Studies on some Javanese Anthocerotaceae. I.

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With Plates XLIV-XLVI.

THE family Anthocerotaceae is a very isolated one, and its relation to the other Archegoniates is by no means clear. The single alga-like chromatophore, found in nearly all of them, the endogenously formed antheridia, and the characteristic sporophyte all distinguish them sharply from the Hepaticae with which they are usually associated. The proposal of Howe (the Hepaticae and Anthocerotes of California; Mem. Torrey, Bot. Club, VII, p. 9, 1899) to separate them from the Hepaticae, as a class Anthocerotes, has been accepted by the writer (Mosses and Ferns, 2nd Edition, 1905), and it is probable that this view will be maintained.

The researches of Hofmeister and the later works of Janczewski and others were mainly concerned with the common European species, Anthoceros laevis, but Leitgeb (Untersuchungen über die Lebermoose, Heft 5, 1879) also made a fairly complete study of several other species of Anthoceros as well as of species of two other genera, Dendroceros and Notothylas. Janczewski (Vergleichende Untersuchungen über die Entwickelungsgeschichte des Archegoniums, Bot. Zeit., 1872) showed that the early divisions of the archegonium were essentially the same as in the true Hepaticae, and Leitgeb confirmed his investigations. The latter writer thought that in Notothylas he had discovered examples where the sporogenous tissue arose from the endothecium, as in the true Liverworts, and he regarded Notothylas as intermediate between the Jungermanniales and the other Anthocerotaceae. This view, however, has not been confirmed by later researches (Mottier, Contributions to the Life History of Notothylas, Ann. of Bot., VIII, 1894; Campbell, Mosses and Ferns, 1st Edition, 1895).

Three genera are usually recognized—Anthoceros, Dendroceros, and Notothylas—which closely resemble each other in the characters of both gametophyte and sporophyte, and they are all obviously closely related. Of these three genera, Anthoceros is almost cosmopolitan. Schiffner (Hepaticae. Engler and Prantl, Nat. Pflanzenfam, I Th., Abt. 3, 1895)

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gives seventy-nine species of Anthoceros, but a number of new species have since been described, and there are doubtless many more, as it is evident that the species have been quite superficially studied from many regions in the tropics where they abound. Dendroceros is credited with fifteen species, all of which are tropical, and Notothylas with nine, occurring both in tropical and temperate regions; but it is probable that the number of species in both of these genera is also greater than Schiffner indicates.

There are certain striking parallelisms in the character of the sexual organs and of the sporophyte between Anthoceros and the lower Pteridophytes, and also the Sphagnaceae, and the question arises whether these parallelisms indicate any true relationships. The writer has been inclined to believe that they do, although fully appreciating the difficulties in the way. The two greatest differences between the Anthocerotaceae and the lower ferns, like Ophioglossum, are the character of the antheridium and spermatozoids and the single chromatophore of the Anthocerotaceae. The former would allow a comparison with Sphagnum or with Lycopodium, but the single chromatophore of Anthoceros is very different from those of either of these forms. In all of the Anthocerotaceae hitherto described, a single large chromatophore is present in each cell, and this often contains a very distinct pyrenoid. The chromatophore thus resembles very much that of certain green Algae like Ulva or Coleochaete. It is not uncommon, however, to find in the larger interior cells of the thallus a division of the chromatophore, but hitherto no species was known in which the cells regularly contained more than one. In the summer of 1897 the writer collected in Jamaica an undetermined species of Anthoceros in which the superficial cells usually contained two chromatophores, but no further study was made of the plant at the time. This material was sterile, and could not be identified. No further discoveries were made until a recent visit to Java, where a similar form was discovered near Buitenzorg and later a second species (possibly more than one) was found at Tjibodas. About this time Goebel (Archegoniatenstudien, Flora 96, 1st Part; p. 195, 1906) noted the presence of multiple chromatophores in A. giganteus from New Zealand. As both of the Javanese forms, as well as A. giganteus, belonged to the section of the genus with spiral elaters and no stomata upon the sporogonium, it was thought that the Jamaica specimen might also belong to the same section.

Through the kindness of Dr. M. A. Howe, of the New York Botanical Garden, material of two American species of this section was secured—A. Vincentianus, Lehm. and Lindenb. from the West Indies, and A. flavens, Spruce, from South America—and in both of these the multiple chromatophores were found. This would seem to show that this character is probably common to all species of Anthoceros belonging to Gottsche's

third section of the genus, and, together with the other characters, would seem to constitute sufficient ground for separating these species as a distinct genus, as Leitgeb (loc. cit. p. 27) believed should be done. The discovery of the multiple chromatophores is important, as it breaks down one of the barriers separating the Anthocerotaceae from the other Archegoniates; but we still believe that the differences are sufficient to warrant the retention of the class Anthocerotes.

MEGACEROS, A NEW GENUS OF ANTHOCEROTACEAE.

Shortly after the writer's arrival in Java, in March, 1906, while on a botanizing excursion to the Tjiapus Gorge at the base of Mount Salak, near Buitenzorg, a number of species of Anthoceros were collected, and among them was one growing among mosses upon a fallen log. As this was a position very similar to that in which the West Indian specimens were collected, it was hoped that this might prove to be a similar form, and an examination after returning to Buitenzorg showed that such was the These specimens were fruiting abundantly, and it was at once evident that the plant belonged to the section with spiral elaters and no stomata, thus approaching Dendroceros. Later collections at Tjibodas showed that a similar species was not rare in the neighbourhood, and plants were repeatedly collected at various points from the immediate neighbourhood of the laboratory (altitude about 1,450 metres) to a short distance below Kandang Badak, which is about 2,500 metres high. comparison of these specimens with those collected on the Salak showed that they were evidently specifically distinct, although much resembling the species from the lower level.

The Tjibodas material showed a good deal of variation as to habitat. Most commonly, as in the specimens first collected, the plants grew on rotten logs; but sometimes they were found upon the earth, and, in a few instances, upon boulders. The latter specimens had a thicker thallus than the others, but otherwise were not perceptibly different, and probably are not specifically distinct. A number of specimens with quite different chromatophores were found in the material when it was examined upon the writer's return to America, but these were sterile, and so could not be compared with the others. It is possible that they represent a third species, but at present this can only be conjectured.

The multiple chromatophores, together with the *Dendroceros*-like type of sporogonium, i. e. spiral elaters, green spores, and the absence of stomata, seems to warrant a separation of the group from the other species of *Anthoceros*, as Leitgeb suggested should be done. The name *Megaceros* is here proposed for all the species of this group. The name is selected on account of the very large size of the sporophyte in the commonest Javanese species. This in some instances reaches a length of 9 cm. and

possibly more, and A. giganteus is also characterized by its very long sporophyte. Stephani has described a species of Anthoceros, A. Stahlii, which evidently belongs to the same group as the species here described. The specimens were collected on Mount Gedeh, where the writer also collected much of his material. The original description was not accessible to the writer, but through the kindness of Dr. Howe a copy of the description was sent him.

