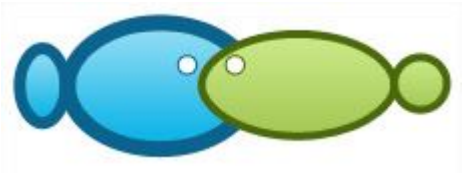

The morphology and morphometric characteristics of the male swimming crab (*Portunus pelagicus*) from the East Sahul Shelf, Indonesia

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Abstract. The swimming crab (*Portunus* sp.) has distinct morphological characteristics. They come in a variety of colors and most have white patterns on their carapaces. The aim of this study is to determine the morphology and morphometric variation of male swimming crabs collected from the East Sahul Shelf in Indonesia. The sample was collected in West Papua, representative of the East Sahul Shelf area. The sampling locations were Sorong (30 crabs), Raja Ampat (45 crabs) and Kaimana (76 crabs). The morphological analysis determined the color, white patterns on carapace and shape of the gonopodium. While the morphometric characteristics were determined by measuring the length and width of the crabs' carapaces and meri. Data regarding the morphometric characteristics were analyzed using *Stepwise Discriminant Analyses*. The distance of genetic variation based on morphology and morphometric characteristics between populations was analyzed using *Predicted Group Membership* and *Pairwise Group Comparison* and the *Test Equality of Group Means* was used to analyze the more specific characteristic of the crab. Results indicated that the morphological characteristics based on color and the white patterns on the carapace of the swimming crab from West Papua were similar to *Portunus pelagicus*. However, based on the shape of the gonopodium, crabs from Sorong, Raja Ampat and Kaimana had characteristics similar to those of the *Portunus armatus* with the percentage of similarity being 30%, 40% and 25%, respectively. Morphometric analysis showed a significant difference between the crabs' morphometric characteristic and their populations from the three locations of sampling ($p < 0.05$). In conclusion, the swimming crabs from West Papua exhibit large variations in morphologic and morphometric characteristics. Thus, it can be concluded that the swimming crab from West Papua shows intraspecific variation.

Key Words: swimming crab (*Portunus* sp.), morphology, morphometry, intraspecific.

Introduction. The swimming crab is a crustacean that has high economic value (Redzuari et al 2012), and a wide geographical distribution (Zainal 2013). They are found in the Indo tropical coastal to West Pacific region (Ikhwanuddin et al 2013). This organism has fast growth rate, attractive morphological characteristic and a delicacy in many parts of the world (Ravi & Manisseri 2012). It is because of all these characteristics that the demand for this crab has increased exponentially over time (Redzuari et al 2012).

The increasing demand for these crabs has caused over-exploitation and a rapidly decreasing population size of wild crabs (Mehanna et al 2013; Kunsook et al 2014). The decreasing population of wild crabs could have a negative effect on genetic variability and, consequently, it could lead to a lowered genetic quality of the crab (de Freitas & Galetti Jr. 2005). To meet the increasing demand for crab meat and to reduce the negative effects of over-exploitation, crab culture is one of the alternative solutions. Crab culture requires that the broodstock has excellent genetic quality. Additionally, the proper hatchery technology must be chosen (Soundarapandian & Tamizhazhagan 2009). However, most crab cultures are still dependent on wild broodstock which is very limited. An alternative solution to this problem is via rigorous broodstock selection that will be used in the hatchery to produce high-quality seeds.

The parameters that determine crab characteristics are their morphological characteristics (Fujaya et al 2016) and their morphometric characteristics (Ravi et al 2008). The copulatory organ is a distinct morphological characteristic in the crab male. This organ is similar to all other crab species. However, there is a part of this organ that is species-specific and different. It is the gonopod (Ewers-Saucedo et al 2015). This organ is found in the *Portunus* species only (Lai et al 2010; Trivedi & Vachhrajani 2012; Padate et al 2013). Morphometric analysis is a strong tool to examine genetic variation (Josileen 2011) in order to increase the accuracy of genetic data (Chang & Hsu 2004). Furthermore, the morphometric characteristic analysis could also be used to determine the phylogenetic relationship of this species with the other organisms (Overton et al 1997; Sangthong & Joundeung 2006).

Previous studies focused on the morphology and morphometric characteristics of female crab broodstock (Ravi et al 2008; de Lestang et al 2003; Johnson et al 2010), while only a few studies have been conducted to examine the reproductive system of the male swimming crab (Ewers-Saucedo et al 2015; Soundaranpandian et al 2014). So far, no study has been conducted to examine the morphology and morphometrics of the male swimming crab. Thus, to provide more data in this area, this study was conducted to examine the morphology and morphometric characteristics of male swimming crab from the East Sahul Shelf in Indonesia.

Material and Method

Study sites. Swimming crab (*Portunus pelagicus*) samples were collected from three locations in West Papua, Indonesia (Sorong, Waiwo (Raja Ampat) and Kaimana) (Figure 1), which contain a significantly high number of crab landings by local fishermen.

