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New Phytologist

Vestured pits and scalariform perforation plate morphology modify the relationships between angiosperm vessel diameter, climate and maximum plant height

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Summary

- Shared ancestry among species and correlation between vessel diameter and plant height can obscure the mechanisms linking vessel diameter to current climate distributions of angiosperms. Because wood is complex, various traits may interact to influence vessel function. Specifically, pit vesturing (lignified cell wall protuberances associated with bordered pits) and perforation plate morphology could alter the relationships between vessel diameter, climate and plant height.
- Using phylogenetically informed analyses, we tested for associations between vessel diameter, climate and maximum plant height across angiosperm species with different pit vesturing (presence/absence) and perforation plate morphology (simple/scalariform and quantitative variation).
- We show significantly larger changes in vessel diameter and maximum plant height across climates for species with vestures and simple perforation plates, compared to nonvestured species and those with scalariform plates. We also found a significantly greater increase in height for a given increase in vessel diameter with lower percentage of scalariform plates.
- Our study provides novel insights into the evolution of angiosperm xylem by showing that vessel pit vesturing and perforation plate morphologies can modify relationships among xylem vessels, climate and height. Our findings highlight the complexity of xylem adaptations to climate, substantiating an integrative view of xylem function in the study of wood evolution.

Introduction

Studies of ecological wood anatomy in angiosperms have demonstrated that xylem traits such as conduit diameter often vary across climate gradients in a predictable manner (Carlquist, 1975, 2001; Medeiros & Pockman, 2014), but climate may not necessarily be the primary factor shaping the evolution of conduit traits (including vessels and tracheids). For example, correlations between vessel diameter and climate could be indirect: vessel dimensions may appear as a trait under selection simply because they are genetically correlated with other traits that actually relate to fitness (Donovan *et al.*, 2011), such as pit dimensions (Cochard *et al.*, 1992; Choat *et al.*, 2008), stem diameter (Noshiro *et al.*, 1995; Olson & Rosell, 2012) or stem length (Noshiro *et al.*, 1995; Wheeler *et al.*, 2007; Olson *et al.*, 2014). Furthermore, many xylem traits can influence the fit between vessel diameter and climate, by either directly or indirectly altering the resistance of xylem to long-distance water transport.

In particular, the joint cell walls where water moves between vessels could directly alter conducting efficiency (Carlquist, 2001) or the spread of embolisms between adjacent conduits (Hacke & Sperry, 2001; Brodribb *et al.*, 2016; Choat *et al.*, 2016). Of particular importance for water transport resistance are bordered pits and perforation plates (Fig. 1), which make up the connection between adjacent vessel walls, and between vessel elements inside individual vessels, respectively. For example, compared to scalariform perforation plates, simple plates should have higher water transport efficiency (Jansen & Nardini, 2014; Lens *et al.*, 2016) because they are associated with larger vessels, and they present a clear, unrestricted pathway inside individual vessels. By contrast, scalariform plates, which provide a more defined barrier between vessel elements, might reduce the spread of embolism, but at the cost of increased resistance to flow (Tyree & Zimmermann, 1983; Schulte & Castle, 1993; Schulte, 1999; Christman & Sperry, 2010). Similarly, pit vesturing, or the presence of tiny lignified, cell wall outgrowths along the pit border, is hypothesized to be a safety feature associated with drought embolism (Jansen *et al.*, 2003, 2004).

Previous studies have addressed these theoretical expectations of safety and efficiency by examining the relationship between

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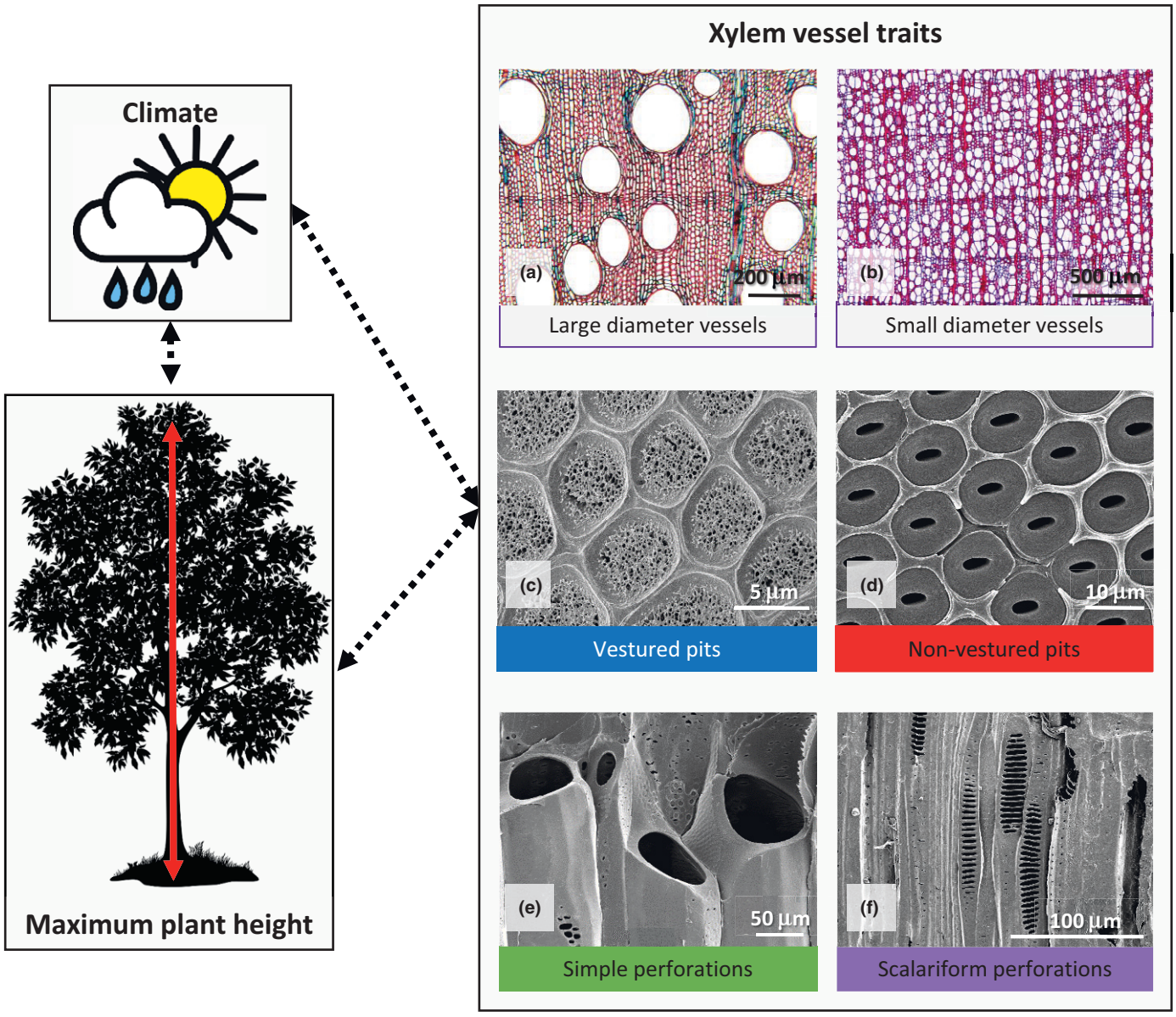


