#### By

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FLORIN in his excellent work deals in detail with the leaf-epidermis of *Coniferae* and *Cordaites*, precisely with the structure of the stomas and he reaches important phylogenetic conclusions. He describes briefly in his paper the leaf-structure of some types of the extinct *Psilophyta* and *Pteropsida*, but he does not deal with the leaf-structure of *Cycadales*, especially the stoma. We get acquainte l with the leaf-epidermis of many extinct *Pteridospermae* and *Cycadales* by the recent paleontologic findings. They are very difficult to identify as we have no synthetic work which deals with the structure of leaf-epidermis of *Cycadales*, at least by photo. This work intends to reterieve the lack, by describing the structure of the leaf-epidermis of living *Cycadales* and wishes to help the paleontologists at the identification of the paleontologic findings.

## The acquisition of the material.

First of all we needed definite material to achieve our aim. We requested material from countries in which these rare woody-plants grow, secondly from Botanic Gardens, where they are kept in hothouses. (Berlin, Kew, etc.) In such a way we get leaves from 8 of the living genera of *Cycadales*. The Australian *Bowenia* is still missing, I am unable to examine the leaf-epidermis of this genus at present. I hope briefly described.

In this dissertation I deal only with the surface-structure of the *Cycadales* leafepidermis, I intend to deal with the internal anatomy of the leaves in a second work. In the present paper the method employed to examine the epidermis of the leaves is briefly described.

We cut a little piece of  $1 \times 1$  cm aut the margin of the studied leaf in order to prepare the final section. The epidermis of the right and back side of the leaf remains after spreading out side by side. Such a preparation immediately proves the hypostomatic or amphystomatic character of the leaf. We put the little piece in an eprouvette, we heat it carefully by *Schultze*'s method, (KClO<sub>3</sub> + HNO<sub>3</sub>) till the leaf's leaves dissolves completely and there remains only the transparent upper and under epidermis, so the margin of the spread leaf keeps together the right and back side. green colour disappears and becomes nearly transparent. The mesophyllum of the

It promotes the exact examination of the details when we treat the epidermis-preparatum by various simple or double colouring methods. For the colouring for instance gentiana-violett, vesuvin, methylen-blue or silvering method are suitable. With a colouring method perfect contrast preparations are obtained, fit for photographing and for detailed studies. It is important to take photograms to observe the quantity, size, arrangement of the stomas in the epidermis and using magnification. minute details can be fixed well too. In order to facilitate the view, x-50 magnification photos of the right and back side of the leaves were taken and the minute details illustrated in x-300 enlarged photos. Sometimes we took two photograms of the same stoma, mainly in species at which the stoma is nearly crater-like, we can observe on the two photos taken, adjusted in the same manner the upper part i. e. the top of the crater and the aperture of the closing-cells located dcep down at the bottom of the crater sharply. (See *Cycas, Encephalartos* photos.)

Among the 9 living Cycadales genus the following 8 genera were examinded:

1. Ceratozamia mexicana Brongn.

- 2. Cycas revoluta Thunbg.
- 3. Dioon edule Lindl.
- 4. Encephalartos altensteinii Lehm.

5. Macrozamia spiralis Miq.

- 6. Mycrocycas calocoma A, DC,
- 7. Stangeria eriopus Kunze.
- 2. Zamia portoricensis Urban.

#### **Detailed** descriptions.

1. Ceratozamia mexicana Brongn. Material obtained from Miami, Florida. (Mr. Stanley C. KIEM.) The leaf is characteristically hypostomatic. On the right side of the leaf the epidermis-cells are narrow and elongated in direction of the longitudinal axis. The single cell is hardly 10 microns wide and the most wide cell is scarcely 16-20 microns. Their length is different too. Certain cells are hardly 25-30 microns long, other 150-200 microns long and their walls link at transverse or at various angles. In some places many hundred microns long cells fit into the longer or shorter cells, which cells have thinner walls than the above mentioned narrow epidermis-cells. On the surface of the epidermis-cells with thicker walls there are tiny warts. The epidermis-cells on the back side of the leaf are of two kinds. Elongated and thinwalled cells fit in the row between the short and thickwalled cells. The stomas line up in longitudinal strips here too, but some strips do not separate from its vicinity firmly, so that in the arrangement of the stomas there is no real system. The crater-like structure can be observed with this genus too, but only to a certain extent. The length of the closing-cells is 50-60microns, the width of both closing-cells is 40-59 microns. The tips of the two closing-cells are cut off straightly in the direction of the soma. Between the concave sides shorter or longer quadrangles are formed, 20-22 microns wide and 30-35 microns high. The closing-cells are sourrounded by one or two rings of accessory cells. There are 23 stomas in 1 mm<sup>2</sup>. (See: Plate I.)

