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High genetic variation of *Portunus pelagicus* from Makassar Straits revealed by RAPD markers and mitochondrial 16S rRNA sequences

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The blue swimmer crab, *Portunus pelagicus*, is presently managed as a single stock. In this study, random amplified polymorphic DNA (RAPD) markers and 16S rRNA sequences were used to understand the genetic diversity and parentage of *P. pelagicus* from Makassar Strait. A total of 150 samples were collected from the sea, grouped by gender and morphological features. DNA genome was performed at approximately 23130 base pairs (bp) and mitochondrial 16S rRNA sequences in the 500 to 600 bp. The percentage of polymorphic band using OPA-5, OPA-11, and OPA-17 primers was high (44.4 to 100%). Genetic identity ranged between 0.7266 and 0.9050 and genetic distance between 0.1008 and 0.3214. Alignment of 16S rRNA sequences shows 96 to 99% homology with *P. pelagicus* available in GenBank. Both RAPD markers and 16S rRNA sequences indicate that there was a high genetic variation observed among population, which formed two clusters. Specific unique bands found at 450 and 600 bp in OPA-11 gives an indication of hybridization among the population. The variation of white spot pattern on the carapace can be used as indicators of differentiation in population and parentage of *P. pelagicus*. Therefore, farmers or hatchery operators can continue to use the population as sources of natural broodstock.

Key words: *Portunus pelagicus*, random amplified polymorphic DNA (RAPD), 16S rRNA, genetic, aquaculture.

INTRODUCTION

The blue swimmer crab (*Portunus pelagicus* is Linnaeus, 1758) is the most commercially important marine species in Indonesia and several countries in the world. This crab is extracted from the sea using traps and gill nets to meet demand of the world market. *P. pelagicus* spread throughout the Indian and West Pacific Oceans: From Japan and Philippines throughout Southeast and East Asiato Indonesia, the East of Australia, and Fidji Islands, and westward to the Red Sea and East Africa. This species occurs also in the

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Mediterranean Sea along the coast of Egypt, Israel, Lebanon, Turkey, the Syrian Arab Republic, Cyprus and the East Southern Coast of Sicily. *P. pelagicus* occupies sandy and sand-muddy in shallow waters between 10 and 50 m depth, including areas near reefs, mangroves, sea grass, and algal beds. The juvenile is commonly occurring in intertidal shallower areas (FAO, 2014).

In Indonesia, this crabs spread almost throughout the coastal island. Several key indicators show that *P. pelagicus* population is in crisis due to overexploitation. Kunsook et al. (2014) reported an increase in fishing mortality to 4.14. The exploitation rate was higher (0.71) than the optimal value (0.38). The size of the mature females has also decreased from 8.10±0.39 cm to 7.52±1.14 cm. Mehanna et al. (2013) reported along the Oman coasts on both Arabian and Oman Seas, the yield per recruit showed that the crab stock is being exploited beyond its maximum biological limit, but the increase of fishing mortality to the level which gives the maximum Y/R (83% of its current value) will be accompanied with a negligible increase in Y/R (2.7%) and a considerable decrease in both biomass per recruit (21.1%) and spawning stock biomass (37.6%). Harris et al. (2014) also reported that since July 2011 the relative abundance of all size classes of the crabs in Shark Bay declined significantly.

The decline in catches of the crabs was also reported from Cockburn Sound, Western Australia since 2000 resulted in closure of the crab fishery in December 2006 (Johnston et al., 2011). Similar phenomenon also occur in Indonesia (personal observation), therefore, Indonesia government recently issued a regulation to protect the crabs population. Aquaculture and stock enhancement is a prospect to solve the problem. Aquaculture refers to the breeding, rearing, and harvesting of aquatic species for food and other human uses, while marine stock enhancement is a set of management approaches involving the release of cultured organisms to enhance or restore fisheries (Lorenzen et al., 2010). Hatchery-produced stocks are used to replenish or supplement wild stocks (Waples and Drake, 2012). Enhancement of wild stocks by release of hatchery-reared seed is one method by which the yield from the hatchery can be improved (Roberts et al., 2007; Altamirano, 2010). But there are important question on how increase in global aquaculture production without treating sustainability of natural populations and the ecosystems for long-term viability. Wang et al. (2012) reported that the cultured croaker had significantly reduced genetic diversity in contrast to the wild populations. These changes may be caused by founder's effects, artificial selection, and random genetic drift.

Genetic status is an essential information in fisheries management through stock enhancement or cultivation. Since *P. pelagicus* is managed as a single stock, but there were a lot of evidence leading to variations in species. According to Lai et al. (2010), *P. pelagicus* is a species complex consisting of four species. Klinbunga et al. (2010) also found that the populations of *P. pelagicus* from the same geographic location of Thailand waters have high genetic diversity. Sienas et al. (2014) estimated that *P. pelagicus* in Philippine waters are cryptic species and consists of at least two species. In Makassar Strait around South Sulawesi Indonesia, *P. pelagicus* found in some different morphologically features especially in color and patterns of white spots in its carapace (personal observation) when compared with *P. pelagicus* reported by Lai et al. (2010). This raises the suspicion that *P. pelagicus* of Makassar Strait may differ, even though Lai et al. (2010) also took samples from Indonesia waters (Sumatra: Padang; West Nusa Tenggara: Lombok; Sulawesi: Manado). The application of DNA markers has allowed rapid progress in aquaculture investigation of genetic variability and inbreeding, parentage assignment, species and strain identification, and the construction of high-resolution genetic linkage maps for aquaculture species (Liu and Cordes, 2004). Random amplified polymorphic DNA (RAPD) analysis has been described as a simple and easy method to detect polymorphisms based on amplification of DNA segment with single primers of arbitrary nucleotide sequences (Williams et al., 1990; Welsh and McClelland, 1990). Suresh et al. (2013) used RAPD markers to study the genetic structure of the population of *Mugil cephalus,* while de Freitas and Galetti (2005) evaluated the genetic diversity of a commercial broodstock line of *Litopenaeus vannamei* shrimp. Klinbunga et al. (2010) suggested that the RAPD technique is simpler and more cost-effective than amplified fragment length polymorphism (AFLP) analysis for monitoring levels of genetic diversity of *P. pelagicus*.