The Tjibodas form, or at least the commonest one, agrees in many respects with Stephani's description of A. Stahlii, and may possibly be the same, but the thallus in the writer's specimens is very much thicker, and the spores and especially the elaters much larger than Stephani describes for A. Stahlii. For this species, the name Megaceros Tjibodensis is proposed. The species from Mount Salak we shall call M. Salakensis. The important points of difference between Megaceros and Anthoceros are the multiple chromatophores of the former, the absence of stomata from the sporogonium, the solitary antheridium, spiral elaters, and green spores. In all of these respects, except the character of the chromatophores, Megaceros resembles Dendroceros more than it does Anthoceros; but the form of the thallus and the apical growth, at least in all of the forms examined by the writer, are like those of a typical Anthoceros. be noted here that Anthoceros Pearsoni, Howe, a common Californian species, has solitary antheridia, and it is interesting to note that the inner thallus cells very commonly show a double chromatophore. It is, therefore, to a certain extent intermediate between the typical species of Anthoceros and Megaceros.

Both *M. Tjibodensis* (Pl. XLIV, Fig. 1) and *M. Salakensis* (Fig. 4) have a relatively large thallus, which in the former may reach a length of 5 cm. or more, with a breadth of about 1 cm. Of the different forms which seem to belong to *M. Tjibodensis*, the largest were growing upon boulders and upon the earth. These have a thicker thallus than the typical form shown in Fig. 1, and may possibly be specifically distinct. In the type the thallus is rather thin, with a conspicuously lobed and strongly laciniate margin (Fig. 6). The laciniate character is somewhat less marked in the stouter rock form (Fig. 7).

M. Salakensis is somewhat smaller in all its parts, and the margin of the thallus is lobed, but does not show the laciniate margin so marked in M. Tjibodensis (Fig. 8). The sporogonium in M. Salakensis is also very much shorter than in M. Tjibodensis. In the latter it may reach a length of 9 cm. (see Fig. 3), while in M. Salakensis the largest specimens found were less than half as long (Fig. 5). In the latter species also, the ripe sporogonium opens along one suture instead of dividing into two valves, which is the rule in M. Tjibodensis. In this respect, as well as in some others, M. Salakensis resembles Dendroceros more nearly than does

M. Tjibodensis. In both species the usual Nostoc colonies occur, but these are not especially noticeable.

The antheridia are produced singly in the usual chamber, and are large enough to be seen with the naked eye, in some cases having a diameter of about 170 μ . Stephani states that in M. Stahlii they do not occur upon the same branches of the thallus as the archegonia, but in all forms studied by the writer they occurred upon the same shoot. The plants are markedly proterogynous, however, unlike most species of Anthoceros, where the antheridia are formed first. It may have been the overlooking of this fact that led Stephani to suppose that they were borne upon special branches.

THE CHROMATOPHORES.

A careful study of the chromatophores of both of the Javanese species was made. These are most different from the ordinary Anthoceros type in M. Tjibodensis. In most species of Anthoceros, as is well known, each cell contains a single large flat chromatophore, in the centre of which is a very distinct pyrenoid, the whole closely resembling the chromatophores of many green Algae, especially many Confervoideae, like Stigeoclonium A careful study of M. Tjibodensis shows that not only or Coleochaete. is the single chromatophore replaced by several, sometimes as many as twelve, but all trace of a pyrenoid has disappeared, so that the chromatophore is in all respects like that found in the other Archegoniates. pyrenoid also seemed to be absent from the chromatophore of M. Vincentianus and M. flavens, of which dried specimens were examined; but in M. Salakensis (Figs. 13 to 17) there was an aggregation of small starch granules about a central area in the larger chromatophores, which had the appearance of a pyrenoid, although it was much less definite than is usually the case in most species of Anthoceros.

M. Tjibodensis, although in a few of the superficial cells a single chromatophore may occur, in most cases has from two to four, while the number is much greater in the inner cells of the thallus. In the superficial cells of the upper side (Fig. 9) there are most commonly two chromatophores. Sometimes there is a single one and sometimes three. In the superficial cells of the ventral side the number ranges from two to four (Fig. 10), and they are smaller, but otherwise resemble those of the dorsal surface. They are in both cases thin flat plates, showing a uniform finely granular appearance, but no trace of anything like a pyrenoid, nor were large starch granules observed. In the cells immediately below the surface there are usually four smaller chromatophores, while in the large inner cells (Fig. 11) they are much more numerous, but of smaller size, and often quite round, closely resembling the typical chlorophyll bodies of higher plants. The nucleus (n) in all the cells is very small,

and not at all conspicuous, but may easily be demonstrated. Fig. 12 shows a cell from the interior of the thallus of a form which was growing with the typical M. Tjibodensis. The specimens were not fertile, and no comparison could be made with the sporogonium of M. Tjibodensis, so that it was not possible to determine certainly whether it was specifically distinct, but the very different form of the chromatophores would indicate that it is a different species. There were seldom more than four chromatophores present even in the largest cells, and these were flattened and irregular in outline, and were more or less completely joined by fine protoplasmic filament, presenting a very different appearance from the small rounded and isolated chromatophores of the typical specimens.

M. Salakensis differs a good deal from M. Tjibodensis in the character of the chromatophores. In the cells of the upper surface there is usually but a single chromatophore (Fig. 13), but this is often constricted, and may be completely divided into two. In the cells of the ventral surface two distinct chromatophores are generally present. These show a central area which is surrounded by numerous starch granules, and there are indications of a pyrenoid, but as we have seen, it is much less evident than in the typical Anthocerotaceae, where it is often very conspicuous. The cells below the epidermal layer contain usually two smaller chromatophores (Fig. 15), which also show the pyrenoid-like centre body. The inner cells of the thallus (Figs. 16 and 17) contain from four to six small rounded chromatophores without any central body, which are connected by delicate protoplasmic threads into a sort of chain.

Through the kindness of Dr. M. A. Howe, dried material of two species from tropical America was secured. An examination of these showed that they also possess multiple chromatophores. These were A. Vincentianus, Lehm. and Lindenb., from Martinique, and A. flavens, Spruce, from the Peruvian Andes. In both of these species (Figs. 18 and 19) the inner cells contained two to four chromatophores, while the superficial cells had usually but a single one, although it was often deeply constricted, and occasionally divided completely in two. From this dried material it was not possible to make a study of the structure of the chromatophore beyond noting that apparently no pyrenoid was present, in which respect they seem to agree with M. Tjibodensis.

While in most other Archegoniates the chromatophores are small and numerous, there are a number of exceptions which approach the condition found in *Megaceros*. The writer has found, as an exceptional occurrence, large flat chromatophores in the prothallial cells of *Osmunda cinnamomea* (Campbell, On the Prothallium and Embryo of Osmunda. Ann. of Bot., VI, 1892), and in a species of Cyathodium collected in Java, apparently somewhat different from the common *C. foetidissimum*, the chromatophores were relatively very large, and in some cases there were only four in a cell.

