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Genetic Diversity Of Giant Mantis Shrimp *Harpiosquilla raphidea* Fabricius, 1798 From Banten Bay and Other Indonesian Waters

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Abstract

The Harpiosquilla raphidea Fabricius, 1798 is one of the essential mantis shrimps in Banten Bay that has unique waters characteristic. Nucleotide sequence analysis of mtDNA COI region of 4 mantis shrimp population dendrogram obtained in 3 main clusters; first cluster consists of Cirebon and Jambi population, Pontianak in the second cluster population and Banten Bay in the third cluster population. Farthest genetic distance value based on mtDNA COI sequences are amongst Banten Bay and Jambi population, while the closest population is Pontianak.

Keywords: Banten Bay; Genetic Diversity; *Harpiosquilla raphidae ;* Indonesia (;).

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1. Introduction

Mantis shrimp is a species of Stomatopoda Ordo having higher value in economy. In Indonesia, Mantis shrimp has its dissemination area in Sumatra, Java, Kalimantan, Sulawesi and Nusa Tenggara [3,13]. Mantis shrimp is a favorite seafood for its delicious meat and very typical, especially it has high enough protein.

This puts mantis shrimp as an exclusive seafood with expensive price relatively (price per 1 mantis shrimp > 15 US Dollar). People consider consuming this type of seafood as prestige, therefore the demand of mantis shrimp continues to increase from year to year. Mantis shrimp has high demand, causing to the increament of its exploitation so that the population is declining (overfishing).

The population of mantis shrimp is likely to decline and causing the effectiveness of poputaion and giving the result of inbreeding so that pushing the "fitness" of the shrimp population will finally cause the extinction of the shrimp [8]. The correct management strategy is necessary to avoid the extinction of mantis shrimp, and for that reason it needs a study covering the population biological aspect and the condition of habitat.

The molecular mark is able to indentify the difference of direct genetics at DNA level as genetics components. The entire characters that shown visible and invisible by one individual animal reflect of genetics character owned by the individual of animal [14]. All information that can be observed at one individual is a genectic mark from the individual. The characteristic of this molecular mark can handle limitation of the use of morfological mark that this mark is free from fenotype and environment influences, so that it can provide more accurate information [10, 12].

One of the molecular marks that is normally used to identify the stock is by analyzing the mtDNA sequence. This is due to the maternal character of mtDNA and generated by its parental without recombination [11], the molecular is compact and the size of length is relatively short (16000-20000 nucleotida). High level of evolution (5--10 times bigger than main DNA) so that can show clearly the difference between the population and familiarity relations [15]. It has big number of copy of 1000-10000 and faster and easy to obtain the result from previous preserved tissue [1].

The area of *Cytochrome-c Oxidase* COI at mtDNA has faster mutation rate comparing to the other mitocondria area, this area is perfect to use for the analysis of animal's diversity, both in species and inter species [10] and often used as a genetic mark [8]. The genetic mark or DNA *barcoding* is considered as a standard system to indentify the entire of eukaryotic taxa accurately and fast.

According to the necessary genetic diversity data as the effort of the conversion of *H. raphidea* mantis shrimp, it shall be done morfological and molecular analysis. The aim of this genetic diversity study is to obtain genetic's diversity based on molecular analysis to: genom isolation and fragment of *Cytochrome oxidase* area sub unit I (COI) mtDNA, distribution and haplotype compositions, diversity of population's genetic and the difference of genetic [9]. The result of this study is to give the advantage for formulating the starting points in the conversion efforts. The effort of conservation through domestication action by conducting hybridization to avoid genetic saturation of *H. raphidea* mantis shrimp, so that the species of *H. raphidea* mantis shrimp can be sustained, both

ecological and economical in Banten Bay particularly and in Indonesia Waters generally.

2. Material and Methods

This study has been done from December 2011 up to October 2013. Molecular Analysis was carried out at the Laboratory of Genetics Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia Depok. Sequencing of mtDNA was carried out at the Laboratory of Macrogen, South Korea through the sequencing services from PT Sciencewerke Jakarta.

