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CUTICLE MICROMORPHOLOGY OF *PINUS KREMPFII* LECOMTE (PINACEAE) AND ADDITIONAL SPECIES FROM SOUTHEAST ASIA¹

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Cuticle micromorphology of the unusual Vietnamese pine, *Pinus krempfii* Lecomte, and three additional endemic southeast Asian species of *Pinus* L. (Pinaceae) is characterized for the first time. Taxa studied include (1) *P. krempfii*, typically placed in its own subgenus *Ducampopinus* (Chevalier) Ferré ex Little & Critchfield; (2) the endemic Vietnamese species *Pinus dalatensis* Ferré and (3) the southeast Asian species *Pinus kwantungensis* Chun ex Tsiang, both of subgenus *Strobos*; and (4) the widespread Asian species *Pinus kesiya* Royle ex Gordon of subgenus *Pinus*. The current and previous studies demonstrate that the genus *Pinus* and its subgenera are delimited by unique combinations of cuticular characters, although some of these characters may occur individually in other conifers. Cuticular micromorphology supports taxonomic assignment of *P. krempfii* to subgenus *Strobos* rather than to its own subgenus, a result that is also indicated by other anatomical studies and recent molecular studies. Sectional affinities of *P. krempfii* are usually with *Parrya*, subsection *Krempfianae*. An alternative classification of *P. krempfii* with subsection *Gerardianae* can be supported by micromorphological characters including broad cuticular bridges between stomata, details of the intercellular flanges of the epidermal cells, and usually an amphistomatic stomatal distribution. Features of other Asian species studied are consistent with their taxonomic assignments. The study demonstrates the utility of cuticle micromorphology to taxonomic delimitation within the family Pinaceae.

Keywords: *Pinus krempfii*, Pinaceae, Vietnam, cuticle micromorphology.

Introduction

Pinus krempfii Lecomte is a rare endemic pine that occurs in the central highlands of Vietnam at about lat. 12°N, long. 108°E. Unusual features that distinguish this pine from other members of the genus *Pinus* are its flat needles, measuring up to 5 mm in width (Lecomte 1921, 1923, 1924; Buchholz 1951; Rollet 1955), its falcate needle shape in cross section (Buchholz 1951), the reported occurrence of juvenile leaves (N. T. Hiep, personal communication, National Centre of Science and Technology of Vietnam, Institute of Ecology and Biological Sciences, Hanoi, 1995), early deciduous fascicle sheaths (Buchholz 1951; Rollet 1955), and the absence of ray tracheids (Rollet 1955; Hudson 1980; Ickert-Bond 1997b).

The taxonomic position of *P. krempfii* has been problematic for many years and has been treated in a variety of ways by different authors. This anomalous pine has been commonly placed in the monotypic subgenus *Ducampopinus* (Chevalier) Ferré ex Little & Critchfield or variously placed under subgenus *Strobos*, in the monotypic subsection *Krempfianae* (Klaus 1980), in the subsection *Balfourianae* (Van der Burgh 1973), or even elevated to generic rank (Chevalier 1944; Landry 1994) as *Ducampopinus krempfii* (Lecomte) Chevalier. However, not all authors recognize subgenus *Ducampopinus*

(Farjon 1996; Farjon and Styles 1997; Price et al. 1998). For example, Van der Burgh (1973) subdivided the genus *Pinus* into eight sections, largely based on wood anatomy. Unlike most authorities (e.g., Chevalier 1944; Ferré 1952; Gausson 1960; Little and Critchfield 1969), he included *P. krempfii* within subsection *Balfourianae* Engelmann in accordance with Shaw (1924) and Pilger (1926). Klaus (1980) assigned *P. krempfii* to section *Parrya* Mayr, subsection *Krempfianae* Little & Critchfield, primarily on the basis of cone scale morphology. Most recent studies based on DNA analysis agree with the division of the genus into two major groups (Strauss and Doerksen 1990; Wang and Szmidt 1993; Liston et al. 1999; Wang et al. 1999, 2000). While some authors suggest *P. krempfii* be placed within subgenus *Strobos*, subsection *Strobi* (Liston et al. 1999), others classify *P. krempfii* outside of *Strobi* and find a closer affinity to subsection *Gerardianae* and its two Asian members *P. gerardiana* and *P. bungeana* (Wang et al. 1999). Wang et al. (1999) further assume *P. krempfii* to be a link between sections *Parrya* and *Strobos*. Molecular data for *P. krempfii* as well as its unusual morphology thus demonstrate the need for reevaluating the taxonomic assignment of this species.

The problematic position of *P. krempfii* has been at least in part due to the limited information available about many of the morphological and anatomical features of this unusual pine. As a whole, the Asian pines are the least well known taxonomically, often due to the inaccessibility of natural populations, a situation that is especially true for Vietnam. Because information about them is not easily available, three additional southeast Asian species are included in this study: *Pinus dal-*

¹ This article represents a portion of Stefanie M. Ickert-Bond's thesis submitted in partial fulfillment of the requirements for the M.S. degree at Arizona State University, Tempe, Arizona.

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Table 1

Pinus Herbarium Material Examined

Voucher	Herbarium ^a	Collection locality	Date
<i>Pinus krempfii</i> subg. <i>Strobos</i> :			
N. C. Ban s.n.	HN	Dalat Flower Garden, Vietnam	Jan. 24, 1983
L. K. Bien 1236	HN	Lam Dong, Lac Duong, Vietnam	May 2, 1980
Ickert-Bond 268	ASU	Dalat Flower Garden, Vietnam	June 14, 1995
Ickert-Bond 278	ASU, GH, B, E	Cong Troi, Dalat, Vietnam	June 16, 1995
Ickert-Bond 300, 304	ASU, GH, B, E	Cong Troi, Dalat, Vietnam	June 21, 1995
Krempf 1537	S	Nhatrang, Vietnam	Not mentioned
Poilane 3426	S	Nhatrang, Vietnam	Not mentioned
Poilane 3461	HN, P	Nhatrang, Vietnam	May 19, 1922
Poilane 4382	HN	W of Nhatrang, Vietnam	July 4, 1922
Poilane 6540	P	N of Nhatrang, Vietnam	May 18, 1923
Poilane 6540	ILL	Ninh-hoa, Nhatrang, Vietnam	March 8, 1923
Poilane 18737	BM	N of Dankia, Langbian Mts., Vietnam	Oct. 29, 1930
Truong Van Len s.n.	IFG, GH, UC, US	Boungia/Dalat, Ankroet area, Vietnam	March 13, 1961
Vu Dung s.n.	FHO	Cong Troi, Lac Duong, Lam Dong, Vietnam	Feb. 15, 1984
Vu Van Dung 71	HN	Lam Dong, Lac Duong, Cong Troi, Vietnam	Nov. 21, 1984
Vu Dung 72	HN	Cong Troi, Lac Duong, Lam Dong, Vietnam	Nov. 24, 1984
<i>P. dalatensis</i> subg. <i>Strobos</i> :			
R. Businsky PO/4/01-14	IFG	Traimat (Dalat), Vietnam	Not mentioned
E. N. Cooling	FHO	Traimat, Vietnam	May 14, 1967
Poilane 32581	IFG	S. of Darlac, Vietnam	April 24, 1941
Truong Van Len s.n.	IFG, GH	Traimat (Dalat), Vietnam	March 17, 1961
<i>P. kesiya</i> subg. <i>Pinus</i> :			
A. Chevalier 30024	S	Dalat, Vietnam	Feb. 12, 1914
E. N. Cooling 365	FHO	N. Dalat, Vietnam	May 14, 1967
Ickert-Bond 269	ASU, GH, B, E	Dalat, Vietnam	June 14, 1995
Ickert-Bond 283	ASU, GH, B, E	Dalat, Vietnam	June 18, 1995
F. Kingdon-Ward 19298	BM	Rima, Assam	April 6, 1950
Truong Van Len s.n.	UC	Dasar (Dalat), Vietnam	April 20, 1961
<i>P. kwantungensis</i> subg. <i>Strobos</i> :			
Davo Shan Team 11185	MO	Road to Jinxiouyu, ?, China	Oct. 3, 1981
East Hainan Team 598	MO	Five Fingers Peak, Hainai, China	Nov. 8, 1954
Ickert-Bond 160	ASU, GH, E, B	Hoa Binh, Pa Ca, Vietnam	June 6, 1995
Zhijian Feng 80831	MO	Yangjiau protection area, Chenjia Peak, Yangshan and Ruyan Cos., Guandong, China	May 5, 1987
Zhiyou Li 879	MO	Ru Yang Forest Bureau, Ruyan Co., Guandong, China	Oct. 17, 1985
Zhiyou Li 951	MO	Chenjia, Yangshan and Ruyan Cos., Guangong, China	Oct. 28, 1985
Unknown	GH	Dalat, Vietnam	Sept. 28, 1935

^a Herbarium acronyms follow Holmgren et al. 1990, except for HN (Hanoi University, Vietnam), which is not listed.

atensis Ferré, *Pinus kesiya* Royle ex Gordon, and *Pinus kwantungensis* Chun ex Tsiang. Whereas *P. dalatensis* is endemic to a small mountain range, the Central Highlands, Vietnam (Ferré 1960; Ngoc-Sanh 1962; Hiep 1994), *P. kwantungensis* and *P. kesiya* have a much wider distribution within southeast Asia (Kha 1965; Critchfield and Little 1966; Mirov 1967; Armitage and Burley 1980). While *P. krempfii* is taxonomically problematic, the other southeast Asian pines in the study are readily assigned to subgenera. Both *P. dalatensis* and *P. kwan-*

tungensis have been easily included in subgenus *Strobos* (Little and Critchfield 1969; Klaus 1980). Little and Critchfield (1969) place these species within section *Strobos*, subsection *Strobi* Loudon, while Van der Burgh (1973) recognizes them in section *Strobos*. Most authors agree on the placement of *P. kesiya* in subgenus *Pinus*, section *Pinus*, subsection *Sylvestres* Loudon (Little and Critchfield 1969; Klaus 1980; Price et al. 1998).

