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ORIGINAL RESEARCH ARTICLE

Morphological and molecular monitoring of fishes in the waters of mangrove ecosystems

M. Basyuni[1,2,*], Irmawati[3], A. Mubaraq[1,5], A. Susilowati[1,2], A.B. Rangkuti[1,2], Desrita[1,4], A.A. Aznawi[1,5], S.S. Al Mustaniroh[1], S.H. Larekeng[6], A.H. Salman[6], E.R. Ardli[7], Y. Isowa[8], T. Kajita[8]

[1] *Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan 20155, Indonesia*

[2] *Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan 20155, Indonesia*

[3] *Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar 90245, Indonesia*

[4] *Department of Aquatic Resources Management, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia*

[5] *Doctoral Program of Agriculture Science, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia*

[6] *Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar 90245, Indonesia*

[7] *Faculty of Biology, Jendral Soedirman University, Purwokerto 53122, Indonesia*

[8] *Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, Taketomi, Okinawa 907-1541, Japan*

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ABSTRACT

BACKGROUND AND OBJECTIVES: Mangroves are primarily found along Sumatra's east coast in Aceh and North Sumatra, Indonesia. Fish communities and other marine life are supported by mangrove forests, which are extremely productive ecosystems. This study used morphological and 16 small subunit ribosomal ribonucleid acid barcodes to track fish diversity in waters surrounding mangroves. The study objectives were to integrate morphological identification with 16 small subunit ribosomal ribonucleic acid deoxyribonucleic acid barcoding to assess fish diversity in mangrove ecosystems along the east coast of North Sumatra and Aceh, Indonesia.

METHODS: Study sites were selected in five locations, namely, Percut Sei Tuan, Belawan, Lubuk Kertang, and Pulau Sembilan in Sumatera Utara coast and Langsa in Aceh coast. Fish specimens were caught using gillnet and danish seines operated by the local fishermen. Morphological identification was performed based on standard taxonomic keys, and deoxyribonucleic acid barcoding using 16 small subunit ribosomal ribonucleic acid deoxyribonucleic acid marker was applied to validate species identification.

FINDINGS: Seventy fish specimens were 70 collected, and 62 species and 39 families were morphologically identified. *Leiognathus equula (Leiognathidae)* was found in all study sites, followed by *Ambassis vachellii (Ambassidae), Pomadasys argenteus (Haemulidae),* and *Eleutheronema tetradactylum (Polynemidae)* in four locations. *Gerres filamentosus (Gerreidae), Terapon jarbua (Teraponidae), Sphyraena qenie (Spyraenoidae),* and *Elops machnata (Elopidae)* were detected in three locations. ribosomal ribonucleic acid barcoding confirmed 17 of the fish species detected. Deoxyribonucleic acid barcoding using 16 small subunit ribosomal ribonucleic acid validated the identification of 17 species, confirming that morphological and molecular approaches are complementary in assessing fish diversity. *Two species, Larimichthys crocea and Thunnus maccoyii, were found to be critically endangered and endangered, respectively.*

CONCLUSION: The combination of field surveys and 16 small subunit ribosomal ribonucleic acid barcoding provides a comprehensive assessment of the changes in fish species composition and abundance over time, allowing for the identification of vulnerable species and the development of effective conservation strategies. The findings highlight the importance of long-term monitoring, habitat conservation, and sustainable fisheries management to protect fish biodiversity in mangrove ecosystems along the east coast of North Sumatra and Aceh.

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*Corresponding Author:
Email: *m.basyuni@usu.ac.id*
Phone: +62853 2577 6785
ORCID: 0000-0003-1120-8571

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INTRODUCTION

Mangrove forests are highly dynamic intertidal ecosystems that support marine biodiversity. These habitats serve as breeding, nursery, and feeding grounds for various fish species, contributing substantially to fisheries and coastal livelihoods (Basyuni *et al.,* 2021; Fekri *et al.,* 2024). Many animals and microorganisms, such as fishes, shrimps, and macrozoobenthos, are closely associated with mangrove ecosystems (Basyuni *et al.,* 2021; Sari *et al.,* 2022). With fisheries and aquaculture becoming key sectors in its regional and national economic development, Indonesia now accounts for approximately 8 percent (%) of global fish production (FAO, 2024). As one of the world's largest archipelagic nations, this country hosts extensive mangrove forests, particularly along the east coast of North Sumatra and Aceh. Despite their ecological and economic importance, mangrove ecosystems are increasingly threatened by anthropogenic activities, such as deforestation, pollution, and overfishing. These disturbances can lead to significant declines in fish populations, affecting biodiversity and fisheries-dependent communities (Islam *et al.,* 2020). Physical and environmental factors shape aquatic ecosystems, and conservation measures are necessary to ensure species can adapt to changing conditions while maintaining ecological balance (Isroni *et al.,* 2023). Mangroves also provide essential ecosystem services that support coastal communities, including shoreline protection, carbon sequestration, and water filtration (Basyuni *et al.,* 2022). The structural complexity of mangrove forests, characterized by supporting roots and pneumatophores, enhances habitat availability and fosters high biodiversity (Nordhaus *et al.,* 2019). Accurate species identification is essential to effectively monitor and conserve fish populations in mangrove ecosystems. Although useful, traditional morphological identification methods often fail to distinguish closely related species, especially in their larval stages. This limitation has led to the increasing use of molecular approaches, such as deoxyribonucleic acid (DNA) barcoding, which provides precise genetic markers for species identification (Afrand *et al.,* 2024). DNA barcoding, particularly using mitochondrial genes such as 16 small subunit ribosomal ribosomal ribonucleic acid (16S rRNA) and cytochrome c oxidase subunit I (COI), has proven effective in identifying fish and shellfish species in mangrove habitats across Indonesia, India, and Bangladesh (Hairani *et al.,* 2023; Habib *et al.,* 2021; Ram *et al.,* 2021). This technique has revealed high genetic diversity within fish and shrimp populations and has been instrumental in resolving taxonomic ambiguities, such as the misidentification of mud crab species in the Sundarbans (Habib *et al.,* 2021). Recent studies have demonstrated the advantages of integrating morphological and molecular techniques for biodiversity assessment in mangrove ecosystems. In the Merbok Estuary, Malaysia, DNA barcoding identified 140 molecular operational taxonomic units (MOTUs), including potential cryptic species, providing a comprehensive fish inventory for the region (Abidin *et al.,* 2022). In Gorontalo, Indonesia, a combination of morphological and molecular methods identified two species of mudskippers (*Periophthalmus argentilineatus* and *P. malaccensis*) in coastal mangrove habitats (Baderan *et al.,* 2023). These findings highlight the importance of integrating multiple identification methods to improve species resolution and enhance biodiversity assessments. The east coast of North Sumatra and Aceh is known for its high fish biodiversity, including commercially and ecologically important species. However, this region faces significant threats from overfishing, habitat degradation, and pollution. Monitoring fish species abundance and composition is crucial for assessing the effect of these threats and developing effective conservation strategies. Combining these approaches can increase the accuracy of species identification by documenting fish distribution patterns and creating a database for future conservation and management initiatives. This study integrated morphological identification with 16S rRNA DNA barcoding to assess fish diversity in mangrove ecosystems along the east coast of North Sumatra and Aceh, Indonesia in 2022.

MATERIALS AND METHODS

Study site and qualification of habitat

The research was conducted for 3 months, from July to September 2022 at five locations, namely, Percut Sei Tuan, Belawan, Lubuk Kertang, Pulau Sembilan, and Langsa. Fish sampling was conducted between July and September 2022 to minimize seasonal variations in species composition. Future studies should incorporate multiseason sampling to assess temporal shifts in biodiversity. The five

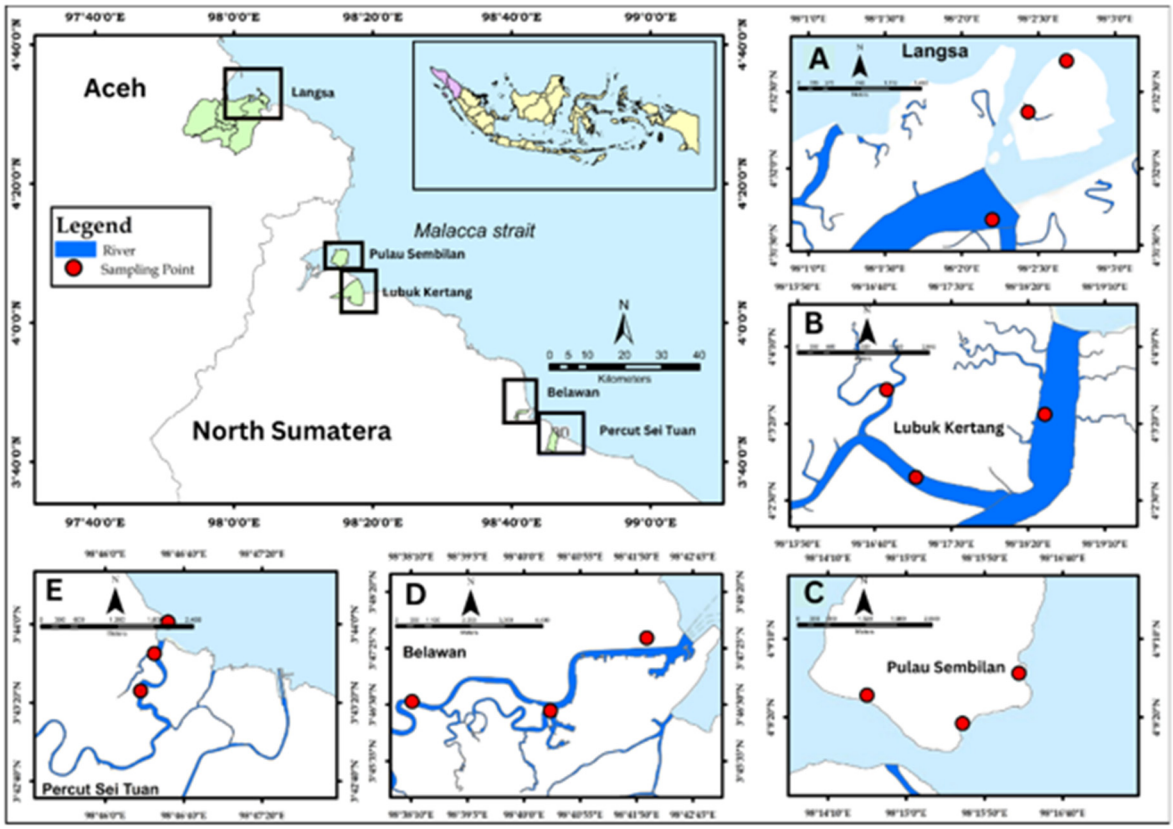

Fig. 1: Geographical location of the study area along with sampling points at Langsa (A) (Aceh Province), Lubuk Kertang (B), Pulau Sembilan (C), Belawan (D), and Percut Sei Tuan (E) of North Sumatra Province, Indonesia

