

PERMINERALIZED PINE CONES FROM THE CRETACEOUS OF VANCOUVER ISLAND, BRITISH COLUMBIA

Selena Y. Smith and Ruth A. Stockey¹

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

Two abraded, cylindrical cone specimens found in calcareous concretions from the Cretaceous Spray Formation (Late Campanian) of Vancouver Island, British Columbia, were sectioned using the cellulose acetate peel technique and characterized anatomically. Their sclerenchymatous pith is surrounded by a ring of separate secondary xylem bundles that lack resin canals. The outer cortical zone is sclerenchymatous and covered in a dense ramentum of trichomes that is also present on ovuliferous scale and bract bases. Vascular traces to the ovuliferous scales and bracts arise independently. The bract, with a terete trace and two lateral resin canals, lacks a distinct abaxial lobe. Ovuliferous scales are sclerotic with resin canals adaxial, abaxial, and between the vascular bundles. The ovuliferous scales have an interseminal ridge that is prominent and thick near the micropylar end of the seed, thins out near the seed chalaza, and attaches to the seed wing tissue. There are two winged seeds per scale, and the edges of the ovuliferous scale turn upward and partly enclose the seeds near the micropylar end. Seeds have a ridged sclerotesta; nucellus, megagametophyte, and embryos with eight cotyledons are preserved. Cone structure most closely resembles fossil Pinaceae of the genus *Pityostrobus*. These cones have a unique combination of characters that distinguish them from the previously described taxa and are described as *Pityostrobus beardii* sp. nov. A phylogenetic analysis using morphological data from the ovulate cones of extant and fossil taxa of Pinaceae with *Cryptomeria japonica* (L. f.) D. Don and *Sciadopitys verticillata* (Thunb.) Siebold et Zucc. as outgroups was undertaken to assess the phylogenetic position of *P. beardii* within Pinaceae. *Pityostrobus beardii* appears to be most closely related to *Pityostrobus hokodzensis* from the Cretaceous of Russia. These cones provide further evidence that the Pinaceae, like the angiosperms, were undergoing a rapid Cretaceous radiation.

Keywords: cones, Cretaceous, *Obiraostrobus*, Pinaceae, *Pityostrobus*, *Pseudoaraucaria*.

Introduction

The Pinaceae are a diverse, almost exclusively Northern Hemisphere conifer family that form a major component of coniferous forests (Farjon 1998). The family also has an extensive and diverse fossil record. While the extant genera *Abies* Mill., *Picea* A. Deitrich, *Larix* Mill., *Pseudolarix* Gordon, and *Tsuga* Carr. are known in the Tertiary, the genus *Pinus* L. is first recorded in the Cretaceous (Alvin 1960; Miller 1976, 1977a; Wehr and Hopkins 1994; Falder et al. 1998; Farjon 1998). Large numbers of fossil taxa of Pinaceae have been described from the Cretaceous, but most are not assignable to any of the modern genera. These are placed within the fossil genera *Pseudoaraucaria* Fliche, with six described species; *Pityostrobus* Nathorst emend. Dutt, with 26 known species; and *Obiraostrobus* Ohsawa, Nishida & Nishida, with two species.

In molecular and morphological phylogenetic analyses, the Pinaceae have appeared in a basal position among extant conifers (Hart 1987; Chaw et al. 1997, 2000; Stefanović et al. 1998; Bower et al. 2000). Relationships between extant genera of the family have been examined only in one study using three genes (Wang et al. 2000), but there have been several attempts to find natural groupings among fossil and extant genera and

within the fossil genera *Pseudoaraucaria*, *Pityostrobus*, and *Obiraostrobus* (Alvin 1988; Ohsawa 1997; Smith and Stockey 2001; C. N. Miller, Jr., unpublished data; C. R. Robison, unpublished data).

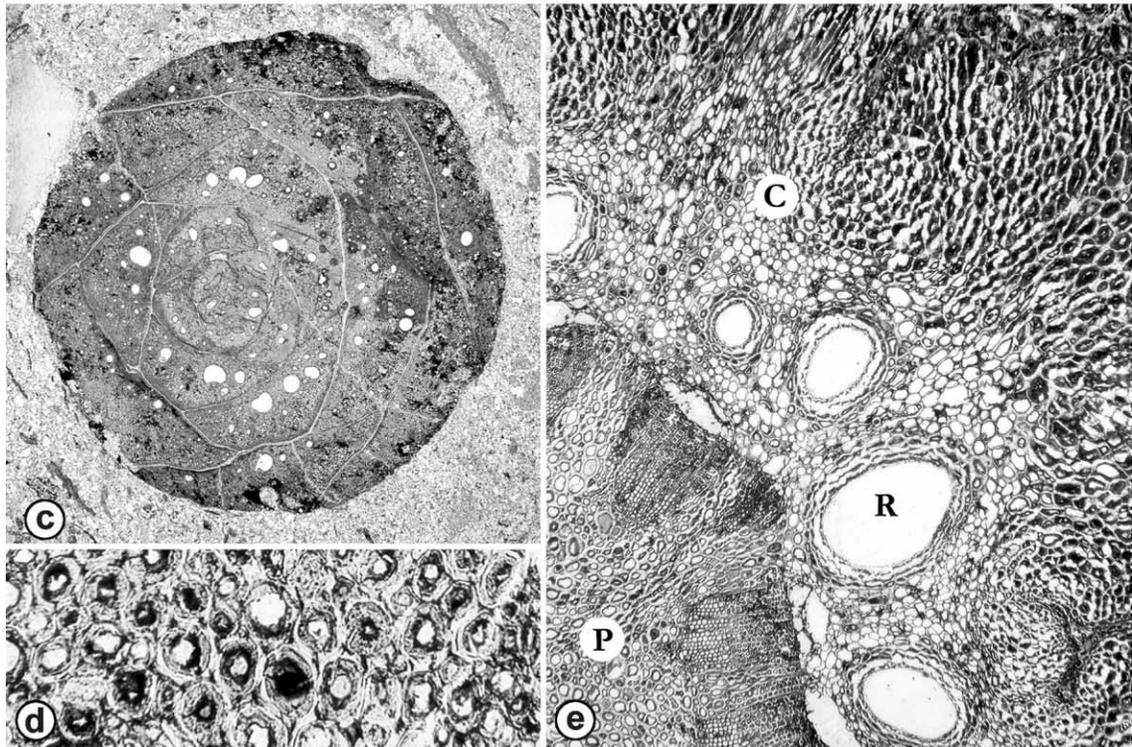
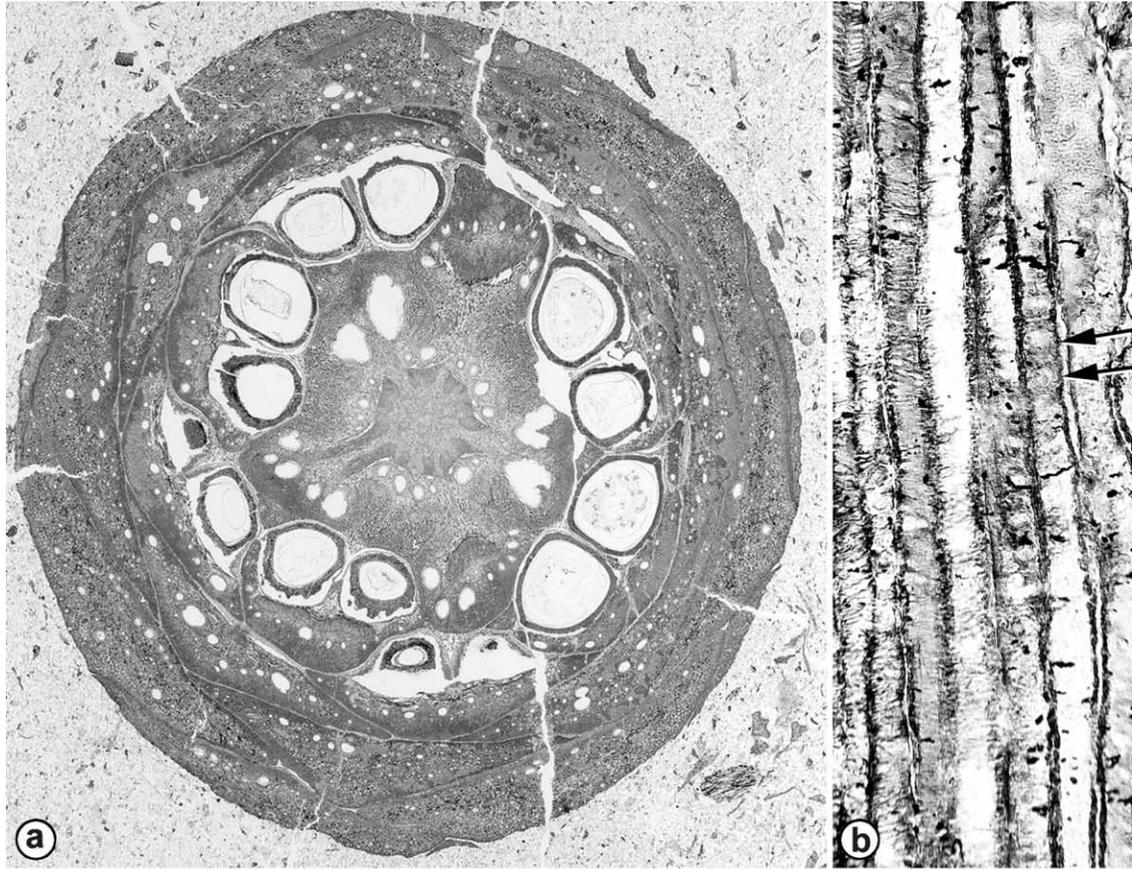
A new species of *Pityostrobus* is described here based on two incomplete, calcitic, permineralized cones from the Late Cretaceous of Vancouver Island, British Columbia. These cones represent the first occurrence of *Pityostrobus* on Vancouver Island, and represent the twenty-seventh species of anatomically preserved *Pityostrobus* described to date. They are interpreted in relation to the other fossil and extant taxa of Pinaceae using a revised data matrix based on ovulate cones (Smith and Stockey 2001).

