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Anatomical Structure of Orchid Species (Orchidaceae) in Pengelengan Hill, Sukasada, Buleleng, Bali

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Abstract. Orchids are among the plants with the greatest diversity in the world. Orchids have several specialized structures, such as velamen and cuticles, which help protect them against unfavorable environmental conditions. The anatomical structure of a plant is closely related to how it adapts to its environment. Anatomical characteristics can support data on previously known species, complementing analyses beyond morphological and molecular data related to taxonomic classification. The aim of this study was to examine the variation in leaf anatomical structures among several orchid species found in the Pengelengan Hills, Sukasada, Bali. Sample collection was conducted using exploratory methods, and leaf anatomical structures were analyzed using cross-sectional and peeling methods. Based on the overall research findings, there were variations in the leaf anatomical structures among the five orchid species found in Pengelengan Hills, Sukasada, Buleleng, Bali. Variations were observed in the paradermal sections, showing differences in the arrangement of epidermal tissues. The variations include epidermal cell arrangement, cell shape, stomatal type, and epidermal derivatives. The arrangement of epidermal cells was categorized into two types: aligned and non-aligned. The shapes of epidermal cells observed include pentagonal, hexagonal, elongated, and irregular. The stomatal types observed include tetracytic and paracytic, and epidermal derivatives such as non-glandular trichomes were found in species *B. hyacinthoides* and *M. oblitterata.*

Keywords: Orchid; Anatomy Structure; Stomata; Pengelengan Hill

I. INTRODUCTION

Orchids are one of the plants with the greatest diversity in the world. Orchid diversity is centered in tropical and subtropical regions with a large distribution [1]. Orchids have several structures that serve as defenses against less suitable conditions. The orchid leaf surface is covered with a waxy layer that protects the plant from pests and diseases [2]. Additionally, orchid roots have a velamen layer that absorbs atmospheric water; it is a form of adaptation for epiphytic plants, particularly orchids [3]. The waxy layer and velamen in orchids are anatomical structures that are not directly visible, unlike morphological characteristics. Anatomical structures include components throughout the plant, from roots to seeds, and are classified into five types: epidermal tissue, vascular tissue, ground tissue, supportive tissue, and secretory tissue [4].

The anatomical structure of a plant is closely related to how it adapts to its environment. This is seen in the *pseudobulbs* of *Cymbidium sinense* orchids, which have a thick cuticle layer that resists water and gases. This structure allows *Cymbidium sinense* orchids to retain about 64% of their water content after 42 days, even under water stress conditions [5]. Furthermore, in other species, the pseudobulbs of Oncidium "Goldiana" also support photosynthesis. The *pseudobulbs* accumulate carbohydrates during the vegetative development, facilitating the easy formation of new shoots [6]. Anatomical structures in plants, aside from their functions and environmental adaptations, can also relate to plant classification. For example, the Holcoglossum genus can be distinguished from other closely related genera by anatomical characteristics of the leaf tissue [7].

Research on the anatomical structures of orchids was conducted on Arachnis, Phalaenopsis, and Vanilla, revealing differences in epidermal tissue structures [8]. Additionally, in Sempu Island, a study showed variations in mesophyll tissue types, either homogeneous or heterogeneous, in four species of epiphytic orchids [9]. Recent research in Bali was conducted at Eka Karya Botanic Garden in Bedugul. This study focused on the diversity of orchid collections based on morphological characteristics and stomata. The results indicated eight orchid species, including epiphytic orchids, with two types of stomata: paracytic and anomocytic [10]. Anatomical characteristics can support existing species data, fit out morphological and molecular data related to taxonomic classification. Pengelengan Hill is the site where the Turyapada Tower will be built. Data on the types of plants that grow naturally in the area, both now and in the future, is crucial to assessing environmental damage in the years following the tower's construction by comparing vegetation diversity and plant structure over time. The aim of this study was to determine the variation in leaf anatomical structures among several orchid species found in Pengelengan hills, Sukasada, Bali.

