

MICROMORPHOLOGY OF LEAF EPIDERMIS OF ELEVEN SPECIES IN *PIMPINELLA* (APIACEAE) FROM CHINA

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Abstract

The present study is the first to investigate the micromorphology of leaf epidermis of 11 species of *Pimpinella* in China by SEM. Several species examined are covered with unbranched epidermal hairs on the adaxial epidermis. Adaxial leaf epidermal cells are usually polygonal or irregular. The patterns of anticlinal walls of the adaxial leaf epidermal cells are straight or arched mostly. Primary waxy ornamentations are mainly dense stripes. In addition, secondary ornamentations of most samples are single or double ridges. Stripe ornamentations are usually distributed on the abaxial epidermis uniformly. Stomatal shape is usually ellipse. Inner margin of smooth outer stomatal ledge is mainly sinuate. In the face of such a complex group which is difficult to understand, comprehensive taxonomic and phylogenetic investigations of *Pimpinella* will undoubtedly benefit from these micromorphology data of leaf epidermis examined.

Key words: Anticlinal wall, Epidermal appendage, Ornamentation, Stomata.

Introduction

Pimpinella L. comprises about 150 species mainly distributed in Europe, Asia and Africa, with 39 species and 2 varieties in China (Pu, 1985). It was revised to include 44 species in this genus in the Flora of China (Pu & Watson, 2005). The usefulness of leaf epidermal micromorphological characters in distinguishing species and taxonomy in some members of Apiaceae has been highlighted (Feng *et al.*, 2009; Li *et al.*, 2017). Leaf epidermal features of eighteen *Pimpinella* species from tropical Africa were examined and it was indicated that the dicytic stomata was common for them (Abebe, 1992). Epidermal characters of *P. puberula* (DC.) Boiss. and *P. thellungiana* H. Wolff were reported and results showed that most of their stomatal complexes were anomocytic (Ostroumova & Kljuykov, 1991). Cuticular characters of leaves of *P. brachycarpa* (Kom.) Nakai and *P. koreana* (Y. Yabe) Nakai were recorded (Kim *et al.*, 1998). Moreover, stomatal types of seven Chinese and Himalayan *Pimpinella* members were examined (Ostroumova & Kljuykov, 2007). Morphological observation of *Pimpinella* species in China is still inadequate, even if the observation of pollen morphology of sixteen *Pimpinella* species from China has been completed (Wang *et al.*, 2012). As the first one to investigate the micromorphology of leaf epidermis of *Pimpinella* species in China by SEM, the major objective of this study is to enrich the systematics and taxonomy of *Pimpinella*.

Material and Methods

We have studied 12 accessions of 11 species of *Pimpinella* distributed in China in the present investigation (Table 1). All vouchers were deposited in the herbarium of Hengyang Normal University (HYNU). After washed in 95% ethanol solution, fragments of leaves were dried naturally. Then they were observed by scanning electron microscopic (SEM) method using JSM-6490LV. Length and width of stomatal apparatus were measured using KaryoType (Altnordu *et al.*, 2016) of ten samples under random field of vision. Leaf micromorphology terminology referred to previous studies (Hong *et al.*, 2001; Li *et al.*, 2017).

Results

Morphological characteristics of adaxial and abaxial epidermis of 12 accessions of *Pimpinella* under SEM are presented in Tables 2 and 3.

Characteristics of adaxial epidermis: Hairy leaves are not common in *Pimpinella* plants presently investigated. There are simple epidermal hairs on the epidermis of several observed plant species (Fig. 1). The adaxial epidermis of *P. rockii* H. Wolff is densely covered with unbranched hairs, it is the same of *P. rubescens* (Franch.) H. Wolff ex Hand.-Mazz.. The adaxial epidermis of *P. coriacea* (Franch.) H. Boissieu is sparsely hairy, mainly concentrated on the vein. It is similar to *P. candolleana* Wight & Arn. but on the abaxial epidermis. Both adaxial and abaxial epidermis of *P. diversifolia* DC. are sparsely covered with simple hairs, moreover, for the population (Shaoyang, Hunan), they are more densely hairy along veins of abaxial leaves. Similarly, there are sparse hairs on all two epidermis of *P. thellungiana*, with concentration on the veins of lower epidermis.

