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Epidermal morphology of *Ligusticum* (Apiaceae) from China

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We examined 15 species of the genus *Ligusticum* (Apiaceae) from China using LM (light microscopy) and SEM (scanning electron microscopy). Different species have irregular and polygonal epidermal cells and four different stomatal types. Almost all species have different types of stomata on the same leaf surface. Ridges and striations on the cuticular membrane are common features in *Ligusticum*, and special striation papillae are found in three species. Regarding its epidermal characters, *Ligusticum* is not supported as a natural genus. *Tilingia* is suggested as being restored from *Ligusticum*. The monophyly of *Ligusticopsis* is not supported and it cannot be segregated from *Ligusticum* by epidermal characters. We confirm a very close relationship between *Ligusticum* and *Conioselinum* and discuss some of the valuable taxonomic characters of *Ligusticum*.

Key words: leaf epidermis, light microscopy, morphology, scanning electron microscopy, taxonomy

Introduction

Ligusticum is a genus in the flowering plant family Apiaceae and has more than 60 species globally. There are 40 species known from China, 35 of which are endemic. A typical north-temperate genus, *Ligusticum* is widely distributed all over Asia, Europe and North America. It has two diversity centers, one in the Himalayas, the other in North America. The majority of the species found in China are distributed in the southwest, in subalpine coniferous forests and forest edges, alpine scrub, and meadows.

Many characters traditionally used to distinguish the genus or species, especially the flowers, vegetative organs and mericarps, are very diverse and gradually varying. These make *Ligusticum* one of the morphologically most difficult genera in the Apiaceae. The circumscription of *Ligusticum* has considerably varied (Drude 1898, Leute 1969). Its relationships with the putatively allied genera *Cnidium*, *Hymenidium*, *Pachypleurum*, *Paraligusticum*, *Rupiphila*, *Selinum*, *Tilingia*, and *Ligusticopsis* are still under study (Pu & Watson 2005), especially whether or not to include *Tilingia* and

Ligusticopsis in *Ligusticum* (Regel 1858, Koso-Poljansky 1916, Hiroe & Constance 1958). The delimitation from *Selinum* and *Conioselinum* was discussed by Pimenov *et al*. (2001, 2003). Current molecular work shows that *Ligusticum* is not a monophyletic genus because its nomenclatural type, *L. scoticum*, is distant from all other *Ligusticum* species (Katz-Downie *et al*. 1999, Downie *et al*. 2000a, 2000b, 2001, 2002, 2004, Spalik *et al*. 2004). However only a small number of *Ligusticum* species were included in those studies, and they did not include the majority of *Ligusticum* species from China, despite the fact that the Chinese flora contains the greatest number of *Ligusticum* species.

The taxonomic significance of epidermal morphology is well documented in some of the botanical literature (Watson 1962, Guyot 1966, 1971, Dehgan 1980). However, such information is scanty for *Ligusticum*. This paper presents epidermal observations on 15 *Ligusticum* species from China. Our main objective was to survey the leaf epidermis for taxonomic characters that might assist in identification and understanding of the systematic position of *Ligusticum* and variations within it. Several species of *Ligusticum* are of medicinal importance and the knowledge of epidermis should be useful for species identification. The epidermal characters of five species are treated for the first time in this paper.

Material and methods

We collected mature leaves of 15 species from herbaria specimens or in the field (*see* Table 1). Voucher specimens were deposited in the Herbarium of Sichuan University (SZ). Because most leaves of the 15 species were very small, complete leaves were taken when the area was smaller than 10 mm^2, and segments were taken from the median area of each leaf when the area was larger than 10 mm^2. Two to five specimens of each species were used, depending on whether the species were from different locations.

All specimens were boiled in 1% NaOH solution for about 5–10 min until they became transparent, rinsed in distilled water, then lightly

Table 1. Specimens of *Ligusticum* and their origins. All specimens are deposited in SZ.

Species	Location	Herbarium sheet	Date	Altitude (m)	Collector
L. acuminatum	Jinyang County, Sichuan Province	00031037	VII.1956	3100	Di-Ping He
L. ajanense	–	00031039	–	–	Yu-Ting Wu
L. angelicifolium	Baoxing County, Sichuan Province	00031040	VII.1954	3000	Zi-Pu Song
	Nanchuan County, Sichuan Province	00031046	VIII.1957	2080	Ji-Hua Xiong
	Leibo County, Sichuan Province	00031049	VII.1983	2190	Ming-You He
L. brachylobum	Leibo County, Sichuan Province	00031051	VIII.1989	2190	Ming-You He
L. daucoides	Emei County, Sichuan Province	00031062	X.1956	3150	Guo-Feng Li
	Muli County, Tibet	00031061	IX.1978	3900	Qing-Shen Zhao
L. franchetii	Muli County, Tibet	00031068	IX.1979	3950	Qing-Shen Zhao
L. involucratum	Muli County, Tibet	00031071	IX.1978	3500	Qing-Shen Zhao
	Fugong County, Yunnan Province	00031073	VIII.1934	4000	Xi-Tao Cai
L. jeholense	Laiyuan County, Hebei Province, 1600 m	00031075	VIII.1959	1600	Yu-Ting Wu
L. multivittatum	Muli County, Tibet	00031079	VIII.1978	4800	Qing-Shen Zhao
	Leibo County, Sichuan Province	00031082	VI.1973	3600	Yu-Ting Wu
L. scapiforme	Kongding County, Sichuan Province	00031116	VII.1987	3800	Zheng-Biao Zhao
	Muli County, Sichuan province	00031104	VII.1978	4600	Qing-Shen Zhao
L. sikiangense	Kangding County, Sichuan Province	00031120	VII.1981	4100	Zheng-Biao Zhao
	Muli county, Sichuan Province	00031125	VIII.1978	4900	Qin-Shen Zhao
L. sinense	Emei County, Sichuan Province	00031149	V.1959	1600	Yu-Hui Tao
	Fengjie County, Sichuan Province	00031144	IX.1964	1200	Hong-Fu Zhou
L. tenuissimum	Songpan County, Sichuan Province	00361560	VIII.2006	3700	Na Sun
L. thomsonii	Seda County, Sichuan province	00031153	IV.1964	3703	Hong-Fu Zhou
L. reptans	Songpan County, Sichuan province	00361561	VIII.2006	3200	Na Sun

scraped with a stainless steel blade to release pieces of epidermis. These were stained in 1% safranin solution, dehydrated in an alcohol series, then mounted in Canada balsam. The slides were examined and photographed with an Olympus BH-2 light microscope.

For statistical analyses, we measured each character 20 times on random samples. We assumed the measurements to be normally distributed, although this might not be very accurate for some characters, such as the epidermal cell width. We calculated the stomatal index (SI) using the formula of Salisbury (1927): $[S/(S + E)] \times 100$, where S denotes the number of stomata per unit area and E denotes the number of epidermal cells in the same unit area. To determine the epidermal cell width, we measured the widest point on each cell. We deposited the slides in the plant taxonomic laboratory, College of Life Sciences, Sichuan University. The material for SEM observation was mounted with double-side adhesive tape, after gold sputtering, and examined and photographed them using a Hitachi-SX-450 scanning electron microscope.

The employed terminology is based on Baranova (1983, 1987, 1992), Stace (1965, 1984), Dilcher (1974) and Wilkinson (1979).

Results

Characters seen with LM (Figs. 1–5)

Epidermal cells (Table 2)

Ligusticum angelicifolium, *L. brachylobum*, *L. jeholense* and *L. scapiforme* had polygonal cells on both adaxial and abaxial leaf surfaces. *Ligusticum franchetii*, *L. multivittatum* and *L. sinense* had polygonal cells intermixed with irregular cells on their adaxial leaf surfaces, which were different from the irregular cells on their abaxial surfaces. Other species had irregular epidermal cells on both leaf surfaces. The anticlinal cell walls were straight to arched in the polygonal epidermal cells, and undulate to sinuous in the irregular epidermal cells.

The number of cells per mm^2 varied between species or even within the same species. *Ligusticum franchetii* had the lowest number of cells on both adaxial (224.9) and abaxial (189.6) leaf surfaces, while the highest numbers on both leaf surfaces were observed in *L. scapiforme*: 2309.8 on the adaxial surface, 2329 on the abaxial surface. Most of the species examined had fewer cells on the adaxial than on the abaxial surface. The largest cells were found in *L. franchetii*, whose average width was 114.8 μm on the adaxial surface and 133.7 μm on the abaxial surface. The smallest cells on the adaxial and abaxial surfaces were found in *L. scapiforme*, with a mean cell width of 32.7 μm on the adaxial and 39.7 μm on the abaxial surface. Most of the species examined had almost equal average cell widths on both surfaces except for *L. ajanense*, which had clearly different-sized epidermal cells on the two leaf surfaces. The mean cell wall thickness ranged from 2.19 μm in *L. ajanense* to 10.21 μm in *L. franchetii* on the adaxial surface, and from 2.62 μm in *L. thomsonii* to 8.78 μm in *L. involucratum* on the abaxial surface.

Stomatal type (Table 3)

Among the species studied, *L. franchetii*, *L. acuminatum*, *L. ajanense*, *L. jeholense* and *L. sinense* are hypostomatic, *L. angelicifolium*, *L. brachylobum*, *L. daucoides*, *L. involucratum*, *L. multivittatum*, *L. tenuissimum*, *L. thomsonii* and *L. reptans* are amphihypostomatic, and *L. scapiforme* and *L. sikiangense* are amphistomatic.

According to the definitions of Dilcher (1974), a total of four types of stomata were found in this study: paracytic (two cells completely enclosing the guard cells with their long axis parallel to the long axis of the guard cells), anisocytic (a single ring of three cells (two larger, one smaller) enclosing the guard cells), anomotetracytic (four cells enclosing the guard cells in an irregular and variable pattern) and anomocytic (five or more cells enclosing the guard cells, cells adjacent to the guard cells not differentiated in any way from the normal epidermal cells).

