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RESEARCH ARTICLE

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Leaf epidermal morphology of Asparagaceae of Taiwan and its systematic significance

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Abstract

The current study analyzed the epidermal morphology of Asparagaceae in detail and assessed its systematic importance. At the familial level, no consistent characteristics were found, but anticlinal wall and stomata morphology provided systematic information of different tribes, especially Ophiopogoneae and Polygonateae. In Ophiopogoneae, *Liriope* and *Ophiopogon* had similar epidermis, which implying a close relationship between them, and was also supported by related studies. The leaves of Polygonateae exhibited rounded and undulate anticlinal wall. *Polygonatum arisanense* var. *formosanum* had a rounded anticlinal wall, whereas other species exhibited undulate anticlinal walls. Different epidemis of *Po. arisanense* var. *formosanum* supported the variety treatment of *Po. arisanense*. The intergeneric relationship was also interpreted based on the anticlinal wall and stomata. Therefore, the epidermis could provide the systematic value of Asparagaceae. The present study also revealed the linkage of stomata and habitat types, though the adaptative significance of epidermal traits needs further study.

Research Highlights

- Anticlinal wall and stomatal morphology had systematic potential on tribal or generic levels of Asparagaceae.
- Stomatal types of Asparagaceae might be linked to environmental factors.

KEYWORDS
light microscope, plant taxonomy, scanning electronic microscope, stomatal index

1 | INTRODUCTION

The epidermis is the outermost layer of plant bodies. It comprises epidermal cells and the stomatal complex (Dilcher, 1974). The primary functions of the epidermis include gas exchange, mechanical protection, and mitigating water loss (Cutler et al., 2007; Evert, 2006). The varied epidermal morphology provides abundant information for studies of plant physiology, ecology, taxonomy, and phylogeny across the different angiosperm taxa (Stebbins & Khush, 1961; Roth-Nebelsick et al., 2001; Hetherington & Woodward, 2003; Sonibare et al., 2005; Melotto et al., 2006; Franks & Beerling, 2009; Casson & Hetherlington, 2010; Fortini et al., 2013; Sack et al., 2013; Adebowale, Naidoo, Lamb, & Nicolas, 2014; Deng et al., 2014; Wang et al., 2014; Wang et al., 2015).

Asparagaceae comprises 118 genera and 3200 species distributed worldwide except the Antarctic region (Christenhusz et al., 2017). The members of Asparagaceae were often treated as belonging to Liliaceae *s.l.* in several classification systems (e.g., Bentham & Hooker, 1883; Bessey, 1915; Krause, 1930; Melchior & Weidermann, 1964; Hutchinson, 1973; Cronquist, 1981; Goldberg, 1989). The tribal-based intrafamilial system of Liliaceae *s. l.* was widely accepted and applied in related studies (e.g., Sterling, 1982; Utech, 1987; Tamura, Chen, & Turland, 1993; Bao et al., 1999; Wang et al., 2016; Wang et al., 2017). However,

this system was not considered in the classification of Asparagaceae in the APG system (APG, 2009, 2016). Asparagaceae was classified into seven subfamilies and several tribes under Agavoideae and Scilloideae (Stevens, 2001). There are 13 genera and 23 species belonging to four subfamilies recorded in Taiwan, including several endemic species (Chao, 2016; Chao et al., 2013; Ying, 2000) (Figures 1–4). Among these, Asparagoideae, Lomandroideae, and Scilloideae comprise only one species. The others belong to Nolinoideae. The members of Nolinoideae are further divided into four tribes, namely Convallarieae, Dracaeneae, Ophiopogoneae, and Polygonateae.

Leaf epidermal studies conducted on Asparagaceae have been limited in scope and sampling thus far. Yi yi and Jewers (1973) studied the epidermal morphology of Agaveae of Agavaceae. Different morphology features were observed among genera. Dai and Liang (1991) studied the epidermal features in Ophiopogonoideae. The results revealed that *Ophiopogon* and *Liriope* had epidermal morphology similar to *Peliosanthes*. Additionally, Dai and Liang (1991) studied the evolution trend of the features of Ophiopogonoideae. Chen et al. (2006) studied the epidermal morphology of five species of *Polygonatum* in the Dabieshan area, Anhui Province, China. The result showed that the size of the epidermal cell, the type of anticlinal wall, and both size and density of the stomata could provide an excellent taxonomic trait for the taxonomy on the generic level. However, few epidermal morphological studies have been conducted in Taiwan. This study aimed to evaluate the systematic values of epidermal morphology.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

For the epidermal observation, leaves of 23 taxa of Asparagaceae were collected from living plants and fixed in 70% alcohol. The leaves prepared for dissection were cut at the central part beside the midrib into pieces with approximately 1×1 cm^2. Linear leaves of *Liriope* and *Ophiopogon* were cut at the central part into pieces with lengths of approximately 5 mm. For *Asparagus cochinchinensis* (Lour.) Merr., cladodes were utilized for the study. The voucher specimens were preserved at the Herbarium Department of Forestry, National Chung-Hsing University (TCF) (Table 1).

3 | STUDY METHODS

3.1 | Epidermal observation

3.2 | Preparation for light microscopy

Leaf dissection was performed following the method of Sun and Jiang (2009). The leaves were placed in a solution of 30% H_2O_2 and acetic acid and maintained under a 65°C dryer or on a heat plate overnight. After the leaves were dissected, a small writing brush was used to remove the residual mesophyll tissue. Each taxon had at least three replicates.

FIGURE 1 Photos of Convallarieae of Taiwan: (a) *Aspidistra attenuata*, (b) *A. daibuensis*, (c) *A. mushaensis*, and (d) *Rohdea fargesii*

FIGURE 2 Photos of Ophiopogoneae of Taiwan: (a) *Liriope graminifolia*, (b) *L. platyphylla*, (c) *L. spicata*, (d) *Ophiopogon intermedius*, (e) *O. reversus*, (f) *Peliosanthes arisanensis*, and (g) *P. kaoi*

The dissected epidermises were placed into 1% safranin stain for 1 min, and then dehydrated with 50%, 70%, 80%, 90%, 95%, and 99.5% EtOH for 3 min each. The epidermis was hardened with wintergreen oil for 3 mins, treated with 99.5% alcohol+xylene solution and xylene for 3 mins, and sealed with Entellan new (Merck, Germany). The prepared specimens were observed using a light microscope (Nikon HFX-DX and Olympus bx51).

3.3 | Preparation for scanning electron microscopy

The leaves were fixed in 70% EtOH and serially dehydrated using 80%, 90%, 95%, and 99.5% EtOH. After critical point drying (QUORAM E3100) and coating with gold sputter (QUORUM SC7620), the specimens were examined and photographed under a scanning electron microscope (HITACHI S-3400 N).

3.4 | Data analysis

Structural analysis of the epidermis, was conducted using the method of Dilcher (1974), included the shape, size, arrangement, anticlinal wall form, stomatal location, stomatal arrangement, stomatal index, stomatal size, and subsidiary cell form. The stomatal index was not calculated for genera in which the stomata form a stomatal band, such as *Liriope* and *Ophiopogon*, due to the non-random distribution of stomata.

FIGURE 3 Photos of Polygonateae of Taiwan: (a) *Disporopsis pernyi*, (b) *Heteropolygonatum altelobatum*, (c) *Maianthemum formosanum*, (d) *M. harae*, (e) *Polygonatum arisanense* var. *arisanense*, (f) *P. arisanense* var. *chingshuishanianum*, and (g) *P. arisanense* var. *formosanum*

4 | RESULTS

Based on the observations, the epidermal morphology of genera of Asparagaceae of Taiwan could be classified by following key:

1. Stomatal bands on the abaxial surface.....*Liriope* and *Ophiopogon*
- No stomatal bands on the abaxial surface.....2
2. Epidermal cell with straight anticlinal wall.....3
- Epidermal cell with round or undulate anticlinal wall.....9
3. Linear epidermal cell.....4
- Rectangular epidermal cell.....5
4. Length of epidermal cells >500 μm.....*Thysanotus*
- Length of epidermal cells <500 μm.....*Dracaena*
5. Length of epidermal cells >250 μm.....*Asparagus*
- Length of epidermal cells <250 μm.....6
6. Subsidiary cells of adaxial surface paracytic form.....*Aspidistra*
- Subsidiary cells of adaxial surface not paracytic form.....7
7. Adaxial surface without stomata.....*Peliosanthes*
- Adaxial surface with stomata.....8
8. Both surfaces with scale-like ornamentation.....*Barnardia*
- Both surfaces without scale-like ornamentation.....*Rohdea*
9. Round anticlinal wall.....10
- Undulate anticlinal wall *Maianthemum*
10. Anticlinal wall of adaxial surface rounded.....*Disporopsis*
- Anticlinal wall of adaxial surface not rounded.....11
11. Adaxial surface with scale-like ornamentation.....*Heteropolygonatum*
- Adaxial surface without scale-like ornamentation.....*Polygonatum*

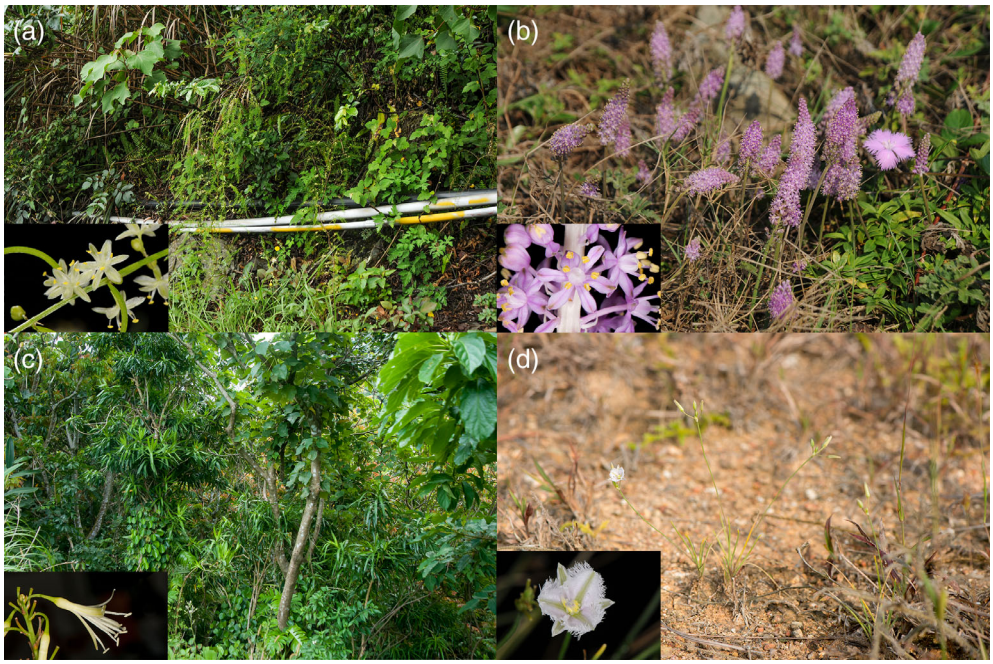


FIGURE 4 Photos of Asparagaceae of Taiwan: (a) *Asparagus cochinchinensis*, (b) *Barnardia japonica*, (c) *Dracaena angustifolia*, and (d) *Thysanotus chinensis*

