

## PIT MEMBRANES IN TRACHEARY ELEMENTS OF ROSACEAE AND RELATED FAMILIES: NEW RECORDS OF TORI AND PSEUDOTORI<sup>1</sup>

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The micromorphology of pits in tracheary elements was examined in 35 species representing 29 genera of Rosaceae and related families to evaluate the assumption that angiosperm pits are largely invariant. In most Rosaceae, pit membranes between fibers and tracheids frequently appear to have amorphous thickenings with an irregular distribution. Although these structures are torus-like under the light microscope, observations by electron microscopy illustrate that they represent “pseudotori” or plasmodesmata-associated thickenings. These thickenings frequently extend from the periphery of the pit membrane and form a cap-like, hollow structure. Pseudotori are occasionally found in few Elaeagnaceae and Rhamnaceae and appear to be related to species with fiber-tracheids and/or tracheids. True tori are strongly associated with round to oval pit apertures and are consistently present in narrow tracheary elements of *Cercocarpus* (Rosaceae), *Planera* (Ulmaceae), and ring-porous species of *Ulmus* and *Zelkova* (Ulmaceae). Vestured pits with homogenous pit membranes are reported for *Hemiptelea* (Ulmaceae). The homoplastic nature of pit membrane characteristics may be related to functional adaptations in terms of safety and efficiency of water transport or may reflect different developmental processes of xylem elements. These observations illustrate that there is more variation in angiosperm pits than previously thought.

**Key words:** fiber; pit; pit membrane; pseudotorus; Rosaceae; Rosales; torus; tracheid.

Pits are among the most conspicuous wood anatomical structures in plants. Their morphology and occurrence are frequently used to define cell types or may help to identify unknown wood samples (Baas, 1986; IAWA Committee, 1989). Although pits in tracheary elements are characteristically bordered (i.e., the pit membrane is overarched by the secondary cell wall), there is great structural variation with respect to pit size, pit shape, pit membrane structure, border configuration, pit-field arrangement, and presence of vestures (Schmid, 1965; Bauch et al., 1972; Jansen et al., 2001). Moreover, because pits allow water transport from vessel to vessel or from tracheid to tracheid, they play a key role in the hydraulic conductivity of tracheary elements, linking water uptake in roots with transpiration in leaves. Recent physiological studies highlight the importance of pit structure in pit function, illustrating that certain pit characters may affect flow resistance and vulnerability to air entry (Tyree and Sperry, 1989; Cochard et al., 1992; Becker et al., 2003; Choat et al., 2003, 2004, 2006). In addition, micromorphological work on pit membranes has potential application in the field of wood technology, including the paper and pulp industry, because pit characters such as tori and vestures affect the drying of wood and the penetration of gases and liquids, which is important, for

instance, for the impregnability and the treatment of commercial timbers (Bailey, 1913; Griffin, 1919; Singh et al., 1999; Usta and Hale, 2006).

While we have gained new insights into the physiological role of pits in relation to water transport processes in the last 20 years, our understanding of the structural variation of bordered pits, including their development, is less advanced, burdened by the perception that (ultra)structural work of pits is generally too difficult to attempt or that the structure of bordered pits is already fully understood. Pit membranes of angiosperms are generally thought to be of uniform thickness, while pit membranes with a central thickening or torus are found to characterize most gymnosperms. After this generalization was established in the literature, no one investigated this matter much further for a long time. This generalization holds true for the majority of angiosperms and gymnosperms, but requires further testing in a modern phylogenetic context with a wide range of species. So far, torus-margo pit membranes have been recorded in narrow tracheary elements of four angiosperm families (i.e., Cannabaceae, Oleaceae, Thymelaeaceae, Ulmaceae), which raises interesting questions about their systematic distribution and functional significance within woody angiosperms (Ohtani and Ishida, 1978; Wheeler, 1983; Dute and Rushing, 1987, 1988, 1990; Dute et al., 2001, 2004; Jansen et al., 2004).

Another character that illustrates our restricted knowledge of variation in angiosperm pit membranes includes the distribution of plasmodesmata-associated thickenings, which have been reported in mainly imperforate tracheary elements of species of *Crataegus*, *Pyrus*, *Prunus*, and *Sorbus* (Parameswaran and Liese, 1981; Barnett, 1982, 1987a, b; Lachaud and Maurousset, 1996). This feature was initially interpreted as

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a torus-like structure, but more detailed observations based on transmission electron microscopy (TEM) showed that the development of this type of pit membrane thickening is not homologous with a torus (Barnett, 1987a, b). In addition to *Ribes sanguineum* Pursh (Grossulariaceae) (Parameswaran and Liese, 1973), all genera in which plasmodesmata-associated thickenings have been reported hitherto belong to Rosaceae, Ericaceae, and Oleaceae (Rabaey et al., 2006). So far, however, there has been no extensive study on pit membranes of tracheary elements covering a wide range of Rosaceae species, nor do any detailed wood anatomical studies of this family report on the structure of pit membranes (Zhang, 1992; Zhang and Baas, 1992).

By focusing on the order Rosales, this work aims to evaluate the assumption that angiosperm pit membranes are largely invariant. We hypothesize that there is more variation than previously thought, because earlier wood anatomical studies on Rosales paid little attention to the ultrastructure of pit membranes or covered only a small number of species. Moreover, the occurrence of pit membrane thickenings such as tori and plasmodesmata-associated thickenings in some Rosales species suggests that these features may not be as rare as previously thought.

## MATERIALS AND METHODS

**Materials**—With respect to Rosaceae, 39 specimens including 35 species and 29 genera were examined. Samples from six related families within the order Rosales were selected. Samples of Dirachmaceae and Elaeagnaceae were used from previous wood anatomical studies, but no detailed attention was paid to pit membranes of tracheary elements in these works (Jansen et al., 2000; Baas et al., 2001). Dried wood samples were taken from the herbaria of the National Botanic Garden of Belgium (BR), the Royal Botanic Gardens at Kew (K), University of California at Davis (DAV, AHUC), Auburn University in Alabama (AUA), and from the wood collection at the Royal Museum for Central Africa at Tervuren (Tw). Samples taken from the Tw collection were usually mature wood from sapwood of the stem, although in some cases the xylem location was unknown. Samples removed from herbarium material (BR, K, DAV, AHUC, and AUA) were usually juvenile wood from thin branches (about 1 cm in diameter). Fresh material was collected from June through August 2005 from the living collections of the Royal Botanic Gardens, Kew and the Botanic Garden of the Hokkaido University (Sapporo). Fresh samples of *Prunus sargentii* were collected at the Tomakomai Experimental Forest of Hokkaido University during October 2003. We examined outer sapwood of young branches for all fresh material. Family classification of the species studied follows APG II (Angiosperm Phylogeny Group, 2003). For the genus *Cercocarpus*, the species delimitation is in agreement with Vanden Heuvel (2002). A list of all specimens investigated is included in Appendix 1.

**Light microscopy (LM)**—All wood samples from the BR herbarium and the collection of Tervuren (Tw) were studied using light microscopy (LM). Thin sections (10–15  $\mu\text{m}$ ) were cut using a sliding microtome (Reichert, Vienna, Austria) and stained with a mixture of safranin and alcian blue (35 : 65, v/v). 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 and mounted in Euparal (Agar Scientific, Essex, UK). Observations were carried out with a Leica DMLB light microscope (Leica, Wetzlar, Germany) and pictures were taken using an AxioCam digital camera (Zeiss, Hallbergmoos, Germany).

