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Leaf Anatomy Profile of *Begonia* spp. Section *Platycentrum-Sphenanthera* Group From Java and Sumatera, Indonesia

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Abstract. *Anatomical studies have been used in the classification and identification of Begonia species. However, the research on Indonesian native Begonia is still limited. This study focused to evaluate the importance of leaves' anatomy character for the identification and classification purpose of the Sphenanthera group in Java and Sumatra. A total of six individuals of four Begonia species used in this study, namely Begonia multangula, B. longifolia, B. pseudoscottii, and B. robusta. This study aimed to describe the anatomical characters of the leaves that are useful in the identification and grouping of Begonia section Platycentrum–Sphenanthera. The anatomical preparations used the paraffin method for the cross-section of leaves and the semi-permanent method for paradermal observations. In general, the epidermal cells of paradermal Begonia leaves are polygonal in shape. Consistently, Begonias have single-type stomata with an anisocytic type which are only present on the abaxial leaves. Begonia Sphenanthera group has a thin cuticle (less than 2 µm), both in the abaxial and adaxial surfaces. The lower epidermis cells are smaller than the upper epidermis cells. The mesophyll is differentiated into palisade and spongy tissue. The number and form of mesophyll tissue are varied, therefore useful for diagnosing an important character in the grouping of Begonias from this section.*

Keywords: *Begonia, leaf anatomy, paraffin method, Platycentrum, Sphenanthera.*

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INTRODUCTION

Genus *Begonia* is the sixth-largest of Angiospermae, comprising approximately 2.119 species in the world (Hughes & Girmansyah, 2011; Hughes et al., 2015). *Begonia*, with attractive leaves, were used for ornamental plants and as a great horticultural interest in the world. More than 10.000 hybrids of *Begonia* have been produced known worldwide (Kiew et al., 2015). *Begonia* is used for medicines due to its bioactive compound, as reported by Hartutiningsih et al. (2018) on *B. baliensis*. Locally, *B. lombokensis* and *B. longifolia* are also eaten and/or used medicine and food (Girmansyah, 2009).

Taxonomically, *Begonia* dividing into 70 sections (Moonlight et al., 2018), of which six sections can be found in Java and Sumatra Islands (Indonesia), namely sect. P*arvibegonia*, sect. *Reichenheimia*, sect. *Jackia*, sect. *Platycentrum*, sect. *Petermannia*, and sect. *Bracteibegonia* (Hughes et al., 2015). A new sectional classification recognized the *Bego-*

nia sect. *Sphenanthera* is synonymised with *Begonia* sect. Platycentrum, and with an informal name "*Sphenanthera group*" of sect. *Platycentrum*, based on their molecular data (Moonlight et al., 2018). *Sphenanthera* group differs with sect. *Platycentrum* by fleshy fruits type, 3-8 locule number, and short-winged, (not capsule type, 2-locular splash-cup, with one enlarged wing) (Doorenbos et al., 1998). Five species of the *Sphenanthera* group were recorded from Java and Sumatra Islands, namely *B. longifolia*, *B. robusta*, *B. multangula*, *B. scottii* dan *B. pseudoscottii* (Hughes & Girmansyah, 2011; Hughes et al., 2015). The taxonomical studies of *Sphenantera* group in Java and Sumatra have been reported by several researchers. Hughes & Girmansyah (2011) reported that *B. sarcocarpa* Ridl. and *B. turbinata* Ridl. as synonyms of *B. longifolia* Blume, as well as *B. trigonocarpa* Ridl. as a synonym of *B. multangula* Blume. A new species of fleshy fruit *Begonia* has been added from northern Sumatra by Hughes et al. (2015), namely *B. pseudoscottii*.

Anatomical studies have been used in the classification and identification of various plant families (Rudall, 2007), such as genera in *Pandanaceae* (Rahayu et al., 2012; Santika et al., 2014), *Euphorbia* (Bercu & Popoviciu, 2015), and genus *Festuca* (Martínez Sagarra et al., 2017). Anatomical studies of *Begonia* have been carried out on *B. fischeri* sect. *Ephemera* (Rodica & Răzvan, 2017) and *Begonia dipetala* sect. *Haagea* (Indrakumar et al., 2013). Previously, Efendi (2019) described the stomata-type of 32 *Begonias* to support taxonomy data on *Begonia* classification. However, there is no comprehensive research on *Begonia* anatomy, especially *Begonia* leaf anatomy of *Spenanthera* group from Java and Sumatra. Java and Sumatra islands have six species from eight species of *Begonia* sect. *Sphenanthera* in Indonesia, and samples have been collected in Cibodas Botanic Gardens for the last ten years. Thus, this study focused to evaluate the importance of leaves' anatomy character for the identification and classification purpose of the *Sphenanthera* group in Java and Sumatra.

