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Leaf morpho-anatomy and taxonomic significance in six *Phalaenopsis* s. l. (Orchidaceae) species from China

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ABSTRACT

Phalaenopsis is well-known as a highly ornamental and rare orchid. The taxonomy and infrageneric relationship of the genus of *Phalaenopsis* s. l. has been highly disputed due to some overlapping morphological features. Leaf morpho-anatomy of six *Phalaenopsis* s. l. species from China were investigated using a microscope and paraffin section to provide new evidence for taxonomic implications. The observation of the study revealed the following: (1) The shape of the ordinary epidermal cells on both sides are polygonal with straight-arched anticlinal wall. (2) Papillae occur on each epidermal cells and are present on both leaf sides, including the uni-papilla on both the adaxial and abaxial epidermis cells in these six species, with an exception of the bi-papillae on adaxial epidermis cells in *P. wilsonii* and *P. braceana*. (3) Orbicular stomata and elliptic stomata occur only on the abaxial epidermis, commonly with co-occurrence of tetracytic and anomocytic stomata. (4) The straight type and the convex type of outline of leaf midrib were observed in six *Phalaenopsis* s. l. species. The thickness of leaf midrib is different greatly, the thickest observed in *P. deliciosa* and the thinnest observed in *P. braceana*. (5) Spherical silica bodies and prismatic and raphide crystals were observed in six *Phalaenopsis* s. l. species. Some leaf morpho-anatomic features were selected and used for species discrimination, including the size of ordinary epidermal cells, type of papillae, stomatal shape and density, thickness and outline of cross-section of a leaf midrib, and crystal type. Meanwhile, it was supported that *Kingidium* should be grouped into *Phalaenopsis* s. l., since *P. deliciosa* (syn. = *K. deliciosum*) and *P. braceana* (syn. = *K. braceanum*) shared common leaf morpho-anatomic features with the other four species of *Phalaenopsis* s. s. In addition, a key to six *Phalaenopsis* s. l. species from China was proposed based on leaf morpho-anatomic features.

1. Introduction

Phalaenopsis s. l. Blume (Aeridinae, Vandeae, Epidendroideae, Orchidaceae) was a small genus, comprising about 45–50 species (Pridgeon et al., 2014), or up to 81 (Liu et al., 2022), distributed from India to Southern China, Thailand, Indochina, Malaysia, and the Philippines (Pridgeon et al., 2014). There were 22 species recorded in China, containing 5 endemic species, mostly occurring in Yunnan (Liu et al., 2022). Members of *Phalaenopsis* s. l. are the most well-known ornamental orchids owing to their attractive and long-lasting flowers (Lawler, 1984; Handini et al., 2016; Liu et al., 2022). However, wild

resources of *Phalaenopsis* have been decreasing due to the native habitat fragmentation and over-harvesting (Pridgeon et al., 2014). Four species from China were listed as the rare and endangered orchids, including *P. lobbii* (Rchb. f.) H. R. Sweet, *P. malipoensis* Z. J. Liu, *P. wilsonii* Rolfe, and *P. zhejiangensis* (Z. H. Tsi) Schuit. (National Forestry and Grassland Administration of China, 2021).

The taxonomy and species delimitation of *Phalaenopsis* has been controversial based on morphological and molecular data available (Tsai et al., 2005, 2010; Chase et al., 2015; Li et al., 2016; Liu et al., 2022). The *Phalaenopsis* s. s. was firstly classified into two categories based on the presence of lip appendages (Reichenbach, 1860), and then

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into four groups according to the size of sepals and petals, the structure of column, and lip tip (Rolfe, 1886). Later, it was revised and divided into five subgenera, with a treatment of some alliance as synonyms, such as *Kingidium* P. F. Hunt and *Doritis* Lindl. (Christenson, 2001). And then, an extended genus of *Phalaenopsis* was classified into four subgenera, with a new adding of *Hygrochilus* Pfitz. and *Ornithochilus* (Lindl.) Wall. ex Benth., based on nucleotide and chloroplast sequences (Cribb and Schuiteman, 2012; Kocyan and Schuiteman, 2014; Li et al., 2016). Therefore, a broadened genus of *Phalaenopsis* (*Phalaenopsis* s. l.) was proposed formally, with a treatment of its alliance as synonyms like the previous research (Pridgeon et al., 2014). However, the infrageneric relationship of *Phalaenopsis* had been controversial greatly due to lack of morphological evidence (Hidayat et al., 2005, 2012). Meanwhile, species discrimination of *Phalaenopsis* s. l. has been problematic because of some overlapping floral features, leading to some easily confused species (Fig. 1), such as *P. stobartiana* Rchb. f. vs. *P. braceana* (Hook. f.) Christenson (syn. = *Kingidium braceanum* (Hook. f.) Seidenf.), *P. stobartiana* vs. *P. wilsonii*, or *P. lobbii* vs. *P. malipoensis* (Dalström, 2006; Chen et al., 1999; Chen and Wood, 2009). In fact, these easily confused species should be recognized as separate taxa based on chloroplast genomic data (Hu et al., 2022; Tao et al., 2023). Therefore, more evidence should be needed to provide a better understanding of taxonomy and phylogeny of *Phalaenopsis* (Hidayat et al., 2005, 2012; Liu et al., 2022).

