Two Little-known Genera of Green Algae (Tetrasporidium and Ecballocystis).[1]

BY

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With Plates VII and VIII and nine Figures in the Text

PART I. *Tetrasporidium javanicum*, Moebius.

THIS alga was described by Moebius from Java in 1893 (11, p. 122), and was again collected by Massart in 1896 (cf. 19, p. 32), but has not since been recorded either from Java or from any other part of the world. In the fairly detailed account published by Moebius many interesting features are, however, overlooked, presumably owing to the scantiness of the material at his disposal. Moreover, his record of the occurrence of sporangia is based on a misinterpretation. A certain amount of interest attaches to this alga, owing to the uncertainty as to its exact systematic position. Wille (20, pp. 30 and 31) placed it in the Tetrasporaceae under 'Wenig bekannte oder unsichere Gattungen', and the same procedure was adopted by Printz (13, p. 79) in 1927, owing to the absence of any further information about this alga. Blackman and Tansley (1, p. 241) also refer it to Tetrasporaceae, while Chodat (4, p. 112) suggests inclusion in the genus *Tetraspora*.

Tetrasporidium javanicum is not uncommon in Madras, and advantage was taken of the large amount of material available to make a careful study of the alga, both in the living and preserved conditions, in the hope of throwing more light on its structure and development, as well as on its systematic position.

Moebius records the alga from a river. In Madras it generally occurs in the small pools formed during the rainy season (October to December) growing on aquatics, such as *Monochoria hastaefolia*, Presl. (*M. hastata*, Solms), and certain grasses; to these it is attached by a broad base, beyond which the thallus floats out on the surface of the water. It was also once found stranded by the current on the sandy banks of the river Nagari in South India,[2] along with *Hydrodictyon reticulatum*.

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[2] I am indebted to Mr. N. Venkatanathan for providing me with material from this locality.

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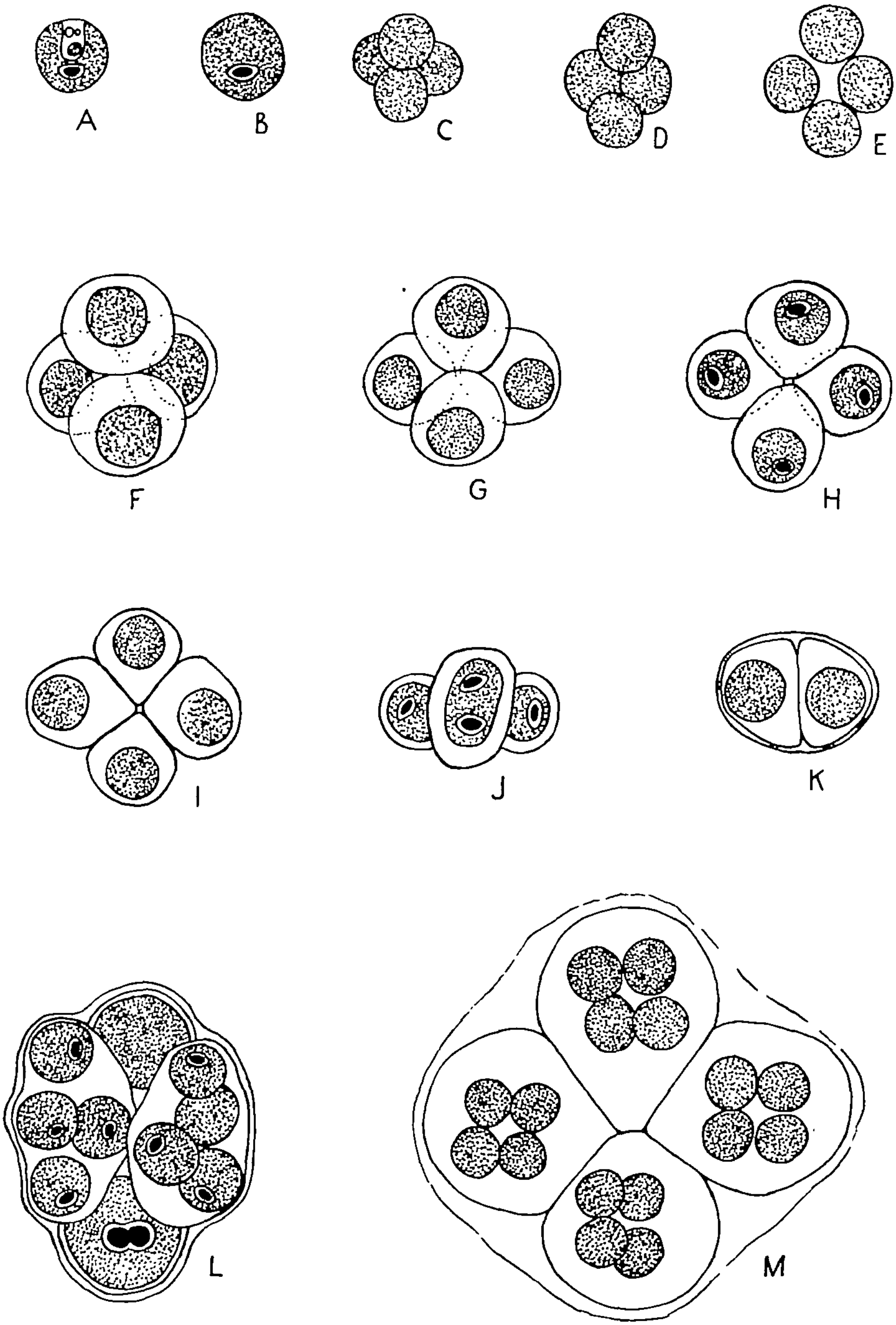
The thallus, when fully grown, is a thin, flat, more or less circular, gelatinous expanse of pale green colour which may reach a diameter of 12–13 cm. (5 inches) (Pl. VII, Fig. 1). The youngest stages seen were about the size of a pea and roughly spherical or oblong in shape. Moebius's specimens must have been young or fragmentary, since he puts the maximum size of the thallus at 2 cm.; he himself, however, suggests that these may have been merely fragments. The young stages found by me were apparently quite solid and contained numerous cells.

When a mature thallus is examined under the lower power of the microscope, it presents a net-like appearance with numerous, round or elliptic, and often large and irregular perforations with a smooth or lobed margin; these perforations vary from a minute hole to a large irregular aperture (Pl. VII, Fig. 3), and their edge is more clearly seen on staining. The numerous cells are embedded in a gelatinous matrix within which at first sight they appear to form a single layer. Closer examination, however, discloses that the thallus really consists of two superposed layers of mucilage, each provided with an independent series of perforations, the two layers being connected with each other at various places by irregular gelatinous processes (cross-connexions) in a rather haphazard manner (Pl. VII, Figs. 2, 4). The impression obtained is that of overlapping of different parts of the thallus as a result of careless mounting, but, if specimens are floated out on a slide from a dish of water, just the same appearance is observed. At the edges of undamaged thalli, it can be seen that the upper and lower layers are continuous with one another, but the edge is not sharply defined and seems to form part of a curved surface. The thallus as a whole appears indeed to represent a hollow sack with a one-layered wall broken by numerous perforations, overlying parts of the wall being joined by the cross-connexions just referred to. This structure is, however, obscured and rendered rather difficult to decipher owing to the numerous perforations and the many connecting branches between the opposite surfaces of the thallus. The way in which the hollow state is arrived at has not been determined with certainty, but it seems probable that the originally solid thallus becomes hollow and that the cells become distributed in a single layer within the mucilage enveloping the hollow. The origin of the perforations and the cross-connexions is dealt with below.

The individual cells closely resemble those of a globular *Chlamydomonas* in structure, the general shape being rounded to rounded-oval; the diameter of the protoplast of young cells is 7–9 μ and of fully grown ones 10–11·5 μ. Each cell has a small round nucleus and a bell-shaped chloroplast with a large median elliptical pyrenoid lying near the posterior end, while in living specimens two contractile vacuoles (max. diam. 1·75 μ), pulsating alternately, were distinctly visible at the anterior end (Text-fig. 1, A). There is no eye-spot. Although various stains and reagents

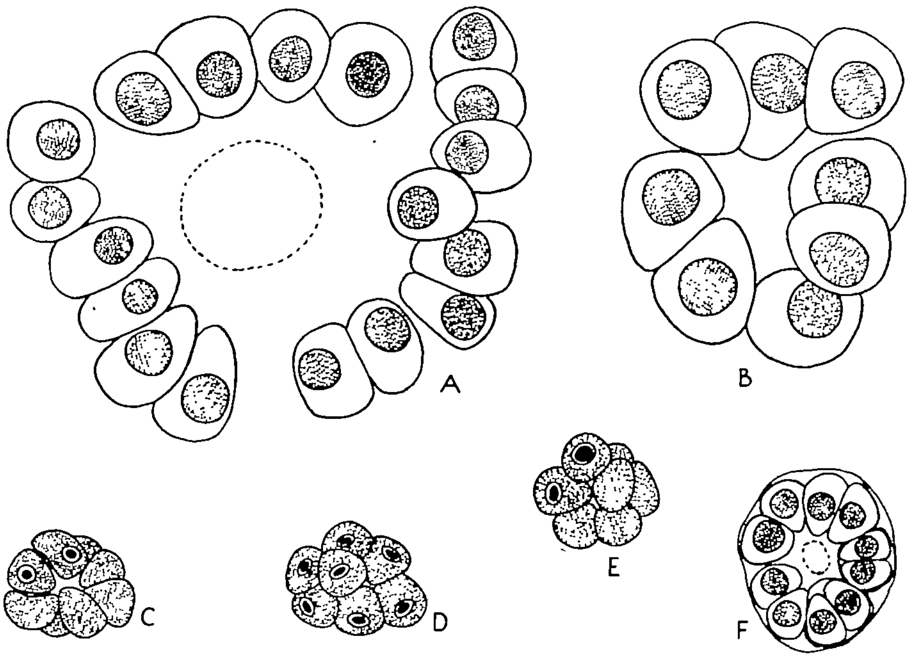


TEXT-FIG. 1. *Tetrasporidium javanicum*, Moeb. A, single cell; B, full-grown cell preparatory to division; C–I, division in two planes, leading to crosswise arrangement shown in I; J, a case of arrested division with one of the cells elongated and with two pyrenoids; K, division into two; L, M, mother-cell walls of three generations intact. Pyrenoids black. A × 920; B–M × 1,050.

(including iodine, safranin, fuchsin, methylene blue, gentian violet, haematoxylin, toluidine blue, and eosin) were used, no pseudocilia could be found. In place of the firm cellulose wall of a *Chlamydomonas* there is around each protoplast a thick, hyaline, but fairly consistent gelatinous envelope which can be clearly seen only after treatment with a dilute aqueous solution of safranin, methylene blue, toluidine blue, or Delafield's haematoxylin (Text-fig. 1, F–K). The general mucilage of the thallus is mainly composed of these envelopes, though there is also a certain amount of homogeneous intervening mucilage, which is seen clearly at the edges of the perforations. There are no protoplasmic connexions between the cells.

The cells multiply by division, either into two (Text-fig. 1, K) or, more commonly, into four. In the latter case the division appears to be successive, first into two and then into four, and most commonly the four daughter-cells lie in the same plane which is parallel with the surface of the thallus. Not infrequently, however, the two successive divisions take place in planes respectively parallel and at right angles to the surface of the thallus. If the plane of the first division is parallel to, and that of the second perpendicular to the surface, we obtain groups of cells exhibiting a crosswise arrangement in two planes (parallel to the surface of the thallus), i.e. there is a pair of upper and a pair of lower cells, the lower alternating with the upper (Text-fig. 1, C, D, F–H). Occasionally one finds one of the two daughter-cells of the first division undivided, such cells being elongate with a pyrenoid at each end (Text-fig. 1, J). If the plane of the first division is perpendicular to the surface and only one of the two resulting daughter-cells then divides in a plane parallel to the surface, three of the daughter-cells lie in one plane and the fourth in another. In such cases, especially if the mucilage be a little displaced, the cells appear to have a tetrahedral grouping, but close scrutiny supports the view of the origin of such groups in the way described. Sometimes division into eight is observed, the daughter-cells exhibiting no very regular arrangement, though more or less radially grouped (Text-fig. 2, C, D, E). Division into eight is, however, comparatively rare, and appears, as explained below, to be connected with the formation of the perforations in the thallus. In all cases, the daughter-protoplasts secrete a gelatinous wall distinct from that of the parent-cell, and the new envelope can be clearly distinguished inside the latter. When the daughter-cells divide again the same process is repeated and, in stained preparations, the envelopes of successive generations can often be clearly, though faintly, seen (Text-fig. 1, L, M).

When division into four takes place, the daughter-protoplasts at first lie close together but, as they become surrounded by their own envelopes, they get pushed apart. This appears to be due to the fact that the secretion of the gelatinous envelope takes place more actively on the side of the cell towards the centre of the group, so that the individual protoplasts come to lie near the outer boundary of each group (Text-fig. 1, F–I, K). When the two divisions have been in different planes, and the pairs of daughter-cells are placed crosswise, the cells appear gradually to become disposed in one plane, though often showing a slight overlapping of the inner edges of their gelatinous walls. The four cells then appear to be separated by



TEXT-FIG. 2. *Tetrasporidium javanicum*, Moeb. A, B, F, beginning of a perforation after division into eight cells; C–E, division into eight. Pyrenoids black. A–E × 870; F × 440.

partition-walls which are arranged crosswise, and such groups can be found abundantly within the thallus. In the centre of each group, there often appears to be a small approximately square space, where the four 'partitions' meet, reminding one of the intercellular spaces of higher plants (Fig. 1, H, I). But these apparent spaces in *Tetrasporidium* are not due to splitting, but to the overlapping of the wall of the cells of each group. It has not been possible to ascertain the course of events in those cases in which the products of division show a pseudo-tetrahedral grouping, but it is to be presumed that here also all the cells come to lie in one plane.