MATERIALS AND METHODS

Preparation of *Begonia* Leaf Samples

Leave samples were collected from six individuals of four *Begonia* species planted in Cibodas Botanic Gardens (Table 1). Mature and healthy *Begonia* leaves were selected to be used as samples, both by paradermal observation and cross-sectional preparation.

Table 1. Samples for anatomical study of *Begonia* sect. *Platycentrum* – *Sphenanthera* group

Scientific Names	Origin
Begonia multangula Blume	Remnant forest, Cibodas Botanic Gardens, Java
Begonia multangula Blume	Remnant forest, Cibodas Botanic Gardens, Java
Begonia longifolia Blume	Cibogo waterfall, Cibodas Botanic Gardens, Java
Begonia longifolia Blume	Kerinci Seblat National Park, Jambi, Sumatra
Begonia robusta Blume	Remnant forest, Cibodas Botanic Gardens, Java
Begonia scottii Tebbitt	Mt. Singgalang, West Sumatra, Sumatra

Paradermal Section of *Begonia* Leaves by Replica Method

Paradermal preparations used the replica method using nail polish. The leaves were cleaned with a paper tissue, then clear nail polish was smeared on the lower leave surface and the leaves were left to dry. Next, the layer of nail polish was pulled slowly to produce a stomata impression. The result was placed on glass objects. The slides were observed using a light microscope merk Nikon AFX-IIA and photographs were taken using a light microscope merk Nikon Eclipse 80i.

Cross-section of *Begonia* Leaves by Paraffin method

Cross-section preparation used the paraffin method according to Sass (1951). The leaves were cut ± 1cm and fixed with a fixative solution (FAA) for 24 hours in a vacuum. Rinsing and dehydration of the sample were carried out by removing the FAA and replacing them with graded concentrations of alcohol (70% to 100%), each for 3 hours. Dealcoholization was carried out by removing the absolute alcohol solution and replacing it with a mixture of alcohol:xylol in a ratio of 3:1, 1:1, 1:3, and then pure xylol solution, twice for 3 hours each. Infiltration of powdered paraffin was carried out by giving powdered paraffin slowly until saturated and then put into an incubator at 58^0 C for 24 hours. Next, infiltration of liquid paraffin by removing the xylol:paraffin mixture gradually every 3 hours. The tissues were embedded into liquid paraffin. The paraffin-embedded tissues were sectioned at the thickness of 17 μm using a rotary microtome. The section tapes were placed on the objective glass smeared with haupt adhesive and a little aquades followed by placing the objective glass on a low-temperature hot plate (40˚C) for approximately 2 days until the slices dry and stuck to the object-glass. The tissues were then stained by inserting a glass objective into a solution of 2% safranin in 70% alcohol and 1% fastgreen in absolute alcohol. The anatomical preparations were observed using a light microscope merk Nikon AFX-IIA and photographs were taken using a light microscope merk Nikon Eclipse 80i.

Data Analysis

The qualitative data of leaf anatomy were analyzed descriptively. Paradermal observation included epidermis cell – form and stomata type. A slide was observed with 10 to 15 repetitions. While, cross-section observation included cuticles size, leaf thickness, epidermis thickness, palisade thickness, the number of palisades, spongy thickness, and Ca oxalate type. A slide was observed with 10 to 15 repetitions.

RESULTS AND DISCUSSION

Paradermal Surfaces of *Begonia* sect. *Platycentrum – Sphenanthera* Group

The paradermal section of the leaf structure on all species of *Begonia* is very uniform. The epidermal cell of *Sphenanthera* group has a polygonal shape. The anticlinal wall of the epidermal cell is straight, as in other *Begonia* species (Indrakumar et al., 2013).

Adaxial and/or abaxial surfaces of *Begonia* sect. *Sphenanthera* were covered by sparse to dense trichomes, such as *B. scottii*, *B. multangula* and *B. robusta*. The glabrous surface of leaves is only found on *B. longifolia*. Although the trichomes' density depends on its environmental condition, such as shade (Herawati et al., 2020), this case can be used for species delimitation in this section. The presence of trichomes in *Begonia* is an interesting trait for breeding, due to their plant resistance function (Herawati et al., 2020) and attractive color, such as in *B. robusta*.