Fig. 1 The relationship between climate, plant height and the xylem vessel morphological traits examined in this study. We investigated the relationships between climate, maximum plant height (m), xylem vessel diameter (µm), pit morphology (blue, vestures present; red, vestures absent) and perforation plate morphology (purple, scalariform perforations present; green, scalariform perforations absent) for woody angiosperms. Species depicted in xylem vessel images include: (a) *Dictyophleba ocrhacea*, (b) *Nyssa sylvatica*, (c) *Cleistocalys ellipticus*, (d) *Combretodendron africana,* (e) *Chrysophyllum perpulchrum* and (f) *Ilex aquifolium*.

perforation plate morphology and climate, but these show that the correlations are not straightforward. The hypothesis that scalariform plates prevent xylem embolism (Hacke & Sperry, 2001; Brodribb *et al.*, 2016; Choat *et al.*, 2016) has been supported by the fact that they are often associated with cooler climates compared to simple plates, but many tropical montane species also have scalariform perforations, and they can occasionally be found in dry environments (Baas, 1973; Baas & Schweingruber, 1987; Carlquist, 2001; Lens *et al.*, 2016). Interestingly, across angiosperms, 'safer' vestured pits are strongly associated with 'more efficient' simple perforation plates (Jansen *et al.*, 2003), while the combination of scalariform perforation plates and vestured pits is rare. Furthermore, studies based on vessel diameter often find only weak evidence for safety–efficiency trade-offs, and there is a preponderance of species with vessel traits that appear to be not particularly efficient or safe (Gleason *et al.*, 2016). This is probably driven, in part, by poor standardization of where along the stem axis vessel traits are measured, but these perplexing results also point to the hypothesis that combinations of traits, rather than single traits, could represent adaptations to complex climate-driven water transport challenges. The potential for climate associations to be driven by interactive effects of vestured pits and perforation plate morphology with other plant traits such as vessel diameter and plant height, however, remains largely untested.

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An additional layer of complexity is added by the fact that vestured pits and scalariform plates, although often presented as categorical traits, exhibit quantitative variation (Jansen *et al.*, 1998, 2001; Carlquist, 2001). The amount of vesturing should influence hydraulic resistance, but defining a scale for degree of vesturing is complex and quantitative data are generally scarce (e.g. Jansen *et al.*, 2008). Investigating the effects of variation in scalariform perforation plates is more tractable across large numbers of species, and these vary in the per cent of plates with scalariform perforations, and in the number of bars crossing the end-wall (Carlquist, 2001), which grades into the 'simple' plated category with zero bars. Based on previous differences observed between species with exclusively scalariform or simple plates, a large number of bars per plate and/or a greater percentage of scalariform plates is hypothesized to be associated with smaller vessel diameters, shorter plant heights, and cooler or drier climates (Baas, 1973; Baas & Schweingruber, 1987; Carlquist, 2001; Lens *et al.*, 2016). The potential for variation in scalariform plate morphology to influence the relationships between vessel diameter and other factors such as plant height and climate, however, remains to be seen.

In addition to physiological interactions between xylem traits, shared ancestry might alter the relationships between traits, as some wood anatomical characters, including perforation plate morphology (Lens *et al.*, 2016) and vesturing (Jansen *et al.*, 2003), are more similar among closely related species than expected by chance. Thus, there could be a poor fit between current-day traits and current-day climate. Alternatively, if the evolution of vesturing or scalariform plates represent solutions to safety–efficiency challenges, then selection on vessel diameter could be relaxed, leading to different vessel–climate-plant height relationships. To better understand the factors causing variation in vessel traits and maximum plant height, we investigated climate associations for species with different pit and perforation plate morphology, while accounting for shared ancestry using phylogenetic information.

Specifically, we addressed the following questions:

- Do vessel diameter, maximum plant height and climate differ across species with different types of pit and perforation plate morphology?
- Do the relationships between vessel diameter and climate, between maximum plant height and climate, or between vessel diameter and maximum plant height differ according to the types of pit and perforation plate morphology?
- Do the relationships between vessel diameter, maximum plant height and climate differ according to quantitative variation in scalariform perforation plate morphology?

Materials and Methods

Data sources and characters examined

We compiled data from the scientific literature and published databases on mean vessel diameter (μm) and maximum plant height (m) for self-supporting angiosperm trees and shrubs. Previous studies have focused on measured plant height. While many outside factors contribute to the actual height of plants at particular locations, by examining maximum plant height this study focuses on the properties of the plants themselves (i.e. developmental patterns or physical properties of the stem such as resistance to mechanical or functional failure). In addition, because the relationship between vessel diameter and plant height approximates to a power law (Olson *et al.*, 2018), mean basal vessel diameter across a wide range of mature plant heights should correlate fairly closely with maximum height. In support of this, within our dataset, the slopes for the relationships between vessel diameter and maximum plant height for species without vestures or scalariform perforations (see the 'Results' section, Table S3) were within the 95% confidence interval estimated for slopes for the relationships between vessel diameter and measured plant height reported in the literature (Olson & Rosell, 2012).

We chose angiosperm species contained in the global angiosperm phylogeny (Zanne *et al.*, 2013, 2014; see 'Data analysis' section below), for which data on perforation plate morphology or vestures were also available. We chose to focus on major clades with relatively abundant data and relatively high variation in each trait (Fig. 2): for vestures, we examined species in the Rosid I and Rosid II clades, and for perforation plates, we examined species within the Asterids, as well as Asterids I and II clades. Although angiosperm xylem could contain both vessels and tracheids, here we focus only on vessels, as these represent by far the larger contribution to hydraulic conductance. Data on vessel diameter and maximum plant height were obtained from the TRY Database (Kattge *et al.*, 2011) and from the literature; the Supporting Information provides the sources of data (Dataset S1), and the names and xylem traits of species examined in this study (Dataset S2). In cases where we had more than one observation for a species, all observations were combined into a single mean for that species. For one database, Zanne *et al.* (2010), we used data on mean vessel area to compute mean vessel diameter based on the mathematical relationship between the diameter and area of a circle.