All five hypostomatic species (*L. franchetii*, *L. acuminatum*, *L. ajanense*, *L. jeholense*, *L. sinense*) had both anisocytic and anomotetracytic stomata on the abaxial surface, while very few anomocytic stomata were also present on the abaxial surface of *L. sinense*. In all other spe-

Table 2. Leaf epidermal-cell characteristics of *Ligusticum* species under LM. Ab = abaxial, Ad = adaxial, Irr = irregular, Pol = polygonal, Sn = sinuous, Ud = undulate, St = straight, Ar = arched. Given are value ranges, and means ± 95% confidence intervals.

Taxa	Surface	Shape of cells	Pattern of anticlinal walls	Number of cells per mm^2	Cell width (μm)	Cell-wall thickness (μm)	Figure
L. acuminatum	Ad	Irr	Sn	549.8–648.5	39.7–92.2	2.00–3.9	1A
				598.7 ± 20.3	65.1 ± 6.2	2.86 ± 0.19	
	Ab	Irr	Sn	590.6–1211.8	37.7–105.7	2.13–3.91	1B
				919.7 ± 74.7	63.2 ± 7.5	3.12 ± 0.20	
L. ajanense	Ad	Irr	Sn	215.3–248.4	65.5–140.5	1.31–3.7	1C
				229.6 ± 7.8	101.3 ± 5.3	2.19 ± 0.23	
	Ab	Irr	Ud-Sn	414.6–745.9	32.8–87.3	3.21–7.00	1D
				626.4 ± 56.1	58.2 ± 7.4	5.31 ± 0.57	
L. angelicifolium	Ad	Pol	St-Ar	443.8–668.0	54.7–91.0	3.33–5.62	1E
				573.1 ± 48.2	65.1 ± 4.8	4.20 ± 0.36	
	Ab	Pol	St-Ar	592.9–780.0	42.5–86.2	2.20–6.04	1F
				682.9 ± 55.9	55.9 ± 5.4	4.11 ± 0.55	
L. brachylobum	Ad	Pol	St-Ar	532.1–662.3	39.6–68.5	1.8–4.9	2A
				609.9 ± 26.0	56.2 ± 5.8	3.15 ± 0.36	
	Ab	Pol	St-Ar	597.4–662.7	30.2–85.7	1.8–5.0	2B
				632.6 ± 13.1	57.6 ± 6	3.37 ± 0.53	
L. daucoides	Ad	Irr	Sn	360.5–410.7	57.4–122.7	2.7–5.8	2C
				382.2 ± 11.1	90.8 ± 8.8	4.01 ± 0.36	
	Ab	Irr	Sn	415.9–543.6	43.7–140.2	4.4–6.6	2D
				488.6 ± 27.9	80.1 ± 9.4	5.69 ± 0.36	
L. franchetii	Ad	Pol-Irr	St-Ar-Ud	200.87–242.3	70.0–163.9	6.0–18.7	2E
				224.9 ± 8.1	114.8 ± 11.1	10.21 ± 1.74	
	Ab	Irr	Ud	151.3–224.3	89.2–217.7	6.9–11.2	2F
				189.6 ± 15.7	133.7 ± 20.3	8.59 ± 0.70	
L. involucratum	Ad	Irr	Ud	390.2–455.0	42.7–83.7	3.0–9.3	3A
				428.3 ± 16.7	69.2 ± 5.9	6.71 ± 0.73	
	Ab	Irr	Ud	527.5–662.9	46.7–80.7	6.9–10.8	3B
				576.8 ± 25.9	58 ± 5.7	8.78 ± 0.61	
L. jeholense	Ad	Pol	St	414.3–525.1	43.7–72.2	5.9–10.5	3C
				455.6 ± 21.3	62.0 ± 4.4	8.19 ± 0.66	
	Ab	Pol	St-Ar	697.1–804.0	36.4–68.8	4.7–10.2	3D
				741.8 ± 20.7	51.5 ± 3.4	6.86 ± 0.71	
L. multivittatum	Ad	Pol-Irr	St-Ar-Ud	814.2–980.5	38.4–63.9	4.7–8.1	3E
				894.2 ± 39.8	51.2 ± 2.9	6.33 ± 0.34	
	Ab	Irr	Sn	777.2–854.9	43.2–66.9	4.1–9.3	3F
				817.4 ± 17.6	57.2 ± 4.7	5.81 ± 0.80	
L. scapiforme	Ad	Pol	St-Ar	1919.1–2786.5	18.0–47.6	2.8–5.7	4A
				2309.8 ± 176.8	32.7 ± 3.9	4.66 ± 0.56	
	Ab	Pol	St-Ar	2133–2541	26.1–49.3	3.4–6.8	4B
				2329 ± 101.3	39.7 ± 3	5.03 ± 0.46	
L. sikiangense	Ad	Irr	Ud	1009.2–1065	32.5–57.9	3.1–5.7	4C
				1028 ± 13.9	46.4 ± 4.3	4.61 ± 0.40	
	Ab	Irr	Ud	806–1025	30.4–66.9	3.3–5.7	4D
				929.5 ± 58.5	46.5 ± 4.7	4.49 ± 0.28	
L. sinense	Ad	Pol-Irr	St-Ar-Ud	488.6–528	43.0–87.6	4.2–5.2	4E
				504.2 ± 9.4	66.1 ± 9.2	4.79 ± 0.25	
	Ab	Irr	Ud	840.8–932.4	43.8–92.5	4.1–6.6	4F
				886.4 ± 20.8	59.0 ± 7.4	5.23 ± 0.31	
L. tenuissimum	Ad	Irr	Sn	1236.9–1421.3	34.1–65.7	3.5–6.3	5A
				1335.1 ± 35.1	43 ± 4.5	4.37 ± 0.52	
	Ab	Irr	Sn	1209.2–1241	31.9–96.2	3.2–4.7	5B
				1225.3 ± 6.7	48.8 ± 8.4	4.26 ± 0.26	
L. thomsonii	Ad	Irr	Ud	1057.5–1220.6	34.4–85.8	2.8–4.5	5C
				1144.4 ± 34.7	48.9 ± 6.9	3.55 ± 0.24	
	Ab	Irr	Ud	1227.7–1252.4	29.1–60.89	1.9–3.0	5D
				1235.8 ± 8	46.3 ± 5	2.62 ± 0.18	
L. reptans	Ad	Irr	Ud	691.2–770.1	41.8–62.7	4.1–5.2	5E
				732.1 ± 15.8	54.2 ± 5.0	4.67 ± 0.23	
	Ab	Irr	Ud	966–1026	33.6–72.2	2.7–4.0	5F
				985 ± 10.2	53.9 ± 9.0	3.48 ± 0.25	

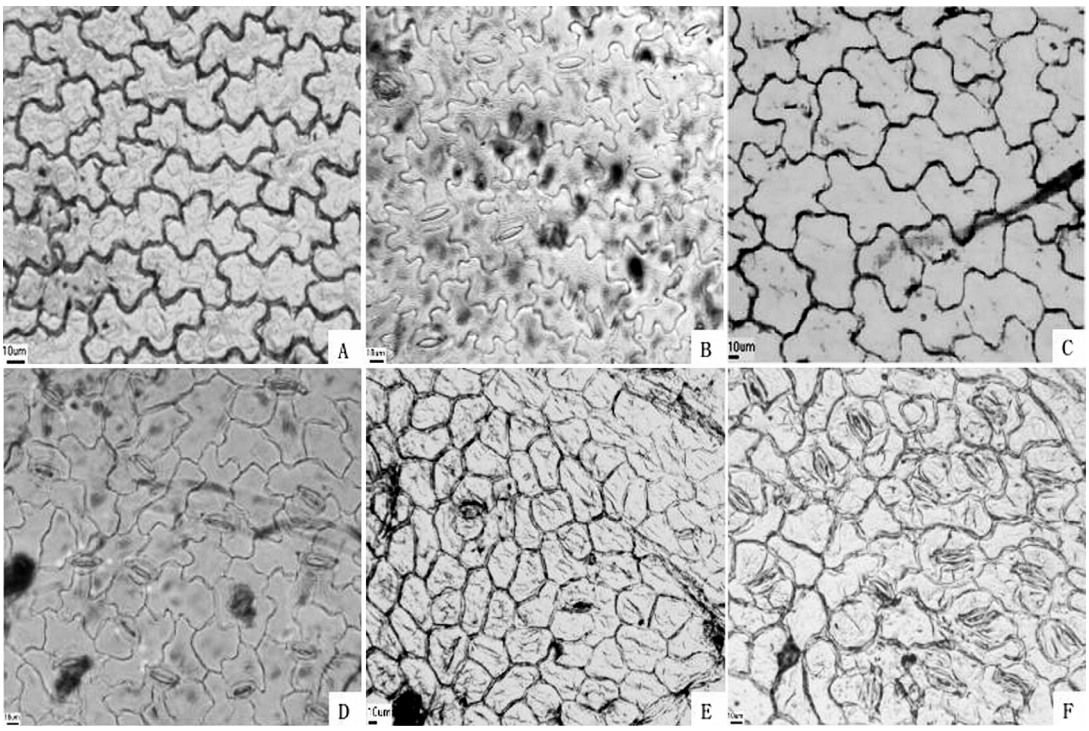

Fig. 1. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A**: *L. acuminatum*. Adaxial surface shows irregular epidermal cells without stomata. — **B**: *L. acuminatum*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **C**: *L. ajanense*. Adaxial surface shows irregular epidermal cells without stomata. — **D**: *L. ajanense*. Abaxial surface shows irregular epidermal cells with anomotetracytic and anisocytic stomata. — **E**: *L. angelicifolium*. Adaxial surface shows polygonal epidermal cells with paracytic and anisocytic stomata. — **F**: *L. angelicifolium*. Abaxial surface shows polygonal epidermal cells with paracytic and anisocytic stomata. Scale bars: **A**–**F** = 10 μm.

cies, anisocytic and anomotetracytic stomata were present on both adaxial and abaxial surfaces. An exception was *L. angelicifolium*, which has paracytic and anisocytic stomata on both surfaces. The number of different types of stomata on the same surface in each species was also different, the types with a larger number are listed before those with a smaller number (Table 3 and Figs. 1–5).

