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Genetic characteristics of lobster *Panulirus versicolor* (Latreille,1804) from bird's head seascape-Papua based on cytochrome oxidase subunit 1 (COI) gene

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Abstract. Bird's Head Seascape of Papua has high biodiversity. This region is a potential area for lobster (*Panulirus versicolor*) to grow and breed. This research aim is to analyze the genetic characteristic and diversity of lobster from Papua. Sample of *P. versicolor* was collected from several locations in BHS, which are Nabire, Wasior, Biak, Cenderawasih Bay, and Raja Ampat. Genomic DNA was isolated and amplified by using Polymerase Chain Reaction methods with primers HCO-02198 and LCO-1490. A total of 684 base pairs of COI genes were sequenced with an average composition of T (32%), C (22%), A (27,8%), and G (18,2%). All sequences are grouped into four haplotypes based on nine polymorphic sites. The Phylogenetic tree shows there is a close relationship among samples from Nabire, Wasior, Teluk Cenderawasih, and Raja Ampat. Based on this result, there is a need for enlarge management of conservation areas and built the marine protected area network in one management. The conservation should not be locally in each region.

1. Introduction

Bird's Head Seascape (BHS) of Papua is a region that has high biodiversity in marine organism including seagrass [1], mangrove [2,3], coral [4], and reef fishes [5,6]. The researcher refers to BHS as the heart of the world coral triangle because of the diversity of the coral near to 75% from the world's coral [4]. The BHS region stretches from Cenderawasih Bay to the Raja Ampat Islands, and southward to Fak-Fak and Kaimana. BHS has an area of more than 180,000 Km² and 2500 islands [7]. Nevertheless, data and information on the genetic diversity of marine life in this region are still limited.

Lobster is one source of seafood that has important economic value [8]. Seven species of the 19 species of *Panulirus* that are commonly found in the world could be found in Indonesia. Papua is one of the potential areas for catching crayfish or lobster [8]. This abundance of marine resources requires us to manage and use it very well. Efforts to conserve and cultivate of lobster (*P. versicolor*) should always be made. Therefore, studying lobster in terms of biology and genetics is very important. Moreover, data on the genetic diversity of *P. versicolor* is still limited.



Deoxyribonucleic acid (DNA) is one of the macromolecules that preserve all of the life information of an organism (blueprint of life) that deliver the genetic information through their offspring. [9,10,11]. Cytochrome oxidase subunit I (COI) is one genetic marker that codes a complex protein of cytochrome oxidase that play a role in respiration and electron transfer chain [12]. This gene is part of mitochondrial DNA (mt-DNA). Compared to nuclear DNA, mt-DNA has many copies and small genome size, that makes it easy to study [13]. Also, mt-DNA is inherited according to maternal lineage so that it can be used to track evolutionary history. Based on some of genes on mt-DNA, the COI gene is relatively stable and evolve slowly [14]. With all of the characteristic of COI gene, in this research, the COI gene was chosen as the topic of analysis.

This research aims to study the characteristic of the COI gene of lobster (*P. versicolor*) from BHS that includes nucleotides sequence and genetic composition (nucleotide percentages and polymorphic site). Also, the phylogenetic relationship tree will be analyzed. The results of this study are expected to provide benefits in studying the genetic diversity of *P. versicolor* so that it can be utilized in the development and business of lobster farming and its conservation. By knowing the nucleotide sequence of COI gene fragments from *P. versicolor*, we can use them for inventory and genetic conservation of *P. versicolor* on the BHS region. Information about the relationship of *P. versicolor* is expected to be able to predict the origin of *P. versicolor* in BHS so that it can be used as a basis for determining inter-regional conservation policies.

2. Material and Methods

2.1. Sampling location

A total of 10 samples were collected from five locations around the Birds Head Seascape of Papua: 1) Nabire, 2) Wasior, 3) Raja Ampat, 4) Cenderawasih Bay, and 5) Biak. The tissue sample was collected from the antenna of *P. Versicolor* and preserved in ethanol 95% [15] until used.

