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The oldest Pinus and its preservation by fire
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Abstract:	Pinus (Pinaceae) is a diverse conifer genus that dominates Northern Hemisphere forests today, and is noteworthy for its fire-adapted traits. Here we describe the oldest known fossils attributable to the genus from the Lower Cretaceous (Valanginian, ~133-140 Ma) part of the Chaswood Formation of Nova Scotia, Canada. Pinus mundayi sp. nov. comprises charred long-shoots, which show a constellation of derived characters including (1) axial resin ducts with thin-walled epithelial cells in the secondary xylem and phloem, (2) fenestriform or pinoid cross-field pits, and (3) helically-arranged short-shoots that pass through growth ring boundaries before distally diverging into two separate needle bases. The fossils, which are interpreted as remains of an evergreen 2-needle pine, provide a new constraint on timetrees of Pinaceae evolution. Their preservation as charcoal and the occurrence of resin ducts, which produce flammable terpenes in modern pines, demonstrate that Pinus has co-occurred with fire since its Mesozoic origin.
Response to Reviewers:	

1   # The oldest *Pinus* and its preservation by fire

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5   **ABSTRACT**

6   *Pinus* (Pinaceae) is a diverse conifer genus that dominates Northern Hemisphere

7   forests today, and is noteworthy for its fire-adapted traits. Here we describe the oldest

8   known fossils attributable to the genus from the Lower Cretaceous (Valanginian, ~133–

9   140 Ma) part of the Chaswood Formation of Nova Scotia, Canada. *Pinus mundayi* sp.

10   nov. comprises charred long-shoots, which show a constellation of derived characters

11   including (1) axial resin ducts with thin-walled epithelial cells in the secondary xylem

12   and phloem, (2) fenestriform or pinoid cross-field pits, and (3) helically-arranged short-

13   shoots that pass through growth ring boundaries before distally diverging into two

14   separate needle bases. The fossils, which are interpreted as remains of an evergreen 2-

15   needle pine, provide a new constraint on timetrees of Pinaceae evolution. Their

16   preservation as charcoal and the occurrence of resin ducts, which produce flammable

17   terpenes in modern pines, show that *Pinus* has co-occurred with fire since its Mesozoic

18   origin.

19   **INTRODUCTION**

20   The Pinaceae is the most diverse and widespread family of conifers, comprising

21   11 genera and c. 230 species (Eckenwalder, 2009). It dominates large areas of the

22   Northern Hemisphere, especially in cool temperate and taiga biomes (Farjon, 2010), a

23   biogeographic pattern established in Early Cretaceous time (Peralta-Medina and Falcon-

24   Lang, 2012). A sister taxon to all other crown-group conifers, molecular studies suggest

25   that stem-Pinaceae diverged in Late Triassic time (Lu et al., 2014) with the origin of the

26   most diverse Pinoideae crown-group (*Pinus*, *Picea*, and *Cathaya*; Gernandt et al., 2011)

27   delayed until Late Jurassic time (Lin et al., 2010). However, accurate calibration of such

28   timetrees is limited by the paucity of well-dated fossils, with the first representative of

29   non-pinoid crown-group conifers (Podocarpaceae) known from Late Triassic time, the

30   first stem-Pinaceae known from Late Jurassic time (Rothwell et al., 2012), and the oldest

31   fossils attributable to extant pinoid genera occurring in Early Cretaceous time (Ryberg et

32   al., 2012; Klymiuk and Stockey, 2012).

33   The evolutionary history of *Pinus* has attracted special interest because, not only

34   is it the most diverse (~115 species) genus of Pinaceae (Farjon, 2010), but also it exhibits

35   fire-adapted ecological traits (Keely, 2012). These are restricted to *P.* subgenus *Pinus*, in

36   which there are two end-member fire strategies: (1) *fire-tolerators* rapidly grow to great

37   height, utilize thick bark, and self-prune lower branches, to maximize resilience to low-

38   intensity surface fires; (2) *fire-embracers* store flammable deadwood, to promote lethal

39   crown fires, but deploy fire-dependent serotinous cones, to ensure post-fire cohort-

40   renewal (Keely, 2012). Molecular studies suggest that these adaptations arose in Early

41   Cretaceous time (He et al., 2012) when atmospheric oxygen levels were elevated

42   (Glasspool and Scott, 2010) and fires were common (Brown et al., 2012). However, Early

43   Cretaceous fossil evidence for *Pinus* being part of a fire-prone community is lacking.

44   In this paper, we describe the oldest known fossils referable to *Pinus*, and

45   emphasize their preservation as charcoal, the product of wildfire (McParland et al., 2007).