In order to further assess the taxonomic position of *Pinus*

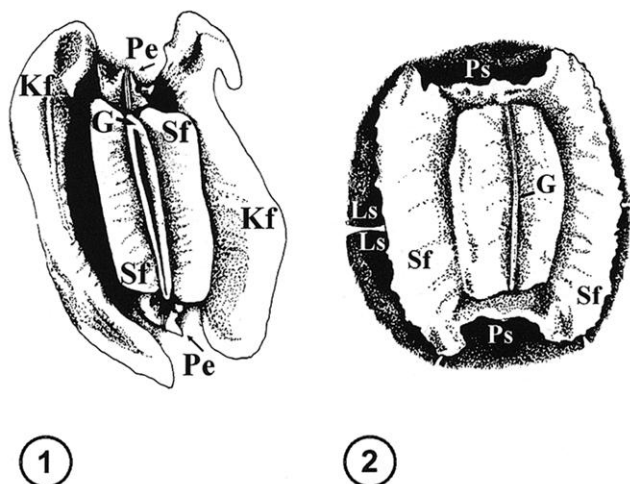


Fig. 1 Comparison of stomatal complex more typical of Pinaceae (fig. 1.1) with *Pinus* (fig. 1.2). Fig. 1.1, Amphicyclic stomatal complex (*Keteleeria*) showing guard cells surrounded by inner ring of two polar and two lateral subsidiary cells, in turn surrounded by outer ring (Kranz subsidiary cells). Inner subsidiary cells are obscured by flange in this figure. Flanges occur between guard cells and between guard and inner subsidiaries and between inner and outer (Kranz) subsidiaries. The inner subsidiary cells can have polar extensions. Fig. 1.2, Monocyclic stomatal complex of *Pinus* showing guard cells surrounded by one ring of subsidiary cells, composed of two polar and four to eight lateral subsidiary cells. Flanges occur between guard cells and between guard and subsidiary cells. There is no Kranz layer. Not to scale. G, guard cell; Kf, flange on Kranz subsidiary cell; Ls, lateral subsidiary cell; Pe, polar extension; Ps, polar subsidiary cell; Sf, flange between guard and subsidiary cell.

krempfii, a study was initiated in 1995 to more fully document field, morphological, and anatomical details of this poorly known but intriguing taxon (Ickert-Bond 1997a). The results of this study will be published in several articles. In this study, internal and external features of cuticle micromorphology for *P. krempfii* and three additional species from Vietnam are characterized for the first time. Micromorphology provides previously unidentified cuticular characters of these leaves unavailable through light microscopy that have taxonomic significance to the classification of Pinaceae at the generic and subgeneric levels. Cuticle micromorphology has been seldom used for pine taxonomy. This feature has been useful in delineation of Southern Hemisphere conifer genera in the Araucariaceae and Podocarpaceae (Stockey and Ko 1986, 1989; Stockey and Atkinson 1993; Stockey and Frevel 1997; Stockey et al. 1998) and in the Cupressaceae (Alvin et al. 1982). While the cuticular features of the Pinaceae have been investigated in detail at the light-microscope level by several authors (Porsch 1905; Florin 1931; Pant and Basu 1977) and in a more general descriptive sense by others (e.g., Doi and Morikawa 1929; Harlow 1931; Laubenfels 1953), relatively few studies have documented micromorphology with scanning electron microscopy (SEM). These include external cuticle surfaces of 51 species of *Pinus* (Yoshie and Sakai 1985), internal cuticle surfaces for 17 species by Hu (1986), and both external and internal cuticular surfaces for 21 species by Kim et al. (1999).

The genus *Cathaya* was studied by Hu and Wang (1984), and additional representative genera of the Pinaceae have been investigated with SEM by Boulter (1971) and Miranda and Chaphekar (1980). From these studies, preliminary comparative data suggest that features of potential taxonomic value include floric ring morphology, stomatal opening size, and intercellular flanges of epidermal cells.

Material and Methods

Leaves of *Pinus krempfii*, *Pinus kwantungensis*, and *Pinus kesiya* were studied from freshly collected samples taken from natural populations in Vietnam; *Pinus dalatensis* was studied from herbarium sheets (table 1). Preparation of material for light microscopy followed Jain (1976). Specimens were permanently mounted in Permount (Fisher Scientific) and viewed under an Olympus BH2 compound light microscope. Preparation of leaves for the SEM was carried out according to techniques outlined in Alvin and Boulter (1974) and Stockey and Ko (1986). Both inner and outer surfaces of cuticles were examined with SEM. Specimens were mounted on stubs with double-sided sticky tape, coated with 200 Å of gold on a Hummer II sputter coater, and viewed with Amray 1000A and Joel 6301 FXE scanning electron microscopes at 20 kV.

Terms for conifer cuticle micromorphology follow Florin (1931, 1951). Guard cells are surrounded by a ring of subsidiary cells composed of polar and lateral subsidiaries (fig. 1). In some conifers, these in turn may be surrounded by a second ring of subsidiary cells composed of polar and lateral subsidiaries called the Kranz layer (Kranzzelle) (fig. 1.1). The Kranz layer is absent in *Pinus* (fig. 1.2). Lateral cuticular extension of the guard cells (flanges) can reach neighboring subsidiary cells. Intercellular flanges of epidermal cells are situated on anticlinal walls of epidermal cells and are also pronounced in *Pinus*. In other genera of Pinaceae, flanges can also occur on encircling cells or as polar extensions on the guard cells.

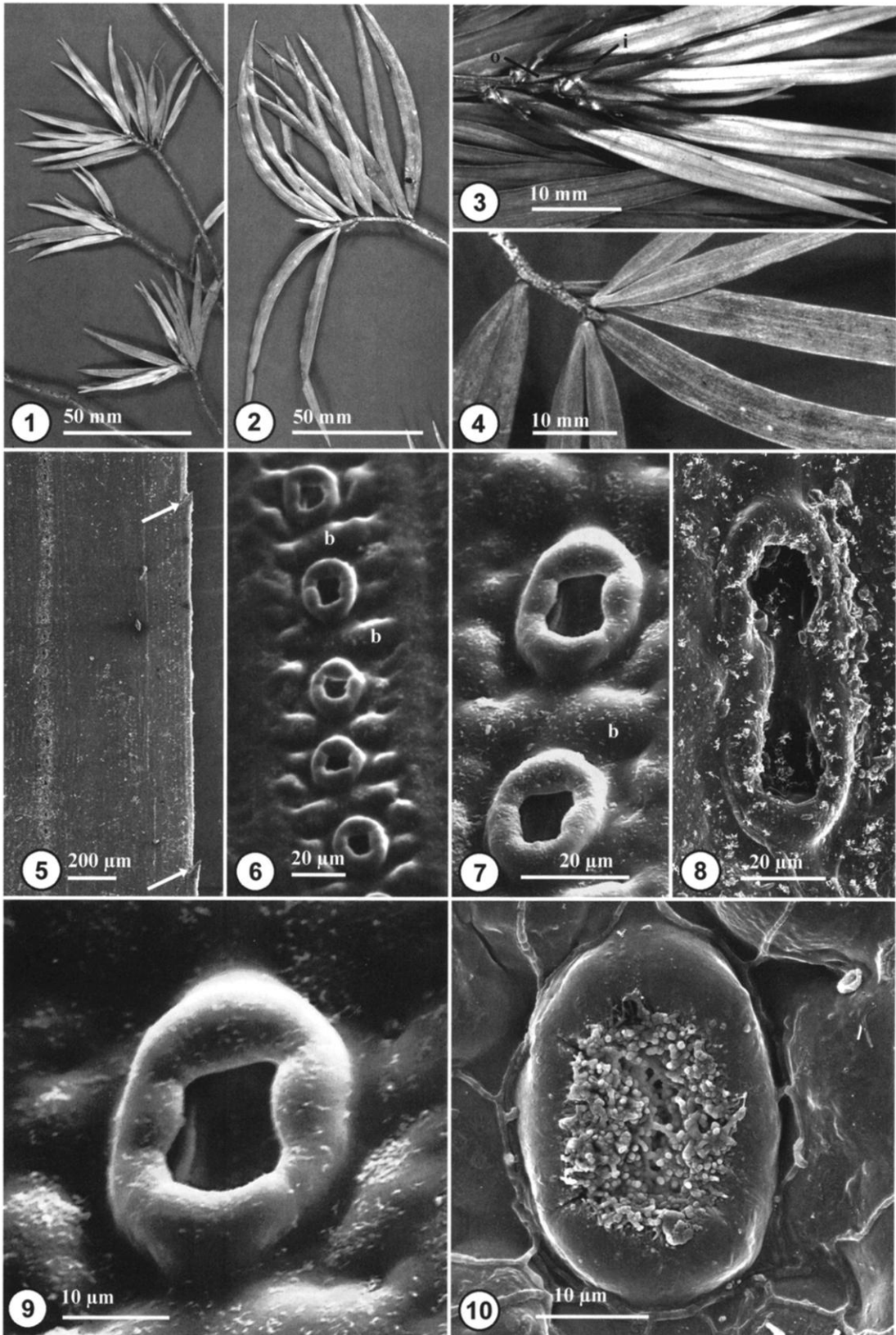
Results

Pinus krempfii Lecomte (Figs. 2, 3)

Pinus krempfii has dwarf and long shoots typical of pines. The dwarf shoots or foliar spurs in pines develop on long shoots, arising in the axils of scale leaves (fig. 2.3) and bear helically arranged flattened leaves in fascicles of two (figs. 2.1–2.4) (Lecomte 1921). Leaves taken from mature trees (fig. 2.1) and juvenile foliage (fig. 2.2) from *P. krempfii* saplings as well as some cotyledons from seedlings were taken from plants growing in natural populations in the Cong Troi area in the Central Highlands of Vietnam at 1800 m and from herbarium specimens (table 1).

