Fairyland is nothing but the sunny country of common sense.

-- G.K. Chesterton

In fact, we were probably wrong about everything, and, no doubt, still are.
Whenever we come to a conclusion, we should just assume we're wrong
and go from there.

-- Steven Brust, P.J.F.

A point of view can be a dangerous luxury when substituted
for insight and understanding.

-- Marshall McLuhan

Since no one is perfect, it follows that all great deeds have been accomplished out of imperfection.
Yet they were accomplished, somehow, all the same.

-- Lois McMaster Bujold

The most exciting phrase to hear in science, the one that heralds new discoveries,
is not "Eureka!" but "That's funny…"

-- Isaac Asimov

The interplay of ideas and the oblique uses of knowledge
are often of extraordinary interest.

--Sir Arthur Conan Doyle

In every object there is inexhaustible meaning; the eye sees in it
what the eye brings means of seeing.

-- Goethe

If all else fails, look at the plant.

-- Gar W. Rothwell

I don't necessarily believe everything I say.

-- Marshall McLuhan

University of Alberta

Fossil Taxa in the Family Pinaceae, and their Phylogenetic Implications

by

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DEDICATION

To Fluffy.

ABSTRACT

Although the peak diversity of Pinaceae is reflected by many Cretaceous seed cones representing extinct genera, the oldest definitive record of the family is attributable to an extant genus. A seed cone discovered at the Valanginian Apple Bay locality extends the record of *Picea* by ~75 Ma, resolving a ghost lineage predicted by molecular dating analyses. However, a pine from the Eocene Princeton Chert indicates that extant genera are themselves relicts of greater historical diversity. *Pinus arnoldii* Miller is reconstructed here as the first organismal concept for an extinct member of Pinaceae, on the basis of anatomical attachments between the seed cones attributable to Subgenus *Pinus*, and vegetation like that of Subgenus *Strobus*. The phylogenetic implications of these fossils are assessed through cladistic analyses, and comparisons with maximum likelihood ancestral states reconstructed on topologies derived from Bayesian cpDNA analysis of extant *Pinus* and *Picea*.

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CHAPTER I: INTRODUCTION TO THE NATURAL HISTORY AND PHYLOGENY OF THE FAMILY PINACEAE

The Pinaceae Lindley is the largest family of conifers, with 232 species assigned to 11 extant genera (Farjon 2010). The family has a widespread Laurasian distribution, and forms an economically and biologically important component of montane and boreal forests (Hosie 1969; Eckenwalder 2009; Farjon 2010). The genus *Pinus* L. is the most species-rich, with 113 accepted taxa, and has the most cosmopolitan distribution, ranging from the sub-arctic to the Mediterranean, central America, and tropics of Thailand and Cambodia (Farjon 2010). The 47 species of *Abies* Miller, the firs, are predominantly montane, as are many of the 38 species of *Picea* A. Dietrich, the spruces. *Picea* and *Larix* Miller (the larches) are the most ubiquitous components of the taiga forests (Hosie 1969), and seem adapted to the widest range of growing conditions, particularly acidic soils and harsh climates (Farjon 2010). *Cedrus* Trew, *Pseudotsuga* Carrière, *Tsuga* Carrière, and *Keteleeria* Carrière contain fewer species which are more geographically restricted; *Pseudolarix* Gordon, *Nothotsuga* Hu ex C.N. Page, and *Cathaya* Chun et Kuang are monotypic genera endemic to China alone (Farjon 2010) in modernity.

In terms of growth architecture, most pinaceous conifers are pyramidal trees, although shrubby forms are not uncommon (Price 1989; Farjon 2010). Although pinaceous conifers are monoecious (Price 1989; Thieret 1993; Farjon 2010), seed cones and pollen cones may be segregated to different branches or levels of the plant. The Pinaceae are united by compound seed cones composed of helically arranged ovuliferous scales that bear two inverted adaxial seeds, and are subtended by bracts which are typically free and small or rudimentary (Hart 1987; Price 1989; Thieret 1993). The seeds of most pinaceous conifers are winged (Price 1989), and these wings are derived from ovuliferous scale tissue (Miller 1988; Thieret 1993). All members of the Pinaceae have spiral phyllotaxy (Price 1989) of evergreen or deciduous leaves which may be directly inserted on long shoots, or arranged in fascicles on short shoots (Price 1989; Farjon 2010). Although such shoot dimorphism is striking in the Pinaceae, it has been noted that it is not a feature restricted to the family (Barnard 1926). Hart (1987) concluded that the absence of phloem fibers and biflavonoids, sperm cells without cell walls, free nuclear division, a thin nucellus at the micropylar end of the seed, and a four-tiered proembryo were synapomorphies that distinguished the family from other groups of conifers.

The phylogenetic affinities of Pinaceae within the conifers have been analysed with both morphological (Hart 1987) and molecular (Chaw et al. 1997, 2000; Stefanović et al. 1998, Bowe et al. 2000) data. In the largest and most recent of molecular analyses, Rai et al. (2008) examined higher-order phylogenetic relationships among exemplars of seven commonly recognized monophyletic families of extant conifers through maximum parsimony (MP) and likelihood (ML) analyses of 15-17 noncontiguous cpDNA loci. The cpDNA sequence data was derived from exemplars of the Araucariaceae, Cephalotaxaceae, Cupressaceae, Podocarpaceae, Taxaceae, and Pinaceae. In these analyses, Pinaceae, which was represented by *Abies*, *Cedrus*, *Pinus*, and *Pseudotsuga*, was resolved as sister to the other conifer families (Rai et al. 2008), which together have been informally termed the cupressophytes.

However, other molecular studies (i.e., Hajibabaei et al. 2006) suggest that Pinaceae is sister to the gnetophytes which include the extant genera *Gnetum* L., *Ephedra* L., and *Welwitschia* Hooker. As such, resolving the phylogenetic affinities of the gnetophytes may be pertinent to understanding higher-order relationships among conifers, as the "gnepine" phylogenetic hypothesis indicates that the conifers, as traditionally defined (Chamberlain 1935; Hart 1987), are a paraphyletic assemblage if the gnetophytes are excluded.

At present, the phylogenetic position of the gnetophytes is a question that stands unanswered. In their maximum parsimony analyses, Rai et al. (2008) resolved a monophyletic assemblage comprising the coniferophytes, *Ginkgo* L., and the Cycadales, to which the angiosperms were sister; in their maximum likelihood approach, however, the conifers were resolved as a sister clade to the angiosperms and cycads + *Ginkgo*. In both ML and MP analyses, the gnetophytes were resolved as basal to all seed plants. Studies sampling substantially more plastid loci (Wu et al. 2007, McCoy et al. 2008) compared *Cycas* L., *Ginkgo* and *Pinus* to *Gnetum* (Wu et al. 2007) and *Welwitschia* (McCoy et al. 2008) and suggested that the gnetophyte taxa were either basal to all seed plants, as in the more taxonomically diverse study (Rai et al. 2008), or that they were sister to *Pinus*. In yet another twist that further compounds the complexity of the problem, Mathews (2009) cites ML and MP analyses of an unpublished supermatrix

(Burleigh and Mathews, unpublished), wherein gnetophytes were resolved as either sister to the cupressophytes, or to all seed plants. However, Burleigh and Mathews' subsequent analyses using a revised supermatrix, which contained fewer taxa but a greater number of shared nucleotides, produced phylogenetic topologies that accorded with the gnepine hypothesis, although parsimony analyses that included outgroup data also found support for the gnetophytes as basal among seed plants (Mathews 2009).

Although the deep nodes of higher-order relationships among conifers remain somewhat tentative, the phylogeny of the family Pinaceae itself has been well resolved with respect to the extant genera. The first comprehensive morphological cladistic analysis (Hart 1987) resolved two distinct lineages. The "pinoid" group was delimited as the genera *Cathaya*, *Picea*, and *Pinus*; the remaining 7 genera (Hart did not recognize *Nothotsuga* as distinct from *Tsuga*) were resolved as a sister "abietoid" lineage. In discussing the results of his cladistic analysis, however, Hart proposed that an abietoid lineage consisting of *Abies*, *Cedrus*, *Keteleeria*, *Pseudolarix* and *Tsuga* was monophyletic. He further suggested that the presence of resin ducts in secondary xylem (Chamberlain 1935) and leaves with an endodermis characterized by a thickened Casparian strip (Yao and Hu 1982) were characters restricted (within Pinaceae) to the pinoid lineage, within which he included *Larix* and *Pseudotsuga*, despite his cladistics analysis resolving these taxa as sister to his abietoid group.

The basic taxonomic division of Pinaceae into two subfamilies (*Abies*, *Cedrus*, *Keteleeria*, *Pseudolarix*, and *Tsuga* comprising *Abietoideae*; *Cathaya*, *Larix*, *Picea*, *Pinus*, and *Pseudotsuga* comprising *Pinoideae*) was first proposed by Van Tiegham (1891), who called the pinoid lineage “les Epixylocèles”, because members of the group exhibit resin canals in the primary root which are adjacent to protoxylem poles. Although Hart’s cladistics analysis did not resolve a monophyletic *Pinoideae*, the circumscription of the abietoid and pinoid lineages was subsequently corroborated by UPGMA immunological comparisons (Price et al. 1987). Of the pinoid group *Picea* and *Pinus* were resolved as sister taxa, as were *Larix* and *Pseudotsuga*; although the study did not include *Cathaya* Price and his colleagues suggested on a morphological basis that it was most appropriately placed within the pinoid group (Price et al. 1987). In the abietoid group, *Tsuga* and *Pseudolarix* were sister to the clade containing *Cedrus*, *Keteleeria*, and *Abies*.

In a subsequent review of subfamilial classifications, Price (1989) suggested that the family was most appropriately divided into two subfamilies, the *Abietoideae* and *Pinoideae*. He did allow that a classification invoking three subfamilies -- a monogeneric subfamily *Pinoideae* and a subfamily *Laricoideae* consisting of *Larix, Pseudotsuga, Picea* and *Cathaya* -- were tenable alternatives. In addition to these latter circumscriptions, Frankis (1989) suggested that a monogeneric *Piceoideae* also be established.

Recent phylogenetic analyses of the chloroplast DNA (cpDNA) *rbc*L and *mat*K coding loci (Gernandt et al. 2008) using Bayesian approaches have produced tree topologies consistent with the division of the family into the subfamilies *Abietoideae* and *Pinoideae*. Maximum parsimony analyses (Gernandt et al. 2008) were, however, discordant with respect to the phylogenetic position of *Cedrus*, which was found to be basal to two clades that otherwise correspond to the abietoid and pinoid lineages. In their Bayesian analysis, *Cedrus* occupied a basal position within a monophyletic abietoid group, which consisted of *Abies* + *Keteleeria* as sister to [*Pseudolarix* (*Nothotsuga* + *Tsuga*)], and was recovered with high support in all cpDNA analyses (Gernandt et al. 2008). In all cpDNA analyses *Larix* and *Pseudotsuga* were resolved as sister taxa, as were *Cathaya* and *Picea*, which were themselves sister to *Pinus*. Parsimony and Bayesian analysis of a combined dataset (a non-molecular dataset concatenated with cpDNA) produced a slightly differing tree topology: *Cathaya* was resolved as basal to *Pinus* + *Picea*.

Divergence rates within the Pinaceae were assessed in simultaneous analyses (Gernandt et al. 2008) using a relaxed molecular clock, fossil-calibrated at the *Pinus-Picea* and *Pseudolarix-Tsuga* nodes. The *Pinus-Picea* fixed calibration point was set with the early Aptian seed cone, *Pityostrobus bommeri* Alvin 1953 (=*P. bernissartensis*, Alvin 1957), at 123 Ma, and yielded younger minimum divergence estimates at all nodes than when the calibration point was set at the *Pseudolarix-Tsuga* node. This latter node was set using the Upper Jurassic (Oxfordian) *Pseudolarix erensis* Krassilov (1982) which has been dated at 156 Ma (Keller and Hendrix 1997; LePage 2003*a*); this compression-impression taxon may therefore represent the earliest fossil record for Pinaceae. Under the *Pinus-Picea* calibration, the Pinaceae crown group was estimated (Gernandt et al. 2008) to have diverged in the early Cretaceous (136 Ma) which is significantly younger than the minimum divergence estimation of 184 Ma, found under the *Pseudolarix-Tsuga* calibration. As Gernandt and his colleagues noted, one of the principle difficulties in estimating divergence within Pinaceae is the paucity of reliably dated fossils that are readily assignable to extant genera (Gernandt et al. 2008).

To date, the earliest putative fossil record for an extant genus is *Pseudolarix erensis*, a compression-impression morphospecies of detached ovulate cone scales from the Gurven-Eren Formation of Mongolia (Krassilov 1982), which is stratigraphically correlative with the Tsagan-Tsav Fm (Khosbayer 1973). Keller and Hendrix (1997) constrained the age of the Tsagan-Tsav to the latest Oxfordian (Upper Jurassic, 156 Ma), by ^{40}Ar/^{39}Ar dating sanidine from tuffs of the Suihent petrified forest. However, because these Jurassic remains are fragmentary and poorly preserved, their taxonomic affinities may be suspect (RAS pers. comm.). At present, *Pseudolarix* is monotypic and restricted to southeast China (LePage and Basinger 1995; Eckenwalder 2009; Farjon 2010), but numerous fossils assigned to *Pseudolarix* have been recovered from Cretaceous through Paleogene strata of Asia, North America, and Europe (LePage and Basinger 1995).

The earliest purported fossil records of other abietoid genera are mostly fossilized woods, and include *Cedrus penzhianaensis* Blokhina et Afonin (2007), *Keteleerioxylon kamtschatkiense* Blokhina, Afonin, et Popov (2006), which were preserved in the Albian - late Albian (Lower Cretaceous) Kedrovskaya Fm of the Russian Kamchatka Peninsula. *Abietoxylon shakhtnaense* Blokhina (2010), an

Abies-type wood morphospecies from the late Chattian-early Aquitanian (Upper Oligocene - Lower Miocene) of Sakhalin, is the earliest representative of the genus *Abies*. A *Tsuga*-type palynomorph (Macko 1963) from the Turonian (Upper Cretaceous) of Poland is the earliest record of the genus; isolated seeds, scales and vegetative remains are not represented prior to the Eocene (LePage 2003*b*).

Pinus belgica Alvin (1960), a seed cone morphotaxon presumed to be from the middle Barremian to early Aptian Wealden Fm of Belgium, has traditionally (Miller 1976) been considered the oldest representative of *Pinus*, although there is uncertainty with respect to its provenance (Gernandt et al. 2008; P. Ryberg pers. comm. 2011). The description of an older seed cone from the Hauterivian-Barremian transition (Lower Cretaceous) Speeton Clay Fm of Yorkshire (Ryberg et al. 2010; Ryberg pers. comm. 2011) will extend the fossil record of *Pinus* to 131-132 Ma.

In comparison to *Pinus*, the fossil records of the other pinoid genera are surprisingly recent. All are well represented in Paleogene floras, except for *Pseudotsuga*. To date, only a seed cone morphospecies, *Pseudotsuga jechorekiae* Czaja (2000) from the Miocene of Germany, has been described, although dispersed anatomically preserved leaves from the early Cretaceous provide an indication that the genus has an older record (Stockey and Wiebe 2006). *Larix altoborealis* LePage et Basinger emend. Jagel, LePage et Jiang, which has been described from fertile and vegetative remains from the Lutetian (middle Eocene) Buchanan Lake Fm of Axel Heiberg Island, is the oldest fossil assignable to the genus (LePage and Basinger 1991; Jagels, LePage and Jiang 2001). The Buchanan Lake Fm also preserves the earliest record of *Cathaya*, a palynomorph, *C. gaussenii* Sivak (Liu and Basinger 2000). Although the genus is at present geographically restricted to China, *Cathaya* pollen has also been described from the Miocene of southwestern France (Sivak 1976), and Austria (Klaus 1984).

Like *Cathaya,* the earliest fossil record for *Picea* is also a palynomorph, *P. grandivescipites*, described from the Danian-Selandian Fort Union Fm (Paleocene) of Montana's Powder River Basin, a stratigraphic correlate to the Williston Basin (Belt et al. 2004). Definitive *Picea* macrofossils (three seed cone morphospecies: *P. sverdrupii*, *P. nansenii*, and *P. palustris*) have to date only been described from the younger (Lutetian) Buchanan Lake Fm (LePage 2001). However, a *Picea*-type wood, *Piceoxylon talovskiense* Blokhina et Alfonin (2009) is known from the Albian of Russia, and a *Picea*-like leaf morphology, *Midoriphyllum piceoides* Stockey et Wiebe (2008) has been described from the late Valanginian of Vancouver Island, Canada. The Cretaceous diversity of *Picea* was once thought to encompass seed cones as well (i.e., Berry 1905; Penny 1947), as the affinities of many seed cones were assessed on the basis of gross external morphology (Miller 1974).

Long, cylindrical cones were often depicted as representatives of *Picea*, but examination of their internal anatomy (Miller 1974, 1976) revealed significant differences. As such, most Mesozoic pinaceous seed cones have been relegated to two morphogenera: *Pseudoaraucaria* Fliche, which is considered a natural genus (Alvin 1957; Miller and Robison 1975, Miller 1976; Smith and Stockey 2001, 2002), and *Pityostrobus* Nathorst emend. Dutt, which has long been recognized as polyphyletic (Miller 1976, Stockey 1981, Falder et al. 1998; Smith and Stockey 2001). There are as many anatomical and morphological differences between any of the 27 *Pityostrobus* morphospecies as there are between extant genera, and it has therefore been suggested that individual *Pityostrobus* specimens are likely representative of extinct genera (Miller 1976, Smith and Stockey 2001, 2002).

The realization that the extant members of Pinaceae are relicts of a much more diverse Mesozoic history was dependent upon three-dimensional anatomical preservation of *Pityostrobus* morphotaxa. Witham (1833) was the first to recognize that permineralization, as a mode of preservation, offered paleobotanists an unprecedented wealth of data. His observations of anatomically preserved Carboniferous flora set a precedent for comparative plant anatomy of fossil taxa (Andrews 1980), and permineralization has been widely recognized as the most informative mode of preservation for fossil plants (Taylor et al. 2009).

Many permineralized plants, including those of Vancouver Island's Apple Bay locality, are preserved as allochthonous phytodetrital components of marine sequences. These assemblages exhibit a high degree of preservational acuity, and permit us to assess the comparative anatomy of fossil plants with respect to extant species. A new pinaceous ovulate cone, from the Lower Cretaceous Apple Bay deposits (Chapter 2), provides just such an opportunity, as its internal anatomy is preserved with a high degree of fidelity. This cone marks the earliest fossil record for pinaceous seed cones, and assessment of its anatomy in a comparative framework indicates that it also provides the earliest record for the extant genus *Picea*.

In some exceptional cases, like the Ypresian-Lutetian (middle Eocene) Princeton Chert, permineralization is coupled with successive preservation of a stable mire community. Such *konservat-laggerstätten* provide the unique opportunity to develop organismal concepts that combine vegetative and reproductive morphotaxa (i.e., Cevallos-Ferriz and Stockey 1988; Stockey and Pigg 1991, 1994; Stockey et al. 1999; Little and Stockey 2003; Little et al. 2004; Smith et al. 2006). Successive deposition and silicification of the Eocene Princeton Chert mire has given us the unique opportunity to also develop the first organismal concept for an extinct species within the family Pinaceae (Klymiuk et al. 2011; Chapter 3). The fossil plant *Pinus arnoldii* Miller was originally described as a seed cone morphotaxon most similar to pines of Subgenus Pinus (Miller 1973; Stockey 1984), associated with vegetation, *P. similkameenensis* (Miller 1973), that is anatomically identical to that seen in a number of species within Subgenus *Strobus*. Anatomical attachments between the seed cones and vegetative organs, as well as associated pollen cones, immature ovulate cones, and ectomycorrhizal coralloid roots allow us insight into this Paleogene pine that is comparable to the level of detail at which most extant members of *Pinus* are known. Whole-plant concepts, like that presented for the extinct pine, *Pinus arnoldii* allow us to make meaningful biological statements about fossil plants, and may offer insights into phylogeny and character evolution when they define a species exhibiting a novel combination of characters.

One of the difficulties plaguing the incorporation of fossils into cladistic analyses of plant relationships is the large amount of missing data (Nixon 1996). It has been suggested (Huelsenbeck 1991) that the inclusion of fossil taxa in morphological analyses is principally of value if the majority of phylogenetically informative characters are known. Topologic resolution is commensurately diminished by missing data, as the number of most parsimonious trees produced by a search algorithm is increased (Huelsenbeck 1991). This suggests that the inclusion of morphotaxa in phylogenetic analyses is of limited value, in comparison to fossil species such as *P. arnoldii*.

The nature of the paleobotanical record, however, is often incompliable with conditions that permit the development of organismal concepts. In addition to preservational biases that decrease the potential for an organism's incorporation into the rock record, the paleobotanical record is further confounded by the fact that plants die in a successive fashion, shedding their vegetative and reproductive organs at different points throughout their life history. Thus, morphotaxa are most frequently the *only* record of an extinct plant. In some cases, morphotaxa seem assignable to extant lineages, and these specimens have been of particular interest to molecular systematists, as they provide calibration points for divergence time estimates (e.g. Willyard et al. 2006; Gernandt et al. 2008). However, as is demonstrated by whole plant reconstructions (Klymiuk et al. 2011; Crane and Stockey 1986) actual biological entities can be composed of morphotaxa that would otherwise be assigned to disparate taxonomic lineages.

The appropriate taxonomic placement of morphotaxa is a complex problem that underlies the use of such specimens in molecular-based hypotheses of divergence, biogeography, and character evolution. When a fossil is suggested to have morphological similarities to a group of extant plants, it may be possible to test such a taxonomic assessment by comparing the fossil's morphological characters to hypothesized ancestral states for the lineage, that are informed by the degree of genetic divergence (evolutionary branch lengths) between extant species (Chapter 4). At present, taxonomic placement of fossils is most frequently accomplished by cladistic analysis of morphological characters across a scaffold provided by molecular data analyses (Hermsen and Hendricks 2008), or by simultaneous analyses of both extant and fossil taxa (Nixon and Carpenter 1996). The methodology proposed here (Chapter 4) may offer a secondary approach to taxonomy of fossil plants, as it appears to reveal phylogenetic position of morphotaxa that is consistent with cladistic analyses for the whole plant that the morphotaxa comprise.

As a repository for novel combinations of morphological characters, the fossil record provides an important source of data for systematic studies of modern plants. By increasing our understanding of past diversity, the fossil taxa described here permit insight into the evolution of modern genera of Pinaceae, and significantly expand the quantity of fossils applicable to systematic investigations of extant pinaceous conifers.

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CHAPTER II: A LOWER CRETACEOUS *PICEA*-LIKE SEED CONE FROM VANCOUVER ISLAND

INTRODUCTION

The family Pinaceae is comprised of 11 extant genera and approximately 225-232 species (Farjon 1998, 2010), many of which are biologically and economically important components of forests throughout the Northern Hemisphere. While phylogenetic analyses of nonmolecular (Hart 1987) and molecular (Wang et al. 2000; Liston et al. 2003; Eckert and Hall 2006; Gernandt et al. 2008) characters for Pinaceae have provided resolution of the relationships between extant genera, the modern genera have consistently shown themselves to be relicts of a much more diverse evolutionary history (Alvin 1988; Miller 1976*a*, 1977*a*, 1988; Miller and Li 1994; Miller and Robison 1975; Robison and Miller 1977; Smith and Stockey 2001, 2002). Divergence estimates (Gernandt et al. 2008) indicate long branches at all basal nodes within Pinaceae, particularly with respect to the genera *Pinus* L., *Picea* A. Dietrich, and *Cathaya* Chun et Kuang. Along with the sister taxa *Larix* Mill. and *Pseudotsuga* Carrière, these genera form the subfamily Pinoideae (Price 1989; Liston et al. 2003), and are suggested to share a minimum divergence within the Late Jurassic or Early Cretaceous under a relaxed fossil-calibrated molecular clock (Gernandt et al. 2008).

During the late Mesozoic, pinaceous conifers were undergoing rapid evolution and radiation, the evidence for which is preserved as the preponderance of seed cone morphologies comprising the natural genus *Pseudoaraucaria* Fliche, which contains six described species, *Obirastrobus* Ohsawa, Nishida et Nishida

(1992), which contains two, and the polyphyletic assemblage *Pityostrobus* Nathorst emend. Dutt. This unnatural group contains at least 28 cone morphotaxa that are as anatomically distinct from one another as are any extant genera (Miller 1976*a*, Stockey 1981; Falder et al 1998). That the majority of the family's morphological diversity has been lost through extinction is a significant hurdle to a complete understanding the phylogeny, taxonomy, and evolutionary history of the family Pinaceae, to say little of the difficulties inherent in determining the evolutionary trajectory of various anatomical features.

Representatives of these earliest lineages within the Pinaceae can only be assessed with respect to morphological and anatomical characters, the study of which has been the subject of two previous inquiries (Smith and Stockey 2001, 2002). The placement of Cretaceous morphotaxa with respect to their evolutionary relationships with modern genera has been tenuous at best. Exceptions include representatives of the genus *Pinus*, for which a number of seed cone and vegetative morphospecies are known (Alvin 1960; Miller 1976*b*; Wehr and Hopkins 1994; Falder et al 1998; Farjon 1998, Karafit 2008, Ryberg et al. 2010; P. Ryberg pers. comm. 2011). Although dispersed seeds, cone scales and leaves have been assigned to *Pseudolarix* Gordon (LePage and Basinger 1995), these fossils are preserved as compression-impressions, and their taxonomic assignment has been argued as questionable (RAS pers. comm. 2011).

Here, I present the oldest record of a fossil seed cone assignable to the family Pinaceae. Its morphology and anatomy are most similar to extant *Picea*, making it the oldest definitive representative of the genus, which is corroborated by the presence of *Picea* pollen within the ovules of the new cone. *Picea*-like leaves (Stockey and Wiebe 2008) are also found at the locality, and may represent the vegetation of this early member of the spruce lineage. However, the development of a whole-plant concept reconciling the new cone with the *Picea*-like leaves is precluded by the nature of the depositional setting of the strata within which these taxa are preserved.

MATERIAL AND METHODS

Geologic and depositional setting. The specimen, an isolated immature seed cone, was collected at the Apple Bay fossil locality of northern Vancouver Island, British Columbia, Canada (50°36’21”N, 127°39’25”W; UTM 9U WG 951068), where sedimentary strata crop out as a rocky beach on the north shore of Holberg Inlet, in Quatsino Sound. The Apple Bay locality is one of several sedimentary basins associated with the Wrangellian terrane, which reflect erosion of the Jurassic Bonanza rhyolites during what has been interpreted as a continental transgressive sequence (Muller et al. 1974; Hammack et al. 1994). Apple Bay was originally regarded as a Lower Cretaceous Longarm Fm equivalent (Jeletzky 1976; Haggart and Tipper 1994), correlative to Jeletzky’s (1976) Barremian Variegated Clastic Unit (Sweet 2000). Recent oxygen isotope analyses, however, have placed the age nearer to the Valanginian-Hauterivian boundary, at approximately 136 Ma (D.R. Gröcke, pers. comm. to RAS).

The 6.2 m thick section is composed of 20 beds, the lithology of which is predominantly clast-supported sandstone cemented with calcium carbonate, although muddy siltstones are also in evidence. Thirteen of the beds contain semi-sideritic calcareous concretions, some of which preserve three-dimensionally permineralized phytodetrital remains. Qualitatively, those plant remains which exhibit the best preservation are found in concretions where the matrix is composed of well-sorted, cryptically bioturbated (*sensu* Pemberton et al. 2008) sandstone. In contrast, plant remains preserved in siltstone concretions tend to show more pyritic replacement of organic materials.

To date, detailed sedimentological and ichnological association assays have not been conducted at Apple Bay. Although some compression-impression fossils (i.e., Stockey et al. 2006) have been recovered from 7 beds lower in the section, collecting endeavors at the locality have focused primarily on retrieval of concretions, most of which have eroded out from host strata. These concretions are subsequently sectioned into ~0.75 cm wafers, which are then examined for the presence of phytodetritus. Sectioning also clarifies sedimentary structure; when cut with respect to bedset orientation, concretions can provide a level of sedimentological detail comparable to that seen in cores. Thus, by taking the composition of these concretions as a reasonable proxy for the stratigraphic succession, some inductions as to depositional setting may be posited on the basis of ichnological inclusions.

The Apple Bay strata are notably impoverished with respect to the archetypal near-shore *Skolithos* ichnofacies, a behavioural suite (Sielacher 1953, 1967) that represents deposit-feeding and sedentary carnivory in a high-energy, wave-dominated setting (MacEachern et al. 2005*a*) -- conditions that might otherwise be inferred from the lithology of the concretions recovered from Apple Bay.

Excepting cryptic bioturbation, which is indicative of relatively stable conditions (Pemberton et al. 2008), the most common identifiable ichnofossil at Apple Bay is *Macaronichnus segregatis*, an unbranched feeding trace in which micaceous grains and clay minerals have been segregated to the lining of the burrow (Clifton and Thompson 1978). In modern depositional settings, these traces are produced by polychaete worms like *Ophelia limacina* and *Euzonus mucronata* which inhabit the foreshore and shallow subtidal deposits associated with high-energy sandy beaches (Pemberton et al. 2001, Nara and Seike 2004). *Macaronichnus* is, however, known to be associated with other depositional regimes (Goldring 1993), including those of wave-dominated (high energy) delta-fronts.

Sandy stacked amalgamated bedsets, like those of the Apple Bay locality, are formed on delta-fronts under hyperpycnal conditions (MacEachern et al. 2005*a*). These conditions may be persistent, as in the case of seasonal storms or floods, which also produce concomitant increases in phytodetrital influx (MacEachern et al. 2005*a*). Stockey et al. (2006) have previously suggested that deposition at Apple Bay occurred under tempestite conditions: the remains of delicate moss and liverwort gametophytes, lycophytes, *Equisetum*, and a preponderance of pteridophytes, including sporelings, as well as fertile remains of numerous osmundaceous and pteridaceous ferns, and the dipteridaceous fern *Hausmannia morinii* combine to form a cohesive picture of a forest-floor flora marginal to a river or stream, which was swept into the marine basin during storm events.

In most depositional settings, tempestite deposits are rapidly recolonized by organisms producing traces of the *Skolithos* ichnofacies (Howard and Frey 1984; Pemberton and Frey 1984). When tempestites are associated with delta-fronts, however, there is “anomalous impoverishment” (MacEachern et al. 2005*a*) of sedentary feeding structures. Instead, “facies-crossing” ichnofossils, including *Macaronichnus*, predominate. This circumstance is explained by invoking high wave energy during storms: Clay is winnowed from sandy substrates, resulting in sedimentological sorting at the seafloor, and sustained turbidity in the overlying watercolumn, which prohibit colonization by suspension-feeders (MacEachern et al. 2005*a*). Thus, the resultant sedimentary fabrics are composed of well-sorted sandstone, extensively bioturbated by facies-crossing members, and impoverished with respect to suspension-feeding structures normally found in *Skolithos* assemblages (MacEachern et al. 2005*a*). Observation of these conditions within the Apple Bay material has lead me to hypothesize that the locality represents tempestite deposition on a wave-dominated delta-front, where infaunal burrowing, and hyperpycnal saline reduction produced localized alterations in chemical-osmotic gradients, permitting the formation of concretions. Individual strata at Apple Bay likely represent discrete depositional events, and the extent to which paraconformities reflect palimpsests or hiatuses is not quantifiable.

Specimen preparation. The specimen was initially exposed in oblique longitudinal section when the concretion was sectioned into wafers. Consecutive

~25 μm sections were prepared from both faces, using the cellulose acetate transfer technique described by Joy et al. (1956). Of the two faces, the more tangential (P16098 Ctop) was peeled to completion for description of vasculature and resin canals throughout the length of the ovuliferous scales. The other face (P16098 Bbot) was reoriented, and consecutive transverse sections were prepared. Cellulose acetate peels of the specimen were mounted on glass slides, using xylene-soluble Eukitt (O. Kindler GmbH, Freiberg, Germany) mounting medium. Images were captured with a Powerphase (Phase One, Copenhagen, Denmark) digital scanning camera mounted on a Leitz Aristophot bellows, a Zeiss WL compound microscope, and a Nikon DS-Ri1 camera mounted on a Nikon Eclipse 80*i* compound microscope, and a Leaf Microlumina System version 1.2 (Leaf Systems, Westborough, Massachusetts, USA), and processed with Adobe Photoshop CS3. Specimens and slides are housed in the University of Alberta Paleobotanical Collection (UAPC-ALTA).

Phylogenetic analyses. Phylogenetic analyses were conducted using a morphological matrix (Table 2-1) for seed cones of pinaceous affinity, adapted from previous studies (Smith and Stockey 2001, 2002). The new cone was compared to extant and fossil taxa with respect to 33 anatomical characters (as defined in Appendix A) derived from CN Miller and CR Robison's unpublished matrices (University of Montana), and subsequently revised by Smith and Stockey (2001).

The Smith and Stockey (2001, 2002) morphological matrix (Table 2-1) was updated for this study to include the new specimen from Apple Bay; the morphospecies *Pityostrobus yixianensis* (Shang et al. 2001), the characters of which were assessed from the published description; and the extinct species *Pinus arnoldii* (Klymiuk et al. 2011). As such, the matrix includes one extinct species, and 37 fossil seed cone morphotaxa representing all known species of *Pseudoaraucaria* Fliche, *Pityostrobus*, and *Obirastrobus* Ohsawa, Nishida et Nishida (1992) for which anatomical details may be observed, as well as the ovuliferous cone *Pararaucaria patagonica* Wieland, which was included in previous studies (Smith and Stockey, 2001, 2002) due to possible affinities with Pinaceae (Stockey 1977).

Extant taxa were scored using the University of Montana Conifer Reference Collection, now residing at Oregon State University, Corvallis. In previous analyses (Smith and Stockey 2001, 2002) the 11 extant genera were scored as composite placeholders, such that characters which varied within a genus were scored as polymorphisms. The use of such composite polymorphic taxa effectively compares genera to individual species and in doing so makes numerous assumptions with respect to the polymorphic characters. To illustrate by example, in the composite placeholder '*Pinus*', the polymorphic coding of the tenth character (cortical resin canal diameter) makes a probabilistic assumption that markedly dilating resin canals are *just as likely* as those of uniform diameter (Table 2-1, Appendix A). In considering the frequency distribution of each of these states among the 36 species of *Pinus* L. for which the character has been scored (Klymiuk et al. 2011), it is apparent that dilating resin canals are present in only 8 species. Clearly, it is inappropriate to weight the two states equally in this taxon, as their frequency of occurrence among extant taxa is not equivalent. However, an appropriate weighting scheme is elusive, as the frequency distribution of states is not known for all extant taxa. Furthermore, the developmental or genetic mechanisms that underpin the expression of this character are not presently understood. Thus, there is no appropriate way to discriminate whether character states should be ordered, as there is no unbiased way to determine how "easy" it is to transition (in a phylogenetic sense) between resin canals of uniform diameter to dilating, or vice versa. Similar difficulties underlie all polymorphic characters, in each of the composite placeholders. In deference to these issues, I have also run independent analyses of the matrix in which representative species for each of the extant genera have been scored, and composite placeholders have been excluded.

In all analyses, *Cryptomeria japonica* (L. f.) D. Don and *Sciadopitys verticillata* (Thunb.) Siebold et Zucc. were defined as outgroups, as per Smith and Stockey (2001, 2002). 5 multiratchet tree-searches were performed under the parsimony ratchet perturbation algorithm (Nixon 1999), in the program TNT (Goloboff et al. 2008) spawned through Winclada (Asado, version 1.1 beta, by K. Nixon, Cornell University). All characters were non-additive, and multistate characters were unordered. In each search, ratchet perturbation sampled 10% of the parsimony-informative characters through 5000 iterations per replication, with three trees held per iteration. Nelson (1979, and see Nixon and Carpenter 1996) consensus phylogenies are reported; bootstrap (Felsenstein 1985) values were produced from 1000 replicates, with ten trees held for each of 100 multiple TBR search replications.

RESULTS

Systematics

Order – Coniferales

Family – Pinaceae Lindley 1836

Genus – *Picea* A. Dietrich 1824

Species – *Picea burtonii* Klymiuk et Stockey, *sp. nov.*

Specific diagnosis. Seed cone, at least 3.2 cm long and 0.5 cm in diameter, cylindrical. Cone scale complexes helically arranged. Pith, 330-350 µm in diameter, parenchymatous with scattered sclereids. Vascular cylinder continuous; 110 µm, 8-10 tracheids wide. Tracheids with helical and scalariform thickenings. Cortex 135-160 µm wide, with 12-15 resin canals, mostly parenchymatous with scattered sclerenchyma fibers. Vascular traces to ovuliferous scale abaxially concave, and terete bract trace, arising separately from vascular cylinder. Bract up to 650-680 µm long, triangular in cross section, with two lateral resin canals; separating from ovuliferous scale at lateral margins. Ovuliferous scale at least 1.13 mm long and borne perpendicular to cone axis; resin canals both abaxial and adaxial to vascular strands throughout, and also between vascular strands distal to seed body. Seeds two per scale, 400 µm long and 170 µm wide; with wings at least 545 µm long.

Holotype hic designatus. Peels, and slides of concretion wafers Bbot, Bxs, and Ctop, cut from specimen P16098, housed in the University of Alberta Paleobotanical Collection (UAPC-ALTA).

Stratigraphy. Longarm Formation equivalent

Age. late Valanginian, Early Cretaceous

Etymology. This seed cone morphotaxon is named for the motion picture storyteller Tim Burton. The ovuliferous scale, when viewed in transverse section (see figs. 2-2E, 2-2F), seems a demonstrable case of 'life imitating art'.

Description

General features. The cone, which measures 3.2 cm in length, and 0.5 cm in width, is nearly complete (fig. 2-1A). The apex, however, has not been preserved, and the exterior bears evidence of abrasion (figs. 2-1A, 2-1B). Some internal tissues have been obscured by pyritization, and there is a small amount of tissue shrinkage due to halitic dehydration.

Cone axis. The pith, which is composed of parenchyma and scattered sclereids, is 330-350 μm in diameter (figs. 2-1B, 2-1C). It is surrounded by a continuous vascular cylinder exhibiting primary and secondary growth (fig. 2-1C) which is occasionally interrupted by divergence of ovuliferous scales (fig. 2-1B). The xylem is 110 μm (8-10 tracheids) wide, and represents a single growth increment (fig. 2-1C). Tracheids, when viewed in longitudinal section (fig. 2-2D) exhibit helical to scalariform secondary wall thickenings. No resin canals are present within the xylem, and phloem is not preserved (fig. 2-1C). The cortex is 135-160 μm wide, and is predominantly parenchymatous, with scattered sclereids throughout (fig. 2-1C). A ring of 12-15 resin canals, 65 μm in diameter, occurs medially within the cortex (fig. 2-1B, 2-1C).