locations can be found on the Eastern coast of North Sumatra and Aceh. The mangroves on Percut Sei Tuan beach are damaged by the conversion of abandoned ponds, those on Belawan beach are damaged by oil palm conversion and ponds, those in Lubuk Kertang are the result of rehabilitation, those on Pulau Sembilan are damaged by oil palm conversion and then restored, and those in Langsa are intact and continuously planted. The research stations were selected based on the characteristics of each location. Each location was studied at three stations, each with three substations. Percut, Belawan, and Langsa consist of downstream rivers, estuaries, and the sea. Lubuk Kertang is represented by estuaries and the sea (Fig. 1). Sampling effort varied slightly across these locations due to logistical constraints, though an equal number of sampling stations were selected per site. Future studies should ensure uniform sampling efforts to minimize potential biases in species richness estimates.

Sampling, preservation, and identification of fishes

Sampling was conducted using the following fishing gears owned and operated by the local fishermen: gill nets (mesh size 1, 1.5, and 3 in) and drag trawls (graduated mesh size 1,1/2 and 1/4 in). Seventy fish specimens were collected.

Fresh fish samples were collected, separated according to type, photographed one by one, and given a paper label as a temporary identity. The samples were preserved using 10% formalin, transferred to the laboratory, and observed for identification using fish identification books (Wahyudewantoro, 2018; Ahn *et al.*, 2020). After identification, the samples were placed into a container with absolute ethanol.

Characteristics of mangrove forests

At each sampling location, three plots of 10 m x 10 m were set to collect fundamental forest stand characteristics. All trees within the plots were identified (Hidayat et al., 2017). The data were

subsequently utilized to compute tree density as individuals per hectare (ha).

DNA extraction and amplification and phylogenetic tree

Total genomic DNA was extracted from fish tissue muscle (fillet) using Miniprep System kit (Promega) following the manufacturer's protocol. The presence and quality of the extracted DNA template were checked through electrophoresis on 1% agarose gel, and the quantity was assessed using a nanodrop spectrophotometer (Thermo Scientific) at an absorbance ratio of 260/280 (Basyuni *et al.,* 2024). The extracted genomic DNA was amplifies using a polymerase chain reaction (PCR) machine (Sensoquest thermal cycler) and a pair of 16s rRNA primers (16s rRNA forward (F):5'-CCTGTTTANCAAAAACAT-3' and reverse (F):5'-AGATAGAAACCAACCTGG-3') (Hairani *et al.,* 2023). Subsequent to gel electrophoresis detection, all the amplification products (PCR products) were sent to Macrogen Inc. (Seoul, Republic of Korea) for further sequencing. GenBank was utilized to perform Basic Local Alignment Search Tool (BLAST) searches for the sequences obtained from the bidirectional sequencing of the fragments. Upon identifying substantial disparities between the sequences and original species, clarifications were pursued and validated through expert consultation until the BLAST results for the sequences and original species aligned in terms of genus or family (Basyuni *et al*., 2024). UGENE version 40.1 software (Salinas *et al.,* 2024) was utilized for splicing and proofreading the sequences. Phylogenetic trees were constructed with molecular evolutionary genetics analysis (MEGA X) software (Kumar *et al.,* 2018) from the 16s rRNA barcodes based on bootstrap analysis performed with 100 permutations.

Data analysis

Local distribution

The neighbor-joining (NJ) method was utilized, and the Kimura two-parameter model served as the foundation for the analysis (Laojun *et al.,* 2023 Phylogenetic trees were constructed using the distinct sequence of barcodes for each specimen and drawn by the interactive tree of life (iTOL) v. 6.7 using Eq. 1 (Letunic and Bork, 2024; Nur *et al.,* 2021):

$$D = \text{Ni.loc}/\text{N.loc} \times 100\% \quad (1)$$

where *D* is the local distribution, and N.loc is the total sampling location where fishes were found.

For the study of structural diversity, the Shannon–Wiener diversity index (H') was calculated using Eq. 2 (Herrera *et al.,* 2023)

$$H' = -\sum_{i=1}^{s} Pi \ln Pi \quad (2)$$

where Pi is ni/N. The value of H' can be 0–1 (indicating low/minimal species diversity), 1–3 (indicating medium species diversity), and 3 or more (indicating high species diversity). According to the Shannon–Wiener index, the higher the diversity index, the better the ecosystem.

For the study of evenness, the evenness index (E) was computed using Eq. 3 (Setiyaningrum and Puspitasari, 2022):

$$E = \frac{H'}{H\,max} \quad (3)$$

where Ln is the experience logarithms, H' is the Shannon–Wiener diversity index, and E is the number of kinds. Using the following criteria, the population's evenness index falls between 0 and 1: High evenness is denoted by $E > 0.6$, medium evenness by $0.4 < E < 0.6$, and poor evenness by $E < 0.4$.

Statistical analysis was carried out using Analysis of variance (ANOVA) and Kruskal–Wallis tests to determine statistically significant differences in fish diversity (Pagano *et al.,* 2022) Null Hypothesis: Fish diversity is not significantly different among the sampled locations. Alternative Hypothesis: Fish diversity significantly differs among the sampled locations.

RESULTS AND DISCUSSION

Characteristics of mangrove ecosystems

Rhizophora apiculata and *Nypa fructicans* are two mangrove species consistently found across all the observation sites. Among the surveyed locations, Pulau Sembilan in North Sumatra Province hosts the highest species diversity, with 14 recorded species, followed by Lubuk Kertang with 10, Belawan and Langsa with 8, and Percut Sei Tuan with 6. The widespread presence of *Rhizophora* species in each site can be attributed to the high salinity tolerance, adaptability, and rapid growth of *R. apiculata*, making it one of the most resilient mangrove species in dynamic coastal environments (Yun *et al.,* 2022; Samal

et al., 2023). Meanwhile, *N. fructicans,* commonly known as the nipa palm, thrives in brackish water forests and along riverbanks near estuaries. Despite its ecological role, *N. fructicans* is considered an invasive species, necessitating careful management to prevent uncontrolled expansion that could alter mangrove ecosystem dynamics (Eddy and Basyuni, 2020; Widodo *et al.,* 2020).

Diversity index (H')

According to the Shannon–Wiener diversity index, the growth rates of seedlings, saplings, and mangrove trees in Pulau Sembilan and Belawan Villages and the tree level in Percut Sei Tuan fall within the medium diversity category, with H' ranging from 1.02 to 1.6 (Fig. 2). By contrast, the seedling, sapling, and tree levels in Langsa City; the seedling and sapling levels in Percut Sei Tuan; and the tree level in Lubuk Kertang Village and Langsa City are categorized as low diversity, with H' ranging from 0.21 to 0.95. Comparative studies on mangrove diversity in different regions reveal variations in their Shannon–Wiener diversity indexes (H'). For instance, in the Angke Kapuk Mangrove Protected Forest, Jakarta, the H' was 1.88 for saplings and 1.18 for seedlings, placing them in the low to moderate diversity category. The predominance of certain species, such as *R. stylosa*, across all growth stages may have contributed to the low diversity observed in this region (Basyuni *et al.,* 2018; Rumondang *et al.,* 2021 By contrast, the Tanakeke Mangrove Forest in South Sulawesi exhibited higher diversity, with 11 species from 8 families. The Shannon–Wiener diversity index was 2.01 for trees, 1.88 for saplings, and 1.18 for seedlings, suggesting a more stable and healthy ecosystem compared with that in the other locations (Setiawan and Mursidin, 2018). These findings highlight the variability of mangrove diversity across different regions and emphasize the importance of assessing local ecological conditions to inform conservation strategies.