Shrimp sampling was carried out at four waters population which were Banten Bay (Banten), Kuala Tungkal (Jambi), Padang Tikar, Pontianak (West Kalimantan), and Gebang, Cirebon (West Java). Location determination of sample was based on the different geografical location (Figure 1).

Sample of mantis shrimp from each population is further packed in dry hybernation technic or declining the temperature by putting ice cubes in boxes so that the mantis shrimps are unconsciously and 10 of each population shall be packed for DNA extraction.

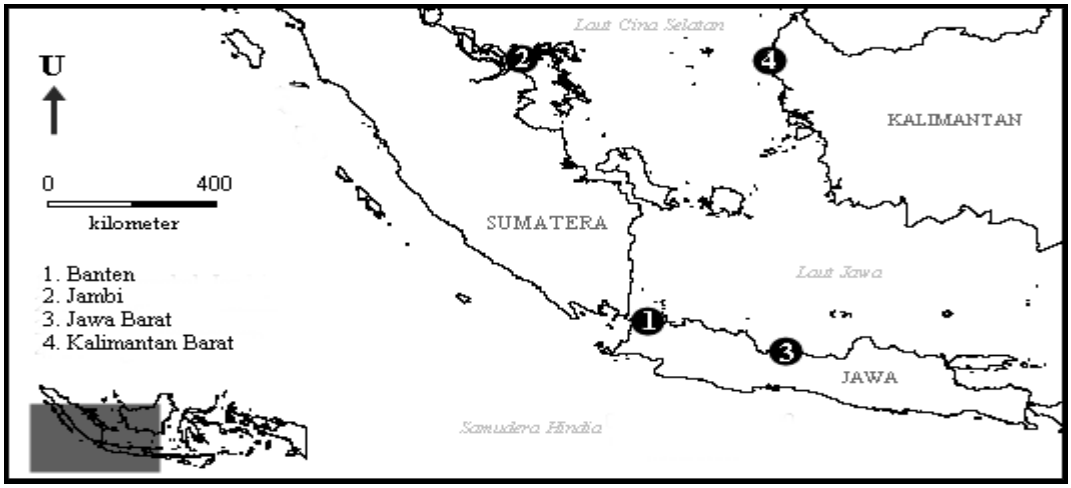

Figure 1: Intake location of *H. raphidea* mantis shrimp sample.

2. 1 Methods of Research

2.1.1 DNA Extraction

DNA was extracted from using Wizard Genomic DNA Purification Kit (Promega).

2.1.2 DNA Amplification and Sequising

The area along 710 double of alkaline has been amplificated by using LCO 1490 and HCO 2198 of universal primer [4, 9]. Every 25 µL reactan 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_20. DNA Amplification consists of denaturation, annealing, and DNA extension was done on PCR machine. The

condition of PCR that has been used was *pre-denature* PCR at 95°C during 3 minutes, PCR period PCR during 35 cycle includes denaturation at 95°C during 35 second, *annealing* at 45°C during 30 second, and extension at 72°C during 50 second. PCR, ending with *post*-PCR at 72°C during 7 minutes.

The result of disquensing amplification done by sequensing services (Macrogen via PT. Sciencewerke) to know the sequence of nucleotida alkaline. The sequence of nucleotida alkaline of each species is compared by using *neighbor-joining* methods (NJ) on MEGA software. The patern of genetic structure was analyzed by using the statistic test *of Molecular Variance* (AMOVA).