Mitochondrial DNA sequences have also been widely used to study genetic variability and relationships in many crustacean groups (Khedkar et al., 2013). The mtDNA localizes to the mitochondrial matrix. Besides protein coding genes, mtDNA also codes for 22 transfer RNAs (tRNAs) and two ribosomal RNAs (12S and16S rRNAs) (Moraes et al., 2002). The rRNA gene in all cells is the most conserved (least variable). This means that sequences from distantly related organism can be precisely aligned, making the true differences easy to measure. An et al. (2005) also reported that the 16S rRNA sequences more conserved than cytochrome c oxidase subunit I (COI) sequences. The important question about genetic variability of *P. pelagicus* in Makassar Strait has not been identified. In this study, specimens from Makassar Strait were described and determine the relationship between morphological appearance (color and spot pattern in carapace) and genetic variation. This information will provide valuable input to the protection, genetic enhancement, and the cultivation of this species in the future.

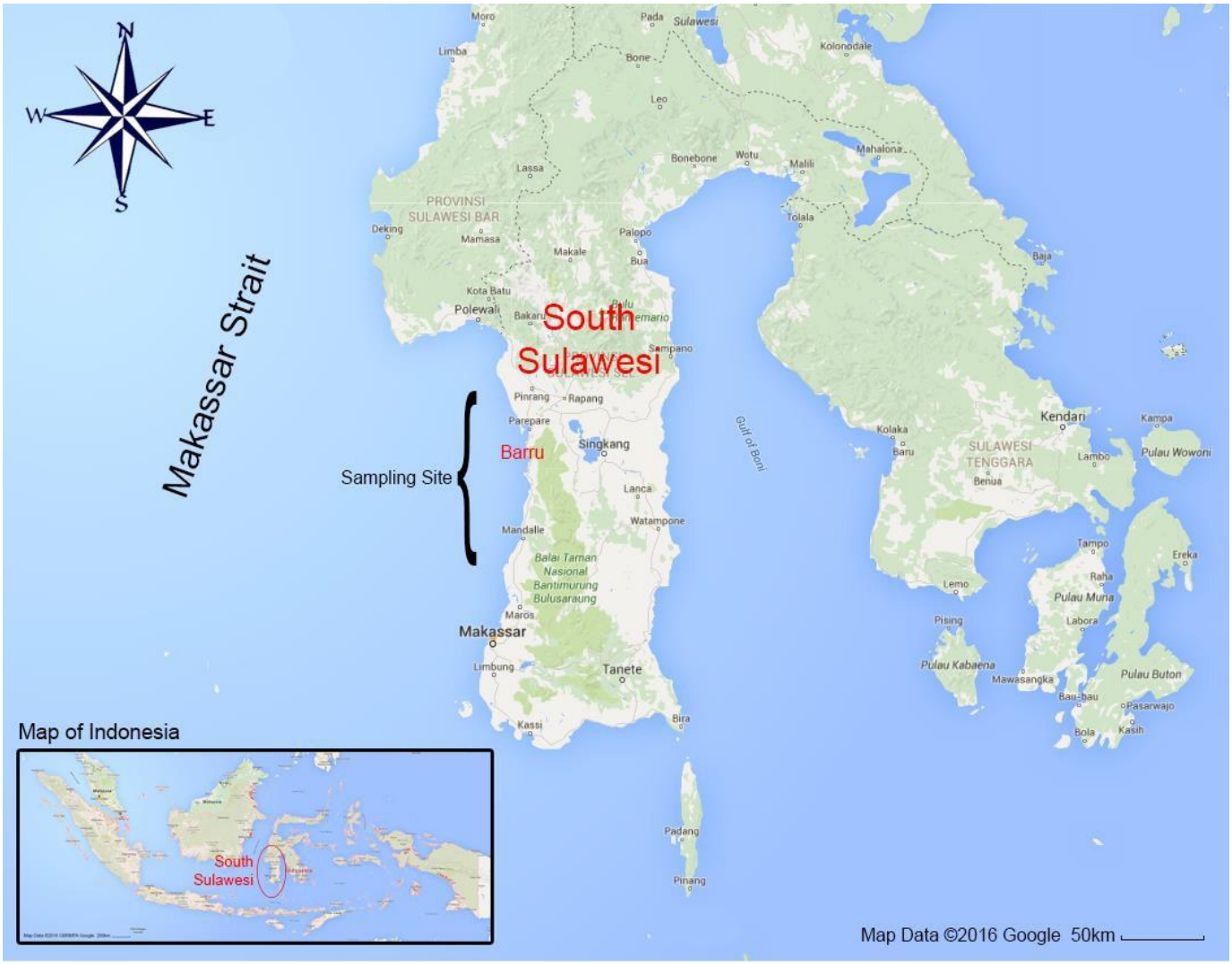

Figure 1. Sampling site of *Portunus pelagicus* specimens in Makassar Strait around. The Barru Regency South Sulawesi.

