

74-21,982

MILINDASUTA, Boon-earn, 1934-  
DEVELOPMENTAL ANATOMY OF CORALLOID ROOTS IN  
CYCADS.

The University of Oklahoma, Ph.D., 1974  
Botany

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

DEVELOPMENTAL ANATOMY OF CORALLOID ROOTS IN CYCADS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

BOON-EARN MILINDASUTA

Norman, Oklahoma

1974

DEVELOPMENTAL ANATOMY OF CORALLOID ROOTS IN CYCADS

APPROVED BY

Norman Boh

H. G. ...  
Eddie Carol Smith

Frank ...

... Kerner

DISSERTATION COMMITTEE

## ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Dr. Norman H. Boke, my major professor, for his advice, encouragement and helpful guidance throughout the course of this work. I am indebted as well to Dr. Howard W. Larsh for his assistance during my graduate study.

I wish to thank the members of my dissertation committee, Dr. Norman H. Boke, Dr. George J. Goodman, Dr. Eddie C. Smith, Mr. Frank A. Rinehart and Dr. John W. Renner for their help in the planning of my graduate program and the preparation of this dissertation. Appreciation is also expressed to Mrs. Karen Bowers for the use of her camera.

Special thanks are given to my wife and my children for their patience and understanding.

Financial support for this graduate work was provided by an AID scholarship and the Royal Thai Government.

## TABLE OF CONTENTS

	Page
LIST OF ILLUSTRATIONS .....	v
Chapter	
I. INTRODUCTION .....	1
II. MATERIALS AND METHODS .....	5
III. OBSERVATIONS AND DISCUSSION .....	7
IV. SUMMARY .....	49
LITERATURE CITED .....	51

## LIST OF ILLUSTRATIONS

Illustration	Page
1-6 .....	22, 23
7-12 .....	24, 25
13-18 .....	26, 27
19-25 .....	28, 29
26-29 .....	30, 31
30-33 .....	32, 33
34-37 .....	34, 35
38-43 .....	36, 37
44-49 .....	38, 39
50-55 .....	40, 41
56-59 .....	42, 43
60-65 .....	44, 45
66-71 .....	46, 47, 48

# DEVELOPMENTAL ANATOMY OF CORALLOID ROOTS IN CYCADS

## CHAPTER I

### INTRODUCTION

In all the genera of cycads there are, in addition to normal roots, apogeotropic roots which extend above ground as numerous, dichotomously branched structures (Chamberlain, 1935). These outgrowths, which are modified perennial lateral roots (Spratt, 1915), are known as coralloid roots. They are not confined to the soil surface but may be found at depths 30 cm or more below ground level (Schneider, 1894; Life, 1901; Chaudhuri and Akhtar, 1931; Wittmann, Bergersen, and Kennedy, 1965).

The coralloid roots are often infected with endophytic blue-green algae which have been differently identified as species of Nostoc or Anabaena (Schneider, 1894; Spratt, 1915; Chaudhuri and Akhtar, 1931). More recently, Bowyer and Skerman (1968) have isolated the endophytes from Macrozamia lucida (Linnaeus) Johnson, Encephalartos

altensteinii Lehm., and Bowenia serrulata Bull. and identified them as species of Nostoc.

Typically, the endophytes are localized in a definite algal zone. Wittmann, Bergersen, and Kennedy (1965) explained that the single layer of cells which forms the algal zone in Macrozamia communis is a transformed epidermis and is enclosed by an outer cortex corresponding to a persistent rootcap. In young coralloid roots without algal infection this zone remains comparatively inconspicuous. Generally the algal zone is continuous except immediately below the lenticels, extending from the base almost to the root apex (Life, 1901; Spratt, 1915). The endophytes make their way through intercellular spaces as radial elongation of the host cells proceeds toward the root apex following algal infection (Wittmann, Bergersen, and Kennedy, 1965).

The presence of an algal zone, together with the outer cortex, gives cycad coralloid roots an unusual appearance not found in normal roots. Mostly, the algal zone has been described as a special layer of cells in the cortex (Schneider, 1894; Spratt, 1915; Chaudhuri and Akhtar, 1931). Although Schneider (1894) reported a rudimentary rootcap in Cycas revoluta, Life (1901) stated



that the coralloid roots of this species do not possess a true rootcap but have a sheath of several cell layers extending over the tip and enveloping the entire root as the outer cortex. Both Schneider (1894) and Life (1901) believe that the vascular cylinder of the coralloid roots is simple, as in ordinary roots. Spratt (1915) did not mention rootcap but explained that the algal zone is enclosed by a very definite phellogen which produces a few layers of parenchymatous cells towards the inside and a few very regular cells on the outside. McLuckie (1922) observed coralloid roots of Macrozamia spiralis and concluded that a rootcap is present and persistent; however, he observed no algal zone in this species.

Life (1901) concluded from his study of Cycas revoluta that coralloid roots could have at least two functions: that of aerating and that of assisting in nitrogen assimilation. Bergersen, Kennedy, and Wittmann (1965) have shown that the algal endophytes in coralloid roots of Macrozamia communis are the agents of nitrogen fixation rather than nitrogen-fixing bacteria, as suggested by Spratt (1915) and McLuckie (1922). Although young coralloid roots without endophytic blue-green algae do not fix nitrogen, the nitrogen fixed in those that have them is available

for the growth of the plant. Since the alga-containing coralloid roots could be found at depths up to 30 cm below the ground, the existence of the alga in the absence of light is problematical. Recently it has been found that a species of Nostoc from coralloid roots of Macrozamia lucida grows heterotrophically in complete darkness under both aerobic and anaerobic conditions (Hoare et al., 1971). Therefore, it was inferred that the algal endophytes grow heterotrophically at the expense of carbohydrates within the cycads and, in turn, assist the cycads in nitrogen assimilation.

No attempt has been made to study the symbiotic relation between the alga and the cycads in this investigation. The purpose of my study is to observe the developmental anatomy of coralloid roots among the available genera of cycads.

## CHAPTER II

### MATERIALS AND METHODS

Root specimens used in this study were collected from cycads growing in the University of Oklahoma greenhouse. The investigation was carried out with materials of Ceratozamia mexicana Brongn., Ceratozamia sp., Cycas circinalis L., Cycas revoluta Thunb., Dioon edule Lindl., Stangeria paradoxa Th. Moore., and Zamia sp. All the plants except Ceratozamia mexicana and Cycas circinalis were grown in pots. The latter were uprooted and the manner of origin of coralloid roots, as well as their external morphology, was observed.

When possible, three types of roots, namely, apogeotropic secondary roots with young coralloid outgrowths, young and mature coralloid masses, and normal lateral roots were collected from each plant. Coralloid roots were cut into 3-mm segments from the tip to the base; tips 5-mm long were cut from normal roots; and apogeotropic secondary roots were cut into 5-mm segments so as to include young

coralloid roots. These materials were immediately fixed separately in Craff V (Sass, 1958). After fixation, they were dehydrated in a tertiary butyl alcohol-ethyl alcohol series (Johansen, 1940) and embedded in paraplast (60 C). Longitudinal and transverse sections were cut at 6-10  $\mu\text{m}$  and stained with safranin (Johansen, 1940) and fast green (Boke, 1952).

The Körper-Kappe concept of Schüepp (1917, quoted by Clowes, 1961) was employed in order to show the presence of a rootcap and the ontogenetic relationships of the rootcap and secondary cortex of coralloid roots.

## CHAPTER III

### OBSERVATIONS AND DISCUSSION

Coralloid roots have been found in every species of cycad so far investigated. Normally they arise as lateral outgrowths from adventitious roots or secondary roots that have become exposed or nearly exposed at the soil surface (Fig. 1, 3, 5, 6). In the early stages of development they appear as modified, yellowish tan, lateral roots with rounded tips and dichotomous branching. By contrast, normal lateral roots are white with pointed tips and no dichotomous branching. Most coralloid roots lack algal endophytes at this stage.

The tips of upward-growing roots that give rise to coralloid outgrowths may protrude above ground and then curve downward, or the root apex may stop growing and become covered by a mass of coralloid roots (Fig. 15). Some of these roots, after reaching the soil surface and giving rise to coralloid lateral outgrowths, may grow parallel to the soil surface for a short distance, then

start growing downward and behave like normal roots as they grow away from the soil surface (Fig. 4).

In Cycas circinalis, Stangeria paradoxa, and Zamia sp., the larger masses of coralloid roots arising from the tips of big adventitious roots are predominant (Fig. 10, 11, 18), but some smaller masses arising from slender upward-growing secondary roots are also present (Fig. 10, 12). Although the fleshy adventitious roots of Stangeria paradoxa and Zamia sp. are quite short, about 15 cm long, they are as big as the tap root. They grow downward from the point of origin 2-3 cm, then curve upward and bear a mass of coralloid roots that covers the tip at about soil level (Fig. 10, 11, 12). Coralloid roots may also originate directly from the surface of the swollen base of the tuberous stem in Stangeria paradoxa (Fig. 8). In Cycas circinalis, masses of coralloid roots have been found to arise from the tips of upward-growing roots as much as 2 m away from the plant. Fleshy adventitious roots are also produced in large numbers by Cycas revoluta, but none were found that bore coralloid masses at their tips as in Stangeria paradoxa or Zamia sp. (Fig. 7). Some of these Cycas roots may grow upward to the soil surface and then turn downward, with only that part of the root surface

that touches soil level giving rise to a few small coralloid lateral outgrowths. The distal parts of those roots which penetrate deeper into the soil bear no more coralloid roots (Fig. 4). Most of the coralloid clusters in Cycas revoluta arise from upward-growing secondary roots (Fig. 4, 7). In Ceratozamia mexicana, Ceratozamia sp., and Dioon edule, they generally arise from apogeotropic secondary roots (Fig. 2, 16).

Thus far, coralloid roots appear to be most numerous at the soil surface. Only in Ceratozamia sp. (Fig. 2) and Cycas revoluta (Fig. 7) did some of them occur at depths of 6 cm and 8 cm, respectively. In both cases the algal endophytes were present. However, the ability to produce coralloid roots is not limited only to roots growing upward to the soil surface. In one plant of Ceratozamia mexicana small lateral roots that grew out from the bottom of the box in which the plant was growing also gave rise to coralloid outgrowths (Fig. 14). These lateral roots originated about 20 cm below the soil level in the box, but as they grew out and became exposed to the atmosphere they were able to produce coralloid roots. Therefore, in this plant of Ceratozamia mexicana there were two layers of coralloid roots: one at the surface of the soil in the

box and the other at the surface of the soil on which the box was resting.

