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Short Communication

Implementation of 16S rRNA Gene for Fish and Shrimp Barcoding in Mangrove Ecosystems in North Sumatra and Aceh, Indonesia

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Abstract

Fish and shrimp are among the species that are vulnerable to high level of disturbance in mangrove ecosystem. This study aimed to investigate the implementation of 16S rRNA gene for fish and shrimp barcoding in mangrove ecosystems in North Sumatra and Aceh, Indonesia. 50 samples fresh fish and shrimp were collected from five mangrove ecosystems in North Sumatra, namely Belawan, Lubuk Kertang, Percut Sei Tuan, and Pulau Sembilan and Langsa of Aceh for DNA extraction process. The research was divided into four activities: sample collection, DNA isolation, PCR amplification, and data analysis. DNA was extracted using kit extraction (kit Reliaprep gDNA Tissue Miniprep System) and with PCR amplification. Genetic parameters were analyzed using Gen Alex 6.51 software and the relationship between sites was examined using the MVSP 3.22. The genetic diversity of fish and shrimp from mangrove habitats was relatively substantial, according to the findings, with a heterozygosity of 0.926, with the exception of Langsa samples, which were only identified in subclass A1. Genetic relationship between sites revealed that all samples clustered into two branches and were randomly dispersed within each site. This work confirmed the 16S rRNA gene worked for fish and shrimp barcoding in mangrove ecosystems, North Sumatra and Aceh, Indonesia and our findings are expected to be useful in developing mangrove conservation and restoration initiatives.

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1. Introduction

Indonesia is recognized for having the largest mangrove biodiversity in the world. Indonesia has 2.7 million ha of mangrove forest in 2020 spread over 34 provinces, mainly spread out in Eastern Indonesia. Papua Province has the largest mangrove with 26.6% and followed by West Papua (17.5%), East Kalimantan (7.5%) and North Kalimantan (5.6%) (Basyuni *et al*., 2022). Over the last 30 years, approximately 800,000 ha of mangrove forests have been removed and converted (Ilman *et al*., 2016). Deforestation and degradation of mangroves, on the other hand, continue to become a problem throughout Indonesia. Land conversion to non-forest, oil palm plantation, tourism, pond development, and excessive logging all contribute to the deforestation and degradation of mangrove forests in Indonesia (Richards and Friess, 2016; Basyuni and Sulistiyono, 2018).

Mangrove species are scattered along the intertidal zone or along the shoreline of the tidal zone in the tropics and subtropics (Balke *et al*., 2015; Fu *et al*., 2018). Mangrove plants are able to adapt in terms of morphology, anatomy, physiology, and molecular complexes that allow them to survive and grow well even in high stress habitats (Srikanth *et al*., 2016; Basyuni *et al*., 2017), such as flooded soil conditions, high salinity and unstable soil conditions (Hogarth, 2015; Woodroffe *et al*., 2016). The interaction between oceans, brackish water, rivers, and land waters is quite large, resulting in a high diversity of marine plants and animals, freshwater species, and land species in the mangrove ecosystem (Rizal *et al*., 2018). Mangrove ecosystems have an important role both ecologically and economically (Basyuni *et al*., 2022) as a home for various marine components such as algae, crabs, bivalves, snails, meiofauna, fish, and invertebrates as well as various animals such as birds that nest in the dense canopy, also serves as a shelter for migratory birds (Almasi *et al*., 2019). Many young fish from nearby coral reefs, such as protected snapper and grouper, crustaceans such as shrimp, crab and lobster also survive and grow in this ecosystem, which proves that mangroves are important for marine life and invertebrates (Winata *et al*., 2017; Zhu *et al*., 2017).

Fish and shrimp in mangrove habitats are diverse and need to be identified. The fish and shrimp are identified using their morphology, while molecular approaches can also be utilized. Previous morphological study conducted by Sitorus *et al*. (2017) in Lubuk Kertang found 16 species fish diversity that belonged to the 5 orders, 15 families, 15 genera. Previous reports in the basic knowledge in fish systematics taxonomy based on morphological characters have been described before studying on genetic level (Fitri *et al*., 2018; Basyuni *et al*., 2021). A common molecular identification technique was DNA barcoding which has various advantages over morphological identification. DNA barcoding can be used to identify fish species from whole fish, fillets, fins, fragments, juveniles, larvae, eggs, or any other properly preserved tissue available, transforming taxonomy for taxa with validated data sets (e.g., fishes) by providing a quick and cost-effective method for identifying eukaryotes (Becker *et al*., 2011).

The chloroplast 16S ribosomal RNA gene (16S rRNA) has been utilized in a number of studies to identify numerous coastal aquatic species and mangroves, including marine fish and shrimp (Galvão *et al*., 2013; Joseph *et al*., 2013; Wilwet *et al*., 2018). In this regard, the 16S rRNA gene sequences of 59 individuals from 43 species from 30 fish families were examined (Habib *et al*., 2021). However, the importance of the application of 16S rRNA gene for fish and shrimp barcoding in Indonesian mangroves were rarely reported. The primer used was 16S primer because 16S rRNA genes, they were utilized to form phylogenetic trees. Based on this consideration, this work aims to investigate the implementation of 16S rRNA gene for fish and shrimp barcoding in mangrove ecosystems, North Sumatra and Aceh, Indonesia.