MATERIALS AND METHODS

Sample collection

The sample of *P. pelagicus* was obtained from fisherman that caught it in the Makassar Strait, South Sulawesi, Indonesia (Figure 1) using traps. 150 samples were collected during March 2013 to May 2014. The samples were grouped by gender and morphological variation (white spot pattern on carapace) (Figure 2). There were 30 male and 30 female crabs randomly selected for DNA analysis. The selected crabs were anesthetized using cold water (8°C) before releasing their first pereiopod (claw) by autotomy. Muscle of the claw was removed from each crab taken as much as 50 mg, preserved using 250 µl TNES-Urea buffer (Tris for 200 ml; 2 ml of 1 M pH 7.5; final concentration: 10 mM NaCl; for 200 ml: 5 ml of 5 M; final concentration: 125 mM ethylenediaminetetraacetic acid (EDTA)-2Na; for 200 ml: 2 ml of 0.5 M pH 7.5; final concentration: 10 mM sodium dodecyl sulfate (SDS); for 200 ml: 10 ml of 10%; final concentration: 0.5% Urea; for 200 ml: 48.05 g; final concentration: 4 M) (Asahida et al., 1996) and stored at room temperature until extraction.

DNA extraction

DNA was extracted based on the phenol-chloroform method described by Parenrengi et al. (2001) as follows: (1) Digestion buffer (0.5 M NaCl, 0.001 M EDTA, 1% SDS, 0.8% Triton-X, and 0.1 Tris-HCl at pH 9.0) were added into 1.5 ml microcentrifuge tube containing 50 mg crab muscle and then 40 µl SDS 10% and 40 µl Proteinase K (20 mg/ml) were added. The tube was shaken gently and incubated at 55°C for 1 to 3 h; (2) the sample was treated with 25 µl of RNase (20 mg/ml) and was left at room temperature for 15 to 30 min; (3) the samples were treated with 500 to 600 µl of phenol:chloroform:isoamyl alcohol (25:24:1) and gently homogenized. The sample was left at room temperature for 10 min before centrifugation at 13,000 rpm for 4 min; (4) the top layer of aqueous were removed and dispersed into the new microcentrifuge tube. The step of adding phenol:chloroform:isoamyl alcohol were repeated three times; (5) the samples were treated with 500 µl of chloroform:isoamyl alcohol (24:1) and were centrifuged at 13,000 rpm for 2 min; (6) The upper aqueous layer was mixed with 1 ml of ice-cold absolute ethanol by rapid inversion of the tubes several times.

