

## Leaf Morphology of Five Hypoxidaceae Species in China and Its Taxonomic Significance: Postprint

**Authors:** Tian Qin, Duan Hanning, Wang Yunqiang, Li Haitao, Li Lu

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### Abstract

To clarify the taxonomic issues concerning intergeneric systematic relationships in the Hypoxidaceae family, this study selected five species from three genera of Chinese Hypoxidaceae as research subjects. Using light microscopy, scanning electron microscopy, and paraffin sectioning techniques, we observed their leaf morphology, leaf epidermal microcharacteristics, and leaf anatomical features. The results are as follows: (1) Leaf morphology exhibits three types: small flat leaves, medium undulate leaves, and large plicate leaves. (2) Leaf epidermal trichomes are unicellular and uniseriate, and can be classified into three types: long pubescent hairs, strigose hairs, and stellate pubescent hairs. (3) Leaf epidermal cells are hexagonal and pentagonal. Stomata are paracytic and elliptical, with an inverse correlation between stomatal size and density. (4) Wax ornamentation comprises four categories: smooth, granular, scaly, and crustose. (5) Leaf midrib cross-sections are of two types: flat and keeled. Vascular bundles are circular and oblong. Leaf epidermal thickness correlates with epidermal cells. Comparative analysis of the leaf morphological and anatomical characteristics of the five species suggests that some character combinations are helpful for understanding intergeneric phylogenetic relationships, while other characters are suitable for interspecific identification.

### Full Text

## Leaf Morphology and Its Taxonomic Significance of Five Species in Hypoxidaceae from China

**Qin Tian<sup>1,2</sup>, Hanning Duan<sup>1</sup>, Yunqiang Wang<sup>3</sup>, Haitao Li<sup>3</sup>, Lu Li<sup>1\*</sup>**

<sup>1</sup>College of Biodiversity Conservation, Southwest Forestry University, Kunming 650224, Yunnan, China

<sup>2</sup>College of Forestry, Southwest Forestry University, Kunming 650224, Yunnan, China

<sup>3</sup>Yunnan Key Laboratory of Sustainable Utilization of Southern Medicinal Resources, Yunnan Branch Institute of Medicinal Plants, Chinese Academy of Medical Sciences, Jinghong 666100, Yunnan, China

## Abstract

To clarify taxonomic issues concerning intergeneric relationships within Hypoxidaceae, we investigated leaf morphology, epidermal microstructure, and anatomical characteristics of five species representing three genera from China using light microscopy, scanning electron microscopy (SEM), and paraffin sectioning. The results revealed: (1) Three distinct leaf morphological types: small-even leaves, medium-undulate leaves, and large-plicate leaves. (2) Trichomes were unicellular and uniseriate, classifiable into three categories: pilose, strigose, and stellate-pubescent. (3) Epidermal cells were hexagonal or pentagonal; stomata were paracytic and elliptical, with an inverse relationship between size and density. (4) Four types of epicuticular wax ornamentation were identified: smooth, granular, scale-like, and crust-like. (5) Midrib cross-sections exhibited either even or carinate profiles, with vascular bundles being either circular or oblong. Leaf epidermal thickness correlated with epidermal cell size. Comparative analysis of leaf morphology and anatomical features among the five species suggests that certain character combinations aid in understanding intergeneric relationships, while other features are applicable for species-level identification.

**Keywords:** Curculigo, Hypoxis, leaf morphology, leaf anatomy, Molineria, taxonomy

## Introduction

Hypoxidaceae comprises approximately 10 genera and 200 species, primarily distributed in the Southern Hemisphere, with some representatives in the Northern Hemisphere, forming two distribution centers in southern Africa and the Indian Ocean region. The family is placed within Asparagales and is considered related to Orchidaceae (Kocyan et al., 2011; APG, 2016). Members of this family are perennial herbs with typical taxonomic features including: (1) basal leaves with persistent leaf bases, petiolate or sessile; lanceolate blades with conspicuous plicate venation and non-glandular integument-type indumentum; (2) solitary or racemose/spicate inflorescences, sometimes with drastically shortened axes forming capitate or corymbose arrangements; actinomorphic flowers with six tepals in two whorls, the outer whorl smaller and dorsally green, the inner whorl larger, petaloid, and golden-yellow; inferior ovary typically with a beak at the apex; and (3) capsular or baccate fruits.