2. Materials And Methods

2.1 Study Area

The study was conducted in five sampling sites in the provinces of Aceh and North Sumatra, Indonesia, which represents different management conditions. The Aceh Province of Langsa city (LGS) has a natural forest, while the mangroves in Percut Sei Tuan Village (PCT), Pulau Sembilan Village (PS) from North Sumatra Province are restored from abandoned and clear-cut aquaculture ponds. Meanwhile, the mangroves in Belawan Village (BLW) and Lubuk Kertang Village (LK), North Sumatra Province are rehabilitated (Table 1) (Figure 1). This site has an average rainfall of 150–200 mm/year and sea surface temperatures range from 26–30ºC.

2.2 Research Materials

A total of 50 fish and shrimp samples were taken from five different locations, namely Percut Sei Tuan, Belawan, Lubuk Kertang, Pulau Sembilan from North Sumatra Province, and Langsa City from Aceh Province, which were then used as DNA genetic material (Table 1) (Figure 1).

DNA isolation was carried out using the *Reliaprep* gDNA Tissue *Miniprep* System kit (Promega).

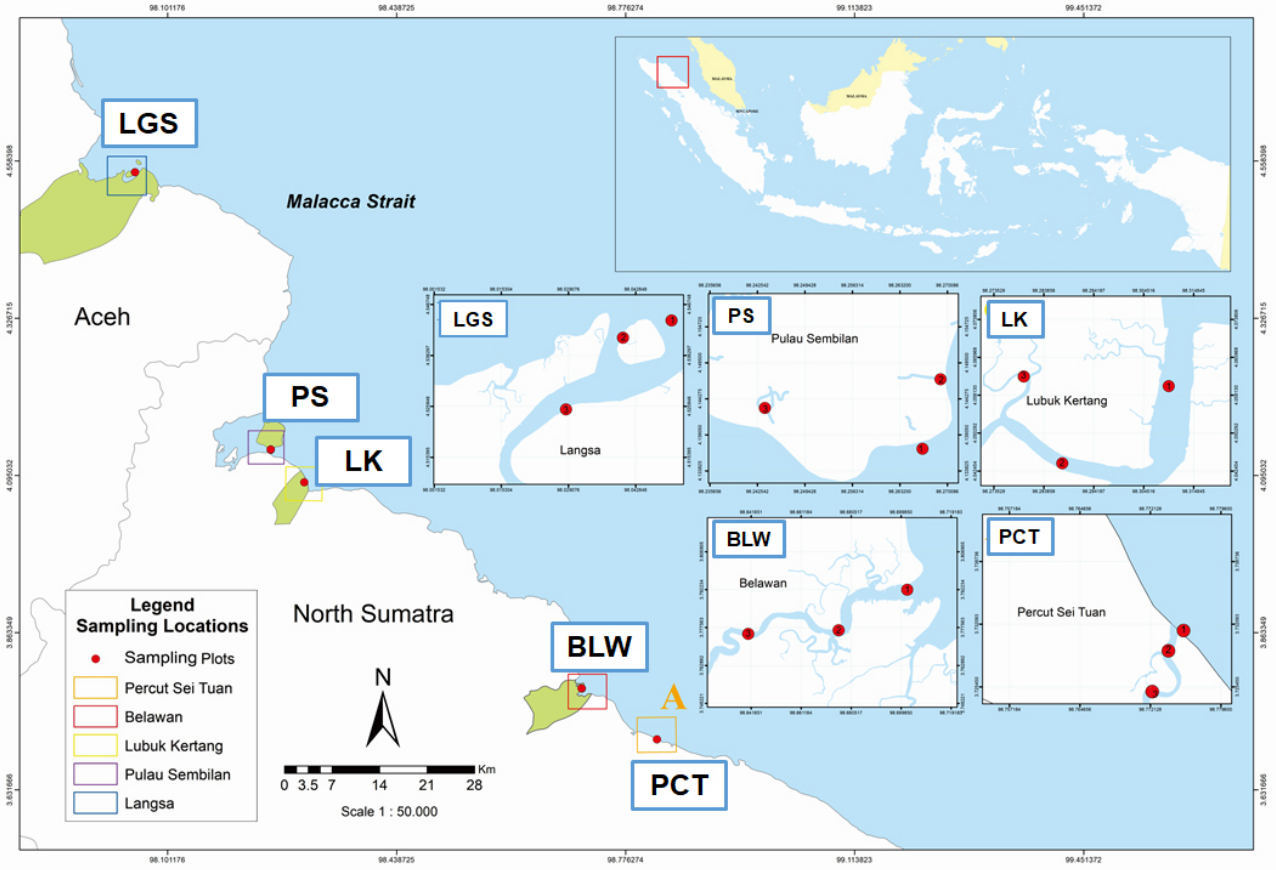

Figure. 1. Sampling sites (red dot) at Percut Sei Tuan (PCT), Belawan (BLW), Lubuk Kertang (LK), and Pulau Sembilan (PS) of North Sumatra, and Langsa of Aceh (LGS)

