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Genetic variation of giant freshwater prawns *Macrobrachium rosenbergii* wild population of South Sulawesi, Indonesia

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Abstract. *Wahidah, Omar SBA, Trijuno DD, Nugroho E, Amrullah, Khatimah K. 2023. Genetic variation of giant freshwater prawns* Macrobrachium rosenbergii *wild population of South Sulawesi, Indonesia. Biodiversitas 24: 3081-3090.* Quality giant freshwater prawn broodstock can be provided using broodstock sources from wild populations with good morphological and genetic performance. This study aimed to analyze the genotypes of the wild population of giant freshwater prawns in South Sulawesi, hence it can be used as basic information for quality broodstock. Evaluation of giant prawn haplotypes, *Macrobrachium rosenbergii*, from 3 rivers in South Sulawesi: 26 samples from the Waelawi River (11 males, 15 females); 25 samples from the Kalibone River (10 males, 15 females); and 26 samples from the Kariango River (12 males, 14 females), so there are 6 population groups. Analysis used mt-DNA and obtained an amplification of 700 bp. The amplified restriction uses four enzymes (Hae III, Mbo I, Rsa I, and Taq I). The identified male and female Kariango populations each had 3 haplotypes: female Kalibone had 2 haplotypes; male and female Waelawi had 1 haplotype; and male Kalibone had 1 haplotype. Female Kariango had the highest haplotype diversity (0.3651), followed by male Kariango (0.1449) and female Kalibone (0.1011). Kariango females have the farthest genetic distance from Kalibone males, Waelawi males, and Waelawi females. The male Waelawi, female Waelawi, and male Kalibone are in one cluster and form a new cluster with the male Kariango, then with the female Kalibone, and the last cluster is formed with the female Kariango. The Kariango female population can be used as potential broodstock, which can be mated with Kalibone males, Waelawi males, or other populations.

Keywords: Breeding program, enzyme restriction, genetic distance, haplotype diversity, mt-DNA RFLP

INTRODUCTION

Freshwater prawns of the *Macrobrachium* genus consist of 200 species worldwide (Liu et al. 2007; Chen et al. 2009). Several species of *Macrobrachium* have been documented their distribution in Indonesia, including *M. scabriculum* Heller, 1862 (Dwiyanto et al. 2017), *M. horstii* (Eprilurahman and Nabil 2018), *M. esculentum* (Goud et al. 2020), *M. idae* (Wahidah et al. 2019) and *Macrobrachium lar*, *M. idae*, *M. lanchesteri*, *M. rosenbergii*, *M. pilimanus*, and *M. javanicum* (Wowor and Choy 2001; Indarjo et al. 2021). Among these *Macrobrachium*, *M. rosenbergii* is a mainly cultivated species. The farmed production of *M. rosenbergii* contributed 51.7% of the global total (New and Neir 2012).

The global production of giant freshwater prawns has not increased significantly (FAO 2020). Compared to Bangladesh, Thailand, and China, the development of giant freshwater prawns cultivation in Indonesia is relatively low (Farook et al. 2019). Although shrimp production has decreased in Indonesia, on the other hand, national shrimp demand has increased. Based on national shrimp production data, there has been an increase in demand for shrimp, including giant prawns. This can be seen from the increase in demand for shrimp from 3.92 million kg in 2020 to 5.33 million kg in 2021 (Badan Pusat Statistik 2022), with an increase in export volume of 36.13% (databoks.katadata.co.id). This data includes data on giant prawn production in 2020 of 456 thousand kg (KKP 2022).

Giant prawn production in Indonesia is supplied by seeds from hatcheries. While the seeds in the hatchery are produced by giant prawns from nature. The quality of natural broodstock will affect the quality of the seeds produced, which will have an impact on the output of cultivated seeds, both in terms of quality and quantity. Therefore, to produce quality seeds, quality broodstocks are also needed, including those with high genetic variation and fast growth.

The activities of giant freshwater prawns production are directly tied to genetic quality and broodstock management in the hatchery. Proper genetic improvement and brood control result in high-quality seeds. These two components are important to boost the yield of aquaculture production. In addition, using broodstocks with high genetic variation can increase genetic variation. Broodstock control can be accomplished by hybridizing between geographically distinct wild populations with substantial genetic variation and long genetic distances. Hybridization is a successful method for improving genetic quality since it employs simple processes, is minimal in cost, requires few facilities, and requires few human resources (Goyard et al. 2008).

Furthermore, it is stated that the availability of genetically different populations, either due to geographic isolation or domesticated, cultivated populations, is a prerequisite for hybridization.

