

Uwe Hacke *Editor*

# Functional and Ecological Xylem Anatomy

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# Preface

Trees and wood have fascinated people for centuries, and this book was born out of this fascination. Trees provide a habitat to countless birds and other animals that live in them. Trees provide us with oxygen, timber, shade, and shelter, and they absorb large amounts of carbon from the atmosphere. Similar statements can be made about the other plant groups and growth forms covered in the book.

Vascular plants, whether woody or not, have remarkably complex conducting tissues. The sugars made by photosynthesis as well as many other molecules are distributed in the phloem. Water and nutrients are transported in the xylem. Phloem and xylem, though fundamentally different in the way they function, are in close proximity to each other, and together they connect plant organs that are often quite distant. According to the cohesion-tension theory, water ascends plants in a metastable state under tension (negative hydrostatic pressure). This transport mechanism has profound implications for the structure of the xylem, as explained in several of the book chapters. In woody plants, xylem also provides structural support to the plant body and serves in storage. Water transport and leaf water supply are vitally important for the sustained growth of forests, agricultural crops, and horticultural plants. Globally, the amount of water moving through plant xylem is enormous. Transpiration represents by far the largest water flux from Earth's continents.

Many of my fellow contributors and I have been inspired by Martin Zimmermann's book *Xylem Structure and the Ascent of Sap* and by Melvin Tyree and M. Zimmermann's 2nd edition of the book, which was published in 2002. While the present book contains many ideas and concepts originally described in *Xylem Structure and the Ascent of Sap*, it is different in terms of its organization and content. A large amount of experimental data is synthesized. New and emerging topics are covered.

The overall objective of the book is to explain what is currently known about functional and ecological xylem anatomy. In addition to serving as a source of information to professionals, instructors, and advanced students in plant science and forestry, I hope that the inherent beauty of xylem that is apparent in many images will be a source of inspiration to readers who are not yet familiar with the topic. The book offers a unique combination of scientific insight based on thorough experimental

work and beautiful images, which often speak for themselves. The beauty of xylem is evident at different scales; from views of striking tree ring patterns and vascular networks in fern fronds, conifer needles, and angiosperm leaves, to microscopic images of developing pits. As we continue to focus on increasingly small structures that are relevant for water transport in plants, we may eventually arrive at aquaporins, membrane-based water channels that impact water flow in living cells of xylem and phloem, and in other tissues. In short, it is my hope that the book will not only be a source of information, but also of inspiration.

The book begins with a discussion of the xylem of seedless vascular plants by J. Pittermann and co-workers. Ferns and lycophytes have existed for 400 hundred million years, yet we know relatively little about the vascular attributes of these ancestral plants. The following chapters describe the ecological xylem function of conifers, lianas, and drought-adapted chaparral species. The xylem of woody model systems (poplar and grapevine) is discussed in detail.

Each of these plant groups has distinct xylem features, with regard to the size of vessels and tracheids, the way in which these conduits are connected, and in terms of pit structure. Pits connect adjacent conduits to allow water flow while also blocking the spread of gas from embolized conduits. Numerous studies have focused on the function of pits, but less is known about their development. R. Dute synthesizes what we currently know about the development, form, and function of torus-bearing pit membranes in seed plants.

Several chapters explore how wood anatomy and hydraulic traits relate to a plant's ability to cope with drought. As temperature and precipitation regimes are changing, drought-induced tree mortality has been observed in many parts of the Earth in recent decades, and is now a major research topic (see Chap. 9 by W. Andereg and F. Meinzer).

Plants respond to different and changing environments by producing distinct phenotypes within their life span. Understanding plastic responses of hydraulic traits may be useful in predicting the impacts of climate change on woody plants. In the past, variation in xylem properties has been mostly studied across different species, but we are now learning more about how the xylem of a single species can adjust to different growing conditions.

Many of the anatomical images show that vessels and tracheids do not exist in isolation. Conduits specialized in axial transport of water are intimately connected with living parenchyma cells. As explained by L. Plavcová and S. Jansen, parenchyma cells play important roles in the xylem. They provide opportunities for radial transport between tissues, they store nonstructural carbohydrates, and they facilitate the exchange of water and solutes between the apoplast and symplast.

Our understanding of whole-plant hydraulic architecture would remain incomplete without considering leaves. The leaf represents a major bottleneck in the whole-plant hydraulic system; leaf hydraulic conductance is variable and it influences photosynthetic gas exchange. L. Sack et al. evaluate the contributions of the vein system and the outside-vein system to leaf hydraulic conductance and to its dynamics with leaf water status.

The chapters are written by leading international scholars; I sincerely thank them for joining the project despite many competing demands on their time. I thank Eric Stannard from Springer for inviting me to edit the book, and for his continued support. Finally, thanks to my wife Irene and my parents for their support.

Edmonton, AB  
December 2014

Uwe Hacke



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# Chapter 1

## The Structure and Function of Xylem in Seed-Free Vascular Plants: An Evolutionary Perspective

Jarmila Pittermann, James E. Watkins, Katharine L. Cary, Eric Schuettpelz,  
Craig Brodersen, Alan R. Smith, and Alex Baer

### 1 Introduction

The evolution of xylem was critical to the success of embryophytic land plants. According to the fossil record, the earliest terrestrial autotrophs were algae followed by bryophytes, simple organisms that lacked the ability to efficiently transport water (Raven 1984; Sperry 2003; Taylor et al. 2009; Pittermann 2010). Like their ancestors, today's non-vascular plants have essentially two options for survival: inhabit perennially wet substrates such as stream sides or exploit poikilohydry, the capacity to recover from near complete desiccation (Niklas 2000; Proctor and Tuba 2002; Sperry 2003). In the absence of xylem, non-vascular plants are subject to the vagaries of