Table 1. Information of sampling sites

No	Site	Longitudinal	Species names and numbers of fish	Species names and numbers of shrimp
1	Percut Sei Tuan District (PS), Deli Serdang Regency, North Sumatra Province	3°43'49.88" E 98°46'26.93" S	*Hexanematichthys sagor* (3), *Epinephelus coioides* (3)	*Macrobrachium equidens* (2), *Penaeus indicus* (2)
2	Belawan District (BLW), Medan City, North Sumatra Province	3°46'39.63" E 98°40'31.40" S	*Elops saurus* (3), *Epinephelus fuscoguttatus* (2)	*Macrobrachium equidens* (3), *Penaeus indicus* (2)
3	Lubuk Kertang Village (LK), West Brandan District, Langkat Regency, North Sumatra Province	4° 3'13.59" E 98°16'33.53" S	*Leptocarpus potamiscus* (2), *Elops hawaiensis* (2), *Thunnus maccoyii* (2)	*Macrobrachium acanthurus* (2), *Penaeus indicus* (2)
4	Pulau Sembilan Village (PS), Pangkalan Susu District, Langkat Regency, North Sumatra Province	4° 8'18.15" E 98°15'36.38" S	*Larimichthys crocea* (3), *Pellona ditchela* (2)	*Macrobrachium acanthurus* (3), *Penaeus longistylus* (2)
5	Langsa City (LGS), Aceh Province	4°32'19.62"E 98° 2'7.32" S	*Leptocarpus potamiscus* (2), *Danio rerio* (2), *Sphyraena jello* (2)	*Penaeus longistylus* (2), *Macrobrachium equidens* (2)

A PCR (Polymerase Chain Reaction) machine (Sensoquest thermal cycler) was used to amplify genomic DNA extraction. A pair of 16S primers (16S rRNA F:5'-CCTGTTTANCAAAAACAT-3' and R:5'-AGATAGAAACCAACCTGG-3') were used to amplify DNA, as previously described by Crandall and Fitzpatrick (1996). The quality of total DNA (200–300 ng) was evaluated using 1% agarose gels and then quantified by a nanodrop spectrophotometer (Thermo Scientific) with an absorbance ratio of 260/280. The following solution components were used to amplify each sample; 2 µl DNA dilute, 1 µl Primer forward and 1 µl primer reserve, 3.5 µl ddH2O, and 2.5 µl Green Go Taq, for a total mixture of 10 µl in one tube. The PCR amplification performed according to protocol included pre-denaturation at 94°C for two minutes, denaturation at 94°C for 30 seconds, followed by 35 cycles of annealing at 56°C for one minute, extension at 72°C for three minutes, final extension at 72°C for seven minutes, and storage at 4°C for 30 minutes. The PCR amplification data were evaluated using electrophoresis in a 1% agarose gel dyed with Gel Red (Biotium) and visualized using Ultraviolet Translation (UVP).

2.3 Data Analysis

UVITEX Cambridge Reader software was used to analyze the bands to measure the molecular weight of DNA, which was then used to create specific band patterns based on the primer employed. The genetic parameters for each population was assessed by GenAlex ver. 6.502 software (Peakall and Smouse, 2006) as the frequency of correlation alleles between individuals in subpopulations (F_{IS}), allele frequency correlation between subpopulations (F_{ST}), allele frequency in population caused by both factors (F_{IT}), number of migrants (Nm), number of alleles (N), number of different allele frequencies > 0.5 percent (Na), and number of active alleles (Ne). Shannon Index Information (I) was calculated for each population and locus as previously reported by Hayati *et al*. (2020). Analysis molecular of variance (AMOVA) and a principal coordinate analysis (PCoA) to examine the linkages between populations were calculated using GenAlex ver. 6.502 (Peakall and Smouse, 2006). Dendrogram analysis was done based on the sites and grouping analysis of fish and shrimp populations using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), and the relation distance was carried out with Multi-Variate Statistical Package (MVSP 3.2.2) program (Hayati *et al*., 2020).

3. Results and Discussion

3.1 PCR Amplification

The appearance of DNA bands produced from the 50 samples indicated the success of PCR amplification. The resulting band pattern, however, has varying DNA thickness; thick, thin, and smear. Variation in DNA concentration is determined by the physical treatment of the extraction process as well as the effectiveness of extraction buffers in breaking down cells (Basyuni *et al*., 2017; Istiqomah *et al*., 2020). The process of physical cell destruction was carried out by crushing a sample which could perfect the extraction buffer in breaking down cells. Amplification of PCR employing a 16S results in bands ranged from 200 to 800 bp.