TABLE 1 Materials used for the study and voucher information

Taxa		Location	Collect no.
Asparagoideae	*Asparagus cochinchinensis* (Lour.) Merr.	Taichung City: Taiping district	Chao 2221
Lomandroideae	*Thysanotus chinensis* Benth.	Kinmen County: Tienpu wetland	Chao 3098
Nolinoideae-Convallarieae	*Aspidistra attenuata* Hayata	Taitung County: Tulanshan	Chao 2109
	Aspi. daibuensis Hayata	Hualien County: Chingshuishan	Chao 2966
	Aspi. mushaensis Hayata	Taichung City: Taiping distrtict	Chao 2320
	Rohdea chinensis (Baker) T. Tanaka	Taichung City: Wuling farm	Chao 2108
Nolinoideae-Dracaeneae	*Dracaenas angustifolia* Roxb.	Taitung County: Lanyu Islet, Shiaotienchih	Chao 3224
Ophiopogonoideae	*L. graminifolia* (L.) Baker	Pingtung County: Lilongshan	Chao 2604
	L. muscari (Decne.) L. H. Bailey	Pingtung County: Tahan forest trail	Chao 3836
	L. spicata (Thunb.) Lour.	Taipei City: Neihu Dist., Liyushan trail	Chao 2449
		Taitung County: Lanyu Islet	Chao 3223
		Pingtung County: Taiwu township	Chao 2374
	Ophiopogon intermedius D. Don	Nantou County: Shanlinhsi	Chao 2907
		Taichung County: Hsuehshan	Chao 2117
	O. reversus C. C. Huang	Nantou County: Nanfeng Village	Chao 2451
	Peliosanthes arisanensis Hayata	Chiayi County: Yuantan	Chao 2720
	Pe. kaoi Ohwi	Kaohsiung City: Tengchi	Chao 2125
Nolinoideae-Polygonateae	*Disporopsis pernyi* (Hua) Diels	Nantou County: Road sign 19 km of highway no. 14A	Chao 3296
		Taoyuan County: Rarashan	Chao 1031
	Heteropolygonatum alte-lobatum (Hayata) Y. H. Tseng, H. Y. Tzeng & C. T. Chao	Hsinchu County: Chienshih township, Lupi trail	Chao 1273
	Maianthemum formosanum (Hayata) LaFrankie	Miaoli County: Tapachienshan	Chao 3483
		Taichung City: Hsuehshan	Chao 972
	M. harae Y. H. Tseng & C. T. Chao	Chiayi County: Tefuyeh ancient trail, Tsuchong section	Chao 1385
	Polygonatum arisanense Hayata var. *arisanense*	Nantou County: Shanlinhsi	Chao 2909
	Po. arisanense var. *chingshuishanianum* S. S. Ying	Hualien County: Chingshuishan	Chao 1424
			Chao 2131
	Po. arisanense var. *formosanum* (Hayata) Masam. & Shimada	Taipei City: Main peak of Tatunshan	
Scilloideae	*Barnardia japonica* (Thunb.) Schult. & Schult. f.	Miaoli County: Tunghsiao town	Chao 2452

5 | EPIDERMAL MORPHOLOGY

This study is the first systematic research on the leaf epidermis of Asparagaceae of Taiwan. Our results revealed that epidermal cell shape, anticlinal wall form, stomatal orientation, and guard cell position could provide systematic value at different taxonomical hierarchies. Epidermal characteristics of the studied taxa are described as follows (Table 2, Figures 5–12).

5.1 | Shape

Leaf epidermal cell shapes were rectangular and linear in Asparagaceae. In most taxa, they were rectangular and were similar on both adaxial and abaxial surfaces; however, *Heteropolygonatum altelobatum* (Hayata) Y. H. Tseng, H. Y. Tzeng & C. T. Chao, *Polygonatum arisanense* Hayata var. *chingshuishanianum* (S. S. Ying) C. T. Chao & Y. H. Tseng, *Po. arisanense* Hayata var. *formosanum* (Hayata) C. T. Chao & Y. H. Tseng, and *Rohdea fargesii* (Baill.) Y. F. Deng exhibited different types between these surfaces (Figure 9j–l 11d–f, 11p–r, and 11s–u). Due to linear epidermal cell shape was not defined by Dilcher (1974), we defined this type as length-to-width ratio ca. 6:1 and sharpening at both ends of the cell. This type was found in *Dracaena angustifolia* (Medik.) Roxb. (Figure 12d–f) and *Thysanotus chinensis* Benth. (Figure 12g and h). Additionally, *T. chinensis* was the only species with a unifacial leaf in this study.

5.2 | Size

The size of epidermal cells ranged from 44 × 19 μm^2 (*Aspidistra daibuensis* Hayata) to 809 × 42 μm^2 (*T. chinensis*), exhibiting wide variations among taxa and different sides of leaves. Most taxa had cells ranging from approximately 40 × 20 μm^2 to approximately 180 × 20 μm^2. Huge cells were found in *Aspa. cochinchinensis* (314 × 12 μm^2) (Figure 8a, b), *Barnardia japonica* (Thunb.) Schult. & Schult. f. (214–266 × 25–27 μm^2) (Figure 12a–c), and *T. chinensis* (809 × 42 μm^2) (Figure 12g, h).

5.3 | Arrangement

Two types of epidermal cell arrangement were recorded: linear and polygonal. The former was found in all species, and the latter was recorded on the abaxial surface of *Di. pernyi* (Hua) Diels (Figure 11a–c), *H. altelobatum* (Figure 11d–f), *Po. arisanense* Hayata var. *arisanense* (Figure 11m–o), *Po. arisanense* var. *chingshuishanianum* (Figure 11p–r), *Po. arisanense* var. *formosanum* (Figure 11s–u), and *R. fargesii* (Figure 9j–l).

5.4 | Anticlinal wall form

Three types of anticlinal walls, viz. straight, round, and undulate, were found in the taxa of Asparagaceae. Most of the taxa had straight form. The round form was found in *Di. pernyi* (Figure 11a–c), *H. altelobatum* (Figure 11d–f), *Po. arisanense* var. *chingshuishanianum* (Figure 11p–r), and *Po. arisanense* var. *formosanum* (Figure 11s–u). The undulate form was found only in *Maianthemum* (Figure 11g–l) and *Po. arisanense* var. *arisanense* (Figure 11m–o).

5.5 | Surface ornamentation

Surface ornamentation was found in most taxa of Asparagaceae, except *R. fargesii* (Figure 9j–l), *Dr. angustifolia* (Figure 12d–f), *M. formosanum* (Figure 11g–i), *M. harae* Y. H. Tseng & C. T. Chao (Figure 11j–l), *Peliosanthes arisanensis* Hayata (Figure 10p–r), *Pe. kaoi* Ohwi (Figure 10s–u), and *T. chinensis* (Figure 12g–h). The surface ornamentations were scale-like or granular. Scale-like epidermal process was found in *B. japonica* (Figure 12a–c), *Di. pernyi* (Figure 11a–c), *H. altelobatum* (Figure 11d–f), *L. graminifolia* (L.) Baker (Figure 10a–c), and all members of *Polygonatum* (Figure 11m–u). Most exhibited sparse coverage except for *H. altelobatum* and *Polygonatum* taxa, which had dense coverage on both surfaces. Granular epidermal process was found in *L. muscari* (Decne.) L. H. Bailey (Figure 10d–f) and *Ophiopogon* (Figure 10j–o).

5.6 | Stomata orientation

Stomata were present on the abaxial leaf surface of all taxa and the adaxial surface in some taxa. Stomatal orientation showed random arrangement and stomatal bands in Asparagaceae. Most taxa showed random arrangement in stomatal orientation, except *Liriope* (Figure 10a–i) and *Ophiopogon* (Figure 10j–o), which exhibited stomatal bands on the abaxial surface. The long axes of the guard cells were parallel to the long axis of the leaf in all studied species.

5.7 | Stomatal index

Stomatal index represents the density of stomata in a specific leaf area. Stomatal indices (Table 2) ranged from 0.97 (*Dr. angustifolia*) to 42.00 (*T. chinensis*). There was a wide divergence between the genera but relative consistency within the genera.

5.8 | Stomata size

Stomatal sizes (Table 2) were between 10 × 5 μm^2 (*M. harae*) and 33 × 23 μm^2 (*Di. pernyi*). The variances in stomatal sizes were smaller than those of stomatal indices, which lacked regularity within the taxonomic hierarchy of Asparagaceae; hence, this cannot be considered a helpful characteristic at the genus level or even at a level higher or lower.

