

# Intervascular pit membranes with a torus in the wood of *Ulmus* (Ulmaceae) and related genera

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## Summary

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- The distribution of intervascular pit membranes with a torus was investigated in juvenile wood samples of 19 species of *Ulmus* and seven related genera.
- A staining solution of safranin and alcian blue (35 : 65) was recommended to distinguish torus-bearing pit membranes using light microscopy.
- Intervascular pit membranes connecting relatively wide vessel elements resembled those of most angiosperms, as they were of uniform thickness. By contrast, bordered pit pairs with round to oval pit apertures and indistinct pit canals that connected narrow (incomplete) vessel elements or vascular tracheids with distinct helical thickenings were frequently characterized by a torus in ring-porous wood samples of *Ulmus* and *Zelkova*. Tori were lacking in diffuse-porous species of *Ampelocera*, *Aphananthe*, *Gironniera*, *Holoptelea*, *Phyllostylon*, *Trema* and *Ulmus*.
- Our observations suggest that tori are more common in cold temperate climates than in warm (sub)tropical environments. This may indicate that narrow tracheary elements with torus-bearing pit membranes provide an auxiliary conducting system which is of low conductivity, but offers greater resistance to freezing-induced cavitation.

**Key words:** *Ulmus* (elm), Ulmaceae, pit membrane, torus, pit structure, wood anatomy, tracheary elements.

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## Introduction

As water moves primarily from vessel to vessel or from tracheid to tracheid, the pit structure plays an important role in water transport in living plants, but it also affects the drying of wood and its treatment with preservatives (Schmid, 1965; Bauch *et al.*, 1972). Micromorphological studies illustrate that there is a wide structural variety associated with pits in tracheary elements, for instance with respect to pit size, shape, depth of pit chamber, pit-field arrangement and presence of vestures (Frost, 1931; Liese, 1965; Schmid, 1965; Jansen *et al.*, 2001). Recent findings highlight the importance of pit membranes in pit function, especially with respect to flow resistance and cavitation vulnerability (Tyree & Sperry, 1989; Cochard *et al.*, 1992; Zwieniecki *et al.*, 2001; Becker *et al.*, 2003; Choat *et al.*, 2003; Sperry, 2003). Pit membranes can be considered to act as finely porous filters, allowing the flow of water and nutrients, but at the same time limiting the passage of air bubbles and pathogens between adjacent tracheary elements.

While the basic structure and function of bordered pit membranes of softwoods is fairly well understood (Bauch *et al.*, 1972; Fengel, 1972; Booker, 1992; Sano *et al.*, 1999), our understanding of angiosperm pit membranes is based on an extremely small fraction of species (Ohtani, 1983; Wheeler, 1983; Sano & Fukazawa, 1994). Therefore structural variation in pit membranes of hardwood species is not fully understood, and additional observations are required.

Pit membranes with a torus, which is defined as the central thickening of an intervascular pit membrane, are known to be a characteristic feature of tracheid pits in conifers. Tori are found in many, but not all, genera of gymnosperms (Bauch *et al.*, 1972). Although pit membranes of angiosperms are of uniform thickness and lack visible openings, tori were reported for the first time in angiosperms in the intervascular pits of *Daphne* (Thymelaeaceae) and *Osmanthus* (Oleaceae) (Schmid, 1965; Schmid & Machado, 1968; Ohtani & Ishida, 1978). This character was found subsequently in two species of *Ulmus* (Ulmaceae) and three species of *Celtis* (Cannabaceae

*sensu* APG II) (Wheeler, 1983; Dute & Rushing, 1990; APG II, 2003). Moreover, 'torus-like' structures were reported in interfibre pits of *Prunus* and *Pyrus* (both Rosaceae) as well as for intervacular pit membranes of *Ligustrum* (Oleaceae) (Parameswaran & Gomes, 1981; Parameswaran & Liese, 1981; Barnett, 1987). Dute & Rushing (1987, 1988, 1990) and Dute *et al.* (1990, 1992, 1996, 2001) have focused specifically on the development and ultrastructure of torus-bearing pit membranes in angiosperms. It is clear that tori have evolved several times in some highly specialized angiosperms, because the feature has been reported in species belonging to Rosales, Malvales and Lamiales according to the APG II classification (APG II, 2003). The homoplastic nature of tori in angiosperms is probably caused by functional adaptations, but the ecological and physiological significance of these tori are not fully understood.