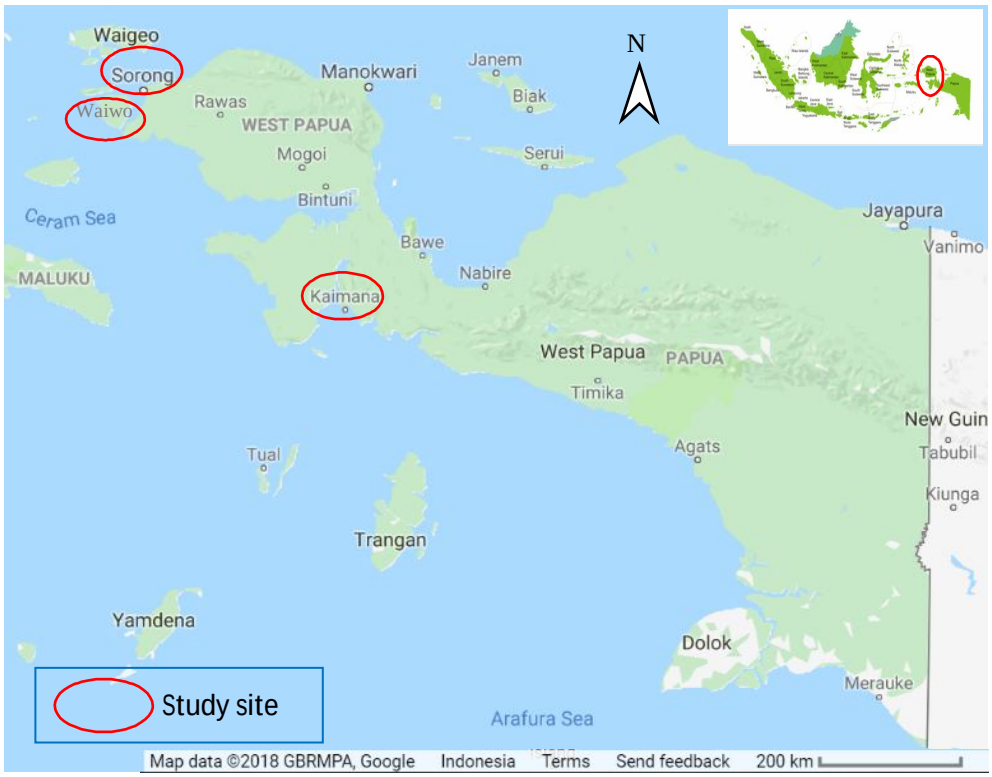

Figure 1. Study sites map (Source: www.googlemap.com [accessed date: April 29, 2018]).

Number of crab samples. Swimming crab samples from this study were collected from Sorong (30 crabs), Raja Ampat Regency (45 crabs) and Kaimana Regency (76 crabs). Thus, a total of 151 crabs were collected and had their morphology and morphometric characteristics measured.

Morphology characteristics. The morphological characteristics of the male crabs were examined. Examination focused specifically on the colors and patterns of white spots on the carapace and on the male gonopod. Group 1 comprised all the samples collected from Sorong city, while Group 2 comprised samples that were collected from the Raja Ampat District. Group 3 comprised all samples from the Kaimana District. Specimens were freshly dead. Color of the crabs were analysed using Toca Color Finder (TCF) Sofware (Ayi et al 2018) and the patterns of white spots on the carapace were described (Table 1). The male gonopods was observed via photographic image taken using a camera (OMAX 9.0MP USB digital camera) that was attached was to a microscope (Olympus CX21). The images were also described.

Table 1

Color scale of TCF

Color	The colour name
	Yellow Olive (RAL 6014)
	Moss Grey (RAL 7003)
	Beige Grey (RAL 7006)
	Green Grey (RAL 7009)
	Brown Grey (RAL 7013)

Morphometric analysis. The morphometric characteristics, which include the length and width of carapace and merus, were measured (Figure 2) (Fujaya et al 2016). Statistical analysis tests were undertaken using SPSS 15.0. Morphometric ratio comparisons between individual males from different groups were carried out by a one-way ANOVA. Then the variation analysis group (population) was determined using Predicted Group Membership. Next, the magnitude of the differences between species genetic distance (dissimilarity) was analyzed using the Pairwise Comparison Group (Lai et al 2010). Furthermore, to identify the difference in characteristics for each sample group the Equality of Group Means Test was used (Lai et al 2010).

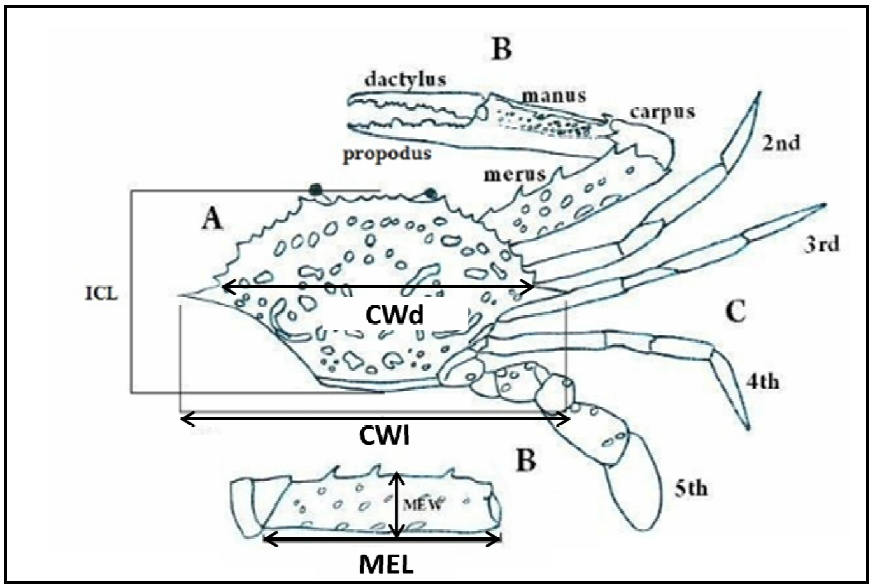

Figure 2. Body parts of *Portunus pelagicus* for morphometric measurement in this study. A. Carapace and appendages; B. Cheliped; C. Pereiopod; CWl = outer carapace width; CWd = deep carapace width; CL = carapace length; MEW = major cheliped merus width; MEL = major cheliped merus length (after Lai et al 2010).