Leaf morpho-anatomic features were varied and possessed taxonomic implications in Orchidaceae (Morris et al., 1996; Carlsward et al., 2006; Stern and Carlsward, 2006; Figueroa et al., 2008; Xie et al., 2024). The broad leaf was common in most taxon of Aeridinae, while the terete leaf occurred in some taxa, such as *Papilionanthe* Schltr., *Luisia* Gaudich., *Cleisostoma* Blume, and *Trichocentrum* Poepp. & Endl. (Chen and wood, 2009; Liu et al., 2011; Fan et al., 2014; Angela et al., 2015). The cuticular wax and stomatal on the abaxial surface were used for discrimination of *Holcoglossum* Schltr. from its related genera, including *Rhynchostylis* Blume and *Vanda* Jones ex R. Br. (Fan et al., 2014). Leaf anatomical characters could be used in species delimitation of plant taxa

when the floral morphology is unavailable or similar, such as four *Vanda* species (Kowsalya et al., 2017) and five *Dendrobium* species (Xie et al., 2024), including epidermal cell shape, stomatal density, leaf midrib outline, vascular bundle, peltate trichome, and crystals. However, there were few data on leaf morpho-anatomy of *Phalaenopsis*, only scattered in some species and simply described, such as *P. deliciosa* Rchb. f. (syn. = *Kingidium deliciosum* (Rchb. f.) Sweet) (Carlsward, 2004; Carlsward et al., 2006; Baishnab et al., 2020) and *P. amabilis* Blume (Bercu et al., 2011).

Therefore, leaf morpho-anatomy of six *Phalaenopsis* s. l. (Subgenus *Parishianae*) species from China were investigated using microscope and paraffin section, including *P. malipoensis*, *P. lobbii*, *P. wilsonii*, *P. stobartiana*, *P. braceana*, and *P. deliciosa*. Among of them, the former four species were always placed in the *Phalaenopsis* s. s., and the latter two species were moved from the traditional *Kingidium* (Chen et al., 1999). The aims of this study are: (1) to observe the leaf morpho-anatomy of six *Phalaenopsis* s. l. species in detail; (2) to select some leaf morpho-anatomic features for the intergeneric and infrageneric taxonomy of *Phalaenopsis* s. l. based on a comparative analysis; (3) to provide some new evidence for species delimitation of *Phalaenopsis* s. l. and for a better understanding of the relationship between *Phalaenopsis* s. s. and *Kingidium*.

2. Materials and methods

Plants of six *Phalaenopsis* s. l. (Subgenus *Parishianae*) species (Table 1 and Fig. 1) have been cultivated for many years and bloom every year in the Orchid Germplasm Resource Nursery of Yunnan Fengchunfang Biotechnology Company Limited, the Teaching Practice Base of Southwest Forestry University, located in Fumin County, Yunnan Province, China (N 25° 20' 01", E 102° 27' 26").

Two mature leaves per species were collected and fixed in FAA (50 % ethanol: formalin: acetic acid = 90: 5: 5) (Johansen, 1940) for over 72 h and then transferred and kept in 70 % ethanol for next observation.



Fig. 1. Floral morphology of six easily confused species from *Phalaenopsis* s. l. A: *P. malipoensis*. B: *P. lobbii*. C: *P. stobartiana*. D: *P. wilsonii*. E: *P. braceana*. F: *P. deliciosa*.

Table 1
Data on six *Phalaenopsis s. l.* species observed.

| Number | Taxa (Liu et al., 2022) | Distribution | Leaf habit |
|--------|--------------------------|---------------------------------|----------------------|
| I | Sect. <i>Parishianae</i> | | |
| 1 | <i>P. malipoensis</i> * | South of China | Bloom with 2 leaves |
| 2 | <i>P. lobbii</i> * | South of China | Bloom without leaves |
| II | Sect. <i>Aphyllae</i> | | |
| 3 | <i>P. stobartiana</i> | South China and Burma | Sometimes leafless |
| 4 | <i>P. wilsonii</i> * | South of China | Sometimes leafless |
| 5 | <i>P. braceana</i> | South of China and Bhutan | Sometimes leafless |
| III | Sect. <i>Deliciosae</i> | | |
| 6 | <i>P. deliciosa</i> | South of China, India, and Laos | Bloom with 2 leaves |

* * Indicating the Chinese endemic species.

To observe leaf epidermal characteristics, samples were prepared following Sun and Jiang (2009). Leaves preserved were cut into small fragments approximately 1 cm × 1 cm from an area between the mid-vein and leaf edge, immersed in 30 % H₂O₂-CH₂COOH (30 % hydrogen peroxide: acetic acid = 1: 1) for 12 - 24 h, and stained with 1 % Safranin for 2 - 3 min. Transverse sections of the leaf blade of each species were investigated for leaf anatomical features. Fragments about 0.5 cm × 0.5 cm from an area between the midvein and edge of the leaf blade were dehydrated using ethanol, embedded in paraffin wax (melting point = 57 °C), and cut at a thickness of 8 - 10 μm using a Leica RM2235 Rotary Microtome. Sections were stained with (1 %) safranin and (1 %) fast green (Gerlach, 1977). Samples of the dissociated epidermis and sections were mounted on glass slides using Canada balsam. The sections were observed and photographed using a Leica DM 750 microscope. Thirty measurements were taken for each documented value of the

lengths and widths of leaf ordinary epidermal cells, stomata, and the thickness of leaf midrib by Image J software. Stomatal index = number of stomata / (number of stomata + number of ordinary epidermal cells) × 100 %. The terminologies used for stomatal complex types were followed by Patel (1979).

Leaf morpho-anatomical features were statistically analyzed using SPSS 25.0 software, and mean values and standard errors were obtained. To evaluate the significant differences in anatomical features among six *Phalaenopsis s. l.* species, the data were tested separately using one way analysis of variance (ANOVA). The means were separated using Duncan's multiple range test, and differences between mean were considered significant with p < 0.05.

