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MIDDLE JURASSIC EVIDENCE FOR THE ORIGIN OF CUPRESSACEAE: A PALEOBOTANICAL CONTEXT FOR THE ROLES OF REGULATORY GENETICS AND DEVELOPMENT IN THE EVOLUTION OF CONIFER SEED CONES¹

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- *Premise of the study:* Triassic and Jurassic fossils record structural changes in conifer seed cones through time, provide the earliest evidence for crown-group conifer clades, and further clarify sister-group relationships of modern conifer families. A new and distinct seed-cone from the Isle of Skye in western Scotland provides the oldest detailed evidence for the ancestral morphology of the phylogenetically contentious family Cupressaceae.
- *Methods:* A single isolated cone was prepared as serial sections by the cellulose acetate peel technique, mounted on microscope slides, and viewed and photographed using transmitted light. The three-dimensional structure of the cone was first reconstructed from the serial sections and then refined through imaging with x-ray microtomography.
- *Key results:* *Scitistrobis duncaanensis*, gen. et sp. nov., is a 7.5 mm-diameter cylindrical seed cone with helically arranged bract–scale complexes in which three scale tips separate from a large bract, each tip bearing one adaxial seed. Seeds are near-inverted, show 180° rotational symmetry, and have a diminutive wing in the major plane.
- *Conclusions:* *Scitistrobis duncaanensis* extends the fossil record for anatomically preserved seed cones of the Cupressaceae backward from the Upper Jurassic to the Aalenian Stage of the Middle Jurassic. The cone displays a previously unknown combination of characters that we regard as diagnostic for seed cones of early-divergent Cupressaceae and helps to clarify the sequence of structural changes that occurred during the transition from ancestral voltzialean conifers to morphologically recognizable Cupressaceae. Hypotheses of homology underpinning such transformational series can be tested by ongoing reciprocal illumination between the morphology of fossil taxa and the morphogenesis and developmental genetics of their extant crown-group relatives.

Key words: bract–scale complex; Coniferales; Cupressaceae; evolutionary–developmental genetics; fossil seed cone; Middle Jurassic; Voltziaceae; x-ray microtomography.

Conifers are among the most diverse and economically important of all seed plants. The clade is represented by a rich fossil record that extends back through 300 Myr to the Pennsylvanian

Period (Rothwell et al., 1996; Taylor et al., 2009) and has contributed a major proportion of the biomass for Mesozoic and Tertiary coals worldwide. The overall pattern of conifer phylogeny is currently the subject of intense interest and study, with several interrelated approaches each contributing to a growing understanding of relationships among extinct stem-group families and, within the crown group, among families with living representatives (e.g., Gadek et al., 2000; Quinn et al., 2002; Rothwell et al., 2005, 2011; Rai et al., 2008; Mathews, 2009; Groth et al., 2011; Escapa et al., 2012; Leslie et al., 2012). However, a long fossil history and extensive extinction has left areas of uncertainty in key regions of the conifer phylogeny.

All of the crown-group families of conifers appear to have originated during the Triassic and Jurassic (Rothwell et al., 2012; see also Leslie et al., 2012), when conifers dominated several biomes. However, our understanding of crown-group

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conifer evolution is constrained by a paucity of well-preserved fossils from which to develop organismal concepts of conifer species that lived during this time interval. As a result, the fossil record thus far has provided no whole-plant organismal concepts based on anatomically preserved fossils from which to infer the evolution of each modern family. On the other hand, recent studies suggest that anatomically preserved conifer seed cones from Triassic and Jurassic deposits may serve as the most valuable single-organ proxy for the whole-plant concepts of extinct conifers badly needed to formulate systematic hypotheses (Bateman and Hilton, 2009; Rothwell et al., 2009). Certainly, megasporangiate cones are the organ category that contains the least homoplastic phylogenetic signal among the rhizomorphic clubmosses (Bateman and Simpson, 1998), and it is reasonable to suspect a similarly low level of homoplasy for conifer seed cones. Ovulate cones provide crucial information about both the homologies of their components and also the divergent sequences of structural changes that have accompanied the evolution of each modern conifer family (Hirmer, 1936; Florin, 1951; Serbet et al., 2010; Rothwell et al., 2011).

In the current study, we characterize and name the oldest anatomically preserved cupressaceous seed cone thus far discovered, *Scitistrobis duncaanensis* gen. et sp. nov. This cone-species extends the fossil record of permineralized Cupressaceae back ~20 Myr from the Kimmeridgian Stage of the Upper Jurassic to the base of the Aalenian Stage of the Middle Jurassic and is the second-oldest fossil occurrence of the family Cupressaceae (Escapa et al., 2008). *Scitistrobis* also displays a novel combination of reproductive characters that falls earlier within the proposed transformational series for evolution of Cupressaceae from within Voltziaceae (Rothwell et al., 2011) than does any of the previously known anatomically preserved fossils, and further increases the known structural diversity for seed cones of early-divergent Cupressaceae.

The newly recognized suite of characters exhibited by *S. duncaanensis* expands our understanding of the structural transformations associated with the origin of Cupressaceae. When coupled with the rapidly growing knowledge of the genetic and developmental regulatory basis for structural changes in conifer cone organs (e.g., Theissen and Becker, 2004; Vazquez-Lobo et al., 2007; Carlsbecker et al., 2013), this increased understanding of the pattern of structural transformations through time provides, in theory, a framework for inferring changes in the regulatory genetics that underlie conifer seed-cone evolution. We review the considerable progress made recently in this arena and suggest further steps that will need to be taken if this potential is to be adequately realized.

MATERIALS AND METHODS

The single isolated seed cone was found by R. M. Bateman in May 1995 in a sandy limestone concretion within the Dun Caan Shale Member of the Berreraig Sandstone Formation, at Berreraig Bay in the Isle of Skye (57°29'56.4"N, 6°08'52.8"W; Fig. 1A, B). From this locality, Bateman et al. (2000) and Dower et al. (2004) previously documented fossil plants anatomically preserved as carbonate permineralizations and charcoal. Speculative correlation of macrofossil organ-species indicates a whole-plant flora that includes one horsetail, four ferns, one cycad, two bennettites, one czekanowskialean and at least two conifers. A modest diversity of anatomically preserved conifer foliage dominates the plant macrofossil assemblages throughout the sequence, and microfossil assemblages (e.g., Riding, 2005) suggest greater whole-plant diversity of conifers, including representatives of both the Cheirolepidiaceae (Bateman et al., 2000) and the Cupressaceae (described herein).

The 14 plant-bearing horizons identified within the Berreraig Sandstone Formation span deposits extending from the middle of the Opalinum Zone of the Dun Caan Shale Member to the top of the Laeviuscula Zone in the Holm Sandstone Member (Fig. 1C; Morton and Hodson, 1995). The Berreraig Sandstone Formation represents a succession of shallow marine sediments, into which fossil plants rafted from surrounding land areas (Bateman and Morton, 1994; Bateman et al., 2000). Once the fossil plants sank to the sea floor, they were permineralized by early-stage calcium carbonate that was remobilized through dissolution of aragonite and calcite from innumerable marine organisms admixed with the plant fossils (Bateman et al., 2000). The formation is undoubtedly of Middle Jurassic (Aalenian–Bajocian) age, based on detailed ammonite and dinoflagellate biostratigraphy (Cox and Sumbler, 2002). The conifer cone described here occurred in the lowermost of the plant-bearing horizons, within the Opalinum Zone of the Aalenian stage, approximately 174.1 ± 1.0 to 170.3 ± 1.4 Ma (Cohen et al., 2013; Fig. 1C). The cone was tentatively ascribed to the Araucariaceae in the brief review of the Berreraig flora published by Bateman et al. (2000).

Taphonomic factors placed some constraints on our interpretation of the cone. The plant fossils at Berreraig were deposited over a ~9 Myr period ~10 km offshore from a major fluvial discharge that sampled taphonomically an extensive hinterland (Bateman et al., 2000). All of the fern macrofossils recovered from the locality were wholly charcoaled, whereas most conifer remains were either unburned or, as was the case with the present cone, lightly singed on the outside. In addition, some diagenetic pyrite is present throughout the sedimentary sequence, often nucleated around plant remains and reaching its acme in a large horsetail pith-cast that was largely replaced by pyrite. The present cone accumulated a considerable concentration of pyrite, particularly in the interior of the cone where, despite its comparatively open architecture, anoxia probably prevailed. The presence of the pyrite challenged each of the analytical approaches employed by us.

The ~5-cm-long concretion was ground smooth on the surface where the cone was exposed in cross section and subsequently cut to expose the cone in longitudinal section. The originally exposed surface was designated as A top, and a subsequent cut obliquely parallel to the A top surface produced surfaces that were designated A bottom and B top. Serial sections of all three surfaces were prepared by the well-known cellulose acetate peel technique (Joy et al., 1956). Peels were mounted under glass coverslips on 25 × 75 mm microscope slides with Eukitt (O. Kindler GmbH, Freiburg, Germany). Images of the acetate peel sections (Figs. 2–28) were captured with a View Finder 7 large-format digital scanning camera system (Better Light, 1200 Industrial Rd. San Carlos, California, USA), focused through a Leitz Aristophot large-format bellows camera and a Zeiss WL compound microscope, and processed with Adobe Photoshop (San Jose, California, USA).

Following the preparation of peel sections, the remaining segment of cone (in slab B) was scanned using x-ray microtomography (XMT) on a Metris X-Tek HMX-ST scanner at the Natural History Museum, London. A tungsten reflection target with 0.25 mm Cu filter at 230 mA/170 kV, 0.5 s exposure time was employed, resulting in 3142 projections with a spatial resolution of 13.8 μm. A three-dimensional false-color model illustrating the different parts of the cone was constructed from the XMT data set using SPIERS software (Sutton et al., 2012; Spencer et al., 2013). Ray-traced images and videos were prepared using the program Blender (Garwood and Dunlop, 2014). Images of the three-dimensional model (Figs. 29–34) were produced in Corel Draw x6 and x7 (Corel, Ottawa, Ontario, Canada).

Specimen segments, peels, and microscope slides are housed in the Palaeontology collections at the Natural History Museum, London, UK (NHMUK) as specimen number V 68522. Each figured microscope slide has been given a unique subset of that number, as indicated in the captions to Figs. 2–28.

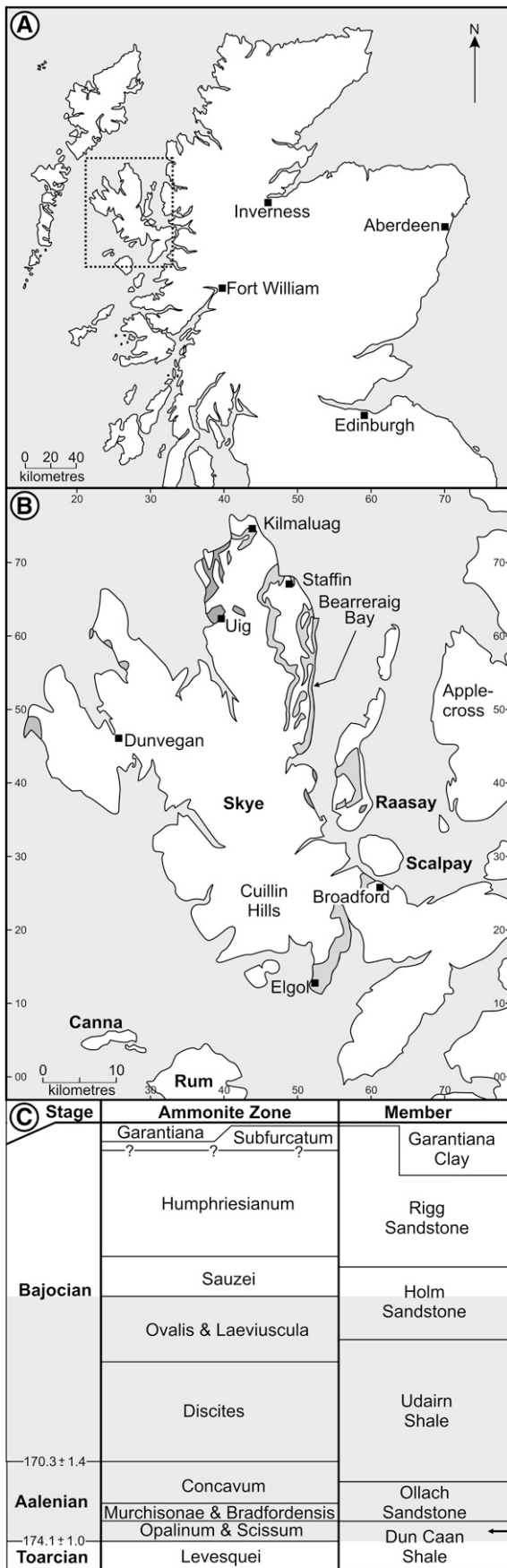
SYSTEMATICS

Order—Coniferales.