Results and Discussion

A. Morphological characteristics

Color and pattern of white spots on carapace. Polymorphism in the same species is commonly found in both invertebrate and vertebrate species (Trivedi & Vachhrajani 2012). Color variation of species is also important in crabs (Chi et al 2010; Martin & Zimmerman 2007). In general, morphological differences in species are often due to the differences in their geographical habitats; particularly, geographically isolated habitats (Lawrence & Morrissy 2000). Species that have a wide distribution usually have a high variation in morphology (Daniels et al 2001). For invertebrate species, morphology variation mostly manifests as difference in the color of the body, which is correlated with avoiding predators, age, feeding habits, parasites on the skin and gene heritage (Trivedi & Vachhrajani 2012). This present study attempted to prove that the same species may have different morphological features as a result of living in different habitats. For this reason, the color of the carapace could be an indicator of which species it belongs to (Metri et al 2016).

Branchyurid species show variation in color, this color variation plays important role in their social interactions, particularly when they need to recognize each other for mating (Silbiger & Munguia 2008). Additionally, this color variation was correlated with crab behavior ecology and physiology (Chi et al 2010). The pattern of color in Branchyurid species was called a complex species, this means that similar color patterns belonged to several species (Martin & Zimmerman 2007). For example, the blue swimming crab previously was identified as *Portunus pelagicus.* However, based on gene and morphometric analysis, blue swimming crab (BSC) was divided into four species namely: *P. pelagicus, P. reticulatus, P. armatus* and *P. segnis* (Lai et al 2010). Fujaya et al (2016) found that color pattern in *P. pelagicus* did not correlate with a habitat characteristic but it was strongly correlated with genes.

Results of this present study showed that there was a significant difference in morphological characteristics between the crab samples from the same location. There was variation in terms of color and white spot patterns between the crab samples from our sampling locations. The crab samples from Sorong, Raja Ampat and Kaimana had 7, 5 and 7 variations, respectively (Figures 3, 4 and 5).

Based on the Toca Color Finder (TCF) scale, S1 variation of the carapace had a yellow olive color with white spot pattern that spread from the posterior and anterolateral parts of the carapace. White spots on carapace were scattered with medium density. The S2 variation of the carapace was green-grey color, and the white spot pattern on the carapace were scattered with minimal density. The S3 variation had beige-grey carapace with white spots that occupied the entire carapace. The S4 variation of the carapace had moss-grey color with white spot pattern that spread across the posterior and cardiac areas of the carapace with medium density. The S5 variation had brown-grey color of the carapace and white spot pattern that are found on the entire carapace. The carapace color and white spot pattern of the S6 variation were similar to those of the S5 variation, however; the size of the white spots on the S6 variation was smaller than those on S5. Lastly, the S7 variation was similar to the S1 variation, the only difference was that the carapace of S7 variation had a rough surface (Figure 3).

Crab samples from the Raja Ampat Regency had five variations of color and white spot patterns. The RA1 variation had a brown-grey carapace with large white spot patterns at posterior area of carapace and small white spots on the anterolateral area of the carapace. The RA2 variation had a green-grey color with large white spots that spread across the entire carapace. The variation of carapace for RA3 was similar to RA2 in terms of the white spot pattern; however, the color of carapace was beige-grey. The RA4 variation had moss-grey carapaces with white spot patterns that were dense in the posterior and anterolateral ends of the carapace. The RA5 variation's carapaces were olive yellow in color with white spots that appeared in a line at the anterolateral and cardiac areas of the carapace (Figure 4).

