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

S Andriyono¹², M J Alam¹, H Pramono², A A Abdillah², H W Kim¹³

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

| Gene | Position | Size (bp) | codon | space (+) overlap (-) |
|------|----------|-----------|-------|-----------------------|
| COX1 | Start | Stop | 1534 | AGA | TA | 1- |
| tRNA Leu | Start | Stop | 1534 | AGA | TA | 1- |
| COX2 | Start | Stop | 1599 | 66 | TAA | 4+ |
| COX3 | Start | Stop | 2291 | 688 | ATG | T-- | 0 |
| tRNA Lys | Start | Stop | 2292 | 67 | TAA | 0 |
| tRNA Asp | Start | Stop | 2388 | 64 | TAA | 0 |
| ATP8 | Start | Stop | 2452 | 159 | ATC | T-- | 7- |
| ATP6 | Start | Stop | 2604 | 678 | ATG | TAA | 1- |
| COX3 | Start | Stop | 3281 | 790 | ATG | TAA | 1+ |
| tRNA Gly | Start | Stop | 4072 | 66 | TAA | 0 |
| ND3 | Start | Stop | 4138 | 352 | ATA | TAA | 1- |
| tRNA Ala | Start | Stop | 4489 | 4553 | 65 | | 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, ND6, 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*.

| Gene     | Position | Size (bp) | codon | space (+) overlap (-) |
|----------|----------|-----------|-------|----------------------|
| 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                  |
| tRNA His | 6625     | 6689      | 65    | 3-                   |
| ND4      | 6687     | 8028      | 1342  | ATT                  |
| ND4L     | 8022     | 8324      | 303   | ATT                  |
| tRNA Thr | 8327     | 8393      | 67    | 1-                   |
| tRNA Pro | 8393     | 8461      | 69    | 1+                   |
| ND6      | 8463     | 8978      | 516   | ATG                  |
| Cyt B    | 8979     | 10113     | 1135  | ATG                  |
| tRNA Ser | 10114    | 10182     | 69    | 1+                   |
| ND1      | 10213    | 11157     | 945   | TTG                  |
| 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                  |
| tRNA Trp | 15508    | 15578     | 71    | 3-                   |
| tRNA Cys | 15576    | 15643     | 68    | 3-                   |
| tRNA Tyr | 15641    | 15707     | 67    | 0                    |
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*.

Acknowledgement
We would like to thank graduate students at Molecular Physiology Laboratory, Pukyong National University, Busan, for their helpful suggestion during this study. We also grateful for Indonesian Endowment Fund for Education (LPDP) and Beasiswa Unggulan Dosen Indonesia-Luar Negeri (BUDI-LN) batch I 2016 scholarship.

References

[1] Chan T 1998 Shrimps and prawns *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific* 2 851-972
[2] KKP 2015 Kelautan dan perikanan dalam angka tahun 2015. Jakarta: Pusat Data Statistik dan Informasi, Kementerian Kelautan dan Perikanan)
[3] Jones C M 2010 *J. Mar. Biol. Ass. India* 52 304-15
[4] Holthuis L B 1991 *Marine lobsters of the world: an annotated catalogue of species of interest to fisheries known to date: Food and Agriculture Organization of the United Nations*)
[5] Chan T-Y 2010 *The Raffles Bulletin of Zoology* 23 153-81
[6] George C, Reuben N and Muthe P 1955 *J Anim Morphol Physiol* 2 14-27
[7] Kagwade P 1988 *Journal of the Marine Biological Association of India* 30 114-20
[8] Radhakrishnan E and Devarajan K 1986 Growth of the spiny lobster *Panulirus polyphagus* (Herbst) reared in the laboratory
[9] Deshmukh S 1966 *Crustaceana* 137-50
[10] Kagwade P 1993 *Indian Journal of Fisheries* 40 63-73
[11] Ptacek M B, Sarver S K, Childress M J and Herrnkind W F 2001 *Marine and Freshwater Research* 52 1037-47
[12] Davis G E and Dodrill J W 1989 *Bulletin of Marine Science* 44 78-88
[13] Boudreau S A and Worm B 2012 *Marine Ecology Progress Series* 469 195-214
[14] Kanciruk P 1980 *The biology and management of lobsters* 2 59-96
[15] Munro J L 1974 *The Biology, Ecology, Exploitation and Management of Caribbean Reef Fishes: Coral reef fish and fisheries of the Caribbean Sea: Zoology Department, University of the West Indies*)
[16] Herrnkind W F 1980 *Biology and management of lobsters. Physiology and behavior* 1 349-407
[17] Wahyudin R A, Hakim A A, Boer M, Farajallah A and Wardiatno Y 2016 *Biodiversity Journal* 7 901-6
[18] Yamauchi M M, Miyata M U and Nishida M 2002 *Gene* 295 89-96
[19] Liu Y and Cui Z 2011 *Molecular biology reports* 38 403-10
[20] Qian G, Zhao Q, Wang A, Zhu L, Zhou K and Sun H 2011 *Zoological Journal of the Linnean Society* 162 471-81
[21] Liang H 2012 *African Journal of Biotechnology* 11 14519-28
[22] Kim G, Yoon T-H, Park W G, Park J Y, Kang J-H, Park H and Kim H W 2016 *Mitochondrial DNA Part A* 27 4576-7
[23] Shen H, Braband A and Scholtz G 2013 *Molecular Phylogenetics and Evolution* 66 776-89
[24] Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A,
Markowitz S and Duran C 2012 Bioinformatics 28 1647-9
[25] Lehwark P and Greiner S 2018 bioRxiv 273441
[26] Lohse M, Drechsel O and Bock R 2007 Current genetics 52 267-74
[27] Kumar S, Stecher G and Tamura K 2016 Molecular biology and evolution 33 1870-4
[28] Ojala D, Montoya J and Attardi G 1981 Nature 290 470-4
[29] Laslett D and Canbäck B 2008 Bioinformatics 24 172-5