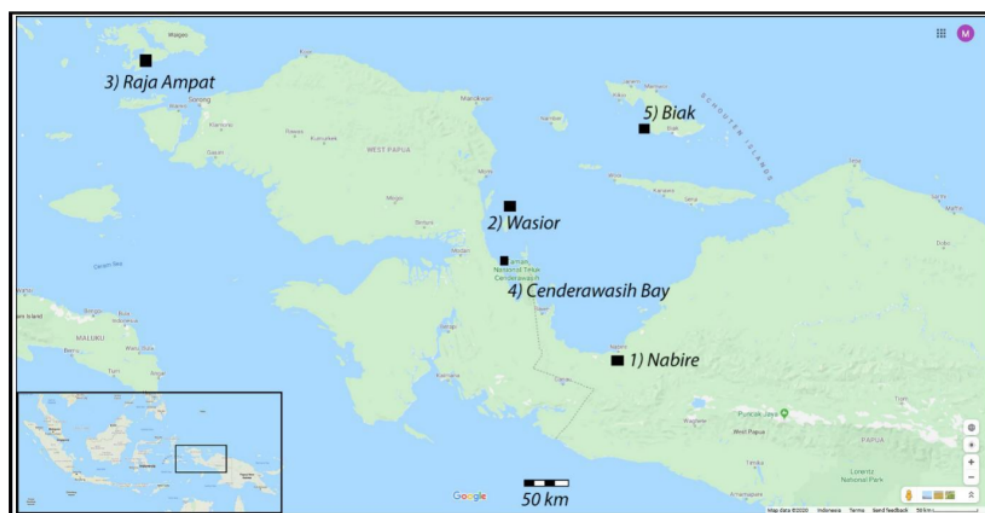


Figure 1. The sampling location of lobster in Bird Head Seascape of Papua Regions with five site sampling: 1) Nabire, 2) Wasior, 3) Raja Ampat, 4) Cenderawasih bay, and 5) Biak

2.2. DNA Isolation

Genomic DNA isolation was done by chelex 10% methods [16] by taking a small part of the antenna tissue (30 mg), then was immersed in a 10% chelex solution. The mixture was vortexed and centrifuged for 10 seconds. The sample was heated at 95°C for 30 minutes. The supernatant was used in the amplification steps by *polymerase chain reaction* (PCR) [17,18].

2.3. COI Gene Amplification

COI gene fragment of *P. versicolor* was amplified by polymerase chain reaction using general forward primer LCO-1490 (5'-ggtaacaatacataagattgg-3') and reverse primer HCO-2198 (5'-taaacctcagggtgaccaaataca-3') [19]. The PCR temperature profile used was pre-denatured at 94°C for 5 minutes, continued with an amplification process with a denaturation temperature of 94°C for 30 seconds, annealing 50°C for 30 seconds, and extension 72°C for 45 seconds. This amplification process was repeated in 37 cycles. The final stage of the PCR process was carried out post-denaturation at 72°C for 5 minutes and cooling at room temperature 32°C for 1 (18).

2.4. Electrophoresis

The amplification results (PCR) were visualized by DNA electrophoresis method with agarose Gel. The gel is made using 1 gram agarose in 100 mL of sodium boric buffer (SB buffer). The electrophoresis process lasts for 30 minutes with a voltage of 100 volts. DNA staining uses ethidium bromide (EtBr), which will glow when exposed to ultraviolet (UV) light. The results are documented using a digital camera.

2.5. Exo-Sap, Cycle sequencing

Then EXO-SAP was carried out with the enzyme exonuclease and shrimp alkaline phosphatase to clean up the remnants of primers and dNTPs. For the sequencing of nucleotides, cycle sequencing is performed by using big dye terminator 3 of Sanger sequencing, and the nucleotide sequence is determined by sending it to Cornell University, California.

2.6. Data Analysis

The DNA sequences obtained were aligned and proofread between the DNA sequences written and the electropherogram that appeared. This process is done by using Sequencher 4.6 software. Nucleotide composition, polymorphism, and phylogenetic tree analyses were carried out using MEGA 4 software [20]. The comparison sequence is obtained by basic local alignment search tools (BLAST) on GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) [21,22].

3. Results and discussion

The results of the *P. versicolor* mitochondrial DNA COI gene fragment amplification stained with ethidium bromide and visualized using a UV transilluminator lamp, are presented in Figure 2.

