



Title	Homoplastic occurrence of perforated pit membranes and torus-bearing pit membranes in ancestral angiosperms as observed by field-emission scanning electron microscopy
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1       **Title:**

2           Homoplastic occurrence of perforated pit membranes and torus-bearing pit  
3           membranes in ancestral angiosperms as observed by field-emission scanning  
4           electron microscopy

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22  
23       **Key words:**

24          pit membrane, interfiber pit, torus, wood fiber, angiosperm

25  
26       **Footnote:**

27          Part of this study was presented at the 60th Annual Meetings of the Japan Wood  
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## 1       **Abstract**

2           Recent studies demonstrated that perforated pit membranes (i.e. pit membranes  
3       with a large opening in their central portion) are commonly present between wood  
4       fibers in core eudicots. It is unclear whether this type of pit membranes might also  
5       occur in ancestral angiosperms. Therefore, structure of interfiber pit membranes  
6       was examined by field-emission scanning electron microscopy in nine species  
7       representing seven families that are located at more ancestral position than core  
8       eudicots. We found perforated pit membranes in three of the nine species. Our  
9       observations indicate that perforated pit membranes are relatively common even in  
10      ancestral groups of angiosperms. In the non-perforated pit membranes of the other  
11      six species, we found a range of structural variations. Thin-walled pit membranes  
12      without apparent intercellular layers were always found in three of the six species  
13      and the porosity of sheet-like pit membranes differed among the three species.  
14      Unlike the thin-walled pit membranes, interfiber pit membranes of *Buxus*  
15      *microphylla* var. *japonica* were thick-walled with obvious intercellular layers, and  
16      in *Schisandra chinensis*, we often observed torus-bearing pit membranes. Such  
17      variations in layered structure of pit membranes and homoplastic occurrence of  
18      torus-bearing pit membranes have not yet been reported for ancestral angiosperms.  
19      Our observations indicate that the structure of interfiber pit membranes might be  
20      more complicated than previous studies might suggest.

## 1       **Introduction**

2           Pit pairs are pathways that allow water to flow between cells in wood. A thin  
3 partition, known as the pit membrane, is present in each pit pair and divides one  
4 cell from an adjacent cell. The structures of pit membranes have a major effect on  
5 the movement of water in living trees and on the permeability of woods. Therefore,  
6 the structure of pits has been the focus of considerable attention. Our  
7 understanding of pit structures increased dramatically with the advent of electron  
8 microscopy. Since the structure of pits was first revealed by the pioneers of  
9 transmission electron microscopic studies of wood structures, most notably Harada  
10 et al. [1], Côté [2] and Schmid [3], large amounts of information have been  
11 accumulated and, as a result, the structure of pits in wood are fairly well  
12 understood. In recent years, there have been many studies of the functions of  
13 intervessel pit membranes of angiosperms and the intertracheary pit membranes of  
14 gymnospermae [e.g. 4-7]. However, attention has still been concentrated on only a  
15 few types of pits and our understanding of the structure and function of certain  
16 types of pits, such as the interfiber pits in angiosperms, remains limited and  
17 fragmentary.

18           Perforated pit membranes between wood fibers were found in some  
19 angiosperms [8, 9] after they had already been reported in *Carya tomentosa* [10]  
20 and *Fraxinus mandshurica* var. *japonica* [11], but this type of pit membrane was  
21 considered to be relatively rare and attracted scant attention. However, recent  
22 studies revealed that (1) perforated pit membranes are common in various taxa of  
23 angiosperms and (2) wood fibers with perforated pit membranes are  
24 non-conductive while wood fibers with sheet-like pit membranes are conductive [8,  
25 9, 12]. In previous studies, the selection of species was limited to core eudicots. It  
26 remained to be determined whether such pit membranes might also be common in  
27 taxonomic groups that are located at more ancestral positions in the angiospermous  
28 clade. Therefore, as described in this report, we examined the structural variations  
29 in interfiber pit membranes of nine species that represented seven families, chosen  
30 from among so-called basal angiosperms and basal eudicots.

## Materials and Methods

### Wood samples

Nine species were selected on the basis of their positions on a phylogenetic tree according to Angiosperm Phyllogeny Group (Fig. 1) [13] and the availability of material. The origins of the materials used are listed in Table 1. Discs of stems or blocks of the outer layer of sapwood were taken from one or two trees of each species. The specimens were stored in 30% ethanol either after fixation in FAA (a mixture of 37% aqueous solution of formaldehyde, acetic acid and 50% ethanol; 7:3:90, v/v) or without such treatment. We found no apparent differences in terms of the structure of interfiber pit membranes between specimens that had been treated with FAA and those that had not been treated with FAA prior to storage in 30% ethanol.