3. Results

3.1. Leaf surface

Ordinary epidermal cell: Ordinary epidermal cells on both leaf surfaces were polygonal with straight-arched anticlinal walls, arranged in longitudinal rows parallel to leaf veins (Figs. 2 and 3). Papillae with varied number were observed on both surfaces (Figs. 2, 3 and Table 2). The uni-papilla (Figs. 2A-D and 3) was observed on the abaxial epidermal cells in the six species, and on the adaxial epidermal cells in four species. However, the bi-papillae (Fig. 2E and F) were observed in *P. wilsonii* and *P. braceana*. Meanwhile, the shape of uni-papilla on the abaxial epidermal cells was less evident in *P. deliciosa* (Fig. 3H), *P. wilsonii* (Fig. 3K), and *P. stobartiana* (Fig. 3Q) than the other three species. The adaxial epidermal cells were conspicuously larger than the abaxial epidermal cells in five species, but smaller in *P. stobartiana* (Table 2). The largest cells on the adaxial epidermis were found in *P. braceana* (Fig. 2F), and on the abaxial epidermis were observed in *P. stobartiana* (Fig. 3Q). Meanwhile, the smallest cells were found in both

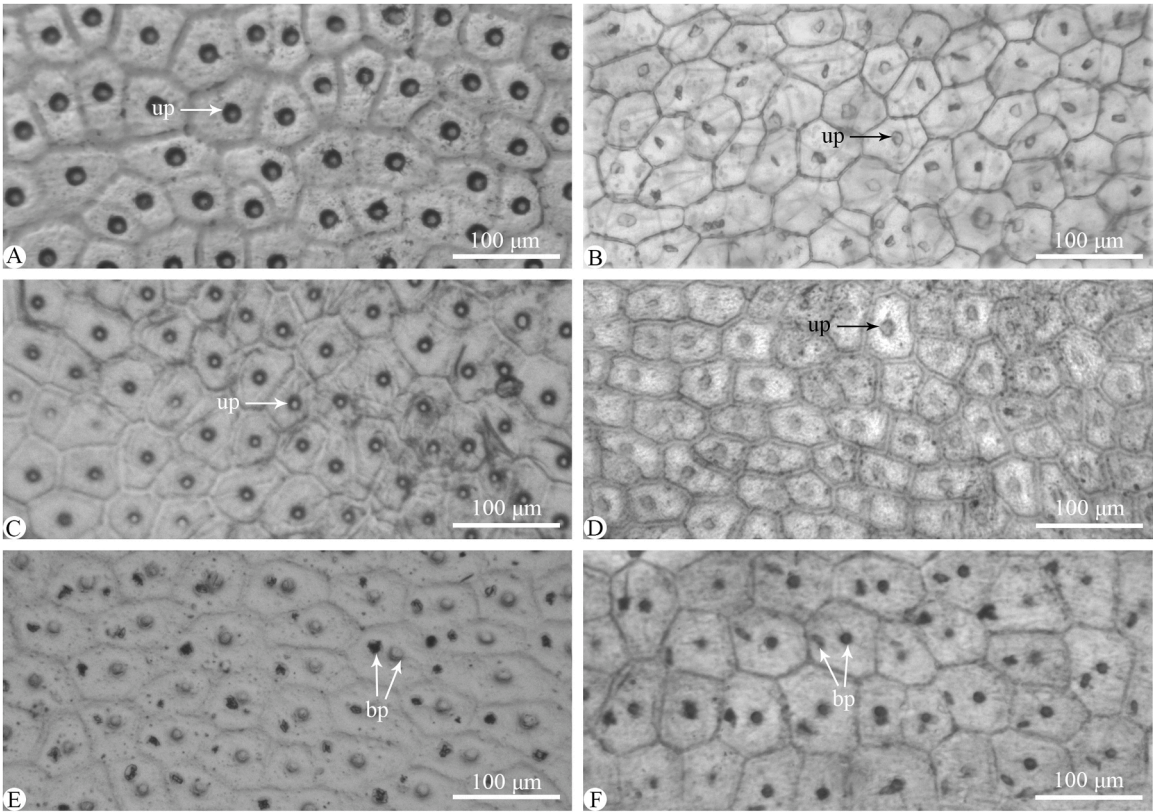


Fig. 2. Micro-morphology of the adaxial leaf epidermis in six *Phalaenopsis s. l.* species. A - D: Uni-papilla (up) on each epidermal cell, including *P. malipoensis* (A), *P. lobbii* (B), *P. stobartiana* (C), *P. deliciosa* (D). E - F: Bi-papillae (bp) on each epidermal cell, including *P. wilsonii* (E) and *P. braceana* (F).

