HARDLY A RELICT: FREEZING AND THE EVOLUTION OF VESSELLESS WOOD IN WINTERACEAE

Taylor S. Feild,[1] Tim Brodribb, and N. Michele Holbrook
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts

Abstract.—The Winteraceae are traditionally regarded as the least-specialized descendents of the first flowering plants, based largely on their lack of xylem vessels. Since vessels have been viewed as a key innovation for angiosperm diversification, Winteraceae have been portrayed as declining relicts, limited to wet forest habitats where their tracheid-based wood does not impose a significant hydraulic constraints. In contrast, phylogenetic analyses place Winteraceae among angiosperm clades with vessels, indicating that their vesselless wood is derived rather than primitive, whereas extension of the Winteraceae fossil record into the Early Cretaceous suggests a more complex ecological history than has been deduced from their current distribution. However, the selective regime and ecological events underlying the possible loss of vessels in Winteraceae have remained enigmatic. Here we examine the hypothesis that vessels were lost as an adaptation to freezing-prone environments in Winteraceae by measuring the responses of xylem water transport to freezing for a diverse group of Winteraceae species as compared to *Canella winterana* (Canellaceae, a close relative with vessels) and sympatric conifer taxa. We found that mean percent loss of xylem water transport capacity following freeze-thaw varied from 0% to 6% for Winteraceae species from freezing-prone temperate climates and approximately 20% in those taxa from tropical (nonfreezing) climates. Similarly, conifers exhibit almost no decrease in xylem hydraulic conductivity following freezing. In contrast, water transport in *Canella* stems is nearly 85% blocked after freeze-thaw. Although vessel-bearing wood of *Canella* possesses considerably greater hydraulic capacity than Winteraceae, nearly 20% of xylem hydraulic conductance remains, a value that is comparable to the hydraulic capacity of vesselless Winteraceae xylem, if the proportion of hydraulic flow through vessels (modeled as ideal capillaries) is removed. Thus, the evolutionary removal of vessels may not necessarily require a deleterious shift to an ineffective vascular system. By integrating Winteraceae's phylogenetic relationships and fossil history with physiological and ecological observations, we suggest that, as ancestors of modern Winteraceae passed through temperate conditions present in Southern Gondwana during the Early Cretaceous, they were exposed to selective pressures against vessel-possession and returned to a vascular system relying on tracheids. These results suggest that the vesselless condition is advantageous in freezing-prone areas, which is supported by the strong bias in the ecological abundance of Winteraceae to wet temperate and tropical alpine habitats, rather than a retained feature from the first vesselless angiosperms. We believe that vesselless wood plays an important role in the ecological abundance of Winteraceae in Southern Hemisphere temperate environments by enabling the retention of leaves and photosynthesis in the face of frequent freeze-thaw events.

Key words.—Angiosperm evolution, Canellaceae, *Drimys*, freezing stress, Winteraceae, xylem evolution.

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For decades, Winteraceae, including five genera (*Drimys*, *Pseudowintera*, *Tasmannia*, *Takhtajania*, and *Zygogynum*) and approximately ~65 spp. of trees and shrubs distributed primarily across wet forested environments in the southern hemisphere (Table 1; Takhtajan 1969; Thorne 1974; Vink 1970, 1977, 1993; Feild et al. 2000), have been regarded as one of the most primitive angiosperm families (Van Tieghem 1900; Bailey and Thompson 1918; Bailey 1953; Takhtajan 1969; Thorne 1974; Ehrendorfer et al. 1979; Gottsburger et al. 1980; Cronquist 1981, 1988). In support, the Winteraceae are among the first angiosperm lineages recognized in the fossil record, with ''winteroid'' pollen extending to the Barremian-Aptian (Early Cretaceous, Walker et al. 1983; Doyle et al. 1990; Doyle 2000). The primary basis for viewing Winteraceae as archaic is their lack of xylem vessels (Van Tieghem 1900; Bailey and Thompson 1918; Bailey 1953; Takhtajan 1969; Thorne 1974; Carlquist 1975, 1983a,b). Xylem vessels are only lacking in a few other extant woody angiosperms, which include the New Caledonian endemic *Amborella* (1 spp.) and the Trochodendrales, including *Tetracentron* (1 spp.) and *Trochodendron* (1 spp.; Bailey and Tompson 1918; Bailey 1953; Carlquist 1975). *Trochodendron*

is native Japan, Taiwan, South Korea, and the Ryukyu Islands while *Tetracentron* occurs in Nepal, China, and northern Myanmar (Smith 1945a).

Xylem vessels are water-conducting tubes that consist of a file of individual, nonliving cells (termed vessel elements) with partially to completely open axial cell walls (Frost 1930; Zimmermann 1983). Tracheids, in contrast, are narrower single-celled conduits with intact axial cell walls (Zimmermann 1983). Vessel elements were derived through modifications of the developmental program giving rise to tracheids (Frost 1930; Bailey 1953; Carlquist 1975). In general, vessel-bearing plants possess greater xylem hydraulic capacity than those with tracheids because vessels provide a more open path for water movement (Zimmermann 1983; Tyree and Ewers 1991; Sperry et al. 1994; Becker et al. 1999; Brodribb and Feild 2000).

The potential for greater hydraulic transport has led to the suggestion that the evolution of the angiosperm vessel is an important innovation that fueled their rise to ecological dominance (Takhtajan 1969; Stebbins 1974; Carlquist 1975; Doyle and Donoghue 1986; Bond 1989; Taylor and Hickey 1996). A corollary of this perspective is that vesselless Winteraceae experience severe hydraulic constraints that have influenced both their ecological range and diversification (Bailey and Nast 1944; Carlquist 1975, 1983a,b; Ehrendorfer

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[1] Present address: Department of Integrative Biology, University of California, Berkeley, California 94720-3140; E-mail: acmopyle@hotmail.com.

TABLE 1.   Current classification, species number, habitat, and geographic distribution of Winterceae genera. Classification for genera is based on Vink (1970, 1977, 1993). Note that Vink (1985, 1993) has circumscribed *Zygogynum* broadly to include the genera *Belliolum, Bubbia, Exospermum,* and *Zygogynum* (sensu Vink 1977). Habitat information for each genus was compiled based on literature sources, collection reports, and personal observations. Winteraceae: *Drimys* (Smith 1943a; Young 1972; Ehrendorfer et al. 1979; Gottsburger et al. 1980; Lusk 1993; Rebertus and Veblen 1993; Feild and Holbrook 2000), *Pseudowintera* (Sampson 1980; Wardle 1985); *Takhtajania* (Schatz 2000; G. S. Schatz, pers. comm. 1999); *Tasmannia* (Smith 1943b; van Stennis 1968; Vink 1970; Hnatiuk et al. 1976; Sampson et al. 1998; Raleigh et al. 1994; Kirkpatrick and Bridle 1999; Barnes et al. 2000; Feild and Brodribb 2001; G. Jordan, pers. comm. 2000); *Zygogynum* (Smith 1943b; Vink 1977, 1985, 1993; Sampson 1983; L. B. Thien, pers. comm. 1999; G. Jordan, pers. comm. 1999).

Taxon	Species no.	Habitat	Geographic distribution
Family Winteraceae			
Drimys	6	Tropical montane rainforests, alpine páramos, windy ''elfin'' montane cloud forests, coastal temperate rainforest	Brazil, Southern Chile, Venezuela, Central America, and north to highlands of Mexico
Pseudowintera	3	Temperate rainforest and temperate subalpine woodlands	New Zealand
Takhtajania	1	Tropical montane cloud forest	Madagascar
Tasmannia	40	Tropical montane cloud forests, coastal temperate rainforest, tropical and temperate alpine shrublands	Australia, Papua New Guinea, Malaysia, Philippines
Zygogynum	15	Tropical montane cloud forest	Australia New Caledonia, Papua New Guinea, Solomon Islands

et al. 1979; Gottsburger et al. 1980). Winteraceae have been portrayed as declining relicts, limited to wet rainforest refugia based on their occurrence in cool to cold (1200–12,000 mm year^{-1}), low evaporative-demand environments, including montane cloud forests, coastal rainforests, and alpine shrublands (Table 1; Smith 1943a,b, 1945b; Vink 1970, 1977, 1983, 1993; Carlquist 1975, 1983a; Ehrendorfer et al. 1979; Gottsburger et al. 1980). This view of Winteraceae as hydraulically limited is reflected in the long-held interpretation of their characteristic stomatal waxy occlusions, referred to as stomatal plugs, as structures essential to compensate for vesselless wood by curtailing maximum transpiration rates (Bailey and Nast 1944; Bailey 1953; Baranova 1972; Carlquist 1975; Cronquist 1981). Nevertheless, the dominance of conifers over large areas of arid, high-latitude, and alpine environments demonstrates that vesselless xylem cannot be viewed necessarily as an ecological liability (Bond 1989; Sprugel 1989).

