik Fenotipe dan Genotipe Ikan Gurami, *Osphronemus goramy*, Strain Galunggung Hitam, Galunggung Putih, dan Hibridanya. Jurnal Riset Akuakultur. 2017; 12(1):99–110.
5. Tave D. Genetics for Hatchery Managers. United States of America: The Avi Publishing Company. Inc; 1986.
6. Mulyadi GK, Bowono dan ID, Subhan U. Analisis Kekerabatan Genetik Hibrid Ikan Nilem (*Osteochilus hasseltii*) dan Ikan Mas (*Cyprinus carpio* L) menggunakan PCR RAPD. Perikanan Dan Kelautan. 2017; 8(1):42–47.
7. Kristanto AH, Subagja J, Cahyanti dan W, Arifin OZ. Evaluasi Variasi Fenotipe Dan Genotipe Populasi Ikan Tambakan Dari Jawa Barat, Kalimantan Tengah, Dan Jambi Dengan Truss Morfometrik Dan Random Amplified Polymorphic Dna (Rapd). Jurnal Riset Akuakultur. 2017; 12(3):203–211.
8. Omer OM, Abdalla AEWH, dan ZN Mahmoud. Genetic Diversity of Two Tilapia Species (*Oreochromis niloticus* and *Sarthrodongalilaeus*) Using Random Amplified Polymorphic DNA. The Open Biology Journal. 2020;8(1):22–28.
9. Yustiati A, Bangkit I, Lili W. Genetic Analysis of Tilapia Nirwana Strain (*Oreochromis niloticus*) Cultured In Lumajang East Java and Wanayasa West Java By Using Random Amplified Polymorphic DNA. Global Scientifix Journals. 2019;7(11):1–9.
10. Ayuningrum PI, Afrianto E, dan Y Mulyani. Keragaman Genetik Rumput Laut *Eucheuma spp.* dari Sukabumi, Jawa Barat Berdasarkan Metode RAPD PCR. Jurnal Perikanan Dan Kelautan Unpad. 2012;3(4):337–345.
11. Mulyani Y, Purwanto A, dan I Nurruhwati. Perbandingan Beberapa MetodeIsolasi DnaUntuk Deteksi Dini Koi Herpes Virus (KHV) Pada Ikan Mas (*Cyprinus carpio L*.). Jurnal Akuatika Indonesia. 2011;2(1):1–16.
12. Adriany DT, AAB dan MIB. Perbandingan MetodeIsolasi DNA Terhadap Nilai Kemurnian DNA untuk Pengujian White Spot Syndrom Virus (WSSV) pada Lobster Bambu (*Panulirus versicolor*). Jurnal Prosiding Simposium Nasional VII. 2020;187–194.
13. Novita H, Mufidah T, dan I. Koesharyani. Perbandingan Penggunaan Berbagai Preservasi RNA Jaringan Dengan Rna Later, Alkohol, dan Alkohol. J. Ris. Akuakultur. 2009;4(3):377–383.

14. Abdullah A, Nurilmala M, dan KP Sitaresmi. DNA Mini-Barcodes Sebagai Penanda Molekuler Untuk Ketertelusuran Label Pangan Berbagai Produk Ikan Layur Asadatun. Jurnal Pengolahan Hasil Perikanan Indonesia. 2019;22(1):33–40.
15. Akbar N, dan R Labenua. Keragaman Genetik Ikan Cakalang (*Katsuwonus pelamis*) di Perairan Laut Maluku Utara. JurnalIlmu-Ilmu Perairan, Pesisir Dan Perikanan. 2018;7(2):164–176.
16. Foo CL, Dinesh KR, Lim TM, Chan WK, dan VPE Phang. Inheritance of RAPD Markers in the Guppy Fish, *Poecilia reticulata*. Zoological Science. 1995;12(5): 535–541.
17. Lathifah AU, Buwono ID, dan U Subhan. Deteksi Keragaman Genotip Hibrid Ikan Lele Sangkuriang, Mutiara Transgenik Dan Mutiara Non Transgenik Pada Keturunan Pertama. Jurnal Perikanan Dan Kelautan Unpad. 2016;7(2):133–141.
18. Shaddock P. Gupi Color Strain Third Edition. ISBN 978-0-9865700-0-1. 2012.
19. Shaddock P. Guppy Color Bank First Edition; 2008.
20. Bias AS, dan RD Squire. The Phenotypic Expression of Purple Body (Pb) in Domestic Guppy Strains of Poecilia reticulata. BioRxiv. 2017;1–23.
21. Adro'I H, Hariyanto S, Ali M, dan B Irawan. Phenotype variation of guppy fish (*Poecilia reticulate* W. Peters, 1859) population from different quality of aquatic environments in Surabaya, Indonesia. AIP Conference Proceedings. 2018;1-10.
22. Küçük S. Estimation of genetic differences among guppies, poecilia reticulata by rapd markers. ADÜ Ziraat Fakültesi Dergisi. 2010;7(1):65-70.
23. Ariyanto, Didik dan R Utami. Evaluasi Laju Pertumbuhan, Keragaman Genetik dan Estimasi Heterosis pada Persilangan Antar Spesies Ikan Patin (*Pangasius*sp). Jurnal Perikanan. 2006;8(1):81-86.
24. Noor R. Genetika Ternak. Jakarta: Penebar Swadaya; 2000.
25. Yustiati A, Hutagalung RM, Andriani Y, Lili W, dan I Bangkit. Genetic Analysis of Tilapia (*Oreochromis niloticus*) Nirwana Strain Cultured in Aceh Besar and Wanayasa by Using Random Amplified Polymorphic DNA Method. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2020;4(3):1–9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68016