Material and Methods

The cones were collected from Shelter Point, south of Campbell River, Vancouver Island, British Columbia. The Shelter Point section consists of six units, and fossil plant remains are found in calcareous concretions within two of these units (Richards 1975). This locality consists of upper Cretaceous rocks from the Spray Formation, Nanaimo Group. Marine invertebrate fossils indicate a latest Campanian age (Richards 1975).

The abraded calcitic cones were cut transversely and peeled using the cellulose acetate peel technique (Joy et al. 1956).

¹ Author for correspondence; e-mail ruth.stockey@ualberta.ca.



Longitudinal sections were made from one slab. Slides were prepared using Eukitt (O. Kindler GmbH, Freiberg, Germany) mounting medium.

The specimens were compared with living and fossil taxa of Pinaceae using the University of Montana Conifer Reference Collection, now housed at the University of Alberta (UAPC-ALTA). They were coded using a previously compiled data matrix for Pinaceae ovulate cones (Smith and Stockey 2001), and a cladistic analysis was performed on the updated matrix. Also, the recently described *Pityostrobus hokodzensis* (Ratzel et al. 2001) was added to the data matrix based on our personal observations of the original material.

Cladistic analyses were performed using a matrix of 33 characters and 48 taxa, including all known species of *Pityostrobus*, *Obirastrobus*, and *Pseudoaraucaria*, based on permineralizations and using composite placeholders for the extant genera of Pinaceae. Polymorphic coding was used for characters that varied within a genus. *Sciadopitys verticillata* (Thunb.) Siebold et Zucc. and *Cryptomeria japonica* (L. f.) D. Don were used as outgroups (Smith and Stockey 2001), and *Pararaucaria patagonica* was also included in the analysis because of its suggested affinities to Pinaceae (Stockey 1977).

Heuristic searches were performed using the maximum parsimony criterion in PAUP* (version 4.0b3; Swofford 2000) with TBR branch swapping and Multrees on. Fifty random addition replicates were performed, with Maxtrees set to 10,000. All character states (appendix) were unordered and equally weighted. Bootstrap analysis was performed using the same general search conditions as above, with a fast heuristic search and 100 bootstrap replicates.

Results

Systematics

Order—Coniferales

Family—Pinaceae

Genus—*Pityostrobus* Nathorst 1897 emend. Dutt 1916

Species—*Pityostrobus beardii* Smith et Stockey sp. nov.

Diagnosis. Ovulate cone, at least 2.8 cm in diameter and 3.0 cm long, cylindrical. Cone-scale complexes helically arranged. Pith, 2.0–3.0 mm in diameter, sclerenchymatous; lacking resin canals. Vascular cylinder moderately dissected; 0.5 mm, up to 27 tracheids, thick; lacking resin canals. Cortex, 1.5–3.0 mm in diameter, with 14–17 resin canals; parenchymatous with sclerenchymatous outer zone 14–20 cells thick. Rammentum of trichomes on cone axis, ovuliferous scale bases,

and bract bases. Traces to ovuliferous scale and bract arising separately from vascular cylinder; ovuliferous scale trace abaxially concave; bract trace terete. Bract sclerenchymatous, free part up to 1.0 mm long, with two lateral resin canals; separating from ovuliferous scale laterally. Ovuliferous scale sclerenchymatous, at least 29 mm long, at an angle of ca. 45° to axis; resin canals abaxial to vascular strands at scale base, becoming abaxial, adaxial, and between vascular bundles distal to seed body. Two winged seeds per scale, ca. 7 mm long and 3.5–4.0 mm wide, irregular sclerotesta; wings at least 29 mm long. Embryo with eight cotyledons.

Holotype. *Hic designatus*, 998.1.689 COP A, B, C Courtenay & District Museum, Courtenay, B. C. (fig. 1a, 1b, 1e; fig. 2a–2e; fig. 3a, 3b, 3d–3f).

Etymology. This cone is named in honor of Graham Beard, who collected and made one of the specimens available for study. His work on plant fossils from Vancouver Island, British Columbia, has increased our knowledge of the biology and evolution of Mesozoic and Cenozoic plants.

Stratigraphy. Spray Formation, Nanaimo Group.

Age. Late Cretaceous (Campanian).

