The Prothallus of Lycopodium clavatum, L.[1]

BY

WILLIAM H. LANG, M.B., B.Sc.,

Lecturer in Botany at Queen Margaret College, and Clark Scholar, University of Glasgow.

With Plates XVI and XVII.

IN no group of the Pteridophyta has information regarding the life-history been accumulated more slowly than in the case of the Homosporous Lycopodiaceae. Both sexual and asexual generations are now known, however, in several species of the genus *Lycopodium*. Had the prothalli of these been found to resemble one another as closely as is the rule in the other genera of Vascular Cryptogams, comparatively little interest would attach to the investigation of the remaining species. But among them several distinct types of prothallus

[1] Since the manuscript of this paper was completed, an important monograph by Professor Bruchmann (Ueber die Prothallien und die Keimpflanzen mehrerer europäischen Lycopodien; Gotha, 1898), dealing with the prothalli and young plants of *Lycopodium clavatum*, *annotinum*, *complanatum*, and *Selago*, has been published. The facts contained in this are considered along with the other knowledge we possess of the gametophyte in this genus, in the concluding portion of the present paper, but the earlier part has been left untouched, save by the addition of a few notes referring to Bruchmann's observations on the same species. I may therefore take this opportunity of pointing out that the description of the facts, though founded on independent observations, must for the most part be regarded as confirmatory of the account previously published by Professor Bruchmann.

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are known, associated with differences in the young sporophyte. The possible systematic importance of these characters of the gametophyte in determining the relationship of the species of *Lycopodium* to one another has been pointed out by Dr. Treub, to whose investigations our entire knowledge of the life-history of tropical species is due; the subject will be further discussed below.

The present paper deals with the prothallus of *Lycopodium clavatum*, a preliminary account[1] of which was read before Section K of the British Association last year. During some weeks spent at Clova in Forfarshire, where all the British species of *Lycopodium*, with the exception of *L. inundatum*, can be found in abundance, the soil in which the plants grew was repeatedly examined in the hope of finding prothalli, but without success. In July of last year, however, a number of young plants of *L. clavatum* were found growing among a patch of *Racomitrium lanuginosum* on a rock shaded by a few trees in Glen Doll. Since the presence of the 'foot' rendered it certain that these plants had been borne on prothalli, the thin layer of peaty soil underlying the moss was examined with great care, every part of it being gently crumbled down. In this way seven prothalli were found, three of which bore young plants of various ages. Careful examination of the ground for a considerable distance around failed to disclose any more young plants, nor were old plants of this species to be found near the spot.

External Form and Structure of the Prothallus.

The size and general appearance of the prothalli and young plants, upon the study of which the following description is founded, will be evident from Fig. 1, which is reproduced from a natural-size photograph of a prothallus seen from above, and of one of the smaller plants; the foot of the latter is still recognizable though the prothallus to which it had been attached had disappeared. Although the material did not

[1] British Association Report, 1898, p. 1050.

allow of the development of the prothallus being followed, the specimens differed from one another in size and shape, and probably illustrate changes of form associated with the later stages of growth ; all conclusions drawn from the comparison of a small number of prothalli must, however, be tentative.

The smallest of the prothalli, which is represented as seen from above in Fig. 2, measured 4 mm. in length by 3 mm. in breadth. It was an almost flat plate of tissue about 0·5 mm. thick, of a dirty white colour. Numerous rhizoids of considerable length projected from the under surface, especially around the margin ; they were absent from the upper surface, which was lighter in tint than the rest of the prothallus. From external examination an apical[1] region could not be distinguished, though in this and all the other prothalli a median plane dividing them into equal and similar halves could be recognized. In this longitudinal direction the prothallus was slightly curved, the upper surface being convex. Owing to the lateral margins being slightly bent up, this surface was also concave from side to side. It will be seen that these curvatures increase in larger and presumably older specimens, but it is of interest to note that in a prothallus of such relatively considerable size they were so slight. In the prothallus represented in Fig. 3 both these curvatures were much more marked. Owing to the increase of the longitudinal curvature, the ends were approximated to one another; while the transverse curvature gives rise to the trough-like form which will be apparent from an examination of the figure. Each of the lateral margins exhibited a secondary fold rather nearer to one end. As in the younger prothallus, the rhizoids, which were here much shorter, were more numerous near to the margin. Another prothallus, which resembled this in shape, save in the absence of the secondary folds, was found. Two

[1] This difficulty is explained, as Bruchmann has shown, by the flattened structure being preceded by a vertical, radially symmetrical stage. The longer diameter of these old prothalli has thus no relation to the true longitudinal axis. On re-examination of serial sections of my prothalli, the conical projection from the under surface, which is all that remains of the earlier stage, was to be found, though small and inconspicuous. It is not visible in any of the figures.

others had a more complicated outline. One of them is represented from the side in Fig. 4, which shows that the increase of the longitudinal curvature has brought the two ends into actual contact, while a single secondary fold is visible. Each lateral margin of the similar prothallus represented in Fig. 1 showed two such secondary folds.

The resemblance in form and habit which these prothalli of *L. clavatum* presented to those of *L. annotinum*[1] rendered it probable that the structure would be similar in the two cases. As will be seen, this similarity was found to exist even in points of detail. In Fig. 5 part of a vertical section across the lateral margin of the prothallus shown in Fig. 3 is represented; the corresponding sections of the ends presented a similar arrangement of tissues. As will be seen from the figure, a number of layers of tissue can be distinguished which agree, in relative thickness and in the characters of the cells composing them, with the layers described by Bruchmann in *L. annotinum*[2]. This distinction of layers appears to stand in relation to the localized distribution of an endophytic Fungus, the characters of which will be described toward the end of this paper. The slightly flattened cells of the outermost layer (*a*, Figs. 5, 6), which bounds the prothallus on the under surface, are quite free from the Fungus, with the exception of some of those prolonged as rhizoids. The outer layer of the free walls of these cells gives the reaction of cuticle; this is especially marked at the base of a rhizoid. The latter structures are as a rule simple extensions of superficial cells, but in some cases the main part of the rhizoid is separated by a transverse wall from the basal portion. Above the lower limiting layer comes a band of cells three or four deep (*b*, Figs. 5, 6), which are extended parallel to the surface of the prothallus. These cells, as well as the succeeding single layer (*c*, Figs. 5, 6), which consists of more or less regular palisade-shaped elements with their long

[1] Fankhauser, Bot. Zeit., 1873, p. 1; Bruchmann, Bot. Centralblatt, xxi, 1885, p. 23.

[2] Loc. cit., 1885.

axes at right angles to the surface, are filled with the mycelium of the Fungus, which is here entirely intracellular. In consequence of this they appear darker than the other regions of the section. Above the layer of palisade-cells comes a region (*d*, Figs. 5, 6) about eight cells deep, from the cell-cavities of which the Fungus is absent. These cells are smaller than those of the preceding layer, but are not infrequently elongated in the same direction. Their walls appear thicker than those of the rest of the prothallus, especially at the angles. Throughout the whole of this region the mycelium of the Fungus can be traced within these thickened walls, but never penetrating into the cavities of the cells. The latter contain a large quantity of starch, which is also present, though less abundantly, in the tissues above, but is practically absent from those in which the mycelium is intracellular. Whether, as in *L. annotinum*, oil was also stored in this region was not determined. The appearance of the four layers of tissue described above is shown in greater detail in Fig. 6. The region of the prothallus above the layer last described usually forms more than half its thickness (*e*, Fig. 5). The cells composing it are thin-walled and possess a scanty protoplasmic lining. Those situated more internally are of large size, but those surrounding the antheridia or archegonia are much smaller. The latter form a fairly definite layer (*f*, Fig. 5) on the upper surface, the origin of which, from the superficial layer of cells at some distance from the growing region, can be followed in Fig. 5. This uppermost layer may be distinguished as the generative layer, while the large-celled tissue beneath may be compared to the cushion of a Fern-prothallus. The prothallus is thus seen to consist structurally of six more or less clearly defined regions, the four lowest of which are, as their structure indicates, concerned with the nutrition of the organism, while the uppermost layer contains the sexual organs.

Great difficulty was experienced in tracing the origin at the growing regions of the different tissues, the mature condition of which has now been described. It may be stated at the outset that the formation of new tissue is not confined to one end, but takes place all round the edge of the prothallus. Leaving aside for the moment the structure of the two ends, the results obtained from the study of sections across the lateral margin may be first considered. The merismatic region to which growth can be referred is situated at the margin of the prothallus toward the upper surface ; its position is indicated by a cross in Fig. 5. Toward the lower surface the tissues of the vegetative half of the prothallus become differentiated, while to the other side the cushion-layer is formed. When the latter, in cases where the sexual organs are numerous and near to the margin, attains a considerable thickness, a marginal groove is the result, which can be recognized in Figs. 5, 8, and 9. In these cases the initial region occupies the upper side of the lower lip of the groove. Periclinal divisions sometimes occur in the superficial cells of the merismatic region, but it is very doubtful whether these cells can be considered to be initials. The comparison of a number of sections rather suggested that the meristem is imperfectly stratified. The superficial layer can be traced on the one hand into the limiting layer of the under surface, the cells of which undergo anticlinal divisions only. On the other side this layer gives rise at a greater or less distance from the margin to the small-celled generative layer with the sexual organs. Below the superficial layer in the merismatic region are a few layers of fairly large cells, which can be traced on the one hand into the band of longitudinally extended cells (*b*), and on the other into the cushion-layer (*e*). Still deeper in is a small-celled meristem, from which the storage-layer (*d*) and the layer of palisade-cells (*c*) are differentiated. This differentiation is complete at a very short distance from the meristem, the whole appearance suggesting that the addition of new cells to the permanent tissue is very slow. This explains the difficulty that was experienced in following the course of divisions in the meristem.

The vertical sections through the ends of the prothalli presented considerable differences in the various specimens examined, and also when the two ends of any one prothallus were compared. In the prothallus represented in Fig. 4, no difference could be made out between sections of either end and those across the lateral margin; in all these regions the appearance of the tissues and the succession of archegonia showed that growth was proceeding. But in the other prothalli the sections of the ends differed from each other and from those across the lateral margin. The nature of these differences will be gathered from Fig. 7 *a* and *b*, which represent the sections through the ends of a prothallus which resembled the one in Fig. 3; the crosses indicate the points in relation to which the tissues seemed to be arranged. The insufficiency of material will not permit of any more detailed statement being made, but the existence of such differences is of interest with regard to the appearance of symmetry presented by these old prothalli.

