

## THE LEPIDODENDROID STOMA

by B. A. THOMAS

**ABSTRACT.** Detailed investigations have been made of the stomata of several lepidodendroid species using cuticle preparations from compressions together with sections and peels from petrified material. The scanning electron microscope has allowed more critical observation of the compression preparations than has been previously possible. Structures are shown within the guard cells which are tentatively interpreted as the remains of lignified wall thickenings. This is the first time such structures have been shown in these plants.

FOR well over a century epidermal characters have been used to help interpret the morphological and taxonomical relationships of many fossil plant organs. More recently such work has been extended to extant plants where it is also proving to be of immense value. Although the same basic ideas govern the study of epidermises from fossil and living material, the actual methods are somewhat different. Living material can be examined in a number of ways, but with fossils the type of preservation controls the method of study. The best possible interpretation of a fossil epidermis is thus often a composite picture achieved by utilizing evidence from several differently preserved specimens.

Arborescent lycopods are suitable for epidermal studies as they are found in large numbers and in various types of preservation. The general features of the lepidodendroid epidermis have been already discussed (Thomas 1966) and used in re-describing certain species of *Bothrodendron*, *Ulodendron*, and *Lepidodendron* (Lacey 1962; Thomas 1967 et seq.). In these studies observations were made on macerated cuticles by transmitted light microscopy which were supplemented by some examination of petrified material. This gave a clear picture of epidermal cell size and stomatal distribution but there were certain internal guard-cell structures which were not fully understood.

Lepidodendroid stomata often possess guard cells sunk in stomatal pits, rather like those of many gymnosperms. The cushion cuticles show structures which have been described as 'dome cells with crests' by Bartlett (1929). This is an apt description for their appearance until it is realized that they project inwards from the general cuticle layer, that is in the same direction as the cuticular ridges of the epidermal anticlinal walls. They are in fact the cuticles from the sides of stomatal pits, the upper walls of the guard cells, and the outer portions of the guard-cell poral walls (Pl. 78, figs. 1, 2). It should not be thought unusual that the poral wall is not continued as a cuticular flange between the fused ends of the guard cells as they appear to be absent in many plants, including some species of the extant *Lycopodium sensu lato*. Some lepidodendroid stomata, however, show extra structures in the form of a very conspicuously thickened central oval area, often with a very prominent poral wall 'crest', and a separate oval band of thickening running around the central thickening. These outer bands may be continuous showing neither break nor change in thickness in the regions where the anticlinal walls once separated the guard cells. Cuticles from

some species habitually show more stomata with these thickenings although these are not always perfectly preserved. This suggested that the maceration process itself was a major factor in their preservation, so attempts were made to observe its effects.

Boulter (1970) showed that maceration affected the preservation of certain guard-cell thickenings in the Taxodiaceae and that it was the alkali clearing treatment which was particularly disruptive. The difficulty encountered in the present study was that *Lepidodendrolean* cushion compressions are more coalified than Boulter's Tertiary *Cryptomeria* material. Oxidation in Schulze solution neither renders cushion compressions transparent nor does it separate the cuticle from the compression. Treatment with dilute alkali is therefore normally necessary. Occasionally, however, specimens can be found with relatively robust and uncracked cuticles which will withstand rougher treatment than usual. After oxidation in Schulze solution the compression was removed from these cuticles with a mounted needle and by ultrasonic-wave treatment using a 'Pulsatron' bath. This naturally damages and destroys much of the cuticle but some usable fragments can be obtained which have thus not been treated with alkali. Very brief immersion in alkali was tried out at this point as an attempt at 'etching' the compression to highlight certain features such as epidermal anticlinal walls.

Optical microscopy has naturally been the traditional means of studying cuticle preparations but recently the scanning electron microscope has been shown to be of immense value for this purpose. Naturally, cuticles are normally observed from their inner surfaces when using the s.e.m. as this side is the one more affected by the underlying epidermal cells. Epidermal cell anticlinal walls are marked by cuticular ridges and their corners by slender cuticular pegs, while inwardly projecting surfaces such as pits or sunken guard cells appear raised.

The cuticles of lycopod leaf cushions were therefore prepared in various ways and examined by light microscopy and by the s.e.m. in an attempt to explain the internal structure of the guard cells. The best results obtained were from specimens of *Lepidodendron veltheimii* Sternberg and *Lepidophloios acerosus* Lindley and Hutton, so these two are described in some detail.

#### DESCRIPTIONS

##### *Lepidodendron veltheimii* Sternberg

*Material.* No. 2411, Kidston collection, Institute of Geological Sciences, London; from immediately beneath the Orchard Limestone, New Brawden Quarry, Giffnock, Renfrewshire. Upper Limestone Group of the Carboniferous Limestone Series, Lower Namurian.