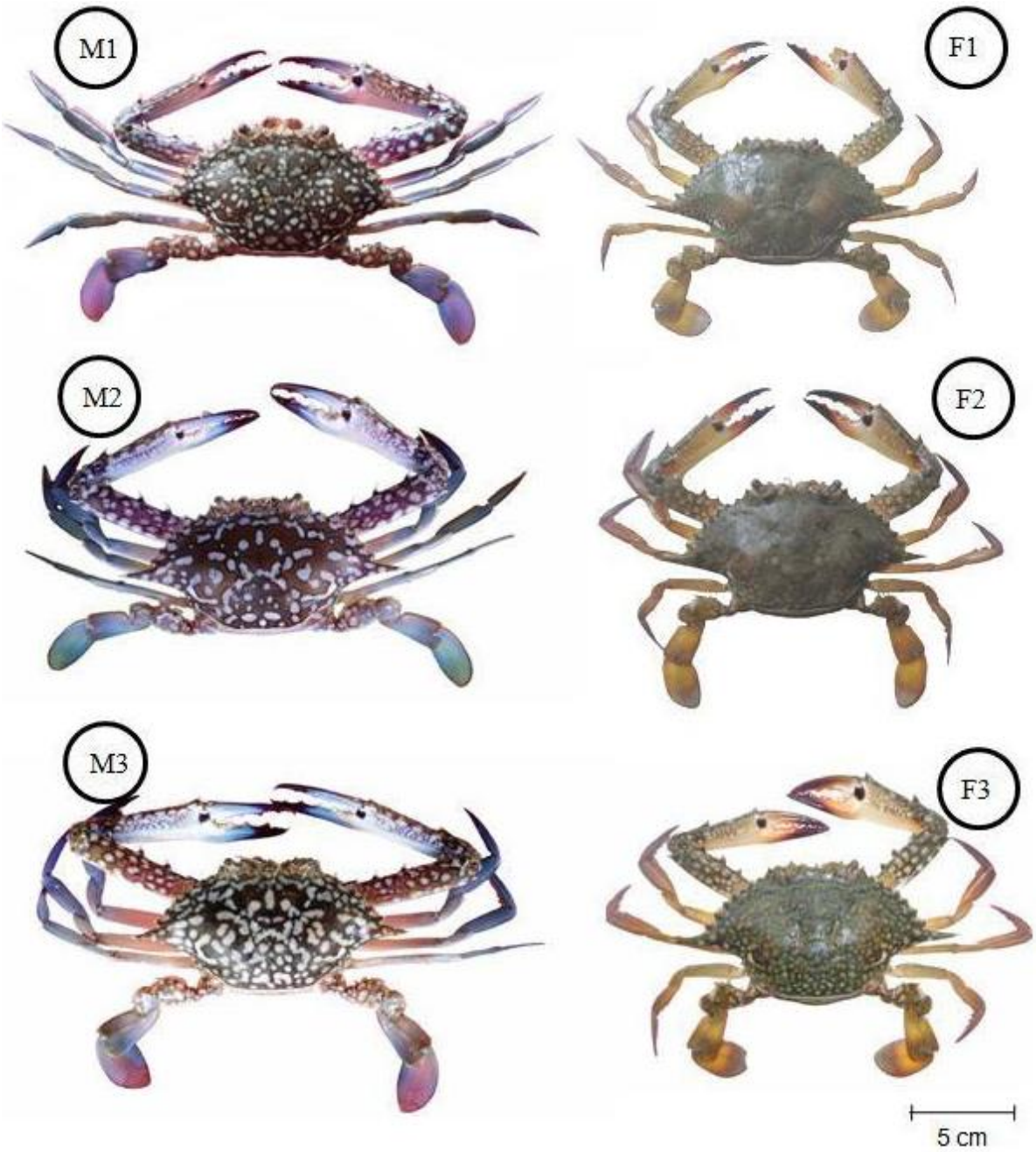

Figure 2. Colors and white spot pattern of *Portunus pelagicus* (males and females) from Makassar Strait. M, Male; F, female; M1, body color is light blue, white spot pattern on carapace is small and fills the entire carapace; M2, Body color is purple, large white spots scatter on carapace with medium density; M3, body color is purple greenish, pattern of white spots on carapace are rarely; F1, body color is greenish without spot; F2, body color is brownish, dark spots each one at the left and right of the carapace; F3, body color is light greenish, faintly visible white spots on the carapace.

Then, centrifuged at 6,000 rpm for 30 min; (7) the precipitated DNA were collected at the bottom tubes as a white pellet and washed with 500 µl of 70% of ethanol and then centrifuge at 6,000 rpm for 15 min; (8) the DNA was allowed to dry at room temperature for 20 min and resuspended with 50 µl sterile distilled water (SDW) for at least 24 h at room temperature to fully dissolved before proceeding to the next step. The samples were purified by electrophoresis in a 0.8% agarose. This DNA genome samples was kept in -20°C to avoid DNA degradation.

RAPD-PCR

Seven RAPD primers: OPA-1 (5'-cag gcc ctt c-3'), OPA-5 (5'-

agg ggt ctt g–3'), OPA-9 (5'-ggg taa cgc c-3'), OPA-10 (5'-gtg tcg ccg t-3'), OPA-11 (5'-caa tcg ccg t–3'), OPA-17 (5'-gac cgc ttg t–3'), and OPA-18 (5'-agg tga ccg t-3') were screened for the amplification success against genomic DNA of representative individual of *P. pelagicus* from each morphological variation and gender. Three primers, OPA-5, OPA-11, and OPA-17 were selected for genetic variation analysis of *P. pelagicus*. Polymerase chain reaction (PCR) was performed in a 25 µl reaction volume containing 1 µl primer (50 pmol/µl), 3 µl DNA template, and 21 µl water free RNAse. PCR reaction using kit PureTaq Ready-To-Go Beads (GE Healthcare, USA) contained 2.5 unit Taq Polimerase; 10 mM Tris-HCl, pH 9; 50 mM KCl; 1.5 mM $MgCl_2$; and 200 µM dNTP-mix and 50 ρmol of each primer. The amplification profiles consisted of predenaturation at 94°C for 2 min followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 36°C for 30 s and extension at 72°C for 60 s. The final extension was carried out at 72°C for 2 min. Three microliters of the amplification reaction was electrophoresed through 2.0% agarose gels and visualized under a UV transilluminator after ethidium bromide staining.

16S rRNA-PCR and sequencing

Amplification of 16S rRNA was carried out using the following primers: forward 16S rRNA-F: 5'- cgc ctg ttt aac aaa aac at -3' and reverse 16S rRNA-R: 5'- ccg gtc tga act cag atc atg t -3'. DNA amplification was performed using PCR System 2700 GeneAmp (Applied Biosystems, USA). PCR reaction using kit PureTaq Ready-To-Go Beads (GE Healthcare, USA) contained 2.5 unit Taq Polimerase; 10 mM Tris-HCl, pH 9; 50 mM KCl; 1.5 mM $MgCl_2$; and 200 µM dNTP-mix and 50 ρmol of each primer. Sterile distilled water (SDW) was added until 25 µl final volume.

Amplification mtDNA was performed using PCR GeneAmp PCR System 2400 (Applied Biosystems, USA) as 1 cycle at 98°C for 1 min; 30 cycle at 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and final extension at 72°C for 5 min. PCR products were purified by electrophoresis in a 1.0% agarose gel using 1x TAE buffer. The gel was stained with ethidium bromide, and the desired DNA band was cut and eluted using kit QIAquick Purification (Qiagen, USA). DNA concentration was measured with Spectrophotometer Bio-Spectro (Shimazu, Japan).

PCR for the 16S rRNA sequencing was done using primers and special reagents for DNA sequences (Big Dye). PCR volume of 10 ml consists of 1 to 1.5 DNA, Big Dye 2 ml, 6 ml H_2O, and 1 ml of primer. PCR cycle consists of 3 stages: 95°C for 15 min, 43°C for 15 min, and 60°C for 240 min. PCR products were purified and denatured, then sequenced with an automated tool ABI Prism 3103-Avant Genetic Analyzer (Applied Biosystems, USA). Sequencing was viewed manually by navigator sequence program (Applied Biosystems). The partial mitochondrial 16S rRNA genes were sequenced from four individuals for each of the male crab morphological variation.

