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PALYNOLOGICAL NOTES ON *BRETSCHNEIDERA SINENSIS* HEMSL.[1]

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Abstract

The systematic position of the monotypic *Bretschneidera* has not been agreed upon in the past, although the placement of this genus in its own family, Bretschneideraceae, has been favored. The recent discovery of this unique plant from northern Taiwan has enabled us to study its pollen, using the scanning electron microscope. A comparison of the pollen morphology of *B. sinensis* with that of Sapindace, Hippocastanaceae, Moringaceae, and Caesalpinioideae from previous surveys is made. The mature pollen of *B. sinensis* is here reported as binucleate.

Key words: *Bretschneidera sinensis*; palynology; binucleate pollen.

Since Hemsley (1901) first described the monotypic genus, *Bretschneidera*, various, systematic placements of the genus have been proposed. There has been no agreement, however, as to whether *Bretschneidera* should be included in Sapindaceae (Hemsley, 1901; Hutchinson, 1926, 1959, 1973) or Hippocastanaceae (Engler and Gilg, 1919; Hutchinson, 1969), or deserves the rank of a separate family, Bretschneideraceae (Radlkofer, 1908; Engler and Gilg, 1924; Tang, 1935; Cronquist, 1981). Recently the last viewpoint has received a certain level of acceptance, basing primarily upon the perigynous flowers, mainly large and pluriseriate wood-rays, and myrosin-cells in the bark of twig, the pedicels as well as the petals (cf. Cronquist, 1981). The affinities of Bretschneideraceae were suggested to be with Capparaceae, Leguminosae (specifically, Caesalpinioideae), and Moringaceae in Engler's Rhoeadales, or with Sapindaceae in Sapidales. Lately, Yang and Hu (1985) reported the chromosome cytology of *B. sinensis* but considered the data insufficient to suggest relationship of the genus.

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Bretschneidera sinensis is distributed in southern and southwestern China as well as northern Vietnam (Fu and Fu, 1984). Although pollen of this unique species was described previously (Erdtman, 1952; Lu *et al.*, 1986), its detailed features under scanning electron microscope have not been well revealed. The recent discovery of *Bretschneidera* in Taiwan (Hsu and Lu, 1984; Lu *et al.*, 1986) has prompted the present palynological survey. This report intended to provide an additional evidence from pollen morphology and from the number of nucleus in the pollen cells in deducing relationship of *Bretschneidera*.

Materials and Methods

Pollen grains were removed from herbarium specimens, *Peng 4765, 9800* (deposited at HAST), collected from Yangmingshan National Park and Chinkuashih respectively, Taipei County, Taiwan. For scanning electron microscopic (SEM) observations, pollen samples were acetolyzed, dehydrated through graded ethanol concentration and acetone, critical point dried, and coated with gold to 200 Å. A Hitachi S-250 SEM was used for examination. Cross section of the exine was obtained for SEM study by spreading pollen on a slide and cutting it with a razor blade prior to acetolysis. The terminology for pollen morphology followed mainly that of Walker & Doyle (1975).

For the observation of nucleus in pollen cells, mature pollen grains were examined with a Nikon Biophot microscope after staining with either FLP orcein (Jackson, 1973) or 1% acetocarmine. It was found that pollen cells stained with FLP orcein gave better differentiation than with acetocarmine. Prolonged staining and heating on a warm plate enhanced results. Besides, applying pressure to rupture the exine made the observation easier.

Results and Discussion

A description of *Bretschneidera sinensis* pollen viewed under scanning electron microscope follows:

Pollen tricolpate and semitectate with reticulate tectum; outline subcircular and slightly trilobed in polar view, suboblate to oblate sphaeroidal in equatorial view, 34-39×42-47 μm (P×E); colpi 32.5-38 μm long, 5-12 μm wide, the margin conspicuously delimited, the ends acute, the sexine baculate to rugulate; exine ca. 4 μm thick in mesocolpate area, with free sexine elements (granula) in the lumen, the nexine as thick as the sexine, without a distinguishable foot layer, thinner at colpate area than at mesocolpate area, the surface of the tectum psilate to sparsely minutely scabrate (Fig. 1).