The trichome type of sect. Sphenanthera is a non-glandular trichomes and is found on *B. multangula*, *B. multangula*, *B. longifolia* Sumatera, and *B. robusta*. The non-glandular trichomes were also documented in some *Be-*

gonia, such as *Begonia dipetala* sect. *Haagea* (Indrakumar et al., 2013), *B. delicata* sect. *Ephemera* (Gregório et al., 2015). Compared to other *Begonia* of sect. *Platycentrum*, namely *B. tamdaoensis* dan *B. sphenantheroides* (Peng et al., 2015) and *B. masoniana* sect. *Coeloncentrum* (Suffan et al., 2021) showed a different type, i.e. glandular trichomes. Therefore, trichomes trait is an important diagnostic character in *Begonia*.

Begonia of *Sphenanthera* group showed a similar stomata type, i.e. anisocytic (Figure1). It is synonymous with helicocytic-type by Prabhakar (2004). Stomata complex is single-type with three-guard cells and has different sizes. The size and density of stomata in the *Sphenanthera* section showed high variation and it was difficult to distinguish between species (Efendi, 2019). Stomata are only found on the adaxial leaves (hypostomatic) with a deeper position (cryptophores). The consequence is inefficiency in water use compared to clustering-type stomata by a modulating stomatal movement and leaf structure (Papanatsiou et al., 2017), for example, *Begonia* sect. *Diploclinium* (Hughes et al., 2011).

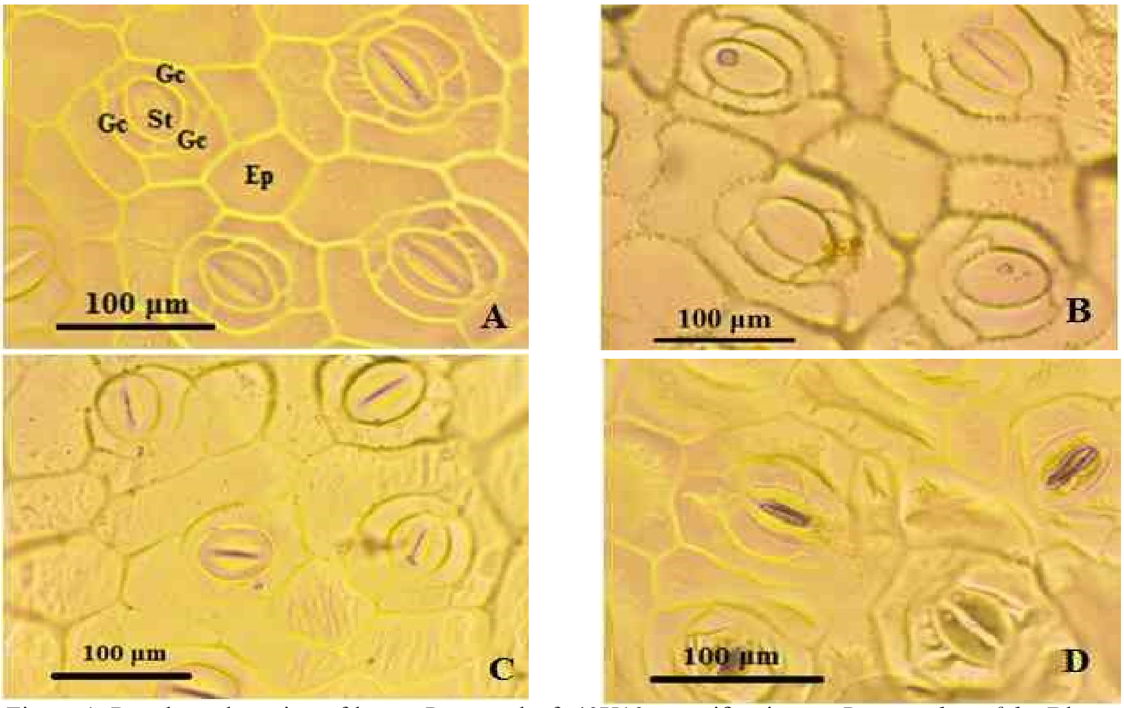


Figure 1. Paradermal-section of lower *Begonia* leaf: 40X10 magnification, a. *Begonia longifolia* Blume, b. *Begonia multangula* Blume., c. *Begonia scottii* Tebbit, d. *Begonia robusta* Blume. Notes: Ep: epidermis, st: stomata, Gc: Guard Cell

