THE STRUCTURE AND LIFE-HISTORY OF \textit{AZOLLA PINNATA} R. BROWN WITH REMARKS ON THE FOSSIL HISTORY OF THE HYDROPTERIDÆ.

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The genus \textit{Azolla} was instituted by Lamarck in 1783 on sterile specimens brought from Magellan by M. Commerson (Griffith, 1849, p. 556). It comprises at least four living species, \textit{A. filiculoides}, \textit{A. caroliniana}, \textit{A. pinnata} and \textit{A. nilotica}; there is a fifth species, \textit{A. rubra}, which Strasburger has kept only as a variety of \textit{A. filiculoides}. There are three species of the genus, \textit{A. tertiaria} Berry (1927, 4–5, Pl. I, Figs. 9, 10), \textit{A. intertrappea} Sahni and Rao (1934, 26, 27), and \textit{A. prisca} Reid and Chandler (1926), which are known only in the fossil state. Both \textit{A. filiculoides} and \textit{A. pinnata} are also known in the fossil state (Florschütz, 1928).

The genus has representatives in all the divisions of the globe at the present day. \textit{A. filiculoides} is confined to the western part of America, being reported from as far south as Chile, and reaching to California at least, and probably beyond (Campbell, 1893, 155). It ascends in the Andes to 16,000 ft. (Baker, 1887, 137). \textit{A. rubra} is found in Australia and New Zealand. \textit{A. caroliniana} is found in the southern United States and California, through tropical America to Buenos Ayres. \textit{A. pinnata} is the type in Australia; the variety, which approximates in habit to \textit{A. caroliniana}, is widely spread in tropical Asia and Africa. \textit{A. nilotica} is exclusively African. The accompanying map shows approximately the distribution of the living and fossil species.

Thus the only living species known in India is \textit{A. pinnata}. This species, with \textit{A. nilotica}, belongs to the section Rhizosperma; all the other living species are included in Euazolla. Of the fossil species \textit{A. intertrappea} belongs to Euazolla, \textit{A. prisca} combines the characters of Euazolla and Rhizosperma, and \textit{A. tertiaria} is of doubtful affinity, being incompletely preserved.

**Historical Review.**

The genus \textit{Azolla} has been described by a number of workers: Griffith,* Mettenius, Mayen, Martius, Strasburger,* Berggren*, Belajeff, Campbell* and others. Of these, the works of Strasburger, Berggren and Campbell are by
far the most important. The results of the earlier workers have been given by Strasburger in his monograph. Of the above-mentioned works only those marked with an asterisk have been available to me.

Griffith's work (Griffith, 1884) has only a historical interest. He has given a large number of drawings of the sporocarps of *A. pinnata*. Obviously the sporocarps were a great puzzle to him. His nomenclature for the various organs is archaic, often faulty. His was the earliest work on the Indian species.

Strasburger's monograph of the genus (Strasburger, 1873) although now sixty-four years old, still commands respect as an authentic record of observations, like most of his other works. The details of structure were elucidated chiefly in *A. filiculoides* though other species were also figured. His work deals exhaustively with the anatomy and histology of the mature sporophyte, and also of the full-grown sporangium and spores. He could not study the development of the spores for lack of material.

Berggren's paper (Berggren) deals with the female prothallium and embryo. His figures are mostly rather diagrammatic. Campbell's work (1893, 1928) largely supersedes this, being more complete. It is very important; he has not only summarised the results of the previous workers, but also worked out in detail the life-history of *Azolla filiculoides*, which is thus the best known species.

Pandit and Mulay (1931, 267) who published a preliminary note on the *Azolla* found at Khandala in the Western Ghats (Bombay Presidency) suggest that their plant is a new species or a new sub-genus. This view is no doubt due to their ignorance of the literature. Their account shows that their plant is in no way different from *A. pinnata*.

Mr. Sud's preliminary note (1934, 189-196) on *A. pinnata* is also incorrect in several points, viz., branching of stem, growth of leaf, the time of fertilisation, and the female prothallus (see below under Discussion).

I took up the investigation of the structure and life-history of the Indian species at the kind suggestion of Prof. B. Sahni. A necessity was felt for a comprehensive account of at least one species, other than *A. filiculoides*, if only to confirm the results arrived at for the latter. *A. pinnata* seemed specially suited for such a study, as it belongs to the second sub-genus Rhizosperma.

My observations, though not so complete as Campbell's on *A. filiculoides*, have, however, shown that *A. pinnata* resembles that species in almost all its salient characters.
The basis on which the two sub-genera Euazolla and Rhizosperma have been formulated is the characters of the float-corpuscles and the glochidia. The diagnosis of Rhizosperma and *A. pinnata* given by Baker is incorrect in one particular. He says the roots are fascicled in *A. pinnata*. Strasburger's diagnosis and drawing show that they are single in *A. pinnata*, which my observations also confirm, and fascicled only in *A. nilotica*.

An important point elucidated in my investigations is that the male prothallia of *A. pinnata* are liberated from the massulae.

**Material and Methods.**

First of all a few specimens from Cuttack sent by Prof. Parija were examined; next I examined material from Poona sent to Prof. Sahni by Mr. B. R. Pandit, some from Sagar, Mysore State, collected by me, and also some from Agra sent by Mr. B. M. Johri. Having ascertained that there is only one species known so far in India, further work was conducted on plants collected at Lucknow. The structure was studied mainly by microtoming the entire plants, the small size of the plants being convenient to handle that way. Dissection under the binocular and dissecting microscopes was illuminating. As fixatives I used chrom-acetic acid and sometimes formalin-acetic-alcohol. To sink the plants in the fixative I had to use the exhaust pump. Safranin and gentian violet gave as good staining results as iron-alum hæmatoxylin.

The megasporocarps gave the greatest trouble in cutting. The spore wall is very tough and hard like the chitin of insects. Softening in hydrofluoric acid was helpful to some extent but the innermost spore-wall was almost always broken.

**Description.**

*Azolla* is common in the United Provinces, occurring abundantly in tanks and pools. The "fronds" are triangular or polygonal in shape, measuring about 10 mm. to 25 mm. on the sides (Figs. 1–4). The stem is dorsi-ventral, bearing dorso-laterally two rows of alternate leaves, which are deeply two-lobed. The branching is in a scorpoid cymose manner. At each forking of the stem generally a root hangs downwards, looking feathered on account of the long root-hairs, whence the specific name. The sporocarps are formed from the ventral lobe of the first leaf of a branch. After a period of vegetative multiplication by fragmentations of the plants, a normal sexual regeneration occurs. The diagnosis of the Indian plant is as follows:—

*Azolla pinnata* R. Brown.—Roots single and conspicuously feathered on account of the root-hairs. Fronds oblong or deltoid, 10 mm. to 25 mm.
long, with numerous crowded primary branches, all simple or the longest with a few crowded primary branches towards the tip. Leaf lobes firm in texture, broad-ovate. Macrospore crowned with nine float-corpuscles, its cuticle finely granular, armed with a few clavate papilae. Massulae more than two in the microsporangium, with only a few weak simple or branched processes on one side (Baker, 1887, 138; Strasburger, 1873, 79).