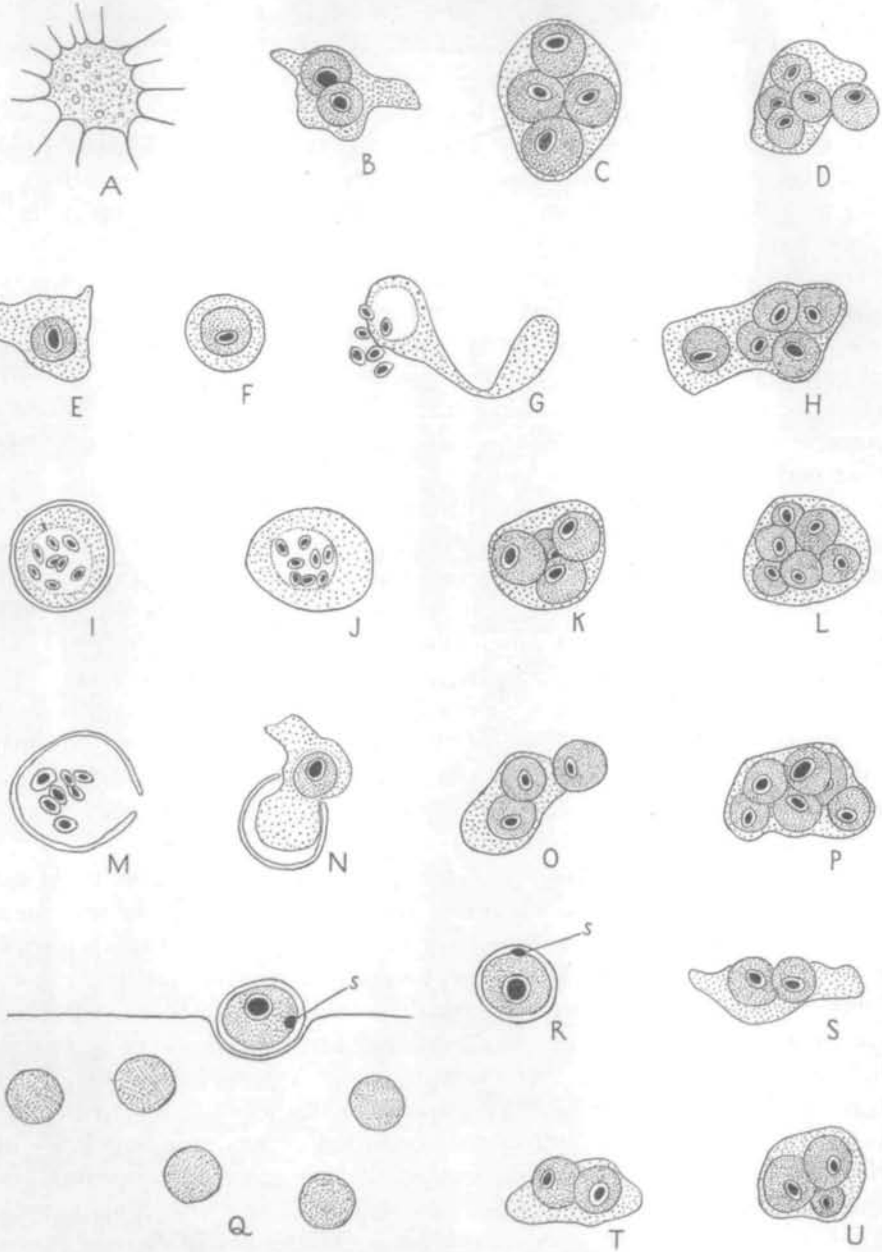
When division into eight takes place (Text-fig. 2, C–E), the enlargement of the daughter-cells after they have formed their gelatinous walls appears invariably to lead to the formation of a tiny rupture between the converging inner ends of the cells. With the further division of the latter, these small spaces are gradually converted into large holes. It seems that the simultaneous enlargement of a considerable number of cells all converging to a single point sets up local strains leading to the rupture of the thallus at such places (Text-fig. 2, A, B, F). The cells round the edges of the perforations are always more densely grouped than elsewhere.

Origin of the cross-connexions between the two layers of the thallus. From the edges of the perforations thus formed there grow out small lobes which, increasing in length, gradually extend across the perforations and finally fuse with some other point on the margin. In other cases these lobes may grow towards the opposite surface of the hollow thallus and become joined up with it at their tips. Similarly, large lobes may arise at any point from the inner surface of one of the two layers of the thallus and, growing towards the other layer, may unite with it at various places in an intricate manner. There does not appear to be any order or regularity in the formation of these cross-connexions, with the help of which the opposite surfaces of the thallus are closely knitted together. The thallus thus superficially resembles an *Ulva*, in which the two layers have split apart and become irregularly perforated, although connected together by numerous cross-branches.[1]

Reproduction. Although the living alga was kept under prolonged observation at Madras, no motile reproductive cells of any kind were noticed. The presence of contractile vacuoles in the ordinary cells, however, renders the occurrence of motile stages probable. Nor were any resting spores seen, and the way in which the alga tides over the dry season when the pools dry up still remains to be established. *Palmodictyon viride*, Kütz., which shows a certain resemblance to *Tetrasporidium*, is known to form hypnospores (16, p. 246), and, here too, swarmers have not been observed.

Moebius (11, p. 122) records sporangia whose contents, as a result of successive division, give rise to a number of spores. Structures similar to those described and figured by Moebius as sporangia occurred frequently in my material, but I am of opinion that they are due to a small protozoon parasite which is found in the gelatinous matrix of the *Tetrasporidium*. The organism in question is no doubt a *Vampyrella*, and is possibly identical with *V. lateritia* (Fresen.), Leidy, but the material was inadequate for an accurate determination of the species. The individuals observed within the mucilage of the alga were brownish-red in colour and approximately spherical, the protoplast being produced into numerous delicate

[1] A small unicellular epiphyte was found growing attached to the surface of the thallus, the spherical cells being about the same size as those of the *Tetrasporidium*. These cells had a well-defined firm cell-wall, and their contents resembled those of a *Chlamydomonas*, though less granular than those of the *Tetrasporidium*, while the large pyrenoid was rounded and not elliptic; there was a very definite eye-spot. This epiphyte was always seated on the outer surface of and opposite a depression in the mucilaginous matrix of the *Tetrasporidium*, being often attached to the edges of the perforations (Text-fig. 3, Q, R). This form resembles *Malleochloris sessilis*, Pascher (Süsswasserfl., iv, 1927, p. 480), but does not possess the short gelatinous attachment of the latter.



TEXT-FIG. 3. A, *Vampyrella* from the mucilage of *Tetrasporidium*; B, C, E, F, H, K, L, P, U, the parasite with ingested cells of *Tetrasporidium*; D, O, S, T, parasite about to ingest a further cell; G, ejection of undigested portions of algal cells; J, parasite with the algal cells (apart from the pyrenoids) completely digested and about to encyst; I, encysted parasite; N, the parasite escaping from its cyst-wall with an algal cell already ingested; M, empty wall of cyst from which the parasite has escaped leaving undigested pyrenoids behind; Q, R, an algal epiphyte (*Malleochloris*, sp.?) on *Tetrasporidium* showing the eye-spot (*s*). Pyrenoids black. A × 810; B–U × 920.

pseudopodia (Text-fig. 3, A). These amoeboid individuals were in the living material seen to attack cells of the alga (Text-fig. 3, D, O, S, T) which became engulfed within the protoplast of the parasite (Text-fig. 3, B–F, H) and were then slowly digested, the undigested portions often being finally extruded from the body of the parasite (Text-fig. 3, G). The pyrenoids could be detected for a long time after the algal cells had been engulfed by the parasite and did not appear to undergo digestion (Text-fig. 3, I, J), since they formed the principal part of the extruded undigested material (Text-fig. 3, G). Usually the remains of a number (up to eight or more) of algal cells were to be found within the body of the parasite. It is not clear whether this is due to the ingestion of successive algal cells or to a number of the parasitic protoplasts uniting to form a plasmodium, as is undoubtedly often the case. It is also possible that the algal cell continues to live for some time within the body of the *Vampyrella*, and may even divide during that period.

Such stages of the parasite with a number of included algal cells soon secrete a definite enveloping wall. Owing to the practically unaltered condition of the pyrenoids, the contained algal cells still appear to be intact, and in this condition the encysted parasite, with its included algal cells, superficially resembles a sporangium with a number of spores inside (Text-fig. 3, C, K, L). The semi-transparent granular body of the parasite surrounding the algal cells it has encompassed resembles a peripheral layer of protoplasm around the 'spores' of the 'sporangium'. Moebius evidently misinterpreted these stages of the parasite as sporangia of *Tetrasporidium*, which are stated to possess a certain amount of periplasm; this latter is, however, actually the protoplasmic body of the parasite.

The systematic position of Tetrasporidium. Since the cells do not possess any pseudocilia, the suggested inclusion of the alga in the genus *Tetraspora* (cf. Chodat and others) cannot be supported. In fact, for this reason, it cannot be included in the Tetrasporaceae and must find a place in the Palmellaceae, an artificial family established for those palmelloid Green Algae that lack the pseudocilia of the Tetrasporaceae. It does indeed appear to come rather close to *Palmodictyon viride*, Kütz, which it resembles in the cells having gelatinous walls which retain their individuality for a long time and in the formation of anastomosing lobes on the thallus. The apparent absence of motile reproductive stages in *Tetrasporidium* constitutes another point of resemblance to *Palmodictyon viride*. There is some uncertainty about the nature of the chloroplasts in *Palmodictyon*, since Lemmermann (10, p. 35) describes the cells as containing several curved disc-shaped chloroplasts without pyrenoids, whilst according to West (16, p. 240), in *P. varium* (Naeg.) Lemm. (*Palmodactylon varium*, Naeg.), there is a parietal irregularly lobed chloroplast.

Moebius largely based his genus on the occurrence of sporangia with a peripheral formation of periplasm, a feature which has been shown above to be due to a parasitic organism. But *Tetrasporidium* exhibits a number of other peculiarities that for the present justify its retention as a separate genus. In its cell-structure *Tetrasporidium* clearly resembles a *Chlamydomonas*, differing only in the absence of an eye-spot and of cilia and in the consistency of the wall. In the possession of the two contractile vacuoles it shows a closer relation to motile types than do most of the other members of the Palmellaceae, although in *Asterococcus*, Scherffel, both contractile vacuoles and eye-spots have been recorded (18, pp. 38 and 40).

The emended diagnosis of the genus rendered necessary by the facts established in this paper is as follows:

Thallus macroscopic, attached by a broad base to other aquatics and forming a gelatinous two-layered expanse, the two layers separated by a hollow, but continuous at the edges, each layer provided with numerous perforations of diverse size; opposite edges of the perforations, as well as the two layers of the thallus, joined by numerous cross-connexions formed by the protrusion of lobes which ultimately anastomose with other parts of the thallus; cells numerous, somewhat remote from one another, embedded in a gelatinous matrix formed largely by the gelatinous cell-membranes, without any protoplasmic connexions, each with a posterior bell-shaped chloroplast containing a single pyrenoid and with two contractile vacuoles at the anterior end, but without cilia, pseudocilia, or eye-spot; cell-wall gelatinous, but fairly consistent, the walls of successive cell-generations remaining distinct for some time and clearly seen after staining; cell-division into two, four, or eight; zoospores, gametes, and resting-spores unknown.

Geographical distribution: Java, S. India.

PART II. The genus *Ecballocystis*, Bohlin.