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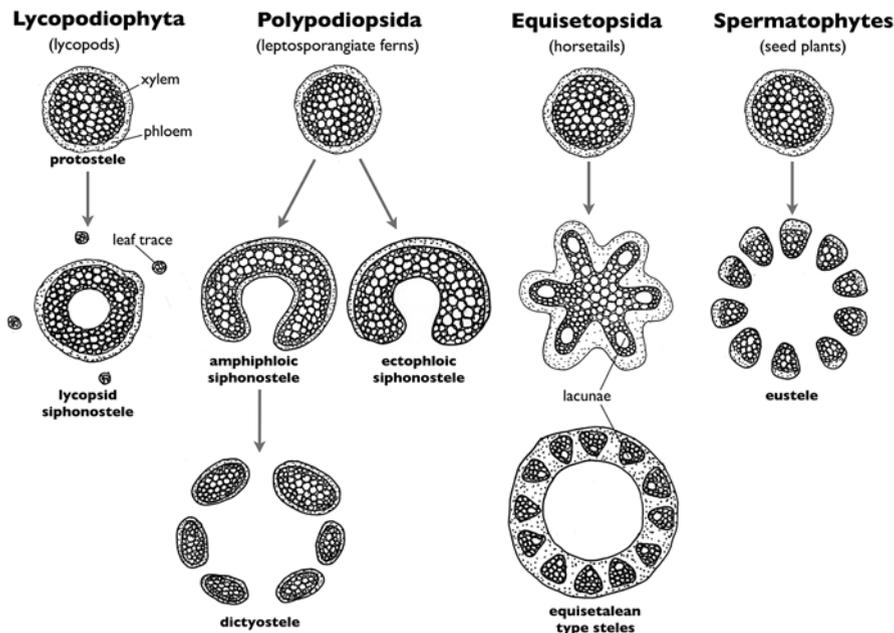
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their habitat and remain small because rehydration is a rapid, metabolically expensive process that supports the rapid recovery of photosynthesis (Proctor and Tuba 2002). Over time, the need for effective spore dispersal combined with increased competition for light selected for progressively taller, homoiohydric vascular plants, peaking with evolution of hydraulically efficient woody xylem across both seed-bearing and extinct seed-free lineages (Niklas 2000; Niklas and Speck 2001; Sperry 2003; Wilson 2013).

Much is known about the structure and function of xylem tissue in trees and shrubs because they are economically important, ecologically dominant, and in the case of angiosperms, highly diverse. However, the xylem in seed-free vascular plants such as ferns and lycopods demands equal scrutiny because (1) ferns and lycopytes have persisted for well over 400 million years—no small feat considering several extinction events, changing climates and the evolution of angiosperm-dominated tropical forests (Rothwell and Stockey 2008; Taylor et al. 2009; Watkins and Cardelus 2012) and (2) many species play critical roles in ecosystem processes and may even control forest growth and regeneration (George and Bazzaz 1999; Watkins and Cardelus 2012). Today, seed-free vascular plants are found on nearly every continent and in a variety of habitats ranging from xeric or salt-water niches up to the tropical forest canopies, which are often dominated by epiphytic ferns (Moran 2008; Watkins and Cardelus 2012). In north temperate regions most ferns are seasonal and terrestrial but in warmer climates, the fern morphospace includes vines, tree ferns, epiphytes, aquatic and floating ferns (Ranker and Hauffler 2008; Mehlreter et al. 2010). Boasting an impressive 12,000+ species, the fern lineage is the second most diverse only after the angiosperms (Ranker and Hauffler 2008). Despite their long history and ecological breadth, little is known about the vascular attributes of these ancestral plants (Pittermann et al. 2013). The goals of this chapter are to provide an overview of xylem structure and function in modern ferns, and to consider the selection pressures that lead to observed macroevolutionary patterns of xylem structure in extinct and extant seed-free vascular plants. The discussion will be restricted to sporophytic shoots and leaves since these structures are the functional equivalents to stems and leaves in seed plants.

## **2 Vascular Tissues in Stems and Leaves of Seed-Free Vascular Plants**

The fossil record shows that the vascular tissues of seed-free vascular plants appeared prior to those of conifers and angiosperms. Because extant pteridophytes do not produce wood, their primary xylem and phloem tissues are packaged in discrete vascular bundles (meristemes) that are surrounded by an endodermis and often a waxy, circum-endodermal band (see stele and vascular patterns in Figs. 1.1 and 1.2). The shape and arrangement of these bundles differs among genera; even within bundles the xylem can adopt a variety of patterns. In contrast to xylem of woody seed plants, which mechanically supports the canopy as well as transports water, the primary xylem of

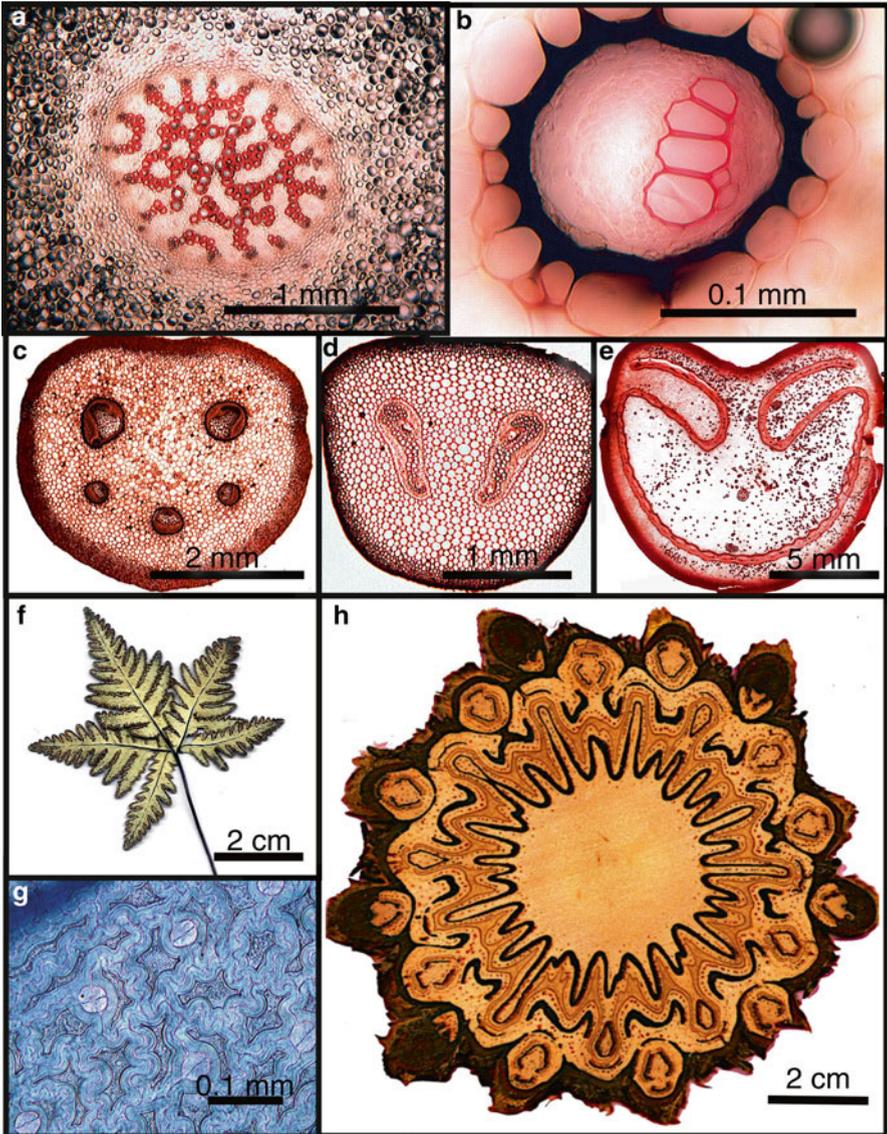


**Fig. 1.1** Stem and rhizome stelar patterns in vascular land plants. All fossil and extant vascular arrangements have their origin in the protostele, the simplest of steles. Drawings based on illustrations in Beck (2010) and Taylor et al. (2009)

ferns and lycophytes serves solely for the purpose of water transport (Pittermann et al. 2011, 2013). Despite their developmental canalization, extant pteridophyte shoots exhibit a surprising diversity of vascular arrangements, suggesting that evolution may have acted on fern xylem in response to numerous selection pressures.

