

On the Sporogonium of *Notothylas*.

BY

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With Plate XXI.

THE only indication of direct relationship between the Anthocerotaceae and any other group of Liverworts has been afforded by Leitgeb's¹ study of the sporogonium of *Notothylas*. This extended and careful investigation was carried out with special thoroughness so far as the material of a number of species allowed, and Leitgeb's observations must be given full consideration, even if regarded as insufficient to support the view of a genetic connexion between the Anthocerotaceae and the Jungermanniaceae, which he only put forward tentatively. Recent researches on satisfactory material of *Notothylas orbicularis* have shown that the well developed columella of this species is not (as Leitgeb had suggested was the case throughout the genus) due to secondary differentiation within the sporogenous tissue, but is defined by the first periclinal divisions in the embryo, as in other Anthocerotaceae. The lack of agreement between the facts of development in this species and the conclusions of Leitgeb leaves us in a state of uncertainty on a number of points which can only be cleared up by the reinvestigation of other species. The present paper is a contribution towards this.

A brief outline of the progress of investigation and opinion on the subject will serve to make the points at issue clear. *Notothylas* as founded by Sullivant² was described as having a columella in its small capsule. Shortly afterwards another form was found by Milde³ in Germany, and, mainly on the absence of a columella, was placed by him in a new genus as *Chamaeceros fertilis*. Gottsche⁴ in 1858 critically discussed the question and collected and grouped the known species. He found that many capsules of Milde's plant possessed a columella, and did not regard it as even specifi-

¹ Untersuchung über die Lebermoose, Heft v, 1879.

² Musci and Hepaticae of the United States, East of the Mississippi River. New York, 1856.

³ Nova Acta, xxvi, 1856, p. 167.

⁴ Bot. Zeit., 1858, Beilage.

cally distinct from *N. valvata*, Sull. He explained the absence of a columella as due to the latter sometimes separating into its constituent cells. In the following year Milde¹ reinvestigated the genus, and (though his drawings do not appear to bear this out) stated that the breaking down of the columella took place sooner or later in every case. The work of these earlier investigators, which was carried out from the systematic standpoint by the dissection of dried material, resulted in the recognition of the fact that a columella might or might not be demonstrable in the mature capsule of any of the species.

The work of Leitgeb², since it took the development of the sporogonium into account, was on another plane, but was hampered by the lack of young stages. He had fresh material of *N. fertilis*, and herbarium material of *N. valvata*, *melanospora* and *Breutelii*, and confirmed the fact that in nearly mature sporogonia of the same species the columella might be present or wanting. By extending his observations to the basal region of the capsule, where the tissue was still continuous, he showed that the columella was really absent, and did not merely dissociate into elater-like cells. When present it was often thin and composed of cells similar to the elaters, but in other cases was as well differentiated as in capsules of *Anthoceros*. From the study of a few young sporogonia of *N. fertilis* and *N. Breutelii* he came to the conclusion that, while the first divisions of the embryo were like those of *Anthoceros*, the central group of cells defined by the first periclinal walls gave rise in *Notothylas* to the sporogenous tissue, within which a sterile columella was subsequently differentiated. While he recognized the possibility that when the columella was well developed and distinct it might have originated as in *Anthoceros*, he regarded it as unlikely that such differences in the laying down of the sporogenous tissue should exist within the genus.

The work of Campbell³ and Mottier⁴ was done on *N. orbicularis*. The capsules were found in all cases to have a well-developed columella, and the delimitation of this in the embryo and the origin of the archesporial layer from the amphithecium proceeded as in the other Anthocerotaceae. Campbell regarded it as unlikely that the other species should differ so radically from the one he investigated as would be the case were Leitgeb's surmise correct.

If the inferences as to the uniform development of the sporogonium throughout the genus, made on the one hand by Leitgeb, and on the other by Campbell, are put on one side, the observations of the various investigators are not readily combined into a consistent account of the sporogonium of *Notothylas*. It is clear that a columella developed as in *Anthoceros* may be present, and this is doubtless the origin of the well-defined columella

¹ Bot. Zeit., 1859, p. 33.

² loc. cit.

³ Mosses and Ferns, 1st Ed., p. 140.

