RESEARCH FINAL REPORT
DIPA BIOTROP 2021

UTILIZATION of eDNA METABARCODING AS A VARIABLE TO EVALUATE MANGROVE ECOSYSTEM CONDITION IN NORTH SUMATRA AND ACEH

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Bogor, 29 November 2021

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1. INTRODUCTION

1.1. Background

Indonesia has the largest mangrove forest in the world with 22.6% of the total global area (Giri et al. 2011). Mangrove forests are disjunct distribution in almost all big islands of Indonesia ranging from Sumatra, Java, Kalimantan, Sulawesi to Papua. Mangrove forests in North Sumatra are concentrated in east coast region of North Sumatra, generally found in Langkat, Deli Serdang, Batu Bara, Tanjung Balai, Asahan, Labuhanbatu to mangrove forest areas in Serdang Bedagai (Basyuni & Sulistyono, 2018; Basyuni et al. 2015).

Mangrove forests are known to provide great benefits to the community as ecosystem services (Fitri et al. 2018; Vo et al. 2015) and globally the mangrove forests are threatened to disappear due to anthropogenic and non-anthropogenic activities (Basyuni & Sulistyono, 2018; Richard et al. 2016; Kraus et al. 2014). Our previous results indicate the important role of mangroves in the context of climate change for reducing greenhouse gas emissions and diversity of terpenoids for defense of salinity (Basyuni et al. 2015, Basyuni et al. 2012; Basyuni et al. 2017).

Related to the importance of mangrove existence, each mangrove species has a different adaptation to various biotic and abiotic stresses. Grouping and species composition can correlate with health and function of mangrove ecosystems that can be detected biochemically and molecularly (Basyuni et al. 2017; Basyuni et al.2018; Ng et.al 2013). Recent developments using environmental DNA metabarcoding technology to evaluate the ecosystem biodiversity have shown satisfactory results and are suitable for species composition studies (Miya et al. 2015; Yamamoto et al. 2016; Suria et al. 2018). Environmental DNA metabarcoding is also able to show differences in species composition that occurs.

As it is known that, the existence of species in an environment can be known by direct observation (direct sign) and indirect observation (indirect sign) (Keeping and Pelletier 2014). For animals species, indirect observation (indirect sign) can be observed through the traces left by the animal, one of which is genetic material (DNA), which is known as Environmental DNA (eDNA). eDNA detection is a technique used to monitor animals in water (Ficetola et al. 2008; Jerde et al. 2011). This technique is based on the fact that all animals that live in water leave DNA through their feces, urine, and skin expulsion (Herder et al. 2014). The use of eDNA has been applied to detect the presence of amphibians, reptiles, fish, crustaceans, water birds, and mammals (Goldberg et al.

2011; Jerde et al. 2011; Olson et al. 2012; Biggs et al. 2015, Minamoto et al. 2015 ; Wilcox et al. 2015). Takahara et al. (2012) also stated that eDNA detection can also be used to calculate biomass and fish abundance (Takahara et al. 2012). Compared to traditional sampling techniques, this technique is considered effective and efficient.

Developing variables to evaluate the function of mangrove ecosystems with environmental DNA metabarcoding is a very important. Meta-barcoding can provide information for evaluating mangrove ecosystems using easy and fast methods, among those developed by Miya et al. 2015 namely MiFish. The MiFish and related methods are non-invasive methods with excellent, simple, fast, and economical capabilities. Using this technique, we can conduct biodiversity analysis based on environmental DNA data (Miya et al. 2015; Suriya et al. 2018; Ushio et al. 2018).

This research focused on screening and microcosm experiment (community and zoning) mangrove in North Sumatra (Langkat) and Aceh (langsa) then ifollowing by in silico testing of sequencing results to determine interspecific variations. Bycomparing biodiversity in different mangrove forest condition (damaged by pond conversion, palm conversion, rehabilitation results, oil palm conversion plants, and natural), we will understand which groups of organisms (including mangrove biota) respond more quickly to environment changes of the mangrove forest, and we can compare them in both directions (decreasing and increasing). However, information on metabarcoding for mangroves in North Sumatra has yet to be obtained. Therefore this study was conducted to fill gaps information regarding the composition of mangrove species in relation to the ecological dynamics that occur. As is known, that the diversity of mangrove forests in North Sumatra and Aceh can be a source of ecological dynamics and new knowledge and also potential sources of mangrove forests, which can open other possible benefits of mangrove forests.

1.2. The objective of research

The objective of this research were :

1. Screening of community and mangrove zoning in North Sumatra (Langkat) and Langsa (Aceh) using the matK, rbcl, ITS primers.
2. Interspecific variations analysis of mangrove species in North Sumatra and Aceh.
3. Formulating genetic and ecological-based conservation strategies for mangroves in North Sumatra

1.3. Expected Output

The expected output in this study were data and information related to mangrove communities in North Sumatra and Aceh and also interspecific variations. The information would be arrange into books, reputable journals and proceedings. In the future, this data can be used for stakeholders in managing mangroves in Indonesia.

2. Methodology

Research flowchart

Mangrove metabarcoding research in North Sumatra and Aceh consist of some activities those were DNA screening of mangrove environments in North Sumatra, e- DNA primary development, genetic markers selection, marker testing, microsomal experiments and in silico evaluation of interspecific variations. The final goal of research was to identify the biodiversity of mangrove environment in North Sumatra, so that species conservation activities and appropriate management for these conditions can be determined. The flow diagram of mangrove e-metabarcoding research in North Sumatra can be presented in Figure 3.1.

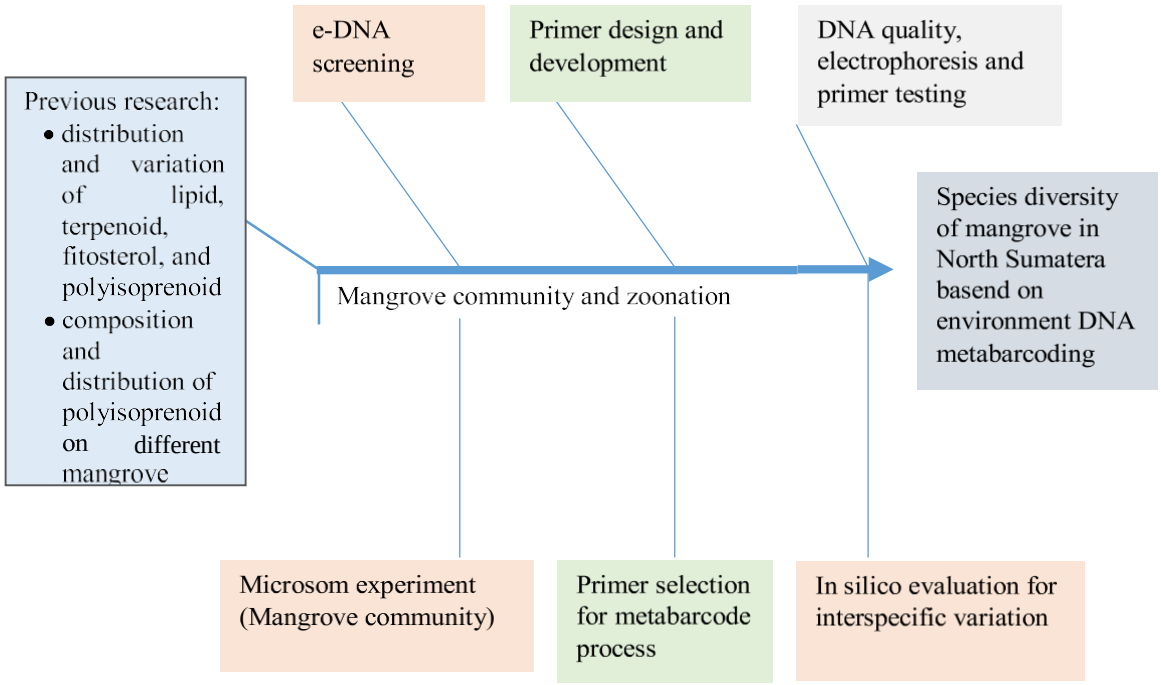


Figure 1. Metabarcoding Research flowchart

Research Methods

In 2021, the research focused on microcosm screening and experimentation (community and mangrove zoning) in North Sumatra (Langkat) and Aceh (Langsa) using the MiFish universal primer, then in silico testing of sequencing results to determine interspecic variations (Figure 2). In detail, the research implementation can be described as follows:

Samples collection

In this study, the main distribution areas of mangroves in North Sumatra and Aceh provinces were selected, namely the mangrove areas in Percut Sei Tuan, Deli Serdang; Pulau Sembilan and Lubuk Kertang of Langkat and Langsa in Aceh as. Detail sampling location information is depicted in Table 1. According to the DNA barcode sample collection specifications, two to three individuals of each mangrove species were sampled. This involved taking fresh leaves and buds to facilitate the extraction of DNA molecular materials. A total of 252 individuals of mangrove plants were collected. The collected sites was depicted in Figure 1.

DNA isolation

Water samples were centrifuged at 7000 rpm for 10 minutes. The precipitate pellet then rinsed with 70% alcohol and then precipitated again. The remaining alcohol in the precipitate process is evaporated using a vacuum desiccator. The obtained pellet was added with an elution buffer (Thermo Fisher Scientific Inc., US) to each sample. The sample extraction in the form of tissue of the targe species was carried out using Tissue / Blood DNA Mini Kit (Geneaid, Taiwan).

Polymerase Chain Reaction (PCR)

The first amplification process was using universal primers developed by Miya et al (2015), and several primer modifications for metabarcoding related to regions in the mitochondrial 12S rSNA gene (median insert length = ~ 171 bp), such as MiMammal- U (Table 1). The primary sequence with the MiSeq adapter (for PCR) is listed in Table 2.

The PCR product was electrophoresed using 1.2% agarose gel and visualized using a UV transluminator. The length target of the first amplification was 710 bp and the second amplification is 394 base pair.