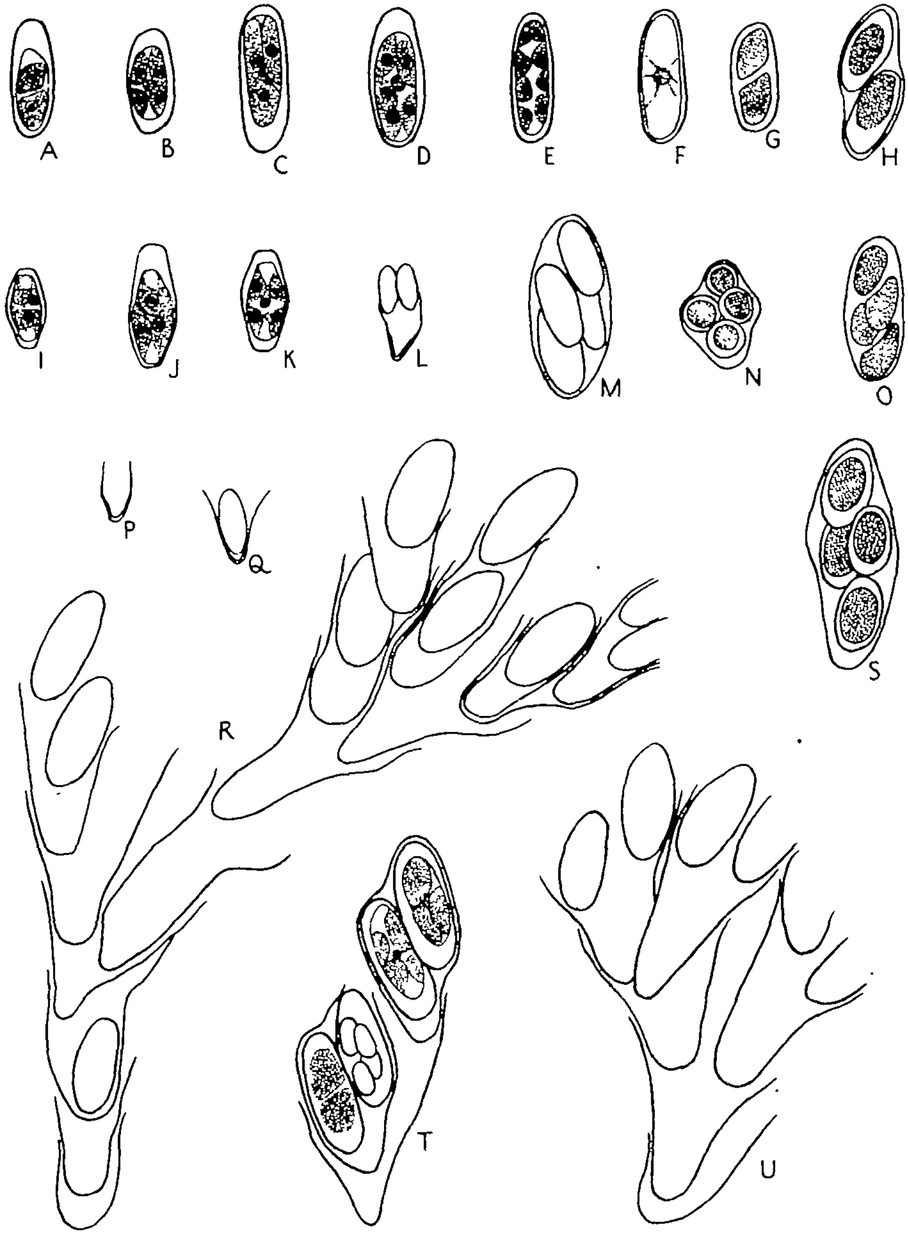
This genus was established by Bohlin in 1897 (2, p. 7) for an alga which occurred in collections from Rio Grande do Sul in Brazil, and which he described under the name of *E. pulvinata*. It formed small macroscopic cushions, attached to stones in a river, composed of numerous elongated cells arranged in a characteristic manner. Bohlin describes this form as follows: 'If one starts with a *single* cell, the mode of division is as follows. The mother-cell divides first by a transverse wall into two daughter-cells, which in the course of their growth very soon become obliquely disposed with respect to each other. Often a second division may take place before the bursting of the membrane of the mother-cell, but usually the membrane breaks already after the first division. In the latter case the upper daughter-cell is pushed obliquely upwards and becomes fastened at its base to the wall of the mother-cell by a cone-shaped secretion from the cell-wall which occupies the free space in the lower part of the cell. Each daughter-cell thereupon divides with repeated rupture of the respective mother-cell walls and attachment of the upper daughter-cell to the same. Generally the divisions take place somewhat more rapidly on one side of the colony. All cells are, however, capable of division, and displacement of the cells takes place in several directions. In this way there arises a slightly branched colony in which one can observe the ruptured membranes of several generations. The remains of the older membranes seem to gelatinize, and in this way even macroscopically visible cushion- or mat-like coverings are formed on the substratum. The chromatophores are pure green and contain starch; for the rest nothing of their structure was to be seen in the available material. The membrane showed no cellulose reaction.'

The essential features of the alga are the oblique arrangement of the daughter-cells, the attachment of the latter to the walls of the mother-cells by a basal secretion of mucilage, and the dendroid colonies with their false branching. In 1903 Yendo (21) referred three marine algae to the genus *Ecballocystis* but, as pointed out by Printz (13, pp. 78, 79), these should be placed in the genus *Collinsiella*. In 1917 Fritsch (7, pp. 494–502) described from S. Africa two species of *Ecballocystis*, *E. ramosa* and *E. simplex*, which are referred to in greater detail below. The writer has met with abundant material of species of *Ecballocystis* in various localities in Southern India, the majority of which differ in important respects from the forms hitherto described. It is thus evident that the genus has a wider geographical distribution than has so far been recognized, and that it displays a considerable range in morphological structure. In the following matter the individual species are first considered separately, and this is followed by a general discussion of the genus.

Ecballocystis Fritschii sp. nov. (Text-fig. 4).

The thalli of this alga are small, irregularly rounded, lobed masses, sometimes somewhat cylindrical, about one-eighth to one-third of an inch wide and about one-eighth to one-third of an inch high. They were found attached to the rocky bed of a small stream. The masses were compact enough and sufficiently firmly attached to resist the force of the fairly strong current. Some of the material was kept under observation in a living condition, while the rest was preserved either directly in 4 per cent. formalin or after fixing in Flemming's weaker chromo-acetic solution and subsequent washing.

The alga resembles *E. pulvinata*, Bohlin, in a general way in the structure of the thallus and the disposition of the cells. Since, however, Bohlin's description and figures do not afford any data as to the cell-structure, the degree of correspondence between the two forms cannot be fully estimated.



TEXT-FIG. 4. A–H, M–O, R–U, *Ecballocystis Fritschii*, sp. nov. A–E, cells showing chloroplasts; F, cell showing only the nucleus; G, division of protoplast; H, formation of two daughter-cells inside mother-cell wall; M, N, O, S, formation of four daughter-cells (in N seen from above); R, T, U, parts of mature colonies, showing dendroid branching. I–K, L, P, Q, *E. Fritschii* var. *pulneyensis* var. nov.; I–K, cells with chloroplasts; L, two daughter-cells at apex of ruptured mother-cell wall; P, empty mother-cell wall; Q, a single daughter-cell remaining at the base of the mother-cell wall. Pyrenoids black. L, P, Q × 450; the rest × 890.

Cell-structure. The cells are elliptic oblong with broadly rounded ends. There is a single central nucleus suspended in the vacuole by radiating cytoplasmic threads (Text-fig. 4, F) and two, four, or eight, more or less curved, discoid, parietal chloroplasts, each containing a single pyrenoid (Text-fig. 4, A–E). The younger cells have two chloroplasts only (Text-fig. 4, A), but in most of the older ones there are four (Text-fig. 4, B, C) and, in a few of the fully grown cells, eight (Text-fig. 4, D, E). In cells with four chloroplasts the latter are arranged in two pairs in the upper and lower halves of the cell respectively, the two chloroplasts of each pair standing opposite to each other; the two pairs of chloroplasts may lie in the same plane (Text-fig. 4, B) or be placed at right angles to one another (Text-fig. 4, C). When there are eight chloroplasts in a cell, they are sometimes arranged in four superposed pairs (Text-fig. 4, E), but the arrangement is not always so regular; often one sees five chloroplasts at one focus and three below (Text-fig. 4, D). The older cells are full of starch. The pyrenoids are very small and inconspicuous in the older cells, but somewhat larger and more distinct in very young cells, especially in those containing two chloroplasts only. There is a thin but definite starch sheath around the pyrenoid. Staining with iron-haematoxylin or Delafield's haematoxylin brings out nuclei, chloroplasts, and pyrenoids clearly. The cell-wall is thin and firm, but very slightly thicker at the basal pole, a fact especially obvious after the rupture of the wall has taken place; the wall is not coloured blue by chlor-zinc-iodide. It envelops the contents closely at all points. The mucilaginous matrix, within which the cells of the colony are embedded, readily takes up various stains such as methylene blue, congo red, &c.

Cell-division and colony-formation. Division of the protoplast takes place obliquely into two (Text-fig. 4, G), and a new wall is soon formed around each of the products, with the result that two somewhat obliquely placed cells are seen inside the membrane of the parent-cell (Text-fig. 4, H). As the daughter-cells increase in size, the latter at first becomes distended, but ultimately undergoes rupture at the apex. In the course of further growth, the daughter-cells come to lie at approximately the same level near the mouth of the ruptured membrane of the parent-cell, as shown in Text-fig. 4, R, U. The daughter-cells in their turn divide, and form grand-daughter-cells whose enlargement leads to a similar rupture of the daughter-cell membranes, and this process continues until a fairly big aggregate is formed. The cells appear to secrete a certain amount of mucilage, while at the same time the membranes of the older cells gradually undergo gelatinization, so that an extensive mucilaginous matrix is produced with a large number of cells embedded in it. Within the gelatinous matrix the remnants of the mother-cells of several generations can always be detected (Text-fig. 4, U). If a small piece of the thallus is stained on the slide for a few minutes with methylene blue or congo red and gently pressed under a cover glass, the arrangement of the old mother-cell walls and the dendroid nature of the thallus can be clearly made out (Text-fig. 4, R). The thallus is seen to be built up of successive 'branchings', each resulting from a cell-division. The conical gelatinous processes at the base of the cells described by Bohlin in *E. pulvinata* are lacking in this form. In Bohlin's form, moreover, the lower ends of the old mother-cell walls are broadly rounded (2, Figs. 2, 3, and 4), whilst in *E. Fritschii* they are funnel-shaped and somewhat constricted below.

Gelatinization of the wall of the mother-cell always seems to commence at the top, where rupture takes place, and from there it spreads slowly to the rest of the wall. The slightly thickened basal portion of the wall is the last to become completely gelatinized. The process of gradual gelatinization from the apical region downwards is readily followed. If a piece of the thallus is stained with dilute aqueous solution of toluidine blue, the gelatinized portion of the wall is stained blue, while the ungelatinized firmer portion becomes bluish-yellow. The upper thinner portion of the ruptured wall is more easily distended by the enlarging daughter-cells than the lower, and this results in the funnel-shaped form previously referred to. The upward movement of the daughter-cells is, moreover, probably in part due to the pressure of the elastic lower part of the wall upon them, although secretion of mucilage inside the mother-cell wall may also play a part. It may be pointed out that the upward movement of the daughter-cells is not due to migration of the protoplast inside the cell, as suggested by Davis (6, p. 377) in *Euglenopsis*, but that the movement is purely passive.

During this passive transference towards the aperture of the ruptured mother-cell wall, the lower of the two daughter-cells glides past the lower end of the upper one and becomes placed alongside of the upper cell at the mouth of the funnel, though very slightly below it. As a consequence both daughter-cells come to lie near the edge of the ruptured mother-cell wall and thus, as the gelatinous thallus develops with repeated cell-division, all the cells become arranged near the external surface. Occasionally it happens that one of the two daughter-cells remains at the base of the parent membrane (Text-fig. 4, R). Such cells do not appear to divide further. Microtome-sections of the thallus likewise show that practically all the living cells are placed near the outer surface with their longitudinal axes roughly at right angles to the latter, while the inner portion of the thallus is occupied mainly by the empty cell-walls and the mucilaginous matrix with a sprinkling of isolated cells (Pl. VIII, Fig. 8). Some of the fully developed thalli are even found to be hollow in the centre, a fact visible with the naked eye. In the detailed structure of the colony therefore this species contrasts markedly with *E. pulvinata*.

Reproduction. The cells usually divide into two daughter-cells only, but division into four is not uncommon. The daughter-protoplasts (Text-fig. 4, O) immediately develop cell-walls of their own, and the resulting group recalls daughter-cell formation in an *Oocystis* (Text-fig. 4, M, N, S). The daughter-cells, except for their much smaller size, are exactly like the parent. The cells thus formed do not appear to add to the bulk of the thallus, and it is not improbable that they escape after rupture of the wall of the parent-cell and serve for purposes of multiplication.

The living material kept in the laboratory was repeatedly searched for possible motile spores, but without success. The alga remained for a month in a fairly healthy condition, but after that began to deteriorate and finally died. The disintegrating and dead thalli were carefully examined for evidence of perennating spores, but none were found.

An alga resembling that just described occurred attached to the rocky bed of a stream above the water-falls at Courtallum in South India. The thalli were smaller and not nearly so abundant, but there were no other noteworthy differences.

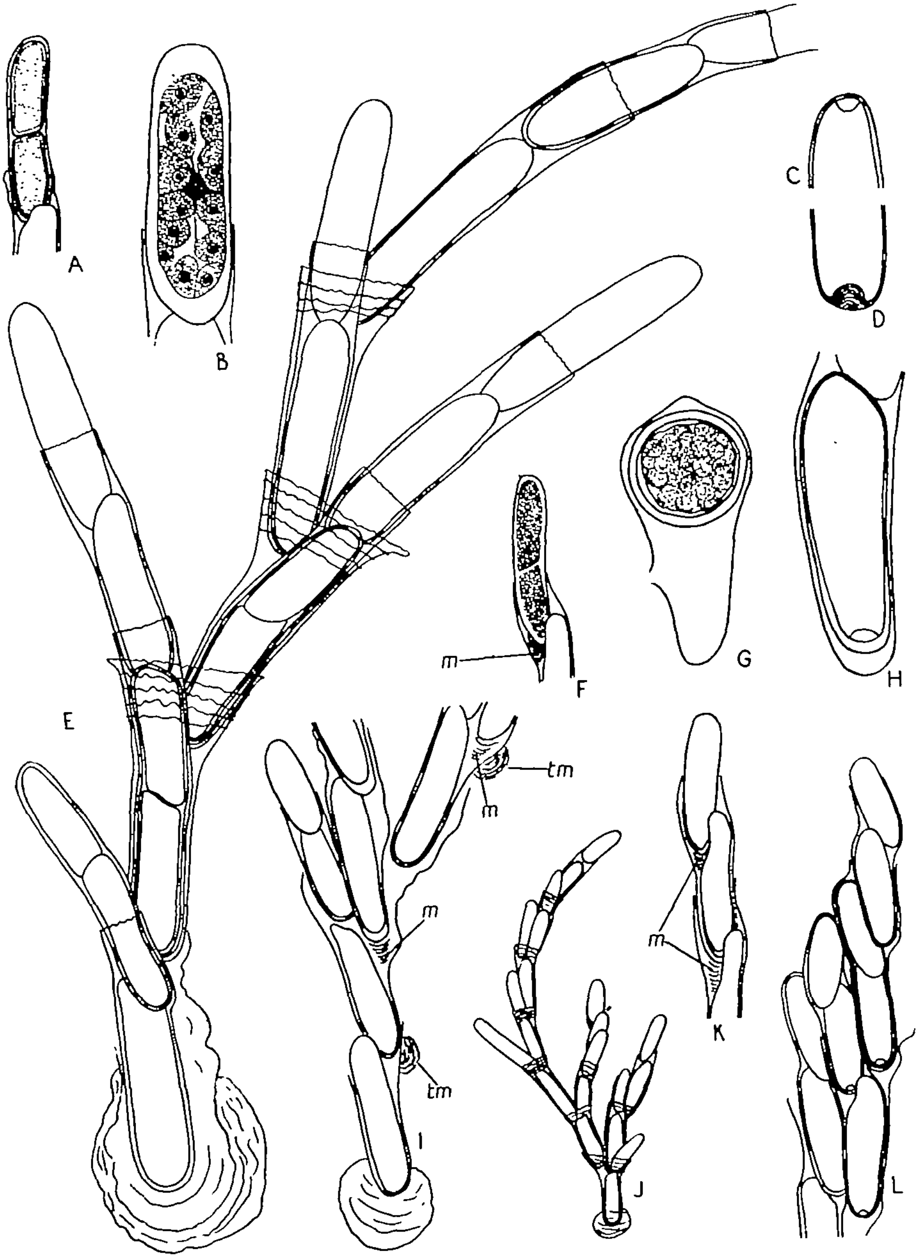
In another form (var. ***pulneyensis*** var. nov.), found attached to the rocky bed of a cold mountain stream at Kodaikanal in the Pulney Hills in South India, the mother-cell walls of only one generation could be detected in the mucilaginous matrix, since there was rapid gelatinization of those of the older generations (Text-fig. 4, L, P, Q). Owing to this the thallus lacked the tough consistency and the compact habit of the type, and appeared as a more expanded cushion. The cells in this form were broader in the middle than at the ends, which appeared somewhat attenuated. The pyrenoids were larger and more distinct, and at each end of the protoplast was a colourless cytoplasmic region not occupied by the chloroplasts (Text-fig. 4, I–K).

