


LEAF ANATOMICAL ADAPTATION OF EXOTIC INVASIVE *MICONIA CRENATA* (VAHL) MICHELANG. ALONG AN ELEVATION GRADIENT: A CASE STUDY OF MOUNT GEDE-PANGRANGO NATIONAL PARK, WEST JAVA, INDONESIA

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ABSTRACT

JUNAEDI, D. I., TIHURUA, E. F., WIDOYANTI & GIRMANSYAH, D. 2024. Leaf anatomical adaptation of exotic invasive *Miconia crenata* (Vahl) Michelang. along an elevation gradient: a case study of Mount Gede-Pangrango National Park, West Java, Indonesia. *Reinwardtia* 23(1): 45–54. — *Miconia crenata* is a widely spread species that occurs in multiple ecosystems. However, there is limited information on *M. crenata* invasion biology, ecology, and anatomy, particularly in the context of mountainous tropical forest and biodiversity management. Therefore, we examined elevation effects upon leaf anatomical structure of exotic invasive *M. crenata* at Mount Gede-Pangrango National Park (MGPNP). We sampled the leaves at four different elevations *i.e.*, 715 m asl, 800 m asl, 900 m asl, and 1,000 m asl. Cross section leaf anatomy specimens of *M. crenata* were obtained by using paraffin method and stained with safranin and fast green. This study found that *M. crenata* leaf anatomy was correlated with elevation shown by changes in leaf tissue thickness and stomata size. Further study is needed of leaf anatomical variation of exotic invasive species along driven by soil properties and the variation across different plant taxa and growth forms. Such studies are important to determine adaptation capacity of invasiveness.

Key words: Biodiversity management, elevation, invasive species, *Miconia crenata*, Mount Gede-Pangrango, tropical forest.

ABSTRAK

JUNAEDI, D. I., TIHURUA, E. F., WIDOYANTI & GIRMANSYAH, D. 2024. Adaptasi anatomi daun asing invasif *Miconia crenata* (Vahl) Michelang. sepanjang gradien ketinggian: sebuah studi kasus Taman Nasional Gunung Gede-Pangrango, Jawa Barat, Indonesia. *Reinwardtia* 23(1): 45–54. — *Miconia crenata* merupakan jenis yang tersebar luas dan dapat hidup di kondisi ekosistem dan ketinggian yang berbeda. Namun demikian, informasi mengenai invasi biologis *M. crenata*, meliputi biologi, ekologi, dan anatomi masih terbatas, terutama yang menyangkut pengelolaan keanekaragaman di hutan pegunungan tropis. Oleh karena itu, studi ini dilaksanakan untuk meneliti variasi struktur anatomi daun jenis asing invasif *M. crenata* pada beberapa ketinggian di Taman Nasional Gunung Gede-Pangrango (TNGGP). Sampel daun jenis tersebut dikumpulkan pada empat ketinggian berbeda yaitu, 715 m dpl, 800 m dpl, 900 m dpl, dan 1.000 m dpl. Irisan melintang daun *M. crenata* didapatkan dengan menggunakan metode parafin dan diwarnai dengan *safranin* dan *fast green*. Studi ini menunjukkan bahwa anatomi daun *M. crenata* berkorelasi dengan ketinggian yang diindikasikan oleh perubahan ketebalan daun dan ukuran stomata pada ketinggian berbeda. Penelitian lebih jauh mengenai anatomi jenis asing invasif pada variasi ketinggian dan habitat, bentuk hidup pada taksa yang berbeda sangat penting dilakukan untuk mengetahui kemampuan adaptasi yang berkaitan dengan tingkat keinvasian suatu jenis.

Kata kunci: Hutan tropis, jenis invasif, ketinggian, *Miconia crenata*, pengelolaan keanekaragaman hayati, Taman Nasional Gunung Gede-Pangrango.