### Field-emission scanning electron microscopy

Specimens were prepared by two techniques. For observations of the surface of pit membranes, we trimmed wood into blocks of approximately  $5 \times 5 \times 5 \text{ mm}^3$  after rinsing in distilled water while wood was still wet. We dehydrated the blocks in a graded ethanol series and air-dried them on the laboratory bench. Then, the wood blocks were split longitudinally along a radial or a tangential plane. To allow observations of complementary images of a divided pit pair, each pair of split samples was mounted on the same aluminum stub with electron-conductive carbon paste.

Sectional views of interfiber pits were also recorded since it was often difficult to confirm the nature of pit pairs on a longitudinal split face. For such observations, specimens were prepared by the method devised by Yumoto et al. [14]. In brief, cubic blocks of approximately  $3 \times 3 \times 3 \text{ mm}^3$  were cut from wood and embedded in methacrylate resin (a mixture of n-butyl methacrylate and methyl methacrylate, 1:1, v/v). A transverse or a tangential plane was exposed on an ultramicrotome with a glass knife. Then the methacrylate resin was removed by

1 immersion in acetone and the specimen was air-dried.

2 Both types of specimen were coated with platinum by vacuum evaporation and  
3 examined with a field emission scanning electron microscope (JSM-6301F; Jeol,  
4 Akishima) at an accelerating voltage of 2.5 kV.

#### 5 6 Anatomical measurements

7 Diameters of interfiber pit membranes were measured for an examination of the  
8 relationship between the type of pit membrane and its size. Twenty-five pits were  
9 selected at random, and vertical and horizontal diameters of each pit membrane  
10 were measured on the monitor of the microscope under the same conditions as  
11 observations of specimens had been made after exposure of pits by splitting. For  
12 species in which we found different types of interfiber pit membranes, we  
13 calculated the frequency of each type of pit membranes. Such calculations were  
14 based on observations of 25 to 100 pit membranes.

#### 15 16 Terminology

17 Classification of the various types of imperforate tracheary elements has been a  
18 matter of debate [15-18] and no consensus has yet been reached [9, 19]. Therefore,  
19 we use the generic term 'wood fiber' to include various types of imperforate  
20 tracheary elements, e.g. fibers with distinctly or minutely bordered pits according  
21 to IAWA Committee [19], or libriform fibers, fiber-tracheids and tracheids  
22 according to IAWA Committee on nomenclature [20, 21].

#### 23 24 **Results**

25 In each species examined, wood fibers were monomorphic in terms of the  
26 extent of pit borders and pit density, which have been traditionally considered as  
27 characters for distinguishing fiber types, except for *Meliosma myriantha*  
28 (Awabuki).

#### 29 Species with perforated pit membranes

30 Perforated pit membranes were found in three of nine species examined (Table

1) The frequency of perforated pit membranes differed among species. In *Cinnamomum camphora* (Kusunoki), approximately 20% of the interfiber pit membranes were perforated (Figs. 2 and 3), while the remaining 80% were densely packed with cell wall materials without any visible openings. In *M. myriantha* (Awabuki), dead fibers and septate fibers coexisted. Perforated pit membranes were occasionally visible between the dead fibers (Figs. 4-6) while pit membranes without visible openings were always present between septate fibers. The frequency of perforated pit membranes between dead fibers was approximately 20%. In *Magnolia obovata* (Ho'onoki), more than 90% of the interfiber pit membranes were perforated (Figs. 7 and 8).

#### Species without perforated pit membranes

In six of the species examined, there were no perforated pit membranes but inter- and intraspecific variations in structure were found among the non-perforated pit membranes.

In *Euptelea polyandra* (Fusazakura), *Illicium anisatum* (Shikimi) and *Platanus* × *acerifolia* (Momijibasuzukakenoki), sheet-like pit membranes were always present between wood fibers (Table 1; Figs. 9-11). These pit membranes were thin-walled and lacked apparent intercellular layers. The porosity of the pit membranes differed among these species. In *E. polyandra* (Fusazakura), microfibrils were evenly deposited throughout the entire region, and minute openings of up to 50 nm in diameter were evenly distributed (Fig. 9). By contrast, microfibrils were sparsely deposited in small areas of the sheet-like pit membranes and openings of up to 200 nm in diameter were found in these areas in some of the interfiber pit membranes of *I. anisatum* (Shikimi) and *P. × acerifolia* (Momijibasuzukakenoki) (Figs. 10 and 11). Such small porous zones tended to be located near the periphery of the pit membranes in *I. anisatum* (Shikimi) (Fig. 10) but no similar tendency was evident in *P. × acerifolia* (Momijibasuzukakenoki) (Fig. 11). The frequency of interfiber pit membranes with these small porous zones was approximately 50% in *I. anisatum* (Shikimi) and approximately 30% in *P. ×*

1 *acerifolia* (Momijibasuzukakenoki).