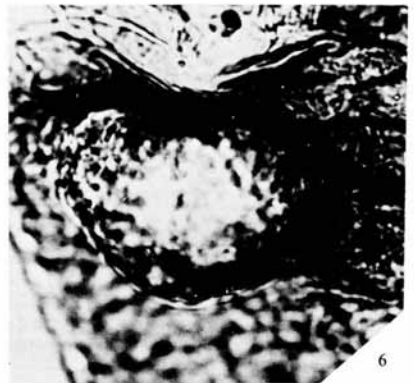
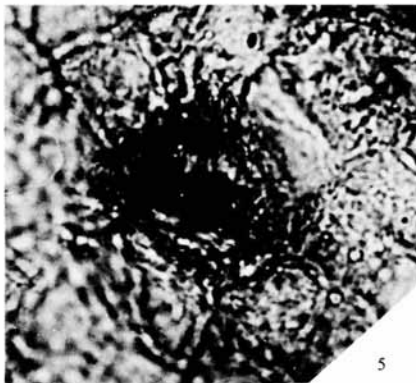
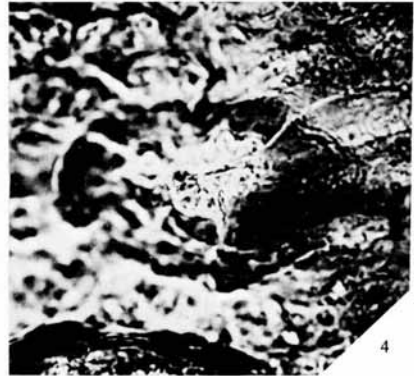
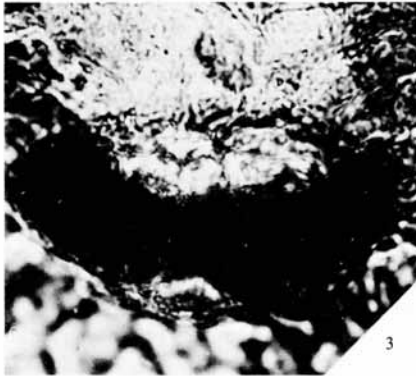
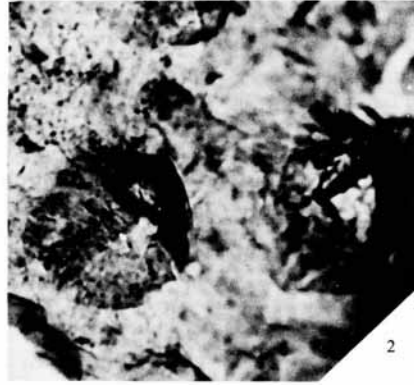
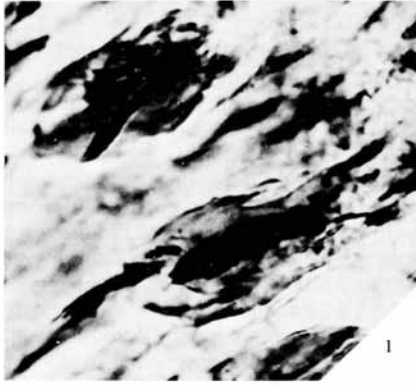
#### EXPLANATION OF PLATE 78

Lepidodendroid stomata as seen with transmitted light.

Figs. 1, 2. The cuticles prepared by Bartlett. Stomata are visible showing their 'dome and crest' appearance as originally described,  $\times 500$ .

Figs. 3, 5. *Lepidodendron veltheimii* Sternberg. Stomata showing the dark walls of their pits,  $\times 800$ . 3, obliquely compressed stoma (compare with Pl. 80, figs. 1, 2). 5, horizontally compressed stoma (compare with Pl. 79, figs. 3-6).

Figs. 4, 6. *Lepidophloios acerosus* Lindley and Hutton. Vertically compressed stomata,  $\times 1200$ . 4, stoma showing remnants of the central 'crest' thickening (compare with Pl. 81, figs. 4, 6). 6, stoma showing remnants of the outer thickening (compare with Pl. 81, figs. 2, 4).



THOMAS, *Lepidodendroid stomata*

This is a specimen which has previously had its cuticle prepared and figured after treatment with Schulze solution and dilute ammonia solution (Thomas 1970, pl. 33, figs. 5, 6; text-fig. 5). The guard cells were described as sunken in pits with some over-arching of the subsidiary cells; an interpretation which is supported by the use of the s.e.m.

Small depressions can be just seen with the naked eye on the surface of the cushion compression and these have been interpreted as stomatal pits. Portions of compression were therefore removed, cleaned with hydrofluoric acid, and mounted for direct viewing with the s.e.m. Such preparations predictably revealed vast numbers of shallow depressions but also showed that these often contained what appears to be a pair of guard cells (Pl. 79, figs. 1, 2). Similar evidence is clearly shown with macerated cuticles viewed from the underside. Those prepared by oxidation and clearing in alkali show the cuticles of the guard-cell outer walls as raised oval areas overlapping the underlying epidermal cell cuticle (Pl. 78, figs. 3, 5; Pl. 79, fig. 3); while if the guard cells are completely lost the stomatal pit wall is exposed as a layer of cuticle recurved over the general epidermal cuticle sheet (Pl. 80, figs. 1, 2). The central guard-cell thickenings seen with transmitted light are usually visible here as flattened oval structures with a marked ridge orientated longitudinally to the guard cells and along the stomatal aperture (Pl. 79, figs. 4, 6). Something, however, is clearly affecting these structures as some appear to be well preserved while others are damaged or virtually destroyed (Pl. 79, fig. 5). The preparation process was the most likely factor involved here and, in making comparisons by varying the times of acid and alkali treatment, it was found that the longer treatments gave the greater amounts of damage.