2. Cycas revoluta Thubg. Material obtained from Botanic Garden Szeged. It has also a typically hypostomatic leaf-structure. On the rightside

of the leaf the cells line up parallel with the longitudinal axis, they are 16-30 microns wide and 100-130 microns long. The shorter wall bends onto the longer rectangular or oblique-angled one, on another occasion it is wavy. The epidermis-cells are in certain cases more or less isodiametric and their walls are waved too. The surface of the epidermis-cells is full of little pores which are arranged in 2-3, or even in 4 longitudinal walls. The back side of the leaf: the stomas are arranged irregularly, in some places they are near each other or there is a breach between, them. The stomas of the Cycas are characteristic and posses a definitely crater-like structure. The upper-aperture of the crater is generally round or elliptic. 10-12 cells located in a threaded direction form the boundery. The two closing-cells lie in the bottom of the crater and between them is the split-like aperture. The crater-cells are bordered by 10-12 crown-cells at their foot in a ring-like manner. The size of the stomas is 70-80 microns, whilst the upper aperture is a circle or an ellipse, with a diameter of 20-30 microns. The size of the crown-cells is 20-22 microns, the width is about the same. The clearness of the photo No 7 is adjusted to the stomas and the crater-cells, the other photo shows in the same size and setting the closing-cells and stomas on the bottom of the crater, This cattle-like stoma is characteristic for the Cycadales, such structure of the stoma is not to be found in the case of other species of the genus Cycas. There are 67 stomas in 1 mm<sup>2</sup>. (See: Plate II.)

3. Dioon edule Lindl. Material obtained from Miami, Florida. (Mr. Stanley C. KIEM.) The leaf is charakteristically hypostomatic. There are no stomas on the right side. The epidermis-cells are elongated parallel with the longitudinal axis. The width of the cells is 18-20 microns, the length is 20-80 microns. They have generally the shape of an elongated oblonge, the shorter sides are perpendicular to the longer ones, or bend towards them at various angles of inclinations. The tips of the cells and the way they join vary too, they terminate here and there like forks or in other forms. On the back side of the leaf the stomas line up in longitudinal strips. The strips of stomas alternate with strips without stomas. In the strips with stomas there are 5-6 gaps situated irregularly, loosely near each other. (See photo No 10) The structure of the single stoma is essentially like, that of the Cycas, i. e. like a crater. The photo No 11 shows the upper aperture of the crater, the photo No 12 the closing-cells on the bottom of the crater. The size of the crater-cells is 40-55 microns, the size of the ellipse-shaped gap of the crater is  $32 \times 16$  or  $18 \times 16$  microns respectively it is interesting that the longitudinal axis of the ellipse-shaped crater-gap is situated transversally to the aperture of the closing-cells. Among the epidermis-cells with thin walls there are in some places cells with thicker walls and varied shape, like real idioblasts or little idioblast-fields. The staining of this cells differs from the other cells: on the side of the crater of the stomas there is no ribbing as at the Cycas. This is the essential difference between it and the Cycas. (See photo No 7) There are 48 stomas in 1 mm<sup>2</sup>. (See: Plate III.)

4. Encephalartos altensteinii Lehm. Material obtained from the Botanic Garden Berlin. (Miss Eva POTSTAL) The leaf is charakteristically hypostomatic. It has no stomas on the right side. The single cells are isodiomet-

ric and have various shapes, triangle, oblong, quadrangle and differently elongated cells join each other in various manners. (See photo No. 14) On the back side of the leaf the stomas are arranged in narrow or wide strips without any definite order. The stomas are slightly elongated, craterlike, generally 30--35 microns wide and 14-16 microns long. The aperture of the crater is nearly as wide as the outline of the closing-cells. As contrasted with *Dioon* it is characterised by the longitudinal axis of the crater-aperture which always runs parallel with the longitudinal axis of the closing-cells and is never transversally situated as in the case of *Dioon*. The tips of the beanshaped closing-cells are obliquely cut off towards the centre or are a little concave. Other crater-cells are slightly raised, but under such crater-apertures the gaps of the closing-cells can be not, or hardly seen. Consequently *Encephalartos* has two kinds of stomas, lying sometimes side by side. (See photo *No 16*) There are 67 stomas in 1 mm<sup>2</sup>. (*See: Plate IV.*)

