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Epidermal Structures of *Sequoia sempervirens* (D. Don) Endl. (Taxodiaceae)

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ABSTRACT: *Sequoia sempervirens* (D. Don) Endl. is the unique living species of genus *Sequoia*. Epidermal structures of *S. sempervirens* were reported divergently. This study focuses on the understanding of variation of the epidermis structures of *S. sempervirens,* based on integral leaves, rather than on leaf fragments. Epidermal cells on both upper and lower surfaces of leaves possess similar features, but their shape and size in non-stomatal areas are different from those in stomatal areas. The amount and distribution of stomata on the lower surface vary indistinctly, but obvious variations of them on the upper surface are described as four situations. Guard cells of stomata are sunken and partly blanketed with subsidiary cells. Each stoma has 4-6, but mostly 4 subsidiary cells, occasionally exceeding 6. Long axes of stomata are mostly parallel to, but sometimes oblique or perpendicular to the mid-vein.

KEY WORDS: Taxodiaceae, *Sequoia sempervirens*, Epidermis, Stomata.

INTRODUCTION

Sequoia sempervirens (D. Don) Endl., the unique living species of the genus *Sequoia* in the family Taxodiaceae, is an endemic species restricted to the ocean fog belt from the extreme southwestern corner of Oregon to about 100 miles south of San Francisco, California (Zheng and Fu, 1978; Benson, 1979), but fossils of the genus were reported in many localities of the Northern Hemisphere from Late Mesozoic to Tertiary time (Mai, 1995; Yu, 1995).

The leaves in some genera of Taxodiaceae show similar features of morphology. Epidermal structures of leaves reflected in the leaf cuticle (Boulter, 1970) provide important evidence to separate the similar leaves of these genera in Taxodiaceae (Sze, 1951; Kerp, 1990). Data of epidermal features in the living plants are also the source of cellular information for fossil plants, for example, to identify the taxa of fossil conifers (Florin, 1931; Kerp and Krings, 1999).

The distribution of stomata on *Sequoia* leaves was described to be amphistomatous (Yao and Hu, 1982; Srinivasan and Friis, 1989) or hypostomatous (Stebbins, 1948; Chaturvedi, 1993; Villar De Seoane, 1998), and the direction of stomata on leaves, as well as their structure, was also reported to be varied (Florin, 1922; Stebbins, 1948; Miki and Hikita, 1949; Yao and Hu, 1982; Srinivasan and Friis, 1989; Chaturvedi, 1993; Villar De Seoane, 1998). In this work, we pay more attention to the former studies on the epidermal features of *Sequoia* and focus on understanding the variations of epidermal structures of *S. sempervirens*.

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MATERIALS AND METHODS

Specimens of *Sequoia sempervirens* were collected from California (No. 583003 from Santa Cruz County, No. 1173131 from Upper Mill Greek Valley, Marin County, 14 miles NW of San Francisco and No. 1589799 from Del Norte County) of USA; Hangzhou (No. 1524948 from the Botanical Garden at Hangzhou) of Zhejiang Province and Luotian (No. 1605246, Institute of Forests at Luotian) of Hubei Province, China. Entire leaves studied in this paper were obtained from top, middle and lower parts of shoots. These specimens are preserved in the Herbarium, Institute of Botany, Chinese Academy of Science, Beijing.

Leaves were placed with their adaxial surface upwards and lacerated at the right edge of leaves by a knife, so that the epidermis is easy to be distinguished between its upper or lower surfaces when the epidermis of leaves is obtained. Prepared with hydrogen peroxide (H_2O_2) and glacial acetic acid mixed at 1:1, in 60℃, 54 pieces of epidermis were obtained from the specimens. Epidermis was stained in 1% safranine solution in water and stain washed out with 100% alcohol. The epidermises were photographed by light microscopy (LM) (Olympus BX50) and scanning electron microscopy (SEM) (Hitachi S570).