Although the details of the merismatic regions will require to be investigated in younger prothalli, the facts of which an account has just been given suggest the explanation of the main changes of form exhibited by these old prothalli. Starting from a flat plate of tissue (Fig. 2), the growth of which is proceeding all round the edge, irregularities of growth would cause the lateral margins to assume the folds seen in Figs. 1, 3, and 4, while the main curvatures of the prothallus may find their explanation in differences in rapidity of growth between the upper and the lower sides of the marginal meristem. A much larger number of prothalli would, however, be needed to enable this to be traced in detail.

Antheridia and Archegonia.

From what is known of the distribution of the sexual organs on the prothalli of other species of *Lycopodium*, it might have been anticipated that archegonia and antheridia would have occurred in numbers upon the same prothallus. It is of course impossible to draw any general conclusion as to the distribution of the sexes in *L. clavatum* from the few prothalli investigated : but the striking fact results from the detailed study of complete series of sections of six prothalli and the external examination of another, that a practically complete separation of male and female sexual organs on old prothalli of similar size and form may occur. Of the seven prothalli six were female and only one male. No archegonia were found on the latter, but on two of the female prothalli a few antheridia were seen among the much more numerous archegonia. In the absence of continuous observations on developing prothalli it was impossible to be certain whether the same prothallus passes through a male and a female stage, or if at an earlier stage both kinds of sexual organs do not co-exist [1].

The archegonia and antheridia occupy corresponding positions : both are confined to the upper surface of the prothallus. In the specimen represented in Fig. 2, the archegonia were scattered over the slightly projecting cushion, the youngest being found nearest to the edge, a distribution that is explained by the marginal growth. In older prothalli the central region bore only old and often almost unrecognizable sexual organs, while the functional and developing archegonia and antheridia were found near the margin, the part of the cushion on which they were situated projecting as a more or less prominent ridge. The position of this ridge will be evident from Figs. 8 and 9, which represent vertical sections across the margins of the prothalli shown in Figs. 4 and 3 respectively. As the figures show, the central region of the upper surface of these prothalli bore only obscure remains of antheridia or archegonia, while on the marginal ridge a succession of these organs can be traced, the youngest being nearest to the marginal groove. In the case of the male prothallus (Fig. 3) this ridge was only present within the lateral margins, while in the largest female prothallus (Fig. 4) the appearance of a vertical section of either end was in-

[1] Bruchmann's observations (loc. cit., 1898) show that as a rule archegonia and antheridia occur together on the prothallus. He does not mention any cases of their separation in old prothalli such as those described in the text.

distinguishable from the section across the lateral margin (Fig. 8). So far therefore as the evidence afforded by these specimens goes, the archegonia and antheridia appear to be formed in regular succession following the marginal growth of the prothalli.

The antheridium is developed from a single superficial cell which divides by a wall parallel to the surface (Fig. 10). In the outer of the resulting cells only anticlinal divisions occur, the free wall of the antheridium being only a single layer of cells in thickness; from the inner by repeated divisions the small-celled tissue, which gives rise to the spermatozoids, is formed (Figs. 11 and 12). The mature antheridium projects very little if at all from the surface of the prothallus. The large ellipsoidal mass of spermatocytes is wholly enclosed within the generative layer of the latter; its form and size will be evident from Figs. 13 and 14, which represent longitudinal and transverse sections of almost mature antheridia. The cells adjoining the spermatocytes are usually more or less flattened, but do not present any distinctive characters. The development of the spermatozoids resembles that in other Vascular Cryptogams, but was not followed in detail. The opening of the antheridium appears to be effected by the breaking down of a small cap-cell, which, with the cells surrounding it, originates from the outer of the two segments into which the mother-cell of the antheridium divides. A sufficient number of sections in the required direction were not available to determine the succession of divisions in this cell, but, as the examples figured (Figs. 15 and 16) show, there is probably some variation in this respect. The spermatozoids were not obtained.

The archegonium also is developed from a single superficial cell which divides by two periclinal walls into a series of three, which are recognizable by their dense protoplasmic contents (Fig. 17.) From the outer of these the neck arises; the other two form the central series of cells. A basal cell does not appear to be present. Older stages, in which the neck as yet projects but little above the surface, are shown in Figs. 18 and

19. In the latter, however, the series of central cells is complete. In the mature archegonium, the neck usually projects considerably from the surface (Fig. 20), though only the upper portion of its wall is a single layer of cells thick. The lower portion appears to be produced by the surrounding cells of the generative layer keeping pace with the enlargement of the archegonium at maturity, and is not derived from the outermost cell of the young archegonium (Fig. 17); this region, which is as a rule more than one layer of cells thick, is thus not strictly comparable to the archegonium-neck in other Vascular Cryptogams. In the archegonium represented in Fig. 21, this division and growth of the cells of the generative layer has on one side not been confined to the cell immediately adjoining the archegonium. The venter of the archegonium and the lower portion of the canal are surrounded by a layer of small cells cut off from those of the generative zone. These cells have dense protoplasmic contents, and their nuclei are frequently to be found against the wall which bounds the canal of the archegonium (Figs. 20 and 21). Within the venter is the large ovum, and the canal is occupied by six to eight canal-cells, seven being the number most frequently observed (Figs. 20 and 21); the lowest of these was not distinguished with any certainty as a ventral canal-cell, since the material was insufficient to allow of the succession of divisions in the two inner cells of the young archegonium being traced. Transverse sections of the young archegonium-neck show that it is typically composed of four rows of cells (Fig. 22), though subsequent divisions in one or more of these may increase the number. In sections a little lower down, the wall of the neck-canal is found to be more than one layer thick, while still lower down the ovum is found embedded in the prothallus. These relations will be sufficiently apparent from the longitudinal section (Fig. 20). The details of the opening of the archegonium-neck were not observed.

The Young Plant.

It has been mentioned already that three of the prothalli bore young plants. These were of different ages, but none were young enough to afford information as to the embryology. Figs. 23 and 24 represent two of the prothalli. The one in Fig. 23 is seen from the end, and shows a young plant, the leafy stem of which had already attained a considerable length, while the first root is still unbranched. In this and another example the plants occupied a position on the marginal ridge corresponding to the younger archegonia in Fig. 8. Here, as in *L. annotinum*, the plants remain attached to the prothallus, which, however, had ceased to produce sexual organs, until they have attained a considerable size. The peculiar form of the prothallus represented as seen from above in Fig. 24, appears to have been due to an injury at an earlier stage of development. One-half appears to have been partially destroyed and had ceased to grow, while from the opposite margin a new growth of semicircular outline had proceeded; the light colour of the latter contrasted with the older portion, which was dark brown in tint. Two of the last-formed archegonia must have been fertilized about the same time, for the young plants were almost exactly similar in form and size. The longer cylindrical body projecting from the ruptured archegonium-wall is the shoot, the scattered scale-leaves of which were inconspicuous, while the primary root is recognizable as the short conical projection at the base of this. It may be noted that the orientation of these two plants with regard to the edge of the prothallus is the same.

No structure comparable to the protocorm of *L. cernuum* was recognizable on external examination of the young plants, and its absence is further shown by the outline drawings in Figs. 25 and 26, which represent sections through the young plants in Fig. 24. In Fig. 25 the section passes in the median plane of the first root, but owing to the shoot being curved to one side, fails to follow the latter for any length;

while in Fig. 26 the section is in the median plane of the shoot, but at right angles to the median plane of the root. The two sections are thus in planes at right angles to one another.

The superficial layer[1] of the foot was characterized by the dense protoplasmic contents of its cells. The extent of this layer, which is possibly concerned in the absorption of organic substances from the prothallus, is indicated by the shading in Figs. 25 and 26; a small portion of a similar section is represented in detail in Fig. 27. These figures and Fig. 28 also show the irregularity of outline of the foot and the thickness of the outer walls of its limiting layer of cells; these outer walls stained very deeply with Bismarck-brown.

The structure of the region of attachment to the prothallus of the older plant shown in Fig. 23 is drawn in detail in Fig. 28. It was ascertained on comparing this section, which is approximately median as regards both shoot and root, with the rest of the series that the vascular system does not extend into the foot; the central cells of the latter, however, seem to be arranged so as to facilitate the conduction of absorbed substances towards the vascular strand. The absorbent layer, though still recognizable, did not present the characteristic appearance seen in the younger plant (Fig. 27). This may be associated with the fact that the plant at the latter stage was devoid of chlorophyll and wholly dependent on the prothallus, whereas the absorbent function of the foot would be of secondary importance in the case of a plant with a shoot-system capable of assimilation. It may be noted that in the specimen represented in Fig. 28 the structure of the foot is not uniform, the region just below the insertion of the root being composed of smaller, thinner-walled cells with more abundant contents; whether this difference possesses any significance is however uncertain.

The large foot persists for a considerable time after the prothallus has disap peared, and wasrecognizable in much

[1] Bruchmann (loc. cit., 1898, p. 44) ascribes to this peripheral layer of the foot the main part in the growth of the latter; the arrangement of the cells in my specimens did not however seem to support such an interpretation.

older plants than that represented in Fig. 1. Its position with regard to the main axis of the plant is variable. The differences probably depend on the inclination of the surface of the prothallus upon which the plant was borne, the young shoot and root of course tending to grow vertically upwards and downwards respectively. Figs. 29 and 30 represent two extreme examples. In the former the position of the foot is distinctly lateral, the surface of the prothallus having in all probability been almost vertical; while in the specimen represented in Fig. 30, which was probably inserted on a horizontal surface, the foot is in the same line as the axis of the shoot, the root arising at the base of the latter. For a variable distance above the position of attachment to the prothallus, the stem bears only small colourless scale-leaves which higher up pass by gradual transitions into ordinary green foliage-leaves (Fig. 1). This doubtless depends on the thickness of soil and moss through which the shoot had to grow before reaching the light.

The Endophytic Fungus.

It has been mentioned above in the general description of the prothallus that its lower half harbours an endophytic Fungus, the distribution of which within the cells of the layers marked *b* and *c* and between those of the layer above (*d*) was described. It will be sufficient in this place to refer to Fig. 6, with regard to which it should, however, be noted that the intercellular mycelium is more uniformly distributed than was demonstrable in the portion of the section drawn. When thicker sections are mounted in Schulze's solution, the filaments of the Fungus swell somewhat and take on a purplish colour; it is then seen that the mycelium is present throughout the entire thickness of the storage-layer.