5. Macrozamia spiralis Miq. Material obtained from Brisbane, Australia. (Mr. WILKES) The leaf-structure is typically hypostomatic. The right side of the leaf has not any stomas. The single epiderm-cells (seen from above) set in longitudinal rows, run generally parallel with the longitudinal axis of the leaves. The shape of the single cells varies to a great extent. They are generally 150-200 microns long and 20-25 microns wide. The cells are elongated in the direction of the longitudinal axis and therefore have various shapes or they are arranged perpendicularly or obliquely to the longitudinal row. This various shape of cells can be seen well on the x 50 and x 300 magnified photo No 17, 18. On the back side of the leaf the shape, size and arrangement of the epidermis-cells are similar to those on the right side. The single stomas are arranged in longitudinal strips, generally 5-6-7 stomas line up side by side in loose order. The single stomas are elongated in the direction of the longitudinal cell rows, the closing-cells are bean-formed, the accessory cells are long-shaped too and they line up close to the closing-cells. The inner side of the closing-cell is concave so that the breach between the concave halves forms a longish oblong, in the middle of which there is the split-like aperture. The tips of the closing-cells are cut off outwards obliquely i. e. the obtuse triangular ends in the four vertexes of the bean-shaped closing-cells can be seen. The length of the closing-cells is generally 70-80 microns, the width of the double ones is 45-50 microns. The oblong-field of the concave side is 204-22 microns, its height is 40-45 microns. - There are 23 stomas in 1 mm<sup>2</sup>. (See: Plate V.)

6. Microcycas calocoma A. DC. Material obtained from M1ami, Florida (Mr. Stanley C. KIEM.) The leaf is characteristically hypostomatic. On the right side of the leaf the cells are elongated in the direction of the longitudinal axis of the leaf. The cells are 25—32 microns wide, 200—220 microns long and they fit into one another, the tips vary to a great extent. On the back side the stomas line up in longitudinal strips, in strips there are 3—6 rows of stomas. In the neighbouring rows there is no definitive system. The closing-cells of stomas are 40—60 microns high, together the width of the two closing-cells is 27—28 microns wide, the height is the same. A typi-

cal feature is that the tips of the closing-cells are concave and not cut off straightly towards the gap, — towards the tip they become sharp. The accessory cells are always well visible beside the closing-cells. There are 40 stomas. in 1 mm<sup>2</sup>. (See: Plate VI.)

7. Stangeria eriopus Kunze. Material obtained from Kew, Royal Botanic Garden, (Sir E. SALISBURY.) The leaf is characteristically hypostomatic. The single cells on the right side of the leaf are slightly elongated, but the most characteristic feature is the wavy margin, nearly serrated. The humps on the one side of the cell fit exactly into the cavities of the other. The second typical feature is that on the surface of the epidermis-cells longitudinal and dense wavy ribs extend. These ribs originate radially from the central-cell and proceed in the riblike structure of the neighboruring cells. (See photo No 26) On the back side of the leaf there are no longitudinal ribs on the surface of the cells, only in strips corresponding to the veins of the leaves. The sidewalls of the cells are winding. The single cells are elongated in the direction of the capillaries, the margin and the surface are less wavy. The stomas line up irregularly, though assemling in longitudinal rows occurs occasionally. The length of the closing-cells is 55-60 microns, the width of the two closing-cells together is 30-35 microns. The sharpe and structure of the closing-cells differ from the above described types, the bean-shaped type of the closing-cells is not cut off or concave, but round and thin. The gap of the concave side of the closing-cells is narrow and short, the length is 10-16 microns, the width is 5-6 microns. It is remarkable that here and there thin spiral or netlike tracheaids penetrate into the point of the little tooth. of the leaf-margin. There are 21 stomas in 1 mm<sup>2</sup>. (See: Plate VII.)

8. Zamia portoricensis Urban. Material obtained from Miami, Florida. (Mr. Stanley C. KIEM.) The leaf is hypostomatic. On the right side the epidermis-cells are elongated in the longitudinal axis, the width is 10-15microns, the length is 250-300 microns, the endings of the cells composeoblique walls or various insertions by which they fit into one another. The walls are thickened in two manners. Some cells have thick walls, others thinnerones. This cellwall-structure differs essentially too. On the back side of the leaf syndetocheil structure of the stomas is visible particularly. The stomas line up in longitudinal strips without any regularity, but we can observe longitudinal and partly oblique rows here and there. The crater-like structure of stomas is observable too. The aperture of the crater is a most. regular circle, the length of the closing-cells is 27-30 microns and the width of the two closing-cells together is nearly the same, 30-32 microns. The combined shape of the two closing-cells is a nearly regular circle or short. ellipse. The tips of the closing-cells are concave and not straight, a short. extension is observable on both sides in the direction of the aperture. Thereare 53 stomas in 1 mm<sup>2</sup>. (See: Plate VIII.)