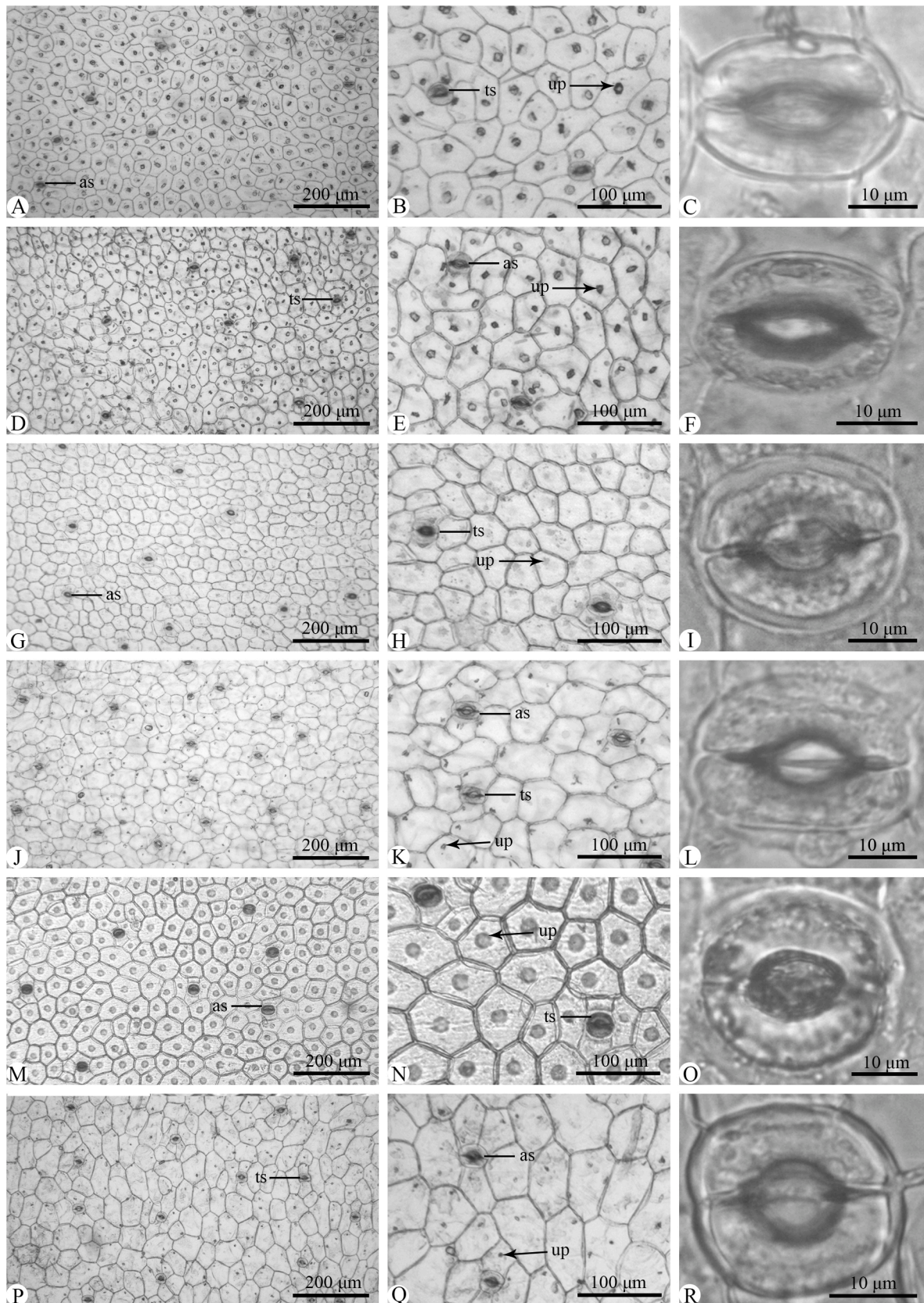


Fig. 3. Micro-morphology of the abaxial leaf epidermis of six *Phalaenopsis* s. l. species. **A - C:** *P. malipoensis*. **D - F:** *P. lobbii*. **G - I:** *P. deliciosa*. **J - L:** *P. wilsonii*. **M - O:** *P. braceana*. **P - R:** *P. stobartiana*. **Noting:** Uni-papilla (**up**) on each epidermal cell and both anomocytic stomata (**as**) and tetracytic stomata (**ts**) co-occurred in the six species. Two types of stomata shape, including the elliptic stomata (C, F, I, L) and the orbicular stomata (O, R).

Table 2Leaf epidermal features in six *Phalaenopsis s. l.* species (Mean \pm SE, $n = 30$).

| Species | Adaxial ordinary epidermal cell | | | Abaxial ordinary epidermal cell | | |
|-----------------------|---------------------------------|-------------------------|------------------|---------------------------------|--------------------------|------------------|
| | Length (μm) | Width (μm) | Type of papillae | Length (μm) | Width (μm) | Type of papillae |
| <i>P. malipoensis</i> | 66.18 $\pm 1.27^d$ | 36.78 $\pm 0.2^d$ | Uni-papilla | 58.89 $\pm 0.31^c$ | 37.53 $\pm 0.27^b$ | Uni-papilla |
| <i>P. lobbii</i> | 71.76 $\pm 1.32^c$ | 39.11 $\pm 0.2^c$ | Uni-papilla | 58.19 $\pm 0.78^c$ | 36.98 $\pm 0.26^{bc}$ | Uni-papilla |
| <i>P. stobartiana</i> | 62.17 $\pm 0.29^e$ | 46.64 $\pm 0.32^b$ | Uni-papilla | 85.52 $\pm 0.92^a$ | 47.62 $\pm 0.2^a$ | Uni-papilla |
| <i>P. wilsonii</i> | 75.59 $\pm 0.57^b$ | 46.68 $\pm 0.42^b$ | Bi-papillae | 64.66 $\pm 0.64^b$ | 35.87 $\pm 0.45^{cd}$ | Uni-papilla |
| <i>P. braceana</i> | 83.32 $\pm 1.53^a$ | 60.00 $\pm 0.6^a$ | Bi-papillae | 85.25 $\pm 0.43^a$ | 46.89 $\pm 0.7^a$ | Uni-papilla |
| <i>P. deliciosa</i> | 56.32 $\pm 0.96^f$ | 35.45 $\pm 0.6^e$ | Uni-papilla | 49.32 $\pm 0.44^d$ | 34.81 $\pm 0.36^{cd}$ | Uni-papilla |

Different letters in the same column indicating statistical difference $P < 0.05$ (ANOVA).

epidermis of *P. deliciosa* (Table 2).

