Flora 317 (2024) 152560

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Leaf morpho-anatomy and taxonomic significance in five *Dendrobium* sect. *Stachyobium* species from China

Yunxi Xie[a,c,1], Yanping Wang[b,1], Lei Tao[a], Wenhao Liu[a], Yan Luo[d,2], Lu Li[a,2,*]

[a] College of Forestry, Southwest Forestry University, Kunming 650224, Yunnan, China
[b] Yunnan Forestry Technological College, Kunming 650224, Yunnan, China
[c] College of Biological Science and Food Engineering, Southwest Forestry University, Kunming 650224, Yunnan, China
[d] Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences & Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, Yunnan, China

ARTICLE INFO

Edited by: Dr Teresa Terrazas

Keywords:
Dendrobium
Leaf epidermal cell
Peltate trichome
Stomata
Vascular bundles
Crystals
Taxonomy
Species identification

ABSTRACT

Dendrobium Sw. is one of the three largest genera in Orchidaceae, which has been disputed due to controversial taxonomy and difficult species identification. Leaf morpho-anatomy of five sect. *Stachyobium* species from China were investigated using microscope and paraffin section to provide anatomic evidence for taxonomic significance. (1) Leaf size and apex of blade were varied among species and could be used for species delimitation. (2) Two shapes of ordinary epidermal cell were recognized, consisting of narrow-polygonal cell and broad-polygonal cell. The adaxial ordinary epidermal cells were conspicuously larger than the abaxial epidermal cell. The anticlinal wall of ordinary epidermal cells was straight-arched, with obvious thickenings. Peltate glandular trichomes occurred on both sides of leaf blade, with higher density on the abaxial surface. (3) Tetracytic stomata was observed only in *D. compactum*, while co-occurrence of tetracytic and anomocytic stomata was found in other four species. (4) Two types of outlines of leaf midrib were recognized, including flat type and carinate type. (5) The bicollateral vascular bundle was only observed in *D. strongylanthum*, but the amphivasal vascular bundle was commonly observed in the rest four species. (6) Two types of peltate trichome were firstly described anatomically, including a uni-celled stalk type and a bi-celled stalk type. Two types of crystals were present in idioblasts, including the spherical silica body in *D. sinominutiflorum*, and prismatic crystal in the rest four species. The monophyletic sect. *Stachyobium* was strongly supported by a comparative morpho-anatomy of leaf. Some morpho-anatomic features were selected and used for species discrimination and taxonomy of *Dendrobium*, including leaf size, leaf shape, apex of blade, the ordinary epidermal cell shape, stomatal density, leaf midrib outline, type of vascular bundle, anatomic type of peltate trichome, and crystals. Additionally, a key to five sect. *Stachyobium* species from China was proposed based on leaf morpho-anatomical features.

1. Introduction

Dendrobium Sw. is one of the three largest genera in Orchidaceae with approximately 1 100 - 1 500 species worldwide, widely distributed in tropical and subtropical regions of Asia to Oceania (Schuiteman and Adams, 2014). There were about 74 - 110 species recorded in China occurred from tropical and subtropical to Qinling Mountains regions (Chen et al., 1999; Zhu et al., 2009; Jin et al., 2019; Li, 2023). Most *Dendrobium* members have been valued greatly as medicinal and horticultural resources, and listed as endangered wild orchids, which needed further investigations (National Forestry and Grassland Administration of China, 2021).

The taxonomy of *Dendrobium* is considered to be one of the most intricate problems in Orchidaceae, mainly focusing on infrageneric relationships, species identification, and conservation and utilization, due to the wide distribution range, the large number of species, the diverse morphology, and some overlapping features between species (Clements, 2003, 2006; Adams, 2011). Since the establishment of *Dendrobium* by Swartz (1799), a number of intergeneric and infrageneric systems have been proposed based on morphological characters. It was initially

* Corresponding author.
E-mail address: lilu@swfu.edu.cn (L. Li).

[1] These authors contributed equally to this work and should be considered as co-first authors.
[2] These authors contributed equally to this work and should be considered as co-corresponding authors.

https://doi.org/10.1016/j.flora.2024.152560
Received 11 March 2024; Received in revised form 15 June 2024; Accepted 24 June 2024
Available online 2 July 2024
0367-2530/

recognized that there were four sections in *Dendrobium*, which were later expanded to ten sections (Lindley and Paxton, 1851). Kranzlin (1910) divided *Dendrobium* into ten subgenera and 27 sections with about 600 species. Schlechter (1982) proposed an infrageneric system of *Dendrobium* with four subgenera and 41 sections, which was followed by latter scholars (Ji et al., 1999). Recently, *Dendrobium* s.l. was proposed by adding of *Epineneium* Gagnep. and *Flickingeria* Hawkes, based on molecular phylogenetic data (Burke et al., 2008; Xiang et al., 2013). The taxonomy of *Dendrobium* in China has undergone similar revisions. They were grouped into 12 sections with 74 species and two varieties (Ji et al., 1999) and later expanded to 14 sections containing 78 species, with a new establishment of sect. *Holochrysa* Lindl. and sect. *Calcarifera* J. J. Smith. (Zhu et al., 2009). Up to now, the number of species in *Dendrobium* s.l. increased to around 110 (Jin et al., 2019). However, the species delimitations and taxonomy in *Dendrobium* had been still controversial and needed more evidences (Zhu et al., 2009; Xiang et al., 2013; Li, 2023).

Leaf morpho-anatomy in *Dendrobium* varied greatly, which played a key role in species identification and taxonomy (Stern et al., 1994; Morris, 1996; Zhu et al., 2009). The unifacial-leaved species of sect. *Rhizobium* Lindl. constituted a distinctive clade based on leaf anatomy (Stern et al., 1994). Sect. *Aporum* (Bl.) Lindl. and sect. *Rhizobium* of *Dendrobium* should be regarded as sister taxa because of synapomorphies in their leaf morphology and anatomy (Carlsward et al., 1997). Some leaf morpho-anatomic features have been selected as significant roles in taxonomy and phylogeny in *Dendrobium*, such as trichomes, shape and size of epidermal cell, number of epidermal cell of stomata (subsidiary and neighboring), stomatal index, size of stomatal pore, and the thickness of cuticle and cross section of a midrib (Yukawa et al., 1992; Stern et al., 1994; Morris, 1996; Carlsward et al., 1997; Pradhan and Bajracharya, 2018, 2020). In addition, leaf anatomic features of *Dendrobium* could be related with potential adaption to water storage and drought (Sun et al., 2014; Metusala et al., 2017; Yang et al., 2016; Qi et al., 2020).

There were about 44 species of *Dendrobium* sect. *Stachyobium* Lindl., distributed in mountainous regions in mainland Asia (Prommanut, 2017). The monophyly of sect. *Stachyobium* was strongly supported based on five DNA markers (Xiang et al., 2013), which were characterized well by its tufted growth habit, fleshy stems enclosed by leaf sheath, lip trilobed, column foot well-developed, and small pollinia covered by a shallow helmet-shaped anther cap (Zhu et al., 2009; Wang et al., 2021a; Wang et al., 2021b). Five species of sect. *Stachyobium* were found in China (Zhu et al., 2009), including *Dendrobium compactum* Rolfe. ex W. Hackett, *D. monticola* P. F. Hunt et Summerh, *D. porphyrochilum* Lindl., *D. strongylanthum* Rchb. F., and a Chinese endemic species of *D. sinominutiflorum* S. C. Chen. The last one is only found in Southern Yunnan, China (Zhu et al., 2009). Most of them have been mistaken or misused as ornamental or medicinal resources in Chinese market due to their similar morphology and problems of identification (Chen et al., 1999). Only certain members of sect. *Stachyobium* have been investigated or mentioned anatomically, these usually as part of broad investigations of particular features rather than as comprehensive studies of specific taxa (Morris et al.,1996; Pradhan and Bajracharya, 2018; Prommanut et al., 2018). There is no systematically based body of information on sect. *Stachyobium*.