Table 1. The information of mangrove samples collected in the North Sumatra and Aceh provinces

No	Species	Family	Status	Life form	Sites	Red List	Herbarium Voucer No.
1	Acrostichum aureum	Pteridaceae	TM	shrub	Percut	LC	2106020001
2	Rhizophora apiculata	Rhizophoraceae	TM	tree	Percut	LC	2106020004
3	Nypa fruticans	Arecaceae	TM	palm	Percut	LC	2106020007
4	Avicennia alba	Acanthaceae	TM	tree	Percut	LC	2106020010
5	Acanthus ilicifolius	Acanthaceae	MA	shrub	Percut	LC	2106020016
6	Avicennia officinalis	Acanthaceae	TM	tree	Percut	LC	2106020025
7	Avicennia marina	Acanthaceae	TM	tree/shrub	Percut	LC	2106020031
8	Rhizophora stylosa	Rhizophoraceae	TM	tree	Percut	LC	2106020042
9	Excoecaria agallocha	Euphorbiaceae	TM	tree/shrub	Percut	LC	2106020043
10	Xylocarpus granatum	Meliaceae	TM	tree	Pulau Sembilan	LC	2106020046
11	Bruguiera parviflora	Rhizophoraceae	TM	tree	Pulau Sembilan	LC	2106020050
12	Nypa fruticans	Arecaceae	TM	palm	Pulau Sembilan	LC	2106020055
13	Acacia auriculiformis	Fabaceae	MA	tree	Pulau Sembilan	LC	2106020058
14	Excoecaria agallocha	Euphorbiaceae	TM	tree/shrub	Pulau Sembilan	LC	2106020067
15	Scyphiphora hydrophyllacea	Rubiaceae	TM	tree/shrub	Pulau Sembilan	LC	2106020070
16	Avicennia marina	Acanthaceae	TM	tree/shrub	Pulau Sembilan	LC	2106020082
17	Avicennia officinalis	Acanthaceae	TM	tree	Pulau Sembilan	LC	2106020085
18	Acrostichum aureum	Pteridaceae	TM	shrub	Pulau Sembilan	LC	2106020088
19	Acanthus ilicifolius	Acanthaceae	MA	shrub	Pulau Sembilan	LC	2106020097
20	Avicennia alba	Acanthaceae	TM	tree	Pulau Sembilan	LC	2106020103
21	Scyphiphora hydrophyllacea	Rubiaceae	TM	tree/shrub	Lubuk Kertang	LC	2106020112
22	Avicennia marina	Acanthaceae	TM	tree/shrub	Lubuk Kertang	LC	2106020115
23	Avicennia alba	Acanthaceae	TM	tree	Lubuk Kertang	LC	2106020122
24	Excoecaria agallocha	Euphorbiaceae	TM	tree	Lubuk Kertang	LC	2106020124
25	Sonneratia alba	Lythraceae	TM	tree	Lubuk Kertang	LC	2106020128

26	Avicennia officinalis	Acanthaceae	TM	tree	Lubuk Kertang	LC	2106020133
27	Acanthus ilicifolius	Acanthaceae	MA	shrub	Lubuk Kertang	LC	2106020139
28	Nypa fruticans	Arecaceae	TM	palm	Lubuk Kertang	LC	2106020142
29	Rhizophora stylosa	Rhizophoraceae	TM	tree	Lubuk Kertang	LC	2106020151
30	Acanthus ilicifolius	Acanthaceae	MA	shrub	Langsa	LC	2106020156
31	Avicennia officinalis	Acanthaceae	TM	tree	Langsa	LC	2106020165

TM = true mangrove, AM = associate mangrove, LC = Least concern

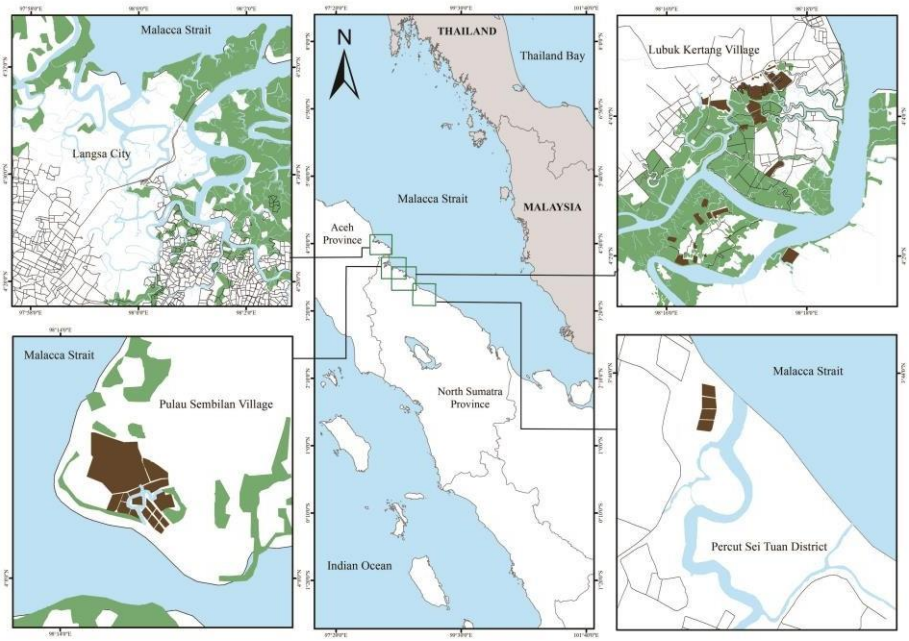


Figure 2. Study sites at Percut Sei Tuan, Pulau Sembilan, Lubuk Kertang of North Sumatra, and Langsa of Aceh

Table 2. The primers used to amplify DNA barcodes and the amplification protocol.

DNA fragment	Primers	Sequences (**5'–3'**)	Amplification procedure	References
rbcl	rbcl F	ATGTCACCACAAACAGAGACTAAAGC	72 °C 1 min, 35 cycles; 72 °C 7 min, 72 ◦C 1 min, 35 cycles; 72 ◦C 7 min	Kress (2009)
	rbcl R	GTAAAATCAAGTCCACCRCG		
matK	Matk F	ACCCAGTCCATCTGGAAATCTTGGTTC	94 °C 3 min; 94 °C 45 s, 51 °C 45 s, 72 °C 1 min, 35 cycles; 72 °C 7 min	Wu et al. (2019)
	Matk R	ACCCAGTCCATCTGGAAATCTTGGTTC		
psbA-trnH	PsbA F	GTTATGCATGAACGTAATGCTC	94 ◦C 3 min; 94 ◦C 30 s, 55 ◦C 1 min, 72 ◦C 1 min,35 cycles; 72 ◦C 7 min	Sang et al. (1997)
	trnH R	CGCGCATGGTGGATTCACAATCC		Tate and Simpson (2003)
ITS	ITS 1	GTCCACTGAACCTTATCATTTAG	94 ◦C 3 min; 94 ◦C 30 s, 55 ◦C 1 min, 72 ◦C 1 min, 35 cycles; 72 ◦C 7 min	White et al. 1990
	ITS 4	TCCTCCGCTTATTGATATGC		

ITS: The internal transcribed spacer of nuclear ribosomal DNA.

3. RESULT AND DISCUSSION

DNA Extraction on Mangrove Leaves

Mangroves are a collection of several species of trees or shrubs that grow around the coastline and can live in high salinity environments. Mangrove forests provide an important role, as a source of livelihood, because they can produce various products of high economic value including fuel (firewood and charcoal), building materials (beams, roofs, etc.), fisheries, food raw materials, medicines, and agro-tourism (Basyuni et al. 2021; Fitri et al., 2018).

Mangrove loss in North Sumatran found in primary mangrove forests significantly decreased 61.21% from 1990 to 2015, main deforestation was from 1990 to 2000 to be secondary mangrove forest and swamp shrub (Basyuni & Sulistiyono, 2018). The main factor in the destruction of mangroves during this period was conversion to ponds. In the next two decades, it is estimated that the expansion of ponds and the expansion of oil palm plantations will still be the main factors for mangrove loss in Indonesia, especially in North Sumatra.

Identification of mangrove species is critical in conserving and utilizing biodiversity, which seems to be hindered by taxonomic expertise. The method of identification of living species has evolved from morphological identification to molecular identification [6]. This study aims to report a DNA extraction and PCR amplification prior was used for the identification of mangroves in North Sumatra.

Table 1 shows the length of DNA fragments viewed with UV-ID after irradiation with UV light. The length of DNA fragments for *Acrosticum aureum* mangroves ranged from 619,58-632,005, *Rhizophora apiculata* 619,588-585,786 bp, *Nypa fruticans* 600- 592,904 bp, *Avicennia alba* species 549,135-533,58 bp, Hibiscus tiliaceus species were not detected, and mangroves *Acanthus ilicifoilus* 480, 316 bp.

Table 3. DNA amplification of mangrove species in Percut Sei Tuan

Line	Mangrove species	Amplicon (bp)
1	*Acrosticum aureum*	632.0
2	*A. aureum*	625.8
3	*A. aureum*	619.6
4	*Rhizopora apiculata*	585.8
5	*R. apiculata*	613.1
6	*R. apiculata*	619.6
7	*Nypa fruticans*	600.0
8	*N. fruticans*	592.9
9	*N. fruticans*	578.6
10	*Avicennia alba*	533.5
11	*A. alba*	549.1
12	*A. alba*	549.1
13	*Hibiscus tiliaceus*	Na
14	*H. tiliaceus*	Na
15	*H. tiliaceus*	Na
16	*Acanthus ilicifolius*	480.2

Na= not amplification

PCR and PCR primers used can help detect mangrove species that are visualized in the form of DNA bands. The rbcL marker is an effective marker for identifying mangroves. According to the research of Saddhe etal., 2016, which tested the markers rbcL and matK to identify mangrove species and they recommended matK to be a suitable candidate marker for the identification of mangrove species. However, the results of other studies showed that rbcL and trnH-psbA have a high success rate for amplification and sequencing, which indicates that these two markers are suitable for species identification in mangrove plants (Wu et. al. 2019).

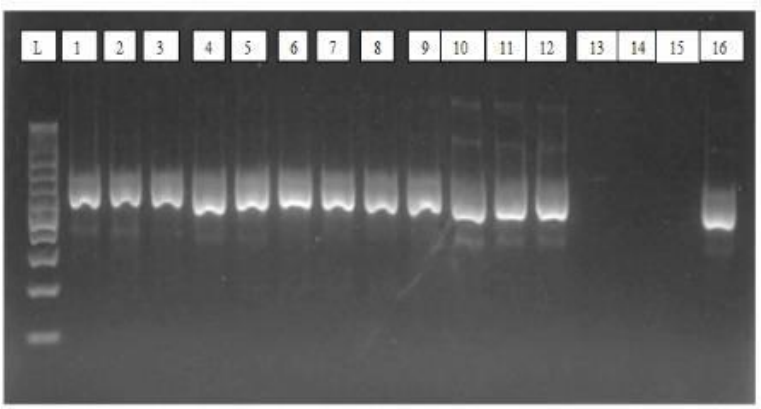


Figure 3. PCR Results of Mangrove Samples (L = Ladder, lines 1-16 = PCR results) documented withUV-1D software (UV-Tex ver v16.09b).

