

AJB REVIEWS

Diversity in conduit and pit structure among extant gymnosperm taxa

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Gymnosperm taxa are incredibly diverse in many traits, including taxa with evergreen or deciduous leaves; broad, compound, needle-like or scale-like leaves; trees, shrubs, and lianas; and taxa with seed cones that range from fleshy to woody. Although less appreciated, xylem conduits are also diverse in structure among extant gymnosperm taxa. Within the xylem of gymnosperms, axial transport occurs predominantly via tracheids, although 10–40% of gymnosperm taxa, particularly within the Gnetophyta and Cycadophyta, also contain vessels. Gymnosperm taxa vary greatly in their inter-conduit pit structure, with different types of pit membranes and pitting arrangements. While some taxa display torus-margo bordered pits (60%), many others do not contain tori (40%), and at least some taxa without tori occur within each of the four extant phyla (Coniferophyta, Cycadophyta, Ginkgophyta, and Gnetophyta). Pit membrane types vary within families but appear relatively conserved within genera. Woody species with torus-bearing pit membranes occur in colder environments (lower mean annual temperature) compared to those without tori; but occurrence does not differ with mean annual precipitation. Detailed descriptions of pit membrane types are lacking for many species and genera, indicating a need for increased anatomical study. Increased knowledge of these traits could provide a unique experimental context in which to study the evolution of conduit networks, the hydraulic implications of conduit and pit structure, and the diverse structural and functional strategies utilized by gymnosperms. There are myriad potential study questions and research opportunities within this unique and diverse group of plants.

KEY WORDS bordered pit; margo; pit membrane; pitting; seriation; torus; tracheid; tracheary elements; vessel; xylem.

Gymnosperms, nonflowering seed plants, represent a morphologically diverse group of our modern mega-flora. Among extant gymnosperms are many of the woody plants that are common to our forests and landscapes, especially the economically important timber trees within *Pinus* (pine), *Abies* (fir), and *Picea* (spruce). The ubiquity of needle-leaved and evergreen gymnosperms often overshadows the many other morphologically diverse members of this group, including broad-leaved, deciduous *Ginkgo*; broad-leaved, tropical lianas of *Gnetum*; large frond-like leaves and palm-shaped bodies of *Cycas*; aridland stem-photosynthetic shrubs of *Ephedra*; and highly unique *Welwitschia* (Fig. 1). Concomitant with this

variation in leaf habit, habitat, and form is diversity in the vascular tissue systems of gymnosperms.

Gymnosperm xylem is composed of axial tracheary elements (conduits) and several other cell types that may include ray tracheids, axial parenchyma, ray parenchyma, and epithelial cells associated with resin ducts and canals (Richter et al., 2004). Axial conduits always include tracheids, but also sometimes include vessel elements that connect into multicellular vessels. Conduits are connected along their radial walls via inter-conduit bordered pits. These pits are composed of an opening within the secondary wall of each connected conduit (pit aperture), a wide pit chamber, and a

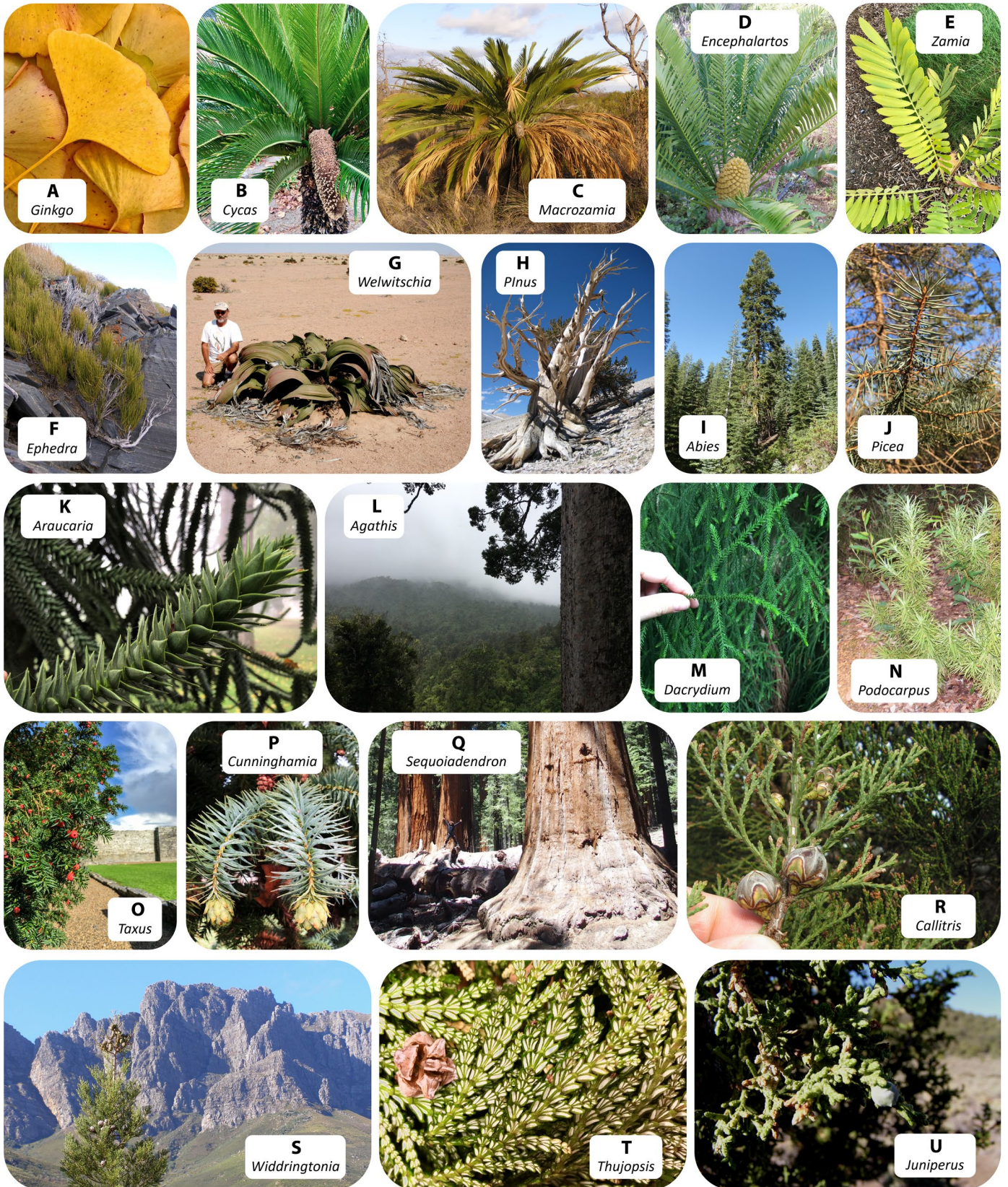


FIGURE 1. Select extant gymnosperm taxa illustrating diversity in habit, leaf morphology, and growth form. Genera are identified within each panel. Image credits: L. M. Moe (G), D. G. Jacobsen (J), A. L. Jacobsen (all other panels).

central pit membrane that is a complex and variable structure composed predominantly of cellulose microfibrils.