Stomatal frequency

The average number of stomata per mm^2 ranged from 24.6 in *L. brachylobum* to 473.8 in *L. scapiforme* on adaxial surfaces, and from 51.7 in *L. franchetii* to 473.1 in *L. scapiforme* on abaxial surfaces. There were always more or almost equal numbers of stomata on abaxial surfaces than on adaxial surfaces.

Stomatal size and index

Ligusticum scapiforme had the smallest stomata on both adaxial and abaxial surfaces, with an average length of 23.54 μm and width of 18.88 μm on the adaxial surface, and an average length of 22.88 μm and width of 18.18 μm on abaxial surfaces. *Ligusticum franchetii* had the largest stomata found on the abaxial surface with an average length of 54.04 μm and width of 32.35 μm, while *L. angelicifolium* had the largest stomata found on the adaxial surface, with an average length of 39.42 μm and width of 28.49 μm. Stomata on abaxial surfaces were normally larger than those on adaxial surfaces. *Ligusticum scapiforme* had the widest stomata on both leaf surfaces and the smallest average stomatal ratio of 1.229 on the adaxial surface and 1.226 on the abaxial surface, while *L. franchetii*

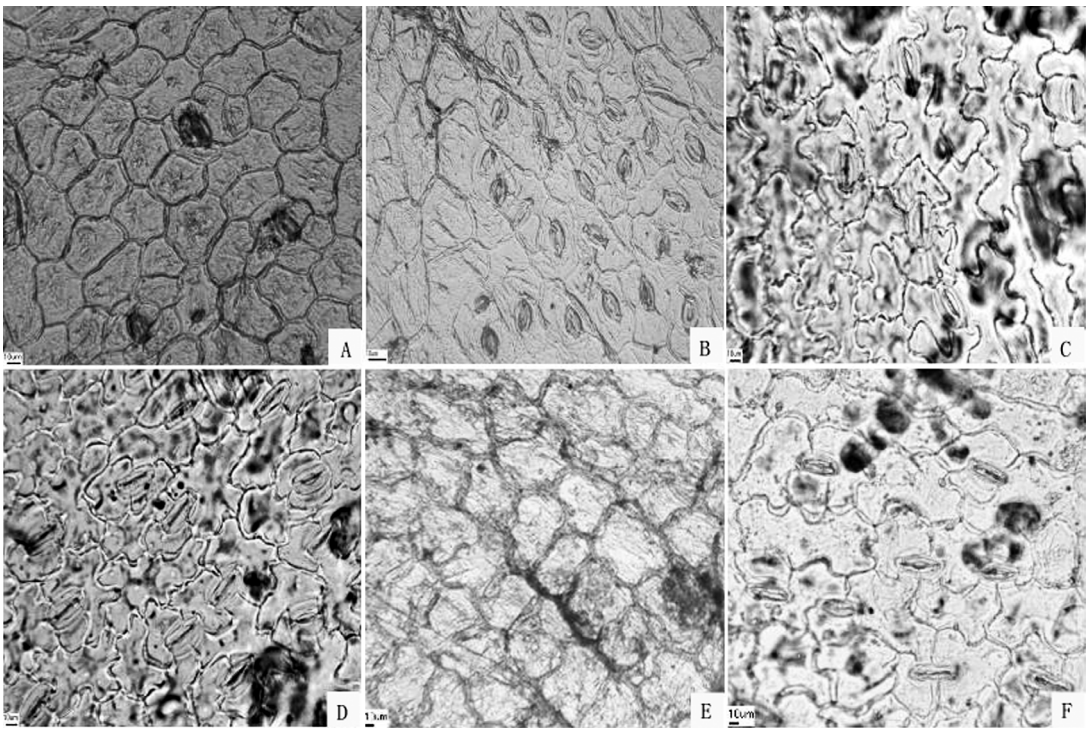

Fig. 2. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A**: *L. brachylobum*. Adaxial surface shows polygonal epidermal cells with anisocytic and anomotetracytic stomata. — **B**: *L. brachylobum*. Abaxial surface shows polygonal epidermal cells with anisocytic and anomotetracytic stomata. — **C**: *L. daucoides*. Adaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **D**: *L. daucoides*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **E**: *L. franchetii*. Adaxial surface shows polygonal to irregular epidermal cells without stomata. — **F**: *L. franchetii*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. Scale bars: **A–F** = 10 μm.

had the narrowest stomata on abaxial surfaces, with the largest average ratio of 1.682. *Ligusticum brachylobum* had the narrowest stomata on adaxial surfaces, with largest average ratio of 1.741. With regard to the stomatal index on adaxial surfaces, *L. brachylobum* had the lowest average value at 3.88 and *L. reptans* had the highest at 24.4. On abaxial surfaces, this value ranged from 15.09 in *L. tenuissimum* to 36.83 in *L. brachylobum*.

Characters seen with SEM
(Table 4 and Figs. 6–11)

Cuticular membranes were ridged in all species, but to different degrees. In general, the degree of ridging on adaxial surfaces was greater than, or almost the same as, that on abaxial surfaces. An exception was *L. sikiangense*, which had heavy ridging on the abaxial surface (Fig. 9K) but normal ridging on the adaxial surface (Fig. 9I). Striation was a common feature for all the species. In species that had fine striation, for example, *L. acuminatum* (Fig. 6A–D), the striae ran parallel on the membrane with irregular orientations and often for long expanses that could even cross the ridges. In other species that had normal striation, such as *L. multivittatum* (Fig. 9A–D), the striae were not as obvious and continuous as were the ones with fine striation; it looked more like a few striae segments separated by ridges on the membrane. Species such as *L. ajanense* (Fig. 6E–G) had coarse striation on the membrane, represented by a few very short striae normally radiating from or cycling around a stomatal area. On the abaxial surface of *L. involucratum*, striation was rarely found. In general, the striations

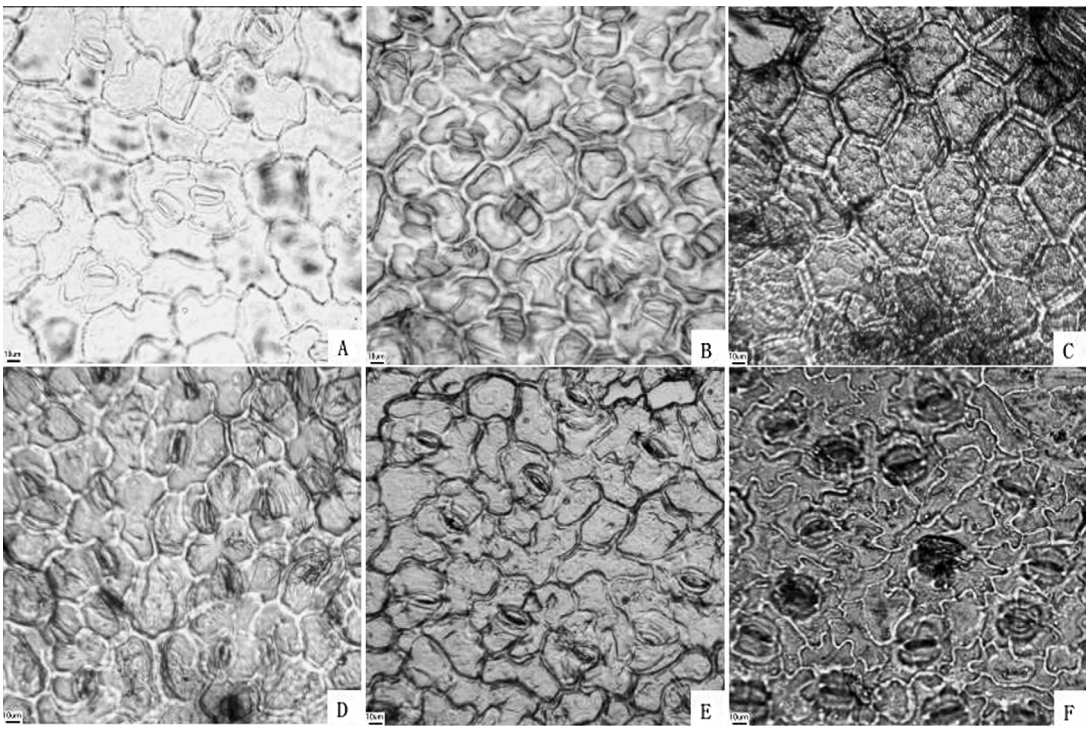

Fig. 3. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A**: *L. involucratum*. Adaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **B**: *L. involucratum*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **C**: *L. jeholense*. Adaxial surface shows polygonal epidermal cells without stomata. — **D**: *L. jeholense*. Abaxial surface shows polygonal epidermal cells with anisocytic and anomotetracytic stomata. — **E**: *L. multivittatum*. Adaxial surface shows polygonal to irregular epidermal cells with anisocytic and anomotetracytic stomata. — **F**: *L. multivittatum*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. Scale bars: **A**–**F** = 10 μm.

on both surfaces were almost the same; otherwise, the striations on abaxial surfaces were more conspicuous and finer than on adaxial surfaces. In three species, *L. acuminatum* (Fig. 6B), *L. jeholense* (Fig. 8G) and *L. sinenses* (Fig. 10A), special striation structures were noted on the adaxial surfaces that looked like papillae generated by twisted radial striae (indicated by '**P**' and an arrow in the figures).

Guard cells

Most of the guard cells were elliptic or narrow-elliptic on both leaf surfaces, but in *L. ajanense* obviously different wide-elliptic (or nearly cyclic) guard cells occurred on the abaxial surface. Almost all of the inner margins of the outer stomatal rim were undulate or between smooth and undulate, except in *L. acuminatum* and *L. involucratum*, which had quite smooth inner margin guard cells on the abaxial surfaces. In addition, filiform waxes were observed in the apertures of *L. reptans*.

Wax ornamentation

Most of the species had granular or flaky wax ornamentation, except for *L. brachylobum* and *L. daucoides*, in which wax ornamentation was invisible on the adaxial surface. On the adaxial surfaces of *L. acuminatum*, *L. jeholense* and *L. sikiangense* and the abaxial sufaces of *L. brachylobum*, *L. jeholense* and *L. sikiangense*, only very few granular wax ornamentations could be observed. In *L. involucratum* very large wax granules were found on the adaxial surface.