INTRODUCTION

Miconia crenata (Vahl) Michelang. (Mabberley, 2017) is included in the Melastomataceae family and is naturally distributed in Mexico to Tropical America. It is considered an exotic species in Indonesia and Asia more widely (Wester & Wood, 1977; Junaedi *et al.*, 2021; Holmes *et al.*, 2023). *Miconia crenata* is one of the 100 worst invasive alien species in the world (GISD, 2023). *M. crenata* has dispersed as an exotic invasive species into many native ecosystems of Asia and Africa (Rojas-Sandoval & Acevedo-Rodríguez, 2014; Judd *et al.*, 2018).

Miconia crenata is perennial and usually grows in the form of a densely-branched shrub. The species' height tends to range from 0.5 m to 3 m but may grow up to 5 m tall. Most part of the plant are hairy and the leaves have prominent veins. The flowers are 0.5 cm to 1 cm in size, the fruits are berry-type, and the weight of 1,000 dried seed is 3.83 grams (Mune & Parham, 1967; Wickens, 1975; Francis, 2004). *Miconia crenata* has become an invasive species in many regions due to its reproductive characteristics. Firstly, *M. crenata* produces many seeds, resulting in a large soil seed bank size (Rojas-Sandoval & Acevedo-Rodríguez, 2014). Secondly, the fruit of this species is commonly eaten and dispersed by many animals, particularly bird species (Weber, 2003).

Miconia crenata invades multiple ecosystems, including those at both high and low elevations. There are currently limited studies on the invasion of *M. crenata* using ecophysiological and anatomical traits. Previous studies of *M. crenata* have mostly focused on their occurrence as an exotic invasive species (Peters, 2001; De Walt *et al.*, 2004a; Le *et al.*, 2018), the biomass allocation and genetic comparison between native and invasive ranges (De Walt *et al.*, 2004b; De Walt & Hamrick, 2004), and seed and seedling studies (Brooks *et al.*, 2018; Wanigasinghe & Gunaratne, 2020; Chandima *et al.*, 2022). Trait-based studies of invasive *M. crenata* have been conducted in the highlands of Java (Junaedi *et al.*, 2021) and lowlands of Borneo (Waddell *et al.*, 2020). Anatomical and morphological explanations of this species' adaptation as a successful invasive remain limited. The ecological, physiological, and anatomical information of *M. crenata* is crucial for invasive species management to protect biodiversity in mountainous tropical forests such as those in Indonesia, which contain many threatened, and endemic species (Higginbottom *et al.*, 2019; Utteridge *et al.*, 2024).

The Mount Gede-Pangrango National Park (MGPNP) is one of the important protected areas in Java, due to its rich and threatened biodiversity (Padmanaba *et al.*, 2017). Previous studies on invasive plant species in MGPNP have focused on

detection of exotic invasive species (Tjitrosodirdjo & Veldkamp, 2008; Uji *et al.*, 2010; Kudo *et al.*, 2014; Padmanaba *et al.*, 2017; Junaedi *et al.*, 2018), invasive species risk assessment (Junaedi & Mutaqien, 2018; Junaedi *et al.*, 2021a), and trait-based invasion ecological studies (Junaedi *et al.*, 2021b). These studies are crucial for providing sufficient information for relevant stakeholders regarding the threat and potential development of invasive plant species in MGPNP. Invasive species are considered as one of the main causes of ecosystem degradation and biodiversity loss (IUCN, 2017; Dueñas *et al.*, 2021; Holmes *et al.*, 2024).

This study aims to examine the altitudinal variation in the leaf anatomical structure variation of exotic invasive *M. crenata*. We conducted a case study at Mount Gede-Pangrango National Park (MGPNP) for two main reasons. First, MGPNP is an important conservation area in Java, containing the typical mountainous forests of the western part of Java. Studying the ecology and biology of invasive species is therefore essential to minimize the risk and impact of invasive species occurrence in MGPNP. Secondly, previous studies have identified that *M. crenata* is a problematic invasive species in both lowland and highland forests of MGPNP (Loke *et al.*, 2023). Plant ecophysiological adaptation to altitudinal variations has been detected by previous studies (Rahman *et al.*, 2020). The anatomical aspect of invasive species studies will support a comprehensive understanding of the invasion mode of *M. crenata* to aid risk assessment and future management. Anatomical aspect provides a proxy to explain invasive plant species adaptation to its invaded habitat condition. The changes in plant anatomical structure and tissues may indicate the existing and ongoing inherent biological and ecophysiological process within the studied invasive plants. Finally, these biological and ecophysiological information can be utilized by relevant stakeholders and management authorities to decide the accurate management strategies to manage these invasive species.

