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Journal of Multidisciplinary Applied Natural Science Vol. 6 No. 2 (2026) https://doi.org/10.47352/jmans.2774-3047.347
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Research Article

Stomata Characterization of Native *Dendrobium* in Liwa Botanical Garden

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Received : October 5, 2025	Revised : November 17, 2025	Accepted : November 29, 2025	Online : January 14, 2026

Abstract
Dendrobium is one of the most commonly collected native orchid genera at the Liwa Botanical Garden, Lampung, Indonesia. In its conservation efforts, the identification of native orchid species through anatomical characterization is essential. The aim of this research is to identify the native *Dendrobium* species collection at the Liwa Botanical Garden based on stomatal anatomical characteristics and to confirm these results with previous morphological and molecular characterization. The research steps involved the collection of leaves from 19 *Dendrobium* accessions at the Liwa Botanical Garden, while the anatomical characterization was conducted by preparing paradermal sections to microscopically observe the stomata. The main anatomical characteristics observed included stomatal aperture width, stomatal length, stomatal width, number of stomata, stomatal density, and stomatal index. The results of the study show that, overall, the stomatal aperture width is 2.88 µm, stomatal length is 12.38 µm, stomatal width is 12.58 µm, stomatal density is 29 stomata/mm², and stomatal index is 0.061%. Phenetic analysis based on the dendrogram divided the different native *Dendrobium* samples into two clusters (A and B) with similarity indices of 1.60 and 0.90, and PCA values (0.170 and 0.044) were found to be greater than 0.02, indicating the contribution of each group. The PCA value of 0.170 reflects the influence of stomatal area, whereas 0.044 reflects the influence of stomatal aperture width, stomatal index, and stomatal density. The anatomical characterization results show a correlation with the identification outcomes based on morphological and molecular characteristics. Furthermore, these findings can serve as a recommendation for the identification of native orchid species and provide a basis for the conservation efforts of native *Dendrobium* at the Liwa Botanical Garden, Indonesia.

Keywords: anatomi characterization, identification, Liwa botanical garden, native *Dendrobium*, stomata

1. INTRODUCTION

Dendrobium is one of the most diverse native orchids, with over a thousand species naturally occurring across various tropical regions, including Indonesia [1][2]. Liwa Botanical Garden is the only regional botanical garden developed by the National Research and Innovation Agency (BRIN) in Lampung Province and has conducted the exploration and collection of 48 accessions [3][4]. In addition to bacterial infections [5], fungi [6], and viruses [7]-[9], species identification remains a primary challenge in the conservation efforts of native *Dendrobium* at Liwa Botanical Garden [10].

Identification can be conducted through morphological characterization [11]-[13], anatomical characterization [14][15], and molecular characterization [16][17]. So far, species identification of native *Dendrobium* in the region has encountered several challenges, particularly in terms of accuracy [4][7], which has traditionally relied on observation techniques through morphological [18] and molecular [19] characterizations. Observations of physical traits such as flower shape, leaf size, and stem structure are often insufficient to distinguish morphologically similar species [20][21]. Additionally, molecular characterization based on analysis of genotypic variation, which may itself be affected by environmental conditions [22][23], has limitations for reliable species identification [24] and its for direct use in conservation [25] and plant breeding programs [26], particularry when applied in isolation.

This study is a continuation of our previous research aimed at complementing the identification of native *Dendrobium* at Liwa Botanical Garden through anatomical characterization. Mahfut [4] explained that the main anatomical characterization is conducted through the observation of stomata, such as shape and size, which are used to understand the diversity within the *Dendrobium* genus. Based on its characteristics, most *Dendrobium* species have stomata only on the lower surface of the leaf (hypostomatic) with a