In later stages of coralloid root development, dichotomous branching becomes more and more prominent and the color darkens noticeably. Lenticels also develop progressively as growth in length continues. When mature, coralloid roots are various shades of brown, with greenish yellow at the active part near the tips; and in Dioon edule, there are numerous dark tannin spots visible on the surface, even at the tip (Fig. 21). The average diameter of individual roots is between 2-3 mm, but the length varies. On the other hand, the diameter of coralloid clusters ranged from less than 1 cm to more than 17 cm (Fig. 17). All mature coralloid roots in every species observed in this study are associated with algal endophytes (Fig. 19-25). This is in partial disagreement with the observations of Spratt (1915), who found no algal zone in coralloid roots of Zamia lindenii and Ceratozamia mexicana. However, her descriptions of the coralloid roots of both species suggest that she may have observed them at a stage too young to be infected by the alga.

A study of transverse and longitudinal sections from apogeotropic secondary roots that have incipient coralloid



roots showed that these are lateral in origin (Fig. 26-35). Typically they arise endogenously from the multiseriate layers of pericycle that lie opposite the protoxylem poles. Quite often they branch dichotomously soon after emerging from the parent roots, which are diarch in Ceratozamia mexicana, Ceratozamia sp., and Cycas revoluta and triarch in Dioon edule and Stangeria paradoxa. However, the number of protoxylem poles may vary from diarch (Fig. 31) to hexarch (Fig. 37), even in the same root, as observed in serial transverse sections of Cycas revoluta. Generally, the primary xylem is exarch, but it may be mesarch in Stangeria paradoxa (Fig. 36).

After emergence from the parent roots, some coralloid roots may become infected by the alga, but some may not. However, alga-free examples were found only in small masses of coralloid roots in their early stages of development. Occasionally, in these young clusters, a few roots were already infected by the endophytes. Growth in length of young coralloid roots is much retarded as compared to normal roots at the same age, but growth in diameter increases noticeably.

Observations on the apical organization of cycad roots and its relation to the development of primary tissues

were made from median longitudinal sections of apices of normal roots (Fig. 38-43), alga-free coralloid roots (Fig. 44-49), and alga-containing coralloid roots (Fig. 50-55). Within a given type, different species of cycads seem to show a common pattern of apical organization which, in most respects, conforms with previous descriptions for that particular kind of root in Macrozamia communis (Wittmann, Bergersen, and Kennedy, 1965).

In normal roots the meristematic region is deeply situated under a massive rootcap. In contrast, the meristematic region in coralloid roots is covered by only a few layers of rootcap. The presence of a rootcap can be shown by an analysis of cell lineages and planes of division, based on the Körper-Kappe concept of Schüepp (1917, quoted by Clowes, 1961). Kappe-type T-divisions occur in the peripheral region of root tips, and root apices appear to have the Körper-Kappe boundary in a distinct layer of cells, which is analogous to the protoderm of normal roots (Fig. 56-59). Transverse divisions at the centre of the cap seem not to happen as frequently in coralloid roots as they usually do in normal roots, and the length of the cap is much less than that in normal roots of the same species. There is

an average of ten cell layers in the cap at the root apex of coralloid roots. Although the columella in coralloid roots is normally inconspicuous, it is very distinct in normal roots (Fig. 38-43).

In young, actively growing coralloid roots, the outer rootcap is usually differentiated into proliferated layers consisting of large, rounded cells loosely connected to each other (Fig. 32, 44-49). These loose masses of cells may soon be sloughed by the activity of periderm which develops acropetally beneath them (Fig. 32, 44). In slow-growing roots, periderm development may extend almost to the tips and may replace all the outer layers of rootcap. However, the inner part of the rootcap that is under the periderm will remain intact and become persistent as secondary cortex outside the tissue arising from the protoderm. The common rows of cells which extend from the centre of the cap into the secondary cortex indicate their ontogenetic relationships (Fig. 56-59).

Various interpretations have been proposed by earlier workers regarding the presence and extent of the rootcap in coralloid roots. According to Schneider (1894) and Chaudhuri and Akhtar (1931), it is represented by a mass

of loosely connected cells covering only the rounded end of the root. Life (1901) is, to my knowledge, the first person who called attention to the common origin of the rootcap and the outer cortex. His observations were later confirmed by McLuckie (1922), who further suggested that the outer cortex appears to be analogous to the velamen on the aerial roots of some orchids. Recently, Wittmann, Bergersen, and Kennedy (1965) have clearly explained the difference in origin between the outer cortex of coralloid roots and the velamen of orchid roots and reconfirmed the origin of the outer cortex from the rootcap. The Körper-Kappe concept which has been employed in this study not only reveals the presence of a rootcap in coralloid roots but also shows the ontogenetic relationship between rootcap and secondary cortex. The results of this investigation further support the earlier reports (Life, 1901; McLuckie, 1922; Wittmann, Bergersen, and Kennedy, 1965) of the common origin of rootcap and outer cortex in coralloid roots of cycads.

The protoderm, a single layer of cells which is destined to become the algal zone in alga-containing coralloid roots, appears to arise from the innermost layer of the rootcap (Fig. 56-59). As described previously, it is

buried under the secondary cortex in later stages of development and, therefore, has never been exposed. Initially, it appears as a continuous row of cells which remains undifferentiated. It keeps pace with cell enlargement, which has already begun in the adjacent cortex and rootcap, by transverse divisions and by gradual cell enlargement. It remains at this stage for a short distance; then it begins to enlarge radially while the cortex and the rootcap are enlarging longitudinally. As a result, the layer becomes wider, but the cells no longer keep pace with the rootcap and the cortex in a longitudinal direction. Therefore, the cells in this layer soon become separated from each other along their radial and transverse walls, leaving the narrow tangential walls in contact with the cortex on one side and the rootcap on the other (Fig. 60, 61). The intercellular spaces that result provide new habitat for the alga, which subsequently enters, extending the algal zone progressively as the root grows. The corresponding cell layer in alga-free coralloid roots is rather inconspicuous.

Normally, the algal endophytes are found living in available intercellular spaces in the algal zone (Fig.

60-62). They seem incapable either of infecting living cells or of making their way through living cells. However, they must enter the roots from outside prior to the formation of the algal zone in young coralloid roots because they do not occur in the parent root. Although large numbers may be washed from the surface of coralloid roots during the preparation processes, algal filaments were occasionally observed outside the roots, and quite often they were found at the root surface (Fig. 63). As described previously, the surface of young coralloid roots is usually covered with loose masses of cells arising from the outer part of the rootcap. It seems likely that the alga may easily travel through the spaces abundantly present between these outer layers (Fig. 63). If, by chance, they could find an opening into the inner layers before the advent of periderm, they should be able to establish themselves inside the roots. Figures 64 and 65 suggest that this may be correct. Certain openings were found in the secondary cortex of young coralloid roots which seem to provide channels for the entry of the alga. These channels were continuous from the root surface to the algal zone and were filled with algal filaments, which suggests that they may enter the

roots in this manner. The breakage in the secondary cortex that permits formation of the algal zone may arise at the point of separation between dichotomous branches (Fig. 64) or at the point where coralloid roots emerge from the parent roots (Fig. 65). Once the algae have established themselves inside the roots, they may in certain ways stimulate the development of the algal zone. However, the exact role of the algae in relation to the initiation of the algal zone is as yet undetermined.

Never before has the mode of entry of the algae into coralloid roots been definitely observed. However, this does not mean that what I have found in this investigation is the definitive one. In fact, it has been previously suggested by Schneider (1894) that the algae may enter through breaks in the dermal layers, but he did not actually observe it. Detection and proof of the mode of entry is not easy. The breaks in the dermal layers are so few that there is only a small chance of obtaining sections of them. Furthermore, the channels may not be straight and they may not be included in a single section. Therefore, they may not appear to be continuous pathways for the algae unless followed in a series of sections.

However, I could not find any evidence, as suggested by Life (1901), Spratt (1915), and Chaudhuri and Akhtar

(1931), that the algae enter coralloid roots through lenticels. There may be some separation of cells in the outer part of lenticels, which may suggest a pathway for the algae, but I have never observed any connection between these breaks and the algal zone. Besides, the algal zone is generally interrupted under the lenticels and is replaced by parenchyma-like cells. This, in my opinion, diminishes the possibility of origin of the pathway to the algal zone under the lenticels. So, it seems unlikely to me that this is the general mode of entry for the algae into the coralloid roots. As far as my observations are concerned, entry through a break in the dermal layers, as initially suggested by Schneider (1894), seems to be the most likely at present.

Beneath the algal zone is the cortex, which consists mainly of parenchyma. The ground tissues of the future cortex divide periclinally, increasing the number of cell layers in early stages of development, then begin to enlarge and undergo vacuolation. Vacuolation and cell enlargement of the cortex proceed rapidly in a centripetal direction. At maturity the cortex consists of about ten layers of cells that occupy most of the cross-sectional area of the root.



The endodermis originates from the innermost layer of the cortex. It becomes recognizable at a distance of 350-1130  $\mu\text{m}$  behind the apical initials (Table 1). Normally, the first Casparian strips were identifiable in the radial walls of the endodermis opposite the phloem regions, and then later appeared in regions opposite the xylem. The endodermis is generally present throughout the length of the root above the region of maturation; however, it appears to be crushed in later stages of development (Fig. 70).

As shown in Table 1, the first sign of differentiation in the procambium of coralloid roots begins with the appearance of vacuolated cells in the central part of procambium 180-330  $\mu\text{m}$  from the apical initials (Fig. 66). However, at about the same level secretory cells were frequently observed in regions destined to become phloem (Fig. 67). Development of the phloem seems to follow the appearance of these secretory cells in most species of cycads except in Stangeria paradoxa, which has no secretory cells in the phloem region. The correlation between the appearance of secretory cells and the development of phloem in coralloid roots of cycads, therefore, remains in doubt. However, the presence of secretory cells was described by

Wilcox (1954) as one of the first signs of differentiation of the phloem and the entire stele in Abies procera. Normally, vacuolation in the phloem region proceeds quite rapidly and is completed in advance of the first protoxylem, although no sieve areas were observed in the phloem region. Therefore, the phloem elements are hardly distinguishable from stelar parenchyma in early stages of development. Later, wall thickening may be detected, and the mature tissue may appear as a band of cells arranged in two or three rows between the ridges of xylem (Fig. 70).

