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Research Article

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Genetic Variations of Two Anguilla Species in the West Coast of West Sumatra and Mentawai Using RAPD

Variasi Genetik Dua Spesies Anguilla di Pesisir Barat Sumatera Barat dan Mentawai Menggunakan RAPD

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ABSTRACT

The existence of *Anguilla* population in the world become reduced. One of the spawning ground in the world at Indian Ocean which is close to the Sumatra island still in progress to study it. Research on the genetic variation of *Anguilla* spp. in the waters of the West Coast of West Sumatra was carried out from February to June 2022. This study used the RAPD (random amplified polymorphic DNA) method.In this study, two *Anguilla* species were found, namely *A. marmorata* and *A. bicolor bicolor*. The highest heterozygosity value of *A. marmorata* in the Tarusan River was 0,2018, and *A. bicolor bicolor* in Mentawai was 0,2340. The values of gene flow (Nm) in *A. marmorata* and *A. bicolor bicolor* were 0.7592 and 1.6318. The values of genetic differentiation (G_{ST}) in *A. marmorata* and *A. bicolor bicolor* were 0.3971 and 0.2345, respectively. This shows that the value of genetic variation of *A. marmorata* and *A. bicolor bicolor* in several rivers on the west coast of West Sumatra is low.

INTRODUCTION

Anguilla spp. is a with a catadromous life cycle that spawns in the deep sea (Arai, 2020). The life cycle of *Anguilla* spp. consists of five stages: Leptocephalus, glass eel, elver, yellow eel, and silver eel. Newly hatched eel larvae are called Leptocephalus (Cresci et al., 2020). The waters of the Indo-Pacific Ocean are considered the origin of the *Anguilla* species (Arai, 2020). The waters of the west coast of Sumatra are directly related to the Indian Ocean. Most of the eel fish found in the islands between the Indian Ocean dan the Mentawai Straits. In Sumatra In the West, especially in the Mentawai, three species and subspecies of eel were recorded, namely *A. marmorata*, *A. bicolor bicolor*, and *A. bicolor pacifica* (Syaifullah et al., 2019).

The size of the eel population continues to decline, a significant decline that has resulted in the European eel (*A. anguilla*) being listed as critically endangered on the IUCN Red List (Pike et al., 2020). Factors that cause the decline of eels in nature include excessive exploitation of glass eels in their natural habitats, as well as decreased quality and quantity of water habitats. Habitat loss and degradation can affect eel populations because freshwater and brackish water are habitats for eel growth (Kaifu et al., 2021).

The decline in eel populations in subtropical areas has increased in demand for eels from other species, especially tropical eel species. The fulfillment of the demands and needs of eels will depend on aquaculture activities. In the development of fish farming, the conservation of genetic diversity is an important aspect in population (Fahmi, 2015). Genetic variation can be described as interspecies variation. Genetic data such as heterozygosity values and the number of alleles per locus can help in understanding the factors that influence genetic diversity in a fish population (Matinez et al., 2018).

The RAPD method has the ability to quickly and efficiently detect polymorphisms at a number of loci (Kumari and Thakur, 2014). RAPD has been used to determine genetic data

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for glass eels on the Mentawai Island (Syaifullah et al., 2019), genetic variations of *A. marmorata* in Vietnam, and to evaluate genetic diversity and conservation of some fish populations (Muhajirah, 2021). RAPD can effectively identify genetic markers to differentiate closely related species (Anggereini, 2008). The RAPD technique is able to work quickly and efficiently for DNA-based polymorphisms and uses a variety of potential primers (Kumari and Tahkur, 2014). This method only required low quantities of template DNA. This study aims to obtain information about the genetic variation of *Anguilla* on the west coast of West Sumatra And some from North Pagai of Mentawai islands

MATERIALS AND METHODS

Sample Collection

Anguilla samples were collected from five locations, namely the Bangek River (Padang), the Tarusan River (Pesisir Selatan), the Antokan River (Lubuk Basung), the Maligi River (Pasaman Barat), and the Mentawai. DNA isolates from Mentawai were obtained from Syaifullah et al. (2019) (Figure 1).