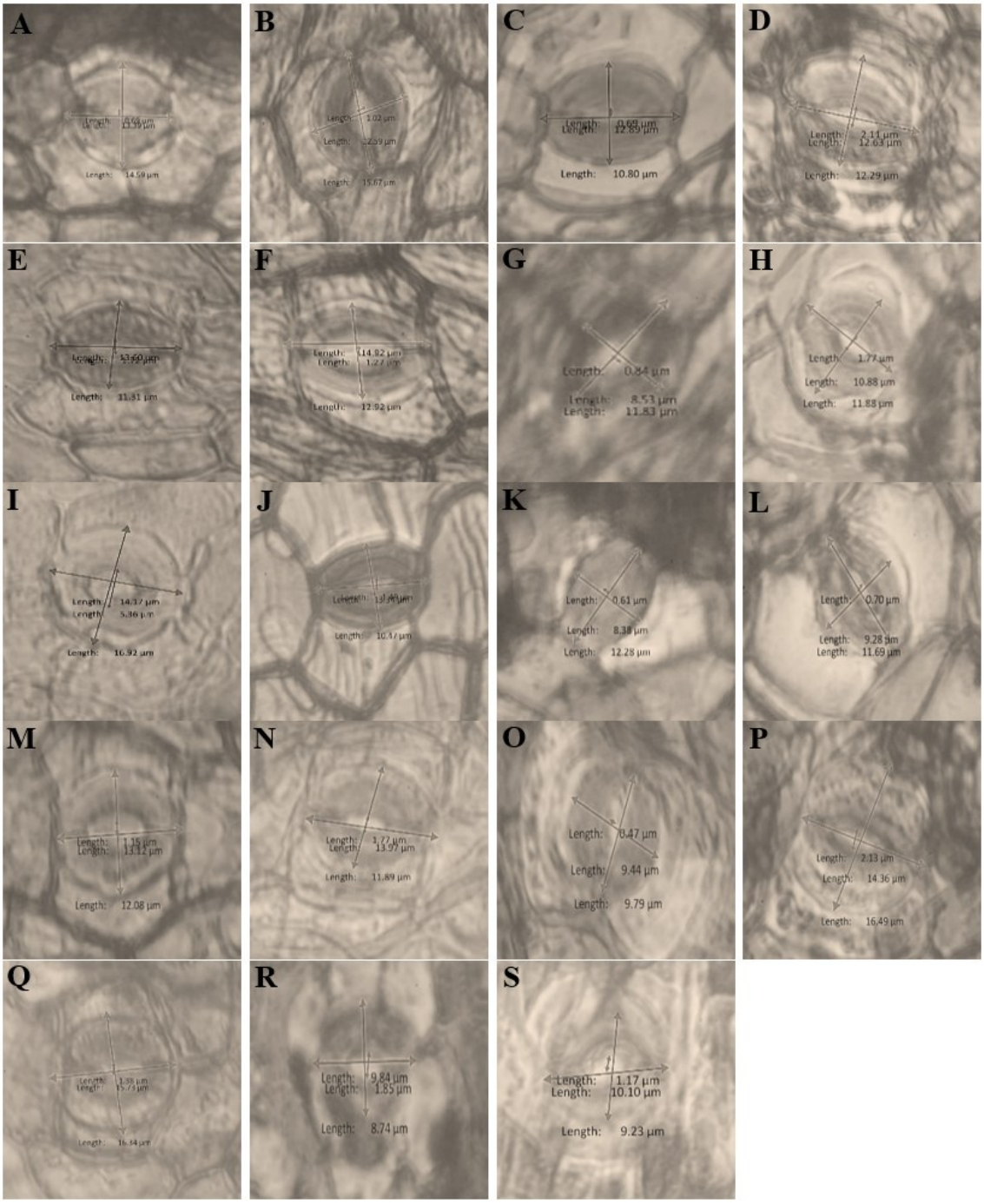

Figure 1. Stomatal anatomy of native *Dendrobium* accessions from Liwa Botanical Garden observed at 100× magnification. A. D1, B. D2, C. D3, D. D4, E. D5, F. D6, G. D7, H. D8, I. D9, J. D10, K. D11, L. D12, M. D13, N. D14, O. D15, P. D16, Q. D17, R. D18, S. D19.