The chromatophores in the assimilative cells of *Targionia hypophylla* are also few in number, and relatively large. The single chromatophore found in some of the species of *Selaginella* may be also mentioned in this connexion. These instances will serve to show that the chromatophores of the Anthocerotes are not so radically different from those of the other Archegoniates as has been generally assumed.

STRUCTURE OF THE THALLUS.

The general form of the thallus in both *M. Tjibodensis* and *M. Sala-kensis* resembles that of a typical *Anthoceros*, and in neither of these species could any trace of a mid-rib such as occurs in *Dendroceros* be seen. In *M. giganteus*, however, Goebel (loc. cit., p. 195) states that the thallus is costate, although not so distinctly so as in *Dendroceros*, which it evidently resembles in habit. This species showed two to four chromatophores, which, like those of *M. Salakensis*, were often joined together. No statement is made by Goebel as to the character of the apical cell, which in *Dendroceros* is quite different from that of *Anthoceros*.

The thallus in both of the species under discussion is decidedly thicker than that of A. Stahlii, to judge from Stephani's description. He states that in the latter the thallus is six cells thick in the middle and only three cells thick in the wings. The specimens of the typical form of M. Tjibodensis are from eight to ten cells thick in the middle, diminishing very gradually toward the margin to four or five. In no case was any part of the thallus seen with less than four cells. The larger form, growing on boulders, in some cases showed twelve cells in the central region. M. Salakensis is slightly thicker than the typical M. Tjibodensis—usually nine to ten cells in thickness. In both species, as in Anthoceros, the superficial cells are flattened, and the chromatophores larger than in the inner ones. The sub-epidermal layer is often quite well defined, the cells being intermediate in size between the shallow superficial ones and the four or five layers of large central ones.

The apical growth is entirely like that of Anthoceros and the large initial cells show a regular succession of dorsally and ventrally arranged segments (Fig. 20). Each segment divides into outer and inner cells, and from the former in the fertile branches the sexual organs arise. In a few exceptional cases an approach to the condition found in Dendroceros was noted. In the latter genus the initial cells in vertical section appear semicircular in outline, and segments are cut off from the inner face which extend the whole depth of the thallus, and are subsequently divided by the median wall into a dorsal and a ventral portion. Fig. 21 shows a section of the apex of the thallus of M. Tjibodensis, in which there is an approach to the condition found in Dendroceros. No intercellular spaces are present

in the thallus of either species, but there are numerous large cells filled apparently with a mucilaginous substance which stains very strongly.

THE SEXUAL ORGANS.

Both of the species of Megaceros under consideration are monoecious. Stephani (loc. cit.) states that in no cases did he find in *M. Stahlii* antheridia and archegonia upon the same branch, and thinks it may be dioecious. In both *M. Tjibodensis* and *M. Salakensis* the two sorts of organs are found upon the same branch, but, unlike most species of *Anthoceros*, the archegonia are developed first, and one may find fresh antheridia upon the younger parts of branches which bear advanced sporogonia. It may be that owing to this marked proterogeny Stephani failed to find antheridia and archegonia together.

THE ANTHERIDIUM.

The antheridia, as has already been noted in *M. Vincentianus* (?) (Leitgeb, loc. cit., p. 17) and *M. Stahlii* (Stephani, loc. cit.), occur singly, and in this respect resemble those of *Dendroceros*. As we have already stated, however, there is one species of typical *Anthoceros*, *A. Pearsoni*, in which solitary antheridia also occur, and this fact, together with the frequent doubling of the chromatophores in the inner cells, suggests an approach to *Megaceros*.

The study of the development of the antheridium is attended with some difficulty owing to the mucilage developed about it in the chamber where it is formed. This mucilage evidently interferes with the penetration of fixing agents, and much of the material that was examined was found to have the younger antheridia so shrunken as to be quite useless for study. This was specially the case with specimens treated with I per cent. chromic acid, which otherwise proved the best fixing agent. Specimens fixed with acetic alcohol (alcohol, 90 per cent.; acetic acid, 10 per cent.) gave much better results, although for the study of the archegonia and embryo the chromic acid yielded much better preparations.

The early stages of the antheridium closely resemble those of Anthoceros Pearsoni (see Campbell, Mosses and Ferns, 2nd Edition, Fig. 57). The superficial cell from which the antheridium arises, as in all other Anthocerotaceae that have been studied, divides by a periclinal wall into an outer and inner cell, the latter in Megaceros, as in Dendroceros and Anthoceros Pearsoni, becoming at once the single antheridium. In the other species of Anthoceros and in Notothylas the inner cell divides by longitudinal walls into (usually) four cells, each of which develops into an antheridium. Before any divisions take place in the young antheridium, there begins to develop the cavity or chamber in which the antheridium

lies. The cells adjoining the mother-cell of the antheridium withdraw slightly from it, apparently owing to a mucilagenous degeneration of the inner layers of the cell walls. This mucilage forms a strongly staining substance outlining the young antheridia in stained sections, and with the growth of the tissue near it the chamber rapidly enlarges and becomes very conspicuous (Figs. 24, 25).

In the youngest stage that could be recognized (Fig. 23) the mothercell had divided into a small basal cell and a large terminal one. Whether this division always occurs is doubtful, but usually traces of the basal cell could be made out in the younger antheridia (Fig. 24 b). The first wall in the antheridium itself is longitudinal (Fig. 24), and this is probably in most cases at least followed by a second longitudinal wall intersecting the first. Following this, transverse walls arise that separate the pedicel from the upper portion. There next arise periclinal walls in each of the upper segments, by which the inner cells that develop into the sperm cells are separated from a single layer of parietal cells. Each of the latter finally develops a conspicuous chromatophore, as in other cases. The further divisions in the central cells follow with a good deal of regularity, and the limits of the early cell divisions are evident, even in the nearly ripe antheridium where the mass of almost cubical spermatocytes is divided into blocks corresponding to the early divisions of the central cell, a phenomenon which is of common occurrence in many other Bryophytes. The small size of the spermatozoids makes them unfavourable subjects for study of spermatogenesis, and no attempt was made to follow out the details. In the adult antheridia the slender curved body of the spermatozoid can be readily seen; but the blepharoplast, which is presumably present, could not be seen. Free spermatozoids were found to resemble those of ordinary Bryophytes, having the usual two cilia, but beyond demonstrating this point no further study was made of them.

The two species studied differed a good deal in the form of the antheridia. In M. Tjibodensis (Fig. 31) the mature antheridium is nearly globular, and has a short stalk inserted near the centre of the floor of the antheridial chamber. In M. Salakensis the insertion of the pedicel is toward the front of the antheridial chamber, not infrequently actually on the anterior wall of the cavity, and the antheridium lies almost horizontal, as it does in Dendroceros (Fig. 29). The pedicel is much longer than in M. Tjibodensis, and strongly bent, in this respect also resembling Dendroceros, although it never reaches the extreme length found in the antheridium of the latter. The pedicel in both species examined has ordinarily four rows of cells, but it is possible that sometimes, as is usually the case in Dendroceros, there may be only two rows. Rarely two antheridia occur in a single chamber.

