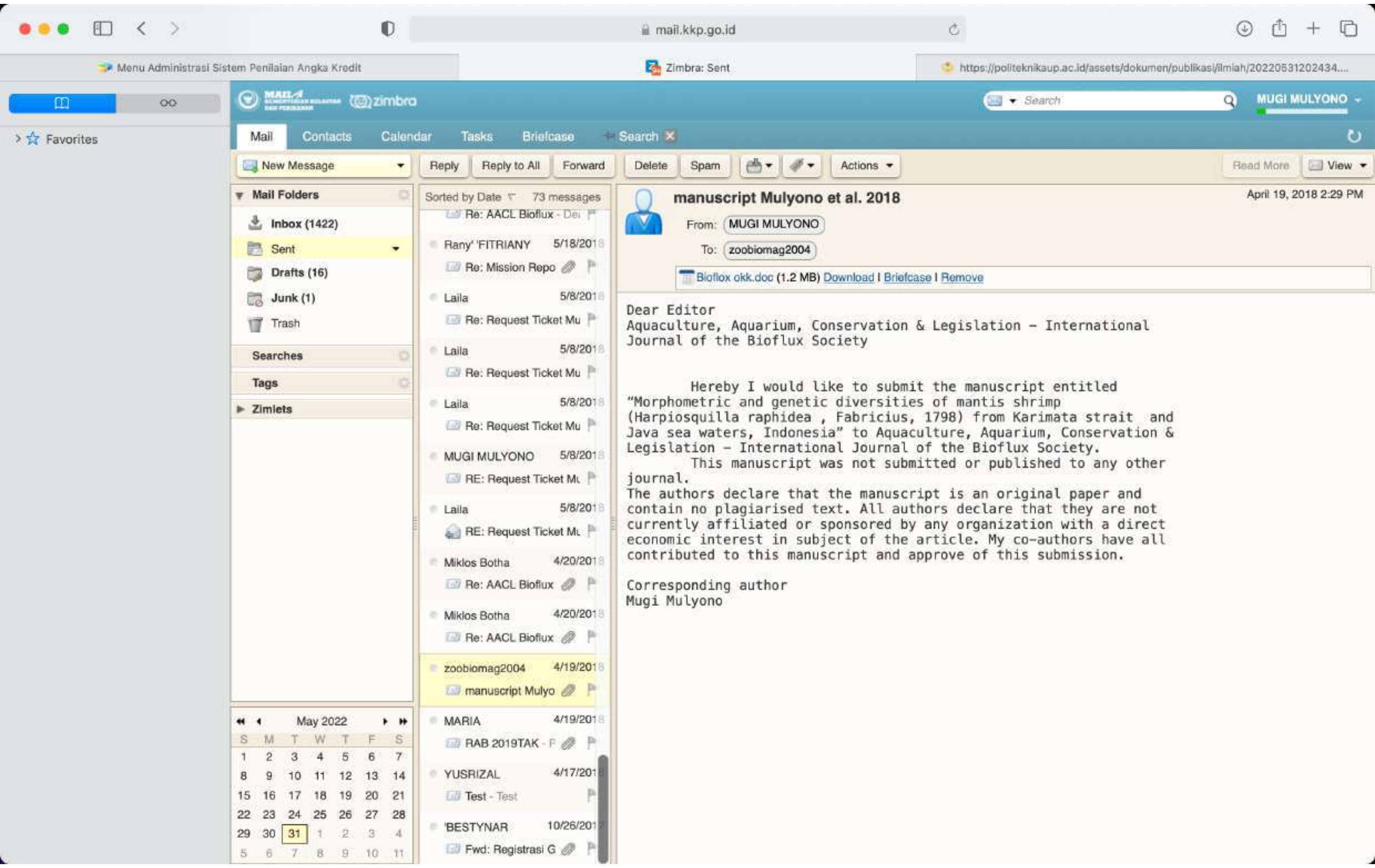

Dear Editor
Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society

Hereby I would like to submit the manuscript entitled "Morphometric and genetic diversities of mantis shrimp (Harpiosquilla raphidea , Fabricius, 1798) from Karimata strait and Java sea waters, Indonesia" to Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society.

This manuscript was not submitted or published to any other journal.
The authors declare that the manuscript is an original paper and contain no plagiarised text. All authors declare that they are not currently affiliated or sponsored by any organization with a direct economic interest in subject of the article. My co-authors have all contributed to this manuscript and approve of this submission.