⁴ Annals of Botany, xxxii, 1894, p. 391.

which Leitgeb observed in some examples. But this does not appear to justify putting on one side the recorded cases in which the columella was thinner and less distinct, or the records of its complete absence in many sporogonia. Leitgeb found that in sections across the basal region of such capsules the centre was occupied by a uniform mass of sporogenous cells. This observation is independent of his explanation of the origin of the sporogenous tissue from the endothecium alone; as based on few and possibly abnormal embryos this is open to doubt, though even here the facts he records are not to be lightly put aside.

The study of the form of *Notothylas* to be described below appears not only to provide some additional facts, but to show how the conflicting statements, which from the foundation of the genus have been made as to the presence or absence of a columella, may be reconciled. My material was collected in the Singapore Botanic Gardens, and since it was in abundant fruit, the small supply preserved in spirit has sufficed for a fairly complete study of the development and structure of the sporogonium.

The form in question has dark spores, which are muriculate on the convex face, and thus belongs to the section *Acantho-Notothylas*, and differs from the species of *Eu-Notothylas* (including *N. javanicus* from the same geographical region), in which the spores are smooth. Specific differences in the genus are slight and indefinite, and from published descriptions and figures this form at first seemed distinguishable from *N. Breutelii*. Comparison with specimens from Cuba¹ showed, however, such close agreement in all definite features that there seems no ground for distinguishing the plant from Singapore even as a variety from *N. Breutelii*. This species, which was first described as *Anthoceros Breutelii* in the Synopsis Hepaticarum, has been examined with regard to the presence or absence of a columella by Gottsche, Milde, and Leitgeb. The material examined by the two former observers showed as a rule a well-marked columella, between which and the wall the elaters extended. Milde, however, believed the columella to ultimately disintegrate, since he sometimes found it intact, sometimes only larger or smaller fragments, while in other cases no trace of it was to be found but a short stump-like portion at the base of the capsule. He figures a very complete columella. Leitgeb found a more or less perfect columella in some sporogonia. In sections of the base of other capsules no sterile columella could be distinguished, and on the evidence afforded by several embryos he regarded the whole sporogenous mass as derived from the endothecium and the columella as differentiated within it. Thus this particular species illustrates the difficulty which has been experienced in the genus as a whole of attaining a clear statement of the facts of structure of the sporogonium.

¹ I am indebted to Professor Weiss for the opportunity of examining authentic specimens in the Herbarium of the Victoria University, Manchester.

The characters of the gametophyte need only be briefly referred to for the purpose of systematic comparison and as introductory to the more detailed account of the sporogonium. The plants grew on the surface of a path, the individual thalli closely overlapping one another. The form of a single complete plant developed from the spore was not ascertained, since the branches were mostly in continuity with older underlying portions. The individual lobes or branch systems attained a length of 5–10 mm., and widened out from a narrow base to a breadth of 12 mm. or less. The thallus thus assumed the usual suborbicular form, and the numerous growing-points in the anterior margin were separated by rounded or spatulate middle lobes (Fig. 1). For the most part the thallus, which had no definite midrib, was six to eight cells thick. Each of the smaller cells of the limiting layer above and below contained a single large chloroplast. The internal cells were large and clear, and some of them contained mucilage; intercellular spaces containing mucilage were absent. In the middle lobes the internal tissue became reduced to a single layer of mucilage cells, and at the extreme margin was wanting, the lobe being composed of two layers of cells. The apical region resembled that of *N. orbicularis*, and numerous mucilage pits, some of which were later occupied by large oval colonies of *Nostoc*, were present. The antheridia and archegonia were situated behind the apical regions on the same individuals. Usually a pair of large antheridia occupied the antheridial cavity, but sometimes three were found, and occasionally the antheridium was solitary. The inconspicuous sunken archegonia resembled those of *N. orbicularis*. Fertilization appears to take place when the archegonia are close to the apex, and as a rule further growth of the thallus is slight, so that the sporogonia are situated close to the margin of the thallus (Figs. 1, 2). Occasionally sporogonia were found further back on the thallus.

