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Stomatal Characteristics in Three Groups of Mangrove Plants: Major, Minor, and Associate

Karakteristik Stomata pada Tiga Kelompok Tumbuhan Mangrove: Mayor, Minor, dan Asosiasi

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ABSTRACT

Three types of mangroves inhabit different environmental gradients and zones within the mangrove ecosystem. These three mangrove types exhibit distinct adaptation strategies. Stomata, as a crucial organ of the plant system, play a significant role in the adaptability of plants. Understanding stomata is essential for comprehending the physiological conditions and responses of plants to environmental conditions. This research aimed to study the stomatal characteristics of three different mangrove types and gain a deeper understanding of their potential adaptive strategies. This research was conducted at the Mangrove Botanical Garden Gunung Anyar, Surabaya, in November 2022. We selected two representative mangrove species from the major, associate, and minor types. Stomata of each mangrove species were observed to identify the stomatal type, density, and size using an Olympus microscope at 100 xs and 400 x magnifications. The results showed there were variations in stomatal type, density, and stomatal length and width among studied mangrove types. Major mangrove species exhibited the lowest stomatal density and the largest size of stomata compared to minor and associated mangroves. The diversity of stomatal traits among major, associate, and minor mangrove types underscores their adaptations and ecological strategies.

Keywords: Adaptations, Mangrove Types, Stomatal Type, Stomatal Density, Stomatal Size

ABSTRAK

Terdapat tiga tipe mangrove yang hidup pada gradient lingkungan dan zonasi yang berbeda pada ekosistem mangrove. Ketiga tipe mangrove ini memiliki strategi adaptasi yang berbeda. Stomata sebagai organ penting pada sistem tumbuhan memiliki peran pada kemampuan adaptasi tumbuhan. Pemahaman tentang stomata penting untuk mengetahui kondisi fisiologis dan respon tumbuhan pada kondisi lingkungan. Penelitian ini bertujuan untuk mempelajari karakteristik stomata pada tiga jenis mangrove yang berbeda untuk mendapatkan pemahaman yang lebih mendalam tentang potensi strategi adaptasi tiga tipe mangrove. Penelitian ini dilaksanakan di Kebun Raya Mangrove Gunung Anyar Surabaya pada bulan November 2022. Kami memilih dua spesies mangrove yang mewakili mangrove mayor, asosiasi, dan minor. Stomata dari setiap jenis mangrove diamati untuk menentukan tipe, densitas dan ukurannya dengan menggunakan mikroskop Olympus dengan perbesaran 100x dan 400x. Hasil penelitian menunjukkan adanya variasi dalam jenis stomata, kerapatan, panjang, dan lebar stomata di antara jenis mangrove yang diteliti. Spesies mangrove mayor menunjukkan kerapatan stomata terendah dan ukuran stomata terbesar dibandingkan dengan mangrove minor dan asosiasi. Keanekaragaman sifat stomata di antara jenis mangrove utama, asosiasi, dan minor menunjukkan adaptasi dan strategi ekologis yang mereka miliki.

Kata Kunci: Adaptasi, Jenis Mangrove, Jenis Stomata, Densitas Stomata, Ukuran Stomata

INTRODUCTION

Indonesia stands as the largest mangrove forest area, constituting 25% of the global mangrove forest and approximately 50% of mangrove ecosystems within the Asian continent (Giesen, 2007; Das-Gupta and Shaw, 2013). These mangroves are dispersed across Indonesia, including the eastern coast of Sumatera, the northern shores of Java, the western and eastern coastlines of Kalimantan, Sulawesi, the archipelago of the Maluku islands, and the southern fringes of Papua.

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Mangrove ecosystems encompass a variety of plant species, including true mangroves, minor mangroves, and associated mangroves. These mangroves exhibit a diverse array of growth forms, ranging from shrubs like Aegiceras to tall, large trees (up to 40 m) such as Rhizophora and Bruguiera mangroves

Mangrove species are known for their exceptional adaptability to the harsh and dynamic conditions of coastal environments. Major mangroves consist of true mangroves that inhabit the tidal zone. Major mangroves can adapt to high salinity levels and naturally form pure stands. There are several species of major mangroves, for instance, Avicennia, Rhizophora, Bruguiera, Ceriops, Kandelia, Sonneratia, Lumnitzera, and Nypa. Minor mangroves, on the other hand, are supporting mangroves that cannot form pure stands and are typically found at the margins of mangrove habitats. Some genera representing minor mangroves include Excoecaria, Xylocarpus, Heritiera, Aegiceras, Aegialitis, Acrostichum, Camptostemon, Scyphiphora, Pemphis, Osbornia, and Pelliciera. Associate mangroves are plants that can grow near both major and minor mangroves but may also be found in habitats other than mangroves. These species serve as transitional vegetation from land to sea and include species from genera such as Cerbera, Acanthus, Derris, Hibiscus, Calamus, Ficus, Casuarina, etc (Tomlinson, 2016; Kusmana et al., 2013).