TABLE 2 Epidermal morphology of Asparagaceae species in Taiwan

Taxa		Epidermal cell ad/ab				
		Shape	Size (μm)	Arrangement	Anticlinal wall form	Surface ornamentation
Asparagoideae	*Asparagus cochinchinensis*	rec	314 × 12	lin	str	—
Lomandroideae	*Thysanotus chinensis*	lin	809 × 42	lin	str	—
Nolinoideae-Convallarieae	*Aspidistra attenuata*	rec/rec	57 × 24/77 × 23	lin/lin	str/str	sca/sca
	Aspi. daibuensis	rec/rec	44 × 19/62 × 17	lin/lin	str/str	−/−
	Aspi. mushaensis	rec/rec	64 × 21/53 × 14	lin/lin	str/str	sca/sca
	Rohdea chinensis	rec/iso	71 × 33/101 × 43	lin/pol	str/str	−/−
Nolinoideae-Dracaeneae	*Dracaena angustifolia*	lin[a]/lin	114 × 13/182 × 19	lin/lin	str/str	−/−
Ophiopogonoideae	*Liriope graminifolia*	rec/rec	51 × 14/34 × 8	lin/lin	str/str	sca/sca
	L. muscari	rec/rec	67 × 26/133 × 22	lin/lin	str/str	gra/gra
	L. spicata	rec/rec	90 × 20/91 × 13	lin/lin	str/str	−/−
	Ophiopogon intermedius	rec/rec	74 × 16/77 × 13	lin/lin	str/str	gra/gra
	O. reversus	rec/rec	85 × 14/109 × 20	lin/lin	str/str	−/gra
	Peliosanthes arisanensis	rec/rec	88 × 25/78 × 16	lin/lin	str/str	−/−
	Pe. kaoi	rec/rec	137 × 30/101 × 32	lin/lin	str/str	−/−
Nolinoideae-Polygonateae	*Disporopsis pernyi*	iso/iso	69 × 10/84 × 47	lin/pol	rou/rou	sca/sca
	Heteropolygonatum alte-lobatum	rec/iso	47 × 19/122 × 35	lin/pol	str/rou	sca/sca
	Maianthemum formosanum	rec/rec	94 × 52/54 × 25	lin/lin	und/und	−/−
	M. harae	rec/rec	84 × 63/115 × 54	lin/lin	und/und	−/−
	Polygonatum arisanense	rec/rec	128 × 24/92 × 39	lin/pol	str/und	−/sca
	Po. chinshuishanianum	rec/iso	92 × 23/95 × 50	lin/pol	str/rou	−/sca
	Po. arisanense var. formosanum	rec/iso	126 × 30/97 × 50	lin/pol	str/rou	−/sca
Scilloideae	*Barnardia japonica*	rec/rec	214 × 25/266 × 27	lin/lin	str/str	sca/sca

	Stomatal complex ad/ab						Subsidiary cell ad/ab	
	Taxa	Orientation	Stomatal index (%)	Size (μm)	Guard cell position	Guard cell size (μm)	Form	Size (μm)
Asparagoideae	*Asparagus cochinchinensis*	A[b]	29.16	24 × 8	lev	31 × 50	ano	—
Lomandroideae	*Thysanotus chinensis*	A	42.00	29 × 50	lev	40 × 11	ano	—
Nolinoideae-Convallarieae	*Aspidistra attenuata*	A/A	3.32/11.83	23 × 6/18 × 6	lev/lev	34 × 8/35 × 13	par/par	78 × 16/78 × 19
	Aspi. daibuensis	A/A	1.61/15.24	20 × 10/22 × 10	lev/lev	30 × 5/27 × 9	par/par	49 × 18/67 × 23
	Aspi. mushaensis	A/A	1.89/10.90	22 × 8/19 × 3	lev/lev	30 × 11/27 × 12	par/par	76 × 17/47 × 17
	Rohdea chinensis	A/A	3.70/11.18	22 × 14/18 × 11	lev/lev	26 × 9/29 × 10	−/ano	−/−
Nolinoideae-Dracaeneae	*Dracaena angustifolia*	A/A	0.97/19.00	18 × 6/22 × 7	lev/lev	21 × 7/23 × 80	−/ano	−/−
Ophiopogonoideae	*Liriope graminifolia*	−/sto	−/−	−/17 × 5	−/sun	−/21 × 4	−/ano	−/−
	L. platyphylla	sto/sto	−/−	25 × 10/19 × 9	lev/lev	28 × 11/32 × 9	−/ano	−/−
	L. spicata	−/sto	−/−	−/22 × 8	−/lev	−/28 × 90	−/ano	−/−
	Ophiopogon intermedius	sto/sto	−/−	14 × 2/15 × 30	sun/sun	16 × 4/16 × 5	−/ano	−/−
	O. reversus	−/sto	−/−	−/13 × 30	−/sun	−/13 × 3	−/ano	−/−
	Peliosanthes arisanensis	−/A	−/14.19	−/31 × 10	−/lev	−/32 × 11	−/ano	−/−
	Pe. kaoi	−/A	−/18.99	−/24 × 11	−/lev	−/31 × 11	−/ano	−/−
Nolinoideae-Polygonateae	*Disporopsis pernyi*	−/A	−/13.04	−/33 × 23	−/lev	−/50 × 16	−/par	−/61 × 35
	Heteropolygonatum alte-lobatum	−/A	−/24.00	−/19 × 11	−/lev	−/30 × 12	−/ano	−/−
	Maianthemum formosanum	−/A	−/25.00	−/16 × 80	−/lev	−/28 × 6	−/ano	−/−
	M. harae	−/A	−/21.05	−/10 × 50	−/lev	−/18 × 6	−/ano	−/−

(Continues)

TABLE 2 (Continued)

	Stomatal complex ad/ab						Subsidiary cell ad/ab	
	Taxa	Orientation	Stomatal index (%)	Size (μm)	Guard cell position	Guard cell size (μm)	Form	Size (μm)
	Polygonatum arisanense	−/A	−/27.27	−/19 × 50	−/sun	−/34 × 13	−/ano	−/−
	Po. chinshuishanianum	−/A	−/34.20	−/21 × 50	−/sun	−/33 × 11	−/ano	−/−
	Po. arisanense var. formosanum	−/A	−/25.00	−/20 × 60	−/sun	−/34 × 13	−/ano	−/−
Scilloideae	*Barnardia japonica*	A/A	17.8/28.26	26 × 12/28 × 12	lev/lev	40 × 12/30 × 8	−/ano	−/−

Abbreviations: ano, anomocytic; iso, isodiametric; lev, ±level; lin, linear; par, paracytic; pol, polygonal; rec, rectangular; rou, rounded; sca, scale-like; sto, stomatal band; str, straight; sun, sunken; und, undulate.

Note: [a]The type was not defined by Dilcher (1974); it is defined here as the length-to-width ratio of 6:1 and sharpened at both apices. [b]A. = long axis of guard cell parallel to long axis of leaf.

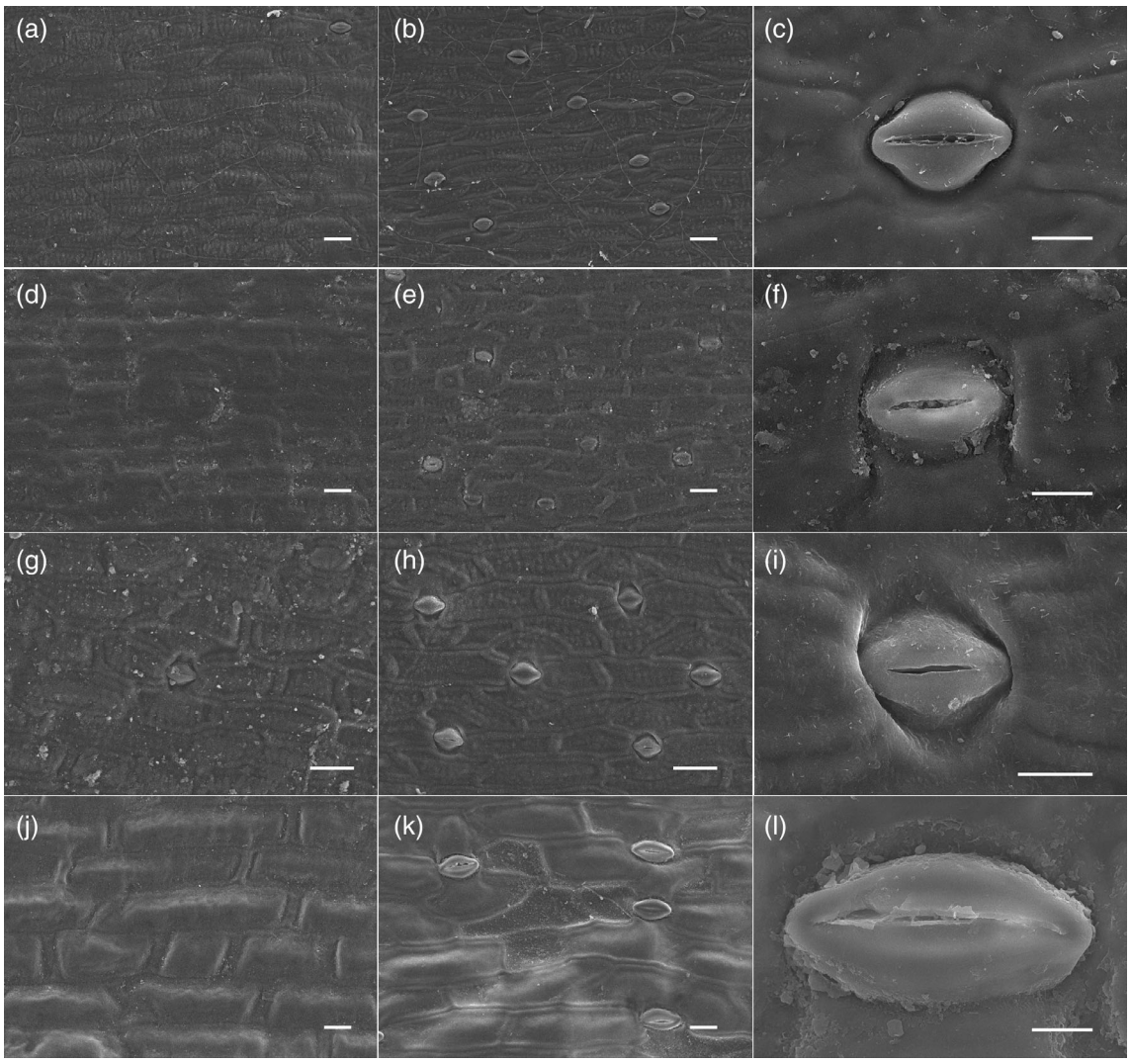


FIGURE 5 Scanning electron microscopy of the leaf epidermal morphology of Convallarieae. (a–c) *Aspidistra attenuata*, (d–f) *A. daibuensis*, (g–i) *A. mushaensis*, and (j–l) *Rohdea fargesii*. (a, d, g, j) adaxial surface (scale bar: 30 μm), (b, e, h, k) abaxial surface (scale bar: 30 μm), and (c, f, i, l) stomata (scale bar: 10 μm)