Eveness index (E)

The evenness index (E) at different growth levels across the five study sites falls within the medium category, ranging from 0.33 to 0.97, except for the sapling level in Langsa City, which is categorized as high with an E of 1.18. In general, E values between 0.33 and 0.97 indicate that individuals of different species are fairly evenly distributed, suggesting no single species dominates the ecosystem. However, the exceptionally high E at the sapling level in Langsa City exceeds the theoretical maximum. According to Gregorius and Gillet (2022), values close to 1 indicate high evenness, and values exceeding 1 are unusual and may result from calculation or data interpretation errors. A study conducted by Sholiqin *et al.* (2021) on the south coast of Pacitan, East Java, Indonesia identified eight dominant mangrove species, with *R. stylosa* being the most abundant. The Shannon–Wiener diversity index classified the mangrove diversity in this region as low to medium. Furthermore, the evenness index (E) varied significantly between the study sites. The Teleng

Table 1: Number of individuals/ha found at the study location

No.	Species	Lubuk Kertang	Pulau 9	Belawan	Percut Sei Tuan	Langsa
1.	*Avicennia alba*		87	427	473	
2.	*A. marina*	147	240	367	713	
3.	*A. officinalis*	213	240	560		373
4.	*Bruguiera gymnorrhiza*	533	7	427		47
5.	*B. sexangula*		240			
6.	*B. cylindrica*				20	
7.	*Excoecaria agallocha*			93	7	
8.	*Ceriops tagal*	267	1.247			67
9.	*Lumnitzera racemosa*		47			
10.	*N. fructicans*	380	187	73	35	7
11.	*R. apiculata*	2.507	1.007	220	227	2.147
12.	*R. mucronata*	53	60			13
13.	*R. Stylosa*		1.387			13
14.	*Scyphiphora hydrophylacea*	240	7			
15.	*Sonneratia alba*	47	487			
16.	*Xylocarpus granatum*	73	147	40		173
	Total	4.460	5.387	2.207	1.673	2.840

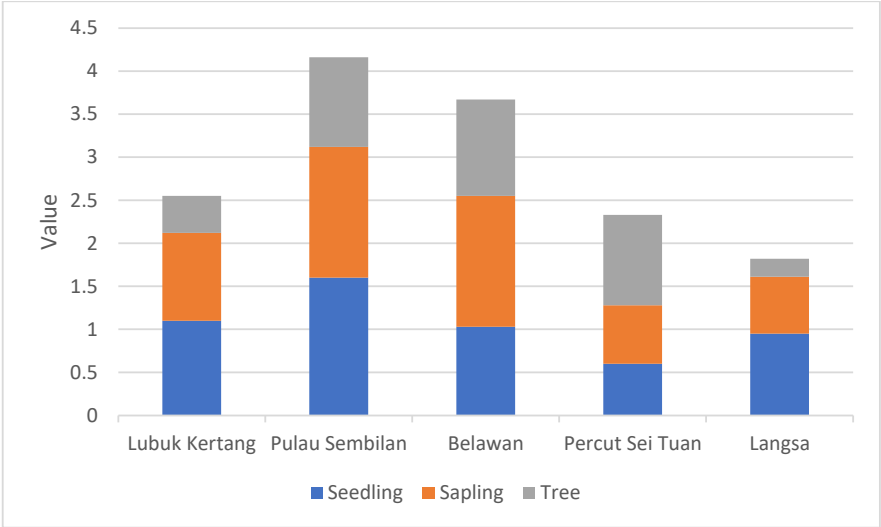

Fig. 2: Diversity index values for mangrove plants at the observation location

Ria Estuary exhibited a higher evenness index than the other locations, which is suggestive of its more balanced species distribution and relatively stable ecosystem.

Index richness (R)

The Margalef species richness index (R) across all observation locations fall within the low category, ranging from 0.67 to 1.63 (Fig. 3). This finding suggests that these mangrove ecosystems have relatively low species richness, indicating limited species diversity. Species richness in mangrove ecosystems is influenced by various ecological and environmental factors, including salinity, tidal fluctuations, substrate composition, and anthropogenic pressures, such as deforestation and pollution (Nagelkerken *et al.,* 2018). The relatively low R values observed in this study suggest that the mangrove ecosystems in the surveyed areas may be experiencing environmental stressors that limit their species diversity. Comparative studies reported varying species richness levels in different mangrove regions. For example, a study in the Segara Anakan Lagoon, Indonesia, found an R value ranging from 2.01 to 3.50, indicating moderate to high species richness due to the presence of diverse mangrove habitats (Jennerjahn *et al.,* 2024). Similarly, mangrove forests in Malaysia's Matang Mangrove Reserve reported R values above 2.5, suggesting a diverse and well-preserved ecosystem (Goessens *et al.,* 2014). The low species richness observed in this study could be attributed to habitat fragmentation, pollution, and mangrove resource overexploitation, all of which have been identified as key threats to mangrove biodiversity in Indonesia (Basyuni *et al.,* 2021; Sari *et al.,* 2022). In addition, the species dominance by a few genera, such as *Rhizophora* and *Avicennia*, may reduce overall species richness by limiting the establishment of other mangrove species (Alongi, 2018).

Distribution and species composition

Sixty-two fish species, representing 38 families, were identified based on morphological characteristics. The most abundant families are Serranidae, Pomacentridae, and Labridae, which are commonly found in mangrove and reef-associated ecosystems. DNA barcoding successfully identified 17 out of the 50 morphologically identified species; the remaining nine species required further molecular analysis for confirmation. Morphological analysis also revealed high genetic diversity within several species, suggesting the presence of multiple subpopulations. However, the overall species diversity and abundance exhibit a decline over the 2-year monitoring period, raising concerns about the long-term stability of fish populations in these ecosystems. These findings highlight the significant discrepancies between morphological and molecular identification,

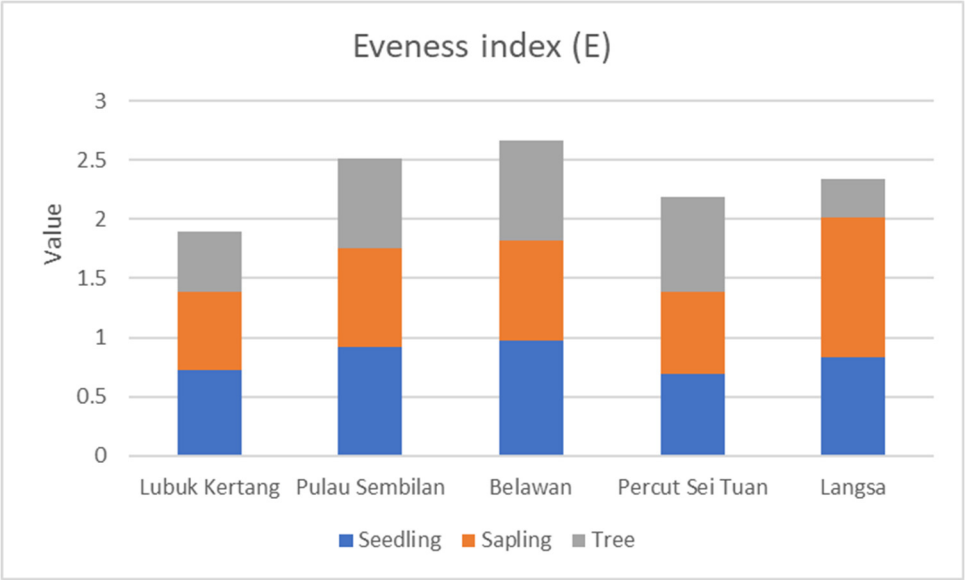

Fig. 3: Evenness index for mangrove plants at the observation location

emphasizing the need for expanded genetic reference databases. The dominance of species X and Y across multiple sites suggests their adaptability to mangrove ecosystems, and the restricted distribution of species Z may indicate specific habitat preferences or localized threats. These patterns underscore the importance of site-specific conservation strategies. For the ANOVA test results: F-statistic = 543.89, p-value = 4.29×10^{-20} (highly significant). For the Kruskal–Wallis test results: H-statistic = 23.08, p-value = 0.00012 (highly significant) (Table 2). Given that both tests yielded extremely low p-values ($p < 0.05$), the null hypothesis is rejected, that is, fish diversity significantly differs across the five locations (Percut Sei Tuan, Belawan, Lubuk Kertang, Pulau Sembilan, and Langsa).

Identification of fish species

Table 2 shows the results of fish field identification in the study sites and confirmation using 16s rRNA barcodes based on BLAST search (identity and query cover). The 16S rRNA barcode sequences reported in this paper has been deposited in the GenBank/ he European Nucleotide Archive (ENA) and the DNA Data Bank of Japan (DDBJ).

Biodiversity assessment is a key aspect of biological conservation. However, current biodiversity assessment methods generally need improvements because they are crucial in the future of biodiversity, forest resources, and aquatic ecosystems. Biodiversity in mangrove ecosystems is affected by river runoff that transports large amounts of nutrients and organic matter (Pasquaud *et al.,* 2015). Fish diversity in mangrove ecosystems is influenced by various ecological and environmental factors, including habitat structure, water quality, and anthropogenic activities, such as overfishing and habitat destruction (Nagelkerken *et al.,* 2018). The dominance of Serranidae, Pomacentridae, and Labridae aligns with a previous study indicating their ability to adapt to mangrove and coral reef environments and their role as key components of coastal food webs (Asriyana *et al.,* 2020). Among the 17 fish species, 10 were confirmed using 16S rRNA with 100% identity and 100% query (Table 3). The sequences from these 14 fish species perfectly match a reference sequence in the database with 100% identity, without mismatches or gaps in the aligned region. This finding suggests that the 16S rRNA gene is highly conserved for these species in the reference database. Two species, *Larimichthys crocea* and *Thunnus maccoyii*i, were found to be critically endangered and endangered, respectively (Table 3), revealing the ability of this technique to facilitate the identification of at-risk species and the formulation of successful conservation plans. The combination of morphological and molecular techniques provides a comprehensive assessment of fish biodiversity.