Ecballocystis courtallensis sp. nov. (Text-fig. 5 and Pl. VIII).

This alga[1] was found, along with an attached species of *Spirogyra*, on a rock which was continuously splashed by a waterfall above. It formed a thin green coating over a small area, and the material had to be scraped off carefully with the knife.

The form of the thallus is definitely dendroid (Text-fig. 5, E, J; Pl. VIII, Fig. 9). The cells are elongate and cylindrical with rounded ends, but the upper end is slightly more broadly rounded than the lower. The cell-contents are most clearly brought out with Heidenhain's iron-haematoxylin or Delafield's haematoxylin. Each cell contains a single central nucleus with a fairly prominent nucleolus and a large number (mostly 16 or 32) of disc-shaped, rounded or roughly elliptical, parietal chloroplasts, each having a minute, though definite pyrenoid (Text-fig. 5, B). The nucleus

[1] I am indebted to Mr. R. V. Narayanaswamy for sending me further material of this alga.



TEXT-FIG. 5. A–F, H, J, K, *Ecballocystis courtallensis* sp. nov. A, formation of two daughter-cells inside the mother-cell wall; B, single cell showing nucleus and chloroplasts; C, apical, D, H, basal nodular thickenings of the cell-wall; E, portion of a colony showing details of branching; F, division of cell-contents; J, colony showing habit; K, elongation of lower daughter-cell past the base of the upper one. G, I, L, *E. courtallensis*, f. *jogensis*. G, swollen cell with contents rounded like a cyst and having an aperture in the cell-wall (see p. 208); I, L, details of colonies; *m*, attaching mucilage; *tm*, mucilage secreted outside wall (see p. 208). Pyrenoids black. A, F × 270; J × 115; K, L × 405; B, C, D, G, H × 800.

appears to be suspended in the centre of a large vacuole by means of a number of radiating cytoplasmic threads. The chloroplasts of the older cells were packed with starch, which made it difficult to see the pyrenoids in them.

The cell-wall is not coloured blue by chlor-zinc-iodide. It is thin at the top, but gradually becomes thicker towards the base of the cell. At each of the two poles there is a small nodular thickening which is only recognizable after careful examination under a high power. The thickening when examined under high magnification appears lamellated, the lamellae evidently belonging to the inner portion of the wall at the two poles of the cell (Text-fig. 5, D). This thickening is somewhat more prominently developed at the lower than at the upper end of the cell, and is conspicuous only in the older cells (Text-fig. 5, C, H, and Text-fig. 5, L for f. *jogensis*).

Cell-division and colony-formation. When the cell has reached a certain size the contents undergo a slightly oblique division across the middle (Text-fig. 5, F), each daughter-protoplast surrounding itself with a wall of its own (Text-fig. 5, A; Plate VIII, Fig. 10). The enlarging daughter-cells soon cause a rupture of the mother-cell wall at the top where it is thinnest and where a certain amount of gelatinization probably takes place. The upper of the two daughter-cells now projects beyond the ruptured end of the mother-cell wall, and the subsequent growth of the lower daughter-cell helps to push the upper one still further out; the lower cell, however, remains at the base of the ruptured parent-membrane. Very soon each of the daughter-cells secretes at its base a very small, more or less conical mucilage pad which seems to attach the cell with some degree of firmness to the inside of the mother-cell wall (Text-fig. 5, F, I,[1] K, *m*). As further elongation of the lower daughter-cell takes place, it pushes past the base of the attached upper cell (Text-fig. 5, K), causing a distension of the elastic mother-cell wall which is later thrown into wrinkles (Text-fig. 5, E, J).

When the daughter-cells have attained their full size a fresh division takes place, and, since the same process is repeated again and again, a richly branched colony is soon established (Pl. VIII, Fig. 9, and Text-fig. 5, E, J). Division is, however, often confined to the upper daughter-cell, the lower one either remaining undivided or dividing only once or twice. At each division one of the daughter-cells becomes attached to the base of the ruptured parent-membrane, while the other comes to be fixed just within the mouth of the latter. The whole colony is firmly attached to the substratum by a thick, approximately circular pad of tough mucilage secreted by the base of the lowermost cell of the colony (Text-fig. 5, E, J). A considerable quantity of tough mucilage is also to be found at the base of some of the oldest cells, even though they are not actually in contact

[1] Fig. 5, I refers to the forma *jogensis*, p. 208.

with the substratum (Text-fig. 5, 1, *tm*). The amount of mucilage in these cases is considerably greater than that on the younger cells, and it seems as though its secretion went on indefinitely. It appears, however, that contact with a firm substratum acts as a special stimulus, since the lowermost cells of few-celled colonies, and, in some cases, even the single cells of first stages, show a large mucilaginous attaching pad.

The cells of this alga display a marked degree of polarity, as exhibited by the restriction of mucilage-secretion to the lower ends, by the slightly greater breadth of the cells at the upper end, by the slightly greater thickening of the cell-wall at the base, by the invariable rupture of the wall at the upper end, and the more conspicuous development of the polar nodule at the basal pole. When single cells get dispersed from a parent-colony—at present the only known method of reproduction—contact with a substratum presumably at once induces profuse secretion of mucilage from one end of the cell, whereby attachment is effected. How the erect position of the cell is assumed is not quite clear. There is probably some kind of adjustment, as shown below in the case of *E. ramosa* (p. 215), due to localized secretion of mucilage. It is open to discussion whether the loose cells already possess polarity or whether the polarity is determined in them only after contact with the substratum is established. This can only be settled by experiment. Free cells show very slight differences in the shape of the two ends, but clear outward evidence of polarity is afforded only in the older cells.

Reproduction. Although numerous specimens were examined at different times, division into more than two cells was never observed. The living alga was kept under observation for some time, but no zoospores were seen. Certain empty cells which exhibit a small round hole in their wall may represent sporangia from which swarmers have escaped, but they may equally well be the result of the presence of some parasite (cf. also the structures described in *E. courtallensis* var. *jogensis*, p. 208). So far, the only certainly established method of reproduction is by the liberation of the ordinary daughter-cells from the colony.

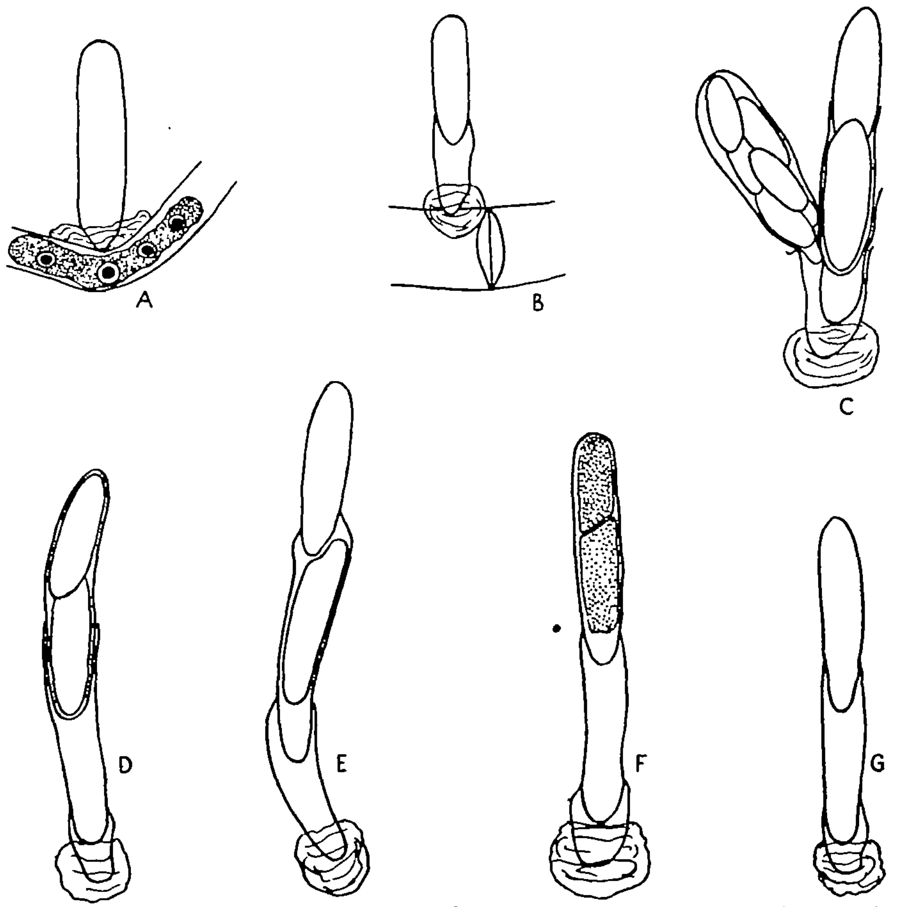
In the character of the cells and the dendroid habit this species shows a certain resemblance to the African *E. simplex* Fritsch (cf. below, pp. 209–10), but the Indian form is composed of far more numerous cells and is profusely branched. In *E. simplex*, moreover, a considerable number of empty cell-walls are found beneath the cells and especially below the lowermost one; this feature is never found in the same degree in the Indian alga. Though numerous specimens of *E. courtallensis* collected at different times during the last seven years have been examined, none showed 'sporangia' with four or more daughter-cells, such as are very commonly found in *E. simplex* (7, Text-fig. 4, D). This indicates that in the Indian alga sporangium-formation is either lacking or of very rare occurrence.

E. courtallensis forma *jogensis* forma nov. (Text-fig. 5, I, L; Pl. VIII, Figs. 5, 6, 7).

Another form of this species (f. *jogensis*) was found abundantly as a thin greenish covering on stones wetted by spray near the famous Jog Falls in the Mysore Province of South India. It is much more profusely branched than the type with more crowded branches, and the slightly smaller cells are placed nearer to each other. It may well prove to be merely a habitat-form of the main species (Text-fig. 5, I, L; Pl. VIII, Figs. 5–7.)

The difference in the habit is due to the slightly different behaviour of the daughter-cells after division, as well as to the shape assumed by the old walls of the parent cells. In this form the upper daughter-cell becomes attached *a little above the middle* of the ruptured wall of the parent-cell (Text-fig. 5, I; Pl. VIII, Fig. 7), and, when the lower cell grows past it, the original membrane becomes distended from the middle portion upwards, whereas in the type it is only the uppermost part that is affected (Text-fig. 5, E). The cell-wall, moreover, does not appear to be so elastic as in the type species, and does not show wrinkling to the same extent. The aperture remains fairly wide open (Text-fig. 5, I, L). As a result of these differences, the cells of the colony lie closer together within the widely opened mother-cell walls and the numerous branches are more closely adpressed to the 'main axis', and arise a little above the middle of the old wall, and not from near its upper end. The 'main axes' often present the appearance of consisting of two or more oblique rows of cells, which is due to the fact that the upper portion of the old mother-cell wall encloses within it the upper portion of the lower daughter-cell and the lower portion of the upper one (Text-fig. 5, L; Pl. VIII, Figs. 6, 7).

The material of this form included many loose cells in which the upper portion of the unruptured envelope was much dilated, while the contents, surrounded by a separate, relatively thick, membrane, formed a spherical mass within the swollen end (Text-fig. 5, G). Similar cells were occasionally also found within the intact thallus. The appearance is that of a sporangium containing a hypnospore serving as a possible means of perennation. Each of these cells, however, shows a definite aperture with slighty protruded edges situated on one side of the basal portion and presenting altogether the appearance of a natural opening formed by the cell itself. Since the edges of this pore are directed outwards, it would seem to be formed by gelatinization of the apex of a papilla after the manner of the formation of the pore in an oogonium of *Oedogonium*, but no such intact papillae were observed. The contents of the spore showed chloroplasts and pyrenoids and appeared quite healthy. In spite of careful examination no trace of a parasite was found, nor does the general character of the structures in question really suggest that they are due to a parasitic organism. A very remote possibility is that we are concerned with fertilized oogonia, but their true nature can only be settled by observation of living material.



