Phylogeographic and molecular characterization of Pronghorn spiny lobster (Panulirus penicillatus Olivier, 1791) in the Southern Coast of Java and Lombok, Indonesia

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2Department of Oceanography, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Jl. Prof. Soedharto, Kampus Undip Tembalang, Semarang 50275, Central Java, Indonesia  

Abstract. Irwani, Wijayanti DP, Satria AW, Sabdono A. 2020. Phylogeographic and molecular characterization of Pronghorn spiny lobster (Panulirus penicillatus Olivier, 1791) in the Southern Coast of Java and Lombok, Indonesia. Biodiversitas 21: 5690-5696. Panulirus penicillatus is one of the most important lobster species in Central Java. It is one of the most populous species in the southern part of Java and Lombok. However, uncontrolled catching causes a decrease in the population of P. penicillatus. Despite such human threats, information about the taxonomic status of this species is limited. Several previous reports on the taxonomy of this species have always been based on the morphological features that cause ambiguous identification. Therefore, this study aimed to establish the relationships of P. penicillatus in southern parts of Java and Lombok by using the molecular technique. Twelve samples were collected from four study areas of the southern coast of Java and Lombok and identified using DNA barcoding technique. DNA barcoding technique was used for the first time to identify P. penicillatus in this region. This study demonstrated that all specimens belonged to P. penicillatus. However, one specimen (PPK-06) showed high intraspecies nucleotide divergence that formed a distinct subclade. Therefore, the specimen (PPK-06) could represent a cryptic species within P. penicillatus that needs to be studied further.

Keywords: Cluster analysis, DNA barcoding, Java, Lombok, Panulirus penicillatus, south coast

INTRODUCTION

The spiny lobster, a member of the Family Palinuridae, is the main target species for commercial fishery that support the economy of various countries (Heyman and Granados-Dieseldorff 2012; Diedrich et al. 2020). Global production of the fishery was valued to reach 260,000 metric tons per year with a landed value of approximately US$500 million (Penn et al. 2015). However, most traded lobsters are obtained from wild-catch due to inadequate contribution of aquaculture sector (Priyambodo et al. 2020; Jones et al. 2019; Jones 2010; Carpenter et al. 2011). During the last two decades, the mean size of wild catch lobsters is decreasing, more likely because of the intense fishing activity and lack of regulatory management. Currently, only Panulirus homarus and P. ornatus are farmed in the sea cages at Lombok, Indonesia, and Vietnam (Jones 2012; Jones et al. 2019).

One of the most important species in commercial fishery is the pronghorn spiny lobster, Panulirus penicillatus which probably has the widest global distribution among all the species of spiny lobster. The species is known to distribute throughout the entire Indo-Pacific regions, eastern and western regions of the Pacific Ocean (Holthuis 1991; Abdullah et al. 2014; Vaitheeswaran 2018), and even is found in the East Pacific (Chow et al. 2011). The species is found on rocky substrates, at shallow water less than 4 m depth, in the outer part of reef slopes and water channels (Cockcroft et al. 2013). The life cycle of individual species of Panulirus involved a very long pelagic stage that can last from several months up to almost 18 months in some species. The phyllosoma was then metamorphosed into puerulus which migrate towards the coast and settle at the shallow waters (Ernawati et al. 2017; Prince et al. 2020). This long pelagic life may facilitate the wide geographic distribution of the spiny lobster (Chow et al. 2011).

The pronghorn spiny lobster is traded as lobster tails in the form of fresh or frozen products with the Philippines and Indonesia are among the main exporter countries (Petersen et al. 2014)). In Indonesia, lobsters were caught from various regions such as along the southern coast of Java (Setyanto et al. 2019); the eastern part of Indonesia (Permana et al. 2014); Aceh Jaya, Kalimantan, Seram, and Raja Ampat (Wahyudin 2018). Indonesia Central Bureau of Statistics (BPS 2019) data showed that in the first quarter of 2014-2019, the value of Indonesia’s lobster commodity exports reached 7.09 million USD. The harvest rate of spiny lobsters in Indian Ocean among 11 WPP (Fisheries Management Area) has almost reached the total allowable catches (TAC) value (Wahyudin 2018).

