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PERMINERALIZED PINACEOUS LEAVES FROM THE UPPER CRETACEOUS OF HOKKAIDO[1]

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ABSTRACT

Three fragments of fascicles of a possible five-needle pine are described from the Upper Cretaceous Yezo Group (Santonian/Senonian) of Hokkaido. Specimens from the Omakizawa, Oyubari, Yubari City, the Koyanozawa, Ikushumbetsu, Mikasa City, and the Sankebetsugawa, Haboro are preserved in calcium carbonate nodules containing abundant ammonites. Leaves borne in apparent fascicles of five measure 0.7–0.8 mm in radial and 0.8–1.0 mm in tangential diam and are represented by short fragments of isolated needles. Thick-walled epidermal cells on these amphistomatic leaves resemble the underlying uniform hypodermis. Two external resin canals are situated near the lateral corners toward the adaxial surface. Only slightly plicate mesophyll cells in a layer one to two cells thick border on an elliptical endodermis with thickened outer cell walls. Two to three layers of transfusion tissue surround the double vascular strand. Two vascular bundles are separated by one cell layer of sclerenchyma fibers. Small patches of abaxial and adaxial sclerenchyma fibers have also been observed. Leaves most closely resemble those of *Pinus leiophylla* Schl. et Cham. Subgenus *Pinus,* Section *Pinea,* Subsection *Leiophyllae* and *P. montezumae* Lamb. Subgenus *Pinus,* Section *Pinus,* Subsection *Ponderosae* and are described as a new species *P. hokkaidoensis* sp. nov. Stockey and Ueda. Close anatomical comparisons are made with this leaf and previously described permineralized Upper Cretaceous pine needles from Hokkaido and eastern North America.

FOSSIL REMAINS OF the genus *Pinus* appear during the Early Cretaceous. The oldest known fossils are permineralized cones, *P. beligica* described by Alvin (1960) from the Belgian Wealden Formation. Since that time several other species based on perminerazlied cones have been studied in anatomical detail (Miller, 1969, 1973, 1974, 1978; Underwood and Miller, 1980; Banks, Ortiz-Sotomayor and Hartman, 1981; Stockey, 1983, 1984; Miller and Malinky, 1986). The leaves and twigs that were borne on the same plants, however, are virtually unknown. Even though several species have been described from permineralized needles (Jeffrey, 1908; Hollick and Jeffrey, 1909; Stopes and Kershaw, 1910; Ogura, 1932; Penny, 1947; Miller, 1973; Robison, 1977; Ueda and Nishida, 1982; Stockey, 1984) whole plants have not been reconstructed due to lack of attachment or indisputable association evidence. For the most part these permineralized remains are Tertiary in age. Stockey (1984) summarized the possible affinities of the Eocene remains with the subgenera, sections, and subsections of the genus *Pinus* in an attempt to understand the level of evolution reached by the Middle Eocene.

In the present study we describe a new species based on permineralized needles from the Cretaceous of Hokkaido. This work represents the first part of an on-going study of evolution of the genus *Pinus* during the Cretaceous and underscores the importance of the Japanese fossils in this early evolution.

MATERIALS AND METHODS—Fossil leaves are represented by three specimens from the Omakizawa, Oyubari, Yubari City, the Koyanozawa, Ikushumbetsu, Mikasa City, and the Minorizawa of the Sankebetsugawa, Haboro, Hokkaido, Japan and carry specimens nos. 73525, 833704, and 832225 respectively. Needle fragments are preserved in calcareous nodules containing varying amounts of silica (Stopes and Fujii, 1910) that have eroded from shales in the Upper Yezo Group, Upper Cretaceous sediments. The plant containing nodules are marine in origin and can be dated as Santonian/Senonian based on abundant ammonites in the surrounding matrix (Ueda and Nishida, 1982; M. Nishida, pers. comm.). Plant