Table 2: ANOVA and Kruskal–Wallis test

Statistical test	Test statistic	P-value	Interpretation
ANOVA	543.89	4.29×10^{-20}	Significant differences exist
Kruskal-Wallis	23.08	0.00012	Significant differences exist

Table 3: Field identification of fish species samples with 16s rRNA barcode data and BLAST search results

ID	Field identification species	Accession	Length	Identity (%)	Query cover (%)	1st Hit	1st Hit Accession	Locality
B1	*Larimichthys crocea*	PV174210	458	100	100	*Larimichthys crocea*	MF004337	Belawan
B18	*Larimichthys crocea*	PV174211	645	100	100	*Larimichthys crocea*	AP006002	Belawan
BLW4_16	*Epinephelus fuscoguttatus*	PV174212	366	100	95.63	*Epinephelus hexagonatus*	HQ840453	Belawan
BLW8_16	*Larimichthys crocea*	PV174213	511	100	99.41	*Larimichthys crocea*	AP006002	Belawan
BLW18_16	*Elops saurus*	PV174215	491	97.36	97.56	*Elops saurus*	NC_005803	Belawan
L30	*Seriola lalandi*	PV174217	490	100	100	*Seriola lalandi*	KX234657	Langsa
LGS6_16	*Sphyraena jello*	PV174219	651	90.02	88.63	*Sphyraena barracuda*	NC_022484	Langsa
GS7	*Larimichthys crocea*	PV174220	560	100	100	*Larimichthys crocea*	GU120089	Langsa
LGS8	*Thunnus maccoyii*	PV174221	350	100	100	*Thunnus maccoyii*	LN558761	Langsa
LK3_16	*Scomberoides commersonnianus*	PV174223	490	99.59	99.59	*Scomberoides commersonnianus*	MN023229	Lubuk Kertang
LK25_16	*Elops hawaiensis*	PV174225	607	99.51	99.51	*Elops hawaiensis*	NC_005798	Lubuk Kertang
P24	*Sillago attenuate*	PV174228	630	100	100	*Sillago attenuata*	MF572044	Pulau Sembilan
PC12	*Cottoperca gobio*	PV174230	560	100	100	*Cottoperca gobio*	AY520102	Percut Sei Tuan
PCT1_16	*Epinephelus coioides*	PV174231	618	94.04	89.32	*Epinephelus coioides*	NC_011111	Percut Sei Tuan
PCT4	*Hexanematichthys sagor*	PV174232	560	94.15	91.96	*Hexanematichthys sagor*	FJ626072	Percut Sei Tuan
PS17_16	*Ilisha elongata*	PV174234	630	99.51	96.19	*Pellona ditchela*	AP011609	Pulau Sembilan
PS28	*Larimichthys crocea*	PV174236	420	100	100	*Larimichthys crocea*	GU120089	Pulau Sembilan

The present work used 16S rRNA barcoding, a well-conserved genetic marker effective for broad taxonomic classification. However, this method has limitations in distinguishing closely related species due to low genetic variability. By contrast, the COI gene is commonly used for species-level identification because it provides greater resolution. Emerging techniques such as environmental DNA metabarcoding offer noninvasive biodiversity monitoring by detecting fish DNA in water samples. Although 16S rRNA barcoding validated 17 species in the current research, some species require additional molecular markers for precise identification. Morphological identification remains an essential tool for field surveys; however, it has limitations in distinguishing cryptic species and early life stages. DNA barcoding using mitochondrial genes, such as 16S rRNA and COI, has proven effective in improving species identification accuracy (Baderan *et al.,* 2023). The present work used 16S rRNA due to its efficiency in resolving phylogenetic relationships among closely related fish species. However, COI and internal

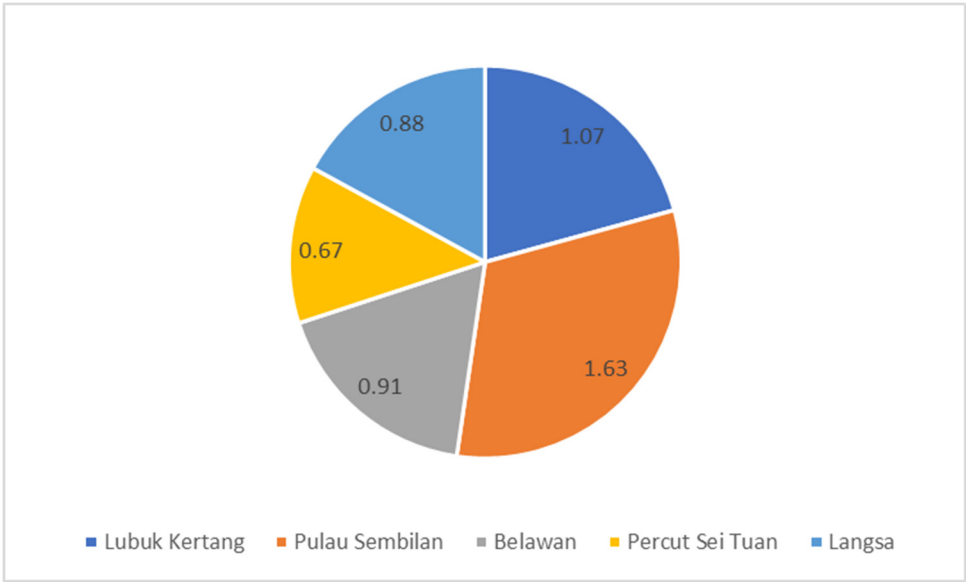

Fig. 4: Richness index of mangrove flora at observation location

transcribed spacer markers are known to provide high taxonomic resolution in some cases (Imtiaz *et al.,* 2017). Future studies should incorporate multiple markers to enhance species-level identification and confirm ambiguous classifications. The discovery of high genetic diversity within several species suggests the presence of localized adaptations and subpopulations, which could have important implications for fisheries management and conservation (Hubert *et al.,* 2015). Although DNA barcoding is already widely used tool, the current study contributed to its further development by applying it to the poorly studied mangrove fish communities along the east coast of Sumatra and Aceh. The integration of morphological and molecular approaches enables an accurate assessment of biodiversity, particularly for cryptic species. Future research should focus on expanding local genetic reference databases to improve taxonomic resolution and enhance conservation efforts.

In this study, 88% of fishes distributed on the eastern coast were identified in the field work (Fig. 5). Molecular work successfully confirmed 17 out of the 50 sequencing data (58%). The most abundant families are Serranidae, Pomacentridae, and Labridae. Morphology analysis also detected the presence of some rare and endangered species, including the humphead wrasse (*Cheilinus undulatus*) and giant grouper (*Epinephelus lanceolatus*). The overall species diversity and abundance have exhibited a decline over the 2-year monitoring period. This finding corresponds with previous research highlighting the benefits and drawbacks of both methodologies. Conventional field identification, although beneficial for swift evaluation, may be affected by observer bias and contextual factors (Barreto *et al.,* 2022). Although DNA barcoding and environmental deoxyribonucleic acid (eDNA) metabarcoding have demonstrated enhanced accuracy, they are frequently constrained by insufficient reference datasets (Keck *et al.,* 2022). The integration of both methodologies improves species detection, particularly for cryptic and immature species that are challenging to identify morphologically (Sheth and Thaker, 2017). Only 17 out of the 62 morphologically identified species were confirmed using 16S rRNA barcoding. This discrepancy may be attributed to the gaps in reference databases, PCR amplification failures, or cryptic species misclassified in morphological assessments (Phukuntsi *et al.,* 2016). Future studies should expand genetic reference libraries and incorporate multiple genetic markers to improve species validation.

This study demonstrates the efficacy of DNA monitoring for assessing fish biodiversity and

