

Genetic biodiversity of spiny lobsters (*Panulirus* spp.) from coastal waters of Southern Java, Indonesia

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Abstract. Haryono FED, Ambariyanto. 2018. Genetic biodiversity of spiny lobsters (*Panulirus* spp.) from coastal waters of Southern Java, Indonesia. *Ocean Life 2*: 73-78. Spiny lobsters of the genus *Panulirus* inhabit the southern Java, however, species identification of early life-stages based on morphological features analysis is equivocal. The condition as result of differences in morphology of the larval stage and the adult, a long period of larval life cycle, impact of oceanographic currents inhibit and restrict the route and direction of larval dispersal that causes genetic changes. However, genetic biodiversity of spiny lobsters from this area has not been investigated. The aim of this/our study was to identified genetic diversity of lobsters in Southern Java waters. The research was conducted from January through to August 2015. A total of 1137 lobsters were collected from seven different locations for morphological analyses and 40 lobsters were taken randomly for genetic analysis. Observations nuclear and mitochondrial DNA, and the relationships of genetic in phylogenetic using Codon of code and MEGA 5.0, and dendogram using Primer_e software. Morphological analysis obtained 6 species of spiny lobsters: *P. homarus*, *P. versicolor*, *P. ornatus*, *P. penicillatus*, *P. polyphagus* and *P. longipes*. Phylogenetic analysis obtained two clades and morphological identification results obtained *P. penicillatus* (spiny rock lobster), but the identification genetically acquired included in a group of genetic *P. homarus* (spiny sand lobster). Such discrepancies are possibly due to a long period of larval life, adult in low light of intensity environment and an extreme of oceanographic conditions with marine oil spill.

Keywords: Biodiversity, genetic, spiny lobster, Southern Java

INTRODUCTION

As an archipelagic and tropic country, Indonesia is known as the center of mega-biodiversity (Brooks et al. 2006; Ambariyanto 2010) biological aspect of marine environment condition shown that marine organisms found are very diverse (Hutomo and Moosa 2005; Allen 2008) and most of that marine organism as high demand of commodity. As a result of the condition exploitation of marine resources is very high. Especially for the resource to be consumed and to be traded mainly have high economic value (Berkes et al. 2006). Various types of marine organisms are important export commodities for Indonesia, which has an important role in the country's economy (Baily 2006; Lambaga 2009).

Lobsters are able to have a special ability in comparison to other marine species, the special ability was able to survive without water for a certain period and this excess used to be distributed under life conditions (Landau 1991), and these advantages lead to high economic value. Spiny lobster is an important species and as one of species that have the support of global food stocks. World stock of lobster as supporting fishery production was currently around 260,000 tons per year (de Lestang et al. 2015). Tropical lobster was consists of several species, commonly known as lobster/mangkara/barong

The study of diversity lobster is not only morphological level, but also genetic diversity (DeBoer et al. 2008;

Starger et al. 2010; Vogler et al. 2012; Barber et al. 2014). Family *Panulidae*, genus *Panulirus* consists of 19 species, all of these species live spread in the marine world environment, seven species live in Indonesia ocean, five species namely *P. homarus* Linnaeus (1758), *P. penicillatus* Olivier (1791), *P. longipes* A. Milne-Edwards (1868), *P. ornatus* Fabricius (1798) and *P. versicolor* Latreille (1804). The lobster is not only live in Indonesian also found in the Pacific Ocean (Reddy 2013). Southern of Central Java lobsters life in Cilacap waters are 4 species, i.e., *P. homarus*, *P. ornatus*, *P. penicillatus*, and *P. ornatus* (Hartoyo et al. 2002). Busono (2008) was found 5 species in southern of Central Java waters. While Haryono and Tjahya (2008) states that the spiny lobster in Cilacap waters based on morphological characteristics obtained 6 species, i.e., the "sand lobster" (*P. homarus*), "pearl lobster" (*P. ornatus*), "bamboo lobster" (*P. versicolor*), "stone lobster" (*P. penicillatus*), "batik lobster" (*P. longipes*), and "brown lobster" (*P. polyphagus*).