Data analysis

RAPD bands were treated as dominant markers. The percentage of monomorphic (>95% of investigated specimens) and polymorphic (<95% of investigated specimens) bands was estimated for each morphological variation and gender. Unbiased Nei's genetic distance between gender and morphological variation was determined. Population genetic parameters (similarity index and genetic distance) were analyzed with Tools for Population Genetic Analyses version 1.3 (TFPGA Ver. 1.3) and genetic distance clusters using Unweighted Pair Group Method of Aritmethic (UPGMA) program. The data of mt-DNA sequencing were analyzed using Genetyx Version 7 program and Basic Local Alignment Search Tool (BLAST). Genetyx program was used to determine nucleotide variation and genetic distances between individual of crab. BLAST program online, especially for BLAST-N was used to determine the similarity (similarity index) of 16S rRNA sequences and relationship among several species of crustaceans which are available in the GenBank.

RESULTS

Random amplified polymorphic DNA (RAPD)

DNA genome of *P. pelagicus* sample was performed at approximately 23130 base pairs (bp). Among seven RAPD primers that were screened, three of them (OPA-5, OPA-11, OPA-17) were successfully amplified genomic DNA of 60 individual of *P. pelagicus*. 162 RAPD fragments ranging from 200 to 1200 bp in length were generated (Table 1). High genetic polymorphism was observed in all population.

The percentage of polymorphic bands for each primer across all population samples was 44.4 to 100%. In the male, the highest percentage of polymorphism was found in M3 (84%) and the lowest in M1 (71%), while in the female, the highest level of polymorphism (80%) was exhibited by the F3 whereas the lowest (73%) was exhibited by the F1 population. The highest and lowest number of RAPD bands was detected for primers OPA-17 (85.45%) and OPA-11 (68.96%) respectively.

Polymorphic bands are essential in identification in two ways; first in generating patterns of banding that are unique to individual species and second in exhibiting or lacking unique band (s) (marker bands) that distinguish an individual from the rest of the population. In this study, specific unique bands were found among population (Figure 3). These fragments were considered as potential species-specific marker for *P. pelagicus*. There are indication that the M2 population was a hybrid between M1 and M3 based on a specific unique band at 450 and 600 bp in OPA-11. Large genetic distances between pairs of population samples were observed.

Similarity index ranged from 0.7266 to 0.9050 and genetic distances from 0.1008 to 0.3214 (Table 2). In the male, the lowest similarity index values obtained were between M1 and M3 population and the highest were among M2 and M3 population, namely 0.9050 and 0.7266, respectively. In the females, the lowest similarity index was between F3 and F2 populations, whereas the highest obtained were between F1 and F3 populations, namely, 0.9011 and 0.7461, respectively. These values indirectly reflected degrees of differentiation in the blue swimming crab population of Makassar Strait. UPGMA dendogram among *P. pelagicus* populations using Nei's genetic distance obtained two main clusters. The first cluster consists of a population of M2, M3, and F2, while the second cluster consists of population M1, F1, and F3 (Figure 4).

The dendogram also explained the parentage of the

Table 1. Pattern of polymorphism between 60 samples of *P. pelagicus*.

Population	Primer	Total of fragment	Total of polymorpic bands	Polymorphism (%)	Fragment Size
	OPA-5	9	6	66.6	250-900
M1	OPA-11	12	8	66.6	200-1100
	OPA-17	10	8	80	200-1200
	OPA-5	8	7	87.5	250-900
M2	OPA-11	9	6	66.6	300-1200
	OPA-17	8	7	87.5	250-1000
	OPA-5	8	8	100	250-900
M3	OPA-11	9	8	88.8	300-1200
	OPA-17	8	5	62.5	300-1100
	OPA-5	8	4	50	250-800
F1	OPA-11	8	5	62.5	200-1200
	OPA-17	10	10	100	200-1200
	OPA-5	9	7	77.7	250-900
F2	OPA-11	9	4	44.4	300-1200
	OPA-17	12	12	100	250-1200
	OPA-5	7	6	85.7	250-900
F3	OPA-11	11	9	81.8	200-1200
	OPA-17	7	5	71.4	250-900

population, that M1 is paired with F1 and F3, while M2 and M3 are paired with F2.

Mitochondrial 16S rRNA

Isolation of mitochondrial 16S rRNA of 12 specimen of *P. pelagicus* representing three different male populations morphologically showed a single band at about 500 to 600 bp. The partial of 16S rRNA gene alignment was 556 bases long, including insertions and deletions. BLAST nucleotide analysis results indicate that the samples have a high similarity (identity) with *P. pelagicus* found in Genbank (Accession number: DQ062734.1, FM208750.1, FJ152161.1, KF220520.1, KF220521.1, KF220519.1, FJ812329.1, DQ388052.1) that is 96 to 99%, while with *Portunus trituberculatus* ranged between 94 and 95% (Accession number: FJ919807.1, AB093006.1, GQ180777.1, DQ062735.1, GU321227.1, GU321228.1, AY303612.1, AY264913.1). The lower similarity was found with *Parablennius sanguinolentus* that is 93 to 94% (Accession number: KF220524.1, KF220522.1, KF220525.1, KF220526.1, KF220523.1). This BLAST analysis results also provide information that there is a 3% intra-species diversity of *P. pelagicus* registered in the genbank.