The morphology and genetic variety of the broodstocks to be utilized for hybridization can be used to assess their quality. Therefore, morphological and genetic performance are important based on phenotype and genotype evaluation. Phenotype measures are associated with morphometric characteristics. Morphometric evaluation of *Macrobrachium* has been carried out (Eniade et al. 2019; Jimoh and Lawal 2020; Khanarnpai et al. 2019; Konan et al. 2017; Pillai et al. 2017; Tizkar et al. 2017), and in Indonesia, it has been carried out by several authors (Eprilurahman and Nabil 2018; Jurniati et al. 2021; Rimalia et al. 2015; Rimalia et al. 2018; Suwartiningsih et al. 2018; Suwartiningsih and Utami 2020; Wahidah et al. 2017; Wahidah and Yusuf 2017; Wahidah et al. 2018). While genotype is associated with a genetic variation assessed using molecular markers, one such method is mitochondrial DNA (mt-DNA) genetic markers. The mt-DNA control region is the mt-DNA section, which consists of a conservative center area with relatively distinct left and right domains and engages 3-5 times faster than other mitochondrial genome segments (Li et al. 2022). Furthermore, mt-DNA mutation types are relatively simple, consisting of base substitutions and long modifications that occur primarily in small non-coding regions; thus, mt-DNA polymorphisms are neutral genetic markers.

The quantity of gene variety can be used to assess genetic quality since considerable genetic variation in a population indicates many seeds with diverse characteristic variants. For example, the production of giant freshwater prawns is affected by genetic variation. Therefore, the quality of the seeds produced by mating giant freshwater prawns with limited genetic variety will be reduced. Hybridization between populations/strains can increase genetic variation in aquaculture activities to avoid inbreeding. Inbreeding pressure can reduce genetic variation, as seen by traits associated with average phenotypic value, such as reproductive capacity (fecundity, egg size, hatchability) or physiological efficiency (seed deformity, growth rate, survival) (Gjerde 2005). Using new broodstocks from nature with high genetic variation is advocated for genetic improvement (Binur and Pancoro 2017). Genetic studies comparing hatchery populations and wild populations have been conducted in Bangladesh (Bala et al. 2017), China (Sui et al. 2019), Myanmar (Yu et al. 2019), Taiwan and the Ryukyu Islands (Han et al. 2022), Cameroon (Makombu et al. 2019), and Indochinese countries (Siriwut et al. 2021).

Therefore, to increase the quality of the seeds produced, the genetic variety of giant freshwater prawns in Indonesia must be evaluated to find new genetic sources that can be used as a genetic source for prospective broodstock in hatcheries. Several Sulawesi rivers have been identified as having wild populations of giant freshwater prawns that could be developed as potential broodstock. Our previous research (Wahidah et al. 2017; Wahidah and Yusuf 2017; Wahidah et al. 2018) and research by Jurniati et al. 2021 analyzed information on the morphology of gigantic prawns in Sulawesi. However, data on the genetic variation of South Sulawesi giant freshwater prawns are still scarce.

The lack of knowledge on the genetic variety of the giant freshwater prawns to be used is the challenge with employing wild populations to supply quality wild broodstock. Therefore, it can be undertaken to assess genetic variance, starting with DNA analysis. Several methods for analyzing DNA are available, including Random Amplified Polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellite DNA, DNA fingerprinting, and mt-DNA. Alam et al. (2017), Chen et al. (2015, 2017), Guerra et al. (2014), and Khanarnpai et al. (2019) have used mt-DNA on *Macrobrachium*. The COI mt-DNA fragments and 16SrRNA D-loop mt-DNA sequences are widely used in population genetic analysis because they do not encode proteins and are unaffected by the selection (Liao et al. 2016; Maltsev et al. 2015). Numerous variable sites and observable haplotypes suggest that the D-loop can be used as a molecular marker to detect genetic variations in *M. nipponense* populations (Chen et al. 2015; Chen et al. 2017). Therefore, this study aims to examine the genotypes of wild populations of giant freshwater prawns in South Sulawesi to provide basic information on the good quality broodstock demands.

MATERIALS AND METHODS

Sampling site

This research was conducted in South Sulawesi Province, Indonesia on the Waelawi River 120º 19' 59.961" E; 2º52'39.7" S (Balease watershed in North Luwu), Kariango River 119º37'21.061" E; 3º52'57.268" S (Sawitto-Kariango-Rappang watershed in Pinrang) and the Kalibone River 119º35'7.014" E; 4º54'9.03" S (Sangkara watershed in Pangkep) (Figure 1).

Procedure sampling

The giant freshwater prawn samples consisted of 26 samples of the Waelawi River (11 males and 15 females), 25 samples of the Kalibone River (10 males and 15 females), and 26 samples of the Kariango River (12 males and 14 females). Catching giant freshwater prawns was conducted based on fishermen's typical fishing techniques for each location. Sample analysis was conducted from August 2014 to February 2015.

Catching giant freshwater prawns on the Waelawi River uses a *kopa* (local name), or prawn trap made of bamboo; on the Kariango River, a *bubu* (local name), or prawn trap made of bamboo; and on the Kalibone River, a casting net, called *jala* by the local peoples. The sample was obtained using the simple random sampling method, where each member of the population has the same probability of being selected (Laewa et al. 2018).

