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THE APPLICATION OF LEAF ANATOMY IDENTIFYING SPECIES OF ORCHIDANTHA N.E.BR. (LOWIACEAE RIDL.) IN MALAYSIA

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ABSTRACT
YUSOFF, N. S. S. M., KALU, M., & SIAM, N. A. 2025. The application of leaf anatomy identifying species of *Orchidantha* N.E.Br. (Lowiaceae Ridl.) in Malaysia. *Reinwardtia* 24(1): 17–25. — Lowiaceae only consists of one genus, *Orchidantha*, which with thirty-four species has the lowest number of species among the eight families within order Zingiberales. Recently, *Orchidantha* has garnered attention for its potential medicinal value in treating fever, cough, chest, and back pain. The species are often only found in a small area. The morphology of the flower of *Orchidantha* is essential to identification but often the flowers are rarely found. As a result, this study aims to investigate vegetative anatomical characteristics of the petiole, midrib, and lamina, which could be used for species identification independent of availability of flowers; by identifying shapes and special characteristic of labellum, stigma, and viscidium. The results of this study shown a marked difference in shape and depth of the petiole grooves, petiole margin, and the number of vascular systems. In conclusion, the study demonstrates that the anatomical–petiole grooves, petiole margin and number of vascular systems, are useful for identifying species.

Key words: Anatomy, diagnostic characters, Lowiaceae, petiole grooves, vascular systems.

ABSTRAK
YUSOFF, N. S. S. M., KALU, M., & SIAM, N. A. 2025. Penggunaan anatomi daun untuk identifikasi jenis *Orchidantha* N.E.Br. (Lowiaceae Ridl.) di Malaysia. *Reinwardtia* 24(1): 17–25. — Lowiaceae hanya terdiri atas satu marga, *Orchidantha yang* beranggotakan 34 jenis dan merupakan suku dengan jumlah jenis paling sedikit diantara suku lain dalam Zingiberales. Saat ini, *Orchidantha* telah mendapat perhatian karena potensinya sebagai tanaman obat untuk demam, batuk, sakit dada, dan sakit punggung. Jenis ini sering ditemui di kawasan kecil. Morfologi bunga *Orchidantha* merupakan karakter penting untuk identifikasi, tetapi bunga ini jarang ditemui. Oleh karena itu, kajian ini bertujuan untuk mengetahui ciri-ciri anatomi vegetatif pada tangkai daun, tulang tengah daun (*midrib*), dan helaian daun, yang dapat digunakan untuk identifikasi jenis tanpa bergantung pada ketersediaan bunga; dengan mempertelakan bentuk dan ciri khas bibir bunga, kepala putik, dan perlengketan. Hasil penelitian ini menunjukkan perbedaan yang jelas pada bentuk dan kedalaman alur pada tangkai daun, tepi tangkai daun, serta jumlah berkas pengangkut. Kesimpulan kajian ini membuktikan bahwa ciri-ciri anatomi–alur tangkai daun, tepi tangkai daun, dan jumlah berkas pengangkut, berguna untuk identifikasi jenis.

Kata kunci: Alur tangkai daun, anatomi, ciri diagnostik, Lowiaceae, sistem berkas pengangkut.

INTRODUCTION

Orchidantha, commonly known as "Lobak Hitam" by locals in Malaysia, is the only genus in the Lowiaceae family and currently consists of thirty-four species (Leong-Škorničková *et al.*, 2021). However, the actual number of species is likely higher due to continuous discoveries. The genus is found abundantly in Malaysia compared to other countries within Southeast Asia (Syauqina *et al.*, 2016) but the species are often narrowly endemic.

Without flower, *Orchidantha* species are hard to identify because their habit would be easily mistaken as any other monocots species, especially *Hanguana* Blume, *Tupistra* Ker Gawl, and *Apidistra* Ker Gawl (Leong-Škorničková, Tillich & Nguyen, 2014). *Orchidantha borneensis* N.E.Br. was the first *Orchidantha* species collected and described by Nicholas Edward Brown in Borneo in 1886 (Holttum, 1970). Brown named the plant *Orchidantha* due to its flower's striking resemblance with an orchid, particularly its large

labellum. Brown temporarily classified *Orchidantha borneensis* in the tribe Museae J.G.Baker, as its flowers and overall characteristics shared similarities with those of the Musaceae Juss. family.