A. nilotica differs from A. pinnata, along with which it is grouped in the sub-genus Rhizosperma, by its wide trailing leafless stem, with dense fascicles of roots from its nodes. The sub-genus Euazolla, under which are grouped A. filiculoides and its var. rubra, and A. carolimana, differs from Rhizosperma in having the macrospores crowned with three float-corpuscles; massulae armed all round with rigid glochidiate processes.

As far as my observations show, the life-history of A. pinnata at Lucknow runs roughly as follows: Fresh embryo plants come up about September or October. They are green at first, but acquire a brownish colour in the cold season on account of a pigment formed in the leaves. The plants produced from the sporocarps mature in spring. By about April the sporocarps are ripe and are separated from the mother plants, which soon die off. During summer the plants are almost completely absent except some which remain vegetating where there is deeper water. A normal sexual method of regeneration occurs after the rains, i.e., at about the end of September. During the vegetative season—September to April—the plants rapidly multiply by fragmentation. This happens both through the mechanical action of the waves, and by the insects, snails and other animals nibbling at the plants. This is further facilitated by the formation of an absciss layer (Fig. 20) at the bases of the branches. There is a similar absciss layer of smaller cells at the bases of the roots (Fig. 27) and hence they are deciduous when mature.

Stem.

The stem apex is curved, upward and backward (Fig. 11) reminding us of the circinate vernation of the fern leaf. There is a two-sided apical cell from which segments arise right and left. The first division of the apical cell is horizontal, i.e., parallel to the upper and lower surfaces (Fig. 11), the second in the sagittal plane. A transverse section through the stem at this stage shows four cells like the quadrants of a circle. The dorsal segments develop the leaves, the ventral ones the lateral branches and roots. Strasburger’s drawings show with great clearness the successive divisions at the stem apex. The third division forms the octant walls. The fourth one is periclinal and lays down the central cell-complex which develops the conducting strands.
The mature stem shows a central vascular bundle (Fig. 10). The structure is very simple, there being only three or four spiral tracheids and a few phloem elements. The endodermis is clearly seen, though the Casparian thickenings are not marked. There are only a few air-spaces in the cortex, which open out by means of stomata (Fig. 12). Aeration of the plants is more efficiently carried out by the leaves.

Branching is extra-axillary, i.e., having no relation with the leaf axils. The lateral branches arise in acropetal order, but not always at equal intervals. Their development is a repetition of the main axis. At the bases of the branches there is an absciss layer composed of smaller cells. This does not seem to have been noticed by the previous workers. On account of this the plants easily fragment at these places (Fig. 20).

The stem, like the dorsal lobes of the leaves, is covered by hairs (Fig. 12). At the stem apex, among the lateral organs, are found a few large hairs (Fig. 11) with a large basal cell, bearing branches at the distal end. Tangles of Anabaena are always found at the stem apex and enter the leaf-cavities while these are being formed.

The dorsal lobes of the leaves so closely overlap each other like the tiles of a roof, that the stem is not visible when viewed from above. The under-surface is similarly covered by the lower lobes (Figs. 2, 4).

**Leaf.**

The leaf arises from the upper segments of the stem quadrant, as described by Strasburger and Campbell in *A. filiculoides*. Only the alternate cells on the right and left produce leaves, resulting in alternate rows of leaves. Thus, though the apical segmentation is regular, there is no constant relation between the formation of the segments and the origin of the appendages. This is the case in all Pteridophytes where the apical segmentation is most regular (Bower, 1908, 177). The mother-cell of a leaf is distinguished by its size and position. The first wall divides it into an inner small cell and an outer larger cell. The latter forms the upper and the former the lower lobe of the leaf. Each leaf-lobes is next divided into an inner and an outer cell. The latter divides into two by a radial wall. Then succeed alternate radial and tangential walls which are repeated with great regularity and can be seen in the young stages of the leaf (Fig. 7). No apical cell in the leaf is produced in *Azolla*, an exceptional feature in ferns.

An invagination is formed in the dorsal lobe at this stage. *Anabaena* cells already begin to enter it. This invagination (Fig. 14) later becomes the *Anabaena*-cavity of the leaf. The wall of the cavity bears a number of characteristic large hairs, which consist of a basal cell like the holdfast of
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an alga, from the bulged distal end of which generally a few-celled branch arises at a rather small angle (Figs. 16-19).

The mature leaf is deeply two-lobed (Fig. 13). The lower lobe covers the stem on the ventral side, and is in contact with the water. It is simple in structure, one-layered for the most part except in the middle near the base, where it becomes a few cells thick and contains pigment. There is a small cavity in the thickness of this lobe near the base. This, however, does not open on the outside, and encloses no Anabæna (Figs. 21, 22). The ventral lobe is broader than the dorsal.

The dorsal lobe of the leaf is aerial. It has a complex structure (Figs. 13, 21, 22). It is ovate in shape, thick and firm. The dorsal lobes overlap each other like the tiles of a roof. The lobe is thick in the middle, with a thin colourless margin, one cell thick and 4 to 5 cells broad. The upper surface of the leaf is covered by turgid, colourless, two-to four-celled hairs, borne obliquely forward and upward. These hairs hold air-bubbles among them, and thus the plants cannot be made to sink at all. Even an exhaust pump does not make the plants sink readily.

Both the upper and lower surfaces of the dorsal lobe bear a number of stomata, whose form is shown in Figs 13 and 15. These are peculiar, simple perforate cells as described by Sud, the pore being slitlike, reminding one of the stomata on the apophysis of Funaria (Campbell, 1928, 410, Fig. 239 b).

There is a peculiar, large, hollow cavity in the dorsal lobe of the leaf, which opens by an aperture on the lower surface (Figs. 13, 14, 21, 22). As mentioned above, it is formed as an invagination in the leaf-lobe into which Anabæna cells creep in. When the leaf matures the cavity becomes larger, its mouth narrows down to a small aperture, somewhat elongated in the longitudinal direction. The aperture is lined by three rings of tooth-shaped cells (Fig. 21). Thus it reminds one somewhat of the stomatal apertures of Marchantia. The wall of the cavity around the aperture is formed of two layers of elongated, colourless cells, radiating from the aperture (Fig. 13). The layer of cells lining the cavity bears a few hairs (Figs. 16–19), which consist of a basal cell like an algal holdfast, the bulged distal end of which bears one or more branches, a few cells in length. These branches hold the Anabæna tangles, which do not immediately line the cavity, but are held a little way towards the centre by these hairs.