Stomatal frequency gives the number of stomata per unit area of leaf surface. In the present paper, the selected area is 0.5×0. 4 mm^2, 0. 4 mm is about equal to width of stomatal area on each side of the mid-vein, and then convert to stomatal numbers per mm^2. One hundred areas were chosen at random from about 15 lower epidermises. Stomatal index = 100S/(E+S), where S = number of stomata per 0.2 mm^2, E = number of epidermal cells per 0.2 mm^2, stomatal index calculated according to the same 100 areas as used in stomatal frequency.

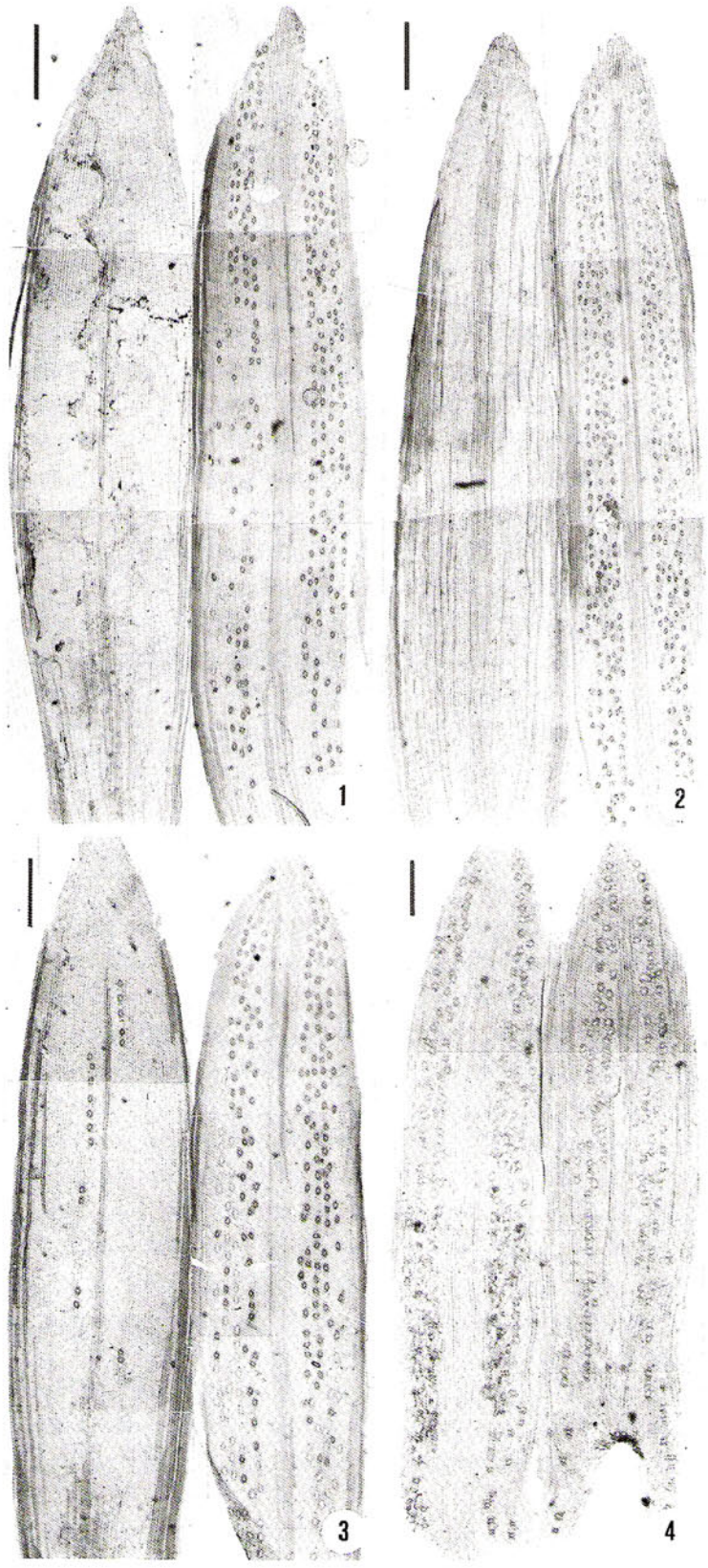
RESULTS

Leaves are dimorphic, linear and scale-like, and arranged spirally on branch. Leaves have entire margins, with the obvious mid-vein seen on the lower surface of leaves. Leaf bases are decurrent and obliquely trending along the branch. The angles between leaves and branch axis are 15-71°. Linear leaves are 0.3-2.3 cm long and 0.7-2.2 mm wide, with L/W from 3.3:1 to 13.5:1. Scale leaves are triangular in shape, about 6 mm long and 4 mm wide.

Epidermal cells on both the upper and lower epidermis of leaves are very similar. The epidermal cells are elongate, with thick and straight walls. Pits are clearly observed from cell walls (Figs. 9, 10). Long axes