So far tori have been observed with light microscopy (LM) and electron microscopy (TEM, SEM) in two species of *Ulmus* (*Ulmus alata* and *Ulmus thomasi*), while the feature was found to be lacking in *Ulmus americana* (Wheeler, 1983; Dute & Rushing, 1990). Although most studies on the wood structure of Ulmaceae did not comment on torus-bearing pit membranes (Sweitzer, 1971; Zhong *et al.*, 1992), tori may be common in numerous representatives of this family. It seems that tori may easily be overlooked during LM observations of wood sections, because micromorphological details of pits are usually difficult to observe accurately. Therefore counterstaining is desirable to maximize the observation of minute pit features with LM. With this consideration in mind, we initiated an investigation of bordered pit pairs of 19 elm species. Also included were five related genera of Ulmaceae, and two genera that were previously placed in Ulmaceae but are now incorporated in Cannabaceae (APG II, 2003). An oil-immersion objective of a light microscope and a simple mixture of two staining solutions were shown to be sufficiently appropriate for LM observation of tori in wood sections. This paper is part of ongoing studies on the micromorphological structure of pits (Jansen *et al.*, 1998b, 2001, 2003).

## Materials and Methods

Young stem segments were collected from dried herbarium material of the National Botanic Garden of Belgium. A specimen of *Ulmus laciniata* was collected from the Arnold Arboretum of Harvard University. The diameter of all wood samples studied was < 1.5 cm. The nomenclature of all species followed the currently accepted species delimitation according to the International Plant Names Index of the Plant Names Project ([www.ipni.org](http://www.ipni.org)).

The following species (with reference to their origin and collector) were investigated: *Ampelocera dichotoma* Mildbr. ex Baehni, Peru, Loreto, Maynas, M. Rimachi Y. 6565; *Aphananthe aspera* Planch., Japan, Kagawa, D. Champluvier 5469; *Gironniera celtidifolia* Gaudich., Philippine Islands, Surigao, C.A. Wenzel

3149; *Holoptelea integrifolia* Planch., Sri Lanka, Thwaites 2381; *Phyllostylon rhamnoides* (Pois.) Taub., Argentina, Palo de Lauza, Corrientes, Troels Myndel Pedersen 2793a; *Trema lamarckiana* Roem. & Schult., Puerto Rico, Sintensis 3902; *Ulmus campestris* L., France, Bourges, A. Déséglise 1763; *U. campestris* L., Greece, Crete, Montagnes de Lelfa Ori, R. Dechamps 9047; *U. campestris* L., France, Côte d'Or, between Pont-de Pany and Arcey, J. Callé s.n.; *U. campestris* L., France, Le Puy, Velay, collector unknown; *Ulmus carpini-folia* Gled., UK, Hertfordshire, Cottered Green, R. Melville s.n.; *Ulmus coritana* var. *coritana* Melville, UK, Oxfordshire, Banbury, R. Melville & J.C. Went 38.68; *Ulmus cornubiensis* Weston var. *goodgeeri* Melville, UK, Hampshire, New Bashley, R. Melville 37.123; *Ulmus davidiana* Planch. var. *japonica* Nakai forma *suberosa* Nakai, Japan, Nagano, Mt. Asama, Kitasaku-gun, T. Makino 19602; *Ulmus diversifolia* Melville, UK, Hertfordshire, Hatfield, R. Melville 36.265; *Ulmus effusa* Willd., France, Bois de Raimes, A. Lelièvre s.n.; *Ulmus fulva* Michx., USA, Missouri, Jackson, Beug Park, B.F. Bush 882; *Ulmus glabra* Huds., Bulgaria, Barzya, Stara Planina, H. Beeckman bu50; *Ulmus japonica* Sarg., China, Chihli, Huai lai, Yang kia ping, J. Hers 2108; *Ulmus laciniata* Mayer, Japan, Hyôgo, Hyônoson, Mikata-kôgen, T. Yamazaki & H. Kanai 10964; *U. laciniata* Mayer, USA, Arnold Arboretum of Harvard University, ASI, 26-NE, accession number 17909-B; *Ulmus lancifolia* Roxb. ex Wall., Indonesia, Lesser Sunda Islands, W. Flores, Manggarai, E. Schmutz 4945; *Ulmus macrocarpa* Hance, China, Shansi, J. Hers 2016; *Ulmus mexicana* (Liebm.) Planch., Mexico, Landa de Matamoros, Queretaro, S. Zamudio s.n.; *Ulmus montana* With., France, Givet, C. Vanden Berghen & D. Duvigneaud s.n.; *Ulmus parvifolia* Jacq., Japan, Tatebayashi, Gumma, T. Makino 19467; *Ulmus pedunculata* Foug., France, Forêt de Mormal, J. Duvigneaud 64F 468; *Ulmus plotii* Druce, UK, Bedfordshire, Pegzodon, R. Melville & H.K. Riry-Shaw 46.12 A; *Ulmus scabra* Mill., Belgium, Vilvoorde, Ecole d'horticulture, collector unknown; *U. scabra* Mill., France, Sainte-Eugrâce, C. Vanden Berghen s.n.; *Zelkova acuminata* Planch., Japan, Hondo, Honaimura, Yehigo, M. Togasi 590; *Zelkova crenata* Spach, Belgium, Nimy, A. Wesmael s.n.