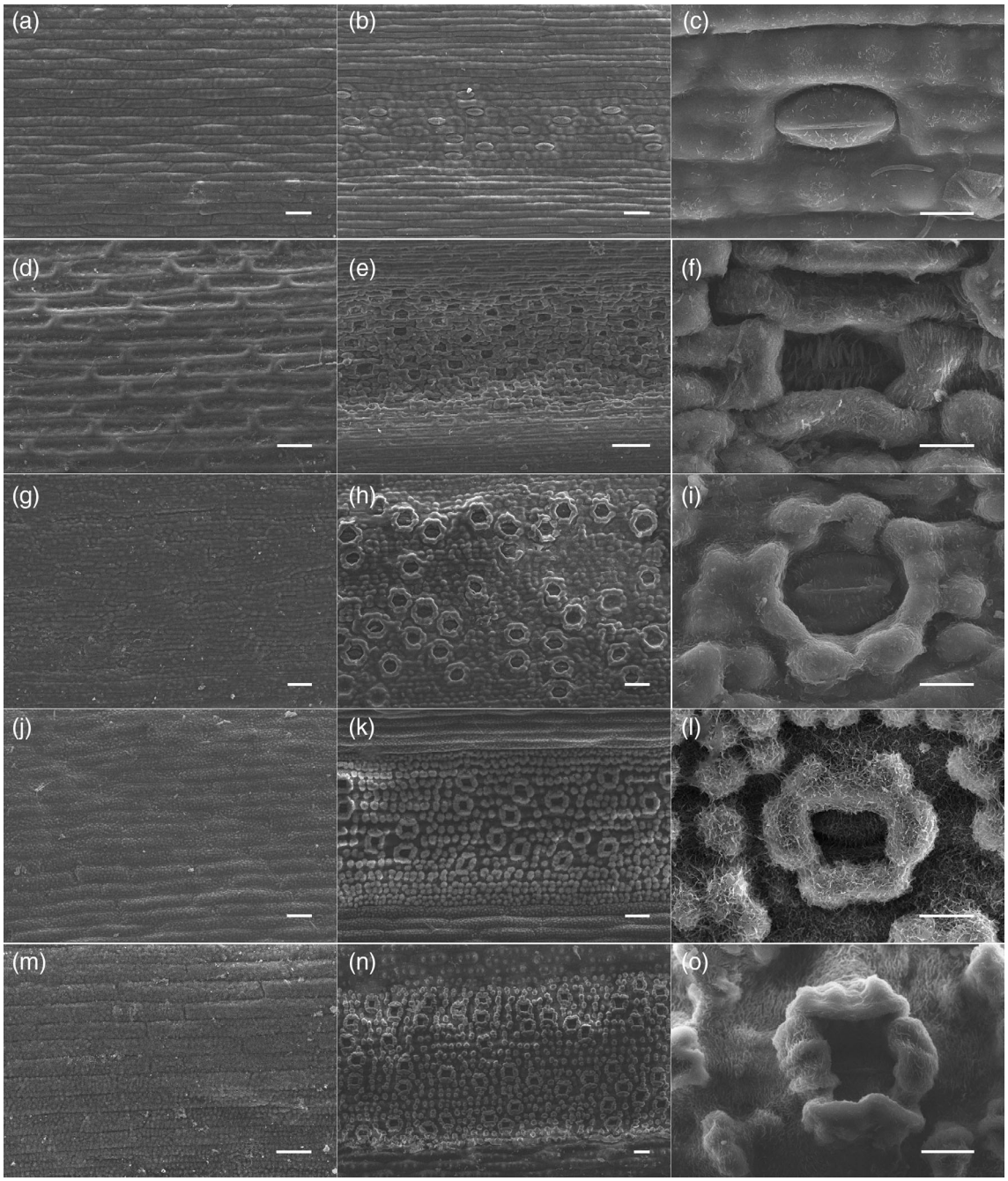


FIGURE 6 Scanning electron microscopy of the leaf epidermal morphology of Ophiopogoneae. (a–c) *Liriope graminifolia*, (d–f) *L. platyphylla*, (g–i) *L. spicata*, (j–l) *Ophiopogon intermedius*, (m–o) *O. reversus*, (p–r) *Peliosanthes arisanensis*, and (S–U) *P. kaoi*. (a, d, g, j, m, p, s) adaxial surface (scale bar: 30 μm), (b, e, h, k, n, p, t) abaxial surface (scale bar: 30 μm), and (c, f, i, l, o, r, u) stomata (scale bar: 10 μm)

5.9 | Guard cell position

The guard cells were found at approximately the same level as the epidermal cells in most species, with sunken guard cells in *L. graminifolia*, *Ophiopogon* (Figure 10j–o), and *Polygonatum* (Figure 11m–u). This characteristic state was consistent among intrageneric taxa within most genera—the only exception was *Liriope* which had two types.

5.10 | Guard cell size

Guard cell ranged from 13×3 μm^2 (*O. reversus* C. C. Huang) to 50×16 μm^2 (*Di. pernyi*) (Table 2). The variations in guard cell sizes were somewhat consistent within genera but overlapped between genera, which would not yield a high systematic value in Asparagaceae.

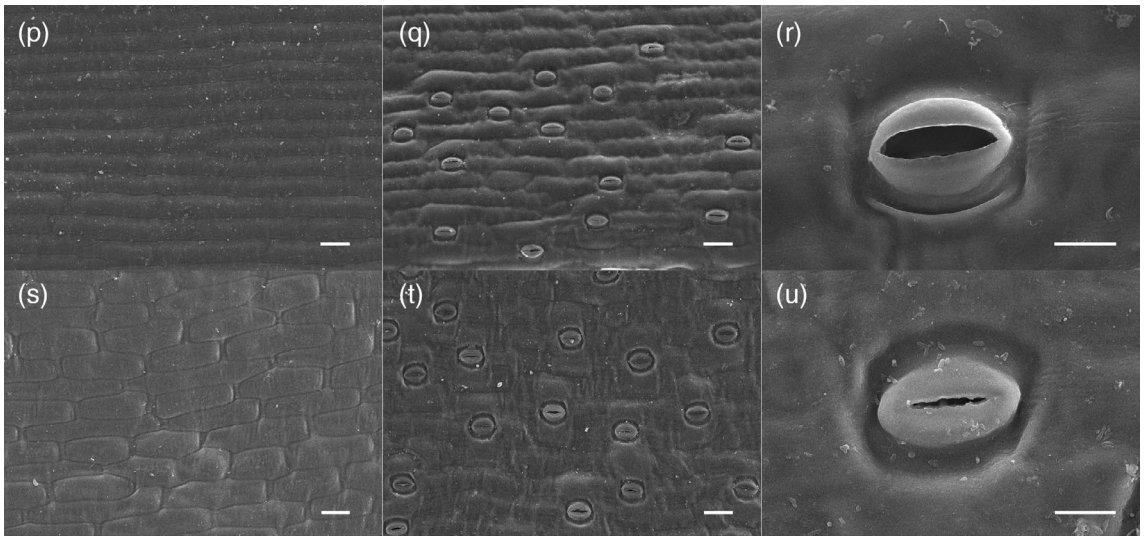


FIGURE 6 (Continued)