2 Sheet-like pit membranes were also observed between wood fibers of *Buxus*  
3 *microphylla* var. *japonica* (Tsuge) and *Lindera triloba* (Shiromoji) (Figs. 12-17).  
4 Unlike the thin-walled pit membranes of the abovementioned three species, the  
5 interfiber pit membranes in these two species were densely packed with cell wall  
6 materials and no openings were visible (Figs. 12, 13 and 16). We observed  
7 intercellular layers in *B. microphylla* var. *japonica* (Tsuge) when we examined  
8 sections from this species (Fig. 14, arrow) but no intercellular layers were apparent  
9 in *L. triloba* (Shiromoji) (Fig. 15). In *L. triloba* (Shiromoji), pit membranes were  
10 often covered with granular and/or amorphous material (Figs. 16 and 17).

11 In *Schisandra chinensis* (Chosengomishi), torus-bearing pit membranes were  
12 commonly found between wood fibers (Figs. 18-20). Such torus-bearing pit  
13 membranes were frequently aspirated, with one side of the pit aperture being  
14 tightly sealed by tori (Fig. 20). The porosity of the margo region varied among  
15 individual pit membranes, and small porous zones resembling those in *I. anisatum*  
16 (Shikimi) were occasionally found. In *S. chinensis* (Chosengomishi), sheet-like or  
17 mesh-like pit membranes without tori were also present (Figs. 21 and 22).

#### 18 19 Diameter of pits

20 The diameters of pits with perforated pit membranes were consistently small  
21 while those of pits with non-perforated pit membranes were variable (Table 1 and  
22 Fig. 23). In *S. chinensis* (Chosengomishi), diameters of torus-bearing pit  
23 membranes were larger than those of pit membranes without tori (Table 1 and Fig.  
24 23). In *C. camphora* (Kusunoki) and *M. myriantha* (Awabuki), diameters of  
25 perforated pit membranes were similar to those of pit membranes without visible  
26 openings (Table 1 and Fig. 23).

27 The results of this study are summarized in Fig. 23.

#### 28 29 **Discussion**

30 The presence of perforated pit membranes between wood fibers has been



1 demonstrated in several taxa of core eudicots (Table 1) [8-12]. The present study of  
2 more ancestral groups of angiosperms revealed that perforated pit membranes  
3 between wood fibers also exist in such ancestral groups, although their frequency  
4 and morphology varied among species examined. Our results suggest that the  
5 presence of perforated pit membranes is not unusual even in ancestral groups of  
6 angiosperms. It is likely that this feature exists and will be found in more and more  
7 species from basal to derived groups of angiosperms.

8 According to previous studies, perforated pit membranes tend to occur in  
9 smaller interfiber pits while homogeneous pit membranes tend to occur in larger  
10 interfiber pits [8, 9]. A similar trend was noted in the case of perforated pit  
11 membranes in the present study. By contrast, it was obvious that homogeneous pit  
12 membranes were not exclusively present in larger pits. Densely packed pit  
13 membranes without any visible openings were always found in *Buxus microphylla*  
14 var. *japonica* (Tsuge) and *Lindera triloba* (Shiromoji) which have very small  
15 interfiber pits with an average vertical diameter of less than 2  $\mu\text{m}$ . Moreover, the  
16 frequency of perforated interfiber pit membranes was lower than in *Magnolia*  
17 *obovata* (Ho'onoki) in both *Cinnamomum camphora* (Kusunoki) and *Meliosma*  
18 *myriantha* (Awabuki), in which diameters of pit membranes are smaller than in  
19 those in *M. obovata* (Ho'onoki). Clearly, the relationship between the type of pit  
20 membrane and the pit diameter is not straightforward.

21 The present study also indicated new finding of phylogenic distribution of  
22 torus-bearing pit membranes. To our knowledge, our observation of torus-bearing  
23 pit membranes in *Schisandra chinensis* (Chosengomishi) is the first record of this  
24 feature in this taxonomic group. Torus-bearing pit membranes in angiospermous  
25 species were first reported by Ohtani and Ishida [22], who found them in species in  
26 the genera *Daphne* (Thymelaceae) and *Osmanthus* (Oleaceae). To date, this kind of  
27 pit membrane has been found in species that belong to Cannabaceae, Oleaceae,  
28 Rosaceae, Thymelaceae and Ulmaceae [23-25]. All these five families are located  
29 within the taxonomic group of core eudicots (Fig. 1). The presence of torus-bearing  
30 pit membranes in basal angiosperms has not previously been reported [26].

