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The Suitable COI Marker for Lobster of Genus *Panulirus*

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Abstract. This study was conducted to analyze the COI (cytchrome oxidase subunit-1) sequences of members of the genus *Panulirus*, and determine the best region to use for species identification through DNA barcoding. Six samples were collected randomly from five sites in Indonesia. The three lobster species collected were *P. femoristriga* (Padang), *P. homarus* (Malang), and *P. versicolor.* A total of 1,516 sequences were analyzed using the software MEGA 6 for covering alignment, nucleotide variation, mutation rates, and genetic distance. Samples classified in the upper region (UR) consisted of 25 *Panulirus* species and 17 undetermined species, while samples classified in the lower region (LR) consisted of 11 *Panulirus* species and 2 undetermined species.

Keywords: crustacea, DNA barcode, genetic distance, genetic marker, mutation rates, *Panulirus*

Introduction

Panulirus (Palinuridae; Decapoda) is a genus of lobster with a varied morphology that is widely distributed in tropical and subtropical marine areas globally (Abdullah *et al*., 2014; Chow *et al*., 2011; Chan, 2010; George, 2006; Holthuis, 1991). *Panulirus* spp. frequently inhabit coral reefs, rocks, soft sediments, and other habitats with crevices and cavities to hide in (Tewfik 2014; Withy-Allen and Hovel 2013). Seven species of *Panulirus* can be found in Indonesia: *P. ornatus, P. homarus, P. longipes, P. versicolor, P. penicillatus, P. femoristriga,* and *P. polyphagus* (Wahyudin *et al*., 2017; 2016; Chan, 2010; Holthuis 1991).

Species identification based on morphological characters is mostly done in adult lobsters. As lobsters grow, their morphology changes; thus, species identification is often difficult during this process. Accordingly, morphological analysis is insufficient for differentiating among all members of this genus (Tsang *et al*., 2009). In addition, the phylogeny of this group has not been resolved. Using morphological characters, *Panulirus longipes* with striped legs were synonymized as *P. longipes femoristriga*. However, an analysis based on mitochondrial cytochrome c oxidase subunit 1 (COI) genes indicated that *P. femoristriga* is a separate species from *P. longipes* (Ravago and Juinio-Menez, 2002).

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Mitochondrial DNA (mtDNA) fragments can be used as genetic markers for phylogenetic studies in animals because they have a simple genome structure. Short sequences of mtDNA that have been standardized for identifying an organism to the species level are called DNA barcodes (Hubert, 2008; Waugh, 2007; Hebert *et al*., 2003). DNA barcoding identifies organisms by comparing the similarity and dissimilarity in their DNA sequences to a set of sequences from reference taxa (Habeeb and Sanjayan, 2011). sequences to a set of sequences from reference taxa (Habeeb and Sanjayan, 2011). DNA barcoding is an important taxonomic tool for identifying species quickly and accurately. An additional advantage of DNA barcoding is that samples can be in the form of small amounts of tissue or blood, so the sampling process does little harm to the organism being studied (Janzen *et al*., 2005).

The COI gene is part of the mitochondrial genome. COI is widely used for DNA barcoding, as it has a universal primer and allows for a greater range of phylogenetic analyses than other mitochondrial genes (Arief and Khan, 2009; Popa *et al*., 2007; Hebert *et al*., 2003). For example, COI has been successfully used in phylogenetic analyses at the species level and at higher taxonomic levels (Alcantara and Yambot, 2014) and for taxonomic studies of diverse animal groups such as fish (Kress and Erickson, 2012; Zhang and Hanner, 2011; Lakra *et al*., 2011; Ward *et al*., 2005; Hebert *et al*., 2003), birds (Hebert *et al*., 2004), and insects (Hajibabaei *et al*.,

2006; Janzen *et al*., 2005; Hebert *et al*., 2003). Specifically, COI has been used to confirm the phylogenetic relationships among many groups of crustaceans (Cunningham *et al*., 1992). Although the COI gene can be used to identify crustaceans at various taxonomic levels, present study was aimed to use COI gene in verifying its potential to distinguish different lobster species.

