Contributions to our Knowledge of the Colonial Volvocales of South India.

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(PLATE 28, and 10 Text-figures.)

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THERE appears to be a general impression among algologists that the colonial members of the Volvocales are pre-eminently cold-water types (West, 1916, p. 429). This impression arises from the fact that so far only very few members of this group have been recorded from the warmer parts of the globe, and it has naturally been assumed that these records represent sporadic occurrences. The object of this paper is to record and describe the colonial Volvocales collected within a small area of Southern India *, and to draw attention to the common occurrence of these forms in a typically tropical area. I have no personal familiarity with the occurrence of the colonial Volvocales in Northern European countries, and am therefore not in a position to compare their numbers there and in the Tropics, but when viewing the abundance of these forms in India have often wondered whether they could ever attain to such profusion in the colder waters of the temperate zones.

Remarks on the Occurrence of the Colonial Volvocales.†

Colonial Volvocales have been met with in many different kinds of waters in Southern India, such as large reservoirs and tanks, ponds and permanent pools, paddy-fields, water-tubs, and brackish water-canals. Though occurring commonly in these habitats, it is in the innumerable temporary rain-water pools found during the wet seasons, both by the roadside and in the open country, that they attain to maximum abundance. There are two wet seasons in India, the summer or South-West monsoon from June to the middle of September, and the winter or North-East monsoon from the middle of October to December. The weather during these rainy months is comparatively mild, and the sky cloudy for long periods during the day.

Numerous Volvocales are found in these small pools, often occurring in such large numbers as to give the water a green colour. The depth of the green colour usually depends upon the density of the algal population, and

* An account of a species of *Volvox* from Lucknow in North India is included in this account.

† I have already mentioned some of the following points in a previous paper (Iyengar, 1920).

is often found to change in the same spot at different times of the day, especially if the day is bright. This is due to the upward and downward movement of the forms involved, in response to the decreasing or increasing intensity of the light. They aggregate near the surface in the morning and evening, and retreat below the surface in the middle of the day when the light becomes too strong. They also appear to move downwards at night, as they are not to be found at the surface during the early morning hours. Smith (1917, p. 178) observed similar upward and downward movements on the part of *Volvox mononae* at different times of the day in Lake Monona, in response to the varying intensity of the light.

These organisms exhibit a somewhat similar behaviour, even in the diffuse light of the laboratory. Kept in a green vessel in the middle of a room lighted from a window on one side, they collect on the side directed towards the light during the morning and evening hours, but, during the middle of the day when the light is strongest, they recede to the side of the vessel farthest from the window. This movement was observed even in material placed in a watch-glass. Many observers have referred to similar phenomena (Oltmanns, 1922–23, pp. 371–372), both in nature and in the laboratory, but it may not be without interest to record these observations made in a tropical environment.

Another set of observations appear to be explicable as being determined by the need for an adequate air-supply. In certain pools in the sandy beach at Madras, numerous Volvocales (*Chlamydomonas* spp., *Gonium pectorale*, *Pandorina morum*) and Diatoms were to be found in the water. But, in addition, the sand was green to a distance of one or two feet from the edge of the pools, forming as it were a green border around them. The first impression obtained was that the algae had been left behind by the drying up of the pool. When some of the green sand was shaken up with water, however, the latter became green, and when a drop of it was examined under the microscope, countless forms identical with those occurring in the pools were observed actively swimming in the water. When a few particles of the wet green sand were mounted under the microscope, large numbers of the algae could be seen swimming in the thin film of water surrounding each particle. It was found that the algae in the pool were not so active as those in the films around the sand-particles, and it seemed that the latter were definitely in a healthier condition. The thin films of water around the sand-particles were, of course, in direct contact with the air in the interspaces of the sandy soil, and it is to be presumed that they were richer in dissolved air than the water of the pool itself. Evidently the algae favoured this situation with its presumably better aeration. Such sand-algae (*Chlamydomonas* and Diatoms) have been observed by Warming (1909, p. 175) on the coasts of Denmark and by Cowles (1899, p. 114) on the coasts of Lake Michigan.