The first protoxylem is visible at 320-620  $\mu\text{m}$  from the apical initials (Table 1). Basically, the development of primary xylem is exarch, but weakly mesarch xylem is found in coralloid roots of all species of cycads (Fig. 69). Coralloid roots are mostly diarch to tetrarch, but variation in number of xylem poles may be found within the same species (Table 1). Tracheary thickening is generally helical (Fig. 71). Occasionally, reticulate thickening is observed, most frequently on the end walls of some of the centrifugal metaxylem elements (Fig. 69, rmx).

Normally the pericycle is multiseriate. However, because of the occurrence of a few centrifugal mesarch primary xylem elements, roots with a locally uniseriate pericycle are occasionally observed.

TABLE 1

SEQUENCES OF DIFFERENTIATION IN CORALLOID ROOTS OF CYCADS AND THE NUMBER OF PROTOXYLEM POLES.

Plant	Distance from apical meristem ( $\mu\text{m}$ )				Number of protoxylem poles
	Vacuolation of metaxylem	Presence of secretory cells	Visibility of protoxylem	Visibility of endodermis	
<i>Ceratozamia mexicana</i>	*	240	340	380	Triarch
<i>Cycas circinalis</i> I	330	330	490	1120	Triarch
<i>Cycas circinalis</i> II	280	280	620	1130	Triarch
<i>Cycas revoluta</i> I	180	240	410	610	Tetrarch
<i>Cycas revoluta</i> II	220	200	380	490	Tetrarch
<i>Dioon edule</i> I	220	200	350	350	Tetrarch
<i>Dioon edule</i> II	*	*	370	370	Triarch
<i>Stangeria paradoxa</i>	220	**	320	900	Diarch*** Triarch Tetrarch
<i>Zamia</i> sp.	270	270	450	740	Triarch*** Tetrarch

\* Not visible due to damage from preparation

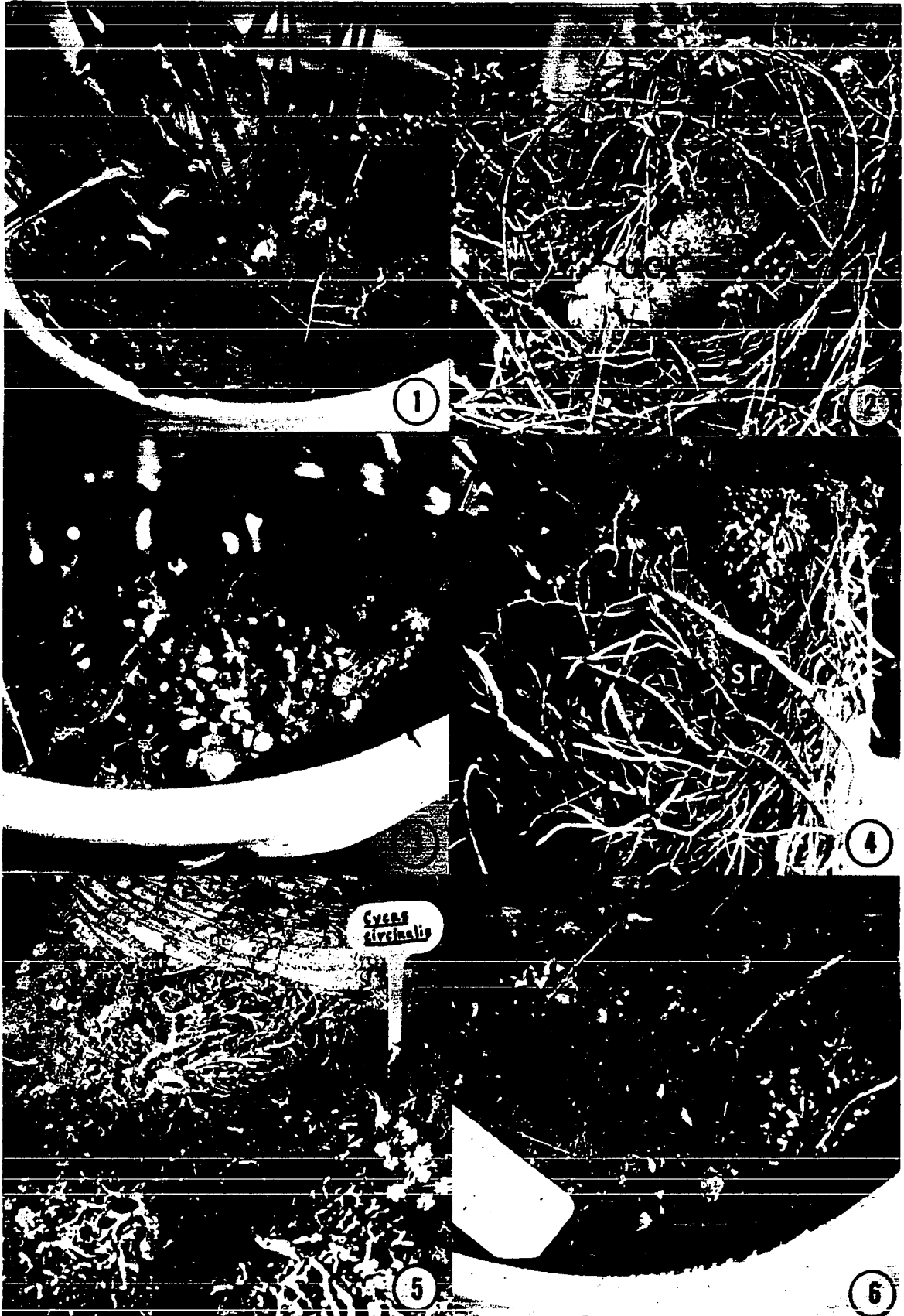
\*\* Not present

\*\*\* Does not represent variation in a given root

## ILLUSTRATIONS 1-6

1. Ceratozamia sp.--coralloid roots arising from apogeotropic secondary roots.
2. Ceratozamia sp.--two masses of underground coralloid roots from a depth of 6 cm.
3. Cycas revoluta--coralloid roots at soil surface.
4. Cycas revoluta--a secondary root that gives rise to coralloid outgrowths while growing upward to the soil surface behaves normally when growing downward.
5. Cycas circinalis--masses of coralloid roots at soil surface.
6. Dioon edule--coralloid roots at soil surface.

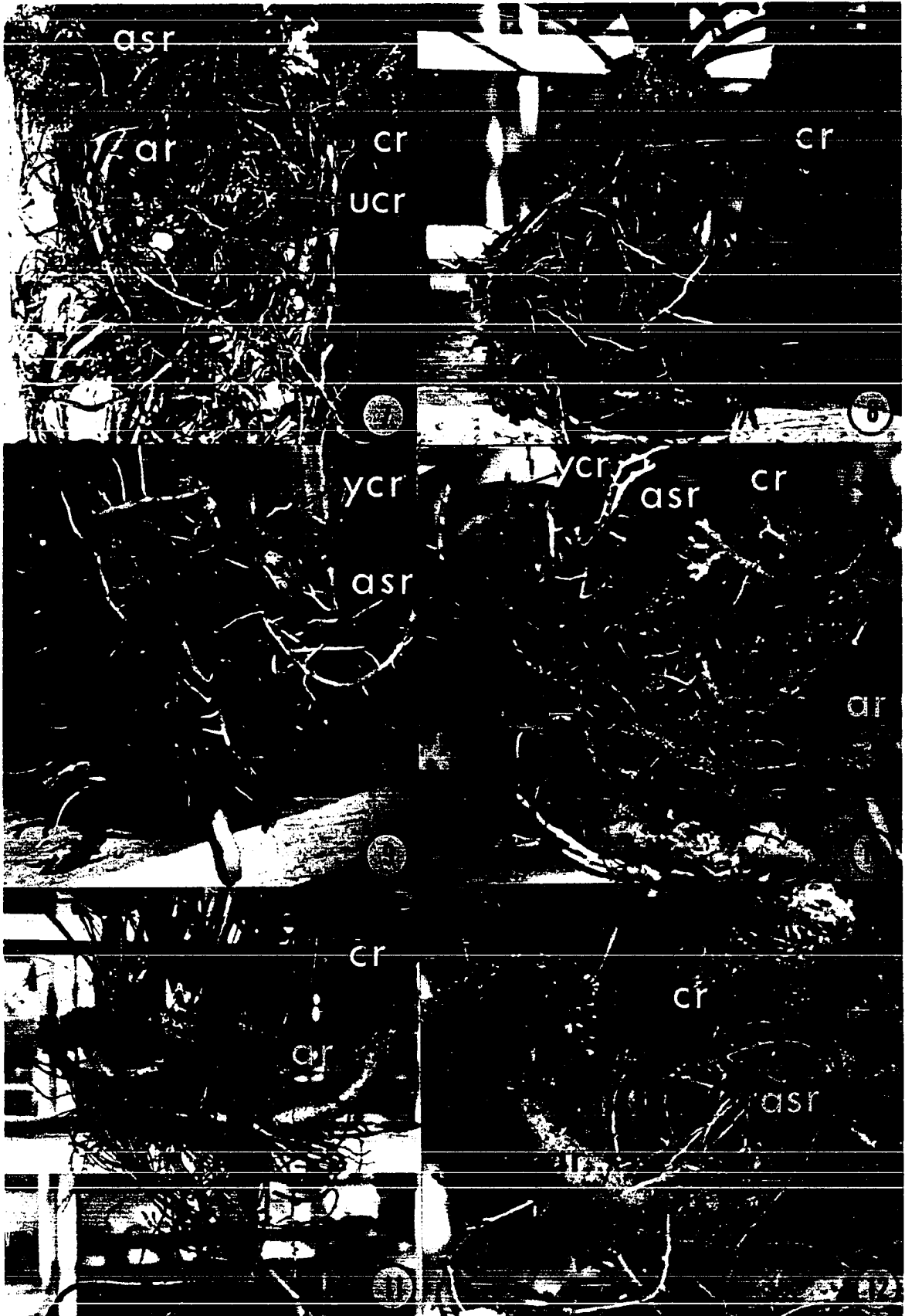
Key to abbreviations: sr, secondary roots; ucr, underground coralloid roots.