Longitudinal and transverse sections (10–15 µm thick) were sectioned from dried stem segments using a sliding microtome (Reichert, Vienna, Austria) (Jansen *et al.*, 1998a). A mixture of safranin and alcian blue (35 : 65) was used as staining solution. This counterstaining solution was found to be most useful for light microscopic observations of pits. The safranin was prepared as a 1% solution in 50% ethanol. The 1% alcian blue stain was dissolved in deionized water. After staining, sections were washed in deionized water, dehydrated in an alcohol series, treated with the clearing agent Parasolve (Prosan n.v., Merelbeke, Belgium) and embedded in Euparal (Agar Scientific Ltd, Essex, UK).

In order to verify the true nature of tori in sections prepared according to the method above, semi-thin sections of

**Table 1** Distribution of intervacular pit membranes with a torus in Cannabaceae and Ulmaceae, with reference to correlated wood anatomical features and their origin

Family/species (number of specimens studied)	Torus	Porosity	HT	VT	Origin of specimens studied
Cannabaceae					
<i>Trema lamarckiana</i> (1)	–	DP	–	–	Puerto Rico
<i>Gironniera celtidifolia</i> (1)	–	DP	–	–	Philippine Islands
Ulmaceae					
<i>Ampelocera dichotoma</i> (1)	–	DP	–	–	Peru
<i>Aphananthe aspera</i> (1)	–	DP	–	–	Japan
<i>Holoptelea integrifolia</i> (1)	–	DP	–	–	Sri Lanka
<i>Phyllostylon rhamnoides</i> (1)	–	DP	–	–	Argentina
<i>Ulmus campestris</i> (4)	+	RP	+	+	France; Crete
<i>Ulmus carpiniifolia</i> (1)	+	RP	+	+	UK
<i>Ulmus coritana</i> var. <i>coritana</i> (1)	+	RP	+	+	UK
<i>Ulmus cornubiensis</i> var. <i>goodgeeri</i> (1)	+	RP	+	+	UK
<i>Ulmus davidiana</i> var. <i>japonica</i> forma <i>suberosa</i> (1)	+	RP	+	+	Japan
<i>Ulmus diversifolia</i> (1)	+	RP	+	+	UK
<i>Ulmus effusa</i> (1)	+	RP	+	+	France
<i>Ulmus fulva</i> (1)	+	RP	+	+	USA
<i>Ulmus glabra</i> (1)	+	RP	+	+	Bulgaria
<i>Ulmus japonica</i> (1)	+	RP	+	+	China
<i>Ulmus laciniata</i> (2)	+	RP	+	+	Japan; cultivated in USA
<i>Ulmus lancifolia</i> (1)	–	DP	–	–	Indonesia
<i>Ulmus macrocarpa</i> (1)	+	RP	+	+	China
<i>Ulmus mexicana</i> (1)	–	DP	+	+	Mexico
<i>Ulmus montana</i> (1)	+	RP	+	+	France
<i>Ulmus parvifolia</i> (1)	+	RP	+	+	Japan
<i>Ulmus pedunculata</i> (1)	+	RP	+	+	France
<i>Ulmus plotii</i> (1)	+	RP	+	+	UK
<i>Ulmus scabra</i> (2)	+	RP	+	+	France; cultivated in Belgium
<i>Zelkova acuminata</i> (1)	+	RP	+	+	Japan
<i>Zelkova crenata</i> (1)	+	RP	+	+	Belgium

Family classification follows APG II (2003); DP, diffuse-porous; RP, ring-porous; HT, helical thickenings confined to narrow tracheary elements; VT, vascular tracheids; +, present; –, absent.