Among the unicellular genera that were collected in South India with more or less frequency are *Chlamydomonas*, *Carteria*, *Chlorogonium*, *Lobomonas*, *Phacotus*, *Pteromonas*, and *Pyramidomonas*, while the colonial members are

represented by *Chlamydobotrys*, *Gonium*, *Pandorina*, *Eudorina*, *Pleodorina*, and *Volvox*. These algae are very usually associated with species of *Euglena*, *Trachelomonas*, and other Flagellata. The present account deals with the colonial forms. It is hoped to publish an account of the unicellular Volvocales at a later date.

Apart from their occurrence in the rain-water pools, the Volvocales are also found in bigger pieces of water, but here they occur sparsely among other algae. *Volvox*, which is of rare and sporadic occurrence in India, has, however, never been collected in the temporary rainwater pools. It seems to prefer deeper and more permanent waters. As regards the other forms, they evidently find, as in other parts of the world, optimum conditions for growth in the fresh rain-water (with its high percentage of dissolved oxygen) and under the diminished light-intensity of the monsoon periods. They occur in greater profusion during the summer monsoon than during the winter monsoon months, which suggests that it is the higher temperature of the former that favours the growth of these algae. During the summer monsoon the highest shade temperatures for Madras in 1919 were 105°·0, 102°·2, 101°·1, and 99°·0 F. for June, July, August, and September respectively, while during the winter monsoon they were 96°·2, 90°·6, 86°·3 F. for October, November, and December respectively. This effect of temperature indicates that the Volvocales are more likely to attain to maximum abundance in the Tropics than in the colder regions of the globe.

The rain-water pools are often very muddy, since buffaloes and other animals tend to wallow in the water during the warmer parts of the day. The muddiness does not seem to affect the algal population, since as the water clears the algae again become visible, and once more impart to it a greenish colour. The churning up of the water by the wallowing animals may help in the solution of the mineral salts, and the muddiness may also afford the algae a certain amount of protection against strong insolation in the middle of the day.