II. METHODS

Study site

This research was carried out from February to April 2024. Orchid leaf samples were collected using a survey method at Pengelengan Hill, Sukasada, Buleleng, which is located at 8°14'13.7 "S; 115°08'55.7"E coordinates. The research on anatomical specimens was conducted at the Plant Structure and Development Laboratory, Department of Biology, Udayana University.

Research Tools and Materials

The tools and materials used were ziplock bags, labels, silica gel, adhesive tape, scissors, a cutter, bleach, distilled water, 70% alcohol, watch glasses, beakers, object glass, cover glass, dropper pipettes, needles, tweezers, 0.1% safranin, a binocular microscope, and an optical lab.

Research Methods

Samples for anatomical observation were taken from the leaf to prepare cross-sections. The leaf sections used were from the middle part of mature leaves (if there are multiple leaves, sections from the 3rd or 4th leaf from the tip were taken). Anatomical characteristics observed included paradermal sections to examine epidermal tissue and its derivatives, and transverse sections to examine the arrangement of mesophyll tissue and vascular bundles.

The method used to view paradermal leaf sections was peeling. The leaf was cut into squares, then boiled using 30% nitric acid (HNO3). After boiling, the leaf was soaked in 70% alcohol and then stained with 0.5% safranin [11]. The method for observing transverse sections followed the freehand section technique. The samples were then soaked in bleach, rinsed with 70% alcohol, stained with 0.1% safranin for 1 minute, and rinsed with distilled water [12]. The samples were placed on glass slides, a drop of water was added, and the slides were covered with cover slips. A sample observation was conducted using a binocular microscope (Yazumi XSZ-107 bn) enhanced with an optical lab to clearly view the leaf's anatomical characteristics [13].sure that work can be repeated according to the details provided. It contains technical information of the study presented clearly. Therefore, readers can conduct research based on the techniques presented. Materials and equipment specifications are necessary. Approaches or procedures of study together with data analysis methods must be presented [5-7].

III. RESULTS AND DISCUSSION

Results

1. Anatomical structure of Bulbophyllum angustifolium (Blume) Lindl.

The transverse section of the leaf through the midrib shows an epidermal tissue layer (Ep) on both the adaxial and abaxial sides of the leaf, each consisting of a single layer. The mesophyll tissue (M) does not differentiate into palisade or spongy tissue. There are vascular bundles (VB) consisting of supportive tissue, in the form of sclerenchyma cells, surrounding the vascular tissue (closed collateral type). The vascular tissue observed includes xylem and phloem, which are not separated by cambium tissue. The anatomical structure of B. angustifolium leaves can be seen in (Figure 1a-1d).

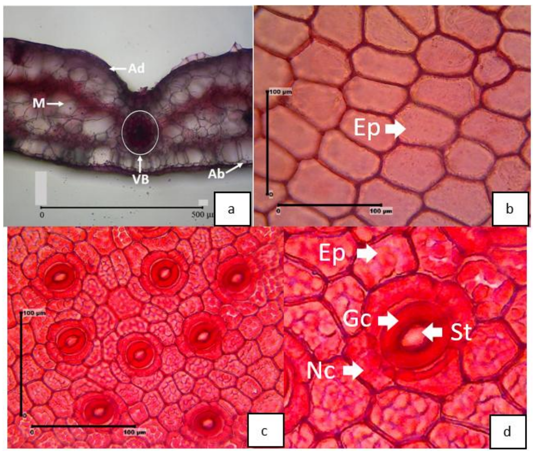

Figure 1. The anatomical structure of *Bulbophyllum angustifolium* (Blume) Lindl. (a) Transverse section of the

leaf through the midrib, 10x10 magnification, 500 µm scale bar (b) Upper epidermis, 40x10 magnification, 100 µm scale bar (c) Lower epidermis, 40x10 magnification, 100 µm scale bar (d) Lower epidermis, showing clearer view of the stomata (Ad: Adaxial Epidermis, Ab: Abaxial Epidermis, M: Mesophyll, VP: Vascular Bundle, Ep: Epidermal cell, Gc: Guard Cells, Nc: Neighboring Cells, St: Stoma).