TEXT-FIG. 6. *Ecballocystis simplex*, Fritsch. A, single cell on a filament of *Mougeotia*; B, D–G, different stages of growth of the colony; C, a slightly branched colony with a sporangium containing eight daughter-cells. Pyrenoids black. × 510.

Ecballocystis simplex, F. E. Fritsch (Text-fig. 6).

Through the kindness of Prof. Fritsch I have been able to examine a slide of this species, and, as a result, have come to the conclusion that the very small stature is not due to immaturity, but to the possession of a very different method of growth from that found in *E. courtallensis*. I am inclined to think that the plants figured by Fritsch may really be full-grown specimens. *E. simplex* occurs attached to water-plants and filaments of *Mougeotia* in flowing water. The plants, which often consist of but one cell (cf. below), arise perpendicularly from the substratum, and are attached by a mucilage pad (Text-fig. 6, A–G). The cell-structure appears to be quite similar to that of *E. courtallensis*, with numerous parietal disc-shaped chloroplasts, each with a pyrenoid. In *E. simplex* it appears that of the two daughter-cells formed at any given division (Text-fig. 6, F, D), only the lower one remains as part of the colony, the upper one being gradually pushed out by the growth in length of the lower daughter-cell, as well as by a more or less pronounced upward movement of the latter inside the mother-cell wall (Text-fig. 6, E). It looks as though the mucilage secreted by the daughter-cells is neither sufficiently copious, nor tough enough, to attach them firmly to the mother-cell wall, as happens in *E. courtallensis*. The mucilage appears to be diffluent and diffuse (cf. 7, Text-fig. 4, A, F). As a consequence, when the lower cell lengthens, it simply pushes the upper one in front of it. The upward movement on the part of the lower cell may be due to the pressure of the elastic mother-cell wall which, after being distended by the enlarging daughter-cells before apical rupture takes place, will tend to shrink to its old size. This upward movement is possibly also aided by the thin mucilage secreted by the cell at its lower end. In some cases this upward movement is not considerable, but often it leads to the lower daughter-cell ultimately shifting so far up that only its conical basal portion remains within the old mother-cell wall (Text-fig. 6, B, E–G).

In this way, therefore, the upper of the two cells formed at each division will tend to become thrust completely out of the colony, so that in general only one daughter-cell will remain as part of the colony. The latter will thus undergo no great increase in size. It appears, however, that very occasionally both daughter-cells may remain adhering to the top of the mother-cell wall (cf. 7, Text-fig. 4, F, G), so that a small dendroid colony is formed. But for this occasional retention of both daughter-cells, the colony would consist of only a single cell with more or less numerous cell-membranes below it. Every division, in fact, adds another empty membrane to the group. To judge from the dimensions of the successive empty membranes, it would appear that the first cell of a colony is relatively small and that the later ones are larger. Fritsch regarded the successive ruptured membranes found at the base of the older colonies as evidence of repeated rejuvenation of the protoplast, but in the light of the experience gained from a study of the Indian species of *Ecballocystis*, I believe that the explanation put forward above is the more probable. A study of living material of *E. simplex* would, however, alone settle the matter conclusively. In view of the fundamental difference in the *method of growth of the colony*, there can be no doubt that *E. courtallensis* is distinct from *E. simplex*, quite apart from other differences. In *E. simplex* division into 4, 8, or 16 daughter-cells is frequent.

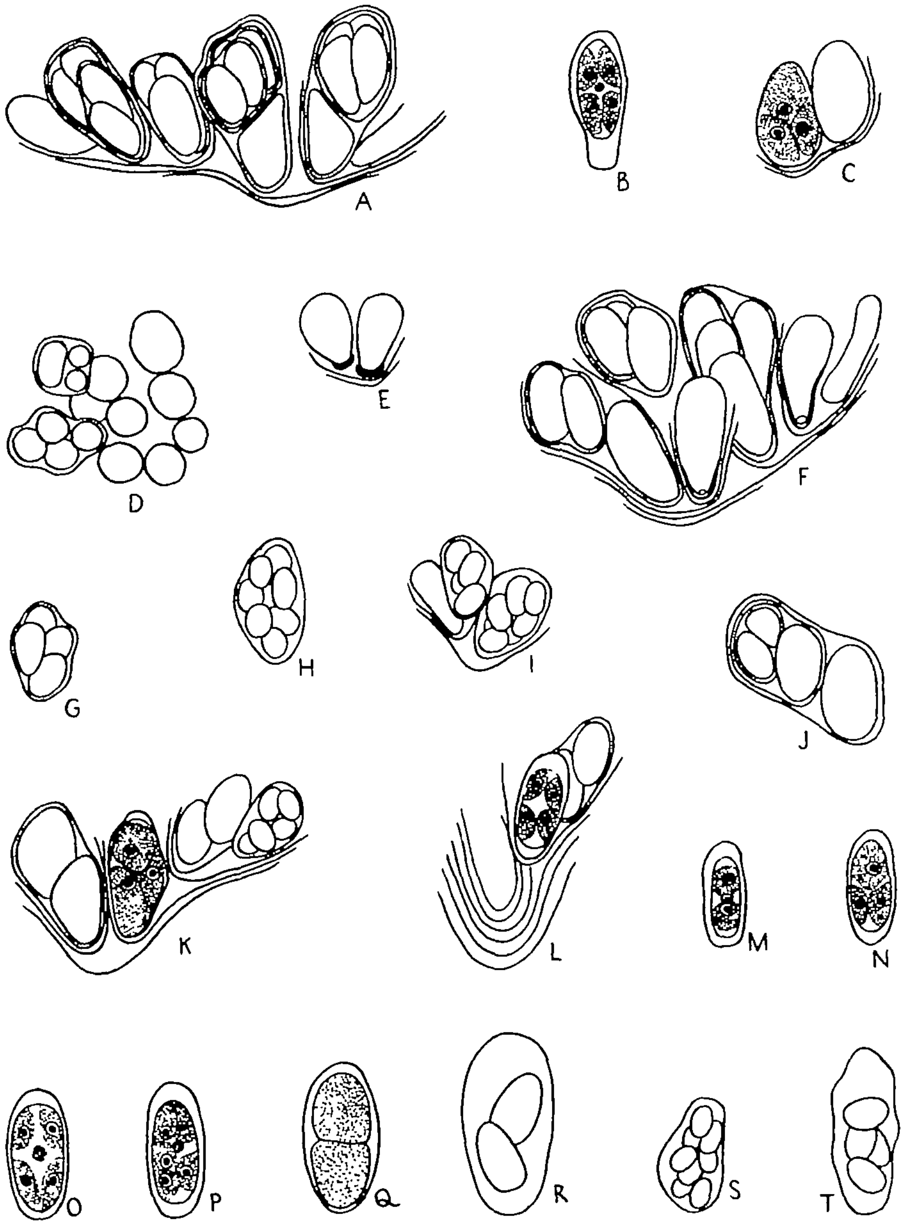
Ecballocystis pulvinata var. *minor* var. nov. (Text-fig. 7, A–L).

The colonies of this alga are very small with closely crowded cells, so that in the intact colony only the rounded ends of the cells seen in vertical view are presented to the observer (Text-fig. 7, D). If a colony is subjected to gentle pressure under a cover-glass, the cells are seen spread out, and if previously stained for a few minutes with Delafield's haematoxylin, Congo red, or methylene blue, the dendroid nature of the alga becomes clear.

The general arrangement of the cells is very similar to that shown in Bohlin's figure of *E. pulvinata* (2, Tab. I, Figs. 1–4), since the thallus as a whole is broadly obconical, and spreads obliquely over the substratum (Text-fig. 7, A, F, K). The conical gelatinous processes at the bases of the cells recorded by Bohlin are, however, replaced by a thin layer of mucilage which serves to attach the cells to the mother-cell wall (Text-fig. 7, E). The old mother-walls undergo gelatinization to some extent and help to attach the thallus to the substratum.

The cells are oblong to irregularly elliptic in shape, but they are often somewhat narrowed towards their lower end and then appear sub-conical with a truncate base and a broadly rounded apex (Text-fig. 7, B). Bohlin was unable to give any details as to the cell-contents in his species, but in the present form, by careful staining with Delafield's haematoxylin, a central nucleus with four parietal disc-shaped chloroplasts, each with a pyrenoid, can be more or less clearly seen (Text-fig. 7, B, C, K, L). The arrangement of nucleus and chloroplasts is similar to that seen in a cell of *E. Fritschii* containing four chloroplasts. In some cells the wall shows a slight polar thickening, rather similar to, but not so distinct as, that found in *E. courtallensis*; when present, this thickening is more pronounced at the lower than at the upper pole (Text-fig. 7, F). Division of the cells takes place in the usual way, but the resulting daughter-cells do not alter their respective positions to any considerable extent subsequent to division, and are, in later stages, found standing side by side at the base of the membrane of the parent-cell, the lower daughter-cell only very slightly below the upper one (Text-fig. 7, C, E, F, J). The upper one is generally situated on the side of the mother-cell directed towards the periphery of the colony, while the lower one is located towards the inside. Further division is usually confined to the upper daughter-cell and, as a result of this and of the fact that this cell is usually placed on the outside, the spreading habit of the colonies is brought about. Moreover, since the daughter-cells remain near the base of the parent-cell, the whole colony is procumbent and does not possess the erect branched habit of the previously described species of *Ecballocystis*.

As in the other cases, the old mother-cell walls of successive generations



TEXT-FIG. 7. A–L, *Ecballocystis pulvinata*, Bohlin var. *minor*, var. nov. A, C, F, K, L, portions of colonies; B, single cell with chloroplasts and nucleus; D, vertical view of cells of a colony; E, two cells showing basal mucilaginous secretion; G, H, I, sporangia with four or eight daughter-cells; J, several cell-generations included in the respective mother-cell walls; M–T, *Oocystis ecballocystiformis*, sp. nov. M–P, cells showing contents; Q, division of the protoplast; R, S, T, formation of autospores. Pyrenoids black. All × 890.

persist for some time and are to be seen in considerable numbers as somewhat swollen distinct lamellae below the cells.

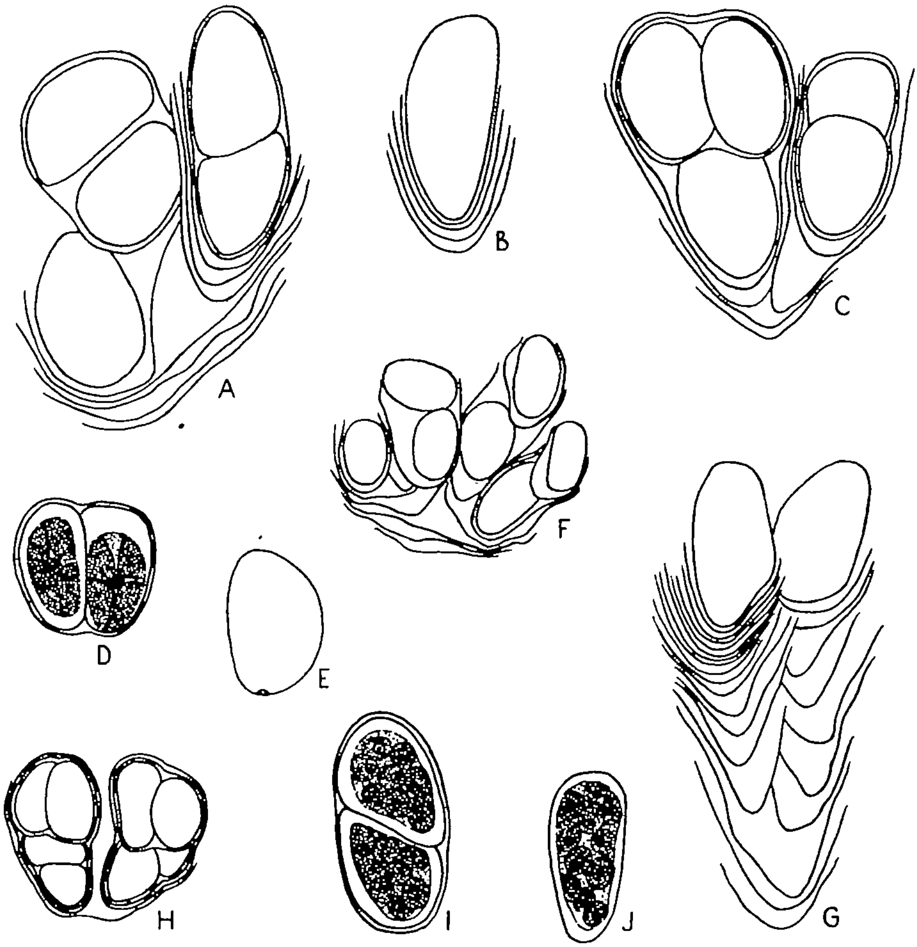
The cell-contents very often divide into four or eight daughter-cells (Text-fig. 7, G–I, K), and any cell of the colony may develop into such a 'sporangium'. Bohlin did not encounter such stages in his material. On the other hand, it is not uncommon to find a number of cell-generations enclosed in the unruptured membrane of the parent (Text-fig. 7, J). This is also shown in Bohlin's figure of the type (2, Fig. 4).

Ecballocystis pulvinata Bohlin var. *diffluens*, var. nov. (Text-fig. 8).