of cells are parallel to the mid-vein. The epidermal cells are 30-300 μm long and 10-45 μm wide, with L/W ratios from 1.1:1 to 17.3:1 in linear leaves (Figs. 1-5), 25-150 μm long and 15-37.5 μm wide, with L/W ratios from 1.0:1 to 10.0:1 in scale leaves (Fig. 6). The shape and size of epidermal cells in stomatal areas are more irregular than those in non-stomatal areas. The L/W ratios of epidermal cells in stomatal areas are smaller than those in non-stomatal areas (Figs. 1-6).

The lower epidermis of leaves has 3-8 rows of stomata located on each side of the mid-vein. The amounts of stomata vary on the lower epidermis (Figs. 1-5). In the stomatal area of the lower epidermis, mean stomatal frequency is 128 per mm^2; mean stomatal index is 13.8.

The distribution of stomata on the upper epidermis is obviously different from that on the lower one. The stomata on the upper epidermis are fewer and irregular in arrangement, comparing to the lower one (Figs. 1-5). There are four situations of distribution of stomata on the upper epidermis of linear leaves.



Figs. 1-4. Leaves epidermis of *Sequoia sempervirens*. The left side of each figure is upper surfaces of leaves, while the right side is lower surfaces. Scale bars = 0.05 cm.

