

# Cenozoic climate change shaped the evolutionary ecophysiology of the Cupressaceae conifers

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The Cupressaceae clade has the broadest diversity in habitat and morphology of any conifer family. This clade is characterized by highly divergent physiological strategies, with deciduous swamp-adapted genera-like *Taxodium* at one extreme, and evergreen desert genera-like *Cupressus* at the other. The size disparity within the Cupressaceae is equally impressive, with members ranging from 5-m-tall juniper shrubs to 100-m-tall redwood trees. Phylogenetic studies demonstrate that despite this variation, these taxa all share a single common ancestor; by extension, they also share a common ancestral habitat. Here, we use a common-garden approach to compare xylem and leaf-level physiology in this family. We then apply comparative phylogenetic methods to infer how Cenozoic climatic change shaped the morphological and physiological differences between modern-day members of the Cupressaceae. Our data show that drought-resistant crown clades (the Cupressoid and Callitroid clades) most likely evolved from drought-intolerant Mesozoic ancestors, and that this pattern is consistent with proposed shifts in post-Eocene paleoclimates. We also provide evidence that within the Cupressaceae, the evolution of drought-resistant xylem is coupled to increased carbon investment in xylem tissue, reduced xylem transport efficiency, and at the leaf level, reduced photosynthetic capacity. Phylogenetically based analyses suggest that the ancestors of the Cupressaceae were dependent upon moist habitats, and that drought-resistant physiology developed along with increasing habitat aridity from the Oligocene onward. We conclude that the modern biogeography of the Cupressaceae conifers was shaped in large part by their capacity to adapt to drought.

cavitation resistance | photosynthesis | plant water transport | xylem structure

Global climate underwent dramatic changes ~33.7 Mya when a warm and almost universally equable world rapidly became much colder and drier (1–3). This shift is attributed in part to a dramatic decrease in atmospheric CO<sub>2</sub> that caused global mean temperatures to drop 3–4 °C in only ~300,000 years (4, 5). As might be expected, the subsequent distribution of plants and animals changed dramatically (5–7), leading to the development of entirely new biomes, such as grasslands (8) and cactus-dominated deserts (9). Other groups of plants became restricted to habitats that reflected the previous tropical conditions of their ranges (10, 11). However, it is rare to find groups that reflect adaptation to both the Cretaceous-Early Paleogene greenhouse and the cooler, drier post-Eocene world. The Cupressaceae is an exception, known for both moisture-loving paleoendemics, such as the Dawn Redwood (*Metasequoia glyptostroboides*), and exceptionally drought-tolerant desert trees, such as *Juniperus californica* and the South African *Widdringtonia cedarbergensis*. Here, we offer an analysis that was aimed at unraveling the physiological underpinnings of these two dramatically different lineages, and show that they were the likely result of Cenozoic climate change.

The Cupressaceae family is remarkable for the diversity of morphology, phenology, and habitat preference exhibited by its members, and it is the only conifer family with a circumglobal distribution. The phylogenetic structure of the Cupressaceae suggests that mesic-

hydric habitats are likely ancestral within this group. Indeed, early-diverging Cupressaceae—such as *Cunninghamia*, *Taiwania*, *Metasequoia*, and *Sequoia*—occupy mesic habitats, and close relatives—such as *Glyptostrobus* and *Taxodium*—can tolerate water-logged soils. In contrast, many members of the two crown clades, the Cupressoids and Callitroids (Fig. 1), are adapted to arid climates across the northern and southern hemispheres, respectively, and also comprise most of the species within the Cupressaceae. Shorter and often shrub-like, these conifers exhibit small, imbricate leaves that minimize water loss (Fig. 1) (12, 13).

Temperature and water availability constrain the distribution of most woody plants, primarily because the xylem must balance efficient water delivery against safety from water stress. For example, prolonged dehydration can cause water potential to become negative enough to cause air to leak into xylem conduits (cavitation). In conifers, cavitation occurs when air is sucked into a water-filled conduit through a broken seal in a pit membrane (14, 15). This air expands (embolism) and replaces the water in the conduit. Air-filled conduits can significantly impair xylem conductivity, so selection favors vascular traits and leaf phenologies that optimize water transport with respect to expected water deficits in any given environment (16–18).

