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Comparative anatomical characters of the genus *Aerides* Lour. (Orchidaceae) in Thailand

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Abstract. *Tokaew W, Krubkratoke D, Payakdet S, Phromprasit P. 2023. Comparative anatomical characters of the genus* Aerides *Lour. (Orchidaceae) in Thailand. Biodiversitas 24: 6641-6651.* The genus *Aerides* Lour. belonging to the subfamily Epidendroideae (Orchidaceae), which are ornamental plants in Thailand. The identification of Thai *Aerides* is mostly based on the flower morphology which is a short period. The vegetative parts of *Aerides* species have similar characters. The anatomical data are also helpful for the identification of the *Aerides* species. Therefore, this study aimed to observe the vegetative stages (leaf and root) of seven species of *Aerides* in Thailand. Permanent slides of the leaf epidermis were prepared by epidermal peeling and stained with safranin. Transverse sections of leaf lamina and root were prepared using the paraffin method with a rotary microtome. They were then stained with safranin and fast green, examined using a light microscope, and their anatomical characteristics were described. The results showed that certain anatomical characteristics can be used for species identification, including distribution of stomata, presence of crystals, types of crystals, xylem arches in the root, shapes of the midrib and margin, and presence of starch grains. The key to species of *Aerides* species in Thailand based on anatomy characters is constructed. Anatomical features can be used for taxonomically important data and provide more information on the anatomical properties of Thai *Aerides*.

Keywords: *Aerides*, leaf characteristics, paraffin method, root characteristics, Thailand

INTRODUCTION

Orchidaceae is one of the biggest families of monocot plants (Christenhusz and Byng 2016). It is composed of five subfamilies, namely Apostasioideae, Cypripedioideae, Orchidoideae, Vanilloideae, and Epidendroideae (Chase et al. 2015), 880 genera, and more than 28,000 species in the world (Christenhusz and Byng 2016). The Epidendroideae is the largest subfamily, comprising approximately 21,160 species or about 76% of the family (Govaerts et al. 2023). Distribution of the subfamily Epidendroideae is common in Thailand (Thaitong 1999).

The genus *Aerides* Lour. belongs to the tribe Vandeae, subfamily Epidendroideae (Pridgeon et al. 2014; Chase et al. 2015). The genus comprises 31 species found in tropical and subtropical Asia (Govaerts et al. 2023). The newly described species is known from India, namely *A. agasthiyamalaiana* (Karuppusamy et al. 2022). It has been found in Southeast Asia, including Papua New Guinea, Manipur, Uttarakhand, West Bengal, Arunachal Pradesh, Malaysia, and Indonesia (Paraste et al. 2023). In Thailand, eight native species of *Aerides* have been reported, namely *A. crassifolia*, *A. falcata*, *A. flabellata* (synonym of *Vanda flabellata*), *A. houlletiana*, *A. krabiensis*, *A. multiflora*, *A. odorata*, and *A. rosea* (Thaitong 1999) and *A. lawrenceae* are reported as introduced species in the country (Pooma and Suddee 2014). The genus is rarely terrestrial and is characterized by monopodial epiphytic herbs, ascending stems, alternate leaves, and racemose or rarely paniculate inflorescence. The flower has a forward-facing spur (Sitthisatham 2006). There is variation in shapes, sizes, and colors for potted plants or cut flowers. Interspecific and intraspecific hybridization between *Aerides* spp. and *Rhynchostylis coelestis* was successful for pod formation, seed germination, and development into plantlets (Jitsopakul 2022). *A. odorata* contains a variety of fungi (mycorrhizae and non-mycorrhizae), antimicrobial efficacy, antioxidant activity, natural medicinal molecules, and therapies with anti-cancer properties (Paraste et al. 2023). It has been used by the people of Bangladesh as a medicinal plant (Pant 2013).

Anatomical characteristics of *Aerides* were reported by several authors. Abraham et al. (2016) stated that *Aerides ringens* was often distributed on lower surface with anomocytic stomata with 13.44% stomatal index. Saensouk and Saensouk (2020) recorded the surface of *A. falcata* was square, rectangle to polygonal with 5-7 sides. Raphide crystals were present on the abaxial surface, and the type of stomata was paracytic, only on the abaxial side. The stomata density was 83±6 per mm^2. Various qualitative and quantitative anatomy characteristics from leaf and root serve as important tools in identifying the Orchidaceae. For the leaf, shape of epidermal cell, length of guard cell, trichome type, presence of stomata, density of stomata

(Saensouk and Saensouk 2020), stomata types, type of cell inclusion (Fan et al. 2014; Saensouk and Saensouk 2020), stomata index, and cuticular ornamentation (Fan et al. 2014), hypodermis characteristics were presented and the root anatomy includes the number of xylem arches (Kowsalya et al. 2017), number of velamen layers, shape of velamen cells in cross-section (Andreota et al. 2015) were reported.

Several anatomical research on Orchidaceae have been published, such as Fan et al. (2014), Abraham et al. (2016), Kowsalya et al. (2017), Saensouk and Saensouk (2020), and Muangsan et al. (2022). However, only leaf epidermis of *A. falcata* has been reported in Thailand (Saensouk and Saensouk 2020). Given *Aerides'* wide morphological changes, anatomical data can aid in plant identification. Therefore, this study aimed to observe the vegetative stages (leaf and root) of seven *Aerides* in Thailand and to provide more information on the anatomical characters of Thai *Aerides*.