The calyptra enclosing the sporogonium was directed forwards, parallel to the surface of the thallus (Figs. 1, 4, 5). It measured from 1.5 to 2.5 mm. in length by about .75 mm. across. The side turned away from the thallus bore a number of longitudinal wing-like ridges, and the calyptra was thicker on this side than the other. The mature sporogonium usually projected only a short distance from the calyptra. Its length varied from 2 mm. to 4.25 mm., but dwarf forms, like those mentioned by Leitgeb, were also seen, in which the total length was under 1 mm. (Fig. 3). The bulbous foot, the superficial cells of which grew out into processes, was separated from the cylindrical slightly curved capsule by a slender stalk (Fig. 4). The mature capsule itself was from 1.5 to 3.75 mm. long. The superficial cells of the wall were more or less rectangular, and their anticlinal walls were strongly thickened and of a brown colour. The line of dehiscence was clearly marked, the margins of the two valves being formed of narrower, reddish-brown cells. The opening of sporogonia attached to the plant was

not observed, but most of the capsules were not quite mature. The occurrence in the underlying soil of intact capsules isolated by decay suggests, however, that in many cases they may not open. It is indeed difficult to see how the small part of the capsule projecting from the calyptra can suffice for effective dehiscence. The cavity of the capsule was occupied for the greater part of its extent by a uniform mass of spores and elaters, a sterile columella being completely absent or only present at the base. The spores measured $40-45\mu$ in diameter: the wall was dark and the convex outer face was studded with short, blunt, almost black projections. The individual sterile cells or elaters, which at first were united into flat transverse plates, measured about $40\mu \times 30\mu$, and had thin walls with yellowish-brown thickenings in the form of more or less complete rings.

The general appearance of an almost mature sporogonium in median longitudinal section is shown in Fig. 4, and Fig. 5 represents a similar section of a smaller and slightly less advanced sporogonium under a somewhat higher power. These figures, together with Figs. 6 and 7, which represent median longitudinal and transverse sections from mature capsules under a high power, demonstrate the absence of a sterile columella from the greater part of the capsule. The cavity of the latter is seen to be filled with a reticulum of sterile cells, in the meshes of which the spores lie. The structure is in fact that described and figured by Leitgeb as occurring in some cases in *Notothylas*, although, as will be shown below, its origin in development is different from what he supposed. When a columella was present it only extended a short distance from the base, and its diameter varied in different capsules.

From this general survey of the structure of the mature sporogonium we may return to trace its development. A sufficient number of young embryos were found to indicate clearly the course of the early segmentations of the fertilized ovum. In Fig. 8 a young embryo is shown in median section as it lies in the venter of the archegonium, and the commencing growth of the surrounding tissue to form the calyptra is evident. The first division is in the line of the axis of the archegonium (Fig. 9 *a*). In the next stage found the embryo consisted of three tiers of four cells each (Fig. 9 *b*); this stage has been found in the embryogeny of all Anthocerotaceae that have been investigated. Comparison with succeeding stages shows that here, as in *Anthoceros*, the two lower tiers go to form the foot, while the capsule and stalk originate from the uppermost tier. The periclinal divisions in the four cells of the latter separate the endothecium from the amphithegium, as in *Anthoceros* (Figs. 8, 9 *c*). After some anticlinal divisions have occurred, and the young capsule has increased in size, the archesporium is cut off from the amphithegium. This begins at the summit of the embryo and extends to the cells below, but, as Fig. 10 shows, not to the base of the region derived from the uppermost tier of cells. The

limits of foot, stalk, and capsule are thus clearly established at this stage, and continue to be traceable throughout the further development of the sporogonium. In the slightly older embryo seen in median section in Fig. 11 the capsule has grown considerably. The whole course of the segmentation of the embryo thus agrees with the very uniform type for the Anthocerotaceae. In these young capsules the regions corresponding to the wall, the archesporium (derived from the amphithecium), and the columella in other Anthocerotaceae, can be recognized. All these regions are present in the zone just above the stalk, from which the intercalary growth proceeds.

The difference between the mature capsule of this form of *Notothylas* and the typical capsule of the Anthocerotaceae depends on the different fate of the cells of the endothecium in the two cases. In *Anthoceros* this is devoted to the formation of the sterile columella. In this *Notothylas* it can only be called the columella on comparative grounds, for, as intercalary growth proceeds, the cells of the endothecium, as well as those of the amphithecial archesporium, give rise to sporogenous tissue. In this way the capsule, as has been shown above, comes to be filled with a uniform mass of spores and elaters.