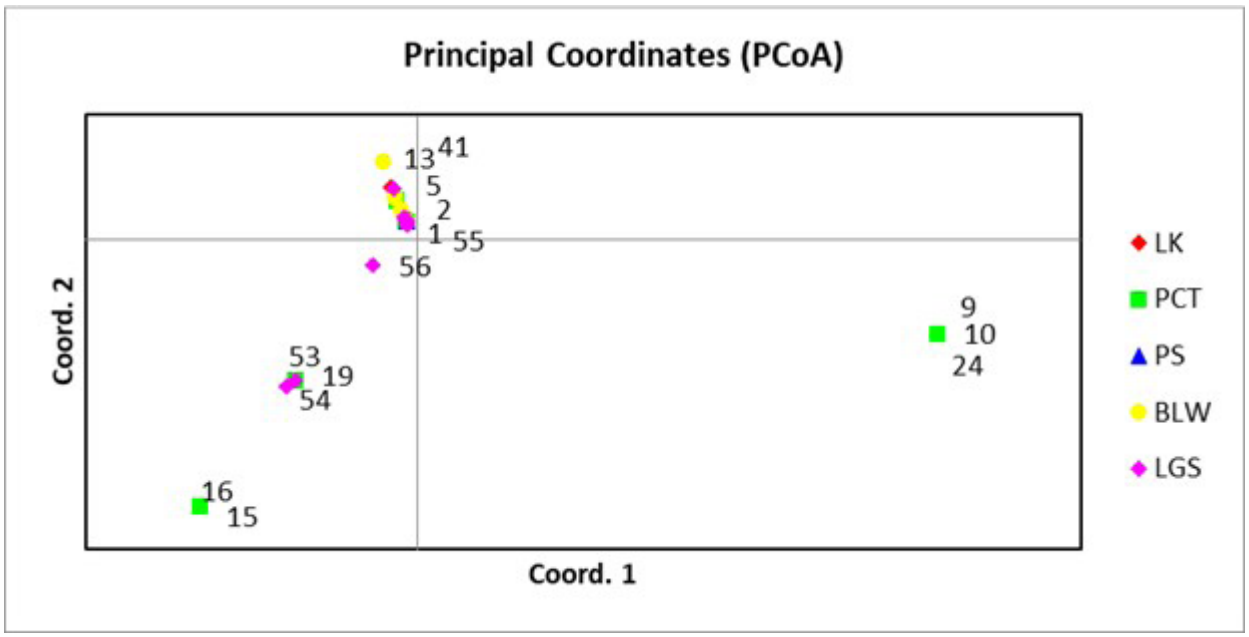

Figure 2. Principal coordinate analysis (PCoA) visualization LK = Lubuk Kertang, PCT = Percut, PS = Pulau Sembilan, BLW = Belawan, LGS = Langsa

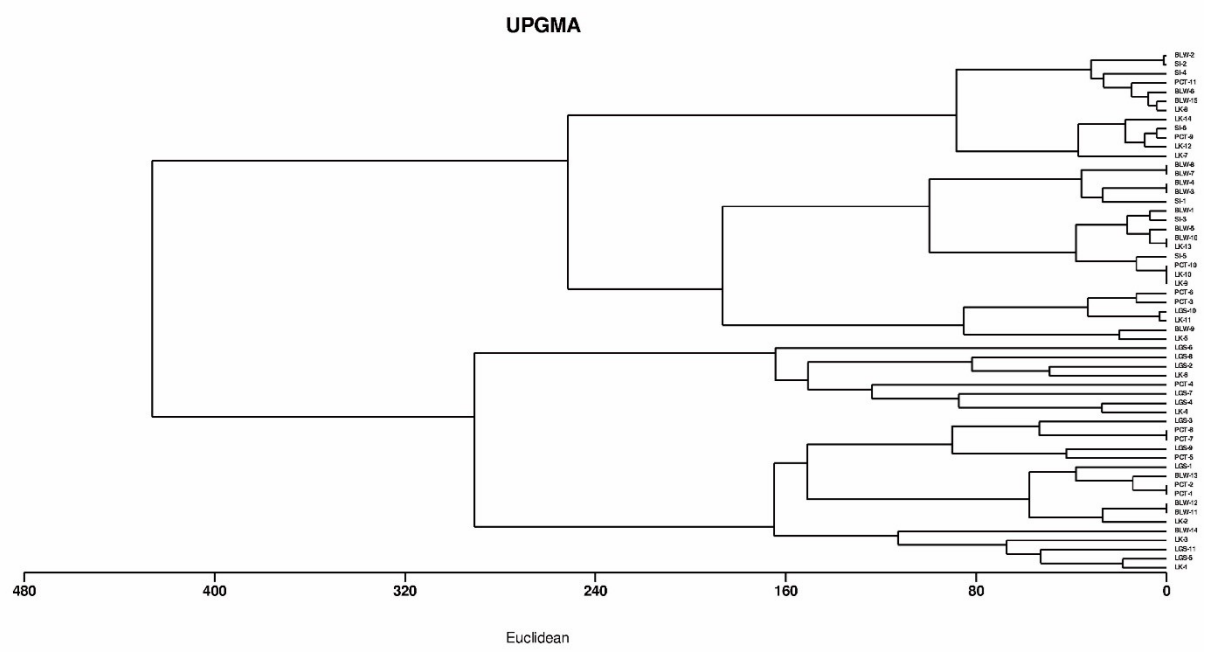

Figure 3. Dendrogram of fish and shrimp kinship relationship using cluster analysis, LK = Lubuk Kertang, PCT = Percut, PS = Pulau Sembilan, BLW = Belawan, LGS = Langsa

