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CASE STUDY

The impact of land use change on morphological and anatomical adaptations of Rhizophora species

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ABSTRACT

BACKGROUND AND OBJECTIVES: The ongoing destruction of mangrove forests highlights the urgent need for mitigation and control efforts. Indonesia, home to a rich diversity of mangrove species, has the potential to provide viable solutions to address this issue. The main objective of researchers is to maintain the existence of all species. It is crucial that research data support control and mitigation strategies to ensure efficient and optimal management of these ecosystems. Analyzing how a species responds to alterations in land use can yield significant understanding of its capacity for adaptation. The ability to adapt can be assessed by analyzing variations in leaf structure and anatomy. This study investigated the morphological and anatomical characteristics of three species in the *Rhizophora*ceae family: *Rhizophora mucronata*, *Rhizophora apiculata*, and *Rhizophora stylosa*, which thrive in three distinct mangrove ecosystems in North Sumatra Province. The purpose was to examine how effectively these species have modified their behaviors and characteristics in relation to shifts in their environmental conditions. This study sought sought to evaluate the extent to which these species have adjusted to the changing environmental conditions in their habitat located in North North Sumatra Province, Indonesia.
METHODS: This study aimed to select sites that illustrated three unique land uses residential areas in Belawan Sub-District, healthy mangrove forests in Sitardas Village, and mangrove areas designated for fish pond activities in Percut Sei Tuan Sub-District. Observations were made on three mangrove species: *Rhizophora apiculata* Blume, *Rhizophora mucronata* Poir, and *Rhizophora stylosa* Griff, to assess how their leaf morphology and anatomy respond to changes in land use within their natural environments. Leaf samples were collected from three separate locations, ensuring that ten trees of each species were included at each site. Each tree had five branches, and from each branch, five healthy leaves were selected for measurement and analysis. Data were gathered on 12 distinct characters. The method of stomatal printing was used to prepare leaf material for the analysis of stomatal characteristics. Data were collected on seven characteristics. Stomatal density and stomatal index were measured across 15 units per leaf. The morphological and anatomical characteristics of *Rhizophora apiculata*, *Rhizophora mucronata*, and *Rhizophora stylosa* were statistically analyzed using Analysis of Variance. Furthermore, a principal component analysis graphic was utilized to depict the environmental parameters, emphasizing the differences observed among the sampling locations.
FINDINGS: The results revealed that the varying land use conditions at each study site affected the three species of *Rhizophora*. *Rhizophora stylosa* was unable to grow at the Medan Belawan study site due to severe damage and pollution. In contrast, the other two sites, Percut Sei Tuan and Sitardas, are lightly polluted environments that are suitable for all three *Rhizophora* species. This finding demonstrates that certain *Rhizophora* species, specifically *Rhizophora apiculata* and *Rhizophora mucronata*, can thrive in habitat conditions and environmental factors that are unsuitable for *Rhizophora stylosa*. Furthermore, there were notable variations in the stomatal index among all three *Rhizophora* species. *Rhizophora apiculata* had a stomatal index of 19.69, *Rhizophora mucronata* had a value of 111.7, and *Rhizophora stylosa* had a value of 24.67. These variations may impact their efficiency in adapting to their respective environments.
CONCLUSION: This study offers insight into how three *Rhizophora* species adapt to changes in land use. Each species demonstrates distinct adaptations influenced by various factors. The outcomes of this study will be instrumental in suggesting species that can effectively contribute to restoration initiatives within mangrove ecosystems.

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INTRODUCTION

Indonesia is home to the largest mangrove forest area in the world, covering approximately 2.7 million hectares (Mha), which represents 22.6 percent (%) of the global mangrove forests as of 2020. Within this vast region, North Sumatra Province hosts around 57,010 hectares of mangrove forests (Basyuni *et al.,* 2022). The unique structural and functional characteristics of mangrove ecosystems contribute significantly to environmental health and support a diverse range of living organisms. These benefits include supporting essential ecological processes (Lee *et al.,* 2014; Primavera *et al.,* 2019; Mitra, 2020), providing important hydrological functions (Perez-Ceballos *et al.,* 2020; Adame *et al.,* 2021), creating habitats for diverse species (Wahyuni *et al.,* 2021; Badave *et al.,* 2023; Bindiya *et al.,* 2023), supplying food sources (Contreras *et al.,* 2018; Muro-Torres *et al.,* 2020), mitigating the impact of natural disasters (Asari *et al.,* 2021; Akram *et al.,* 2023), biogeochemical cycles including nitrogen that contribute to mitigating greenhouse gas emissions (Mubaraq *et al.,* 2024), and serving as potential areas for ecotourism (Azis *et al.,* 2018; Abdillah *et al.,* 2020). Remarkably, mangrove forests prosper in settings that are typically severe and challenging (Akram *et al.,* 2023). The organisms that inhabit these forests have adapted to cope with high salinity, strong wind, elevated temperature, anaerobic muddy soil (Koh *et al.,* 2018), and the frequent disturbances caused by tidal changes in seawater (Friess *et al.,* 2020). The global decline of mangrove ecosystems is occurring at an alarming pace, primarily due to intense human activities that exert significant pressure on these vital habitats. The pressures arise from several elements, including an increasing population, shifts in land use practices, and diverse sources of pollution caused by human activities (Ferreira and Lacerda, 2016; Carugati *et al.,* 2018; Bryan-Brown *et al.,* 2020). The Food and Agriculture Organization (FAO) reported that the total global mangrove area in 2020 is estimated at around 14.8 Mha. Between 1990 and 2020, there was a total loss of 1.04 million hectares, with the annual loss rate decreasing from 46,700 hectares per year during 1990–2000 to 21,200 hectares per year during 2010–2020 (FAO, 2020). As per the 2022 findings released by the Republic of Indonesia's Ministry of Environment and Forestry, 2.32 million hectares of the total 3.48 million hectares of mangrove land are deemed healthy, with a tree density surpassing 1,500 per hectare. In contrast, 1.16 million hectares are identified as damaged. Annually, around 200,000 hectares (ha) of mangroves are damaged in Indonesia, primarily due to land conversion for agriculture (Ilman *et al.,* 2016; Rahman *et al.,* 2020). Human activities and climate change are the primary drivers behind the degradation of coastal vegetation, including mangroves (Wang and Gu, 2021). Examples of human activities that negatively impact the landscape include land use changes, habitat loss, mangrove logging, industrial activities, the invasion of alien species, and the overexploitation of biological resources (Cahyaningsih *et al.,* 2022). Significant degradation is occurring in the mangrove forests of North Sumatra, primarily due to shifts in land use and land cover that often fail to take environmental factors into account. Study conducted by Bryan *et al.* (2013), Sasmito *et al.,* (2019), and Basyuni *et al.,* (2021) indicates that the mangrove forests in Percut Sei Tuan Sub-District, Deli Serdang Regency, as well as those studied in Medan Belawan Sub-District, Medan City, have experienced a significant decline over the past fourteen years. Through mapping and analyzing changes in mangrove forest cover using remote sensing, geographic information system (GIS), and analytical hierarchy process (AHP), one study concluded that prioritizing economic reasons is essential to halt further changes (Rahmawaty *et al.,* 2022). In contrast, the mangrove forest in Sitardas Village, located in Central Tapanuli, is well-preserved (Muhtadi *et al.,* 2020; Rangkuti *et al.,* 2024). Land-use activities play a significant role in climate change, and shifts in land-cover patterns are one of the ways the effects of climate change become evident. The relationship between land use and climate change encompasses the effects of modifications in land use on atmospheric carbon dioxide (CO2) concentrations, as well as the resulting implications for the climate system (Barati *et al.,* 2023). Climate change poses a significant threat to the survival of plants. Understanding plant anatomy and morphology offers valuable insights into their resilience, adaptation, resource efficiency, carbon dynamics, and ecosystem interactions. By examining the anatomy and morphology of plants, traits associated with tolerance to climate stress can be identified, which can help to better understand how plants adapt to changing conditions. Such knowledge can facilitate the conservation of at-risk

plant species and bolster breeding efforts designed to produce more robust plant variants, in addition to fostering sustainable management in an evolving ecological landscape. Although many studies have focused on how land use changes affect anatomy and morphology, there are still few references available on how these changes lead to environmental changes that influence the adaptation process, particularly concerning the morphological and anatomical characterizations of mangrove species. Therefore, this study examined the morphological and anatomical traits of three Rhizophoraceae species, namely *Rhizophora mucronata*, *R. apiculata*, and *R. stylosa,* growing in three distinct mangrove ecosystems in North Sumatra Province. The aim of the current study is to determine how well these species adapted to changing habitat environmental conditions. This study was carried out in North Sumatra Province, Indonesia, in 2023.