Family—Cupressaceae sensu Eckenwalder (2009).

Genus—*Scitistrobis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. nov.

Generic diagnosis—Conifer seed cones with numerous, helically arranged bracts and axillary ovuliferous scales. Scale



fused to bract up to mid-region of bract–scale complex, diverging laterally and separating into three short free tips, each tip bearing one adaxial seed attached near its apex. Bract–scale trace diverging as single prominent bundle, separating laterally into three strands, each dividing vertically to produce terete scale trace and larger bract trace. Bract traces extend to near bract tip; scale trace terminating at base of seed. Resin canals absent from cone axis; three originating de novo at base of bract–scale complex, increasing in number distally, one entering each free scale tip and one extending at center of bract to near tip. Seeds reflexed toward cone axis, oval in transverse sections with subdued wing, showing 180° rotational symmetry. Nucellus adnate to integument to near apex, with prominent pollen chamber.

Etymology—Referring to the collecting locality on the Isle of Skye (Latin *Scitis*), western Scotland, and cone (Latin *strobilus, pro parte*).

Type species—*Scitistrobus duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, sp. nov.; Figs. 2–35, Appendices S1, S2 (see Supplemental Data with the online version of this article).

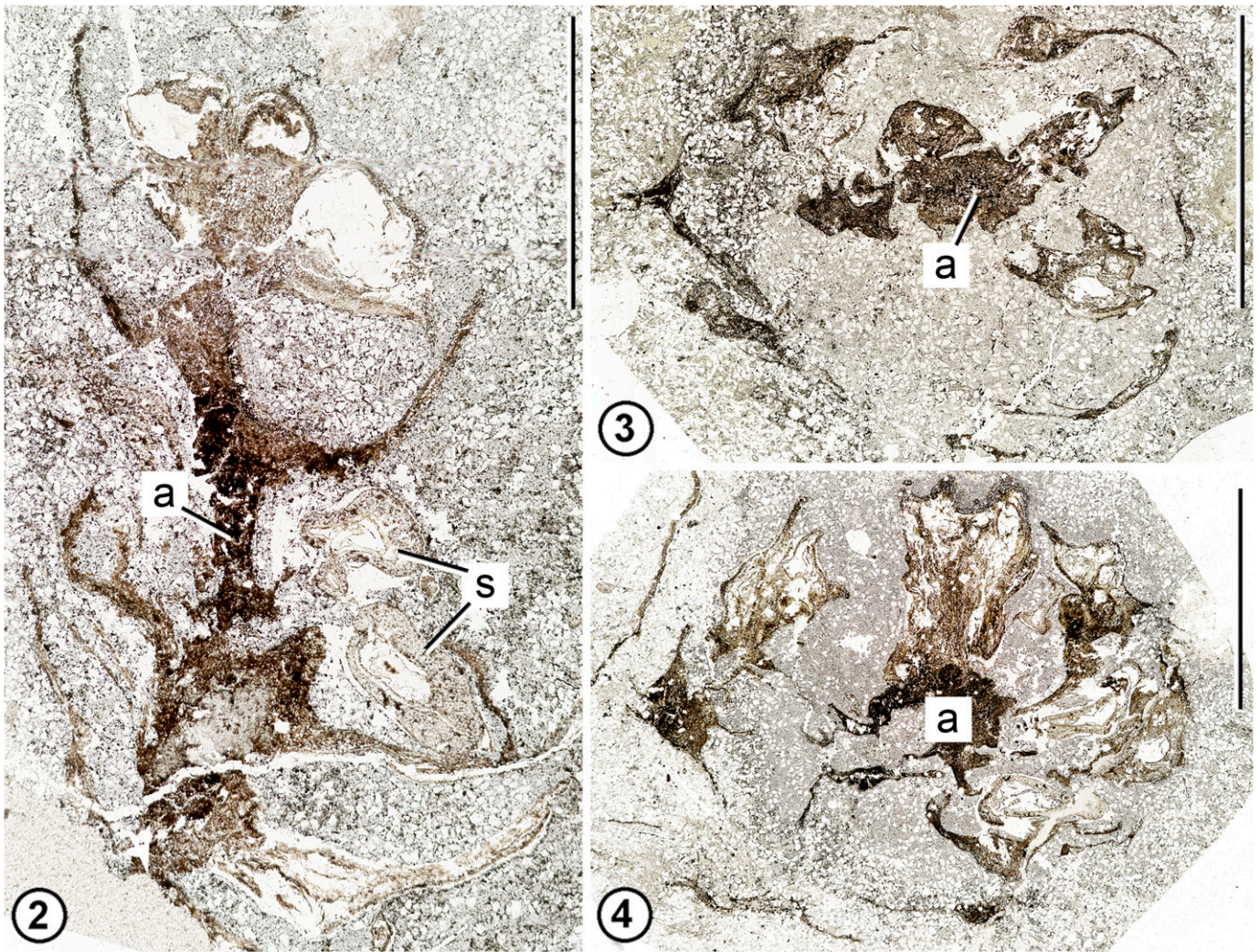
Specific diagnosis—Cylindrical cone up to 7.5 mm in diameter and >3 cm long, with axis ca. 3 mm wide. Cone axis with parenchymatous pith, woody cylinder, and parenchymatous cortex; bract–scale trace diverging as single bundle. Bract–scale complex ca. 5 mm long, narrowly attached, rhomboidal with an acuminate tip; diverging from axis at 80–90°, bending gently toward apex to nearly parallel cone axis; lateral margins of bract bending adaxially to partially surround free scale tips and ovules. Ovuliferous scale tips separating from bract at mid-level of complex, each bearing one inverted seed adaxially, immediately below apex. Seeds oval in transverse section with diminutive wing; ca. 2.1 mm in major plane, 1.2 mm in minor plane.

Holotype here designated—Slabs, peels, and slides of permineralized specimen No. 95/1, housed in the Palaeontology collections, Natural History Museum, London, UK (NHMUK) as acquisition number V 68522 (Figs. 2–35; Appendices S1, S2).

Collecting locality—Cliff face above Berreraig Bay on the Isle of Skye, western Scotland.

Stratigraphic position and age—Dun Caan Shale Member of the Berreraig Sandstone Formation, Aalenian Stage at the base of the Middle Jurassic.

Fig. 1. Location of Berreraig Bay and stratigraphic relationships of the Berreraig Sandstone Formation. (A) Outline map of northern Britain with boxed area showing position of the Isle of Skye in western Scotland. (B) Enlargement of boxed area from (A) showing Skye and adjacent islands; outcrops of Middle Jurassic age are shown in dark gray. (C) Lithostratigraphic and biostratigraphic relationships of the members of the Berreraig Sandstone Formation; position of the conifer cone indicated by arrow, and span of the plant-bearing horizons shown in gray in Fig. 1B. Figure modified from figs. 6.1 and 6.44 of Cox and Sumblar (2002), radiometric ages from the International Chronostratigraphic Chart v2013/01 (International Union of Geological Sciences).



Figs. 2–4. *Scitistrobus duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. (holotype, NHMUK, V 68522). 2. Near-radial section of cone showing divergence of bract–scale complexes from cone axis (a) in longitudinal view, and (at top) oblique cross section of one complex immediately proximal to divergence of scales from bract. Note two seeds (s) above bract–scale complex at lower right. A Bot #20 ×10 (NHMUK, V 68522\$1). Scale bar = 5 mm. 3. Cross section of cone at level where histological preservation is incomplete, showing divergence of bract–scale complexes from axis (a) and relatively distal sections of bracts at periphery. Note the large percentage of empty space within the cone at this level. A Top #40 ×10 (NHMUK, V 68522\$2). Scale bar = 5 mm. 4. Cross section of cone with better preservation at slightly more proximal level than Fig. 2, showing divergence of several bract–scale complexes from axis (a). Note the large area of interior cone space with this quality of preservation. A Top #110 ×6 (NHMUK, V 68522\$3). Scale bar = 5 mm.

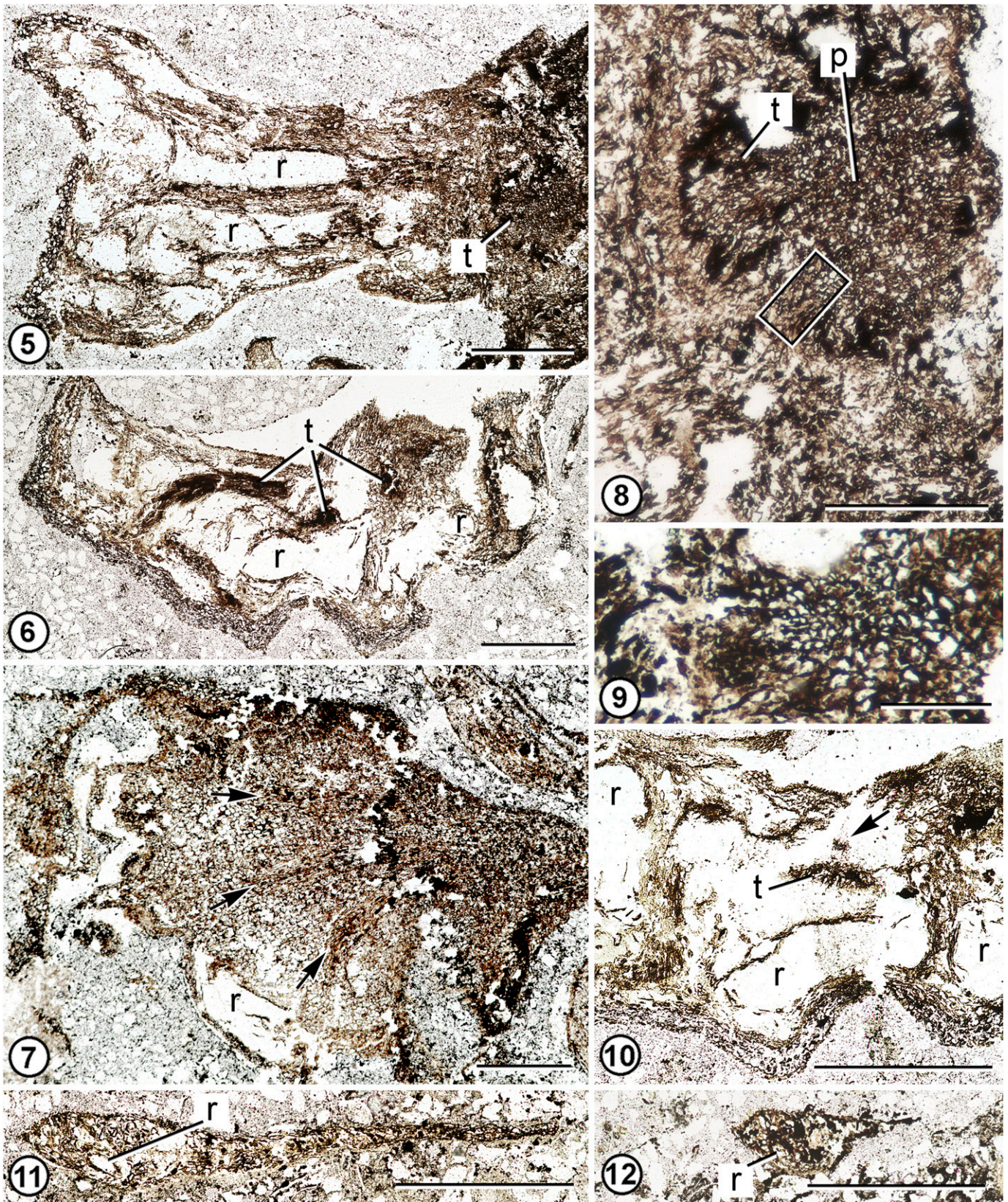
Etymology—Referring to the lithostratigraphic source unit, Dun Caan Shale, from which the specimen was collected (the Shale is in turn named after the highest topographical point on the adjacent island of Raasay).