The pollen sculpture described by Erdtman (1952) using a light microscope is

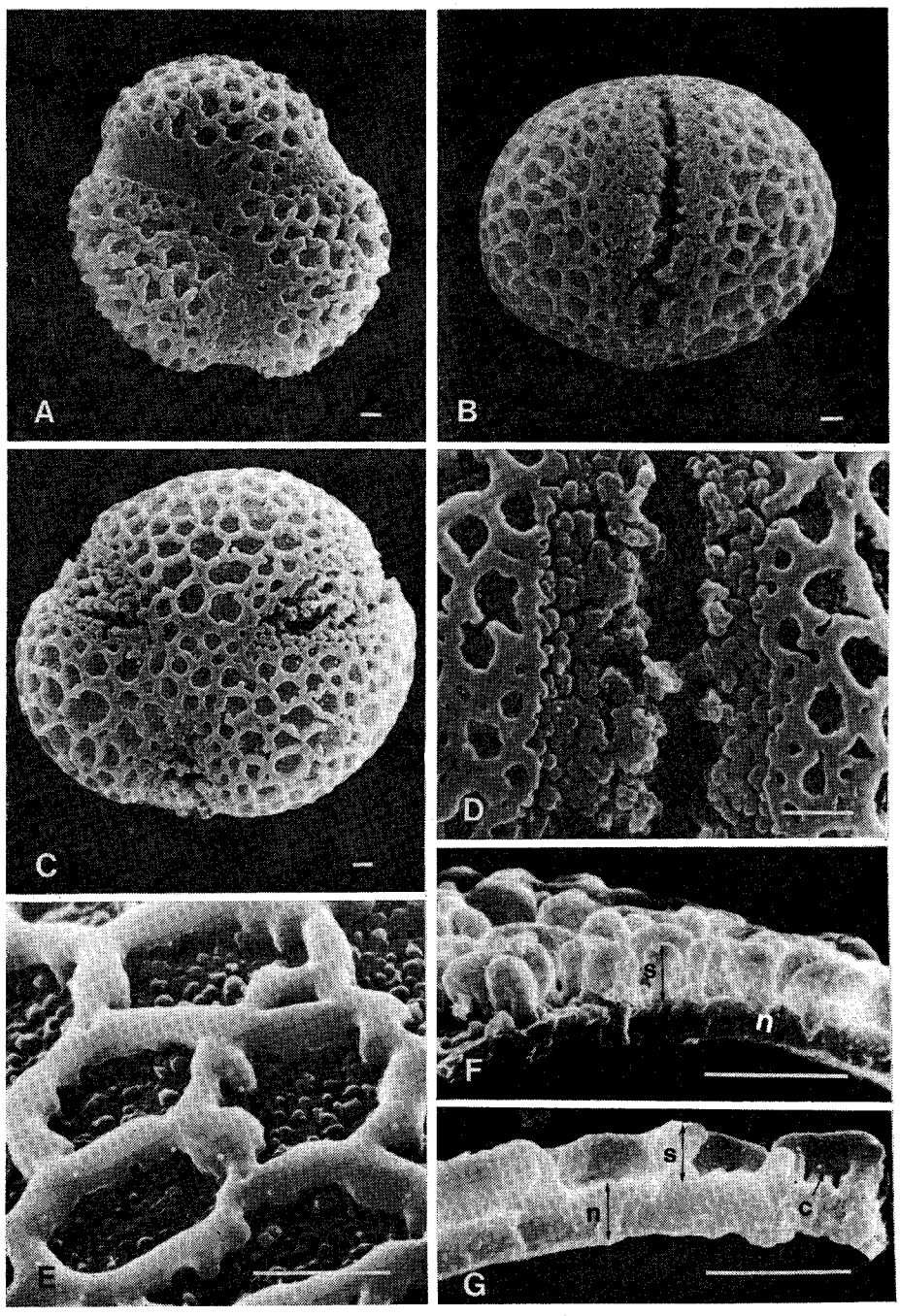

Fig. 1. ***Bretschneidera sinensis*** (from ***Peng 4765***). A. nearly mature pollen; B-G. mature pollen. A. polar view; B. equatorial view; C. polar view; D. detail of colpate area; E. detail of exine surface; F. cross section at colpate area showing exine stratification; G. cross section at meso-colpate area showing exine stratification. Scale bars=2.5 μm; c=columella, n=nexine, s=sexine.

confirmed, though we have noted a considerably smaller pollen size, 34-39×42-47 μm, as compared to 45×52 μm. While Lu *et al.*'s report (1986) of *Bretschneidera* pollen as being tricolporate appears to be erroneous. Erdtman noted that the pollen is different from that of Hippocastanaceae and Moringaceae but without pointing out the detail.

