Cunninghamia beardii sp. nov. (Cupressaceae: Cunninghamioideae), Anatomically Preserved Pollen Cones from the Eocene of Vancouver Island, British Columbia, Canada

CUNNINGHAMIA BEARDII SP. NOV. (CUPRESSACEAE: CUNNINGHAMIOIDEAE), ANATOMICALLY PRESERVED POLLEN CONES FROM THE EOCENE OF VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA

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Premise of research. A large pollen cone cluster attached to a cunninghamioid twig and surrounded by leaves has been identified from Eocene calcium carbonate marine concretions from the Appian Way locality on Vancouver Island, British Columbia, Canada. The cluster preserves 18 cones but probably bore at least 24 pollen cones based on cone placement in the cluster.

Methodology. Specimens were studied using the cellulose acetate peel technique, and reconstructions were made with ImageJ visualization software. Pollen was examined using SEM.

Pivotal results. Cones are helically arranged around the tip of an ultimate leafy branch that terminates in scale leaves, each showing a central resin canal. Vegetative leaves on the twig are amphistomatic, showing typical cunninghamioid anatomy, with a large central resin canal abaxial to the vascular bundle, an elongate zone of transfusion tissue, a nonplicate mesophyll, and a hypodermis three to four cells thick. Each pollen cone is produced in the axil of a bract and has three scale leaves surrounding the base of the cone axis. Individual pollen cones have helically arranged microsporophylls, each with three elongate abaxial pollen sacs. While the cones are immature, pollen sacs with pollen are present in several cones. The exine is scabrate, with numerous orbicules, and no papilla is evident.

Conclusions. This cluster provides the first detailed anatomically preserved fossil evidence for the pollen cones of *Cunninghamia*. It shows a large number of similarities to the pollen cone clusters of extant *Cunninghamia lanceolata* and *Cunninghamia konishii* and the Late Cretaceous *Cunninghamia taylorii*, strengthening hypotheses for a basal position of cunninghamioids within the Cupressaceae and further demonstrating that some characters of *Cunninghamia* have remained relatively unchanged since at least the mid-Cretaceous.

Keywords: conifers, *Cunninghamia*, Cupressaceae, Eocene, fossil, pollen cones.

Introduction

Cupressaceae Gray has one of the longest and most species-rich fossil records of all conifer families (Stockey et al. 2005; Escapa et al. 2008; Spencer et al. 2015). Based on seed cone structure of both living and extinct species, the family also shows the widest range of morphologies and the most well-documented transformational series from which to infer familial-level evolution of all conifers (Schulz and Stützel 2007; Rothwell et al. 2011; Atkinson et al. 2014a, 2014b). Cupressaceae consists of a morphologically diverse basal taxodioid grade with worldwide distribution through time, which consists of subfamilies Cunning-

hamioideae, Taiwanioideae, Athrotaxoideae, Sequoioideae, and Taxodioideae as well as a cupressoid clade of two terminal clades including Northern Hemisphere Cupressoideae and Southern Hemisphere Callitrideae (Farjon 2005; Leslie et al. 2012; Mao et al. 2012). The most ancient Cupressaceae are either assignable to or extremely similar to the Cunninghamioideae (Stockey et al. 2005; Escapa et al. 2008; Spencer et al. 2015), and the fossil record for that clade is extremely rich throughout the Cretaceous (Atkinson 2014b).

Although the genus *Cunninghamia* R. Br. ex Rich. does not appear in the fossil record until the Late Cretaceous (Brink et al. 2009; Serbet et al. 2013), it is the only surviving representative of the earliest diverging lineage of Cupressaceae, subfamily Cunninghamioideae, and thereby has proven to be a crucial genus for understanding the evolution of the family (Stockey et al. 2005; Brink et al. 2009; Serbet et al. 2013). The earliest records of *Cunninghamia* are of permineralized leafy shoots and a whole plant from the Late Cretaceous: *Cunninghamia hornbyensis* Brink, Stockey, Beard & Wehr (2009)

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and *Cunninghamia taylorii* Serbet, Bomfleur, & Rothwell (2013), respectively. The genus *Cunninghamia* was widespread in the Cenozoic in North America, Europe, and Asia (Miki 1941; Matsuo 1954, 1966, 1970; Lakhanpal 1958; Szafer 1958; Tanai and Onoe 1961; Givulescu 1972; Kimura and Horiuchi 1978; Meng et al. 1988; Walther 1989; Dillhoff et al. 2005, 2013; Dolezych and Schneider 2007; Du et al. 2012; Yabe and Yamakawa 2012) and appears to have had a Northern Hemisphere distribution throughout the past.

Typically, studies of the evolution of *Cunninghamia* and cunninghamioid plants have focused on seed cones and vegetative morphology and anatomy (Brink et al. 2009; Serbet et al. 2013; Klymiuk et al. 2015). Although pollen cones of *Cunninghamia* display a wealth of systematically informative characters, well-preserved pollen cones are far less common in the fossil record. Therefore, up to the present, they have played a far less important role in resolving the evolution and phylogeny of Cupressaceae.

In this article, we describe *Cunninghamia beardii* sp. nov., an Eocene fossil conifer shoot terminating in large numbers of scale leaves that surround a cluster of at least 18 helically arranged pollen cones. Cones were preserved at various stages of immaturity at the time of preservation in the calcium carbonate marine nodule but show helically attached microsporophylls and pollen sacs containing nonsaccate scabrate pollen. These pollen cones contribute additional evidence that some characters of *Cunninghamia* species may have remained relatively unchanged for at least 70 Myr (Serbet et al. 2013).

Material and Methods

A single twig with an attached pollen cone cluster was collected from the Appian Way locality (lat. 49°54′42″N, long. 125°10′40″W; UTM 10U CA 5531083N, 343646E) on the east coast of Vancouver Island, British Columbia, on the northern periphery of the Tertiary Georgia Basin (Mustard and Rouse 1994). Permineralized fossil plants, gastropods, echinoderms, and bivalves are found in calcareous concretions embedded in a silty mudstone matrix representing a shallow marine environment. Molluscs, decapods (Schweitzer et al. 2003), and shark teeth indicate that the concretions are of Eocene age (Haggart et al. 1997; Cockburn and Haggart 2007). Sweet (2005) studied the pollen from the site and found both late Paleocene and early Eocene signatures. Stratigraphy of the area is currently being examined (J. W. Haggart, personal communication). The Appian Way beds unconformably overlie rocks of the late Cretaceous Nanaimo Group strata in the area and are estimated to be ca. 50 Ma (Mindell et al. 2014).