DESCRIPTION

The holotype is incomplete, missing both base and apex of the cone. The preserved central region is clearly cylindrical, measuring 3 cm long and ca. 7.5 mm in diameter. Numerous bract–scale complexes are attached to the axis in a helical arrangement (Figs. 2–4, 29–31, 35A), diverging from the axis almost perpendicularly, each bending sufficiently near the cone surface (Figs. 2, 29, 31–35) to become nearly parallel to the cone axis at the bract apex (Fig. 29). Bract–scale complexes consist of a rhomboidal bract (Figs. 5, 7) with an acuminate tip (Figs. 12, 29–35) and a

less conspicuous axillary ovuliferous scale (Figs. 2–4, 29–32, 35B, 35C). The ovuliferous scale is attached to the bract up to the mid-region (Fig. 13), where it separates and bends toward the cone axis (i.e., away from the bract), forming three short tips (Figs. 14–22, 35C). The bract–scale complex bears three more-or-less inverted seeds, one attached to the adaxial surface of each free scale tip (Figs. 18–22, 35C). Seeds occur close to the scale tip and distal to the level of scale separation from the bract (Figs. 18–22, 30–32, 35B). A small, somewhat raised scar marks the point where seeds separate from the scale (Figs. 26, 31A, 31B).

The cone axis is ca. 3 mm in diameter (Figs. 2–4, 29). It consists of a parenchymatous pith (Fig. 8) surrounded by an incompletely preserved cylinder of radially aligned cells (Figs. 8, 9) that represent a complete cylinder of wood, and an outer zone of cortex that shows no evidence of resin canals (Figs. 5, 8). Cells of the cortex also are incompletely preserved, but appear to be uniformly thin-walled parenchyma (Figs. 5, 8).



Figs. 5–12. *Scitistrobis duncaensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. (holotype, NHMUK, V 68522). 5. Cross section of cone showing divergence of bract–scale complex from cone axis, with large resin canals (r) extending from near cone axis to cone periphery where complex bends distally. Note divergence of single bract–scale trace (t) from axis. A Top #122 × 17 (NHMUK, V 68522\$4). Scale bar = 1 mm. 6. Cross section near base of bract–scale complex showing large resin canals toward abaxial surface and bract–scale vascularization at level where bundle is adaxial to resin canals (r) and has divided

Bracts are narrowly attached and expand in width from the base to the margin of the cone (Figs. 2, 4, 5, 7, 29–32). Each bends distally, narrows toward the apex, and terminates in an acuminate tip (Figs. 2, 11, 12, 29–35). Bract tissue is largely parenchymatous (Figs. 5, 7), but somewhat larger and thicker-walled cells near the epidermis suggest the presence of a one- or two-layered sclerotic hypodermis on both the abaxial and adaxial sides (Figs. 5 at left, 6, 10).

The bract trace diverges from the cone axis as an oval bundle (Fig. 8 at left) and almost immediately separates laterally into three strands (Figs. 6, 7 at arrows). Proximal to cone-scale separation each of the strands divides vertically into a prominent bract trace (Fig. 10 at t) and a minute scale trace (Fig. 10 at arrow). More distally, the three traces converge and broaden out into a discontinuous bundle (Figs. 14, 15) that extends somewhat farther before reaching a point where tracheids can be recognized only at the center of the bract. The narrow bract trace extends distally to near the apex, where it enters the acuminate tip. Two to three resin canals originate *de novo* near the base of the bract–scale complex (Fig. 5) and abaxial to the scale traces (Figs. 6, 7, 10). At the level of scale separation about four resin canals are evident, one entering each scale tip (Figs. 14–17, 19–21) and one continuing into the acuminate bract tip (Figs. 11, 12).

Separation of the ovuliferous scale from the bract begins at the margins (Figs. 13–15) and immediately divides laterally into three free tips (Figs. 13–22, 31). Some scale tips appear to separate from each other before separating from the bract, whereas others do not (Figs. 14–22; Appendix S1). Three-dimensional images reveal that the scale tips bend away from the bract and toward the cone axis (Figs. 20–32, 35B, Appendix S2). In oblique sections, each scale tip typically shows two horns on the adaxial side (Figs. 14–17, 19–22). These features suggest that the position of ovule attachment is adaxial, immediately below the scale tip, a suspicion that has been verified by the three-dimensional images of the cone (Figs. 30–32, 35B, Appendix S2). Light-colored cells in this region (Figs. 15, 23, 26) are interpreted as closing tissue associated with seed abscission. Transverse and oblique sections show that each scale tip consists of a large resin canal surrounded by a narrow zone of thin-walled cells (Figs. 15–17). There also is some evidence of the terete scale trace on the adaxial side of the resin canal, but tracheids are sufficiently hard to identify in this region to suggest that the scale trace terminates before reaching the base of the seed.

Seeds are attached to some scales (Figs. 18–22, 26, 30, 32) but are absent from others (Figs. 13–17, 31, 33), though dislocated seeds often remain within the cone (Fig. 2 at s). Each seed is oriented out of the plane of the scale tip to which it is attached, revealing that the micropyle is somewhat recurved toward the axis (Figs. 18–22, 35B). However, in acetate peels the

seeds do not appear to be as completely inverted as they are in many conifer cones, an observation that was confirmed by the three-dimensional views (Figs. 30, 32, Appendix S2). The closing layer of golden-colored cells at the base of each seed (Figs. 20, 23, 26) reveals that the seeds abscised at maturity, an inference supported by the absence of seeds from many of the scale tips (Figs. 14–17, 32–34, Appendices S1, S2).

Seeds are oval and flattened in transverse section (Figs. 27, 28, 30, Appendix S2), 2.1 mm in major plane and 1.2 mm in minor plane. A diminutive lateral wing can be identified in some sections (Fig. 28). Seed length is difficult to determine from acetate peels, as complete longitudinal sections are unavailable. However, the longest oblique sections reach 2.1 mm, which suggests that the width and length are approximately equal. In the three-dimensional visualizations, some seeds appear to be slightly wider than long, but others appear to be near-isodiametric or slightly longer than wide (Fig. 35C, Appendix S2). The narrow micropylar canal is open and surrounded by incompletely preserved cells in most specimens (Figs. 24, 25), but in some it is closed by elongated cells (Fig. 23 at arrow), a condition characteristic of the postpollination stage of ovule development.

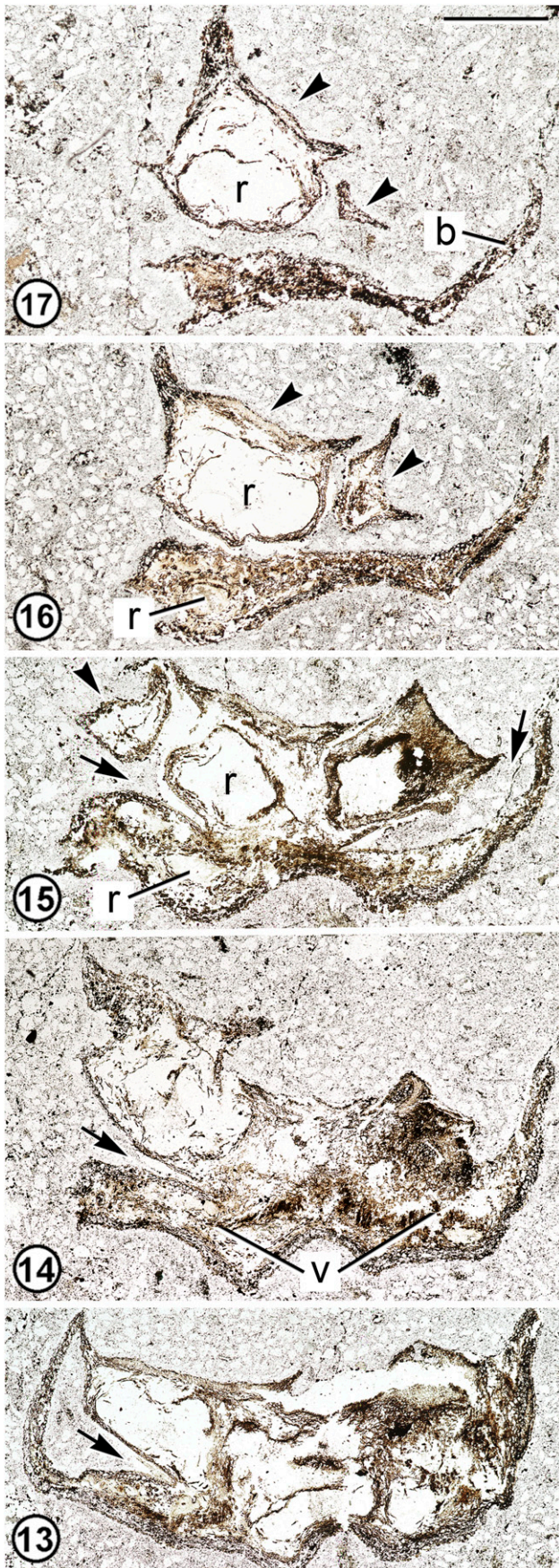
The integument measures 200–250 μm thick in the minor plane of the seed mid-region, consisting of several layers of cells that are more-or-less differentiated into zones. The innermost one or two layers of cells are thin-walled, have no internal contents, and form an endotesta (Figs. 24, 27, 28 at E). Most of the thickness of the integument consists of cells that either have somewhat thickened walls (Figs. 27, 28) and/or dark cellular contents, often giving areas of the integument a dark brown color (Figs. 19, 26, 28). In some seeds there is a thin outer zone of incompletely preserved cells that may represent sarcotesta (Fig. 27).

The nucellus is adnate to the integument up to about 75% of the length of the seed cavity (Fig. 25) and surrounds a hollow area (Figs. 24, 25). Some seeds exhibit a cellular apex of the nucellus that shows evidence of beginning to break down to form a pollen chamber (Fig. 25), whereas others clearly have already developed a well-formed pollen chamber (Figs. 18, 24). In the mid-region of the seed the nucellus ranges in thickness from a single layer (Fig. 24) to as many as four or five cell layers thick (Fig. 25). A distinct megaspore membrane is evident within the nucellar cavity of most specimens (Figs. 24, 25, 27).

DISCUSSION

Conifer systematics and clade age estimations—Figure 36 summarizes at family/subfamily level the results of eight recent

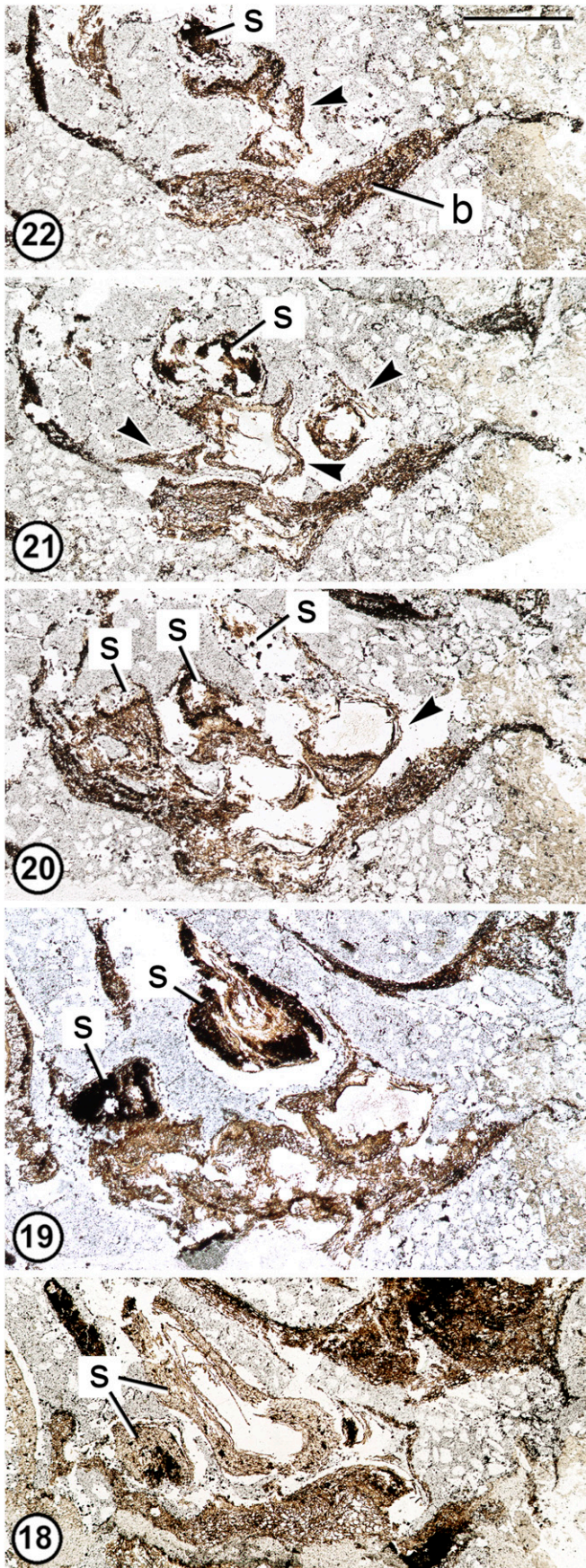
← into three traces (t). Note trace at left is extending toward abaxial surface at this level. A Top #95 \times 16 (NHMUK, V 68522\$5). Scale bar = 1 mm. **7.** Cross section of cone showing diverging bract–scale complex in peridermal view. At this level, three traces (at arrows) are diverging from each other near adaxial surface. Note more abaxial resin canal (r), and lobed ovuliferous scale proximal to separation from bract (at left). A Bot #13 \times 16 (NHMUK, V 68522\$6). Scale bar = 1 mm. **8.** Cross section of cone axis showing parenchymatous pith (p) and cortex, scale trace (t) and position (at rectangle) of incompletely preserved tracheids of vascular cylinder. A Top #115 \times 55 (NHMUK, V 68522\$7). Scale bar = 500 μm . **9.** Enlargement of incompletely preserved tracheids of vascular cylinder from position of rectangle in Fig. 8. A Top #113 \times 185 (NHMUK, V 68522\$8). Scale bar = 100 μm . **10.** Cross section of bract scale complex at level where vascular bundle to seed base (at arrow) diverges from larger bract–scale trace (t). Note vascular tissue is still adaxial to resin canals (r) at this level. A Top #89 \times 30 (NHMUK, V 68522\$9). Scale bar = 1 mm. **11.** Slightly oblique cross section of bract near tip, where there is a single resin canal (r) in mid-rib, and narrow lamina extends from slightly more proximal side. A Top #63 \times 30 (NHMUK, V 68522\$10). Scale bar = 1 mm. **12.** More distal cross section of bract than in Fig. 11, where tip is terete and the central resin canal can be identified. A Top #57 \times 30 (NHMUK, V 68522\$11). Scale bar = 1 mm.