Leaves, occurring in pairs and arranged in a scissor-like fashion (Rollet 1955) (figs. 2.1–2.4), are borne in the axils of six to eight brown scale leaves (fig. 2.3). The scales are plicately folded around the edges of the leaves (fig. 2.3). The exterior scales measure 10 mm in length, while the two innermost scales are remarkably elongated, measuring up to 30 mm (fig. 2.3). Once the leaves are fully enlarged, the fascicle sheath falls off, exposing rough scale scars on the twig (fig. 2.4).

Mature leaves are oblong-lanceolate, flattened (Buchholz



1951), and 4.5–7.0 cm long and 3.5–4.1 mm wide, with short, slightly twisted petioles (figs. 2.1, 2.4). Margins are minutely serrulate with teeth ca. 480 μm apart (fig. 2.5; table 2). Leaves are amphistomatic. Stomata were found on both the abaxial and adaxial surfaces of the cotyledons but are mostly concentrated on adaxial surfaces of juvenile foliage and adult leaves, with occasional short stomatal lines on the abaxial surface (table 2).

The external cuticle surface is undulating with sunken stomata in eight to 12 rows (figs. 2.6, 2.7) with stomatal apparatus oriented parallel to the leaf axis. Each stoma is surrounded by an encircling ridge called a Florin ring (Buchholz and Gray 1948) (figs. 2.6–2.10), although sometimes fusion of the Florin rings of two neighboring stomata occurs (fig. 2.8). Typically, broad cuticular bridges *sensu* Yoshie and Sakai (1985) alternate with stomata (fig. 2.6). Florin rings are sunken at the base and rise steeply and sharply (figs. 2.6–2.9). The shape of the opening is circular to square, measuring 247 μm^2 (12.3 \times 20.1 μm) in diameter (fig. 2.9; table 2). This type of Florin ring and its stomatal size are referred to as “type D,” typical of subgenus *Pinus* (Yoshie and Sakai 1985). The fresh material had stomatal plugs composed of short fused rods *sensu* Yoshie and Sakai (1985) (fig. 2.10; table 2), but these were rarely found on herbarium specimens (fig. 2.9).

The internal cuticle surface shows stomata arranged in continuous rows with stomatal apparatus oriented parallel to the long leaf axis (fig. 3.1) and occasionally in discontinuous rows (fig. 3.2). The stomatal apparatus varies slightly in shape, depending on the number of subsidiary cells and the presence of shared subsidiary cells (figs. 3.2, 3.3). Most commonly, two polar and up to eight lateral subsidiary cells are present (fig. 3.4; table 3). Cuticle on subsidiary cell surfaces is thicker on the abaxial surface. The cuticular flange between guard and subsidiary cells is very extensive and irregular in outline (figs. 3.3, 3.6). An extension of this flange sometimes approaches the edge of the opposite wall of the subsidiary cell cuticle (fig. 3.3). Removal of the flange allows for observation of the subsidiary cells (fig. 3.4). The surface of the subsidiary cell cuticle is slightly pitted but obscurely striate (fig. 3.6). Cuticle thins in the polar region and is nearly smooth with a small extension (fig. 3.3). The cuticular flange between guard cells is thick and smooth to slightly rugose with an irregular margin.

Epidermal cells are rectangular on adaxial leaf surfaces. Those between stomatal rows are longer than broad; those within stomatal rows, broader than long (table 3). Cell outlines are deeply sinuous and end walls are typically oblique (fig. 3.5). Cuticle surfaces have protrusions and depressions; the depressions run together, forming a reticulum (fig. 3.7).

Pinus dalatensis Ferré (Figs. 4, 5)

Adult needle leaves were examined from herbarium specimens (table 1). In this species, leaves are needle-like, 5.6–5.9 cm long, 0.5 mm wide, and are found in fascicles of five (fig. 4.1), ensheathed by deciduous cataphylls (fig. 4.2). Margins are minutely serrulate with teeth 569 μm apart (fig. 4.3; table 2). Stomata are mainly restricted to the adaxial surfaces of the leaves, although short stomatal lines are found on the abaxial surfaces as well (table 3).

The external cuticle surface is undulating and bears sunken stomata in closely spaced rows (figs. 4.3–4.5). Florin rings rise sharply from the general cuticle surface (figs. 4.3–4.7), and cuticular bridges are absent. Low ridges and furrows occur between stomatal rows (figs. 4.3, 4.4). Occasionally, Florin rings lacking a stoma occur (fig. 4.5). The stomatal opening is rectangular and medium sized, measuring ca. 218 μm^2 (7.2 \times 30.1 μm) (fig. 4.7; table 2). Stomatal plugs composed of granular rods of wax *sensu* Yoshie and Sakai (1985) were found on herbarium specimens (fig. 4.6; table 2).

The internal cuticular surface shows stomata arranged in continuous rows and oriented parallel to the long leaf axis (figs. 5.1, 5.2). Occasionally, within the rows, a group of four subsidiary cells is present and guard cells are lacking (fig. 5.2). These structures correspond with small Florin rings on the outer cuticular surface (fig. 4.5). Stomata are regular in shape, reflecting a consistent number of subsidiary cells, commonly four (fig. 5.3; table 3). Occasionally, a lateral subsidiary cell may be divided into two (fig. 5.4). Cuticle of subsidiary cells is pitted. Cuticular flanges between guard and subsidiary cells are thick and subentire in outline (fig. 5.3). Thin extensions of the cuticular flange over subsidiary cells occur but are often not obvious. The cuticular flange between guard cells is thick and smooth (fig. 5.3).

Epidermal cells are rectangular with straight end walls on both adaxial and abaxial cuticle surfaces. On abaxial surfaces epidermal cells are extremely elongate between and much broader within rows of stomata (table 3; fig. 5.5). Cell outlines are sinuous with thin intercellular flanges that lack buttresses or cavities (fig. 5.5). Cuticle on epidermal cell surfaces is pitted to granular (table 3), showing protrusions and depressions, and the depressions run together forming a reticulum (fig. 5.6).

Pinus kesiya Royle ex Gordon (Figs. 6, 7)

Adult leaves were taken from herbarium specimens (table 1). Each fascicle is composed of three acicular leaves ensheathed by persistent scale leaves (figs. 6.1, 6.2). Mature leaves are needle-like, 15–17.5 cm long, and 0.1 cm wide. Leaves are minutely serrulate with teeth 415 μm apart (fig.

Fig. 2 *Pinus krempfii*, outer views. Fig. 2.1, Branch of mature tree showing arrangement of dwarf shoots toward the end of branches. Fig. 2.2, Branch of sapling showing extremely long and wide leaves of juvenile foliage. Fig. 2.3, Detail of dwarf shoots showing plicately folded scale leaves surrounding shoot. Fig. 2.4, Detail of leaf fascicle showing leaves in pairs and arranged in a scissor-like fashion. Note that the fascicle sheath has fallen off. Fig. 2.5, Adaxial surface showing stomatal rows with Florin rings (at left) and teeth on leaf margin (arrows). Fig. 2.6, Adaxial surface showing Florin rings and broad cuticular bridges alternating with stomata. Fig. 2.7, Adaxial surface showing Florin rings and cuticular bridges. Fig. 2.8, Adaxial surface showing a fused Florin ring, surrounding two stomata. Fig. 2.9, Adaxial surface showing a Florin ring with a circular to square stomatal opening. Fig. 2.10, Adaxial surface showing a Florin ring with stomatal plug composed of short fused rods. *b*, cuticular bridges; *i*, innermost scale leaf; *o*, outer scale leaf.

Table 2

External Cuticular Features on Leaves of *Pinus* from Vietnam

Species	Leaf dimension (length × width) (mm)	Serration (μm)	Stomatal distribution	Stomatal plug morphology	Stomatal opening	
					Shape	Size (μm)
<i>Pinus krempfi</i>	45[–70] × 3.5[–4.1]	480	Amphistomatic	Short fused granular rods	Circular to square	12.3 × 20.1
<i>P. dalatensis</i>	56[–59] × 0.5	569	Amphistomatic	Granular rods	Rectangular	7.2 × 30.1
<i>P. kwantungensis</i>	43[–49] × 0.9	835	Epistomatic	Fused rods	Rectangular	9.7 × 17.4
<i>P. kesiya</i>	150[–175] × 1.1	415	Amphistomatic	Thick granular rods	Oval	17.9 × 27.3

6.3; table 2). Stomata occur on both the adaxial and the abaxial surfaces of the leaves (table 2).

The external cuticle surface is slightly undulating and bears stomata in six rows abaxially and four rows adaxially. Florin rings rise gently to the general surface of the leaf (figs. 6.3–6.5). The stomatal opening is elliptic to oval and large, measuring 488 mm^2 ($17.9 \times 27.3 \text{ mm}$) (figs. 6.4, 6.5; table 2). Stomatal plugs composed of thick granular wax rods were found in herbarium specimens (fig. 6.6; table 2).