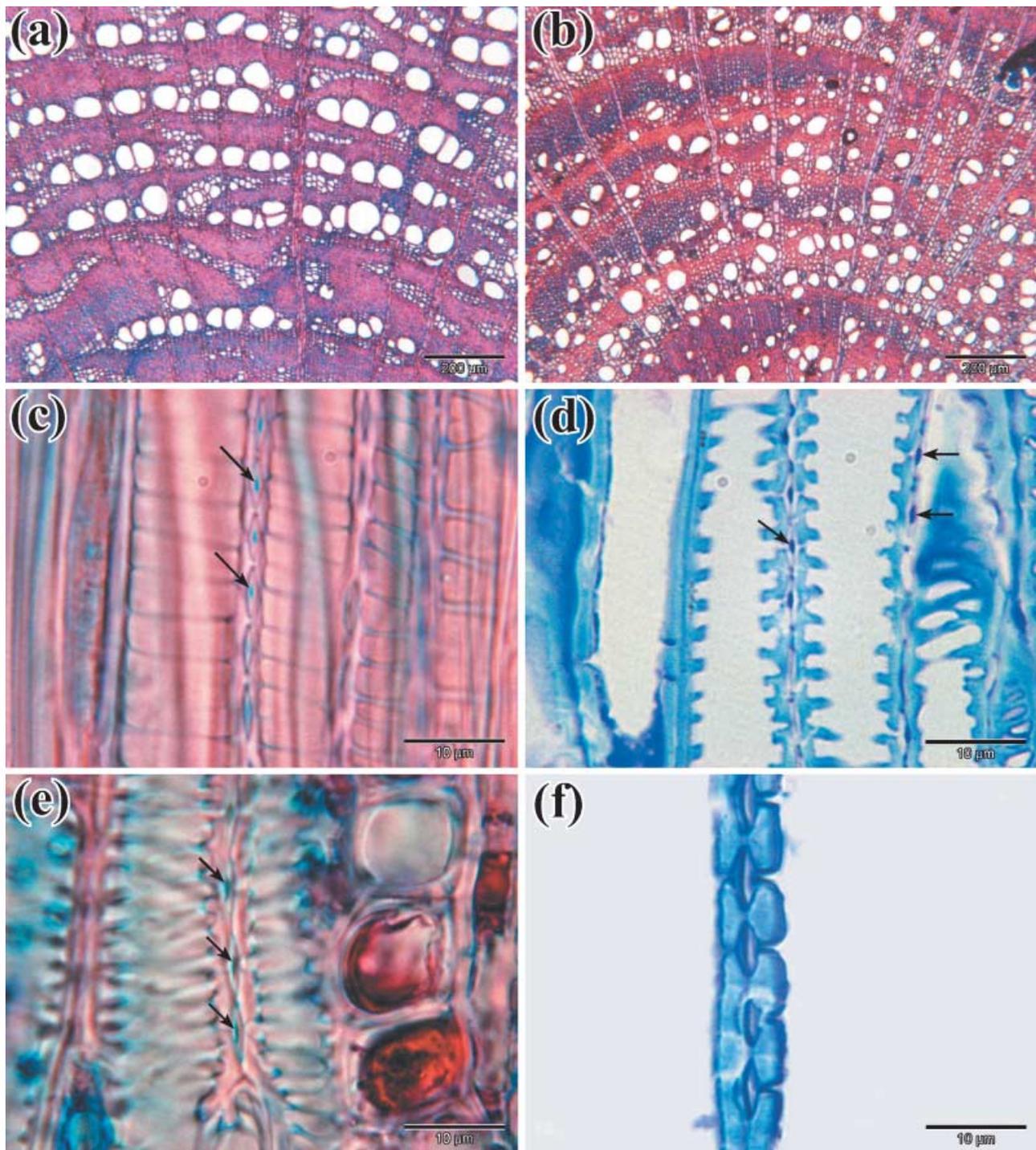
*U. campestris*, *U. laciniata*, *U. lancifolia*, *U. parvifolia* and *Z. crenata* were prepared of material embedded in LR White Resin (Polysciences Inc., Warrington, PA, USA). Young stem segments were fixed with 2% glutardialdehyde at pH 7.3 and buffered with 0.05 M sodium cacodylate. Before embedding in LR White Resin, the material was dehydrated in a graded ethanol series and block-stained with 1% phosphotungstic acid in 100% ethanol. Semi-thin ( $\pm 4 \mu\text{m}$ ) sections were cut with glass knives using an Ultracut E microtome (Reichert, Vienna, Austria) and stained with 0.1% thionin–0.1% methylene blue.

Observations were carried out using a Dialux 20 light microscope (Leitz, Wetzlar, Germany). In order to provide detailed information about the pit geometry, measurements of *U. laciniata* were conducted on LM images using CARNOY 2.0 software (Schols *et al.*, 2002). Quantitative data for sliding microtome sections of this species were based on counts of 50 measurements. The ratio of the short to long axes of pit apertures and pit borders was used as a measure of circularity. Pictures were taken using a DP50-CU digital camera (Olympus, Hamburg, Germany). Terminology follows the International

