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Molecular identification and potency of scalloped spiny lobster (*Panulirus homarus*) study from Kodang Merak Beach, South Malang

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Abstract. Lobster is one of the commodities from the fisheries sector that has high economic value. The problem faced is that there is still limited biological information on lobster species in Indonesia, including identifying genetic diversity. The purpose of this study was to determine the potential ofscalloped spiny lobster. Lobster identification was carried out morphologically and molecularly on the most dominant lobster species at Kondang Merak Beach, Malang. The method in this study used observation and experimental methods. The results of recording data on natural lobster catch from local fishermen from Kondang Merak,scalloped spiny lobster is the most dominant species compared to other types of lobster (painted spiny lobster, ornate spiny lobster). Morphologically, the most dominant lobster sample (MAL-01) obtained from this research location was *Panulirus homarus*. The lobster has a color pattern and meristic characteristics that are in accordance with the morphological identification reference used. Molecular identification of scalloped spiny lobster (DNA barcoding) has shown that the lobster specimen is a scalloped spiny lobster (*Panulirus homarus*) which has the same sequence as KF548574 (100%) in the BLASTN results with the Genbank Database.

1. Introduction

Kondang Merak is one of the coastal areas in South Malang, whose overall economic income depends on the wealth of marine resources in the area. Some of the exploration and exploitation activities carried out by the community are lobster catching [1]. Lobsters are large crustaceans that are found in tropical and subtropical seas. Lobsters in the *Panurilus* genus have a wide distribution area and belong to the Palinuridae family and are commonly referred to as spiny lobsters [2]. Sand lobster (*Panulirus homarus*) is one type of lobster that is commonly found in Indonesian waters, especially in the waters of the Indian Ocean [2].

Lobsters or crayfish generally live in rocky places, such as on coral (both on live and dead coral) and can also be found in fine rocky sand. Lobsters like sheltered areas and calm waters to live in and often hide among the corals. Lobsters enter into nocturnal animals, during the day lobsters take refuge in between or

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coral caves and at night come out of their hiding places and look for food. Lobsters have an important ecological role, namely as benthic predators in marine waters, lobsters eat benthic animals from the mollusk group (bivalves and gastropods) and echinoderms (sea urchins, sea cucumbers, starfish, and sea lilies [3].
The importance of balance and the existence of lobster stocks in nature and can result in stock declines, imbalanced ratios between lobster sexes, to the extinction of sand lobster species. Therefore, it is necessary to identify the molecular and reconstruct the phylogenetic tree of the existing lobster resources. One of the popular molecular identification techniques that can be used in the identification of an organism from species to subspecies that is carried out accurately on various species that are difficult to distinguish morphologically is the DNA barcoding technique. DNA barcoding is a system designed for fast and accurate species identification as a species marker . While morphological identification is the identification of species based on the structure, shape, and external body parts of a living creature. The initial development of the taxonomy of a species is based on morphological characteristics, namely by distinguishing lobster species with several characters [4]. Identification based on DNA barcodes has been agreed globally with various advantages and is very simple and uses universal tools covering all animals in both fresh samples and processed products [5]. Branching relationships that show evolutionary relationships between various sequences of living things based on similarities and differences in physical and genetic characteristics that are inherited from their parents as a logical approach to showing evolutionary relationships between organisms are called phylogenetic diagrams. The results obtained can be applied to make biological systematics, look for the function of a gene or protein, medical research, epidemiology to evolutionary studies. To determine the phylogenetic tree, it is necessary to sequence the data from the sequencing results [6]. Identification of spiny lobster genetic diversity is very important in the management of lobster resources, both in stock enrichment, quarantine interests, conservation and sustainable management as well as information to related institutions in managing limited lobster fishery resources [4]. Based on the problems above, it is necessary to conduct research on the Identification and Reconstruction of Phylogenetic Trees of Sand Lobster (*Panulirus homarus*) at Kondang Merak Beach, South Malang.