MATERIALS AND METHODS

Plant materials

Seven *Aerides* species in Thailand were investigated. The specimens were obtained from the collection of *Aerides* at the Korat Orchid Society, Thongchai Nuea Subdistrict, Pak Thong Chai District, Nakhon Ratchasima Province, Thailand (14°46′38.7″ N 102°00′30.2″ E). The living samples were collected from Loei, Mukdahan, Ubon Ratchathani, and Nakhon Ratchasima provinces. The materials from leaves and roots were fixed in FAA 70. Then, the identification of the species followed Seidenfaden (1988). Voucher specimens were deposited in the Biology Program, at Nakhon Ratchasima Rajabhat University, Thailand. The source of *Aerides* species and vouchers are shown in Table 1 and Figure 1.

Table 1. *Aerides* species in Thailand used in the study

Species	Local name	Locality	Habitat	Collector number
A. crassifolia C.S.P.Parish ex Burb.	Ueang kulap daeng	Loei	Mi	D. Krubkratoke 01
A. falcata Lindl. & Paxton	Ueang kulap krapao poet	Mukdahan	Mi	D. Krubkratoke 02
A. houlletiana Rchb.f.	Ueang kulap khorat	Nakhon Ratchasima	Ev	D. Krubkratoke 03
A. krabiensis Seidenf.	Ueang kulap phuang chomphu	Loei	Ev	D. Krubkratoke 04
A. multiflora Roxb.	Ueang phuang malai	Ubon Ratchathani	Mi	D. Krubkratoke 05
A. odorata Lour.	Ueang kulap krapaopit	Mukdahan	Mi	D. Krubkratoke 06
A. rosea Lodd. ex Lindl. & Paxton	Ueang kulap aiyarawan	Loei	Ev	D. Krubkratoke 07

Note: Mi: mixed deciduous forest, Ev: evergreen forest

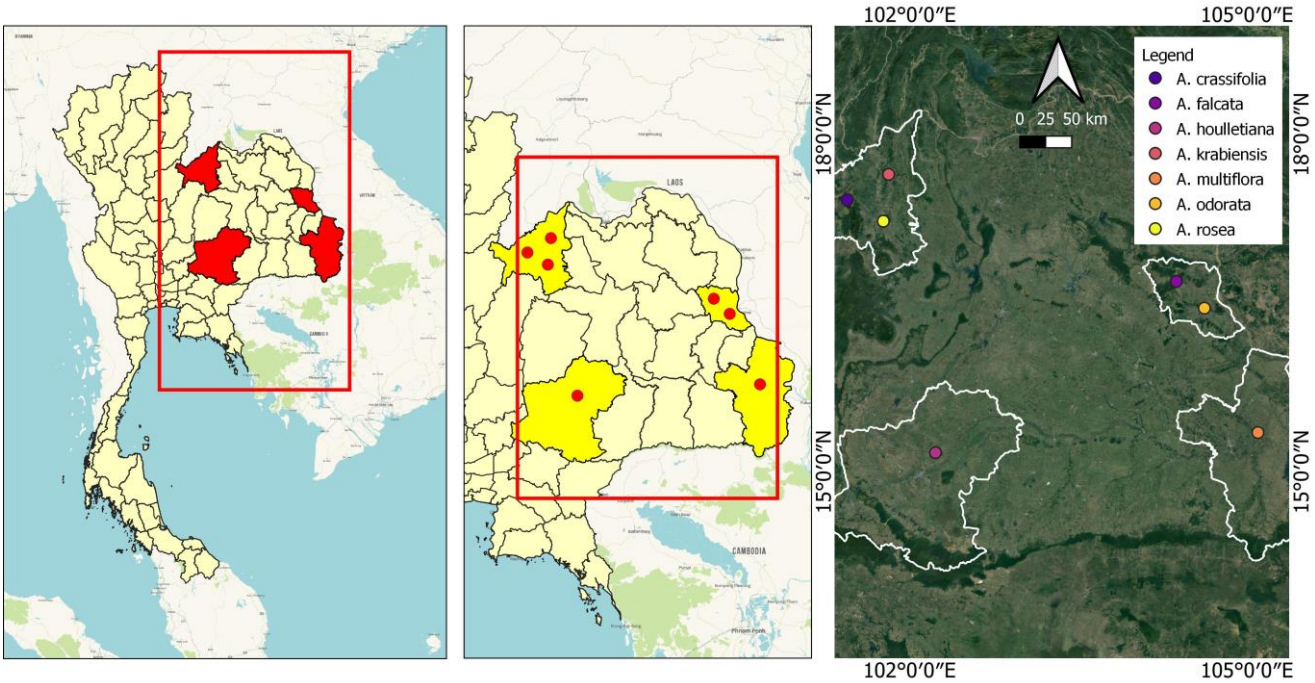


Figure 1. Location of sample collection of the studied species in Thailand

Procedures

The leaf anatomical characteristics of seven *Aerides* species in Thailand were investigated using leaf epidermal peeling and paraffin method. The root anatomical characteristics were investigated using the paraffin method described by Johansen (1940) with some modifications. The samples were examined at 100× and 400× magnification using a Leica DM750 light microscope and photographed with a Leica ICC50 W (Leica Camera AG, Wetzlar, Germany). The characters of cuticle types, epidermal cell shape, stomatal types, crystal types, and trichome types were recorded in leaf epidermal observation (Kermanee 2017). For leaf and root cross-section observation, shapes and number of epidermal cell and velamen layers, shapes of mesophyll, number of vascular bundles, appearance of water storage, starch grains, and crystal types were recorded.

Leaf epidermal observation

Leaf epidermal anatomy was determined using the peeling method. The adaxial and abaxial epidermis of mature leaf lamina, midrib, and margin were peeled. The samples were stained with 1% safranin for 24 hours and dehydrated with ethanol series of 30%, 50%, 70%, and 95%. Complete dehydration was accomplished with absolute ethanol. Then, the samples were transferred to a mixture of equal parts of absolute ethanol and xylene at least 10-15 minutes was allowed at each step before the samples were cleared with pure xylene for at least 3 hours and mounted with DePeX as modified by Johansen (1940).