Figure 1 showed the amplification of mangrove plant samples, from 16 samples, only 3 mangrove samples were not detected. Each sample has a different length of the fragment depending on mangrove species. These samples were further investigated to clarify the correct identification using sequence.
The rbcL and trnH-psbA primers could be used as a DNA barcode in identifying mangrove species in North Sumatra Province.

3.2. DNA Extraction on Crab and Macrobenthos

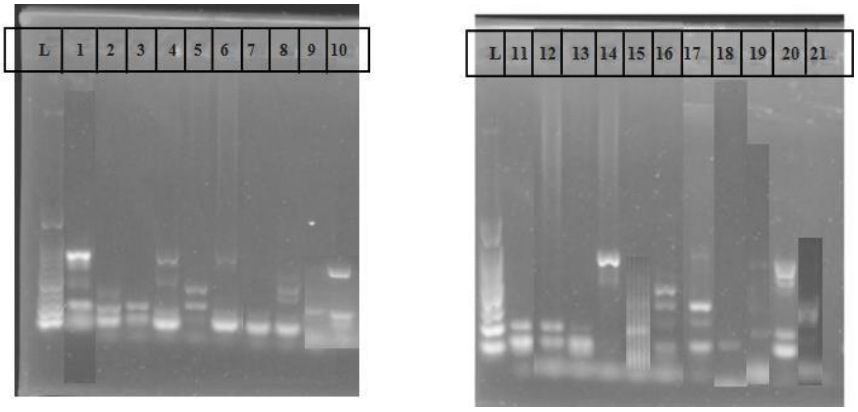
Table 2 shows the length of DNA fragments viewed with UV-ID after irradiation with UV light.

The length of DNA fragments for crab and macrobentos from forest mangroves ranged from 206-678.

Table 4. DNA amplification of Crab and Makrobentos from Mangrove Forest of North Sumatera and Aceh

Line	Acces Code	Amplicon (bp)
1	LGS 1	489
2	LGS 2	277
3	BLW 6	292
4	LGS 3	247
5	PCT 5	300
6	LGS 5	289
7	LK 5	230
8	PS 5	269
9	PS 2	341
10	PS3	284
11	LK 4	234
12	LGS 9	219
13	PCT 7	206
14	PS 6	678
15	BLW 9	423
16	LGS 8	360
17	PCT 3	227
18	BLW 10	227
19	BLW 8	378
20	PS 7	489
21	PC 4	388

Description : LGS (Langsa), PCT (Percut), BLW (Belawan), PS (Pulau Sembilan), LK (Lubuk kertang).



a **b**

Figure 4. PCR Results of Crab DNA and Macrobentos from Mangrove Forest, North Sumatera

(a) L = Ladder, lines 1-10 = PCR results (b) L = Ladder, lines 11-21 = PCR results documented withUV-1D software (UV-Texver v16.09b).

Visualization of PCR amplification results with primer16S of crab samples and macrobento produces thin ribbons and bright and clear ribbons with different values from each sample. The amplification stage produces a low DNA band with a size of 206 bp and a height of 678 bp. DNA amplification from crabs and macrobentos obtains a single and multi band where a single band indicates that primary used is the primary that specifics to amplify crab DNA and Macrobentos (Sabdono et al. 2006). DNA bands produced through electrophoresis can be analyzed to see the genetic diversity of a group organism (Sulistiwati & Widyamoko, 2017). DNA crab and macrobentos were successfully amplified using primary 16s which produced a number of different DNA patterns in each sample ranging from 206 678 bp and further sequenced for further analysis.

3.3 Sequence analysis

Sequence statistics were calculated for 253 individuals of 31 species of mangrove plants (Table 3). A total of 75 squences of mangrove plants from the Percut area were obtained. The highest amplification success rate was rbcL followed by trnH-psbA, ITS, and matK. The highest success rate for squencing was rbcl with 82%, followed by trnH-psbA 72%, ITS 66%, and matK 60%. From the Pulau Sembilan area, 89 sequences of mangrove plants were obtained. The highest amplification success rates were primers rbcL, trnH-psbA, ITS, and matK. The highest success rates were rbcL 90%, trnH-psbA 86%, ITS 75% and matk 57%. In the Lubuk Kertang area, 65 squences of mangrove plants were obtained with the success rate of amplification, namely rbcL followed by ITS, trnH-psbA, and matK. The highest success rate of squencing was rbcL and ITS as much as 83%, followed by trnH-psbA 80%, and matK 60%. For the Langsa area, 24 sequences of mangrove plants were obtained. The highest amplification success rates were rbcL, trnH-psbA, ITS, and matK. The success rate

of the sequence of the four primers.

Table 5. The success rates of PCR amplification and sequencing of the four barcoding fragments in the four mangrove sites.

Sites	Primer	Amplicon success rate (%)	Sequence success rate (%)	Individual
Percut	rbcL	100	82	25
	matK	71	60	12
	trnh-psbA	89	88	22
	ITS	85	66	16
Pulau Sembilan	rbcL	100	90	30
	matK	63	57	12
	trnh-psbA	87	86	25
	ITS	87	75	22
Lubuk kertang	rbcL	100	83	20
	matK	62	60	9
	trnh-psbA	83	80	16
	ITS	100	83	20
Langsa	rbcL	100	50	6
	matK	75	50	6
	trnh-psbA	100	50	6
	ITS	100	50	6
Total				253

Table 4-7 showed the BLAST results for matkK, rbcl, trnH-psbA, and ITS primers. All the samples corresponded to the respective species in the genebank.

Table 6. BLAST result on matK primer

No.	Sampel code	Species	E-Value	Similarity (%)	Accession	Sites
1	M7	Nypa fruticans	0.0	93.79	KT312925.1	Percut
2	M8	Nypa fruticans	2E-177	95.69	JX903670.1	Percut
3	M9	Nypa fruticans	0.0	98.49	AM114552.1	Percut
4	M10	Avicennia alba	0.0	90.61	KF848318.1	Percut
5	M11	Avicennia alba	6E-111	94.72	KF848318.1	Percut
6	M12	Avicennia alba	0.0	90.92	KF848318.1	Percut
7	M17	Acanthus ilicifolius	0.0	85.02	KX231339.1	Percut
8	M18	Acanthus ilicifolius	0.0	9359	MW752129.1	Percut
9	M25	Apiciena officinalis	0.0	88.00	KX231343.1	Percut
10	M26	Apiciena officinalis	0.0	88.82	KF848319.1	Percut
11	M27	Apiciena officinalis	0.0	91.26	KX231343.1	Percut
12	M31	Avicennia marina	0.0	93.34	MF064270.1	Percut

13	M55	Nypa fruticans	0.0	90.21	DQ182343.1	P. Sembilan
14	M56	Nypa fruticans	9E-153	92.33	KT312925.1	P. Sembilan
15	M57	Nypa fruticans	0.0	93.90	JX903670.1	P. Sembilan
16	M58	Acacia auriculiformis	0.0	99.04	KX518644.1	P. Sembilan
17	M59	Acacia auriculiformis	0.0	99.18	KX518644.1	P. Sembilan
18	M60	Acacia auriculiformis	0.0	91.42	KJ510954.1	P. Sembilan
19	M70	Scyphiphora hydrophyllacea	0.0	98.78	MN390972.1	P. Sembilan
20	M71	Scyphiphora hydrophyllacea	0.0	96.54	KJ815667.1	P. Sembilan
21	M72	Scyphiphora hydrophyllacea	0.0	98.43	KF848321.1	P. Sembilan
22	M97	Acanthus ilicifolius	0.0	98.84	MW752129.1	P. Sembilan
23	M99	Acanthus ilicifolius	0.0	99.12	KX231339.1	P. Sembilan
24	M105	Avicennia alba	0.0	93.84	KF848318.1	P. Sembilan
25	M112	Scyphiphora hydropgyllacea	0.0	95.02	MN390972.1	L. Kertang
26	M113	Scyphiphora hydropgyllacea	0.0	89.78	KJ815667.1	L. Kertang
27	M114	Scyphiphora hydropgyllacea	4E-152	96.96	KY378668.1	L. Kertang
28	M121	Avicennia alba	0.0	90.99	KF848318.1	L. Kertang
29	M122	Avicennia alba	0.0	95.43	KF848318.1	L. Kertang
30	M123	Avicennia alba	0.0	92.73	KM255082.1	L. Kertang
31	M139	Acanthus ilicifolius	0.0	97.87	KX231339.1	L. Kertang
32	M140	Acanthus ilicifolius	0.0	89.97	MH332531.1	L. Kertang
33	M141	Acanthus ilicifolius	0.0	91.50	MW752129.1	L. Kertang
34	M156	Acanthus ilicifolius	4E-121	96.65	KP976102.1	Langsa
35	M157	Acanthus ilicifolius	3E-132	97.86	MW752129.1	Langsa
36	M158	Acanthus ilicifolius	0.0	94.70	KX231339.1	Langsa
37	M165	Avicennia officinalis	0.0	93.96	KX231343.1	Langsa
38	M166	Avicennia officinalis	0.0	95.60	KF848319.1	Langsa
39	M167	Avicennia officinalis	0.0	87.60	KM255084.1	Langsa

Table 7. BLAST results from rbcl primers

No	Sampel Code	Species	E-value	Similarity (%)	Accession	Sites
1	R1	Acrosticum aureum	0.0	99,64	MT657438.1	Percut
2	R2	Acrosticum aureum	0.0	97,69	AB246703.1	Percut
3	R3	Acrostichum aereum	0.0	99.81	LC604224.1	Percut
4	R4	Rhizophora apiculata	0.0	97.93	KM255076.1	Percut
5	R5	Rhizophora apiculata	0.0	98.54	KP697362.1	Percut
6	R6	Rhizophora apiculata	0.0	99.17	KP697362.1	Percut
7	R7	Nypa fruticans	0.0	93,96	MG437529.1	Percut
8	R8	Nypa fruticans	0.0	94,79	AY012471.1	Percut
9	R9	Nypa fruticans	0.0	98,35	JX903253.1	Percut
10	R10	Avicennia alba	0.0	97,27	KU748517.1	Percut
11	R11	Avicennia alba	0.0	99,32	KM255067.1	Percut
12	R12	Avicennia alba	0.0	97.71	MG970419.1	Percut
13	R16	Acanthus ilicifolius	0.0	98.59	KX231351.1	Percut
14	R17	Acanthus ilicifolius	0.0	89.59	KX231351.1	Percut