3.2 Genetic Parameters

Genetic variety within and across populations was found in order to select populations responsible for the majority of current variation. If genetic variety is concentrated within a population, fewer populations are required to retain and perpetuate overall variation across a species' geographic range. If genetic variety is generally retained between populations, then a larger number of populations that make up evolutionary units should be safeguarded (Szczecińska *et al.*, 2016). The entire population obtained a higher Na value than the Ne value in the LGS population with a Na value of 16 and Ne of 13, while the lowest value was obtained in the PS population with a Na value of 5 and a Ne value of 5 (Table 2). According to Karabağ *et al.* (2016), genetic variation is more likely to be detected if the mean Ne is high, which is defined as the inverse of homozygosity. The values of Na and Ne obtained in the PS population are identical. Frankham *et al.* (2017) stated that the frequency of alleles is in equilibrium if the number of effective alleles (Ne) is equal to or almost equal to the number of alleles observed (Na).

This finding was supported by previous studies. Basyuni *et al.* (2021) reported that Langsa had the largest fish biomass compared to Lubuk Kertang (Fitri *et al.*, 2018), followed by Pulau Sembilan and Percut Sei Tuan. Furthermore, Langsa was characterized as natural mangrove forest which experienced no deforestation and degradation, in contrast to Lubuk Kertang, Pulau Sembilan, and Percut Sei Tuan, all of which experienced deforestation and degradation (Basyuni and Sulistiyono, 2018), and restoration activity has been progressed in these places (Basyuni *et al.*, 2022).

Agglomerative hierarchical clustering (AHC) analysis revealed that mangrove sites were clustered by site attribute similarity clustering, with Langsa and Pulau Sembilan within group 1, and Jarin Halus was more related to group 1 compared to group 3, which includes mangroves at Kampai Island and Percut Sei Tuan. Physicochemical parameters and stand density were the variables with the most weight in the clustering (Fitri *et al.*, 2018).

Shannon's diversity index has been used in various studies to estimate genetic uniformity. Shannon's diversity index values are also utilized to describe genetic variation at different population levels from single nucleotides to polymorphism (SNP) through whole species or larger taxonomic units to ecosystems. The highest diversity index was obtained in the LGS population of 2,685, while the lowest diversity index value was in the PS population of 1,609.

Average heterozygosity of expectation (He) was understandable considering the population's demographic history and genetic structure (Wang, 2018). The highest He value was in the LGS population at 0.926, while the lowest value is in the PS population at 0.8 (Table 1). The value of heterozygosity is one of the parameters that can be used to assess the genetic diversity

of a population. Heterozygosity values range from 0–1. The value of heterozygosity is said to be low when it approaches 0, whereas the heterozygous value is said to be high when the value is close to 1. In this study, the heterozygosity value obtained from the entire population is close to 1, indicating that the genetic diversity in the population is relatively high. The observed heterozygosity value (Ho) is greater than the expected heterozygosity value (He) (P>0.05) (Table 2). Inbreeding, concealed population subdivision, and genotyping artefacts could all contribute to the apparent divergence from Hardy-Weinberg equilibrium if He > Ho (e.g., null alleles, allelic dropouts). If He > Ho, apparent deviation from Hardy-Weinberg equilibrium could imply that the population has been hybridized or admixed (Wang, 2018).

The wealth of information on which markers, otherwise known as Polymorphism Information Content (PIC) reported in this study, is very interesting. The PIC value can also be used to determine the absence of a polymic allele for a gene in a population. The PIC value generated from this research is 0.845–0.897. With the PIC value obtained, the 16S primer is suitable for use as a molecular marker in fish and shrimp. Previous studies reported PIC values of 0.387 to 0.899, with a mean a value of 0.750, which reveals that the marker has the necessary properties to be used in diversity studies (Zhu *et al*., 2017). The closer the PIC value is to 1, the more useful it is in the identification of diversity (Kwak *et al*., 2020). The mean value of PIC is determined by several parameters, including the breeding behavior of the species, genetic diversity within the collection, length of the collection, sensitivity of genotyping methods, and the location of the marker genome (Chen *et al*., 2017).

Table 3. F-Statistics and Nm estimates for all population observed using 16S rRNA

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
16S rRNA	0.729	0.754	0.092	2.46

Description: Fis = inbreeding coefficient, FIT= overall inbreeding coefficient, FST= fixation index, Nm = number of migrants

Statistical analysis of F (F_{IS}, F_{IT}, and F_{ST}) is an important indicator in the analysis of genetic populations (Table 3). F_{IS}, F_{IT}, and F_{ST} values indicate inbreeding in subpopulation, inbreeding in total population, and population differentiation, respectively (Maharani *et al.*, 2017). The magnitude of inbreeding in the subpopulation of (F_{IS}) is 0.729, while the rate of inbreeding in the total population (F_{IT}) is 0.7534 (Table 3). F_{IS} levels that are negative suggest an excess of heterozygotes, whereas F_{IS} values that are positive imply a shortfall (Parreira *et al.*, 2020). Inbreeding that occurs in these populations results in a decreased uniformity in the long term, causing extinction. As the inbreeding degree of individuals decrease, genetic differences between them also increase.