The southern coast of Java is one of the main sources of spiny lobster production. The region contributes up to 10% of the national catch. The fishery in southern Java is
operated using two different gears, namely a tangled trap and an inshore bottom gillnet (Milton et al. 2014). There are 6 main targeted species of the fishery with *P. homarus* and *P. penicillatus* are the major catches. Despite being one of the main sources of the spiny lobster production, there is no report on the population distribution of *P. penicillatus* in Southern Java. A study on the exploitation rate of the species based on biological factors and the mortality rates suggested that the species is under overexploitation (Larasati et al. 2018). Therefore, it is necessary to investigate the genetic relationship among the pronghorn spiny lobster populations in Southern Java to obtain data on the sustainability of the species as well as to provide information on larvae sources through the current model which transport the larvae through the region. Oceanographic current presumably involves in producing the geographic barrier and directions of larval dispersal (Johannesson et al. 2019; White et al. 2010; Sanvicente-Añorve et al. 2018). In this study, we analyzed the highly polymorphic marker, mitochondrial DNA (mtDNA) control region of *P. penicillatus* to determine whether genetic diversity could be detected in Southern Java and Lombok populations and the relationship among populations by modeling the current.

**MATERIALS AND METHODS**

**Lobster sampling**

Sampling was done by getting the catches of fishermen who have fishing activities on the southern coast of Java and Lombok. After landing in the port, the result of lobster’s catch from the fishermen was sampled randomly. A total of 12 individuals of *P. penicillatus* were examined morphologically, then placed in the ice-cool box and brought directly to Marine Science Laboratory for further analyses. Sampling site was shown in Figure 1.

**Molecular technique**

Total genomic DNA was extracted from each sample using a modified Gopal protocol (Gopal et al. 2006). Muscle tissue samples for mitochondrial extraction (mtDNA) were taken from the leg of the first pleopod with a size of 0.2 mm. Then the muscle was ground using a mortar and pestle, placed in a 1.5 ml mini tube that contains a 100 µL Aliquot 20% chelex (DNA extractor) and closed, then vortex for 10-15 seconds. To separate the supernatant, the sample was centrifuged at a speed of 10,000 rpm for 1 minute. Furthermore, the samples were incubated at 95 °C for 20 minutes, vortexed again for 10-15 seconds, and re-centrifuged for 10-15 minutes at 10,000 rpm. The resulting supernatants containing the genomic DNA were transferred to a new tube.

Supernatant produced from DNA extraction in the microtube was then added with 2 µL universal primer (R) (LCO-ph): 5’- GTCAACAAATCATAAGATATTGG-3’ and Primer Forward (F) (HCO-ph): 5’- TAACCTCAGGGTGACCAAAAAATCA -3’, 6 µL dd H2O, 2, 5 µL DNA template and 12.5 µL Green Master Mix, in total 25 µL PCR mix solution. PCR products were purified using the Qiagen gel cleanup system, separated by using electrophoresis, and visualized by using a UV transilluminator, and the good quality PCR products were selected and sent to PT. Indolab, Jakarta, Indonesia for nucleotide sequencing.

**Construction of phylogenetic-tree**

Phylogenetic-tree was constructed according to the method of Sabdono et al. (2019). The CLUSTAL X and the PAUP*4.0 software package were used to establish the tree. The DNA sequences were submitted to the GenBank database with accession numbers from MT750271 to MT750282. The COI sequences of *Acanthacaris tenuimana* KF828006.1 was used as an Outgroup.