1 However, it is not surprising that this feature is found in basal angiosperms because  
2 of the homoplastic nature of tori in angiosperms [27].

3 In view of recent examinations of the anatomical features of imperforate  
4 tracheary elements that are associated with their conductive properties, we can  
5 postulate that the wood fibers of the three species with sheet-like pit membranes  
6 contribute to water conduction while the wood fibers of the three species with  
7 perforated pit membranes do not [9]. Indeed, it has been reported that all or some  
8 of the xylem elements that surround vessels contribute to water conduction in  
9 *Euptelea polyandra* (Fusazakura), *Illicium anisatum* (Shikimi) and *L. triloba*  
10 (Shiromoji), whereas such xylem elements do not contribute to water conduction in  
11 *M. myriantha* (Awabuki) and *Magnolia obovata* (Ho'onoki) [28, 29]. Wood fibers  
12 of *B. microphylla* var. *japonica* (Tsuge) and *S. chinensis* (Chosengomishi) might  
13 contribute to water conduction but no relevant data are available, to our knowledge.  
14 In these two species, pit pairs were commonly found between vessel elements and  
15 wood fibers (data not shown). This anatomical feature is also closely associated  
16 with the conductive nature of wood fibers [9].

17 Wood fibers with perforated pit membranes are dehydrated after the cells are  
18 formed, whereas wood fibers with sheet-like pit membranes retain water in their  
19 lumina after formation [9]. It is likely that the presence of larger pores in the  
20 perforated pit membranes is an important factor of the dehydration, because the  
21 larger the pores of pit membranes, the more easily cavitation progresses from a  
22 cavitated cell to a water-filled cell, according to air-seeding [30]. The dehydration  
23 from lumina of wood fibers obviously results in reduction in weight of tree body.  
24 This is advantageous to relieve mechanical stresses like strong winds. Perforated  
25 pit membranes between wood fibers might help trees to grow higher.

26 Carlquist [31] regarded wood fibers of *Schisandra* species as "tracheids"  
27 according to IAWA Committee on nomenclature [20, 21] because their lumina are  
28 wide, their pits are large, and the pit borders are extended. Wood fibers of *E.*  
29 *polyandra* (Fusazakura) and *I. anisatum* (Shikimi) are also regarded as tracheids  
30 because they also have similar morphological features of those of *S. chinensis*

1 (Chosengomishi). By contrast, wood fibers of *L. triloba* (Shiromoji) are regarded  
2 as libriform fibers according to IAWA Committee on nomenclature [20, 21] on the  
3 basis of the morphological features of their pits. In spite of this classification based  
4 on morphological features, it is indicated that wood fibers of *E. polyandra*  
5 (*Fusazakura*), *I. anisatum* (Shikimi) and *L. triloba* (Shiromoji) are water  
6 conductive [28]. The relationship between the morphology of pits and conductive  
7 capacity in wood fibers of vessel-bearing angiosperms might be rather complicated.  
8 More information about the structural variations among fiber pits and their  
9 association with conductive capacity might clarify issues related to the  
10 classification of wood fibers.

11 In the literature, anatomical descriptions of the wood fibers in *M. obovata*  
12 (Ho'onoki) are inconsistent. Some sources note that septate fibers are present [32]  
13 while others do not mention such fibers [33, 34]. Alternatively, Saiki [35] regarded  
14 septa that are found in wood fibers of *M. obovata* (Ho'onoki) as tyloses on the basis  
15 of their morphological features and infrequent occurrence in sapwood. In the  
16 present study, we found neither septa nor tylosis-like partition in wood fibers of *M.*  
17 *obovata* (Ho'onoki) during our observations by scanning electron microscopy.  
18 Therefore, we should not regard this feature as important in the identification of  
19 the wood of this species. Careful studies using sapwood and heartwood from  
20 various are needed to reveal the true nature of the septa found in wood fibers of *M.*  
21 *obovata* (Ho'onoki).

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27

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## 21 **Legends to Figures**

22 **Fig. 1** Phylogeny of angiosperms from the Angiosperm Phylogeny Group (APG III  
23 2009), showing the taxonomic groups that include species with each type of pit  
24 membrane. Underlining indicates groups that include species examined in the  
25 present study. Circles and triangles indicate groups that include species with  
26 perforated pit membranes and species with torus-bearing pit membranes,  
27 respectively. Black symbols indicate groups that were examined for the first time  
28 in the present study and white symbols indicate previously examined groups.  
29 Abbreviations in parentheses are those given in the legend to Table 1. Details of  
30

1 monocots are omitted.