Identification of lobster based on morphological resulting many discrepancies. The conditions based on the differences in morphology of larval stage to the adult morphology (Chow et al. 2006). A result of a long period of lobster larval life cycle, oceanographic currents and limit the impact that inhibits the larval dispersal routes and directions causing genetic changes with lobster in other regions (Abdullah et al. 2014). Genetic diversity and population structure of marine species due to long life of

larval stage (Palero et al. 2008), and the present species was product of long history of models of the effects of past and oceanographic processes. Another cause of the irregularity was result of intermarriage causes of genetic diversity (Palero et al. 2009).

Liquid and solid wastes have polluted extremely in Southern Java waters will affect benthic organism, including spiny lobster. Biophysical conditions such as oceanographic conditions in relation to the behavior of the larvae, contribute significantly to population genetic differentiation for marine and pelagic phase, genetically distinct effect on the development of non-pelagic (Laurenzano et al. 2013). Some of the causes of disruption of oceanographic parameters that have occurred in the southern waters of Java, among others, the batik industry in Yogyakarta and Surakarta with chemical wastes in the process of dyeing fabrics leading to coastal waters. Extreme pollution also occurs with many ship incidents containing crude oil polluting the sea, among others Alisa XVII on November 1989 loading 11.000 drums of asphalt, MT King Fisher loaded full of crude oil in April 2000. Crude oil and bitumen spill show the distribution toward the northwest when highest tide, otherwise, the oil spread toward southeast when lowest tide (Widhayanti et al. 2015). Banana VI and a ship were leaked with loaded 5000 tons of airplane fuel on January 15, 2010, MT Alisa XVII 18.101 GT aground and leaked, including a lot of fishing boats. During period 2006-2008 had been accidents four of tanker as shipwreck (Fitri 2008). The latter MT HHC have sunk loaded bulk of asphalt and MT Harmony Seven sank October 17, 2015, loaded of 33,000 tons of diesel.

Such conditions have implications for fisheries management related to the stock lobster for evaluation of

long-term management of lobster. Species in deeper water more sensitive overexploitation, and long period of overexploitation impacting genetic diversity (Palero et al. 2010). Irregularity of species organ such as communication organ for lobster was the result of evolution and based on morphological data, there is two adult morphology of organ in the same species. Based on that irregularities need a special of lobster management. The aim of the study was identification genetic diversity of lobster in Southern coastal of Java water, Indonesia.

MATERIALS AND METHODS

Study area

Coastal waters of Southern Java is known as an area where high of lobster catches. Study area especially in Southern waters of Central Java Province and Special Region of Yogyakarta Province, Indonesia, which Southern Central Java waters consist of four districts, namely Cilacap, Kebumen, Purworejo, and Wonogiri. Special Region of Yogyakarta consist tree districts, namely Kulonprogo, Bantul, and Gunungkidul. Lobsters were captured from the southern waters of Java with lobster data collection stations covering lobsters living in Teluk Penyus waters of Cilacap where the tourism coast Teluk Penyus as Station 1, Station 2 in the outside the Cilacap Ocean Fishing Port (PPSC) waters, Station 3 and 4 in the Kebumen water with landing center at Pantai Ayah and Karang Duwur, 5th, 6th and 7th stations are waters of Yogyakarta with concentration of catching in Depok-Bantul, Baron and Sadeng-Gunungkidul waters.