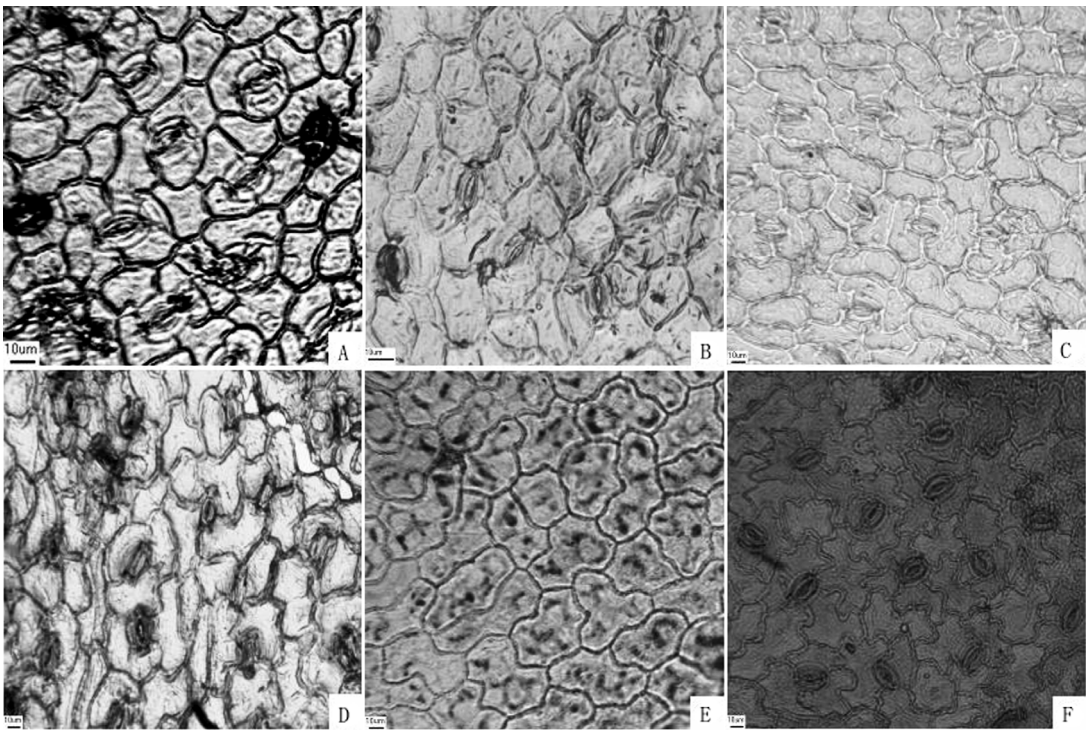

Fig. 4. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A**: *L. scapiforme*. Adaxial surface shows polygonal epidermal cells with anomotetracytic and anisocytic stomata. — **B**: *L. scapiforme*. Abaxial surface shows polygonal epidermal cells with anomotetraytic and anisocytic stomata. — **C**: *L. sikiangense*. Adaxial surface shows irregular epidermal cells with anomotetracytic and anisocytic stomata. — **D**: *L. sikiangense*. Abaxial surface shows irregular epidermal cells with anomotetracytic and anisocytic stomata. — **E**: *L. sinense*. Adaxial surface shows polygonal to irregular epidermal cells without stomata. — **F**: *L. sinense*. Abaxial surface shows irregular epidermal cells with anomotetracytic, anisocytic and anomocytic stomata. Scale bars: **A**–**F** = 10 *μ*m.

Trichomes

Unicellular, nonglandular, simple hairs with narrow apiculate tips were found on the adaxial surfaces of *L. involucratum*, *L. jeholense* and *L. sinense* and on the abaxial surface of *L. involucratum*.

Discussion

Classification

Almost all of the leaf epidermal characters were quite variable and inconsistent among the species studied. The epidermal cells had both irregular and regular shapes while the anticlinal walls varied from straight, arched to undulate or sinuous. The epidermal cell frequencies ranged from hundreds to thousands, stomatal frequencies from tens to hundreds. Other characters such as stomatal index and size also had very broad ranges. Moreover, the ridges and striations were quite different on the epidermal membranes of all species, changing from smooth to heavy ridging and fine striations. Therefore, the epidermal characters do not support *Ligusticum* as a natural genus. This conclusion also matches the results from other morphological or molecular work.

Regel (1858) established *Tilingia* with the type species *T. ajanensis*, which has conspicuous calyx teeth, and normally one vitta in each furrow of the mericarp. Koso-Poljansky (1916) placed *T. ajanensis* in *Ligusticum*. Regarding the epidermal characters, *L. ajanense* is quite different from the other *Ligusticum* species studied here. For example, the number of cells per mm^2 on the abaxial surface of *L. ajanense* is

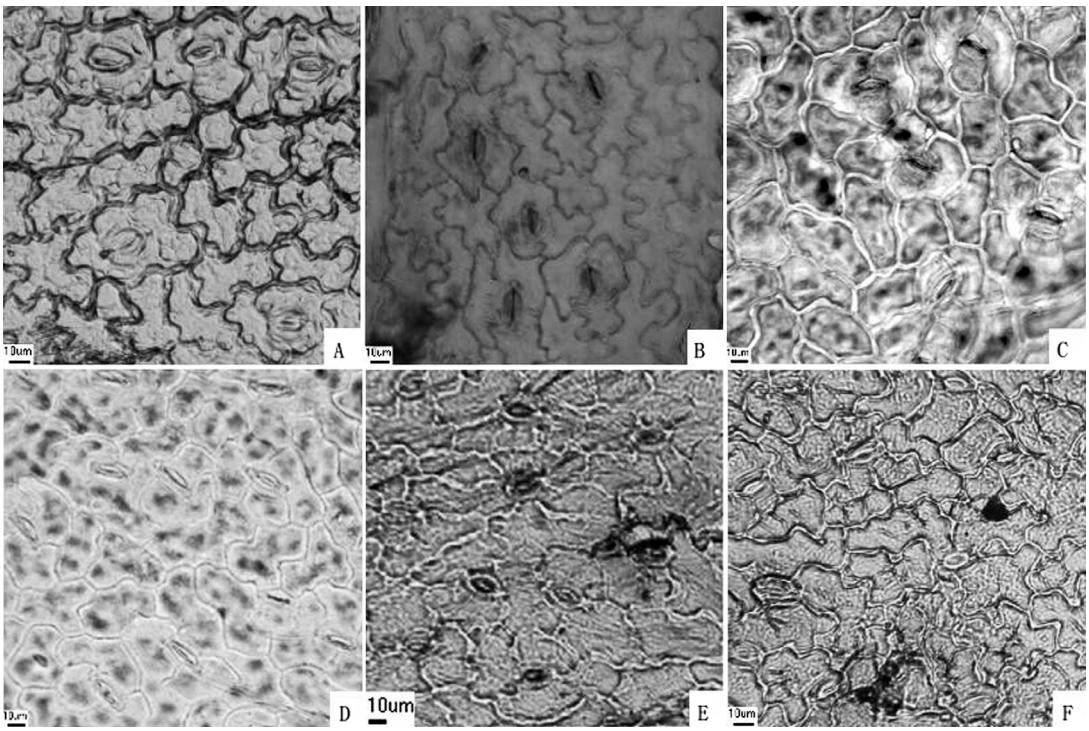

Fig. 5. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A**: *L. tenuissimum*. Adaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **B**: *L. tenuissimum*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **C**: *L. thomsonii*. Adaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **D**: *L. thomsonii*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **E**: *L. reptans*. Adaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. — **F**: *L. reptans*. Abaxial surface shows irregular epidermal cells with anisocytic and anomotetracytic stomata. Scale bars: **A**–**F** = 10 μm.

about triple the number on its adaxial surface. *Ligusticum ajanense* also has the thinnest cell wall measured on the adaxial surfaces. Moreover, the nearly round stomata (Table 4) and its smallest stomatal ratio (Table 3) also distinguish *L. ajanense* from the other *Ligusticum* species studied here. Recently, *Tilingia* has been restored by not only molecular but also morphological research (Pimenov *et al*. 2003, Valiejo-Roman *et al*. 2006). Though the differences between *L. ajanense* and other *Ligusticum* species need to be confirmed against other *Tilingia* species, the epidermal characteristics reported here support the segregation of *L. ajanense* from *Ligusticum* back to *Tilingia*.

Leute (1969) segregated the species in *Ligusticum* that have conspicuous calyx teeth as a new genus, *Ligusticopsis*. In the species studied by us, 7 out of the 15 have been placed in *Ligusticopsis* (*L. acuminatum*, *L. angelicifolium*, *L. brachylobum*, *L. daucoides*, *L. franchetii*, *L. multivittatum* and *L. scapiforme*). These 7 species contain almost all of the most different characters among the 15 species studied; for example, the striation papillae on adaxial surface of *L. acuminatum*, the paracytic type stomata on both surfaces of *L. angelicifolium*, the low epidermal cell frequency and large epidermal cell size of *L. franchetii* and the high epidermal cell density of *L. scapiforme*. The states of each of these epidermal characters of *Ligusticopsis* species varied strongly, for example, the epidermal cell density of *L. scapiforme* was even 10 times higher than that of *L. franchetii*. This wide variation in the epidermal characters, and the degree to which they differ in these *Ligusticopsis* species, suggest that these species do not form a natural grouping to be segregated from *Ligusticum*.

Table 3. Leaf stomata characteristics of *Ligusticum* species under LM. Ab = abaxial, Ad = adaxial, Irr = irregular, Pol = polygonal, Sn = sinuous, Ud = undulate, St = straight, Ar = arched. Given are value ranges, and means ± 95% confidence intervals.