The genotype analysis was conducted at the Integrated Biotechnology Laboratory, Faculty of Animal Husbandry, Universitas Hasanuddin (Unhas), Makassar, Indonesia. In addition, the DNA quality analysis was conducted at the Nechri Laboratory, Unhas Teaching Hospital.

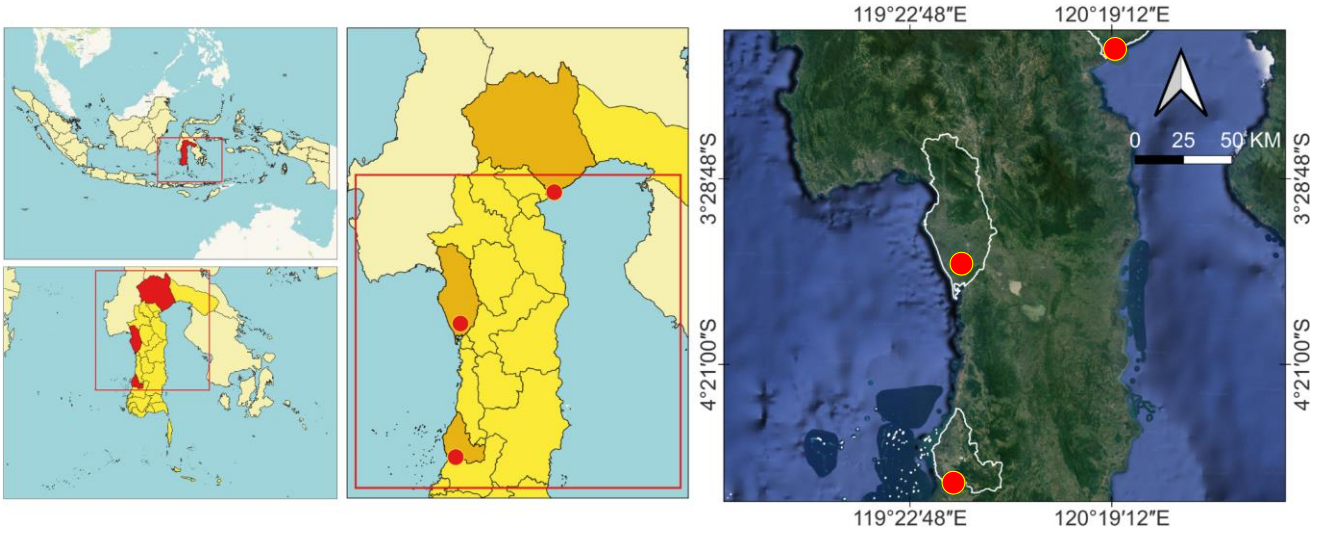

Figure 1. Sampling locations in South Sulawesi Province, Indonesia on the Waelawi River, Kariango River, and Kalibone River

DNA sample

The mitochondrial DNA technique can be applied immediately using fresh samples frozen or stored in alcohol. Samples were taken from as much as 1 gram of the swimming leg (pleopod) of giant prawns and then preserved or soaked in 70% alcohol. Sample handling technique refers to Nugroho (2002).

DNA extraction

The extraction and purification of the genomic DNA method are based on the Thermo Scientific GeneJET Genomic DNA Purification Kit. First, DNA extraction was carried out by weighing the sample, which is 15-20 mg each. Next, the sample was cleaned of fixative solution (alcohol) by adding 1,000 µL of distilled water (DW), vortexed, and allowed to stand for about 5 minutes. Next, the sample was centrifuged at 8,000 rpm for 5 minutes, and the DW was removed using a micropipette. Subsequent extraction applications followed the Thermo Scientific procedure GeneJET Genomic DNA Purification Kit.

DNA purification

The purity of the DNA products was measured by Pro Spectrophotometer Genesys 10S UV/Vis Reader.

Amplification

Mitochondrial sequence amplification used forward primer (LCO1490), namely 5' GGTCAACAAATCATAA AGATATTGG-3' and reverse. (HC02198) was 5'-TAAAC TTCAGGGTGACCAAAAAATCA-3'. Amplification was carried out using the PCR method with the composition of materials: 0.15 µL primer forward, 0.15 µL reverse, 2 µL DNA; that was mixed in 0.3 µL dNTP, 1 l MgCl, 2.5 µL 10X Buffer, 0.1 µL Taq and 18.8 µL water. The samples were put into the PCR machine Sensoquest, Labcycler 48 - Gradient Thermocycler (PCR) type with a cycle: one initial denaturation cycle at 94ºC for 3 minutes, 36 cycles consisting of denaturation of 94ºC for 45 seconds, annealing 50ºC for 45 seconds, and elongation 72ºC for 1 minute. The final elongation of 1 cycle was 72ºC for 30 minutes. Furthermore, the PCR products were checked on 2% agarose gel that was electrophoresed and then observed on a UV illuminator.