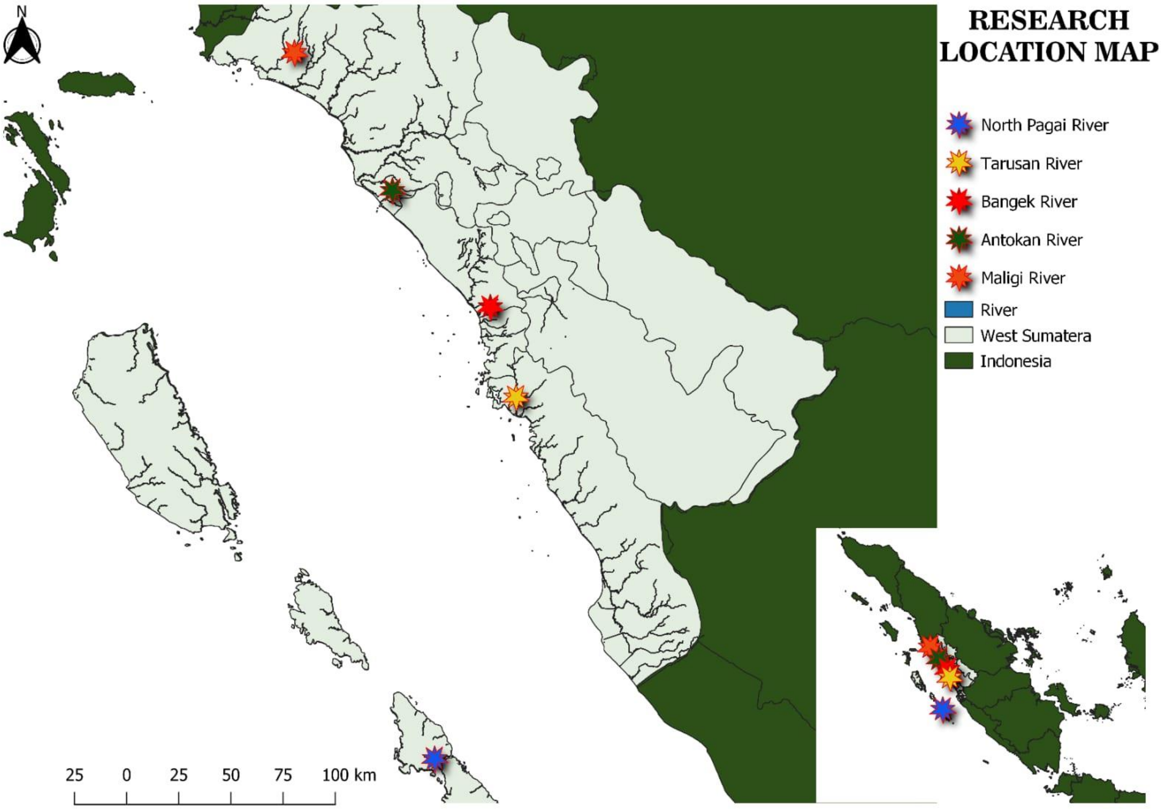

Figure 1. Depicts the location of *Anguilla* spp. sampling in several rivers on West Sumatra's west coast.

DNA Extraction and RAPD-PCR Test

An Invitrogen DNA isolation kit was used to isolate genomic DNA from liver tissue of *Anguilla* spp. individuals in each population. Primer selection was done by amplifying the sample DNA isolates using several primers to obtain suitable primer types having high polymorphism. The primer tested is a primer used in previous research by Yoon (2015) using BION primer on *Anguilla* fish. The primers used were BION-30 (5' '-GCCACCTCCT-3'), BION-35 (5'-AGCGGCTAGG-3'), and BION-40 (5'-GAAACGGGTG-3'). DNA amplification was carried out regarding the study of Jia and Li (1999). The success of primer amplification was tested by electrophoresis on agarose gel at 2% (w/v), then visualized using UV light and photographed with a digital camera.

RESULTS AND DISCUSSION

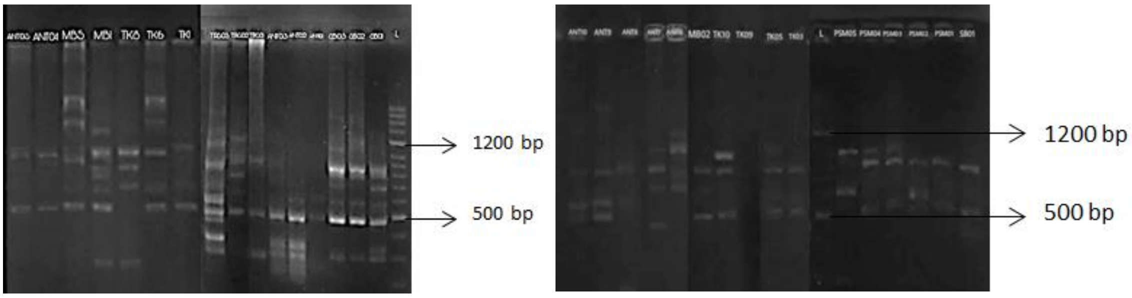

Figure 2. DNA bands amplified using primers BION-40. A*) A. marmorata*, B) A. *bicolor bicolor*