## ILLUSTRATIONS 7-12

7. Cycas revoluta--fascicled root system, showing coralloid roots arising only from secondary roots, not from tips of upward-growing adventitious roots.
8. Stangeria paradoxa--coralloid roots arise directly from the surface of the swollen base of the tuberous stem.
9. Stangeria paradoxa--apogeotropic secondary roots bearing young coralloid outgrowths.
10. Stangeria paradoxa--coralloid roots originate both from tips of upward-growing adventitious roots and apogeotropic secondary roots.
11. Zamia sp.--habit of origin of coralloid roots from tips of upward-growing adventitious roots.
12. Zamia sp.--coralloid roots originate both from tips of adventitious root and apogeotropic secondary roots.

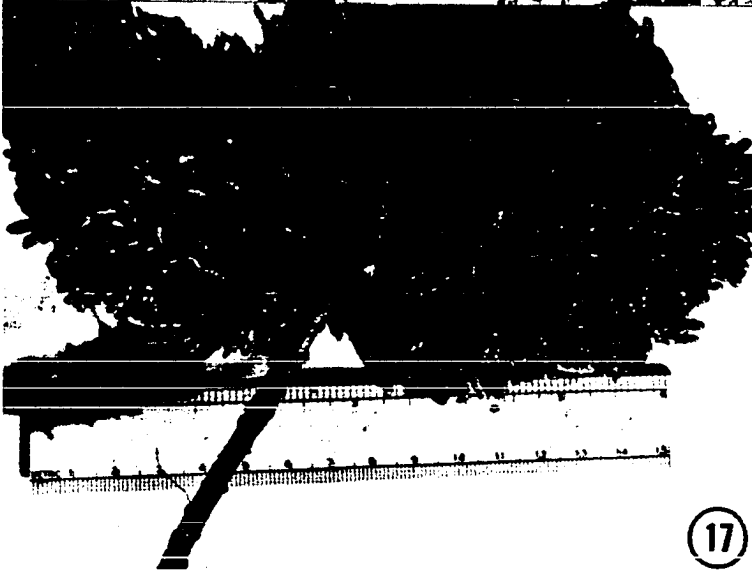
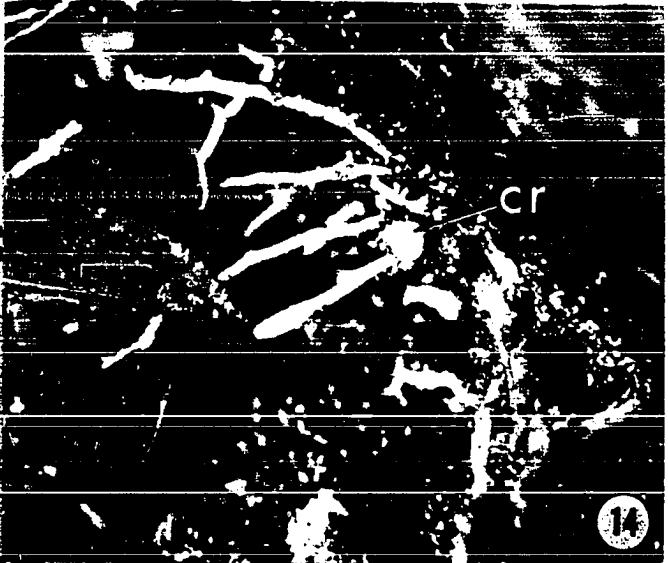
Key to abbreviations: asr, apogeotropic secondary roots; ar, adventitious roots; cr, coralloid roots; ucr, underground coralloid roots; y-cr, young coralloid roots.



## ILLUSTRATIONS 13-18

13. Ceratozamia mexicana--apogeotropic secondary roots with young coralloid outgrowths.
  14. Ceratozamia mexicana--coralloid outgrowths from a secondary root at the bottom of the box.
  15. Dioon edule--habits of apogeotropic secondary root tips after giving rise to coralloid outgrowths.
  16. Dioon edule--masses of coralloid roots at ground level.
  17. Cycas circinalis--a mass of coralloid roots.
  18. Cycas circinalis--mode of origin of a coralloid mass.
- Key to abbreviations: cr, coralloid roots.

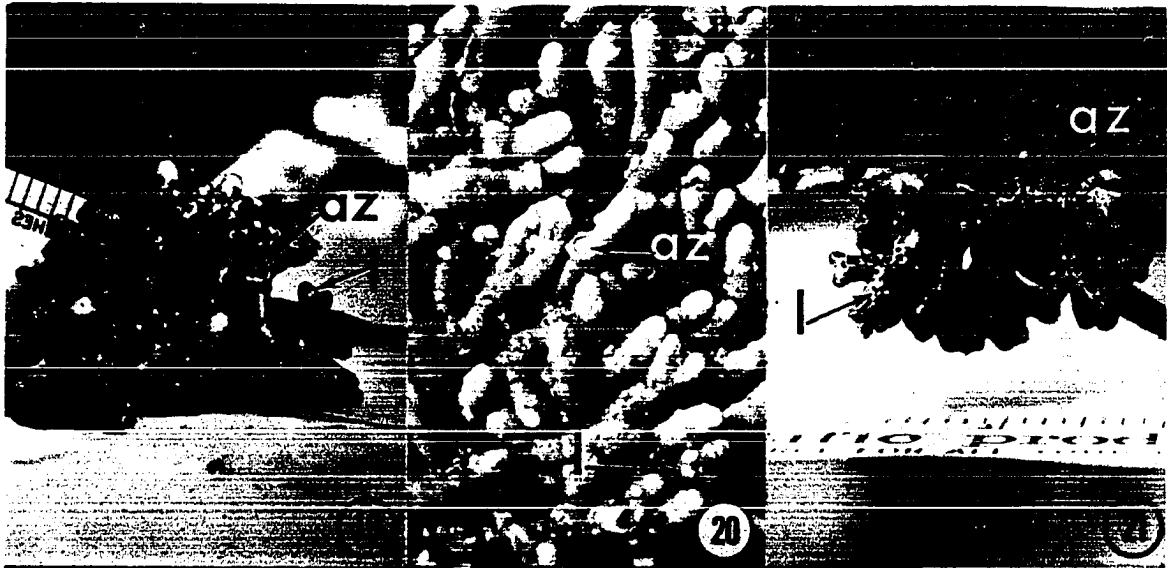




## ILLUSTRATIONS 19-25

19. Ceratozamia mexicana--a coralloid mass, showing the algal zone and lenticels.
20. Cycas circinalis--surface view of a mass of coralloid roots, showing the algal zone and lenticels.
21. Dioon edule--a coralloid mass, showing the algal zone, lenticels, and numerous dark tannin spots.
22. Ceratozamia sp.--a coralloid mass, showing the algal zone and lenticels.
23. Zami sp.--two masses of coralloid roots originating from different kind of parent roots. The larger mass with the algal zone and lenticels arises from tip of an adventitious root. The smaller mass without the algal zone and lenticels arises from an apogeotropic secondary root.
24. Cycas revoluta--a coralloid mass, showing the algal zone and lenticels.
25. Stangeria paradoxa--a coralloid mass, showing the algal zone and lenticels.

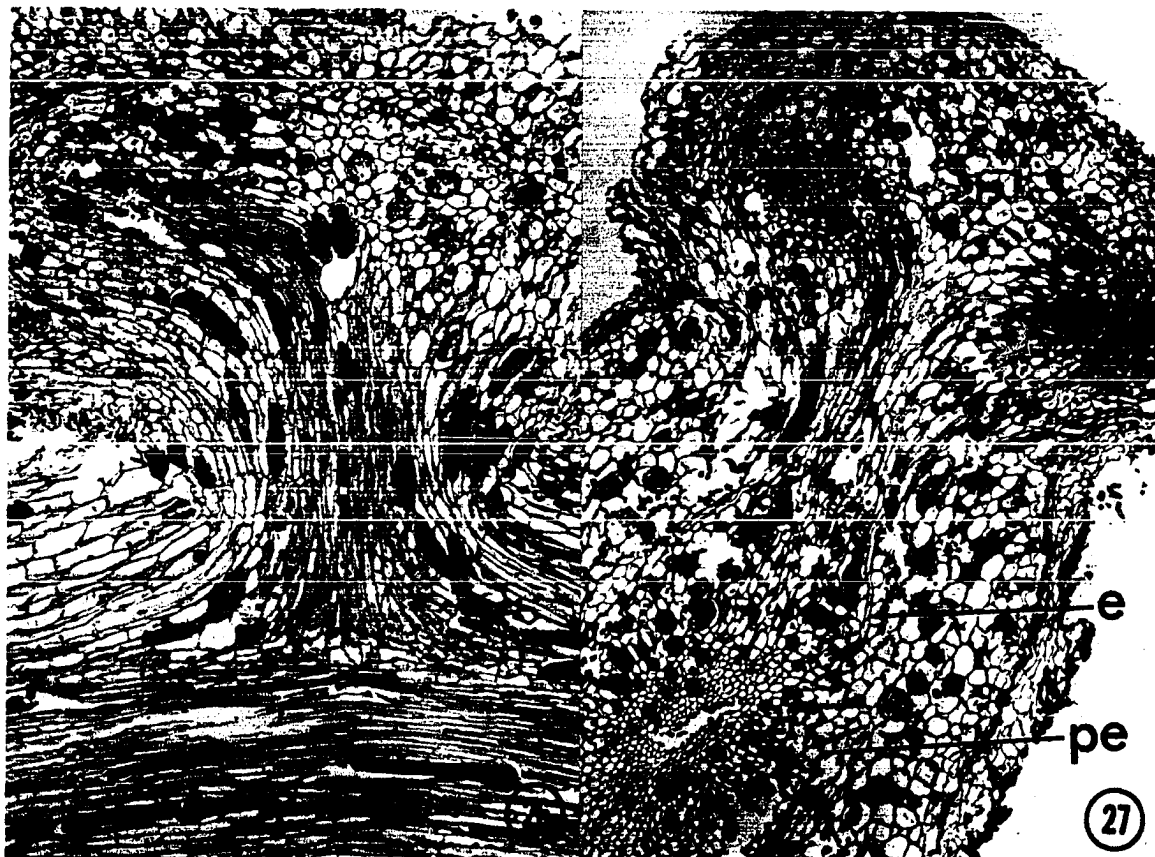
Key to abbreviations: l, lenticels; asr, apogeotropic secondary roots; ar, adventitious roots; az, algal zone.