The internal cuticle surface shows stomata arranged in continuous rows oriented parallel to the long axis of the leaf (figs. 7.1, 7.2). The stomatal apparatus varies slightly in shape, depending on the number of subsidiary cells and the occurrence of shared polar cells. Most commonly, two polar cells and six lateral subsidiary cells are present (fig. 7.1). Occasionally, two of the lateral cells may divide again, resulting in eight lateral cells (fig. 7.1; table 3). Up to 12 subsidiary cells were counted in this species (fig. 7.1). The cuticular flange between guard and subsidiary cell is extensive and regular in outline (figs. 7.3, 7.4). It is thick and smooth, sometimes with a triangular extension (fig. 7.4). Cuticle of the guard cells thins in the polar region and is nearly smooth with two long extensions (fig. 7.4). Epidermal cells are elongated with cell flanges being thick and the outlines sinuous (fig. 7.5) with cavities present at the base of the flange. They have straight end walls. Cuticle on epidermal cell surfaces is granular (fig. 7.6; table 3) with protrusions clustered together.

Pinus kwantungensis Chun ex Tsiang (Figs. 8, 9)

Adult leaves were taken from fresh material (table 1). Fascicles of five leaves are ensheathed by deciduous scale leaves (figs. 8.1, 8.2). Mature leaves are needle-like, 6–8 cm long, and 0.15 cm wide (fig. 8.1). Margins are minutely serrulate with teeth 834.5 mm apart (fig. 8.3; table 2). Stomata are restricted to the adaxial surface of the leaves.

The external cuticle surface is undulating and bears sunken stomata in rows (figs. 8.3, 8.4). Florin rings are sunken at their base and rise steeply and sharply to the general surface of the leaf (figs. 8.4–8.6), as seen in *Pinus krempfi*. Ridges are well developed between stomatal rows (figs. 8.3, 8.4) and are more pronounced than in *P. dalatensis* (figs. 4.3, 4.4). The opening of the Florin ring is rectangular and medium sized (fig. 8.6), measuring 188 mm^2 ($9.7 \times 17.4 \text{ mm}$) (table 2). Several radial striations delimiting individual epidermal cells occurring across Florin rings are sometimes visible (fig. 8.6), as in *Pinus par-*

viflora var. *glauca* (Yoshie and Sakai 1985). Stomatal plugs composed of fused wax rods were found on herbarium specimens (fig. 8.5; table 2).

The internal cuticle surface has stomata arranged in continuous rows oriented parallel to the long axis of the leaf (figs. 9.1, 9.2). Stomatal apparatus are regular, due to the consistent number of subsidiary cells, most commonly six (fig. 9.4; table 3). Cuticle of subsidiary cells is pitted (fig. 9.6). Cuticular flanges between guard cells and subsidiary cells are extensive and subentire in outline (fig. 9.3) and thin and smooth (figs. 9.3, 9.4). Prominent subsidiary cell flanges are visible (figs. 9.3, 9.4). Epidermal cells are rectangular, both on adaxial and abaxial cuticle surfaces, and have oblique end walls. On the abaxial surface, epidermal cells are extremely elongated between rows (fig. 9.5) but much broader within rows of stomata (table 3). Cell outlines are sinuous with thin cuticular coverings (fig. 9.5). Cuticle on epidermal cell surfaces is granular (fig. 9.7; table 3), showing protrusions and depressions, with the depressions running together to form a reticulum.

Discussion

Cuticle Micromorphology in Pinus

Cuticular characters of the genus *Pinus* as a whole include (1) continuous stomatal rows oriented parallel to the long axis of the leaf (Florin 1931), (2) two polar subsidiary cells and usually two to eight (up to 14) lateral subsidiary cells (Florin 1931), (3) stomatal apparatus of the monocyclic type (Florin 1931), (4) needle margin with highly cutinized teeth, (5) undulating outer cuticle surfaces, (6) Florin rings that are raised above the general cuticle surface to varying degrees (Yoshie and Sakai 1985), (7) stomatal plugs, (8) rectangular epidermal cells, (9) intercellular flanges that are sinuous with varying degrees of buttressing (Hu 1986), and (10) granular epidermal cell surfaces. Of these characters the first three are the only ones obtainable by light microscopy alone. The rest of the characters can be known only from SEM studies. Scanning electron microscopy studies have been done in part for only about two-thirds of the genus *Pinus*, with some characters known for approximately 60 out of 100 species (Miranda and Chaphekar 1980; Yoshie and Sakai 1985; Hu 1986; Kim et al. 1999). Although some of these cuticular features are individually present in other conifers, this combination is unique within Pinaceae to the genus *Pinus*.

Other genera in the Pinaceae differ considerably from this

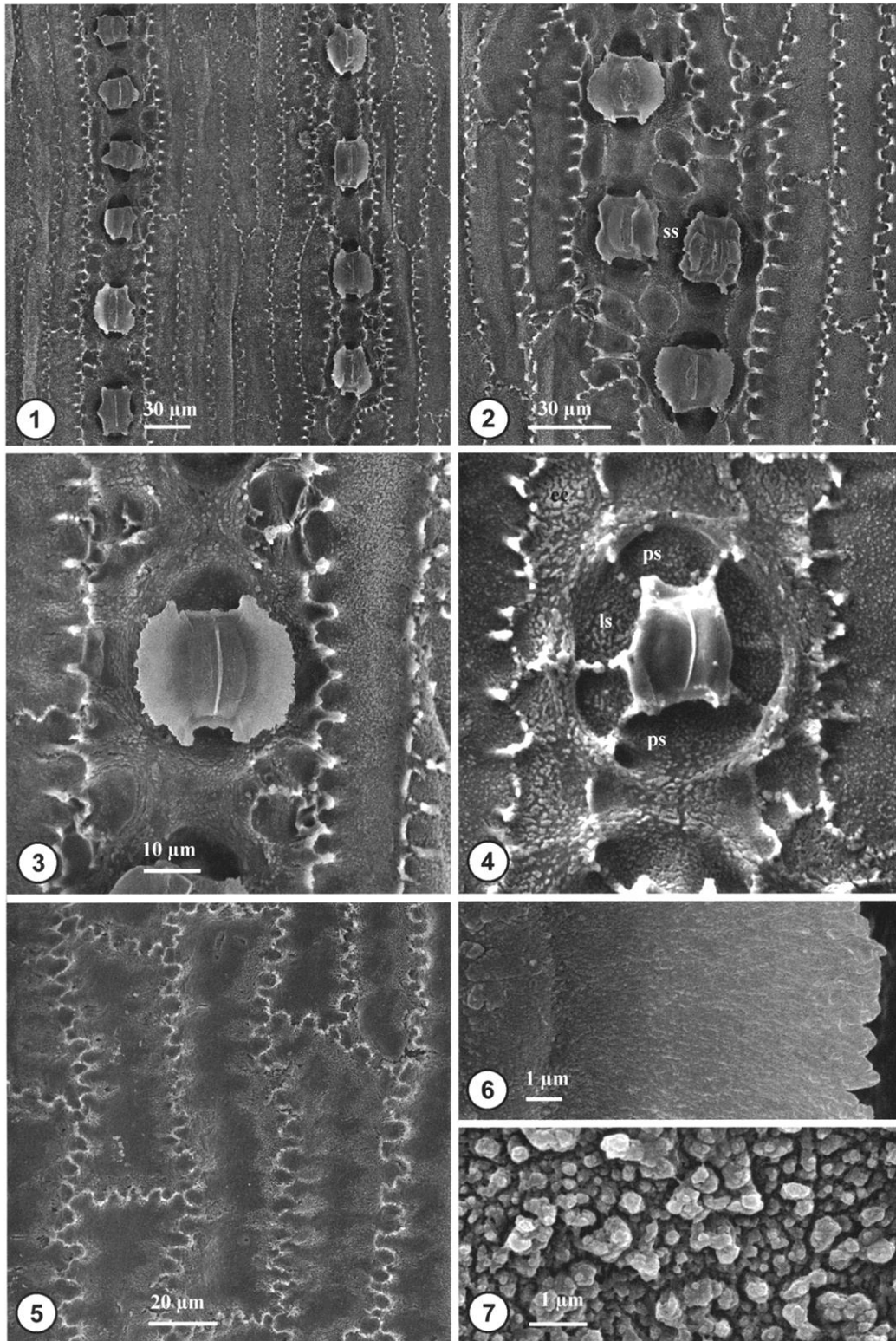


Fig. 3 *Pinus krempfii*, inner views. Fig. 3.1, Adaxial surface showing stomatal rows. Note elongated epidermal cells between stomatal rows. Fig. 3.2, Adaxial cuticle showing discontinuous rows. Fig. 3.3, Adaxial cuticle showing stomatal apparatus. Intact flange obscures views of subsidiary cells. Fig. 3.4, Adaxial surface showing stomatal apparatus. Flange is removed, revealing subsidiary cell number and position. Fig. 3.5, Adaxial surface showing sinuous epidermal cell outlines with oblique end walls. Fig. 3.6, Adaxial surface showing detail of guard cell (at left) and subsidiary cell (at right). Note pits and slight striations on surface and the irregular edge of the subsidiary cell at right. Fig. 3.7, Adaxial surface showing detail of cuticle on epidermal cell surfaces. *ec*, epidermal cell; *ls*, lateral subsidiary cell; *ps*, polar subsidiary cell; *ss*, shared subsidiary cell.