SYSTEMATIC ENUMERATION OF THE COLONIAL VOLVOCALES OF SOUTHERN INDIA

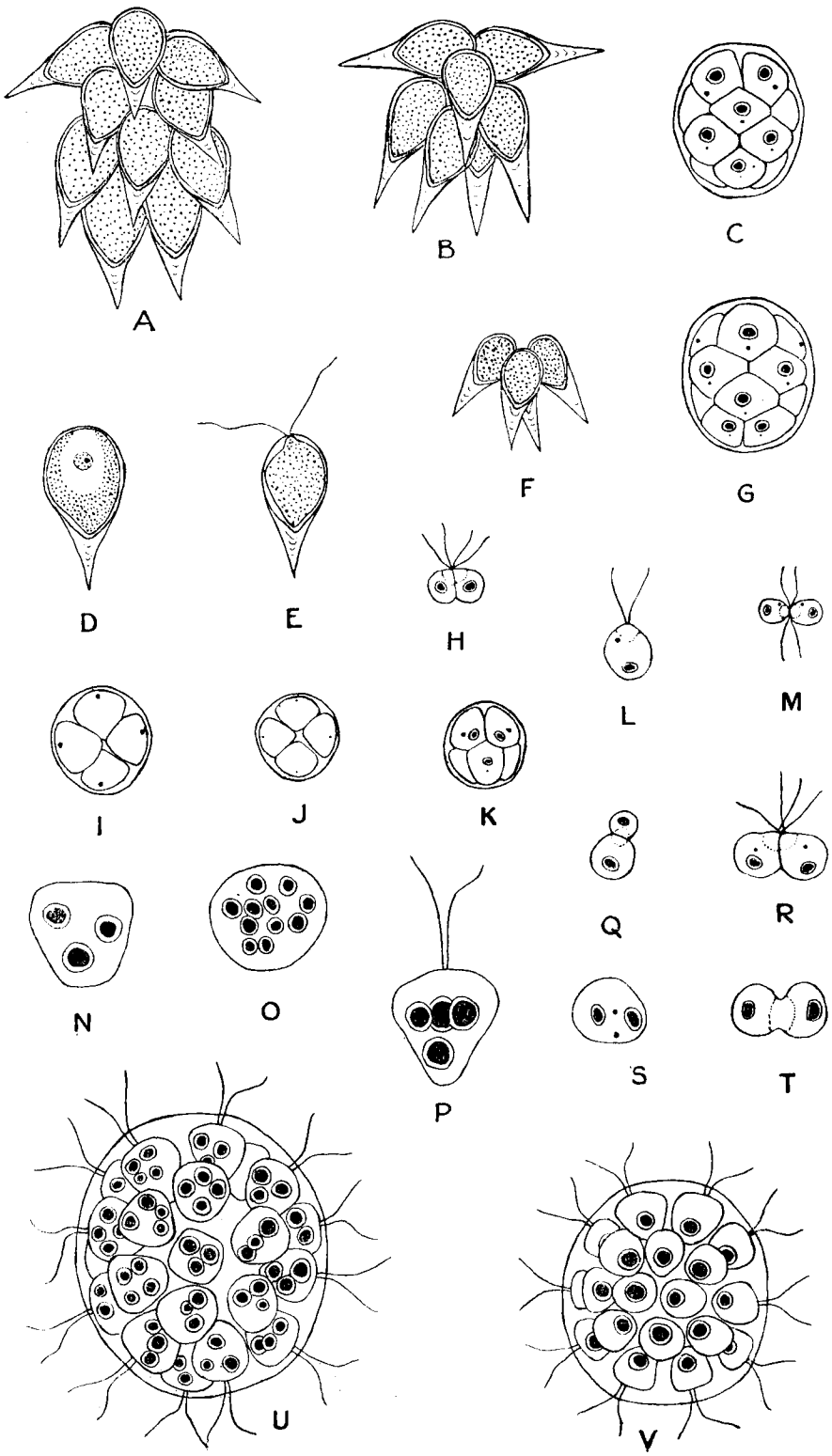
The following account deals with the colonial Volvocales, both from the taxonomic and morphological points of view. No complete description of the previously known forms is given, but under each species a number of independent observations are described, which either confirm earlier work or appear to extend our knowledge of the form in question. The preliminary observations were usually made on the living algae, although the investigations were subsequently completed on preserved material.

SPONDYLOMORACEAE.

CHLAMYDOBOTRYS STELLATA Korschikoff. (Text-fig. 1, A, B, D–F.)

This alga, which has not been before recorded from India, is very rare. It was collected near Bangalore by Dr. Sampathkumaran, who kindly handed

TEXT-FIG. 1.



EXPLANATION OF TEXT-FIG. 1.

A, B, D–F. *Chlamydobotrys stellata* Korschikoff.

A, 16-celled colony; B, 8-celled colony; D, single cell showing nucleus and chloroplast; E, single cell showing beaked protoplast and two cilia; F, 4-celled colony.

C, G–M, Q–T. *Pandorina morum* Bory.

C, G, 16-celled colonies showing the arrangement of the cells when the colonies are viewed in longitudinal planes at right-angles to one another; I and J, colony seen from the anterior and posterior ends respectively, showing position and size of eye-spots; K, 8-celled colony; L, gamete; H, M, Q–T, conjugation between gametes of various sizes.

N–P, U, V. *Pandorina morum* Bory f. *major* n. f.

U, mature, and V, young colony; N, P, cells of older colonies; O, cell of a very old colony with numerous pyrenoids. Pyrenoids black.

A, B, F, N–P, × 1060; C, G–M, Q, T, × 390; D, × 1700; U, V, ×580.

me the material. I subsequently found the same species on two occasions in rain-water pools in Madras.