Within the order Zingiberales the Zingiberaceae Martinov is widely known to have medicinal values (Sunandar & Kahar, 2017). The useful aspects of *Orchidantha* are not an exception. Indigenous people in Perak use shoots of *O. longiflora* (Scort.) Ridl. for medicinal purposes and their healing properties (Ong *et al.*, 2012), urinary incontinence in children, and rituals (Lau & Saw, 2015). In addition, Danh *et al.* (2022) stated that in ancient traditional Chinese treatment, the whole plant of *O. chinensis* T.L.Wu was used to treat fever and cough while the leaf of *O. fimbriata* Holttum was used to relieve chest and back pain. Recently, the fresh areal part of leaf *O. vietnamica* K.Larsen was found to have the presence of essential oil metabolites (Danh *et al.*, 2022). These uses could be followed up by providing more phytochemical evidence as the exact medicinal properties of this genus is poorly known.

The anatomy of the vegetative organs of *Orchidantha* have been poorly studied but may be useful for identification especially of species occurring in low abundance. Morphologically, Lowiaceae Ridl. is closely related to Musaceae Juss., Strelitziaceae Hutch., and Heliconiaceae Vines which is also supported by molecular evidence. Lowiaceae has, however, an anatomy distinct from the other three families (Tomlinson, 1961). The epidermal layer of the petiole of *Orchidantha* has one layer alternately with numerous fiber bundles encircling the petiole outline shape similar to all Zingiberales except for Strelitziaceae, which has no fiber bundles (Tomlinson, 1956). This paper describes the unique anatomical features of nine species of *Orchidantha*.

MATERIALS AND METHODS

Sample specimen

Nine *Orchidantha* species were freshly collected in Penang and Sarawak. Five of the species grow cultivated at Suriana Garden, Balik Pulau (*O. siamensis* K.Larsen, *O. maxillarioides* (Ridl.) K.Schum, *O. fimbriata* Holttum, *O. longiflora* (Scort.) Ridl., and *O. lengguani* Škorničk.), while the other four grow naturally in the area respectively (*O. inouei* Nagam. & S.Sakai, *O. ranchanensis* Syauqina & Meekiong, *O. sarawakensis* Syauqina & Meekiong and *O. micrantha* Škorničk. & A.D.Poulsen). Voucher specimens of *Orchidantha* species have been deposited in the Herbarium of Universiti Malaysia Sarawak. Herbarium samples were collected for species identification in the Herbarium of Universiti Malaysia Sarawak.

Petiole anatomy slide preparation

Five replications of five individual matured stems were collected from different clumps. The middle part of the petiole was cut into 1 cm each and sliced using a sliding microtome. The petiole cross-section is transferred into a petri dish and soaked with 50% of safranin for three minutes. The material was then dehydrated for three minutes in each three ethanol concentrations (70%, 80%, and 90%). The process was continued in two series of absolute ethanol for 10 minutes each. Steps were repeated for 50% toluidine blue. Petiole cross-sections were placed on a clean slide. A few drops of Canada Balsam (for gluing) were added to the slide and the slide was covered with a cover slip. After completely drying, the anatomical characters of petioles and midrib were examined under compound system 35 microscope model Olympus Microscope (BX51).

Epidermal cell slide preparation

The middle part of each leaf was cut into four sets; each of which had two slices measuring 5 cm × 5 cm. One for viewing adaxial and another for abaxial. Leaves were abraded using a dissecting blade until a layer of translucent tissue was left. The tissue was placed on a petri dish containing 5% acetic acid for 30 seconds. The tissues were washed with distilled water and placed in petri dishes containing Sodium hypochlorite (NaOCl) until decolorized or turned translucent white. The tissues were washed again with distilled water and placed in a petri dish containing 50% ethanol for 2 minutes. After that, the tissues were transferred to another petri dish containing 50% Safranin (staining) for about 3 minutes. Subsequently, the tissues were dehydrated in a series of alcohol concentrations (50%, 70%, 80%, 90%, and 100% of ethanol) for permanent slide preparation. Then, the tissues were placed on a clean slide. A few drops of Canada balsam (for gluing) were added to the slide and the sample was covered with a cover slip. All of the prepared slides were placed in an oven set at 60°C for three days or more until completely dry. Each slide was observed under the light microscope and the images of leaf structure were taken using the compound system microscope model Olympus Microscope (BX51).