Categorical data on pit and perforation plate morphology

We compiled data on pit morphology (presence/absence of vestured pits; 425 species) and perforation plate morphology (presence/absence of scalariform perforations; 252 species). Most of these observations were from the Xylem Functional Traits Database (Choat *et al.*, 2012) and the Global Wood Anatomy Database accessed through the TRY Database (Kattge *et al.*, 2011). We also obtained data on perforation plate pore morphology from the literature (see Dataset S1) and some original data (S. Jansen *et al.*, unpublished). We used the R package 'GGTREE' to visualize categorical trait data on the phylogeny (Yu *et al.*, 2017).

Quantitative data on variation in scalariform perforation plate morphology

To address the potential effects of quantitative variation in scalariform perforation plates, we examined data on the average

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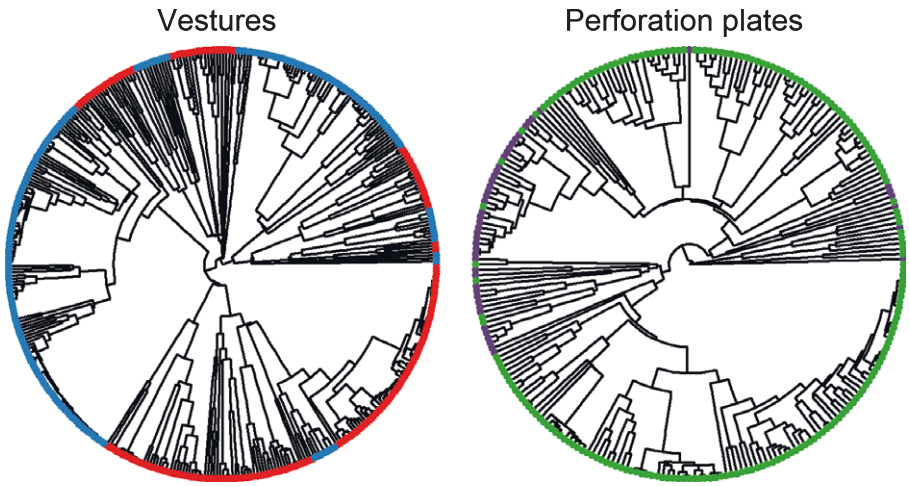


Fig. 2 Occurrence of vestured pits and scalariform perforation plates in woody angiosperm species. Vestured vs nonvestured pits are shown on the left (blue, vestures present; red, vestures absent) and scalariform vs simple perforation plates are shown on the right (purple, scalariform perforations present; green, scalariform perforations absent).

number of bars per plate (66 species; these same species were also included within the 252 species examined for the categorical analysis described above) or percentage of plates with scalariform perforations (37 species; these species have mixed occurrence of scalariform and simple perforation plates, and thus were not included in the categorical analysis described above). These data were obtained from the Global Wood Anatomy Database accessed through the TRY Database (Kattge *et al.*, 2011). For this analysis, we only included species in which: the average number of bars per plate was >0 (i.e. only scalariform species), and the percentage of plates with scalariform perforations was <100 and >0 (i.e. only species with mixed occurrence of scalariform plates).

Climate data

For each of the 714 species, we used phylogenetically informed principal component analysis (phyl.pca in the R package PHYTOOLS) to construct climate indices which incorporated all available information on the climate, including: mean values (e.g. mean annual temperature), indicators of variability (e.g. temperature annual range), and interactions between temperature and precipitation (e.g. precipitation in the coldest quarter).

For each species, we retrieved location records from the Global Biodiversity Information Facility (www.gbif.org, accessed 13 January 2016). Up to 500 locations were retrieved for each species. To avoid including erroneous location information we removed records that fit the following criteria: numeric latitude $<-90°$ or $>90°$, longitude $<-180°$ or $>180°$, either coordinate exactly equal to zero, latitude exactly equal to longitude. Duplicate records were removed using the GBIF 'key' field. Duplicate observations of latitude and longitude for the same species were removed. Records within 50 km of GBIF headquarters (Copenhagen, Denmark: 55.68°N, 12.59°E) were removed to minimize records with coordinates corresponding to where the data were stored rather than where the plant was actually located. Records with content in the GBIF field 'issues' were checked for accuracy by hand, and those that were inconsistent with all other observations for a species were removed. Cleaned locations used to generate climate estimates are shown in Fig. 3(a).

Based on these locations, we used Bioclim GIS layers (bio_1 to bio_19, 30 s grids; Hijmans *et al.*, 2005) to obtain interpolated climate data for each occurrence of a species. Bioclim interpolations are based on actual monthly average minimum, mean and maximum temperature and precipitation recorded at thousands of weather stations worldwide from 1970 to 2000. Interpolated values were averaged by species, and these averages were passed to the principal component analysis, resulting in two climate scores for each species, representing temperature (PC1) and precipitation (PC2), which are discussed in the Results section.

Data analysis

Before analysis, binomials in each data set were checked for current accepted usage using the R package 'TAXONSTAND' (Cayuela & Oksanen, 2015). To account for the effects of nonindependence due to shared ancestry on the outcome of statistical analyses, we used phylogenetic generalized least squares (PGLS) regression. We used PGLS to examine the relationships between xylem vessel traits, maximum plant height and climate (two principal component axes describing temperature (PC1) and precipitation (PC2), which are described in detail in the Results section) across species with different types of pit and perforation plate morphology. Phylogenetic relationships among species in each analysis were obtained from a large-scale multi-gene angiosperm molecular phylogeny (Zanne *et al.*, 2013, 2014) with fossil-calibrated branch lengths. We estimated λ, or the magnitude of nonindependence due to phylogenetic relationships among species, using maximum likelihood and assuming a Brownian model of evolution. Lambda varies from 0 (indicating complete independence between residual error of trait covariances and the phylogenetic variance covariance matrix) and 1 (indicating complete dependence between residual error of trait covariances and the phylogenetic variance covariance matrix; Freckleton *et al.*, 2002). One important advantage of PGLS is that