MATERIALS AND METHODS

Study Area

The leaf samples and specimens of *M. crenata* were collected from 17 to 24 October 2022 from Mount Gede-Pangrango National Park (MGPNP), at Bodogol Resort Area from four sampling locations at different elevations (-6.77631 E, 106.85574 S). These elevations are: 715 m asl, 800 m asl, 900 m asl, and 1,000 m asl. The leaf samples were collected from the area around the hiking trail because *M. crenata* occurs in relatively open area at MGPNP. The plants sampled were detected within 0–10 m of the hiking trail. Two individuals were sampled from each elevation. For each individual sample, at least four leaves were sampled for ana-

tomical examination and specific leaf area measurement. The leaves were sampled following Perez-Harguindeguy *et al.* (2016), ensuring the leaves were healthy, not too young and not too old. Before being processed in the laboratory, the leaf samples were stored in 70% alcohol solution.

Anatomical Preparation

The cross-section leaf anatomy specimens of *M. crenata* were obtained using the modified paraffin method (Sass, 1951). Leaf specimens of 1 cm × 0.5 cm size were fixed in FAA solution (Ethanol 96%, Formalin, Acetic Acid, and Aquades). The fixed specimens were then dehydrated by using ethanol absolute, serial mix solution ethanol:tert-Butanol (3:1), ethanol:tert-Butanol (1:1), ethanol:tert-Butanol (1:3), and absolute tert-Butanol. The samples were further infiltrated by liquid paraffin with a melting point of 56–58°C, then embedded with paraffin (with similar melting point). The embedded samples were cut with a rotary microtome with a thickness of 14–16 µm. The sections were stained using 1% of safranin in 70% ethanol and 2% of fast green in absolute ethanol. Finally, the slides were covered by cover glass in mounting entellan media. The leaf surface micro morphology specimens were obtained by dipping the leaf sample with 1 cm × 1 cm in size in a warmed HNO₃ solution (Cutler, 1978) until the upper and lower surfaces of *M. crenata* leaves were separated. The mesophyll was then cleaned and dyed with safranin. Then, samples were placed on an object glass with the addition 1–2 drops of glycerin. Finally, the specimens were covered by using cover glass.

Anatomical Observation

The observation and measurement of anatomical characters of *M. crenata* leaves were conducted using Nikon eclipse 80i microscope. The observed leaf tissue anatomical parts included the thickness of upper and lower epidermis tissue layer, leaf palisade tissue, sponge tissue, stomata size and stomata density. Observation measurements for each leaf tissue part were repeated 10 times for each observed individual specimens, resulting in a total of 40 observation for each trait at every elevation.

Anatomical Traits vs Habitat

The correlation of all collected anatomical data variables was calculated using Pearson correlation analysis that was conducted in R studio and R 4.3.0 (R Core Team, 2020). Then we checked and choose suitable variables that were included in the linear model. We choose the variable in a way that highly correlated explanatory variables were not included in the model simultaneously to avoid confounding factor in the model. A regression model between significantly correlated anatomical variables and elevation was performed using linear regression analysis, also in Rstudio and R 4.3.0 (R

Core Team, 2020). The visualization of the correlation matrix and results of Pearson correlation analysis was conducted in “corrplot” package (Wei & Simko, 2021).