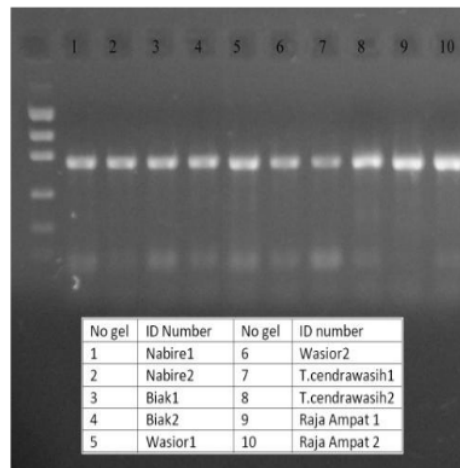


Figure 2. Electrophoresis gel photo. The left band is a low mass ladder (DNA Marker, Invitrogen), and band 1-10 is the amplicon of the COI gene from the sample.

Based on these results, it appears that all samples were successfully amplified with primers HCO2198 and LCO1490 and have the same fragment size. According to Folmer [19], this universal primer pairs could amplify for 710 base pairs of the COI gene. HCO and LCO respectively are an acronym of high (weight) and *light* of *cytochrome oxidase*. The primer pairs were used to amplify of COI gene from many organisms, sea urchin *Tripneustes gratilla* [23], *Octopus cyanea* [24], lobster *P. versicolor* [25] including some others invertebrates such as worm, marine mollusk, freshwater mollusk, Arthropoda, and parasite [19].

The sequence of the amplicon resulted in 684 bp of nucleotides sequenced. The length of the nucleotide sequence is in a normal range, targeted by Folmer [19]. The genetic composition of the COI gene from all samples is displayed in Figure 3.

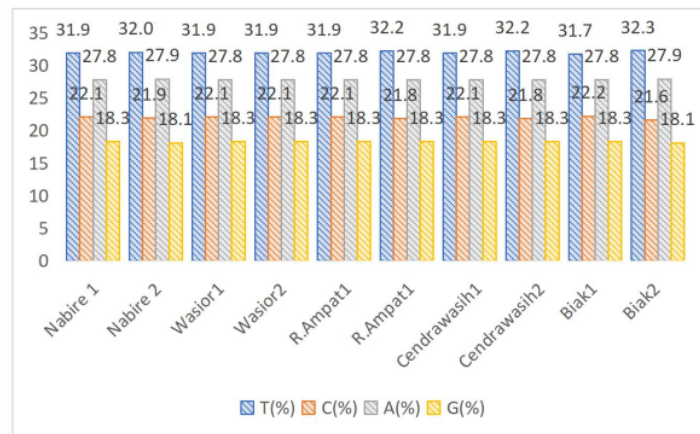


Figure 3. Nucleotide compositions A (Adenine), G (Guanine), C (Cytosine), T (Tymin) of *P. versicolor* collected from Bird Head Seascape of Papua.

All samples have an average of nucleotide percentages of T (32%), C (22%), A (27,8%), and G (18,2%). Besides, from 684 bases, there are nine polymorphic sites, as shown in Table 1.

Table 1. Polymorphic site of COI gene of *P. versicolor*

Sample ID	Nucleotide Number									Haplotype
	87	126	438	483	501	543	606	609	669	
Nabire 1	G	C	C	C	C	C	C	G	T	Haplotype 1
Nabire 2	G	C	C	C	C	T	C	A	T	Haplotype 2
Wasior1	G	C	C	C	C	C	C	G	T	Haplotype 1
Wasior2	G	C	C	C	C	C	C	G	T	Haplotype 1
R.Ampat1	G	C	C	C	C	C	C	G	T	Haplotype 1
R.Ampat2	G	T	C	T	C	C	C	G	T	Haplotype 3
Cendrawasih1	G	C	C	C	C	C	C	G	T	Haplotype 1
Cendrawasih2	G	T	C	T	C	C	C	G	T	Haplotype 3
Biak 1	G	C	C	C	C	C	C	G	C	Haplotype 4
Biak 2	A	C	T	C	T	C	T	G	T	Haplotype 5