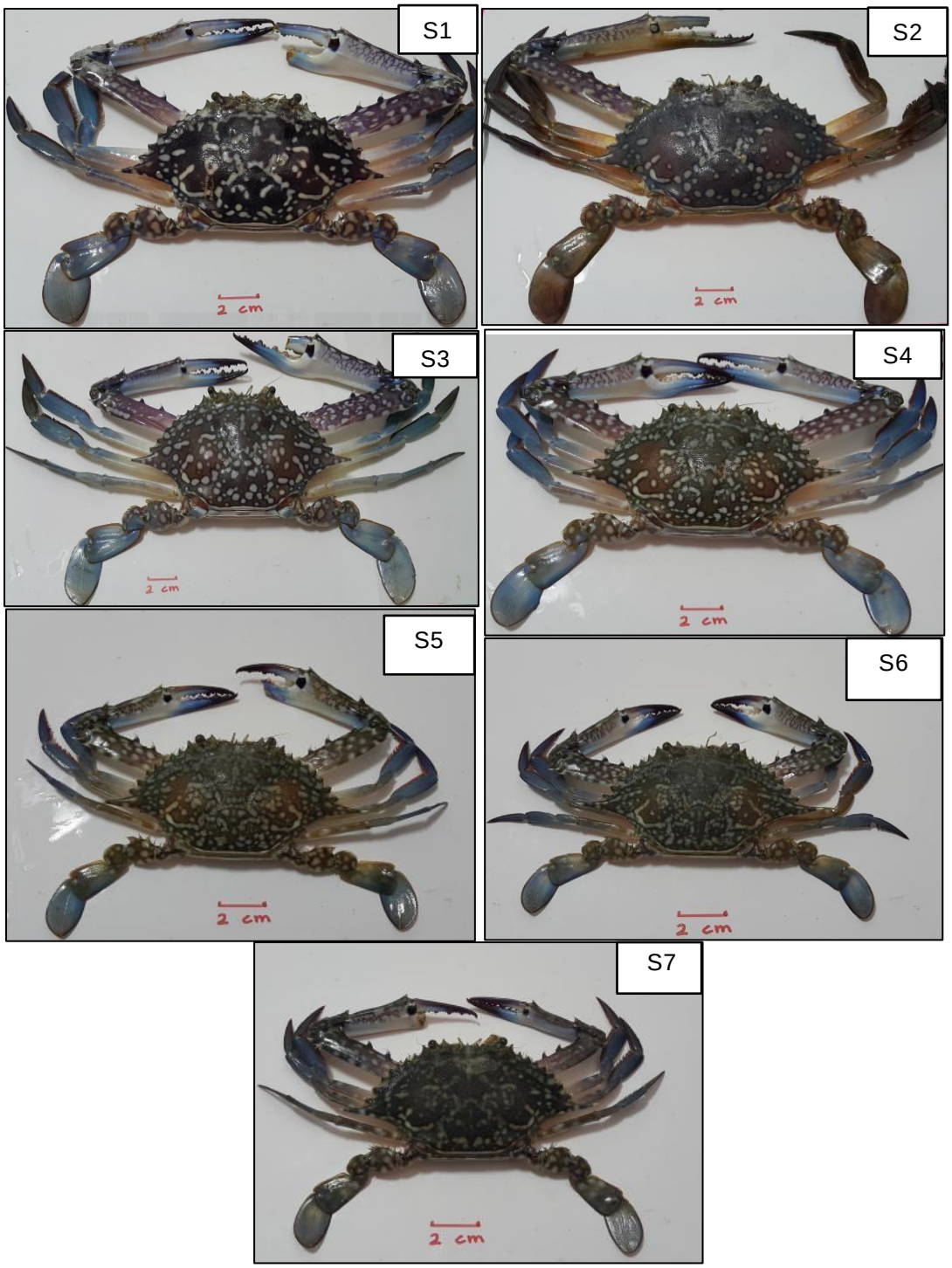

Figure 3. Color of the carapace and pattern of the white spots of the male *Portunus* sp. collected from Sorong City, (S indicating the sampling location (Sorong) and Number 1 – 7 indicating the variation of color and white spot pattern).

