UNIVERSITY OF TASMANIA

T H E S I S

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MASTER OF SCIENCE

by

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S T U D I E S
O F T H E L I F E H I S T O R I E S
A N D M O R P H O L O G Y
O F T A S M A N I A N C O N I F E R S

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C O N T E N T S

LIST OF CONIFERS NATIVE TO TASMANIA

Family PODOCARPACEAE

Podocarpus alpinus R. Br.

Phyllocladus rhomboidalis Rich. (P. asplenifolius Hook.)

Microcachrys tetragona Hook.

Dacrydium franklinii Hook.

Pherosphaera hookeriana Arch.

Family TAXODIACEAE

Athrotaxis selaginoides Don.

Athrotaxis laxifolia Hook.

Athrotaxis cupressoides Don.

Family CUPRESSACEAE

Diselma archeri Hook. (Fitzroya archeri Benth. & Hook.)

Callitris tasmanica Baker & Smith. (C. rhomboidea R. Br. var. tasmanica Benth.)

Callitris oblonga Rich.

FOREWORD

Within its small area, Tasmania presents a remarkable profusion of endemic plants, a number of them belonging to genera which are confined to the state. Among the most interesting are the conifers, a list of which appears on page 3. Of these the genera Microcachrys, Athrotaxis, and Diselma are entirely confined to Tasmania, while the native species of Phyllocladus, Dacrydium, Pherosphaera, as well as Callitris oblonga, are all endemic.

Modern research on conifer morphology has revealed a remarkable diversity in the order as a whole. The lack of information on some genera is obscuring our understanding of the interrelationships of the main groups. These genera are mostly found in isolated areas and have not been extensively cultivated; consequently few of them have been investigated. This is specially true of the Tasmanian conifers, our knowledge of most of which is very meagre.

Microcachrys has been the subject of several investigations. Thomson (1908, 1909) described the pollen. Lawson (1923a) gave an account of the life history, which is not complete, but describes megasporogenesis, the mature gametophytes and the male gametes, and figures a pollen tube pressing down on several archegonia of the archegonial complex. From the dates Lawson gives, one learns that pollination occurs early in December (10th Dec. 1922) which is the time of megasporogenesis, and that the male gametes are formed early in February (16th Feb. 1923). The plant is a widespread component of the fell-field community among the cushion plants and herbs on the exposed mountain tops and plateaus.

Our knowledge of the morphology of Dacrydium is very limited. The New Zealand species D. cupressinum has been the most frequently studied, but even here, the complete life cycle has not been worked out, and a Dacrydium with the cone type of D. franklinii has never been examined.

Phyllocladus, on the other hand, is tolerably well understood, although a few points are still obscure, e.g. the pollination of the erect ovule (cf. Doyle, 1945). The chief papers on the genus are those by Young (1910), Holloway (1937), and Buchholz (1941), all of whom used

New Zealand material. The Tasmanian species has not been investigated, but one would not expect it to differ essentially from the other species.

Nothing whatever is known of the life history of Diselma. Only one paper has been published on the species (Doyle, 1934). This drew attention to the presence in the cone of a columella, mention of which had been omitted in various systematic works. The genus is generally considered closely related to Fitzroya, in which it was at one time included (cf. Rodway, 1903). The life history of Fitzroya has been investigated by Doyle and Saxton (1923). Their account shows Fitzroya has many characters which would give it a place among the more primitive members of the Callitris group. Diselma is included by Pilger (1926) in the sub-family Thujoideae of the Cupressaceae, along with Actinostrobus, Callitris, Tetraclinis, Widringtonia, Fitzroya, Thujopsis, Thuja, Libocedrus, and Fokienia. Buchholz (1933b) includes it provisionally in his Cupressoideae, which comprises" Cupressus, Chamaecyparis, Libocedrus, Biota, and possibly some of the following: Thujopsis, Fokienia, Fitzroya, and Diselma." Diselma is one of the several shrubby conifers of the Tasmanian mountains, and is widely spread in the centre and west of the state.

The Callitris spp. stand apart from the other Tasmanian conifers in their occurrence in the east and north-east of the state, while the others are confined to the mountainous areas of the centre and west of the island. They belong to the Australian element ofthe flora, whereas the other genera are best considered as part of the Sub-Antarctic flora. C. tasmanica occurs along the east coast. There can be few plants which have a more restricted distribution than C. oblonga. It is confined to the South Esk valley. The life history of Callitris is fairly well understood. It has been investigated by Saxton (1910) who worked on C. verrucosa. Further details were added for C. rhomboidea by Looby and Doyle (1940). Baird has investigated two Western Australian species, C. robusta, and C. roei (unpublished).

Apart from a description of the stem apex(Cross, 1943), the only morphological work on Athrotaxis is the very incomplete account by Saxton and Doyle (1929), which refers to several important phases of the life history; but their work is not complete enough, nor in sufficient detail

and more information is required on all aspects of the morphology of the genus.

Lawson (1923b) investigated the life history of Pherosphaera but here again the account is not full enough, and in some respects is misleading. Many features of Pherosphaera are unusual, and Lawson's work is an inadequate basis for reaching any definite conclusions on the taxonomic and phylogenetic position of the genus. A subsequent paper by Saxton (1930) dealt with the root nodules, and with anatomy, and referred to the problem of its ecology.

Of the works on the Australian conifers as a whole, which naturally include the Tasmanian species, Baker and Smith's monumental volume (1910) deals only with the commercially important species, so that Diselma recieves scant attention, and Pherosphaera hookeriana, Podocarpus alpinus, Athrotaxis cupressoides and A. laxifolia, and Microcachrys are dismissed in a few lines. Dacrydium franklinii, Phyllocladus rhomboidalis, and Athrotaxis selaginoides and the two Callitris spp. are investigated in the field covered by the work. Patton (1927) described the anatomy of all the Tasmanian species.

This thesis presents the results of 2½ years work, during which time a morphological investigation of three Tasmanian conifers has been undertaken, Work on Pherosphaera hookeriana, Podocarpus alpinus and Athrotaxis cupressoides is described. The first part of the thesis deals with Pherosphaera. The gametophytes and development of the embryo are described, and with these newly elucidated facts it has been possible to arrive at more definite conclusions regarding the relationship of Pherosphaera to other conifers than has been possible up to now, The second part deals with Podocarpus alpinus, which has an important cone type. An attempt is made to correlate cone morphology and embryology in the genus Podocarpus. The third part of the thesis describes work on Athrotaxis. Many phases of

the life cycle of A. cupressoides have been investigated but large gaps remain at critical points. In each case an attempt has been made to describe the habit and ecology of the plants. A more detailed discussion than has been given above of prevoius work relevant to the subject preceeds each section.

It was much desiredt to investigate the embryogeny of Microcachrys, but fertile material was not found either in 1946 or 1947. On the Acropolis (Lake St.Clair National Park) on 26th February 1947 male cones were found sheddinh pollen. Cones with the characteristic red scales can be found from February on, but the reddening is quite independent of fertility. Attempts were also made to collect material of Diselma for a morphological investigation, but the cones are extreemly small and difficult to distinguish from vegetative tips, and no results were obtained.

Most of the work was done during tenure of a Commonwealth Research Grant in the University of Tasmania. Collecting of material was done almost entirely by myself, and has involved much travelling and hiking beyong the areas served by commercial transport. I wish to acknowledge my indebtedness to Professor H.D. Gordon, who suggested to me the problem of the Tasmanian conifers. My thanks are due also to Miss W.M. Curtis and Mr. E. Matthaei for their advice on drawing and photography respectively, and to Professor Gordon and Mr. C.E. Fowefaker for specemins of New Zealand conifers.

TECHNIQUE

The material for these studies was in most instances fixed in the field in formalin-acetic-alcohol, the formula used being

Commercial formalin	5 ccs.
Glacial acetic acid	5 ccs
70% ethyl alcohol	90 ccs.

(Johansen, 1940, p.41). Methylated spirit used in place of ethyl alcohol was found to be in no way inferior. If anything, the subsequent staining was more brilliant. This fixative was used on account of its convenience. Slight shrinkagewas caused, but because of the small size of the structures under investigation, and the nature of the stages studied most intensively, this was not a serious objection.

In the examination of the material two techniques were used - microtome sections by the paraffin method, and dissection of embryo systems.

Sectioning: When sections of the prothalli alone were required, the integuments were dissected off, and the nucellus and gametophyte only embedded. This made penetration by the wax very easy. Butyl alcohol was found to be a very convenient and entirely satisfactory infiltrating agent. The ovules were dissected in 70% alcohol, and the material passed through Johansen's series of solutions (Johansen, 1940, p.130), ½ - 2 hours in each with 1 - 3 hours in butyl alcohol. The objects were embedded in waxes of melting points 48-54°C. Microtome sections of Pherosphaera were cut 8 microns thick, while those of Athrotaxis and Podocarpus were usually 12 microns thick.

Staining: Haidenhain's iron alum-haematoxylin was used and excellent results were obtained with it for all material. Bismark Brown was used as counter stain. The sections were placed in 4% aqueous iron alum to mordant for 4 - 8 hours, and stained in 1% haematoxylin overnight. They were then differentiated in 2% iron alum. Bismark brown was used as 2% solution in 70% alcohol. 1 minute in the stain was adequate, but overstaining was impossible.

Dissection and Staining of Embryos: The technique of dissection of conifer embryos and their staining in phloxine, developed by Buchholz, has been described in one of his papers (Buchholz, 1938). In the present study fixed prothalli were dissected in 70% alcohol. Fresh material of Podocarpus was dissected in 15% sucrose as recommended by Buchholz, and fixed in formalin-acetic-alcohol. However, the resinous nature of the epimatium and integument made the solution very cloudy, and it was preferred to use prevoiusly fixed material. The embryos were transferred by pipette to the phloxine solution (a trace of phloxine in 10% solution of glycerine in 50% alcohol), and left in the stain 24 - 72 hpurs. A dissicator was not used, the staining being done simply in a covered crystallizing dish. Direct mounting from absolute alcohol to euparal was too severe, as was even preliminary transfer to Buchholz's "sandarac solvent" directly. Thefollowing proceedure was therefore found necessary:- The embryos were removed from the stain and washed in 70% alcohol (10 minutes), transferred to 90% alcohol (5 minutes) by pipette, and then to absolute alcohol (10 minutes). All these operations were performed under a binocular dissecting microscope. The sandarac solvent (1 part paraldehyde : 2 parts eucalyptus oil) was then added drop by drop to the embryos in absolute alcohol. At first the drops were added at the rate of about 1 per 15 seconds, but later more quickly, until the concentration of solvent was about 70%. The embryos were then transferred to pure sandarac solvent (10 minutes) and finally mounted in euparal.

The staining in phloxine was not all that might be desired. It did not differentiate between structures sufficiently, all protoplasm staining rather evenly. Nuclear membranes were not always readily distinguished, and although the nucleolus stained more intensely, it was difficult in many cases to be sure how many nuclei there were in a cell, However, no other stain was used extensively, and confirmation of the conclusions reached from examining dissected embryos was sought in sections.

Illustrations in this Thesis: The photomicrographs were taken with a Bausch and Lomb microscope and a Leitz plate camera, using Kodak Orthochromatic plates. The drawings of whole cones and similar structures were done on graph paper while viewing the cone lying on graph paper through a binocular microscope. The drawings of embryo systems are from camera lucida outlines.

PHEROSPHAERA HOOKERIANA Archer[1]

I N T R O D U C T I O N

The genus Pherosphaera, established by Archer in 1850, consists of two species of conifers whose true relation to other forms is not yet known with any certainty, and has been the subject of considerable speculation. Stiles (1912) considered that because of the morphologically erect ovules and simple strobilus and absence of an epimatium, the genus was closely related to Phyllocladus, which also has a simple strobilus, but in which the epimatium is symmetrical like an aril. It was not until the work of Lawson (1923b) however that we had any knowledge of the life history. He investigated both species but did not distinguish between them in his account. Lawson found that the three-winged pollen grains have two nuclei only - the tube and generative nuclei. Three megaspores are formed and free nuclear divisions occur in all of them, the middle one even enlarging considerably; the innermost survives. A tapetum is present. The megaspore membrane is very thin. Archegonia number three or four; their necks open laterally on the prothallus some distance from the apex. There are four neck cells. There is a shallow depression over each archegonium. A jacket layer is present. The fate of the ventral canal nucleus was not determined. The male cells were not observed, but Lawson assumed they were unequal. Lawson did not observe fertilization, nor did he describe the development of the embryo in detail. He concluded that "in the gametophyte structures and embryo of Pherosphaera there are no features which justify our classifying this genus among the Podocarpineae," to which group it had always been considered to belong. Saxton (1930) however claimed the root nodules to be of the podocarpean type. Doyle and Looby (1939) considered that if Pherosphaera is a podocarp at all, it "would appear to be a very aberrant one." They pointed out the incompleteness of our knowledge, but suggested that Pherosphaera might provisionally be regarded as "an advanced derivative of the Phyllocladus line."

(1) The introduction sections on embryogeny and discussion of this part of the the thesis are taken largely from a paper (Elliott 1948) published by the Linnean Society of New South Wales. The remaining sections describe work completed since that paper was written.

Doyle (1945) subsequently favoured the view that it may be related to Microcachrys.

In its cone morphology Pherosphaera resembles no family of conifers other than the Podocarpaceae, and the problem would then seem to be whether Pherosphaera has any true affinity with the podocarps, or whether it is so far removed from them to warrant segregation altogether into a family of its own. The present investigation leads to the conclusion that Pherosphaera has indeed true podocarp affinities, especially with Dacrydium. These two genera are closely related on the basis of cone morphology, and the general features of the embryogeny are similar. Moreover in the details of development of the gametophytes Pherosphaera resembles podocarps in which these features have been thoroughly investigated. In the nature of the embryo initials Pherosphaera differs strikingly from the other podocarps, but the difference can be correlated with the general condition so as to demonstrate the podocarpean nature of the plant even more conclusively, as will be shown later.

E C O L O G Y

Pherosphaera hookeriana is a much branched shrub up to 6 feet in height. Its habit is in the mountains of the centre and west of Tasmania over 3000 feet, where the rainfall is high. The ecological factors controlling its distribution are unknown, and their investigation would be a most interesting problem. The plants are found only near running water, either beside small tarns, along streams or in depressions where water flows after rain. This need for running water restricts its distribution. However there are localities where this condition seems to be fulfilled where Pherosphaera does not occur. Near Lake St. Clair I have found the species only on the Labyrinth, a plateau with numerous small tarns about 3800 feet high north of Mt. Gould, (See Map 2) and on a trip through the headwaters of the Nive and Mersey rivers, where there is a multitude of lakes, not a single Pherosphaera was recognised. On the other hand Diselma, which it closely resembles in habit, was abundant.

It is not known in what respect the juvenile foliage

is different from the adult. Seed has been planted, but it failed to germinate. The mature leaves are very small, keeled and imbricating, and appressed to the stem. Their anatomy has been described by Saxton (1930). The plants are dioecious. The cones are terminal on the short branches which bear them. The male cones are erect, but the female cones are reflexed, a fact of considerable phyletic interest. The female cone (Fig. 48) consists of a number of closely aggregated bracts (br.) rather distinct from the leaves, to the number of 5 - 9, most commonly 7, the lower members of which subtend ovules in their axils. There are always two sterile bracts at the top of the cone, and sometimes a third (Table 1).

Material for these investigations was collected in the Mt. Field National Park (Map 1), where it is abundant. In Tables 2 - 4 data for the fertility of some of the material is given. For the purpose of these tables a fertile ovule is simply one that contains a large well developed prothallus.

TABLE 1

No. of bracts to cone	No. of cones with n ovules				Total no. of cones
	n = 3	4	5	6	
5	1	-	-	-	1
6	8	6	-	-	14
7	2	18	12	-	32
8	-	7	3	2	12
9	-	1	-	-	1
Totals	11	32	15	2	60

TABLE 2

	No. of cones examined	Total No. of ovules	% fertile	% abortive	% unpollinated ovules aborting early	late
1(2)	62	213	25.0	75.0	86.25	13.75
2	20	90	36.7	63.3	73.7	26.3
3	20	84	45.2	54.8	26.1	73.9

(2) For explanation of material described in Tables 2 - 4, see text.

All the material was at a comparable stage of development. The first

lot of material was collected on 22 March 1947 at 4000 feet on Mt. Mawson. The site is just above the tree line on a shelf below the top of the range, affording a slight, but not very great amount of protection from the prevailing westerly weather. The second lot was collected on 13 February 1948. The winter of 1946 was unusually severe, while that of 1947 was of average intensity. As Table 2 shows, the fertility of the material in 1947 was less than in 1948. The third lot of material was collected on 15 February 1948 at Eagle Tarn, a small lake 3390 feet nestling at the foot of Mt. Mawson in a sheltered position. The fertility was ~~much~~ but not significantly so higher. Lawson does not give the date when pollination occurs and I have not observed it myself. It presumably takes place about the time of megasporogenesis. Unpollinated ovules abort and do not develop large prothalli. The nucellus and embryo sac and surrounding integument in such ovules may enlarge for a time however before ceasing to grow. The stage at which they abort is also indicated in Table 2. Some abortive ovules were very small. Others were larger; but both contained a mere shell of a nucellus, or very degenerate prothallus. The two sizes intergrade but a rough distinction could be drawn at about half the normal size of the fertile ovule. It will be seen that in the exposed situation a much higher proportion of ovules aborts at an early stage than at the sheltered Eagle Tarn, and we can conclude that the weather conditions directly affect the amount of growth which takes place in the absence of the stimulus of a pollen tube.

TABLE 3

	No. of cones	No. of cones with n total ovules						
		n = 0	1	2	3	4	5	6
1	62	-	-	6	28	23	5	-
2	20	-	-	-	1	10	7	2
3	20	-	-	-	1	14	5	-

TABLE 4

	No. of cones	No. of cones with m fertile ovules						
		m = 0	1	2	3	4	5	6
1	62	22	28	10	2	-	-	-
2	20	3	5	8	4	-	-	-
3	20	5	3	4	6	-	-	-

Tables 3 and 4 compare the total numbers of ovules and the numbers of fertile ovules per cone. It can be seen that the distribution of fertile ovules among the cones is not comparable with the distribution of the total number of ovules, and therefore ovules are not pollinated at random. Some cones are in a more favourable situation than others in respect to the passing pollen grains. This would be expected where pollen is blown in a cloud.

Lawson's (1923b) Figs. 7 - 9 show pollen grains in the funnel-shaped micropyle. The exact mechanism of pollination has not been studied, but there is the definite orientation of the cone with respect to gravity which is characteristic of conifers with a pollination drop. Micropylar closing cells are well developed, but conspicuous lips to the integument remain in the mature ovule (Fig. 1). Germination takes place on the nucellus (Lawson's Figs. 6, 10, 11, 19).

THE GAMETOPHYTES

A. The Male Gametophyte

Lawson (1923b) described the pollen grains as having two nuclei, the tube nucleus and the generative cell. The latter divides early to give the body cell and stalk nucleus. The stage at which this takes place is not indicated, but according to his account, well organised body cells are found in pollen tubes which have penetrated half way through the nucellus.

Numerous preparations showed the three male nuclei. In Fig. 2 they can all be seen in the one section. The body cell has as usual a densely staining sheath, and the nucleus is large and clear with a big nucleolus. As Lawson said, the nucleus is slightly excentric. In Fig. 2 the stalk nucleus is very close to the body cell, and in Fig. 3a it is in close contact with, and decidedly flattened against, the body cell. Fig. 3b shows the tube nucleus of this male complex. The close association between the body and stalk is also seen in Fig. 4. The division of the body cell nucleus, and the male gametes, have not been observed. The structure in Fig. 5 may be the prophase of the division of the body cell nucleus, but it

stains more like fragments of a nucleolus. In Saxegothea (Looby and Doyle, 1939, Text- Fig. 4) the nucleolus does not disappear until the male gametes are well organised.

There has been a great deal of confusion over the identification of male cells in podocarps, and Looby and Doyle's (1939) discussion (pp. 110-1) shows that a small nucleus adherent to the body cell has often been interpreted as one of the unequal male cells. Fig. 3a of this thesis is similar to Sinnott's (1913) figures of Podocarpus dacrydioides (Pl.9, Fig. 50) and P. ferrugineus (Pl. 5, Fig. 2) and to Looby and Doyle's for Saxegothea (their Pl.3, Fig. 22). In the latter case this nucleus is sterile, and is very different from the male cells. Even when the male cells are unequal (as in P. andinus) there is a definite cytoplasmic sheath round the smaller nucleus (Looby and Doyle, 1944a, Pl. 9, Figs. 58, 59), it being doubtful if this is ever extruded from the body cell (p. 231). The adherence of a sterile nucleus to the body cell does not appear to be a regular occurrence in P. andinus, though their Pl. 9, Fig. 56 evidently shows such a condition. In Phyllocladus, Young's (1910) Figs. 32 and 34 show nuclei adherent to the gametes. In Pherosphaera the small and definite number of male nuclei makes the situation easier to interpret. No more than three nuclei have ever been seen in these preparations, and if a stalk nucleus is formed (there being no evidence to the contrary) the nucleus thus associated with the body cell must be the stalk nucleus. The adherence of the stalk to the body cell in this fashion seems to be a podocarpean feature, and is certainly an interesting point of resemblance between Pherosphaera and other podocarps.

B. The Female Gametophyte

One of the most significant features of the female gametophyte of Pherosphaera hookeriana is that the mature archegonia are deeply buried by growth of the prothallus. This was not specially noted by Lawson, although his Figs. 26 and 27 show buried necks. Lawson said that "each archegonium has its own small and shallow

depression over the neck" (pp. 511-2). This feature is an important resemblance between Pherosphaera and the other podocarps, in all of which this occurs.

The most superficial archegonium found is illustrated in Fig. 6 in which the neck is clearly visible. The megaspore membrane can be seen passing over the top, and the cells of the prothallus are just beginning to encroach over the neck cells. This is very similar to Looby and Doyle's (1939) Pl. 2, Fig. 10 of Saxegothea. A slightly later stage is indicated in Fig. 7 where the two cells concerned have nearly touched. In Fig. 8 the neck is just buried. All this occurs at an early stage. The archegonium, as Fig. 8 shows, has a very large vacuole and only a slight amount of peripheral cytoplasm. The megaspore membrane is curiously buckled (Figs. 6, 8). As growth continues the neck becomes more deeply buried and the amount of cytoplasm increases, resulting in numerous small vacuoles (Figs. 9, 2). The nucleus has a characteristic structure in the young archegonium. It has a comparatively large and conspicuously staining nucleolus, and little else can be seen in it. In Podocarpus andinus the nucleus has a highly vesicular structure, but as far as can be seen in Pherosphaera there is simply a central area which does not stain so heavily. Asteriods are not evident.

The ventral canal division was not observed, but the ventral canal nucleus was seen in several preparations of which Fig. 11 is one. Looby and Doyle (1944a) say the division takes place when the nucleolus has reached its full size (p.227), but in Saxegothea the nucleolus of male gametes degenerates after the male cells are organised. Before the ventral canal division the nucleus of the archegonium is close to the neck (Figs. 8, 9). Afterwards however, the egg nucleus is found in the centre of the upper part of the archegonium (Figs. 10, 11, 12, 14). In Fig. 11 the egg nucleus has its characteristic mature appearance, such as is seen also in Figs. 12, 14. No nucleolus is visible, and the nuclear material as here fixed and stained is evenly mottled. In Figs. 10, 13 some

granular material is to be seen, which perhaps represents the nucleolus or fragments thereof, though this is uncertain. There is no definite sheath round the egg nucleus. In this respect Pherosphaera differs from Pod. andinus, P. spicatus and P. dacrydioides, and resembles Saxegothea and presumably also Dacrydium (Sinnott, Pl. 5, Fig. 3). In Figs. 11, 14 the cytoplasm radiating from the egg nucleus has a highly fibrillar appearance. In old unfertilized archegonia, the egg nucleus becomes highly lobed, as in the two archegonia in Fig. 13. As in other podocarps the jacket cells do not extend quite to the neck, shown especially in Fig. 10.

There can be little doubt that as in Podocarpus andinus the archegonia are buried before fertilization. No preparations have been secured showing definitely the relation between the growing pollen tube and the archegonium. In the ovule of Figs. 1 and 2 the pollen tube definitely seems to be burrowing through the prothallus, and in Fig. 14 the end of a pollen tube expanded over the neck can be seen. In Fig. 15, the pollen tube pursued a course between the prothallus and nucellus before passing inwards to the archegonium, but the relation to the megaspore membrane is not clear. According to Lawson, the pollen tube grows between the nucellus and megaspore membrane (p. 504) to the position of the archegonium, which he apparently considered still superficial. As Fig. 14 shows, the pressure of the pollen tube on the archegonium results in the "suspension" of the neck cells, as has been described for other podocarps. Comparison of Figs. 12 and 14 shows how the neck is pushed downwards.

The entry of the male cells into the archegonium has not been seen, but they find their way between the neck cells, many preparations showing the ruptured neck (Figs. 15, 17, 19, 22).

EARLY EMBRYOGENY

Stages of fertilization were not observed. However the first division of the zygote was found, and is illustrated in Fig. 16, although the photograph does not do justice to this splendid preparation. The division is intranuclear, and the arrow points to

the nuclear membrane. It is probable that a certain amount of male cytoplasm enters the archegonium. The dark mass in the centre of the archegonium in Fig. 17 is so interpreted. Only a limited number of free nuclear stages was obtained in the material sectioned. In Fig. 18 two nuclei of the 4-nucleate stage are shown. Fig. 19 is apparently a 16-nucleate stage in which the division to give this number has just occurred, as evidenced by the close association of the nuclei in pairs. Some of these pairs seem to be enclosed by a common membrane which does not appear in the photograph. Fig. 19 shows clearly what proportion of the archegonium is taken up by the proembryo, and the ruptured neck can be seen above.

Relict nuclei are a frequent feature of the proembryo of Pherosphaera. Fig. 20, a 16-nucleate stage, shows a large relict nucleus enclosed in a sheath of cytoplasm. The nuclei in the dense embryonic area, which seems to be more condensed than in Fig. 19, are arranged in three tiers which have approximately 1, 4, and 10 nuclei each respectively. In Fig. 21, the "cell" above the dense portion contains 2 nuclei. In Fig. 22 the "cell" seems to be dividing into 3 or 4, and a nucleus can be seen in one of the segments. Fig. 23 shows apparently persistent nuclei from the open cell tier, and numerous other bodies reminding one of Looby and Doyle's remark that in Saxegothea there are "deeply staining cytoplasmic areas, 'protein vacuoles,' which are sometimes hard to distinguish from true nuclei; while other cytoplasmic aggregations of striae and granules may present a strikingly nucleus-like appearance." (p. 112). This feature makes it impossible to decide how many male nuclei enter the archegonium, or how long they persist.

Wall formation is in progress in Fig. 24. The cells below already have walls. They are uninucleate. Above, cleavage planes are extending upwards though they have not yet reached the top, and are delimiting the cells which divide (Figs. 25, 26) to give the open cell tier and the prosuspensor cells. The fully organised proembryo of Pherosphaera hookeriana consists of a tier of 6 to 10 prosuspensor cells and a number of embryo initials arranged more or less in two tiers. The lowest tier contains one or two of the units (embryo initials) and the others are in

the tier above, next below the prosuspensors. In Fig. 25 however, there seems to be only one tier of 3 embryo units, and 11 or 12 prosuspensor cells. The fact that there is only one tier of embryo units would be correlated with their small number. In Fig. 29, where there are 5 units, the lowest tier has only one unit, while in Fig. 28 with the unusually large number of 9 units there are 2 in the lowest tier. Most proembryos have from 4 to 6 embryo units. This organisation would arise in a proembryo with 16 free nuclei. One embryo system was found with 13 - 15 units (it was impossible to count the exact number). This presumably arose from a proembryo in which a further division to give 32 free nuclei had taken place.

