RECONSIDERING RELATIONSHIPS AMONG STEM AND CROWN GROUP PINACEAE: OLDEST RECORD OF THE GENUS *PINUS* FROM THE EARLY CRETACEOUS OF YORKSHIRE, UNITED KINGDOM

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This study describes a specimen that extends the oldest fossil evidence of *Pinus* L. to the Early Cretaceous Wealden Formation of Yorkshire, UK (131–129 million years ago), and prompts a critical reevaluation of criteria that are employed to identify crown group genera of Pinaceae from anatomically preserved seed cones. The specimen, described as *Pinus yorkshirensis* sp. nov., is conical, 5 cm long, and 3.1 cm in maximum diameter. Bract/scale complexes are helically arranged and spreading. Vasculature of the axis forms a complete cylinder with few resin canals in the wood, and the inner cortex is dominated by large resin canals. Bracts are short, with two resin canals, and separate from the scale base laterally. Distally, the ovuliferous scales broaden and thicken to form a rhomboidal apophysis with a dorsal umbo, characters found only in the genus *Pinus* among living conifers. Resin canals enter the ovuliferous scale abaxial to the vascular tissue, which divides distally to form a row of adaxially convex bundles. A short interseminal ridge separates two inverted and winged seeds on the adaxial surface of the ovuliferous scale. Seeds contain megagametophyte tissue and polycotyledonary embryos. Numerical cladistic analysis of anatomically preserved seed cones yields a well-resolved phylogeny of crown and stem group Pinaceae that is roughly concordant with the results of analyses that include living species only. All of the included species of *Pinus* form a clade with three very *Pinus*-like species that currently are assigned to the stem genus *Pityostrobus*. These results call to question the utility of traditional methods for assigning fossil seed cones to *Pinus*, resolve relationships among stem and crown group genera, and highlight the unnatural circumscription of *Pityostrobus*. This suggests that some species of *Pityostrobus* may actually represent the genus *Pinus*, and it demonstrates that the evolutionary diversification of Pinaceae began earlier than previously recognized from fossil evidence.

Keywords: Early Cretaceous, fossil, phylogeny of Pinaceae, *Pinus*, seed cone, Yorkshire.

Introduction

Pinaceae is the most species rich family of conifers (Chamberlain 1935; Farjon 1990; Eckenwalder 2009) and the sister group to all other living conifers (Liston et al. 2003; Gernandt et al. 2008; Rai et al. 2008). The family also resolves as the sister group to Gnetales in the results of some systematic analyses of living species (i.e., gnepine hypothesis; Chaw et al. 2000; Magallón and Sanderson 2002; Mathews 2009) and is therefore a crucial component of studies that focus on the overall pattern of seed plant phylogeny (Rothwell et al. 2009). Systematic relationships among stem group species and the 11 living genera of Pinaceae have become increasingly resolved over the past several years (Ohsawa 1997; Wang et al. 2000; Smith and Stockey 2001, 2002; Liston et al. 2003; Gernandt et al. 2008, 2011) through the implementation of numerical cladistic analysis, but the evolutionary origins for the modern genera of Pinaceae and of the overall pattern of phylogeny for the family remain incompletely understood.

Anatomically preserved seed cones contribute some of the most important data from the fossil record for improving our understanding of conifer evolution and phylogeny (Florin 1951; Miller 1976; Ohsawa 1997; Smith and Stockey 2001; Rothwell et al. 2005, 2011; Escapa et al. 2010, 2012; Gernandt et al. 2011). Within the Pinaceae, permineralized stem group seed cones traditionally have been assigned to one of three genera: *Pseudoaraucaria* Fliche, *Obirastrobus* Ohsawa Nishida et Nishida, or *Pityostrobus* Nathorst sensu Dutt (Ohsawa et al. 1992; Smith and Stockey 2001, 2002; Gernandt et al. 2008, 2011). Whereas, *Pseudoaraucaria* is a well-defined monophyletic genus of a few species, *Pityostrobus* constitutes a large heterogeneous group (Miller 1976) of nearly 30 species in which the two species of *Obirastrobus* typically are nested and that are distributed throughout the family as a polyphyletic assemblage (Smith and Stockey

2001, 2002; Gernandt et al. 2008, 2011) sensu Gernandt et al. (2011). Because different species of *Pityostrobus* are the sister to at least 6 of the 11 living pinaceous genera (Gernandt et al. 2011), improving our understanding of evolution within the Pinaceae relies heavily on a clear recognition of structural relationships between individual *Pityostrobus* species and the most ancient fossil seed cones from crown groups.

This study focuses on one such anatomically preserved fossil pinaceous seed cone from the Lower Cretaceous of County Yorkshire in the United Kingdom. This fossil shows marked similarities to the seed cones of living *Pinus* species but is older than all previously described seed cones that have been assigned to the genus with confidence. The specimen is, therefore, extremely important both for potentially extending the well-documented fossil record of the genus *Pinus* and for focusing attention on those criteria that are most useful for determining the systematic relationships of fossil pinaceous seed cones. In addition, this cone provides valuable data for identifying character transformations that accompany the evolutionary origin of *Pinus*.

In this study, we describe the anatomically preserved fossil seed cone as *Pinus yorkshirensis* sp. nov. Our recognition of this ancient cone as a species of *Pinus* highlights the growing disparity between traditional criteria that have been employed for designating fossils either as species of *Pinus* or the stem group genus *Pityostrobus* (Miller 1976) and the results of cladistic analyses that focus on the pattern of phylogenetic relationships among extinct and extant pinaceous seed cones (Gernandt et al. 2011). The predictive value of each of these contrasting approaches for recognizing the generic relationships of fossil seed cones is reevaluated, a cladistic approach for identifying fossil pinaceous seed cones to genus is employed, and a well-resolved overall pattern of phylogeny for Pinaceae is presented.

Material and Methods

The specimen (BU4737) was found in the Lapworth Geological Museum of the University of Birmingham with a label that reads "Yorkshire Jurassic cone (BIRUG60832)." The cone is preserved within a weathered, red-orange-colored sideritic marine concretion that had been fractured into six fragments (recorded as fragments a–f for the purposes of identification), and each surface of the fragments was drawn and photographed. Sediment was removed from around the specimen and used in palynological analysis to accurately date the cone. The specimen was reassembled and embedded in BioPlastic (Wards, Rochester, NY). The cone was then cut on a small slab saw in cross section through the midregion. By the end of preparation there were three segments of the cone that were labeled A–C from the apex to the base of the specimen. Each segment was cut into wafers, and serial sections of several wafer surfaces were prepared using the cellulose acetate peel technique (Joy et al. 1956). Due to partial fusainization and heterogeneous mineralization of the specimen, examination of both wafers and peels was required to reveal all of the preserved histological details. Wafers were studied and photographed using reflected light, while peels were viewed and photographed using transmitted light.

Wafers were affixed to frosted microscope slides with epoxy. Four wafers were made of the middle segment of the cone (i.e., wafers B1–B4), and an additional four wafers were cut from the basal segment of the cone (i.e., wafers C1–C4). The apicalmost segment was cut into six longitudinal wafers (i.e., wafers A1–A6). Peels are identified by the wafer surface from which they were made and were numbered sequentially. Peels were mounted on microscope slides with the mounting medium Eukitt (O. Kindler, Freiburg, Germany).

Due to the highly porous nature of the sediment, several procedures were implemented to retard the formation of air bubbles in the preparations. Each wafer was vacuum-embedded in Eukitt before mounting on a microscope slide, and a coverslip was affixed using additional Eukitt. A few of the sections required additional processing before they were permanently affixed to microscope slides. After the initial embedding in Eukitt they were temporarily affixed to slides for photography. Those sections were then removed from the slide and ground down with a slurry of 600 grit carborundum and oil. Oil was used in the grinding process instead of water to prevent a reaction with the Eukitt that generates a cloudy appearance. Once the specimens were ground down, additional oil was used to remove the carborundum, and then the sections were wiped clean of oil before they were again embedded in Eukitt and put on microscope slides for photography. This process continued until the specimens were too delicate for additional grinding, and then each was permanently mounted. The remaining segments of specimen along with wafers and peels generated from the specimen are housed in the Lapworth Geological Museum at the University of Birmingham under the acquisition number BU4737.

A cladistic analysis was performed by modifying the 54-character conifer seed cone morphological matrix of Gernandt et al. (2011; deposited in TreeBASE as study number 10553). *Pinus yorkshirensis* was added to the matrix. Several characters of *Pityostrobus milleri* Falder, Rothwell, G. Mapes, R.H. Mapes & Doguzhaeva (1998) were recoded after reexamination and reinterpretation of some characters from holotype slides for that species, and several character codings for *Pararaucaria patagonica* Wieland were recoded as reinterpreted by Escapa et al. (2012). Codings of characters 1–54 for *P. yorkshirensis* are 000100100? 10110000110001110110110101211100010000100?. The revised codings of characters 1–54 for *P. millerii* are 00010010101011?000110?01110110111101101110100010?? 01???, and the revised codings for characters 1–54 for *P. patagonica* are 10000?1-0010-1000010-0000--------0010? 20-0-1000000(01)01-.

Two new characters were also added, which resulted in a matrix of 51 taxa and 56 characters. Character 55 is "Scale Apex (0) entire, (1) lobed" and is coded as "0" for all taxa except *P. patagonica* and *Cryptomeria japonica* (Thunb. ex L. f.) D. Don, which are coded as "1." Character 56 is "Adaxial Tissue Covering Seed (0) absent, (1) present," and is coded as "0" for all taxa except *P. patagonica*, which is coded as "1." The tree is rooted with *Sciadopitys verticillata* (Thunb.) Siebold & Zucc.