Taxa	Surface	Type	Frequency per mm^2	Length (μm)	Width (μm)	Length/width ratio	Index (%)	Figure
L. acuminatum	Ad	–	–	–	–	–	–	1A
	Ab	At/Ai	144.88–266.0	29.3–33.4	18.4–24.0	1.33–1.85	14.5–21.7	1B
			197.4 ± 15.2	31.21 ± 0.62	20.89 ± 0.58	1.504 ± 0.063	17.79 ± 0.77	
L. ajanense	Ad	–	–	–	–	–	–	1C
	Ab	At/Ai	85.7–189.6	27.3–36.7	23.2–28.8	1.14–1.39	16.9–23.7	1D
			162.7 ± 20.4	31.82 ± 1.72	25.85 ± 1.54	1.234 ± 0.047	20.48 ± 1.30	
L. angelicifolium	Ad	Pa/Ai	19.8–39.1	36.6–43.4	25.1–32.7	1.29–1.56	3.4–7.2	1E
			33 ± 4.6	39.42 ± 1.46	28.49 ± 1.48	1.388 ± 0.046	5.147 ± 0.74	
	Ab	Pa/Ai	186.3–277.1	31.4–38.0	18.5–25.0	1.34–1.81	20.5–27.3	1F
			221.5 ± 23.4	33.89 ± 1.17	21.15 ± 1.03	1.613 ± 0.097	24.523 ± 1.95	
L. brachylobum	Ad	Ai/At	19.7–31.9	25.2–26.3	12.6–16.3	1.56–2.06	3.0–5.0	2A
			24.6 ± 2.3	25.83 ± 0.5	14.82 ± 0.89	1.741 ± 0.111	3.88 ± 0.34	
	Ab	Ai/At	344.6–404.9	22.1–26.9	12.2–18.6	1.41–2.0	34.2–39.7	2B
			368.9 ± 11.2	24.51 ± 1.1	14.82 ± 1.06	1.666 ± 0.097	36.83 ± 1.03	
L. daucoides	Ad	Ai/At,	100.2–137.3	32.7–42.2	22.7–28.8	1.22–1.63	19.7–27.6	2C
			119.9 ± 7.4	36.95 ± 1.24	26.05 ± 0.96	1.422 ± 0.043	23.9 ± 1.48	
	Ab	Ai/At	124.6–160.6	37.9–44.3	21.7–27.9	1.38–1.92	20.5–25.0	2D
			143.8 ± 10.2	40.89 ± 1.12	24.88 ± 1.31	1.655 ± 0.077	22.73 ± 0.95	
L. franchetii	Ad	–	–	–	–	–	–	2E
	Ab	Ai/At	34.9–66.7	46.1–67.1	25.2–40.7	1.47–1.97	18.4–26.1	2F
			51.7 ± 6.1	54.04 ± 3.14	32.35 ± 2.43	1.682 ± 0.080	21.38 ± 1.59	
L. involucratum	Ad	Ai/At	39.6–66.6	30.7–39.2	20.5–26.9	1.18–1.81	8.0–12.5	3A
			46.3 ± 5	35.14 ± 1.35	24.56 ± 1.14	1.444 ± 0.103	9.76 ± 0.91	
	Ab	Ai/At	110.8–159.7	30.2–35.9	18.5–21.9	1.43–1.89	16.0–22.3	3B
			128.5 ± 10.6	32.55 ± 1.27	20.33 ± 0.94	1.610 ± 0.113	18.19 ± 1.11	
L. jeholense	Ad	–	–	–	–	–	–	3C
	Ab	Ai/At	147.2–215.8	33.2–41.4	22.2–27.4	1.36–1.53	16.9–23.6	3D
			182.5 ± 13.3	35.97 ± 2.39	24.82 ± 1.66	1.451 ± 0.050	19.73 ± 1.33	
L. multivittatum	Ad	Ai/At	143–176.7	31.5–36.1	21.0–28.3	1.28–1.61	13.3–17.3	3E
			158.5 ± 7.6	33.18 ± 0.78	23.27 ± 1.15	1.433 ± 0.050	13.60 ± 3.06	
	Ab	Ai/At	184.9–215.9	29.6–39.2	19.6–26.2	1.36–1.64	18.0–21.7	3F
			199.8 ± 7.1	34.22 ± 2.12	23.17 ± 1.36	1.479 ± 0.062	19.64 ± 0.68	
L. scapiforme	Ad	At/Ai	430.6–511.4	21.7–25.4	17.5–20.6	1.13–1.37	13.4–21.0	4A
			473.8 ± 16.1	23.54 ± 0.75	18.88 ± 0.53	1.229 ± 0.065	17.19 ± 1.30	
	Ab	At/Ai	452.8–491.1	20.5–24.9	16.1–20.0	1.03–1.44	15.9–18.7	4B
			473.1 ± 8.8	22.88 ± 0.98	18.18 ± 0.86	1.226 ± 0.077	17.2 ± 0.68	
L. sikiangense	Ad	At/Ai	196.3–236.5	29.1–34.5	21.0–26.7	1.23–1.60	15.5–18.99	4C
			210.5 ± 9.9	32.8 ± 1.04	23.68 ± 1.26	1.394 ± 0.075	17.1 ± 0.83	
	Ab	At/Ai	201.5–238.8	31.4–35.2	20.7–25.2	1.29–1.57	17.5–20.9	4D
			222.5 ± 7.3	33.36 ± 0.85	23.17 ± 0.96	1.444 ± 0.053	19.4 ± 0.78	
L. sinense	Ad	–	–	–	–	–	–	4E
	Ab	At/Ai/Ac	154.1–217.8	27.5–30.7	20.9–23.5	1.21–1.44	14.9–19.3	4F
			185.1 ± 11.6	29.0 ± 0.72	22.4 ± 0.44	1.296 ± 0.043	17.25 ± 0.74	
L. tenuissimum	Ad	Ai/At	84.7–143.9	24.6–30.8	19.0–23.6	1.19–1.47	6.4–9.5	5A
			111.4 ± 13.2	27.38 ± 1.49	21.23 ± 0.88	1.290 ± 0.051	7.66 ± 0.72	
	Ab	Ai/At	198.4–234.8	23.6–31.0	20.0–23.5	1.09–1.55	14.0–15.9	5B
			217.8 ± 7.6	27.48 ± 1.41	21.38 ± 0.58	1.289 ± 0.084	15.09 ± 0.41	
L. thomsonii	Ad	Ai/At	191.2–196.6	24.9–29.1	18.1–21.9	1.19–1.40	13.5–15.7	5C
			193.3 ± 1.3	26.57 ± 0.75	20.33 ± 0.73	1.310 ± 0.039	14.49 ± 0.47	
	Ab	Ai/At	259.0–311.5	24.1–32.4	14.5–23.4	1.26–1.92	17.2–19.9	5D
			286.3 ± 11	28.04 ± 1.22	19.12 ± 1.13	1.481 ± 0.087	18.8 ± 0.60	
L. reptans	Ad	Ai/At	201.8–278.7	23.4–27.9	17.3–19.5	1.25–1.61	20.8–27.3	5E
			237.2 ± 16.9	25.48 ± 0.88	18.58 ± 0.42	1.374 ± 0.066	24.4 ± 1.3	
	Ab	Ai/At	256.9–331.9	20.7–27.6	14.9–17.3	1.35–1.67	21.0–25.3	5F
			295.1 ± 17.3	24.5 ± 1.4	16.63 ± 0.62	1.475 ± 0.075	23.0 ± 0.93	

According to Pimenov *et al*. (2003), three of the species studied here (*L. jeholense*, *L. sinense*, *L. acuminatum*) belong in *Conioselinum*, due to carpoanatomical criteria, such as narrow (sometimes very narrow) commissures, winged ribs, and the localization of vascular bundles in the distal parts of the ribs, etc. The epidermal characters of these three species are also different from other *Ligusticum* species because they all have hypostomatic leaves. In addition, these three species have many other similar epidermal characters, for example, the very fine striation and striation papillae on the epidermal membrane. Both *L. sinense* and *L. jeholense* have unicellular trichomes only on the adaxial epidermal surface. Apart from these three species, there are two hypostomatic species studied here: *L. ajanense* is suggested to belong to *Tilingia*, and *L. franchetii* has very similar epidermal features to *L. sinense*, suggesting that it may be another candidate to be moved to *Conioselinum* from *Ligusticum*.

Some taxonomically valuable leaf epidermal micro-characters

Stomatal type

A total of 10 out of the 15 studied species were investigated previously by Ostroumova and

Table 4. Leaf epidermal characteristics of *Ligusticum* species (from SEM). Ab = abaxial; Ad = adaxial; Nor = normal; Sl = alight; Hv = heavy; Fi = fine; Co = coarse; In = invisible; W = wide; E = elliptic; N = narrow; Sm = smooth; Ud = undulate; Gr = granular; Fl = flake; Uc = unicellular. [1]Few small granules, almost invisible, [2]Small flakes, many big granules.