Therefore, leaf morpho-anatomic investigation of five sect. *Stachyobium* species from China was carried out and aimed to: (1) observe leaf morpho-anatomic features of these five species in detail; (2) select some taxonomic traits for species identification based on a comparative leaf morpho-anatomy; (3) provide a better understanding of taxonomy of *Dendrobium* based on leaf morpho-anatomic data.

2. Materials and methods

Plants of five *Dendrobium* sect. *Stachyobium* species have been cultivated for many years and bloom in summer and autumn 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″).

The length and width of fully expanded leave were measured in the field with the help of a glass scale ruler and graph paper. All observations of leaf morphological features were taken from three fully expanded leaves from each of ten plants per species as sample. Mature leaves were collected and fixed in FAA (Johasen, 1940) and then preserved in 50% ethanol.

To observe leaf epidermal characteristics, samples were prepared following Sun and Jiang (2009). Five leaves of each species were cut into small fragments approximately 1 cm × 1 cm from an area between the midvein and leaf edge, immersed in 30% H_2O_2—CH_2COOH for 12 - 24 h, and stained with 1% Safranin for 2 - 3 min. Transverse sections of five leaves of each species were investigated for anatomical features. Fragments about 0.5 cm × 0.5 cm from an area between the midvein and edge of leaf were dehydrated using ethanol, embedded in paraffin wax (melting point = 57 °C), and cut at a thickness of 8 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 samples were observed and photographed using a Leica DM 750 microscope. 30 measurements were taken for each documented value of the lengths and widths of leaf ordinary epidermal cells, stomata, and the thickness of leaf by ImageJ 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).

The morphological and anatomical features were statistically analyzed using SPSS 25.0 software, and mean values and standard errors were obtained. To evaluate the significant differences in morpho-anatomical features among five species of sect. *Stachyobium*, 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 morphology

Five *Dendrobium* sect. *Stachyobium* species have simple leaves with parallel venations like other monocots. They showed alternate phyllotaxy and were exstipulate (Fig. 1). Leaf size, leaf shape, and apex of blade were varied from species to species (Table 1, Fig. 1). The smallest leaf occurred in *D. compactum* (1 - 2.5 cm × 0.4 - 0.6 cm, $L \times W$), that was oblong, papery, with apex obtuse and unequally bifid (Fig. 1A). The largest leaf was featured in *D. strongylanthum* (4 - 10 cm × 1.7 cm, $L \times W$), that was long lanceolate (Fig. 1E). The smaller leaves were observed in *D. sinominutiflorum* (1.5 - 5.5 cm × 0.4 - 0.7 cm, $L \times W$) (Fig. 1B), *D. monticola* (2.5 - 4.5 cm × 0.5 - 0.6 cm, $L \times W$) (Fig. 1C), and *D. porphyrochilum* (4.5 cm × 0.6 - 1 cm, $L \times W$) (Fig. 1D). Two types of leaf texture were found: leathery leaves in *D. strongylanthum* and *D. monticola*, and papery leaves in other three species. Two shapes of leaf apex were observed, containing apex obtuse in *D. compactum* and *D. sinominutiflorum*, and acute apex in other three species.

3.2. Leaf surface

Ordinary epidermal cells: Epidermal cells on both leaf surfaces were polygonal, arranged in longitudinal rows parallel to leaf veins (Figs. 2 and 3). The adaxial epidermal cell were conspicuously larger than the abaxial epidermal cell. Anticlinal walls of ordinary epidermal cells were straight-sided and thickened significantly. Two shapes of epidermal cells were recognized (Table 2), consisting of narrow-polygonal cells (1.40 - 2.10 μm, L/W) and broad-polygonal cells (0.90

Fig. 1. Plants of five *Dendrobium* sect. *Stachyobium* species, indicating leaf morphology. **A:** *D. compactum*. **B:** *D. sinominutiflorum*. **C:** *D. monticola*. **D:** *D. porphyrochilum*. **E:** *D. strongylanthum*.

Table 1
Leaf morphology of five *Dendrobium* sect. *Stachyobium* species.

Number	Species	Size ($L \times W$)/cm	Shape	Texture	Apex
1	*D. compactum*	1 - 2.5 × 0.4 - 0.6	Oblong	Papery	Obtuse
2	*D. sinominutiflorum*	1.5 - 5.5 × 0.4 - 0.7	Oblong	Papery	Obtuse
3	*D. monticola*	2.5 - 4.5 × 0.5 - 0.6	Oblong	Leathery	Acute
4	*D. porphyrochilum*	4.5 × 0.6 - 1	Oblong	Papery	Acute
5	*D. strongylanthum*	4 - 10 × 1.7	Long lanceolate	Leathery	Acute

- 1.40 µm, L/W). Accordingly, the narrow-polygonal epidermal cells on both leaf surface were observed in *D. compactum* (Fig. 2A-B and 3A) and *D. sinominutiflorum* (Fig. 2C-D and 3D), while the broad-polygonal epidermal cells on both leaf surface were present in *D. porphyrochilum* (Fig. 2G-H and 3 G), and co-occurrence of both shapes were found in *D. monticola* (Fig. 2E-F and 3F) and *D. strongylanthum* (Fig. 2I-J and 3I).

Stomata: Elliptic stomata was observed only abaxial surface, in which the long axis of the stomatal apparatus parallel to the veins and scattered randomly in five species. Tetracytic stomata was found in *D. compactum* (Fig. 3B), mostly tetracytic with occasionally anomocytic stomata was mixed in *D. sinominutiflorum* (Fig. 3D), *D. monticola* (Fig. 3F), *D. porphyrochilum* (Fig. 3H) and *D. strongylanthum* (Fig. 3J). There were significant differences in stomatal size (mean ± SE of polar axis and equatorial axis. The stomatal index remained around 21, while stomatal density was different between species (Table 3, Fig. 3). The highest stomatal density was found in *D. monticola* (Fig. 3E), and the lowest stomatal density in *D. strongylanthum* (Fig. 3I).

Trichomes: Trichomes occurred on both leaf surfaces, with higher density on the abaxial. (Fig. 2 and 3). The trichomes was peltate, which was swollen and morphologically obvious different from other epidermal cells and stomata (Fig. 2 and 3).

3.3. *Leaf section*

Leaf showed common monocot anatomical features, consisting of cuticle, epidermis, mesophyll, and vascular bundles. Leaf lamina of all five species had a single layer cuticle and an epidermis on both surfaces.

Leaf midrib outline: The leaf midrib outline demonstrated varied shapes in transversal sections, and could be recognized as flat and carinate types. Flat type had an angle of 120° - 180°, found in *D. sinominutiflorum* (Fig. 4A) and *D. monticola* (Fig. 4C). Carinate type possessed an angle of $0^\circ - 120^\circ$, found in *D. compactum* (Fig. 4E), *D. porphyrochilum* (Fig. 4G), and *D. strongylanthum* (Fig. 4I).

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

Trichomes: There were two types of peltate trichome that could be characterized by number of stalk cells. One type of uni-celled stalk was composed by a single celled stalk and a peltate head. The uni-celled stalk trichome was observed and varied in four species, which could be further divided into two subtypes. The apex of stalk cell was peltate-like in *D. sinominutiflorum* (Fig. 5A), *D. monticola* (Fig. 5B), *D. porphyrochilum* (Fig. 5C), while the apex of stalk cell was capitate-like in *D. strongylanthum* (Fig. 5D). On the contrary, the other type of bi-celled stalk was comprised by two-celled stalk with a peltate head, which was observed only in *D. compactum* (Fig. 5E).