Data analysis

The stomata index was calculated based on a formula by Paul *et al*. (2017).

$$\text{Stomata Index} = \frac{\text{No. of stomata}}{\text{No. of stomata + no. of epidermal cells}} \times 100$$

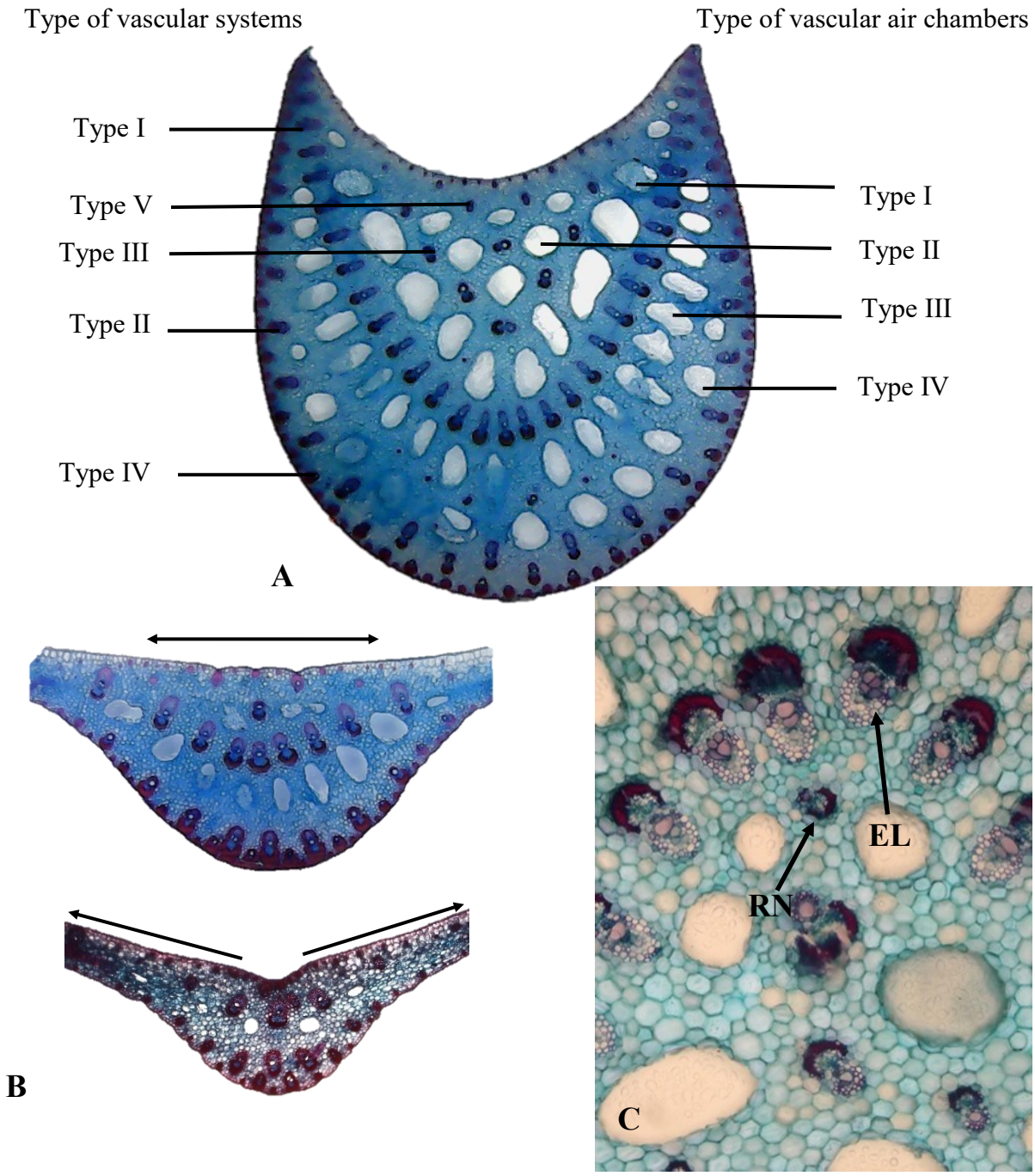

Fig. 1. Cross sections. A. Elliptic petiole cross-section with type of vascular systems and type of air chambers. B. Midrib crosssections, show flat induplicate and erect induplicate. C. Two shapes of vascular bundle: **RN**=rounded and **EL**=elongated.

RESULTS

Petiole. Size in an average of 0.16 × 0.65 mm to 8.93 × 6.22 mm. *Adaxial outline*. Grooved with various shallow and deep V and U shapes (Fig. 1). *Abaxial outline*. All species are ⅓ elongated in shape making the whole shape of the petiole elliptic with two kinds of margin which are entire and undulate. *Vascular tissue*. Two shapes of vascular bundle are found which are elongated and rounded (Fig. 1). The vascular bundle is arranged in a line called the vascular system (Table 1) and forms a shape like an arc. There are five types of vascular systems (Fig. 2). Type I: main vascular bundle (lining in the middle of petiole from edge to edge), Type II: second main vascular system (located near abaxial outline), Type III: lining above Type I, Type IV: lining above epidermal cell and Type