Cross-section of *Begonia* Leaves

Leaf shape of all samples of *Begonia* sect. *Sphenanthera* observed was dorsiventral. In general, the *Begonia* leaf cross-section consists of a cuticle layer, upper epidermis, palisade tissue, spongy tissue, vascular bundle, and lower epidermis (Figure 2). Ca-oxalate was found in several types of *Begonia* leaves.

A cross-section of the *Begonia* leaf showed a single-layered epidermis covered by thin cuticles, both on the upper and lower surfaces. The upper epidermis (ca. 10.68–31.32µm) is larger than the lower epidermis (ca. 4.18-30.80 µm). *Begonia robusta* has the highest epidermal thickness, while *B. longi-*

folia Sumatra has the lowest epidermal thickness. Although it varies amongst species in this section, the epidermal thickness cannot be used to distinguish them since it is an adaptive response to environmental variables such as shade, temperature, and humidity (Suffan et al., 2021). Like as in the stomata's role, the epidermal thickness supports water loss regulation, particularly on the lower surface (Cuéllar Cruz et al., 2020; Suffan et al., 2021). In addition, the cuticle and epidermal tissue also play a role in increasing the strength of the cell surface and protecting internal tissues from ultraviolet radiation (Cuéllar Cruz et al., 2020).

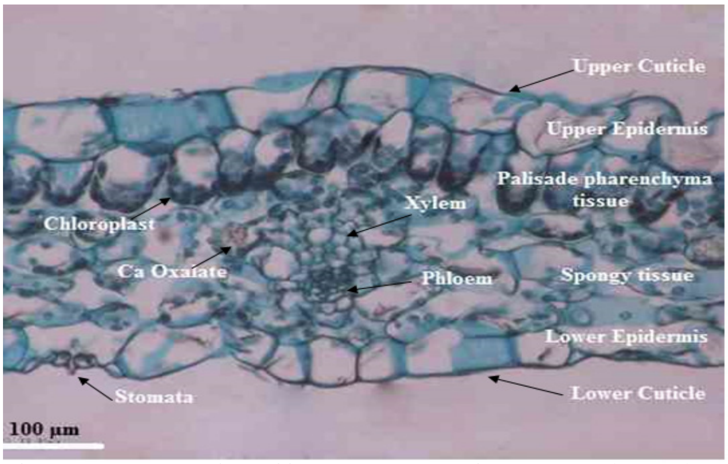


Figure 2. Cross-section of leaves anatomy of *Begonia Sphenanthera* group: 20X10 magnification (Photo: *Begonia longifolia* Blume)

The leaf thickness of *Begonias* observed were varied. *Begonia longifolia* (Cibodas, Java) has the thickest leaves, not different from *B. multangula* (Cibodas, Java). Meanwhile, the thinnest leaves were found in Sumatran *B. longifolia*. Leaf thickness depends on the arrangement of mesophyll tissue that is different in composition (Figure 3). The thickness of mesophyll tissue increases the rate of photosynthesis due to the high quantity of chlorophyll (Lailaty & Nugroho, 2021).

Mesophyll tissue in *Begonia* sect. *Sphenanthera* were differentiated into palisade and spongy tissue. It could be distinguished based on its shape and size. *Begonia multangula* Blume, *B. multangula* (remnant forrest), and *B. longifolia* Sumatra have one layer of palisade tissue. Meanwhile, the two layers of palisade are found in *B. longifolia* Cibogo, *B. scottii* West Sumatra, and *B. robusta* (Figure 4). *Begonia fischeri* sect. *Diploclinium* has 7-8 layers of palisade tissues (Rodica & Răzvan, 2017). It can be a useful diagnostic characteristic among sections in *Begonia*.