**Field-emission scanning electron microscopy (SEM)**—All samples were prepared for SEM as described by Sano (2004). In brief, samples were cut into small cubes (5 mm<sup>3</sup>) and air-dried after dehydration in an ethanol series (50%, 70%, 90%, 100%). The dried blocks were split along a tangential or radial plane, and the split samples were affixed to aluminum stubs with electron-conductive carbon paste. They were coated with gold/palladium using an

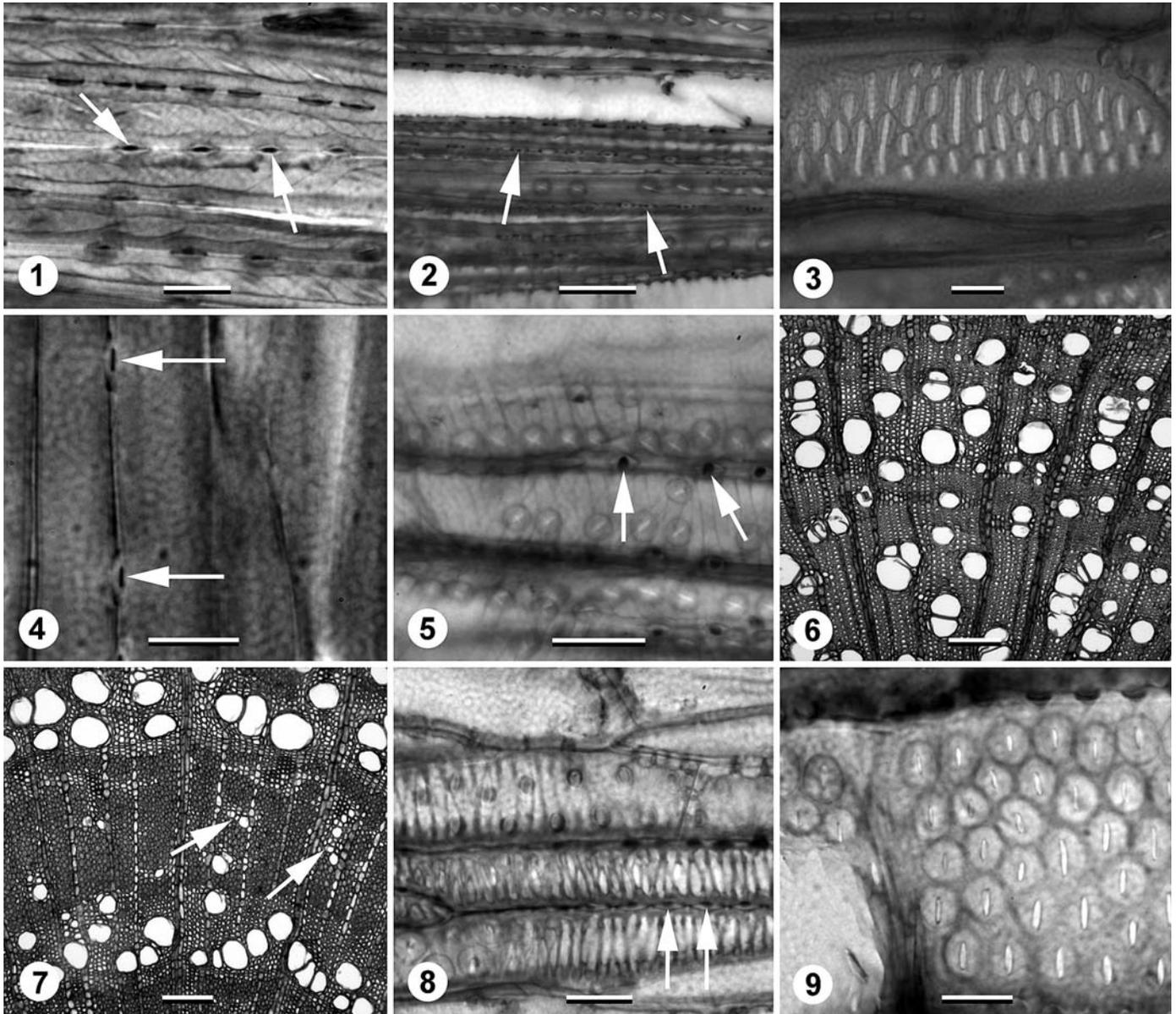
Emitech K550 sputter coater (Emitech, Ashford, UK) and with platinum using a JEOL JEE-5B vacuum evaporator (JEOL, Tokyo, Japan). Observations were carried out with a Hitachi S-4700 (Hitachi High Technologies Corp., Tokyo, Japan) at the Royal Botanic Gardens, Kew and with a JSM-6301F field-emission scanning electron microscope (JEOL, Tokyo, Japan) at Hokkaido University. For *Prunus sargentii*, we also examined interfiber pits in sections because it was often difficult to confirm the nature of pit pairs on a split face. Samples of *P. sargentii* were taken at breast height from the outer sapwood of a mature tree in October 2003. Surfaces of samples were exposed by the method devised by Yumoto et al. (1982). Small cubes of wood (2 mm<sup>3</sup>) were cut and embedded in methacrylate resin (a mixture of *n*-butyl methacrylate and methyl methacrylate, 1:2, v/v). Transverse or tangential surfaces were planed on an ultramicrotome with a glass knife. Then, the methacrylate resin was removed by soaking in acetone. The samples were subsequently air-dried and affixed with electron-conductive carbon paste to aluminum stubs. After being coated with carbon and gold/palladium by vacuum evaporation or with osmium by plasma polymerization (Sano et al., 1999), the samples were examined with the SEM as described. Quantitative characters were measured using ImageJ software (freeware available from <http://rsb.info.nih.gov/ij/>) and were based on 25 counts unless otherwise mentioned. The size of pits and pores in pit membranes was measured at the broadest point.

**Transmission electron microscopy (TEM)**—Fresh material of *Cercocarpus ledifolius*, *Malus yunnanensis*, and *Rosa sikangensis* was taken from the living collection of the Royal Botanic Gardens, Kew in December 2005. These samples were from 1- to 2-yr-old branches. Preparation of fresh TEM samples was based on Chaffey (2002). Small segments from thin branches were cut into 2-mm<sup>3</sup> pieces and fixated overnight in Karnovsky's fixative at room temperature (Karnovsky, 1965). After washing in a 0.05 M phosphate buffer, the specimens were postfixed in 1% buffered osmium tetroxide for 4 h at room temperature, washed again, and dehydrated through a graded ethanol series. The ethanol was gradually replaced with LR White resin (London Resin Co., Reading, UK) over several days. The resin was polymerized at 60°C and 1000 mm Hg for 18 to 24 h. Embedded samples were trimmed and sectioned on an ultramicrotome (Ultracut, Reichert-Jung, Vienna, Austria). One- and 2- $\mu\text{m}$  sections cut with a glass knife were heat-fixed to glass slides, stained with 0.5% toluidine blue O in 0.1 M phosphate buffer, and mounted in DPX (Agar Scientific, Stansted, UK). Resin-embedded material also was prepared for TEM observations by cutting ultrathin sections between 60 and 90 nm using a diamond knife. The sections were attached to formvar grids and stained with uranyl acetate and lead citrate using an LKB 2168 ultrastainer (LKB-Produkter AB, Bromma, Sweden). Observations were carried out using a JEOL JEM-1210 (JEOL, Tokyo, Japan) TEM at 80 kV accelerating voltage, and digital images were taken using a MegaView III camera (Soft Imaging System, Münster, Germany).

Samples of *Cercocarpus montanus* and *C. betuloides* were taken from dried herbarium material and prepared for TEM. Thin transverse segments of stems were cut with a razor blade and placed into vials containing absolute ethanol (two changes of fluid over 1 h). The ethanol was replaced by absolute acetone, remaining overnight in the latter. Specimens were gradually infiltrated with and polymerized in Spurr's resin (Spurr, 1969). Ultrathin sections of 70–90 nm were cut with an MT-2b ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Zeiss EM-10 TEM (Carl Zeiss, Thornwood, New York, USA) at 60 kV accelerating voltage. Photographic negatives were produced, and the images stored digitally using Adobe (San Jose, California, USA) Photoshop. Dried herbarium material of *Elaeagnus umbellata* and *Ulmus americana* was embedded for TEM using the same protocol. The samples were sectioned and examined as described for fresh material.

## RESULTS

**LM observations**—Pit membrane thickenings were observed in most species of Rosaceae (Figs. 1, 2). However, their presence or absence could not always be confirmed using LM, and it was usually impossible to determine whether these thickenings represent true tori or plasmodesmata-associated thickenings as previously reported in some Rosaceae. Pit membrane thickenings were irregularly distributed in pits of fiber-tracheids and/or vasicentric/vascular tracheids, i.e., they were only present in part of the pits in fiber-tracheids and tracheids. In some samples, more