15	R18	Acanthus ilicifolius	0.0	91.18	KP697352.1	Percut
16	R25	Avicennia officinalis	0.0	94.37	KX231355.1	Percut
17	R26	Avicennia officinalis	0.0	99.03	KP697351.1	Percut
18	R27	Avicennia officinalis	0.0	97.19	KM255069.1	Percut
19	R31	Avicennia marina	0.0	98.19	NC_047414.1	Percut
20	R32	Avicennia marina	0.0	100	KM255068.1	Percut
21	R33	Avicennia marina	0.0	97.09	KX231354.1	Percut
22	R42	Rhizophora stylosa	1e-54	79.37	LC498757.1	Percut
23	R43	Excoecaria agallocha	0.0	99.10	KP697360.1	Percut
24	R44	Excoecaria agallocha	0.0	92.70	MG970488.1	Percut
25	R45	Excoecaria agallocha	0.0	91.40	KP697359.1	Percut
26	R47	Xilacorpus granatum	0.0	99.40	KM895563.1	P.Sembilan
27	R48	Xylacopus granatum	0.0	100	MH348155.1	P.Sembilan
28	R50	Bruguira parvivolia	0.0	91.22	AF127692.1	P.Sembilan
29	R55	Nypa fruticans	0.0	100	AY012471.1	P.Sembilan
30	R56	Nypa fruticans	0.0	83.76	MG437529.1	P.Sembilan
31	R57	Nypa fruticans	0.0	99.6	AJ404778.1	P.Sembilan
32	R58	Acacia auriculiformis	0.0	99.39	GU135162.1	P.Sembilan
33	R59	Acacia auriculiformis	0.0	99.24	KX385911.1	P.Sembilan
34	R60	Acacia auriculiformis	0.0	98.97	FJ716655.1	P.Sembilan
35	R67	Excoecaria agalocha	0.0	93.78	KP697360.1	P.Sembilan
36	R68	Excoecaria agalocha	0.0	98.84	MG970488.1	P.Sembilan
37	R69	Excoecaria agalocha	0.0	97.79	KY501144.1	P.Sembilan
38	R70	Scyphiphora hydropgyllacea	0.0	99.16	MN390972.1	P.Sembilan
39	R71	Scyphiphora hydropgyllacea	0.0	93.90	KY378668.1	P.Sembilan
40	R72	Scyphiphora hydropgyllacea	0.0	99.32	NC_049078.1	P.Sembilan
41	R82	Avicennia marina	0.0	100	KP697351.1	P.Sembilan
42	R83	Avicennia marina	0.0	99.41	KM255068.1	P.Sembilan
43	R84	Avicennia marina	0.0	92.78	AY008832.1	P.Sembilan
44	R85	Avicennia officinalis	0.0	99.82	KM255069.1	P.Sembilan
45	R86	Avicennia officinalis	0.0	99.81	KP697351.1	P.Sembilan
46	R87	Avicennia officinalis	7e-131	92.41	MG970432.1	P.Sembilan
47	R88	Acrosticum aereum	0.0	100	LC604224.1	P.Sembilan
48	R89	Acrosticum aereum	0.0	100	LC604224.1	P.Sembilan
49	R90	Acrosticum aereum	0.0	100	AB246703.1	P.Sembilan
50	R97	Acanthus iliifolius	0.0	99.38	KP697343.1	P.Sembilan
51	R98	Acanthus iliifolius	0.0	99.80	KM255065.1	P.Sembilan
52	R99	Acanthus iliifolius	0.0	100	KM255065.1	P.Sembilan
53	R103	Avicennia alba	0.0	99.65	KP697348.1	P.Sembilan
54	R104	Avicennia alba	0.0	99.81	KU748517.1	P.Sembilan
55	R105	Avicennia alba	5e-127	98.50	KF848222.1	P.Sembilan
56	R112	Scyphiphora hydropgyllacea	0.0	99.15	MN390972.1	L.Kertang
57	R113	Scyphiphora hydropgyllacea	0.0	100	KY378668.1	L.Kertang
58	R115	Avicennia marina	0.0	91.67	KP697350.1	L.Kertang
59	R116	Avicennia marina	0.0	98.83	NC_047414.1	L.Kertang
60	R122	Avicennia alba	0.0	98.82	KU748517.1	L.Kertang
61	R123	Avicennia alba	0.0	99.20	KP697348.1	L.Kertang
62	R128	Sonneratia alba	0.0	90.73	MH105772.1	L.Kertang
63	R129	Sonneratia alba	0.0	93.33	KU748520.1	L.Kertang
64	R133	Avicennia officinalis	0.0	99.45	KP697352.1	L.Kertang

65	R134	Avicennia officinalis	0.0	97.89	KM255069.1	L.Kertang
66	R135	Avicennia officinalis	5E-110	98.30	KX231355.1	L.Kertang
67	R139	Acanthus ilicifolius	0.0	99.43	KX231351.1	L.Kertang
68	R140	Acanthus ilicifolius	0.0	99.49	MW752129.1	L.Kertang
69	R141	Acanthus ilicifolius	2e-128	92.42	KP697342.1	L.Kertang
70	R142	Nypa fruticans	9e-160	99.68	MG970498.1	L.Kertang
71	R143	Nypa fruticans	6e-39	82.53	MG437529.1	L.Kertang
72	R144	Nypa fruticans	2e-149	97.48	MG437529.1	L.Kertang
73	R151	Rhizopora stylosa	1e-154	89.24	AY050564.1	L.Kertang
74	R152	Rhizopora stylosa	2e-167	85.47	AF127686.1	L.Kertang
75	R153	Rhizopora stylosa	0.0	92.69	AF127686.1	L.Kertang
76	R156	Acanthus ilicifolius	0.0	99.66	NC_054308.1	Langsa
77	R157	Acanthus ilicifolius	0.0	99.82	KX231351.1	Langsa
78	R158	Acanthus ilicifolius	0.0	99.45	KP697342.1	Langsa
79	R165	Avicennia officinalis	0.0	99.15	KM255069.1	Langsa
80	R166	Avicennia officinalis	0.0	99.66	KP697352.1	Langsa
81	R167	Avicennia officinalis	0.0	99.48	KX231355.1	Langsa

Table 8. BLAST results from trnH-psbA primers

No	Sampel Code	Species	E-value	Similarity (%)	Accession	Sites
1	P1	Acrostichum aureum	0.0	98.97	KY099881.1	Percut
2	P2	Acrostichum aureum	0.0	98.97	LC604852.1	Percut
3	P3	Acrostichum aureum	0.0	97.94	LC604870.1	Percut
4	P4	Rhizophora apiculata	0.0	87.65	NC_057465.1	Percut
5	P5	Rhizophora apiculata	0.0	89.26	NC_057465.1	Percut
6	P6	Rhizophora apiculata	8E-59	74.18	NC_057465.1	Percut
7	P7	Nypa fruticans	0.0	99.05	KT312925.1	Percut
8	P8	Nypa fruticans	0.0	98.83	KT312925.1	Percut
9	P10	Avicennia alba	0.0	99.31	EU352162.1	Percut
10	P11	Avicennia alba	0.0	98.84	EU352162.1	Percut
11	P16	Acanthus ilicifolius	8E-137	88.86	MW752129.1	Percut
12	P17	Acanthus ilicifolius	7E-94	94.78	MW752129.1	Percut
13	P18	Acanthus ilicifolius	6E-15	95.3	MW752129.1	Percut
14	P25	Avicennia officinalis	3E-72	98.12	KP697352.1	Percut
15	P26	Avicennia officinalis	1E-45	87.57	KT453644.1	Percut
16	P27	Avicennia officinalis	1E-21	79.89	KT453644.1	Percut
17	P31	Avicennia marina	2E-108	95.31	NC_047414.1	Percut
18	P32	Avicennia marina	0.0	92.87	NC_047414.1	Percut
19	P33	Avicennia marina	0.0	96.59	MN307164.1	Percut
20	P43	Excoecaria agallocha	0.0	95.60	LC604863.1	Percut
21	P44	Excoecaria agallocha	0.0	99.84	LC604863.1	Percut
22	P45	Excoecaria agallocha	5E-75	91.90	MK084735.1	Percut
23	P46	Xylocarpus granatum	0.0	94.43	NC_039925.1	P.Sembilan
24	P47	Xylocarpus granatum	0.0	97.90	NC_039925.1	P.Sembilan
25	P48	Xylocarpus granatum	0.0	94.95	NC_039925.1	P.Sembilan

26	P55	Nypa fruticans	0.0	99.86	KT312925.1	P.Sembilan
27	P56	Nypa fruticans	0.0	90.12	KT312925.1	P.Sembilan
28	P57	Nypa fruticans	2E-105	100	KT312925.1	P.Sembilan
29	P58	Acacia auriculiformis	2E-83	83.06	MH621761.1	P.Sembilan
30	P59	Acacia auriculiformis	0.0	95.59	MH621761.1	P.Sembilan
31	P60	Acacia auriculiformis	4E-162	90.47	KM191776.1	P.Sembilan
32	P67	Excoecaria agallocha	0.0	96.35	LC604863.1	P.Sembilan
33	P68	Excoecaria agallocha	0.0	99.73	LC604863.1	P.Sembilan
34	P70	Scyphiphora hydropgyllacea	1E-170	99,12	MN390972.1	P.Sembilan
35	P71	Scyphiphora hydropgyllacea	3E-107	98.25	MK607942.1	P.Sembilan
36	P72	Scyphiphora hydropgyllacea	2E-168	98,82	KY378668.1	P.Sembilan
37	P82	Avicennia marina	0.0	100	MN307164.1	P.Sembilan
38	P83	Avicennia marina	0.0	97.79	KT161360.1	P.Sembilan
39	P88	Acrosticum aureum	0.0	97.87	KY099881.1	P.Sembilan
40	P89	Acrosticum aureum	0.0	96.48	LC604852.1	P.Sembilan
41	P90	Acrosticum aureum	0.0	98.41	LC604870.1	P.Sembilan
42	P97	Acanthus ilicifolius	7E-102	99.07	MW752129.1	P.Sembilan
43	P98	Acanthus ilicifolius	7E-101	98.17	NC_054308.1	P.Sembilan
44	P99	Acanthus ilicifolius	3E-99	97.71	MW752129.1	P.Sembilan
45	P103	Avicennia alba	0.0	100	EU352162.1	P.Sembilan
46	P104	Avicennia alba	0.0	98.81	MZ405094.1	P.Sembilan
47	P105	Avicennia alba	0.0	98.35	MN117552.1	P.Sembilan
48	P112	Scyphiphora hydropgyllacea	1E-143	97.11	MN390972.1	L.Kertang
49	P113	Scyphiphora hydropgyllacea	1E-142	98.63	KY378668.1	L.Kertang
50	P114	Scyphiphora hydropgyllacea	7E-146	98.99	NC_049078.1	L.Kertang
51	P115	Avicennia marina	0.0	98.66	MZ405088.1	L.Kertang
52	P121	Avicennia alba	0.0	97.65	EU352162.1	L.Kertang
53	P122	Avicennia alba	0.0	95.37	MZ405095.1	L.Kertang
54	P123	Avicennia alba	5E-159	94.85	MN117553.1	L.Kertang
55	P124	Excoecaria agallocha	0.0	99.30	LC604863.1	L.Kertang
56	P125	Excoecaria agallocha	0.0	99.31	LC604863.1	L.Kertang
57	P126	Excoecaria agallocha	0.0	98.63	LC604863.1	L.Kertang
58	P128	Sonneratia alba	0.0	99.75	LC604868.1	L.Kertang
59	P129	Sonneratia alba	0	98.88	MH105772.1	L.Kertang
60	P135	Avicennia officinalis	8E-60	86.70	MZ405097.1	L.Kertang
61	P139	Acanthus ilicifolius	4E-109	88.06	MW752129.1	L.Kertang
62	P140	Acanthus ilicifolius	1E-99	97.72	NC_054308.1	L.Kertang
63	P141	Acanthus ilicifolius	1E-109	88.10	MW752129.1	L.Kertang
64	P156	Acanthus ilicifolius	2E-133	88,71	MW752129.1	Langsa
65	P157	Acanthus ilicifolius	1E-129	88,13	NC_054308.1	Langsa
66	P158	Acanthus ilicifolius	3E-109	78.96	GQ141552.1	Langsa
67	P165	Avicennia officinalis	0.0	99.56	MH243946.1	Langsa
68	P166	Avicennia officinalis	4E-83	87.76	MZ405097.1	Langsa
69	P167	Avicennia officinalis	5E-80	86.64	MN117563.1	Langsa