RESULTS

Miconia crenata stomata are found exclusively on the lower surface of the leaf (hypostomatic). The stomata type of *M. crenata* is anomocytic (Fig. 1B), meaning that the stomata are surrounded by cells that closely resemble the epidermal cells. The epidermis at the upper leaf side exhibit variety in anticlinal cell wall, namely straight, undulate, and sinuous wall (Figs. 1A–C). On the other hand, the lower side of the leaf's epidermis has anticlinal sinuous wall (Figs. 1A & 1B). Non-glandular trichomes (shown in Fig. 1E) and glandular trichomes (shown in Fig. 1F) throughout the upper and lower surfaces of the leaf. The non-glandular trichomes (Fig. 1E) are stellate hair and simple and multicellular structure. The glandular trichomes (Fig. 1F) are pilate consisting of a long stalk with multicellular head cells.

Miconia crenata possesses a single layer of epidermis tissue on both surfaces of the leaf. The leaf mesophyll is composed of palisade tissue at the upper part and sponge tissue at the lower part (Fig. 2). The palisade tissues that collected from an elevation 715 m asl tend to have a cylindrical shape (Fig. 2A) and consist of 1–2 layers. On the other hand, *M. crenata* leaves collected from elevations of 800, 900, and 1,000 m asl showed one layer palisade tissue with funnel shaped (Figs. 2B–D). The *M. crenata* leaf also contains druse-shaped CaCO₃ crystals which are scattered throughout the leaf mesophyll (Fig. 2A).

Anatomical differences in the leaves of *M. crenata* were observed at varying elevation. These differences did not arise in the tissue structure, but rather in the size of the tissues in the leaves. There are notable variations in the thickness of the upper epidermis, mesophyll, and the size of stomata (Table 1).

The stomata of *M. crenata* at an elevation of 715 m asl are the shortest, measuring with range 15.46 and 20.38 µm. In contrast, *M. crenata*, which resides at an elevation of 1,000 m asl, has the longest stomata, ranging from 16.52 to 22.16 µm. At the highest altitude *M. crenata* possess the narrowest measuring between 13.65 and 18.34 µm, whereas the widest are owned by individuals at the lowest altitude (13.98–19.51 µm). The stomatal density of *M. crenata* tend to decrease as the altitude increases (Fig. 3). The *M. crenata* at highest altitude have a stomatal density ranging from 159 to 334 stomata/mm², while at the lowest altitude have the highest stomatal density ranging from 298 to 754 stomata/mm².