The differentiation of the tissues within the wall can be followed even in sections of advanced sporogonia, so that the absence of some of the intermediate stages from the material has been of little importance. Fig. 12 is a detailed drawing of the median section of the sporogonium represented in Fig. 5, and includes the region from the stalk to the base of the capsule, where the distinction of elaters and spore-mother-cells is becoming established. In the stalk the endothecium appears as two rows of cells bounded by the amphithecium, consisting of a single layer. Just above this the amphithecium is seen to consist of two layers, the outer of which is continuous when traced upwards with the wall of the capsule; the inner can be followed upwards as a definite layer adjoining the endothecium, and is the archesporial layer. Though different in origin, the four rows of cells seen within the wall in Fig. 12 behave alike. Their cells undergo an ultimate transverse division, the upper segment forming one or more spore-mother-cells, while the lower gives rise to sterile elaters. In the figure the spore-mother-cells are shaded. Comparison with Fig. 6 will make it clear how the reticulum of sterile cells found in the mature capsule is derived. The relation of the uniform sporogenous tissue to the meristematic zone below in Fig. 12 demonstrates its origin from series of cells which correspond in other Anthocerotaceae, both to the archesporium and the columella. Further evidence of the complete absence of a sterile columella from such capsules is afforded by transverse sections of the basal region. In Fig. 13 the limits of the four rows composing the endothecium and the surrounding groups of cells derived from the archesporium can be traced in the uniform

sporogenous tissue. These limits continue to be traceable, even in the zone at which the spore-mother-cells are differentiated, and Fig. 14 *a* and *b*, which are two successive sections of the sporogenous tissue at this level, afford additional proof of the sporogenous nature of the endothecium.

For the greater part of the intercalary growth in all the sporogonia examined the endothecium and archesporium behave alike as described above. In many sporogonia a sterile columella appears towards the close of growth. This results from the intercalary meristem of the endothecium becoming in whole or part sterile. The change is apparent as soon as the sporogenous cells acquire the dense appearance by which they contrast with the cells of the wall of the capsule. When one or more quadrants of the endothecium have become sterile, they also stand out in contrast to the sporogenous cells (Fig. 15 *d, e*; Fig. 16). The series of transverse sections in Fig. 15 illustrates a case in which one quadrant of the endothecium had become sterile, while the other three were sporogenous. This series extends from the stalk to the region where the elaters and mother-cells are distinguishable, and may be compared with the longitudinal section in Fig. 12. In the longitudinal section through the base of another capsule (Fig. 16) a short columella, derived from the whole thickness of the endothecium, is shown. Such short columellas were found at the base of some fully mature capsules, and did not appear to undergo any disintegration into separate cells. In the numerous capsules examined the sterilization only supervened shortly before the completion of intercalary growth. The arrest in growth was in no way dependent on decay of the stalk, and Leitgeb's suggestion to this effect seems to be without proper foundation. It seemed, on the other hand, to be associated with the maturing of the spores at the tip of the capsule. When these have acquired their dark-coloured walls, a change which extends downwards towards the base of the capsule, active growth appears to cease.

We may sum up the chief features of the development and structure of the sporogonium in this form, by saying *that the embryogeny conforms to the usual type for the Anthocerotaceae, but that the endothecium, instead of being devoted to the formation of a sterile columella, forms sporogenous tissue for the greater part of the intercalary growth of the sporogonium. In a considerable proportion of cases, however, it produces sterile tissue towards the close of development.*

Other investigators have shown that in *N. Breutelii* there is sometimes a complete sterile columella, and this doubtless arises by the endothecium being sterile from the first. The range of variation in the development of the internal tissues of the embryo may fairly be assumed to be dependent on physiological factors which vary from individual to individual, and not in this case to be of value as an indication of specific difference. That the majority of the sporogonia in a single small collecting should show the same

degree of development of the columella is quite consistent with this. Not only do Leitgeb's observations on this species agree with what has been shown above as to the complete absence of a sterile columella in some capsules, but his few observations on arrested embryos suggest that a further grade of simplification may occur, in which only the endothecium is sporogenous. Were no amphithecial archesporium cut off, the sporogenous tissue would be traceable downwards to four rows of cells in the stalk, as he describes and figures it. It would be of great interest to have these observations, the possibility of which must be admitted, verified. Unfortunately satisfactory preparations were not obtained of the two dwarf capsules found in my material, and in all the other embryos and sporogonia examined it was clear that both endothecium and amphithecium had contributed to the sporogenous tissue. If such extreme forms occur they would seem to be infrequent and exceptional.