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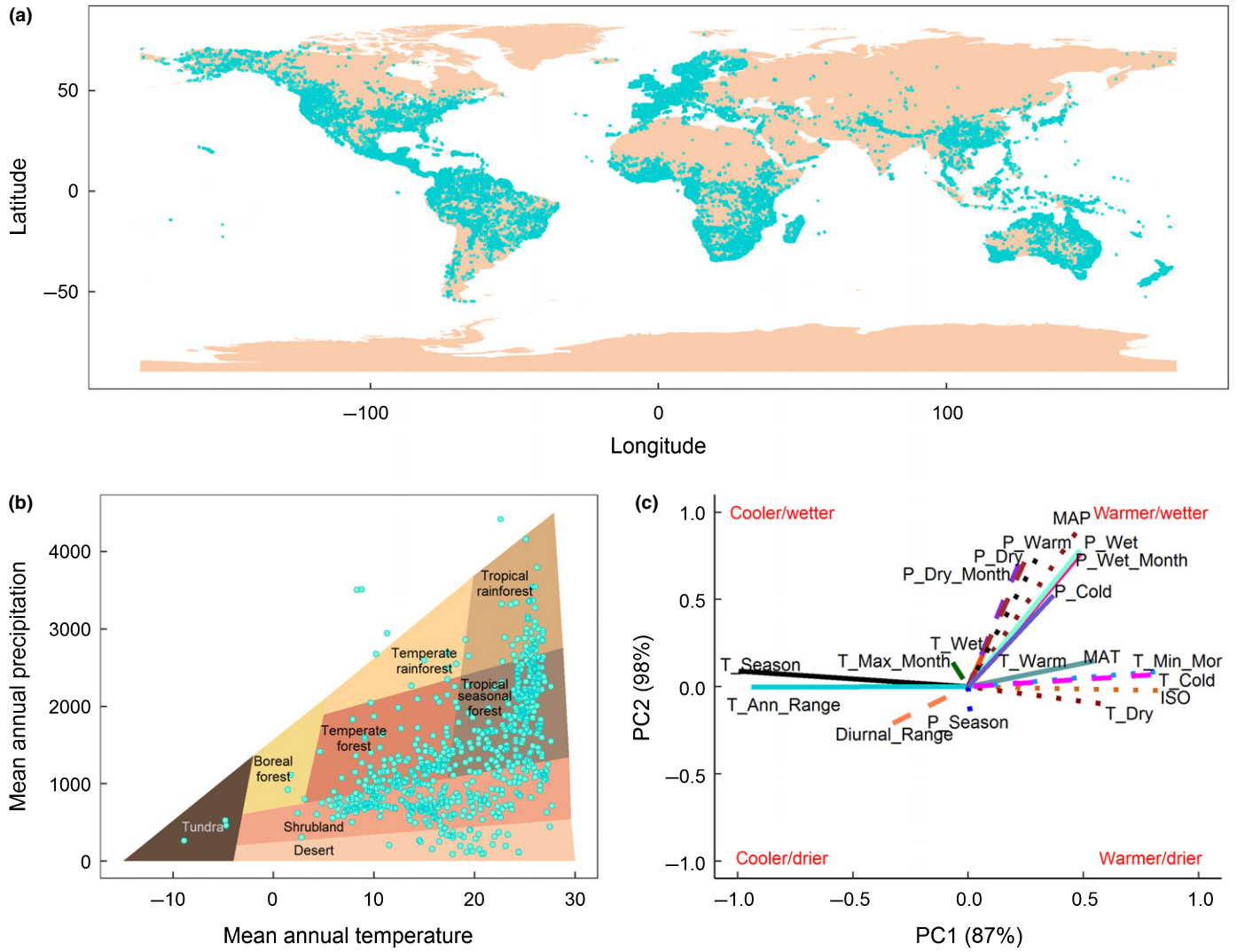


Fig. 3 Geographic and climatic features for 714 woody angiosperm species. (a) Observations of latitude and longitude used to generate climate estimates (blue circles), (b) estimated mean annual temperature (°C) vs estimated mean annual precipitation (mm, blue circles) for all 714 species showing representation in all eight Whittaker biomes (Whittaker, 1975), and (c) variable loadings for phylogenetic principal components (PC) analysis showing Bioclim variables included in climate estimates. Points that fall above the classical biome space include four species from New Zealand, and one from the Andean region of South America. Dashed and solid lines are used for clarity. Bioclim variables used in our analysis included: annual mean temperature (MAT), mean diurnal range (Diurnal_Range), isothermality (ISO), temperature seasonality (T_Season), maximum temperature of the warmest month (T_Max_Mon), minimum temperature of the coldest month (T_Min_Mon), temperature annual range (T_Ann_Range), mean temperature of the wettest quarter (T_Wet), mean temperature of the driest quarter (T_Dry), mean temperature of the warmest quarter (T_Warm), mean temperature of the coldest quarter (T_Cold), annual precipitation (MAP), precipitation of the wettest month (P_Wet_Mon), precipitation of the driest month (P_Dry_Mon), precipitation seasonality (P_Season), precipitation of the wettest quarter (P_Wet), precipitation of the driest quarter (P_Dry), precipitation of the warmest quarter (P_Warm) and precipitation of the coldest quarter (P_Cold). PC1 was composed primarily of variables describing temperature regime, with higher scores indicating warmer, less variable climates and lower scores indicating cooler, more variable climates. PC2 was composed mostly of variables describing precipitation regime, with higher values indicating wetter climates with less variable precipitation, and lower values indicating drier climates with more variable precipitation.

because coefficients from each analysis are adjusted for nonindependence due to shared ancestry, they represent the best unbiased estimate of relationships among variables. PGLS models and maximum likelihood estimation of λ were implemented in the R package 'CAPER' (Orme *et al.*, 2015), which accommodates both continuous and categorical predictor variables, as well as their interactions. In some cases, the maximum likelihood function did not converge, resulting in an estimated λ of 0. All variables were log transformed before analysis. We conducted two types of analysis:

- We tested for the effect of vestured pits (presence/absence) or scalariform perforation plates (presence/absence and quantitative variation) using vessel diameter, maximum plant height or climate (PC1 or PC2) as the dependent variable.
- We examined whether pit or perforation plate morphology modified relationships between vessel diameter, maximum plant

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height and climate. For this we tested for an interaction between perforation plate morphology and vestured pit occurrence with climate (PC1 or PC2), and maximum plant height, using vessel diameter as the dependent variable. We also examined the interaction between perforation plate morphology and vestured pit occurrence with climate using maximum plant height as the dependent variable.