Description

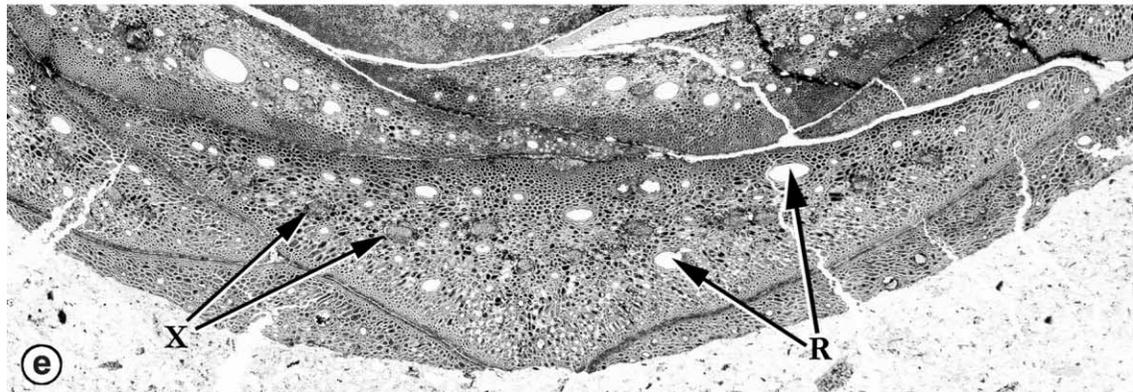
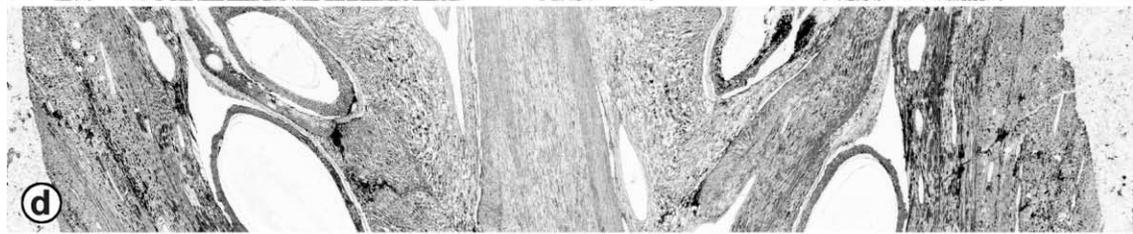
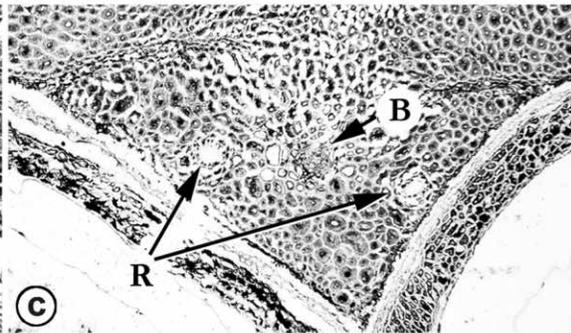
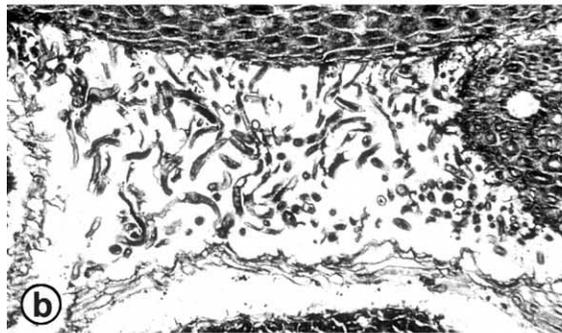
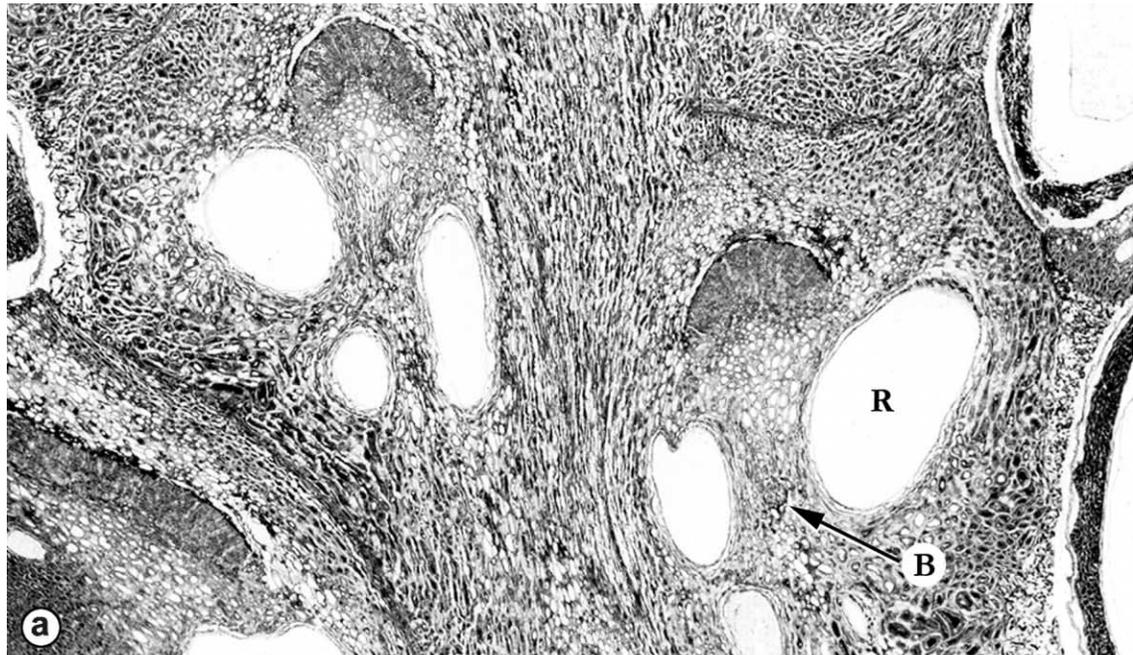
General features. The cones are cylindrical, measuring at least 2.8 cm in diameter and at least 3.0 cm in length with helically arranged cone-scale complexes (fig. 1a, 1c). One is a partial specimen missing basal and apical regions (fig. 1a), while the second specimen represents a more apical section of the cone (fig. 1c). The outer surface of both cones is abraded.

Cone axis. The pith of the cone axis is 2.0–3.0 mm in diameter and composed of sclerenchyma (fig. 1d, 1e), with some scattered parenchyma present in the basal sections of the cone. There are no resin canals found in the pith.

The vascular cylinder is dissected in midsections of the cone due to the large number of traces to cone-scale complexes (fig. 1a, 1e). This cylinder is more complete near the apex, where there are fewer scales (fig. 1c). The secondary xylem is 0.5 mm (up to 27 tracheids) thick and contains uniseriate rays and tracheids with uniseriate, circular bordered pits (fig. 1b). Tracheids of the primary xylem show scalariform pitting. Only one growth increment is present, and no resin canals occur (fig. 1e).

The cortex, 1.5–3 mm in diameter, is composed of an inner parenchymatous zone with occasional sclerenchyma cells and a thick outer sclerenchymatous zone, 14–20 cells thick (fig. 1a, 1e). A ring of 14–17 resin canals, 0.3–1.0 (usually 0.5) mm in diameter, occurs in the cortex near the vascular cylinder (fig. 1a, 1e). Large numbers of elongate trichomes are present on the cone axis, ovuliferous scale bases, and bract bases (fig. 2b).

Fig. 1 *Pityostrobus beardii* sp. nov. a, Transverse section of cone axis showing abraded surface, helically arranged cone scales, dissected secondary xylem cylinder, and two seeds per scale. Holotype C&DM 998.1.689 COP, C top #34 × 3.8. b, Longitudinal section through secondary xylem showing uniseriate, circular bordered pits (arrows). Holotype C&DM 998.1.689 COP, B₁ #158 × 256. c, Transverse section of cone axis showing abraded surface of specimen, helically arranged cone scales, and inflated scale apophyses. Paratype VIPM SH488, B bot #7 × 3.8. d, Sclerenchymatous pith. Paratype VIPM SH488, A #22 × 212. e, Transverse section of cone axis showing pith, dissected secondary xylem, parenchymatous inner cortex with resin canals, and outer sclerenchymatous cortical zone at right. Holotype C&DM 998.1.689 COP, C top #51 × 26. P = pith, R = resin canal, C = cortex.



Cone-scale complex. Vascular traces to the ovuliferous scale and bract diverge separately from the cone axis stele (fig. 2a). The ovuliferous scale traces are abaxially concave (horseshoe shaped) in cross section, 0.5 mm high and 1.0 mm wide (fig. 2a), and the large amount of intervening parenchyma results in the large gaps seen in stele cross sections (fig. 1a, 1e). The angle of divergence is ca. 45° (fig. 2d).

The resin canals to the cone-scale complex are derived from the cortical resin canal system. In the inner cortex, the two vertical resin canal systems branch, which gives rise to the two resin canals that accompany the bract trace. More distally, these two canals branch again to form the two canals that are associated with (and abaxial to) the ovuliferous scale trace (fig. 2a). The resin canals in the ovuliferous scale continue to divide and remain abaxial to the xylem. Distal to the seed body, they become scattered throughout the scale.

The bract lacks a distinct abaxial lobe. The portion of the bract that is free is up to 1.0 mm long and is 1.5–2.0 mm wide at the base. It separates from the ovuliferous scale laterally first (fig. 2c). The bract trace is terete; it enters the free bract and is accompanied by two lateral resin canals (fig. 2c).

Ovuliferous scales are at least 29 mm long and 2.8–5.5 mm wide at their base to at least 15 mm wide distally. Scales are 1.5 mm thick at the base to 3 mm thick at the apex; however, scale tips have been lost due to abrasion prior to burial (fig. 1a, 1c; fig. 2d, 2e). Ovuliferous scales are almost completely sclerotic (fig. 2e). Distally, the xylem breaks up into 11–12 strands. There is a small interseminal ridge measuring 0.5–1.5 mm high and 0.3–0.8 mm wide that is continuous with the adaxial ovuliferous scale tissue surrounding the seed body (fig. 3a, 3b). Edges of the ovuliferous scales curve up around the seeds (fig. 3a) and leave the seed bodies completely enclosed in a thin scale tissue. This tissue is continuous with the seed wing in more distal scale sections.

Seeds. Two winged seeds occur on each adaxial ovuliferous scale surface (figs. 1a, 3a), with slit-shaped micropyles facing the cone axis (fig. 3c). Seed bodies measure 3.5–4.0 mm wide and ca. 7 mm long. The sclerotesta, composed of thick-walled sclereids, 34.3–73.5 mm in diameter, has irregular ridges on the seed surface that is in contact with the ovuliferous scale. Seed wings are at least 29 mm long and are formed from the upper ovuliferous scale tissue. The nucellus is present in most seeds and has a wavy apex (fig. 3d). Megagametophyte cell walls are not distinguishable; however, apparent cell contents are preserved (fig. 3e, 3f). These appear granular and may represent starch grains. Embryos are found within several seeds, and have eight cotyledons (fig. 3e, 3f).