Corresponding author
Mugi Mulyono

Morphometric and genetic diversities of mantis shrimp (*Harpiosquilla raphidea* , Fabricius, 1798) from Karimata strait and Java sea waters, Indonesia

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Abstract. Study on morphometric variations and genetic diversities of the mantis shrimp (*Harpiosquilla raphidae*) from Karimata strait and Java sea waters has been conducted from January 2013 to April 2014. The mantis shrimp samples were collected from six locations i.e. Teluk Jakarta, Cirebon, Semarang, Tanjung pandan, Pontianak and Jambi waters. The total of 360 individual mantis shrimp 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 mantis shrimp population (84.16%) was in Teluk Jakarta, while the lowest one was in Pontianak (56%). According to the multiple alignment analyses, there were 10 haplotpes distributed from Karimata strait (Jambi, Tanjung Pandan, Pontianak) and from Java sea waters (Teluk Jakarta, Cirebon, Semarang). The results showed the results of the classification of six populations into three groups among the population, based on the analysis of genetic distance.

Key words : Teluk Jakarta, *Harpiosquilla raphidae*, genetic diversity, morphometric diversity

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

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 (Liu et al, 2007). 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 (Nei, 1987). 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 (Moosa, 1989).

Some information about morphologic and genetic characteritics, such can provide helpful insights for management and conservation of this species. Until now, neither morphologic nor genetic diversities of giant Harpiosquillid from Karimata strait and Java sea have been studied ever before. Accordingly, this paper presents the preliminary results of morphologic and genetic diversity of mantis shrimp, *Harphiosquilla 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 mantis shrimp samples were identified using Moosa (2000) and Ahyong (2012). A total of 22 characters of morphometric (Figure 1), the measurements were done on leftside of mantis shrimp sample (Table 1).

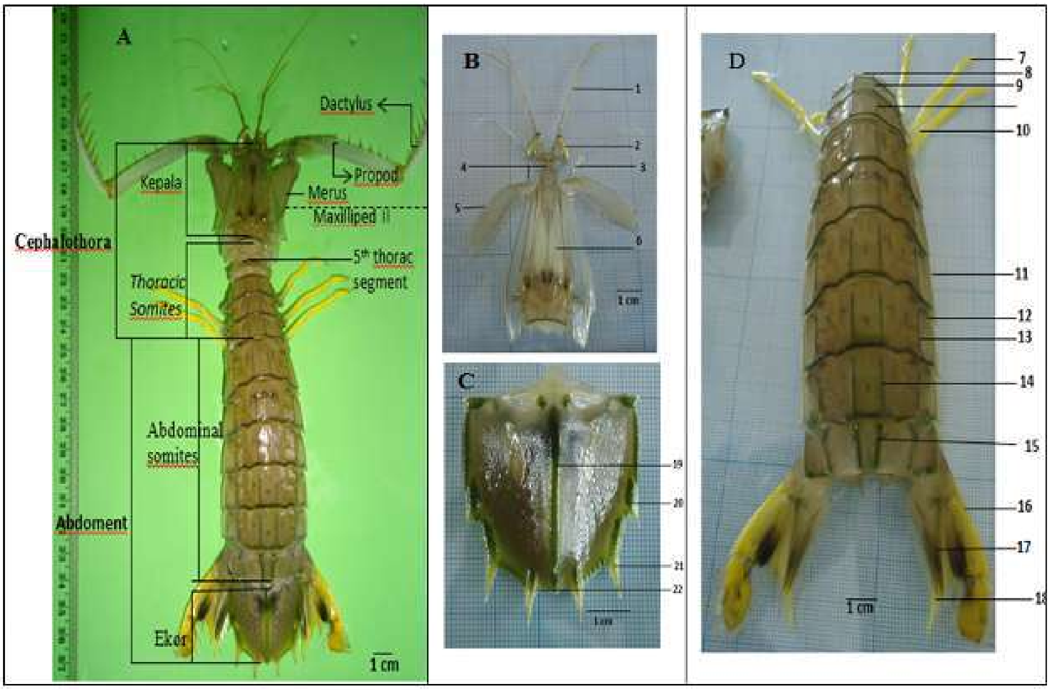

Figure 1. Morphology of giant Harphiosquillid (*Harphiosquilla raphidea)*.

Morphologic analysis. Morphometric characteristics were measured by according to Mori *et al.* (2010) with 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 Sequising. The area along 710 double of alkaline has been amplificated by using LCO 1490 and HCO 2198 of universal primer (Ferguson et al, 1995; Miller and Austin, 2006). 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/µl), and 7,25 µL ddH Amplification consists of denaturation, annealing, and DNA extension was done on PCR machine.

The DNA 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.

Table 1. The morphometric characters of *H. 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

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 (1994).

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). 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.