46 Findings extend the antiquity of this genus, provide a new constraint for molecular

47 timetrees, and demonstrate that *Pinus* has co-occurred with fire since its Mesozoic origin.

48 **GEOLOGICAL CONTEXT**

49   The *Pinus* fossils were obtained from Bailey Quarry, near Windsor, Nova Scotia,

50 Canada (45°01′10′′N; 64°03′31′′W). At this site, deposits of the 'lower member' of the

51 Cretaceous Chaswood Formation unconformably infill hollows in the weathered top of a

52 gypsum unit assigned to the Carboniferous Windsor Group. The Cretaceous beds

53 comprise pebbly sandstone units showing low-angle cross-stratification and trough cross-

54 bedding, interpreted as the deposits of braided rivers that flowed through 'tower karst'

55 topography. These fluvial deposits contain charred mesofossils as concentrated lag

56 deposits and also yield an associated palynoflora of conifers (including many bisaccate

57 forms), ginkgos, bennettites, cycads, ferns and lycopsids (Falcon-Lang et al., 2007).

58   The palynoflora contains *Aequitriradites verrucosus*, *Distaltriangulisporites*

59 *perplexus* and *Trilobosporites canadensis* indicating a Cretaceous (Valanginian–

60 Hauterivian) age compared with North American sections (Burden and Hills, 1989).

61 Based on the Last Appearance Datum of *T. canadensis* that determination can be refined

62 to an early Valanginian age compared to more proximal sites within the Atlantic Rift

63 (Taugourdeau-Lantz, 1988). This placement is consistent with the diversity of

64 *Cicatricosisporites* spore types, which suggests proximity to the Jurassic–Cretaceous

65 boundary (Abbink et al., 2001). A younger age is unlikely because *Plicatella* spores are

66 rare (one specimen found), *Appendicisporites* spores are absent, and angiosperm pollen is

67 also lacking. Younger Barremian palynofloras at this paleolatitude (30 – 40ºN) typically

68 show greater abundance and diversity of *Plicatella* and *Appendicisporites* species

69 (Burden and Hills, 1989) and contain angiosperm pollen (Heimhofer et al., 2005).

70 **METHODS**

71 Bulk samples were digested in 40% HF to release charcoal, and the residue

72 washed in distilled water. Charred conifer axes (up to 20 mm long) were fixed to

73 aluminum stubs using Electrodag 550 (nickel acrylic colloid), coated in 150 Å of gold

74 palladium using an ISI Sputter coater and viewed at 15 kv with a Hitachi S-3500N

75 Scanning Electron Microscope. The exterior of each specimen was imaged before being

76 removed from the stub, dissected with a scalpel under a binocular microscope, and

77 remounted in order to image internal anatomy. Half of each specimen was retained intact,

78 while the other half was fractured and imaged along transverse (TS), radial longitudinal

79 (RLS), and tangential longitudinal (TLS) surfaces to allow direct comparison with

80 illustrations in the literature. When compared with extant conifer material, measurements

81 were corrected to account for charring-induced contraction using published coefficients

82 (Falcon-Lang et al., 2012).

83 **CHARRED *PINUS* MESOFOSSILS**

84 The *Pinus* fossils show features indicative of preservation as charcoal, the product

85 of wildfire, such as homogenization of adjacent tracheid cell walls, elevated reflectance,

86 and fire-cracks (McParland et al., 2007). Fossils are described as a new species.

87 ***Pinus mundayi* sp. nov., Falcon-Lang, Mages, Collinson (Fig. 1)**

88 Diagnosis: Eustelic long shoots with endarch primary xylem patches, axial resin ducts

89 with $6-10$ thin-walled epithelial cells in both the secondary xylem and phloem,

90 fenestriform or pinoid cross-field pits, and helically-arranged short-shoots (3/8

91   phyllotaxic fraction) that pass through growth ring boundaries and distally diverge as two

92   separate needle bases; ray tracheids are absent.