The cells containing the Fungus, although their protoplasmic body was occupied by closely packed hyphae, appeared to be perfectly healthy. The nucleus presented a normal appearance (Fig. 32), though occasionally it may be altered in shape by the pressure of the adjoining hyphae. It was usually found close to the vacuole, but sometimes against the cell-wall. No indication of septa in the hyphae has been seen, though a large number of sections have been examined with this object in view. Their infrequency may therefore be considered to be probable; though owing to the difficulty of examining a long filament of the Fungus, their absence cannot be definitely asserted. Small oval nuclei occur at intervals in the filament. The intercellular hyphae resembled those found within the cell-cavities.

The appearance of the endophyte was found to present differences according to the region of the prothallus in which it occurred, and differences of a similar nature were noted when fertilized prothalli were compared with those in which marginal growth continued. The important factor common to both these cases appears to be the age of the tissue in which the Fungus is found.

Taking first the case of an unfertilized prothallus, the appearance of the Fungus may be described as it is seen in a section on passing from the margin towards the more central regions of the prothallus. For a short distance from the small-celled meristem at the margin, the tissues are free from Fungus. In the corresponding cells a little further from the margin, fungal filaments are found in the protoplasmic body, running for the most part parallel to one another. None but these vegetative hyphae are present in this region, and both the cells and the mycelium appear to be quite healthy. Passing to the cells slightly further from the margin, in addition to the ordinary hyphae, bodies of peculiar form are found in them; the nature of these will have to be discussed later, but they may be termed *multinucleate vesicles*. A number of these are to be seen in the portion of a vertical section represented in Fig. 6. They were found to be most numerous in the band of tangentially extended cells, but they occur occasionally in the cells of the palisade-layer; they have not been observed between the cells. Early stages of these vesicles are represented in Fig. 31 *a* and *b*, and an isolated older vesicle, in which a number of nuclei are already present, in Fig. 31 *c*. The vesicle appears to originate by the swelling of the end of a hypha, and at first contains only one nucleus. Intermediate stages have been seen between these small swellings and the large vesicles containing many nuclei. The connexion of the larger vesicles with the mycelium is usually not apparent (Fig. 32); but by examining series of very thin sections, examples such as that represented in Fig. 33 can be found which show that they are borne on hyphae. Whether or not a septum is present between the cavity of the hypha and that of the multinucleate vesicle could not be definitely determined. On passing to the older cells of these layers, the vesicles are seen to increase in size, the number of nuclei within them to become greater, and their wall to thicken somewhat, though it never attains any great thickness; the protoplasmic body fills, or almost fills, the cavity of the vesicle. Up to this stage the mycelium appears to be healthy, its nuclei staining readily, as does the nucleus of the cell of the prothallus.

But in the region succeeding this, about the same distance from the margin in all the prothalli examined, a change in the appearance of the mycorhizal tissue occurs. The cells with their nuclei still appear healthy, and the latter stain as well as before; but the fungal filaments have lost the regular arrangement they exhibited before and, what is still more significant, show merely the stained wall, the nuclei, and presumably the other contents, having disappeared. The multinucleate vesicles persist in a healthy condition in fairly old cells, their nuclei staining as before. But besides these vesicles of normal appearance, more or less collapsed ones were found, some of which were empty while others had a portion of their contents remaining. These, however, were difficult to detect, though there is no reason to doubt that this tissue formerly resembled that nearer to the margin, in which numerous multinucleate vesicles occur. It has been found impossible to arrive at any definite conclusion as to the way in which the disappearance of the contents of the vesicles comes about.

No perforation in the wall has been seen in any specimen, and it is quite possible that the contents of these dilatations of the mycelium are absorbed in the same way as from the filamentous portion. On the other hand, it is not impossible that in some instances at least the contents may escape into the cell-cavity. In this connexion another characteristic of the region of mycorhizal tissue in which the vesicles are mostly empty must be referred to, although its significance is still doubtful. There are frequently to be found in the cells of this region small round or oval bodies, the walls of which stain deeply with Bismarck-brown ; a cell containing these spore-like bodies is represented in Fig. 34. They may be distributed through the mycelium, with which they sometimes appear to be connected, or may form a more or less compact group. Though appearances suggestive of an origin of these ' spores ' from the contents of the multinucleate vesicles have been seen, it has been found impossible to demonstrate any connexion between the two structures. The question must therefore be left open. In the case of prothalli the marginal growth of which had ceased, the changes described above as affecting the older mycorhizal cells extend to those close to the margin until in these also the contents of the hyphae have disappeared ; the nuclei and protoplasm of the vesicles persist longer.

The multinucleate vesicles described above appear to be the same as the ' Sphaeromen ' described by Bruchmann [1] in the prothallus of this and other species of *Lycopodium*. The considerable differences between the above description and that given by Bruchmann may be accounted for by the fact that the methods employed in the present investigation have demonstrated the contents of these bodies more clearly. They appear also to agree closely with the 'vesicules' found by Janse [2] in a large number of examples of mycorhiza. The organs of the endophyte, termed by that author 'sporangioles,'

[1] Bot. Cent., xxii. 1885, p. 312, and loc. cit., 1898, pp. 19 and 23.
[2] Annales du Jard. Bot. Buitenzorg, xiv. p. 53.

have not been observed in the case of the Fungus inhabiting the prothallus of *L. clavatum.*

Neither the structure of the mycelium nor the presence of the multinucleate vesicles, the nature of which is uncertain, afford sufficient indication of the group to which this endophytic Fungus belongs. The appearance is quite consistent with the position, as one of the Peronosporeae, which has been commonly assigned to the similar endophytes found in the prothalli of other species of *Lycopodium*; but fuller knowledge of its reproductive organs and life-history is needed before the question of its systematic position can be settled.

The Fungus comes into relation with the surrounding soil by means of hyphae which are present in many of the rhizoids. One or more hyphae, the continuity of which with the mycelium in the underlying tissue can be traced, may be found running almost parallel within the cell-cavity of a rhizoid (Fig. 35). In passing through the basal wall of the latter, the hypha is surrounded by a short sheath derived from the perforated cell-wall. The presence of similar sheaths has been described and discussed by Jeffreys[1] in the case of the Fungus inhabiting the prothallus of *Botrychium virginianum*; but there the sheaths follow the hypha as it passes *inwards* through the outer cell-walls. In *L. clavatum*, on the other hand, the sheaths project *outwards* into the cavity of the cell which bears a rhizoid (Fig. 36). This difference is not without meaning, for it affords indirect evidence that the hyphae penetrated from the interior of the prothallus into the rhizoid, and are therefore not to be regarded as infecting hyphae. Probably after passing along the rhizoid, the filaments of the Fungus penetrate its wall and ramify on the soil, as has been described for *L. annotinum*[2].

In concluding this account of the prothallus of *L. clavatum*, one or two biological points which can be inferred from the study of its structure may be referred to, though they can at

[1] Trans. Canadian Institute, 1896–7, p. 273.
[2] Bruchmann, loc. cit., 1885.

present only be suggested as possibilities. The flattened and still more the trough-like form which these older prothalli present may be an adaptation to facilitate fertilization. Whether this be the case or not, it must, in cases where both kinds of sexual organs are present on the upper surface of the same prothallus, serve this purpose by arresting the water percolating through the peaty soil and causing it to run over the surface of the prothallus before sinking deeper. The complete absence of chlorophyll shows that the prothallus is a total saprophyte, and, as is almost invariably the case in the higher plants, a Fungus is found living as a symbiont in the tissues. The few prothalli found did not unfortunately permit of a determination of the relation which the two organisms bear to one another ; such a comparatively simple example of mycorhiza presents a problem of great interest. We may however assume with probability that the Fungus acts in some way as an intermediary in rendering the organic material, absorbed from the soil by the numerous rhizoids, and possibly by the hyphae which extend to the soil, available as food for the prothallus. The appearance of the mycelium in different regions of the prothallus further indicates that at a certain stage the whole of the contents of the Fungus are made use of by its host. Since the young plant is not inhabited by the Fungus, it must be wholly dependent on the prothallus until it reaches the light. This, owing to the depth at which the prothalli grew, does not take place until the plant has attained a considerable size, a fact which may be put in relation with the large foot which the embryo possesses.

Comparative Remarks.

In this portion of the paper an attempt will be made to gather together the facts that are known regarding the gametophyte of *Lycopodium*, and to consider their bearing on the relationship of the species included in that genus to one another and to other groups of Vascular Cryptogams. The full account of the prothallus of a number of European species given by Bruchmann in his recent work has rendered this more possible: but it will be evident that all that can be done at present is to estimate the direction in which the facts appear to point; further investigation both of the sporophyte and the gametophyte is needed before definite conclusions can be drawn.

A natural classification of the Bryophyta and Pteridophyta, in which both sexual and asexual generations attain considerable complexity and more or less complete physiological independence, must present special difficulty. The characters of both generations must be taken into account in ascertaining affinities; and in both, recent adaptations to the conditions of life must be distinguished from long-inherited characters which indicate common descent and the degree of relationship. While the chances of error are increased, however, the additional evidence available in the case of those plants, neither generation of which is rudimentary or extremely reduced, cannot be neglected. In the Pteridophytes the classification has been founded almost entirely on the characters of the highly developed sporophyte, the differences in the sexual generation affording additional evidence in the recognition of the larger subdivisions. The resulting arrangement would in most cases be little affected by taking the characters of the gametophyte further into account, mainly because the latter exhibits such ready adaptability to its environment; while those of its characters which may be regarded as of morphological importance, are for the most part closely similar throughout the large groups.

The genus *Lycopodium* appears, however, to be to some extent exceptional among Vascular Cryptogams, since in it the sporophyte conforms closely to a general type, while great differences exist among the gametophytes. Thus the possible use of the characters of the sexual generation in arranging the species of this genus cannot be overlooked. The examination of an arrangement of the species of *Lycopodium* based on the external characters of the sporophyte, such as that given in Baker's Fern-Allies, shows clearly that some at least of the characters upon which the systematist is forced to depend are not such as render it probable that the groups distinguished by their means will be those expressing common descent. So slight are these differences that it may be said that there is nothing in the external characters of the sporophyte to suggest that *Lycopodium* is not a natural genus, the species of which have come to differ somewhat in habit from one another.

All that need be said here with regard to the geological history of the genus, which is unfortunately imperfectly known, is to point out that there is sufficient evidence to show that even in Palaeozoic times Lycopodineous plants of the general habit and size of *Lycopodium* and *Selaginella* existed along with the larger Lepidodendreae. The comparative anatomy of the stele of the existing species of *Lycopodium* is at present too little known to be available as an indication of relationship. It may be hoped that the investigations which are at present being made on this subject will afford a valuable means of checking the conclusions as to affinity drawn from the study of the gametophytes.