In *B. angustifolium* (Figure 1), the epidermal cells on both the adaxial and abaxial surfaces are arranged in a non-uniform manner. The epidermal cells vary in shape, with some being elongated and others irregular. No intercellular spaces are observed in the epidermal layer. The only epidermal derivative found in this orchid is stomata, which are present solely on the abaxial surface (hypostomatic). The stomata in the leaves of *B. angustifolium* are of the tetracytic type, where four neighboring cells (Nc) are present. Two neighboring cells are aligned parallel to the guard cells (Gc), while the other two are oriented perpendicular to them.

2. Anatomical structure of Bulbophyllum odoratum (Blume) Lindl.

The anatomical structure of *B. odoratum* leaves can be seen in Figure 2a-d. The anatomical structure of *B. odoratum* leaves through the midrib includes a single layer of epidermal tissue (Ep) on both the adaxial and abaxial sides of the leaf. The entire leaf is predominantly composed of homogeneous mesophyll tissue. The mesophyll tissue (M) throughout the leaf does not differentiate into palisade or spongy tissue. Within the mesophyll, vascular bundles (VB) consist of supportive sclerenchyma cells surrounding the vascular tissue (closed-collateral type). The vascular tissue observed includes xylem and phloem, which are not separated by cambium tissue.

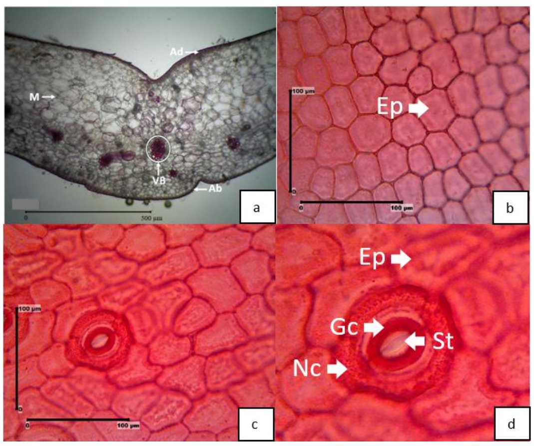

Figure 2. The anatomical structure of *Bulbophyllum odoratum* (Blume) Lindl. (a) Transverse section of the leaf through the midrib, 10x10 magnification, 500 µm scale bar (b) Upper epidermis, 40x10 magnification, 100 µm scale bar (c) Lower epidermis, 40x10 magnification, 100 µm scale bar (d) Lower epidermis, showing clearer view of the stomata (Ad: Adaxial Epidermis, Ab: Abaxial Epidermis, M: Mesophyll, VP: Vascular Bundle, Ep: Epidermal cell, Gc: Guard Cells, Nc: Neighboring Cells, St: Stoma).

In *B. odoratum*, the epidermal cells on both the adaxial and abaxial side surfaces are arranged in a non-uniform structure. The epidermal cells vary in shape, with some elongated, hexagonal, and others irregular. No intercellular spaces were observed in the epidermal layer. Epidermal derivatives include stomata, which are only found on the abaxial surface or hypostomatic. The stomata of *B. odoratum* leaves are of the tetracytic type, where four neighboring cells (Nc) are present (Figure 2a-d).

3. Anatomical structure of Bryobium hyacinthoides (Blume) Y.P.Ng & P.J.Cribb

The transverse section of *B. hyacinthoides* leaves through the midrib includes a single layer of epidermal tissue (Ep) on both the adaxial and abaxial sides of the leaf. The central part of the leaf is composed of mesophyll tissue (M), which does not differentiate. Within the mesophyll, vascular bundles (VB) consist of supportive sclerenchyma cells surrounding the vascular tissue (closed-collateral type). The vascular tissue includes xylem and phloem, which are not separated by cambium tissue.