molecular phylogenies of extant conifers, together with approximate molecular-clock estimates of divergence dates gleaned from the literature (cf. Mao et al., 2012; Yang et al., 2012). Although molecular clock-based node dating is currently remarkably popular, serious questions have been raised regarding the accuracy of phylogeny hypotheses upon which most applications of the molecular clock methods are based (i.e., hypotheses generated without inclusion of fossils; Huelsenbeck, 1991). This statement particularly applies to the deep internal nodes that help establish the overall pattern of phylogeny for ancient clades in which there has been extensive extinction (e.g., Rothwell and Nixon, 2006; Tomescu, 2009; Hipsley and Muller, 2014, and references cited therein). Even when molecular clock-based analyses report “total evidence” methodology (Sun et al., 2002), such analyses frequently do not employ total evidence in phylogeny resolution (i.e., they resolve trees from living species only, and then attach extinct species to the tree a posteriori; e.g., Mao et al., 2012). Therefore, the accuracy of the overall pattern of phylogeny represented by such results remains in serious question. Also, such results often are either directly contradicted by fossil evidence, or when considered to be hypotheses of clade ages, are falsified by testing with paleontological data (e.g., Equisetaceae; Stanich et al., 2009). As a result, many paleontologists view molecular clock dating with considerable skepticism, considering such results to often be less reliable than specimen-based minimum ages of clades determined by the oldest known well-documented fossils of densely sampled clades (e.g., Wilf and Escapa, 2014).

Because both approaches to clade age assessment rely on fossil-based date calibrations, confident assignment of the calibrating fossils to a specific clade is essential for providing reliable data for all calculations and modeling exercises (Bell, 2015). For the current study, the most critical data for dating basal nodes of the Cupressaceae are contingent on confident identification of the oldest fossils that post-date the divergence of Pinaceae from all other crown-group conifers (for conifer tree and references see Leslie et al., 2012; Wilf and Escapa, 2014). The clade age of this crucial node is most frequently calibrated by the Late Triassic conifer, *Compsostrobus neotericus* Delevoryas & Hope (Delevoryas and Hope, 1973, 1987; Leslie et al., 2012). This calibration relies on the confident assignment of *C. neotericus* to the Pinaceae, but that assignment is actually far from certain. Although some workers do accept the assignment of *C. neotericus* to the Pinaceae, others (including the original authors) consider the species to

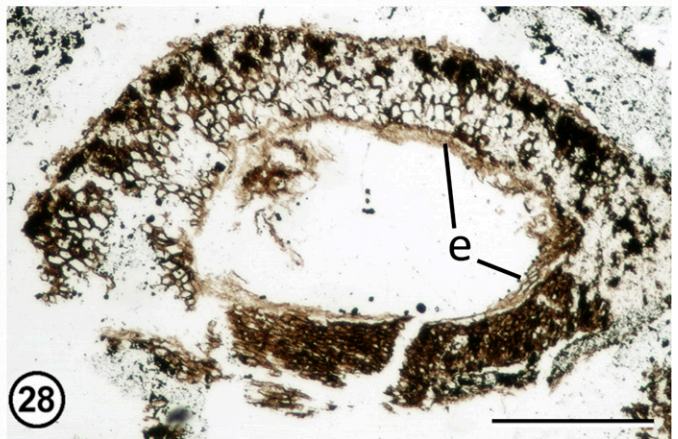
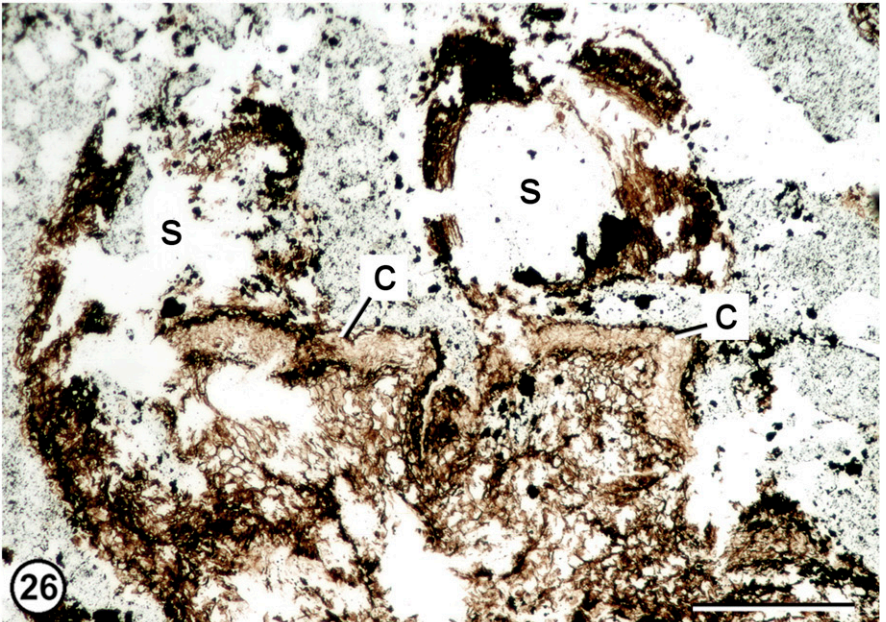
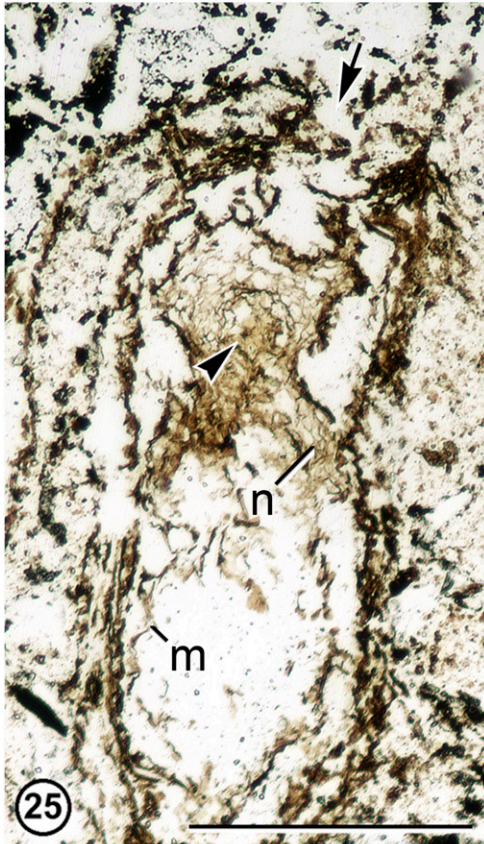
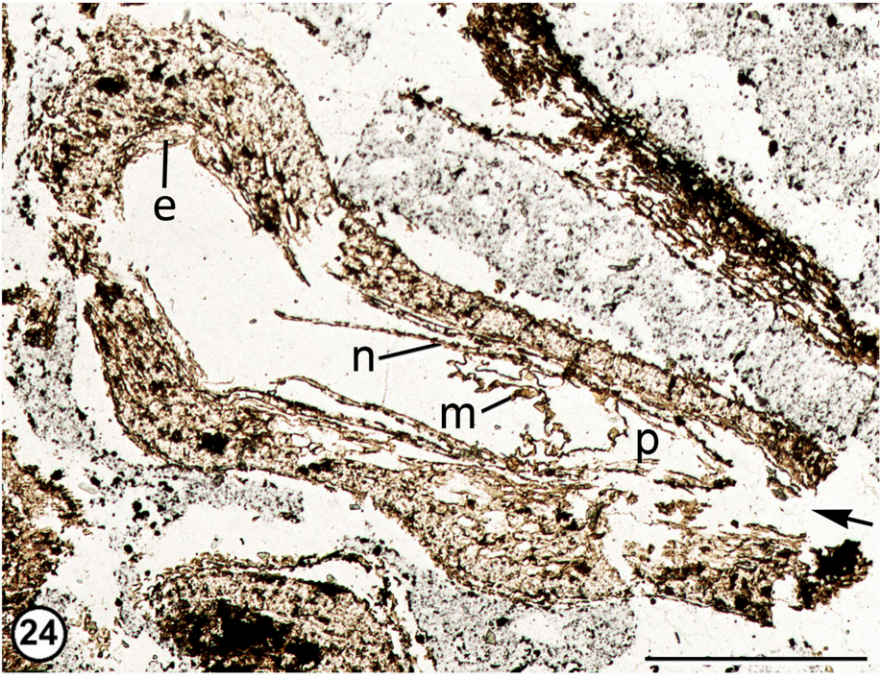
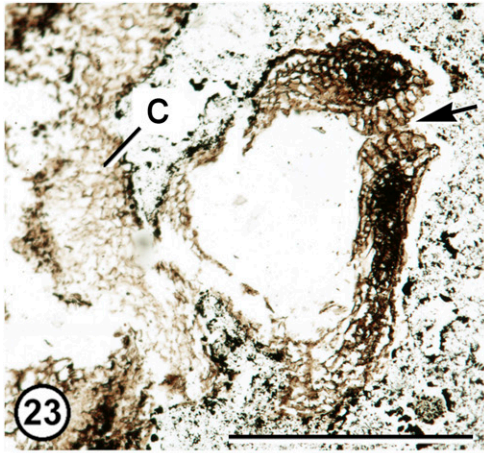
Figs. 13–17. *Scitistrobus duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. (holotype, NHMUK, V 68522). Serial sections of bract–scale complex proceeding distally (Figs. 13–17) through region where scale tips separate from the bract and from each other. No seeds are attached to this complex. Note resin canal (r) in central scale tip (Figs. 15–17). All figs. $\times 15$. Scale bar = 1 mm. **13.** Most proximal level, where bract and scale are beginning to separate from margin (at arrow). Bract and scale margins are in contact at left, but are not adnate at this level. A Top #89 (NHMUK, V 68522\$9). **14.** Level where one scale tip is separating from bract (arrow at left). Note vascular tissue of bract at v. A Top #85 (NHMUK, V 68522\$12). **15.** Level where scale tip at left (arrowhead) has separated and central scale tip is beginning to separate from bract (arrow). Note central resin canal in bract (r at bottom). A Top #81 (NHMUK, V 68522\$13). **16.** Level where scale tips (arrowheads) have separated. Note central resin canal of bract (r at bottom). A Top #75 (NHMUK, V 68522\$14). **17.** Most distal level of bract–scale complex showing two free scale tips (arrowheads) adaxial to bract (b). A Top #71 (NHMUK, V 68522\$15).



be of alternative or uncertain familial affinities (e.g., Delevoryas and Hope, 1973; Rothwell et al., 2012). Subsequent clarification of some features of *C. neotericus* (Delevoryas and Hope, 1987) nonetheless left several others that deviate considerably from those considered typical of Pinaceae (Rothwell et al., 2012). Additional features of *C. neotericus* that are either not characteristic of, or not found elsewhere in, Pinaceae include (1) lax cone architecture resembling that of voltzialean stem-group conifers, (2) seeds with an attenuated microphyllar tube, and (3) seeds that lack a membranous wing of ovuliferous scale derivation (Delevoryas and Hope, 1973, 1987; Rothwell et al., 2012). Additional concerns about assigning *C. neotericus* to Pinaceae derive from the Pinaceae-like leafy shoots and pollen cones that are associated with, but not physically attached to, the seed cones. Although those associated remains could be parts of the *C. neotericus* plant, they also could have been produced by the plants that bore two other conifer seed cones occurring in the same fossil assemblage (i.e., *Voltzia andrewsii* Delevoryas & Hope and *Metridiostrobus palissyaeoides* Delevoryas & Hope; Delevoryas and Hope, 1973, 1975, 1981). Therefore, the equivocal familial relationships of *C. neotericus* render that species an unreliable fossil upon which to calibrate the age of the stem-group node of Pinaceae. If *Compsostrobus* is not assignable to the Pinaceae, then the oldest fossil for calibration for a minimum age of this crucial node (arrowed in Fig. 36) is the Early Jurassic *Austrohamia minuta* Escapa, Cuneo & Axsmith (ca. 189 Ma; Cuneo et al., 2013), significantly pre-dating the age of *A. minuta* (i.e., 164.2 Ma) accepted by Mao et al. (2012) and post-dating the calibrated nodes for the more highly derived cupressaceous clades presented in Fig. 36.