Given the well-resolved phylogeny (12, 13, 19, 20), deep fossil record (21–24), global distribution, and morphological diversity of the Cupressaceae, this family presents a rare opportunity to investigate how comparative physiology evolved with respect to the Cenozoic paleoclimate in a woody plant lineage. Here, we use a “common garden” approach, standardizing the plants’ growing environment by sampling the hydraulic and gas-exchange response of individuals growing in San Francisco Bay Area arboreta (Table 1 and Table S1). By analyzing standardized physiological data within a phylogenetic framework, this study fills an important gap in our mechanistic understanding of how selection shaped the biogeography of the Cupressaceae since the Mid-Jurassic, and demonstrates that retention of ancestral character traits may explain the present limited distribution of the early-diverging Cupressaceae.

## Results

We first characterized the climate affinities of all extant Cupressaceae according to detailed descriptions of species habitat (12). Eighty-one percent of *Cupressus* and 69% of *Juniperus*, the two most speciose genera, are found in climates with arid conditions during the growing season (Fig. 1, subclade I). Similarly, 57% of species within the crown Callitroids (Fig. 1, subclade V) have xeric associations. Of the remaining Callitroid and

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**Table 1. Study species, figure abbreviations, and species' natural history**

Species	Abbr.	Phenology, native habitat
<i>Athrotaxis laxifolia</i>	AL	E, Montane forests, Tasmania
<i>Austrocedrus chilensis</i>	AC	E, The Andes, Chile and Argentina
<i>Calocedrus decurrens</i>	CD	E, Montane forests, United States–Mexican Pacific Coast
<i>Chamaecyparis lawsoniana</i>	CL	E, Mixed forests, Oregon to northern California
<i>Callitris macleayana</i>	CM	E, Mesic-dry forests, eastern Australia
<i>Calocedrus decurrens</i>	CD	E, Mixed forests, Oregon to northern Mexico
<i>Cryptomeria japonica</i>	CJ	E, Mixed evergreen forests, Japan
<i>Cunninghamia lanceolata</i>	CL	E, Mixed broad-leaved forests of southeast Asia
<i>Cupressus forbesii</i>	CF	E, Chaparral, southern California, northern Mexico
<i>Fitzroya cupressoides</i>	FC	E, Evergreen rainforest, Chile
<i>Glyptostrobus pensilis</i>	GP	D, Riparian, southern China
<i>Juniperus californica</i>	JC	E, Desert, southern California to northern Mexico
<i>Libocedrus plumosa</i>	LP	E, Mixed conifer rainforests, New Zealand
<i>Metasequoia glyptostroboides</i>	MG	D, Mesic mixed forests, central China
<i>Sequoiadendron giganteum</i>	SG	E, Sierra Nevada, California
<i>Sequoia sempervirens</i>	SS	E, northern coastal California
<i>Taxodium distichum</i>	TD	D, Riparian regions in southeastern United States
<i>Taxodium mucronatum</i>	TM	D, Southern Texas, Mexico, Central America
<i>Taiwania cryptomeroides</i>	TC	E, Cool temperate forests, Asia
<i>Thuja plicata</i>	TP	E, Mixed coniferous forests, United States Pacific northwest
<i>Thujopsis dolabrata</i>	TO	E, Coastal and montane Japan
<i>Widdringtonia cedarbergensis</i>	WC	E, Fynbos, South Africa
<i>Sciadopitys verticillata</i> (Sciadopityaceae)	SC	E, Temperate moist forests, Japan
<i>Taxus baccata</i> (Taxaceae)	TB	E, Broadly distributed across Europe

D, deciduous; E, evergreen.

grown under common conditions, species'  $P_{50}$  values reflected the water availability of their native habitats. For example, the riparian *Glyptostrobus pensilis* had a  $P_{50}$  of  $-2.8 \pm 0.62$  MPa (mean  $\pm$  SD), but *W. cedarbergensis*, a xeric species, showed extreme cavitation resistance of  $-11.27 \pm 3.52$  MPa (Fig. 2). A strong genetic influence was also observed in the relationship between  $P_{50}$  and the minimum growing-season precipitation of these species' native ranges ( $r^2 = 0.34$ ,  $P = 0.0025$ ) (Fig. S1). We would expect a stronger correlation between  $P_{50}$  and climate if the hydraulic measurements were performed on plants collected from their native habitats.