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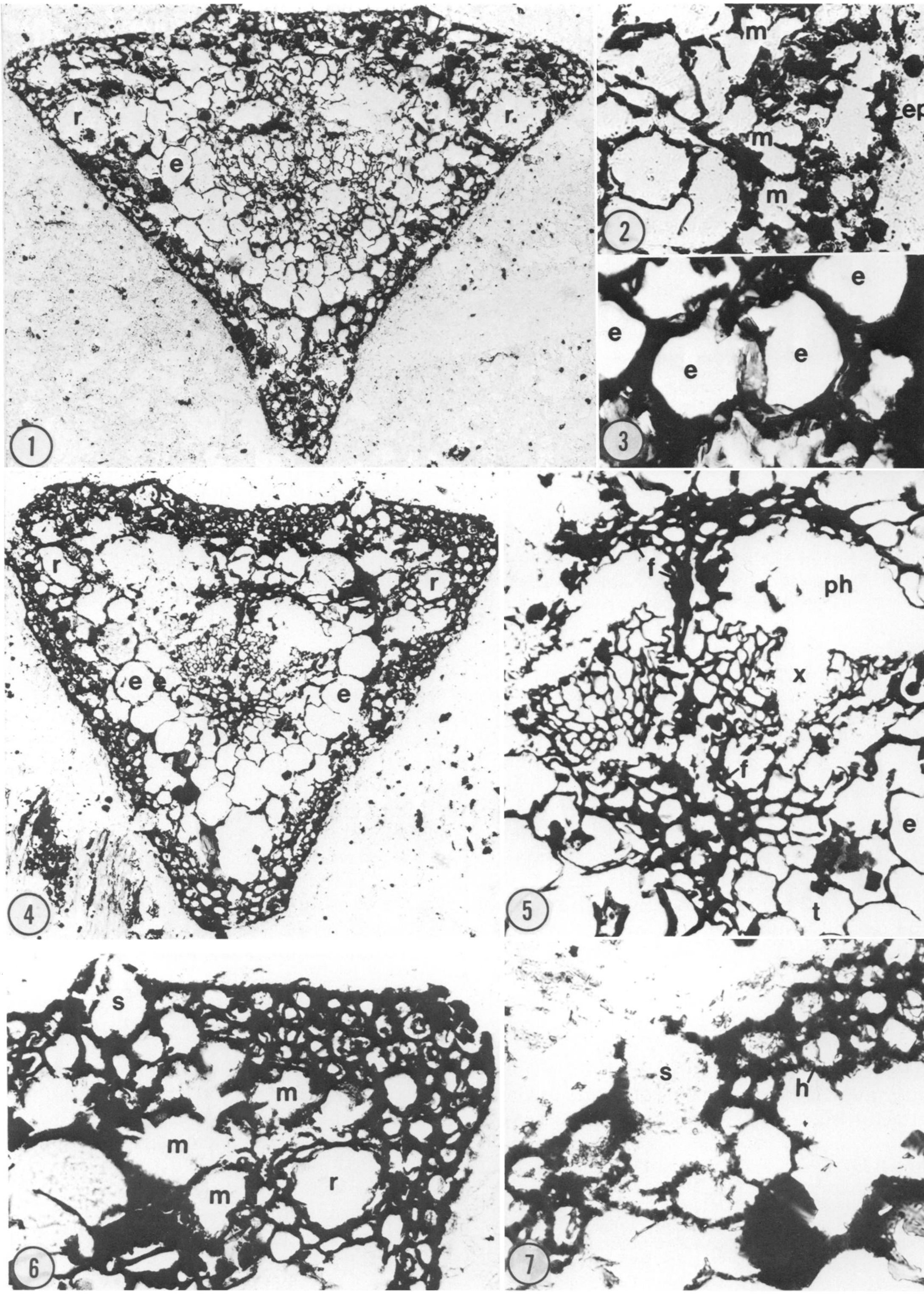

Fig. 1–7. *Pinus hokkaidoensis* sp. nov. **1.** Transverse section interpreted as from near the base of a leaf. 73525. sl. #1. ×100. **2.** Mesophyll cells showing plications. 73525. sl. #1. ×300. **3.** Endodermal cells with simple pits on radial walls. 73525. sl. #3. ×320. **4.** Transverse section of leaf. 832225 C top #33. ×100. **5.** Transverse section of double vascular strand showing "I" shaped band of fibers. 832225 C top #33. ×260. **6.** Leaf corner showing resin canal and beaked epidermal cells around stoma. 832225 C top #33. ×240. **7.** Transverse section of leaf showing hypodermis and

material found is debris that may have been carried a considerable distance from its original growth habitat. Only short segments of foliage are usually found associated with bored wood fragments. Often cuticle and sometimes epidermis and hypodermis are missing from plant parts.