Plants, including abraded wood and fruits representing numerous taxa, are well preserved in the concretions (Mindell 2008; Mindell et al. 2014). Juglandaceae fruits (Elliott et al. 2006), cupules and nuts of Fagaceae (Mindell et al. 2007*a*, 2009), endocarps of Icacinaceae (Rankin et al. 2008), inflorescences of Platanaceae (Mindell et al. 2006*a*), flowers of Lauraceae (Atkinson et al. 2015), schizaeaceous (Trivett et al. 2006) and gleicheniaceous (Mindell et al. 2006*b*) fern remains, leafy liverworts (Steenbock et al. 2011), and shelf and cup fungi (Smith et al. 2004; Mindell et al. 2007*b*) have already been described from the locality. Terminal, solitary, globose, taxodi-

aceous pollen cones have been described from Appian Way as *Homalcoia littoralis* Hernandez-Castillo, Stockey & Beard (2005). However, much of the flora still remains to be described (Mindell et al. 2014).

Concretions were cut and peeled using the cellulose acetate peel technique (Joy et al. 1956). Microscope slides were made using Eukitt (O. Kindler, Freiberg, Germany) mounting medium. Images were captured using a Better Light digital scanning camera (Placerville, CA) and processed using Photoshop (Adobe, San Jose, CA). Reconstructions were made using Pixelmator 2.0 (Vilnius, Lithuania), Photoshop, and ImageJ visualization software (Rasband 1997–2014).

Pollen was either examined on inverted peels or removed from cellulose acetate peels for SEM using a modified Daghlian and Taylor (1979) technique under vacuum on a Millipore filter (Bedford, MA). Stubs were coated with 100 A° Au on a Nanotek sputter-coater and examined using a JEOL 6301F SEM at 5 kV.

Pollen cone–bearing branches of *Cunninghamia lanceolata* were obtained from a tree in Corvallis, Oregon (Ninth and Buchanan Streets) in February and April 2015 and dissected, and one section was stained with saffranin O to increase visibility of pollen cones and scale leaves on the shoot tip. All fossil specimens and microscope slides are housed in the Royal British Columbia Museum, Victoria.

Results

Systematics

Order—Coniferales

Family—Cupressaceae

Subfamily—Cunninghamioideae

Genus—Cunninghamia *R. Br. ex Rich.*

Species—Cunninghamia beardii *Buczkowski, Stockey, Atkinson et Rothwell sp. nov. (Figs. 1–4, 6A)*

Species diagnosis. Cluster of at least 18 pollen cones, subterminal on leafy branch, helically arranged. Ultimate leafy branch terminating in scale leaves, significantly increasing in diameter where pollen cones attached. Leafy twig with parenchymatous pith surrounded by cylinder of secondary xylem, parenchymatous cortex with numerous resin canals, hypodermis of thick-walled cells. Vegetative leaves amphistomatic, with large central resin canal abaxial to vascular bundle, elongate zone of transfusion tissue, nonplicate mesophyll, hypodermis three to four cells thick. Pollen cones at least 2 mm in diameter, produced in axil of bract; three scale leaves surrounding base of cone axis. Microsporophylls helically arranged. Pollen sacs abaxial, three per microsporophyll, elongate. Pollen subspheroidal; exine scabrate, orbiculate, no papilla evident.

Holotype hic designatus. *Cunninghamia beardii* Buczkowski, Stockey, Atkinson et Rothwell sp. nov. AW 358 A bot, B_1 top, B_1 bot.

Repository. Royal British Columbia Museum, Victoria, British Columbia, Canada.

Type locality. Appian Way (lat. 49°54′42″N, long. 125°10′40″W; UTM 10U CA 5531083N, 343646E), Vancouver Island, British Columbia, Canada.

Age. Eocene.

Etymology. The species is named in honor of Graham Beard (Vancouver Island Paleontological Museum, Qualicum Beach, British Columbia, Canada), who has collected and prepared large numbers of fossil plant specimens, particularly from the Appian Way locality, and generously provided the plants for study.

Description. A single large pollen cone cluster has been identified in the calcium carbonate concretion from Appian Way (fig. 1*A*). The cone is present on one face, while the leafy branch to which it was attached appears on the other side of the saw cut and on the opposite face of the adjacent slab. Cone axes of 18 individual pollen cones have been mapped on the first peel of the sequence (figs. 1*A*, 6*A*). Based on symmetry of the cone cluster, however, we estimate that approximately 24 pollen cones were produced in the cluster. There are numerous terminal scale leaves at the center of the pollen cone cluster, each with a centrally located resin canal (figs. 1*A*, 2*A*). This indicates that the pollen cones are actually subterminal on the twig.

Each pollen cone of the cluster is subtended by a large bract (fig. 1*A*, 1*B*) at least 3 mm wide and 0.2 mm thick that has a central resin canal and an abaxial hypodermis about three cells thick. Three helically arranged scale leaves surround the base of each pollen cone axis (figs. 1*B*, arrowheads, 6*C*), each containing a single centrally located resin canal, and in some sections, three resin canals, one large central and two lateral smaller, appear. The outermost scale leaf is 1.8–2.0 mm wide, the second is 1.1–1.5 mm wide, and the innermost scale leaf is 1.1–1.25 mm wide (fig. 1*B*). Preservation of these scale leaves is inadequate to enable a detailed description of their internal tissues. The bract and scale leaves almost completely surround (figs. 1*B*, 6*C*) and enclose the apex of the smallest cones, indicating that such cones were immature at the time of preservation.

Pollen cones are variable in size and apparently at different stages of maturity. The largest are near the periphery of the cluster (2 mm wide), while the smallest (0.9 mm) and most immature are near the center (fig. 1*A*, 1*B*). Cone axes are not well preserved, but a ring of 14–16 resin canals can be seen in the cortex of some of the cone axes (fig. 1*B*, 1*C*). Microsporophylls helically arranged around the cone axis are 0.3 mm long with a lamina about 0.3 mm wide (figs. 1*C*, 6*C*–6*E*). Each sporophyll extends toward the periphery of the cone as a terete stalk, turns distally, and expands into the sporophyll lamina (figs. 1*C*, 6*D*). There is a centrally located resin canal in each of the microsporophylls (figs. 1*C*, 2*B*). Pollen sacs are elongate, 0.7–1.0 mm long, 0.24–0.34 mm wide (fig. 2*B*, 2*C*), and abaxially attached to the sporophyll at the base of the upturned lamina (fig. 2*B*, 2*C*). Two of the pollen sacs are situated slightly above the third, which is more abaxial (fig. 2*B*, 2*C*). Pollen sac walls have dark-colored contents and are incompletely preserved (fig. 2*B*–2*D*).

Pollen in all cones is immature, 24–27 μm in diameter (mean = 26 μm, $n = 10$), compressed together, and closely packed (figs. 2*D*, 3*A*, 3*B*). Grains are folded, and apertures have not been identified. As is characteristic of many taxodioid-grade Cupressaceae, the exine consists of a relatively uniform nexine (fig. 3*B*, 3*C*, *bottom*) and a scabrate sexine with numerous orbicules (fig. 3*C*, *top*). It is possible that grains are in a late tetrad stage, but this could not be determined with certainty.

