Isolation and genomic amplification of fish and shrimps from mangrove ecosystems in North Sumatra

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Abstract. Mangrove is a very typical ecosystem with a high level of diversity of flora and fauna. However, mangrove ecosystems are also one of the most vulnerable locations to extinction due to the high disturbances that occur. Some of the species that are susceptible to such disturbances are fish and shrimp. The use of barcode DNA is considered a fairly effective way to evaluate the condition of fish and shrimp populations in North Sumatra. Therefore, this study aims to obtain information on the success of kit methods and amplification patterns of barcoding marks 16S for fish and shrimp in mangrove ecosystems North Sumatra. The results showed that the kit extraction method produced a pattern of band that varied thick, thin and even smear. PCR amplification results with the 16S gene showed a good band pattern and indicated that the 16S primer used can detect fish and shrimp species that are visualized in the form of DNA bands.

1. Introduction

Indonesia has a high biodiversity that is dubbed the Mega biodiversity country. One of Indonesia's biodiversity is mangrove ecosystem. Estimated mangrove forest area around the world reaches > 14 million hectares. Of the 14 million hectares, 3.1 million hectares or 22.6% of mangrove forests are in Indonesia [1] However, mangrove damage continues to occur in Indonesia. During the period 1980-2005, Indonesia has lost more than 30% of mangrove forests. The loss of mangrove forests in Indonesia is caused by things such as land transfer into settlements, tourism, pond development and excessive logging [2,3].

The ecological function of mangroves is as a habitat for shelter and foraging for animals such as primates, reptiles and aves. Mangrove ecosystem is also a habitat of fish and shrimp as a foster area, a place the natural habitat (spawning, nursery and feeding grounds) for various species of fish, shrimps and crabs [4,5]. The diversity of fish and shrimp in mangrove ecosystems is quite high. Therefore, identification of one of them is required through molecular approach.
Based on these considerations, this study aimed to fill the gap in information related to the identity of fish and shrimp species in the mangrove ecosystem of North Sumatra through the use of barcoding markers of the 16S gene. With the knowledge of this information is expected to accelerate the introduction of a species either named or not named and monitor changes in biodiversity of fish and shrimp in the world.

2. Material and Methods

2.1. Fish and Shrimp Material
The genetic material used was DNA from fish and shrimp obtained from Percut, Belawan, Lubuk Kertang, Pulau Sembilan and Langsa, North Sumatra Province. The primer used was 16S primer because 16S rRNA genes are identical in each organism, can change according to their evolutionary distance, have conservative parts and hyper variable parts to build phylogenetic trees [6,7]

2.2. Method

2.2.1. DNA extraction. DNA isolation using the kit Reliaprep gDNA Tissue Miniprep System. Previously samples of fish and shrimp have been preserved in a 50 ml tube using ethanol. First the sample is crushed and doused with liquid nitrogen until flour-shaped and then inserted into a 1.5ml tube. Tail Lysis Buffer (TLA) 100 \( \mu l \) and Proteinase K Solution 20 \( \mu l \) into the sample and then divortex for 10 seconds. After mixing, the sample was given an additional Cell Lysis Buffer (CLD) of 200 \( \mu l \) and then divortex for 10 seconds. The next sample is incubated for 30 minutes in the oven and divortex for 10 seconds every 10 minutes. After the deep oven process is complete, the sample is given an additional RNAse A Solution 20 \( \mu l \) and centrifuged at a speed of 10,000 rpm for 10 minutes. After the centrifugation process, the sample is separated part of the liquid with the settle garbage. Next, a 250 \( \mu l \) Binding Buffer (BBA) was added, and centrifuged again at 10,000 rpm for 5 minutes. The sample is then transferred the top of the liquid to the filter tube and centrifuged at a speed of 10,000 rpm for 2 minutes. After the process, the sample was given an additional Column Wash Solution (CWD) 500 \( \mu l \) then centrifuged at a speed of 10,000 rpm for 2 minutes. The liquid in the tube is then disposed of and added CWD 500 \( \mu l \) back. This activity is carried out as many as 3 repetitions. Cwd fluid in the tube is discharged to leave the filter tube only. Then put tube 1.5 ml under filter tube, then added Nuclease Free Water 100 \( \mu l \), then centrifuged at a speed of 12,000 rpm for 1 minute. The insulation results are stored in the freezer for 1 night as previously reported [8].