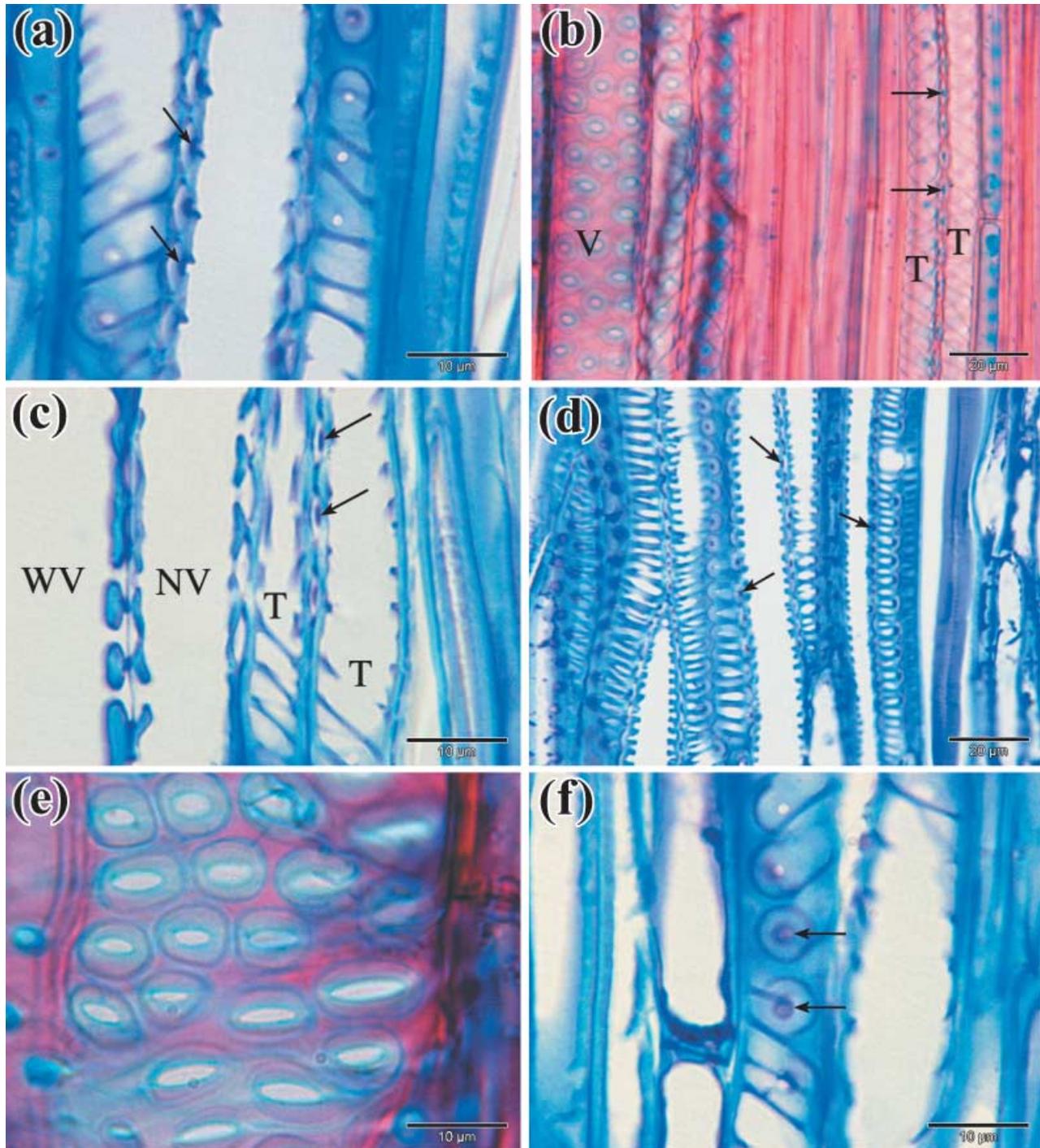
Association of Wood Anatomists' list of microscopic features for hardwood identification (IAWA Committee, 1989).

## Results

Intervascular pit membranes with a torus were present in 17 ring-porous wood species of *Ulmus* and two ring-porous species of *Zelkova* (Table 1; Fig. 1a,c–e). Although the resolution of micromorphological pit structures was usually higher in semi-thin sections (Fig. 1d) than in relatively thick sections prepared with the sliding microtome (Fig. 1c), the presence or absence of tori could be determined in all sections prepared with the sliding microtome. The staining solution of safranin and alcian blue generally resulted in a dark blue colour of tori in contrast to the margo, which was practically invisible. Tori were most distinctly present in *U. laciniata* (Fig. 2a–c,f), *U. montana*, *U. scabra* (Fig. 1c) and most specimens of *U. campestris* (Fig. 1d), while they could hardly be seen in longitudinal sections of *U. cornubiensis* var. *goodgeeri*. Species of *Ulmus* lacking tori included *U. lancifolia* (Fig. 1f) and *U. mexicana* (Fig. 1b), which showed diffuse-porous wood



**Fig. 1** Light microscopic illustrations of wood of *Ulmus* and *Zelkova*. All sections are 10–15  $\mu\text{m}$  thick and stained with safranin and alcian blue, except for (d) and (f) which are semi-thin sections, stained with thionin-methylene blue. (a) Transverse section showing ring-porous wood of *Ulmus diversifolia* with wide vessels in earlywood and narrow tracheary elements in latewood. (b) Transverse section of *Ulmus mexicana* showing diffuse-porous wood and indistinct growth rings. (c) Radial longitudinal section of narrow tracheary elements with helical thickenings and tori (arrows). (d) Radial longitudinal section of *Ulmus campestris* showing intervascular pit membranes with tori (arrows) associated with bordered pits of narrow tracheary elements. (e) Tangential longitudinal section of *Zelkova crenata* with tori (arrows) in intervascular pits of two narrow tracheary elements. (f) Tangential longitudinal section of *Ulmus lancifolia*. Note the presence of a relatively thick vessel wall, pit canal and intervascular pit membranes without a torus.