parasitic type and higher stomatal density on the lower surface compared to the upper surface [27]. The large number of stomata on the underside can increase the rate of transpiration [28], which is an adaptive mechanism for plants in environments with high humidity, and *Dendrobium* exhibits good transpiration and photosynthesis efficiency [29]. Studies on various *Dendrobium* species from Nepal [30] and Indonesia [31] have shown that quantifiable traits like stomata type, density, size, and position, as well as epidermal cell shape, provide distinguishing characteristics. Mahfut *et al*. [32] also reported that the analysis of diversity using principal component analysis (PCA) showed that only two of the six characteristics, stomata aperture width (LBS) and stomata cell length (PS), had the most influence on the observed variation. Characterization of stomata anatomical is a valid

approach for complementing morphological and molecular methods in the accurate identification of *native Dendrobium* species [33]. Additionally, this study aims to confirm the results of previous identifications based on morphological and molecular characterization. Furthermore, the findings of this research may serve as a recommendation for identification efforts and provide a strong foundation for more accurate identification, as well as offering recommendations for the conservation of native *Dendrobium* species at Liwa Botanical Garden, Lampung, Indonesia.

2. MATERIALS AND METHODS

2.1. Materials

A survey and sample collections were conducted on 19 native *Dendrobium* collections at the greenhouse of the Liwa Botanical Garden, which were subsequently labeled D1–D19. The collected samples consisted of 1–2 leaves per collection, with the sampling process following the previous methods [4][30]. The collection process began with selecting healthy and representative leaves from each *Dendrobium* species to ensure that the obtained samples could represent genetic variation. The samples were stored and further analyzed in the laboratory.

2.2. Methods

2.2.1. Stomata Anatomical Characterization

Leaf stomatal characterization was performed by making paradermal cuts of the leaf following the method of Mahfut [4]. The leaf was cleaned using 70% ethanol, then the lower leaf surface section was placed on a slide, coated with transparent nail polish, and dripped with glycerine, followed by covering with a cover glass. Observations were made using a light microscope (Olympus, 10× ocular, 40× objective) to analyze stomatal characteristics, including stomatal aperture width,

Table 1. Average size and stomatal index of native *Dendrobium* at Liwa botanical garden.

Sample code	Stomatal length (µm)	Stomatal width (µm)	Stomatal aperture width (µm)	Stomata density (stomata/mm^2)	Stomata index (%)
D1	13.34	14.52	0.69	49	0.07
D2	15.59	12.56	1.04	27	0.05
D3	12.61	10.40	0.62	35	0.07
D4	12.48	12.56	2.29	19	0.06
D5	13.54	11.36	1.74	34	0.06
D6	14.77	12.77	1.23	31	0.06
D7	11.76	8.60	0.78	40	0.07
D8	10.81	11.72	1.81	32	0.09
D9	16.84	14.22	5.33	19	0.05
D10	13,51	10.48	1.51	34	0.09
D11	12.34	8.38	0.63	28	0.08
D12	11,69	9.36	0.71	28	0.09
D13	13.12	12.07	1.16	29	0.05
D14	13.86	11.84	1.82	10	0.03
D15	9.71	9.54	0.42	11	0.04
D16	14.39	16.56	2.16	12	0.04
D17	15.70	16.42	1.42	13	0.05
D18	9.06	8.72	1.88	18	0.05
D19	10.28	9.24	1.21	9	0.03

Table 2. Similarity index of native *Dendrobium* samples at Liwa botanical garden.

