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Genetic Diversity of *Panulirus Versicolor* in the Waters of Barrang Caddi Island, Makassar Strait, South Sulawesi, Indonesia

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Abstract*—Barong shrimp or lobster (*Panulirus versicolor*) is a fishery commodity with high economic value and great demand. This study aims to determine the genetic characteristics of *Panulirus versicolor* at the fishing location in the waters of Barrang Caddi Island, Makassar Strait, using primers LCO-1490F and HCO-2198R. Genomic DNA extraction was performed using a Quick-DNA Tissue Miniprep Kit for total isolation. For DNA purity, the elution buffer utilized in this research was specifically selected for its suitability for PCR amplification with KOD FX Neo, providing optimal conditions for efficient genetic analysis. The primary genes used were LCO-1490F with primers GGT CAA CAA ATC ATA AAG ATA TTG G, and HCO-2198R with TAA ACT TCA GGG TGA CCA AAA AAT CA. The study utilized a 1% agarose gel in a 1X TBE buffer at 50 voltage for 45 minutes to move the total DNA and PCR products. The analysis results at sampling stations 0.2-03 showed increasingly smaller genetic distances. Based on the results of BLASTn analysis, each station had the same type of *Panulirus versicolor* with a similarity of 99-100%. The morphological characteristics included a carapace with a black spot and black and white lines on each abdominal segment. The analysis of genetic variation in the lobster population on Barrang Caddi Island using primers LCO-1490F and HCO-2198R obtained a DNA fragment of 700 bp. The population of *Panulirus versicolor* lobster on Barrang Caddi Island showed a high level of similarity or low diversity.

***Keywords*—Panulirus versicolor; Barrang Caddi Island; genetic diversity; BLAST amplification.**

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I. INTRODUCTION

"Barong shrimp" refers to a collection of species belonging to the *Panulirus* genus. One of these species is Panulirus versicolor. This species of spiny lobster exclusively inhabits tropical reefs within the Indo-Pacific region [1]. Spiny lobsters are nocturnal and solitary. They hide in reefs or under coral during the day and eat carrion, arthropods, crustaceans, and sometimes small fish. "Barong shrimp" includes several species of the Panulirus genus, such as Panulirus versicolor. The sex ratio is typically 1.83:1.00, and 3.40% of females carry eggs [2].

Barong shrimp or lobster (Panulirus versicolor) is a precious commodity in the fishery industry and is highly sought after due to its high economic value and great demand [3]. The domestic and export market demand for this commodity has consistently increased in countries such as Hong Kong, Taiwan, Singapore, Japan, and China [4]. However, lobster fishing practices employed in the industry often involve environmentally unfriendly methods and tools [5]–[7], which leads to detrimental effects on the lobster habitat and causing disturbances to the fragile coral environment. These unsustainable practices threaten the long-term survival of the lobster population in its natural habitat. Moreover, despite the high demand, barong shrimp and lobster cultivation expansion through aquaculture methods remains limited [8].

The population of Panulirus versicolor in Makassar Strait waters has shown signs of over-exploitation due to intensive and unsustainable fishing [9]. The resource utilization status in Spermonde Islands Makassar Strait indicated growth overfishing, evident by the value comparison of the Lc. and Lm. in crayfish (Lc.< Lm.). 2014 Panulirus versicolor was classified as fully exploited, indicating that the stock of crayfish resources had been overly exploited, nearing the MSY (maximum sustainable yield). This condition is further corroborated by the smaller size of the lobsters caught in these waters. Panulirus versicolor species live in coral reef waters at 1-4 meters deep, sheltered between coral rocks, and rarely live in groups. To prevent population decline due to high

fishing intensity, information about Panulirus versicolor resources that support its conservation and development, including aspects of genetic diversity is needed. Therefore, basic knowledge of gene population diversity is necessary as a reference in fisheries resource management [10], [11] and domestication [12].