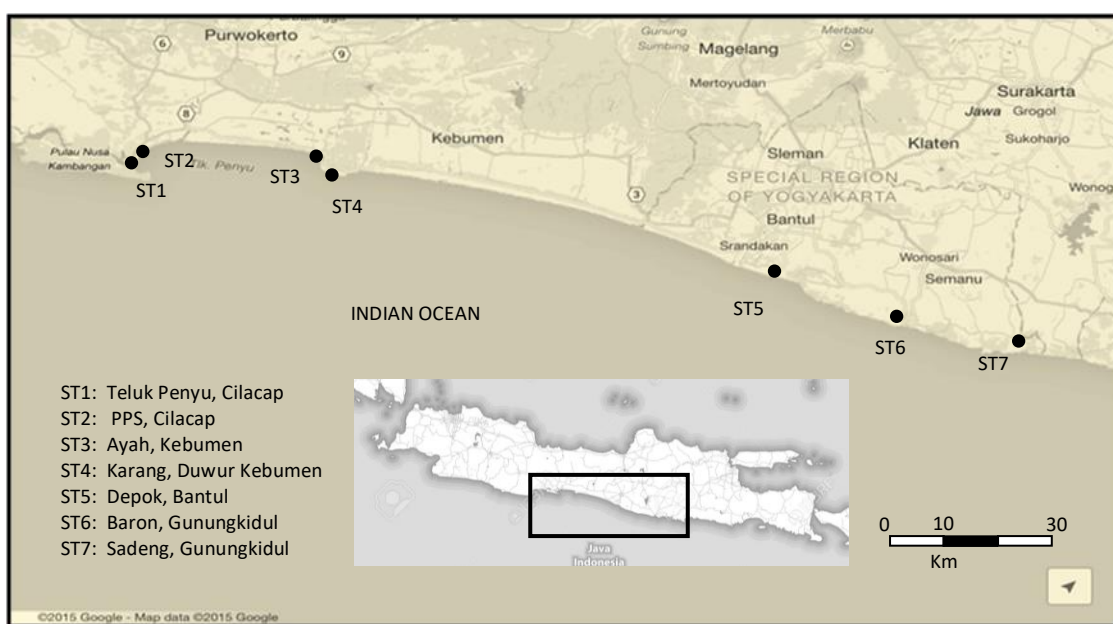


Figure 1. Station site (ST), Southern water of Java, Indonesia

Procedure

Lobster sampling was done randomly at the Southern Central Java waters (Cilacap, Kebumen, Purworejo districts), and Southern of Special Region of Yogyakarta waters (Kulonprogo, Bantul and Gunung Kidul districts). These locations are known as high lobster fishing areas. Sampling was conducted on February to August 2015 using lobster gillnet with mesh size ¾ inch. Sampling was performed six times at the locations. All lobsters captured were put in a cool box to be identified morphologically in situ based on Carpenter and Niem (1998). Genetic analyses of the sampled lobsters were carried out at the Integrated Laboratory of Diponegoro University, Semarang, Indonesia.

Lobster DNA was extracted in five steps. First, 1 g of leg tissue was crushed and placed in a mini tube containing DNA extractor. The solution was then centrifuged for 10 minutes, incubated at 95°C for 20 minutes and centrifuged for 10 minutes. Extracts of each sample were transferred to a microtube. The amount of mtDNA (in base pairs, bp) was determined using a Nanodrop and the number of its base pairs that are used as a basis of comparison with its standard lobster base pairs (bp) 566-571 (Abdullah et al 2014). The solution was diluted sample extract DNA template if more than bp standard and vice versa. Amplification of mtDNA base pairs molding method Polymorphic Chain Reaction (PCR) based methods Lavery et al. (2014). Nucleotide base pairs multiplication process begins by mixing a lobster leg muscle extract results from a DNA template preparation by PCR solution, namely primary brand Promega releases (LCOI) and reserve (HCOI) each at a concentration of 2 uL and 2.5 uL and added solution PCR 25 uL, add 18.5 uL H₂O, the overall

concentration is 50 uL (DNA extraction Promega). PCR method with the first phase template DNA sample was heated 95°C for 4 minutes, 94°C for 10 min, 20 min 47°C, 72°C 30 minutes, 72°C for 5 minutes. Overall the DNA template is heated 35 times, last indefinitely heating temperature 40°C. Separation of samples of genetic cellular molecules with the electrophoresis process is to form a mtDNA fragment. Soaked in a solution of ethidium bromide, and the solution will infiltrate into the DNA, which in turn serves to visualization as it will fluoresce when irradiated with uLtraviolet light.