The upper epidermis of the dorsal lobe, as mentioned above, bears stomata. These open into air-spaces interspersed in the palisade tissue. The latter is usually one layer thick. Its cells contain chloroplasts lining its walls. Below the palisade is the mesophyll tissue, which also lines the
Anabaena cavity. There is only a feeble development of conducting elements in the leaf, represented by only a few spirally thickened tracheids.

The leaves are green early in the season. By about November the plants turn brownish red on account of the red pigment produced in the cells of the leaf.

Root.

The root is produced from the lower segments of the stem, resulting from the quadrant division. The root initial cuts off a cell superficially which again divides into two. There is an oblique wall formed in the initial, cutting off the apical cell. After the next three divisions, the three-sided pyramidal apical cell is already formed, with the outer wall bulging out. When only two of these divisions have taken place, a transverse section of the apical cell is approximately 4-sided.

Azolla is distinct from other Filices in the mode of development of the root-cap (Strasburger, 1873, 44-46, and Figs. 53-72, Pls. 4 and 5). The first superficial cell cut off by the root initial forms two root-sheaths, the inner of which disorganises. Before the disorganisation, however, the apical cell cuts off two layers of cells, so that at this stage the apical cell is enveloped by four layers which are later reduced to three by the disorganisation of the inner root-sheath (Figs. 23, 29, 31). Strasburger too showed this feature clearly.

In the nearly adult root two root-sheaths are still found covering the root-tip, but they were broken off at the base and carried forward with the root-tip as the root elongated. There is a tube-like collar at the base of the adult root, which is the remnant of the torn-off outermost root-sheath (Fig. 4).

The initials of the root-hairs arise just behind the apex and under the innermost root-sheath (Figs. 26-27). As the root completes its development, the apical cell itself becomes divided in several parts, each one piliferous (Fig. 28). The mature root-hairs are 2-3 mm. long, with a nucleus near the tip. The tip is lined by granular contents. The root-hairs have a bulge at the base and a characteristic knee-like bend (Fig. 28). In a young root the tangential section of the root-hairs gives a remarkably regular pattern (Fig. 24).

The root is formed on a constant trimerous plan. The transverse section shows that the cells are in multiples of three. The endodermis cells are a multiple of three, so also the root-hairs themselves. The vascular structure is very simple as in the stem, there being only two or three spiral tracheidal elements. When a root is seen under the microscope the air
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contained in the tracheids shows as dark lines, like the mercury line of a thermometer.

The mature roots are deciduous. After they attain a length of 40-50 mm, they drop off. The root-hairs provide accommodation for a large number of protozoa, algae and soil particles. The roots contain some green pigment when young. The older roots look white. The deciduous roots come off with a neat end, owing to the presence of a basal absciss layer (Fig. 27) as in the case of the deciduous branch (Strasburger, 1873, Fig. 71, Pl. 5).

Sporocarps.

The sporangia are borne inside the so-called sporocarps, which are generally regarded as basipetal indusiate sori on the analogy of the Gradate ferns. The sporocarps arise from the ventral lobe of the first leaf of a branch, the dorsal lobe forming an involucre enveloping the sporocarps (Figs. 33, 40, 41). The sporocarps are borne in pairs, either both of the same sex, or one male and the other female (micro- and mega-sporocarps). At their base are found a number of filamentous hairs (Figs. 34, 41, 43). The sporocarps receive a vascular bundle which penetrates a short way up the stalk (Fig. 43).

The leaf-lobe, when its first median division occurs, at once begins to develop the two sporocarps. The two sporocarp initials grow by means of a three-sided apical cell for a short time. When three series of segments are cut off a ring-shaped projection arises about their base (Fig. 35). This is the beginning of the indusium (Figs. 37, 41, etc.). The apical cell of the sporocarp now cuts off an opercular cell and thus becomes a terminal megasporangium (Fig. 35). The ring-like outgrowth at its base becomes two-layered and grows up simultaneously with the sporocarp, so that by the time the primary tapetal cells are formed, the indusium stands as high as the sporangium. Now from the base of the sporangium arise all round a number of papillate outgrowths (Fig. 36), the cells containing dense protoplasm like the tapetal cells and the central cell of the sporangium. These are the beginnings of the microsporangia. The sporangial capsule forms the one-layered tapetum and eight megaspore mother-cells. Shortly after the microsporangial protuberances appear, the walls of the tapetal cells of the megasporangium dissolve. The tapetal nuclei lie around the now separated and rounded off spore mother-cells (Figs. 36, 37).

The megaspore mother-cells divide and form eight tetrads. The indusium now grows up to enclose the megasporangium, all except at the top. Anabahana cells enter through this opening and lie on the top of the megasporangium (Figs. 39, 41, etc.). From this point onwards the two kinds of sporocarps begin to be differentiated.
Megasporocarp.

In the megasporangium, thirty-one of the megaspores abort, while only one (Figs. 38, 42), which usually holds the central position, continues to develop. The megasporangium increases in size. Its stalk ceases growth, as also the young microsporangia at the base of the megasporangium. As the latter grows in size, it completely fills the sporocarp and the microsporangia are squeezed down against the stalk until they become hardly recognizable.

The functional megaspore increases in size (Fig. 42). It is surrounded by densely granular protoplasm in which can be seen the tapetal nuclei. A beak-like projection forms on the top of the spore. This is the beginning of the conical body at the top of the spore, which gets surrounded by the float-corpuscles.

The ripe female sporocarp (the megasporocarp) is about 1.5 mm. × 1 mm. It is pointed at the tip. The cells of the upper half of the indusium become hardened, lignified, and dark-coloured, so that after the lower part decays, these upper, dark-coloured cells remain as a conical cap at the top of the spore, until it is pushed aside by the growth of the embryo (Figs. 58, 64, 66). The thickening of the cell-walls of the inner layer of the cap is peculiar (Fig. 65). It takes the form of hair-like growths into the cavity of the cells.

A longitudinal section of the mature sporocarp (Fig. 45) shows that the spore with its float-corpuscles fills the sporangium completely, and that the latter is in close contact with the resting cells of Anabaena. The megasporangial wall is at this place represented by the membrane which bears numerous hairy appendages, and which becomes cup-shaped (Fig. 64) when the indusial cap is pulled off.

The ripe megaspore is more or less globular, with a firm yellowish exospore, which in sections is seen to be radially striated (Fig. 45). Outside this are two more epispore walls the middle one of which is granular in section, which at the top of the spore forms the spongy conical structure, which is surrounded by the float-corpuscles. The outermost epispore wall, which has a densely granular structure, bears curious, rigid, fluffy-looking bodies (Fig. 47), which later become rod-shaped (Figs. 48, 49). Thread-like outgrowths arise from the bases of these rods. (For the origin of these episporic appendages see the Discussion, p. 189.)