This polymorphism site occurs due to a substitution mutation. In nucleotides, number 87 and 609, mutations from bases G to A are called transversion, as well as nucleotides 126, 438, 483, 501, 543, 606, and 669 also occur in transversion point mutations (from C to T). By looking at the polymorphic side pattern, we can group all the samples into five different haplotype groups. Haplotype 1 consists of

Nabire 1 samples, Wasior 1 and 2, Raja Ampat 1, and Cenderawasih 1. While Haplotype 2 only has Nabire 2 sample. Haplotype 3 made of Raja Ampat 2 and Cenderawasih 2, then Haplotype 4 only has Biak 1 and haplotype 5 only Biak2. Each location has two different haplotypes, except for the location of the wasior; the lowest genetic diversity is found in wasior areas. The distribution of haplotypes at the five study sites is shown in Figure 4.

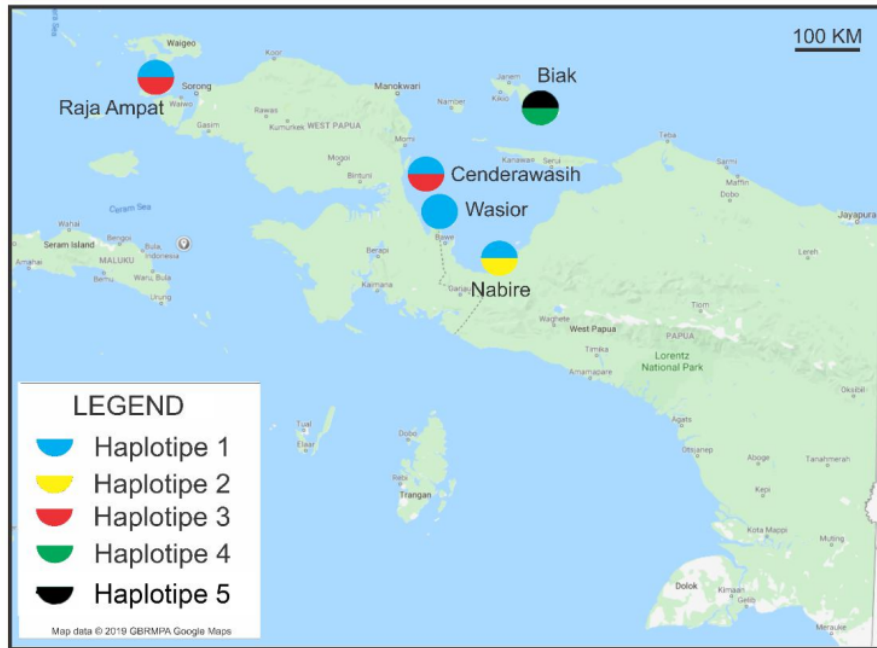


Figure 4. A haplotype map of *P.versicolor* from Bird Head's Seascape of Papua Area

The COI gene is a gene that encodes an important protein, so it is relatively stable and undergoes a slight mutation in nucleotides. According to da Fonseca et al. [14], the COI gene has a low potential mutation rate compared to the cytochrome b gene. Despite the changes in nucleotides, the translation of amino acids is still in the same order and composition. This translation is caused by mutations occurring in the second or third nucleotide positions of each codon.

The BLAST results on the gene bank, provide information that all ten sequences, are the COI gene of *P.versicolor* with query coverage 94%, max indent 99%, and max score 1166. COI Gene is a gene that is used for species identification through DNA barcoding [26]. This gene is a standard gene for barcode DNA [27] that plays as a reference in genetic identification. This quarry coverage is less than previous research [25]. One sequence the most closely related to the samples is the sequence of *P. versicolor* from Palau Island with accession number AF3394721(Ptacek et al., 2001) that has length 643 bp. This sequence we used as a reference in the construction of the phylogenetic tree. As an outgroup, the sequence of *P. simpsoni* from Hongkong (accession number AF339471.1) [28] with a length of 643 bp was used.

The phylogenetic tree is shown in Figure 5, and the pairwise distance analysis is displayed in Table 2.

