Genetic Diversity and Community Structure of Macrozoobenthos from Five Mangrove Forests in North Sumatra and Aceh, Indonesia

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Abstract

Mangrove forests play an important role in macrozoobenthos ecology, assisting them in foraging, sheltering, and reproduction. In order to better understanding the genetic diversity and population genetic structure of macrozoobenthos, the mitochondrial 16S subunit (16S rRNA) gene was used. Present study aimed to determine genetic diversity and community structure of macrozoobenthos in the North Sumatra and Aceh mangrove forests, Indonesia. The reliaprep gDNA tissue miniprep system kit was used to extract 50 samples. To determine the PCR product's molecular weight, UVITEX software was employed. Genetic polymorphism was examined with GenAlex version 6.502, and each community underwent principal coordinates analysis (PCoA). MVSP 3.2.2 software and the unweighted pair group method with arithmetic mean (UPGMA) were used to construct the dendrogram. The 16S rRNA gene revealed a band size in the range of 370–500 bp. The PCoA showed that approximately all individual macrozoobenthos from each community structure were localized in the same quadrant. Genetic variation was varied by 3% between populations and 97.5% within individuals according to the analysis of molecular variance (AMOVA), with the dendrogram classifying the populations into two major clusters. Dendrogram analysis showed low genetic differentiation between macrozoobenthos populations of North Sumatra and the Aceh mangroves, implying the low ability of individuals in a population to adapt. The high polymorphic information content (PIC) value (0.886) reported was consistent with the number of alleles and size of the population's heterozygosity value. Present findings provide important information that will assist in formulation of mangrove conservation and restoration approaches.

Keywords: *Genetic diversity, macrozoobenthos, mangrove forest, population structure, restoration.*

Introduction

Mangroves provide a variety of marine species with food, shelter, spawning and breeding grounds (Setiawan, 2015; Chen *et al*., 2017; Prasetya *et al.,* 2017; Hasibuan *et al*., 2021). Ecologically, mangrove areas have high productivity for supporting the surrounding environment because they are rich in nutrients and have temperature, light, pH, oxygen, and optimum salinity, as well as conditions which make them a suitable habitat for crustaceans (Hogarth, 2015). The damage to mangrove forest vegetation has an impact on the survival of marine species, one of which is macrozoobenthos. Macrozoobenthos including crabs and snails that live in habitats such as epifauna, infauna, arboreal, brackish water, intertidal, and sediment mudflats; they play an important role in the mangrove ecosystem (Rahayu *et al*., 2018; Basyuni *et al*., 2022) in the process of overhauling organic matter and mineralization processes (Jawad, 2021).

Macrozoobenthos is one of the most significant groups in ecosystem waters due to their position as a vital biota in the food web and as an organic material degradator (Zabbe and Uyi, 2014). This creature serves as a detritivore on the mangrove substrate, hence macrozoobenthos populations can be utilized as an indication of mangrove ecosystem balance (Chen *et al*., 2017). Mangrove habitat conditions, including species composition and density, will impact physical, chemical, and biological

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features of waterways, which will determine the community structure of organisms connected with mangroves (Zabbey *et al*., 2021). Previously, 8 macrozoobenthos species were found in Lubuk Kertang mangrove forest of North Sumatra (Basyuni *et al*., 2018); 16 species, 12 genera, and 7 families of bivalves in Tanjung Balai Asahan (Susetya *et al*., 2018); and 7 macrozoobenthos species, 6 genera, and 4 families from Percut Sei Tuan (Hasibuan *et al*., 2021). Recently, 21 macrozoobenthos species, 17 genera, and 9 families have been described in North Sumatra and Aceh, and this has been shown as a key indicator of the restoration success (Basyuni *et al*., 2022).