PCR restriction

The mt-DNA restriction was carried out by mixing 1 µL of restriction enzyme, 1 µL of Enzyme buffer, 3 µL of DNA products, and 5 µL of water in an Eppendorf tube. The mixture was incubated at 37ºC for 24 hours. The enzymes used were Hae III, Rsa I, Mbo I, and Taq I,

The restriction products were separated by 2% agarose gel electrophoresis, with the wells filled with a mixture of 10 µL (1 µL of restriction enzyme + 1 µL of Enzyme buffer + 3 µL of DNA products + 5 µL of water) of restricted mt-DNA with 2 µL of loading buffer added. Each electrophoresis process used 4 µL marker 100 bp that can detect base pairs from 100 to 10,002 bp and contains 20 DNA fragments. The results of the mt-DNA restriction electrophoresis were observed on an ultraviolet illuminator and documented.

The presence of genomic DNA is determined by electrophoresis. First, 3 µL of extracted DNA was added with 1 µL of loading buffer on 1% agarose gel inserted into the electrophoresis well. Furthermore, the electrophoresis bath was supplied with electricity with a programmed voltage and current that was programmed automatically. The electrophoresis process was stopped after the DNA migrating from the negative pole to the positive pole reached three-quarters of the gel length. The presence of DNA in the gel can be observed using an ultraviolet illuminator.

Next, 1% agarose gel was prepared by mixing 0.5 g agarose with 50 ml tris borate EDTA (TBE 1%) solution. The solution was heated until clear, poured into a mold, and a comb was installed to form a well. Furthermore, the frozen gel can be directly used for electrophoresis or stored/stocked by immersion in 1% TBE solution.

Data analysis

The purity of DNA samples was analyzed to determine DNA quality by calculating the ratio of DNA260/DNA280. Analysis of the genetic variation of giant freshwater prawns from six different populations was carried out by calculating the haplotype diversity (h) in a population based on the equation[2] Nei and Tajima (1981), namely:

$$h = 2n (1 - \sum xi^2) / (2n - 1)$$

Where:
h : haplotype diversity
n : sample size
xi : sample haplotype frequency

The haplotype arrangement of each restriction enzyme collected was analyzed according to the method of Raymond and Rousset (1995) using the TFPGA (Tool for Population Genetic Analysis) program version 1.3 (Miller 1997). Relationships between populations were analyzed using genetic distance, based on Rogers's (1972) modified UPGMA (Unweighted Pair Group Method with Arithmetic) program from TFPGA software. The resulting data was in phylogenetic tree construction and presented as a dendrogram.

RESULTS AND DISCUSSION

Amplification

The results of giant freshwater prawn amplification using primers forward (LCO 1490) and reverse (HC0 2198) obtained a band with a length of about 700 bp (Figure 2) with DNA purity 1.7702 - 2.1883.

Enzyme restriction sites and types

The restriction of amplification products with the enzyme Hae III, Mbo I, Taq I, Rsa I, Hind III, Hinf I, Nde I, Alu I, and Msp I, were observed. It showed four enzymes with restriction sites: Hae III, Mbo I, Taq I, and Rsa I (Table 1, 2, 3, and Figure 3). Alignment of the restriction results (Table 4) showed variations in the restriction sites and the length of the fragments. Different fragment sizes gave different cuts (haplotypes) (Table 5).

Genetic type

The genetic distance calculation (Table 6) showed three populations with the closest genetic distance: Male Waelawi and Male Kalibone populations, Male Waelawi with Female Waelawi, and Male Kalibone and Female Waelawi. Meanwhile, the farthest genetic distance was found in the Female Kariango population with Male Waelawi, Female Kariango with Male Kalibone, and Female Kariango with Female Waelawi. The data were mapped in a genetic distance dendrogram using the UPGMA program, as shown in Figure 4.

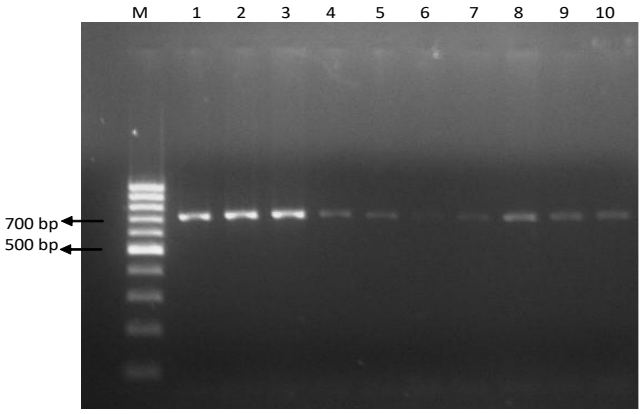

Figure 2. The results of DNA amplification of giant freshwater prawns *Macrobrachium rosenbergii* population in the Waelawi River. M = Markers; 1,2,3,4,5,6,7,8,9,10 = sample of giant freshwater prawns from the Waelawi population. *The marker used is a 100-bp marker that can detect base pairs from 100 to 10,002 bp and contains 20 DNA fragments (Sambrook and Russel 1989)