Complete, or almost complete, avoidance of the alkali stage gave rather different results. The compression is naturally left intact and must be mechanically removed with mounted needles or by immersion in an ultrasonic bath, but as such a method is difficult to duplicate variable results are obtained. Some cuticles retain a layer of cracked compression covering all features except the guard cells (Pl. 80, fig. 5), while others show the remnants of isolated pairs of guard cells raised up above the remaining compression (Pl. 80, fig. 6). Damage of the lower parts of the guard cells unfortunately often occurs during such preparations presumably accompanying the mechanical removal of the anatomically underlying compression. Limited immersion in alkali has no effect on some stomata (Pl. 80, fig. 4), while it partially destroys others (Pl. 80, fig. 3). Slightly longer alkali treatment dissolves away more of the guard cells and also more of the remaining compression revealing faint outlines of the epidermal cells.

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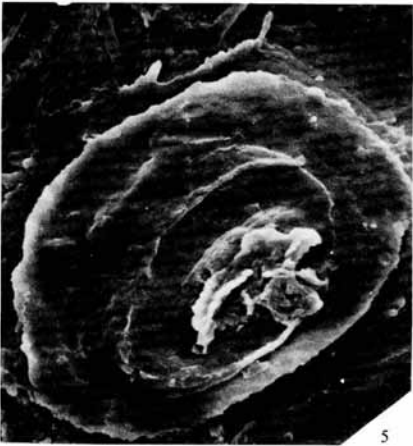
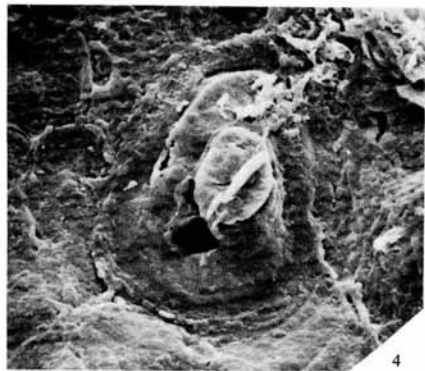
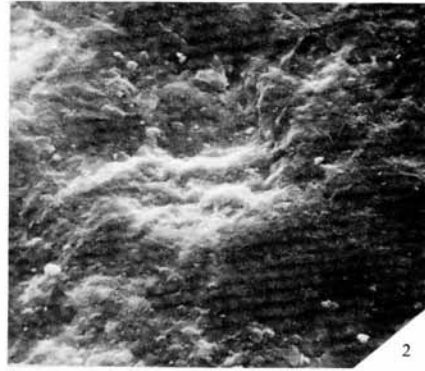
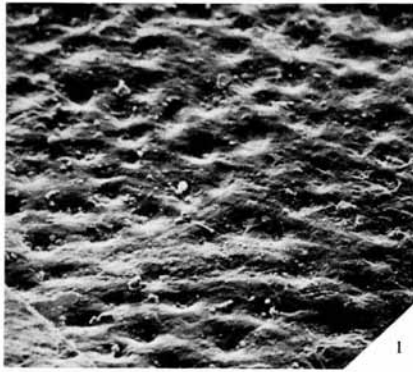
EXPLANATION OF PLATE 79

*Lepidodendron veltheimii* Sternberg.

Figs. 1, 2. Unmacerated cushion compression in surface view. 1, showing stomatal pits; photographed at 45°, × 100. 2, single pit photographed at 25° to the horizontal, × 1000.

Figs. 3, 5. Cushion cuticles prepared by oxidation with Schulze solution and clearing with dilute ammonia solution; vertical photographs. 3, epidermal cells and three stomata are visible. The guard-cell cuticles overlap the epidermal cuticle and show remnants of their central thickenings, × 500. 5, single stoma enlarged from fig. 3, × 1400.

Figs. 4, 6. Stomata showing well-preserved central thickenings; vertical photographs, × 1400.



THOMAS, Lepidodendroid stomata

The central portions of the guard cells seem to be selectively dissolved away while the persistent walls show limited disjunction in their terminal regions presumably representing two areas of fusion of the two cells (Pl. 81, figs. 1, 3). An outer layer therefore seems to have been removed by the alkali revealing a more resistant inner layer which is presumably thickened in some way.

*Lepidophloios acerosus* Lindley and Hutton

*Material.* No. 764, Kidstone collection, Institute of Geological Sciences, London; from above the Kiltongue Coal, Foxley, near Glasgow, Lanarkshire; *communis* Zone, Westphalian A.

*Lepidophloios* differs from *Lepidodendron* in having a vast majority of its leaf cushions bulging outwards and downwards in such a manner that they all partially overlap other cushions below them on the shoot. Therefore, only the cushion surfaces above the leaf scars are normally visible in compression fossils. In *L. acerosus* there is one further difference in that stomata are restricted to the upper cushion surfaces while the lower obscured surfaces have only elongated epidermal cells and no stomata (Pl. 82, fig. 6). No details are given here of specific epidermal characters. These will be better dealt with in a comparative account including details of other *Lepidophloios* species.