## ILLUSTRATIONS 26-29

26. Ceratozamia mexicana--longisection of apogeotropic secondary root, showing the origin of a coralloid root. X 54.
27. Ceratozamia mexicana--transection of apogeotropic secondary root, showing the origin of a coralloid root. Parent root with diarch stele. X 54.
28. Ceratozamia sp.--longisection of apogeotropic secondary root, showing the origin of a coralloid root. X 54.
29. Ceratozamia sp.--transection of apogeotropic secondary root, showing the origin of a coralloid root. Parent root with diarch stele. X 54.

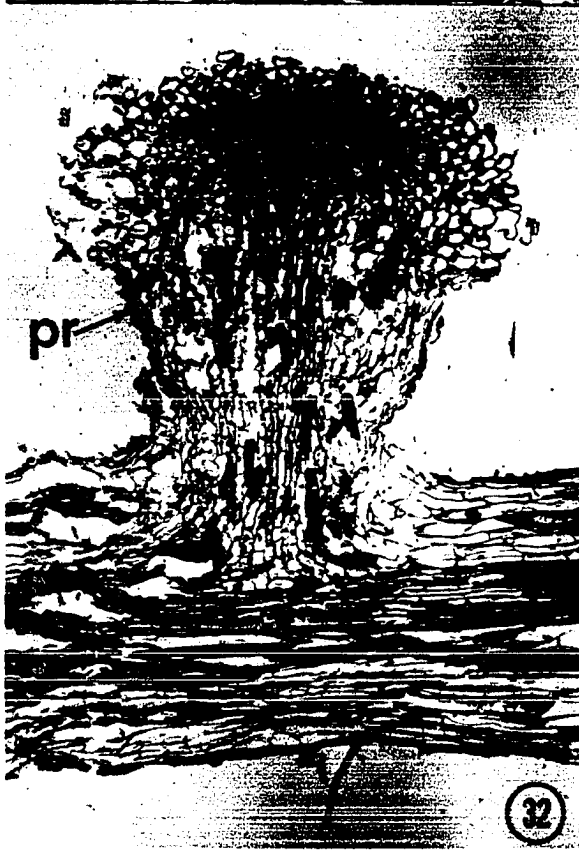
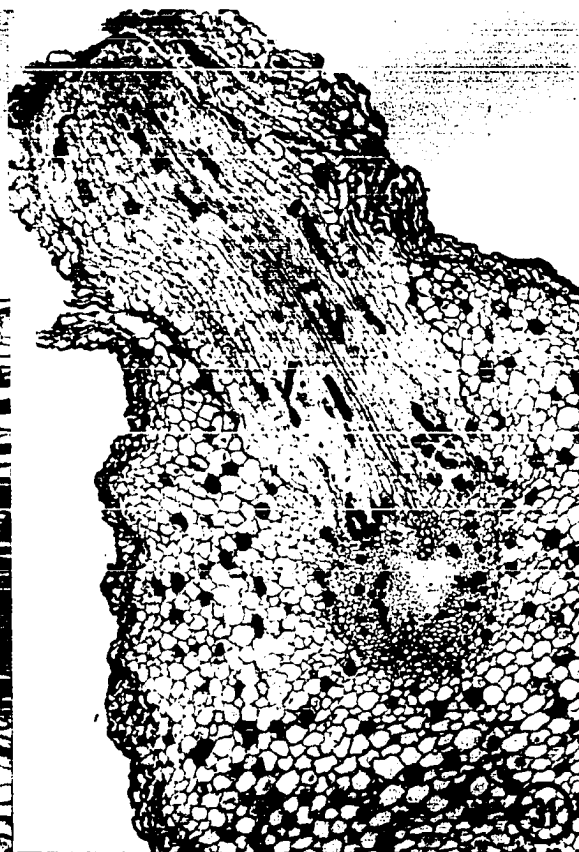
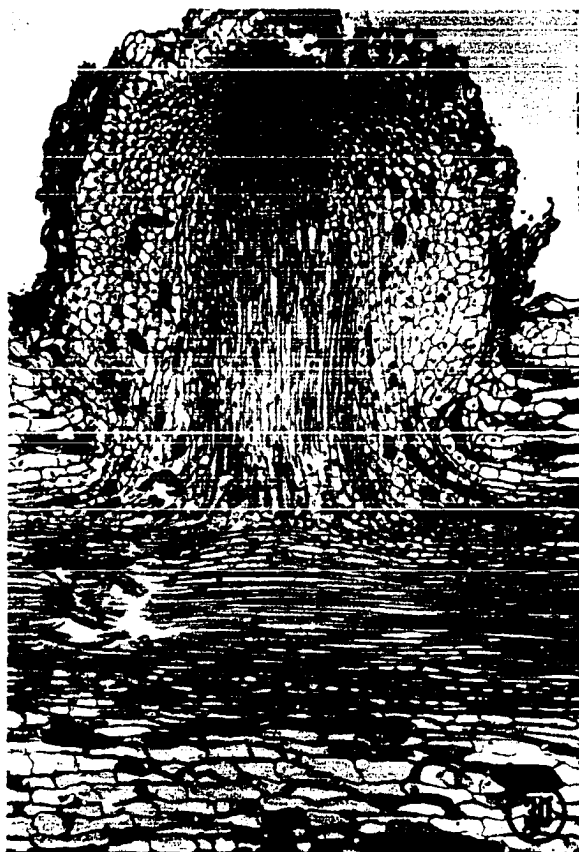
Key to abbreviations: e, endodermis; pe, pericycle.



## ILLUSTRATIONS 30-33

30. Cycas revoluta--longisection of apogeotropic secondary root, showing the origin of a coralloid root. X 54.
31. Cycas revoluta--transection of apogeotropic secondary root, showing the origin of a coralloid root. Parent root with diarch stele. X 54.
32. Dioon edule--longisection of apogeotropic secondary root, showing the origin of a coralloid root. X 54.
33. Dioon edule--transection of apogeotropic secondary root, showing the origin of a coralloid root. Parent root with triarch stele. X 54.

Key to abbreviations: pr, periderm.



32

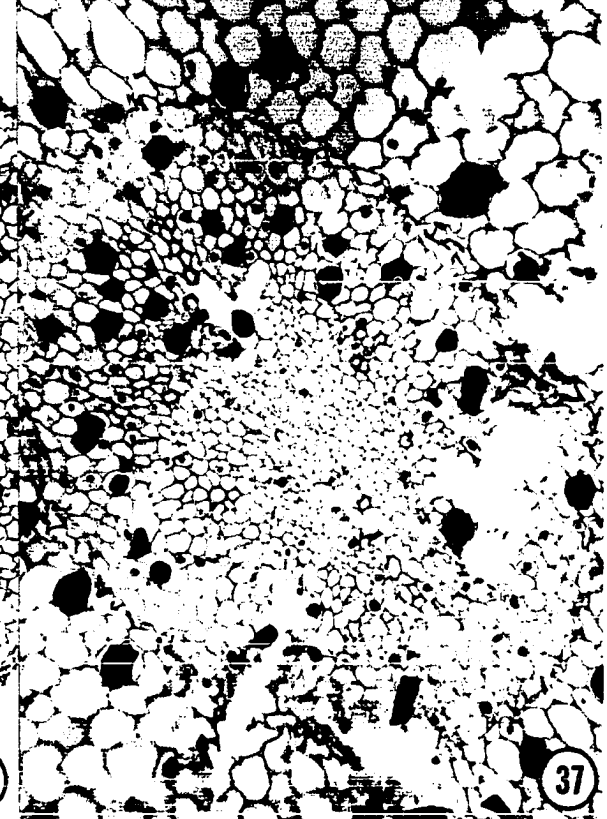
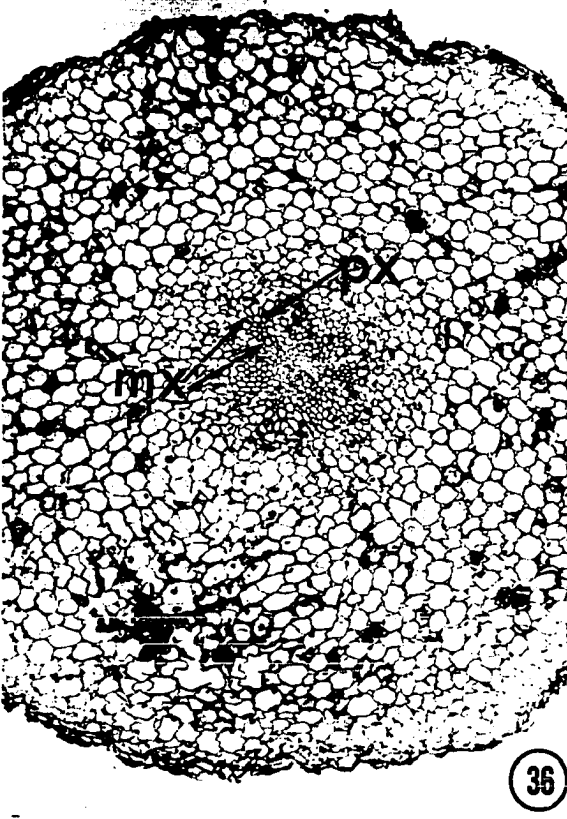
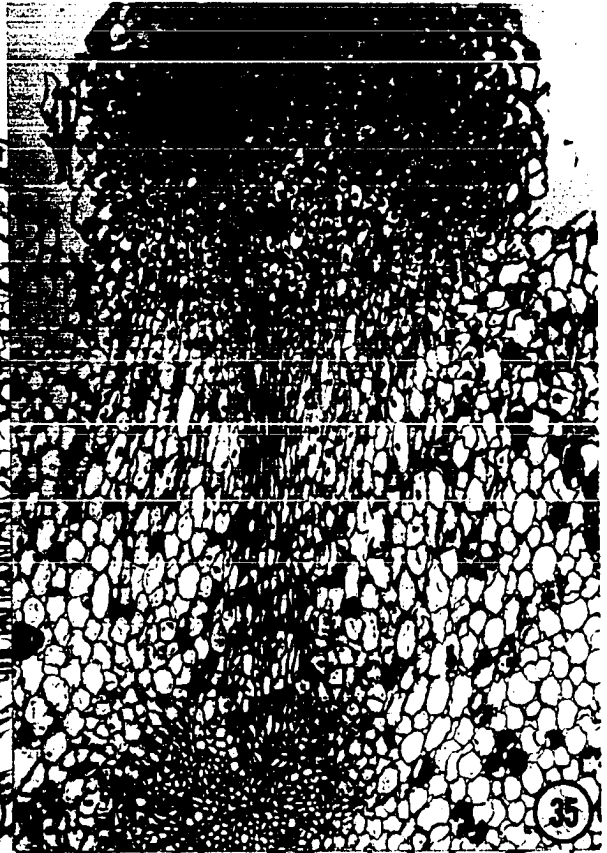
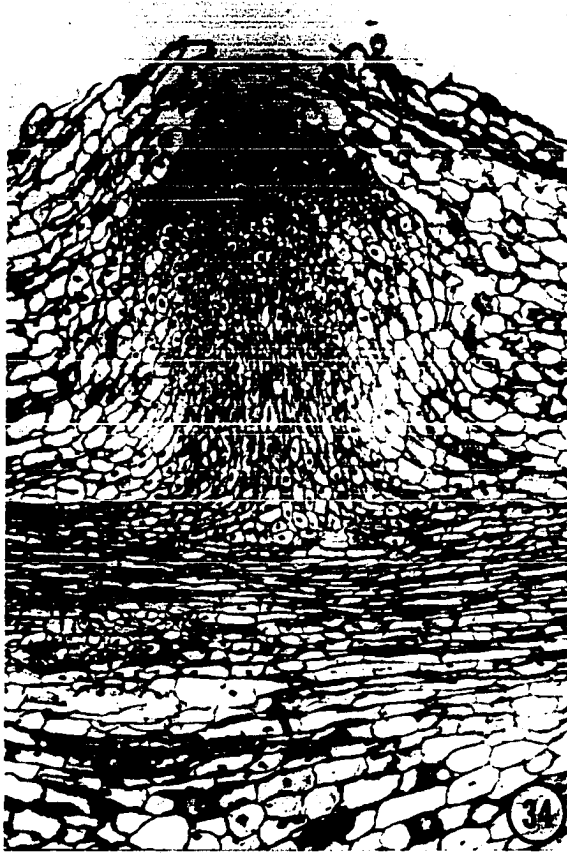
33