The analyses were conducted with all characters treated as nonadditive and also with the same five characters treated as "additive" as in the analyses of Gernandt et al. (2011). In the
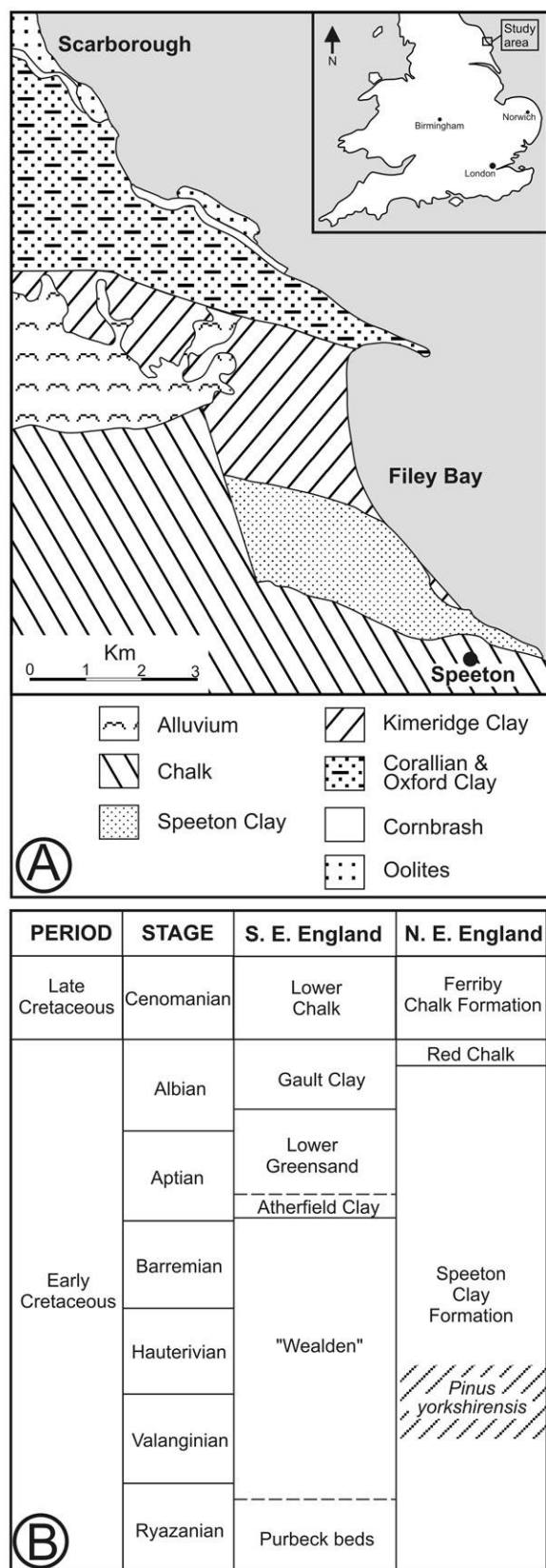
current matrix the additive characters are 13, 16, 43, 51, and 54. Maximum parsimony phylogenetic analyses (i.e., parsimony ratchet analyses, 100,000 iterations; Nixon 1999) were performed on a Dell Optiplex 990 desktop computer using the program NONA (Goloboff 1999), spawned through Winclada (Asado, ver. 1.1 beta, by K. Nixon; http://www.cladistics.com). Branch support was estimated with 1000 jackknife replicates saving 10 trees per addition sequence replicate. The published matrix is deposited at Morphobank www.morphobank.org as "*Pinus yorshirensis* published matrix (matrix 1636)."

Palynology, Stratigraphy, and Locality Information

Palynological preparation was conducted by C. H. Wellman at the University of Sheffield, and prepared slides were analyzed at the British Geological Survey in Nottingham, UK. Preservation of palynomorphs was poor to fair and consisted of dinoflagellate cysts and spores. Some Jurassic dinoflagellate cysts of ?*Endoscrinium galeritum* Deflandre, *Gonyaulacysta jurassica* subsp. *adecta* Deflandre, *G. jurassica* subsp. *jurassica* Deflandre, and *Rigaudella aemula* Deflandre are present but occur in relatively small numbers, indicative of contamination or reworking (Riding 2005). Cretaceous dinoflagellate cysts include *Callaiosphaeridium asymmetricum* Deflandre & Courteville, ?*Cribroperidinium confossum* Duxbury, *Cyclonephelium distinctum* Deflandre & Cookson, *Odontochitina operculata* Deflandre, *Oligosphaeridium* spp., and *Spiniferites* spp.

The included dinoflagellates with the narrowest geological range are *C. asymmetricum*, *O. operculata*, and *C. confossum*, which constrain the sediments to the late Hauterivian or early Barremian stages of the Early Cretaceous (Duxbury 1977). The range base of *C. asymmetricum* is of mid-Hauterivian age (the *S. inversum* zone; Davey 1979; Costa and Davey 1992). However, the key taxa are *C. confossum* and *O. operculata*. The range top of *C. confossum* is of latest Hauterivian age (*S. variabilis* Zone), and the range base of *O. operculata* is early Barremian (*H. rarocinctum* Zone; Davey 1979; Costa and Davey 1992; Heilmann-Clausen 1987; Duxbury 1977). Hence, these taxa are indicative of the Hauterivian-Barremian transition (i.e., the latest Hauterivian-earliest Barremian *S. variabilis*–*H. rarocinctum* zones). No intra-Hauterivian or Aptian and Albian marker taxa were observed. The occurrence of the spore genera *Appendicisporites* and *Cicatricosisporites* in significant numbers is entirely consistent with an Early Cretaceous age.

Therefore, we conclude that the cone was deposited during the Hauterivian-Barremian transition, ~131–129 Ma. Yorkshire deposits of that age crop out north of the town of Speeton (fig. 1A) as part of the Speeton Clay Formation (fig. 1B), which includes Early Cretaceous sediments of the Hauterivian and Barremian. The Speeton Clay Formation is ~100 m thick and comprises marine mudstones, cementstones, sporadic bentonites, and sideritic nodule horizons (Mitchell and

Fig. 1 Geography and stratigraphy. *A*, Location of Speeton Clay Formation (modified from Duxbury 1977); *B*, stratigraphy of Speeton Clay Formation (modified from Hart 1987), indicating stratigraphic location of *Pinus yorkshirensis*.

Underwood 1999). As the conifer cone was deposited during the Hauterivian-Barremian transition and occurs in a sideritic nodule, the biostratigraphical and lithological evidence indicates that it was derived from the middle part of the Speeton Clay Formation.

Systematic Description

Order—Coniferales Florin

Family—Pinaceae Lindley

Genus—Pinus *L.*

Species—Pinus yorkshirensis *Ryberg, Stockey, Hilton, Mapes, Riding et Rothwell, sp. nov.*

Species diagnosis. Incomplete seed cone conical, 5 cm long, 2.6–3.1 cm in diameter. Cone-scale complexes helically arranged. Pith 2 mm wide; secondary xylem forming complete cylinder 0.7 mm wide without growth ring; resin canals few. Cortex 1.3–1.7 mm wide, inner zone with resin canals, outer zone sclerenchymatous. Ovuliferous scale up to 1.7 cm long, 5–9 mm wide, up to 3 mm thick, with abaxial band of sclerified tissue 0.3–0.6 mm thick; adaxial ground tissue parenchymatous, 0.3–0.6 mm thick. Vascular tissue of ovuliferous scale forming row of adaxially arched vascular bundles alternating with resin canals. Interseminal ridge low, sclerified; apophysis rhomboidal with dorsal umbo. Seeds inverted, winged, two per scale, 3–5 mm long, 1–1.6 mm in diameter; embryo with ~6 cotyledons.

Locality. North of the town of Speeton, Yorkshire, UK.

Stratigraphy. Speeton Clay Formation, Lower Cretaceous.

Age. Early Cretaceous, Barremian-Hauterivian.

Holotype hic designatus. Specimen BU4737, Lapworth Geological Museum of the University of Birmingham, Birmingham, UK (figs. 2–5).

Etymology. The species epithet *yorkshirensis* is named after the county of Yorkshire in which the cone is found.

Description

General Features

The cone was found as seven fragments, and when reassembled it was observed that most of the cone axis had eroded away before collecting. In addition to the fossilized specimen being partially eroded, the cone itself appears to have been weathered before fossilization. The surface of the scales and cone axis appear to have been abraded, somewhat obscuring surface features (fig. 2A, 2B). The specimen is also partly fusainized (fig. 2A) and somewhat pyritized, and it has some coalification (fig. 2A–2C). The incomplete cone is conical in shape, measuring 5 cm long and 2.6–3.1 cm in diameter (fig. 2A, 2B). Numerous helically arranged cone-scale complexes, each with two adaxially attached and inverted seeds, surround the axis (fig. 2A–2C). The free portion of the bract is small (fig. 3A, 3B), and the ovuliferous scales that extend away from the cone axis are spreading (fig. 2A, 2B). Each ovuliferous scale is distinctly inflated distally forming an apophysis with a dorsal umbo (figs. 2A, 2B, 3C, 5A). Fragments of other plant structures, including needle-like leaves, are preserved within the sediment that is located among the spreading ovuliferous scales.

Cone Axis

The cone axis (fig. 2C) is up to 4.4 mm in diameter with a pith 2 mm in diameter consisting mostly of a gray material that does not show good cellular detail (fig. 2C–2E). The presence of resin canals and sclereids in the pith cannot be determined, but such structures were probably absent as sclerotic tissue and resin canals are preserved throughout the rest of the specimen (figs. 2, 3C, 4A–4C). Along the margin of the pith, parenchyma cells are preserved (fig. 2D) and measure 27–39 μm in diameter. The secondary xylem of the axis, 0.7 mm wide, forms a complete cylinder, interrupted only by pith tissue extending into the ovuliferous scales at levels of vascular tissue to cone-scale complexes (fig. 2D, *upper left*). Although incomplete, general features of the woody cylinder can be determined from preserved tracheids and uniseriate rays (fig. 2D). Tracheids are up to 30 μm in diameter. A growth ring is absent from the xylem and resin canals are few (fig. 2D, arrowhead).