The nuclei of the cells below the prosuspensor tier, it is believed, immediately divide again to give a binucleate cell. Binucleate cells in proembeyos of the age have not been seen, but their existence is discussed in the next paragraph. The division (?prometaphase) is seen in Fig. 26.

Some of the embryo units are single binucleate cells, as are three of them in Fig. 28, and one of them in Fig. 32. Generally however, each unit comprises two uninucleate cells. In Fig. 28 the large terminal embryo shows a wall running obliquely, but no sign of a wall can be seen in the plane of focus of the two nuclei, which do not appear to be separated even by a cleavage plane, nor is there any sign of persistent spindle fibres. This suggests that a wall is being laid down on a furrow which has not yet reached the centre of the cell. Looby and Doyle (1944b) find that in Podocarpus andinus the first walls formed in the proembryo are laid down on cleavage planes, and so are these terminating the binucleate state in the embryo initials. In Pherosphaera it will be shown that walls form by this method in slightly later stages, as are the first walls (Fig. 24), so that it seems certain that the walls between the two cells of a 2-celled unit are laid down in furrows in binucleate cells. There is no definite orientation for the wall between the two cells of a unit. In Fig. 29 it is transverse to the axis of the suspensors in two units (only one of which is seen), parallel to that axis in two of them, and oblique in the terminal unit. Generally the shape and position

of the two cells of a unit make it obvious they are sister cells. The formation of the 2-celled units takes place early. In the smallest embryo system dissected out (Fig. 35; its embryo units are illustrated on a larger scale in Fig. 27) all the units are two-celled; the second cell of the terminal cell is behind the one drawn. The embryo system from which Fig. 28 was taken has quite long suspensors; the development of cell walls in some units was evidently delayed.

During the early development of the embryo wall formation also takes place on cleavage planes after the nuclei have returned to the resting condition. Fig. 30 shows a condition frequently found. Two embryos are visible, one with 2 cells, the other with 3. In the right hand embryo, the two cells of the embryo unit can be recognised. One is uninucleate, the other binucleate, the latter condition having arisen by division of the nucleus without wall formation. There is no suggestion of spindle fibres between the two nuclei. The more advanced left hand embryo has one binucleate cell, and two uninucleate, this having come about by development of a wall on a cleavage plane between the two nuclei of a binucleate cell. In Fig. 31 both binucleate and uninucleate cells are seen. In the terminal embryo the cells a and b are evidently sister cells. The cell a is binucleate; the nuclei are in a resting state, and a wall was presumably about to develop between them. The mother cell of a and b would similarly have been a sister of cell c which is still uninucleate. The cells a, b and c are derived from the one cell of a 2-celled unit.

In Fig. 33 the nucleus of the uppermost cell (indicated by "b" arrow) is in the telophase stage of division, but there is no trace of a wall being formed across the equator of the spindle, which is itself scarcely evident. Figs 34a and b (two photographs of the same section in different planes of focus) show 2 embryos. The left hand one is a 2-celled unit. Each of the two cells (? of a 2-celled unit) in the right hand embryo is binucleate. In the right hand cell however, a furrow is being formed on the outside of the protoplast, but has not yet reached the centre. In Fig. 34a the furrow appears as a definite cleavage plane right across the centre of the cell. In b, in a lower plane of focus

(that of one of the nuclei), the furrow is not nearly so evident, and does not extend between the nuclei. At least three other examples of this process were seen, but this is perhaps the best. The fixative used would tend to enlarge, but not to create, such furrows. I regard them as significant and tangible evidence of cleavage plane formation.

The presence of binucleate cells in young developing embryos as distinct from binucleate embryo initials has not been recorded hitherto as far as I am aware. Binucleate cells are shown in Fig. 38 which has embryonal tubes beginning to elongate. Probably they do not occur in embryos older than this, although definite evidence on this point is lacking. In Podocarpus andinus, Looby and Doyle's (1944b) Pl. 14, Fig. 70, shows cells which appear to be binucleate.

No definite evidence is available concerning the possibility of the binucleate cell phase, which occasionally persists for some time, giving rise to 4-celled units, instead of 2-celled units as normally.

LATER EMBRYOGENY

As is usual, the prosuspensors elongate considerably before any further development takes place in the embryo initials (Fig. 36). During their elongation, prosuspensor cells are often left behind. Such a cell is indicated by fb. in Fig. 36. In Fig. 39 is another in which the nucleus has divided several times, and walls are probably about to be laid down. At pe. in Fig. 39 this formation of cellular tissue has taken place. Similar conditions are illustrated in Fig. 38 and Fig. 42. Fig. 37 depicts a more extensive development of cellular tissue - this figure represents the end of a single prosuspensor cell from a system with embryos similar to but larger than Fig. 42.

In young embryos cells are frequently seen in the position of apical cells; e.g. Fig. 30 (B), Figs. 38 and 39. Some resemble the condition illustrated by Buchholz (1933a) for Dacrydium, where it is claimed that growth by the divisions of an apical cell occurs. However,

I could find no evidence to enable me to state definitely whether or not apical growth takes place in Pherosphaera.

Figs. 38 and 42 show early stages in the development of embryonal tubes (et) which are produced by every embryo, their growth pushing the embryos apart. Since there is an embryo in the terminal position which will become the definitive embryo, the embryogeny is of the type which Buchholz (1933a) calls Determinate Cleavage Polyembryony. Where there are two embryo units in the lowest tier, the condition is perhaps somewhat indeterminate (fig. 42). Embryo systems similar to Fig. 42 were dissected in which there were two quite large embryos on secondary suspensors of equal length. There is no primary suspensor. The prosuspensor plays no part in the separation of the embryos. Although the free prosuspensor cells form "embryos" the remaining cells do not separate to carry the embryo units apart. The embryos are separated solely by the growth of the secondary suspensor.

What is properly called a rosette tier is absent in Pheropphaera, as in most conifers where a prosuspensor is present. Nevertheless, cells are delimited above the prosuspensors, and these may divide to form groups of cells. Similar "rosette" cells are known in many other cases, but their origin is uncertain. It has been suggested (Buchholz, 1940) that they may arise (1) by division of a prosuspensor cell at the upper end, or (2) from the relict nuclei or from the open cell tier. In Pherosphaera all the "rosette" embryos (r.) are above the thin basal plug (b.p.) of the archegonium which develops between the prosuspensors and relict nuclei (Coker, 1902), as in Fig. 40 - 42, and hence in this case the first alternative is ruled out. Although these figures are drawn from embryo systems dissected and mounted so that the parts may be separated somewhat, in most cases the prosuspensors, basal plug and rosette cells doubtless remain in their true relative positions. There was no suggestion that cells are ever cut off from the upper end of the prosuspensors. Reference has been made above (p. 19) to the frequent presence of relict nuclei in Pherosphaera, and there can be no doubt that they

give rise to these "rosette" embryos. Fig. 40 represents a group of 10 cells which are probably "rosette" cells. In Fig. 41 one of the "rosette" embryos is binucleate; the other has 6 cells. Fig. 42 shows similar embryos with 2 and 4 cells respectively. Embryos with up to 8 cells were observed. The fate of these "rosette" embryos in later stages was not determined.

Fig. 43 illustrates very well the determinate nature of the cleavage. The terminal embryo has a large secondary suspensor, and the three smaller embryos higher up each have embryonal tubes. At this stage the beginning of internal differentiation can be seen in the tendency of the cells to be orientated round the future root apex. As yet no extensive "columnar tissue" is formed. Fig. 44 shows an embryo system with two smaller embryos (emb.). The terminal embryo has developing cotyledons(c) between which can be seen the large stem apex(st. a.). The large size of the stem apex in the early stages is conspicuous. There are two cotyledons. They develop as outgrowths of the embryo, the tip of which remains round, and becomes the definitive stem apex. This seems to be a general condition in conifers (cf. Allen, 1947; Buchholz, 1919).

DISCUSSION

A. The Podocarpean Affinities of Pherosphaera

Binucleate embryo initials are regarded as characteristic of the Podocarpaceae. It has been shown by Tahara (1941) for Podocarpus nagi, by Looby and Doyle (1944b) for P. andinus, and by the same authors (1939) for Saxegothea that the binucleate condition arises by division of the nucleus in an original cell of the proembryo withput subsequent formation of a wall, and this is sure to be true of the whole family. In Pherosphaera binucleate cells occur, but more commonly the embryo units in the proembryo are 2-celled.

Evidence suggests that the 2-celled units arise from binucleate cells by formation of walls on cleavage planes after the nuclei have returned to the resting stage. Looby and Doyle (1944b) show that in Podocarpus andinus the binucleate units give rise to 4 uninucleate cells by formation of walls on cleavage planes after the next division of the two nuclei, and they suggest that this process may be as characteristic of podocarps as the binucleate cells themselves. In Pherosphaera this process may possibly operate occasionally, but the presence of 4-celled units has not been confirmed. The usual 2-celled unit in Pherosphaera is derived from the normal podocarp condition through the precocious development of a wall generally delayed until after the next nuclear division. This would be true both in ontogeny and phylogeny. Thus the nature of the embryo initials, binucleate and bicellular, indicates podocarpean affinities for Pherosphaera.

In such features as the adherence of the stalk nucleus to the body cell, and especially in the burial of the archegonia by growth of prothallus tissue and subsequent "suspension" of the neck cells, Pherosphaera agrees with podocarps which have been fully investigated, and these resemblances are to be considered very good evidence of the podocarpean nature of the genus.

Wilde (1944) has made use of the concept of the primary fertile branch, a shoot which bears fertile units (male cones, or female shoots reduced to single ovules) in the axils of its bracts. She concludes (pp. 9, 34) that the primitive fertile branch had a proximal vegetative portion bearing leaves and a distal fertile portion with bracts. She shows that in the genus Podocarpus there are two lines of evolution. One involves reduction of the fertile branch system, large leaves being retained. In the other the richly branched habit associated with numerous fertile branches is retained, and the leaves are reduced, being thick, keeled, imbricating and appressed to the stem. The latter group includes the sections Dacrycarpus and Microcarpus. Dacrydium and Pherosphaera both resemble the latter group, having richly branched habits and the characteristic leaf form

at maturity. Their fertile branches consist of a basal vegetative portion, and a differentiated terminal fertile region in which there are generally, in the female, a number of fertile bracts (according to the species), while in the male this is represented (as in Dacrycarpus) by a single unit (cone).

Buchholz (1933a) studied the embryogeny of Dacrydium cupressinum, and described it as the type of Determinate Cleavage Polyembryony. Pherosphaera resembles Dacrydium in the arrangement of embryo units and occurrence of determinate cleavage. Buchholz' Fig. 9 (1933a) shows the terminal embryo with a long secondary suspensor while the other embryos have small embryonal tubes only. In Pherosphaera embryonal tubes are produced by all the embryos about the same time (see Figs. 38, 42 of this thesis). In Dacrydium free prosuspensors forming embryos do not occur, and Buchholz did not observe "rosette" embryos.

The absence of an epimatium in Pherosphaera may be regarded as the culmination of an evolutionary sequence towards erectness of ovule and reduction of epimatium illustrated by species of Dacrydium. D. bidwillii (Fig. 45) has a fertile branch with two or three bracts (br.) subtending ovules, which are inverted at maturity, and completely enclosed by the epimatium, as in Podocarpus [3].

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(3) Sahni and Mitra (1927) have argued on the basis of cone anatomy that D. bidwillii should be referred to Podocarpus. This view however ignores the fundamental organisation of the fertile branch system. In Dacrydium (including D. bidwillii) there is a basal vegetative portion with small leaves, keeled and imbricating, while in most Podocarpus sp. this is lacking. Sahni and Mitra do not indicate in which section of the genus Podocarpus they would place D. bidwillii. Rather beaause of the diversity of fertile branch morphology, it is Podocarpus which should be split. If D. bidwillii has the integument fused with the epimatium for part of its length, this is merely an example of an organism being advanced in some respect while remaining primitive in others.

In D. cupressinum (Fig. 46) , and in D. intermedium and D. colensoi, the ovule is inverted when young, but grows into an erect position, and the epimatium is merely a thin sheath round the base of one side of the ovule (Sinnott, 1913; Sahni and Mitra, 1927). The terminal portion of the fertile branch constitutes a rather definite receptacle bearing a number of sterile bracts (ster. br.) and a single fertile bract (br.). D. franklinii (fig. 47) is more primitive in having numerous fertile bracts which are separated by considerable internodes. The axis and bracts are somewhat fleshy. It is advanced in that the ovules are erect and only partly enclosed by the epimatium. The fertile branch is strongly flexed at the base of the fertile portion, so that the micropyles still point downwards. Finally in Pherosphaera (Fig. 48) the vegetative part of the fertile branch is curved, making the cone pendulous, and the ovules are morphologically erect. The lower bracts are fertile, and in the material I have examined at least two of the uppermost bracts are sterile. The axis of the cone is considerably shortened so that the bracts are crowded. There is no epimatium. As in Dacrydium the nucellus is free from the integument. It is interesting to recall that some taxonomists, e.g. Eichler (1889) included Pherosphaera in Dacrydium.

Unfortunately it is not possible at present to institute a detailed comparison between the gametophytic structures of Pherosphaera and Dacrydium. The work on Dacrydium of Young (1907), who described the male gametophyte, of Stiles (1911), who described the mature female gametophyte and the male gametes, which he said were unequal, and of Sinnott (1913) who gave an outline of the development of the gametophytes and embryo, leaves us without such details, as of gamete formation and fertilization, on which a comparison might be made to establish a close relation between the genera. A detailed study of the development of Dacrydium is urgently needed, and more details of Pherosphaera are still required. In view of the considerable differences in cone structure manifest in the different species of Dacrydium, investigation of several species would be desirable.

The evidence of embryology does nor support the view that Phyllocladus is related to Pherosphaera. In Phyllocladus the embryo units are more numerous and differently arranged, and simple polyembryony occurs (Buchholz, 1941). Moreover, the fertile branches have no vegetative portion, and the leaves are flat scales, reduced in association with the development of cladodes, and have not the keeled imbricating form as in Dacrydium and Pherosphaera.

It is regrettable that Looby abd Doyle have referred to the 4 cells formed from binucleate cells in podocarps as a tetrad. The use of this term ought to be confined to the 4 cells formed by meiosis from a spore mother cell. The terminology I would use is as follows:- The cells formed when wall formation first takes place in podocarps are "primary embryo units". From these develop "binucleate embryo units", which give rise to "2-celled units" or "4-celled units". The two cells resulting from the first division of the uninucleate embryo initials in any conifer also constitute 2-celled units.

B. The Taxomomic Status of Pherosphaera

Pilger (1926) divided the Podocarpaceae into 3 subfamilies: Pherosphaeroideae, containing only Pherosphaera; Podocarpoideae, including Microcachrys, Saxegothea, Dacrydium, Acmopyle, and Podocarpus; and Phyllocladoideae, with Phyllocladus. More recently Buchholz (1933b) has proposed a family Pherosphaeraceae, containing the one genus Pherosphaera.

Although Pherosphaera has some characters which are very different from those of other podocarps, a case has been made put for regarding it as developed from Dacrydium-like forms. If our classification is to reflect phylogenetic relationships, Pherosphaera should be placed in the same group as Dacrydium, and not in a group of equal independent status.

The greatest similarity between Dacrydium and Pherosphaera lies in the nature of cone and fertile branch. The

organisation of the fertile branch in these two genera is essentially the same as that originally described by Wilde (1944) for Dacrycarpus and Microcarpus. The same type of organisation can be shown to occur in Microcachrys, which has a fertile branch with a proximal portion which is vegetative, having small keeled imbricating leaves arranged in 4 ranks, and a distal fertile portion in which the bracts are spirally arranged and form a definite cone. The bracts become very fleshy. Acmopyle (Sahni, 1920) would also appear to have a fertile branch in which the basal part is vegetative and bears small leaves.

Thus the nature of the primary fertile branch seems to provide the basis for a natural scheme of classification, and I therefore suggest that one subfamily of the Podocarpaceae should include Dacrydium, Pherosphaera, Microcachrys, Acmopyle, and possibly the genus proposed by Wilde, comprising the present Dacrycarpus and Microcarpus sections of Podocarpus together with Podocarpus vitiensis and P. minor. The subfamily could be called the Dacrydioideae. It would be characterised by the richly branched habit associated with retention of the complete fertile branch system, and by the fertile branches having a well developed basal vegetative portion in which the leaves are typically keeled and imbricating.

The validity of this proposed grouping is a matter which should be investigated further. In particular, the position of the Podocarpus spp. should be examined. The receptacle of Dacrycarpus is composed of the fleshy bases of the bracts (Gibbs, 1912), and the lamina of the fertile bract is fused to the epimatium of the ovule. Thus the receptacle is not entirely homologous with that of Eupodocarpus, and it is concievable that it arose independently. P. dacrydioides (Dacrycarpus) resembles Pherosphaera and Microcachrys and differs from other Podocarpus spp. in having a pollen grain with 3 wings (Sinnott, 1913).

This proposed group of genera with fertile branches in which the basal vegetative part is well developed is to be contrasted with other podocarps in which the fertile branch is reduced, or in which the basal portion is not well developed. Even if thespecies at present included in Podocarpus be not included, Dacrydium, Pherosphaera, and Microcachrys at least constitute a natural group, which, if diverse in some respects, is closely united on the basis of cone and fertile branch morphology.

C. Binucleate Cells and Primary Suspensors

The most distinctive feature of the embryogeny of Pherosphaera is the usual occurrence of 2-celled units instead of the binucleate cells which are general for the Podocarpaceae. The genus will therefore be important in any discussion on the significance of these binucleate cells. When the two cells in Pherosphaera are superposed, they recall the corresponding stages in conifers such as Chamaecyparis and Biota (Buchholz, 1932a) and Sciadopitys (Buchholz, 1931) in which the upper cell of a 2-celled unit elongates as a primary suspensor. In the case of Sciadopitys Buchholz states (p. 260) that "the small embryos are variously oriented and begin to elongate in all directions", corresponding to the haphazard direction of the wall in the 2-celled unit of Pherosphaera as noted above (p. 20). It may be then that the absence of a primary suspensor in Podocarpaceae is correlated with the occurrence of binucleate cells dividing into 4-celled units, the two courses being mutually exclusive. The condition seen in Pherosphaera is probably not primitive for the family, but derived, as are its other pecularities, e.g. absence of male cells and of epimatium, reduced megaspore membrane (Lawson, 1923b).

Buchholz (1931) regards the embryogeny of Sciadopitys as a type from which those of the Cupressineae and Taxodineae may have been derived, and he points out also the resemblance, "at least in general organisation", to Podocarpus spicatus, a member of

the Stachycarpus group. The proembryo of Sciadopitys was described briefly by Lawson (1910). Wall formation takes place with 16 free nuclei (4). Below the prosuspensor tier is a single tier of cells which "divide repeatedly, forming three or four rows which taper to a point" (p. 416). The cells of these tiers are the embryo initials. In Podocarpus andinus (Looby and Doyle, 1944b) on the other hand, a similar final result arises by a different process. There are 32 free nuclei at the base of the archegonium, and walls develop on cleavage planes giving rise to the cells which are the primary units. If the presence of binucleate cells in developing embryos as in Pherosphaera is demonstrated in more typical species, it would indicate a tendency in podocarps to delay wall formation unrelated to primary suspensor formation. It would seem possible that in the most primitive conifers wall formation was not specifically related to nuclear division. There might well have been an extensive free nuclear stage such as at present occurs in Ginkgo. The protoplasm cleaved up into protoplasts with several nuclei, and wall formation took place on the resultant interfaces. Nuclear divisions probably continued in such cells, again without wall formation. In the modern conifers however this process has become more standardized. In Stachycarpus the walls form round uninucleate protoplasts, in which two subsequent divisions take place before walls are laid down again, while in Pherosphaera normally only one further division takes place. In Podocarpus dacrydioides and P. totara, Buchholz (1941) figures spindle fibres between the two nuclei of the binucleate cell. Thus it would appear that in the more advanced podocarps wall formation on cleavage planes, probably related to their delay in formation, is being eliminated. I suggest that that two different ways in which a comparable final result is achieved in Stachycarpus and Sciadopitys result from this variation in the time at which walls are laid down. The 2-celled

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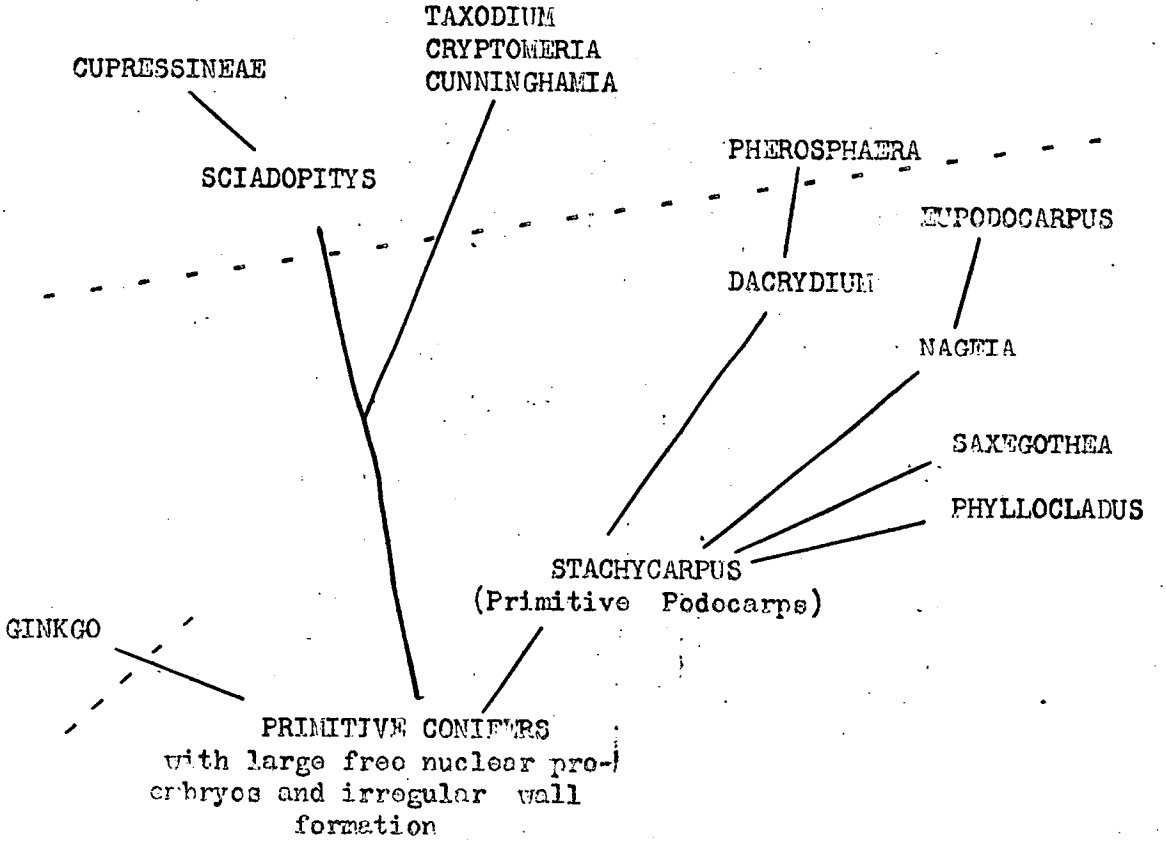
(4) Tahara, quoted by Buchholz (1940), says there are 32 free nuclei.

embryo initials being a derived state in Pherosphaera we can postulate that the presence of 2-celled units in Sciadopitys and elsewhere is similarly a derived condition.

In Pherosphaera both cells of the 2-celled unit contribute to a single embryo, and this is true also of Cunninghamia, Cryptomeria and Taxodium (Buchholz, 1932b; 1940; Coker, 1903) which have no primary suspensors. In the evolution of Sciadopitys, however, it is suggested that one of the cells of the 2-celled unit became the primary suspensor, and the definitive embryo grew from the other cell.



Suggested relationships between different types of Embryogeny in the Taxodiaceae and Podocarpaceae. The genera above the broken line possess 2-celled units.

SUMMARY

1. Ovules are not pollinated at random.

2. Archegonia are buried by growth of the prothallus, as in podocarps.

3. There are 16 free nuclei.

4. Proembryo has a tier of prosuspensors and generally two irregular tiers of embryo units.

5. Embryo units pass through a binucleate phase, which gives rise to a 2-celled unit of 2 uninucleate sister cells. This is considered to be derived from the typical podocarpean condition wherein a further division takes place giving 4 nuclei and a 4-celled unit.

6. Each embryo unit gives rise to a separate embryo. The development is an example of determinate cleavage polyembryony.

7. Binucleate cells regularly occur in developing embryos.

8. Rosette embryos develop from relict nuclei.

9. There are two cotyledons.

10. The structure of the cone is the highest development of an evolutionary line towards reduction of epimatium and erectness of ovule illustrated by species of Dacrydium.

11. A natural subfamily of the Podocarpaceae comprises those genera and species with unreduced fertile branch systems, in which the fertile branch has a basal vegetative portion bearing small keeled imbricating leaves.

12. Two-celled units occur in all conifers except typical podocarps. Each of the two cells may contribute to a single embryo (primitive condition) or one cell may elongate as a primary suspensor.

P O D O C A R P U S A L P I N U S R.Br.

ECOLOGY

Podocarpus alpinus Has the widest distribution within the island of any of the Tasmanian conifers, and it is the only one of the mountain forms which extends beyond the state, occurring on the Bogong High Plains, and elsewhere in the Victorian Alps. In Tasmania it grows prostrate on the dolerite talus which covers considerable areas of the mountains. There is a small patch of it on Mt. Wellington, being the only conifer on that mountain (apart from a few that have been planted). As far as I have observed it is confined to talus in Tasmania, but on the Bogong High Plains, as well as being on basalt talus, it occurs in creek beds, where it grows more erect, resembling Pherosphaera in habit.

The life cycle of Eupodocarpus sp. is generally stated to occupy a single season. On Mt. Wellington pollen being shed has been observed on 3rd December 1946. Pollen was being shed on the Snowies on 2nd March 1947. On Mt. Mawson, prothalli with archegonia still in the initial stage were found on 8th December 1945. Embryos with binucleate units were found on Mt. Wellington on 20th April 1946, and also on 24th August, and still even on 29th September. On 20th October 1946 multicellular embryos and cleavage of suspensors were observed. It is by no means certain however that a two-season life cycle is general in this species.

CONE MORPHOLOGY

According to Wilde (1944), P. alpinus exhibits stages transitional between those species in which the male cones are in "Primary Clusters", and those with "Secondary Clusters". "While the Stachycarps group and Nageia section of the genus,"

she says (p. 14), "are characterised by reduction of the primitive fertile branch to primary clusters and finally to solitary cones, the Eupodocarpus section posses in general secondary clusters. The male fertile branch has likewise been reduced to the extreem of a single, terminal fertile branch unit on a bracteate pedicel varying in length and representing with the few remaining sterile bracts the vestige of the fertile branch. These reduced fertile branches, however, have been brought together into secondary clusters by reduction in the internodes of the vegetative branch on which they are borne. While the secondary clusters are found only rarely in isolated cases on individual plants in the Stachycarpus group, they are a constant and characteristic feature in the Eupodocarpus section. Furthermore, the secondary clusters are reduced here in some species to single cones by a subsequent loss of reduced fertile branches from the secondary cluster. In some species it has become constant, in others both clusters and single cones occur."