Similar methods of preparation were attempted here as were used on the specimen of *L. veltheimii*. Comparable results were generally obtained although in some ways the guard-cell structures revealed were more spectacular. The more fragile nature of these cuticles prevented their preparation without alkali treatment as they were always virtually destroyed during attempts to mechanically remove the adhering compression. However, limited immersion in alkali gave useful preparations in which the stomata appear entire and almost unaffected even though the epidermal cell anticlinal walls are clearly visible (Pl. 82, fig. 5). In contrast, complete maceration with extensive alkali treatment entirely destroyed the guard cells except for the cuticles of their upper walls. This left the stomatal apertures as faint longitudinal ridges or as narrow slits in the guard-cell cuticles (Pl. 82, fig. 6). No stomatal pit cuticles are visible so the guard cells were apparently superficial.

Immersion for about one or two minutes removed most of the cell structure but left some parts of the guard cells undamaged. This treatment revealed two structures from within the guard cells. An oval plate with a central ridge (Pl. 78, fig. 4) and a ridge-like structure in the vicinity of the outer edge of the guard cells (Pl. 78, fig. 6). However, very few stomata were seen with both structures as variation in preservation was apparent in every cuticle fragment (Pl. 81, figs. 2, 4-6; Pl. 82, fig. 1).

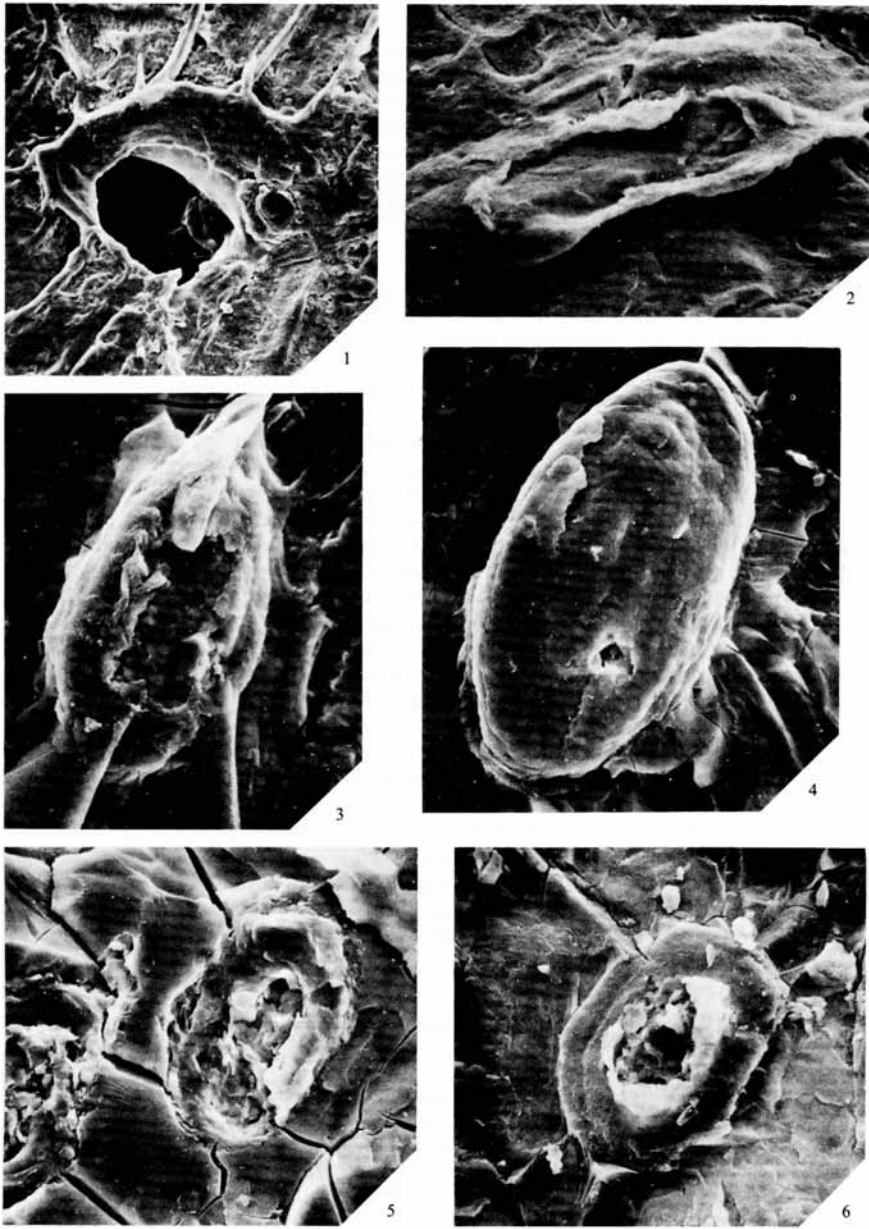
EXPLANATION OF PLATE 80

*Lepidodendron veltheimii* Sternberg.

Figs. 1, 2. Overmacerated stomata which have lost their guard-cell cuticles. Stomatal pit cuticles can be seen overlapping the epidermal cuticles,  $\times 700$ . 1, vertical photograph. 2, photographed at  $45^\circ$ .