This alga formed a minute cushion-like colony about 0·5–1·5 mm. in diameter, and was found growing along with *E. courtallensis* f. *jogensis* on stones constantly wetted by spray from the Jog Falls. It shows some resemblance to *E. pulvinata* var. *minor* in its general appearance. The colony consists of numerous short, more or less erect, dendroid branches closely aggregated together. Owing to the dense growth, the structure is not easily made out, but if a colony is gently squeezed under a cover-glass, the erect branches are spread out, and some of them may be seen very clearly at the edges where they happen to lie clear of the others. The base of the whole colony is obconical or broad (Text-fig. 8, A, C, F–H). The arrangement of the cells is somewhat similar to that of *E. pulvinata* var. *minor*, but there are marked differences in the shape and size of the cells, as well as in the structure of the cell-contents. The cells are broadly and often irregularly elliptic, sometimes narrowed at the base and broadly rounded above. They possess a central nucleus and 4, 8, or 16 parietal disc-shaped chloroplasts each with a small pyrenoid (Text-fig. 8, D, I, J). In some of the fully-grown cells a very minute nodular thickening of the wall can occasionally be seen at the base (Text-fig. 8, E), although never discernible at the upper pole.

The walls of the older generations undergo gelatinization relatively quickly, with the result that the thallus is more diffuse than in var. *minor*. Moreover these walls are thin and delicate, and only to be detected with difficulty, so that the thallus, unless carefully stained, appears as a structureless mass of cells, and its dendroid nature is not at first apparent. Cell-division takes place as in *E. pulvinata* var. *minor*, but only two daughter-cells are formed. Division into four or more daughter-cells has not been observed, but as in var. *minor*, two or three cell-generations are often found enclosed in the unruptured parent membrane (Text-fig. 8, H). In the upper portions of the dendroid branches, one of the daughter-cells is often seen to have escaped, probably serving for purposes of propagation (Text-fig. 8, F). It is not unusual to find single cells with numerous old walls below them (Text-fig. 8, B, G) at the ends of some of the branches indicating that, as in *E. simplex*, repeated cell-division has occurred, accompanied by liberation of the upper daughter-cell.

The alga just described differs from *E. pulvinata* var. *minor* in the



TEXT-FIG. 8. *Echallocystis pulvinata*, Bohlin, var. *diffluens*, var. nov. A, C, F, H, portions of colonies; B, cell with many old membranes below it; D, I, J, cells with contents (in I showing cell-division); E, cell with nodular thickening; G, portion of colony with two cells having numerous old membranes below each. Pyrenoids black. All × 950.

larger size and different shape of its cells, in forming only two daughter-cells at each division, and in the presence of 4, 8, or 16 chloroplasts in the cells, whereas var. *minor* never has more than eight. It agrees with *E. pulvinata* Bohlin in forming only two daughter-cells during cell-division, but differs from it in several other respects.

Ecballocystis ramosa Fritsch (Text-fig. 9).

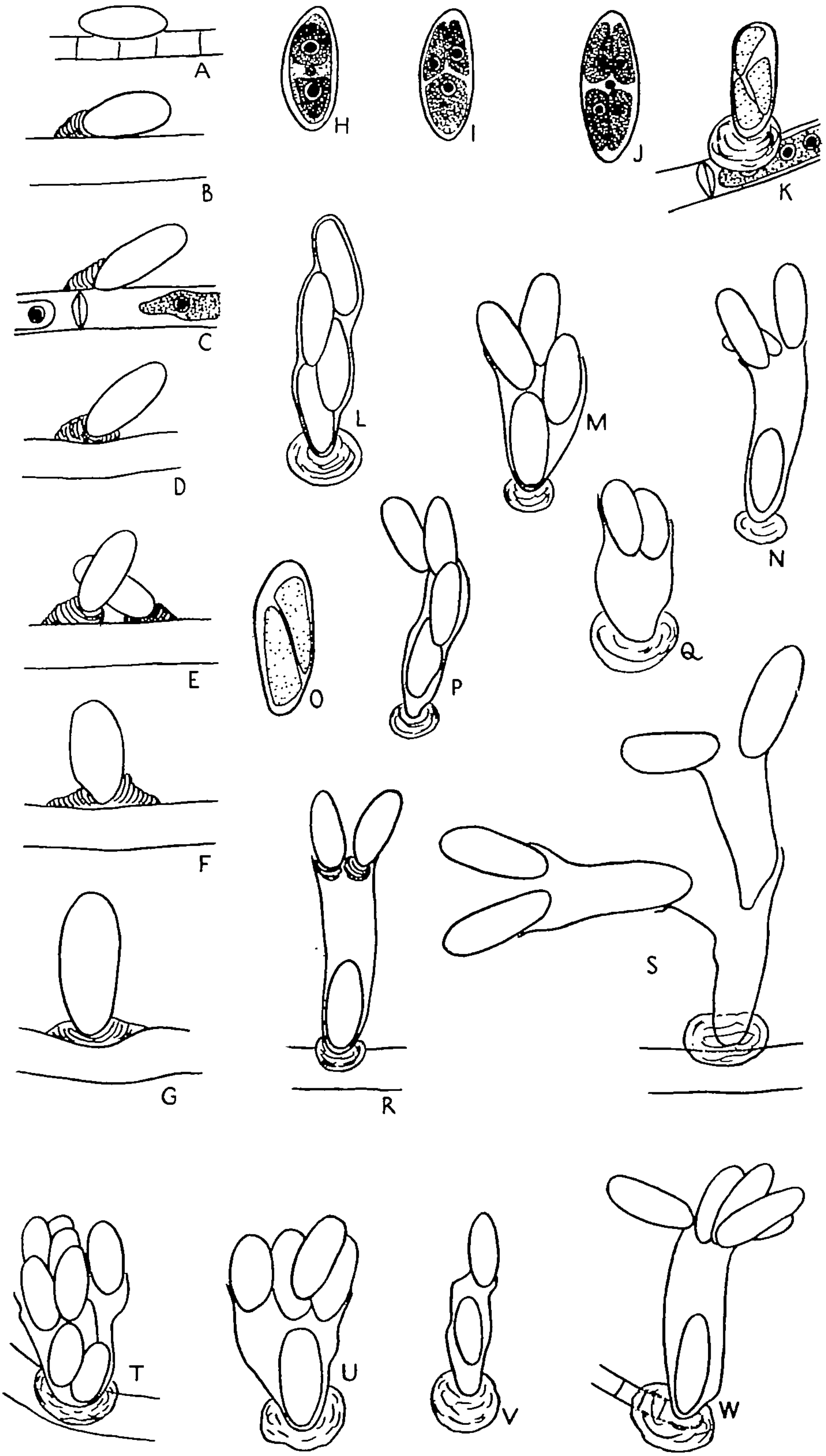
This African species was found by Fritsch [1] epiphytic upon attached filaments of *Mougeotia*, *Ulothrix oscillarina*, and *Hormidium subtile* in flowing water. Compared to the other species of the genus, *E. ramosa* has a very simple thallus. The colony begins as a single erect cell attached by a well-developed mucilage pad. This cell usually gives rise to four daughter-cells (Text-fig. 9, L) which, as they increase in size, distend the mother-cell wall so that it finally ruptures at the apex (Text-fig. 9, M, P). One daughter-cell usually stays at the base of the ruptured membrane, while it appears that one of the remaining three is generally pushed out of the parent-membrane. The other two become attached to the aperture of the ruptured wall by means of mucilage, well seen in stained preparations (Text-fig. 9, R). Sometimes three daughter-cells are found attached at the aperture (Text-fig. 9, N). In other cases division into eight occurs (Text-fig. 9, T). In such cases, one daughter-cell seems to remain at the base of the ruptured parent-membrane, a few are extruded and the remainder become attached at the aperture (Text-fig. 9, U, W). Division into two is not uncommon (Text-fig. 9, O) and, in that case, one cell may remain at the base, while the other either becomes attached to the aperture of the ruptured wall (Text-fig. 9, V) or escapes to the outside; just as commonly, however, both daughter-cells are found attached to the aperture (Text-fig. 9, Q).

The daughter-cells divide again to form a small dendroid colony, with two or three generations of cells, though not so compact as in most other species of the genus (Text-fig. 9, S). The whole remains quite small, possibly owing to the temporary nature of the substratum.

Multiplication appears to be effected by means of the daughter-cells, which are set free and float away. These cells are often somewhat conical at one end and broadly rounded at the other, i.e., they exhibit a clear polarity before attachment to the substratum is effected. They are always found attached by the conical ends. Such cells commonly exhibit a slanting position upon the threads of the algae which serve as substratum; some lie very nearly parallel to them (Text-fig. 9, B). In the former case most of the basal mucilage is found to be secreted on the side of the cell away from the substratum, and very little on the opposite side. In cells exhibiting a more erect position a larger quantity of mucilage is found on the side adjacent to the substratum, while in the fully erect specimens the amount of mucilage is equal on both sides. All transitions are to be found from a very acute angle, with the cell almost parallel to the substratum, to a definitely erect position.

These various stages (Text-fig. 9, B–G) suggest that, when such loose

[1] Prof. Fritsch very kindly allowed me to examine some of his material of *E. ramosa* from S. Africa.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cells happen to be floated on to a suitable filament, the stimulus of contact induces a secretion of attaching mucilage from the conical pole which at first ensues on the outer side only. Later secretion of mucilage also takes place on the side adjacent to the substratum, and the cell gradually assumes an erect position. Single loose cells were often found lying flat on the algal filaments, a position which might be due to the accident of mounting (Text-fig. 9, A). In view of the many instances of oblique attachment, however, in which in every case there was a definite secretion of mucilage from the basal pole, these cases may possibly represent the first stages in the establishment of the alga upon the filaments.

These observations on *E. ramosa* may indicate the manner in which the cells of the other species of *Ecballocystis* become attached to and erected upon the substratum.

The contents of the cells of this species resemble those of *E. Fritschii.* There is a central nucleus and 2, 4, or 8 parietal disc-shaped chloroplasts each with a pyrenoid (Text-fig. 9 H, I, J). The chloroplasts are very delicate and lie closely crowded, so that they are not very clearly defined.

The Affinities of Ecballocystis.

The systematic position of *Ecballocystis* is very uncertain. Bohlin (2), when he first established the genus, placed it among the Tetrasporaceae, and suggested that it was nearly related to the Flagellata, and especially to the genera *Euglenopsis* (6), *Chlorangium* (15) and *Prasinocladus* (9). He considered that it stood nearer to the two last-named genera, in which the cells are attached by broad mucilaginous stalks secreted at their lower ends, and suggested that *Ecballocystis* might be regarded as a *Chlorangium* in which the mucilaginous stalks remained undeveloped.

Wille (20, pp. 27, 28) likewise referred it to Tetrasporaceae (tribe Hauckieae), including in it Setchell and Gardner's *Collinsiella*; West (17, pp. 185–189) and Printz (19, p. 69), on the other hand, place it in the tribe Chlorangieae of the Tetrasporaceae, and retain *Collinsiella* as a distinct genus in the tribe Palmophylleae. Oltmanns (12, p. 138) includes *Ecballocystis*, together with *Chlorodendron* and *Prasinocladus*, in the new family Chlorodendraceae of Volvocales (cf. also 12*a*, p. 240). Lemmermann (10, p. 25) places it in the family Chlorangiaceae of the group Tetrasporales. Fritsch (7, pp. 494–502), in describing the species *E. ramosa* and *E. simplex*, lists the genus under Chlorodendrales, which is ranked as a group of equal status to the Tetrasporales and Chlamydomonadales, the three

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TEXT-FIG. 9. *Ecballocystis ramosa*, Fritsch. A–G, stages in the attachment of cells to the substratum; H–K, cells with contents (in K showing division); L, formation of four daughter-cells; M, N, P, R, different types of attachment of daughter-cells to the ruptured mother-cell wall; Q, V, the same in a cell with two daughter-cells; T, U, W, the same in a cell with eight daughter-cells; O, division of protoplast into two; S, loose dendroid colony. Pyrenoids black. All × 560.

being regarded as representing different evolutionary lines of the Volvocales (cf. also 18, p. 67). He regards *Ecballocystis* as quite distinct from *Collinsiella.* Printz (13, pp. 78, 79) very rightly refers the three species, described by Yendo (21) under *Ecballocystis*, to *Collinsiella.*

All authorities are thus agreed in placing *Ecballocystis* among the simpler Chlorophyceae, but while some regard it and the related genera merely as a tribe of the Tetrasporaceae, Oltmanns and Fritsch include them in a group with a higher status, the latter even regarding the Chlorodendrales as a distinct evolutionary line (cf. also 8, p. 109). The latter authority, moreover, considers the genera grouped in Chlorodendrales to be closely related to the motile forms from which they probably originated. Of the divers botanists who have dealt with the systematic position of the genus, only Bohlin and Fritsch actually investigated it, and both had scanty preserved material at their disposal in which the details of cell-structure were not easily decipherable. The possession of chloroplasts with pyrenoids, and the presence of starch in the cells of the forms here described fully confirms the reference to Chlorophyceae.

As regards the relation of *Ecballocystis* to the other genera of Chlorodendraceae, the writer's investigations have shown that in its possession of several or many parietal chloroplasts, each with a pyrenoid, it diverges markedly from the others. While there is a general resemblance embodied in the dendroid habit, the detailed manner of colony-formation is not the same. And in the third place the apparent absence of motile reproductive stages, considered more fully below, removes *Ecballocystis* from the other three genera with which it has usually been associated. In view of the rather decided differences *Ecballocystis* would perhaps best be placed by itself in a separate family of Chlorodendrales. In the writer's opinion its nearest allies are to be sought elsewhere, as will be explained in the following.

Emphasis has just been laid on the non-discovery of spores. Fritsch (7) searched for zoospores in his material, but in every case the loose cells found in the sediment were clothed with a wall. He suggested that this might be due to any zoospores present having formed a wall during the interval between collection and preservation of the material. It has been possible to study the Indian members of the genus in a living condition, both in nature and in the laboratory, on *many different* occasions, but no motile stages have ever been observed. There is thus reason to suspect the absence of motile spores. Such negative evidence is not, however, sufficient to dismiss the possibility of an occasional formation of swarmers, the more so as many empty cells from which the contents had apparently escaped were often to be found in the colonies.