The leafy branch that bears the pollen cone cluster (fig. 4*A*) has a diameter of 4 mm and swells and increases in size to a diameter of 7 mm just below the pollen cone cluster (fig. 4*B*). The pith of the stem is about 1 mm wide proximally (fig. 4*A*) and increases to 2.45 mm wide near the pollen cone cluster (fig. 4*B*). The pith and cortex are parenchymatous and have scattered cells with amber contents, some of which appear to be sclereids (fig. 4*C*). The pith is surrounded by a cylinder of radially aligned secondary xylem tracheids, five to six cells wide, that is interrupted by pith rays (fig. 4*A*–4*C*). The cortex contains a ring of numerous resin canals with an epithelial lining that enter the bases of vegetative leaves (fig. 4*A*–4*E*). The stem periphery shows a narrow sclerotic hypodermis that extends into the leaf bases.

The cross section of the stem nearest the pollen cone cluster shows a larger number of attached leaves (fig. 4*B*). Leaves are amphistomatic and 2.0–2.4 mm wide at the base and contain a single vascular bundle with an abaxial resin canal and an elongate zone of transfusion tissue (fig. 4*D*–4*F*). Transfusion tracheids are short and have circular-bordered pits (fig. 4*F*). The mesophyll is nonplicate, and the hypodermis is three to four cells thick (fig. 4*D*, 4*E*).