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19
D1	1.00																		
D2	0.75	1.00																	
D3	1.00	0.75	1.00																
D4	0.50	0.25	0.50	1.00															
D5	0.75	0.50	0.75	0.75	1.00														
D6	0.75	0.50	0.75	0.75	1.00	1.00													
D7	0.75	0.50	0.75	0.25	0.50	0.50	1.00												
D8	0.75	0.50	0.75	0.75	1.00	1.00	0.50	1.00											
D9	0.25	0.50	0.25	0.75	0.50	0.50	0.00	0.50	1.00										
D10	0.75	0.50	0.75	0.75	1.00	1.00	0.50	1.00	0.50	1.00									
D11	0.75	0.50	0.75	0.25	0.50	0.50	1.00	0.50	0.00	0.50	1.00								
D12	0.75	0.50	0.75	0.25	0.50	0.50	1.00	0.50	0.00	0.50	1.00	1.00							
D13	0.50	0.75	0.50	0.50	0.75	0.75	0.25	0.75	0.75	0.75	0.25	0.25	1.00						
D14	0.25	0.50	0.25	0.75	0.50	0.50	0.00	0.50	1.00	0.50	0.00	0.00	0.75	1.00					
D15	0.25	0.50	0.25	0.25	0.00	0.00	0.50	0.00	0.50	0.00	0.50	0.50	0.25	0.50	1.00				
D16	0.25	0.50	0.25	0.75	0.50	0.50	0.00	0.50	1.00	0.50	0.00	0.00	0.75	1.00	0.50	1.00			
D17	0.25	0.50	0.25	0.75	0.50	0.50	0.00	0.50	1.00	0.50	0.00	0.00	0.75	1.00	0.50	1.00	1.00		
D18	0.00	0.25	0.00	0.50	0.25	0.25	0.25	0.25	0.75	0.25	0.25	0.25	0.50	0.75	0.75	0.75	0.75	1.00	
D19	0.00	0.25	0.00	0.50	0.25	0.25	0.25	0.25	0.75	0.25	0.25	0.25	0.50	0.75	0.75	0.75	0.75	1.00	1.00

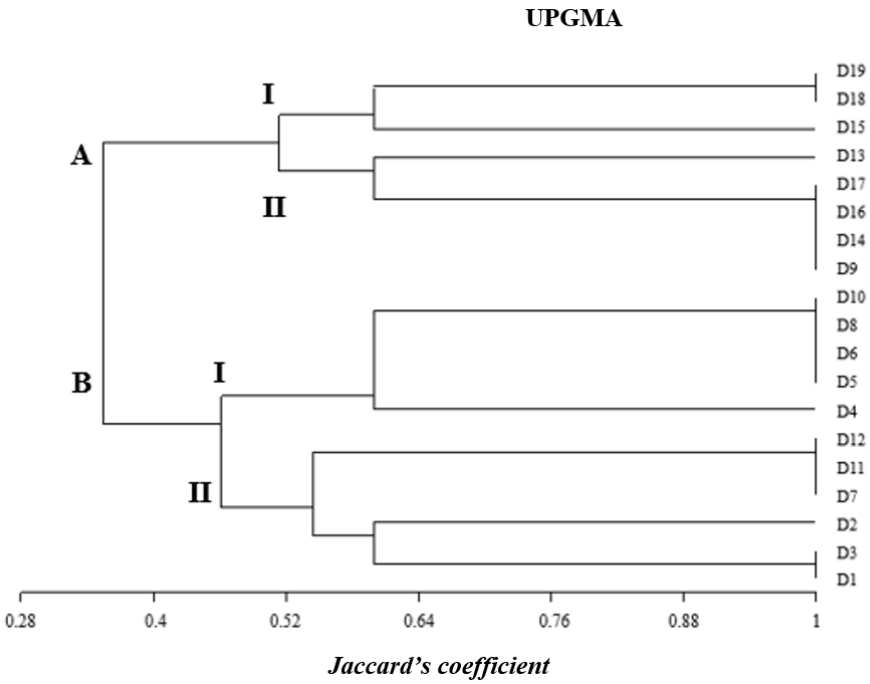

Figure 2. Phenetic analysis dendrogram of native *Dendrobium* at Liwa botanical garden.

length, width, number, density, and stomatal indeks [32][34]. Each characteristic was measured three times within a wide field of view of approximately 0.16 mm² at 400× magnification [31].