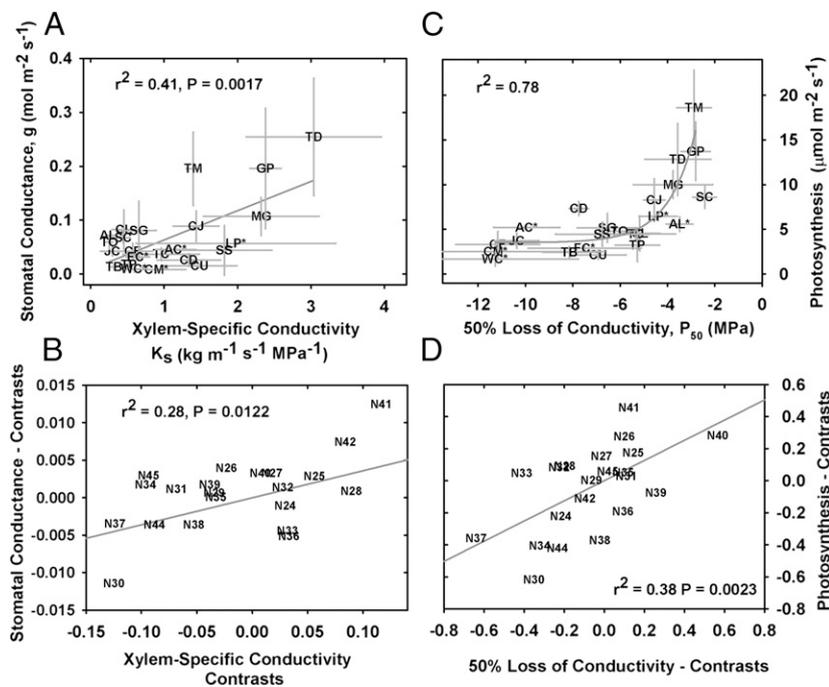
In north-temperate conifers, a key cost of xylem adaptation to drought stress is the need for increased wood density, which represents a significant carbon investment per unit height gained (Fig. 2A) (25, 26). Dense wood arises from the need for increased conduit implosion resistance  $[(t/D)_h]^2$  to prevent conduit collapse under very negative xylem tensions. Increased  $(t/D)_h^2$  is achieved through both thicker conduit walls ( $t$ ) and reduction in hydraulic conduit diameter ( $D_h$ ), although  $D_h$  typically plays a larger role (Fig. 2B and C) (26). As a result, cavitation-resistant species typically show a greater number of smaller, thick-walled conduits per unit xylem area, resulting in denser, more costly wood (Fig. S2).

Given that drought-resistance necessitates the development of smaller conduits, it follows that xylem-specific conductivity ( $K_s$ ) declines with  $P_{50}$  (Fig. 2C and D), representing a significant transport cost associated with adaptation to arid habitats. Fig. 2 shows the correlations between  $P_{50}$ ,  $D_h$ , and  $K_s$  for the combined northern and southern hemisphere Cupressaceae, but the strength of these relationships increases considerably if only the north temperate taxa are considered (for example,  $P_{50}$  vs.  $D_h$ :  $r^2 = 0.51$ ,  $P = 0.017$ ;  $P_{50}$  vs.  $K_s$ :  $r^2 = 0.43$ ,  $P = 0.019$ ). This distinction was observed for many of the anatomical and physiological data presented here, as well as in other studies concerned with conifer xylem structure and function, but the reasons for this remain unclear (26, 27).