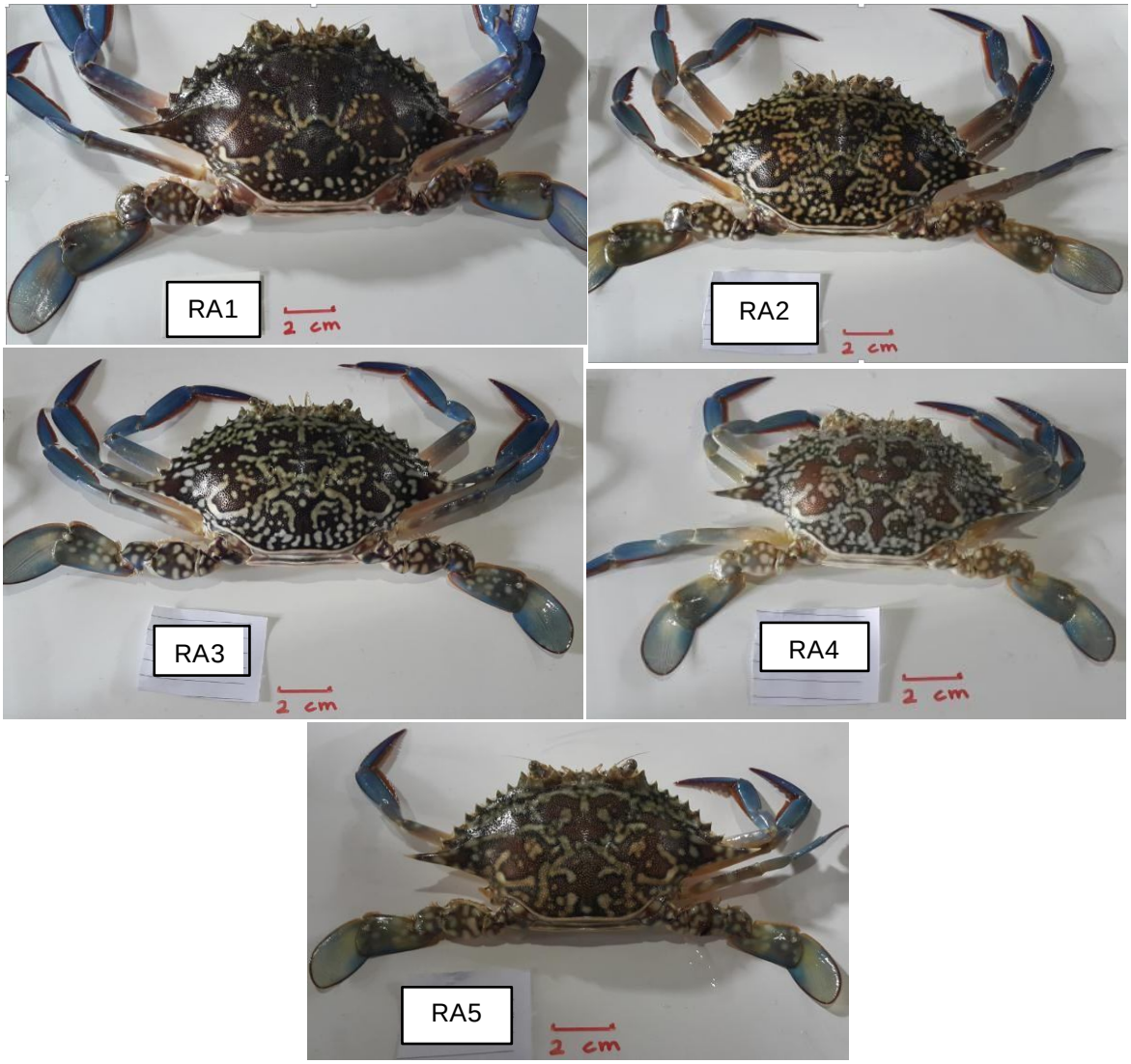

Figure 4. Color of the carapace and pattern of the white spots of the male *Portunus* sp. collected from the Raja Ampat District, (RA indicating the sampling location, i.e., Raja Ampat, and the Numbers 1 – 5 indicating the variation of color and white spot patterns).

The crab samples from the Kaimana District had seven variations of color and white spot patterns on their carapaces. The K1 variation had a beige-grey color with large white spots that spread across the entire carapace with the distance between white spot being large. The K2 variation had was olive yellow in color with more white spots in the posterior area than in the anterolateral area of the carapace. The K3 variation was brown-grey in color with white spots that in the anterolateral and posterior areas of the carapace. The K4 variation was green-grey in color with large white spots that spread across the entire carapace. However, the white spots appeared in a line in the anterolateral area of the carapace. The K5 variation was similar to K4 in color. However, the white spot pattern showed variation in size. The color and white spot pattern on the K6 variation was similar to the K3 variation. However, the size of the white spots on the K6 was larger than those on the K3. The K7 variation was brown-grey in color with white spot patterns similar to those on the K5 variation. However, the K7 variation had a smaller number of white spots than the K5 variation (Figure 5).