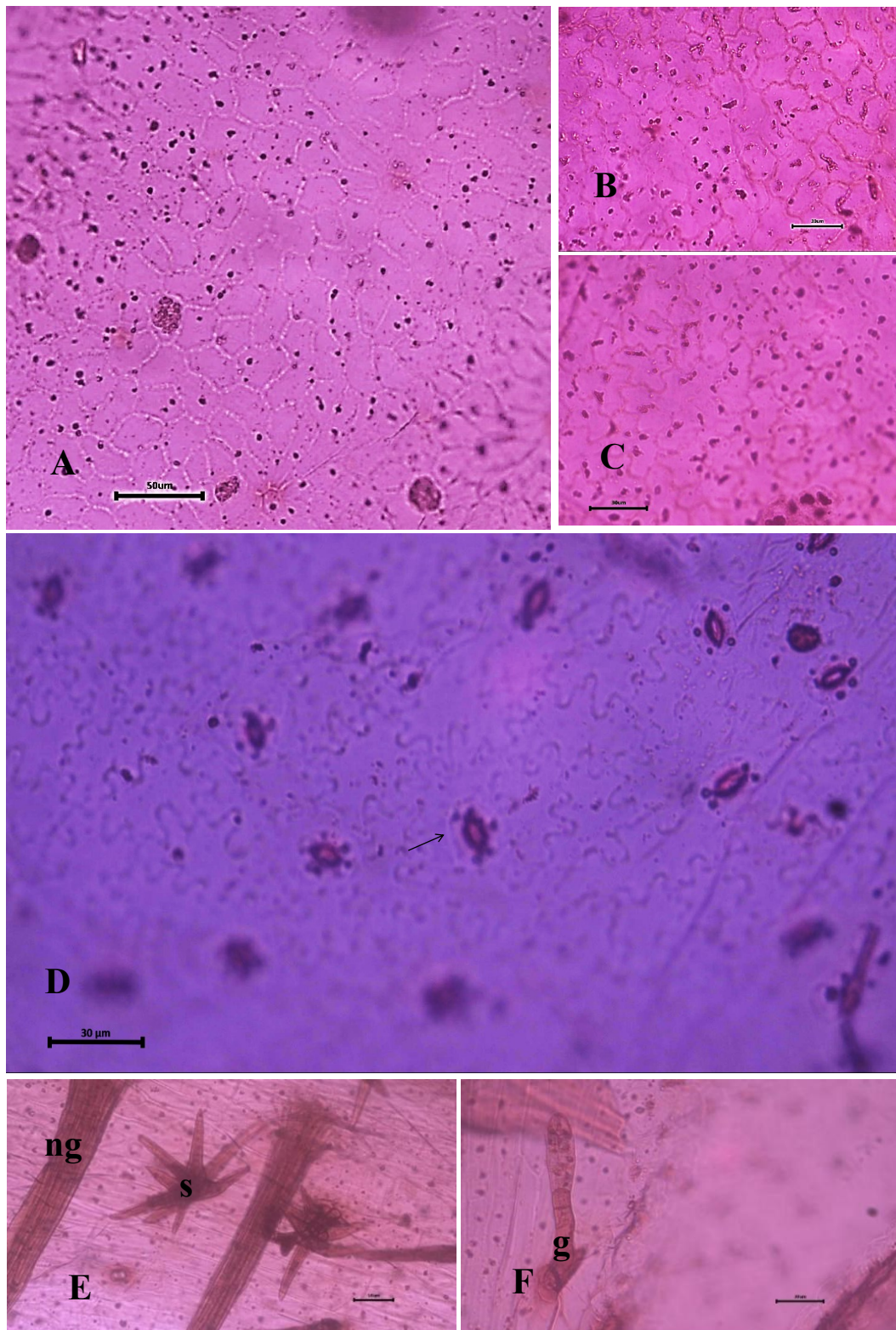


Fig. 1. Leaf surfaces of *Miconia crenata*. A-C. Upper leaf surface. A. Straight anticlinal cell wall. B. Undulate anticlinal cell wall. C. Sinuous anticlinal cell wall. D. Sinuous anticlinal cell wall in the lower surface of the leaf. E-F. Trichomes of *M. crenata*. Anomocytic stomata (arrow sign) that only occur at the lower side of the leaf of *M. crenata*. Non-glandular trichomes with stellate shape (s), simple and multicellular type (ng), and glandular trichomes, pilate type (g). Scale bar: 30 μm (B, C, D & F), 50 μm (A & E).