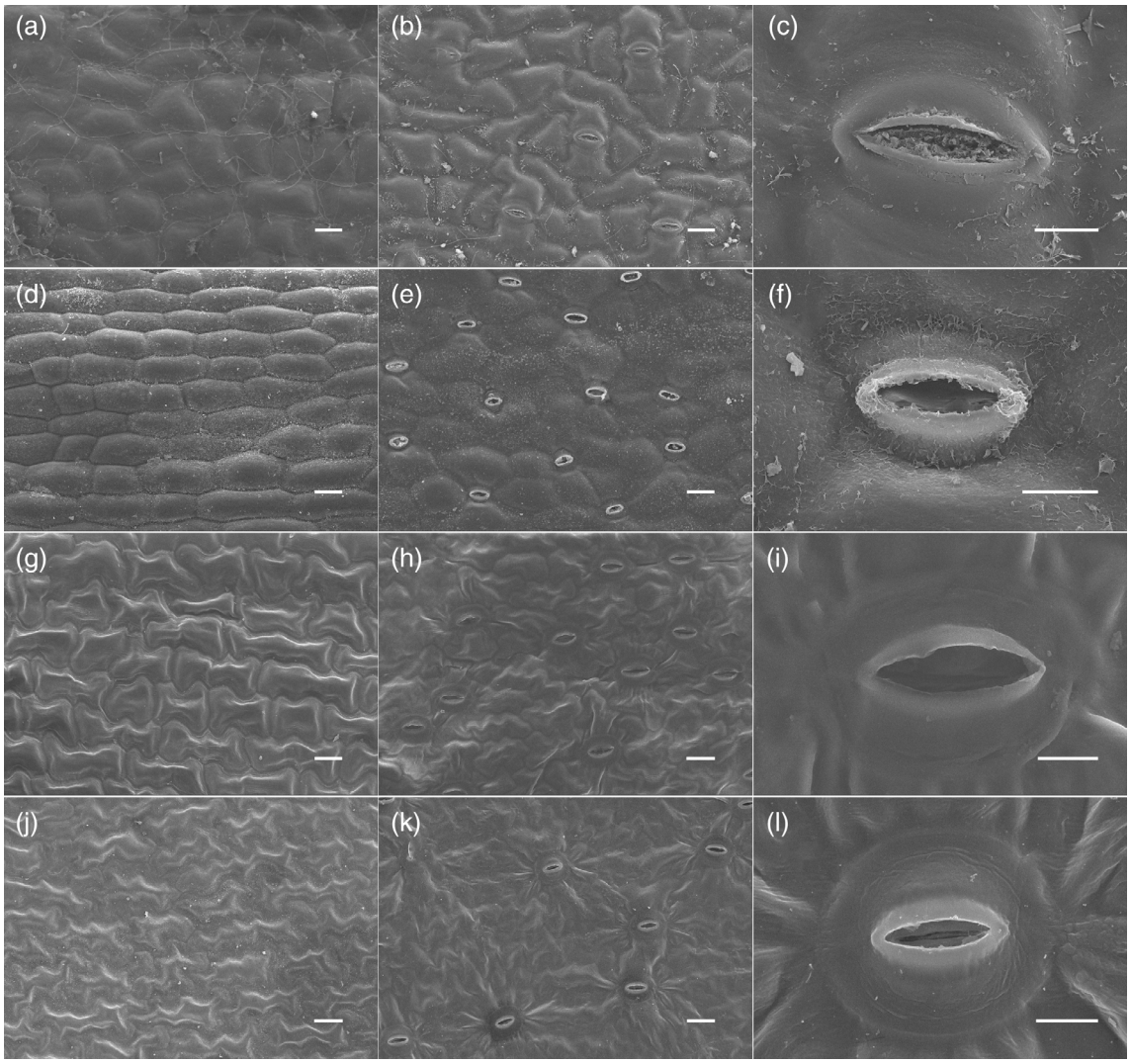


FIGURE 7 Scanning electron microscopy of the leaf epidermal morphology of Polygonateae. (a–c) *Disporopsis pernyi*, (d–f) *Heteropolygonatum altelobatum*, (g–i) *Maianthemum formosanum*, (j–l) *M. harae*, (m–o) *Polygonatum arisanense*, (p–r) *P. arisanense* var. *chingshuishanianum*, and (s–u) *P. arisanense* var. *formosanum*. (a, d, g, j, m, p, s) adaxial surface (scale bar: 30 μm), (b, e, h, k, n, q, t) abaxial surface (scale bar: 30 μm), and (c, f, i, l, o, r, u) stomata (scale bar: 10 μm)

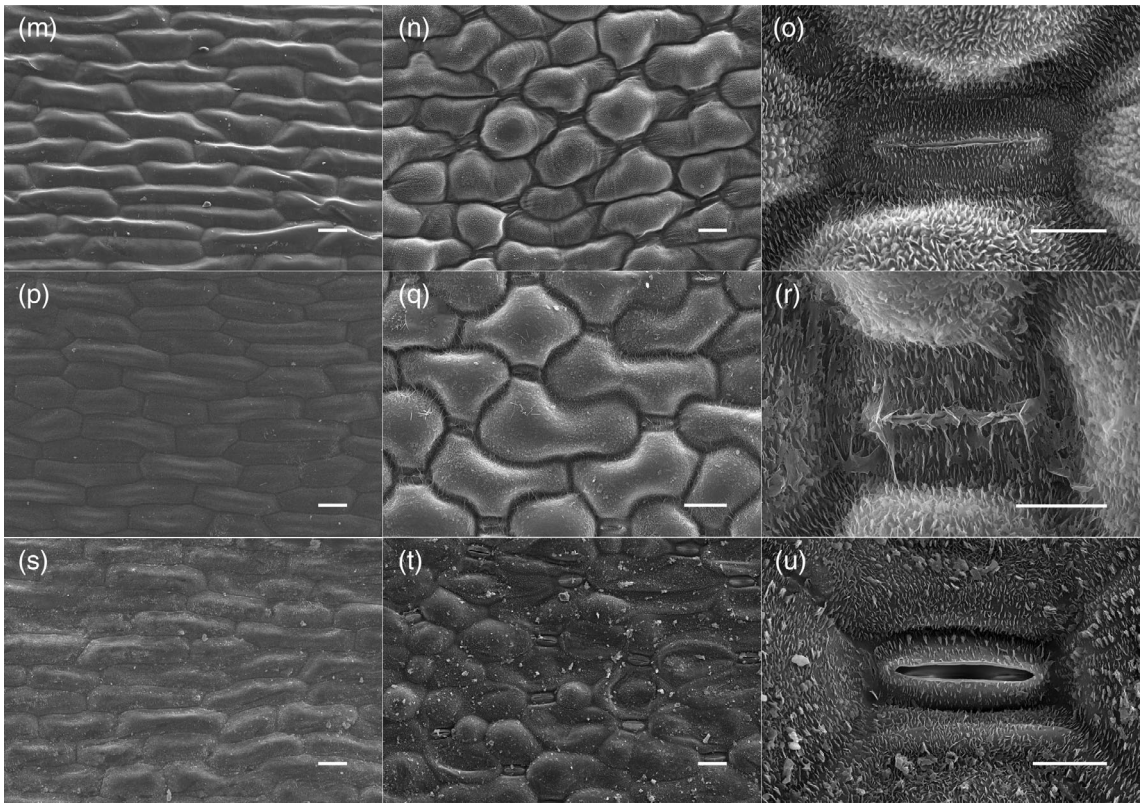


FIGURE 7 (Continued)

5.11 | Subsidiary cell type

Two subsidiary cell types were recorded, namely paracytic and anomocytic. The paracytic type was found only in *Aspidistra* (Figure 9a–i) and *Di. pernyi* (Figure 11a–c); all the other species belonged to the anomocytic type. Although the subsidiary cell types exhibited clear differences within some taxa, this characteristic would provide only limited systematic value.

5.12 | Subsidiary cell size

The subsidiary cell was between $47 \times 17\ \mu m^2$ (*Aspi. mushaensis* Hayata) and $78 \times 19\ \mu m^2$ (*Aspi. attenuata* Hayata). The subsidiary cell sizes of *Aspidistra* and *Di. pernyi* could not be differentiated.

6 | DISCUSSION

Most taxonomic study of Asparagaceae of Taiwan was based on morphology (Ying, 2000, included in Liliaceae *s.l.*) or karyotype analysis (Chang & Hsu, 1974; Wang, 1996, 1997). The epidermal morphology of Asparagaceae showed high heterogeneity and without any consistent characteristic that could be separated from related families. Some taxa had similar epidermal morphology to another family; for example, *Maianthemum* had an undulate anticlinal wall and anomocytic subsidiary cell similar to those of *Disporum* species of Colchicaceae (Kim et al., 2021). Thus, the results of epidermal morphology provided weak support on the familial level. However, at the generic level, several genera had unique epidermal morphology and exhibited conspicuous and stable differences, such as stomatal bands found only in *Liriope* and *Ophiopogon*. These findings were similar to those of Dai and Liang (1991).

Convallarieae comprises 10 genera distributed mainly in eastern and southeastern Asia (Conran & Tamura, 1998). Two genera, that is, *Aspidistra* and *Rohdea*, were found in Taiwan with five to six species. Based on the epidermal features, *Rohdea* was different from *Aspidistra* by having an isodiametric epidermal cell shape on the abaxial surface (vs. rectangular), larger cell size, and anomocytic subsidiary cells (vs. paracytic ones). Such epidermal morphology was also found in *Tupistra* (Ma & Hong, 1990). However, three species of *Aspidistra* could not be separated based on epidermal morphology. Wang et al. (2007) studied the epidermal morphology of nine *Aspidistra* species of China, which showed conspicuous differences in stomatal characteristics. However, these features were relatively similar to those of Taiwanese taxa. These findings implied that Taiwanese taxa were closely related.

The genera *Liriope*, *Ophiopogon*, and *Peliosanthes* comprised seven species in Taiwan. These genera are treated as a natural group in Asparagaceae due to their unusual fruit morphology (Dahlgren et al., 1985). Different systems have treated them as a single tribe, that is, Ophiopogoneae (Melchior & Weidermann, 1964), or two