Epidermis: Leaf lamina of all five species had a single layer epidermis on both surfaces. All of the species had a uniseriate epidermis composed of square to rectangular ordinary epidermal cells with external periclinal walls thicker than their internal walls. Ordinary epidermal cells of the adaxial surface were conspicuously larger than ordinary epidermal cells of the abaxial surface, up to 1/3 the height of the abaxial epidermal cells in *D. compactum* (Table 4, Fig. 4E), or up to 1/2 the height of the abaxial epidermal cells in the other four species (Table 4, Fig. 4A, C, G, I).

Vascular bundles: The vascular bundles were always arranged in one row across the width of a blade. The midvein vascular bundle was large with smaller vascular bundles to either side. The midvein vascular bundle was partially surrounded by bundle sheath. The amphivasal vascular bundle was observed in *D. sinominutiflorum* (Fig. 4B),

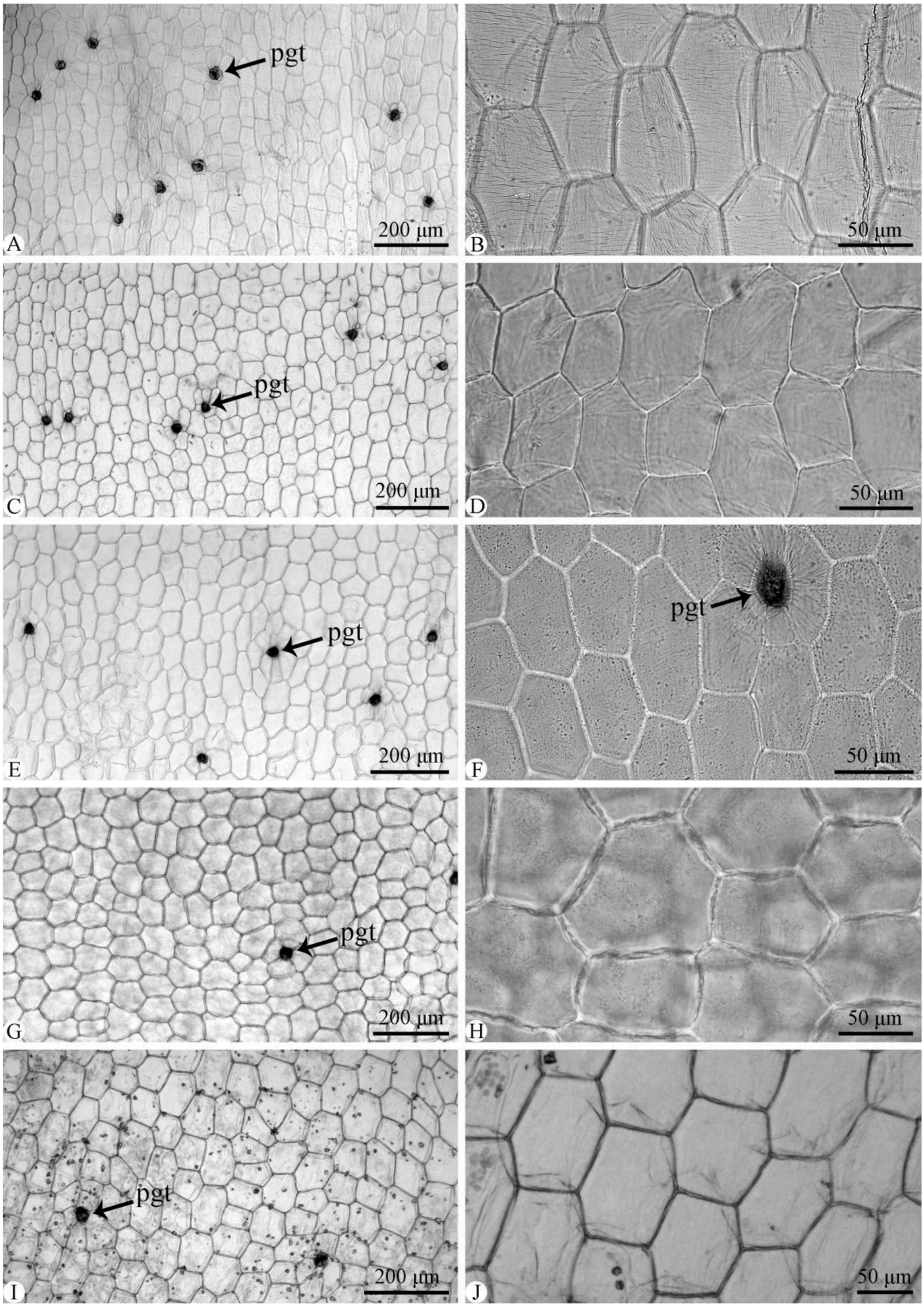

Fig. 2. Micro-morphology of leaf adaxial epidermis of five *Dendrobium* sect. *Stachyobium* species. **A - F:** Narrow polygonal type of ordinary epidermal cells, *D. compactum* (A, B), *D. sinominutiflorum* (C, D), *D. monticola* (E, F). **G - J.** Broad polygonal type of ordinary epidermal cells, *D. porphyrochilum* (G, H), *D. strongylanthum* (I, J). **pgt** = peltate glandular trichome.

D. monticola (Fig. 4D), *D. compactum* (Fig. 4F) and *D. porphyrochilum* (Fig. 4H). However, the vascular bundle has a bicollateral organization in *D. strongylanthum* (Fig. 4J), differing from those of other four species.

Mesophyll: Undifferentiated. The mesophyll was homogeneous, composed of 5 - 8 layers of elliptic and rounded parenchyma cells with varied shapes (Fig. 4).

Crystals: Crystals in idioblasts were observed and varied morphologically in five species. Two types of crystals were characterized, including the spherical silica body crystals in *D. sinominutiflorum* (Fig. 5F) and the prismatic crystals in *D. compactum* (Fig. 5G), *D. monticola* (Fig. 5H), *D. porphyrochilum* (Fig. 5I), *D. strongylanthum* (Fig. 5J).