## 2.1 Vascular Arrangement

The primary transport tissues are arranged in central zones of roots or stems known as steles. Stelar theory has traditionally pertained to stems and roots (Beck et al. 1982) but in this discussion, the nomenclature is applied to the arrangement of primary tissues in the main axes of fern leaves (fronds) and less frequently, the stems of lycophytes. One or several vascular traces, each containing xylem and phloem, can constitute a stele and these bundles may be variously situated in ground parenchyma or sclerenchyma tissues (Beck 2010; see Figs. 1.1 and 1.2a–h). Of extant and fossil stelar patterns, the protostelic arrangement characteristic of lycophytes is considered the most ancestral (Fig. 1.1; Beck et al. 1982; Taylor et al. 2009). In what is thought to be the very first protostele (also termed a haplostele), the xylem is in the centre of the vascular cylinder and is surrounded by the phloem (Beck et al. 1982). In contrast,



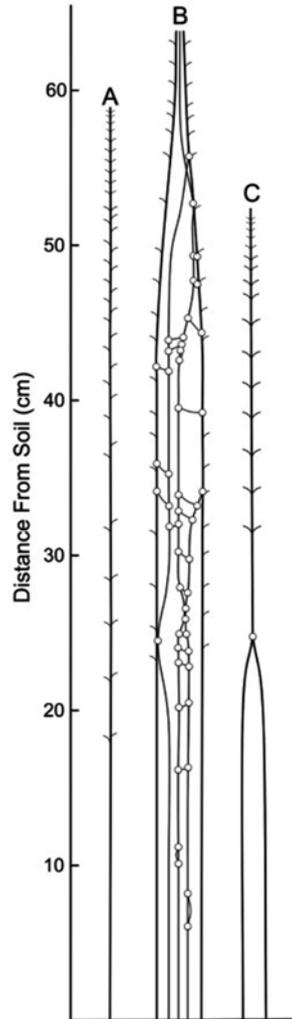
**Fig. 1.2** Stelar arrangements and leaf characteristics of lycophytes and ferns. (a) The mixed protostele of *Phlegmariurus squarrosus*, an erect lycophyte. Xylem tissue is stained red while phloem and parenchyma remain unstained. (b) A close-up on a vascular bundle of *Platycerium bifurcatum* (Cav.) C. Chr. showing red-stained xylem and clear phloem surrounded by an endodermis with a thick circumendodermal band. (c) The dictyostele of *Dryopteris arguta* (Kaulf.) Watt comprising two large vascular bundles and three smaller auxillary ones; the fibrous, peripheral sterome is also stained red. (d) The dictyostele of *Athyrium filix-femina* (L.) Roth. (e) An omega-shaped vascular strand in *Pteris livida* Mett. (f) The underside of a frond of *Notholaena standleyi* Maxon, a desiccation-tolerant cheilantheid fern found in rocky, dry outcrops of the American Southwest; the underside of the leaf is covered with a pale yellow farina that is exposed when the leaf curls while desiccating. (g) Stomatal and epidermal patterns on the underside of pinnae in *Pellaea truncata* Goodd., a dry adapted cheilantheid fern with sclerophyllous leaves. (h) Cross section of the trunk of *Dicksonia antarctica* Labill. showing sclerenchyma tissue (dark brown) adjacent to a contiguous strand of primary xylem (dark beige); leaf traces are evident on the periphery of the trunk. (Panel h by Christopher Rico)

the more derived protostele of *Phlegmariurus squarrosus* (G. Forst. Á. Löve and D. Löve; Huperziaceae) exhibits mixed elements of xylem and phloem (see micrograph in Fig. 1.2a; Beck et al. 1982). In the rhizomes and shoots of many lycopods and leptosporangiate ferns, the protostele appears to have given rise to the more derived siphonostele arrangement, which is a hollow or parenchyma-filled cylinder with ectophloic (external) phloem tissue (Fig. 1.1; Beck et al. 1982; Beck 2010). In typical siphonosteles, a diverging leaf trace is evident as an interruption of the cylinder, giving it the resemblance of an arch, but in the absence of leaf traces, the siphonosteles form complete cylinders with phloem that can be on both sides of the xylem (amphiphloic) or simply on the external face (ectophloic) (Fig. 1.1; Beck 2010). The dictyostele is the most dissected and derived of the leptosporangiate fern steles, formed by numerous vascular strands that may or may not come into contact over the length of the rhizome or the frond axis (Figs. 1.1 and 1.2b–d). Unusual among extant steles, Equisetalean stelar arrangements incorporate characteristic air-filled lacunae (Fig. 1.1). Lastly, the primary stem tissues of seed plants form the characteristic eustele, a cylindrical arrangement of numerous vascular bundles (Fig. 1.1).

The three-dimensional arrangement of the vascular network is much less well characterized than the stelar arrangements in transverse sections. The detailed representations of Ogura (1972) and Beck et al. (1982) were probably created from numerous cross sections and careful dissections, but the imaging methods pioneered by Zimmermann and Tomlinson (1974) and Zimmermann and Tyree (2002) improved both efficiency and precision by using sequential photographs of serial stem cross sections to assemble, frame by frame, three-dimensional reconstructions of vessel or vascular bundle arrangements. This cinematographic approach was limited to small pieces of tissue but it provided important insight into the longitudinal and three-dimensional positioning of vascular tissues in both woody and monocot plants (Zimmermann and Tyree 2002). White and Weidlich (1995) used it to document the longitudinal, interdigitated pattern of meristeles in semi-arborescent members of the fern genera *Diplazium* and *Blechnum*, demonstrating that comparable phyllotaxis has evolved convergently in ferns and seed plants. A newer method in which resins and polymers are directly injected into xylem tissue has proven extremely useful for understanding angiosperm vessel networks and has been especially revealing about vessel-to-vessel pit connections (Mauseth and Fujii 1994; Kitin et al. 2004). This approach is also valuable for measurements of conduit length in seed-free vascular plants because tracheids seed-free vascular plants because tracheids are often longer than a few millimetres and frequently resistant to standard solutions of hydrochloric acid and hydrogen peroxide, making tissue macerations problematic (Wheeler et al. 2005; Pittermann et al. 2011).