Bract-scale complex. The bract-scale complexes are helically arranged and borne at right angles to the cone axis (figs. 2-1D, 2-1E, 2-2A). The vascular traces to the ovuliferous scale and bract, as in other non-*Pinus* members of Pinaceae, are separate at their origin from the vascular cylinder (figs. 2-2B, 2-2C). The vascular trace to the ovuliferous scale initially derives from the vascular cylinder as two separate bundles, from either side of the gap, which unite within the inner cortex to form a single abaxially concave strand (fig. 2-2C). Within the ovuliferous scale, the vascular tissue rapidly divides, forming multiple strands, no more than 3-5 cells in diameter.

The resin canal system of the bract-scale complex (fig. 2-4) is derived from perpendicular branching of the axial resin canals within the inner cortex (fig 2-2A). The resultant two resin canals (fig. 2-2B) accompany the vascular tissue (fig. 2-2C) to the base of the bract-scale complex whereupon they become associated with the bract after ramifying adaxially to produce the two resin canals ultimately associated with the interseminal ridge (fig. 2-2B, 2-2E). A subsequent ramification of these latter canals produces an additional four abaxial to the vascular tissue of the ovuliferous scale (figs. 2-2B, 2-2E). At the level of the seed body, these canals divide laterally (fig. 2-2E). Distal to the seed body, they undergo a further division to form two abaxial series (fig. 2-2F, 2-2G).

The two resin canals associated with the bract accompany the terete bract trace, which enters the free part of the bract (fig. 2-2A). Separation of the bract from the ovuliferous scale occurs at the lateral margins (figs. 2-2E, 2-2F). Bracts are 650-680 μm long, and 190 μm wide, triangular in transverse section, and exhibit basal lobes (figs. 2-2A, 2-2E).

The ovuliferous scales are at least 1.13 mm long, and vary in width from 760 μm at their base to 670 μm immediately distal to the seed (figs. 2-1D, 2-2A, 2-2E, 2-2F). Near the cone axis, ovuliferous scales are 360 μm thick, thinning to 200 μm at their tips. The abaxial surface of the ovuliferous scale is sclerotized (fig. 2-2E), and a zone of parenchymatous tissue above the vascular tissue extends up between the paired seeds to form an interseminal ridge (figs. 2-2E, 2-2F), 83 μm high and 145 μm wide. It extends no more than half the height of the seeds (fig. 2-2E). The tissue forming the interseminal ridge is continuous with the scale tissue, from which the seed wings are derived (fig. 2-2F).

Seeds. The adaxial surface of each ovuliferous scale bears two inverted, winged ovules (figs. 2-2A, 2-2E, 2-3A), oriented with micropyles (fig. 2-3B) facing the cone axis. The seed bodies are 400 μm long, 90 μm high, 170 μm wide, and are partially enclosed by ovuliferous scale tissue, which is thickest at the chalazal end of the seed (fig. 2-2F), before thinning distally to form the wing (fig. 2-3A). Seed wings are at least 545 μm long, and at least 18 μm thick distal to the seed (fig. 2-3A). The seeds exhibit endotestal, sarcotestal and sclerotestal integumentary layers (fig. 2-2E, 2-3A), and some contain nucellar tissue (fig. 2-3A), which is attached to the integument at the chalazal end of the seed (figs. 2-3A, 2-3C). Megagametophyte tissue can also be observed in a few seeds (fig. 2-3A), as well as bisaccate pollen grains, several of which can be observed embedded within nucellar tissue (fig. 2-3C). A greater number are present within the micropyles of numerous seeds (fig. 2-3B).

Pollen. Grains are bisaccate, and exhibit distinct irregular verrucate endoreticulations, which become finer with proximity to the corpus (fig. 2-3D). Sacci are 44-48 µm long, and 20-28 µm wide. Sacci are borne low with respect to the proximal aspect of the corpus, and their broad attachment describes an obtuse angle (fig. 2-3D, at arrow). The suboblate corpus is 36-42 µm wide, 48-52 µm long. The cappa, or proximal surface of the corpus, is psilate to scabrose, but not otherwise ornamented. The cappula, or distal surface, exhibits sculptured ridges which largely occlude the germinal furrow (fig. 2-3E, at arrow)

Phylogenetic Analysis

Two separate analyses of 51 taxa were performed using a revised version of Smith and Stockey's (2001, 2002) morphological matrix for pinaceous seed cones (Table 2-1). When extant genera were scored as polymorphic composite placeholders (fig. 2-5A), the analysis produced 2636 most parsimonious trees (tree length [TL] = 204, consistency index [CI] = 0.28, retention index [RI] = 0.64). When composite placeholders were replaced with representative species of extant genera, 1723 most parsimonious trees (TL = 213, CI = 0.27, RI = 0.61) were recovered (fig. 2-5B). Neither of the analyses recovered clades with significant (>70) bootstrap support.

The results of these analyses are presented as Nelson consensus cladograms, where clades are retained if they are present in all most-parsimonious trees. If support was ambiguous (the topology was not represented in all ratchet searches), the clade was collapsed into polytmous "clusters". If clusters from separate multiratchet searches were combinable, they were retained in the final consensus. In both analyses, the morphogenus *Pseudoaraucaria* was recovered as a monophyletic clade. The analyses also recovered sister-taxon relationships between *Pityostrobus bommeri* and *P. hueberi* Robison et Miller, *P. hallii* Miller and *P. villerotensis* Alvin, *P. hautrageanus* Alvin and *P. macrocephalus* Lindley et Hutton, and between the extant taxa *Cedrus* Trew and *Pseudolarix* Carrière.

When polymorphic composite placeholders were utilized to represent extant taxa (fig. 2-5A), the analysis resolved a terminal clade wherein *Pseudoaraucaria* nested within a monophyletic assemblage comprised of *Tsuga* Carrière, *Pityostrobus cornetii* (Coemans) Alvin, and *Abies* Mill.; this clade is itself sister to *Pityostrobus leckenbyi* (Carruthers) Seward + *P. oblongus* (Lindley et Hutton) Seward. None of these relationships were recovered when extant genera were represented by individual species (fig. 2-5B). Instead, this latter analysis resolved sister-taxon relationships between *Pityostrobus beardii* Smith et Stockey and *P. hokodzensis* Ratzel, Rothwell, Mapes, Mapes et Doguzhaeva, *P. mcmurrayensis* Stockey and *P. pubescens* Miller, and between the two *Obirastrobus* morphospecies. This analysis also recovered *Sciadopitys* and *Pararaucaria* as basal to the pinaceous assemblage -- in contrast, the phylogenetic position of these outgroup taxa was not resolved when composite placeholders were utilized.

DISCUSSION

The pinaceous affinities of the Apple Bay cone are evident in the helical arrangement of the bract-scale complexes, the ovuliferous scales of which bear two seeds upon their adaxial surfaces. As in all members of the Pinaceae, these seeds possess wings derived from ovuliferous scale tissue. The cone itself is slender, cylindrical, and elongate; this gross morphology is typical of pinaceous cones (Miller 1976*a*; Farjon 1998).

Pinaceous fossil seed cones have traditionally been placed in one of three morphogenera: *Pseudoaraucaria*, *Pityostrobus*, or *Obirastrobus*. Of the three, only *Pseudoaraucaria* is perceived as a natural, monophyletic group (Alvin 1957a, 1988; Miller and Robison 1975; Miller 1976*a*, 1977*a*, Smith and Stockey 2001, 2002), although the two taxa comprising the genus *Obirastrobus* (Ohsawa et al. 1992) appear to be closely related. A sister-taxon relationship between the *Obirastrobus* species is resolved in the cladistics analysis wherein extant genera are represented by exemplars as opposed to polymorphic composite taxa. However, the phylogenetic position of the *Obirastrobus* cones remains uncertain with respect to other fossil or living taxa. The final morphotaxon, *Pityostrobus*, has long been acknowledged as a polyphyletic assemblage; each taxon ascribed to it most likely represents a distinct genus (Miller 1976*a*, 1988; Smith and Stockey 2001, 2002).

Cladistic analyses of these fossil cones have produced little resolution of their phylogenetic affinities. Most members of the *Pityostrobus* assemblage, including the new specimen, fall within an unresolved polytomy that includes several of the extant genera. Rigorous parsimony analyses of the revised Smith and Stockey (2001, 2002) morphological matrix do not even resolve the fossil species *Pinus arnoldii* Klymiuk, Stockey, et Rothwell as a sister to either *Pinus thunbergii* de Candolle, or the composite polymorphic placeholder representing the genus *Pinus* when it is scored as a polymorphic composite. As such, the anatomical characters assessed by the morphological matrix for pinaceous seed cones seem to be of little utility in unravelling terminal or crown group relationships when unordered and unweighted. The sister-taxon relationships suggested by the analyses presented here may, in fact, be only spurious results, as none are supported by significant bootstrap values (BS<50%). In order to increase the utility of these morphological characters, it will be necessary to hypothesize appropriate weighting schemes to capture the phylogenetic signal embodied within their expression.

Because I have been unable to resolve the phylogenetic position of the new cone in a cladistic framework, determination of its taxonomic affinities is as a consequence necessarily predicated upon direct comparisons with other pinaceous taxa (Table 2-1). The Apple Bay cone does exhibit a combination of characters that is distinct from that seen in all previously described fossil morphotaxa. The only taxon that the Apple Bay cone directly compares with, in terms of the 33 anatomical characters defined for pinaceous seed cones (Miller 1976*a*, Smith and Stockey 2001, 2002, Table 2-1), is the extant genus *Picea* A. Dietrich.

General features. Although the specimen has been subject to taphonomic effects, including abrasion of its exterior, it is obvious that the apex of the ovuliferous scale thins distally; thus, I am confident that the scale did not support an umbo. Nor does it seem likely that the scales bore any similarity to the distally-thickened condition observed in extant *Pseudolarix* Gordon, or as in some morphospecies of *Pseudoaraucaria* or *Pityostrobus* (namely, *P. andraei* (Coemans) Seward, *P. argonnensis* (Fliche) Creber, *P. beardii*, *P. hueberi*, *P. jacksonii* Creber, *P. kayei* Miller et Robison, *P. lynnii* (Berry) Miller, *P. milleri*, *P. palmeri* Miller., or the presumed sister taxa *P. hautrageanus* and *P. macrocephalus*). Preservation of the cone is also more than sufficient to determine that, as in most pinaceous cones, the distal portion of the scale (beyond the level of the seed body) does not turn sharply upward, as in extant *Cedrus*, *Abies*, and *Keteleeria* Carrière, or as in the pseudoaraucarian cones or *Pityostrobus yixianensis*.

Features of the ovuliferous scale differentiate the Apple Bay cone from many taxa. The presence of an interseminal ridge between the paired seeds may be observed in all extant genera except *Abies* and some species of *Cedrus*, and is present in all fossil taxa, except *P. bommeri*, *P. cornetii,* and the sister taxa *P. hautrageanus* and *P. macrocephalus*. The expression of the interseminal ridge within the natural genus *Pseudoaraucaria* is distinct, in that it overarches the seed body in transverse section, a condition not found in any other taxon, including the Apple Bay cone, where the interseminal ridge is no higher than half the height of the seed body. Furthermore, the integuments of seeds from the new cone do not exhibit resin vesicles, as are found in four pseudoaraucarian morphospecies, and the morphotaxa *Pityostrobus oblongus*, *P. makahensis* Crabtree et Miller, *P. leckenbyi*, *P. cornetii*. Among extant genera, the presence of resin vesicles within the seed integument is a character that is restricted to the subfamily *Abietoideae* (*sensu* Gernandt et al. 2008). Finally, the abietoid genera *Abies*, *Cedrus*, and *Pseudolarix* all have ovuliferous scales which abscise from the cone axis to facilitate seed release. In all other members of Pinaceae, including the new Apple Bay cone, seed release is accomplished via spreading of the ovuliferous scales.

Cone axis. The pith of the new cone contains scattered sclerenchyma, a condition which is broadly distributed across both fossil morphotaxa and extant members of the family, and is likely of little phylogenetic value. In most pinaceous conifers, the secondary xylem of the vascular cylinder is complete, or dissected very little. A vascular cylinder comprised of separate strands occurs within the abietoid genera *Cedrus*, *Pseudolarix*, and *Abies*; the presence of a dissected stele within some species of *Pseudoaraucaria* lends some credence to the cladistic topology wherein *Pseudoaraucaria* is nested within an abietoid lineage, but several other fossil taxa also exhibit dissected steles, including *Obirastrobus*, *Pityostrobus milleri*, and [*P. beardii* + *P. hokodzensis*] and [*P. mcmurrayensis* + *P. pubescens*] which are resolved as sister taxa in analyses excluding composite placeholders. In previous analyses (Smith and Stockey 2001, 2002) the composite placeholder for *Picea* was coded as polymorphic with respect to dissection of the vascular cylinder (state 0=complete, state 1=dissected). Having re-examined the Miller Conifer Reference Collection, I have determined that none of the represented exemplars exhibit stele dissection to the degree seen in the taxa mentioned previously. Where the stele is slightly dissected, as in the new Apple Bay cone, the specimens appear to be immature.

Within the cortex of the new cone, resin canals are uniform in diameter, except when they diverge laterally to accompany a vascular trace, in which case they may be slightly dilated. The condition is similar to that seen in most *Pityostrobus* morphospecies, excepting *P. andraei*, *P. californiensis*, *P. lynnii*, *P. makahensis*, *P. milleri*, *P. oblongus*, and *P. ramentosa* Miller, which have markedly dilated resin canals (Table 2-1); dilating resin canals may also be observed in *Keteleeria,* and in some species of *Abies* and *Pinus*.

Bract. In terms of gross morphology**,** the Apple Bay cone is like most pinaceous conifers, in that its bracts are shorter than the ovuliferous scales. Bracts that are longer than the scales they subtend are generally exceptions, but do occur within some species of *Abies*, *Larix* Mill.*,* and *Tsuga*, and the genus *Pseudotsuga*, which is notable for its exserted trifurcate bracts (Silba 1986). The bracts of the new cone are also abaxially lobed; although the feature is observed in only two other fossil cones, *Obirastrobus nihongii* Ohsawa, Nishida et Nishida and *Pityostrobus mcmurrayensis*, such bracts are common to *Cathaya* Chun et Kuang, *Picea*, and *Keteleeria*, and some species of *Abies*, *Larix*, and Tsuga (Table 2-1).

The new cone has two resin canals which accompany the bract trace, which is typical for Pinaceae, although in *Pseudolarix* and *Cedrus* there are none. The extant genera *Picea* and *Larix* are polymorphic in this regard, as individual species may have two, or more than two, resin canals within the bracts. *Pityostrobus makahensis* is also polymorphic in this respect (Crabtree and Miller 1989), as several bracts contain 1-2 additional resin canals, although these are not typical of most in the specimen. In contrast, *Pityostrobus jacksonii* and both *Obirastrobus* species have bracts that invariably contain more than two resin canals.

In the Apple Bay cone, the vascular trace enters the free part of the bract, unlike the condition seen in the extant genera *Cedrus*, *Cathaya*, and *Keteleeria*, as well as some species of *Larix*, and in the fossil cones *Pityostrobus argonnensis*, *P. cornetii*, *P. hokodzensis*, *P. leckenbyi*, *P. milleri*, *P. palmeri*, *P. shastaensis,* [*P. hautrageanus* + *P. macrocephalus*], and [*P. bommeri* + *P. hueberi*]. In these taxa, vascular trace terminates prior to the separation of the bract from the ovuliferous scale (Table 2-1).

As is the case for most other taxa, the bract and scale of the Apple Bay cone undergo separation along their lateral margins. Those taxa which have medial separation of the bract and scale include three *Pityostrobus* morphospecies (*P. halliii*, *P. lynnii*, and P. *villerotensis*), and both morphospecies of *Obirastrobus*; the condition can also be observed in the extant genera *Larix*, *Abies*, and *Tsuga*. In *Pityostrobus hokodzensis*, the bract appears to separate all at once (Ratzel et al. 2001), as opposed to gradually separating. Conversely, *P. argonnensis* and *P. milleri* retain their bracts entirely (Table 2-1).

Vascular architecture of the scale. As in most non-*Pinus* members of the family, the vascular traces to the bract and ovuliferous scale are not united at their origin from the vascular cylinder in the new cone. Because united bract and scale traces are seen only in *Pinus* among living genera, the presence of this character in the fossil taxa *Pityostrobus hallii*, *P. leckenbyi*, *P. lynnii*, *P. oblongus*, *P. ramentosa*, *P. villerotensis*, and *P. virginiana* Robison et Miller (Table 2-1) suggests that these *Pityostrobus* cones may represent plants closely affiliated with the *Pinus* lineage. These species were among some of the first to be described in terms of their anatomy, which prompted Miller to suggest that *Pityostrobus* showed a "strong *Pinus* influence" (Miller 1976*a* pp. 114). Smith and Stockey (2001) have noted that with the inclusion of more taxa, the assemblage as a whole actually shows more similarities to non-*Pinus* members of the family.

When considering a tangential section taken within the inner cortex of the new cone, the vascular trace to the ovuliferous scale can be seen to derive from the axial vascular cylinder as two lateral strands, which is true for all extant members of Pinaceae, and for the majority of seed cone morphospecies. The fossils *Pityostrobus palmeri*, *P. virginiana*, and the putative sister taxa *P. hautrageanus* and *P. macrocephalus*, as well as the cones assigned to the *Pseudoaraucaria* clade, differ from all other members of the family in that their ovuliferous scale traces appear to initially diverge as a single abaxially concave strand (Table 2-1). The phylogenetic import of this distinction is uncertain; however, in most pinaceaous conifers, the two initial lateral strands do combine within the inner cortex to form an abaxially concave trace which enters the ovuliferous scale. This is not the case in *Keteleeria*, and the fossils *P. cornetiii* and *P. hueberi*, where the vascular tissue forms a complete cylinder (Table 2-1) as it enters the scale.

Resin canal architecture of the scale. Miller (1976a) demonstrated that the origin and architecture of the system of resin canals associated with the ovuliferous scale were diagnostic for extant constituents of Pinaceae. He suggested that comparative assessment of fossil morphotaxa for these characters would also yield phylogenetically informative distinctions.

In most pinaceous cones, including the new specimen from Apple Bay, the pair of resin canals that accompany the vascular trace to the ovuliferous scale are derived from the vertical resin canal system from either side of the diverging cone-scale complex. Although some species of *Pseudoaraucaria* exhibit a similar resin canal conformation, this condition may have been independently derived, as the resin canals of the most basal member, *P. gibbosa* (Coemans) Alvin, have four separate origins from the axillary system. This is similar to the resin canal architecture of the extant genera *Cedrus* and *Tsuga*, and is also seen in *Obirastrobus kokubunii*, although not in its sister taxon, *O. nihongii*, where resin canals have more than 4 separate origins (Table 2-1). *Pityostrobus leckenbyi* and *P. oblongus* also differ from the typical pinaceous condition, in that they exhibit 3 separate origins of the resin canals accompanying the vascular tissue of the bract-scale complexes.

At the base of the ovuliferous scales within the outermost cortex of the Apple Bay cone, the resin canals of the scale are adaxial to the vascular tissue. The basal-most of these canals become associated with the abaxial surface, once the vascular tissue has undergone lateral divisions within the ovuliferous scale proper. This precise conformation is seen only in extant *Picea*, and the fossil *Pityostrobus oblongus* (Table 2-1). Close affinities between the Apple Bay cone and this latter taxon may be confidently dismissed, as *P. oblongus* has cortical resin canals which are markedly dilating, the vascular traces of the bract and scale are united at their origin, the resin canals of the bract/scale complex have three separate origins, the bract lacks an abaxial lobe, and there are resin vesicles within the seed integument (Table 2-1).

When the new specimen is assessed at a level distal to the seed body, the resin canals are found both ab- and adaxial to the vascular strands (which are exceedingly thin, as in extant *Picea*), as well as between the vascular bundles. This particular distribution of resin canals at this level of the ovuliferous scale may be symplesiomorphic within the family, as it is common to most taxa. In considering those taxa which differ, the resin canals of *Keteleeria*, *Pityostrobus hueberi*, and *P. yixianensis* do not course between vascular strands. In *Cedrus* the resin canals remain adaxial and, in *Abies*, they are adaxial to and between vascular bundles. In *P. pubescens,* resin canals occur only between vascular bundles, and in *P. palmeri*, they are abaxial to, and between vascular bundles.

Affinities. The new Apple Bay cone accords best with the extant genus *Picea* in the combination of characters it exhibits. Pollen grains associated with the cone provide corroborative evidence for an affinity with *Picea*, as they can be definitively attributed to the genus (AR Sweet, pers. comm. 2010).

Picea pollen grains are generally larger than those of other genera that possess bisaccate grains, with the exception of *Abies* (Kapp 1969). *Abies* pollen is distinguished from that of the subfamily Pinoideae by the triradiate streak upon its cappa (Bagnell 1975); the cappa of the Apple Bay pollen is scabrose to psillate. Similarly, the sacci of the Apple Bay pollen are borne low with respect to the corpus. This is typical of pollen in the subfamily Pinoideae, whereas in *Abies*, the sacci are borne nearly lateral to the corpus (Bagnell 1975). The pollen found within the micropyles of seeds contained in the Apple Bay cone is ~75µm in total length, and thus accords with the lower end of the *Picea* size range (Kapp 1969). As in *Picea*, the corpus of the Apple Bay pollen is ellipsoidal and suboblate. The juncture formed between the corpus and sacci describes an obtuse angle, which is characteristic of *Picea* and *Cathaya* (Kapp 2000; Saito et al. 2000; Liu and Basinger 2000); in *Pinus*, this angle is distinctively acute (Kapp 1969, 2000). Finally, the sculptured cappula ridges, which largely occlude the germinal furrow on the ventral, or distal, surface of the grain, are diagnostic for *Picea* (Bagnell 1975).

While the new Apple Bay cone provides the oldest record of a complete pinaceous seed cone, the presence several pinaceous leaf morphologies at the Apple Bay locality, which include *Tsuga*-, *Pseudotsuga*-, *Pinus*-, *Picea*- and *Abies*-like forms, indicates that the family was already highly diverse in the late Valanginian (Stockey and Wiebe 2008). Depending upon the points chosen for calibration, relaxed fossil-calibrated molecular clock estimates for divergences of crown groups within the family Pinaceae (Gernandt et al. 2008) have suggested that the subfamilies *Abietoideae* and *Pinoideae* diverged prior to the Aptian (Early Cretaceous, ~125 Ma). Thus far, corroborative Mesozoic fossil records of extant pinaceous genera have been limited to *Pseudolarix* (Krassilov 1982, LePage and Basinger 1995, LePage 2003) and *Pinus* (Alvin 1960, Ryberg et al. 2010). Although the palynomorph *Picea grandivescipites* has been described from the Paleocene of Montana (Wilson and Webster 1946), the oldest spruce macrofossil remains have been found in the Lutetian (Eocene, 41.3-47.5 ma) strata of Axel Heiberg Island in Canada's north (LePage 2001). Accepting the new Apple Bay cone as an early representative of the *Picea* lineage extends the fossil record of *Picea* by ~75 Ma.

A *Picea*-like leaf, described as *Midoriphyllum piceoides* Stockey and Wiebe (2008), provides further evidence for the presence of *Picea*-like plants at the Valanginian Apple Bay locality. The morphology of the vascular bundle and resin canals of *Midoriphyllum*, and the presence of a thickened hypodermis associated with leaf angles, are all similar to extant *Picea*, although the pronounced plicate mesophyll is more like that of *Pinus* (Stockey and Wiebe 2008). Transfusion tissue is similar in *Picea*, *Pinus* (Hu and Yao 1981), and *Midoriphyllum* (Stockey and Wiebe 2008). *Cathaya*, which some analyses (e.g., Gernandt et al. 2008) suggest is sister to *Picea*, has transfusion tissue that is more similar to the basal members of the subfamily Pinoideae (*sensu* Gernandt et al. 2008), *Pseudotsuga* and *Larix* (Stockey and Wiebe 2008). The "*Pseudotsuga*-type" (Hu and Yao 1981) of transfusion tissue exhibited by *Cathaya* is characterized as being predominantly abaxial to lateral, and the transfusion tracheids exhibit spiral thickenings and bordered pits, whereas the "*Pinus*-type" (which, among extant genera, is shared only by *Picea* and *Pinus*) of transfusion tissue completely surrounds the vascular bundle, and contains only bordered pits.

Although *Midoriphyllum* bears striking similarities to extant *Picea*, the cross-sectional leaf shape, which encompasses hexagonal, pentagonal, quadrangular, and triangular forms, is more diverse than that of any extant genus (Stockey and Wiebe 2008). If *Midoriphyllum* and the new cone are vegetative and reproductive organs, respectively, of the same plant, we may infer that the anatomical and morphological features of seed cones are, in evolutionary context, highly conserved in comparison to vegetative features. However, reconciliation of the new *Picea* cone with the vegetative morphospecies *Midoriphyllum piceoides* as a biologically meaningful whole-plant reconstruction is unlikely, owing to the allochthonous nature of the sedimentary strata which comprise the Apple Bay locality. Because the concretions containing *Midorphyllum* specimens and the new cone were collected without regard to specific provenance within the stratigraphic succession preserved at Apple Bay, it cannot be assumed with certainty that these morphotaxa are penecontemporaneous. The likely depositional setting of the Apple Bay strata increases uncertainty with respect to paraconformities between individual beds, which reflect periods of non-deposition or deposition and subsequent erosion, and therefore represent unknown amounts of time.

However, it is obvious that pinaceous diversity in the Early Cretaceous has been undersampled and underestimated to date. As older representatives of extant

-- and extinct -- lineages within Pinaceae are discovered, we may be able to better determine polarity of individual characters. Coupled with novel combinations of characters, this may allow us to untangle the early evolutionary history of this family.

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TABLES

Table 2-1 Morphological matrix for pinaceous seed cones and their stratigraphic context. Characters are defined in Appendix A, and are scored in accordance with Smith and Stockey (2001, 2002). (?) indicates uncertainty of stratigraphic provenance.

	I	XI	XXI	XXXI	Stratigraphic Context (ICS 2009)	Reference
Abies	0(01)101(01)011(01)	(01)(01)1011(01)110	0117001121	001	Chattian – Aquitanian	*Abietoxylon shakhtnaense,* Blokhina 2010
Abies firma	01101?011?	1?1011?110	0117001121	001		
†Apple Bay cone	0010000110	0010101010	0125010020	001	late Valanginian	Klymiuk and Stockey 2010
Cathaya argyrophylla	0000000010	0010??1011	02?5010020	001	Lutetian	*C. gaussenii*, Liu and Basinger 2000
Cedrus	0000101(01)10	(01)010300101	02004(01)1121	001	Albian	*C. penzhianensis*, Blokhina and Afonin 2007
Cedrus deodara	0000101110	1010300101	02004?1121	001		
Cryptomeria japonica	0210000110	00??000020	?344400010	002	mid-Miocene	palynomorph Tsukada 1982
Keteleeria	0000000011	(01)011101111	(01)222011021	001	Albian	*Keteleerioxylon kamtschatkiense*, Blokhina et al. 2006
Keteleeria davidiana	0000000011	1011101111	0222011021	001		
Larix	0(01)100(01)0(01)10	101011(01)0(12)(01)	(01)1150(12)0020	001	Lutetian	*L. altoborealis*, LePage and Basinger 1991
Larix decidua	0010010010	1010110021	0115010020	001		
†*Obirostrobus kokobunii*	0000110111	00(01)0310020	0235020020	001	Turonian-Campanian (?)	Ohsawa et al. 1992
†*O. nihongii*	0000010011	0(01)10411020	0225020020	001	Turonian-Campanian (?)	Ohsawa et al. 1992
Picea	00(01)00(01)0(01)10	(01)0101010(12)0	(01)12(57)4(12)0020	001	Selandian	*Picea grandivescipites,* Wilson and Webster 1946
Picea sitchensis	0000000010	0010101010	0125410020	001		
Pinus	10(01)001(01)(01)1(01)	0110100(01)10	(01)0(03)5410020	001	Hauterivian-Barremian transition	*Pinus* spp. Ryberg et al. 2010
Pinus thunbergii	1010010011	0110100110	?005410020	001		
†*Pinus arnoldii*	1000010010	0110100110	0055410020	001	late Ypresian to early Lutetian	Klymiuk et al. 2011
†*Pararaucaria*	1010000?12	1010500000	0344601020	00(01)	middle – late Jurassic	Stockey 1977
†*Pityostrobus andraei*	10000?01?1	00101?0???	0005000020	001	mid- Barremian – early Aptian	Seward 1919, Creber 1967
†*P. argonnensis*	1010000110	0010130??1	0005010020	001	Albian	Creber 1967
†*P. beardii*	1010100110	1010100010	0005410020	101	late Campanian	Smith and Stockey 2001
†*P. bommeri*	0011000000	0010100?01	0002100020	001	mid- Barremian – early Aptian	Alvin 1953
†*P. californiensis*	0011010111	0010100010	1235020020	001	late Aptian	Smith and Stockey 2002
†*P. cliffwoodensis*	?000010010	0010??0?10	0035110020	001	Santonian-Campanian	Miller 1978
†*P. cornetii*	0010000110	0011?00111	0235100021	001	mid- Barremian – early Aptian	Coemans 1866, Alvin 1953
†*P. hallii*	0000000010	0110110010	00554?0020	001	Santonian	Miller 1974
†*P. hautrageanus*	1010000110	0000??00?1	0055100020	101	mid- Barremian – early Aptian (?)	Alvin 1960
†*P. hokodzensis*	0010100110	1010120011	0005410020	101	late Aptian	Ratzel et al. 2001
†*P. hueberi*	1010000100	1011100101	0052110020	001	mid- Albian – early Cenomanian	Robison and Miller 1977
†*P. jacksonii*	1000010000	0010?00?20	00?511002?	?01	Aptian	Creber 1956, 1967
†*P. kayeii*	1010010000	0000100010	0005000020	001	Santonian	Miller and Robison 1975
†*P. leckenbyi*	0000000???	0110200011	0115(13)10021	001	Aptian – early Cenomanian	Creber 1960
†*P. lynnii*	1000010011	01101100?(01)	000511002?	?01	Thanetian	Miller 1977*b*
†*P. macrocephalus*	1010000110	000?1?0?11	00?5000020	101	Ypresian – Lutetian	Dutt 1916
†*P. makahensis*	0010010011	00101100(12)0	0025010021	001	Priabonian – Chattian	Crabtree and Miller 1989
†*P. mcmurrayensis*	0010100000	0010101010	002?210020	001	Albian	Stockey 1981
†*P. matsubarae*	0010000110	0000100010	0235010020	001	Turronian-Campanian	Ohsawa et al. 1991
†*P. milleri*	1000110011	101013?111	005501002?	?01	late Aptian	Falder et al. 1998
†*P. oblongus*	0010000111	0110200010	0125010021	001	Albian	Fliche, 1896, Creber 1960 Louvel 1960
†*P. palmeri*	1010011110	0(01)00100011	0026410020	001	Santonian	Miller 1972
†*P. pubescens*	0000100000	1010101010	0013420020	001	Cenomanian	Miller 1985

	I	XI	XXI	XXXI	Stratigraphic Context (ICS 2009)	Reference
†*P. ramentosa*	0000000011	1110?00010	022502002?	?01	late Barremian – early Albian	Miller 1976*b*
†*P. shastaensis*	?010010110	0010100011	00?5010020	001	late Barremian – early Albian	Miller 1976*b*
†*P. villerotensis*	0000000000	0110110?10	0035410020	001	mid- Barremian – early Aptian (?)	Alvin 1957*a*
†*P. virginiana*	00000?0000	1100?00010	0000010020	001	mid- Albian – early Cenomanian	Robison and Miller 1977
†*P. yixianensis*	0010010010	0010100010	02224(01)1020	001	Aptian	Shang et al. 2001
†*Pseudoaraucaria arnoldii*	0000100011	000010002(01)	0115531021	011	Santonian	Miller and Robison 1975
†*P. benstedii*	?000100?1?	0000?00?10	0?1?131021	011	Aptian	Alvin 1957*b*
†*P. gibbosa*	1010100111	0000300?10	0215031020	011	mid- Barremian – early Aptian (?)	Alvin 1960
†*P. heeri*	1000100010	0000100110	0112031021	011	mid- Barremian – early Aptian (?)	Alvin 1957*a*
†*P. loppinetti*	1000100010	0000100010	111?131020	011	Albian	Alvin 1957*a*
†*P. major*	1000100010	0000100011	1115431021	011	mid- Barremian (?) – Albian	Alvin 1988
Pseudolarix	1010100110	1010?00100	?235610121	001	Oxfordian-Kimmeridgian	*P erensis*, Krassilov 1982
Pseudolarix amabilis	1010100110	1010?00100	?235610121	001		
Pseudotsuga	0110010(01)10	(01)010100010	(01)115010020	001	middle Miocene	*P. jechorekiae*, Czaja 2000
Pseudotsuga menziesii	0110010010	0010100010	0115010020	001		
Sciadopitys verticillata	0200100000	1000100020	0117200010	002	Cenomanian-Coniacian	*Sciadopityostrobus kerae*, Saiki 1992
Tsuga	0(01)10000(01)(01)0	(01)01031(01)110	0115010021	001	Turonian	palynomorph, Macko 1963
Tsuga mertensiana	0010000100	0010310110	0115010021	001		

FIGURES

Fig. 2-1 *Picea burtonii* sp. nov. Holotype UAPC-ALTA P16098. *A*, Oblique longitudinal section through ovulate cone, showing helically arranged bract-scales complexes. Bbot #2, scale = 1 mm. *B*, Transverse section near base of cone. Note ovuliferous scale bearing two ovules on its adaxial surface (at arrows). Bxs #92, scale = 1 mm. *C*, Transverse section of cone axis, showing sclerotic pith (p), vascular cylinder, and cortex with resin canals (r). Bxs #80, scale = 0.5 mm. *D*, Longitudinal section of cone showing attachment of ovule-bearing bract-scale complexes at right angles to cone axis. Bbot #13, scale = 1 mm. *E*, Tangential longitudinal section of cone showing bract attachment at base of bract-scale complexes. Bbot #9, scale = 1 mm.

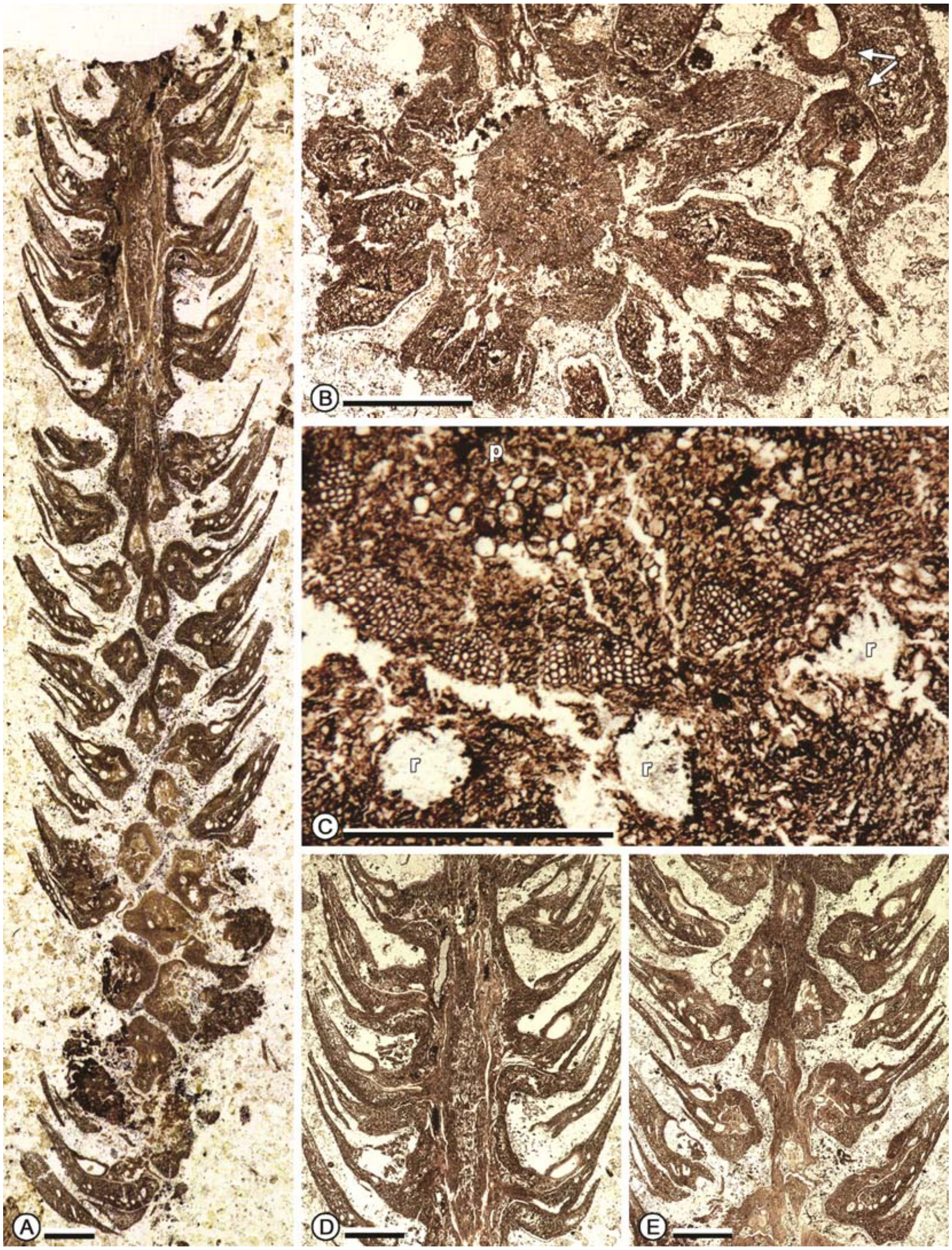
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Fig. 2-2 *Picea burtonii* sp. nov. Holotype UAPC-ALTA P16098. *A*, Radial longitudinal section of cone showing ovuliferous scale (os) and bract (b). Note inverted winged ovule borne on adaxial surface of ovuliferous scale. Bbot #15, scale = 500 µm. *B*, Tangential longitudinal section through cone axis, showing proximal arrangement of vascular tissue and resin canals to the ovuliferous scales (os) and bracts (b) at base of bract-scale complex, i.e., within outer cortex. Bbot #9, scale = 1 mm. *C*, Tangential longitudinal section of cone axis, showing proximal arrangement of abaxially concave vascular trace to ovuliferous scale (os), and terete bract trace (b). Bbot #26, scale = 50 µm. *D*, Secondary xylem showing tracheids with helical to scalariform thickenings. Bbot #13 x 460. *E*, Transverse section through bract-scale complex at level of seed body. Note lateral separation of bract from ovuliferous scale and interseminal ridge. Ctop #2, scale = 500 µm. *F*, Transverse section through bract-scale complex at terminus of seed body. Ctop #40, scale = 500 µm. *G*, Transverse section through bract-scale complex distal to seed. Ctop #51, scale = 500 µm.