Cortex of the cone axis is 1.3–1.7 mm wide and is divided into two zones (fig. 2E). The inner cortex, 0.6–1.6 mm in diameter, is dominated by resin canals 400–700 μm in radial diameter. Two resin canals enter each scale (fig. 2E). Tissue around the resin canals is incompletely preserved, similar to that of the pith, suggesting that the inner cortex is parenchymatous. The outer cortex is sclerenchymatous and 0.4–0.9 mm thick (fig. 2E).

Bract

Bracts are small (fig. 3A, 3B), up to 2 mm wide, and separate from the ovuliferous scale at the level where the cone-scale complex first creates a radial bulge in the surface of the cone axis (fig. 3A). Bracts separate from the ovuliferous scale at the margins first (fig. 3B, arrowhead), and in cross section show a single terete vascular bundle. There appear to be two lateral resin canals within the bract tissue (fig. 3A, arrowheads). Bracts were only observed in cross sections of the cone, so bract length could not be measured, but the free tip is present in only three or four serial peel sections, so they must be very short.

Ovuliferous Scale

Ovuliferous scales are helically arranged around the cone axis (fig. 2A–2C), diverging from the cone axis at a 90° angle in the midregion of the cone, and then arching upward near the chalazal end of the seeds (fig. 2B). Distally the ovuliferous scales spread and extend away from the axis at angles that range between 70° near the base of the cone to 10° toward the apex of the cone. Although tissue of the xylem is incompletely preserved at the level of divergence from the stele of the cone axis, it can be identified as it extends radially, traverses the cortex, and enters the scale base (figs. 2E,
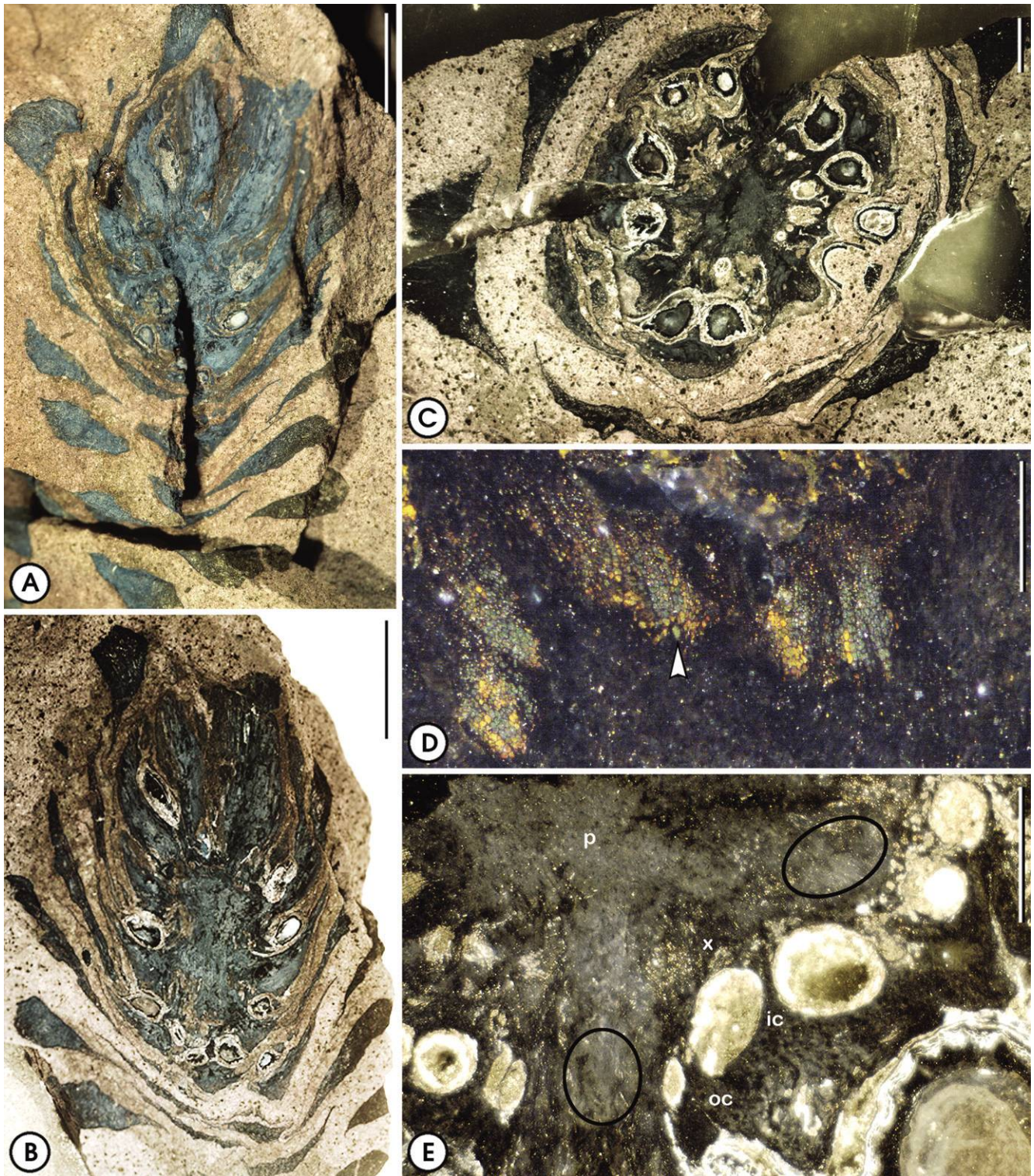
Fig. 2 *Pinus yorkshirensis* Ryberg, Stockey, Hilton, Mapes, Riding et Rothwell sp. nov. Holotype (BU4737). *A*, Surface view of whole cone after seven fragments reassembled, showing combination of permineralization, fusainization, and coalification of cone ×1.7. Scale bar = 1 cm. *B*, Surface of cone in oblique section. Fragment d bottom (bot) ×2 ; scale bar = 1 cm. *C*, Cross section of cone showing axis and helically arranged bract/ovuliferous scale complexes, each with two adaxial seeds. Wafer B3 top ×4.3; scale bar = 2 mm. *D*, Cross section of cone axis with preserved wood tissue, pith parenchyma, and one possible resin canal (arrowhead). Wafer B1 top ×45; scale bar = 0.5 mm. *E*, Cross section of cone axis with pith (p), vasculature (x), inner cortex with resin canals (ic), outer cortex (oc), and positions of scale xylem (circles). Note seed with irregular margin of sclerotesta at bottom right. Wafer B3 bot ×23; scale bar = 1 mm.
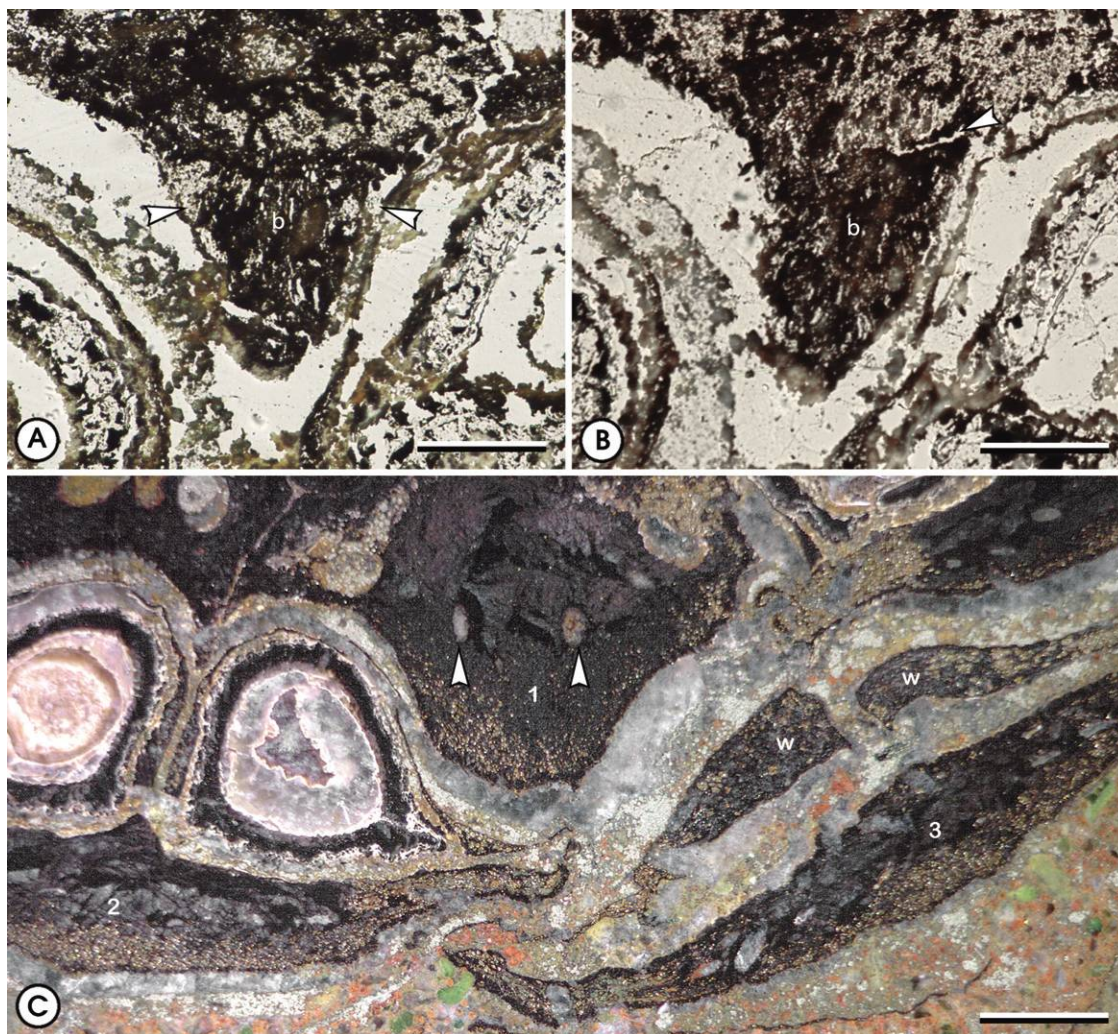
Fig. 3 *Pinus yorkshirensis* Ryberg, Stockey, Hilton, Mapes, Riding et Rothwell sp. nov. Holotype (BU4737). *A*, Cross section of cone showing bract (b) below level of separation. Arrowheads identify resin canals. Peel B4 bottom (bot) #27 ×40; scale bar = 0.5 mm. *B*. Same bract (b) as in fig. 2*A* at slightly more distal level, showing beginning of lateral separation (arrowhead) on right side. Peel B4 bot #31 ×40; scale bar = 0.5 mm. *C*, Cross section showing cone scales at three different levels: (1) lowest level of scale with two abaxially located resin canals (white arrowheads); (2) scale at level of seeds; (3) scale at level distal to seeds showing seed wings (w). Wafer B2 top ×20; scale bar = 1 mm.