2. Materials and methods

This research is an observational and experimental research. Analysis and presentation of data using descriptive methods, descriptive research using the object of scalloped spiny lobster (*Panulirus homarus*) at Kondang Merak Beach, South Malang with a molecular approach. The molecular approach was carried out by looking at the DNA sequences of the COI gene markers. The DNA samples that have been obtained from the isolation process will be amplified using the PCR technique and visualized by electrophoresis. After that the DNA is then sequenced. Sequencing results will be analyzed qualitatively and quantitatively. Qualitative analysis was carried out by reconstructing phylogenetic trees while quantitative analysis was carried out by looking at the genetic distance (p-distance) between species using MEGA-X software.
This research activity was carried out from September 2020-April 2021 in Kondang Merak Beach, South Malang, East Java Province. The main ingredient used in this research is scalloped spiny lobster (*Panulirus homarus*) taken from the catch of local fishermen at Kondang Merak Beach, South Malang. Other ingredients are frozen ice and sterile distilled water. The materials used for DNA extraction are 70% alcohol, 90% ethanol, andThe NEXprep™ Cell/Tissue DNA Mini Kit consists ofbuffer GT1-2, buffer W1-2, ellution buffer and Proteinase K. Primary dilution using TAE (Tris Acetate EDTA) material. The materials used for PCR were PCR mastermix (Promega M750), distilled water and DNA template. Electrophoresis using TAE buffer and 2% agarose gel. The materials used for sequencing were pure DNA, FishF1R1 primer, QIAquick PCR Purification Kit (Qiagen 28104), 70% ethanol, 90% ethanol, buffer and distilled water.

3. Results and discussion

3.1. Lobster Catch Data at the Kondang Merak Beach

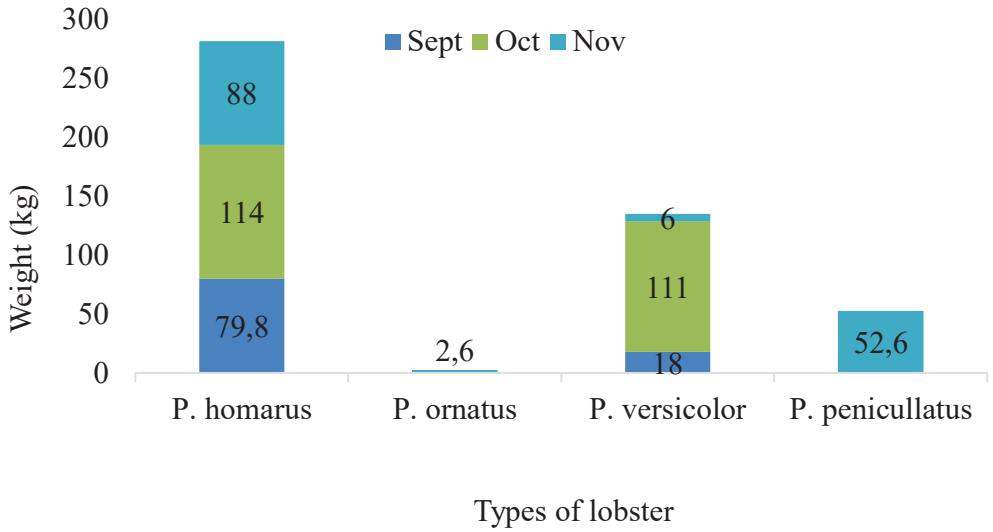

Figure 1. Result of lobster catch data at the Kondang Merak Beach

Based on observational data obtained from the catch of traditional fishermen at Kondang Merak Beach, South Malang (Figure 1), it shows fluctuations in each species. Observational data obtained from September to November 2020, *Panulirus homarus* is the type of lobster with the highest number of catches and for 3 months of observation its availability is always there compared to other types of lobster.

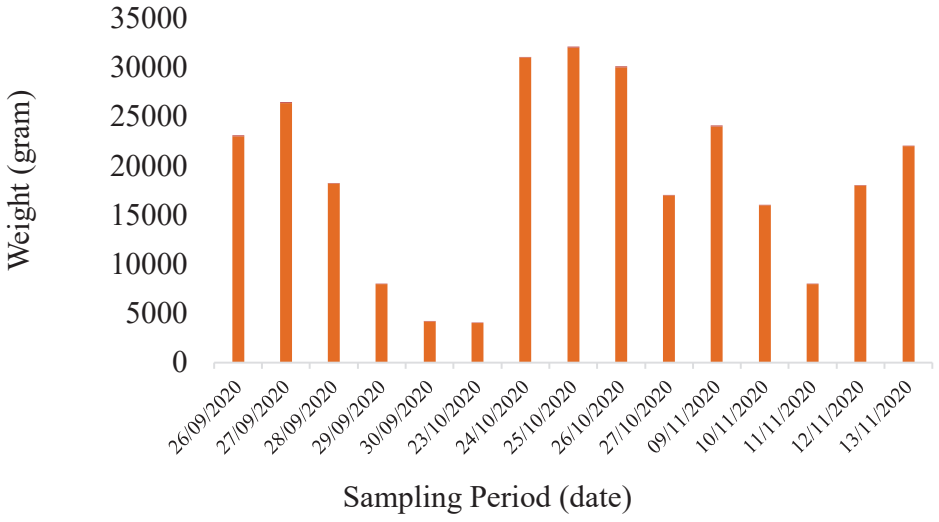

Figure 2. Graph of data on observation of scalloped spiny lobster catches at Kondang Merak Beach, South Malang.