F_{ST} analyzes genetic variance in allelic frequencies among demes and describes the mean reduction in heterozygosity of a deme compared to the whole, due to genetic drift and genetic differentiation (Parreira *et al*., 2020). The F_{ST} is 0.092, and this value indicates that there is no genetic difference between fish and shrimp populations from Lubuk Kertang, Percut, Pulau Sembilan, Belawan and Langsa (Holsinger and Weir, 2009).

Table 2. Genetic diversity parameters in five fish and shrimps' populations of the mangrove ecosystems of North Sumatra and Aceh

Pop	N	Na	Ne	I	Ho	He	F	PIC
LK	14	12	10.3	2.404	0.214	0.903	0.763	0.89
PCT	11	11	8.6	2.284	0.364	0.884	0.589	0.88
PS	5	5	5	1.609	0	0.8	1	0.84
BLW	14	11	9.8	2.342	0	0.898	1	0.89
LGS	13	16	13.5	2.685	0.615	0.926	0.335	0.89
Mean	11.4	11	9.4	2.265	0.239	0.882	0.737	

Description: N = Number of samples; Na = Average number of alleles; Ne = Average number of effective alleles; Ho = Average heterozygosity of observation, He = Average heterozygosity of expectation, I = Shannon's information index, F = fixation index , LK = Lubuk Kertang, PCT = Percut, PS = Pulau Sembilan, BLW = Belawan, LGS = Langsa

If the F_{ST} is small, it means that the frequency of alleles in each population is the same; if it is large, the frequency of the allele is different. An F_{ST} value < 0.05 indicates that its genetic differentiation is small. The rate of fundamental evolutionary processes (migration, mutations, and deviations) has a predictable relationship with the level of genetic differentiation among populations (Holsinger and Weir, 2009). With an Nm value of 2,460 and a paired F_{ST} value implying poor genetic differentiation and moderate genetic differentiation between pairs of other populations and is supported by an Nm value higher than 1.0 (Biba *et al*., 20217).

3.3 Genetic Structure through Molecular Analysis of Variations (AMOVA) and Principal Coordinate Analysis (PCoA)

The diversity among individuals in the populations were higher compared to those within individuals (Table 3). According to Groeneveld *et al.* (2010), the presence of genetic diversity that is rich and at a sufficient level in a species enables the formation of new populations according to the changing conditions and needs.

Two-dimensional insecurity in Principal Coordinate Analysis (PCoA) showed five populations spread over four quadrants (Figure 2). Populations that were in the quadrant showed that the species had similar special characteristics. The results of the PCoA analysis showed that almost all individuals from each population of Lubuk Kertang (LK), Percut (PCT), Pulau Sembilan (PS), Belawan (BLW), and Langsa (LGS) were in the same quadrant. This was because these individuals were geographically close together, and genetic flow from each sample population was also the same. The closer the geographic distance of a population was, the closer its genetic distance would be. However, there were some individuals from the LGS and PCT population which were in different quadrants. Coordinate differences in PCoA analysis are caused by differences in individuals' genetic flow of a population influenced by their abilities to adapt.

3.4 Discussion

Phylogenetic relationships are not necessarily determined by geographical distance. Information based on phenotypic and molecular genetic features, as well as geographic and historical information from the population studied, must be included to get superior phylogenetic results (Hariyono *et al*., 2019). The genetic kinship dendrograms of five fish and shrimp populations were grouped based on amplification results using primary 16S (Figure 3). The genetic kinship dendrograms divided 30 fish and shrimp samples into two main clusters and four sub-clusters. Each cluster was divided into several sub-clusters consisting of individual populations. The classification showed the magnitude of the variation between these species. It may be a consequence of migration between two different geographic populations (Shokoohmand *et al*., 2018).

Based on UPGMA, the population was divided into two main clusters and four sub-clusters (Figure 3). In sub-cluster A1, no LGS species was found which was caused by several factors. In accordance with Consuegra *et al.* (2021) who stated that in addition to elevation and water velocity, barrier density and its interplay with cumulative barrier height determine a species' presence/absence.

In summary, amplification of PCR using primary 16S provides information and can be recommended for use in further molecular analysis. Primer 16S belongs to a specific primer where the PIC value obtained ranges from 0.845–0.897. The findings of a genetic diversity analysis utilizing many criteria revealed that the genetic diversity of fish and shrimp from mangrove habitats was high, with He value of 0.926. Our findings are useful in developing mangrove conservation and restoration initiatives (Basyuni *et al*., 2017).

4. Conclusion

The genetic diversity of fish and shrimp from mangrove habitat is relatively large, the entire population obtains a higher Na value than the Ne value. PCR amplification using 16S produced bands with sizes ranging from 200 to 800 bp. The highest Na and Ne values were found in the LGS population. F_{ST} value of 0.092 indicates that there is no genetic difference between fish and shrimp populations from the five observation locations. Present work provides the developing mangrove conservation and restoration initiatives.