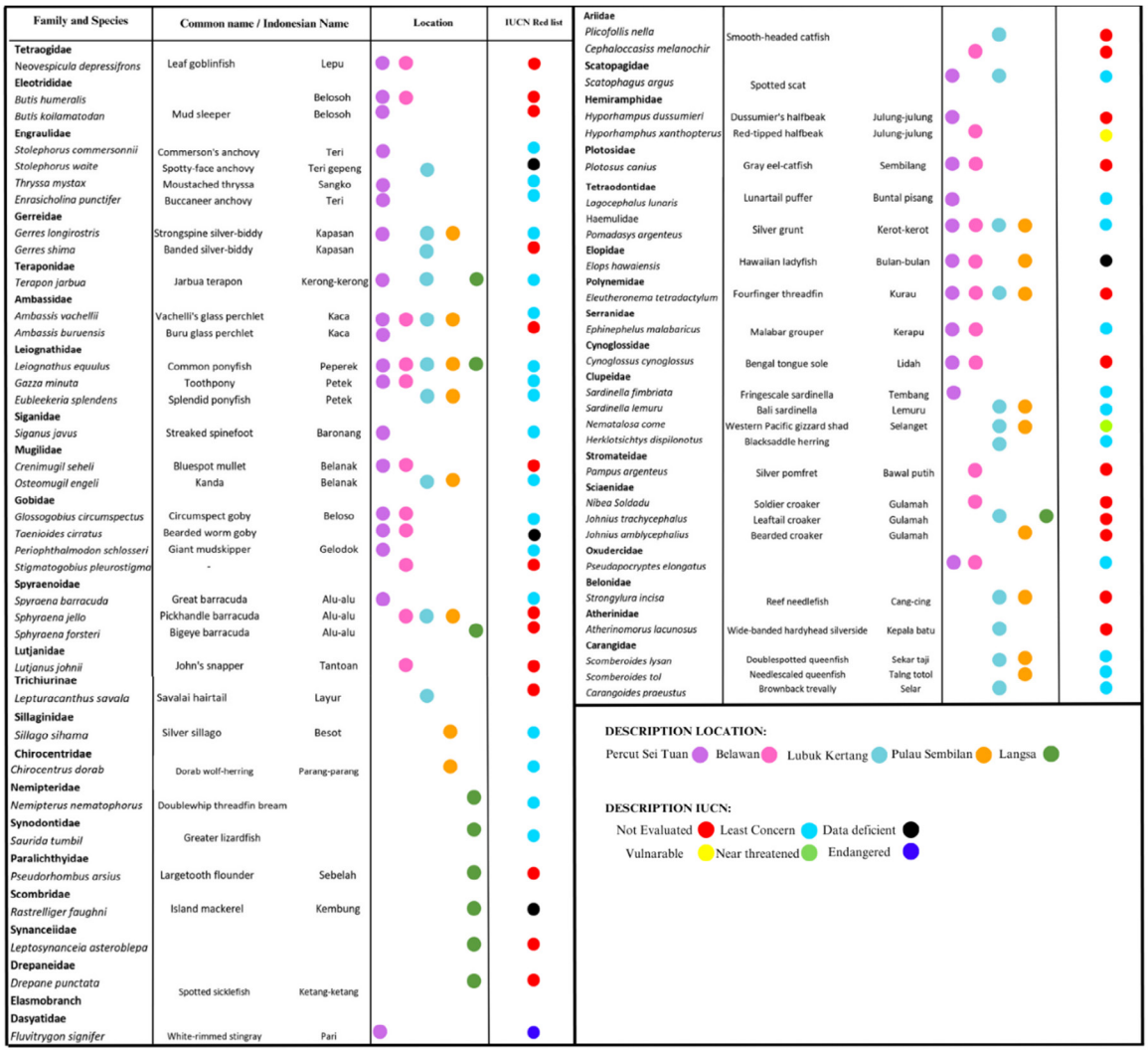

Fig. 5: Identification of family, species fish diversity, and International Union for Conservation of Nature (IUCN) status through field work on the study sites

abundance in the east coast of North Sumatra and Aceh, Indonesia (Fig. 6). The decline in species diversity and abundance over the 2-year monitoring period highlights the urgent need for conservation efforts in the region. In addition, the detection of rare and endangered species using DNA monitoring underscores the importance of protecting these species and their habitats. DNA monitoring can provide significant results.

The results of this study demonstrate the efficacy of DNA monitoring for assessing fish biodiversity and abundance in the east coast of North Sumatra and Aceh, Indonesia (Fig. 7). The Shannon–Wiener Index (H') value shows significant differences among the locations, indicating that the diversity of fish from each location vary and is strongly influenced by environmental factors. Meanwhile, the evenness index (E) tends to be high at each location, indicating that fishes are generally uniformly distributed within each location. Confidence interval (CI) values were added to ensure that the values are accurate and confirm the variation in fish species diversity among the sites. DNA monitoring can also provide significant results. The dominance of Serranidae (groupers),

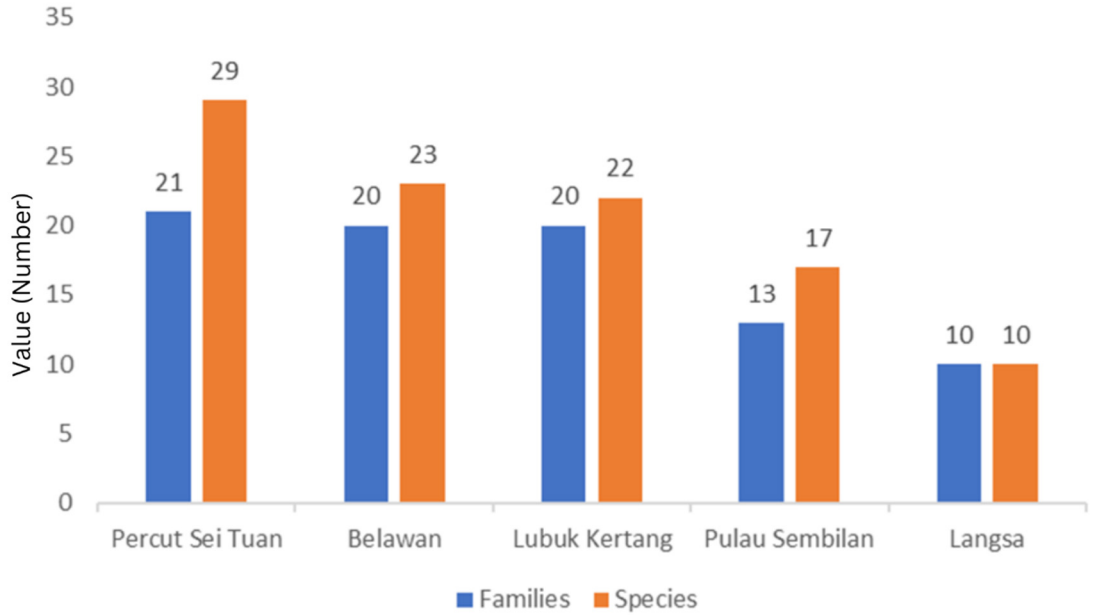

Fig. 6: Distribution of fish in the east coast of North Sumatera and Aceh, Indonesia

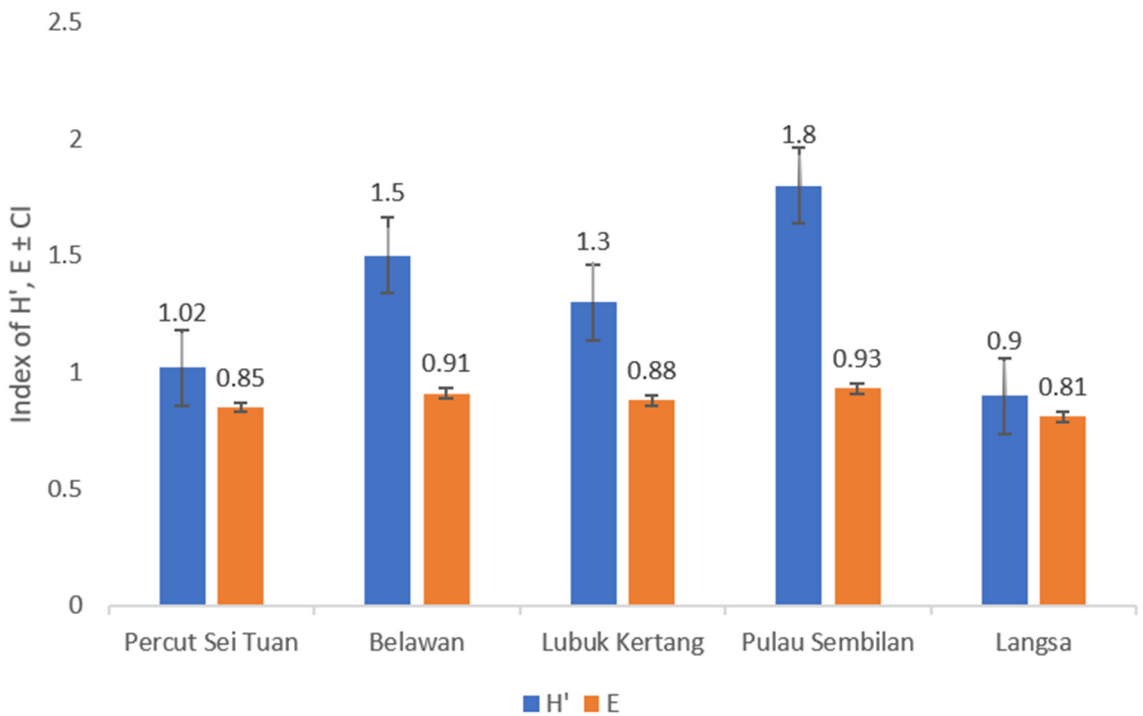

Fig. 7: Indices of diversity and evenness of fish in the research sites

Pomacentridae (damselfish), and Labridae (wrasses) is consistent with previous studies in tropical reef and coastal habitats (Du *et al.,* 2020). These families play significant ecological roles: Serranidae includes top predators that regulate fish populations, Pomacentridae are key reef inhabitants influencing coral health, and Labridae contribute to reef maintenance by controlling algal growth (Seemann *et al.,* 2018). The presence of these families suggests that the area supports a functional mangrove ecosystem. In general, the fish diversity in the mangrove ecosystems of North Sumatra and Aceh is influenced by various environmental factors such as water salinity, which in turn is affected by tides and rainfall. Optimal salinity allows various fish species to grow and reproduce, and extreme salinity fluctuations can limit species distribution. Salinity levels differ at each location where mangroves grow in North Sumatra and Aceh (Basyuni *et al.,* 2019), causing variations in the composition of mangrove species that grow. The density of vegetation determines the composition of fish that provide ideal habitat for protection, spawning, and food sources. In addition, the distribution of fish diversity varies because each species exhibits a specific type of salinity tolerance per habitat (Hairani *et al.,* 2023).