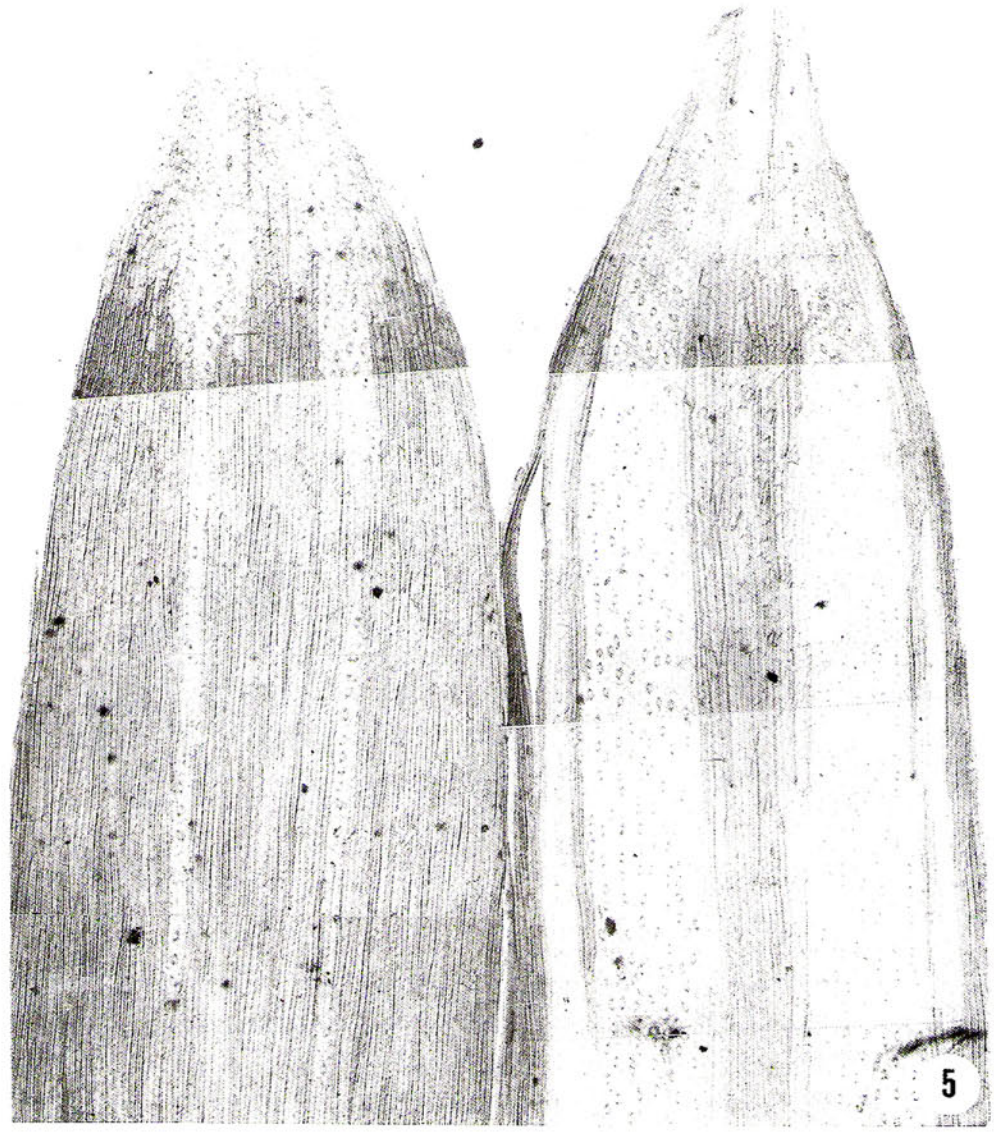


Fig. 5. Top parts of leaf epidermis of *Sequoia sempervirens*. The left side of figure is upper surface of leaf, while the right side is lower surface. No stomata are found downward on upper epidermis. Scale bar=0.05 cm.

1) Almost no stomata are found at the upper epidermis (Figs. 1 & 2). Leaves in this situation are 9 in number and occupy about 16.7% of the total obtained epidermis of 54.
2) Only 1 to 2 rows of stomata on upper epidermis are discontinuously distributed on each side of the mid-vein (Fig. 3). This kind of leaf is 14 in number, about 25.9% of the 54 epidermises.
3) Most stomata are located at the top of the upper epidermis and are decreased in number and finally absent downwards (Fig. 5). This kind is 15 in number and occupies about 27.8% of the epidermises.
4) The distribution of stomata on the upper epidermis is most similar to the lower one (Fig. 4). This kind of leaves is 16 in number and occupies about 29.6% of the 54 epidermises.

The stomata on the upper epidermis of scale leaves are located on each side of the mid-vein and are a little fewer in number than those on the lower one (Fig. 6).