The paradermal section of *B. hyacinthoides* leaves shows cells that are arranged neatly and uniformly on both the adaxial and abaxial surfaces. Most epidermal cells are uniform in shape, being hexagonal, with few irregularly shaped cells. No intercellular spaces are observed in the epidermal layer. The stomata in this species are considered hypostomatic, as they are found only on the abaxial surface of the leaf. The stomata of *B. hyacinthoides* leaves are of the tetracytic type, where four neighboring cells (Nc) surround the guard cells (Gc). Another epidermal derivative found in this species is non-glandular trichomes. The anatomical structure of *B. hyacinthoides* leaves can be seen in (Figure 3a-3d).

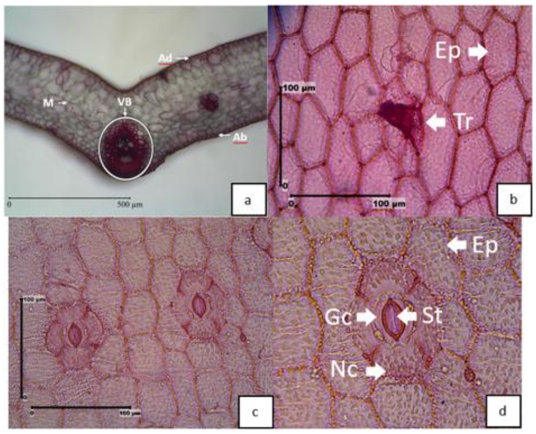

Figure 3. The anatomical structure of *Bryobium hyacinthoides* (Blume) Y.P.Ng & P.J.Cribb (a) Transverse

section of the leaf through the midrib, 10x10 magnification, 500 µm scale bar (b) Upper epidermis, 40x10 magnification, 100 µm scale bar (c) Lower epidermis, 40x10 magnification, 100 µm scale bar (d) Lower epidermis, showing clearer view of the stomata (Ad: Adaxial Epidermis, Ab: Abaxial Epidermis, M: Mesophyll, VP: Vascular Bundle, Ep: Epidermal cell, Gc: Guard Cells, Nc: Neighboring Cells, St: Stoma).

4. Anatomical structure of Mycaranthes oblitterata Blume

The anatomical structure of *M. oblitterata* leaves through the midrib includes a single layer of epidermal tissue (Ep) on both the adaxial and abaxial sides of the leaf. The central part of the leaf is predominantly composed of mesophyll tissue (M), which does not differentiate into palisade or spongy tissue. Within the mesophyll, vascular bundles are present, consisting of xylem and phloem that are not separated by cambium tissue. There are supportive sclerenchyma cells surrounding the vascular tissue, forming closed, collateral-type vascular bundles. The anatomical structure of *M. oblitterata* leaves can be seen in (Figure 4a-4d).