Table 3

Internal Cuticular Features on Leaves of *Pinus* from Vietnam (μm)

Species	Stomatal dimension (avg. polar \times lateral)	Subsidiary cell		Epidermal cell dimension (polar \times lateral)		Epidermal cell	
		Number	Edge	Between rows	Within rows	Surface	End walls
<i>Pinus krempfii</i>	15.8 \times 31.4	Up to 8	Irregular	22.8 \times 132.9	30.4 \times 19.8	Granular	Oblique
<i>P. dalatensis</i>	14.8 \times 29.8	4–6	Subentire	22.1 \times 205.0	32.8 \times 20.3	Granular	Straight
<i>P. kwantungensis</i>	18.1 \times 27.8	6	Subentire	18.3 \times 240.0	24.4 \times 18.7	Granular	Oblique
<i>P. kesiya</i>	20.1 \times 28.4	Up to 12	Regular	21.1 \times 271.3	34.4 \times 17.2	Granular	Straight

combination of characters. The stomatal apparatus is usually amphicyclic within the family, as in *Cedrus*, *Keteleeria* (fig. 1.1), *Cathaya*, *Tsuga*, *Picea*, and *Abies* (Florin 1931; Pant and Basu 1977), while it is monocyclic in *Pinus* (fig. 1.2), *Larix*, *Pseudolarix*, and *Pseudotsuga*. *Larix*, *Pseudolarix*, and *Pseudotsuga* can be distinguished from *Pinus* by the lack of prominent teeth on the leaf margin and by straight rather than wavy anticlinal walls of the epidermal cells (Florin 1958; Miranda and Chaphekar 1980). *Pinus* also differs from these three genera in that neighboring stomata generally do not share lateral subsidiary cells.

Taxonomic Value of Cuticle Micromorphology at the Subgeneric Level

Cuticle micromorphological characters that are of taxonomic use to separate the subgenera *Strobos* and *Pinus* include (1) distribution of stomata, (2) Florin ring micromorphology, (3) intercellular flanges on epidermal cells, (4) number of subsidiary cells (Florin 1931; Yoshie and Sakai 1985; Hu 1986; Kim et al. 1999), and an additional character described by Kim et al. (1999), the “bristle,” that has not been observed in this study. In subgenus *Pinus*, stomata are consistently amphistomatic (Florin 1931; Farjon and Styles 1997), while in subgenus *Strobos*, they are most often epistomatic. However, in some species of subgenus *Strobos*, short lines of stomata are found on the abaxial surface as well. Of the species from Vietnam of subgenus *Strobos*, one, *P. dalatensis*, is amphistomatic while the other, *P. kwantungensis*, is epistomatic. In contrast, *P. kesiya* has an amphistomatic distribution, as is characteristic of subgenus *Pinus*.

Distribution of stomata of *P. krempfii* is also variable but differs from all other pines. Lecomte (1923) originally described leaves in *P. krempfii* as typically amphistomatic. He proposed the variety *polanei* Lecomte for specimens with extremely large leaves (up to 70 \times 5 mm) that were either epi- or amphistomatic (Lecomte 1923, 1924). In reevaluating this character in this study, the widened leaves that I examined are clearly amphistomatic (fig. 2.2). This type of stomatal distribution is related to the way leaves are attached in fascicles and how they are exposed to the light. Leaves occur in fascicles of two and are arranged in a scissor-like fashion (Lecomte 1923) with slightly twisted petioles (fig. 2.4). One leaf in the fascicle is exposed with the adaxial side; the other, abaxially. When stomata are found on the opposing surfaces in each leaf, most have short stomatal lines on the opposite surface and are therefore amphistomatic. From my field observations, wide leaves of the dimensions described by Lecomte (fig. 2.2) were

consistently borne by smaller trees or saplings (N. T. Hiep, personal communication, National Centre of Science and Technology of Vietnam, Institute of Ecology and Biological Sciences, Hanoi, 1995). Although Hiep referred to the large leaves on young saplings as “juvenile leaves” (*les feuilles jeunes*, N. T. Hiep, personal communication, 1995), these leaves might also be interpreted as shade leaves since they occur on saplings that germinate in the shade of parent plants and other canopy trees of the forest. Mature *P. krempfii* trees lack such large leaves. As trees increase in stature and grow above the canopy, leaves produced are increasingly smaller, and mature trees bear consistently smaller “sun” leaves (fig. 2.1) on crowns that extend above all the other canopy trees. The situation in *P. krempfii* may also be compared to some extent with anisophylly *sensu* Goebel (1900), as seen in *Abies pectinata*, where the leaves vary in size with respect to their position on the stem.

A second character of subgeneric value Florin ring morphology was recognized with six morphological types of Florin rings described by Yoshie and Sakai (1985) and reevaluated by Kim et al. (1999). In this study, in *P. kwantungensis*, Florin rings are sunken at the base and rise sharply to the general surface with well-developed ridges and furrows between stomatal rows, corresponding to “type E” of Yoshie and Sakai (1985) and conforming with subgenus *Strobos*, subsection *Strobi* (Yoshie and Sakai 1985). In *P. dalatensis*, Florin rings are similar, but ridges are less well developed, corresponding to “type F,” also typical of subgenus *Strobos*. *Pinus krempfii* has well-developed Florin rings with cuticular bridges that alternate with stomata. Yoshie and Sakai (1985) report this type only from *P. sylvestris* L. and *P. densiflora* Sieb. & Zucc. within subgenus *Pinus*, subsection *Sylvestres*. In another study, similar stomata were reported in *P. gerardiana* and *P. bungeana* of subgenus *Strobos*, section *Parrya* (Hu 1986). Florin rings rise gently to the general surface in *P. kesiya* corresponding to type B, a feature exclusively found in subgenus *Pinus*.

Another cuticular character of taxonomic value is the intercellular flange between epidermal cells as delimited by Hu (1986). All three types described by Hu (1986) are found in the four species examined here. *P. kesiya* demonstrates the “oil pine type” with thick and balustrading intercellular flanges. This type is also illustrated in *P. ponderosa* of subgenus *Pinus* (Miranda and Chaphekar 1980). In *P. dalatensis* and *P. kwantungensis* the flanges are less pronounced, characteristic of the “red pine type” of Hu (1986), a type characteristic of subgenus *Strobos* (Miranda and Chaphekar 1980; Hu 1986; Kim et al. 1999). Those of *P. krempfii* are indicative of the “white pine

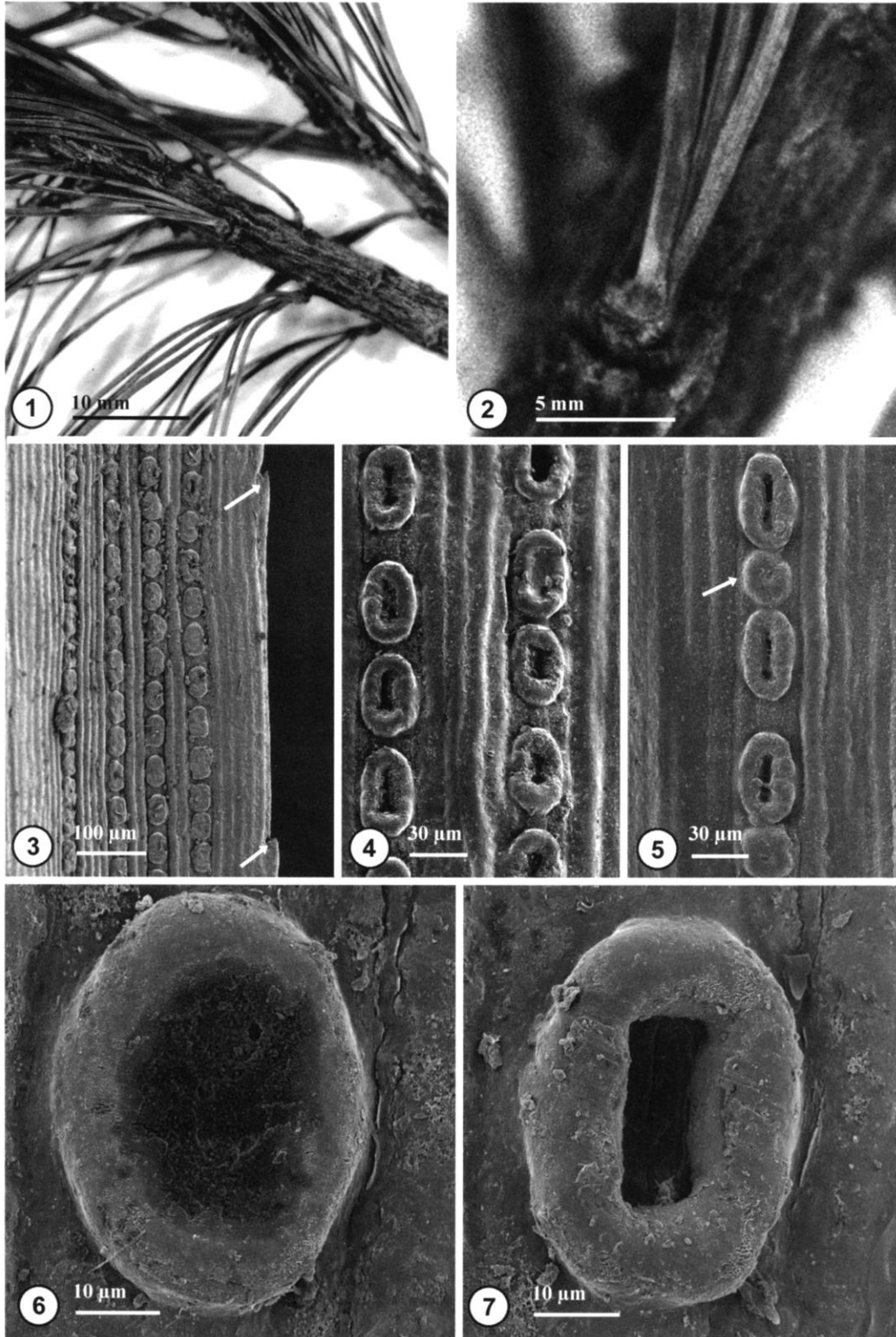


Fig. 4 *Pinus daltensis*, outer views. Fig. 4.1, Branch showing distribution of fascicles. Fig. 4.2, Detail of fascicle showing position of deciduous fascicle sheath. Fig. 4.3, Adaxial surface showing stomatal rows with Florin rings and undulating epidermal cell surface. Note teeth on the leaf margin (arrow). Fig. 4.4, Adaxial surface showing Florin rings and underlying epidermal cell outline. Fig. 4.5, Adaxial cuticle on stomatal rows. Note "added" Florin ring with underlying stomatal apparatus missing (arrow). Fig. 4.6, Adaxial surface showing Florin ring with stomatal plug. Fig. 4.7, Adaxial surface showing Florin ring with rectangular stomatal opening.