A heatmap representation showing the distribution of fish species and families across the five different study sites. Purple color indicates the presence of a species or family at a site, with darker color intensity indicating higher abundance (Fig. 8). Some species are found to be widespread in several locations, while others only occur in certain locations. In total, 62 species were identified exclusively using morphology, and 17 species were identified exclusively through DNA barcoding. This study represents the fish species detected across five different sampling locations: Percut Sei Tuan (28 species), Belawan (23 species), Lubuk Kertang (23 species), Langsa (10 species), and Pulau Sembilan (17 species). The total number of species per site using DNA Barcoding was 3 for Percut Sei Tuan, 5 for Belawan, 2 for Lubuk Kertang, 4 for Langsa, and 3 for Pulau Sembilan. In this study, 62 fish species belonging to 39 families were identified using morphological characteristics. DNA barcoding successfully confirmed 17 species, with some

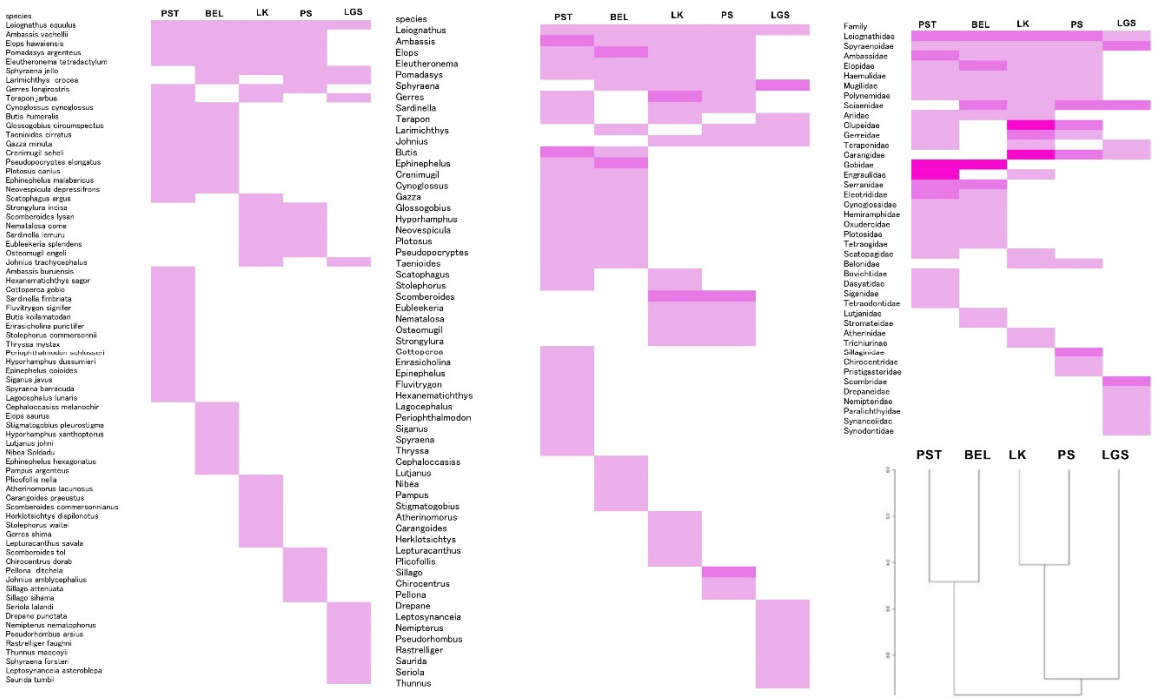

Fig. 8: Heatmap analysis of species and family distribution patterns from each site, Langsa (LGS), Lubuk Kertang (LK), Pulau Sembilan (PS), Belawan (BEL), and Percut Sei Tuan (PST)

requiring further genetic analysis for precise identification. Identification using the combination of morphology and DNA barcoding revealed 62 fish species identified morphologically and 17 fish species successfully validated using DNA barcoding. Molecular analysis of the phylogenetic tree revealed genetically distinct lineages. According to their IUCN status, two fish species were found to be threatened with extinction, namely, *L. crocea* and *T. maccoyii* categorized as endangered and critically endangered, respectively. This result shows the potential of the mangrove ecosystem as a good protection habitat for these vulnerable species. Other species such as *Elops* sp. that appear in some locations indicate the possibility of the hidden diversity of these fishes, which require further exploration. This finding is consistent with the previous study of (Hubert *et al.* 2015), which highlighted the ability of DNA barcoding to uncover cryptic diversity and differentiate morphologically similar species. The differences in species composition between suggest variations in species detectability (Fig. 8), possibly due to seasonal fluctuations, habitat degradation, or fishing pressure (Winn *et al.,* 2021). Traditional morphological identification has long been used in fish biodiversity assessments; however, it faces several limitations. Phenotypic plasticity, cryptic species, and ontogenetic variations can lead to misidentification, particularly in species with overlapping morphological traits. Molecular techniques, such as 16S rRNA and COI barcoding, provide an accurate and reproducible approach by relying on genetic markers that remain stable across different life stages and environmental conditions. Moreover, DNA-based methods enable the detection of cryptic diversity and rare species that may be overlooked in conventional surveys. DNA barcoding and phylogenetic studies allow for accurate species identification, especially cryptic species that are difficult to distinguish morphologically. These methods also help us understand the genetic diversity within mangrove populations, which is important in determining the level of adaptation to environmental changes and external threats, such as climate change and pollution (Gostel and Kress, 2022). Nevertheless, morphological analysis remains necessary to understand the structure and growth of mangrove plants in relation to environmental factors, such as salinity, substrate, and tides. The combination of these two methods provides a comprehensive picture of the condition of mangrove ecosystems and supports science-based decision-making in conservation. In long-term conservation planning, the integration of molecular and morphological methods is helpful in monitoring the health of ecosystems and the effectiveness of mangrove rehabilitation. Knowing the genetic variation between populations allows the development of restoration strategies that consider species' resilience to specific environmental stresses. In addition, genetic mapping can help in determining priority conservation zones to protect mangrove populations with high genetic diversity, which make them resilient to climate change and anthropogenic pressures. Studies on fish diversity in other ecosystems also showed that mangrove ecosystems can be potential habitats for various fish species. Henc, species richness varies from one location to another, indicating that the environmental factors in each location are influential. The Shannon–Wiener Index (H') in the whole research area (0.9–1.8) tends to be higher than that in the restored mangrove ecosystem in Malang, Indonesia (H'<1) (Ridlo *et al.,* 2020). The relatively low diversity of the latter indicates that restoring this mangrove forest requires a long time to rebuild the fish community and the full function of the mangrove ecosystem. Research conducted on natural mangrove conditions in Qinzhou mangrove forest, China revealed the presence of a semi-closed estuary system that allows stable environmental conditions; 67 fish species were found in this location, which is also known as a large and protected mangrove forest (Huang *et al.,* 2016). A high level of diversity was also recorded in the mangrove forests of Oman with 177 fish species; these mangrove forests are in active conservation and have a close relationship with the open ocean system (Al Jufaili *et al.,* 2021). Seasonal variations significantly affect the composition and abundance of fish species in mangrove waters. Indonesia has two seasons, so each seasonal change alters the environmental conditions, such as salinity, temperature, and water flow; fish species can grow and develop under low salinity, which is optimal in finding nursery habitats (Purwanto *et al.,* 2022). Climate change plays a role in biodiversity dynamics by affecting fish migration patterns, reproductive cycles, and food availability. Some species such as baronang (Siganidae), mullet (Mugilidae), and snapper (Lutjanidae) may use mangrove areas

seasonally as spawning and rearing grounds before migrating to offshore waters. The high species richness observed in Percut Sei Tuan and Belawan aligns with findings from fish diversity studies in coastal areas, where estuarine and mangrove-associated habitats serve as important nursery grounds (Sheaves *et al.*, 2015). The low species richness in Pulau Sembilan and Langsa may be attributed to habitat fragmentation or inadequate sampling efforts (Basyuni *et al.,* 2022). The observed decline in species diversity and abundance over the 2-year monitoring period highlights the urgency of conservation efforts in the region. Previous research documented mangrove deforestation, coastal pollution, and overfishing as key drivers of fish population decline (Carlson *et al.,* 2021). To address this problem, future research should focus on longitudinal monitoring programs (Bhowmik *et al.,* 2022) and the expansion of genetic reference databases for accurate species identification. Moreover, advancements in eDNA metabarcoding and remote sensing technologies can provide noninvasive and efficient biodiversity assessments (Zhang *et al.,* 2023Expanded genetic reference databases can help improve the accuracy of species identification through DNA barcoding methods by providing a large amount of comparison data to match DNA sequences with previously identified species. With many species documented in the database, identification errors due to lack of references can be minimized (Gostel and Kress, 2022). This step is especially important for the taxonomy of species that are difficult to distinguish morphologically. In addition, an extensive database enables the detection of new or invasive species that may not have been conventionally recognized, thus contributing to accurate conservation efforts and ecosystem monitoring (Imtiaz *et al.,* 2017). In future biodiversity studies, a complete genetic database will serve as an important tool for species monitoring and protection. For example, in mangrove ecosystems and surrounding waters, widely referenced DNA barcoding methods can be used to quickly and noninvasively identify organisms without having to capture or damage them. This approach allows for an efficient and real-time monitoring of ecosystems, aiding in scientific-based conservation decision-making. Obstacles in the form of limitations related to taxonomy and databases were encountered in the present study. Several taxa of identified species showed low sequence similarity with existing GenBank references. Molecular-scale studies, such as through DNA barcoding, are warranted to validate morphological species identification. The limitation of using one molecular marker resulted in the limited resolution of mangrove ecosystem fish taxonomy. To overcome these challenges, future research can be carried out by integrating several molecular markers, such as COI with high discriminatory power for identification. In addition, technological advances with eDNA metabarcoding applications can be an opportunity for monitoring fish diversity.

Conservation implications

The observed decline in species richness and abundance over the 2-year study period underscores the pressing need for conservation measures. The detection of rare and endangered species, such as *C. undulatus* (humphead wrasse) and *E. lanceolatus* (giant grouper), further highlights the ecological significance of these mangrove ecosystems. Strengthening habitat protection, enforcing sustainable fishing practices, and promoting community-based conservation programs are essential for mitigating biodiversity loss in these coastal areas.