The classification of major and minor mangrove types is based on several characteristics, including the presence of aerial roots, plant mechanisms for salinity tolerance, and non-dormant seeds, including viviparous and recalcitrant seeds. Previous research mentioned that major mangroves, for instance, can excrete salt from their systems as an adaptation mechanism. This ability enables major mangrove plants to thrive in high-salinity zones and tidal fluctuations, allowing them to form pure stands (Setiawan et al., 2015; Martuti, 2013; Tomlinson, 2016). In addition to these characteristics, mangroves in different zones exhibit anatomical differences such as variations in cuticles, epidermis, and thick leaves as adaptations to salinity (Tihurua et al., 2020).

Stomata play a fundamental role in the life of plants. It governs the essential processes of gas exchange, photosynthesis, and water management in plants. The adaptability and responsiveness to environmental conditions make understanding stomatal characteristics crucial in the study of plant physiology and adaptation. The objective of this study is to observe the stomatal characteristics of mangrove plants, with a specific focus on three distinct groups: major, minor, and associated mangroves. In the unique ecosystem of mangroves, where plants inhabit the interface between land and sea, understanding stomatal characteristics holds particular significance in studying the adaptation mechanisms to challenging environmental conditions. Furthermore, understanding the intricacies contributes to our broader comprehension of mangrove ecology.

MATERIALS AND METHOD

Mangrove species selection

This study was conducted in the Mangrove Botanical Garden in Gunung Anyar, East Java which is located at 7°19'50.6"S 112°48'56.2"E. Observations of stomatal characteristics were focused on representative species from the 3 mangrove groups: major, minor, and associate mangrove found in the Mangrove Botanical Garden of Gunung Anyar (KRM GA) in Surabaya. There are 55 mangrove species recorded at KRM GA, Surabaya, consisting of major, minor, and association mangroves. These mangrove species are distributed in the estuarine area, along riverbanks, and towards the edges of the botanical garden. Six mangrove species were chosen as representatives of major, minor, and association mangroves for further study. These selected mangrove species naturally grow in the KRM GA area, Surabaya. The list of these mangrove species is provided in Table 1.

Table 1. The selected mangrove species, their classification, and growth form

No	Species	Mangrove yype	Growth form
1	*Rhizophora mucronata* Poir.	Major	Tree or shrub
2	*Cerbera manghas* L	Associate	Tree or shrub
3	*Xylocarpus moluccensis* (Lam.) M.Roem.	Minor	Tree
4	*Excoecaria agallocha* L	Minor	Tree
5	*Acanthus ilicifolius* Lour.	Associate	Shrub
6	*Sonneratia caseolaris* (L.) Engl.	Major	Tree

Stomatal characterization

We selected three individual plants for each species of mangrove and sampled three mature leaves per individual. Samples for stomatal analysis were collected by creating stomatal replicas using transparent nail polish. The nail polish was carefully applied to two distinct 1cm square areas on the abaxial surfaces of the leaf, avoiding the leaf edge and areas close to the midrib. After it had dried, the cast was detached using clear adhesive to get the stomata replicas. Subsequently, these stomatal replicas were examined under an Olympus microscope camera with magnification of 100x and 400x, and high-resolution images were captured. The images captured were subjected to analysis using Image J software to quantify various stomatal parameters, including stomatal type, stomatal length (SL), stomatal width (SW), and stomatal density (SD).

Statistical analysis

The data were analyzed using one-way ANOVA, followed by post hoc testing with Tukey's test. Before analyzing variance (ANOVA), a normality test using the Kolmogorov-Smirnov test was performed.

RESULT AND DISCUSSION

The stomatal type, density, length, and width of stomata vary among the six mangrove species. The smallest stomatal density was found in major mangrove species (*R. mucronata* and *S. caseolaris*), and the highest is found in *X.moluccensis* at 229 mm/mm^2 (Table 2). Additionally, the *R.mucronata* had the largest stomata in terms of both length and width among the six mangrove species studied. Conversely, the smallest stomata were found in *X.moluccensis*.