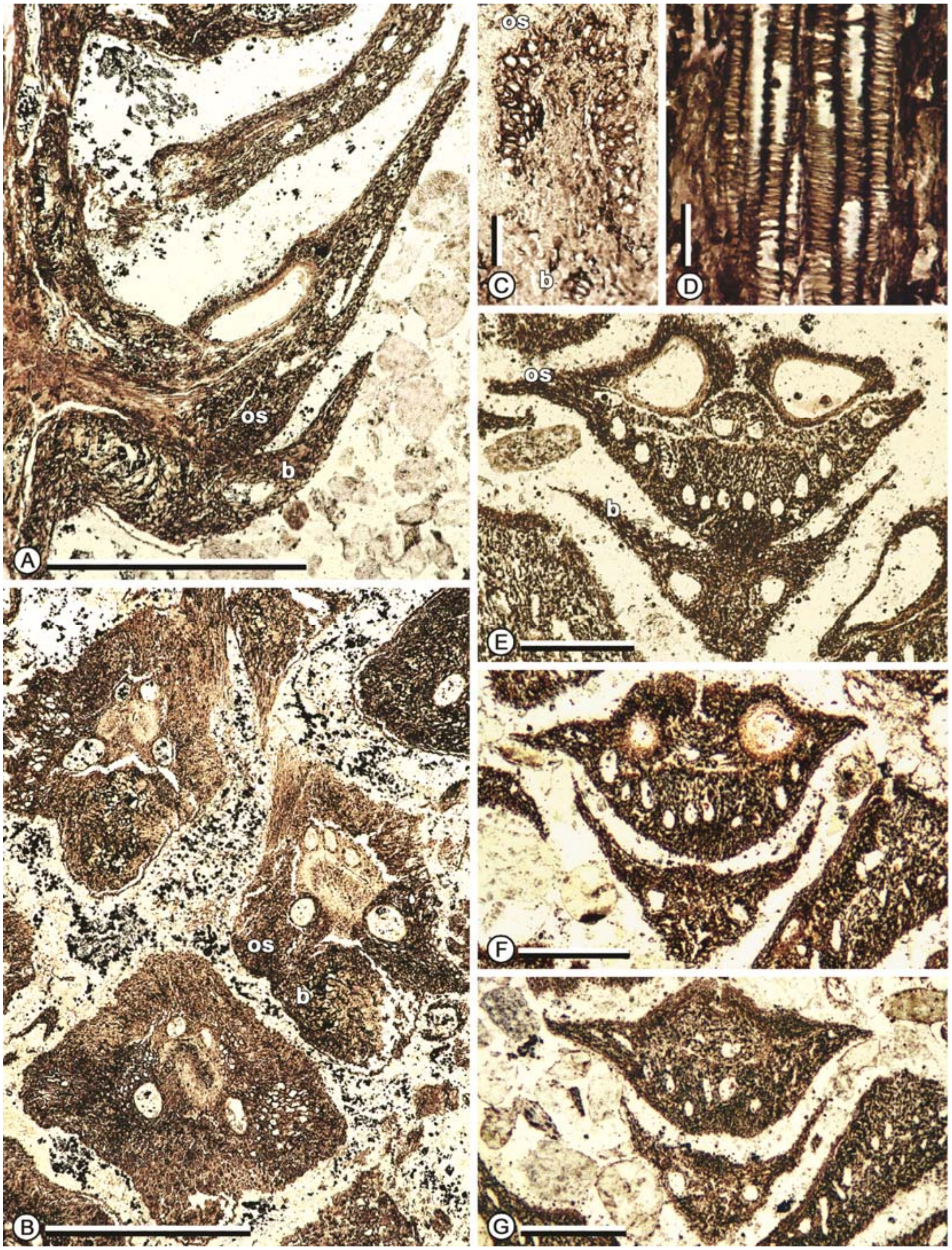
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Fig. 2-3 *Picea burtonii* sp. nov. Holotype UAPC-ALTA P16098. *A*, Longitudinal section through ovuliferous scale showing inverted winged ovule, with micropyle (m) oriented toward cone axis. Note presence of megagametophyte (mg) and nucellar (n) tissue and bisaccate pollen (p). Bbot #13, scale = 500 µm. *B*, Longitudinal section through micropyle of ovule, containing several bisaccate pollen grains (p). Bbot #12, scale = 50 µm. *C*, Longitudinal section through chalazal end of ovule, showing attachment of nucellus (n) to endotesta, and bisaccate pollen (p) embedded in apex of nucellus. Bbot #12, scale = 50 µm. *D*, Bisaccate pollen grains in lateral and dorsal view. Sacci are borne at obtuse angle (arrow) to corpus, and exhibit prominent endoreticulations which become finer at juncture of sacci with corpus. Bxs #72, scale = 25 µm. *E*, Bisaccate pollen grains. Sacci associated with corpus (in ventral view, at arrow) broken away, but note germinal furrow (at arrow). Bxs #72, scale = 25 µm.

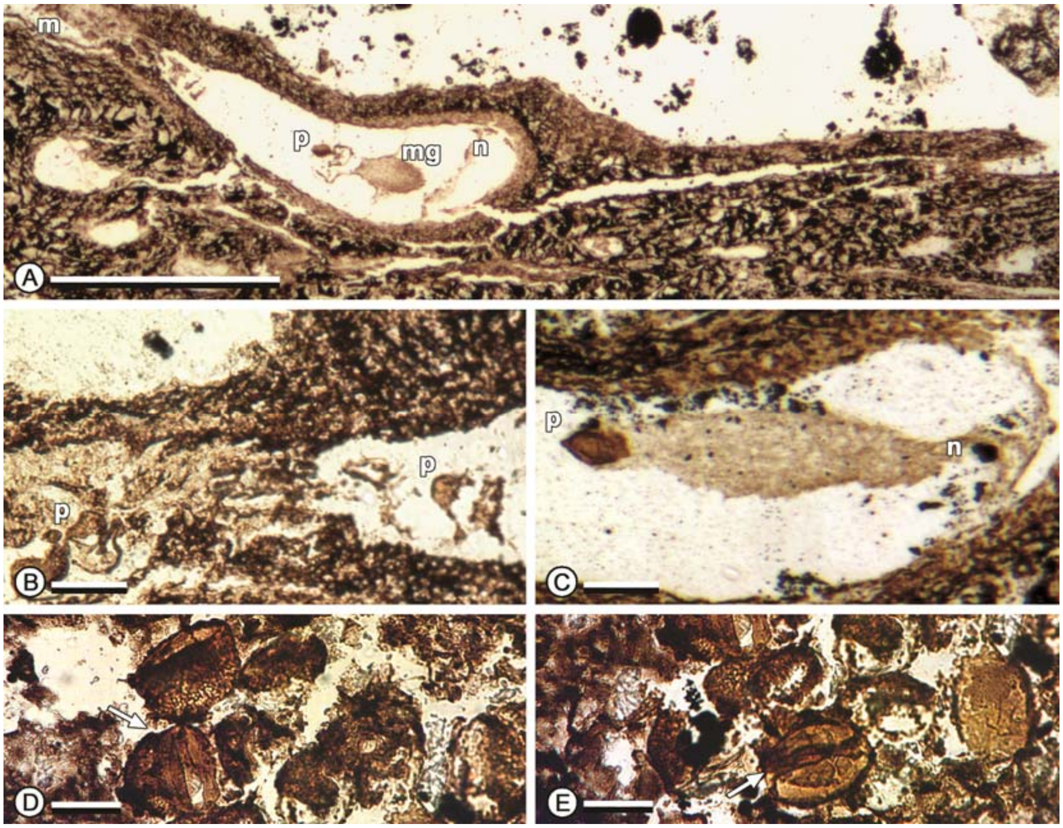
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Fig. 2-4 Origin and architecture of lateral resin canal and vascular systems within base of bract-scale complexes. Abaxially concave trace to ovuliferous scale and terete bract trace (dark grey), surrounded by vertically branching resin canals (light grey) derived from cortical resin canal system.

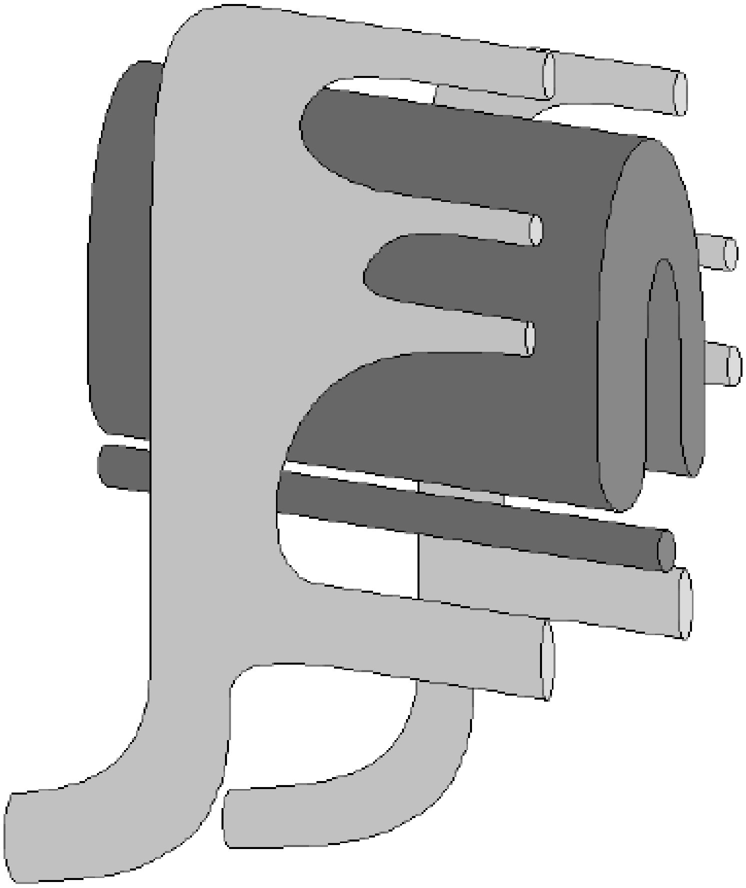
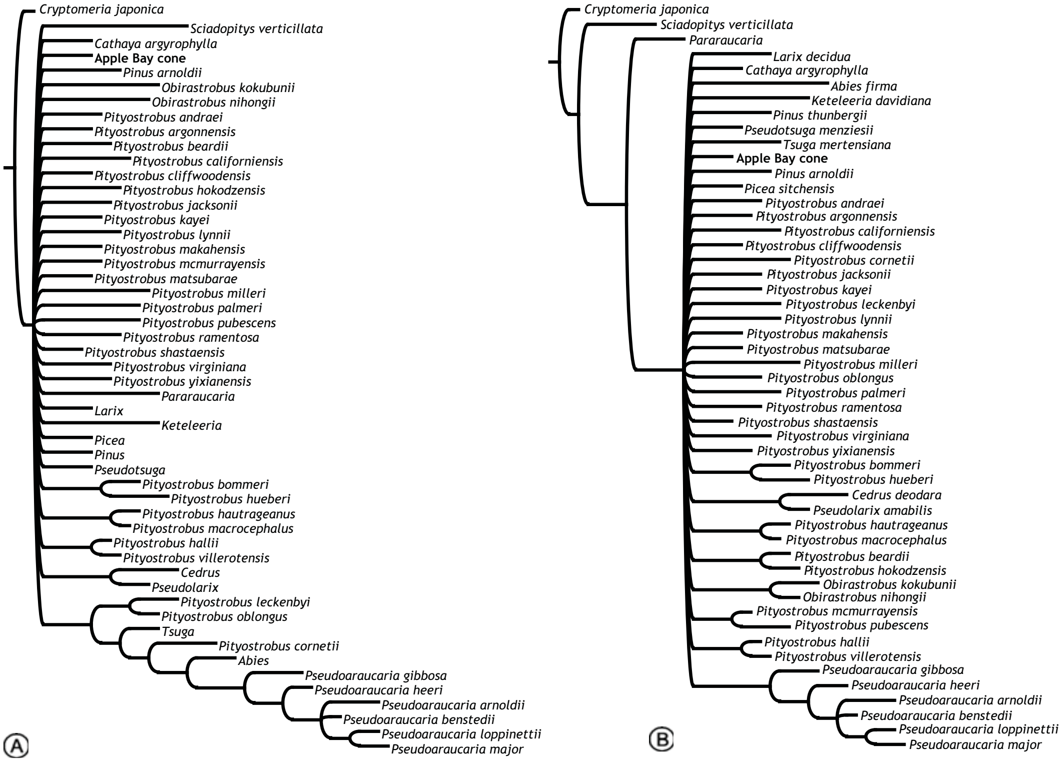


Fig. 2-5 Nelson consensus trees produced from analysis of 33 morphological characters using the parsimony ratchet perturbation algorithm (Nixon 1999). *A*, Nelson consensus of 2636 most parsimonious trees (tree length [TL] = 204, consistency index [CI] = 0.28, retention index [RI] = 0.64) produced when extant genera were scored as polymorphic composite placeholders. *B*, Nelson consensus of 1723 most parsimonious trees (TL = 213 steps, CI = 0.27, RI = 0.61) produced when extant genera were represented by individual species.


Cryptomeria japonica
Sciadopitys verticillata
Cathaya argyrophylla
Apple Bay cone
Pinus arnoldii
Obirastrobus kokubunii
Obirastrobus nihongii
Pityostrobus andraei
Pityostrobus argonnensis
Pityostrobus beardii
Pityostrobus californiensis
Pityostrobus cliffwoodensis
Pityostrobus hokodzensis
Pityostrobus jacksonii
Pityostrobus kayei
Pityostrobus lynnii
Pityostrobus makahensis
Pityostrobus mcmurrayensis
Pityostrobus matsubarae
Pityostrobus milleri
Pityostrobus palmeri
Pityostrobus pubescens
Pityostrobus ramentosa
Pityostrobus shastaensis
Pityostrobus virginiana
Pityostrobus yixianensis
Pararaucaria
Larix
Keteleeria
Picea
Pinus
Pseudotsuga
Pityostrobus bommeri
Pityostrobus hueberi
Pityostrobus hautrageanus
Pityostrobus macrocephalus
Pityostrobus hallii
Pityostrobus villerotensis
Cedrus
Pseudolarix
Pityostrobus leckenbyi
Pityostrobus oblongus
Tsuga
Pityostrobus cornetii
Abies
Pseudoaraucaria gibbosa
Pseudoaraucaria heeri
Pseudoaraucaria arnoldii
Pseudoaraucaria benstedii
Pseudoaraucaria loppinettii
Pseudoaraucaria major
A
Cryptomeria japonica
Sciadopitys verticillata
Pararaucaria
Larix decidua
Cathaya argyrophylla
Abies firma
Keteleeria davidiana
Pinus thunbergii
Pseudotsuga menziesii
Tsuga mertensiana
Apple Bay cone
Pinus arnoldii
Picea sitchensis
Pityostrobus andraei
Pityostrobus argonnensis
Pityostrobus californiensis
Pityostrobus cliffwoodensis
Pityostrobus cornetii
Pityostrobus jacksonii
Pityostrobus kayei
Pityostrobus leckenbyi
Pityostrobus lynnii
Pityostrobus makahensis
Pityostrobus matsubarae
Pityostrobus milleri
Pityostrobus oblongus
Pityostrobus palmeri
Pityostrobus ramentosa
Pityostrobus shastaensis
Pityostrobus virginiana
Pityostrobus yixianensis
Pityostrobus bommeri
Pityostrobus hueberi
Cedrus deodara
Pseudolarix amabilis
Pityostrobus hautrageanus
Pityostrobus macrocephalus
Pityostrobus beardii
Pityostrobus hokodzensis
Obirastrobus kokubunii
Obirastrobus nihongii
Pityostrobus mcmurrayensis
Pityostrobus pubescens
Pityostrobus hallii
Pityostrobus villerotensis
Pseudoaraucaria gibbosa
Pseudoaraucaria heeri
Pseudoaraucaria arnoldii
Pseudoaraucaria benstedii
Pseudoaraucaria loppinettii
Pseudoaraucaria major
B

CHAPTER III: THE FIRST ORGANISMAL CONCEPT FOR AN EXTINCT SPECIES OF PINACEAE: *PINUS ARNOLDII* MILLER

INTRODUCTION

Our understanding of plant phylogeny is dramatically enhanced by the fossil record (e.g., Huelsenbeck, 1991; Kenrick and Crane, 1997; Rothwell et al., 2009), but the impact of paleontology on systematics is often limited by an incomplete understanding of extinct species. Most fossil plants are known from isolated organs that are described as morphotaxa. Furthermore, we know that plant organs may evolve at different rates (Crane and Stockey 1986). The reconstruction of whole plants from the fossil record is essential to our understanding of extinct species, to provide the data necessary for studies of trends in plant evolution, and to resolve the overall pattern of phylogeny for major clades.

The Middle Eocene Princeton Chert locality of southern British Columbia, Canada, reflects a predominantly autochthonous assemblage, in which extinct species of permineralized plants are represented by numerous *in situ* vegetative and fertile organs (Cevallos-Ferriz et al. 1991; Pigg and Stockey 1996). Thus, the Chert provides the opportunity for a number of whole-plant reconstructions. The filicalean fern *Makotopteris princetonensis* Stockey, Nishida et Rothwell (1999) is represented by rhizomes, roots, vegetative fronds, sporangia, and spores, and represents the most completely known extinct species within the Athyriaceae. Similarly, a blechnaceous fern, *Trawetsia princetonensis* Smith, Stockey, Nishida

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et Rothwell (2006), has been reconstructed on the basis of interconnected rhizomes, roots, and vegetative fronds. The Chert also preserves two eudicots, *Eorhiza arnoldii* Robison et Person (Robison and Person 1973; Stockey and Pigg 1991, 1994) and *Decodon allenbyensis* Cevallos-Ferriz et Stockey (1988; Little and Stockey 2003; Little et al. 2004), which have been largely reconstructed on the basis of organic attachment among both vegetative and fertile organs. Of the conifers present in the Princeton Chert, *Metasequoia milleri* Rothwell et Basinger (Cupressaceae) is the only species that has previously been reconstructed on the basis of ovulate and pollen cones, leaves, wood stems, and roots bearing *Paris*-type vesicular-arbuscular mycorrhizal associations (Rothwell and Basinger 1979; Basinger 1981, 1984, Stockey et al. 2001).

In this study, we develop a similar organismal concept for another Princeton Chert conifer, *Pinus arnoldii* Miller. *Pinus arnoldii* was originally described as an ovulate cone (Miller 1973), and subsequently amplified (Stockey 1984) to include details of seed morphology and the presence of megagametophytes and embryos. Miller (1973) also described *P. similkameenensis* Miller from the same locality, as a five-needled pine known from leaves, woody stems, and dwarf shoots. Here, we document organic attachment of *P. arnoldii* ovulate cones to the woody stems of *P. similkameenensis* to demonstrate that it comprises the vegetative morphology for *P. arnoldii*. Pinaceous pollen cones, as described by Phipps et al. (1995) and ectomycorrhizal coralloid root masses (LePage et al. 1997) are interpreted as constituent organs of *P. arnoldii* on the basis of spatial association, and provide evidence for *in situ* growth of the species at this locality. We also provide an assessment of the developmental sequence for ovulate cones, including the pollination stage. Consequently, an organismal concept is developed for this extinct species of pine, in which the morphology and anatomy of all vegetative and fertile organs, gametophytes, and embryos, as well as growth habit, ecology, and several facets of development are characterized.

This reconstruction yields a phylogenetically important combination of characters within the genus *Pinus*. Among extant genera, ovulate cones most similar to *P. arnoldii* (Miller 1973) are classified within the subgenus *Pinus*, section *Pinus*. The leaves of *P. similkameenensis* resemble those of species classified within subgenus *Strobus*, section *Quinquefoliae*, while the wood anatomy resembles that seen in subgenus *Strobus*, section *Parrya* (Miller 1973). To date, phylogenetic analyses of *Pinus* have predominantly focused on molecular data (Gernandt et al. 2005, 2008; Parks et al. 2009), with only minor contributions from morphological data sets in some studies (Gernandt et al. 2005). This limits the resolving power of such studies to extant taxa, leaving the internal nodes of the tree less confidently resolved than if extinct species were included. Phylogenetic analyses which include fossil species such as *P. arnoldii* provide an opportunity to develop stronger and/or more accurate resolution at the deeper nodes, thereby improving our understanding of phylogeny as evidenced through morphology.

MATERIAL AND METHODS

All of the organs of *Pinus arnoldii* co-occur within permineralized peat, that crops out as ca. 70 discrete and anastomosing chert layers (Smith et al. 2006) interbedded with sub-bituminous coal seams, stratigraphically 630 m above the Princeton-Black Coal Seam (Boneham 1968). Most of the materials examined in this study derive from layers 16, 18, and 20; a small number of specimens were obtained from dislodged blocks collected at the base of the exposure.

The Princeton Chert is part of the Allenby Formation, Princeton Group, (Hills and Baadsgaard 1967) and has been K-Ar dated to 48.7 million years bp (Baadsgaard pers. comm. to RAS 1999). The locality is a single inclined exposure that crops out along the east bank of the Similkameen River, approximately 8.4 km southwest of Princeton, British Columbia (UTM 10U 678057 5472372; 49°22’40” N, 120°32’48” W).

Chert blocks were cut into ca. 2 cm slabs. Sections of plant organs were prepared with the cellulose acetate peel technique (Joy et al. 1956) as modified for 48% hydrofluoric acid (Basinger and Rothwell 1977; Basinger 1981). Specimens, organ attachments, and organ associations were identified on peels from ca. 2600 slabs; the most informative of these specimens were serially sectioned to identify organ attachments, and to characterize morphological features of individual organs. The study material includes 351 seed cones, 17 of which are immature, and 56 pollen cones ranging from immature through mature to senescent. Twigs, leaves, and roots are typically ubiquitous throughout the slabs containing specimens of *P. arnoldii*. Histological features of the specimens were studied from peels mounted on microscope slides prepared using the xylene-soluble mounting medium Eukitt (O. Kindler GmbH, Freiburg, Germany). Images were captured with a Powerphase (Phase One, Copenhagen, Denmark) digital scanning camera mounted on a Leitz Aristophot bellows, a Zeiss WL compound microscope, and a Nikon DS-Ri1 (Nikon, Tokyo, Japan) camera mounted on a Nikon Eclipse 80*i* compound microscope, and a Leaf Microlumina System version 1.2 (Leaf Systems, Westborough, Massachusetts, USA), and processed with Adobe Photoshop CS2. All specimens, peels, and slides are housed in the University of Alberta Paleobotanical Collection (UAPC-ALTA).

Design of the phylogenetic analysis performed in this study follows established practices wherein morphological characters of living and extinct species are added to gene sequence matrices to test tree topology hypotheses, and to place the extinct species upon the tree (e.g., Jud et al. 2008). Phylogenetic analyses were performed using modified matrices (Appendix B) that utilize *mat*K and *rbc*L nucleotide sequences (Gernandt et al. 2005, archived at TreeBASE) for 101 extant pines, and massively parallel sequenced cpDNA (Parks et al. 2009) for 31 extant pines, in combination with morphological characters (adapted from Gernandt et al. 2005; Farjon and Styles 1997; Smith and Stockey 2001, 2002). In this study, the extant pines and the extinct species *P. arnoldii* were scored for nine of the characters (Appendix C) assessed in the morphological matrix presented by Gernandt et al. (2005). The tenth character was excluded from the analysis as it relates to biogeography and is not phylogenetically informative. In addition, all taxa in the analyses were scored for 31 seed cone characters (Appendix C), in accordance with the Smith and Stockey (2001; 2002) morphological matrix for pinaceous seed cones; where representative specimens of extant pines were unavailable, polymorphic characters were scored as unknown. Tree-searches were performed under the parsimony ratchet perturbation algorithm (Nixon 1999), utilized in the program TNT (Goloboff et al. 2008) spawned through Winclada (Asado, version 1.1 beta, by K. Nixon, Cornell University). Branches were collapsed if the support was ambiguous, all characters were non-additive, multistate characters were unordered. In each search, ratchet perturbation sampled 10% of the parsimony-informative characters through 5000 iterations per replication, with five trees held per iteration. Only strict consensus phylogenies are reported; bootstrap (Felsenstein 1985) values were produced from 1000 replicates, with ten trees held for each of 100 multiple TBR search replications.

RESULTS

Systematics

Order – Coniferales

Family – Pinaceae

Genus – Pinus L.

Species – Pinus arnoldii Miller

Amplified diagnosis

Ovulate cones long-conical, base slightly asymmetrical, 5-7.5 cm long by 1.8-2.8 cm at widest diameter; axis 6-12 mm in diameter, surrounded by numerous cone-scale complexes. Vascular cylinder of axis ca. 5 mm in diameter; pith 2 mm in diameter, constructed of large thick-walled parenchyma cells, many with dark contents. Primary xylem endarch; secondary xylem with resin canals abundant in one ring, rays mostly uniseriate. Cortex 1.5-3 mm thick, with thin inner layer of small parenchyma cells, thick middle layer of large parenchyma cells with 14-22 resin canals, broad outer layer of thick-walled sclereids. Cone-scale complex trace separating from vascular cylinder of cone axis as circular to oval unit, dividing in outer part of middle cortex to form terete bract trace and abaxially concave scale trace. Bract 5 mm long, free from scale. Ovuliferous scale 20 mm long, 15 mm wide, ca. 1 mm thick, borne at angle of 5°-10° to cone axis, containing up to 15 vascular strands. Resin canals of scale base abaxial to vascular strand, forming up to 24 canals apically, most abaxial to vascular strands but some interfascicular. Ovuliferous scale apex ca. 2 mm thick; apophysis rhomboidal, 5-8 mm high, 8-12 mm wide; umbo dorsal, centrally located on apophysis. Seeds two per scale, 5 mm long, 3 mm wide, winged. Seed wings ca. 1 cm long; wing cells 70 μm wide, up to 600 μm long. Sclerotesta of isodiametric thick-walled sclereids 40 μm in diameter. Sarcotesta of rectangular parenchymatous cells 30 μm high. Immature ovulate cones, sheathed in scale leaves, 1.3 mm wide, 2.2 mm long. Ovulate cones at pollination stage, 3 mm in diameter, at least 6 mm long; pith 0.9 mm in diameter; vascular strands of 6-9 tracheids, discrete bundles; resin canals conspicuous, 0.17-0.37 mm in diameter; cortex narrow, only outer sclerotic layer present; ovuliferous scales 1.1-1.3 mm long, widest at apophysis, 1.2 mm wide, 0.27 mm thick; distinct hypodermis and epidermis visible on apophysis; pollen bisaccate.

Stems with well-developed growth rings. Pith of large thick-walled parenchyma cells. Primary xylem endarch, tracheids with annular and helical thickenings. Secondary xylem with tracheids 10-30 µm in diameter, bearing uniseriate circular bordered pits. Resin canals abundant, with single layer of thin-walled epithelial cells. Rays uniseriate, 1-8 cells high, those with resin canals biseriate, 10-16 cells high. Ray tracheids rare, marginal, non-dentate, with 1-4 pits per tracheid. Ray parenchyma with 1-5 pinoid cross-field pits. Cortex of large thin-walled parenchyma cells, numerous resin canals. Trace to short shoot originating from two bundles that fuse to become circular in x.s., ca. 0.5 mm in diameter, surrounded in cortex by several cell layers of small parenchyma cells, short shoot separating from stem in axil of cataphyll.

Short shoots 1-1.5 mm in diameter, 2 mm long. Fascicle sheath of 6-8 scale leaves, 70-120 µm thick, deciduous with senescent fascicle. Fascicles five-needled. Leaves at least 2.3 cm long, 0.4-0.6 mm wide; vascular strand single; resin canals two, dorsal, external, lacking hypodermal sheath; mesophyll 2-4 cells thick, cell margins undulating not plicate; hypodermis mostly one cell layer thick, infrequently 2 layers, fibers smaller than epidermal cells. Stomata slightly sunken, in 2-3 rows on each abaxial surface.

Pollen cones elongate, ellipsoidal, 2.8-6.9 mm long, 1.6-3.5 mm in diameter, ensheathed by scale leaves when immature. Cone axis 0.3-0.6 mm in diameter, bearing numerous helically arranged microsporophylls. Pith parenchymatous, surrounded by 14-18 discrete vascular bundles. Tracheids with scalariform thickenings and uniseriate circular bordered pits; cortex parenchymatous, containing resin canals. Microsporophyll parenchymatous, with single vascular trace, resin canals 2, pollen sacs 2, abaxial, exterior cell walls with endothecial thickenings; grains bisaccate, psilate to scabrate, endoreticulate, 50-70 µm; corpus 27-43 µm wide, 23-50 µm long, 30-33 µm high; sacci 27-40 µm wide, 10-23 µm long, 20-30 µm high.

Lateral roots triarch and tetrarch, 3-4 mm diameter, with attached diarch coralloid roots. Coralloid roots dichotomously branched, 0.3 mm diameter; cortex parenchymatous, with dense extracellular hyphal network forming Hartig nets, mantle hyphae pseudoparenchymatous, simple-septate.

Holotype. University of Michigan Museum of Paleontology (UMMP) No. 60482.

Paratypes. University of Michigan Museum of Paleontology (UMMP) No. 60483. University of Alberta Paleobotanical Collection (UAPC-ALTA) No. P1126, P1143, P1302, P1618, P1981, P2449, P2480, P2566, P4800.

Description

Several organs that represent the *P. arnoldii* plant, as described here, have been described previously, including the seed cones (Miller 1973, Stockey 1984), pollen cones (Phipps et al. 1995), stems with attached fascicles of leaves (Miller, 1973), and coralloid mycorrhizal root systems (LePage et al 1997). Therefore, this study summarizes previous work and describes newly discovered characters, developmental data, and organ attachments, derived from a large suite of new specimens.

Seed cone, general characters. The seed cones are long-conical, 5-7.5 cm long by 1.8-2.8 cm at their widest diameter (figs. 3-1A, 3-1B). The cones are asymmetrical at the base, (fig. 3-1C), open at maturity, and bear umbos which lack mucros.

Cone axis. The pith of the cone axis is 2.6-2.9 mm in diameter near the base of the cone, gradually tapering towards the apex (fig. 3-1D). Cells are parenchymatous, polygonal to circular in transverse section, and 15-75 µm in diameter. No resin canals or sclereids are associated with the pith.

The vascular cylinder is composed predominantly of secondary xylem, with strands of endarch primary xylem projecting into the pith (figs. 3-1A, 3-1B, 3-1D). The vascular tissue forms an entire cylinder, up to 5 mm in diameter at the cone base, and narrowing to ca. 1 mm near the cone apex. Phloem is rarely preserved, but may be inferred by a narrow, mineralized band surrounding the secondary xylem (fig. 3-1D). Tracheids are polygonal to rectangular in transverse section, 15-45 µm in diameter. In radial longitudinal section, the tracheids exhibit sparse uniseriate circular bordered pits. In tangential section, the secondary xylem exhibits rays that are 5-10 cells high.

Resin canals are associated with the earliest wood of the vascular cylinder, and many are opposite a primary xylem strand (fig. 3-1D). At the cone base, they form a ring of 34-40; at the apex, this number decreases to 14-16. A smaller number of canals are scattered throughout late wood secondary xylem. The canals range in diameter from 45-85 µm, and are lined with a single layer of 6-12 thin-walled epithelial cells.

The cortex consists of three distinct layers (fig. 3-1D), and is 1.8-3.0 mm thick basally, thinning to 0.7 -1.5 mm towards the apex. The innermost zone of small-diameter parenchymatous cells is 0.1- 0.2 mm thick at the cone base, rapidly thinning and disappearing towards the apex. The middle cortex, 0.5 – 0.65 mm thick basally, is comprised of larger-diameter parenchyma and resin canals (fig. 3-1D). As many as 22 resin canals occur at the base of the cone (fig. 3-1D); this number diminishes to no more than four near the apex. The outer cortical layer, 1.0-2.0 mm thick basally, contains thick-walled, highly sclerotic cells that are the same size as those of the middle cortex (fig. 3-1D).

Cone scale complex. Vascular traces to the cone-scale complexes diverge from the axis as cylinders, which appear circular to oval in transverse section (fig. 3-1D). The vascular trace is accompanied by two resin canals originating within the middle cortex (fig. 3-1D). At the boundary between the middle and outer cortex, the vascular trace assumes an abaxially concave configuration as a result of the divergence of the bract trace (fig. 3-1E). At this position, the resin canals also branch: two canals remain associated with the terete bract trace, while the primary branches of the canals accompany the ovuliferous scale trace, and undergo successive branching (figs. 3-1E, 3-1F, 3-1G). As many as ten resin canals may be found abaxial to the vascular trace when it enters the ovuliferous scale as a horizontal band.

Bracts are 4-5 mm long, and 3-4 mm high and wide at the point of separation from the axis. The bract is entirely free from the scale, with its base decurrent on the axis (fig. 3-1B). The ground tissue of the bract is comprised of sclereids continuous with the outer cortex of the axis and a single layer of thin-walled parenchyma forms the epidermis. The vascular strand diminishes in diameter once inside the bract. It is flanked by two resin canals which persist only a short distance beyond the terminus of the vascular strand.

The ovuliferous scale is 16.5-20 mm long, 4.5-6.0 mm wide, and 1.5-2.2 mm thick. It projects from the axis at a right angle for ca. 3 mm, or until distal to the seed body, after which it bends towards the cone apex for 13-16 mm (figs. 3-1B, 3-1C). At the base of the ovuliferous scale, the vascular tissue occupies the adaxial half of the scale in an unbroken band which is concave beneath the seed cavities (fig. 3-1F). There is a thin zone of parenchyma immediately adaxial to the vascular tissue which contains 7-10 resin canals (fig. 3-1F), and extends ca 0.7 mm between the seed cavities, forming a subtle interseminal ridge (fig. 3-1F). The abaxial side of the scale base has a zone of sclerenchymatous tissue that extends laterally, forming the margins of the scale. (figs. 3-1F, 3-1G).

At the point of attachment of the seed wing, the ovuliferous scale briefly broadens to a width of 10 mm as it bends towards the cone apex (figs. 3-1A, 3-1G). The vascular tissue is dissected into 10-15 discrete strands that occupy a medial position with respect to the scale surfaces (fig. 3-1G). The adaxial parenchyma layer is thick, while both the abaxial parenchyma and sclerenchyma layers are thin (fig. 3-1G). At the level of the seed wing, the abaxial parenchyma contains up to 24 resin canals which are predominantly abaxial to the vascular bundles, but occasionally occur between them. The ovuliferous scale is largely parenchymatous beyond the level of the wing (fig. 3-1G).

The rhomboidal apophysis is 5-8 mm high, 8-12 mm wide, and bears an umbo that projects 0.5 - 1.3 mm (figs. 3-1B, 3-1C, 3-4A). Ten vascular strands pass into the apophysis, but terminate 0.5 mm from the umbo. The umbo does not bear a mucro, as evidenced by epidermal cells preserved along the entire margin (figs. 3-1C, 3-4A).

Seeds. Each ovuliferous scale bears two seeds, 5 mm long and 3 mm wide, on its adaxial surface (figs. 3-1A, 3-1F, 3-2A). The endotesta is thin and often degraded, but the sarcotesta and sclerotesta are preserved in many specimens (fig. 3-2A). The sarcotesta is 1-3 cells thick, (fig. 3-2A), and the sclerotesta is comprised of 5-7 thick-walled, isodiametric cells, 37-40 µm wide (fig. 3-2A). The abaxial surface of the seed is slightly undulating, but not rugose or ridged (figs. 3-1F, 3-2A). Seed wings (fig. 3-2A, at arrows) are derived from the ovuliferous scale tissue, and are up to 1 cm long, 7-14 cells thick, thinning to a single cell layer distally (figs. 3-1F, 3-2A).

Nucellar tissue is commonly preserved within seeds, and several specimens preserve remnants of the megagametophyte and/or a developing embryo (figs. 3-1F, 3-3A, 3-3C). However, degradation of these tissues prevents further description. The number of cotyledons is unknown.

Immature ovulate cones. Immature cones are known from 17 specimens, and exhibit several different developmental stages. The earliest stage is represented by an immature cone, 1.3 mm in diameter, and 2.2 mm long, that is still in bud (i.e., completely sheathed by scale leaves; fig. 3-2C) and displays developing bract and ovuliferous scale primordia.

The most complete specimens are those first-year cones preserved at the pollination stage (figs. 3-2B, 3-2D). They are ca. 3 mm in diameter, and at least 6 mm long, and exhibit cone structure comparable to that of extant pines at the pollination stage (figs. 3-3C, 3-3D). Ovuliferous scales are 1.1-1.3 mm long. Where they diverge from the cone axis, they are very narrow (0.1 mm), and are widest at the inflated apophysis (fig. 3-2B). In transverse section, the umbo is 1.2 mm wide and 0.27 mm thick. The distinctive cortical zonation of mature cones, wherein the outer cortex is densely sclerotic, is almost entirely absent at the pollination stage (fig. 3-2B), but the exterior tissue of the apophysis is distinctively zonate (figs. 3-2B, 3-2D). The inner ground tissue of the ovuliferous scale is continuous with that tissue which forms the outer cortex of the cone axis. The abaxial surface of the apophysis has a hypodermis of thin-walled parenchyma, up to two cell layers thick (fig. 3-2B). The ovuliferous scale bears two inverted ovules, with micropyles oriented towards the cone axis (fig. 3-2B). The seed integument has not differentiated, and the micropyle is open (figs. 3-2B, 3-3A, 3-3B). Several bisaccate grains, identical in size and morphology to those found in the pollen cones (figs. 3-6D, 3-6E), are preserved on the apex of the nucellar tissue (figs. 3-3A, 3-3B).

Seed cone attachment. Two specimens preserve anatomical attachment between mature *Pinus arnoldii* seed cones, and woody stems (figs. 3-4A, 3-4B). These stems are identical in all respects to those previously described as *Pinus similkameenensis* (Miller 1973). These seed cones are flexed at an angle from that of the stem, but otherwise appear to be terminal. Another specimen, cut in oblique longitudinal section, shows an immature cone at the pollination stage attached at the tip of a woody stem (fig. 3-2D), which is 7 mm long, and bears several scale leaves, or cataphylls, 0.4-0.6 mm long. A similar configuration occurs in extant pines (for example, *P. mugo* L., figs. 3-2E, 3-2F), after abscission of pollen cones, which are borne laterally in the axils of cataphylls (fig. 3-2E), and branches are often terminated by immature ovulate cones.

