An Observation of the Scalariform Perforation Plate of the Vessel in Some Hardwoods, Using Scanning Electron Microscopy*

By

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走査型電子顕微鏡による数種広葉樹材道管の階段せん孔板の観察

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CONTENTS

Introduction

The perforation plate, which is defined as the area of the wall involved in the coalescence of two members of a vessel (IAWA) varies, widely in its structure, from simple (with a single large opening) to multiple (with a number of small openings). During the course of a study on wood structure using scanning electron microscopy (SEM), the authors found a peculiar substructure between the bars of the scalariform perforation plate, which is a type of multiple perforation plate. This has been already briefly reported together with other structures found on the examination of wood by SEM (ISHIDA, 1969, 1970).

Perforation plates are formed during the differentiation of the individual vessel members (ESAU, 1965). Recently, YATA, ITOH and KISHIMA (1970) reached a conclusion on the formation of the perforation plates and bordered pits in

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differentiating vessel elements by the use of transmission electron microscopy. Using SEM, BUTTERFIELD and MEYLAN (1972), MEYER and MUHAMMAD (1971), MEYLAN and BUTTERFIELD (1972), MEYLAN and BUTTERFIELD (1973) and PARHAM (1973) have reported valuable information about the morphology and development of the plate.

The authors have made some additional observations on the scalariform perforation plate of Katsura, as well as Keyamahannoki, Shirakanba and Mizuki, resp., to confirm the substructure mentioned above. This paper deals with these observations and some discussions.

Materials and methods

Wood samples were collected for this study from living trees of the following species:

Katsura, *Cercidiphyllum japonicum* SIEB. et ZUCC.
Keyamahannoki, *Alnus hirsuta* TURCZ.
Shirakanba, *Betula platyphylla* SUKAT. var. *japonica* HARA
Mizuki, *Cornus controversa* HEMSL.

Fresh materials were removed from the sapwood at breast height of a mature tree trunk of each species (D. B. H.; 20–25 cm), grown in Tomakomai College Experimental Forest, Hokkaido University. Specimens were finished to the shape of $7 \times 7 \times 2$ (mm) and dried at room conditions. They were then mounted on specimen stubs with electric conductive paste. The longitudinal radial surface was coated with gold for SEM examination, which was carried out at 9–25kV mainly with a JSM-2 microscope.

Results and discussion

I. Basic structure of the scalariform perforation plate.

Typical scalariform perforation plates were found in all species examined, and are illustrated in photos 1–5, showing striking similarities in its structure among species. The perforation bars are constant in width ranging 2 to 3 microns and situated at regular intervals, especially in the center region of the plate. In the margin (lateral edges and vertical ends) of the plate, the bars usually become wider than in the center. The corresponding bars in two contiguous vessel elements are similar in interval, shape and size, thus forming a regular pair as shown in photo 6 (Katsura) in detail. In this photograph there are two pairs of bars, of which the right halves of the top-vessel bars have been torn away from the middle lamella. There are no borders on the bars. In the margin, however, the bars are always bordered even in the typical perforation plate.

No substructures are found between the bars in the center region of the typical plate. In the lateral edges, on the contrary, fibrillar strands are often found as shown in photos 7 (Katsura) and 8 (Keyamahannoki). Photo 7 shows reticulate microfibrillar webs or membranous substances, limited to the edges of some elongated perforations, where a bordered structure can be seen. Photo 8 illustrates also the reticulate webs, characteristic of this species, consisting of a number of fibrillar strands.

At the plate ends, perforations are found to grade into pits, similar to the intervessel pits on the longitudinal wall, as shown in photos 9–14. Photo 9 (Keyamahannoki) represents two perforation plates specially showing their vertical ends, where a number of pit-like perforations or same pits as the intervessel, especially in the left one. In photo 10 (Katsura) which shows one end of the perforation plate, the bars become gradually wider in its width, simultaneously accompanied by gradual thickening of the membranous partitions of the perforation. Outer side (as seen from the middle lamella) of such portion as in this photograph is illustrated in photo 11 which shows clearly a bordered structure just like in the intervessel pits. Photos 12 (Keyamahannoki), 13 (Shirakanba) and 14 (Mizuki) all show grading of perforation into pit structure quite similar to that of the intervessel, although there are some differences among the species.

