5-2010

Early Vessel Evolution and the Diversification of Wood Function: Insights from the Malagasy Canellales

Patrick Joseph Hudson
University of Tennessee - Knoxville, phudson2@utk.edu

Recommended Citation

Hudson, Patrick Joseph, "Early Vessel Evolution and the Diversification of Wood Function: Insights from the Malagasy Canellales. " Master's Thesis, University of Tennessee, 2010.
https://trace.tennessee.edu/utk_gradthes/632

To the Graduate Council:

I am submitting herewith a thesis written by Patrick Joseph Hudson entitled "Early Vessel Evolution and the Diversification of Wood Function: Insights from the Malagasy Canellales." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Taylor S. Feild, Major Professor

We have read this thesis and recommend its acceptance:

Randall Small, C. Darrin Hulsey

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Patrick Joseph Hudson entitled “Early vessel evolution and the diversification of wood function: Insights from the Malagasy Canellales.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Taylor Feild , Major Professor

We have read this thesis
and recommend its acceptance:

Randall Small

C. Darrin Hulsey

Accepted for the Council

Carolyn R. Hodges
Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Early Vessel Evolution and the Diversification of Wood Function: Insights from the Malagasy Canellales

A Thesis Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Patrick Hudson
May 2010

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. Taylor Feild for his support and guidance during my tenure at the University of Tennessee- Knoxville, and to Dr. Randy Small, Dr. Darrin Hulsey and Dr. Stan Wullschleger for serving as my committee.

I must recognize the Malagasy government agencies (ANGAP, the Direction des Eaux et Foret) and PBZT for granting permission for field research. I owe thanks to L. Andriamehefarivo, C. Birkinshire, and the staff of the Missouri Botanical Garden in Antananarivo, Madagascar, for invaluable assistance in logistical planning and permit preparation. In the field, Mr. M. Ramahazaka and the porters of Befingotra, as well as J. Ratsimilaka and the porters of Manatenaina, are recognized for assistance in transporting equipment and in identifying plants. I thank the people of the village of Befingotra for their warm and gracious reception. Transportation to Andapa, Befingotra and Manatenina was provided by Mr. J. Dedieu Tsilanizara and his son, to whom I am especially grateful. I also acknowledge Mr. E. Mathieu for a welcome lift and information about *Takhtajania*. Many thanks to N. Rowe, T. J. Brodribb, H. S. Feild, C. T. Feild, and D. S. Chatelet for their helpful comments on the manuscript. Finally, I would like to thank my parents for their continued support of my pursuit of education.

This research was supported by a NSF grant (IOB-0714156).

ABSTRACT

Xylem vessels have long been proposed as a key innovation for the ecological diversification of angiosperms by providing a breakthrough in hydraulic efficiency to support high rates of photosynthesis and growth. However, recent studies demonstrated that angiosperm woods with structurally ‘primitive’ vessels did not have greater whole stem hydraulic capacities as compared to vesselless angiosperms. As an alternative to the hydraulic superiority hypothesis, the heteroxylly hypothesis proposes that subtle hydraulic efficiencies of primitive vessels over tracheids enabled new directions of functional specialization in the wood. However, the functional properties of early heteroxyllous wood remain unknown. We selected the two species of Canellales from Madagascar to test the heteroxylly hypothesis because Canellaceae (represented by *Cinnamosma madagascariensis*) produces wood with vessels of an ancestral form, while Winteraceae, the sister-clade (represented by *Takhtajania perrieri*) is vesselless. We found that heteroxylly correlated with increased wood functional diversity related mostly to biomechanical specialization. However, vessels were not associated with greater stem hydraulic efficiency or increased shoot hydraulic capacity. Our results support the heteroxylly hypothesis and highlight the importance integrating a broader ecological context to understand the evolution of vessels.

TABLE OF CONTENTS

LIST OF FIGURES

This thesis is revised based on a paper published by Patrick Hudson, Jaqueline Razanatsoa and Taylor Feild:

Hudson, P.J., J. Razanatsoa, and T.S. Feild. 2010. Early vessel evolution and diversification in wood function: Insights from the Malagasy Canellales. *American Journal of Botany* **97**(1): 80-93.

My primary contributions include (i) assistance in development of the questions addressed and hypotheses tested, (ii) travel to research localities and collection of physiological data, (iii) collection of anatomical data, (iv) data analysis, (v) assistance in figure construction, writing and editing.

INTRODUCTION

The early evolution and rise to ecological dominance by flowering plants (angiosperms) during the Mesozoic (130 to 65 million years ago) marked a profound change in the water- and carbon-use capacity of global vegetation (Bond, 1989; Becker, 2000; Feild and Arens, 2005, 2007; Boyce *et al.*, 2009; Feild *et al.*, 2009a). Specifically, the vast majority of ecologically important angiosperms today function at rates of leaf CO_2 and transpiration that are considerably greater than all other known living and extinct land plant clades (Bond, 1989; Brodribb and Feild, 2000; Pammenter *et al.*, 2004; Brodribb *et al.*, 2007; Boyce *et al.*, 2009). The high productivity and transpiration that angiosperm leaves can supply has (1) founded diverse biotas that directly or indirectly require angiosperm productivity, (2) may have entrained increased rates of forest turnover, and (3) irreversibly changed rates of global biogeochemical cycles through increased nitrogen use and weathering (Knoll and James, 1987; Volk, 1989; Grimaldi, 1999; Moreau *et al.*, 2006; Crepet, 2009).

How and when angiosperms evolved their emblematic high rates of photosynthesis and growth remain enigmatic. However, the prerequisite that high CO_2 uptake requires high transpiration means that understanding how the water conducting pipeline diversified during early angiosperm evolution represented a critical factor that enabled the evolution of angiosperm hyperproductivity (Sperry, 2003; Pammenter *et al.*, 2004; Sperry *et al.*, 2006; Brodribb *et al.*, 2007; Feild *et al.*, 2009a). In this vein, xylem vessels have been viewed as an important feature of the vascular network that helped spark the rapid ecological sweep of angiosperms across the planet during the Cretaceous (Bailey, 1944; Carlquist, 1975; Doyle and Donoghue, 1986; Bond, 1989; Crane and Lidgard, 1989; Lupia *et al.*, 1999; Sperry, 2003; Pammenter *et al.*, 2004; Brodribb *et al.*, 2005; Feild and Arens, 2005; McElwain *et al.*, 2005). Vessels, by virtue of their larger conduit sizes, furnish a greater capacity to conduct water than tracheids—their evolutionary antecedents (Brodribb and Feild, 2000; Sperry, 2003; Sperry *et al.*, 2006). In addition, increases in the efficiency of the xylem by vessels may enable the same hydraulic flow for a given amount of transpiring leaf area supported, but with less investment in wood (Sperry, 2003; Brodribb *et al.*, 2005; Feild and Arens, 2007). Thus, vessels have been pointed to as innovations that allowed early angiosperms to take advantage of falling atmospheric CO_2 during their Cretaceous radiation as compared to vesselless plants with more costly stems (McElwain *et al.*, 2005). The hypothesis that angiosperms vessels resulted in increased flow capacity has in large part formed a conceptual cornerstone for an influential hypothesis that early angiosperms outcompeted most nonangiosperms during the Mesozoic (Doyle and Donoghue, 1986; Bond, 1989; Becker, 2000; Lupia *et al.*, 1999; Pammenter *et al.*, 2004; Feild and Arens, 2005; McElwain *et al.*, 2005; Lusk *et al.*, 2007).

Though viewed as appearing early in angiosperm evolution (Doyle and Donoghue, 1986; Bond, 1989; Pammenter *et al.*, 2004; McElwain *et al.*, 2005), extensive anatomical evidence suggested that highly efficient vessels, defined as those consisting of long tubes with thousands of short and wide elements interconnected by simple perforation plates, were rare at the base of the extant angiosperm phylogeny as well as during the Cretaceous (Frost, 1930; Bailey, 1944; Carlquist, 1975, 2004; Wheeler and Baas, 1991; Carlquist and Schneider, 2002). Instead, vessels of many basal lineages exhibited a range of small anatomical departures from vesselless angiosperm tracheids. Such vessels, referred to as "basal vessels" (Sperry *et al.*, 2007), possessed incompletely lysed pit membranes, had little differentiation between lateral pitting and endwall pitting of vessel elements, and contained elongated scalariform perforation plates. Compared to the vessels of most derived angiosperm clades, basal vessels appear to be less streamlined for water flow (Bailey, 1944; Carlquist, 1975, 2004; Carlquist and Schneider, 2002). Supporting that hypothesis, measured hydraulic capacities of woods with basal vessels overlapped with those of vesselless angiosperm woods, and these capacities were much lower than woods of derived eudicots with vessels bearing simple perforation plates (Sperry *et al.*, 2007). Hydraulic capacity in these comparisons was defined as hydraulic conductivity (K_H) divided by the cross-sectional area of xylem ($K_S = K_H$/sapwood area; Sperry *et al.*, 2007).

If early vessels did not provide a leap in hydraulic efficiency over vesselless wood at the whole stem level, then what processes favored early vessel evolution in angiosperms? One untested hypothesis is that primitive angiosperm vessels permitted new developmental potential for xylem specialization (Feild and Arens, 2007; Sperry *et al.*, 2007; Feild *et al.*, 2009a). For example, on a per xylem conduit basis, hydraulic measurements indicated that basal vessels were on average more efficient than angiosperm tracheids (Hacke *et al.*, 2007; Sperry *et al.*, 2007). Therefore, fewer hydraulic cells appear to be necessary for a given K_S in woods with basal vessels. Fewer cells dedicated to hydraulics in a given volume may permit the wood to function in other ways because K_S would not be decreased by a loss of hydraulically dedicated wood volume. New wood functional properties promoted by vessels could result from the evolution of new cell types and/or altered proportions of cells dedicated to storage, radial transport, and mechanical functions relative to vesselless plants (Feild *et al.*, 2009a). The manifestation of this effect, which vessels are a part of, is the broad phylogenetic trend of increasing heteroxylly with angiosperm diversification (Bailey, 1944; Carlquist, 1975, 2004; Wheeler and Baas, 1991; Baas and Wheeler, 1996; Carlquist and Schneider, 2002). However, the specific functional features in the wood that were involved during the early evolution of heteroxyllous wood remain unknown.

The magnoliid lineage, Canellales, consisting of vessel-bearing Canellaceae (five to six genera, ~21 species) and its vesselless sister-group Winteraceae (four genera, ~65 species, Feild *et al.*, 2000; Karol *et al.*, 2000; Salazar and Nixon, 2008), represents a unique comparative system to dissect the complex web of functional responsibilities of wood that were involved in early vessel evolution (Feild *et al.*, 2002; Baas *et al.*, 2004; Rowe and Speck, 2004; Pratt *et al.*, 2007; Chave *et al.*, 2009). A comparative analysis of vessel structure–function evolution conducted within a specific lineage offers the advantage of reducing the number of independent variables bearing on wood evolution to potentially expose a finer decomposition of the functional processes involved in trait evolution among closely related taxa (Edwards and Donoghue, 2006; Edwards, 2006; Bhaskar *et al.*, 2007; Pratt *et al.*, 2007). Although extant Canellaceae and Winteraceae radiated in divergent biogeographic realms (Doyle, 2000; Karol *et* *al.*, 2000; Salazar and Nixon, 2008), the eastern tropical montane rainforests of Madagascar represent the only region where the distributions of Canellaceae (*Cinnamosma*) and Winteraceae (*Takhtajania*) come into close ecological contact (Schatz, 2000; Feild *et al.*, 2002). The exceptional nature of the range overlap in Madagascar suggested that a comparative study of Canellales from the region might provide insights on the ecophysiological consequences of early vessel evolution. Such a suggestion was made because occurrence in the same climatic regime and biogeographic context may permit a clearer view of the possible ecophysiological advantages of early vessels (Feild *et al.*, 2002). Although some systematic anatomy has been presented on Malagasy Canellales (Wilson, 1960; Carlquist, 2000), no comparative data exist on how the functional responsibilities of the xylem relate to tracheary element structure and ecological preference.