At present our attention will be confined to the evidence afforded by the gametophyte which is now known in *L. cernuum*[1], *inundatum*[2], *salakense*[3], *clavatum*[4], *annotinum*[5], *complanatum*[6], *Selago*[7], *Phlegmaria*[8], *Hippuris*[9], *nummularifolium*[9], and *carinatum*[9]. Before those of *L. complanatum* and *L. Selago*, which have only recently been discovered, were known, three types unconnected by intermediate forms appeared to exist ; these had been termed the *cernuum*, *Phlegmaria*, and *annotinum* types. The further information regarding the earlier stages of the prothalli of *L. clavatum* and *annotinum* given by Bruchmann, and his description of the prothalli of *L. complanatum* and *Selago*, necessitates

[1] Treub, Ann. Jard. Bot. Buitenzorg, iv. p. 107; viii. p. 1.
[2] Goebel, Bot. Zeit., 1887, p. 161. [3] Treub, Ann. Jard. Bot. Buit., vii. p. 141.
[4] Bruchmann, loc. cit., 1898.
[5] Fankhauser, loc. cit.; Bruchmann, loc. cit., 1885 and 1898.
[6] Bruchmann, loc. cit., 1898. [7] Ibid.
[8] Treub, Ann. Jard. Bot. Buit., v. p. 87. [9] Ibid. vii. p. 146.

a reconsideration of the importance to be attached to the types of gametophyte within the genus *Lycopodium.* This has been done briefly by Bruchmann, with whose conclusions, however, the author is unable to entirely agree. He recognizes five distinct types, represented by the prothalli of *L. clavatum* (i), *L. complanatum* (ii), *L. Selago* (iii), *L. inundatum* (iv), and *L. Phlegmaria* (v) respectively, and expresses the opinion that the differences distinguishing them warrant the subdivision of *Lycopodium* into a corresponding number of genera. After enumerating the main characters in which the above types agree and differ from one another, Bruchmann thus sums up his conclusion:—'It follows from the above facts that the groups of *Lycopodium* characterized especially by means of their sexual generation do not stand in close relationship to one another, especially not such as one would expect in species of plants which have found their position together in one genus. This knowledge leads to a separation of the Lycopodiums into groups, or still better into genera, to which it would be quite in place to give new names. There arise as many groups as the sexual generation allows types to be distinguished. Thus, for example, the six European species would be separable into four groups (genera), of which only those of the types i. and ii. are represented by two species each, those of types iii. and iv. by one species each. These still existing [groups of] Lycopodiums, now poor in species, are the much reduced survivors of a family of plants which in earlier time played a prominent part, and the origin of which from a common stock cannot be gainsaid; still, their very considerable differentiations point to a long course of independent evolution, and thus to a separation at a very ancient period [1].'

It must be admitted that this view is a quite possible one; but when all the facts bearing on the question are considered, another mode of regarding the diversity in the sexual generation of *Lycopodium* appears to be more probable. Whether this will prove to be the case or not can only be seen as our

[1] Bruchmann, loc. cit., 1898, p. 108.

knowledge of the life-history is extended to other species, but it appears advisable to state it. In order to do so it will be necessary to briefly discuss the characteristics of the *Lycopodium* prothalli now known. It would be tedious to compare them point by point in every case, but it may be stated that in drawing conclusions the germination of the spore, form and structure of prothallus, position of meristem, position and structure of the sexual organs, and the embryogeny have been taken into account. Further, the results have been put into relation with the habit of the prothallus and that of the sporophyte. Those prothalli which belong to the type of *L. cernuum* will in the first place be considered, and those of the other species afterwards compared with this type.

The prothallus of *L. cernuum*, with which those of *L. inundatum* and *L. salakense* agree in all important respects, is a small body of cylindrical form which grows upright on the surface of the soil. On the germination of the spore an oval or spherical mass of tissue (the primary tubercle) is formed, from the surface of which one or more short filaments originate. By the further development of one of these the cylindrical portion of the prothallus is formed. Sometimes the primary tubercle can be recognized at the base of the cylindrical portion of a prothallus which has attained its full size. In other specimens these two regions are not to be distinguished on external examination. The latter state of affairs is the usual one in *L. inundatum*, while in the other two species the tubercle is as a rule recognizable. The merismatic region of the young prothallus is not at first localized. Ultimately, however, a zone of merismatic tissue can be distinguished extending completely round the upper part of the cylindrical portion of the prothallus, but not terminal. As the cells of the zone become converted into permanent tissue, they contribute on the lower side to the growth of the cylindrical portion; while above the meristem green expanded lobes are formed, at the bases of which the sexual organs are situated. These lobes may be well-developed structures, as in *L. cernuum* and *L. inundatum*, but in *L. salakense* they are rudimentary or entirely absent. Thus, in the fully developed prothallus of this type, three regions can be more or less clearly distinguished: the primary tubercle, the cylindrical portion, and the terminal region bearing the sexual organs and assimilating lobes. Since development proceeds from a zone between the two latter regions, the youngest lobes and sexual organs are to be found towards the periphery next the meristem.

The structure of the prothallus is very simple, and exhibits little differentiation of tissues. Chlorophyll is most abundantly present in the terminal lobes and the upper portion of the cylindrical part, while the rhizoids are borne on the lower portion of the latter. The more internal cells are somewhat longer than those of the superficial tissue, but are not markedly elongated in the direction of the longitudinal axis. An endophytic Fungus is almost constantly present; this occupies the cavities of the outer cells of the primary tubercle, and extends between the internal cells of this region and into the cylindrical portion. Both antheridia and archegonia take origin from a single superficial cell. The former hardly project from the surface of the prothallus, and the free portion of their wall presents a triangular cover-cell. The spermatozoids developed from the large mass of spermatocytes are biciliate. The mature archegonium consists of a short projecting neck of four rows of cells, and of the central series of ovum, ventral canal-cell, and a single neck canal-cell.

The embryo at an early stage consists of the suspensor, which usually remains unicellular, and of two tiers of cells borne on this. The small foot proceeds from the whole of the tier adjoining the suspensor; the terminal tier gives origin to a small tuber-like structure (the protocorm), which becomes attached to the soil by numerous rhizoids, and to the first leaf. As the protocorm develops, other leaves of simple form and structure are produced from it. Not until a number of these have appeared is the apex of the stem differentiated; this grows into the leafy shoot of the *Lycopodium* plant, the first root arising exogenously from the protocorm near to it.

There are several reasons which appear to indicate that the type of prothallus, the main characters of which, as described by Treub and Goebel, have been summarized above, is in some degree a primitive one. Whether it is to be regarded as primitive in relation to the other types of *Lycopodium* prothallus is a further question that will be considered later. In the first place the prothalli of the *L. cernuum* type possess chlorophyll, and are thus capable of assimilation, though the presence of an endophytic Fungus probably indicates that some degree of saprophytism is possible in addition. But more direct evidence is supplied by the form of the young sporophyte. The parts of this which have been mentioned, viz. protocorm, leaves borne on the protocorm, leafy shoot and exogenous first root, afford when taken together characteristic marks which may fairly be used for phylogenetic purposes. They are repeated exactly, not only in all young plants arising from the fertilized ovum, but in those which originate vegetatively. These are known in the case of *L. inundatum* to arise from leaves separated from the young sporophyte, and in *L. cernuum* from the 'root-tubercles,' the further development of which agrees with that of a young protocorm. Further, a close correspondence can be traced between the development of plants of *L. cernuum* from the root-tubercles and the vegetative propagation of *Phylloglossum Drummondii* [1], which may on other grounds be regarded as a primitive form. Even in the absence of any information as to the gametophyte in this genus, this resemblance affords important evidence that the form of the young plant of *L. cernuum*, &c., is not to be regarded as recent and adaptive, but as possessing an important phylogenetic bearing.

The prothalli and young plants of *L. Selago*, *complanatum*, and *clavatum*, and those which present the type of *L. Phlegmaria*, may now be compared with the *L. cernuum* type. It will be convenient to deal in the first place with the external form, sexual organs, &c.; then to consider the differences of anatomical differentiation; and lastly to compare

[1] Bower, Phil. Trans., 1885, p. 675.

the embryos and young plants. The question upon which this comparison may throw light may be stated at the outset. All the other *Lycopodium* prothalli known differ considerably from the *L. cernuum* type. Is there anything to indicate that they have been derived by modification of a form of gametophyte similar to that of *L. cernuum*, or do their characters rather suggest that, owing to the similarity of the mature sporophytes, a number of groups of species derived from independent stocks of ancient Lycopodiaceous plants have been grouped together in the genus *Lycopodium*? The latter conclusion, which practically amounts to regarding the genus as an artificial one, is that at which Professor Bruchmann arrives. It may be as well to add that this is distinct from the question whether the differences in the gametophyte are such as to justify the separation of the species of *Lycopodium* into several *closely related* genera.

The prothallus of *L. Selago* may be taken first, since it is in several respects less specialized than those of the other species. In form it may resemble the *L. cernuum* type, being a short, upright cylindrical body, growing by means of a merismatic zone beneath the terminal region on which the sexual organs are borne. Being as a rule situated a short distance below the surface of the soil, this prothallus is devoid of chlorophyll, which is, however, developed in considerable amount on natural or artificial exposure to light. Leafy expansions are absent from the terminal portion, which may be distinguished as generative in distinction to the lower vegetative half. The germination of the spore is not known, but no distinct region comparable to the primary tubercle of *L. cernuum* is recognizable in the fully grown prothallus; in uninjured specimens this terminates below in a conical point, the structure of which may in some respects be compared with the primary tubercle. Besides this form of prothallus, the resemblance of which to the *L. cernuum* type will be evident, elongated cylindrical forms which originate by growth becoming localized in one portion of the merismatic zone are found. The interest of these will be seen in connexion with the *L. Phlegmaria* type of prothallus. As in *L. cernuum*, the sexual organs of *L. Selago* are confined to the terminal region, the youngest being found nearest to the merismatic zone. Associated with them, however, are found numerous hairs (the paraphyses) consisting of a single row of cells; these structures, which are known in all the saprophytic *Lycopodium* prothalli, are not found in those of the *L. cernuum* type. The sexual organs themselves do not appear to differ fundamentally from those of that type, the antheridia being closely similar, while the archegonium has a slightly longer neck, the canal being occupied by a central series consisting of the ovum and not more than six canal-cells.