The position of *Notothylas* among the Anthocerotaceae, and of the group as a whole in relation to the other Hepaticae, may in conclusion be briefly considered in the light of the facts as at present known. The point that has emerged from the study of this form of *N. Breutelii*, that the endothecium must be regarded as potentially sporogenous, is of considerable theoretical interest. It increases the justification for considering the central group of cells, which in all other Anthocerotaceae is wholly devoted to the formation of a sterile columella, as the original sporogenous tissue and the amphithecial archesporium as of secondary origin. The duty of producing spores would seem to have been transferred from the central to a more superficial set of cells. This may, I think, be regarded on comparative grounds as probable, whether or not sporogonia are met with in which the amphithecial archesporium is absent. Leitgeb's deduction that the columella was to be looked on as originating by gradual differentiation within the sporogenous tissue does not, on the other hand, properly represent the facts. The causes of the change from the fertile to the sterile condition of the products of the endothecium must be looked for in influences acting on the primary meristematic tissue of the embryo, or on the intercalary zone of secondary meristem. The idea of a grouping of elaters in a central position to form the columella is not in this case in accord with the facts. The diverse results of the development of the similar embryos of the Anthocerotaceae suggest the influence of nutritive factors acting on the young embryo when it is enclosed and dependent like a foetus in the uterus on the tissue of the gametophyte.

The interest of the potentially sporogenous nature of the region usually developed as columella is independent of the question as to whether the species of *Notothylas* are to be regarded as primitive or reduced in comparison with the Anthocerotaceae with larger sporogonia. I am inclined to regard the evidence as pointing to their being reduced forms. The appear-

ance of the archesporial layer derived from the amphithecium, even when the central cells are fertile, suggests an origin from forms with a sterile columella. In other words, the fertility of the endothecium in some cases seems better explained as the resumption of a lost power than as a persisting primitive feature. An origin from forms with a larger capsule seems also to be indicated by the preparations for splitting of the wall of the capsule into two valves extending to the base, even though the sporogonium is usually almost enclosed by the calyptra and effective dehiscence rendered impossible. I incline to look upon *Notothylas* as a rather artificial genus, including a number of forms derived by reduction in size of sporogonia from one or more *Anthoceros*-like forms. Accompanying the reduction are the changes in development of the regions of the embryo; these, though becoming manifest in reduced forms, may have an important bearing on the morphology of the Anthocerotaceous type of sporogonium.

The possibility or probability that the ancestral forms of the Anthocerotales had, like the other Hepaticae, a central sporogenous tissue derived from the endothecium does not lead us far in the search for the ancestry of this very natural and isolated group. In considering this question, all the peculiarities of cell structure, of the gametophyte and sexual organs, and of the sporogonium must be taken into account. There appears to be no indication at present of any natural relationship being established between the Anthocerotales and any particular series of Hepaticae. Whether they were derived from primitive forms of the same stock as the other Hepaticae, or independently, but by corresponding steps from what we provisionally assume as the unknown Algal ancestral forms, or whether on the other hand reduction has played a part in the origin of the group as we now have them, seem to be at present open questions.

EXPLANATION OF FIGURES IN PLATE XXI.

Illustrating Dr. Lang's Paper on the Sporogonium of *Notothylas*.

Fig. 1. Outline of a branch system of the thallus of *Notothylas Breutelii* bearing sporogonia of various ages. $\times 5$.

Fig. 2. Calyptra enclosing an almost mature sporogonium, the tip of which projects: the ridges on the calyptra are indicated. $\times 16$.

Fig. 3. A dwarf sporogonium drawn to the same scale as the one in the preceding figure.

Fig. 4. Median longitudinal section of a similar sporogonium to that in Fig. 2, showing its relation to the thallus, the regions of the sporogonium, the gradual ripening of the spores from above downwards, and the absence of a sterile columella except at the extreme base. $\times 20$.

Fig. 5. Longitudinal section of a similar but slightly less advanced sporogonium, which is completely enclosed within the calyptra. $\times 45$.

Fig. 6. Small portion of a median longitudinal section of a mature capsule, showing the arrangement of elaters and spores and the absence of a sterile columella. $\times 110$.