In China, Hypoxidaceae includes approximately 4 genera and 10 species (Liu et al., 2012; Wang et al., 2019): *Hypoxis* L., *Curculigo* Gaertn., *Molineria* Colla., and *Sinocurculigo* Z. J. Liu, L. J. Chen & K. Wei Liu (Liu et al., 2012). *Hypoxis* contains about 100 species widely distributed in tropical regions, with only one species in China—*Hypoxis aurea*—a dwarf perennial herb occurring in southern

regions. *Curculigo* comprises approximately 20 species distributed globally in tropical and subtropical areas, with two species in China: *Curculigo glabrescens* and *C. orchioides*, found in southern and southwestern China. *Molineria* contains about 10 species in tropical regions, with six species in China: *Molineria capitulata*, *M. breviscapa*, *M. crassifolia*, *M. gracilis*, *M. sinensis*, and *M. prainiana* (Wang et al., 2019). *Sinocurculigo* is a monotypic genus established based on morphological characteristics including absence of longitudinal seed ridges, hairy stigma, and parietal placentation, combined with molecular evidence, containing only *Sinocurculigo taishanica* from Taishan City, Guangdong Province (Liu et al., 2012).

Intergeneric relationships within Hypoxidaceae have long been complex and require clarification (Kocyan et al., 2011). A prominent taxonomic issue concerns the unclear boundary between *Curculigo* and *Molineria*. In traditional classifications, members of the latter have often been placed within *Curculigo* (Qian, 1985; Ji & Merrow, 2000). Molecular systematics also suggests that species delimitation between these two genera is difficult, with species that should belong to *Curculigo* often clustering with *Molineria*, and vice versa (Kocyan et al., 2011). Although some studies argue that they differ significantly in stem, flower, fruit, and seed characteristics, warranting their separation (Henderson, 1987), key taxonomic characters are unstable and show overlapping variation between genera (Kocyan et al., 2011). Early research distinguished the genera based on presence (*Curculigo*) or absence (*Molineria*) of a fruit beak (Nel, 1914). However, this character proved unstable, as some species placed in *Molineria* also possess fruit beaks (Kocyan et al., 2011). Later scholars proposed a seemingly more stable but less easily observed anatomical character: anther cross-section symmetry, with symmetrical anthers in *Curculigo* and asymmetrical in *Molineria* (Hilliard & Burt, 1978). Subsequent research demonstrated that this character, like the fruit beak, shows both symmetrical and asymmetrical patterns within some *Molineria* species, leading to the reclassification of two *Molineria* species back into *Curculigo* (Kocyan & Endress, 2001b; Kocyan, 2007). Therefore, additional morphological data are needed to differentiate these genera. Currently, the most reliable distinguishing characters are seed beak presence and seed coat ornamentation: *Curculigo* seeds possess a beak (an extension of the funiculus) with striate seed coat patterns, while *Molineria* seeds lack a beak, are smaller, and may or may not have striate patterns (Henderson, 1987; Wiland, 1997; Nordal, 1998; Kocyan et al., 2011).

Furthermore, since both *Curculigo* and *Molineria* are non-monophyletic in molecular phylogenetic trees (Kocyan et al., 2011), and *Sinocurculigo* is also nested within the intermingled clades of these two genera (Liu et al., 2012), the relationships among these three genera require clarification. Additionally, in molecular systematics of Hypoxidaceae (Kocyan et al., 2011), the widely distributed genus *Hypoxis* (approximately 100 species) appears non-monophyletic, with species from different regions falling into different clades. Many species cluster with the African genus *Rhodohypoxis* Nel in the Hypoxis Clade, while two Australian species group with *Pauridia* Harv. and *Saniella* in the Pauridia

Clade.

Hypoxidaceae is a relatively understudied family with limited morphological research, primarily focused on Southern Hemisphere taxa (Shah & Gopal, 1970; Thompson, 1976, 1978, 1979), providing evidence for systematics of related genera and the allied family Asteliaceae (Rudall et al., 1998). The taxonomic value of morphological characters in Hypoxidaceae has been discussed primarily in descriptions of new taxa (Zona et al., 2009; Sanchez-Ken, 2010; Kocyan & Szymanska, 2016; Roy & Vijayan, 2016; Gore & Gaikwad, 2018; Gaikwad, 2019) and taxonomic revisions (Zimudzi, 1994; Snijman & Kocyan, 2013; Snijman, 2014; Tiwari, 2020). Notably, research on Chinese Hypoxidaceae is also limited, comprising mainly new taxon descriptions (Liu et al., 2012; Wang et al., 2019), medicinal plant identification (Li, 2005), seed morphology of five *Curculigo* species (Dong & Zhang, 1998), and leaf morphology of five Amaryllidaceae species (excluding Hypoxidaceae) (Qin et al., 2003). Both *Curculigo* and *Hypoxis* contain rich medicinal chemical compounds and represent traditional medicinal plant resources (Qian, 1985; Perus et al., 2018). Recent pollination biology studies indicate that floral morphological features in *Hypoxis* have important ecological significance for UV reflectance (Klomberg et al., 2019) and evolution of disjunctly distributed species between China and North America (Ren et al., 2019).