Taxa	Surface	Cuticular membrane			Shape of guard cells	Inner margin of outer stomatal rim	Wax ornamentation	Trichome type	Figure
		Ridged	Striation	Papillae					
L. acuminatum	Ad	Nor	Fi	Yes	–	–	Gr[1]	–	6A–B
	Ab	Nor	Fi	No	N, E	Sm	Gr, Fl	–	6C–D
L. ajanense	Ad	Nor	Co	No	–	–	Gr, Fl	–	6E
	Ab	Sl	Co	No	W, E	Sm, Ud	Fl	–	6F–G
L. angelicifolium	Ad	Nor	Nor	No	N, E	Sm, Ud	Gr	–	6H–I
	Ab	Sl	Fi	No	N, E	Sm, Ud	Gr	–	6J–K
L. brachylobum	Ad	Hv	Nor	No	N, E	Sm, Ud	In	–	7A–B
	Ab	Hv	Fi	No	N, E	Sm, Ud	Gr[1]	–	7C–D
L. daucoides	Ad	Nor	Co	No	N, E	Sm, Ud	In	–	7E–F
	Ab	Nor	Co	No	N, E	Ud	Gr	–	7G–H
L. franchetii	Ad	Nor	Co	No	–	–	Gr, Fl	–	7I
	Ab	Sl	Co	No	E	Ud	Fl	–	7J–K
L. involucratum	Ad	Nor	Co	No	N, E	Sm, Ud	Gr-Fl[2]	Uc	8A–B
	Ab	Sl	In	No	N, E	Sm	Fl	Uc	8C–E
L. jeholense	Ad	Nor	Fi	Yes	–	–	Gr[1]	Uc	8F–G
	Ab	Nor	Fi	No	E	Ud	Gr[1]	–	8H–I
L. multivittatum	Ad	Hv	Nor	No	E	Ud	Gr	–	9A–B
	Ab	Nor	Nor	No	E	Ud	Gr	–	9C–D
L. scapiforme	Ad	Sl	Nor	No	E	Ud	In	–	9E–F
	Ab	Sl	Nor	No	N, E	Sm, Ud	Gr, Fl	–	9G–H
L. sikiangense	Ad	Nor	Co	No	E	Ud	Gr[1]	–	9I–J
	Ab	Hv	Co	No	E	Ud	Gr[1]	–	9K–L
L. sinense	Ad	Nor	Nor	Yes	–	–	Gr	Uc	10A–B
	Ab	Sl	Fi	No	N, E	Sm, Ud	Gr, Fl	–	10C–D
L. tenuissimum	Ad	Nor	Fi	No	E	Sm, Ud	Fl	–	10E–F
	Ab	Nor	Fi	No	E	Sm, Ud	Fl	–	10G–H
L. thomsonii	Ad	Nor	Co	No	E	Sm, Ud	Gr, Fl	–	10I–J
	Ab	Sl	Co	No	E	Sm, Ud	Gr, Fl	–	10K–L
L. reptans	Ad	Hv	Co	No	N, E	Ud	Gr, Fl		11A–B
	Ab	Hv	Nor	No	N, E	Ud	Gr, Fl		11C–D

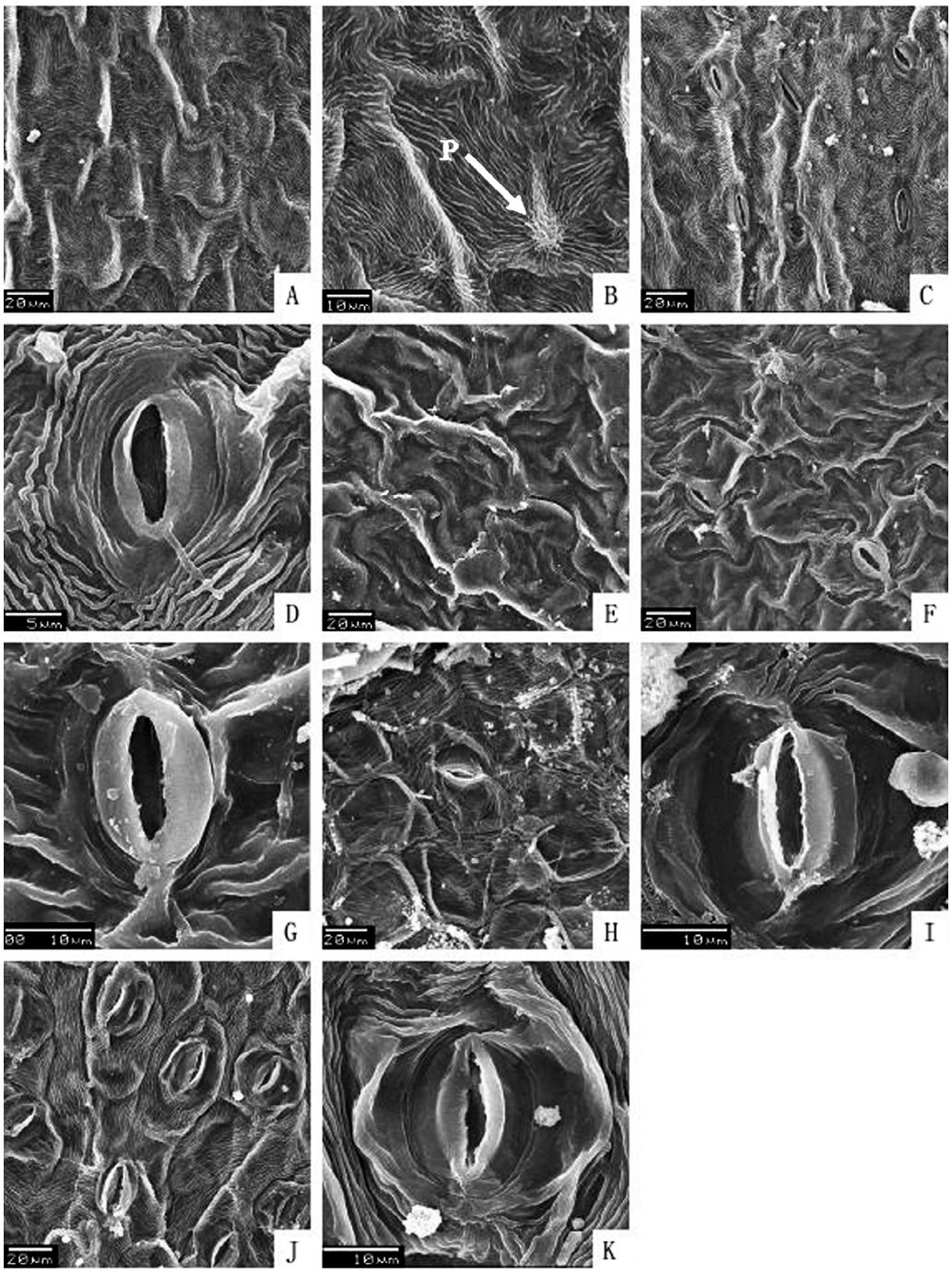

Fig. 6. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A** and **B**: *L. acuminatum*. Adaxial surface shows normal ridges, very fine striation and striation papillae (indicated by arrow) on membrane. — **C** and **D**: *L. acuminatum*. Abaxial surface shows normal ridges, very fine striation and narrow-elliptic stomata. — **E**: *L. ajanense*. Adaxial surface shows normal ridges and coarse striation on membrane. — **F** and **G**: *L. ajanense*. Abaxial surface shows slight ridges, coarse striation and wide-elliptic stomata. — **H** and **I**: *L. angelicifolium*. Adaxial surface shows normal ridges, fine striation with narrow-elliptic stomata. — **J** and **K**: *L. angelicifolium*. Abaxial surface shows slight ridges, fine striation and narrow-elliptic stomata. Scale bars: **A**, **C**, **E**, **F**, **H**, **J** = 20 μm; **B**, **G**, **I**, **K** = 10 μm; **D** = 5 μm.

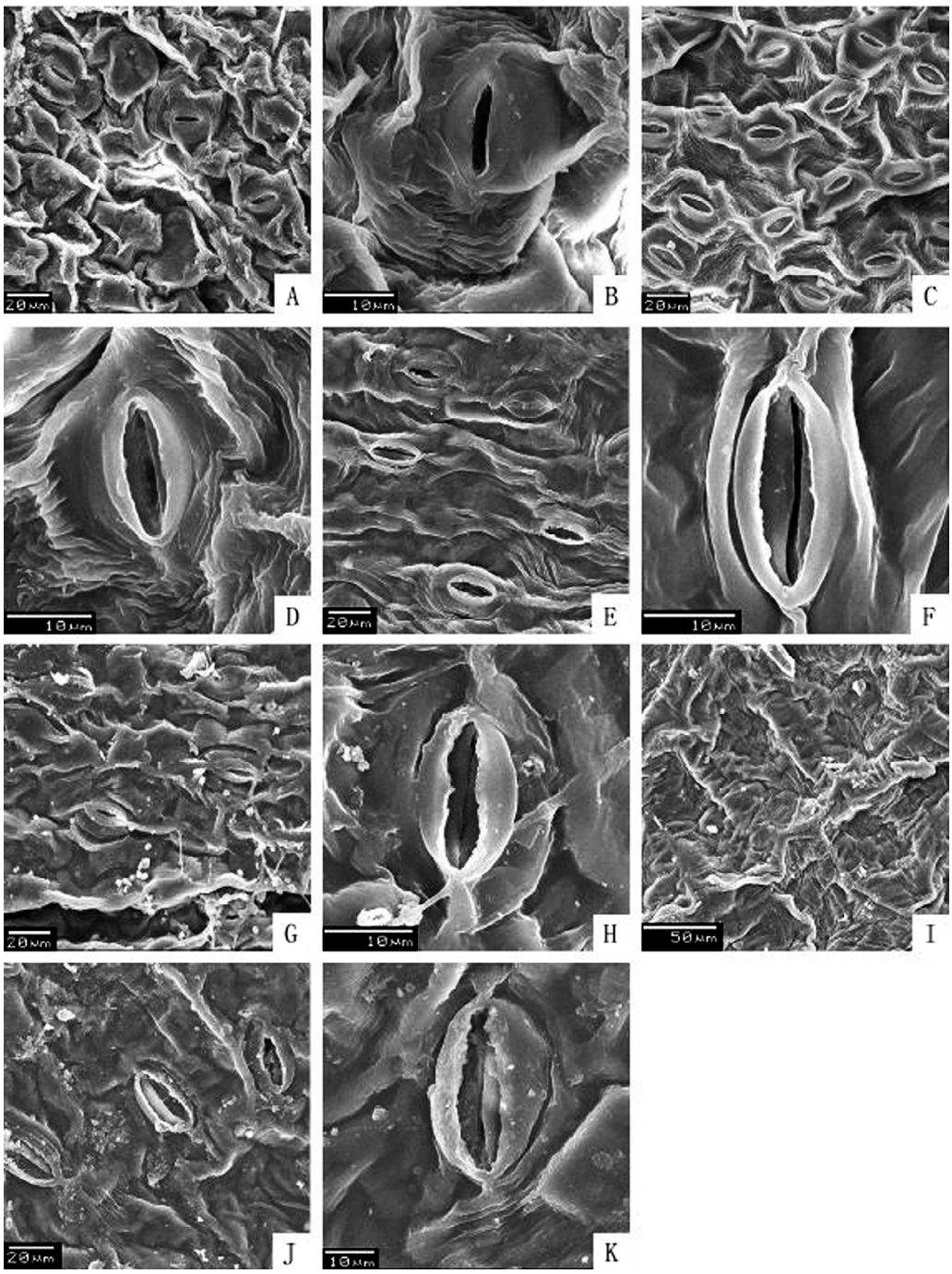

Fig. 7. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A** and **B**: *L. brachylobum*. Adaxial surface shows heavy ridges and normal striation and narrow-elliptic stomata. — **C** and **D**: *L. brachylobum*. Abaxial surface shows heavy ridges with fine striation and narrow-elliptic stomata. — **E** and **F**: *L. daucoides*. Adaxial surface shows normal ridges and coarse striation and narrow-elliptic stomata. — **G** and **H**: *L. daucoides*. Abaxial surface shows normal ridges, coarse striation and narrow-elliptic stomata. — **I**: *L. franchetii*. Adaxial surface shows normal ridges and coarse striation on membrane. — **J** and **K**: *L. franchetii*. Abaxial surface shows slight ridges, coarse striation on membrane and elliptic stomata. Scale bars: **I** = 50 μm; **A**, **C**, **E**, **G**, **J** = 20 μm; **B**, **D**, **F**, **H**, **K** = 10 μm.