Pit membranes are important structures in the water transport pathway. During periods of water stress, pit membranes serve as a barrier to prevent spread of gas emboli from one conduit to the next, thereby limiting losses of conductivity associated with embolism. When sap moves from one water-filled conduit to the next, pit membranes act as resistors that slow the flow of water and reduce the maximum rates of hydraulic transport. Hypothesized trade-offs between xylem hydraulic safety and efficiency are based upon pit membrane structure and the influence of pit membranes in both preventing embolism spread and adding resistance to the hydraulic pathway (Hacke et al., 2005).

PIT MEMBRANE STRUCTURE IN INTER-CONDUIT BORDERED PITS

The structure of inter-conduit pit membranes is complex, with distinct regions of the membrane that vary in organization and composition (Liese, 1965; Bauch et al., 1972; Sano et al., 1999). Some pit membranes have a distinct central region that contains a thickened plate termed the torus. Definitions of tori vary slightly among practitioners, but generally include the requirement that this structure meet all three of the following criteria: The structure must be (1) thicker than the adjacent pit membrane and matrix materials (Richter et al., 2004), (2) added during cellular development through apposition (Dute et al., 2008), and (3) chemically distinct from the rest of the pit membrane and matrix material (Bauch and Berndt, 1973). The International Association of Wood Anatomists (IAWA) suggest that tori be observed in stained tissue with light microscopy (Richter et al., 2004), which reveals both the thickened torus structure and histological features of the torus that permit determination of whether it is chemically distinct from other pit membrane components. Transmission electron microscopy (TEM) is a powerful tool to examine cross sections of pit membranes for determining whether a thickened central torus is present, especially when combined with histological techniques to evaluate the chemical composition of the structure.

Pit membranes may vary in the orientation of their cellulose microfibrils, the density of matrix material deposited in between microfibrils, and the visibility of pores within the membrane (Fig. 2). A pit membrane composed of radially oriented microfibrils with large pores is referred to as a margo-type pit membrane. Alternatively, the microfibrils may be present in a much more disorganized arrangement in a divergent orientation, which is typical of angiosperm-type pit membranes and sometimes referred to as a homogeneous pit membrane structure. Pit membrane microfibril organization is often examined using scanning electron microscopy (SEM; Bauch et al., 1972; Meylan and Butterfield, 1972; Dute et al., 2008).

Different schemes for classification of gymnosperm pit membrane structural types, representing the common suites of microfibril orientation, matrix material, and central region traits, have been developed (Fig. 2). These include a 5-category system proposed by Liese (1965) and a 6-category system proposed by Bauch et al. (1972). The key difference among these classifications was that Liese (1965) included a single category of *Pinus*-type pit membranes that Bauch et al. (1972) divided into two separate *Pinus* types: Type 1 pit membranes (earlywood) and Type 2

pit membranes (latewood). In both schemes, Type 3 (*Araucaria*-type) and 4 (*Thujaopsis*-type) pit membrane types were often found together within the same tissue sample and were indicative of earlywood and latewood tracheids for some species. For the present review, examining traits across extant genera, these classification systems have been simplified to include just three categories of pit membrane structure:

Type A: Pit membranes that include both a margo and torus (torus-margo pit membranes) (Types 1 and 2 according to Bauch et al. [1972]; *Pinus*-type according to Liese [1965]);

Type B: Margo pit membranes that lack a thickened torus but that may have a distinct unthickened central matrix region (Types 3 and 4 according to Bauch et al. [1972]; *Araucaria* type and *Thujaopsis* type according to Liese [1965]);

Type C: Divergently organized pit membranes that lack both a margo and torus (Types 5 and 6 according to Bauch et al. [1972]; *Gnetum* type and *Cycas* type according to Liese [1965]). This type of pit membrane has also been referred to as “hardwood type,” i.e., angiosperm type (Bauch et al., 1972; Yin and Xiao-Mei, 1992).

These broader categories, Types A, B, and C, have the benefit of being consistent across both earlywood and latewood within a sample, thus permitting a single pit membrane type classification for the xylem of species that contain distinct growth rings.

LITERATURE REVIEW AND EXAMINATION OF GYMnosperm PIT TRAITS

A review of the literature was conducted to compile pit membrane traits as scored using either the Liese (1965) or Bauch et al. (1972) classifications. In some cases, pit membrane traits were not explicitly reported within studies, but examination of included micrographs permitted the determination of pit membrane type or other conduit traits. For some taxa, published data were verified by the examination of conduit and pit membrane traits from fresh samples and wood blocks using light microscopy of both unstained and stained (0.5% w/v astra blue and 1% w/v safranin O) tissue (Schweingruber, 2007). All anatomical figures shown are original to the present review. No newly observed samples contradicted prior reported pit membrane type classification. Additional data were collected on the presence of vessels, inter-conduit pitting seriation (uni-, bi-, or multiseriate), and inter-conduit pitting arrangement for conduits that displayed bi- or multiseriate pitting (opposite, alternate), with an emphasis on collecting data for these additional traits in species for which pit membrane type data were also documented. Pit traits were scored from the radial walls of conduits.

Pit trait data were compiled for extant gymnosperm taxa resulting in 583 reports for 281 species from 63 of the 84 extant genera (Appendix S1). Where both tracheids and vessels co-occurred in the xylem and these conduit types differed in their pit membrane types, both pit membrane types were recorded (for *Gnetum* only and discussed more below). All traits were examined in vessel elements or axial tracheids of secondary xylem earlywood of the bole or branches, except for in some of the nonwoody taxa, such as *Cycadophyta* and *Welwitschia*, in which roots and petioles were examined.

A cladogram of extant gymnosperm genera was compiled using Phylomatic (v. 3, <https://phylodiversity.net/phyloomatic/>; accessed 23 November 2019), which assembles published phylogenies into