MATERIALS AND METHODS

Study area

This study was conducted from April to December 2023 in three mangrove ecosystem locations in North Sumatra Province, Indonesia. The study sites included Percut Sei Tuan Sub-District in Deli Serdang Regency, the Medan Belawan Sub-District in Medan City, and Sitardas Village in Central Tapanuli Regency (Fig. 1). Data collection and material observations were conducted over the course of the year. Nevertheless, the consistent and stable nature of air temperature and humidity in tropical regions ensured that these factors did not compromise the accuracy of the data gathered (Noer *et al.,* 2022). The mangrove ecosystems in Percut Sei Tuan and Medan Belawan Sub-Districts represent mangrove ecosystems that have been degraded (Fig. 2a and 2b), while the mangrove ecosystem in Sitardas Village represents a mangrove ecosystem that is still well-preserved

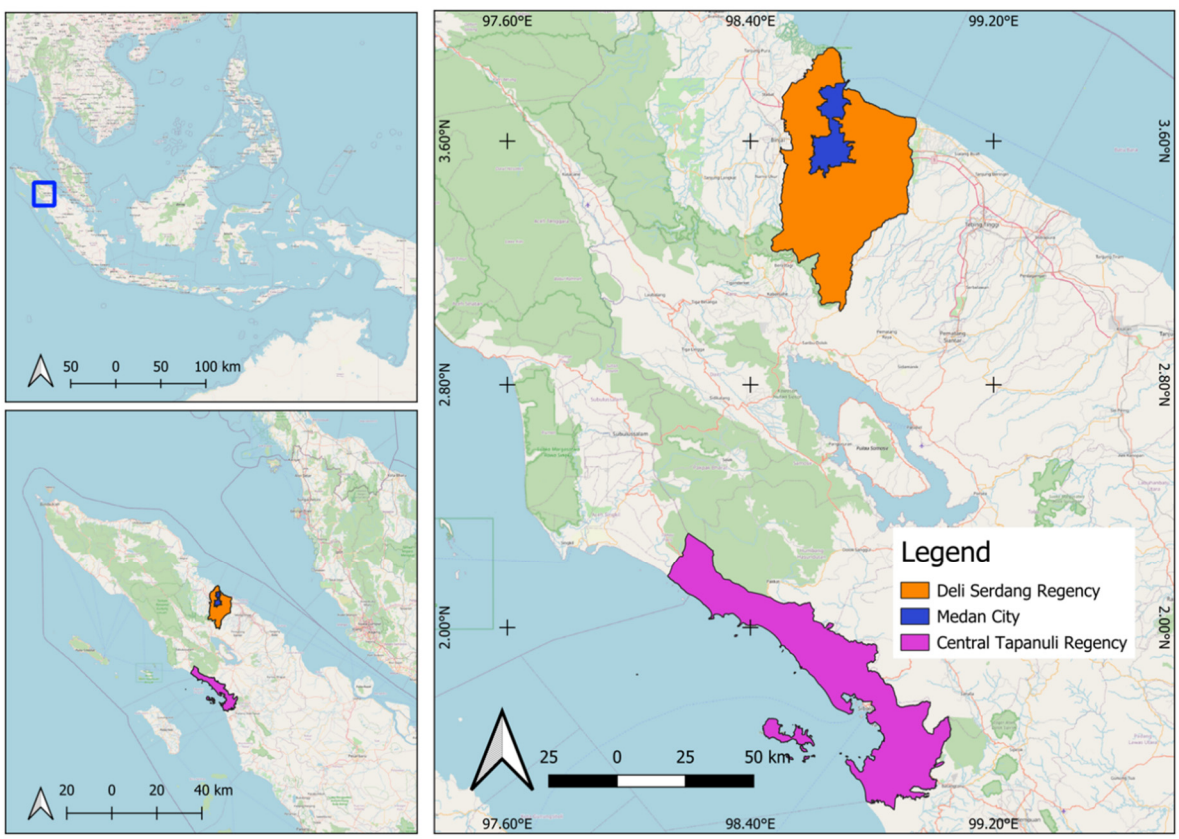

Fig. 1: Geographical location of the study area in Deli Serdang Regency, Medan City, and Central Tapanuli Regency in North Sumatra Province, Indonesia

(Fig. 2c). As of 2016, the alteration of mangrove ecosystems for different land purposes in the Percut Sei Tuan Sub-District had resulted in over 80% of these ecosystems being identified as facing moderate to high degrees of degradation (Sulistiyono *et al.,* 2019). These areas predominantly have a substrate of sandy mud (Hasibuan *et al.,* 2021). The most severe damage to mangrove ecosystems occurred in the Medan Belawan Sub-District of Medan City, which was characterized by roily seawater and elevated nitrate and phosphate levels exceeding water quality standards (Widiani *et al.,* 2021). This implies that the aquatic environments and ecosystems in the Medan Belawan Sub-District are suffering from pollution caused by human actions, including the disposal of waste, domestic refuse, and oil spills from the port of Belawan port (Harefa *et al.,* 2022). Generally, the substrate around the Medan Belawan Sub-District