93   Holotype and repository: NSM016GF004.001, Nova Scotia Museum, Halifax, Canada

94   Other material: NSM016GF004.002–004.

95   Locality: Bailey Quarry, near Windsor, Nova Scotia, Canada

96   Stratigraphy and age: Chaswood Formation (Cretaceous, Valanginian)

97   Etymology: epithet in honor of Derek and Mary Munday of Bodowen, Barmouth

98   **Description of Long-Shoots**

99   Long-shoots, 3.6–4.2 mm diameter and <17 mm long, preserve the pith, primary

100   vasculature, secondary xylem, and (locally) phloem (Fig. 1A–B). The stellate pith, 1.15

101   mm diameter (Fig. 1B), is composed of axially-elongate parenchyma, cells 100–120 μm

102   high and 20–25 μm diameter, with profuse pits on all walls. The eustele comprises ~20

103   endarch primary xylem patches (Fig. 1C), composed of scalariform tracheids, ~10 μm

104   diameter. The secondary xylem, 1.2–1.4 mm in radius, comprises one or two rings of

105   growth, each ring being composed of thin-walled earlywood tracheids, 10–25 μm

106   diameter, that pass centripetally into thick-walled latewood, 10–15 μm diameter.

107   Earlywood tracheids show 1–2-seriate, circular, bordered pits, 10–15 μm, with circular

108   apertures (Fig. 1M), and opposite arrangement where biseriate. Latewood tracheids lack

109   pits or show sparse, circular bordered pits only. Rays are uniseriate, 1–11 cells high (Fig.

110   1K), and lack ray tracheids. Cross-fields of ray parenchyma show 1–4 fenestriform or

111   pinoid pits (Fig. 1N). A few scattered axial parenchyma strands also occur close to axial

112   resin ducts. A thin layer of secondary phloem, <350 μm radius (Fig. 1I), locally adheres

113   to the outermost part of the shoot; periderm is not preserved.

114 **Description of Short-Shoot Bases**

115   Prominent short-shoot bases, 0.7–1.1 mm diameter, are helically arranged on the

116 preserved exterior of the long-shoot with a 3/8 phyllotaxic fraction (Fig. 1A). In cross-

117 section, short-shoot bases are oval in the proximal part of their course, comprising a pith

118 and a concentric xylem cylinder (Fig. 1L). More distally, they diverge into two separate

119 kidney-shaped bundles of secondary xylem that represent needle bases (Fig. 1H). In more

120 mature specimens (NSM016GF004.004), traced from the pith, short-shoots pass through

121 one or two subtle growth ring boundaries in the secondary xylem (Fig. 1D).

122 **Description of Resin Ducts**

123   Resin ducts occur within the secondary xylem and phloem of long-shoots. In the

124 secondary xylem, axial resin ducts, 60–90 µm diameter, locally tylose-filled, and

125 surrounded by 6–10 epithelial cells, are common in the earlywood (Fig. 1C, E) but reduce

126 in frequency and size (30 µm diameter) toward the latewood. Radial resin ducts, 40–50

127 µm diameter, with 6–8 epithelial cells (Fig. 1J) are present in some fusiform rays with a

128 density of 2.6 per mm^2 (based on limited observations of small areas). In the phloem,

129 axial resin ducts, 40 µm diameter, occur (Fig. 1I). Resin ducts also occur within short-

130 shoot bases, where typically 1–4 axial ducts, 40–110 µm diameter, with 6–9 epithelial

131 cells, occur (Fig. 1L). In all cases, epithelial cells are thin-walled, a characteristic that can

132 be ascertained by comparing half the double-wall width of fused tracheid cell walls with

133 those of epithelial cells (Fig. 1F). Epithelial cell walls lack pits except where in contact

134 with vasicentric axial parenchyma.

135 **Diagnostic Characters of *Pinus* Seen in Fossils**

136   A noteworthy character of the long-shoots is the presence of resin ducts (Fig. 1C).

137   Where axial ducts occur in secondary xylem, wood anatomists distinguish

138   schizogenously formed 'normal' ducts (tubular, and generally solitary) from 'traumatic'

139   ducts (cyst-like and typically in tangential series) triggered by environmental shock (Lin

140   et al., 2002). Although traumatic ducts occur in the secondary xylem of several of the

141   eight families of the Pinales, especially in Cupressaceae and Pinaceae (Cleary and

142   Holmes, 2011), normal axial ducts, as seen in the Bailey Quarry long-shoots, are

143   confined to five genera of Pinaceae: *Pinus*, *Picea*, *Cathaya*, *Larix*, and *Pseudotsuga* (Wu

144   and Hu 1997). Normal ducts occasionally occur in the secondary xylem of *Nothotsuga*

145   (Lin et al., 1995) and *Keteeleria* (Lin et al., 2000), but they are absent from juvenile

146   shoots so those genera are unlike the fossils.