Woody stems. Stems preserved in the Chert, including those to which are attached fascicles of leaves and seed cones, range in size from 3 mm to 4 cm in diameter, with up to 91 growth increments recorded thus far (figs. 3-4C, 3-4G, 3-5A). The pith is composed of thick-walled parenchymatous cells, 15-65 µm in diameter (figs. 3-4G, 3-5A). There are typically 8 primary endarch xylem strands at the pith margin. Protoxylem tracheids are 10-15 µm in diameter, with annular or helical secondary wall thickenings. Secondary xylem tracheids are 10-30 µm in diameter, and have large uniseriate circular bordered pits (figs. 3-4D, 3-4F) on radial walls, and smaller uniseriate pits on tangential walls of summer wood.

Rays are numerous, uniseriate and 1-8 cells high, except where they contain a resin canal, where they are biseriate and 10-16 cells high (figs. 3-4E, 3-4F). Ray tracheids, although rare, can be seen in radial section at the margins of ray parenchyma; they are 15-26 µm in diameter, and 35-80 µm long, with adentate (smooth) walls. Ray parenchyma cells have a similar size and shape, but have 1-5 pinoid cross-field pits (fig. 3-4F). Many are filled with dark contents.

Resin canals are numerous within the secondary xylem of woody stems (figs. 3-4C, 3-4D, 3-4F). They are 60-90 µm in diameter, a measurement that includes the epithelial layer. The epithelial layer is infrequently preserved, but where evident is composed of a single layer of thin-walled cells (fig. 3-4D). Within the cortex, resin canals are numerous, and evenly distributed (fig. 3-5A).

Young stems (2-6 years) tend to exhibit good preservation of the cortex, which is parenchymatous (figs. 3-4G, 3-5A). Some specimens also preserve radial files of periderm, 4-6 cells in height; individual cells are 40-50 µm in length and 8-15 µm high (fig. 3-4G). Most stems in which cortical tissues are preserved also show vascular cylinders of short shoots (figs. 3-4G, 3-5A, 3-5B, 3-5C, 3-5D).

Short shoots and leaves. Short shoots are vascularized by a narrow, woody stele 0.3 mm in diameter, which encloses a small pith, 70-80 µm in diameter (figs. 3-5C, 3-5D). As is characteristic of seed plant axillary branching (Rothwell 1975), the vascular cylinder is derived from a pair of strands diverging from two cauline bundles of the stem that flank the radius of leaf trace divergence (fig. 3-5B). This pair of branch traces fuse within the cortex to form a cylinder (figs. 3-4G, 3-5A, 3-5C). In successive acropetal sections, the epidermis, cortical tissues, and resin canals associated with the incipient short shoot form a bulge at the periphery of the cortex, which separates from the stem (figs. 3-4G, 3-5A). A small leaf trace of 6-8 tracheids can be seen on the abaxial side of the short shoot cylinder. This trace vascularizes the cataphyll, or scale leaf, which subtends the short shoot.

At the base of the short shoot the vascular cylinder is surrounded by five resin canals (fig. 3-5D), which laterally ramify to form the resin canals of the cataphylls. Six to eight persistent cataphylls enclose the base of the leaves. Each cataphyll contains 6-8 resin canals, is 70-120 μm thick, and is densely sclerotic (fig. 3-5F).

Immediately below the visible divergence of the leaves (fig. 3-5G, at line e), the vascular trace divides into five strands, each of which is accompanied by two resin canals (fig. 3-5E). At this level, the mesophyll and epidermal tissue of the leaves is undifferentiated from the parenchymatous ground tissue of the short shoot. The margins of the leaves differentiate first; the leaves remain basally constricted for some distance (fig. 3-5E).

The majority of leaves appear to be dispersed, a function of taphonomy, and of the orientation and level at which they have been cut. Despite this, articulated fascicles of five leaves, including those attached to *P. arnoldii* stems, are extremely common (fig. 3-5F), suggesting that the dispersed *P. similkameenensis*-type leaves found in the Princeton Chert were produced by *P. arnoldii* plants. Five four-needled fascicles have been found to date, but because they number among literally thousands of five-needled fascicles, and are identical with respect to pertinent anatomy, we consider them to be a normal variant of *P. arnoldii*.

In transverse section, the leaves of the fascicle approximate equilateral triangles (fig. 3-5F). Leaves are 0.4-0.6 mm wide and at least 2.3 cm long. Each leaf contains a single vascular strand (fig. 3-5F). The endodermis has a circular outline in transverse section, and encloses a layer of thin-walled transfusion tissue, two cells thick (fig. 3-5F). The mesophyll is composed of slightly undulating but not plicate parenchyma, 2-4 cells thick. The two external resin canals contact the hypodermal fibers, but are not surrounded by them. The hypodermis is 1-2 cell layers thick, with fibers 10-25 µm in diameter. The epidermis is a single layer of rectangular cells 15 µm wide. Stomata occur only on the adaxial surfaces, and have long axes parallel to that of the leaf. They occur in 2-3 rows, and are slightly sunken, to the level of the hypodermis (fig. 3-5F).

Pollen cones. Pollen cones are numerous; 56 have been included in this study. They are almost always found in association with *P. similkameenensis*-type leaves, and frequently occur within rocks containing abundant *P. arnoldii* seed cones, vegetative shoots and roots. Groups of pollen cones (fig. 3-6A), are striking because they are often preserved in rows, and occur in the same orientation, which is suggestive of attachment at another level in the chert block (i.e., a level that was lost in the saw cut). These data are consistent with the cones having been borne in fertile zones similar to those of some extant species (e.g., fig. 3-2E), and are also in agreement with the specimen in Fig. 3-2D, which suggests possible attachment of pollen cones in the axils of scale leaves that subtend seed cones. However, at this time no organic attachment has been found between *P. arnoldii* stems and pollen cones.

Pollen cones are cylindrical, 2.8-6.9 mm long by 1.6-3.5 mm in diameter (fig. 3-6B). At their base, some cones are sheathed by scale leaves (fig. 3-6C) 0.6-5 mm long. The morphology and anatomy of these scale leaves are consistent with those of the scales subtending other organs of *P. arnoldii* (figs. 3-2C, 3-5F, 3-5G, 3-6B, 3-6C).

The pollen cone axis is 0.3-0.6 mm in diameter, and contains parenchymatous pith, surrounded by 14-18 discrete vascular traces in transverse section (fig. 3-6C). Each vascular trace contains 9-18 tracheids with scalariform thickenings or circular bordered pits. The cortex is composed of thick-walled parenchyma, and contains 16-20 longitudinally oriented resin canals, which branch into the microsporophylls.

The helically-arranged microsporophylls, 0.25 mm long by 0.17 mm wide, are composed of thick-walled parenchyma (fig. 3-6C, 3-6D). Each microsporophyll contains a single vascular trace, associated with two resin canals. There are two pollen sacs borne on the abaxial surface (fig. 3-6C, 3-6D). Pollen sacs are 0.2 mm long, and exhibit distinct endothecial thickenings (fig. 3-6D). Pollen grains are bisaccate, and have external sculpture that is psilate to scabrose (fig. 3-6E). The grains exhibit endoreticulation on the inner walls of the sacci. Grains are 50-70 µm wide; the corpus is 27-43 µm wide by 23-50 µm long, and 30-33 µm high. The sacci measure 27-40 µm in width, 10-23 µm in length, and 20-30 µm high (fig. 3-6E).

Roots. Pinaceous roots are represented in many of the chert blocks studied, but are particularly abundant in chert containing bands of densely compressed peat. Lateral triarch and tetrarch roots (fig. 3-7A) are 3-4 mm diameter, and have attached coralloid rooting systems (fig. 3-7B). Coralloid roots measure 0.3 mm in diameter, and exhibit as many as four dichotomies (fig. 3-7B). Intercellular spaces throughout the cortex and epidermal tissues are host to a dense network of ectomycorrhizal fungi, the hyphae of which form Hartig nets (fig. 3-7C). The coralloid roots entirely lack root hairs; instead, the hyphae form a pseudoparenchymatous mantle (fig. 3-7D).

Phylogenetic Analysis

Two separate series of phylogenetic analyses were conducted, wherein the addition of morphological characters provided a test of phylogenetic hypotheses generated from separate molecular studies. The molecular matrices comprised combined *mat*K and *rbc*L genomic sequences for 101 extant species of pine and 5 outgroup taxa, assembled by Gernandt et al. (2005), and whole plastome sequences for 34 extant pines and 4 outgroups, produced by massively parallel sequencing cpDNA (Parks et al. 2009). To ascertain the congruence of our results with those produced using different search methodologies (Gernandt et al. 2005, Parks et al. 2009), both of the molecular data sets were assessed for maximum parsimony (figs. 3-8, 3-9A) using the parsimony ratchet perturbation algorithm (Nixon 1999); this “island-hopping” algorithm was then utilized for all subsequent analyses.

Cladistic topologies generated from molecular data were tested by the addition of morphological characters (Appendix B) to the cpDNA matrices in subsequent analyses (figs. 3-8, 3-9B). Characters pertaining to ovulate cone morphology and vegetation were adapted from those scored by Gernandt et al. (2005); all taxa were also scored for ovulate cone characters as per Smith and Stockey (2001; 2002). Finally, the extinct species *Pinus arnoldii* was scored for all morphological characters, and the data were reanalyzed to place *P. arnoldii* upon the tree (figs. 3-8, 3-10).

A difficulty inherent in the inclusion of fossil organisms within analyses of predominantly extant plants is a paucity of morphological characters in the face of large molecular datasets. The parsimony ratchet (island-hopping) algorithm (Nixon 1999) was utilized in these analyses to optimize the recruitment of weak phylogenetic signals in the morphological matrix. The algorithm alternates tree bisection with phases of random unequal weighting of phylogenetically informative characters, thereby maximizing the probability that replicates derive from different islands. This increases the heterogeneity of tree topologies, increasing the likelihood that optimum trees with weak signals are captured (Nixon 1999).

Full Plastome Sequences

The parsimony ratchet algorithm was applied to full plastome sequences generated from massively parallel sequenced cpDNA (Parks et al. 2009), containing 11376 informative characters. Ratchet searching recovered a single most parsimonious tree (L=19321, CI = 0.71, RI= 0.92), the topology of which was identical to that of Parks et al. (2009). Tree topology was unaltered by the addition of morphological characters, 24 of which were parsimony-informative; this second analysis produced one most-parsimonious tree (L = 19403 , CI = 0.71, RI = 0.92). When *Pinus arnoldii* was added to the analysis, it was found to occupy a position at the base of the subgenus *Pinus* (fig. 3-8), but the topology of the single most-parsimonious tree was otherwise unchanged (L = 19405, CI = 0.71, RI =0.92). Bootstrap support was diminished at all nodes (fig. 3-8) when morphological characters and the fossil species were analyzed in conjunction with the molecular data.

***rbc*L and *mat*K Sequences**

An analysis of combined *rbc*L and *mat*K sequences containing 278 parsimony-informative characters produced 67800 most parsimonious trees with a length of 574 steps (CI = 0.58, RI= 0.93), the strict consensus of which (fig. 3-9A) had a length of 638 steps (CI = 0.52, RI = 0.91). The addition of 41 morphological characters (Appendix B) increased the number of parsimony-informative characters to 301, resulting in 94587 most parsimonious trees (Length = 694, CI = 0.46, RI = 0.89), the strict consensus of which (fig. 3-9B) had a length of 784 steps (CI = 0.46, RI = 0.89). When the extinct species *Pinus arnoldii* was analyzed in concert with both the molecular and morphological data, 94789 most parsimonious trees (Length = 574, CI = 0.58, RI = 0.93) were generated. The strict consensus (fig. 3-10) had 776 steps (CI = 0.47, RI = 0.89).

As with other search algorithms (i.e., Gernandt et al. 2005), parsimony ratchet searching of the *rbc*L and *mat*K sequences recovered the two clades that represent the recognized subgenera, *Strobus* and *Pinus* (fig. 3-9A; bootstrap support = 100). Similarly, all four recognized sections of the genus *Pinus* (fig. 3-9A, 3-10) were supported as monophyletic, although bootstrap support values for sects. *Pinus* and *Quinquefoliae* were slightly lower than those reported for heuristic searching (Gernandt et al. 2005). Of the four sections, all except sect. *Trifoliae* had topologies in congruence with those of Gernandt et al. (2005). Tree topologies were altered by the addition of morphological characters (fig. 3-9B);

these alterations are summarized below. The addition of the extinct species, *Pinus arnoldii,* did not further alter tree topology for the living species (fig. 3-10), but bootstrap support values for all clades were reduced. *Pinus arnoldii* occurred at a polytomy with the two subgeneric clades at the base of the genus (fig. 3-10).

Section *Parrya* – The addition of morphological characters altered tree topology (fig. 3-9B) by establishing *Pinus rzedowskii* Sánchez et Deloya as the basal-most taxon in the monophyletic group corresponding to subsect. *Cembroides* (bootstrap = 78). The sister taxa *Pinus maximartinezii* Rzedowskii and *P. pinceana* Gordon were resolved as the next most-basal clade, sister to the remainder of the subsect. *Cembroides*.

Section *Quinquefoliae* – Only the subsect. *Strobus* was topologically altered by the addition of morphological characters (fig. 3-9B). The strict consensus of combined *rbc*L and *mat*K sequences suggested two sister group relationships (*Pinus koraiensis* Siebold et Zuch. + *P. wallichiana* A.B. Jacks and *P. strobus* L. + *P. chiapensis* (Martinez) Andresen), but these groupings were not upheld under the addition of morphological characters. *Pinus morrisonicola* Hayata, *P. fenzeliana* Hand.-Mazz., and *P. bhutanica* Grierson, Long et Page received some support (bootstrap = 49) as a monophyletic clade, and an affinity was suggested (bootstrap = 31) between *P. sibirica* Du Tour, *P. koraiensis*, *P. pumila* (Pall.) Regel, and *P. cembra* L..

Section *Pinus* – Support for several groups was weakened by the addition of morphological characters (fig. 3-9B). The subsect. *Pinaster* was collapsed into a polytomy at the base of the section. Notably, a sister relationship between *Pinus* *roxburghii* Sargent and *P. canariensis* C. Smith was erected under assessment with morphological characters (bootstrap = 81); this relationship was not rigorously supported with molecular data alone. In the subsect. *Pinus*, *P. densiflora* Siebold et Zucc., *P. sylvestris* L., *P. nigra* J.F. Arnold, *P. resinosa* Aiton, and *P. tropicalis* Morelet were collapsed into a polytomy at the base of the subsection, but all other relationships remained stable.

Section *Trifoliae* – In contrast to the heuristic analysis performed by Gernandt et al. (2005), a strict consensus of trees generated by ratchet searching (fig. 3-9A) resulted in the collapse of the subsect. *Ponderosae*. When molecular data are analyzed in isolation, the sect. *Trifoliae* forms an unresolved grade; the species ascribed to the subsect. *Ponderosae* form an unresolved polytomy, nested as a monophyletic group (bootstrap = 88) within a basal polytomy comprised of the species ascribed to the subsect. *Australes*. Three monophyletic clades within the subsect. *Australes* were recovered, but received weaker bootstrap support than reported by Gernandt et al. (2005) for heuristic searches of the same dataset. The subsequent addition of morphological characters (fig. 3-9B) resulted in further loss of support for the monophyly of one of these groups (*P. rigida* Mill., *P. taeda* L., *P. serotina* Michx., and *P. pungens* Lamb.) but did re-erect the subsect. *Australes* as a monophyletic lineage (bootstrap = 43).

DISCUSSION

Whole-plant Reconstruction

The seeds cones of *P. arnoldii*, along with the stems, short shoots, and needles described as *Pinus similkameenensis* and the *Pinus* pollen cones and roots, are among the most common fossils in Princeton Chert layers 16-20. When Miller (1973) described *P. arnoldii*, only 8 seed cones were known; Stockey's (1984) reinvestigation incorporated 121 cones, and the current study included more than 350 specimens. In addition, large numbers of woody stems and thousands of five-needled fascicles of the *P. similkameenensis* type have been identified. *Pinus similkameenensis* leaves are always present in rock slabs bearing mature and immature seed cones, and in those which contain pollen cones and large numbers of pine roots. Close spatial associations between the leaves and seed cones prompted Miller (1973) to suggest that the vegetative organs described as *P. similkameenensis* could belong to the *P. arnoldii* plant. In extant pines, however, *P. similkameenensis*-type needles are restricted to the haploxylon, or "soft" pines (Subgenus *Strobus*), whereas the ovulate cone of *P. arnoldii* bears morphological similarities to the Subgenus *Pinus*, the diploxylon, or "hard" pines (Miller 1973, Critchfield and Little 1963). Thus, the cones and vegetative materials were described as separate morphotaxa (Miller 1973).

While the sheer abundance of these pine organs within the Chert is highly suggestive of biological affinity (Miller 1973, Stockey 1984, Phipps et al. 1995), these are not the only pine morphotaxa described from the Chert. Stockey (1984) described a second seed cone, *P. princetonensis*, from two specimens. *Pinus princetonensis* is similar to *P. arnoldii* in many respects, having inflated apophyses bearing dorsal umbos, and a densely sclerotic outer cortex (Stockey 1984). However, it is histologically distinct in that all vascular tissue contains a double ring of resin canals, and the pith is composed of thin-walled cells that lack contents. Stockey (1984) also described *Pinus andersonii*, a vegetative morphotaxon represented by a single fascicle of three leaves. The leaves contain two separate vascular strands, with a heavy band of fibers on their adaxial side. Transfusion tissue is 1-4 cells thick, and the endodermis describes a broad ellipse (Stockey 1984). The mesophyll of *P. andersonii* is distinctly plicate, unlike that of *P. similkameenensis*, and there are five medial resin canals. Stomata are distributed on abaxial and adaxial surfaces. Stockey (1984) suggested that this morphology is similar to some diploxylon pines (Doi and Morikawa 1929, Stockey 1984), most closely resembling that of *P. jeffreyi* A. Murray or *P. ponderosa* Dougl. (Harlow 1931, Stockey 1984).

Stratigraphically equivalent chert from another locality, the Princeton-Tulameen Mine dump at the John Allison schoolyard, Princeton, B.C., contains another leaf morphotaxon, described as *Pinus allisonii* Stockey (1984). This two-needled pine is known from numerous specimens, including complete fascicles. Like *P. andersonii*, *P. allisonii* contains two vascular strands, the plicate mesophyll is distinct, and leaves are amphistomatic (Stockey 1984). In *P. allisonii*, the vascular bundles are separated by only a thin zone of fibers, and the mesophyll contains 6-9 (but more commonly 8-9) medial resin canals (Stockey 1984).

The presence of these other pine morphotaxa within the Princeton Chert and its stratigraphic equivalents precludes a whole-plant reconstruction based on association alone. The organismal concept developed in this study is based on anatomical attachment between the seed cones and vegetative organs as well as several forms of associational data for the other organs of the plant. Our study conclusively demonstrates that the morphospecies *P. similkameenensis* comprises the vegetative organs of the fossil species *Pinus arnoldii* (*P. arnoldii* takes taxonomic precedence over *P. similkameenensis*; Miller 1973). Three *P. arnoldii* seed cones, one of which is immature, exhibit anatomical attachment to woody stems with *P. similkameenensis*-type wood anatomy. As such, the organismal concept for the *P. arnoldii* plant incorporates the mature seed cones and woody stems producing five-needled fascicles of leaves, as originally suggested by Miller (1973), and is amplified to include immature ovulate cones, providing further insight into the ontogeny of this species. *Pinus* pollen cones described by Phipps et al. 1995) and ectomycorrhizal coralloid rooting systems (LePage et al. 1997) are also inferred, by several lines of evidence, to be constituent organs of the *Pinus arnoldii* plant. Immature ovulate cones at the pollination stage frequently contain pollen grains identical to those preserved in the *Pinus* pollen cones described by Phipps et al. (1995), and these pollen cones often have non-random orientations reflecting attachment. Similarly, pine rooting systems (LePage et al. 1997) are preserved *in situ*, as evidenced by intact extraradical mycelia of the ectomycorrhizae. This localized growth strengthens the case for inclusion of the rooting systems into the organismal concept. At this time, the organography of this extinct pine is almost entirely known, and many aspects of growth architecture, habit, ontogeny, pollination biology, and ecology may be inferred.

Several lines of evidence permit inferences regarding growth architecture and phenology of the *Pinus arnoldii* plant. Because tangential tracheid diameter is correlative to tracheid length, which is itself directly correlated to tree height (Bannan 1965; Carlquist 1975), a comparison of wood anatomy with that of extant pines suggests that *P. arnoldii* could have achieved heights of at least 25 m.

A woody stem bearing a terminal immature ovulate cone provides further insight into the organography of the *P. arnoldii* plant: the cone is subtended by a length of stem that bears cataphylls but no fascicles of leaves. This morphology is consistent with that of living pines in which pollen cones replace fascicles of leaves in the axils of cataphylls, often subtending a terminal seed cone (fig. 3-2E); the pollen cones abscise a few weeks after development, leaving only the cataphylls attached to the stem (fig. 3-2F). *Pinus arnoldii* exhibits similar morphology below the immature seed cone (fig. 3-2D), which indicates that pollen cones were borne in a similar position on this extinct species. Although the pollen cones themselves have yet to be found in anatomical attachment, a cluster of cones in identical orientation (eg. 3-6A) is independently suggestive of the same morphology. Thus, it is apparent that at least some of the branches upon which the *P. arnoldii* plant bore seed cones produced pollen cones in preceding seasons. In contrast, many extant pines (i.e., *P. strobus* L., *P. contorta* Douglas ex Loudon) bear pollen cones and ovulate cones on separate branches, often on different parts of the tree.

The presence of such ephemeral structures as pollen cones (Phipps et al. 1995), including undehisced immature specimens, still sheathed by scale leaves, evidences swift preservation of these organs, under minimal transport flow regimes. This autochthonous taphonomic profile gains further support from the presence of articulated coralloid ectomycorrhizal rooting systems (LePage et al. 1997), which are ubiquitous throughout all of the chert layers assessed in this study. The ectomycorrhizal fungi associated with these roots form Hartig nets – hyphal growth between cortical parenchyma cells. On the external surface of the root, ectomycorrhizal growth takes the form of a dense pseudoparenchymatous mantle, with an extensive extraradical mycelium, suppressing and functionally replacing root hairs (Peterson et al. 2004). Such well-preserved coralloid roots are most often associated with densely compressed bands of peat. This homogenously-textured compressed peat most probably represents aerobic decay of plant tissues, and thus may reflect regions of relatively low inundation (i.e., a shoreline), or intermittent periods of drying. Evidence for *in situ* growth, coupled with low transport, indicates that the extinct *Pinus arnoldii* plant grew immediately adjacent to the aquatic system preserved as the Princeton Chert *konservat laggerstätte*, shedding its seed cones, pollen cones, short shoots, and stems into the wetland.

Phylogeny

As a whole plant, *Pinus arnoldii* exhibits a novel combination of characters that are broadly distributed among the subgenera and sections to which extant species of *Pinus* belong. Miller (1973) suggested that the inflated scale apex and dorsal umbo of the cone indicated affinity with the subgenus *Pinus*, but noted that the long-conical to cylindrical shape of the cone is more typical of basal species in the subgenus *Strobus* (i.e., sects. *Nelsoniae*, *Balfourianae*, *Krempfianae* and *Gerardianae, sensu* Gernandt et al. 2005, and this study). Of these pines, Miller observed that all excepting *P. aristata* Engelm. and *P. balfouriana* Grev. et Balf. have larger seeds than *P. arnoldii* (Miller 1973). Close affinities with *Pinus aristata* and *P. balfouriana* were deemed unlikely, as these extant species differ from the fossils with respect to cellular features of the pith, vascular strand architecture within the cortex, and composition of the outer cortex. Instead, Miller concluded that the seed cone morphotaxon *Pinus arnoldii* likely represented a species with affinities to the subgenus *Pinus*, section *Pinus*, citing further evidence in the form of a densely sclerotic outer cortex, apparent cone symmetry (examination of a larger number of specimens reveals the cones to be somewhat assymetrical), and lack of serotinous habit (Miller 1973).

The vegetative organs of *P. arnoldii* (originally described as *Pinus similkameenensis*; Miller 1973) have a combination of characters that are, in extant taxa, restricted to separate sections of the subgenus *Strobus*. Miller (1973) noted that the needles are anatomically identical to those of *Pinus monticola* Douglas ex D. Don, *P. peuce* Griseb., *P. parviflora* Siebold et Zucc., and *P. strobus*, of the sect. *Strobus*, while the wood anatomy more closely resembles that seen in the sect. *Parrya*, particularly *P. aristata*, *P. bungeana* Zucc. ex Endl., and *P. cembroides* Zucc..

This broad distribution of characters would seem to indicate that the features of vegetative and seed cone anatomy exhibited by the *Pinus arnoldii* plant represents one of the “ancestral” combinations of characters that no longer are found among living pines. Calibrated node estimation (Gernandt et al. 2008) suggests that the subgenera of *Pinus* were distinct no more than 72 Ma; the modern diversity of the genus reflects a Paleogene radiation. In our analyses, the phylogenetic position of *Pinus arnoldii* was resolved as either a basal member of the subgenus *Pinus* (Fig. 3-8), or occurs in a polytomy with the two subgenera (Fig. 3-10). Both are reasonable phylogenetic hypotheses, and suggest that during the Eocene, the modern subgenera of *Pinus* were not yet distinct, or, more plausibly, that the genus contained species with combinations of characters that are no longer represented among the living species.

Subgenera within the genus *Pinus* are largely resolved by molecular systematics, but several internal nodes remain ambiguous. Due to the rapid radiation of the genus and disproportionate segregation of phylogenetic information at the *ycf*1 and *ycf*2 loci, whole plastome sequences (i.e., Parks et al. 2009) may offer greater phylogenetic resolution than partial sequence sets. Simulation studies have shown that external branch length is decreased when taxon sampling from within monophyletic groups is increased (Rannala et al. 1998), thereby improving the accuracy of a phylogeny. Thus, relationships between plastid lineages at low taxonomic levels are likely to be further resolved as a larger number of taxa are fully sequenced. Such plastid phylogenies, if corroborated by those obtained from nuclear markers (i.e., Liston et al. 1999, Syring et al. 2005), may permit further elucidation of crown group phylogeny amongst extant *Pinus*.

However, it has recently been stressed (Hörandl 2010) that a cladogram is only a hypothesis for the phylogeny of a group, and that if the evolution of a lineage has progressed by reticulation or budding, a strictly cladistic approach is likely to be inappropriate. Given that many species of pine exhibit high rates of hybridization in sympatric populations (e.g. Lanner 1974, Panetsos 1975, Politov et al. 1999, Xu et al 2008, Zavarin et al. 1980), the probability of reticulate evolution within or between clades should offer a caution to attempts at reconstructing ancestral conditions from phylogenetic analyses of extant taxa alone. Thus, the inclusion of fossil species provides data for testing hypotheses of the overall phylogeny of clades, and furthermore may help to polarize character suites, as fossil taxa near basal nodes better represent the ancestral conditions, by virtue of having had less time to diverge from the ancestral state (Huelsenbeck 1991).

Testing a molecular hypothesis by the inclusion of fossil taxa is necessarily reliant upon datasets composed entirely of morphological characters. For morphological datasets, the inclusion of fossil taxa provides greater resolution of trees when the fossils are temporally close to the ancestral state; however incompleteness with respect to phylogenetically informative characters can overwhelm any increase in resolution (Huelsenbeck 1991) by increasing the number of most parsimonious trees produced by a search algorithm, regardless of temporal position. This suggests that the inclusion of morphotaxa is of limited value. Huelsenbeck (1991) asserts that the addition of fossil species involves a tradeoff between temporal position and completeness; a temporally advantageous taxon, if incomplete, may resolve a phylogeny no better than an extant taxon (Huelsenbeck 1991). As such, the importance of organismal concepts for extinct plants cannot be overstated. Those species for which a large number of characters have been determined provide both novel character combinations and the level of character scoring that can increase phylogenetic resolution.

The extinct species *P. arnoldii* cannot be attributed to either of the modern subgenera, as it exhibits a novel combination of characters that is not encompassed by the extant diversity of the genus. Unlike all other fossils of pinaceous affinity, however, this fossil pine is now known as a complete organism, with a level of character scoring rivalling that of extant species. *Pinus arnoldii* is thus an invaluable addition to morphological datasets for future studies.

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FIGURES

Fig. 3-1 *Pinus arnoldii* seed cones (UAPC-ALTA). *A*, Transverse section of mature seed cone with attached seeds. P1981 Ftop #2, scale = 5 mm. *B*, Olique longitudinal section of mature seed cone. P2453 Etop (b), scale = 5 mm. *C*, Oblique longitudinal section of mature seed cone showing curvature of cone axis. P4800 Gbot #2, scale = 5 mm. *D*, Transverse section near base of seed cone, showing pith, eustele, cylinder of wood, and cortical tissues. Note circular bract/scale trace and cortex differentiated into inner (i), middle (m), and outer zones (o). P1297 Bbot #9 x 23. *E*, Tangential section through middle cortex of cone showing vascular tissue of ovuliferous scale (os) and bract (b). P2442 Dbot #2, scale = 1 mm. *F*, Transverse section through mid-region of ovuliferous scale showing winged seeds with megagametophyte (mg) and embryo (at arrow). P1981 Ftop #0, scale = 1 mm. *G*, Transverse section through ovuliferous scale distal to seeds. P6136 Gbot #11, scale = 1 mm.

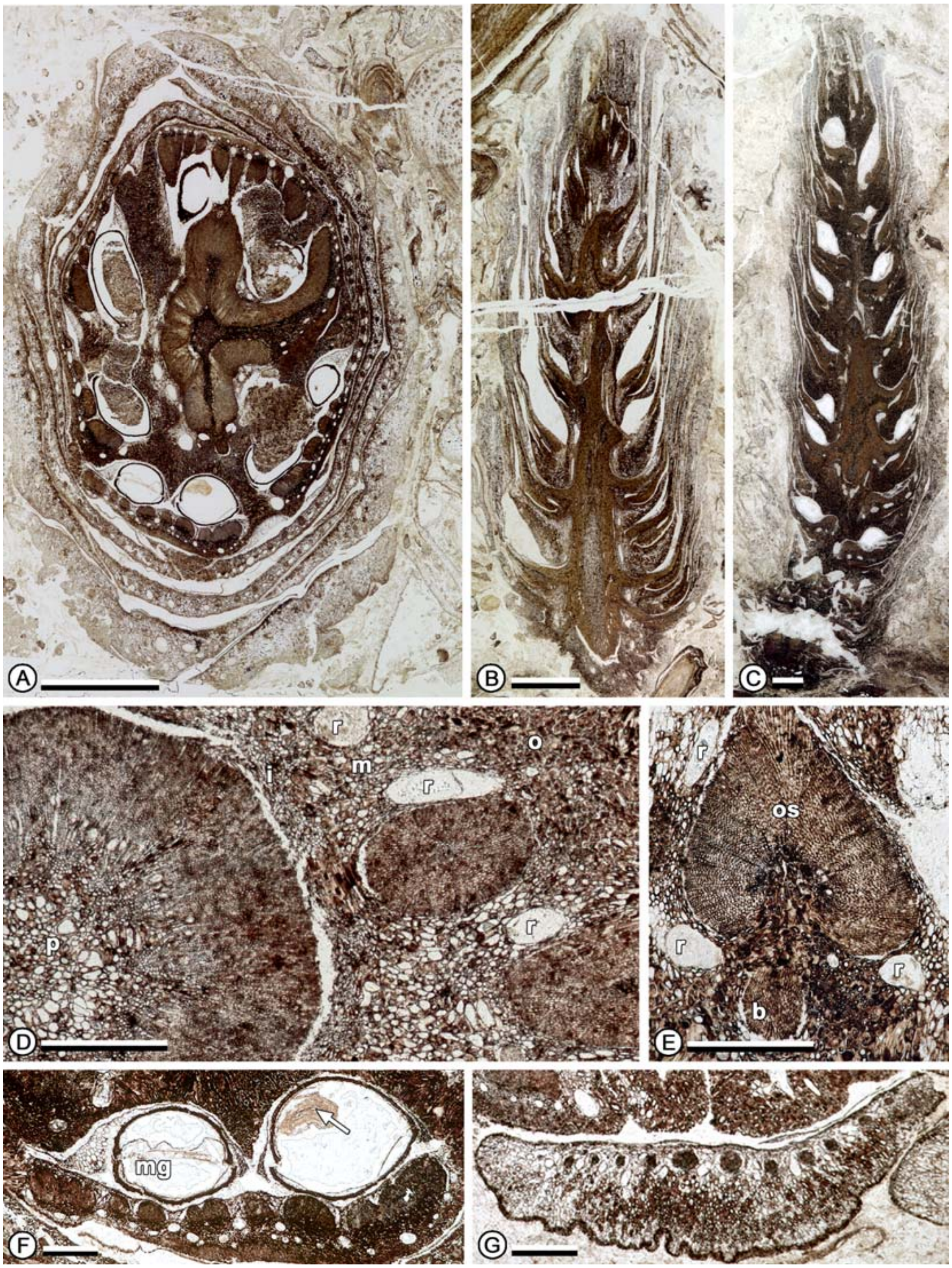
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Fig. 3-2 *Pinus arnoldii* seed cones (UAPC-ALTA) and *P. mugo* branches. *A*, Transverse section through winged seeds showing integument, nucellus (n) and megagametophyte (mg). Note differentiation of sclerotesta (sc) and sarcotesta (sa). An arrow identifies seed wing. P2593 Btop #3, scale = 1 mm. *B*, Transverse section of immature ovulate cone at pollination stage showing ovuliferous scales diverging from cone axis. Note inverted immature ovule at arrow. P3599 Btop #3, scale = 1 mm. *C*, Radial section of immature ovulate cone still enclosed in bud. P5864 H_1top #1, scale = 1 mm. *D*, Longitudinal section of immature ovulate cone at pollination stage, attached to stem. Note scale-like leaves subtending cone. P2731 B #1, scale = 1 mm. *E*, *Pinus mugo* Turra. branch showing immature ovulate cone subtended by zone of fascicles distal to zone of pollen cones in axils of scale-like leaves. *F*, *Pinus mugo* branch showing zone from which pollen cones have abscised. Note persistent scale-like leaves similar to *P. arnoldii* branch in Fig. 2D.


sa
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Fig. 3-3 Ovules of *Pinus arnoldii* (A, B; UAPC-ALTA) and extant *Pinus mugo* (C, D) at pollination stage. *A*, Longitudinal section through ovule with pollen grains at tip of cellular nucellar apex. P3599 Btop #5, scale = 100 µm. *B*, Enlargement of micropylar region of Fig. 3-3A, showing 3 pollen grains at tip of nucellus. P3599 Btop #5, scale = 50 µm. *C*, Longitudinal section through ovule of extant pine at pollination stage for comparison to *P. arnoldii* (Fig. 3-3A). *D*, Enlargement of micropylar region in Fig. 3-3C for comparison to *P. arnoldii* (Fig. 3-3B).

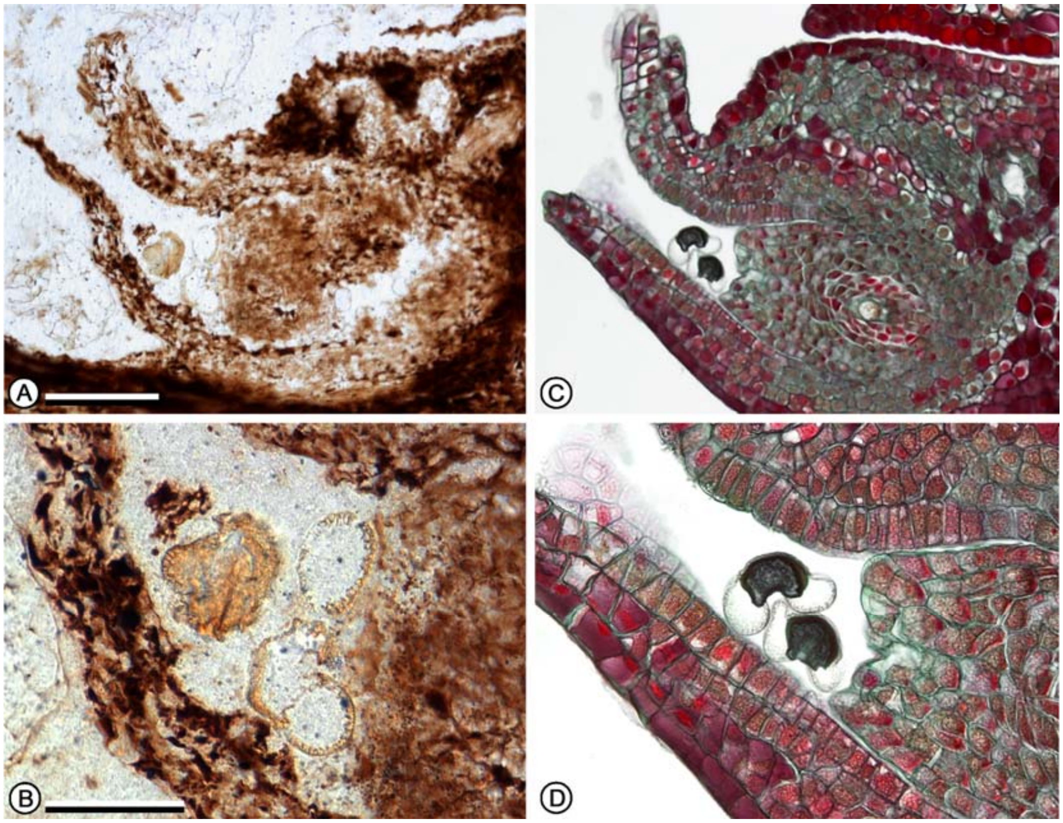
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Fig. 3-4 *Pinus arnoldii* seed cone attachments, stems, and wood (UAPC-ALTA). *A*, Oblique longitudinal section near base of seed cone and stem to which it is attached. The relative orientation of stem and cone base reflects angle of attachment and cone asymmetry. P4800 Etop #3, scale = 2 mm. *B*, Stem with attached seed cone base. P6667 Hbot #1, scale = 2 mm. *C*, Transverse section through mature stem showing 18 prominent growth increments and resin canal distribution. P2601 Cbot #3, scale = 5 mm. *D*, Transverse section of wood showing a single growth increment. P1123 $N_{2\ SL2698}$, scale = 100 µm. *E*, Tangential longitudinal section showing uniseriate and biseriate ray parenchyma. P1123 $N_{2\ SL2699}$ scale = 100 µm. *F*, Radial section showing resin canal (at left), uniseriate pitting of tracheids, and crossfield pits of ray cells (at right). P1123 $N_{2\ SL2700}$,scale = 50 µm. *G*, Transverse section through four year-old stem, showing features of pith, wood, and primary cortex. Note vascular trace to short shoot (st). P2656 Gbot #1, scale = 1 mm.