Calcium oxalate/raphides were found in mesophyll tissue, namely solitary druses type, as reported by Cuéllar Cruz et al. (2020) on *Begonias*. Calcium oxalate crystals occur in more than 215 higher plant families with different shapes, sizes, and numbers of crystals that show variations among taxa. Various biological, chemical, and physical parameters may affect the location, size, and other properties of crystals in plants, such as light, temperature, pH, ion concentration, and herbivory. Calcium oxalate crystal formation is under genetic control, meanwhile, its shape and location within a taxon are often very specific and may be represented as a taxonomic character. Many functions have been reported for calcium oxalate crystals in plants such as participating in calcium homeostasis, storage of calcium, removal of excess oxalate, metal detoxification, tissue support, light gathering and reflection, and protection against insects and foraging animals (Konyar et al., 2014). This mechanical effect of needle-like crystals is assumed as the plant defense, especially in *Begonia*. Chloroplasts are scattered in the mesophyll tissue, which is more abundant in palisade compared to sponge tissues. The vascular bundle is found in the lamina and the midrib. The type of vascular bundle is bicollateral or closed collateral.

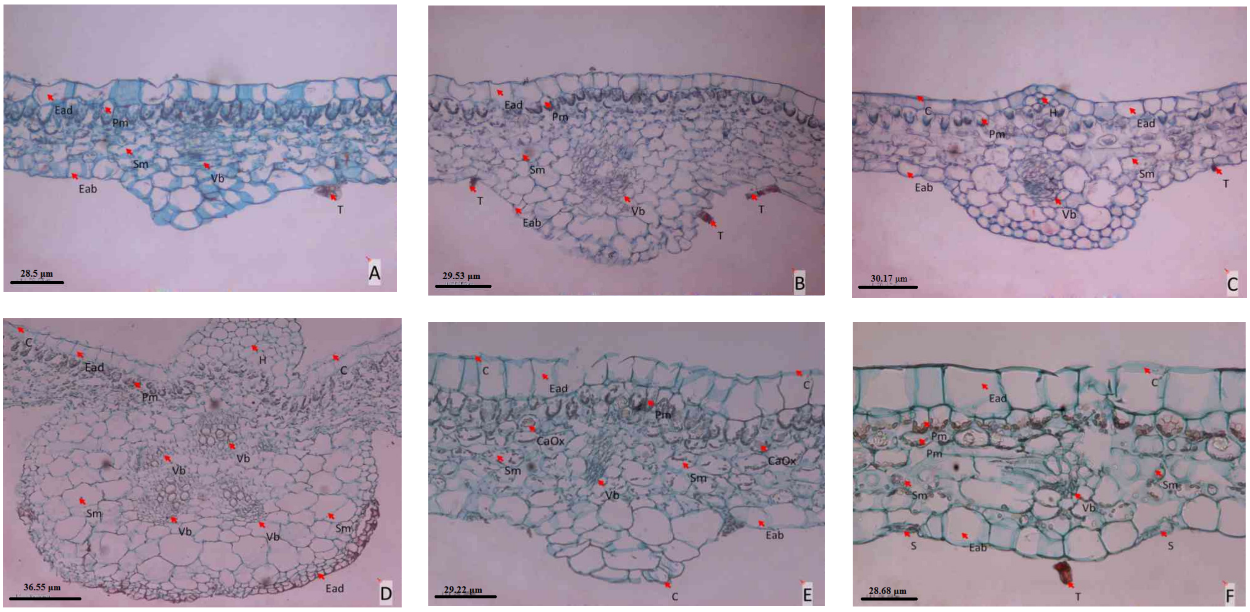
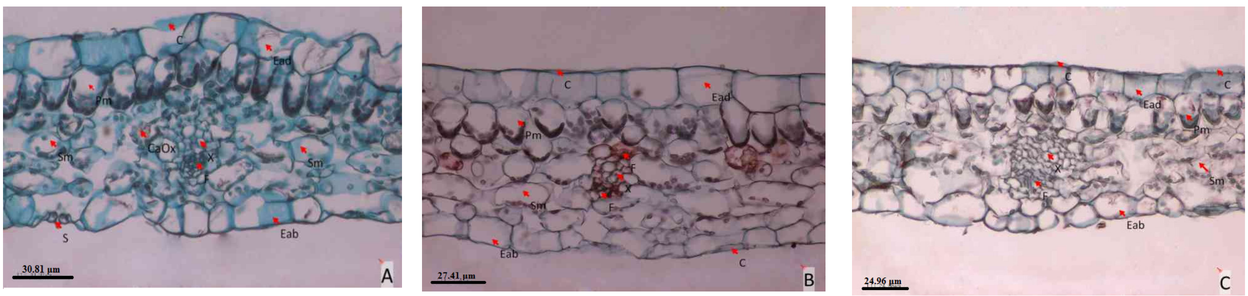


Figure 3. Cross-section of midrib *Begonia* leaves, A-D: 10x10 magnification, E-D: 20x10 magnification, (A). *B. multangula* Blume, (B). *B. multangula* hutan lumut, (C). *B. longifolia* sumatera, (D). *B. longifolia* cibogo, (E). *B. scotii* Sumatra Barat, (F). *B. robusta*.