Newer methods may overcome some of the problems associated with xylem network imaging. Recently, high resolution computed tomography (HRCT) along with straightforward manual frond xylem extractions have revealed interesting differences in the vascular organization of ferns (Brodersen et al. 2012). The HRCT method relies on a synchrotron-generated source of focused X-rays to provide micrometre-range resolution of vascular tissues in three dimensions (Brodersen et al. 2011) and can be used for visualization of water movement and droplet formation within the xylem as well as for reconstruction of tissue arrangements (Brodersen et al. 2010, 2012). Unlike serial sectioning and macerations,

**Fig. 1.3** Vascular arrangements along the frond primary axis in *A Osmundastrum cinnamomeum* (L.) C. Presl, *B Polystichum acrostichoides* (Michx.) Schott, *C Onoclea sensibilis* L. Open circles represent the location of connections between vascular bundles



HRCT is non-destructive, but at present, processing time and computing power preclude the imaging of tissues longer than a few centimetres.

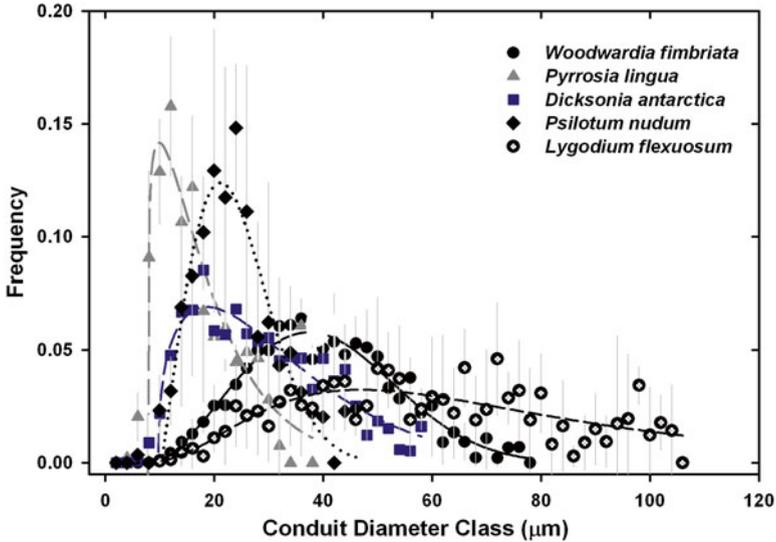
Dissections and meristele extractions have revealed that protosteles, such as those in the lycopod *Phlegmariurus squarrosus* and the whisk fern *Psilotum nudum* (L.) P. Beauv. (Psilotaceae) remain invariable along the shoot axis (Pittermann unpublished data), but similar patterns can be seen in *Osmundastrum cinnamomeum* (L.) C. Presl (Osmundaceae) in which a C-shaped stele remains intact over the length of the frond (see xylem map in Fig. 1.3). In more derived pteridophyte clades, the number of vascular bundles as well as their frequency of contact may vary considerably. For example, a 110-cm-long frond of *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) has anywhere from 23 to 29 vascular bundles at the base of the stipe and between 3 and 6 bundles at the tip of the rachis, with well over 100 bundle

connections throughout the main axis of the frond (Brodersen et al. 2012). This is an intricate network and probably an extreme example of stelar dissection. By contrast, only 4–5 basal bundles fuse to a single strand at the tip of the rachis in the simpler network of *Woodwardia fimbriata* Sm. (Blechnaceae) and *Polystichum acrostichoides* (Michx.) Schott (Dryopteridaceae; Brodersen et al. 2012). There is some variation between the base of the frond (petiole) and the rachis region which gives rise to the pinnae (Fig. 1.3). Lateral veins emerge from the large, main vascular strands in the rachis of all three species but network complexity increases in *P. acrostichoides*, whereas it simplifies in the more derived *Onoclea* (Onocleaceae). How selection drives xylem network variation is currently unclear.

## 2.2 Xylem and Phloem Ultrastructure

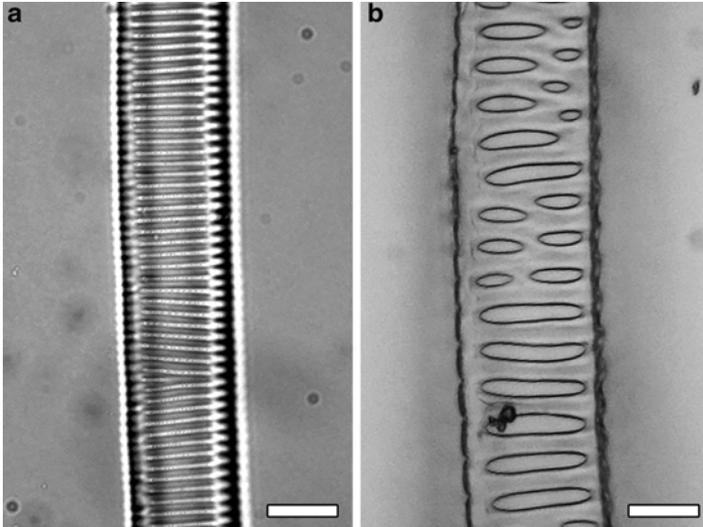
Phloem function is closely coupled with water transport, and Münch's pressure flow hypothesis posits that mass flow of phloem sap depends in part on water influx from adjacent xylem cells (Pickard and Abraham-Shrauner 2009). Recent models of leaf gas exchange show plant productivity to be coupled to the dynamics of xylem and phloem pressure gradients, and phloem has also been implicated in the recovery of hydraulic function after a water deficit (Salleo et al. 2004; Nikinmaa et al. 2014). The rapid wounding response of phloem makes it a difficult tissue to study but the use of aphid stylets, scanning electron microscopy and models has enabled insight into its functional attributes (Gaupels et al. 2008; Mullendore et al. 2010; Jensen et al. 2012). Unfortunately, studies on phloem tissue in seed-free vascular plants are rare. Early studies of *Equisetum* revealed that the phloem tissue comprises sieve elements and perhaps also parenchyma cells (Agashe 1968). In *Blechnum orientale* L. (Blechnaceae), sieve elements have sieve areas with numerous pores and possibly callose tissue but the presence of companion cells is not clearly established (Fotedar and Shah 1975). Clearly, phloem function in early-derived plants deserves more study in contemporary plant biology.

