Morphometric and genetic diversity of mantis shrimp Harpiosquilla raphidea

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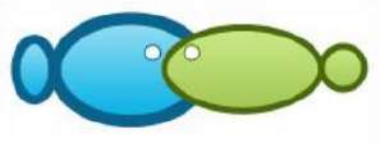

Morphometric and genetic diversity of mantis shrimp *Harpiosquilla raphidea* from Karimata strait and Java Sea waters, Indonesia

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Abstract. Study on morphometric variations and genetic diversity of the mantis shrimp (*Harpiosquilla raphidea*) from Karimata strait and Java sea waters has been conducted from January 2013 to April 2014. *H. raphidea* samples were collected from six locations i.e. Teluk Jakarta, Cirebon, Semarang, Tanjung pandan, Pontianak and Jambi waters. A total of 360 *H. raphidea* individuals have been collected from Karimata strait and Java sea waters. Based on Canonical Discriminant Univariate Statistics Analyses, 20 out of 22 morphometric characters were significantly different ($P<0.01$). The highest internal diversity of *H. raphidea* population (84.16%) was in Teluk Jakarta, while the lowest one was in Pontianak (56%). According to the multiple alignment analyses, there were 10 haplotypes distributed from Karimata strait (Jambi, Tanjung Pandan, Pontianak) and from Java sea waters (Teluk Jakarta, Cirebon, Semarang). The results showed a classification of six populations into three groups among the *H. raphidea* population, based on the analysis of genetic distance.

Key Words: Teluk Jakarta, morphometric diversity, morphometric characters, population, genetic distance, giant harpiosquillid.

Introduction. Indonesian giant harpiosquillid or mantis shrimp (*Harpiosquilla raphidea*, Fabricius 1798) is an indigenous species in Indonesian marine waters which is of a very important economic value. The species can get extinct if it is overexploited. Furthermore, the extinction of the species also can be caused by inbreeding depression. Therefore, some efforts were needed in order to avoid the extinction of this species. Morphological and genetic diversity study of the *H. raphidea*, is one of the alternative solutions to obtain genetic information on the population of the Western Indonesian marine waters.

The population of *H. raphidea* is likely to decline and causing inbreeding so that pushing the "fitness" of the shrimp population will finally cause the extinction of the species (Lui et al 2007). The correct management strategy is necessary to avoid the extinction of *H. raphidea*, and for that reason it needs a study covering the population biological aspect and the condition of habitat.

The molecular mark is able to identify the difference of direct genetics at DNA level as genetics components. All characters displayed are seen (phenotype) and invisible (genotype) by one individual reflects the animal's genetic character possessed by individual animals (Nei 1987). All informations that can be observed at one individual is a genetic mark of the individual. Molecular characteristics can handle limitations on the use of morphological (phenotypic) characters that are often influenced by the environment, so that by analyzing phenotypic and genotypic characters will provide more accurate informations (Moosa 1989).

Some information's about morphologic and genetic characteristics, can provide helpful insights for management and conservation of this species. Until now, neither morphologic nor genetic diversities of *H. raphidea* from Karimata strait and Java Sea have been studied ever before. Accordingly, this paper presents the preliminary results of morphologic and genetic diversity of *H. raphidea* from Karimata strait and Java Sea.

Material and Method. Three hundred and sixty mantis shrimps (130-330 mm in total length) were collected from Jambi, Tanjung Pandan, Pontianak, Teluk Jakarta, Cirebon and Semarang waters. The *H. raphidea* samples were identified using Moosa (2000) and Ahyong (2012). A total of 22 morphometric characters were observed (Figure 1), the measurements were performed done on left side of *H. raphidea* sample (Table 1).