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Authors' Contributions

The contribution of each author is as follow, M.B. and A.S.; conceptualized the manuscript. H.H., M.B., R.A. and Y.B.; conducted the methodology. H.H., M.B., I.E.S., and A.S.; validated, formal analyzed, and investigated the data. M.B. T.K.; provided resources. M.B.; curated the data. H.H. and M.B.; wrote original draft preparation. M.B., R.A., A.S., T.K., and I.E.S.; reviewed and edited the manuscript. H.H., Y.B.; visualized the data. M.B., I.E.S., and A.S.; supervised the

research. M.B.; managed the project administration. M.B.; acquired funding. All authors have read, agreed, discussed, and contributed to the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

Almasi, M., Milow, P., & Zakaria, R. M. (2019). The study of participatory mangrove forest ecosystem management in Malaysia, the case of the Matang mangrove forest. *World Journal of Environmental Biosciences*, 8(4):23-34.

Balke, T., Swales, A., Levelock, C. E., Herman, P. M. J., & Bouma, T. J. (2015). Limits to seaward expansion of mangroves: Translating physical disturbance mechanisms into seedling survival gradients. *Journal of Experimental Marine Biology and Ecology*, 467:16-25.

Basyuni, M., & Sulistiyono, N. (2018). Deforestation and reforestation analysis from land-use changes in North Sumatran Mangroves, 1990-2015. IOP Conference Series: *Materials Science and Engineering*, 309(1):012018.

Basyuni, M., Baba, S., & Oku, H. (2017). Microsatellite analysis on genetic variation in two populations of red mangrove *Rhizophora mangle* L. (Rhizophoraceae) and its implication to conservation. In IOP Conference Series: *Materials Science and Engineering*, 180(1):012243.

Basyuni, M., Slamet, B., Sulistiyono, N., Munir, E., Vovides, A., & Bunting, P. (2021). Physicochemical characteristics, nutrients, and fish production in different types of mangrove forests in North Sumatra and the Aceh Provinces of Indonesia. *Kuwait Journal of Science*, 48(3):1-14.

Basyuni, M., Sasmito, S. D., Analuddin, K., Ulqodry, T. Z., Saragi-Sasmito, M. F., Eddy, S. & Milantara, N. (2022). Mangrove biodiversity, conservation and roles for livelihoods in Indonesia. In S. C. Das, Pullaiah, & E. C. Ashton (Ed.), Mangroves: Biodiversity, livelihoods and conservation. (pp. 397-445). Singapore: Springer.

Biba, A., Hoda, A., Bozgo, V., & Mali, S. (2017). Genetic diversity of *Cyprinus carpio* of natural lakes in Albania estimated by microsatellite loci. *Endocytobiosis and Cell Research*, 28(1):1-8.

Becker, S., Hanner, R., & Steinke, D. (2011). Five years of FISH-BOL: brief status report. *Mitochondrial DNA*, 22(1):3-9.

Chen, W., Hou, L., Zhang, Z., Pang, X., & Li, Y. (2017). Genetic diversity, population structure, and linkage disequilibrium of a core collection of *Ziziphus jujuba* assessed with genome-wide SNPs developed by genotyping-by-sequencing and SSR markers. *Frontiers in Plant Science*, 8(575):1-14.

Consuegra, S., O'Rorke, R., Rodriguez-Barreto, D., Fernandez, S., Jones, J., & de Leaniz, C. G. (2021). Impacts of large and small barriers on fish assemblage composition assessed using environmental DNA metabarcoding. *Science of the Total Environment*, 790:148054.

Crandall, K. A., & Fitzpatrick, Jr. J. F. (1996). Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology*, 45(1):1-26.

Fitri, A., Basyuni, M., Wati, R., Sulistiyono, N., Slamet, B., Harahap, Z. A., Thorste, B., & Bunting, P. (2018). Management of mangrove ecosystems for increasing fisheries production in Lubuk Kertang village, North Sumatra, Indonesia. *Aquaculture, Aquarium, Conservation & Legislation Bioflux*, 11(4):1252-1264.

Frankham, R., Ballou, J. D., Ralls, K., Eldridge, M., Dudash, M. R., Fenster, C. B., Lacy, R. C., & Sunnucks, P. (2017). Genetic management of fragmented animal and plant populations. Oxford: Oxford University Press.

Fu, H., Wang, W., Ma, W., & Wang, M. (2018). Differential in surface elevation change across mangrove forests in the intertidal zone. *Estuarine, Coastal and Shelf Science*, 207:203-208.

Galvão, M. S. N., Pereira, O. M., & Hilsdorf, A. W. S. (2013). Molecular identification and distribution of mangrove oysters (*Crassostrea*) in an estuarine ecosystem in Southeast Brazil: implications for aquaculture and fisheries management. *Aquaculture Research*, 44(10):1589-1601.

Groeneveld, L. F., Lenstra, J. A., Eding, H., Toro, M. A., Scherf, B., Pilling, D., Negrini, R., Finlay, E. K., Jianlin, H, Groeneveld, E, Weigend, F, & The GLOBALDIV Consortium. (2010). Genetic diversity in farm animals–a review. *Animal*

Genetics, 41:6-31.

Habib, K. A., Neogi, A. K., Rahman, M., Oh, J., Lee, Y. H., & Kim, C. G. (2021). DNA barcoding of brackish and marine water fishes and shellfishes of Sundarbans, the world's largest mangrove ecosystem. *Plos one*, 16(8):e0255110.