Phylogenetic Analysis

The analysis of all 48 taxa resulted in 2096 most parsimonious trees (in five islands) of 197 steps (consistency index

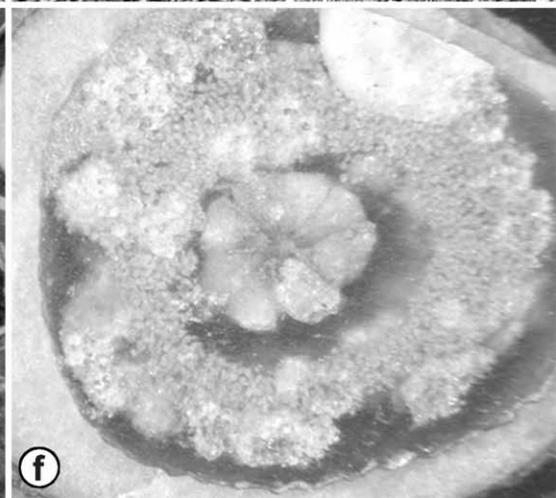
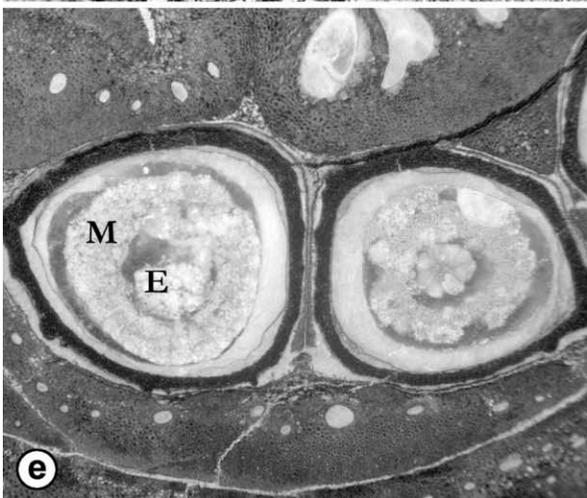
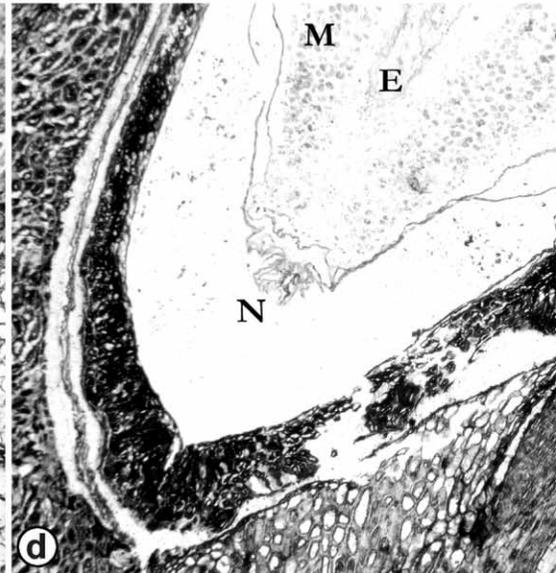
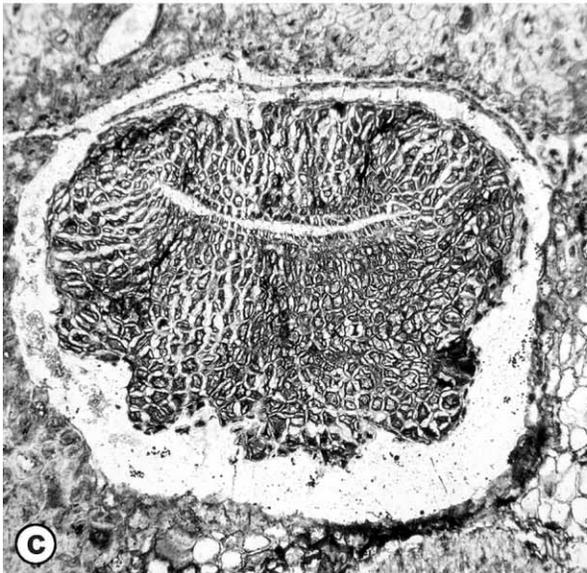
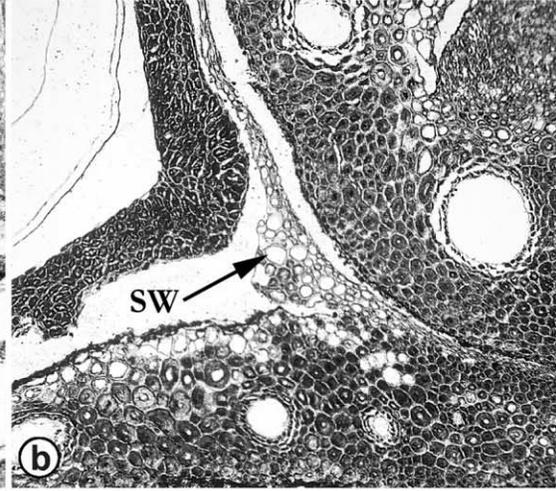
[CI] = 0.299, retention index [RI] = 0.558) (fig. 4). The species of *Pseudoaraucaria* resolve as a clade found in all most parsimonious trees, and they are part of a larger monophyletic group containing *Abies*, *Pityostrobus corneti*, *Tsuga*, *Pityostrobus leckenbyi*, and *Pityostrobus oblongus*. Several clusters of *Pityostrobus* species are found in all 2096 trees, such as (*P. leckenbyi* + *P. oblongus*); (*P. mcmurrayensis* + *P. pubescens*); (*P. hallii* + *P. villerotensis*); (*P. hautrageanus* + *P. macrocephalus*); (*P. bommeri* + *P. hueberi*); and (*P. hokodzensis* + *P. beardii*). *Pararaucaria* is sister to a monophyletic Pinaceae in all most parsimonious trees. ((*Cedrus* + *Pseudolarix*) (*P. bommeri* + *P. hueberi*)) form a basal clade in Pinaceae. In 75% of the most parsimonious trees found, there is a clade consisting of *Pinus* and five species of *Pityostrobus* (fig. 5). A clade of *Picea* and four species of *Pityostrobus* is seen in 77% of the shortest trees. *Obirastrabus* appears as a monophyletic group in only 69% of the most parsimonious trees (fig. 5).

Discussion

The pinaceous affinities of these cones are clearly demonstrated by the presence of helically arranged cone-scale complexes, a small free bract, and two winged seeds per ovuliferous scale. Scale apices, although abraded, appear to be inflated like those in *Pinus* cones (table 1). However, the Shelter Point cones have vascular traces that arise separately, like the non-*Pinus* species of Pinaceae (Miller 1976; table 1). Also unlike cones of *Pinus* as well as those of *Cathaya*, *Larix*, *Keteleeria*, *Pseudotsuga*, and *Tsuga*, the fossil cones have dissected secondary xylem in the cone axis, as in the cones of *Abies*, *Cedrus*, *Picea*, and *Pseudolarix* (table 1). The lack of resin canals in the secondary xylem is a character shared with all the extant taxa except *Pinus* and *Pseudotsuga* (table 1). The pattern of distribution of resin canals to the ovuliferous scale as seen at the scale base is considered to be an important diagnostic character (Miller 1976). In the Shelter Point cones, the resin canals are abaxial to the ovuliferous scale trace in the cortex as in *Pinus* species (Miller 1976). The cones are very sclerenchymatous, and a similar distribution of sclerenchyma is seen in some cones of *Pinus* and *Picea* (table 1). The abaxially ridged sclerotesta of the seeds observed in the fossil specimens has not been seen in any of the extant taxa examined (table 1). We therefore conclude that these cones do not show a sufficiently similar suite of characters to any of the extant taxa of Pinaceae to be placed in one of these genera.

Fossil pinaceous cones that cannot be placed in the modern taxa have traditionally been placed in one of three genera, *Pseudoaraucaria*, *Obirastrabus*, and *Pityostrobus*. *Pseudoaraucaria* is considered to be a natural genus (Alvin 1957b; Miller and Robison 1975; Miller 1976; Smith and Stockey 2001) with six described species. Features uniting these cones are a

Fig. 2 *Pityostrobus beardii* sp. nov. Holotype C&DM 998.1.689 COP. *a*, Tangential longitudinal section through cortex of cone showing transverse section of vascular traces to cone-scale complexes and associated resin canals. B₁ #114 × 19. *b*, Elongate trichomes on cone axis and scale base. B₁ #79 × 41. *c*, Transverse section of bract showing abundant sclerenchyma, terete bract trace, two lateral resin canals, and lateral separation. A #89 × 19. *d*, Radial longitudinal section of cone showing abraded scale tips and cone scale attachment. B₁ #158 × 4.7. *e*, Transverse section of ovuliferous scale showing abundant sclerenchyma, numerous vascular bundles, and resin canals scattered throughout scale. A #130 × 9.4. B = bract trace, R = resin canal, X = xylem.



large, overarching interseminal ridge; a parenchymatous pad at the seed chalaza; ovuliferous scale traces arising from two lateral strands; a dissected vascular cylinder in the cone axis; and scales at right angles to the cone axis (Alvin 1957a, 1957b, 1960; Miller and Robison 1975). Of these diagnostic characters, the Shelter Point cones show only a dissected vascular cylinder, a character that appears in several different extant genera as well as a few species of *Pityostrobus* (table 1).