Leitgeb (loc. cit., p. 19) has called attention to the occasional occur-

rence of superficial antheridia, but he recognizes that these are not normal cases. Two instances of a condition approaching this were seen in the course of these investigations. One of them is shown in Fig. 27, where the cavity containing the antheridium was open on one side so as to expose the top of the young antheridium. Apparently in this instance the primary cover cell of the antheridial cavity had been very early pulled away from the adjacent surface cells and the cavity thus became open, and no doubt, by the further lateral growth of the superficial cells, the antheridium would soon have stood in a shallow depression such as Leitgeb describes in the specimens seen by him. More recently Lampa (Untersuchungen an einigen Sitzungsber. der Kais. Akad. der Wiss., Wien, cxi, pp. 477, 489, 1902) has described exogenously-formed antheridia in Anthoceros; but Howe has criticized her work, and thinks that the structures described as antheridia were tubers, as the figures do not agree with the structure of normal antheridia (Howe, Torreya, iv, p. 175, 1904).

From a study of the development of the antheridium it is quite impossible to say whether or not the endogenous origin is primary or secondary, nor do the exceptional cases where the origin is superficial throw much light on the question. Such a condition as that shown in Fig. 27 is not unlike what is found in Sphaerocarpus or Riccia, where the antheridium is surrounded by an involucre; but whether this is any indication of a possible relationship between these forms and the Anthocerotaceae is another question. There can be no doubt that in all the Anthocerotaceae the antheridia are normally of endogenous origin.

THE ARCHEGONIUM.

The researches of Janczewski (loc. cit.) and those of Leitgeb, show that although the form of the archegonium is apparently quite different from that of the true Hepaticae, nevertheless in its essential structure it agrees closely with the other Bryophytes. All of the genera are much alike in the development of the archegonium, and Megaceros shows no marked differences when compared with the others. youngest stages are not easily recognizable, as the archegonium does not project at all above the level of the thallus at first, and the mother-cell is not usually markedly different in appearance from the neighbouring cells (Fig. 20 Q). Transverse sections of the young archegonium (Fig. 38) show that the axial row of cells is cut out by three intersecting walls as in the typical bryophytic archegonium, and longitudinal sections of the younger stages (Figs. 33-6) present an appearance not very different from similar sections of archegonia of true Hepaticae, except that in the latter the whole archegonium is free, while in the Anthocerotaceae only the upper surface is exposed. Nevertheless the limits of the neck cells are pretty well

defined, and in cross section (Fig. 39 a) the archegonia usually show the same number, six, that is characteristic of the lower Hepaticae.

The first division of the axial cell is transverse (Fig. 33) and usually there are no longitudinal walls formed; but in some cases the outer cell divides by a longitudinal wall (Fig. 34) before any further transverse divisions appear. In such cases the neck canal-cells are consequently much narrower than usual, and the central cell relatively broader. cases the breadth of all the cells of the axial row is approximately equal. The next transverse division is in the outer cell, and divides it into the cap-cell, and the primary neck canal-cell. The former divides by a vertical wall into two nearly equal cells, which may remain undivided, or one or both of them may divide again, so that the final number of cap-cells The canal-cell divides by a series of transverse walls into is two to four. four or sometimes five cells. In no case was a larger number found. Janczewski states that as many as twelve neck canal-cells may be formed in Anthoceros laevis, but the writer has never found more than six in any species of Anthoceros, Notothylas, or Dendroceros that he has studied. As in other cases that have been observed, the cover cells are thrown off when the archegonium opens, and the upper neck cells which project considerably above the thallus diverge more or less. As in the other Anthocerotaceae the central canal-cell is nearly or quite equal in size to its sister cell, the egg (Figs. 37, 40). No marked difference was observed between the archegonia of the two species studied. Of the three genera that have been examined hitherto, Notothylas has the axial cells relatively broadest, approaching in this respect the lower Pteridophytes. Dendroceros is intermediate between Notothylas and Anthoceros in the form of the archegonia, and Megaceros resembles it closely in this respect.

THE EMBRYO.

The very small size of the spermatozoids makes the Anthocerotaceae unsuited to a study of the details of fertilization, and no attempt was made to investigate these in *Megaceros*. The egg-cell at the time of fertilization does not fill the central cavity, and its nucleus is small. After fertilization it grows until it fills the venter before the first division occurs.

In the early divisions of the embryo Megaceros more nearly resembles Dendroceros than it does Anthoceros. As in both of these forms the first wall is longitudinal and the second transverse; but whereas in Anthoceros the transverse walls are below the middle of the young embryo, this being very marked in A. fusiformis (Mosses and Ferns, 2nd Edition, Fig. 69), in Dendroceros they are nearly median, and the young embryo is divided into nearly equal quadrants. The same is true in Megaceros, although the two lower quadrants are slightly smaller (Figs. 41, 48). Of the two species,

M. Tjibodensis and M. Salakensis, the former (Fig. 41) has a broader embryo, and the lower tier of cells very early begins to send out the root-like outgrowths which become so prominent a feature of the foot in the older embryo. This early development of these root-like processes was noted by Leitgeb in M. Vincentianus. The next divisions are vertical ones, and these octant walls are followed by a second series of transverse walls, as in Anthoceros and Dendroceros. Both of the lower tiers form the foot, as in Anthoceros, but the differentiation into amphithecium and endothecium extends almost to the base of the foot. In the lower tier of cells the divisions are less regular and this seems to be especially the case in M. Salakensis (see Fig. 50 b), where the quadrant divisions may be only very imperfectly indicated.

The formation of the columella or endothecium and the outer tissue or amphithecium follows much as in the other forms that have been investigated, but this can be clearly traced to the lowest tier of cells in *Megaceros*, while in *Anthoceros* it is not usually evident below the base of the uppermost tier which marks the boundary of the foot. The cutting off of the endothecium may in some cases apparently be brought about by periclinal walls directly, but to judge from a study of series of transverse sections (Fig. 51) these anticlinals may sometimes at least be formed before the development of the periclinal walls which finally delimit the endothecium. This is very like what is sometimes found in some mosses, e.g. Funaria, where there is not absolute uniformity in the succession of walls in the young segments of the embryo.

While the very young embryo of *M. Tjibodensis* is broader than the corresponding stage of *M. Salakensis*, this is not true in the latter stages, where in the latter species the embryo is noticeably broader at the base than in *M. Tjibodensis*. This can be seen by comparing Figs. 43, 52 with Figs. 49, 55. In the first species (Fig. 52) the young sporogonium is slightly constricted, while in *M. Salakensis* the base is broader than the region above it.