Results

Distribution of scalariform perforation plates and vestures across angiosperm clades

Vestured pits and scalariform perforation plates were not evenly distributed across angiosperm clades (Fig. 2). For pit morphology, we found that major clades were strongly represented by either vestured or nonvestured species, and even more so for perforation plate morphology, few transitions were observed within major clades. Also, within our dataset vestures were virtually mutually exclusive with scalariform perforation plates.

Representation of species across major biomes and resulting climate indices

A plot of mean annual temperature vs mean annual precipitation obtained from the Bioclim grids shows that our dataset contains species representing every major terrestrial biome (Fig. 3b). The principal component analysis recovered two indices, together explaining 98% of the variation in climate among species (Fig. 3c). The first principal component, PC1, was composed primarily of variables describing temperature, with higher scores indicating higher, less variable temperatures, and lower scores indicating lower, more variable temperatures. The second component, PC2, was composed mostly of variables describing precipitation, with higher values indicating wetter climates with less variable precipitation, and lower values indicating drier climates with more variable precipitation.

Pit and perforation plate morphology and vessel diameter, maximum plant height and climate

We found that vestured species had significantly larger vessel diameter compared to nonvestured species (Table S1a; Fig. 4a). Nonvestured species showed a markedly high number of species with low vessel diameter, while vestured species were more evenly distributed across the range of vessel diameter values. Despite differences in vessel diameters, the height of vestured species did not differ significantly from that of nonvestured species (Fig. 4b), although vestured species did have significantly higher mean PC1 scores (warmer climates with less variable temperature, Fig. 4c) and significantly lower PC2 scores (drier climates with more variable precipitation, Fig. 4d) than nonvestured species.

Considering perforation plate morphology, we found that species with scalariform plates had significantly smaller vessel diameters (Table S1b; Fig. 4e), shorter maximum height (Fig. 4f), lower PC1 scores (lower, more variable temperatures; Fig. 4g) and higher PC2 scores (higher, less variable precipitation; Fig. 4h), compared to species with simple plates. Species with scalariform plates were more evenly distributed across PC1 (associated with a wider range of temperature conditions) compared to species with simple plates.

Pit and perforation plate morphology and vessel–climate–plant height relationships

Vestured species showed significantly larger increases in vessel diameter and maximum height with increasing PC1 (temperature) and PC2 (precipitation), compared to nonvestured species (significant interaction term, Table S2; Fig. 5a,b). We also found that maximum plant height increased significantly more quickly across PC2 for vestured species compared to nonvestured species (Fig. 5c). Furthermore, when these relationships were examined separately for vestured and nonvestured species we found that the relationship between maximum plant height and PC2 (precipitation) was significant for vestured species but not for nonvestured species (Table S3a).

Compared to species with scalariform plates, species with simple plates showed a significantly faster increase in vessel diameter across PC1 (temperature; Table S4a; Fig. 6a), and a similar pattern was observed for maximum plant height (Fig. 6b). We also observed a faster increase in vessel diameter with increasing PC2 (precipitation; Table S4a). In addition, the relationships of vessel diameter and maximum plant height to PC1 and PC2 were significant for species with simple plates but not for species with scalariform plates (Table S3b). Lastly, we found that species with simple plates showed a significantly greater increase in vessel diameter as height increased, both when examined as interactive effects (Table S4a; Fig. 6c) and when species with exclusively scalariform and simple plates were examined separately (Table S3b).

Effects of quantitative variation in perforation plate morphology

The average number of bars per plate was significantly positively related to PC2 (higher number of bars associated with higher, less variable precipitation; Fig. S1a; Table S1c). We also found that species with many bars per plate (i.e. relatively more scalariform) showed a significantly weaker increase in height moving from cool to warm climates, compared to species with a low number of bars per plate (i.e. relatively more simple; Table S4b; Fig. S1b).

We found significantly lower percentages of scalariform plates in warmer climates and in species with larger vessels (Table S1d), but we also found significant interactive effects. Species with 75% or greater scalariform plates exhibited a smaller increase in vessel diameter moving from cooler to warmer climates (Fig. 7a) and from drier to wetter climates (Table S4c; Fig. 7b), compared to species with 25% or less scalariform plates. The same pattern was observed for significant interactions between maximum plant height and PC1 (Fig. 7c), and between vessel diameter and maximum plant height (Fig. 7d); for both of these, a higher percentage of scalariform plates was associated with a smaller change across climate and height, respectively.

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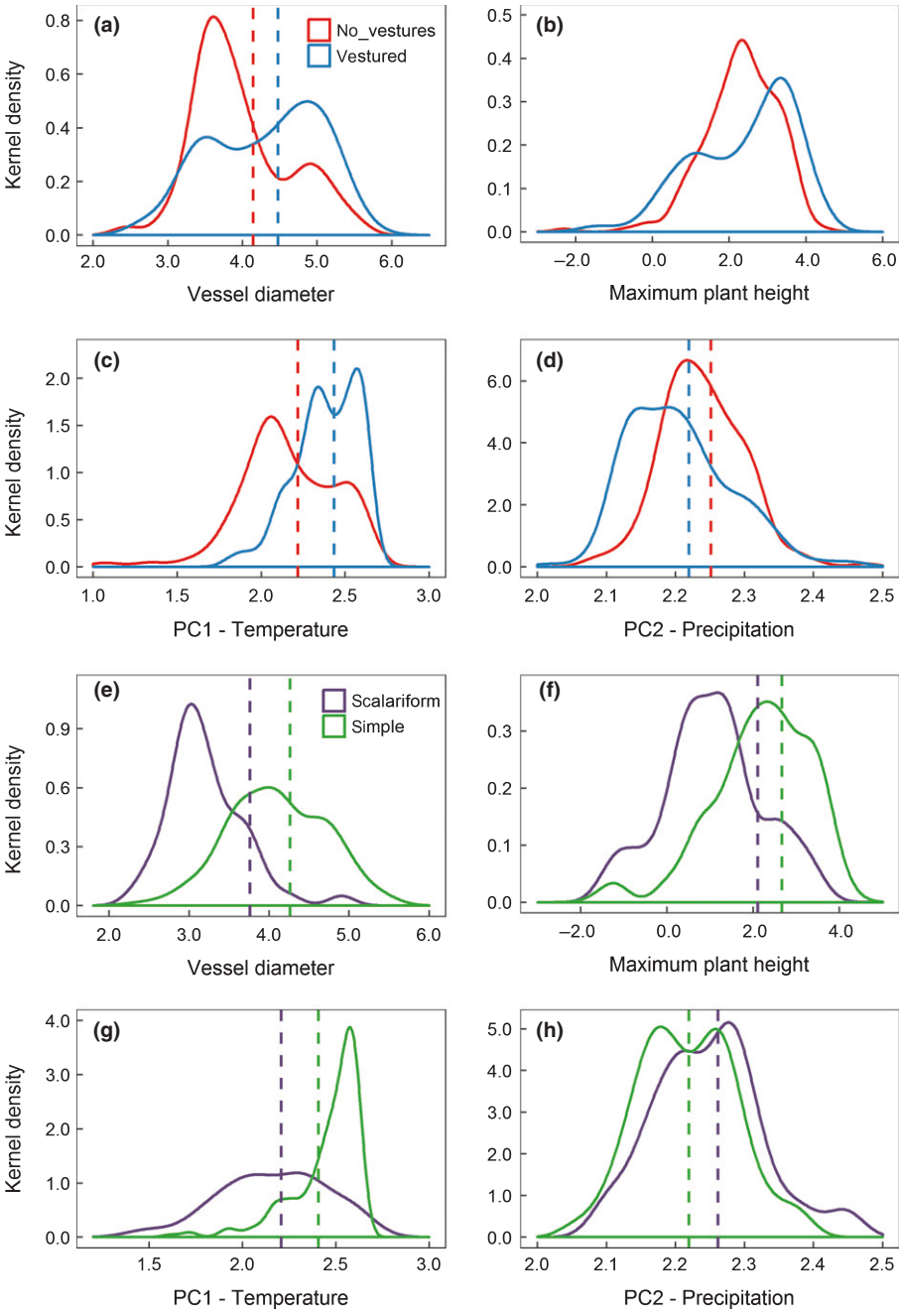