2  
3 **Figs. 2 & 3** A complementary pair of fractured planes between wood fibers of  
4 *Cinnamomum camphora* (Kusunoki). **Figs. 4 & 5** A complementary pair of  
5 fractured planes between wood fibers of *Meliosma myriantha* (Awabuki). **Fig. 6**  
6 Cross-sectional view of an interfiber pit of *M. myriantha* (Awabuki). **Figs. 7 & 8** A  
7 complementary pair of fractured planes between wood fibers of *Magnolia obovata*  
8 (Ho'onoki). **Figs. 9 - 11** Interfiber pit membranes of *Euptelea polyandra*  
9 (Fusazakura), *Illicium anisatum* (Shikimi) and *Platanus × acerifolia*  
10 (Momijibasuzukakenoki), respectively. Scale bars in Figs 2 through 11 indicate 1  
11  $\mu\text{m}$ .

12  
13 **Figs. 12 & 13** A complementary pair of fractured planes between wood fibers of  
14 *Buxus microphylla* var. *japonica* (Tsuge). **Figs. 14 & 15** Cross-sectional views of  
15 an interfiber pit of *B. microphylla* var. *japonica* (Tsuge) and *Lindera triloba*  
16 (Shiromoji), respectively. An arrow indicates intercellular layer. **Figs. 16 & 17** A  
17 complementary pair of fractured planes between wood fibers of *L. triloba*  
18 (Shiromoji). **Figs. 18 - 22**. Interfiber pits of *Schisandra chinensis* (Chosengomishi).  
19 **18** Torus-bearing pit membranes. **19 & 20** Cross-sectional views of an intact and an  
20 aspirated interfiber pit membrane, respectively. **21 & 22** A complementary pair of  
21 fractured planes between wood fibers showing mesh-like pit membranes without  
22 tori. Scale bars in Figs 12 through 22 indicate 1  $\mu\text{m}$ .

23  
24 **Fig. 23** Average vertical diameters of each type of pit membranes ( $n = 25$ ). Black  
25 circles, data from the present study; white circles, data from previous studies (Sano  
26 and Jansen 2006, Sano et al. 2011). A, sheet-like pit membranes; B, sheet-like pit  
27 membranes without apparent intercellular layers (including pit membranes without  
28 visible openings in *Mm* and *Cc*); C, sheet-like pit membranes with apparent  
29 intercellular layers; D, torus-bearing pit membranes; E and F, perforated pit  
30 membranes. For abbreviations, see legend to Table 1.