In the differentiation of the columella and in the origin of the sporogenous tissue, the two species agree closely and resemble *Dendroceros* more than they do *Anthoceros*. Comparisons of longitudinal and transverse sections show very clearly the origin and extent of these tissues. Before the cutting off of the sporogenous layer (Figs. 49, 51) the endothecium (columella) forms a cylindrical mass of tissue occupying the centre of the embryo and surrounded by a single layer of amphithecial cells. It can be traced to the base of the second tier of cells, thus including the upper part of the foot. The latter is clearly limited by the second transverse walls in the young embryo, and there is very little displacement of these walls, so that there is a sharp line between the foot and the base of the capsule. In *Anthoceros* (Campbell, Mosses and Ferns, 2nd Edition, Fig. 70) the

sporogenous tissue does not extend to the base of the capsule, but there is a clearly defined basal zone where it is absent. In *Megaceros*, on the contrary, the sporogenous layer can be traced to the line dividing the foot from the capsule. In this respect *Megaceros* exactly resembles *Dendroceros* (Campbell, loc. cit., Fig. 79). As in all the other Anthocerotaceae the sporogenous layer arises from a series of periclinal walls in the amphithecium, and there is thus formed a single layer of cells investing the columella (Figs. 43-5). These do not form simultaneously (see Fig. 43), but very soon the sporogenous layer is complete and is then seen to extend to the base of the capsule.

The columella of the young embryo seen in cross section forms a nearly regular square of four cells. Subsequently, these are further divided (Fig. 45 b), and in the older sporophyte there may be sixteen cells arranged in regular quadrant fashion, or not infrequently (Fig. 58) there may be further divisions in some of the cells. This also recalls the larger species of *Dendroceros*. In longitudinal sections there usually appear four rows of cells in the columella except toward the apex, where the columella becomes narrow, this being especially marked in *M. Tjibodensis*, where it tapers to a point (Fig. 52).

While in the early development of the embryo and in the details of the adult sporogonium Megaceros most nearly resembles Dendroceros, the sporogenous tissue is much more extensive and may best be compared with that of Notothylas. In Dendroceros and in some species of Anthoceros the sporogenous layer remains simple throughout nearly its whole extent. In the larger species of Anthoceros, e.g. A. fusiformis, it becomes double, while in Notothylas it is three to four cells thick, occupying relatively a very much larger part of the sporophyte. In the extent of the sporogenous tissue Megaceros (Figs. 54, 57) is to some extent intermediate between Anthoceros and Notothylas. When the divisions are complete it is three to four cells in thickness, but the cells are smaller than in Notothylas, and hence the mass of sporogenous tissue is relatively less; but it is very decidedly greater than in any species of Anthoceros that has yet been studied.

While in Anthoceros the portion of the sporogenous layer above the apex of the columella has the same thickness as that at the sides, there is in Megaceros the development of a considerable amount of sporogenous tissue in the amphithecial cells lying above the layer in contact with the columella (Figs. 53, 56). This is especially conspicuous in M. Tjibodensis, where it is quite as marked as it is in Notothylas. A trace of this increase of the sporogenous tissue at the apex of the sporogonium is also seen in Dendroceros, but it is very much less marked. The development of sterile tissue in the apical region of the sporogonium of Megaceros is also greater than in the other genera.

The sporogonium may become very long, this being especially so in M. Tjibodensis, where it may attain a length of q cm. or possibly more. In M. Salakensis it is much shorter, but in both it is stout and the amount of sterile tissue large. To correspond with this development of the assimilative tissue the foot becomes very large, and at an early period develops extensively branched rhizoid-like outgrowths (Figs. 42, 47), which penetrate between the adjacent cells of the thallus. Sections through the older sporogonium show the different stages of development of the sporogenous tissue, as there is of course the same basal zone of meristem by which the growth of the sporogonium is brought about. Transverse sections, where the sporogenous layer is first clearly differentiated (Fig. 58), show the square columella, its cells more or less rounded, and exhibiting small intercellular Surrounding this is the single clearly defined row of archisporial The sterile amphithecial tissue shows four or five rows of cells outside the archesporium. Higher up the archesporium divides into two layers, and still higher up these divide again more or less completely, so that there are three or four layers of cells (Fig. 54). In M. Salakensis the development of the archesporial tissue is somewhat less than in M. Tjibodensis, and there are seldom more than three layers of cells in the completed archesporium (Fig. 57).

Soon after the final divisions of the archesporial tissue are complete the differentiation of the sporogenous cells and the elaters begins. There seems to be no definite relation of these to the divisions of the archesporium, such as can be seen in *Notothylas*, and in some species of *Anthoceros*. Certain cells (el) are longer than the other (sp). The latter soon begin to become rounded off and to separate, while the sterile cells rapidly increase in length with the growth of the sporogonium, and push their way between the young spore-mother-cells which thus lie in the meshes of a net-work formed by the coherent elongated sterile cells (Figs. 60, 61). The separation of the spore-mother-cells is due to a disintegration of a part of the original cell-wall, which evidently becomes mucilaginous, and this mucilage stains very strongly, and makes the sporogenous tissue extremely conspicuous in stained sections.

The young spore-mother-cells are nearly round, with a small nucleus which does not stain very strongly. At an early period the single chromatophore can be easily seen. Owing to its small size the divisions of the nucleus are difficult to study, and no attempt was made to follow out the details of the nuclear division. They probably do not differ much from what has been observed in *Anthoceros* (Davis, The Spore Mother Cells of Anthoceros. Bot. Gazette, xxviii, 1899). Davis found four chromosomes in the nucleus of Anthoceros. As in *Anthoceros*, the division of the chromatophore occurs before the nucleus divides. The nucleus of the young cell lies near the centre, more or less enclosed by the cup-shaped chromatophore

(Fig. 63 a). In the next stage (b) the chromatophore divides into two, which assume an oval form, and show clearly a number of starch granules imbedded in them. The two chromatophores are in Megaceros connected by a strand of fine fibrils, which Davis does not seem to have seen in About this time there is a marked appearance of synapsis in the nucleus (Fig. 63 b), and Davis says that this is a constant phenomenon, and regards it as entirely normal. The two chromatophores divide again and assume a position marking the tetrad arrangement of the future spores. They are connected, as in the case of the two chromatophores, by strands of fibrils, and have very much the effect of the four nuclei connected by fibrils which are seen in the ordinary type of spore division. The nucleus now divides, as Davis has shown, by successive mitoses, with a resting-stage between, and the four nuclei arrange themselves at the four points occupied by the chromatophores, after which the cell-wall separating the four spores of the tetrad are developed. It was noted that before the division began there was already a slight lobing of the cell, such as is so common in the Jungermanniales, marking the position of the spores. This has also been observed by the writer in Targionia.

The outer membrane of the spore becomes somewhat thickened, but remains thinner than is the case in *Anthoceros* and *Notothylas*. In this respect *Megaceros* approaches *Dendroceros*, and the spores agree with those of *Dendroceros* also in the development of chlorophyll, which is wanting in the ripe spores of all typical species of *Anthoceros*.

The elaters, as in Dendroceros, are as a rule composed of several cells, and are simply larger or smaller fragments of the net of sterile cells which This becomes especially clear in M. Salakensis, surrounds the spores. where the elaters are usually branched (Fig. 72). In M. Tjibodensis they are rather more slender and seldom show any branches. The characteristic thickened spiral upon the walls of the elaters first becomes evident about the time that the division of the spores is complete. In both species the ripe spores show fine spinules or papillae upon the outer surface, these being more numerous, finer, and more regular in M. Salakensis. superficial cells of the sporogonium develop thick walls and assume a brownish colour as the sporogonium ripens, but no trace of the stomata characteristic of the typical Anthoceros sporogonium is developed. two species examined differ in the form of the superficial cells of the sporogonium, which are about twice as long in M. Tjibodensis as in M. Salakensis. In the former species there are two to six chromatophores in each cell of the assimilative tissue of the sporophyte, the commonest number being four.