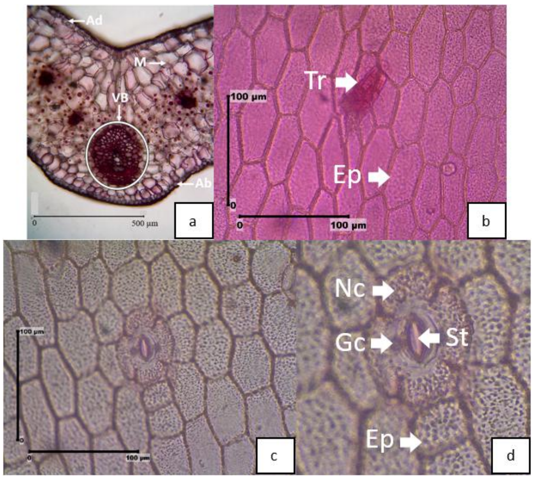

Figure 4. The anatomical structure of Mycaranthes oblitterata Blume (a) Transverse section of the leaf through the midrib, 10x10 magnification, 500 µm scale bar (b) Upper epidermis, 40x10 magnification, 100 µm scale bar (c) Lower epidermis, 40x10 magnification, 100 µm scale bar (d) Lower epidermis, showing clearer view of the stomata (Ad: Adaxial Epidermis, Ab: Abaxial Epidermis, M: Mesophyll, VP: Vascular Bundle, Ep: Epidermal cell, Gc: Guard Cells, Nc: Neighboring Cells, St: Stoma).

The paradermal section of *M. oblitterata* shows epidermal cells on both the adaxial and abaxial surfaces arranged neatly and uniformly, with the stomata aligned in position. The epidermal cells are mostly homogeneous in shape, being either hexagonal or elongated. No intercellular spaces are visible in the epidermal layer. The epidermal derivative found is stomata, which are only present on the abaxial surface and are considered hypostomatic. The stomata of *M. oblitterata* leaves are of the tetracytic type, with four neighboring cells (Nc) surrounding the guard cells. Another epidermal derivative found in this species is non-glandular trichomes.

5. Anatomical structure of Coelogyne carnea (Blume) Rchb.f.

The anatomical structure of this species shows a single layer of epidermal tissue (Ep) on both the adaxial and abaxial sides of the leaf. The central part of the leaf, in addition to the vascular bundles, is predominantly composed of mesophyll tissue (M). In the entire leaf of the epiphytic orchid sample, the mesophyll tissue does not differentiate into palisade or spongy tissue. In the central part of the transverse section, the vascular bundles (VB) consist of several layers of sclerenchyma cells surrounding the vascular tissue (closed collateral type). The vascular tissue observed includes xylem and phloem, which are not separated by cambium tissue.

In the paradermal section of *C. carnea*, the epidermal cells on both the adaxial and abaxial surfaces are arranged neatly and uniformly. The epidermal cells of this species exhibit the greatest variety in shape, including pentagonal, hexagonal, elongated, and irregular forms. No intercellular spaces are visible in the epidermal layer. The epidermal derivative found in the orchid is the stomata, which are only present on the abaxial surface and are classified as hypostomatic. The stomata of *C. carnea* leaves are of the paracytic type, where two neighboring cells (Nc) are aligned parallel to the guard cells (Gc). The overall anatomical structure of *Coelogyne carnea* (Blume) Rchb.f. leaves are shown in Figure 5.

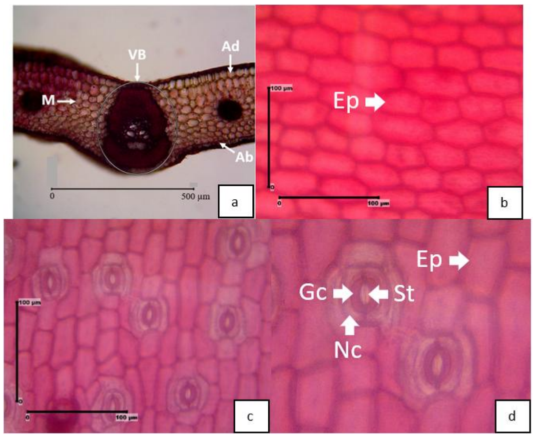

Figure 5. The anatomical structure of *Coelogyne carnea* (Blume) Rchb.f. (a) Transverse section of the leaf through the midrib, 10x10 magnification, 500 µm scale bar (b) Upper epidermis, 40x10 magnification, 100 µm scale bar (c) Lower epidermis, 40x10 magnification, 100 µm scale bar (d) Lower epidermis, showing clearer view of the stomata (Ad: Adaxial Epidermis, Ab: Abaxial Epidermis,

M: Mesophyll, VP: Vascular Bundle, Ep: Epidermal cell, Gc: Guard Cells, Nc: Neighboring Cells, St: Stoma).