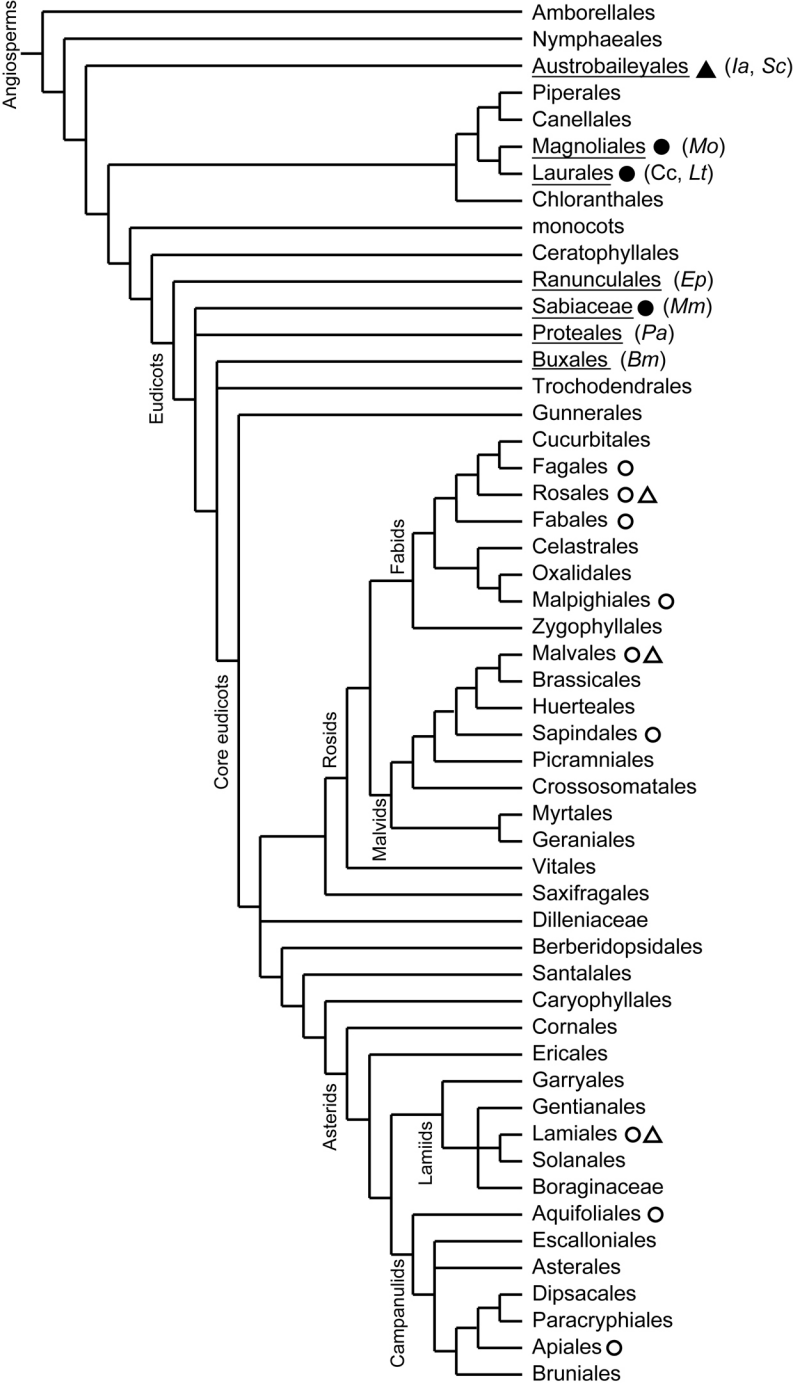
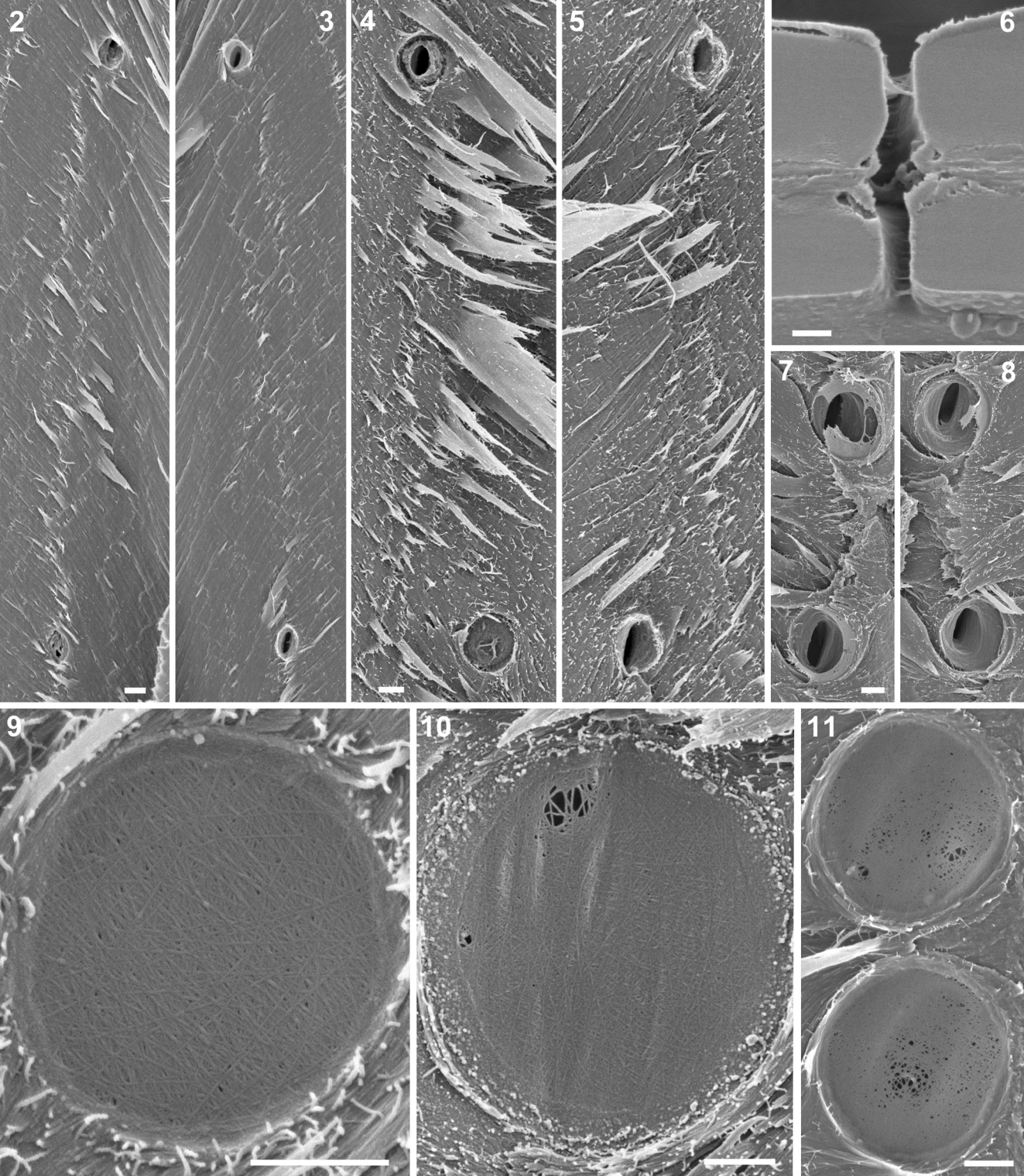