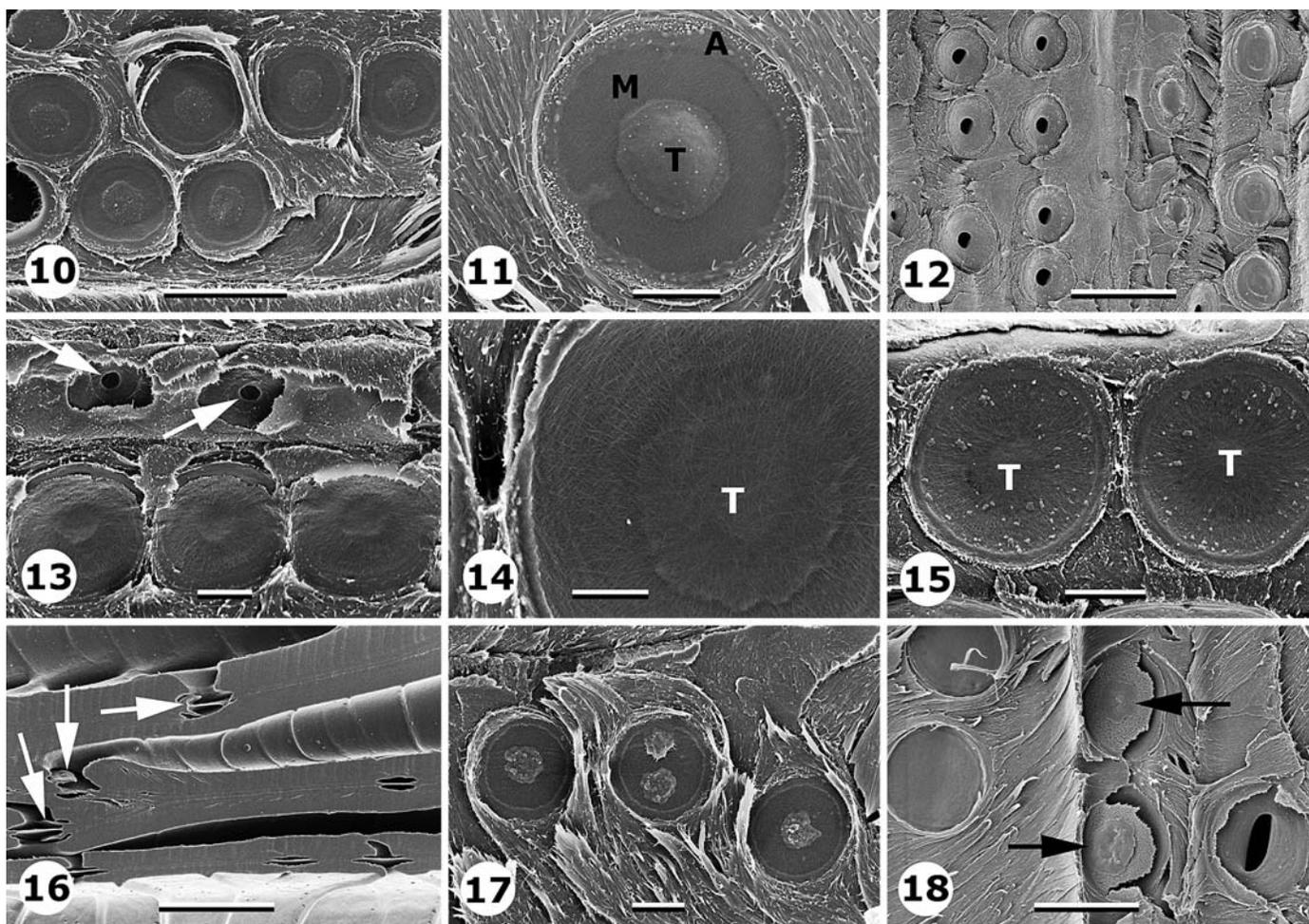


Figs. 1–9. LM images of Rosaceae (Figs. 1–4) and related families (Figs. 5–9). **1.** *Cotoneaster integerimma*, tangential longitudinal section (TLS), fiber-tracheids showing pit membranes with pseudotori (arrows), bar = 10  $\mu\text{m}$ . **2.** *Lindleya mespiloides*, TLS, fiber-tracheids with distinctly bordered pits and pseudotori (arrows), bar = 20  $\mu\text{m}$ . **3.** *Ribes petraea*, radial longitudinal section (RLS), intervessel pits with homogenous pit membranes and slit-like apertures, bar = 10  $\mu\text{m}$ . **4.** *Cercocarpus ledifolius*, TLS, fiber-tracheids with torus-bearing pit membranes (arrows), bar = 10  $\mu\text{m}$ . **5.** *Rhamnus utilis*, RLS, pits between narrow tracheary elements with helical thickenings and pseudotori (arrows), bar = 20  $\mu\text{m}$ . **6.** *Celtis philippensis*, transverse section (TS), diffuse-porous wood with indistinct growth ring, bar = 100  $\mu\text{m}$ . **7.** *Celtis australis*, TS, ring-porous wood with narrow tracheary elements in latewood (arrows), bar = 100  $\mu\text{m}$ . **8.** *Celtis australis*, TLS, narrow tracheary elements in latewood with helical thickenings and aspirated torus-bearing pit membranes (arrows), bar = 10  $\mu\text{m}$ . **9.** *Zelkova serrata*, RLS, intervessel pits with homogenous pit membranes and slit-like, narrow apertures, bar = 10  $\mu\text{m}$ .

than one thickening appeared to be associated with a single pit membrane. In general, intervessel pits were not found to show pit membrane thickenings (Fig. 3). The general appearance of the bead-like structures attached to pit membranes was transparent, slightly brownish to darkly stained. Pit membrane thickenings visible in species of *Cercocarpus* (Rosaceae) were observed as dark lines when using the oil-immersion objective and did not have a glass-like appearance as in most other Rosaceae species (Fig. 4). Moreover, the shape of the pit apertures associated with

thickened pit membranes in species of *Cercocarpus* appeared circular to oval, while all other Rosaceae genera studied had more slit-like pit apertures. The pit membrane thickenings in species of *Cercocarpus* also seemed to be consistently present in tracheids and were occasionally associated with intervessel pit membranes.

In addition to Rosaceae, pit membrane thickenings were found in all Elaeagnaceae studied (except for *Hippophae salicifolia*) and in *Rhamnus utilis* (Rhamnaceae; Fig. 5). Thickenings of the pit membrane were absent in diffuse- to



Figs. 10–18. SEM images of intervacular pits in Rosaceae (Figs. 10–12, 16–18) and related families (Figs. 13–15). **10.** *Cercocarpus ledifolius*, tangential longitudinal section (TLS), torus-bearing pit membranes in narrow tracheary element, bar = 10  $\mu$ m. **11.** *Cercocarpus ledifolius*, TLS, detail of pit membrane, bar = 2  $\mu$ m. **12.** *Cercocarpus betuloides*, TLS, narrow tracheary elements with round pit apertures (left) and tori (right), bar = 5  $\mu$ m. **13.** *Celtis australis*, TLS, aspirated pit membranes with tori between narrow tracheary elements and round pit apertures (arrows), bar = 2  $\mu$ m. **14.** *Ulmus americana*, TLS, detail of a pit membrane in a narrow vessel element, bar = 1  $\mu$ m. **15.** *Zelkova serrata*, TLS, tori in a narrow tracheary element, bar = 2  $\mu$ m. **16.** *Prunus sargentii*, TLS, pit pairs between narrow tracheary elements with helical thickenings and pseudotori (arrows), bar = 10  $\mu$ m. **17.** *Kerria japonica*, TLS, pseudotori in fiber-tracheid, bar = 2  $\mu$ m. **18.** *Cotoneaster integerimma*, pseudotori (arrows) in narrow tracheary element, bar = 5  $\mu$ m. *Figure abbreviations:* A, annulus; M, margo; T, torus.

semi-ring-porous species of *Celtis* (Cannabaceae; Fig. 6), but clearly present in narrow tracheary elements in the ring-porous wood of *Celtis australis* (Figs. 7, 8), *Ulmus americana*, *Planera aquatica*, *Zelkova cretica*, and *Z. serrata* (Ulmaceae). The nature and presence of pit membrane thickenings were frequently doubtful. They were most frequently associated with pit membranes of fibers and tracheids, while intervessel pits usually had homogenous pit membranes with narrow, slit-like apertures (Fig. 9).

**SEM observations**—Two different types of pit membrane thickenings were distinguished: (1) true torus-bearing pit membranes (Figs. 10–15; Table 1) and (2) amorphous thickenings that usually do not take a central position on the pit membrane (Figs. 16–29; Table 2).

Torus-bearing pit membranes were consistently found in all species of *Cercocarpus* studied (Figs. 10–12). They were present in narrow tracheary elements, i.e., narrow vessel

elements and tracheids. Quantitative data of tori in *Cercocarpus* are summarized in Table 3. Pits in imperforate tracheary elements and outer pit apertures were circular to oval in shape (Figs. 10–12). Pit membrane pores could not be detected in the majority of torus-bearing pit membranes of *Cercocarpus* (Figs. 10, 11) and were only clearly visible in five of circa 250 pit membranes observed in more than 50 narrow tracheary elements of *C. ledifolius*. The average size of the pit membrane pore size in this species was 57 nm ( $N = 610$ ), with values ranging from 5 to 1386 nm. A size distribution of the pit membrane pore size in narrow tracheary elements of *C. ledifolius* is shown in Fig. 40. In *C. montanus* var. *paucidentatus* pores were found in the torus in no more than two pit membranes. These pores were on average 65 nm, ranging from 44 to 78 nm.