**Figure 1.** Sampling site locations of *Panulirus penicillatus* on south coast of Java and Lombok islands, Indonesia
Dataset

We used surface current data to investigate the possible mechanisms of the genetic distribution of *P. penicillatus*. Surface current data was obtained from the monthly Global Reanalysis Ensemble Product from Mercator Ocean at 1/4° resolution (Gounou et al. 2020) from December 2017 to November 2018. This dataset was produced by combining numerical ocean models constrained with data assimilation of satellite and in situ observations (i.e., GLORYS2V4 from Mercator Ocean (France), ORAS5 from ECMWF, GloSea5 from Met Office (UK) and C-GLORS05 from CMCC (Italia)). The multi-model ensemble approach provides a more reliable dataset than the individual reanalysis product since the estimation of the uncertainties or error bars in the ocean state can be estimated. This dataset can be downloaded at https://resources.marine.copernicus.eu/?option=com_csw&view=details&product_id=GLOBAL_REANALYSIS_PHY_001_031. The analysis was conducted based on season i.e., southeast monsoon and northwest monsoon season. We calculated June, July, and August (December, January, and February) data to construct a southeast (northwest) monsoon mean map.

RESULTS AND DISCUSSION

Morphological characteristics within the lobster of *Panulirus penicillatus*

A total of 12 individual species of *P. penicillatus* were used in this research. Samples were collected from four study areas of the south coast of Java and Lombok namely Cilacap, Kebumen, Gunung Kidul, and Lombok. Lobster’s samples of *P. penicillatus* from the four locations showed a slight morphological difference in colors (Figure 2). The lobster’s body colors ranged from yellowish green to blue (black) depending on habitat and morphometry (spines on the antennular plate grooves on the abdomen and exopod of the flagellum).

### Table 1. Details of *Panulirus penicillatus* samples from 4 different locations

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Sampling location</th>
<th>Sex</th>
<th>Carapace length (cm)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPC-04</td>
<td>Cilacap</td>
<td>Female</td>
<td>6.45</td>
<td>186</td>
</tr>
<tr>
<td>PPC-05</td>
<td>Cilacap</td>
<td>Male</td>
<td>5.50</td>
<td>134</td>
</tr>
<tr>
<td>PPC-06</td>
<td>Cilacap</td>
<td>Male</td>
<td>4.82</td>
<td>90</td>
</tr>
<tr>
<td>PPC-07</td>
<td>Gunungkidul</td>
<td>Female</td>
<td>4.98</td>
<td>103</td>
</tr>
<tr>
<td>PPC-08</td>
<td>Gunungkidul</td>
<td>Male</td>
<td>4.50</td>
<td>74</td>
</tr>
<tr>
<td>PPK-06</td>
<td>Kebumen</td>
<td>Female</td>
<td>5.48</td>
<td>141</td>
</tr>
<tr>
<td>PPK-07</td>
<td>Kebumen</td>
<td>Male</td>
<td>5.39</td>
<td>133</td>
</tr>
<tr>
<td>PPK-08</td>
<td>Kebumen</td>
<td>Male</td>
<td>5.09</td>
<td>114</td>
</tr>
<tr>
<td>PPL-06</td>
<td>Lombok</td>
<td>Female</td>
<td>6.17</td>
<td>216</td>
</tr>
<tr>
<td>PPL-07</td>
<td>Lombok</td>
<td>Female</td>
<td>6.89</td>
<td>279</td>
</tr>
<tr>
<td>PPL-08</td>
<td>Lombok</td>
<td>Female</td>
<td>6.65</td>
<td>255</td>
</tr>
<tr>
<td>PPL-09</td>
<td>Lombok</td>
<td>Female</td>
<td>6.60</td>
<td>236</td>
</tr>
</tbody>
</table>

Table 1 showed that carapace length of the lobsters varied between 4.50 and 6.89 cm, while the carapace weight was between 74 and 279 g, respectively. The sex determination demonstrated that samples contain more female than male lobster. Previous studies reported that the weight and length intervals of carapace spiny lobsters of Bantul and Cilacap were varied between 6.5 and 771.7 g, and 2.7 to 10.3 cm (Haryono et al. 2016). While, Onkgers et al. (2014) reported that the interval weight of carapace spiny lobsters of Latulahat, Ambon ranged from 101 to 1130 g, and the carapace length ranged from 6.0 to 14.1 cm.