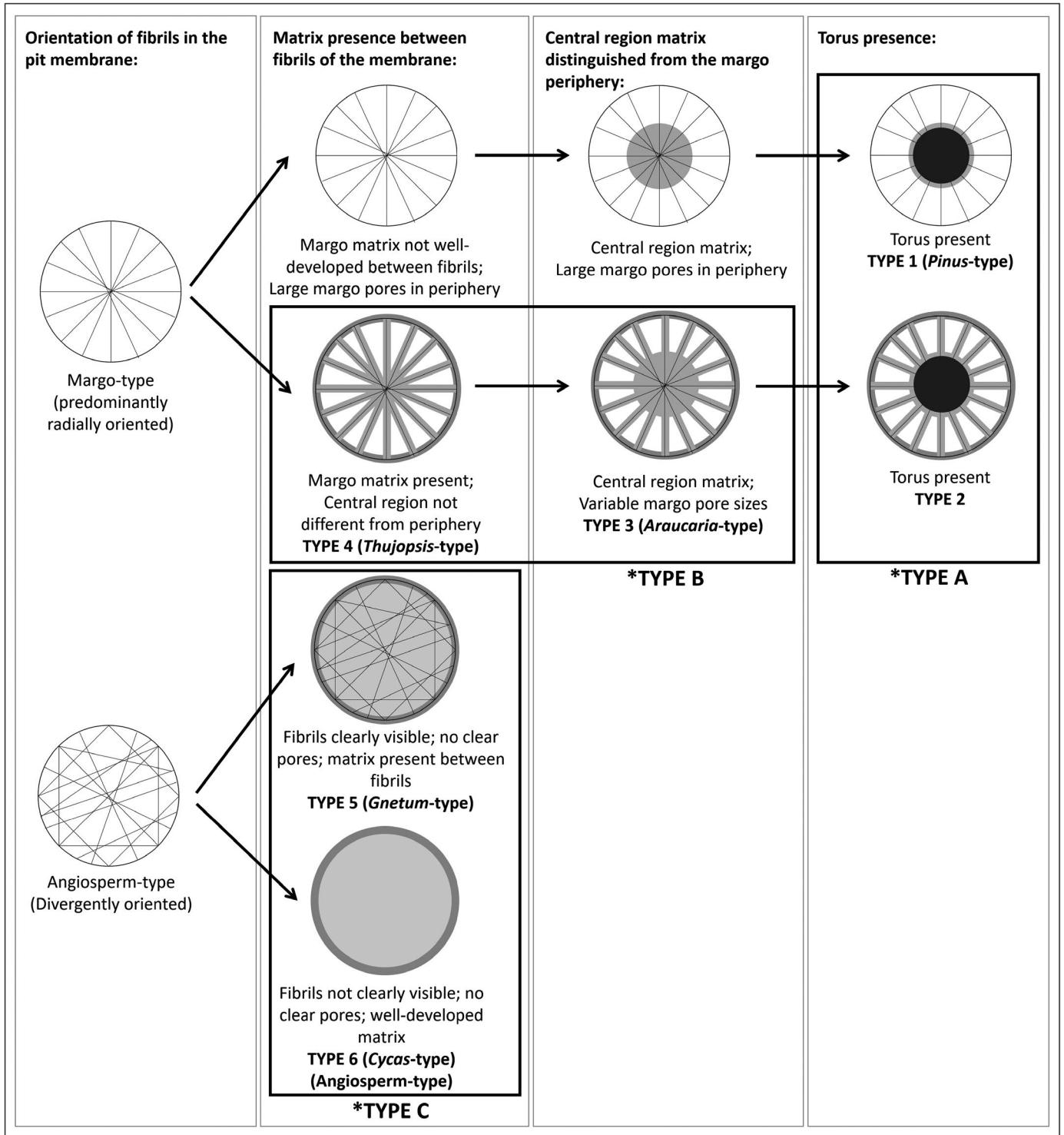


FIGURE 2. Classification of pit membrane types based on the orientation of microfibrils, the presence and distribution of matrix material between the fibrils, and the presence of a central thickened torus. Different proposed classifications schemes are indicated, including the genus-type classification of Liese (1965), the Types 1–6 classification of Bauch et al. (1972), and the simplified Type A, B, and C classification used in the present review (*).

a “mega-tree”. All taxa were updated to reflect current taxonomic classifications and the number of extant genera and species as reported by Kew Science (<http://powo.science.kew.org/>) and the International Plant Names Index (<https://www.ipni.org/>) (both

accessed repeatedly November 2019–September 2020 during the compilation of study data). Hydraulic functional traits, mean annual temperature (MAT), and mean annual precipitation (MAP) for species with reported pit membrane types were extracted from

data reported in Choat et al. (2012); these data were only available for woody tree species. Reported values were mostly for species with Type A pit membranes with limited reports for those with type B pit membranes and no reports for species with Type C pit membranes.

VESSEL-BEARING GYMNOSPERMS

Vessels are present within some gymnosperm taxa, including all members of the Gnetophyta, and possibly the Cycadophyta, representing 10–40% of extant gymnosperm species (Fig. 3). Vessel elements are connected via simple perforation plates in *Gnetum* and *Welwitschia* (Fig. 4) and via foraminate perforation plates in *Ephedra* (Fig. 5). All display multiserial alternate pitting in their vessel elements.

Reports of vessel occurrence within the Cycadophyta were variable. Purported vessel elements are connected via scalariform perforation plates at end walls, which are morphologically distinct from round bordered pits on lateral walls (Bailey, 1925). Controversy over the classification of these cells as vessel elements versus tracheids is based on differing interpretations of structures as being either scalariform perforation plates in vessels (Huang and Zhang, 1999; Huang et al., 2010) or scalariform pits in tracheids (Schneider et al., 2007). These differences are based on alternate interpretations of the sparse microfibril strands that are retained across the plate/pit openings. Although these openings may developmentally differ from true perforation plates, functionally it is likely that these plates/pits would behave as perforation plates because the very large openings between the few microfibril strands are too large to resist air-seeding between axially connected conduits (Sperry and Tyree, 1988). The use of perfusion experiments, as done by Zhang et al. (2017), could be valuable in identifying whether conduits are functionally connected in a manner consistent with the presence of vessels.

Vessel-bearing taxa vary in their pit membrane types. Pit membranes in *Welwitschia* are Type C. Pit membranes in *Gnetum* are variable in structure, with some Type C pit membranes present (Liese, 1965; Muhammad and Sattler, 1982) and Type A pit membranes also present between tracheids