2.1.3 Data Analysis

Sequensing data of sequence partial nucleotida of oxidase cytochorome sub unit I mtDNA was edited by the assistance BIO *software* and was done by *multiple alignment* through the previous sequensing which was provided at GEN Bank and NCBI BLASTN at nukleotida level http://blast.ncbi.nlm.nih.gov/blast.cgi. Multiple Alignment was done by the assistance of Clustal W. While filogenetic analysis was done by the GENETYX software GENETYX version 7 and UPGMA method through MEGA program version 4,0 and *Neighbour joining* method (5)

3. Result and

3.1 The result of Genom Isolation of Mantis Shrimp (H. raphidea)

The result of *elektroforesis* to the genom of mantis shrimp (Figure 2.) showing DNA isolation against the sample of pereopod was succss. The result also shows that pereopod has potential as sources of DNA genomic for the mould and for the amplification of mitocondria DNAim that shall be done further.

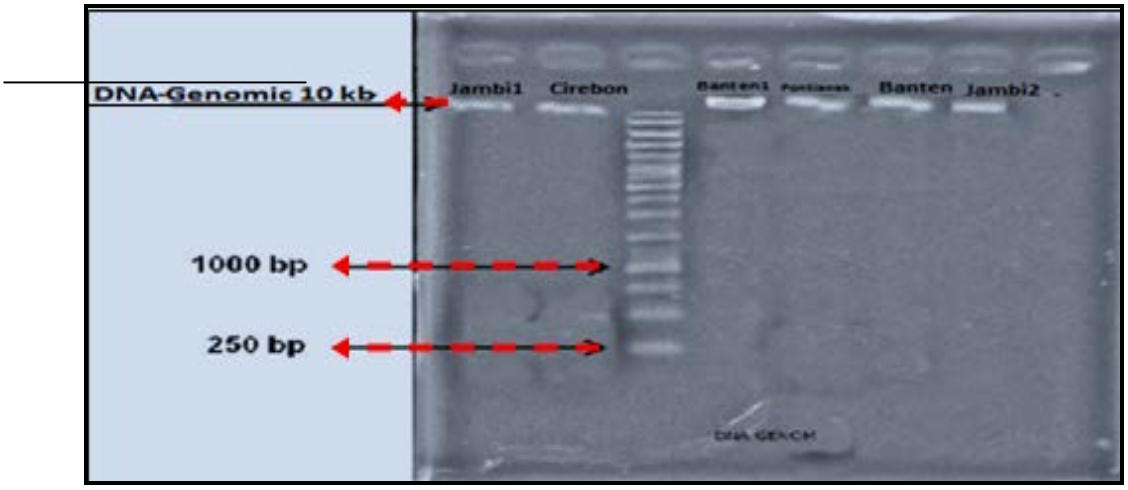

Figure 2: The result of genomic electroforesis on four population of mantis shrimp on 1% gel agarose.

Beside doing the electroforesis, the result of DNA isolation was done and conducted the DNA ratio measurement with protein using *spectrophotometer*, for the measurement result, the *genomic* DNA shall be obtained in normal limitation at ratio 1 : 1,8--2,0 so that further process can be continued which is DNA amplification.

Fragment *cytochrome oxidase* sub unit 1 (COI) mtDNA which was isolated from mantis shrimp (*H. raphidea*) through the PCR technic was shown at the position around 690--710 bp (Figure 3.).