Leaf stomatal conductance ( $g$ ) is closely coupled with  $K_s$  in the Cupressaceae (Fig. 3A) because xylem efficiency places a limit on

water availability to the leaf, thus constraining maximum leaf water loss (Fig. S3) (28). Leaf and stem capacitance can modulate the stomatal response in situ (29, 30), but our measurements support the hydraulic coordination of xylem and leaf-level water transport, with cavitation-resistant species exhibiting lower rates of  $g$  because of narrow, implosion-resistant conduits. Analysis of phylogenetically independent contrasts (PICs) shows evolutionary coordination of  $g$  and  $K_s$  ( $r^2 = 0.28$ ,  $P = 0.0122$ ) (Fig. 3B). We observed a dramatic reduction in  $\text{CO}_2$  assimilation rates in more cavitation-resistant Cupressaceae (Fig. 3C) ( $r^2 = 0.78$ ) because of the coupling between  $g$  and photosynthesis ( $r^2 = 0.79$ ,  $P < 0.0001$ ) (Fig. S4). PIC analyses suggest that  $\sim 40\%$  of variation can be explained by coevolution between  $P_{50}$  and photosynthesis ( $r^2 = 0.38$ ,  $P = 0.0023$ ) (Fig. 3D).

The rich Cupressaceae macrofossil record offers the opportunity to examine these results in the context of deep-time approximations of clade divergence (Table S2), allowing us to present a hypothesis for the evolution of physiological traits against a backdrop of Cenozoic climate. Ancestral reconstruction of  $P_{50}$  on the phylogenetic topology recovered here indicates that adaptation to mesic and riparian environments with high water availability is ancestral within the Cupressaceae. This reconstruction also suggests at least two separate evolutions of adaptations to arid environments, one within the Cupressoids (Fig. 1, subclades I–IV) and one within the Callitroids (Fig. 1, subclades V and VI). The earliest-diverging members within each of these clades have more mesic habitat preferences than the crown groups, supporting this conclusion. The 95% highest posterior density interval for the divergence between *Juniperus* and *Cupressus* (node 35) is 38.68–31.98 Mya, indicating that diversification within these two groups began after that time. Diversification of the arid-adapted Callitroids begins at node 38, which has a 95% highest posterior-density interval of 52.58–33.92 Mya. Both time intervals include the Eocene-Oligocene boundary ( $\sim 33.7$  Mya), suggesting that the relatively high species diversity observed in each clade developed after this time.



**Fig. 3.** The relationship between leaf- and xylem-level traits in the Cupressaceae. Leaf stomatal conductance is positively correlated with stem water supply,  $K_s$  (A; means  $\pm$  SD). Reduced photosynthesis rates arise from the inverse relationship between  $P_{50}$  and  $K_s$ , and by extension,  $g$  (C; see text for details). Asterisks indicate Cupressaceae taxa endemic to habitats in the southern hemisphere. PIC analyses (B and D) demonstrate the correlated evolution of leaf and xylem traits. Each independent contrast corresponds to trait divergence at a specific node; node numbers shown here (labeled "N") correspond to nodes, or phylogenetic divergence events, in Fig. 1.

## Discussion

The macrofossil record indicates that the morphology of the Cupressaceae has remained mostly static for the past 70 million years (21–24). Both the coupling of form and function, and the ancestral reconstructions presented here, support the view that the physiology of early-diverging clades is to a large degree conserved. In contrast, the Cupressoid and Callitroid clades show adaptive shifts in xylem and leaf-level physiology that are consistent with expansion into increasingly arid, late Cenozoic habitats.

Reconstructions of Cretaceous climates concur that warm temperatures, elevated levels of atmospheric  $CO_2$ , and minimal glaciation characterized this period in the Earth's history, as well as much of the Paleocene and Eocene climates (1–4, 31, 32). The Cupressaceae have their origins in the mid-Mesozoic, with taxa such as *Elatides williamsonii* appearing in the Jurassic and *Sphenolepis kurriana* appearing in the Cretaceous. Consistent with this, ancestral reconstructions suggest that early members of the clade likely exhibited increased vulnerability to drought-induced cavitation, and affinities for habitats with higher precipitation (Figs. 1–3, Fig. S5, and *SI Text*).