These caveats notwithstanding, we consider it important to evaluate *Scitistrobus* in relation to molecular clock clade-age calculations to provide an opportunity to relate our approach to the most popularly applied methodologies and to employ fossils in the testing of clade-age hypotheses. The molecular clock estimates of divergence dates for nodes of the Cupressaceae (Fig. 36) suggest that the Bearreraig cone—dated to 173 ± 1 Ma—should post-date all of the family divergences and all of the subfamily divergences within Cupressaceae s.l. other than the separation of Cupressoideae from Callitrioideae and arguably also the divergence of Taxodioideae (Fig. 36). On the other hand, if the family divergences are calibrated by *Austrohamia minuta* then *Scitistrobus* is relatively close to the basal family divergence node, probably pre-dating all of the cupressaceous subfamily divergence nodes estimated in Fig. 36. Fortunately, although inferred absolute nodal dates are most likely subject to considerable errors, the relative timings of lineage separation inferred from molecular topologies are more consistent, confidence in their reliability being enhanced by the large proportion of major coniferous lineages that are still represented in the extant flora. Clearly, it is important to compare

Figs. 18–22. *Scitistrobus duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. (holotype, NHMUK, V 68522). Serial sections of bract–scale complex with seeds (s) attached to scale tips, proceeding distally (Figs. 18–22) through region where scale tips (arrowheads) separate from the bract (b) and from each other. All figs. $\times 15$. Scale bar = 1 mm. 18. A Top #106 (NHMUK, V 68522\$16). 19. A Top #100 (NHMUK, V 68522\$17). 20. A Top #95 (NHMUK, V 68522\$5). 21. A Top #92 (NHMUK, V 68522\$18). 22. A Top #89 (NHMUK, V 68522\$9).



Scitistrobus duncaanensis with most coniferous crown groups, as well as with several exclusively fossil groups.

Morphological comparison of *Scitistrobus* with other cupressaceous taxa—Among crown-group conifers that are characterized by seed cones constructed of a large number of helically arranged bract–scale complexes, *Scitistrobus* shares a conspicuous bract and less obvious ovuliferous scale with the three earliest-diverging lineages of the Cupressaceae (epitomized by *Cunninghamia*, *Taiwania*, *Athrotaxis*), estimated to have originated sequentially between ca. 220–190 Ma by Mao et al. (2012) and ca. 200–185 Ma by Yang et al. (2012). However, these estimates were based substantially on the questionable assignment of *Compsostrobus neotericus* to Pinaceae. By contrast, species of the Pinaceae have a large conspicuous ovuliferous scale and typically a smaller bract, and species of both the Araucariaceae and Sciadopityaceae have a bract and scale that are approximately equal in size (Miller, 1977; Stockey, 1982; Farjon, 2005). In Pinaceae, Araucariaceae, and Sciadopityaceae, the ovuliferous scale is a single essentially undivided unit. The extinct stem-group conifer family Cheirolepidiaceae also has a large number of helically arranged bract–scale complexes in which the bract and scale are of similar size. However, in that family the scale is divided into several separate tips (Clement-Westerhof and Van Konijnenburg-Van Cittert, 1991; Escapa et al., 2012; Steart et al., 2014), a feature shared by some other extinct conifers of uncertain familial affinities (e.g., *Bancroftiastrobus digitata* G.W.Rothwell, G.Mapes, Stockey & J.Hilton and *Schizolepopsis* spp.; Leslie et al., 2013). Seed cones of the other crown-group conifer families (i.e., Podocarpaceae and the sister-group pairing of Taxaceae plus Cephalotaxaceae) typically are smaller in size and/or have a much smaller number of bract–scale complexes that bear seeds (Sporne, 1965; Knopf et al., 2012).

Among the Cupressaceae, *Scitistrobus duncaanensis* is relatively unusual in having a distinctly cylindrical cone shape (Figs. 35a), whereas most other seed cones of that family are typically globose/ovoid to ellipsoidal in shape (Farjon, 2005). Only the extinct cupressaceous species *Cunninghamiostrobus yubariensis* Stopes & Fujii (Stopes and Fujii, 1909; Ohana and Kimura, 1995) and *Hubbardiostrobus cunninghamioides* B.A. Atkinson, G.W. Rothwell & Stockey (Atkinson et al., 2014) share a cylindrical shape with *S. duncaanensis* (Ohana and Kimura, 1995; Atkinson et al., 2014). The free ovuliferous scale tips of *S. duncaanensis* are reminiscent of extant *Cunninghamia lanceolata* (Lamb.) Hook. and *C. konishii* Hayata (Serbet et al., 2013), as well as several extinct cunninghamioid species (Atkinson et al., 2014; Shi et al., 2014). Free scale tips are absent from most living and extinct species of *Athrotaxis* and *Taiwania* (Klymiuk et al., 2015), and also from the extinct *Cunninghamiostrobus yubariensis* (Atkinson et al., 2014). Where

present, the scale tip (or tips) of those extinct species separate from the bract distal to the level of seed attachment (e.g., *Elatides zhoui* G. Shi, A.B. Leslie, Herend., Ichinnorov, Takahasi, Knopf & P.R. Crane; Shi et al., 2014).

Among early-divergent Cupressaceae with free scale tips, all species of *Cunninghamia*, *Cunninghamiostrobus hueberi* Miller, *C. goedertii* Miller (Miller and Crabtree, 1989), *Parataiwania nihongii* M. Nishida, Ohsawa & H. Nishida (Nishida et al., 1992), and *Acanthostrobus edenensis* Klymiuk, Stockey & G.W. Rothwell (Klymiuk et al., 2015) have a single free tip per ovuliferous scale. By contrast, *Scitistrobus duncaanensis*, *Hughmillerites juddii*, *H. vancouverensis*, and *Hubbardiostrobus cunninghamioides* all have a separate free scale tip for each of the three seeds per bract–scale complex. *Elatides zhoui* is variable in both number of seeds per bract–scale complex (four to six) and in the morphology of the free scale tip(s). Whereas some complexes of *E. zhoui* have a single free tip per seed, in others there is only one tip per complex (Shi et al., 2014).

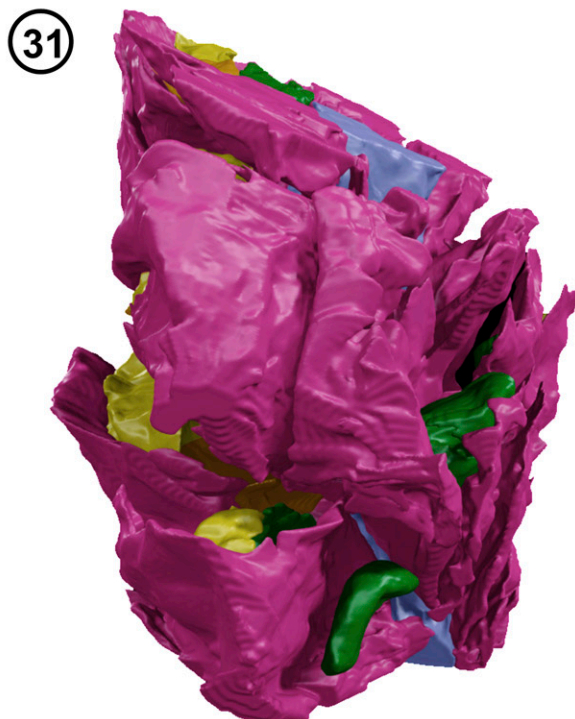
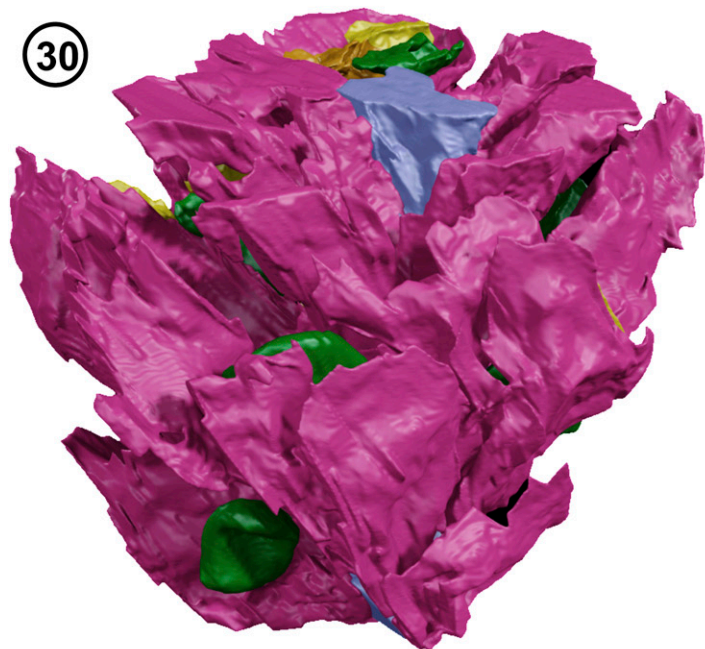
The only living cupressaceous conifer that possesses several scale tips is *Cryptomeria japonica* (L.f.) D. Don, a genus consistently placed by molecular phylogenetic studies as the earliest-divergent extant genus within the more derived subfamily Taxodioideae (Fig. 36). In *C. japonica* several seeds are produced near the base of the ovuliferous scale, below the separation of robust free scale tips that protrude beyond the apex of the bract (Eckenwalder, 2009). *Scitistrobus duncaanensis* has larger and more prominent scale tips than all other previously described early-divergent cupressaceous seed cones. Perhaps more importantly, only in *S. duncaanensis* are the seeds attached to the free scales distal to the level of scale separation from the bract (Figs. 13–22, 31, Appendix S2). In all the species of early-divergent extant lineages of Cupressaceae (i.e., species of Cunninghamioideae, Taiwanioidae, and Athrotaxoideae sensu Farjon, 2005: fig. 36), either no free tips are developed or the seeds are attached to the bract–scale complex proximal to the level where the scale tips separate from the bract. Moreover, in all other cupressaceous species except *Cryptomeria japonica*, the free scale tips consist only of a membranous structure that is pressed closely to the adaxial surface of the bract. By contrast, each scale tip of *S. duncaanensis* is a much more robust structure that bears a seed and may be vascularized. The free scale tips of *S. duncaanensis* also curve away from the adaxial surface of the bract (Figs. 31, 35b) and overarch the adaxially attached ovule, thereby contributing to orientation of the seed micropyle toward the cone axis.