Figure 5 shows the relationships among the *P. pelagicus* population inferred from their partial 16S rRNA sequences. Two clusters were formed, of which cluster 1 showed a higher variation in the appeal cluster 2. This clustering gives a strong indication that there is a high variation among *P. pelagicus* from Makassar Strait and may consists of two species or strain.

Morphological performance

Morphological performance of *P. pelagicus* from Makassar Strait was different from *P. pelagicus* shown by Lai et al. (2010). They reported that the blue swimming crab found in Indonesia is *P. pelagicus* with morphological characteristics are as the following.

Carapace colour is a dark blue-green with purple-blue chelipeds. Both males and females possess white spots on the carapace, often merging into broad almost banded reticulations, in particular on the posterior and branchial regions. While *P. pelagicus* found in Makassar Strait possess different white spot pattern (Figure 2).

This distinction reinforces the notion that *P. pelagicus* was found in Makassar Strait in contrast to those

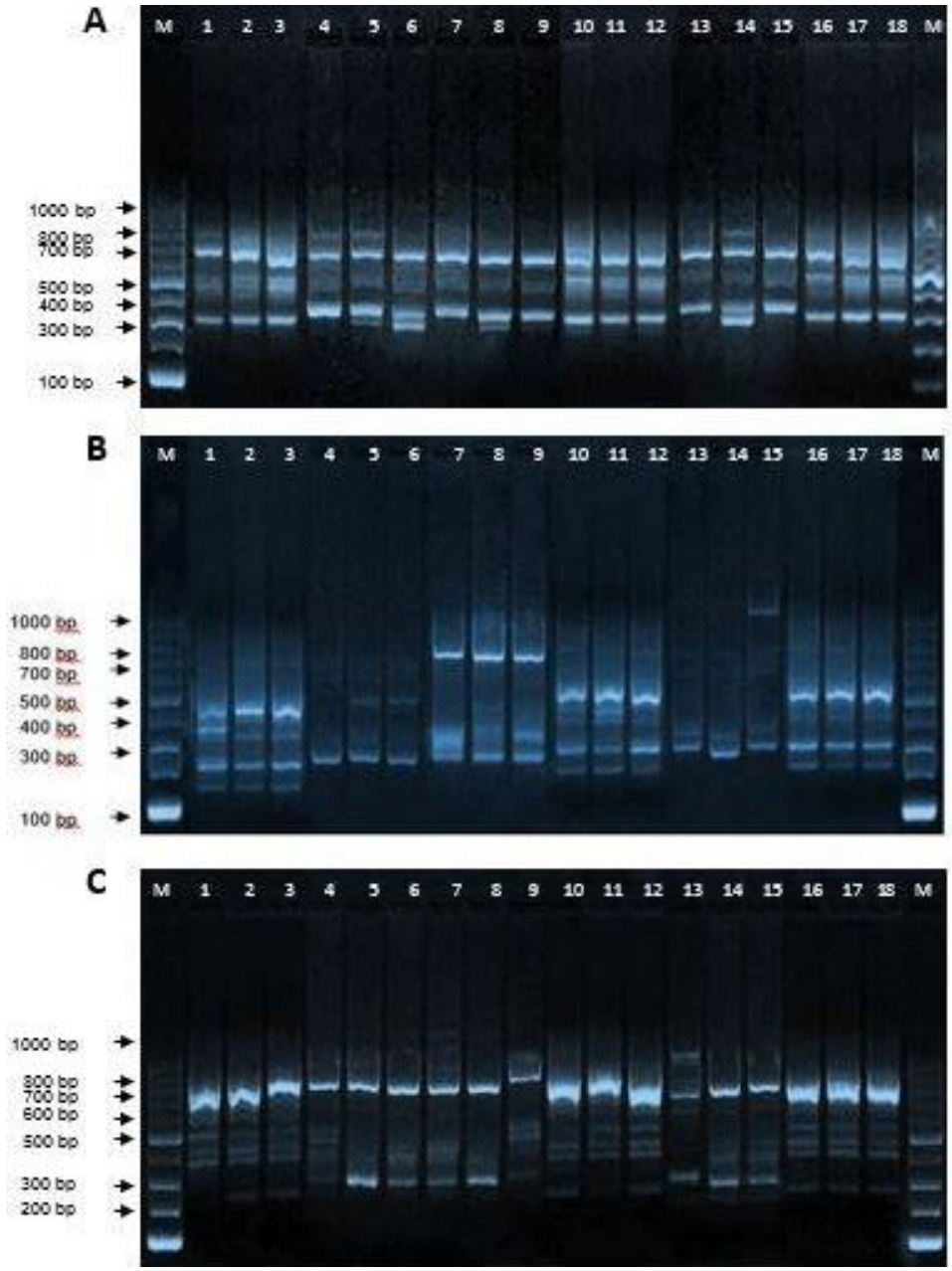

Figure 3. Random amplified polymorphic DNA fragment pattern generated of *Portunus pelagicus* from Makassar Strait using OPA-5. **(A)**, OPA-11. **(B),** OPA-17. **(C)** primer. M=Marker; 1-3 = M1; 4-6 = M2; 7-9 = M3; 10-12 = F1; 13-15 = F2; 16-18 = F3.

Table 2. Nei's genetic distance (below diagonal) and identity (above diagonal) among population of *Portunus pelagicus* from Makassar Strait.