Leaf morphological characters hold significant value in plant classification systems, particularly for resolving taxonomically difficult groups such as *Actinidia* and *Clematoclethra* in Actinidiaceae (He et al., 2000; Yang et al., 2020), *Quercus* in Fagaceae (Luo & Zhou, 2001), *Impatiens* in Balsaminaceae (Zhang et al., 2013), and *Rosa* in Rosaceae (Zeng et al., 2017). Addressing the taxonomic problems and limited research foundation in Hypoxidaceae, this study selected five species from three Chinese genera, employing light microscopy, SEM, and paraffin sectioning to compare and analyze leaf morphology, ultrastructural features, and cross-sectional anatomy, providing morphological evidence for understanding intergeneric and interspecific relationships.

## Materials and Methods

**1.1 Materials** Experimental materials of five species from three genera of Hypoxidaceae in China were collected from southern Yunnan Province between 2019 and 2021. Three species (*Hypoxis aurea*, *Curculigo orchioides*, and *Molineria crassifolia*) were collected from Wuliang Mountain, Jingdong County (voucher specimens TQ36, TQ37, TQ38, respectively). The remaining two species (*M. breviscapa* and *M. capitulata*) were collected from Hekou County and Mengla County (Lilu20190004 and Lilu20190003, respectively). Voucher specimens are deposited at the Herbarium of Yunnan Branch Institute of Medicinal Plants, Chinese Academy of Medical Sciences (IMDY).

**1.2.1 Leaf Morphology of Whole Plants** In the field, 5–10 individuals of each species were randomly selected. From each plant, 2–5 mature leaves

were collected, totaling 30 leaves per species. A ruler was used to measure petiole length and maximum values of leaf polar axis (length) and equatorial axis (width). Photographs documented trichome presence, and the number of plicate veins was counted to determine leaf type.

**1.2.2 SEM Observation of Leaf Epidermal Ultrastructure** Naturally air-dried mature leaves were collected from herbarium specimens. Along the midrib, 2 mm × 2 mm sections were cut (four per species). Both abaxial and adaxial surfaces were fixed to sample stages with conductive adhesive, sputter-coated with gold, and observed using a Zeiss Evo LS10 scanning electron microscope (Germany). Epidermal trichomes, epidermal cells, and stomatal inner/outer aperture covers were examined. Terminology and classification of epidermal ultrastructure followed Barthlott et al. (1998) and Wilhelm et al. (2008).

**1.2.3 Light Microscopy of Leaf Epidermal Morphology** Freshly collected mature leaves were fixed in FAA solution (90% ethanol:acetic acid:formalin = 90:5:5) for over 24 hours. The acetic acid-hydrogen peroxide method (Sun & Jiang, 2009) was used for epidermal observation. Sections (1 cm × 1 cm) were cut along the midrib and soaked in a 1:1 mixture of 30% acetic acid and hydrogen peroxide at 60°C for 12–24 hours. After mesophyll cells separated from epidermal cells, mesophyll was gently brushed away with a writing brush, mounted on slides as temporary preparations. Five samples per species were examined under a Leica DM 750 microscope, with five fields of view per sample photographed to document trichome types, epidermal cells, and stomatal types on both surfaces. ImageJ software was used to measure dimensions, with 30 random measurements recorded and averaged. Stomatal index = (stomatal count / (stomatal count + epidermal cell count)) × 100%. Stomatal density = number of stomata per mm<sup>2</sup>.

**1.2.4 Paraffin Sectioning of Leaf Cross-Sectional Anatomy** Five mature leaves fixed in FAA were sampled along the midrib (2 mm × 2 mm). Samples underwent graded ethanol dehydration, xylene clearing, and paraffin embedding. Routine paraffin sections (8 μm thickness) were dried, stained with safranin-fast green, and mounted with Canada balsam. After drying, sections were observed and photographed under a Leica DM 750 microscope. Cross-sectional features were examined from five samples per species, including upper/lower epidermal cells, vascular bundle size, and mesophyll cells. ImageJ software measured leaf thickness, upper/lower epidermal thickness, and vascular bundle dimensions.

## Results

**2.1 Leaf Morphology of Five Hypoxidaceae Species** Leaf size and plicate vein number showed significant correlation with plant size (Table 1, Plate I). Dwarf herbs with small stature produced smaller leaves with fewer veins, such as

*Hypoxis aurea* and *Curculigo orchioidea* (Plate I: 1–6). Conversely, larger plants produced larger leaves with more veins, as seen in the three *Molineria* species (Plate I: 7–15). Second, trichome types were diverse and could be classified into three categories: pilose, strigose, and white tomentose, with significant interspecific differences in density. Pilose trichomes were slender, the longest reaching 5 cm, sparsely distributed along leaf margins and veins on the abaxial surface, occurring in *H. aurea* and *C. orchioidea* (Plate I: 2–6). Strigose trichomes were the shortest, less than 1 mm, sparsely distributed on raised veins of both surfaces, occurring in *M. capitulata* and *M. brevicauda* (Plate I: 7–12). White tomentose trichomes were intermediate in length (2–3 mm), densely covering the abaxial surface as a fluffy layer, occurring only in *M. crassifolia* (Plate I: 15).