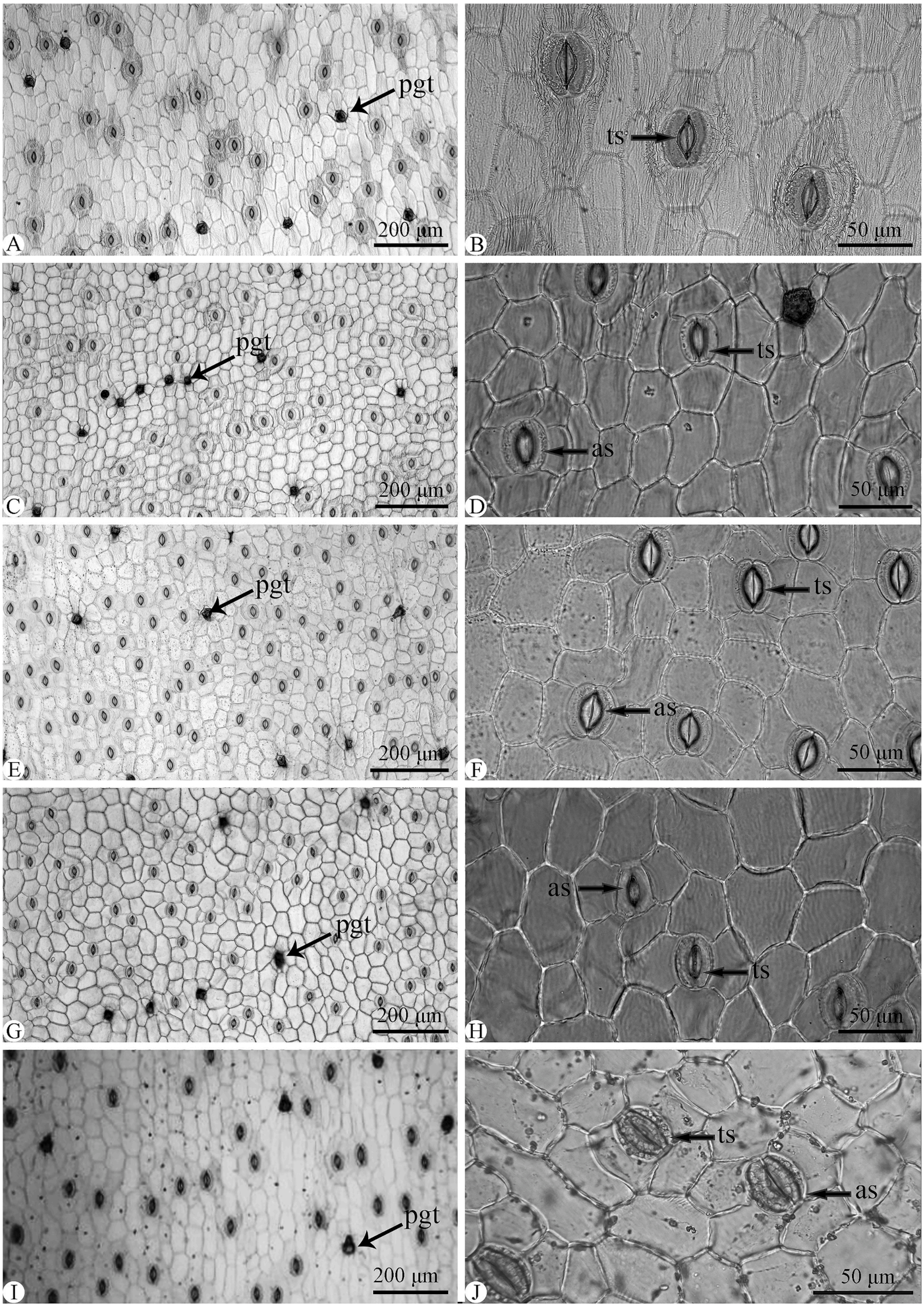

Fig. 3. Micro-morphology of leaf abaxial epidermis of five *Dendrobium* sect. *Stachyobium* species. **A - B:** Tetracytic stomata, *D. compactum*. **C - J:** Mostly tetracytic with few anomocytic stomata, *D. sinominutiflorum* (C, D), *D. monticola* (E, F), *D. porphyrochilum* (G, H) and *D. strongylanthum* (I, J). **as**= anomocytic stomata. **ts** = tetracytic stomata. **pgt** = peltate glandular trichome.

Leaf thickness: Leaf thickness was almost consistent in the five species observed, with minor variations (Table 4). The smaller thickness, less than and around 300 µm, was observed in the papery leaves of *D. compactum, D. sinominutiflorum*, and *D. monticola*. The larger thickness between 320 - 350 µm was found in the leathery leaves of *D. porphyrochilum* and *D. strongylanthum*.

4. Discussion

4.1. Leaf morpholoy

Leaf morphology was used as a key to classification of Chinese *Dendrobium* members, including trichomes, leaf shape, leaf size, and leaf

Table 2
Leaf epidermal features in five *Dendrobium* sect. *Stachyobium* species (Mean ± SE, $n = 30$).

Species	Adaxial epidermis					Abaxial epidermis				
	Ordinary epidermal cells length (μm)	Ordinary epidermal cells width (μm)	L/W	Shape of epidermal cells	Anticlinal wall	Ordinary epidermal cells length (μm)	Ordinary epidermal cells width (μm)	L/W	Shape of epidermal mcells	Anticlinal wall
D. compactum	86.14 ± 2.68^{a}	54.27 ± 1.81^{c}	1.59	Narrow - polygon	Straight - sided	83.49 ± 3.55^{a}	41.21 ± 1.44^{b}	2.03	Narrow - polygon	Straight - sided
D. sinominutiflorum	84.41 ± 2.81^{a}	60.23 ± 1.63^{b}	1.40	Narrow - polygon	Straight - sided	50.84 ± 2.33^{b}	37.32 ± 0.99^{c}	1.36	Narrow - polygon	Straight - sided
D. monticola	87.23 ± 1.78^{a}	52.25 ± 1.32^{c}	1.67	Narrow - polygon	Straight - sided	54.38 ± 1.84^{b}	39.95 ± 1.34^{bc}	1.36	Broad - polygon	Straight - sided
D. porphyrochilum	81.90 ± 2.2^{a}	83.34 ± 2.15^{a}	0.98	Broad - polygon	Straight - sided	58.28 ± 2.34^{b}	45.81 ± 1.07^{a}	1.27	Broad - polygon	Straight - sided
D. strongylanthum	84.02 ± 2.28^{a}	83.01 ± 1.23^{a}	1.01	Broad - polygon	Straight -sided	90.12 ± 2.56^{a}	43.13 ± 1.44^{ab}	2.09	Narrow - polygon	Straight - sided

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

Table 3
Stomatal characteristics and their variations in five *Dendrobium* sect. *Stachyobium* species. (Mean ± SE, $n = 30$).

Species	Stomata size (μm)			Shape	Stomatal index (%) SI	Stomatal density (mm^{-2}) SD	Stomatal type
	Polar axis(P)	Equatorial axis(E)	P/E				
D. compactum	38.58 ± 0.63^{b}	38.05 ± 0.56^{a}	1.01	elliptic	19.40 ± 0.95^{a}	23 ± 1.92^{b}	tetracytic
D. sinominutiflorum	34.03 ± 0.55^{c}	30.05 ± 0.58^{c}	1.13	elliptic	20.79 ± 0.54^{b}	34 ± 1.64^{c}	tetracytic and anomocytic
D. monticola	36.72 ± 0.51^{b}	31.57 ± 0.38^{b}	1.16	elliptic	23.54 ± 0.46^{c}	39 ± 1.30^{d}	tetracytic and anomocytic
D. porphyrochilum	31.79 ± 0.32^{c}	29.97 ± 0.28^{c}	1.06	elliptic	22.61 ± 0.44^{c}	34 ± 0.58^{c}	tetracytic and anomocytic
D. strongylanthum	51.66 ± 0.96^{a}	37.47 ± 0.52^{a}	1.38	elliptic	18.52 ± 0.60^{a}	18 ± 0.38^{a}	tetracytic and anomocytic

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

texture (Zhu et al., 2009; Li, 2023). Sect. *Stachyobium* is strongly supported as monophyletic by plastid and nuclear sequences (Xiang et al., 2013). This section had tufted growth habit, small grassland-like plants with leathery or papery, oblong, dorsiventral, and glabrous leaves from the current year's and usually immature stem (Zhu et al., 2009). The papery, smooth and dorsoventral leaf in sect. *Stachyobium* could be distinguished from those in other *Dendrobium* sections, such as bi-facial leaf with black hairs in sect. *Formosae* (Benth. & Hook. f.) Hook. f., leaf laterally compressed and shortly ensiform in sect. *Aproum*, leaf subulate-cylindric or subcylindric in sect. *Strongyle* Lindl. (Zhu et al., 2009). Moreover, leaf morphology of sect. *Stachyobium* were similar to those of sect. *Grastidium* (Bl.) J. J. Smith, including leathery and grass-like leaves (Zhu et al., 2009). Notably, *D. strongylanthum* were greatly distinct from the rest four species by the largest leaf, which was long lanceolate and leathery.