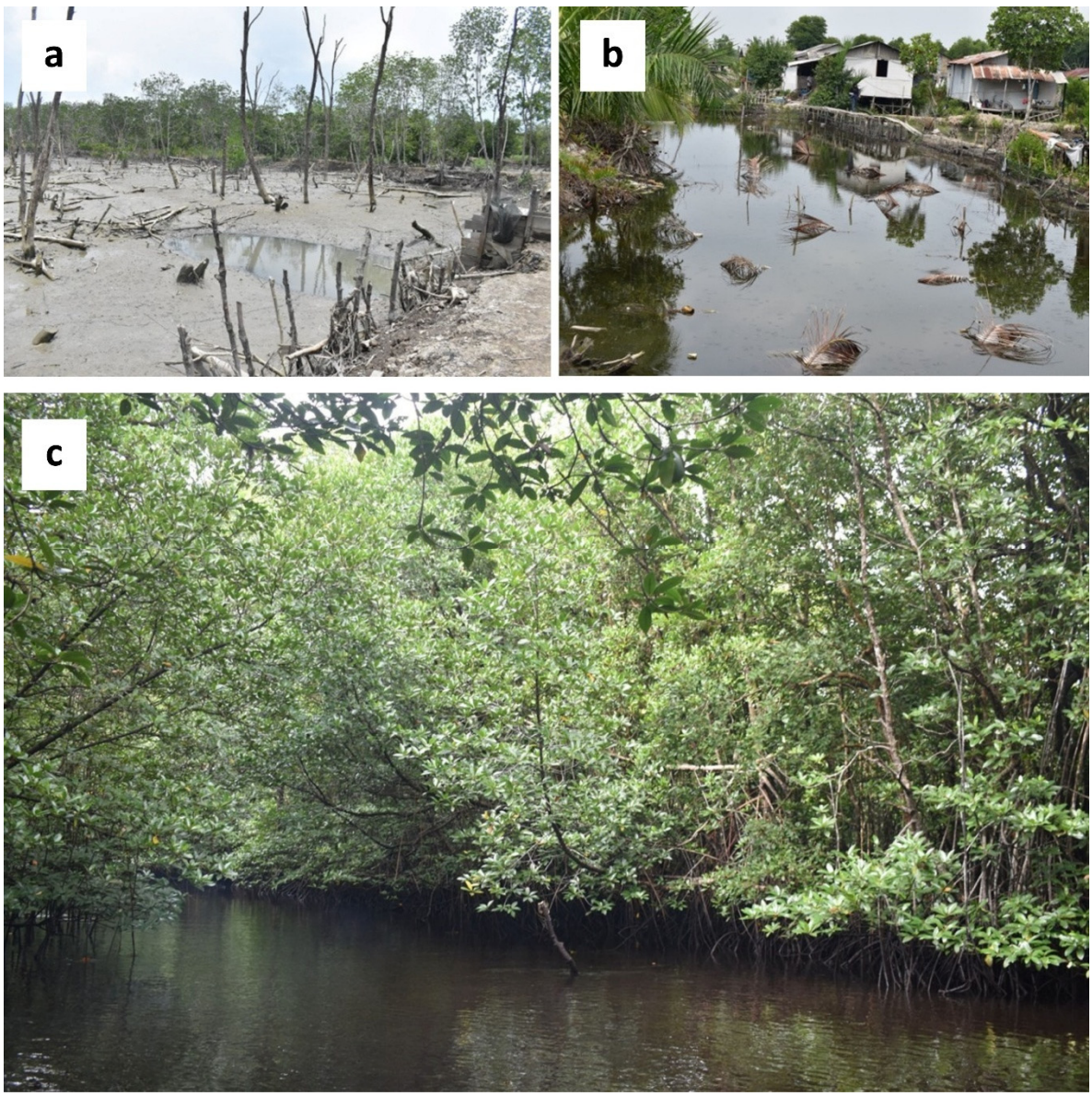

Fig. 2: Overview of the study site at the three locations: a. Percut Sei Tuan, b. Medan Belawan, and c. Sitardas

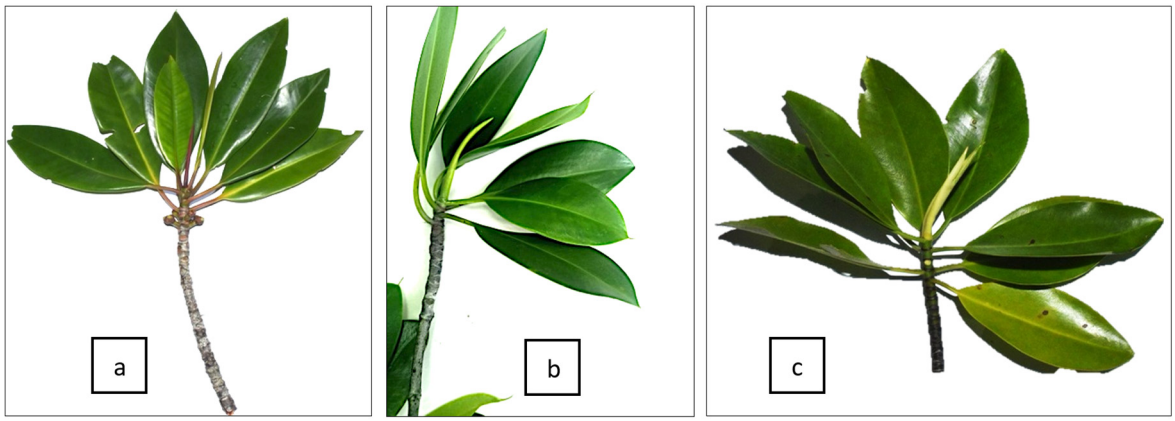

Fig. 3: Leaves sample of *Rhizophora apiculata* (a), *R. mucronata* (b), and *R. stylosa* (c).

consists of muddy clay, which contributes to a higher abundance of microplastics in mangrove sediments compared to the water flow in estuaries (Leidonald *et al.,* 2024). In contrast, the mangrove ecosystem in Sitardas Village is distinguished by a substrate that is predominantly made up of sandy soil, coral fragments, and patches of sandy mud. This ecosystem is also found adjacent to seagrass and coral reef ecosystems, which remain in good condition to this day (Muhtadi *et al.,* 2020).

Data collections

In this study, the morphology and anatomy of the leaves of three commonly found mangrove species in the Indo-Malayan region, namely R*hizophora apiculata* Blume, *R. mucronata* Poir, and *R. stylosa* Griff (Setyawan *et al.,* 2014), were observed in order to understand how these species adapt to changes in land use in their habitat (Fig. 3).

A targeted sampling method was employed to collect leaf twigs for the analysis of their morphological and stomatal characteristics. The collected leaf twigs were in healthy and intact conditions (Baroga and Buot, 2014). Data were gathered on 12 distinct characters, which included 7 qualitative and 5 quantitative characters. The observed qualitative traits encompassed the shape of the leaves, the coloration of both the upper and lower surfaces, the types of leaf tips and bases, the nature of the leaf edges, and the type of leaf reinforcement. Meanwhile, the quantitative characteristics measured consisted of stipule length (SL), lamina length (LL), leaf width (LW), petiole length (PL), and leaf point (LP). The color of the adaxial and abaxial of *Rhizophora* spp. Leaves were characterized using the Mussell Chart. A total of 50 leaf twigs from each species were observed and measured, with five twigs collected from ten trees for each species. To observe stomatal characteristics, the leaf material was prepared using the stomatal printing technique (Purnomo *et al.,* 2023). The procedure begins with selecting mature leaves from each species studied. Transparent nail polish is then applied to the upper surface of the leaf (adaxial side). Once the nail polish had dried, transparent tape was applied over it. The sample was then carefully placed onto the prepared glass. The printed epidermal layer is subsequently observed using an Olympus CX23 digital microscope connected to an Otilab camera and a computer screen. Data was gathered based on seven distinct characteristics, comprising three qualitative and four quantitative characters. The qualitative characteristics observed included stomata type, epidermis shape, and anticlinal wall type. Furthermore, the quantitative characteristics observed were: 1) The number of stomata in the field of view. 2) The length and width of stomata. 3) The number of epidermises in the field of view. Stomatal density and stomatal index were observed in 15 units per leaf. Each unit leaf area was observed under a microscope with 20x magnification and in a field of view measuring 382,900 micrometers (µm) x 722,285 µm. Additionally, the length and width of stomata were observed on 25 stomata with 40x magnification in a field of view measuring 355,674 µm x 188,420

µm. The methodology established by Dilcher (1974) was utilized to identify the characteristics of stomata.

Data analysis

A descriptive analysis of stomatal density and index was conducted, utilizing both qualitative and quantitative observations. Calculations of stomatal density and stomatal index values were analyzed using Eqs. 1 and 2 (Paul *et al.,* 2017):

$$\text{Stomatal density} = \frac{Number\ of\ stomata}{Field\ of\ view under\ microscope} \quad (1)$$

$$\text{Stomatal index} = \frac{Number\ of\ stomata}{Number\ of\ stomata + number\ of\ epidermal\ cells} \times 100\% \quad (2)$$

Statistical analysis

The morphological and anatomical characteristics of *R. apiculata*, *R. mucronata*, and *R. stylosa* were statistically analyzed using analysis of variance (ANOVA). Following the identification of statistically significant differences in the ANOVA results, additional analysis was conducted utilizing the Tukey test for post-hoc comparisons. This approach helped to identify significant differences among *R. apiculata*, *R. mucronata*, and *R. stylosa* collected from Percut Sei Tuan, Medan Belawan, and Sitardas. This analysis took into account various environmental factors including wind speed (meter per second (m/s)), light intensity (lux), humidity (%), temperature in degrees Celsius (°C), potential of hydrogen (pH), total dissolved solids (TDS), parts per million (ppm), electrical conductivity (EC), milisiemens per centimeter (mS/cm), and salinity (ppm), all of which may influence mangrove growth. These parameters were subsequently depicted in a principal component analysis (PCA) graphic to demonstrate the differences among the study locations: Percut Sei Tuan, Medan Belawan, and Sitardas. All analyses were conducted using paleontological statistics (PAST) 4.17 software (Hammer and Harper, 2024).