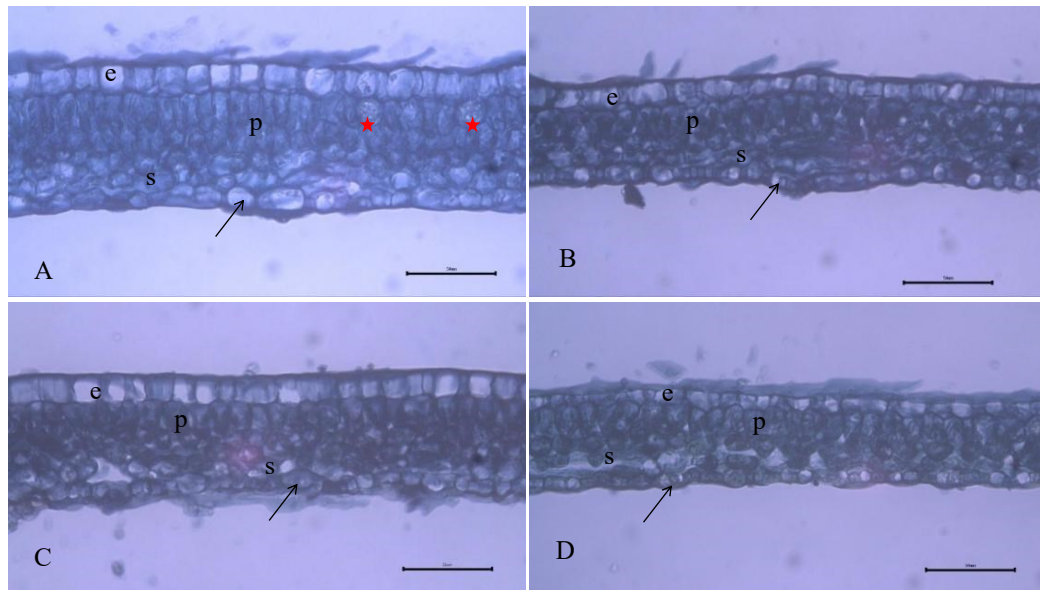


Fig. 2. Cross section of *Miconia crenata* leaf samples from four elevation. A. 715 m asl. B. 800 m asl. C. 900 m asl. D. 1000 m asl. ea: upper epidermis; p: palisade tissue; s: sponge tissue; red arrow: lower epidermis; star: druse crystal. Scale bar: 50 μ m.

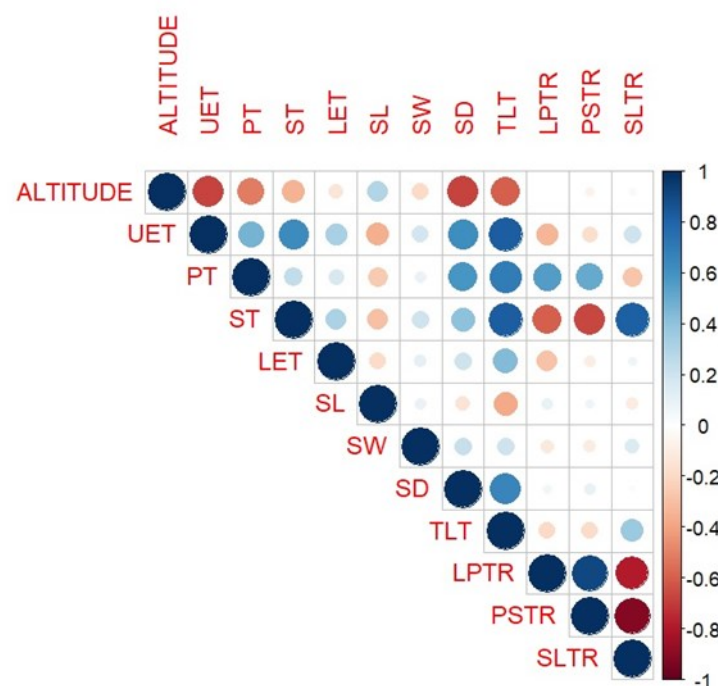


Fig. 3. Correlation among the anatomical tissue parameters. UET: upper epidermis thickness, PT: palisade thickness; ST: sponge thickness; LET: lower epidermis thickness; SL: stomata length; SD: stomata density; SW: stomata width; TL: total leaf thickness; LPTR: leaf thickness-palisade thickness ratio; PSTR: palisade thickness-sponge thickness ratio; SLTR: sponge thickness-leaf thickness ratio.

Table 1. Values of measured anatomical parameter (with the standard deviation value) of *Miconia crenata* leaves along different elevation: 715, 800, 900, and 1,000 m asl. Number of measurements for each individual: 10 measurements, total measurements: 40 measurements per elevation.

Anatomical parameter	Elevation (m asl)			
	715	800	900	1,000
Length of stomata (μm)	18.10 \pm 1.17	19.08 \pm 1.35	18.45 \pm 1.43	19.58 \pm 1.44
Width of stomata (μm)	16.32 \pm 1.21	15.79 \pm 1.19	16.25 \pm 1.38	15.45 \pm 1.01
Stomata density (Σ stomata/ mm^2)	506 \pm 99	338 \pm 67	332 \pm 82	271 \pm 46
Upper epidermis thickness (μm)	18.21 \pm 2.31	11.54 \pm 2.12	14.91 \pm 2.29	8.59 \pm 1.49
Lower epidermis thickness (μm)	10.59 \pm 1.85	9.34 \pm 1.28	11.17 \pm 2.09	9.20 \pm 1.47
Palisade thickness (μm)	32.36 \pm 7.74	19.58 \pm 1.86	14.91 \pm 2.29	20.91 \pm 1.83
Sponge thickness (μm)	30.09 \pm 8.10	16.45 \pm 2.39	27.91 \pm 6.20	17.40 \pm 3.56