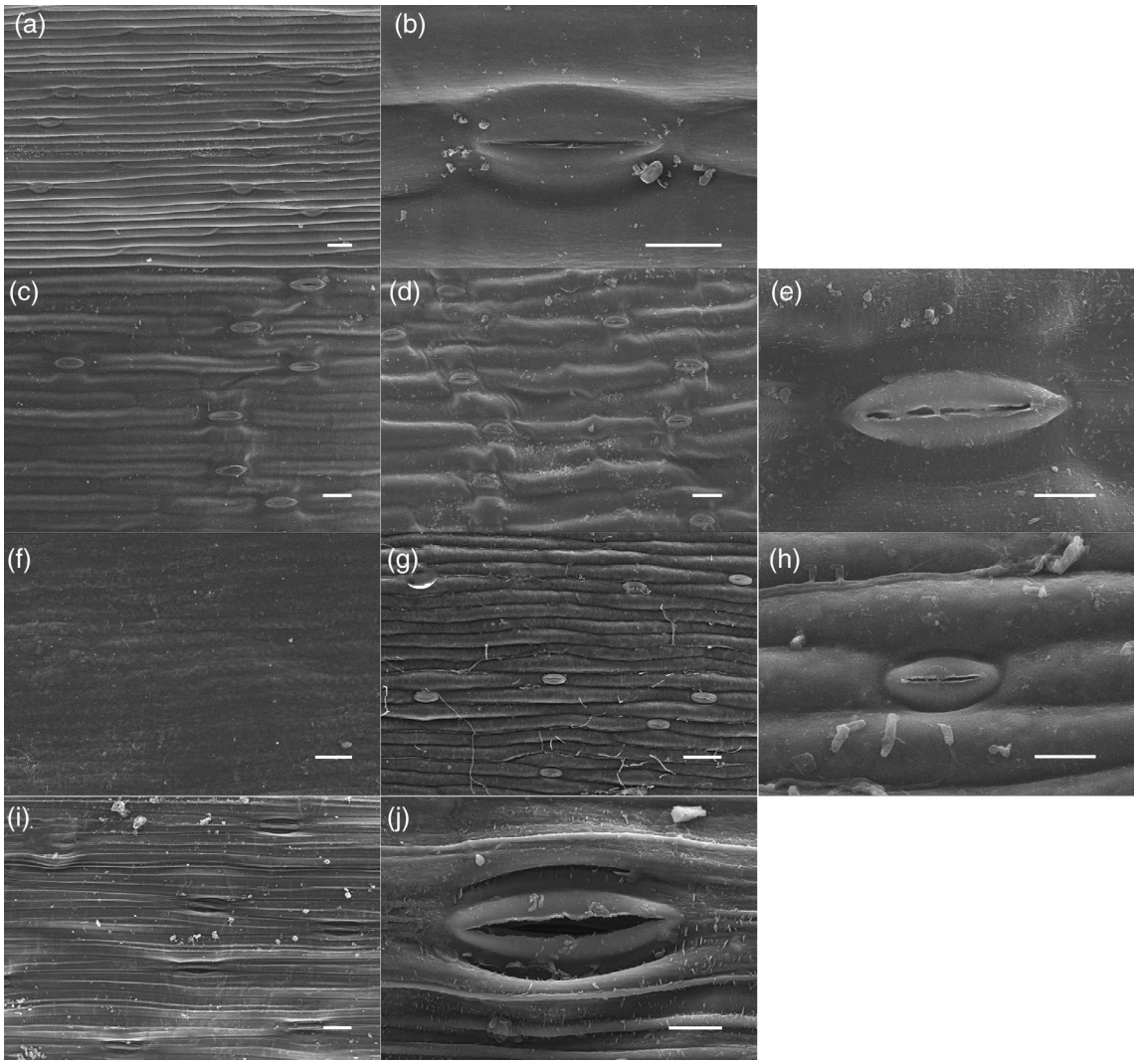


FIGURE 8 Scanning electron microscopy of the leaf epidermal morphology of other Asparagaceae taxa. (a, b) *Asparagus cochinchinensis*, (c–e) *Barnardia japonica*, (f–h) *Dracaena angustifolia*, and (i–j) *Thysanotus chinensis*. (b, e, h, j) stomata (scale bar: 10 μm), (c, f) adaxial surface (scale bar: 30 μm), (d, g) abaxial surface (scale bar: 30 μm), (a, i) cladode and leaf surface (scale bar: 30 μm)

tribes, that is, Ophiopogoneae (including *Ophiopogon* and *Liriope*) and Peliosantheae, of Liliaceae *s.l.* (*Peliosanthes*) (Hutchinson, 1973). Some other systems treated them as a single family, that is, Ophiopogonaceae, with two tribes (Takhtajan, 1997); or as a subfamily, that is, Ophiopogonoideae, of Convallariaceae with two tribes (Takhtajan, 2009). Among them, *Liriope* and *Ophiopogon* had stomatal bands which was absent in *Peliosanthes*. Such morphology implied that *Ophiopogon* and *Liriope* were more closely related than with *Peliosanthes*. Our results were supported by taxonomic treatments by Hutchinson (1973). *Ophiopogon* and *Liriope* were subsequently placed in a group independent from that of *Peliosanthes* following the treatment of Hutchinson (1973) and Takhtajan (1997, 2009) and the results of Dai and Liang (1991), Sang (1995), and Kim et al. (2010).

In Taiwan, Polygonateae includes four genera, *Disporopsis*, *Heteropolygonatum*, *Maianthemum*, and *Polygonatum*, and eight taxa. The four genera contain two distinct anticlinal forms (rounded and undulate). Rounded anticlinal wall was found in *Di. pernyi*, *H. altelobatum*, and *Polygonatum* (except *Po. arisanense* var. *arisanense*). Undulate anticlinal wall was found in *Maianthemum* and *Po. arisanense* var. *arisanense*.

The genus *Polygonatum* had three species treated by Ying (2000). The taxon distributed in Yangmingshan National Park of Northern Taiwan is often identified as *Po. odoratum* (Mill.) Druce var. *pluriflorum* Ohwi or *Po. arisanense* (Hayata, 1920; Ying, 2000) and was described as a new species *Po. formosanum* by Masamune and Simada (1936). Recently, this taxon was treated as a variety, *Po. arisanense* var. *formosanum*, in the revisional study of *Polygonatum* of Taiwan (Chao & Tseng, 2019). Such taxonomic opinion was also supported by epidermal morphology, *Po. arisanense* var. *formosanum* has a rounded anticlinal wall, which differs from its autonym with undulate one.

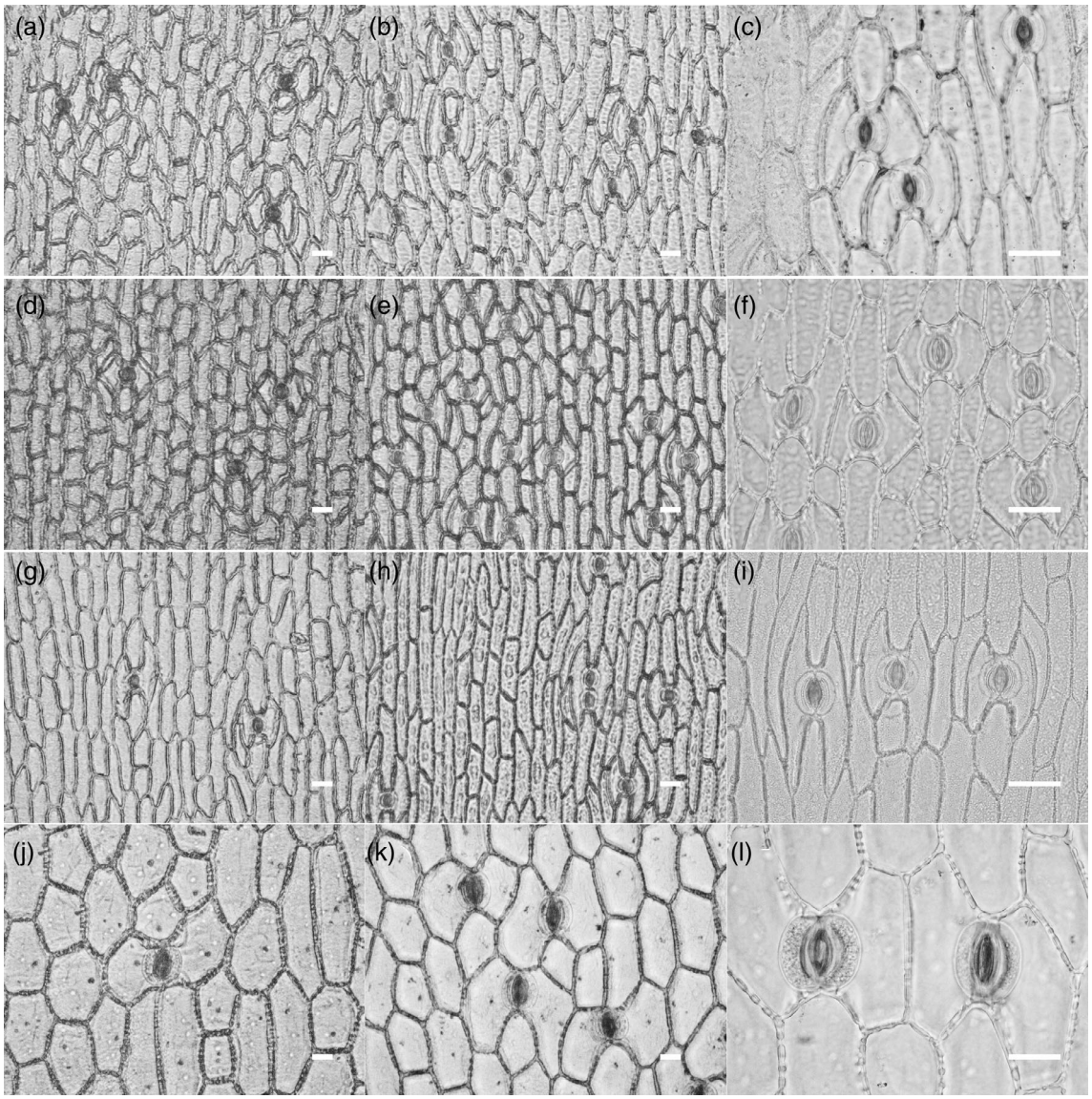


FIGURE 9 Light microscopy of the leaf epidermal morphology of Convallarieae. (a–c) *aspidistra attenuata*, (d–f) *a. daibuensis*, (g–i) *a. mushaensis*, and (j–l) *Rohdea chinensis* (scale bar: 30 μm)

Tamura (1993) classified *Polygonatum* into two sections based on staminal filaments and karyotypes. All Taiwanese species belonged to sect. *Polygonatum*. Lü et al. (2000) studied the epidermal morphology of 32 species in China, and the results led to classification into two groups. The first group was the alternate phyllotaxis taxa (sect. *Polygonatum*), characterized by isodiametric epidermal cell shape and undulated or arched anticlinal wall. The second group comprised opposite or verticillate phyllotaxis taxa (sect. *Verticillatum*), which had epidermal morphology of rectangular or rhomboid cell shape and straight or arching anticlinal wall. Nevertheless, some species with intermediate types, such as *Po. kingianum* (Lü et al., 2000) and *Po. arisanense* var. *formosanum*, were found; hence the intrageneric classification of *Polygonatum* is still a work in progress.

Heteropolygonatum was described by Tamura, Ogisu, and Xu (1997) as having different morphology and karyology, and most species were included in *Polygonatum* or *Maianthemum* (*Smilacina*) (Tamura et al., 2000; Tamura, Ogisu, & Xu, 1997). The genus was similar to *Disporopsis* or *Polygonatum* in morphology; all these three genera had axillary inflorescences, floral tubes, and berry fruits. *Heteropolygonatum alteobatum* was a new combination designated by Chao et al. (2013); this species was treated in *Polygonatum* in its protologue (Hayata, 1915). The anticlinal wall was rounded form, which may have a closer relationship to *Di. pernyi* than to *Maianthemum*. This finding was similar to the results of the molecular study of Tamura, Schwarzabh, Kruse, and Reski (1997), which revealed that epidermal morphology could reflect the phylogeny of Polygonateae to a certain extent.