147   The wall thickness of epithelial cells that surround 'normal' axial ducts in the

148   secondary xylem (Fig. 1F) is a further key feature for fossil diagnosis (Wu and Hu,

149   1997). *Pinus* shows thin-walled (unlignified) epithelial cells that are thinner than the

150   walls of adjacent tracheids, whereas the other Pinaceae genera have thick-walled

151   (lignified) epithelial cells similar to tracheids (Lin et al., 2002). This distinction is not

152   completely clear-cut because 9% of the ~115 extant *Pinus* species have thick-walled

153   epithelial cells (Esteban et al., 2004) and some juvenile *Picea* shoots show of a mixture

154   of thin- and thick-walled epithelial cells (Lin et al., 2002). Applying this criterion, the

155   long-shoots from Bailey Quarry, which show exclusively thin-walled epithelial cells, are

156   probably *Pinus*, although referral to *Picea* cannot be completely excluded based on these

157   characters alone because the fossil material is juvenile.

158   However, other anatomical characters collectively confirm placement of the

159   fossils in *Pinus* and rule out other possible taxa with resin ducts as follows:

160   (1) Axial resin ducts occur in the secondary phloem (Fig. 1I), a feature that is never seen

161   in *Cathaya*, *Picea* or *Pseudotsuga* but is typical of *Larix* and *Pinus* (Lin et al., 2002);

162   (2) Axial resin duct diameter is always >60 µm (after correction; Fig. 1E), a feature

163   characteristic of almost all *Pinus* species, but absent elsewhere in Pinaceae (Esteban

164   et al., 2004);

165   (3) Radial duct density is ~1.3 per mm^2 (after correction), a value that is within the

166   normal range for *Pinus* (0.5 – 2), but uncharacteristic of *Cathaya* (0.7), *Larix* (0.15 –

167   0.7), *Picea* (0.3 – 0.4), or *Pseudotsuga* (0.15 – 0.2) (Lin et al., 2002);

168   (4) Cross-field pits are fenestriform or pinoid (Fig. 1N), typical of *Pinus* but

169   uncharacteristic of the piceoid pits of *Pseudotsuga*, *Larix*, *Picea*, and *Cathaya*

170   (Esteban et al., 2004) even when distortion due to charring is taken into account

171   (Gerards et al. 2007); and

172   (5) Divergent secondary xylem bundles in the more distal course of short-shoots suggest

173   they bore two needles per fascicule (Fig. 1H; Dörken et al., 2010), a feature that is

174   strongly suggestive of *Pinus* because other Pinaceae (with the exception of *Larix*)

175   bear single leaves directly attached to main shoots (Farjon, 2010).

176   **DISCUSSION**

177   Based on anatomical analysis, *Pinus mundayi* sp. nov., reported here, is the oldest

178   known representative of the genus (~133–140 Ma; Valanginian). *Pinus yorkshirensis*, the

179   former oldest recognizable member of the genus, is based on material of uncertain

180   provenance, but palynology of attached sediment indicates an origin in rocks close to the

181   Hauterivian–Barremian (~129 Ma) boundary (Ryberg et al., 2012). The new fossils

182   therefore extend the record of *Pinus* by 4–11 myrs, meaning that it is of similar antiquity

183   to the oldest known *Picea*, which is also of Valanginian age (Klymiuk and Stockey,

184   2012). Although pinaceous cones of *Pityostrobus*-type are abundant from Barremian time

185   onward (~129 Ma), their systematic position within the Pinaceae is poorly resolved

186   (Rothwell et al., 2012). The new fossils are therefore only one of three pre-Albian

187   occurrences identifiable to an extant genus of Pinaceae, and improve calibration of

188   molecular timetrees of the family (Lin et al., 2010). In addition, the occurrence of two

189   needles per fascicle suggests an affinity with *Pinus* subgenus *Pinus* (Farjon, 2010) and

190   implies that subgeneric diversification may have occurred earlier than previously thought

191   (Gernandt et al., 2011). Further, based on its unusual short-shoot/long-shoot syndrome, it

192   has been hypothesized that ancestral *Pinus* was deciduous (Dörken et al., 2010);

193   however, the passage of short-shoots through > 2 growth rings demonstrates that the

194   fossil short-shoots were evergreen as seen in extant species.