She goes on to describe P. alpinus, in which "the male cones are usually in loose clusters of three or sometimes more at the end of short branches about 5 mm. or less in length. These branchlets sometimes bear a few ordinary vegetative leaves other than the crowded, terminal ones subtending cones. Ordinarily, the internodes on such branchlets are shorter and the leaves a little smaller than those on the rest of the plant. Towards the extremity of the branch the internodes are completely eliminated and four or more leaves are brought together surrounding the cluster of, usually, three cones. One cone is always in a terminal position on the branchlet; no case of a persistent apical bud was found.... A cone pedicel... is almost eliminated in P. alpinus. Each individual cone axis, however, bears at its base three or four membranous bracts and represents a fertile branch reduced to its single terminal unit.

"In some cases the lower leaves on the vegetative cone-bearing branchlets are completely lost and the upper ones surrounding

the clusters have been reduced to scales. Moreover, various stages in the shortening of the branchlet occur, from the length already described to 2 or 3 mm. With loss of lower leaves, the bases of the upper scales entirely sheathe the branchlet, now actually a peduncle. When this occurs the clusters resemble those in other species of the section that no longer show the steps leading to this reduction. This species therefore,... forms an important transition to more advanced conditions prevalent in the rest of the section." (pp. 15, 17).

In all the material I have examined I have not found the small leaves described by Wilde, all the branches being naked (Fig. 49). These branches are subtended by a scale leaf. The leaves subtending the fertile branches are rarely completely whorled, but generally arise fairly close together. One very interesting group of cones was found (Fig. 50). As usual, three reduced fertile branches were represented. One of these fertile branches in the axil or a leaf bore a single terminal cone (c_1). Another leaf subtended a fertile branch with three cones , the lateral ones in the axil of a membranous bract ($c_{2a,b,c}$); there was quite a long internode below the lowest bract. The third fertile branch - the terminal one - had a terminal cone (c_{3a}), with a second smaller one below it (c_{3b}). This illustrates very well the way primary clusters are reduced and gathered together to give secondary clusters. One fertile branch in this case comprised a primary cluster of three cones, the second a cluster of two cones, while the third was reduced to a single cone. At the other extreem a single cone in the axil of a vegetative leaf was found. The cone was at the end of a short peduncle, and had a number of scale leaves clustered round the base.

The female cone is not described by Wilde, nor by Gibbs (1912), although a description without figures is given by Brooks and Stiles (1910). The branch bearing the cone is subtended

by a scale leaf or occasionally by a vegetative leaf (Figs. 51,52). The peduncle may be up to 2 mms. long, or reduced almost to vanishing point. At the top of the peduncle, Brooks and Stiles described 2 small scale-like bracts, although in my material there are none, agreeing with Pilger's observations quoted by Brooks and Stiles. There are 2 fleshy bracts, one of which normally subtends the ovule. Rarely 2 ovules are present (Fig. 51); this might be a genetically controlled character, since all the cones on one plant were found to have 2 ovules. A third bract is sometimes found (Fig. 52). Brooks and Stiles give the impression that it is a general feature, which is not so in my experience. It is often difficult to decide whether the structure is a bract or the tip of the stem of the branchlet. The latter interpretation often seems more likely.

Thus although P. alpinus has typical Eupodocarpus features in its female cone, it forms an important "transition form" in respect to its male cone. For this reason these following observations on the embryogeny may be of value, since Buckholz (1941) has recommended study to correlate external features and embryogeny.

EMBRYOGENY

The proembryo consists of a tier of prosuspensor cells, below which are binucleate embryo units, in this case usually two in number (Figs. 54, 57), though sometimes one only (Fig. 55) and occasionally other numbers. Their relative frequencies are given in Table 5. The embryo units are arranged normally in a single tier, but in the case of 5 embryo units there were several tiers. Table 6 gives the numbers of prosuspensors counted.

In the development of the embryo there is a high degree of cleavage polyembryony. Even at such an early stage as Fig. 54, not all the prosuspensors are in contact with the embryos.

TABLE 5

x	No. of embryo systems observed with x binucleate units
1	6
2	21
3	3
4	1
5	1

TABLE 6

y	No. of embryo systems with y prosuspensors
6	1
7	2
8	5
9	5
10	3

As they elongate, cells are cut off at their ends. In Fig. 53 the early telophase of such a division is seen, and a wall is already beginning to develop on the equator of the spindle. In Fig. 56 several such cells are shown, and in Fig. 60 one of the embryos in a prosuspensor is 2-celled.

As well as this very common phenomenon of cutting off of walls from the free ends of the prosuspensors, cells may be delimited from the centre of such a cell. One is seen in Fig. 53, and several are in Fig. 57. The upper one is binucleate, and in the lower, immediately below it, the nucleus is seen in division. In addition, cells may be cut off at the top end of the prosuspensors. Such a cell is shown in Figs. 53, 61. Another is in Fig. 58, in which the relation to the archegonium is clear - the "rosette" cell is below the basal plug of the archegonium.

Concerning the development of the embryos themselves, little information has been obtained. In Fig. 59 embryos from three systems are drawn. The two lowest embryos appear to come from different systems which had a single unit at their tips, while the upper two seem to be derived from an embryo system with two units. It appears that the number of embryos which develop is equal to the number of binucleate units.

DISCUSSION

The embryo of Podocarpus alpinus resembles in some respects that of P. macrophyllus and P. totara, and in others P. urbanii (Buchholz, 1941). In P. macrophyllus there is normally only one binucleate embryo unit, and 8 - 15 prosuspensors. (Buchholz says 14 - 15 for the subspecies maki. Tahara (1941) gives 8 - 14, though it is not clear whether he included that subspecies, or was concerned with the typical form only.) In P. macrophyllus there is extensive separation of the cells of the prosuspensor. In P. totara, as in P. hallii and P. nivalis, there is normally only one binucleate unit, and 7 - 10 prosuspensors, which apparently do not cleave up much. P. urbanii has 11 - 13 prosuspensors and 2 - 3 units. P. glomeratus, P. coriaceus, and P. purdeanus are apparently similar. In P. urbanii there is very extensive cleavage.

We may distinguish two methods whereby the embryos are separated when there is cleavage. In Dacrydium (Buchholz, 1933a) and Pherosphaera (Elliott, 1948) the embryos are separated by the growth of the secondary suspensors produced by each embryo. On the other hand, in Cryptomeria (Buchholz, 1932b) and in Taxodium, the embryos are separated by cleavage of the prosuspensor cells. This matter is not especially concerned with the determinate or indeterminate nature of the cleavage. In P. nagi (Tahara, 1941) the embryo units, 7 - 9 in number, are all in one tier, and cleavage is thus indeterminate, but Tahara's figures show clearly that the embryos are not separated until the growth of the secondary suspensors forces them apart. Buchholz' (1941) decription of P. amarus, which probably has a similar arraggement of embryos to P. gracilior (l.c.) and P. usambarensis (Buchholz, 1936), indicates a massive secondary suspensor produced by a large terminal embryo, with several smaller embryos further up the suspensor, which could not have been separated by prosuspensor cleavage. In P. urbanii separation of embryos by secondary suspensor growth is the rule, though in 10% of cases

prosuspensor cleavage takes place.

It is regrettable therefore that information was not obtained as to the details of embryonic cleavage in P. alpinus, since it would be interesting to know how the embryos are separated in this form, with normally two embryo units and extensive cleavage of prosuspensors.

Buchholz (1941, p. 20) observes that the American Eupdocarpus spp. have about 12 prosuspensor cells, and the Australian ones about 8, and P. alpinus is no exception.

At first sight there seems to be no correlation between embryology and cone morphology. In embryogeny, P. glomeratus and P. coriaceus resemble P. urbanii (Buchholz, 1941), a type which is more primitive than P. alpinus. But P. glomeratus has pedunculate secondary clusters, and P. coriaceus has single cones (Wilde, 1944) - see Table 7. However, it is possible evolution has taken place independently in different geographical areas, a plausible supposition if they have remained isolated for long enough. Considering the American forms, there seems to have been little varation in the embryo attendant on fertile branch reduction. In the Australian region, P. alpinus with a transitional cone type has a less specialized embryo than the New Zealand species in which secondary clusters are a constant feature. P. macrophyllus from China and Japan falls in a group by itself. It is possible that when further knowledge is available, a more definite correlation between cone morphology, embryogeny, and geographical distribution will be evident.

SUMMARY

1. Reference is made to the morphology of the cones of Podocarpus alpinus.

2. In the embryo there are normally 2 binucleate units,

TABLE 7

Type of arrangement of male cone [1]	Embryonic organization		Geographical distribution [2]
	No. of prosuspensors	No. of binucleate units	
TRANSITIONAL			
P. alpinus	7 - 10	(1) - 2 - (5) [3]	Victoria, Tasmania
SECONDARY CLUSTERS WITH PEDUNCLES			
P. totara, P. nivalis, P. hallii	7 - 10	1 - (2) [4]	New Zealand
P. glomeratus	11 - 13	2 - 3 [4]	Peru Ecuador
SESSILE SECONDARY CLUSTERS			
P. macrophyllus	8 - 15	1 - (2) [4,5]	China, Japan
SOLITARY CONES			
P. coriaceus	11 - 13	2 - 3 [4]	Colombia Venezuela West Indies

(1) Wilde, 1944; (2) Dallimore and Jackson, 1923; (3) Present investigation; (4) Buchholz, 1941; (5) Tahara, 1941.

and 6 - 10 prosuspensors. A high degree of cleavage polyembryony occurs, and cells may be cut off either end and in the centre of the prosuspensors.

3. A distinction is drawn between separation of embryos by growth of secondary suspensors and cleavage of prosuspensor cells.

4. In considering the relation between cone morphology and embryology it would seem that geographical distribution must also be taken into account.

A T H R O T A X I S

INTRODUCTION

The genus Athrotaxis was established by Don for his two species A. selaginoides (the King William Pine, named after the King William Range where it was first observed), and A. cupressoides (Pencil Pine). J.D. Hooker described the third species, A. laxifolia. The name has sometimes been spelt Arthrotaxis, and Campbell (1940) has perpetrated Arthrotaxus. Baker and Smith (1910) pointed out that Don's spelling was derived from the Greek for "crowded".

Baker and Smith (1910) described A. selaginoides in some detail - the leaf and wood anatomy, and the leaf oil. This species has been the subject of the only morphological work published on the genus - Saxton and Doyle's (1929) fragmentary account of the life history, and Cross's (1943) description of the stem apex. The desirability of confirming and amplifying Saxton and Doyle's account, and of working out the embryogeny, was the reason for including Athrotaxis in the present study, which has been practically confined to A. cupressoides.

A. cupressoides is the commonest species of the genus, with a very wide range over the central highlands. Its habitat is generally by the side of tarns and along streams, where it forms small groves. The whole aspect of the tree (which may grow to 60 feet in height) is most pleasing. A. selaginoides on the other hand is a forest tree, 100 feet in height. In the Lake St. Clair National Park, at the head of Pine Valley, there is a magnificent forest which must be unique, since both the dominant - A. selaginoides - and the chief subdominant - Richea pandanifolia - are confined to Tasmania. Baker and Smith refer to the habit of the King Billy to form a dense crown with a long unbranched trunk, and hence cones are usually quite inaccessible. However, when trees grow isolated as they do occasionally (e.g. at Lake Skinner in the Snowy Mountains, and at Lake Rhona in the Denison Range) the habit resembles that of the pencil pine, and cones are generally within reach. A. laxifolia

is rare, and occurs only as single trees. I know of some half dozen specimens - on the Acropolis, and between the Guardians and Mt. Gould, near Lake St. Clair. (Map 2) In the herbarium at the University of Tasmania there are specimens of A. laxifolia from the Tarn Shelf, Mt. Field National Park, but though I have been there many times I have never found any of these trees there. A. cupressoides is very common in the Mt. Field area, especially round Lake Dobson. In 1946-47 this species formed no female cones in that locality, although there was plenty of pollen. Strangely enough, the few trees of A. selaginoides in the area (there are a few near Lake Fenton, and I have found one on the Tarn Shelf) set fertile seed, which germinated in Melbourne after having been kept in an envelope for more than 10 months. There were plenty of female cones of A. cupressoides in the Lake St. Clair district in 1946-47, but their fertility was poor, only about half the ovules having been pollinated. In 1947-48 numerous cones were formed by the trees near Lake Dobson.

The status of the species in the genus is interesting. All the characters of A. laxifolia are intermediate between those of A. cupressoides and A. selaginoides. Dallimore and Jackson (1923) suggest that the three forms "might well be regarded as gradations of one species." But the two common forms are really quite distinct, and are held by all botanists in Tasmania to be good species. The possibility that A. laxifolia is a hybrid is widely discussed. The variability and intermediate nature of its characters, together with its rarity and occurrence near both of the other two species, suggest a hybrid origin. No proof of this view is available however. Cytological examination has not so far been fruitful, and it has not yet been possible to attempt to cross the two common species artificially owing to their relative inaccessibility. The most convenient approach would be to collect seed from the same tree of A. laxifolia for several years, and attempt to grow it to see if any segragation took place. Cones with nearly mature seed were found on the Acropolis on 26th February 1947, but I foolishly fixed all my material, and it was not found possible to revisit the locality during the ensuing

monthd.

ATHROTAXIS CUPRESSOIDES Don.

DEVELOPMENT OF THE MALE CONES

About the third week of February the tips of the branches which will bear the male cones can be distinguished from the purely vegetative tips, which is impossible a month earlier. A section through the cone at this stage shows the sporophylls fully differentiated. Fig. 71 is such a section. It is not median, but has been selected for the two sporophylls on the left which show the lamina and sporangia weal. There are two pollen sacs (Fig. 64). Rarely the sporophylls near the top have three (Fig. 63). The lowest sporophylls are of course larger that the upper ones. The uppermost sporophyll sometimes has no lamina at all (Fig. 65). As usual there is a median resin sac.

Fig. 72 shows one of the sporangia from Fig. 71 at a higher magnification. The archesporial tissue occupies a relatively small volume in the centre. It may be distinguished by the size of the nuclei, and thier more frequent division, and the loose arrangement of the cells, as compared with the outer wall and tapetal tissues, where the cells are in more orderly rows.

By the middle of May the sporangia are fully grown and contain spore mother cells. When material is placed in a jar of water after picking its development continues. Male cones collected at the Great Lake on 18th May 1946 were left in a jar of water in the laborotary in Hobart, and were fixed on 29th May. Although meiosis does not take place till August, examination of this material showed that it had already begun in a number of spore mother cells, although none had advanced beyond pachytene. Fig. 73 shows a number of these cells, showing pronounced synezesis. The wall of the sporangium consists at this stage of an outer layer of cells, and two or three narrow cells within it. The cells immediately outside the sporogenous cells

in Fig. 73, though resembling them somewhat in appearance, are not functioning as such, and some somatic mitoses are seen in them. These cells may be designated tapetum.

Material was next collected at Lake Dobson on 25th August 1946. Material fixed in the field was found to contain microspores, though in some cones the uppermost sporangia contained quadrinuclear protoplasts, and a few examples (which the arrows point to) of Telophase II. (Fig. 74). Unfortunately no metaphases were observed. Thus meiosis occurs in about the 2nd and 3rd weeks of August. Fig. 75 shows stages of cleavage into microspores, and in Fig. 76 most of the microspores, still unwalled, are completely separated. At this stage the sporangium wall consists of one or two layers of narrow cells inside the epidermal layer, and the tapetal cells can be seen adhering to the wall (Figs. 74, 75). In Fig. 73 the tapetal cells seem adherent to the sporogenous tissue, which may have been due to the abnormal treatment, or to the use of a different fixative (Navaschin's). At the stage of Fig. 74 vascular tissue in the cone axis does not extend higher than the level of the lowest sporophyll. Above the end of the tracheids there is a conspicuous tract of procambial tissue. After the material had been left in a jar of water for 12 days, tracheids were found to have differentiated in the stalks of the sporangia. The strand of vascular tissue bifurcates, and passes to each side of the resin sac.

By this time pollen grains have long since been organised. The inner layers of the sporangium wall become disorganised, so that the wall consists merely of a single layer, the walls of which cells have bars of thickening deposited on them. (Figs. 77, 78). At the time of meiosis the sporophylls overlap, and the lowest are enclosed by the uppermost leaves (Fig. 62), but as the pollen matures, the cone axis elongates, and the sporangia emerge.

POLLEN

It is well known that the pollen grains of the Taxodiaceae and Cupressaceae have no air bladders, nor are there any male prothallial cells. It is commonly stated that the exine is very thin, while the intine is thick and gelatinous, and when the mature pollen grain comes in contact with water the intine swells throwing off the exine (Wodehouse, 1935; Doyle, 1945). During the course of theis morphological study of A. cupressoides, attempts were made, both in 1946 and 1947 to grow pollen in sucrose solutions of various concentrations. As far as the growth of pollen tubes was concerned the experiments were a failure (as might have been expected considering the normally slow growth of conifer pollen tubes), but they were interesting in that they showed that the wall of the pollen grain has not two, but three, layers.

As stated above, meiosis occurs about the third week of August. The pollen is ready to be shed about a month later. I have not observed the shedding of the pollen in the field. However, male cones collected at Lake Dobson on 9th September 1946 and placed in a jar of water in the laborotary in Hobart shed pollen there on 17th September. Also, cones collected at the Hugel Lakes (Lake St. Clair National Park) on 14th September 1947 shed pollen in the lab. on 18th September.

The pollen of A. cupressoides is spherical or subspherical and devoid of any furrow or germ pore. The surface of the exine is ornamented with minute granules. The pollen of Athrotaxis thus resembles that of Cunninghamia [1] and the Cupressaceae, and differs markedly from that of Cryptomeria, Taxodium, Glyptostrobus, Sequoia and Wellingonia (Wodehouse, 1935; Erdtman, 1943).

Pollen prepared by the usual acetolysis methods (Erdtman, l.c., p. 27) has an average diameter of 27.3 microns (extreems 22.5 - 31). Owing to the thinness of the exine, many of the acetolosed grains collapsed.

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(1) According to Wodehouse, Cunninghamia has no germ pore, but Erdtman figures a small one, although there is no trace of any germinal papilla

When mounted in water the grains become turgid, and the avergge diameter is 30 microns. The exines are thrown off in from 2 to 4 minutes. The contents of the grain is now seen to be surrounded by a gelatinous "halo", spherical in shape, and of average diameter 38 microns. The "cell" inside is pear shaped, the narrower end being the generative cell end. The wall separating the generative cell can be seen in some of the grains in Figs. 79 and 80.

After 4 days in water the condition shown in Fig. 81 is reached. The protoplast has enlarged and become vacuolate, and the starch, which is densely packed in the pollen grain, has been used up to a large extent. The tube and generative nuclei are clearly visible in most cases. Although the shape of the protoplast is irregular, the "halo" surrounding it is still spherical except where the protoplast is actually forcing it out of position. The protoplast within the "halo" is seen to have a wall of its own. With time, it emerges from the "halo". In water this may already have happened in 4 days, and in Fig. 81 one of the cells is seen to be without a "halo". Generally by the end of one week the greater part of the "halos" has been lost. In Figs. 82 and 83 the generative and tube cells are emerging from the "halo", and a definite wall (here somewhat swollen) is seen around them. The "halo" also has a definite thickness.

Thus the pollen grain has 3 walls - the cutinous one thrown off first, the gelatinous "halo", and the innermost wall. These may be designated ectexine, intexine, and intine respectively. Such a 3-layered wall has not been recorded before in this group of conifers, but probably would not be noticed where the grains germinate normally on the nucellus. Looby and Doyle (1942) say that in Wellingtonia "no features of special interest appear" in the early changes in the pollen grain (p. 49).

FEMALE CONES

The female cones of Athrotaxis cupressoides are distinguishable in the middle of September before pollination.

Eames (1913, pp. 32, 33) has referred to the arrangement of the vascular bundles in the cone scales of Athrotaxis. The three species differ in the extent to which the so-called "ovuliferous scale" is developed. In A.cupressoides it attains its highest elaboration in the genus. In the young cone, and even at the time of fertilization the "bract" is very conspicuous (Fig. 66). In older cones with mature seeds, the tip of the bract has increased in size very little, but the "ovuliferous scale" has grown to become relatively much larger than at the early stage. (Fig. 67) The ovuliferous scale in the young cone forms a comparatively small cushion round which the ovules are attatched. The ovules are in a single row, are winged from the earliest stages, and have their micropyles directed towards the cone axis.

Larvae of a fly have been found in cones collected in January and February 1947, at Liaweenie, Pine Valley and the Hugel Lakes. The larvae appeared to be eating the ovules, and infested cones were often deformed. As many as 9 grubs have been found in a single cone. It would be interesting to know how long, and when, the imago is active. Presumably eggs are laid when the cone scales are open at pollination, and the pupae may remain in the cone until the scales separate to let the seeds drop out.

THE FEMALE GAMETOPHYTE

The youngest material examined was collected on 14th December 1946, and contained free nuclear stages. Fig. 84 is a longitudinal section of an ovule at such a stage, and shows very well the nature of the integuments, the micropyle, and attatchment of the ovule to the cone scale. Fig. 85 is a transverse section of a similar ovule. An important feature is that the nuclei are not distributed round the embryo sac evenly in a single layer, but are congregated at the lower end

where they are two deep. In this respect A. cupressoides resembles Sequoia sempervirens and differs from Wellingtonia gigantea (Looby and Doyle, 1942). Saxton and Doyle's (1928) Fig. 8 of A. selaginoides shows nuclei distributed round the embryo sac in a single layer, though more densely packed lower down. It is now well established (Looby and Doyle, 1942) that in Wellingtonia alveolation proceeds evenly all round the embryo sac. In Sequoia however, while alveoli are formed against the central vacuole, in the lower part where nuclei are numerous and not in a single layer, walls form cutting out cells of irregular arrangement. In A. selaginoides, the arrangement of cells in Saxton and Doyle's Fig. 12 shows that wall formation takes place by the method in Sequoia sempervirens. Their Fig. 18 shows alveoli being formed at the vacuolar edge of the basal portion.

According to Saxton and Doyle, the upper half or three quarters of the embryo sac in A. selaginoides does not form cellular tissue, but remains vacuolate. In A. cupressoides my sections of prothalli with young embryos shows walls in the tip, but the cells are of very irregular form.

In connection with the alveolar origin of archegonia, Looby and Doyle mention the possibility that nuclei may remain in a single layer against a pollen tube, while elsewhere at the same level the nuclei are several deep. This is perhaps illustrated at several points round the embryo sac of Fig. 85, although pollen tubes have not been identified for certain in this ovule. The excessive shrinkage on fixation makes certainty on these points difficult.

According to Saxton and Doyle's account the pollen tube of Athrotaxis grows over the surface of the nucellus, entering it later, to grow against the megaspore membrane. If this is so, it is another feature in which Athrotaxis resembles Sequoia and not Wellingtonia. In Fig. 86 the male complex is seen to be inside two layers of nucellus, and in Fig. 88 it is well inside, though the relation to the megaspore membrane is not clear. In Fig. 89 there is a hole in the nucellus

opposite the body cell, which is perhaps where the pollen tube entered.

It is quite evident from my material that as Saxton and Doyle stated, the nucellus is consumed up to a thickness of one cell at an early stage. This is shown in Figs. 84 and 86 which are free nuclear stages, and it is obvious in later stages with embryos.

In Fig. 87 dividing nuclei are seen. Since only a very few seen seen were not in late anaphase, it would appear that all nuclei divide simultaneously in the early stages(this is the 8th division in the embryo sac), though how irregular they become in later stages I am unable to say.

It was impossible to collect any more material for over a month, by which time the prothalli contained young embryos. Although a full series of developing embryos has been obtained, their interpretation is difficult owing to lack of proembryo stages.

EMBRYOGENY

The essential points in the embryogeny of A. cupressoides are:

1. Simple polyembryony.
2. Rosette embryos are always present, though their development is variable.
3. The suspensor consists of a prosuspensor and a secondary suspensor.
4. Two cotyledons.

Saxton and Doyle (1929) reported that the proembryo completely filled the archegonium. This however is an error, as Looby and Doyle (1937, p. 471) have pointed out. Owing to the orientation of the archegonia it is of course very easy to cut sections which would omit the archegonium while passing through the three tiers of the proembryo. Saxton and Doyle's Fig. 19 seems to include the archegonium, at least in the right hand embryo.

The proembryo of Athrotaxis can be interpreted with reference to that of Wellingtonia (Looby and Doyle, 1937), which in common with Cunninghamia (Buchholz, 1940) and Taxodium (Coker, 1903), has 8 free nuclei. In Wellingtonia the 8 free nuclei are arranged in two tiers of 2 and 6, or 3 and 5. After wall formation the upper open tier then divides, thus giving rise to prosuspensor and rosette tiers. In position in Athrotaxis may be similar. The 8 nuclei are regularly arranged in two tiers of 4 each. The division of the upper tier then gives rise to prosuspensor and rosette tiers.

The 4 primary units of the embryo have been seen only once in a section. However, numerous 8-celled embryos have been dissected. They arise by the division of the lowest 4 cells in a plane at right angles to the axis of the suspensors. The walls may be inclined towards the outer corners (Fig. 93). The next division increases the number of cells in each tier. In Fig. 94 the last of such divisions is taking place in the upper tier, while the next division takes place only in the lower tier and adds a third tier of cells to the embryo, as the orientation of the spindle in the lower tier of Fig. 94 suggests. The result is seen in Fig. 95. Thereafter the divisions are less regular, but they result in a single embryonic mass, which may show some lobing (Fig. 96) and whose origin from 4 primary cells can generally be made out in sections (Fig. 97). In only one case however was the independent development of the 4 quadrants observed (Fig. 99), which is clearly abnormal.

Normally the 4 prosuspensors elongate in unison and remain attatched to the embryo. Occasionally it would appear that only 2 of the prosuspensors have elongated, and Fig. 90 is so interpreted although the 4 primary embryo units seem accounted for in the embryo.

The development of the rosettes is highly variable. It is difficult to make out the arrangement of walls in the dissected embryo systems mounted in euparal, and sections have not been much help. The commonest condition seems to be 4 nuclei in a single cell covering the whole of the 4 prosuspensors. (Fig. 92). This cell evidently has a wall

above, as an unwalled "cell" could not be dissected out so consistently. Such a 4-nucleate cell becomes divided into 4 cells (? or the 4 cells are there from the first) (Fig. 98) or at least into 2 (Fig. 91), in which further divisions take place, which may or may not give rise to walled cells. In Fig. 101 from a section, 2 rosette cells are seen which have just divided into 4. In Fig. 90 there seems to be merely a single uninucleate rosette cell. A thick plug is sometimes seen above the rosettes, much like that that which develops above the prosusupensors in podocarps. There does not seem to be any definite correlation in the stage of development of the rosettes with that of the embryo proper.

Embryo systems are frequently found in pairs, the result presumably of the fertilization of 2 adjacent archegonia by the two male cells.

A massive secondary susoensor is produced by the embryo. An early stage of development of embryonal tubes is seen in Fig. 100. As the suspensor elongates, the upper portion becomes folded up together with the nucellus and rather indefinite prothallus tissue and all these form a compact mass at the micropylar end of the seed (Fig. 102). As the embryo grows the gametophyte rapidly enlarges in its basal portion, and nuclear divisions are frequently seen in it. Unfortunately in no case could the chromosome number be counted.

In material collected late in February the seed is practically mature. The growth of the embryo takes only a little over a month, and the whole cycle from megasporogenesis is completed in a remarkably short period (3 - 4 months). There are two cotyledons. The stem apex is laterally compressed by the cotyledons as is shown by Figs. 103 and 104, two views approximately at right angles. Cross (1943) described the group of apical cells in the stem apex, giving rise below to sub-apical initials. These may be identified tentatively in the shoot apex of the embryo. It is

interesting to speculate whether these apical cells are the direct descendants of the 4 primary embryo cells. Growth by a single apical cell does not occur in Athrotaxis, but if segmentation continues as it appears to take place in the early stages, there would still be 4 cells or some derivative thereof in the apical position in the mature embryo. Specific data on this point was not obtained. Some fusion of cotyledons may take place to form a "cotyledonary tube", and in the embryo cut transversely from which Fig. 105 was drawn the cotyledons were united for some distance on one side. One of these cotyldons contains the primordia of 2 vascular strands, the other only one.