The thickness of upper epidermis of *M. crenata* tend to decrease as the altitudinal increases (Fig. 3). Nevertheless, this pattern is not consistent (Table 1). A comparable trend was observed in lower epidermis, sponge and leaf thickness of *M. crenata*. Conversely, the thickness of the palisade of *M. crenata* decreased as altitude increase from 715 to 900 m asl, with corresponding thickness values of 32.36 μm , 19.58 μm , 14.91 μm . Then increasing at an altitude of 1,000 m asl (20.91 μm) (Table 1).

Leaf thickness of *M. crenata* is strongly correlated with the upper epidermis, sponge, and palisade, indicating that the majority of leaf thickness is contributed by the sponge and palisade thickness (Fig. 4). In addition, there is positive correlation between leaf thickness and stomata density, however it is not very strong. Similar findings were also achieved regarding the association between stomata density and upper epidermis thickness.

DISCUSSION

Miconia crenata is an exotic plant in MGPNP that has become invasive due to its highly successful dispersal technique and reproductive strategies, resulting in the high number of seed production. Invasive plants species employ a vegetative approach by adapting to many types of environments and rapidly developing their vegetative as a fast-growing species (van Kleunen *et al.*, 2010). In this study, we detected this vegetative adaptation due to its leaf anatomical variation along elevation. The value of stomatal density and leaf upper epidermis thickness of *M. crenata* were getting smaller along the increase of the habitat elevation.

The negative correlation of elevation and the stomata density was consistent with a relevant study by Putri *et al.* (2022) that suggested that the stomata density value of an exotic species *Bartlettina sordida* at MGPNP tends to get lower along the increase of altitude. The same results are found by Li *et al.* (2006) and Nautiyal & Purohit (1980). Stomata have the function of regulating the exchange of O_2 and CO_2 , and water (Hetherington & Woodward, 2003; He & Liang, 2018; Lawson & Matthews, 2020). However, this finding contradicts the finding of Liu *et al.* (2020) who reported a positive correlation between stomatal density and elevation. At higher altitude, the availability of CO_2 and O_2 is generally reduced. Consequently, plants should enhance their stomatal density to optimize their CO_2 and O_2 intake. However, stomatal density has positively correlate with light intensity (Liu *et al.*, 2020). Hence, we speculate that the leaf of *M. crenata* at higher altitude are having smaller stomatal density because of potentially lower light intensity. This is due the fact that most of the examined specimens grew under the shade of forest canopies.

Yang *et al.* (2022) and Yang *et al.* (2023) have found that the upper and lower epidermis thickness tend to decrease as altitude increase. This reduction enhances the efficiency of gas exchange between leaves and the external environment. There was a negative correlation between the thickness of *M. crenata* palisade tissue and altitude. This finding is in concordance with previous studies conducted by Yang *et al.* (2022), Yang *et al.* (2023), and Nautiyal & Purohit (1980) which stated that the palisade thickness tends to decrease as altitude decreases. According to their studies, the reduction of the palisade, sponge and leaf thick-