4.2. Leaf micro-morphology

Five species observed shared some common leaf morpho-anatomical features, including the ordinary epidermal cells with polygonal to irregular and straight-sided anticlinal walls, the parallel arrangement, the adaxial ordinary epidermal cells larger than the abaxial ordinary epidermal cells, the hypodermis absent. These features could be used as diagnostic characters of sect. *Stachyobium* from other *Dendrobium* sections. The polygonal epidermal cells with straight-sided anticlinal walls were different from those in sect. *Formosae* and sect. *Aporum*, which appeared to be irregular cells with curved anticlinal walls (Morris et al., 1996; Sathapatayanon, 2008; Carlward et al.,1997). Hypodermis was absent, like those observed on the leaves of sect. *Monanthos* C. Y. Wu et R. C. Fang and sect. *Grastidium*, but different from those in sect. *Rhizobium* and sect. *Aporum*, because the latter had hypodermis on abaxial surface (Stern et al. 1994; Carlward et al.,1997). The epidermal cell shape was varied between the five species observed here. The narrow-polygonal epidermal cells were observed in *D. compactum* and *D. sinominutiflorum*, while the broad-polygonal type was present in *D. porphyrochilum*, and co-occurrence of both shapes were found in *D. monticola* and *D. strongylanthum*.

The stomata occurred on the abaxial surface, which varied in shape, size and density among the five species. Their stomatal pore was mostly elliptic rather than round, and stomatal type was mostly tetracytic and occasionally anomocytic, which was consistent with other data on representative of sect. *Stachyobium* (Morris et al., 1996; Pradhan and Bajracharya, 2018). Stomata tetracytic occurred only in *D. compactum*, but co-occurrence of tetracytic and anomocytic type was observed in other four species. Meanwhile, *D. strongylanthum* could be discriminated from the rest four species by possessing the lowest stomatal density with the larger stomata.

4.3. Leaf anatomical features

Anatomically, there were some distinct features among five species, including the outline of cross sections of midrib leaf, the thickness of a blade, vascular bundle, trichome type, and crystals.

Two types of leaf midrib outline were observed, which could be used in discrimination of two similar species, such as carinate type in *D. compactum*, but flat type in *D. sinominutiflorum*. These two species often have been mistaken and misused because both have small grass-like plants (Zhu et al., 2009; Jin et al., 2019).

The thickness of the adaxial ordinary epidermal cells and abaxial ordinary epidermal cells could be used to distinguish *D. compactum* from five species of sect. *Stachyobium* observed. The adaxial ordinary epidermal cells up to 1/3 the height of the abaxial ordinary epidermal cells in *D. compactum*, were greatly distinguished from those of the others four species that adaxial ordinary epidermal cells up to 1/2 the height of the abaxial ordinary epidermal cells.

The vascular bundles of the five species investigated here were embedded in the middle of mesophyll tissues, with primary xylem oriented toward leaf axis, which conformed to the relevant data (Morris et al., 1996; Pradhan and Bajracharya, 2020). Vascular bundles arrangement in one row in the mesophyll in five sect. *Stachyobium* species observed here, could be distinguished from those of sect. *Aporum* and sect. *Rhizobium*, that were symmetrically arranged within the

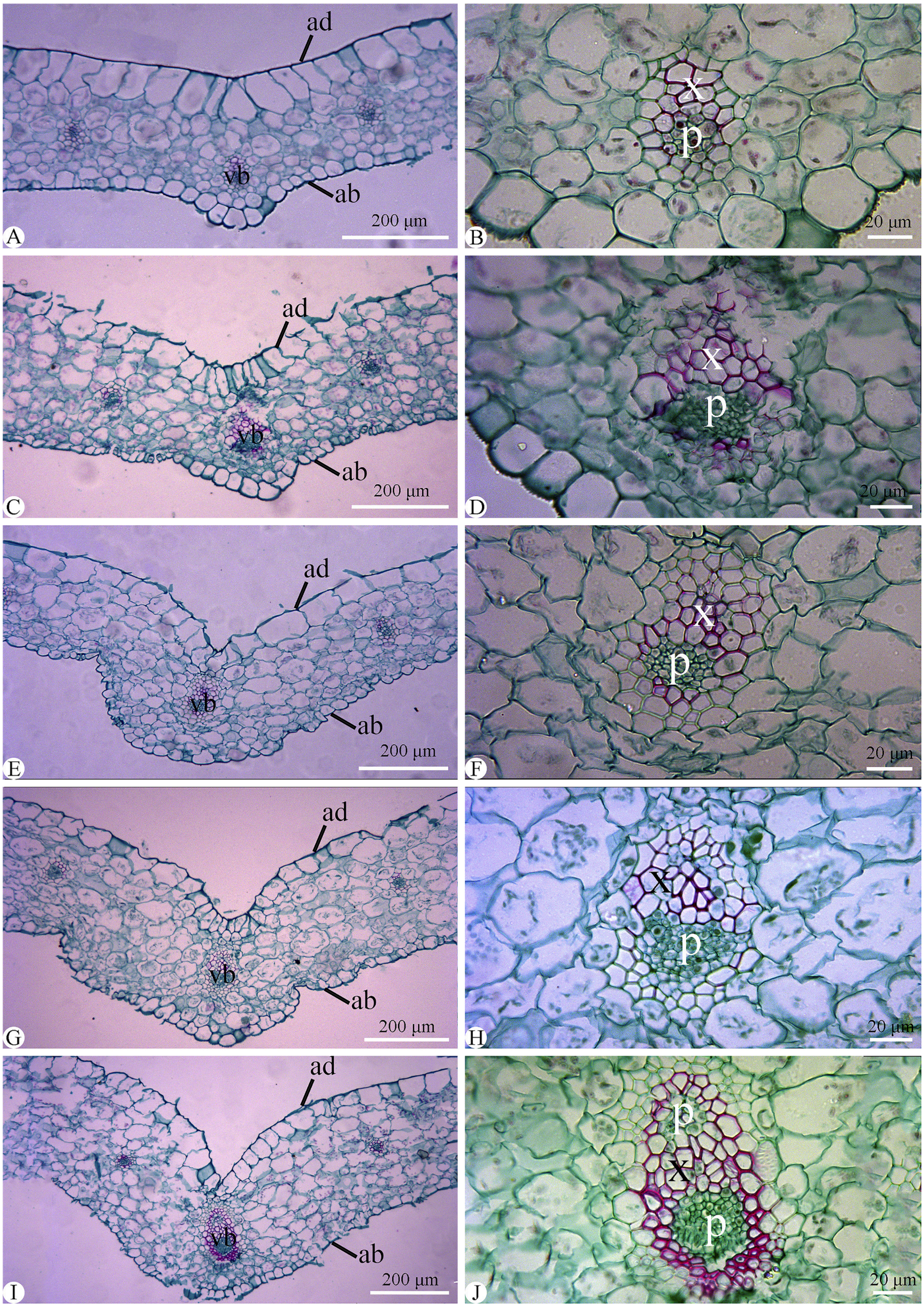

Fig. 4. Transverse section of a leaf midrib in five *Dendrobium* sect. *Stachyobium* species. **A - B:** *D. sinominutiflorum*. **C - D:** *D. monticola*. **E - E:** *D. compactum*. **G - H:** *D. porphyrochilum*. **I - J:** *D. strongylanthum*. **ab** = abaxial surface. **ad** = adaxial surface. **p** = phloem. **vb** = vascular bundle. **x** = xylem.

mesophyll (Carlsward et al., 1997). In present study, arrangement of xylem and phloem could be used for distinction of *D. strongylanthum* from other four species. The vascular bundle possessed a bicollateral organization in *D. strongylanthum*, but the amphivasal vascular bundle in other four species.