Data analysis

DNA sequencing was performed at the PT. Genetic Center Indonesia. Philo-genetic analysis using codons code and software MEGA5.0 and Dendrogram use Primer_e software.

RESULTS AND DISCUSSION

A total of 1137 *Panulirus* spp. individual lobsters were collected, i.e. Cilacap (243 individual lobsters), Kebumen (129), Purworejo (13), Kulonprogo (55), Bantul (275), Gunungkidul (422), and Wonogiri not obtained data. Morphological identification of spiny lobster from Southern water of Java obtained six species of lobster, namely *P. homarus*, *P. versicolor*, *P. ornatus*, *P. polyphagus*, *P. penicillatus*, and *P. longipes*. Genetic analysis of 40 leg muscles of lobsters sample with distribution from Cilacap, Kebumen, Kulonprogo, Bantul and Gunung Kidul districts (Table 1).

Table 1. Number of *Panulirus* species collected per districts and sequencing analysis codes

Districts	Spesies	Code	Districts	Spesies	Code
Cilacap	<i>P. homarus</i> 0	CHP0	Bantul	<i>P. homarus</i> 1	BHP 1
	<i>P. homarus</i> 1	CHP 1		<i>P. homarus</i> 2	BHP2
	<i>P. homarus</i> 2	CHP 2		<i>P. homarus</i> 3	BHP3
	<i>P. homarus</i> 3	CHP 3		<i>P. homarus</i> 4	BHP4
	<i>P. homarus</i> 4	CHP 4		<i>P. homarus</i> 5	BHP5
	<i>P. homarus</i> 5	CHP 5		<i>P. homarus</i> 6	BHP6
	<i>P. ornatus</i> 1	CM 1		<i>P. penicillatus</i> 1	BBT1
	<i>P. versicolor</i>	CB		<i>P. penicillatus</i> 2	BBT2
	<i>P. polyphagus</i>	CP		<i>P. penicillatus</i> 3	BBT3
	<i>P. polyphagus</i> 1	CP 1		<i>P. ornatus</i>	BM
	Kebumen	<i>P. homarus</i>		KHP	Sadeng (Gunung Kidul)
<i>P. penicillatus</i>		KBT	<i>P. homarus</i>	SHP	
<i>P. ornatus</i>		KM	<i>P. homarus</i> 1	SHP1	
<i>P. polyphagus</i>		KP	<i>P. homarus</i> 2	SHP2	
<i>P. versicolor</i>		KBB	<i>P. homarus</i> 3	SHP3	
Kulon Progo	<i>P. homarus</i> 0	KPHP0		<i>P. polyphagus</i> 1	SBT1
	<i>P. homarus</i> 1	KPHP1		<i>P. polyphagus</i> 2	SBT2
				<i>P. polyphagus</i> 3	SBT3
				<i>P. polyphagus</i> 4	SBT4
				<i>P. versicolor</i>	SBB
			<i>P. longipes</i>	SBS	

Mitochondrial DNA (mtDNA) lobster sample of PCR process results, then optimized through the electrophoresis stage. The electrophoresis process, the band fraction that was not visible at the base pair (bp) reading under ultraviolet rays was 6 samples (Table 1 bold letters), i.e., CP, CP1, KBB, SBS, BHP4 and SBT1. We suspect the consequences of leg tissue lobster with less good condition.