Tori were also found in narrow tracheary elements of *Celtis australis* (Fig. 13), *Planera aquatica*, *Ulmus americana* (Fig. 14), *Zelkova cretica*, and *Z. serrata* (Fig. 15), but were absent

TABLE 1. List of families and species with torus-bearing pit membranes occurring in tracheary elements within the order Rosales. Classification follows APG II (APG, 2003). Species printed in boldface have been surveyed in this study using SEM to examine for tori. Additional species for Ulmaceae and Cannabaceae are based on data from Wheeler (1983), Dute and Rushing (1990), and Jansen et al. (2004).

Family	Tori present	Tori absent
<b>Cannabaceae</b>	<i>Celtis australis</i> , <i>C. laevigata</i> , <i>C. occidentalis</i> , <i>C. reticulata</i>	<i>Celtis iguanaea</i> , <i>C. philippensis</i> , <i>C. tala</i> , <i>Gironniera celtidifolia</i> , <i>Trema lamarckiana</i>
<b>Rosaceae</b>	<i>Cercocarpus intricatus</i> , <i>C. ledifolius</i> , <i>C. montanus</i> , <i>C. traskiae</i>	All other Rosaceae species examined in this study
<b>Ulmaceae</b>	<i>Planera aquatica</i> ; <i>Ulmus alata</i> , <i>U. americana</i> , <i>U. campestris</i> , <i>U. carpinifolia</i> , <i>U. coritana</i> var. <i>coritana</i> , <i>U. cornubiensis</i> var. <i>goodgeeri</i> , <i>U. davidiana</i> var. <i>japonica</i> forma <i>suberosa</i> , <i>U. diversifolia</i> , <i>U. effusa</i> , <i>U. fulva</i> , <i>U. glabra</i> , <i>U. japonica</i> , <i>U. laciniata</i> , <i>U. macrocarpa</i> , <i>U. montana</i> , <i>U. parvifolia</i> , <i>U. pedunculata</i> , <i>U. plotii</i> , <i>U. scabra</i> , <i>U. thomasi</i> , <i>Zelkova acuminata</i> , <b><i>Z. cretica</i></b> , <b><i>Z. serrata</i></b>	<i>Ampelocera dichotoma</i> , <i>Aphananthe aspera</i> , <b><i>Hemiptelea davidii</i></b> , <i>Holoptelea integrifolia</i> , <i>Phyllostylon rhamnoides</i> , <i>Ulmus lancifolia</i> , <i>U. mexicana</i>

in all other species of Rosaceae studied as well as in *Celtis iguanaea*, *C. philippensis*, and *C. tala* (Table 1). Quantitative information about torus-bearing pit membranes is provided in Table 3. Although the torus was more or less circular in shape, its circumference was frequently irregular (Fig. 14; Table 3). The vertical torus diameter was higher than the horizontal torus diameter in all species with tori, and the circularity ratio (short axis divided by the long axis) was consistently higher for tori than for outer pit apertures (Table 3). The mean size of the tori was larger than that of the outer pit apertures (Table 3). The outline of pits with tori was circular to oval (Figs. 13, 15). Pores in the pit membrane surrounding the torus were on average 21 nm (8–49 nm range, *N* = 50) in *U. americana* and were either too small or too infrequent to be detected using SEM in the other species with torus-bearing pit membranes.

Thickenings associated with pit membranes of fibers and tracheids were found in all species of Rosaceae studied (Figs. 16–26), except for *Aruncus sylvestris*. The frequency of these thickenings varied among species. In none of the species, however, were the thickenings consistently present. The shape of the thickenings was amorphous and did not correspond to the outline of the slit-like pit aperture (Fig. 25). Moreover, the feature did not always take a central position on the pit membrane, and two thickenings were infrequently associated with a single pit membrane in most species (Figs. 17, 26). Although the eccentric position of the thickenings was inconsistent among pit pairs, the position almost always corresponded within each pit pair (Fig. 16). In many specimens a hollow structure could be seen, which appeared tongue-like or cap-like when extending from the periphery of the pit membrane and (partly or entirely) overhanging the pit membrane (Figs. 16, 19, 28). The size of the thickenings ranged from very small (0.1 μm) to circa 3 μm and varied

considerably within a single specimen. Most thickenings had a long axis between 1 and 2.5 μm. Similar pit membrane thickenings were found in narrow tracheary elements in Elaeagnaceae (Figs. 27, 28) and Rhamnaceae (Fig. 29).

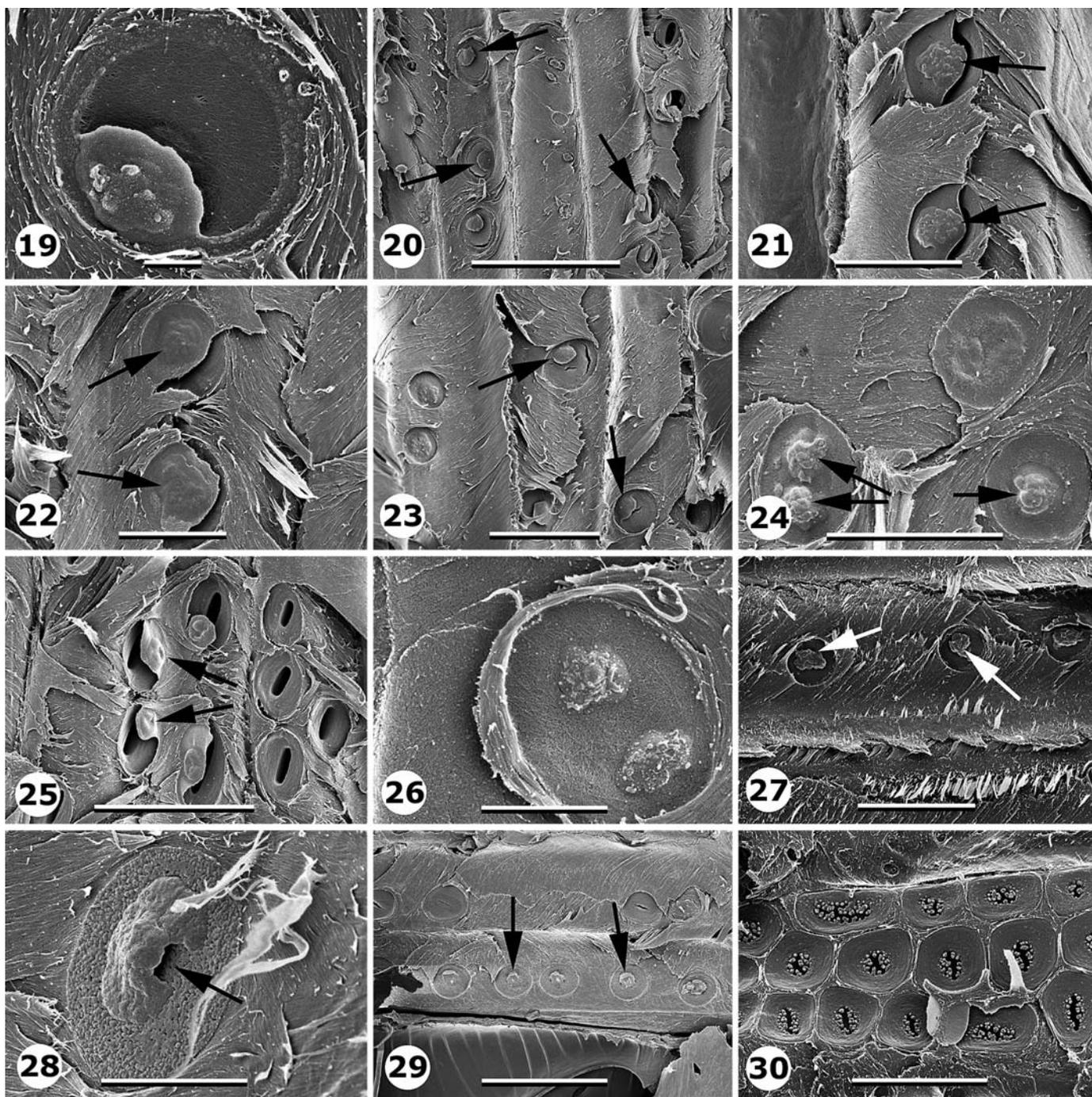
Vestured pits were observed in *Hemiptelea davidii*, both in vessels and imperforate tracheary elements (Fig. 30). Vestured pits were also common in the narrow tracheary elements of Elaeagnaceae.