2.2.2. Data Analysis

Stomata anatomical characteristics were analyzed both quantitatively (stomata aperture width, length, and width, number of stomata, stomata density, and stomata index) and qualitatively (stomata type) in a descriptive format to examine variations in stomatal features. The multivariate statistics package (MVSP) software v.3.2 was used to generate a phenetic dendrogram and perform PCA. A phenetic dendrogram was generated using the Jaccard coefficient and the unweighted pair-group method with the arithmetic average (UPGMA) method for genetic distance analysis, while PCA scatter plots were created with the Euclidean Distance algorithm. Stomatal density and index were calculated following the methods of Asadudin et al. [14] and Putera et al. [15], respectively.

3. RESULTS AND DISCUSSIONS

3.1. Stomata Anatomical Characterization

The stomata observations on all native *Dendrobium* samples revealed anomocytic stomata type. This stomatal type is characterized by guard cells surrounded by a number of neighboring cells similar to the surrounding epidermal cells [4]. The results of the stomatal structure observations on 19 *Dendrobium* leaf samples are shown in Figure 1.

This anomocytic stomatal type is commonly found in orchids and reflects the physiological condition in response to the environment [35], such as *Phalaenopsis* and *Dendrobium* species [36]. These stomata are crucial for gas exchange including CO_2 uptake, water loss, and dynamically respond to environmental cues like light, CO_2, temperature, and water availability, a physiological adaptation to environmental stress [37]. The stomata index analysis was performed by calculating the number of stomata in a field of view divided by the number of epidermal and stomatal cells. The average size and stomatal index of 19 *Dendrobium* samples showed varying values, as presented in Table 1.

The stomatal size in 19 native *Dendrobium* samples varied, with the longest stomatal length observed in D17 (15.70 µm) and the shortest in D18 (9.06 µm). The widest stomata were found in D16 (16.56 µm), while the narrowest were in D18 (8.60 µm). The average stomatal length on the lower surface of native *Dendrobium* leaves is 7.46 mm [38]. Stomatal length is categorized into short (< 20

μm), long (20–25 μm), and very long (> 25 μm) [39]. The results obtained from 19 native *Dendrobium* samples show that their stomatal length is < 20 μm, thus categorizing them as having short stomata. The average stomatal length and width varied across samples due to environmental factors during growth [36]. These differences in stomatal size may be caused by genetic variation and environmental conditions such as humidity, temperature, and light, which affect the plant's physiological processes [40]. Xie [31] also stated that larger stomata are generally found in species growing in environments with low humidity. This is considered an adaptation that increases the transpiration rate to maintain water balance. These findings align with studies showing stomatal variation in the *Dendrobium* genus, which is associated with specific environmental adaptations [27][28].

The stomatal aperture width showed considerable variation among the samples, ranging from a minimum of 0.42 μm in D15 to a maximum of 5.33 μm in D9. The smallest value was observed in D15, while the largest was in D9. Other samples varied as follows: D1 had 0.69 μm, D2 had 1.04 μm, D3 had 0.62 μm, and D4 had 2.29 μm. The average values ranged from 0.42 to 5.33 μm, with most samples falling between 0.5 and 2.5 μm. The variation in stomatal aperture size among the samples suggests possible environmental or genetic influences, as variations in stomatal traits can affect plant transpiration and water use efficiency [33].

Stomata density in the samples also showed variation, with the highest value recorded in D1 at 49 stomata/mm^2 and the lowest in D19 at 9 stomata/mm^2. Samples D2 to D10 showed fluctuations with values ranging from 19 to 40 stomata/mm^2. The second lowest value was recorded in D14 at 10 stomata/mm^2. Meanwhile, D3, D5, D6, D7, D8, D11, and D12 exhibited relatively stable densities, ranging from 28 to 40 stomata/mm^2. These differences in stomatal density may be influenced by genetic and environmental factors [4]. The decrease in stomatal density in several samples, such as D14 and D19, may reflect adaptation to less favorable environmental conditions or could be related to specific characteristics of each accession [30]. In contrast, the higher density observed in accession D1 may enhance transpiration and photosynthetic capacity under favorable conditions, whereas lower stomatal density can be advantageous in water-limited environments by reducing water loss [33].