The genetic variation of a lobster population is an illustration of intraspecies differences. Information obtained from genetic variation will provide an initial picture of the diversity and genetic kinship of *Panulirus versicolor*. This information can be used as a management consideration for the conservation, restocking, and utilization of resources in related locations by policymakers. Genetic variations and differences within or between taxa are usually calculated from the presence or absence of emerging DNA bands based on changes in sequences for each locus [13]. The genetic characteristics of *Panulirus versicolor* was determined at the fishing location in the waters of Barrang Caddi Island, Makassar Strait, using primers LCO-1490F and HCO-2198R. The selection of CO1 markers for DNA sequencing is based on their origin in mitochondrial DNA, which has undergone evolutionary changes and is considered essential for cellular function.

The COI gene is one of the target regions in DNA barcoding techniques because it is considered adequate for use as an animal group discriminant [14]–[19]. It has been used to distinguish various animal species, including mantis shrimp, shellfish, silkworms, insects, cattle, and several fish species. COI is one of the most helpful methods for identifying kinship in animal and fish species [20]–[25]. Besides that, the COI genes could help bioidentification systems in various types of animals and provide answers to kinship relationships within species up to 100% [26].

II. Materials and Methods

A. Research Setting

Locally known as "bubu," a shrimp-catching tool was used to sample *Panulirus versicolor* in the waters of Barrang Caddi Island, Makassar City, South Sulawesi, Indonesia, in January 2020 (Fig.1). The samples were promptly stored in a 96% ethanol solution, immediately frozen, and expeditiously dispatched to PT Genetika Science in Jakarta for rigorous analysis.

B. DNA Isolation

We utlized Quick-DNA Tissue/Insect Microprep Kit for total isolation in genomic DNA extraction. Quick-DNA Tissue/Insect Microprep Kit is quicker and easier to use [27]. The DNA purity was determined with absolute certainty using an elution buffer that was specifically optimized for PCR amplification with KOD FX Neo [28].