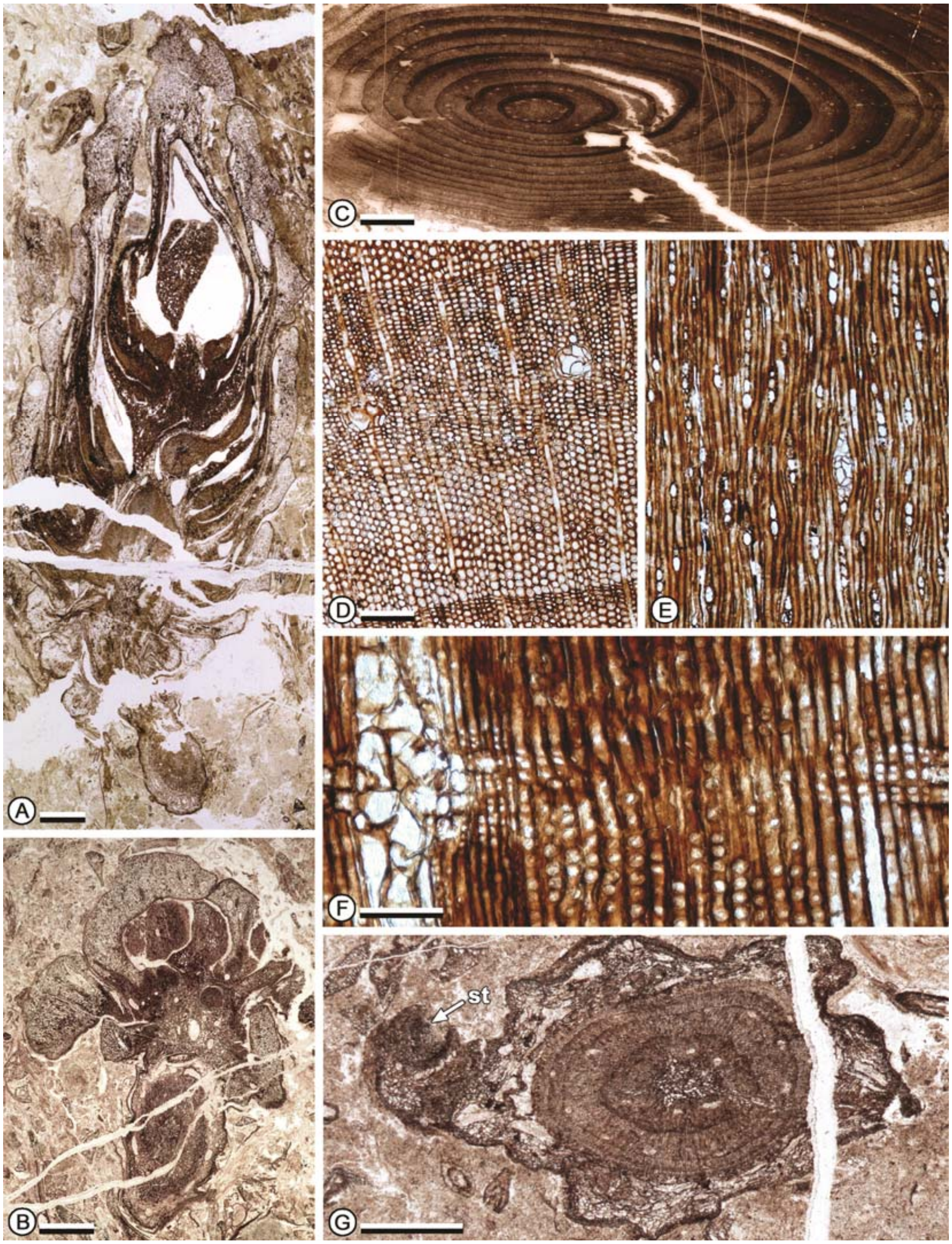
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Fig. 3-5 *Pinus arnoldii* short shoots and leaves (UAPC-ALTA). *A*, Oblique transverse section through a three year-old stem showing base of short shoot in cortex (st). P4801 Jtop (b) #5, scale = 1 mm. *B*, Transverse section of young stem at the level of branch trace divergence to short shoot. Note xylem to short shoot consists of two traces (at arrows) separated by parenchymatous pith ray (p). P2656 Gbot #1, scale = 200 µm. *C*, Transverse section of short shoot stele within stem cortex, enlarged from Fig. 3-5A. Note cylindrical shape and presence of both primary and secondary tracheids at this level. P4801 Jtop (b) # 4, scale = 200 µm. *D*, Transverse section of short shoot stele, corresponding to position acropetal to Fig. 3-5C, at level indicated (d) in Fig. 3-5G. Note presence of pith (p) surrounded by cylinder of tracheids and incompletely preserved vascular cambium and phloem. P1143 Bbot (d) #7, scale = 200 µm. *E*, Transverse section acropetal to Fig. 3-5D, at level indicated (e) in Fig. 3-5G. Note vascular tissue has separated into leaf traces and leaves are partially separated from one another. P1143 B_1bot (b) #6, scale = 200 µm. *F*, Transverse section through fascicle at level where leaves have separated from one another, but are still enclosed within sheath of persistent bud scales, at level indicated (f) in Fig. 3-5G. Note single vascular trace in each needle, sunken stomata (s) and external position of resin canals (r). P5298 Gtop #12, scale = 100 µm. *G*, Longitudinal section of fascicle showing short shoot base within bud scale at left, and five needles. Lines indicate relative position of corresponding transverse sections (d, e, f). P4801 Jtop (a) #1, scale = 500 µm.

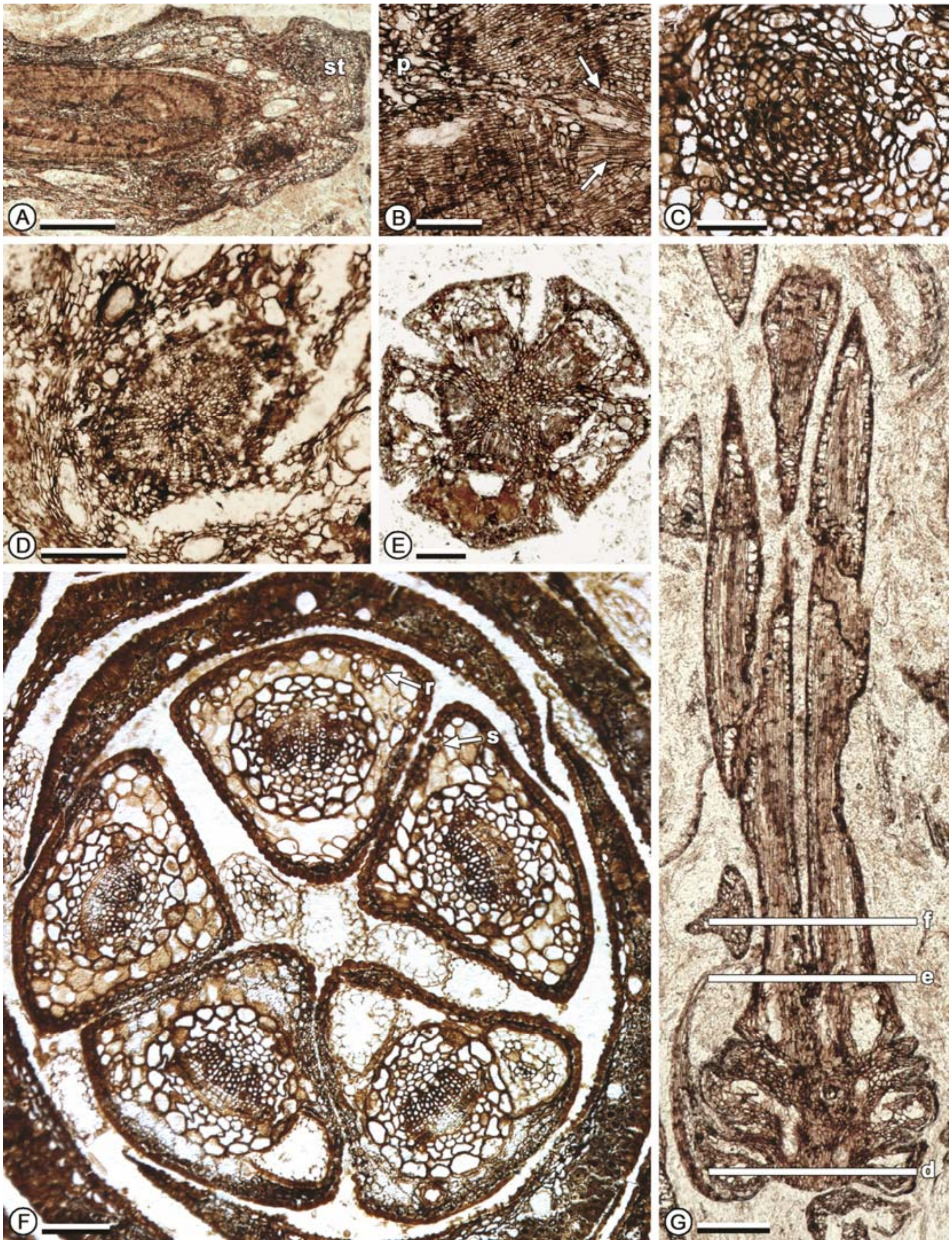
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Fig. 3-6 *Pinus arnoldii* pollen cones and pollen (UAPC-ALTA). *A*, Row of five pollen cones oriented as if attached to stem in same arrangement as *Pinus mugo* (Fig. 3-2E). P1173 C #12, scale = 1 mm. *B*, Longitudinal section of senescent pollen cone showing size and shape at this developmental stage. P1173 B_2top #5, scale = 1 mm. *C*, Transverse section of pollen cone near base, showing axis and microsporophylls with distal laminae, each bearing two pollen sacs containing full-sized pollen grains. Note that cone is sheathed by bud scales. P4800 Mbot #11, scale = 500 µm. *D*, Transverse section of cone showing attachment of pollen sac with full-sized pollen grains, attached to microsporophyll. P1143 Dbot #1, scale = 100 µm. *E*, Full-sized, apparently mature, bisaccate pollen within pollen sac. P1143 Dbot #1, scale = 50 µm.

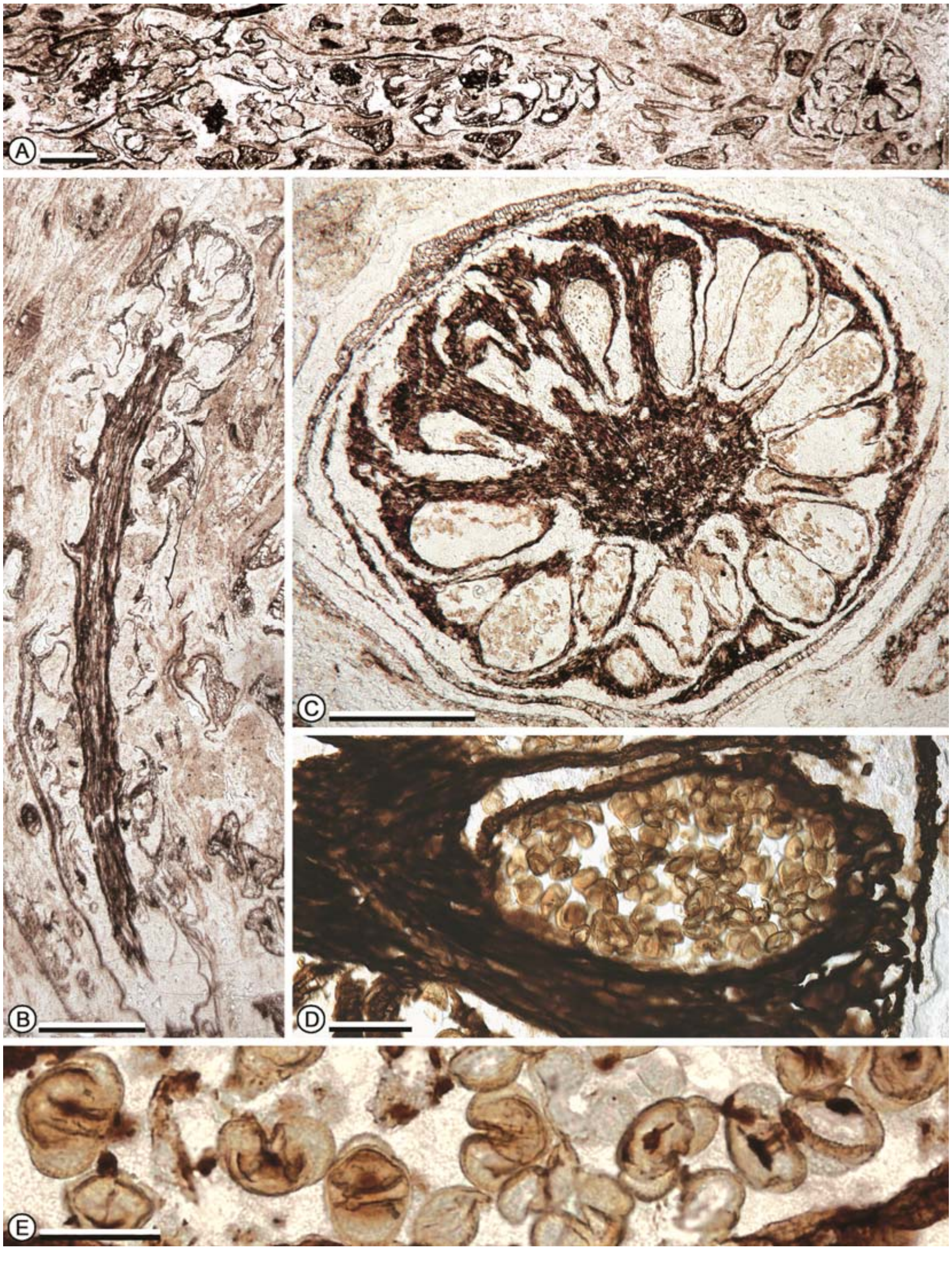
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Fig. 3-7 *Pinus arnoldii* roots and ectomycorrhizal associations (UAPC-ALTA). *A*, Two year-old tetrarch lateral root with attached system of coralloid roots. P5294 Etop (a) #4, scale = 1 mm. *B*, Coralloid root system showing up to four dichotomies. P1143 B_1bot (g) #1, scale = 1 mm. *C*, Cortex of coralloid root, showing fungal hyphae of Hartig net (at arrow). P1126 Ctop #14a, scale = 100 µm, Surface of coralloid root with attached hyphal mantle (at arrow) and extraradical mycelium. P1126 Ctop #14a, scale = 100 µm.

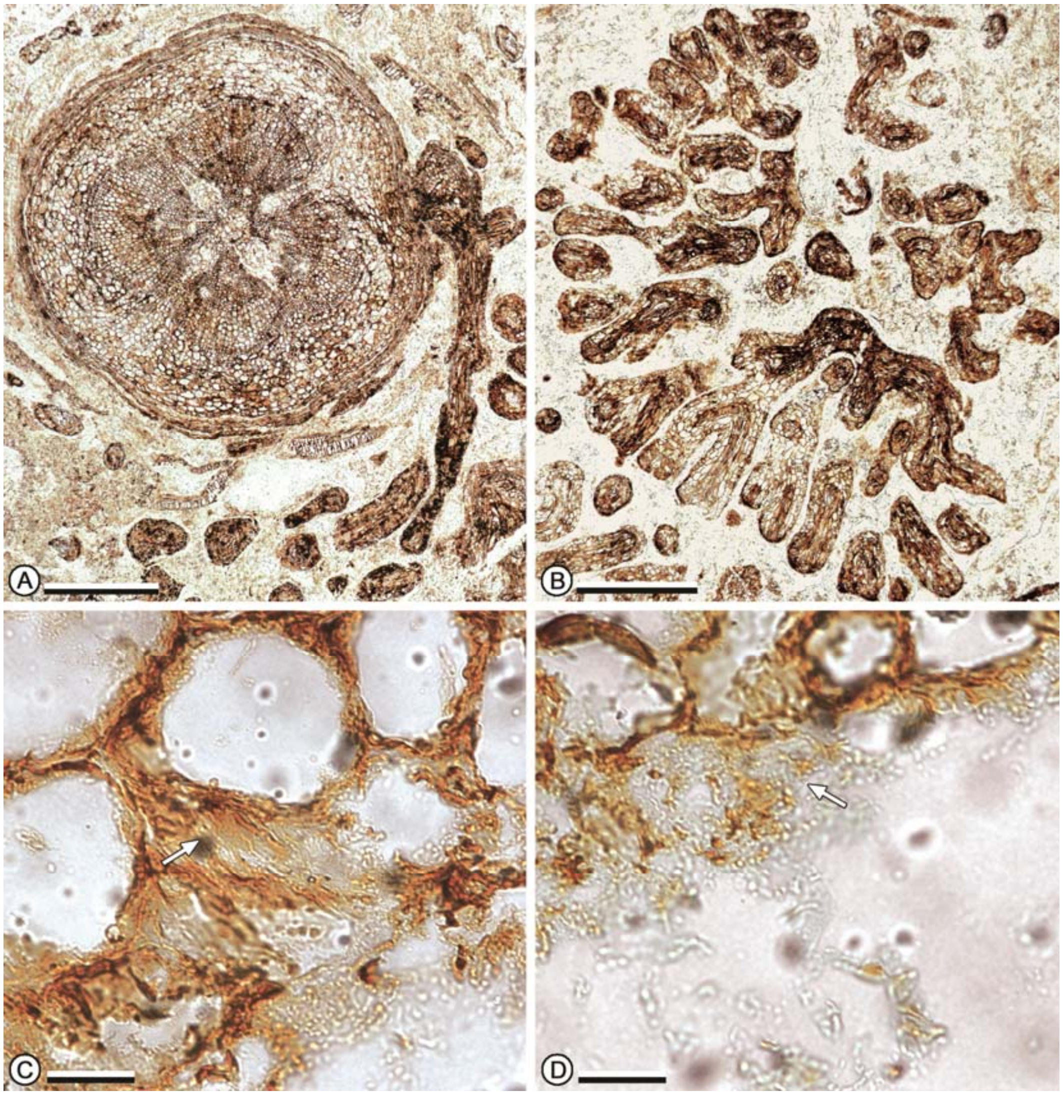
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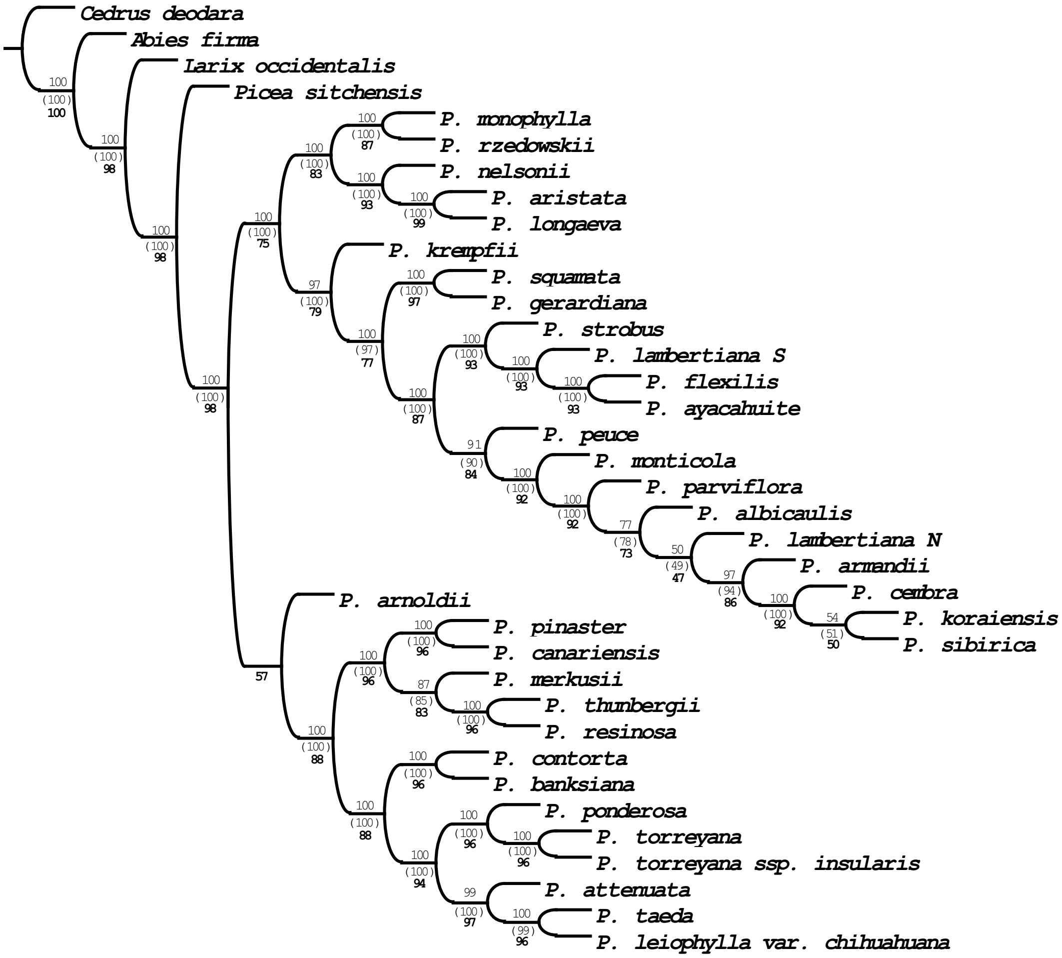
Fig. 3-8 Single most-parsimonious tree produced from a combined matrix of full plastome sequences (Parks et al. 2009) and morphological characters (adapted from Gernandt et al. 2005; Smith and Stockey 2001, 2002) for 38 extant taxa, and the extinct species *Pinus arnoldii*, derived under parsimony ratchet perturbation (L =19405, CI = 0.71, RI = 0.92). Tree topology was identical when molecular data was analyzed alone (L=19321, CI = 0.71, RI = 0. 92; bootstrap support above node), and in conjunction with morphological characters, but excluding the fossil taxon (L = 19403, CI = 0.71, RI = 0.92; bootstrap support below node, in brackets).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Larix occidentalis
Picea sitchensis
P. monophylla
P. rzedowskii
P. nelsonii
P. aristata
P. longaeva
P. krempfii
P. squamata
P. gerardiana
P. strobus
P. lambertiana S
P. flexilis
P. ayacahuite
P. peuce
P. monticola
P. parviflora
P. albicaulis
P. lambertiana N
P. armandii
P. cembra
P. koraiensis
P. sibirica
P. arnoldii
P. pinaster
P. canariensis
P. merkusii
P. thunbergii
P. resinosa
P. contorta
P. banksiana
P. ponderosa
P. torreyana
P. torreyana ssp. insularis
P. attenuata
P. taeda
P. leiophylla var. chihuahuana
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100 (100) 88
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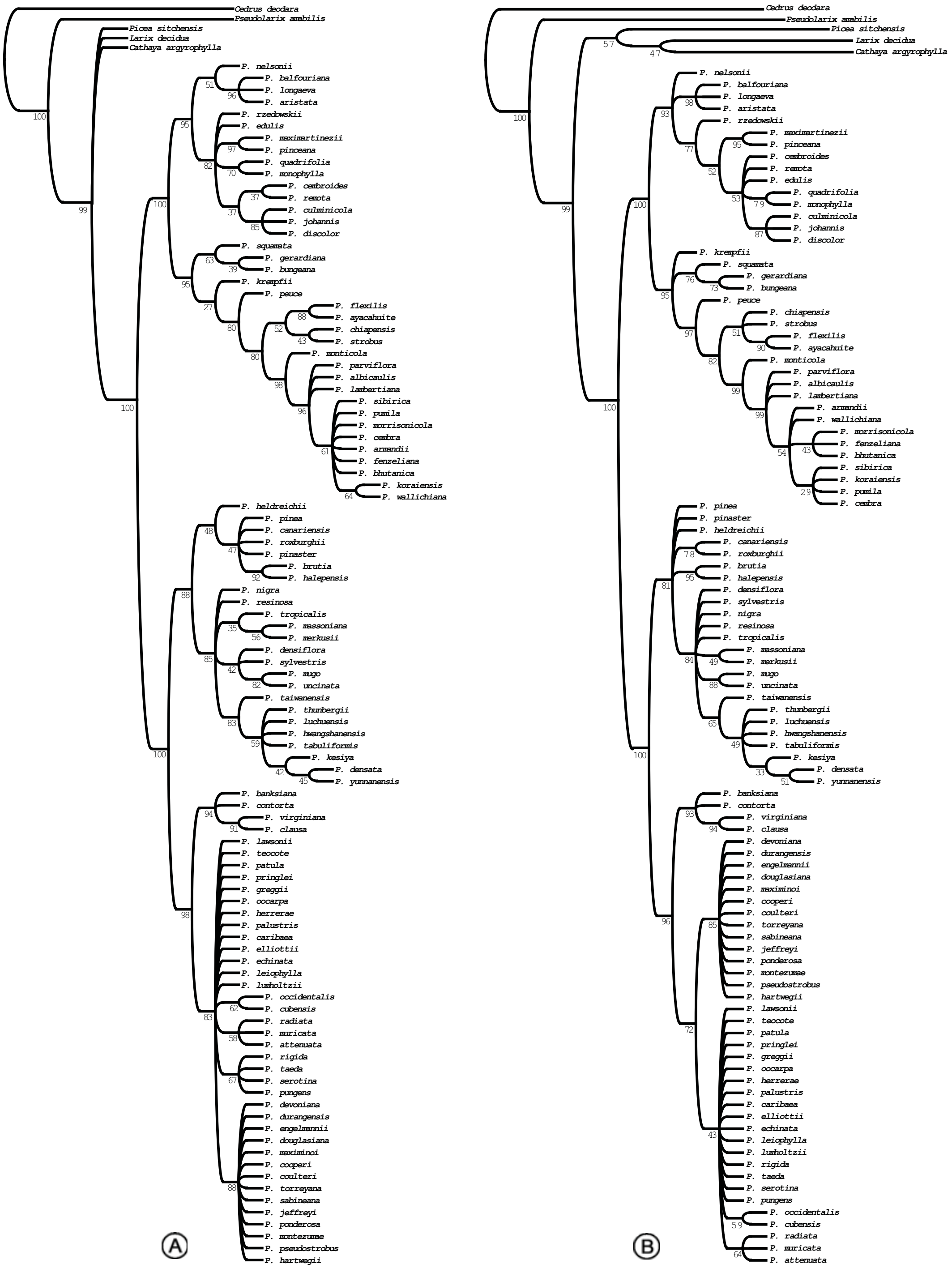
100 (100) 94
99 (100) 97
100 (99) 96

Fig. 3-9 Cladistic analyses of *rbc*L and *mat*K sequences for 101 extant species in the genus *Pinus* and 5 outgroup taxa. *A*, Strict consensus phylogeny of 70953 most parsimonious trees (L =638, CI = 0.52, RI = 0.91) with 278 parsimony-informative characters, derived from ratchet perturbation searches of *rbc*L and *mat*K sequences (Gernandt et al. 2005). *B*, Strict consensus phylogeny of 39910 most parsimonious trees (L = 784, CI = 0.46, RI = 0.89) derived from addition of morphological characters to the molecular matrix (301 parsimony-informative characters). Bootstrap support indicated below nodes; branch lengths do not reflect divergence.


Cedrus deodara
Pseudolarix amabilis
Picea sitchensis
Larix decidua
Cathaya argyrophylla
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P. nelsonii
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P. aristata
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P. rzedowskii
P. edulis
P. maximartinezii
P. pinceana
97
82
P. quadrifolia
P. monophylla
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P. cembroides
P. remota
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P. culminicola
P. johannis
P. discolor
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P. squamata
P. gerardiana
P. bungeana
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P. krempfii
P. peuce
P. flexilis
P. ayacahuite
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P. chiapensis
P. strobus
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P. parviflora
P. albicaulis
P. lambertiana
P. sibirica
P. pumila
P. morrisonicola
P. cembra
P. armandii
P. fenzeliana
P. bhutanica
P. koraiensis
P. wallichiana
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61
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P. heldreichii
P. pinea
P. canariensis
P. roxburghii
P. pinaster
P. brutia
P. halepensis
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P. nigra
P. resinosa
P. tropicalis
P. massoniana
P. merkusii
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P. densiflora
P. sylvestris
P. mugo
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P. taiwanensis
P. thunbergii
P. luchuensis
P. hwangshanensis
P. tabuliformis
P. kesiya
P. densata
P. yunnanensis
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P. banksiana
P. contorta
P. virginiana
P. clausa
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91
P. lawsonii
P. teocote
P. patula
P. pringlei
P. greggii
P. oocarpa
P. herrerae
P. palustris
P. caribaea
P. elliottii
P. echinata
P. leiophylla
P. lumholtzii
P. occidentalis
P. cubensis
62
P. radiata
P. muricata
P. attenuata
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P. rigida
P. taeda
P. serotina
P. pungens
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P. devoniana
P. durangensis
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Larix decidua
Cathaya argyrophylla
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P. occidentalis
P. cubensis
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P. radiata
P. muricata
P. attenuata
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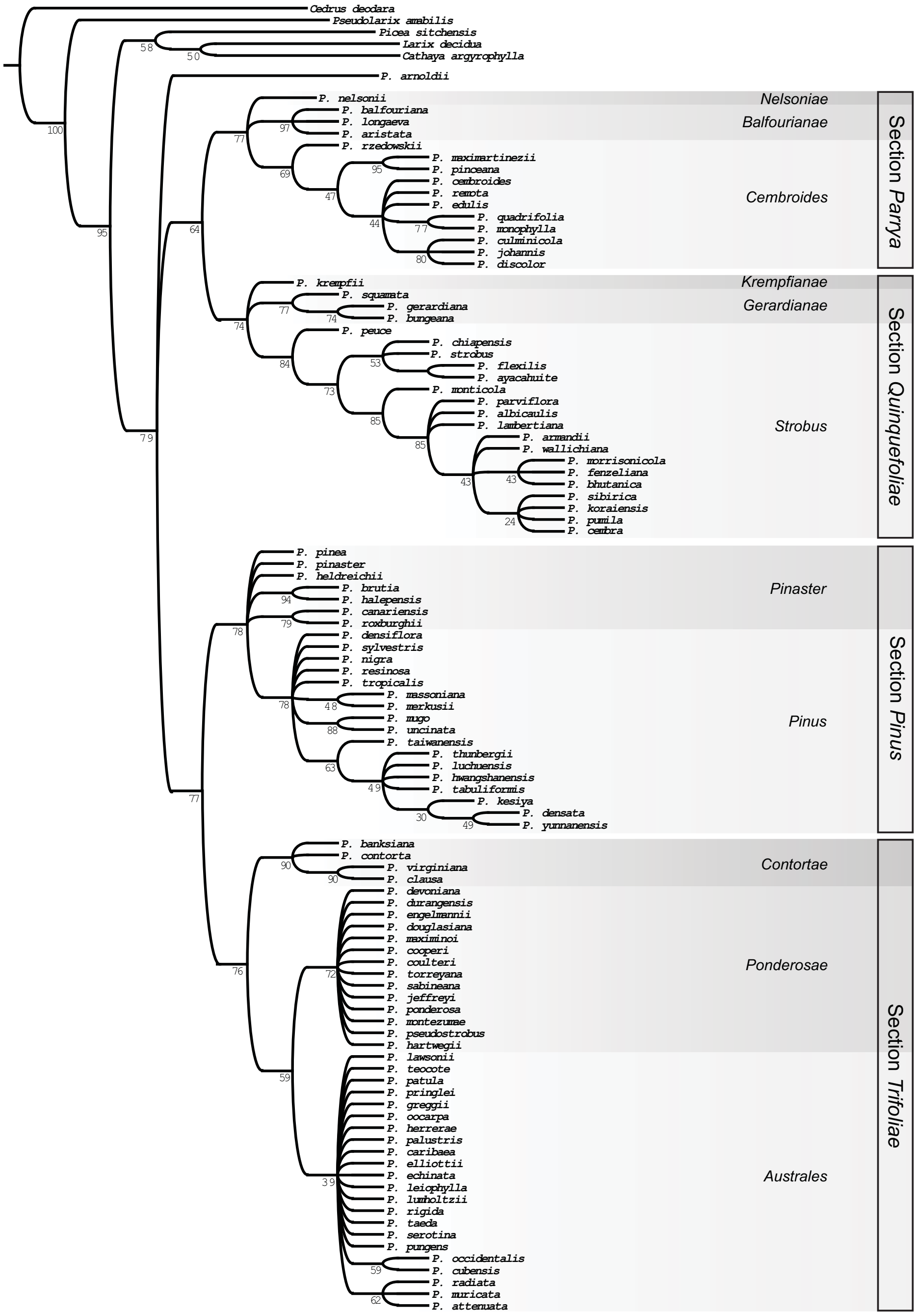
Fig. 3-10 Strict consensus phylogeny of 94789 most parsimonious trees, derived from parsimony ratchet searches of combined molecular and morphological matrices for 106 extant taxa, and the extinct species *Pinus arnoldii* (301 informative characters, L = 776, CI = 0.47, RI = 0.89; branch length does not reflect divergence). *rbc*L and *mat*K sequence characters are derived from Gernandt et al. (2005), and morphological characters are adapted from Gernandt et al. (2005) and Smith and Stockey (2001, 2002). Classification after Gernandt et al. (2005). Bootstrap support values indicated below nodes.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Balfourianae
Cembroides
Section Parrya
Krempfianae
Gerardianae
Strobus
Section Quinquefoliae
Pinaster
Pinus
Section Pinus
Contortae
Ponderosae
Australes
Section Trifoliae

CHAPTER IV: INDUCTIVE APPROACH TO FOSSIL TAXONOMY USING MAXIMUM LIKELY ANCESTRAL STATE RECONSTRUCTION

INTRODUCTION

Pinaceae has an extensive fossil record, and is well represented in Mesozoic and Paleogene floras. The family, which may have originated during the Jurassic (LePage 2003; Miller 1988; Gernandt et al. 2008), reached its peak morphological diversity during the Cretaceous (Miller 1976*a*, 1977*a*, 1988), which is represented by at least 28 different seed cone morphospecies (Alvin 1957*a*, 1957*b*, 1960, 1988; Crabtree and Miller 1989; Creber 1956, 1960, 1967; Dutt 1916; Falder et al. 1998; Louvel 1960; Klymiuk and Stockey 2010; Miller 1972, 1974, 1976*b*, 1977*b*, 1978, 1985; Miller and Li 1994; Miller and Robison 1988; Ohsawa et al. 1991, 1992; Ratzel et al. 2001; Robison and Miller 1977; Smith and Stockey 2001, 2002; Stockey 1981) assigned to the monophyletic groups *Pseudoaraucaria* Fliche and *Obirastrobus* Ohsawa, Nishida et Nishida, and the polyphyletic morphogenus *Pityostrobus* Nathorst emend. Dutt. This unnatural group contains at least 28 fossil seed cone morphologies (Smith and Stockey 2001, 2002; Klymiuk and Stockey 2010) that are as anatomically different from one another as are any extant genera (Miller 1976*a*). As such, a complete understanding the phylogeny, taxonomy, and evolutionary history of Pinaceae is significantly hampered by the fact that not only has most of the family's historical diversity been lost through extinction, but representatives of those earliest lineages can only be assessed with anatomical characters that do not offer cladistic resolution.

The paucity of phylogenetically informative morphological characters for pinaceous conifers is a significant stumbling block to resolving the phylogenetic position of fossils, even where complete species are known (Klymiuk et al. 2011). Where only seed cone morphotaxa are available, this problem has been demonstrated to be intractable in a 'Fitch parsimony'-based cladistic approach (Smith and Stockey 2001, 2002; and see Chapter 2). Although it is possible to make inductions regarding phylogenetic affinity on the basis of close similarity to extant genera (Klymiuk and Stockey 2010, Chapter 2), there are too few morphological characters to support these hypotheses in a strictly cladistic framework. Thus, even fossils that appear to be closely related to extant genera cannot be confidently placed in phylogenetic context using established parsimony methodologies.

The phylogenetic affinities of members of the *Pityostrobus* assemblage have been the subject of two previous studies (Smith and Stockey 2001, 2002) in which all anatomically preserved (permineralized) *Pityostrobus* species were scored for anatomical and morphological characters. In the preceding study (Chapter 2), I reanalyzed the *Pityostrobus* matrix, to both resolve the phylogenetic position of the new seed cone morphotaxon described therein, and to assess what effect the addition of two new fossil taxa (the new Apple Bay cone and *P. yixianensis*; Shang et al. 2001) would have upon topology and resolution. My analyses, unlike those of Smith and Stockey (2001, 2002), utilized the parsimony ratchet algorithm (Nixon 1999), and extant genera were represented by exemplar species, as opposed to composite polymorphic placeholders as in previous analyses (Smith and Stockey 2001, 2002). Nelson consensus topologies produced from my analyses exhibited fewer clades than were found in previous studies, and no new clades were resolved by my analyses. Thus, it appears that the addition of more taxa to these analyses, as suggested by Smith and Stockey (2002), will not increase the resolving power of this ovulate cone matrix.

The placement of any of the Cretaceous seed cone morphotaxa within the context of evolutionary affinities to modern genera has been tenuous at best, with the exception of *Pseudolarix* Gordon and *Pinus* L. Detached ovulate cone scales, seeds and vegetation from the late Jurassic of Mongolia have been assigned to *Pseudolarix* (Krassilov 1982; Keller and Hendrix 1997; LePage 2003). However, this taxonomic assignment has been called into question (RAS pers. comm.), as the specimens are dispersed compression-impression fossils. In comparison, anatomically preserved *Pinus* remains are known from two early Cretaceous morphospecies, *P. belgica* Alvin (1960, but see Gernandt et al. 2008), and an older Hauterivian-Barremian representative from Yorkshire (P. Ryberg et al. 2010, P. Ryberg pers. comm. 2011). Today, *Pinus* contains two monophyletic subgenera (Little and Critchfield 1969; Gernandt et al. 2005), but it is likely that much of the earlier diversity of the genus has been lost. An Eocene species, *Pinus arnoldii* Miller emend. Klymiuk, Stockey et Rothwell, is currently the only fossil plant of pinaceous affinity for which an organismal species concept has been developed (Klymiuk et al. 2011). *Pinus arnoldii* has an unresolved position in phylogenetic analyses, indicating that during the Eocene, there existed members of the genus *Pinus* that exhibited morphological diversity not encompassed by extant pines (Klymiuk et al. 2011) – it is the *Pityostrobus* problem in microcosm.

Because the only characters available to us in resolving the relationships of fossil taxa are morphological in nature, resolution of the affinities of *Pityostrobus* species is necessarily dependent upon understanding the phylogenetic signals of the morphological characters for which these seed cones can be assessed. As the application of Fitch parsimony (unordered, unweighted characters) to this problem yields minimal resolution of the group, alternative approaches are necessitated. However, because the polarity of evolutionary signal is not understood for most conifer characters and character states (Hart 1987; Rothwell et al. 2011), character weighting and ordering would invoke as many unsupported, *a priori* hypotheses of phylogeny as are inherent in using Fitch parsimony. Consequently, I have sought to determine whether molecular sequence data can be used to inductively reason affinity between members of the *Pityostrobus* assemblage and extant genera, by reconstructing most-likely ancestral conditions for deep nodes within extant genera, and comparing these hypothetical ancestral states to fossil taxa. The results of such comparisons could allow better assessment of phylogenetic position for fossil data. This method may therefore prove to be a viable avenue for further research in character polarity within the context of extant members of the family Pinaceae, and as such may help to guide us in unravelling the affinities of some Cretaceous members of the polyphyletic *Pityostrobus* assemblage.