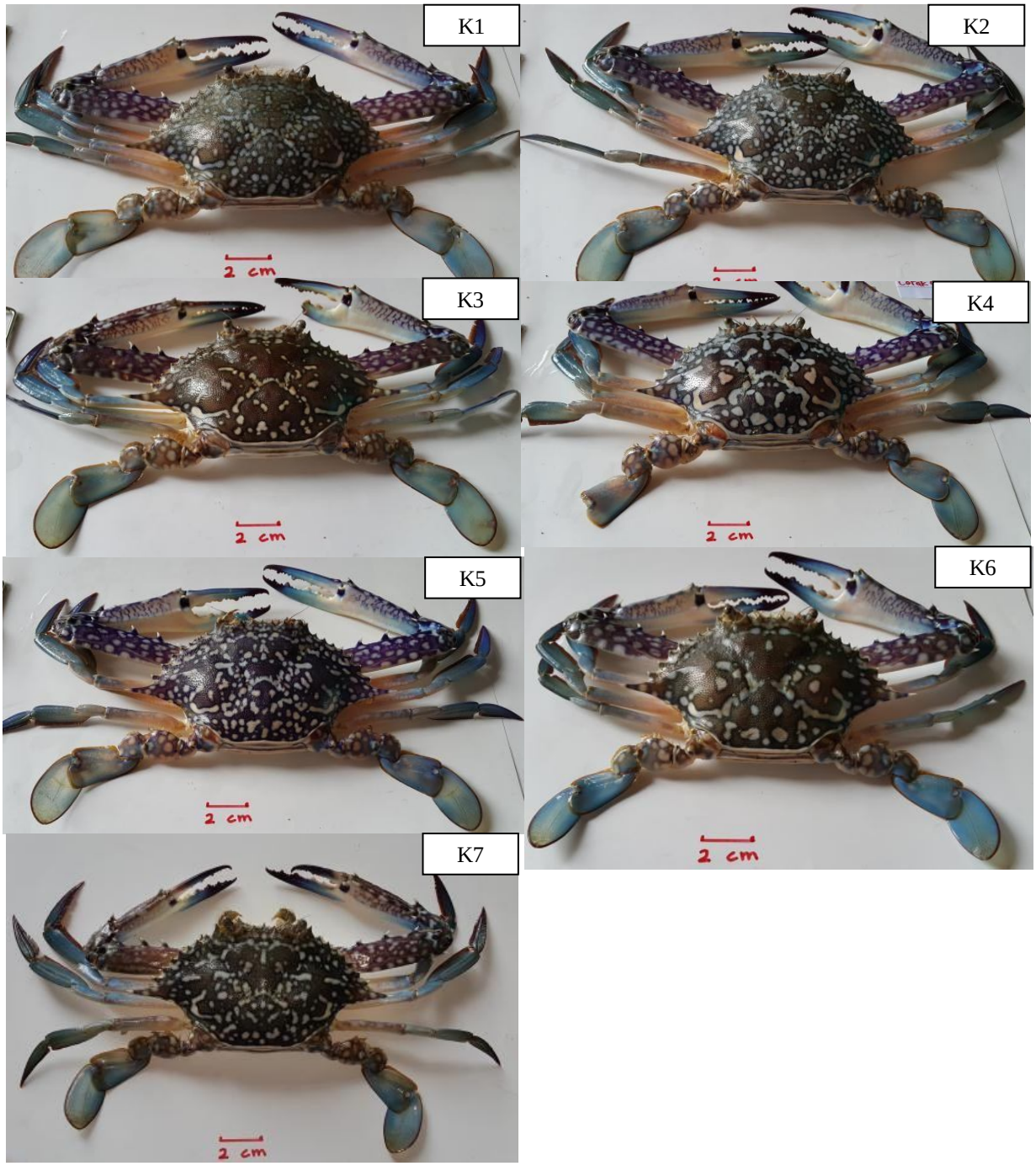

Figure 5. Color of the carapace and pattern of the white spots of the male *Portunus* sp. collected from the Kaimana District (K indicating a sampling location (Kaimana) and Number 1 – 7 indicating the variation of color and white spot patterns).

In this present study, the *Portunus pelagicus* from West Papua showed a high variation in color pattern and white spots on the carapace and these morphological characteristics were different from what Lai et al (2010) had studied. Lai et al (2010) found that the male *P. pelagicus* had a blue-greenish color with purple-bluish chelipeds and white spots on the carapace were seen as lines, particularly in the posterior and branchial areas. The *P. pelagicus* of our samples showed a similar characteristic with *P. pelagicus* and *P. armatus* with the color of the carapace being brown. Our results also showed that there was a variation in color pattern and white spots on the carapaces of the *P. pelagicus*. This finding was supported by a previous study (Fujaya et al 2016) which found that the *P. pelagicus* from Makassar Strait could be divided into three populations based on color pattern and white spots on

the carapaces for both male and female crabs. Our findings also indicated that it was possible that hybridization had occurred naturally between *P. pelagicus* and *P. armatus*. Lai et al (2010) also found that there was a natural hybridization between *P. pelagicus* with *Phrynocephalus reticulatus* or *P. reticulatus* in Bengal Bay.

It is commonly known that *P. pelagicus* is a monophyletic species (Mantelatto et al 2007). Moreover, in several countries, this species is known as a single stock (Klibunga et al 2010; Sienes et al 2014). Sienes et al (2014) regarded the *P. pelagicus* to be a cryptic species; it has similar morphological characteristics but genetically it was a different species. The majority of previous studies of Blue swimming crab identification used the color patterns on the carapace. However, there were a few studies that focused on white spots on carapace for the purpose of Blue swimming crab identification. Fujaya et al (2016) posited that the different white spot patterns on the carapaces of the *P. pelagicus* correlated with gene interaction; moreover, this could be used as an indicator for species identification in a population.

Morphology of the gonopod organ. One of the most distinct characteristics of the Portunidae family is the presence of the gonopod organ. The other morphological characteristic for species identification of Branchyura was the male gonopode (Daniels et al 2001; Lai et al 2010; Trivedi & Vachhrajani 2012; Devi et al 2017). The Portunidae has a pair of gonophode (G1 and G2), but in order to identify a species of Portunidae, taxonomists usually use the distal part of gonopods 1 (G1) structure (Figure 6). The gonopod is an accessory organ that consists of two pairs and is used in mating (Ravi et al 2014; Soundarapandian et al 2013). The first gonopode (G1) was in a tabulate form and is usually as a species identifier (Lai et al 2010) and the second gonopode (G2) is a stem form (G2) (Ewers-Saucedo et al 2015).