## ILLUSTRATIONS 34-37

34. Stangeria paradoxa--longisection of apogeotropic secondary root, showing the origin of a coralloid root. X 56.
35. Stangeria paradoxa--transection of apogeotropic secondary root, showing the origin of a coralloid root. Parent root with triarch stele. X 56.
36. Stangeria paradoxa--transection of apogeotropic secondary root, showing mesarch development of primary xylem. X 56.
37. Cycas revoluta--transection of apogeotropic secondary root, showing hexarch stele. X 128.

Key to abbreviations: mx, metaxylem; px, protoxylem.





## ILLUSTRATIONS 38-43

Median longisections of normal root tips. All X 74.

38. Ceratozamia mexicana.

39. Cycas circinalis.

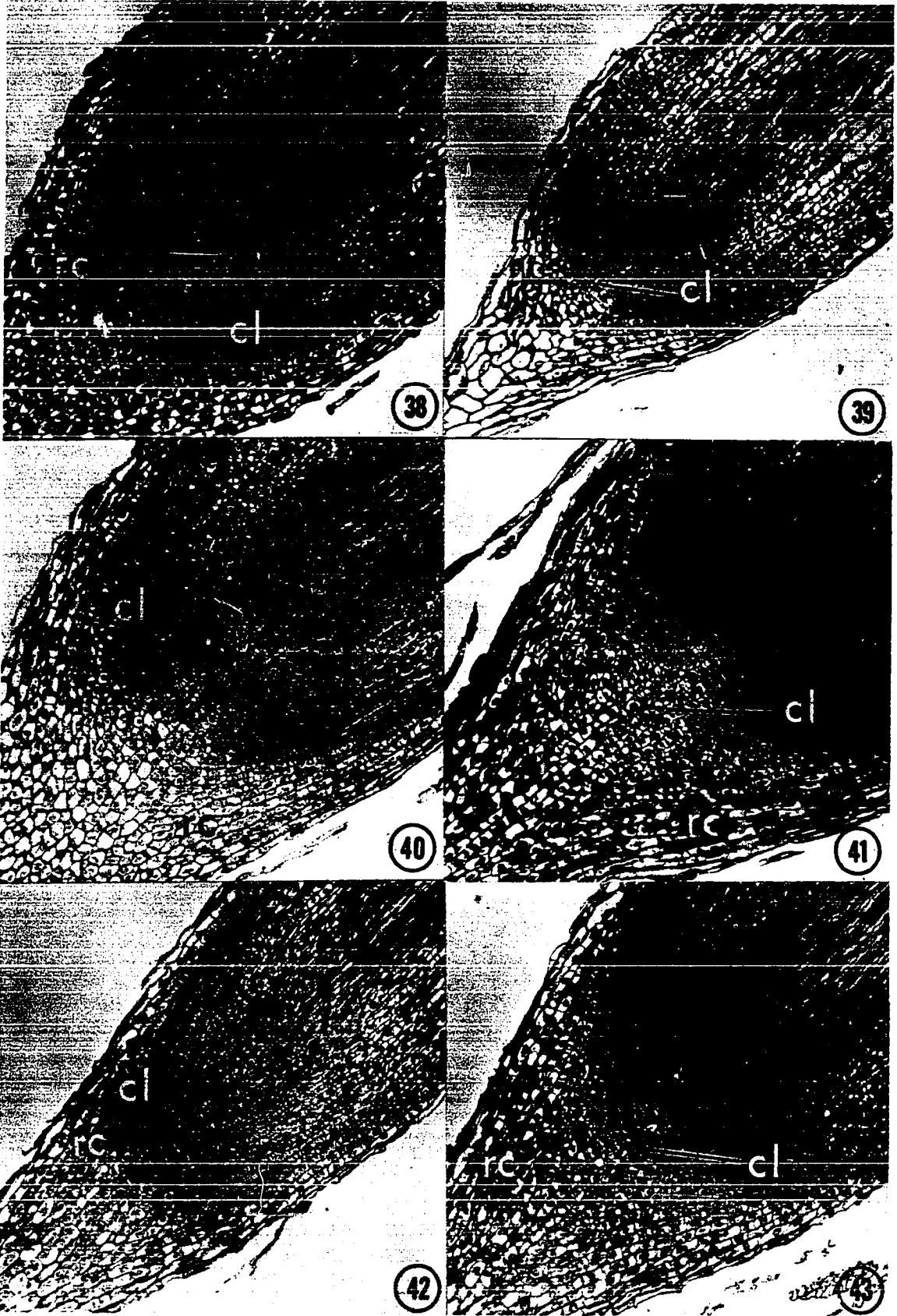
40. Cycas revoluta.

41. Dioon edule.

42. Stangeria paradoxa.

43. Zamia sp.

Key to abbreviations: cl, columella; rc, rootcap.



## ILLUSTRATIONS 44-49

Median longisections of alga-free coralloid root tips.

All X 74.