The megaspore is capped by the float-corpuscles, which form the "Swimming apparatus" of Strasburger (Strasburger, 1873, 66, and Campbell, 1893, 163). These consist of nine parts in three groups, i.e., a ring of six below and of three above. The lower floats are slightly sunk in shallow
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Cavities in the sides of the central, spongy, conical structure. From the latter, as well as from the apex of the floats, filaments, like those growing from the papillae on the surface of the spore, are produced in large numbers. The compartments of the floats which look like the cells of a honeycomb, are not cellular in nature. They arise by vacuolation in the protoplasm of the megasporangium. The similarity in structure between the massulae and the epispore of the megaspore warrants the conclusion that the two are homologous. The threads attached to the epispore may "morphologically as well as physiologically be compared to the glochidia" (Campbell, 1893, 164).

Sometimes a cleft may be seen extending upward part way through the central conical mass, as Campbell (1893, 164) also states of A. filiculoides.

The megasporocarps become ripe by about April and are liberated from the mother plants. They float about for some days and then sink to the bottom. At this stage the indusial wall disorganises, all but the cap. The megasporocarps remain at the bottom till September.

Microsporocarp.

We have now to trace the development of the microsporocarp from the stage at which the male and female sporocarps were yet undifferentiated. As above stated, a terminal megasporangium is developed in both the sporocarps. In the microsporocarp, however, this degenerates. This happens only after the tetrad divisions of the megaspore mother-cells have taken place. Thus there is no doubt that this is an abortive megasporangium, as Strasburger and Pfeiffer (1907) concluded from their study of A. filiculoides. Its contents shrivel up, to the form of a few granular condensations (Figs. 43, 57). This structure has been referred to by some authors as the columella (Pfeiffer, 1907, 451), but this is confusing because this term was originally applied by Strasburger to the elongated receptacle on which the microsporangia arise (Strasburger, 1889, 8, and Fig. 1 ma, Pl. 1).

As in the case of the megasporocarp, papillae are formed on the stalk of the megasporangium at its base, the beginnings of the microsporangia. Their growth was arrested in the megasporocarp; but in the microsporocarp, many more of these are formed in basipetal succession, and develop into the microsporangia.

A young microsporocarp, soon after it has begun to develop into one, can be distinguished as it is broader than the megasporocarp of about the same age. The Anabena cells creep in at the top of the indusium in the usual way. The indusium is two layers thick as in the female sporocarp. Its tip is thickened and hard as in the female sporocarp, but here it is short and abrupt. The body of the mature sporocarp is globular, only slightly
losing its shape by being pressed against its fellow, during the increase in size of both. The microsporocarp is more than twice the size of the female (Fig. 46). Like the latter it receives a vascular bundle into its short stalk.

The microsporangium initial has dense contents. Its development proceeds in the typical leptosporangiate manner. The first division is oblique (Figs. 50–52), producing an apical cell which after forming two further cells becomes a three-sided apical cell. The next division is periclinal, cutting off an opercular cell enclosing the apical cell. The latter then cuts off a layer of tapetal cells (Fig. 52), and itself undergoes division till a 16-celled tissue is built up. These sixteen cells are the microspore mother-cells. They undergo tetrad-divisions; the tapetal cells lose their walls and their nuclei divide into a large number and help in the nutrition of the sporogenous cells.

In the meantime the sporangial stalk elongates. It is composed of a row of cells, two cells in thickness (Fig. 53). Branching of the microsporangial stalk is sometimes seen, the branch bearing either another microsporangium or a sterile cellular filament (Fig. 53), which might be regarded as a sterile microsporangium.

Sixty-four microspores are developed. They remain thin-walled, with very little of granular contents. A clear triradiate mark is visible on their surface. The ripe spore is about .035 mm. in diameter.

A very large number of microsporangia are produced in the microsporocarp, a median section of a full-grown specimen showing as many as thirty.

When the spores are nearly mature the formation of the massulae begins (Fig. 56). In each sporangium there are formed 3–6 of them. They arise by vacuolation of the hardened protoplasm, resulting in a foamy appearance. The tapetal nuclei lie around the massulae, and they later develop—partly at least—the glochidiate processes on the inner sides of the massulae, *i.e.*, along the faces where adjacent massulae are in contact, as shown by Strausburger (1889, 17) in *A. filiculoides*. The glochidia themselves partake of the vacuolate structure of the massulae. They are of various shapes; some simple processes, others branched and hooked (Fig. 55).

The ripe microsporocarps are liberated from the mother plant. They are red when shed but they soon lose the colour. They float about for some time and then sink to the bottom. The wall disorganises, and the bunches of microsporangia can be seen for a long time, at the bottom of the water. Later the sporangia get detached, their walls also disorganise and the massulae containing the spores are set free. Massulae were found both
floating and sunken in September. The floating ones examined did not show the germination of the microspores. Some of them, however, were quite empty of spores, indicating a liberation of antheridia.

Germination of the megaspores.

The megasporocarps remain at the bottom of the ponds during the summer. They germinate after the rains.

As stated above, the indusium of the sporocarp disorganises all but the thickened tip which remains as a mitre on the top of the float-corpuscles.

The ripe megasporocarp is full of oily contents which escape as a cloudy mass when a sporocarp is punctured under water. The oil, no doubt, is the food reserve.

I have not seen how the first divisions take place in the development of the female prothallus. From sections examined of the mature prothallus it is found that it fills only the upper part of the spore-cavity. Berggren's observations (Berggren, Pl. 1., Figs. 13, 15, 16, 19 and 21) on \textit{A. caroliniana}, and Campbell's (1893, Pl. 8, Figs. 43–49) on \textit{A. filiculoides} show that in both these species the condition is as here described in \textit{A. pinnata}. Mr. Sud's (1934, 195, Fig. 9) statement that in \textit{A. pinnata} the prothallium fills the whole cavity of the megaspore is incorrect. I have not been able to ascertain when the germination of the megaspore begins: whether before the detached sporocarps sink to the bottom or after.

When the prothallus is fully developed and the archegonia are formed, the sporocarp floats up to the surface. The enlarging prothallus makes its way through the tri-radiate opening at the top of the spore. It pushes aside the floats and thus all the nine of them become separated from one another and project outwards, the lower six forming a ring below (Figs. 58–62, 64) showing six floats symmetrically placed, and the upper three in a ring above. The indusial cap is still at the top in an erect position.

I put some sporocarps in Knop's solution* for algal culture, together with some microsporangia and massulae. The sporocarps floated up to the surface after about fourteen days. And within three days of this the young embryo plants had already developed in the same dishes.

* KNOP'S SOLUTION.