ATHROTAXIS SELAGINOIDES Don.

SEEDLING

The seed of A. selaginoides was found near Lake Fenton on 16th May 1947. The seed was kept in an envelope, and planted in Melbourne on 5th April 1948. The proportion of germination was very high. In 3 weeks the cotyledons were above ground, generally bringing the integument with them (Fig. 68), althpugh when the integument was left below ground growth was as a rule more vigourous (Fig. 69). Growth during the winter was slow, and about 10 leaves were produced by 21st August (Fig. 70). Their arrangement is decussate in contrast to the spiral arrangement of the mature leaves. Germination thus follows the epigeal type of conifers generally. The high fertility of the seed is noteworthy. It is unknown whether the seed naturally has a dormant period.

DISCUSSION

The Relation of Athrotaxis to Other Conifers

To determine the relationships of Athrotaxis is very difficult, partly beacuse of the nature of the problem itself, but more beacuse of the incompleteness of information. Its affinities clearly lie with the Taxodiaceae rather than the Cupressaceae, its cone morphology having little in common with that of the latter family. The spiral arrangement of cone scales points to Taxodiaceae, the Cupressaceae having typically opposite or whorled arrangement. Moreover, the scales in the cones of A. selaginoides significantly resemble thaose of say Cryptomeria. Cross (1943) finds that the stem apex of Athrotaxis resembles that of other Taxodiaceae. However, as Buchholz (1940) points out, the "Sequoias" and Athrotaxis have gametophytic structures very different from those of the rest of the Taxodiaceae: Cryptomeria, Cunninghamia, Taxodium, and presumably Taiwania and Glyptostrobus.

To begin with we may list those characters in which Athrotaxis resembles Sequoia and Wellingtonia? and those in which it differs or is unique.

Similarities to Sequoia sempervirens:

Method of wall formation in embryo sac,
Mode of growth of pollen tube,
Thin megaspore membrane,
Ovules in a single row,
Life cycle completed in a single season,
Two cotyledons.

Similarities to Wellingtonia gigantea:

Leaves of one kind only,
Archesporium,
Presumed development of proembryo,
Presence of rosette cells,
Presence of prosuspehsor.

Features in which Athrotaxis resembles neither Sequoia nor Wellingtonia:

Type of pollen grain,

Digestion of nucellus up to one cell,

Simple polyembryony,

Absence of primary suspensor,

In practically every respect Wellingtonnia may be considered more primitive than Sequoia - its chromosome number, method of formation of prothallus tissue, thick megaspore membrane, proembryo and presence of prosuspensor, may be mentioned as the most outstanding. Athrotaxis seems more advanced than Wellingtonia in method of forming prothallial cells, in the specialized mode of arrangement of cells in the proembryo, in the presence of one row of ovules only on the cone scale, and - if one accepts Buchholz' (1919) view that polycotyledony is primitive - in having only two cotyledons. Buchholz (1939b, p. 254) has placed Athrotaxis in a position intermediate between Wellingtonia and Sequoia. In the nature of the proembryo and in development of the spore mother cell, Athrotaxis seems definitely more primitive than Sequoia. But in the reduction of the nucellus to a thickness of one cell, Athrotaxis seems more advanced than either of the other two genera.

Wodehouse (1935, p. 248) considers that Taxodium with its small germinal protuberance has the primitive type of pollen grain for the family, and that the prominent papilla of Sequoia, Wellingtonia and Cryptomeriais a specialization of this. Cunninghamia is considered a reduced form, and since Athrotaxis resembles it, this is evidence that Athrotaxis is either further advanced than, or not closely related to, Wellingtonia and Sequoia.

There remains the question of the primitive status of simple polyembryony. Buchholz (1939b, and elsewhere) holds that simple polyembryony is advanced. He says (1939b, p. 255) "the Big tree with its extensive cleavage polyembryony (nearly a dozen embryos per zygote) is the more primitive species, and the Redwood with a reduced form of cleavage polyembryony (four embryos per zygote)

is the more specialized. The next step in evolution would be only one embryo per zygote, thus ending the series in simple polyembryony." Although the type of proembryo in Athrotaxis seems more specialized than that of Wellingtonia, it is certainly less so than that of Sequoia and the type of proembryo giving rise to a single embryo in Athrotaxis cannot be derived from the Sequoia tyoe which seems destined to give rise to several embryos from the nature of the first divisions. It has been suggested (Elliott, 1948) that the condition wherein both cells of a 2-celled unit contribute to a single embryo is primitive. In Athrotaxis the 2-celled units are 4 groups of 2 cells at the ends of the prosuspensors at the 8-celled embryo stage. In Wellingtonia the 2-celled units are formed at the ends of elongating prosuspensors, and one of them becomes the primary suspensor (Buchholz, 1939a). In Sequoia the 2-celled units are formed in the archegonium itself, as in Callitris, and one cell elongates as a suspensor. (Buchholz, 1939b). In the Podocarpaceae it would seem certain that simple polyembryony is primitive, since the species exhibiting it are those considered primitive on the nature of their gametophytes (Doyle and Looby, 1939) and in having unreduced fertile branch systems (Wilde, 1944). Furthermore, the other genera of the Taxodiaceae have 2-celled units which contribute to complete embryos.

To sum up, many characters of Athrotaxis are intermediate between those of Wellingtonia and Sequoia; but the simple polyembryony of Athrotaxis is possibly more primitive than the condition in the other two genera, and the one-cell-thick nucellus represents a character more advanced than in either of them. In the pollen grain, and in the cone scales of A. selaginoides, there is no resemblance either to Wellingtonia or Sequoia. The conclusion is that these genera are related but not closely. We can envisage a group of plants, in whose characters there existed the potentiality to evolve, say, towards cleavage polyembryony, an embryo sac in which the nuclei are congregated at the base, a pollen grain with a very pronounced germinal papilla, and so on. These potentialities were realized independently in different lines, some progressing far in one or two respects while remaining primitive in others. Wellingtonia,

Athrotaxis and Sequoia represent survivors of three distinct branches of this complex.

SUMMARY

1. The male cones of Athrotaxis cupressoides are first distinguishable in February. The winter is passed in the spore mother cell stage, and meiosis occurs in the 2nd or 3rd week of August. Pollen is ready to be shed a month later.

2. Three layers may be recognised in the wall of the pollen grain, of which the middle one is the gelatinous layer.

3. The arrangement of nuclei in the embryo sac resembles that in Sequoia sempervirens.

4. The proembryo has three tiers: rosette, prosuspensor, and embryo units. There is no primary suspensor, and simple polyembryony occurs. There are two cotyledons.

5. Free nuclear stages in the megaspore are found in mid-December, and the embryo is nearly mature late in February.

6. Wellingtonia, Athrotaxis and Sequoia represent divergent developments of a common stock, but are not directly related.

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LOCALITIES WHERE MATERIAL WAS COLLECTED

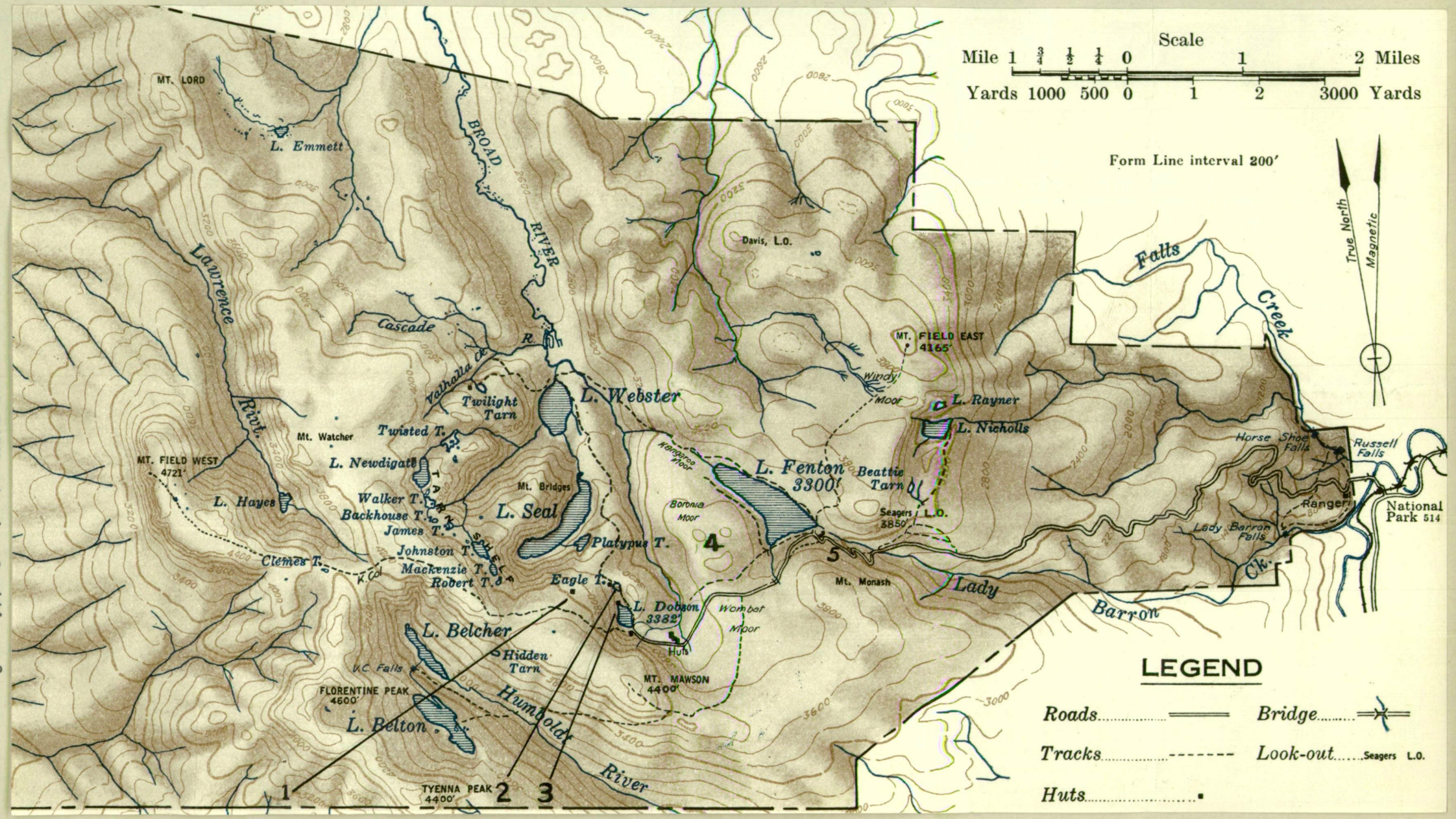
This list gives the localities where material was collected from which the preparations were made which have been used as illustrations in this thesis. In the captions the numbers in brackets preceding the date of collection correspond to the following numbers:-

1. Mt. Mawson, 4000 feet.
2. Eagle Tarn, 3390 feet.
3. Lake Dobson, 3382 feet.
4. Boronia Moor, about 3500 feet.
5. Lake Fenton, about 3200 feet.

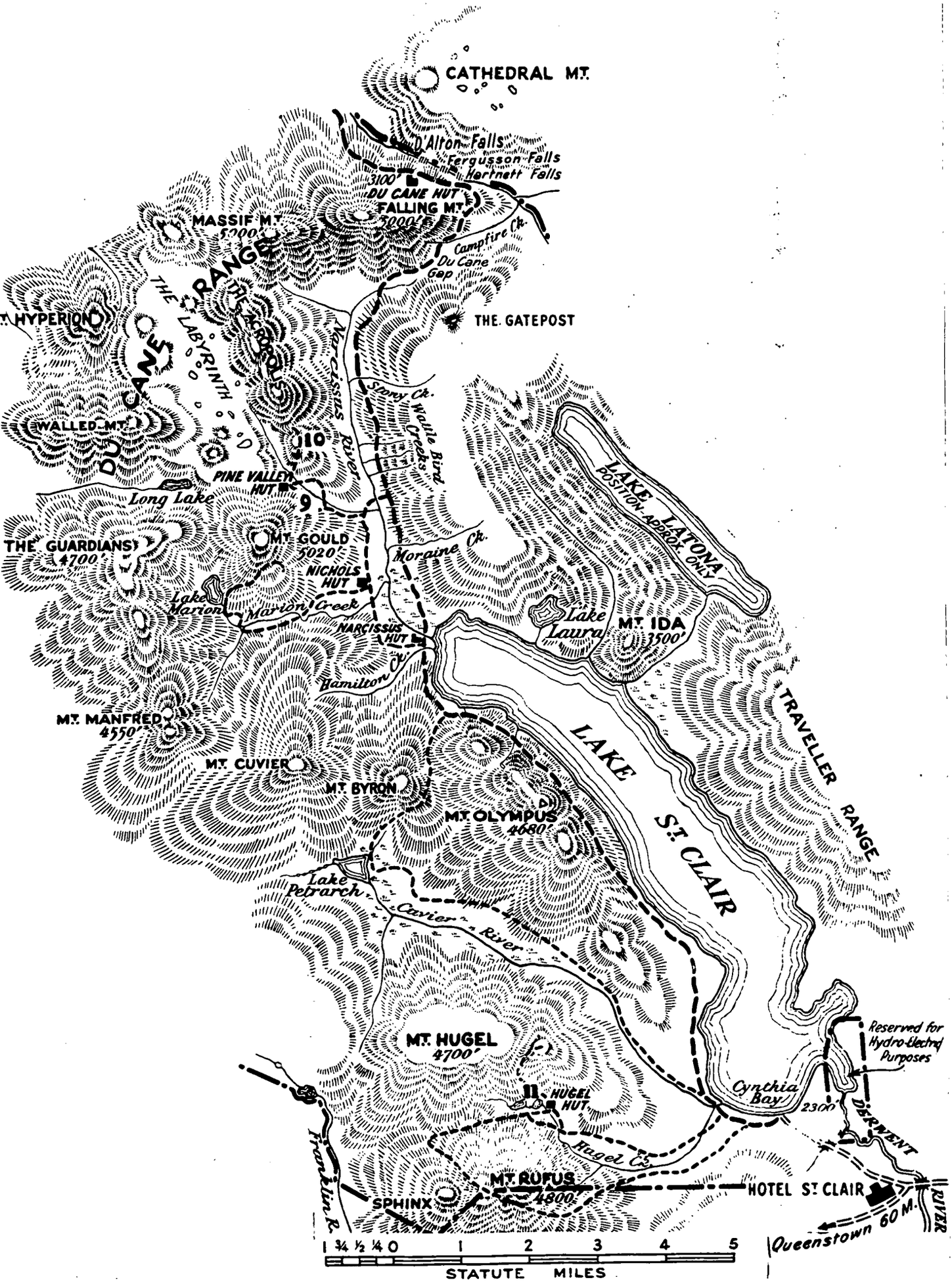
MT. FIELD NATIONAL PARK. See Map. 1.

6. Mt. Wellington, about 3800 feet.
7. Great Lake.
8. Liaweenie.
9. Pine Valley, about 3000 feet.
10. The Acropolis, 3900 feet.
11. Hugel Lakes, about 300 feet.

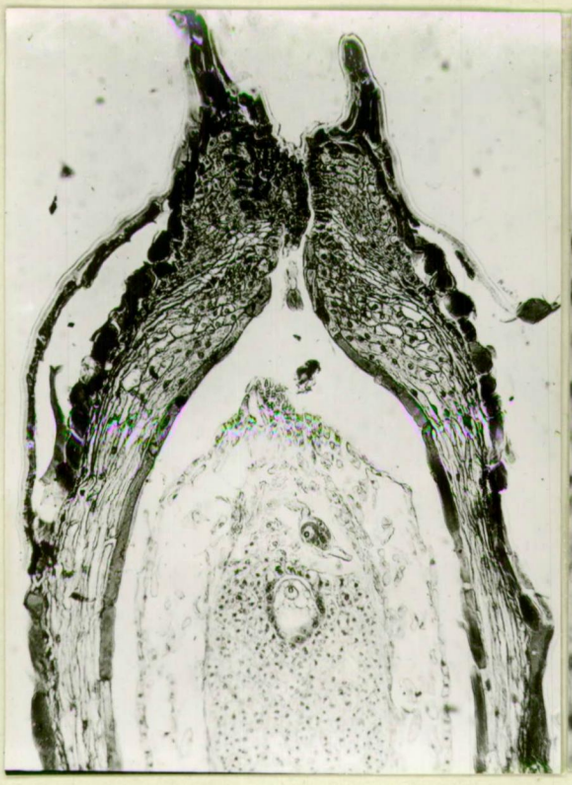
LAKE ST. CLAIR NATIONAL PARK. See Map 2.



MAP 1. MT. FIELD NATIONAL PARK, showing position of collecting localities 1 - 5. Lands and Surveys Department, Hobart.



Map 2. Part of the Lake St.Clair National Park, showing collecting localities 9 - 11.



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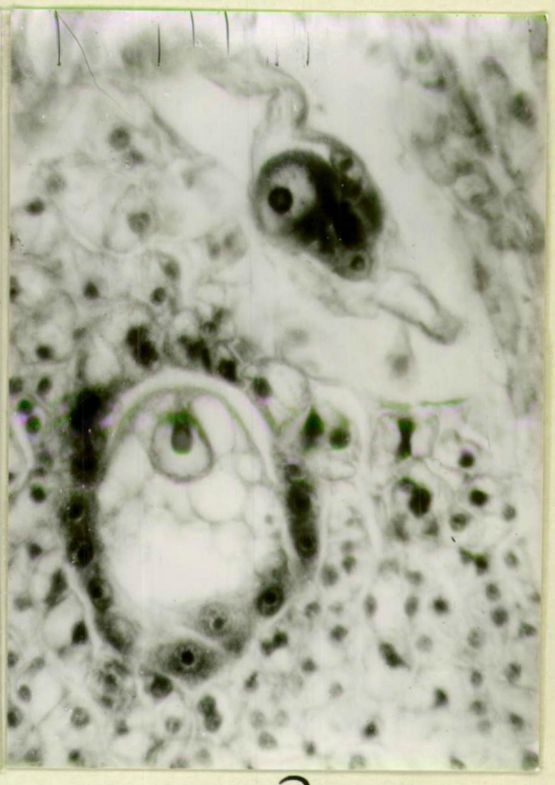

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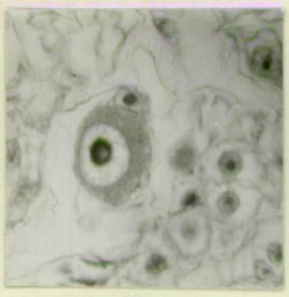

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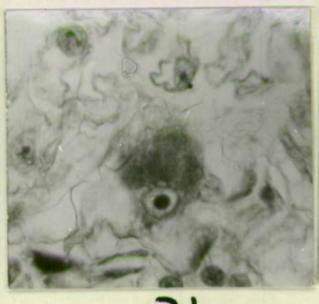

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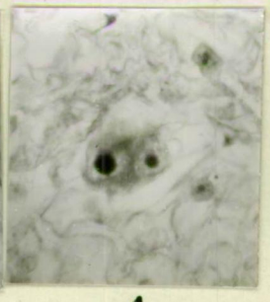

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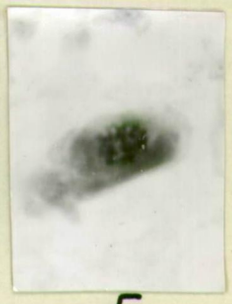

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Pherosphaera hookeriana

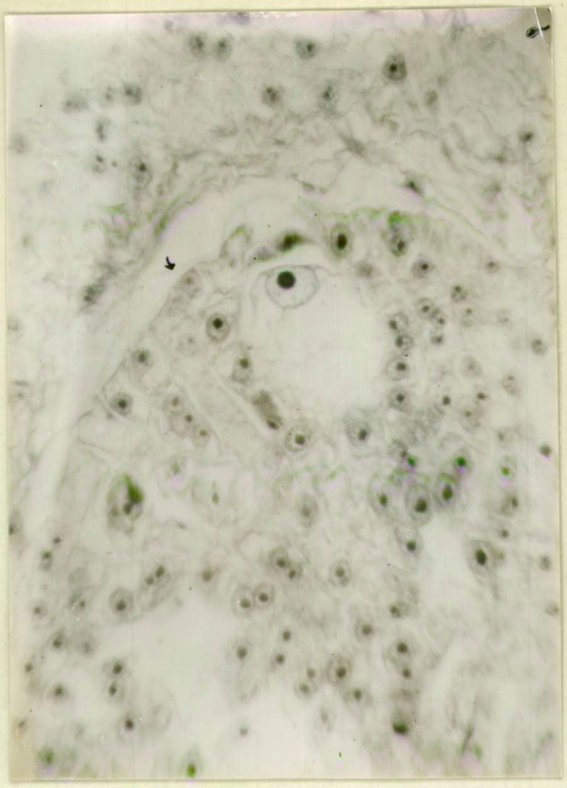
1. Gen. view of ovule, showing integument with lips, micropyle, nucellus with pollen tube, prothallus with archegonium. (3) Jan. 1942. x 80.

2. Part of above, showing body cell, stalk and tube nuclei, archegonium with nucellus and vacuolate protoplasm. x 380.

3 a & b. Two sections through same pollen tube. 3a shows body cell with stalk nucleus adherent to it. 3b, tube nucleus. (1) 13 Feb. '48. x 380.

4. Body cell and stalk nucleus closely adherent. (1) 13 Feb.'48. x 380.

5. Body cell, ? prophase of division to form gametes. (1) 13 Feb. '48. x 380.



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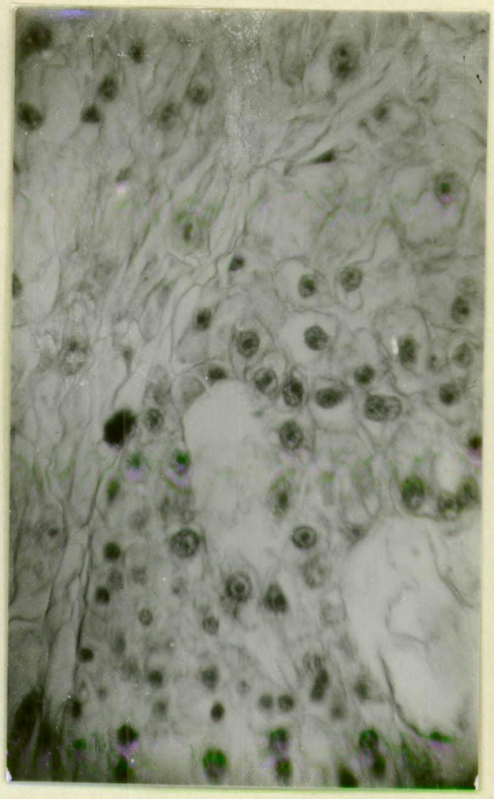

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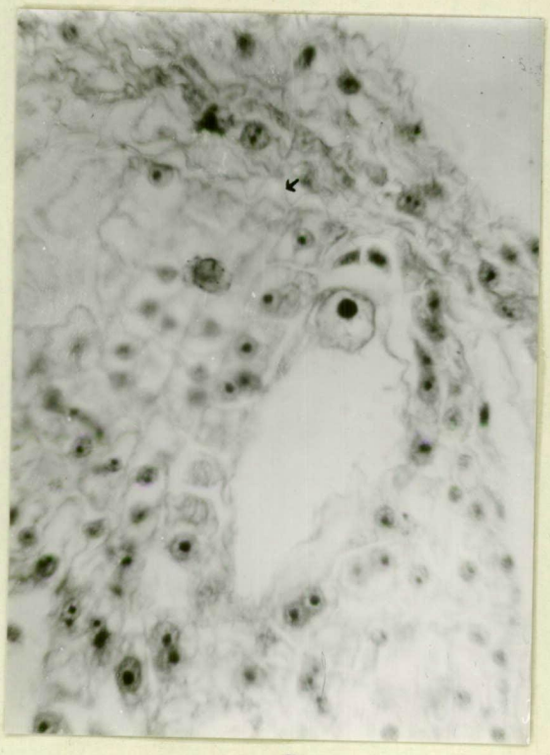

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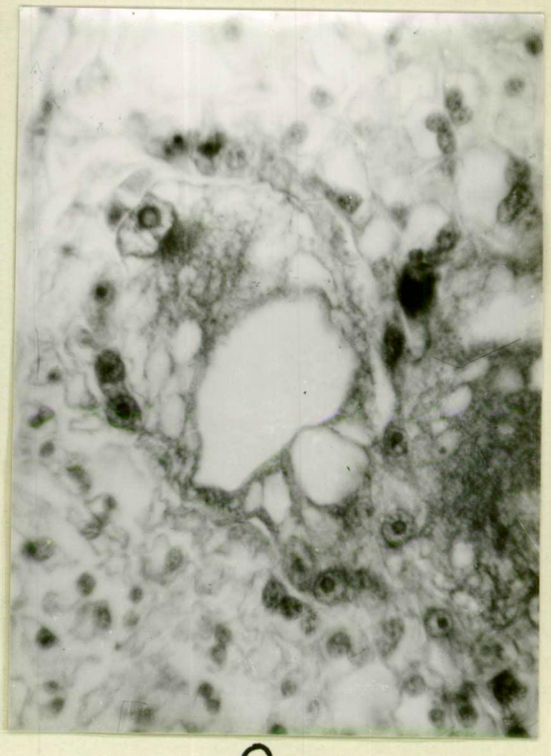

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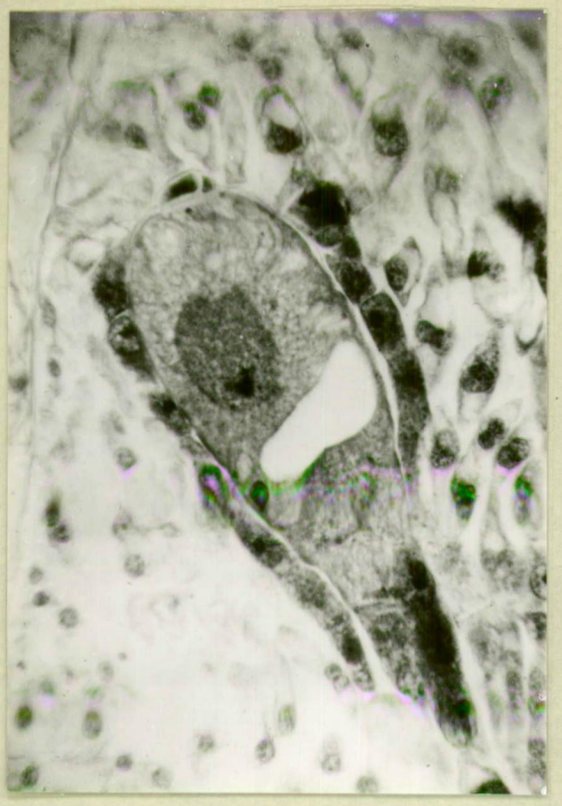
6. Archegonium with nucleus, and neck not yet covered by prothallial cells. Shows megaspore membrana well. (1) 13 Feb. '48. x 380.

7. Neck of archegonium being overgrown by prothallial cells. (1) 13 Feb. '48. x 380.

8. Young archegonium showing nucleus, central vacuole and small amount of peripheral cytoplasm, early stage of development of jacket; megaspore membrane indicated by arrow. (1) 13 Feb.'48. x 380.