Hu et al. [28] state that plants with high stomatal density will increase transpiration rates through the leaves, whereas plants with low stomatal density will reduce the transpiration rate. This mechanism serves as an adaptation for plants living in arid environments to conserve water within their tissues [37]. *Dendrobium* species with high stomatal density experience higher water loss through transpiration, making them less efficient in adapting to dry climates [38]. Stomata index also shows variation across the samples, with the highest

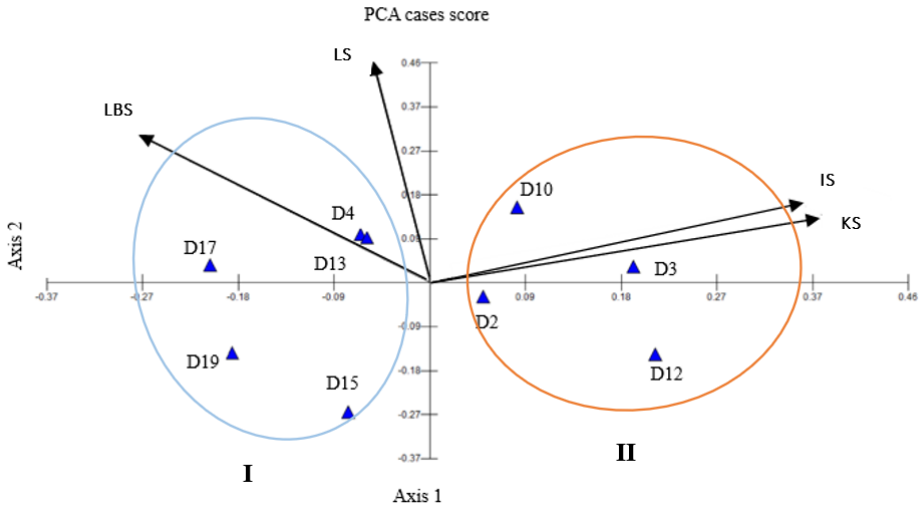

Figure 3. Grouping of PCA results based on anatomical characteristics.

Table 3. Stomata characteristics involved in the clustering of native *Dendrobium* at Liwa botanical garden.

Character	PC1	PC2
Aperture Width	0.479	-0.631
Index	0.454	0.491
Density	0.486	0.519
Area	0.572	-0.302
Eigenvalues	0.170	0.044
Percentage	73.346	19.088
Cum. Percentage	73.346	92.434

values recorded in D8 and D10 (0.09%) and the lowest in D14 and D19 (0.03%). D1, D3, and D7 have an index of 0.07%, while D2, D9, D13, D17, and D18 range between 0.05% and 0.06%. D4, D5, and D6 show slightly lower indices (0.06%). The variation in stomatal index is influenced by environmental factors, particularly light intensity and humidity. Higher light intensity leads to an increase in stomatal index and density on both leaf surfaces. These changes reflect the plant's adaptive mechanisms to its environment, as higher light intensity also raises the surrounding temperature [4] [33].

3.2. Phenetic Analysis

The results of calculating morphology character data for the 19 native *Dendrobium* showed similarity index values ranging from 0.0–1.00 (Table 2). Furthermore, the results of the phenetic analysis revealed a relationship between *Dendrobium* accessions, which were divided into two main groups: group A and group B (Figure 2).

The phenetic dendrogram based on anatomical characteristics shows that group A divided into subcluster I (Ia: D19 and D18, Ib: D15, similarity index: 0.75) and subcluster II (IIa: D13, IIb: D17, D16, D14, and D9, similarity index: 0.75). Group B consists of subcluster I (Ia: D10, D8, D6, D5; Ib: D4) and subcluster II (IIa: D12, D11, D7, IIb: D2, and D3-D1). This clustering indicates genetic relationships among native *Dendrobium* species at Liwa Botanical Garden, which may be influenced by genetic variation and environmental factors. Similarity indices of 0.75 suggest that the species within each subcluster share notable morphological or anatomical traits, supporting previous findings that anatomical features play a role in species classification [31]. The findings also highlight the importance of anatomical analysis for understanding the diversity within the *Dendrobium* genus [4][32].