Recent findings on the phylogeny of angiosperms and functional physiology of Winteraceae challenge the view that this group represents a relictual lineage from the first angiosperms by calling into question the primitive status of their vesselless xylem (Young 1981; Donoghue 1989; Donoghue and Doyle 1989; Feild et al. 1998; Mathews and Donoghue 1999; Doyle and Endress 2000; Feild and Holbrook 2000). Although *Amborella*, a vesselless tropical shrub, is placed at the base of the angiosperms, Winteraceae is nested among taxa with vessels (Fig. 1; Mathews and Donoghue 1999; Qiu et al. 1999, 2000; Soltis et al. 1999; Doyle and Endress 2000). Winteraceae is linked with Canellaceae (six genera; 16 spp. of vessel-bearing tropical dry forest and lowland rainforest trees) and are collectively the sister-group to Magnoliales or Piperales. Both of these phylogenetic positions appear to be several nodes up from the angiosperm root (Qiu et al. 1999, 2000; Doyle and Endress 2000; Karol et al. 2000). Thus, phylogenetic analyses indicate that it is more parsimonious to assume that Winteraceae returned to a tracheid-based vasculature, rather than vesselless xylem being retained along the line from *Amborella* to Winteraceae, because assuming

irreversible vessel evolution requires nine (Fig. 1, Doyle and Endress 2000) to ten (Qiu et al. 2000) independent vessel origins. Furthermore, recent work demonstrates that stomatal plugs, at least in *Drimys* taxa, cannot be used as evidence that winteraceous plants are primitively vesselless because these structures do not aid in drought avoidance (Feild et al. 1998; Feild and Holbrook 2000). Specifically, stomatal plugs modify the humidity response of the leaf guard cells such that under high evaporative demand, *Drimys winteri* leaves with plugs lost water at a faster rate than leaves from which the plugs were experimentally removed (Feild et al. 1998). Instead, these cuticular structures appear to be related to the occurrence of Winteraceae in areas that are generally wet (i.e., cloud forests) through enabling photosynthetic activity under conditions of excess water on the leaf surface. Stomatal plugs are hydrophobic and decrease leaf wettability which prevents the formation of a continuous water film that would otherwise impede diffusion of CO_2 into the leaf and leaf carbon gain (Feild et al. 1998). Similar functions have been discussed for stomatal plugging in conifers, but the presence of stomatal plugs in other groups may be related to drought resistance (in some Papaveraceae and possibly Epacridaceae) or avoidance of fungal infection (Brodribb and Hill 1998). However, the hypothesis that Winteraceae lost vessels has received considerable skepticism, primarily on the grounds that no mechanism could drive the loss of vessels because this would require a deleterious shift to an inefficient hydraulic system (Bailey 1953; Carlquist 1983a,b).

The selective forces acting on xylem evolution are not limited to hydraulic efficiency. Two additional functions of the xylem include mechanical support and maintenance of a continuous vascular pipeline in the face of stresses (Niklas 1992; Sperry et al. 1994; Tyree et al. 1994; Sperry 1995; Canny 1997). Trade-offs in biomechanical performance between possession of a vascular system composed solely tracheids for water conduction and support versus one where support and hydraulic functions are differentiated between two cell types (i.e., fibers and vessels, Niklas 1992; Carlquist 1988a) may exist which could potentially influence the di-
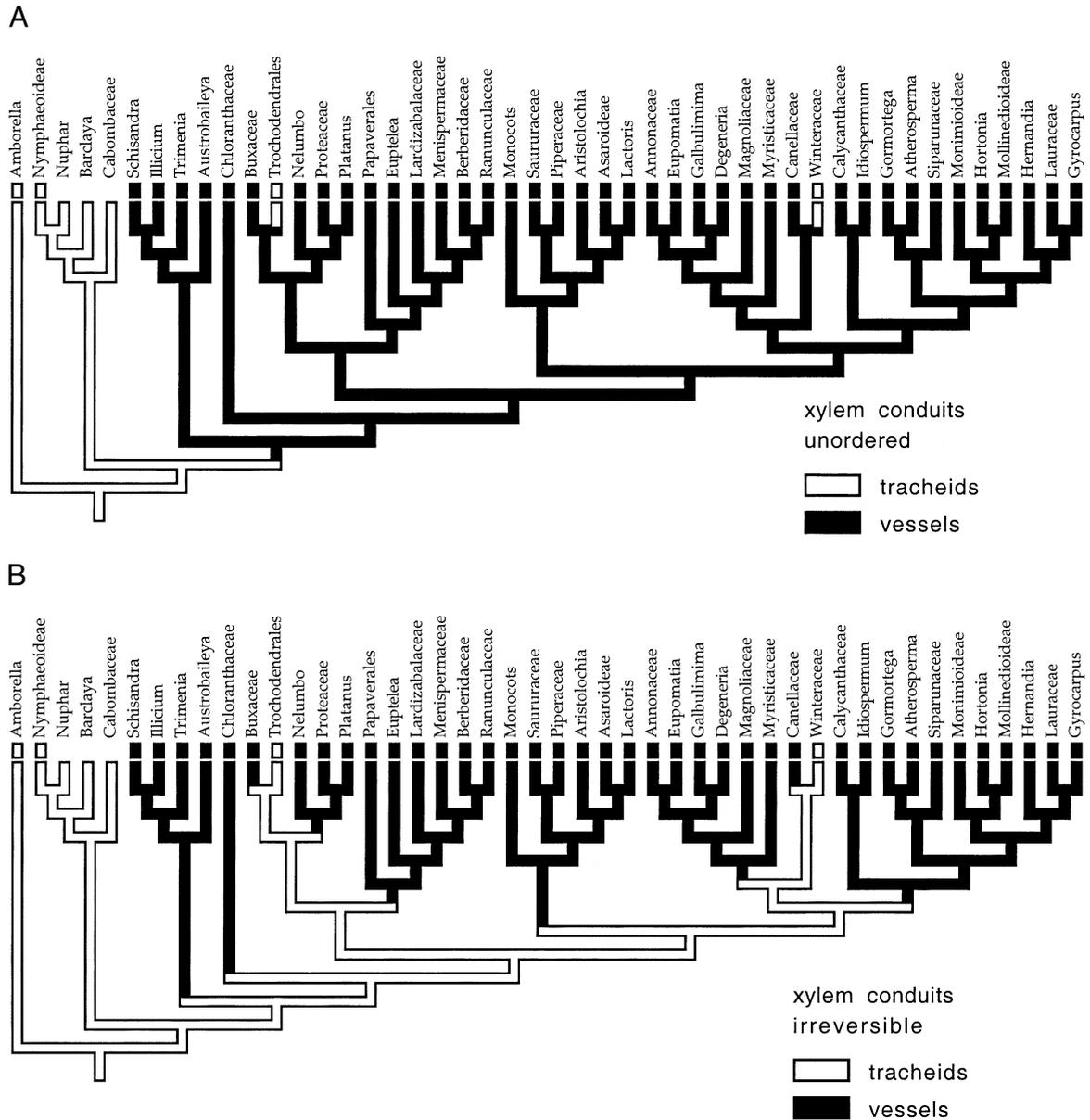
FIG. 1. The most parsimonious phylogenetic tree of Doyle and Endress (2000) from the combined analysis of three genes (*rbc*L + 18S ribosomal DNA + *atp*B, data set from Soltis et al. 1999) and morphology data matrix for early-diverging angiosperms showing the evolution of xylem vessels, assuming that vessel evolution is a reversible (A) or that it is irreversible (B). Character overlaying was accomplished using MacClade 3.2 (Maddison and Maddison 1992).

rection of xylem evolution in Winteraceae (Givnish 1995). At present, no information is available on the magnitude of these trade-offs and their potential importance in the evolution of Winteraceae, and Winteraceae are not preferentially associated with environments characterized by high mechanical stress, such as windy or riparian habitats (Givnish 1995).

However, with respect to xylem hydraulic resilience to freezing stress, there is abundant evidence that tracheids offer some advantages over xylem vessels in that they avoid xylem embolisms due to freezing (for a recent review, please see Pearce 2001; Sperry et al. 1994; Tyree et al. 1994; Sperry 1995; Davis et al. 1999). Compared to vessels, the smaller volume of tracheids lowers the likelihood that bubbles frozen

out of xylem sap will, upon thawing, expand to form embolisms that block water transport (Hammel 1967; Zimmermann 1983; Wang et al.1992; Sperry and Sullivan 1992; Sperry et al. 1994; Sperry 1995; Davis et al. 1999; Feild and Brodribb 2001). The stability of water flow through the vessel-free wood of conifers following freeze-thaw conditions is likely to enable leaf retention and some photosynthesis over winter conditions (Hammel 1967; Sprugel 1989; Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999; Feild and Brodribb 2001). In contrast, many vessel-bearing angiosperms in the Northern Hemisphere embolize extensively from a single freeze-thaw event such that water supply to leaves cannot be maintained and these plants shed their

leaves during autumn (Wang et al. 1992; Sperry and Sullivan 1992; Sperry et al. 1994; Sperry 1995; Davis et al. 1999; Feild and Brodribb 2001). In spring, stem water transport is restored either by the allocation of carbon to produce new mega-porous vessels or through expenditures of energy to generate root pressure for refilling embolized vessels before leaves can be expanded (Wang et al. 1992; Sperry et al. 1994). Importantly, Winteraceae are evergreen and a common characteristic of their current distribution and ecological abundance is a strong association with cool and cold, wet environments in the Southern Hemisphere that characterized by relatively mild (frequently between -5 and $-10°C$ and generally not lower than $-20°C$), but frequent nightly freezing events that are followed by relatively warm temperatures during the day which permit growth year-round (van Stennis 1968; Vink 1970; Wardle 1985; Markgraf et al. 1995; Kirkpatrick and Bridle 1999; Feild et al. 2000; Feild and Brodribb 2001). Thus, selection for the avoidance of xylem dysfunction arising from freezing stress may represent a viable mechanism selecting for a return to a tracheid-based vascular system in Winteraceae (Groom 1910; Doyle et al. 1990; Sperry 1995; Doyle 2000; Feild et al. 2000). The hydraulic responses of Winteraceae xylem to freezing and how these functional constraints relate to their current ecology and the evolution of their unusual vascular system remain unexamined.