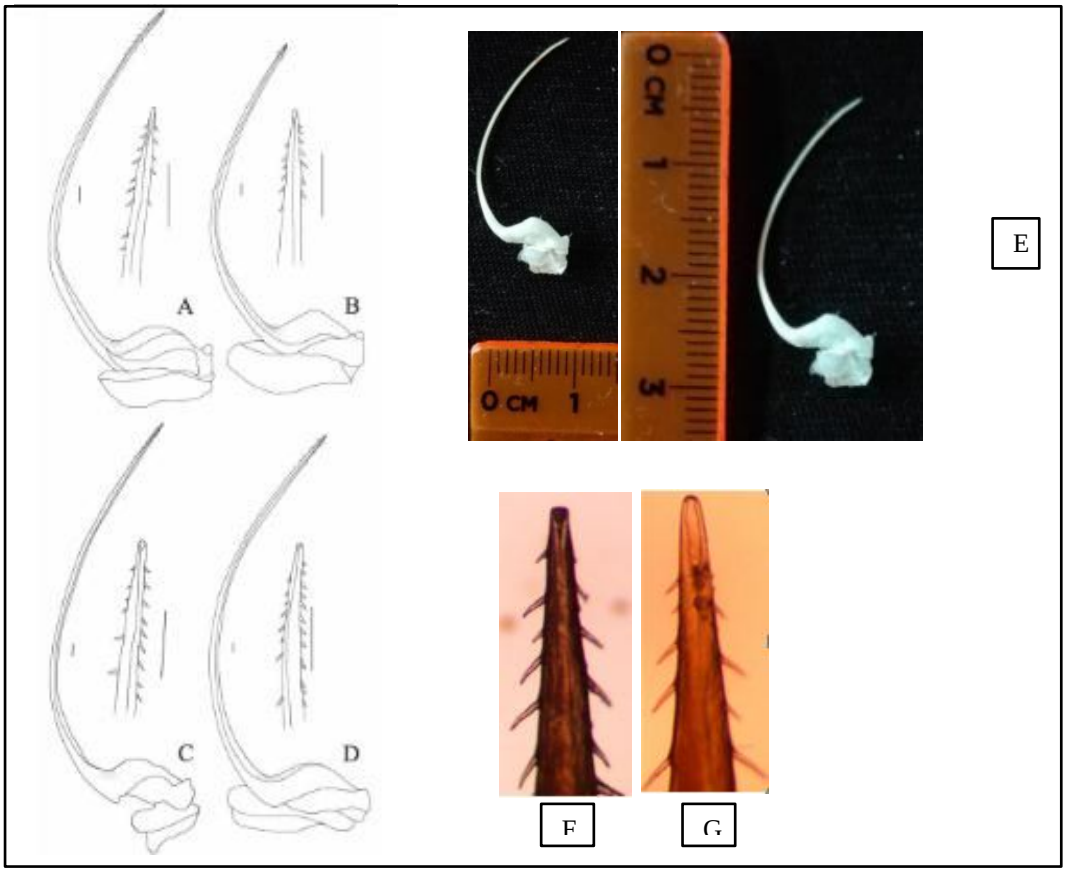

Figure 6. G1 and the distal part of the G1 structure. (A) *Portunus pelagicus*, (B) *Portunus segnis*, (C) *Portunus reticulatus* dan (D) *Portunus armatus* (Figure adapted from Lai *et al*., 2010). (E) G1 from our sample. (F) The distal part of the G1 structure of our sample for *P. pelagicus* and (G) Distal part of the G1 structure of our sample for *P. armatus*.

The gonopode was taken and analyzed from the same sample that we analyzed in terms of color and white spot patterns on the carapace. In general, Sorong's crab samples had the same G1 structure; however, the distal part of the G1 structure was different. Based on the distal structure, the crab samples from Sorong were differentiated into two species of *Portunus*: *P. pelagicus* and *P. armatus* (Figure 6F and 6G).

Our findings showed that crab samples have G1s that are similar to those in the *P. armatus*. The main characteristic of *P. armatus* was the basal part of G1 was rounded (Figure 6G), while *P. pelagicus*, *P. segnis,* and *P. reticulatus* had a round basal spur part of the G1 (Figure 6F). This finding has strongly suggested the possibility that natural hybridization had occurred between the *P. pelagicus* and *P. armatus* in West Papua.

B. Morphometric analysis. The morphometric study of species was used mainly to determine the sex and species, the description of morphological variation, and the classification and prediction for phylogenetic correlation (Fujaya et al 2016). Our study has successfully described a blue swimming crab morphological variation from three locations. *Canonical Discriminant Functions* (CDF) and *Predicted Group Membership* (PGM) results showed that the longest distance of blue swimming crab genetic was a BSC population from Raja Ampat and Kaimana. The shortest distance of blue swimming crab genetic was in the samples from Sorong and Kaimana. This finding indicated that blue swimming crab populations from Sorong and Kaimana share similar morphological characteristics. This finding was also supported by the *Pairwise Group Comparison* (PGC) results that showed the level of genetic distance was high among blue swimming crab populations from Raja Ampat and Kaimana.

Canonical Discriminant Functions (CDF) results showed that there was a clear grouping of male crab samples (Figure 7). Morphometrically, male crab samples from three different locations (Sorong, Kaimana and Raja Ampat) showed different characteristics. Based on *Predicted Group Membership* (PGM) result, the male crabs from Sorong had 40% similarity with the Kaimana samples. Furthermore, the Raja Ampat samples were 13% characteristically similar to the Sorong sample and 17.8% similar to the Kaimana samples. Male crab samples from Kaimana were 42.1% similar to the Sorong samples accounting and were 5.3% similar to the Raja Ampat samples (Table 2). *Pairwise Group Comparison* (PGC) results showed that there was a significant genetic distance between the crab samples from Kaimana and Raja Ampat districts; the genetic distance was found to be 46.23% (Table 3).