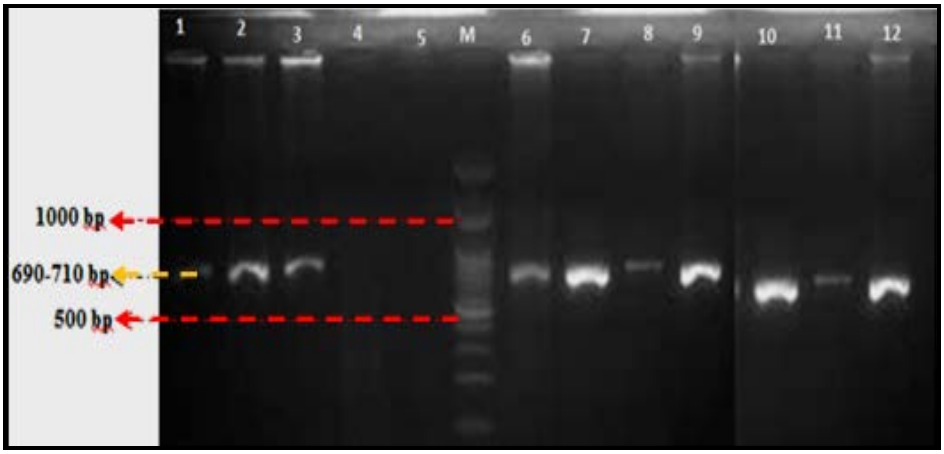

Figure 3: Single fragment of COI mtDNA area, which was amplificated from the mtDNA of *H. raphidea*. Mantis shrimp M = sign 100 bp (QIAGEN), 1--12 = mt DNA *H. Raphidea* mantis shrimp.

Fragment of COI mtDNA uses the same primer, disequencing and one of the samples of fragmen Chromatography of COI mtDNA along (Figure 4). The result of reading is completely. The long sequence read from sequencing result was 647-655 nucleotida, then the multiple alignment was conducted with sequence data of COI mtDNA of other mantis shrimp provided at the Gen Bank.

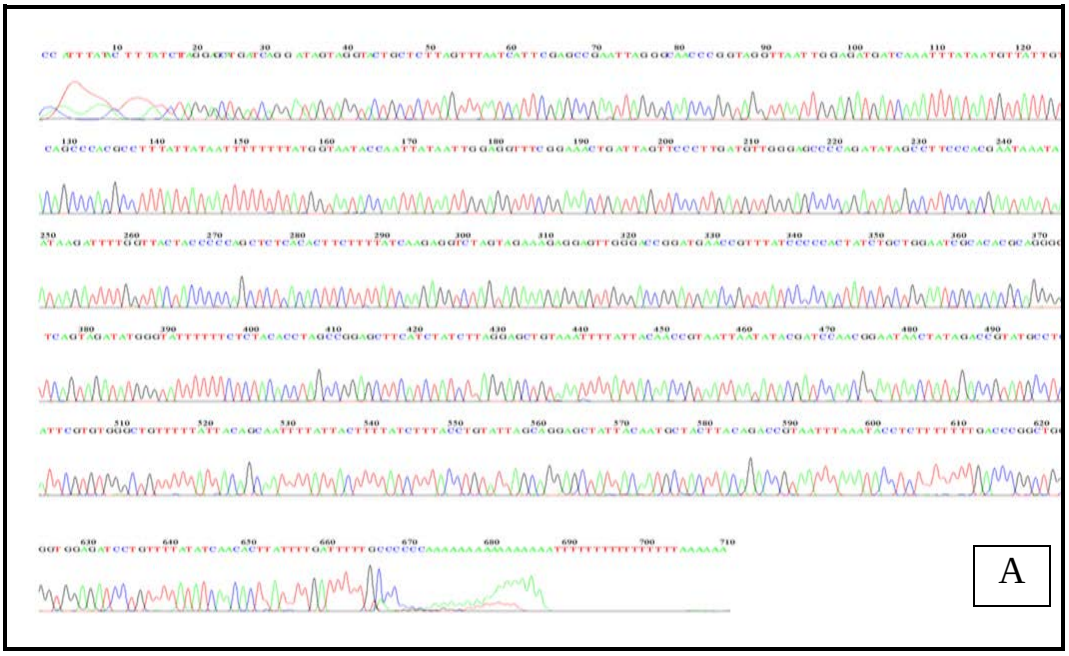

Figure 4: Chromatography (A) and fragment sequence of COI mtDNA of *H. raphidea* (B) mantis shrimp.

3. 2 Distribution and Haplotype Composition

The result of analysis study from 4 population of mantis shrimp, describing the existence of 8 haplotype (Figure. 5).