The xylem structure of seed-free vascular plants has received considerably more attention than phloem. The tracheids of ferns may be substantially longer and wider than those of conifers, reaching widths well over 100  $\mu\text{m}$  and lengths exceeding 1 cm (Veres 1990; Pittermann et al. 2011; see conduit diameter distributions in Fig. 1.4). By comparison, conifer tracheids rarely exceed 40  $\mu\text{m}$  in diameter and 2 mm in length, particularly in stems (Pittermann et al. 2006), with narrower, shorter tracheids found in stiffer wood (Panshin and de Zeeuw 1980). In the absence of a mechanical function, fern tracheids serve solely to transport water, and thus exhibit none of the hydraulic constraints imposed by smaller lumina, relatively thicker walls, and short cells evident in conifer tracheids (Pittermann et al. 2006; Sperry et al. 2006). Several fern genera are believed to have vessel elements in their rhizomes (*Astrolepis* (Pteridaceae), *Marsilea* (Marsileaceae), *Woodsia* (Woodsiaceae) and *Pteridium*; Carlquist and Schneider 2007), with silicon injections confirming vessels in excess of 20 cm in length in the petiole of *Pteridium aquilinum* (Wheeler et al. 2005; Pittermann et al. 2011).



**Fig. 1.4** Conduit diameter frequencies in five fern species (reprinted from Pittermann et al. 2011)

Conduits must be perforated by inter-conduit pit membranes to allow water to flow from one conduit to another. As in other vascular attributes, the pit membrane structure in seed-free vascular plants differs substantially from that of conifers and angiosperms. Angiosperms possess what is known as a homogenous pit membrane, which is a partially digested primary wall protected by an overarching, secondary wall border (Choat and Pittermann 2009). These membranes typically occupy radial vessel walls and may be grouped near vessel-to-vessel junctions or located sporadically along the full length of the vessel wall. Conifers, on the other hand, possess a torus–margo pit membrane in which water moves through the porous margo region while the torus, a thickening in the centre of the membrane, serves to protect conduits from the spread of air throughout the xylem (Choat and Pittermann 2009; see Chaps. 2 and 3). The vast majority of ferns and lycopods possess homogenous pit membranes that span the length of the tracheid in a scalariform arrangement and are located on nearly all the tracheid walls (see micrographs in Fig. 1.5a, b). Ranging in thickness from 150 to 350 nm (Brodersen et al. 2014), pteridophyte pit membranes appear less variable than those of angiosperms, in which range from 70 to 1,892 nm (Jansen et al. 2009). Similar pit membrane arrangements have been observed in seed-free vascular plants since the Early Devonian (Kenrick and Crane 1997; Taylor et al. 2009). Interestingly, torus–margo pit membranes are present in ferns, but only in the anomalous secondary growth in rhizomes in the genus *Botrychium* (Ophioglossaceae; Morrow and Dute 1998; Rothwell and Karrfalt 2008). The presence of conifer-like pit membranes in *Botrychium* as well as bordered pits in *Psilotum* indicates that ferns have the genetic potential to evolve xylem that is functionally comparable to higher plants, but either selection acted on attributes of fern physiology or life history that override the importance of pit membranes, or the genetic or developmental capacity to capitalize on these traits is absent.



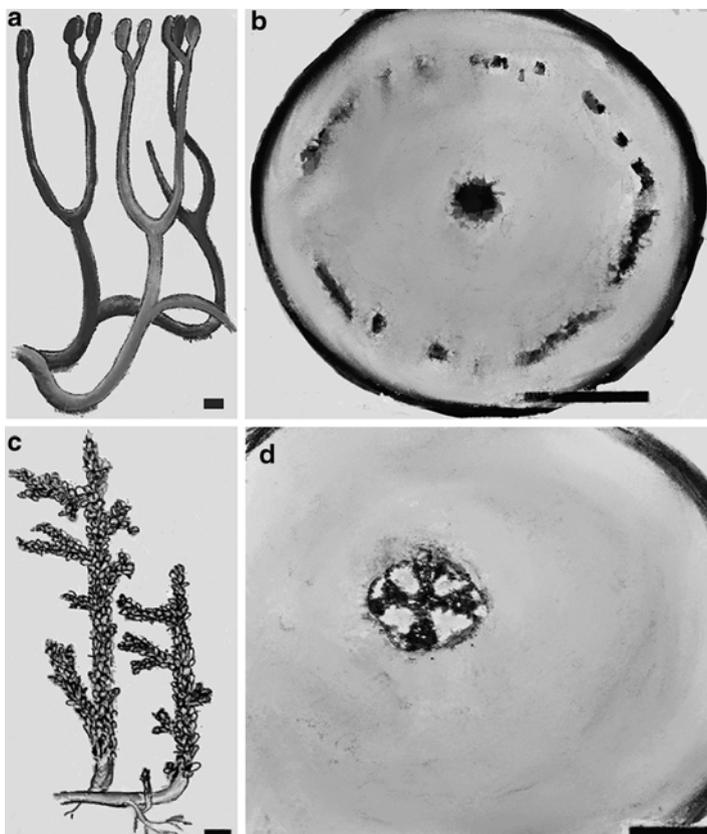
**Fig. 1.5** Tracheids of (a), *Selaginella pallescens* (C. Presl) Spring (air-seed pressure=3.8 MPa) and (b), *Phlebodium aureum* (L.) J. Sm. (air-seed pressure=2.12 MPa). Tracheid walls are perforated by scalariform (*S. pallescens*) and reticulate (*P. aureum*) pit membranes. Scale bar=5  $\mu$ m

### 3 The Xylem of Seed-Free Vascular Plants in the Fossil Record

The fossil record opens a window into the fascinating morphological diversity of early land plants, and also into some unusual excursions in plant structure and function. Many of these botanical experiments have failed but others have persisted into the modern flora for reasons that may have little to do with physiology. Aside from physiological attributes, ecological, reproductive and other life history traits certainly have played a role in taxon persistence. In fact, the disappearance of variously woody progymnosperms, sphenopsids and isoëtopsids demonstrates that having secondary xylem is no guarantee against extinction.