Fig. 4 Kernel density estimates of vessel diameter, maximum plant height and climate for woody angiosperm species with vestured and nonvestured pits (a–d) for species with scalariform and simple perforation plates (e–h). Vertical dashed lines indicate the mean value of the trait in cases where species groups were significantly different. All data are presented on a log scale. Two principal component (PC) axes describe climate: higher values of PC1 indicate warmer climates with less variable temperature regime, while lower scores indicate cooler climates with more variable temperature. Higher values of PC2 indicate wetter climates with less variable precipitation, and lower values indicate drier climates with more variable precipitation.

Discussion

Our study provides novel insights into the evolution of angiosperm xylem by showing that pit vesturing and perforation plate morphologies are associated with different vessel–climate–height relationships. Because resistance to water flow increases with greater total path length (Giordano *et al.*, 1978; Hacke & Sperry, 2001), as plants grow taller the overall diameter of vessels must increase in order to maintain a tip-to-bole gradient from narrower to wider conduits (Olson *et al.*, 2014). Our work expands on this by showing that vessel diameter is indeed strongly related to maximum plant height, and that scalariform plates and vesturing can explain some of the variation observed in vessel–height relationships (Olson *et al.*, 2014, 2018; Rosell & Olson, 2014; Morris *et al.*, 2018). By including maximum plant height in our analyses of vessel dimeters across species with different pit and perforation plate morphologies, we provide support for the idea that selection may act, to some extent, on either trait independently.

By accounting for phylogenetic nonindependence, our study also strengthens the conclusions of previous studies showing that warm and dry environments have driven the evolution of vestured pits (Jansen *et al.*, 2003, 2004). Including phylogenetic information is necessary given that vesturing is strongly conserved (Jansen *et al.*, 1998, 2000; Jansen & Smets, 2000; Baas *et al.*, 2003; Wheeler *et al.*, 2007). Vesturing of pits has been

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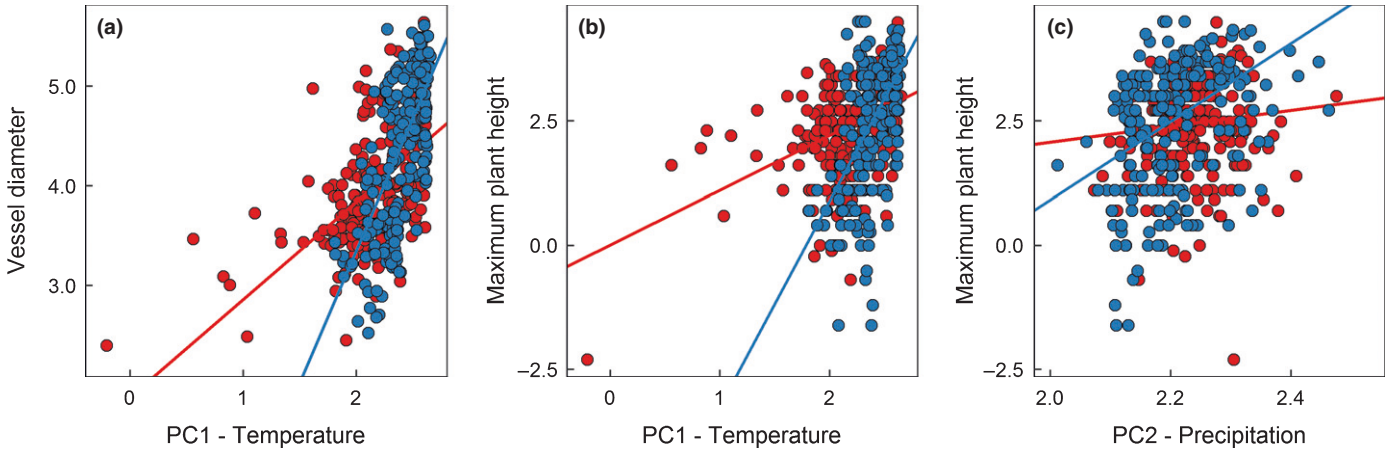


Fig. 5 Effect of pit morphology (vestured (blue) vs nonvestured (red)) on the relationships between vessel diameter, maximum plant height and climate for woody angiosperms. Solid lines indicate the phylogenetically correct slope in cases where there was a significant interaction term, including: (a) vessel diameter vs PC1, (b) maximum plant height vs PC1, and (c) maximum plant height vs PC2. All data are presented on a log–log scale. Two principal component (PC) axes describe climate: higher values of PC1 indicate warmer climates with less variable temperature regime, while lower scores indicate cooler climates with more variable temperature. Higher values of PC2 indicate wetter climates with less variable precipitation, and lower values indicate drier climates with more variable precipitation.