9. Archegonium with nucleus before ventral canal division, showing increasing amount of cytoplasm with small vacuoles but large central vacuole. (1) 13 Feb. '48. x 380.



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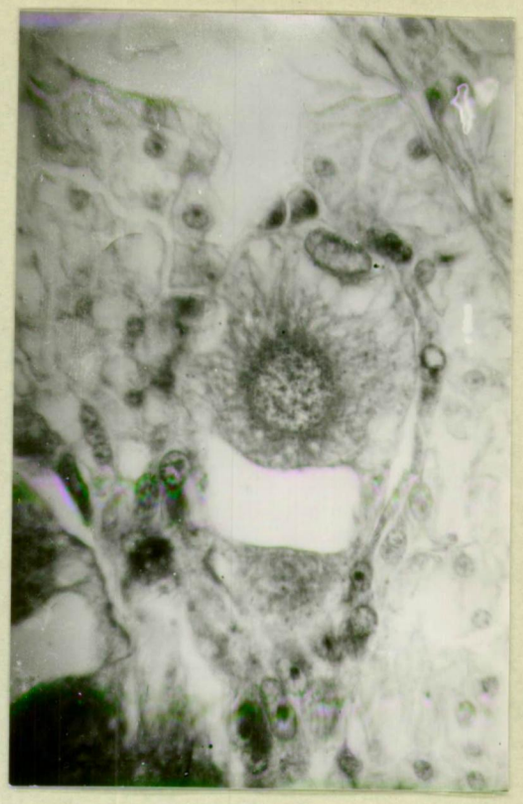

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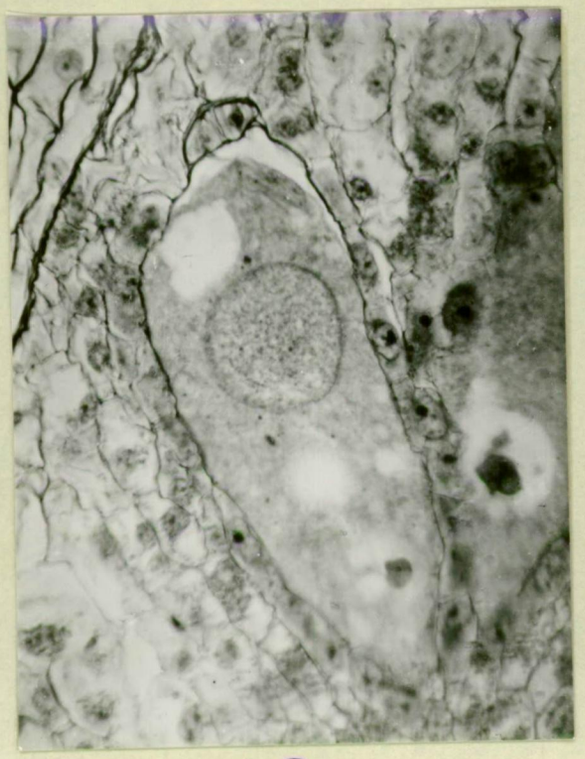

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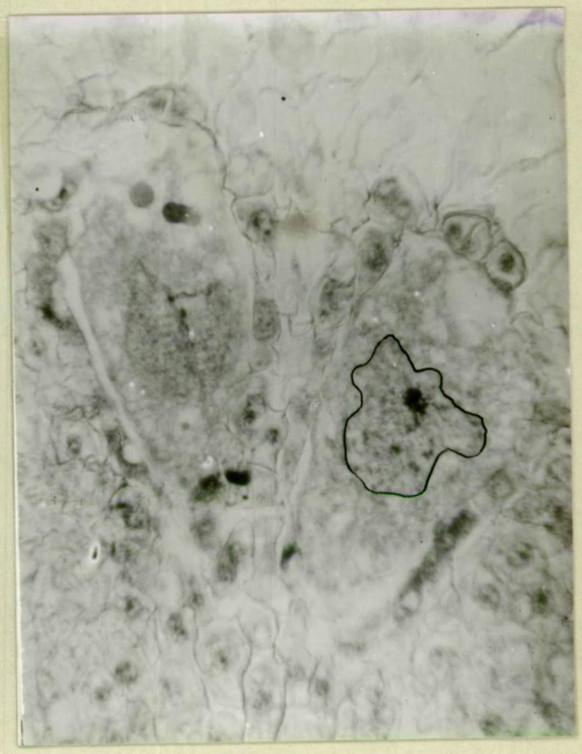

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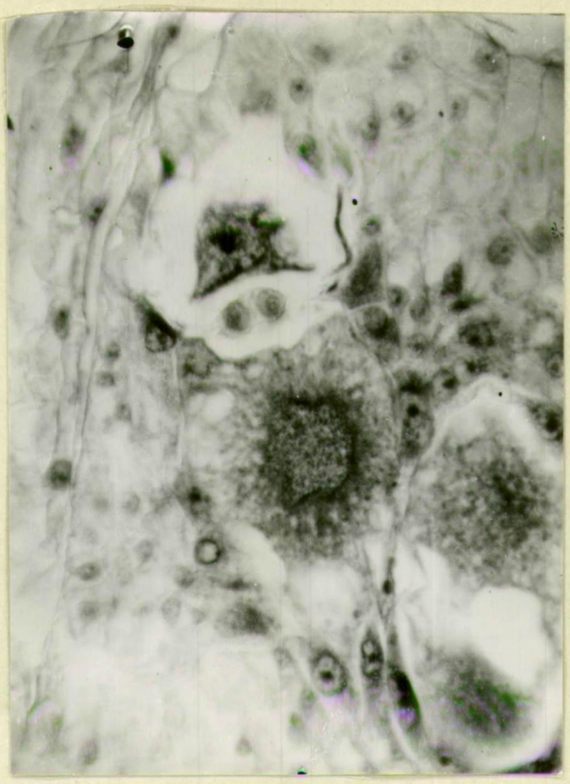
Pherosphaera hookeriana

10. Archegonium with buried neck, egg nucleus, ? with remains of nucleolus, jacket layer not extending to neck cells. (1) 13 Feb. '48. x 380.

11. Egg and ventral canal nuclei. (1) 13 Feb. '48. x 380.

12. Genaral form of archegonium, showing buried neck and egg nucleus. (2) 15 Feb. '48. x 380.

13. Two unfertilized archegonia with large lobed nuclei. (2) 15 Feb. '48. x 380.



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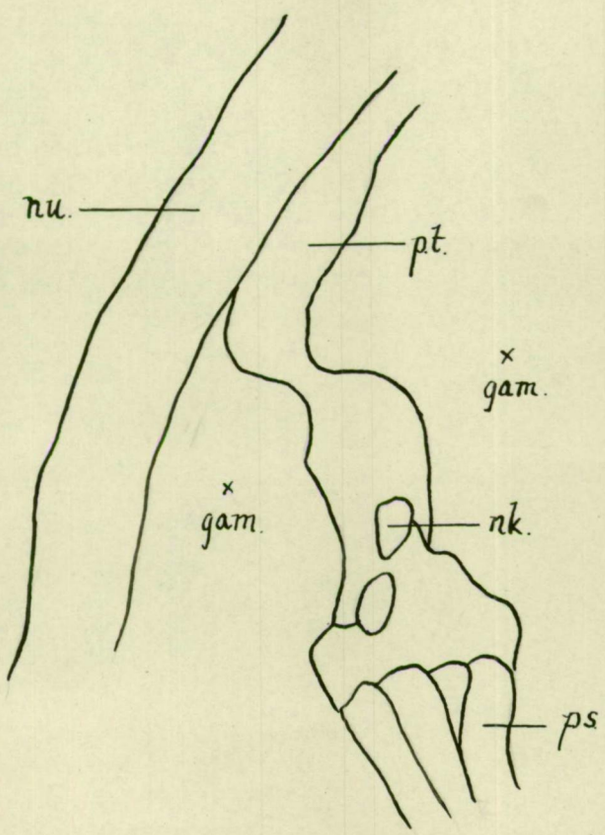

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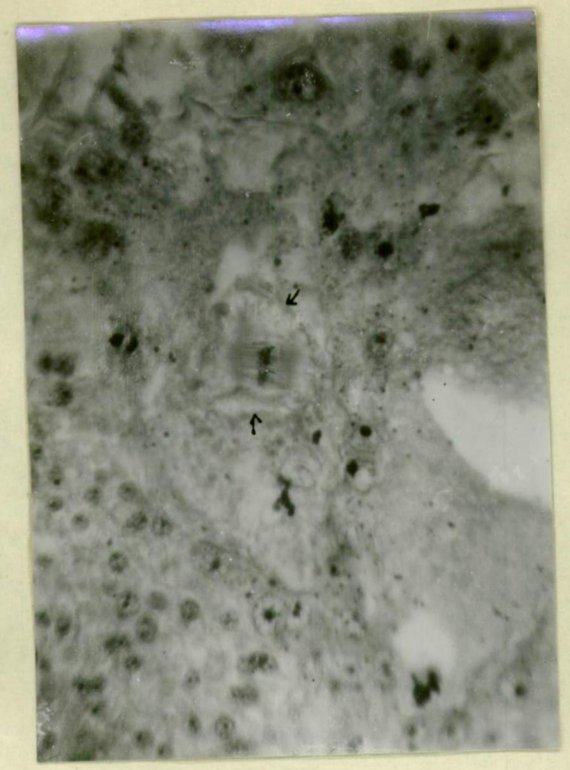
Pherosphaera hookeriana

14. Pollen tube pressing down on archegonium, resulting in suspension of neck cells. (1) 13 Feb. '48. x 380.

15. Camera lucida sketch showing relationship between pollen tube (p.t.), nucellus (nu.), prothallus (gam.), ruptured neck cells (nk.), and top of prosuspensors (ps.). (4) 13 Feb. '48. x 400.



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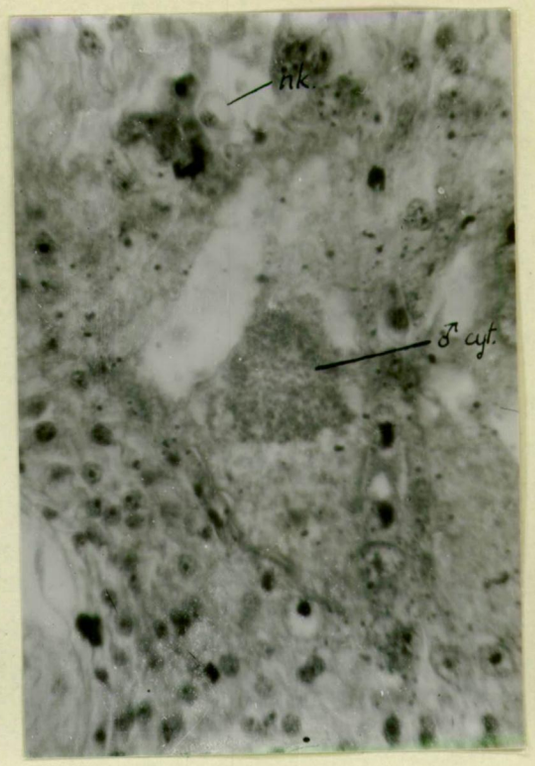

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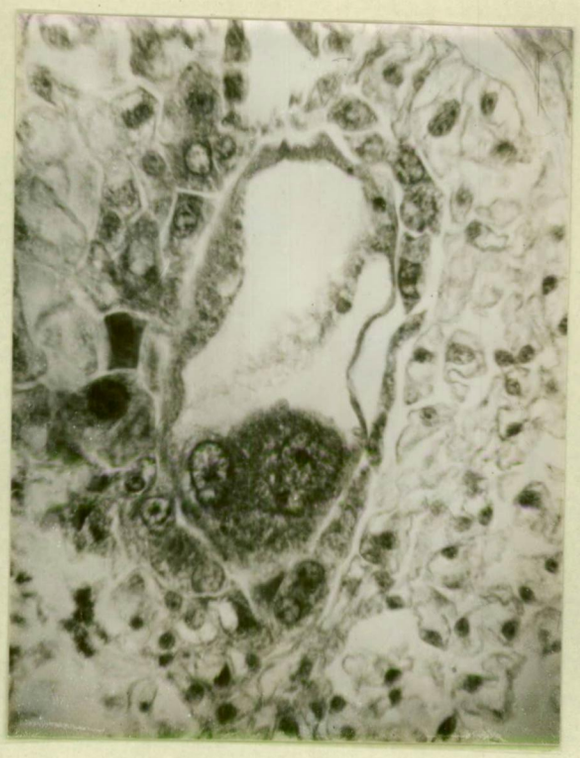

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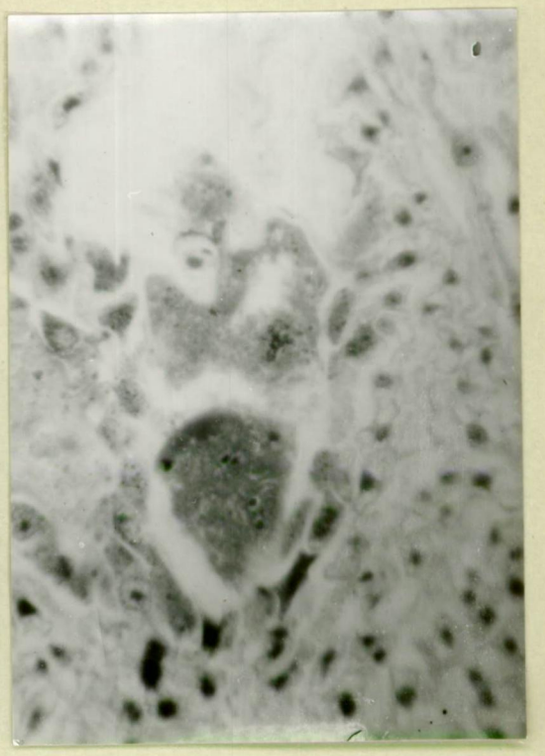

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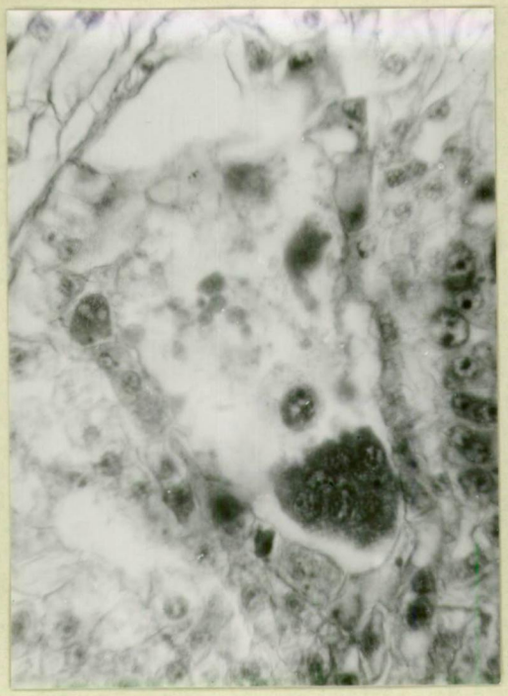
Pherosphaera hookeriana

16. First division of zygote. Arrow indicates nuclear membrane. (1) 13 Feb. '48. x 380.

17. Another section from series through same archegonium, showing ruptured neck, and ? residual male cytoplasm. (1) 13 Feb. '48. x 380.

18. Two of the 4 free nuclei, with vacuole above proembryo. (1) 13 Feb. '48. x 380.

19. 16-free nuclear stage, with nuclei still arranged in pairs after division. Ruptured neck of archegonium above. (4) 13 Feb. '48. x 380.



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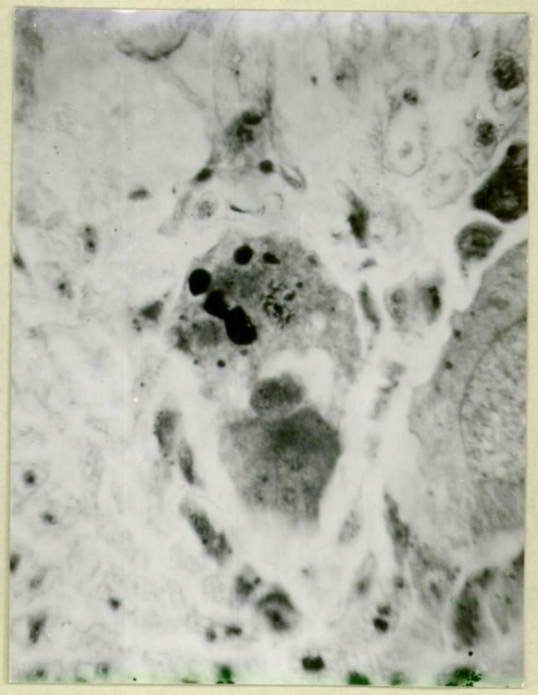

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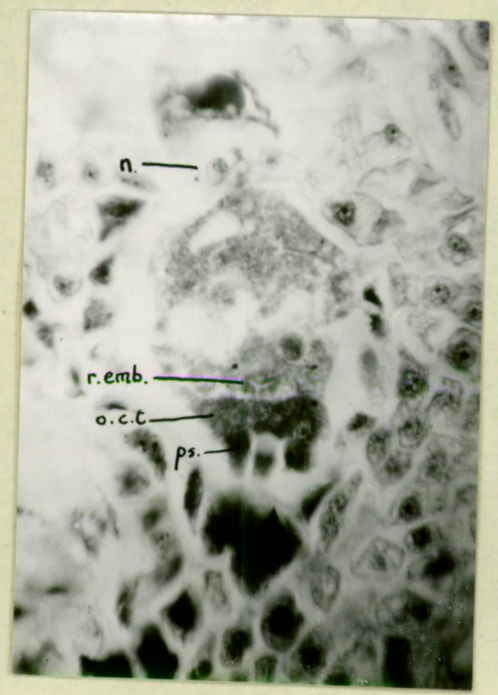

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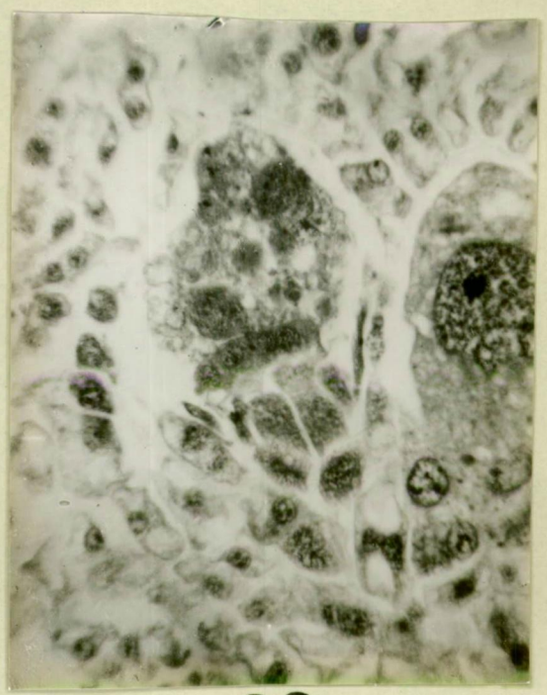


23

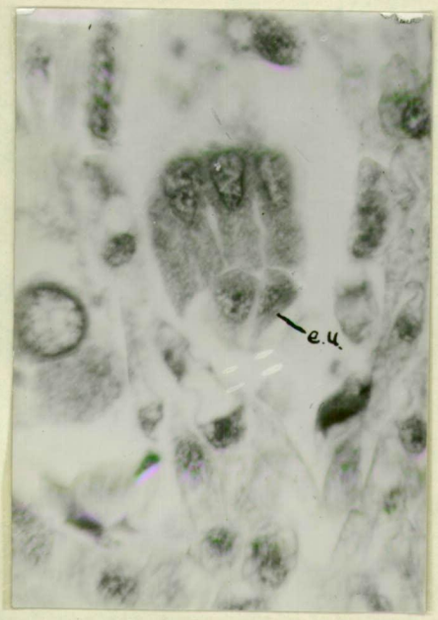
Pherosphaera hookeriana

20. 16-free nuclear stage showing one relict nucleus. (2) 15 Feb. '48 x 380.

21. Several relict nuclei. Protein vacuoles and nucleus-like artifacts in residual protoplasm. (4) 13 Feb. '48. x 380.

22. Relict nucleus forming 3-celled embryo (r.emb.); ruptured neck (n.), prosuspensors (ps.), and open cell tier (o.c.t.) visible. (4) 13 Feb. '48. x 380.

23. Numerous nucleus-like artifacts. (1) 13 Feb. '48. x 380



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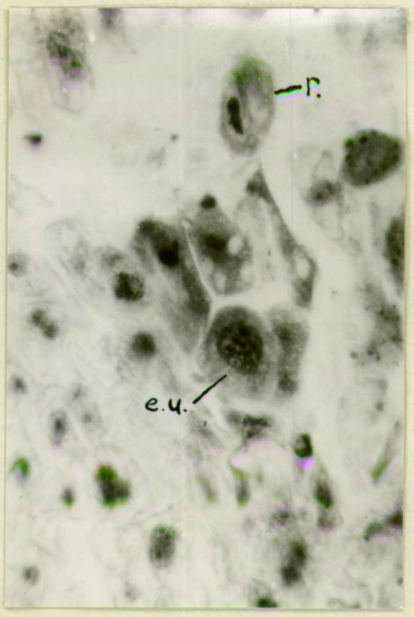

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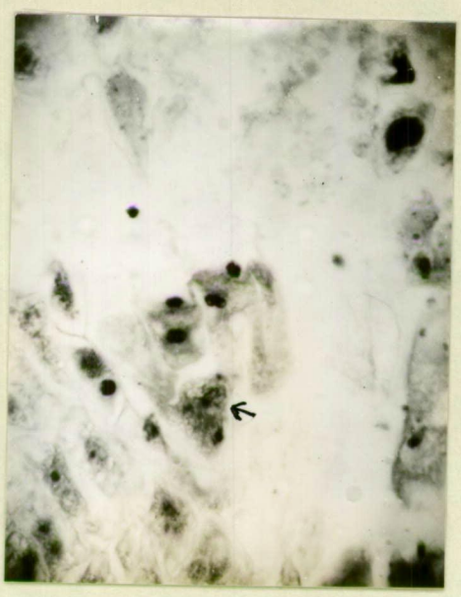


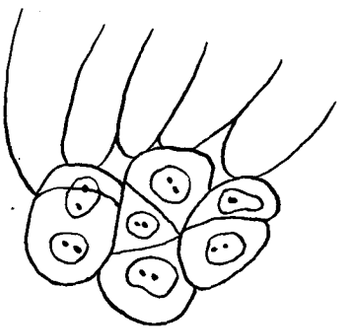
26

Phæosphaera hookeriana

24. Primary embryo units (e.u.) organised, with cleavage planes extending upwards to delimit future prosuspensors. (2) 15 Feb. '48. x 485.

25. Telophase of division to form prosuspensors and open cell tier. Primary unit (e.u.) below. Rosette cell (r.) top right. (4) 13 Feb. '48. x 485.

26. Another section of same series showing prometephase of division to give binucleate units (arrow). (4) 13 Feb. '48. x 485.



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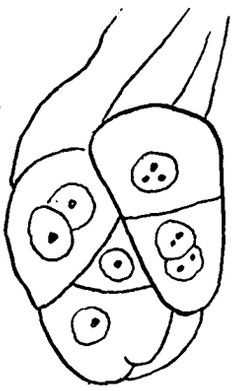

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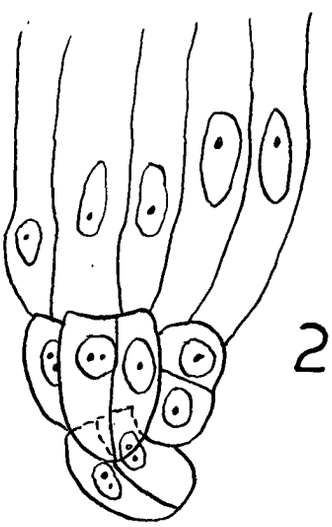

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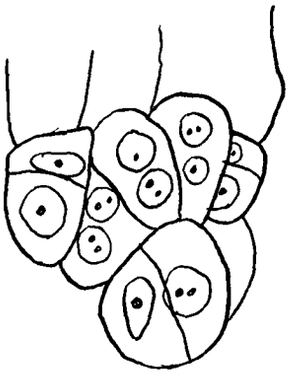
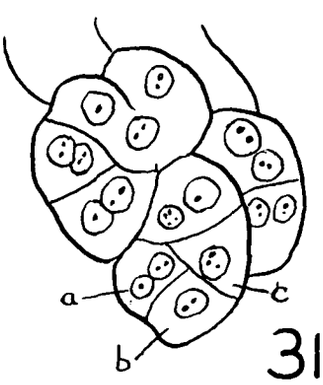




31

Pherosphaera hookeriana

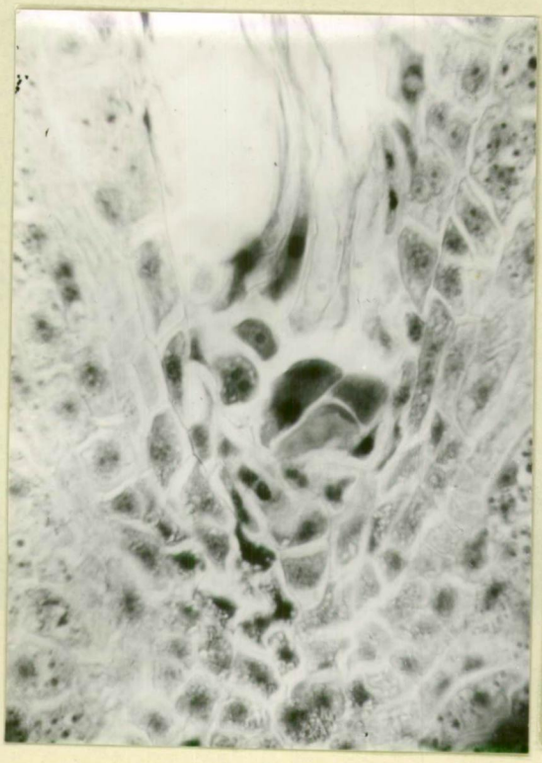
27. Embryo system showing 4 2-celled units, the second cell of terminal unit being hidden by one drawn. (1) 22 Mar. '47. x 550.

28. Embryo system of four binucleate units and two 2-celled units. The terminal unit has wall extending obliquely in high focus but not in plane of nuclei. (1) 22 Mar. '47. x 550.

29. Four 2-celled units, showing variation in orientation of wall separating the 2 cells of 2-celled unit. (1) 22 Mar. '47. x 550.

30. Two embryos in a single tier, the right hand one with 2 cells, one of them binucleate; the left hand one with 3 cells, one of them binucleate. (1) 22 Mar. '47. x 550.