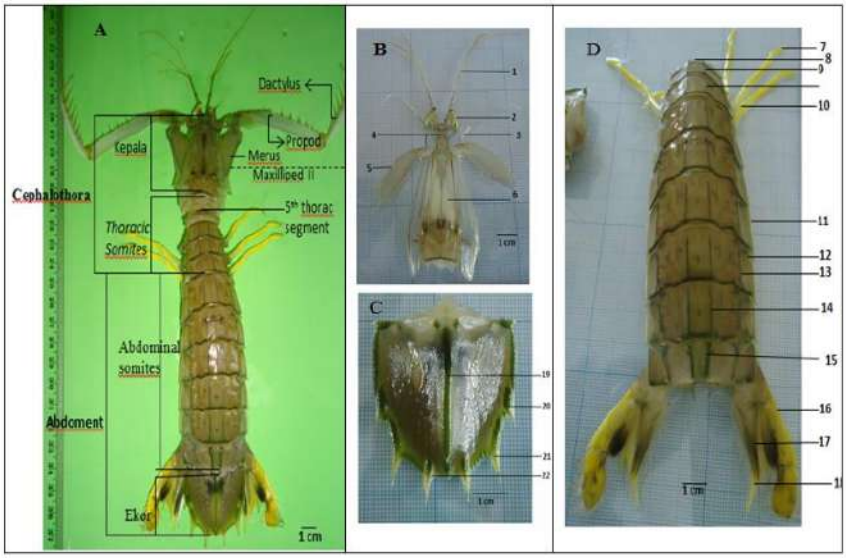

Figure 1. Morphology of *Harphiosquilla raphidea* A. Overview; B. Carapace; C. Telson; D. Abdoment (Mulyono et al 2016). The morphometric characters (1-22) are described in Table 1.

Table 1

The morphometric characters of *Harphiosquilla raphidea* measured in the study

No	*Code*	*Morphometric character*
1	PTO	Distance to the tip end of the telson carapace
2	PST	Distance edge telson innermost shell and front end
3	PBD	Distance edge down bottom up abdomen carapace
4	PKP	Distance edge carapace and carapace rear limit torac
5	LKP	Distance between carapace width fence from right to left edge
6	PTS	Distance between limit up limit belly carapace
7	PAS	Distance between limit somite torac to telson rear end up front
8	ASS	Distance segment first abdominal somite
9	ASD	Distance segment second abdominal somite
10	AST	Distance segment third abdominal somites
11	ASE	Distance segment fourth abdominal somites
12	ASL	Distance segment fifth abdominal somites
13	ASN	Distance segment sixth abdominal somites
14	PLA	Distance between stomach fence width from right to left edge
15	TLS	Distance between telson deepest abdominal limit up rear
16	PMI	Distance between edge maxilliped until end dactylus left side
17	LMI	Distance segment propondus up down left edge part differences
18	PMA	Distance between dactylus maxilliped up to the right
19	LMA	Distance segment propondus edge down right up part differences
20	PUI	Distance uropod until end of lists the left
21	PUA	Distance long edge of base uropod up to the right
22	LTL	Distance telson up of middle depth part differences before the sixth abdominal somites

Morphologic analysis. Morphometric characteristics were measured according to Mori et al (2009) with slight some modifications. The data collected were analyzed by Kruskal Wallis test and continued with Mann Whitney U test using SPSS ver. 19. We also analyzed the data with the Principle Component Analysis (PCA) using MVSP 3.1 and Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

DNA amplification and sequencing. The area along 710 double of alkaline protease has been amplified by using LCO 1490 and HCO 2198 of universal primer (Miller & Austin 2006). Every 25 µL reactant of amplification contains 12, 5 µL PCR Ready mix (KAPA 2G Robust), 1 µL primer LCO 1490 and HCO 2198 (20 mM), 4 µL DNA template (40 ng/uL), and 7.25 µL ddH. Amplification consists of denaturation, annealing, and DNA extension was performed on PCR machine.

The DNA condition of PCR that has been used was pre-denature PCR at 95°C during 3 minutes, PCR period during 35 cycles includes denaturation at 95°C during 35 second, annealing at 45°C during 30 seconds, and extension at 72°C during 50 seconds. PCR ending with post-PCR at 72°C during 7 minutes. The result of the sequencing amplification done by sequencing services (Macrogen via PT. Sciencewerke) to know the sequence of alkaline nucleotide.

Genetic analysis. Genomic DNA was extracted from pleopod using Wizard® Genomic DNA Pufication Kit (Promega). The Cytochrome Oxydase subunit 1 (COI) was amplified using the universal primers (LCO1490 and HCO 2198) according to modified method of Folmer et al (1994).

The sequence of alkaline nucleotide of each species is compared by using neighbor-joining methods (NJ) on MEGA software. The pattern of genetic structure was analyzed by using the statistic test of Molecular Variance (AMOVA). Sequencing data of partial nucleotide sequence of oxidase cytochorome sub unit I mtDNA was edited by the assistance BIO software and was done by multiple alignment through the previous sequencing which was provided by GEN Bank and NCBI BLASTN at nucleotide level (http://blast.ncbi.nlm.nih.gov/blast.cgi).

Alignment was done by the assistance of Clustal W., while phylogenetic analysis was done by the GENETYX software version 7 and UPGMA method through MEGA program version 4.0 and Neighbour joining method.

Results and Discussion

Morphologic diversity. Twenty out of twenty two morphometric characters were significantly different ($P<0.01$), while the other two (ASN and ASN) were not significantly different ($P>0.05$).