**Fig. 2** Light microscopic illustrations of wood of *Ulmus*. All sections are  $\pm 4 \mu\text{m}$  thick and stained with thionin-methylene blue, except for (b) and (e) which are  $10\text{--}15 \mu\text{m}$  thick and stained with safranin and alcian blue. (a) Radial longitudinal section of narrow tracheary elements with helical thickenings and tori in *Ulmus laciniata*. Arrows indicate pit aspiration. (b) Radial longitudinal section of *U. laciniata*. Vessel element (V) without tori at left. Tori (arrows) are associated with bordered pits connecting two vascular tracheids (T). (c) Radial longitudinal section of *U. laciniata*. Tori (arrows) are associated with bordered pits connecting a wide vessel element (WV) and narrow vessel element (NV). Tori (arrows) are restricted to very narrow tracheary elements (T) with helical thickenings. (d) Radial longitudinal section of *Ulmus campestris* showing narrow vessel elements and vascular tracheids with distinct helical thickenings and tori (arrows). (e) Radial longitudinal section of *U. laciniata* with surface view of bordered pits of a relatively wide vessel element. Note the elliptical to slit-like apertures. (f) Radial longitudinal section of *U. laciniata*. Surface view of bordered pits with a torus extending beyond the pit aperture (arrows).

samples. Tori were also absent in *Ampelocera*, *Aphananthe*, *Gironniera*, *Holoptelea*, *Phyllostylon* and *Trema*.

Tori were centrally located in the pit border, although pit membranes were frequently aspirated, and in such instances the torus occluded the pit aperture (Fig. 2a). The distribution of tori was closely associated with ring porosity and varied with the tracheary elements of the species involved. A torus was usually lacking in pit membranes of vessel elements with a tangential diameter > 20 µm wide (Fig. 2b,c). The inter-vascular pits of these relatively wide vessel elements were sometimes polygonal, with slit-like pit apertures (Fig. 2e). By contrast, torus-bearing pit membranes were frequently visible in longitudinal sections in pits connecting narrow tracheary elements, which mainly occurred in latewood. These elements were either narrow vessel elements showing one or two very small perforations (so-called 'fibriform' vessel elements), or vascular tracheids, which intergraded with narrow vessel elements (Fig. 2b–d). Helical thickenings were densely present throughout the body of these narrow tracheary elements (Figs 1c, 2a,b,d). The tangential diameter of tracheary elements with tori was usually approx. 10 µm, varying from 5 to 20 µm.

The mean longitudinal diameter of tori in *Ulmus laciniata* was 2.03 µm, varying from 1.31 to 2.83 µm (Table 2). The mean horizontal diameter of tori in this species was 1.92 µm, with 1.05 and 2.66 µm as minimum and maximum values, respectively. The horizontal diameter of the pit border was found to be smaller in pits with tori than in those without ( $P < 0.0001$ ), with mean values of 5.72 and 7.33 µm, respectively. Also, the horizontal and vertical diameter of the pit aperture was much smaller in pits with tori (mean values of 1.64 and 0.80 µm) compared with pits without tori (mean values of 3.29 and 1.89 µm) ( $P < 0.0001$ ). While no difference was found in the circularity ratio of the pit borders ( $P = 0.499$ ), the shape of the pit apertures was more circular or elliptical in pits with tori than in those without ( $P < 0.0001$ ) (Table 2; Fig. 2e,f).

## Discussion

Our LM observations illustrate the presence of torus-bearing pit membranes in ring-porous species of *Ulmus* and *Zelkova*,

while tori appear to be lacking in diffuse-porous species of *Ampelocera*, *Aphananthe*, *Gironniera*, *Phyllostylon*, *Trema* and *Ulmus*. The observation of tori in *Zelkova* represents a new record for this genus. Moreover, our data support the idea that tori are common in numerous elm species (Wheeler, 1983; Dute & Rushing, 1990). Although there is no question that TEM observations are most useful to investigate ultrastructural details of pit membranes, the staining technique applied in this study allows us to explore the distribution of tori following a simple but adequate method. Hence LM observations may form a welcome addition to SEM or TEM observations, especially where comparative studies are concerned, as LM observations are less time-intensive and more cost-effective than reviewing large amounts of material using SEM or TEM. Ohtani (1983) surveyed intervascular pits in 197 Japanese species (110 genera, 50 families) based on SEM observations, but reported tori to be lacking in *U. laciniata*, *U. davidiana* var. *japonica*, *Z. acuminata* (Ulmaceae), *Celtis sinensis* var. *japonica* (Cannabaceae), and *A. aspera* (Cannabaceae). However, the observations of tori in *U. laciniata*, *U. davidiana* and *Z. acuminata* reported in this study cast doubt on Ohtani's interpretation of these species.