Our goal in the current study was to understand how early vessel evolution was integrated with overall wood function by comparing the growth habits, stem hydraulic and biomechanical structure–function relations, as well as shoot ecophysiological performances of two species of Canellales with and without vessels. We investigated three questions: (1) Do xylem vessels enable greater hydraulic efficiency relative to tracheids? (2) What are the functional consequences of an early experiment in heteroxylous vasculature evolution? (3) How are vesselless and vesselled woods related to leaf function? We discuss how our results bear on the early selective pressures for the tracheid–vessel element developmental transition in early angiosperms.

MATERIALS AND METHODS

Field sites and study species—

We studied two species: vessel-bearing *Cinnamosma madagascariensis* Danguy (Canellaceae) and vesselless *Takhtajania perrieri* (Capuron) Baranova & J.-F. Leroy (Winteraceae). These species were investigated at two relatively close (~40 km) sites in northeastern Madagascar and from one more distant site located in the central highlands of Madagascar (Fig. 1, Table 1). *Takhtajania perrieri* is the only member of the genus and is endemic to Madagascar (Schatz, 2000). There are at least three species of *Cinnamosma* in Madagascar (Schatz, 2001). We focused on *C. madagascariensis* because the species occurs in wet, humid, evergreen, tropical forests along the eastern mountains of Madagascar, which are climatically most similar to the known populations of *Takhtajania* (Fig. 1) (Schatz, 2001). The other *Cinnamosma* species occur only in drier or warmer lowland, evergreen, tropical habitats (Schatz, 2001). However, some populations of *C. madagascariensis* occur in littoral tropical rainforests that are warmer and more evaporative than the sites we sampled (Table 1) (Bollen *et al.*, 2004). We sampled two populations having climatic conditions that came closest to the climatic zone of *Takhtajania* (Table 1). Because of logistical constraints, we only conducted stem hydraulic measurements (see below) on the population of *C. madagascariensis* from Réserve Spécial Ambohitantely (Table 1). The full complement of ecophysiological observations outlined below was made on *C. madagascariensis* from Parc National Marojejy and *T. perrieri* at Réserve Spécial Anjanaharibe-Sud (Table 1).

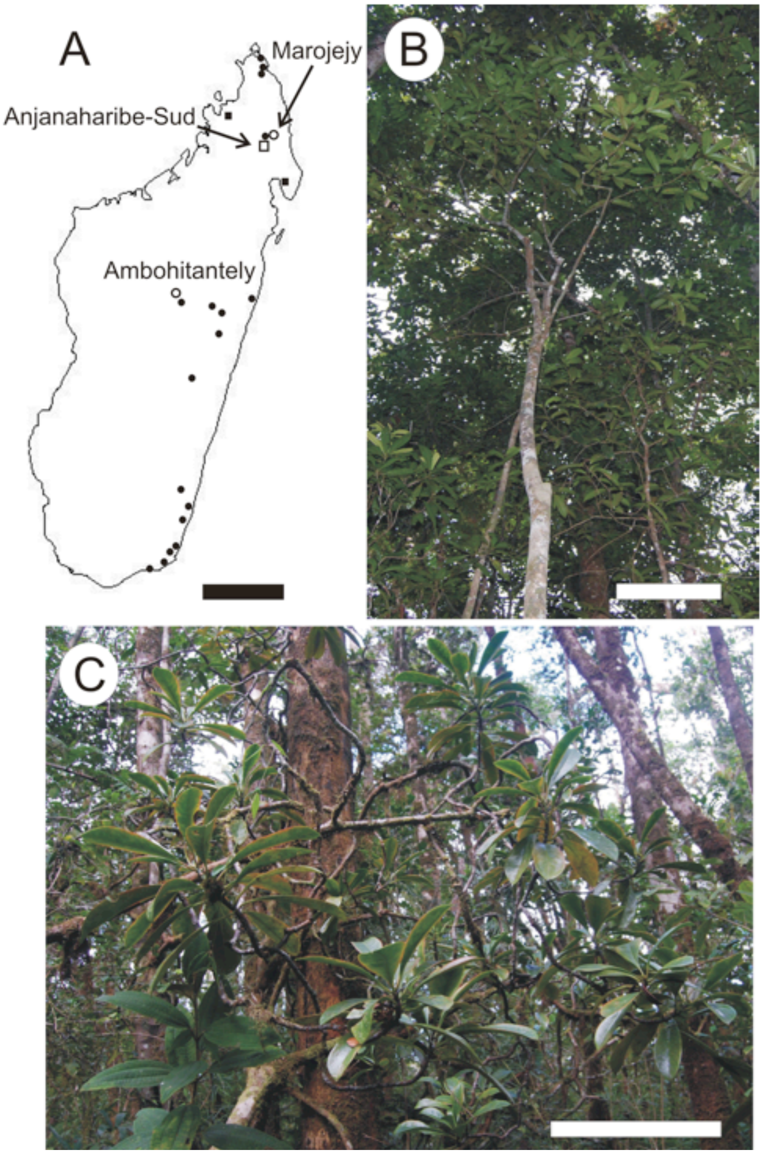

Figure 1. Study site locations and growth forms of the two species investigated. (A) Vesselless *Takhtajania perrieri* was sampled at Réserve Spécial Anjanaharibe-Sud Reserve (denoted as an open square, 14°44′S, 49°28′E, 1100 m a.s.l., 10 km from Andapa). Vessel-bearing *Cinnamosma madagascariensis* from two sites (denoted by open circles): Réserve Spécial Ambohitantely, 18°9′S 47°16′E, 1500 m a.s.l., Central Highlands and Parc National Marojejy, 14°26′S 49°45′E, 12 km from the village of Manatenina, 400–700 m a.s.l. The known distributions of *T. perrieri* (filled squares) and *C. madagascariensis* (filled circles) are provided (GBIF Data Portal 2009). (B) Growth habit of an adult *C. madagascariensis* tree from PN Marojejy. (C) Growth habit of an adult *T. perrieri* at RS Anjanaharibe-Sud. Scale bar: (A) 150 km, (B) 50 cm, (C) 30 cm.

Table 1. Details of field sites of the two species investigated. References for climatic and vegetation characteristics were: Ambohitantely (Pareliussen *et al.*, 2006), Anjanaharibe-Sud (Goodman, 1998), and Marojejy (Goodman, 2000; Raxworthy *et al.*, 2008).

Locality Information	*Cinnamosma madagascariensis* Population 1	*Cinnamosma madagascariensis* Population 2	*Takhtajania perrieri*
Locality			
Site	PN Marojejy	RS Ambohitantely	RS Anjanaharibe-Sud
Elevation (m a.s.l.)	400–700	1500	1100
Climate			
MAT (°C)	25	16.5	16
Rainfall (mm·yr^{-1})	2200	1823	3600
Cloud Input	low	moderate	high
Humidity	high	moderate	high
Vegetation characteristics	Tropical premontane rainforest	Ttropical montane rainforest	Tropical montane cloud forest
Forest Type	20–25	15–20	
Canopy Height (m)			10–15

Notes: MAT, mean annual temperature

Stem hydraulic conductivity—

We measured stem hydraulic conductivity (K_H; kg·m^{-1}·s^{-1}·MPa^{-1}) using a portable flowmeter (Brodribb and Feild, 2000). K_H was determined by measuring the pressure drop across a PEEK capillary tube (Upchurch Scientific, Oak Harbor, Washington, USA) of known conductance with the stem under a positive head pressure. We measured stems under 0.075 to 0.09 MPa using an air-filled captive air tank pressurized with a hand pump. We used an electronic manometer (± 0.001 MPa resolution; model 840081, SPER Scientific, Scottsdale, Arizona, USA) to measure the head pressure and pressure drop across the stem segment during flow. The flowmeter was filled with filtered (to 0.2 mm), degassed water from nearby streams.

Potassium and calcium ions can significantly influence stem K_H (Zwieniecki *et al.*, 2001). We were unable to control ionic concentrations for K_H measurements. However, we found that greenhouse plants of *Drimys winteri* (Winteraceae) and *Canella winterana* (Canellaceae) both displayed approximately a 25% increase in stem K_H to perfusing solutions of KCl ranging from 15 to 100 mM as compared to distilled water (T. S. Feild, unpublished observations, 2001). These results suggest that our lack of control of ionic solution concentration would not have altered relative hydraulic differences between *C. madagascariensis* and *Takhtajania*.

We cut undamaged branches with fully expanded leaves from the subcanopy. We checked for native embolism by flushing several stem segments of each species with 0.2 MPa pressurized, degassed, and filtered water using a syringe. We then observed whether bubbles were pushed out of the xylem. When plants were sampled on wet, cloudy mornings (0700–0830 hours) (Feild and Balun, 2008), we found no evidence of native embolism in both species. We cut stem segments from branches while underwater. We checked that all measured stems were longer than the longest functional conduit (i.e., 20 cm in *C. madagascariensis* as measured by air injection; Brodribb and Feild, 2000). *Takhtajania* stems, although vesselless, were measured at the same dimensions to facilitate comparisons. For *Takhtajania* and the two populations of *C. madagascariensis*, we sampled five individual plants and two branches from each individual for hydraulic measurements.

From hydraulic flux data, we calculated sapwood-area specific hydraulic conductivity (K_S; kg·m^{-1}·s^{-1}·MPa^{-1}) as K_H divided by the sapwood cross-sectional area with the pith area subtracted (Feild and Balun, 2008). Sapwood cross-sectional areas were determined on portions of measured stem tissue preserved in 50% ethanol using a Zeiss SterREO Discovery.V8 dissecting microscope (Carl Zeiss MicroImaging, Gottingen, Germany) and ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). We calculated conduit-area specific hydraulic conductivity (K_C; kg·m^{-1}·s^{-1}·MPa^{-1}) as stem K_H divided by the cross-sectional area of hydraulic lumens in a stem (Sperry *et al.*, 2007). We calculated per capita conduit specific conductivity, defined as the average K_H attributable to vessels and tracheids in *C. madagascariensis* as well as tracheids of *Takhtajania*. In these calculations, we assumed that fibers did not participate in hydraulic flow. Next, we divided K_H by the number of conduits in a measured stem that were counted using criteria described below to specify cell types. We determined the leaf-area specific hydraulic conductivity (K_L, kg·m^{-1}·s^{-1}·MPa^{-1}) as K_H divided by the surface area of leaves distal to the cut end of the segment. Using digital image analysis software on scanned images of leaves (ImageJ, NIH Image, Bethesda, MD, USA), we quantified leaf area. Shoot Huber value (HV) was calculated as sapwood area divided by the distal leaf area.

Xylem anatomy—

We isolated tracheary elements using acetic acid, hydrogen peroxide, and heat (Ruzin, 1999). Macerated wood was stained in 1% aqueous safranin, vortexed, and tracheary cells were pipetted onto slides. We imaged cells with an upright microscope (Axio Imager M1, Zeiss), and cells were analyzed for anatomical characteristics using ImageJ. The observations described below were made on three stems for each species.