Population	M1	M2	M3	F1	F2	F3
M1	-	0.7569	0.7266	0.7861	0.7857	0.7924
M2	0.2785	-	0.9050	0.7922	0.9041	0.8254
M3	0.3193	0.0998	-	0.7252	0.8472	0.8166
F1	0.2407	0.2329	0.3214	-	0.7774	0.9011
F2	0.2412	0.1008	0.1658	0.2517	-	0.7461
F3	0.2327	0.1919	0.2026	0.1042	0.2929	-

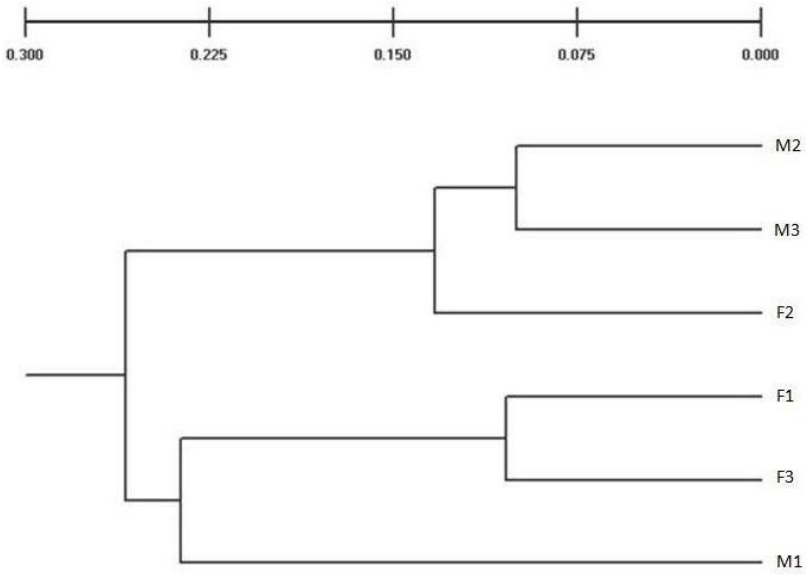

Figure 4. The UPGMA dendogram using Nei's genetic distance. M: Male; F: Female.

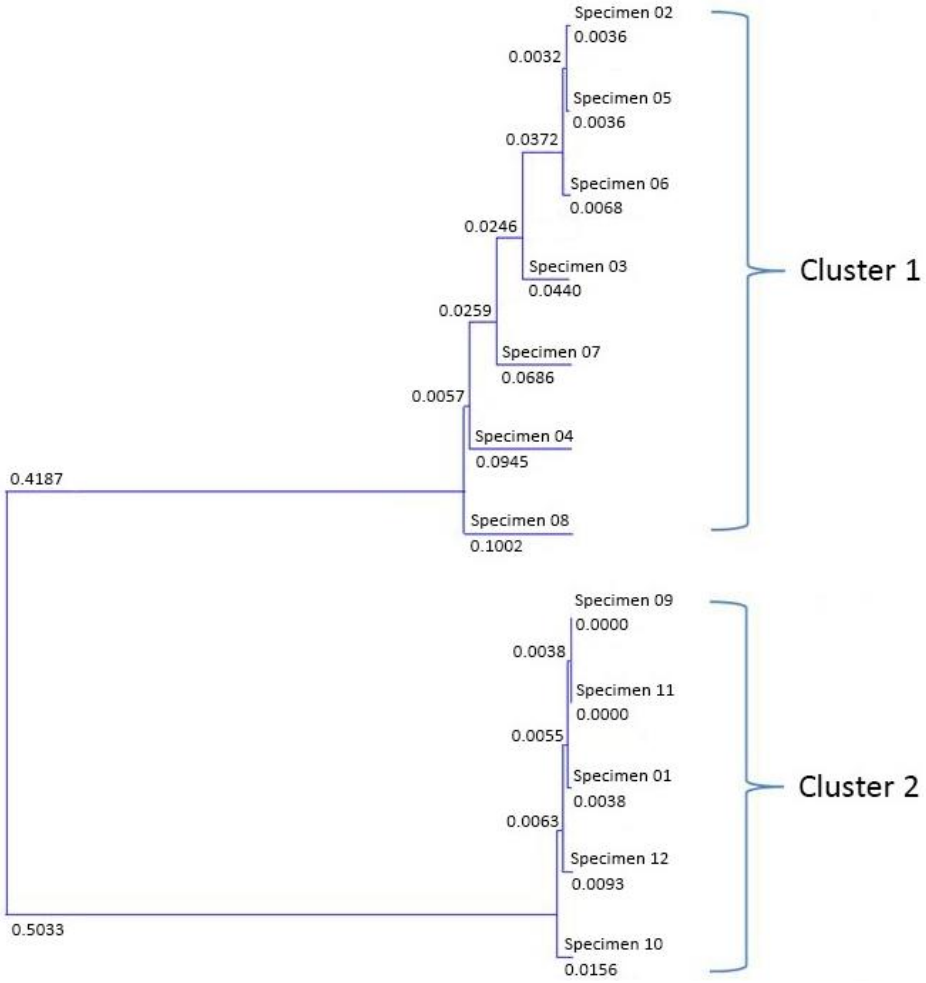

Figure 5. Phylogenetic relationship among 12 specimens of *P. pelagicus* population from Makassar Strait based on 16S rRNA sequences.

reported by Lai et al. (2010). The results also explained that the differences in the white spot pattern in the carapace of *P. pelagicus* are influenced by genetic. Thus, the variation of white spot pattern on the carapace can be used as indicators of differentiation in population of *P. pelagicus*.