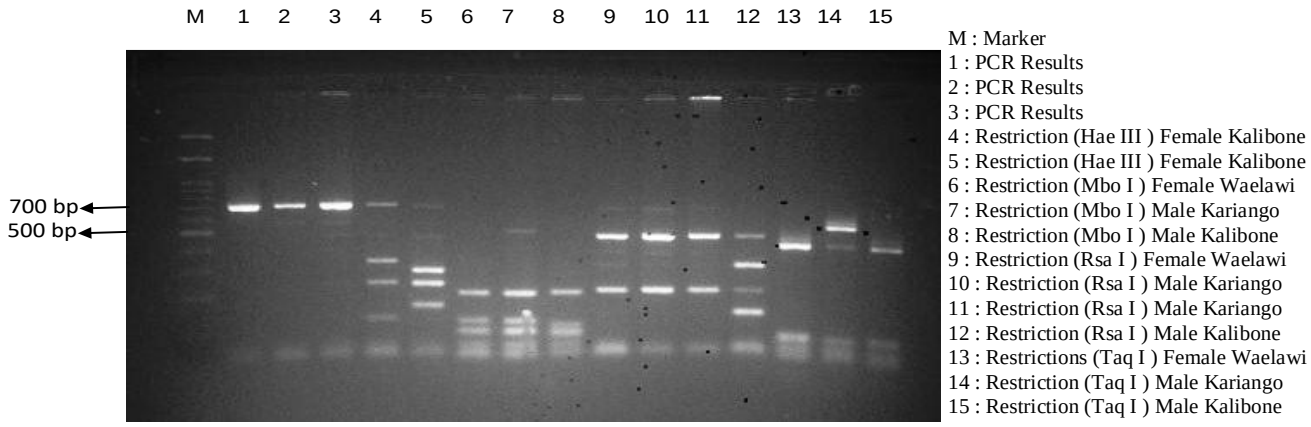

Figure 3. Results of DNA amplification and restriction enzymes in giant freshwater prawns *Macrobrachium rosenbergii* population of Waelawi River, Kariango River, and Kalibone River

Table 1. Site and type of restriction of Hae III, Mbo I, Rsa I, and Taq I enzymes in male and female *Macrobrachium rosenbergii* prawns from the Waelawi population

Waelawi population	(Enzyme) haplotype	The length of the fragment (bp)								Total (bp)
		50	75	125	150	200	250	475	500	
	(*Hae* III)									
Male	A				--				--	650
Female	A				--				--	650
	(*Mbo* I)									
Male	A	--	--	--	--		--			650
Female	A	--	--	--	--		--			650
	(*Rsa* I)									
Male	A					--			--	700
Female	A					--			--	700
	(*Taq* I)									
Male	A	--		--				--		675
Female	A	--		--				--		675

Note: -- The location of the site in the restricted fragment

Table 2. Site and type of restriction of Hae III, Mbo I, Rsa I, and Taq I enzymes in male and female *Macrobrachium rosenbergii* prawns from the Kariango population

Kariango population	(Enzyme) haplotype	The length of the fragment (bp)													Total (bp)
		50	75	100	125	150	225	250	325	350	375	400	475	600	
	(*Hae* III)														
Male	A			--										--	700
Female	A			--										--	700
	(*Mbo* I)														
Male	A	--	--		--	--		--							650
Male	B	--			--	--					--				700
Female	A	--	--		--	--		--							650
Female	B	--			--	--					--				700
	(*Rsa* I)														
Male	A						--						--		700
Male	B						--					--			625
Female	A						--						--		700
Female	B								--	--					675
	(*Taq* I)														
Male	A		--		--								--		675
Female	A			--										--	700
Female	B		--		--								--		675

Note: -- The location of the site in the restricted fragment

Table 3. Site and type of restriction of Enzyme Hae III, Mbo I, Rsa I, and Taq I in male and female *Macrobrachium rosenbergii* prawns from the Kalibone population

Kalibone population	(Enzyme) haplotype	The length of the fragment (bp)											Total (bp)
		50	75	100	125	150	175	250	275	325	375	475	
	(*Hae* III)												
Male	A				--			--		--			700
Female	A				--			--		--			700
Female	B						--	--	--				700
	(*Mbo* I)												
Male	A	--	--		--	--		--					650
Female	A	--	--		--	--		--					650
	(*Rsa* I)												
Male	A		--				--				--		625
Female	A		--				--				--		625
	(*Taq* I)												
Male	A			--	--							--	700
Female	A			--	--							--	700