Figs. 3, 4. Macerated stomata after very brief alkali treatment,  $\times 1400$ . 3, partially destroyed guard cells. 4, complete guard cells.

Figs. 5, 6. Macerated stomata after no alkali treatment. The compression has been removed mechanically which has also partially destroyed the guard cells; vertical photographs,  $\times 700$ .



THOMAS, Lepidodendroid stomata

Some of the central structures show stages of disintegration like those in *L. veltheimii* (Pl. 82, figs. 3, 4) but in the majority of stomata they are either perfectly preserved or they are absent. They appear to be less firmly attached to the guard cells than in *L. veltheimii* because they nearly all appear to be undercut at their edges. This might be due to the selective removal of alkali soluble material which eventually leads to their dislodgement before they are destroyed by the maceration. The outer circular ridges are similarly affected so that the majority appear to be undercut.

#### DISCUSSION

These cuticle studies demonstrate the complex structure of the Lepidodendrolean stomata. Their guard cells have internal thickenings which appear to be made of some other substance than cutin as they react differently to maceration and alkali treatment. Many other fossil and living plants have guard-cell thickenings which have been described as lignin. Such lignin has been demonstrated in the guard cells of most gymnosperms and some vascular cryptogams and angiosperms (Thomas and Bancroft 1913; Kaufman 1927; Florin 1931; Boulter 1970).

Boulter has also illustrated, in his work on the Taxodiaceae, how such thickenings can be lost during cuticle preparations and in particular during immersion in alkali. If the alkali treatment was omitted the thickenings remained attached to the guard-cell cuticles and Boulter suggested that this was due to the fact that lignin is soluble in alkali as shown by Isherwood (1965). However, what is equally or probably more important is that the cellulose, or partially lignified cellulose, between the cuticle and the lignin is even more readily soluble in alkali. This removal of an intermediate soluble layer would then lead to a subsequent detachment of the lignin.

Cuticle preparations are fortunately not the only means by which lepidodendroid stomata can be examined. There are numerous specimens of petrified stems which have stomata preserved in their leaf cushion epidermises. The angle of cut of the stem section is naturally important as it determines the type of stomatal section that is visible. A horizontal or radial longitudinal stem section of a *Lepidodendron* stem will be cut at right angles to the cushion surface giving vertical stomatal sections, while tangential longitudinal stem sections will yield nearly all horizontal stomatal sections cut at varying depths from the epidermal surface. Sections of *Lepidophloios* are not, however, so easily definable due to the bulging and drooping nature of the leaf

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#### EXPLANATION OF PLATE 81

*Lepidodendron veltheimii* Sternberg.

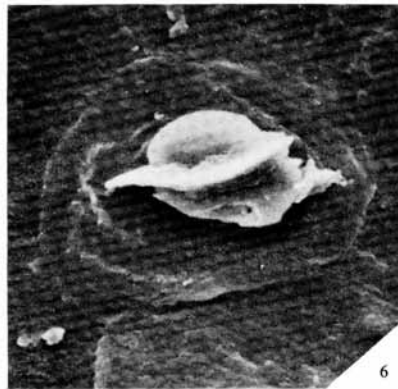
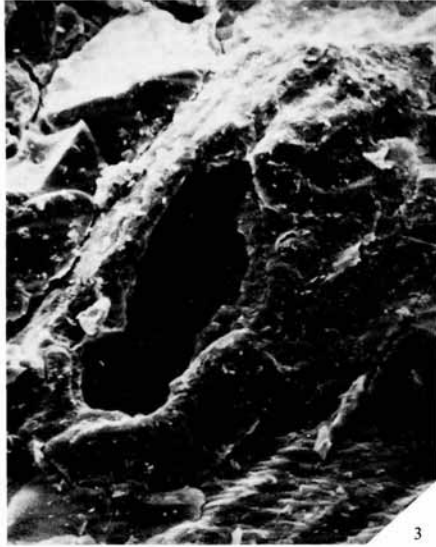
Figs. 1, 3. Macerated cuticle after limited alkali treatment; photographed at 45°. 1,  $\times 200$ . 3,  $\times 1000$ .

*Lepidophloios acerensus* Lindley and Hutton. Stomata after maceration and alkali treatment.

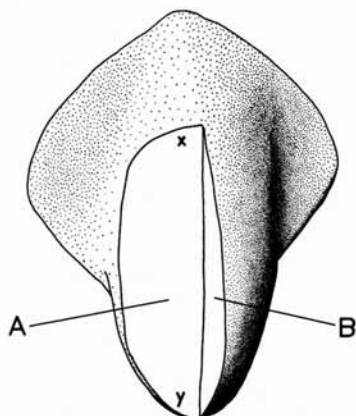
Figs. 2, 4. Stomata showing remnants of outer bands of thickening and of their stomatal apertures,  $\times 1500$ . 2, photographed at 45°. 4, photographed at 25° to the vertical.

Figs. 5, 6. One stoma showing a central thickening partially detached from the guard-cell cuticles. 5, photographed at 45°,  $\times 3000$ . 6, vertical photograph,  $\times 2000$ .