Discussion

Based on the observations of transverse sections through the midrib, the anatomical structure of the leaves of the five orchid species examined shows similarities. The fundamental tissue, the mesophyll, consists of parenchyma cells with polygonal shapes that contain chlorophyll. The mesophyll tissue does not differentiate into palisade and spongy tissue, indicating that, in orchids, it does not [14]. The epidermis primarily protects the underlying tissues from radiation, extreme temperatures, and the heat of sunlight. A thicker epidermal layer in a plant can reflect more heat and can also contribute to water storage [9,15].

Anatomical differences among orchid species begin to emerge in the paradermal sections, showing the epidermal tissue. Two species, *B. angustifolium* and *B. odoratum*, exhibit epidermal cells arranged in a non-uniform or irregular manner, whereas the other three species, *B. hyacinthoides*, *M. oblitterata*, and *C. carnea*, have neatly and uniformly arranged epidermal cells. The shapes of epidermal cells in the five species vary widely, including pentagonal, hexagonal, elongated, and irregular forms. Two species, *B. hyacinthoides* and *M. oblitterata*, have relatively homogeneous epidermal cell shapes, mostly hexagonal with a few irregular forms. The clear component of epidermal cells is the cell walls, with other cellular components not visible. Unlike in another research, where the paradermal sections of *Dendrobium*, *Phalaenopsis*, and *Vanilla* orchids showed visible cell nuclei due to the living nature of the epidermal cells [8].

Two types of stomata were observed in the samples: tetracytic and paracytic. The tetracytic type was found in four species: *B. angustifolium*, *B. odoratum*, *B. hyacinthoides*, and *M. oblitterata*. The paracytic type was found only in *C. carnea*. The paracytic type of stomata is commonly found in orchids, including many *Dendrobium* species [13, 16]. Another research report states that tetracytic, cyclosytic, and anomalous stomata types are also found in some orchid species. The tetracytic type is observed in *the Cymbidium and Eulophia genera, while anomalous stomata are found in several Dendrobium species* [17]. There is no specific research on stomata in the species found at the location. However, the paradermal section shows the stomata types: the tetracytic type, characterized by four neighboring cells surrounding the guard cells, and the paracytic type, characterized by two neighboring cells parallel to the guard cells [18, 19]. All orchid species found have hypostomatic stomata, with stomata present only on the abaxial surface of the leaf. This indicates that all orchid species found at the location have hypostomatic stomata [13]. This proves that most stomata in plants are on the underside of leaves [20]. This stomatal placement is an adaptation to minimize water loss, which would occur more rapidly if stomata were on the upper surface exposed to sunlight.

Another structure found in orchid epidermis is trichomes. Trichomes were observed in two species: *B. hyacinthoides* and *M. oblitterata*. The trichomes are present on both the adaxial and abaxial surfaces of the leaves and are of the non-glandular type. This is different from some research, which found trichomes mainly on the abaxial side of leaves [21]. However, non-glandular trichomes found on both leaf surfaces may indicate a role in preventing excessive transpiration more efficiently rather than in secreting secondary metabolites [22,23].

IV. CONCLUSION

Based on the overall results of the study, variations in leaf anatomical structure among the five orchid species found in Pengelengan hills were observed in paradermal sections, which showed the arrangement of epidermal tissue. These variations include differences in epidermal cell arrangement, cell shapes, stomatal types, and epidermal derivatives.

The arrangement of epidermal cells was categorized into two types: uniform and non-uniform. The shapes of epidermal cells varied, including pentagonal, hexagonal, elongated, and irregular forms. The types of stomata observed include tetracytic and paracytic types. Additionally, epidermal derivatives such as non-glandular trichomes were found in *B. hyacinthoides* and *M. oblitterata*.

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