Macrozoobenthos has an important function in the mangrove ecosystem, one of which is environmental preservation. The existence of macrozoobenthos in mangrove ecosystems must be maintained and protected. Environmental pollution caused by unfavorable industrial activities in the neighborhood of mangrove forests affects the mangrove ecosystem (Denisenko *et al*., 2003). Disturbance to the mangrove habitat has an impact on marine organisms. If the mangrove ecosystem is degraded, the protection offered to macrozoobenthos from predators and currents will be lost, as will habitat functions, leading to food shortages, disturbance of the reproductive process, and decreasing water quality. Based on those considerations, a study that can quantify the condition of macrozoobenthos from various distribution locations, representing natural forest, rehabilitated forest, and degraded forest/converted forest such as in Percut Sei Tuan, Belawan, Lubuk Kertang, Pulau Sembilan in North Sumatra and Langsa in Aceh, is required, one way of doing which is by using a molecular marker. In this research the 16S rRNA was used as a marker for macrozoobenthos diversity and population. The 16S-rRNA gene is found in all prokaryotes and has a highly variable section or sequence as well as other sequences (Klindworth *et al*., 2013).

The 16S rRNA gene analysis has been used to construct phylogenetic relationships at the species level as a universal, representative, and practical molecular systematic parameter (Benítez-Páez *et al*., 2016). The amplified ribosomal DNA restriction analysis (ARDRA) technique can be used to examine the DNA profile of the 16S rRNA gene resulting from PCR amplification in a fast, simple, and inexpensive manner. The ARDRA technique involves amplifying the 16S rRNA gene with primers tailored to the DNA sample being tested. (Marchesi *et al*., 1998) created primers 63f and 1387r for amplification of the 16S rRNA gene, allowing them to estimate the diversity of macrozoobenthos originating in the environment. Understanding of the application and utilization of genetic diversity for conservation should be increased. This can simplify the determination of what and where to protect, and it will increase our understanding of the taxonomy, origin, and development of the importance of macrozoobenthos species. There are three strategies to maintain genetic diversity, namely preventing marine ecological harm, reducing inbreeding, and implementing integrated coastal management (Reynold *et al*., 2012; Beermann *et al*., 2018. Despite the importance of macrozoobenthos genetic diversity and structure, there has previously been no information on the mitochondrial 16S rRNA gene of macrozoobenthic genetic variation and structure from North Sumatran and Aceh mangroves. Therefore, the purpose of this research was to determine genetic diversity and provide a clear population structure of macrozoobenthos in the North Sumatra and Aceh mangrove forests, Indonesia.

Materials and Methods

The research was initiated by a series of activities starting with sampling macrozoobenthos, which were carried out in five different mangrove forest locations; four locations were in North Sumatra Province (Percut Sei Tuan District (PCT), Belawan District (BLW), Lubuk Kertang Village (LK), and Pulau Sembilan Village (PS)) and one location in Aceh Province (Langsa City (LGS)). Details are provided in Figure 1 and Table 1. A total of 50 samples of macrozoobenthos, species names of snails and crabs were mentioned in Table 1 and stored in a 50 ml centrifuge tube, and ethanol was added as a preservative before analysis.

DNA extraction

The reliaprep gDNA Tissue Miniprep System kit (Promega) was used in accordance with the manufacturer's instructions to extract DNA from muscle tissues (50 mg) of crabs and snails using the non-organic adoption extraction approach. The total quality of DNA (100–350 ng) was determined using 1% agarose gels and quantified using a Thermo Fisher Scientific Nanodrop Spectrophotometer with a 260/280 absorbance ratio. In DNA dilution, a DNA quantity test was employed as a reference (Perwitasari *et al*., 2020). Using a purification kit, we purified DNA concentration (Promega).

Polymerase Chain Reaction (PCR) amplification

Using a set of 16S-rRNA primers with the principal configurations of 16S rRNA Forward (5-CCTGTTTACAAAAACAT-3) and 16S rRNA Reverse (5 AGATAGAAACCAACCTGG-3) as previously published, DNA was amplified by polymerase chain reaction (PCR) (Sensoquest thermal cycler) (Crandall *et al*., 1996). To obtain distinct bands, we used PCR gradients.