Peltate trichomes occurred on both sides of a blade in five species, with higher density on the abaxial surface, which was observed also in certain members of sect. *Stachyobium* (Morris et al., 1996; Prommanut et al., 2018). Peltate trichome was featured by a bi-celled stalk in *D. compactum*, but peltate trichome with a uni-celled stalk was characterized in the rest four species. Moreover, the apex of stalk cell was capitate-like in *D. strongylanthum*, but peltate-like in the rest four members. Peltate trichomes were ever investigated in detail by microscope and SEM (scanning electronic microscope) and varied in two sect.

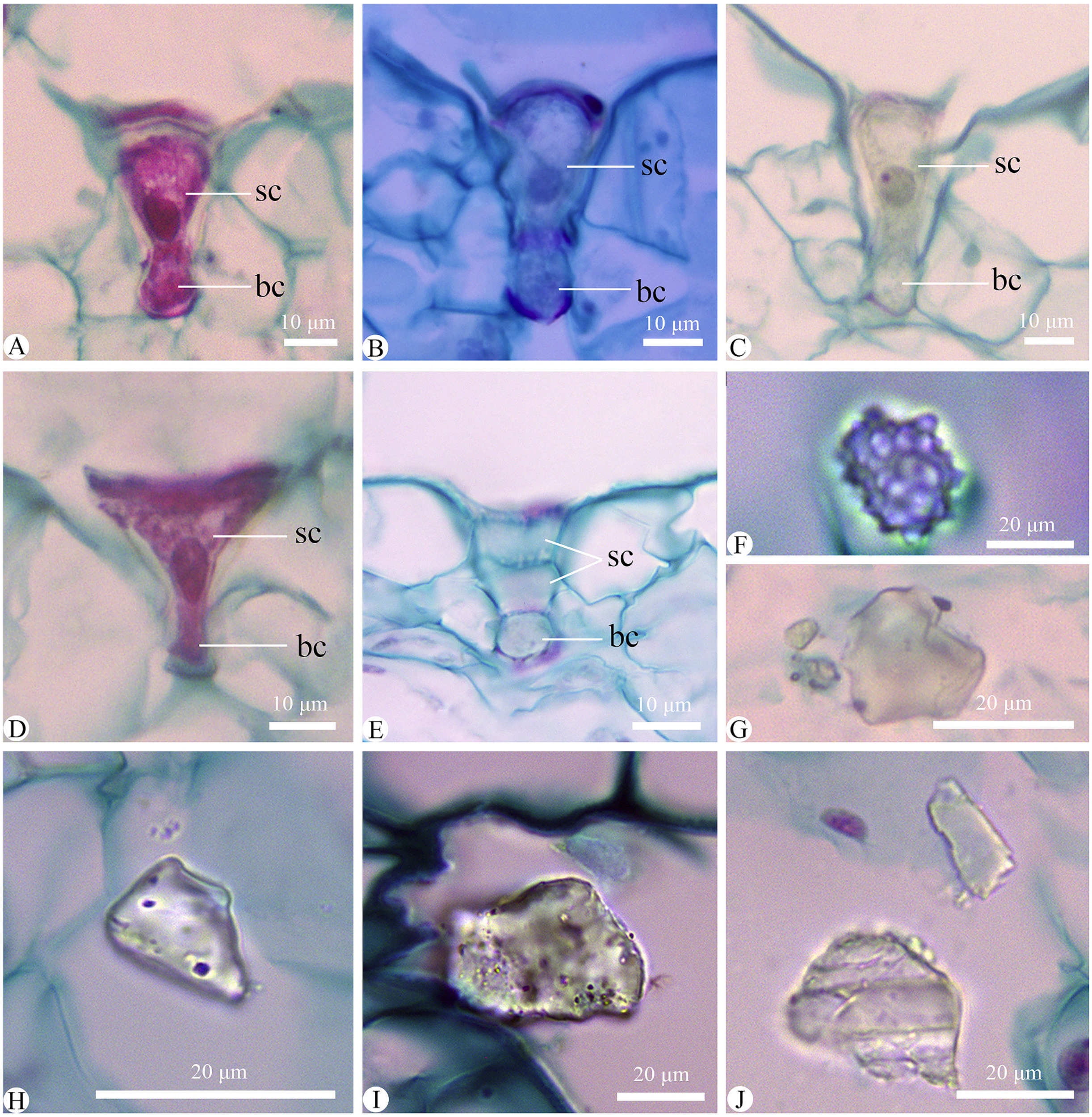

Fig. 5. Two types of peltate trichomes and crystals observed in five *Dendrobium* sect. *Stachyobium* species. **A – D:** Uni-celled stalk type, indicating the apex of stalk cell was peltate-like in *D. sinominutiflorum* (A), *D. monticola* (B), *D. porphyrochilum* (C) and the apex of stalk cell was capitate-like in *D. strongylanthum* (D). **E:** Bi-celled stalk type in *D. compactum*. **F - J:** Two types of crystals, spherical silica body crystals in *D. sinominutiflorum* (F) and prismatic crystals in *D. compactum* (G), *D. monticola* (H), *D. porphyrochilum* (I), and *D. strongylanthum* (J). **bc** = basal cell. **sc** = stalk cell.

Table 4
Leaf anatomic features of five *Dendrobium* sect. *Stachyobium* species. (Mean ± SE, $n = 30$).

Spaces	TL/μm	TDE/μm	TBE/μm	TUE/TLE	Types of trichomes	Type of crystals
D. compactum	273.22 ± 2.38^{d}	54.44 ± 1.19^{b}	20.14 ± 0.57^{d}	2.70	Uni-celled stalk cell	prismatic
D. sinominutiflorum	280.35 ± 0.87^{d}	73.36 ± 1.27^{a}	30.66 ± 1.57^{b}	2.39	Bi-celled stalk cell	spherical silica body
D. monticola	300.55 ± 2.61^{c}	55.23 ± 1.65^{b}	34.24 ± 1.35^{a}	1.61	Bi-celled stalk cell	prismatic
D. porphyrochilum	324.22 ± 6.08^{b}	47.65 ± 2.12^{ac}	29.55 ± 1.61^{b}	1.61	Bi-celled stalk cell	prismatic
D. strongylanthum	346.66 ± 3.03^{a}	52.39 ± 3.84^{bc}	23.96 ± 0.89^{c}	2.18	Bi-celled stalk cell	prismatic

Note: TL. Thickness of leaf blade; TDE. Thickness of adaxial epidermal cells; TBE. Thickness of abaxial epidermal cells.
Different letters in the same column indicating statistical difference $P < 0.05$ (ANOVA).

Stachyobium species, which was useful in species delimitation (Prommanut et al., 2018). Two types of peltate trichome were described, including one composed by a swollen, smooth head and a muti-celled stalk, and the other by a flat and rough head and a single-celled stalk. These two types of peltate trichomes could distinguish *D. obchantiae* Promm., Suddee & Kidyoo from *D. incurvum* Lindl. (Prommanut et al., 2018). Therefore, anatomic and ultrastructure data on peltate trichomes sect. *Stachyobium* needed more investigation for further taxonomic significance.

Two types of crystals were observed in the five species investigated here, including the spherical silica body in *D. sinominutiflorum* and prismatic crystal in the rest four species. The calcium oxalate crystals were widely spread in monocotyledons, but silicate crystals occasionally presented (Prychid, 1999). The crystals were mostly raphide bundles in leaf of Orchidacaeae (Holtzmeier et al., 1998; Şenel et al., 2019). Raphides crystal were observed in *Dendrobium* sect *Rhizobium* and sect *Aporum*, and druse-like structures also were found in *D. aloifolium (Blume) Rchb. f.* (Stern et al., 1994; Carlsward et al., 1997). In this study, prismatic crystals were observed in the four species, which was different from raphides in sect *Rhizobium* and druse-like structure in sect *Aporum* (*D. aloifolium*). In addition, the spherical silica body crystals were only observed in *D. sinominutiflorum*, which was seldom recorded in *Dendrobium*.