Similarly, close relatives of the Taxodioids and Sequoioids were most abundant during the Cretaceous and Paleocene, with populations occupying the once humid, warm climates of the high northern latitudes (21–24, 33). Most early-diverging Cupressaceae, such as *M. glyptostroboides* and *Sequoia sempervirens*, have experienced dramatic range contractions since the Paleocene, and their present habitats are thought to be relictual (12, 21–23). These range reductions have been attributed to the cooling and drying of the global climate that followed the early Eocene climatic optimum (4, 5, 23). Consistent with this finding, paleosol evidence documents a transition from forest to woodland to grassland during the Eocene and Oligocene in the Eastern Rockies (7, 34), as well as Old and New World vegetation shifts during the Miocene (6, 8, 9).

With its rich fossil record, the once-abundant *Metasequoia* is a useful model for understanding the climate-related biogeographical patterns of early-diverging Cupressaceae. In its narrow present-day range in central China, *Metasequoia* occupies a moderate climate with an average 950–2,300-mm annual precipitation

(21, 23). This finding is consistent with this species' vulnerability to cavitation ( $P_{50} = -3.76 \pm 1.67$  MPa). Planted *Metasequoia* trees grow well in Alaska and parts of Canada, which suggests that this species is tolerant of freezing temperatures, although cool temperatures may affect seed germination (21, 23). We suggest, therefore, that the drought sensitivity of *Metasequoia*, like that of other basal Cupressaceae, may explain in part the dramatic diminution of its population range since the Late Cretaceous (21–23).

In contrast, the geographic expansion and diversification of the arid-adapted Cupressoids and Callitroids occurred over the same time period (12, 13, 22, 35). We hypothesize that the physiological shift toward greater drought tolerance, specifically cavitation resistance, observed in these lineages (15, 36, 37) allowed them to radiate into increasingly dry environments during the Mid- to Late-Cenozoic. Although this trend was observed in both Northern and Southern Hemisphere Cupressaceae, the expansion and speciation of *Juniperus* and *Cupressus* across North America, Europe, and North Africa is particularly dramatic, with over 70% of subclade I species exhibiting xeric affinities (Fig. 1) (12). *Juniperus* is the most widely distributed member of the Cupressaceae in North America, and its rapid diversification during the Miocene can be attributed in some part to its inherent drought resistance (19, 36). In contrast, early-diverging mesic or hydric taxa within each of the Cupressoid and Callitroid clades have also been restricted to climatically favorable refugia: examples include *Chamaecyparis*, *Fitzroya*, and *Thuja*, all of which once occupied much broader ranges (12, 22).

The drought tolerance of the Cupressoid and Callitroid lineages came at the expense of reduced hydraulic efficiency and lower rates of photosynthesis (Figs. 1–3), and these costs arise from anatomical, tissue, and whole-plant adjustments. At the level of the interconduit pit membrane, increasing cavitation resistance is coupled with smaller pit apertures, the cost of which is up to a sixfold reduction in pit conductivity (15). Within the xylem tissue, a more negative  $P_{50}$  exerts significant carbon and hydraulic penalties, both of which contribute to reduced photosynthetic capacity. Finally, imbricate leaves are favored in drier habitats over bilaterally flattened foliage, a trend that is consistent with adjustments in sapwood to leaf-area ratios that prevent xylem

tensions from becoming low enough to cause cavitation (38). Taken together, these data indicate that the cost of survival in arid environments is slow growth, a trend reflected by a reduction in stature from the tall early-diverging Cupressaceae to the often shrubby, arid-adapted Callitroids and Cupressoids (Fig. 1).

It is less clear how other conifer lineages responded to Cenozoic climate change, but some were apparently excluded from the newly developed arid habitats. In the southern hemisphere for example, members of the Podocarpaceae were subject to selective pressures arising from increasing aridity as well as greater ecological competition from angiosperms (10, 11, 39). Several of the drought-intolerant Podocarp lineages disappeared during the mid-to-late Cenozoic, and others found refugia in mesic tropical forests by evolving flattened leaves that allowed them to compete with angiosperms for light (10, 11, 39). Although the Podocarpaceae retreated, we speculate that Callitroid lineages may have succeeded in colonizing available arid habitats. In the northern hemisphere, the Oligocene saw a dramatic reduction in the relative abundance of *Metasequoia* (and perhaps other mesic Cupressaceae) as the Pinaceae became increasingly dominant (21, 23). Indeed, the Pinaceae is a family whose members exhibit fast growth and high reproductive output combined with a tolerance to freezing temperatures, drought, and fire (40, 41). There is evidence that post-Eocene climate deterioration shaped the biogeography of all conifer families, not just the Cupressaceae, and that conifer persistence depended on proximity to refugia, shade tolerance, and in the case of the Pinaceae, aggressive life-history strategies. The extent to which competition, nutrient acquisition, dispersal, and geologic events have shaped the relative abundances of the Earth's conifers will require further study.