Based on plant size, leaf dimensions, plicate vein number, and trichome types, leaf morphology of the five species was categorized into three types: even-small leaves, undulate-medium leaves, and plicate-large leaves.

**Type 1: Even-small leaves**—Petiole indistinct; leaves small, narrow-linear, 8–30 cm long, less than 1 cm wide; even and nearly smooth, without plicate secondary veins, but with conspicuous primary veins; sparse pilose trichomes on margins and primary veins; plant height approximately 10 cm. Observed in *Hypoxis aurea* (Plate I: 1–3), growing in open alpine grasslands.

**Type 2: Undulate-medium leaves**—Distinct petiole less than 10 cm, notably shorter than the blade; leaf blade linear-lanceolate, up to 40 cm long, less than 2 cm wide; blade even and nearly smooth with conspicuous primary veins and 8–12 secondary veins not forming plicate folds; sparse pilose trichomes on margins and midrib; plant height up to 50 cm. Observed in *Curculigo orchioidea* (Plate I: 4–6), distributed in forest understories or forest-margin grasslands.

**Type 3: Plicate-large leaves**—Distinct petiole with grooves, shorter than or equal to the blade, reaching 80 cm; leaf blade broadly lanceolate, up to 150 cm long and 10–30 cm wide; blades with conspicuous plicate venation, prominent primary veins, and 10–20 secondary veins forming plicate ridges; adaxial surface nearly smooth, abaxial surface smooth or tomentose; plant height up to 2 m. Observed in *Molineria capitulata*, *M. brevicauda*, and *M. crassifolia* (Plate I: 7–15), all growing under tropical and subtropical evergreen broad-leaved forests.

**Table 1** Leaf morphology of five species in Hypoxidaceae

Species	Plant Height (cm)	Petiole Length (cm)	Leaf (Length×Width) (cm×cm)	Secondary Veins (Number)	Trichome Type
<i>Hypoxis aurea</i>	< 20	0	(8 <sub>30</sub> )×(0.2–0.8)	0	Pilose, occasionally
<i>Curculigo orchioidea</i>	25–38	< 10	(40 <sub>80</sub> )×(0.8–2.5)	8–12	Pilose, occasionally

Species	Plant Height (cm)	Petiole Length (cm)	Leaf (Length×Width) (cm×cm)	Secondary Veins (Number)	Trichome Type
<i>Molineria capitulata</i>	40~80	50~120	(50 <sub>120</sub> )×(10 <sub>26</sub> )	10~20	Pubescent, occasionally
<i>M. brevica</i>	< 200	50~78	(92 <sub>118</sub> )×(9 <sub>15</sub> )	10~20	Strigose, occasionally
<i>M. crassifolia</i>	40~112	(40 <sub>112</sub> )×(2.5 <sub>8</sub> )	10~16	White tomentose, densely	

**Plate I** Leaf morphology of five species in Hypoxidaceae. 1-3. *Hypoxis aurea*; 4-6. *Curculigo orchiooides*; 7-9. *Molineria capitulata*; 10-12. *M. brevica*; 13-15. *M. crassifolia*. Left: whole plants; Middle: abaxial (upper) and adaxial (lower) leaf surfaces; Right: abaxial surfaces. ab = abaxial epidermis; ad = adaxial epidermis.

**2.2 Microscopic Features of Leaf Epidermal Trichomes** Leaf morphology results showed significant interspecific differences in trichome type and density. To further clarify the taxonomic significance of trichome characters, we examined leaf epidermal trichome micromorphology using light microscopy and SEM.

Under light microscopy, all five species possessed trichomes of varying densities on leaf epidermis, all being unicellular and uniseriate, solitary or clustered, with swollen bases and conspicuous follicles. Four species showed concentrated distribution on leaf margins and abaxial midribs (Plate II: 3, 6, 9, 12), while only *M. crassifolia* had dense coverage on petioles and abaxial surfaces (Plate II: 15). Based on length and morphology, trichomes were classified into three types: pilose, stellate, and strigose. **Type 1: Pilose**—unicellular, uniseriate trichomes 1-2 mm long, slender and linear-tortuous, sparsely appearing on leaf margins and abaxial veins, appearing nearly glabrous; common in *H. aurea*, *C. orchiooides*, and *M. capitulata* (Plate II: 3, 6, 9). **Type 2: Stellate**—forked pilose trichomes arising from the same follicle base, densely covering the abaxial surface and appearing as a thick white tomentose layer under macroscopic observation; observed in *M. crassifolia* (Plate I: 14-15; Plate II: 15). **Type 3: Strigose**—unicellular, uniseriate trichomes less than 20 μm long, relatively robust, tapering from base to apex in a needle-like form, usually solitary or in clusters of two; observed on leaf margins and midribs of *M. brevica* (Plate II: 12). SEM observation focused on ultrastructural features of stellate pilose

trichomes in *M. crassifolia*, revealing their slender, tortuous nature with smooth outer walls and scale-like ornamentation (Plate III: 14).