**TEM observations**—The presence of torus-bearing pit membranes could be confirmed in *Cercocarpus* (Figs. 31–33). They are on average 500 nm in thickness, varying between 300 and 800 nm (*N* = 10). In pit pairs between relatively wide tracheary elements (tangential lumen diameter > 30 μm) and narrow tracheary elements (tangential lumen diameter < 30 μm), a torus thickening is deposited only on the side of the narrow tracheary element (Fig. 32). In many instances, pit membranes separating a parenchyma cell and a tracheid had a small deposit of torus material on the pit membrane surface of the tracheid. This deposit sometimes could be a very thin coating layer and easily overlooked. Intervessel pit membranes without tori were on average 130 nm (90–170 nm range, *N* = 10) in thickness. The occurrence of torus-bearing pits was also confirmed in *Ulmus americana* (Fig. 34). The thickness of tori in *U. americana* was on average 170 nm (117–256 nm range; *N* = 10).

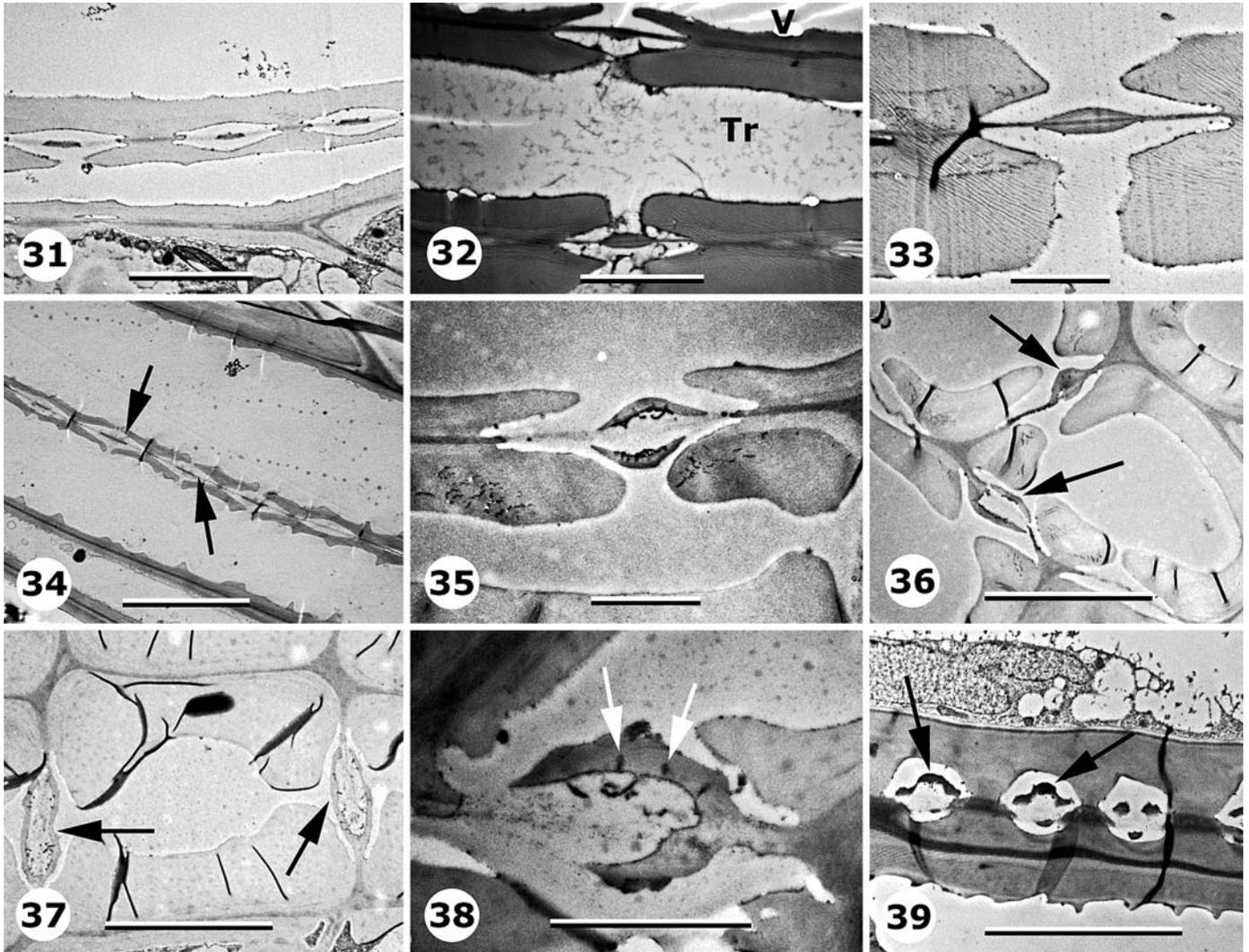
Pit membrane thickenings in narrow tracheary elements of both *Malus yunnanensis* (Figs. 35, 36) and *Rosa sikagensis* (Figs. 37, 38) showed a structure that was very different compared to true tori. The thickenings were found to be part of the primary wall containing plasmodesmata. The thickenings were mainly found in pits between fiber-tracheids, but also in pits connecting a tracheid and vessel element. Based on counts of 10 measurements, the swollen region of the primary wall

TABLE 2. List of taxa within the order Rosales showing the distribution of pseudotori associated with pit membranes in tracheary elements. Classification follows APG II (APG, 2003). All samples have been surveyed in this study using SEM to examine for pseudotori.

Family	Pseudotori present	Pseudotori absent
<b>Cannabaceae</b>	—	<i>Celtis</i>
<b>Dirachmaceae</b>	—	<i>Dirachma</i>
<b>Elaeagnaceae</b>	<i>Elaeagnus</i> , <i>Hippophae rhamnoides</i> , <i>Shepherdia</i>	<i>Hippophae salicifolia</i>
<b>Moraceae</b>	—	<i>Maclura</i>
<b>Rhamnaceae</b>	<i>Rhamnus</i>	<i>Ceanothus</i> , <i>Colletia</i> , <i>Condalia</i>
<b>Rosaceae</b>	<i>Amelanchier</i> , <i>Amygdalus</i> , <i>Chaenomeles</i> , <i>Chamaebatia</i> , <i>Cotoneaster</i> , <i>Cowania</i> , <i>Crataegus</i> , <i>Eriobotrya</i> , <i>Holodiscus</i> , <i>Kerria</i> , <i>Lindleya</i> , <i>Lyonothamnus</i> , <i>Malus</i> , <i>Mespilus</i> , <i>Micromeles</i> , <i>Neviusia</i> , <i>Photinia</i> , <i>Physocarpus</i> , <i>Potentilla</i> , <i>Prunus</i> , <i>Purshia</i> , <i>Pyracantha</i> , <i>Pyrus</i> , <i>Rosa</i> , <i>Rubus</i> , <i>Spiraea</i> , <i>Stephanandra</i>	<i>Aruncus</i> , <i>Cercocarpus</i>
<b>Ulmaceae</b>	—	<i>Ulmus</i> , <i>Hemiptelea</i> , <i>Planera</i> , <i>Zelkova</i>



Figs. 19–30. SEM images of intervacular pits in Rosaceae (Figs. 19–26) and related families (Figs. 27–30). **19.** *Prunus sargentii*, tangential longitudinal section (TLS), pit membrane of fiber with tongue-like thickening, bar = 10  $\mu$ m. **20.** *Chaenomeles speciosa*, TLS, narrow tracheary elements with pseudotori (arrows), bar = 20  $\mu$ m. **21.** *Cowania mexicana*, radial longitudinal section (RLS), pit membranes in narrow tracheary element with pseudotori (arrows), bar = 5  $\mu$ m. **22.** *Chamaebatia foliolosa*, RLS, pseudotori (arrows) associated with bordered pits of a narrow tracheary element, bar = 5  $\mu$ m. **23.** *Crataegus wilsonii*, TLS, fiber pits with pseudotori (arrows), bar = 10  $\mu$ m. **24.** *Holodiscus dumosus*, RLS, bordered pits of narrow tracheary elements with pseudotori (arrows), bar = 5  $\mu$ m. **25.** *Lyonothamnus asplenifolius*, RLS, narrow tracheary element with bordered pits, elongated pit apertures and pseudotori (arrows), bar = 10  $\mu$ m. **26.** *Stephanandra incisa*, TLS, detail of bordered pit in narrow tracheary element with two pseudotori, bar = 2  $\mu$ m. **27.** *Hippophae rhamnoides*, TLS, narrow tracheary element with pseudotori, bar = 10  $\mu$ m. **28.** *Elaeagnus argentea*, TLS, fiber pit membrane with a granular appearance and a hollow pseudotorus (arrow), bar = 3  $\mu$ m. **29.** *Rhamnus utilis*, TLS, narrow tracheary element with several pseudotori (arrows), bar = 20  $\mu$ m. **30.** *Hemiptelea davidii*, RLS, vessel outer pit apertures with small, unbranched vestures near the pit aperture, bar = 10  $\mu$ m.