For tracheary elements (including fibers of *C. madagascariensis*), we quantified mean length, lumen diameter, wall thickness, and the percentage of surface area devoted to pits (Hacke *et al.*, 2007). Diameters and wall thicknesses of *T. perrieri* and *C. madagascariensis* conduits were derived from transverse sections from three stem segments of each species imaged from a microscope and 250 to 300 measurements of each variable per species (Hacke *et al.*, 2007). Three cross sections of three stems for each species were imaged at 200× to estimate areas of cell types. Pit area was assessed on 50 cells for each class at 400× to 600×. To quantify tracheary element diameter, we measured each element's lumen in a stem cross section was traced in ImageJ to determine the area of the conduit lumen. Then, the diameter of the circle with equal area was calculated to produce the lumen diameter for all cell types in both species. Tracheary element diameters were analyzed for frequency distribution in diameter classes of 5-mm increments for the relative contribution of each diameter class to the sum of all conduits raised to the fourth power of the radius. The sum was proportional to xylem hydraulic conductance, assuming that xylem conduits acted as ideal capillaries (i.e., Hagen–Poiseuille equation; Feild *et al.*, 2002). Mean hydraulic diameters of tracheary elements were calculated as described previously (Hacke *et al.*, 2007).

We distinguished vessel elements, fibers, and tracheids in *C. madagascariensis* on the basis of statistical differences in wall thicknesses that correlated with tracheary element pitting such that we could categorize the frequencies of cell types based on thickness measurements. Distinguishing tracheids from fibers is difficult because of blurry distinctions in pitting between the two cell types (Carlquist, 1975, 2004). However, we found that such a situation did not occur in *C. madagascariensis*. Tracheids had consistently different wall thicknesses and pitting characteristics from fibers. Using these assessments, we were able to normalize the densities of cell types to a 1-mm^2 of sapwood for both species. With these results, we expressed K_C in *Cinnamosma* on the basis of vessel- and tracheid-conduit areas. Finally, we quantified cross-sectional differences in ray tissue and xylem parenchyma between the two species on digital images (Pratt *et al.*, 2007).

***Wood density and stem biomechanics*—**

Wood density (ρ_{wood}) was calculated as dry mass of stem wood divided by fresh volume ($g \cdot cm^{-3}$) using volumetric displacement. We cut in half and removed the pith and bark of five 3-cm long and 1-cm thick pieces of wood. Next, a syringe needle was used to submerge each sample in a 60 mL beaker on a field portable balance (± 0.001 g resolution, Denver Instruments, Denver, Colorado, USA), and the displacement of water measured. We dried the samples at 100°C in an oven overnight and measured them for dry mass. We accounted for the effect of temperature on the density of water by normalizing values to 20°C.

The elastic modulus of wood from each species was calculated using four points bending tests (Chapotin *et al.*, 2006). The modulus of elasticity (E, GPa) was

$$E = [Fa(3L^2 - 4a^2)/(24yI)]$$

where F was the force (N) applied to each point, L (m) was the distance between the two supports, a (m) was the distance between the support and the point of force application, I was the second moment of area of the stem section (m^4), and y (m) was the vertical deflection at the center of the segment. I was calculated by approximating the stem cross section as an ellipse (Niklas, 1992). An appropriate force was used such that vertical deflection was small with respect to the length of the stem segment (less than 5%). We selected five straight stems for each species, cut the stems to 15 cm in length, and stripped them of bark before bending tests. Bark was removed to limit measured E to a mechanical property of the wood. Stem diameters along the length were then measured with a micrometer to the nearest 0.1 mm. Pith was ignored in our calculations, as it should have only a minimal contribution to the mechanical properties of the stem (Niklas, 1992). To relate biomechanical measurements to measured stem hydraulic capacities, we conducted our bending tests on the most distal branches for both species.

Measurements of xylem energy content—

Wood samples used for ρ_{wood} were burned in an LECO AC-350 isoperibol calorimeter (LECO, St. Joseph, Michigan, USA) to determine their heat of combustion. Samples were ground to a fine powder and then dried again at 75°C to a constant mass. Approximately 0.5 g of sample was combusted in the bomb calorimeter in accordance with the ASTM D240-02 (2007) standard test method for heat of combustion. Heat of combustion was expressed on a fresh volumetric basis, and dry mass basis represented a measure of wood construction cost. Five samples for energetic content from five different stems were measured for each species.

Leaf ecomorphic traits—

Because of the remoteness of our field sites, we were unable to measure leaf gas exchange performances. Therefore to infer relative differences in leaf gas-exchange capacity, we used a well-supported anatomical proxy of leaf water loss and CO_2 uptake (Parlange and Waggoner, 1970; Brodribb *et al.*, 2007; Boyce *et al.*, 2009; Kaiser, 2009). We inferred maximum leaf water vapor conductance (mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) from the structure of stomatal system and a previously published one-dimensional diffusion equation as

$$g_{smax} = 1/\{[(d/\pi ab) + \ln(4a/b)/\pi a]/DN\}$$

where a = guard cell pore length/2; b = guard cell pore width/2; d = guard cell pore depth; D = diffusivity of water vapor in air; N = guard cell density (Parlange and Waggoner, 1970; Brodribb and Hill, 1997). Measurements of d were made on cross sections of formalin-acetic acid-alcohol (FAA, made by mixing 1 part formalin, 0.5 parts acetic acid and 5 parts ethanol in 3.5 parts DI water) fixed leaves embedded in a plastic resin (JB-4, Polysciences, Warrington, Pennsylvania, USA). The resin was polymerized according to the manufacturer's instructions. We sectioned samples (7 mm thick) using a tungsten carbide blade on a rotary microtome (RM2245, Leica Microsystems, Wetzlar, Germany). Twenty guard cell pores from each species were imaged at 400× and measured with Image J. We determined a and N on safranin-stained, macerated cuticles that were isolated by using an accepted procedure (Feild *et al.*, 2009b). Fifty guard cells were imaged at 400× and measured with Image J, and ten 4 mm × 4 mm sheets of cuticle were counted for N at 200×. The value b was taken as one-third of the guard cell pore length (Osborne *et al.*, 2004). Geometric criteria for measuring guard cell pore length and depth followed Lawson and colleagues (1998).

The value g_{smax} calculated from stomatal pore dimensions correlates well with CO_2 uptake and leaf hydraulic conductance (Brodribb and Hill, 1997; Kaiser, 2009). However, this equation does not take into account other resistors, such as intercellular conductance, the presence of internal cuticle, and ornamentations to the stomatal pore complex that pare back effective diffusional aperture (Brodribb and Hill, 1997; Kaiser, 2009). Because *C. madagascariensis* and *T. perrieri* did not possess stomatal plugs (Bongers, 1973; Feild *et al.*, 2000), g_{smax} based on stomatal pore dimensions is likely to be a relatively good approximation of leaf maximum gas-exchange capacity (Brodribb and Hill, 1997; Kaiser, 2009).

In addition, we made observations on the cross-sectional anatomy of leaves to test for differences in leaf structure related to the optical processing of photosynthetically active radiation, including mesophyll cell structure and leaf thickness (Smith *et al.*, 1997; Smith and Hughes, 2009). Finally, leaf mass per area (LMA, $g \cdot m^{-2}$) and leaf size (cm^2) were determined for 20 leaves of each species. All leaves measured were undamaged and fully expanded. Leaves were scanned on a portable scanner, measured using ImageJ, and dry mass determined after drying for 2 d at 65°C.

Pressure–volume analysis of drying leaves—

Pressure isopleths (bulk leaf water potential [Ψ_W] vs. relative water content [RWC]) of leaves were assessed by repeated measures (four to six observations per leaf) of mass and Ψ_W using accepted procedures (Sack *et al.*, 2003). We collected seven fully expanded and undamaged leaves for each species at maximum field hydration before sunrise (0600 to 0700 hours), and checked Ψ_W to verify that it was greater than 0.05 MPa before beginning a pressure–volume (PV) curve. From the initial point, leaves were desiccated, and alternately weighed and Ψ_W measured until the leaf was well below the turgor loss point. We weighed leaves on a field portable balance, and we determined Ψ_W with a pressure chamber. Our field-portable pressure chamber system consisted of a pressure chamber (PMS-1000 chamber, Corvallis, Oregon, USA), a digital pressure gauge (± 0.01 bar, Ashcroft, Stratford, Connecticut, USA), and 1-L volume stainless steel cylinder (Swagelok, Olon, Ohio, USA) in series with a high-pressure hand pump (three-stage pump PCP, Axsor, Sweden, up to 7 MPa). A hand lens (10×) was used to observe the cut end of the petiole for the exact moment of sap exudation. We determined the leaf dry mass after 48 h of drying at 65°C. Fresh masses and dry mass values were used to calculate RWC (Sack *et al.*, 2003). We determined the Ψ_W and RWC at the turgor loss point using accepted curve-fitting procedures of PV data (Sack *et al.*, 2003) in the program Sigma-Plot (Jandel Scientific, SPSS, Chicago, Illinois, USA).

Statistical analyses—

All data were analyzed using a statistical analysis program (PASW Statistics 17, SPSS). Mann–Whitney *U* tests were applied to the ecophysiological and structural data to test for statistically significant differences between species.

RESULTS

Growth forms—

Cinnamosma madagascariensis occurred as a treelet to small tree up to 8 m tall with a single stem bole up to 13 cm diameter at breast height (dbh). No evidence of basal sprouting was observed in either of the populations of *C. madagascariensis* that we studied. The canopies of *C. madagascariensis* trees were wide and deep with several interlacing, distichously branched distal branches (3 to 5 mm in diameter on most distal branches with little pith; Fig. 1B). Leaves were retained along much of the length of terminal shoots. Internodes of *C. madagascariensis* (1 to 2 cm long) were longer than *T. perrieri*. Plants occurred on flat areas of forested slopes in the understory to subcanopy and away from gaps.

In contrast, *Takhtajania perrieri* formed a shorter tree up to 5 m tall with stems up 10 cm dbh. All adult plants we observed consisted of multiple trunks. Most plants sprouted from the base, with one to eight basal and pithy shoots. Plants occurred near trails, on cyclone-influenced ridge crest forests, and gap-phase vegetation. The canopies formed by *T. perrieri* were irregular due to leaning trunks, and the canopies were sparsely leaved with irregularly oriented candelabriform branches bearing leaves in tufted whorls at the tips (Fig. 1C). The terminal branches of *Takhtajania*, from 8 to 15 mm, were thicker than *C. madagascariensis* and consisted primarily of pith. Internodes of *T. perrieri* were short (0.4 to 0.9 mm long), and numerous leaf scars subtended each whorl.

Stem xylem anatomy and tracheary element structure—

In transverse section, we found, as reported earlier, that the xylem of *Takhtajania perrieri* was homoxylous, with a dominance by tracheids of uniform lumen diameter and wall thickness (Fig. 2A, Table 2); (Carlquist, 2000). In contrast, transverse sections indicated that the wood of *Cinnamosma madagascariensis* was more heteroxylous (Fig. 2B) (Wilson, 1960). Compared to *T. perrieri*, axial cell types ranged widely in mean lumen diameter (from 8.9 μm in fibers to 36.7 μm in vessels) and mean wall thicknesses (1.5 μm in vessels to 4.3 μm in fibers; Table 2). In *Takhtajania*, we confirmed previous observations that tracheid endwalls had uniseriate and biseriate circular pits as well as occasionally dense scalariform pitting at the endwalls (Fig. 1C) (Carlquist, 2000). In *C. madagascariensis*, vessels were characterized by variably shaped, elongated, many-barred scalariform perforation plates, which accounted for on average 64.4% ± 13.8 SD of element length.