## 2.3 Leaf Epidermal Ultrastructure of Five Hypoxidaceae Species

Light microscopy and SEM revealed significant interspecific differences in leaf epidermal microstructure (Table 2, Plates II–IV), including: anticlinal walls of epidermal cells were straight-arcuate; epidermal cells were of two types: hexagonal (elongated rectangular) and pentagonal (short rectangular); stomata were paracytic and elliptical, with slight interspecific variation in size, stomatal index, and density.

**2.3.1 Epidermal Cell Characteristics** Under both microscopes, epidermal cell morphology and size were generally consistent between adaxial and abaxial surfaces within species but differed significantly among species. Anticlinal wall patterns were consistently straight-arcuate (Table 2, Plates II–IV). Based on cell size and outline, two types were recognized: **Hexagonal**—flat, elongated hexagonal cells forming long rectangles; larger cells averaging  $89.30\text{ }\mu\text{m} \times 32.63\text{ }\mu\text{m}$  with a length-width ratio of 2.70; observed in *H. aurea* and *C. orchioides* (Plate II: 1–2, 4–5; Plate III: 1–6). **Pentagonal**—slightly sunken, short pentagonal cells forming short rectangles; smaller cells averaging  $27.47\text{ }\mu\text{m} \times 18.82\text{ }\mu\text{m}$  with a length-width ratio of 1.26; observed in three *Molineria* species (Plate II: 7–15; Plate III: 7–9). Statistical analysis showed the two cell types differed by more than twofold in size (Table 2).

**2.3.2 Stomatal Types** Stomata were distributed on abaxial surfaces in all species, occasionally on adaxial surfaces, being paracytic and elliptical in outline, with interspecific variation in size, stomatal index, and density (Table 2, Plates II, IV). Stomatal index remained relatively stable across the five species, all below 10 with a mean of 7.78. Two categories were recognized based on size and density: **Large stomata-low density**—stomata averaging  $35.00\text{ }\mu\text{m} \times 28.88\text{ }\mu\text{m}$  with low density (mean 5.56); observed in the two small-statured species with small leaves (*H. aurea* and *C. orchioides*) (Plate II: 1–6; Plate IV: 1–6). **Small stomata-high density**—stomata averaging  $22.94\text{ }\mu\text{m} \times 17.38\text{ }\mu\text{m}$  with high density (mean 20.90); observed in the three *Molineria* species (Plate II: 7–12; Plate IV: 7–13).

Thus, leaf epidermal cell and stomatal features showed clear interspecific variation, allowing classification into two groups: **Group 1**—hexagonal epidermal cells (elongated rectangular), large stomata with low density, observed in the two dwarf species with small leaves (*H. aurea* and *C. orchioides*). **Group 2**—pentagonal epidermal cells (short rectangular), small stomata with high density, observed in the three large-statured *Molineria* species with large leaves.

**2.3.3 Epicuticular Wax Ornamentation** Under SEM at low magnification ( $\times 400$ – $1000$ ), epidermal cell morphology and size and stomatal types were observable, but wax ornamentation was not conspicuous. At high magnification

( $\times 2000$ – $5000$ ), wax features became clearly visible. Wax types and distribution varied considerably between adaxial and abaxial surfaces within and among species (Plates III, IV), with four types identified: smooth, scale-like, crust-like, and granular. **Smooth**—thin, inconspicuous wax layer with smooth cell surfaces and occasional granular debris; observed on adaxial surface of *H. aurea* (Plate III: 1–3). **Scale-like**—irregular flaky protrusions scattered like wax scales, densely distributed on adaxial surface of *C. orchioides* (Plate III: 6) and both surfaces of *M. capitulata* (Plate IV: 5, 6), sparsely distributed on abaxial surface of *M. capitulata* (Plate IV: 7–9). **Crust-like**—irregular crustaceous protrusions of varying thickness; observed on adaxial surfaces of *M. capitulata* and *M. breviscapa* (Plate III: 7–11). **Granular**—circular or square granular protrusions of various sizes, sparsely appearing on adaxial surface of *M. crassifolia* (Plate III: 12) or mixed with other types on abaxial surfaces of all five species (Plate IV).