31. Three embryos, the leading one with 2 binucleate cells and 2 uninucleate cells. (1) 22 Mar. '47. x 550.



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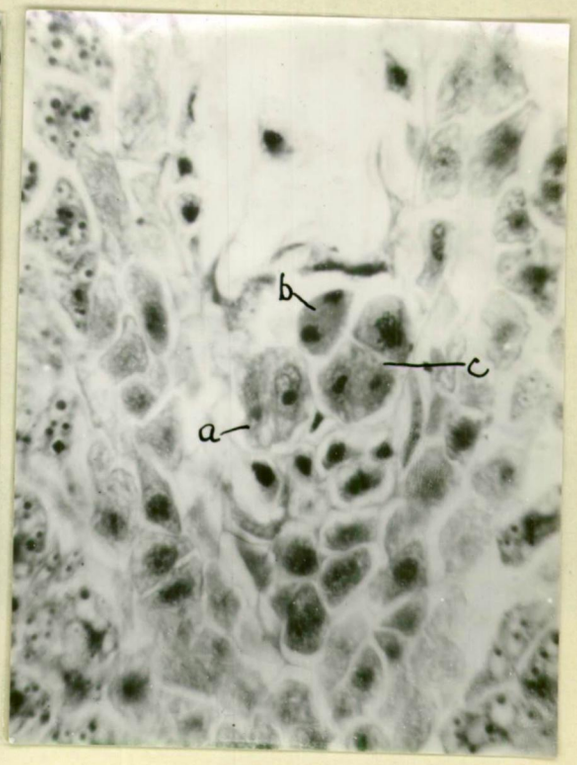

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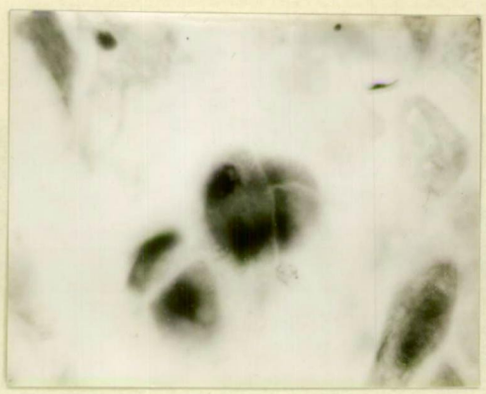
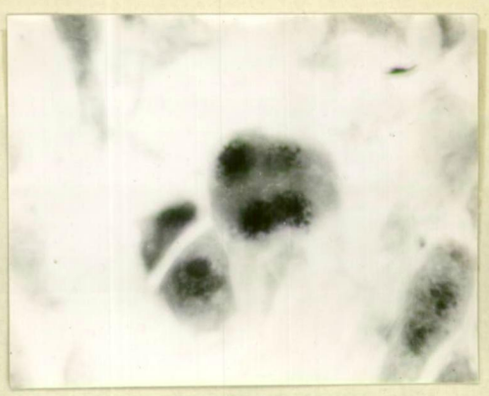


34 a



34 b

Pherosphaera hookeriana

32. Embryos system with two 2-celled units and ? binucleate unit. (4) 13 Feb. '48. x 485.

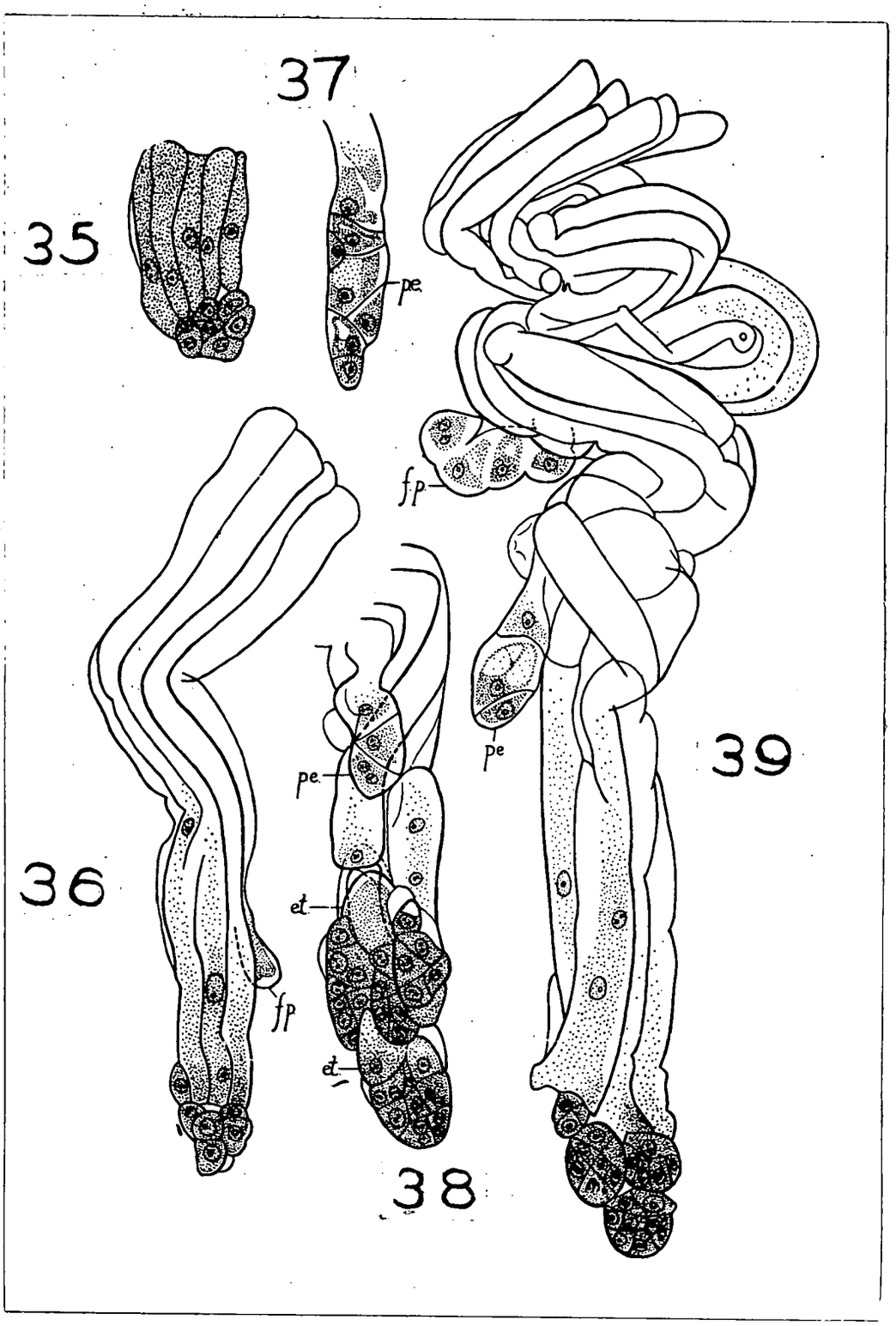
33. One 2-celled unit (a), cell of 2-celled unit showing telophase without wall formation (b), and part of 3-celled embryo showing prometaphase in one cell (c). (4) 13 Feb. '48. x 485.

34 a and b. Two views of same section in different planes of focus. To the left, a 2-celled unit; to the right 2-celled embryo with binucleate cells, in the left hand of which a cleavage plane is visible in a, but has not extended as far in as plane of focus of b. (4) 13 Feb. '48. x850.

Explanation of Figs. 35 - 39 continued

39. Complete embryo system showing 4 developing embryos without secondary suspensors. (1) 22 Mar. '47. x 240.

e.t., embryonal tubes; f.p., free prosuspensor cells; p.e., embryos formed in prosuspensor cells.



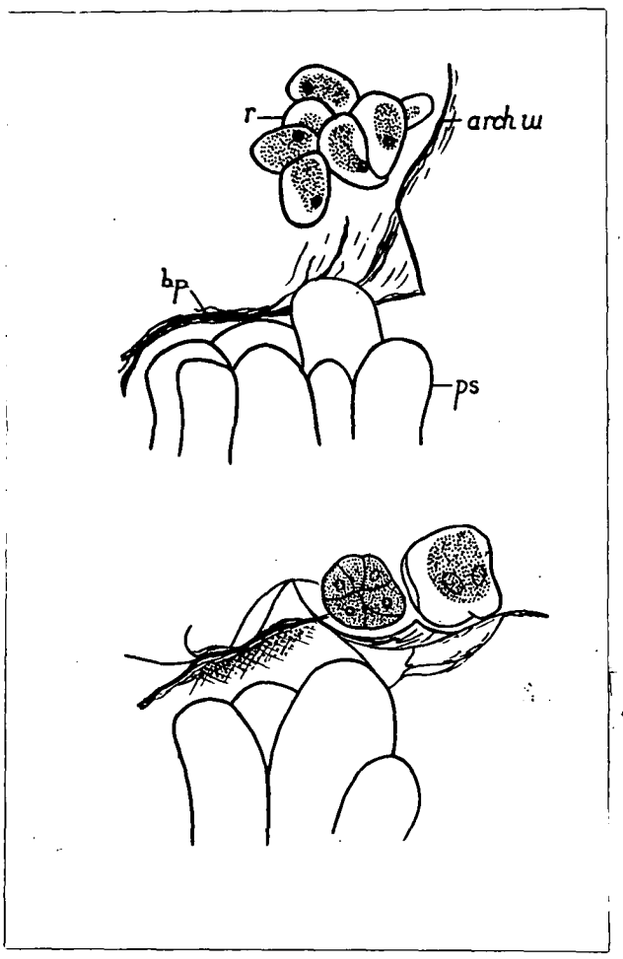
Pherosphaera hookeriana

35. Smallest embryo system dissected out. See Fig. 27. (1) 22 Mar. '47. x 240.

36. Elongating prosuspensors, some of which have lost contact with embryo units. (1) 22 Mar, '47. x 240.

37. Free prosuspensor cell with cellular tissue. (1) 22 Mar. '47. x 240.

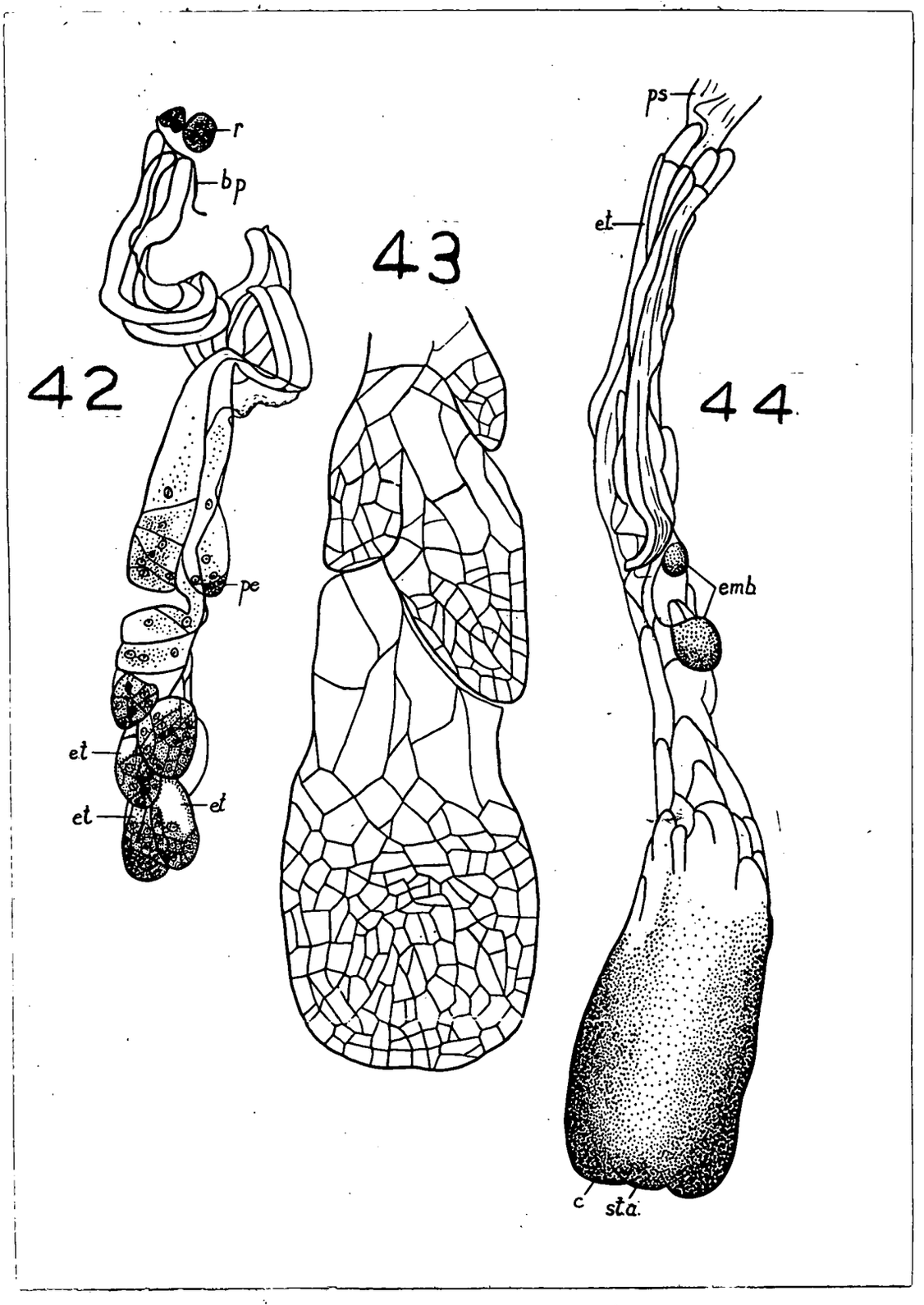
38. Embryos with embryonal tubes beginning to elongate. Some cells of embryo binucleate. (1) 22 Mar. '47. x 240.



Pherosphaera hookeriana

40. Part of a group of 10 "rosette" cells (r.) above basal plug of archegonium (b.p.). (1) 22 Mar. '47. x 335.

41. "Rosette" embryos, one with 6 cells visible, the other binucleate. (1) 22 Mar. '47. x 335.

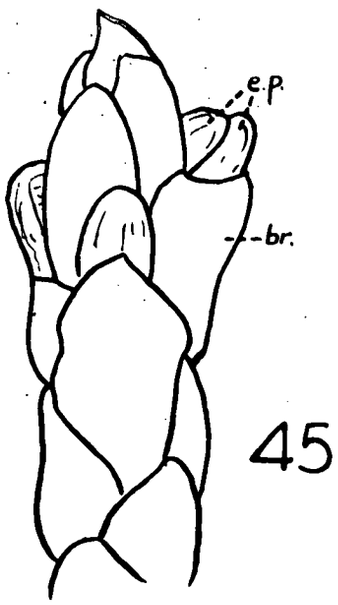


Pherosphaera hookeriana

42. Embryo system showing embryos with very small secondary suspensors; free prosuspensors and "rosette" embryos. (1) 22 Mar. '47. x 140.

43. Section of embryos system showing terminal embryo with large secondary suspensor, and smaller embryos with secondary suspensors. (1) 21 May '47. x 265.

44. Older embryos with primordia of cotyledons. Two smaller embryos higher up suspensor. (1) 16 June '47. x 140.



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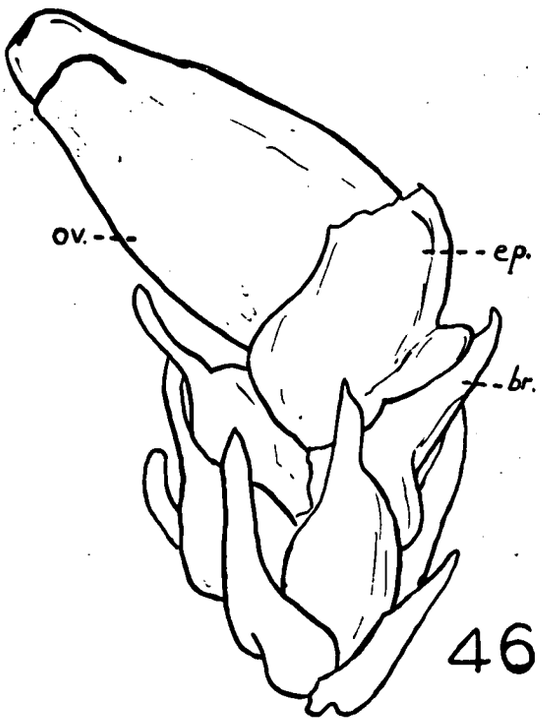

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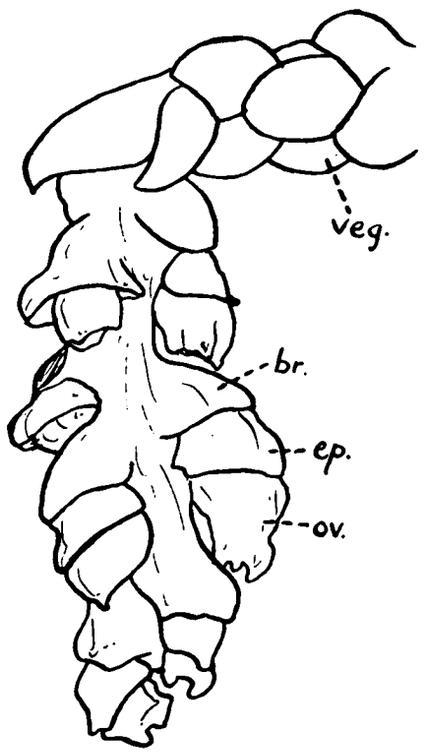

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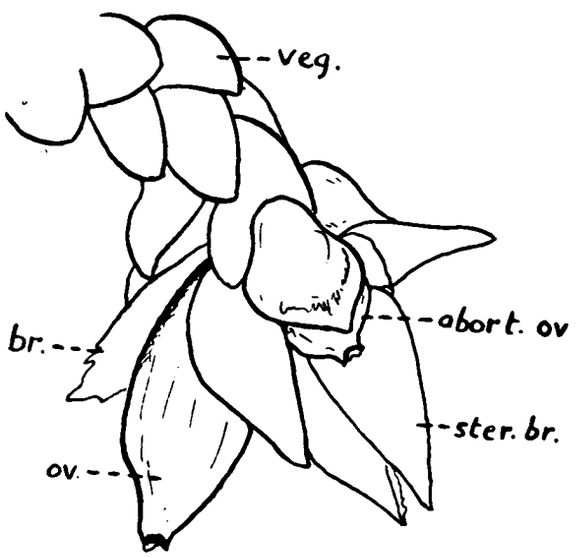


48

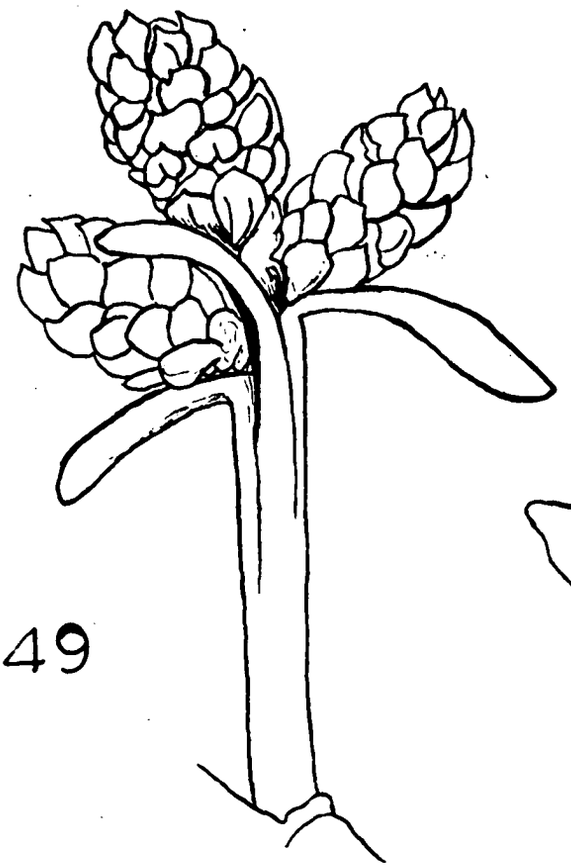
45. Dacrydium bidwillii. Young female cone. x 15.

46. Dacrydium cupressinum. Nearly mature cone. x 15.

47. Dacrydium franklinii. Young cone. x 15.

48. Pherosphaera hookeriana. (1) 22 Mar. '47. x 20.

abort.ov., abortive ovule; br., fertile bracts; ep., epimatium; ov., fertile ovule with integument showing; ster.br., sterile bract; veg., basal vegetative portion of fertile branch.



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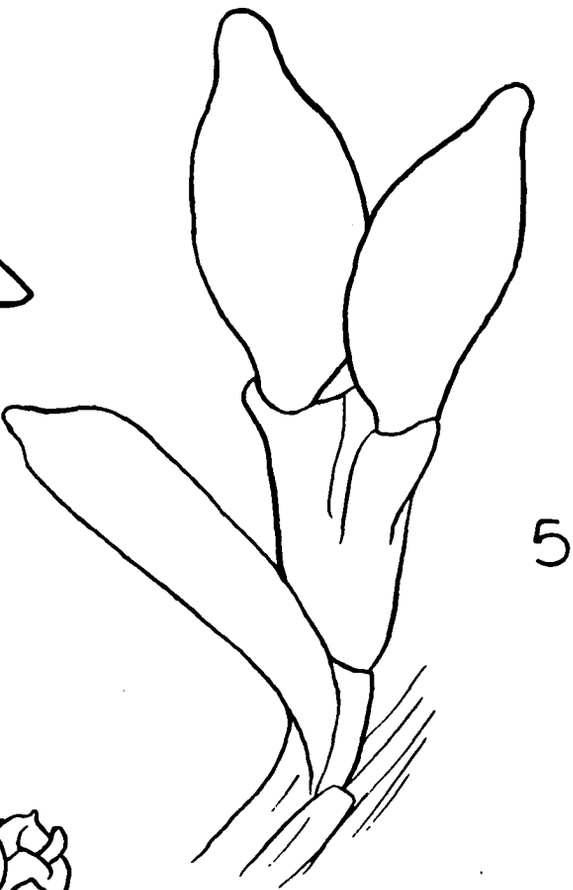

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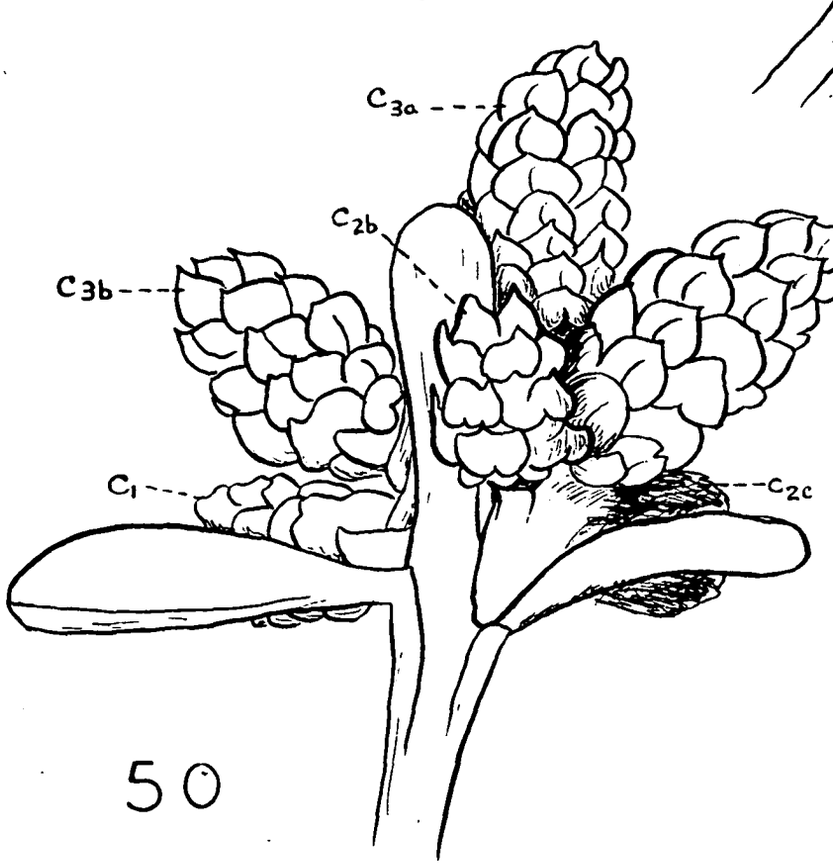


50

52

Podocarpus alpinus

49. Secondary cluster of three male fertile branches. x 10.

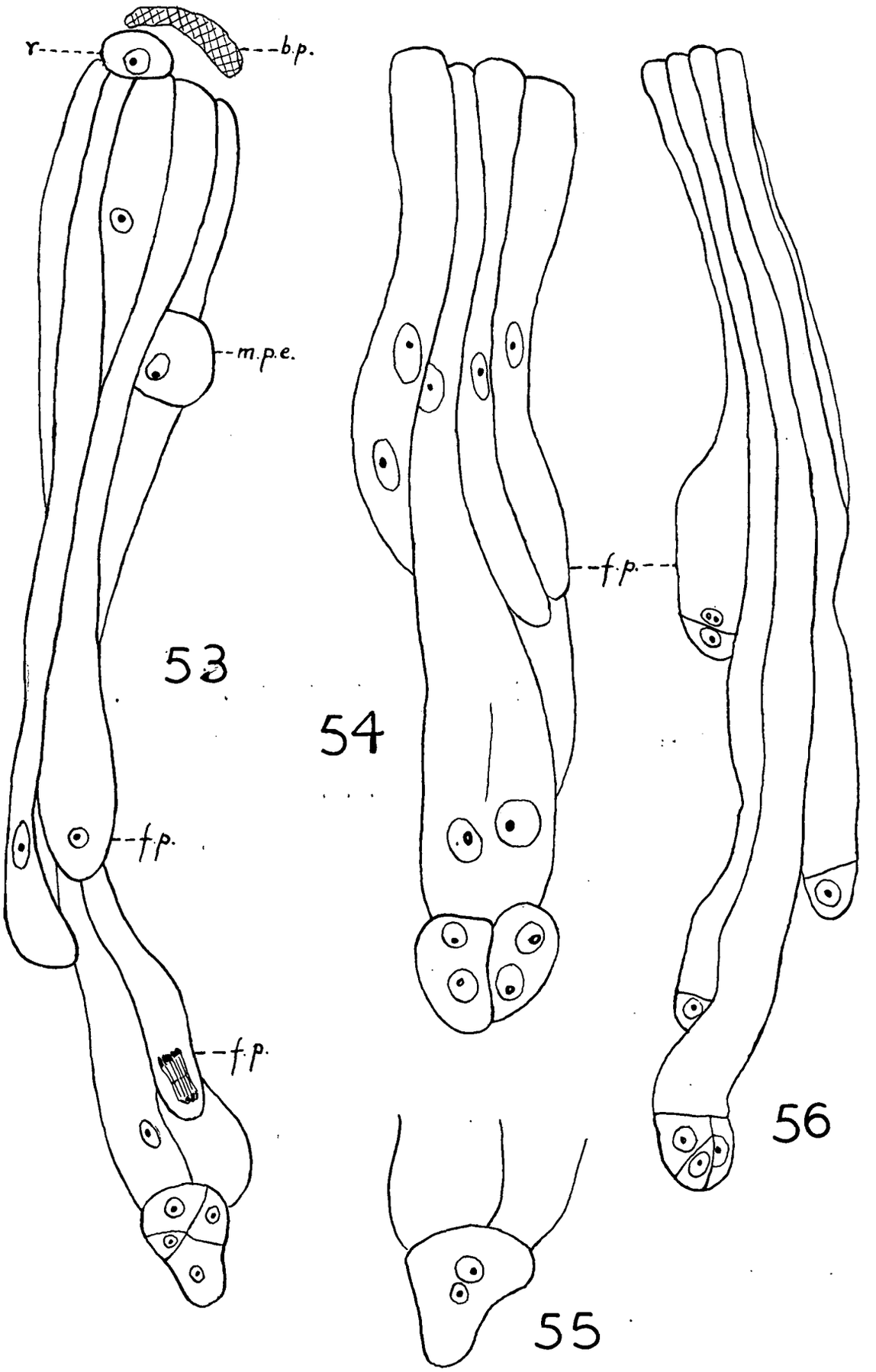
50. Group of three male fertile branches showing transition from primary cluster to single cones and aggregation in secondary cluster. c_1, single cone of fertile branch; $c_{2a,b,c}$, primary cluster of 3 cones; $c_{3a,b}$, 2 cones of the 3rd fertile branch. x10.