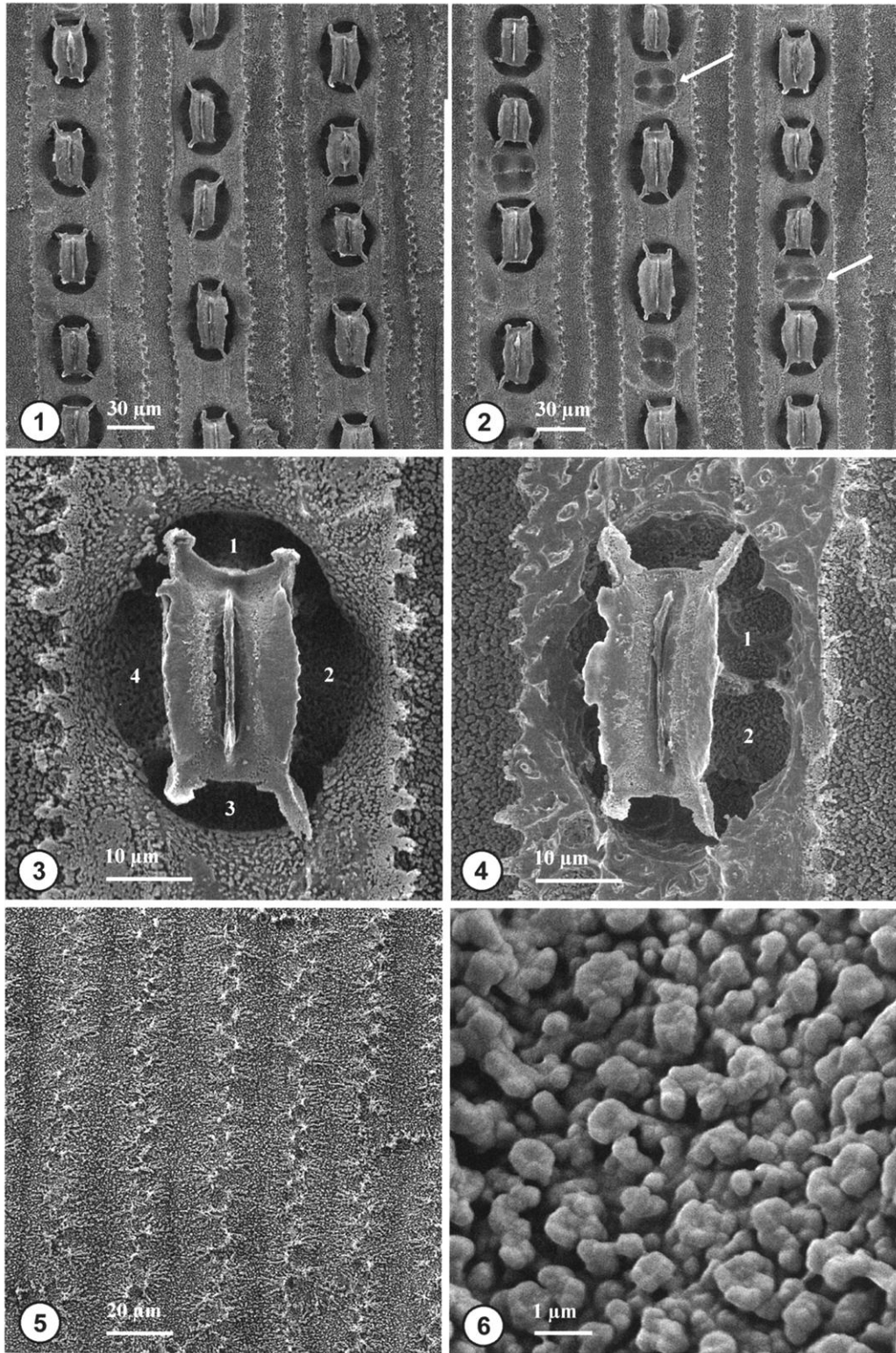


Fig. 5 *Pinus dalatensis*, inner views. Fig. 5.1, Adaxial surface showing stomatal rows, alternating with elongate epidermal cells. Fig. 5.2, Adaxial surface showing stomatal rows. Note stomatal apparatus occasionally missing (arrows). Fig. 5.3, Adaxial surface on stomatal apparatus showing position of four subsidiary cells (1-4). Fig. 5.4, Adaxial surface showing two lateral subsidiary cells (1, 2). Fig. 5.5, Adaxial cuticle showing epidermal cells with sinuous outlines. Fig. 5.6, Adaxial cuticle on detail of epidermal cell surface.

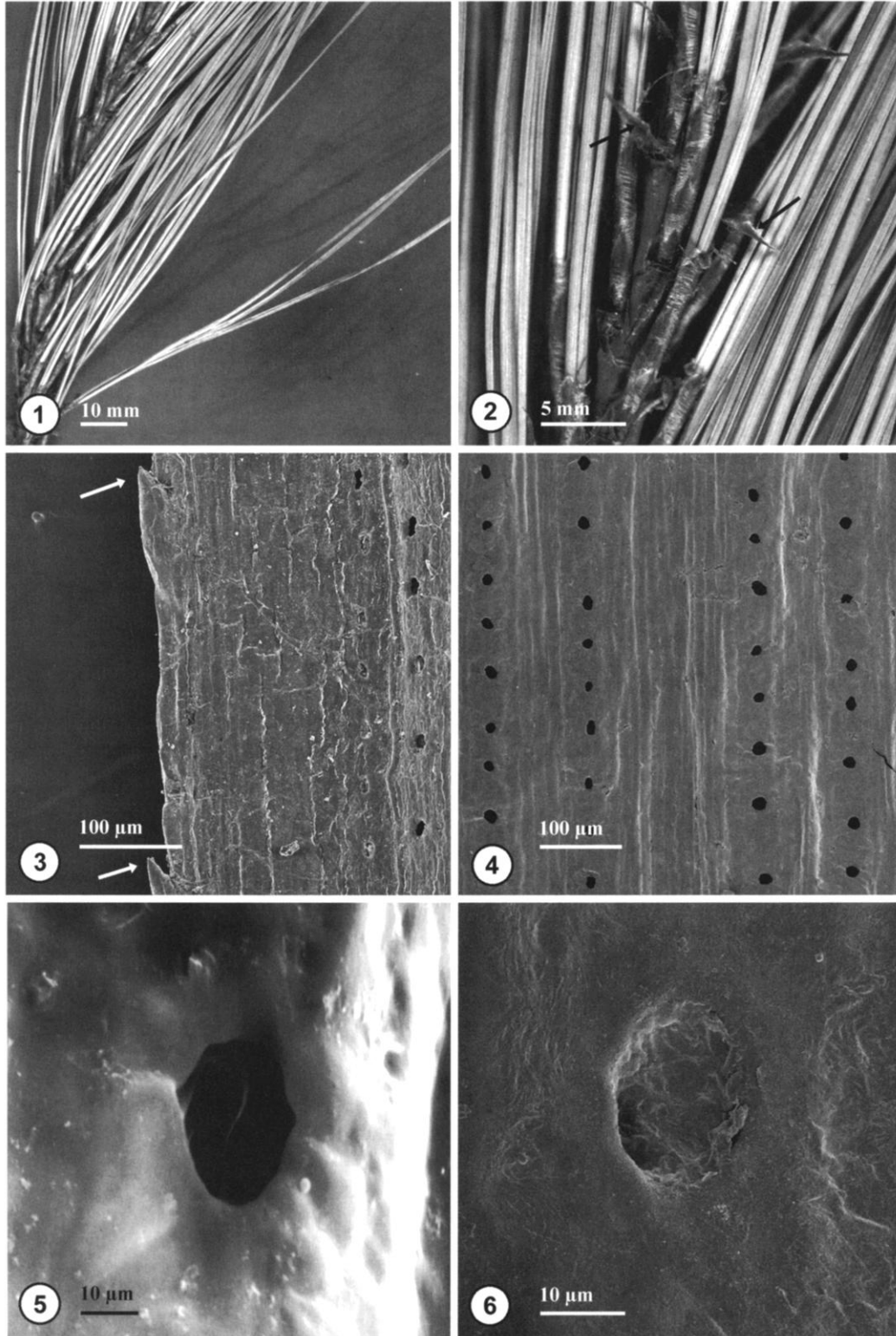


Fig. 6 *Pinus kesiya*, outer views. Fig. 6.1, Branch with dwarf shoots distributed evenly over the branch. Note fascicles of three. Fig. 6.2, Detail of leaf fascicle with persistent fascicle sheaths (arrows). Fig. 6.3, Abaxial surface showing stomatal rows (at right) and teeth on leaf margin (arrows). Fig. 6.4, Abaxial surface, stomatal rows showing Florin ring with little relief. Fig. 6.5, Abaxial cuticle showing Florin ring and circular stomatal opening. Fig. 6.6, Abaxial surface showing stoma with plug components.