Table 1. Information of sampling sites

No	Site	Latitude and longitude	Species names and numbers of snails	Species names and numbers of crabs
1	Percut Sei Tuan District (PCT), Deli Serang Regency, North Sumatra Province	3°43'49.88" E 98°46'26.93" S	*Chicoreus capucinus* (2 samples), *Perna viridis* (2 samples), *Littoraria intermedia* (1 samples)	*Scylla paramamosain* (2 samples), *Etisus laevimanus* (2 samples), *Clibanarius symmetricus*(1 samples)
2	Belawan District (BLW), Medan City, North Sumatra Province	3°46'39.63" E 98°40'31.40" S	*Nassarius reeveanu* (2 samples), *Littoraria intermedia* (2 samples), *Littoraria scabra* (1 samples)	*Charybdis* sp (2 samples), *Varuna litterata* (1 samples), *Paromola japonica* (2 samples)
3	Lubuk Kertang Village (LK), West Brandan District, Langkat Regency, North Sumatra Province	4° 3'13.59" E 98°16'33.53" S	*Cerithidea alata* (1 samples), *Melampus castaneus* (2 samples), *Nerita balteata* (2 samples)	*Charybdis* sp (3 samples), *Clibanarius longitarsus* (1 samples), *Varuna litterata* (1 samples)
4	Pulau Sembilan Village (PS), Pangkalan Susu District, Langkat Regency, North Sumatra Province	4° 8'18.15" E 98°15'36.38" S	*Cerithidea alata* (2 samples), *Melampus castaneus* (2 samples), *Nerita balteata* (1 samples)	*Charybdis* sp (2 samples), *Clibanarius symmetricus* (2 samples), *Coenobita* sp (1 samples)
5	Langsa City (LGS), Aceh Province	4°32'19.62"E 98° 2'7.32" S	*Nerita planospira* (2 samples), *Nerita balteata* (2 samples), *Littoraria scabra* (1 samples), ,	*Charybdis lucifera* (2 samples), *Clibanarius rutilus* (2 samples), *Tubuca rosea* (1 samples)

2 l of DNA, 1 ul of forward and reverse primers, 3.5 l of ddH2O, and 2.5 l of Green Go Taq were combined to make a PCR mix in 10 l. The PCR amplification took place for 35 cycles for 1 min at 56°C, extension for 3 min at 72°C, final extension for 7 min at 72°C, and storage for 30 min at 4°C. The PCR took place for 2 min at 94°C for predenaturation, 30 sec at 94°C for denaturation, and 35 cycles for 1 min at 56°C (Basyuni *et al*., 2017; Hayati *et al*., 2020).

Data analysis

The PCR product was electrophoretically stained with GelRed® (Biotium) and then observed using a UV-Transluminator. Binary data were generated from band profiles. The UVITEC Cambridge Reader software was used to determine the size of the base fragment in the PCR product (Hayati *et al*. 2020). Using GenAlex ver. 6.502 software (Peakall *et al*., 2013), the genetic polymorphism in each population was evaluated as the average number of alleles (Na) and anticipated heterozygosity (He). The approach developed by Hayati *et al*. (2020) was used to determine the polymorphic information content (PIC). The principal coordinates analysis (PCoA) was used to visualize the macrozoobenthos populations from mangrove forests using GenAlex ver. 6.502 software. GenAlex ver. 6.502 were used for estimated the genetic structure of population molecular through the molecular variance analysis (AMOVA). Based on macrozoobenthos population location and grouping analysis, the unweighted pair group method with arithmetic mean (UPGMA) was used to perform dendrogram analysis, and the genetic distance was determined using the MVSP 3.2.2 program Hayati *et al*. (2020).