51. Female fertile branch with 2 ovules subtended by vegetative leaf. x 10.

52. Female fertile branch subtended by scale leaf. Tip of fertile branch visible. x 10.

Explanation of lettering in Figs. 53 - 6.

b.p., basal plug of archegonium; f.p., free prosuspensor cells; m.p.c., cell cut off in middle of prosuspensor cell; r., rosette cell.



Podocarpus alpinus

53. Embryo system with single 4-celled unit, several free prosuspensors in end of one of which cell is being cut off. Cell delimited in middle of prosuspensor (m.p.e.), and rosette cell, and basal plug shown. (6) 29 Sept. '46. x 400.

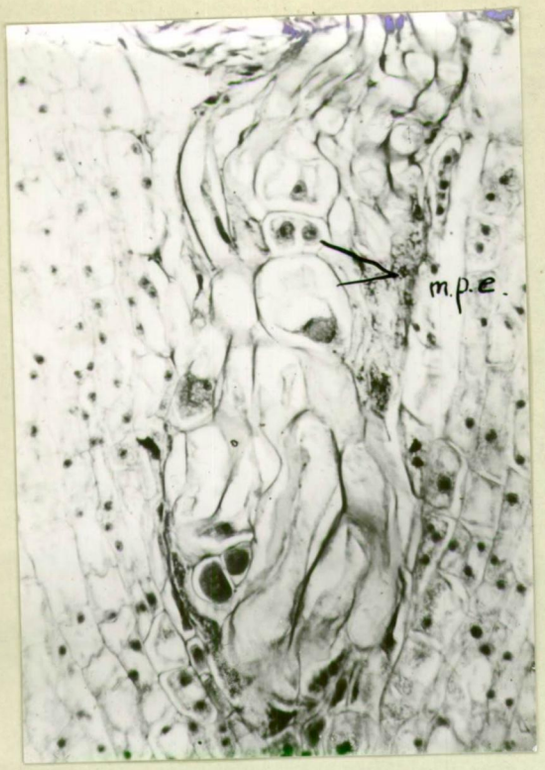
54. Embryo system with 2 binucleate units and free prosuspensor cells. (6) 29 Sept. '46. x 400.

55. Single binucleate unit of embryo system. (6) 29 Sept. '46. x400.

56. Embryo system with numerous free prosuspensors. (6) 20 Oct. '46. x 400.

Explanation of lettering in Figs. 57 - 61.

arch., upper part of archegonium not occupied by proembryo; b.p., basal plug of archegonium; m.p.e., cells cut off in middle of prosuspensor cells; r., rosette cell; ps., prosuspensor.



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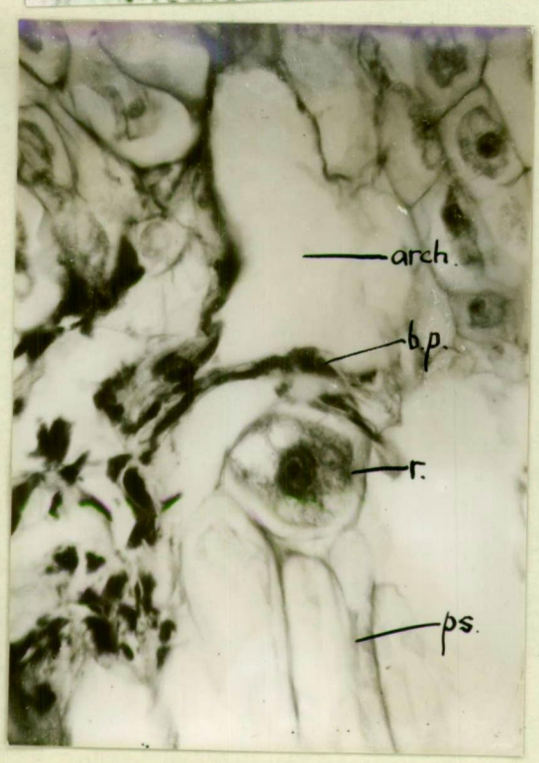

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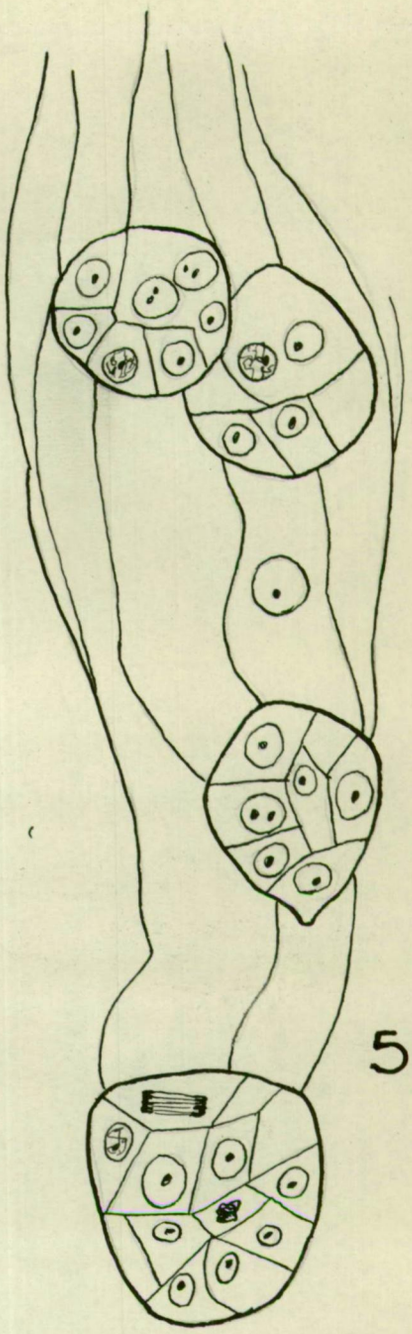

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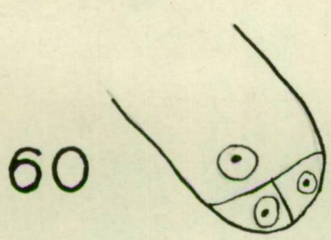

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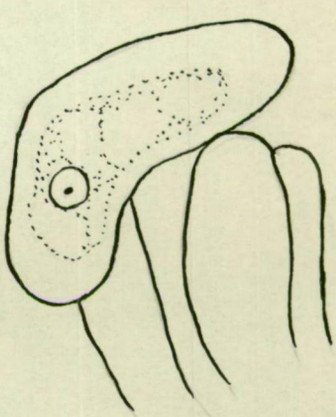


61

Podocarpus alpinus

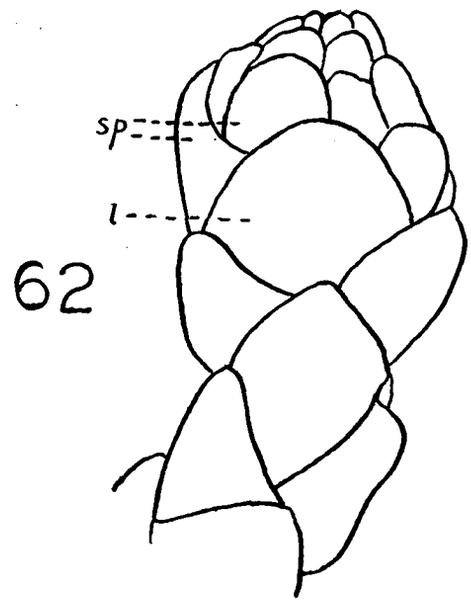
57. Section through 2 embryo systems. The left has 2 binucleate units. The right shows several cells cut off in middle of prosuspensor. (6) 4 Aug. '46. x 280.

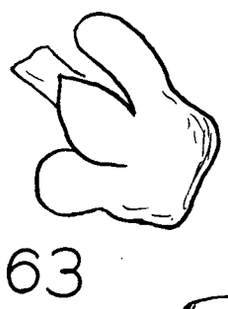
58. Section showing relation of rosette cell to archegonium and prosuspensor. (6) 4 Aug. '46. x 380.

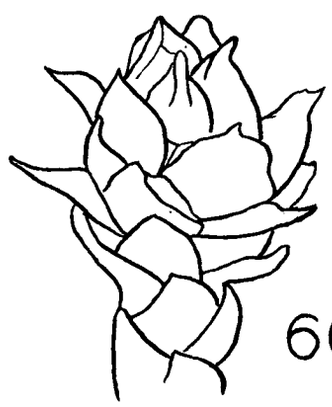
59. Embryos from 3 embryo systems. (6) 20 Oct. '46. x 400

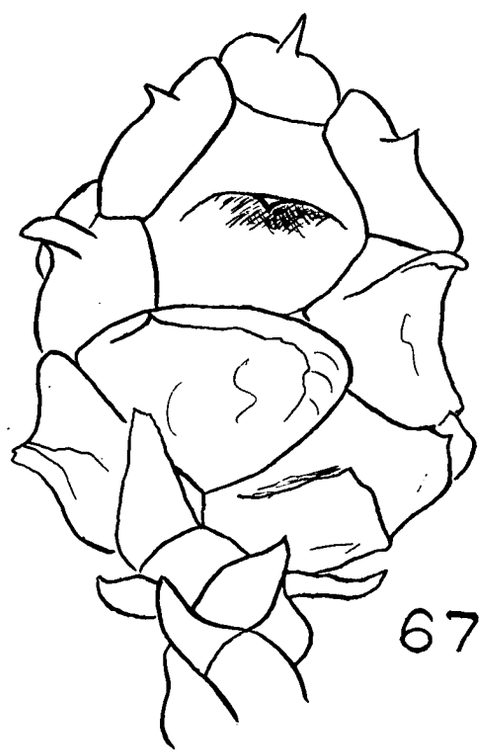
60. "Embryo" in end of prosuspensor cell. (6) 2 Dec. '46. x 400.

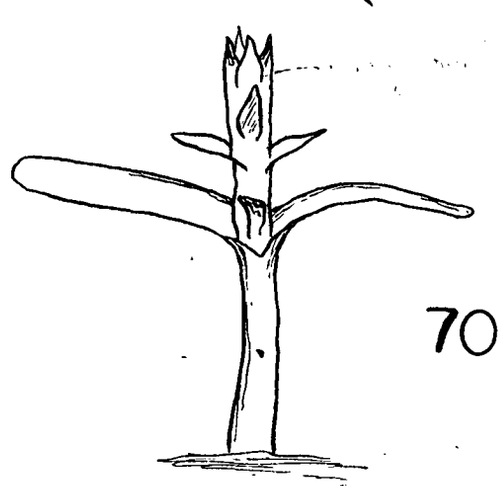
61. Large "rosette" cell. (6) 29 Sept. '46. x 400.











Athrotaxis cupressoides

62. Male cone about the time of meiosis. sp., laminae of sporophylls; l., uppermost leaf. (3) 25 Aug.'46. x 10.

63. Sporophyll from near top of cone with 3 pollen sacs. x 20.

64. Normal sporophyll. x 20.

65. Terminal sporophyll of cone without lamina. x 20.

66. Female cone about the time of fertilization. (9) 14 Dec.'46. x 5.

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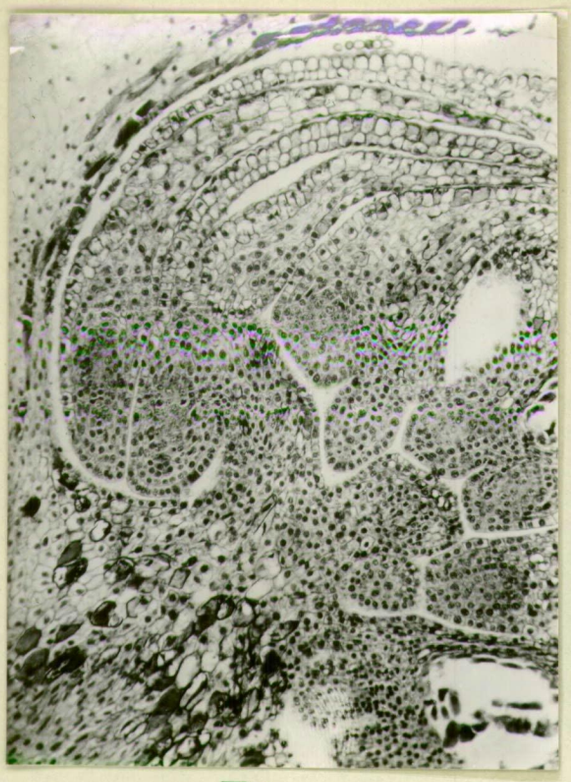

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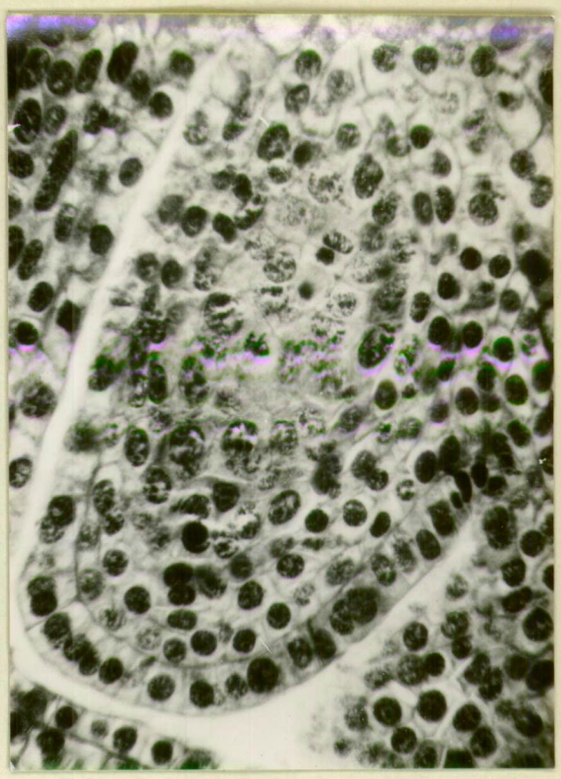

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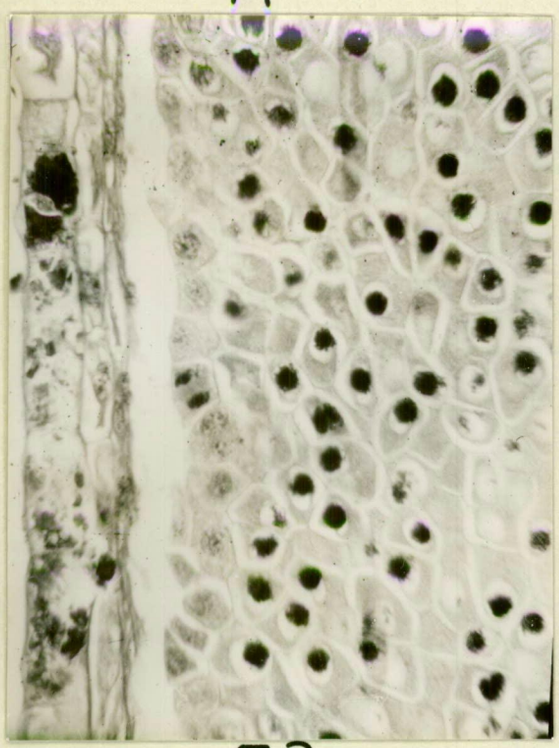

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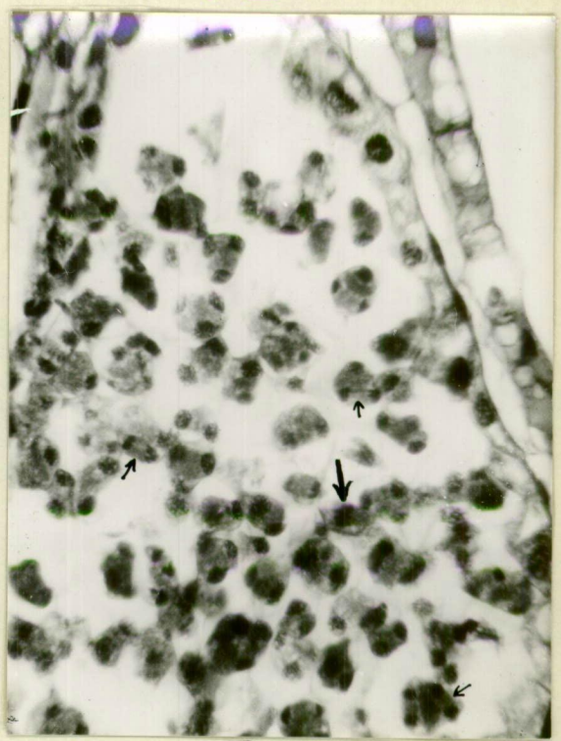

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Athrotaxis cupressoides

71. Tangential section through male cone. (11) 24 Feb.'47. x 80.

72. Sporangium from section of Fig. 71. x 380.

73. Spore mother cells at zygotene showing synezesis. (7) 18 May '46, fixed 29 May. x 380.

74. Examples of Telophase II (small arrows) and one Metaphase II (large arrow) with quadrinucleate protoplasts. (3) 25 Aug.'46. x 380.

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67. Cone with nearly mature seed. (11) 24 Feb.'47. x 55 ?

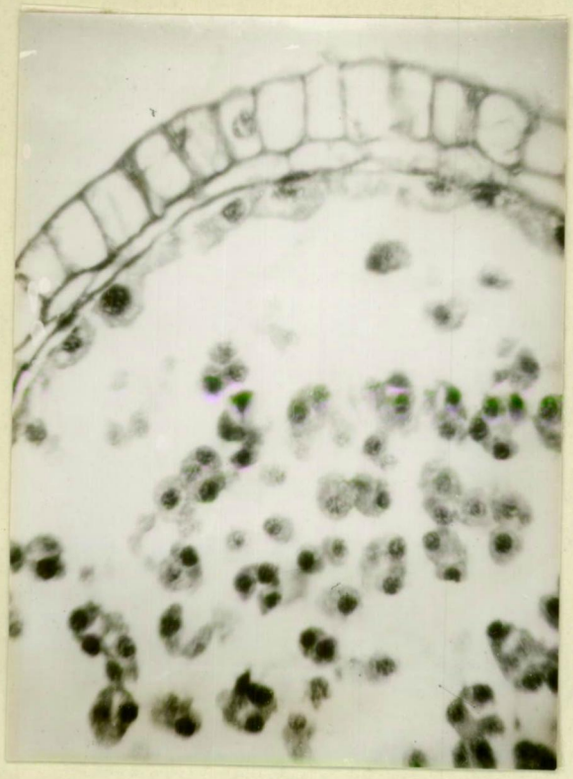
Athrotaxis selaginoides

Seedlings from material collected (5) 16 May'47, planted 5 Apr.'48.

68. Seedling just above ground, carrying integument. 3 May '48. x 5.

69. Seedling with cotyledons not yet expanded. 30 Apr. '48. x 5.

70. Seedling with 10 plumular leaves. 21 Aug.'48. x 5.



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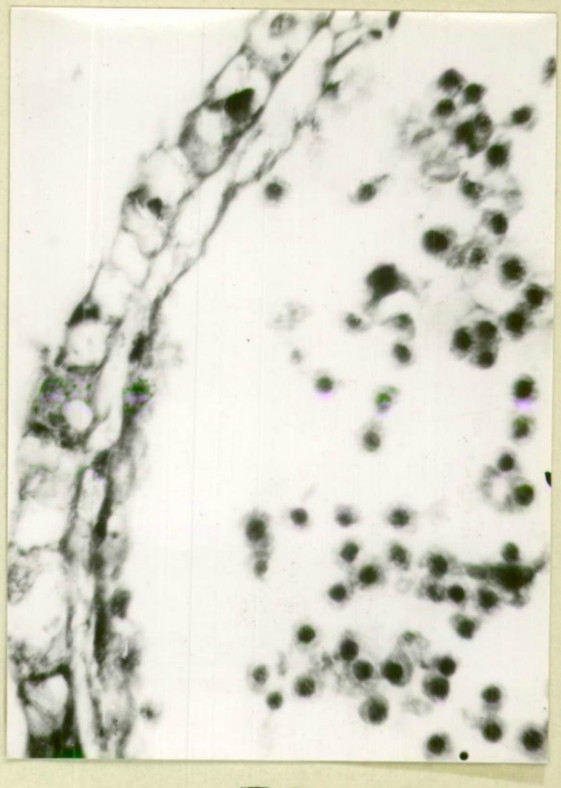

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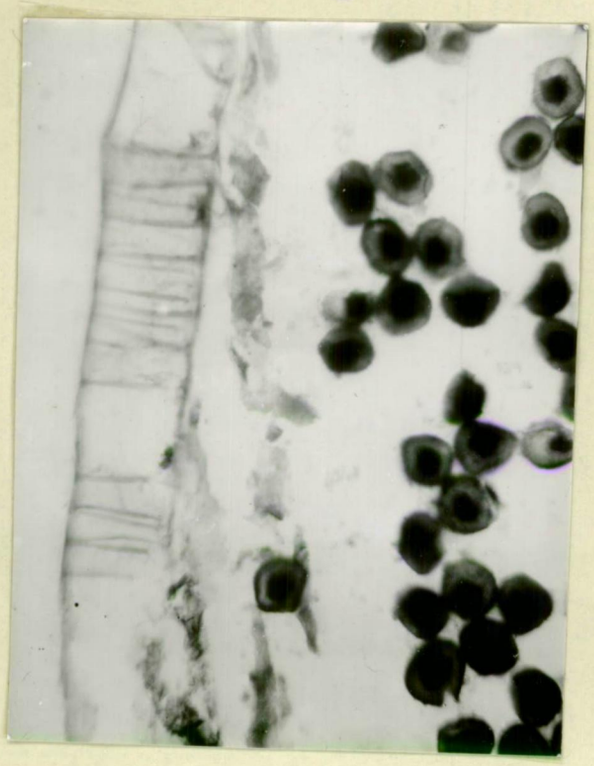

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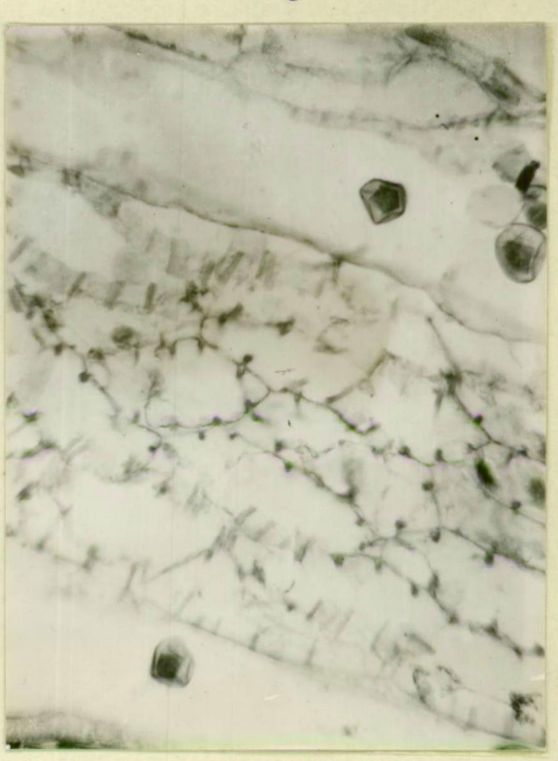


78

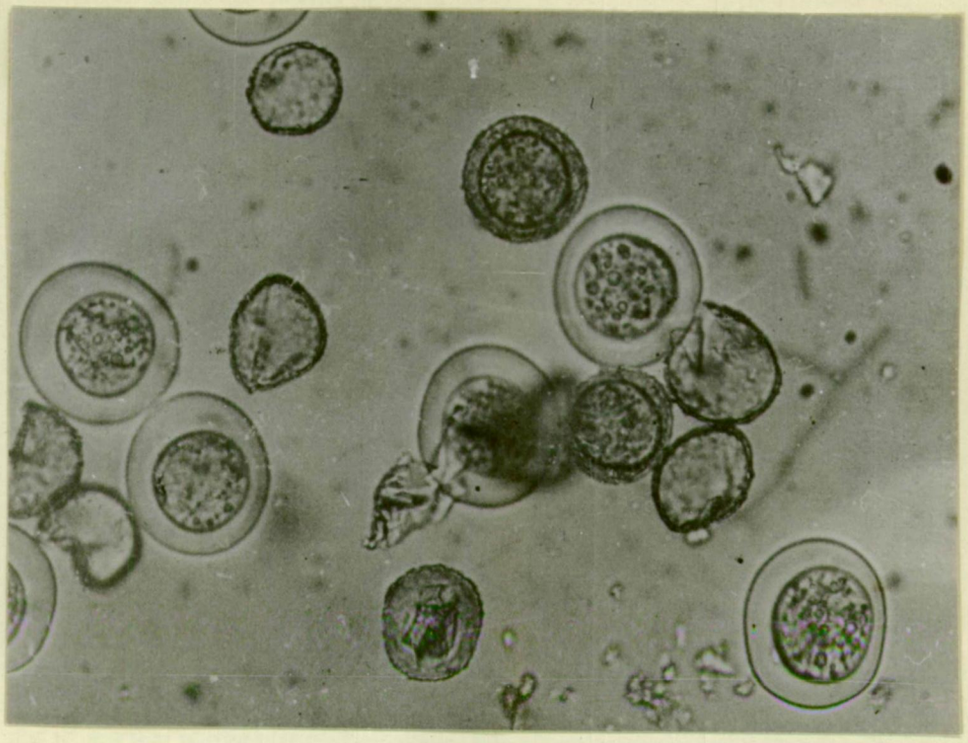
Athrotaxis cupressoides

75. Stages of cleavage into microspores. Shows tapetum adhering to wall of sporangium. (3) 25 Aug. '46. x 380.

76. Microspores nearly rounded off. (3) 25 Aug. '46. x 380.

77. Bars of thickening in wall of sporangium seen in longitudinal section. Stained with safranin. Collected (3) 25 Aug. '46, fixed 9 Sept. x 380.