Comparative analysis revealed that adaxial wax ornamentation was relatively uniform within species and clearly distinguishable among species, with four distinct types (smooth, scale-like, granular, crust-like). For example, the first three types appeared sequentially in *H. aurea* (Plate III: 1–3), *C. orchioides* (Plate III: 6), and *M. crassifolia* (Plate III: 9, 12), while crust-like ornamentation occurred in two *Molineria* species (*M. capitulata* and *M. breviscapa*) (Plate III: 7, 10; 8, 11). However, abaxial wax ornamentation was more complex, representing mixtures of two types that were difficult to differentiate among species. For instance, granular and crust-like waxes were sparsely mixed in *H. aurea* (Plate IV: 1–3), densely mixed in *C. orchioides* (Plate IV: 4–6), and variously combined with scale-like ornamentation in the three *Molineria* species (Plate IV: 7–12). Therefore, adaxial wax ornamentation showed significant interspecific differences and could serve as a diagnostic character, whereas abaxial patterns appeared irregular.

**Table 2** Characteristics of leaf epidermis of five species in Hypoxidaceae

Species	Adaxial Epidermis	Abaxial Epidermis
	Cell Size (L $\times$ W )	Cell Size (L $\times$ W )
<i>Hypoxis aurea</i>	(38.87–105.40) $\times$ 34.23	(43.16–126.52) $\times$ 35.41
<i>Curculigo orchioides</i>	(21.11–114.66) $\times$ 29.38	(24.43–56.42) $\times$ 31.50
<i>Molineria capitulata</i>	(15.84–36.95) $\times$ 18.09	(12.33–21.56) $\times$ 19.55
<i>M. breviscapa</i>	(19.06–32.16) $\times$ 18.85	(14.56–27.21) $\times$ 18.70
<i>M. crassifolia</i>	(14.66–63.19) $\times$ 17.08	(13.25–22.64) $\times$ 19.64

Note: *L* = length of adaxial epidermal cell; *W* = width of adaxial epidermal cell; *L* = length of abaxial epidermal cell; *W* = width of abaxial epidermal cell; *L* = stomatal length; *W* = stomatal width; *SI* = stomatal index; *SD* = stomatal density. Unit: *m*.

**2.4 Leaf Cross-Sectional Anatomy of Five Hypoxidaceae Species** To reveal interspecific differences in leaf cross-sectional anatomy, we examined midrib and lateral vein sections. Results showed that anatomical features were generally similar across species, all composed of three distinct tissues: epidermal layer, mesophyll tissue, and vascular bundles. However, midrib cross-sectional profiles, upper/lower epidermal cell sizes, leaf thickness, mesophyll thickness, and vascular bundle morphology differed significantly among species (Table 3, Plate V).

First, midrib cross-sectional profiles varied slightly among species. Based on the angle formed by leaf cross-sections on both sides of the midrib, two types were recognized: **even** and **carinate**. Even type had angles of 160°–180°, appearing nearly straight; observed in three species across three genera (*H. aurea*, *C. orchioides*, and *M. crassifolia*). Carinate type (V-shaped) had angles of 90°–160°, appearing V-shaped; observed in two *Molineria* species (*M. capitulata* and *M. breviscapa*).

Second, statistical analysis revealed that leaf thickness and mesophyll/midrib thickness showed significant positive correlation but negative correlation with leaf size, allowing clear classification into two groups. **Group 1: Small leaf-thick leaf type** occurred in two species from two genera with dwarf stature and small leaves (*H. aurea* and *C. orchioides*). Their leaf thickness exceeded 120  $\mu\text{m}$  (156.51, 123.13; mean 139.82), mesophyll thickness approximately 80  $\mu\text{m}$  (87.56, 84.73; mean 86.14), and midrib thickness 190  $\mu\text{m}$  (190–320; mean 254.65). **Group 2: Large leaf-thin leaf type** occurred in three *Molineria* species with large stature and leaves (*M. capitulata*, *M. breviscapa*, and *M. crassifolia*). These had leaf thickness around 90  $\mu\text{m}$  (90.65, 92.86, 102.37; mean 95.30), mesophyll thickness around 65  $\mu\text{m}$  (68.54, 61.30, 64.22; mean 64.68), and midrib thickness around 160  $\mu\text{m}$  (160.25, 166.18, 159.56; mean 161.99). Thus, leaf thickness correlated with leaf size.

Third, comparison of cross-sectional profiles and statistical analysis revealed significant differences in upper and lower epidermal cell size and thickness within species, particularly prominent at the midrib (Plate V: 2, 5, 8, 11, 14), allowing classification into two types. **Type 1: Isodiametric epidermal cells**—upper and lower epidermal cells nearly equal in size (ratio 1.10–1.50), observed in two species from two genera: *H. aurea* (1.37) (Plate V: 1–3) and *C. orchioides* (1.11) (Plate V: 4–6). **Type 2: Anisodiametric epidermal cells**—upper epidermal cells more than 1.5 times larger than lower epidermal cells (1.50–3.50), observed in three *Molineria* species: *M. capitulata* (2.72) (Plate V: 7–9), *M. breviscapa* (3.27) (Plate V: 10–12), and *M. crassifolia* (1.54) (Plate V: 13–15).