Leaf and root cross-section observation

The paraffin method was applied for preparing cross-sections of leaves and roots. The mature laminas, midribs, and margins of the leaf and root were sliced into thin pieces of 10-15 µm with a rotary microtome and dehydrated with TBA series. Next, they were stained with 1% safranin and fast green, and permanent slides were made. They were dehydrated with absolute ethanol, transferred to a mixture of equal parts absolute ethanol and xylene, then allowed to steep for at least 10-15 minutes at each step. The cross-sections were cleared with pure xylene for at least 3 hours and mounted with DePeX modified by Johansen (1940).

Data analysis

The qualitative data of leaf and root anatomy were analyzed descriptively. All slide collections are kept in the Biology Program, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand.

RESULTS AND DISCUSSION

Anatomical characters

The results revealed the anatomy of *A. crassifolia*, *A. falcata*, *A. houlletiana*, *A. krabiensis*, *A. multiflora*, *A. odorata*, and *A. rosea* from Thailand, as determined with a light microscope. Summaries of the leaf and root anatomical characteristics observed in this study are presented in Tables 2-3 and Figures 2-4.

***Aerides crassifolia* C.S.P.Parish ex Burb.**

Leaf anatomical features (Figure 2E)

The surface of the leaf showed smooth cuticle on adaxial and abaxial surfaces. Epidermal cells on both surfaces were irregular square, rectangular, or polygonal with 4-7 sides. The paracytic stomata were found on both sides of the lamina. The leaf lamina in cross-section showed uniseriate epidermal cells, composed of elongate to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were surrounded by a sclerenchyma sheath with fibers. The midribs on the adaxial surfaces occurred in cleft. The shape on the abaxial surface was inflated. Numerous fibers surrounded the vascular bundles. The leaf margin was decurved. Raphides crystals were present on the margin.

Root anatomical features (Figure 4E)

The root outline in the cross-section was quite round. Roots were velamentous with 4-6 layers that usually consist of isodiametric cells to elongated cells without clear differentiation between exovelamen and endovelamen. The exodermis was thin-walled with an O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells (Figure 4E). The cortex was multi-layered. The endodermis cell walls were thickened in one or two O-shaped layers with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate. The vascular cylinders were 21-arched. Piths were comprised of parenchymatous and water storage cells. Starch grains were absent.

***Aerides falcata* Lindl. & Paxton**

Leaf anatomical features (Figures 2A, 3C)

The leaf surface showed smooth cuticle on adaxial and abaxial surfaces. Epidermal cells on the adaxial surface was rectangular with 4-5 sides. Epidermal cells on the abaxial surface was irregular square, rectangular, or polygonal with 4-7 sides. The paracytic stomata were found only abaxial surface. No stomata on the adaxial surface (Figure 2A). Dark red inclusions accumulated in epidermal cells were commonly present. Leaf lamina in cross-section showed uniseriate epidermal cells, composed of elongated to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were surrounded by a sclerenchyma sheath with fibers. The midribs on the adaxial surfaces occurred in emarginate (Figure 3C). The shape on the abaxial surface occurred in inflate. Numerous fibers surrounded the vascular bundles. Leaf margins were decurved. Raphides crystals were absent on the margin.

Root anatomical features (Figures 4I, 4J)

The root outline in the cross-section was round to almost elliptic. Roots were velamentous with 4-5 layers. Exovelamen was isodiametric. Endovelamen was isodiametric and angular

to radially elongate. The exodermis was thin-walled with an O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells. The cortex was multi-layered. The endodermis cell walls were thickened in one O-shaped layer with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate to biseriate. The vascular cylinders were 28-arched. Piths were comprised of parenchymatous and sclerenchymatous. Raphides crystal and druse crystal were present in the cortex (Figure 4I). Starch grains were present in the pith (Figure 4J).

***Aerides houlletiana* Rchb.f.**

Leaf anatomical features (Figures 2F, 3A, and 3B)

The surface of the leaf showed a smooth cuticle on the adaxial and abaxial surfaces. Epidermal cells on the adaxial surface were rectangular with 4-5 sides. Epidermal cells on the abaxial surface was irregular square or polygonal with 4-6 sides. The paracytic stomata were found on both sides of the lamina (Figure 2F). Dark red inclusions accumulated in epidermal cells were commonly present. Leaf lamina in cross-section showed 1-2 seriate epidermal cells, composed of elongated to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were surrounded by a sclerenchyma sheath with fibers. The midrib on the adaxial surface was cleft (Figure 3A). The shape on the abaxial surface was inflated. Numerous fibers surrounded the vascular bundles. Leaf margins appeared in straight (Figure 3B). Raphides crystals were absent on the margin.

Root anatomical features (Figure 4A)

The root outline in cross-section was quite round. Roots were velamentous with 5-6 layers (Figures 4A-B). The exovelamen layer was larger than the endovelamen layer. Exovelamen was isodiametric. Endovelamen was isodiametric, angular to radially elongate. The exodermis was thin-walled with an O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells. The cortex was multi-layered. The endodermis cell walls were thickened in one O-shaped layer with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate. The vascular cylinders were 25-arched. Piths were comprised of parenchymatous and water storage cells. Raphides crystals were present in the cortex. Starch grains were present in the pith.