Tracheids of *Takhtajania* and *Cinnamosma* differed in structure (Table 2, Fig. 2C and D). *Takhtajania*'s tracheids were twice as long, 27% wider in lumen diameter, and had approximately 50% more surface area devoted to pits compared with *C. madagascariensis* tracheids (Table 2). Tracheid wall thicknesses of both species tracheids were similar (Table 2). Unlike *Takhtajania* tracheids, however, no *C. madagascariensis* tracheids possessed scalariform pitting. Compared to vessel elements and fibers, *C. madagascariensis* tracheids occupied an intermediate position in wall thickness as well as the area of an individual element devoted to pits (Table 2). The lateral pitting of these tracheids was most often uniseriate, with some biseriate pitting (Fig. 2D).

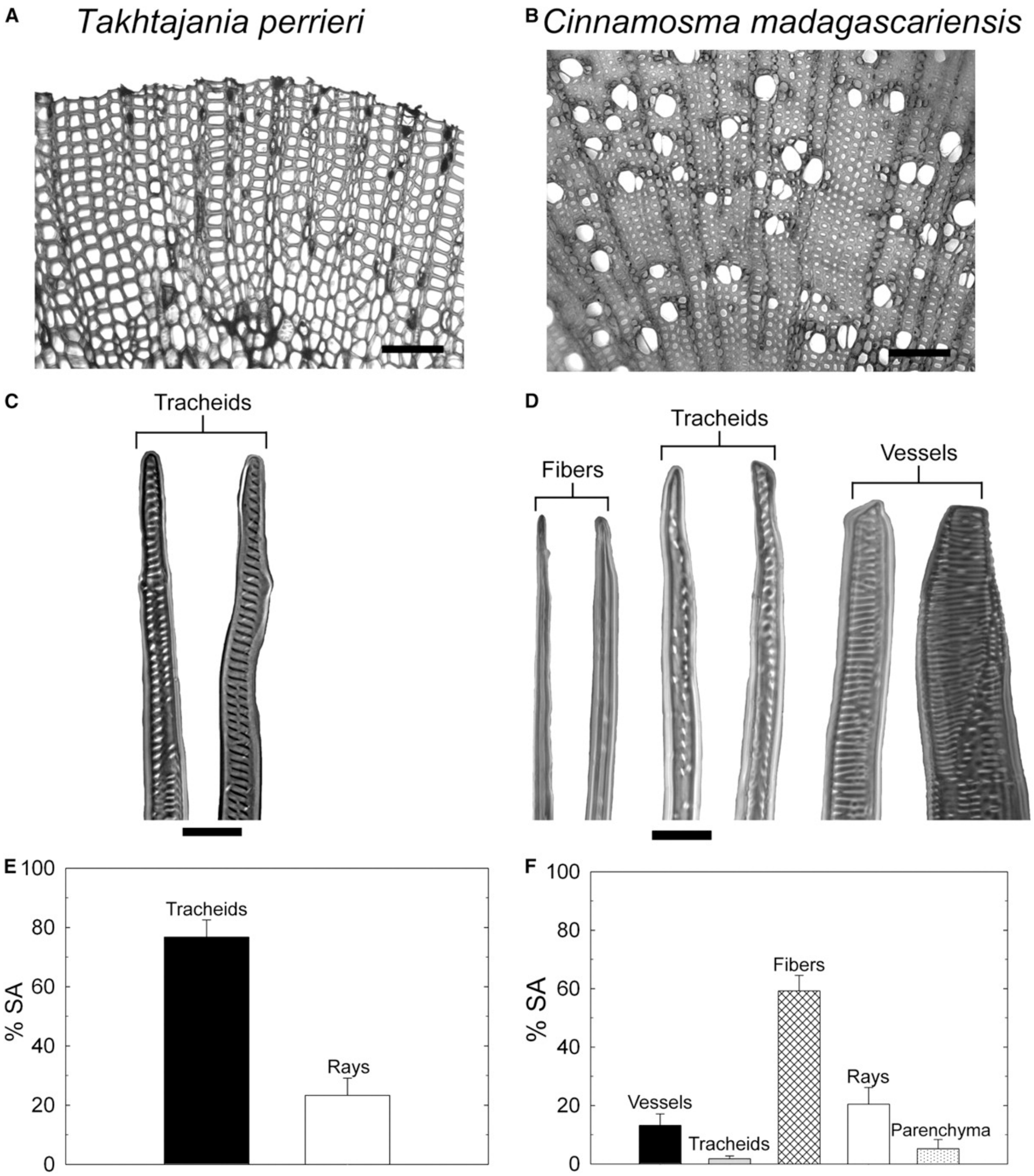

Figure 2. Comparative cross-sectional anatomy, tracheary element anatomy, and cell contents of *Takhtajania perrieri* and *Cinnamosma madagascariensis*. (A) Transverse section of *T. perrieri* wood, showing tracheids of nearly uniform diameter. (B) Transverse section of *C. madagascariensis* wood illustrating the greater variation tracheary element diameters. (C) Endwalls of macerated *T. perrieri* tracheids. The two tracheids illustrate the extremes of endwall pitting anatomies that we observed. (D) Variations in endwall pitting of fibers, tracheids, and vessel elements in *C. madagascariensis*. (E, F) The percentages of the transverse surface area (SA) contributed by different cell types for (E) *T. perrieri* and (F) *C. madagascariensis*. We classified cell types in *C. madagascariensis* using wall thickness measurements as described in the Materials and Methods. Scale bar: (A, B) 100 μm, (C, D) 40 μm.

Table 2. Comparative quantitative anatomical characteristics of the stem secondary xylem from vessel-bearing *Cinnamosma madagascariensis* (Marojejy population only; Table 1) and vesselless *Takhtajania perrieri*. Letters denote significance of difference among variables of at least $P < 0.01$ (Mann-Whitney U), and errors are standard deviations about the mean. Sample sizes for individual means are reported in the Material and Methods section. NA denotes not applicable.

	Cinnamosma madagascariensis			*Takhtajania perrieri*
Tracheary Element Trait	Vessels	Tracheids	Fibers	Tracheids
Element Length (μm)	948 ± 135^{A}	840 ± 127^{A}	815 ± 173^{A}	1779 ± 291^{B}
Mean hydraulic diameter (μm)	37.0 ± 1.9^{A}	16.4 ± 1.7^{A}	NA	21.2 ± 1^{A}
K_H per conduit ($\times 10^{-10}$)	71.0 ± 1.6^{A}	6.6 ± 2.4^{B}	NA	6.36 ± 1^{B}
Element diameter (μm)	37 ± 9.5^{A}	13.4 ± 2.5^{B}	8.9 ± 0.8^{C}	18.2 ± 4.1^{D}
Element density (number mm^{2})	160 ± 41^{A}	76 ± 38^{B}	2446 ± 617^{C}	1421 ± 137^{D}
Wall thickness (μm)	1.5 ± 0.1^{A}	2.5 ± 0.4^{B}	4.3 ± 0.2^{C}	2.64 ± 0.2^{B}
% Wall area with pits	25.1 ± 5^{A}	18.1 ± 4^{B}	4.1 ± 2^{C}	33.2 ± 7^{D}
Pit membrane area (μm^{2})	Perforation plate: 35.5 ± 5.0 NA Lateral pitting: 26.4 ± 10.7^{A}	30.9 ± 7.3^{A}	17.7 ± 5.5^{B}	56.8 ± 13.9^{C}
Perforation plate length (μm)	296.2 ± 85.3	NA	NA	NA
Number of bars per perforation plate	88 ± 26.3	NA	NA	NA

Tracheids composed 77% ± 6 SD of the transverse surface area of *Takhtajania*'s stem xylem, with 23% of the surface area occupied by rays (Fig. 2E). In contrast, we found that fibers dominated transverse xylem surface area (65% ± 5 SD) in *C. madagascariensis*, with rays (20 ± 6% SD), water-conducting cells (15% total; 13% ± 4 SD, vessels; 2% ± 1 SD, tracheids), and axial parenchyma (5% ± 3) at smaller fractions (Fig. 2F). Vasicentric parenchyma surrounded each vessel or vessel group (Fig. 2F).

Stem hydraulics, biomechanics, and wood energy content—

Takhtajania possessed an approximately 65% greater average K_L and HV than both populations of *C. madagascariensis* (Fig. 3A, B). However, we found that average K_S of *C. madagascariensis* from Marojejy was statistically indistinguishable from that of *T. perrieri* (Fig. 3C). Mean K_S, HV, and K_L values from both *C. madagascariensis* did not differ significantly (Fig. 3). However, mean K_S in branches from the *Cinnamosma* population from Ambohitantely was ~20% lower than *T. perrieri* (Fig. 3C). When normalized to conduit area, mean K_C of the two populations of *C. madagascariensis* were both nearly three times greater than *T. perrieri* (Fig. 3D). Mean K_C did not differ between the two populations of *C. madagascariensis* (Fig. 3). Vessels accounted for the majority (96%) of K_C in both populations of *C. madagascariensis* (Fig. 3E). On a per capita basis, vessels possessed an order of magnitude greater conductivity than tracheids (Table 2).

For *T. perrieri*, the largest proportion of tracheids (mean = 39% ± 14 SD) fell in the 15–20 μm diameter class (Fig. 4A). By comparison, most of the vessels (30% ± 20 SD) in