Finally, vascular bundle morphology differed significantly among species. Based on xylem and phloem outlines, two types were recognized: **Circular vascular bundles**—in midrib cross-sections, phloem concentrated around xylem, isolated from upper/lower epidermal cells by distinct mesophyll tissue, forming a closed ring; observed in three species from two genera (*H. aurea*, *M. capitulata*, and *M. breviscapa*) (Plate V: 2, 8, 11). **Oblong vascular bundles**—in midrib cross-

sections, phloem extending to upper and lower epidermal surfaces, with more cells near the upper epidermis than the lower, forming an irregular shape wider at the top; observed in two species from two genera (*C. orchioides* and *M. crassifolia*) (Plate V: 5, 14).

Thus, leaf cross-sectional anatomy showed significant interspecific variation. Based on midrib profile, leaf thickness, and upper/lower epidermal cell size, two groups were recognized: **Group 1**—even midrib, thick leaves, isodiametric epidermal cells, occurring in two species from two genera (*H. aurea* and *C. orchioides*). **Group 2**—carinate midrib, thin leaves, anisodiametric epidermal cells, occurring in three *Molineria* species (*M. capitulata*, *M. breviscapa*, and *M. crassifolia*).

**Table 3** Leaf anatomy of five species in Hypoxidaceae

Species	TL ( m)	Tm ( m)	TM ( m)	TUE ( m)	TLE ( m)	TUE/TLE
<i>Hypoxis aurea</i>	156.51	87.56	190.00	28.31	20.64	1.37
<i>Curculigo orchioides</i>	123.13	84.73	320.00	25.07	22.64	1.11
<i>M. capitulata</i>	90.65	68.54	160.25	31.72	11.65	2.72
<i>M. breviscapa</i>	92.86	61.30	166.18	19.04	5.82	3.27
<i>M. crassifolia</i>	102.37	64.22	159.56	19.50	12.67	1.54

Note: TL = leaf thickness; TM = midrib thickness; Tm = mesophyll thickness; TUE = upper epidermal thickness; TLE = lower epidermal thickness.

**Plate V** Anatomical features of leaf transection of five Hypoxidaceae species. 1-3. *Hypoxis aurea*; 4-6. *Curculigo orchioides*; 7-9. *Molineria capitulata*; 10-12. *M. breviscapa*; 13-15. *M. crassifolia*. mvb = midrib vascular bundle; vb = vascular bundle of secondary veins.

## Discussion

Addressing the limited research and controversial systematic classification of Hypoxidaceae, this study examined leaf morphology, epidermal microstructure, and cross-sectional anatomy of five species from three Chinese genera using light microscopy, SEM, and paraffin sectioning. We compared intergeneric and interspecific differences in leaf morphological features and evaluated their taxonomic significance, focusing on five main points.

**First**, interspecific differences in leaf morphology can serve as a basis for intergeneric delimitation. Previous studies of three Southern Hemisphere genera (*Spiloxene*, *Empodium*, *Pauridia*) categorized leaf morphology into four types based on leaf form and midrib vascular bundle characteristics: carinate, canaliculate, terete, and plicate (Thompson, 1976). The first three types occur in *Spiloxene* for species identification (Garside, 1942), while the plicate type characterizes *Empodium*. In taxonomic revisions of Hypoxidaceae, *Hypoxis* is typically described as having small, petiole-less, non-plicate leaves (Rudall et al., 1998), while *Curculigo* and *Molineria* are recorded as having large, petiolate, plicate leaves (Zimudzi, 1994; Ji & Mellow, 2000; Wang et al., 2019). Thus, plicate venation characteristics reflected by secondary vein number have taxonomic value. The three leaf types observed in this study can be used for intergeneric classification: *Hypoxis* (*H. aurea*) has even-small leaves; *Curculigo* (*C. orchioides*) has undulate-medium leaves; and *Molineria* (three species) has plicate-large leaves.

**Second**, a combination of five characters—epidermal cell morphology, stomatal type, midrib features, leaf thickness, and epidermal layers—shows distributional patterns at the generic level. This five-character complex can be distinctly divided into two groups occurring in different genera. **Group 1** comprises elongated rectangular (hexagonal) epidermal cells, large-low-density stomata, even midribs, thick leaves, and isodiametric epidermal cells; this combination occurs in two genera with small stature and leaves: *Hypoxis* and *Curculigo*. **Group 2** comprises the opposite states: short rectangular (pentagonal) epidermal cells, small-high-density stomata, carinate midribs, thin leaves, and anisodiametric epidermal cells; this combination characterizes three *Molineria* species. These results support the independence of *Molineria* from *Curculigo* (Henderson, 1987) and suggest a close relationship between *Hypoxis* and *Curculigo*, providing new clues for the hypothesis that *Hypoxis* is polyphyletic (Kocyan, 2011). Indeed, *Hypoxis* exhibits diverse epidermal cell types, predominantly pentagonal and hexagonal, occasionally tetragonal (Wiland-Szymańska, 2009).

