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by Sapto Andriyono

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Next-generation sequencing yields the complete mitochondrial genome of mud spiny lobster, *Panulirus polyphagus* (Crustacea: Decapoda) from Madura water

S Andriyono[1,2*], M J Alam[1], H Pramono[2], A A Abdillah[2], H W Kim[1,3]

[1]Interdisciplinary Program of Biomedical, Mechanical and Electrical Engineering, Pukyong National University, Busan, 48513, Republic of Korea
[2]Department of Marine, Fisheries and Marine Faculty, C Campus Jl. Mulyorejo Surabaya 60115. Universitas Airlangga, Surabaya, East Java, Indonesia
[3]Department of Marine Biology, Pukyong National University, Busan 48513, Republic of Korea

[*]Email: sapto.andriyono@fpk.unair.ac.id

Abstract. The circular mitochondrial genome of the mud spiny lobster, *Panulirus polyphagus* was determined by next-generation sequencing (NGS) platform. The mitochondrial genome of *P. polyphagus* was 15,707 bp in length, which comprised 13 protein-coding genes, 22 tRNAs, two ribosomal RNAs (12S and 16S). A non-coding putative control region (739 bp) was located between 12S ribosomal RNA and tRNA-*Ile*. Except for COX1, 13 protein-coding genes initiated with the conventional start codon (ATG). The phylogenetic analysis with the mitogenomes in family Palinuridae showed *P. polyphagus* was clustered together with four congener species forming a clade, and *Panulirus versicolor* is closest to *P. polyphagus*.

1.Introduction

Lobster is a well-known commodity in the fishery industry, especially the crustacean global fishery market [1]. In Indonesia, the exploitation of this species in 2014 is 3,179 ton or US$ 42,096,000 [2]. The exploitation causes some areas to experience over-exploitation of Lobster species, including this type of spiny lobster [3]. The genus *Panulirus* is characterized by vibrant colors with habitats that are shallow sea waters to a depth of 683 m [1]. The distribution of *Panulirus polyphagus* habitat covering Pakistan to Vietnam, the Philippines, Indonesia, north-west Australia, and the Gulf of Papua [4]. Within the 21 known species of Panulirus, seven of them are found in the Indonesia water, and one of them is *P. polyphagus* (Herbst 1973) [4, 5]. Several of the biological aspects of this aspect of spiny lobster have been widely used, among others, digestive systems [6], fecundity [7], larvae growth under laboratory condition [8, 9], and assessment of *P. polyphagus* in north-west coast of India [10]. General information of spiny lobster buffer has received more attention because this species is an essential commodity in aquaculture [3]. Furthermore, there is a lack of knowledge regarding DNA information of this lobster species. Previous research on molecular details of the genus *Panulirus* has been done on the region of COI and 16S ribosomal RNA [11].

Ecologically, *Panulirus* has a role as a benthic and carnivore animal [12]. They were influences of the structuring of benthic habitat by food web cycles and also occasionally importance role by suppressing herbivorous animal and or space competitors [13]. They feed various benthic organisms such as gastropod molluscs and pelecypod, echinoid and asteroids, crustaceans, and some feed contain

calcareous materials, including sponges [14]. Other researcher reported that Palinurids also feeding partially algae [15]. Although as a carnivore, lobsters also become prey of several marine carnivores fish species such as sharks, skates, large snappers, groupers, jewfish, octopus or marine mammals including dolphins and marine reptile loggerhead turtles [16]. Then humans constitute the most significant threat of declining lobster populations in Indonesia due to being valuable fisheries commodity and exclusive consumption for several communities [17].

In this study, we report the complete mitochondrial genome of Spiny Lobster *P. polyphagus* (Herbst 1973). In addition, we perform phylogenetic tree analysis with six *Panulirus* complete mitochondrion genome. Based on the GenBank database, only six species of *Panulirus* found, there are *P. japonicus* NC004251 [18], *P. stimpsoni* NC014339 [19], *P. ornatus* NC01485 [20, 21], *P. cygnus* NC028024 [22], *P. homarus* NC016015, and *P. versicolor* NC028627 [23].

2. Material and Methods

2.1. Samples collection and extraction

Five *P. polyphagus* specimens were collected from at Sumenep local market in Madura Island (7°02'40"S and 113°56'52"E) in Indonesia. Collected tissue was directly stored in 96 % ethanol (J.factory, Korea) and kept at -20°C until used for further analysis. Lobster muscle from claw was dissected and homogenized by the tissueLyser II (Qiagen). Genomic DNA was isolated using AccuPrep® Genomic DNA genomic extraction kit (Bioneer, Daejeon, Republik of Korea) according to the manufacturer's protocol. Purified genomic DNA was quantified by the Nanodrop spectrophotometer (Thermofisher Scientific D1000) and stored at the -70°C until used for PCR.