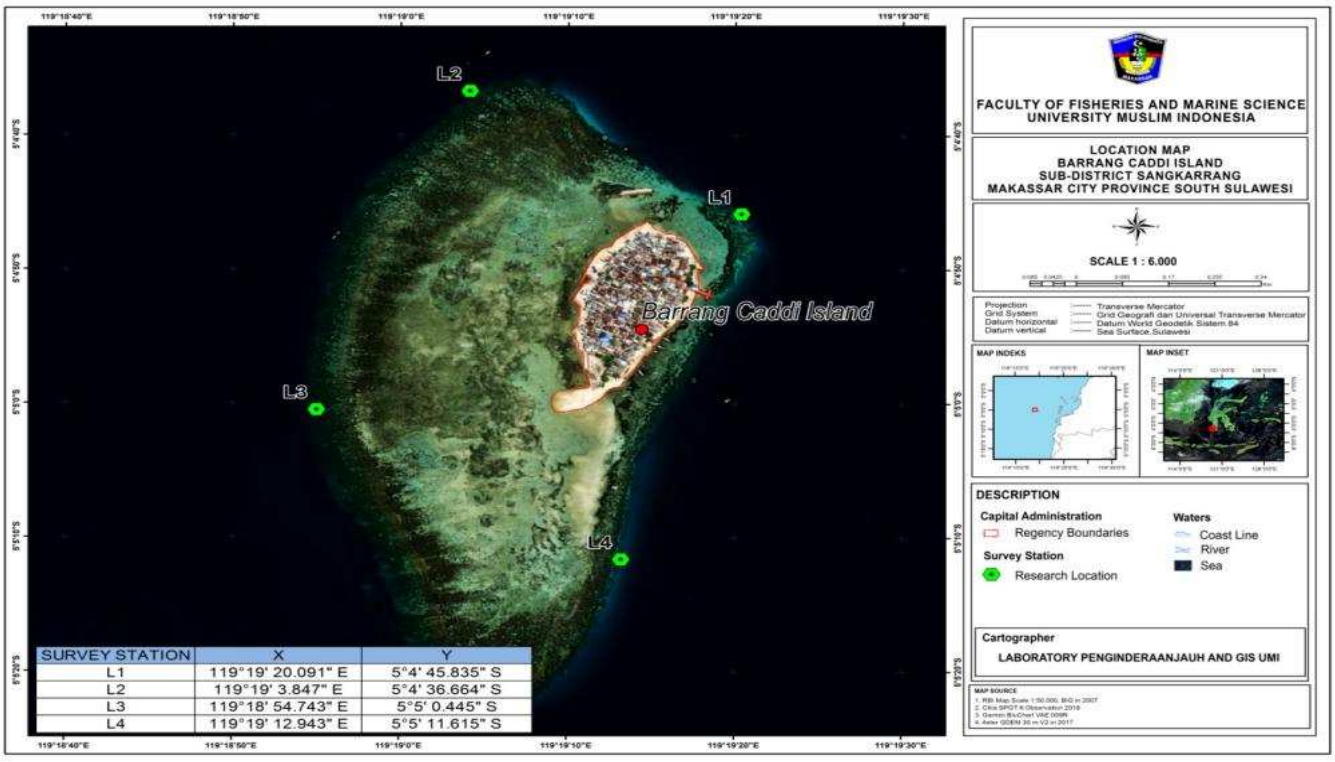

Fig. 1 Sampling location of P. versicolor in Barrang Caddi Island

C. Polymerase Chain Reaction (PCR)

The main genes remained LCO-1490F with primers GGT CAA CAA ATC ATA AAG ATA TTG G, and HCO-2198R with TAA ACT TCA GGG TGA CCA AAA AAT CA [29]. It is essential to follow these instructions strictly to ensure accurate results. The reaction mixture includes dd H2O, PCR buffer KoD FX neo, dNTPs, two different plate numbers, DNA template, and KOD FX Neo. The PCR cycling program includes several steps, including denaturation and annealing, that must be followed carefully. It is criticial to pay close attention to these instructions to ensure the best possible outcome. The product purification was performed with Zymoclean™ Gel DNA Recovery Kit [30]. An efficient and

trouble-free method has been provided for the high-yield recovery of pure DNA from agarose gels. Additionally, the success of PCR amplification can be promptly detected through electrophoresis techniques.

D. Electrophoresis

In this study, Electrophoresis was performed with utmost precision to determine the success of DNA and PCR isolation. The method used in the study involved transferring the total DNA and PCR products to a 1% agarose gel in a 1X TBE buffer. Electrophoresis was then performed at 50 voltage for 45 minutes to analyze the results. The DNA tape was stained with 5 µg mL-1 of ethidium bromide to ensure optimal visualization under a UV transilluminator lamp, and finally, a photograph was taken using a UV-filtered digital camera to record the results with utmost accuracy.

E. DNA Sequencing

PT. Genetika Science, Jakarta conducted the DNA sequencing, while gene purification and sequencing were performed at 1st Base in Malaysia. The PCR product size was 40 µL, and each primer size was 30 µL.

F. Data Analysis

The study utilized BioEdit7 software to align the DNA sequence data obtained from the forward and reverse primers. The similarity of the DNA COI sequences with those in the GenBank database was analyzed using the Basic Local Alignment Search Tool (BLAST) for computationally efficient purpose [31]. The sequences with high similarity were downloaded to create phylogenetic trees using the MEGA version 6.06 software and the analysis was conducted using the Kimura 2-parameter model and UPGMA with 1000 bootstrap [32]–[34].

III. Results and Discussion

A. Results

1) DNA Fragments: The fragment length of PCR amplification results on *P. versicolor* in Barrang Caddi Island using primers LCO-1490F: GGT CAA CAA ATC ATA AAG ATA TTG G and HCO-2198R: AA ACT TCA GGG TGA CCA AAA AATCA showed 700 bp with 4 samples as shown in Fig. 2.