Stomata: Stomata were observed only on the abaxial leaf epidermis with co-occurrence of anomocytic and tetracytic stomata in six species (Fig. 3). However, the stomatal shape and size were varied among species with a significant difference (Table 3). The elliptic stomata ($P/E \geq 1.2$) were observed in *P. malipoensis* (Fig. 3C), *P. lobbii* (Fig. 3F), *P. deliciosa* (Fig. 3I), and *P. wilsonii* (Fig. 3L), and the orbicular stomata ($P/E < 1.2$) were presented in *P. braceana* (Fig. 3O) and *P. stobartiana* (Fig. 3R). The largest stomata occurred in *P. braceana*. While the smallest one was found in *P. lobbii*. The stomatal index was varied between species (Table 3), around 4.5 in four species (*P. malipoensis*, *P. lobbii*, *P. braceana*, and *P. deliciosa*), but around 12 in *P. wilsonii* and *P. stobartiana*. Stomatal density was different between species (Fig. 3 and Table 3). The highest stomatal density ($SD \geq 30 \text{ mm}^{-2}$) was found in *P. wilsonii* (Fig. 3J), and the lowest stomatal density ($SD < 30 \text{ mm}^{-2}$) in *P. braceana* (Fig. 3M).

3.2. Transverse section of leaf blade

Transverse of the leaf midrib of leaf blade showed the common monocot anatomical structure, consisting of the cuticle, the epidermis, the mesophyll, and the vascular bundles, but the outline and thickness of cross section of the midrib (Fig. 4), as well as the crystals, were varied greatly among the six species (Fig. 5). These anatomic features were described in detail as below.

Cuticle: Thin, smooth, inconspicuous on both adaxial and abaxial surfaces (Fig. 4).

Epidermis: Both the adaxial and abaxial epidermis were uniseriate, and equally composed of some square or rectangular cells, as well as

some papillae (Fig. 4).

Vascular bundles: The vascular bundles were always arranged in one row across the width of a leaf blade, which was less evident, even in the midrib vascular bundles (Fig. 4A, D, G, J, M, P).

Mesophyll: Undifferentiated. The mesophyll was homogeneous, which was composed of several layers of elliptic and rounded parenchyma cells, together with unregular cells (Fig. 4).

Leaf midrib outline: The outline of a leaf midrib was varied, including the straight type (Fig. 4A) in *P. malipoensis*, and the convex type (Fig. 4D, G, J, M, P) in the other five species.

Leaf midrib thickness: The thickness of leaf midrib was varied greatly among species (Fig. 4 and Table 4), including the thinnest ($\leq 450 \mu\text{m}$) in *P. braceana* (Fig. 4D) and the thickest ($\geq 1000 \mu\text{m}$) in *P. deliciosa* (Fig. 4P). Moreover, the medium thickness ($450 - 1000 \mu\text{m}$) of midrib was observed in *P. malipoensis* (Fig. 4A), *P. lobbii* (Fig. 4G), *P. wilsonii* (Fig. 4J), and *P. stobartiana* (Fig. 4M).

Crystals: Three types of crystals in idioblasts were observed, including prismatic and spherical silica bodies and raphide crystals (Fig. 5 and Table 4). Each type of crystals commonly occurred accompanied with other one or two types in five species, but only one type of prismatic in *P. malipoensis* (Fig. 5A and Table 4). Prismatic (Fig. 5A–F) were common in six species, and spherical silica bodies (Fig. 5G–K) was presented in five species but absent in *P. malipoensis*. Raphide crystals (Fig. 5L–N) was observed in *P. lobbii*, *P. wilsonii*, and *P. deliciosa*.

4. Discussion and conclusion

4.1. Taxonomic significance of leaf morpho-anatomy in *Phalaenopsis s. l.* and its alliance

There were some distinguished features of leaf morpho-anatomy in *Phalaenopsis s. l.* from its alliance, including papillae on the epidermal cell, the shape of epidermal cell, and the stomata type. Firstly, papillae were observed on leaf epidermal cells in the six *Phalaenopsis s. l.* species, which was also found in *P. amabilis* (Bercu et al., 2011) and *P. deliciosa* (Baishnab et al., 2020), but without further attention on its taxonomic implications. The type of papillae on adaxial epidermal cell was varied between species, which could be recognized as the bi-papillae in *P. braceana* and *P. wilsonii*, and uni-papilla in the other species. The papillae on leaf epidermal might be used in species determination and taxonomy at the levels of tribe and genus. Papillae was ever observed in tribe Neottieae (Epidendroideae), including *Cephalanthera* Rich., *Limodorum* L., and *Stenisiella* Schltr. (Şenel et al., 2019). However, it was absent in other members of *Aerides-Vanda* of tribe Vandae, such as *Papilionanthe*, *Ascocentrum*, *Luisia*, *Holcoglossum* (Fan et al., 2014) and *Vanda* (Kowsalya et al., 2017). Secondly, the leaf epidermal cell was polygonal on both sides in the six species, which was similar to those of two species observed in *Phalaenopsis s. l.* that *P. amabilis* and *P. deliciosa* (Bercu et al., 2011; Baishnab et al., 2020), as well as of most Orchids (Şenel et al., 2019; Muangsan et al., 2022; Xie et al., 2024). However, there were quadrilateral epidermal cell observed in its related taxon, such as *Papilionanthe biswasiana* (Ghose & Mukerjee) Garay, *Ascocentrum*

Table 3Stomatal characteristics in six *Phalaenopsis s. l.* species (Mean \pm SE, $n = 30$).