Extant *Cunninghamia lanceolata*

Collections of branches bearing pollen cones of *C. lanceolata* show that pollen cones can remain on the tree for at least 4 yr (fig. 5*A*). When the cone clusters first mature, they appear to be terminal. However, there are terminal vegetative leaf primordia at the center (i.e., apex) of each pollen cone cluster (fig. 6*B*) that grow out during the next year to produce additional leafy branches with subterminal pollen cones (fig. 5*A*). Pollen cones are tightly packed in clusters when they are immature (fig. 5*B*, 5*C*), with cones elongating and separating from the cluster at maturity (fig. 5*D*). They are helically arranged around a vegetative stem with a developmental gradation, the older cones occurring farthest from the center (i.e., being most basal; figs. 5*B*, 5*C*, 6*B*). Immature cones in clusters at a comparable stage to those seen in the Appian Way fossil (fig. 5*B*, 5*C*) are 2–5 mm in diameter (mean = 2.57, $n = 60$). The immature cones are 3–9 mm long (mean = 4.63, $n = 60$). Mature pollen cones are 8–22 mm long (mean = 13.4, $n = 60$). Each cone is subtended by an elongate bract (fig. 5*C*, 5*E*); three smaller scale-like leaves occur at the base of the cone axis (fig. 5*E*). Cones are borne on the axis subterminally, and the apical portion of the stem bears numerous helically arranged scale leaves (fig. 6*B*), as in the Appian Way cone (fig. 6*A*). These axes routinely grow out in the subsequent year, producing new vegetative shoot growth and then terminating in a new subapical pollen cone cluster (fig. 5*A*). There are two to four pollen sacs per microsporophyll, with most sporophylls having three elongate pollen sacs on their abaxial surfaces (mean = 2.91, $n = 100$), that are attached near the base of the sporophyll lamina. Two
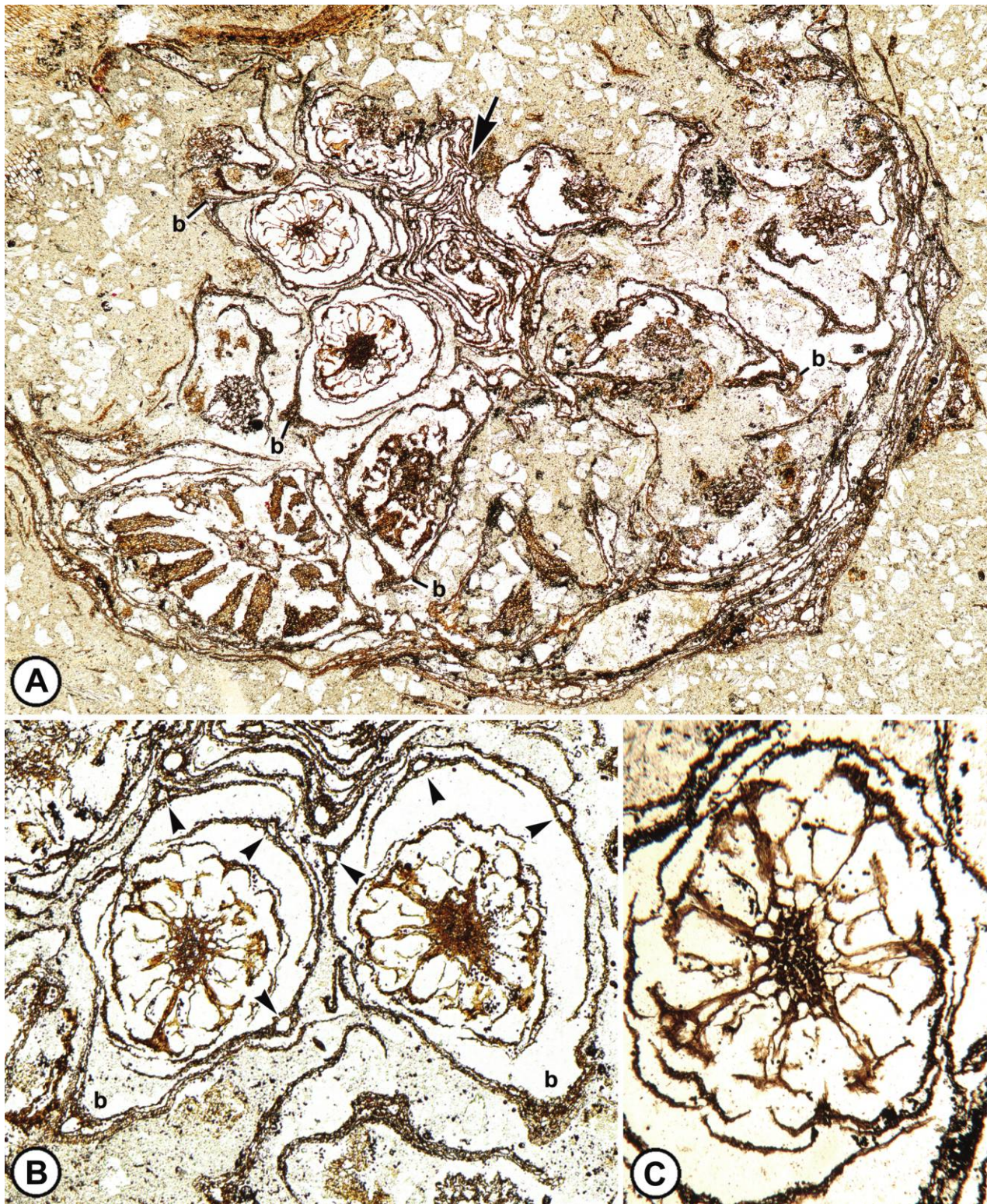
Fig. 1 *Cunninghamia beardii* sp. nov. Holotype, AW 358. *A*, Pollen cone cluster with subtending leaves at periphery, immature leaves at center (arrow), and pollen cones between. Note each pollen cone in axil of bract (b) and that basalmost pollen cones (near periphery) are larger and have more mature pollen sacs than cones closer to center (i.e., more apical). A bot #8 ×19. *B*, Two immature pollen cones, each in the axil of bract and with three scalelike leaves (arrowheads). Note helically arranged sporophylls with narrow stalk and distal lamina. A bot #6 ×43. *C*, Enlargement of immature pollen cone showing resin canals in cortex of axis and single resin canal in stalk and distal lamina of each sporophyll. A bot #16 ×100.
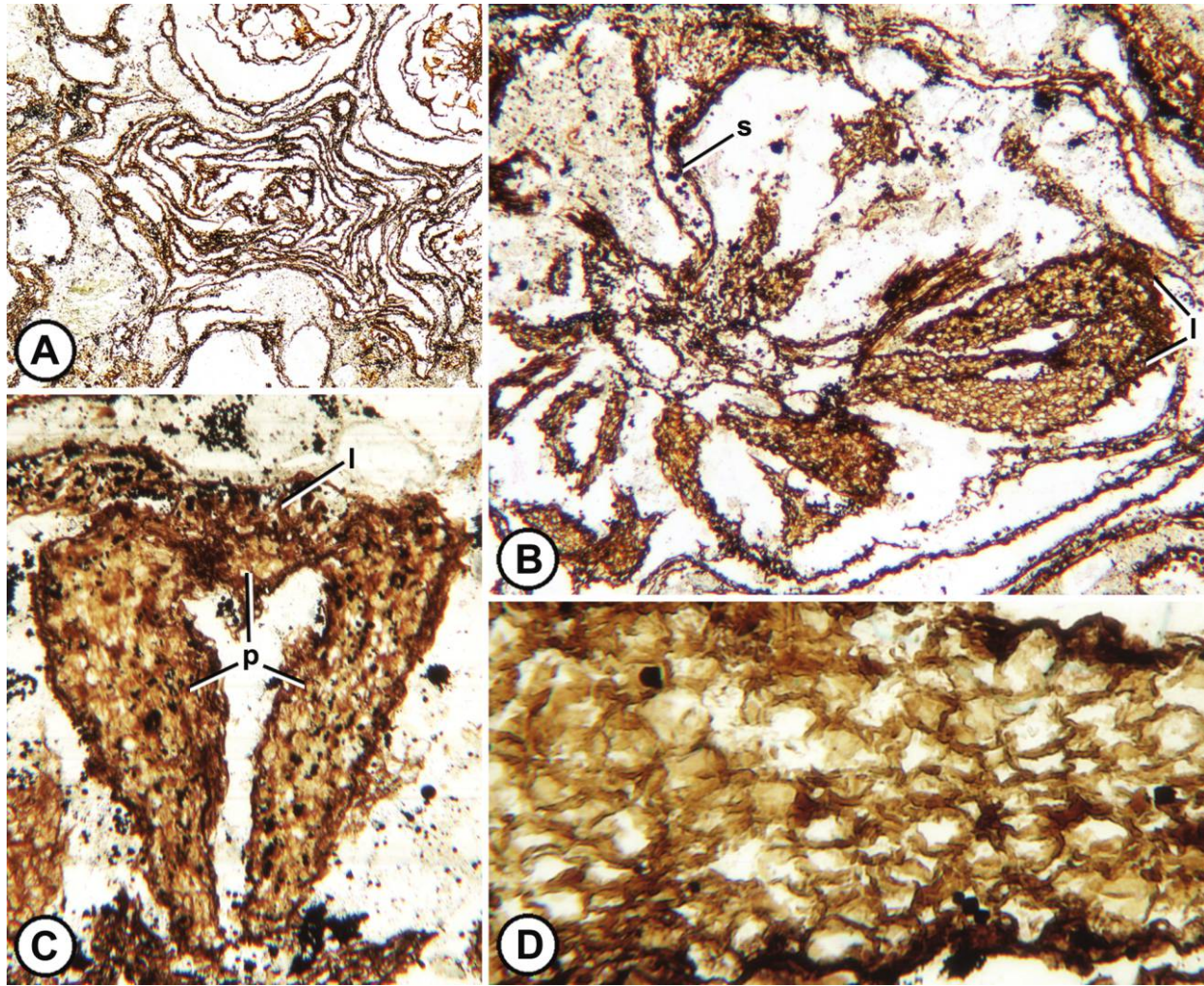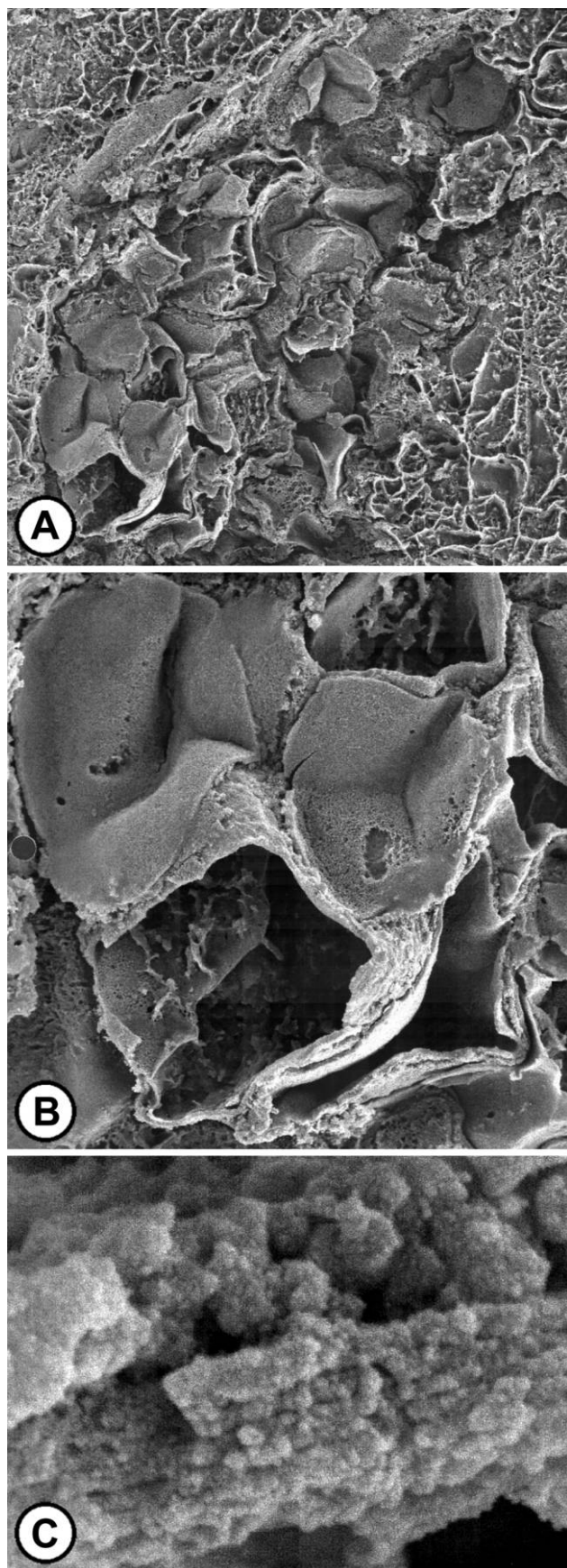
Fig. 2 *Cunninghamia beardii* sp. nov. Holotype, AW 358. *A*, Central leafy zone of pollen cone cluster. Note that each immature scalelike leaf has a central resin canal. A bot #6 × 30. *B*, Cross section of single pollen cone showing sporophylls diverging from cone axis. Note single resin canal in sporophyll stalk (s) and three pollen sacs (*right*) attached to lamina (l). A bot #8 × 61. *C*, Cross section of single pollen cone showing sporophyll lamina with three attached pollen sacs (p), two of which show elongation toward cone axis. A bot #16 × 124. *D*, Enlargement of somewhat immature pollen sac showing closely packed pollen grains. A bot #10 × 440.