The physiological data presented here accord well with other data suggesting that the Cenozoic was an era of large-scale habitat sorting that saw angiosperm success in climatically favorable regions, while conifers competed more effectively in marginal habitats (42–44). The tremendous success of the angiosperms can be partially attributed to their developmentally flexible and hydraulically efficient xylem, which supports a high degree of morphological variation and rapid growth, something the conifers never have been able to match (42–44). Because of their low tolerance to drought and limited ability to compete with angiosperms, we suspect that the continued presence of the early-diverging Cupressaceae may be attributed to their longevity and to once-abundant distributions that increased the probability of their occurrence in refugial habitats.

In conclusion, our data indicate that drought-adapted lineages within the Cupressaceae have undergone profound vascular structure-function adjustments consistent with their presumed radiation into the increasingly dry habitats of post-Eocene climates. This radiation was coupled with greater cavitation resistance but at the cost of increased investment in xylem and reduced rates of photosynthesis. In contrast, ancestral reconstruction suggests that the narrowly distributed Taxodioids and Sequoioids have retained their ancestral leaf and xylem features, and that the physiology of these plants has changed little. Consequently, we propose that the dramatic range reduction of the mesic Cupressaceae in response to Cenozoic climate change can be explained in part by physiological limitations, such as low resistance to drought-induced cavitation.

It is rare that a single group of related organisms offers an opportunity to examine physiology on both sides of a dramatic shift in global climate. The Cupressaceae, with its combination of morphologically static mesic paleoendemics and its radiation into newer arid environments, provides a chance to look at evolution in progress. Future generations may witness the limits of adaptive flexibility in this ancient plant lineage as greenhouse gas-induced climate change brings warmer temperatures and altered hydrologic regimes to the Anthropocene world (45, 46).

## Materials and Methods

**Plant Material.** Twenty-one Cupressaceae species, plus *Sciadopitys verticillata* and *Taxus baccata*, were collected from arboreta on California's central coast (Table S1). Specimens grew in close proximity to scheduled irrigation systems but were not watered preferentially. The climate is characterized by mild temperatures, wet winters, abundant summer fog, and very rare freezing events [San Francisco, mean annual temperature (MAT),  $13 \pm 0.23$  °C, mean annual precipitation (MAP),  $502 \pm 150$  mm; Berkeley MAT:  $13.9 \pm 0.23$  °C, MAP:  $599 \pm 196$  mm; Santa Cruz MAT:  $13.8 \pm 0.25$  °C, MAP:  $763 \pm 243$  mm]. Juvenile stems ranging from 5 to 8 mm in diameter were collected from sun-exposed regions of mature tree canopies from heights of 1.5–4 m. Five to eight branches were clipped from two to six individuals (depending on permission). Species were randomly sampled from April to August from 2006 to 2009.

**Hydraulic Measurements.** Hydraulic measurements ( $K_s$ ) were made according to standard plant hydraulic protocols (15, 18, 26). Species' cavitation resistance ( $P_{50}$ ) was determined using the centrifuge method and the curves fit with a Weibull Function (26, 27). Conduit diameter distributions and  $(t/D)_h^2$  measurements were performed according to standard anatomical methods (18, 25, 26).

**Gas-Exchange Measurements.** Gas-exchange measurements were performed in June 2010 on the same individuals sampled for hydraulic and anatomical data. We found no significant differences in conductivity or  $P_{50}$  in a subsample of the plants sampled in 2010 vs. 2006–2009.