2.2. Mitochondrial DNA extraction

Species identification of the specimen was confirmed by both its morphological characteristics and DNA sequence identity (99. 17%) in the COI region to GenBank database (JN418939). Mitochondrial DNA was extracted by a commercial kit ab65321 (Abcam, UK), followed by the fragmentation of the purified mitochondrial DNA into smaller sizes (~350 bp) by Covaris M220 Focused-ultrasonicator (Covaris Inc., Woburn, MA, USA). A library for sequencing was constructed by TruSeq® RNA library preparation kit V2 (Illumina Inc., San Diego, CA, USA) and its quality and quantity were analyzed by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). DNA sequencing was performed by MiSeq sequencer (Illumina, San Diego, CA) and the complete mitogenome of *P. polyphagus* was assembled by Geneious v 11.0.2 [24].

2.3. The tRNA and Gene Map Phylogenetic Analysis

After assembled of sequence, sequin file and GenBank file format was create using Sequin Application version 15.50 [25]. The tRNAs were predicted by ARWEN, then gene map was usingOGDraw [26]. Phylogenetic trees complete mitochondrial genome sequences were constructed by MEGA 7 program [27].

Table 1. The mitochondrial DNA organization of the *Panulilus polyphagus*

Gene	Position		Size (bp)	codon		space (+) overlap (-)
	Start	Stop		Start	Stop	
COX1	1	1534	1534	AGA	TA	1-
tRNA Leu	1534	1599	66			4+
COX2	1604	2291	688	ATG	T–	0
tRNA Lys	2292	2358	67			30
tRNA Asp	2388	2451	64			0
ATP8	2452	2610	159	ATC	T–	7-
ATP6	2604	3281	678	ATG	TAA	1-
COX3	3281	4070	790	ATG	TAA	1+
tRNA Gly	4072	4137	66			0
ND3	4138	4489	352	ATA	TAA	1-
tRNA Ala	4489	4553	65			0

Gene	Position		Size (bp)	codon		space (+) overlap (-)
	Start	Stop		Start	Stop	
tRNA Arg	4554	4619	66			1
tRNA Asn	4621	4686	66			0
tRNA Ser	4687	4754	68			1-
tRNA Glu	4754	4825	72			1+
tRNA Phe	4827	4897	71			5-
ND5	4893	6621	1729	ATA	TAA	3+
tRNA His	6625	6689	65			3-
ND4	6687	8028	1342	ATT	TAA	7-
ND4L	8022	8324	303	ATT	TAA	3+
tRNA Thr	8327	8393	67			1-
tRNA Pro	8393	8461	69			1+
ND6	8463	8978	516	ATG	TAA	0
Cyt B	8979	10113	1135	ATG	T–	0
tRNA Ser	10114	10182	69			1+
ND1	10213	11157	945	TTG	TAA	26+
tRNA Leu	11184	11258	75			0
16S-rRNA	11259	12601	1343			0
tRNA Val	12602	12673	72			2+
12S-rRNA	12676	13524	849			35+
D-Loop	13560	14298	739			0
tRNA Ile	14299	14365	67			4-
tRNA Gln	14362	14430	69			11+
tRNA Met	14442	14508	67			0
ND2	14509	15502	994	ATT	TA	6+
tRNA Trp	15508	15578	71			3-
tRNA Cys	15576	15643	68			3-
tRNA Tyr	15641	15707	67			0

3. Results and Discussion

The complete mitochondrial genome of *P. polyphagus* (MK503959) was 15,707 bp in length, which comprised 13 protein-coding genes, 22 tRNAs, two ribosomal RNAs (12S and 16S). A non-coding putative control region/D-Loop (739 bp) was located between 12S ribosomal RNA and tRNA-*Ile*. Nine protein-coding genes (COX1, COX2, COX3, ATP6, ATP8, ND2, ND3, ND6, and *Cyt b*) and 14 tRNAs were encoded in the H strand, and the remaining four protein-coding genes (ND1, ND4, ND4L, and ND5), two ribosomal RNAs (12S and 16S) and eight tRNAs were encoded in the L strand (Figure 1). Two ribosomal RNAs were 848 and 1343 nucleotides long. Except for the COX1 gene, twelve protein-coding genes initiated with the conventional start codon (ATG). The incomplete stop codons were identified in COX1, COX2, COX3, ATP8, ND2, ND3, ND4, ND5, and *Cyt b* genes (Table 1). Those are putatively completed via post-transcriptional poly-adenylation [28]. The secondary structures of all tRNAs were predicted by ARWEN [29] and found typical clover-leaf structures, except for the tRNA-*Ser*$^{(TCT)}$.