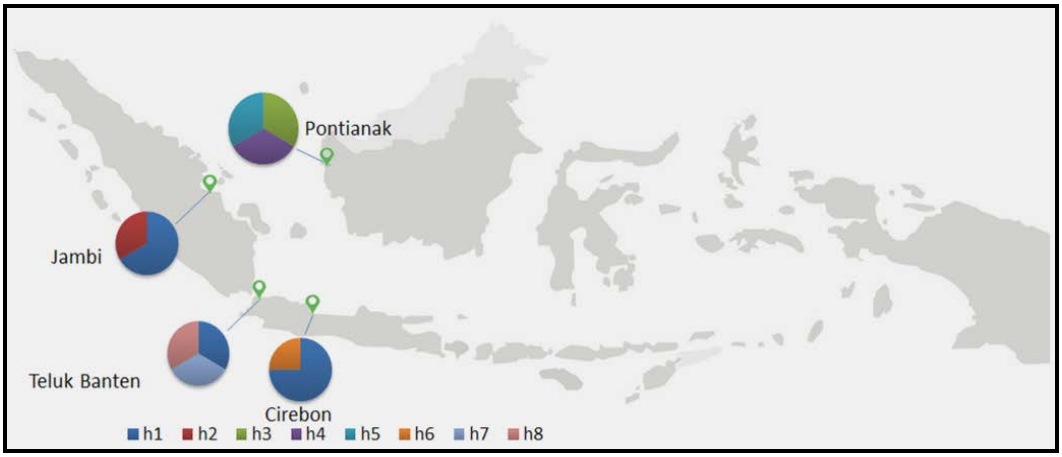

Figure 5: Haplotype distribution map of *H. raphidea* mantis shrimp in Banten Bay, Jambi,Cirebon and Pontianak.

Remarks = h1: haplotipe 1, h2: haplotipe 2, h3: haplotipe 3, h4: haplotipe 4, h5: haplotipe 5, h6: haplotipe 6, h7: haplotipe 7, h8: haplotipe 8

Composition of haplotype 1, was found 33% at the population in Banten Bay, 67% in Jambi population and 75% in Cirebon population. Population in Pontianak is a distinguish haplotype composition comparing with the three populations in Banten Bay, Jambi dan Cirebon, based on the ownership of the distinguished haplotype especially haplotype 3, 4 and 5. Haplotype 2 only found in Jambi population about 33% and haplotype 6 only exists in Cirebon population about 25%, while haplotype 7 and 8 only in Banten Bay population and each site about 33% (Table 1).

Table 1: Composition and haplotype frequence of mantis shrimp origin from Banten Bay, Jambi, Cirebon and Pontianak.

Type of Haplotype		Frequence of haplotype			
		Banten Bay	Jambi	Cirebon	Pontianak
h1	ATAGACCCGTTGGT	0,333	0,667	0,75	0
h2	ATAAACCCATTGGT	0	0,333	0	0
h3	GTAGACCCGTTGGT	0	0	0	0,333
h4	GTAAACCCATTGGT	0	0	0	0,333
h5	ATAAATTCATTGGT	0	0	0	0,333
h6	ATAGACCAGCACCA	0	0	0,25	0
h7	GAGAGCCCATTGGT	0,333	0	0	0
h8	ATAAATCCATTGGT	0,333	0	0	0

3.3 Diversity of Population Genetic and Genetic Difference based on Molecular

AMOVA Analysis shows from total proportion of genetic diversity, 6 % is inter-population diversity (sample of mantis shrimp amongst the sampling location) and the rest of 94% is inter-population diversity (sample inside of sampling location) (Table 2.). AMOVA result indicating that there is a real genetic distinguish amongst the 4 location of the observed mantis shrimp sample.

Table 2: Molecular Analysis (AMOVA) for *H. raphidea* mantis shrimp sample based on COI mtDNA fragment

Sources Variation	d.f.	Number of quadrate	Component Variation	Percentage Variation
amongst Population	3	5,551	0,097	6%
Inside Population	9	13,833	1,537	94%
Total	12	19,385	1,634	100%

The difference genetic amongst the mantis shrimp population can be seen by using dendrogram. Analysis result of familiarity inter population showing that mantis shrimp population which was observed divided into 3 main

cluster with the first cluster from Jambi and Cirebon, second cluster from Pontianak and third cluster from Banten Bay (Figure 6).