#### Summary

On studying the structure of leaf-epidermis of the characteristic species of the above treated 8 genera more precisely that of the structure of the

stoma located among the epidermis-cells thoroughly, it can be stated, that the genera of *Cycadales* can be differentiated from one another according to the structure of the leaf-epidermis. Their epidermis or stoma-structure resembles that of the *Coniferales*. There are some types e. g. *Cycas*, which have no similarity with *Coniferales*, the stoma-structure of which resembles that the air-gaps exhibiting craterous structure of *Marchantia*. The resemblance is really striking! On the other hand, there are some genera which show similarity with the stoma-structure of the extinct *Pteridospermae*, e. g. *Lyginodendron oldhamium*. The aim of this short paper is to draw conclusion from the comparison of the structure of the leaf-print in fossils concerning the genetic relation with living forms.

The aim of the following detailed communication will be to study not only the structure of the leaf epidermis of *Cycadales*, but the structure of the leaf's and stem«s internal organisation too. I hope to publish it soon.

#### References

- Arnold, Ch. A.: Origin and relationships of the Cycads. Phytomorphology 3. 51-65 (1953).
- (2) Boureau, E.: 1954. Anatomie vegetale, Paris.
- (3) Cookson, I. C.: On Macrozamia hopeites an early tertiary Cycad from Australia. -Phytomorphology 3, 306–312 (1953).
- (4) Eames, A. I. et Mac Daniels: 1951. An introduction to plantanatomy London.
- (5a) Florin, R.: 1931. Untersuchungen zur Stammesgeschichte der Coniferales und Cordaitales. Almquist et Wiksels, Stockholm.
- (5b) Florin, R.: (1933) Studien über die Cycadales des Mesozoikums nebst Erörterungen über die Spaltöffnungsapparate der Bennetitales. Almquist et Wicksels, Stockholm.
- (6) Gothan, W.-Weyland, H.: 1954. Lehrbuch der Paläobotanik, Akademie Verlag, Berlin.
- (7) Hofmann, E.: (1934) Paläohistologie der Pflanzen. J. Springer, Wien.
- (8) Jeffrey, E. Ch.: (1917) The anatomy of woody plants. University of Chicago, Press Chicago.
- (9) Laubenfels, D. I. de: (1953) The external morphology of coniferous leaves, Phytomorphology, 3, 1-20.
- (10) Linsbauer, K.: (1930) Die Epidermis. In Handbuch der Pflanzenanatomie. Gebr. Bornträger, Berlin.
- (11) Mägdefrau, K.: (1956) Paläobiologie der Pflanzen. Veb Gustav Fischer Verlag, Jena,
- (12) Pilger, R.: (1926) Cycadaceae. In Engler-Prantl: Die nat. Pflanzenfam. Leipzig.



1. Right side x-50



2. Right side x-300



3. Back side x-50



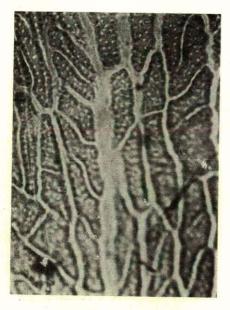
4. Back side x-300

Ceratozamia mexicana Brongn.

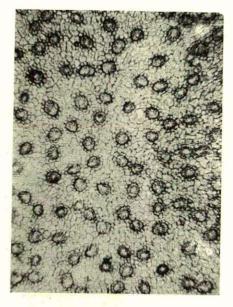
157

Plate I.

Plate II.



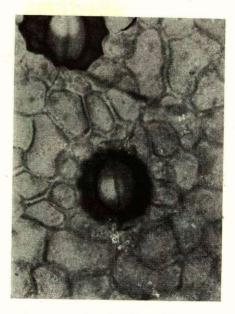
5. Right side x-300



6. Back side x-50



7. Back side x-300



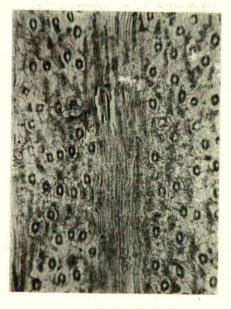
8. Back side x-300

Cycas revoluta Thunb.

Plate III.