44. Ceratozamia mexicana.

45. Cycas circinalis.

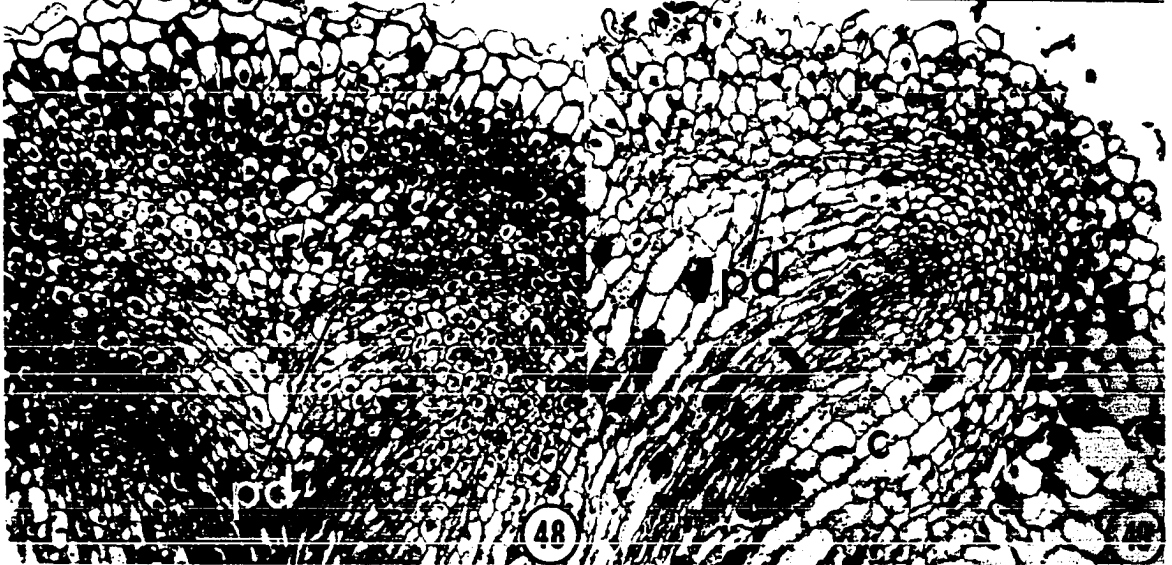
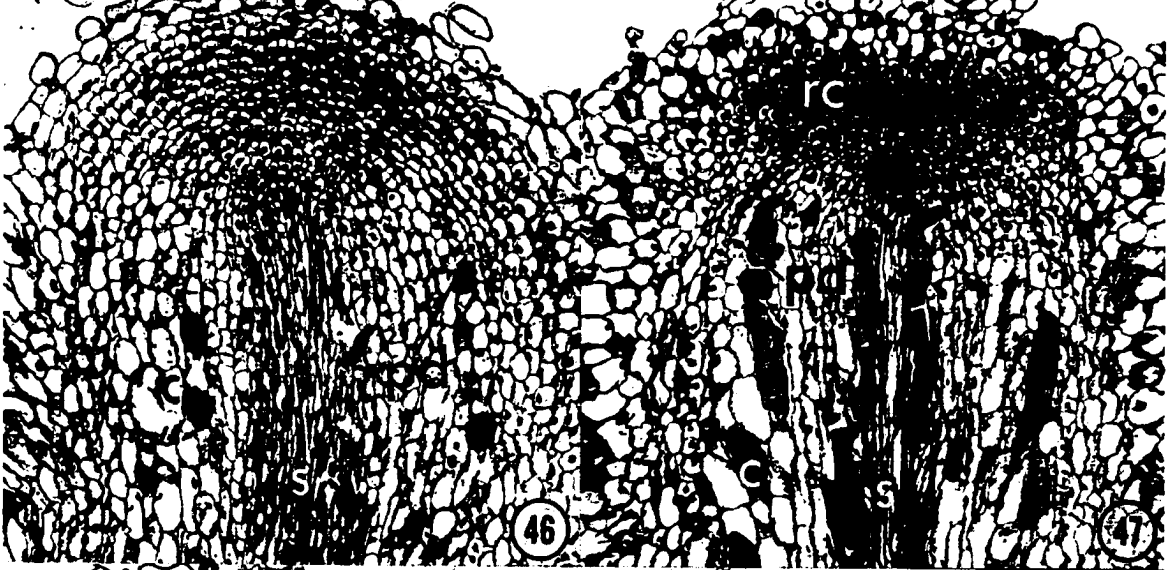
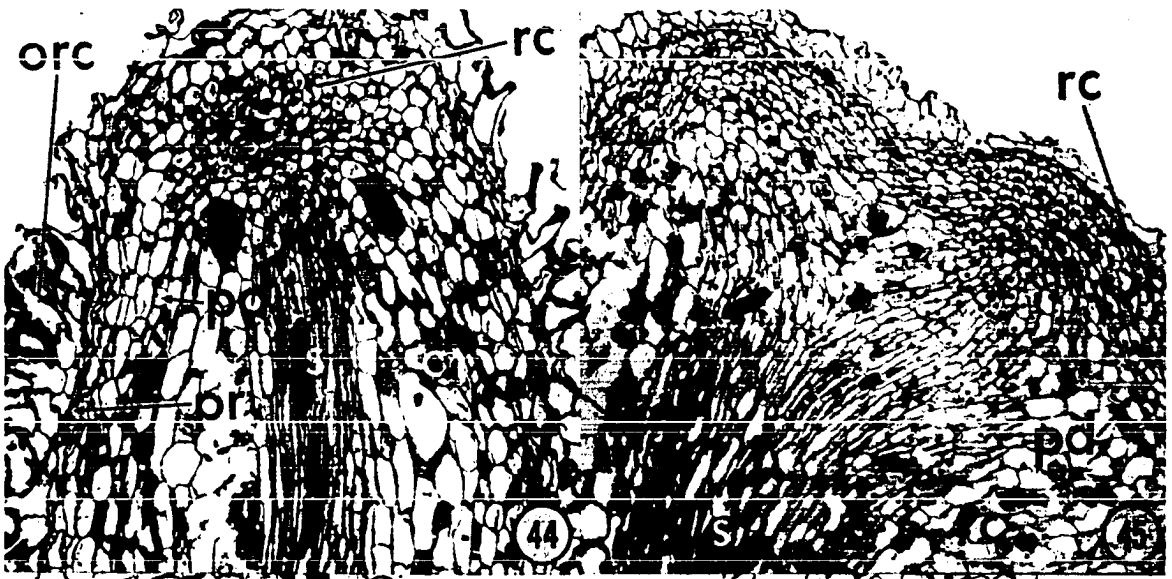
46. Cycas revoluta.

47. Dioon edule.

48. Stangeria paradoxa.

49. Zamia sp.

Key to abbreviations: c, cortex; orc, outer part of rootcap; pd, protoderm; pr, periderm; rc, rootcap; s, stele.



## ILLUSTRATIONS 50-55

Median longisections of alga-containing coralloid root tips. All X 74.

50. Ceratozamia mexicana.

51. Cycas circinalis.

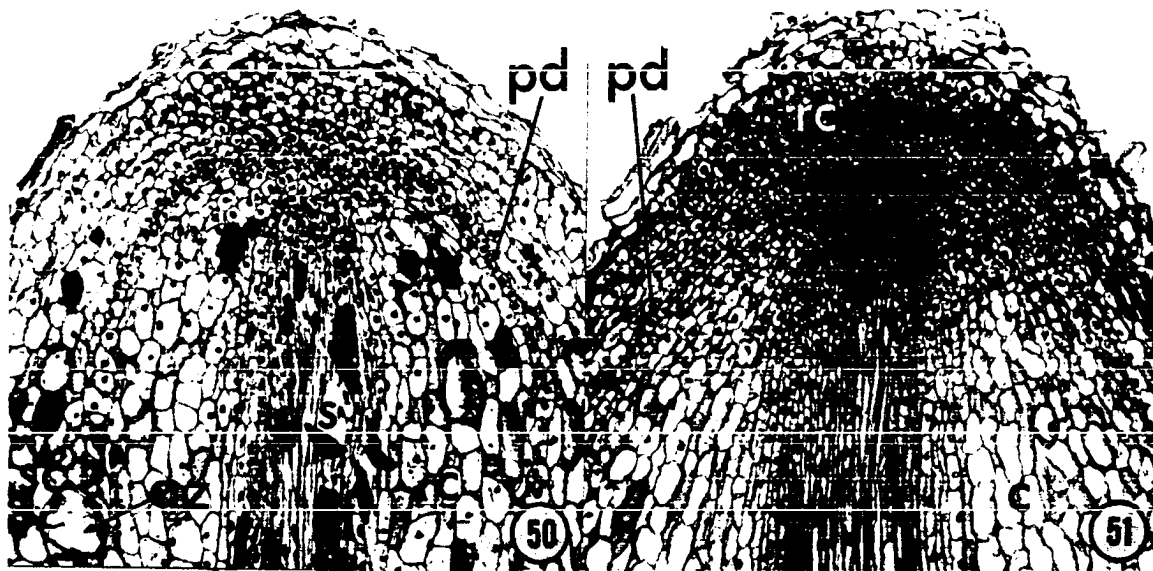
52. Cycas revoluta.

53. Dioon edule.

54. Stangeria paradoxa.

55. Zamia sp.

Key to abbreviations: az, algal zone; c, cortex; pd, protoderm; rc, rootcap; s, stele; sc, secondary cortex.



## ILLUSTRATIONS 56-59

Median longisections of coralloid root, showing Körper-Kappe boundary. All X 129.

56. Ceratozamia sp.

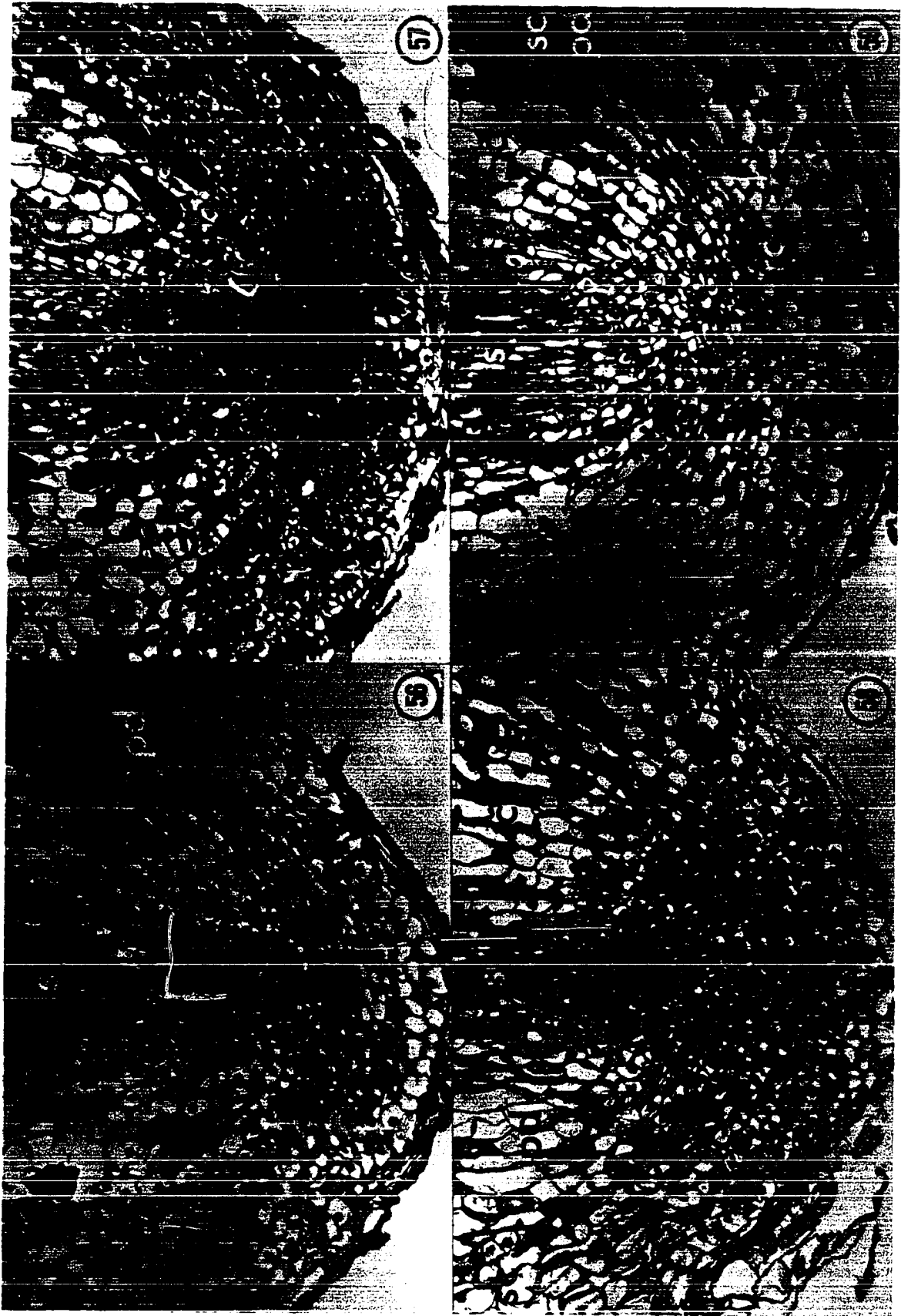
57. Dioon edule.