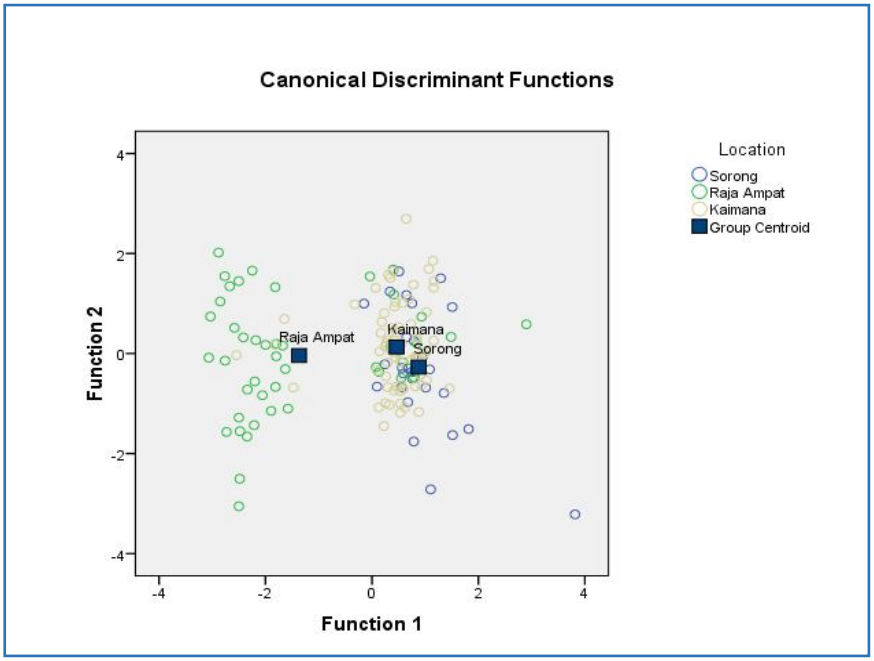

Figure 7. Scatter plot CDF between crab samples from three locations (Sorong, Raja Ampat and Kaimana).

Table 2

PGM results and population diversity of the male *Portunus pelagicus* from Sorong, Raja Ampat, and Kaimana

Classification		*Population*	*Predicted group membership*			*Total*
			Sorong population	*Raja Ampat population*	*Kaimana population*	
Cross-validated	Count	Sorong	17	0	13	30
		Raja Ampat	6	31	8	45
		Kaimana	32	4	40	76
	%	Sorong	56.7	0.0	43.3	100.0
		Raja Ampat	13.3	68.9	17.8	100.0
		Kaimana	42.1	5.3	52.6	100.0

Noted: 58.9 % original data as have been grouped properly 58.3% cross-validated data have been grouped properly. Validation value ≥ 50% was valid.

Table 3

Genetic Distance PGC results based on the male *Portunus pelagicus* morphometric data from Sorong, Raja Ampat and Kaimana

Male		*Sorong population*	*Raja Ampat population*	*Kaimana population*
Sorong population	F Sig.		45.612 $3.88x10^{-16}$	2.312 0.103
Raja Ampat population	F Sig.	45.612 $3.88x10^{-16}$		46.231 $2.65x10^{-16}$
Kaimana population	F Sig.	2.312 0.103	46.231 $2.65x10^{-16}$	

Based on the results of the *Equality of Group Means* (EGM) test, it was found that there were four (4) specific characters of crab morphometrics from three locations of crab samples. However, there was only one character non-significant difference between three locations of crab samples, which was the width of the outer carapace and the width of the inner carapace ratio ($p > 0.05$) (Table 4).

Table 4

Specific Character based on the *Test of Equality of Group Means* results from Sorong, Raja Ampat and Kaimana

Population		*Wilks' Lambda*	*F*	*df1*	*Sig.*
Male	CL/CWl	0.923	6.140	2	0.003
	CL/CWd	0.848	13.285	2	$4.94x10^{-6}$
	CWl/CWd	0.979	1.603	2	0.205
	MEL/MEW	0.587	52.125	2	$7.31x10^{-18}$

Note: CL = carapace length; CWl = outer carapace width; CWd = deep carapace width; MEL = major cheliped merus length; MEW = major cheliped merus width.

The width of carapace and meri are discriminant characters of the three male crab populations in East Sahul Shelf, Indonesia. The largest carapace width was found in the crab samples from Sorong and Kaimana. However, the longest merus was found in the crab samples from Raja Ampat accounting for 0.264 (Table 5).

Table 5

Discriminant character of male crab from Sorong, Raja Ampat and Kaimana base on ratio average

Population	Discriminant character	Description	Ratio average (population)		
			Sorong	Raja Ampat	Kaimana
Male	CL/CWl	Ratio of carapace length to the width of the outer carapace	0.472	0.465	0.473
	CL/CWd	Ratio of carapace length to deep carapace width	0.588	0.576	0.585
	MEL/MEW	The ratio of merus length to width merus	0.249	0.264	0.233

Note: CL = carapace length; CWl = outer carapace width; CWd = deep carapace width; MEL = major cheliped merus length; MEW = major cheliped merus width.

The *Equality of Group Means* results showed that there were three significant and specific characteristics, namely the ratio of carapace length to inner carapace width, the ratio of the carapace lengths to the outer carapace width and the ratio of merus length to merus width. These specific characteristics could be used a discriminant tool between blue swimming crab populations. These three specific characteristics are almost similar to those used in identifying the *P. pelagicus* which has an intraspecific variation. Our findings indicated that blue swimming crab from West Papua had a high variation in morphological and morphometrical characteristics and are different from the findings of Lai et al (2010).

Conclusions. Based on morphological characteristic findings, particularly those related to the gonopode characteristic, the *P pelagicus* from West Papua showed a similar gonopods morphological characteristic to *P. armatus*. This finding was strongly suggests that the blue swimming crab from West Papua is a species complex. To confirm this finding, it is important to conduct further research through analysis of the DNA of the *P. pelagicus* from West Papua.

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