As a preliminary test of this methodology, I reconstructed maximum likelihood ancestral states for the genera *Pinus, Picea* A. Dietrich, and *Cathaya* Chun et Kuang, which, as previously noted (Chapter 1), are resolved as a monophyletic clade within the subfamily Pinoideae in both molecular and non-molecular analyses of the extant genera (Hart 1987; Price 1989; Liston et al. 2003; Eckert and Hall 2006; Gernandt et al. 2008). The choice of these genera was directly correlated to two questions of appropriate taxonomic placement for the fossils *Pinus arnoldii* (Klymiuk et al. 2011), and the Lower Cretaceous seed cone described in Chapter 2, which represents the oldest fossil record for the extant genus *Picea*.

As a complete taxon, *Pinus arnoldii* combines vegetative features restricted to Subgenus *Strobus*, but seed cone characters that are most similar to the diversity found within Subgenus *Pinus* (Miller 1973; Stockey 1984; Klymiuk et al. 2011). However, when the fossil was assessed for phylogenetic position in two separate total-evidence combined molecular and morphological analyses (following the methodology employed by Jud et al. 2008), its placement was ambiguous. When assessed in the context of *rbc*L and *mat*K sequence data, the fossil species was placed in a basal polytomy with the two extant subgenera. An alternate analysis assessed the fossil in the context of massively parallel sequenced data for the whole plastome (from Parks et al. 2009), for a smaller taxonomic sampling of *Pinus*. In the latter analysis, *P. arnoldii* was found to be most closely related to the subgenus *Pinus*. Thus, although it is tempting to consider *P. arnoldii* a potential candidate for an ancestral condition of the genus

Pinus, on the basis of the combination of characters it exhibits, cladistic analyses have not been entirely conclusive as to its phylogenetic position. Therefore, I sought specifically to assess whether ancestral state reconstruction could exclude one of these topological hypotheses, as a test of the resolving power of this inductive method. *Pinus arnoldii* makes a good case example for the potential utility of the seed cone morphological characters to assess phylogenetic position, because it can be definitively ascribed to a genus with extant representatives, which, as noted, is not the case with most fossil seed cones.

The Lower Cretaceous seed cone described in Chapter 2 was discovered at the Apple Bay locality of Vancouver Island, British Columbia. This succession of marine sand- and siltstones was originally regarded as a Lower Cretaceous Longarm Fm equivalent, of roughly Barremian age (Jeletzky 1976) but recent oxygen isotope analyses have placed it within the latest Valanginian, at approximately 136 Ma (GW Rothwell pers. comm. 2009; Sweet 2000, Klymiuk and Stockey 2010). The stratigraphic age of the Apple Bay deposits is of significance to studies of phylogeny and divergence in the family Pinaceae, as the divergence of the sister taxa *Picea* and *Cathaya* from the genus *Pinus* is suggested to have occurred (Gernandt et al. 2008), minimally, between 113-123 Ma (when calibrated using the fossil taxon *Pityostrobus bommeri* Alvin), or at 142-155 Ma (when calibrated with *Pseudolarix erensis* Krassilov). The Apple Bay cone, if confidently placed in phylogenetic context to the extant genera *Cathaya, Picea* and *Pinus*, would provide an additional, and more stratigraphically restricted, calibration point for molecular dating of the extant lineages in the family Pinaceae.

MATERIALS AND METHODS

cpDNA. Sequence data for *Picea* were assembled from 29 species, with loci comprising the *trn*K intron which includes the maturase-K coding region, and from the tRNA-Thr (*trn*T), tRNA-Leu (*trn*L), tRNA-Phe (*trn*F) intergenic spacer regions. These data were kindly provided by Joselle Germano and colleagues (CS Campbell pers. comm. 2010), under the auspices of the Gymnosperm AToL Project, and comprise Genbank accessions AF156800-AF156809, AH008018 (Clouser et al. 1999), AY035193-AY035204 (Germano et al. 2001), AF133915, AF133919, AF133923 (Germano and Klein, 1999), AB045053-AB045072 (Kobayashi et al. 2000), and from unaccessioned sequences provided by CS Campbell (University of Maine, pers. comm. 2010).

Phylogenetic analyses of the genus *Pinus* were performed utilizing two coding loci, *rbc*L and *mat*K, and partial *trn*K intron sequence reads associated with the *mat*K gene. Data for 101 species of pine derived from codon-aligned sequences (Gernandt et al. 2005), which are accessioned on TreeBASE under Legacy Study ID 1229.

I aligned cpDNA sequence data for *Picea* and *Pinus* to 10 outgroup taxa, which were chosen in accordance with those pinaceous genera utilized by Smith and Stockey (2001, 2002). Taxa were taken from studies accessioned on Genbank, and included *Pinus thunbergii* Parlatore (D17510, Tsudzuki et al.

1992), *Cathaya argyrophylla* (AF143435, Wang et al. 2000; AY013740, Zhou et al. 2000), *Pseudolarix amabilis* (J Nelson) Rehder (AY013739, Zhou et al. 2000; DQ987889, Wang et al. 2006; EF395571, Havill et al. 2008), *Abies firma* Siebold et Zucc. (AB015647, Tsumura and Suyama 1998; AB029659, Suyama et al. 2000), *Larix decidua* Mill. (AB019826, AB019863, Wang et al. 1999; DQ087943, Gos-Louis et al. 2005), *Sciadopitys verticillata* (Thunberg) Siebold et Zucc. (L25753 Brunsfeld et al. 1994; AB023994, Cheng et al. 2000; AB029872, AB030068, Kusumi et al. 2000), *Tsuga mertensiana* (Bongard) Carrière (AF143434, Wang et al. 2000; DQ153004, Eckert and Hall 2006), *Pseudotsuga menziesii* (Mirbel) Franco (AF143439, Wang et al. 2000; AY664856, Rai et al. 2004), and *Keteleeria davidiana* (Bertrand) Beissner (EU269027, EU269031, Gernandt et al. 2008).

Outgroup sequences were codon-aligned to ingroup taxa using ClustalW2 (Chenna et al. 2003) and were visually inspected with Mesquite v2.74 (Maddison and Maddison 2001). Indels corresponding to missing data at the ends of sequence reads were excluded from all analyses. *Cedrus deodara* (D. Don.) G. Don. could not be reliably aligned to the coding regions for *Pinus*, so the alignment of outgroup taxa was performed excluding this taxon. No further adjustments to the ClustalW2 alignments were performed.

Morphology. Characters for seed cone morphology (as defined in Appendix A) were scored in accordance with Smith and Stockey (2001). Seed cone characters for the extant genera *Pinus* and *Picea* were scored for all species represented in the University of Montana Conifer Reference Collection; the collection contains exemplars of roughly half the recognized species for both genera. Scorings for *Pinus* were adapted for this study from Klymiuk et al. (2011) and character scorings for *Picea* are shown in Appendix D.

Phlyogenetic analyses. The morphological matrices for both genera were analyzed in a parsimony framework to assess whether known clades of extant species could be resolved through analyses of their morphological diversity. The matrices were analysed in a maximum parsimony (MP) framework, using the ratchet perturbation algorithm (Nixon 1999), in TNT (Goloboff et al. 2008), which was spawned through Winclada (Asado, version 1.1 beta, by K. Nixon, Cornell University). Characters were non-additive and unordered. Ten sequential ratchet searches were performed in each analysis, with 10% character sampling, 5000 iterations per replication, and 5 trees held per iteration. Nelson consensus phylogenies are reported, with bootstrap (Felsenstein 1985) support derived from 1000 pseudoreplicates, with ten trees held for each of 100 multiple TBR search replications.

Total-evidence tree topologies for both *Pinus* and *Picea* were generated using maximum likelihood (ML) as implemented in Garli v0.96b8 (Zwickl 2006), under optimal models of evolution (GTR+I+Γ and GTR+Γ, respectively). The choice of models was informed by the Akaike Information Criterion (Akaike 1974), and models were selected using jModelTest v0.1.1. (Posada 2008), which utilizes the PhyML algorithm (Guindon and Gascuel 2003). Resultant nucleotide distribution frequencies, transition to transversion ratios, gamma-distribution shape parameters, and proportions of invariant sites, where applicable, were userdefined in all maximum likelihood analyses. The ML topologies were assessed for replicability (Felsenstein 1985) using 500 bootstrap pseudoreplicates generated in Garli v0.96b8.

Cladistic topologies for both genera were also constructed under Bayesian inference, using MrBAYES v3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Data partitions (for *Pinus* these comprised *rbc*L, *mat*K, and partial noncoding sequence reads flanking the genes; for *Picea*, they comprised the *trn*K intron exclusive of *mat*K, *mat*K, and the *trn*T-L-F intergenic spacer) were unlinked, and assigned models of evolution as selected by mrModelTest v2 (Nylander 2004) under AIC. Metropolis-coupled Markov chain Monte Carlo (MCMCMC) simulations were conducted under default flat Dirichlet priors, and heated chains had a temperature of 0.1. Bayesian analyses were run to 10,000,000 generations, with sampling every 500 generations; 20% were discarded as burn-in. Convergence of MCMCMC chains was confirmed by the standard deviation of split frequencies (*Picea* = 0.001687, *Pinus* =0.00796), and potential scale reduction factor (PSRF, Gelman and Rubin 1992) which reached 1.000 for both analyses. Stationarity of MCMCMC chains was also confirmed by visual inspection.

Likelihood-based ancestral state reconstruction (Schluter et al. 1997; Pagel 1999; Pagel et al. 2004) was conducted in Mesquite v.2.74, where character history was traced over the Bayesian tree topologies which included branch lengths as generated in MrBAYES. Terminal character states were defined in the morphological matrices, as previously discussed, and likelihood reconstruction employed the one parameter Markov-k probability model, which corresponds to Lewis' (2001) equal-probability model (wherein character states are unordered, and therefore equally probable if branch lengths are equivalent). Default optimization intervals were utilized, and most probable states were judged under the default decision threshold (T=2.0).

RESULTS

Morphological analyses. When species comprising the extant genera *Picea* and *Pinus* were scored in accordance with the Smith and Stockey seed cone characters (Appendix A), and analyzed using ratchet perturbation, no internal nodes within the genera were resolved in either a strict, or Nelson consensus. The Nelson consensus of 7436 MP trees (tree length [TL]=84, consistency index [CI]=0.48, retention index [RI]=0.60) generated by analysis of the morphology of *Picea* collapsed the genus into a broad polytomy (fig. 4-1A). Only relationships between representatives of the genus *Pinus* were resolved with robust bootstrap support (BS=87). In a similar consensus of 29443 MP trees (TL=81, CI=0.49, RI=0.70) for *Pinus* (fig. 4-1B), the monophyly of the genus received slightly weaker, but still significant support (BS=76). *Cathaya* and *Keteleeria* were resolved as sister taxa in both analyses, and representatives of the genera *Tsuga*, *Abies*, *Cedrus*, and *Pseudolarix* formed a monophyletic assemblage in both analyses. The basal node of this "abietoid" clade was ambiguous in the *Picea* analysis.

cpDNA analyses for *Picea*. Bayesian analyses of unlinked partitions comprising the *trn*K intron (which includes *mat*K) and the *trn*T-L-F intergenic spacer explicitly demonstrated the monophyly of the genus, and several monophyletic assemblages were resolved within it (fig. 4-2). *Picea sitchensis* (Bongard) Carrière and *P. breweriana* S. Watson were resolved as sister to the other members of the genus. The ingroup (fig. 4-2) comprises the "omorika" clade (after Germano et al. 2002), and a polytomous assemblage consisting of *P. schrenkiana* Fischer et CA Meyer + *P. smithiana* (Wallich) Boisier, *P. pungens* Engelmann, the "purpurea" clade, and the "abies" and "glauca" clades, the latter of which are sister lineages with strong support (posterior probability [PP]=100, BS=81). The Bayesian topology (fig. 4-2) is consistent with the optimum ML tree, and those clades for which the optimum likelihood topology evidenced BS support values greater than 50 are indicated on the Bayesian consensus tree (fig 4-2).

The principle differences between the Bayesian consensus and the maximum likelihood trees are in terms of clade support. Although the monophyly of the "abies" clade is well supported, several internal nodes had high posterior probability support (>95%), but received little bootstrap support under maximum likelihood. In most respects, posterior probabilities were higher for all clades than ML support values, with the exception of a sister-group relationship between *Picea purpurea* Masters and *P. maximowiczii* Regel ex Carrière, which was highly supported in the optimum ML tree (BS=78) but had only 50% PP support in a Bayesian framework.

Branch lengths between all species of *Picea* are short (fig. 4-3); the branch between *Picea* and its presumed sister taxon *Cathaya* is significantly longer than any found in the monophyletic clade corresponding to Subfamily *Abietoideae* (*sensu* Liston et al. 2003; Gernandt et al. 2008), as is the branch defined by *Pinus thunbergii*.

cpDNA analyses for *Pinus*. As in analyses of *Picea,* the Bayesian and ML analyses *Pinus mat*K and *rbc*L coding data produced trees topologically similar trees with differing clade support (fig. 4-4). In both analyses, the subgenera of *Pinus* (*Pinus* and *Strobus*) were resolved as monophyletic (BS=100, PP=100). Support was also high for the monophyly of all sections.

Several terminal sister groups were also resolved with high PP support (>95), but did not have similarly high BS support values. These clades included *P. koraiensis* Siebold et Zucc. + *P. wallichiana* A.B. Jackson, *P. cubensis* Grisebach + *P. occidentalis* Swartz, *P. echinata* Mill. + *P. palustris* Mill., *P. densata* Masters + *P. yunnanensis* Franchet, and *P. massoniana* Lambert + *P. merkusii* Junghuhn et de Vriese. All of these crown groups exhibit very short branch lengths (fig. 4-5). Branches defining the two subgenera are significantly longer than those of terminals, with the branch of Subgenus *Pinus* slightly longer than that of *Strobus*.

In these analyses, *Picea sitchensis* is resolved as sister to the genus *Pinus*, but the high posterior probability (PP=90) conflicts with bootstrap support (BS<50). *Cathaya argyrophylla* is resolved as basal to the *Picea-Pinus* pair, with moderate support (PP=69, BS<50). *Pseudotsuga* and *Larix* are unambiguously resolved as sister taxa, as are *Abies* and *Keteleeria*. The placement of the members of the abietoid group differed from the topology produced with the

Picea dataset, in that *Pseudolarix* and *Tsuga* were placed in a basal polytomy. Furthermore, *Abies*+*Keteleeria*, and *Cedrus* were basal to Subfamily *Pinoideae* (*Larix*, *Pseudotsuga*, *Cathaya*, *Picea* and *Pinus*; sensu Gernandt et al. 2008). However, excepting of the monophyly of *Pinoideae*, these backbone topologies do not receive significant bootstrap support.

Ancestral state reconstruction. *Picea* (Appendix D) and *Pinus* (Klymiuk et al. 2011) are polymorphic with respect to several morphological features. Likelihood-based reconstructions of these characters (figs. 4-6, 4-7) were assessed for the basal node of the genus *Picea*, and for the node formed by the divergence between *Picea* and *Cathaya* (indicated in fig. 4-2), as well as for the basal nodes of the subgenera *Pinus* and *Strobus*, and for the base of the genus *Pinus* (indicated in fig. 4-4).

Most characters were reconstructed as equally likely on both Bayesian consensus topologies. Three characters for which *Picea* was assessed proved to be exceptions to this: trichomes are most likely to be absent at the nodes (defined in fig. 4-2) under consideration, and the bract is most likely to contain two resin canals. It appears that the presence of an interseminal ridge is also an ancestral condition for *Picea* and the common ancestor of *Picea* and *Cathaya*, although the height of the ridge with respect to the seed body is ambiguous. Similarly, at the nodes for which ancestral states in *Pinus* were assessed, it appears that a parenchymatous inner cortex, sclerotic bract, lack of a vascular ray in the bract, and the presence of resin canals abaxial to the vascular tissue of the ovuliferous scale at the level of the seed are the most likely ancestral conditions.

The reconstructed ancestral states were compared to character states exhibited by the Cretaceous seed cone from the Apple Bay locality, which is presumed to have affinities to *Picea* (Klymiuk and Stockey 2010), and to the extinct Eocene species *Pinus arnoldii* (Klymiuk et al. 2011). For all states, the fossils were comparable to the likely ancestral states. It is of particular interest that although *Pinus arnoldii* does not conflict with the protential ancestral states of character 23 for either the ancestor of the subgenus *Pinus* or the genus itself, neither does it represent the most likely condition for any of the nodes.

DISCUSSION

Concordance of tree topologies with previous studies. Previous non-molecular systematic studies of the extant genera (i.e., Hart 1987; Price et al. 1987) have resolved two subfamilial lineages, *Abietoideae* and *Pinoideae*, within the family Pinaceae. In contrast to the systematic affinities proposed by Price (1989) and subsequently supported by several molecular analyses (Wang et al. 2000; Eckert and Hall 2006; Gernandt et al. 2008), previous analyses of the seed cone matrix that have excluded fossil taxa (Smith and Stockey 2001) indicate that *Pseudotsuga* and *Larix* are basal to an abietoid group that excludes *Keteleeria*. In their 2001 analyses, Smith and Stockey found a weakly supported (BS=57) sister-taxon relationships between *Keteleeria* and *Cathaya*. Use of the parsimony ratchet algorithm in this study also resolves a *Cathaya* + *Keteleeria* clade, but support for this relationship was low (BS<50). Although the morphological data based on the seed cone characters appears to be less useful than molecular characters, its utility at the generic level may be increased if homologous and homoplasious characters can be ascertained. As the genetic mechanisms underpinning conifer structure and development (i.e., Rutledge et al. 1998) become better-understood, it will be possible to assess questions of character homology.

The genus *Pinus*, which contains 113 known species (Farjon 2010), contains two subgenera and four sections, the taxonomy and phylogeny of which is generally well-resolved by morphological data, when species diagnoses contain characters pertaining to leaf and wood anatomy, as well as growth architecture, in addition to seed cone characters (Farjon 2010). Most of the internal nodes within *Pinus* have also been well-resolved by previous analyses of the cpDNA dataset utilized in this study (Gernandt et al. 2005). With one exception, the topology of the genus found by Bayesian inference in this study does not conflict with previous parsimony-based analyses (Gernandt et al. 2005, Klymiuk et al. 2011) of these data. In a strict consensus of MP trees derived from ratchet perturbation (Klymiuk et al. 2011), *Pinus rzdeowskii* Sánchez et Deloya occupied a taxonomic position within a terminal polytomy that comprised Subsection *Balfourianae*, but in the analyses presented here, as in the parsimony analysis performed by Gernandt et al. (2005), the species is a basal member of Section *Parrya,* Subsection *Cembroides*.

The genus *Picea* contains approximately 38 species, the majority of which are found only in Asia (Farjon 2010). There are a scant eight North American and two European species (Farjon 2010). Phylogenetic relationships within the genus have recently been subject to molecular analysis (Germano et al. 2002; Ran et al.

2006; CS Campbell pers. comm. 2010). Ran et al. (2006) reconstructed the phylogeny of *Picea* using *trn*T-*trn*F and *trn*C-*trn*D cpDNA regions and the maternally-inherited mitochondrial *nad*5 intron. As in my analyses, and those of Germano et al. (2002), *Picea sitchensis* and *P. breweriana* were resolved as sister to the remaining members of the genus, which some authors (Sigurgeirsson and Szmidt 1993; Ran et al. 2006) suggest indicates a North American origin for *Picea*. In the Bayesian topology presented here, *P. breweriana* and *P. sitchensis* are resolved as sister taxa with high support (BS=81, posterior probability=89); in their analyses of the same dataset (albeit with fewer outgroup taxa), Germano et al. (2002) found that *P. sitchensis* was basal to the clade (BS=57).

Curiously, several of the regional variants of *P. likiangensis* (Franchet) E Pritzel that were sequenced by Ran et al. (2006) showed affinities to *P. schrenkiana* and *P. smithiana*, while in the analyses presented here, as in those performed by Germano et al. (2002), *P. likiangensis* was resolved as a constituent of the "abies clade". Although these discordant results may be due to introgression, it has been demonstrated that cpDNA haplotypes in *Picea* are typically species-specific (Du et al. 2009). Similarly, the taxonomic placement (Ran et al. 2006) of *P. wilsonii* Masters was also discordant with topologies generated from the *trn*K, *trn*T-L-F dataset, which suggested the *P. wilsonii*, like *P. likiangensis*, has its closest taxonomic affinities within the "abies clade". With respect to this "abies clade", the Bayesian topology I present does differ from that of Germano et al. (2002), in that my analyses resolved *P. crassifolia* Komarov, *P. koraiensis* Nakai, *P. koyamae* Shirasawa, *P. likiangensis*, and *P. wilsonii* as a nested polytomy within the remainder of the clade, although support was weak (PP=79, BS<50).

The other notable conflict between topologies produced by Ran et al. (2006), and analyses of the *trn*K, *trn*T-L-F dataset involved two North American species (*P. mariana* (Mill.) Britton Sterns, et Poggenburg and *P. rubens* Sargent) and *P. omorika* (Panèiæ) Purkyne, which is native to the Balkan Peninsula (Farjon 2010). These species were resolved as a strongly supported monophyletic clade in my phylogeny and that of Germano et al. (2002), but were ambiguously placed in the Ran et al.(2006) study, with low BS support in both MP and ML analyses.

The generic relationships among outgroup taxa in Bayesian and ML topologies of the *Picea* data set agree with other phylogenies of the family Pinaceae (Wang et al. 2000; Liston et al. 2003; Gernandt et al 2008). These analyses recovered a monophyletic *Abietoideae* clade, and found *Larix* sister to *Pseudotsuga*. Similarly, the addition of taxa to the *mat*K and *rbc*L datasets for *Pinus* brought several phylogenetic relationships into better accordance with other studies. For instance, previous analyses of this data set (Gernandt et al. 2005; Klymiuk et al. 2010) suggested a close relationship between *Cathaya* and *Larix*; the addition of representatives of *Picea* and *Pseudotsuga* produce topologies more congruent with those generated in other studies (Liston et al. 2003; Gernandt et al. 2008). However, the relationships of the abietoid taxa remain obscure when assessed with these two genes (*rbc*L and *mat*K), although *Keteleeria* and *Abies* are resolved as sister taxa, which accords with other analyses.

Implications for further research. Most of the ancestral state reconstructions performed in this study yielded equally-likely reconstructions, which are of little informative value. The most pressing problem plaguing this attempt at reconstructing ancestral conditions at basal nodes is likely to be the incomplete character scoring of extant taxa for both *Pinus* and *Picea*. Nearly half of all of the species assessed in this study were not represented in the Miller Conifer Reference Collection, and as a consequence were scored as unknown.

Another potential problem with this analysis is the preponderance of missing sequence data, particularly with respect to outgroup taxa. Molecular matrices containing ambiguous data, resulting from incomplete datasets and equivocal sites such as insertions or deletions, have been shown (Lemmon et al. 2009) to incorrectly estimate among-site variation and therefore produce inaccurate topological reconstructions with respect to both phylogeny and branch lengths, in both ML and Bayesian frameworks. Li et al. (2008) suggest that when branch lengths are not known with a high degree of accuracy, it is more appropriate to reconstruct basal or root nodes using character states defined only for those taxa close to the node of interest. This may be a potential solution to the problem of both missing sequence and morphological data, and merits further investigation.

The use of the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) search methodology, as employed in MrBAYES, does have a distinct advantage over maximum likelihood estimations of tree topology. The Bayesian method integrates uncertainty across the tree, such that phylogenetic uncertainty, branch lengths, and model parameters are accounted for (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck 2003). Huelsenbeck and Bollaback (2001) suggest, however, that ancestral state reconstruction is made “more uncertain” when this method is employed.

Despite uninformative reconstruction of some character states at basal nodes, the inductive method I utilize here is not without merit. The Cretaceous Apple Bay cone exhibits character states that are not in conflict with the most-likely reconstructions for the ancestral conditions of *Picea* and the most-recent common ancestor of *Picea* and *Cathaya*. Reconstruction of deeper nodes may serve to delimit this taxon to the subfamily Pinoideae, or to a most-appropriate placement along the stem lineage of *Picea* and *Cathaya*. As such, ancestral state reconstruction can provide an independent test for phylogenetic hypotheses where characters are not amenable to cladistic analysis.

Of even greater immediate utility is the comparison of ancestral reconstructions of basal nodes of *Pinus* to characters exhibited by the extinct species *Pinus arnoldii*. The fossil fell within the range of variation of all nodes, but with respect to the course of resin canals in the ovuliferous scale, the state exhibited by the fossil does not accord with the most-likely ancestral state for either subgenus. Thus, despite the fact that its seed cone morphology is similar to cones of Subgenus *Pinus* (Miller 1973), *P. arnoldii* likely does not represent an ancestor of the diploxylon pines, thereby refuting this particular hypothesis of appropriate taxonomic placement for *P. arnoldii*. As such, the inductive comparative method delineated here shows promise as a method by which competing taxonomic hypotheses can be tested.

Comparison of fossil taxa to most-likely ancestral states may be an appropriate method to apply in cases of taxonomic uncertainty. Further tests are necessary, and ought to be conducted with data that limit uncertainty: cpDNA sequence data should be comprehensive and as complete as possible, morphological characters should be scored for all taxa, and the generic affinities of the fossil taxa under consideration should be certain.

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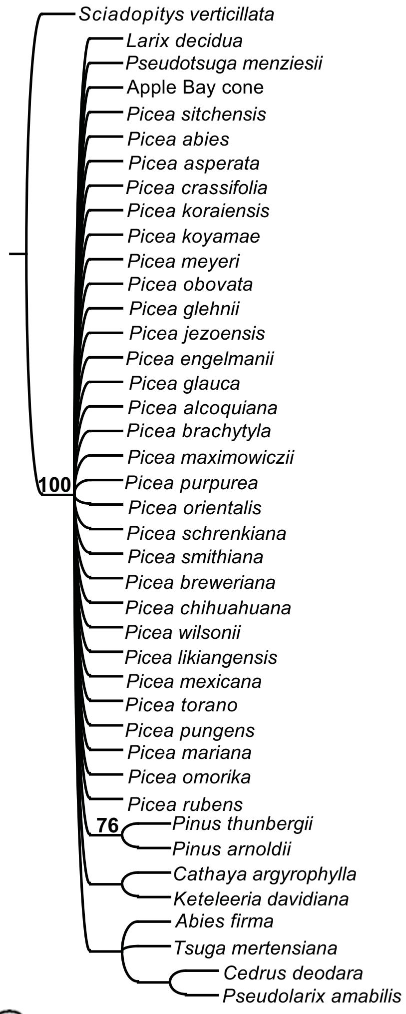
FIGURES

Fig. 4-1 Nelson consensus phylogenies derived from parsimony ratchet perturbation (Nixon 1999) of morphological characters for pinaceous seed cones (adapted from Smith and Stockey 2001, 2002). *A*. Nelson consensus of 7436 most parsimonious trees (MPTs), produced from analysis of 29 extant species of *Picea*, 10 outgroup taxa, and the fossil species *P. arnoldii* (33 informative characters, TL = 81, CI = 49, RI = 70). *B*. Nelson consensus of 29443 MPTs, produced from analysis of 101 extant species of *Pinus*, 10 outgroup taxa, and the fossil species *P. arnoldii* (33 informative characters, TL = 207, CI = 0.28, RI = 0.55). Bootstrap support values are indicated above branches.


Sciadopitys verticillata
Larix decidua
Pseudotsuga menziesii
Apple Bay cone
Picea sitchensis
Picea abies
Picea asperata
Picea crassifolia
Picea koraiensis
Picea koyamae
Picea meyeri
Picea obovata
Picea glehnii
Picea jezoensis
Picea engelmanii
Picea glauca
Picea alcoquiana
Picea brachytyla
Picea maximowiczii
100
Picea purpurea
Picea orientalis
Picea schrenkiana
Picea smithiana
Picea breweriana
Picea chihuahuana
Picea wilsonii
Picea likiangensis
Picea mexicana
Picea torano
Picea pungens
Picea mariana
Picea omorika
Picea rubens
76
Pinus thunbergii
Pinus arnoldii
Cathaya argyrophylla
Keteleeria davidiana
Abies firma
Tsuga mertensiana
Cedrus deodara
Pseudolarix amabilis
A

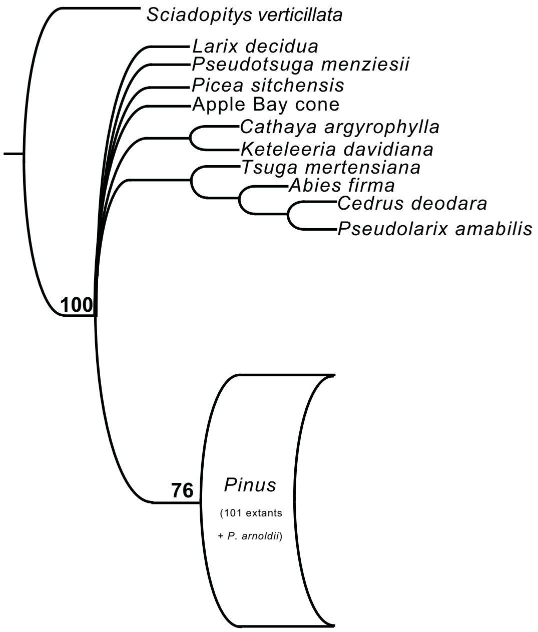
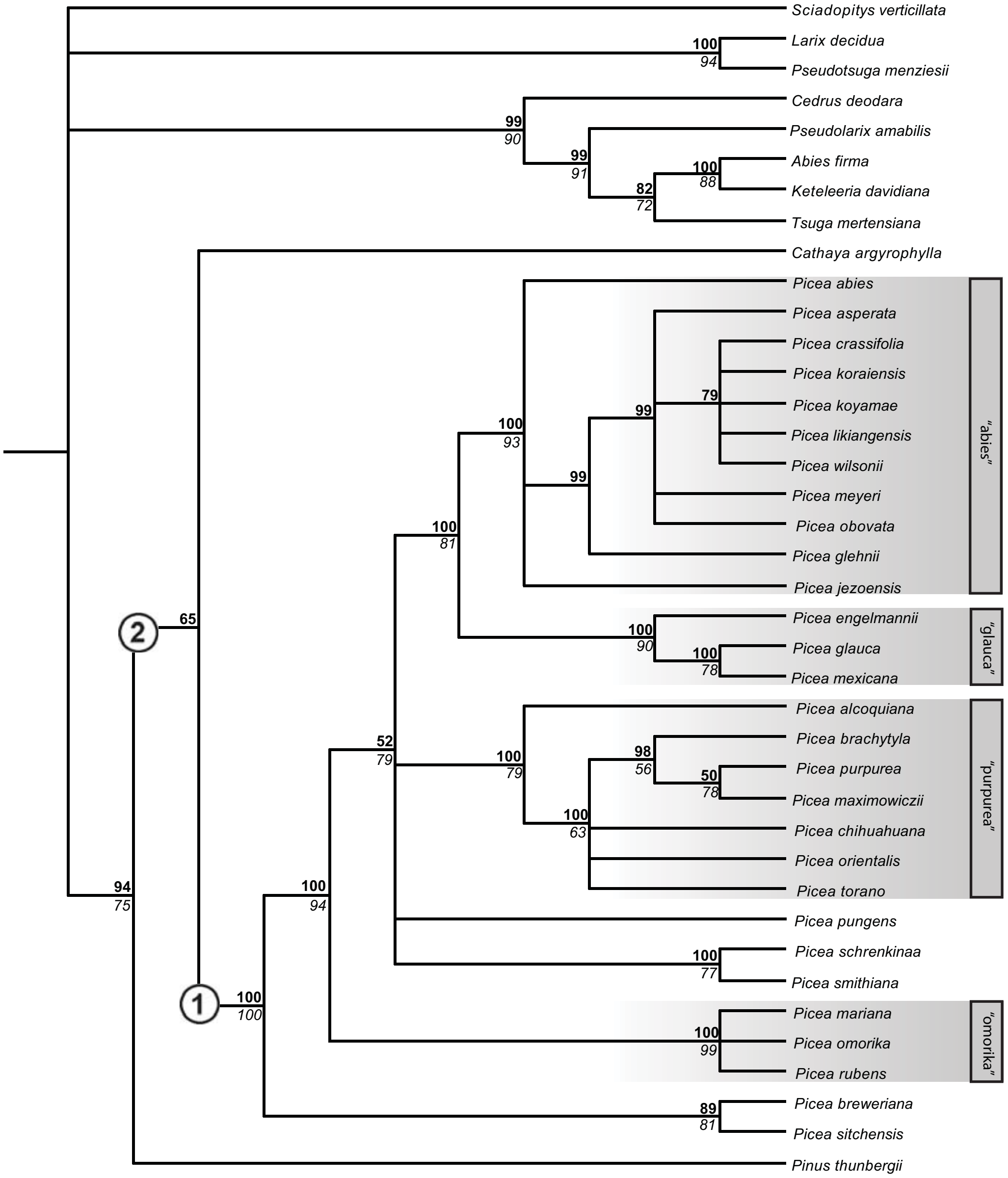

Sciadopitys verticillata
Larix decidua
Pseudotsuga menziesii
Picea sitchensis
Apple Bay cone
Cathaya argyrophylla
Keteleeria davidiana
Tsuga mertensiana
Abies firma
Cedrus deodara
Pseudolarix amabilis
100
76
Pinus
(101 extants
+ P. arnoldii)
B

Fig. 4-2 Bayesian consensus of unlinked molecular data partitions (*trn*K intron exclusive of *mat*K, *mat*K, and *trn*T-L-F intergenic spacer) for 29 species of *Picea* and 10 outgroup taxa, assessed with separate models of evolution chosen with AIC, and default flat Dirichlet priors. Posterior probabilities given above branches; bootstrap support values (below) derived from likelihood analyses of full molecular dataset formed from concatenated partitions. Numbered nodes correspond to Figure 4-6. Clade indications are after Germano et al. (2002).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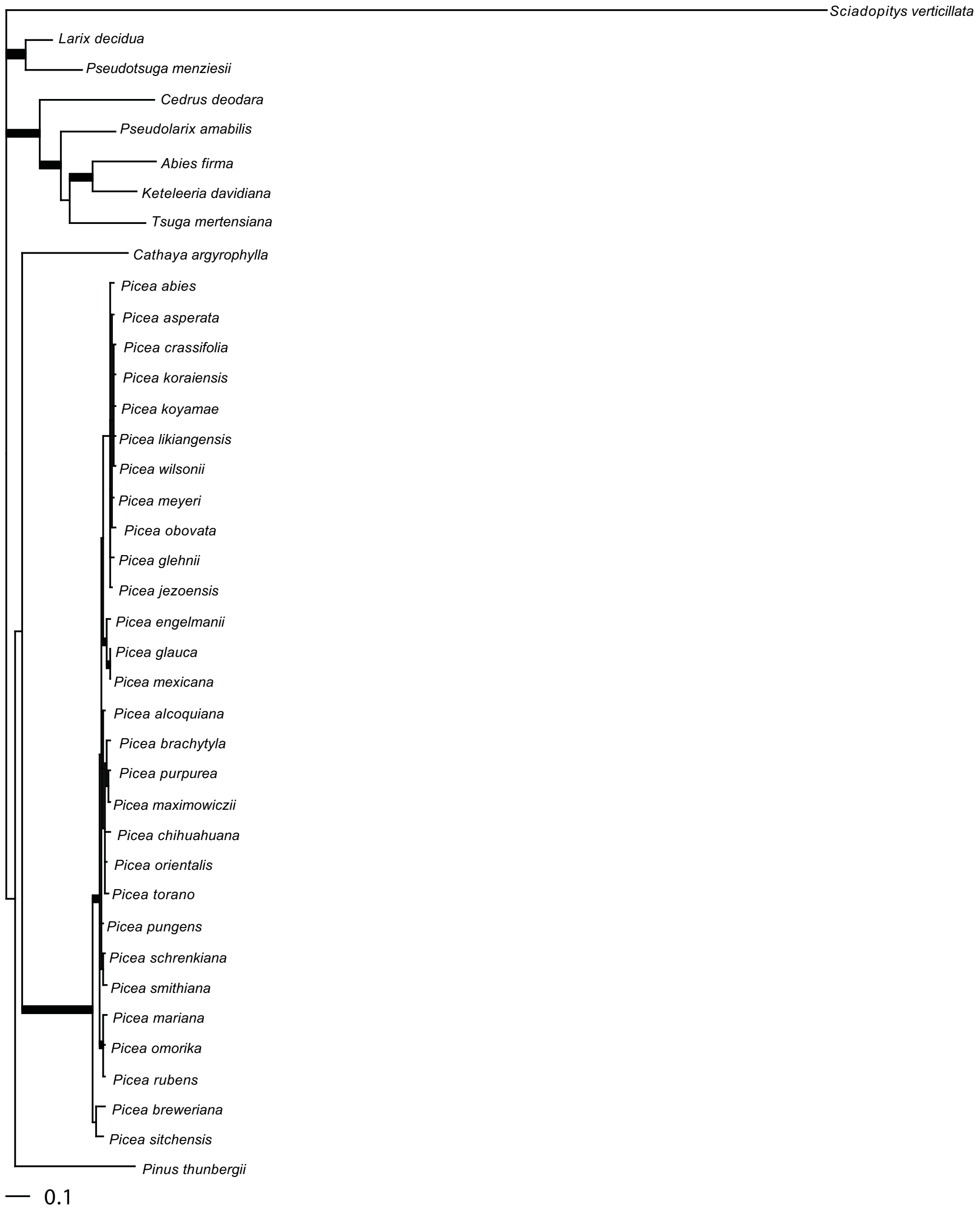
100
94
Larix decidua
Pseudotsuga menziesii
99
90
Cedrus deodara
99
91
Pseudolarix amabilis
100
88
Abies firma
82
72
Keteleeria davidiana
Tsuga mertensiana
Cathaya argyrophylla
Picea abies
Picea asperata
Picea crassifolia
Picea koraiensis
79
Picea koyamae
99
Picea likiangensis
Picea wilsonii
100
93
99
Picea meyeri
Picea obovata
Picea glehnii
100
81
Picea jezoensis
"abies"
65
2
Picea engelmannii
100
90
Picea glauca
100
78
Picea mexicana
"glauca"
Picea alcoquiana
52
79
98
56
Picea brachytyla
100
79
50
78
Picea purpurea
Picea maximowiczii
100
63
Picea chihuahuana
Picea orientalis
"purpurea"
94
75
100
94
Picea torano
Picea pungens
100
77
Picea schrenkinaa
Picea smithiana
1
100
100
Picea mariana
100
99
Picea omorika
Picea rubens
"omorika"
89
81
Picea breweriana
Picea sitchensis
Pinus thunbergii

Fig. 4-3 Bayesian consensus of unlinked molecular data partitions (*trn*K intron exclusive of *mat*K, *mat*K, and *trn*T-L-F intergenic spacer) for 29 species of *Picea* and 10 outgroup taxa, assessed with separate models of evolution chosen with AIC, and default flat Dirichlet priors. Weighted branches indicate those clades which receive posterior probability support >95%. Branch lengths reflect divergence.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Abies firma
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Tsuga mertensiana
Cathaya argyrophylla
Picea abies
Picea asperata
Picea crassifolia
Picea koraiensis
Picea koyamae
Picea likiangensis
Picea wilsonii
Picea meyeri
Picea obovata
Picea glehnii
Picea jezoensis
Picea engelmanii
Picea glauca
Picea mexicana
Picea alcoquiana
Picea brachytyla
Picea purpurea
Picea maximowiczii
Picea chihuahuana
Picea orientalis
Picea torano
Picea pungens
Picea schrenkiana
Picea smithiana
Picea mariana
Picea omorika
Picea rubens
Picea breweriana
Picea sitchensis
Pinus thunbergii
0.1

Fig. 4-4 Bayesian consensus of unlinked molecular data partitions (*mat*K, *rbc*L, and partial reads of associated noncoding sequences) for 101 species of *Pinus* and 10 outgroup taxa, assessed with separate models of evolution chosen with AIC, and default flat Dirichlet priors. Posterior probabilities given above branches; bootstrap support values (below) derived from likelihood analyses of full molecular dataset formed from concatenated partitions. Numbered nodes correspond to Figure 7. Classification after Gernandt et al. (2005).