Table 2. Comparison of stomatal characteristics between 6 species of mangrove

Mangrove Classification	Species	Stomatal type	SD (mm/mm^2)	SL(µm)	SW (µm)
Associated	*A. ilicifolius*	diacytic	122 ± 3	2.41 ± 0.31	1.14 ± 0.12
	C. manghas	anomocytic	132 ± 8	1.89 ± 0.26	0.89 ± 0.13
Major	*R. mucronata*	cyclocytic	63 ± 6	4.38 ± 0.26	2.26 ± 0.14
	S. caseolaris	cyclocytic	58 ± 7	2.68 ± 0.23	1.43 ± 0.11
Minor	*E. agallocha*	diacytic	98 ± 42	2.66 ± 0.26	1.09 ± 0.23
	X. moluccensis	parasytic	229 ± 18	1.72 ± 0.09	0.15 ± 0.10

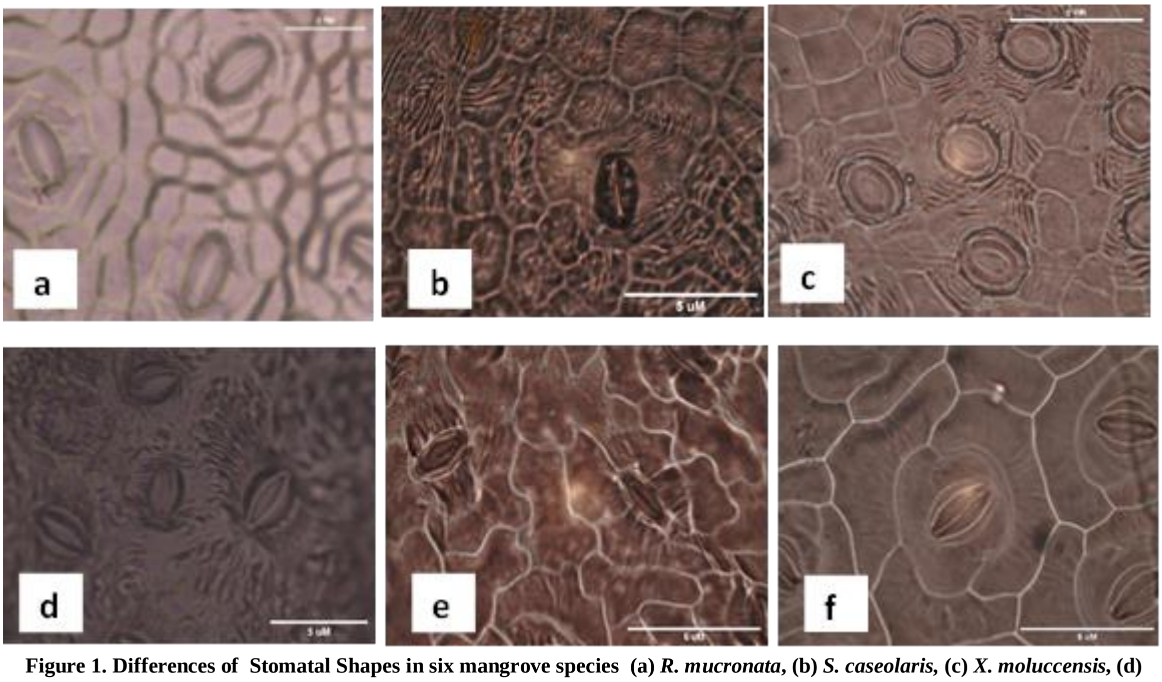

Figure 1. Differences of Stomatal Shapes in six mangrove species (a) *R. mucronata*, (b) *S. caseolaris*, (c) *X. moluccensis*, (d) *E.agallocha*, (e) *C. manghas* dan (f) *A.illicifolius*. The white bar line indicates a scale of 5µm.

The variation in stomatal characteristics in plants is influenced by both genetics and the growth environment. Studies have suggested there is a trade-off between stomatal size and density (Bertolino et al., 2018; Rudall et al., 2017). Larger stomata generally exhibit lower densities compared to their smaller ones, a pattern consistently observed in our result. Two species of mangrove major *R. mucronata* and *S. caseolaris* had the lowest stomatal density but the largest size of stomata. Stomatal density has a significant influence on two vital plant processes: transpiration and photosynthesis. Plants with higher stomatal densities tend to have increased transpiration rates compared to plants with lower stomatal densities. Moreover, the study suggested that smaller stomata demonstrate quicker response times and faster opening and closing rates than larger-sized stomata. This implies that the combination of a high stomatal density and reduced size enhances stomatal conductance under favorable conditions while optimizing the diffusion rate of carbon dioxide (CO_2), thereby reducing stomatal conductance in unfavorable environments (Hong et al., 2018). Based on our results, *X.molucensis* showed the best adaptation mechanism among other mangrove species.