Note: -- The location of the site in the restricted fragment

Table 4. Restriction type and mt-DNA fragment size of giant freshwater prawns *Macrobrachium rosenbergii* restricted by Hae III, Mbo I, Rsa I, Taq I enzymes

Population	Enzyme Type			
	(*) *Hae* III (**)	(*) *Mbo* I (**)	(*) *Rsa* I (**)	(*) *Taq* I (**)
Male Waelawi	A 150 + 500 (650)	A 50 + 75 + 125 +150 + 250 (650)	A 200 + 500 (700)	A 50 + 125 + 475 (675)
Male Kariango	A 100 + 600 (700)	A 50 + 75 + 125 + 150 + 250 (650) B 50 + 125 + 150 + 375 (700)	A 225 + 475 (700) B 225 + 400 (625)	A 75 + 125 + 475 (675)
Male Kalibone	A 125 + 250 + 325 (700)	A 50 + 75 + 125 + 150 +250 (650)	A 75 + 175 + 375 (625)	A 100 + 125 + 475 (700)
Female Waelawi	A 150 + 500 (650)	A 50 + 75 + 125 +150 + 250 (650)	A 200 + 500 (700)	A 50 + 125 + 475 (675)
Female Kariango	A 100 + 600 (700)	A 50 + 75 + 125 + 150 + 250 (650 B 50 + 125 + 150 + 375 (700)	A 225 + 475 (700) B 325 + 350 (675)	A 100 + 600 (700) B 75 + 125 + 475 (675)
Female Kalibone	A 125 + 250 + 325 (700) B 175 + 250 + 275 (700)	A 50 + 75 + 125 + 150 + 250 (650)	A 75 + 175 + 375 (625)	A 100 + 125 + 475 (700)

Note: * Restriction type, ** Total fragment size

Table 5. Genetic variation of six populations of giant freshwater prawns *Macrobrachium rosenbergii* populations of the Waelawi River, Kariango River, and Kalibone River based on the haplotype frequency of mt-DNA restricted by enzyme Hae III, Mbo I, Rsa I, and Taq I

Haplotype	Population					
	Male Waelawi	Male Kariango	Male Kalibone	Female Waelawi	Female Kariango	Female Kalibone
AAAA	1.000 (11)	0.667 (8)	1.000 (10)	1.000 (15)	0.571 (8)	0.733 (11)
AABA		0.167 (2)				
ABAA		0.167 (2)				
AAAB					0.071 (1)	
ABBB					0.357 (5)	
BAAA						0.267 (4)
Total haplotype (6)						
Total sample	11	12	10	15	14	15
Total alel	1	3	1	1	3	2
Haplotype diversity	0.0000	0.1449	0.0000	0.0000	0.3651	0.1011

Table 6. Rogers Genetic distance of six populations of giant freshwater prawns *Macrobrachium rosenbergi*i in the Waelawi River, Kariango River, and Kalibone River based on Rogers modification

Population	Genetic distance of population					
	Male Waelawi	Male Kariango	Male Kalibone	Female Waelawi	Female Kariango	Female Kalibone
Male Waelawi	-	-	-	-	-	-
Male Kariango	0.0122	-	-	-	-	-
Male Kalibone	0.0000	0.0122	-	-	-	-
Female Waelawi	0.0000	0.0122	0.0000	-	-	-
Female Kariango	0.1195	0.0786	0.1195	0.1195	-	-
Female Kalibone	0.0175	0.0363	0.0175	0.0175	0.1660	-

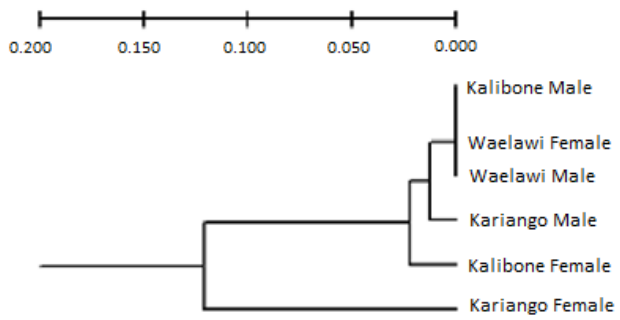

Figure 4. Genetic distance dendrogram of giant freshwater prawns *Macrobrachium rosenbergii* population in Waelawi River, Kariango River, and Kalibone River based on the UPGMA program

Discussion

Evaluation of genetic variation of giant freshwater prawns population in South Sulawesi based on mt-DNA RFLP method obtained haplotype diversity and genetic distance that showed genetic differences of giant freshwater prawns population based on their wild habitat. The highest haplotype diversity was in the Female Kariango population, with the farthest genetic distance between the other three populations (Male Kalibone, Male Waelawi, and Female Waelawi).