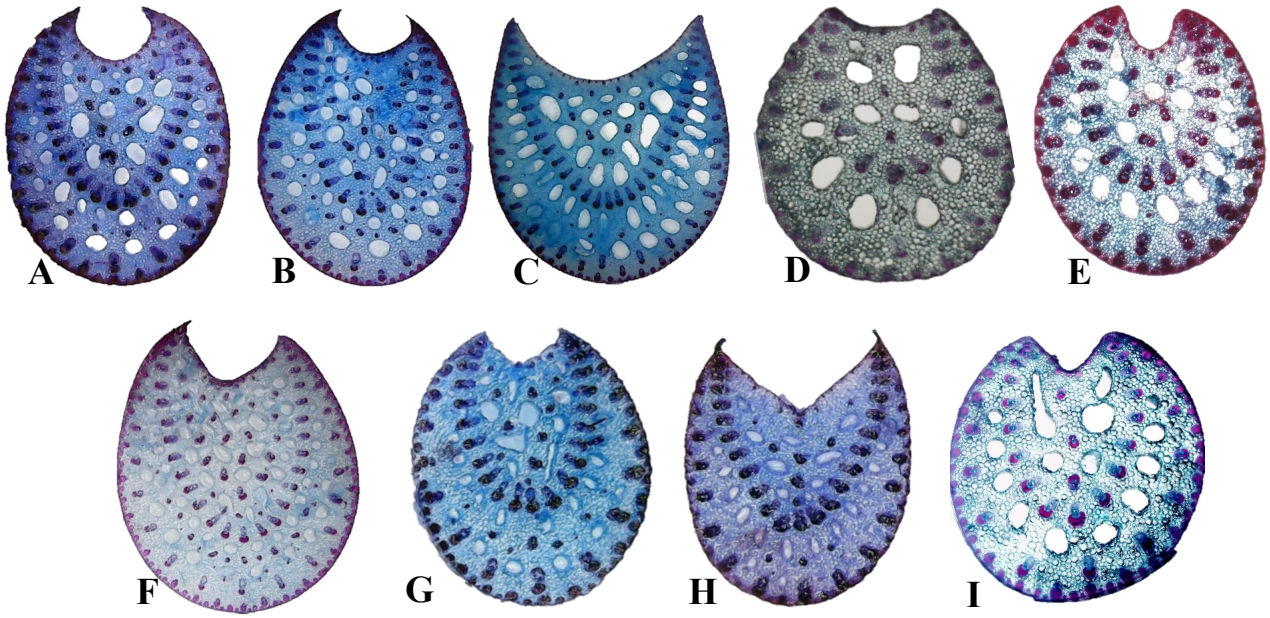

Fig. 2. Variations shapes of *Orchidantha* petiole groove. A. Absolute-U shape, *Orchidantha inouei*. B. Small widely-U shape, *O. fimbriata*. C. Widely-U shape, *O. sarawakensis*. D. Flat shape, *O. maxillarioides*. E. Narrowly-U shape, *O. lengguani*. F. Absolute-V shape, *O. longiflora*. G. Small, undulate absolute V shape, *O. micrantha*. H. Wide-V shape, *O. ranchanensis*. I. Narrowly-V shape, *O. siamensis*.

Table 1. *Orchidantha* petiole characteristic.

Species	Av. petiole size (mm)	Outline shape		Margin	No. of vascular bundle					Av. size (mm)	No. of the air chamber				Av. size (mm)
		Adaxial	Abaxial		I	II	III	IV	V		I	II	III	IV	
O. maxillarioides	0.16 × 0.65	Flat	Rounded	Slightly undulate	14	10	1	10	-	0.21 × 0.10	4	6	-	-	0.36 × 0.26
O. lengguani	0.53 × 0.43	Deep-U	Rounded	Slightly undulate	21	16	5	9	-	0.03 × 0.02	6	11	-	-	0.12 × 0.13
O. siamensis	1.00 × 0.88	Deep-V	Rounded	Entire	18	12	1	8	-	0.06 × 0.03	4	10	-	-	0.12 × 0.07
O. ranchanensis	1.63 × 1.33	Shallow-V	Narrow	Sparsely undulate	24	-	4	17	-	0.13 × 0.05	8	12	1	5	0.12 × 0.13
O. micrantha	2.56 × 3.36	Absolute V	Narrow	Deeply undulate	21	13	5	-	-	0.22 × 0.02	8	12	1	5	0.12 × 0.13
O. sarawakensis	6.73 × 5.68	Shallow-U	Broad	Entire	32	20	9	19	3	0.35 × 0.16	13	21	5	11	0.54 × 0.34
O. inouei	8.93 × 6.22	Absolute-U	Narrow	Entire	29	16	9	12	3	0.45 × 0.31	10	15	4	9	0.67 × 0.50
O. fimbriata	8.73 × 6.22	Shallow-U	Narrow	Entire	29	19	11	11	4	0.03 × 0.02	13	15	7	9	0.19 × 0.04
O. longiflora	8.93 × 6.22	Absolute-V	Narrow	Entire	30	18	12	16	6	0.34 × 0.16	17	19	10	11	0.45 × 0.43