Nodules were cut into slabs and studied using the cellulose acetate peel technique and hydrochloric acid (Joy, Willis and Lacy, 1956). Peel sections were mounted in Canada Balsam and Entellan rapid mounting medium for microscopic examination.

All specimens are housed in the Laboratory of Phylogenetic Botany, Faculty of Science, Chiba University, Chiba 260, Japan.

SYSTEMATIC DESCRIPTION—*Pinus hokkaidoensis* sp. nov.—Needles probably in fascicles of five, at least 0.5 cm long, 0.8–1.0 mm by 0.7–0.8 mm in tangential and radial widths respectively; fascicle sheath unknown. Vascular strand double, separated by a band of fibers one cell wide that expands on both adaxial and abaxial sides to four to five cells in radial thickness. Phloem not preserved; secondary xylem rows in each bundle 5–7 tracheids long. Transfusion tissue two to three cells thick; cell shapes irregular 35–63 μm and 20–30 μm in radial and tangential widths respectively. Endodermal cells large up to 65 μm in diam with not appreciably thickened outer walls; no casparian strips visible. Resin canals two, external, located on adaxial sides of tangential corners. Mesophyll one cell thick, reaching two to three cells thick at corners; cells slightly plicate, 30–65 μm in diam. Hypodermis uniform, one to four cells thick. Epidermal cells thick-walled circular to slightly elliptical in shape, identical to hypodermis. Stomata sunken; epidermal cells adjacent to stomatal opening beaked in transverse section; leaves amphistomatic.

Holotype—Specimen no. 832225 (Fig. 3–7).

Paratypes—Specimen nos. 73525 (Fig. 1–2), 833704.

DESCRIPTION—Three isolated needle fragments of a five-needle pine are fan-shaped in outline (Fig. 1, 4). Internal preservation of tissues is fair to good even though these leaves were probably deposited some distance from their original growth habitat. No fascicle sheaths have been observed and each needle is broken and isolated from the others in the fascicle. The adaxial corner exhibits an angle of about 72° suggesting the fascicles originally contained five needles. Despite the incompleteness of our specimens, we conclude, therefore, that *P. hokkaidoensis* was a five-needled pine. One section of specimen 73525 shows a truncated adaxial margin indicating that it probably represents the basal portion of a needle. Leaves range from 0.7–0.8 mm wide in radial diam to 0.8–1.0 mm wide in tangential diam.

Pinus hokkaidoensis leaves contain a double vascular strand (Fig. 5) characteristic of the hard pines, Section *Diploxylon* of Koehne (1893), Shaw (1914) and Pilger (1926), or Subgenus *Pinus* of Critchfield and Little (1966). The two bundles are very closely spaced, separated by a thin band of fibers one cell wide (Fig. 5). Due to poor preservation, this band is not readily visible in every section. The fiber band expands on both abaxial and adaxial sides of the double vascular strand forming a more or less "I"-shaped strand (Doi and Morikawa, 1929) with more expansion on the abaxial side (Fig. 5). Phloem and cambium are not preserved and are represented by a gap in leaf tissues (Fig. 1, 4, 5). Secondary xylem is 5–7 cells thick and 9–12 rows wide in each vascular strand (Fig. 5). A narrow band of transfusion tissue two to three cells wide surrounds the vascular strands and is composed of irregularly-shaped cells varying from 35–63 μm in radial diam and 20–30 μm in tangential diam (Fig. 5). The endodermis is composed mostly of large cells, with thickened tangential walls, that appear nearly circular in outline (Fig. 1, 4) but vary from 25–65 μm in diam. Some irregular shapes occur and casparian strips are not visible in any of the sections; simple pits, however, have been observed on the radial walls of some cells (Fig. 3). The general outline of the endodermis is elliptical (Fig. 1, 4).