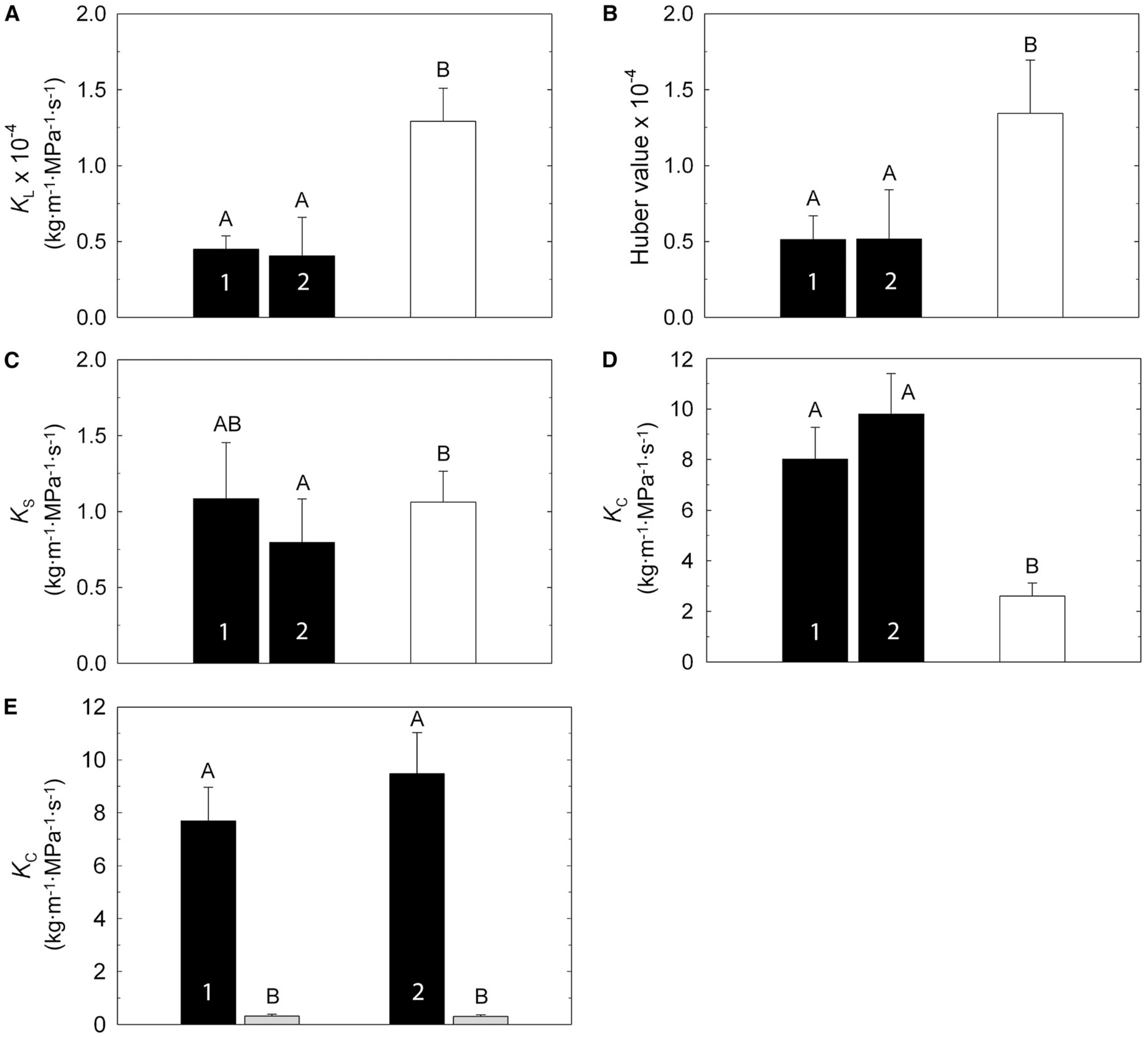

Figure 3. Comparative hydraulic performances of two populations of *Cinnamosma madagascariensis* (black plots subtended by numbers referring to population sites, Table 1) and *Takhtajania perrieri* (white plots). The following traits are depicted: (A) leaf-area specific hydraulic conductivity (K_L), (B) Huber value (HV, sapwood area divided by distal leaf area), (C) sapwood-area specific hydraulic conductivity (K_S), (D) Conduit-area specific hydraulic conductivity (K_C). In (E), the breakdown of K_C by vessel (black bars) and tracheid (gray bars) contributions for the two populations of *C. madagascariensis* are depicted. Letters denote significance at least $P < 0.05$ (Mann–Whitney *U*). Sample sizes are provided in the Materials and Methods.

C. madagascariensis occurred in the 25–30 μm diameter class (Fig. 4B). When modeled as pipes, tracheids of the 15–20 μm size class was responsible for the largest proportion of hydraulic flow (34% ± 15 SD, Fig. 4C). The highest proportion of flow (33% ± 22 SD, Fig. 4D) occurred through vessels in the 30–35 μm diameter class. Vessels accounted for 96% ± 3 SD of idealized hydraulic flow, and tracheids were responsible for 4% ± 3 SD. (Fig. 4D).

Both populations of *C. madagascariensis* possessed a 45% greater mean ρ_{wood} as compared to *T. perrieri* (Fig. 5A). We measured a 35% greater *E* in *C. madagascariensis* from Marojejy relative to *T. perrieri* (Fig. 5B). Consistent with this finding, we observed that branches bent to 40° relative to the stem axis snapped in *T. perrieri*, whereas twigs of *C. madagascariensis* did not snap with 90° bending.

The mean heat of combustion of *C. madagascariensis* dry wood on a mass basis was approximately 3% less than that of *T. perrieri* (Fig. 5C). However, when expressed on a volumetric basis, the amount of energy per volume of fresh wood in *C. madagascariensis* was 41% greater than *T. perrieri* wood (Fig. 5D).

Leaf functional traits—

Both species had similar guard cell densities, guard cell pore depth, but the mean guard cell length of *T. perrieri* was larger (Table 3). Consequently, calculated g_{smax} based on guard cell dimensions and diffusional theory of C. *madagascariensis* was 39% lower than *T. perrieri* (Table 3). In cross section, we observed that *C. madagascariensis* developed a single layer of palisade parenchyma in the mesophyll, and some cells below the layer were columnar (Fig. 6A).

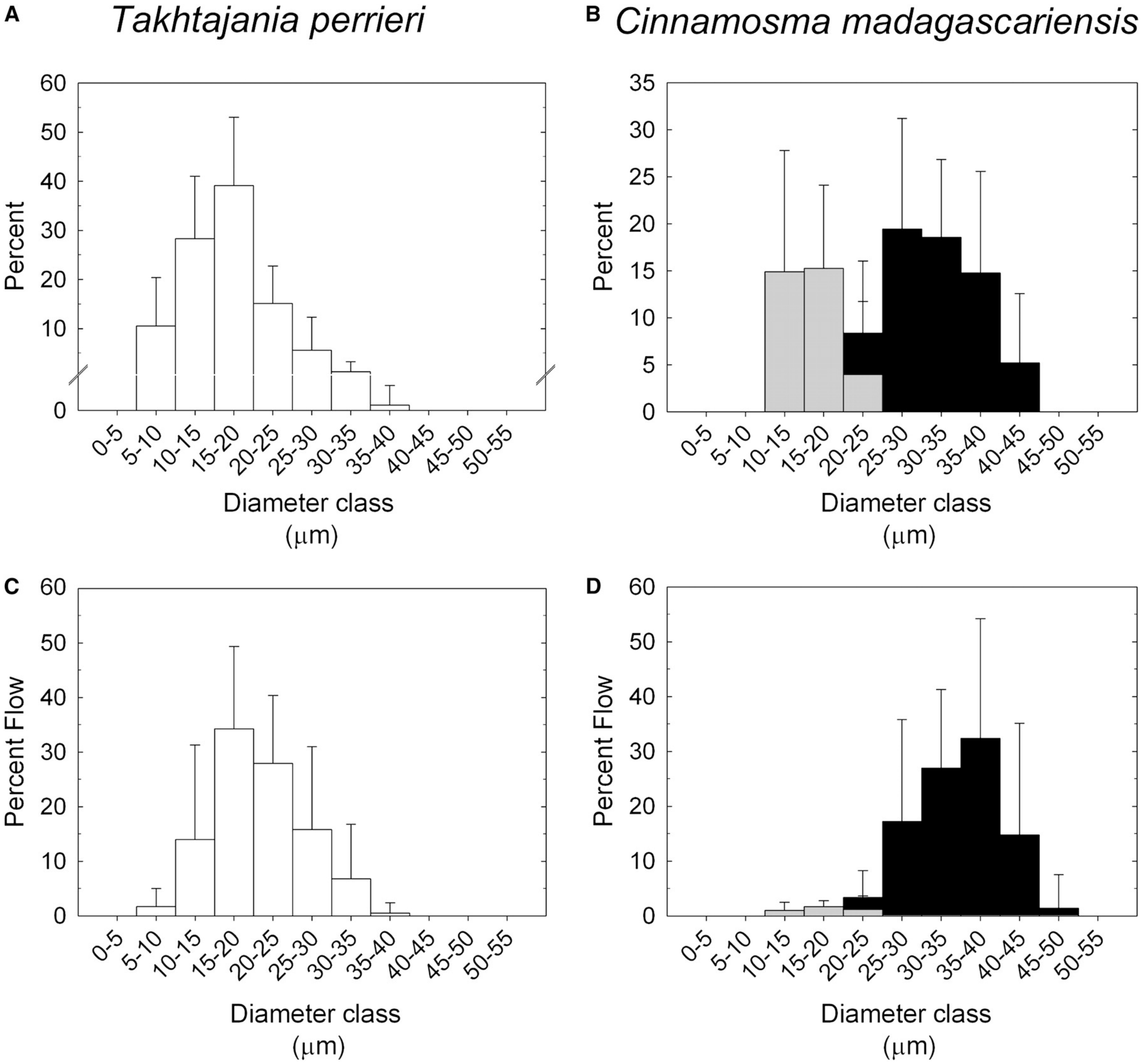

Figure. 4. Comparisons of frequency distributions and hydraulic contributions of tracheary elements in *Cinnamosma madagascariensis and Takhtajania perrieri*. Distributions of conduit diameter frequencies for (A) *T. perrieri* tracheids and (B) tracheids (gray bars) and vessels (black bars) in *C. madagascariensis*. Distributions of conduit diameter frequencies and percent contribution to calculated hydraulic flows for (C) *T. perrieri* tracheids and (D) *C. madagascariensis* tracheids (gray bars) and vessels (black bars). Sample sizes and hydraulic calculations using the Hagen–Poiseuille equation are provided in the Materials and Methods.

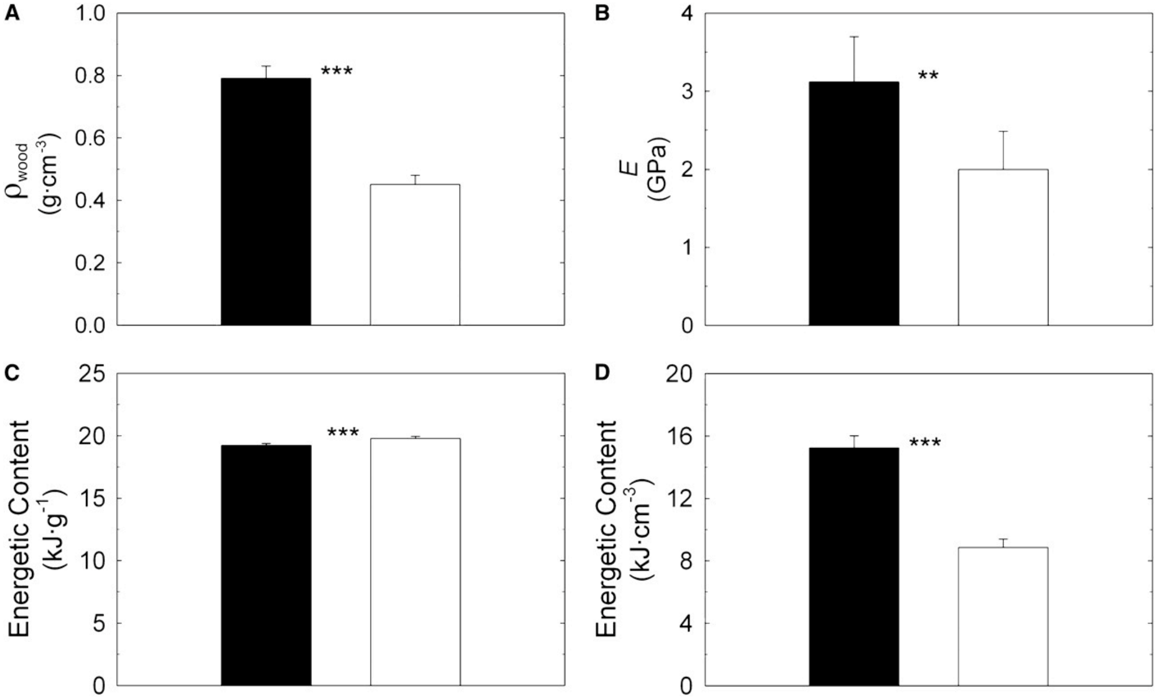

Figure 5. Wood density, biomechanical performances, and energetic contents of stem wood in *Cinnamosma madagascariensis* (black plots) and *Takhtajania perrieri* (white plots). (A) Wood density (ρ_{wood}) was greater in *C. madagascariensis* than *T. perrieri*. (B) *C. madagascariensis* stems had a greater Young's elastic modulus (*E*). (C) On a mass basis, wood of *Takhtajania* had a 3% greater energetic content than *Cinnamosma*. (D) On a fresh volumetric basis, wood of *C. madagascariensis* had a higher energetic content than *Takhtajania*. Asterisks denote significance of difference between species means: $^{**}P < 0.01$ and $^{***}P < 0.001$ (Mann–Whitney *U*). Sample sizes are provided in the Materials and Methods.