Various kinds of unusual perforation plates, reported by MEYLAN and BUTTERFIELD (1973), were observed also in the present study. Photo 15 (Shirakanba) shows an example characteristic of this species complicated by an abnormal orientation of the bars.

II. Substructures of the perforation plate, esp., in its center region.

In Katsura and Mizuki, the scalariform perforation plate, in which fibrillar strands stretch orthogonally between the bars in the center region, is sometimes observed as shown in photos 16 (Katsura) and 17 (Mizuki). Other species examined, Shirakanba and Keyamahannoki, were observed to have no strands in the center. In the perforation plates with such fibrillar strands in the center, the plate bars are thicker than those without strands there, and usually bordered, as shown in photo 18.

In photo 19 (Katsura), the fibrillar strands are apparently thicker and denser than those in photo 16, and they are embedded in partially membranous substances. In the photograph the borders are clearly seen. On the contrary, only a few strands between the bars can be seen in photo 20 (Katsura). Most of the strands illustrated in photos 16 and 20 are extremely thin. Such thin fibrillar strands of Katsura were treated with sodium chlorite-acetic acid. SEM and TEM (using the direct carbon replica method) examinations showed no distinct differences between both the treated and untreated materials. Since a single strand as shown in photo 16, taken by SEM, measures about 400 Å in diameter, the real thickness of an intact strand must be calculated at ca. 200 Å, because of additional thickness of ca. 200 Å due to the coating of gold. According to these evidences concerning a certain chemical nature and thickness of the strand the existing unit strand is considered to be a cellulose microfibril or a similar structure to it (Kollmann and Côté, 1968), and it may easily gather in a more thicker strand, actually observed frequently under the SEM.

In all the species examined the perforation was found, sometimes, more or less closed with a membranous substances, as shown in photos 21–24. Pores in various sizes and in shape round to oval are found in these photographs. In Katsura (photo 21), there are a thin membranous part and several fibrillar strands, suggesting a process of development from the membrane to the strands. Photo 22 (Mizuki) represents about the same fact as the former photograph, although both are quite different from each other in appearance. Photo 23 (Katsura) illustrates a perforation plate covered with membranous materials. This type of perforation plate was often found in the terminal zone of an annual ring, while the other type, as in photo 21, was never found in the terminal. In the case of Shirakanba (photo 24), the membrane is characteristic of the species in appearance, and it was difficult to discover its fibrillar structure.

Conclusion

The formation of the substructure between the bars of the scalariform perforation plate is closely related to the process of cell wall development of the vessel elements. Occurrence of vertical strands connecting opposite borders of elongated pittings has been observed in the fossil plant, and in relation to this, investigations have also been carried out on certain modern higher plants (BARGHOORN et al, 1958). Valuable information about the development of the perforation plate has been recently reported as stated above, According to this information, including the observations of the present writers, all sorts of substructures must be a remnant of the compound middle lamella at various stages of enzymatic degradation during differentiation of the vessel elements. The completion of the degradation results in a typical perforation, i.e., clear opening, in the plate. The degradation may depend on the species, the season of the vessel formation (and therefore the position of the vessel within the annual ring), the position to be perforated within the plate, and also on the position within the elongated perforation … etc. Thus, in the case of Katsura, a fully mature, typical perforation occurs usually in the center region of an individual perforation plate of those vessels which are situated from earlywood to middle-latewood, but in the terminal of the annual ring.

Two matters must be noticed here: one is bordering in the bar, the other is fibrillar orientation of the strands within a perforation, each relating to the substructure formation. The center region of the typical perforation is clearly open and non-bordered, while in the margin there often appear various partitions and borders. It is probably said that no borders of the bar are usually accompanied by no partitions, with the exception in the terminal zone of the annual ring where noticeable partitions appear between the bars having hardly any borders. Consideration should be paid both to existing borders consisting of lignified S2 and to the compound middle lamella regarding its morphological and chemical nature, to clarify formation of the substructure. As stated above, the nearly parallel orientation of fibril strands is often found in the center of the plate in Katsura and Mizuki, while in the margin it is basically reticulate. During the degradation of the compound middle lamella, its matrix substances are first removed, and part of cellulose microfibrils of the primary wall remains depending on the conditions of the degradation. The remnant fibrils may be more or less re-oriented. Supposedly, this process partly depends on the position within the individual perforation, perforation plate and within the annual ring. Besides this, the primary wall texture itself is considered to be of importance for the fibrillar substructure formation. This must be a problem further to study.