#### 3.1 Xylem in Early Land Plants

Fossil spore and possibly micro-fossil records suggest that vascular plants made their appearance in the Silurian (Niklas and Smocovitis 1983; Kenrick and Crane 1997; Taylor et al. 2009), with the Lower Devonian giving rise to a diversity of plants with transport tissues (Niklas 1985). Of these proto-vascular plants, the Rhyniophytes (so-named for the Early Devonian Rhynie Chert locality) are probably the best characterized. Genera such as *Rhynia*, *Aglaophyton* and *Cooksonia* are rhizomatous, with dichotomizing, stomatal-bearing shoots up to 18 cm tall,



**Fig. 1.6** Reconstructions and stem cross sections *Aglaophyton major* (a and b) and *Asteroxylon mackiei* (c and d). Scale bars equal 1 cm in the plant reconstructions and 1 mm in the cross sections. Figure adapted from Boyce et al. (2003)

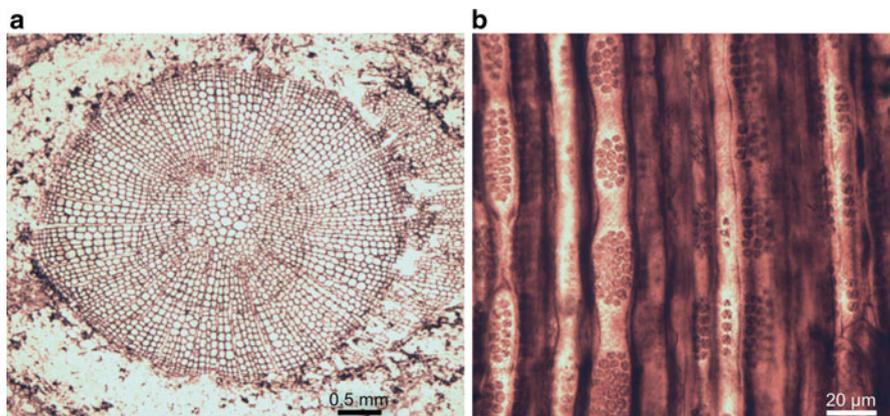
possessing variously developed central conducting strands (Edwards 1986; Taylor et al. 2009; see illustrations of *Aglaophyton* in Fig. 1.6a, b). The vascular tissues of *Rhynia gwynne-vaughanii* and *Aglaophyton major* are described as hybrids between the hydroids of bryophytes and the xylem of more derived plants (Edwards 1986; Kenrick and Crane 1991) but chemical analysis of fossil material suggests that the vascular tissue was not lignified (Boyce et al. 2003). Bearing a close resemblance to bryophytes, *A. major* is categorized as a pro-tracheophyte (see Boyce et al. 2003; Fig. 1.6a, b), with *R. gwynne-vaughanii* falling closest to the next most-derived group, the tracheophytes (Kenrick and Crane 1997; Boyce et al. 2003). *Asteroxylon mackiei*, a more derived eutracheophyte from the Lower Devonian Rhynie Chert, has well-developed lignified primary xylem, the presence of which is consistent with the need to hydrate enations (small flaps of photosynthetic tissue) that cover its stems and increase its evaporative surface area (Boyce et al. 2003; Taylor et al. 2009; see illustrations of *Asteroxylon* in Fig. 1.6c, d).

The conduit structure of early land plants may have been more diverse than that of extant seed-free vascular plants. Moss hydroids are unligified and, aside from plasmodesmata-derived pores, they lack additional structural features (Kenrick and Crane 1997; Beck 2010). By comparison, conduits of more derived rhyniophytes show greater complexity and reinforcement against collapse. In *Rhynia*, for example, the transport cells have spongy helical wall thickenings (Kenrick and Crane 1991). In Early Devonian *Goslingia* and *A. mackiei*, the tracheid walls comprise a two-layered cell wall with a chemically resistant inner layer (Kenrick and Crane 1991; Taylor et al. 2009). The P-type tracheids of *Psilophyton*, a Lower- to Mid-Devonian eutracheophyte, are probably the most structurally robust with what appears to be a higher wall fraction and degradation-resistant cell layer (Kenrick and Crane 1991, 1997). Taken together, the fossil record during the early Devonian trends toward an increase in xylem content and tracheid size, as well as tracheid reinforcement. Wall perforations resembling pit membranes become evident in the eutracheophytes as hydraulic demand must meet increasing plant size.

The Cladoxylopsids, early euphyllous fern-like plants with various degrees of xylem organization, make their appearance in the Middle to Upper Devonian, along with the more derived Rhachophytales, Stauropteridales and Zygopterids (extinct taxa with large, frond-like leaves; Taylor et al. 2009). Collectively, these groups appear to be related to ferns but it is unclear whether they are ancestral or sister taxa. Many of these plants have xylem arrangements that not featured in today's flora (Rothwell and Stockey 2008). For example, the star-shaped protostele of the Cladoxylopsids is defined as an actinostele and is unlike any extant stelar arrangement (Rothwell and Stockey 2008; Taylor et al. 2009; Beck 2010). Some specimens such as *Pseudosporochnus* may have been small and arborescent, but others such as *Eospermatopteris* and *Pietzschia schulleri* reached heights in excess of 8 m (Taylor et al. 2009). In Cladoxylopsids such as the Iriopteridales, the main axes measured over 5 cm in diameter, with cross sections showing dissected cross-shaped or bifurcating star-shaped protosteles that supported leaves with a single vascular trace (Taylor et al. 2009).

### 3.2 Secondary Xylem in Seed-Free Vascular Plants

Increased competition for light and other resources selected for taller plants with more developed canopies in the early evolution of eutracheophytes. Higher rates of transpiration necessitated increased hydraulic demand that was satisfied by greater xylem fraction and wider (and presumably longer) conduits. Fossils show that tracheid diameter increased by an order of magnitude during the Devonian from the 8  $\mu\text{m}$  tracheids of the primitive *Cooksonia* to the 140  $\mu\text{m}$  metaxylem conduits of *Stenomylon primaevum*, an early Carboniferous seed fern (Niklas 1985). Although early vascular plants such as *Rhynia* and *Asteroxylon* were herbaceous, the recent identification of true secondary xylem in putative relatives of *Psilophyton* and *Armoricaphyton* suggests that this trait evolved much earlier than originally thought (Gerrienne et al. 2011; Strullu-Derrien et al. 2014). It appears that *Psilophyton* had a bifacial vascular cambium in which cell initials divide both radially and tangentially.



**Fig. 1.7** Cross section of a root (**a**) and (**b**) a longitudinal section of stem tissue belonging to *Callixylon-Archaeopteris*, a Devonian progymnosperm. Clustered pit membranes with cruciform apertures are evident in panel **b**. (Material courtesy of Dr. Diane Erwin, UC Berkeley Museum of Natural History)

Secondary xylem, whether unifacial (only tangential cell divisions) or bifacial, is now believed to have evolved on at least five separate occasions, and by the Late Devonian, secondary vascular tissues were present in many dominant seed-free vascular plant lineages including the Lepidodendrales, Sphenopsids, pro-gymnosperms, the Zygopterids and Devonian–Carboniferous seed ferns (Barghoorn 1964; Beck 1970; Cichan 1985a, b; Sperry 2003; Taylor et al. 2009). Unifacial vascular cambia were present in arborescent lycopods such as the Lepidodendrales. Indirect analysis of xylem development suggests that cambial initials divided centrifugally with no anticlinal (radial) division, such that any increase in trunk circumference was accommodated by progressively larger fusiform initials (Cichan 1985a). Similar patterns of development probably occurred in the Carboniferous horsetail relatives including *Sphenophyllum* and *Arthropitys* species (Cichan 1985b).