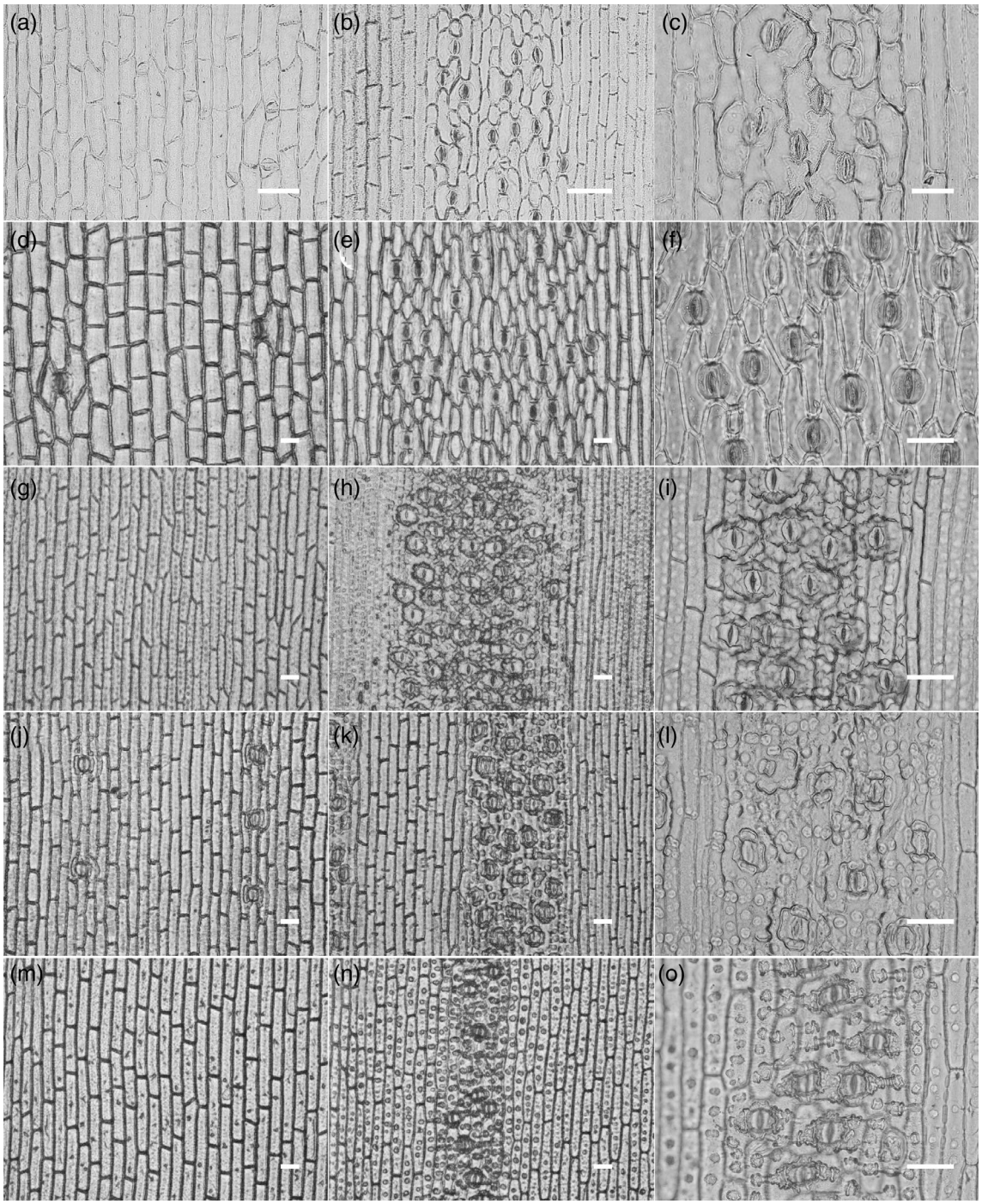


FIGURE 10 Light microscopy of the leaf epidermal morphology of Ophiopogoneae. (a–c) *Liriope graminifolia*, (d–f) *L. muscari*, (g–i) *L. spicata*, (j–l) *Ophiopogon intermedius*, (m–o) *O. reversus*, (p–r) *Peliosanthes arisanensis*, and (s–u) *P. kaoi* (scale bar: 30 μm)

Dracaena angustifolia was the only species of Dracaeneae in Taiwan. The epidermal features of this species were similar to the observations of Klimko and Wiland-Szymańska (2008), which had anomocytic subsidiary cell and glabrous leaves. According to the study of Klimko and Wiland-Szymańska (2008), the stomata of xerophytic *Dracaena* species often had cuticle appendages and high-density stomata on both surfaces of leaves. These features were different from *Dr. angustifolia*, which only had stomata on the abaxial surface and without cuticle appendages around the stomata. Compared to the phylogenetic study of *Dracaena* (Lu & Morden, 2014), the species with similar epidermal traits did not in a monophyletic group. Therefore, the epidermal morphology of *Dracaena* might relate to the habit type rather than the phylogenetic relationship.

Genera *Asparagus*, *Barnardia*, and *Thysanotus* all had only one species in Taiwan and had only limited epidermal reports. The epidermis of cladodes of *Asparagus* was described for the first time, and the stomata

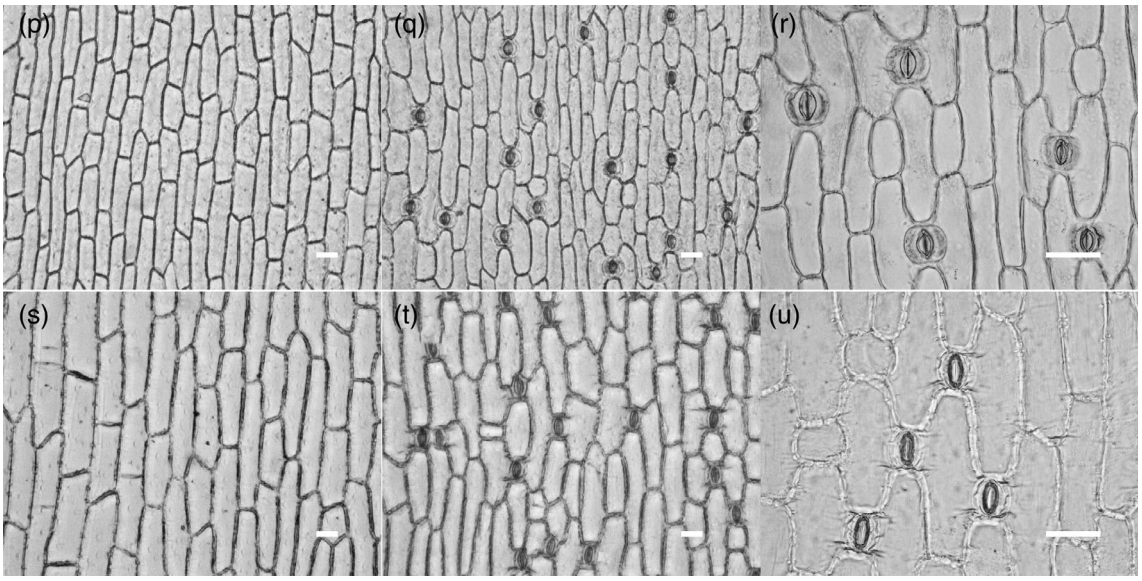


FIGURE 10 (Continued)