Based on our observations, the types of stomata also vary among the six mangrove species. For example, major mangrove species like *R.mucronata* and *S.caseolaris* have cyclocytic stomata, where four or more subsidiary cells surround the guard cell (see Figure 1). In the cyclocytic type, the subsidiary cells tend to be located above the guard cells resulting in the formation of sunken stomata. Meanwhile, the paracytic stomata type is found in the *X. moluccensis* species. In the paracytic type, the stomata consist of lateral subsidiary cells arranged parallel to the guard cells. Both *E.agallocha* and *A.illicifolius* mangroves have diacytic stomata types, where the guard cells are surrounded by two subsidiary cells with cell walls forming right angles to the longitudinal axis of the stoma. Both of these species also have sunken stomata, which serve to mitigate direct light exposure and consequently lower the rate of transpiration (Gray et al., 2020). *C.manghas* has an anomocytic stomata type. Anomocytic stomata are characterized by the absence of subsidiary cells. Typically, this type of stomata features large and relatively plump guard cells, which experience minimal expansion or lateral movement when the stomata open (Franks and Farquhar, 2006).

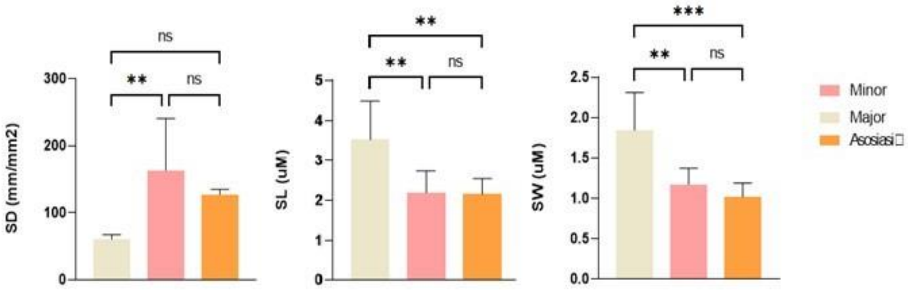

Figure 2. Comparison of stomatal density, length, and width in major, minor, and associated mangrove species.
(ns P > 0.05; ** P ≤ 0.01; *** P≤ 0.001; **** P≤0.0001)

When comparing each type of mangrove major, minor, and associated mangrove significant differences were observed in stomatal density. In contrast to stomatal density (SD), the highest stomatal length (SL) and width (SW) are found in both major mangrove types, while the smallest measurements are observed in associate and minor mangroves (Figure 2). Major mangroves exhibited the lowest stomatal density and the largest stomatal size. Despite major mangrove species being found in high light and temperature environments without shade, they exhibit low stomatal density due to their growth in high salinity conditions. Major mangrove species could thrive in coastal saline areas with frequent inundation compared to minor and associated mangroves, which inhabit areas closer to the mainland. As a result, major mangrove species have developed larger stomata sizes reducing the stomatal density. The previous study in several species of mangroves also demonstrated a reduced stomatal density in the species found in the higher salinity zonation (Ariyanto, 2018).

In plant systems, Abscisic acid (ABA) plays a significant role in modulating stomatal aperture, serving as a critical signal for drought stress and promoting rapid stomatal closure. Some plant species have evolved adaptive responses to drought stress, including the reduction in both the size and number of stomata. Some plant species adapt to drought stress by implementing strategies that include reducing both the size and number of stomata. Nevertheless, the study has also documented that under conditions of high salinity, mangrove species undergo anatomical changes, such as a reduction in stomatal density (Ball and Farquhar, 1984). This response underscores the intricate equilibrium that mangrove ecosystems have evolved to establish, balancing the optimization of gas exchange for photosynthesis with the conservation of water resources in their challenging coastal habitats. Furthermore, mangrove species also possess stomatal types that enable them to minimize water loss. These observed variations in stomatal characteristics among major, minor, and associated mangroves underscore the distinct ecological strategies employed by different mangrove species to thrive in their specific environmental contexts.

CONCLUSION

In conclusion, our study has highlighted the significant variations in stomatal characteristics across the six mangrove species, each equipped with unique stomatal types to cope with their specific environmental challenges. Major mangrove species, for instance, *R.mucronata* and *S.caseolaris*, had the lowest stomatal density coupled with the largest stomatal size, distinguishing them from their minor and associated mangrove. These observed stomatal traits offer insights into the delicate equilibrium that mangrove species have evolved to maintain, striking a balance between optimizing gas exchange and conserving water resources in their system environments.

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