1. Magnesium sulphate .. 0.25 gm.
2. Calcium nitrate .. 1.00 gm.
3. Potassium phosphate .. 0.25 gm
4. Potassium chloride .. 0.12 gm.
5. Ferric chloride .. 1 drop.
6. Water .. 1\,000 c.c.
A large number of archegonia are produced in *A. pinnata* (Fig. 73), unlike in *A. filiculoides* (Campbell, 1893, Figs. 49–51, 54, 55, 60 and 61), or *A. caroliniana* (Berggren, Pl. 1, Figs. 4–6, 10, 11, 13 and 15). More than thirty were noted in one case (Fig. 73). The oldest archegonia are at the centre, where the prothallus is somewhat depressed. The venter is rather large, and adjacent archegonia are often separated by only a single layer of cells (Fig. 63). The neck is formed of two rings of four cells each which are radially senate (Fig. 73) so that when seen from above they look like two concentric circles traversed by a cross. The contents of the archegonia were not preserved in all the preparations. Only one of them seems to show a fertilisation stage (Fig. 76).

The prothallial cells are thin-walled and contain chlorophyll (Fig. 67).

**Male Prothallus.**

I have not been able to follow the germination of the microspores. Mature male prothalli, however, are seen in large numbers in sections of the female prothallus developing the young embryo. The male prothalli were lying freely on the surface of the female prothallus, in the neighbourhood of the archegonia (Fig. 68).

An important point which my investigations have brought out is about the male prothallus. In *A. pinnata* the prothalli escape from the massulae which get hooked on to the megasporocarp (Fig. 64), and come to lie about the female prothallus. Campbell (1893, 169) who studied the germination of the microspore and the development of the antheridia, did not see the dehiscence of the antheridia. He says that the ripe prothallium remains completely embedded in the substance of the massulae, and conjectures that probably the spermatozoids escape by a softening of the outer surface of the massula. Judging from the case in *Salvinia*, we should expect that in *Azolla* also the male prothalli escape as a whole from the massula as they do in *Salvinia*, where they are said to be easily detachable (Campbell, 1928, 400). The facts observed in *A. pinnata* fulfil this anticipation and suggest that the same is the case in all the other species of *Azolla*.

**Fertilisation.**

Fertilisation most probably takes place at the surface, after the female sporocarp has floated up. The archegonia of the prothallus which just came up to the surface showed no fertilisation. Campbell (1928, 414) says the term "Swimming apparatus" applied by Strasburger to the float-mass is a misnomer, as the sporocarps sink to the bottom when they are shed from the mother plants. If this were true, it would be hard to explain their presence. I think that the term is still applicable, but that the floats become really
functional when the female prothallus has developed and the archegonia are ready for fertilisation.

Embryo.

I could not follow the development of the embryo plant in its early stages for lack of material. As the embryo develops it pushes aside the indusial cap, which at this stage is thus tilted at 90° (Figs. 58-62, 64, 66). The megasporocarp remains attached to the young plant till it has produced 8-10 leaves. The "foot" remains embedded in the archegonium. The lower walls of the cells of the foot, i.e., where it is in contact with the floor of the archegonium, becomes somewhat thickened (Fig. 70).

The development of the stem-tip of the embryo plant is similar to that of the adult plant. I could not study the development of the first leaf or "cotyledon". The first root of the embryo grows obliquely on one side (Fig. 74). The lateral branches are formed by the young plant before it has produced 8 or 10 leaves.

The resting cells of Anabena present at the tip of the indusium get into the cavities of all the leaves, except the cotyledon. As Campbell describes (Campbell, 1893, 182) "they assume the blue-green colour of the active cells, elongate and divide rapidly by a series of transverse walls into short filaments that at first look like Oscillaria". The cells are then rounded off, the heterocysts are formed and the typical form of the ordinary filaments is attained.

Discussion.

In his preliminary note on A. pinnata Sud (1934, 190) says that his observation about branching of the stem differs from that given by Engler and Prantl (1902, 1 Teil, 4 Abt., 401). He has mistaken the term "extra-axillary" and says "that is, here, a lateral branch is said to rise opposite a leaf". The term only means that branching is not in relation to the axil of a leaf, as Sud himself has observed (Sud, 1934, 190, Fig. 1). He also says that the growth of the leaf, at least in the earlier stages, is by means of an apical cell. I have been able to confirm Strasburger's (1873, 41) statement that there is no apical cell in the leaf of Azolla. The growth is by means of divisions of the marginal cells of the leaf, a kind of growth seen also in the development of the indusial wall. In a section of a young leaf, one of these marginal cells is necessarily found at either end of a section (Sud's Fig. 5b); these marginal cells Sud has mistaken for apical cells.

Sud's drawing of the female prothallus is incorrect. He says that the prothallial cells fill the whole of the spore-cavity (Sud, 1934, 195, Fig. 9): "in the lower part the cells are smaller in size and in the upper they become
elongated and narrow. The latter are similar to the prothallus cells of *A. caroliniana* figured by Berggren". The fact is that the upper part alone is the true prothallus. The lower part, as his drawing shows, is not cellular tissue at all. He has mistaken the granular condensations of protoplasm and food matter for cells.

Sud did not observe massulae attached to the megasporangia (Sud, 1934, 195) and conjectures that the glochidia are rudimentary and do not serve for attachment. My observation shows (Fig. 64) that the massulae get attached to the megasporangia as is known in *A. filiculoides* (Campbell, 1893, Fig. 75, Pl. IX) and *A. caroliniana* (Berggren, Fig. 2, Pl. 1).

The stoma of the mature leaf in *A. pinnata* is a simple perforate cell. In *A. filiculoides* Strasburger has shown two septa across the guard-cells. Haberlandt (1914, 469) says that in *A. caroliniana* also the 'pore is elongated at right angles to the plane of the septa between the two guard-cells and these septa finally become partially or entirely obliterated'. I could not see any such septa in my preparations of *A. pinnata*. It may be that I did not come across young enough stages.

The constant association of *Anabaena* with *Azolla* is interesting. I have never seen a leaf-cavity or a megasporocarp of *Azolla* which does not contain *Anabaena*. But the physiological aspect of the relation between the two plants needs critical investigation. Neither of the plants seems to suffer by this association. Whether they are mutually beneficial is not known. Judging by the presence of *Anabaena* about the extreme apex of the stem and of an early development of an invagination in the dorsal lobe of the leaf, it would seem as if the *Anabaena* induces a stimulus to the formation of the cavity. The *Anabaena* has become closely adapted in conformity with the life-history of *Azolla*; the cells entering the sporocarps become resting cells just as the sporocarps are also resting organs. The resting cells of the *Anabaena* germinate when the embryo plant develops.