Sun-exposed twigs (5–15 mm diameter) were clipped from the tree, re-clipped under water, and the cut ends smoothed with a razor blade. To ensure maximum hydration, the twigs were placed in water overnight, uncovered. Water potentials ranged from  $-0.03$  to  $-0.75$  MPa when gas-exchange measurements were obtained (11:00 AM–3:00 PM).

A Li-Cor LI-6400XT instrument (Li-Cor Biosciences) with an opaque  $2 \times 3$  cm LED-lighted chamber was used to obtain leaf gas-exchange measurements. Each measurement took between 4 and 6 min with leaf temperature at  $22.2 \pm 0.8$  °C, the light level at  $2,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and a flow rate of  $300 \text{ mL}\cdot\text{min}^{-1}$  with the  $\text{CO}_2$  mixer set to  $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Projected leaf area measurements were used across all species.

**Species' Habitat Characterization.** The precipitation regimes for each of the sampled Cupressaceae represent averaged data collected from climate stations located within each species' known range, detailed with maps and observations by Farjon (12) (Fig. S1). The coordinates of species' distributions were manually identified using Google Maps and reconciled with the global-regional climate database of Peel et al. (47) (<http://www.hydrol-earth-syst-sci.net/11/1633/2007/hess-11-1633-2007-supplement.zip>; last accessed in July 2011), who also categorize climate according to standard Köppen–Geiger criteria. This database contains long-term monthly precipitation and temperature values reported alongside station coordinates and elevation. Elevation data, slope, and aspect for the Cupressaceae observations are not provided, and Peel et al.'s (47) climate data may not be from stations in close proximity to these locations. Species may naturally occur outside of our conservative estimates of their climate envelope.

We also relied on Peel et al.'s climate criteria (47) and detailed species' habitat descriptions (12) for species' assignments of arid- or mesic/hydric-adapted Cupressaceae in Fig. 1.

**Phylogenetic Reconstruction.** Sequences for two molecular markers (matK and rbcL) were downloaded from GenBank (see Table S3 for accession numbers). Sequences were aligned with ClustalW using default settings and concatenated in Mesquite (48, 49). Topology and branch lengths were reconstructed simultaneously using BEAST and interpreted using associated programs (50–52). Each gene was included in a separate partition. Substitution models were allowed to vary separately for each partition. A general time-reversible model of nucleotide substitution with a four-category  $\gamma$  + invariant model of site heterogeneity was used, with empirical base frequencies. A single linked uncorrelated lognormal clock model was estimated for both partitions jointly. Speciation was estimated from a combined birth-death model. Two separate runs of 10,000 samples each from 2,500,000 generations were combined to generate the final phylogeny.

Fossil calibrations drew on a variety of sources (Table S2). Fossils with high resolution dating were rarely available and the average length of the date range was  $\sim 19$  Mya. At the same time, the ecological habit of several extant and many fossil Cupressaceae is near freshwater swamps, a taphonomic environment with a relatively high likelihood of preservation. For several specimens, there is therefore a high probability of rapid incorporation into the fossil record, and a relatively large uncertainty about the exact time of incorpora-

tion; in light of this, we chose to use normally distributed calibration priors with a mean centered on the middle of the geological period from which the fossil was recovered, and a SD of one-half of the length of the period.

**Comparative Phylogenetic Analyses.** The phylogeny was imported into the R statistical environment (R Development Core Team, 2008) using the APE package (54) and the physiological data presented in this article were analyzed using PICs (53). No contrast was calculated for differences between *Taxodium mucronatum* and *Taxodium distichum* because of the very short branches diverging from this node. Only data for *T. distichum* was included in the PIC analysis and figures presented. PICs compare the amount of divergence in trait values that has occurred between branching events. Each value calculated corresponds to the difference in trait values associated with a particular node. In figures, we label each contrast with the number corresponding to its associated node in the phylogeny shown in Fig. 1. Further statistical analysis was conducted on the resulting contrasts using

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APE and Picante (55). R scripts used are available from S.A.S. upon request. Ancestral trait reconstruction of  $P_{50}$  values was conducted in Mesquite, using a maximum parsimony model. Reconstructed ancestral states are shown as the color of branches leading to each node in Fig. 1.

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