Electrophoresis under ultraviolet light was shown that DNA base pair lobster sample at 600 bp. Two clades were obtained out of the 34 sequenced individuals. Clad 1 consisted of ten species lobster *P. homarus* (8) and *P. penicillatus* (2), namely CHP2, KBT, KPHP1, BHP3, SHP2, BHP1, KHP1, SBT3, SHP0, and SBT2. Clad 2 consists of 8 species, namely BBT2, BBT3, BBT1, SBB, CB, KM, BHP4, BM1. Kinship, morphological identification of lobster in clad 1 were identified as rock lobster *P. penicillatus* (SBT and KBT2). Phylogenetic group, common ancestor by lobster *P. homarus* (even being a clad and in groups of lobster *P. homarus*). Conditions of aquatic habitat of seabed fishing ground on Gunung Kidul district was rocks. Identification of spiny lobster morphologically was *P. penicillatus* (rock lobster), and genetic analysis was result as *P. homarus* (sand lobster). The condition presumed that identification of spiny lobster morphologically as *P. penicillatus* and identified genetically as *P. homarus*, the condition were estimate that an adaptation form in rock environment. Laveri et al. (2014) state that diversity of geographic pattern of genetic diversity resulted in an evolution of *P. homarus*. Base on color of spiny lobster Tlusty and Hyland (2005) state that phenotype color of lobster influenced by genetic aspects and mechanisms of diet. Variability color is a tool to identify lobster and differences of lobster color genetically difference by the phenotype of lobster. Seabed conditions Gunungkidul fishing ground as rock and color of lobster darker.

Spiny lobster has a complex life cycle and bio-oceans (Diniz et al. 2010), a tropical species with a very wide distribution (Lavery et al. 2014), aspects of evolution, distribution, ecology and biology of lobster and how they relate to genetic aspects (Reddy. 2013), but the association between the environment and the gradient is important as identifying the impact of natural selection (Sork and Waits 2010).

Diversity genetics can occur in different individuals within the same species (intra-specific), may also occur in some species (interspecific), and also diversity genetically between genus to another, and between families with each other family (Hoshino et al. 2012). Genetic variation spatially significant contribution to the genetic mapping and genetic maps effects on genetic variations that can be used as identification and restriction of gene flow (Sork and Waits 2010). The loss of genetic diversity can contribute to the collapse of the lobster fishery, cause ecological and social impacts on ecosystems and fisheries (Worm et al. 2006).

Discussion

We identified six spiny lobster species in southern Java waters. These were the *lobster pasir* (*P. homarus*), *lobster*

mutiara (*P. ornatus*), *lobster bambu* (*P. versicolor*), *lobster batu* (*P. penicillatus*), *lobster batik* (*P. longipes*) and *lobster cokelat* (*P. polyphagus*). Lobster catches in Cilacap, Kebumen, Purworejo, Bantul and Kulon Progo districts were dominated by *P. homarus*, whereas catches in the Gunung Kidul district were composed especially of *P. polyphagus*. Additionally, *P. homarus* was the most abundant species caught in muddy sand substrate while *P. homarus* was the most abundant species in the rock substrate.

Morphological identification of specimens collected in the Gunung Kidul district resulted in two different species, the rock spiny lobster and the sand spiny lobster. However genetic analyses clustered them into the same clad. The condition assumed as adaptation to the environment and low of light intensity.

Spiny lobster *Panulirus* sp. have a very complex life cycle and bio-oceans (Diniz et al. 2010), as its terms of tropical species that live with a very wide distribution (Lavery et al. 2014), aspects of evolution, distribution, ecology and biology lobster associated with genetic aspects (Reddy 2013), but the relationship between the environment and the gradient is important because it identifies the impact of natural selection (Sork and Waits. 2010). Genetic diversity may occur in individuals which are different even though the same species (intra-specific), also can occur in several species (interspecific), and genetic diversity can also occur when there is a cross between a genus one genus to another, and between the families one with other family (Hoshino et al. 2012). The loss of genetic diversity can contribute to the collapse of the lobster fishery, causing ecological and social impacts on ecosystems and fisheries Significant genetic variations also as a result of the contribution spatially, so the genetic condition of a species is important for genetically mapped, the genetic map is important because it can be used as identification and restriction of gene flow (Sork and Waits. 2010).

Species of *Panulirus* spp. in Southern Java waters were obtained of six species, namely *P. homarus*, *P. ornatus*, *P. versicolor*, *P. penicillatus*, *P. longipes*, and *P. polyphagus*. Species *P. homarus* dominant were caught in Cilacap, Kebumen, Purworejo, Bantul and Kulon Progo districts and the dominant of lobster were caught in Gunungkidul *P. penicillatus*. The dominant lobster caught in the muddy sand seabed substrate were lobster *P. homarus* and substrate rock *P. penicillatus*.