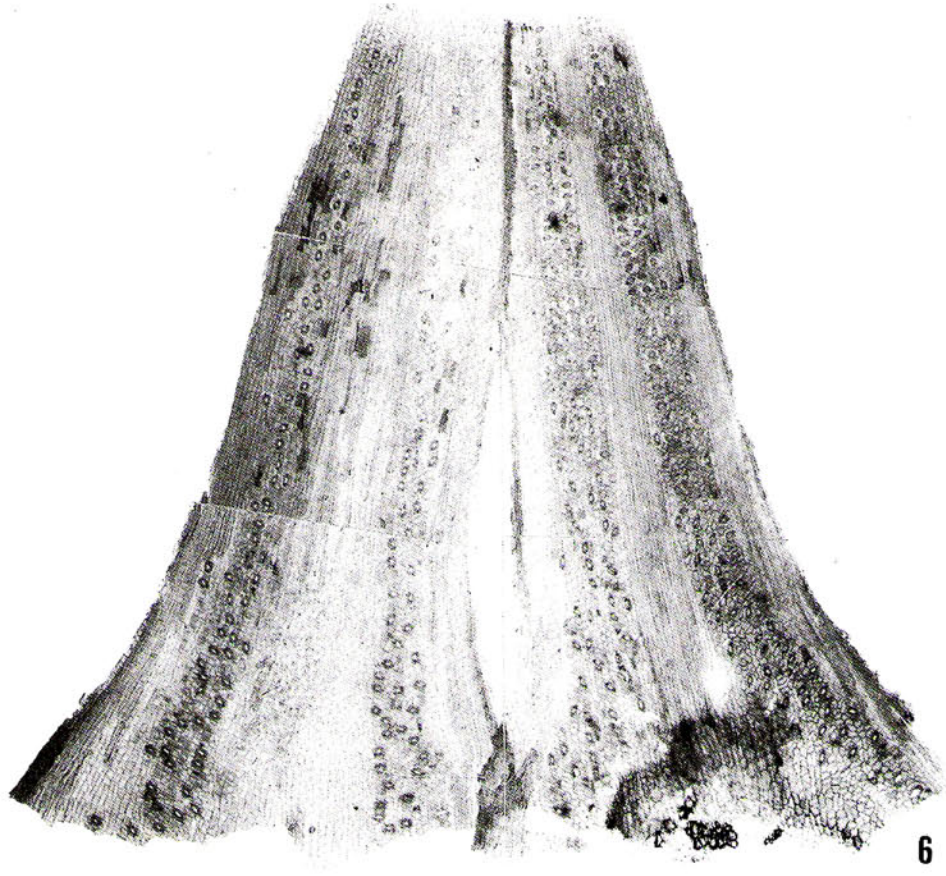


Fig. 6. Epidermis of scale leaf of *Sequoia sempervirens*. The left side of figure is upper surfaces of leaf, while the right side is lower surfaces. Scale bar=0.05 cm.

The two guard cells of the stoma are sunken, 35-70 μm long, 7-20 μm wide. The guard cells have thickened walls, which are prolonged towards the two poles forming polar lamellae (Figs. 9 & 10). Each stoma has 4-6 subsidiary cells, occasionally exceeding 6, but mostly 4 subsidiary cells. Subsidiary cells are arched over the guard cells and cover part of the guard cells (Figs. 9 & 10). Pores of stomata are elliptic, sometimes elongate, 15-27 μm in length and 4-10 μm in width (Figs. 7, 8 & 9). The long axes of pores are mostly parallel to the mid-vein, sometimes oblique or perpendicular to the mid-vein (Figs. 1-6).

DISCUSSION

The distribution of stomata on leaves of *S. sempervirens* was reported on the lower surface of leaves (Stebbins, 1948; Chaturvedi, 1993; Villar De Seoane, 1998), or on both upper and lower surfaces, but more on the lower ones (Yao and Hu, 1982; Srinivasan and Friis, 1989). Linear leaves possess the discontinuous rows of stomata on the upper surface and two white bands of stomatal rows on the lower one (Zheng and Fu, 1978). The scale leaves were described as having the rows of stomata only on the upper surface (Zheng and Fu, 1978).

After carefully examining the stomata on both upper and lower epidermis, we describe four situations of stomatal distribution on the leaves of *S. sempervirens*. These four situations cover the formal reports of stomatal distribution of this species. As the distribution of stomata

Figs. 7-10. Epidermis of *Sequoia sempervirens*. Fig.7: Outer surface of leaf (SEM). Scale bar=40 μm. Fig. 8: Inner surface of leaf (SEM). Scale bar=40 μm. Figs. 9-10: A epidermis in different focus, showing two guard cells of stomata are sunken and subsidiary cells mulch part of guard cells. Scale bars=30 μm.

on the upper epidermis of leaves is not stable obviously (Figs. 1-5), we took the data of stomatal frequency and index based only on the distribution of stomata on the lower epidermis.