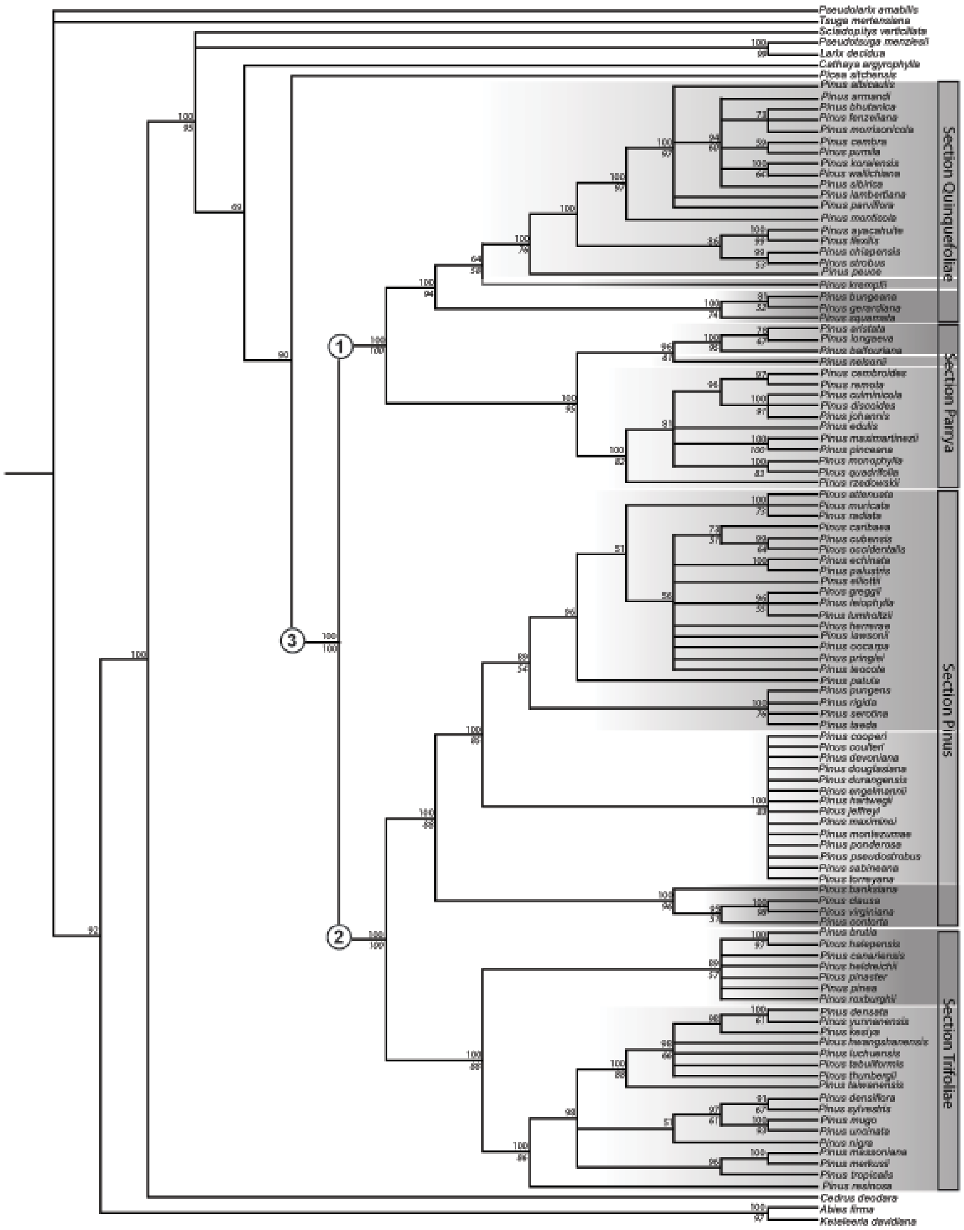

Pseudolarix amabilis
Tsuga mertensiana
Sciadopitys verticillata
Pseudotsuga menziesii
Larix decidua
Cathaya argyrophylla
Picea sitchensis
Pinus albicaulis
Pinus armandi
Pinus bhutanica
Pinus fenzeliana
Pinus morrisonicola
Pinus cembra
Pinus pumila
Pinus koraiensis
Pinus wallichiana
Pinus sibirica
Pinus lambertiana
Pinus parviflora
Pinus monticola
Pinus ayacahuite
Pinus flexilis
Pinus chiapensis
Pinus strobus
Pinus peuce
Pinus krempfii
Pinus bungeana
Pinus gerardiana
Pinus squamata
Pinus aristata
Pinus longaeva
Pinus balfouriana
Pinus nelsonii
Pinus cembroides
Pinus remota
Pinus culminicola
Pinus discolor
Pinus johannis
Pinus edulis
Pinus maximartinezii
Pinus pinceana
Pinus monophylla
Pinus quadrifolia
Pinus rzedowskii
Pinus attenuata
Pinus muricata
Pinus radiata
Pinus caribaea
Pinus cubensis
Pinus occidentalis
Pinus echinata
Pinus palustris
Pinus elliottii
Pinus greggii
Pinus teocote
Pinus lumholtzii
Pinus herrerae
Pinus lawsonii
Pinus oocarpa
Pinus pringlei
Pinus leiophylla
Pinus patula
Pinus pungens
Pinus rigida
Pinus serotina
Pinus taeda
Pinus coulteri
Pinus cooperi
Pinus devoniana
Pinus douglasiana
Pinus durangensis
Pinus engelmannii
Pinus hartwegii
Pinus jeffreyi
Pinus maximinoi
Pinus montezumae
Pinus ponderosa
Pinus pseudostrobus
Pinus sabiniana
Pinus torreyana
Pinus banksiana
Pinus clausa
Pinus virginiana
Pinus contorta
Pinus brutia
Pinus halepensis
Pinus canariensis
Pinus heldreichii
Pinus pinaster
Pinus pinea
Pinus roxburghii
Pinus densata
Pinus yunnanensis
Pinus kesiya
Pinus hwangshanensis
Pinus luchuensis
Pinus tabuliformis
Pinus thunbergii
Pinus taiwanensis
Pinus densiflora
Pinus sylvestris
Pinus mugo
Pinus uncinata
Pinus nigra
Pinus massoniana
Pinus merkusii
Pinus tropicalis
Pinus resinosa
Cedrus deodara
Abies firma
Keteleeria davidiana
Section Quinquefoliae
Section Parrya
Section Pinus
Section Trifoliae
1
2
3

Fig. 4-5 Bayesian consensus of unlinked molecular data partitions (*mat*K, *rbc*L, and partial reads of associated noncoding sequences) for 101 species of *Pinus* and 10 outgroup taxa, assessed with separate models of evolution chosen with AIC, and default flat Dirichlet priors. Weighted branches indicate those clades which receive posterior probability support >95. Branch lengths reflect divergence.

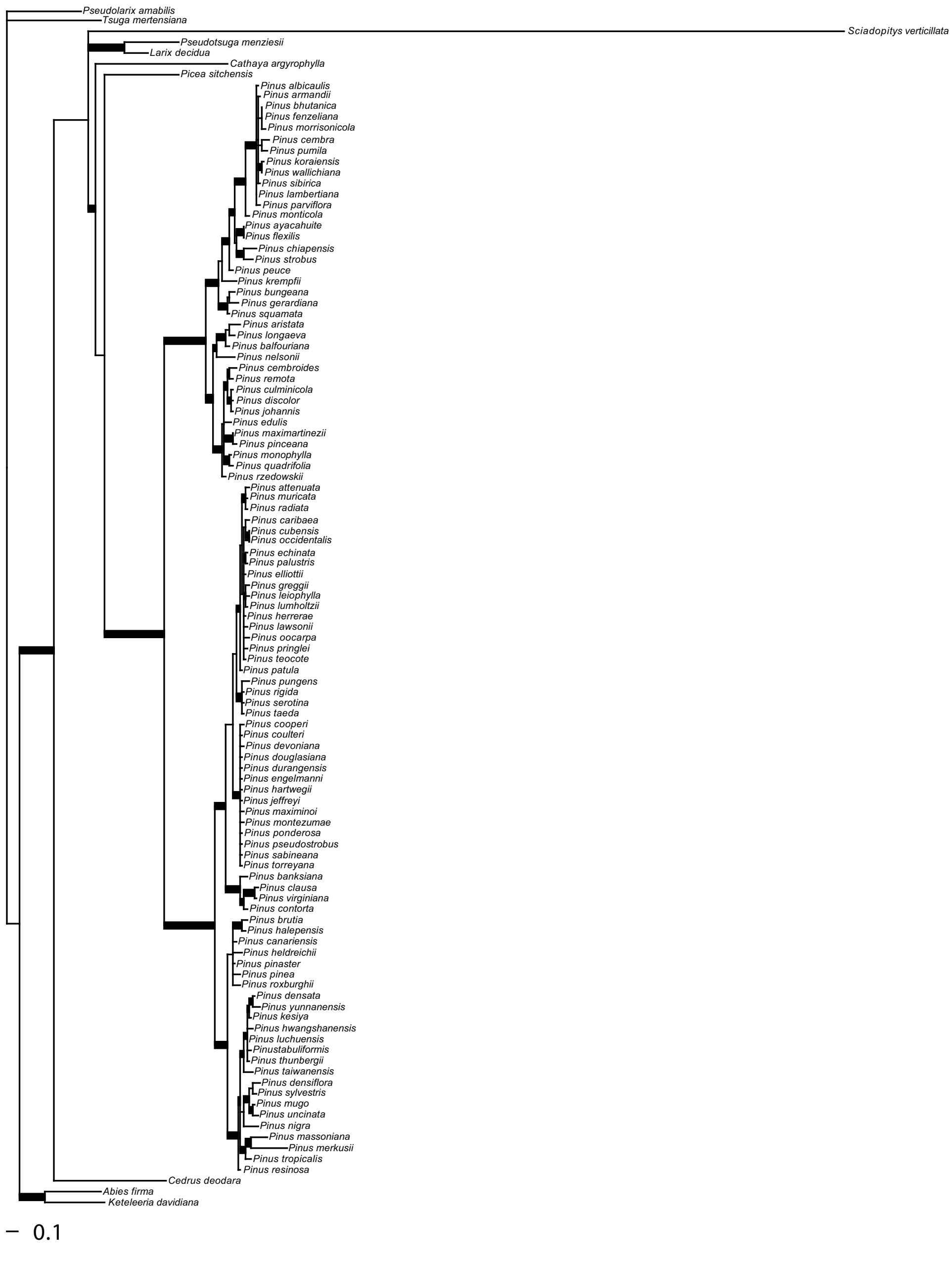

Pseudolarix amabilis
Tsuga mertensiana
Sciadopitys verticillata
Pseudotsuga menziesii
Larix decidua
Cathaya argyrophylla
Picea sitchensis
Pinus albicaulis
Pinus armandii
Pinus bhutanica
Pinus fenzeliana
Pinus morrisonicola
Pinus cembra
Pinus pumila
Pinus koraiensis
Pinus wallichiana
Pinus sibirica
Pinus lambertiana
Pinus parviflora
Pinus monticola
Pinus ayacahuite
Pinus flexilis
Pinus chiapensis
Pinus strobus
Pinus peuce
Pinus krempfii
Pinus bungeana
Pinus gerardiana
Pinus squamata
Pinus aristata
Pinus longaeva
Pinus balfouriana
Pinus nelsonii
Pinus cembroides
Pinus remota
Pinus culminicola
Pinus discolor
Pinus johannis
Pinus edulis
Pinus maximartinezii
Pinus pinceana
Pinus monophylla
Pinus quadrifolia
Pinus rzedowskii
Pinus attenuata
Pinus muricata
Pinus radiata
Pinus caribaea
Pinus cubensis
Pinus occidentalis
Pinus echinata
Pinus palustris
Pinus elliottii
Pinus greggii
Pinus leiophylla
Pinus lumholtzii
Pinus herrerae
Pinus lawsonii
Pinus oocarpa
Pinus pringlei
Pinus teocote
Pinus patula
Pinus pungens
Pinus rigida
Pinus serotina
Pinus taeda
Pinus cooperi
Pinus coulteri
Pinus devoniana
Pinus douglasiana
Pinus durangensis
Pinus engelmanni
Pinus hartwegii
Pinus jeffreyi
Pinus maximinoi
Pinus montezumae
Pinus ponderosa
Pinus pseudostrobus
Pinus sabineana
Pinus torreyana
Pinus banksiana
Pinus clausa
Pinus virginiana
Pinus contorta
Pinus brutia
Pinus halepensis
Pinus canariensis
Pinus heldreichii
Pinus pinaster
Pinus pinea
Pinus roxburghii
Pinus densata
Pinus yunnanensis
Pinus kesiya
Pinus hwangshanensis
Pinus luchuensis
Pinustabuliformis
Pinus thunbergii
Pinus taiwanensis
Pinus densiflora
Pinus sylvestris
Pinus mugo
Pinus uncinata
Pinus nigra
Pinus massoniana
Pinus merkusii
Pinus tropicalis
Pinus resinosa
Cedrus deodara
Abies firma
Keteleeria davidiana
0.1

Fig. 4-6 Likelihood ancestral state reconstruction for basal nodes of *Pinus*, with comparison to extinct species *Pinus arnoldii*. Reconstructions performed in Mesquite under mk-1 with default optimization intervals. Most probable states (*) assessed under default decision threshold (T=2.0).

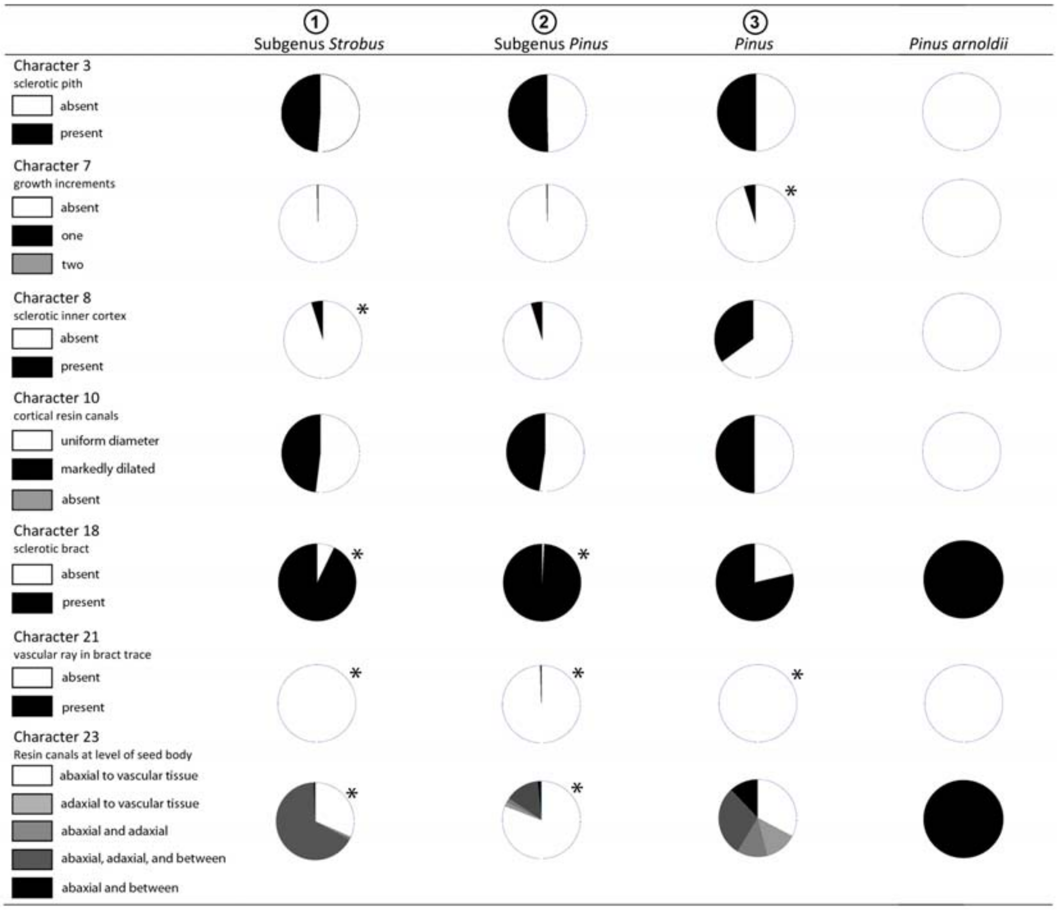
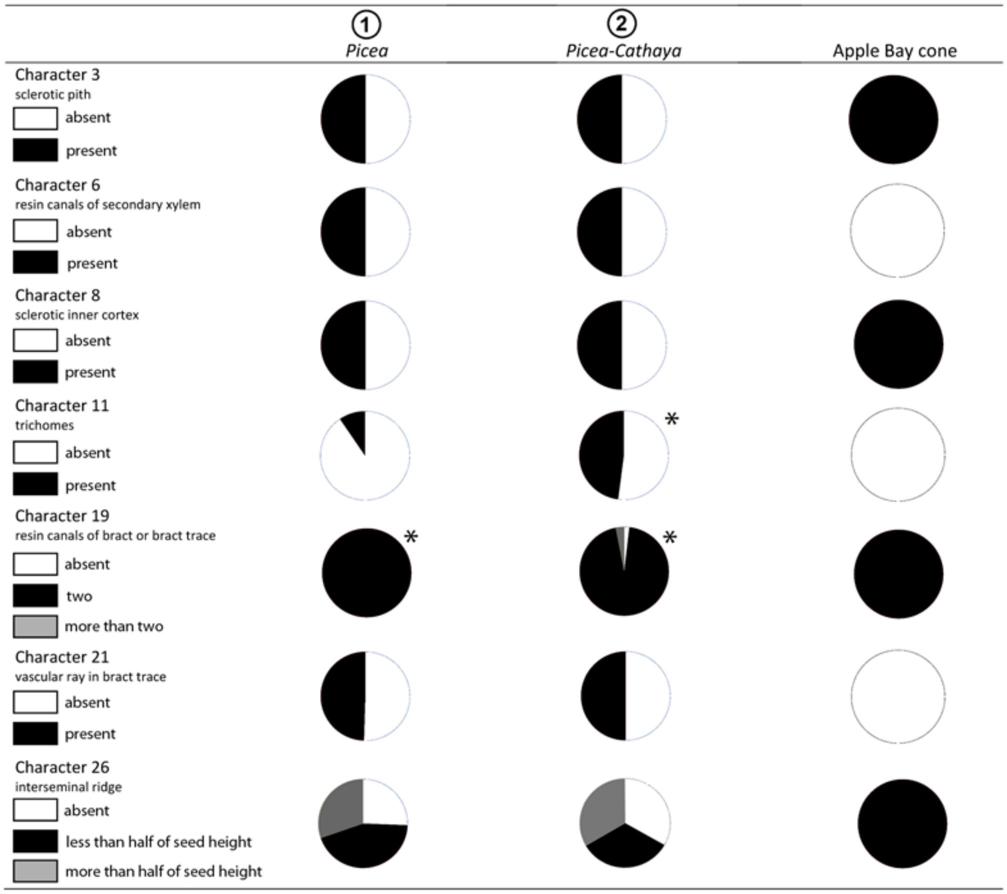

①
Subgenus *Strobus*
②
Subgenus *Pinus*
③
Pinus
Pinus arnoldii
Character 3
sclerotic pith
absent
present
Character 7
growth increments
absent
one
two
Character 8
sclerotic inner cortex
absent
present
Character 10
cortical resin canals
uniform diameter
markedly dilated
absent
Character 18
sclerotic bract
absent
present
Character 21
vascular ray in bract trace
absent
present
Character 23
Resin canals at level of seed body
abaxial to vascular tissue
adaxial to vascular tissue
abaxial and adaxial
abaxial, adaxial, and between
abaxial and between
*

Fig. 4-7 Likelihood ancestral state reconstruction for basal node of *Picea*, and MRCA of *Picea* and *Cathaya*, with comparison to an Early Cretaceous seed cone from Apple Bay, Vancouver Island. Reconstructions performed in Mesquite under mk-1 with default optimization intervals. Most probable states (*) assessed under default decision threshold (T=2.0).


①
Picea
②
Picea-Cathaya
Apple Bay cone
Character 3
sclerotic pith
absent
present
Character 6
resin canals of secondary xylem
absent
present
Character 8
sclerotic inner cortex
absent
present
Character 11
trichomes
absent
present
*
Character 19
resin canals of bract or bract trace
absent
two
more than two
*
*
Character 21
vascular ray in bract trace
absent
present
Character 26
interseminal ridge
absent
less than half of seed height
more than half of seed height

CHAPTER V: EVOLUTIONARY CONTEXT AND PHYLOGENETIC IMPLICATIONS OF NEWLY-DESCRIBED PINACEOUS TAXA

Pinaceae, with 225-232 extant species in 11 genera (Farjon 1998, 2010), has been historically divided into two subfamilies (i.e., van Tiegham 1891, Chamberlain 1935), which have been supported by cladistic analyses of non-molecular (Hart 1987) and molecular data (Price et al. 1987; Wang et al. 2000; Liston et al. 2003; Eckert and Hall 2006; Gernandt et al. 2008). Of the extant genera, *Cedrus* is either basal to both subfamilies, or the basal member of Subfamily Abietoideae, which also includes [*Abies* + *Keteleeria*] and [*Pseudolarix* (*Nothotsuga* + *Tsuga*)] (Gernandt et al. 2008). Subfamily *Pinoideae* includes the sister taxa *Larix* and *Pseudotsuga*, as well as *Cathaya* Chun et Kuang, *Picea* A. Dietrich, and *Pinus* L., which comprise a monophyletic group (Liston et al. 2003, Gernandt et al. 2008).

The family is thought to have evolved during the late Jurassic (LePage 2003; Miller 1988; Gernandt et al. 2008) or early Cretaceous (Gernandt et al. 2008). Separate relaxed-clock molecular divergence analyses using two different fixed-point fossil calibrations have yielded minimum divergence estimates for the family at either 184 or 136 Ma (Gernandt et al. 2008). Gernandt and his colleagues noted that one of the principle difficulties with such analyses is the paucity of reliably dated fossils that are readily assignable to extant genera.

The earliest putative fossil record for Pinaceae, *Pseudolarix erensis* Krassilov (1982), is a compression-impression morphospecies of detached ovulate cone scales, dispersed seeds, and vegetative remains from Mongolia, which have been dated to the Upper Jurassic by stratigraphic correlation with ^{40}Ar/^{39}A -dated sanidine tuffs (Khosbayer 1973; Keller and Hendrix 1997). However, the fragmentary nature of these remains, coupled with compression-impression preservation, suggest that their taxonomic affinities may be suspect (Stockey pers. comm. 2011). This indicates that the use of this taxon by Gernandt et al. (2008) as a calibration point may have inaccurately inflated estimates of the family's age.

Plant fossils permineralized by silica or calcium carbonate frequently preserve highly detailed internal anatomy, and therefore permit more definitive comparative anatomical assessments of taxonomic affinities than do compression-impression fossils. As such, permineralization has been widely recognized as one of the most informative modes of preservation for fossil plants (Crane et al. 2004; Taylor et al. 2009). Permineralized *Pinus* seed cones have been considered the oldest definitive representatives of an extant genus. *Pinus beligica* Alvin, a seed cone presumed to have been collected at a Wealden (middle Barremian to early Aptian) locality in Belgium (Alvin 1960), has historically been considered the oldest fossil pine. However, the description of a new seed cone from the Hauterivian-Barremian transition (Lower Cretaceous) Speeton Clay Fm of Yorkshire (Ryberg et al. 2010; P. Ryberg pers. comm. 2011) has extended the known record of *Pinus* to 131-132 Ma.

In comparison to *Pinus*, for which a number of Cretaceous seed cone and vegetative morphospecies are known (Alvin 1960; Falder et al 1998; Karafit 2008, Miller 1976*b* Ryberg et al. 2010; P. Ryberg pers. comm. 2011; Stockey and

Wiebe 2006; Wehr and Hopkins 1994), the fossil records of the other pinoid genera are surprisingly recent, and sparse. The earliest seed cone record for *Pseudotsuga* is Miocene (Czaja 2000), although *Pseudotsuga*-like leaves have been documented from the early Cretaceous (Stockey and Wiebe 2006). Fertile and vegetative remains assignable to *Larix* have been described from the middle Eocene (LePage and Basinger 1991; Jagels et al. 2001), as have *Cathaya* palynomorphs, which comprise the fossil record for *Cathaya* (Liu and Basinger 2000; Sivak 1976; Klaus 1984). Until now, the oldest recognized representative of *Picea* was a Paleocene palynomorph, although *Picea*-like wood is known from the Albian of Russia (Blokhina and Alfonin 2009), as are *Picea*-like leaves ,from the Valanginian of Vancouver Island (Stockey and Wiebe 2008). Until this study, definitive *Picea* macrofossils, however, had only been described from the middle Eocene of Canada's high Arctic (LePage 2001).

Thus, there is a stratigraphic disparity between fossils of *Picea*-like vegetation and cones attributable to the genus, which indicates that *Picea* is under-represented in paleobotanical collections. Divergence estimates produce corroborative ghost lineages, as long branches are evident at all basal nodes, particularly those subtending the genera *Pinus*, *Picea*, and *Cathaya* (Gernandt et al. 2008). Although the Cretaceous diversity of *Picea* was once thought to encompass many seed cones (i.e., Berry 1905; Penny 1947), these early diagnoses were based on external morphology (Miller 1974). Where permineralization permitted examination of their internal anatomy, these long, cylindrical cones were found to lack anatomical similarity to *Picea* (Miller 1974, 1976*a*).

Most Mesozoic pinaceous seed cones have been relegated to three morphogenera: *Pseudoaraucaria* Fliche, which is considered a natural genus with six known species (Alvin 1957; Miller and Robison 1975, Miller 1976a; Smith and Stockey 2001, 2002), *Obirastrobus* Ohsawa, Nishida et Nishida (1992), which contains two, and *Pityostrobus* Nathorst emend. Dutt, within which the majority of seed cones are classified. *Pityostrobus* has long been recognized as polyphyletic (Miller 1976*a*, Stockey 1981, Falder et al. 1998; Smith and Stockey 2001). There are as many anatomical and morphological differences between any of the 28 *Pityostrobus* morphospecies as there are between extant genera, and it has therefore been suggested that each of these morphotaxa may represent an extinct genus (Falder et al. 1998; Miller 1976*a*, Smith and Stockey 2001, 2002).

The polyphyletic *Pityostrobus* assemblage provides evidence for the rapid evolution and radiation of pinaceous conifers during the late Mesozoic. Pinaceae reached its peak morphological diversity during the Cretaceous (Miller 1976*a*, 1977, 1988), and the modern genera have consistently shown themselves to be relicts of a much more diverse evolutionary history (Alvin 1988; Miller 1976*a*, 1977, 1978, 1988; Miller and Li 1994; Miller and Robison 1975; Robison and Miller 1977; Smith and Stockey 2001, 2002). Seed cones representing these early pinaceous lineages have been traditionally assessed with respect to 33 morphological and anatomical characters (Miller 1976*a*; Miller and Robison, unpublished data; Smith and Stockey 2001, 2002). When these data are analyzed in a cladistic framework using Fitch parsimony (unweighted and unordered characters and character states), the evolutionary relationships between

Cretaceous morphotaxa and extant genera cannot be resolved (Smith and Stockey 2001, 2002; Chapter 2).

Furthermore, several pinaceous leaf morphologies are preserved at the Valanginian Apple Bay locality of Vancouver Island, British Columbia, Canada (Stockey and Wiebe 2006, 2008), which is the oldest locality at which anatomically preserved (permineralized) representatives of the family have been found. The Apple Bay leaf morphologies include *Tsuga*-, *Pseudotsuga*-, *Pinus*-, *Picea*- and *Abies*-like forms (Stockey and Wiebe 2006, 2008), indicating that not only was the family already very diverse in the late Valanginian, but also that plants belonging to extant lineages had already evolved by this time.

Early Cretaceous Record for **Picea**

The *Picea*-like leaf morphology found at Apple Bay was described as *Midoriphyllum piceoides* Stockey and Wiebe (2008), and exhibits a plexus of characters which, although most similar to *Picea*, also exhibit some commonalities with *Pinus*. The vascular bundle and resin canal anatomy of *Midoriphyllum*, as well as the presence of a thickened hypodermis associated with leaf angles, are very similar to extant *Picea*, although the presence of pronounced plicate mesophyll is more typical of *Pinus* (Stockey and Wiebe 2008). Finally, the cross-sectional leaf shape, which encompasses hexagonal, pentagonal, quadrangular, and triangular forms, is more diverse than that exhibited by any extant genus (Stockey and Wiebe 2008), and there has been suggestion that these needles may have been borne in loose fascicles (RAS pers. comm.). In their concluding remarks, Stockey and Wiebe (2008) suggested that if these leaves represented an early member of the *Picea* lineage, there ought to be corresponding *Picea*-like cones.

Comparative assessment of the internal anatomy of the new pinaceous seed cone described here reveals that it exhibits a combination of features that accord only with extant *Picea*. Designation of the new cone as the earliest representative of the *Picea* lineage is corroborated by the presence of *Picea* pollen within several ovules. *Picea* pollen grains are generally larger than those of other genera that possess bisaccate grains (Kapp 1969), and the pollen found within the Apple Bay cone (~75µm in total length) conforms to the lower end of the *Picea* size range. Furthermore, the obstuse angle formed between the corpus and sacci is characteristic of *Picea* and *Cathaya* (Kapp 2000; Saito et al. 2000; Liu and Basinger 2000). Finally, the sculptured cappula ridges, which largely occlude the germinal furrow, are diagnostic for *Picea* (Bagnell 1975).

The preservation of pollen grains within ovules of pinaceous cones is rare. Aside from the Apple Bay cone, palynological associations have only been reported in *Pityostrobus yixianensis* (Shang et al. 2001; He et al. 2004; Jiang and Sha 2006) from the Aptian of NE China (He et al. 2004). The pollen preserved in *P. yixianensis* ovules is said to be similar to *Picea*, but the diagnostic ridges of the cappula are not apparent, and the cone itself differs with respect to several anatomical features, including the course of resin canals, lack of a keeled bract, and architecture and divergence of vascular strands.

As the Apple Bay cone is consistent with all "*Picea*" features, and contains *Picea* pollen, I contend that it not only marks the earliest fossil record for pinaceous seed cones, but is the earliest representative of the *Picea* lineage, extending the fossil record of *Picea* by ~75 Ma. The leaf morphotaxon, *Midoriphyllum piceoides* may represent the vegetation associated with this early spruce. However, the reconciliation of the *Midoriphyllum* vegetation and the new *Picea* cone as an organismal concept is almost certainly precluded by the nature of the depositional setting of the strata within which these taxa are preserved.

Plant fossils at the Apple Bay locality are preserved as allochthonous phytodetrital components of marine sequences. Marine strata are the most likely to produce anatomically preserved botanical remains, as hyperpycnal conditions associated with seasonal storms produce influxes of plant material, and result in localized changes in chemical-osmotic gradients, allowing the formation of calcium carbonate concretions containing permineralized plants. However, such sedimentary assemblages are often products of time averaging, (Flessa et al. 1993; Kidwell 2002; Kowalewski 1996), where strata are frequently palimpsests of cycles of deposition and reactivation (e.g. Slatt 1974). As the strata at Apple Bay likely represent tempestite deposits (Stockey et al. 2006), paraconformities between the 20 individual beds may reflect periods of non-deposition or deposition and subsequent erosion, and therefore represent unknown amounts of time. Because the concretions containing *Midorphyllum* specimens and the new cone were collected without regard to provenance within the stratigraphic succession preserved at Apple Bay, these morphotaxa may not be penecontemporaneous.

If *Midoriphyllum* and the new cone *do* represent vegetative and reproductive organs, respectively, of the same plant, it can be inferred that the morphology and anatomy of seed cones are more evolutionarily conserved than vegetative features. The hypothetical organismal concept for this plant is complicated by the fact that these leaves exhibit features not seen among living members of the genus. As such, this late Valanginian spruce illustrates an important difficulty inherent in assessing the biological meaning of fossil taxa. Although morphotaxa like the new seed cone may seem attributable to extant lineages, the biological species that these plant organs represent may in fact exhibit novel combinations of characters, or unique features that are not encompassed within the diversity of living taxa. Morphotaxa are most often the only record of such extinct plants, due to the fact that plants die successively, shedding their vegetative and reproductive organs at different points throughout their life history. While such specimens may seem assignable to extant lineages, it is also possible that the complete taxa they represent could be composed of separate morphotaxa that would otherwise be assigned to disparate taxonomic lineages, as has been demonstrated by whole plant reconstructions (i.e., Klymiuk et al. 2011; Crane and Stockey 1986).

Pinus arnoldii ***Miller, an Organismal Concept***

Unequivocal organismal concepts for extinct plants are dependent upon anatomical attachments between the constituent organs. While the possibility exists that attachments could be discovered in allochthonous marine tempestites, terrigenous sediments that exhibit low or only localized transport, are more probable in terms of providing the opportunity to develop whole-plant reconstructions. The Ypresian-Lutetian (middle Eocene) Princeton Chert *konservat-laggerstätten* of southern British Columbia is a classic example of an optimal locality in this respect. At the Chert, a stable mire community has been subject to successive preservation via silica permineralization. A number of organismal concepts that combine vegetative and reproductive morphotaxa (i.e., Rothwell and Basinger 1979; Basinger 1981, 1984; Cevallos-Ferriz and Stockey 1988; Stockey and Pigg 1991, 1994; Stockey et al. 1999; Little and Stockey 2003; Little et al. 2004; Smith et al. 2006; Klymiuk et al. 2011) have been developed from this locality.

The description of *Pinus arnoldii* (Klymiuk et al. 2011) is the most recent organismal concept developed from the Princeton Chert flora, and is the first fossil taxon in Pinaceae for which a whole plant reconstruction has been developed. This Eocene pine was first described from seed cones most similar to those borne by species within the subgenus *Pinus* (Miller 1973, Stockey 1984). Along with stems, short shoots, and leaves described as *Pinus similkameenensis* (Miller 1973), they are among the most common fossils preserved in the Princeton Chert. The five-needled fascicles of leaves described as *P. similkameenensis* are ubiquitous, and always present in chert samples bearing mature and immature seed cones, pollen cones (Phipps et al. 1995), and coralloid ectomycorrhizal rooting structures (LePage et al. 1997). Miller was the first to recognize the close spatial associations between the *P. similkameenensis* vegetation and *P. arnoldii*, and suggested that these taxa could comprise the vegetative and fertile organs of a single species. Among extant pines, however, needles like those described as *P. similkameenensis* are restricted to the haploxylon, or "soft" pines (Subgenus *Strobus,* Critchfield and Little 1966, Miller 1973). Anatomically, these leaves are very similar to those of *Pinus monticola* Douglas ex D. Don, *P. peuce* Griseb., *P. parviflora* Siebold et Zucc., and *P. strobus* L. (Miller 1973), classified within sect. *Strobus* (Subgenus *Strobus*). The stems that give rise to these fascicles have wood anatomy that closely resembles that seen in the sect. *Parrya* (Subgenus *Strobus*), particularly *P. aristata*, *P. bungeana* Zucc. ex Endl., and *P. cembroides* Zucc. (Miller 1973). As such, *P. similkameenensis* shows a combination of features that, among extant taxa, are segregated to different sections of Subgenus *Strobus*. The *P. arnoldii* ovulate cones, however, are most similar to those of Subgenus *Pinus*, in that they exhibit an inflated scale apex and dorsal umbo, are not serotinous, and have a densely sclerotic outer cortex (Critchfield and Little 1966, Miller 1973). Because these morphotaxa were most similar to living pines that are taxonomically disparate, and attachments between them were lacking, Miller (1973) classified them separately. Although the sheer abundance of these pine organs is highly suggestive of biological affinity (Miller 1973, Stockey 1984, Phipps et al. 1995), other pine morphotaxa have been described from the Princeton Chert and its stratigraphic equivalents (Stockey 1984), precluding a whole-plant reconstruction based on association alone.

Through anatomical attachments between seed cones and vegetative organs, the organismal concept developed in this study conclusively demonstrates that the morphospecies *P. similkameenensis* comprises the vegetative organs of the fossil species *Pinus arnoldii*. Mature and immature *P. arnoldii* seed cones are attached to stems exhibiting *P. similkameenensis*-type wood anatomy. As such, the organismal concept for the *P. arnoldii* plant incorporates the mature seed cones and woody stems producing five-needled fascicles of leaves, as originally suggested by Miller (1973), and has been amplified to include immature ovulate cones.

Several immature ovulate cones have been preserved at the pollination stage, and contain pollen grains identical to those preserved in the *Pinus* pollen cones described by Phipps et al. (1995). These pollen cones are often oriented in such a way as to reflect attachment, and although a potential attachment between several cones was lost in the saw cut, the specimen exhibits orientation that compares to the organography of many extant pines. Similarly, pine rooting systems (LePage et al. 1997) are preserved *in situ*, and localized growth strengthens the case for inclusion of the rooting systems into the organismal concept. At this time, the organography of this extinct pine is almost entirely known, and many aspects of growth architecture, habit, ontogeny, pollination biology, and ecology may be inferred. As such, the extinct plant *Pinus arnoldii* is known at a level of detail that is comparable to (and often surpasses that of) extant pines.