A hypodermis was present above the palisade layer (Fig. 6A). In contrast, the leaf cross section of *Takhtajania* consisted only of spongy mesophyll tissue (Fig. 6B). Leaves of *C.madagascariensis* were 25% thinner than *Takhtajania* (Table 3). Leaves of *C. madagascariensis* were smaller in leaf mass per area as compared to *Takhtajania* (Table 3), but leaves of *Takhtajania* were larger than those of *C. madagascariensis* (Table 3). In addition, the leaves of *C. madagascariensis* were more resistant to desiccation in comparison to *T. perrieri*. *Cinnamosma madagascariensis* lost turgor pressure at Ψ_W of −2.1 MPa and RWC of 86.1% vs. Ψ_W of −1.2 MPa and 93.4% RWC for turgor loss in *T. perrieri* (Fig. 6C,D).

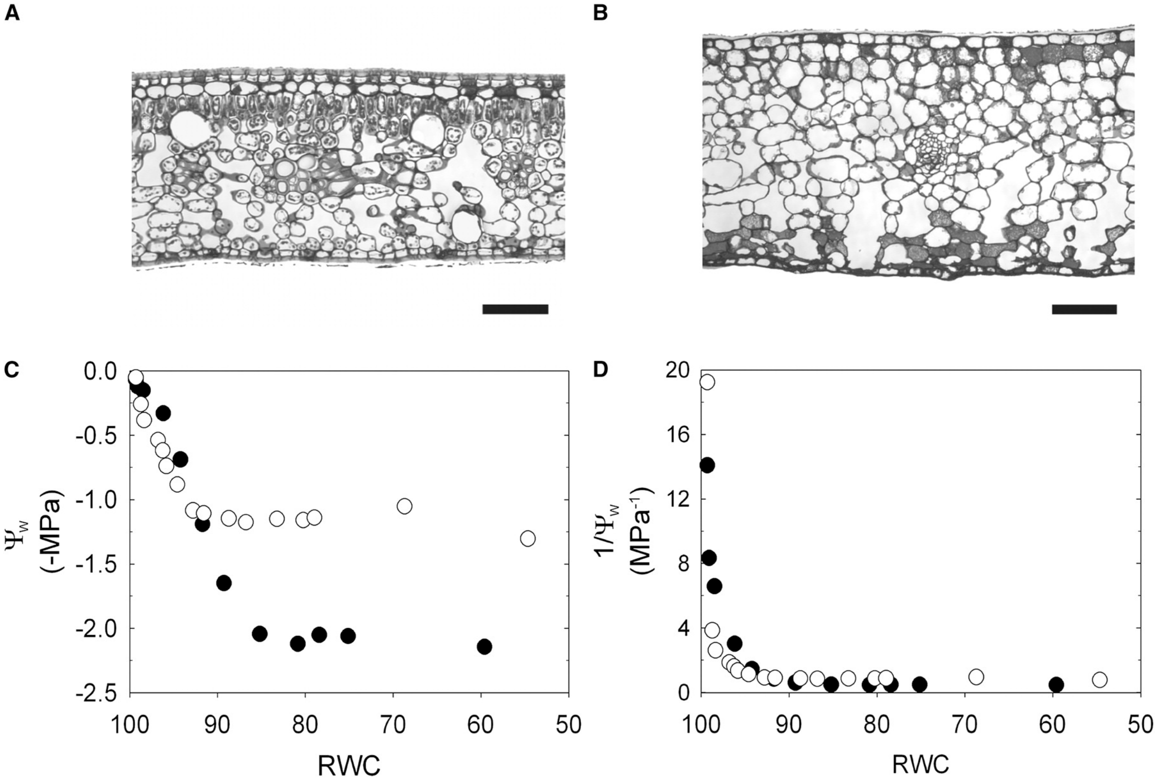

Figure 6. Comparative leaf anatomy and water relations in *Cinnamosma madagascariensis* and *Takhtajania perrieri*. In cross section, (A) *C. madagascariensis* leaves had a palisade layer as well as a hypodermis, whereas (B) *T. perrieri* leaves were composed of spongy mesophyll tissue. Scale bars: = 100 µm. (C) Moisture release and (D) pressure–volume curves demonstrate that leaves of *C. madagascariensis* (black circles) have a greater ability to maintain turgor under drought conditions than *T. perrieri* (white circles). Sample sizes are provided in the Materials and Methods.

Table 3. Comparative functional trait values (mean ± SD) of *Cinnamosma madagascariensis* and *Takhtajania perrieri* leaves. Asterisks denote significance of difference between species means: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Mann–Whitney *U*). NS denotes not significant at $\alpha = 0.05$. Sample sizes for individual means are reported in the Material and Methods.

Leaf Trait	*C. madagascariensis*	*T. perrieri*
Stomatal density (mm^{-2})	148.3 ± 7.4	147.8 ± 17.3 NS
Guard cell size (length × width, μm)	25.7 ± 2.4 x 21.2 ± 1.3	29.1 ± 2.2 x 22.5 ± 1.7***, **
Pore length (μm)	6.48 ± 0.85	8.32 ± 0.89***
Pore depth (μm)	10.16 ± 2.07	9.76 ± 0.92 NS
g_{smax} (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	125.49	203.23
Leaf size (cm^2)	32.2 ± 5.3	58.1 ± 18.3***
Leaf thickness (μm)	312.9 ± 7.7	404.5 ± 21.4***
LMA ($g \cdot m^{-2}$)	100 ± 0.1	111 ± 0.1**

DISCUSSION

We found that vessels of an ancestral form, characterized by long and low-angle scalariform perforation plates with up to 120 pit bars (Frost, 1930; Bailey, 1944; Carlquist, 1975, 2004; Carlquist and Schneider, 2002), were associated with substantial divergence in wood function as compared to a relative without vessels. Vessels of *C. madagascariensis* provided approximately 3-fold greater K_H normalized to the cross-sectional area of conduit lumens (K_C) in comparison to the vesselless wood of *T. perrieri*. Increased K_C decreased by 75% the amount of wood cross-sectional area devoted to axial hydraulic flow as compared to *T. perrieri*. Although *C. madagascariensis* possessed tracheids (Wilson, 1960), we found that vessels were responsible for the vast majority of K_C. Consistent with greater conduit efficiency, the vessel elements of *C. madagascariensis* possessed several anatomical differences involved in greater flow efficiency in spite of a large decrease in the numerical density of hydraulic cells in the wood. These features included increased pit membrane area by perforation plates, ability to develop wider lumens, and thinner walls than *T. perrieri* tracheids. Although we were unable to determine the vessel length distribution in the field, we note that maximum vessel length in *C. madagascariensis* was ~20 cm by air injection. This observation suggests another significant increase in conduit efficiency of vessels relative to tracheids (Pittermann *et al.*, 2006; Sperry *et al.*, 2006, 2007; Hacke *et al.*, 2007).

However, increased hydraulic efficiencies of basal vessels relative to tracheids occurred only at the conduit level because hydraulic efficiencies of whole stem (i.e., the sapwood level, K_S) of *C. madagascariensis* and *T. perrieri* were equivalent (Table 2, Fig. 3). The functional distinction between K_C and K_S is important because differences in K_S reflect overall wood allocation costs to supply transpiring leaves with water (Brodribb and Feild, 2000; Sperry, 2003; Baas *et al.*, 2004; Sperry *et al.*, 2006). In addition, vessels of *C. madagascariensis* were associated with lower capacity of the wood to supply transpiring leaves with water (lower K_L) in *C. madagascariensis* (Fig. 3). Instead of reducing overall shoot costs, we found that greater K_C furnished by basal vessels increased the area available in the wood to function differently from vesselless Winteraceae wood.

Functional divergence of Cinnamosma and Takhtajania wood—

Our results indicated that the majority of xylem cross-sectional area "freed up" by the basal vessels of *C. madagascariensis* was allocated to biomechanical function. This conclusion was supported by a dominance of sapwood cross-sectional area in *C. madagascariensis* by fibers having greater wall thicknesses as compared to vessels and tracheids (Table 2). The development of mechanically specialized fibers in *C. madagascariensis* most likely was responsible for the greater ρ_{wood} and increased E as compared to *Takhtajania* (Hacke *et al.*, 2001; Jacobsen *et al.*, 2005).

It is unknown if the E and mechanically associated traits (e.g., wall thickness, pit area fraction) of fibers in *C. madagascariensis* represent properties extending outside the ranges capable by vesselless vasculatures in the Winteraceae. We note, however, that average ρ_{wood} values of Canellaceae, including *C. madagascariensis*, *C. fragrans* (mean = $0.928\ g{\cdot}cm^{-3} \pm 0.06$ SD; $N = 10$), and *Pleodendron costaricense*, were 9, 23, and 22% greater, respectively, than the reported maximum ρ_{wood} found in 16 species of Winteraceae (Hacke *et al.*, 2007; Moya-Roque *et* *al.*, 2007; P. J. Hudson and T. S. Feild, personal observations, 2007, 2008). Because basal vessels furnished higher K_C, our results suggest that a greater ρ_{wood} and E can be achieved in *C. madagascariensis* for a given allocation of sapwood cross-sectional area in contrast to vesselless woods of Winteraceae (Hacke *et al.*, 2007). The option for increased cellular specialization toward mechanics and increased ρ_{wood} at a given K_S could afford several advantages. These advantages include increased wood longevity and pathogen resistance as well as increased mechanical strength (Hacke *et al.*, 2001; Jacobsen *et al.*, 2005; Preston *et al.*, 2006; Swenson and Enquist, 2007; Chave *et al.*, 2009). Mechanical traits also have consequences for the evolution of growth forms and regeneration ecologies (see below, Sterck *et al.*, 2001; Rowe and Speck, 2004; Osunkoya *et al.*, 2007).

Increased ρ_{wood}, however, entails significant energetic cost because the cell walls of tracheary elements probably explain the majority of wood construction cost (Hacke *et al.*, 2001). Our results supported this hypothesis because differences in ρ_{wood} and the energetic cost on a volumetric basis between *C. madagascariensis* and *T. perrieri* were nearly proportional (Fig. 5). Interestingly, we found that the basal vessels in *C. madagascariensis* were not associated with major differences in the energy content of wood on a dry mass basis. Although we could not differentiate the energy content of specific cell types, our results suggest that vessel-fiber based wood was not intrinsically more expensive than vesselless wood. Future studies, however, are needed to examine how vessels and the evolution of heteroxyly may have varied stem maintenance respiration costs. Such research is needed because we found evidence that the fraction of living cells (rays plus axial parenchyma) was greater in *C. madagascariensis* than *T. perrieri* (Fig. 2).

Vessels in relation to ecological divergence—

We observed that vessels related to the ecologies of the two species in unexpected ways. First, we observed that basal vessels and the biomechanical specialization of *C. madagascariensis* were associated with a preference for densely shaded forest understory habitats. Greater mechanical strength can be advantageous in low light understories to develop thin diameter stems that support a broad leaf area to efficiently collect light (Sterck *et al.*, 2001; Rowe and Speck, 2004; Osunkoya *et al.*, 2007). Stems with greater E for a given diameter can be more resilient to breakage, which can increase the probability that a costly investment in wood production is not lost by understory disturbance (Jacobsen *et al.*, 2005; Rowe and Speck, 2004). Consistent with greater ρ_{wood} and E, the growth form of *C. madagascariensis* appeared to be more demanding of mechanical support as compared to *Takhtajania*'s (Fig. 1). *Cinnamosma madagascariensis* grew with a single and taller erect trunk that supported a broader and deeper canopy of thinner, densely leaved terminal branches (Fig. 1B). Also in line with an apparent understory preference, the values of K_{S}, K_{L}, and HV in both populations of *C. madagascariensis* nested in the ranges reported for shade-demanding woody plants from tropical forests (Brodribb and Feild, 2000; Feild and Holbrook, 2000; Santiago *et al.*, 2004; Lusk *et al.*, 2007; Campanello *et al.*, 2008; Feild and Balun, 2008).