Among the plant species observed, the outline of epidermal cells on leaves of most species is clear (except *P. rubescens* and *P. yunnanensis* (Franch.) H. Wolff), and the shape of cells is polygonal or irregular (such as *P. purpurea* (Franch.) H. Boissieu, *P. smithii* H. Wolff, *P. thellungiana* and *P. vallecuculosa* K.T. Fu). It is shown that the cell contours of *P. purpurea* and *P. vallecuculosa* are concave to the upper epidermis, compared to convex ones of other accessions. Anticlinal walls of polygonal epidermal cell are straight or arched, and them of irregular cell are sinuate, sinuous or sinuate.

Most of the primary wax ornamentation patterns on the upper epidermis are dense strips, and a few (*P. coriacea*, *P. rhomboidea* Diels and *P. vallecuculosa*) are sparse strips. Most of the secondary ornamentation types are continuous ridges (except *P. rubescens* and *P. thellungiana*), and some of them (*P. coriacea* and *P. rhomboidea*) are continuous double ridges.

Table 1. Plant accessions from which micromorphology of leaf epidermis were investigated in this study.

Taxon	Location	Voucher no.
<i>Pimpinella candolleana</i> Wight et Arn.	Yunnan, China	w190804
<i>P. coriacea</i> (Franch.) de Boiss.	Yunnan, China	w19080201
<i>P. diversifolia</i> DC.(1)	Hunan, China	w150827
<i>P. diversifolia</i> DC.(2)	Sichuan, China	LCY2019081802
<i>P. purpurea</i> (Franch.) de Boiss.	Yunnan, China	w19080202
<i>P. rhomboidea</i> Diels	Sichuan, China	w19080801
<i>P. rockii</i> Wolff	Yunnan, China	w19080101
<i>P. rubescens</i> (Franch.) Wolff ex Hand.-Mazz.	Yunnan, China	w19080102
<i>P. smithii</i> Wolff	Sichuan, China	w19080802
<i>P. thellungiana</i> Wolff	Shanxi, China	w19082501
<i>P. vallecucosa</i> K. T. Fu	Chongqing, China	w190806
<i>P. yunnanensis</i> (Franch.) Wolff	Yunnan, China	w190803

Characteristics of abaxial epidermis: There are many stomata in the abaxial epidermis of leaves of each accession examined. Several species even own sparse stomata on the adaxial epidermis at the same time, such as *P. candolleana*, *P. coriacea*, *P. rockii*, *P. rubescens*, *P. yunnanensis*.

The stomatal ornamentation of most species is radiating, and only it of *P. candolleana* is surrounding. In terms of the distribution types of ornamentation, them of most species are evenly distributed on the abaxial epidermis, and only a few species (*P. purpurea*, *P. rhomboidea*, *P. rubescens* and *P. smithii*) own ornamentation distributed around the stomata only.

P. yunnanensis owns the relatively bigger stomata size among accessions investigated, with length of 21.09 μm and width of 12.41 μm . In addition, *P. rhomboidea* has the largest L/M ratio of 2.17.

Most of the stomata are elliptic, and only a few species (*P. rhomboidea* and *P. rubescens*) have spindle shaped stomata. Inner margins of outer stomatal ledge of accessions presently investigated is usually sinuate or nearly smooth. Moreover, there is no any ornamentation on the surface of outer stomatal ledge.

Discussion

For Apiaceae, a natural group which is famous for fruit characteristics, but difficult to classify below the family level, it is necessary to promote and improve the systematic classification and evolution study of it by comprehensive measures and methods. In recent years, micromorphology studies of leaf epidermis have been widely applied in many taxa, and provided important evidence for taxonomy and phylogeny (Feng *et al.*, 2009; Guo *et al.*, 2016; Guo *et al.*, 2017; Li *et al.*, 2017).

The micromorphological characteristics of accessions examined are consistent to a certain extent, including polygonal or irregular adaxial leaf epidermal cells with straight or sinuate anticlinal walls, generally striate waxy ornamentations on both leaf sides (similar to findings obtained from tropical African species (Abebe, 1992)) and ellipse stomata mainly present on the abaxial epidermis. They can provide reference for genus-related taxonomic and systematic research. At the same time, some interesting results have attracted our attention.