The clear separation between clusters A and B indicates that combinations of stomatal traits play an important role in cluster formation. Differences in mean stomatal density, stomatal number, and stomatal index among clusters not only generate the clustering pattern mathematically but also reflect distinct physiological strategies for regulating gas exchange and water loss. Accessions in cluster A generally exhibit more homogeneous patterns of stomatal density and size, whereas accessions in cluster B show more diverse combinations of stomatal traits, resulting in further separation into smaller subclusters. Thus, variation in stomatal size, density, and index contributes substantially to cluster differentiation and can serve as anatomical indicators for distinguishing groups of *Dendrobium* accessions [4][31][32].

Variation in the combination of stomatal size and density between clusters A and B reflects adaptive responses to differences in microhabitat conditions, such as light intensity, temperature, and water availability in Liwa Botanical Garden. Plants with higher stomatal density or smaller stomata tend to be more efficient in controlling water loss, whereas plants with fewer but larger stomata tend to have a greater capacity for gas exchange under certain environmental conditions. Therefore, clustering based on stomatal traits not only illustrates patterns of relatedness but also reveals differences in physiological adaptation strategies among *Dendrobium* accessions [4][31].

The resulting clusters can be used to identify groups of accessions that are closely related and

possess relatively stable stomatal characteristics. These groups have the potential to serve as practical taxonomic units as well as priority genetic resources for conservation and breeding programs. Integrating stomatal anatomical information with morphological data and, at a later stage, molecular data will strengthen the basis for classification and support more systematic management of native *Dendrobium* collections in Liwa Botanical Garden [31][32]. This multicharacter approach is consistent with the recommendations of various orchid taxonomic studies that emphasize the integration of morphological, anatomical, and genetic traits to understand diversity and phylogenetic relationships within the genus *Dendrobium*.

3.3. PCA

PCA was performed following the dendrogram construction to better visualize groupings and assess inter-group distances [31]. PCA effectively highlights the contribution of anatomical traits to the grouping process. In this study, PCA results are presented through a scatter plot and correlation table [14][15]. The PCA analysis of 19 native *Dendrobium* leaf samples from Liwa Botanical Garden based on anatomical characteristics, is shown in Figure 3.

Stomata aperture width, stomata index, stomata density, and stomata area influence the variation in the data explained by the first principal component (PC1) and the second principal component (PC2). Stomatal area has an impact on the main variation in the data (PC1), indicating that larger stomatal area is associated with similarity in several characteristics. Plants with higher stomatal density tend to exhibit more similar patterns, such as the number of stomata on the leaf surface. Moreover, plants with higher stomatal index values also show similar stomatal distribution patterns, suggesting a positive correlation between these two characteristics. Meanwhile, stomatal aperture width contributes differently to the secondary variation explained by PC2. This analysis underscores the importance of stomatal traits, such as aperture width and index, in understanding the ecological and adaptive strategies of plants. Higher stomatal density and index are often linked to improved water regulation and gas exchange, which are crucial for plant adaptation to varying environmental conditions [34].

The distribution pattern of accessions in the PCA biplot supports the clustering results obtained from the dendrogram. Accessions belonging to Cluster B (e.g., D3, D10, and D12) tend to have higher PC1 scores and are strongly associated with the vectors for stomatal density and stomatal index. This pattern indicates that the separation of Cluster B from Cluster A is primarily driven by relatively high values of stomatal density and stomatal index. In contrast, several accessions in cluster A (such as D15, D17, and D19) occupy positions closer to the vectors for stomatal area and stomatal aperture width, suggesting that this cluster is more strongly characterized by variation in stomatal size than by stomatal density. Accordingly, PC1 represents a gradient of stomatal density–stomatal index, whereas PC2 represents a gradient of stomatal size, and together these principal components explain a consistent separation pattern among the clusters [4][31].