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要　約

カツラ，ケヤマハンノキ，シラカンバ，ミズキの道管の階段せん孔板の形態を走査型電子顕微鏡によって観察した。得られた結果を要約すれば次のとおりである。

1. 観察した4樹種ともその道管には Photo 1～5 に示されるような，定義どおり (IAWA) の階段せん孔板が観察された。すなわち，道管軸に対してほぼ直角で，細長く平行な多数のせん孔が存在し，Bar はほぼ等間隔に位置している。また，上下に隣接する道管要素のそれぞれの Bar の間隔，形，大きさは等しく，それらは整然とした対をなしている (Photo 6)。このようなせん孔板では，その上下両端に近くなると Bar は多少とも有縁になるが (Photo 7)，中央部では有縁でない。せん孔の側方縁辺部も有縁であり，そこにはフィブリル状のストランドや薄膜状のものが存在することがしばしば観察された (Photo 7, 8)。

2. 極めてまれに，特異な，やや奇形的なせん孔板がそれぞれの樹種に認められた。その1例（シラカンバ）を Photo 15 に示す。

3. せん孔板の上下両端部には，道管要素細胞の側膜にある道管相互膜孔と極めて類似した膜孔が存在する。この上下両端部から中央部の方へゆくにつれて膜孔の孔口は徐々に大きくなり，それとともに膜孔膜は次第に消失してゆく（Photo 10～14)。

4. カツラ，ミズキの階段せん孔板では，フィブリル状のストランドがせん孔板の中央部にも認められることがあった（Photo 16～22)。これらの配向や密度は変化に富むが，カツラではBar に対してほぼ直角に配向する場合が多い（Photo 16, 19～21)。また，このようなせん孔板の Bar は一般に有縁であり，広い（Photo 19)。

カツラの脱リグニン処理材におけるフィブリル状ストランドの TEM および SEM による観察結果，および SEM により観察測定されたフィブリル状ストランドの直径から判断すればこれらのストランドは，太さ約 200 Å で，セルロースミクロフィブリルより成るユニットストランドであると推定される。

5. カツラでは1生長期の終期に形成される道管，すなわち，年輪のターミナル付近にある道管の階段せん孔板は，膜状物によりそのせん孔が閉ざされている場合が多い（Photo 23)。これらの膜状物には，多数の，大きさの異なる小孔が認められる。膜状物によりせん孔が閉鎖されているせん孔板は，シラカンバ（Photo 24)，ケヤマハンノキにも認められ，それらが現われる位置は必ずしも年輪のターミナルとは限らなかった。

6. 階段せん孔板の Bar の間にあるストランドや膜状物は，崩壊過程にある複合間層の残留物とみなされる。酵素によるこの崩壊は樹種，細胞の形成時期（したがって材の年輪内位置)，せん孔板内の位置，せん孔内位置，……などによってその質や程度がことなると考えられる。現実に，膜孔膜に近いものから，消失して完全な開孔状態のものまで，いろいろな段階のものが観察された。Border の存在とストランド（あるいは膜状物）の存在との間に密接な関連のあることが明らかになったが，その理由はまだ明らかでない。階段せん孔の中央部にみられるストランドの平行配列は，1次膜構造の崩壊過程でのセルロースミクロフィブリルの再配列によるものと考えられるものの，それが，縁辺部の網状構造のストランドと同様な1次膜構造に基因するものかどうかは問題のあるところである。これらについてはなお今後の研究が必要である。

Explanation of photographs

Photo 1. A scalariform perforation plate as seen in the transverse surface in Katsura.

Photo 2. A typical scalariform perforation plate of Katsura.

Photo 3. A typical scalariform perforation plate of Keyamahannoki.

Photo 4. A typical scalariform perforation plate of Shirakanba.

Photo 5. A typical scalariform perforation plate of Mizuki.

Photo 6. A part of two bars in the center region of a typical scalariform perforation plate in Katsura. The right halves of the top-vessel bars have been removed from the middle lamella.

Photo 7. Katsura. Fibrillar strands are present in perforations in the lateral margin of a scalariform perforation plate.

Photo 8. Keyamahannoki. Membrane consisting of dispersed fibrils remains between bars in the lateral margin of a scalariform perforation plate.

Photo 9. Scalariform perforation plates of two vessels in Keyamahannoki, showing the vertical ends of them.