| Species | Size (μm) | | | Shape | Stomatal Index (%) | Density (mm^{-2}) |
|-----------------------|-------------------------------|-------------------------------|------|-----------|-------------------------------|------------------------------|
| | Polar axis (P) | Equatorial axis (E) | P/E | | | |
| <i>P. malipoensis</i> | 30.99 \pm 0.34 ^b | 21.14 \pm 0.23 ^c | 1.46 | elliptic | 4.88 \pm 0.69 ^c | 21 \pm 0.16 ^d |
| <i>P. lobbii</i> | 23.11 \pm 0.17 ^d | 18.03 \pm 0.14 ^d | 1.28 | elliptic | 4.58 \pm 0.64 ^c | 26 \pm 0.42 ^c |
| <i>P. stobartiana</i> | 23.31 \pm 0.26 ^d | 22.64 \pm 0.16 ^b | 1.03 | orbicular | 12.01 \pm 0.26 ^a | 42 \pm 0.46 ^b |
| <i>P. wilsonii</i> | 33.14 \pm 0.27 ^a | 22.43 \pm 0.16 ^b | 1.48 | elliptic | 11.74 \pm 0.14 ^a | 51 \pm 0.57 ^a |
| <i>P. braceana</i> | 25.36 \pm 0.29 ^c | 23.02 \pm 0.27 ^b | 1.10 | orbicular | 5.26 \pm 0.12 ^b | 15 \pm 0.27 ^e |
| <i>P. deliciosa</i> | 33.2 \pm 0.25 ^a | 25.35 \pm 0.23 ^a | 1.31 | elliptic | 3.58 \pm 0.32 ^d | 26 \pm 0.28 ^c |

Different letters in the same column indicating statistical difference $P < 0.05$ (ANOVA).

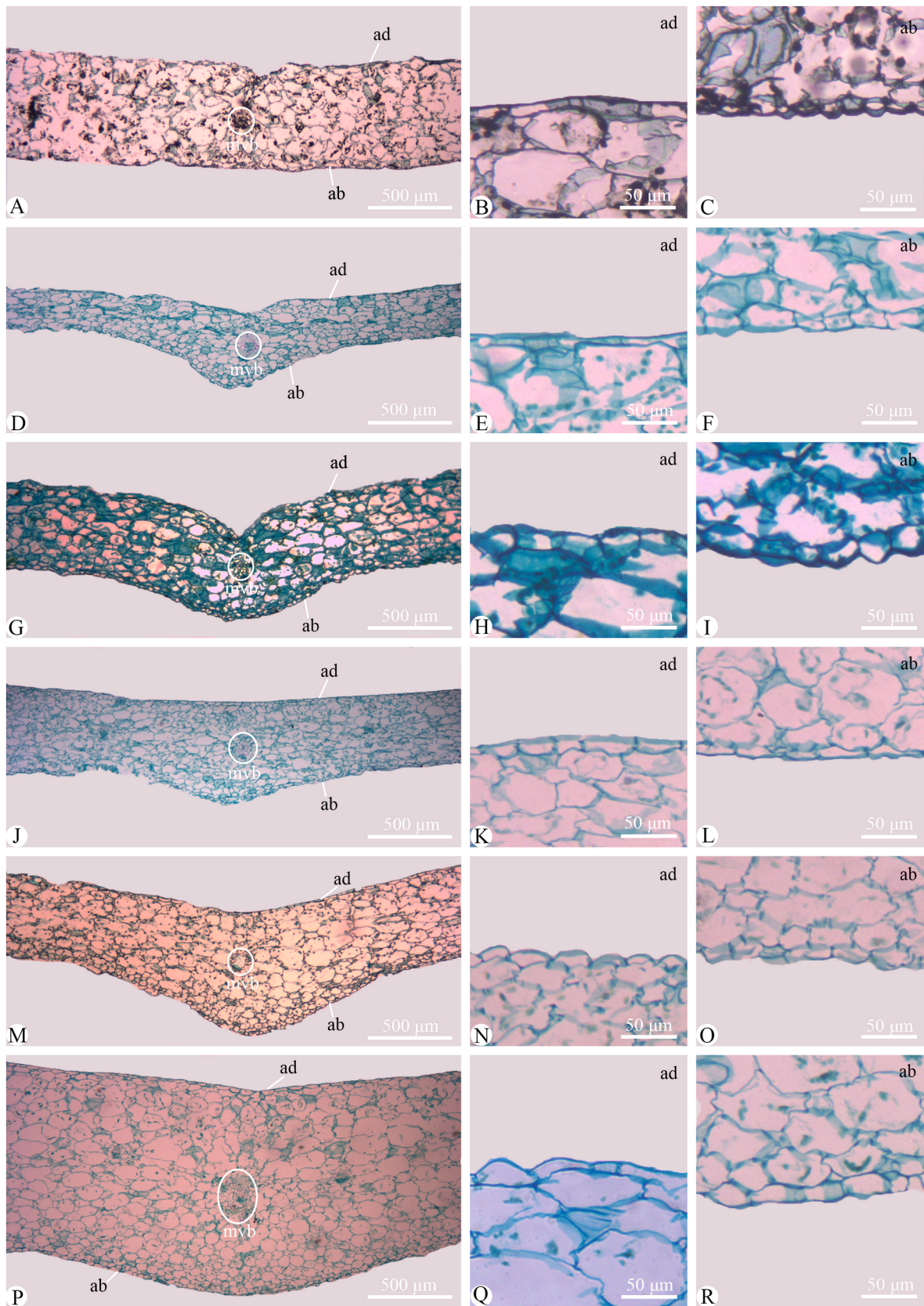


Fig. 4. Transverse section of a leaf blade midrib in six *Phalaenopsis* s. l. species. **A - C:** *P. malipoensis*. **D - F:** *P. braceana*. **G - I:** *P. lobbii*. **J - L:** *P. wilsonii*. **M - O:** *P. stobartiana*. **P - R:** *P. deliciosa*. **ab** = abaxial epidermis surface. **ad** = adaxial epidermis surface. **mvb** = midrib vascular bundle.