Results and Discussion

Genetic diversity

The PCR amplification of 50 samples was carried out successfully and could be scored using UVITEX software. This was preceded by DNA analysis of macrozoobenthos, which was performed repeatedly with adjustment of annealing temperature during the amplification process. The band size of the rRNA 16S gene obtained from 50 samples used in the study was in the range of 370–500 bp. Utilizing the number of alleles (Na), predicted heterozygosity (He), and polymorphic information content (PIC), the genetic parameter value of each population was calculated.

In a population, the number of alleles and heterozygosity are the parameters used to measure the genetic diversity level. Based on Table 2, Na value ranges from 6.0–17.0. The highest Na value was found in the LGS population (17.0), whereas the lowest was found in LK (6.0). The average expected heterozygosity (He) was identified as 0.894, with the value ranging from 0.813 (LK) to 0.934 (LGS). The

lowest He was derived from the LK population (0.813), and the highest from the Langsa population (0.934). The lowest He value was from the LK population (0.813), and the highest from the LGS population (0.934). Based on (Chesnokov *et al*., 2015), the He value of all populations observed in this study is high. The higher the He value of a population, the more likely it is to inbreed. This will have an impact on increasing the He genotype (Markiyanova *et al*., 2020). A low value of He will endanger the survival of a species or population, low value of genetic diversity also reflects a smaller population in the ecosystem (Ribeiro and Lopes, 2013), there are several causes of decreased genetic diversity of marine invertebrates such as overexploitation. Anthropogenic pollution, oil pollution, global warming, and tourism activities (Macher *et al*., 2016). High genetic diversity can be designed to estimate population size, because population size affects genetic diversity (Cao *et al*., 2018), differences between many orders of magnitude variation in population size and a much narrower distribution of diversity levels, related selection plays an important role both in Overall genetic diversity of a species as well as in the variation of diversity within the genome, genetic diversity also seems to be predictable from the life history of a species (Ellegren and Galtier, 2016).

Indicators of genetic diversity among genotypes in populations include the He and PIC values. Both values describe the evolutionary pressure on alleles and the potential historical mutation rate at a site (Markiyanova *et al*., 2020; Luo *et al*., 2019). He represents gene diversity for haploid markers and gives an estimate of the average heterozygosity and genetic distance among individuals in a population, whereas PIC values represent the effectiveness of markers for linkage analysis when determining inheritance between offspring and parental genotypes (Salem *et al*., 2016). A population of aquatic organisms with a high genetic variety may be better able to adapt to environmental changes, pathogen infections, and other selection processes (Liu *et al*., 2020). In this study, the PIC values ranged from 0.855 (LK) to 0.897 (PS). The high PIC value of all observed populations is related to the number of alleles and the magnitude of the heterozygosity value of a population, the PIC value for all markers used in this study is more than 0.5, indicating that this microsatellite marker can be used effectively. for molecular characterization, genome mapping. and genetic diversity studies, a marker with a PIC value of 0.5, showed that the marker was highly informative (Liu *et al*., 2017; Amareswari *et al*., 2018).