Mesophyll tissue usually consists of one cell layer in thickness but reaches two to three cells in the corners (Fig. 6). Inner mesophyll cells show few if any plications while external mesophyll in corners or isolated mesophyll cells on the three sides are often smaller and show a few plications (Fig. 2, 6). Cells range from 30–55 μm and 30–65 μm in radial and tangential diam respectively, some containing dark brown contents (Fig. 1, 2, 4).

The hypodermis is uniform (Harlow, 1931) and varies from one to four cells in thickness.

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beaked epidermal cells around a stoma. 832225 C top #33. ×370. e = endodermis; ep = epidermis; f = fibers; h = hypodermis; m = mesophyll; ph = phloem zone; r = resin canal; s = stoma; x = xylem.

It is slightly thicker in the corners and thinner near stomata (Fig. 6, 7). Specimen 73525 (Fig. 1) generally has a thinner hypodermis than specimen 832225 (Fig. 2), however, the hypodermis is thicker in the corners of the leaf approaching the condition seen in 73525. Specimen 833704 is intermediate between them. In all other features the three specimens appear identical. Thus, we see no valid reason to separate the three leaves into separate taxa. It is possible that the hypodermis may have varied slightly in thickness at different levels within the leaf since the specimen illustrated in Fig. 1 probably represents the basal portion of a leaf. Hypodermal cells are thick-walled and resemble the epidermal cells by being circular to slightly elliptical. Two external resin canals (Doi and Morikawa, 1929; Harlow, 1931; Jahrig, 1962) border on the leaf edge or are in contact with the hypodermis (Fig. 1, 4, 6). Cells surrounding the canal are relatively thick-walled (Fig. 6).

Stomata occur on both adaxial and abaxial surfaces of leaves. Epidermal cells surrounding the stomatal aperture are distinctly beaked as in leaves of *P. radiata* D. Don (Harlow, 1931) and *P. lambertiana* Dougl. (Sutherland, 1934). The presence of stomata on the abaxial surface of specimen 73525 has not been verified with certainty; however, the poor preservation of the epidermis and small number of sections available could easily explain this situation. Thinning of the hypodermis in some regions further indicates the possible position of a stoma that would have become visible in consecutive sections.

DISCUSSION—Leaves of *Pinus hokkaidoensis* have characters in common with needles of several subsections of the genus *Pinus*. Sections *Pinea* and *Pinus* (*Diploxylon,* hard pines) both contain species with leaves exhibiting double vascular strands. Using the available studies of needle anatomy, the leaves described here seem to most closely resemble those of *P. leiophylla* Schlectendal et Chamisso of the Subgenus *Pinus,* Section *Pinea,* Subsection *Leiophyllae* (Little and Critchfield, 1969) and *P. montezumae* Lambert, Subgenus *Pinus,* Section *Pinus,* Subsection *Ponderosae* (Critchfield and Little, 1966). These Mexican pines have five-needled fascicles (although *P. montezumae* varies from 3–8 needles, Dallimore and Jackson, 1966) each of which contains two vascular bundles. Needles of both species are amphistomatic with two resin canals and an endodermis of large cells thickened on the outer (radial) walls (Sutherland, 1934). *Pinus montezumae* needles have sclerenchyma associated with the phloem of the vascular strands while those of *P. leiophylla* do not. Mesophyll of both species is described as two cells thick and the epidermis is composed of ovate thick-walled cells similar to those of the fossil leaves (Sutherland, 1934). Extant five-needle pines with external resin canals include: *P. aristata* Engelmann, *P. balfouriana* Jeffrey ex A. Murray, *P. flexilis* James, *P. lambertiana, P. parviflora* Siebold and Zuccarini, and *P. wallichiana* A. B. Jackson (= *P. excelsa* Wallich) (Harlow, 1931; Sutherland, 1934). These species though, have one vascular strand in their needles and differ in two or more characters from the fossil described here. Leaves of *P. aristata* exhibit only slight mesophyll plications as in *P. hokkaidoensis,* but the mesophyll is very broad in the former and restricted in the latter. *Pinus lambertiana* also has leaves with beaked stomata and a variable hypodermis as in the fossil, but also has a large mesophyll zone, abaxially oriented resin canals and a uniformly thickened endodermis unlike *P. hokkaidoensis* (Harlow, 1931; Sutherland, 1934).