The latter features of *S. duncaanensis* are especially reminiscent of ovuliferous scale morphology in voltzian Voltziales (sensu Rothwell et al., 2011)—the group from which Cupressaceae has frequently been hypothesized to have evolved (e.g., Schweitzer, 1963, 1996; Rothwell et al., 2011). A broad range

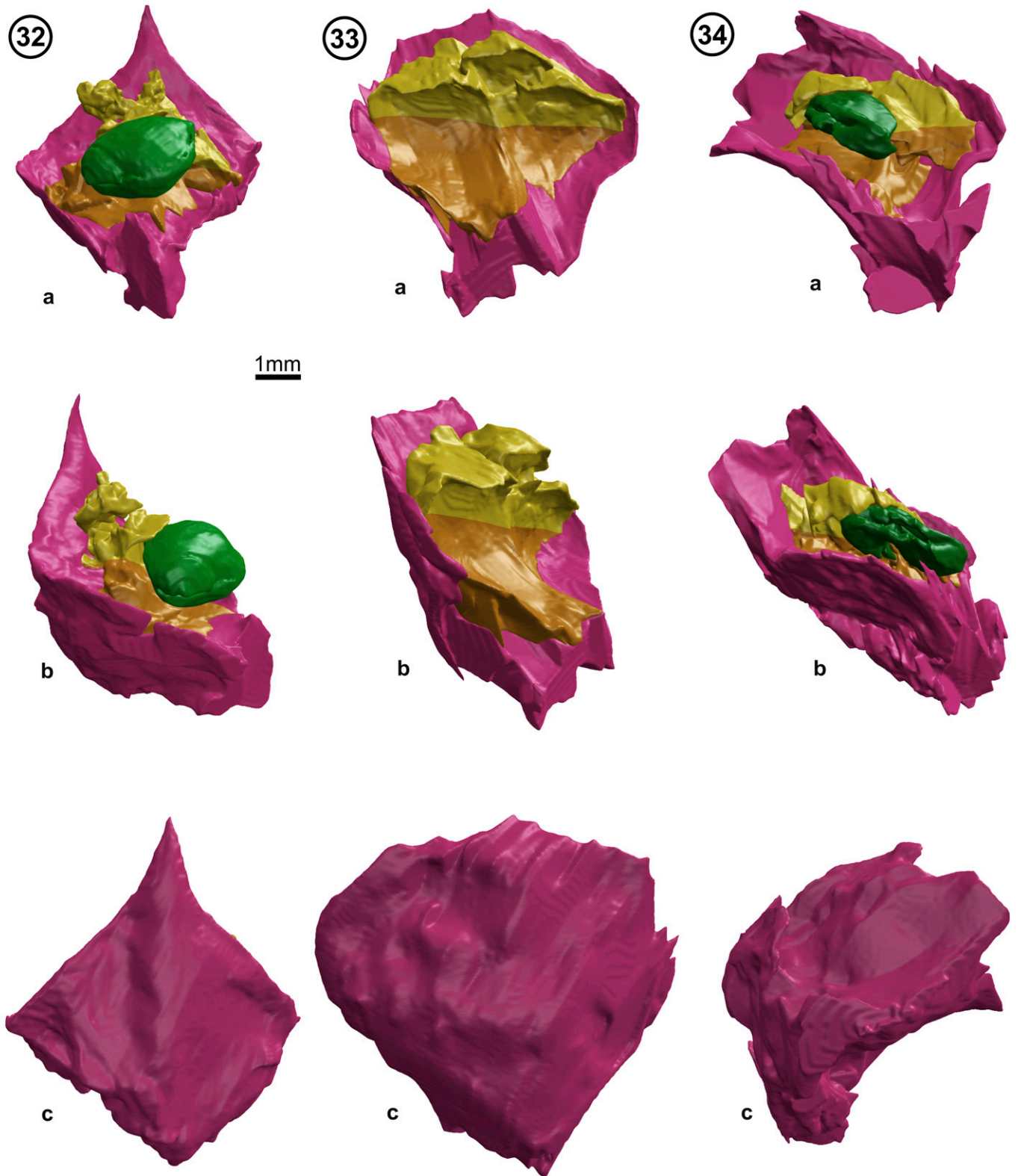
← Figs. 23–28. *Scitistrobus duncaanensis* A.R.T. Spencer, G. Mapes, J. Hilton & G.W. Rothwell, gen et sp. nov. (holotype, NHMUK, V 68522). Features of seeds and seed attachment. All scale bars = 500 μm. **23.** Oblique distal section of attached seed showing histology of integument at micropyle (arrow) and closing tissue (c) of scale tip at position of seed abscission. A Top #22 X60 (NHMUK, V 68522\$19). **24.** Longitudinal section of seed showing features of integument, endotesta (e), nucellus (n), and megaspore membrane (m). Note large pollen chamber (p) below micropyle (arrow). A Top #106 X55 (NHMUK, V 68522\$16). **25.** Oblique section near apex of seed at less mature stage than in Fig. 24, showing nucellus (n) and megaspore membrane (m) within integument. Note nucellar apex below micropyle (arrow) where cells in central area (arrowhead) appear to be breaking down to form pollen chamber. A Bot #32 X70 (NHMUK, V 68522\$20). **26.** Oblique section of bract–scale complex showing closing tissue (c) of abscission zones below two attached seeds (s). A Top #70 X40 (NHMUK, V 68522\$21). **27.** Oblique cross section of distorted seed showing histology of integument, endotesta (e), and megaspore membrane (m) within nucellus. Note cells of integument at bottom left are smaller than in other areas of integument. A Bot #6 X40 (NHMUK, V 68522\$22). **28.** Mid-region of seed showing shape in cross section and histology of integument (e = endotesta). Note small cells with dark walls, similar to those evident in Fig. 27, in one region of integument (at bottom). A Top #99 X40 (NHMUK, V 68522\$23).



1mm



Figs. 29–31. *Scitistrobis duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. Whole cone visualizations based on a lateral external view of cone fragment, with Fig. 30 rotated by ca. 120° from Fig. 29, and Fig. 31 rotated by an additional 120°. Blue = cone axis, orange = ovuliferous scales, yellow = free scale tips, green = seeds, magenta = bracts. All are figures of holotype (NHMUK, V 68522) at same magnification.



Figs. 32–34. *Scitistrobus duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. Visualizations of three bract–scale complexes in (a) abaxial, (b) lateral, and (c) adaxial views. Colors as in Figs. 29–31. All are figures of holotype (NHMUK, V 68522) at same magnification.

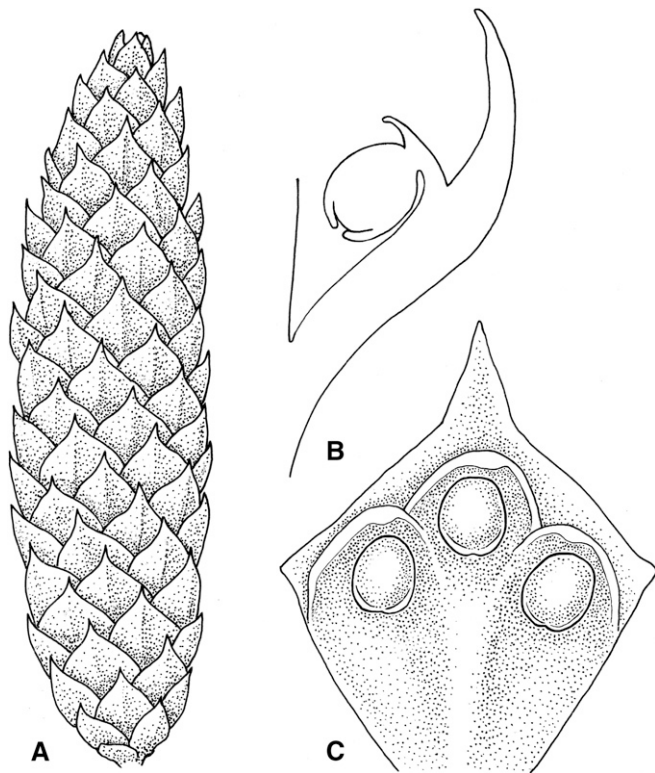


Fig. 35. *Scitistrobus duncaanensis* A.R.T.Spencer, G.Mapes, J.Hilton & G.W.Rothwell, gen. et sp. nov. Line-diagram reconstructions of (A) cone exterior, (B) bract-scale complex with adaxially attached seeds in lateral mid-section view, and (C) bract-scale complex with attached seeds in adaxial view.

of morphological diversity is documented among voltzian conifer seed cones. Some voltzian cones from Lower and Mid-Permian sediments have comparatively intricate morphologies of their bract-scale complexes (= secondary fertile shoots; e.g., *Ortiseia* Florin; Clement-Westerhof, 1984), approaching those of the more ancient (and more primitive) cordaites and walchian conifers (Rothwell et al., 2005). Other voltzians from mid-Upper Permian and Triassic sediments have fewer, and presumably more derived, structures constituting their bract-scale complexes [e.g., *Voltzia hexagona* (Bischoff) Geinitz]. Genera from the Lower Permian sediments, such as *Ortiseia* and *Dolomitia* Clement-Westerhof, tend to approach radial construction and have relatively large numbers of vegetative scales. By contrast, those from the more recent sediments of the mid-Upper Permian and Triassic (e.g., *Voltzia hexagona*, *V. libeana* sensu Schweitzer, *Lebowskia* Looy, and *Aethophyllum* Brongniart; Grauvogel-Stamm, 1978; Schweitzer, 1996; Looy, 2007) tend to be more clearly dorsiventral structures and have fewer vegetative scales. Homologies of the seed-bearing structures of voltzians remain ambiguous because such structures are not clearly comparable with either the ovulate sporophylls of antecedent cordaites and walchian conifers or the ovuliferous scales of modern conifers (Mapes, 1987; Mapes and Rothwell, 1991; Serbet et al., 2010).

The bract-scale complexes of *Scitistrobus duncaanensis* are similar to those of many voltzians in having their seeds attached to the scale distal to the level where the scale separates from the bract (Clement-Westerhof, 1988), but there are fewer segments to the ovuliferous scale in *S. duncaanensis* than there are in the

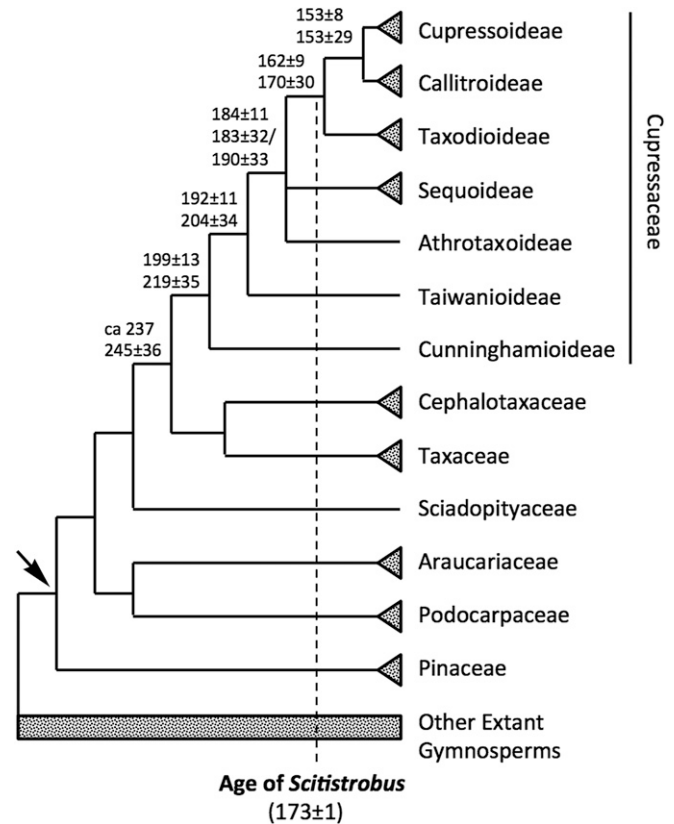


Fig. 36. Crude consensus tree summarizing relationships of extant families (and, within Cupressaceae, subfamilies) implied by trees constructed from eight substantial sequence matrices (Gadek et al., 2000; Kusumi et al., 2000; Quinn et al., 2002; Rai et al., 2008; Graham and Iles, 2009; Wu et al., 2011; Mao et al., 2012; Yang et al., 2012). Calculated molecular clock dates of nodes (in Ma) are derived above from fig. 7 of Yang et al. (2012) and below from fig. 1 plus table 1 of Mao et al. (2012). See text for details of alternative dating hypotheses.

voltzians, and there are no vegetative scale tips in *S. duncaanensis*. Moreover, there are typically five or more segments to the ovuliferous scale in the voltzians, whereas *S. duncaanensis* has only three. *Scitistrobus* resembles in this feature several Mesozoic conifers of uncertain affinities, including *Cycadocarpidium* Nathorst spp., *Swedenborgia tyttosperma* Stanislavsky, *S. megasperma* Stanislavsky, and two species of *Schizolepidopsis* (Braun) Doweld (Zhang et al., 2011; Leslie et al., 2013).