Hariyono, D. N. H., Maharani, D., Cho, S., Manjula, P., Seo, D., Choi, N., Sidadolog, J. H. P., & Lee, J. H. (2019). Genetic diversity and phylogenetic relationship analyzed by microsatellite markers in eight Indonesian local duck populations. *Asian-Australasian Journal of Animal Sciences*, 32(1):31-37.

Hayati, R., Basyuni, M., & Chalil, D. (2020). Genetic diversity, sequence and bioinformatic analysis of Ganoderma boninense isolates. *International Journal of Agriculture & Biology*, 23(4):763-770.

Hogarth, P. J. (2015). The biology of mangroves and seagrasses. Oxford: Oxford University Press.

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. *Nature Reviews Genetics*, 10(9):639-650.

Ilman, M., Dargusch, P., Dart, P., & Onrizal. (2016). A historical analysis of the drivers of loss and degradation of Indonesia's mangroves. *Land Use Policy*, 54:448-459.

Istiqomah, M. A., Basyuni, M., & Hasibuan, P. A. Z. (2020). Development of DNA extraction on mangrove leaves. *International Journal of Scientific & Technology Research*, 9(4):139-141.

Joseph, A. V., Sasidharan, R. S., Nair, H. P., & Bhat, S. G. (2013). Occurrence of potential pathogenic Aeromonas species in tropical seafood, aquafarms and mangroves off Cochin coast in South India. *Veterinary World*, 6(6):300-306.

Karabağ, K., Balcıoğlu, M. S., Karlı, T., & Alkan, S. (2016). Determination of genetic diversity using 15 simple sequence repeats markers in long term selected Japanese quail lines. *Asian-Australasian Journal of Animal Sciences*, 29(12):1696-1701.

Kwak, Y. H., Kim, K. R., Kim, M. S., & Bang, I. C. (2020). Genetic diversity and population structure of the endangered fish *Pseudobagrus brevicorpus* (Bagridae) using a newly developed 12-microsatellite marker. *Genes & Genomics*, 42(11):1291-1298.

Maharani, D., Hariyono, D. N. H., Cho, S., Manjula, P., Seo, D., Choi, N, Sidadolog, J. H. P, & Lee, J. H. (2017). Genetic diversity among Indonesian local duck populations in Java Island assessed by microsatellite markers. *Journal of Animal Breeding and Genomics*, 1(2):136-142.

Parreira, B., Quéméré, E., Vanpé, C., Carvalho, I., & Chikhi, L. (2020). Genetic consequences of social structure in the golden-crowned sifaka. *Heredity*, 125(5):328-339.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1):288-295.

Richards, D. R., & Friess, D. A. (2016). Rates and drivers of mangrove deforestation in Southeast Asia, 2000–2012. *Proceedings of the National Academy of Sciences*, 113(2):344-349.

Rizal, A., Sahidin, A., & Herawati, H. (2018). Economic value estimation of mangrove ecosystems in Indonesia. *Biodiversity International Journal*, 2(1):98-100.

Shokoohmand, M., Zolgharnein, H., Mashjoor, S., Laloi, F., Foroughmand, A. M., & Savari, A. (2018). Analysis of genetic diversity of white shrimp (*Metapenaeus affinis*) from the Northwest of the Persian Gulf using microsatellite markers. *Turkish Journal of Fisheries and Aquatic Sciences*, 18(3):385-394.

Sitorus, H., Lesmana, I., & Tarigan, R. (2017). Relationship of mangrove density with fish diversity in the waters of mangrove area in Lubuk Kertang Village, Langkat District of North Sumatera. *International Journal of Fisheries and Aquatic Studies*, 5(5):266-271.

Srikanth, S., Lum, S. K. Y., & Chen, Z. (2016). Mangrove root: adaptations and ecological importance. *Trees*, 30(2):451-465.

Szczecińska, M., Sramko, G., Wołosz, K., & Sawicki, J. (2016). Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) Mill in East Central Europe. *PloS one*, 11(3):e0151730.

Wang, J. (2018). Effects of sampling close relatives on some elementary population genetics analyses. *Mol Ecol Resour*, 18(1):41-54.

Wilwet, L., Jeyasekaran, G., Shakila, R. J., Sivaraman, B., & Padmavathy, P. (2018). A single enzyme

PCR-RFLP protocol targeting 16S rRNA/tRNA^{val} region to authenticate four commercially important shrimp species in India. *Food chemistry*, 239:369-376.

Winata, A., Yuliana, E., & Rusdiyanto, E. (2017). Diversity and natural regeneration of mangrove vegetation in the tracking area on Kemujan Island Karimunjawa National Park, Indonesia. *Advances in Environmental Sciences*, 9(2):109-119.

Woodroffe, C. D., Rogers, K., McKee, K. L., Lovelock, C. E., Mendelssohn, I. A., & Saintilan, N. (2016). Mangrove sedimentation and response to relative sea-level rise. *Annual Review of Marine Science*, 8:243-266.

Zhu, K., Yu, W., Huang, J., Zhou, F., Guo, H., Zhang, N., Jiang, S., & Zhang, D. (2017). Parentage determination in black tiger shrimp (Penaeus monodon) based on microsatellite DNA markers. *Aquaculture international*, 25(2):827-836.