Fig. 7. Transverse section of a similar sporogonium. $\times 110$.

Fig. 8. Longitudinal section of a lobe of the thallus, showing an embryo in position; the endothecium and amphithecium are separated in the upper tier. $\times 190$.

Fig. 9 *a, b, c*. Young embryos in median section, showing successive stages of segmentation. $\times 530$.

Fig. 10. Median section of more advanced embryo, in which all the regions of the sporogonium have been laid down. $\times 375$.

Fig. 11. Slightly older stage showing the rapid growth of the terminal group of archesporial cells. $\times 375$.

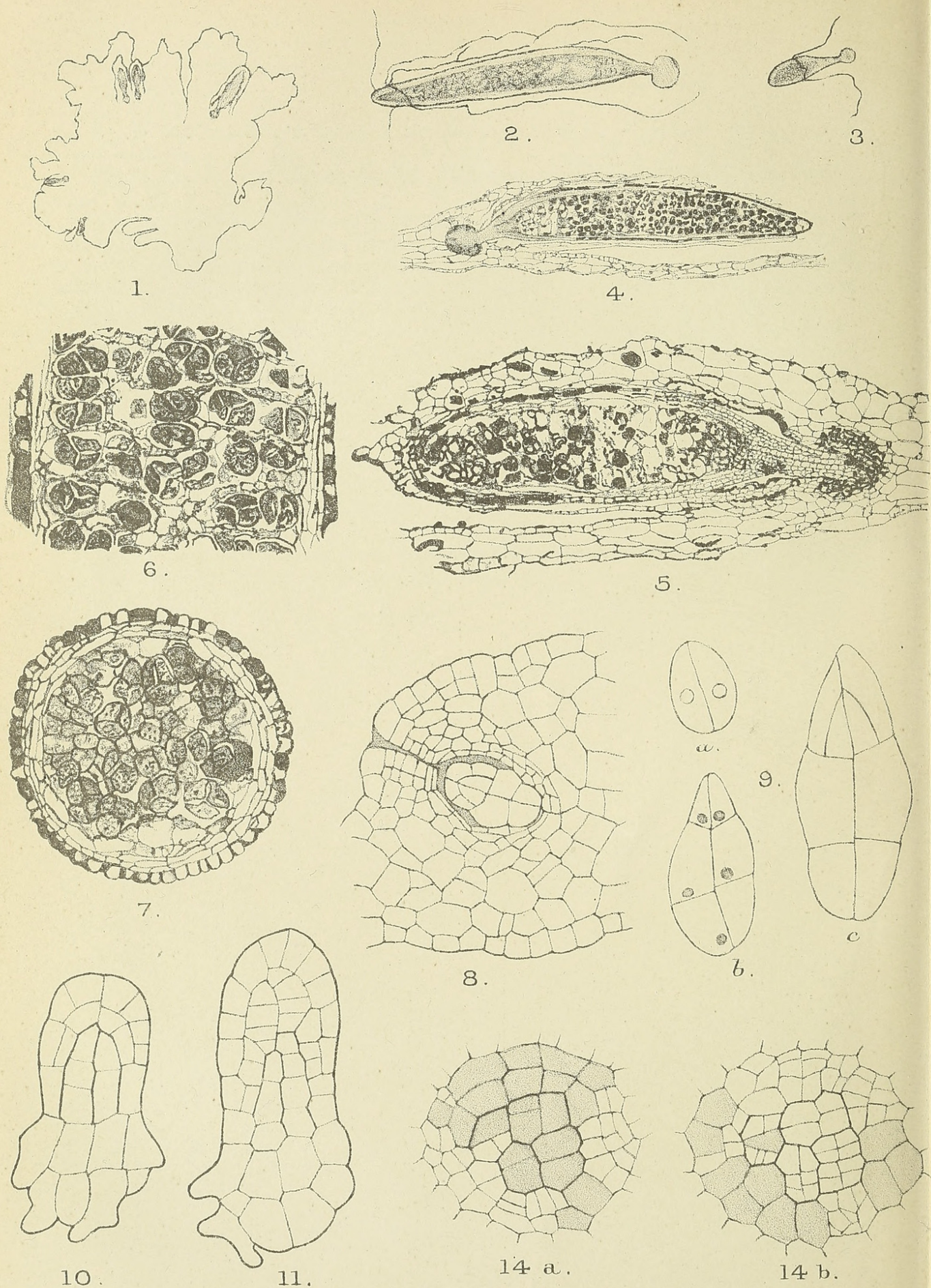
Fig. 12. Median longitudinal section of the lower portion of the sporogonium in Fig. 5. In the upper part the wall of the capsule can be distinguished from the sporogenous tissue, the cells of which are differentiated into spore-mother-cells (shaded) and elaters. Traced downwards the central portion of the sporogenous tissue is continuous with the endothecium, while the peripheral portion comes from a layer of cells which have a common origin with the wall. $\times 250$.