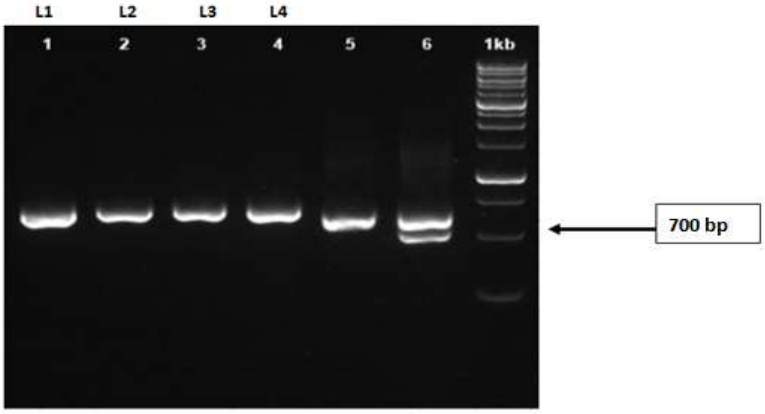

Fig 2 DNA amplification results of Pannulirus versicolor population in Barrang Caddi Island using primers LCO-1490F and HCO-2198R

2) Genetic Distance: The analysis results at sampling stations 0.2-03 showed increasingly smaller genetic distance values as described in Fig. 3. This represented the closeness of genetic distance between *P. versicolor* populations at the sampling station location. Based on the low significance of the difference in genetic distance between locations, it was estimated that the lobster population belonged to the same stock. The genetic distance value correlates with kinship between populations.

L1 (Station 1)													
L2 (Station 2)	0.002												
L3 (Station 3)	0.002	0.003											
L4 (Station 4)	0.002	0.003	0.003										
Panulirus_versicolor_NBFGR-CHN-PV3	0.000	0.002	0.002	0.002									
Panulirus_versicolor_JSPVW4	0.000	0.002	0.002	0.002	0.000								
Panulirus_versicolor_LPP	0.000	0.002	0.002	0.002	0.000	0.000							
Panulirus_versicolor_BIN_0032	0.000	0.002	0.002	0.002	0.000	0.000	0.000						
Panulirus_versicolor_BIN_0031	0.000	0.002	0.002	0.002	0.000	0.000	0.000	0.000					
Panulirus_homarus_homarus_NBFGR-CHN-PHH-KL5	0.164	0.164	0.162	0.166	0.164	0.164	0.164	0.164	0.164				
Panulirus_homarus_homarus_NBFGR-CHN-PHH-KL7	0.166	0.166	0.164	0.164	0.166	0.166	0.166	0.166	0.166	0.021			
Panulirus_stimpsoni	0.144	0.144	0.142	0.146	0.144	0.144	0.144	0.144	0.144	0.173	0.175		
Panulirus_inflatus	0.150	0.150	0.148	0.153	0.150	0.150	0.150	0.150	0.150	0.139	0.141	0.171	
Panulirus_ornatus_24	0.178	0.178	0.176	0.178	0.178	0.178	0.178	0.178	0.178	0.141	0.134	0.162	0.144

Fig. 3 The genetic distance value of *P. versicolor* at the sampling station location on Barrang Caddi Island

3) BLASTn and Phylogenetic Analysis: Based on the results of BLASTn analysis, each station (See from Table 1 to Table 4) had the same type of *P. versicolor* with a similarity of 99-100%. The morphological characteristics included a carapace with a black spot, as well as black and white lines on each abdominal segment. Furthermore, the eyes were prominent and also had 33 whip-like antennae and 4 antennal plates. The position of the 2 front spines was longer than the 2 rear spines located near the eyes, and there was a black spot at the base. The samples also had horns curved forward, with a white spot, and the spines on the carapace were slightly pointed. Antennules were biramous and white, while antennal segments were alternately colored black and white. There was no spine between the 5th pereiopod, and the spine on the telson was blunt. Morphometrically, the total body length was 17.5 cm, with a carapace length of 7.9 cm and an abdominal length of 9.6 cm (abdominal plate + tail length); hence, the ratio of carapace length to the abdomen was 1.7 cm.