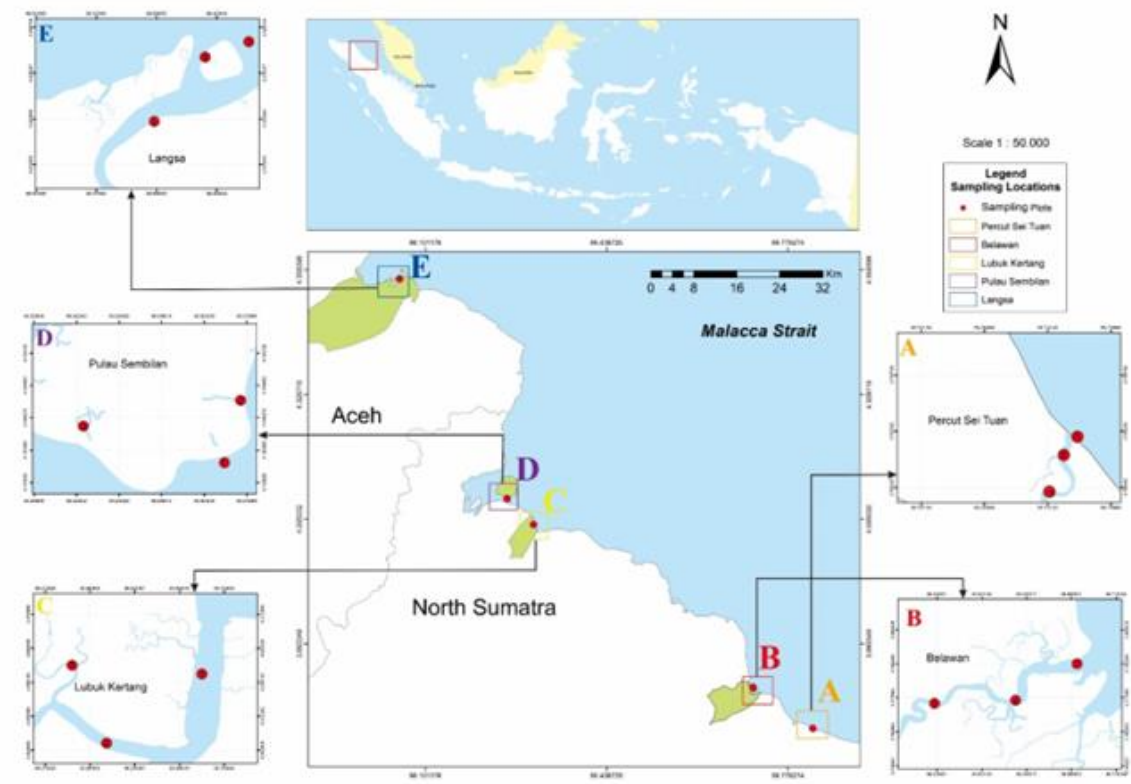

Figure 1. Sampling sites at North Sumatra Provinces: Percut Sei Tuan, Belawan, Lubuk Kertang, and Sembilan Island (A–D), and Langsa, Aceh (E). The red dots represent 3–4 samples per plot.

Table 2. Genetic parameters of macrozoobenthos in each population.

Population	Na	He	PIC
PCT	12.0	0.906	0.892
BLW	10.0	0.891	0.888
LK	6.0	0.813	0.855
PS	15.0	0.925	0.897
LGS	17.0	0.934	0.898
Mean	12.0	0.894	0.886
SE	1.93	0.02	

Na = average number of alleles, He = average heterozygosity of expectation, PIC = polymorphism information content, PCT = Percut Sei Tuan, BLW = Belawan, LK = Lubuk Kertang, PS = Pulau Sembilan Village, LGS = Langsa

This finding supports our previous results on the macrozoobenthic community assemblage in Langsa City (Basyuni *et al*., 2022), which was higher than in Lubuk Kertang Village (Zabbe *et al*., 2014). In contrast to Lubuk Kertang Village, which experienced deforestation and degradation (Basyuni *et al*., 2018), and where restoration action has been expanded, the Langsa City population was described as a natural mangrove forest (Basyuni *et al*., 2022). The existence of the macrozoobenthos community is related to its geographical location. Natural zones dominated by mangroves (Langsa City and Jaring Halus Village) have close distances between locations within the same location and smaller confidence interval ellipses (Basyuni *et al*., 2022). However, based on the similarity of their macrozoobenthic community assemblages, cluster analysis identified three primary groupings, each of which was dominated by management circumstances (Basyuni *et al*., 2018).