Thus as regards external form and sexual organs, the prothallus of *L. Selago* might reasonably be regarded as having originated by modifications in relation to its wholly saprophytic mode of life from a type of gametophyte resembling that possessed at the present day by *L. cernuum*, &c. The prothalli of the other European species, which are all subterranean saprophytes, present greater differences in these respects, which can be mentioned more briefly. Those of *L. clavatum* and *annotinum* are, in a young stage, not unlike that of *L. Selago*. As the activity of the merismatic zone between the vegetative and generative halves proceeds, however, the upper portion of the prothallus becomes greatly extended horizontally, the conical portion of the young prothallus appearing as an insignificant projection from this flattened part which later becomes more or less irregularly folded. The description in the earlier part of this paper will show the similarity of arrangement of the sexual organs in this species to that in *L. Selago*. It must be added to this, however, that paraphyses develop when the prothalli are grown at the surface of the soil; under such circumstances chlorophyll is also formed. It has also been seen that the increased length of the archegonium-neck in this species is mainly due to a growth of surrounding cells; the central series consists of ovum and seven canal-cells.

The prothallus of *L. complanatum* (with which that of

L. alpinum appears to correspond closely) does not become extended horizontally in this manner. It maintains an elongated conical shape, and the strongly convex generative region bears antheridia resembling those of the other species, and archegonia which have still longer projecting necks than those of *L. clavatum.* Here also the true neck is not very long, and the increased length is almost entirely due to its being elevated upon a 'false neck' of considerable height. Besides the ovum, 8–14 canal-cells are present.

There is considerable uniformity in the appearance of the prothalli of the other tropical species at present known, *L. Phlegmaria*, *carinatum*, *Hippuris*, and *nummularifolium*, the main differences being in the thickness of the cylindrical branches, of which they consist. These ramify in all directions through the dead bark in which the prothalli grow. Thicker branches, on the upper surface of which the sexual organs are situated, are also found. This form suggests comparison with the modification of the prothallus of *L. Selago* referred to above, which was seen to arise by the localization of growth in one part of the merismatic zone. The early stages of the *L. Phlegmaria* prothallus are not known, and evidence is wanting to show whether they ever are radially symmetrical with a merismatic zone; but the growth of the branches which bear the sexual organs shows a distinction of upper and lower surfaces comparable to that seen in *L. Selago.* The sexual organs themselves, which are accompanied by well-developed paraphyses, also resemble those of the latter species, the projecting archegonium-neck being entirely a product of the outer segment of the archegonium-mother-cell; the central series consists of ovum and 3–5 canal-cells.

It will be evident from the above description of the external form and the sexual organs of the known *Lycopodium* prothalli that they cannot on these grounds be separated into types unconnected by intermediate forms. The similarity in ground-plan of the prothalli would appear rather to indicate that they are all more or less profound modifications of a type not unlike that of *L. cernuum.* The two forms of prothallus found in *L. Selago* give the clue to the more specialized saprophytic types which, in the deeper-growing subterranean species, retain the radial symmetry while becoming modified in shape. On the other hand, the type of prothallus growing in rotting wood has lost the radial symmetry, and consists of cylindrical but more or less clearly dorsiventral branches. The variability of several characters, such as the presence or absence of leafy lobes, the distinctness of the primary tubercle, and the passage from radial to dorsiventral symmetry within the limits of the *L. cernuum* type, when taken in conjunction with the varieties of form of the prothallus of *L. Selago*, appears to justify such a view as that suggested above. The differences between the archegonia in the saprophytic forms are all such as suggest later modifications rather than deep morphological distinctions.

The importance to be attached to the differences in anatomical differentiation in the vegetative regions of these prothalli has now to be considered. The generative half of the prothallus consists in every case of a parenchymatous tissue into which the endophytic Fungus does not penetrate. In *L. cernuum*, &c., the lower half has been seen to be almost equally undifferentiated save by the distribution of the endophyte, which occupies the cavities of the outer layer of cells of the primary tubercle and penetrates between the cells of the internal tissue. In the case of all the prothalli in which the oldest region adjoining the spore is known, the endophyte is present in the limiting layer of cells of this region only ; as suggested above this may be regarded as the representative in these prothalli of the distinct primary tubercle of *L. cernuum* and *salakense*. In the vegetative portion of the prothallus of *L. Selago*, which has been seen to approach those of the *L. cernuum* type most closely in external form, the structure is correspondingly simple. The cells of the limiting layer, with the exception of those prolonged as rhizoids, are free from Fungus. This is found within the cells of a broad band of tissue internal to this, the cells of which show no alteration of their form. This tissue surrounds a central strand of elongated cells which broadens out above where it is continuous with the cushion of the generative half. In this prothallus the endophyte is never found between the cells, and the reserve materials are stored within the cells occupied by its hyphae. The differences presented by the other subterranean prothalli almost entirely concern the band of tissue in which the endophyte is found; in all of them there is a central region of more or less elongated cells, and the external limiting layer is free from the mycelium. In *L. complanatum* a few layers of oval cells containing the Fungus within their cavities are present within the latter, while between them and the central strand is found a single layer of cells enormously elongated at right angles to the surface; the mycelium extends between these cells, but is absent from their cavities which contain reserve materials. The differentiation of the corresponding region of the prothallus of *L. clavatum* has been described in the earlier part of this paper. In this type several layers of oval cells with intracellular mycelium are found within the limiting layer, then a single layer of palisade-cells also containing the endophyte, while next to the central tissue is a broad band of smaller cells stored with reserve materials between which the hyphae extend. Finally, in the case of the prothalli of the *L. Phlegmaria* type, the endophyte is absent from the outermost layer, but is found in the cavities of most of the cells within this; in the thicker branches a central strand of elongated elements can be recognized. This simplicity of structure, which is associated with the thin cylindrical branches of which these prothalli consist, finds its nearest analogue in the much thicker dorsiventral branches of the prothallus of *L. Selago*, the tissues of which are, however, more sharply differentiated.

In considering these structural differences it must be borne in mind that they concern the vegetative region of the prothallus, those tissues which there is reason to believe are occupied in the assimilation of the absorbed plant-food and its storage in the form of starch and oil. Our ignorance of the physiological processes taking place in such organisms as these renders any explanation of the differences in the arrangements of tissue in the several species at present impossible; but as regards their morphological value, they may fairly be considered as comparable to variations in the details of arrangement of the assimilating tissue of leaves in closely related plants. The furthest degree of separation of the types that they justify us in assuming is that these types represent independent modifications of less differentiated prothalli, something like those of *L. cernuum*, which have enabled them to become wholly saprophytic. Such close similarity in the arrangement of the various layers as is seen in *L. clavatum* and *annotinum* on the one hand, and in *L. complanatum* and *alpinum* on the other, probably indicates comparatively recent origin of these pairs of species from ancestors the prothalli of which were already adapted to saprophytic life. The close relationship here indicated by the gametophyte is borne out by the similarity of the sporophytes of the species named. With regard to the *L. Phlegmaria* type of structure, the most probable view would appear to be that, along with the reduction in thickness of the branch, the structure had remained or become greatly simplified. The dorsiventral cylindrical branches of the prothallus of *L. Selago* afford a suggestion of how such a state of things may have come about, whether the *L. Selago* type be regarded as in any way related to that of *L. Phlegmaria* or not. It will be evident that if the complexity of structure of some of the saprophytic forms of *Lycopodium* has arisen as an adaptation to the mode of life, it cannot be regarded as evidence of the descent of the *Lycopodium* gametophyte from highly differentiated ancestors as Bruchmann suggests[1]. The similarity of the regions into which the tissues harbouring the mycelium are divided in the *Lycopodium* prothalli to those which Janse[2] has been able to distinguish in the roots of many plants containing a mycorhizal Fungus, affords considerable support to the point of view advocated above.

[1] Loc. cit., 1898, p. 111. [2] Ann. Jard. Bot. Buit., xiv. p. 53.

Lastly the species of *Lycopodium* with saprophytic prothalli must be compared with the *L. cernuum* type in respect to their embryology, before the attempt is made to estimate the phylogenetic significance of all the resemblances and differences that have been mentioned taken together.

A well-developed protocorm is as yet known only in those Lycopods the prothalli of which belong to the *L. cernuum* type, though indications of it have been recognized by Treub in some of the young plants of *L. Phlegmaria*. It is thus confined to those species whose prothalli grow on the surface of the soil, with which the young plant soon comes into direct relation by means of the rhizoids of the protocorm, and thus early becomes independent of nutritive supplies from the gametophyte. In all the other cases known as yet, the prothalli being buried in the soil or in rotting wood, the young plants have to attain a more or less considerable size before they are capable of assimilation and become independent of the reserve materials in the prothallus. The larger size of all the saprophytic prothalli may be put in relation with this need. The very young embryo in all the Lycopods of which the embryology has been followed in detail, exhibits a stage in which it consists of a suspensor and two tiers of four cells each. Slight differences in the succession of the divisions by which this result is brought about are known, the two tiers being sometimes separated by the second division in the cell from which the embryo will be formed, in other cases by the third; little importance can, however, be attached to such a distinction when the similarity of the result is borne in mind. From the whole of the tier of cells adjoining the suspensor the foot is derived in all cases. In *L. Phlegmaria* and the other species with this type of prothallus, the foot remains comparatively small, though much larger than the same structure in *L. cernuum*. From the terminal tier there originate the stem-apex, the first leaf, and the first root; in some examples, as mentioned above, the appearance of the root is delayed, and a rudimentary protocorm can be recognized. By the elongation of the hypocotyledonary stem the first leaf is brought to the light, and is capable of carrying on the work of assimilation ; it is almost immediately followed by the second leaf, which is apparent before the embryo has broken through the prothallus. The embryology of *L. Selago* agrees in detail with that of *L. Phlegmaria*, save in the period of formation of the wall separating the two tiers ; no appearance of a protocorm is recorded for this species.

The main differences then between the embryology of these species with saprophytic prothalli, which grow a short distance below the surface of the substratum, and that of the *L. cernuum* type, are accounted for by the absence of a protocorm. The reasons given above in favour of the protocorm being a primitive organ, taken together with the similarity between the *L. Selago* prothallus and those of the *L. cernuum* type, support the view that this stage has been omitted from the development of these young plants. Some direct evidence in favour of this is further afforded by the occurrence of a rudimentary protocorm bearing rhizoids in *L. Phlegmaria*[1]. While the absence of a protocorm stands in relation with the subterranean habit of the prothallus, the comparatively small foot must be put into relation with the fact that the first leaf is able to reach the light, and by its assimilation renders the young plant independent of the prothallus.