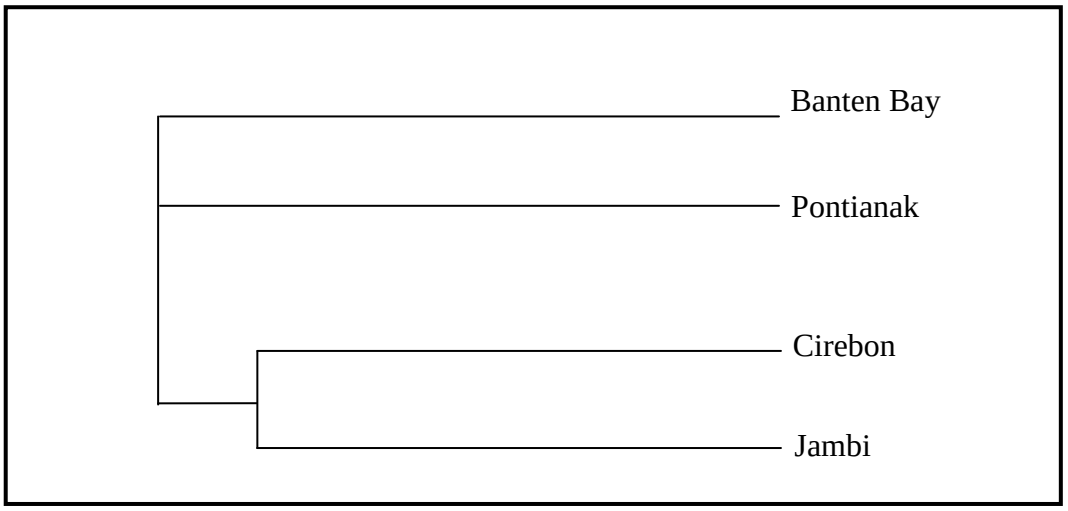

Figure 6: UPGMA Dendrogram based on nucleotida on COI mtDNA area of *H. raphidea* mantis shrimp with size of 690--710 bp origin of Banten Bay, Jambi, Cirebon and Pontianak.

4. Discussion

Genom Isolation determines the result in genetic diversity study, the accurated genom isolation can be able to help in further process to the final process in molecular analysis. According to the isolation research study from its leg muscle can be obtained the ratio between protein and genom DNA which was between 1.8--2 , this assumed that the muscle tissue of shrimp's leg is the tissue which is used as motoric tools. Besides that, the use of pereopod in mantis shrimp can make shrimp survival, and so if it will be used as living sample, it is not necessary to kill the sample.This statement was reinforced by the opinion of [6] stated that to determine sample in genom isolation, can be used from a part or several parts from shrimp's organ.

The genetic's distance between mantis shrimp in the farthest Banten Bay population and the population in Jambi. The genetic difference between *H. raphidea* mantis shrimp in Banten Bay and the population in Cirebon, Pontianak and Jambi. This is caused by geographical position from *H. raphidea* mantis shrimp and the distingusih of genetic by its factor of environmental condition. According to [2] the genetic difference is also influenced by geographical factor and the previous period of *Haptosquilla pulchella* mantis shrimp larve around Krakatau volcano to the waters area in Sulawesi [7], the genetic distinguish of white shrimp between the population in Bengkulu, NTB and Java Sea which was determined by geographical distance.

Homological result conducted using *blastN* analysis for study case with other mantis shrimp species in *Gene Bank* obtaining 74% for *H. harpax*, 98% for *Oratosquilla oratoria*, it means that familiarity relations with mantis shrimp at the G*ene Bank* is very closed. There is no Nucleotida Sequence Data of *H. raphidea* mantis shrimp at the *Gene Bank*, that was why *H. harpax* mantis shrimp from Vietnam was used as a comparaison [5].