Colonies with eight and with sixteen cells were usual (text-fig. 1, B, A), the former possessing two tiers and the latter having four tiers of four cells each. The cells of successive tiers alternate with one another. In each tier, however, the four cells are not at the same level, one diagonally opposite pair of cells being placed at a slightly higher level than the other diagonal pair. Very occasionally four-celled colonies are found (text-fig. 1, F). Each cell has two cilia and two contractile vacuoles. In one preserved specimen in which a good view of the anterior end was obtained, the protoplast appeared to be slightly beaked, the two cilia arising from this point (text-fig. 1, E). No pyrenoids were evident. The cells are pear-shaped, with the broader end anterior, the narrow, backwardly directed posterior end being very sharply conical. This conical posterior end was clearly seen on staining with dilute aqueous methylene-blue. The first impression obtained is that the contents of the cells have contracted away from the posterior extremity, but careful examination shows that the apparently empty area is solid, the thickening here showing some lamellation (text-fig. 1, A, B, D–F). Previous authors who have dealt with this form have assumed that the hyaline area at the posterior end was due to contraction of the protoplast.

The colonies during their forward movement rotate on their axes, the rotation being clockwise from right to left. No reproductive stages were observed.

A sixteen-celled colony measured 30·5 μ broad and 39 μ long, the cells being 9 μ broad and 12·5 μ long.

VOLVOCACEAE.

GONIUM PECTORALE Müller.

This alga is fairly common, especially in fresh rain-pools in the sandy beach at Madras. The colonies are composed of four, eight, or sixteen cells.

PANDORINA MORUM Bory. (Text-fig. 1, C, G–M, Q–T.)

This occurs very commonly in rain-water pools, often intermingled with other motile algae, but is also found in larger pieces of water. The forward movement of the colonies, accompanied by the usual rotation, takes place with a series of very slight jerks, the posterior portion of the colony oscillating slightly from side to side, while the anterior portion remains steady.

The colonies, though usually sixteen-celled, quite frequently consisted of only eight cells. They were very slightly broader at the anterior than at the posterior pole. The cells were arranged in alternating zones of four cells each, there being four in sixteen-celled and two in eight-celled colonies. The somewhat pear-shaped cells were arranged very compactly in the living specimens, so that they were slightly angular through mutual pressure. In living material the enveloping mucus-sheath is well seen in young coenobia, but in the older ones it appears to fit closely over the cells, so that it is sometimes difficult to see it clearly. In preserved formalin material, on the other hand, when the cells through contraction appear more rounded, the mucilage is equally evident in both young and old specimens.

There was usually a single pyrenoid in each cell, but in some *very old* colonies the cells contained from one to four pyrenoids. The eye-spots were very large in the front zone cells and gradually diminished in size in the others (text-fig. 1, C, G). In one coenobium the eye-spots in the front zone were 3 μ in diameter, in the second zone 2 μ, in the third zone about 1 μ, and in the fourth zone a hardly visible speck. The eye-spots are round in surface-view, but when seen in profile are concavo-convex with the concavity directed outwards. In the individual cell the eye-spot is situated close to the hyaline anterior region where the cilia arise, but on the side remote from the anterior end of the colony. The eye-spots of the four anterior cells, as seen from the end, are placed more or less equidistantly with reference to the circular contour of the colony (text-fig. 1, I). This position enables them to have good exposure, not only to the light from the front, but also to that coming from the side. The eye-spots of the four posterior cells occupy a similar position as seen from the posterior end (text-fig. 1, J).

Conjugation was observed once (text-fig. 1, H, L, M, Q–T). The naked gametes were of different sizes, the smaller measuring on the average 12 μ and the larger 16 μ in diameter. Conjugation took place in one of three ways, viz. (1) between a large and a small gamete (text-fig. 1, Q), (2) between two large gametes (text-fig. 1, R), or (3) between two small ones (text-fig. 1, H, M). These observations agree with those of previous workers. The zygotes with four cilia, two eye-spots and two pyrenoids, remained motile for some time, and, finally, came to rest (text-fig. 1, R. S); their further development was not observed.