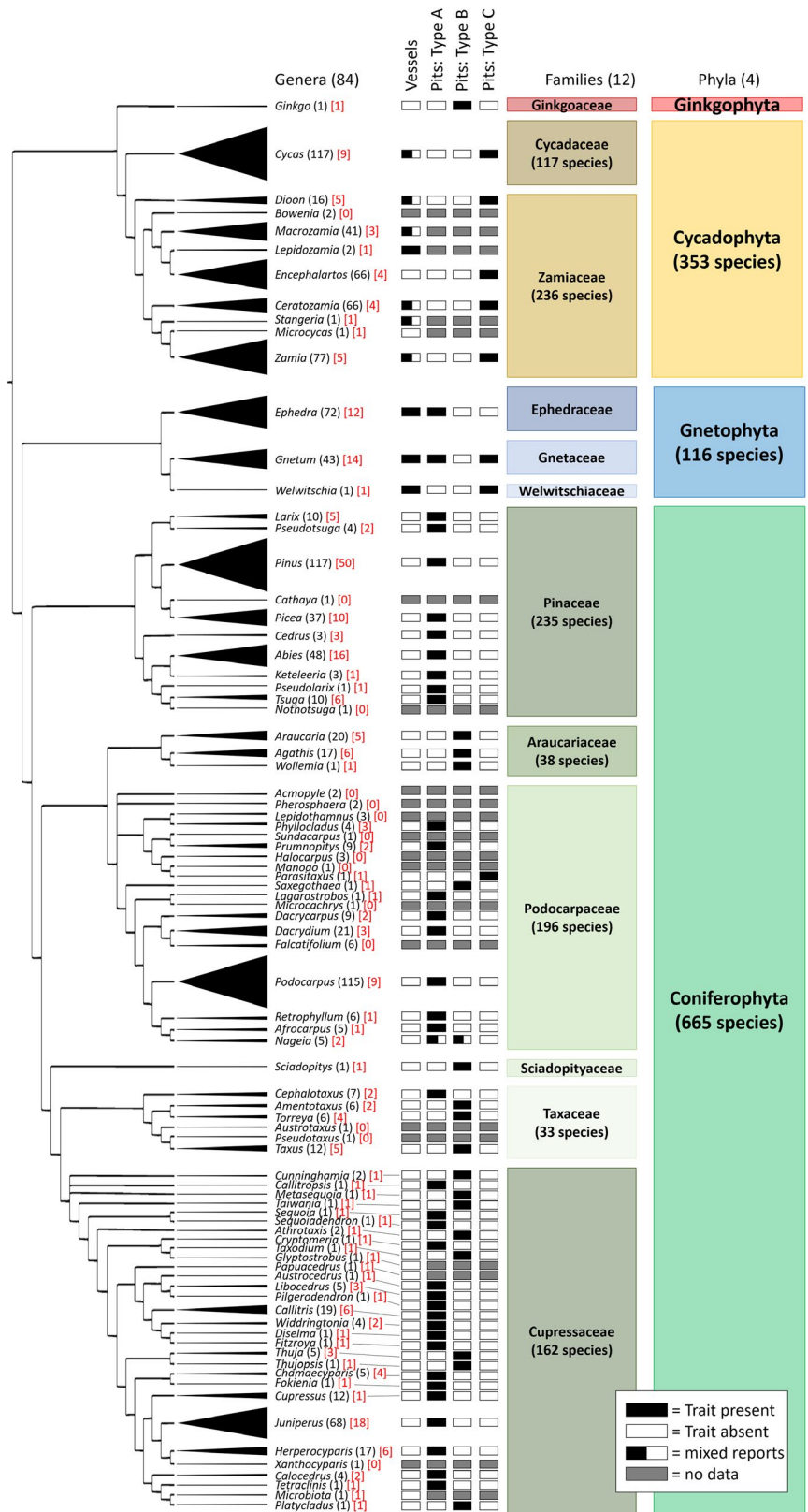


FIGURE 3. Cladogram showing the relationships between extant gymnosperm genera. The number of extant species within each genus, family, and phyla are included parenthetically. Tip width corresponds to number of extant species. The number of species for which vessel presence and/or pit membrane type data were included is indicated in brackets (red text) following each genus. Additional information on the number of published reports, sources, and other pit traits are included in Appendix S1.

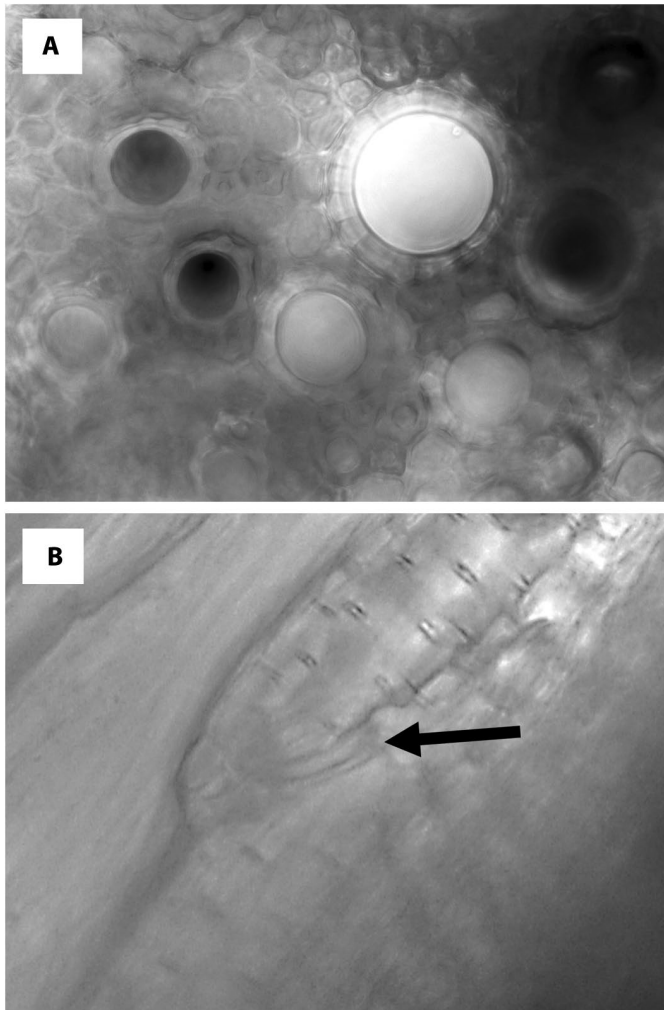


FIGURE 4. Xylem vessels from *Welwitschia mirabilis* shown in transverse (A) and longitudinal section (B). Simple perforation plates connect vessel elements as indicated by arrow (B) and multiseriate alternate pitting of the vessel is visible. Image credit: A. L. Jacobsen.

and in vessel-to-tracheid pits in some species (Carlquist, 1996). Additionally, the pit apertures and chambers of some *Gnetum* have vestures, which are extensions of the cell wall that protrude

from the edges of the pit chamber (Muhammad and Sattler, 1982; Carlquist, 1996). In *Ephedra*, pit membranes generally are Type A; however, Type B pit membranes are present in large pits that occur near perforation plates (Carlquist, 1990). Growth ring porosity varies, with scattered diffuse-porous vessels within *Gnetum* (Fisher and Ewers, 1995) and ring porosity in *Ephedra* (Fig. 4; Schweingruber et al., 2006). Taxa within Cycadophyta contain Type C pit membranes.

INTER-CONDUIT PITS VARY IN STRUCTURE AND ARRANGEMENT AMONG TAXA

Pit membrane types varied among extant gymnosperm genera (Fig. 3). Data on pit membrane types (A, B, and C) were available for 63 of the 84 extant genera and included observations for most species-rich genera; genera with pit membrane type reported include 1061 of 1135 extant gymnosperm species. There were clear classifications for 61 genera after removal of *Nageia*, for which reports of pit membrane type were inconsistent, and *Gnetum*, which contain different pit membrane types depending upon the conduit type (see above). For *Nageia*, *N. wallichiana* was reported to have Type A pit membranes (originally reported as Type 1 pits in *Podocarpus blumei*; Bauch et al., 1972), but *N. nagi* has been reported to lack tori (originally reported as *P. nagi*; Schulte and Gibson, 1988). For the remaining genera, pit membrane types were consistent among all reported species within each genus. Additional observations of more species, particularly within species-rich genera, are needed to determine whether this intrageneric pattern is robust.

Across genera, 37 genera displayed Type A (60.7%), 17 Type B (27.9%), and eight Type C (11.5%) pit membranes. Some of these genera are very species-rich, while others are not, so species-level values are not equivalent to genera-level ratios of pit membrane types, particularly for B and C. Assuming pit membrane type is consistent within a genus and applying this to genera with known pit membrane types, 626 species likely have Type A (61.8%), 79 species likely have Type B (7.8%), 308 species likely have Type C (30.4%) pit membranes. It was not possible to infer pit membrane types for the remaining genera or species, because pit membrane types showed variability within families, especially within Podocarpaceae and Cupressaceae. Based on the available data, approximately 60% of gymnosperm taxa likely display torus-margo pit membranes, while the remaining taxa do not.

