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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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Authors' full names, affiliations, and addresses:

Yuzou Sano [1], Yasuhiro Utsumi [2], Ryogo Nakada [3]

[1] Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

[2] Kyushu Research Forest, Kyushu University, Sasaguri 811-2415, Japan

[3] Forest Tree Breeding Center, Forestry and Forest Products Research Institute, Hitachi 319-1301, Japan

Corresponding author:

Yuzou Sano

E-mail address: pirika@for.agr.hokudai.ac.jp

Tel: +81-(0)11-706-3859

Fax: +81-(0)11-706-3859

Key words:

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

Footnote:

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Abstract

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

Introduction

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

Perforated pit membranes between wood fibers were found in some angiosperms [8, 9] after they had already been reported in *Carya tomentosa* [10] and *Fraxinus mandshurica* var. *japonica* [11], but this type of pit membrane was considered to be relatively rare and attracted scant attention. However, recent studies revealed that (1) perforated pit membranes are common in various taxa of angiosperms and (2) wood fibers with perforated pit membranes are non-conductive while wood fibers with sheet-like pit membranes are conductive [8, 9, 12]. In previous studies, the selection of species was limited to core eudicots. It remained to be determined whether such pit membranes might also be common in taxonomic groups that are located at more ancestral positions in the angiospermous clade. Therefore, as described in this report, we examined the structural variations in interfiber pit membranes of nine species that represented seven families, chosen 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 × 5 × 5 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 × 3 × 3 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

immersion in acetone and the specimen was air-dried.

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

Anatomical measurements

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

Terminology

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

Results

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

Species with perforated pit membranes

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.* ×

acerifolia (Momijibasuzukakenoki).

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

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

Diameter of pits

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

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

Discussion

The presence of perforated pit membranes between wood fibers has been

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

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

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

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

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

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

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

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

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

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Legends to Figures

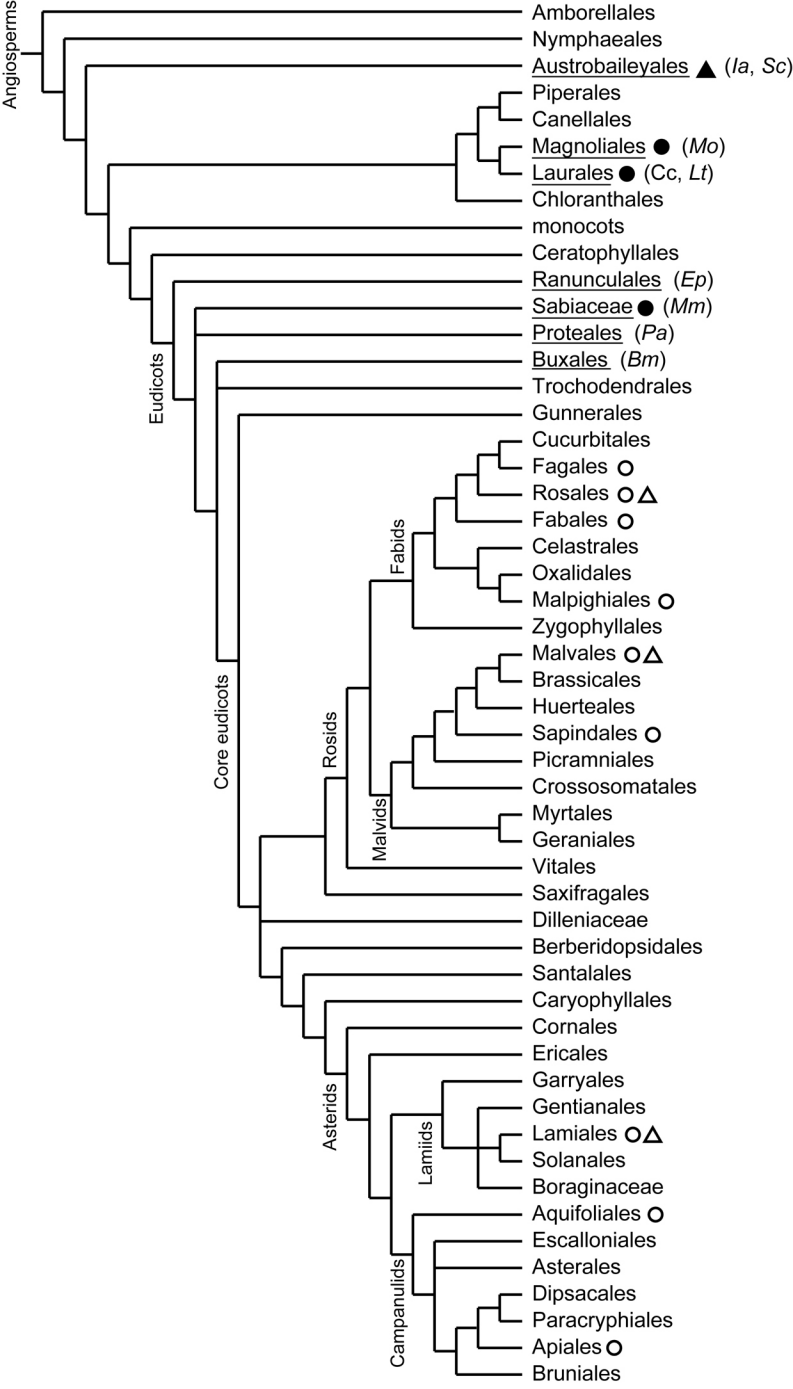
Fig. 1 Phylogeny of angiosperms from the Angiosperm Phylogeny Group (APG III 2009), showing the taxonomic groups that include species with each type of pit membrane. Underlining indicates groups that include species examined in the present study. Circles and triangles indicate groups that include species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