Table 2. Leaf transverse sections and leaf surface of seven species of *Aerides* studied

Species	Midrib		Leaf margin		Type of crystal in lamina	Type of stomata	
	Shape on the adaxial surface	Shape on the abaxial Surface	Shape	Type of crystal		Adaxial surface	Abaxial surface
A. crassifolia	Cleft	Inflate	De	RA	RA	PA	PA
A. falcata	Emarginate	Inflate	De	–	RA	–	PA
A. houlletiana	Cleft	Inflate	St	–	RA	PA	PA
A. krabiensis	Emarginate	Inflate	St	RA	RA	PA	PA
A. multiflora	Cleft	Inflate	In	–	RA	PA	PA
A. odorata	Not concave	Two ridges	St	–	RA	PA	PA
A. rosea	Emarginate	Inflate	De	–	RA	PA	PA

Note: (–): absent; De: decurved; In: incurved; RA: raphides crystal; PA: paracytic stomata; St: straight

Table 3. Root transverse sections of seven species of *Aerides* studied

Species	Root on transverse section	No. of velamen layers	Xylem: No. of poles	Type of crystal in cortex	Starch grains		Pith	
					Pith	Cortex	Parenchyma	Sclerenchyma
crassifolia	RO	4-6	21	–	–	–	+	–
A. falcata	RO, EL	4-5	28	RA, DC	+	–	+	+
A. houlletiana	RO	5-6	25	RA	+	–	+	–
A. krabiensis	RO	3-4	12	RA	–	–	–	+
A. multiflora	RO	4-5	15	RA	–	–	+	–
A. odorata	RO	5-6	22	RA	–	–	+	–
A. rosea	RO, EL	3-4	18	RA	+	+	+	–

Note: (–): absent; (+): present; DC: druse crystal; PA: paracytic stomata; RA: raphides crystal; RO: rounded; EL: elliptic

***Aerides krabiensis* Seidenf.**

Leaf anatomical features (Figures 2H, 3E, and 3F)

The surface of leaf showed a striated cuticle on the adaxial and abaxial surfaces. Epidermal cells on both surfaces were irregular square, rectangular, or polygonal with 4-6 sides. The paracytic stomata were found on both sides of the lamina (Figures 2E and 2H). Leaf lamina in cross-section showed 1-2 seriate epidermal cells, composed of elongated to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were incompletely surrounded by a sclerenchyma sheath with fibers. The midrib on the adaxial surface was emarginate (Figure 3E). The shape on the abaxial surface was inflated. Numerous fibers surrounded the vascular bundles. Leaf margins were straight (Figure 3F). Raphides crystals were present on the margin.

Root anatomical features (Figures 4C, 4D, and 4F)

The root outline in the cross-section was quite round. Roots were velamentous with 3-4 layers (Figure 4C). The exovelamen layer was larger than the endovelamen layer. Exovelamen was isodiametric. Endovelamen was isodiametric, angular to radially elongate. The exodermis was two thick-walled layers (outer and inner) with O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells (Figure 4F). The cortex was multi-layered. The endodermis cell walls were thickened in one O-shaped layer with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate. The vascular cylinders were 12-arched (Figure 4D). Piths were comprised of sclerenchymatous. Raphides crystals were present in the cortex. Starch grains were absent.

***A. multiflora* Roxb.**

Leaf anatomical features (Figures 2B, 2C)

The surface of the leaf showed a striated cuticle on the adaxial and abaxial surfaces. Epidermal cells on both surfaces were irregular square, rectangular, or polygonal with 4-7 sides. The paracytic stomata were found on both sides of the lamina (Figures 2C and 2B). The leaf lamina in cross-section showed uniseriate epidermal cells, composed of elongate to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were surrounded by a sclerenchyma sheath with fibers. The midrib on the adaxial surface was cleft. The shape on the abaxial surface was inflated. Numerous fibers surrounded the vascular bundles. Leaf margins were incurved (Figure 3H). Raphides crystals were absent on the margin.

Root anatomical features (Figure 4H)

The root outline in cross-section was quite round. Roots were velamentous with 4-5 layers that usually consist of isodiametric cells to elongated cells without clear differentiation between exovelamen and endovelamen. The exodermis was two thick-walled layers outer and inner) with O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells. The cortex was multi-layered. The endodermis cell walls were thickened in one O-shaped layer with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate. The vascular cylinders were 15-arched. Piths were comprised of parenchymatous and water storage cells. Raphide crystals were present in the cortex (Figure 4H). Starch grains were absent.

***A. odorata* Lour.**

Leaf anatomical features (Figures 2G, 2D, 3D, and 3I)

The surface of the leaf showed a striated cuticle on the adaxial and smooth cuticle on the abaxial surface. Epidermal cells on both surfaces were irregular square, rectangular, or polygonal with 4-6 sides. The paracytic stomata were found on both sides of the lamina (Figures 2G and 2D). The leaf lamina in cross-section showed uniseriate epidermal cells, composed of elongated to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were surrounded by a sclerenchyma sheath with fibers. The midribs on the adaxial surfaces were not concave. The shape on the abaxial surface had two ridges. Numerous fibers surrounded the vascular bundles (Figure 3I). Leaf margins were straight (Figure 3D). Raphides crystals were absent on the margin.

Root anatomical features (Figure4B)

The root outline in cross-section was quite round. Roots were velamentous with 5-6 layers. The exovelamen layer was larger than the endovelamen layer. Exovelamen was isodiametric to radially elongate. Endovelamen was isodiametric, angular to radially elongate. The exodermis was thin-walled with O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells. The cortex was multi-layered. The endodermis cell walls were thickened in one an O-shaped layer with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate to biseriate. The vascular cylinders were 22-arched. Piths were comprised of parenchymatous and water storage cells. Raphides crystals were present in the cortex. Starch grains were absent.