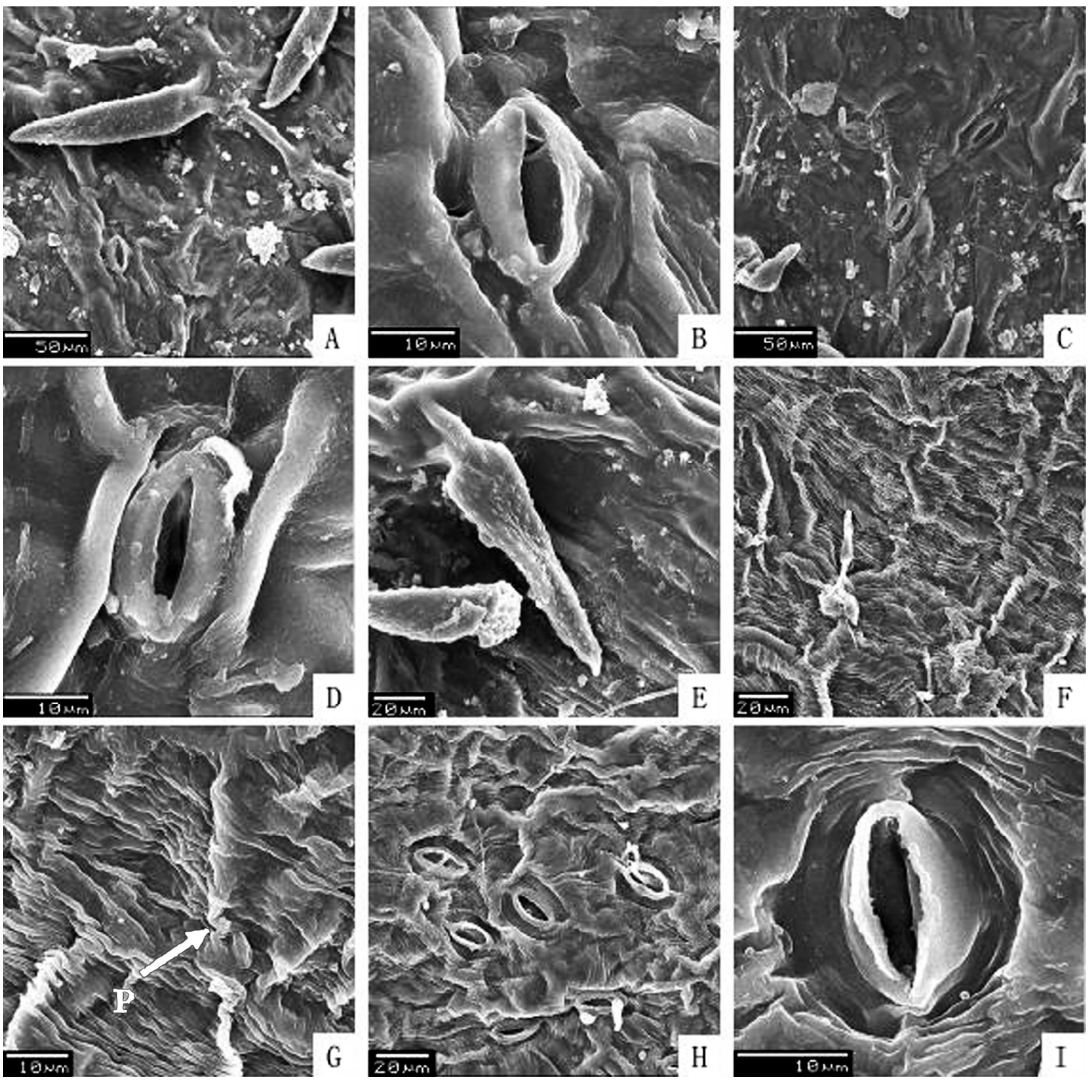

Fig. 8. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A** and **B**: *L. involucratum*. Adaxial surface shows normal ridges, coarse striation on membrane, unicellular trichome, narrow-elliptic stomata, and very big granular wax. — **C–E**: *L. involucratum*. Abaxial surface shows slight ridges, almost invisible striation on membrane, unicellular trichome and narrow-elliptic stomata. — **F** and **G**: *L. jeholense*. Adaxial surface shows normal ridges, fine striation and striation papillae (indicated by arrow) on membrane. — **H** and **I**: *L. jeholense*. Abaxial surface shows normal ridges, fine striation and elliptic stomata. Scale bars: **A**, **C** = 50 *μ*m; **E**, **F**, **H** = 20 *μ*m; **B**, **D**, **G**, **I** = 10 *μ*m.

Lavrova (1991) and Ostroumova and Kljuykov (2007). In the current paper, the most common stomatal types were Anisocytic and Anomotetracytic (stomata with 3 or 4 surrounding cells). Stomatal complexes with 5 or 6 surrounding cells (anomocytic) were also observed in some species, such as *L. sinense*, and the proportion of anomocytic stomata was much lower than that seen for anisocytic and anomotetracytic stomatal types, which supports observations by the afore-mentioned authors on hundreds of Apiaceae species. Moreover, within the species studied, only *L. angelicifolia* had a significant proportion of paracytic stomata (two surrounding cells) while other species studied had very few stomata of that type. These findings also

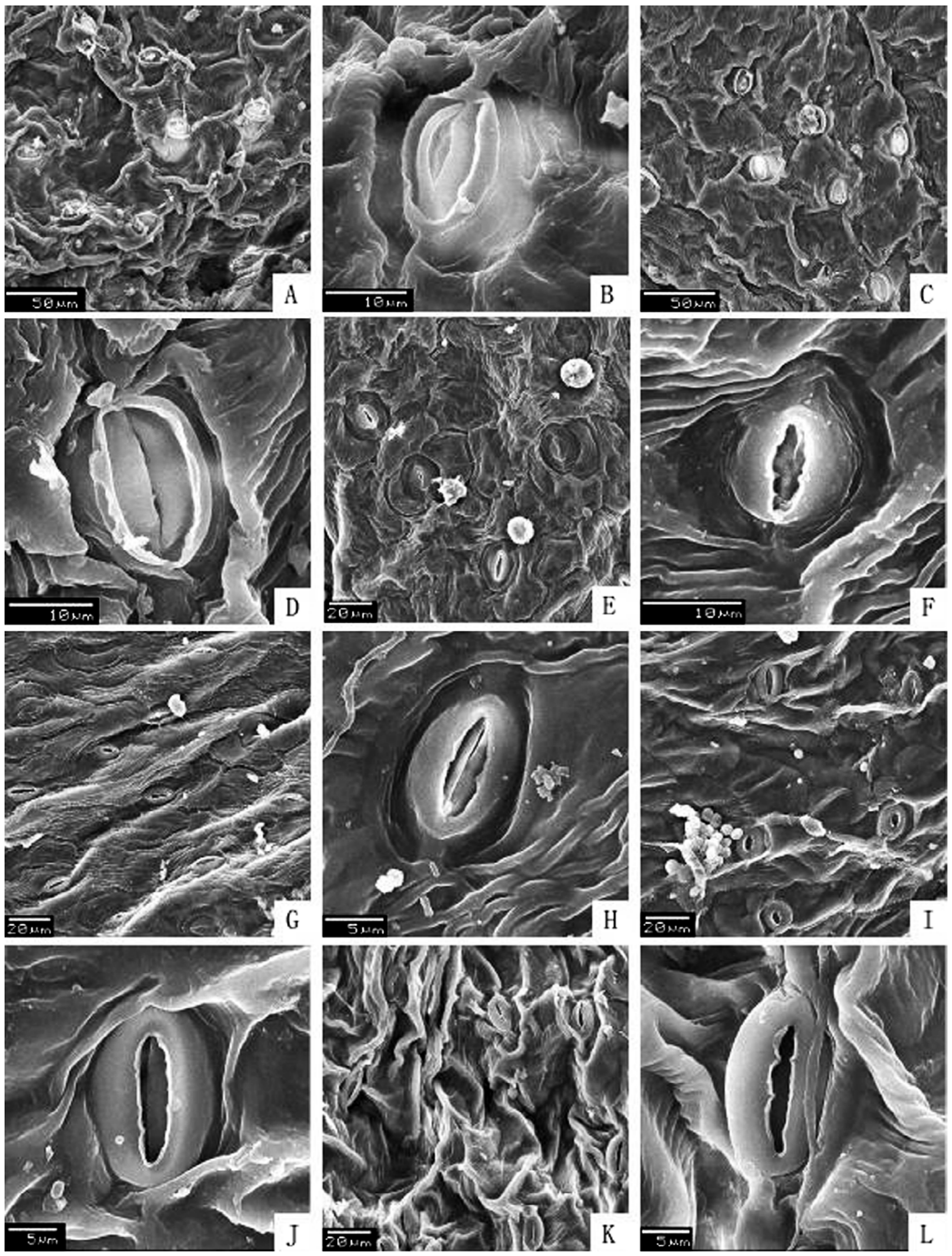

Fig. 9. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A** and **B**: *L. multivittatum*. Adaxial surface shows heavy ridges, normal striation on membrane and elliptic stomata. — **C** and **D**: *L. multivittatum*. Abaxial surface shows normal ridges and normal striation on membrane, elliptic stomata. — **E** and **F**: *L. scapiforme*. Adaxial surface shows slight ridges and normal striation on membrane, elliptic stomata. — **G** and **H**: *L. scapiforme*. Abaxial surface shows slight ridges and normal striation on membrane, narrow-elliptic stomata. — **I** and **J**: *L. sikiangense*. Adaxial surface shows normal ridges and coarse striation on membrane and elliptic stomata. — **K** and **L**: *L. sikiangense*. Abaxial surface shows heavy ridges and coarse striation on membrane, elliptic stomata. Scale bars: **A**, **C** = 50 μm; **E**, **G**, **I**, **K** = 20 μm; **B**, **D**, **F** = 10 μm; **H**, **J**, **L** = 5 μm.