Figs. 31–39. TEM pictures of intervacular pits in Rosaceae (Figs. 31–33, 35–38) and some related families (Figs. 34, 39). **31.** *Cercocarpus ledifolius*, TLS, three torus-bearing pit membranes between narrow tracheary elements, bar = 5  $\mu\text{m}$ . **32.** *C. ledifolius*, TLS, pit between a narrow tracheary element (Tr) and a vessel element (V) with a torus on the tracheid side, torus-bearing pit membrane in lowest pit between narrow tracheary elements, bar = 5  $\mu\text{m}$ . **33.** *C. ledifolius*, TLS, bordered pit with a torus between two narrow tracheary elements, bar = 2  $\mu\text{m}$ . **34.** *Ulmus americana*, TLS, torus-bearing pit membranes (arrows) between pits of narrow tracheary elements, bar = 10  $\mu\text{m}$ . **35.** *Malus yunnanensis*, TS, pit between narrow tracheary elements with pseudotorus with cap-like thickenings and plasmodesmata remnants, bar = 2  $\mu\text{m}$ . **36.** *M. yunnanensis*, TS, fiber-tracheid with two pseudotori (arrows), bar = 5  $\mu\text{m}$ . **37.** *Rosa sikagensis*, TS, fiber-tracheid with two pseudotori (arrows), bar = 5  $\mu\text{m}$ . **38.** *R. sikagensis*, TS, pit membrane with pseudotorus consisting of two cap-like (electron dense) structures, plasmodesmata remnants (arrows) and a hydrolyzed central part of the primary wall thickening, bar = 2  $\mu\text{m}$ . **39.** *Elaeagnus umbellata*, RLS, pits between narrow tracheary elements with pseudotori (arrows), bar = 10  $\mu\text{m}$ .

was on average 1260 nm and 1380 nm thick in *M. yunnanensis* and *R. sikagensis*, respectively, while pit membranes without any thickening were between 120 nm and 240 nm thick in *R. sikagensis* and between 60 nm and 130 nm in *M. sikagensis*. In most cases the pit membrane was clearly traversed by several branched plasmodesmata, which came to a broad focus at the middle lamella (Figs. 35, 37). The plasmodesmata sometimes appeared to end blindly in the pit membrane thickening in some pits and seemed to be occluded by the cap-like structure (Fig. 37), but in some cases plasmodesmata were seen crossing the cap (Fig. 38). While the central part of the pit membrane was clearly affected by the autolysis (enzymatic removal of most of the primary wall under the cap), the cap remained

dense and unaltered. In a pit membrane between a tracheary element and a parenchyma cell, the plasmodesmata were found only on the side of the former. The thickenings developed similarly on the tracheary cell side, while a protective layer formed on the parenchyma cell side. Similar observations of plasmodesmata associated thickenings were observed in *Elaeagnus umbellata* (Fig. 39).

## DISCUSSION

The observations illustrate that pit membrane thickenings are very common in tracheary elements of Rosaceae. Two types of

TABLE 3. Quantitative pit characters of the taxa studied within the Rosales showing torus-bearing pit membranes. All data are based on counts of 25 measurements for each species; minimum and maximum values are given with mean values between parentheses; average and variation range are given for the species of *Cercocarpus* and *Zelkova* studied. The mean circularity ratio represents the short axis divided by the long axis. Data for *Planera aquatica* are based on measurements according to Dute et al. (2004).

Species studied	<i>Cercocarpus intricatus</i> , <i>C. ledifolius</i> , <i>C. montanus</i> , <i>C. traskiae</i>	<i>Celtis australis</i>	<i>Planera aquatica</i>	<i>Ulmus americana</i>	<i>Zelkova cretica</i> , <i>Z. serrata</i>
Vertical torus diameter (µm)	1.2–(2.3)–3.6	1.3–(1.5)–1.7	1.7–(2.1)–2.5	1.7–(2.4)–3.2	1.8–(2.1)–2.6
Horizontal torus diameter (µm)	0.9–(2.1)–3.0	1.1–(1.3)–1.7	1.3–(1.9)–2.3	1.4–(2.3)–3.1	1.5–(1.8)–2.5
Mean circularity ratio of torus	0.70–(0.88)–1	0.70–(0.80)–0.93	0.64–(0.89)–1	0.70–(0.87)–0.96	0.67–(0.77)–0.92
Vertical diameter of outer pit aperture (µm)	1.3–(1.5)–1.7	0.7–(1.0)–1.3	0.9–(1.5)–2.0	0.8–(1.0)–1.5	0.6–(0.9)–1.4
Horizontal diameter of outer pit aperture (µm)	0.9–(1.2)–1.5	0.9–(1.4)–1.9	0.7–(1.1)–1.4	0.6–(0.9)–1.6	0.6–(1.0)–1.5
Circularity ratio of outer pit aperture	0.61–(0.80)–1	0.54–(0.70)–1	0.62–(0.76)–1	0.51–(0.77)–1	0.47–(0.79)–1
Horizontal diameter of pit chamber (µm)	3.5–(5.2)–6.7	4.2–(5.5)–5.8	3.4–(4.5)–6.5	3.3–(5.7)–7.5	3.4–(4.3)–5.8

thickenings can be distinguished: true tori and plasmodesmata-associated thickenings. Both features are rather difficult to characterize when using LM, and clear morphological differences can only be observed when using SEM and TEM. This may explain why previous studies on the wood anatomy of Rosaceae did not mention pit membrane thickenings (e.g., Zhang, 1992). Also, pit membrane thickenings seem to be more difficult to detect in wood sections that are relatively thick (20–30 µm) compared to 10 µm sections and especially semithin sections of 1–2 µm (Jansen et al., 2004). Main differences between both membrane thickenings include the distribution, shape, and association with other pit characters. The following criteria appear to be useful for the identification of tori: (1) a central position on the pit membrane, (2) a consistent distribution in pits of narrow tracheary elements (fibriform-like vessel elements, tracheids or fiber-tracheids), and (3) a correlation with round to oval pit apertures, whose diameters are also slightly less than those of tori. The relationship between tori and round to oval pit apertures seems to support their functional role in pit aspiration as has been suggested previously (Wright, 1928; Beck et al., 1982; Dute et al., 1996, 2001; Jansen et al., 2004). The second type of pit membrane thickenings is characterized by an irregular distribution and is associated with the occurrence of plasmodesmata. Genuine tori in angiosperms typically do not have plasmodesmata at any time during their ontogeny (Dute and Rushing, 1988).

**Torus-bearing pit membranes in Rosaceae and related families**—Most thickenings within the Rosaceae do not represent true tori. A new record of tori, however, was found in all species of the genus *Cercocarpus*, adding another genus and family to the list of torus-bearing angiosperms. Although this illustrates the homoplasious nature of tori in angiosperms, it is remarkable that tori seem to be restricted to a single genus within Rosaceae and that this feature is absent in related genera such as *Chamaebatia* or *Purshia*. The latter genera are suggested to belong to the same phylogenetic group as *Cercocarpus* and are often placed in the tribe Dryadeae, which used to be in the subfamily Rosoideae (e.g., Schulze-Menz, 1964). More recent studies suggest that the position of a redefined Dryadeae, which is characterized by nitrogen-fixing genera (including *Cercocarpus*, *Chamaebatia*, *Cowania*, *Dryas*, and *Purshia*), is uncertain and forms a weakly supported clade as sister to the remaining members of the family outside the Rosoideae (Evans et al., 2002; Potter et al., 2002). Although *Chamaebatia*, *Purshia*, and *Cercocarpus* include xerophytes and occur in deserts, chaparral, and

montane areas in southwest regions of the USA and northern Mexico, it is unclear why tori seem to have evolved in only one single genus within Rosaceae.