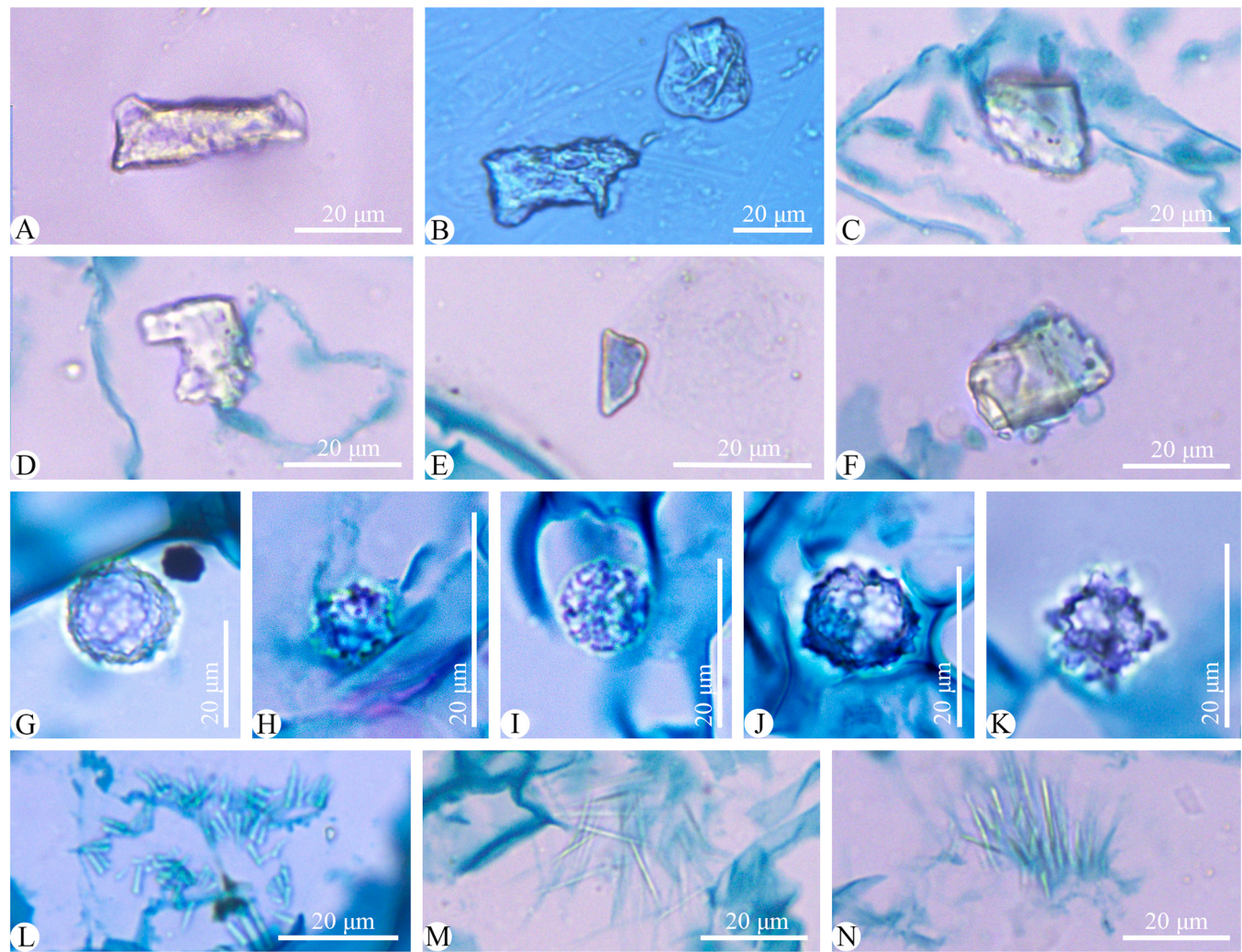


Fig. 5. Three types of crystals were observed in six *Phalaenopsis s. l.* species. **A - F:** Prismatic in *P. malipoensis* (A), *P. lobbii* (B), *P. stobartiana* (C), *P. wilsonii* (D), *P. braceana* (E), and *P. deliciosa* (F). **G - K:** Spherical silica bodies in *P. lobbii* (G), *P. stobartiana* (H), *P. wilsonii* (I), *P. braceana* (J), and *P. deliciosa* (K). **L - N:** Raphide in *P. lobbii* (L), *P. wilsonii* (M), *P. deliciosa* (N).

Table 4
Leaf anatomic features of six *Phalaenopsis s. l.* species (Mean \pm SE, $n = 30$).

| Species | TL (μm) | Leaf blade midrib outline | Type of crystal |
|-----------------------|--------------------------------|---------------------------|-------------------------------|
| <i>P. malipoensis</i> | 548.26 \pm 0.72 ^d | straight | Prismatic |
| <i>P. lobbii</i> | 528.75 \pm 0.87 ^e | convex | Prismatic, spherical, raphide |
| <i>P. stobartiana</i> | 721.66 \pm 0.75 ^b | convex | Prismatic, spherical |
| <i>P. wilsonii</i> | 611.17 \pm 0.75 ^c | convex | Prismatic, spherical, raphide |
| <i>P. braceana</i> | 415.43 \pm 0.61 ^f | convex | Prismatic, spherical |
| <i>P. deliciosa</i> | 1206.5 \pm 0.6 ^a | convex | Prismatic, spherical, raphide |

Noting: TL. Thickness of leaf blade. Different letters in the same column indicating statistical difference $P < 0.05$ (ANOVA).

ampullaceum (Roxb.) Schltr., *Vanda pumila* Hook. f., *Luisia magniflora* Z. H. Tsi & S. C. Chen, seven *Holcoglossum* species (Fan et al., 2014), and *Vanda tessellata* (Roxb.) Hook. ex G. Don (Kowsalya et al., 2017). Thirdly, the stomata were observed only abaxial surface in six species, but it was present on both leaf surfaces in *Rhynchostylis retusa*,

Ascocentrum ampullaceum, and eight *Holcoglossum* species (Fan et al., 2014). Meanwhile, co-occurrence of tetracytic and anomocytic stomata was observed in the six species, but the hexacytic type stomata in *Ascocentrum ampullaceum*, *Holcoglossum lingulatum*, *H. flavescens*, and *H. weixiense* (Fan et al., 2014).