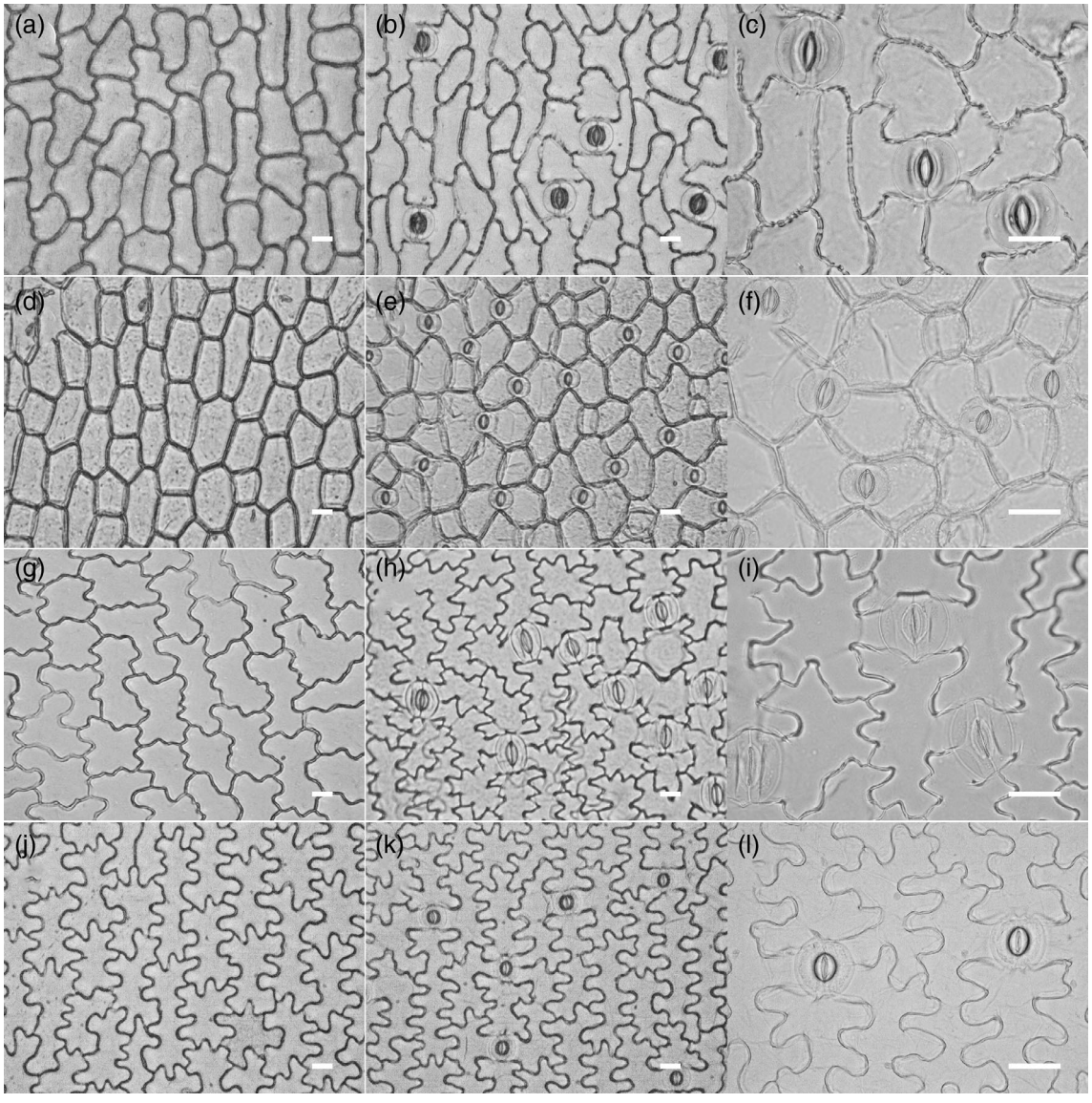
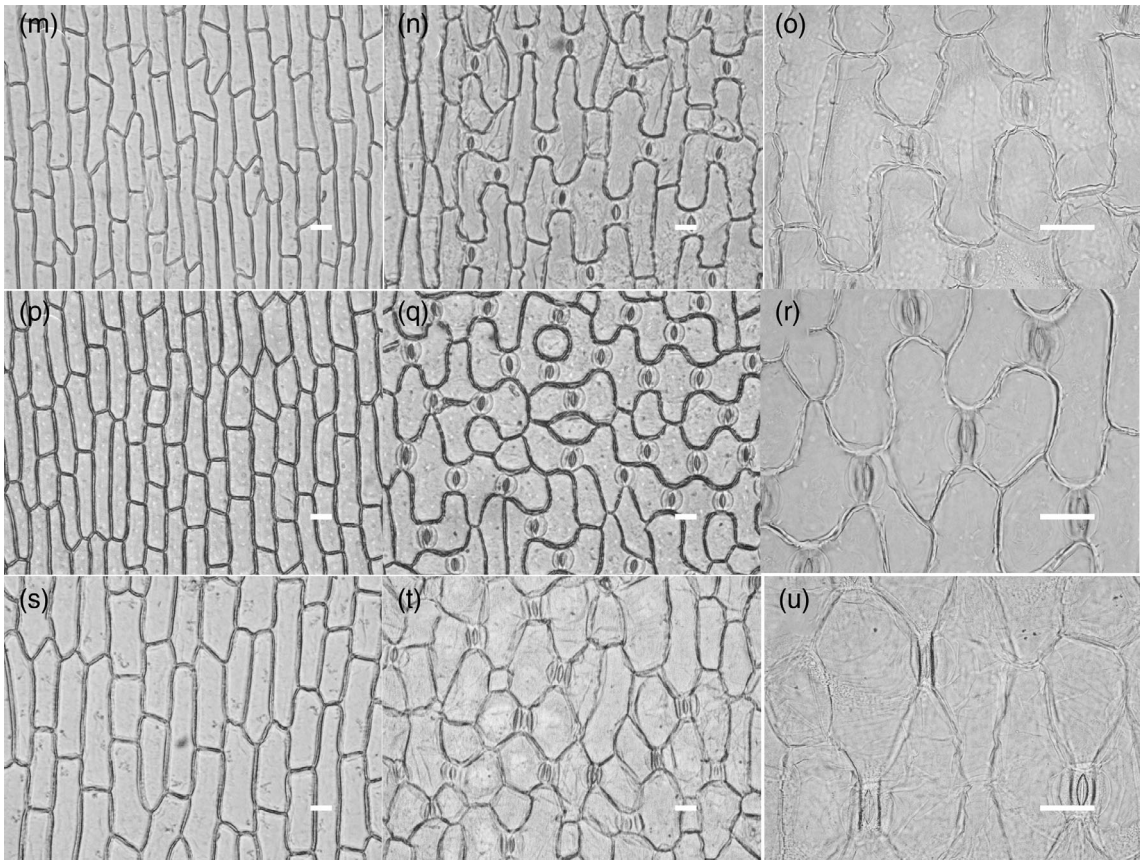


FIGURE 11 Light microscopy of the leaf epidermal morphology of Polygonateae. (a–c) *Disporopsis pernyi*, (d–f) *Heteropolygonatum altelobatum*, (g–i) *Maianthemum formosanum*, (j–l) *M. harae*, (m–o) *Polygonatum arisanense* var. *arisanense*, (p–r) *P. arisanense* var. *chingshuishanianum*, and (s–u) *P. arisanense* var. *formosanum*. (a, d, g, j, m, p, s) adaxial surface, (b, e, h, k, n, q, t) abaxial surface, (c, f, i, l, o, r, u) stomata (scale bar: 30 μm)



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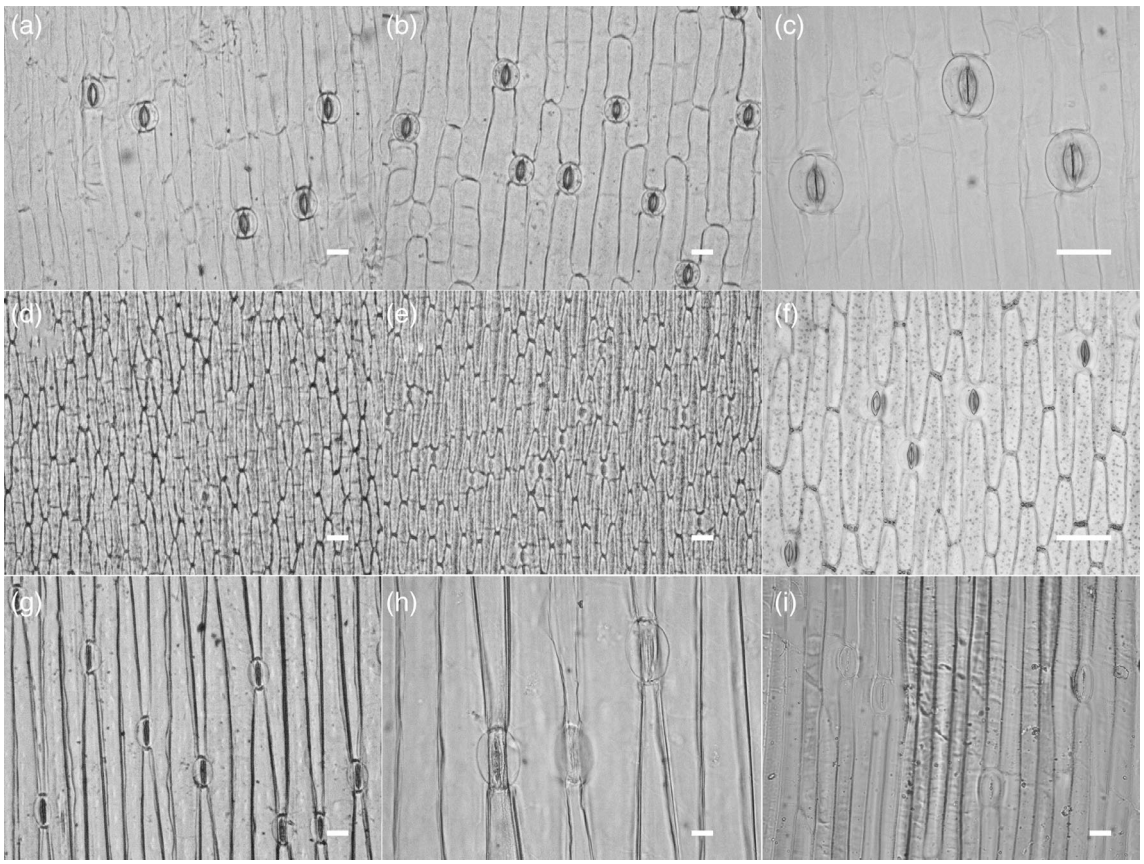

FIGURE 12 Light microscopy of the leaf epidermal morphology of other Asparagaceae taxa. (a–c) *Barnardia japonica*, (d–f) *Dracaena angustifolia*, (g, h) *Thysanotus chinensis*, (i) *Asparagus cochinchinensis*. (a, d) adaxial surface, (b, e) abaxial surface, (c, f) stomata, (h, i) leaf and cladode surface (scale bar: 30 μm)

were sunken in this species, such character was often found in some xerophyte species (Denk et al., 2014; Lyshede, 1979), which was meeting the habit of this species. The related studies revealed the high variation of cladode morphology and habitat type. Therefore, the pattern of epidermal variation of *Asparagus* needs further study to elucidate.

Barnardia japonica was the only species of this genus distributed in East Asia. Compared to the other species of Hyacinthaceae (=Scilloideae sensu APG IV), the epidermal traits were similar to that of *Drimiopsis* (Lynch et al., 2006), *Scilla* (Kandemir et al., 2016), and *Ledebouria* species (Venter, 1993), which had rectangular epidermal cells and with few papillae on epidermal cells. These findings might imply that the epidermal morphology could not apply the systematic value of Scilloideae. The epidermal morphology variation might be related to the xeric habitats and leaf morphology (Lynch et al., 2006).

The epidermal morphology of *T. chinensis* confirmed the observation of Brittan (1970), which had anomocytic subsidiary cells and sunken stomata. Although some traits might link with the habitat, for example, sunken stomata, only a few species had been observed, the pattern of epidermal characters of the genus needs further study.

The epidermal morphology and habitat type of Asparagaceae showed high variation among the different taxa. Considering the position and function of the epidermis, the epidermal morphology may give insights into adaptation to different environments; however, the relationship between specific morphology and climate is not always predictable (Haworth & McElwain, 2008). For example, sunken stomata were regarded as a trait of xerophyte species. However, some species (e.g., *Ophiopogon* or *Polygonatum*) in the present study with such type of stomata did not always grow in an arid environment. Therefore, additional studies are needed to understand the relationship between the epidermis and habitat.

7 | CONCLUSION

The results reveal that epidermal features of Asparagaceae provide limited value at the familial level, but the morphological traits of the anticlinal wall and stomata still provide systematic value at tribe and genus levels. The high variation of epidermis and habitat types also implied the relationship between them, especially when the stomatal variation often linkage with environmental factors (e.g., humid or arid habitat), the significance of adaptation and evolution of epidermis to Asparagaceae is needed further study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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