4*A*). Such tissue is round to oval in cross sections (identified by ovals in fig. 2*E*) as it diverges from the stele of the axis, and it appears to have been in a closed cylinder at the level of divergence from the stele of the cone axis. However, preservation of the xylem at these levels is insufficient to demonstrate that the bract and scale traces are united into a cylinder at the level of divergence (i.e., in the inner cortex; fig. 2*E*). As the vascular tissue enters the scale it expands laterally and divides several times (fig. 4*A*, arrowheads), forming a line of bundles as seen in cross sections (fig. 4*B*, arrowheads). Two resin canals located abaxial to the scale trace (fig. 3*C*, white arrowheads) extend into the scale and then divide to form a line (fig. 4*B*, between white arrowheads). At the level of the seed body and more distally, the resin canals alternate with and are slightly abaxial to the vascular bundles (figs. 3*C*, 4*A*–4*C*).

Ovuliferous scales are 1.2–1.7 cm long and 5–9 mm wide (figs. 2*B*, 3*C*, 4*B*). The base of the scale measures 1.4–1.6 mm thick, increasing to 3 mm thick, including a small (~0.6 mm) interseminal ridge, at the level of the seeds (fig. 4*B*). Distally, the scale tapers to 1.3 mm and then expands into a swollen apophysis (fig. 5*A*). The apophysis is rhomboidal (fig. 5*A*) and 2.1–2.7 mm thick. The umbo is located in the dorsal region of the apophysis (fig. 5*A*, arrowheads).

As described above, vascular bundles form a row in which they alternate with resin canals through the distal portion of the scale (figs. 3*C*, 4*B*, 4*C*). The resin canals extend slightly more abaxially than do the vascular bundles (figs. 3*C*, 4*B*, 4*C*). Tracheids of the bundles are not easy to see in the thick sections because of preservation, but their location and shape are indicated by dark areas that alternate with the resin canals (figs. 3*C*, 4*A*–4*C*). As is characteristic of cones assign-
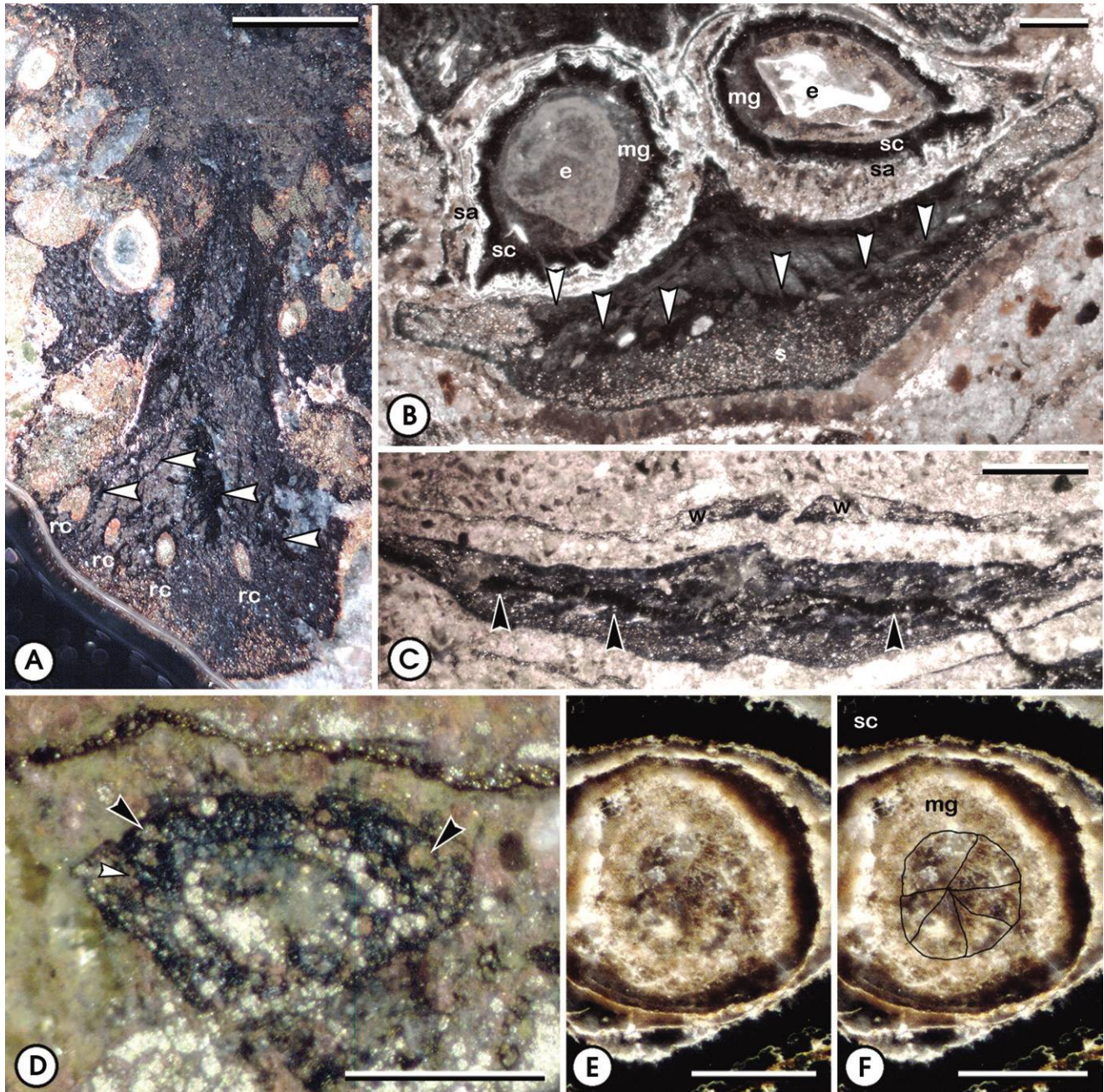
Fig. 4 *Pinus yorkshirensis* Ryberg, Stockey, Hilton, Mapes, Riding et Rothwell sp. nov. Holotype (BU4737). *A*, Ovuliferous scale diverging from axis with dividing vasculature (arrowheads) and row of resin canals (rc). Wafer B4 top ×18; scale bar = 1 mm. *B*, Ovuliferous scale with two adaxial seeds, sclerified abaxial region (s), alternating vascular bundles (arrowheads) and resin canals, and small interseminal ridge. Seeds with sarcotesta (sa), sclerotesta (sc), megagametophyte (mg), and embryo (e). Wafer B3 bottom (bot) ×21; scale bar = 0.5 mm. *C*, More distal level of ovuliferous scale than in fig. 3*C*, showing alternating resin canals and vascular bundles (arrowheads) and wings of two seeds (w). Wafer B3 top ×17; scale bar = 1 mm. *D*, Cross section of pine needle showing distinctive triangular shape, hypodermal layer (outside), mesophyll cells with possibly sinuous margins (white arrowhead), abaxially located resin canals (black arrowheads), and distinctive endodermis. Wafer B2 bot ×65; scale bar = 0.5 mm. *E, F*, Enlargements of seed at left in fig. 3*C*, showing megagametophyte tissue and multicotyledonary embryo in cross section. Embryo sectioned through cotyledons. Megagametophyte tissue (mg) labeled and cotyledons outlined in *F*. Wafer B2 top ×40; scale bar = 0.5 mm.