There remains for consideration the type of embryology presented by *L. clavatum*, *annotinum*, and *complanatum*, which all agree in this respect. The early segmentation is the same as in *L. Selago*, and here also the suspensor and two tiers are recognizable. The stem-apex, first leaves, and first root originate from the tier furthest from the suspensor ; this, however, remains for a considerable period as a small-celled meristem in which no differentiation of members is apparent. Before this takes place, the foot, derived from the middle tier, has undergone great enlargement so that the embryo for a time is almost spherical. With the appear-

[1] Treub., Ann. Jard. Bot. Buit., viii. p. 32.

ance of the rudiments of the members of the young plant in the region derived from the terminal tier, another difference of detail is manifest. For in this case the apex of the stem is first apparent, followed by a pair of leaf-rudiments the position of which is not constant; these are succeeded by a second pair at right angles to the first. Later the first root appears. These differences seem to stand in relation to the depth at which the prothallus grows. The young plant, on account of this, is dependent on the prothallus until it has attained a much greater size than in *L. Selago* and *L. Phlegmaria.* This explains the need for the large spherical foot with its special absorbent layer. Further the arrangement of the first leaves covering the apex serves the purpose of protecting the latter on its way to the surface.

The known forms of *Lycopodium* prothallus having thus been compared with one another, the evidence which the resemblances and differences between them afford as to the relationship of the species of that genus must now be considered. In doing this it is necessary to bear in mind that the problem is complicated by the need of adaptive modifications of the prothalli to the diverse conditions under which they live. In the preceding pages the relation that appears to exist between the conditions of existence and particular characters of the prothallus and young plant has been repeatedly referred to. The importance to the species in the Vascular Cryptogams of satisfactory adaptation of the gametophyte to its environment can hardly be overestimated. For the establishment of the sporophyte in a new situation is entirely dependent on the gametophyte having been able to come to maturity and produce sexual organs, so that the localities of the plants are determined more by their suitability for the gametophyte than for the sporophyte, except in so far as the latter is spread by vegetative propagation. Since the struggle for existence tells largely upon plants in their attempts to seize upon fresh situations, it becomes all-important in the case of a Vascular Cryptogam that favourable modifications should take place in the prothallus. It is thus on *a priori* grounds quite comprehensible that a genus of this group, which is known to have had a long geological history, might retain the spore-bearing generation but slightly modified, and owe its survival almost entirely to the adaptations of the gametophyte to new conditions. The uniformity of the sporophyte in the genus *Lycopodium* suggests that this has actually been the case with regard to it, and, as has already been shown, the main differences between the prothalli can be put into relation with the conditions under which they live. These considerations appear to lead to some such view of the relationship of the species of *Lycopodium*, of which the life-history is known, as the following.

In the *L. cernuum* type the primitive form of prothallus and young plant has been most completely retained. From forms like these others have been derived, in which the gametophyte is adapted to the saprophytic mode of life. In all of these the protocorm is undeveloped, and changes in form of the prothallus, &c., have taken place. In those in which the first leaf is able to reach the light, whether the prothallus grows in rotting wood or just below the surface of the soil, the chief difference in the embryology is the absence of a protocorm, and the foot remains small. Under this head come the *L. Selago* type and the *L. Phlegmaria* type which may be looked upon as somewhat similar independent derivations from the primitive form. Further, several types of prothallus adapted to life at a considerable depth below the surface have probably arisen from the primitive type independently of one another. In the embryos of these the foot has attained much greater development and the first leaves arise in pairs protecting the apex. This includes the *L. clavatum* and *L. complanatum* types which differ mainly in the structure of the prothallus. On this view several biological types would be recognized that may or may not characterize related groups of species.

If the habit of the sporophytes, the prothalli of which are known, be now taken into consideration, a further confirmation of this view will be obtained. For of the twelve species the three which present the *L. cernuum* type, are terrestrial plants not specially attached to soil containing humus. The plants of the *L. Selago, complanatum,* and *clavatum* types of prothallus live for the most part on moors or in open woods, places where the soil is rich in humus. The rarity of young plants, unless in exceptional circumstances due to human interference, is an index of the difficulty encountered before the spore can succeed in developing into a prothallus, a disadvantage only compensated by the capacity for vegetative reproduction of the sporophyte by a creeping habit or by means of bulbils. Lastly, all the species which possess the *L. Phlegmaria* type of prothallus are epiphytes.

It would be strange if the genetic affinities of the species of *Lycopodium* were found to coincide exactly with these biological divisions. More probably when all the facts are known such a parallelism will be found to be disturbed by instances of independent adaptation to similar conditions. Only the investigation of the remaining species can confirm or disprove the correctness of this mode of regarding the differences of the gametophyte and young plant ; it appears to the author to be indicated on the imperfect data we at present possess. Until further facts are available its general discussion as one possible interpretation of the facts will be sufficient. It follows from such a view that, while a complete critical study of the gametophyte may afford valuable assistance in determining affinities within the genus *Lycopodium*, the characters of the prothallus cannot be safely taken by themselves, since the variations in them have been shown to stand in such close relation to the conditions of life, and especially to the saprophytism of certain species. The characters of the less modified sporophyte must be used to check those derived from the gametophyte and should have greater weight. On these grounds it seems inadvisable to subdivide the genus *Lycopodium* as suggested by Bruchmann.

It may be hoped that the discovery of the prothallus in *Phylloglossum*, *Psilotum*, and *Tmesipteris* will cast further light on the relationship of the existing Homosporous Lycopodiaceae. But any serial relationship between the few surviving groups is highly improbable. This is at least equally the case when *Lycopodium* and *Selaginella* are compared, where such direct connexion is frequently assumed to exist. While these two genera are rightly grouped together, there appears to be no sufficient evidence for the view that they constitute a direct series. In connexion with this, Bruchmann justly points out the 'far-reaching separation' between them. With that author's further conclusion, I am however unable to agree. He says, 'according to such comparison of the two families of *Lycopodium* and *Selaginella*, their mutual relationship does not appear to be a close one, and in my opinion the distinction between the Selaginellas and the Gymnosperms would be less than that between the Selaginellas and Lycopodiums[1].'

The relationship of *Lycopodium* and *Selaginella* is none the less clear because the connexion lay far back in the history of the Lycopodiaceae. For all that we know to the contrary, these two genera may have persisted with but slight modifications since Carboniferous times, having escaped the extinction which was the fate of all the larger forms. That the Coniferae arose from some heterosporous forms, whether Pterictophyta or more primitve Gymnosperms, about that period may be regarded as probable; but little or nothing points to a close relationship between *Selaginella* and that group, though the life-history of the former aids us in picturing the passage-forms from the Vascular Cryptogams to the Conifers.

The only other group of Vascular Cryptogams that need be referred to is the Ophioglossaceae. Without entering into the general question of the relationship between that family and the Lycopodiaceae which has been suggested[2] on grounds afforded by a detailed comparison of the sporophyte, it may be pointed out that the fuller information regarding

[1] Loc. cit., 1898, p. 110.

[1] Bower, Studies in the Morphology of Spore-producing Members: II. Ophioglossaceae. London, 1896.

the gametophyte recently obtained in both groups does not afford support to such a common origin. For the resemblance between the prothallus of *Lycopodium clavatum* for instance and that of *Botrychium virginianum*[1], which appears at first very striking, seems to be entirely homoplastic and to stand in relationship with the subterranean saprophytic life. It is in form and texture, position of the sexual organs, and the large foot of the embryo, that the likeness exists ; while the prothalli differ in symmetry, in the details of the sexual organs, in the spermatozoids, and in the embryology, points which when taken together must be regarded as of great weight. The resemblances between the prothalli of these two plants are of interest, as indicating how an appearance of affinity may follow from the modifications related to subterranean life of the prothallus, and afford an additional reason why the species of *Lycopodium* which possess similar saprophytic prothalli should not, on that evidence alone, be regarded as closely related and forming a sub-genus.

The differences between the prothalli of the great groups of Vascular Cryptogams are indeed so striking, even when we confine the comparison to those which live in similar situations, as to be quite in accord with an independent origin of all those groups from Algal or Bryophytic forms. Within any one group, and especially within a narrow circle of affinity such as the genus *Lycopodium*, aid may doubtless be obtained from a consideration of the characters of the gametophyte. The critical attitude which has been taken up in the preceding pages towards the immediate use of the gametophyte for the purpose of subdividing the genus *Lycopodium*, is not intended to imply any doubt of the value of such characters, but to contribute to the separation of homoplastic from homogenetic resemblances, which must form the preliminary to any such use of the gametophyte in groups where it has become adapted to several different kinds of environment.

[1] Jeffreys, loc. cit.

EXPLANATION OF FIGURES IN PLATES XVI AND XVII.

Illustrating Mr. W. H. Lang's paper on The Prothallus of *Lycopodium clavatum*, L.

PLATE XVI.

Fig. 1. Photograph of a prothallus of *Lycopodium clavatum* and of a young plant free from the prothallus. Natural size.

Fig. 2. The smallest prothallus found, seen from above. × 7.

Fig. 3. Prothallus bearing numerous antheridia on ridges just within the lateral margins: *a* from the side, *b* from above. × 7.

Fig. 4. The largest prothallus found, seen from the side. The two ends are in contact. × 7.

Fig. 5. Part of a transverse section of the prothallus in Fig. 3 including the margin: *a–f*, the layers of tissue described in the text; × position of the marginal meristem. The shaded cells are those within which the endophytic Fungus was present. × 80.

Fig. 6. Lower portion of a similar section of the same prothallus including cells of the layers *a*, *b*, *c*, and *d*. × 375.

Fig. 7 *a*, *b*. Median sections through the ends of a prothallus to show the difference of outline that may exist between them, and for comparison with sections across the lateral margin: × position of meristem. × 25. In this and in the other outline figures of sections through the prothallus (Figs. 8, 9, 25 and 26) the darker shading indicates the region in which the mycorhizal Fungus is intracellular, the lighter shading the layer in which it is intercellular.

Fig. 8. Vertical section across the margin of the prothallus in Fig. 4 to show the marginal ridge and succession of archegonia. × 35.

Fig. 9. Similar section of the prothallus in Fig. 3 showing the succession of antheridia on the marginal ridge. × 35.

Figs. 10–12. Stages in the development of antheridium; the cells which give rise to the spermatozoids are shaded. × 530.

Fig. 13. Longitudinal section of an almost mature antheridium. × 375.

Fig. 14. Transverse section of an almost mature antheridium. × 375.

Figs. 15, 16. Arrangement of cells forming the free part of the antheridium-wall. The triangular cell in the centre has broken down to allow of the escape of the spermatozoids. × 375.