Table 9. BLAST results from ITS primers

No	Sampel Code	Specimen	E-value	Similarity (%)	Accesion	Sites
1	T4	Rhizophora apiculata	0.0	100	KX231337.1	Percut
2	T5	Rhizophora apiculata	0.0	100	HQ337908.1	Percut
3	T6	Rhizophora apiculata	0.0	89.98	HQ337920.1	Percut
4	T7	Nypa fruticans	1E-157	80.33	MH244012.1	Percut
5	T10	Avicennia alba	0.0	99.45	EU528876.1	Percut
6	T11	Avicennia alba	0.0	100	MG880032.1	Percut
7	T12	Avicennia alba	0.0	98.29	MH243934.1	Percut
8	T25	Avicennia officinalis	0.0	95.42	MH243946.1	Percut
9	T26	Avicennia officinalis	0.0	97.65	MH243945.1	Percut
10	T27	Avicennia officinalis	0.0	99.28	MH243946.1	Percut
11	T31	Avicennia marina	0.0	100	MF063712.1	Percut
12	T32	Avicennia marina	0.0	100	MF063710.1	Percut
13	T33	Avicennia marina	0.0	95.74	MH243938.1	Percut
14	T43	Excoecaria agallocha	3E-123	91.57	MH243997.1	Percut
15	T44	Excoecaria agallocha	0.0	96.05	MH243997.1	Percut
16	T45	Excoecaria agallocha	0.0	96.27	MH243998.1	Percut
17	T46	Xylocarpus granatum	0.0	96.32	KY429956.1	P. Sembilan
18	T47	Xylocarpus granatum	0.0	97.93	MH244038.1	P. Sembilan
19	T48	Xylocarpus granatum	2E-153	87.58	MH243997.1	P. Sembilan
20	T55	Nypa fruticans	3E-19	83.33	MH244012.1	P. Sembilan
21	T57	Nypa fruticans	1E-12	87.18	MH244011.1	P. Sembilan
22	T58	Acacia auriculiformis	1E-41	88.12	JX856395.1	P. Sembilan
23	T59	Acacia auriculiformis	2E-39	95.45	KC955519.1	P. Sembilan
24	T67	Excoecaria agallocha	0.0	99.16	MH243997.1	P. Sembilan
25	T68	Excoecaria agallocha	0.0	99.44	MH243998.1	P. Sembilan
26	T69	Excoecaria agallocha	0.0	98.88	MH244003.1	P. Sembilan
27	T70	Scyphiphora hydropgyllacea	2E-43	95.00	MK607942.1	P. Sembilan
28	T71	Scyphiphora hydropgyllacea	2E-107	98.25	MK607942.1	P. Sembilan
29	T82	Avicennia marina	0.0	98.66	KX641591.1	P. Sembilan
30	T83	Avicennia marina	3E-28	94.51	MH243939.1	P. Sembilan
31	T84	Avicennia marina	6E-145	91.05	MH243939.1	P. Sembilan
32	T85	Avicennia officinalis	2E-153	95.16	MG880050.1	P. Sembilan
33	T86	Avicennia officinalis	3E-62	75.86	MH243946.1	P. Sembilan
34	T97	Acanthus ilicifolius	7E-110	78.99	GQ141552.1	P. Sembilan
35	T99	Acanthus ilicifolius	2E-105	79.96	GQ141552.1	P. Sembilan
36	T103	Avicennia alba	0.0	99.18	MH243934.1	P. Sembilan
37	T104	Avicennia alba	0.0	99.13	MH243937.1	P. Sembilan
38	T105	Avicennia alba	0.0	94.31	MH243936.1	P. Sembilan

39	T112	Scyphiphora hydropgyllacea	0.0	97.85	MK607942.1	L. Kertang
40	T113	Scyphiphora hydropgyllacea	3E-92	93.97	MK607942.1	L. Kertang
41	T115	Avicennia marina	0.0	99.55	MH243938.1	L. Kertang
42	T116	Avicennia marina	0.0	97.60	MH243939.1	L. Kertang
43	T117	Avicennia marina	0.0	98.35	MH243940.1	L. Kertang
44	T121	Avicennia alba	0.0	99.71	MH243934.1	L. Kertang
45	T122	Avicennia alba	0.0	99.28	MH243935.1	L. Kertang
46	T123	Avicennia alba	0.0	99.85	KF848261.1	L. Kertang
47	T124	Excoecaria agallocha	4E-137	88.00	MH243997.1	L. Kertang
48	T125	Excoecaria agallocha	0.0	98.03	MH243998.1	L. Kertang
49	T126	Excoecaria agallocha	0.0	91.01	LC604848.1	L. Kertang
50	T127	Sonneratia alba	0.0	99.58	LC604851.1	L. Kertang
51	T128	Sonneratia alba	0.0	99.39	MH553463.1	L. Kertang
52	T129	Sonneratia alba	0.0	98.37	KF848277.1	L. Kertang
53	T133	Avicennia officinalis	0.0	99.10	MH243947.1	L. Kertang
54	T134	Avicennia officinalis	0.0	99.55	MH243946.1	L. Kertang
55	T135	Avicennia officinalis	1E-164	94.32	MH243947.1	L. Kertang
56	T142	Nypa fruticans	1E-12	89.86	MH244012.1	L. Kertang
57	T143	Nypa fruticans	2E-14	93.44	MH244012.1	L. Kertang
58	T153	Rhizophora stylosa	5E-80	95.83	MZ735369.1	L. Kertang
59	T156	Acanthus ilicifolius	4E-107	78.71	GQ141552.1	Langsa
60	T157	Acanthus ilicifolius	3E-109	78.96	GQ141552.1	Langsa
61	T165	Avicennia officinalis	0.0	99.56	MH243946.1	Langsa
62	T166	Avicennia officinalis	3E-173	92.52	MH243945.1	Langsa
63	T167	Avicennia officinalis	0.0	96.05	MH243944.1	Langsa

Phylogenetic analysis

Phylogenetic trees were constructed in using the PhyML 3.1/3.0 aLRT method (http://www.phylogeny.fr/) based on the bootstrap 100 permutation. Phylogenetic trees were constructed with the individual fragments and the average node support rate was calculated. Phylogenetic trees were fan-shaped, with one branch of the same or similar species as shown in Figure 2-5. The average node support rate for mangrove phylogenetic trees in the four regions was 89.66% ± 18.50% in Zhanjiang, 88.49% ± 17.25% in Huizhou, 86.85% ± 15.60% in Shenzhen, and 80.33% ± 19.89% in Shantou.