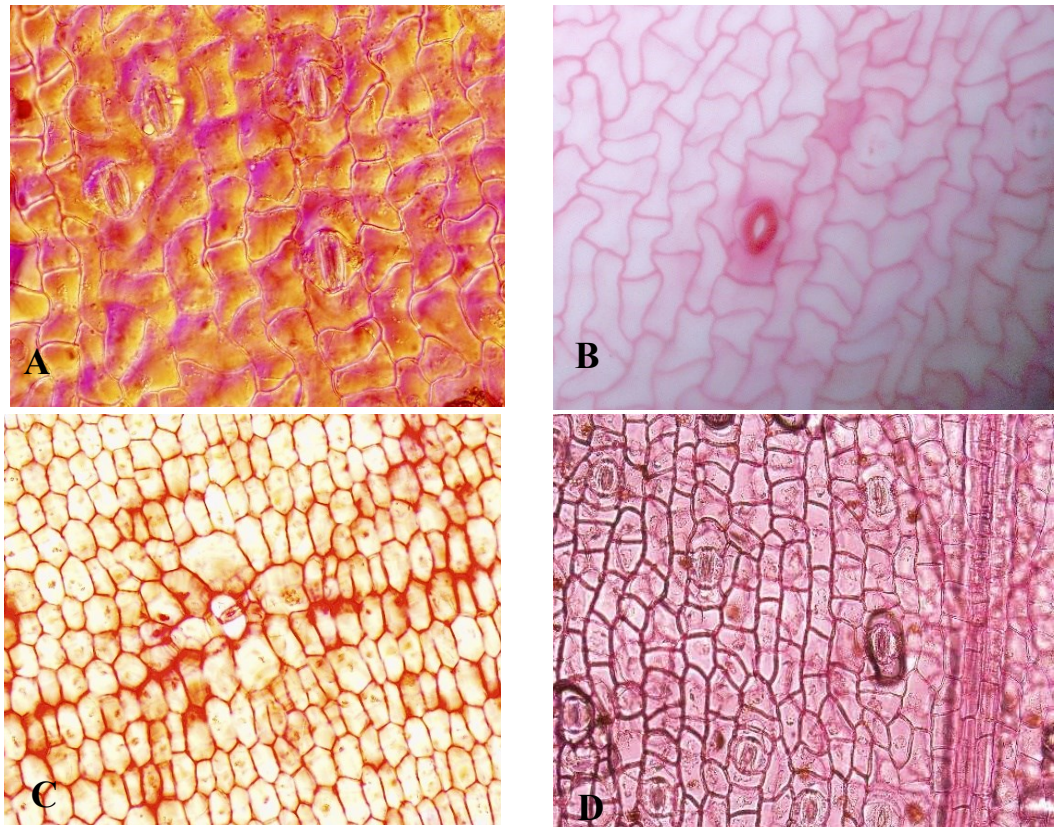

Fig. 3. Varieties of *Orchidantha* epidermis cell shape, and anticlinal wall. A. *Orchidantha lengguani* abaxial leaf with polygonal epidermis cell, curved anticlinal wall. B. *Orchidantha longiflora* abaxial leaf with elongated cell, straight to curved anticlinal wall. C. *Orchidantha maxillarioides* abaxial leaf with polygonal epidermis cell, straight anticlinal wall. D. *Orchidantha sarawakensis* abaxial leaf with elongated epidermis cell, straight anticlinal wall.

V: scattered vascular bundle near adaxial forming circle shape. *Epidermis cell*. One to two layers with compact round cells and protected with a thin layer of cuticle. *Ground cell*. Parenchyma cells are round and compact near the epidermis cells. It starts to get bigger towards the middle petiole and is modified into an aerenchyma cell (air chamber). Aerenchyma cell is arranged in a line (Table 1) forming a shape like an arc. There are four types of air chambers (Fig. 1). Type I: lining above Type I vascular system, Type II: lining above Type I air chamber, Type III: lining below Type I vascular system, and Type IV: lining below Type III air chamber.