PLATE XVII.

Figs. 17–19. Stages in the development of archegonium. × 375.

Fig. 20. Longitudinal section of a mature archegonium. × 375.

Fig. 21. Similar section of a mature archegonium in the development of which the divisions in the generative layer forming the 'false neck' are seen to extend to adjoining cells on the right-hand side of the figure. × 375.

Fig. 22. Transverse section of the neck of an opened archegonium. × 375.

Fig. 23. Prothallus bearing a young plant, seen from the end. × 7.

Fig. 24. Prothallus, the irregular shape of which is probably the result of an injury, bearing two young plants of similar age; from above. × 7.

Fig. 25. Section of one of the young plants in Fig. 24 in the median plane of the root. × 25.

Fig. 26. Similar section of the young plant in Fig. 24 in a plane at right angles to that of the root. × 25.

Figs. 25 and 26 demonstrate the absence of the protocorm; in both the extent of the absorbent layer of the foot is indicated by shading.

Fig. 27. Portion of the foot of one of the young plants in Fig. 24, showing the absorbent layer of cells bounding it; these are seen to be densely filled with protoplasm. × 200.

Fig. 28. Section through the region of attachment of the young plant in Fig. 23 to the prothallus in the plane of root and shoot. The tissue of the prothallus is uniformly shaded. × 80.

Figs. 29, 30. The region of attachment to the prothallus of two young plants to show the differences in form and position of the foot. × 7.

Fig. 31 *a*, *b*, *c*. Stages in the origin of the 'multinucleate vesicles' from the ends of hyphae of the mycelium within the cells of the prothallus. × 1,000.

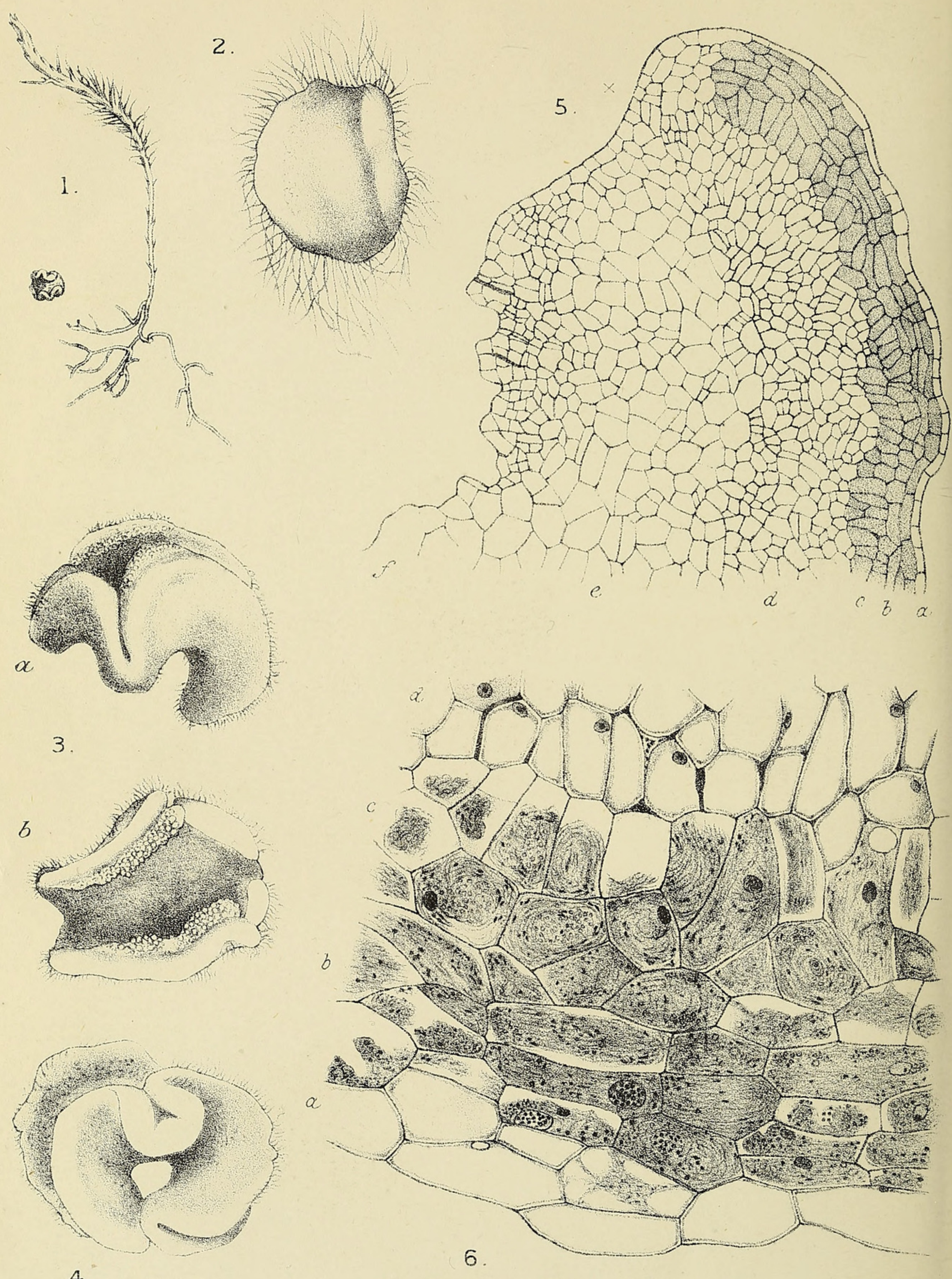
Fig. 32. Cell from layer *b* of the prothallus, showing a multinucleate vesicle, close to which the nucleus of the cell is seen presenting a healthy appearance. × 1,000.

Fig. 33. Cell from the same layer, showing coarser hyphae, two of which are seen to expand into multinucleate vesicles. × 1,000.

Fig. 34. Older cell from the same layer, showing the disorganized mycelium in which bodies resembling spores are visible. × 1,000.

Fig. 35. Basal portion of a rhizoid in optical section, showing two hyphae passing into the cavity of the rhizoid from the tissue beneath. × 375.

Fig. 36. Section through the basal portion of a rhizoid, showing the entrance into its cavity of two hyphae which receive short tubular sheaths on passing through the wall. × 750.



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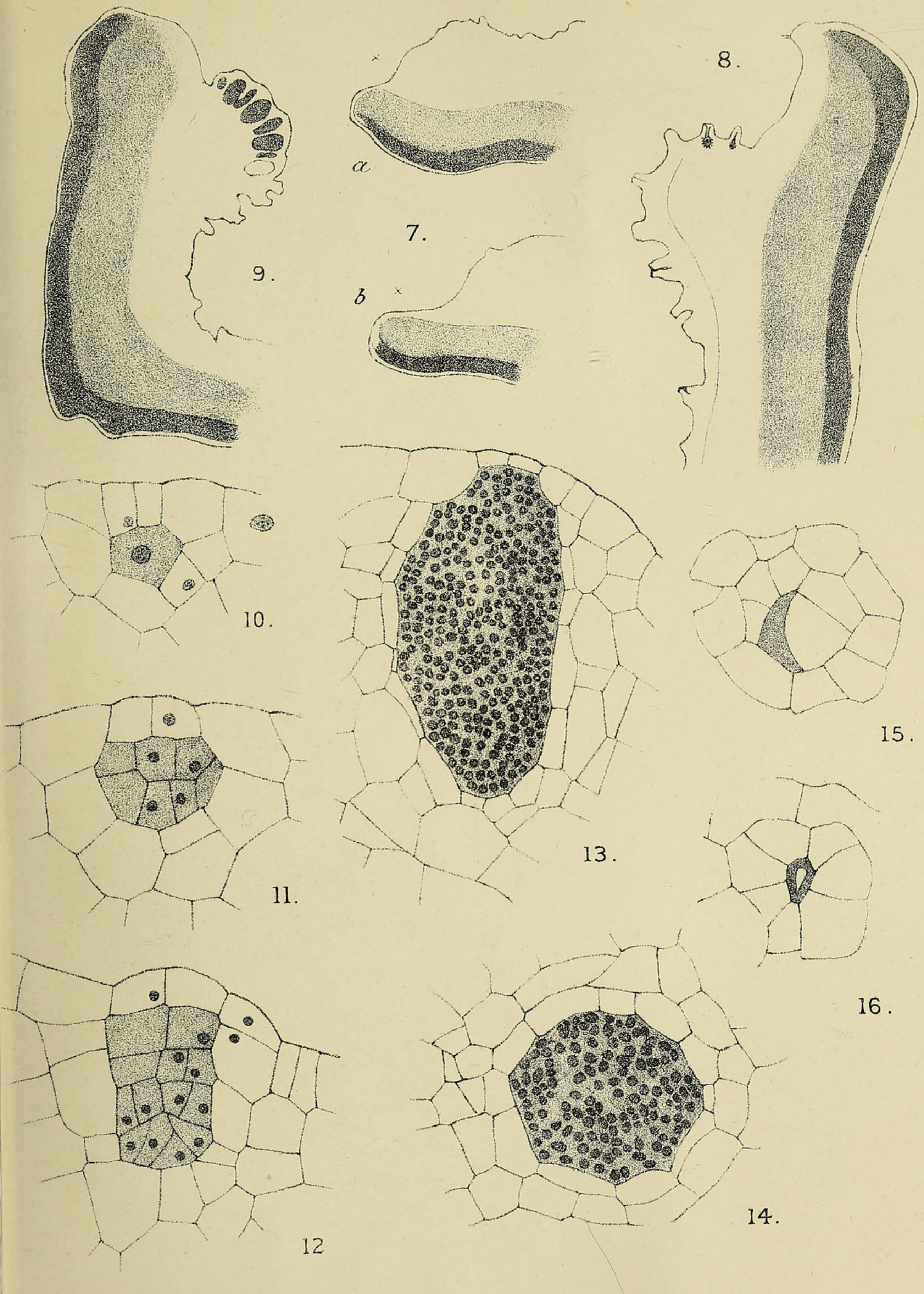