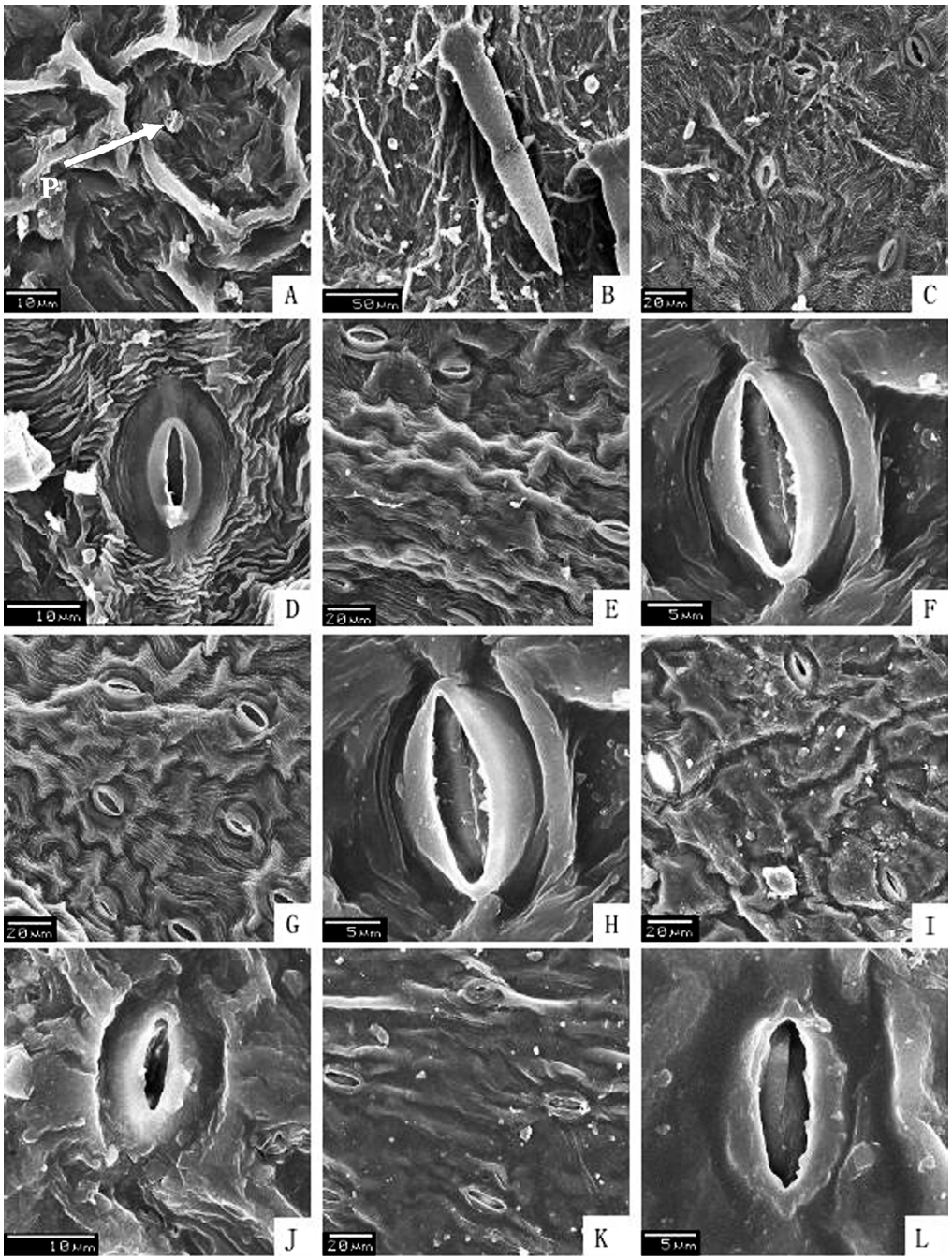

Fig. 10. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A** and **B**: *L. sinense*. Adaxial surface shows normal ridges, normal striation and striation papillae (indicated by arrow) on membrane, also some unicellular trichomes. — **C** and **D**: *L. sinense*. Abaxial surface shows slight ridges and fine striation on membrane and narrow-elliptic stomata. — **E** and **F**: *L. tenuissimum*. Adaxial surface shows normal ridges and fine striation on membrane, narrow-elliptic stomata. — **G** and **H**: *L. tenuissimum*. Abaxial surface shows normal ridges and fine striation on member, elliptic stomata. — **I** and **J**: *L. thomsonii*. Adaxial surface shows normal ridges and coarse striation on membrane, elliptic stomata. — **K** and **L**: *L. thomsonii*. Abaxial surface shows slight ridges and coarse striation on membrane, elliptic stomata. Scale bars: **B** = 50 μm; **C**, **E**, **G**, **I**, **K** = 20 μm; **A**, **D**, **J** = 10 μm; **F**, **H**, **L** = 5 μm.

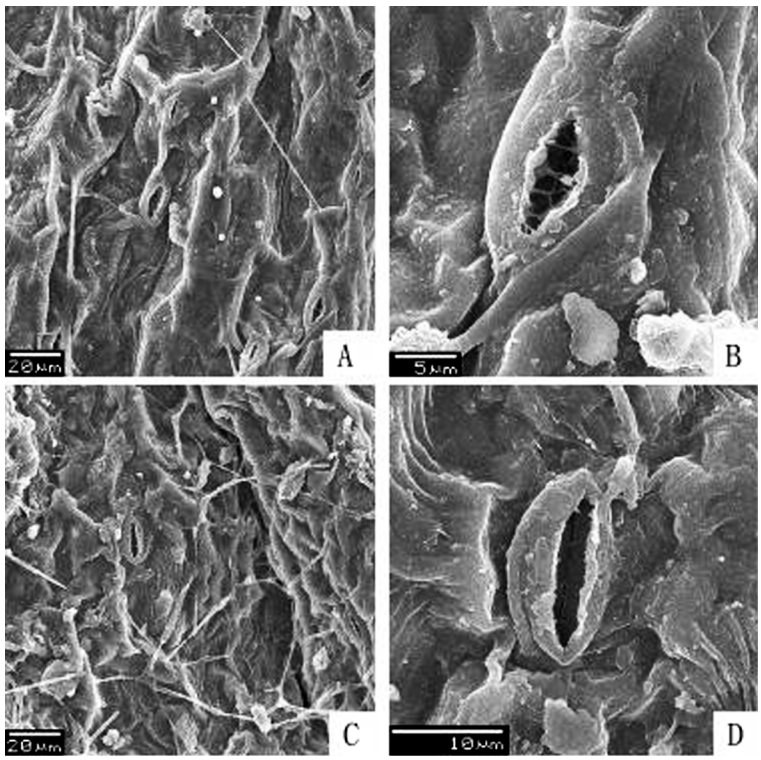

Fig. 11. Photomicrographs of leaf surfaces of *Ligusticum* (epidermal cells and stomata). — **A** and **B**: *L. reptans*. Adaxial surface shows heavy ridges and coarse striation on membrane, narrow-elliptic stomata with filiform wax in stomata aperture. — **C** and **D**: *L. reptans*. Abaxial surface shows heavy ridges and normal striation on membrane, narrow-elliptic with filiform wax in stomata aperture. Scale bars: **A**, **C** = 20 *μ*m; **B** = 5 *μ*m; **D** = 10 *μ*m.

match the results from Ostroumova and Lavrova (1991) and Ostroumova and Kljuykov (2007) for this special species. Because the papers just cited give descriptions of stomatal types only, without information on the stomatal sizes and indexes, as well as without description of the epidermal microstructure under SEM, further comparisons are not possible.

Frequency and size of epidermal cells and stomata.

According to our observations, species with higher epidermal cell frequency, or smaller epidermal cell sizes or stomata, had more stomata per unit area. This was true especially for the abaxial surfaces. *Ligusticum franchetii* is easily separated from the other species because it has a much lower number of epidermal cells per mm^2 and much larger epidermal cells and stomata sizes. *Ligusticum scapiforme* is distinguished by its number of epidermal cells and stomata per mm^2 on both leaf surfaces, which are much higher than in the other species.

Ridges and striations on epidermal cell membranes

Striations are usually of value in specific diagnoses (Solereder 1908). Among the species studied here, most have ridges on the epidermal surface and have striations between or across the ridges. *Ligusticum acuminatum*, *L. angelicifolium*, *L. brachylobum*, *L. jeholense*, *L. sinense* and *L. tenuissimum* have very fine striations on one or both leaf surfaces. Among these six species, striation papillae separate *L. acuminatum*, *L. jeholense* and *L. sinense* from the other three. All three of these species are also hypostomatic. However, the other two hypostomatic species, *L.*

ajanense and *L. franchetii*, have coarse or almost invisible ridges and striations on the membrane. Other species, such as *L. multivittatum* and *L. scapiforme*, have normal ridges or striations on their leaf surfaces.

Other potentially important characters

The taxonomic value of trichomes in angiosperms is well documented in the botanical literature (Theobald *et al*. 1979, Batterman & Lammers 2004). However, because trichomes are absent in most of the species studied, these may be of little diagnostic value in *Ligusticum*. Among the studied species, trichomes do occur only in *L. involucratum*, *L. jeholense* and *L. sinense*.

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