Notes: Ead: adaxial epidermis, H: hypodermis, Pm: palisade mesophyll, Sm: Spongy mesophyll, Vb: vascular bundle, Eab: abaxial epidermis, C: cuticles, S: stomata, T: trichomes, CaOx: calcium oxalate, red dots in cells.



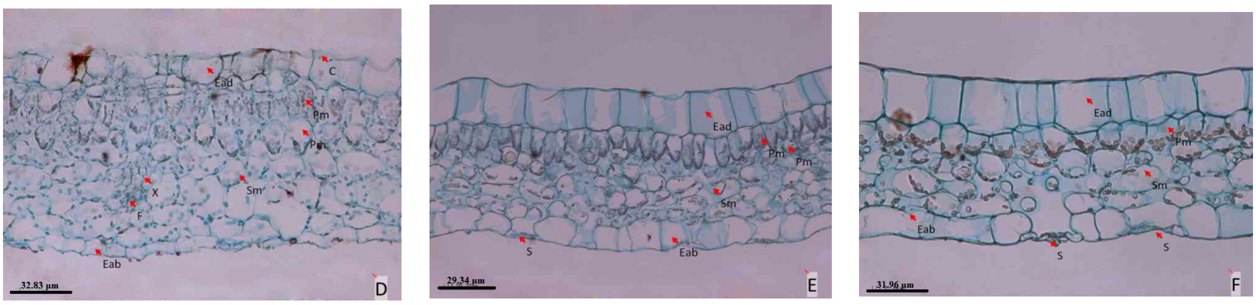


Figure 4. Cross-section of lamina *Begonia* leaves with 20x10 magnification, (A). *B. multangula* Blume, (B). *B. multangula* hutan lumut, (C). *B. longifolia* sumatera, (D). *B. longifolia* Cibogo, (E). *B. scotii* Sumatra Barat, (F). *B. robusta.*

Notes: Ead: adaxial epidermis, Pm: palisade mesophyll, Sm: Spongy mesophyll, X: xillem, F: floem, Eab: abaxial epidermis, C: cuticles, S: stomata, CaOx: calcium oxalate.

***Begonia multangula* Blume Originated from Cibodas Botanic Gardens, Java**

The adaxial surface covering with sparse to dense trichomes, and a polygonal epidermis. An anisocytic-stomata with three-guard cells, stomata complex single and cryptophore type were distributed in the abaxial surface. Cross-section leaf ca. 106.70±6.03 µm thick; thin cuticles (less than 2 µm), epidermis single-layered on both surfaces, adaxial surface ca. 19.71±3.24 µm thick, and abaxial surface 15.57±2.70 µm thick; palisade tissue 1-celled layered, funnel-shape, ca. 26.90±5.08 µm thick; spongy tissue ca. 53.10±6.82 µm long, 4–6 cell-layered. Abaxial surface with sparse, minute glandular hairs and multiseriate trichomes. Ca oxalate druse crystal type, solitary spread in mesophyll tissue.

***Begonia multangula* Blume Originated from Cibodas Botanic Gardens, Java**

The adaxial surface covering with glabrous to sparse trichome, polygonal epidermis. An anisocytic-stomata with three-guard cells, stomata complex single and cryptophore type were distributed in the abaxial surface. Cross-section leaf ca. 101.55±4.48 µm thick; thin cuticles (less than 2 µm), epidermis single-layered on both surfaces, adaxial surface ca. 20.35±2.26 µm thick, and abaxial surface 11.74±2.40 µm thick; palisade tissue 1-celled layered, funnel-shape, ca. 23.48±2.44 µm thick; spongy tissue ca. 42.73±3.71 µm long, 4–6 cell-layered. Abaxial surface with sparse, minute glandular hairs and multiseriate trichomes. Ca oxalate druse crystal type, solitary and distributed in mesophyll tissue. The vascular bundle is bicollateral type.