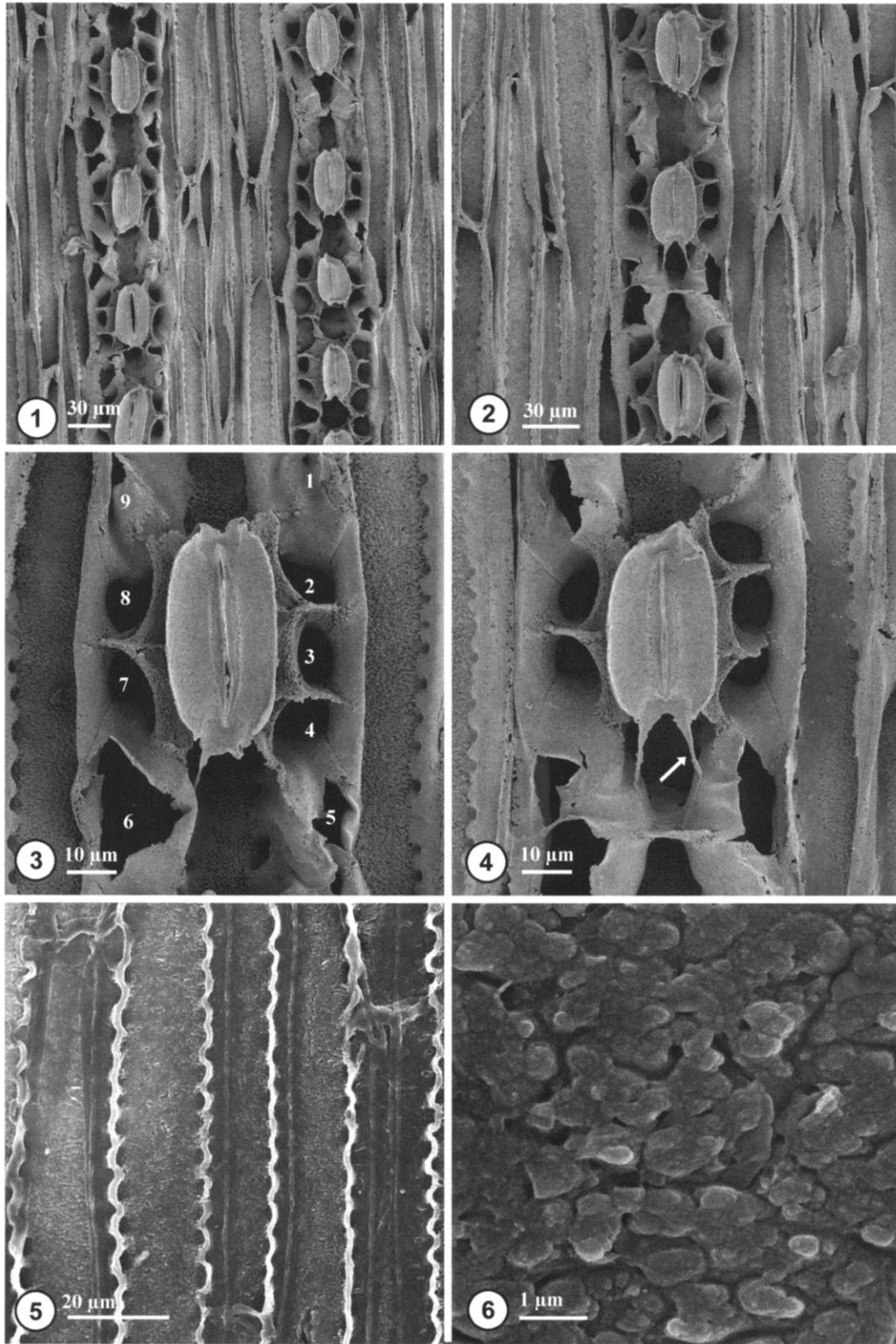


Fig. 7 *Pinus kesiya*, inner views. Fig. 7.1, Abaxial surface showing stomatal rows with differing numbers of subsidiary cells (up to 12 at top left stomatal complex) and alternating elongate epidermal cells. Fig. 7.2, Abaxial surface showing stomatal rows with varying numbers of subsidiary cells. Fig. 7.3, Abaxial cuticle of stomatal apparatus. Note nine lateral subsidiary cells (1-9). Fig. 7.4, Abaxial cuticle on stomatal apparatus with guard cell extensions over subsidiary cell wall (arrow). Fig. 7.5, Abaxial cuticle showing sinuous epidermal cell outline with deep cavities. Fig. 7.6, Detail of abaxial cuticle of epidermal cell surface.

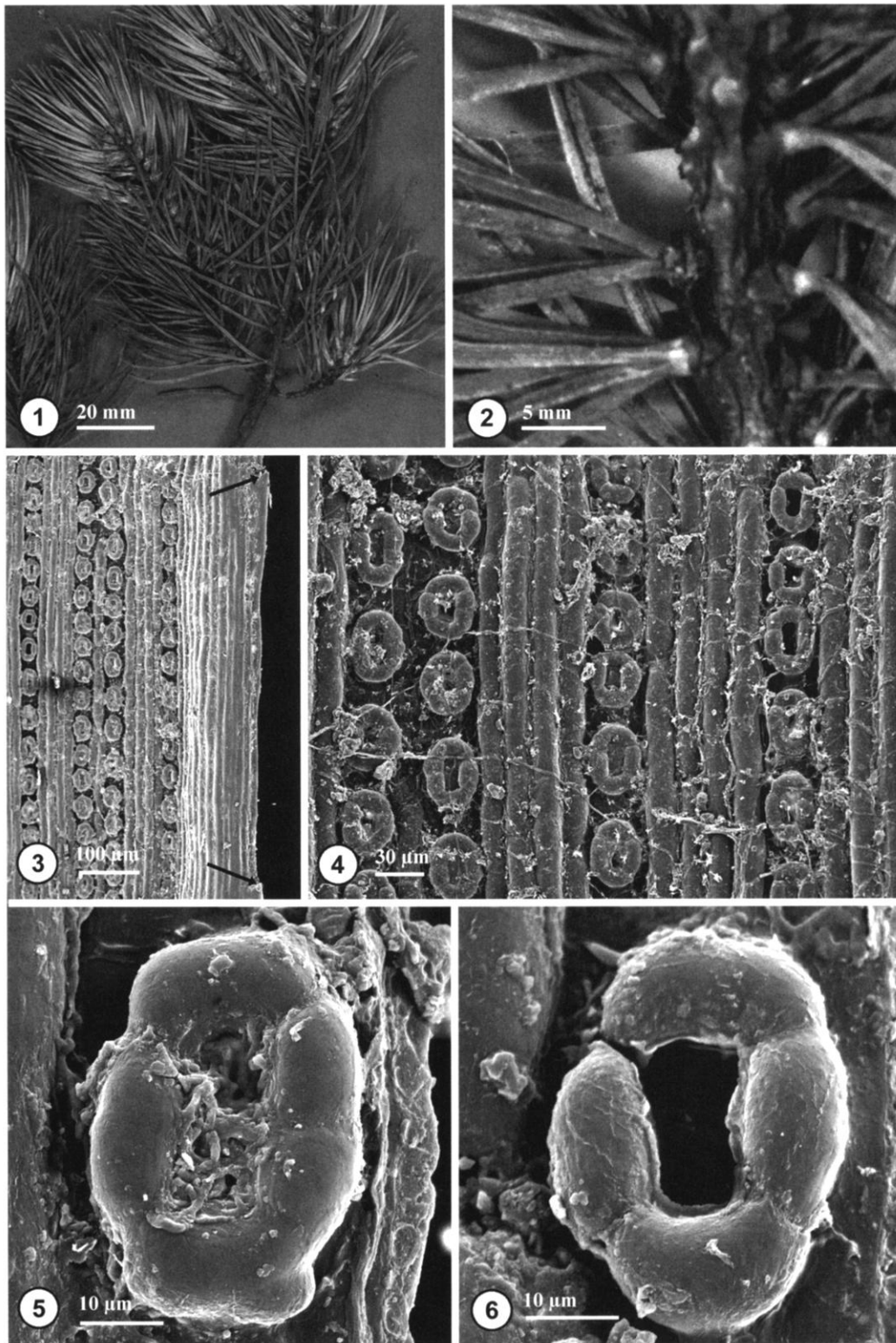


Fig. 8 *Pinus kwantungensis*, outer views. Fig. 8.1, Branch showing even distribution of fascicles. Fig. 8.2, Detail of fascicle showing five needles per fascicle and position of deciduous fascicle sheaths. Fig. 8.3, Adaxial cuticle showing stomatal rows with Florin rings, alternating with elongated epidermal cells. Note teeth on the leaf margin (arrows). Fig. 8.4, Adaxial cuticle showing Florin rings and underlying epidermal cell outline. Fig. 8.5, Adaxial cuticle showing Florin ring and stomatal plug components. Fig. 8.6, Adaxial cuticle showing Florin ring with rectangular stomatal opening.