pollen sacs are situated above the third, as in the Appian Way pollen cones.

Discussion

The pollen cone cluster from Appian Way clearly represents a taxodioid-grade cupressaceous conifer with helically arranged leaves and typical taxodioid pollen. Previously described pollen cones of *Homalcoia littoralis* from Appian Way are terminal and globose, with numerous subtending scale leaves and ellipsoidal pollen sacs. Pollen is 11–20 μm in diameter and papillose at maturity. While the pollen of *Cunninghamia beardii* is immature, it is larger at this stage of development when compared to *H. littoralis* and lacks any discernible papilla. *Homalcoia* pollen cones are solitary and not borne in clusters, as in the pollen cones described here.

Similarities of *C. beardii* to extant *Cunninghamia* spp. are striking. Using extant *Cunninghamia lanceolata* (Lamb.) Hook. and *Cunninghamia konishii* Hayata for comparison to the Appian Way fossil, all three species have subterminal pollen cone clusters with helically arranged pollen cones surrounding a vegetative stem that has terminal scale leaves and the capacity to grow through the next year (Farjon 2005). The Appian Way cones were immature at the time of preservation. This can be seen in the tightly spiraled nature of the cones of the cluster as well as the immature pollen contained in the pollen sacs, and in these features, they compare closely with the immature *C. lanceolata* pollen cone clusters in figure 5B, 5C. The smallest Appian Way cones have bracts and scale leaves that extend up around the pollen cone apex, a feature that is characteristic of immature conifer pollen cones that have yet to grow beyond their basal bud scales. This character suggests that the least ma-

ture fossil cones were younger than the most immature extant cones illustrated here (fig. 5C, 5E).

Among extant conifers, pollen cone clusters are reported in several pinaceous genera including *Keteleeria* Carrière and *Pseudolarix* Gord. (Schulz et al. 2014), in which the clusters are borne terminally or laterally on branches (Farjon 1990); however, leaf anatomy, pollen type (bisaccate), and two pollen sacs per microsporophyll preclude assignment of the Appian Way cones to the Pinaceae. Likewise, *Podocarpus* L'Héritier ex Persoon, *Retrophyllum* C. Page, *Acmopyle* Pilger, and *Nageia* J.Gaertner in the Podocarpaceae are also reported to bear pollen cones in clusters (Schulz et al. 2014), but podocarpaceous pollen cone clusters tend to be borne on small, specialized lateral shoots that have widely spaced pollen cones. The leaf anatomy, pollen type (bisaccate), and presence of two pollen sacs per microsporophyll (Sporne 1965; Woltz 1986; Eckenwalder 2009) clearly differentiate those podocarpaceous pollen cone clusters from the cone cluster described here.

Among all conifer families, pollen cone clusters are most common in fossil and living species of Cupressaceae. Pollen cones of the most ancient cupressaceous fossils from the Early Jurassic of Argentina, *Austrohamia minuta* Escapa, Cúneo & Axsmith, occur in terminal clusters of up to seven, while those of the Middle Jurassic *Sewardiodendron laxum* (Phillips) Florin are borne in terminal clusters of seven to eight (Yao et al. 1998). Shi et al. (2014) report clusters of up to nine pollen cones attached to twigs in their reconstruction of the cunninghamioid *Elatides zhoui* Shi, Leslie & Herendeen, lignitic fossils from the Early Cretaceous (Aptian-Albian) of Mongolia. Those cones are located along the stem near the shoot apex and occur in the axils of modified bracts, which are much broader and more triangular than the vegetative leaves. Therefore, they form a less dramatically expanded cluster at the shoot apex (Shi et al. 2014) than seen in *C. beardii* (i.e., fig. 2 of Shi et al. 2014). Among extant species of Cupressaceae, *Taiwania cryptomerioides* Hayata bears pollen cones in terminal clusters of up to seven, and *Amentotaxus* Pilger in the Taxaceae also has clusters of pollen cones (Schulz et al. 2014). *Cunninghamia beardii* is easily distinguished from all of these species by the much larger number of pollen cones (~24) that occur in clusters of a much larger size.