***Begonia longifolia* Blume Originated from Kerinci Seblat National Park, Sumatra**

Adaxial surface glabrous, epidermis polygonal. An anisocytic-stomata with three-guard cells, stomata complex single and cryptophore type were distributed in the abaxial surface. Cross-section leaf ca. 83.11±4.34 µm µm thick; thin cuticles (less than 2 µm), epidermis single-layered on both surfaces, adaxial surface ca. 16.46±2.60 µm thick, and abaxial surface 12.30±2.79 µm thick; palisade tissue 1-celled layered, funnel-shape, ca. 19.88±2.90 µm thick; spongy tissue ca. 36.22±5.82 µm long, 3–5 cell-layered. Abaxial surface with sparse, minute glandular hairs and multiseriate trichomes. Ca oxalate type is like crystal sand or prisms and is distributed in mesophyll tissue. The vascular bundle is collateral.

***Begonia longifolia* Blume Originated from Cibogo Waterfall, Cibodas Botanic Gardens, Java**

Adaxial surface glabrous, epidermis polygonal. An anisocytic-stomata with three-guard cells, stomata complex single and cryptophore type were distributed in the abaxial surface. Cross-section leaf ca. 107.61±3.9 µm thick; thin cuticles (less than 2 µm), epidermis single-layered on both surfaces, adaxial surface ca. 15.16±2.38 µm thick, and abaxial surface 8.00±3.60 µm thick; palisade tissue 2-celled layered, funnel-shape, ca. 32.44±5.10 µm thick; spongy tissue ca. 48.60±3.92 µm long, 3–6 cell-layered. Abaxial surface with sparse, minute glandular hairs and multiseriate trichomes. Ca oxalate druse crystal type, solitary and distributed in mesophyll tissue. In the midrib, several collateral-type vascular bundles were found.

Begonia scotii **Tebbitt Originated from Mt. Marapi, West Sumatra**

The adaxial surface covering with sparse to dense trichomes, epidermis polygonal. An anisocytic-stomata with three-guard cells, stomata complex single and cryptophore type were distributed in the abaxial surface. Cross-section leaf ca. 87.04±2.37 µm thick; thin cuticles (less than 2 µm), epidermis single-layered on both surfaces, adaxial surface ca. 22.41±2.78 µm thick, and abaxial surface 13.58±2.26 µm thick; palisade tissue 2-celled layered, funnel-shape, ca. 16.83±2.08 µm thick; Spongy tissue ca. 34.88±4.94µm long, 4–6 cell-layered. Abaxial surface with sparse, minute glandular hairs and multiseriate trichomes. Ca oxalate druse crystal type, solitary and distributed in mesophyll tissue.

Begonia robusta **Blume Originated from Cibodas Botanic Gardens, Java**

Adaxial surface glabrous, epidermis polygonal. An anisocytic-stomata type with three-guard cells, stomata complex single, and cryptophore type was distributed in the abaxial surface. Cross-section leaf ca. 103±7.45 µm thick; thin cuticles (less than 2 µm), epidermis single-layered on both surfaces, adaxial surface ca. 27.97±1.68 µm thick, and abaxial surface 23.89±4.94 µm thick; palisade tissue 2-celled layered, funnel-shape, ca. 12.82±2.23 µm thick; spongy tissue ca. 43.32±4.24 µm long, 4–6 cell-layered. Abaxial surface with sparse, minute glandular hairs and multiseriate trichomes. Ca oxalate druse crystal type, solitary and distributed in mesophyll tissue. The vascular bundle is collateral type.

CONCLUSION

Begonias have single-type stomata with an anisocytic type which are only present on the abaxial leaves. *Begonia* section of *Platycentrum-Sphenanthera* group has a thin cuticle (less than 2 µm), both in the abaxial and adaxial surfaces. The lower epidermis cells are smaller than the upper epidermis cells. The mesophyll is differentiated into palisade and spongy tissue. The number, size, and form of mesophyll tissue are varied, therefore useful for diagnosing an important character in the grouping of *Begonias* from this section. The vascular bundles are scattered in the midrib and lamina. At least, there are two types of calcium oxalate found in the *Begonia* section of *Sphenanthera*, namely the druse type and crystal sand or prisms.

AUTHOR CONTRIBUTION

M.E. and I.Q.L. have similar contribution as main contributor in designed the research, collected and analized data, wrote and confirmed the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest in this research and manuscript preparation.

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