Here the first experimental data are presented on the responses of xylem water transport to freezing for a diverse group of Winteraceae taxa compared to *Canella winterana* (Canellaceae), a close relative with vessels (Karol et al. 2000) and several sympatric conifer taxa. In addition, comparative anatomical and hydraulic analyses of Winteraceae and Canellaceae vascular systems are presented to examine the hydraulic cost of vessel loss. These data demonstrate that the vesselless vascular system of most Winteraceae species is resilient to freezing-induced embolism. In light of recent phylogenetic analyses of Winteraceae (Suh et al. 1993; Karol et al. 2000) and their fossil record (Walker et al. 1983; Doyle et al. 1990; Doyle 2000; Poole and Francis 2000), we suggest that the evolution of vesselless wood in Winteraceae is more likely to be connected to the group's migration into cooler, wet temperate climates with frequent freezing events than a retained feature from the first flowering plants.

MATERIALS AND METHODS

Plant Materials and Field Sites

Nine Winteraceae species, representing four of the five genera, were studied from naturally occurring populations and greenhouse-cultivated plants (Tables 1 and 2). Taxa were chosen to encompass the range of Winteraceae's environments. Species studied under greenhouse conditions were raised from cuttings or obtained from local nurseries. Plants were grown under greenhouse conditions (e.g., day/night temperatures 25/20°C; 200–500 μmol photons m^{-2} s^{-1} irradiance for 10 to 12 h, relative humidity 60–85%). Plants were grown under these conditions for at least two years before experiments. Species were represented by a minimum of five cuttings that were obtained from five different parent plants. In addition, five plants of *Canella winterana* were raised from seed obtained from a population on Key Largo,

Florida. We sampled several species of temperate and tropical conifers that co-occur with Winteraceae. In Tasmania (on the summit of Mt. Field, ~1200 m), we sampled *Diselma archeri* (Hook.f.) (Cupressaceae) and three podocarps (*Microstrobus niphophilus* J. Garden and L. A. S. Johnson; *Phyllocladus aspleniifolius* [Labill.] Rich ex Hook.f.; *Podocarpus lawrencei* [Hook.f.]) from a mixed-coniferous alpine heath community. From tropical Queensland and New Caledonia, plants of *Podocarpus dispermis* C. T. White and *Retrophyllum comptonii* (J. Buchholz) C. N. Page were examined.

Xylem Hydraulic Conductivity and Anatomical Measurements

Stem xylem hydraulic conductivity (K_H), defined as the mass flow rate of water through an excised stem segment per pressure drop per unit length (kg m MPa^{-1} s^{-1}), was measured in the field using methods and procedures described previously (Feild and Holbrook 2000). A steady-state flowmeter method was used which capitalizes on the pressure drop across a tube of known conductance to determine water efflux from a stem segment. Briefly, an elevated reservoir (100–150 cm) of filtered water (to 0.2 μm particle size) was used to perfuse water through a piece of capillary tubing (PEEK, Upchurch Scientific, Seattle, WA) and the stem segment arranged in series. To control for ion-mediated effects on xylem hydraulic conductance (Zwieniecki et al. 2001), the osmolarity of the perfusing solution was standardized using bottled spring water (Evian and Mont Dore Spring Water Sources). All measurements were conducted out of direct sunlight to minimize temperature heterogeneity across the flowmeter and between the measured stem and hydraulic head. We found that temperature differences between the measured stem segment and the capillary tube, which were monitored using a copper-constantan thermocouple (30 American wire gauge) reader, were less than 1°C. Thus, measurement errors of hydraulic flux related to viscosity were lower than approximately 2% under these experimental conditions (Nobel 1988).A pressure transducer (PX136, 0.03 MPa/5 mV range; Omega Engineering, Stamford, CT) was fitted so that the pressure of the hydraulic head and at the stem fitting could be easily read with a four-digit range multimeter. The conductance of each tube was calibrated as described previously (Feild and Holbrook 2000).

Leaf-bearing branches were collected for xylem hydraulic measurements from five individual trees of each species. Branches were sampled early in the morning (0800–0930 h) on wet, overcast days. For taxa examined under greenhouse conditions, plants were watered to saturation and bagged in plastic overnight to minimize transpiration. In the field, sampled branches were severed with stem clippers, triple-bagged in plastic, and transported to a laboratory. Stem segments were then cut from branches underwater at a standardized length (50 mm) and diameter (3–5 mm) to control for stem diameter-dependent variation in vascular conduit geometry and hydraulic conductance (Carlquist 1975). For *Canella winterana*, stem segments were cut to a length longer the maximum vessel length (150 mm long in 3–5 mm diameter stems), which was determined using low pressure (0.01 MPa) air-injection. Both ends of the stem segment were shaved

with a fresh razor blade underwater. Sapwood-area specific conductivity (K_S; kg MPa^{-1} m^{-1} s^{-1}) was determined by dividing K_H by the sapwood cross-section area (m^2). Sapwood cross-section was measured with a micrometer along the major and minor diameters of the stem end supporting the leaf area, with the surface area contributed by the pith subtracted (Sperry et al. 1988).

Conduit diameters of conifer and Winteraceae tracheids as well as Canellaceae vessels and tracheids were determined from cross-sections from three stem segments of each of the species that were measured for freeze-thaw responses. In *Canella*, vessels were easily distinguished from tracheids based on their thinner wall thicknesses (1–3 μm) compared to tracheids (4–6 μm, Wilson 1960). A sector of xylem was photographed at 250× and enlarged proportionally to fit an area of 620 cm^2. The major diameter of all tracheids and vessels present in a given sector were measured using a ruler calibrated with an ocular micrometer. Sectors were located randomly around the stem cross-section, excluding regions of compression wood in conifers and tension wood in angiosperms because these are known to conduct very little water (Spicer and Gartner 1998) For each sector, 300–500 conduits were measured, and a mean diameter was determined for each species ($n = 3$ stems for each species). Diameters were then analyzed for their frequency distribution in 5 μm diameter classes for the relative contribution of each diameter class to the sum of all conduits raised to the fourth power of the radius (Sperry et al. 1994). This sum is proportional to xylem hydraulic conductance, assuming that xylem conduits can be modeled as ideal capillary tubes and that flow through them is laminar (Hagen-Poiseuille law, Zimmermann 1983).

Freeze-Thaw Treatment and Changes in Stem Hydraulic Conductivity

Five branches from the same plants sampled for xylem hydraulic conductivity were used in experiments on freeze-thaw hydraulic responses. Before freeze-thaw treatment, leaf-bearing branches were dehydrated to a moderate stem xylem tension (Ψ_{stem}; −0.5 MPa), approximating the lowest water potential that Winteraceae plants would be likely to experience in the field during a freeze-thaw cycle (T. Feild, unpubl. data). Drought-stress vulnerability curves for *Drimys winteri* and *Tasmannia lanceolata*, which span the wettest to driest limits of Winteraceae's natural ecological range (Feild et al. 2000), indicated that no embolism should be induced by dehydration to a Ψ_{stem} of −0.5 MPa (T. Feild, unpubl. data). Ψ_{stem} was inferred by measuring the leaf water potential with a Scholander pressure bomb after a dehydrated branch was placed into a humid plastic bag in the dark for 30 min (Melcher et al. 1998). Branches were frozen in humid plastic bags in the field to −6.5°C at a controlled freezing rate of 1°C/min using a portable freezer (Engel 15, Engel Refrigeration, Melbourne, Australia) powered by a car battery. Freezing exotherms, which occurred between −2 and −6°C in all species, were measured on portions of the branches not used for hydraulic measurements by inserting a copper-constantan thermocouple between the bark and wood. A minimum freezing temperature of −6.5°C was selected to ensure that samples underwent freezing but avoiding freezing-in-

duced damage to living xylem cells that can reduce K_H in ways independent of xylem embolism (Pockman and Sperry 1997). Measurements of the relation between the loss of stem K_H (relative to an initial value before treatment, see below) and freezing temperature for *Tasmannia lanceolata* species suggests that damage to living vascular tissues did not occur until freezing temperature dropped below −15°C (Feild and Brodribb 2001). Thus, at a freezing temperature of −6.5°C, we can reasonably assume that decreases in K_H following freeze-thaw result from xylem embolism. After 2 h, bagged branches were thawed to room temperature (16–18°C), which required approximately 150 min. Rates of freezing and thawing were comparable to those observed in the field (Feild and Brodribb 2001) and on high tropical mountains with frequent frosts (Melcher et al. 2000).