In the absence of swarmers attachment to the substratum is not easily visualized. In flowing water, or in the littoral zone of lakes where the water is agitated, the cells of Zygnemaceae, however, become attached without possessing motility. And the same may be true of *Ecballocystis*. In either case a ready secretion of attaching mucilage must take place. Assumption of the erect position must depend upon some erecting sense, and is evidently brought about by a localized secretion of mucilage (cf. the data above given for *E. ramosa*, p. 215).

During cell-division in *Ecballocystis* the contents divide obliquely into two, and very soon new membranes are formed around the daughter-protoplasts, which, by further growth, ultimately rupture the wall of the parent-cell, usually at its upper end. This is seen in Bohlin's type-species, in the two species described by Fritsch, as well as in all the species collected by me. In *E. ramosa*, Fritsch, *E. simplex*, Fritsch, *E. Fritschii* and *E. Fritschii* f. *pulneyensis*, the cell-contents occasionally divide into more than two daughter-cells (four, eight, or sixteen) without the rupture of the mother-cell wall. And in all such cases the daughter-protoplasts surround themselves with their own individual cell-walls soon after their production. Such a formation of daughter-cells recalls to a very marked extent an *Oocystis* forming 'autospores' inside it, although oblique division is not recorded in this genus. The daughter-cells in *Ecballocystis*, even when there are only two, may in fact be regarded as *autospores*. The formation of 2, 4, 8, or 16 autospores in the cells of different species of *Ecballocystis* is fully paralleled among the species of *Oocystis*. The ordinary cell-multiplication of other Chlorodendraceae is likewise of the nature of 'autospore formation', although none of the other genera except *Chlorangium* are recorded as showing division into more than two daughter-cells.

The cell-contents in the Indian species of *Ecballocystis* are very similar to those of certain species of *Oocystis*. Thus in a species of *Oocystis* (which comes near to *O. crassa*, Wittrock), collected from an artificial tank in the University Botanical Garden at Heidelberg last September, the cells contain a central nucleus and a number (2, 4, or 8) of parietal disc-shaped chloroplasts with a single pyrenoid. The same arrangement of the chloroplasts is found in *O. Borgei*, Snow and *O. lacustris*, Chodat (4, Pl. XXII, Figs. 4, 8, 9, and 13). *O. eremosphaeria*, Smith (14, p. 113, Pl. XXIII, Figs. 1 and 2) has numerous chloroplasts with a pyrenoid in each. In another species of *Oocystis* collected near the Jog Falls some years ago, the resemblance is so close that the cells might readily be mistaken for the free cells of *E. Fritschii*. The species in question may be named

Oocystis ecballocystiformis sp. nov. (Text-fig. 7, M–T).

This species occurred as a light-green floating scum on the surface of the water occupying a depression in the rock, about thirty yards from the spot where *E. courtallensis* f. *jogensis* and *E. prostrata* were found. This scum consisted of numerous free-floating cells which were in all respects (including size) exactly like the loose cells of *E. Fritschii*. They were oblong-elliptic in shape, with a central nucleus and 2, 4, or 8 parietal disc-shaped chloroplasts, each with a pyrenoid (Text-fig. 7, M–P). There was no difference in the thickness of the cell-wall at the two ends. The cell-division was not oblique, but nearly transverse, as in *Oocystis* (Text-fig. 7, Q). Occasionally two, four, or eight daughter-cells were found loosely enclosed within the much distended mother-cell wall (Text-fig. 7, R–T. The daughter-cells evidently become free by the dissolution of the latter, but actual instances of this were not available in the material.

It is possible that these are actually loose cells of *E. Fritschii*, and that this is the form in which this alga reproduces. On the other hand, the normal *E. Fritschii* has not been met with in the neighbourhood of the Jog Falls, its nearest recorded localities being more than two hundred miles away. In view of this fact and of the other distinctive features, viz., absence of polarity, transverse division, and the occurrence of cells lying loosely within the distended mother-cell wall, it would seem best for the present to regard it as a species of *Oocystis*, with cells very similar to those of *Ecballocystis*.

The large number of chloroplasts found in the dendroid species of *Ecballocystis* is no doubt due to the larger size of the cells.

In some of the species of *Ecballocystis* here described the wall is often somewhat thickened at the lower end, although this thickening is often seen only in the older cells. Definite polar thickenings, like those of many species of *Oocystis*, are only seen in *E. courtallensis*. The presence of such polar thickenings in this genus may be of significance, since even in *Oocystis* there are quite a number of species which lack this feature. In two important respects, therefore, in the mode of formation of daughter-cells and in the structure of the cells, there are considerable resemblances between *Ecballocystis* and certain species of *Oocystis*.

On the other hand, there are marked points of difference, such as the constant oblique division in *Ecballocystis* and the pronounced difference between base and apex in the cells. This finds its expression in the greater thickening of the membrane in the basal part,[1] the almost invariable rupture of the membrane in the apical part, and the frequent secretion of special mucilage pads only from the lower ends of the cells.

In connexion with my investigations on *Ecballocystis Fritschii* it was observed that, as the hill stream dwindled with the onset of the dry season, the plants in the tiny pools of water remaining at the sides became less compact and more diffluent, as if they were undergoing gradual dissolution.

[1] One species of *Oocystis*, *O. apiculata*, W. West, shows a certain amount of polarity, the cells having only one polar thickening (cf. 16, p. 125, and Fig. 97).

There were plenty of loose cells in the material, though they did not present an unhealthy appearance. Evidently the conditions in the stagnant water were not favourable for a dendroid growth, and the colonies were breaking up into the individual cells.

If such cells became adapted to life in stagnant water, and lost the faculty of dendroid growth, though still retaining the same method of daughter-cell formation (2, 4, 8, or 16 'autospores' within the mother-cell wall), there would be a close approach to *Oocystis*. Under such new conditions of life the cells might be expected ultimately to lose their polarity, and would be indistinguishable from an *Oocystis*. Though most species of *Oocystis* are non-colonial, *O. gloeocystiformis*, Borge, shows the retention of several generations of daughter-cells within the membranes of successive generations. Similarly, in the closely allied genus *Nephrocytium*, *N. ecdysiscepanum*, W. & G. S. West forms a diffuse colony in which 'several generations are aggregated in a fan-shaped manner, owing to ecdysis, but incomplete dissolution of the old mother-cell walls' (17, p. 197). This latter species recalls *E. Fritschii* in the arrangement of the old mother-cell walls (cf. 18, Fig. 36, B). It is not impossible that the two algae just mentioned represent transitional stages between an *Ecballocystis* (dendroid) condition and an *Oocystis* (free, unicellular) condition.

On the other hand, the dendroid *Ecballocystis* condition might be derived from unicellular forms like *Oocystis* as an adaptation to life in rapidly flowing water. If an *Oocystis* acquired polarity, and developed the capacity to secrete mucilage at one end of the cell, whereby attachment to a substratum became possible, and, if the daughter-cells, after the rupture of the mother-cell wall, secreted mucus in the same way and remained attached to the latter, a dendroid colony like that of *E. ramosa* or *E. courtallensis* would result. While, if all the old walls underwent gelatinization, a form like *E. Fritschii* would be obtained. Forms like *Oocystis gloeocystiformis* and *Nephrocytium ecdysiscepanum* would fit well into such a scheme.

This second alternative does not, however, appear likely for the following reasons. Polarity is probably an earlier condition associated with the presumably primitive motile habit. Whatever the mode of propagation of *Ecballocystis* may be at the present day, it is extremely probable that it originated from a motile form which settled down by attaching itself to some substratum by the anterior end. Of two forms, one with and one without polarity, the former may be considered as standing nearer to the motile ancestral type. And it is easier to conceive of the loss of an already existing polarity than the acquisition of polarity by a form devoid of it. The derivation of an *Ecballocystis* with its pronounced polarity from *Oocystis* therefore seems very improbable. The oblique division of the protoplast seen in *Ecballocystis* marks a more primitive condition than the transverse one of *Oocystis*.

DIAGNOSIS OF THE NEW FORMS.

Ecballocystis pulvinata var. *minor* var. nov.

Thallus forming a minute procumbent expanse on the substratum; cells densely crowded (the ends alone visible in the intact colony), oblong to irregularly elliptical, each with a central nucleus and four parietal chloroplasts containing a minute pyrenoid, cell-wall occasionally with polar thickenings visible only under very high magnifications, that at the lower pole more marked than that at the upper; cell-division oblique, the two daughter-cells subsequently standing nearly erect and side by side at the base of the membrane of the parent-cell, the lower daughter-cell being only very slightly below the upper one; further division usually confined to the upper cell, so that successive divisions lead not to an erect branched growth, but to an horizontal, somewhat procumbent, broadly obconical thallus; successive mother-cell walls undergoing slight gelatinization and found in considerable numbers as somewhat swollen distinct lamellae at the base of the younger cells; secretion of mucus confined to a small mass at the base of each cell; division into four and eight very common.

Dimensions of the fully grown cells: $14 \times 8{\cdot}75\ \mu$, $15 \times 9\ \mu$, $16 \times 10\ \mu$, $18 \times 8{\cdot}5\ \mu$, $12{\cdot}25 \times 8{\cdot}5\ \mu$, $11{\cdot}5 \times 7\ \mu$.

Hab. Growing along with *E. courtallensis* on a rock continuously splashed by the Shenbagadevi Falls at Courtallum, South India.

Ecballocystis pulvinata, Bohlin, var. *diffluens* var. nov.

Thallus forming minute diffluent cushions on the substratum, in which the densely crowded cells can be seen arranged in numerous dendroid systems growing closely together side by side. Cells elliptic-oblong to broadly or irregularly elliptic, often narrowed at the base and broadly rounded above; each with a single nucleus and 4, 8, or 16 closely packed parietal disc-shaped chloroplasts with a single pyrenoid. Cell-division into two daughter-cells only observed; walls of older generations gelatinizing rapidly and hard to distinguish; sporangia with four or more cells not observed.

Dimensions of cells: $19 \times 12\ \mu$, $18{\cdot}8 \times 12{\cdot}8\ \mu$, $19 \times 11{\cdot}8\ \mu$, $20 \times 14\ \mu$, $21 \times 12\ \mu$, $21 \times 14\ \mu$, $21 \times 15\ \mu$, $22{\cdot}5 \times 13\ \mu$, $23 \times 9\ \mu$, $24{\cdot}5 \times 14\ \mu$.

Hab. On stones wetted continuously by spray from the Jog Falls in Mysore Province, South India, growing together with *Ecballocystis courtallensis* f. *jogensis*.

Ecballocystis Fritschii, sp. nov.

Thallus macroscopic, irregularly rounded, lobed, sometimes somewhat cylindrical, the two-daughter-cells usually formed at each division both passing to the mouth of the ruptured parent-membrane, with the result that in the older thalli nearly all the living cells are to be found at the periphery, while the inner part consists mostly of mucilage and empty cell-membranes; cells devoid of conical gelatinous processes at their lower ends, the older mother-cell walls conical or funnel-shaped at the base; cells 16–25 μ long and 6–11 μ broad. Cells occasionally dividing into four daughter-protoplasts, which clothe themselves with cell-walls while still inside the mother-cell wall, and possibly serve for reproduction.

Dimension of cells: $16 \times 7{\cdot}5\ \mu$, $17 \times 8\ \mu$, $17{\cdot}5 \times 6\ \mu$, $17{\cdot}5 \times 7\ \mu$, $18 \times 7{\cdot}5\ \mu$, $19 \times 8{\cdot}5\ \mu$, $20 \times 9\ \mu$, $22 \times 11\ \mu$, $29 \times 9\ \mu$. Newly formed cells inside the mother-cell wall: $12 \times 5{\cdot}75\ \mu$, $13{\cdot}75 \times 5{\cdot}5\ \mu$.

Hab. Growing attached in crowded masses to the sloping rocky bed of a small mountain stream just above a waterfall, Mamandur, South India. February, 1930.

Ecballocystis Fritschii var. *pulneyensis* var. nov.

Thallus macroscopic, forming minute, spreading, approximately round or elliptic-oblong gelatinous cushions, less compact than those of the type, about one-quarter to half an inch broad and about one-eighth to one-sixth of an inch in thickness; mother-cell walls of only one generation distinguishable, owing to rapid gelatinization of the older walls; cells broader in the middle than at the somewhat attenuated and occasionally sub-truncate ends; chloroplasts similar to the type in number and arrangement, but not so sharply marked off from one another; pyrenoid larger and distinct, with a clear starch-sheath; protoplast with a colourless cytoplasmic area at each end. Cells usually dividing into two, occasionally into four.

Dimensions of cells: $17 \times 8\ \mu$, $16 \times 5{\cdot}75\ \mu$, $15 \times 6\ \mu$, $17 \times 7{\cdot}5\ \mu$, $19 \times 7\ \mu$, $18 \times 7{\cdot}25\ \mu$, $14{\cdot}5 \times 7{\cdot}8\ \mu$, $17 \times 7{\cdot}25\ \mu$; the width being that of the broadest part of the cell.

Hab. Attached to the rocky bed of a cold mountain stream with a fairly rapid current at Kodaikanal in the Pulney Hills in South India. The depth of the flowing water was about six inches.

Ecballocystis courtallensis, sp. nov.