PCR amplification obtained a total of 47 bands, consisting of one monomorphic band and 46 polymorphic bands for *A. marmorata* samples, with an average of 15.67 bands three primer. Meanwhile the DNA amplification profile of *A. bicolor bicolor* results in, a total of 41 bands, consisting of two monomorphic bands and 39 polymorphic bands, with an average of 13.67 bands per primer. Based on the amplification results, the highest percentage of polymorphic bands of *A. marmorata* was 100% and the lowest was 92%, with an average of 97.33% for the four populations. The highest percentage of polymorphic bands of *A. bicolor bicolor* was 100% and the lowest was 91.6%, with an average of 95.2% in the three populations (Table 2). This shows overall that *Anguilla* spp. has high polymorphism (<50% polymorphic band), and there is genetic variation in the species, but it does not necessarily show high genetic variation. Roesma et al. (2017) stated that the number of polymorphic bands can provide an overview of genetic variation within a species.

In Table 3, the population of *A. marmorata* in the Bangek River has the lowest heterozygosity value of 0.0925 and in the Tarusan River has the highest heterozygosity value of 0,2018. From the four locations, it can be seen that the genetic variation of *A. marmorata* is low, and it is suspected that *A. marmorata* comes from the same spawning ground.

The population of *A. bicolor bicolor* in Mentawai had the highest heterozygosity value of 0.2340 (Table 3). The high heterozygosity value in the Mentawai population is thought to be influenced by the relatively high allele variation and that the Mentawai population comes from two locations, namely the mouth of the Taikako River and the mouth of the Maibola River. The results of the research by Syaifullah et al. (2019) showed that the value of intra-population genetic variation of *A. bicolor bicolor* was highest in the glass eel population of the Taikako river estuary. *A. bicolor bicolor* in the Maligi River population had the lowest genetic variation with a heterozygosity value of 0.1638.

Table 1. Types of *Anguilla* spp. found in several rivers on the West Coast of West Sumatra

No	Sampling Location	*A. marmorata*	Long size (cm)	*A. bicolor bicolor*	Long size (cm)	Collection time
1	Bangek River	3	60, 41, 42	-	-	February 2021
2	Tarusan River	3	48, 34, 30	-	-	June 2021
3	Maligi River	-	-	5	52, 39, 60, 38, 39.3	March 2021
4	Sungai Antokan	5	38, 42, 41.2, 58.3, 56	5	44, 44, 38, 55, 50	March 2021 January 2022
5	Mentawai (Syaifullah et al., 2017)	5	Glass eel	5	Glass eel	September 2016
	Total	16		15		

Table 2. Percentage of primary polymorphic RAPD markers produced in *Anguilla* spp. samples

No	Primer	Total DNA Bands	Number of Monomorphic Bands	Number of Polymorphic Bands	Polymorphic Band Percentage (%)
A. marmorata					
1.	BION-40	19	0	19	100
2	BION-35	15	0	15	100
3.	BION-30	13	1	12	92
	Average	15.67	0.33	15.33	97.33
A. bicolor bicolor					
1.	BION-40	17	1	16	94
2.	BION-35	12	0	12	100
3.	BION-30	12	1	11	91.6
	Average	13.67	0.67	13	95.2

Table 3. Values of intrapopulation genetic variation of *A. marmorata* and *A. bicolor bicolor*

Species	Location	Number of Samples	H	I	N	PP%
A. marmorata	SB	3	0.0925	0.1391	12	25.53
	ANT	5	0.1621	0.2474	23	48.94
	TRS	3	0.2018	0.3028	26	55.32
	MTW	5	0.2004	0.2986	26	55.32
A. bicolor bicolor	PSM	5	0.1638	0.2430	18	43.90
	MTW	5	0.2340	0.3341	25	60.98
	ANT	5	0.2142	0.3244	26	63.41

Remarks: H: Heterozygosity value, I: Shannon diversity index, N: Number of polymorphic loci, PP: Percentage of polymorphic loci. SB: Bangek River, ANT: Antokan River, TRS: Tarusan River, PSM: Maligi River, MTW: Mentawai.