The loss of K_H following freeze-thaw treatment was determined using two methods. The paired-segment method was used for measuring the response of vesselless xylem to freezing (Sperry and Sullivan 1992; Sperry et al. 1994). When branches reached the desired Ψ_{stem}, two similar segments, approximately five cm in length and with one cm between them (which is three to ten times longer than the longest tracheid in Winteraceae and conifers) were marked on a leaf-bearing branch. One stem segment was excised in air, trimmed underwater to remove embolized tracheids by the first cut, and then measured while the remaining portion of the branch was frozen under tension. After freeze-thaw treatment, K_H was measured and compared to the K_H of the first stem segment. The difference in K_H of the first and second stem segments gave the percentage loss in K_H resulting from freeze-thaw (Sperry and Sullivan 1992; Sperry et al. 1994). In the second method (which was used on *Canella winterana*), K_H was measured after freezing and then compared with a maximum stem K_H produced after the stem was treated with three 20-min 175 kPa flushes of filtered water to calculate percentage loss in K_H (PLC, Sperry et al. 1988).

RESULTS

Stem xylem hydraulic capacities (K_S, hydraulic conductivity normalized to xylem surface area, m^2) of Winteraceae ranged from 0.08 kg MPa^{-1} m^{-1} s^{-1} in alpine shrubs of *Tasmannia lanceolata* to 0.40 kg MPa^{-1} m^{-1} s^{-1} in *Zygogynum queenslandiana*, a subtropical cloud forest tree (Table 2; Fig. 2). These values were generally lower than most vessel-bearing taxa (which vary from 0.3–10 kg MPa^{-1} m^{-1} s^{-1}; Sperry et al. 1994; Tyree and Ewers 1991, 1996; Brodribb and Feild 2000; Feild and Brodribb 2001), but fell within the range of values for the six species of conifers investigated (Table 2; Fig. 2). Tropical Winteraceae from regions with no frost possessed greater K_S than temperate Winteraceae taxa (tropical mean = 0.32 kg MPa^{-1} m^{-1} s^{-1}, SD = 0.08 vs. temperate mean = 0.19 kg MPa^{-1} m^{-1} s^{-1}, SD = 0.06). The xylem hydraulic capacity of *Canella winterana* stems was two-fold greater than that of all tropical Winteraceae and as much ten times greater than that of temperate Winteraceae taxa (e.g., *Tasmannia lanceolata* from alpine zones; Fig. 2).

Mean tracheid diameters of temperate Winteraceae species overlapped broadly with the range of conifers (Fig. 1B). However compared to temperate taxa, Winteraceae species from

TABLE 2. Habitat, distribution, ecology, and climatic associations of Winteraceae and Canellaceae species included in hydraulic studies. Nomenclature for species is based on Vink (1970, 1977, 1993). Frost frequency classes are: 0, never below 0°C; 1, freezes possible, once every one to seven years; 2, freezing frequent, 20 to 300 events a year.

Species	Habitat	Growth form	Frost frequency	Distribution and sampling locality
Winteraceae				
Drimys winteri var. *andina* Reiche	Valdivian temperate rainforest to subalpine heath	Subcanopy to alpine shrub	1–2	Chile, greenhouse-raised
Drimys winteri var. *chilensis* (DC) A. Gray	Temperate coastal rainforest and sub-Antarctic moorland	Subcanopy to canopy tree	1–2	Chile, greenhouse-raised
Pseudowintera colorata (Raoul) Dandy	Temperate lowland rainforest to alpine heaths and meadows	Subcanopy tree to alpine shrub	1–2	New Zealand, greenhouse-raised
Takhtajania perrieri (Capuron) Baranova and J.-F. Leroy	Tropical premontane cloud forest	Understory shrub to small tree	0	Madagascar, wood sample; P. J. Rakotomalaza et al. 1342 (MO)
Tasmannia insipida R. Br. Ex DC	Temperate sclerophyll forest, open montane scrub	Subcanopy tree	1–2	Eastern Australia, Styx River Valley, New South Wales
Tasmannia lanceolata (Poir.) A.C. Smith	Temperate lowland rainforest to alpine heath and meadows	Subcanopy tree to alpine shrub	1–2	Southern Australia, Mt. Field, Tasmania, Australia, greenhouse-raised
Tasmannia membranea (F.v.M.) A.C. Smith	Tropical montane cloud forest	Subcanopy treelet to small-exposed tree	0–1	Northern Australia and Papua New Guinea, Mt. Bartle Frere, Queensland
Zygogynum balansae Tiegh.	Dense evergreen montane rainforest	Subcanopy tree	0	New Caledonia. Mt. Dzumac
Zygogynum pancheri (Baill.) Vink	Dense evergreen montane rainforest	Understory to subcanopy treelet	0	New Caledonia, Plateau de Dogny
Zygogynum queenslandiana Vink	Tropical premontane cloud forest	Understory to subcanopy treelet	0	Northern Australia, Mt. Bartle Frere, Queensland
Canellaceae				
Canella winterana Gaertn.	Tropical to subtropical dry forest	Subcanopy to canopy	0	Caribbean Islands, Key Largo, Florida, USA

tropical environments developed larger tracheids and possessed greater variability in tracheid sizes (Fig. 3A–C). Based on tracheid diameter distributions, the xylem of *Takhtajania perrieri* is comparable to other tropical Winteraceae (i.e., *Zygogynum queenslandiana*; Fig. 3A–C). In *Canella winterana*, xylem vessels were sporadically distributed in a background of tracheids. Although tracheids dominated numerically (92%), they accounted for only 23% of stem hydraulic conductance when modeled as ideal capillaries (Fig. 3D). This is due to the larger mean diameter of vessels compared to the co-occurring tracheids (vessels = 28.4 µm, SD = 7.66; tracheids = 10.5 µm, SD = 2.6, Fig. 3D), and thus, as anticipated, vessels are likely to conduct more water than an equal cross-section of tracheids (Zimmermann 1983; Tyree and Ewers 1991).

Average percent loss of xylem hydraulic conductivity (PLC) following freeze-thaw was between 0 and 6% in stems of Winteraceae species from temperate climates with numerous freezing events, as well as in *Tasmannia membranea* that occurs in exposed areas of tropical montane cloud forests that experience occasional frost (Fig. 2A). Similarly, the vascular systems of the six conifer species exhibited almost no decrease in xylem hydraulic conductivity following freezing (Fig. 2). PLC values for Winteraceae species from tropical environments (no freezing) were greater compared to temperate taxa, ranging from 20% in *Zygogynum baillonii* to 23% in *Zygogynum queenslandiana*. In contrast, xylem hydraulic conductivity of vessel-bearing *Canella winterana* decreased approximately 85% after freeze thaw (Fig. 2).

DISCUSSION

Freeze-Thaw Xylem Resistance and the Temperate Ecology of Winteraceae

Measurements on Winteraceae species from frost-prone environments demonstrate that their vessel-free wood avoids freezing-induced losses in stem xylem hydraulic capacity (Fig. 2). Additionally, Winteraceae taxa from frost-free tropical habitats, including *Zygogynum* members and probably *Takhtajania perrieri* (based on a similar frequency distribution of tracheid diameters to *Zygogynum queenslandiana*, see Fig. 3A,B), exhibit low xylem vulnerability to freeze-thaw (Fig. 2). Xylem water transport of *Canella winterana*, a close relative with xylem vessels, is also substantially inhibited by freezing (Fig. 2), a potentially general result if, as noted by Wilson (1960), that all canellaceaous genera possess similar or larger (to 55 µm) average vessel diameters than *Canella* (see below). Overall, these results support earlier work demonstrating a tradeoff between xylem hydraulic efficiency and vulnerability to freeze-thaw embolism, with tracheids possessing a low probability of freeze-thaw embolism because smaller diameters limit bubble expansion during thawing at a given xylem tension (Hammel 1967; Wang et al. 1992; Sperry and Sullivan 1992; Sperry et al. 1994; Sperry 1995; Davis et al. 1999; Feild and Brodribb 2001).