able to the genus *Pinus*, scale bundles are adaxially convex at distal levels of the cone scale (fig. 4*C*, center arrowhead). Abaxial to the vasculature of the scale is a region of sclerified tissue 0.3–0.6 mm thick (figs. 3*C*, 4*A*–4*C*). Toward the lat-

eral margins of the scale the entire internal portion of the scale is sclerotic (fig. 3*C*). The interseminal ridge also consists of sclerenchyma tissue (fig. 4*B*). Up to eight resin canals 1–2 mm in diameter are present in the distal portion of the ovuliferous
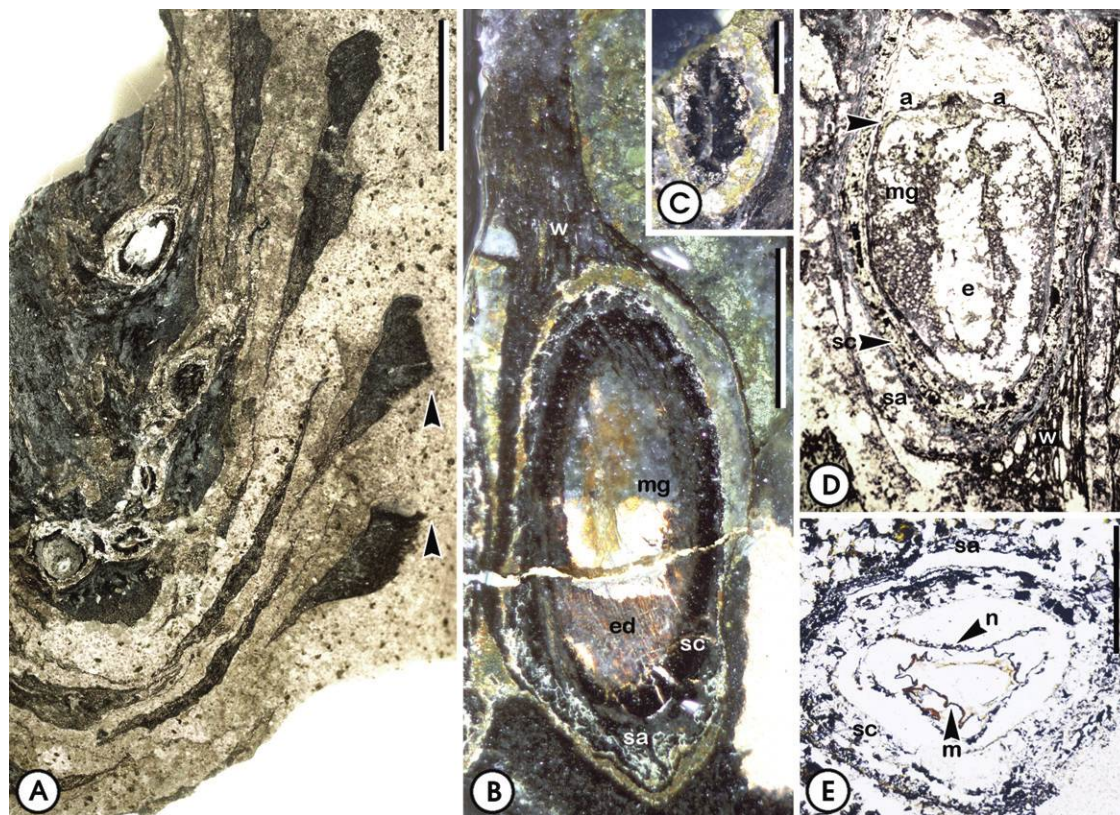
Fig. 5 *Pinus yorkshirensis* Ryberg, Stockey, Hilton, Mapes, Riding et Rothwell sp. nov. Holotype (BU4737). *A*, Surface view of cone fragment in oblique longitudinal section showing divergence of spreading ovuliferous scales with inverted ovules, each scale with apophysis containing dorsal umbo (arrowheads), Fragment e top ×4; scale bar = 2 mm. *B*, Oblique longitudinal section of inverted seed with micropyle toward base of image, lateral wing toward top. Sarcotesta (sa), sclerotesta (sc), probable endotesta (ed), megagametophyte (mg), seed wing (w). Wafer A1 top ×25; scale bar = 1 mm. *C*, Cross section of seed with micropyle appearing as a slit. Note sinuous margin of sclerotesta. Wafer B4 top ×22; scale bar = 0.5 mm. *D*, Longitudinal section of seed with wing (w) at bottom and micropyle toward top, sarcotesta (sa), sclerotesta (sc with black arrowhead), nucellus (n with black arrowhead), archegonial chambers (a), cellular detail of megagametophyte (mg), and position of embryo (e). Peel A2 top #2 ×19; scale bar = 1 mm. *E*, Cross section of seed with sarcotesta (sa), sclerotesta (sc), nucellus (n with black arrowhead), megaspore membrane (m with black arrowhead). Peel C1 bottom #13 ×20; scale bar = 1 mm.

scale. A region of incompletely preserved tissue, 0.4–0.6 mm thick, probably representing parenchyma cells, occurs adaxial to the resin canals (figs. 3*C*, 4*B*, 4*C*).

Seeds

Two inverted seeds are attached on the adaxial surface of each ovuliferous scale (figs. 2*B*, 2*C*, 3*C*, 4*B*, 5*A*). The seeds are 3–5 mm long and 1–1.6 mm in diameter, and oval (fig. 5*C*) to teardrop-shaped (figs. 2*C*, 3*C*, 4*B*) in cross sections. Seed bodies are seen in the basalmost portion of the scale (figs. 2*A*–2*B*, 5*A*, 5*B*). On the sides of the seed near the ovuliferous scale margin and toward the chalaza, the sarcotestal tissue of the integument is attached to the seed wing (figs. 3*C*, 4*B*, 5*B*, 5*D*). The wing is constructed of scale tissue that separates with the seed (figs. 4*C*, 5*B*, 5*D*). Beyond the chalazal end of the seed, the wing is 2–4 mm wide and extends for two-thirds the length of the scale. Wing tissue is several cell layers thick near the seed body (fig. 3*C*, right), narrowing to only one or two cell layers laterally and toward the distal end of the ovuliferous scale (fig. 4*C*).

A layer of elongated cells that represents either the endotesta or inner fibrous layer of the sclerotesta is located at the inner margin of the integument (fig. 5*B*, ed). The sclerotesta consists of several layers of dark brown fibers with a smooth inner margin and an uneven outer margin (figs. 3*C*, 4*B*) that results from incomplete preservation of the outermost sclerotestal cells. The uneven outer surface appears both in cross and longitudinal sections of the sclerotesta (figs. 3*C*, 4*B*, 5*B*). The sarcotesta, 73–87 μm thick, consists of one to three cell layers with an outer cuticle. In cross sections the sarcotesta frequently shows a sinuous outer margin (figs. 3*C*, 4*B*) that more or less follows the uneven surface of the sclerotesta. The integument forms a micropyle that in cross section appears as a slit (fig. 5*C*).

The nucellus appears to be one or two cells thick and is adnate to the integument (fig. 5*E*) except at the apex (fig. 5*D*). In cross sections near the apex of some seeds, the nucellar cuticle is pulled away from the sclerotesta, and the nucellar apex shrunken (fig. 5*E*). In other sections attachment of the nucellus to the integument is less clear, but this appears to be taphonomic in origin. The megaspore membrane forms

a golden-colored thin layer, 8–10 μm thick, most visible near the nucellar apex (fig. 5E).

Megagametophyte tissue is composed of small isodiametric cells 13–20 μm in diameter. Such cells are gray as seen in peel sections under transmitted light (fig. 5D) but are typically light yellow to gray in reflected light (fig. 4E, 4F). One seed in longitudinal section shows two lateral depressions in the apex of the megagametophyte (fig. 5D, a) that appear to represent archegonial chambers.

Embryo cavities or cellular embryos are preserved in several seeds (figs. 3C, 4B, 4E, 4F, 5D, 5E). These seeds appear to have been infected by fungi, making embryo structure difficult to observe at higher magnifications. However, by focusing up and down through thick sections the overall morphology of embryos can be determined. One specimen shows what appear to be six or seven cotyledons (fig. 4E, 4F). Embryos measure 300 μm in transverse section of the hypocotyl region.

Associated Plant Material

Most of the dispersed plant fragments preserved within the sediment located among the spreading ovuliferous scales of the cone (fig. 2C) consist of coniferous wood and unidentified fragments of ground tissue, but two are short segments of needle-like leaves with features that are characteristic of the genus *Pinus*. One is oriented in cross section (fig. 4D) and the other in longitudinal view. The segment cut in longitudinal section (not figured) is 2.3 mm long and ~0.5 mm wide. The epidermis is largely absent. Preserved tissue consists of an outer hypodermis to which small fragments of epidermal cell walls adhere, one to three layers of mesophyll cells, a uniseriate endodermis, and a few tracheids of the vascular bundle. The other leaf is triangular in cross section, measuring ~1.0 mm wide and 0.6 mm thick (fig. 4D). It has a convex abaxial side and two straight adaxial surfaces that meet at an angle of 105°–110°. This indicates that the leaf was produced in a fascicle of either three or four needles.

As with the leaf fragment preserved in longitudinal section, the outer epidermis is largely absent, the outermost preserved layer consisting of a hypodermis of small, closely spaced cells. There are three to four layers of parenchymatous mesophyll cells in which two laterally positioned resin canals can be identified adjacent to the abaxial hypodermis of the leaf (fig. 4D, black arrowheads). Margins of the mesophyll cells appear to be somewhat sinuous (fig. 4D, white arrowhead) but that feature is subtle and inconsistent, and difficult to verify because the heterogeneous mineralization of the specimen may have caused some cell shrinkage. A uniseriate endodermis of variable-sized cells encloses a roughly oval area (fig. 4D). The vascular bundle is incompletely preserved.

Phylogenetic Analysis

The analysis, with all characters designated nonadditive, yielded 15 trees of 250 steps (CI = 0.26, RI = 0.60). The strict consensus of those results resolves a well-defined phylogeny of Pinaceae, with only six collapsed nodes on the tree (fig. 6). The analysis with five additive characters yielded 48 most parsimonious trees of 257 steps (CI = 0.26, RI = 0.60). Nine nodes collapse in the strict consensus tree of that analy-

sis (not figured) producing a tree of comparable topology but with three more collapsed nodes than in the previous results. Both trees resolve a *Pseudoaraucaria* clade, a polyphyletic *Pityostrobus* assemblage of 27 species, and an *Obirastrobus* clade of two species that is nested among species of *Pityostrobus* (fig. 6).