The highly distinctive and systematically isolated living species *Sciadopitys verticillata* (Thunb.) Siebold & Zucc. also produces clusters of apparently terminal to subterminal pollen cones that are superficially similar to those of *C. beardii* (Farjon 2005), but individual cones of *Sciadopitys* are tightly packed and form an ovoid cluster, and the branch tip does not expand as greatly as in *Cunninghamia* (R. A. Stockey and G. W. Rothwell, personal observation). Pollen cones of *Sciadopitys* are larger in diameter (Farjon 2005) and more distinctive within each cluster than

Fig. 3 *Cunninghamia beardii* sp. nov. Holotype, AW 358. SEMs of pollen sac with somewhat immature pollen. All A bot #7. *A*, Closely packed pollen within incompletely preserved pollen sac wall (*upper left*). × 850. *B*, Enlargement of possible tetrad at lower left in *A*. Note that the pollen wall consists of two zones. × 2750. *C*, Cross section of pollen wall showing nexine (*bottom*) and scabrate sexine with orbicules (*top*). × 72,000.
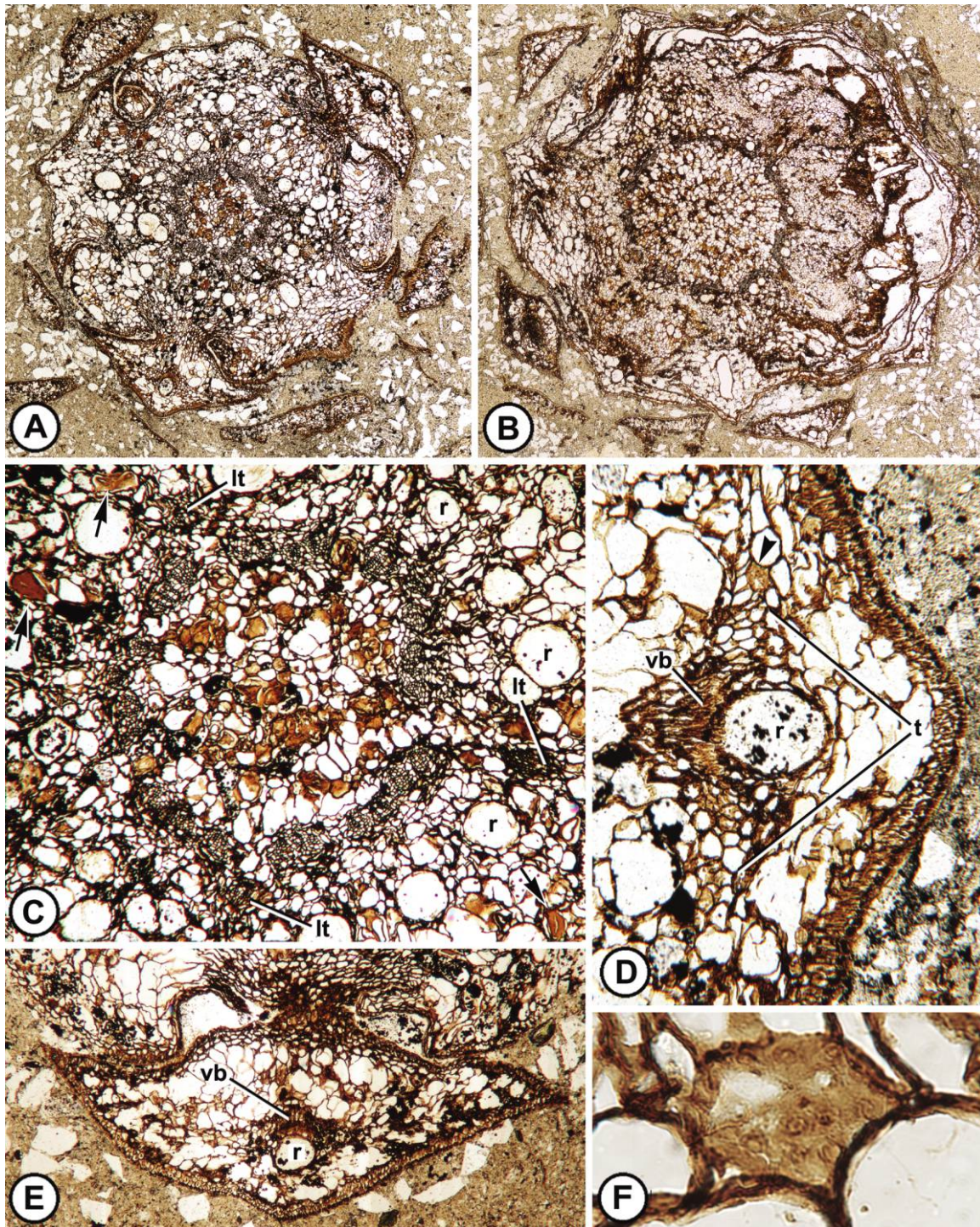
Fig. 4 *Cunninghamia beardii* sp. nov. Holotype, AW 358. *A*, Shoot-bearing pollen cone cluster showing helically arranged leaves, stem with pith, stele with diverging leaf traces, and parenchymatous cortex. Note resin canals in cortex, single resin canal in leaf bases, and narrow sclerotic hypodermis on diverging leaves. B_1 bot #1 ×17. *B*, Cross section at base of pollen cone cluster, with increased stem size, enlarged pith, and larger number of leaves. Note ring of small resin canals at outer margin of stele and slightly larger resin canals scattered within cortex. B top #2 ×12. *C*, Histological features of stem bearing pollen cone cluster, showing pith and cortical parenchyma with empty lumens and scattered cells with amber contents, some that appear to be sclereids (arrows). Note radially aligned tracheids of stelar xylem, diverging leaf traces (lt), and resin canals (r) with epithelial lining. B_1 bot #2 ×65. *D*, Diverging leaf base at periphery of stem showing vascular bundle (vb), resin canal (r), and band of transfusion tissue (t). Note sclerotic hypodermis on abaxial surface