Photo 10. The vertical end of a scalariform perforation plate in Katsura.

Photo 11. The vertical end of a scalariform perforation plate as seen from the outer surface wall in Katsura (cf. photo 10).

Photo 12. The vertical end of a scalariform perforation plate in Keyamahannoki.

Photo 13. The vertical end of a scalariform perforation plate in Shirakanba.

Photo 14. The vertical end of a scalariform perforation plate in Mizuki.

Photo 15. A part of an abnormal perforation plate in Shirakanba.

Photo 16. The center region of a scalariform perforation plate of Katsura. Fibrillar strands run between bars almost at a right angle to them.

Photo 17. Mizuki. Fibrillar strands are present between bars in any perforations not only in the margin but also in the center region of the scalariform perforation plate.

Photo 18. A part of a scalariform perforation plate of Mizuki. Border of each bar which had been existed in this side is removed. Border of bars in the opposite side is clearly seen.

Photo 19. The center region of a scalariform perforation plate of Katsura. Fibrillar strands are denser than in photo 16. Compare with photo 16.

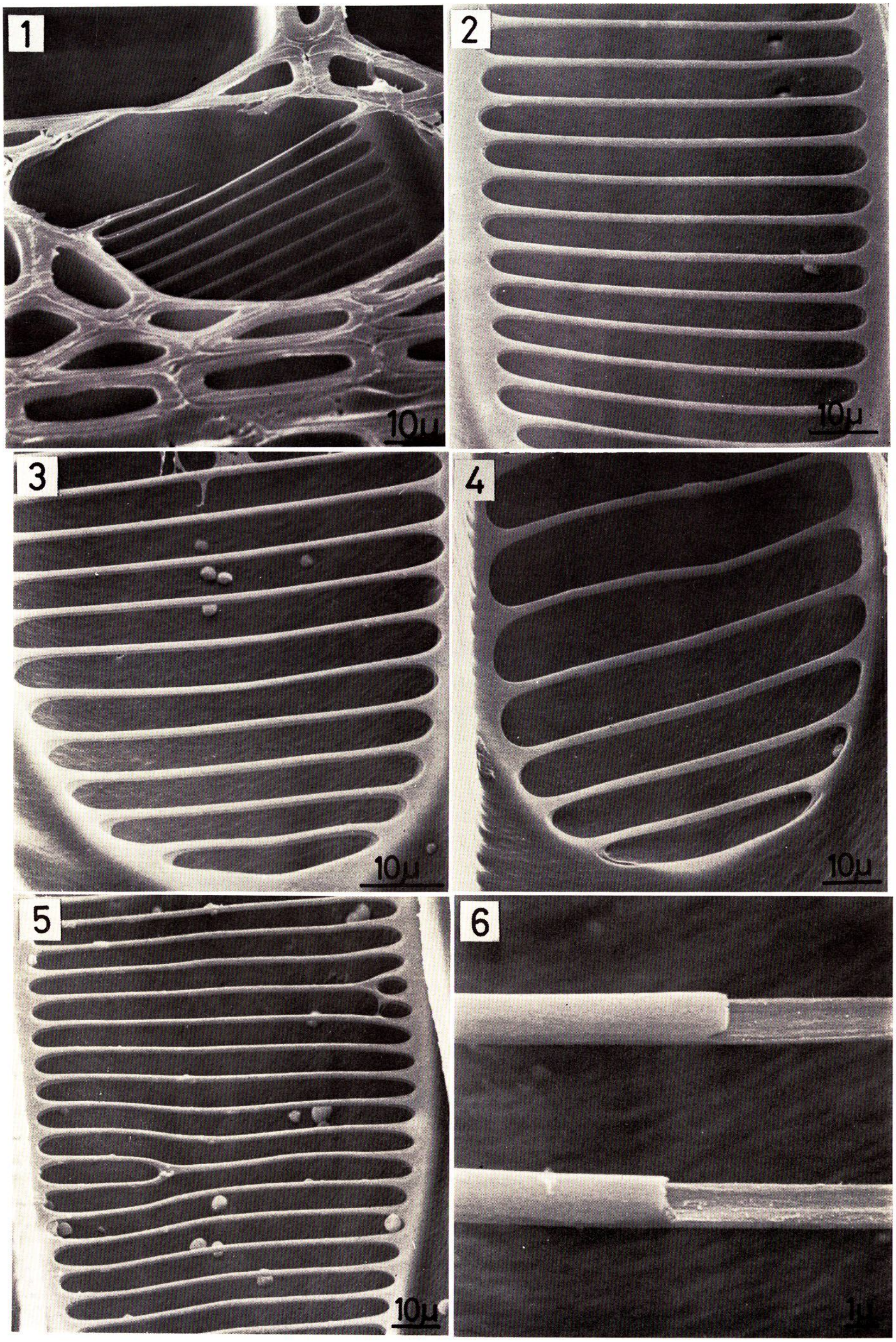
Photo 20. A few fibrillar strands remaining between bars which have nearly no borders. The strand branches at the end respectively.