Midrib. *Adaxial outline*. Leaf attachment is induplicate, either flat induplicate or erect induplicate (Fig. 1). *Abaxial outline*. ½ circle shape and always prominent. The thickness of the midrib depends on the leaf load. *Vascular tissue*. Arranged in a lining forming like an arc shape. Two types of vascular systems are found, in the middle and near the abaxial with additional vascular systems sometimes found near the adaxial outline (Fig. 1). *Epidermis cell*. A thin layer with compact small round cells. *Ground cell*. Parenchyma cells are round, small, and compact below the epidermis layer and the cell is developed into aerenchyma cells towards the middle. Aerenchyma cells are not as porous as in the petiole. They only forming one to two linings of aerenchyma cells (air chamber).

Leaf cuticular epidermal cell. *Epicuticular waxes*. Thick waxes at both adaxial and abaxial surfaces with glabrous surface. *Epidermal cell*. The average size among nine species is H (6.83–44.63 μm) × W (4.29–17.66 μm), elongated cell arranged in a vertical longitudinal line parallel to the veins. Epidermal cell shape (Fig. 3) is either elongated cells, polygonal cells, or a mixture of two (random). *Anticlinal wall*. Thin area ornamentation, the average size of epidermal cell on the abaxial is larger than the adaxial side. The anticlinal wall is either straight, curved, or straight to curved (Fig. 3). In some species, abaxial and adaxial epidermal cell shapes and anticlinal walls are different (Table 2).

Stomata. Can be found on both the adaxial and abaxial and abundantly count on the abaxial compared to the adaxial surface (Fig. 4). Stomata and epidermal cells are arranged in the same row. *Orientation*. Parallel to the long axis on both the abaxial and adaxial sides. Distance between sto-

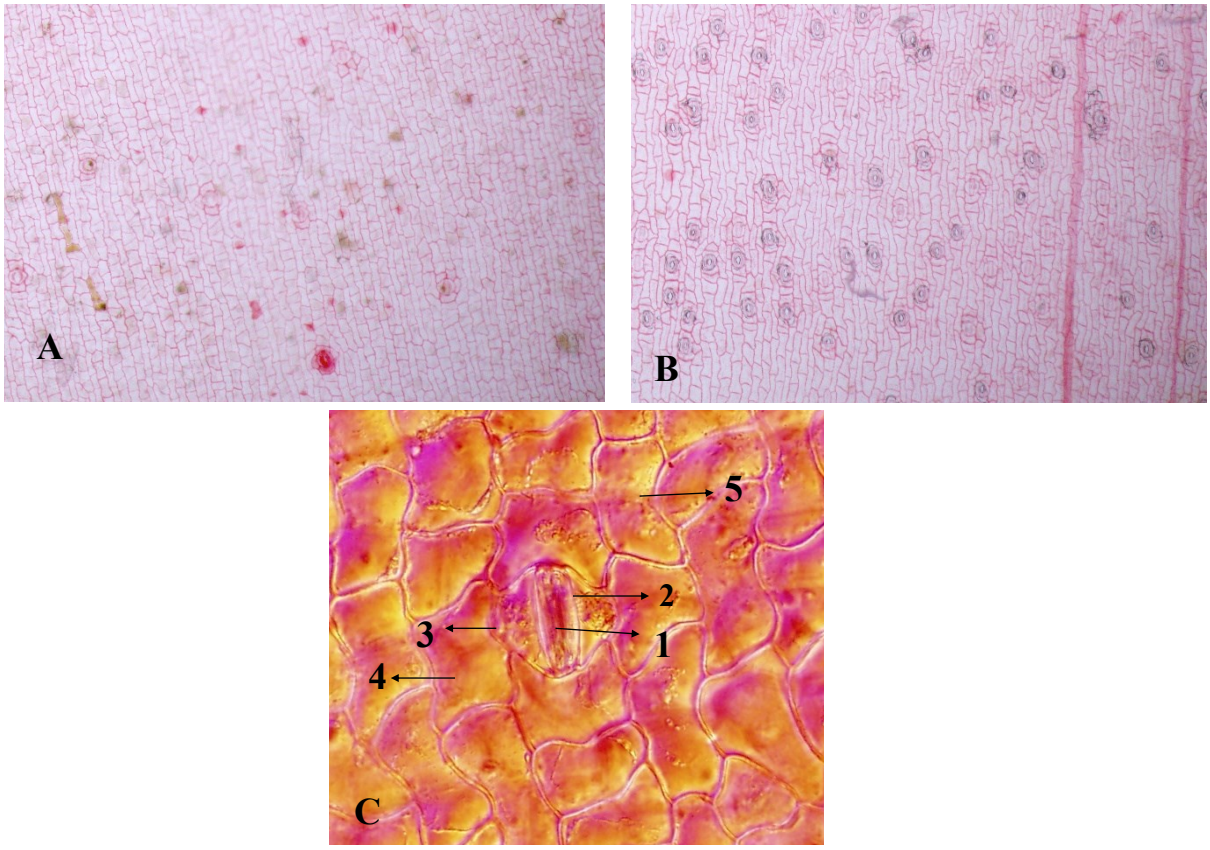

Fig. 4. Stomatal distribution and characteristics. A. *Orchidantha* stomata distribution on an adaxial leaf. B. *Orchidantha* stomata distribution on abaxial leaf. C. Stomata. 1: stomatal pore, 2: guard cell, 3: lateral subsidiary cell, 4: polar subsidiary and 5: epidermal cell.