The origin of the involucre enveloping the sporocarps has been a subject of controversy. Strasburger held that the sori represent the transformed leaf-lobes, and conceived the involucre as the lower lobe of a leaf, while Campbell came to the conclusion that the whole of the ventral lobe goes to form the sori and that the involucre is derived from the whole of the dorsal lobe. Goebel (1918, 1133), who examined this point again, says that the sporophyll results from a division of the lower lobe, which occurs early; the whole of the upper lobe does not form the involucre, but remains as before, receives a vascular strand, and possesses an *Anabaena* cavity; and at its base it produces a wing-like flap which envelopes the sori. My observations confirm those of Goebel (Fig. 40).
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Strasburger calls only the outermost sheath around the root as the root-sheath (Strasburger, 1873, 45) while the two inner he calls the root-cap proper. In all typical roots the root-hairs arise behind the root-cap. In Azolla the so-called root-cap envelopes the whole root, root-hairs and all. I think it would be more appropriate to call all the three layers by the name root-sheaths. The root-sheath is a peculiar analogue of the root-cap, but is not a true root-cap. The older workers called the two inner sheaths a root-cap proper and the outermost a root-sheath as if for fear of saying that there is no root-cap developed in Azolla, while they were aware that the whole structure was unique.

The appearance of a primary megasporangium in both mega- and microsporocarps shows that perhaps originally the sporocarps were bisexual. The degenerate megasporangium in the microsporocarp also indicates that the sporangial primordia about the megasporangium are microsporangia (Pfeiffer, 1907, 451).

The microsporangial stalk is seen branching in some cases, the branch bearing either another microsporangium or a sterile cellular filament which may be considered an abortive sporangium. This occasional feature recalls the branched sporangial stalks of Salvinia.

There is no trace of an annulus in the sporangia. This is perhaps to be attributed to the aquatic habit of the plant. This is the case in all the Hydropteridaceae.

In the megasporangium the spore embedded in the epispore and capped by the spongy conical body, and the float-corpuscles are to be homologised to the massular divisions in the microsporangium. The conical body may be regarded as the homologue of the tri-radiate mark. As Campbell (1893, 164) says “the threads attached to the epispore may morphologically as well as physiologically be compared to the golchidia”. The spore capped by the conical structure and surrounded by the epispore is to be regarded as the single functional spore of the single fertile massula. The floats may be regarded as sterile massulae.

Strasburger and Campbell agree that the episporic thread-like appendages are formed by the tapetal nuclei and protoplasm. A reference to our Fig. 65 showing the peculiar thread-like thickenings arising from the cell-walls of the indusial cap suggests that the appendages have perhaps partly at least been contributed by the thickening in the megasporangial wall.

Several botanists have suggested an analogy between the megasporocarp of Azolla and the seed of the spermaryophytes. Eichler (in Englcr and Prantl, 1889, Teil 2, Abt. 1, p. 16) has used the term “monangic sorus” for an ovule
and regards the integument as homologous with the indusium. The seed-like modification of the megasporocarp is a much more modern contrivance than the seed (Arber, 1906, 229).

The present study has shown that while on the whole the structure and life-history of *A. pinnata* are very similar to those of the other species described, there are important differences in detail.

The genus *Azolla* holds a somewhat isolated position. Its nearest ally is unquestionably *Salvinia* with which it is placed in the family *Salviniaceae*. The *Salviniaceae* have their affinities with the homosporous Filices, or the lower members of the leptosporangiate series, and probably with the Hymenophyllaceae—Gradate Marginales of Bower (1928, Vol. 3, p. 262). The *Salviniaceae* and Marsileaceae, both heterosporous Leptosporangiatae, have had a special line of development, differing from that of the other Filices, as a consequence of their aquatic or amphibious habitat.

**Fossil History of the Hydropterideae.**

It may not be out of place here to give a sketch of the fossil history of the Hydropterideae. It will be of interest on account of the extreme specialisation of the group.

*Salviniaceae.*—*Salvinia* is represented by several Tertiary species. Florin (1919) has given a list of ten such species, nine of them ranging from Eocene to Mio-Pliocene, and one from what may be the Upper Cretaceous rocks of Carbonado, Washington (Seward, 1910, Vol. 2, p. 262). There are several others in addition to these (Berry, 1930, Kirchheimer, 1929, 1930, 1931 and 1932). The species for the most part have been founded on leaves only, a few on sporocarps alone, and a very few on complete specimens.

Three extinct species of *Azolla* have been described, *A. intertrappea* Sahni and Rao (Sahni and Rao, 1934, Part 4, pp. 26-27) from the Deccan Intertrappeans of India, *A. tertiaria* Berry (1927, 4-5) from Western Nevada and *A. prisca* Reid and Chandler (1926, 407), from the Oligocene of the Isle of Wight. Both *A. pinnata* and *A. filiculoides* have been recorded in the fossil state from Pleistocene deposits in Holland (Florschütz, 1928). These two present-day species have not been found in rocks earlier than the Pleistocene. The Indian species *A. intertrappea*, which as Sahni (1934 a, 134-136) has shown is probably of Eocene age and therefore the oldest known species of the genus, shows characters resembling the species assembled under *Euazolla*, viz., in having three "floats" and anchor-like glochidia. This supports the view above expressed that the present-day Indian species, *A. pinnata* has been derived from the Tertiary species which resembles *A. filiculoides*. Also, the nine-floated "swimming apparatus" seems to be more
recent than the three-floated condition. *A. prisca* Reid and Chandler (1926) combines the characters of the two sections of *Azolla*, *Euazolla* and *Rhizospermae*. The occurrence of glochidia and the tubercled filament-bearing macrospore shows that the fossil is related to the *Euazolla*—more particularly to the species *A. fliculoides*. But in the presence of nine floats to the macrospore it resembles the *Rhizospermae*. Probably, therefore, it represents an ancestral type in which features now distributed were combined. Reid and Chandler, however, say that it must belong to an earlier stage of evolution than the two sections.

As regards the Palaeozoic genera *Traquairia* Carr. (Mrs. Scott, 1911, 459-467, Pls. 39-40) and *Sporocarpon* Will., Prof. Seward says that comparisons have been made with the reproductive organs of *Azolla*, but that these rest on a wholly insufficient basis (Seward, 1910, Vol. II, 476). Similarly in the case of *Protosalvinia* Dawson and *Chorionopteris* Corda a relation with the Hydropteridaceae has been suggested on insufficient evidence (Seward, 1910, Vol. II, 476).

*Marsiliaceae.*—The fossil history of the Marsiliaceae is more uncertain. The Wealden genus *Marsilidium* Schenk (Seward, 1910, Vol. II, 474) cannot be regarded as satisfactory evidence of the family in the early Cretaceous flora. The Cretaceous *Marsilia Andersoni* Hollick (Seward, 1910, Vol. II, 474) is too fragmentary to be accorded that generic name. The fragment figured by Heer from the Tertiary rocks of Oeningen as *Pilularia pedunculata* is too small to determine with reasonable accuracy (Seward, 1910, Vol. II, 475). A few other fossils are still more doubtfully assigned to *Marsilia*.