4.2. Taxonomic implication for *Kingidium*

Kingidium P. F. Hunt was an independent genus, comprising about 3 - 4 species (Chen et al., 1999), but was ever moved into *Phalaenopsis* (Sweet, 1980; Christenson, 2001), which was supported and sunk into *Phalaenopsis* by molecular data (Padolina et al., 2005; Tsai et al., 2005, 2010). *Kingidium* was distinguished from members of *Phalaenopsis* by its spurred or saccate lip (Chen et al., 1999). However, it was showed that two species of *Kingidium*, namely, *Phalaenopsis deliciosa* (syn. = *K. deliciosum*) and *P. braceana* (syn. = *K. braceanum*), should be grouped into *Phalaenopsis* based on some similar leaf morpho-anatomic features, such as the presence of the papillae on the polygonal epidermal cell, the stomata on the abaxial leaf epidermis, and the hairs absent. These features were also observed in the previous data on *P. deliciosa* (Carlsward et al., 2006; Baishnab et al., 2020). It was also noted that three types of crystals (spherical silica bodies, prismatic and raphide crystals) were observed in *P. deliciosa* in this study, but only spherical silica bodies was recorded in the previous study (Carlsward et al., 2006).

4.3. Taxonomic implication for species delimitation of *Phalaenopsis* s. l.

For a long time, species delimitation in *Phalaenopsis* has been mainly on flower morphology (column, lip, callus, and the number of pollinia) (Chen et al., 1999; Chen and Wood, 2009; Christenson, 2001; Liu et al., 2022). However, species delimitation has been problematic in some easily confused species of *Phalaenopsis* due to overlapping floral features, such as *P. stobartiana* and *P. braceana*, *P. malipoensis* and *P. lobbii* (Dalström, 2006; Chen and Wood, 2009). It was revealed that some leaf morpho-anatomy features were varied from species to species, including the type of papillae on the epidermal cell, stomatal index and density, outline and thickness of a leaf midrib, and crystal type.

It seemed that there were two pairs of easily-confused species of *Phalaenopsis* because of some overlapping floral features between them (Dalström, 2006; Chen and Wood, 2009), which needed to be clarified, including *P. stobartiana* vs. *P. braceana* and *P. malipoensis* vs. *P. lobbii*. However, two species of each pair were independent and formed a sister clade with strong support, respectively placed in sect. *Aphyllae* and sect. *Parishianae*, based on molecular data (Kocyan and Schuiteman, 2014; Liu et al., 2022). Here, there were some selected features of leaf morpho-anatomy, which could be used for species delimitation of them. *Phalaenopsis stobartiana* could be distinguished from *P. braceana* by the uni-papillae and the higher density of stomata on the adaxial leaf epidermis, but the latter was bi-papillae and the lower stomatal density. Meanwhile, *P. malipoensis* could be delimited by the straight outline of cross section of leaf midrib with only one type of crystals, prismatic, while the latter possessed a convex outline and three types of crystals.

In addition, *Phalaenopsis braceana* and *P. deliciosa* were traditionally placed in *Kingidium* (Chen et al., 1999). However, these two species were not closely related and sunk into sect. *Aphyllae* and sect. *Deliciosae* respectively, even after members of *Kingidium* were merged into *Phalaenopsis* s. l. (Liu et al., 2022). *P. braceana* could be characterized by bi-papillae on the adaxial epidermis, the orbicular stomatal, and the thinner thickness of leaf midrib. On the contrary, *P. deliciosa* was diagnosed by the uni-papilla, the elliptic stomatal, and the thicker thickness of a leaf midrib up to three times of the former.

Accordingly, a taxonomic key to these six *Phalaenopsis* s. l. species was given below based on leaf morpho-anatomic features observed, in order to provide a better understanding of species delimitation of them.

A key to six *Phalaenopsis* s. l. species from China based on leaf morpho-anatomic features

| | |
|--|-----------------------|
| 1. Bi-papillae on leaf adaxial epidermal cells | 2 |
| 2. Elliptic stomata with higher stomatal density ($\geq 30 \text{ mm}^{-2}$) | <i>P. wilsonii</i> |
| 2. Orbicular stomata with low stomatal density ($< 30 \text{ mm}^{-2}$) | <i>P. braceana</i> |
| 1. Uni-papilla on leaf adaxial epidermal cells | 3 |
| 3. Straight outline of the cross section of leaf midrib | <i>P. malipoensis</i> |
| 3. Convex outline of the cross section of leaf midrib | 4 |
| 4. The adaxial ordinary epidermal cell is smaller than the abaxial epidermal cells, orbicular stomata with higher density ($\geq 30 \text{ mm}^{-2}$), prismatic and spherical silica bodies in idioblasts | <i>P. stobartiana</i> |
| 4. The adaxial ordinary epidermal cell is larger than the abaxial epidermal cells, elliptic stomata with low stomatal density ($< 30 \text{ mm}^{-2}$), prismatic and spherical silica bodies and raphide crystals in idioblasts | 5 |
| 5. Thin leaf midrib ($528.75 \pm 0.87 \mu\text{m}$) | <i>P. lobbii</i> |
| 5. Thick leaf midrib ($1205.33 \pm 0.97 \mu\text{m}$) | <i>P. deliciosa</i> |

CRedit authorship contribution statement

Wenhao Liu: Methodology, Data curation, Writing – original draft, Writing – review & editing. Yunxi Xie: Methodology, Writing – review & editing. Le Zou: Writing – review & editing. Yan Luo: Investigation, Writing – review & editing. Lu Li: Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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