9. Right side x-300



10. Back side x-50



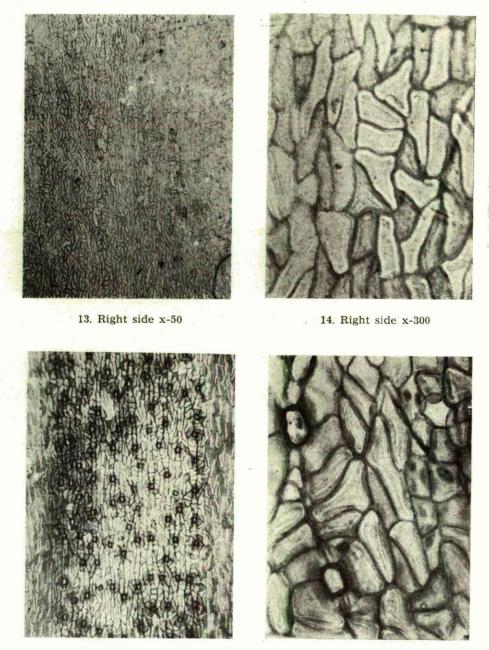
11. Back side x-300



12. Back side x-300

Dioon edule Lindl.

Plate IV.

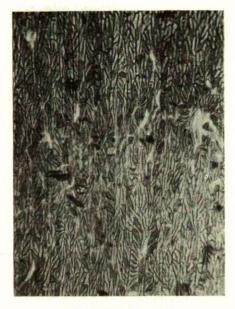


15. Back side x-50

16. Back side x-300

Encephalartos altensteinii Lehm.

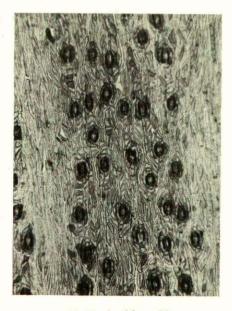
Plate V.



17. Right side x-50



18. Right side x-300



19. Back side x-50



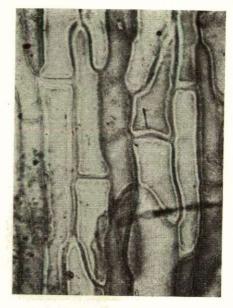
20. Back side x-300

Macrozamia spiralis Miq.

Plate VI.



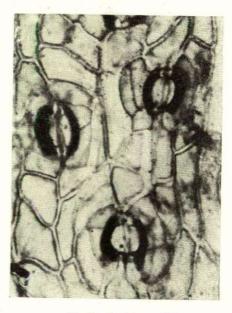
21. Right side x-50



22. Right side x-300



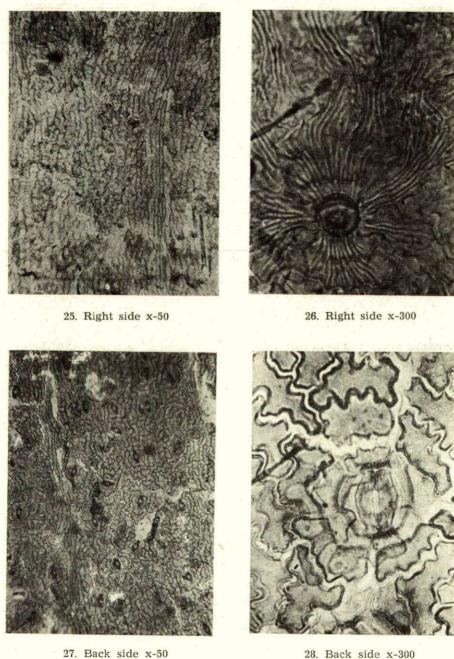
23. Back side x-50



<sup>24.</sup> Back side x-300

Microcycas calocoma A. Dc.

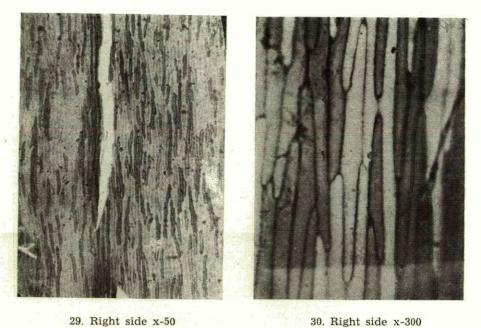
Plate VII.



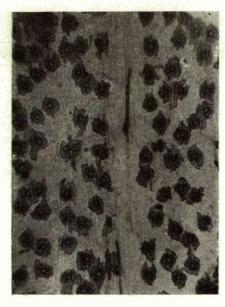
28. Back side x-300

Stangeria eriopus Kunze

Plate VIII.



30. Right side x-300



31. Back side x-50



32. Back side x-300

Zamia portoricensis Urban