The Mesozoic genus *Sagenopteris* seems to have had a long-standing claim to be compared with *Marsilia*. It has, however, recently been shown by Hamshaw Thomas (1925) that *Sagenopteris* leaves are in all probability the foliage of plants which bore reproductive organs indicating affinity to the *Caytoniales* (Seward, 1931, 309, 367, etc.).

According to Harris (1931, 139) "The small ovoid fossil *Hydropteridangium marsilioides* Halle, which Halle suggested was a sporocarp of the Marsiliaceae, is altogether doubtful. Its structure is inconclusive and the associated *Marsilia*-like leaves—*Sagenopteris*—are now thought to belong to the *Caytoniales". From his own study of better preserved Rhaetic material from East Greenland Harris (1932, 122-127, Pl. 9, Pl. 10, Figs. 3-8, Pl. 11, Figs. 1, 2, 15; Text-Fig. 52) concludes that the relationship between *Hydropteridangium* and the Hydropteridaceae is out of the question. He says that it must belong to a seed plant (Gymnosperm) and its spores must be microspores.
The families Salviniaceae and Marsiliaceae are probably not at all related together. The latter seems to have affinities with the Schizaceae, while the former is perhaps nearest related to the Hymenophyllaceae (Bower, 1908, 551; and 1928, Vol. III, 262). They are generally grouped together on account of their heterospory and aquatic habit.

We may conclude this brief summary with the authoritative opinion of Professor Seward: "There is no evidence contributed by fossil records which indicates a high antiquity for the Hydropteridaceae. It is unsafe to base any conclusions on the absence of any undoubted palaeozoic representatives of this group; but the almost complete absence of records in pre-Tertiary strata is a fact which may be allowed some weight in regard to the possible evolution of the heterosporous filicales at a comparatively late period in the earth's history" (Seward, 1910, Vol. II, 477).

Summary.

Work on *Azolla pinnata* was taken up as a necessity was felt for a comprehensive account of at least one species other than the well-known *A. filiculoides*. *A. pinnata* seemed specially suitable as it belongs to the second sub-genus Rhizosperma.

A short historical review of the previous work is given. In the structure and development of stem, leaf, root, sporocarps, gametophytes, etc., *A. pinnata* is essentially similar to the other well-known species, while there are important differences in detail. The general course of the life-history is as follows: fertilisation takes place in September or October. The resulting fresh plants mature in Spring. By about April the sporocarps ripen, and are shed. The spores rest during Summer. The megasporocarp with the attached massulæ floats up before fertilisation.

The Discussion takes notice of the incorrect observations of Sud and other workers. Goebel's view of the nature of the involucre has been confirmed. The advisability of employing the term root-sheaths, instead of both root-sheaths and root-cap is suggested. The author has observed an occasional branching of the microsporangial stalk, bearing more sporangia than one, or the sporangia represented by sterile filaments. A peculiar mode of thickening in the cells of the indusial cap is noticed.

An important point brought out is that the male prothalli of *A. pinnata* are detachable. They escape from the massulae which get hooked on to the megasporocarp, and come to lie about the female prothalli. It is suspected that the same occurs in the other species.

A brief summary of the fossil history of the Hydropteridaceae is given. Literature on the fossil species of *Azolla* shows that the *A. filiculoides* type
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(viz., A. intertrappea) is the oldest known, viz., in the Eocene; a synthetic type combining the characters of the two sub-genera is found in the Oligocene, and both A. filiculoides and A. pinnata are known from the Pleistocene. There is no evidence in the fossil records for a high antiquity for the Hydropteridæ: the oldest authentic records are Tertiary.

Further work on the germination of the spores and early development is hoped to be taken up.

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EXPLANATIONS OF PLATES.

ABBREVIATIONS USED.

\begin{itemize}
\item \textit{a} . . . . Point of attachment.
\item \textit{ab} . . . . Absciss layer.
\item \textit{amg} . . . . Abortive megasporangium.
\item \textit{ar} . . . . Archegonium.
\item \textit{c} . . . . Collar around base of root.
\item \textit{d.l.} . . . . Dorsal lobe of leaf.
\item \textit{e} . . . . Endodermis.
\item \textit{f} . . . . Float.
\item \textit{f.m.} . . . . Fragment of megaspore wall.
\item \textit{h} . . . . Hair.
\item \textit{h.a.} . . . . Hairy appendages.
\end{itemize}
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*i.e.* .. Indusial cap.
*in* .. Involucr.e.
*m* .. Megaspore.
*ma* .. Massula.
*mg.w.* .. Megasporangial wall.
*mi* .. Microsporangium.
*m.p.* .. Male Prothallus.
*o.a.c.* .. Opening of Anabaena cavity.
*p* .. Pericycle.
*ph* .. Phloem.
*r* .. Root.
*r.h.* .. Root-hair.
*r.s.* .. Root-sheath.
*s* .. Stoma.
*st* .. Stem.
*th* .. Thickening.
*t.n.* .. Tapetal nucleus.
*v.l.* .. Ventral lobe of leaf.
*x* .. Xylem.

**PLATE XIII.**

Fig. 1.—A sporophyte plant of *Azolla pinnata*, showing the root with the characteristic root-hairs. × 6.

Fig. 2.—Plant seen from above. × 6.

Fig. 3.—Plant seen from below showing the sporocarps. Their involucres are shown black. × 6.

Fig. 4.—Plant seen from below. Note the collar around the base of the root. × 6.

Figs. 5 & 6.—Stem apex, with the beginnings of the lateral organs. × 300. See also Fig 8.

Fig. 7.—A young leaf-lobe showing the regular tangential and radial cell-divisions. × 480.

Fig. 8.—Stem apex. × 266. See (5) and (6).

Fig. 9.—Shows the margin of the lower leaf-lobe. × 80.

Fig. 10.—Stele of the stem, slightly oblique. × 480.

Fig. 11.—Sagittal section through the apex of the stem showing the first division in the apical cell and an young root with three root-sheaths. × 480.

Fig. 12.—T.S. of part of the stem, showing stomata and hairs. × 300.

**PLATE XIV.**

Fig. 13.—A leaf. The underside of the dorsal lobe is seen with the opening of the Anabaena cavity, with radiating cells around it. Note the stomata. The shaded part is thick. The clear margin of 4-5 cells is one-cell thick. × 48.

Fig. 14.—Section of an young dorsal-lobe of leaf showing the already formed Anabaena cavity. × 123.

Fig. 15.—Section of a stoma of leaf. × 480.
Figs 16-19.—Hairs in the *Anabena*-cavity of leaf. × 300.