Consistent with the widespread resistance to freeze-thaw embolism, three of the five major clades resolved by molecular phylogenetic analyses, including *Drimys*, *Pseudowintera*, and *Tasmannia* (Suh et al. 1993; Karol et al. 2000), have members that occur as exposed plants in regions with nu-
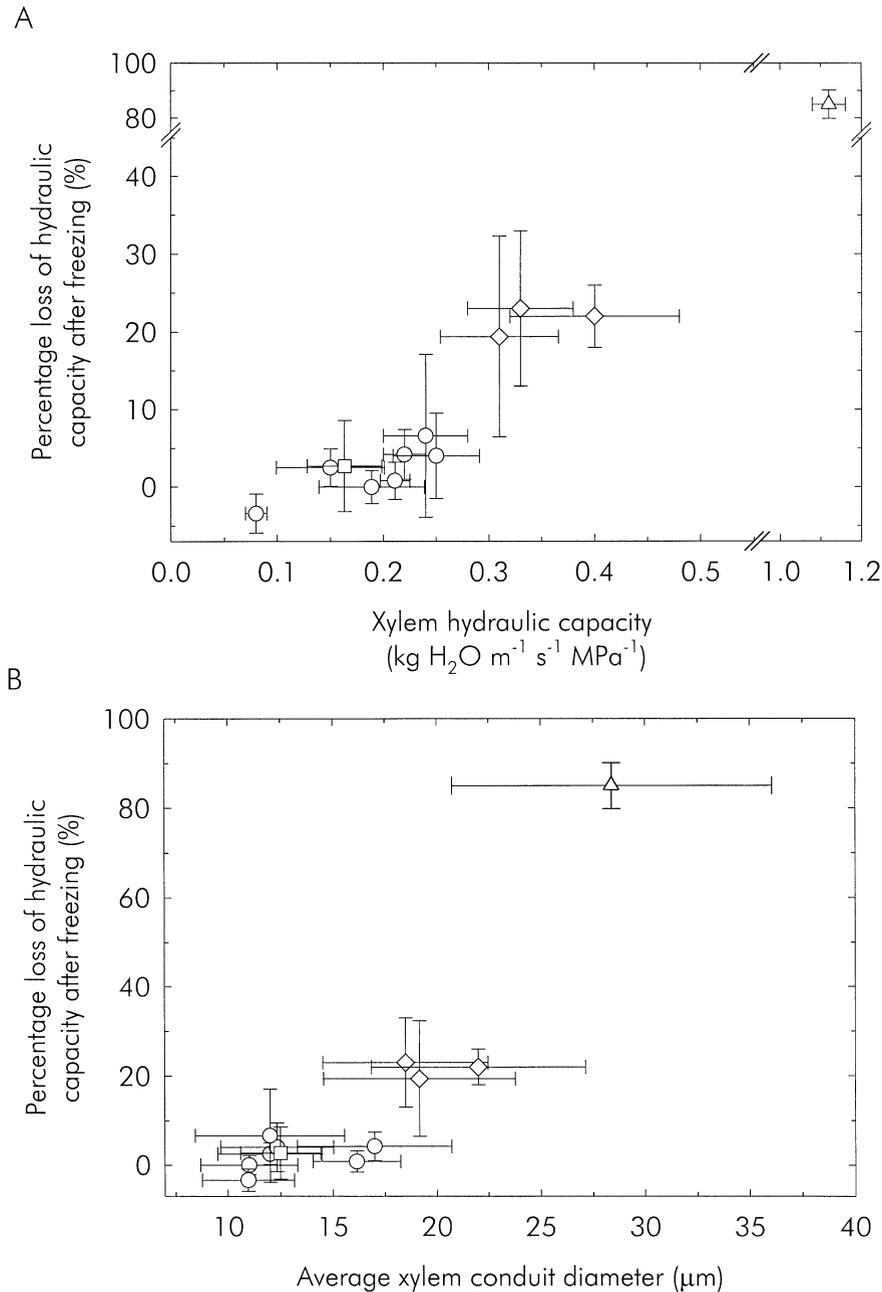
FIG. 2. Relation between percentage loss of xylem hydraulic conductivity following freeze-thaw (PLC) with xylem hydraulic capacity (K_S, A) and average xylem conduit diameter (μm, B) for temperate (circles) and tropical (nonfreezing; diamonds) Winteraceae, average value for six conifer species (square, see Materials and Methods section), and *Canella winterana* (triangle). The average conduit value presented for *Canella* includes vessels only. Sample sizes for each point are five stems from five different plants, with error bars illustrating the standard deviation.

merous freezing events (Table 1; Smith 1945b; Vink 1970; Sampson 1980; Feild et al. 2000). For example, *Drimys winteri* reaches its peak abundance in cold, high-latitude (48° to 56°S) Magellanic rainforests in Chile where it occurs as an exposed, medium-sized tree (to 15 m) forming dense, pure stands (Young 1972; Lusk 1993; Rebertus and Veblen 1993). In spite of prevailing near-freezing temperatures and frosts that can occur year round, *D. winteri* maintains greater relative growth rates than co-occurring conifers and *Nothofagus*

trees (Young 1972). In addition, several *Tasmannia*, *Drimys*, and *Pseudowintera* species occur as abundant colonizing shrubs in frosty alpine heaths, that experience 100 or more freeze-thaw cycles a year, and also as small- to medium-sized trees in cool, temperate rainforests (Vink 1970; Wardle 1985; Barnes et al. 2000; Feild et al. 2000; Feild and Brodribb 2001). Other *Tasmannia* species and *Drimys granadensis* are common colonizers of burnt tree fern scrubs at tropical timberlines in Papua New Guinea, and shrubs in páramos of
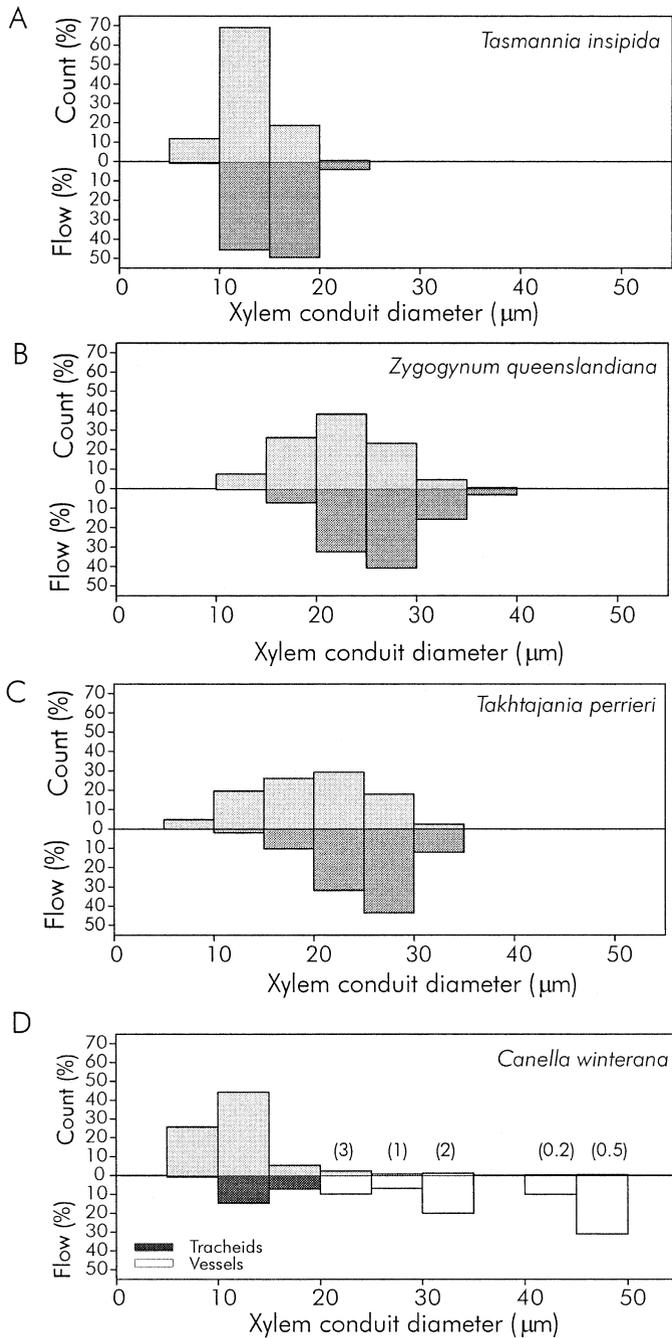
FIG. 3. Frequency distributions of xylem conduit diameter and percentage contribution to xylem hydraulic conductance when idealized as capillaries (see Materials and Methods) for Winteraceae occurring in temperate (A, *Tasmannia insipida*) and tropical, non-freezing (B, C, *Zygogynum queenslandiana* and *Takhtajania perrieri*, respectively) environments compared to *Canella winterana* (D). Numbers above bars denote the percentage of vessel conduits.

South and Central America, respectively, which are regions described as "winter every night, summer every day," in being characterized by nightly freezes as low as −5°C and daytime temperatures as high as 25°C (Vink 1970; Hnatiuk et al. 1976; Barry 1980).

We believe that the possession of vesselless wood plays

an important role in explaining the ecological importance of Winteraceae in Southern Hemisphere temperate environments that are characterized by a climatic regime that is different from the continental climates of North American and Eurasian temperate and boreal zones. Unlike Northern Hemisphere temperate and boreal forests which are generally dominated by deciduous angiosperms and evergreen conifers, temperate forests and tropical alpine woodlands in the Southern Hemisphere are dominated by woody, evergreen angiosperms and contain few species of winter-deciduous trees (Axelrod 1966; Wardle 1971; Sprugel 1989; Chabot and Hicks 1982; Markgraf et al. 1995). One explanation for this large difference in vegetation structure is the prevailing influence oceanic climates present today, and in the past, on Southern Hemisphere land masses which dramatically reduce seasonal temperature variation compared to more continental climates (Axelrod 1966; Wardle 1971; Sprugel 1989; Markgraf et al. 1995). The attenuation of winter-minimum and summer-maximum temperatures appears to have resulted in the evolution of lower frost tolerance of Southern Hemisphere plants, and thus treelines in these regions are lowered, on average 1000 m below those in the Northern Hemisphere at equivalent latitudes (Daubenmire 1954; Sakai et al. 1981; Wardle 1985; Sprugel 1989; Markgraf et al. 1995; Feild and Brodribb 2001). Although the strong oceanic influence means that amplitudes of temperature variation are dampened, in montane areas or at high-latitudes temperature, excursions below freezing (not generally lower than −20°C; Markgraf et al. 1995) can occur at any time of the year, and rapidly in the wake of fast-moving weather fronts that characterize many southern landmasses. Thus, a critical difference between most Northern Hemisphere temperate and boreal areas versus those in the Southern Hemisphere where Winteraceae are most abundant is that almost every productive day of leaf's lifetime lies behind a freeze-thaw event, whereas in temperate climates of the Northern Hemisphere few days do.