The more highly resolved tree (fig. 6) derived from the analysis with no additive characters is rooted by *Sciadopitys verticillata* (Sciadopityaceae). *Cryptomeria japonica* (Cupressaceae), and *Pararaucaria patagonica*, which recently has been assigned to the Cheirolepidiaceae (Escapa et al. 2012), are attached to the stem of the tree at successive nodes with *P. patagonica* forming the sister to Pinaceae (fig. 6). The Pinaceae consists of a basal grade that is roughly equivalent to the Abietoid clade of previous analyses of the family (Wang et al. 2000; Gernandt et al. 2011), and a much larger Pinoid clade (fig. 6). The Abietoid grade is arranged with *Pityostrobus leckenbyi* + *P. oblongus* at the basal node on the stem, followed successively by *Tsuga* and then a clade that consists of *Abies* + (*Cedrus* + *Keteleeria*) + (*Pseudoaraucaria heeri* + (*P. loppineti* + *P. major*) + (*P. gibbosa* + (*P. arnoldii* + *P. benstedii*))).

The Pinoid clade includes *Pseudolarix*, *Cathaya*, *Picea*, *Larix*, *Pseudotsuga*, and *Pinus*, as well as 25 species of *Pityostrobus* and the *Obirastrobus* clade. Species are arranged as three smaller clades on the stem of the tree (fig. 6), with the lowest clade on the stem consisting of *Pityostrobus matsubarae* + (*Pityostrobus californiensis* + (*Obirastrobus kokubunii* + *O. nihongii*)). The next clade consists of *Pityostrobus mcmurrayensis* + (*Pseudolarix amabilis* + (*Pityostrobus beardii* + *Pityostrobus hokodzensis*)) (fig. 6). The apical clade on the tree forms two smaller clades (fig. 6), one of which consists of *Pityostrobus makahensis* + (*P. pubescens* + *P. virginiana*) + (*P. cliffwoodensis* + (*P. hallii* + *P. villerotensis*) + (*Cathaya argyrophylla*, *Picea*, *Pityostrobus ramentosa*, (*Larix* + *Pseudotsuga*))).

The second apical clade consists of 10 "pine-like species" of *Pityostrobus* attached to the stem singly and in pairs at seven nodes forming a roughly pectinate arrangement, plus a terminal clade of seven species that includes all four species of *Pinus* in the analysis (fig. 6). These are arranged as *Pityostrobus cornetii* + *P. hueberi* + (*P. palmeri* + *P. shastaensis*) + (*P. argonnensis* + (*P. hautrageanus* + *P. macrocephalus*) + (*P. bernissartensis* + (*P. kayeii* + (*P. andraei* + (*Pinus ponderosa*, *Pinus nelsonii*, *Pinus belgica*, *Pityostrobus lynnii*, *Pityostrobus jacksonii*, (*Pinus yorkshirensis* + *Pityostrobus milleri*))))). It is interesting to note that the *Pityostrobus* species in the terminal *Pinus* clade include *P. lynnii* (fig. 6), which was originally described as a species of *Pinus* (Berry 1934).

Discussion

Pinus yorkshirensis displays a large number of features that are characteristic of seed cones assignable to the genus *Pinus* (Miller 1976; Farjon 2005; Gernandt et al. 2011). The cone has numerous helically arranged cone-scale complexes consisting of a short narrow bract and much longer ovuliferous scale. Bracts have a single vascular bundle, two lateral resin canals,
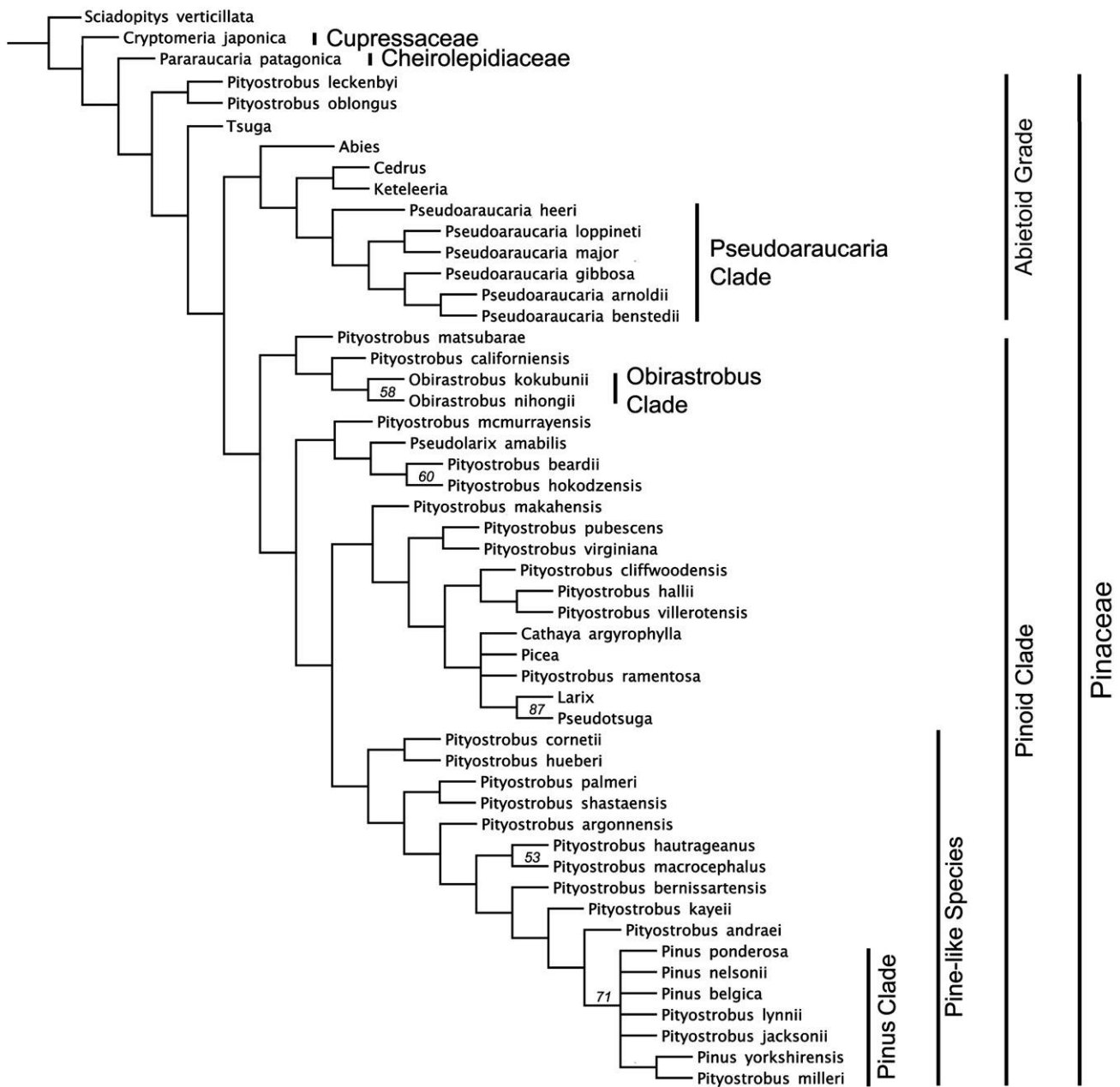
Fig. 6 Strict consensus of 15 most parsimonious trees ($L = 250$, consistency index = 26, retention index = 60) resolving the phylogeny of Pinaceae using a morphological matrix of 56 characters for living and fossil seed cones. Jackknife values above 50% are indicated on the appropriate branches. See text for details.

and a short free tip that separates beginning from the lateral margins. Ovuliferous scales are spreading, inflated toward the apex forming an apophysis, and each displays a dorsal umbo. Two inverted, winged seeds are attached on the adaxial surface of each ovuliferous scale and are separated by a low interseminal ridge that extends less than half of the diameter of the seeds. Internally, the cone axis forms a continuous cylinder of wood with a small number of resin canals. Two resin canals diverge to the base of each ovuliferous scale on the abaxial side of the vascular tissue. Distally within the

ovuliferous scale the vascular bundles are adaxially convex. Preservation of the xylem at the base of each cone-scale complex is inadequate to determine for certain whether the vascular tissue diverges as a single unit.

The diagnostic suite of characters that distinguishes *P. yorkshirensis* from other species of the genus (table 1) consists of conical cones ~5 cm long and 2.5–3.3 cm wide with a pith that is 2 mm in diam. and a vascular cylinder 0.7 mm thick with few resin canals and no growth ring. The cortex is 1.3–1.7 mm wide with large resin canals throughout

and sclerenchyma toward the outside. Ovuliferous scales are 1.2–1.7 cm long, 0.5–0.9 cm wide, and 1–3 mm deep, and show a low interseminal ridge that is less than half the diameter of seeds. Seeds are 3–5 mm long, 1.0–1.6 mm wide, and with a wing that extends two-thirds the length of the scale.

Pinus belgica Alvin (1960) from the Early Cretaceous of Belgium was previously the oldest widely recognized species of *Pinus* (Taylor et al. 2009). Morphologically, *P. yorkshirensis* shares with *P. belgica* a conical shape and a similar size. However, the larger size of cortical resin canals in *P. yorkshirensis* as well as the much larger seeds of *P. belgica* (9 mm vs. 3–5 mm) reveal that these cones are not of the same species (table 1). *Pinus cliffwoodensis* Miller et Malinky (1986) from the Late Cretaceous of North America and *Pinus matthewsii* McKown, Stockey et Schweger (2002) from the Pliocene of North America share numerous characters with *P. yorkshirensis* such as cone shape and size, scale length, and seed size, but the absence of a growth ring and paucity of resin canals in the wood distinguish *P. yorkshirensis* from those two species. The stratigraphic and geographic occurrence of *P. yorkshirensis* also are widely separated from both of those species (table 1).