Fig. 20.—Absciss-layer of branch of stem. × 80.

Fig. 21.—Showing the dorsal and ventral leaf lobes, the *Anabena*-cavity in the former and a simple cavity in the latter. Note the mouth of the *Anabena*-cavity. × 123.

**Plate XV.**

Fig. 22.—See Fig. 21. × 80.

Fig. 23.—L.S. of root. Note the three root-sheaths and the apical cell. × 266.

Fig. 24.—Tangential section of root, across the root-hair papillae. The three root-sheaths are indicated. Note the regular pattern of the section. × 266.

Fig. 25.—L.S. of leaf to show the *Anabena*-cavity and its opening. × 300.

Fig. 26.—Young root-hairs, with the intervening cells. The latter increase in number during the growth of the root. × 300.

Fig. 27.—L.S. of part of root. Note the three root-sheaths, the absciss layer of smaller cells at the base of the root, and the tracheid from the stem, supplying the root. × 300.

**Plate XVI.**

Fig. 28.—Tip of root after the root-sheath has been slipped off. × 80.

Fig. 29.—L.S of young root, showing the four root-sheaths of which the second from outside is disorganising. × 123.

Fig. 30.—Root-sheath slipped off from the adult root, the tip of which is figured in Fig. 29. × 80.

Fig. 31.—L.S. of young root, a stage later than the one seen in Fig. 29. × 300.

Fig. 32.—T.S. of root. × 480.

**Plate XVII.**

Fig. 33.—L.S. of a pair of very young sporocarps, with their involucre arching over them. × 266.

Fig. 34.—Hairs at the base of the sporocarps. × 266.

Fig. 35.—L.S. of very young sporocarps. × 266.

Fig. 36.—L.S. of young megasporocarp, showing the separating spore tetrads. Note the papillae at the base of the megasporangium. × 300.

Fig. 37.—L.S. of megasporocarp, showing tetrad formation in megasporangium. × 300.

Fig. 38.—An oblique section of an young megasporocarp showing the single megaspore developing and three disorganising spores. The smaller nuclei are tapetal. × 300.

Fig. 39.—L.S of megasporocarp at an earlier stage than Fig. 38. × 300.

Fig. 40.—Shows the flap of the dorsal lobe of leaf which forms the involucre of the sporocarps. × 106.

**Plate XVIII.**

Fig. 41.—L.S. of young megasporocarps showing the divisions in the sporogenous tissue. The tapetum is partly disorganising. × 433.

Fig. 42.—L.S. of young megasporocarp showing the functional megaspore with a beak on its top. It is the beginning of the conical body at the top of the spore which gets surrounded by the float-mass. × 300.
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**Fig. 43.**—L.S. of an young microsporocarp with the terminal abortive megasporangium \( \times 300 \).

**Fig. 44.**—L.S. of megasporocarp showing the megaspores and tapetal nuclei. From this stage the single functional megaspore develops further and the others abort \( \times 480 \).

**PLATE XIX.**

**Fig. 45.**—L.S. of mature megasporocarp partly reconstructed. The innermost spore-wall has broken and curled inwards. \( \times 106 \).

**Fig. 46.**—Involucre surrounding the sporocarps. The microsporocarp (A) has been detached, its point of attachment being seen at 'a'. \( \times 10 \).

**Figs 47-49.**—Processes on the surface of the megaspore. Fig. 47 shows an earlier stage than Figs. 48-49. In Fig. 48 are seen the points of attachment of the thread-like appendages. Fig. 47 \( \times 480 \); Fig. 49. \( \times 300 \).

**Figs. 50-52.**—Three stages in the development of the microsporangium. \( \times 480 \).

**Fig. 53.**—Stalk of microsporangium, two-cells thick. Note the sterile filamentous branch. Sometimes another microsporangium replaces the sterile filament \( \times 80 \).

**Fig. 54.**—Stellate hairs which form the wall of the microsporangium. \( \times 80 \).

**Fig. 55.**—A glochidium. Note its vacuolated nature. \( \times 480 \).

**Fig. 56.**—A massula with microspores and glochidia. \( \times 80 \).

**Fig. 57.**—An oblique section of a microsporocarp with the terminal abortive megasporangium \( \times 253 \).

**PLATE XX.**

**Fig. 58.**—An young embryo plant, showing the two rings of floats, the female prothallus, the first leaf and the indusial cap tilted at right angles. See also Fig. 66. Diagrammatic.

**Figs. 59-62.**—Young embryo plants with a few leaves only developed.

**Fig. 63.**—T.S. of female prothallus showing nine archegonia, adjacent ones often separated by only one layer of cells. \( \times 300 \).

**Fig. 64.**—Sporocarp with female prothallus. Note the massulae (shaded) attached to the sporocarp. There are seven of them. The floats are not shaded. See the cup-like structure at the top of the sporocarp from which a number of thread-like appendages arise. The indusial cap is tilted on one side; a few of its cells are shown rather diagrammatic. \( \times 80 \).

**Fig. 65.**—L.S. of the indusial cap. Note the peculiar thickening of the cell-wall projecting into the cavity of the cells, the resting *Anabaena* cells and the mass of thread-like appendages. \( \times 48 \).

**Fig. 66.**—The embryo plant shown in figure 58 seen from above. Note the two rings of floats and the indusial cap. The margin of the cotyledon is shown black. Diagrammatic.

**PLATE XXI**

**Fig. 67.**—L.S. of female prothallus showing two archegonia. The prothallial cells contain chloroplasts. \( \times 48 \).

**Fig. 68.**—A male prothallus. \( \times 1000 \).

**Fig. 69.**—T.S. of sporocarp with the female prothallus cut near its base. As the prothallus arches over the cavity of the megaspore, at this level, a section shows a clear
space in the middle. The six lower floats are arranged symmetrically all round. Note the hairy appendages and part of the megaspore-wall (black). \( \times 123 \).

**Fig. 70.**—L.S. of embryo plant embedded in the female prothallus. The wall of the 'foot' cells in contact with the base of the archegonium is somewhat thickened (black). \( \times 80 \).

**PLATE XXII.**

**Fig. 71.**—Photograph of *Azolla* plants closely covering the surface of the water. Nat. size.

**Fig. 72.**—Photograph of a megasporocarp with the prothallus developed. Seven massulae are found hooked on to the sporocarp. See also Fig. 64.

**Fig. 73.**—Photograph of the female prothallus seen from above, showing more than thirty archegonia.

**Fig. 74.**—Photograph of a number of embryo plants at various stages of development. Note the obliquely borne roots of the older plants.

**Fig. 75.**—T.S. of the female prothallus showing a number of archegonia and a few male prothalli.

**Fig. 76.**—L.S. of the female prothallus showing an archegonium at the fertilization stage. Note the two nuclei.