The phylogenetic tree constructed using 16S rRNA molecular markers represents the evolutionary relationships among the fish species identified in a mangrove ecosystem or estuarine habitat in North Sumatra and Aceh (Fig. 9). DNA barcoding successfully identified 17 out of the 62 morphologically identified species; 9 species required further investigation. The bootstrap value on the phylogenetic tree varies with different CIs of 0.63–1. Bootstrap values of more than 0.70 (70%) indicate strong support for the clade in the construction of the phylogenetic tree. The clades formed from each species and clustered indicate that the species have a high relationship and confirm the accuracy of the use of molecular markers. According to the phylogenetic tree, *L. crocea* (critically endangered) and *T. maccoyii* (endangered) are in a specific clade, indicating their unique evolutionary history different from the other species. The high genetic diversity within some species indicates subpopulation structures, which are critical for conservation planning in mangrove habitats. Some species, such as *Elops* sp. and *Pellona ditchela*, appear

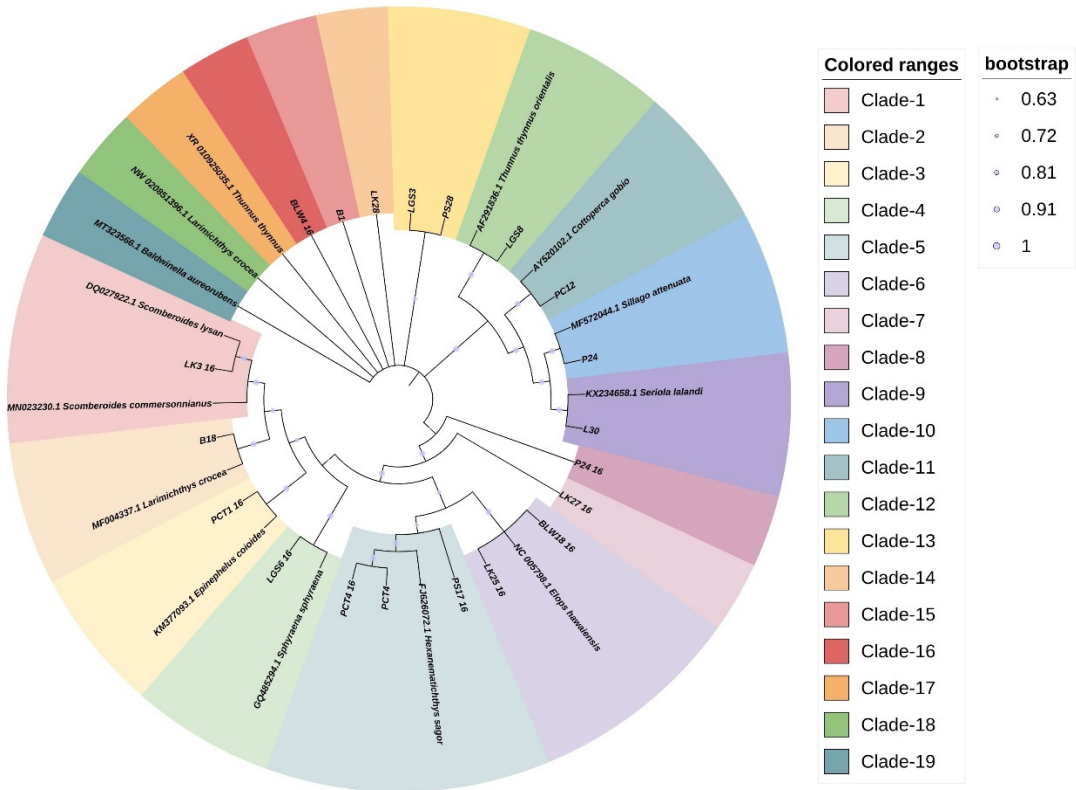

Fig. 9: Phylogenetic diversity of fishes from five sites derived from DNA barcode data

multiple times, which may indicate cryptic diversity (different species that look similar morphologically but are genetically distinct). Mangrove forests serve as nurseries and feeding grounds for many of these species, particularly juvenile fish and crustaceans. The high fish diversity in these mangrove ecosystems can be a reference for sustainable fish management. These mangrove ecosystems can be a natural nursery, hence requiring active conservation efforts. Policy recommendations include protecting estuarine mangrove habitats by limiting fishing in spawning areas, especially for endangered fish species. Conservation measures for endangered fish must be strengthened based on the results of studies at each location, such as *L. crocea* and *T. maccoyii* being threatened with extinction based on the IUCN. Furthermore, ecosystem-based fish management must be carried out by looking at the ecological considerations of each identified species. Community involvement can be invoked by integrating scientific knowledge and traditional knowledge from the community. The presence of commercially important fish (*Epinephelus fuscoguttatus*) highlights the economic value of mangrove ecosystems for local fisheries (Kadim *et al.,* 2022This phylogenetic analysis provides critical insights into fish biodiversity in mangrove ecosystems. For strengthened conservation efforts, long-term monitoring using eDNA metabarcoding is recommended to detect species shifts due to environmental changes. Restoration programs should focus on preserving key mangrove habitats that support nursery grounds and fisheries. Further genetic studies are needed for species requiring advanced taxonomic classification (Wasik and Pattison, 2024). Mangrove ecosystems support the early life stages of many fish species. Their complex roots provide a protective habitat for larvae and young fish from predators and strong currents (Hammerstein *et al.,* 2024). These structures also serve as spawning grounds for many fish species,

providing an environment rich in food resources, such as plankton, detritus, and small invertebrates, which are essential for the growth of young fish. Ecologically, mangrove ecosystems are critical to maintain the viability of fish populations and the biodiversity of coastal waters. Mangroves act as a nursery for fish that will later migrate to open waters and as a provider of organic carbon that supports the marine food chain. Therefore, the conservation and rehabilitation of mangrove forests are necessary to ensure the continuity of these ecological functions and support the natural regeneration of various fish species. Rehabilitation efforts are carried out through the Community-Based Mangrove Management (CBMM) program, which aims to restore degraded mangrove forests and protect coastal areas in an effort to conserve biodiversity (Damastuti *et al.,* 2022). In addition, the success of mangrove forest rehabilitation through restoration has significance in forest conservation efforts. This goal is in line with the government's ambition to restore 600,000 ha of mangrove forests in Indonesia in a sustainable manner (Sasmito *et al.,* 2023). The important role of community governance is to maintain and preserve the integrity of restored mangrove forests and their biodiversity. Mangrove forest restoration and conservation efforts should consider the importance of poverty alleviation through alternative livelihood options and community awareness activities related to optimal forest resource management (Rejeki *et al.,* 2021). Mangrove forest rehabilitation in Indonesia has been carried out with a comprehensive goal of recovering lost biodiversity through sustainable forest restoration strategies. One important approach is to strengthen community-based mangrove management. As key stakeholders in forest conservation, local communities mut be supported with sustainable livelihood programs, such as aquaculture and ecotourism, to reduce their dependence on exploiting forest resources (Khan *et al.,* 2020). Exploitation of fisheries resources by using trawling or netting small fish causes a decline in fish populations. The development of coastal areas, such as the power plant in Lubuk Kertang and the conversion of land into ponds, narrows the living space and hinders the growth of mangrove ecosystems. Therefore, strict conservation strategies must be applied to protect biodiversity in mangrove ecosystems without triggering conflicts with neighboring communities. One approach is sustainable fisheries management, such as the Silvofishery system that has been applied to various mangrove forest areas. This conservative measure will be an addition to restoration, so that the ecological function of mangroves can be stabilized (Pandiangan *et al.,* 2024). Other strategies include the formation of forest farmer groups and increased community participation in conservation. Mangrove rehabilitation through restoration activities with local environmental conditions is essential to restore ecological functions, such as providing habitat for fish and other organisms. In addition, the implementation of protection zones and restrictions on fishing activities in vulnerable areas will help maintain the balance of fish populations. The use of environmentally friendly fishing gear and the enforcement of no-take periods during the spawning season are also effective in ensuring the survival of fish species that depend on mangrove ecosystems. Involving local communities in conservation programs, such as mangrove-based ecotourism and environmental education activities, can increase their awareness and concern for protecting these ecosystems. Through a holistic and scientific-based approach, biodiversity in mangrove ecosystems can be protected in the long term while providing economic and ecological benefits to the surrounding communities. The health of a mangrove ecosystem can be seen in the variability of fish diversity living in the mangrove ecosystem over time. Long-term monitoring is needed to ensure the value of sustainable fish diversity. This program can be maximized by updating technology in monitoring fish diversity. The eDNA method, which identifies fish diversity using metadata from water samples collected in mangrove ecosystems, also increases taxonomic resolution. Advanced technologies, such as underwater camera monitoring and hydroacoustic surveys, can be employed to minimize disturbance. Regular monitoring can also be carried out by involving communities such as local fishermen who live around mangroves to increase data coverage and conservation efforts from the community. However, the challenges to long-term monitoring include seasonal fluctuations and difficulty in identifying morphologically similar species. In addition, ongoing funding, ecological and taxonomic expertise, and cross-disciplinary collaboration are required for implementation. Collaborative engagement is needed

for every stakeholder who plays a role in this continuous monitoring to overcome these challenges.