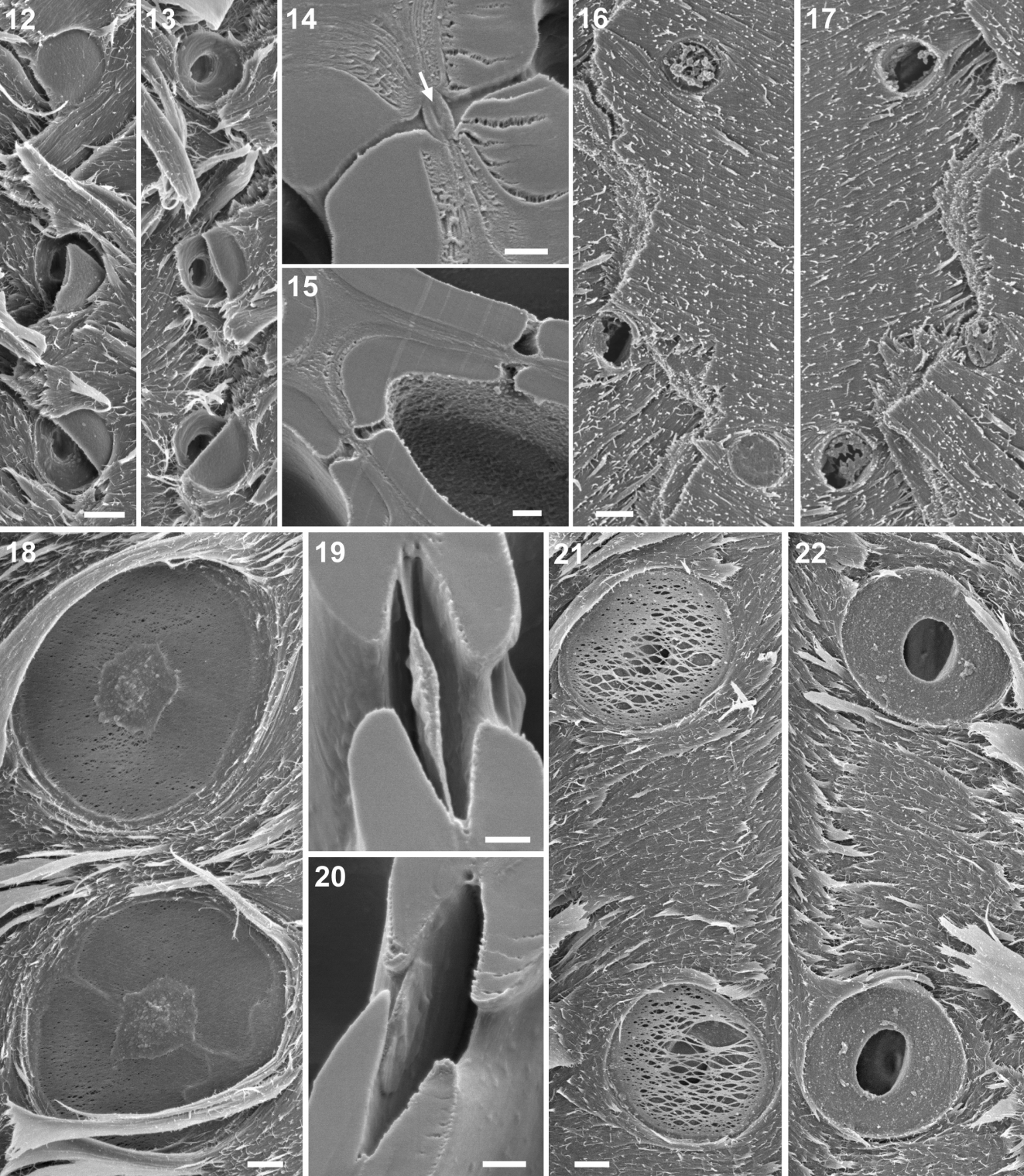
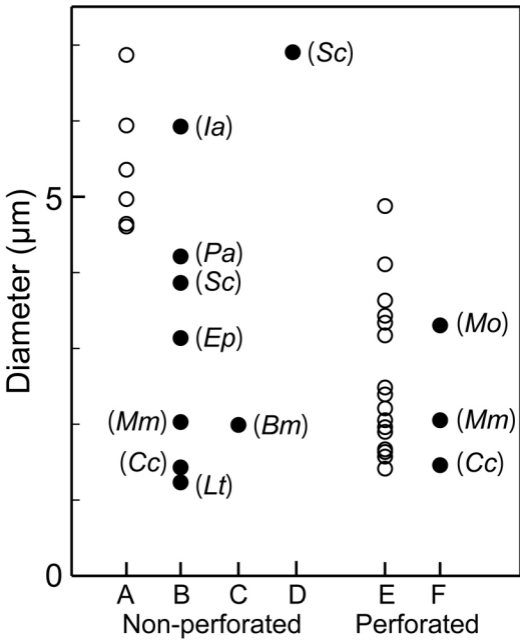