Materials and Methods

Six samples were collected randomly from five sites in Indonesia (Fig. 1). The three lobster species collected were *P. femoristriga* (Padang), *P. homarus* (Malang), and *P. versicolor* (Aceh Jaya, Banten, Trenggalek, Malang). The periopod of each lobster was cut for DNA extraction, and the rest of the body was kept as a voucher specimen in the Molecular Laboratory, Department of Aquatic Resources Management. DNA was extracted using a DNA extraction kit (Promega, USA). The COI gene was then amplified using two primer sets. The first primer set forward AF215 5'-TTCAACAAATCAT-AAAGATATTGG-3'and reverse AF216 5'-TAAA-CTTCAGGGTGACCAAAAAATCA -3' was used to amplify the upper region (UR) of COI and the second primer set was used to amplify the lower region (LR) (Fig. 2). The UR and LR are considered the most commonly used regions for analyses of the COI gene. Amplification with both primer sets was performed in the ESCO thermal cycler machine using the protocol of the GoTaq® Green Master Mix (Promega): initial denaturation at 95 °C for 2 min, denaturation at 95 °C for 30 sec, followed by 30 cycles of annealing at 55 °C for 1 min and elongation at 72 °C for 45 sec with an additional extension step of 5 min at 72 °C. Amplicons were visualized using polyacrylamide gel electrophoresis (PAGE) with silver staining (Byun *et al* 2009). The amplicons were then sent to First BASE Laboratories, Malaysia, for bidirectional sequencing following the manufacturer's protocol.

In addition to the sequences obtained from the six samples in this study, 1,509 COI sequences were extracted for the genus *Panulirus* from list all database (NCBI), and one sequence from Scyllaridae (*Parribacus antarticus*) to be used as an outgroup. A total of 1,516 sequences were analyzed using the software MEGA 6 for covering alignment, nucleotide variation, mutation rates, and genetic distance. Clustal W v.1.8 was used to align the sequences. Clustal W v.1.8 was used to align the sequences. The results were then compared

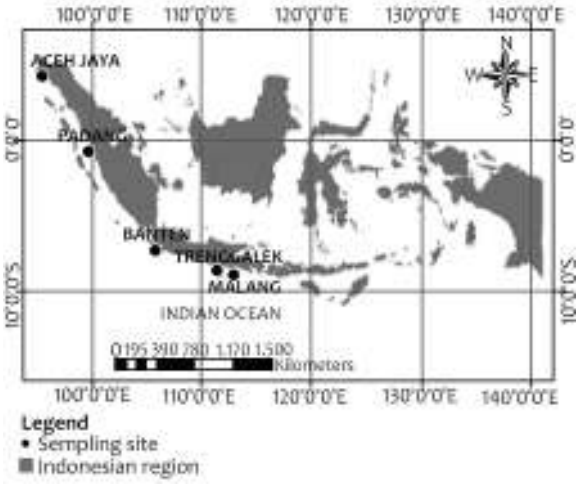

Fig 1. The collection locations for the lobster samples in Indonesia are indicated by circles.

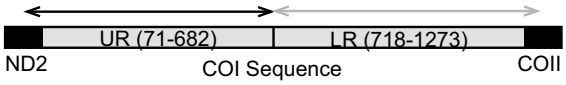

Fig 2. The two sequence regions encompassing the groups of COI subregions used in lobster phylogenetic analyses

between each group of COI sequences. The transition (si)/ transversion (sv) value of each region was also compared using the t-test.

Results and Discussion

Regions of the mitochondrial genome have potential for taxonomic identification through DNA barcoding. This study categorized two potential barcoding regions in the COI sequence, the upper region (UR) and lower region (LR), using primers that were previously designed based on sequences available in public databases (NCBI). The sequences in NCBI had different base lengths, as used for identification of species delineating has been recorded for lobster species (Inoue *et al*., 2007; Seinen *et al*., 2006; Ptacek *et al*., 2001;). The COI sequences extracted from NCBI can be categorized into six groups. Four groups belonged to the UR and two groups to the LR. The six samples collected from Indonesia were also included in the group analysis (Table 1). For this sequence library, 1,239 sequences (81.7%) belonged to the UR at base position 71 to 682, and 277 sequences (18.3%) belonged to the LR at base position 718 to

Table 1. Groups of COI sequences for the UR and LR from lobster samples (including samples collected from Indonesia)

Group	Region	start	end	Species included	Total entries
1	UR	71	682	25 17 *Panulirus* sp. (undetermined species)	186+6*
2	UR	149	618	12	625+6*
3	UR	153	612	8	353+6*
4	UR	204	667	7	51+6*
5	LR	718	1140	11 2 *Panulirus* sp. (undetermined species)	223+6*
6	LR	718	1273	11 2 *Panulirus* sp. (undetermined species)	42+6*

* = sample collection from Indonesia, UR = upper region; LR = lower region.