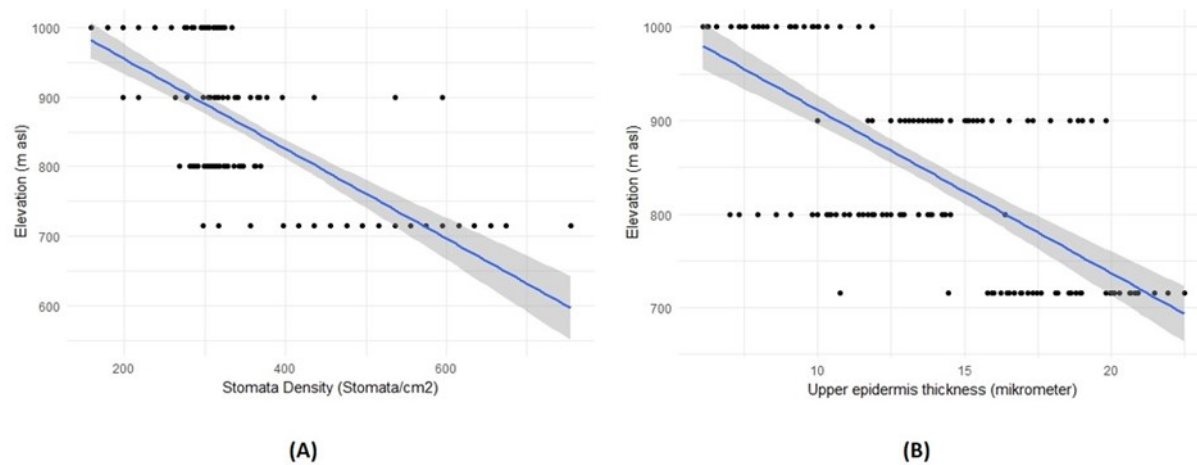


Fig. 4 The linear regression model that indicate the significant negative correlation between elevation stomata density (A) and elevation and upper epidermis (B) of the *Miconia crenata* leaves. Number of samples are 160. The correlation coefficient between altitude and the density of the stomata and upper epidermis thickness are -0.39 ($p\text{-value} = 7.07 \times 10^{-9}$) and -10.95 ($p\text{-value} = 2.15 \times 10^{-9}$) respectively. The adjusted r square for the model is 0.56.

ness indicates a plant adaptation to drought stress and to increase CO_2 intake. However, Liu *et al.* (2020) and Li *et al.* (2006) discovered a contradictory result that suggested a positive correlation between palisade thickness and habitat elevation. Palisade is the leaf tissue that responsible for carrying out photosynthesis. Palisades are known as plastic tissue because of their adaptability to the environment. A thicker palisade tissues could be associated to enhanced photosynthetic efficiency and could be an indication of the amount of chloroplast.

However, this study discovered an abnormality in the leaf thickness and palisade of *M. crenata* at an altitude of 900 m alt. This anomaly may be caused by plant phenotypic plasticity factor. The flexibility of plant tissue is determined by environmental factors such as temperature, humidity, CO_2 content, and even wind (Nautiyal & Purohit, 1980; Jahdi *et al.*, 2020). These environmental factors are not the sole determinants of plant tissue and its plasticity. Therefore, any deviation in the examined individuals in this study may be impacted by one or several environmental factors that differ or fluctuate to some extent at each altitude.

According to the fundings of this study, the ability of *M. crenata* to adjust to different light conditions was relatively noticeable. This species was observed in several environmental conditions characterized by diverse levels of light intensity and shade. In the tropical forest context, *M. crenata* is recorded as an exotic species capable of thriving in the innermost area of the undisturbed tropical forest as a result of its adaptation (Wad-

dell *et al.*, 2020). In this study, we did not collect light data on the parameter of light. However, considering the state of the sampling sites, the plots were located in close proximity or directly along the hiking trail road, exhibiting a similar trees canopies density and condition to some extent. Therefore, we assumed that there is negligible difference in the light condition among the sampling plots. We acknowledge that this assumption is a limitation of this study. Hence, further research is required for leaf anatomical variation of exotic invasive species in relation to various seasonal and environmental factors such as light intensity, pH, temperature, and humidity. Furthermore, it is necessary to compare the structure variation across different plant taxa and growth forms.

CONCLUSIONS

The adaptation of *M. crenata* leaves towards altitude in Gunung Gede-Pangrango National Park involves alterations in the stomata size, stomata density, and thickness of mesophyll tissue and leaves. The higher the altitude, the thinner of upper and lower epidermis, palisade, sponge and leaf of *M. crenata*. In higher altitudes, *M. crenata* exhibited lower stomatal density and longer stomatal size.

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