Through enabling a freeze-thaw, embolism-free vascular system, vesselless wood potentially allows for more rapid resumption of leaf photosynthesis following exposure to sub-freezing temperatures once branches thaw because bubbles frozen out of xylem sap collapse easily in small-diameter conduits (Fig. 2; Hammel 1967; Sucoff 1969; Zimmermann 1983; Wang et al. 1992; Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999). In addition, if embolisms form, these will be contained in a single cell rather than spreading to disable a larger vessel-mediated hydraulic path (Zimmermann 1983). These vascular properties are especially important in Southern Hemisphere alpine and temperate zones where freezing can occur at any time of the year but day-time temperatures are mild enough for growth (Feild and Brodribb 2001). Furthermore, *Drimys winteri* and *Tasmannia lanceolata*, which are two temperate species, appear to lack root pressure (T. Feild, unpubl. obs.), which suggests that Winteraceae may be unable to readily reverse embolisms (unless these are refilled under tension, see Holbrook and Zwieniecki 1999) and therefore sidestepping the hydraulic constraints arising from freeze-thaw embolism through the production of vesselless wood may be of special ecological significance. In this sense, Winteraceae are analogous to evergreen conifers from temperate zones which also possess

limited ability for root pressure (Hammel 1967; Sucoff 1969; Zimmermann 1983; Sprugel 1989; Wang et al. 1992; Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999; Feild and Brodribb 2001). Coupled with a freezing-resistant hydraulic system, leaves and buds of *Drimys winteri* and *Tasmannia lanceolata* were reported to be more frost-tolerant than some co-occurring conifers and vessel-bearing angiosperms (Sakai et al. 1981; Alberdi et al. 1985; Feild and Brodribb 2001).

The two remaining Winteraceae clades (Karol et al. 2000), including the basal lineage represented solely by *Takhtajania perrieri* and the *Zygogynum* clade (Vink 1993), appear to be specialized for shady, understory habitats in tropical montane cloud forests that are frost-free (Carlquist 1981, 1983a,b, 2000; Vink 1977, 1983, 1993; Feild et al. 2000). *Takhtajania perrieri* occurs as a spindly shrub to a treelet, and *Zygogynum* taxa range from multiple-branched shrubs, treelets, or sub-canopy trees under dense, closed-canopy cloud forests (Vink 1977, 1983; Sampson 1983; Feild et al. 2000; Schatz 2000). Unlike their temperate and alpine relatives, populations of *Takhtajania* and *Zygogynum* species tend to be small (Smith 1943b; Sampson 1983; Feild et al. 2000; Schatz 2000), consisting of a few sporadically distributed individuals, or occurring in small isolated forest pockets (in particular *Takhtajania*; Schatz 2000). Consistent with their occurrence in low-light environments, *Takhtajania* and *Zygogynum* share a suite of morphological characters that are likely to increase light-harvesting and to facilitate establishment under light-limited conditions. For example, leaves of *Takhtajania* and many species of *Zygogynum* are large (15–30 cm long) and dark green with a poorly differentiated leaf palisade layer, which are characters that may increase the capture efficiency of diffuse understory light (Vogelmann 1993; Feild et al. 2000; Keating 2000). In addition, these taxa produce abundant stem sprouts and larger seed sizes than the temperate and tropical timberline Winteraceae (Schatz 2000; Tobe and Sampson 2000). These are traits that appear to be important in recovering from traumas induced by falling forest canopy debris and seedling recruitment under dense rainforest canopies, respectively (Greig 1993; Turner 2001). Shade-tolerance in members of these two clades may be further enhanced by the development of larger tracheids, and thus greater xylem hydraulic efficiency (Fig. 2), which, all else being equal, lowers the allocation of wood required to support a given leaf area (Zimmermann 1983). Small investments in wood production may lower respiratory carbon losses, allowing for efficient growth under low light (Givnish 1984, 1995). However, this adjustment apparently entails some sensitivity to freeze-thaw embolism (Fig. 2).

Cretaceous Cold-Adaptation and the Loss of Vessels in Winteraceae

How do these observations bear on the possible loss of vessels in Winteraceae which is supported by phylogenetic analyses? Today Winteraceae are well known for their ''Gondwanan'' or ''Antarctic'' distribution, occupying cool to cold, wet areas of Australia, New Caledonia, New Zealand, Papua New Guinea (with a presumably recent extension of one species to the Philippines), and South America (extend-

ing up the Andes into the Central American highlands), with one species (*Takhtajania perrieri*) in Madagascar (Table 1; Smith 1945b; Raven and Axelrod 1974; Thorne 1986). Numerous Late Cretaceous and Tertiary records of Winteraceae pollen, assignable to *Drimys*, *Pseudowintera*, *Tasmannia*, and *Zygogynum*, point to a similar distribution on Southern Gondwanan landmasses, including Australia, New Zealand, South America, and South Africa (Fig. 3; see Mildenhall 1980; Coetzee and Pragowski 1988; Dettmann and Jarzen 1990; Dettmann 1994; Doyle 2000). Additionally, vesselless winteraceous wood (*Winteroxylon jamesrossi*, Poole and Francis 2000), which appears to be anatomically most similar to *Zygogynum* and possibly *Takhtajania*, first appears in this region (James Ross Island, Antarctica) during the Late Cretaceous (see Fig. 4; Carlquist 2000). The distribution of these Winteraceae fossils is well matched to the wet temperate Early Cretaceous Southern Gondwana province (sensu Brenner 1976), that comprises the present-day areas of southern South America, South Africa, India, Madagascar, Australia, and Antarctica. These areas were dominated by conifers (Podocarpaceae, Araucariaceae), and joined in the Late Cretaceous by the austral angiosperms *Nothofagus* and Proteaceae (*Macadamia*, *Knightia*) that persist with Winteraceae in the same regions today (Herngreen et al. 1981; Askin 1989; Dettmann 1994; Pole 1994; Srivastava 1994; Askin and Spicer 1995).

Considerably older (30 to 40 Mya before the appearance of Winteraceae pollen in Australia) and more primitive ''winteroid'' pollen types, designated as the fossil genus *Walkeripollis*, which are not assignable to any modern clade (e.g., on the stem lineage, see Walker et al. 1983; Doyle et al. 1990; Brenner 1996; Doyle 2000), were discovered in late Barremian to early Aptian sediments from several sites located in what was Northern Gondwana (e.g., Gabon, Brazil, Israel, and Saudi Arabia; see Doyle 2000 for a review). These areas were situated in the Early Cretaceous equatorial tropical belt, a region with a semiarid and warm climate, based on the high abundance of pollen produced by xerophytic Cheirolepidiaceae conifers (*Classopollis*), ephedroid pollen and thick salt deposits (Brenner 1976, 1996; Doyle et al. 1977, 1982; Doyle 2000). One interpretation of the *Walkeripollis* pollen pattern is that the Late Cretaceous Winteraceae and their extant descendents represent a temperate offshoot of an initially tropical lineage that invaded temperate high-latitude Southern Gondwana rainforests (Doyle et al. 1990; Doyle 2000).

A Northern Gondwanan origin of the Winteraceae stem lineage is also in line with the ecology and distribution of their extant sister group, Canellaceae. Canellaceae occur as shrubs and small- to medium-sized trees in habitats where extant Winteraceae are absent, including dry tropical forests and scrubs to lowland tropical rainforests (Wilson 1960; Kubitzki 1993). An intriguing exception is the understory tropical cloud forest plant, *Cinnamosma madagascariensis*, which occurs with *Takhtajania perrieri* in northeast Madagascar (Karol et al. 2000). Compared to the Southern Gondwanan diversification of Winteraceae, phylogenetic relationships and limited fossil evidence of Canellaceae point to a ''reciprocal'' Northern Gondwana pattern of migration and radiation (Karol et al. 2000). Basal Canellaceae clades, including *Canella* and a lineage containing *Pleodendron* and

Cinnamodendron, occur in northern South America and the West Indies (Wilson 1960). A clade comprised of *Capsicodendron*, *Warburgia*, and *Cinnamosma* display a southeastern Brazil-African-Malagasy disjunction (perhaps resulting from the Early Cretaceous biogeographic separation of South America from Africa; Goldblatt 1993; Karol et al. 2000). Putative canellaceous fossils, with the earliest record from the lower Campanian, occur in North America and Puerto Rico, well outside of Southern Gondwana (Graham and Jarzen 1969; Van Borskik 1997). Possible out-groups to Winteralean clade, including Magnoliales and Piperales, also appear to be composed largely of tropical and subtropical lowland rainforest taxa which suggests that the Winteraceae are nested among lineages with this sort of climatic regime (Doyle 2000).