1273. Samples classified in the UR consisted of 25 *Panulirus* species and 17 undetermined species, while samples classified in the LR consisted of 11 *Panulirus* species and 2 undetermined species.

Transition and transversion are the types of mutation that used to determine stability of a DNA sequence. Mutation rates were calculated using two parameters, Kimura 2-Parameter and Tamura 3-Parameter, with all positions containing gaps; missing data eliminated. Mutation rates from both regions based on transition (P-value = 0.78, t-test) and transversion (P-value = 1, t-test) were almost similar. The result indicated that COI sequence UR or LR region had same accuration. On the other hand, UR had lower mutation rates (Table 2). Low mutation rates indicate more stable nucleotide sequence (Kini and Chinnasamy, 2010). The UR was more stable nucleotide sequence.

The UR of COI in lobsters is a conserved region that is used to distinguish between species of the family Palinuridae. COI regions that are highly conserved are suitable for use in primer design (Folmer *et al*., 1994). In addition, there are currently more sequences available for analysis from the UR (81.7%) than the LR (18.3%). Sequences grouped in the UR were extracted from 25 *Panulirus* species and 17 undetermined species, while LR sequences came from 11 *Panulirus* species and 2 undetermined species. Using 650 bp COI sequences, Costa *et al*., (2007) determined that the COI region is a barcoding standard, as this region is able to resolve species lineages in crustaceans at high rates. The interspecific genetic distance in the UR (0.030–0.205) was higher than in the LR (0.091– 0.190, Table 3). The UR is thus more suitable for use as a barcoding region. The UR also had lower mutation rates and higher genetic distances than the LR. In contrast, the si/sv value was not significantly different between both regions (P-value > 0.05). Thus, the UR of COI has ideal characteristics for designing a DNA barcode, including an abundance of publicly available data.

Barcoding can be used to identify a species in all stages of its life cycle. Identification of larvae is often difficult because morphological characters can be similar among different species at this stage. The phyllosoma larval stage in lobsters is pelagic (Matsuda *et al*., 2006) and larval distribution is strongly influenced by ocean currents. DNA barcoding can facilitate the monitoring of larval dispersal in the oceans. The accurate identification of larvae to species is essential for developing management strategies. In addition, DNA

Table 2. Mutation rates of UR and LR COI sequences from lobster samples (including samples collected from Indonesia)

Group	Region	Estimate site by site		Estimate gamma parameter	
		K2P	T3P	K2P	T3P
1	UR	0.2431	0.2321	0.2431	0.2321
2	UR	0.2352	0.2501	0.2352	0.2501
3	UR	0.2163	0.2073	0.2163	0.2073
4	UR	0.2103	0.1942	0.2103	0.1942
5	LR	0.2258	0.2180	0.2258	0.2180
6	LR	0.5183	0.4979	0.5183	0.4979

K2P = Kimura 2-Parameter, T3P = Tamura 3-Parameter.

Table 3. Genetic distance of UR and LR COI sequences from lobster samples (including samples collected from Indonesia)

Group	Region	d	S.E
1	UR	0.205	0.013
2	UR	0.159	0.014
3	UR	0.030	0.003
4	UR	0.090	0.008
5	LR	0.091	0.008
6	LR	0.190	0.014

d = genetic distance, S.E = standard error.

barcoding can be used for species identification in cases where visual identification is not possible, such as for processed materials or packaged seafood (Eischeid *et al*., 2016; Galimberti *et al*., 2013). Determining the authenticity of seafood enhances fair trade practices and transparency in both domestic and global fisheries. Inaccurate labeling of marine seafood products can negatively impact fisheries, stock management, species conservation, and consumer health. As DNA barcoding can be used to detect mislabeled seafood products (Chin *et al*., 2016), it is important for ensuring food safety, and for identifying commercial fraud in both local and international trade.