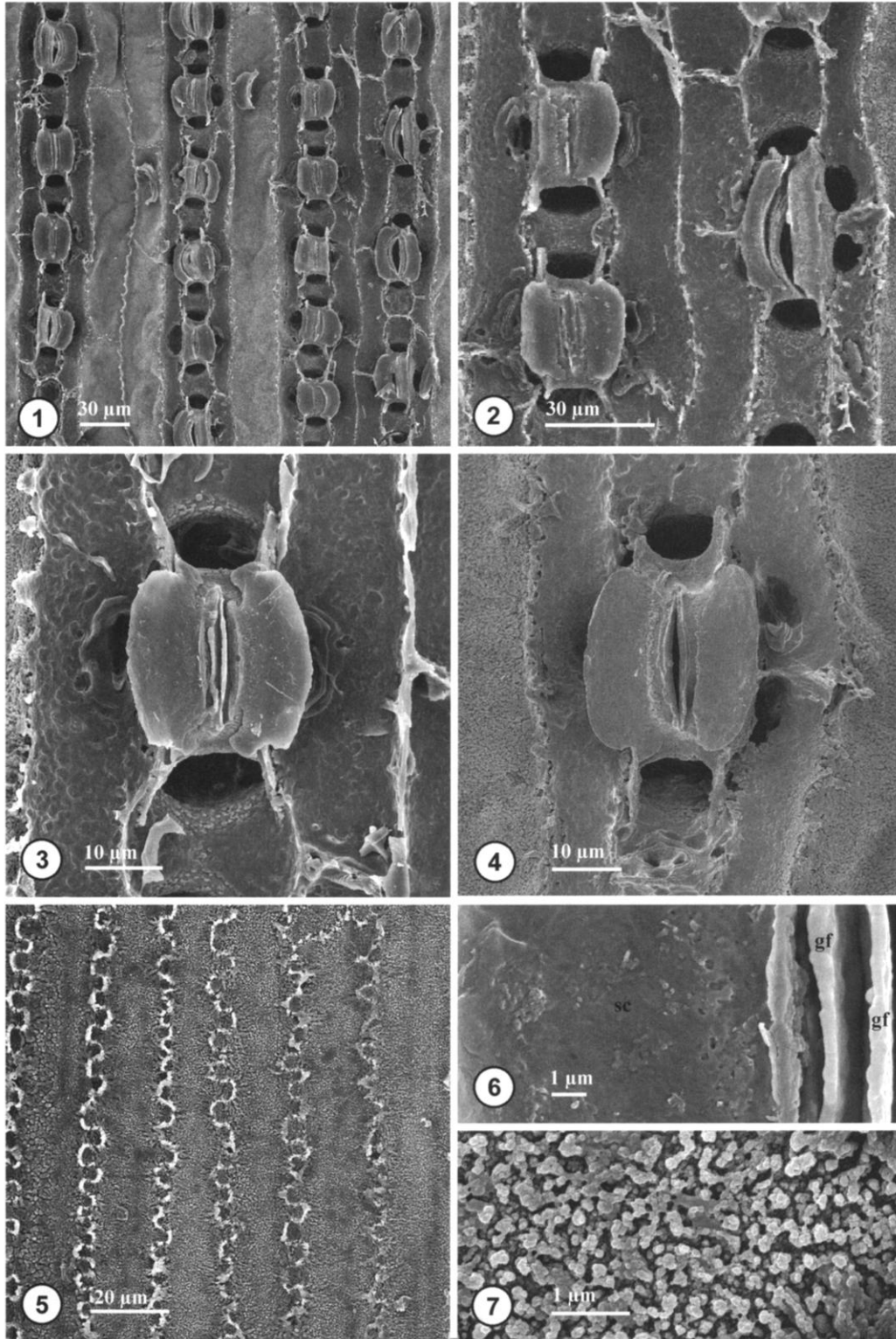


Fig. 9 *Pinus kwantungensis*, inner views. Fig. 9.1, Adaxial cuticle showing stomatal rows alternating with elongate epidermal cells. Fig. 9.2, Adaxial surface showing stomatal rows. Note offset right row from left row. Fig. 9.3, Adaxial cuticle showing stomatal apparatus with flange obscuring lateral subsidiary cells. Fig. 9.4, Adaxial cuticle on stomatal apparatus, note two lateral subsidiary cells on the right. Fig. 9.5, Adaxial cuticle showing epidermal cells with undulating intercellular flanges lacking buttresses and cavities and oblique end walls (top right). Fig. 9.6, Adaxial cuticle on guard and subsidiary cell surfaces, note pitted subsidiary cell surface. Fig. 9.7, Detail of adaxial cuticle on epidermal cell surface. *gf*, guard cell flange; *sc*, subsidiary cell.

type” of Hu (1986) with the characteristic reticulum forming on the epidermal cell surface and flanges that are more pronounced than in *P. dalatensis* and *P. kwantungensis*.

The fourth feature is the number and arrangement of subsidiary cells. With the small sample size in this study, a trend of four to 10 subsidiary cells in subgenus *Strobos* (three species examined) and of 10–12 subsidiary cells in subgenus *Pinus* (one species examined) was observed (table 3).

Other cuticular characters that have been proposed are either less consistently distributed within subgenera or are not well documented. These features include pattern of epidermal cell surface sculptures, number of stomatic bands, epidermal cell size, and marginal teeth spacing. Results of my study are in accordance with findings by Alvin et al. (1982), who demonstrated a strong correlation between leaf form and some size correlation features such as epidermal cell and leaf size within the Cupressaceae. Longer leaf dimension is correlated with larger size of elongated epidermal cells between stomatal rows. All four species show prominent teeth on the leaf margin, but the spacing of such teeth differs (table 2).

Significance of Cuticle Micromorphology to *Pinus krempfii* Taxonomy

Based on cuticle micromorphology, this study supports the commonly used binary division of the genus *Pinus* (Parlatore 1868; Shaw 1924; Klaus 1980; Farjon and Styles 1997), and it further emphasizes the inclusion of *P. krempfii* within subgenus *Strobos*. This study does not support recognition of a monotypic subgenus *Ducampopinus* for *P. krempfii* as erected by Ferré (1948) and maintained by Little and Critchfield (1969). Additional work in progress on *P. krempfii* that supports these conclusions includes details of the ovulate cone structure and wood anatomy (Ickert-Bond 1997a; S. Ickert-Bond, unpublished manuscript).

Sectional affinities of *P. krempfii* are usually with *Parrya* (Van der Burgh 1973; Klaus 1980; Farjon 1984; Price et al. 1998). The features that *P. krempfii* shares with other species in this section include (1) one to five leaves in a fascicle, (2) no clear distinction between hypodermis and epidermis in the leaves, and (3) cones with a dorsal umbo that usually ends in a mucro (Little and Critchfield 1969; Klaus 1980). Because of these distinctive features, as well as other features examined for *P. gerardiana* and *P. bungeana* of section *Parrya*, subsection *Gerardiana*, Hu (1986) has suggested that this section be elevated to the level of subgenus.

Although the species in section *Parrya* appear united on the basis of these distinctive features, and molecular data tends to support this group, there are several reasons to question the assignment of all currently recognized species to this section. One problem in recognizing *Parrya* as a coherent section is that it includes wide ranges of character distributions for certain features, such as primary leaf retention on seedlings, needle number, and seed and bark characters. Species in subsections *Cembroides* and *Rzedowskiana* retain primary leaves for several years on the seedling, while other subsections do not exhibit such a character state (Ferré 1952; Klaus 1989). While species in subsection *Balfouriana* have five needles in a fascicle, those of *Gerardiana* have three, subsection *Cembroides* ranges from one to five needles per fascicle, subsection

Rzedowskiana from three to five, and those of subsection *Krempfiana* have two needles per fascicle (Little and Critchfield 1969; Farjon and Styles 1997). Both winged and non-winged seeds and thin papy to thick-plated bark occur within this section (Kwei and Lee 1963; Van der Burgh 1973; Farjon 1984; Vidakovic 1991; Kindel 1995; Ickert-Bond 1997a). It is not clear whether these types of features are of sectional or subsectional value. In addition, several species placed in this section have not been well surveyed for a number of morphological and cuticular features. Molecular data has also shown that section *Parrya* may not be monophyletic (Liston et al. 1999; Wang et al. 1999). While this study has provided new information about *P. krempfii* that supports its inclusion in this group, several other species need further attention. While species in *Parrya* occur today in two biogeographically distinct regions, Asia and North America, this distribution may be explained by the probable antiquity of this group (Van der Burgh 1973; Millar 1998).

Within the section *Parrya*, five subsections are recognized, *Krempfiana*, *Balfouriana*, *Gerardiana*, *Rzedowskiana*, and *Cembroides*. Subsection *Krempfiana* is monotypic, based on *P. krempfii* (Little and Critchfield 1969; Farjon 1984; Price et al. 1998), and the American subsection *Rzedowskiana* is based on *P. rzedowskii*. Subsection *Gerardiana* is Asian and is composed of two species, *P. gerardiana* and *P. bungeana*, and may also include the newly discovered Chinese *P. squamata* (Price et al. 1998). Subsection *Balfouriana*, the Bristlecone pines, comprises three species, and *Cembroides* has around 11 species. The last two subsections are North American in distribution.

Although *P. krempfii* has been placed in its own monotypic subsection *Krempfiana* by most authors (Little and Critchfield 1969; Klaus 1980; Farjon 1984; Price et al. 1998), an alternative classification with *Gerardiana* is also possible. Unique features of *P. krempfii* that support the distinctiveness of subsection *Krempfiana* are the dimorphic mesophyll, the falcate needle cross section, unusual phenolic compounds of the heartwood, and the absence of ray tracheids (Ferré 1948; Buchholz 1951; Rollet 1955; Erdtman et al. 1966; Hudson 1980; Ickert-Bond 1997a). Features that are shared between *P. krempfii* and subsection *Gerardiana* include broad cuticular bridges between stomata, details of the intercellular flanges of the epidermal cells, usually an amphistomatic stomatal distribution, a large number of resin canals in the leaves, an increased amount of transfusion tissue, bark unlike the typical thick-plated pine bark, and characters of the ray tracheids (Hudson 1960; Jählig 1962; Kwei and Lee 1963; Hu and Yao 1981; Hu 1986; Wu and Hu 1997; Ickert-Bond 1997a). Molecular studies including data from *P. krempfii* and subsection *Gerardiana* showed a close relationship between these two subsections (Wang et al. 1999, 2000). While there are a number of features that are distinct in both subsections, it is clear that these two subsections are closely related, with subsection *Gerardiana* being the more advanced of the two (Van der Burgh 1973).

The distinctive leaf morphology of *P. krempfii* with its broad leaf dimension and early deciduous fascicle sheaths is unusual among pines and has sometimes been confused with *Podocarpus neriifolius* D. Don, which is sympatric with *P. krempfii*. Although no fossil record is currently known for *P. krempfii*,

recognition of fossils with distinctive cuticular features here described could aid considerably in the evolutionary understanding of this interesting pine.

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