4.4. Species identification

For a long time, species delimitation within *Dendrobium* has been based mainly on inflorescence and flower morphology, and variation in anther cap or pollinia features (Chen et al., 1999; Zhu et al., 2009; Wang et al., 2021a, Wang et al., 2021b; Li, 2023). However, leaf morphology was rarely used in species delimitation (Zhu et al., 2009). Some leaf morpho-anatomic features were emphasized for species delimitation of *Dendrobium* from Laos, including shape, reinforcement, arrangement, texture, symmetry, and some anatomic characters (Mahfut et al., 2021). It was proposed that *D. strongylanthum* was well supported within sect. *Stachyobium* by molecular data (Xiang et al., 2013). *D. strongylanthum* was greatly distinguished from the other four species, by possessing the largest size of a leaf and ordinary epidermal cells, broad polygonal ordinary epidermal cells in adaxial epidermis and long polygonal in the abaxial epidermis, the lowest stomatal index and stomatal density, the capitate-like in apex of stalk cell, and a bicollateral organization of vascular bundle. These features were not observed in the other four species.

D. compactum and *D. sinominutiflorum* could be considered as confused species by sharing pale green flower and small and grass-like leaf (Zhu et al., 2009). However, leaf of *D. compactum* was nearly thin and papery, with large stomata, and low stomatal density, while the leaves of *D. sinominutiflorum* were thin and leathery, with small stomata, and high stomatal density. Moreover, the carinate type, two-celled stalk cell, and prismatic crystals were observed in *D. compactum*, the flat type, one-celled stalk cell, and spherical silica body crystals were only found in *D. sinominutiflorum*. The adaxial ordinary epidermal cells in *D. compactum* were found to be up to 1/3 the height of the abaxial cells. However, the adaxial ordinary epidermal cells in *D. strongylanthum* were found to be up to 1/2 the height of the abaxial cells. Therefore, these two species could be clearly distinguished by the stomatal size and density, the leaf midrib outline, and the different thicknesses between the adaxial and abaxial epidermis of leaf.

In summary, sect. *Stachyobium* was strongly supported as a stable monophyly by leaf morpho-anatomical features investigated here. Some morpho-anatomical characters could be selected and used for species discrimination in *Dendrobium* sect. *Stachyobium*, such as stomatal type, leaf midrib outline in transversal section, type of vascular bundles, anatomic type of peltate trichome, and crystals. Accordingly, a taxonomic key to the five species of *Dendrobium* in sect. *Stachyobium* in China was given as below, in order to provide a better understanding of taxonomy of *Dendrobium* based on leaf morpho-anatomical features.

Key to five *Dendrobium* sect. *Stachyobium* species from China based on leaf morpho-anatomic features

1. The size of fully expanded leaves is small (1 - 5.5 cm × 0.4 −0.7 cm, *L* × *W*), and the apex of leaves is obtuse. The narrow-polygonal epidermal cells (1.40 - 2.10 µm, L/W) on both leaf surface.
    2. The leaf midrib outline was carinate (0° - 120°), with tetracytic stomata and low stomatal density (< 25 mm^{-2}). The stalk cell of trichomes had two cells, with prismatic crystals in idioblasts. Ordinary epidermal cells of the adaxial surface conspicuously larger than ordinary epidermal cells of the abaxial surface, up to 1/3 the height of the abaxial epidermal cells..*D. compactum*
    2. The leaf midrib outline was flat (120° - 180°), with tetracytic and anomocytic stomata and higher stomatal density (> 30 mm^{-2}). The stalk cell of trichomes had a single cell, with spherical silica body crystals in idioblasts. Ordinary epidermal cells of the adaxial surface conspicuously larger than ordinary epidermal cells of the abaxial surface, up to 1/3 the height of the abaxial epidermal cells... *D. sinominutiflorum*
1. The size of fully expanded leaves is larger (2.5 - 10 cm × 0.5 −1.7 cm, *L* × *W*), and the apex of leaves is acute. The narrow-polygonal epidermal cells (1.40 - 2.10 µm, L/W) not on both leaf surface.
    3. Stomatal density is lower (< 20 mm^{-2}). The apex of stalk cell was capitate-like and the vascular bundle has a bicollateral organization... .... *D. strongylanthum*
    3. Stomatal density is higher (≥ 20 mm^{-2}). The apex of stalk cell was peltate-like and the organization type of the vascular bundle was amphivasal vascular bundle.
        4. The narrow-polygonal epidermal cells (1.40 - 2.10 µm, L/W) and the broad-polygonal epidermal cells (0.90 - 1.40 µm, L/W) on both leaf surface, and the leaf midrib outline was flat (120° - 180°) .... *D. monticola*
        4. The broad-polygonal epidermal cells (0.90 - 1.40 µm, L/W) on both leaf surface, and the leaf midrib outline was carinate (0° - 120°) .... *D. porphyrochilum*

Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Yunxi Xie: Writing – review & editing, Writing – original draft, Investigation. **Yanping Wang:** Writing – review & editing, Funding acquisition. **Lei Tao:** Writing – review & editing. **Wenhao Liu:** Writing – review & editing. **Yan Luo:** Writing – review & editing. **Lu Li:** 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.

Data availability

No data was used for the research described in the article.

Acknowledgments

This study was supported by the National Nature Science Foundation of China (NSFC 32060049) and by a project from Yunnan Forestry Technological College (KY(QN)202323). We also thank Mr. Zhi-feng Xu and Mrs. Xiao-yun Wang in the Orchid Conservation Center of Yunnan

Fengchunfang Biotechnology Co., Ltd, for providing sample materials. We are grateful to Miss Huimin Liu from Southwest Forestry University for helping with the experiment. This research was carried out with the help from Yunan Academy of Biodiversity, Southwest Foresty University.

References

Adams, P.B., 2011. Systematics of Dendrobiinae (Orchidaceae), with special reference to Australian taxa. Bot. J. Linn. Soc. 166 (2), 105–126. https://doi.org/10.1111/j.1095-8339.2011.01141.x.

Burke, J.M., Bayly, M.J., Adams, P.B., Ladiges, P.Y., 2008. Molecular phylogenetic analysis of *Dendrobium* (Orchidaceae), with emphasis on the Australian section *Dendrocoryne*, and implications for generic classification. Aust. Syst. Bot. 21 (1), 1–14. https://doi.org/10.1071/sb07038.

Carlsward, B.S., Stern, W.L., Judd, W.S., Lucansky, T.W., 1997. Comparative Leaf Anatomy and Systematics in *Dendrobium*, Sections *Aporum* and *Rhizobium* (Orchidaceae). Int. J. Plant Sci. 158, 332–342. https://doi.org/10.1086/297445.

Chen, X.Q., Ji, Z.H., Luo, Y.B., 1999. Chinese Wild Orchid Plant Color Illustration. Science Press, Beijing.

Clements, M., 2003. Molecular phylogenetic systematics in the Dendrobiinae (Orchidaceae), with emphasis on *Dendrobium* section *Pedilonum*. Telopea 10 (1), 247–272. https://doi.org/10.7751/telopea20035619.

Clements, M., 2006. Molecular phylogenetic systematics in Dendrobieae (Orchidaceae). Aliso 22, 465–480. https://doi.org/10.5642/ALISO.20062201.37.

Gerlach, D., 1977. Botanische Mikrotechnik. Georg Thieme, Stuttgart vol. 2.