As the sporophyte develops, rhizoids are formed in great numbers from the lower surface of the thallus below the foot of the sporophyte, the growth of which in *M. Tjibodensis* causes a marked swelling upon the lower

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side of the thallus. This is less marked in M. Salakensis. In this species also many rhizoids are formed immediately below the sporophyte. These are presumably concerned in supplying the growing sporophyte with water and possibly with food. It would not be surprising if sometimes the rhizoid-like outgrowths of the foot itself might penetrate into the substratum, but no demonstration of this could be made. The spores of both of the species of Megaceros under consideration are small-much smaller than in most species of the other genera. The contrast is especially great when compared with the large spores of Dendroceros with which Megaceros otherwise most nearly agrees in the character of the sporophyte, as well as in the thin walls and green chromatophore of the spores. In both species examined the ripe spore contains a bright green chromatophore and germinates very promptly. In size the spores are about equal in the two species, measuring about 28 \(\mu \) in diameter. Those of M. Tjibodensis (Figs. 66, 69) are less regular in outline than those of M. Salakensis (Figs. 73, 75), and the surface papillae are coarser and less uniform in size. The elaters of the two species also differ, as we have seen, those of M. Tjibodensis being rather more slender and more seldom branched. They may reach a length of 300μ , or more, and are usually composed of about three cells, although small unicellular ones (Fig. 68) are sometimes found. In M. Salakensis the elaters are almost always markedly branched, and the ends of the branches are often blunt, showing where they have broken away from others, and indicating clearly that the elater is only a part of an extensive net of sterile cells surrounding the spores (Figs. 71, 72).

GERMINATION.

Experiments were made in germinating the spores of *M. Tjibodensis*. The spores germinate promptly, the first signs of germination being usually evident within two days. Within three days the first cell division occurs. Most of the cells of the young plant have a single chromatophore, but occasionally two are found in a cell. No germ tube is found, and the young thallus develops directly from the spore. The first rhizoid is formed earlier than is usually the case in *Anthoceros*.

THE RELATION OF MEGACEROS TO THE OTHER ANTHOCEROTACEAE.

Megaceros shows points of contact with all three of the other genera. The form of the thallus (at least in the species under consideration) is that of a typical Anthoceros, and this is true also of the apical growth which, however, resembles also that of Notothylas. The archegonium is perhaps most like that of Dendroceros, and this is true also of the large solitary antheridium. The sporophyte resembles most nearly in form and in its large size Anthoceros, but in the early divisions of the embryo and the

origin of the sporogenous tissue it is more like *Dendroceros*, with which it agrees also in the green spores, the spiral elaters, and in the absence of stomata. The great development of the sporogenous tissue, as well as the large amount of sporogenous tissue developed in the apical region of the sporophyte, are more like *Notothylas*.

SUMMARY.

- 1. Probably all of the species of *Anthoceros* in which stomata are absent from the sporogonium, and which have spiral elaters, should be separated as a new genus. The name *Megaceros* is proposed for this.
- 2. Two species from Java were carefully studied; these appear to be undescribed, and the names *M. Tjibodensis* and *M. Salakensis* are proposed for them; the former is probably closely allied to *M. Stahlii* (Steph.), but seems to be distinct.
- 3. Both species show multiple chromatophores, and this peculiarity is probably common to all members of the genus. Two tropical American species, M. Vincentianus and M. flavens, show the same character, and it is also known in M. giganteus. In M. Tjibodensis as many as twelve chromatophores may be found in a cell. Pyrenoids were absent in all of the forms studied except M. Salakensis where there was some evidence that a pyrenoid was present.
- 4. The form of the thallus and the apical growth are like those of the typical Anthoceros.
- 5. Both of the Javanese species are monoecious, but strongly proterogynous; the archegonia resemble those of *Anthoceros*, but the large solitary antheridium is more like that of *Dendroceros*.
- 6. The early divisions in the embryo and young sporophyte most nearly resemble *Dendroceros*; the extent of the sporogenous tissue, however, is much greater, and most like *Notothylas*.
- 7. There is no evident relation between the early divisions of the sporogenous tissue and the distribution of the fertile and sterile cells. The latter form an irregular net enclosing the spore-mother-cells.
- 8. As in *Anthoceros* the primary chromatophore of the spore-mother-cell divides into four before the nucleus divides; there is a slight lobing of the mother-cell before division takes place; the spores are small, thin-walled, with fine papillae or spinules upon their surface, and contain a large chloroplast.
- 9. The elaters are multicellular, and closely resemble those of *Dendroceros*; they are branched in *M. Salakensis*, but unbranched in *M. Tjibodensis*.
- 10. There is a large amount of green tissue developed in the sporophyte, but no stomata are present; in M. Tjibodensis the cells of the sporophyte

contain 2-6 chromatophores; the dehiscence of the capsule is either by two valves (M. Tjibodensis), or along one suture (M. Salakensis).

11. The foot is very large, and the surface cells are developed into extensively branched rhizoid-like outgrowths; rhizoids develop in great numbers from the lower surface of the thallus immediately below the foot.

Megaceros, gen. nov.

Thallus large in all of the Javanese species, closely resembling Anthoceros, both in form and in the apical growth; chromatophores several (2-12) in the inner cells, and usually more than one in the superficial ones; pyrenoids are usually absent. Plants (always?) monoecious; antheridia large, solitary; sporophyte large, epidermis without stomata; spores small, thin-walled, papillate or echinulate, at maturity containing a single large chloroplast; elaters either branched or unbranched, multicellular, with a distinct spiral band like that of Dendroceros.

Probably all of the species of Anthoceros in Gottsche's third section of the genus should be transferred to Megaceros.

M. Tjibodensis, sp. nov.

Thallus 2-5 centimetres long; ecostate, 8-12 cells thick in the middle. Margin strongly lobed, and laciniately fringed; monoecious, but strongly proterogynous; antheridia nearly globular, upright, about 135 μ in diameter, short stalked; involucre 10-15 mm. long; sporophyte very long, 6-9 c.; spores 28 \(\mu, \) somewhat irregular in shape, papillate: elaters long and slender, sometimes 300 μ in length, unbranched; epidermal cells of sporophyte long and narrow. Growing on logs, upon the ground, and upon Tjibodas; also below Kandang Badak on the slopes of Mount boulders. Gedeh, Java; April, May, 1906.

Probably near to M. (Anthoceros) Stahlii (Steph.), but differs in the larger size and greater thickness, the larger spores, and much longer elaters.