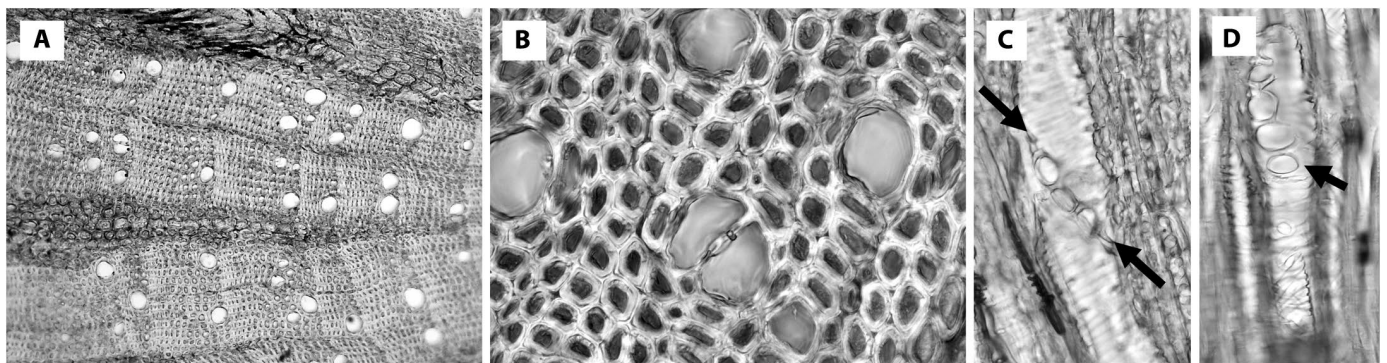


FIGURE 5. Xylem vessels from *Ephedra nevadensis* (A, C) and *E. viridis* (B, D) shown in transverse (A, B) and longitudinal section (C). Growth rings display ring porosity (A). Foraminiferous perforation plates connect vessel elements as indicated by arrows (C, D). Multiseriate alternate pitting is also visible (C). Image credit: A. L. Jacobsen.

Pitting arrangements were variable, with taxa showing uni-, bi-, and multiseriate pitting and both alternate and opposite pit arrangements. Seriation was variable within species and genera, and values were simplified to the most common arrangement reported for a genus (Table 1, Fig. 6). The most common type of pit membrane and seriation, as epitomized by *Pinus* xylem (Fig. 7), contains Type A pit membranes with some biseriate pitting in earlywood but predominantly uniseriate pitting. Among taxa with biseriate pitting, opposite pitting was common (Table 1). Multiseriate pitting was associated with Type C pit membranes and alternate pitting arrangements (Table 1).

INTERMEDIATE TYPES AND THE USE OF TORUS PRESENCE/ABSENCE IN SOFTWOOD IDENTIFICATION

Data were collected on torus presence or absence, even when reported independently of pit membrane type (A, B, C) classification. These reports were sometimes difficult to interpret because details on how torus presence was evaluated and microscopy technique were often lacking. This contrasts with reports that were used to evaluate pit membrane type classification, for which these details were reported and multiple sources of microscopic and histological information used. For Type A and C pit membranes, presence/absence reports generally agreed with more detailed pit membrane type classifications; however, taxa with Type B pit membranes were routinely reported with different and contradictory torus presence/absence reports across studies (contradictory reports within 11 of 17 genera). Such contradictions are perhaps not surprising given the intermediate structure of this type of pit membrane.

The cases of *Ginkgo* (Ginkgoaceae) and *Metasequoia* (Cupressaceae) are particularly illustrative of classification contradictions. Some studies have reported tori in *Ginkgo* (Dute, 1994, 2015). When viewed with light microscopy and the staining technique described above, it is clear that there is no chemically distinct torus or central region thickening in *Ginkgo*, and studies using light microscopy have reported that no tori are present (Schulte and Gibson, 1988; Hacke et al., 2004). An SEM- and TEM-based study, looking at both full and side views of pit membranes also reported no tori in *Ginkgo* (Timell, 1978), and other studies have also reported Type B pits for *Ginkgo* (Liese, 1965; Yin and Xiao-Mei, 1992). Similarly, *Metasequoia* lacks tori (Bauch et al., 1972), although some studies have reported the presence of tori (Delzon et al., 2010; Pittermann et al., 2010; Román-Jordán et al., 2017). In a study

TABLE 1. Number of extant gymnosperm genera displaying different pit membrane types and arrangements for those with predominantly uniseriate, those some biseriate (but which may also contain uniseriate), and those that contain some conduits that display multiseriate (3 or more) pitting.

Pit membrane type or arrangement	Category	Rows of pits (-seriate)			Total
		Uni-	Bi-	Multi-	
All		11	43	13	67
Pit membrane type					
	A	7	25	1	33
	B	2	11	3	16
	C	0	0	5	5
Pitting arrangement					
	Alternate	—	3	11	14
	Opposite	—	19	1	20

that combined SEM and TEM, authors were able to correctly identify that thickened tori were not present within *Metasequoia*, *Thujaopsis*, and *Torreya*, although, in this study, the central matrix region of the membranes in these pits was still analyzed as representing tori (Jansen et al., 2012).

It seems likely that it is difficult to separate matrix material from a thickened torus in dried specimens that are prepared for SEM observation and only viewed radially to observe the full pit membrane surface area. Light microscopy and histological techniques are valuable additional sources of information in interpreting these SEM images. Similarly, the combination of SEM radial views with TEM side views and histological examination may be informative because central region thickness and chemistry can be determined and, thereby, torus presence or absence.

At least some of the confusion in the classification of Type B pit membranes may stem from the classification system utilized for softwood (gymnosperm) identification, which includes only the dichotomy of torus presence/absence (Trait 56) (Richter et al., 2004), rather than a more nuanced division that captures variability in pit membrane types and the possibility of a non-torus central matrix region. Pit membrane types, either as proposed in the present review or as suggested by Liese (1965) or Bauch et al. (1972) could be an informative modification of traditional wood identification traits and keys. Inclusion of these multiple potential pit membrane categories, including intermediate types, could prevent confusion associated with trying to force complex pit membrane types into a strictly dichotomous key. Additionally, pit membrane type may be a taxonomically informative trait, especially since it seems conserved within genera but not families.

FUTURE RESEARCH DIRECTIONS AND OUTSTANDING QUESTIONS

Although there have been a few attempts to compile diverse pit membrane type data across taxa (e.g., Bauch et al., 1972) and there have been many subsequent studies that report data for smaller numbers of species, data are still limited for a large number of gymnosperm species and some genera. A clear conclusion from the present review is that there is still need for examination of pit membranes within many taxa that, to date, have not been examined or reported within the peer-reviewed literature. With increasing study of pit membrane structure, the following questions may also be examined, and these have potential to elucidate both the evolution and function of pit membranes in gymnosperms.