Fig. 1









**Table 1.** List of species studied, with their origins and the features of their interfiber pit membranes.

Botanical name	Abbreviation	Family (order)	Japanese name	Origin <sup>a</sup>	DBH in cm	Pit membrane type	Average horizontal diameter in $\mu\text{m}$ (SD) <sup>b</sup>	Average vertical diameter in $\mu\text{m}$ (SD)
<i>Cinnamomum camphora</i>	Cc	Lauraceae (Laurales)	Kusunoki	3	24	Perforated	1.33 (0.32)	1.46 (0.39)
						Without openings	1.34 (0.28)	1.41 (0.29)
<i>Magnolia obovata</i>	Mo	Magnoliaceae (Magnoliales)	Ho'onoki	4	10 <sup>c</sup> 9 <sup>c</sup>	Perforated	3.17 (0.91)	3.31 (0.79)
<i>Meliosma myriantha</i>	Mm	Sabiaceae	Awabuki	1	6	Perforated	1.83 (0.32)	2.04 (0.38)
						Without openings	1.92 (0.35) <sup>d</sup>	2.03 (0.32) <sup>d</sup>
<i>Euptelea polyandra</i>	Ep	Eupteleaceae (Ranunculales)	Fusazakura	1	8	Sheet (thin-walled)	2.93 (0.25)	3.13 (0.22)
<i>Illicium anisatum</i>	Ia	Schisandraceae (Austrobaileyales)	Shikimi	1	25	Sheet (thin-walled)	5.39 (0.43)	5.94 (0.30)
<i>Platanus</i> $\times$ <i>acerifolia</i>	Pa	Platanaceae (Proteales)	Momijibasuzukakenoki	2	40	Sheet (thin-walled)	3.52 (0.19)	4.20 (0.26)
<i>Buxus microphylla</i> var. <i>japonica</i>	Bm	Buxaceae (Buxales)	Tsuge	3	(1) <sup>e</sup>	Sheet (densely-packed)	1.84 (0.14)	2.00 (0.21)
<i>Lindera triloba</i>	Lt	Lauraceae (Laurales)	Shiromoji	1	7	Sheet (densely-packed)	1.24 (0.24)	1.23 (0.23)
<i>Schisandra chinensis</i>	Sc	Schisandraceae (Austrobaileyales)	Chosengomishi	4	0.5 <sup>c</sup> 1 <sup>c</sup>	Torus-bearing	6.89 (0.61)	6.83 (0.61)
						Without tori	3.86 (0.86)	4.28 (0.91)

<sup>a</sup> 1, Shiiba Research Forest of Kyushu University (Shiiba); 2, Botanical Garden of Hokkaido University (Sapporo); 3, Forest Tree Breeding Center of Forestry and Forest Products Research Institute (Hitachi); 4, Tomakomai Experimental Forest of Hokkaido University (Tomakomai).

<sup>b</sup> SD=Standard deviation.

<sup>c</sup> Two trees were examined for these species.

<sup>d</sup> These values are probably based on both pits of dead fibers and septate fibers because it was difficult to distinguish the pit membranes of dead fibers from those of septate fibers in our observations of split specimens.

<sup>e</sup> Young tree of which height is 1.2 m. Value for sample piece taken at lower height.