of leaf base (*right*). Arrowhead on transfusion tracheid enlarged in *F*. B_1 bot #2 ×97. *E*, Cross section of leaf base at level of separation from stem, showing position of vascular bundle (vb), resin canal (r), parenchymatous mesophyll, and sclerotic hypodermis. B_1 bot #2 ×45. *F*, Enlargement of transfusion tracheid with circular-bordered pits. B_1 bot #2 ×690.
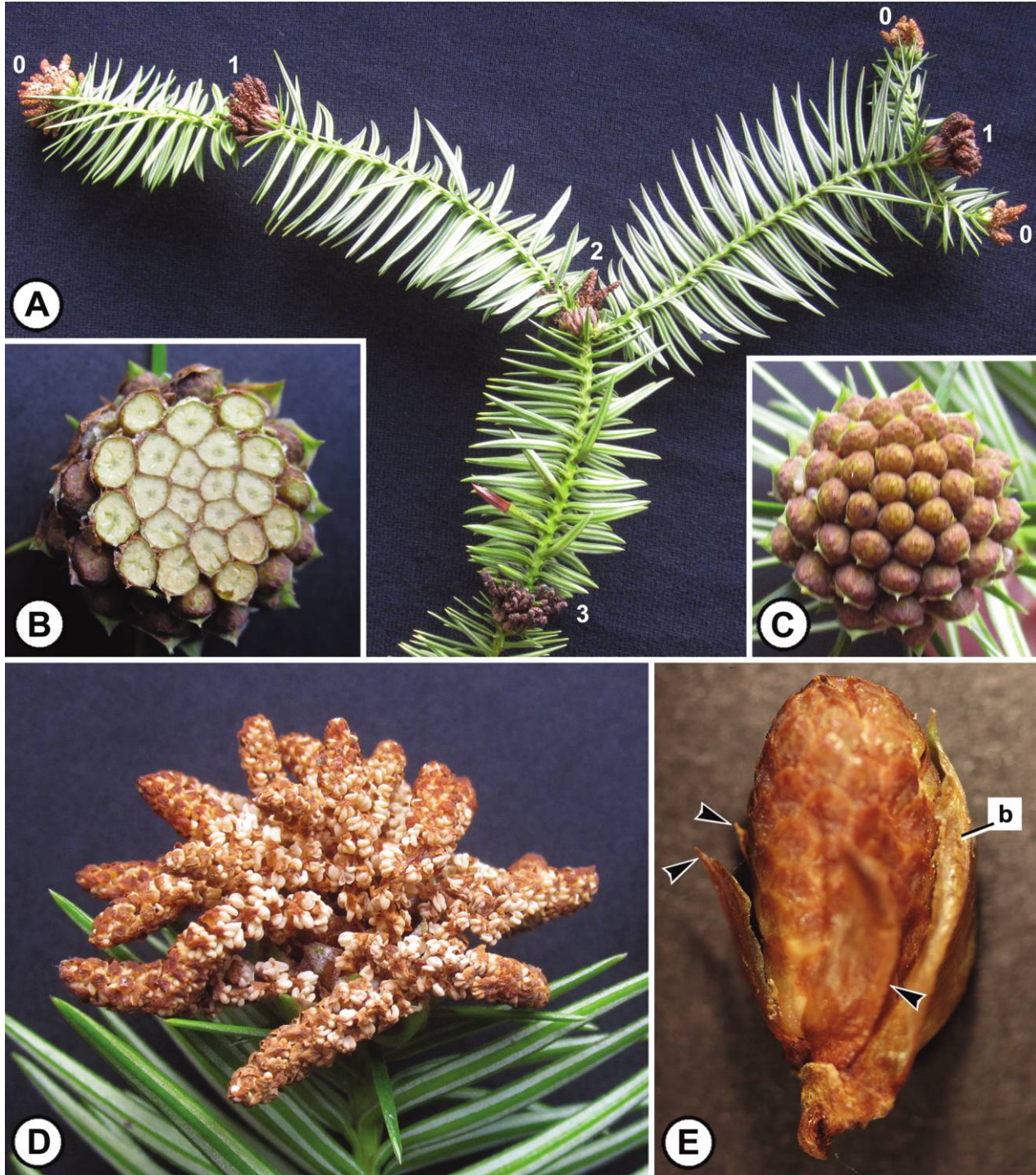
Fig. 5 Extant *Cunninghamia lanceolata*. *A*, Leafy shoot showing positions of pollen cone clusters from current year (0), 1 yr ago (1), 2 yr ago (2), and 3 yr ago (3). × 0.5. *B*, Pollen cone cluster sectioned below apex showing somewhat immature pollen cones crowded within subtending leaves. Note that pollen cones appear to occupy stem apex at this level. × 4. *C*, Pollen cone cluster showing somewhat immature cones, each subtended by bract-like leaf. As in fig. 4*B*, cones appear to occupy apex of branch in this view. × 3. *D*, Shoot tip showing cluster of mature pollen cones. Note that cones elongated at maturity to expose pollen sacs. × 2. *E*, Immature pollen cone with subtending bract (b) at right and three scale-like leaves (arrowheads) at cone base. × 14.
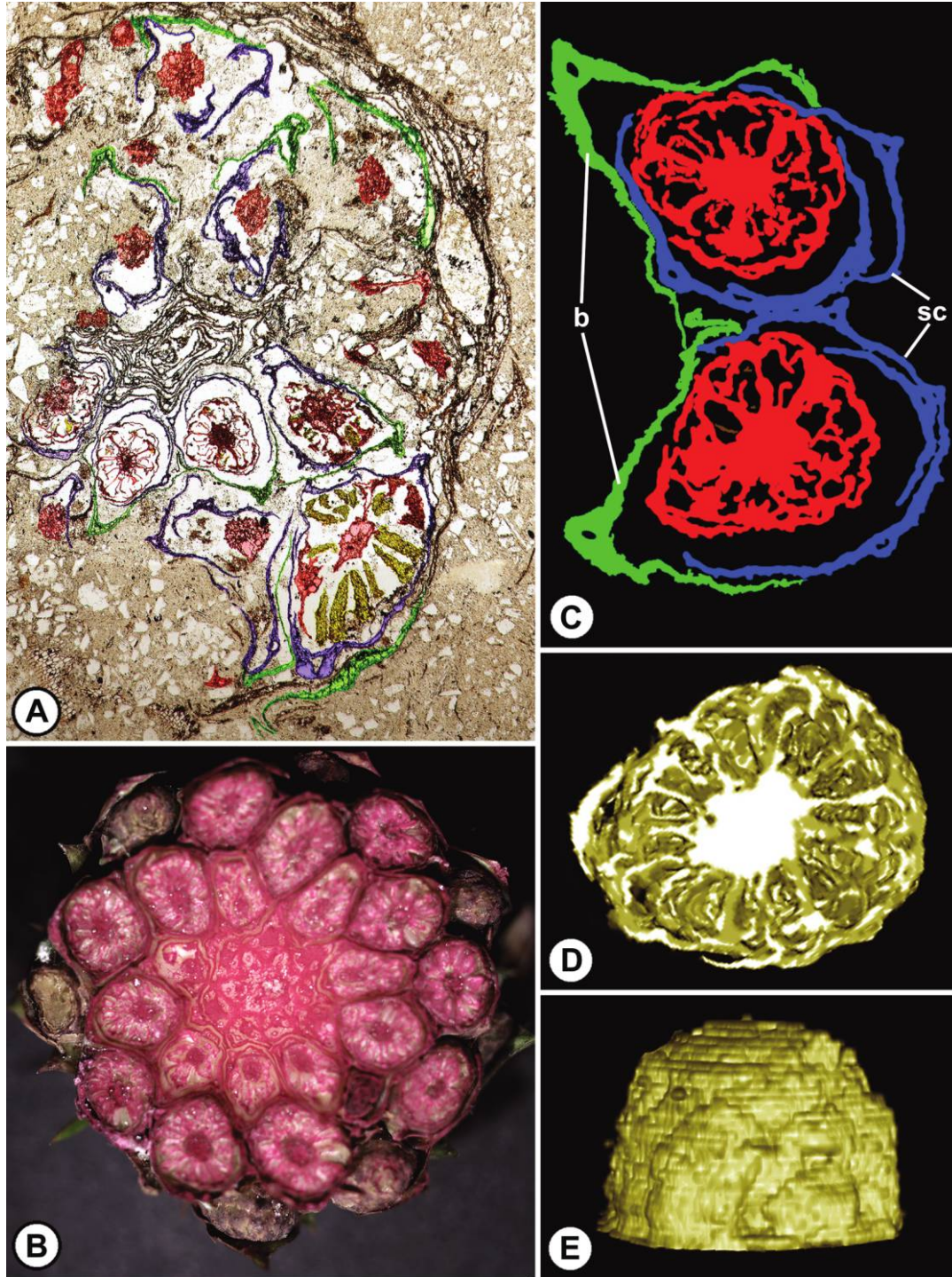
Fig. 6 *Cunninghamia beardii* sp. nov. and *Cunninghamia lanceolata* pollen cone clusters in cross section just distal to stem apex. *A*, *Cunninghamia beardii* with bracts (green), scale leaves at base of cones (blue), cone axes and sporophylls (red), and pollen sacs (yellow). AW 358 A bot #1. Holotype. × 18. *B*, *Cunninghamia lanceolata* at comparable level to fossil in *A*, showing zone of slightly immature cones within peripheral subtending leaves and central zone of vegetative leaf bases. × 7. *C*, *Cunninghamia beardii*, diagram illustrating the relationships among pollen cones (red), scale leaves (sc; blue), and subtending bract (b; green). AW 358 A bot #6. Holotype. × 60. *D*, *Cunninghamia beardii*, three-dimensional reconstruction of one pollen cone as viewed from cut surface. × 65. *E*, *Cunninghamia beardii*, three-dimensional reconstruction of apex of cone surface showing tightly appressed microsporophyll heads. × 55.