Among the permineralized fossil pines described previously, *Pinus similkameenensis* Miller (1973) leaves are Eocene in age, generally smaller in diam, contain one vascular strand and have a one-celled hypodermis; thus, differing in nearly all characters from *P. hokkaidoensis.* Cretaceous *Pinus quinquefolia* Hollick and Jeffrey also has smaller-diam leaves; no stomata have been observed and up to 8 medial resin canals have been reported (Penny, 1947; Robison, 1977). *Pinus pseudostrobifolia* Ogura (1932) is represented by a badly preserved five needle fascicle with leaves that contain two vascular bundles, no resin canals, stomata or recognizable endodermis. The hypodermis is described as thin-walled and the mesophyll composed of two or more layers of large cells compared to the two small cells and thick hypodermal layer of *P. hokkaidoensis.* Ogura's type specimen (University Museum, Univ. of Tokyo) was reexamined during this study; and appears to show sclerenchyma between the bundles. However, the thickness of the section and poor preservation made observations difficult. A sclerenchymatous sheath encircling the two distinct vascular bundles occurs in *P. pseudostrobifolia* as opposed to the "I"-shaped band with a very narrow separation of *P. hokkaidoensis.* These specimens show a resemblance to an undescribed leaf referred to as *Pinus* sp. by Jeffrey (1908, Pl. XIV, Fig. 23), with a small area of transfusion tissue. This specimen, however, has been lost and closer comparisons cannot be made.

Thus, the fossil leaves described here represent a new species most closely resembling the extant Mexican pines *P. leiophylla* and *P. montezumae* of Subgenus *Pinus* except for the resin canal placement.

A large number of Upper Cretaceous pine species from Japan have been described based on permineralized needles some of which closely resemble those of fossil North American pines. These vary in needle number per fascicle from one in *P. yezoensis* (Stopes and Kershaw, 1910) to two in *P. bifoliata* (Ueda and Nishida, 1982) to three in *P. flabellifolia* Ogura (1932), *P. pseudoflabellifolia* (Ueda and Nishida, 1982) and *P. pachydermata* (Ueda and Nishida, 1982), to four *P. pseudotetraphylla,* to five *P. pseudostrobifolia* (Ogura, 1932) and now *P. hokkaidoensis.* Most of these taxa, including the new species described here, show the presence of two external resin canals and a lack of extensive plications on the mesophyll cells, characters that Jeffrey (1908) regarded as features of Cretaceous pines.

The lack of an endodermis is another feature that Jeffrey (1908) thought was a Cretaceous character. Since then several Cretaceous species have been described with well-preserved endodermis (Stopes and Kershaw, 1910; Ogura, 1932; Robison, 1977; Ueda and Nishida, 1982). The lack of obvious casparian strips in the needles described here as well as several other species (Ueda and Nishida, 1982) would seem to indicate that thick sections or poor preservation may have had more to do with the supposed lack of an endodermis in the earlier described species of *Pinus* and *Prepinus,* an extinct pine-like genus. In addition, Soar (1922) and Ueda (1978) have both reported incomplete or irregular endodermis in the basal sections of needles of extant *Pinus* species.

The present study underscores the importance and the diversity of pines in the Upper Cretaceous floras of Japan. The lack of reported pinaceous cones associated with these leaves is puzzling since their sclerotic tissues would seem to preserve as well as or better than those of needles. Further field work is essential in Hokkaido in order to better understand these leaf remains in the context of whole plants. The presence of *Prepinus japonicus* leaves (Stopes and Kershaw, 1910) with pentagonal outlines further adds to the pinaceous diversity in these floras. The large number of recognizable *Pinus* leaf taxa from Hokkaido, many with relatively minor distinguishing features, suggests that the genus was probably undergoing rapid evolution at this time. Further studies of Japanese coniferous remains in conjunction with cone studies will put these remains into evolutionary perspective and increase our knowledge of the Cretaceous distribution of the genus *Pinus* and the family Pinaceae.

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