Scitistrobus duncaanensis shares the feature of ovule attachment to free scale tips with the voltzians and with the genera of uncertain affinities noted above. In other features, *S. duncaanensis* clearly is more closely allied with the Cupressaceae, a conclusion supported by the results of a preliminary cladistic analysis of conifer seed cones (G. W. Rothwell et al., unpublished data). Voltzian seed cones typically are quite lax and elongated structures, whereas those of *S. duncaanensis*, Cupressaceae, and most other crown-group conifer families (particularly Pinaceae, Sciadopityaceae, and Araucariaceae) are much more compact (Leslie, 2011). The bract and scale of *S. duncaanensis* and those of early-divergent Cupressaceae are adnate to the mid-region of the bract-scale complex, unlike those of many voltzians (in other voltzians the degree of bract-scale fusion remains unclear). Also, *S. duncaanensis* shares with species of the Cupressaceae a well-developed system of

resin canals in the bract–scale complexes, a feature that either is absent from (Yao et al., 1997) or unknown for voltzians.

General evolutionary trends in conifer seed-cone morphology through time—The most ancient of the Pennsylvanian Voltziales are characterized by lax cylindrical cones with large numbers of relatively open parts, consisting of clearly recognizable bracts with axillary fertile shoots that were relatively radial and that displayed both a large number of vegetative scales and small number of sporophylls (Florin, 1951; Mapes and Rothwell, 1991; Rothwell et al., 2005; Leslie, 2011). By the Permo-Triassic, voltziales cones had evolved ovuliferous short shoots with fewer parts. Some showed pronounced bilateral symmetry, and others produced only a single seed in the axil of the bract (Archangelsky and Cúneo, 1987; Rothwell et al., 2005; Serbet et al., 2010). During the Triassic and Jurassic, there was a structural transition to cones that were more compact (Leslie, 2011) with ovuliferous scales that were progressively simplified (Escapa et al., 2010; Rothwell et al., 2012). Following the origin of crown-group conifers, diverging morphological trends characterized each modern family.

Cephalotaxaceae and Taxaceae show progressive reduction in number of bract–scale complexes, size and complexity of ovuliferous scales, and elimination of bracts, until only a single terminal ovule is present in the axil of a vegetative leaf (viz., *Taxus* spp.; Sporne, 1965; Mundry, 2000). Within most genera of Podocarpaceae, the number of fertile bract–scale complexes is most often reduced to one or two, with only a single seed per complex (e.g., Kelch, 1998; Knopf et al., 2012). Within the Araucariaceae, the bract and scale become more-or-less fused into single unit, and in some species the seed becomes partially or completely enclosed (Escapa and Catalano, 2013).

Focusing on the Cupressaceae sensu Eckenwalder (1976, 2009), seed cones display the greatest diversity of morphologies in a single conifer family but nonetheless show several trends in structural changes from the base to the tips of the phylogenetic tree (Farjon, 2005; Schulz and Stützel, 2007). In terms of general morphology, the early-divergent subfamilies Cunninghamioideae, Taiwanioidae, and Athrotaxoideae (each apparently surviving with only a relictual distribution today; e.g., Farjon, 2005) share with *Scitistrobis* a more compact seed cone than those of voltziales, possessing a lower length to width ratio than the highly elongated but similarly cylindrical cones of the Voltziaceae (Leslie, 2011). Among the more derived extant subfamilies of Cupressaceae (i.e., Sequoioideae, Taxodioideae, Callitrioidae, Cupressoideae: Fig. 36), the number of bract–scale complexes is reduced further, and the cones tend to be ellipsoidal to spheroidal in outline. All are highly compact before maturity, and in some genera the ovuliferous scale is so highly reduced that the seed cones appear to be of simple construction (i.e., with seeds that have the superficial appearance of being either axillary or attached directly to the adaxial surfaces of bracts; Tomlinson et al., 1993; Schulz and Stützel, 2007). In addition, most and possibly all of these subfamilies are predicted from sequence data to have originated later than the Bajocian (Fig. 36).

Homology challenges in ovulate cones of conifers—Conifers are most likely to appear in textbooks of plant evolution in the context of the classic hypothesis of homology of pollen and seed cones most famously synthesized (though not originated) by Florin (1951, 1954). Florin's hypothesis is best described (albeit

simplistically) by comparing the reproductive organs of Pennsylvanian conifer-relatives of the Cordaitales with those of extant conifers, which are most commonly represented in studies of all kinds by Pinaceae. In both cases, the reproductive shoots are monosporangiate, a condition that is so prevalent as to be nearly ubiquitous among gymnosperms (reviewed by Bateman et al., 2011). In the case of cordaites, the pollen cones and seed cones are similar in size and architecture, allowing confident identification of homologies between them. Both “genders” of cone are widely accepted as compound fructifications, the elongated primary shoot bearing helically arranged determinate secondary shoots, each of which is subtended by a modified leaf termed a bract. The secondary shoots bear in similarly helical arrangements further modified leaves, in this case termed scales. Most of the numerous scales are sterile, but concentrated toward the distal end of the secondary shoot are fertile scales (i.e., sporophylls) that terminate in either pollen sacs or ovules.

Florin's hypothesis views the seed cone of Pinaceae as a condensed and partially fused homolog of the cordaite cone, the bract–scale complex being the precise homolog of the determinate lateral shoot of the cordaite “inflorescence” and the cone thereby determined as compound. By contrast, the pollen cones of Pinaceae are smaller and bear microsporophylls directly on a determinate axis in a spiral arrangement; thus, they are traditionally viewed as being simple rather than compound and homologs of a single secondary branch of a cordaite inflorescence (e.g., Mapes and Rothwell, 1998; Hernandez-Castillo et al., 2001). In other words, the evolutionary transition(s) from cordaite to pine pollen cones involved structural simplification via loss of an order of branching. Returning to the ovulate cone, the transition from cordaite to pine cone obviously required much morphological modification: the ovuliferous scale has become at least partially (presumably congenitally) fused with, and at maturity exceeds, the subtending bract. These two categories of organ retained separate vascular supplies from the cone axis, indicating the compound nature of the bract–scale complex. The trace in the ovuliferous scale divides to supply the ovules, which are partially embedded and recurved such that the inversion causes the micropyle of each ovule to face the cone axis. The resulting bract–scale complex is strongly bilaterally symmetrical.

Understandably, there has long been considerable enthusiasm for testing these fundamental hypotheses of homology. One obvious test is to simply expand the logic from which the hypothesis was originally derived and apply it in field situations, asking the question “Can fossil conifers be found with character combinations that allow phylogenetic interpolation between fossil cordaites and modern conifers?” Examples such as the Voltziales provide an unequivocal and positive answer to this question (cf. Clement-Westerhof, 1984; Mapes, 1987; Mapes and Rothwell, 1991; Rothwell and Mapes, 2001; Rothwell et al., 2005, 2011; Serbet et al., 2010). In the realm of extant conifers, useful evidence has also been derived from ontogenetic studies—initially from microscopic examination of contrasting developmental stages, but later from more integrative studies of developmental genes and their expression within developing cones. We will now briefly review this evidence, with the aim of better interpreting the mature morphology of the *Scitistrobis* ovulate cone and its putative relatives.

Placing *Scitistrobis* and its relatives within an evolutionary-developmental context—Obviously, with respect to *Scitistrobis*, the most useful evolutionary-developmental genetic

data would originate from comparatively early-divergent lineages among the extant Cupressaceae (Fig. 36). Unfortunately, most of the relevant data obtained thus far have originated from Pinaceae, most commonly *Picea* (e.g., Theissen and Becker, 2004; Vazquez-Lobo et al., 2007; Carlsbecker et al., 2013; Gramzow et al., 2014), and the remainder are from more derived Cupressaceae (including *Cryptomeria*: Groth et al., 2011) or from contrastingly “fleshy-fruited” conifers representing the Podocarpaceae and Taxaceae (Vazquez-Lobo et al., 2007).

Much of the early research in this area adopted a top-down perspective, effectively treating gymnosperms as modified angiosperms rather than vice versa (Bateman et al., 2006). It was inevitable that the now famous ABC(D)E model of developmental control of the angiosperm flower would be superimposed on conifers. It rapidly became clear that the non-ABC gene *LEAFY* fulfilled at least one consistent function across all seed plants, dictating the transition from indeterminate to determinate growth and thus to reproduction. Early evidence suggested that the B-class genes responsible for differentiating petals from stamens in angiosperms are expressed only in the microsporophylls of pollen cones of monosporangiate gymnosperms (e.g., Theissen and Becker, 2004). By contrast, the C, D, and E class genes required for ovule development in angiosperms were seemingly represented in conifers by only a C-D class precursor that is expressed in both seed and pollen cones (e.g., Theissen and Becker, 2004). And unsurprisingly, the A class genes that specify sepal identity in angiosperms appeared absent from gymnosperms. However, comparative studies such as that of the expression of *NEEDLY* and its functionally divergent ortholog *LEAFY* in *Picea*, *Podocarpus*, and *Taxus* performed by Vazquez-Lobo et al. (2007) rapidly exposed more complex scenarios, in which expression zones of these developmentally crucial genes altered greatly in extent and location during cone ontogeny, both among and within particular organs.

These and other interpretational uncertainties encouraged considerable anticipation regarding clarification to be gained via the earliest results obtained through next-generation sequencing of conifers. Gramzow et al. (2014) recently surveyed both “whole genome” and “whole transcriptome” data sets from not only *Picea abies* but also *Picea glauca* and *Pinus taeda*. They found MADS-box genes to be far more numerous in seed plants than in pteridophytes. In comparison with angiosperms, these model Pinaceae were generally richer in pseudogenes; among functional genes, conifers had by far the greater ratio of type II MADS-box genes that are widely expressed across the plant bauplan compared with type I MADS-box genes whose expression is largely confined to the megagametophyte and ovule. Also, they uncovered surprisingly little evidence of genome duplications (a process increasingly viewed as driving macroevolution in angiosperms: e.g., De Bodt et al., 2005), which in turn implies limited opportunities in conifers for neofunctionalization (divergence of one paralogous copy of a duplicated gene from its ancestral to a novel function).

Among the type II MADS-box genes, at least seven transcribed MIKC*-P genes were detected by Gramzow et al. (2014) that together were expressed in vegetative organs and both pollen cones and seed cones. By contrast, only one MIKC*-S gene was found, and it was expressed exclusively in pollen cones. C class genes were few and widely expressed. Among E class genes, *AGL2* was expressed in vegetative organs plus pollen cones, whereas *SQUA* was expressed in vegetative organs plus seed cones. Among B class genes, *DEF/GLO*-like genes are typically expressed in pollen cones, but *GGM13*-like genes are expressed

more widely across the bauplan, though in *Picea abies* they appeared to be confined to the seed cone. Genes of the TM8 clade evolved rapidly, individual genes similarly being divided between pollen and seed cones. Lastly, within the TM3 clade, *DAL3* and *DAL19* were expressed in both pollen and seed cones of *P. abies* but at contrasting developmental stages, hinting at the likely complexity of expression patterns and thus the difficulty of making valid comparisons across conifer families. Retaining the theme of *Picea abies*, and moving from taxonomic comparisons that are exceptionally broad to those that are exceptionally narrow, Carlsbecker et al. (2013) extended earlier work comparing wild-type and mutant conifer cones (reviewed by Bateman et al., 2011). Specifically, they explored the expression of several categories of gene (inevitably including the major categories of MADS-box genes) in wild-type and *acrocona* mutant cones of *P. abies*. Wild-type *Picea* cones have a typical Pinaceae organization, whereas in the *acrocona* mutant they are reminiscent of fossil voltzialean in being far less condensed and are believed to be homeotic transformations of leafy shoots. Structures resembling ovuliferous scales initiate in the axils of bract-like needles, often partially fused and bearing multiple ovules that, contrary to the wild-type morph, are not inverted toward the shoot axis (Carlsbecker et al., 2013). Their study showed that both morphs had ovuliferous scales with distinct internal domains. The lateral parts of the scale that generate the ovules showed strong expression of *LEAFY* and an A class gene allied to *AP2*, whereas the sterile central-abaxial zone saw strong expression of *DAL21*; other genes, such as *DAL2*, were expressed throughout the scale. In the *acrocona* mutant, the transition to determinate growth partially fails. The only wild-type gene showing little or no expression in the mutant was *DAL14*, a member of the AGL6/SEP class of genes that are known to influence both floral meristem behavior and organ identity in angiosperms.