The adaxial epidermis of *Pimpinella rockii* and *P. rubescens* are both densely covered with unbranched hairs, possibly related to the evolutionary strategies adopted by plants to adapt to alpine environment. *P. rockii* usually grows in the crevice of rock wall or alpine meadow at an elevation of 2800-3500 meters. Similarly, *P. rubescens* always grows on the slope of valley or the rock beside the ditch at an elevation of 3000-3600 meters (Pu, 1985). Leaf epidermal hairs can reflect sunlight, maintain temperature and reduce water transpiration. It is obviously important for the alpine plants (Feng *et al.*, 2009).

According to the vertical wall pattern of leaf epidermal cells, the evolution trend is from straight to sinuate, and then to sinuate (Hou *et al.*, 2015). In the present investigation, anticlinal walls of adaxial epidermal cell of most accessions are nearly straight (consistent with their tropical African relatives stated (Abebe, 1992)) or sinuate (e.g. *Pimpinella thellungiana*, which is investigated similarly as curved (Ostroumova & Kljuykov, 1991)). It is interesting to notice that anticlinal walls of adaxial epidermal cell of *P. smithii* and *P. vallecucosa* are sinuate (which is recorded as straight in Ostroumova & Kljuykov, 2007) and sinuous separately. In previous molecular phylogenetic analysis (Wang *et al.*, 2014), *P. smithii* and *P. vallecucosa* were not presented in the tribe Pimpinelleae but the tribe Selineae. Based on the molecular data, they are always closer to the genus *Angelica* L., even if they have typical different fruit types from *Angelica* species (Pu & Watson, 2005). More efforts are expected to clarify relationship between these two species and genera allied closely.

It is not surprised to find that *P. purpurea* owns some unusual leaf epidermal characters. The cell contours are concave to the upper epidermis, and these cells own continuous secondary waxy ornamentation of conspicuous ridges. In terms of morphology, the purple petals of *P. purpurea* have base shortly clawed, apex mucronate, and without incurved lobule (Pu & Watson, 2005), different from others of the same genus. Moreover, this species was reported to own smaller pollen grains and unique nearly rhombic pollen grain shape, compared to other *Pimpinella* members (Wang *et al.*, 2012). Another thing is that *P. purpurea* falls into the East Asia clade, which is far from type species of *Pimpinella* based on the molecular data (Wang *et al.*, 2014). It is expected to make clear the proper taxonomic place of it soon.

Table 2. Morphological characteristics of adaxial epidermis of 12 accessions of *Pimpinella* under SEM.

No.	Taxon	Primary waxy ornamentation	Secondary ornamentation	Epidermal appendage	Cell contour	Cell shape	Anticlinal walls of epidermal cells	Occurrence of stomata
1.	<i>P. candolleana</i>	Dense stripes	Continuous ridges	No occurrence	Clear(convex)	Polygonal	Straight or arched	+
2.	<i>P. coriacea</i>	Sparse stripes	Continuous double ridges	Unbranched epidermal hairs	Clear(convex)	Polygonal	Straight or arched	+
3.	<i>P. diversifolia</i> (1)	Dense stripes	Continuous ridges	Unbranched epidermal hairs	Clear(convex)	Polygonal	Straight or arched	-
4.	<i>P. diversifolia</i> (2)	Dense stripes	Continuous ridges	Unbranched epidermal hairs	Clear(convex)	Polygonal	Straight or arched	-
5.	<i>P. purpurea</i>	Dense stripes	Continuous ridges	No occurrence	Clear(concave)	Irregular	Sinuolate	-
6.	<i>P. rhomboidea</i>	Sparse stripes	Continuous double ridges	No occurrence	Clear(convex)	Polygonal	Sinuolate	-
7.	<i>P. rockii</i>	Dense stripes	Continuous ridges	Unbranched epidermal hairs	Clear(convex)	Polygonal	Straight or arched	+
8.	<i>P. rubescens</i>	Dense stripes	Discontinuous ridges	Unbranched epidermal hairs	Unclear		Sinuolate	+
9.	<i>P. smithii</i>	Dense stripes	Continuous ridges	No occurrence	Clear(convex)	Irregular	Sinate	-
10.	<i>P. thellungiana</i>	Dense stripes	Discontinuous ridges	Unbranched epidermal hairs	Clear(convex)	Irregular	Sinuolate	-
11.	<i>P. vallecuculosa</i>	Sparse stripes	Continuous ridges	No occurrence	Clear(concave)	Irregular	Sinuuous	-
12.	<i>P. yunnanensis</i>	Dense stripes	Continuous ridges	No occurrence	Unclear		Sinuolate	+

Table 3. Morphological characteristics of abaxial epidermis of 12 accessions of *Pimpinella* under SEM.