M. Salakensis, sp. nov.

Thallus smaller than M. Tjibodensis, about 2-3 centimetres long, 8-10 cells thick in the middle, crenately lobed but not laciniate; chromatophores 1-2 in the superficial cells, 2-6 in the inner ones, a pyrenoid probably present in the larger ones; monoecious, proterogynous; antheridia oval, about 180 μ in length exclusive of the curved pedicel which is much longer than in M. Tjibodensis, and attached at the forward part of the antheridial chamber; sporophyte much shorter than in M. Tjibodensis, 3-4 centimetres long; spores 28 \(\mu\), nearly round, and finely echinulate; elaters branched. somewhat thicker than in M. Tjibodensis, epidermal cells of the sporophyte about half as long as in M. Tjibodensis. On a dead log, among mosses; Tjiapus Gorge, foot of Mount Salak, Java; March, 1906.

EXPLANATION OF PLATES XLIV-XLVI.

Illustrating Professor Campbell's paper on Anthocerotaceae.

PLATE XLIV.

Fig. 1. A rather small plant of the typical Megaceros Tjibodensis, with two young sporophytes, sp. × 2.

Fig. 2. An older sporophyte, showing the long involucre, in. \times 2.

Fig. 3. A ripe sporophyte removed from the involucre. Natural size.

Fig. 4. A plant of M. Salakensis, with two young sporophytes. \times 2.

Fig. 5. Ripe sporophyte of M. Salakensis. \times 2.

Fig. 6. Margin of the thallus of the typical M. Tjibodensis. × 25.

Fig. 7. Margin of the thallus of a larger form of the same. x 25.

Fig. 8. Margin of thallus of M. Salakensis. × 25.

Fig. 9. Cells from upper surface of M. Tjibodensis, showing the chromatophores. x 280.

Fig. 10. Cells from the lower surface of the same species.

Fig. 11. Cell from the interior of the thallus of M. Tjibodensis, showing twelve chromatophores. \times 280.

Fig. 12. Cell from the interior of the thallus of a form growing with M. Tjibodensis, but showing a different type of chromatophore. \times 280.

Figs. 13-17. Chromatophores of *M. Salakensis*. × 280. Fig. 13, cells from upper surface; 14, from lower surface; 15, a sub-epidermal cell; 16, 17, inner cells. *n*, the nucleus.

Fig. 18. Cell from the interior of the thallus of M. flavens (Spruce). x about 500.

Fig. 19. Cell from the interior of the thallus of M. Vincentianus (Lehm. and Lindenb.). x about 500.

Fig. 20. Longitudinal section of the apex of the thallus of M. Tjibodensis. × 280. q, a young archegonium.

Fig. 21. Apical region of M. Tjibodensis, showing an approach to the type of Dendroceros.

Fig. 22. Longitudinal section of the thallus of M. Tjibodensis, showing the relative position of the antheridium, δ , and the archegonium, ϕ ; the latter contains a young embryo; N, Nostoc colony. $\times 25$.

Figs. 23-5. Development of the young antheridium of M. Salakensis, seen in longitudinal section. \times 480. b, the basal cell.

Fig. 26. The outer cells of the antheridium shown in Fig. 25.

Fig. 27. Young antheridium of M. Salakensis in which the antheridial chamber is open on one side. \times 480.

Fig. 28. An older stage of the same species. x 280.

Fig. 29. Two sections of a nearly ripe antheridium of M. Salakensis. x 280.

Fig. 30. Nearly ripe spermatocytes. x about 900.

PLATE XLV.

Fig. 31. Median section of a nearly ripe antheridium of M. Tjibodensis. × 280.

Fig. 32. Cross-section of the pedicel of the antheridium.

Figs. 33, 34. Two very young archegonia of *M. Tjibodensis*, longitudinal sections. × about 600. In Fig. 34 the outer cell had divided by a longitudinal wall before the separation of the primary neck canal cell had taken place.

Figs. 35, 36. Two older stages of the archegonium. \times 480. In the one shown in 36 there were four cover cells.

Fig. 37. A nearly ripe archegonium of the same species. x 280.

Fig. 38. Cross-section of the venter of a young archegonium. x 480.

Fig. 39. Two cross-sections of the upper part of the neck of a nearly ripe archegonium. \times 280. Three cover cells, d, are present.

Fig. 40. A mature archegonium of M. Salakensis. x 280.

Fig. 41. Longitudinal section of a fertilized archegonium of *M. Tjibodensis*, containing an eight-celled embryo. × 280.

Fig. 42. An older embryo of the same species. \times 285. *I-I*, the primary wall; *II-II*, the second wall.

Figs. 43, 44. Median sections of older embryos of M. Tjibodensis. \times 280. The nuclei of the archesporial cells are shown.

Figs. 45-7. A series of four transverse sections of an embryo of *M. Tjibodensis*, of about the same age as the one shown in Fig. 44; a is the apex of the embryo.

Fig. 48. Longitudinal section of a fertilized archegonium of M. Salakensis, containing a four-celled embryo. x 280.

Fig. 49. An older embryo of M. Salakensis. × 280.

Fig. 50. Two cross-sections of a very young embryo of *M. Salakensis*. \times 280. The divisions in the lower section, b, are very irregular.

Fig. 51. Series of three transverse sections of an embryo of M. Salakensis. \times 280. The primary wall, I, is indicated in the upper section, a.

Fig. 52. Median section of a young sporophyte of M. Tjibodensis. x 110. The archesporial tissue is shaded.

Fig. 53. The upper part of Fig. 52. × 280.

Fig. 54. Part of a median longitudinal section of an older sporophyte of *M. Tjibodensis*, showing the extent of the archesporial tissue, sp; the columbla, col; and the wall-tissues, w. \times 280.

Fig. 55. Median section of young sporophyte of M. Salakensis. x 110.

Fig. 56. Upper part of Fig. 55. x 280.

Fig. 57. Part of median section of older sporophyte of M. Salakensis. x 280.

PLATE XLVI.

Fig. 58. Cross-section of the sporophyte of *M. Salakensis* at a point where the archesporium consists of a single layer of cells; the nuclei are shown in the archesporial cells. \times 280.

Fig. 59. Archesporium of *M. Tjibodensis*, showing the first differentiation of the sterile cells, el, and the spore-mother-cells, sp. \times 280.

Figs. 60, 61. More advanced stages in the segregation of fertile and sterile cells; Fig. 61 is a tangential section. \times 280.

Fig. 62. Section of the sporophyte of M. Tjibodensis, showing nearly ripe spore-tetrads, sp, and elaters, el. × 280. col, columella; w, wall-cells.

-Fig. 63. Spore-mother-cells of *M. Tjibodensis*, showing the division of the chromatophores. x about 900.

Fig. 64. First nuclear division in spore-mother-cell; the cell is slightly lobed, indicating the position of the spores.

Fig. 65. Two ripe spores of M. Tjibodensis. x 280.

Fig. 66. A single spore, more highly magnified.

Figs. 67, 68. Elaters of M. Tjibodensis. x 280.

Fig. 69. Section of a ripe spore of M. Tjibodensis. x about 900.

Fig. 70. Part of the surface of the spore.

Figs. 71, 72. Elaters of M. Salakensis. × 280.

Fig. 73. Two ripe spores of M. Salakensis. x 280.