Fig. 13. Transverse section of a sporogonium at about the level *aa* in Fig. 12. The uniform sporogenous tissue (shaded) can be seen to be composed of four central groups of cells (endothecium) and a surrounding layer derived from the amphithecium. $\times 250$.

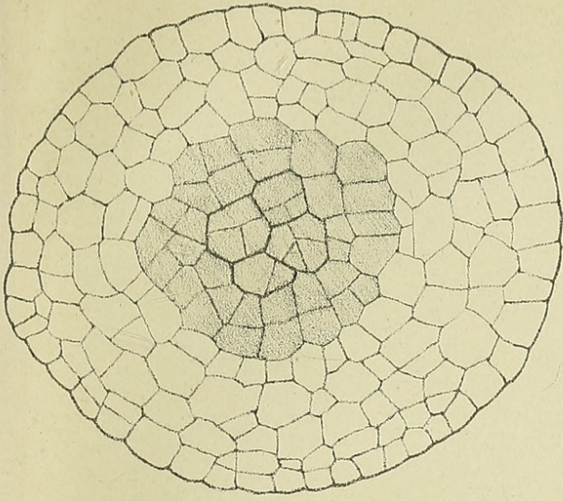
Fig. 14. Two successive sections of the sporogenous tissue at a level corresponding to *b, b* in Fig. 12, to demonstrate the absence of a sterile columella and the relation between the elaters and spore-mother-cells. $\times 250$.

Fig. 15 *a, b, c, d, e*. Successive transverse sections of a sporogonium, in which one of the four rows of cells of the endothecium is forming a sterile columella. In *d* and *e* the sporogenous cells are shaded, the sterile quadrant being left clear. The origin of the amphithecial archesporium can be followed. $\times 250$.

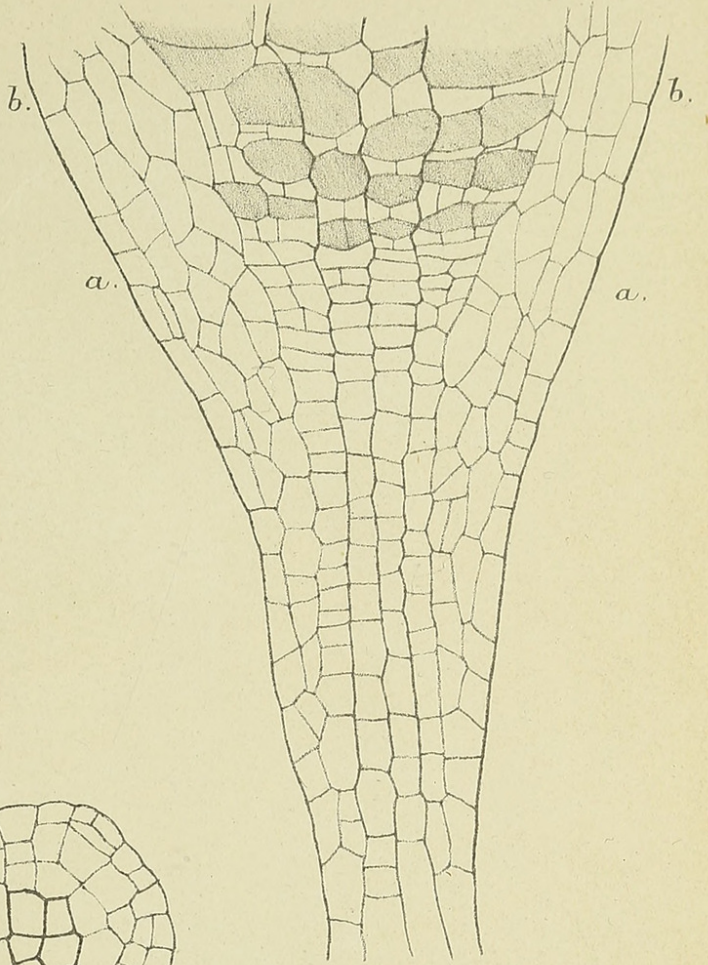
Fig. 16. Longitudinal section of the basal region of an almost mature capsule, showing a short sterile columella derived from the endothecium. $\times 190$.