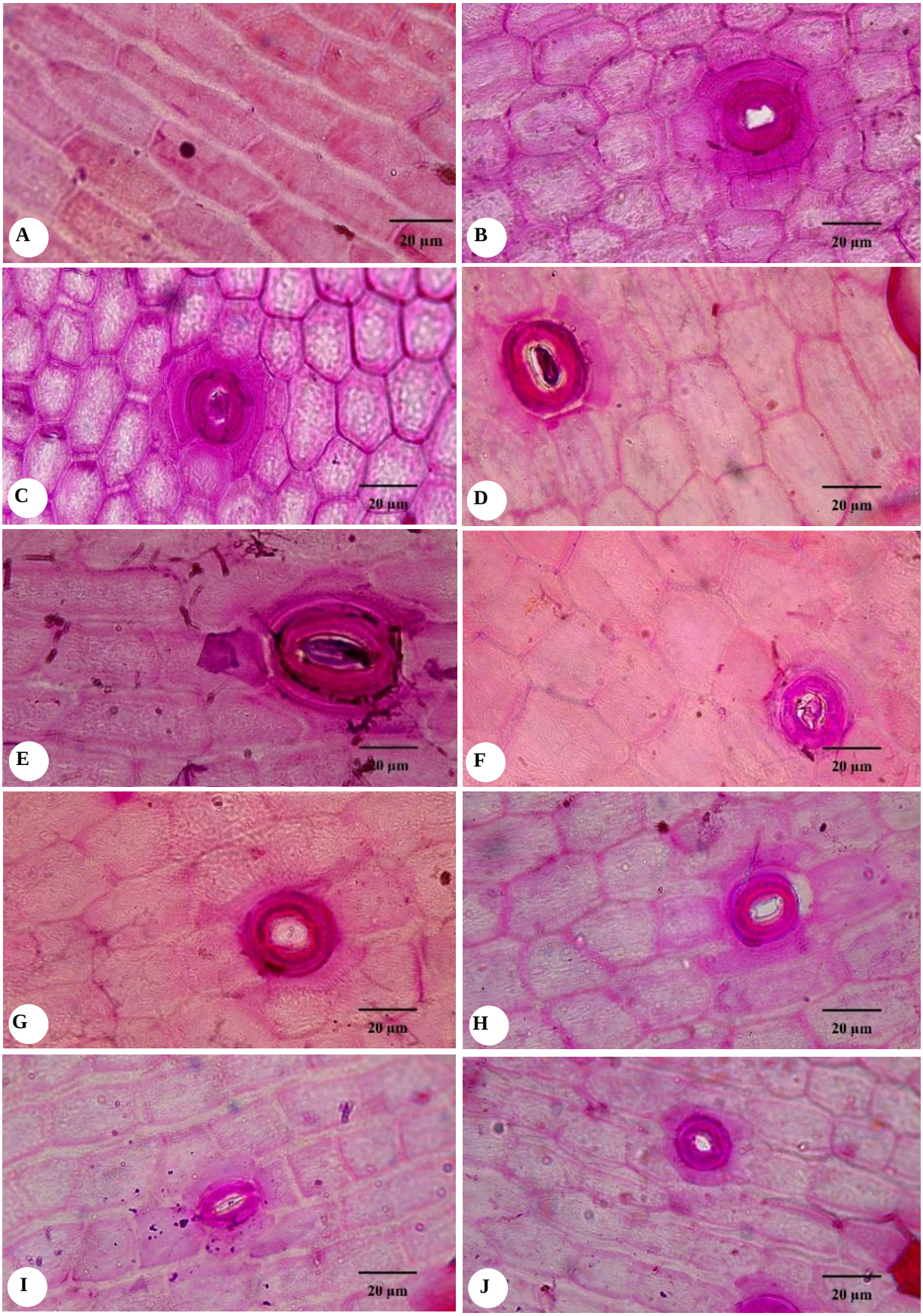


Figure 2. Leaf epidermal anatomy. A. No stomata on adaxial surface (*A. falcata*); Paracytic stomata on adaxial surface, C. *A. multiflora*, E. *A. crassifolia*, G. *A. odorata*, I. *A. rosea*; Paracytic stomata on abaxial surface: B. *A. multiflora*, D. *A. odorata*, F. *A. houlletiana*, H. *A. krabiensis*, J. *A. rosea*