RESULTS AND DISCUSSION

Environmental factors affecting the mangrove ecosystem

The PCA graphic presented in this study illustrates the variance within a dataset. The x-axis (PC1) accounts for 79.28% of the variance, while the y-axis (PC2) represents 12.16%. Nearly all the variability in the dataset can be attributed to these two components, which makes them particularly suitable for comprehending and analyzing data trends. The data points are classified into three groups: Percut Sei Tuan, Medan Belawan, and Sitardas. The representation of each group is distinct, with dots for Percut Sei Tuan, squares for Medan Belawan, and diamonds for Sitardas (Fig. 4).

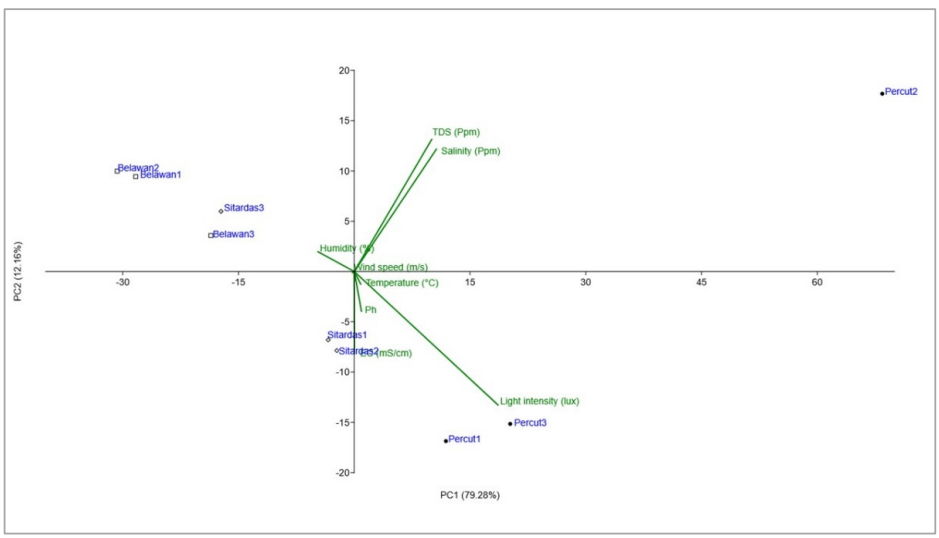

Fig. 4: Principal component analysis for three study sites

The distribution of points revealed significant differences between the various group locations. The Percut Sei Tuan data, particularly along PC1, display notable variation, with Percut-2 positioned far to the right. This indicates a specific profile in the dataset, showcasing an additional type of uniqueness when compared to the other locations. In contrast, the Belawan and Sitardas locations cluster closely to the origin, which signifies that these groups are more similar to each other and exhibit limited variance along both PC1 and PC2. The differentiation between the groups reveals important variations in their core attributes. The environmental elements in the Percut Sei Tuan group appear to be more diverse, while the environmental factors in the Medan Belawan and Sitardas groups are more uniform. Percut-2 may reflect significant variability in environmental factors due to its remote position on the plot, highlighting distinctive features not observed in other locations. The results of the PCA highlight evident groupings and illustrate that the first principal component is responsible for the largest portion of variance in the dataset, positioning it as the key axis for interpreting differences across the locations. The dataset presents an analysis of environmental parameters across three locations: Percut Sei Tuan, Medan Belawan, and Sitardas, highlighting distinct variations among the sites. Wind speed ranged from 0.53 m/s in Medan Belawan to 2.44 m/s in Sitardas, with Sitardas consistently exhibiting higher values. Light intensity demonstrated substantial variability, peaking at 86.33 lux in Percut Sei Tuan and reaching its lowest point at 11.27 lux in Medan Belawan, indicating that Medan Belawan generally experienced reduced light exposure. Sitardas experienced the highest humidity levels at 77.00%, in contrast to Percut Sei Tuan, which had the lowest at 56.20%. Throughout the observations, Sitardas consistently exhibited greater relative humidity throughout the measurements. Temperature measurements showed limited fluctuation, ranging from 30.04°C in Medan Belawan to 33.96°C in Percut Sei Tuan, with Percut Sei Tuan displaying slightly elevated temperatures compared to the other sites. The pH levels varied within a neutral to slightly alkaline range, with the highest value of 20.70 observed in Percut Sei Tuan and the lowest at 6.65 in Medan Belawan. There was notable variation in total dissolved solids (TDS), with the maximum concentration found in Percut Sei Tuan at 54.67 ppm and the minimum in Sitardas at 10.00 ppm, highlighting the increased variability in Percut Sei Tuan. Similarly, Electrical Conductivity (EC) was highest in Percut Sei Tuan (34.40 mS/cm) and lowest in Sitardas (10.64 mS/cm). Salinity ranged from 11.70 ppm in Medan Belawan to 58.29 ppm in Percut Sei Tuan, highlighting significantly elevated salinity in Percut Sei Tuan.

Morphological characteristics of R. apiculata, R. mucronata, and R. stylosa

Field observations conducted in three study sites revealed that *Rhizophora apiculata* and *R. mucronata* naturally grow in Percut Sei Tuan, Medan Belawan, and Sitardas, while *R. stylosa* is only found in Percut Sei Tuan and Sitardas. This distribution could be linked to the compromised and polluted state of the mangrove environment ecology in Medan Belawan. *Rhizophora apiculata* and *R. mucronata* thrive in intertidal mangrove environments characterized by muddy substrates and tidal influences, demonstrating adaptations to high salinity levels and harsh environmental conditions (Shamin-Shazwan *et al.,* 2021). Conversely, *R. stylosa* is usually situated in coastal mangrove habitats and is often subject to (Chen and Wang, 2017). Both *R. apiculata* and. *R. mucronata* are common mangrove species found in various regions of Indonesia and are extensively utilized by local communities. These species assist in the preservation of embankments, and their wood is valuable for making fish traps and providing firewood. Furthermore, they are distinguished by their high levels of total tannins (Setyawan *et al.,* 2019; Hilmi *et al.,* 2021). These two mangrove species often grow together, making it difficult for the community to distinguish between them due to their similar morphological characteristics (Shamin-Shazwan *et al.,* 2021). It is intriguing that both species can create natural hybrids in their respective (Ng and Chan, 2012). In this study, the three *Rhizophora* species observed showed overall similarities in leaf morphology, particularly in terms of margin and venation. Specifically, *R. apiculata* and *R. mucronata* exhibited similarities in leaf apex and base, while *R. apiculata* and *R. stylosa* shared similarities in leaf shape and color (Table 1).

The effects of land-use changes on the quantitative morphological characteristics of three *Rhizophora* species: *R. apiculata*, *R. mucronata*, and *R. stylosa,*

Table 1: Qualitative leaf morphology of *Rhizophora* spp.

Species	Shape	Adaxial colour	Abaxial colour	Apex	Base	Margin	Venation
R. apiculata Blume	Elliptic	2.5 GY 6/6	5.0 GY 7/6	Cuneate	Convex	Entire	Pinnate
R. mucronata Poir.	Oblong	10.0 GY 6/10	2.5 GY 7/8	Cuneate	Convex	Entire	Pinnate
R. stylosa Griff.	Elliptic	10.0 GY 5/8	5.0 GY 7/8	Convex	Rounded	Entire	Pinnate

Table 2. Quantitative leaf morphology of *Rhizophora* spp.