DISCUSSION

The high degree of polymorphism and genetic distances on the population studied showed that the genetic diversity of *P. pelagicus* live in Makassar Strait is quite high. Similar result was reported by Suresh et al. (2013) that genetic distance of *M. cephalus* from Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu in India varied from 0.3717 ± 0.1460 (Gujarat population) to 0.5316 ± 0.1720 (Maharashtra population). A dendrogram based on Nei's genetic distance also showed two clusters. The Maharashtra and Gujarat populations appear in one cluster, while the Tamil Nadu and Andhra Pradesh populations formed the other cluster. A high degree of polymorphism suggested a high degree of genetic variability between the samples (Prasad, 2014). According to Amavet et al. (2007), the presence or the absence of the polymorphism in PCR-RAPD profile is either caused by nucleotide sequence divergence in primer sites or by insertions or deletions in the amplified segment of the template DNA.

The indication of hybridization is even more interesting among the population through the result of unique RAPD bands analysis. These population-specific unique bands can be used to detect any possible mixing of these populations, especially during selective breeding programs (Ferguson et al., 1995). Lai et al. (2010) also reported a phenomenon of natural hybridization between *P. pelagicus* and *Phrynocephalus reticulatus* in the Bay of Bengal.

The result of a combination of RAPD markers and 16S rRNA sequence was mutually reinforcing and complementary that the *P. pelagicus* have a high genetic variation. This indication was reinforced by the formation of two clusters based on RAPD as well as 16S rRNA sequences. The 16S rRNA is one region in mitochondrial genome. The mtDNA in animals is maternal inheritance (Rawson and Hilbish, 1995; Castro et al., 1998; Miller et al., 2005). Miller et al. (2005) suggests that characteristics of mitochondrial genome are maternal inheritance, lack of intermolecular recom-bination, and relatively rapid mutation rate.

Some studies have indicated that the sequence of 16S rRNA accumulates mutations more rapidly than the nuclear rDNA genes and can infer relationships beneath the family level within insects (Simon et al., 1994). Therefore, groups of animals that were descended from the same maternal line have a high similarity index unless there has been a mutation or gene flow. In this study, mutations were shown in the results of 16S rRNA sequencing, because the sequence of 16S rRNA is precise to study the mutation rate of the population as a result of aquaculture and stock enhancement.

A better understanding of population genetic structure is important to the effective fisheries management and conservation of genetic resources in exploited marine organism (Bert et al., 2007). Stock enhancement and cultivation can reduce the probability. Admixed populations will undergo damaging genetic alteration in the event of a decrease in genetic variation, fitness, and effective population size, because maintaining the genetic diversity of admixed populations and their wild-population components first requires managing both the genetic variability (e.g., numbers of alleles) and the genetic composition (frequencies of alleles) in the broodstocks and the broods (Bert et al., 2007). According to Waples et al. (2012), the major genetic risks of aquaculture include loss of genetic diversity within and among the populations and loss of fitness caused by the use of low genetic diversity and small numbers of brood stock. This situation can lead to inbreeding which in turn has implications for the quality of fry, a symmetrical survival of 'families' causing declines in stock quality.

However, this study failed to determine whether the two clusters found were distinct species or a sub species based on 16S rRNA sequences, because the data available on GenBank is only for *P. pelagicus* as a single species. Using COI sequences may help to identify the species complex of *P. pelagicus* in the Makassar Strait. The use of mitochondrial COI gene region in identifying the species *P. pelagicus* have been done by Lai et al. (2010) and Sienas et al. (2014).

Lai et al. (2010) reported, based on morphometric and mt DNA (COI) analysis that *P. pelagicus* in the world is a species complex consisting of four species, namely, *P. pelagicus* (Linnaeus, 1758), *P. reticulatus* (Herbst, 1799), *Petrolisthes armatus* (Milne-Edwards, 1861) and *Portunus segnis* (Forskal, 1775). The species complex is distributed in different geographic locations. *P. pelagicus* is widespread across Southeast and East Asia and is sympatric with *P. armatus* in the Northern Territory, Northern Australia. *P. armatus* is found around most of Australia and East to New Caledonia.

P. reticulatus occurs in the Eastern Indian Ocean. *P. segnis* appears to be confined to the Western Indian Ocean from Pakistan to South Africa, and is a Lessepsian migrant into the Mediterranean from the Red Sea. While Sienas et al. (2014) reported that *P. pelagicus* occuring across the entire Philippine archipelago potential consists of 2 species. Li et al. (2009) also used COI gene to study historical events of chinese shrimp (*Feneropenaeus chinensis*) in the Yellow Sea and Bohai Sea.

The conclusion of this study was the high genetic variation of *P. pelagicus* from Makassar Straits. This study suggests that farmers or hatchery operators can continue to use the population as sources of natural broodstock. Farmers can easily identify the genetic differences of the crab population by color and spot pattern in carapace. Morphologically, male and female were also distinguished and that is where they mate. This information also will be helpful in developing superior strain for aquaculture through selective breeding and formulating stock specific management for conservation of the species.

Conflict of Interests

The authors have not declared any conflicts of interest.

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Abbreviations

RAPD, Random amplified polymorphic DNA; **OPA,** operon teknologi kit A; **PCR,** polymerase chain reaction; **SDS,** sodium dodecyl sulfate; **16S rRNA,** ribosomal RNA region 16S; **COI,** cytochrome oxidase sub unit I; **TFPGA,** tools for population genetic analyses; **UPGMA,** unweighted pair group method of arithmetic.

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