In the observation data of scalloped spiny lobster catches at Kondang Merak Beach, South Malang, shows that the availability of scalloped spiny lobster is always available. In the sampling in September 2020, the smallest weight obtained was 4.2 kg and the largest was 26.4 kg. Then in October the smallest was 4 kg and the largest was 32 kg. Meanwhile, in November the smallest scalloped spiny lobster was 8 kg and the largest was 24 kg. During the sampling period the average total weight of scalloped spiny lobster ranged from 4 kg to 32 kg (Figure 2).

3.2. Morphological Identification

(a) (b)

Figure 3. Scalloped spiny Lobster Morphology (*P. homarus*), (a) Sample code MAL01 (Personal documentation) and (b) *P. homarus* images according to Setyanto and Halimah (2019).

Identification of scalloped spiny lobster species was carried out morphologically and molecularly. Morphological identification carried out included observing the pattern, color of all parts of the lobster body, morphometric characteristics and meristic characteristics. The morphometric characters measured included total length, carapace length, abdominal segment length, and tail length. Meristic characters observed included spina on the antennae, eyes, horns, spina carapace, antennae pattern, and walking legs (Figure 3). Scalloped spiny lobster species obtained from Kondang Merak Beach, South Malang can be seen at Table 1.

3.3. Molecular Identification

DNA barcoding is one of the molecular identification techniques used in identifying species. Molecular identification of scalloped spiny lobster species starts from DNA extraction, PCR and electrophoresis which is then sequenced at PT. Genetics Science Indonesia to generate sequences of nucleotide bases. The data sequences that have been obtained are then processed using MEGA X software. MEGA X software is used to analyze and match the sequence data obtained with the sequences contained in Genbank at NCBI (National Center For Biotechnology Information) using BLASTN (Basic Local Alignment Search Tool Nucleotide) based on the degree of similarity. The sequence data obtained were edited with alignment tools in MEGA X Software and aligned with the Clustal W method to see the diversity of nucleotide bases.

Alignment results are used to determine the type of species obtained. Subsequently, a phylogenetic tree was created to see the level of relationship between species and to determine the genetic distance of each species. The neighbor joining tree method selects sequences which when combined will provide the best estimate of the branch length that most closely reflects the real distance between the sequences [7]. The 1000x bootstrap analysis aims to test the accuracy of branching phylogenetic trees. The results of the phylogenetic reconstruction of the scalloped spiny lobster at Kondang Merak Beach can be seen in Figure 3.

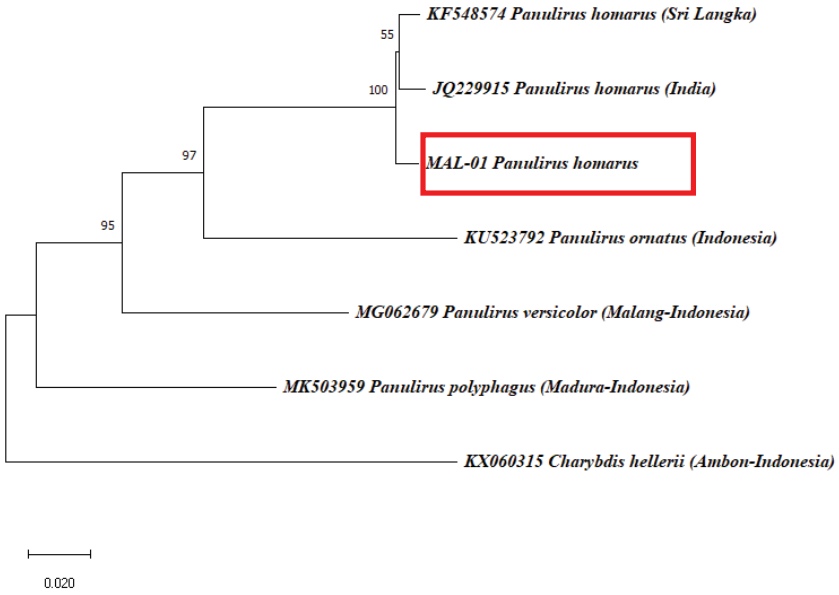

Figure 4. Results of Reconstruction Phylogenetic Tree of Scalloped spiny lobster (*P. homarus*) Sample Sequences with Sequences from Genbank.