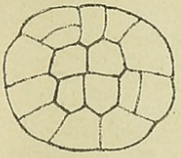
W.H.L., del.



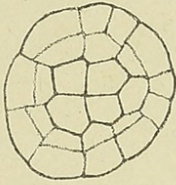
13.



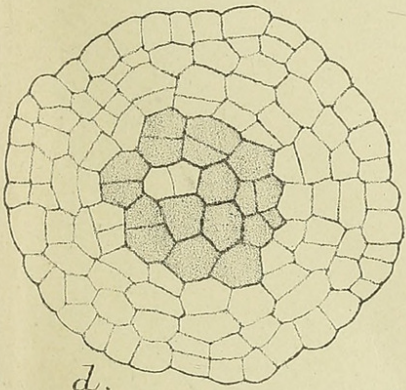
12.



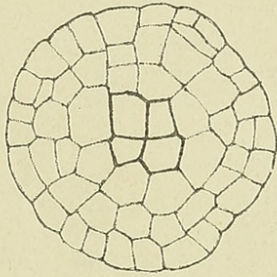
a.



b.

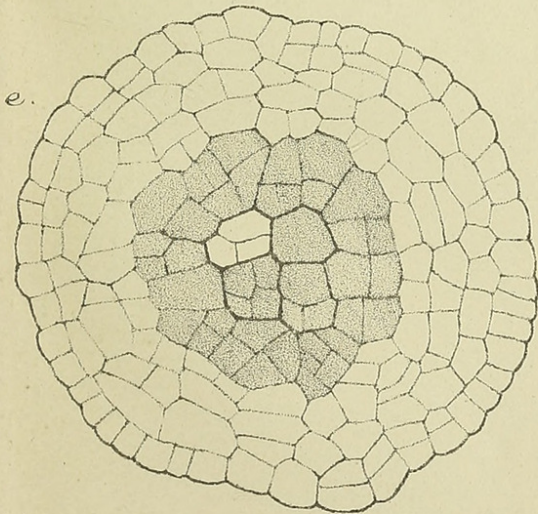


d.

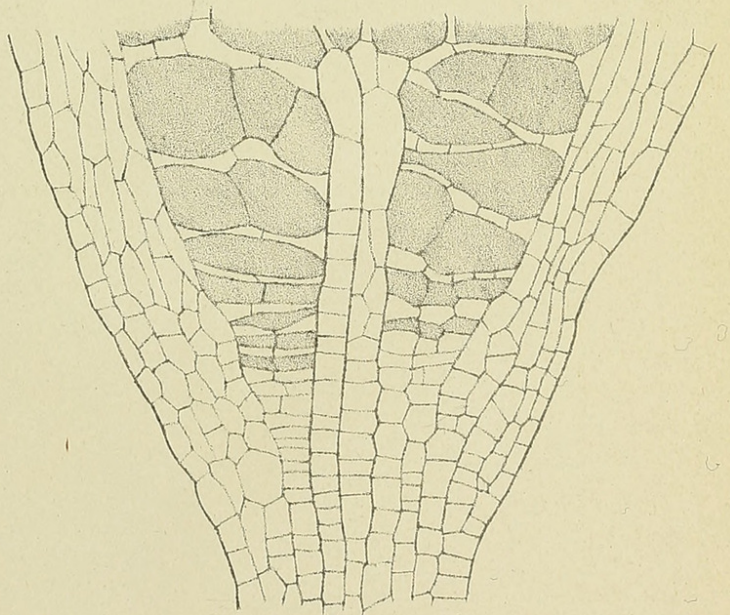


c.

15.



e.



16.



Lang, W. H. 1907. "On the sporogonium of *Notothylas*." *Annals of botany* 21, 201–210. <https://doi.org/10.1093/oxfordjournals.aob.a089130>.

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