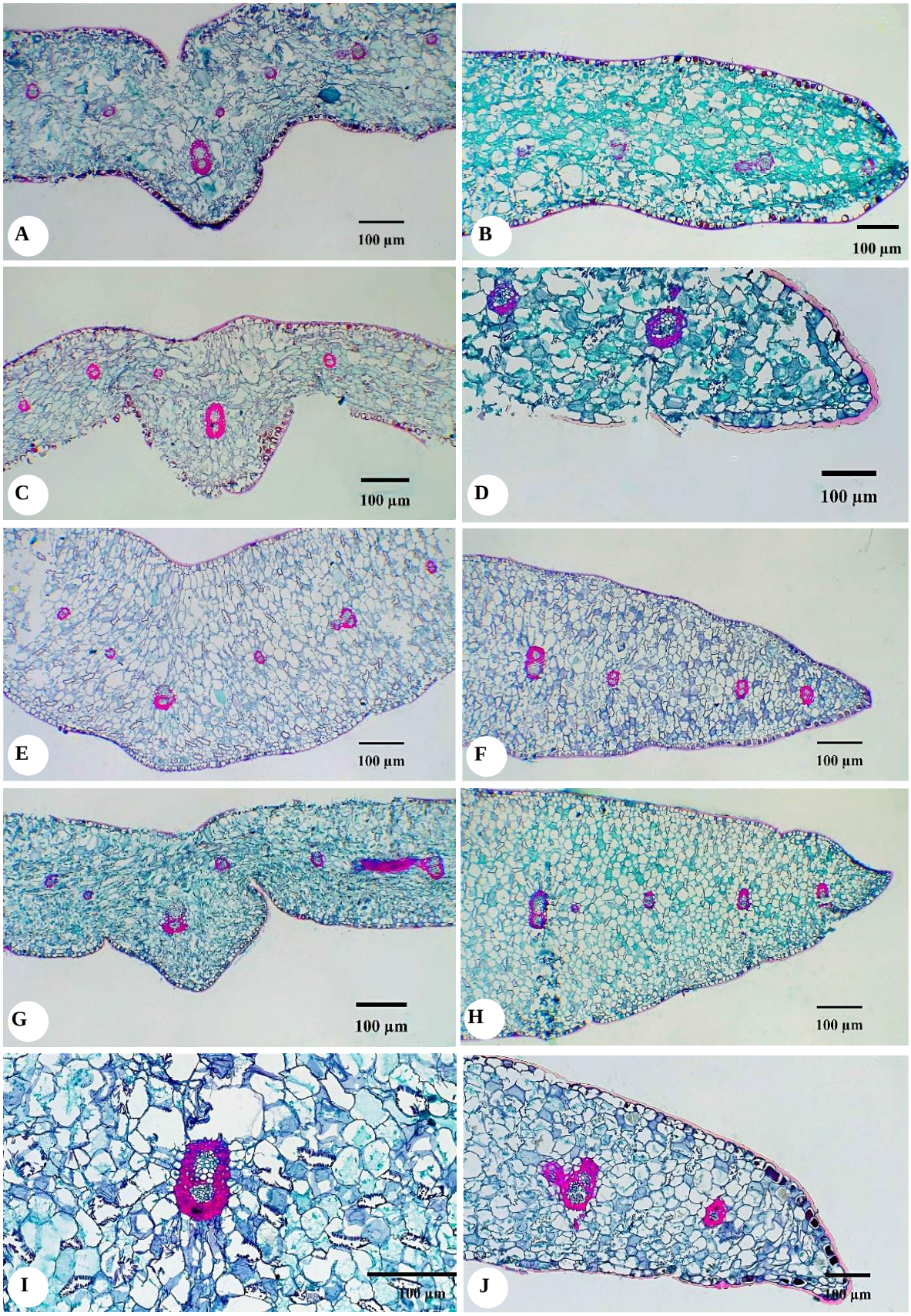


Figure 3. Leaf transverse section. A. Cleft on adaxial surface midrib (*A. houlletiana*); Emarginate on adaxial surface midrib: C. *A. falcata*, E. *A. krabiensis*, G. *A. rosea*; I. Numerous fibers surrounded the vascular bundle (*A. odorata*); straight margin, B. *A. houlletiana*, D. *A. odorata*, F. *A. krabiensis*; H. incurved margin (*A. multiflora*); J. decurved margin (*A. rosea*)

***Aerides rosea* Lodd. ex Lindl. & Paxton**

Leaf anatomical features (Figures 2I, 2J, and 3G)

The surface of the leaf showed a striated cuticle on the adaxial and abaxial surfaces. Epidermal cells on both surfaces were irregular square, rectangular, or polygonal with 4-6 sides. The paracytic stomata were found on both sides of the lamina (Figures 2I and 2J). The leaf lamina in cross-section showed uniseriate epidermal cells, composed of elongate to square-shaped cells. The homogeneous mesophyll had a palisade shaped like spongy parenchyma. The mesophyll consisted of large thin-walled polygonal to round cells. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were incompletely surrounded by a sclerenchyma sheath with fibers. The midrib on the adaxial surface was emarginate (Figure 3G). The shape on the abaxial surface occurred inflate. Numerous fibers surrounded the vascular bundles. Leaf margins were decurved (Figure 3J). Raphides crystals were absent on the margin.

Root anatomical features (Figure 4G)

The root outline in cross-section was quite round to almost elliptic. Roots were velamentous with 3-4 layers that usually consist of isodiametric cells to elongated cells without clear differentiation between exovelamen and endovelamen. The exodermis was thin-walled with an O-shaped wall thickening. In the cortex, the layers consisted of parenchymal tissue and water storage cells. The cortex was multi-layered. The endodermis cell walls were thickened in one O-shaped layer with passage cells. The Casparian strips were observed in the anticlinal walls of endodermis cells. Pericycle was uniseriate. The vascular cylinders were 18-arched. Piths were comprised of parenchymatous and water storage cells. Raphides crystals were present in the cortex (Figure 4G). Starch grains were present in the pith and cortex.

Determination key to *Aerides* species in Thailand

A key to species identification was constructed based on these anatomical characteristics of the leaf and root as follows:

Key to species

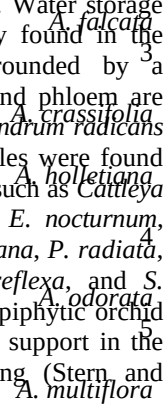
1a. Stomata present on both surfaces..... 2
1b. Stomata present on abaxial surface..... *A. falcata*
2a. Crystal present on transverse sections of root..... 3
2b. Crystal absent on transverse sections of root ..... ….. *A. crassifolia*
3a. Raphides and druse crystals present on transverse sections of root ..... *A. holletiana*
3b. Only raphides are present on transverse sections of root 4
4a. Metaxylem of root with >20 vascular bundles .... *A. odorata*
4b. Metaxylem of root with <20 vascular bundles 5
5a. Cleft midrib with raphides crystal absent on margin ..... *A. multiflora*
5b. Emarginate midrib with raphides crystal present on margin 6
6a. Straight margin with starch grains absent on root ..... ……..... *A. krabiensis*
6b. Decurved margin with starch grains present on root ..... *A. rosea*

Discussion

The seven species of *Aerides* studied can be characterized by several vegetative, such as leaf and root features as follows: smooth and striated cuticle, paracytic stomata, homogeneous mesophyll (a palisade shaped like spongy parenchyma), velamens in root anatomy, exodermis and endodermis cell of root with O-shaped wall thickening, vascular cylinders in the root with polyarchy in 12-28 layers, fiber cells occurring around the vascular elements, water storage cells, and raphides crystal.