monocots are omitted.

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

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

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


Angiosperms
Amborellales
Nymphaeales
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Piperales
Canellales
Magnoliales ● (Mo)
Laurales ● (Cc, Lt)
Chloranthales
monocots
Ceratophyllales
Ranunculales (Ep)
Sabiaceae ● (Mm)
Proteales (Pa)
Buxales (Bm)
Trochodendrales
Gunnerales
Eudicots
Core eudicots
Cucurbitales
Fagales ○
Rosales ○△
Fabales ○
Celastrales
Oxalidales
Malpighiales ○
Zygophyllales
Fabids
Malvales ○△
Brassicales
Huerteales
Sapindales ○
Picramniales
Crossosomatales
Myrtales
Geraniales
Vitales
Saxifragales
Rosids
Malvids
Dilleniaceae
Berberidopsidales
Santalales
Caryophyllales
Cornales
Ericales
Garryales
Gentianales
Lamiales ○△
Solanales
Boraginaceae
Lamiids
Asterids
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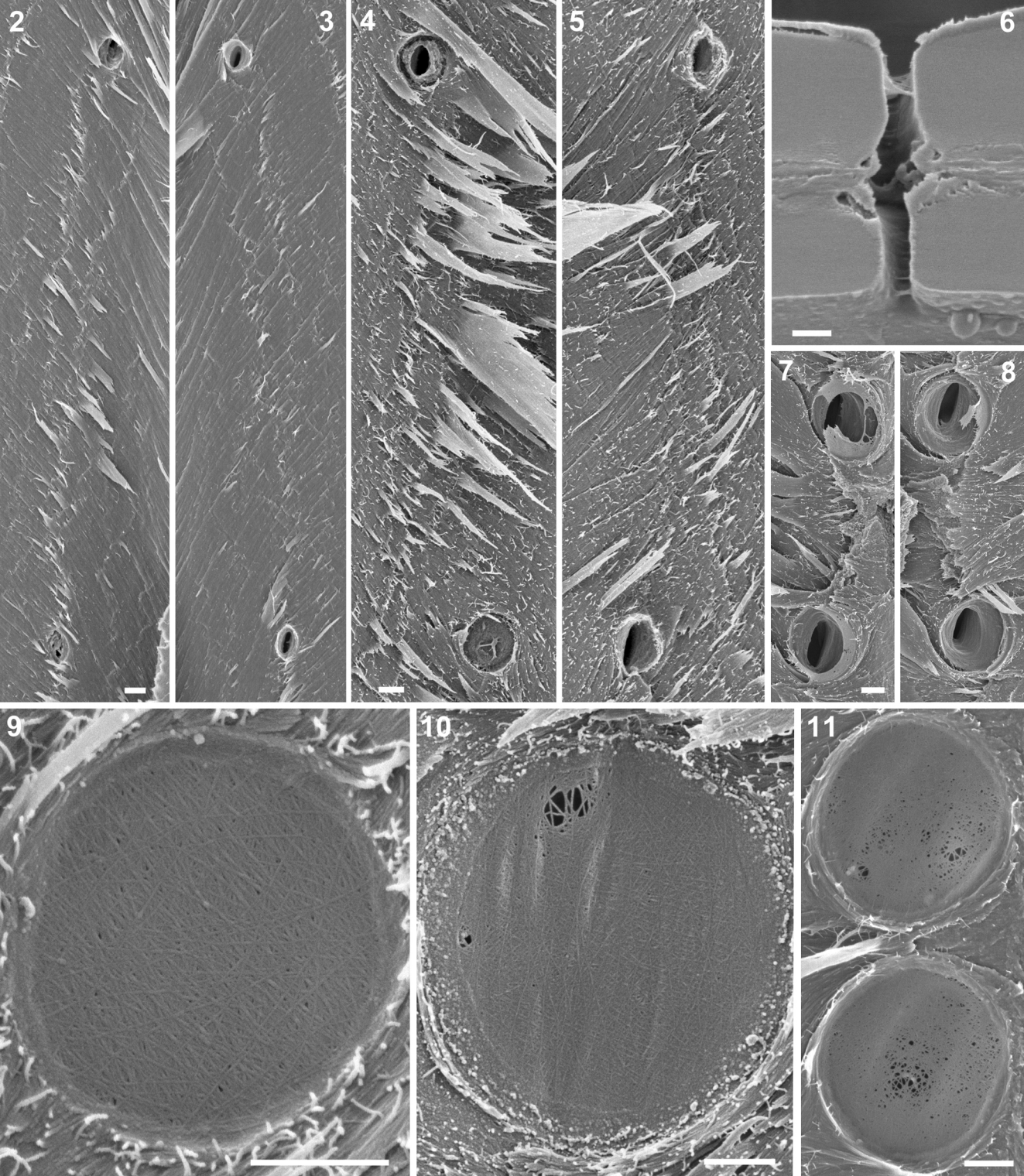
Asterales
Dipsacales
Paracryphiales
Apiales ○
Bruniales
Campanulids