The stoma of *S. sempervirens* was described as being surrounded by 6 (Chaturvedi, 1993), 4-5 (Florin, 1922; Villar De Seoane, 1998) or 4-6 (Yao and Hu, 1982; Srinivasan and Friis, 1989) subsidiary cells. We observed that each stoma usually has 4-6 subsidiary cells and occasionally more than 6. Two different appearances of stomata in *S. sempervirens* were

described (Ma *et al*, 2000a), but were caused by the chemical treatment of cuticles. When the chemical macerates more liginified walls of guard cells, the stomata display changes in their outline. Guard cell walls of Taxodiaceae have polar lamellae (Boulter, 1970; 1971); polar lamellae were also found in *S. sempervirens* (Fig. 10).

The long axes of stomata of *S. sempervirens* are mostly parallel to the mid-vein (Figs. 1-3 & 5), sometimes oblique or perpendicular to it (Figs. 4 & 6) (Chaturvedi, 1993; Villar De Seoane, 1998). Srinivasan & Friis (1989) described the irregularity of orientation of stomata. On the mature leaves the stomatal orientation is longitudinal in the decurrent part of leaves, mostly transverse in the basal and central part and rather irregular in the apical part. Such variation of orientation of stomata was not observed from the material in this study.

Morphological features of branches and leaves of some genera in Taxodiaceae are similar to each other, especially *Sequoia* and *Metasequoia* (Stebbins, 1948; Chaney, 1951; Christophel, 1976; Srinivasan and Friis, 1989; Ma and Gu, 2000; Ma *et al*, 2000b). The undulate walls of epidermal cells of *M. glyptostroboides* (Sterling, 1949; Liu *et al*, 1999) are not present in the leaves of *S. sempervirens*.

Epidermal analysis is a valuable method for the identification and classification of fossil remains of gymnosperms (Kerp, 1990). The intact epidermis of leaves is difficult to obtain from the fossil specimens of Taxodiaceae. Usually only fragments of epidermis are obtained, because the specimens are very brittle (Kerp and Krings, 1999). As we know that the distribution of stomata on leaves are variable on different leaves or different parts of a leaf of *S. sempervirens*, we need to collect intact fossil leaves of Taxodiaceae, in order to reach the correct analysis of their epidermis.

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LITERATURE CITED

Benson, L. 1979. Plant classification. Heath and Company, D.C.

Boulter, M. C. 1970. Lignified guard cell thickenings in the leaves of some modern and fossil species of Taxodiaceae (Gymnospermae). Biol. J. Linn. Soc. **2**: 41-46.

Boulter, M. C. 1971. Fine details of some fossil and recent conifer leaf cuticles. In: Heywood V. H. (ed.). Scanning Electron Microscopy. pp. 211-236. Academic Press, London and New York.

Chaney, R. W. 1951. A revision of fossil *Sequoia* and *Taxodium* in western North America based on the recent discovery of *Metasequoia*. Am. Phil. Soc. NS. **40**: 171-263.

Chaturvedi, S. 1993. Morphological, cuticular and anatomical studies of some members of Taxodiaceae. Bionature **13:** 127-131.

Christophel, D. C. 1976. Fossil floras of the Smoky Tower Locality, Alberta, Canada. *Palaeontographica* Abt. B **157**: 1-43.

Florin, R. 1922. Zur alttertiäeren flora der süedlichen Manschurei. Palaeonto. Sin. Ser.A **1**: 1-52.

Florin, R. 1931. Untersuchungen zur Stammesgeschicnte der Coniferales und Cordaitales. K. svenska. Vetensk. Akad. Handl. Ser. 5. **10**: 1-588.

Kerp, H. 1990. The study of fossil Gymnosperms by means of cuticular analysis. Palaios **5:** 548-569.