Conflict of Interest. The authors declare no conflict of interest.

References

Abdullah, M.F., Alimuddin, Muththalib, M., Salama, A.J., Imai, H. 2014. Genetic isolation among the northwestern, southwestern, and central-eastern Indian ocean populations of the pronghorn spiny lobster *Panulirus penicillatus*. *International Journal of Molecular Science*, **15:** 9242-9254.

Alcantara, S.G., Yambot, A.V. 2014. DNA barcoding of commercially important grouper species (Perciformes, Serranidae) in the Philippines. *Mitochondrial DNA*, Part A: *DNA Mapping Sequencing and Analysis*, **27:** 3837-3845.

Arief, I.A., Khan, H.A. 2009. Molecular markers for biodiversity analysis of wildlife animals: a brief review. *Animal Biodiversity and Conservation*, **32:** 9-17.

Byun, S.O., Fang, Q., Zhou, H., Hickford, J.G. 2009. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Analytical Biochemistry*, **385:** 174-175.

Chan, T.Y. 2010. Annotated checklist of the world's marine lobsters (Crustacea: Decapoda: Astacidea, Glypheidea, Achelata, Polychelida). *The Raffles Bulletin of Zoology*, **23:** 153-181.

Chin, T.C., Adibah, A.B., Hariz, Z.A.D., Azizah, M.N.S 2016. Detection of mislabelled seafood products in Malaysia by DNA barcoding: improving transparency in food market. *Food Control*, **64:** 247-256.

Chow, S., Jeffs, A., Miyake, Y., Konishi, K., Okazaki, M., Suzuki, N., Abdullah, M.F., Imai, H., Wakabayasi, T., Sakai, M. 2011. Genetic isolation between the western and eastern Pacific population of pronghorn spiny lobster *Panulirus penicillatus*. *Plos One*, **6:e** 29280.

Costa, F.O., deWaard. J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M., Hebert, P.D.N. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences*, **64:** 272-295.

Cunningham, C.W., Blackstone, N. W., Buss L.W. 1992. Evolution of king crabs from hermit crab ancestors. *Nature*, **355:** 539-542.

Eischeid, A.C., Stadig, S.R., Handy, S.M., Fry, F.S., Deeds, J. 2016. Optimization and evaluation of a method for the generation of DNA barcodes for the identification of Crustaceans. *LWT-Food Science and Technology*, **73:** 357e367.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytrocrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3:** 294-299.

Galimberti, A., Mattia, F.D., Losa, A., Bruni, I., Federici, S., Casiraghi, M., Martellos, S., Labra, M. 2013. DNA barcoding: a new tool for food traceability. *Food Research International*, **50:** 55-63.

George, R.W. 2006. Tethys Sea fragmentation and speciation of *Panulirus* spiny lobsters. *Crustaceana,* **78:** 1281-1309.

Habeeb, S.K.M., Sanjayan, K.P. 2011. Sequencing and phylogenetic analysis of the mitochondrial cytochrome c oxidase subunit I of *Oxycarenus laetus* (Hemiptera: Lygaeidae). *International Journal of Plant, Animal and Environmental Science IJPAES*, **1:** 85-92.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., Hebert, P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *PNAS*, **103:** 968-971.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak,T.S., Francis, C.M. 2004. Identification of birds through DNA barcodes. *PloS Biology*, **2:**1657-1663.

Hebert, P.D.N., Cywinska, A., Ball, S.L, deWaard, J.R. 2003. Biological identifications through DNA barcodes. *Proceedings Biological Science*, **270:** 313-321.

Hubert, N. 2008. Identifying Canadian freshwater fishes through DNA Barcodes. *PloS One* **3:** 2490.

Holthuis, L.B. 1991. *FAO Species Catalogue.* vol. **13,** Marine Lobster of the World, Rome (IT), Food and

Agricultural Organization of United Nations, Rome, Italy.