The H_T value (0.2724) of *A. marmorata* from all locations was higher than the H_S value (0.1642). The D_{ST} value (0.1081) was lower than the H_S value (0.1642) (Table 4). In the population of *A. bicolor bicolor* in three locations, the H_T value (0.2664) was higher than the H_S value (0.2040). The D_{ST} value (0.0625) is lower than the H_S value (0.2040). The higher H_S value than the D_{ST} value indicates that the genetic variation between populations is lower than the genetic variation within the population. Roesma et al. (2017) stated that the D_{ST} value (0.088) of *Tor douronensis* is lower than the H_S value (0.1184), which indicates that the genetic variation between populations of *T. douronensis* is lower than the genetic variation within the population. This value is supported by the G_{ST} value, which shows that 42.66% of the total genetic variation is among populations and 57.34% is within the population.

The low G_{ST} value (0.3971) in the population of *A. marmorata* was thought to be because *A. marmorata* came from the same spawning ground location. The low G_{ST} value is supported by a high gene flow value of 0.7592. The high value of gene flow (Nm) can be assumed to be caused by random mating between populations from several locations, which causes similar genetic structures and small genetic differences. According to Islam et al. (2011), increased gene flow results in high genetic similarity in a population, so the level of genetic difference is low. As supported by the statement of Culley et al. (2012), high Nm values and low G_{ST} values cause low values of genetic variation between populations.

Table 4. Value of interpopulation genetic variation of *Anguilla* spp. in several rivers on the West Coast of West Sumatra

Species	Number of Samples	H_T	H_S	D_{ST}	G_{ST}	Nm
A. marmorata	16	0,2724	0,1642	0,1081	0,3971	0,7592
A. bicolor bicolor	15	0,2665	0,2040	0,0625	0,2345	1,6318

Remarks: H_T: Total heterozygosity, H_S: Sub-population heterozygosity, D_{ST}: Inter-population heterozygosity values, G_{ST}: Genetics of Differentiation, Nm: gene flow.

The farthest genetic distance between the Bangek River and Mentawai populations in *A. marmorata* is 0.2657 (Table 5). The closest genetic distance between the populations of the Tarusan River and the Antokan River is 0.0353 which indicates that individuals in the populations of these two rivers have close kinship relationships compared to other populations. This can be seen from the dendogram *of A. marmorata* (Figure 3), in which the Antokan River and the Tarusan populations are River are in the same cluster.

Table 5. Matrix of genetic distance of *A. marmorata* in several rivers in the West Coast of West Sumatra

No	Population	1	2	3	4
1.	Bangek River	-			
2.	Antokan River	0,1885	-		
3.	Tarusan River	0,1583	0,0353	-	
4.	Mentawai	0,2657	0,1552	0,1508	-

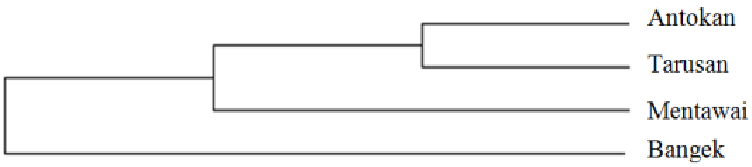

Figure 3. Dendrogram of *A. marmorata* population in several rivers in the West Coast of West Sumatra

Dendogram *A. bicolor bicolor* shows that there are two population groups, the first group consists of the Mentawai and Antokan River populations, meanwhile the Maligi river population forms different group. (Figure 4). The first grouping resulted in a small genetic distance, as shown in the matrix table (Table 6), the smallest genetic distance between the Mentawai population and the Antokan River with a value of 0.0612 indicates that the two populations have genetic similarities. The small genetic distance can be linked to the catadromous life cycle of *Anguilla* species, which spends its larval and adult stages in freshwater on land before migrating to the sea to reproduce. This is probably because the populations of the Mentawai and Antokan Rivers have the same migration period. In addition, the close genetic distance between two populations from far geographical distances can be due to larval transport influenced by ocean currents. According to Arai (2020) this form of larval transport results in large-scale *Anguilla* dispersal, which is influenced by shifts in ocean currents and may result in larval migration outside their typical species range.