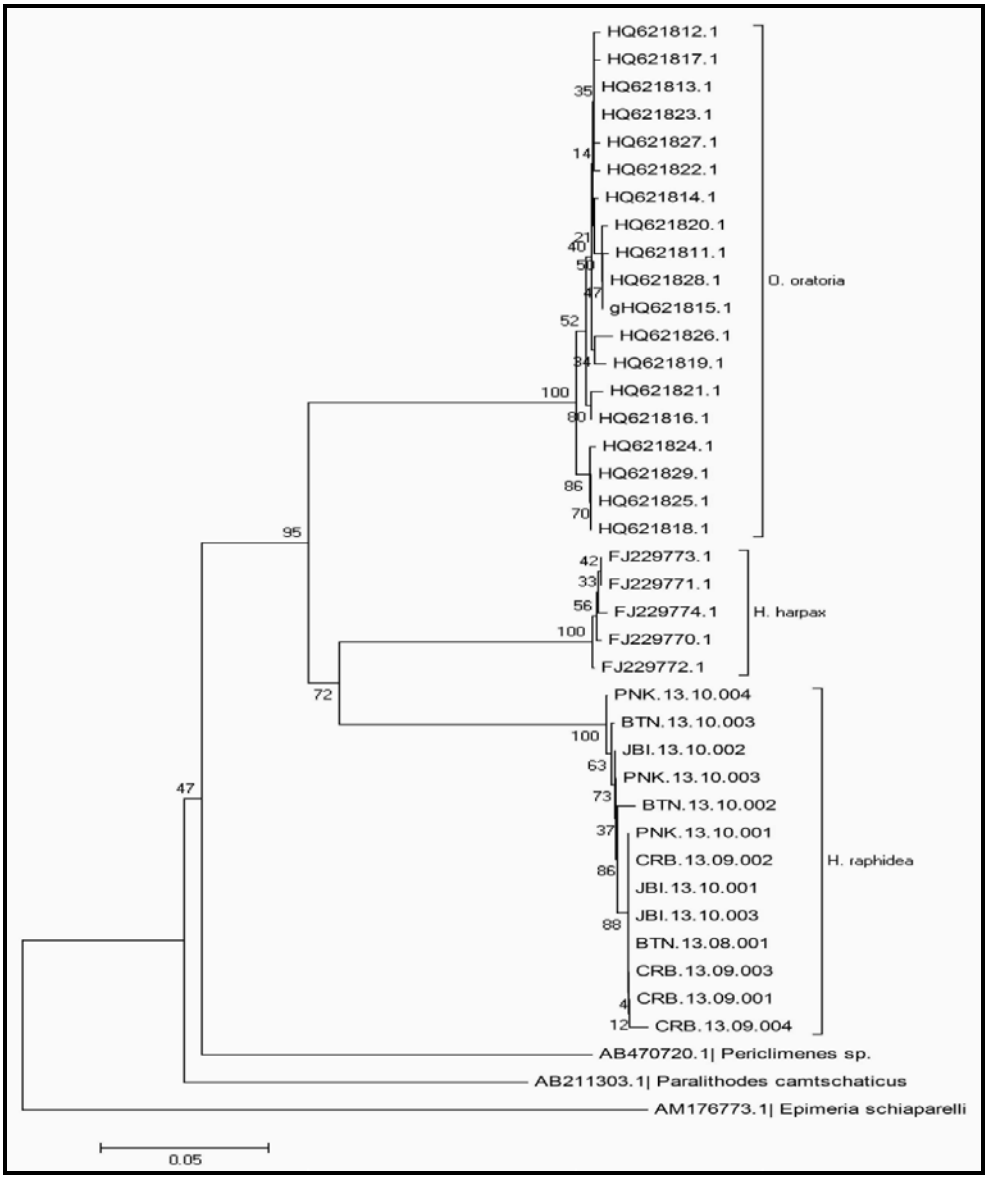

Figure 7: Study's result using Dendrogram for other species of mantis shrimp from *Gene Bank* according to the Nukleotida Sequence of CO1 mtDNA

To know more specific regarding study's result of the familiarity mantis shrimp with other mantis shrimp species, sequence analysis of COI mtDNA 647--655 bp has been conducted to some shrimps provided at the *Gene Bank*. According to the *multiple alignment* result of *H.raphidea, H. harpax and Oratosquilla oratoria* we obtained several differences of their nucleotida order, so we can use to make the difference inter species. Mitocondria DNA is a mark based on the haplotype lineage in each individual.