THOMAS, *Lepidodendroid stomata*



TEXT-FIG. 1. Diagrammatic reconstruction of a leaf cushion of *Lepidophloios*. Two types of longitudinal section are shown: A being tangential, and B radial, to the stem axis. Section A cuts the epidermis vertically at y but nearly horizontally at x.

cells are sectioned horizontally (Pl. 83, figs. 6-8) and sometimes when the cut is vertical (Pl. 83, fig. 4). In other examples, however, they may not be so obvious. The whole cell may appear dark (Pl. 83, fig. 3) possibly because the cut has been made at the ends of the cells taking in the curvature of the outer thickening, or the section may be along a guard cell thereby missing nearly all the internal thickenings (Pl. 83, fig. 5).

The *Lepidodendralean* stomata appear to have some comparable form of lignin thickening to gymnosperms, although our knowledge of their detailed structure is not so complete. The central oval structures bearing the 'crests' probably consist of one separate thickening from each guard cell, which became fused during the compression of the plant material. Each guard cell would therefore appear to have a band of thickening in the region of curvature from the outer to the poral walls of the cell. The 'crest' is apparently solid on all the specimens that I have examined with the s.e.m.

#### EXPLANATION OF PLATE 82

*Lepidophloios acerosus* Lindley and Hutton.

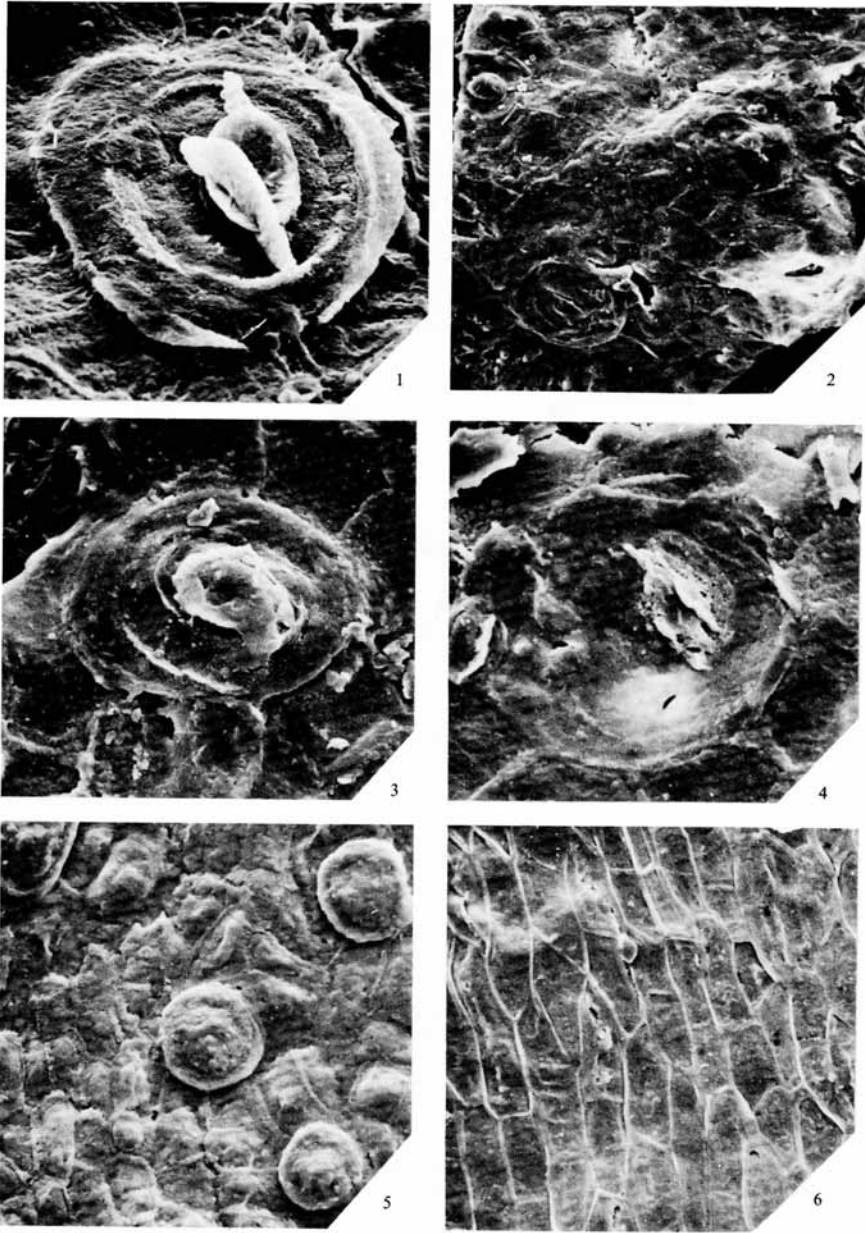
Fig. 1. Stoma showing a central thickening and a remnant of the outer thickening; photographed at 45°, × 3000.

Fig. 2. Overmacerated cuticle showing stomata with no remnants of thickenings; vertical photograph, × 500.

Figs. 3, 4. Stomata showing partially destroyed central thickenings; vertical photographs, × 1500.