Table 6. Matrix of genetic distance of *A. bicolor bicolor* in several rivers in the West Coast of West Sumatra

No	Populasi	1	2	3
1.	Maligi River	-		
2.	Mentawai	0,0776	-	
3	Antokan River	0,1486	0.0612	-

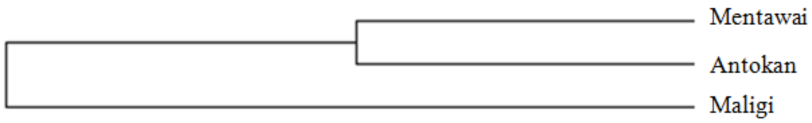

Figure 4. Dendrogram of *A. bicolor bicolor* population in several rivers in the West Coast of West Sumatra

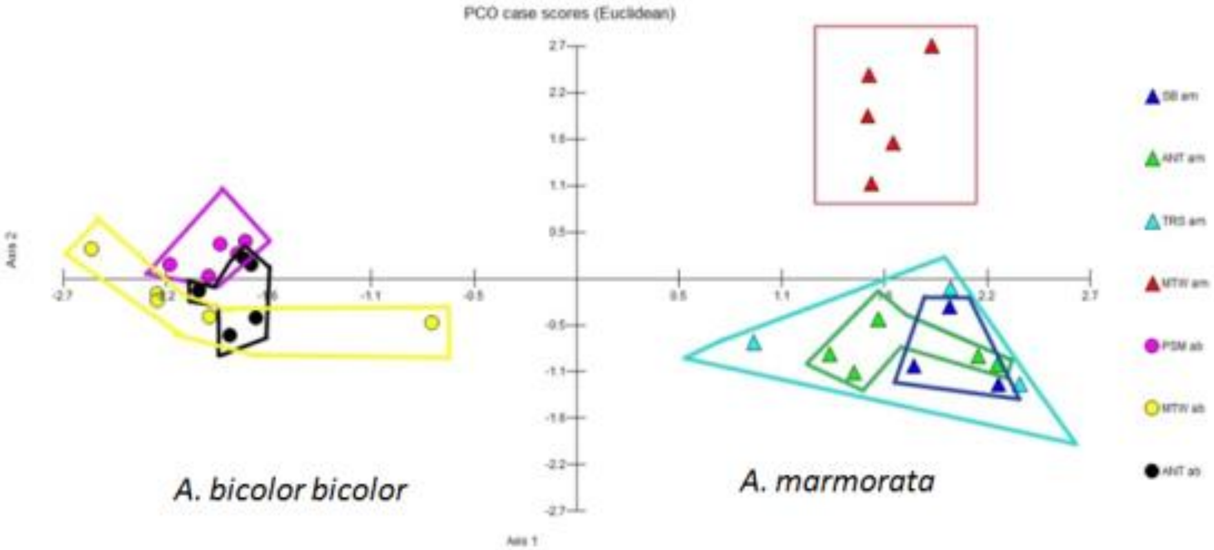

Figure 5. Grouping pattern of *Anguilla* spp. on the West Coast of West Sumatra based on PCoA.

The results of the Principal Coordinates Analysis (PCoA) test on *Anguilla* spp. populations in several rivers on the West Coast of West Sumatra based on the quantification of the amplification results can be seen in Figure 5. Separate *Anguilla* groupings based on species is found for *A. marmorata* (on the right) and *A. bicolor bicolor* (on the left). The PCoA result show the clustered pattern which tends to overlap. This grouping indicates that there is no relationship between geographic distances of the populations and its clustering. Roesma et al. (2017) stated that in *T. douronensis* the high Nm and low G_{ST} values are in line with the overlapping PCoA display which shows that there is no relationship between geographic distance and grouping of populations. The PCoA grouping pattern showed conformity with the heterozygosity value (Table 3). The population of *A. marmorata* has a higher heterozygosity value than the population of *A. bicolor bicolor*, so the grouping pattern has a wider distribution. Individuals of *A. marmorata* from the Tarusan River population, and *A. bicolor bicolor* individuals from the Mentawai population have the widest distribution pattern. From the research that has been done, it was found that the genetic variation of *A. marmorata* and *A. bicolor* at several locations in West Sumatra was low. These results can be used as a source of information and a reference for the scientific community regarding the cultivation potential of *Anguilla* spp. in West Sumatra.

CONCLUSIONS

The genetic variation of *A. marmorata* and *A. bicolor bicolor* populations in the West Coast of West Sumatra (Bangek River, Antokan River, Maligi River, Tarusan River and Mentawai) has low genetic variation based on the value of heterozygosity, genetic differentiation and gene flow.