195   The preservation of long-shoots as charcoal, also, may be significant. Fire-

196   adaptive traits are widespread within *Pinus*, and especially in *P.* subgenus *Pinus* (Keely,

197   2012), an ecology that is hypothesised to have originated in Early Cretaceous time based

198   on molecular clock estimates (He et al., 2012). However, testing of this hypothesis is

199   hampered by the fact that neither *Pinus* nor securely identified Pinaceae have hitherto

200   been identified in Cretaceous charcoal assemblages (Brown et al., 2012). While our

201   charred long-shoot fossils show, for the first time, that *Pinus* burned in Early Cretaceous

202   fires, and probably, crown fires, this does not prove fire-adaption as all plants

203   occasionally burn. Crown fires are characteristic of fire-embracer pines (Keely, 2000),

204 but the fossils lack evidence, such as serotinous cones, to demonstrate adaption. The only

205 fossil feature that is noteworthy is the occurrence of axial resin ducts. In extant *Pinus*,

206 these ducts secrete terpene, a highly flammable, unsaturated hydrocarbon that contributes

207 to fire ecology (He et al., 2012). Therefore, the occurrence of resin ducts and preservation

208 as charcoal in *P. mundayi* are consistent with He et al. (2012)'s hypothesis that fire-

209 adaptive traits of *Pinus* originated in Early Cretaceous time, but do not provide

210 confirmation.

211 **ACKNOWLEDGMENTS**

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302

303 FIGURE CAPTIONS

304

305 Figure 1. *Pinus mundayi* (all images of holotype, NSM016GF004.001, except D,

306 NSM016GF004.004) from the Cretaceous (Valanginian) of Nova Scotia, Canada. A.,

307 Lateral view of long-shoot showing helically arranged short-shoot bases (ss), and

308 prominent fire cracks (fc); scale: 2 mm. B., Cross-section of long-shoot showing stellate

309 pith (pi), short-shoot traces (sst), secondary xylem (sx) and fire-cracks (fc); TS, scale:

310 400 µm. C., Detail from (B) showing pith (pi), endarch primary xylem patches (arrows),

311 short shoot traces (sst), and axial resin ducts (ard, arrow); TS, scale: 150 µm. D., Growth

312 ring boundary (grb) in secondary xylem (sx); TS, scale: 250 µm. E., Cross section of an

313 axial resin duct (ard) and its epithelial cells (ec) in secondary xylem (sx); TS, scale: 30

314 µm. F., Detail from (D) showing thin-walled epithelial cells (ec) of an axial resin duct

315 (ard). Adjacent cells include thin-walled parenchyma (p) and thick-walled tracheids (tr)

316 for comparison, also note absence of wall layering in tracheid cell walls, a characteristic

317   of charcoal; TS, scale: 15 μm. G., Longitudinal section of axial resin duct (ard) in

318   secondary xylem (sx); RLS, scale: 100 μm. H., Cross-section through distal part of short

319   shoot base showing diverging secondary xylem bundles (sx) of two needle bases, and

320   fire-cracks (fc); TS, scale: 250 μm. I., Outer edge of secondary xylem (sx) and phloem

321   (ph) containing an axial resin duct (ard, arrow); TS, scale: 75 μm. J., Radial resin duct

322   (rrd) with epithelial cells (ec) within fusiform ray (fr); epithelial cells are thin-walled but

323   coated with a volatile residue; oblique TLS, scale: 25 μm. K., Short, uniseriate rays in the

324   secondary xylem close to the pith; TLS, scale: 75 μm. L., Cross-section through proximal

325   part of short shoot base showing pith (pi) and concentric secondary xylem (sx) cylinder

326   with axial resin ducts (ard, arrow); TS, scale: 250 μm. M., Circular, uniseriate, bordered

327   pits in secondary xylem; RLS, scale: 25 μm. N., Fenestriform to pinoid cross-field pitting

328   in secondary xylem; RLS, scale: 50 μm.