Kerp, H. and M. Krings. 1999. Light microscopy of epidermis. In: Jones, T. P. and N. P. Rowe. (eds.). Fossil Plants and Spores: Modern Techniques. pp. 52-56. The Geological Society, London.

Liu, Y.-J., C.-S. Li and Y.-F. Wang. 1999. Studies on fossil *Metasequoia* from north-east China and their taxonomic implications. Bot. J. Linn. Soc. **130:** 267-297.

Ma, Q.-W. and F.-Q. Gu. 2000. Comparative studies on morphological features of some genera in Taxodiaceae. Chinese Bull. Bot. **17**(Special issue): 161-164. (In Chinese with English abstract).

Ma, Q.-W., J.-B. Zhang, Y.-F. Wang and C.-S. Li. 2000a. Studies on cuticle of *Sequoia sempervirens*. In: Palaeobotanical committee of Palaeontological and Botanical Society, China. (eds.). Abstract of the sixth conference of international organization of palaeobotany. pp. 81.

Ma, Q.-W., J.-X. Xu, Y.-F. Wang and C.-S. Li. 2000b. First evidence of *Sequoia* in the Miocene of Yunnan Province, China. Acta Bot. Sin. **42:** 438-440. (In Chinese with English abstract).

Mai, D.-H. 1995. Tertiare vegetations geschichte Europas. Gustav Fascher, Jena. pp. 248.

Miki, S. and S. Hikita. 1949. Probable Chromosome Number of Fossil *Sequoia* and *Metasequoia* Found in Japan. Science **113:** 3-4.

Srinivasan, V. and E. M. Friis. 1989. Taxodiaceous conifers from the Upper Cretaceous of Sweden. Bio. Skrifter. **35:** 5-57.

Stebbins, G. L. 1948. The chromosomes and relationships of *Metasequoia* and *Sequoia*. Scicnce **108:** 95-98.

Sterling, C. 1949. Some features in morphology of *Metasequoia*. Am. J. Bot. **36:** 461-471.

Sze, H.-C. 1951. Structure of cuticle and small stomata of modern *Metasequoia* leaves. Sci. in China. **2:** 239-243. (In Chinese).

Villar De Seoane, L. 1998. Comparative study of extent and fossil conifer leaves from the Baquero Formation (Lower Cretaceous), Santa Cruz Province, Argentina. Rev. Palaeobot. Palynol. **99:** 247-263.

Yao, B.-J. and Y.-X. Hu. 1982. Comparative anatomy of conifer leaves. Acta. Phyto. Sin. **20:** 275-294. (In Chinese with English abstract).

Yu, Y.-F. 1995. Origin, evolution and distribution of the Taxodiaceae. Acta. Phyto. Sin. **33:**362-389. (In Chinese with English abstract).

Zheng, W.-J. and L.-G. Fu. 1978. China Vegetation (Vol. 7). Science Press, Beijing. pp. 309-312. (In Chinese).

北美紅杉表皮特徵分析

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摘　　要

北美紅杉 (*Sequoia sempervirens* (D. Don) Endl.) 是紅杉屬現在僅存的一種植物。有關北美紅杉表皮特徵的報導存在分歧。本文觀察包括原產地及不同產地的北美紅杉的完整葉片的表皮特徵。結果顯示其葉片上下表皮細胞特徵相似，但存在於氣孔區與非氣孔區的表皮細胞形態與大小不同。不同葉片的氣孔數量和分佈在下表皮變化不明顯，在上表皮變化較大。根據氣孔在上表皮的分佈特點，將氣孔分布情況分為四種。氣孔保衛細胞下陷，副衛細胞覆蓋部分保衛細胞，副衛細胞數目 4-6 個，但多數為 4 個，偶有超過 6 個。氣孔口長軸多數與中脈平行，也存在氣孔口長軸與中脈垂直或斜向的情況。

關鍵詞：杉科、北美紅杉、表皮、氣孔。

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