No.	Taxon	Stomatal ornamentation	Ornamentation distribution	Stoma					
				Length/ μ m	Width/ μ m	L/W	Shape	Inner margin of outer stomatal ledge	Ornamentation of outer stomatal ledge
1.	<i>P. candolleana</i>	Surrounding	Uniform	18.36	9.39	1.95	Ellipse	Sinuolate	Smooth
2.	<i>P. coriacea</i>	Radiating	Uniform	19.68	11.49	1.72	Ellipse	Nearly smooth	Smooth
3.	<i>P. diversifolia</i> (1)	Radiating	Uniform	19.32	11.90	1.63	Ellipse	Sinuolate	Smooth
4.	<i>P. diversifolia</i> (2)	Radiating	Uniform	20.24	11.02	1.84	Ellipse	Sinuolate	Smooth
5.	<i>P. purpurea</i>	Radiating	Partial	14.31	8.90	1.61	Ellipse	Sinuolate	Smooth
6.	<i>P. rhomboidea</i>	Radiating	Partial	14.66	6.76	2.17	Fusiform	Sinuolate	Smooth
7.	<i>P. rockii</i>	Radiating	Uniform	17.67	9.39	1.90	Ellipse	Nearly smooth	Smooth
8.	<i>P. rubescens</i>	Radiating	Partial	17.40	8.40	2.08	Fusiform	Sinuolate	Smooth
9.	<i>P. smithii</i>	Radiating	Partial	16.67	10.36	1.62	Ellipse	Nearly smooth	Smooth
10.	<i>P. thellungiana</i>	Radiating	Uniform	19.21	12.12	1.60	Ellipse	Nearly smooth	Smooth
11.	<i>P. vallecuculosa</i>	Radiating	Uniform	17.72	8.81	2.03	Ellipse	Sinuolate	Smooth
12.	<i>P. yunnanensis</i>	Radiating	Uniform	21.09	12.41	1.70	Ellipse	Nearly smooth	Smooth