2.2.2. PCR amplification. Genomic DNA extraction is amplified using a PCR machine. DNA amplification is performed using a pair of 16S primers as previously reported [11, 12]. Each sample is amplified with solution components consisting of: DNA dilute 2 \( \mu l \), Primer forward 1 \( \mu l \) and reserve 1 \( \mu l \), ddH2O 3.5 \( \mu l \) and Green Go Taq 2.5 \( \mu l \), so that the total mixture in one tube is 10 \( \mu l \). The amplification program consisted of pre denaturation at 94° for 2 minutes, denaturation at 94°C for 30 seconds, annealing at 56° for 1 minute, extension at 72°C for 3 minutes, final extension at 72°C for 7 minutes and storage at 4°C for 30 minutes.
2.2.3. **Visualization of PCR amplification.** The results of PCR amplification were analyzed by electrophoresis in a 1% agarose gel stained with a Red and visualized by Ultraviolet translation (UVP) [9].

2.2.4. **Data analysis.** Data analysis by software UV-1D (UV-Tex ver v16.09b). Bands are analyzed to obtain base pair numbers so that specific tape patterns are obtained based on a specific primary.

3. **Results and Discussion**

Figure 1 show PCR amplification success is shown by the presence of DNA tape formed from 30 samples used. However, the resulting ribbon pattern has a different DNA tape thickness. The resulting ribbon is a thick, thin and smear tape. Differences in DNA concentration isolation results are determined by physical treatment of the extraction process and the ability of the extraction buffer in breaking down cells [9,10].

PCR can detect fish and shrimp and primer used to produce various size of PCR products. The 16S primer has been reported has successfully amplified 99 individuals from 100 samples of coral fish members of the family Pseudochromidae (dottyback) [11], cork fish [12].

![](image)

**Figure 1.** The results amplification PCR of fish and shrimp documented with UV-1D software

PCR amplification using a 16S primer produces tapes of varying sizes, ranging from 200 bp to 800 bp (Table 1). The presence of DNA tapes produced in the visualization indicates that the sample has a sequence that is complementary to the primary [13].

**Table 1.** DNA amplification of fish and shrimp.

| Line | Material Genetic | Amplification DNA (Base pair) |
|------|------------------|-------------------------------|
| 1    | LK 25            | 636                           |
2  LK 27    636
3  LGS 2    543
4  PCT 20   532
5  LGS 3    600
6  LGS 8    624
7  LGS 1    624
8  PCT 1    632
9  PCT 4    200
10 LGS 6    489
11 PS 17    565
12 BLW 17   690
13 LGS 7    667
14 BLW 4    466
15 BLW 8    491
16 BLW 10   859
17 PCT 25   241
18 LK 6     500
19 BLW 18   474
20 BLW 12   474
21 BLW 6    219
22 PS 28    522
23 LK 2     274
24 PCT 28   741
25 LK 1     752
26 PCT 13   446
27 BLW 15   800
28 LK 28    380
29 LK 3     723
30 PS 22    578

4. Conclusion
The 16S primers could be used as a DNA barcode in identifying fish and shrimp in the Mangrove ecosystem of North Sumatra and further investigation is required to sequence these samples.

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References
[1] Giri C, Ochieng E, Tieszen L L, Zhu Z, Singh A, Loveland T, Masek J and Duke N 2010 *Global Ecology and Biogeography* 20 154
[2] Food and Agriculture Organization of The United Nations 2007 *The World’s Mangroves 1980-2005* (FAO: Rome)
[3] Basyuni M and Sulistiyono N 2018 *IOP Conference Series: Materials Science and Engineering* 309 012018
[4] Noor Y R, Khazali M and Suryadiputra I N N 1999 *Panduan Pengenalan Mangrove di Indonesia* (PHKA/WI-IP: Bogor)
[5] Basyuni M, Gultom K, Fitri A, Susetya I E, Wati R, Slamet B, Balke T and Bunting P 2018 *Biodiversitas* 19 311
[6] Bybee S M, Bracken-Grissom H D, Hermansen R A, Clement M J, Crandall K A and Felder D L 2011 *Journal of Comparative Zoology* 250 497
[7] Ficetola G F, Boyer F, Valentini A, Bonin A, Meyer A, Dejean T and Taberlet P 2021 *Molecular Ecology* 30 3189
[8] Basyuni M, Baba S and Oku H 2017 *IOP Conference Series: Materials Science and Engineering* **180** 012243

[9] Hayati R, Basyuni M, Afandi D, Arifianto D, Bimantara Y and Syahputra I 2019 *IOP Conference Series: Earth and Environmental Science* **305** 012039

[10] Mulyani Y, Purwanto A and Nurruhwati I 2011 *Jurnal Akuatika* **2** 1

[11] Pertiwi N P D, Mahardika I G N K and Watiniasih N L 2015 *Jurnal Biologi* **19** 1

[12] Kombong C B S and Arisuryanti T *Jurnal Perikanan Universitas Gadjah Mada* **20** 57

[13] Sulistyawati P and Widyatmoko A Y P B C 2017 *Jurnal Pemuliaan Tanaman Hutan* **11** 67