329

330   [1]GSA Data Repository item 2016xxx, xxxxxxxx (Phylogeny teaching slide and

331   justification of new species), is available online at www.geosociety.org/pubs/ft2015.htm,

332   or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box
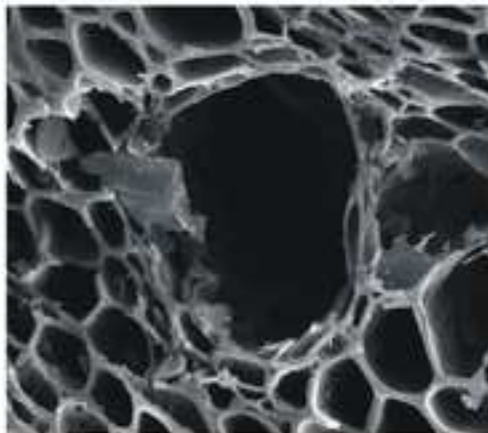
333   9140, Boulder, CO 80301, USA.
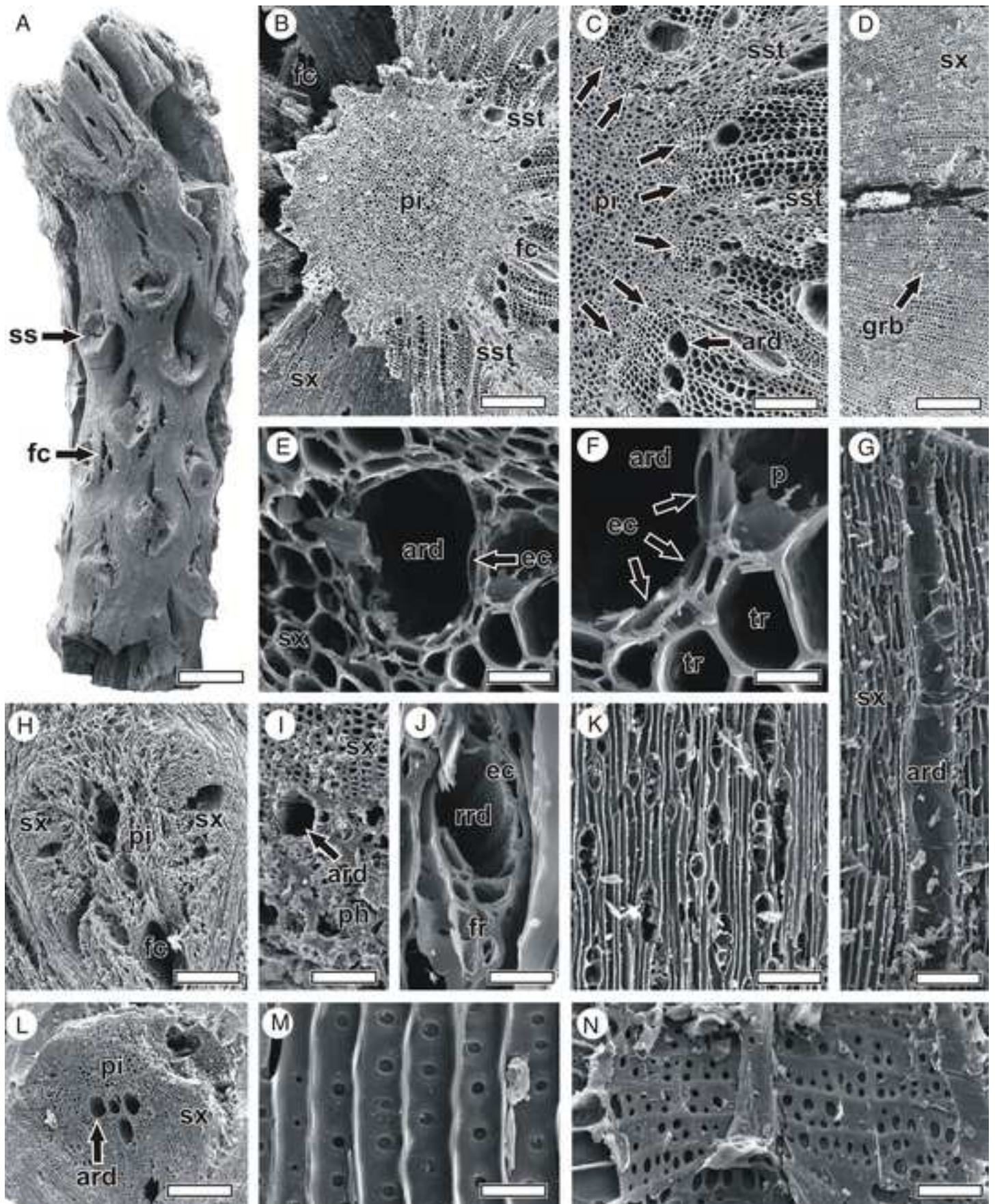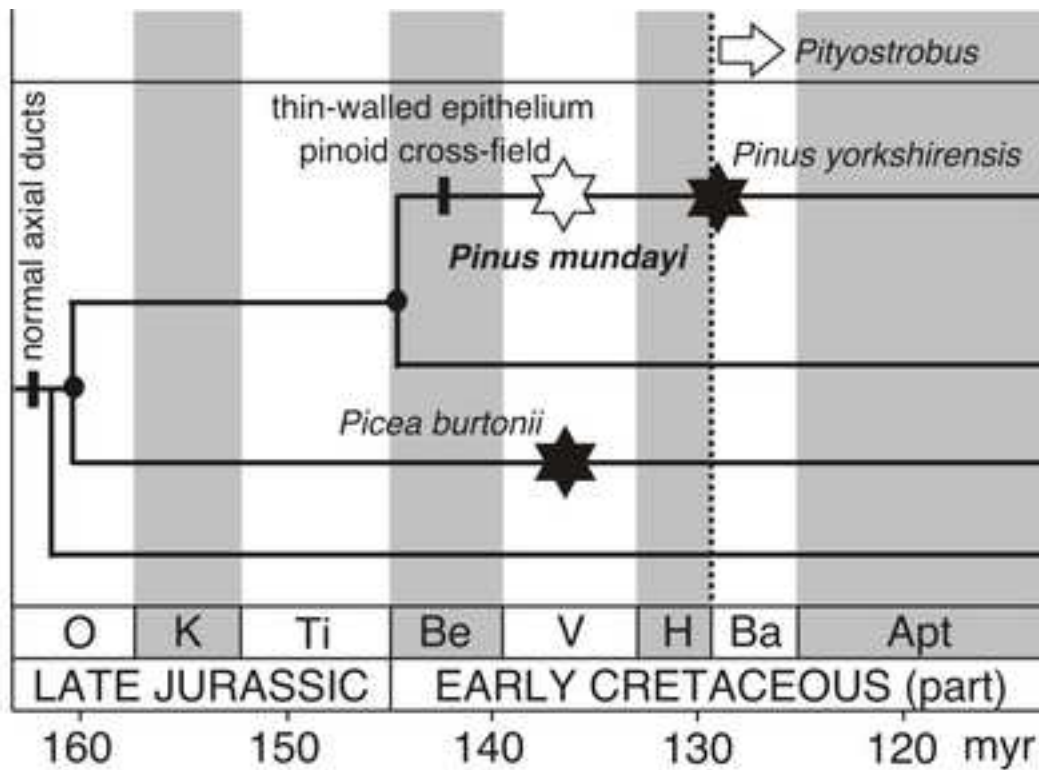
Figure 1