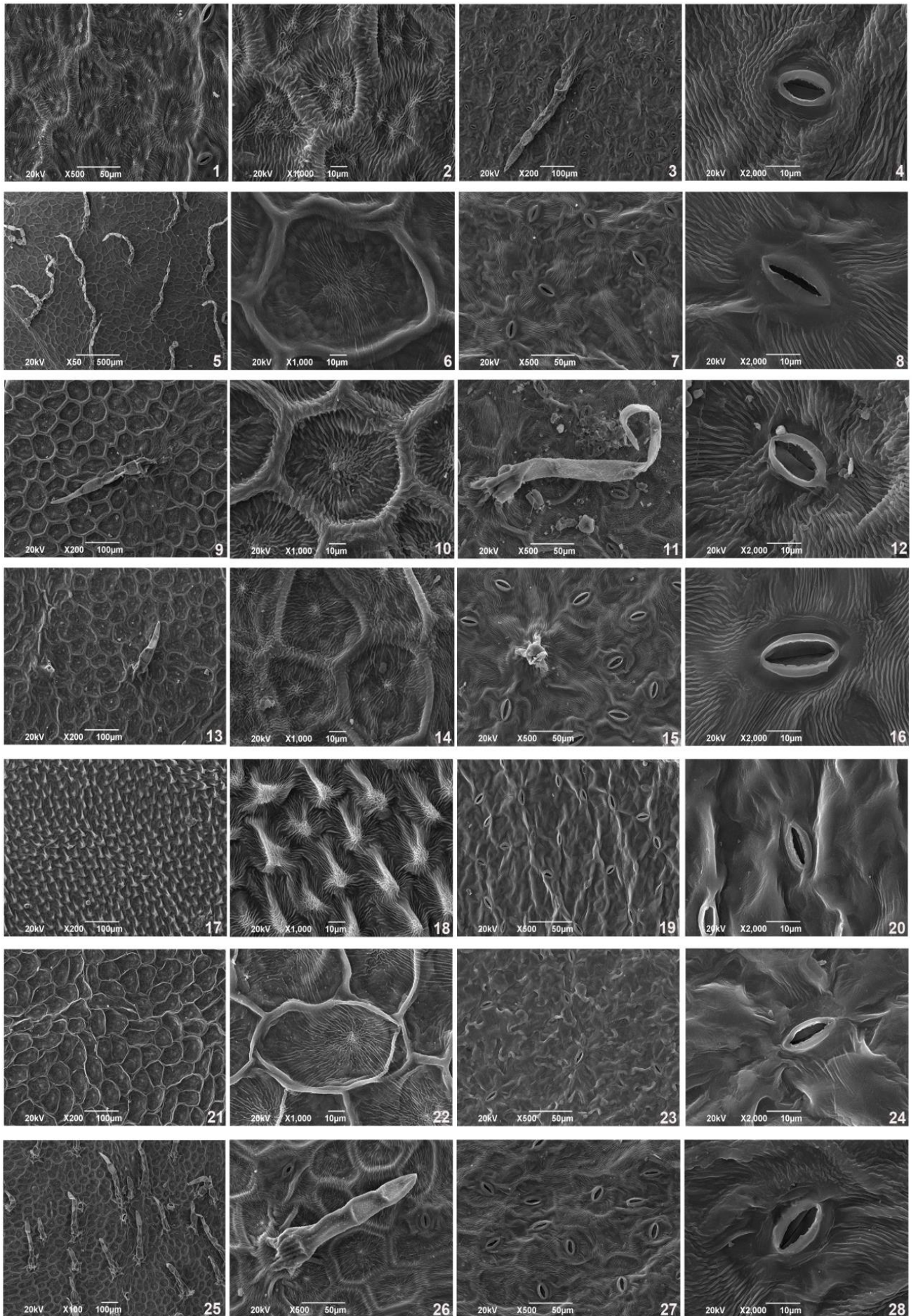


Fig. 1 (Cont'd.)