Fig. 1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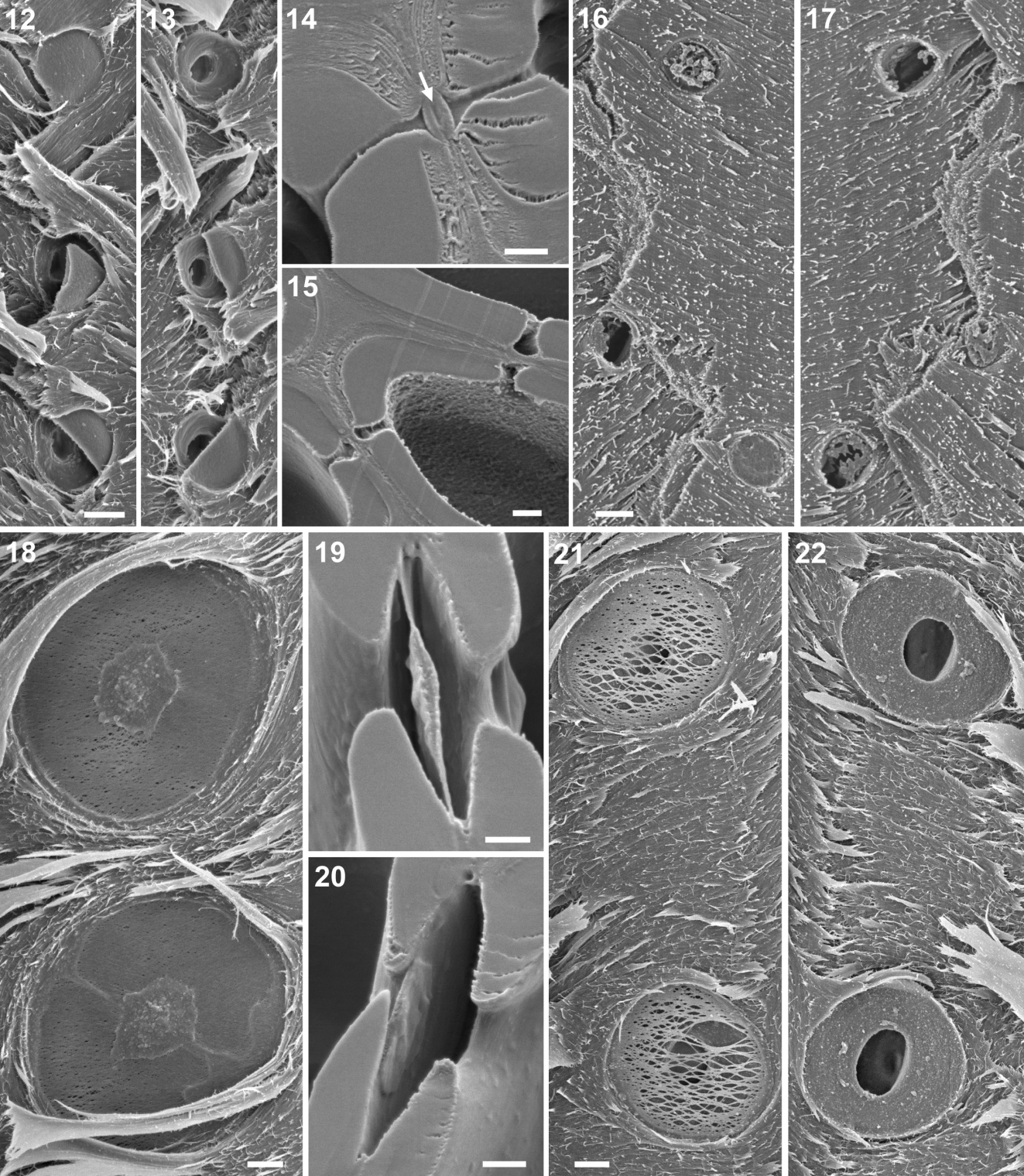
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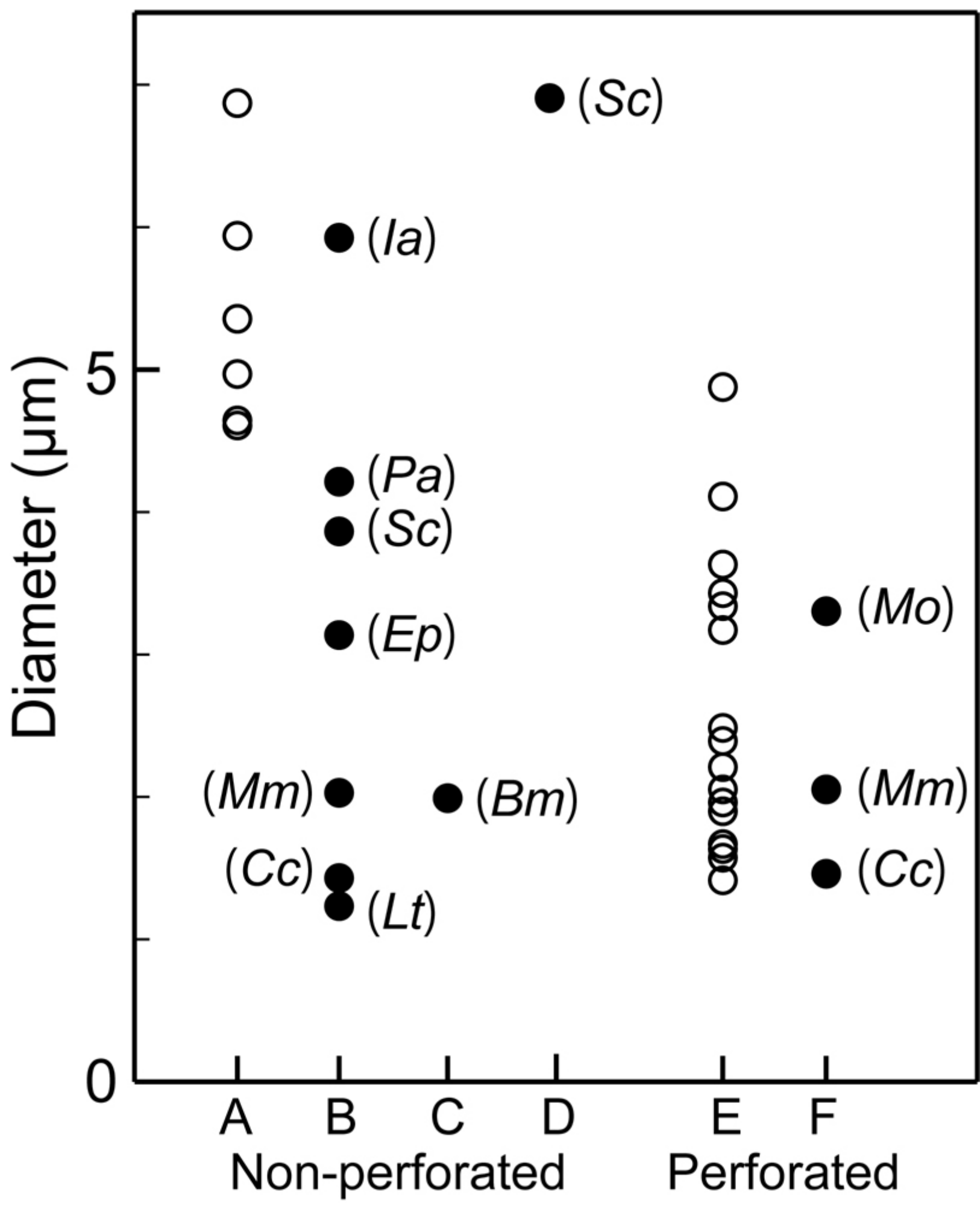
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(Ia)
(Pa)
(Sc)
(Ep)
(Mm)
(Bm)
(Cc)
(Lt)
(Mo)
(Mm)
(Cc)
A
B
C
D
E
F
Non-perforated
Perforated

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

[a] 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).

[b] SD=Standard deviation.

[c] Two trees were examined for these species.

[d] 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.

[e] Young tree of which height is 1.2 m. Value for sample piece taken at lower height.