Molecular identification of *Panulirus penicillatus*

The sequences of *P. penicillatus* COI region were analyzed homologically by using BLAST (Altschul et al. 1990). The result showed that the sequences of specimens collected in this study were similar to the deposited sequences on GenBank and recorded as *P. penicillatus* (Access no. MT750271 to MT750282).

Even there were slight differences in body’s colors of lobster’s samples (Figure 2), however, DNA sequenced analyses showed that the specimens used in this study indeed all belonged to *P. penicillatus* (Table 2).

### Table 2. Homology analyses of *Panulirus penicillatus* from 4 different locations

<table>
<thead>
<tr>
<th>Sample code</th>
<th>BLAST Identification</th>
<th>Voucher</th>
<th>Base Pair</th>
<th>Per ident.</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPC-04</td>
<td><em>Panulirus penicillatus</em></td>
<td>Cilacap</td>
<td>674</td>
<td>99.54%</td>
<td>MT750271</td>
</tr>
<tr>
<td>PPC-06</td>
<td><em>Panulirus penicillatus</em></td>
<td>Cilacap</td>
<td>674</td>
<td>99.85%</td>
<td>MT750272</td>
</tr>
<tr>
<td>PPC-05</td>
<td><em>Panulirus penicillatus</em></td>
<td>Gunungkidul</td>
<td>674</td>
<td>99.85%</td>
<td>MT750273</td>
</tr>
<tr>
<td>PPC-07</td>
<td><em>Panulirus penicillatus</em></td>
<td>Gunungkidul</td>
<td>674</td>
<td>100%</td>
<td>MT750274</td>
</tr>
<tr>
<td>PPK-06</td>
<td><em>Panulirus penicillatus</em></td>
<td>Kebumen</td>
<td>674</td>
<td>99.55%</td>
<td>MT750275</td>
</tr>
<tr>
<td>PPK-07</td>
<td><em>Panulirus penicillatus</em></td>
<td>Kebumen</td>
<td>640</td>
<td>98.75%</td>
<td>MT750276</td>
</tr>
<tr>
<td>PPK-08</td>
<td><em>Panulirus penicillatus</em></td>
<td>Kebumen</td>
<td>674</td>
<td>99.85%</td>
<td>MT750277</td>
</tr>
<tr>
<td>PPL-06</td>
<td><em>Panulirus penicillatus</em></td>
<td>Lombok</td>
<td>674</td>
<td>99.84%</td>
<td>MT750278</td>
</tr>
<tr>
<td>PPL-07</td>
<td><em>Panulirus penicillatus</em></td>
<td>Lombok</td>
<td>674</td>
<td>99.85%</td>
<td>MT750279</td>
</tr>
<tr>
<td>PPL-08</td>
<td><em>Panulirus penicillatus</em></td>
<td>Lombok</td>
<td>674</td>
<td>99.70%</td>
<td>MT750280</td>
</tr>
<tr>
<td>PPL-09</td>
<td><em>Panulirus penicillatus</em></td>
<td>Lombok</td>
<td>674</td>
<td>99.85%</td>
<td>MT750281</td>
</tr>
</tbody>
</table>
Discussion

Color variation in crustaceans is a complex subject because of the variety of mechanisms that might cause the changes (Stevens et al. 2007). Lobsters come in many different colors because of the pigment chromatophores in their shells. Lobsters come in many different colors because of the pigment chromatophores in their shells. Color variation is one of the many spatial differences in the biology of Lobster. Colour plays an important role in grading and marketing the Lobster (Bryars & Geddes 2005). In Southern rock lobster, Jasus edwardsii, the red color of the lobster was influenced by the depth where the lobsters were fished. The number of red colors will decrease with depth while the paler lobster mostly is dominated by the deeper depth (Chandrapavan et al. 2009). Although lobster color is also controlled genetically, the mechanism by which phenotypic color is controlled has not yet been revealed (Tlusty et al. 2009; Duarte et al. 2017).

Some previous studies reported that the coloration of many crustacean tissues was controlled by the type and amount of carotenoids ingested and a number of environmental factors, such as the color of the background substrate, light intensity, photoperiod, and temperature, which may also produce physiological color changes in crustaceans (Wade et al. 2005; Tlusty et al. 2009).