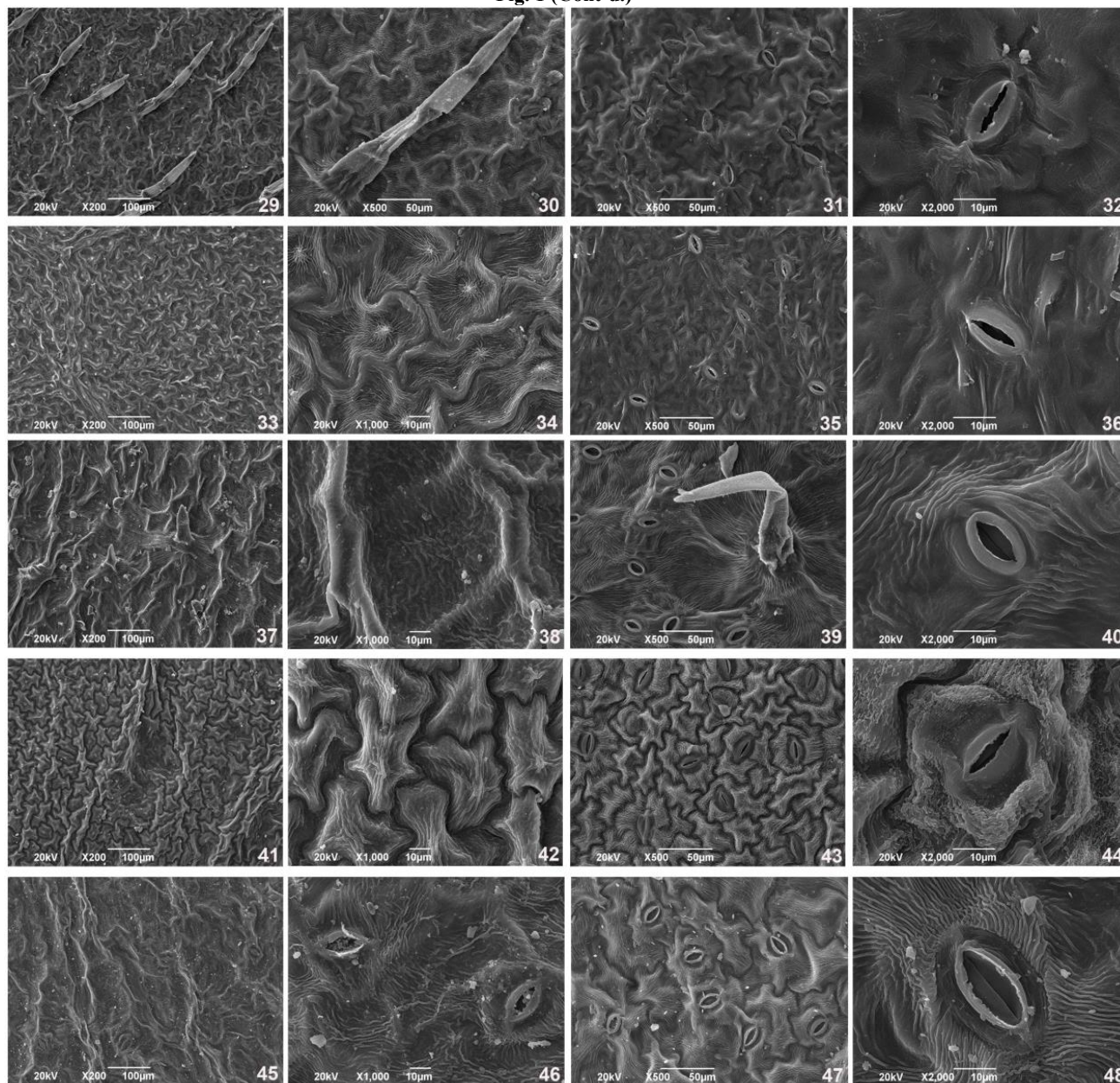


Fig.1 Epidermis of 11 *Pimpinella* species under the scanning electron microscope (adaxial epidermis; adaxial epidermis; abaxial epidermis; abaxial epidermis)
 1-4 *Pimpinella candolleana*; 5-8 *P. coriacea*; 9-12 *P. diversifolia* (Shaoyang, Hunan);
 13-16 *P. diversifolia* (Maoxian, Sichuan); 17-20 *P. purpurea*; 21-24 *P. rhomboidea*; 25-28 *Pimpinella rockii*; 29-32 *P. rubescens*; 33-36 *P. smithii*; 37-40 *P. thellungiana*;
 41-44 *P. valleculosa*; 45-48 *P. yunnanensis*

Several species including *P. candolleana*, *P. coriacea*, *P. rockii*, *P. rubescens*, *P. yunnanensis* were clustered in the Clade II of *Pimpinella* “core group” (Wang *et al.*, 2014). Similar to the closely related species, the sparse stomata on the adaxial epidermis and densely distributed stomata on the abaxial epidermis of these species may be adaptation to the environment. As for *P. diversifolia*, also one of the Clade II of *Pimpinella* “core group”, two populations in present investigation own only stomata on the abaxial epidermis. *P. diversifolia* is pretty widely distributed in China, even in the Central, East and Southeast Asia (Pu & Watson, 2005), combined with the results of such presence or absence of stomata from the

adaxial epidermis of samples geographically distributed (Ostroumova & Kljuykov, 2007), additional samplings are required in the subsequent work.

Conclusions

The micromorphology of leaf epidermis of 11 species of *Pimpinella* in China was observed by SEM herein. The results show that five species examined are covered with unbranched epidermal hairs on the adaxial epidermis, compared with other six species without hairy appendage. The shapes of adaxial leaf epidermal cells are usually polygonal or irregular. The patterns of anticlinal walls of the

adaxial leaf epidermal cells are straight or arched, and sinuate, sinuous or sinuate. Primary waxy ornamentations are mainly dense stripes. Secondary ornamentations of most samples are single or double ridges. The stomatal apparatuses are present only on the abaxial epidermis of seven accessions (except for *P. candolleana*, *P. coriacea*, *P. rockii*, *P. rubescens* and *P. yunnanensis*). Stripes ornamentations are usually distributed on the abaxial epidermis uniformly. Stomatal shape is usually ellipse. Inner margin of smooth outer stomatal ledge is mainly sinuate or nearly straight. It may be suggested the micromorphology of leaf epidermis will be useful for comprehensive taxonomic and phylogenetic studies of complicated genus *Pimpinella*.

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