Location	SL	LL (cm)	LW (cm)	PL (cm)	LP (cm)
Rhizophora apiculata Blume					
Medan Belawan	5.49±1.38^{a}	14.29±2.02^{a}	5.03±1.04^{a}	2.16±0.55^{b}	6.62±1.13^{a}
Percut Sei Tuan	4.79±0.90^{a}	13.31±1.49^{a}	4.35±0.60^{a}	1.59±0.38^{a}	5.78±0.79^{a}
Sitardas	5.31±1.08^{a}	13.27±1.45^{a}	5.45±0.78^{b}	2.40±0.84^{b}	6.21±0.73^{a}
Rhizophora mucronata Poir					
Medan Belawan	6.49±1.23^{b}	17.07±2.39^{b}	9.04±1.58^{b}	3.15±0.65^{b}	7.84±1.25^{b}
Percut Sei Tuan	5.34±1.22^{a}	10.46±2.51^{a}	5.88±1.04^{a}	2.24±0.59^{a}	2.64±0.91^{a}
Sitardas	6.00±1.46^{a}	15.45±2.18^{b}	8.32±1.30^{b}	3.04±0.58^{b}	7.76±1.23^{b}
Rhizophora stylosa Griff					
Percut Sei Tuan	5.58±0.97^{a}	10.33±1.27^{a}	5.39±0.77^{a}	2.65±0.50^{a}	4.96±0.73^{a}
Sitardas	5.45±0.75^{a}	12.84±1.70^{b}	5.18±0.86^{a}	2.14±0.45^{b}	6.29±0.92^{b}

Note: SL = Stipule Length; cm = centimeter; LL = Lamina Length; LW = Leaves Width; PL = Petiol Length; LP = Leaves Point.
Different notations indicate significant differences between groups based on post hoc analysis ($p < 0.05$).

are detailed in Table 2. An analysis using the post hoc Tukey method on leaf morphological features, namely stipule length (SL), leaf length (LL), leaf width (LW), petiole length (PL), and leaf point (LP), demonstrates significant variations in these characteristics across diverse locations and species.

Feng *et al.* (2016) discovered that water stress can diminish leaf size by inhibiting cell division and expansion. In the present study on *R. apiculata*, it was found that stipule length (F=1.092) and lamina length (F=0.7907) were not significantly affected by habitat conditions. It was found that leaf width experienced a significant impact (F=10.31), petiole length had a moderate influence (F=8.647), and the leaf point was affected to a lesser extent (F=3.131). Notably, the Sitardas population exhibited a significant leaf width (LW: 5.45 ± 0.78 cm) compared to the Medan Belawan and Percut Sei Tuan populations. In comparison, petiole length was significantly longer in Medan Belawan (PL: 2.16 ± 0.55 cm) and Sitardas (PL: 2.40 ± 0.84 cm) populations than in the Percut Sei Tuan population (PL: 1.59 ± 0.38 cm). The diversity in leaf width of *R. apiculata* highlights the importance of habitat conditions in its growth, as these plants adapt their morphology to improve photosynthesis, light absorption, and evapotranspiration (Tamimi *et al.*, 2021). In the present study on *R. mucronata*, stipule length is only weakly affected (F=5.693), whereas lamina length (F=45.43), leaf width (F=34.04), and leaf point (F=24.64) were strongly affected. While most characters displayed considerable differences, petiole length in the Medan Belawan and Sitardas populations remained an exception, as it was significantly elevated compared to that in the Percut Sei Tuan population. Belawan displayed the highest values for stipule length (SL: 6.49 ± 1.23 cm), lamina length (LL: 17.07 ± 2.39 cm), leaf width (LW: 9.04 ± 1.58 cm), and leaf point (LP: 7.84 ± 1.25 cm), while Percut Sei Tuan consistently recorded the lowest values, particularly for leaf point (LP: 2.64 ± 0.91 cmAdditionally, recent findings reveal that *R. mucronata* leaves can function as bioindicators for high copper concentrations, with a noticeable decrease in average leaf area corresponding to increased copper levels (Perez *et al.*, 2021). *Rhizophora stylosa* is primarily found in seaside mangrove forests and is often subjected to tidal inundation. This species demonstrates a strong ability to compete and thrive in ecosystems with high salinity and prolonged inundation periods (Chen and Wang, 2017; Chen *et al.*, 2022). Findings from the statistical analysis demonstrated that there are only minor morphological changes in

response to differing ecosystem conditions. Notably, lamina length (F=28.68) and leaf point (F=24.71) are strongly affected, while stipule length is weakly affected (F=2.81), petiole length is moderately affected (F=9.979), and leaf width showed no significant impact (F=0.6409). Significant differences were identified in lamina length and petiole length between Percut Sei Tuan and Sitardas, with the Sitardas recording a longer lamina length (LL: 12.84 ± 1.70 cm) and Percut Sei Tuan a greater petiole length (PL: 2.65 ± 0.50 cm). However, no significant differences were found in SL, LW, or LP between the two locations, as indicated by the shared notation "a." The differences in leaf structure can be linked to the diverse environmental conditions that each species experiences in its specific habitats. For instance, *R. mucronata* showed greater morphological plasticity, which may enhance its adaptability to changing environmental conditions (Fox *et al.,* 2019). Adaptability plays a key role in the survival of species within dynamic coastal ecosystems, where human activities can lead to significant fluctuations in factors such as salinity, nutrient availability, and hydrology (Cloern *et al.,* 2016). Moreover, the distinct differences in leaf features among the species imply that each has undergone evolutionary changes that enable them to maximize resource efficiency in their particular habitats. For example, variations in leaf width and length can influence photosynthetic efficiency and water retention, which are vital for survival in mangrove habitats (Iqbal *et al.,* 2024; Qie *et al.,* 2024). The ability of these species to adjust their morphology in response to land-use changes highlights the importance of considering species-specific characteristics in conservation and restoration efforts. Additionally, the effects of these morphological transformations go beyond individual species and impact the overall ecosystem. Mangroves are vital for safeguarding coastlines, capturing carbon, and offering habitats for numerous marine organisms. Therefore, changes in the morphological characteristics of *Rhizophora* species can affect their overall health and functionality, potentially leading to shifts in ecosystem services provided by mangrove forests (Sanchez *et al.,* 2021; Inoue *et al.,* 2024). Changes in leaf area or modifications in leaf structure may lead to a decline in the photosynthetic potential of these trees, impacting their growth rates and ability to sequester carbon, both of which play a vital role in climate change mitigation.

Anatomical characteristics of R. apiculata, *R. mucronata*, and *R. stylosa*

An evaluation of the anatomical characteristics of the leaves from *R. apiculata*, *R. mucronata*, and *R. stylosa* revealed that variations in land use can influence leaf anatomy, including differences in stomatal type, stomatal density, stomatal index, and stomatal length. The differences in leaf anatomical characteristics found among three distinct mangrove locations demonstrate that leaf anatomy is notably affected by environmental variations. The findings of this study demonstrate that there is substantial variation in the stomatal shape of *Rhizophora* species across the three sites examined. The stomata in *R. apiculata* and *R. stylosa* primarily exhibit an anomocytic type, with undulating or undulating to rounded anticlinal margins in *R. apiculata* and rounded anticlinal margins in *R. stylosa* (Table 3). Conversely, *R. mucronata* mainly shows the desmocyctic, paracytic, and pericytic types, which feature rounded anticlinal margins; however, the anomocytic type is also observed (Table 3). Several types of stomata found in the studied *Rhizophora* species are presented in Fig. 5. The structure of the anticlinal stomata plays a vital role in maintaining both structural and physiological functions of stomata, allowing them to operate effectively under various environmental conditions. Additionally, there is considerable variation in the shape of epidermal cells among three *Rhizophora* species observed at three distinct study sites (Table 3).