Surprisingly, we found that vesselless *Takhtajania* possessed a much greater K_{L} than vessel-bearing *C. madagascariensis* (Table 1, Fig. 2). Consistent with greater K_{L}, calculated g_{smax} values were higher in *T. perrieri*, largely because guard cell pores were larger relative to *C. madagascariensis* (Table 3). Relative to other Winteraceae, the xylem hydraulics of *T. perrieri* were unusually high in capacity. For example, *T. perrieri* possessed 30% greater K_{S} than the previously observed maximum in Winteraceae (Feild and Holbrook, 2000; Feild *et al.*, 2002; Hacke *et al.*, 2007). Supporting the hypothesis that hydraulic conductance and mechanics are closely traded off in vesselless angiosperms and conifers (Pittermann *et al.*, 2006; Feild and Arens, 2007; Hacke *et al.*, 2007; Feild *et al.*, 2009a), *T. perrieri* possessed the lowest ρ_{wood} measured and one of the highest mean conduit lumen diameters for all Winteraceae measured thus far.

The growth habit of *T. perrieri* suggests that this species may be functioning near the biomechanical limits to vesselless wood hydraulic capacity. *Takhtajania* plants consisted of multiple, shorter, and leaning trunks produced by repeated bouts of pithy and brittle shoots from the base of the plant. In addition, *Takhtajania* developed soil-hugging shoots that extended up to a meter from the base. However, reiteration by relatively cheap growth appears to be an effective regeneration mode in the context of generally open, gap-phase habitats of *Takhtajania* on north-facing cloud forest ridges that suffer frequent cyclonic damage (Birkinshaw, 2007). Although a broad comparative survey of Winteraceae leaf-gas-exchange capacities is unavailable, the absence of stomatal plugs in *Takhtajania* that are otherwise wide spread in Winteraceae (Bongers, 1973; Feild *et al.*, 1998, 2000), combined with high K_{L}, suggest that this species possesses an unusually high gas exchange capacity for Winteraceae. Thus, we did not find evidence that basal vessels in the wood were associated with greater capacities of leaf gas-exchange.

Finally, leaf cross-sectional anatomical differences suggested that the two species were structurally tuned to differing light climates. For example, the presence of a single palisade layer combined with a hypodermis and thin construction are consistent with our observations of *C.*

madagascariensis in low-light understory habitats punctuated by sunflecks of bright collimated light (DeLucia *et al.*, 1996). The dominance of the leaf cross section by spongy mesophyll in *Takhtajania* was more consistent with its occurrence in a light climate dominated by diffuse light under prolonged cloud immersion (Lawton, 1990; Smith *et al.*, 1997; P. J. Hudson, personal observations, 2008).

Conclusions—

Our study demonstrated that xylem vessels in *C. madagascariensis* were not linked to increases in overall shoot hydraulic capacity, and vessels were not associated with enhanced leaf photosynthetic or shoot hydraulic capacities. Rather, we found that a significant and early evolving functional consequence of vessel evolution was the uncoupling of hydraulic and mechanical responsibilities in the wood by increasing K_C for a given K_S. Such uncoupling resulted in biomechanics related to the growth habits in two species of Canellales. Thus, our results support the heteroxylly hypothesis for the early evolutionary advantages of vessels as innovations promoting functional diversification of the wood (Carlquist, 1975, 2004; Feild and Arens, 2007; Sperry *et al.*, 2007; Feild *et al.*, 2009a). Our results suggest that biomechanical specialization, perhaps leading to new options for growth form diversification, may have been an important functional consequence of early increases in conduit hydraulic efficiencies by basal vessels. Future studies will be necessary to determine how other important components of heteroxylly evolution, such as parenchyma and rays, diversified and coevolved with hydraulics and biomechanics during early angiosperm evolution. The diversity of xylem vasculatures across extant basal angiosperms offers a diverse range of evolutionary “experiments” and considerable growth form variation to tease apart these interactions (Carlquist, 1975, 2004; Carlquist and Schneider, 2002; Feild and Arens, 2005, 2007; Feild *et al.*, 2009a).

A limitation of our study is that recent phylogenetic studies make it clear that *Takhtajania* and *C. madagascariensis* have come to inhabit somewhat similar geographic and climatic envelopes through different biogeographic histories (Karol *et al.*, 2000; Salazar and Nixon, 2008). While *Takhtajania* is placed phylogenetically as the sister to the remaining genera of Winteraceae, *C. madagascariensis* is well nested within Canellaceae, which results in considerable phylogenetic distance between the two taxa (Karol *et al.*, 2000; Salazar and Nixon, 2008). Thus, a future broader comparative analysis of hydraulic and biomechanical coordinated evolution in relation to Canellales' habitats and growth forms will be needed to resolve how heteroxylly evolution relates to ecological diversification.

Although we did not measure resistance of the scalariform perforation plates, we posit that those of *C. madagascariensis* will be relatively high because of their long lengths with numerous pit bars (Sperry *et al.*, 2007; Christman and Sperry, 2009). Because we found that vessels did not decrease K_S relative to vesselless wood, our results support an emerging hypothesis that vessels only drove major cuts in the cost of K_L through increased K_S once elongate scalariform perforation plates became consolidated into a single aperture (Sperry *et al.*, 2007; Christman and Sperry, 2009; Feild *et al.*, 2009a). Therefore, understanding how early angiosperms acquired such high rates photosynthetic productivity will require a specific focus on perforation plate form and functional diversification, rather than vessels per se (Feild and Arens, 2007; Sperry *et al.*, 2007; Feild *et al.*, 2009a). Instead of devices immediately enabling access to high rates of water transport capacity and therefore photosynthesis, our results highlight the concept of vessels as traits that potentially enabled new properties of wood function.

LITERATURE CITED

Bailey, I. W. 1944. The development of vessels in angiosperms and its significance in morphological research. *American Journal of Botany* **31**: 421–428.

Baas, P., and E. A. Wheeler. 1996. Parallelism and reversibility in xylem evolution—A review. *International Association of Wood Anatomists Journal* **17**: 351–364.

Baas, P., F. W. Ewers, S. D. Davis, and E. A. Wheeler. 2004. Evolution of xylem physiology. In A. R. Hemsley and I. Poole [eds.], The evolution of plant physiology, 273–295. Elsevier, New York, New York, USA.

Becker, P. 2000. Competition in the regeneration niche between conifers and angiosperms: Bond's slow seedling hypothesis. *Functional Ecology* **14**: 401–412.

Bhaskar, R., A. Valiente-Banuet, and D. D. Ackerly. 2007. Evolution of hydraulic traits in closely related species pairs from mediterranean and nonmediterranean environments of North America. *New Phytologist* **176**: 718–726.

Birkinshaw, C. 2007. The effects of cyclone Hudah on the forest of Masoala Peninsula, Madagascar. *Madagascar Conservation and Development* **2**: 17–20.

Bond, W. J. 1989. The tortoise and the hare—Ecology of angiosperm dominance and gymnosperm persistence. *Biological Journal of the Linnean Society* **36**: 227–235.

Bongers, J. M. 1973. Epidermal leaf characteristics of the Winteraceae. Blumea 21: 381–411.

Bollen, A., L. Van Elsacker, and J. U. Ganzhorn. 2004. Tree dispersal strategies in the littoral forest of Sainte Luce (SE-Madagascar). *Oecologia* **139**: 604–616.

Boyce, C. K., T. J. Brodribb, T. S. Feild, and M. A. Zwieniecki. 2009. Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proceedings of the Royal Society, B, Biological Sciences* **276**: 1771–1776.

Brodribb, T., and T. S. Feild. 2000. Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell & Environment* **23**: 1381–1388.

Brodribb, T. J., T. S. Feild, and G. J. Jordan. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**: 1890–1898.

Brodribb, T., and R. S. Hill. 1997. Imbricacy and stomatal wax plugs reduce maximum leaf conductance in Southern Hemisphere conifers. *Australian Journal of Botany* **45**: 657–668.

Brodribb, T. J., N. M. Holbrook, and R. S. Hill. 2005. Seedling growth in conifers and angiosperms: impacts of contrasting xylem structure. *Australian Journal of Botany* **53**: 749–755.

Campanello, P. I., M. G. Gatti, and G. Goldstein. 2008. Coordination between water-transport efficiency and photosynthetic capacity in canopy tree species at different growth irradiances. *Tree Physiology* **28**: 85–94.

Carlquist, S. 1975. Ecological strategies of xylem evolution. University of California Press, Berkeley, California, USA.

Carlquist, S. 2000. Wood and bark anatomy of *Takhtajania* (Winteraceae): Phylogenetic and ecological implications. *Annals of the Missouri Botanical Garden* **87**: 317–322.

Carlquist, S. 2004. Comparative wood anatomy, 2nd ed. Springer-Verlag, Berlin, Germany.

Carlquist, S., and E. L. Schneider. 2002. The tracheid-vessel element transition in angiosperms involves multiple independent factors. *American Journal of Botany* **89**: 185–195.

Chapotin, S. M., J. H. Razanameharizaka, and N. M. Holbrook. 2006. A biomechanical perspective on the role of large stem volume and high water content in baobab trees (*Adansonia* spp.; Bombaceae). *American Journal of Botany* **93**: 1251–1264.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Toward a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.

Christman, M. A., and J. S. Sperry. 2009. Single-vessel measurements indicate scalariform plates confer higher flow resistance than previously estimated. Botany & Mycology 2009: Annual meeting of Botanical Society of America, Snowbird, Utah, USA [Abstract 174, website http://2009.botanyconference.org/engine/search/index.php?func=detail&aid=174].

Crane, P. R., and S. Lidgard. 1989. Angiosperm diversification and paleolatitudinal gradients in Cretaceous florisitic diversity. *Science* **246**: 675–678.

Crepet, W. L. 2009. The fossil record of angiosperms: Requiem or renaissance? *Annals of the Missouri Botanical Garden* **95**: 3–33.

Delucia, E., H. K. Nelson, T. C. Vogelmann, and W. K. Smith. 1996. Contribution of intercellular reflectance to photosynthesis in shade leaves. *Plant, Cell & Environment* **19**: 159–170.

Doyle, J. A. 2000. Paleobotany, relationships, and geographic history of Winteraceae. *Annals of the Missouri Botanical Garden* **87**: 303–316.

Doyle, J. A., and M. J. Donoghue. 1986. Seed plant phylogeny and the origin of angiosperms: An experimental cladistic approach. *Botanical Review* **52**: 321–431.

Edwards, E. J. 2006. Correlated evolution of stem and leaf hydraulic traits in *Pereskia* (Cactaceae). *New Phytologist* **172**: 479–489.

Edwards, E. J., and M. J. Donoghue. 2006. *Pereskia* and the origin of the cactus life-form. *American Naturalist* **167**: 777–793.

Feild, T. S., and N. C. Arens. 2005. Form, function, and environments of the early angiosperms: Merging extant phylogeny and ecophysiology with fossils. *New Phytologist* **166**: 383–408.

Feild, T. S., and N. C. Arens. 2007. The ecophysiology of early angiosperms. *Plant, Cell & Environment* **30**: 291–309.