Genetic differentiation among population

DNA amplification through analysis of molecular variance showed the genetic structure of macrozoobenthos (Table 3.). The level of genetic diversity between populations was 3%, genetic diversity within individuals in the populations was 97%, and there was no genetic diversity among individuals. A population's relationship may be determined from its genetic distance due to the limited genetic variety between populations, which revealed that the macrozoobenthos species observed at each sample site have essentially identical genetic variants (Rowe and Zanatta, 2015). Low genetic differences between regions indicate genetic mixing between these regions, which leads to similarities in genetic structure (Nurtjahtjaningsih *et al*., 2014). Additional epigenetic markers or persistent inheritance information that cannot be explained by differences in the DNA sequence accompany such genetic information (Xavier *et al*., 2019).

Understanding genetic diversity and predicting future association studies can be assisted by studying population structure. A positive relationship between markers and characteristics is very indicative of the presence of population structure in the mapped population (Eltaher *et al*., 2018). The separation of macrozoobenthos populations as determined by the Bayesian technique was often supported by principal coordinate analysis (PCoA) based on Nei's unbiased genetic distance. Figure 2 shows the visualization of macrozoobenthos populations from mangrove forests of North Sumatra and Aceh with the PCoA, where the PCoA map of individuals, based on two main coordinates, is divided into four quadrants.

One coordinate point represents one individual, and the same color indicates that the individual is from the same population (Nurulloh *et al*., 2019). Almost every individual macrozoobenthos from each population of LGS, PCT, BLW, PS, and LK were indicated in the same quadrant by PCoA. Based on these findings, the value of genetic distance between the five sample locations has no relationship with geographic distance. The results of this genetic pattern are somewhat surprising, given that the geographical proximity of the samples does not indicate genetic closeness. Genetic flow is often associated with geographic distance of a population, different genetic flows within individuals within a population that are influenced by individual adaptive capacity, are the cause of differences in location coordinates in PCoA studies (Kuester *et al*., 2015; Nurtjahtjaningsih *et al*. 2014). The genetic distance of a population is inversely proportional to the geographical distance, however, a number of macrozoobenthos individuals from the LGS, PCT, BLW, and PS populations are scattered between the quadrants. According to (Chust *et al*., 2016) the genetic structure of a population is influenced by several factors such as the environment, natural conditions, population size, reproductive methods, and natural selection, and can also be influenced by mutations, gene flow, and genetic drift.

Population structure

The dendrogram analysis of macrozoobenthos from North Sumatran mangrove forests obtained using MVSP software produced dendrograms that form two clusters or main groups. The first cluster comes from the populations of PS and BLW, and the second cluster consists of the populations of LK, LGS, BLW, PCT, and PS. The second cluster is divided into two subclusters, where the first subcluster consists of the populations of LK, LGS, PCT, BLW, and PS. The second subcluster consists of LGS, PCT, BLW, and PS.

Dendrogram analysis showed low genetic differentiation between macrozoobenthos populations of North Sumatra Province and the Aceh Province mangrove forests. Based on (Kusmini *et al*., 2015), the proximity of genetic distance between populations indicates that there is a genetic flow (gene flow) between those populations, as well as the existence of genetic relationships from reproduction. As a result of this study, the Macrozoobenthos population showed limited genetic differences based on geographical distance values, but it still represented one population. Gene flow, a lack of significant differences between sampling sites, a high level of allelic richness across the board, and the absence of genetic barrier evidence all point to this. Rowe *et al*. (2015) carried out a similar study in the Laurentian Great Lake. Geographic distance and habitat complexity influenced gene flow and geographic isolation of some of the marine species. Genetic similarities might also exist due to the similarity of habitats in each population. There is no barrier that can prohibit sexual reproduction between populations through outcrossing. Over short spatial scales, marine invertebrates with high dispersion capacities and life cycles that include pelagic phases and vast populations should show substantial gene flow level and minimal structure of population genetic (Hedgecock, 1986; Cerca *et al*., 2018). Information about genetic diversity is very important at each population level of an organism, to provide information about the genetic quality of the observed