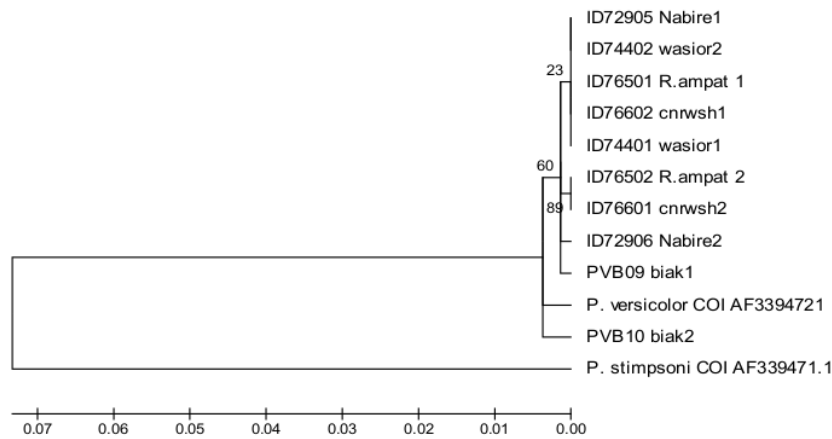


Figure 5. Phylogenetic trees of *P. versicolor* from BHS, Papua was also compared with GenBank sequence data. This tree was created using the Neighbor-joining method with two parameters kimura and 1000 bootstrap replication.

Table 2. Pairwise Genetic distance of *P. versicolor* from Bird Head Seascape of Papua

No	Sample ID	1	2	3	4	5	6	7	8	9	10	11
1	Nabire 1											
2	Nabire 2	0.003										
3	Wasior1	0.000	0.003									
4	Wasior2	0.000	0.003	0.000								
5	R.Ampat1	0.000	0.003	0.000	0.000							
6	R.Ampat2	0.003	0.006	0.003	0.003	0.003						
7	Cendrawasih1	0.000	0.003	0.000	0.000	0.000	0.003					
8	Cendrawasih2	0.003	0.006	0.003	0.003	0.003	0.000	0.003				
9	Biak 1	0.002	0.005	0.002	0.002	0.002	0.005	0.002	0.005			
10	Biak 2	0.006	0.009	0.006	0.006	0.006	0.009	0.006	0.009	0.008		
11	<i>P.versicolor</i> AF3394721	0.006	0.009	0.006	0.006	0.006	0.009	0.006	0.009	0.008	0.006	
12	<i>P. stimpsoni</i> AF339471.1	0.195	0.194	0.195	0.195	0.195	0.195	0.195	0.195	0.192	0.188	0.194

COI genes are widely used in phylogenetic trees, genetic diversity, evolutionary history, genetic distance analysis, and populations genetic [17,25,29,30,31,32]. The phylogenetic tree confirms that the species of all ten samples were *P. versicolor* and grouped into one large group, while the *P. stimpsoni* (out-group) is in a distant clade. This grouping is supported by the genetic distance data (Table 3) between *P. stimpsoni* and other samples having an average distance of 0.194. In the first clade (*P. versicolor* clade), there are three sub-clade that separates the sample Biak 2 with *P. versicolor* COI AF3394721 with the other nine sample groups. The sample Biak 2 has the most distant relationship from the other nine samples with an average genetic distance of 0.007, while the genetic distance with *P. versicolor* AF3394721 is 0.006. Based on phylogenetic trees, we see that all samples from Wasior are in one clade with samples from Nabire, Raja Ampat, and Cenderawasih, this indicates that there is a gene flow between lobster populations in the four regions. Thus, there needs to be a global conservation area, which management must cover the entire BHS region, Papua.

In this study, we could not see further about the population structure of lobsters from each region because there are only a small number of samples. The hope, research can be continued with a larger number of samples (at least 20 samples per location) from all regions in the bird's head region.

4. Conclusion.

All ten samples were grouped into five haplotypes, with the lowest number of the haplotype is samples from wasior. The sample was closely related to the *P. Versicolor* from Palau. There is no population structure of *P. Versicolor* found in these five regions based on this sample data, so the conservation management should be a global conservation area of *P. versicolor* on this BHS regions.

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