As the thin wood samples studied do not include heartwood, this indicates that the tori observed could not be formed as a by-product of the ageing process during the transition of sapwood into heartwood. Moreover, coating materials that cover the pit membrane have been noted from samples collected in winter and as a result of wounding (Rickard *et al.*, 1979; Barnett *et al.*, 1993; Sano & Fukazawa, 1994; Schmitt *et al.*, 1997; Morrow & Dute, 1999). The fact that no deposition was found on the pit membranes of large-diameter vessel members indicates that these coatings (wound or season-dependent) are not responsible for the formation of the tori. Dute & Rushing (1990) illustrated that torus initiation in *U. alata*, *Celtis laevigata* and *Celtis occidentalis* began very early in cell differentiation as an elaboration of the primary walls of the pit membrane. The characteristic blue colour of tori observed in *Ulmus* and *Zelkova* suggests that tori in these genera are rich in pectins and cellulose, and have a chemical composition that is essentially similar to that of the primary wall. While the removal of material by sodium chlorite was

Pit character	Pits without tori	Pits with tori
Horizontal diameter of pit border*	7.33 µm (5.62–10.66)	5.72 µm (3.34–7.55)
Horizontal diameter of pit aperture*	3.29 µm (1.84–7.32)	1.64 µm (0.91–2.46)
Vertical diameter of pit aperture*	1.89 µm (1.18–3.6)	0.80 µm (0.38–1.18)
Horizontal diameter of torus	(torus absent)	1.92 µm (1.05–2.66)
Vertical diameter of torus	(torus absent)	2.03 µm (1.31–2.83)
Circularity ratio† of pit border	0.87 (0.56–0.99)	0.88 (0.69–0.99)
Circularity ratio† of pit aperture*	0.61 (0.25–0.97)	0.85 (0.53–1)

\* $P < 0.0001$ . †Circularity ratio defined as ratio of short to long axis. All data based on 50 measurements.

**Table 2** Mean values of pit characters of *Ulmus laciniata* (minimum and maximum values in parentheses)

interpreted to indicate the presence of lignin in the torus of *U. alata* (Wheeler, 1983), Coleman *et al.* (2004) have recently suggested that tori in *Ulmus* and *Celtis* are not lignified.

Interestingly, angiosperms with tori appear to show the following characteristic features: (1) a relatively small pit aperture that is circular to elliptical in outline; (2) an indistinct pit canal that is very short or entirely lacking; and (3) the prominent occurrence of helical thickenings throughout the body of narrow tracheary elements. The association between the evolution of the torus and small and circular pit apertures was previously suggested in gymnosperms, and further illustrated in Thymelaeaceae (Wright, 1928; Beck *et al.*, 1982; Dute *et al.*, 1996, 2001). Hence a torus is not found in association with linear, slit-like apertures. The converse, however, need not be true – not all species with small and circular pit apertures form torus-bearing pit membranes (Dute *et al.*, 2001). Although quantitative data on the circularity ratio in *U. laciniata* overlap between pits with a torus and those lacking a torus, our data support the above correlation (Table 2). It should be emphasized that tori appear to be closely associated with ring porosity. Except for *U. mexicana*, all species without tori do not show very narrow vessel elements or vascular tracheids with distinct helical thickenings. Based on these correlations, it is likely that tori could be found in two other genera of the Ulmaceae, namely *Planera* and *Hemiptelea*, as ring-porous wood with vascular tracheids and narrow vessel elements with distinct helical thickenings are recorded in these two genera (Sweitzer, 1971; Zhong *et al.*, 1992). The association of tori with other pit characters may indicate that the development of tori is inherited as a functional complex in woody plants (Carlquist & Robinson, 1995).