Study's result of genetic diversity, shows that cluster separation between *H. harpax* and *H. raphidea* has been divided but still in one genus. Sequencing data of *H. raphidea* accomplished data base at NCBI (*Gene Bank*) and can be used as the references access for mantis shrimp study of stomatopoda genus in general and *Harpiosquilla* genus in particular.

The application genetic study in conservation was based on the population genetic theory, which study the factors detemining the genetic composition of a population [11, 16]. Basic Information needed in the conservation and in the efforts of preservation are dissemination and population stock in the nature. In this relations, genetic diversity is absolutely necessary as the basic effort of conservation. The study of genetic diversity gives the useful information in the resources management program of *H. raphidea* mantis shrimp through the breeding and domestication efforts in the future.

The analysis result of morfometric gives the difference relative result from the nucleotida sequencing analysis in the area of COI mtDNA mantis shrimp. The population of *H. raphidea* mantis shrimp at Banten Bay has the closest familiarity relations according to the fenotype with the population in Pontianak, Cirebon and the farthest with the population in Jambi, apart from the familiarity relations based on genotype that population in Banten Bay has the closest familiarity relations with the population in Cirebon and the fathest with population in Jambi. This is in accordance with the Theory of [17] that fenotype of an individual is determined by the genetic and environment where the individual lives and breeds. Further [16] described that the sightings of fenotype diversity at quantitative character mostly influenced by the adaptation of environment, not only by the genetic component. The difference of cluster based on the genetic distance between the morfometric (fenotype) and the molecular (genotype) was caused by the difference measurement indicators [4]. So at the genetic diversity of *H. raphidea* mantis shrimp that was analysed by molecular, becomes a positive control and clarification genetically..

Analysis of nucleotida sequence in the area of COI mtDNA mantis shrimp from 4 population has obtained the dendrogram in 3 clusters; first cluster consists of population Jambi and Cirebon, second cluster consists of population Pontianak and third cluster consists of Banten Bay. The distance value of the farthest genetic based on the sequence of COI mtDNA is between the population in Banten Bay and the population in Jambi, while the closest is with the population in Pontianak. The sequence of COI mtDNA can be used to indentify the difference and to cluster mantis shrimp in Indonesia, both for intra and inter species. *H. raphidea* mantis shrimp of 4 population which were observed having high COI mtDNA homology of 74 % with the *H. harpax* origin from Vietnam (Figure 7).

The population of mantis shrimp from Banten Bay can be used as *selective breeding* in the efforts of conservation on further domestication activity. As the efforts of conservation in the domestication activity, it can be conducted by doing the interbreeding of the mantis shrimp population between the population in Banten Bay and population in Jambi based on their genetic distance.

5. Conclusion

The result presented here clearly demonstrate the diversity *Harpiosquilla raphidae* from the Banten bay waters and Western Indonesia waters, based on genetic characteristics. Nucleotide sequence analysis of mtDNA COI region of 4 mantis shrimp population dendrogram obtained in 3 main clusters; first cluster consists of Cirebon and Jambi population, Pontianak in the second cluster population and Banten Bay in the third cluster populationThe sequence data of from *Harpiosquilla raphidea* has been established.

6. Recommendations

The research and development of the region other than the western Indonesian waters because of the vast ocean waters Indonesia. In order to do also with some other marker genes for the perfection of the data and enrich the diversity of the mantis shrimp

Aknowledgements

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