The genus *Obiraostrobus* contains two species from Japan and is not considered to be very distinct from the other fossil cones placed in *Pityostrobus* (Smith and Stockey 2001). These cones differ from our specimens in having thinning scale apices, an absence of sclerenchyma in the pith, presence of resin canals in the secondary xylem, cortical resin canals that dilate prior to branching, a lack of trichomes, a resin canal system that is both abaxial and adaxial at the scale base, more than two resin canals per bract, nests of sclerenchyma in the ovuliferous scales that are abaxial and adaxial, and seeds without an abaxially lobed sclerotesta (Ohsawa et al. 1992). Therefore, our cones are unlikely to represent cones of *Obiraostrobus*.

The Shelter Point cones are best placed within the large genus *Pityostrobus*, but they are different from those of any previously described species. Characters found in *Pityostrobus beardii* that are not common within the genus are a dissected cone axis; trichomes on cone axes, scales, and/or bract bases; sclerenchyma in the ovuliferous scale that is abaxial, adaxial, and between the vascular strands; and seeds with ridged sclerotesta. Only three cones of the 26 previously described species of *Pityostrobus* show two or more of the above-mentioned features: *Pityostrobus milleri*, *Pityostrobus pubescens*, and *Pityostrobus hokodzensis*. *Pityostrobus pubescens* Miller (1985), of the Cretaceous of New Jersey, like our cones, has the uncommon characters of a dissected cone axis, trichomes, and sclerenchyma in the ovuliferous scale that is abaxial, adaxial, and between the vascular tissue but lacks several features shown in *P. beardii* such as inflated scale apices, sclerenchyma in the pith and cortex, absence of an abaxial bract lobe, presence of a small interseminal ridge, and seeds with a ridged sclerotesta (Miller 1985). Of these four taxa, only *P. hokodzensis* is indicated as being closely related to *P. beardii* (fig. 4).

There have been two cones described from the Aptian Hokodzens locality in Russia: *P. milleri* Falder et al. (1998) and *P. hokodzensis* Ratzel et al. (2001). *Pityostrobus milleri* shares the rare characters of a dissected cone axis and trichomes with our cone but differs in several respects. It has little or no sclerenchyma in the pith, inner cortex, bract, or ovuliferous scales; it has resin canals in the secondary xylem, a bract that does not separate from the ovuliferous scale, and the seeds do not have an irregular sclerotesta (Falder et al. 1998). These dif-

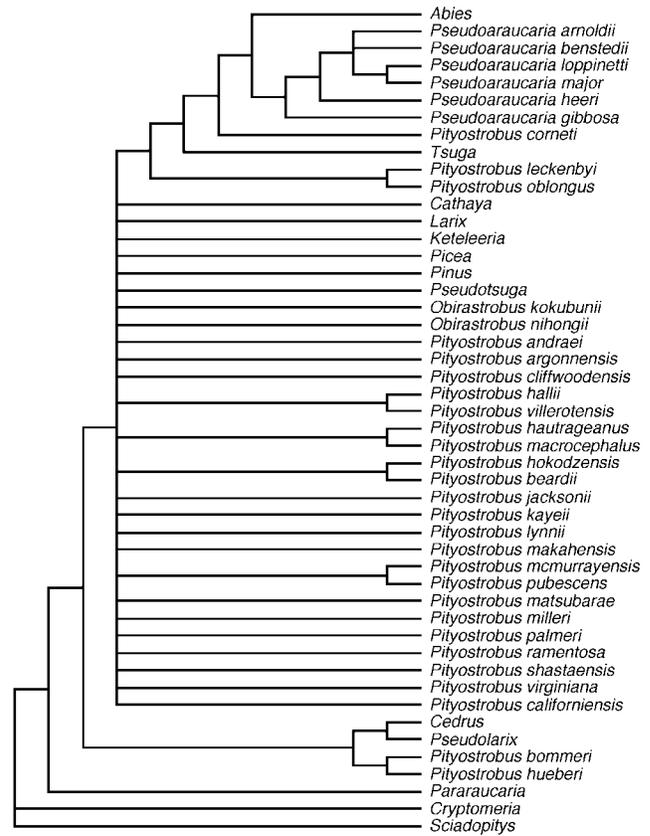


Fig. 4 Strict consensus of 2096 most parsimonious trees. The tree includes all 48 taxa (197 steps, CI = 0.299, RI = 0.558). *Pityostrobus beardii* resolves as the sister to *Pityostrobus hokodzensis* in all most parsimonious trees.

ferences indicate that the Vancouver Island specimens are not conspecific with *P. milleri*. *Pityostrobus hokodzensis* Ratzel et al. has all four features seen within the Shelter Point specimen that are rarely found within *Pityostrobus*: dissected cone axis; trichomes; sclerenchyma abaxial, adaxial, and between vascular tissue in the ovuliferous scales; and seeds with an irregular sclerotesta (Ratzel et al. 2001). *Pityostrobus hokodzensis* forms a clade with *P. beardii* that is supported by the synapomorphic character of having dissected secondary xylem in the cone axis (table 1). However, *P. hokodzensis* differs from the Shelter Point cone in having thinning scale apices and a bract separating both laterally and medially (simultaneously) (Ratzel et al. 2001). Also, *P. beardii* has a greater amount of

Fig. 3 *Pityostrobus beardii* sp. nov. *a*, Transverse section of ovuliferous scale showing abaxial resin canals and parenchymatous interseminal ridge with attachment to ovuliferous scale. Two seeds per scale are enclosed in parenchymatous tissue and show lobed sclerotesta. Holotype C&DM 998.1.689 COP, C top #34 × 12.3. *b*, Seed wing continuous with and separating from upper surface of ovuliferous scale. Holotype C&DM 998.1.689 COP, A #120 × 23. *c*, Slit-shaped micropyle in seed. Paratype VIPM SH488, A #22 × 49. *d*, Longitudinal section of seed showing megagametophyte tissue and wavy nucellar apex. Holotype C&DM 998.1.689 COP, B₁ #131 × 23. *e*, Transverse section of ovuliferous scale showing two seeds per scale and megagametophyte tissue and embryo. Holotype C&DM 998.1.689 COP, C top #62 × 10.5. *f*, Close-up view of cellular megagametophyte and embryo with eight cotyledons. Holotype C&DM 998.1.689 COP, C top #62 × 33. *E* = embryo, *IR* = interseminal ridge, *M* = megagametophyte, *N* = nucellus, *S* = seed, *SW* = seed wing.

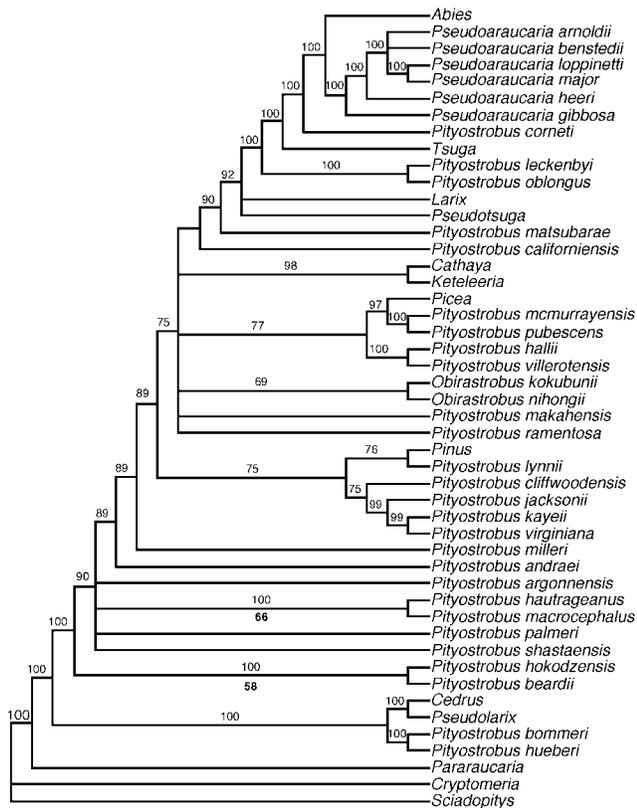


Fig. 5 Majority rule consensus of 2096 most parsimonious trees shown in fig. 4. Bootstrap values are indicated in bold below the line. Numbers above the line indicate values from majority rule consensus.

sclerenchyma in its tissues than does *P. hokodzensis*. Sclerenchyma in the pith of *P. hokodzensis* occurs as scattered nests, in contrast to the nearly completely sclerotic pith of the Shelter Point specimens; the same is true for the ovuliferous scales (Ratzel et al. 2001). Resin canals to the bract arise *de novo* in *P. hokodzensis* (Ratzel et al. 2001), unlike the Shelter Point cones, where they are obviously derived from the cortical canal system. Based on these differences, the Shelter Point specimens are here considered to represent a new taxon, *Pityostrobus beardii* Smith et Stockey sp. nov.