Photo 21. The center region of a scalariform perforation plate of Katsura. Both membranous substances and fibrillar strands are seen between bars.

Photo 22. A part of a scalariform perforation plate of Mizuki. Border of bars which had been existed in this side is removed. Membrane consisting of dispersed fibrils is seen between bars.

Photo 23. A scalariform perforation plate at the terminal zone of annual ring in Katsura. Perforations are closed with membranous substances. Many pores are often observed in the membrane as shown in this photo.

Photo 24. Membranous substances on a scalariform perforation plate in Shirakanba.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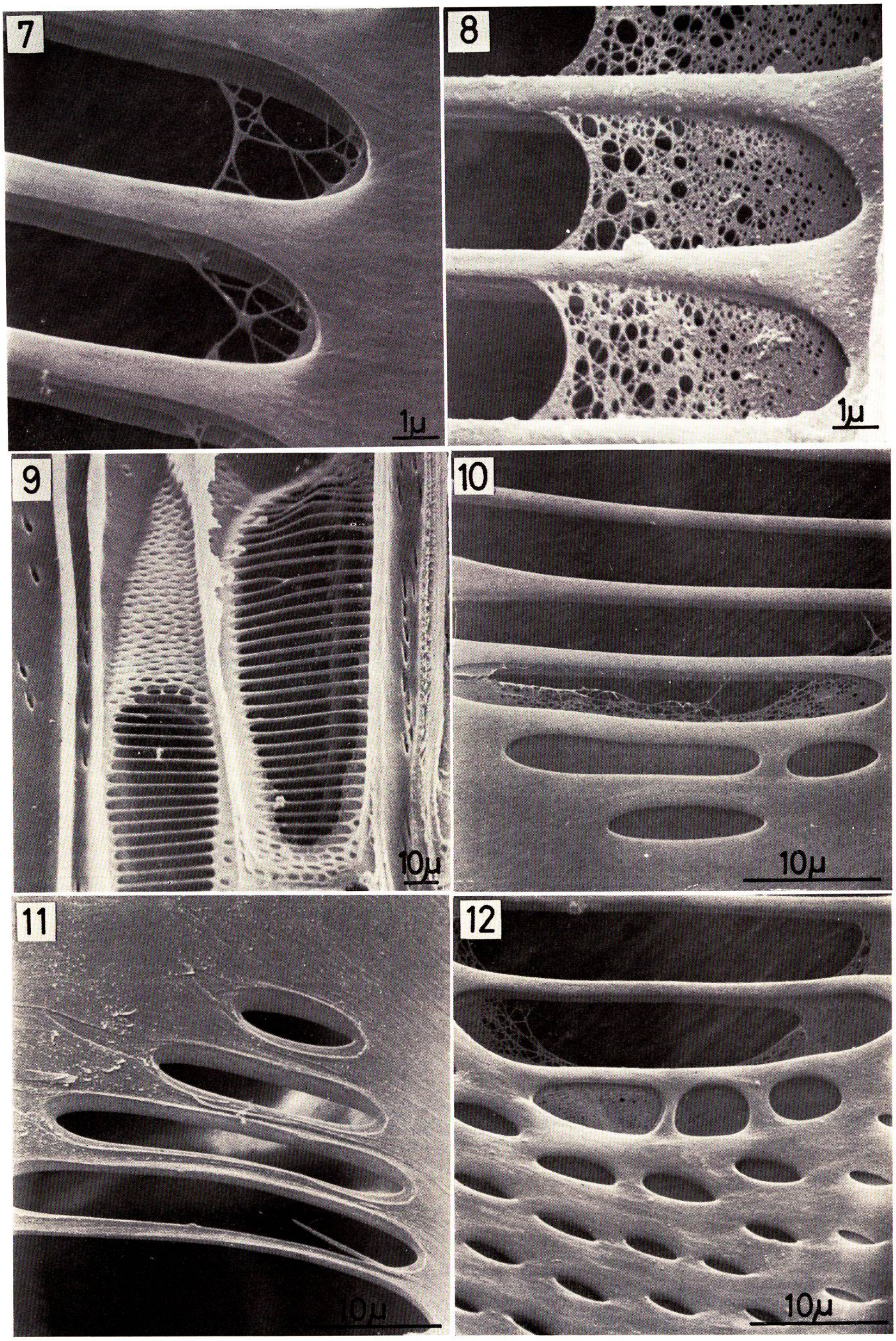
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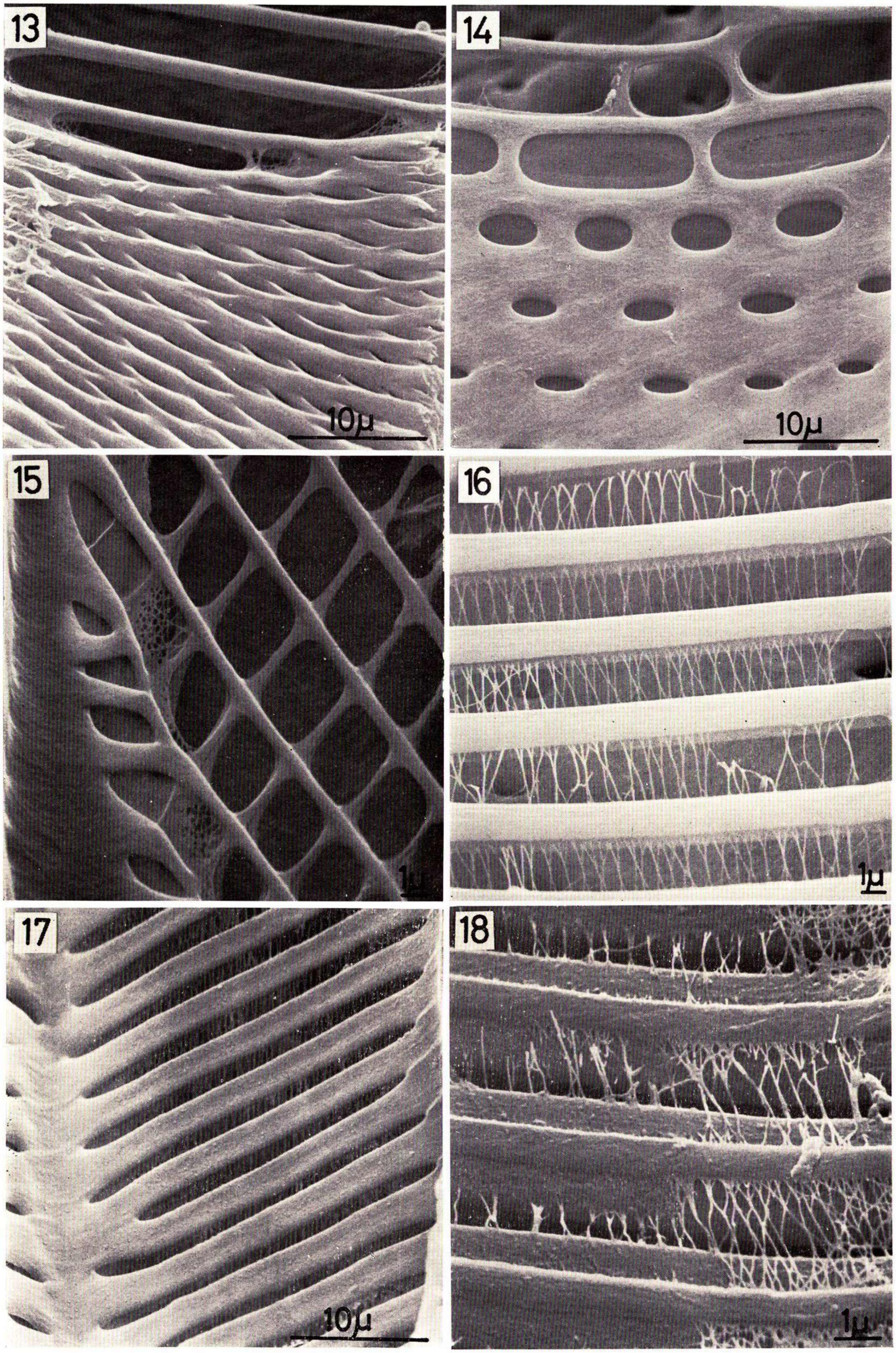
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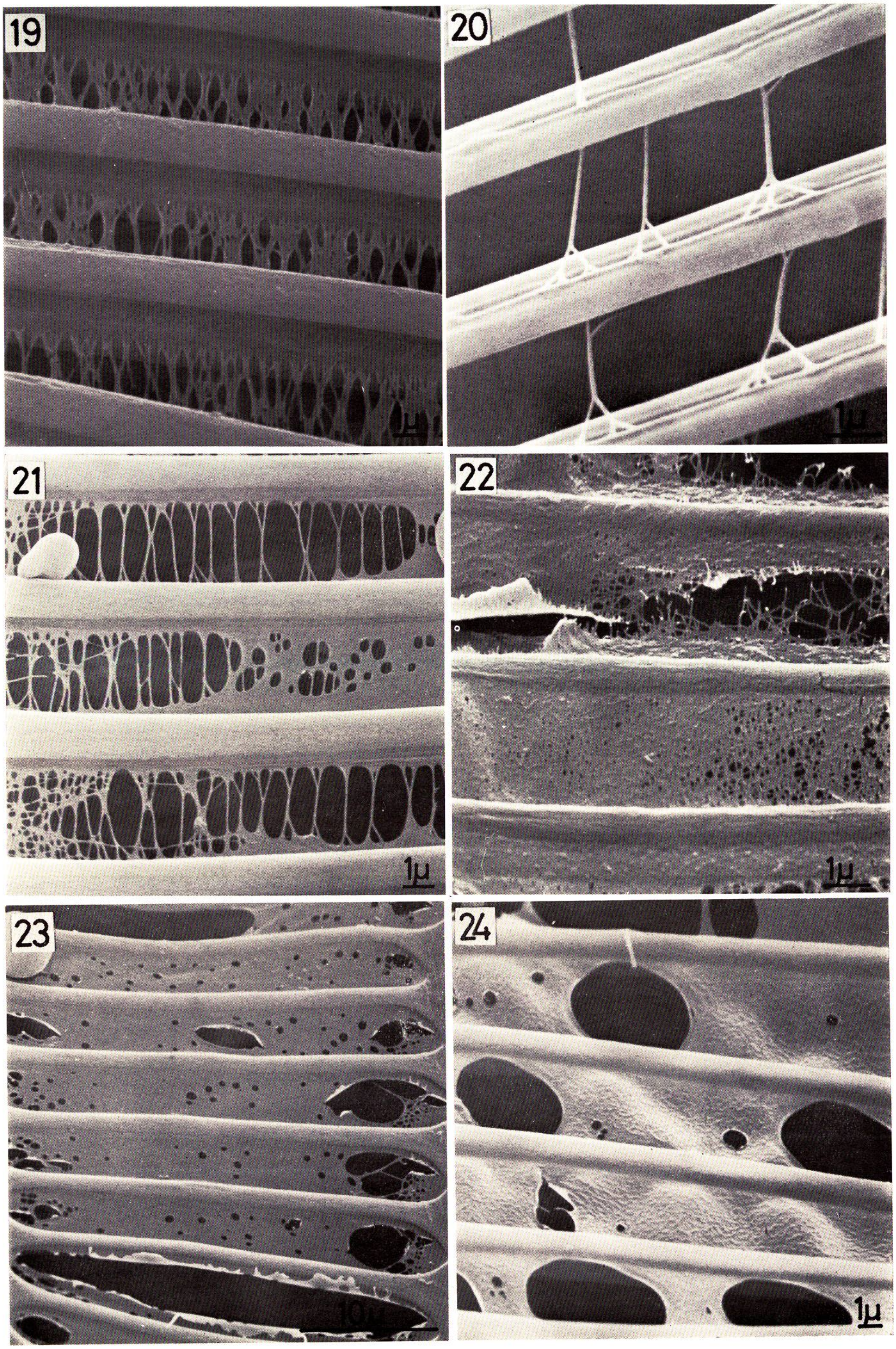
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