Of these leaf characters, two types of cutin sculpturing, smooth and striated were recorded. Other epiphytic orchids were also reported with striate cuticle in *Ascochilus emarginatus* and *Dendrobium subulatum*, while smooth cuticle in *Thrixspermum subulatum* and *T. acuminatissimum* (Rindyastuti et al. 2018). Another papillose cuticle was reported in *Angraecopsis parviflora* (Carlsward et al. 2006). The cuticle plays an important role in reducing the transpiration of epiphytic orchids with thicker cuticles losing water slowly when they encounter water stress (Yang et al. 2016). In this study, the stomata types were paracytic. The paracytic stomata in *A. falcata* is similar to study conducted by Saensouk and Saensouk (2020). The paracytic stomata were also reported in *A. crispa* and *A. maculosa* (Bukhari and Velip 2023). Those stomata were found in various orchids, such as some species of *Cymbidium*, *Dendrobium*, *Geodorum*, *Grammatophyllum*, and *Rhynchostylis* (Saensouk and Saensouk 2020). Moreover, many types of stomata were also found in orchids, such as anomocytic and tetracytic in *Ascochilus emarginatus* and *T. subulatum*, cyclocytic in *Dendrobium subulatum*, and anomocytic in *T. acuminatissimum* (Rindyastuti et al. 2018).

Uniseriate epidermal cells composed of elongate to square-shaped cells were presented in the cross-section. The mesophyll was homogenous not differentiated into palisade and spongy cells (Thangavelu and Muthu 2017; Muthukumar and Shenbagam 2018) similar to this study. Water storage cells and raphide crystals were commonly found in the mesophyll. Vascular bundles were surrounded by a sclerenchyma sheath with fibers. Xylem and phloem are covered by a sclerenchymatous cap in *Epidendrum radicans* (Thangavelu and Muthu 2017). Fiber bundles were found in many species of Laeliinae (Orchidaceae), such as *Cattleya forbesii*, *C. skinneri*, *Epidendrum anceps*, *E. nocturnum*, *Laelia anceps*, *L. lyonsii*, *Prosthechea boothiana*, *P. radiata*, *Scaphyglottis coriacea*, *S. prolifera*, *S. reflexa*, and *S. sincorana*. This character occurs in many epiphytic orchid species. The function of fiber bundles was support in the leaves and has little phylogenetic meaning (Stern and Carlsward 2009).