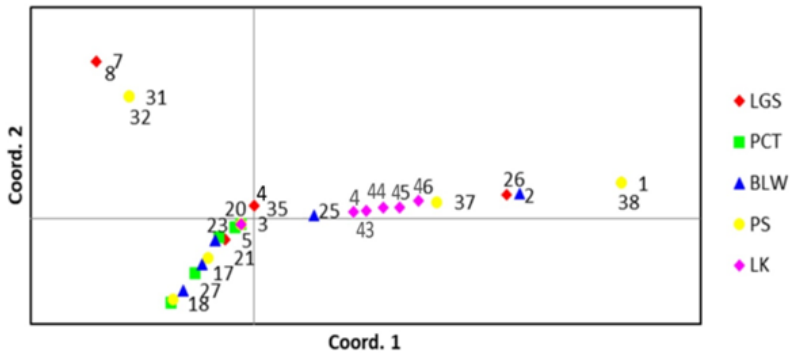

Figure 2. Principal coordinates analysis of macrozoobenthos from mangrove forests of North Sumatra and Langsa of Aceh. Note: PCT = Percut Sei Tuan, BLW = Belawan, LK = Lubuk Kertang, PS = Pulau Sembilan Village, LGS = Langsa

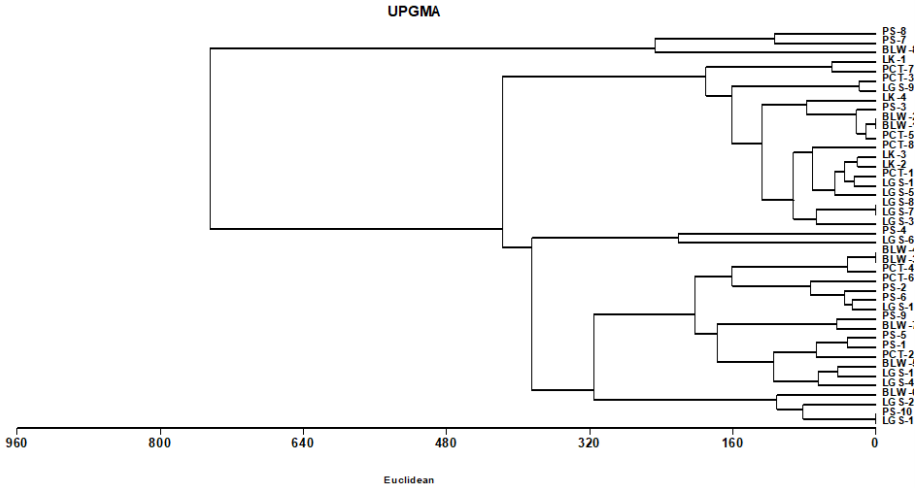

Figure 3. Dendrogram relationship of macrozoobenthos fr om mangrove forests. PCT= Percut Sei Tuan, BLW= Belawan, LK= Lubuk Kertang, PS= Pulau Sembilan Village, LGS= Langsa

population; low genetic diversity causes the low ability of individuals in a population to adapt (Indriatmoko *et al*., 2020).

Conclusion

The application of 16S-rRNA for macrozoobenthos in this study resulted in a genetic diversity value of 3% between populations and 97% within individuals, and the dendrogram divided the sample population into two main clusters or groups, the low genetic diversity between populations indicates that the macrozoobenthos species present in each sample location sampling has almost the same genetic diversity, so that the relationship of a population can be seen from its genetic distance. Visualization of macrozoobenthos populations from mangrove forests of North Sumatra and Aceh with PCoA, where individual PCoA maps based on two main coordinates are divided into four quadrants. The value of genetic distance between the five sample locations has no relationship with geographic distance. Understanding the genetic diversity was seen in macrozoobenthos populations will assist in designing future breeding programs and provide useful data for maintaining and monitoring the genetic variation necessary for successful breeding programs.

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