The results of the 700 bp giant freshwater prawn DNA amplification (Figure 2) are consistent with those obtained by Jimoh et al. (2013) using the same primer. In addition, other species had ranges that were similar to the results of the DNA amplification, such as 680 bp in *Macrobrachium amazonicum* and *Macrobrachium jelskii* (Guerra et al. 2014), 710 bp in *Macrobrachium lanchesteri* (Khanarnpai et al. 2019), and 650 bp in *Macrobrachium* and *Caridina* (Udayasuriyan et al. 2017).

The purity of the DNA solution was determined based on the A260/A280 ratio. The purity of DNA ranged from 1.7702 to 2.1883. It showed that the DNA used for further analysis had a good quality. Sambrook and Russel (1989) stated that the pure DNA required for molecular analysis was 1.8 - 2.0, indicated by the ratio A260/A280.

The restricted mt-DNA analysis with four enzymes (Hae III, Mbo I, Rsa I, and Taq I) on 77 giant freshwater prawns total samples (Table 5) from the six populations were obtained from six haplotype composites. The exact number of haplotype composites (six haplotype composites) was also obtained by Nugroho et al. (2005) on giant freshwater prawns originating from Musi (South Sumatra), Barito (Central of Kalimantan), and GI Macro that were restricted with four enzymes (Hae III, Mbo I, Rsa I, and MSp I). Khanaranpai et al. (2019) obtained nine composite haplotypes on *Macrobrachium lanchesteri* De Man 1911, in Northeastern Thailand using an enzyme (Dde I, Alu I, Hinf I, Bgl II, and Hae III).

The same number of haplotypes in the Musi and Barito populations is possible because these two populations are in the western region for the distribution of flora and fauna in Indonesia. As previously known, the giant freshwater prawn population in Indonesia is divided into three parts: western, central and eastern. The Wallacea line separates the two groups. As a result, the gene pool of gigantic prawns from the western region differs from that of giant freshwater prawns from the eastern zone (Hadie and Hadie 2012). This condition allows the population of the central region to be distinct from the populations of the western and eastern regions or the population of the central region to become a part of one of Indonesia's existing population dichotomies (joining the western region group or the eastern region group). Aliah et al. (2022) discovered different findings from a study that examined three wild populations, including the Peurlak River (Aceh), the Bengawan Solo River (Central Java), and the Tabuk River (South Kalimantan), as well as one hatchery population (SIRATU). These three wild populations are the populations of the western region. The Tabuk populations are a Central Indonesian region with five haplotypes. These haplotypes are smaller than in the Musi and Barito populations. Meanwhile, the population of Bengawan Solo and Peurlak, have 8 and 15 haplotypes, respectively. The Peurlak population has the highest number of haplotypes in Indonesia's westernmost region.

While the total number of haplotypes in the South Sulawesi population is six, consisting of Waelawi males, Kalibone males, and Waelawi females, each with 1 haplotype; Kalibone females have 2 haplotypes; and Kariango males and Kariango females each have 3 haplotypes. The lower number of haplotypes in the South Sulawesi population could be due to the smaller number of samples being evaluated compared to the Musi, Barito, Peurlak, Bengawan Solo, and Tabuk populations. Even though the number of haplotypes in the South Sulawesi population is lower, morphologically, the giant freshwater prawns from the six populations have large sizes (Wahidah et al. 2017) and large edible traits in the Kariango population (Wahidah and Yusuf 2017).

The haplotype composite obtained in the population of South Sulawesi giant prawns can be used to increase genetic variation in giant prawns, namely by mating giant prawns with the farthest genetic distance and with different haplotype variations (between populations of South Sulawesi with populations outside South Sulawesi), which can improve the quality of the offspring produced. This condition will support the breeding program's goal of producing superior seeds by increasing measurable superior traits.

All six groups had haplotype AAAA (Table 5), with frequencies ranging from 0.571 to 1.000. This wide range of haplotype types suggests it is concentrated (dominant) in the entire population. Male and female Waelawi populations and male Kalibone had haplotype frequencies of 1,000, indicating that all three populations have monomorphic restriction outcomes. Aside from the AAAA haplotype, other varieties were only detected in one population. AABA and ABAA haplotypes were found with a frequency of 0.167 in the Kariango male population. AAAB and ABBB haplotypes were detected in Kariango females at a frequency of 0.071 and 0.357, respectively. Meanwhile, the BAAA haplotype was found with a

frequency of 0.267 in Kalibone females. Apart from the AAAA haplotype, the distribution of haplotype types that are only concentrated/found based on population suggests that each population has its unique haplotype type.