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those of *Cunninghamia*. Individual pollen cones of *Sciadopitys* are subtended by a bract such as that in *Cunninghamia*, but each cone lacks basal scale leaves (R. A. Stockey, personal observations). Likewise, two pollen sacs per microsporophyll, inaperturate pollen, and highly distinctive short shoots that appear to be leaves in *Sciadopitys* easily distinguish that genus from *C. beardii*.

In position on the shoot, size, shape, number of pollen cones, microsporophyll structure, pollen type, and morphology and anatomy of subtending leaves and stems, the Appian Way pollen cone cluster is much more similar to those of the genus *Cunninghamia*. The only previously known occurrence of *Cunninghamia* pollen cones in the fossil record was described by Serbet et al. (2013) for *Cunninghamia taylorii* Serbet, Bomfleur & Rothwell from the Upper Cretaceous (Campanian) Horseshoe Canyon Formation near Drumheller, Alberta, Canada. Those pollen cones are borne in clusters of up to 17 that appear to terminate vegetative shoots and are surrounded by scale leaves that are anatomically similar to those of both *C. beardii* and living species of *Cunninghamia* (Serbet et al. 2013). The pollen cones of *C. taylorii* are represented by only the cone axis and the basal scalelike leaves (microsporophylls, pollen sacs, and pollen having been lost from those specimens). While the pollen cone clusters of *C. taylorii* are similar to those of *C. beardii* in most known features, the two species can easily be distinguished by the number of scale leaves at the base of each cone, three in *C. beardii* and five or six in *C. taylorii* (Serbet et al. 2013).

Cunninghamia beardii is most similar to the pollen cone clusters of the living *Cunninghamia* species (i.e., *C. konishii* Hayata and *C. lanceolata* [Lamb.] Hook.; Farjon 2005; Serbet et al. 2013). Similarities include size, overall shape, and pollen cones that appear to be terminal (but are actually subterminal), all having the potential to grow out in subsequent years to produce an additional plastichron of vegetative/fertile growth. Extant *Cunninghamia* species and *C. beardii* have similar numbers of pollen cones of relatively similar sizes in each cluster, and each cone produces three basal scalelike leaves. Moreover, as far as is known, leaf histology is comparable in the two.

Given the structural similarities between *C. beardii*, *C. lanceolata*, and *C. konishii*, one may be tempted to assign the fossil to one of the living species. However, with more careful consideration it becomes clear that there are no characters to determine to which of the two species the fossils would be assigned. More importantly, we have excellent evidence from other fossil taxodioid-grade Cupressaceae that species differences are often extremely subtle and that confident species identifications often cannot be made until ranges of variation for a wide variety of organs have been determined. For example, compression fossils of the genus *Metasequoia* Miki extend from the Cretaceous to

the recent, with as many as 21 species having been described, often from relatively small numbers of specimens and few organs (Liu et al. 1999; Stockey et al. 2001). However, a comprehensive review of fossil *Metasequoia* has revealed that all fossils may be assignable to only three species (Liu et al. 1999). So subtle are the differences between species of Cretaceous, Paleogene, and Neogene *Metasequoia* that more than 10,000 specimens of branches, leafy shoots, pollen cone–bearing shoots, pollen cones, pollen, seed cones, seeds, and seedlings were required to clearly distinguish *Metasequoia foxii* Stockey, Rothwell & Falder (2001) from the other fossil and living species.

Due to our growing understanding of mosaic character evolution within the Cupressaceae, it appears that some reproductive organs have undergone a higher rate of evolution compared to the vegetative organs than have others (Atkinson et al. 2014*a*). Many extinct species have similar leaf anatomy and morphology in many organs, while ovulate cones show novel combinations of characters that are not typically found in living cunninghamioids (Atkinson et al. 2014*a*). Conifer pollen cones are relatively uncommon in the post-Paleozoic fossil record, and the diversity of extinct cupressaceous pollen cones is virtually unknown. If *C. beardii* is characteristic of extinct cupressaceous pollen cones in general, then pollen cone evolution has been far less dramatic than seed cone evolution within the family, as has been suggested for conifers in general (Leslie 2011). Within this context, *C. beardii* provides important data for understanding the evolution of pollen cones within this group.

Although *C. beardii* closely resembles the pollen cone clusters of both living species of *Cunninghamia* (i.e., *C. lanceolata* and *C. konishii*), the most prudent taxonomic placement for the Appian Way fossil is in a new species. This decision actually represents a conservative taxonomic judgment that is concordant with recently modeled evolutionary dynamics for the family Cupressaceae (Leslie et al. 2012), in which the cladogenic event leading to the origin of *C. lanceolata* and *C. konishii* from a putative common ancestor occurred in the Neogene, at least 30 Myr after fossilization of the Appian Way flora (Leslie et al. 2012; Mindell et al. 2014). Whether that putative common ancestor is the species represented by *C. beardii* or some other as of yet to be recognized extinct species of *Cunninghamia* awaits discovery of the unknown vegetative and fertile organs of *C. beardii* and much denser sampling of Cretaceous and Tertiary Cupressaceae. However, in either case, exquisitely preserved fossils from carbonate marine concretions will undoubtedly play a major role in both fleshing out the patterns of evolution for crown group conifers and testing hypotheses of conifer phylogeny generated by analyses of living species using molecular characters and phylogenetic modeling exercises that rely on those hypotheses as sources of data.

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