One interesting character seen in *P. beardii* is the enclosed nature of the seeds. The upper (adaxial) ovuliferous scale surface encloses the seed bodies in a tissue several cells thick that attaches to and becomes the seed wing toward the chalaza. This scale tissue extends over the surfaces of seeds even at the micropylar end (fig. 3c). The tissue attaches to the interseminal ridge, which leaves each seed body almost completely enclosed. This type of tissue is also seen in some extant Pinaceae (Clement-Westerhof and van Konijnenburg-van Cittert 1991; S. Y. Smith and R. A. Stockey, personal observation); however, in *P. beardii*, there is also a pronounced upturning of the ovuliferous scale itself, which further protects and surrounds the seeds. Such an upturning of the lateral margins of the ovuliferous scale has also been reported in *Pityostrobus californiensis* (Smith and Stockey 2001), *P. hokodzensis* (Ratzel et

al. 2001), and the two species of *Obiraostrobus* (Ohsawa et al. 1992).

The intimate association of seed and scale suggest to us a similarity to some members of the Cheirolepidiaceae. This extinct group of conifers was common from the upper Triassic to Cretaceous in Europe and Argentina (Watson 1988). Seeds in cheirolepidiaceous ovulate cones were enclosed in complex ovuliferous scales (Jung 1968; Watson 1988; Clement-Westerhof and van Konijnenburg-van Cittert 1991). Jung (1968) stated that a flap of tissue covered the seeds in *Hirmeriella* Hörhammer. Harris (1979) suggested that the seeds may have been borne in a cutinized sac because of the large number of cuticles obtained during maceration in the material from the Jurassic of Yorkshire. Clement-Westerhof and van Konijnenburg-van Cittert (1991) summarize the previous interpretations of this tissue and interpret the surrounding tissue as an epimatium (outgrowth of the ovuliferous scale).

While Clement-Westerhof and van Konijnenburg-van Cittert (1991) compare the covered ovules of Cheirolepidiaceae to those of araucarians and podocarps (since they shed their scales at maturity), they view the Pinaceae as different in cone construction. In Araucariaceae, however, the bract and ovuliferous scale are always shed together, unlike those of the Cheirolepidiaceae, where the stout bracts remain attached and scales are shed (Jung 1968). Some Podocarpaceae also shed the whole cone-scale complex (Clement-Westerhof and van Konijnenburg-van Cittert 1991). While most Pinaceae shed seeds with attached ovuliferous scale tissue (wings), some also shed the ovuliferous scales, e.g., *Cedrus* and *Abies*. We have observed scale tissue covering the seeds in both of these genera.

While the Cheirolepidiaceae share the character of two seeds per scale with Pinaceae, their pollen is distinct (Alvin 1982). Further fossil evidence is needed to determine whether they may have come from the same ancestral stock before any sort of relationship between these two families can be suggested. However, as Watson (1988) suggests, permineralized ovulate cones of this group would provide the most useful data. If Cheirolepidiaceae did give rise to Pinaceae, this might explain the appearance of Pinaceae as basal to all conifers in cladistic analyses based on molecular characters (Chaw et al. 1997, 2000; Stefanović et al. 1998; Bowe et al. 2000), while their oldest undisputed fossil record extends back only to the Cretaceous (Miller 1977a). This possibility is supported by the basal position of *Cedrus* within the Pinaceae (e.g., Wang et al. 2000), which shares the character of deciduous cone scales with Cheirolepidiaceae. If this scenario is correct, then Cheirolepidiaceae would represent the ghost lineage that accounts for the early divergence of the Pinaceae in the results of molecular analyses.

The phylogenetic analysis done here shows more monophyletic groupings than in previous studies (Smith and Stockey 2001). The addition of *P. beardii* and *P. hokodzensis* to the ovulate-cone-data matrix for Pinaceae (Smith and Stockey 2001) has provided slightly more resolution to the tree. With continued study, and as more taxa are described, the evolutionary relationships among Pinaceae will be better understood. However, this study and a previous one (Smith and Stockey 2001) demonstrate that it may be difficult to determine relationships robustly using cone data alone.

Pseudoaraucaria has long been thought to be a monophy-

Table 1
Pinaceae Ovulate Cone Data Matrix: Taxa and Characters

	1	11	21	31
<i>Abies</i>	0(01)101(01)011(01)	(01)(01)1011(01)110	0117001121	001
<i>Cathaya</i>	000000010	0010??1011	02?5010020	001
<i>Cedrus</i>	0000101(01)10	(01)010300101	02004(01)1121	001
<i>Larix</i>	0(01)100(01)0(01)10	101011(01)0(12)(01)	(01)1150(12)0020	001
<i>Keteleeria</i>	0000000011	(01)011101111	(01)222011021	001
<i>Picea</i>	00(01)0(01)(01)0(01)10	(01)0101010(12)0	(01)12(57)4(12)0020	001
<i>Pinus</i>	10(01)001(01)(01)1(01)	0110100(01)10	(01)0(03)5410020	001
<i>Pseudolarix</i>	1010100110	1010?00100	?235610121	001
<i>Pseudotsuga</i>	0110010(01)10	(01)010100010	(01)115010020	001
<i>Tsuga</i>	0(01)10000(01)(01)0	(01)01031(01)110	0115010021	001
<i>Pseudoaraucaria arnoldii</i>	0000100011	000010002(01)	0115531021	011
<i>Pseudoaraucaria benstedii</i>	?000100?1?	0000?00?10	0?1?131021	011
<i>Pseudoaraucaria gibbosa</i>	1010100111	0000300?10	0215031020	011
<i>Pseudoaraucaria heeri</i>	1000100010	0000100110	0112031021	011
<i>Pseudoaraucaria loppinetti</i>	1000100010	0000100010	111?131020	011
<i>Pseudoaraucaria major</i>	1000100010	0000100011	1115431021	011
<i>Obirastrobus kokubunii</i>	0000110111	00(01)0310020	0235020020	001
<i>Obirastrobus nihongii</i>	0000010011	0(01)10411020	0225020020	001
<i>Pityostrobus andraei</i>	10000?01?1	00101?0???	0005000020	001
<i>Pityostrobus argonnensis</i>	1010000110	0010130??1	0005010020	001
<i>Pityostrobus beardii</i>	1010100110	1010100010	0005410020	101
<i>Pityostrobus bommeri</i>	0011000000	0010100?01	0002100020	001
<i>Pityostrobus californiensis</i>	0011010111	0010100010	1235020020	001
<i>Pityostrobus cliffwoodensis</i>	?000010010	0010??0?10	0035110020	001
<i>Pityostrobus corneti</i>	0010000110	0011?00111	0235100021	001
<i>Pityostrobus hallii</i>	0000000010	0110110010	00554?0020	001
<i>Pityostrobus hautrageanus</i>	1010000110	0000??00?1	0055100020	101
<i>Pityostrobus hokodzensis</i>	0010100110	1010120011	0005410020	101
<i>Pityostrobus hueberi</i>	1010000100	1011100101	0052110020	001
<i>Pityostrobus jacksonii</i>	1000010000	0010?00?20	00?511002?	?01
<i>Pityostrobus kayeii</i>	1010010000	0000100010	0005000020	001
<i>Pityostrobus leckenbyi</i>	0000000???	0110200011	0115(13)10021	001
<i>Pityostrobus lynnii</i>	1000010011	01101100?(01)	000511002?	?01
<i>Pityostrobus macrocephalus</i>	1010000110	000?1?0?11	00?5000020	101
<i>Pityostrobus makahensis</i>	0010010011	00101100(12)0	0025010021	001
<i>Pityostrobus mcmurryensis</i>	0010100000	0010101010	002?210020	001
<i>Pityostrobus matsubarae</i>	0010000110	0000100010	0235010020	001
<i>Pityostrobus milleri</i>	1000110011	101013?111	005501002?	?01
<i>Pityostrobus oblongus</i>	0010000111	0110200010	0125010021	001
<i>Pityostrobus palmeri</i>	1010011110	0(01)00100011	0026410020	001
<i>Pityostrobus pubescens</i>	0000100000	1010101010	0013420020	001
<i>Pityostrobus ramentosa</i>	0000000011	1110?00010	022502002?	?01
<i>Pityostrobus shastaensis</i>	?010010110	0010100011	00?5010020	001
<i>Pityostrobus villerotensis</i>	0000000000	0110110?10	0035410020	001
<i>Pityostrobus virginiana</i>	00000?0000	1100?00010	0000010020	001
<i>Cryptomeria</i>	0210000110	00??000020	?344400010	002
<i>Pararaucaria</i>	1010000?12	1010500000	0344601020	00(01)
<i>Sciadopitys</i>	0200100000	1000100020	0117200010	002