The occurrence of tori in members of Cannabaceae, Ulmaceae, and Rosaceae suggests that this feature has evolved independently in at least three different groups within Rosales (Table 1). Moreover, the mixed occurrence of species with tori and homogenous pit membranes in relatively large genera such as *Celtis* and *Ulmus* demonstrates the plasticity of this feature. Support for this is found here within the genus *Celtis*: the absence of tori in *C. iguanaea*, *C. philippensis*, and *C. tala* agrees with similar observations within *Ulmus*, suggesting that tori are absent in diffuse-porous wood from (sub)tropical areas and are largely restricted to narrow tracheary elements in the late wood of ring-porous species from areas with more distinct seasons (Jansen et al., 2004). This may explain why tori are restricted within Ulmaceae to *Planera*, *Zelkova*, and some species of *Ulmus* and do not occur in diffuse-porous genera (Dute et al., 2004). Interestingly, Ulmaceae are supposed 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 from North America to Eurasia (Manchester and

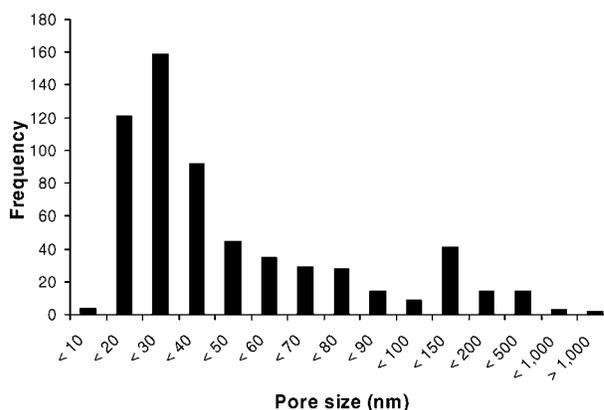


Fig. 40. Distribution of pore size measured at the broadest point in torus-bearing pit membranes of *Cercocarpus ledifolius* (Rosaceae). Measurements were based on 610 counts from five pit membranes in which pores were clearly visible using SEM. Note that (1) by far the majority of the pit membranes observed in all species of *Cercocarpus* did not show any obvious pores under SEM (see Figs. 10, 11), and (2) a wider range of pore size is used for the higher categories (150–200 nm, 200–500 nm, 500–1000 nm, >1000 nm).

Tiffney, 2001). Within Cannabaceae, *Celtis* appears to be the only genus with at least some of its species having ring-porosity (Sweitzer, 1971). Due to the strong correlation between ring-porosity and tori, it has been suggested that tori are not present in diffuse-porous genera such as *Gironniera*, *Parasponia*, *Pteroceltis*, and *Trema* (Jansen et al., 2004). However, torus-margo pits would be expected to be present in the monospecific and ring-porous genus *Hemiptelea* (Ulmaceae). Our observations, however, illustrate homogenous pit membranes for this genus and a new record of vestured pits for Ulmaceae. We are confident that the sample studied by SEM was correctly identified, because vestured pits could also be seen in LM sections of other specimens of *Hemiptelea davidii* from the Jodrell Laboratory slide collection when using the oil-immersion objective. It could be suggested that tori and vestured pits are mutually exclusive in *Hemiptelea*. Similar observations have been found within Thymelaeaceae, in which tori and vestured pits have been reported in several species of *Daphne* and *Wikstroemia*, but both features do not seem to occur in the same species (Dute et al., 1992, 1996, 2001). Additional evidence is required to verify this correlation. As far as we understand the development and chemical characterization of tori in angiosperms, we suggest, based on observations by Coleman et al. (2004), that tori in *Celtis* and *Ulmus* develop early in cell ontogeny (i.e., prior to secondary wall initiation) and are not lignified, but mainly composed on pectins and cellulose. Therefore, the absence of safranin staining of tori in species examined in this study could be caused by an absence of lignin.

**New records of pseudotori**—Pit membrane thickenings in narrow tracheary elements frequently correspond to previous observations of plasmodesmata-associated thickenings in *Pyrus*, *Prunus*, *Crataegus*, and *Sorbus* (Parameswaran and Liese, 1981; Barnett 1982, 1987a, b; Lachaud and Maurousset, 1996). We suggest describing these structures as “pseudotori” because they may give the false impression of tori under the light microscope. In addition to their irregular distribution, pseudotori are usually thicker than tori and are not associated with circular or oval pit apertures. Moreover, this feature is not similar to encrustations or secondary changes that are associated with pit membranes in the inner sapwood or heartwood of some angiosperms and gymnosperms (Wheeler, 1981, 1983; Sano and Fukazawa, 1994; Sano and Nakada, 1998). These secondary deposits are thought to be composed of lignified or phenolic components, while pseudotori are formed in differentiating tracheary elements prior to removal of the cytoplasm. Indeed, previous work by Barnett (1987a, b) and Lachaud and Maurousset (1996) on the ontogeny of this feature illustrated that the hollow, cap-like structure of pseudotori is the result of hydrolysis of the central part of the primary wall thickening, which takes place at the end of cell maturation.

Besides Rosaceae, Elaeagnaceae, and Rhamnaceae, there are observations of pseudotori in Grossulariaceae (Parameswaran and Liese, 1973), Oleaceae (Parameswaran and Gomes, 1981), and various Ericaceae (Rabaey et al., 2006). *Cercocarpus* and *Aruncus* appear to be the only genera of 29 genera of Rosaceae studied that do not show any pseudotori. In case of *Cercocarpus*, this can be explained by the presence of tori. The absence of pseudotori in *Aruncus sylvestris* is likely due to the absence of fibers with distinctly bordered pits in this predominantly herbaceous species. This may suggest a correlation between pseudotori and (fiber-)tracheids.

**Functional aspects of pit membrane thickenings**—It is known that torus-bearing pit membranes are more efficient in optimizing a safety vs. efficiency trade-off. Indeed, torus-margo pits in conifers are found to achieve on average 59 times lower flow resistance per membrane area than homogenous pit membranes, and this advantage of torus-bearing pit membranes is even greater at a higher pressure drop across the membrane (Hacke et al., 2004, 2005; Sperry and Hacke, 2004; Pittermann et al., 2005; Wheeler et al., 2005). Because xylem elements with torus-bearing pit membranes in gymnosperms are unicellular tracheids, which are much shorter than a vessel, the occurrence of tori in gymnosperms can be explained as a mechanism to compensate for their short tracheid length. With respect to tori in the latewood of certain angiosperm species, however, these elements do not have pit membranes with a porous margo as in most gymnosperms. Indeed, with respect to the hydraulic conductivity of a pit, the torus is not as important as the porous margo in providing the flow resistance advantage. Most SEM observations indicate that pores in the margo of angiosperm pit membranes cannot be clearly distinguished and are likely to be as narrow as in homogenous pit membranes. Because membrane conductivity declines sharply with narrower pores required for higher air-seeding pressure, this would mean that the conductive capability of narrow torus-bearing elements in angiosperms is rather limited. Only a few pits of *Cercocarpus ledifolius* are found to have pores in the pit membrane surrounding the torus. Although the largest pores in this species are more than 1000 nm, 88% and 69% of all pores are smaller than 100 nm and 50 nm, respectively (Fig. 40), illustrating that relatively large pores are very rare. It has also been suggested that in some cases SEM observations of pores may not be accurate because of artifactual increases in pore size during SEM preparation, especially from dehydration, coating, and observation under vacuum (Choat et al., 2003, 2006). Observation of wet pit membranes using atomic force microscopy or physiological experiments using fresh material would be required for more detailed measurements (Choat et al., 2003, 2004; Pesacreta et al., 2005).

In addition, torus-bearing pit membranes in angiosperms are found both in tracheids as well as in narrow “fibriformlike” vessel elements, which have one or two very small (simple) perforation plates. It could be suggested that latewood tracheary elements are more adaptive in providing greater membrane strength in narrow vessels and tracheids with a lower cavitation vulnerability and greater air-seeding pressure compared to homogenous pit membranes. This additional strength would especially be functional across the pit aperture where a stretched pit membrane is not supported by the pit border. Why then are tori not also in the hydraulically important earlywood? This remains an intriguing question that is yet to be resolved. Dute et al. (1996, 2001, 2004) suggested that narrow latewood elements with tori serve as a backup water-conducting system to wider, earlywood vessels. Narrow latewood elements in ring-porous wood of *Ulmus americana* appear functional in up to 4-yr-old wood, while the wider and more hydraulically significant earlywood vessels function only for one growth season (Ellmore and Ewers, 1986). Further experimental work on torus-bearing angiosperms is required to test functional ideas with respect to tori. Interestingly, the plasticity associated with the distribution of tori in angiosperms seems to correspond to some extent with the variation found in gymnosperms because it is known that the size and number of margo pores and the thickness of the torus in gymnosperm pit membranes may vary within a growth ring and may change according to the height

and position of tracheids in gymnosperms (Bauch et al., 1972; Sano et al., 1999; Domec et al., 2006).