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 giant Harpiosquillid 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 giant Harpiosquillid 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 the 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 Giant Freshwater Prawn study, genetic distance analysis 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	*

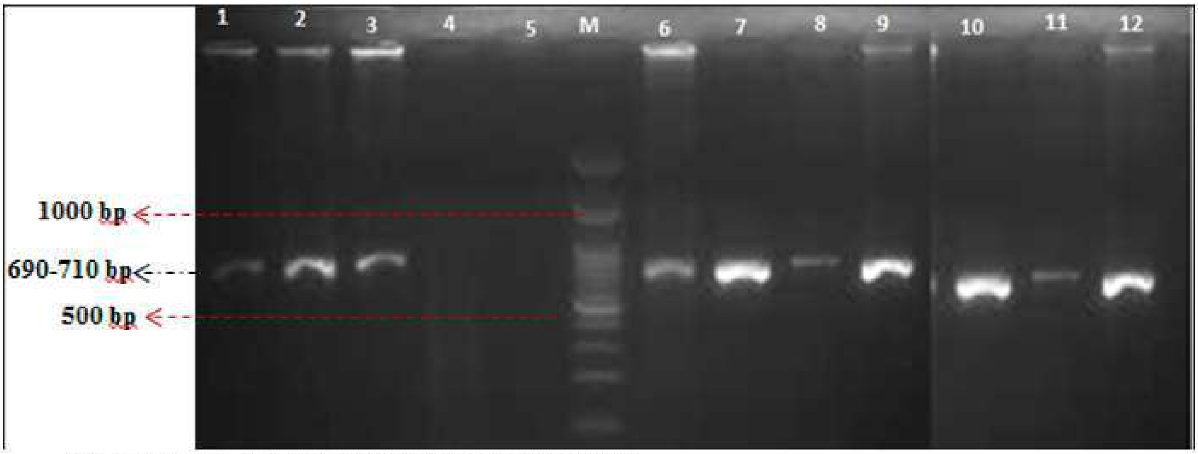

Figure 2. **the amplified** *COI* on 1.2 % agarose

Genetic diversity: Figure 2 showes electrophoresed PCR product 690-710 bp on 1.2% agarose. among the sequenced specimens of *Harpiosquilla raphidae*, there were 10 haplotypes (Table 3). Based on genetic distance analysis, there were three clusters among *Harpiosquilla raphidae* 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 grouped in a different cluster (Fig. 3).

Table 3. Haplotipes distance of *Harpiosquilla raphidae* from Karimata strait (Jambi, Tanjung Pandan, Pontianak) and from Java sea waters (Semarang, Cirebon, Teluk Jakarta).

Nucleotide base composition		Semarang	Cirebon	Teluk Jakarta	Jambi	Tanjung Pandan	Pontianak
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

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

The genetic distance between mantis shrimp in the farthest population is from the waters of Teluk Jakarta. The genetic differences between *H. raphidea* mantis shrimp in the Karimata Strait and the Java Sea show that there is a population that is a mixed or 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* mantis around Krakatau Mountain to Sulawesi waters (Kusrini 2008), the genetic differences between white shrimp among populations in Bengkulu, NTB and Java Sea are determined by geographical distance .

The homology results performed using blastN analysis for case studies with other mantis shrimp species at Gene Bank obtained 74% for, 98% for Oratosquilla oratoria, it means that the harmonic relationship of *H. harpax* with mantis shrimp at Bank Gene is very closed. No Data of Nucleotide Sequence of Grasshopper Shrimp *H. raphidea* at Bank Gene, that is why *H. Harpax* mantis prawns from Vietnam are used as comparaison (Miller & Austin 2006).

Conclusions

The results presented here clearly demonstrate the diversity of *Harpiosquilla raphidae* from the Karimata strait and Java sea waters, based on morphologic and genetic characteristics. The sequence data of *COI* from *Harpiosquilla raphidea* has been established.