As regards functional aspects, tori are suggested to serve as a closure valve in case of pit aspiration, but may also provide additional strength to the pit membrane, and thus prevent rupture during membrane displacement (Wheeler, 1983; Dute *et al.*, 1990, 2001; Morrow & Dute, 1998). As the diameter of the torus is greater than that of the pit aperture in *U. laciniata* (Table 2), the torus-bearing pit membrane would be more effective than one of uniform thickness in sealing off a pit aperture and therefore confining air embolism. Also, differences observed in pit geometry may have consequences for the stress experienced by the membrane during displacement (Hart & Thomas, 1967; Jansen *et al.*, 2003; B.C. and co-workers, unpublished). Sperry (2003) suggested that greater pit conductivity per air-seed pressure could explain the persistence of torus–margo pits in conifers and their convergent evolution in certain angiosperms. As Carlquist (1996) reported for Gnetales, the development of tori in Ulmaceae may represent simply the promotion of conductive safety, which differs between wide vessels and narrow tracheary elements (Carlquist, 1984, 1985). Vulnerability to freeze-induced embolism is well correlated with conduit diameter (Sperry *et al.*, 1994; Davis *et al.*, 1999). Also, there is a correlation between drought-induced embolism and

conduit diameter within one tree (Hargrave *et al.*, 1994), but this does not hold true at the interspecies level (Tyree & Zimmerman, 2002). Narrow vessels formed in the latewood of *U. americana* are suggested to be nearly as resistant as tracheids to embolism formation (Ellmore & Ewers, 1985).

Species commonly containing narrow tracheary elements ('fibriform' vessel elements and vasicentric tracheids *sensu* Carlquist) in combination with helical thickenings frequently occur in areas that are dry or cold (Baas, 1973; van den Oever *et al.*, 1981; Carlquist, 1984, 1985, 2001). Based on the taxa examined in this study, the distribution of tori suggests that this feature tends to occur in cold temperate climates of Eurasia and North America. Hence the absence of tori in species such as *U. mexicana* and *U. lancifolia*, distributed in Mexico and Indonesia, respectively, could be caused by the relatively warmer climate as compared with the other elm species investigated. A similar conclusion could be suggested for the absence of tori in the other genera of Ulmaceae (*Ampelocera*, *Aphananthe*, *Holoptelea*, *Phyllostylon*) and Cannabaceae (*Gironniera*, *Trema*) studied. Interestingly, Ulmaceae are suggested to have a South or Central American origin, with a subsequent dispersal of the Northern Hemisphere taxa (including *Hemiptelea*, *Planera*, *Ulmus*, *Zelkova*) from Asia to Europe and North America, or an initial radiation of these taxa in North America before their spread to Eurasia according to a second phylogeographic model (Manchester & Tiffney, 2001). Although further observations are required on the ecological distribution of tori, most other angiosperm genera with tori (*Daphne*, *Wikstroemia*, *Ulmus*, *Celtis* and *Osmanthus*) tend to occur in temperate to boreal regions. The development of tori could therefore indicate a functional adaptation to relatively cold climates where freezing-induced cavitation may occur. As the highest frequencies of vestured pits occur in tropical, seasonal woodlands and deserts, where drought-induced cavitation is common, vestures could substitute for a torus as regards protection for the pit membrane in order to avoid embolism (Dute *et al.*, 2001; Jansen *et al.*, 2003). So far, vestures and tori appear to be mutually exclusive as they co-occur in the same pits in very few families (e.g. Thymelaeaceae). However, the morphology of these vestures is unlikely to reduce pit membrane deflection, as they are generally minute, simple, and do not point towards the pit membrane (Jansen *et al.*, 2000).

Although the genus *Ulmus* is relatively well defined, the delimitation of species and their taxonomic affinities are subject to major conflicting taxonomic treatments (Armstrong & Sell, 1996). Our data on the distribution of tori do not suggest any systematic implication within *Ulmus*. Tori occur in the two subgenera, *Oreoptelea* and *Ulmus*, as well as all sections distinguished by Wiegrefe *et al.* (1994). Moreover, the absence of tori in *U. mexicana* and *U. lancifolia* suggests that this feature is at least partly determined by environmental aspects. At a higher taxonomic level, *Ulmus* is suggested to form a clade with the genera *Planera*, *Hemiptelea* and *Zelkova* (Ueda *et al.*, 1997; Wiegrefe *et al.*, 1998; Manchester & Tiffney,

2001). The observation of tori in *Ulmus* and *Zelkova* and the possible occurrence of this feature in *Hemiptelea* and *Planera* could support this clade. It would be interesting to investigate whether tori are present in the latter two genera. Tori were also reported to occur in three species of *Celtis*, which was previously included within Ulmaceae (Wheeler, 1983; Dute & Rushing, 1990). As recent classifications place *Celtis* within either Celtidaceae or Cannabaceae, this would suggest that tori have developed independently in *Celtis* and *Ulmaceae* (Judd *et al.*, 2002; APG II, 2003).

A better understanding of pit characters such as tori and vestures, including their systematic and ecological distribution, could be important in order to comprehend characters that contribute to conductive safety or efficiency in secondary xylem. We are currently surveying other angiosperm families for the distribution of tori. Special attention should be paid to plant groups that show narrow tracheary elements with helical thickenings, intervacular pits with circular and small pit apertures, and an indistinct pit canal. We hope that additional studies may help to piece together the evolution of this interesting feature.

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