Prior to the discovery of true secondary xylem in *Psilophyton*, the progymnosperms were thought to represent the earliest known class of plants with true wood derived from a bifacial cambium (Gerrienne et al. 2011). This group of plants possessed the unusual combination of coniferous, pycnoxylic wood with a canopy comprising free-sporing foliage, superficially similar to those of ferns (Beck 1970; Taylor et al. 2009). Growth rings, ray parenchyma and clustered, bordered pits with offset, cruciform apertures were present in both root and trunk xylem of the Archaeopteridales, of which the spore-bearing Devonian/Carboniferous *Archaeopteris-Callixylon* is best studied (Meyer-Berthaud et al. 1999; see micrographs of *Callixylon* xylem in Fig. 1.7a, b). Although it was seed-free, *Archaeopteris* was crowned “the Earth’s first tree” because it demonstrates several developmental attributes of more derived plants, including perennial lateral branches (Meyer-Berthaud et al. 1999, 2000).

### 3.3 *Fern Xylem in the Fossil Record*

Ferns are functionally defined as megaphyllous plants with spores on the underside of their leaves (Rothwell and Stockey 2008, but see also Smith et al. 2006 for a more thorough treatment), but as *Archaeopteris* and *Psilophyton* demonstrate, specimens from the fossil record can certainly stretch this definition. For clarity, we define ferns as vascular, spore-bearing, megaphyllous plants with only primary growth. The fossil record shows that modern ferns, including the Psilotaceae, Ophioglossaceae, Equisetaceae, Marattiaceae and the leptosporangiate ferns have their origins in the Devonian (Rothwell and Stockey 2008). Although some extinct taxa exhibit stelar patterns that are unlike those of modern ferns today, the vasculature of the true ferns has remained relatively conserved over deep time.

Three major diversification events characterize the evolution of the leptosporangiate ferns, the first of these occurring during the climatically mild Carboniferous period (Rothwell and Stockey 2008). Fossilized petioles (stipes) of Botryopteridaceae and Kaplanopteridaceae fronds show variable tripartite and C-shaped steles surrounded by a thick, fibrous sterome layer (Rothwell and Stockey 2008). Similarly, fossil leaf scars of Marattialeen tree ferns (representatives of which are extant in today's tropics) indicate a variety of stelar arrangements including C-shapes as well as more complex, possibly dictyostelic patterns. Stelar arrangements can vary along the length of the frond, but it is difficult to comment on the overall arrangement of the vascular strands with limited fossil material.

The Mesozoic gave rise to the second significant radiation of ferns, and included the Gleicheniales and Schizaeales, descendants of which are found in today's fern flora. Features of other Mesozoic taxa fall within the range of trait variation found in extant plants, as discovered in the Cretaceous remains of *Osmundastrum cinnamomeum*, in which the stipe stelar arrangement (a C-shape) resembles that of modern representatives (Serbet and Rothwell 1999). The steles in the petioles of modern leptosporangiates range from highly dissected, as in the Marattiaceae, to variously C-shaped; given the conserved anatomy of *Osmunda* over deep time, it may be reasonable to suppose that stelar arrangements in the other leptosporangiates with modern representatives are similarly conserved.

The third well-characterized fern radiation probably has origins in the upper Jurassic, with the diversification of the Eupolypod I and II clades (Schneider et al. 2004; Schuettpelz and Pryer 2009), even though most of the fossil diversity is apparent during the Cretaceous. Many taxa are now extinct but the fossil record suggests conserved dictyostelic vascular architecture in the petioles. For example, two large vascular strands resembling those of modern taxa are evident in Eocene specimens of Blechnaceae and Athyriaceae (Rothwell and Stockey 2008; Fig. 1.2c, d). Interestingly, this pattern is prevalent in the most recently derived fern clades, the Eupolypods I and II, a large fraction of which are epiphytic (Schneider et al. 2004; Schuettpelz and Pryer 2009). Characterizing and understanding the biotic and abiotic filters that selected for various vascular attributes over deep time will be a multidisciplinary undertaking requiring expertise in paleobotany, paleoecology, and plant structure and function.

## 4 Xylem Function in Modern Pteridophytes

Water transport in ferns and other seed-free vascular plants presents an interesting conundrum because in the absence of traits such as a bifacial cambium, vessels and torus–margo pit membranes, one might suppose that seed-free vascular plants are at a competitive disadvantage relative to conifers and angiosperms (Pittermann et al. 2011, 2013). However, spore-bearing lineages have thrived since the Devonian, and their physiology certainly warrants a closer look. Indeed, the mechanisms by which ferns and lycophytes compensate for the absence or loss of these features makes for an interesting discussion because it bears not only on the limits of their so-called morphospace but also their evolutionary trajectory over time.

### 4.1 Hydraulic Efficiency in Ferns and Lycophytes

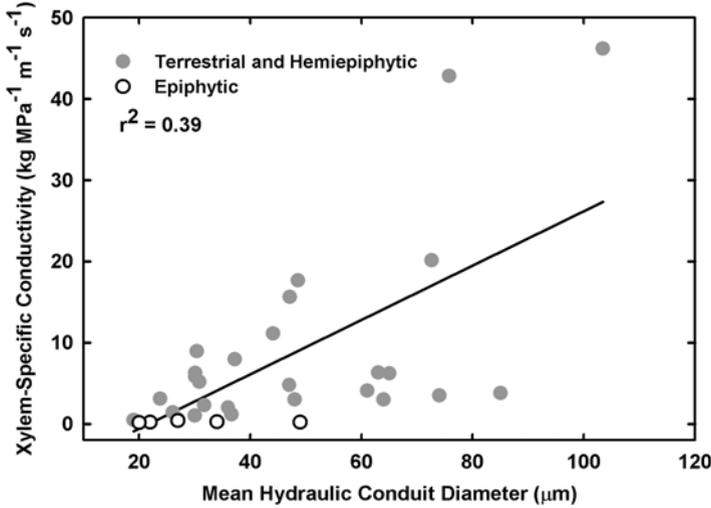
Of all the “missing” traits, the absence of a cambial layer in modern ferns and lycophytes is perhaps the most costly because it severely limits transport capacity and prohibits lateral branching. Consequently, ferns cannot develop complex canopies or occupy the massive ecological footprints of woody plants (Rowe et al. 2004; Rowe and Speck 2005). Ferns can, however, be dominant elements in tropical and subtropical systems in places such as Hawaii, where the tree fern *Sphaeropteris cooperi* (Hook. ex F. Muell.) R. M. Tryon (Cyatheaceae) has become invasive (Robinson et al. 2010). Native tree ferns are ecologically important in the South American and South Pacific floras where they frequently emerge after disturbances (Coomes et al. 2005; Robinson et al. 2010). In arborescent genera, such as *Dicksonia* and *Cyathea*, fibrous overlapping leaf traces form a several centimetre-thick sheath around the trunk and fibrous roots can also serve a supportive function (Sharpe and Mehlreter 2010; Fig. 1.2h). A second, less conspicuous constraint arising from the absence of wood is an inability to increase canopy size and complexity with age—the tree fern trunk simply elevates the canopy but the number of fronds remains relatively constant. There is little if any room for developmental flexibility when only a limited amount of primary xylem serves the canopy.