The combination of stomatal traits also suggests two predominant adaptive strategies in native *Dendrobium* accessions from Liwa Botanical Garden. Accessions with high PC1 scores and strong contributions from stomatal density and stomatal index (mainly in cluster B) are likely associated with a water-saving strategy and a response to microhabitat conditions with higher light intensity. In contrast, accessions more strongly influenced by stomatal area and stomatal aperture width (predominantly in cluster A) may reflect a strategy that prioritizes greater gas-exchange capacity under specific environmental conditions. From a taxonomic perspective, this pattern indicates that the combination of stomatal density, stomatal index, and stomatal size can serve as informative anatomical characters for distinguishing groups of *Dendrobium* accessions and for defining practical taxonomic units as well as priority genetic resources for conservation and breeding programs [31][32]. The stomatal characters that play a role in the clustering of native *Dendrobium* are presented in Table 3.

The contribution of PC1 and PC2 accounts for 92.434%, making anatomical characteristics the primary components for analyzing the entire sample. The key characteristic contributing to the grouping of native *Dendrobium* in PC1 is stomatal

area. In PC2, the contributing factors are stomatal aperture width, stomatal index, and stomatal density. The C1 has an eigenvalue of 0.17, explaining 73.346% of the total morphological variation, while PC2 has an eigenvalue of 0.044, explaining 19.088%. The variation in the number, size, and density of stomata across the 19 native *Dendrobium* leaf samples is influenced by environmental factors such as light intensity and humidity. All samples exhibited stomata smaller than 20 µm, with variation in stomatal density, reflecting the plants' adaptation to their environmental conditions. These findings suggest that stomatal characteristics are key to understanding plant responses to environmental stressors. The variation in stomatal traits supports the hypothesis that environmental factors, especially light and humidity, drive morphological plasticity in plants [4][27].

The correlation of the overall results of native *Dendrobium* identification at Liwa Botanical Garden, based on characterizations of leaf morphological, stomatal anatomy, and molecular reveals congruent relationships across all accessions. The dendrogram analysis divides the samples into two main groups, with subclusters indicating different levels of genetic relatedness [19]. The proximity of these clusters reflects their evolutionary relationships and phenotypic similarities between species or subspecies [18]. Supporting this, PCA analysis indicates that several leaf morphological traits and stomatal anatomical characteristics are key factors explaining the variation among samples, with a total contribution of more than 92%. This suggests that the combining leaf morphological characteristics, stomatal anatomy, and molecular characterization provides a useful phenetic for grouping the native *Dendrobium* accessions D1-D19, although species identities and evolutionary relationships remain unverified and wound require dedicated phylogenetic analysis.

4. CONCLUSIONS

The phenetic analysis based on stomatal anatomical characteristics revealed distinct groupings among native *Dendrobium* accessions at Liwa Botanical Garden, aiding conservation planning. This clustering, driven by variations in stomatal traits such as aperture width, density, and index, highlights the ecological and adaptive diversity within the species. The results emphasize the importance of stomatal anatomy as a reliable tool for species identification, complementing traditional morphological and molecular methods. Despite the lack of phylogenetic confirmation for the species identities of accessions D1–D19, the findings provide a valuable foundation for enhancing conservation strategies and supporting future breeding initiatives.

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Conceptualization, Investigation, Data Curation, Supervision, Project Administration, Funding Acquisition, Writing – Original Draft Preparation, and Writing – Review & Editing, S. W.; Software, and Formal Analysis, L. A.; Methodology, Resources, and Visualization, M. M.; Validation, S. W., M. M., and L. A.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors thank the Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM), Universitas Lampung which has funded the completion of this research through the Penelitian

Terapan DIPA BLU 2025, with the contract number of 706/UN26.21/PN/2025. This research is an implementation of the Cooperation Agreement with the Liwa Botanical Garden partner through the Environmental Agency of West Lampung Regency under Agreement No. 660/246/III.14/2021 and 1605/UN.26.17/KS.00/2021.

DECLARATION OF GENERATIVE AI

Not applicable.

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