Holtzmeier, M.A., Stern, W.L., Judd, W.S., 1998. Comparative anatomy and systematics of Senghas's cushion species of *Maxillaria* (Orchidaceae). Bot. J. Linn. Soc. 127, 43–82. https://doi.org/10.1111/j.1095-8339.1998.tb02087.x.

Johansen, D.A., 1940. Plant Microtechnique. McGraw Hill, New York.

Ji, Z.H., Chen, X.Q., Luo, Y.B., 1999. Flora Reipublicae Popularis Sinicae: *Dendrobium*. Science Press, Beijing, pp. 67–146 (vol 19).

Jin, X.H., Li, J.W., Ye, D.P., 2019. Primary Color Map of Wild Orchids in China. Henan Science and Technology Press, Zhengzhou, pp. 492–594.

Kranzlin, F., 1910. Orchidaceae-Monandrae-Dendrobiinae. Verlag von Wilhelm Engelmann, Leipzig, pp. 210–219.

Li, L., 2023. Floral Morphology of *Dendrobium* in China. Yunnan Science and Technology Press, Kunming.

Lindley, J., Paxton, J., 1851. The transparent dendrobium. Paxton's Flower. Garden 1, 133–136.

Mahfut, M., Handayani, T.T., Wahyuningsih, S., Sukimin, S., 2021. Identification of *Dendrobium* natural orchids in Liwa Botanical Garden based on leaf morphological character. Jurnal Pemberdayaan 5 (1), 38–44. https://doi.org/10.12928/JP.V5I1.2938.

Metusala, D., Supriatna, J., Sopandie, D., 2017. Comparative leaf and root anatomy of two *Dendrobium* species (Orchidaceae) from different habitat in relation to their potential adaptation to drought. In: AIP Conf. Proc., 1862, 030118. https://doi.org/10.1063/1.4991222.

Morris, M.W., Stern, W.L., Judd, W.S., 1996. Vegetative anatomy and systematics of subtribe Dendrobiinae (Orchidaceae). Bot. J. Linn. Soc. 120 (2), 89–144. https://doi.org/10.1111/J.1095-8339.1996.TB00483.X.

National Forestry and Grassland Administration, 2021. List of National Key Protected Wild Plants in China. https://www.forestry.gov.cn/c/www/lczc/10746.jhtml (Accessed 3 November 2024).

Patel, J.D., 1979. New morphological classification of stomatal complexes. Phytomorphology 29 (3–4), 218–229.

Pradhan, B.M., Bajracharya, D.M., 2018. Taxonomic significance of stomatal complex in fifteen species of *Dendrobium* Swartz (Orchidaceae) of Nepal. J. Nat. Hist. Mus. 30, 28–38. https://doi.org/10.3126/jnhm.v30i0.27400.

Pradhan, B.M., Bajracharya, D.M., 2020. Anatomical study of *Dendrobium* (Orchidaceae) of Nepal. Ann. Plant Sci. 9 (7), 3916–3948. https://doi.org/10.21746/aps.2020.9.7.1.

Prommanut, P., 2017. Taxonomic Revision of *Dendrobium* Sw. Section *Stachyobium* Lindl. (Orchidaceae) in Thailand. Chulalongkorn University, Bangkok, p. 11. https://doi.org/10.58837/chula.the.2017.57.

Prommanut, P., Suddee, S., Kidyoo, M., 2018. A narrow endemic new species of *Dendrobium* sect. *Stachyobium* from Thailand (Orchidaceae: Malaxideae). Phytotaxa 348 (2), 090–098. https://doi.org/10.11646/PHYTOTAXA.348.2.3.

Prychid, C., 1999. Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. Ann. Bot. 84, 725–739. https://doi.org/10.1006/anbo.1999.0975.

Qi, Y., Huang, J., Zhang, S., 2020. Correlated evolution of leaf and root anatomic traits in *Dendrobium* (Orchidaceae). AoB Plants 12 (4), 1–13. https://doi.org/10.1093/aobpla/plaa034.

Sathapatayanon, A., 2008. Taxonomic Revision of Orchids in the Genus *Dendrobium* Sw. Section *Formosae* (Benth. and Hook. f.) Hook. f. (Orchidaceae) in Thailand and Adjacent Areas. Chulalongkorn University, Bangkok, pp. 29–43.

Schlechter, R., 1982. The Orchidaceae of German New Guinea. The Australian Orchid Foundation, Melbourne, p. 1180.

Schuiteman, A., Adams, P.B., 2014. Genera Orchidacearum 6, Epidendroideae 3. Oxford University Press, Cambridge, pp. 89–91.

Şenel, G., Akbulut, M.K., Süngü Şeker, Ş., 2019. Comparative anatomical properties of some Epidendroideae and Orchidoideae species distributed in NE Turkey. Protoplasma 256, 655–668. https://doi.org/10.1007/s00709-018-1326-x.

Stern, W.L., Morris, M.W., Judd, W.S., 1994. Anatomy of the thick leaves in *Dendrobium* section *Rhizobium* (Orchidaceae). Int. J. Plant Sci. 155, 716–729. https://doi.org/10.1086/297210.

Sun, M., Yang, S.J., Zhang, J.L., Bartlett, M., Zhang, S.B., 2014. Correlated evolution in traits influencing leaf water balance in *Dendrobium* (Orchidaceae). Plant Ecol. 215 (11), 1255–1267. https://doi.org/10.1007/s11258-014-0383-2.

Sun, T.X., Jiang, X.S., 2009. H_2O_2-CH_2COOH maceration, a simple and perfect method for leaf epidermis. Guihaia 29 (1), 44–47.

Swartz, O., 1799. Dendrobium. Nova Acta Regiae Soc. Sci. Upsaliensis Series 2 (6), 82–85.

Wang, Y.P., LI, L., Yang, C.X., Luo, Y., Li, Q.Q., 2021a. Pollinia morphology of 14 species in *Dendrobium* (Orchidaceae) and taxonomic significance. Bull. Bot. Res. 41 (01), 12–25. https://doi.org/10.7525/j.issn.1673-5102.2021.01.003.

Wang, Y.P., Li, C.R., Luo, Y., Li, Q.Q., LI, L., Wang, X.X., 2021b. Preliminary study on morphology and taxonomic significance of anther cap in nine *Dendrobium* species (Orchidaceae) from China. Plant Sci. 39 (04), 367–378. https://doi.org/10.11913/PSJ.2095-0837.2021.40367.

Xiang, X.G., Schuiteman, A., Li, D.Z., Huang, W.C., Chung, S.W., Li, J.W., Zhou, H.L., Jin, W.T., Lai, Y.J., Li, Z.Y., Jin, X.H., 2013. Molecular systematics of *Dendrobium* (Orchidaceae, Dendrobieae) from mainland Asia based on plastid and nuclear sequences. Mol. Phylogenet. 69, 950–960. https://doi.org/10.1016/j.ympev.2013.06.009.

Yang, S.J., Sun, M., Yang, Q.Y., Ma, R.Y., Zhang, J.L., Zhang, S.B., 2016. Two strategies by epiphytic orchids for maintaining water balance: thick cuticles in leaves and water storage in pseudobulbs. AoB Plants 8, 1–11. https://doi.org/10.1093/aobpla/plw046.

Yukawa, T., Ando, T., Karasawa, K., Hashimoto, K., 1992. Existence of two stomatal shapes in the genus *Dendrobium* (Orchidaceae) and its systematic significance. Am. J. Bot. 79, 946–952. https://doi.org/10.1002/J.1537-2197.1992.TB13678.X.

Zhu, G.H., Ji, Z.H., Wood, J.J., 2009. Flora of China: *Dendrobium*. Science Press, Beijing, pp. 367–397 (Vol. 25).