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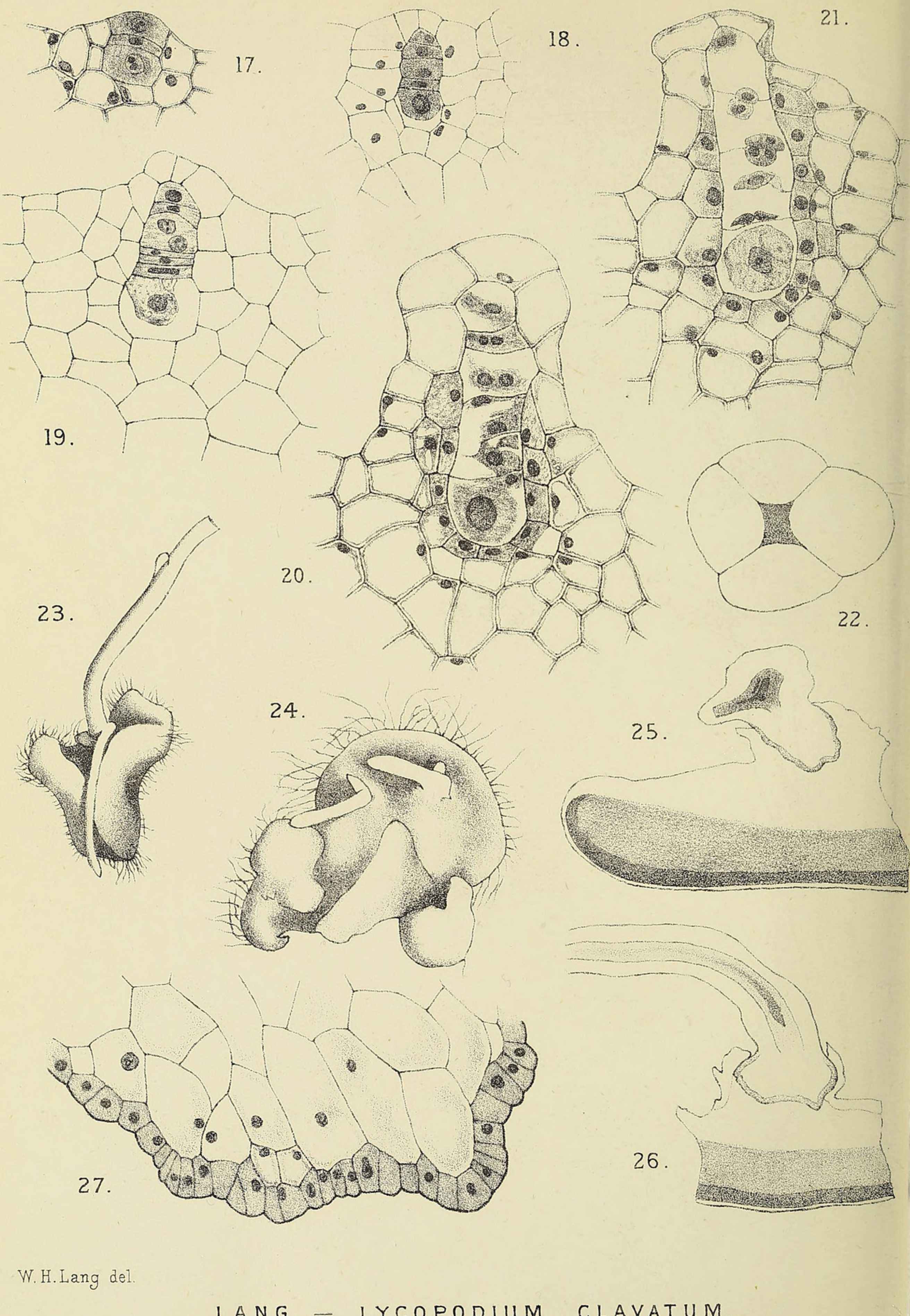

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LANG. — LYCOPODIUM CLAVATUM.

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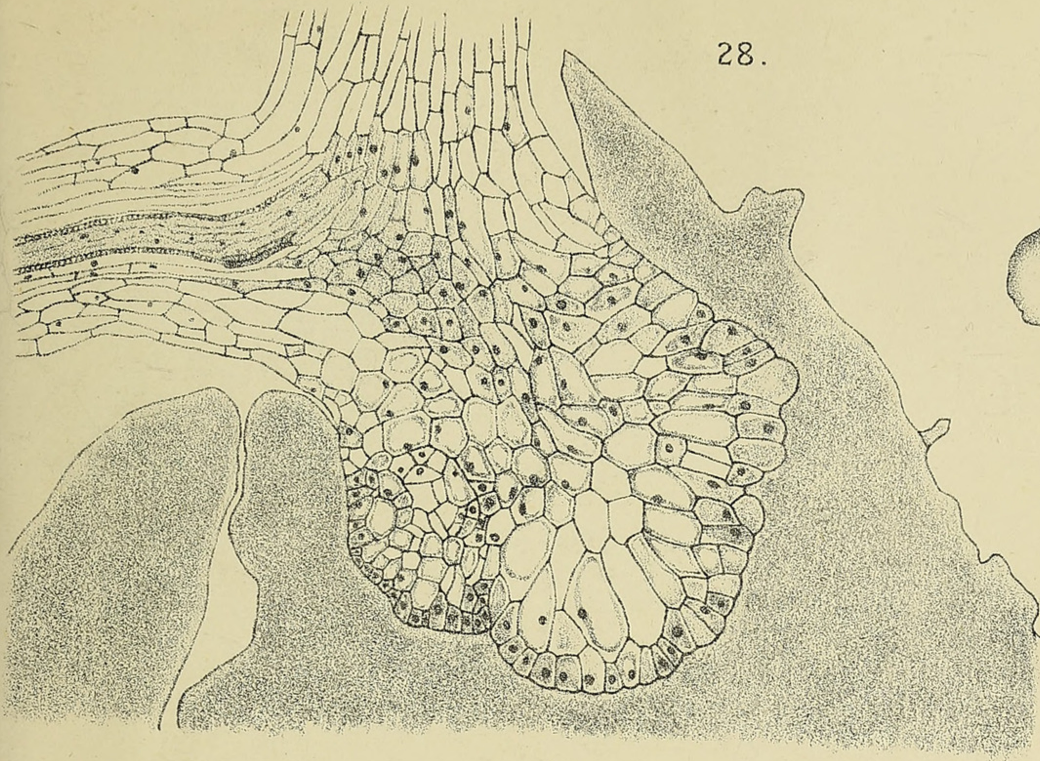

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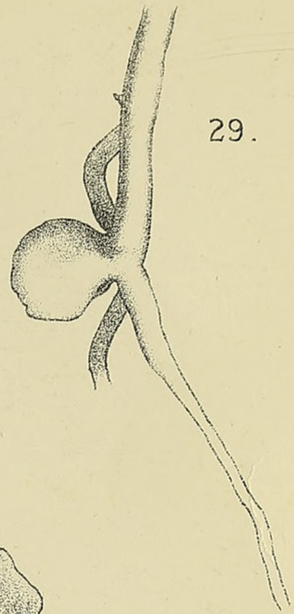

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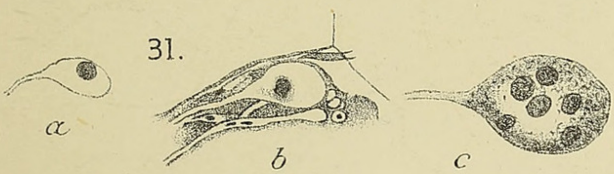

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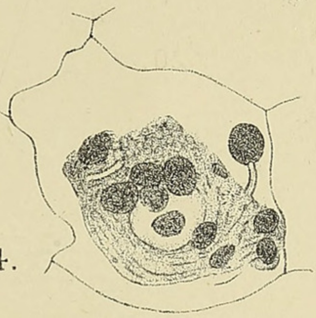

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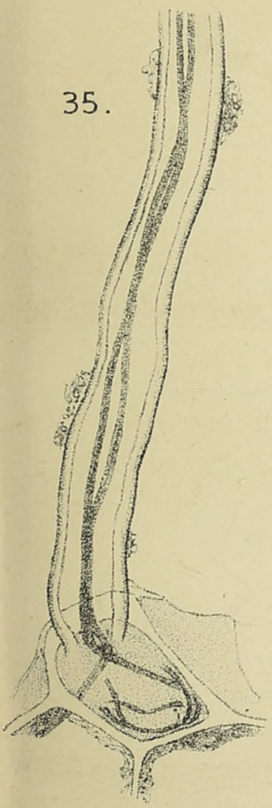

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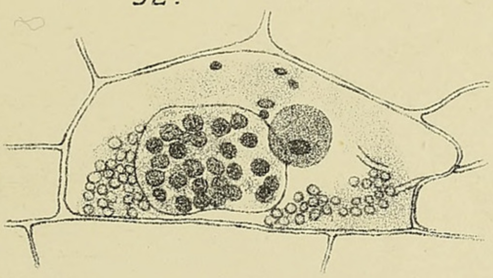

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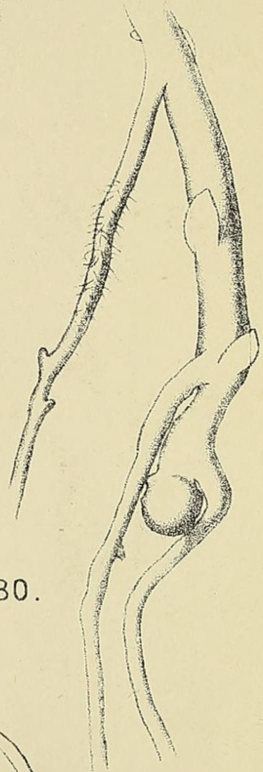

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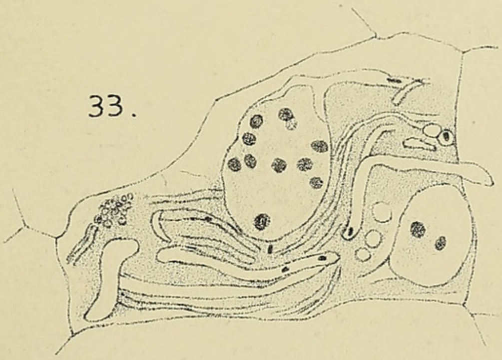

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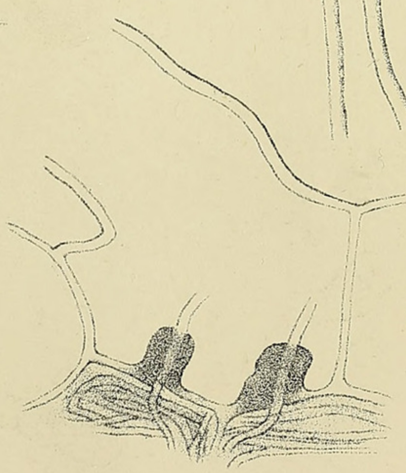

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