Inoue, N., Watanabe, H., Kojima, S., Sekiguchi, H. 2007. Population structure of Japanese spiny lobster *Panulirus japonicus* inferred by nucleotide sequence analysis of mitochondrial COI gene. *Fisheries Sciences*, **73:** 550-556.

Janzen, D.H., Hajibabaei, M., Burns, J.M., Hallwachs, W., Remigio, E., Hebert, P.D.N. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA Barcoding. *Philosophical Transaction of the Royal Society of London. Series B, Biological Sciences*, **360:** 1835-1845.

Kini, R.M., Chinnasamy, A. 2010. Nucleotide sequence determines the accelerated rate of point mutations. *Toxicon*, **56:** 295-304.

Kress ,W.J., Erickson, D.L. 2012. DNA barcodes: Methods and protocols. *Methods in Molecular Biology*, **858:** 3-8.

Lakra, W.S., Verma M.S., Goswami, M., Lal, K.K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K.V., Ward, R.D., Hebert, P.D.N. 2011. DNA barcoding Indian marine fishes. *Molecular Ecology Resources*, **11:** 60-71.

Matsuda, H., Takenouchi, T., Goldstein, J.S. 2006. The complete larval development of the pronghorn spiny lobster *Panulirus penicillatus* (Decapoda: Palinuridae) in culture. *Journal of Crustacean Biology*, **26:** 579-600.

Popa, L.O., Gargarea, P., Murariu, D. 2007. Sequence analysis of the 5' COI gene region from Dama dama (Linnaeus, 1758) (Mammalia: Cervidae). *Travaux du Museum National d'Historie Naturelle*, pp. 537-542.

Ptacek, M.B., Sarver, S.K., Childress, M.J., Herrnkind, W.F. 2001. Molecular phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). *Marine and Freshwater Research*, **52:** 1037-1047.

Ravago, R.G., Juinio-Menez, M.A. 2002. Phylogenetic position of the striped-legged forms of *Panulirus longipes* (A. Milne-Edwards, 1868) (Decapoda, Palinuridae) inferred from mitochondrial DNA sequences. *Crustaceana*, **75:** 1047-1059.

Seinen, C., Nobuaki, S., Hideyuki, I., Taku, Y. 2006. Molecular species identification of spiny lobster phyllosoma larvae of the Genus *Panulirus* from the Northwestern Pacific. *Marine Biotechnology*, **8:** 260-267.

Tewfik, A. 2014. The influence of waves on landing patterns within a diverse Sumatran spiny lobster (*Panulirus* spp.) fishery. *New Zealand Journal of Marine and Freshwater Research*, **48:** 245-255.

Tsang, L.M., Chan, T.Y., Cheung, M.K., Chu, K.H. 2009. Molecular evidence for the Southern Hemisphere origin and deep-sea diversification of spiny lobsters (Crustacea: Decapoda: Palinuridae). *Molecular Phylogenetics and Evolution*, **51:** 304-311.

Wahyudin, R.A., Wardiatno, Y., Boer, M., Farajallah, A., Hakim, A.A. 2017. A new distribution record of the mud-spiny lobster, *Panulirus polyphagus* (Herbst, 1793) (Crustacea, Achelata, Palinuridae) in Mayalibit Bay, West Papua, Indonesia. *Biodiversitas*, **18:** 780-783.

Wahyudin, R.A., Hakim, A.A., Boer, M., Farajallah, A., Wardiatno, Y. 2016. New records of *Panulirus femoristriga* Von Martens, 1872 (Crustacea Achelata Palinuridae) from Celebes and Seram Islands, Indonesia. *Biodiversity Journal*, **7:** 901-906.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. *Philosophical Transaction of the Royal Society of London. Series B, Biological Sciences*, **360:** 1847-1857.

Waugh, J. 2007. DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays*, **29:** 188-197.

Withy-Allen, K.R., Hovel, K.A. 2013. California spiny lobster (*Panulirus interruptus*) movement behaviour and habitat use: implications for the effectiveness of marine protected area. *Marine and Freshwater Research*, **64:** 359-371.

Zhang, J.B., Hanner, R. 2011. DNA barcoding is a useful tool for the identification of marine fishes from Japan. *Biochemistry Systematics and Ecology* **39:** 31-42.