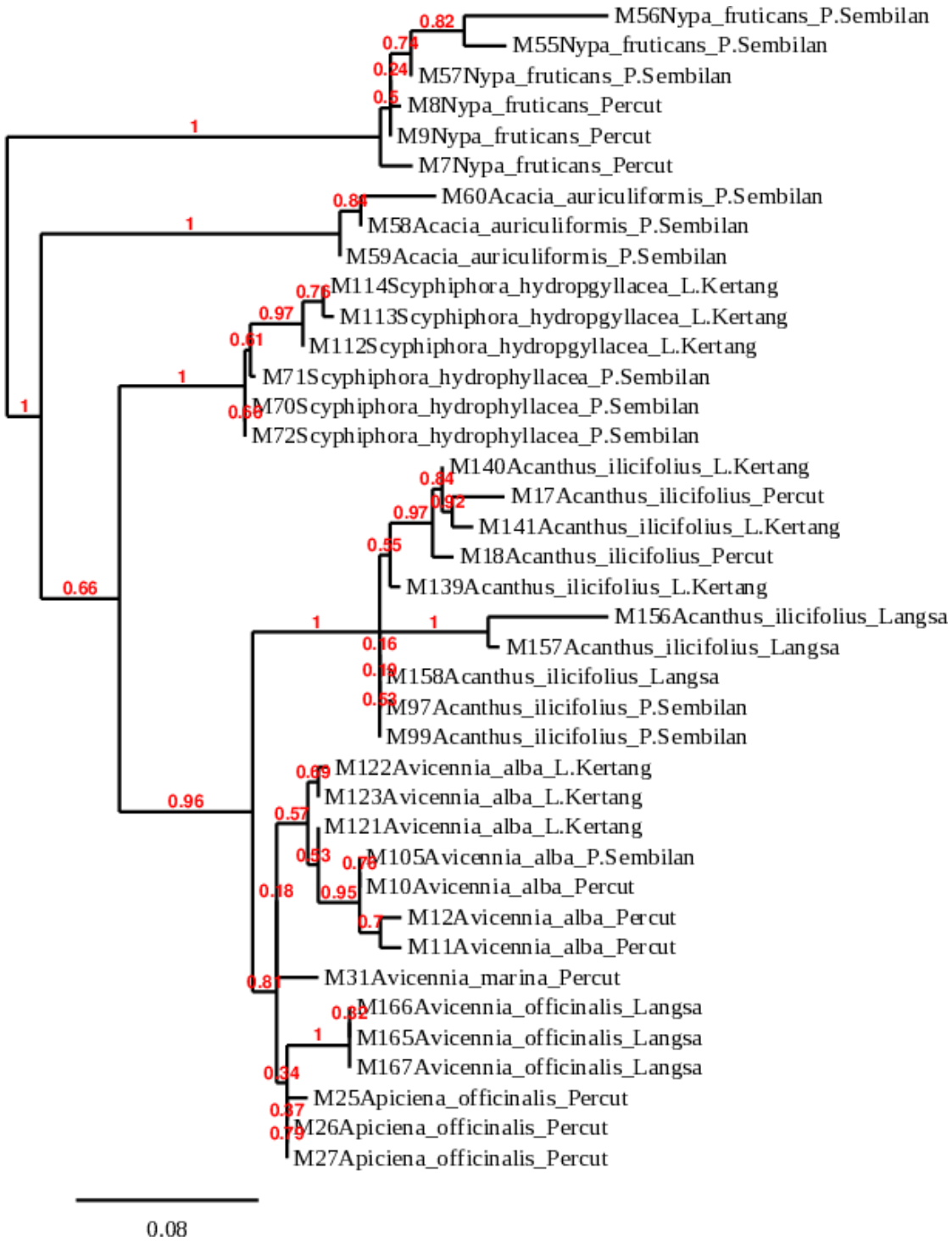


Figure 5. The phylogenetic tree of mangroves in North Sumatra and Aceh using fragment of matK

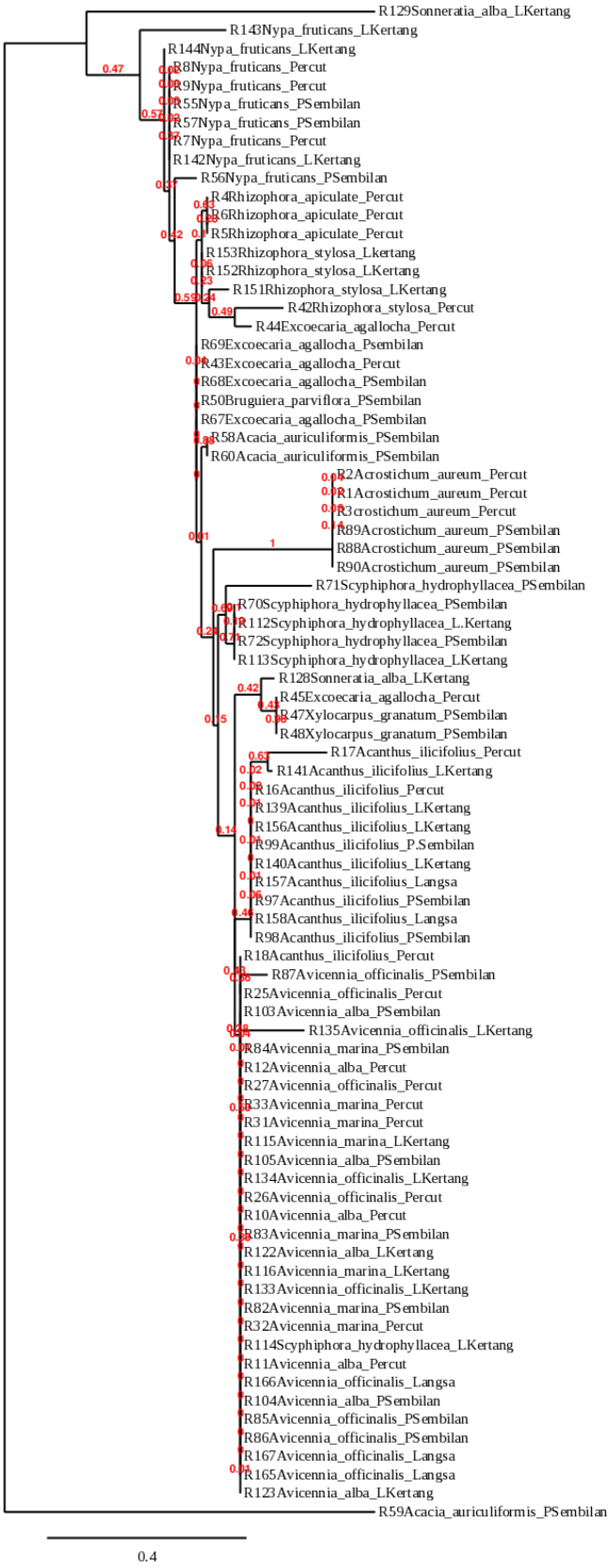


Figure 6. The phylogenetic tree of mangroves in North Sumatra and Aceh using fragment of rbcl

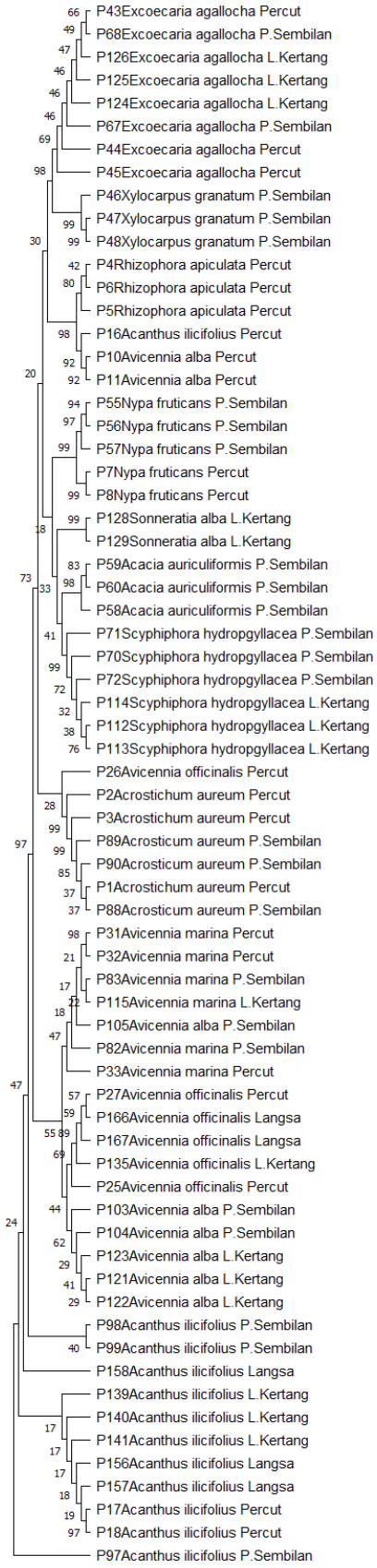


Figure 7. The phylogenetic tree of mangroves in North Sumatra and Aceh using fragment of trnH-psbA

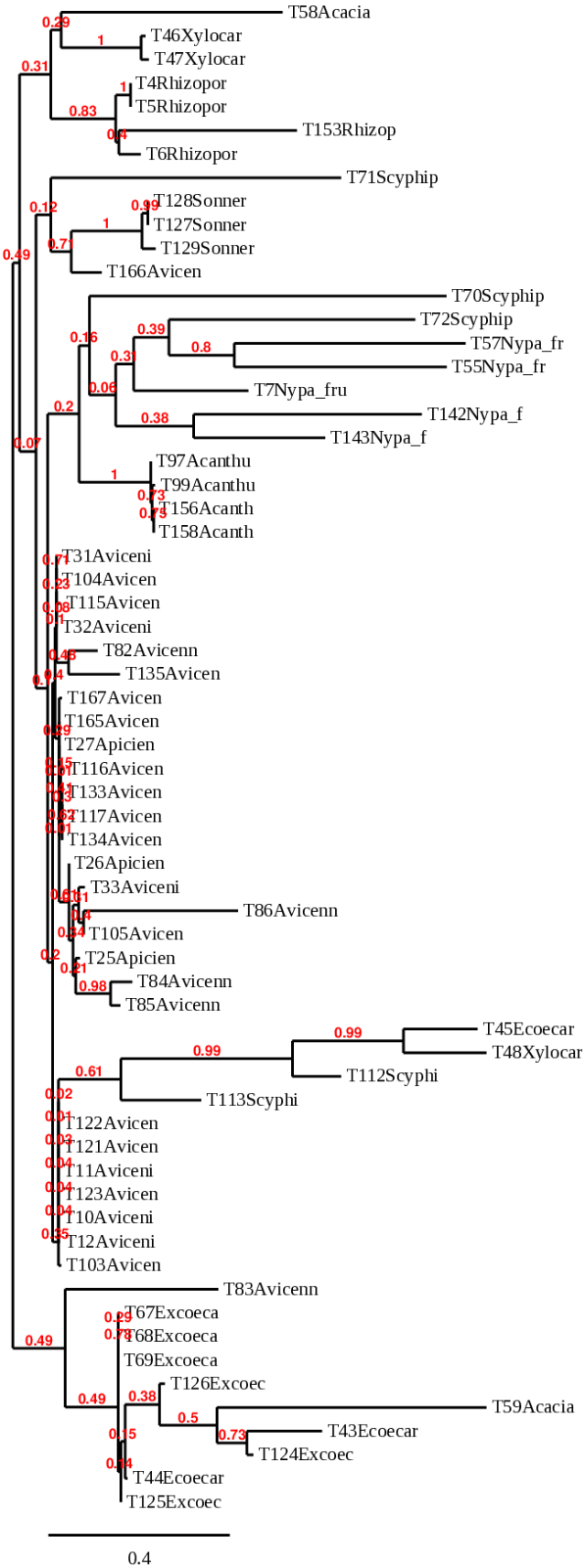


Figure 8. The phylogenetic tree of mangroves in North Sumatra and Aceh using fragment of ITS

5. CONCLUSION

The primer of matK, rbcL, trnH-psbA, and ITS could be used as a DNA barcode in identifying mangrove species in North Sumatra and Aceh Provinces. Our study investigated mangrove plants in the North Sumatra and Aceh provinces using DNA barcoding technology.