Thallus microscopic, forming a dendroid, many-celled colony, consisting of a false axis with a number of short laterals, the whole attached to the substratum by a prominent mucilaginous pad secreted at the lower end of the basal cell; the two daughter-individuals formed in cell-division become attached to the mother-cell wall by a basal secretion of mucus, the lower one near the base, the upper one near the aperture; subsequent division often confined to the upper daughter-cell, although the lower one may divide once or twice and give rise to a false branch. Cells elongate-cylindrical with rounded ends, $9\cdot5$–16×37–$84\ \mu$, the upper end slightly broader than the lower, with a central nucleus and many rounded or sub-elliptic disc-shaped parietal chloroplasts, each with a small pyrenoid ; cell-wall slightly thicker at the base than elsewhere, with nodular thickenings at either pole conspicuous in the older cells. Reproduction apparently only by cells detached from the colonies ; no swarmers observed.

Dimensions of cells ; $11 \times 63\ \mu$, $12 \times 47\ \mu$, $13 \times 53\ \mu$, $14 \times 57\ \mu$, $14 \times 59\ \mu$, $14 \times 66\ \mu$, $14 \times 84\ \mu$, $16 \times 70\ \mu$.

Hab. On a rock splashed by water below the Shenbagadevi Falls at Courtallum, Tinnevelly District, South India ; first collected in October, 1924.

Ecballocystis courtallensis, forma *jogensis*, forma nov.

Thallus very similar to that of the type, but with more profuse and more closely adpressed branches, the upper daughter-cell after every division becoming attached *a little above the middle* of the mother-cell wall which, as a result of the further enlargement of the lower cells, becomes distended from the middle upwards, and not only at the very top as in the type. Cells slightly smaller.

Dimensions of cells : $10\cdot5 \times 36\cdot75\ \mu$, $10\cdot5 \times 31\cdot5\ \mu$, $10\cdot5 \times 45\cdot5\ \mu$, $11\cdot5 \times 47\cdot25\ \mu$, $12\cdot25 \times 49\ \mu$, $11\cdot5 \times 59\cdot5\ \mu$.

Hab. On stones constantly wetted by spray from the Jog Falls, Mysore Province, South India.

Oocystis ecballocystiformis, sp. nov.

Cells oblong-elliptic, with broadly rounded ends ; cell-wall thin and devoid of polar thickenings ; chloroplasts 2, 4, or 8, parietal, disc-shaped, each with a minute pyrenoid ; 2, 4, or 8 autospores formed inside the distended mother-cell wall.

Dimensions of cells : $18 \times 7\ \mu$, $19\cdot6 \times 8\cdot9\ \mu$, $19\cdot75 \times 8\cdot75\ \mu$, $24 \times 9\cdot1\ \mu$, $20\cdot5 \times 9\ \mu$; young cell, $16 \times 5\cdot5\ \mu$.

Hab. In a small pool occupying a depression in a rock near the Jog Falls in Mysore Province, South India.

Artificial Key to the Species of Ecballocystis.

A. Colony with free branched axes not embedded in mucilage, often microscopic.

I. Colony composed of few cells only, microscopic, one or more of the daughter-cells formed in division commonly thrust out of the colony.

(*a*) Colony a small dendroid branch system, cells relatively short, not exceeding $48\ \mu$ in length, with 2–8 somewhat angular disc-shaped chloroplasts whose limits are indistinct; cell-division usually into four, occasionally into two or eight . . . *E. ramosa.*

(*b*) Colony commonly composed of a single cell surmounting a a number of empty membranes, cells longer, up to 67 μ in length, cylindrical with many disc-shaped distinct chloroplasts (up to 32); cell-division usually into two . . . . . *E. simplex.*

II. Colony well-branched, composed of numerous cells; cell-division only into two, both daughter-cells usually remaining attached to the mother-cell wall . . . . . . *E. courtallensis.*

(i) Upper daughter-cell attached near the ruptured apex of the mother-cell wall, often bending outwards . . . f. *typica.*

(ii) Upper daughter-cell attached a little above the middle of the mother-cell wall, nearly parallel to the lower . . f. *jogensis.*

B. Colony relatively large, appearing as a lobed or cushion-shaped gelatinous mass, macroscopic, with closely crowded branched axes, sometimes embedded in mucilage.

I. Colony lobed, cells embedded in the mucilage formed by the gelatinization of the old walls; daughter-cells attached to the mouth of the funnel-shaped mother-cell wall . . . *E. Fritschii.*

(i) Colony relatively compact, cells elliptic-oblong, not broadened in the middle; walls of several generations visible . var. *typica.*

(ii) Colony somewhat diffuse, cells elliptic, broadened in the middle; walls of only one generation visible, the others gelatinizing very quickly . . . . var. *pulneyensis.*

II. Colony minute, cushion-shaped, with fairly firm mother-cell wall, not gelatinizing quickly, daughter-cells not attached to the mouth of the obconical or widely cup-shaped mother-cell; old mother-cell walls of many generations lying close together *E. pulvinata*, Bohlin.

(i) Daughter-cells attached by conical gelatinous processes secreted at their lower end; cell-divisions only into two; chloroplasts unknown . . . . var. *typica.*

(ii) Daughter-cells attached by a broad gelatinous secretion from their lower end; cell-division into two to eight daughter-cells; chloroplasts four or eight, in the form of angular discs var. *minor.*

(iii) Daughter-cells not attached by any definite secretion; walls delicate and rapidly gelatinizing; cells larger and more rounded, irregularly elliptical; chloroplasts many, up to sixteen, in the form of rounded discs . . . . var. *diffluens.*

SUMMARY.

In the first part of the paper an account of the structure and development of *Tetrasporidium javanicum* is given. The thallus consists of two

perforated layers of cells, provided with numerous connecting processes. The sporangia described by Moebius are shown to be due to the attack of of a species of *Vampyrella.* In view of the absence of pseudocilia the genus cannot be referred to Tetrasporaceae, but must find a place among Palmellaceae.

The second part of the paper deals with the genus *Ecballocystis*, a number of species of which are described from various parts of India, while two South African species are also taken into consideration. The diverse form of the colony in the different species is related to the varying behaviour of the daughter-cells after division, which is invariably oblique. The cells contain from two to many discoid parietal chloroplasts with pyrenoids, and usually exhibit a marked polarity. No evidence of motile stages or resting spores has been obtained. The normal method of reproduction would appear to be by means of detached daughter-cells, already enveloped by a membrane at the time of liberation. In some species such cells are produced in considerable numbers within the mother-cell. When floated on to a suitable substratum, the cell secretes mucilage at one pole and gradually becomes erected at right angles to the substratum. It is pointed out that *Ecballocystis* is not closely related to the other genera of Chlorodendrales with which it has usually been associated, and a possible affinity with *Oocystis* is indicated.

The author wishes, in conclusion, to express his great indebtedness to Professor F. E. Fritsch for the plentiful advice he has given during this investigation, and for assistance in preparing the paper for press. He also wishes to acknowledge the help afforded by Dr. N. Carter and Miss F. Rich.

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DESCRIPTION OF PLATES VII AND VIII.

Illustrating Professor Iyengar's paper on Two Little-known Genera of Green Algae (*Tetrasporidium* and *Ecballocystis*)

PLATE VII.

Tetrasporidium javanicum, Moeb.

Fig. 1. Photograph of a thallus floated in water in a Petri dish (about half nat. size).

Figs. 2, 3, 4. Photomicrographs of portions of the thallus. Fig. 2 shows the two perforated layers, the lower seen faintly below the upper, unstained. × 75.

Fig. 3. Part of a single layer of the thallus, lightly stained with safranin. × 150.

Fig. 4. Portion of the thallus showing the two layers with cross-connexions, lightly stained with safranin. × 150.

PLATE VIII.

Ecballocystis, Bohlin.

Figs. 5 and 6. Complete colonies of *Ecballocystis courtallensis* f. *jogensis*. Fig. 5 × 60; Fig. 6 × 110.

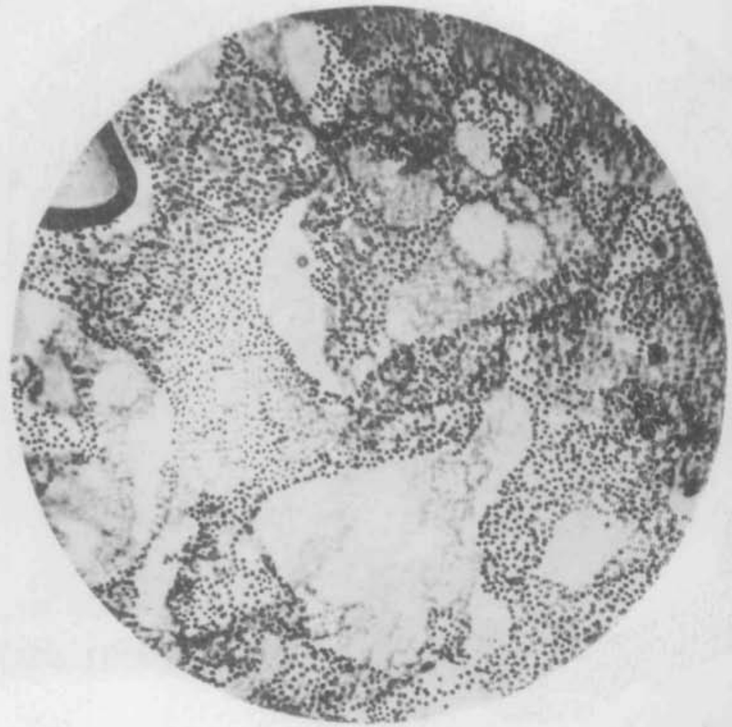
Fig. 7. A portion of the colony shown in Fig. 2, more highly magnified. × 380.

Fig. 8. Photomicrograph of a microtome section of *E. Fritschii* showing the cells crowded near the periphery of the thallus. × 65.

Fig. 9. Young colonies of *E. courtallensis* f. *typica*. × 100.

Fig. 10. Three cells of *E. courtallensis* occupying the end of a branch; the upper two cells have been produced by division of a sister-cell of the lowest which has not divided; the chloroplasts are clearly seen in the upper two cells. × 600.

1



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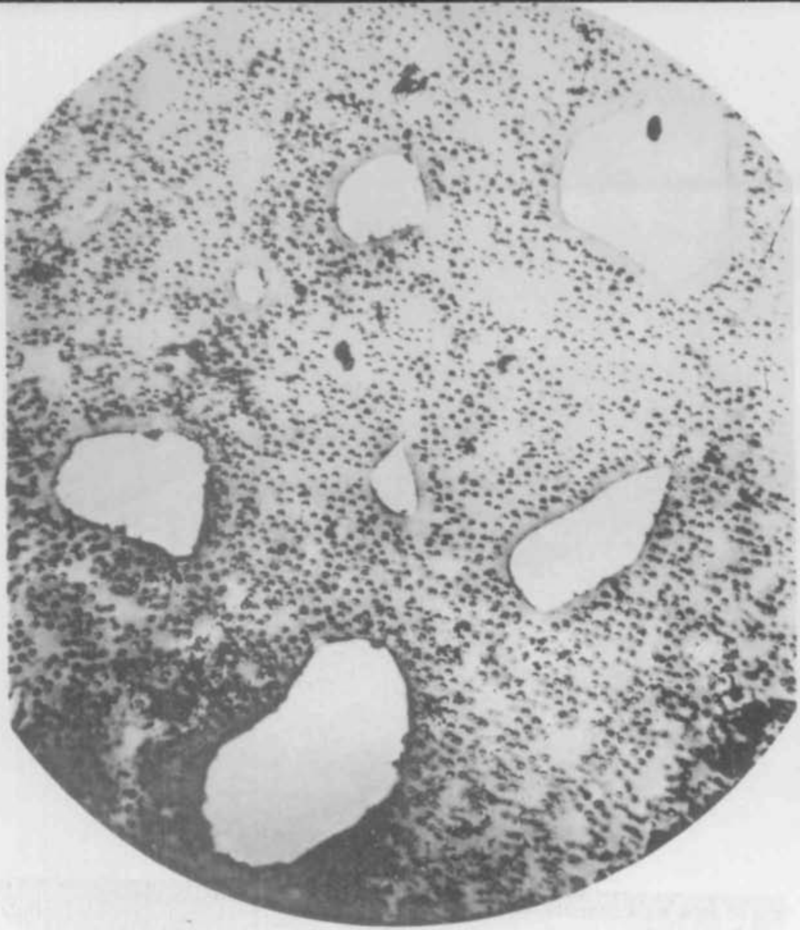

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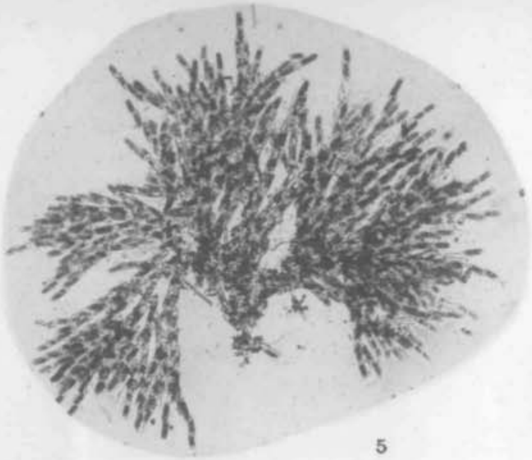
IYENGAR — TETRASPORIDIUM.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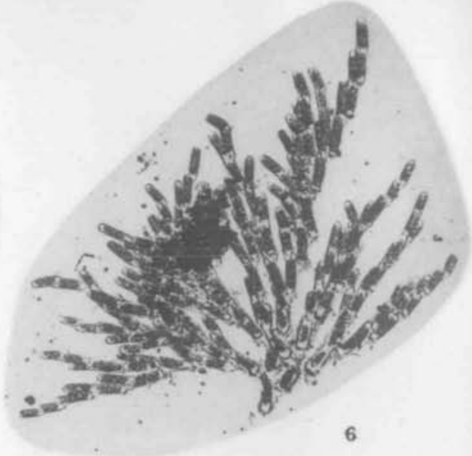
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IYENGAR — ECBALLOCYSTIS.