Combined with the new finding that vesselless Winteraceae wood is generally immune to freezing-induced xylem embolism, it appears possible that vessels were lost as ancestors of Winteraceae shifted from tropical, nonfreezing Northern Gondwanan climates into the wet southern Gondwanan temperate zone (Fig. 4; see Fig. 5A; Donoghue and Doyle 1989; Doyle et al. 1990; Doyle 2000; Feild et al. 2000). Given the energetic costs required to replenish embolized vessels by freezing, through cambial growth and root pressure, the loss of vessels, which entails increased reliance on smaller diameter conduits and greater hydraulic redundancy with a cost of hydraulic efficiency, may be particularly relevant in the context of the clade including Canellaceae and Winteraceae (i.e., Winterales). For example, *Canella winterana*, in addition to the two species of temperate Winteraceae mentioned earlier, also appears to be unable to produce root pressure (T. Feild, unpubl. obs.), which suggests that Winteralean members would be unable to readily remove embolisms induced by freeze-thaw. However, more information on the phylogenetic distribution of root pressure within Winterales and in their sister group are urgently needed. Because all Canellaceae and Winteraceae are evergreen (Vink 1970; Kubitzki 1993), maintenance of this habit while migrating into freezing-prone habitats may require the development of a resilient vascular system to ensure adequate leaf hydration.

Paleoclimatic reconstructions and high-latitude fossil biotas indicate a prevailing wet (as indicated by abundant coals), cool to cold temperate climate in Southern Gondwana (Brenner 1976; Herngreen et al. 1981; Frakes and Francis 1988; Rich et al. 1988; Askin 1989; Dettmann 1994; Srivastava 1994; Askin and Spicer 1995). In particular, Early Cretaceous Southern Gondwanan climates, latest Barremian to Albian, are the coldest, with possible occurrences of soil freezing, glacial development, and certainly prolonged winter darkness given the high-latitude position of Southern Gondwana (Frakes and Francis 1988; Rich et al. 1988; Dettmann 1994; Ditchfield et al. 1994; Quilty 1994; Dingle and Lavelle 1998). Modeling of Cretaceous climates demonstrates that even during cool to warm temperate periods, when surrounding oceanic Gondwanan fragments were frost free (i.e., Cenomanian to Maastrictian, Francis 1986), the interiors of high-latitude austral continents remained seasonally cold with winter freezing (Frakes and Francis 1988; Quilty 1994). Thus, there appears to have been great potential for freezing across Southern Gondwana from the earliest appearance of the Win-

teraceae stem lineage, represented by *Walkeripollis* (Barremian-Aptian, Doyle et al. 1990) and the vesselless state in Winteraceae (*Winteroxylon*, Santonian-Campanian) from Antarctica (Fig. 4). Alternatively, Winteraceae ancestors could have first entered freezing-prone environments closer to the equator by shifting into mountainous regions flanking the rift valley sequence formed between South America and Africa as they separated in the Early Cretaceous.

An inconsistency with the hypothesis that selection for freezing tolerance drove vessel loss in Winteraceae is that the basal clade, *Takhtajania perrieri*, occurs in a frost-free, tropical cloud forest habitat (Fig. 5B, Carlquist 2000; Schatz 2000). Assuming vessels were lost rather than primitively absent, one could suggest that Winteraceae reverted to tracheid-based xylem via genetic drift associated with a transition from tropical dry forest or lowland rainforest environments, like those of Canellaceae and perhaps *Walkeripollis*-parent plants, into upland cloud forest understory habitats where the hydraulic consequences of vessel loss would be low (Donoghue 1989; Donoghue and Doyle 1989; Doyle et al. 1990; Doyle 2000). According to this scenario, as Winteraceae migrated into the austral-temperate zone, vesselless wood represented a preadaptation allowing the occupation of freezing-prone environments (Fig. 5B).

The lack of direct evidence for the timing and location for the origin of the branch containing *Takhtajania* makes it difficult to resolve the selective regime and sequence of ecological events associated the possible loss of vessels in Winteraceae (Fig. 5). However, indirect functional and biogeographic evidence suggests that *Takhtajania perrieri* may not retain the ancestral ecology of the Winteraceae crown-group. In the frost-free, shady environments where *Takhtajania* occurs, one might expect that vessels would be favored over tracheids by decreasing the amount of wood required to support leaves hydraulically and mechanically, and thereby increase carbon-use efficiency under light-limited conditions (Zimmermann 1983; Givnish 1984, 1995). The co-occurrence of *Takhtajania* with *Cinnamosma madagascariensis* (Canellaceae), which is clearly derived from a tropical, dry-forest lineage (Karol et al. 2000) and possesses vessels plus tracheids, is consistent with the idea that ecologically shifting into a tropical, cloud-forest understory would not drive vessel loss.

Biogeographically, Madagascar may not have been a stable refuge for the *Takhtajania* lineage. During the Early Cretaceous, Madagascar (including South Africa) was part of the temperate Southern Gondwana province (Herngreen et al. 1981; Srivastava 1994) and therefore may have experienced frequent or seasonal freezing. Since this time, Madagascar has rifted northward into warmer, tropical latitudes that devastated its Cretaceous podocarp-dominated temperate flora (Rabinowitz et al. 1983; Coetzee and Muller 1984; Coetzee and Pragowski 1988). If the *Takhtajania* clade originated during the Early Cretaceous in the Malagasy-South African region, then it may have arisen in a considerably colder climate than it occurs in today. A similar climatic transition appears plausible for the lineage containing *Zygogynum*. Dettmann and Jarzen (1990) reported the first appearance of *Zygogynum* (designated as *Belliolum/Bubbia* in their study) pollen from the Late Cretaceous of temperate southern Australia. How-
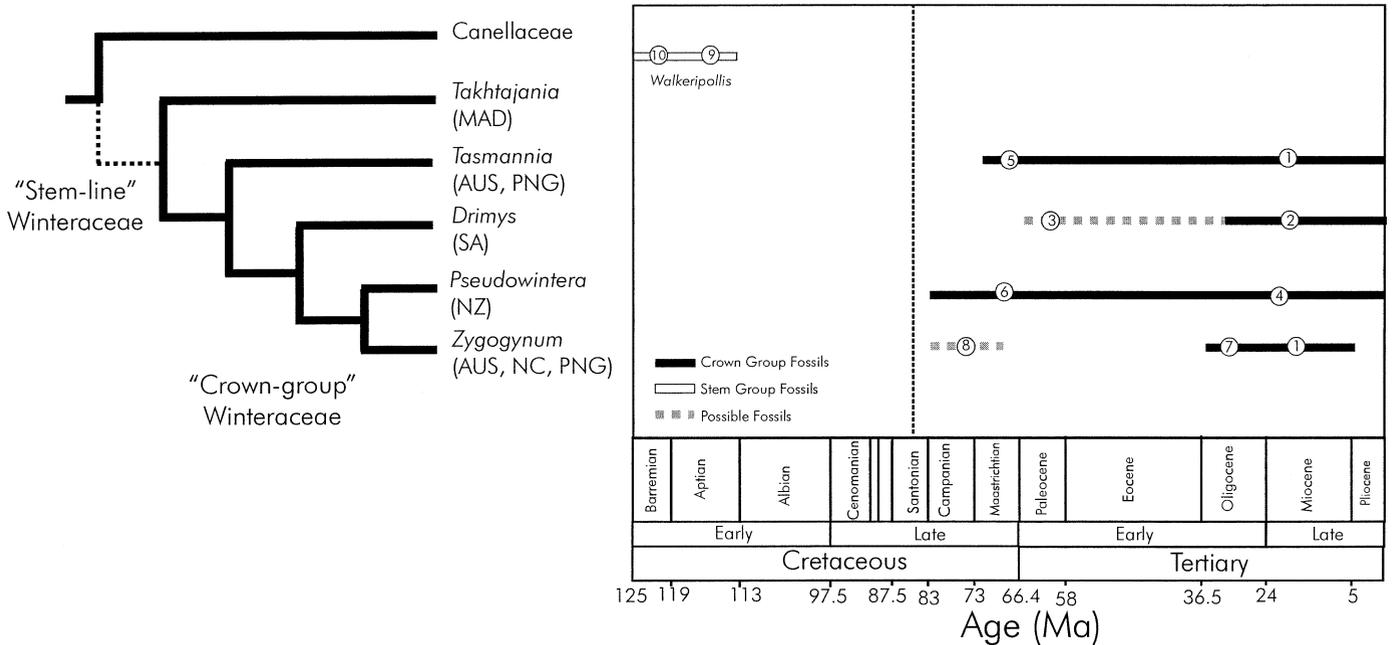
FIG. 4. Summary of the temporal distribution of Winteraceae fossil pollen records. Phylogeny based on Karol et al. (2000) and geographic distribution of genera are denoted below taxon names, abbreviated as: AUS, Australia; MAD, Madagascar; NC, New Caledonia; NZ, New Zealand; PNG, Papua New Guinea; SA, South America. The dotted line denotes the first appearance of vesselless winteraceous wood, as represented by *Winteroxylon jamesrossi* discovered from Antarctica (Poole and Francis 2000). References for taxonomic assignments for fossil pollen are denoted by number the following: (1, Coetzee and Pragowski 1988; 2, Barreda 1997; 3, Baldoni 1988; 4, Mildenhall and Crosbie 1979; 5, Krutzsch 1970, Stover and Partridge 1973, Martin 1978; 6, Mildenhall 1980; 7, Mildenhall and Crosbie 1979; 8, Dettmann and Jarzen 1990; 9, Walker et al. 1983; 10, Doyle et al. 1990; Brenner 1996).

ever, today, *Zygogynum* taxa occur in low- to middle-elevation, cloud forests, on post-Gondwanan remnants generally near the equator (Vink 1977, 1983, 1993). New discoveries of winteroid fossil pollen from the Early Cretaceous time interval and careful analyses of their climatic associations could greatly clarify this situation.