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Appendices

PAPER • **OPEN ACCESS**

DNA extraction and pattern of crab and macrobentos from North Sumatran mangrove forest, Indonesia

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DNA extraction and pattern of crab and macrobentos from North Sumatran mangrove forest, Indonesia

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Abstract. Mangrove forest ecosystem is one of the most productive and unique ecosystems that serves to protect coastal areas from various disturbances, as well as provide habitat for various animal species. The large number of crab species and macrobentos in mangrove ecosystems results in frequent errors in the naming of species that have similarities in morphological features. This problem can be solved through a comprehensive approach by combining morphological analysis with genetic analysi.This study aims to report a DNA extraction and PCR amplification prior was used for the identification of crab and macrozoobentos from mangrove forest, North Sumatra. Primer 16S has a conserved area so it is appropriately used in Polymerase Chain Reaction (PCR) and sequencing analysis to determine taxonomy, phylogeny and diversity between specie. Visualization of PCR amplification results with primer16S from crab samples and macrobentos resulting a low DNA band with a size of 206 bp and a high of 678bp

1. Introduction

Mangrove forest ecosystem is one of the most productive and unique ecosystems that serves to protect coastal areas from various disturbances, as well as provide habitat for various animal species [1]. Especially detritivor animals that live and associate in mangrove ecosystems [2]. The large number of crab species and macrobentos in mangrove ecosystems results in frequent errors in the naming of species that have similarities in morphological features. This resulted in the establishment of a different species of the same name.

The phenomenon of cryptic species in marine biota is a problem that needs to be solved through a comprehensive approach by combining morphological analysis with genetic analysis [3]. Identification of organisms was originally based solely on morphological characteristics, but this time has led to molecular taxonomy in which an organism is grouped based on its gene similarity [4]. This study aims to report a DNA extractionand PCR amplification prior was used for the identification of crab and macrobentos from mangrove forest, North Sumatra.

2. Materials and methods

2.1. Crab and macrozoobentos material

The genetic material used is crab DNA and macrobentos from mangrove forest obtained from Percut, Belawan, Lubuk Kertang, Pulau Sembilan and Langsa, North Sumatera Province. The specific primer used is16S primer. 16S has a conserved area so it is appropriately used in polymerase chain reaction (PCR) and sequencing analysis to determine taxonomy, phylogeny and diversity between species [4].

2.2. DNA Extraction

DNA isolation using Reliaprep gDNA Tissue Miniprep System kit protocol. Briefly, in the extraction process the parts used are meat from crab samples and macrobentos. The part is then added liquid nitrogen and then ground using mortar. The sample was transferred to a 1.5 ml tube then adds 100 µl Tail Lysis Buffer and 20 µl Proteinase K Solution, then vortex fort 10 sec. After that, added 200 µl Cell Lysis Buffer and vortex for 10 sec. The sample was incubated at 56 C for 30 minutes, the sample alternates every 10 minutes. The 20 µl RNase A and vortex and sentrifuged 10,000 rpm for 10 minutes. After that separated part of the liquid with the garbage that settles. Then added 250 µl Binding Buffer , then disentrifuged 10,000 rpm for 5 minutes. is then transferred the top of the liquid to the filter tube, then centrifuge 10,000 rpm for 2 minutes. After that added 500 µl Column Wash Solution and then disentrifuge 10,000 rpm for 2 minutes.This activity was carried out as many as 3 repetitions. Column Wash Solution fluid in the tube.Then put tube 1.5 ml under the filter tube, then added 100 µl Nuclease Free Water sentrifuge 12,000 rpm for 1 minute. The extraction results are stored in the freezer for 1 night [5].

2.3. Polymerase Chain Reaction (PCR)

PCR mix was made with a combined solution component of dna dilute 2 µl, Primer forward and reserve respectively 1 µl, ddH2O 3.5 µl and Green Go Taq 2.5 µl. Amplification is done with PCR machine. PCR program is divided into: pre denaturation for 2 minutes at 94°, denaturation for 30 seconds at 94°C, annealing for 1 minute at 56°, extension for 3 minutes at 72°C, final extension for 7 minutes at 72 ° C and storage for 30 minutes at 4 ° C as previously described [6].

2.4. Agarose Gel Electrophoresis

PCR products were analyzed with electrophoresis in performed by voltage 70 V, current 220 A for 45 minutes in a 1% agarose gel stained with a Red Gel. Electrophoresis results are visualized using uv transmilators.

2.5. Data analysis

Amplification of DNA readings was analyzed by software UV-1D (UV-Tex verv16,09b) as earlier suggested [7]

3. Results and Discussion

Table 1 shows the length of DNA fragments viewed with UV-ID after irradiation with UV light. The length of DNA fragments for crab and macrobentos from forest mangroves ranged from 206-678.

Table 1. DNA amplification of Crab and Makrobentos from Mangrove Forest of North Sumatera.

Line	Acces Code	Amplicon (bp)
1	LGS 1 (Crab)	489
2	LGS 2 (Crab)	277
3	BLW 6 (Makrobentos)	292
4	LGS 3 (Makrobentos)	247
5	PCT 5 (Makrobrntos)	300
6	LGS 5 (Crab)	289
7	LK 5 (Makrobentos)	230
8	PS 5(Makrobentos)	269
9	PS 2 (Makrobentos)	341
10	PS3 (Crab)	284
11	LK 4 (Makrobentos)	234
12	LGS 9 (Makrobentos)	219
13	PCT 7 (Makrobentos)	206
14	PS 6 (Makrobentos)	678
15	BLW 9 (Makrobentos)	423
16	LGS 8 (Crab)	360
17	PCT 3 (Crab)	227
18	BLW 10 (Crab)	227
19	BLW 8 (Makrobentos)	378
20	PS 7 (Makrobentos)	489
21	PC 4 (Makrobentos)	388

Description : LGS (Langsa), PCT (Percut), BLW (Belawan), PS (Pulau Sembilan), LK (Lubuk Kertang).

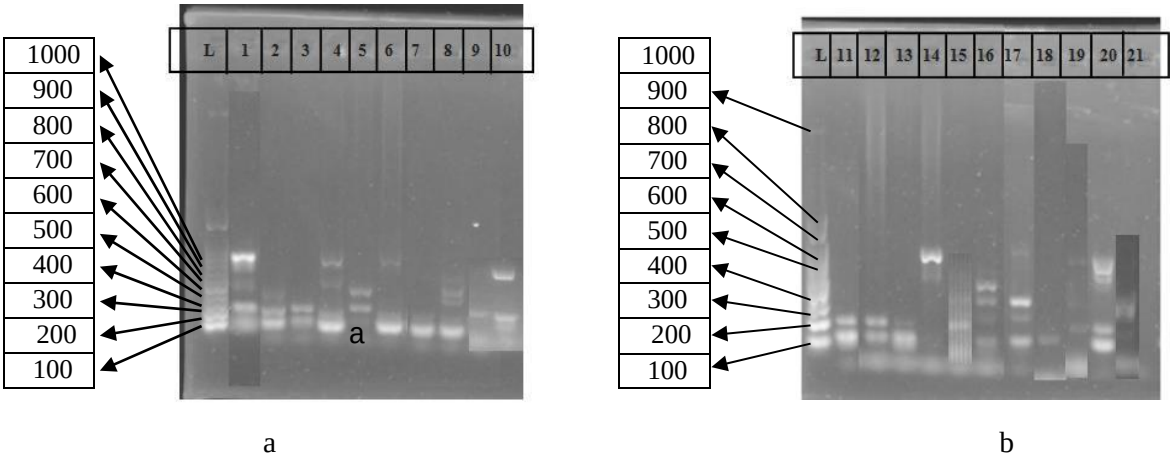

Figure 1. PCR Results of Crab DNA and Macrobentos from Mangrove Forest, North Sumatera L = Ladder, lines 1-10 = PCR results (b) L = Ladder, lines 11-21 = PCR results documented withUV-1D software (UV-Texver v16.09b).

Visualization of PCR amplification results with primer16S of crab samples and macrobentos produces thin ribbons and bright and clear ribbons with different values from each sample (Figure 1). The amplification stage produces a low DNA band with a size of 206 bp and a height of 678bp.DNA amplification from crabs and macrobentos obtains a single and multi band where a single band indicates

that primary used is the primary that specifics to amplify crab DNA and Macrobentos [8]. While, multiband or double tape is suspected because that primer used is not specific to crabs and makrobentos [9]. DNA bands produced through electrophoresis can be analyzed to see the genetic diversity of a group organism [10]

4. Conclusion

DNA crab and macrobentos were successfully amplified using primary 16S which produced a number of different DNA patterns in each sample ranging from 206 -678 bp and further sequenced for further analysis.

Acknowledgment

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PAPER • **OPEN ACCESS**

Isolation and amplification of mangrove plants using DNA barcode in Percut Sei Tuan, North Sumatra, Indonesia

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Isolation and amplification of mangrove plants using DNA barcode in Percut Sei Tuan, North Sumatra, Indonesia

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Abstract. Mangroves are a collection of several species of trees or shrubs that distribute around the coastline and can survive in high salinity environments. Around 60% of mangrove forests in North Sumatra are reported to have been damaged, the main factors of this damage being the mangrove forests conversion into ponds and the expansion of oil palm plantations. Identification of mangrove species is very important in protecting and applying the biodiversity of mangrove forests. Identification of living things has evolved from morphological charcetrization to molecular identification. This study aims to explain the DNA isolation and PCR methods to identify mangrove species in North Sumatra. The results suggested that the rbcL primer used can detect mangrove species that were visualized in the form of DNA bands. The length of DNA fragments of mangrove species *Acrosticum aureum* ranged 632.0-619.6 bp, species *Rhizophora apiculata* 619.6-585.8 bp, species *Nypa fruticans* 600- 592.9 bp, species *Avicennia alba* 549.1-533.5 bp, species *Hibiscus tiliaceus* was not detected, and mangrove species *Acanthus ilicifolius* 480.3 bp.

1. Introduction

Mangroves are a collection of several trees or shrubs species that distribute around the coastline and can live in high salinity environments. Mangrove forests provide an important role, as a source of livelihood, because they can produce various products of high economic value including fuel (firewood and charcoal), building materials (beams, roofs, etc.), fisheries, food raw materials, medicines, and agro-tourism [1-4].

Mangrove loss in North Sumatran found in primary mangrove forests significantly reduced 61.21% between 1990 and 2015, main deforestation was from 1990 to 2000 became secondary mangrove forest and swamp shrub [5]. The main factor in the destruction of mangroves during this period was conversion to ponds. In the next two decades, it is estimated that the expansion of ponds and the expansion of oil palm plantations will still be the main factors for mangrove loss in Indonesia, especially in North Sumatra [5].

Identification of mangrove species is important to conserve and utilize the biodiversity, which seems to be deterred by taxonomic expertise. The method of identification of living species has evolved from morphological identification to molecular identification [6]. The primer used was rbcL primer. The rbcL

gene has a low mutation rate compared to other barcode genes so that this gene provides an advantage for in-depth studies of interspecies genetic and phylogenetic variation [7]. This study aims to report a DNA extraction and PCR amplification prior was used for the identification of mangroves in North Sumatra.