In the present study, the results of post-hoc analyses describe the stomatal characteristics of three *Rhizophora* species (*R. apiculata*, *R. mucronata*, and *R. stylosa*) across different locations in Medan Belawan, Percut Sei Tuan, and Sitardas (Table 4). The data includes the value of stomatal density (stomata per square millimeter), stomatal index (percentage), stomatal length (in micrometers), and stomatal width (in micrometers). Accompanying each value is its standard deviation, while letters signify the significant differences observed among the means values for each species. A prior study emphasized that that salinity stress significantly impacts stomatal density as an anatomical characteristic (Kiani-Pouya *et al.,* 2020; Ibrahim *et al.,* 2021; Chen *et al.,* 2022). In high salinity conditions, plants exhibit a reduction in

Table 3: Qualitative stomatal characteristics of *Rhizophora* spp.

Location	Type of stomatal	Epidermis cell shape	Type of anticlinal
Rhizophora apiculata			
Medan Belawan	Anisocytic Anomocytic* Brachyparatetracytic	Pentagonal Hexagonal	Undulate
Percut Sei Tuan	Anomocytic*	Pentagonal Hexagonal	Undulate to rounded
Sitardas	Anisocytic Anomocytic* Anomotetracytic	Pentagonal Hexagonal Square	Undulate to rounded
Rhizophora mucronata			
Medan Belawan	Anomotetracytic Desmocyctic* Paracyctic	Oval Square Pentagonal	Rounded
Percut Sei Tuan	Anisocytic Anomocytic Anomotetracytic Paracytic*	Square Pentagonal	Rounded
Sitardas	Anomocytic Paracytic Pericytic*	Oval Square	Rounded
Rhizophora stylosa			
Percut Sei Tuan	Anomocytic* Anomotetracytic Staurocytic	Square Pentagonal Hexagonal	Rounded
Sitardas	Anomocytic* Anomotetracytic Anisocytic Desmocyctic	Square Pentagonal Hexagonal	Rounded

Note: *dominant

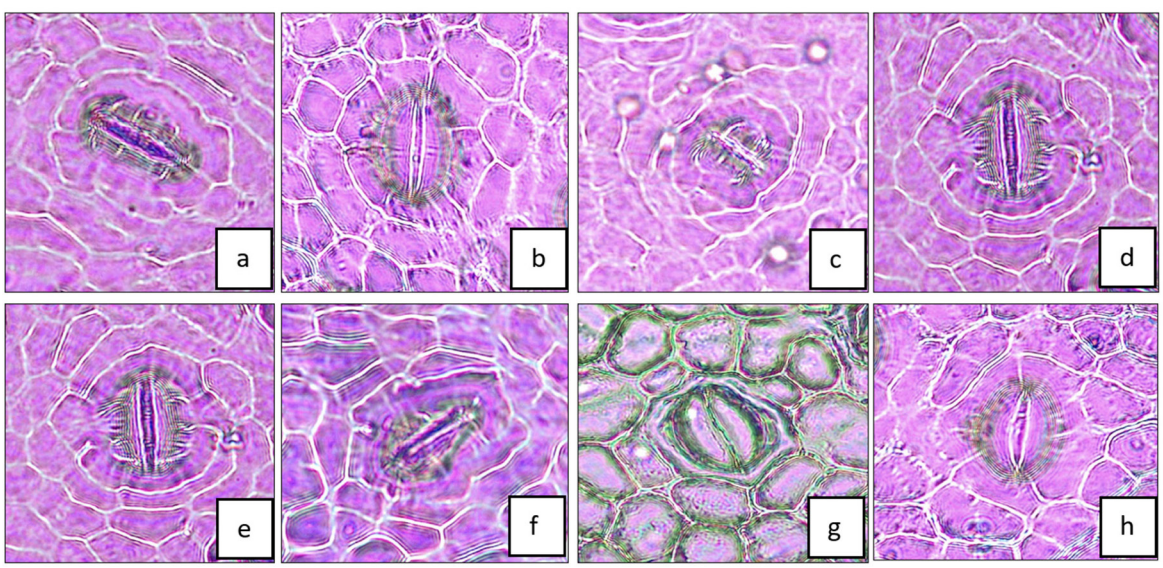

Fig. 5: Stomatal type. a. Anisocytic; b. Anomocytic; c. Anomotetracytic; d. Brachyparatetracytic; e. Desmocyctic; f. Paracytic; g. Pericytic; and h. Staurocytic.

Table 4: Quantitative stomatal characteristics of *Rhizophora* spp.

Location	Stomatal density (stomata/ milimeter (mm^2)	Stomatal index (%)	Stomatal length (µm)	Stomatal width (µm)
Rhizophora apiculata Blume				
Medan Belawan	109±7[a]	3.956±0.463[a]	55.50±6.99[a]	45.29±7.39[a]
Percut Sei Tuan	108±11[a]	3.035±0.344[c]	40.91±3.96[c]	27.57±5.25[c]
Sitardas	103±11[a]	3.456±0.393[b]	44.92±3.64[b]	36.93±7.75[b]
Rhizophora mucronata Poir				
Medan Belawan	101±11[a]	8.173±0.858[a]	64.29±6.06[a]	49.72±5.32[a]
Percut Sei Tuan	100±9[a]	4.832±0.759[b]	52.07±3.76[b]	38.27±2.95[b]
Sitardas	86±7[b]	4.644±0.525[b]	50.71±3.81[c]	39.85±3.42[b]
Rhizophora stylosa Griff				
Percut Sei Tuan	97±13[a]	4.062±0.788[a]	52.16±4.67[a]	40.04±2.74[a]
Sitardas	109±9[b]	2.907±0.225[b]	48.54±4.24[a]	36.06±4.83[b]

Different notations indicate significant differences between groups based on post hoc analysis ($p < 0.05$).

stomatal density, responding by improving water use efficiency without greatly affecting overall yield (Chen *et al.,* 2022). According to the salinity level observed at the study sites, Percut Sei Tuan has the highest salinity, averaging 33.06 ppm, while Medan Belawan and Sitardas have salinity levels of 12.67 ppm and 13.36 ppm, respectively. In this study, the trend of lower stomata density is found only in *R. stylosa*, which shows the stomatal density in the Percut Sei Tuan area is lower than in Sitardas with medium effects (F=8.612). Meanwhile, *R. apiculata* and *R. mucronata* show contradictive results (Percut Sei Tuan has a lower stomatal density than Medan Belawan but has a higher density than Sitardas) with no significant difference (F=1.489) and medium effect (F=12.61), respectively. As a combined residential and harbor zone, Medan Belawan is particularly susceptible to pollution, primarily because of the substantial quantity of chemical waste produced by households. Meanwhile, Percut Sei Tuan is the fish pond area threatened by logging and clearing mangrove areas for opening the fish pond. Furthermore, the similar stomatal density of *R. apiculate* indicated a level of consistency in this characteristic, even with changes in habitat conditions. The quantity of stomata is impacted by the level of pollution found in the mangrove ecosystem. Higher salinity levels can cause a decreased number of stomata (Samiyarsih *et al.,* 2016; Ulfaturrohmah *et al.,* 2024). The findings of this study indicated a relationship between the quantity of stomata and the stomatal index. Results indicated that *R. apiculata* and *R. mucronata* exhibited the lowest stomatal indices in Percut Sei Tuan, while *R. stylosa* had the highest. A comparable trend was noted across the species, indicating that the stomatal indices at Medan Belawan surpassed those recorded at Sitardas. Statistical analysis revealed a significant difference in the stomatal index of *R. apiculata* (F=19.69), indicating that this species is highly responsive to its growing environment. This capacity for adaptability could bolster the survival of R. apiculata amid changing conditions, yet it emphasizes the critical need to monitor environmental elements that may affect its growth. In contrast, *R. mucronata* demonstrated a greater sensitivity to anatomical changes, as evidenced by the strong effects on the stomatal index (F=111.7). This increased sensitivity suggests that *R. mucronata* may be more vulnerable to environmental stressors, which could affect its overall health and functionality in mangrove ecosystems. The ability of *R. mucronata* to respond to changes in its environment may be linked to its ecological role, as it often occupies areas with varying salinity and nutrient levels (Greaver *et al.,* 2016; Alongi, 2018). In addition, *R. stylosa* also exhibited a significant impact on its stomatal index (F=24.67), along with a medium effect on stomatal density and stomatal width. Despite these changes, *R. stylosa* demonstrated greater resilience to land-use changes compared to *R. apiculata* and *R. mucronata* in both Percut Sei Tuan and Sitardas. The ability to retain functional characteristics that aid in survival under changing conditions contributes to this resilience, playing a vital role in the maintenance of mangrove ecosystems (Srikanth *et al.,* 2016; Quadros *et al.,* 2021). The various land uses also influence