Evolutionary gain and loss of the torus

The ancestral lineages to modern gymnosperms had homogenous pit membranes that lacked tori (Schmid, 1967; Cheng-Hong, 1981; Beck et al., 1982). The complex pit membrane structures, including the torus, of extant taxa are derived traits that evolved only in some lineages; however, taxa with and without tori occur throughout the extant gymnosperm taxa, including among close relatives (Fig. 3). Study of pit membrane traits for extant taxa not currently reported in the literature combined with values from extinct taxa could elucidate patterns of torus gain and loss. Tori have evolved multiple times, including in some angiosperm lineages (Jansen et al., 2007; Rabaey et al., 2008; Dute, 2015), and there has been much focus on the gain of tori. Examination of features associated with taxa that

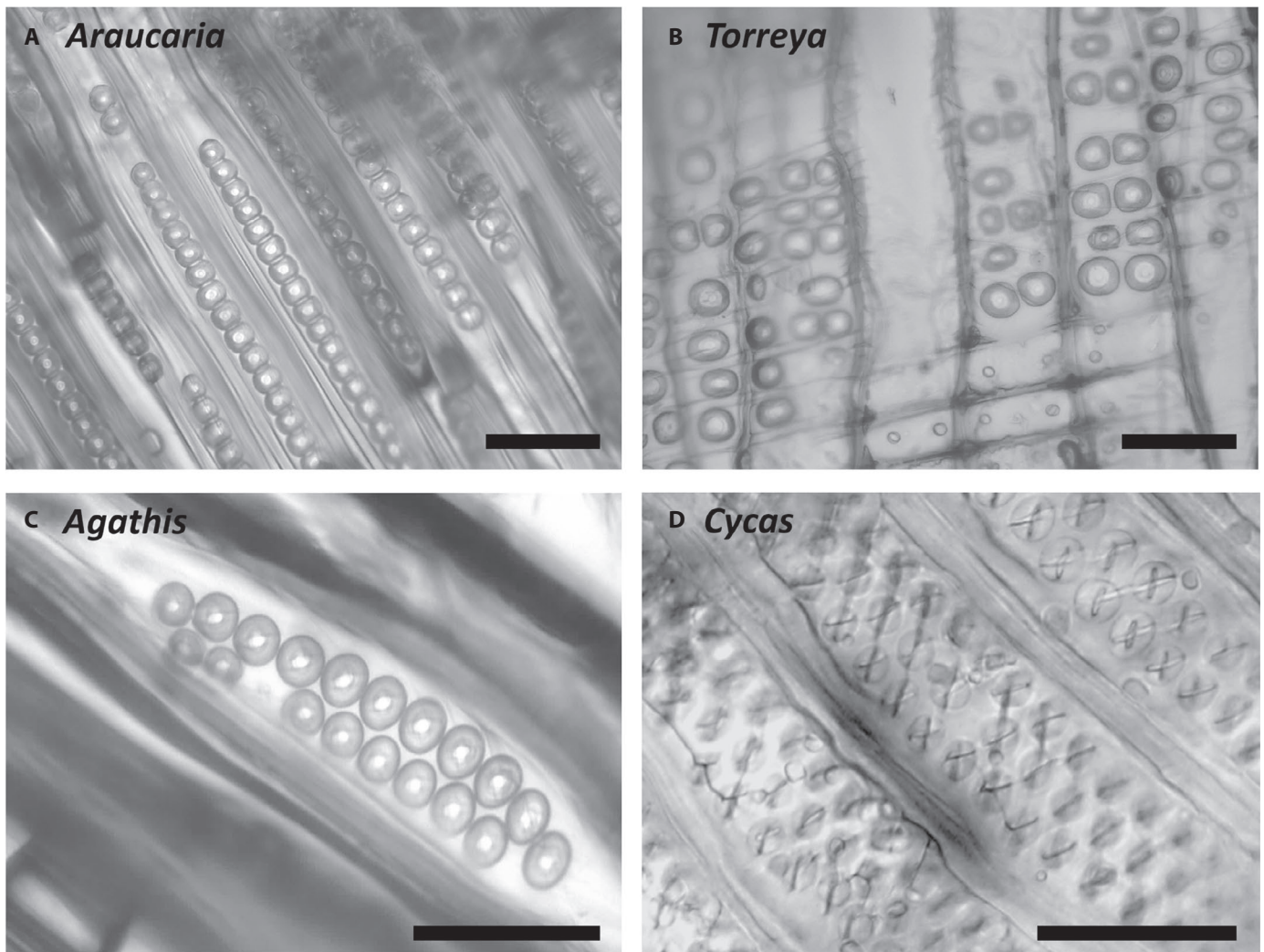


FIGURE 6. Variation in pitting seriation and arrangement among gymnosperm taxa, including uniseriate (A), biseriate opposite (B), biseriate alternate (C), and multiserial alternate (D). Samples shown in panels, A, B, and C all have Type B pit membranes, and panel D has Type C pit membranes. Genera are identified within the panels. Scale bar in the lower right of each panel indicates 50 μm . Image credit: A. L. Jacobsen.