Given that *Pinus arnoldii* exhibits a unique combination of characters that are, among extant species, seen in different sections of the two subgenera, it is reasonable to infer that the *Pinus arnoldii* plant represents a member of the *Pinus* lineage that is ancestral to the modern subgenera. In cladistic analyses of concatenated morphological (adapted from Gernandt et al. 2005, to include seed cone characters after Smith and Stockey 2001), *rbc*L, and *mat*K data (as assembled by Gernandt et al. 2005), *P. arnoldii* was resolved as either a basal member of the subgenus *Pinus*, or was placed in a polytomy with the two subgenera. This suggests either that during the Eocene, the modern subgenera of *Pinus* were not yet distinct, or that the genus was far more diverse than it is at present, and contained lineages without modern issue. Fossil-calibrated node estimation indicates the subgenera of *Pinus* were distinct no more than 72 Ma (Gernandt et al. 2008); therefore the modern diversity of the genus reflects a Paleogene radiation, and both are reasonable hypotheses. While the extinct species *P. arnoldii* cannot be attributed to either of the modern subgenera, it is an important benchmark for systematic studies of Pinaceae. Unlike all other fossils of pinaceous affinity, this extinct pine is now known as a complete organism, and it thus constitutes a critical advance in understanding the biological context of other pinaceous fossils.

In order to develop an understanding of the evolutionary history of Pinaceae, it is imperative that the phylogenetic implications of the Cretaceous *Pityostrobus* morphotaxa be understood, as this group represents the maxima of pinaceous diversity. However, one of the difficulties of incorporating fossils into cladistic analyses of plant relationships is the large amount of missing data (Nixon 1996). For morphological datasets, the inclusion of fossil taxa provides greater resolution of trees when the fossils are temporally close to an ancestral node, but the inclusion of such taxa in morphological analyses is only of value if the majority of phylogenetically informative characters are known (Huelsenbeck 1991). Resolution in topology is diminished commensurately by missing data, as the number of most parsimonious trees is increased (Huelsenbeck 1991). This suggests that the inclusion of morphotaxa in phylogenetic analyses is of limited value, in comparison to fossil species such as *Pinus arnoldii*. Certainly, it has been demonstrated that the relationships between extant taxa and the Cretaceous morphospecies are an intractable problem when assessed using traditional Fitch parsimony (Smith and Stockey 2001, 2002; Chapter 2). By determining the evolutionary polarity of characters as they are expressed in extant taxa, it should be possible to contextualize some of the extinct members of the family.

Comparison with ancestral states, reconstructed under maximum likelihood across topologies independently indicated by analysis of molecular data (Chapter 4), may be an appropriate measure to institute when attempting to understand the evolutionary context of seed cone morphotaxa. As noted previously, combined morphological and molecular parsimony analyses of extant *Pinus* and the extinct species *Pinus arnoldii* (Klymiuk et al. 2011; Chapter 3) indicated that the species is either an early-diverging member of the clade corresponding to Subgenus *Pinus*, or is representative of a more diverse, wholly extinct group of pines. The latter hypothesis is more consistent with what we know of this pine, given that the organismal concept developed for this species demonstrates a novel combination of morphological characters. It is important to note, however, that comparative assessment of the seed cone of *P. arnoldii*, when taken without consideration of the vegetation, indicates affinity to the diploxylon pines. Thus, the seed cone of *P. arnoldii* can be used as a proxy when testing the concept that ancestral state reconstruction can permit more appropriate taxonomic assessments than comparative anatomy, or cladistic analyses alone. Several seed cone characters which are polymorphic within *Pinus* were reconstructed across a consensus topology generated by Bayesian analysis of the *rbc*L and *mat*K coding regions (Chapter 4). When the seed cone of *P. arnoldii* was compared to these hypothesized ancestral states, it did not entirely accord with ancestral states at either the node defining Subgenus *Pinus*, or the basal node of *Pinus* itself (Chapter 4), despite the fact that comparative anatomy of this cone indicates close affinity with Subgenus *Pinus* (Miller 1973). It therefore seems that this method may reveal phylogenetic context where straightforward comparative anatomical and morphological methods are inaccurate. On this basis, it merits further attention and study, as it may provide a means of more judiciously utilizing morphotaxa.

Applications of these Taxa in Molecular Systematics

The appropriate taxonomic placement of morphotaxa is a complex problem that underlies the use of such specimens in molecular-based hypotheses of divergence, biogeography, and character evolution. Because the fossil record provides a line of evidence for testing hypotheses of evolution developed from molecular sequence analyses, fossils have become increasingly important to molecular systematists, particularly with respect to calibrating molecular divergence estimates. Molecular dating is an integral aspect of many studies that seek to investigate mechanisms and processes of evolution on a genetic and organismal scale (Rutschmann 2006). The incorporation of fossil data in molecular dating analyses, as opposed to the earlier, tautological, practise of using dates generated by previous studies, has improved the accuracy of such estimates (Benton and Ayala 2003). The resultant dates, particularly within lineages, are generally concordant with the known fossil record (Benton and Ayala 2003).

It is, however, widely understood that there are still numerous problems plaguing attempts to understand lineage divergence under the molecular clock hypothesis (Hillis et al. 1996; Sanderson 1998; Yang and Yoder 2003; Magallón 2004; Pulquério and Nichols 2007). Changes in the overall rate of genetic evolution (Ohta 1992), and the tendency of different genes exhibit different rates and types of substitution (e.g. Langley and Fitch 1974, Gillespie 1991; Xia 1998; Adams et al. 2002) are problems that have received the most attention (Rutschmann et al. 2007), and may be the least pernicious. Rate heterogeneity can be dealt with by excluding genes and lineages that exhibit differing rates of change (Takezaki et al. 1995, Hedges et al. 1996) rate smoothing (Sanderson 1997, 2002; Yang 2004; Aris-Brosou 2007), applying different evolutionary models to branches (Rambaut and Bromham 1998; Yoder and Yang 2000), or modelling changes in the rate of evolution by Bayesian methods (Thorne et al. 1998, Huelsenbeck et al. 2000; Kishino et al. 2001; Drummond et al. 2006; Yang and Rannala 2006; Rannala and Yang 2007). By comparison, there are numerous intractable problems inherent in the use of fossil taxa to calibrate molecular divergence estimates across a tree (Rutschmann et al. 2007; Ho and Phillips 2009).

The appropriate placement of a fossil taxon is a first-order consideration. At the simplest end of the spectrum, a fossil may be incorrectly placed owing to erroneous identification (Lee 1999). Less conspicuously, the use of fossils as point calibrations (e.g. Gernandt et al. 2008) implies a direct ancestor-descendent relationship between the taxon and a crown group (Ho and Phillips 2009), and can produce dramatically different divergence time estimates (Doyle and Donoghue 1993; Magallón and Sanderson 2001; Forest et al. 2005). Finally, the fact that the vast majority of plant fossils are morphotaxa for which biological correlation is an assumption, and not a default, is an issue that has not been widely addressed in the literature of molecular systematics.

The use of fossils in molecular dating analyses is also confounded by stratigraphic imprecision, as many sedimentary successions have not been reliably dated, and by outright errors, including radiometric dating errors, and incorrect stratigraphic correlation (Conroy and Van Tuinen 2003). Collection data and published stratigraphic descriptions of collecting localities are also lacking for many fossils (i.e., Alvin 1960). As such, the importance of collecting localities like Apple Bay (Jeletzky 1976, Haggart and Tipper 1994, D.R. Gröcke pers. comm. to RAS) and the Princeton Chert (Hills and Baadsgaard 1967, Baadsgaard pers. comm to RAS), which are stratigraphically well-bracketed, cannot be overstated.

Multiple fossil calibration points have been suggested as a method by which to reduce the effects of errors of stratigraphic context or incorrect placement of a calibration taxon (Conroy and Van Tuinen 2003, Soltis et al. 2002; Graur and Martin 2004, Near et al. 2005; Marshall 2008). Near and Sanderson (2004) note, however, that this practise provides a pool of fossil data that is an average of accurate and inaccurate data, necessitating a method by which to assess the credibility of a fossil calibration. At present, such methods involve cross-validation to remove "inconsistent" fossil taxa (Near et al. 2005; Near and Sanderson 2004), and the use of "likelihood checkpoints", which assess the validity of a system of multiple calibration points by comparison with the known fossil record (Pyron 2010). Although Pyron's methodology is flexible in that it permits evaluation of an entire system of calibrations, as opposed to simply indicating calibrations that may be erroneous as in the methodology proposed by Near et al. (2005), both are ultimately reliant upon an excellent fossil record, thus limiting applicability to those groups which have extensive fossil records that are attributable to extant lineages, and are therefore currently of little use in studies of Pinaceae. The two fossil taxa described here are likely to be of particular interest to molecular systematists as additional calibration points, as they are stratigraphically well-bracketed, and anatomically preserved with high acuity, allowing confident assessement of their taxonomic affinities.

Even with the most extensive palaeontological record, however, the oldest fossil it contains must necessarily be younger than the origin of the group (Benton and Ayala 2003, Magallón 2004). Because fossils can provide only minimum constraints, it is common practise to assess lineage divergences with unconstrained maxima, under the assumption that an older fossil taxon could theoretically be found (Marshall 1990, Benton and Ayala 2003). This elastic upper bound results in overestimation of divergence at temporally remote targets (Benton and Ayala 2003). Furthermore, Marshall (2008) has demonstrated that maximum age constraints play a dominant role in assessments of absolute divergence times, while internal minimum divergence calibrations have significantly less effect (Marshall 2008), providing further indication that some estimate of upper bounds ought to be assessed with as much precision as possible.

The establishment of an upper bound for a calibration is typically based on a consensus of palaeontological opinions, which are empirically difficult to justify, even if apt (Marshall 2008). Paleogeographic data may be utilized when the calibration of interest can be correlated with some datable tectonic event (Marshall 2008; Ho and Phillips 2009), although this method may invoke inaccurate *a priori* assumptions about the evolutionary processes affecting the lineage (e.g. Barker et al. 2007). Marshall (2008) posited a quantitative method to assess maximum constraints, by deriving a scaling factor from a “calibration lineage” corresponding to a group whose fossil record encompasses the greatest temporal coverage. The more temporally complete the fossil record, the smaller the maximum age confidence interval will be, and the more precise the subsequent estimation of divergence times. The new taxon described in Chapter 2 as the earliest known member of *Picea* makes this genus a strong candidate for

Marshall's calibration lineage, and may therefore serve to increase the accuracy of divergence time estimates for extant members of Pinaceae.

Further Research

Although there are difficulties inherent in incorporating fossil evidence into modern systematic studies, fossil taxa like the extinct species *Pinus arnoldii* and seed cone morphotaxon that I consider the oldest fossil record for the extant genus *Picea* offer the opportunity to further investigations into the evolutionary divergence of Pinaceae, and the processes of evolution within this family of conifers. Although these fossil taxa are important additions to our understanding of the early diversity of Pinaceae, their role in elucidating the origins of extant genera is dependent upon more detailed assessment of the anatomy and morphology of living members of the family. It has been noted (Crane et al. 2004) that comparative anatomy for living plants has been only sporadically investigated. The Miller Conifer Reference Collection is the most complete and diverse compilation of anatomical sections of pinaceous cones, yet encompasses only a fraction of the extant species. Fewer than half of the living members of *Pinus* and *Picea* have been anatomically assessed; further examination of extant species is imperative if we are to understand character evolution in Pinaceae.

It is also obvious that pinaceous diversity in the Early Cretaceous has been undersampled and underestimated to date. Continuing collection endeavors at Apple Bay are likely to reveal other pinaceous seed cones that match the *Abies*-, *Pseudotsuga*-, *Tsuga*-, *Larix*-, and *Pinus*-like leaf morphologies. Detailed sedimentological studies at Apple Bay will also permit a more comprehensive understanding of the depositional settings and taphonomic processes that result in the preservation of marine successions that preserve phytodetritus. These observations will then have application in the identification of other candidate localities dating from the Kimmeridgian to Berriasian, within which older representatives of Pinaceae might be found. There are also several other significant stratigraphic gaps in the fossil record of the family: for instance, only *Obirastrobus* cones have been described from the Turonian-Coniacian, and as they were collected from washout along a riverbed, their stratigraphic provenance is uncertain. Nor have any pinaceous seed cones been described from the Maastrichtian. Given that many of the modern genera were highly diverse by the Eocene, the absence of a Maastrichtian record of at least extant pinaceous conifers is puzzling. Similarly, the diversity of Pinaceae appears to have dramatically diminished in the Paleogene. Only a single fossil taxon, *Pityostrobus makahensis* (latest Eocene to Oligocene), is not assignable to an extant lineage. Whether this is a sampling bias, or reflects the true record of the decline of the family's diversity is at present unknown. The Paleocene-Eocene Thermal Maximum (Kennett and Stott 1991), which may have been caused by tectonic uplift of epicontinental seaways (Higgins and Schrag 2006), saw global temperatures increase by 5-8°C within 10-30 ky. It is possible that the PETM may be implicated in the decrease of pinaceous diversity. Further investigation of early Paleogene localities is warranted, to better understand the point at which "*Pityostrobus*" conifers decline, and what implications, if any, these extinctions had for the subsequent radiation and diversification of modern genera in Pinaceae.

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APPENDIX A

Concepts of Characters for Pinaceous Seed Cones

adapted from characters defined by Smith and Stockey (2001, 2002).

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1. Ovuliferous scale apex.
    0 = thinning distally
    1 = thickening distally (or has umbo)
    Ovuliferous scales of cones of *Pinus* have an umbo, whereas most other Pinaceae do not. In fossil cones, scale tips are often abraded, but distal thickening or thinning of ovuliferous scales can usually be seen. The presence of an umbo or a distal thickening ovuliferous scale results in a coding of 1; extensive abrasion resulted in a question mark.
2. Bract length.
    0 = bract shorter than ovuliferous scale
    1 = bract longer than ovuliferous scale
    2 = bract and ovuliferous scales equal in length.
3. Sclerenchyma in pith.
    0 = absent
    1 = present
    Includes only sclereids or fibers; thick-walled or sclerotic parenchyma are excluded.
4. Resin canals in pith.
    0 = absent
    1 = present
    Canals do not include those in primary xylem; they must be separated by at least a few pith cells from the xylem.
5. Secondary xylem of cone axis.
    0 = forming continuous cylinder (or little dissected)
    1 = in separate strands
    Steles described as “moderately” dissected were coded as continuous because these are usually interrupted only at point of trace departure.
6. Resin canals in secondary xylem.
    0 = absent
    1 = present
7. Number of growth increments in secondary xylem of cone axis.
    0 = one
    1 = two.
8. Sclerenchyma in inner cortex.
    0 = absent
    1 = present
    The inner cortex may be entirely sclerotic or contain sclereid nests, fiber nests, or isolated sclerotic cells; all are coded as 1.
9. Sclerenchyma in outer cortex.
    0 = absent
    1 = present

Includes cones with an outer layer that is completely sclerotic as well as those with isolated sclerenchyma cells or nests (as above).

10. Cortical resin canals.
    - 0 = uniform in diameter
    - 1 = dilated markedly near points of branching
    - 2 = absent

    Cortical canals are usually uniform or somewhat dilated near branching points, and either condition is coded as 0. Those that are significantly dilated code as 1.
11. Trichomes on the cone axis, scale, or bract base.
    - 0 = absent
    - 1 = present

    This includes “glandular epidermis.”
12. Bract and scale traces.
    - 0 = separate at origin
    - 1 = united at origin

    This is viewed in tangential longitudinal sections through the inner cortex of the cone axis.
13. Scale trace.
    - 0 = clearly derived from two lateral strands
    - 1 = derived as single abaxially concave strand

    In some ovulate cones, such as those of *Pseudoaraucaria*, *Obirastrobus kokubunii*, and some *Pityostrobus*, two strands of vascular tissue are initially derived from either side of the gap, and they later fuse together to form an abaxially concave horseshoe-shaped trace. These are coded as 1 for character 13 and 0 for character 14.
14. Scale trace.
    - 0 = abaxially concave
    - 1 = becoming cylindrical after divergence

    This is examined in the inner cortex of the cone axis.
15. Resin canals to cone-scale complex arising from cortical canals.
    - 0 = as a single branch
    - 1 = two origins
    - 2 = three separate origins
    - 3 = four separate origins
    - 4 = more than four separate origins
    - 5 = no resin canals

    This number is the total number of branches, from both sides of the scale trace, that are formed as the cortical axial system branches to form the resin canals that will enter bracts and ovuliferous scales.
16. Bract/ovuliferous scale separation.
    - 0 = separates laterally first
    - 1 = separates medially first
    - 2 = separates all at once
    - 3 = do not separate

    If the middle region of the bract becomes free of the ovuliferous scale first, this is often described as having a medial pouch before separation.

17. Abaxial lobe of bract base (or cone-scale complex).
    0 = absent
    1 = present
18. Sclerenchyma in bract.
    0 = absent
    1 = present
19. Resin canals in bract (or accompanying bract trace).
    0 = absent
    1 = two
    2 = more than two
20. Bract trace.
    0 = entering bract
    1 = terminating before entering free part of bract
21. Vascular ray in bract trace.
    0 = absent
    1 = present
22. Resin canals to ovuliferous scale at scale base.
    0 = abaxial to vascular tissue
    1 = adaxial to vascular tissue
    2 = both adaxial and abaxial to vascular tissue
    3 = resin canals absent
    This character refers to the distribution of resin canals relative to the ovuliferous scale trace only and is determined in tangential longitudinal sections through the outer cortex of the cone axis. Resin canals to the bract are not included in the coding of this character.
23. Resin canals to ovuliferous scale at level of seed body (not under seed wing).
    0 = abaxial to vascular tissue
    1 = adaxial to vascular tissue
    2 = both adaxial and abaxial to vascular tissue
    3 = abaxial and adaxial and between vascular bundles
    4 = no resin canals
    5 = abaxial and between vascular bundles
24. Resin canals to ovuliferous scale distal to seed body (includes under wing and more distal sections).
    0 = abaxial to vascular tissue
    1 = adaxial to vascular tissue
    2 = both abaxial and adaxial to vascular tissue
    3 = between vascular bundles
    4 = no resin canals
    5 = abaxial and adaxial and between vascular bundles
    6 = abaxial and between vascular bundles
    7 = adaxial and between vascular bundles.
25. Sclerenchyma in ovuliferous scale.
    0 = both abaxial and adaxial to vascular tissue
    1 = abaxial to vascular tissue
    2 = adaxial to vascular tissue

3 = absent
4 = abaxial and adaxial and between vascular bundles
5 = abaxial and between vascular bundles
6 = adaxial and between vascular bundles.
This includes distribution of sclereids and fibers relative to the vascular tissue of the whole ovuliferous scale and not only at scale origins.

26. Interseminal ridge.
0 = absent between seeds
1 = extending less than half of seed diameter
2 = extending more than half of seed diameter
3 = extending between and overarching seeds.
In *Pseudoaraucaria* cones, the interseminal ridge overarches the seeds so that they appear almost embedded in the ovuliferous scale tissue; these are coded as 3. Interseminal ridges that are large but not overarching are coded as 2.

27. Scale at right angles to cone axis for length of seed body with sharply upturned distal portion.
0 = absent
1 = present
Some taxa such as *Pityostrobus pubescens* and *Pityostrobus californiensis* have ovuliferous scales that are at right angles to the cone axis but have a more gradually upturned distal portion. Only those with sharply upturned distal portions are coded as 1.

28. Method of cone seed release.
0 = cone spreading
1 = scale abscission from cone axis

29. Seed wings.
0 = absent
1 = sarcotestal wing(s)
2 = wing formed from ovuliferous scale tissue

30. Resin vesicles/cavities in integument (seed coat).
0 = absent
1 = present

31. Ridged sclerotesta.
0 = absent
1 = present
These ridges have usually been found on the lower surfaces of seeds (the part in contact with the ovuliferous scale) and are independent of resin cavities in the integument.

32. Enlarged parenchyma pad or cushion at chalazal end of seed.
0 = absent
1 = present
This structure is in addition to seed wing tissue, which is separate.

33. Number of ovules/ovuliferous scale.
0 = one
1 = two
2 = three or more

Although Takaso and Tomlinson (1991) report from one to 12 seeds per scale in *Sciadopitys*, theirs was a developmental study examining even the most apical scales of cones. The usual seed number reported by these authors is eight to nine per scale. This number is usually reported from lower cone scales. Therefore, we have keyed *Sciadopitys* as having greater than three seeds per scale.

APPENDIX B

Morphological Character Scorings for *Pinus*

based on representative taxa contained within the University of Montana Conifer Reference Collection, now housed at Oregon State University, Corvallis.

	1	11	21	31	
Abies firma	2--20112-0	2101?011?1	?1011?1100	1170012100	1
Cathaya argyrophylla	1-930113-0	0000000100	010??10110	2?50102000	1
Cedrus deodara	1-930013-1	0100101100	110100?10?	0?54102000	1
Larix decidua	1-920113-0	0100100101	0101100210	1150102000	1
Larix occidentalis	1-930113-0	1100000101	0101110100	1150102000	1
Picea sitchensis	1--30112-0	0000000100	0101010100	1274?02000	1
Pseudolarix amabilis	1-930113-1	0101001101	010?00100?	2356102100	1
Pinus albicaulis	1153021011	0000100100	110100?10?	0354102000	1
P. aristata	1153110301	0000110100	110100010?	0054102000	1
P. armandii	1153011(12)11	0000100100	110100?10?	0?54102000	1
†*P. arnoldii*	10520(12)1301	0000100100	1101001100	0554102000	1
P. attenuata	2032120301	0100100100	110100?10?	0?54102000	1
P. ayacahuite	1153011211	0?001??1?0	110100?10?	0?54102000	1
P. balfouriana	1153110301	0000100100	110100?10?	0?54102000	1
P. banksiana	2022121301	0100100100	110100110?	0?54102000	1
P. bhutanica	115(23)011211	0?001??1?0	110100?10?	0?54102000	1
P. brutia	2023111301	0?001??1?0	110100?10?	0?54102000	1
P. bungeana	1133110301	0000100110	110100?10?	0?54102000	1
P. canariensis	2033111201	0?001??1?0	110100?10?	0?54102000	1
P. caribaea	20(2345)1110301	0?001??1?0	110100?10?	0?54102000	1
P. cembra	115(23)021(01)11	0?001??1?0	110100?10?	0?54102000	1
P. cembroides	11(2345)3011001	0?001??1?0	110100?10?	0?54102000	1
P. chiapensis	1153011211	0?001??1?0	110100?10?	0?54102000	1
P. clausa	2022110301	0?001??1?0	110100?10?	0?54102000	1
P. contorta	2022120301	0100100100	110100110?	0?54102000	1
P. cooperi	20(345)2110301	0?001??1?0	110100?10?	0?54102000	1
P. coulteri	203(12)1(12)0301	0000100100	110100?10?	0?54102000	1
P. cubensis	202(01)110301	0?001??1?0	110100?10?	0?54102000	1
P. culminicola	11(456)3011001	0?001??1?0	110100?10?	0?54102000	1
P. densata	20(23)(23)11(01)301	0?001??1?0	110100?10?	0?54102000	1
P. densiflora	2023111301	0100100100	110100110?	0054102000	1
P. devoniana	20(456)2110301	0?001??1?0	110100?10?	0?54102000	1
P. discolor	11(2345)3011001	0?001??1?0	110100?10?	0?54102000	1
P. douglasiana	20(456)2111301	0100101100	110100?10?	0?54102000	1
P. durangensis	20(45678)2110301	0?001??1?0	110100?10?	0?54102000	1
P. echinata	20(23)(12)11(01)301	0000100100	110100?10?	0054102000	1
P. edulis	11(123)3011001	0100100110	1101001100	0354102000	1
P. elliottii	20(23)1110301	0000100100	110100?10?	0054102000	1
P. engelmannii	20(2345)2110301	0000100100	110100?10?	0354102000	1
P. fenzeliana	1153011211	0?001??1?0	110100?10?	0?54102000	1
P. flexilis	1153011(12)11	0000110110	1101001100	0054102000	1
P. gerardiana	1133110301	0?001??1?0	110100?10?	0?54102000	1
P. greggii	203(12)121301	0?001??1?0	110100?10?	0?54102000	1
P. halepensis	2023111301	0100101110	110100?10?	0354102000	1

P. hartwegii	20(3456)2110301	0?001??1?0	110100?10?	0?54102000	1
P. heldreichii	2022111301	0?001??1?0	110100?10?	0?54102000	1
P. herrerae	203(12)110301	0?001??1?0	110100?10?	0?54102000	1
P. hwangshanensis	2022111301	0?001??1?0	110100?10?	0?54102000	1
P. jeffreyi	2032110301	0?001??1?0	110100?10?	0?54102000	1
P. johannis	11(2345)3011001	0?001??1?0	110100?10?	0?54102000	1
P. kesiya	20(23)(23)111301	0100110100	110100?10?	0354102000	1
P. koraiensis	1152021011	0?001??1?0	110100?10?	0?54102000	1
P. krempfii	1123111301	0?001??1?0	110100?10?	0?54102000	1
P. lambertiana	115(23)011211	0000100100	110100?10?	0354102000	1
P. lawsonii	20(345)(12)111301	0?001??1?0	110100?10?	0?54102000	1
P. leiophylla	21(2345)(23)110301	0?001??1?0	110100?10?	0?54102000	1
P. longaeva	1153110301	0?001??1?0	110100?10?	0?54102000	1
P. luchuensis	202(23)111301	0?001??1?0	110100?10?	0?54102000	1
P. lumholtzii	213(12)111301	0?001??1?0	110100?10?	0?54102000	1
P. massoniana	2023111301	0?001??1?0	110100?10?	0?54102000	1
P. maximartinezii	1153111001	0?001??1?0	110100?10?	0?54102000	1
P. maximinoi	20(456)2111301	0?001??1?0	110100?10?	0?54102000	1
P. merkusii	202(12)111301	0?001??1?0	110100?10?	0?54102000	1
P. monophylla	1113011001	0000100100	110100?10?	0?54102000	1
P. montezumae	20(3456)211(01)301	0?001??1?0	110100?10?	0?54102000	1
P. monticola	1153011211	0000100110	110100?10?	0354102000	1
P. morrisonicola	1153011211	0?001??1?0	110100?10?	0?54102000	1
P. mugo	2023110301	0?001??1?0	110100?10?	0?54102000	1
P. muricata	2022120301	0100100100	110100?10?	0054102000	1
P. nelsonii	1033111001	0?001??1?0	110100?10?	0?54102000	1
P. nigra	2022110301	0000100100	1101001101	0054102000	1
P. occidentalis	20(234)(01)110301	0?001??1?0	110100?10?	0?54102000	1
P. oocarpa	20(345)(01)11(01)301	0?001??1?0	110100?10?	0?54102000	1
P. palustris	20(35)1110301	0000100100	110100?10?	0054102000	1
P. parviflora	1153011211	0100110100	110100?10?	0354102000	1
P. patula	20(345)(12)121301	0?001??1?0	110100?10?	0?54102000	1
P. peuce	1153011211	0?001??1?0	110100?10?	0?54102000	1
P. pinaster	2022111301	0?001??1?0	110100?10?	0?54102000	1
P. pinceana	1133111001	0?001??1?0	110100?10?	0?54102000	1
P. pinea	2023121301	0?001??1?0	110100?10?	0?54102000	1
P. ponderosa	20(2345)2110301	0100100100	1101001100	0354102000	1
P. pringlei	20(345)(01)111301	0?001??1?0	110100?10?	0?54102000	1
P. pseudostrobus	20(456)2111301	0100100100	110100?10?	0354102000	1
P. pumila	1153021011	0?001??1?0	110100?10?	0?54102000	1
P. pungens	20(23)2120301	0100101100	110100?10?	0054102000	1
P. quadrifolia	11(345)3011001	0?001??1?0	110100?10?	0?54102000	1
P. radiata	20(23)(12)120301	0?001??1?0	110100?10?	0?54102000	1
P. remota	11(23)3011001	0?001??1?0	110100?10?	0?54102000	1
P. resinosa	2023111301	0100100100	110100110?	0054102000	1
P. rigida	203(12)110301	0?001??1?0	110100?10?	0?54102000	1
P. roxburghii	2033111201	0?001??1?0	110100?10?	0?54102000	1
P. rzedowskii	11(345)3110301	0?001??1?0	110100?10?	0?54102000	1
P. sabineana	203(12)110301	0?001??1?0	110100?10?	0?54102000	1
P. serotina	20(34)1110301	0000100110	110100110?	0354102000	1
P. sibirica	1153021011	0?001??1?0	110100?10?	0?54102000	1

P. squamata	11(45)3111301	0?001??1?0	110100?10?	0?54102000	1
P. strobus	1153011211	0000100110	110100010?	0354102000	1
P. sylvestris	202311(01)301	0100100100	110100110?	0054102000	1
P. tabuliformis	20(23)(23)111301	0?001??1?0	110100?10?	0?54102000	1
P. taeda	203(12)110301	0000101100	110100110?	0054102000	1
P. taiwanensis	2023111301	0?001??1?0	110100?10?	0?54102000	1
P. teocote	20(2345)(12)11(01)301	0?001??1?0	110100?10?	0?54102000	1
P. thunbergii	2022110301	0100100110	110100110?	0054102000	1
P. torreyana	2052120301	0?001??1?0	110100?10?	0?54102000	1
P. tropicalis	2020111301	0?001??1?0	110100?10?	0?54102000	1
P. uncinata	2023110301	0?001??1?0	110100?10?	0?54102000	1
P. virginiana	2022110301	0000100110	110100110?	0054102000	1
P. wallichiana	115(23)011211	0?001??1?0	110100?10?	0?54102000	1
P. yunnanensis	20(23)3110301	0?001??1?0	110100?10?	0?54102000	1

APPENDIX C

Concepts of Characters Scored for *Pinus*

adapted from characters defined by Smith and Stockey (2001, 2002) and by Gernandt et al. (2005).

1. Fibrovascular bundles per leaf.
    - 1 = one
    - 2 = two
2. Fascicle sheath.
    - 0 = persistent
    - 1 = deciduous
3. Leaves per fascicle.
    - 1 = one
    - 2 = two
    - 3 = three
    - 4 = four
    - 5 = five
    - 6 = six
    - 7 = seven
    - 8 = eight
    - 9 = more than eight
4. Resin canal position in leaf.
    - 0 = septal
    - 1 = internal
    - 2 = medial
    - 3 = external
5. Cone scale.
    - 0 = thin
    - 1 = thick
6. Mature cones.
    - 0 = disaggregate at maturity
    - 1 = open
    - 2 = closed
7. Mucro (umbo prickle).
    - 0 = variably present
    - 1 = absent
8. Seed wing.
    - 0 = absent
    - 1 = rudimentary
    - 2 = adnate
    - 3 = articulate
9. Umbo position.
    - 0 = dorsal
    - 1 = terminal
10. Ovuliferous scale apex.
    - 0 = thinning distally

1 = thickening distally (or has umbo)

11. Bract length.
    0 = bract shorter than ovuliferous scale
    1 = bract longer than ovuliferous scale
    2 = bract and ovuliferous scale equal in length

12. Sclerenchyma in pith (includes only sclereids and fibers; thick-walled parenchyma excluded).
    0 = absent
    1 = present

13. Resin canals in pith (excludes resin canals in primary xylem).
    0 = absent
    1 = present

14. Secondary xylem of cone axis.
    0 = forming a continuous cylinder (or little dissected)
    1 = in separate strands

15. Resin canals in secondary xylem.
    0 = absent
    1 = present

16. Number of growth increments in secondary xylem of cone axis.
    0 = one
    1 = two

17. Sclerenchyma in inner cortex.
    0 = absent
    1 = present (includes sclereid/fiber nests and isolated cells)

18. Sclerenchyma in outer cortex.
    0 = absent
    1 = present (includes sclereid/fiber nests and isolated cells)

19. Cortical resin canals.
    0 = uniform in diameter (may be slightly dilated near branching points)
    1 = dilated markedly near points of branching
    2 = absent

20. Trichomes on cone axis, scale, or bract base.
    0 = absent
    1 = present (includes “glandular epidermis”)

21. Bract and scale traces.
    0 = separate at origin
    1 = united at origin

22. Scale trace.
    0 = clearly derived from two lateral strands
    1 = derived as a single abaxially concave strand

23. Scale trace (examined in the inner cortex of the cone axis).
    0 = abaxially concave
    1 = becoming cylindrical after divergence

24. Resin canals to cone-scale complex arising from cortical canals (total number of branches from both sides of the scale trace).
    0 = as a single branch

  1 = two origins
  2 = three separate origins
  3 = four separate origins
  4 = more than four separate origins
  5 = no resin canals

25. Bract/ovuliferous scale separation.
  0 = separates laterally first
  1 = separates medially first (often described as having a "medial pouch")
  2 = separates all at once
  3 = does not separate

26. Abaxial lobe of bract base (or cone-scale complex).
  0 = absent
  1 = present

27. Sclerenchyma in bract.
  0 = absent
  1 = present

28. Resin canals in bract (or accompanying bract trace).
  0 = absent
  1 = two
  2 = more than two

29. Bract trace.
  0 = entering bract
  1 = terminating before entering free part of bract

30. Vascular ray in bract trace.
  0 = absent
  1 = present

31. Resin canals to ovuliferous scale at scale base (does not include resin canals of the bract).
  0 = abaxial to vascular tissue
  1 = adaxial to vascular tissue
  2 = both abaxial and adaxial
  3 = resin canals absent

32. Resin canals of ovuliferous scale at level of seed body (not under wing).
  0 = abaxial to vascular tissue
  1 = adaxial to vascular tissue
  2 = both abaxial and adaxial to vascular tissue
  3 = abaxial, adaxial, and between vascular bundles
  4 = no resin canals
  5 = abaxial and between vascular bundles

33. Resin canals of ovuliferous scale distal to seed body (under seed wing, and more distal sections).
  0 = abaxial to vascular tissue
  1 = adaxial to vascular tissue
  2 = both abaxial and adaxial to vascular tissue
  3 = between vascular bundles
  4 = no resin canals

5 = abaxial, adaxial, and between vascular bundles
6 = abaxial and between vascular bundles
7 = adaxial and between vascular bundles

34. Sclerenchyma in ovuliferous scale (includes sclereids and fibers relative to the vascular tissue of the entire scale, not only at scale origin).
0 = both abaxial and adaxial to vascular tissue
1 = abaxial to vascular tissue
2 = adaxial to vascular tissue
3 = absent
4 = abaxial, adaxial, and between vascular bundles
5 = abaxial and between vascular bundles
6 = adaxial and between vascular bundles

35. Interseminal ridge.
0 = absent between seeds
1 = extending less than half of seed diameter
2 = extending more than half of seed diameter
3 = extending between and overarching seeds (i.e., like *Pseudoaraucaria*)

36. Scale at right angles to cone axis for length of seed body with sharply upturned distal portion.
0 = absent
1 = present (does not include cones with gradually upturned distal portions)

37. Seed wings.
0 = absent
1 = sarcotestal wing(s)
2 = wing formed from ovuliferous scale tissue

38. Resin vesicles/cavities in integument (seed coat).
0 = absent
1 = present

39. Ridged sclerotesta (usually found on lower surface of seeds and is independent of resin cavities in integument).
0 = absent
1 = present

40. Enlarged parenchyma pad or cushion at chalazal end of seed (structure in addition to seed wing tissue).
0 = absent
1 = present

41. Number of ovules per ovuliferous scale.
0 = one
1 = two
2 = three or more

APPENDIX D

Morphological Character Scorings for *Picea*

based on representative taxa contained within the University of Montana Conifer Reference Collection, now housed at Oregon State University, Corvallis.

	1	11	21	31
Abies firma	01101?011?	1?1011?110	0117001121	001
Apple Bay cone	0010000110	0010101010	0125010020	001
Cathaya argyrophylla	0000000010	0010??1011	02?5010020	001
Cedrus deodara	0000101110	1010300101	02004?1121	001
Keteleeria davidiana	0000000011	1011101111	0222011021	001
Larix decidua	0010010010	1010110021	0115010020	001
Picea abies	0000000010	0010101010	0125410020	001
Picea alcoquiana	00?0??0?10	?0101010?0	?12?4?0020	001
Picea asperata	0000010110	0010101010	1125410020	001
Picea brachytyla	00?0??0?10	?0101010?0	?12?4?0020	001
Picea breweriana	0010000110	0010101010	1127410020	001
Picea chihuahuana	0010010010	0010101010	1125410020	001
Picea crassifolia	00?0??0?10	?0101010?0	?12?4?0020	001
Picea engelmanii	0000000010	0010101010	0125420020	001
Picea glauca	0000000010	0010101010	1127410020	001
Picea glehnii	00?0??0?10	?0101010?0	?12?4?0020	001
Picea jezoensis	00?0??0?10	?0101010?0	?12?4?0020	001
Picea koraiensis	00?0??0?10	?0101010?0	?12?4?0020	001
Picea koyamai	00?0??0?10	?0101010?0	?12?4?0020	001
Picea likiangensis	0000000110	0010101010	11254?0020	001
Picea maximowiczii	00?0??0?10	?0101010?0	?12?4?0020	001
Picea mariana	0000010110	0010101010	1125410020	001
Picea mexicana	0010010010	0010101010	0127410020	001
Picea meyeri	00?0??0?10	?0101010?0	?12?4?0020	001
Picea obovata	00?0??0?10	?0101010?0	?12?4?0020	001
Picea omorika	0010010110	0010101010	1125420020	001
Picea orientalis	00?0??0?10	?0101010?0	?12?4?0020	001
Picea pungens	0000010010	0010101020	0125410020	001
Picea purpurea	00?0??0?10	?0101010?0	?12?4?0020	001
Picea rubens	0000010010	1010101010	1125420020	001
Picea schrenkiana	00?0??0?10	?0101010?0	?12?4?0020	001
Picea sitchensis	0000000010	0010101010	0125410020	001
Picea smithiana	00?0??0?10	?0101010?0	?12?4?0020	001
Picea torano	0010000010	0010101010	0125420020	001
Picea wilsonii	0010000010	0010101010	1127410020	001
Pinus arnoldii	1000010010	0110100110	0055410020	001
Pinus thunbergii	1010010011	0110100110	?005410020	001
Pityostrobus yixianensis	0010010010	0010100010	0224(01) 102	001
Pseudolarix amabilis	1010100110	1010?00100	?235610121	001
Pseudotsuga menziesii	0110010010	0010100010	0115010020	001

Sciadopitys verticillata	0200100000	1000100020	0117200010	002
Tsuga mertensiana	0010000100	0010310110	0115010021	001