the stomatal size, both in length and width (Table 4). Rodriguez *et al.* (2018) explained that the response of stomatal adaptation (in terms of stomatal density and size) to cross-growing area conditions depends on each species. Smaller stomata confer mechanical advantages that improve the operational efficiency of guard cells. When the stomata are smaller, the turgor pressure from epidermal cells required to open the guard cells is also reduced, which in turn minimizes energy requirements. In this study, the stomatal length of *R. apiculata* showed a significant effect (F=26.19), along with its stomatal width. Similar findings were observed in *R. mucronate*, where the stomatal length (F=36.98), and stomatal width (F=27.34) also exhibited strong effects. The notable variations in stomatal length and width observed in both *R. apiculata* and *R. mucronata* suggest a pronounced sensitivity to morphological and anatomical changes resulting from alterations in their environmental growth conditions. Conversely, in *R. stylosa*, the stomatal width (F=10.42) exhibited medium effects, while the stomatal length showed weak effects (F=2.63). Comprehending these responses is vital for creating successful conservation and management strategies, particularly in the context of ongoing changes in land use and climate variability. The diverse reactions of these species to environmental shifts underscore the necessity of implementing species-specific management strategies in restoration and conservation initiatives, ensuring that the distinct adaptations of each species are considered.

Previous findings indicate that *R. stylosa* possesses superior anatomical features, suggesting that it has a good tolerance to both salinity and pollution conditions. The absence of *R. stylosa* in Medan Belawan is particularly noteworthy, given the species' known adaptability to varying salinity levels and polluted environments. This absence brings to light critical ecological issues concerning the interplay between environmental conditions and the distribution of species. The growth and establishment of *R. stylosa* are strongly influenced by several factors, including habitat characteristics, the broader ecosystem dynamics, and the specific properties of the substrate. Notably, *R. stylosa* has been reported to thrive on sandy substrates and coral beds, as highlighted by Kalasuba *et al.* (2023). The absence of this species in Medan Belawan could suggest that there are limiting factors associated with substrate composition or ecosystem conditions that prevent its establishment, even though it is highly adaptable. Delving deeper into these environmental and ecological aspects could enhance our comprehension of the determinants affecting the distribution of *R. stylosa*.

Conservation and restoration of mangrove ecosystem

The morphology and stomatal structure of three *Rhizophora* species have been significantly influenced by alterations in land use. Among these, *Rhizophora mucronata* displayed the greatest sensitivity to these changes, while *R. stylosa* showed higher resistance. The alterations in stomatal characteristics, such as the stomatal index, length, and width, highlighted the ways in which these species adjust to varying environmental conditions. It is important to consider appropriate conservation policies based on the sensitivity of each species to land use change in their respective habitats. Overall, the findings of this study provide valuable insights into the different environmental factors influence the stomatal morphology and characteristics of *Rhizophora* species. These insight can lead to the formulation of more impactful conservation strategies for coastal zones and mangrove forests. By analyzing the specific characteristics of each species alongside the health of their ecosystems, multiple conservation and restoration strategies can be recommended.

Ecological characteristics and conservation practiced

On Sumatra Island, there are 171 species of mangrove, of which 38 are classified as true mangroves, primarily represented by *R. mucronata* and *R. apiculata* (Rahman *et al.,* 2024). *Rhizophora* spp. is part of the true mangrove category and is found in the second layer of the zonation of the mangrove ecosystem. The outermost layer consists of *Avicennia* spp. and *Sonneratia* spp., which thrive in flooded conditions, while *Rhizophora* spp. occupies the next layer, located in areas that are inundated during high tides. *Rhizophora's* adaptation mechanism has proven effective in enduring a range of inundation conditions and various types of substrates. Ecologically, the three *Rhizophora* species found in Indonesia prefer similar growth habitats, although their tolerance to specific limiting factors varies slightly. Mangrove species are impacted by

three primary tolerance limits: the characteristics of the soil, the salinity of the environment, and their resilience against currents and surf conditions. T the ability to germinate, as well as the level of resistance and adaptation to environmental changes, differs among species. Certain species are restricted by their particular environmental requirements (stenocous), while those that possess a greater tolerance (eurycous) experience fewer challenges in their growth and development. For example, *R. mucronata* is sterile when growing in highly saline sea environments; however, it regularly produces fruit when growing along sedimentary stream banks. *Rhizophora apiculata* has growth preferences similar to those of *R. mucronate* but has a lower tolerance for environmental variations. In contrast, *R. stylosa* exhibits accelerated growth at moderate salinity levels (Inoue *et al.,* 2024).

a. Restoration practiced

Numerous studies have reviewed the restoration efforts of the mangrove ecosystem in Indonesia (Gerona-Daga and Salmo, 2022). A comprehensive assessment of mangrove restoration initiatives in Medan Belawan and Percut Sei Tuan has been performed, accompanied by ongoing progress monitoring (Hanggara *et al.,* 2021). However, collaborative efforts to ensure the recovery and sustainability of mangrove ecosystems in Medan Belawan and Percut Sei Tuan must continue, including preserving mangrove ecosystems around Sitardas and surrounding islands. To promote ecosystem recovery and shield the coastline from erosion and other environmental impacts, it is vital to implement planting initiatives that include true mangrove species in addition to *Rhizophora* spp. Facilitating the natural regeneration of mangrove vegetation necessitates an extended period and the management of multiple internal and external challenges that may hinder germination, growth, and development processes. Restoration practices for mangrove ecosystems are critical for enhancing their resilience and functionality, particularly considering the physiological variations observed among species such as *R. apiculata*, *R. mucronata*, and *R. stylosa*. The information regarding stomatal characteristics, such as density, index, length, and width, highlights the importance of choosing suitable species and comprehending their unique adaptations to local environmental conditions in restoration initiatives. For instance, *R. mucronata*, which exhibits a higher stomatal index and density under optimal conditions, may be prioritized in restoration projects aimed at improving carbon sequestration and habitat provision (Lovelock *et al.,* 2022). Additionally, the inclusion of a wide range of species can bolster biodiversity and enhance ecosystem resilience, given that various species respond differently to environmental challenges (Murray *et al.,* 2018). Effective restoration strategies must also consider the hydrological and sedimentary dynamics of the site, as these factors significantly influence the growth and survival of mangrove species (Krauss *et al.,* 2016). Incorporating these insights into restoration efforts can enhance the resilience of mangrove ecosystems, enabling them to better endure the effects of climate change and anthropogenic activities. Habitat restoration techniques, pollution control, salinity management, ongoing monitoring, community education, and technology adoption are essential strategies for managing the mangrove ecosystem, particularly those involving *Rhizophora* species adaptation. These strategies contribute to the sustainability of *Rhizophora* species while simultaneously enhancing the health and functionality of mangrove ecosystems, which in turn makes them better equipped to adapt to changing environmental circumstances. This study advocates for the use of wild species in restoration efforts. Specifically, the ability of *R. mucronata* and *R. apiculata* to adapt to changes in land use makes them highly recommended for it improving the success of habitat restoration initiatives.