**Third**, trichome characters can serve as diagnostic features for Hypoxidaceae species. For example, *H. aurea*, *C. orchioides*, and *M. capitulata* share nearly glabrous leaves with pilose trichomes on margins and abaxial midribs. *M. breviscapa* has nearly glabrous leaves with strigose trichomes sparsely distributed on margins and midribs. Notably, *M. crassifolia* exhibits distinctive dense white tomentose indumentum on the abaxial surface, which under microscopy appears as forked stellate pilose trichomes—a rare and highly diagnostic feature among Chinese Hypoxidaceae species. Importantly, all trichomes observed in the three genera were unicellular and uniseriate, contrasting sharply with the multicellular, multiseriate, forked stellate trichomes commonly reported for Southern Hemisphere Hypoxidaceae (Thompson, 1986; Rudall et al., 1998; Wiland-Szymańska, 2001). These findings provide new data for classification of East Asian Hypoxidaceae. However, *Hypoxis* shows diverse trichome features, including both multicellular-multiseriate pilose and unicellular-uniseriate forked stellate forms (Rudall et al., 1998; Wiland-Szymańska, 2001), warranting further investigation

of trichome taxonomic value.

**Fourth**, epicuticular wax ornamentation on abaxial surfaces was complex, appearing as various combinations of granular, scaly, and crustaceous types that were difficult to differentiate among species. However, adaxial wax ornamentation showed clear interspecific differences and could serve as a species diagnostic: smooth in *H. aurea*, scale-like in *C. orchioides*, granular in *M. capitulata*, and crust-like in *M. breviscapa* and *M. crassifolia*. This suggests a closer relationship between the latter two species compared to the other three.

**Fifth**, vascular bundle morphology in leaf cross-sections (circular vs. oblong) showed clear interspecific differences within genera and could be used for species identification. For example, two *Molineria* species (*M. capitulata* and *M. breviscapa*) have circular vascular bundles, while *M. crassifolia* has oblong bundles. The remaining two species (*H. aurea* and *C. orchioides*) also have oblong bundles. Notably, both types are common within *Hypoxis* (Wiland-Szymańska, 2001).

In summary, leaf morphology and anatomical features of the five Chinese Hypoxidaceae species examined show significant intergeneric and interspecific differences with taxonomic value. The five-character leaf complex (leaf morphology + epidermal cells + stomatal type + leaf thickness + upper/lower epidermal cell thickness) clearly differentiates genera and can be used for intergeneric delimitation. Other characters—including trichome types, epicuticular wax ornamentation, and midrib vascular bundle morphology—show species-specific patterns suitable for species identification. Based on these findings, we present a taxonomic key to the three genera and five species of Chinese Hypoxidaceae based on leaf morphological and anatomical characters.

### Key to Genera and Species of Chinese Hypoxidaceae Based on Leaf Morphology and Anatomy

1. Plants small, height 50 cm; leaves small, maximum width <2.5 cm, length 40 cm; petiole indistinct; margins and midribs with long unicellular trichomes; epidermal cells pentagonal; stomata large, low density; midrib even, leaves thick, upper and lower epidermal cells isodiametric ..... 2
2. Rhizome globose; petiole absent; epidermal cells elongated-elliptic; adaxial wax ornamentation smooth; midrib vascular bundle circular ..... *Hypoxis aurea*
3. Rhizome elongate; distinct petiole; epidermal cells rectangular; adaxial wax ornamentation scale-like; midrib vascular bundle oblong ..... *Curculigo orchioides*
4. Plants large, height up to 200 cm; leaves large, maximum width up to 20 cm, length up to 200 cm; woody petiole nearly equal to blade

length; trichomes diverse; epidermal cells hexagonal; stomata small, high density; midrib carinate, leaves thin, upper and lower epidermal cells anisodiametric .....  
*Molineria* Colla (3)

5. Petiole and leaf blade densely covered with white tomentose indumentum; trichomes forked stellate pilose; adaxial wax ornamentation crust-like; midrib vascular bundle oblong ..... *M. crassifolia*
6. Petiole and leaf blade nearly glabrous; trichomes simple; adaxial wax ornamentation granular or crust-like; midrib vascular bundle circular .....4
7. Scape long, infructescence elevated above ground; margins and abaxial midrib with sparse unicellular pilose trichomes; abaxial wax ornamentation mixed granular and scale-like .....  
*M. capitulata*
8. Scape extremely shortened, infructescence clustered at root; margins and abaxial midrib with sparse unicellular short strigose trichomes; abaxial wax ornamentation sparsely granular .....  
*M. breviscapa*

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