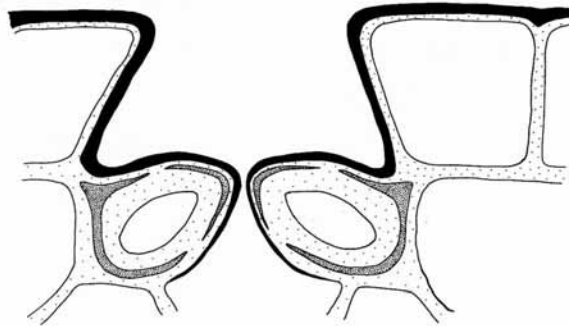
Fig. 5. Macerated cuticle with limited alkali treatment showing entire stomata; photographed at 20° to the vertical, × 550.

Fig. 6. Cuticle from the lower surface of the leaf cushion showing elongated epidermal cells and no stomata; vertical photograph, × 500.



THOMAS, Lepidodendroid stomata

although some appear to be raggedly split at their ends when seen with transmitted light. Indeed, one might always expect a narrow gap representing the stomatal aperture. Perhaps a more realistic suggestion would be that the 'crest' consists of the poral walls of the guard cells; thus being made of cuticle, cellulose, and lignin—all having become fused together during fossilization. This would then explain the undercutting of the outer edges of these inner thickenings by the removal of the intermediate cellulose wall. The outer ring-like structure can then be interpreted as separate thickenings which are adjacent to the outer walls of the guard cells, although this shape as seen in maceration preparations will have been previously altered during compression. The areas attached to the cuticle are broader than the 'upstanding ridges' which suggest that they were keeled curved bands similar to the type found in many gymnosperms. Compression will, however, have shortened the upstanding ridges of these thickenings.



TEXT-FIG. 2. Diagrammatic reconstruction of a vertical section through a lepidodendroid stoma. Cuticle is shaded black and lignin is densely stippled.

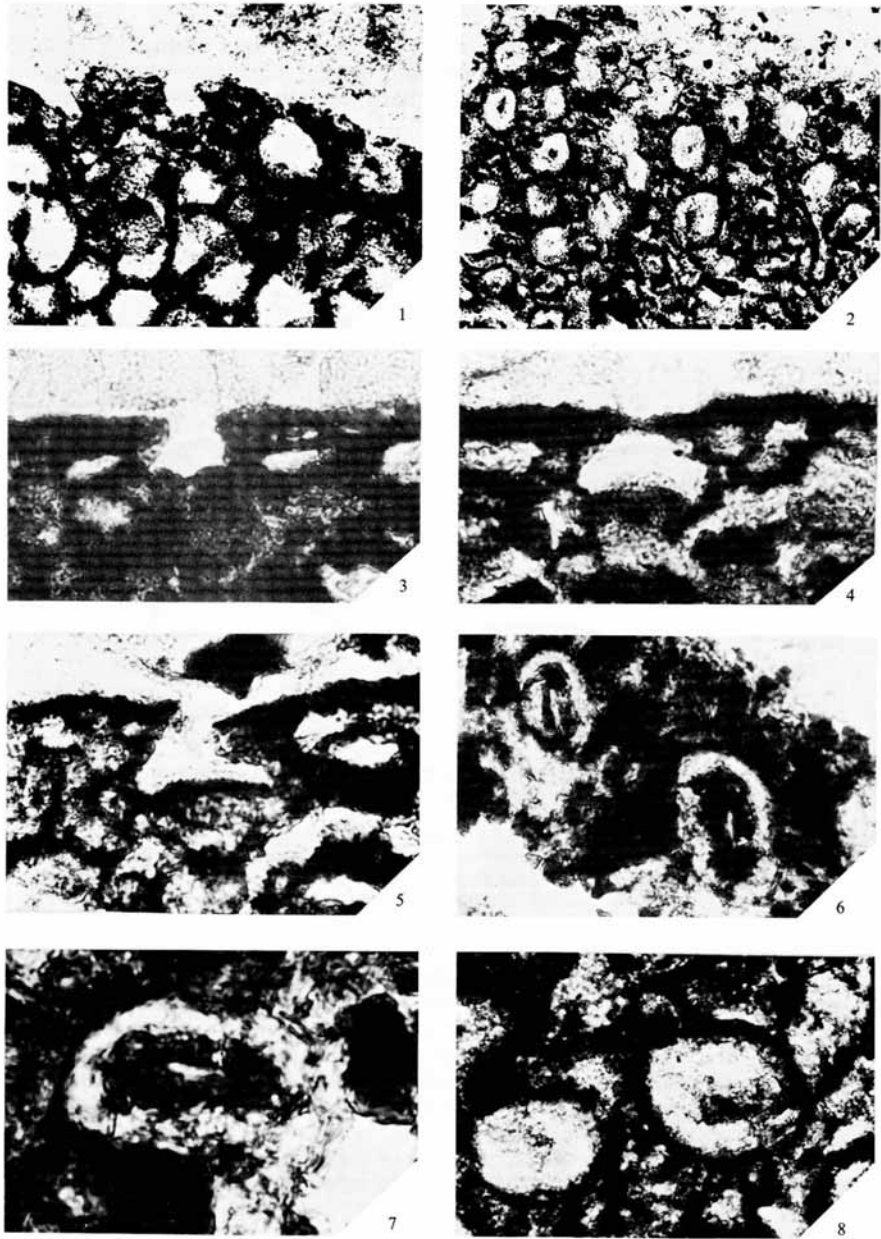
#### EXPLANATION OF PLATE 83

Petrified leaf cushions of *Lepidophloios*.