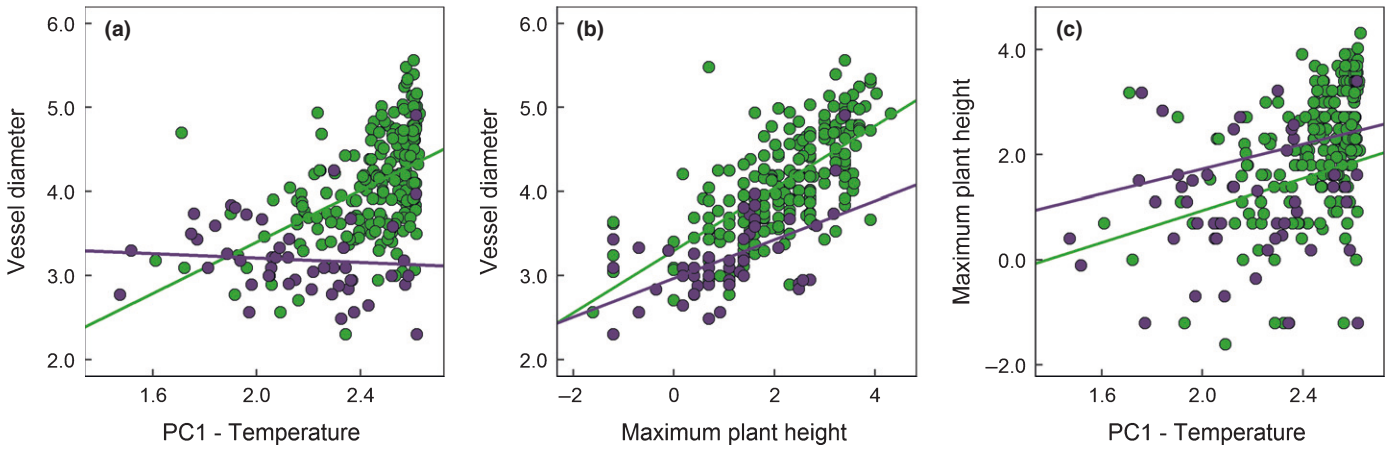


Fig. 6 Effect of perforation plate morphology (scalariform (purple) vs simple (green)) on the relationships between vessel diameter, maximum plant height and climate for woody angiosperms. Solid lines indicate the phylogenetically correct slope in cases where there was a significant interaction term, including: (a) vessel diameter vs PC1, (b) vessel diameter vs maximum plant height, and (c) maximum plant height vs PC1. All data are presented on a log–log scale. Two principal component (PC) axes describe climate: higher values of PC1 indicate warmer climates with less variable temperature regime, while lower scores indicate cooler climates with more variable temperature. Higher values of PC2 indicate wetter climates with less variable precipitation, and lower values indicate drier climates with more variable precipitation.

hypothesized to improve embolism resistance by trapping air bubbles (Carlquist, 1982) or by preventing pit membrane rupture (Zweypfenning, 1978), although more recent work suggests that these hypotheses need substantial revision (Choat *et al.*, 2004). We do not know how embolism resistance could be affected by the presence of vestures, whether or not vesturing affects pit membrane mechanics, air-seeding or pit membrane thickness, and little is known about the mechanical properties of pit membranes (Tixier *et al.*, 2014).

Vestured species in our study also had larger vessel diameters compared to nonvestured species, but we found no significant differences in maximum plant height between them. A post-hoc test determined that vestured species had significantly higher wood density than nonvestured species (Table S5), suggesting that reduced mechanical strength is not the reason for their shorter-than-expected heights, given their larger vessel diameters. There are few proposed hypotheses concerning functional relationships between vestures and maximum plant height, but vestures do have high lignin content (Ohtani *et al.*, 1984; Jansen *et al.*, 1998, 2003). In many Myrtales (e.g. Myrtacea, Vochysiaceae, Melastomataceae) and Gentianales, a network of large and highly branched vestures occupy the entire pit chamber, reducing the pit aperture to a slit-like opening that is difficult to see in scanning electron micrographs (see Fig. 1c). If extensive vesturing is present, this could reduce the resources available to grow taller, while at the same time increasing wood density. Under the drier, more variable precipitation conditions where we observed vestures to be most prevalent, higher wood density could improve

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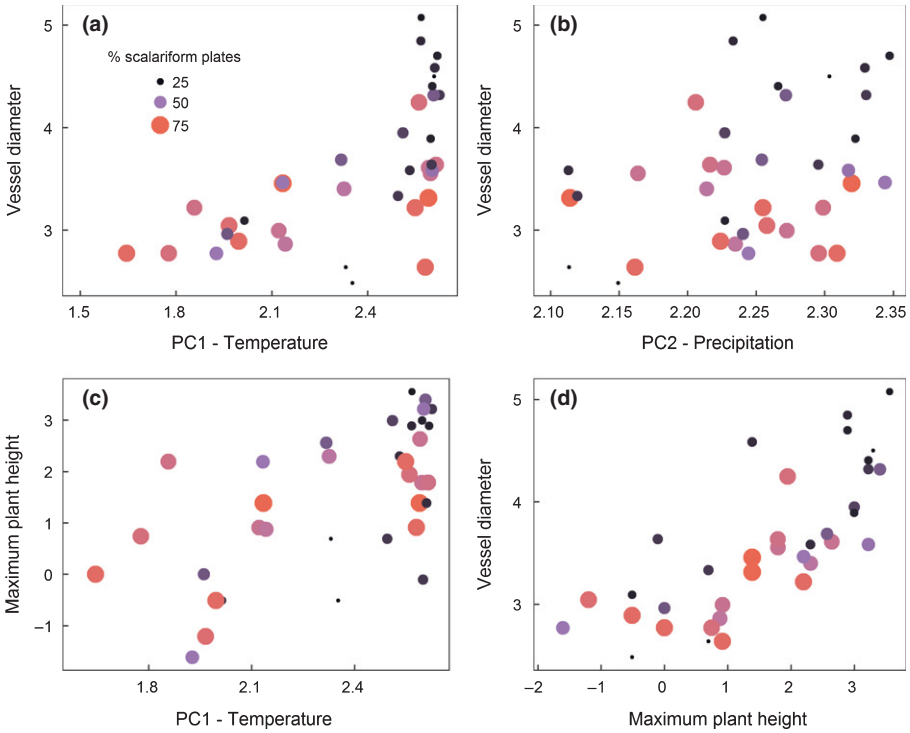


Fig. 7 Effect of per cent scalariform perforations on the relationships between vessel diameter, maximum plant height and climate for woody angiosperms. Relationships exhibiting a significant interactive effect of per cent scalariform perforations are shown, including: (a) vessel diameter vs PC1, (b) vessel diameter vs PC2, (c) maximum plant height vs PC1, and (d) vessel diameter vs maximum plant height. Data on per cent scalariform plates are quantitative, but for ease of interpretation the legend is presented here using bins, with mid-points of 25%, 50% and 75%. All data are presented on a log–log scale. Two principal component (PC) axes describe climate: higher values of PC1 indicate warmer climates with less variable temperature regime, while lower scores indicate cooler climates with more variable temperature. Higher values of PC2 indicate wetter climates with less variable precipitation, and lower values indicate drier climates with more variable precipitation.

their survival through better drought embolism resistance, but as drought alternates with brief windows of high water availability, their larger vessel diameters could improve maximum water transport capacity, allowing faster growth compared to nonvestured species (Cohen, 1970).