A phylogenetic tree is a description of a tree-like lineage of living things that is used as a logical approach to show evolutionary relationships between organisms [8]. Phylogenetic analysis can be strengthened by the value of the calculation of genetic distance (pairwise distance). Genetic distance was carried out using the Kimura-2-Parameter analysis method. The alilysis is used because it considers the level of transition and transversion substitution so that it is effective for DNA Barcoding. The genetic distance between scalloped spiny lobster species can be seen in Table 1.

Table 1. BLASTN Results from Genbank NCBI

No.	Sample Code	Species Name	Query Cover (%)	Identity (%)	Genbank No.
1.	MAL-01	*Panulirus homarus*	100%	98.46%	KF548574
2.	MAL-01	*Panulirus homarus*	99%	98.46%	JQ229915

The sample code MAL-01 shows a 100% similarity to the scalloped spiny lobster species (*Panulirus homarus*). The results of the BLASTN analysis of *P. homarus* samples from Kondang Merak Beach, South Malang can be seen in Table 2. Table 2 shows that the identified scalloped spiny lobster samples have a high degree of similarity, namely 98.46%. The higher the similarity in the BLASTN analysis, the more accurate the results are because there is a match between the sample and the data on Genbank.

Table 2. Genetic Distance of Scalloped spiny Lobster (*Panulirus homarus*) compared to other Species Sequences from Genbank

No.	Species Name	1	2	3	4	5	6
1	MAL-01 *P. homarus*						
2	KF548574 *P. homarus* (Sri Lanka)	0.0156					
3	JQ229915 *P. homarus* (India)	0.0173	0.0156				
4	MG062679 *P. versicolor* (Malang, Indonesia)	0.1657	0.1727	0.1681			
5	MK503959 *P. polyphagus* (Madura, Indonesia)	0.1965	0.1990	0,2014	0.1857		
6	KU523792 *P. ornatus* (Indonesia)	0.1516	0.1496	0.1563	0.1842	0.2146	
7	KX060315 *Charybdis hellerii* (Ambon, Indonesia)	0.2897	0.2847	0.2795	0.2495	0.2335	0.2850

The phylogenetic tree was formed from scalloped spiny lobster sequences that had been obtained from Kondang Merak Beach, South Malang and added with sequences from Genbank as many as 2 individuals in the *Panulirus homarus* species, 1 individual in the P. ornatus species, 1 individual in the P. versicolor species and 1 individual in the P. versicolor species. P. polyphagus species with access numbers and locations are presented. Sequence data downloaded from Genbank was retrieved which had a strong relationship with the sequences obtained, as evidenced by the 99-100% BLAST yield percentage rate. Genetic distance analysis was performed using MEGA X software (Table 3). The results of the analysis showed that the sample code MAL-01 *P. homarus* obtained from Kondang Merak Beach, South Malang had an intragenetic distance of 0.01560-0.01737, while the farthest intragenetic distance was with P. polyphagus (0.19652).

4. Conclusions

Scalloped spiny lobster or *Panulirus homarus* is the most common type of lobster caught by traditional fishermen at Kondang Merak Beach, Malang. The results of recording lobster catches show that from September to November 2020, the type of scalloped spiny lobster reached 281.8 kg. Morphologically, the MAL-01 sample obtained from Kondang Merak Beach, South Malang is *Panulirus homarus*. This lobster has a color pattern and meristic characteristics that match the reference used. Molecular identification of scalloped spiny lobster (DNA barcoding) has shown that the lobster specimen is a scalloped spiny lobster (*Panulirus homarus*) which has the same sequence as KF548574 (100%) on the results of BLASTN with the Genbank Database. Based on the phylogenetic reconstruction, the scalloped spiny lobster *Panulirus homarus* and NCBI sekeun indicate that the scalloped spiny lobster species *P. homarus* has a close relationship with *P. ornatus*. Research related to the genetics of sand lobster is a new thing, so studies related to genetics of lobsters of different types with a larger number are needed as preliminary genetic data of lobsters that can then be used in phylogenetic mapping.

5. References

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