Figs. 1, 2, 6-8. *Lepidophloios scottii* Gordon from the Lower Carboniferous, Pettycur Limestone, Fife, Scotland. No. PB 123, Institute of Geological Sciences, London.

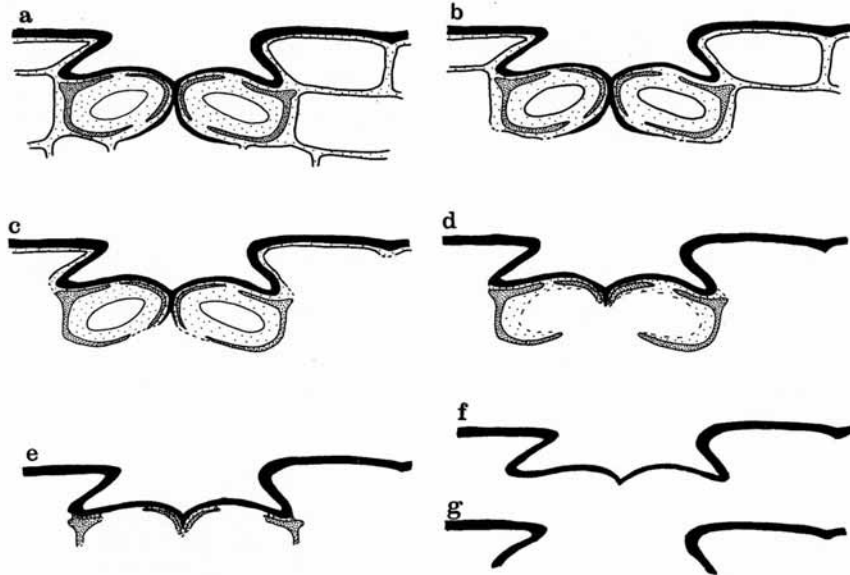
Figs. 3-5. *Lepidophloios fuliginosum* Williamson from the Westphalian of Shore, Lancashire. No. V 52017, British Museum (Natural History), London.

1, oblique vertical section through the outer layers of a leaf cushion. Two stomatal pits are visible opening on to the cushion surface, while a third pit is not cut through its aperture,  $\times 400$ . 2, oblique horizontal section through the epidermis and sub-epidermal cells of a leaf cushion. Many guard-cell pairs are cut showing varying remnants of their internal thickenings,  $\times 400$ . 3, vertical section through a stoma showing two guard cells sunken in a pit,  $\times 800$ . 4, oblique vertical section through a stoma showing the sunken guard cells with their internal thickenings,  $\times 800$ . 5, vertical section through a stoma showing a sunken guard cell cut longitudinally,  $\times 800$ . 6, horizontal section through a pair of sunken guard cells. The outer ring-like thickening appears darker than the rest of the cell walls,  $\times 1600$ . 7, 8, both are horizontal sections through the guard cells showing remnants of the central thickenings,  $\times 800$ . Fig. 7 appears to be cut at a lower level than Fig. 8 as it shows what appears to be a 'crest' in section.



THOMAS, Lepidodendroid stomata

Following the reasoning outlined above I have attempted to construct a tentative diagrammatic vertical section through a stoma as it might have appeared in life (text-fig. 2) and have given a series of sections showing the probable sequence of breakdown during maceration (text-fig. 3).



TEXT-FIG. 3. A series of diagrammatic sections showing the effects of maceration on lycopod stomata. (a) Stoma after vertical compression before maceration has begun. (b) Loss of the sub-epidermal tissues and the commencement of guard-cell disintegration. (c) Epidermal cell loss and breakdown of the guard-cell walls to expose the underlying lignin. (d) Continued breakdown of the guard cells and the exposure of the epidermal cell cuticles. (e) Reduction of the lignin to remnants attached to the guard-cell cuticles. (f) Removal of all cellulose and lignin from the guard cells. (g) Loss of the guard-cell cuticles exposing the stomatal pit cuticle.

The lignin would probably affect the way in which the stomatal aperture opened and closed but it is not yet perfectly clear how it did this. I would, however, tentatively suggest that the combined rigidity of the two separate lignin thickenings held the central parts of the guard cells relatively firm during turgor pressure and volume changes. Turgor pressure increase would presumably lead to an increase in volume tending to force apart the guard-cell walls but the central unfused portions would remain almost unaltered in shape. This would result in an opening of the stomatal aperture. Conversely turgor pressure decrease would result in a closure of the aperture. Such a mechanism is very reminiscent of that found in many monocotyledons where the dumb-bell shape of the guard cells reflects the extreme rigidity of their central portions.

*Acknowledgements.* I would like to thank the Keepers of Palaeontology of the British Museum of Natural History and the Institute of Geological Sciences for the loan of their specimens. The Scanning Electron Microscope part of the work was made possible by a grant from the Central Research Fund of the University of London.

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B. A. THOMAS  
Department of Biological Sciences  
University of London Goldsmiths' College  
New Cross  
London, S.E. 14.

Revised typescript received 12 October 1973