Identification morphologically obtained different species, but genetically in same of group. Such as, morphologically identification the lobster that dominant life at Gunungkidul and life in rock substrate, namely *P. penicillatus*. Genetically was same clad with *P. homarus*. The condition was assumed adaptation to the environment and low of intensity. The condition also important to review the conditions associated with the impact caused by environmental pollution in beach as habitat of lobster. Which within a few years has happened ship tanker with a cargo full of raw fuel oil have to sink and pollute the Southern waters of Java, also events ship tanker leaks that occur shipping channel into the crude oil refinery in Cilacap.

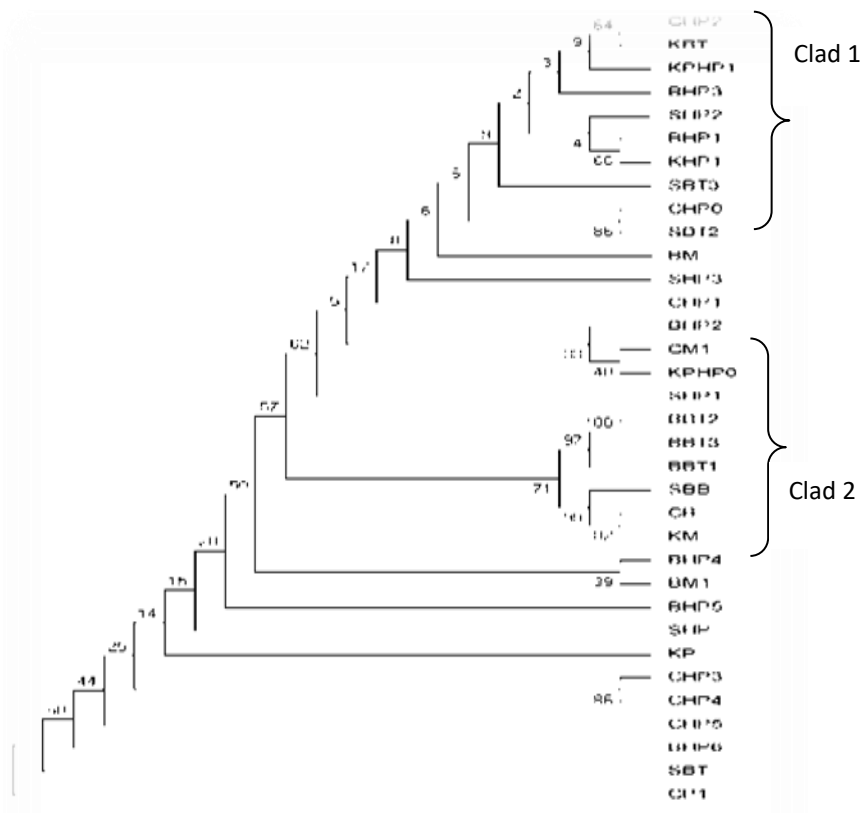


Figure 1. Phylogenetic of spiny lobster (*Panulirus* sp.) from Southern water of Java

Liquid and solid wastes extremely have polluted Southern Java waters. Risk levels of hydrocarbon in Cilacap sediment ranged from low to medium-low and were obtained location which has a greater impact on the ecological risk for marine organism (Syakti et al. 2015). Sewage disposal outlets produced a decrease in population density and changes in size spectra (de-la-Ossa-Carretero et al. 2010). Impact of Cilacap refinery site stations obtained heavy metal Cr, Ni, and Zn concentrations found in sediments and may cause the adverse effect to occur over a wider range of organisms and can contribute to a more serious harmful effect (Syakti and Hidayati 2015). Coastal areas of Cilacap has sensitive and very sensitive to the presence of oil spills (Wibowo et al. 2008).

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