The available recent surveys of pollen types of Sapindaceae (Huang, 1972; Muller and Leenhouts, 1976; Muller, 1985), Hippocastanaceae (Faegri and Iversen, 1975; Heath, 1984), Moringaceae (Ferguson, 1985), and Caesalpinioideae (Tsukada, 1963; Huang, 1972; Senesse, 1980; Graham and Barker, 1981) have contributed much valuable data for comparison in the present study. In general, the remarkable oblate, tricolpate pollen of *Bretschneidera* suggests remote morphological homologies with those of the above taxa, which have predominantly prolate to perblate, and mainly tricolporate to occasionally syncolpate pollen. In view of its tectate exine with bacula on the sexine (Graham and Barker, 1981), however, the tribe Caesalpinieae of Caesalpinioideae seems likely to be allied with *Bretschneidera*. Nevertheless, the prominantly developed endoaperture (pore) frequently found in Caesalpinieae immediately disputes this superficial resemblance.

In the pollen cells of *Bretschneidera sinensis* only one ellipsoidal to spindle-shaped generative nucleus was stained (Fig. 2), which suggests that the pollen is binucleate. This situation is not unusual among other groups of angiosperms. According to the studies of Brewbaker (1967) and Tobe and Raven (1984), the chromatin of the vegetative nucleus is frequently diffuse and often degraded to the extant that it is refractory to stain. Very occasionally, however, two similar, comparatively smaller nuclei could be seen in the pollen of *B. sinensis*. This may represent that the generative nucleus has undergone an early division, rather than that the pollen contains a vegetative and a generative nucleus.

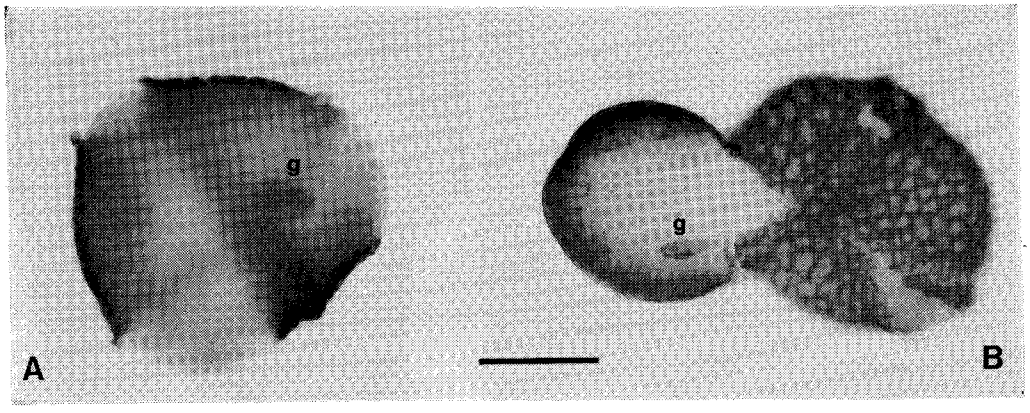

Fig. 2. FLP orcein-stained pollen grains of *Bretschneidera sinensis* (from *Peng 4765*). Both A and B (ruptured) show only one generative (g) nucleus in the pollen. Scale bar=5 μm.

An important contribution that extends our understanding of the phylogenetic significance of binucleate and trinucleate pollen grains in angiosperms was made by Brewbaker (1967), who correlated the distribution of binucleate and trinucleate taxa with the ordinal family tree of angiosperms. The fact that pollen of Sapindaceae, Hippocastanaceae, Moringaceae and Caesalpinioideae (as Caesalpiniaceae) are all binucleate (Brewbaker, 1967), suggests *Bretschneidera* to be related to them. However, approximately 70% of the angiosperms studied release pollen in a binucleate stage (Brewbaker, 1967). It would, therefore, appear that nuclear type can provide little toward the clarification of the relationship of *Bretschneidera*.

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鐘萼木之花粉記要

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鐘萼木（*Bretschneidera sinensis* Hemsl.）分佈於中國大陸南部、西南地區，以及越南北部；最近在臺灣北部低海拔山區亦發現其少量族羣。鐘萼木屬爲單型屬植物，然學者對其分類地位仍有異議。本研究以電子顯微鏡觀察其花粉、描述形態，並與前人提議的本屬近緣植物：無患子科（Sapindaceae）、七葉樹科（Hippocastanaceae）、辣木科（Moringaceae）及豆科之蘇木亞科（Caesalpinioideae）等之花粉做一比較。此外，本文首次報導其成熟花粉爲二核型（binucleate）。