mata is closer on the abaxial. The average size of stomata is H (5.20–32.13 µm) × W (2.74–31.28 µm). *Type of lateral subsidiary cell surround stomata*. Paracytic (Fig. 4). *Stomatal index*. The highest stomata index on the adaxial surface is produced by *O. fimbriata* (5.40) while the lowest is produced by *O. ranchanensis* (0.49) (Table 2). Meanwhile, the highest stomata index on the abaxial surface is produced by *O. inouei* (9.09) while the lowest is produced by *O. maxillarioides* (4.83). Guard cells are in dumbbell shape (Fig. 4) and they are either sunken or raised. The average guard cell size among species is H (3.12–23.66 µm) × W (1.84–12.59 µm) (Table 2). Both adaxial and abaxial guard cells show no variations.

DISCUSSION

The result of this study shows all *Orchidantha* species have a common petiole cross-section shape which is elliptic with grooves. Significant in *Orchidantha* petiole grooves along with petiole margin and their vascular system characteristics are useful in species identifications.

In general, vascular bundles found in *Orchidantha* are collateral, and end closed by a double sheath. These are common elsewhere in Zingiberales (Sunandar & Kahar, 2017). In *Orchidantha*, the vascular bundle is scattered near the adaxial petiole and neatly arranged in line assembling, an arc towards the abaxial petiole comparable with *Heliconia* L. species (Simão & Scatena, 2001), *Zingiber* Mill. species (Tomlinson, 1956), and *Maranta* Plum. ex L. species (Anderson, 1998).

Those four families have a number of vascular systems ranging from three to four arcs at most, indicating plant maturity (Tomlinson, 1956). However, there is some uncertainty regarding the number of vascular systems in *Orchidantha* and whether it can be considered a sign of species maturity, as the development of vascular system arcs increases with plant growth. From the result, *Orchidantha* has the highest number of vascular system (five arcs) in the Zingiberales.

Type I and Type III seem to be the main vascular systems and the others are developing along with plant growth.

In responsible buoyancy balance in harsh environments, supporting the petiole flexibility (Pasini & Mirjalili, 2006), parenchyma in *Orchidantha* developed an aerenchyma cell (air chamber). The

Table 2. Epidermal cell and stomatal characteristics on the adaxial and abaxial leaf surface of *Orchidantha*.

Species		Epidermal cell		Average size (μm)	Stomata index	Guard cell average size (μm)
		Shape	Anticlinal wall			
O. micrantha	Adaxial	Polygonal	Straight	8.01 × 7.85	1.90	9.26 × 2.57
	Abaxial	Elongated	Straight	9.11 × 5.57	5.88	7.29 × 2.41
O. fimbriata	Adaxial	Elongated	Curved	7.35 × 5.18	5.40	5.38 × 2.30
	Abaxial	Elongated	Curved	6.83 × 4.29	5.70	5.46 × 1.94
O. inouei	Adaxial	Elongated	Straight	27.72 × 16.70	3.44	18.31 × 6.09
	Abaxial	Elongated and polygonal	Curved	17.11 × 31.11	9.09	12.20 × 3.35
O. lengguani	Adaxial	Elongated	Curved	6.72 × 4.56	4.34	3.12 × 2.78
	Abaxial	Polygonal	Curved	7.59 × 3.41	6.48	4.13 × 1.84
O. longiflora	Adaxial	Elongated and polygonal	Curved	28.43 × 32.74	0.59	20.63 × 12.59
	Abaxial	Elongated	Curved	45.18	5.21	23.66 × 11.53
O. maxillarioides	Adaxial	Polygonal	Straight	10.02 × 5.15	0.60	4.52 × 4.23
	Abaxial	Elongated	Straight to curved	24.59 × 28.57	4.83	18.88 × 9.87
O. ranchanensis	Adaxial	Elongated and polygonal	Straight to curved	33.21 × 17.48	0.49	19.35 × 9.11
	Abaxial	Elongated	Straight to curved	44.63 × 17.66	5.00	20.14 × 9.53
O. sarawakensis	Adaxial	Polygonal	Straight	23.15 × 8.34	0.84	9.78 × 4.13
	Abaxial	Elongated	Straight	8.32 × 6.81	8.51	8.08 × 2.30
O. siamensis	Adaxial	Polygonal	Straight	22.41 × 11.45	0.59	12.79 × 10.60
	Abaxial	Elongated	Straight	38 × 13.97	8.65	20.47 × 9.27