NJ clustering analysis showed that there were different clades among the sequences of P. penicillatus in the southern parts of Java and Lombok, India, Australia, and the Philippines (Figure 3). It seems that there were no apparent geographical barriers among the sampled regions. This is likely related to its life cycle and the high dispersal capacity of its planktonic larvae. Previous studies on the free-floating phyllosoma larval phase (Matsuda et al. 2006) reported that spawning and early larval stages of P. penicillatus in oceanic waters probably exceeded 8-11 months. These early larval stages exhibit high dispersal capacity, often more related to ocean currents and winds.

Figure 4 shows the distribution of surface current patterns on the southern coast of Java. Alternating current direction is obtained on the southern coast of Java known as the South Java Coastal Current (SJCC). SJCC is the main current system on the southern coast of Java generated by the local wind forcing and the wind-forced equatorial Kelvin waves (Utari et al. 2019). During the northwest monsoon season, the SJCC moves eastward while during the southeast monsoon season, the westward current dominates in this region. The magnitude of westward SJCC is greater than eastward SJCC due to the influence of Indian Throughflow (ITF) which flows in the same direction as the westward SJCC. As reported by Sprintall et al. (2010) SJCC is regulated by ITF that exits from the Lombok Strait. Furthermore, the weak southward current is observed along the onshore area during the southeast monsoon season. This weak southward current corresponds to the occurrence of coastal upwelling which has been reported by many researchers (e.g. Iskandar et al. 2009; Wirasatriya et al. 2020). Cyclonic eddy is also detected at the eastern part of the southern coast of Java. This indicates an upwelling occurrence induced by negative Ekman pumping as has been demonstrated by Wirasatriya et al. (2020). The alternating current pattern along the southern coast of Java, Bali, and Lombok may cause the genetic similarity of the lobsters distributed in those areas. However, some questionable points are still obtained from the phylogenetic tree as shown in Figure 3. The lobsters in southern Java and Lombok have different subclades from the lobsters in India, Australia, and the Philippines. The present study revealed that there is no ongoing gene flow between the southern coast of Java and Lombok and Indian, Australian, and the Philippines P. penicillatus populations. In contrast, very little evidence of
population structuring was observed within southern coast of Java and Lombok. It is unusual that PPK-06 species found in Kebumen waters showed different sub-subclades from the other species of southern coast Java and Lombok in the studied population. In general, it could be said that the presence of lobsters on the southern coast of Java and Lombok is supported by the entry of lobsters from eastern Indonesia, local spawning lobsters, and possible input of lobsters from western Indonesia. Despite the long pelagic larval period common to all spiny lobster species, a narrow distribution range is observed in several species of Panulirus. Chow et al. (2011) stated that spiny lobster species having very wide geographic distributions may show regional variation in population structure. The result should be investigated further in more detail using larger sample sizes and more variable genetic markers. Further current pattern analysis for the wider region should also be conducted to examine this problem.

Our findings suggest that *P. penicillatus* consist of a monophyletic clade within its known distribution range on southern coast of Java and Lombok, and challenge current models for the predicted dispersal of the species. Additional sampling of *P. penicillatus* populations in this area are required to fully understand the diversity of this group.

![Figure 3](image1.png)

**Figure 3.** Phylogenetic tree based on comparative COI mtRNA gene sequence analysis of *Panulirus penicillatus* species showing the phylogenetic affiliation of the southern coast of Java and Lombok, Indonesia. *Acanthacaris tenuimana* KF828006.1 was used as an outgroup.

![Figure 4](image2.png)

**Figure 4.** Map of the mean surface current pattern along the southern coast of Java during a) Southeast monsoon season (June, July, August) and b) Northwest monsoon season (December, January, February)
ACKNOWLEDGEMENTS

This work was supported by a dissertation grant from Diponegoro University, Semarang, Indonesia. The authors are grateful to Sakti and Abi for helping in laboratory work.

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