One of the most obvious characters of *P. yorkshirensis* is the dominance of resin canals in the cortex. Among the fossil pines, only *P. yorkshirensis*, and the Eocene taxa, *Pinus princetonensis* (Stockey 1983), and *Pinus arnoldii* (Miller 1973; Stockey 1984; Klymiuk et al. 2011), display large resin canals that dominate the inner cortical region. *P. princetonensis*, *P. arnoldii*, and seven other species of permineralized ovulate pinecones have a cylindrical or long-conical shape (table 1) with scales departing the cone axis at angles between 5° and 35°, and there is no difference in the angle of divergence from the basalmost scale to the apicalmost scale in those species. In contrast, the angle of divergence of the scale from the cone axis varies from ~70° near the base of the cone to ~10° at the apex of the cone in *P. yorkshirensis*, thus further differentiating *P. yorkshirensis* from all of those species (table 1).

Among the extinct fossil genera of pinaceous seed cones (i.e., *Pseudoaraucaria*, *Pityostrobus*, and *Obirastrobus*), *P. yorkshirensis* is easily distinguished from species of *Pseudoaraucaria* Fliche. Species of *Pseudoaraucaria* have an interseminal ridge that overarches the seeds, a parenchymatous pad at the seed chalaza, an ovuliferous scale trace arising from two lateral strands, and a dissected vascular cylinder in the cone axis (Miller 1976; Smith and Stockey 2002). These characters are absent from *P. yorkshirensis* and contribute to species of *Pseudoaraucaria* resolving as a monophyletic group located within the Abietoid grade on the tree, which is quite distant from the position of species of *Pinus* (fig. 6). In the results of our analyses the *Pseudoaraucaria* species are nested in a clade that also includes *Abies*, *Cedrus*, and *Keteleeria*. By contrast, the characters of *P. yorkshirensis* summarized above place it solidly within the "Pinoid clade" of the family Pinaceae (fig. 6).

The two species of *Obirastrobus* are sisters nested within the Abietoid clade of Gernandt et al. (2011), and they occur at the basal node on the stem of the Pinoid grade, in the results of our analysis (fig. 6). Therefore, they are easily distinguished from *P. yorkshirensis* by several characters (see

Gernandt et al. 2011 for relevant character mappings on the tree in fig. 4 of that analysis).

There is no distinctive character or suite of characters that unites species of *Pityostrobus* (Dutt 1916; Miller 1976; Smith and Stockey 2001), and this structural heterogeneity is reflected by species of *Pityostrobus* being distributed across the Pinaceae tree as a polyphyletic assemblage (fig. 6; fig. 4 in Gernandt et al. 2011). However, species that nest within the pine-like species clade of Pinaceae (fig. 6) show varying degrees of greater similarity to species of *Pinus*, and form a paraphyletic assemblage within the Pinoid clade of Pinaceae (fig. 6). In general, species of *Pityostrobus* positioned successively closer to the *Pinus* clade on the tree share progressively larger numbers of common characters with *P. yorkshirensis* and other species of *Pinus* (fig. 6; table 1).

Traditional Methodology for Systematic Placement of Fossil Pinecones

Our understanding of structural variations among pinaceous genera and species relies heavily on the comparative studies of C. N. Miller Jr., who developed a large reference collection of sectioned seed cones that he used to characterize the features of each modern genus in the Pinaceae. Miller analyzed the systematic usefulness of several anatomical characters of seed cones, among which there is a set of four that he considered to be most important for identifying living species of *Pinus* (Miller 1976). Anatomically preserved fossil seed cones from Cretaceous strata are almost always identified to genus by comparison to Miller's criteria (Falder et al. 1998; Smith and Stockey 2001, 2002; Gernandt et al. 2011), with the presence of all four characters routinely considered to be diagnostic of the genus *Pinus*. According to Miller, the most important anatomical characters for recognizing the genus *Pinus* are the combination of (1) resin canals in the wood of the axis, (2) resin canals that diverge abaxial (and only abaxial) to the vascular tissue to the cone-scale complex, (3) vascular tissue to the cone-scale complex that diverges as a single unit, and (4) vascular bundles that are adaxially convex in the distal region of the ovuliferous scale (Miller 1976).

Miller's combination of shared characters has been useful for identifying fossil pinaceous seed cones. However, his reference collection does not contain all of the living species of the genus *Pinus* (Miller 1976), so there remains the possibility that some living species of the genus may display as yet unknown variation in one or more of the pertinent characters. Miller also made one exception to the application of those criteria when he removed from *Pinus* an extinct species that has all four of the anatomically diagnostic characters of the genus (Miller 1977). That cone has ovuliferous scales that form an apophysis with an umbo (Berry 1934; Miller 1977) and was originally described as *Pinus lynnii* Berry (1934). Nevertheless, in his study of *P. lynnii*, Miller (1977) transferred the species to the genus *Pityostrobus* (i.e., *Pityostrobus lynnii* [Berry] Miller), because it has medial separation of the bract from the scale and it displays a small bract trace that does not always enter the free bract tip (Miller 1977). That systematic opinion reveals that Miller considered his four anatomical criteria as guides for recognizing species of *Pinus*, not as dicta that must be followed in generic placement of species.

Table 1

Morphological Features of Anatomically Preserved Fossil *Pinus* Seed Cones

	P. yorkshirensis	*P. belgica*	*P. mutoi*	*P. cliffwoodensis*	*P. princetonensis*	*P. driftwoodensis*	*P. arnoldii*
Age	Hauterivian/Barremian	Barremian-Aptian	Coniacian	Santonian	Eocene	Eocene	Eocene
Locality	Yorkshire	Belgium	Hokkaido	New Jersey	British Columbia	British Columbia	British Columbia
Citation	This article	Alvin 1960	Saiki 1996	Miller and Malinky 1986	Stockey 1984	Stockey 1983	Klymiuk et al. 2011
Cone:							
Cone shape	Conical	Ovoid-conical	Cylindrical	Ovoid-conical	Cylindrical	Cylindrical	Long-conical
Morphology:							
Cone length (cm)	5	4.5	20	3–4.5	4–4.8	3–4	5–7
Cone diameter (cm)	2.5–3.1	3	6	2.2–2.3	1.5–2	2.7	1.8–2.8
Axis:							
Pith diameter (mm)	2	5	?	2–5	2	4.5	2
Vasculature diameter (mm)	.7	2	?	1.1–2	2	.6	1.5
Growth ring	Absent	Absent	?	Present	Present	Absent	Present
Wood resin canal	Few or none	Near pith	?	Just distal to growth ring	2 rows	Near pith	Near pith
Cortical diameter (mm)	1.3–1.7	5	2	1–2	1–2.5	?	1.5–3
Cortical resin canals	Large, throughout cortex	Ring present	Inner ring	Inner ring	Large, throughout cortex	Inner ring	Midregion
Outer cortical cells	Sclerenchymatous	?	Sclerenchymatous	Parenchymatous	Sclerenchymatous	Parenchymatous	?
Ovuliferous scale:							
Length (cm)	1.2–1.7	2	3.5–4	.8–1.2	1.5–1.8	1.8	2.0
Width (cm)	.5–.9	.9	1.0	.8–.9	1.4	1.2	1.5
Depth (mm)	1–3	?	1–2	.5–2	1–2	?	1
Interseminal ridge	>half seed diameter	Absent?	Absent	Absent	>half seed diameter	Absent	Absent
Seed:							
Ovule length (mm)	3–5	9	5–6	3	8	?	5
Ovule width (mm)	1–1.6	4	5	?	1.5–1.8	2	3
Wing length	2/3 of scale	2/3 of scale	1/2 of scale	?	2/3 of scale	?	1/2 of scale

	P. wolfei	*P. escalantensis*	*P. avonensis*	*P. buchananii*	*P. burtii*	*P. mattheusii*
Age	Eocene	Oligocene	Oligocene	Oligocene	Miocene	Pliocene
Locality	Washington	British Columbia	Montana	Washington	Massachusetts	Yukon
Citation	Miller 1974	Banks et al. 1981	Miller 1969	Underwood and Miller 1980	Miller 1978	McKown et al. 2002
Cone:						
Cone shape	Long-conical	Cylindrical	Long-conical	Long-conical	Long-conical	Ovoid-conical
Morphology:						
Cone length (cm)	7.4	12	5.2–5.5	7–8	11	3.4–4.4
Cone diameter (cm)	4.5	.7–1.1	2.3–2.5	3	4	2.8–3.4
Axis:						
Pith diameter (mm)	2–6	6	2.5–4	4	5	6.3
Vasculature diameter (mm)	.8–2	1.5–2	.7–2	1.3	2.3	1.8
Growth ring	Absent	Present	Absent	Present	Possible	Present
Wood resin canal	Near pith	Near pith	Rare	Near pith	Central (growth ring?)	Near pith
Cortical diameter (mm)	2–3	2–3	2–5	4–4.5	2–5	?
Cortical resin canals	Inner ring	Inner ring	Inner ring	Inner ring	Inner ring	Inner ring
Outer cortical cells	Sclerenchymatous	Parenchymatous	Parenchymatous	Parenchymatous	Sclerenchymatous	Parenchymatous
Ovuliferous scale:						
Length (cm)	2.2	3.5	1.8	2.0	4.5	2
Width (cm)	1.0–1.5	.8–1.2	.8–1.0	1.4	2–2.5	1
Depth (mm)	1–2.5	2.5–5	1.5	2	3–5	?
Interseminal ridge	>half seed diameter	>half seed diameter	Absent	Absent	>half seed diameter	>half seed diameter
Seed:						
Ovule length (mm)	5–6	None	3	5	10	2.7–3.9
Ovule width (mm)	2.3–4	None	1–2	1–2.5	5	2.1–2.5
Wing length	1/4 of scale	None	?	3/4 of scale	1/2 of scale	3/4 of scale

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From a Mechanistic Approach to a Cladistic Approach for Inferring Relationships among Fossil Pinecones

In the results of our cladistic analyses, *P. yorkshirensis* nests within a clade that also includes all of currently recognized species of the genus *Pinus* that are included in the study (fig. 6). This highlights the divergence of methodology employed in this study from that traditionally used for identifying anatomically preserved pinaceous seed cones. On the one hand, Miller's classical set of diagnostic anatomical characters has been applied for many years, has proven to be useful, and is widely accepted as comprising valid methodology for identifying Cretaceous fossil seed cones of the genus *Pinus*. On the other hand, there now is solid evidence that those criteria may not be as defensible in a phylogenetic context as commonly thought. Moreover, those criteria are not commonly applied to fossil pinecones from Tertiary sediments (especially compressions), where specimens are almost always assigned to the genus *Pinus* without first determining the relevant states for those four anatomical characters (Axelrod 1986; Erwin and Schorn 2006; Xing et al 2010).