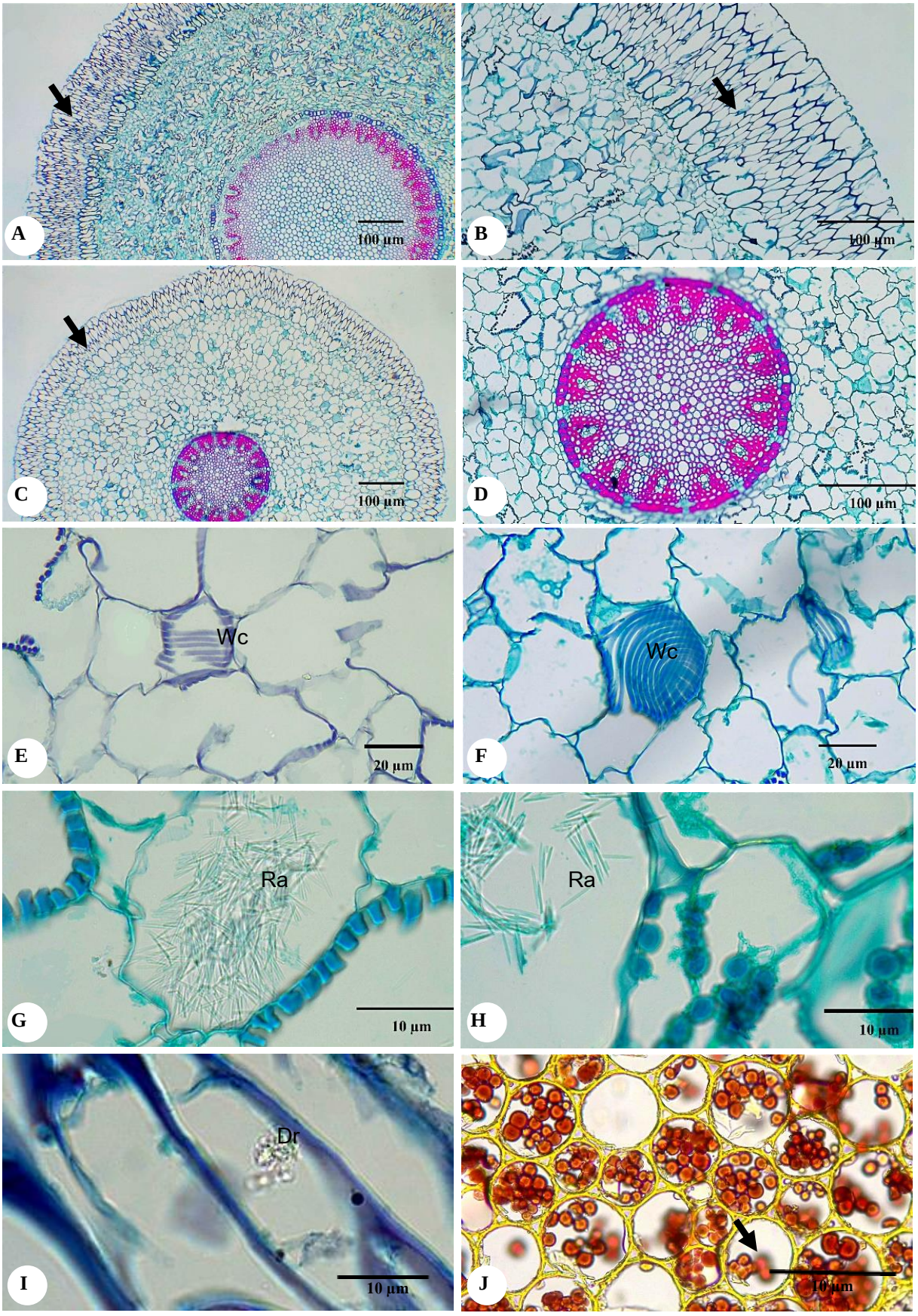


Figure 4. Root transverse section and inclusion in tissue: A. velamen in *A. houlletiana*; B. velamen in *A. odorata*, (C-D) velamen and stele in *A. krabiensis*; E. water storage cell in *A. crassifolia*; F. water storage cell in *A. krabiensis*; G. raphides in *A. rosea*; H. raphides in *A. multiflora*; I. druse in *A. falcata*); J. starch grain in *A. falcata*. Note: Dc: Druse; Ra: raphides; Wc: water storage cell

The root in all of the studied species had 3-6 layers of velamen. *A. crassifolia*, *A. multiflora*, and *A. rosea* usually consist of isodiametric cells to elongated cells without clear differentiation between exovelamen and endovelamen. The velamen shapes were polygonal, rectangular, or elliptical. The velamen was found in other 23 families of monocots in approximately 240 genera, and they are also present in 162 orchid genera (Zotz et al. 2017), epiphytic orchid species (Blanco et al. 2021), and tribe Vandeae in the subfamily Epidendroideae (Kowsalya et al. 2017). The function of velamen is the protection of photosynthetic orchid roots against UV-B damage and the absorption of water and nutrients (Chomicki et al. 2015).

Idioblasts with raphid crystals were common in the root cortex of all studies species, except *A. crassifolia.* This result is similar to the study reported by Andreota et al. (2015) in root of *Cranichis candida*, *Microchilus arietinus*, *Prescottia oligantha*, *Zeuxine strateumatica* (subtribe Goodyerinae), *Cyclopogon apricus*, *C. congestus*, *C. variegatus*, *Mesadenella cuspidata*, *Pteroglossa roseoalba*, and *Sauroglossum nitidum* (subtribe Spiranthinae). The main functions of calcium oxalate crystal in plants include high-capacity calcium regulation and protection against herbivory (Franceschi and Nakata 2005).

The presence of water storage cells was commonly found in the cortex of roots in all species studied. Piths were comprised of water storage cells in all species, except *A. falcata* and *A. krabiensis*. Water storage cells were reported in many species of orchids, such as *Cleisostoma filiforme*, *Oberonia oklongensis*, *Papilionanthe teres*, *Papilionanthe vandarum*, and *Schoenorchis gemmata* (Angela et al. 2015). Water storage cells provide support for the drought tolerance and drought avoidance hypothesis (Hartzell et al. 2017). In addition to the occurrence of water storage cells, multilayered velamen, thick cuticle, and dimorphic exodermis revealed adaptations to moisture stress conditions (Thangavelu and Muthu 2017) and common in epiphytic orchids.

In order to clarify the taxonomy status of those seven *Aeride*s species, a leaf and root anatomical study was carried out. The presence of stomata only on abaxial surface leaves is used to separate *A. falcata*. Stomata of the other six species are present on both surface leaves and these are classified by types of pith tissue, inclusion (type of crystal and starch grains), and number of metaxylem in the root. There are two types of pith tissue, namely: (i) pith comprised of sclerenchyma tissue, which this presence in *A. krabiensis*, and (ii) pith comprised only parenchyma tissue (*A. crassifolia, A. houlletiana, A. multiflora, A. odorata, A. rosea*). For the second, the parenchymatous tissue presence raphides and starch grains in *A. houlletiana* (starch grains in pith) and *A. rosea* (starch grains in cortex and pith), the parenchymatous tissue presence only starch grains in *A. multiflora* (Metaxylem of root with <20 vascular bundles) and *A. odorata* (metaxylem of root with >20 vascular bundles), and the parenchymatous tissue absence raphides and starch grains in *A. crassifolia*.

In conclusion, the generalized leaf anatomical characteristics of *Aerides* studied species were paracytic stomata. The homogeneous mesophyll was found. Velamens in root anatomy had three to six layers. The vascular cylinders in the root were polyarchy in 12-28 layers. Water storage cells were commonly found. Leaf and root anatomy can be used to identify *Aerides* species in Thailand. Leaf anatomical characteristics including stomata distribution, midrib shapes, and presence of crystal on the margin can be used for species identification for *A. falcata* and *A. multiflora*. For root characteristics, the presence and types of crystal, xylem arches, and presence of starch grains can be used for species identification for *A. crassifolia*, *A. holletiana*, *A. odorata*, *A. krabiensis*, and *A. rosea*.

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