The presence of scalariform plates should also reduce maximum water transport capacity, as these can easily double xylem resistance compared to simple plates (Christman & Sperry, 2010). In keeping with this, we did see that the majority of species with simple plates were found in warmer climates, where high evapotranspiration rates or steep competition with other plants might favor high maximum water transport capacity. However, our data also show, however, that scalariform species are broadly distributed across a wide range of temperatures. This is contrary to previous hypotheses that scalariform plates are a component of adaptation to cooler, more seasonal climates (Baas, 1973; Carlquist, 1975; Jansen *et al.*, 2003). Furthermore, we found that an increase in the average number of bars per plate was associated with wetter climates. This indicates that species with scalariform plates may do best only under conditions where low transpiration rates do not present a strong impedance for growth and survival (i.e. cooler, wetter conditions, Baas & Schweingruber, 1987; Jansen & Nardini, 2014; Lens *et al.*, 2016). Taken together, this points to the idea that scalariform plates may not provide an advantage at any particular temperature, and that the transition to simple plates may actually represent the adaptation, in response to warmer climates. In addition, environments with larger seasonal and daily changes in vapor pressure deficit might favor simple plates; we do not have data to test this hypothesis, and therefore this possibility should be addressed in future work.

We also found that vessel–climate and height–climate relationships differ according to pit and perforation plate morphology. Specifically, unlike nonvestured species, vestured species increased their vessel diameter and maximum height more rapidly moving from cooler to warmer, and from drier to wetter climates. This could be related to increased hydraulic resistance, if vestures do, in fact, impact resistance as hypothesized above. If resistance to flow drives these differences in slope between vestured and nonvestured species, and if scalariform plates increase hydraulic resistance over simple plates, then scalariform species should have steeper trait–trait and trait–climate slopes compared to those with simple plates. In fact, we saw just the opposite: groups of species with simple plates responded more strongly to climate gradients, and showed a greater increase in vessel diameter for a given increase in maximum plant height, compared to those with scalariform plates. While vestures may alter the resistance to flow between adjacent vessels, scalariform plates increase resistance to flow between elements of an individual vessel. Interestingly, the resistance offered by the

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perforation plate was found to be more or less equal to the lumen resistance (i.e. the resistance offered by the inner conduit walls and bordered pit resistance; Christman & Sperry, 2010). If the balance between vessel lumen resistance and scalariform perforation plate resistance changes as vessels become larger, this could effectively diminish the benefit of increased vessel size in the presence of scalariform plates. On the other hand, species with simple plates could realize a strong benefit from an increase in vessel diameter, with a more or less constant ratio of lumen resistance to perforation plate resistance.

Our kernel density estimates show considerable overlap of scalariform and simple-plated species at intermediate values of climate and vessel traits, which could suggest equivalent relative fitness of these two alternative states under mesic conditions. Our analysis of quantitative variation in scalariform perforations, however, suggests otherwise. We found that species with a higher percentage of scalariform plates showed smaller increases in vessel diameter across cool to warm climates, and with increasing maximum plant height. This provides additional strong support for the conclusion, based on our categorical analysis, that perforation plate morphology alters the relationships between vessel diameter and maximum plant height, with consequences for climate affinity. Vestures also exhibit variation (Jansen *et al.*, 1998, 2001), but investigation into this phenomenon is hindered by a lack of quantitative metrics and by a lack of data on such variation (e.g. Jansen *et al.*, 2008), leaving this question open for future study.

Our study suggests that the evolution of different pit and perforation plate morphologies may have been important traits allowing xylem function and maximum plant height to be fine-tuned to different environmental conditions over evolutionary time. We were also able to show interesting effects of variation in perforation plate morphology, but one drawback of this study using species means is our inability to address the many other potentially important components of xylem variation, such as sampling height, or differences within and among individuals of a species, so future work should focus on investigating variation in these traits. In addition, of 284 species for which quantitative data on perforation plate morphology have been reported, only 66 are included in the global angiosperm phylogeny. Therefore, more inclusive phylogenies will also be needed to further tease apart the, perhaps subtle, roles of vestures and scalariform plates in navigating water transport safety–efficiency trade-offs, especially given the strong evidence for phylogenetic nonindependence in these traits. The functional hypotheses suggested by our broad-scale analyses must also be tested at a variety of scales to identify causality. First, new physiological experiments are needed, for example elucidating the effects of vestures on phenomena such as pit membrane shrinkage and pore sizes (Zhang *et al.*, 2017). Second, microevolutionary potential needs to be addressed, for example observing the consequences of experimentally manipulated xylem genotypes within populations (e.g. Voelker *et al.*, 2011). Lastly, macroevolutionary studies, for example using dated phylogenies and reconstruction of ancestral character states and paleoclimatic conditions, should be undertaken to define the circumstances under which major shifts have occurred.

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Author contributions

JSM, SJ and HM designed the study, JSM, SJ and FL collected data, JSM and HM conducted the data analysis and all authors contributed to preparation of the manuscript.

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Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Dataset S1 Published literature and databases used in this study.

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Dataset S2 The species examined, their maximum heights and their xylem traits.

Fig. S1 Effect of average number of bars per perforation plate on the relationships between vessel diameter, maximum plant height and climate.

Table S1 *T*-values from one-way PGLS testing for the effect of pit pore vestures and perforation plate morphology on xylem traits, maximum plant height and climate regime.

Table S2 *T*-values from two-way PGLS testing for the effect of pit pore vesturing on the relationships between vessel diameter, maximum plant height and climate.

Table S3 Slopes and intercepts for one-way PGLS used to visualize differences across intervessel pit, and perforation plate morphology.

Table S4 *T*-values from two-way categorical PGLS testing for the effect of perforation plate morphology on the relationships between vessel diameter, maximum plant height and climate.

Table S5 *T*-value for one-way PGLS for effects of vestures on wood density for 357 angiosperm species.

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