78. Sporangium wall in tangential section showing bars of thickening. Same material as Fig. 77. x 380.



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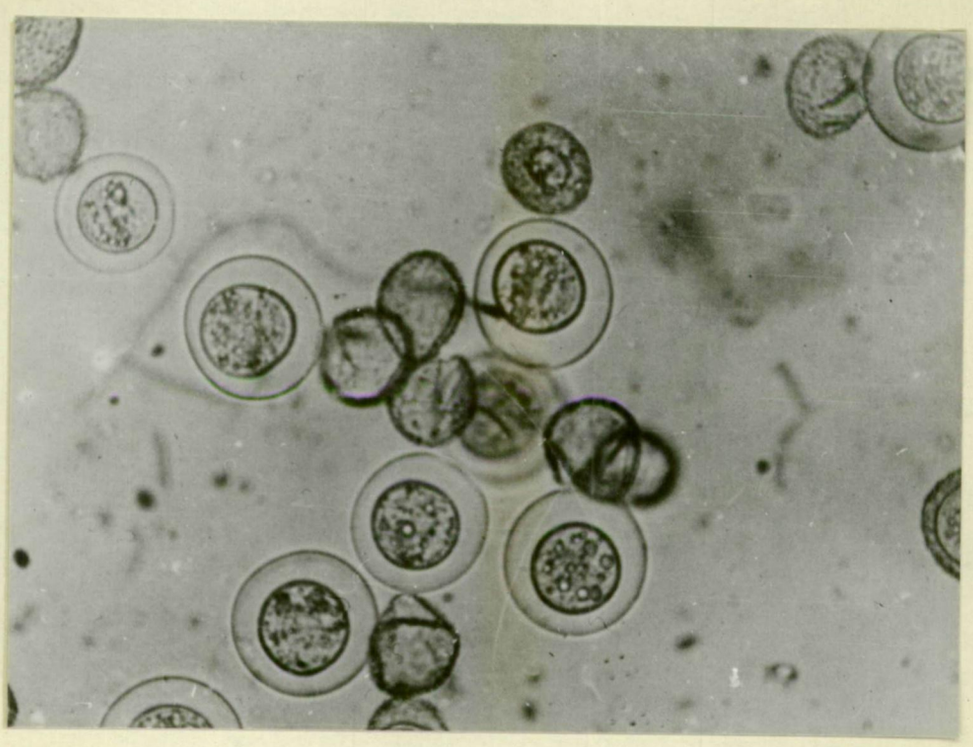
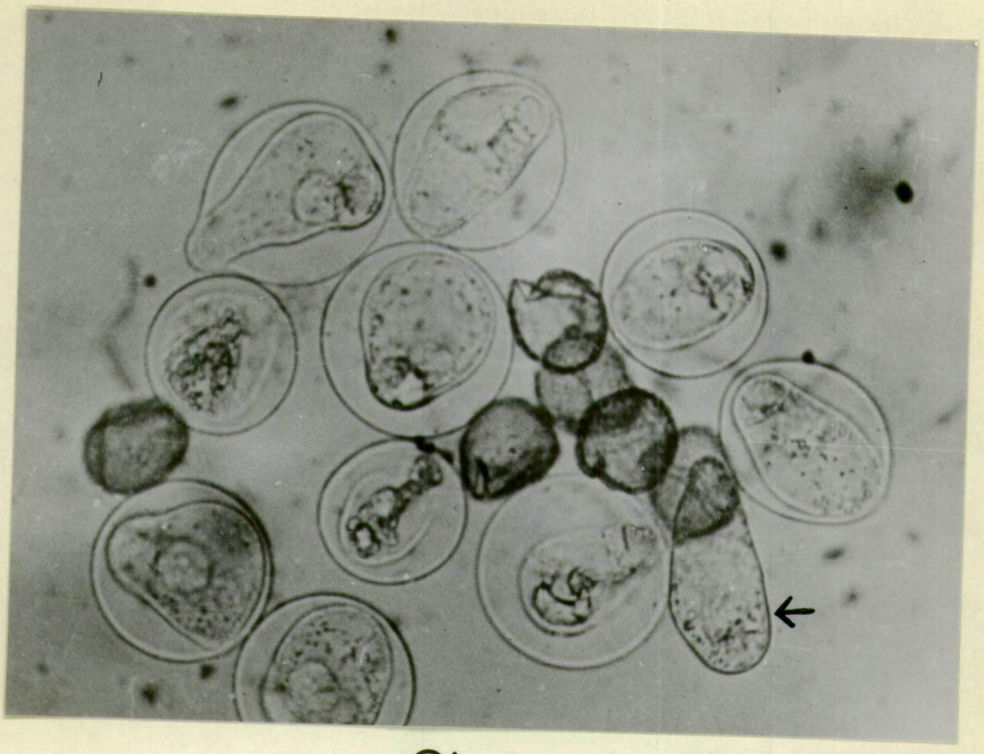


80

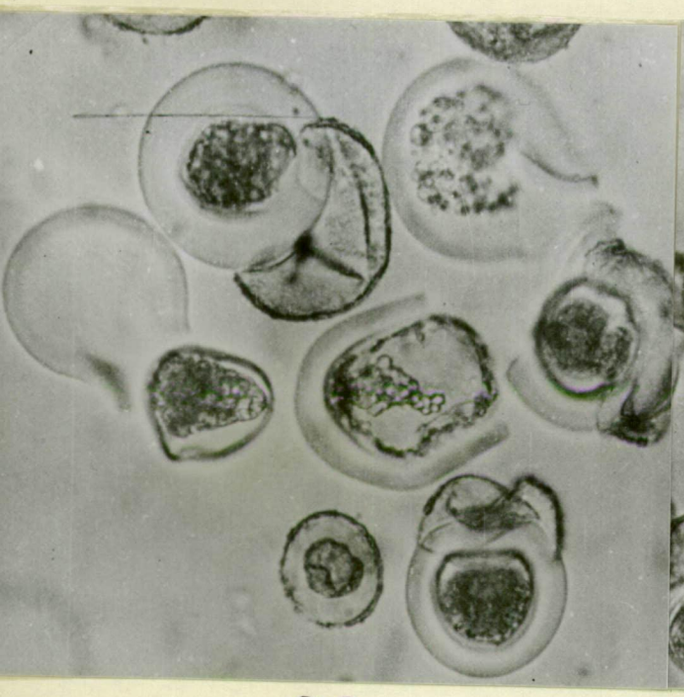
Athrotaxis cupressoides

79. Pollen freshly mounted in water. The exine has not been shed from 2 grains, but can be seen lying free beside several other grains with their halo-like intexines. x 500.

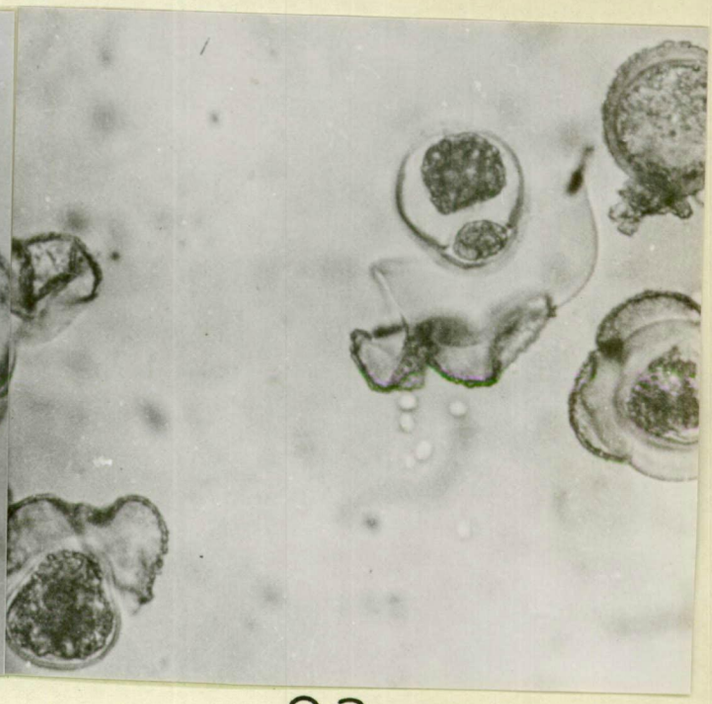
80. Similar to the last. Several grains, especially the one out of focus, show the wall dividing off the generative cell. x 500.



81



82

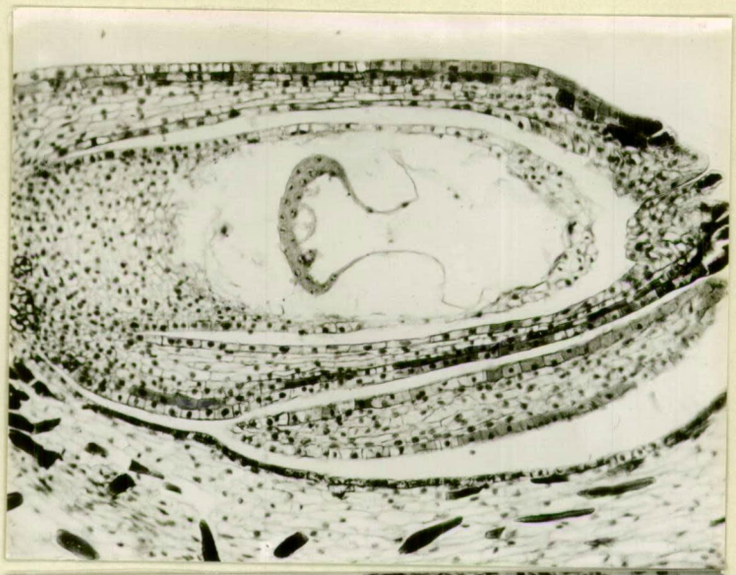


83

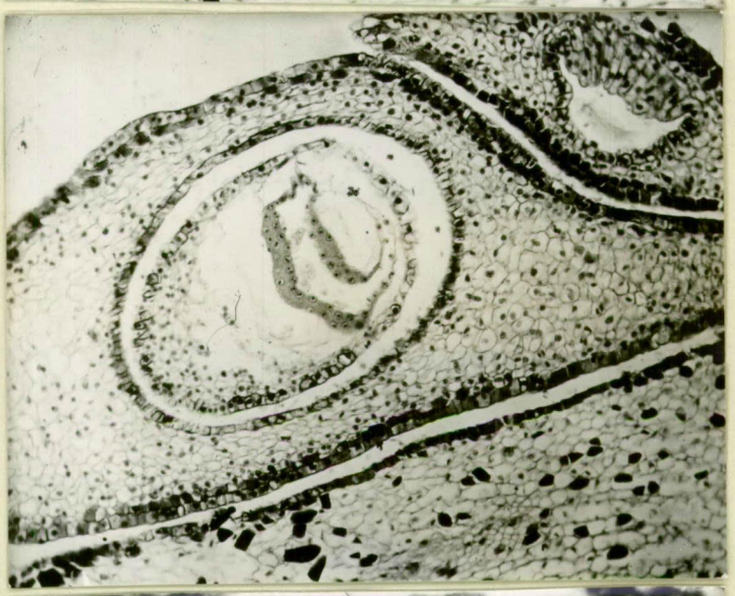
Athrotaxis cupressoides

81. Pollen after 4 days in water. The tube and generative nuclei can be seen in several pollen grains. Shows the more or less spherical intexine, which has been shed by one grain. x 500.

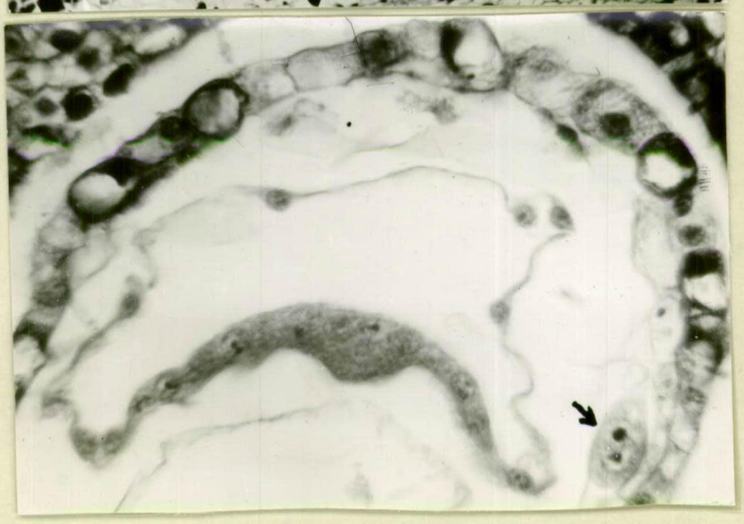
82, 83. Pollen after 8 days in M/4 sucrose. The plasmolysed protoplasts are emerging from intexines, and each is surrounded by a somewhat thickened intine, seen especially in Fig. 83. x 500.



84



85



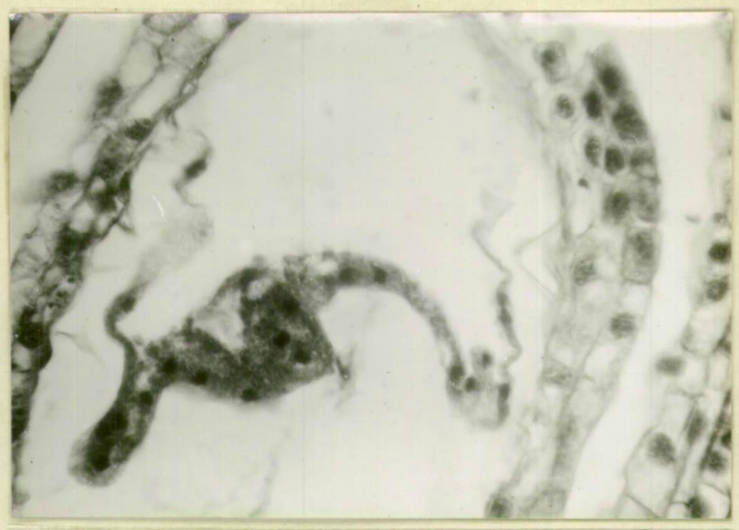
86

Athrotaxis cupressoides

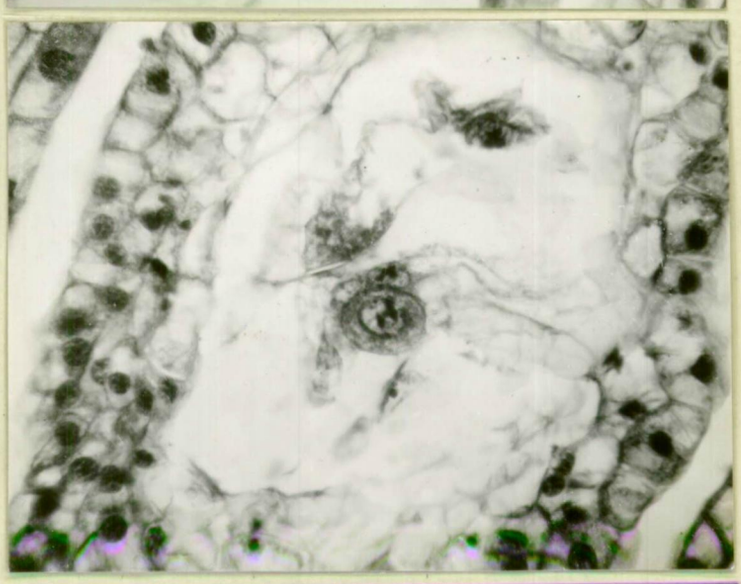
84. L.S. ovule showing integuments, micropyle, one-cell-thick nucellus and embryo sac with free nuclear stage showing nuclei congregated at the base in double layer. (9) 14 Dec. '46. x 80.

85. T.S. similar ovule to the above. (9) 14 Dec. '46. x 80.

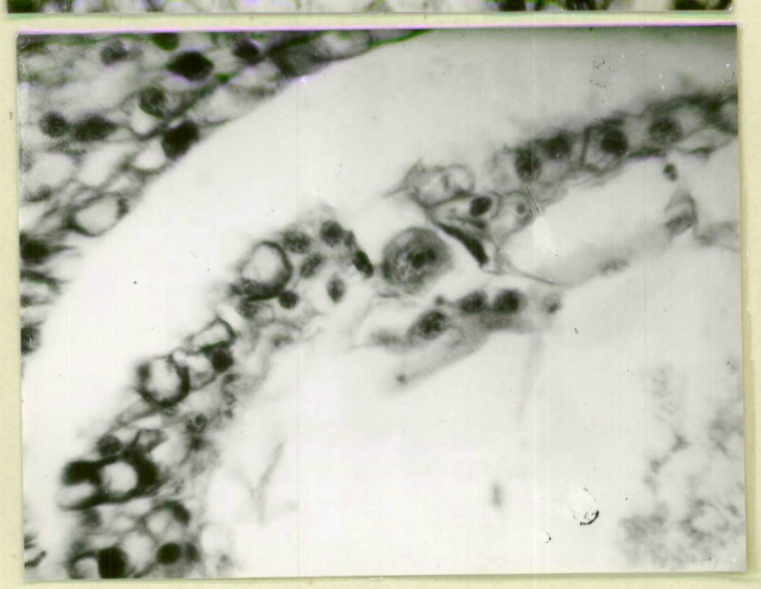
86. L.S. ovule showing free nuclei and one-cell-thick nucellus with male gametophyte in one corner (arrow). (9) 14 Dec. '46. x 380.



87



88



89

Athrotaxis cupressoides.

87. L.S. ovule with nuclei of embryo sac in division. (9) 14 Dec. '46. x 380.

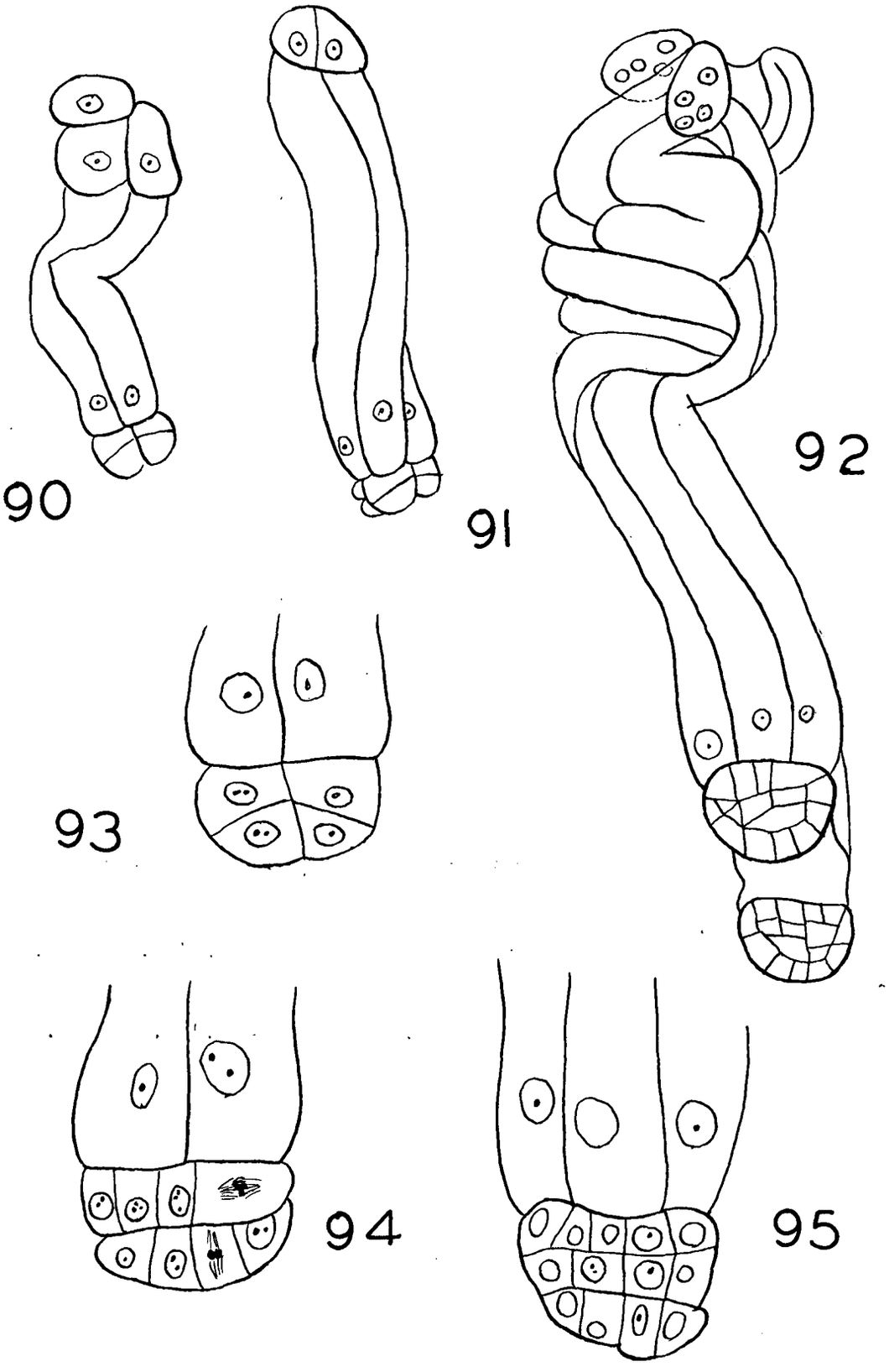
88. Body cell, stalk and tube nuclei. (9) 14 Dec. '46. x 380.

89. Body cell presumably just having entered through nucellus. (9) 14 Dec. '46. x 380.

Explanation of Figs, 90 - 95 continued -

94. Embryo with 2 multicellular tiers, and first division to form third tier taking place. (10) 27 Jan. '47. x 420.

95. Embryo with 3 tiers of cells. (10) 27 Jan. '47. x 420.



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90. 8-celled embryo at end of 2 prosuspensor cells; 2 unelongated prosuspensors and a rosette cell above. (10) 27 Jan. '47. x180.

91. 8-celled embryo at end of 4 prosuspensor cells, with 2 rosette cells above. (10) 27 Jan. '47. x 180.

92. Embryo system with developing embryos without secondary suspensors. Rosette tier apparently of a single cell with 4 nuclei. (8) 19 Jan. '47. x 180.

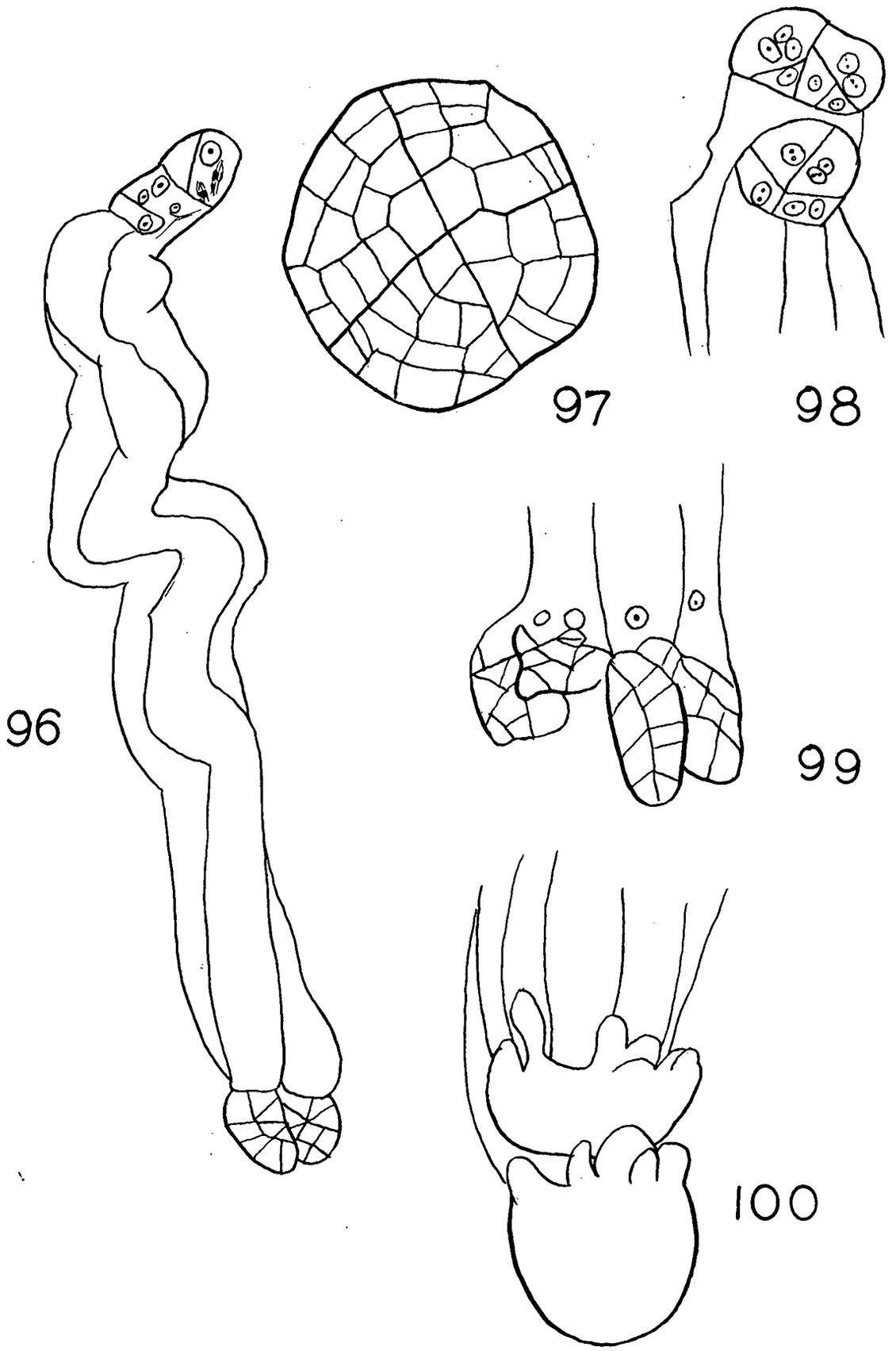
93. 8-celled embryo showing orientation of walls. (10) 27 Jan. '47. x 420.

Continued on opposite page.

Explanation of Figs. 96 - 100 continued

99. Cleavage polyembryony resulting from independent development of 4 primary cells. (8) 26 Jan. '47. x 250.

100. Single embryos from 2 systems, showing early development of embryonal tubes. (9) 26 Jan. '47. x 180.



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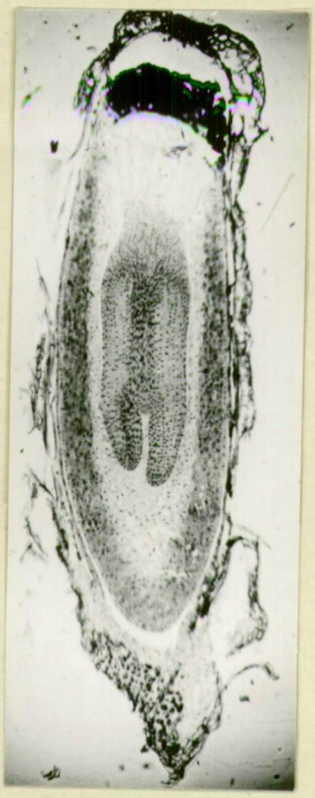
96. Embryo system with lobed embryos and irregularly developed rosettes. (8) 19 Jan. '47. x 180.

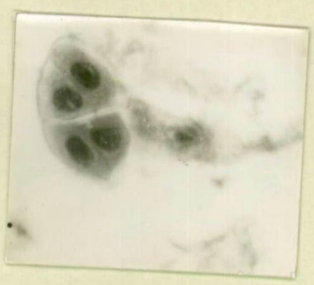
97. T.S. embryo showing 4 quadrants from original 4 cells. (9) 26 Jan. '47. x 420.

98. Rosette cells of 2 embryo systems showing development from 2 primary rosette cells. (8) 19 Jan. '47. x 250.

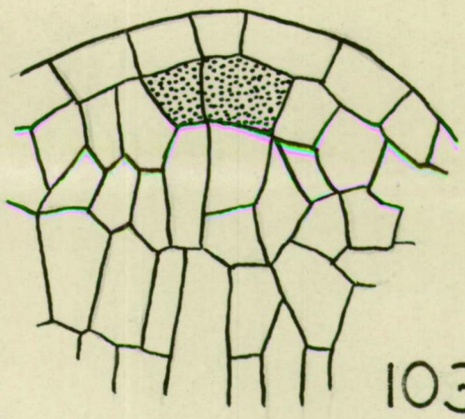
Continued on opposite page



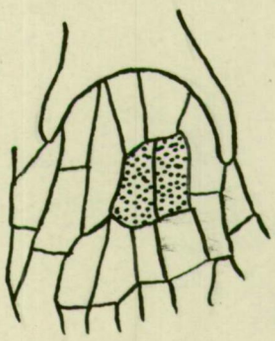
102



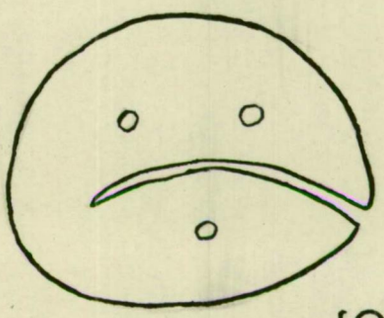
101



103



104



105

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101. Section through two rosette cells, each of which has divided once. (8) 19 Jan. '47. x 380.

102. Section of nearly mature seed. (11) 24 Feb. '47. x 26.

103. Stem apex of em bryo in plane between the cotyledons. (11) 24 Feb. '47. x 400.

104. Stem apex of embryo in plane passing through cotyledons. (11) 24 Feb. '47. x 400.

Sub-apical initials shaded in Figs. 103 - 4.

105. T.S. embryo through cotyledons, showing fusion of cots on one side. (11) 24 Feb. '47. x 77.