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DAFTAR PUSTAKA

Allendorf, F. W., & Luikart, G. H. 2007. *Conservation And The Genetics of Populations*. Blackwell Publishing. USA. 51-55.

Anggereini, E. 2008. Random Amplified Polymorphic DNA (RAPD), Suatu Metode Analisis DNA Dalam Menjelaskan Berbagai Fenomena Biologi. *Biospecies*. 1(2): 73-76.

Arai, T. 2020. Ecology and evolution of migration in the freshwater eels of the genus *Anguilla* Schrank, 1798. *Heliyon*. 11(803) : 1-11.

Cresci, A., Durif, C. M., Paris, C. B., Shema, S. D., Skiftesvik, A. B.., & Browman, H. I. 2020. A comprehensive hypothesis on the migration of European glass eels (*Anguilla anguilla*). *Biol Rev Camb Philos Soc*. 2(366): 1-9.

Culley, T.M., L.E. Wallace, K.M. Gengler-Nowak dan D.J. Crawford. 2002. A Comparison of Two Methods of Calculating Gst, A Genetic Measure of Population Differentiation. *American Journal of Botany*. 89(3):460-465.

Fahmi, M. R. 2015. Short Communication: Conservation Genetic of Tropical Eel in Indonesian Waters Based on Population Genetic Study. *Pros Sem Nas Masy Biodiv Indon*. 1(1): 38-43.

Huyen, K. T., Hue, H. T., & Linh, N.Q., 2021. Genetic Diversity Of Giant Mottled Eel *(Anguilla Marmorata* Quoy & Gaimard, 1824) By RAPD In Thua Thien Hue Province, Vietnam. *Hue University Journal of Science: Techniques and Technology*. 130(2B): 29–40.

Islam, M.N., A. Basak, Ashrafullah., M.S. Alam. 2011. Genetic Diversity in Wild and Hatchery Populations of Stinging Catfish (Heteropneustes fossilis Blonch) Revealed by RAPD Analysis. *J.bio-sci*. 19:81-87.

Khaifu K., Yakouchi, K., Miller. M. J., & Washitani, I. 2020. Management of glass eel fisheries is not a sufficient muasure to recover a local Japanese eel population. Marine Police. 134; 1-11.

Kumari, N., & Takhur, S. K. 2014. Randomly Amplified Polymorphic DNA- A.Brief Review. *American Journal of Animal and Veterinary Sciences*. 9(1): 6-13.

Martinez, A. S., Willoughby, J.R., & Christine, M. R. 2018. Genetic Diversity in Fishes is Influenced by Habitat type and life-history variation. *Ecology and Evolution*. 8: 12022-12031.

Pike, C., Crook V., Gollock M. 2020. *Anguilla Anguilla*. The IUCN Red List of Threatened Species.

Roesma, D. I., Tjong, D. H., Munir, W., Agesi, A. V. & Chornelia, A. 2017. Genetic diversity of *Tor douronensis* (Pisces: Cyprinidae) in West Sumatra, Indonesia. *Biodiversitas*. 18(3): 1018-1025.

Setiati, N., Partaya, & Hidayah, N. 2020. The use of two pairs primer for CO1 gene amplification on traded stingray at fish auction Tasik Agung Rembang. Journal of Physics: *Conference Series*. 1567: -5.

Syaifullah, S., Luqyana, S. Tjong, D. H., Zakaria, I. J. & Roesma, D. I. 2022. Haplotype network of Three species of *Anguilla* (freshwater eels) in West Sumatra, Indonesia , based on cytochrome. *AACL Bioflux*. 2(15): 774-787.

Syaifullah, S., Sari, N. P. M., Tjong, D. H. & Muchlisin, Z. A. 2019. Species Composition of Eels Larvae (Anguillidae) in Mentawai Island waters, Indonesia based genetic data. *IOP Conf. Ser.: Earth Environ. Sci.* 348: 1-6.

Yeh, F. C., Yang, R.C.B.J., Boyle, Z. H. & Mao, J. X. Y. 1999. POPGENE 1.31. The User Friendly Shareware for populations Genetics Analysis. *Molecular Biology and Biotechnology Centre*.University of Alberta. Canada. 1-8.

Yoon, J. M. 2015. Differences and Variations among *Anguilla* japonica, Muraenesox cinereus and Conger myriaster from the Yellow Sea, Dev. *Reprod*. 19(3): 163– 166.