Given these recent insights that the expression of key developmental genes in conifer cones varies greatly in degree of localization, and that their expression frequently overlaps in space and/or ontogenetic time within the bauplan of the conifer in question, conflicting views are inevitable regarding issues such as whether the multifunction gene *LEAFY* is upregulated in the axillary meristems of conifers (cf. Vazquez-Lobo et al., 2007; Moyroud et al., 2010). And conclusively demonstrating nonexpression of particular genes in particular organs throughout their ontogeny is even more difficult. Moyroud et al. (2010) further noted that we do not even yet know how *LEAFY* and *NEEDLY* function at a molecular level.

Nor can we be confident that sequence-level changes underpin these morphological transitions. The assertion of Carlsbecker et al. (2013) that the voltzialean-like ovulate cones that characterize the *acrocona* mutant of *Picea abies* may differ from the wild type only in lacking *AGL6* expression leaves us to speculate that this radical difference in cone morphology could simply reflect methylation rather than strict mutation. Certainly, methylation of cytosine or chromatin offers the most likely explanation of ectopic ovule expression occasionally recorded on leaves of *Ginkgo biloba* (e.g., Bateman et al., 2011).

More generally, it is becoming increasingly evident that cytoplasmic feedback to the genome can induce myriad alternative combinations of enhancers and promoters, which in turn permit great flexibility of transcription patterns in different tissues (e.g., Shapiro, 2011); Jablonka and Raz (2009) were able to identify five major ways in which epigenetic changes could significantly influence evolution. To cite one example, Paun et al. (2010) used epigenome scans of the polyploid-rich orchid

genus *Dactylorhiza* to pinpoint epiloci operating under divergent selection that correlated with ecologically significant variables, encouraging persistent ecological differences that presumably facilitated subsequent accumulation of species-specific genetic contrasts. With regard to extant conifers, some authors have suggested that the large average size of conifer genomes relative to those of (diploid) angiosperms could render them comparatively vulnerable to epigenetically mediated evolution. Epigenetic memory has recently been demonstrated in *Picea abies* (Yakovlev et al., 2012), and studies of *Pinus contorta* have revealed few angiosperm-like 24-nt RNAs but an extensive array of conifer-specific 21-nt RNAs (Dolgorhiema et al., 2015), suggesting that at least some fundamental differences may separate the epigenetic systems of conifers and angiosperms. In short, epigenetic insights are helping to explain why genomic data alone are insufficient to allow us to understand at a genuinely causal level the kinds of morphological transformational series evident among conifer cones.

The crucial role of morphogenesis in determining homologies in conifer cones—The question of why the 21st century discipline of evolutionary-developmental genetics has not yet yielded unambiguous answers to key questions of homology among both pollen and seed cones is best answered by reconsidering the significance of earlier ontogenetic studies of extant conifers that focused on morphogenesis, particularly those that described contrasting ontogenetic stages of the same cone species using scanning electron microscopy. Fortunately, this data set is remarkably complete for Cupressaceae s.l., observations having been gathered from *Sciadopitys* (Takaso and Tomlinson, 1991), *Cunninghamia* (Farjon and Ortiz-Garcia, 2003), *Taiwania* (Farjon and Ortiz-Garcia, 2003), *Athrotaxis* (Jagel, 2002), multiple Sequoioideae (Jagel, 2002; Takaso and Tomlinson, 1992), multiple Taxodioideae (Takaso and Tomlinson, 1989, 1990), and multiple Callitroideae plus Cupressoideae (Tomlinson et al., 1993; Jagel and Stützel, 2001; Farjon and Ortiz-Garcia, 2002; Schulz et al., 2003). The overall trends have been summarized by Jagel (2002) and Schulz and Stützel (2007).

Reviewing the range of mature morphologies exhibited by Cupressaceae and their closer relatives emphasizes the relevance of classic categories of morphological evolution. The overall architecture of the cones is largely determined by a combination of partial homology—the relative proportions of leaf-derived bracts and leaf-derived ovuliferous scales—and serial homology—the absolute and relative numbers of each of these organ types. The structure of individual ovule-bearing bract–scale complexes largely reflects the relative degrees of development of the bract and ovuliferous scale. As noted by Vazquez-Lobo et al. (2007), it is tempting to view the remarkable variation in this feature among conifers as purely the result of some classic examples of various categories of heterochrony, both paedomorphic and peramorphic (cf. Gould, 1977; Alberch et al., 1979; Rothwell, 1987; Rothwell et al., 1996). However, of equal importance is the presence and degree of fusion (presumably congenital) between the two organs. Vestigial remains in some taxa suggest that ovule numbers are readily reduced by organ suppression, with downstream consequences for vascular patterning and also sometimes for lobing of the ovuliferous scales in particular. And the equally striking variation in the orientation of the ovules at maturity depends largely on the degree of fusion of the bract and ovuliferous scale and, if fused, the degree of differential dorsiventral growth available to permit adaxial recurvature of the ovule-subtending organ. Ontogenetically,

ovules of most taxa are initiated early and in the axil of the bract, but their mature position can differ radically, depending on the amount of intercalary growth undergone during ontogeny by various elements of the bract–scale complex.

Fully realizing the high level of ontogenetic change in the structure of conifer cones within developmental trajectories of extant species, and between the trajectories of contrasting species, helps to explain why gene expression patterns and evolutionary-developmental genetic studies have yielded at best only partial answers to homology questions among conifer cones.

The combination of differential reduction in complexity and/or size of features of the bract–scale complex, together with a tendency for increased fusion and/or embedding of organs, reduces organ identity and hence renders homologies more obscure. Moreover, neofunctionalization and subfunctionalization tend to have the same effect at the level of gene expression (e.g., Carlsbecker et al., 2013). Inevitably, the overall trend in extant conifers is one of increasing localization of gene expression (e.g., Bateman et al., 2011).

Several contrasting analytical approaches have all yielded data indicating that many aspects of conifer cone development are subtle and metastable, reflected in a general difficulty of identifying level of gene expression and its consequences. For example, Bateman et al. (2011) noted that, despite the wide range of gender control mechanisms inferred in contrasting groups of conifers, gender control appears readily perturbed. Mutant bisexual cones exhibiting developmental “hybrid zones” that support both megasporophylls and microsporophylls (Bateman et al., 2011; Flores-Renteria et al., 2011; Rudall et al., 2011) suggest that even gender expression may reflect hormonal clines; these are likely to be subject to tipping points at particular hormonal concentrations.

Perhaps the best analogous developmental conundra outside of the gymnosperms lie in the flowers of pre-eudicot extant angiosperms, where several hypotheses advanced to explain their relatively high diversity of floral form all rely on some form of overlap of expression zones, yielding the competing shifting border, sliding border, and fading borders models (e.g., Soltis et al., 2009). A particularly apt analogy can be made between the at least partially fused bract–scale complex of conifers and the most characteristic feature of orchids, the gynostemium. This structure consists of intimately fused seed-producing and pollen-producing organs, the majority of the latter being suppressed during ontogeny. Despite much effort, the evolutionary-developmental genetics underlying these crucial ontogenetic fusions in the orchid gynostemium have not yet been satisfactorily elucidated, whereas the genetics underpinning morphogenesis of the orchid perianth are now well understood (Mondragón-Palomino and Theissen, 2011; Rudall et al., 2013), presumably because it consists of three categories of discrete organs. Compared with orchid flowers, unraveling molecular-level homologies among the reproductive organs of conifers such as *Scitistrobis* is even more challenging.

CONCLUSIONS

Recent years have seen DNA sequence data derived from extant species rise to dominance in phylogeny reconstruction. Rather than being treated as a near-independent test of phylogenetic hypotheses derived from morphological data sets, molecular phylogenies have come to be viewed as superior to, and hence have largely supplanted, morphological phylogenies. If

they consider morphological characters at all, neontological analysts typically score a narrow range of such characters considered a priori to be of greatest interest among the chosen taxa and then map them across a topology dictated by molecular data. Mapping inevitably reveals some degree of homoplasy that is then used immediately to reinforce the argument that morphological data are inferior to molecular data. Moreover, both the morphological and molecular phylogenetic communities widely consider that the recognition of widespread morphological homoplasy has fatally compromised earlier precladistic notions of transformation series that were often driven by unique combinations of morphological characters reported in particular fossils—a pertinent example being the crucial role of Carboniferous cordaites in prompting Florin's (1951, 1954) influential hypotheses of homologies with pollen and seed cones of conifers. In effect, it is the improbable simplicity, apparent lack of testability, and above all the resistance of paleontologically based hypotheses to either proof or falsification that has led to the spectacular fall from grace of transformation series as an explanatory tool in evolution.

However, we suggest that recent evidence shows clearly that neontological approaches have also proven routinely simplistic. There is no shortage of complicating factors in molecular phylogenetics, and gene expression studies are riddled with both technical and interpretational uncertainties. One popular oversimplification is to state that only genetic data derived from extant organisms can inform on process rather than simply on pattern. Yet we have seen here that, at a fundamental level, *evodevo* data are also informing on pattern rather than process. None of the presently available approaches is, in truth, able to unequivocally prove or falsify any homology statement. Rather, all of the available approaches to interpreting morphological evolution can ultimately be reduced to probability estimates that, if done sufficiently well, can crudely assess the relative merits of competing hypotheses. Under these circumstances, the strongest probability statements are likely to emerge from congruence *between* data sets—in other words, from reciprocal illumination between contrasting approaches used to generate and test hypotheses of homology and thereby of relationship.

Returning to *Scitistrobis*, we argue that the novel combination of morphological features interpreted by us as being exhibited by this well-preserved ovulate cone is at least close to unequivocal. Admittedly, reaching this level of confidence has proven time consuming. For example, for much of this study we suspected that the micropyle of the *Scitistrobis* ovules faced away from, rather than toward, the cone axis; the correct interpretation of this character became clear only when our x-ray microtomography data set was used to produce a three-dimensional model with a sufficiently high level of spatial resolution. Despite the considerable levels of homoplasy evident in conifer phylogenies, *Scitistrobis* presents a compelling combination of character states that are more typical of either primitive, early-divergent voltzians or early-divergent extant Cupressaceae subfamilies (Cunninghamioideae, Taiwanoideae, and even in some characters early-divergent Taxodiaceae). Its morphology fits well the overall trends of sporadic organ reduction and/or suppression and fusion that characterize conifer evolution (e.g., Florin, 1954; Rothwell et al., 2011), and it is of an appropriate age to constitute the earliest-known stem-group Cupressaceae.

Our next project will include this newly described fossil in a morphological cladistic analysis of a taxonomically broad representation of conifer seed cones that will be informed by, but

not dictated by, studies of the ontogeny and evolutionary-developmental genetics of extant cones (G. W. Rothwell et al., unpublished data). While recognizing the dangers involved in generating phylogenies from single organs (cf. Bateman and Simpson, 1998; Schulz and Stützel, 2007; Bateman and Hilton, 2009), the present limited data mean that this remains the only practical approach to integrating extant and fossil taxa, and—as we have illustrated here—conifer seed cones are especially rich in phylogenetically useful characters. Admittedly, homology assessments among fossil cones will continue to be challenging, as will detecting whether identified changes are fully representative of evolutionary or transformational change. Further fossil discoveries would be helpful, especially when correlation proves possible between pollen cones and seed cones of the same extinct conceptual whole-plant species, or when multiple developmental stages of the same cone-species are detected.

In the meantime, research on extant conifers is likely to encompass more species, and more ontogenetic stages within those species. Exponential increase in next-generation sequencing should refine our current knowledge of the diversity of different categories of gene, though much greater progress would ensue if at least one extant conifer were to prove transformable and so become the gymnospermous model organism analogous to *Arabidopsis* (Ruelens and Geuten, 2013). Until we have available the yardstick of a genuinely well-understood processes underlying the ontogeny of both pollen cones and seed cones in at least one conifer, comparisons between species will remain speculative (Bateman et al., 2011). Otherwise, the all-important subtleties of gene expression, such as gradation of expression within the cones and through ontogenetic time, combined with pleiotropic expression patterns and major epigenetic effects (e.g., Jablonka and Raz, 2009; Shapiro, 2011), are likely to preclude definitive homology statements for the foreseeable future. For now, we will continue to emphasize the immense value of reciprocal illumination between the living and the fossil.

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