Source. Modified from table 1 in Smith and Stockey (2001; see source note therein for additional references).

letic group (Alvin 1957a, 1988; Miller and Robison 1975; Miller 1976, 1977a), and the current analysis provides more support for this idea. However, the other fossil taxa *Pityostrobus* and *Obirastrobus* are not resolved as monophyletic. This is consistent with the idea that these are not natural groups (Miller 1976; Smith and Stockey 2001). The results of this analysis show different groupings of *Pityostrobus* with the extant taxa than found in previous studies (Smith and Stockey 2001). The clade of *Pseudoaraucaria* and *Abies* is now found

consistently in all of the 2096 most parsimonious trees, whereas only the clade of *Pseudoaraucaria* species and the clade of (*Pityostrobus loppinetti* + *Pityostrobus major*) were found in all most parsimonious trees in Smith and Stockey (2001).

As vegetative remains are rarely associated with the cones, it is unlikely that we will be able to reconstruct the whole plants and better understand their relationships from many localities. Working with isolated organs is problematic because

of the limited number of characters available compared with the number of taxa, which can result in limited phylogenetic resolution. However, the addition of two taxa in this study has resulted in more resolution of the tree shown in Smith and Stockey (2001), which emphasizes the potential value of new taxa in clarifying relationships using pinaceous ovulate cones. Only three fossil cones of *Pityostrobus* are known from outside the Cretaceous. *Pityostrobus makahensis* Crabtree and Miller (1989), *Pityostrobus macrocephalus* Lindley and Hutton (Dutt 1916), and *Pityostrobus lynnii* (Berry) Miller (1977b) are all described from Tertiary specimens. The description of *P. beardii* from the Cretaceous of Vancouver Island and the complex phylogenetic relationships demonstrated among fossil and extant taxa provide more support for the idea that the Pinaceae, like the angiosperms, were undergoing a Cretaceous radiation.

Acknowledgments

We thank Joe Morin and the Courtenay and District Museum, Courtenay, British Columbia, and Graham Beard, Vancouver Island Paleontology Museum, Qualicum Beach, British Columbia, for the loan of the specimens; Jim Haggart, Geological Survey of Canada, for help with geologic data; Gar W. Rothwell, Ohio University, for allowing the examination of *Pityostrobus hokodzensis* and providing a copy of the manuscript (Ratzel et al. 2001); Sean W. Graham, University of Alberta, for useful discussion on and help with phylogenetic analyses. This work was supported in part by Natural Sciences and Engineering Research Council of Canada grant (A-6908) to R. A. Stockey.

Appendix

Concepts of Characters Used in the Phylogenetic Analysis

- Ovuliferous scale apex. 0 = thinning distally; 1 = thickening distally (or has umbo). Ovuliferous scales of the cones of *Pinus* have an umbo, whereas most other Pinaceae do not. In fossil cones, scale tips are often abraded, but distal thickening or thinning of ovuliferous scales can usually be seen. The presence of an umbo or a distal thickening ovuliferous scale results in a coding of 1; extensive abrasion resulted in a question mark.
- Bract length. 0 = bract shorter than ovuliferous scale; 1 = bract longer than ovuliferous scale; 2 = bract and ovuliferous scales equal in length.
- Sclerenchyma in pith. 0 = absent; 1 = present. Includes only sclereids or fibers; thick-walled or sclerotic parenchyma are excluded.
- Resin canals in pith. 0 = absent; 1 = present. Canals do not include those in primary xylem; they must be separated by at least a few pith cells from the xylem.
- Secondary xylem of cone axis. 0 = forming a continuous cylinder (or little dissected); 1 = in separate strands. Steles described as "moderately" dissected were coded as continuous because these are usually interrupted only at the point of trace departure.
- Resin canals in secondary xylem. 0 = absent; 1 = present.
- Number of growth increments in secondary xylem of cone axis. 0 = one; 1 = two.
- Sclerenchyma in inner cortex. 0 = absent; 1 = present. The inner cortex may be entirely sclerotic or contain sclereid nests, fiber nests, or isolated sclerotic cells; all are coded as 1.
- Sclerenchyma in outer cortex. 0 = absent; 1 = present. Includes cones with an outer layer that is completely sclerotic as well as those with isolated sclerenchyma cells or nests (as above).
- Cortical resin canals. 0 = uniform in diameter; 1 = dilated markedly near points of branching; 2 = absent. Cortical canals are usually uniform or somewhat dilated near branching points, and either condition is coded as 0. Those that are significantly dilated code as 1.
- Trichomes on the cone axis, scale, or bract base. 0 = absent; 1 = present. This includes "glandular epidermis."
- Bract and scale traces. 0 = separate at origin; 1 = united at origin. This is viewed in tangential longitudinal sections through the inner cortex of the cone axis.
- Scale trace. 0 = clearly derived from two lateral strands; 1 = derived as single abaxially concave strand. In some ovulate cones, such as those of *Pseudoaraucaria*, *Obirastrobus kokubunii*, and some *Pityostrobus*, two strands of vascular tissue are initially derived from either side of the gap, and they later fuse together to form an abaxially concave horseshoe-shaped trace. These are coded as 1 for character 13 and 0 for character 14.
- Scale trace. 0 = abaxially concave; 1 = becoming cylindrical after divergence. This is examined in the inner cortex of the cone axis.
- Resin canals to cone-scale complex arising from cortical canals. 0 = as a single branch; 1 = two origins; 2 = three separate origins; 3 = four separate origins; 4 = more than four separate origins; 5 = no resin canals. This number is the total number of branches, from both sides of the scale trace, that are formed as the cortical axial system branches to form the resin canals that will enter bracts and ovuliferous scales.
- Bract/ovuliferous scale separation. 0 = separates laterally first; 1 = separates medially first; 2 = separates all at once; 3 = do not separate. If the middle region of the bract becomes free of the ovuliferous scale first, this is often described as having a medial pouch before separation.
- Abaxial lobe of bract base (or cone-scale complex). 0 = absent; 1 = present.
- Sclerenchyma in bract. 0 = absent; 1 = present.
- Resin canals in bract (or accompanying bract trace). 0 = absent; 1 = two; 2 = more than two.
- Bract trace. 0 = entering bract; 1 = terminating before entering free part of bract.
- Vascular ray in bract trace. 0 = absent; 1 = present.
- Resin canals to ovuliferous scale at scale base. 0 = abaxial to vascular tissue; 1 = adaxial to vascular tissue; 2 = both adaxial and abaxial to vascular tissue; 3 =