TABLE I
STATION 1 LOCATION

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panulirusversicolor voucher NBFGR-CHN-PV3 COI gene, partial cds; mitochondrial	1209	1209	97%	0.0	100%	JQ229882.1
Panulirusversicolor clone AU2 COX1 gene, partial cds; mitochondrial	1193	1193	99%	0.0	99%	JN418936.1
Panulirusversicolor voucher JSPVW4 COI gene, partial cds; mitochondrial	1153	1153	93%	0.0	100%	KF548584.1
Panulirusversicolor voucher JSPVW3 COI gene, partial cds; mitochondrial	1148	1148	93%	0.0	99%	KF548583.1
Panulirusversicolor COI gene, partial cds; the mitochondrial gene for mitochondrial product	1142	1142	93%	0.0	99%	AF339472.1
Panulirusversicolor voucher LPvP COI gene, partial cds; mitochondrial	1137	1137	91%	0.0	100%	KT001513.1

TABLE II
STATION 2 LOCATION

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panulirusversicolor voucher NBFGR-CHN-PV3 COI gene, partial cds; mitochondrial	1202	1202	97%	0.0	99%	JQ229882.1
Panulirusversicolor clone AU2 COX1 gene, partial cds; mitochondrial	1189	1189	99%	0.0	99%	JN418936.1
Panulirusversicolor voucher JSPVW4 COI gene, partial cds; mitochondrial	1148	1148	93%	0.0	99%	KF548584.1

TABLE III
STATION 3 LOCATION

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panulirusversicolor voucher NBFGR-CHN-PV3 COI gene, partial cds; mitochondrial	1216	1216	97%	0.0	99%	JQ229882.1
Panulirusversicolor clone AU2 COX1 gene, partial cds; mitochondrial	1186	1186	97%	0.0	99%	JN418936.1
Panulirusversicolor voucher JSPVW4 COI gene, partial cds; mitochondrial	1148	1148	91%	0.0	99%	KF548584.1
Panulirusversicolor voucher JSPVW3 COI gene, partial cds; mitochondrial	1144	1144	91%	0.0	99%	KF548583.1

TABLE IV
STATION 4 LOCATION

Description	Max score	Total score	Query cover	E value	Ident	Accession
Panulirusversicolor voucher NBFGR-CHN-PV3 COI gene, partial cds; mitochondrial	1204	1204	97%	0.0	99%	JQ229882.1
Panulirusversicolor clone AU2 COX1 gene, partial cds; mitochondrial	1189	1189	99%	0.0	99%	JN418936.1
Panulirusversicolor voucher JSPVW4 COI gene, partial cds; mitochondrial	1148	1148	92%	0.0	99%	KF548584.1
Panulirusversicolor voucher JSPVW3 COI gene, partial cds; mitochondrial	1144	1144	92%	0.0	99%	KF548583.1
Panulirusversicolor COI gene, partial cds; the mitochondrial gene for mitochondrial product	1137	1137	93%	0.0	99%	AF339472.1

The phylogenetic analysis to determine the kinship of *the P. versicolor lobster population in Barrang Caddi Island was conducted using the Neighbor-Joining Method with Kimura-2-parameter model and 1000 times bootstrap,* as shown in Fig. 4.