The highest haplotype diversity was Female Kariango (0.3651), followed by Male Kariango (0.1449) and Female Kalibone (0.1011). The diversity of this haplotype was lower than *Macrobrachium rosenbergii* de Man (0.73-0.79) in Thailand (Karaket et al. 2011); *Macrobrachium amazonicum* (0.00-0.67) in Brazil (Vergamini et al. 2011); *Macrobrachium vollenhovenii* and *Macrobrachium macrobrachium* (0.994) in Southern Nigeria (Jimoh et al. 2013), *Macrobrachium olfersii* (0.05-0.10) (Rossi and Mantelatto 2013) and *Macrobrachium nipponense* (0.956) in East Asia (Chen et al. 2017). These low values of haplotype diversity in the six populations evaluated could be caused by inbreeding. Furthermore, intense inbreeding pressure can be caused by the very high utilization of giant freshwater prawns, so the opportunities for individuals with a role in reproductive activities decrease. In addition, the decrease in the number of individuals in a population causes individuals to have a slight chance of mating randomly. Another aspect that may influence the significance of haplotype diversity is the isolated or unrelated geographic position of the three giant freshwater prawn habitats, which prevents population mixing.

The number of discovered haplotypes and the frequency of each haplotype in a population strongly influence haplotype diversity. According to Nei (1978), the genotype diversity index ranges from 0.1 to 0.4 for low diversity, 0.5 to 0.7 for moderate diversity, and 0.8 to 1.0 for great diversity. Female Kariango prawns have close to 0.4 haplotype diversity, while the other populations are regarded as low. The haplotype diversity value can be attributed to a decrease in population owing to fishing. In addition, the competition for giant freshwater prawns is fierce in all three places.

Moreover, the closer the kinship or the smaller the genetic distance number, the more similar the haplotype of the giant freshwater prawn population. Table 6 shows six giant freshwater prawn populations' genetic distance values and matrices. Male Waelawi and male Kalibone have the closest genetic distances, as do male Kalibone and female Waelawi and male Waelawi and female Waelawi. Female Kariango and female Kalibone have the most significant genetic distance, with a value of 0.1660. Conversely, the Waelawi (male and female) group has the shortest genetic distance (Figure 4). This condition could be induced by the two populations in the same environment, as Alam et al. (2017) discovered in giant freshwater prawns from the same or adjacent rivers in Bangladesh. This condition is very likely to occur because gene flow is quite low. Furthermore, the close genetic distance between the Kalibone male and Waelawi populations is likely due to non-random inbreeding. According to Freeland (2005), genetic bottlenecks, genetic drift, natural selection, and reproduction (inbreeding or outbreeding) all impact the loss of some alleles or a decrease in genetic variation in a population.

Three populations (male Waelawi, female Waelawi, and male Kalibone) had the same genetic distance (Figure 4). The three populations will establish a new cluster with the male Kariango population, then with the female Kalibone population, and finally with the female Kariango population. It means the three groups in the first cluster (male Waelawi, female Waelawi, and male Kalibone) are genetically the most distant from the female Kariango population. When observed by sex difference, the male population of Waelawi and the female population of Kariango, as well as the male population of Kalibone and the female population of Kariango, are population pairs that can be crossed due to the difference in genetic distance (Table 6) between the two population pairs that are the farthest (0.1195). Genetic distance differences between populations can be used to increase genetic variation in giant freshwater prawns by crossover.

Genetic improvement results for giant freshwater prawns can produce good-quality seeds if broodstock management is done correctly. Several genetic variation comparisons have been conducted between wild and cultivated populations to predict the inbreeding effect on cultivated populations whose seeds are produced in hatcheries. For example, Nguyen et al. (2015) investigated mt-DNA diversity in Chinese and Vietnamese cultivated shrimp populations. According to the findings, wild populations have a higher genetic variety than cultivated ones. In addition, Bala et al. (2017) also found that the genetic variation of giant freshwater prawn populations of natural origin in Bangladesh was higher than in hatcheries.

On the other hand, the genetic variation in giant freshwater prawn populations in four rivers in Peninsular Malaysia decreased, and inbreeding developed due to overfishing (Atin et al. 2017). Binur and Pancoro (2017) also investigated the variety of four microsatellite DNA loci at four giant freshwater prawn hatchery locations on Java Island. The results showed that inbreeding was moderate at the post-larvae stage. Hence using postlarvae from these four hatcheries for potential broodstock was not suggested. Therefore, identifying genetic quality first will make the crossing results more effective. This condition serves as the foundation for the genetic examination of the South Sulawesi population. Furthermore, the results serve as a reference for good crosses between South Sulawesi with other populations as the best results.

In conclusion, giant freshwater prawns from three natural habitats (rivers) have genetic variations, with a composite haplotype of six. Genetic differences in wild populations of South Sulawesi giant freshwater prawns can identify suitable broodstock candidates. Based on the most significant genetic gap, the preferred population pairs for crossing include male Waelawi populations, female Kariango populations, male Kalibone populations, and female Kariango populations. Identifying superior-quality local giant freshwater prawn populations is required to assist breeding and farming results. Regulations are also needed for collecting, protecting, and using germplasm resources suitable for *M. rosenbergii*, as the South Sulawesi wild population is a superior indigenous shrimp species.

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