CONCLUSION

The findings of this study highlight significant variations in fish biodiversity across different mangrove sites. The integration of morphological and molecular approaches provided a comprehensive assessment of species diversity, revealing 62 species detected through morphological identification, 17 of which were confirmed through DNA barcoding. This discrepancy underscores the need for expanding genetic reference databases, as some species may lack adequate sequences for comparison. In addition, the differences in species composition across sites suggest that habitat quality, environmental stressors, and anthropogenic activities shape fish communities. The dominance of *Leiognathus equula*, *Ambassis vachellii*, and *Pomadasys arg*enteus across multiple locations indicates their adaptability to mangrove ecosystems. Meanwhile, the limited distribution of certain species suggests their specific habitat dependencies or localized threats. The decline in overall species diversity over the 2-year monitoring period raises concerns about habitat degradation and the effectiveness of current conservation strategies. Moreover, the detection of endangered and critically endangered species (*L. crocea* and *T. maccoyii*) underscores the urgency of targeted conservation measures. Future research should focus on several key areas to enhance conservation and biodiversity monitoring efforts. Expanding genetic reference databases is crucial to improving species identification accuracy through DNA barcoding. Long-term monitoring programs should also be established to track changes in fish biodiversity and assess ecosystem health over time. Habitat protection and restoration efforts, such as mangrove reforestation and enforcement of sustainable fishing practices, must be prioritized to mitigate habitat loss. Community engagement is also vital in strengthening biodiversity conservation. Increasing local participation through education programs and citizen science initiatives can foster awareness and encourage responsible resource management. The integration of advanced technologies, including eDNA metabarcoding and remote sensing, offers efficient and noninvasive methods for biodiversity assessment, allowing for a comprehensive and real-time monitoring of fish populations. With these strategies, biodiversity conservation in mangrove ecosystems can be strengthened to ensure the sustainability of fisheries and the resilience of coastal ecosystems in the face of environmental challenges. The limitations of this study are as follows: closely related species may still have identical 16S rRNA sequences, leading to possible taxonomic uncertainty at the species level. Some species may have identical or highly similar sequences, leading to taxonomic uncertainty. Future studies should incorporate COI or whole-genome sequencing (WGS) to refine species identification. The reference database lacks certain fish species or has incomplete records, and the remaining 13 fish specimens may have low identity matches or ambiguous identifications. A collaborative approach involving researchers, policymakers, local communities, and conservation organizations is essential for ensuring the long-term sustainability of fisheries and the protection of coastal biodiversity. Forest rehabilitation through restoration aims to repair the diversity of mangrove ecosystems. The involvement of external organization and communities is crucial to the success of national and regional mangrove rehabilitation projects. In addition to the legal safeguards and sanctions in place for fishing practices that damage the ecosystem, government policies concerning mangrove rehabilitation must include ways to communicate and educate the public on the importance of managing natural resources and protecting forests. Continued research and monitoring efforts will be key to understanding the dynamics of fish populations and addressing the ongoing challenges posed by environmental changes and human activities. Another limitation of this study is the exclusive use of 16S rRNA barcoding, which may not differentiate closely related species.

AUTHOR CONTRIBUTIONS

M. Basyuni, the corresponding author, contributed to this conceptualizing this study, applying for funding, prepared the manuscript text. Irmawati contributed to this collected data and analysis data. A. Mubaraq contributed to acquisition of data and writing manuscript. A. Susilowati helped to writing manuscript and input data. A.B. Rangkuti contributed writing the manuscript and revised manuscript. Desrita contributed to writing manuscript and reviewing the manuscript. A.A. Aznawi analyzing data

and writing manuscript. S.S. Mustaniroh contributed to data analysis and reviewing the manuscript. S.H. Larekeng statistical and writing the initial manucript. A.H. Salman contributed to spasial analysis and writing manuscript. E.R. Ardli contributed to obtaining funding dan supervision. Y. Isowa designing the experimental work and data analysis. T. Kajita performed a part of data analyses.

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CONFLICT OF INTEREST

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

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ABBREVIATIONS

%	percent
∑	Sigma
ANOVA	Analysis of variance
BEL	Belawan
BLAST	Basic local alignment search tool
BP	Base pairs
CBMM	Community-based mangrove management
CI	Confidence interval
COI	Cytochrome oxidase Subunit
D	Distribution
DDBJ	DNA Data Bank of Japan
DNA	Deoxyribonucleic acid
E	The evenness index
ENA	European Nucleotide Archive
eDNA	Environmental deoxyribonucleic acid
F	Forward
H'	The diversity index
H'max	The maximum of diversity index
ha	Hectare
ID	Identifier
IUCN	International union for conservation of nature
iTOL	interactive tree of life
ITS	Internal Transcribed Spacer
LGS	Langsa
LK	Lubuk Kertang
M	Meter
MEGA X	Molecullar Evolutionary Genetics Analysis
MOTUs	Molecular Operational Taxonomic Units
N	Total number of individuals
Ni	Number of individuals of species i
N.loc	Total sampling locations
Ni.loc	Total number of locations
NJ	Neighbor-joining
PCR	Polymerase chain reaction
PCT	Percut Sei Tuan

Pi	The ratio of individuals of species
PS	Pulau Sembilan
rRNA	Ribosomal ribonucleic acid
R	Reverse
(R)	Richness index
S	Total number of species
WGS	Whole-genome sequencing

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AUTHOR (S) BIOSKETCHES

Basyuni, M., Ph.D., Professor, Center of Excellence for Mangrove, Universitas Sumatera Utara, Jalan Perpustkaan No. 3A, Medan 20155, Indonesia.
- Email: *m.basyuni@usu.ac.id*
- ORCID: 0000-0003-1120-8571
- Web of Science ResearcherID: D-8537-2015
- Scopus Author ID: 15055287200
- Homepage: https://puimangroveusu.id/

Irmawati, Ph.D., Lecturer, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar 90245, Indonesia.
- Email: *irmawati@unhas.ac.id, trif.ahwa@gmail.com*
- ORCID: 0009-0002-9739-3199
- Web of Science: JWO-1574-2024
- Scopus ID: 57194702752
- Homepage: https://fikp.unhas.ac.id/2010/03/dosen-prodi-msp/

Mubaraq, A., Ph.D. Candidate, Doctoral Program of Agriculture Science, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia.
- Email: *alfian@students.usu.ac.id*
- ORCID: 0009-0005-9810-3896
- Web of Science: JTU-5714-2023
- Scopus ID: 59122664800
- Homepage: https://www.usu.ac.id/

Susilowati, A., Ph.D., Professor, Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan 20155, Indonesia
- Email: *arida.susilowati@usu.ac.id*
- ORCID: 0000-0001-9608-4787
- Web of Science: AAF-6384-2021
- Scopus ID: 56102433700
- Homepage: https://www.usu.ac.id/id/direktori/arida-susilowati

Rangkuti, A.B., M.Sc., Lecturer, Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan 20155, Indonesia.
- Email: baiqunirangkuti@usu.ac.id
- ORCID: 0000-0002-3019-6038
- Web of Science: GPK-3826-2022
- Scopus ID: 57211799613
- Homepage: https://www.usu.ac.id/id/direktori/ahmad-baiquni-rangkut

Desrita., M.Si, Ph.D. Candidate, Department of Aquatic Resources Management, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia.
- Email: *desrita@usu.ac.id*
- ORCID: 0000-0002-1935-8294
- Web of Science: AFR-6165-2022
- Scopus ID: 57156632300
- Homepage: https://fp.usu.ac.id/id/dosen/desrita

AUTHOR (S) BIOSKETCHES

Aznawi, A. A. Ph.D. Candidate, Doctoral Program of Agriculture Science, Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia.
- Email : aznanaznawi@students.usu.ac.id
- ORCID : 0009-0000-5801-4702
- Web of Science ResearcherID: NA
- Scopus Author IDE: 59123096500
- Homepage: https://www.usu.ac.id/

Mustaniroh, S.S., S.Si., Researcher, Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan 20155, Indonesia.
- Email: shofiyahsabilah@gmail.com
- ORCID: 0009-0000-0370-1759
- Web of Science: NA
- Scopus ID: 57972470900
- Homepage: https://www.usu.ac.id/

Larekeng, S.H., Ph.D., Professor, Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar 90245, Indonesia.
- Email: sittihalimah@unhas.ac.id
- ORCID: 0000-0002-9749-9500
- Web of Science: NA
- Scopus ID: 57200256258
- Homepage: https://bachelor.forestry.unhas.ac.id/dosen/

Salman, A.H., S.Hut., Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar 90245, Indonesia .
- Email: alihasansalman@unhas.ac.id
- ORCID: 0009-0003-7113-1606
- Web of Science: NA
- Scopus ID: NA
- Homepage: https://forestry.unhas.ac.id/

Ardli, E.R., Ph.D., Associate Professor, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto Utara, Banyumas 53122, Central Java, Indonesia**.**
- Email: erwin.ardli@unsoed.ac.id
- ORCID: 0000-0003-0407-0017
- Web of Science: AAD-9177-2021
- Scopus ID: 25227455500
- Homepage: http://v2.pasca.unsoed.ac.id/dr-rer-nat-erwin-riyanto-ardli-s-si-m-sc/

Isowa, Y., Ph.D., Research Associate, Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, Taketomi, Okinawa, 907-1541, Japan.
- Email: isowa@lab.u-ryukyu.ac.jp
- ORCID: 0009-0009-5733-4355
- Web of Science: NA
- Scopus ID: 55341544900
- Homepage: https://tbc.skr.u-ryukyu.ac.jp/iriomote/staf

Kajita, I., Ph.D., Professor, Iriomote Station, Tropical Biosphere Research Center, University of Ryukyus, Takenomi, Okinawa, 907-1541, Japan.
- Email: kajita@mail.ryudai.jp
- ORCID: 0000-0003-1232-4179
- Web of Science: ABB-2784-2020
- Scopus ID: 7006291795
- Homepage: http://nesseiken.info/

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