According to the phylogenetic analysis with the mitogenomes in family Palinuridae, *P. polyphagus* were clustered together with other four species including *Panulirus versicolor* (84.83%), *Panulirus stimpsoni* (84.28%), *Panulirus homarus* (84.15%), *and Panulirus ornatus* (84.43%) forming a clade (Figure 2). The other three species including *Panulirus argus, Panulirus japonicus,* and *Panulirus cygnus* [22] formed the other clade within the genus *Panulirus.*

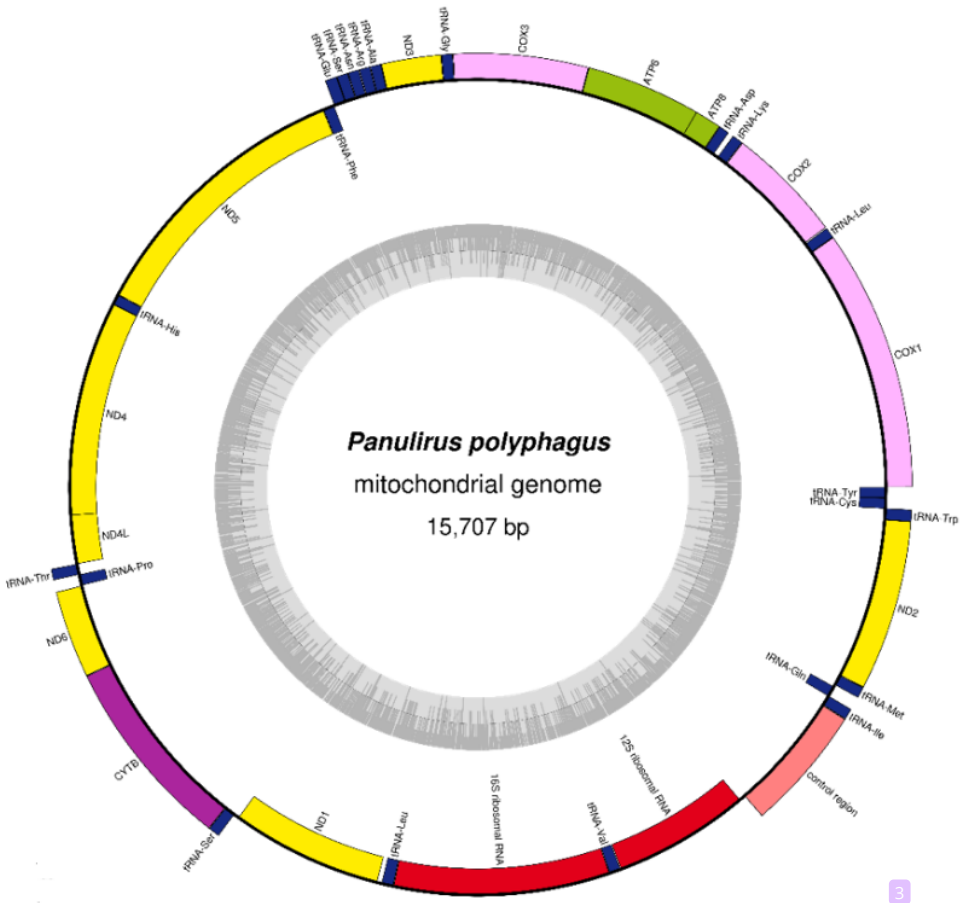

Figure 1. The complete mitochondrial DNA gene map of *P. polyphagus* was constructed by OGDraw (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html)

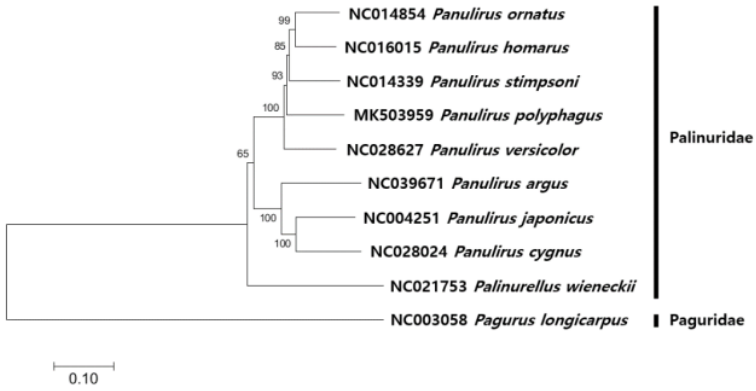

Figure 2. Phylogenetic tree of *Panulirus polyphagus* within Palinuridae

Phylogenetic tree of *Panulirus polyphagus* complete genome was constructed by MEGA7 software with Minimum Evolution (ME) algorithm with 1000 bootstrap replications. Each species scientific name followed by the GenBank accession numbers and furthermore *Pagurus longicarpus* (NC03058) family of Paguridae used as an outgroup species.

4.Conclusion

Molecular identification has successfully identified *Panulirus polyphagus* gathered from Sumenep, East Java. This identification clarifies phylogenetic tree position of *P. polyphagus* between Palinuridae and also the first time of complete mitochondrial genome database in GenBank which is very useful in study genetic population and related topic. Phylogenetic analysis shows that the *P. polyphagus* closest with *P. vercolor*.

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