- resin canals absent. This character refers to the distribution of resin canals relative to the ovuliferous scale trace only and is determined in tangential longitudinal sections through the outer cortex of the cone axis. Resin canals to the bract are not included in the coding of this character.
23. Resin canals to ovuliferous scale at level of seed body (not under seed wing). 0 = abaxial to vascular tissue; 1 = adaxial to vascular tissue; 2 = both adaxial and abaxial to vascular tissue; 3 = abaxial and adaxial and between vascular bundles; 4 = no resin canals; 5 = abaxial and between vascular bundles.
 24. Resin canals to ovuliferous scale distal to seed body (includes under wing and more distal sections). 0 = abaxial to vascular tissue; 1 = adaxial to vascular tissue; 2 = both abaxial and adaxial to vascular tissue; 3 = between vascular bundles; 4 = no resin canals; 5 = abaxial and adaxial and between vascular bundles; 6 = abaxial and between vascular bundles; 7 = adaxial and between vascular bundles.
 25. Sclerenchyma in ovuliferous scale. 0 = both abaxial and adaxial to vascular tissue; 1 = abaxial to vascular tissue; 2 = adaxial to vascular tissue; 3 = absent; 4 = abaxial and adaxial and between vascular bundles; 5 = abaxial and between vascular bundles; 6 = adaxial and between vascular bundles. This includes distribution of sclereids and fibers relative to the vascular tissue of the whole ovuliferous scale and not only at scale origins.
 26. Interseminal ridge. 0 = absent between seeds; 1 = extending less than half of seed diameter; 2 = extending more than half of seed diameter; 3 = extending between and overarching seeds. In *Pseudoaraucaria* cones, the interseminal ridge overarches the seeds so that they appear almost embedded in the ovuliferous scale tissue; these are coded as 3. Interseminal ridges that are large but not overarching are coded as 2.
 27. Scale at right angles to cone axis for length of seed body with sharply upturned distal portion. 0 = absent; 1 = present. Some taxa such as *Pityostrobus pubescens* and *Pityostrobus californiensis* have ovuliferous scales that are at right angles to the cone axis but have a more gradually upturned distal portion. Only those with sharply upturned distal portions are coded as 1.
 28. Method of cone seed release. 0 = cone spreading; 1 = scale abscission from cone axis.
 29. Seed wings. 0 = absent; 1 = sarcotestal wing(s); 2 = wing formed from ovuliferous scale tissue.
 30. Resin vesicles/cavities in integument (seed coat). 0 = absent; 1 = present.
 31. Ridged sclerotesta. 0 = absent; 1 = present. These ridges have usually been found on the lower surfaces of seeds (the part in contact with the ovuliferous scale) and are independent of resin cavities in the integument.
 32. Enlarged parenchyma pad or cushion at chalazal end of seed. 0 = absent; 1 = present. This structure is in addition to seed wing tissue, which is separate.
 33. Number of ovules/ovuliferous scale. 0 = one, 1 = two, 2 = three or more. Although Takaso and Tomlinson (1991) report from one to 12 seeds per scale in *Sciadopitys*, theirs was a developmental study examining even the most apical scales of cones. The usual seed number reported by these authors is eight to nine per scale. This number is usually reported from lower cone scales. Therefore, we have keyed *Sciadopitys* as having greater than three seeds per scale.

Literature Cited

- Alvin KL 1957a On *Pseudoaraucaria* Fliche emend., a genus of fossil pinaceous cones. *Ann Bot, NS*, 21:33–51.
- 1957b On the two cones *Pseudoaraucaria heeri* (Coemans) nov. comb. and *Pityostrobus villerotensis* nov. sp. from the Wealden of Belgium. *Inst R Sci Nat Belg Mem* 135:1–27.
- 1960 Further conifers of the Pinaceae from the Wealden Formation of Belgium. *Inst R Sci Nat Belg Mem* 146:1–39.
- 1982 Cheirolepidiaceae: biology, structure and paleoecology. *Rev Palaeobot Palynol* 37:71–98.
- 1988 On a new specimen of *Pseudoaraucaria major* Fliche (Pinaceae) from the Cretaceous Isle of Wight. *Bot J Linn Soc Lond* 97:159–170.
- Bowe LM, G Coat, CW de Pamphilis 2000 Phylogeny of seed plants based on all three genomic compartments: extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. *Proc Natl Acad Sci USA* 97:4092–4097.
- Chaw S-M, CL Parkinson, Y Cheng, TM Vincent, JD Palmer 2000 Seed plant phylogeny inferred from all three plant genomes: monophyly of extant gymnosperms and origin of Gnetales from conifers. *Proc Natl Acad Sci USA* 97:4086–4091.
- Chaw S-M, A Zharkikh, H-M Sung, T-C Lau, W-H Li 1997 Molecular phylogeny of extant gymnosperms and seed plant evolution: analysis of nuclear 18S rRNA sequences. *Mol Biol Evol* 14: 56–68.
- Clement-Westerhof JA, JHA van Konijnenburg-van Cittert 1991 *Hirmeriella munsteri*: new data on the fertile organs leading to a revised concept of the Cheirolepidiaceae. *Rev Palaeobot Palynol* 68: 147–179.
- Crabtree DR, CN Miller Jr 1989 *Pityostrobus makahensis*, a new species of silicified pinaceous seed cone from the middle Tertiary of Washington. *Am J Bot* 76:176–184.
- Dutt CP 1916 *Pityostrobus macrocephalus*, L. and H.: a Tertiary cone showing ovular structures. *Ann Bot* 30:529–549.
- Falder AB, GW Rothwell, G Mapes, RH Mapes, LA Doguzhaeva 1998 *Pityostrobus milleri* sp. nov., a pinaceous cone from the lower Cretaceous (Aptian) of southwestern Russia. *Rev Palaeobot Palynol* 103:253–261.
- Farjon A 1998 World checklist and bibliography of conifers. Royal Botanical Gardens, Kew. 298 pp.
- Harris TM 1979 The Yorkshire Jurassic flora. V. Coniferales. British Museum (Natural History), London.
- Hart J 1987 A cladistic analysis of conifers: preliminary results. *J Arnold Arbor Harv Univ* 68:269–307.
- Joy KW, AJ Willis, WS Lacy 1956 A rapid cellulose acetate peel technique in paleobotany. *Ann Bot* 20:635–637.
- Jung WW 1968 *Hirmeriella munsteri* (Schenk) Jung nov. comb., eine bedeutsame Konifere des Mesozoikums. *Palaeontogr Abt B Palaeophytol* 122:55–93.
- Miller CN Jr 1976 Early evolution in the Pinaceae. *Rev Paleobot Palynol* 21:101–117.
- 1977a Mesozoic conifers. *Bot Rev* 43:217–280.
- 1977b *Pityostrobus lynnii* (Berry) comb. nov., a pinaceous

- seed cone from the Paleocene of Virginia. Bull Torrey Bot Club 104: 5–9.
- 1985 *Pityostrobus pubescens*, a new species of pinaceous cones from the Late Cretaceous of New Jersey. Am J Bot 72: 520–529.
- Miller CN Jr, CR Robison 1975 Two new species of structurally preserved pinaceous cones from the Late Cretaceous of Martha's Vineyard Island, Massachusetts. J Paleontol 49:138–150.
- Ohsawa T, M Nishida, H Nishida 1992 Structure and affinities of the petrified plants from the Cretaceous of northern Japan and Saghalien. XII. *Obiraastrobus* gen. nov., petrified pinaceous cones from the upper Cretaceous of Hokkaido. J Jpn Bot 66:356–368.
- Ohsawa TA 1997 Phylogenetic reconstruction of some conifer families: role and significance of permineralized cone records. Pages 61–95 in K Iwatsuki, PH Raven, eds. Evolution and diversification of land plants. Springer, Tokyo.
- Ratzel SR, GW Rothwell, G Mapes, RH Mapes, LA Doguzhaeva 2001 *Pityostrobus hokodzensis*, a new species of pinaceous cone from the Cretaceous of Russia. J Paleontol 75:895–900.
- Richards BC 1975 *Longusorbis cuniculosus*: a new genus and species of upper Cretaceous crab; with comments on Spray Formation at Shelter Point, Vancouver Island, British Columbia. Can J Earth Sci 12:1850–1863.
- Smith SY, RA Stockey 2001 A new species of *Pityostrobus* from the lower Cretaceous of California and its bearing on the evolution of Pinaceae. Int J Plant Sci 162:669–681.
- Stefanović S, M Jager, J Deutsch, J Broutin, M Masselot 1998 Phylogenetic relationships of conifers inferred from partial 28S rRNA gene sequences. Am J Bot 85:688–697.
- Stockey RA 1977 Reproductive biology of the Cerro Cuadrado (Jurassic) fossil conifers: *Pararaucaria patagonica*. Am J Bot 64: 733–744.
- Swofford DL 2000 PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b3. Sinauer, Sunderland, Mass.
- Takaso T, PB Tomlinson 1991 Cone and ovule development in *Sciadopitys* (Taxodiaceae-Coniferales). Am J Bot 78:417–428.
- Wang X-Q, DC Tank, T Sang 2000 Phylogeny and divergence times in Pinaceae: evidence from three genomes. Mol Biol Evol 17: 773–778.
- Watson J 1988 The Cheirolepidiaceae. Pages 382–447 in CB Beck, ed. Origin and evolution of conifers. Columbia University Press, New York.
- Wehr W, DQ Hopkins 1994 The Eocene orchards and gardens of Republic, Washington. Wash Geol 22:27–34.