2. Materials and Methods

2.1. Plant material

The plant material used was DNA from mangrove leaves obtained of spesies *Acrosticum aureum*, *Rhizophora apiculata*, *Nypa fruticans*, *Avicennia alba*, *Hibiscus tiliaceus*, *Acanthus ilicifolius* from Percut Sei Tuan, North Sumatra Province, Indonesia as depicted in Table 1.

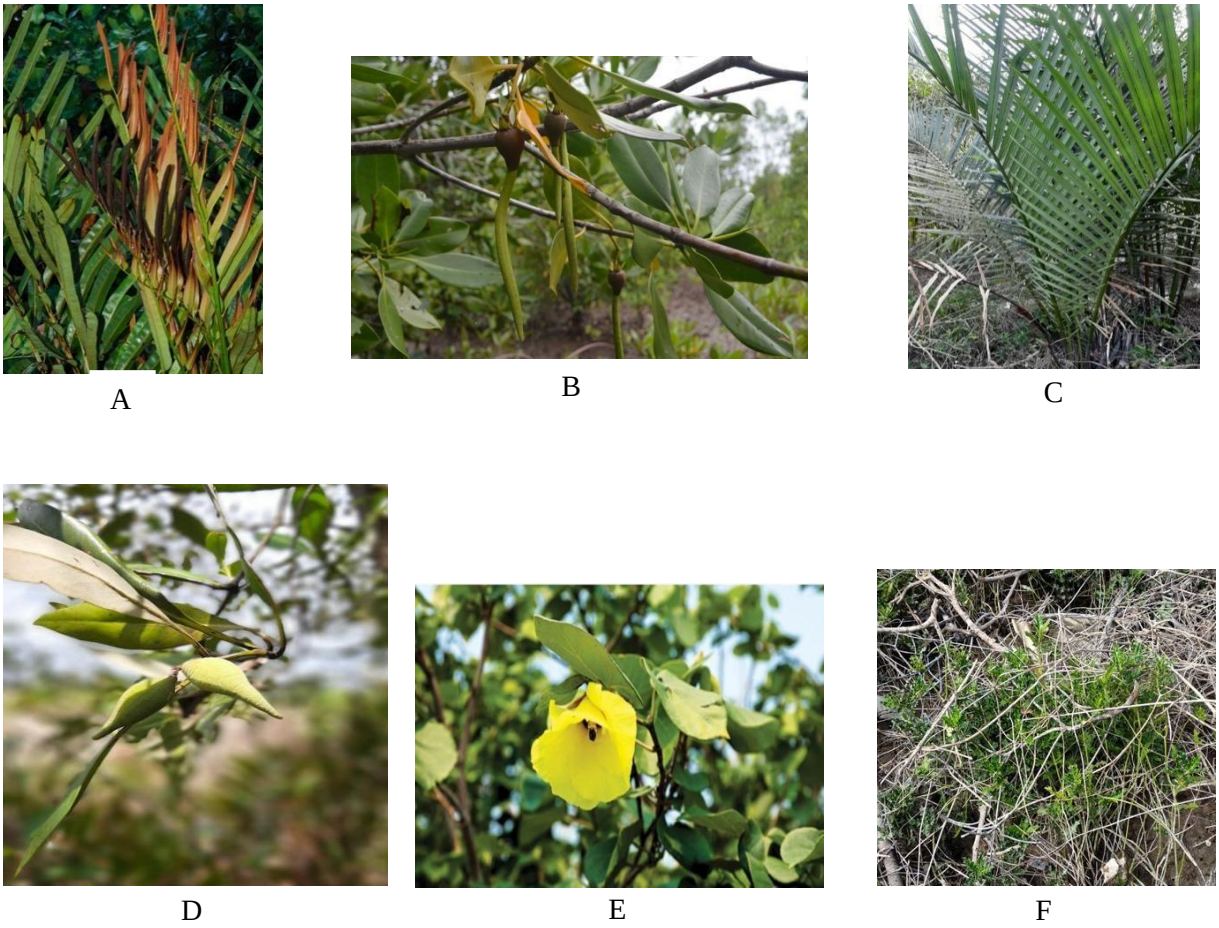

Figure 1. Sample of mangrove leaves (A= Acrosticum aureum, B= Rhizophora apiculata, C= Nypa fruticans, D= Avicennia alba, E= Hibiscus tiliaceus, F= Acanthus ilicifolius).

2.2. Method

2.2.1. DNA Extraction. DNA isolation using the Genomic DNA Mini Kit (Plant). Briefly, before the leaves are used, the leaves are first cleaned with 70% ethanol. 50 mg of leaves were cut and froze with liquid nitrogen, then ground the leaves using a mortar. The sample was transferred to a 1.5 ml tube then

added 400µl Buffers GP1 and 5 µl RNase, then vortex. The sample was incubated at 60°C for 10 minutes, alternating samples every 5 minutes. 100µl buffer added GP2 and vortexed and then incubated on ice for 3 minutes. Samples were centrifuged at 13,000 rpm for 6 minutes. The supernatant was transferred into the filter column and then centrifuged at 15,000 rpm for 2 minutes. Next step buffer GP3 is added into the 1.5 ml tube as much as 700µl as previously described [8].

Then the supernatant was transferred into a 1.5 ml tube that has been filled with Buffer GP3 mix supernatant then transfer 700 µl supernatant that has been mixed into the GD Column then centrifuged at 15,000 rpm for 2 minutes. The filter was removed and then transferred the remaining mixture into the GD Column, centrifuged at 15,000 rpm for 2 minutes, and discarded the filter. 400µl buffer W1 was added into the GD Column and then centrifuged at 15,000 rpm for 30 seconds then discarded the filter results. Then 600µl wash buffer was added then centrifuge 15,000 rpm for 30 minutes, discarded the filter, centrifuged 15,000 rpm for 3 minutes to dry the matrix column. The top of the GD Column was transferred to a new 1.5 ml tube. 100µl added the heated elution buffer to the center of the matrix column. Samples were left for 3-5 minutes then centrifuged at 14,000 rpm for 1 minute. Finally, the DNA was stored in the freezer (-20°C).

2.2.2. PCR amplification. Mix PCR was made with 3.6 µl of ddH2O composition, GoTaq 2.5 µl, primer of 0.5 µl, and DNA of 1 µl, along with rbl and trnH-psbA primers. Amplification was carried out with a PCR machine. Amplification program was performed at 94 °C preheat cycle for 3 minutes, denaturation cycle for 94°C at 30 seconds, annealing at 53°C for 1 min, and elongation at 72°C for 1 min, final heating at 72°C for 7 minutes [9]

2.2.3. Visualization of PCR amplification. Visualization of the results of PCR amplification was carried out by electrophoresis of the PCR product produced by making 2% agarose gel by dissolving 0.8 gram of agarose with 40 mL of TAE 1 X, heated in a microwave for 2 minutes, and visualized by Ultraviolet (UPV).

2.2.4. Data analysis. The DNA amplicon was determined with UV-1D software (UV-Tex ver v16.09b) as previously reported [10].

3. Results and Discussion

Table 1 shows the length of amplicon fragments viewed with UV-ID after irradiation with UV light. The length of PCR product for A. aureum mangroves ranged from 619.6-632.0, R. apiculata 619,588-585,786 bp, N. fruticans 600- 592.9 bp, A. alba species 549,135-533,58 bp, H. tiliaceus species were not detected, and mangroves A, ilicifoilus 480.3 bp.

Table 1. DNA amplification of mangrove species in Percut Sei Tuan.

Line	Mangrove species	Amplicon (bp)
1	*Acrosticum aureum*	632.0
2	*A. aureum*	625.8
3	*A. aureum*	619.6
4	*Rhizopora apiculata*	585.8
5	*R. apiculata*	613.1
6	*R. apiculata*	619.6
7	*Nypa fruticans*	600.0
8	*N. fruticans*	592.9
9	*N. fruticans*	578.6
10	*Avicennia alba*	533.5
11	*A. alba*	549.1
12	*A. alba*	549.1
13	*Hibiscus tiliaceus*	Na
14	*H. tiliaceus*	Na
15	*H. tiliaceus*	Na
16	*Acanthus ilicifolius*	480.2

Na= not amplification

PCR and PCR primer used can detect mangrove species that are visualized in the form of DNA bands. The *rbc*L primer is an effective marker for identifying mangroves, (Harisam et al., 2019). According to the research [11], which tested the primer *rbc*L and *mat*K to identify mangrove species and they recommended *mat*K to be a worthy candidate primer for identifying mangrove species. However, other studies found that *rbc*L and *trn*H-*psb*A have a high amplification and sequencing success rate, indicating that these two markers are suitable for species identification in mangrove plants. [11, 12].

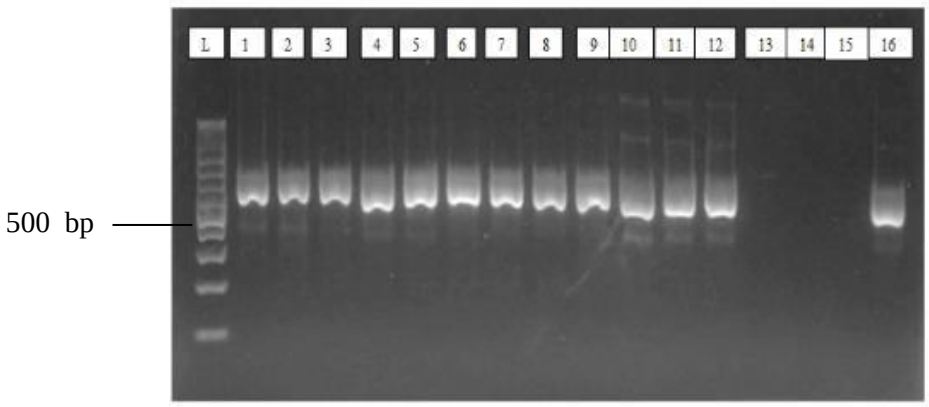

Figure 2. PCR Results primer rbcL of Mangrove Samples (L = Ladder, lines 1-16 = PCR results) documented withUV-1D software (UV-Tex ver v16.09b).

Figure 2 showed the amplification of mangrove plant samples, from 16 samples, only 3 mangrove samples were not detected. Each sample has a different length of the fragment depending on mangrove species. These samples were further investigated to clarify the correct identification using sequence.

4. Conclusion

The primer rbcL could be used as a DNA barcode in identifying mangrove species in North Sumatra Province.

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