The absence of vessels in the vast majority of ferns precludes the leaps in hydraulic efficiency achieved by flowering plants (Brodribb and Feild 2010; Feild et al. 2011). Vessels evolved several times across several lineages including angiosperms, ferns, and possibly in several extinct taxa but for the vast majority of ferns, which are tropical, the xylem remains tracheid based (Calkin et al. 1985; Sperry 2003; Pittermann 2010; Watkins et al. 2010; Watkins and Cardelus 2012). *Pteridium aquilinum*, an aggressive weed, is a conspicuous exception: vessels have been identified in fronds on several occasions, making this species one of the most hydraulically efficient ferns amongst those surveyed (Wheeler et al. 2005; Pittermann et al. 2011; Brodersen et al. 2012). Unlike most pteridophytes, *P. aquilinum* is tolerant of both low light and full sun environments and can support transpiration and photosynthesis rates up to  $0.5 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $15 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , respectively, which are on

par with those of angiosperms (Page 2002; Marrs and Watt 2006; Pittermann et al. 2011; Brodersen et al. 2012). Over 60 species of pteridophytes have managed to invade both intact and disturbed ecosystems, often outcompeting and even smothering native angiosperms and conifers (Robinson et al. 2010). The reasons behind the ferns' competitive edge are complex but a physiological approach may help explain their rapid rates of spread, as well as the mechanism by which these species push their xylem function and overall physiology beyond the norm. It is possible that vessels may be present in a greater number of fern species than we currently know.

Given that the vast majority of ferns have xylem comprised exclusively of tracheids, the absence of conifer type torus–margo pit membranes seems to add yet another handicap that pteridophytes failed to solve during the course of evolution. Torus–margo pit membranes are found primarily in conifer xylem and are about 60 times more permeable than the average angiosperm homogenous pit membrane (Pittermann et al. 2006; Sperry et al. 2006). This is because water moves from tracheid to tracheid through the highly porous margo region of the pit rather than the dense network of microfibrils and hydrogels characteristic of homogenous pit membranes (Sperry et al. 2006; Domec et al. 2008; Pittermann et al. 2010). Functionally, the increased permeability of torus–margo pit membranes compensates for xylem that consists of otherwise short, single-celled, high-resistance tracheids (Pittermann et al. 2006). If torus–margo pit membranes were replaced with an average angiosperm-type homogenous pit membrane, the tracheid-based vascular system would be 38× less efficient (Pittermann et al. 2005; Sperry et al. 2006).

If woody plants represent paragons of hydraulic optimization, it seems remarkable that ferns manage to transport any water at all with what appears to be the least desirable combination of xylem traits. And yet, fronds of *Angiopteris evecta* (G. Forst.) Hoffm. (Marattiaceae) and *Woodwardia fimbriata* can exceed 1.5 m in length with little more than a few strands of primary xylem! The notion that ferns are constrained by primitive, inefficient xylem prevails because the bulk of published data has been generated on tropical taxa, which experience neither high evapotranspirative stress nor a high demand for water. For example, a survey of hydraulic function in tropical terrestrial and epiphytic ferns revealed  $K_s$  to be no higher than 8 kg MPa<sup>-1</sup> m<sup>-1</sup> s<sup>-1</sup> (Watkins et al. 2010). These values are corroborated by gas exchange and leaf hydraulic data, which show that ferns barely achieve 20 % of the physiological capacity of angiosperms (Brodrribb et al. 2007). However, fern habitats and ecological niches are nearly as varied as those of angiosperms, so when non-tropical taxa are considered, the picture of fern hydraulics changes dramatically. The  $K_s$  has consistently been found to be higher than 20 kg MPa<sup>-1</sup> m<sup>-1</sup> s<sup>-1</sup> in the stipes of the seasonally deciduous *P. aquilinum*, the perennial *W. fimbriata* and the stems of climbing *Lygodium japonicum* (Thunb.) Sw. (Lygodiaceae; Pittermann et al. 2011, 2013; Brodersen et al. 2012). Figures 1.8 and 1.9 show numerous species that have  $K_s$  in excess of 10 kg MPa<sup>-1</sup> m<sup>-1</sup> s<sup>-1</sup>, exceeding the hydraulic efficiency of temperate ring- and diffuse-porous species by at least a factor of 2 (McCulloh et al. 2010; Lens et al. 2011; see Chaps. 2 and 4). Temperate ferns should be sampled more intensively because these values may be the norm rather than the exception for seasonally deciduous species.



**Fig. 1.8** Xylem-specific conductivity as a function of mean hydraulic conduit diameter in terrestrial, hemiepiphytic, and epiphytic ferns from tropical and temperate habitats. Data from Watkins et al. (2010) and Pittermann et al. (2011, 2013)

Three attributes may explain potential high specific conductivities in ferns. First, fern tracheids can explore a surprisingly broad anatomical and functional morphospace because shoot biomechanics are largely dependent on the sterome. Indeed, their central position in the stipe-rachis ensures that they occupy a mechanically neutral zone that is subject to neither the tension nor the compressive stresses imposed on the hypodermal sterome by external loads (Niklas 1992). Hence, species' hydraulic conduit diameters range from 20 to 100  $\mu\text{m}$ , exceeding the tracheid and vessel sizes of several tropical and temperate woody taxa (see fern tracheid dimensions in Figs. 1.4 and 1.10; Veres 1990; McCulloh et al. 2010). Hydraulic efficiency of conduits scales to the fourth power of the lumen diameter (Tyree et al. 1994), so even a modest number of large tracheids can disproportionately contribute to the overall transport capacity to the xylem. A second important consideration is conduit arrangement within the fern vascular bundle. Despite devoting less than 30 % of frond cross-sectional area to xylem tissue, hydraulic efficiency is achieved in part by the close packing of conduits within the vascular bundles, much like tracheids in conifer xylem (see fern xylem structure in Fig. 1.2b–d). In this way, fern xylem combines the best of both worlds: water moves through wide, long, angiosperm-sized conduits that are tightly packed like those of conifers (Pittermann et al. 2013). Lastly, pit membrane attributes also contribute to high  $K_s$  in seed-free vascular plants (see pit membranes in Fig. 1.5a). Several studies have shown that the permeability of pteridophyte pit membranes to water is surprisingly similar to