So far, the function of pseudotori remains unknown. Because pseudotori are strictly correlated with the distribution of plasmodesmata, it seems reasonable to assume that their function is related to the role of plasmodesmata during and/or after cell differentiation and that their function may change with different stages of cell development (Lachaud and Maurousset, 1996). Plasmodesmata are suggested to allow for communication and transport of water, various nutrients, and other molecules between plant cells (Epel, 1994; Oparka and Roberts, 2001; Haywood et al., 2002; Roberts and Oparka, 2003). Pits have been thought for a long time to occur in areas of primary walls that are pierced by plasmodesmata and which are known as primary pit fields (Kerr and Bailey, 1934). It has been illustrated, however, that plasmodesmata are not essential for pit formation in tracheids and vessels, while their presence in developing pit membranes of fibers and parenchyma cells has been found in many species (Barnett and Harris, 1975; Yang, 1978; Barnett 1981, 1982). Because some of the plasmodesmata observed in Rosaceae end blindly in the wall thickening, this shows that they do not prevent deposition of secondary wall over the pit membrane. Alternative ideas regarding the function of the pseudotori are an increased resistance to attack by enzymes, resulting probably in the retention of the protoplast. Evidence for this idea could be found in the apparent correlation between living or nucleated fibers and the distribution of pseudotori. The formation of a resistant cap is suggested to block off plasmodesmata and to prevent passage of autolytic enzymes from a dying cell to its living neighboring cells (Barnett, 1987b). Lachaud and Maurousset (1996), however, noticed that some plasmodesmata in *Sorbus torminalis* appeared to perforate the cap-like structure and that the cap does not seem to seal the plasmodesmata.

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APPENDIX 1. List of specimens investigated with reference to their origin and collector. A dash indicates information was not available. Specimens were obtained from the following institutes: AHUC = UC Davis Herbarium, AUA = John D. Freeman Herbarium, BR = National Botanic Garden of Belgium, DAV = UC Davis Herbarium, K = Royal Botanic Gardens Kew, Tw = wood collection of the Museum for Central Africa (Tervuren).

**Taxon**—Herbarium, Voucher, Geographic origin.

**Cannabaceae**—*Celtis australis* L.—BR, Bornmüller 1807, Kurdistan, Mount Kuh-Sefin. *C. iguanaea* (Jacq.) Sargent—BR, Marismas et al. 704, Mexico. *C. philippensis* Blanco—BR, Mangold 2134, New Guinea, Res. Manokwari, Oransbari. *C. tala* Gillies ex Planch.—BR, Pedersen 16224, Uruguay, Tucuaembo.

**Dirachmnaceae**—*Dirachma socotrana* Schweinf. ex I.B.Balfour—K, Smith & Lavros 210 and 593, People's Democratic Republic of Yemen, Socotra.

**Elaeagnaceae**—*Elaeagnus argenta* Pursh—Tw35080, Forstl. Bundesversuchsanstalt Vienna 0200, Austria. *E. conferta* Roxb.—BR, Xing et al. 042, China, Guangzhou. *E. latifolia* Thunb.—BR, Ngong T in Lo Shan & Taam 330, China. *E. orientalis* L.—BR, V. Vašák BR-S.P. 808 003, Russia, Gugarac, Dilishan. *E. umbellata* Thunb.—Tw43308, Forest Research Institute 554, South-Korea. **Hippophae rhamnoides** L.—Tw47246, —, Poland. *H. salicifolia* D. Don—BR, Kathi et al. 2, India, Kumaon. *Shepherdia argentea* (Pursh) Nutt.—Tw21656, Sulerud 413, USA. *S. canadensis* (L.) Nutt.—Tw30305, Thieret 4871, Canada.

**Moraceae**—*Maclura pomifera* (Raf.) C.K.Schneid.—BR, Moldenke 12304, USA, Ohio, Wills Creek, Coshocton Co.

**Rhamnaceae**—*Ceanothus fendleri* A.Gray—BR, Dechamps 4242, USA, California, N.E. of Middletown. *Colletia weddelliana* Miers—BR, Bang 793, Bolivia, Talca Chugiaguillo. *Condalia spathulata* A. Gray—Tw42521, —, USA. *Rhamnus utilis* Decaisne—BR, Hers 413, China, Tsi Ling Ping, Ju Hu Lu Tsi.

**Rosaceae**—*Amelanchier canadensis* M.Roem—BR, A. Taton 2109, France, Gréolières. *Amygdalus spartioides* Spach—BR, Polunin & Khudairi 56, Iraq, Zawita, Mosul Liwa. *Aruncus sylvestris* Kostel.—BR, Lamb 1142, USA, Western Washington Chehalis County. *Cercocarpus betuloides* Nutt.—AUA, Crampton 7776, USA, California, Mendocino County. *C. betuloides* Nutt.—K, Clokey & Templeton 4596, USA, California, Mandeville Canyon, Santa Monica Mountains. *C. intricatus* S.Wats—DAV, Levin 1248, USA, California, San Bernardino County. *C. ledifolius* Nutt.—K, Cult., accession number 1980-6418. *C. montanus* Raf.—K, Baker et al. 392, USA, Southern Colorado, foothills south of Mancos. *C. montanus* Raf. var. *argenteus* (Rydb.) F.L.Martin—AUA, Drost 72, USA, New Mexico, Taos County. *C. montanus* Raf. var. *paucidentatus* (S.Wats.) F.L. Martin—K, Rehder 397, USA, Fresno Canyon, Sacramento Mountains. *C. montanus* Raf. var. *paucidentatus* (S.Wats.) F.L. Martin—K, Rehder 56, USA, Arizona, Oak Creek Canyon. *C. traskiae* Eastw.—AHUC, Crampton s.n., USA, California, Yolo County. *Chaenomeles speciosa* Nakai—K, Cult., accession number 1996-225. *Chamaebatia foliolosa* Benth.—K, Bigelow s.n., USA.

*Cotoneaster integerrimus* Medik.—BR, Gosseye s.n., Switzerland, Grisons, Roua. *C. microphyllus* Lindl.—K, Cult., accession number 1950-10604. *Cowania mexicana* D.Don—K, Lester s.n., USA, Arizona, Colorado Plateau. *C. mexicana* D.Don—K, Leemam 2176, Mexico, Sierra Madre, N.W. of Mexico. *Crataegus wilsonii* Sarg.—K, Cult., accession number 1923-6105. *Eriobotrya japonica* Lindl.—K, Cult., accession number 1996-436. *Holodiscus dumosus* (Nutt. ex Hook.) A.Heller—BR, Dechamps 4041, USA, New Mexico, National Park of Magdalena, Socorro. *H. dumosus* (Nutt. ex Hook.) A.Heller—BR, Lamb 4, USA, 10 miles E of Santa Fe. *Kerria japonica* DC.—Japan, Cult. at the Botanic Garden of Hokkaido University, Sapporo. *Lindleya mespiloides* Kunth—BR, Pringle 2310, BR-S.P. 863 731, Mexico, State of Coahuila. *Lyonothamnus asplenifolius* Greene—K, Cult., accession number 166-91.0084. *Malus yunnanensis* C.K. Schneid.—K, Cult., accession number 1995-1554. *Mespilus germanica* L.—K, Cult., accession number 1998-4111; *Micromeles caloneura* Stapf—K, Cult., accession number 1985-8400; *Neviusia alabamensis* A.Gray—K, —, USA, Cult. in Madison, Wisconsin. *Photinia serratifolia* (Desf.) Kalkman—BR, Lu 18138, BR-S.P. 920 350, Taiwan, Hualien County, Chingshuishan. *Physocarpus opulifolius* (L.) Maxim.—K, Cult., accession number 1973-21761. *Potentilla fruticosa* L.—K, Cult., accession number 239-12.23906. *Prunus sargentii* Rehder—Japan, natural forest of the Tomakomai Experimental Forest of Hokkaido University. *P. virginiana* L.—BR, Epling et al. s.n., USA, California, San Diego County, Palomar Mountain. *Purshia tridentata* DC.—K, —, USA, Oregon. *Pyracantha fortuneana* (Maxim.) H.W.Li—K, Cult., accession number 2001-1602. *Pyrus takhtadzhianii* Fed.—BR, Vašák s.n., Caucasus, Montes "Gegamski khrebet," distr. Ararat. *Rosa sikangensis* T.T.Yu & T.C. Ku—K, Cult., accession number 1994-854. *Rubus crataegifolius* Bunge—K, Cult., accession number 308-55.30801. *Spiraea dumosa* Nutt. ex Hook.—BR, Dechamps 4041, USA, New Mexico, National Park of Magdalena, Socorro. *S. dumosa* Nutt. ex Hook.—BR, Lamb 4, USA, 10 miles E of Santa Fe. *S. japonica* Raf.—K, Cult., accession number 1995-759. *Stephanandra incisa* Zabel—K, Cult., accession number 2004-2112.

**Ulmaceae**—*Hemiptelea davidii* (Hance) Planch.—BR, Cult., BR-S.P. 808894. *Planera aquatica* J.F.Gmel.—BR, Leonard & Radford 1226, USA, South Carolina, Richland County, Swamp forest at Congaree River. *Ulmus americana* L.—BR, Thompson et al. 2256, USA, Pennsylvania, ESE of Majorsville, West Virginia. *Zelkova cretica* Spach.—BR, Reverchon 264, Crete, Amalos. *Z. serrata* Makino—BR, Cult., BR-S.P. 808 858.