air chamber in *Orchidantha* is arranged in line forming an arc. The position of aerenchyma alternates with the vascular system. This character is significantly different to some other families in the Zingiberales. Air chambers exist in Musaceae, Strelitziaceae, and Heliconiaceae. The biggest air chamber is in Musaceae with packed stellate cells forming a shape like a rib cage (Ennos *et al.*, 2000). *Heliconia* species (Simão & Scatena, 2001) possess air chamber-like arc shapes but form compartments defined by diaphragm while *Strelitzia* Banks species (Tomlinson, 1956) have numerous small air chambers forming net-like shapes.

The cross-section of the midrib has variation within the *Orchidantha* species sampled here and also significant compared to other family members in Zingiberales. The weight; width and length, of the leaves affected the midrib characteristic. Species with wider leaves have flat induplicate midribs (*O. sarawakensis, O. inouei, O. ran-chanensis,* and *O. micrantha*) while taper, long leaves have erected induplicate midribs (*O. siamensis, O. lengguani, O. maxillarioides, O. fimbriata,* and *O. longiflora*).

The evidence of epidermal cell characteristics is insufficient for species identification among *Orchidantha* species or family members. From these studies, all nine species of *Orchidantha* show no marked difference in epidermal cells except for slight differences in cell shape and anticlinal wall.

Stomata were found abundantly on the abaxial side compared to the adaxial side. It was arranged longitudinally alongside with epidermal cell. Guard cells are dumbbell-shaped, which is very distinct from other Zingiberales family member which has kidney shapes (Sumardi & Wulandari, 2010). The subsidiary cell in *Orchidantha* is paracytic which is similar in *Canna* (Sultana *et al.*, 2019), *Maranta*, and *Costus* species (Andersson, 1998; Riyas & Mathew, 2018). In contrast, the stomata in *Zingiber* (Zahara, 2020) and *Heliconia* species (Leite *et al.*, 2023) are mostly tetracytic whereas those in, *Musa* are anomosytic (Sumardi

Key to anatomical identification of *Orchidantha* species.

1 a. Petiole grooves V-shaped ……2
b. Petiole grooves U-shaped ……5
2 a. Petiole margin undulate ……...3
b. Petiole margin entire ……4
3 a. Absent Type II and Type IV of vascular systems……..… *O. ranchanensis*
b. Absent Type IV and Type V of vascular systems…...…….. *O. micrantha*
4 a. Absent only Type V of vascular systems…… *O. siamensis*
b. Presence of all five types of vascular systems (I–V) ……..*O. longiflora*
5 a. Petiole margin undulate ……..……...6
b. Petiole margin entire ……...……...…….....7
6 a. Petiole grooves deeply U-shaped ……...……..... *O. lengguani*
b. Petiole grooves wide almost without grooves ……..……*O. maxillarioides*
7 a. Average petiole 6.73 × 5.68 mm broadly abaxial …...…. *O. sarawakensis*
b. Average petiole 8.93 × 6.22 mm narrowly abaxial ……..…….8
8 a. Petiole grooves shallowly U-shaped ……*O. fimbriata*
b. Petiole grooves normal U-shaped …….……*O. inouei*

& Wulandari, 2010).

The stomatal index is influenced by both the formation of stomata and the growth of epidermal cells (Royer, 2001). In higher plants, anatomical features like the type of stomata and the stomatal index can be utilized to determine systematic classifications. In *Orchidantha*, the stomatal index on the abaxial samples shows no significant variations while there are distinct variations in the adaxial ones.

CONCLUSION

Lowiaceae has anatomical features different from other Zingiberales families. This anatomical data plays an important role for plant identification this is because, infertile, Lowiaceae species could be identified by slashing their petiole to see a pattern of petiole grooves and also further confirmation with the number of vascular bundles under microscopic view.

The present study found that the petiole groove shape (U and V) and depth (deep and shallow) is variable between species of the *Orchidantha*. The variation in petiole groove depths is affected by the size of leaves and height of plants as they respond to environment conditions, such as wind force, and insect weight (Ennos *et al.*, 2000; Pasini & Mirjalili, 2006). Our results show that U-shaped petiole grooves were found in plants higher than 1.5 m while shorter plants had variously V-shaped petiole grooves. It is noticeable, that shorter *Orchidantha* possessed undulate petiole margin.

Hence, key of identification using anatomical features of *Orchidantha* is provided.

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