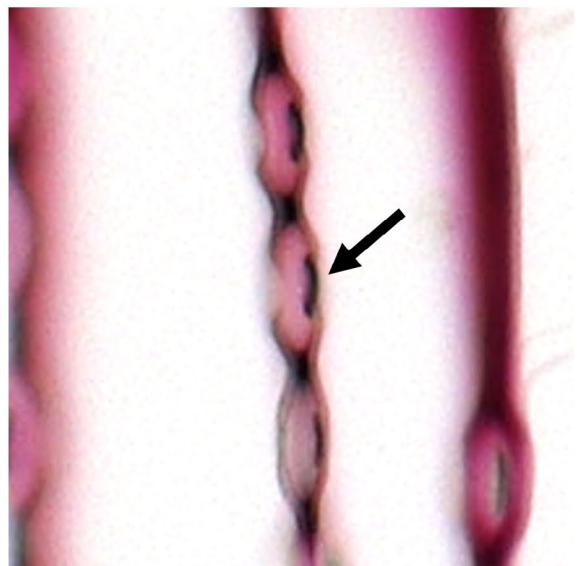
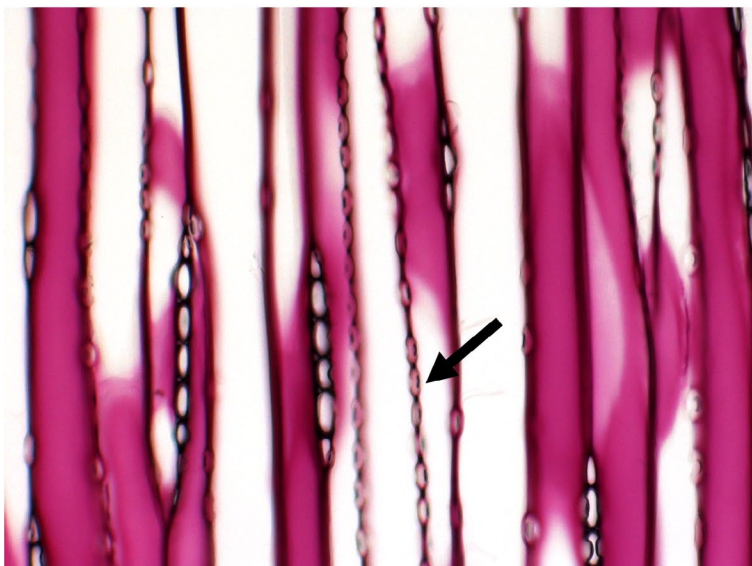
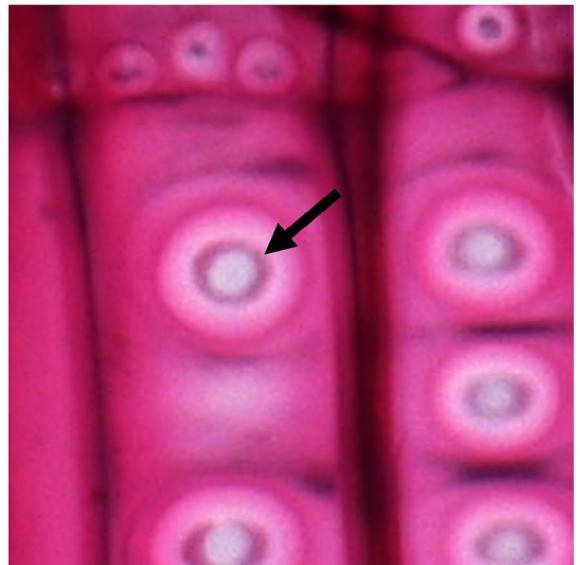
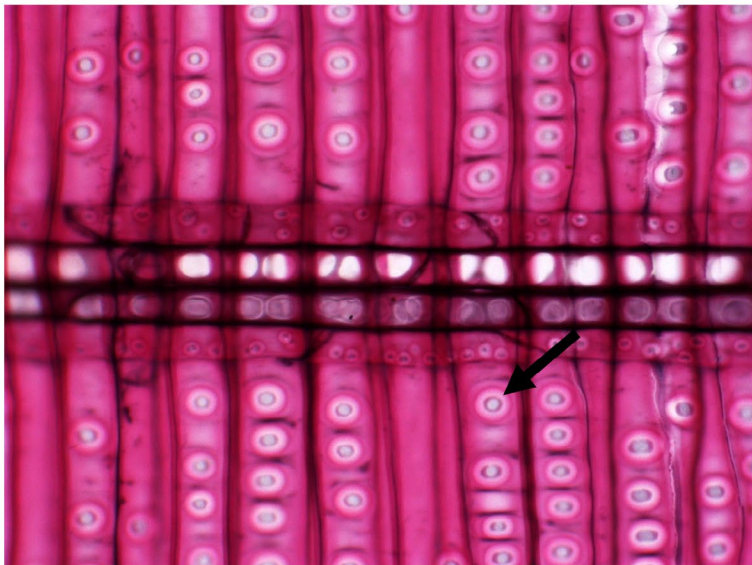
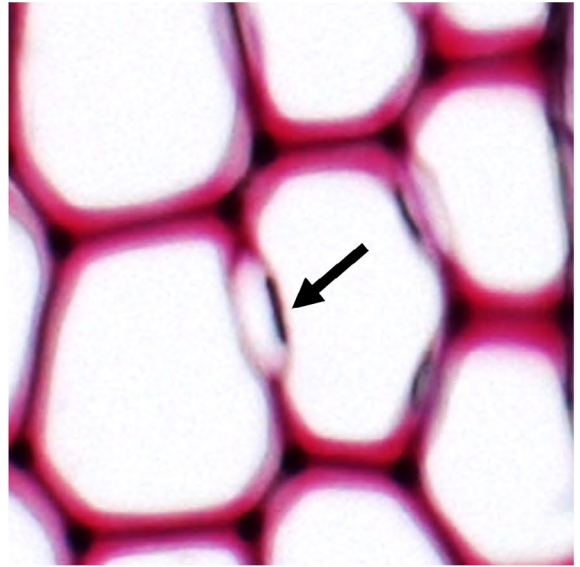
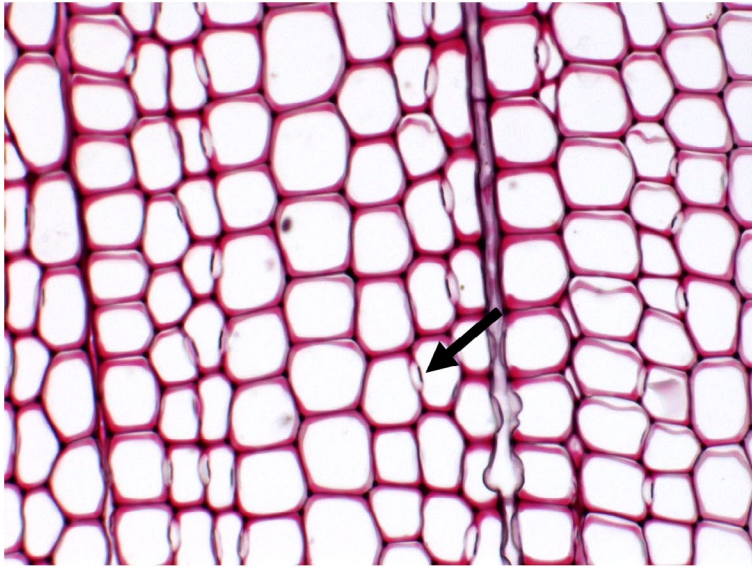
appear to have lost tori have not been discussed much but could be informative. Currently, it appears that some taxa have lost tori, such as in *Thuja* and *Thujopsis* whose related taxa all have tori. What selective pressures might favor the loss of tori? What fossil taxa contain or lack tori?

Hydraulic function in species with unique pit morphologies

Gymnosperm hydraulic studies have often focused on species with torus-margo bordered pits (Hacke et al., 2004; Delzon et al., 2010; Bouche et al., 2014; Schulte and Hacke, 2020; however, see Schulte and Gibson, 1988). This limited focus may fail to capture important hydraulic functional differences and mechanisms of

embolism resistance among taxa. For instance, many studies have focused on torus overlap and pit aspiration (Delzon et al., 2010; Pittermann et al., 2010), but in species without tori, such as *Thuja occidentalis*, pit membranes do not aspirate (Liese and Bauch, 1967). Additionally, focusing on torus aspiration as a key trait likely also results in a failure to fully appreciate other potential pit membrane morphologies, such as in species that have torus extensions, which are thickened segments of the pit membrane that connect the torus to the pit chamber wall and that are found in *Abies* (Sano et al., 1999; Dute et al., 2008), *Tsuga* (Liese, 1965), and *Widdringtonia* (Pittermann et al., 2010); or in species with vestures, such as found in some *Gnetum*, although thus far tori and vestures have not been described as co-occurring within a single *Gnetum* pit membrane

FIGURE 7. The most common pit membrane type and pitting arrangement is exemplified by *Pinus* xylem and is represented by tracheids with mostly uniseriate pitting, some biseriate pitting within the earlywood (not shown), and Type A pit membranes. Stained *Pinus* sections showing these structures were evaluated using light microscopy and are shown in transverse section (upper panels), radial section (middle panels), and tangential section (lower panels). Tori are present and indicated with arrows. Image credit: A. L. Jacobsen.



(Carlquist, 1996). Both torus extensions and vestures would either prevent torus aspiration or prevent sealing of the pit aperture by the torus. What is the function of the torus within these types of pits?

Hydraulic consequences of pit membrane structure

Hydraulic studies have often contrasted species bearing archetypal angiosperm and gymnosperm pit membranes when examining impacts of pit traits on hydraulic function (Hacke et al., 2005, 2015; Pittermann et al., 2005; Sperry et al., 2006; Choat et al., 2008). Within angiosperms, the hydraulic impact of vessels has been examined by comparing vessel-bearing and non-vessel bearing taxa, which can be a powerful comparative approach to evaluate evolutionary trends and the selective advantages of different traits (Hacke et al., 2007; Trueba et al., 2019). Similar experimental designs, comparing among gymnosperm taxa and controlling for other conduit features, could be valuable in understanding the hydraulic consequences of different pit membrane types or the impact of differences in pitting seriation. Such information could also be used to better understand species distributions, drought tolerance, and different hydraulic strategies among gymnosperm taxa.