Vessel Loss Appears to be Hydraulically Possible in the Context of the Winteralean Clade

The view that vessel loss would result in a catastrophic shift to an ineffective vascular system is not supported by measurements of xylem conduit dimensions of Winteraceae taxa and *Canella winterana*. Although vessel-bearing wood of *Canella winterana* possesses considerably greater xylem hydraulic capacity than Winteraceae (Figs. 1 and 2), only 20% of xylem hydraulic conductance remains if the proportion of hydraulic flow through vessels, when modeled as ideal capillaries, is subtracted out (Fig. 3D). Consistent with this finding, approximately 20% of the initial xylem hydraulic conductivity remains in *Canella winterana* stems following freezing, which suggests emboli are localized to xylem vessels while tracheids remain conductive (Fig. 2). Indeed, tracheids of Canellaceae, possess full pit borders which indicates that they are likely to be conductive (Carlquist 1988a,b). The average tracheid diameter of *Canella winterana*, is nested in the range found for some temperate Winteraceae, and therefore these tracheids would not be predicted to become embolized by freezing (Fig. 2B). Similar vascular systems, with *relatively* low proportional xylem conductance contrib-

uted by vessels (65–85%), are widespread in Canellaceae, including *Capsicodendron* and *Cinnamosma* taxa, as well as *Cinnamodendron axillare* (Endl.) (T. Feild, unpubl. data). Utilizing canellaceaous xylem as an analog for the ancestral condition of the Winterales, it is clear that vessel loss would result in a considerable decrease in hydraulic capacity. However, hydraulic capacity would be comparable to that present in temperate Winteraceae taxa (e.g., *Pseudowintera colorata*, *Tasmannia lanceolata*, Fig. 2), and therefore virtually immune to freeze-thaw embolism.

Conclusions

Vessel loss, if it has indeed occurred during Winteraceae evolution, must be exceedingly rare among other angiosperms because in spite of the numerous transitions that angiosperms (including evergreen lineages) have made from warm, tropical climates into freezing-prone tropical and temperate environments, there are very few woody, vesselless taxa (Carlquist 1975, 1988; Cronquist 1988). The only other possible instance of vessel loss in angiosperms may be in the Trochodendrales, which like Winteraceae are also well nested among clades with vessel-bearing xylem (Fig. 1; Soltis et al. 1999; Doyle and Endress 2000; Qiu et al. 2000). Although little is known about the past climatic associations of plants belonging to this clade, a rich fossil record of ''trochodendroid'' plant parts, including the fossil fruit and infructescence genus *Nordenskioldia* and leaf genus *Zizyphoides*, indicates that trochodendraceous plants first appeared in the Early Cretaceous tropical/subtropical zone in Eastern
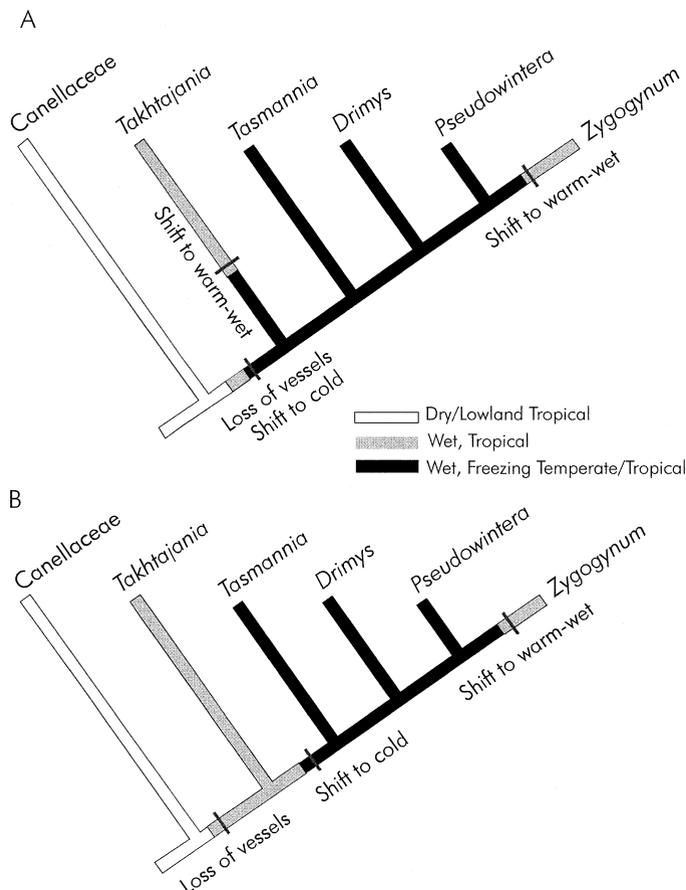
FIG. 5. Phylogenetic scenarios for the loss of vessels in Winteraceae. Phylogeny is based on the single most likely tree based on combined ITS/*trn*L-F data provided by Karol et al. (2000), with all clades supported by bootstrap values with at least 98%. (A) Selection for freezing tolerance, in which case vessel loss occurred concomitantly with a transition from a nonfreezing, dry or tropical lowland environment to a freezing-prone climate. (B) Alternatively, vessels may have been lost by chance, associated with a shift to wet, tropical cloud forest environments and then later the vesselless state represented a preadaptation for frost tolerance.

North America and then spread northward and became abundant at high-latitude boreal forests in the Tertiary across the Northern Hemisphere (Crane et al. 1991; Manchester et al. 1991; Manchester 1999; Pigg et al. 2001). Whether or not ancestors of this clade possessed vessels and then returned to tracheids in response to freezing is not clear because paleoclimatic associations of trochodendroid fossil leaves and wood are poorly known and little ecological data is available on the extant environmental circumstances of *Trochodendron* or *Tetracentron*. However, preliminary xylem hydraulic measurements on *Trochodendron aralioides* Sieb and Zucc. in response to freeze-thaw indicate that its vesselless wood is resistant to frost which may relate to its occurrence in subalpine forests in Japan (Smith 1945a; T. Feild, unpubl. ms.).

If there are functional advantages to be reaped by returning to a vesselless vascular system, then why is this transition so infrequent? Numerous groups of angiosperms (e.g., some Ericaceae, Myrtaceae, Epacridaceae, and Rosaceae, Carlquist 1988a), that possess both vessels and tracheids have clearly migrated into regions subjected to freezing, yet vessels are developed. Although there are no specific cases that have been investigated in an explicit phylogenetic context, it does appear qualitatively that shifting from frost-free to freezing-prone climates selects for greater hydraulic redundancy, favoring the retention or reinvention of tracheids from fibers, coupled with reductions in vessel size and frequency (Carlquist 1988a). Indeed, in the gymnosperm *Ephedra* (Gnetales), some species from cold deserts exhibit a virtual absence of vessels, with stems composed almost entirely of tracheids (Carlquist 1988b). We suggest, in a similar vein as Donoghue (1989), that the rarity of vessel loss might be explained by the evolution of traits dependent on the presence of vessels which increase the developmental and as functional ''burden'' on vessels which constrain this character's reversibility. For instance, in the examples of angiosperm families mentioned above with both vessels and tracheids, the vessels that are produced are relatively more specialized in form compared to those that occur in Canellaceae. Such specializations include, but are not limited to: (1) large differences in vessel element and tracheids lengths which implying size disparities in the cambial initials; (2) possession of simple (open) perforation plates or strongly-sloped (and fewer barred) scalariform perforation plates; and (3) the modifications in the types and frequency of lateral vessel pitting (Bailey 1953; Carlquist 1975; 1988a). Also, with the origin of vessels it becomes possible to optimize mechanical (via fiber evolution) and hydraulic functions of wood separately, which may further spur the evolution of other xylem morphological characters, including in the ray and xylem parenchyma systems, which may increase burden (Carlquist 1975; Zimmermann 1983). Additionally, the appearance of vessels may alter whole plant physiological function, changing the functional constraints (e.g., xylem vulnerability, carbon allocation) associated with evolution of drought and shade tolerance and possibly the regulation of leaf photosynthetic rates (Brodribb and Feild 2000). These developmental and functional factors are likely to interact to restrict the evolutionary flexibility of vessels soon after they appear. Thus, vessel loss appears only plausible in lineages where the selective forces against vessel possession have acted at an early stage of evolution, when vessels were at a low stage of evolutionary specialization (perhaps not so different from tracheids) and potentially few in number which would limit the physiological dependence on them (Donoghue 1989; Donoghue and Doyle 1989). This study suggests that the Winteralean clade may indeed represent such a transition. However, further work is needed to understand the developmental processes and their coordination in the vascular cambium that determine formation of vessels versus tracheids. Our work emphasizes the importance of making direct use of physiological and functional information, in the context of historical information, to inform our perspective on the likelihood of a particular evolutionary transition.

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Corresponding Editor: T. Givnish