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The oldest *Pinus*

axial resin ducts with thin-walled epithelial cells

short-shoots that separate into two needle bases
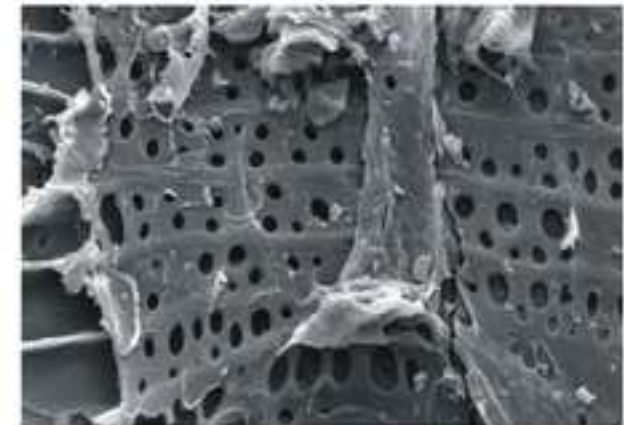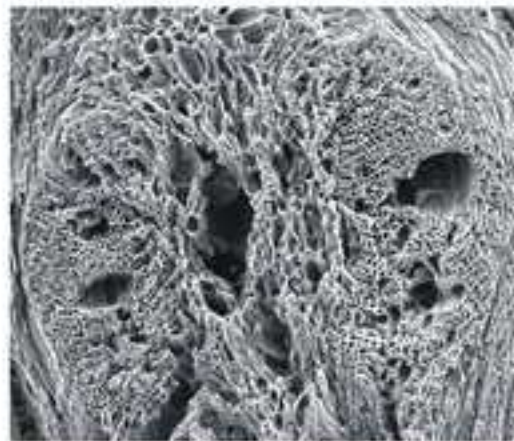
pinoid and fenestriform cross-field pits

Falcon-Lang et al. 2016. The oldest Pinus and its preservation by fire. Geology, v. 44, p. xxx-xxx.

Supplementary discussion: Justification for a new species

Plant fossils are usually preserved in a disarticulated state, and new taxa are described based on individual organs; there are very few taxa reconstructed as 'whole-plants'. The new *Pinus* fossils described here comprise remains of partial twigs, with short shoot bases, lacking fertile remains. We note that there is a paucity of anatomically-preserved pinaceous twigs in the Cretaeous fossil record. Prior to this paper, the oldest described twig material is from the Cretaceous (Cenomanian) of the Czech Republic, some 40 million years younger than our new *Pinus* fossils; however, that material is assigned to family level only, and all other Cretaceous fossils that are similar to modern pines are based on secondary xylem only, and therefore assigned to genera such as *Pinuxylon* (Peralta-Medina and Falcon-Lang, 2012) rather than *Pinus sensu stricto*. Based on the antiquity, rarity, good preservation, and genus-level identification of the new material, it is advantageous to apply a binomial name, to allow its discussion in the literature.

As the material is assignable to the extant genus, *Pinus*, it is also necessary to consider how the fossil differs from the c. 115 extant species. *Pinus* is subdivided into two subgenera, *Pinus* Pinus and *Pinus* Strobus (Farjon, 2010). *Pinus* subgenus Strobus shows, typically, five needles per fascicle, whereas *Pinus* subgenus Pinus, shows, variably, 1 to 8 needles per fascicle (Farjon 2010). Our new *Pinus* fossils consistently show two needles per fascicle suggesting an affinity with *Pinus* subgenus Pinus (Farjon, 2010), and implying that the two subgenera diverged earlier than previously thought (Gernandt et al., 2011).

In *Pinus* subgenus Pinus, the number of needles per fascicle is species-specific in the range of (1) 2 – 5 (8) needles per fascicle (Farjon 2010). Two needles per fascicle is a stable characteristic of the 19 species of so-called 'Old World Pines' only: *P. densata, P. densiflora, P. hawnshanensis, P. kesiya, P. latteri, P. luchuensis, P. massoniana, P. merkusii, P. mugo, P. nigra, P. resinosa, P. sylvestris, P. tabuliformis, P. taiwanensis, P. thunbergii, P. tropicalis, P. uncinata, and P. yannanensis* (Farjon, 2010). A few additional species may show two needles per fascicle but in these taxa, the number of needles per fascicle is highly variable (1-5), unlike the stable two needle state observed in our material (Farjon, 2010).

Another important character state of the new *Pinus* fossils is their 3/8 phyllotaxic fraction. The most common phyllotaxic fractions in *Pinus* subgenus Pinus approximate to 1/3, 2/5, 3/8, and 5/13 (Farjon, 2010). Of the classic 'Old World Pines' that show two needles per fascicle, the most common fractions are 2/5 and 5/13 (Farjon, 2010). Only *P. sylvestris* and *P. nigra* has a phyllotaxic fraction that commonly and closely approximates to 3/8 (i.e., 135°) (Farjon, 2010). According to the analysis of the wood of 352 conifer species compiled by Esteban et al. (2004), the new fossil *Pinus* differs from *P. sylvestris* and *P. nigra*, and most other 'Old World Pines' based on the absence of alternate ray tracheids. Therefore, the fossil material can justifiably be treated as a new species.