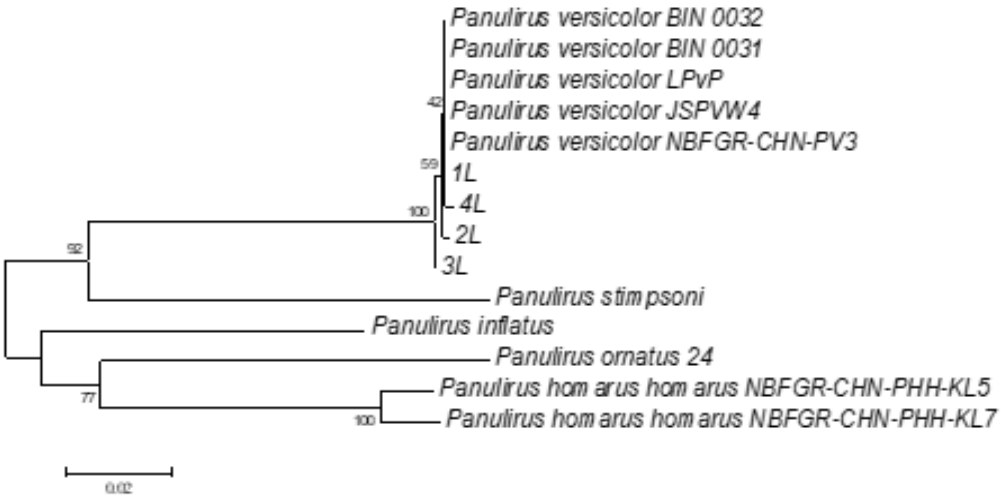

Fig. 4 The reconstructed phylogenetic tree of Panulirus versicolor from Barrang Caddi Island using the Neighbor-Joining Method

B. Discussion

Genetic diversity plays a crucial ecological role [35], [36]. It is closely linked to species diversity, which can show the cause-and-effect relationship between changes in biological resources and environmental conditions. It also serves as a measure of genetic variability within a population [37]. A fragment length of 750 bp found by [38] indicated the primer's success in amplifying the targeted gene [29]. This study was conducted to obtain sequence data using primers LCO-1490F and HCO-2198R from Barrang Caadi Island as well as data from NCBI (See Fig. 1). The mitochondrial COI was instrumental in analyzing the phylogeny of Crustacea at the species level [39]. CO1 was a secondary marker for analyzing phylogenetic relationships between eukaryotic organisms. The use of COI markers was carried out on *Panulirus ornatus* [11]. Thus, the main pairs of LCO1490 and HCO2198 constantly amplified the 710 bp on *Pannulirus versicolor* [38].

Genetic distance refers to the degree of gene (genomic) differences within a population or species [40]–[42]. Meanwhile, genetic diversity is important for population stability, adaptability, and resilience [43], [44]. For example, it can prevent individual fitness loss caused by inbreeding, which can lead to extinction due to uniformity. Low genetic diversity negatively affected essential traits such as the survival of an organism, reduced growth and size diversity, and decreased adaptability. Furthermore, the failure of inherited variety will lessen species ability to adapt to ecological changes. Individuals with high genetic diversity would have a significant fitness component [45], including growth rate, fecundity, viability, and resistance to environmental changes and stress.

The results obtained in this study showed that the genetic variation of *P. versicolor* in Barang Caddi island was relatively low. Meanwhile, information on genetic diversity in one or several populations of specific organisms is essential to provide an overview of the genetic quality. Low genetic diversity can lead to decreased fitness of individuals in the adaptation process due to environmental pressures [46]–[48]. Factors that potentially affect the level of gene variation include migration [49]. Moreover, low inherited variety will modify the species ability to respond to artificial or natural ecologically changes. Each combination of genes has a different contribution to ecological changes, hence, varieties of genes will provide the opportunity for a better response. The low heterozygote value observed in the *P. versicolor* population was consistent with the environmental damage suffered by Barrang Caadi Island. This situation will increase the chance of inbreeding, culminating in low gene variation and the emergence of specific genes.

The BLAST analysis and dendrogram results showed that all four samples were *P. versicolor* because they had the same haplotype. This suggested a close genetic relationship and a common ancestor within the population. In other words, the sample population on Barrang Caddi Island had a single genetic descendant. Fig. 4 shows the number of similarity or kinship levels of each branching between member species. Therefore, the genetic characteristics of this species are important information that needs to be considered for conservation, stock enhancement, and cultivation purposes. Phylogenetics combines molecular biology techniques with statistics to reconstruct kinship relationships. It is one of the most frequently used methods in systematics to understand the diversity of creatures, evolution level, and the species kinship.

IV. Conclusion

Based on the results and discussions presented, several key conclusions can be drawn, shedding light on the genetic characteristics and implications for conservation of the *P. versicolor* lobster population on Barrang Caddi Island, namely The analysis of genetic variation in the lobster population on Barrang Caddi Island using primers LCO-1490F and HCO-2198R obtained a DNA fragment of 700 bp. Population of *P. versicolor* lobster on Barrang Caddi Island showed a high level of similarity or low diversity.

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