Within this context it is perhaps prudent to view Miller's set of four criteria as a hypothesis that his combination of characters reliably place fossil seed cones within the genus *Pinus*. As conceived by Miller (1976) that hypothesis could be paraphrased as "cones that display all four of the appropriate character states represent the genus *Pinus*, whereas those that display alternative character states for one or more of the four characters are part of the ancestral complex of species that are assignable to *Pityostrobus*" (sometimes including *Obirastrobus*; fig. 6).

There are also two implicit hypotheses embedded within Miller's proposal. These are that (1) the four diagnostic character states of the genus *Pinus* have accumulated through time until all four were present in the common ancestor of a monophyletic *Pinus* and (2) that it is systematically useful to define the genus *Pityostrobus* by the absence of characters rather than by a diagnostic combination of derived characters (i.e., synapomorphies). All four species of *Pinus* included in the current analysis (including *P. yorkshirensis*) are nested within a clade that also includes three species of *Pityostrobus* (i.e., *Pinus* clade; fig. 6), thus forming a monophyletic group. It is interesting to note that the species that Miller excluded from *Pinus* despite having all four of the putatively diagnostic anatomical characters for the genus (Miller 1977), also is nested within that clade (fig. 6). This further emphasizes that Miller's set of anatomical characters for recognizing all species of *Pinus* (and only species of *Pinus*; Miller 1976) are useful but not universally definitive.

The second hypothesis implied by Miller's criteria is that the recognition of stem species (i.e., *Pityostrobus* spp.) by the absence of specific characters is systematically useful (see Smith and Stockey 2001, 2002 for data). Although numerous authors have adopted Miller's methodology, that approach has not helped to resolve the pattern of phylogeny for Pinaceae. Indeed, one could argue that approach has actually retarded progress toward resolution of pinaceous systematics by (1) viewing as a single taxonomic entity an extremely large and heterogeneous assemblage of cones and thereby (2) diverting attention from transformational series of characters and character combinations. As a result, relationships of the various *Pityostrobus* species have been more difficult to understand either with respect to each other or with respect to the crown group genera of the family to which they are hypothesized to have given rise.

Systematic Relationships of P. yorkshirensis

Our inclusion of *P. yorkshirensis* within the genus *Pinus* represents somewhat of a departure from traditional practice with respect to circumscription of species of anatomically preserved fossil pinaceous cones. The results of our phylogenetic analyses (fig. 6) both highlight the unnatural systematics of stem group Pinaceae (i.e., with *Pityostrobus* being polyphletic but with some species being paraphyletic to *Pinus*), and focus attention on those characters that are associated with evolution of *Pinus* seed cones. Results of that analysis support the hypothesized monophylesis for genus *Pinus*, place *P. yorkshirensis* within the *Pinus* clade (fig. 6), and provide a framework for recognizing character changes that are associated with evolution of the genus *Pinus*. In this regard it is important to reiterate that among the three species of *Pityostrobus* that also resolve within that clade, *P. lynnii* was originally described as a species of *Pinus* (Berry 1934). Although a reevaluation is outside the scope of this article, these results imply that *P. lynnii* needs to be reevaluated for possible return to the genus *Pinus* and leaves open the possibility that the other "*Pityostrobus*" species nested within that clade (fig. 6) also could be unrecognized species of *Pinus*.

With respect to broader relationships of *P. yorkshirensis*, the *Pinus* clade is nested within a larger clade of pine-like species, where it is subtended by 10 species of *Pityostrobus* (fig. 6). *Pityostrobus corneti* and *P. hueberi* are sister to each other at the basal node of that clade (fig. 6). They share a cylindrical shape of the scale trace and the thinning of the scale distal to the seeds so that neither apophysis nor an umbo is formed. *Pityostrobus hautrageanus* and *P. macrocephalus* form a clade attached to the stem of the tree one node closer to the *Pinus* clade. Those species have no interseminal ridge on the ovuliferous scale and a terminal rather than a dorsal umbo. The small clade consisting of *Pityostrobus palmeri* + *P. shastaensis* occurs closer to the *Pinus* clade. Those species have no sclerenchyma in the bract and the vascular bundle does not enter the free bract, as it does in all species (where known) of *Pinus* (fig. 6).

Species that are attached to the stem of the tree at the two closest nodes to the *Pinus* clade (fig. 6; i.e., *Pityostrobus andraei* and *P. kayeii*) lack a united bract and scale trace at origin (character 10) and resin canals in wood of the axis (character 6) among the four anatomical characters that are considered to be systematically diagnostic of *Pinus* by many previous authors (Miller 1976). The scale trace in all these species is horseshoe shaped and the bract trace is small and abaxial to the scale trace (Creber 1967; Miller and Robison 1975; Miller 1976).

All the species that nest within the *Pinus* clade in the results of our analysis (fig. 6), display an inflated scale apex and a dorsal umbo and two, three, or all four of the anatomical characters considered to be systematically diagnostic of the genus *Pinus* by previous authors (Miller 1976; Gernandt

et al. 2011). *Pityostrobus jacksonii* and *Pityostrobus milleri* do not have resin canals in the wood, and they also lack bract and scale traces that are united at the level of origin from the cone stele. The configuration of the bract/ovuliferous scale trace at the level of divergence from the cone stele is unknown for *Pinus yorkshirensis*, but the other pertinent characters show the "*Pinus*" character state. *Pityostrobus lynnii*, *Pinus ponderosa*, *Pinus nelsonii*, and *Pinus belgica* display all four of the anatomical characters used by many authors to designate anatomically preserved fossil *Pinus* cones from Cretaceous strata (fig. 6; Miller 1976).

The cone we describe herein as *P. yorkshirensis* is known to display only three of the four putatively diagnostic characters for the genus *Pinus* (Miller 1976). However, that uncertainty is due to incomplete preservation of the fourth character (namely, cone-scale trace diverging as a single unit), not to the presence of an alternative character state. Also, *P. yorkshirensis* has an apophysis with an umbo, resin canals in the wood of the axis, resin canals that diverge to the ovuliferous scale abaxial to the vascular bundle, and adaxially convex vascular bundles in the scale. Those character combinations occur within all living species of *Pinus* for which the characters are known and are found in no other crown group genera of Pinaceae, and they thereby strongly imply that *P. yorkshirensis* belongs to the genus *Pinus*. The dispersed conifer needle fragments that conform to a three- or four-needled variety of *Pinus* and are preserved in close association with the *P. yorkshirensis* cone further support the conclusion that *Pinus* is present in these Lower Cretaceous sediments from which *P. yorkshirensis* was recovered.

Systematic resolution of the pattern of phylogeny for Pinaceae has been materially strengthened by numerical cladistic analysis (Gernandt et al. 2008, 2011; fig. 6). For example, the monophyly of *Pseudoaraucaria* and *Obirastrobus* have been confirmed, and their positions within the Pinaceae have been clarified by recent cladistic analyses (fig. 6; Gernandt et al. 2011), and this has allowed us to recognize the sequence of character transformations leading to the origin of the crown group clades (i.e., modern genera). These same

studies have also confirmed polyphylesis of the genus *Pityostrobus* and have clarified relationships both among various groups of *Pityostrobus* species and between various groups of *Pityostrobus* species and crown group genera (fig. 6). While it is beyond the scope of this study to do so, these relationships will be more easily recognized after the various species of *Pityostrobus* are segregated to discrete genera that are each circumscribed by sets of shared derived characters.

Pinus yorkshirensis is the fourth permineralized pinecone to be described from the Cretaceous but only the second recovered from the Early Cretaceous of Europe. Geologically, dated as latest Hauterivian or earliest Barremian, *P. yorkshirensis* is the oldest known *Pinus* cone described to date. *Pinus belgica*, which comes from the Wealden of Belgium, previously was considered the oldest known fossil pinecone. However, the Wealden appears to be time transgressive, as the Wealden of Belgium is dated as Barremian-Aptian, at least 5 million but perhaps as many as 18 million years younger than the Wealden of Yorkshire, which yields *P. yorkshirensis* (Dejax et al. 2007). Regardless of the exact extension of the stratigraphic range of the genus *Pinus* revealed by the discovery of *P. yorkshirensis*, fossil evidence now demonstrates that the family Pinaceae originated in the Jurassic (Rothwell et al. 2012) and that evolutionary diversification of the genus *Pinus* was well under way by the Early Cretaceous.

Acknowledgments

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