Aknowledgements

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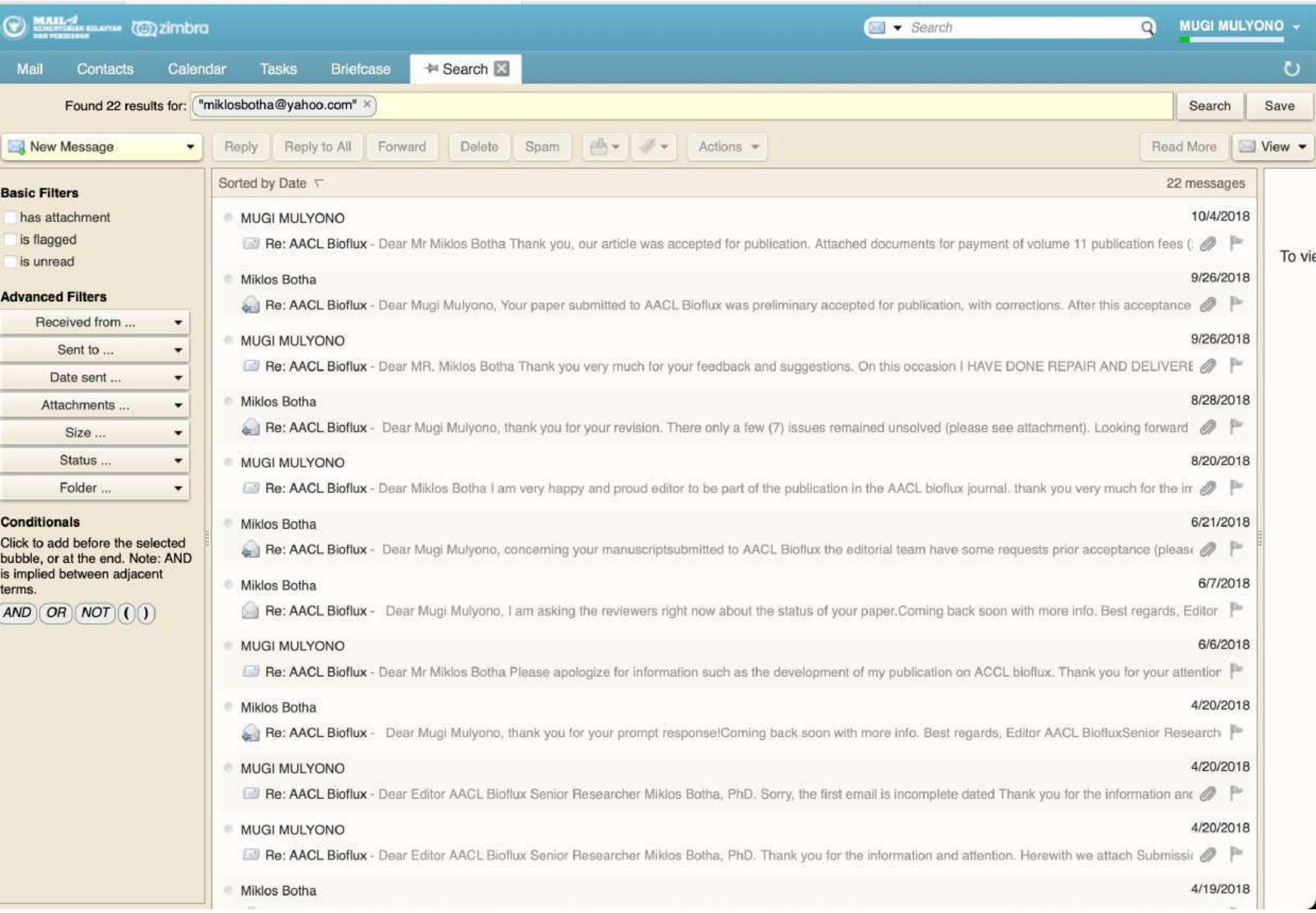

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Figure 3. Genetic distance tree reconstructed based on the genetic distance matrix between *Harpiosquilla raphidae* populations.

The genetic distance between mantis shrimp in the farthest population is from the waters of Teluk Jakarta. The genetic differences between *H. raphidea* mantis shrimp in the Karimata Strait and the Java Sea show that there is a population that is a mixed or 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* mantis around Krakatau Mountain to Sulawesi waters (Kusrini 2008). The genetic differences between white shrimp among populations in Bengkulu, NTB and Java Sea are determined by geographical distance.

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Conclusions. In the present study *H. raphidea* from Karimata strait and Java Sea waters the highest internal diversity of mantis shrimp 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 haplotpes distributed from Karimata strait (Jambi, Tanjung Pandan, Pontianak) and from Java sea waters (Teluk Jakarta, Cirebon, Semarang). The analysis result of morfometric gives the difference relative result from the nucleotida sequencing analysis in the area of COI mtDNA mantis shrimp.

In accordance with the theory that the phenotype of an individual is determined by the genetic and environmental conditions in which the lives of individuals and offspring. The appearance of the phenotype diversity in quantitative characters is largely influenced by environmental adaptation, not only by genetic components. Cluster differences based on genetic distance between morphometrics (phenotype) and molecular (genotype) are caused by differences in measurement indicators, so in genetic diversity *H. raphidea* mantis shrimp that are molecularly analyzed, become positive control and genetic clarification.

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Aknowledgements

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