b. Environmental impacts of mangrove conservation on society

The conservation of mangrove ecosystems yields a range of benefits and effects for both the ecological landscape and the communities that inhabit the area. Multiple reviews of conservation efforts in Southeast Asia have recommended practical approaches, including designating mangrove areas as marine protected areas, promoting collaborative management among stakeholders, encouraging private sector involvement in management and protection, and implementing payment for ecosystem services (PES) mechanisms (Friess *et al.,* 2016). The urgent restoration of mangrove ecosystems in Medan Belawan is crucial for assisting local residents in

managing the tidal flooding that regularly impacts the area (Sarveleni *et al.,* 2023). Compared to other study sites, Medan Belawan requires more comprehensive management from various parties due to the significant land use changes that occurred between 2005 and 2019, which have resulted in considerable mangrove habitat destruction and negatively impacted the surrounding ecosystem (Rahmawaty *et al.,* 2023). Alterations in land use surrounding mangrove ecosystems, including the transformation into ponds or residential developments, can significantly impact the long-term viability of species such as *Rhizophora* spp. The consequences of these changes include the loss of habitat for mangrove flora and fauna, habitat fragmentation, changes in forest structure, and changes in hydrology. Nevertheless, further study is necessary to explore these issues in greater detail. The findings of a study by Basyuni *et al*. (2021) indicate that selecting the right species and employing effective planting techniques can markedly increase seedling survival rates and hasten the restoration of ecosystems. Additionally, using techniques that create optimal conditions for mangrove growth, such as developing waterways to improve water circulation and reduce excessive salinity, has proven beneficial. Moreover, the study by Sasmito *et al.* (2019) employed the integrated coastal zone management (ICZM) method to encourage local community involvement in decision-making related to coastal resource management. Engaging in this process is crucial to guarantee that policies are tailored to meet local needs and circumstances. This method further underscores the need for sustainable management of natural resources, including fisheries and tourism, to ensure that economic gains do not adversely affect mangrove ecosystems. These strategies may bolster the resilience of Rhizophora species in areas facing alterations in land use. Land use changes significantly impact *Rhizophora* species, affecting their growth, distribution, and ecological functions. Urbanization, agriculture, and industrial development often lead to habitat loss and fragmentation, disrupting the natural dynamics of mangrove ecosystems. Such changes can result in a reorganization of species dominance, with more adaptable species potentially outcompeting others, which can contribute to a decrease in biodiversity. Furthermore, alterations in hydrology and sedimentation patterns due to land use changes can affect the growth conditions of *Rhizophora*. This ultimately impacts their ability to sequester carbon and provide essential ecosystem services such as coastal protection and habitat for marine life (Alongi, 2018; Booi *et al.,* 2022). Furthermore, changes in land use play a crucial role in influencing the resilience of Rhizophora species amid the challenges posed by climate change. These species are often sensitive to variations in salinity, temperature, and nutrient availability, making their physiological responses vulnerable to human activities (Alongi, 2018; Sanchez *et al.,* 2021). The reduction of freshwater inflow caused by land development can lead to heightened salinity levels, which may stress mangrove species, ultimately resulting in decreased growth and increased mortality rate. This highlights the importance of implementing effective management and restoration strategies that consider the specific ecological requirements of *Rhizophora* species. Such measures are crucial for enhancing their resilience and ensuring the sustainability of mangrove ecosystems (Ellison *et al.,* 2020; Ferreira *et al.,* 2023).

CONCLUSION

An examination of leaf morphology among three species of *Rhizophora* indicated that *R. apiculata* and *R. mucronata*, which frequently coexist in mangrove environments, display comparable leaf shapes and coloration. This study assessed the impacts of land use changes on the morphological characteristics of *R. apiculata*, *R. mucronata*, and *R. stylosa*. The analysis indicated that habitat conditions significantly influence certain morphological characteristics of *R. mucronata*, with lamina length (F=45.43) and leaf width (F=34.04) showing strong effects, while petiole length was moderately affected (F=13.64) and weakly influenced (F=5.693). *Rhizophora stylosa* was only found in Percut Sei Tuan and Sitardas, with its absence in Medan Belawan attributed to unfavorable substrate and habitat conditions. The statistical analysis indicated that there were only slight morphological variations among the various ecosystems, with the exceptions of lamina length (F=28.68) and leaf tip (F=24.71). In contrast, petiole length (F=2.81) and leaf width (F=0.6409) did not demonstrate any significant effects. Observations of stomatal anatomy revealed that variations in land use influence leaf structure, particularly in terms of density, stomatal index, and stomatal length. The

stomatal type in *Rhizophora* spp. predominantly exhibits anomocytic forms in *R. apiculata* and *R. stylosa*, characterized by circular anticlinal walls. In *R. apiculata*, stomatal density (F=1.489) was not significantly affected, whereas stomatal index (F=19.69), length (F=26.19), and width (F=37.84) showed significant effects, indicating high sensitivity to growth area differences. In terms of morphological and anatomical changes, *Rhizophora* mucronata was more sensitive than *R. apiculata*, while *R. stylosa* proved to be more resilient to land use changes occurring in Percut Sei Tuan and Sitardas. Salinity levels varied across study sites, with Percut Sei Tuan recording the highest average salinity at 33.06 ppm, compared to lower levels in Medan Belawan (12.67 ppm) and Sitardas (13.36 ppm). The results of the PCA indicated considerable variability in the data from Percut Sei Tuan, particularly along the first principal component, which suggests a distinct profile relative to other sites. Meanwhile, Medan Belawan and Sitardas were closely clustered, reflecting a greater similarity and reduced variation between them. This study concludes that *R. stylosa* shows limited tolerance to environmental changes, *R. mucronata* possesses significant morphological and anatomical flexibility, and *R. apiculata* is highly responsive to environmental variations without necessitating changes in leaf shape and structure.

AUTHOR CONTRIBUTIONS

A.B. Rangkuti, the corresponding author, has contributed in conceived and designed the experiments, analyzed and interpreted the data, wrote the manuscript draft, reviewed and finalized the final draft. P. Peniwidiyanti conceived and designed the experiments, supervised morphological and anatomical data collection, analyzed and interpreted the data, and wrote the manuscript draft. M. Basyuni reviewed and finalized the final draft. D. Elfiati reviewed and finalized the final draft. A. Susilowati analyzed and interpreted the data, wrote the manuscript draft, and reviewed and finalized the final draft. A. Mubaraq visualization, reviewed and finalized the final draft. M.R. Hariri conceived and designed the experiments, supervised morphological data collection, analyzed and interpreted the data, and wrote the manuscript draft. A.P. Dewi conceived and designed the experiments, supervised anatomical data collection, analyzed and interpreted the data, and wrote the manuscript draft. F. Indriani prepared the manuscript and organized the text. F.G. Dwiyanti reviewed the manuscript draft. B. Slamet conceived and designed the experiments. R.A. Syahputra reviewed the manuscript draft. All authors have approved the final manuscript submitted.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
µm	Micrometer
±	Plus-minus
AHP	Analytical hierarchy process
ANOVA	Analysis of variance
cm	Centimeter
CO_2	Carbon dioxide
EC	Electro conductivity
F	F-test
FAO	Food and Agriculture Organization
GIS	Geographic information system
GY	Green yellow
Ha	Hornblende Andesite
ha	Hectare
ICZM	Integrated coastal zone management
LL	Lamina length
LP	Leaves point
LW	Leaves width
m/s	Meter per second
Mha	Million hectares
mm	Milimeter
mS/cm	Milisiemens per centimeter
PAST	Paleontological statistics
PC	Principal components
PCA	Principal component analysis
PES	payment for ecosystem services
pH	Potential of hydrogen
PL	Petiole length
ppm	Parts per million
R. apiculata	Rhizophora apiculata
R. mucronata	Rhizophora mucronata
R. stylosa	*Rhizophora stylosa*
SL	Stipule length
TSD	Total disolved solid
VPD	Vapor pressure deficit

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