Based on the Principal Component Analysis (PCA), the highest internal diversity of *H. raphidea* population (84.16%) was in Teluk Jakarta, while the lowest morphologic diversity was in Pontianak (56%). On the other hand, the highest external diversity of the *H. raphidea* population was among Tanjung Pandan and Pontianak populations (14.87%), while the lowest one (1.61%) among Tanjung Pandan and Cirebon population (Table 2).

In a study of morphological diversity in *Penaeus semisulcatus* (Parenrengi et al 2007) it was shown that the value of intra-population diversity was 67.8-93.1%, whereas the inter-population diversity score was 0-30.5%. On the other hand, Hadie et al (2002) reported that the value of intra-population diversity in *Macrobrachium rosenbergii* was 68.3-90%, whereas the value of diversity between populations was 5-26.7%, the study aimed to obtain an overview of genetic distance based on morphometric characterization.

The results of another study on genetic distance analysis of *M. rosenbergii* showed that Barito River has different characteristics from Kintap and Pagatan sectors, Kintap and Pagatan sizes still have similar characteristics (Kisworo 2014).

Table 2

Canonical Discriminant Univariate Statistics analyses of morphometric characters

No.	*Morphometric characters*	*Total STD*	*Pooled STD*	*Between STD*	*R^2*	*F*	*Pr > F*	*Significantly*
1	PTO	0.118	0.114	0.038	0.080	6.850	0.0002	*
2	PBD	0.082	0.079	0.026	0.076	6.500	0.0003	*
3	LBA	0.009	0.007	0.006	0.342	41.060	0.0001	*
4	PKP	0.018	0.010	0.007	0.139	12.700	0.0001	*
5	LKP	0.045	0.040	0.021	0.173	16.510	0.0001	*
6	PTS	0.019	0.010	0.008	0.135	12.340	0.0001	*
7	PAS	0.016	0.015	0.005	0.080	6.880	0.0002	*
8	ASS	0.005	0.004	0.003	0.368	45.830	0.0001	*
9	ASD	0.005	0.003	0.002	0.265	28.500	0.0001	*
10	AST	0.003	0.002	0.003	0.293	32.610	0.0001	*
11	ASE	0.003	0.003	0.003	0.275	29.930	0.0001	*
12	ASL	0.001	0.001	0.000	0.009	0.740	0.5271	none
13	ASN	0.001	0.001	0.000	0.004	0.330	0.8013	none
14	PLA	0.010	0.020	0.006	0.269	29.930	0.0001	*
15	PTL	0.010	0.020	0.003	0.070	5.930	0.0006	*
16	PMI	0.033	0.020	0.025	0.443	62.720	0.0001	*
17	LMI	0.029	0.020	0.014	0.176	16.720	0.0001	*
18	PMA	0.033	0.012	0.024	0.417	56.430	0.0001	*
19	LMA	0.029	0.012	0.015	0.209	20.870	0.0001	*
20	PUI	0.013	0.012	0.009	0.393	51.070	0.0001	*
21	PUA	0.015	0.012	0.010	0.331	38.980	0.0001	*
22	LTL	0.033	0.012	0.033	0.741	226.46	0.0001	*

Genetic diversity. Figure 2 shows the electrophoresis PCR product 690-710 bp on 1.2% agarose. Among the sequenced specimens of *H. raphidea*, there were 10 haplotypes (Table 3).

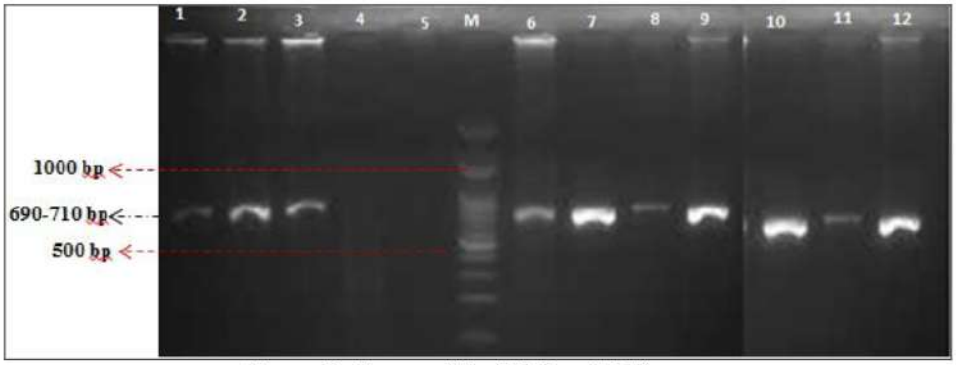

Figure 2. The amplified COI on 1.2% agarose.