Fig. 74. Spore of the same, more highly magnified.

Fig. 75. Sections of ripe spores, \times about 900. b shows the surface sculpturing.

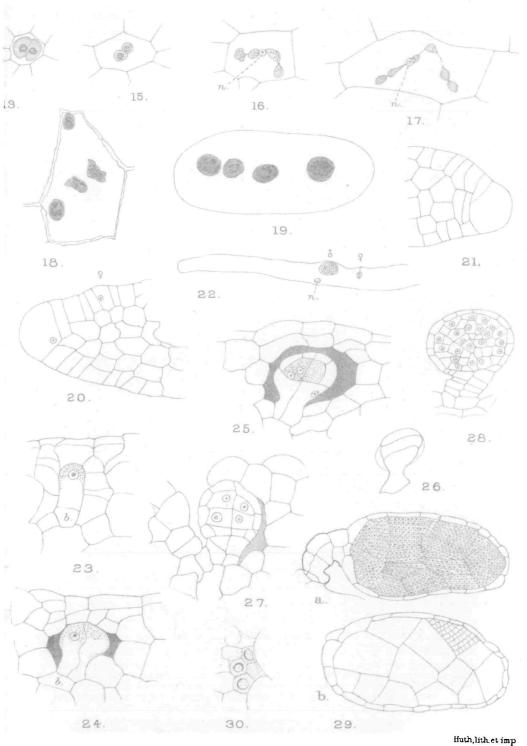
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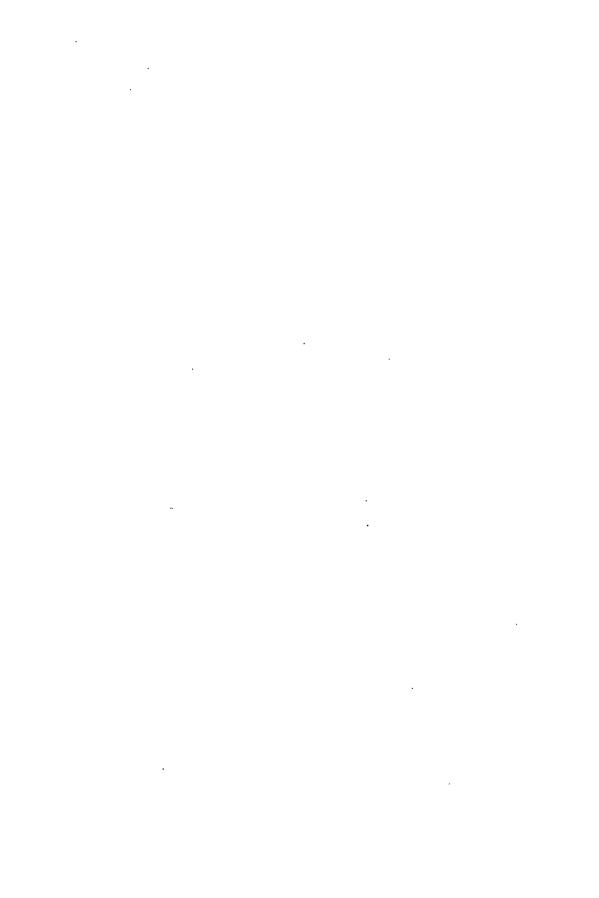


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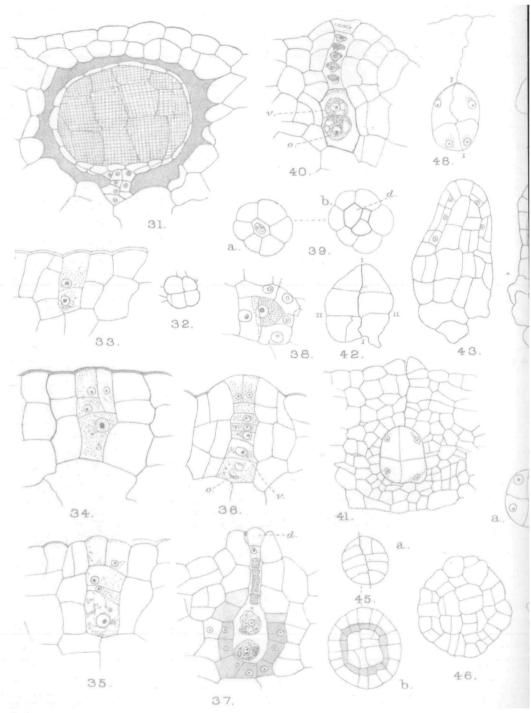
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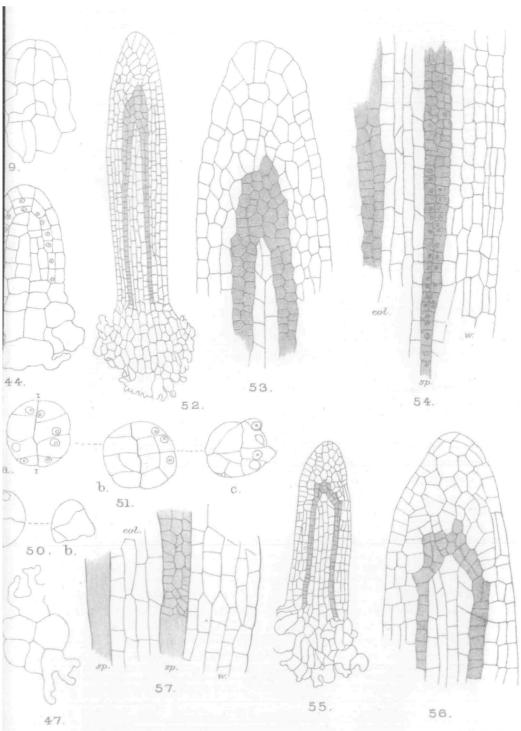
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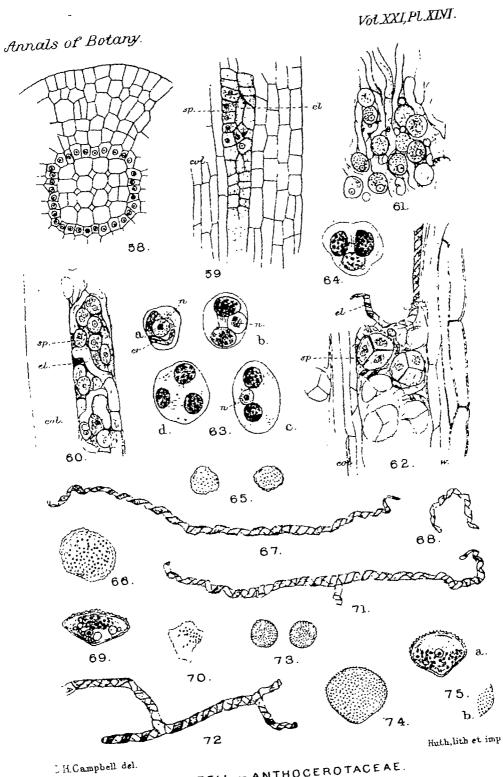
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