Feild, T. S., and L. Balun. 2008. Xylem hydraulic and photosynthetic function of *Gnetum* (Gnetales) species from Papua New Guinea. *New Phytologist* **177**: 665–675.

Feild, T. S., T. Brodribb, and N. M. Holbrook. 2002. Hardly a relict: freezing and the evolution of vesselless wood in Winteraceae. *Evolution; International Journal of Organic Evolution* **56**: 464–478.

Feild, T. S., D. S. Chatelet, and T. J. Brodribb. 2009a. Ancestral xerophobia: a hypothesis on the whole plant ecophysiology of early angiosperms. *Geobiology* **7**: 237–264.

Feild, T. S., D. S. Chatelet, and T. J. Brodribb. 2009b. Giant flowers of southern magnolia (*Magnolia grandiflora*, Magnoliaceae) are hydrated by the xylem. *Plant Physiology* **150**: 1587–1597.

Feild, T. S., and N. M. Holbrook. 2000. Xylem sap flow and stem hydraulics of the vesselless angiosperm *Drimys granadensis* (Winteraceae) in a Costa Rican elfin forest. *Plant, Cell & Environment* **23**: 1067–1077.

Feild, T. S., M. A. Zwieniecki, M. J. Donoghue, and N. M. Holbrook. 1998. Stomatal plugs of *Drimys winteri* (Winteraceae) protect leaves from mist not drought. *Proceedings of the National Academy of Sciences, USA* **95**: 14256–14259.

Feild, T. S., M. A. Zwieniecki, and N. M. Holbrook. 2000. Winteraceae evolution: An ecophysiological perspective. *Annals of the Missouri Botanical Garden* **87**: 323–334.

Frost, F. H. 1930. Specialization in the secondary xylem of dicotyledons. I. Origin of vessel. *Botanical Gazette* **89**: 67–94.

GBIF Data Portal, www.gbif.net. 2009-07-28. Missouri Botanical Garden, 41 records.

Goodman, S. 1998. A floral and faunal inventory of the Réserve Spéciale d'Anjanaharibe-Sud, Madagascar: With reference to elevational variation. *Fieldiana: Zoology* **90**: 1–246.

Goodman, S. 2000. A floral and faunal inventory of the Parc National de Marojejy, Madagascar: With reference to elevational variation. *Fieldiana: Zoology* **97**: 1–286.

Grimaldi, D. 1999. The co-radiations of pollinating insects and angiosperms in the Cretaceous. *Annals of the Missouri Botanical Garden* **86**: 373–406.

Hacke, U. G., J. S. Sperry, T. S. Feild, Y. Sano, E. H. Sikkema, and J. Pittermann. 2007. Water transport in vesselless angiosperms: Conducting efficiency and cavitation safety. *International Journal of Plant Sciences* **168**: 1113–1126.

Hacke, U. G., J. S. Sperry, W. T. Pockman, S. D. Davis, and K. A. Mcculloch. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**: 457–461.

Jacobsen, A. L., F. W. Ewers, R. B. Pratt, W. A. Paddock, and S. D. Davis. 2005. Do xylem fibers affect vessel cavitation resistance? *Plant Physiology* **139**: 546–556.

Kaiser, H. 2009. The relation between stomatal aperture and gas exchange under consideration of pore geometry and diffusional resistance in the mesophyll. *Plant, Cell & Environment* **32**: 1091–1098.

Karol, K. G., Y.-B. Suh, G. E. Schatz, and E. A. Zimmer. 2000. Molecular evidence for the phylogenetic position of *Takhtajania* in the Winteraceae: Inference from nuclear ribosomal and chloroplast gene spacer sequences. *Annals of the Missouri Botanical Garden* **87**: 414–432.

Knoll, M. A., and W. C. James. 1987. Effect of the advent and diversification of vascular land plants on mineral weathering through geologic time. *Geology* **15**: 1099–1102.

Lawson, T., W. James, and J. Weyers. 1998. A surrogate measure of stomatal aperture. *Journal of Experimental Botany* **49**: 1397–1403.

Lawton, R. O. 1990. Canopy gaps and light penetration into a wind-exposed tropical lower montane rainforest. *Canadian Journal of Forest Research* **20**: 659–667.

Lupia, R., S. Lidgard, and P. R Crane. 1999. Comparing palynological abundance and diversity: Implications for biotic replacement during the Cretaceous angiosperm radiation. *Paleobiology* **25**: 305–340.

Lusk, C. H., M. Jiménez-Castillo, and N. Salazar-Ortega. 2007. Evidence that branches of evergreen angiosperms and coniferous trees differ in hydraulic conductance but not Huber values. *Canadian Journal of Botany* **85**: 141–147.

McElwain, J. C., K. J. Willis, and R. Lupia. 2005. Cretaceous CO2 decline and the radiation and diversification of angiosperms. In J. R. Ehleringer, T. E. Cerling, and M. D. Dearing [eds.], A history of atmospheric CO2 and its effects on plants, animals, and ecosystems, 133–165. Springer-Verlag, New York, New York, USA.

Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. *Science* **312**: 101–104.

Moya-Roque, R., M. Morales-Salazar, M. C. Wiemann, and L. A. Poveda. 2007. Wood anatomy of *Pleodendron costaricense* (Canellaceae) from southern Pacific, Costa Rica. *Brenesia* **68**: 25–28.

Niklas, K. J. 1992. Plant biomechanics: An engineering approach to plant form and function. Chicago University Press, Chicago, Illinois, USA.

Osborne, C. P., D. J. Beerling, B. H. Lomax, and W. G. Chaloner. 2004. Biophysical constraints on the origin of leaves inferred from the fossil record. *Proceedings of the National Academy of Sciences, USA* **101**: 10360–10362.

Osunkoya, O. O., K. Omar-Ali, N. Amit, J. Dayan, D. S. Daud, and T. K. Sheng. 2007. Comparative height-crown allometry and mechanical design in 22 tree species of Kuala Belalong rainforest, Brunei, Borneo. *American Journal of Botany* **94**: 1951–1962.

Pammenter, N. W., G. F. Midgley, and W. J. Bond. 2004. Xylem hydraulics and angiosperm success: A testing using broad-leafed species. In A. R. Hemsley and I. Poole [eds.], The evolution of plant physiology, 259–271. Elsevier, New York, New York, USA.

Pareliussen, I., E. G. A. Olsson, and W. S. Armbruster. 2006. Factors limiting the survival of native tree seedlings used in conservation efforts at the edges of forest fragments in upland Madagascar. *Restoration Ecology* **14**: 196–203.

Parlange, J. Y., and P. W. Waggoner. 1970. Stomatal dimensions and resistance to diffusion. *Plant Physiology* **46**: 337–342.

Pittermann, J., J. S. Sperry, J. K. Wheeler, U. G. Hacke, and E. H. Sikkema. 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant, Cell & Environment* **29**: 1618–1628.

Pratt, R. B., A. L. Jacobsen, F. W. Ewers, and S. D. Davis. 2007. Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytologist* **174**: 787–798.

Preston, K. A., W. K. Cornwell, and J. L. Denoyer. 2006. Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *New Phytologist* **170**: 807–818.

Raxworthy, C. J., R. G. Pearson, N. Rabibisoa, A. M. Rakotondrazafy, J.-P. Ramanamanjato, A. P. Raselimanana, S. Wu, R. A. Nussabaum, and D. A. Stone. 2008. Extinction vulnerability of tropical montane endemism from warming and upslope displacement: A preliminary appraisal for the highest massif in Madagascar. *Global Change Biology* **14**: 1703–1720.

Rowe, N., and T. Speck. 2004. Hydraulics and mechanics of plants: Novelty, innovation, and evolution. In A. R. Hemsley and I. Poole [eds.], The evolution of plant physiology, 299–325. Elsevier, New York, New York, USA.

Ruzin, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press, New York, New York, USA.

Sack, L., P. D. Cowan, N. Jaikumar, and N. M. Holbrook. 2003. The ‘hydrology’ of leaves: Co-ordination of structure and function in temperate woody species. *Plant, Cell & Environment* **26**: 1343–1356.

Salazar, J., and K. Nixon. 2008. New discoveries in the Canellaceae in the Antilles: How phylogeny can support taxonomy. *Botanical Review* **74**: 103–111.

Santiago, L. S., G. Goldstein, F. C. Meinzer, J. B. Fisher, K. Machado, D. Woodruff, and T. Jones. 2004. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* **140**: 543–550.

Schatz, G. E. 2000. The rediscovery of a Malagasy endemic: *Takhtajania perrieri* (Winteraceae). *Annals of the Missouri Botanical Garden* **87**: 297–302.

Schatz, G. E. 2001. Generic tree flora of Madagascar. Royal Botanic Gardens, Kew, UK.

Smith, W. K., T. C. Vogelmann, E. H. Delucia, D. T. Bell, and K. A. Shepherd. 1997. Leaf form and photosynthesis. *Bioscience* **47**: 785–793.

Smith, W. K. and N. M Hughes. 2009. Progress in coupling plant form and photosynthetic function. *Castanea* **74**: 1–26.

Sperry, J. S. 2003. Evolution of water transport and xylem structure. *International Journal of Plant Sciences* **164**: S115–S127.

Sperry, J. S., U. G. Hacke, T. S. Feild, Y. Sano, and E. H. Sikkema. 2007. Hydraulic consequences of vessel evolution in angiosperms. *International Journal of Plant Sciences* **168**: 1127–1139.

Sperry, J. S., U. G. Hacke, and J. Pittermann. 2006. Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany* **93**: 1490–1500.

Sterck, F. J., F. Bongers, and D. M. Newberry. 2001. Tree architecture in a Bornean lowland rain forest: Intraspecific and interspecific patterns. *Plant Ecology* **153**: 279–292.

Swenson, N. G., and B. J. Enquist. 2007. Ecological and evolutionary determinants of a key plant functional trait: Wood density and its community-wide variation across latitude and elevation. *American Journal of Botany* **94**: 451–459.

Wheeler, E. A., and P. Baas. 1991. A survey of the fossil record for dicotyledonous wood and its significance for evolutionary and ecological wood anatomy. *International Assoication of Wood Anatomists Bulletin* **12**: 275–332.

Wilson, T. K. 1960. The comparative morphology of the Canellaceae. I. Synopsis of genera and wood anatomy. *Tropical Woods* **112**: 1–27.

Volk, T. 1989. Rise of angiosperms as a factor in long-term climatic cooling. *Geology* **17**: 107–110.

Zwieniecki, M. A., P. J. Melcher, and N. M. Holbrook. 2001. Hydrogel control of xylem hydraulic resistance in plants. *Science* **291**: 1059–1062.

VITA

Patrick Hudson was born in Peoria, IL, to parents Joe and Marty Hudson. He has one younger sister, Alena Poe. After graduating high school, Patrick continued his education at Truman State University, where he began his research career in botany investigating pollen ontogeny in the giant water lily *Victoria* under the tutelage of Dr. Jeffrey Osborn. Patrick left Truman State with both a Bachelors of Science and a Bachelors of Arts in Biology in 2004, and accepted a graduate teaching assistantship with the Biology Department at the University of Nebraska-Omaha. There he studied paleobotany in the lab of Dr. Lisa Boucher, and completed a Masters of Science degree in 2006. Seeing an opportunity to transition into the realm of living plants, Patrick came to the University of Tennessee in the fall of 2007 in order to learn plant physiological ecology. Patrick graduated with a second Masters of Science in May of 2010. He will continue his pursuits in botany at the University of New Mexico, where he has accepted a research assistantship.