58. Stangeria paradoxa.

59. Zamia sp.

Key to abbreviations: c, cortex; pd, protoderm; rc, rootcap; s, stele; sc, secondary cortex.





## ILLUSTRATIONS 60-65

60. Zamia sp.--longisection of algal zone, showing the presence of alga in intercellular spaces. X 256.
61. Cycas revoluta--transection of algal zone, showing the presence of alga in intercellular spaces. X 256.
62. Ceratozamia mexicana--tangential section of algal zone, showing the presence of alga in intercellular spaces. X 256.
63. Zamia sp.--algal filaments on root surface. Note the breakage of the outer part of rootcap and the presence of algae inside the break. X 256.
64. Ceratozamia mexicana--breakage in the secondary cortex leading to the formation of channel into the algal zone. X 128.
65. Zamia sp.--breakage in the secondary cortex leading to the formation of a channel where algal filaments travel into the algal zone. X 128.

Key to abbreviations: a, alga; ap, algal pathway; az, algal zone; c, cortex; orc, outer part of rootcap; pr, periderm; sc, secondary cortex; te, transformed epidermal cells.

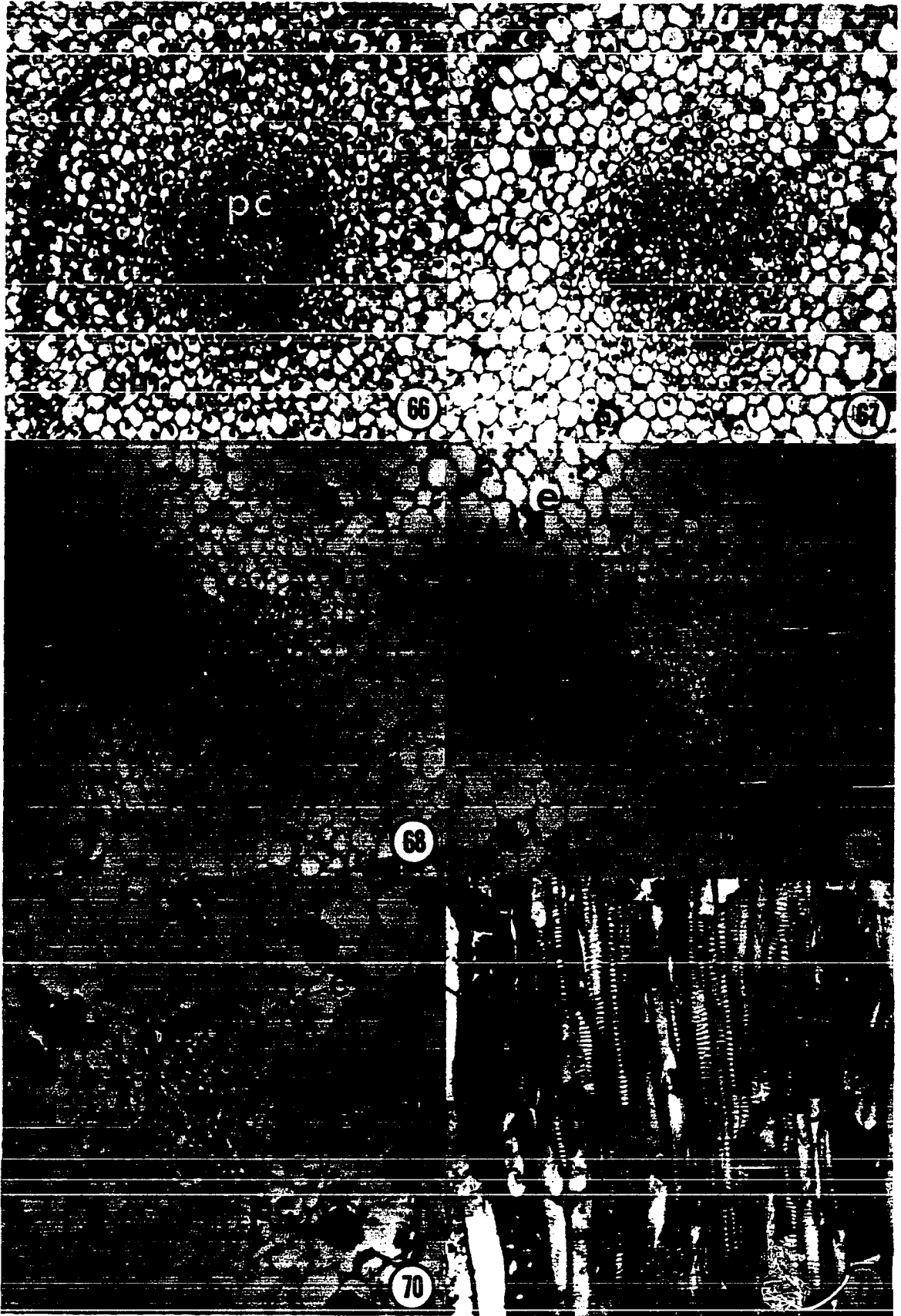


## ILLUSTRATIONS 66-71

66. Cycas revoluta--transection of a coralloid root, showing primary meristematic tissues. Note the vacuolation in the central part of procambium and centripetal development of the cortex. X 128.
67. Cycas revoluta--transection of a coralloid root, showing the presence of secretory cells in region destined to become phloem. X 128.
68. Cycas revoluta--transection of a coralloid root, showing the first protoxylem of each pole. Note the correlation between secretory cells and the phloem region. X 128.
69. Cycas revoluta--transection of a coralloid root, showing mesarch development of primary xylem. Note reticulate thickening on the end wall of a centrifugal metaxylem. X 128.
70. Cycas revoluta--transection of a coralloid root at about 1.2-1.5 cm from the tip, showing exarch development of primary xylem and the crushing of endodermis. X 128.
71. Ceratozamia mexicana--median longisection through the stele of a coralloid root, showing helical thickening of the xylem. X 256.

## ILLUSTRATIONS 66-71 (Continued)

Key to abbreviations: c, cortex; e, endodermis; gm, ground meristems; mx, metaxylem; p, phloem; pc, procambium, pd, protoderm; pe, pericycle; px, protoxylem; rmx, reticulate metaxylem; sv, secretory cells.



## CHAPTER IV

### SUMMARY

Coralloid roots have been found to occur in all species of cycads. They are lateral outgrowths from adventitious roots or secondary roots that have become exposed or nearly exposed to soil surface and are susceptible to infection by an alga, which appears to enter from the soil through breaks in the secondary cortex. Once inside the roots, the alga seems able to stimulate the initiation of a special zone within which it later occupies available intercellular spaces.

The algal zone appears to arise from a layer of cells analogous to the protoderm of normal roots. At maturity it is surrounded by the secondary cortex, which is the persistent part of the rootcap. Coralloid roots develop few layers of rootcap as compared to normal roots. However, the presence of a rootcap in coralloid roots has been shown by an analysis of cell lineages and planes of division based on the Körper-Kappe concept of Schüepf

(1917, quoted by Clowes, 1961). The common origin of the secondary cortex and the rootcap confirmed the concept of a persistent rootcap proposed by earlier workers (Life, 1901; McLuckie, 1922; Wittmann, Bergersen, and Kennedy, 1965).

The apical organization of the root apices of coralloid roots in all species of cycads is similar to the descriptions of coralloid root apices of Macrozamia communis as described by Wittmann, Bergersen, and Kennedy (1965). The development of the cortex and vascular cylinder of coralloid roots is as simple as in ordinary roots.



#### LITERATURE CITED

- Bergersen, F. J., G. S. Kennedy, and W. Wittmann. 1965. Nitrogen fixation in the coralloid roots of Macrozamia communis L. Johnson. Aust. J. Biol. Sci. 18: 1135-1142.
- Boke, N. H. 1952. Leaf and areole development in Coryphantha. Amer. J. Bot. 39: 134-145.
- Bowyer, J. W., and V. B. D. Skerman. 1968. Production of axenic cultures of soil-borne and endophytic blue-green algae. J. Gen. Microbiol. 54: 299-306.
- Chamberlain, C. J. 1935. Gymnosperms: structure and evolution. The University of Chicago Press, Chicago.
- Chaudhuri, H., and A. R. Akhtar. 1931. The coral-like roots of Cycas revoluta, Cycas circinalis and Zamia floridana and the alga inhabiting them. J. Ind. Bot. Soc. 10: 43-59.
- Clowes, F. A. L. 1961. Apical meristems. Blackwell Scientific Publication, Oxford, England.

- Hoare, D. S., et al. 1971. Dark heterotrophic growth of an endophytic blue-green alga. Arch. Mikrobiol. 78: 310-321.
- Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York.
- Life, A. C. 1901. The tuber-like rootlets of Cycas revoluta. Bot. Gaz. 31: 265-271.
- McLuckie, J. 1922. Studies in symbiosis. II. The apogeotropic roots of Macrozamia spiralis and their physiological significance. Proc. Linn. Soc. N. S. W. 47: 319-328.
- Sass, J. E. 1958. Botanical microtechnique. 3rd ed. Iowa State College Press, Ames.
- Schneider, A. 1894. Mutualistic symbiosis of algae and bacteria with Cycas revoluta. Bot. Gaz. 19: 25-32.
- Spratt, E. R. 1915. The root-nodules of the Cycadaceae. Ann. Bot. 29: 619-626.
- Wilcox, H. 1954. Primary organization of active and dormant roots of noble fir, Abies procera. Amer. J. Bot. 41: 812-821.
- Wittmann, W., F. J. Bergersen, and G. S. Kennedy. 1965. The coralloid roots of Macrozamia communis L. Johnson. Aust. J. Biol. Sci. 18: 1129-1134.