Carefully controlled experiments could be designed to compare hydraulic function of pit membrane types and pit arrangements among gymnosperm taxa, while controlling for many other conduit traits. There are numerous pairs of closely related genera that differ in pit membrane types, but that are similar in other traits, such as conduit type, pitting seriation, etc. This framework can be used to evaluate many interesting questions: Does having a margo-type pit membrane without a torus make a species hydraulically efficient, but more vulnerable to embolism? Does having more multiseriate pitting overcome the reduction of pit membrane pore size between species with Type C compared to Type B pit membranes? Within a pit membrane type, are there hydraulic consequences to having predominately uni-, bi-, or multiseriate pitting?

Preliminarily, there is evidence that pit membrane type may be important in hydraulic function. Using previously published data allowed for the comparison of hydraulic traits among species with Type A and B pit membranes, although there were relatively few reports available for species with Type B pit membranes. Xylem did not differ in hydraulic conductivity (K_s) between these pit membrane types ($F_{1,225} = 0.74$, $P = 0.392$), but there was a trend for species with Type A pit membranes to be more resistant to embolism compared to those with Type B pit membranes ($F_{1,387} = 3.57$, $P = 0.060$). This trend is remarkable given the lack of controls and use of different hydraulic methods in these previously published data. Additional data on hydraulic function in species with Type B and C pit membranes and carefully controlled studies to match comparisons for methodological and other pit traits could provide for stronger and more robust comparisons of hydraulic functional differences among different pit membrane types and pitting arrangements.

Pit membrane structure and the environment

Gymnosperm taxa occur in diverse habitats and environments (Fig. 1), including tropical understories, temperate rainforests, arid deserts, and at altitudinal and latitudinal tree line. Presumably, conduit structure also varies across these highly different environments. Examining associations between climate and conduit structure requires the compilation of species-specific pit membrane type data that is more detailed than currently available, so that pit traits can be

linked to species distributions. What habitats and climates are more likely to contain taxa with tori and which ones are likely to contain taxa without them? Do co-occurring torus and non-torus bearing taxa differ in their growth, photosynthesis, or drought tolerance?

Preliminary analysis of mean annual precipitation (MAP) and mean annual temperature (MAT) for species included within the present review suggests that temperature, but not precipitation, may be an important driver of torus presence. Among woody taxa, there was no difference in MAP for locations where species with Type A and B pit membrane types occurred ($F_{1,379} = 2.12$, $P = 0.146$). In contrast, there was a strong MAT difference for locations where species with Type A and B pit membranes occurred ($F_{1,377} = 2.12$, $P < 0.001$), with mean MAT for torus-bearing species significantly lower (8.9°C) than non-torus bearing species (13.7°C). Tori may be important in limiting spread of freeze-thaw-induced emboli, and further examination of their role in response to this stress is warranted (Robson et al., 1988; Sperry et al., 1994; Sparks and Black, 2000; Pittermann and Sperry, 2006).

Pit arrangement and membrane structure within roots and compared to stems

Pit membrane structure and pitting arrangements of root xylem from woody species has not been extensively examined, especially for woody gymnosperm species. Functionally, gymnosperm root xylem has been found to be less resistant to embolism, have higher hydraulic conductivity, and have tracheids and pits that are wider in diameter when compared to stems (Kavanagh et al., 1999; Pittermann and Sperry, 2003; Hacke et al., 2004; Domec et al., 2009; Johnson et al., 2016). Are pit membrane types consistent throughout the secondary xylem tissue of the plant body or does this differ between roots and shoots? Pit seriation may be linked to conduit diameter, with multiseriate pitting present when tracheids are wide (Jane et al., 1970). Does this seriation prediction apply to the wide diameter tracheids of roots compared to the narrower conduits of shoots within a tree? Research questions and opportunities are extensive within this area of research and the use of intra-organismal study systems can provide unique experimental opportunities to test links between hydraulic function and conduit structure (Jacobsen et al., 2018).

Hydraulic function when vessels and tracheids co-occur within xylem

Several gymnosperm taxa have vessels and tracheids that co-occur within their xylem. The occurrence of vessels seems like a clear advantage for some of these taxa, for instance, within the xylem of tropical lianas in *Gnetum*. Long transport pathways in lianas would select for wide and long conduits with reduced hydraulic resistance and, in the tropics, nonfreezing temperatures would prevent freeze-thaw embolism from limiting conduit diameter (Langan et al., 1997; Pittermann and Sperry, 2006). Indeed, some *Gnetum* have very wide and long vessels (some vessels exceed 500 μm in diameter; Fisher and Ewers, 1995) and have increased hydraulic conductance compared to non-vessel-bearing conifers (Field and Balun, 2008).

The advantage of vessels seems less clear for other taxa, such as aridland shrubs. *Ephedra* species occur in dry habits, some of which are incredibly stressful (Fig. 1F shows *Ephedra* growing in Death Valley, California, USA; Fig. 5 shows vessels from this species.). Large-volume xylem conduits are more susceptible to embolism than smaller ones (Jacobsen et al., 2019), and vessels in *Ephedra*

are likely not hydraulically functional for much of the year when conditions are very hot and dry. It may be that vessels are advantageous during rain pulses and brief periods of favorable conditions, which would also be consistent with high seasonality selecting for ring porosity that enables maximum earlywood productivity during “booms” and resistant latewood conduits to maintain the plant during “busts.”

As with *Ephedra*, the combination of vessels and tracheids also occurs within some aridland angiosperm taxa (Carlquist and Hoekman, 1985; Carlquist, 1987; Pratt et al., 2015). Some of these angiosperms also contain tori, such as *Cercocarpus* (Dute et al., 2010), although torus-bearing angiosperms do not contain a margo within their pit membranes. Co-occurring tracheids and vessels represent an interesting convergence among aridland shrubs within both angiosperm and gymnosperm taxa. This type of conduit network could prevent emboli from isolating water within the network, allowing plants to extract more stored water during periods of drought (Jacobsen and Pratt, 2018) or could maintain transport pathways (Carlquist and Hoekman, 1985; Carlquist, 1987; Pratt et al., 2015). Studies looking at these and other potential advantages of a dual tracheid–vessel network in seasonally dry environments could increase understanding of the role of conduit networks in conferring drought tolerance.

CONCLUSIONS

The conduits found within the xylem of extant gymnosperm genera vary in their type, pitting arrangements, and pit membrane structure. Although these differences have long been known, they have rarely been the focus of study. Increased knowledge of these traits could provide a unique experimental context in which to study the evolution of conduit networks, the hydraulic implications of conduit and pit structure, and the diverse structural and functional strategies utilized by gymnosperms. There are myriad potential study questions and research opportunities within this unique and diverse group of plants.

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DATA AVAILABILITY

Data for this article was compiled from previously published sources. Data are reported for extant genera along with their sources within Appendix S1.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Summary of pit membrane traits and pitting arrangement for extant gymnosperm genera and the references from which these traits were compiled.

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