Table 3

Haplotypes distance of *Harpiosquilla raphidea* from Karimata strait (Jambi, Tanjung Pandan, Pontianak) and from Java Sea waters (Semarang, Cirebon, Teluk Jakarta)

Nucleotide base composition		*Sem*	*Cir*	*Teluk Jakarta*	*Jambi*	*Tanjung Pandan*	*Pont*
h1	ACAAACTGCCATTGGT	0	0	0	0.333	0	0
h2	ACAAATTGTCATTGGT	0	0	0	0	0	0.333
h3	ACAAGCCGCCATTGGT	0	0	0.5	0	0.5	0
h4	ACAGACTACCGTTGGT	0	0	0	0	0.5	0
h5	ACAGACTGCAGCACCA	0	0.25	0	0	0	0
h6	ACAGACTGCCGTACCA	0.25	0	0	0	0	0
h7	ACAGACTGCCGTTGGT	0.75	0.75	0	0.667	0	0
h8	AGCAGCTGCCATTGGT	0	0	0.5	0	0	0
h9	GCAAACTGCCATTGGT	0	0	0	0	0	0.333
h10	GCAGACTGCCGTTGGT	0	0	0	0	0	0.333

Sem – Semarang; Cir –Cirebon; Pont –Pontianak.

Based on genetic distance analysis, there were three clusters among *H. raphidea* populations. Population from Semarang, Cirebon, and Jambi was established as one cluster, while populations from Tanjung Pandan and Pontianak grouped in one cluster. On the other hand, population from Teluk Jakarta was grouped in a different cluster (Figure 3).

The genetic distance between *H. raphidea* in the farthest population is from the waters of Teluk Jakarta. The genetic differences between *H. raphidea* in the Karimata Strait and the Java Sea show that there is a population that is a mixed or they are connecting population. This is due to the geographic position of these waters and the genetic factors as well as the environmental conditions. According to Barber & Erdmann (2000), the genetic differences were also influenced by geographical factors and previous periods of shrimp larvae of *Haptosquilla pulchella* around Krakatau Mountain to Sulawesi waters (Kusrini 2008). The genetic differences between white shrimps among populations in Bengkulu, NTB and Java Sea are determined by geographical distance.

A homology research performed using BLASTN analysis for case studies with other mantis shrimp species at Gene Bank obtained 74%, 98% for *Oratosquilla oratoria*, it means that the harmonic relationship of *Harpiosquilla harpax* with *H. raphidea* at Bank Gene is very closed. There are no data of nucleotide sequence of *H. raphidea* at gene

bank, that is why *H. harpax* mantis prawns from Vietnam are used as comparison (Miller & Austin 2006).

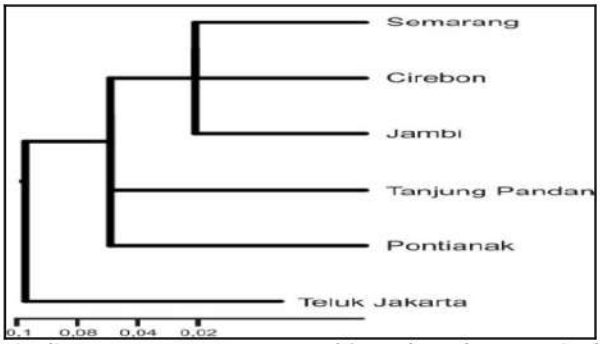

Figure 3. Genetic distance tree reconstructed based on the genetic distance matrix between *Harpiosquilla raphidea* populations.

Conclusions. The results presented here clearly demonstrate the diversity of *H. raphidae* from the Karimata strait and Java Sea waters, based on morphologic and genetic characteristics. The sequence data of COI from *H. raphidea* has been established. In the present study *H. raphidea* from Karimata strait and Java Sea waters showed the highest internal diversity of the population (84.16%) in Teluk Jakarta, while the lowest one in Pontianak (56%). According to the multiple alignment analyses, there were 10 haplotypes distributed from Karimata strait (Jambi, Tanjung Pandan, Pontianak) and from Java sea waters (Teluk Jakarta, Cirebon, Semarang). The morphometric analysis result gives the relative differences result from the nucleotide sequencing analysis in the area of COI mtDNA mantis shrimp. Our results are in accordance with the theory that the phenotype of an individual is determined by the genetic condition and the environmental factors in which the individual lives. The appearance of the phenotype diversity in quantitative characters is largely influenced by environmental adaptation, not only by genetic components. The difference in genetic characters between morphometrics (phenotype) and molecular level (genotype) is caused by differences in measurement methods, therefore the genetic diversity of *H. raphidea* mantis shrimp analyzed molecularly becomes an important and appropriate part in assessing genetic diversity.

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