The full-grown sixteen-celled colonies measured 61×48 μ, 52×44 μ; dimensions of the cells, 16×24 μ, 11×16 μ, 18×22 μ.

One can distinguish two forms in the previous records of *Pandorina morum*;

the one with globular coenobia and somewhat rounded cells and a broad gelatinous margin to the envelope ; the other with oblong-ellipsoidal coenobia, the cells very compactly aggregated and angular, and with a narrow, but firm margin to the envelope (cf. the figures of Smith and Conrad reproduced in Pascher, 1927, figs. 387 and 388 respectively). The Indian alga belongs to the latter type, which may be named f. *oblonga* (cf. Stein, 1859–83, whose Tab. 16, figs. 14 & 15, may be regarded as f. *oblonga*), the former being described as f. *typica*.

PANDORINA MORUM, forma MAJOR, n. f. (Text-fig. 1, N, O, P, U, V ; Pl. **28.** fig. 7.)

This alga was found on one occasion in enormous numbers, giving the water of a rain-water pool a green colour. The colonies were fairly big for *Pandorina*, and normally contained thirty-two cells, though a few with sixteen cells were also found. The chief characteristic lay in the invariable presence of three or four pyrenoids in each cell, no matter whether the colony contained thirty-two or sixteen cells (text-fig. 1, N, P, U). The pyrenoids are formed *de novo* (cf. p. 330). The colonies were ellipsoidal with rounded ends, the anterior pole being broadly rounded, while the posterior one was slightly truncate. The cells were arranged in five zones comprising 4, 8, 8, 8, and 4 cells respectively, the cells of the successive zones alternating with one another. This is the arrangement found in *Eudorina elegans*, but here the cells were pear-shaped (text-fig. 1, P) with the pointed end directed inwards, and were placed very compactly, so that in a surface-view, in a living specimen, they had an angular appearance. In the preserved material from which the drawings were made, the cells were somewhat contracted, and were more rounded at the corners (text-fig. 1, U, V).

Playfair (1915, pp. 336–7 and pl. 44. fig. 18) has described a similar form under the name of var. *tropica*. He did not recognize the *Eudorina*-like arrangement of the cells. He describes them as being arranged in a ' central ring of 10, above and below which is a rosette of 6 plus 1 ' (in planes parallel to the longitudinal axis of the colony). His figure, when examined carefully, however, shows the arrangement found in *Eudorina elegans* and in the alga here described. There are, in his figure, two cells at either end which represent the two upper cells of the anterior and the posterior tiers of four cells. The second, third, and fourth rows (lying at right angles to the longitudinal axis) consist of four or five cells each, being the cells visible from above of the second, third, and fourth tiers, each of which consists of eight cells.

Pandorina morum has been described (Stein, 1859–83, Abth. iii. 1, tab. 18, fig. 1 ; Pascher, 1927, p. 423, footnote) * as sometimes consisting of thirty-two cells, and the occurrence of more than one pyrenoid in each cell has also been

* Stein figures a thirty-two-celled colony with one pyrenoid in each cell. Pascher states that occasionally the number of cells in the colony is eight or thirty-two, or very rarely even four or two.

reported. But in the alga just described the normal number of cells is thirty-two, and more than one pyrenoid is always to be found in the cells of the full-grown colony. Playfair (1915) does not give any details of the cell-structure of var. *tropica*, and it is not clear from his figures how many pyrenoids there were in each cell. The constancy of these features in my material inclines me to consider it as a distinct form of *Pandorina morum*. Specimens of the type-species (with a single pyrenoid in the cell) did not occur in the collection. Similarly, in the gatherings of the type-species, not a single individual of this form was encountered. In such collections, however, certain very old specimens had two to four pyrenoids in the cells. In the same way, in very old specimens of the form under consideration, I found cells with as many as six to eight, and in one case even twelve to thirteen pyrenoids (text-fig. 1, O). In such specimens the cells were very nearly rounded, and these colonies possessed a definite *Eudorina*-like appearance.

Perfectly young colonies of the form under discussion always had a single pyrenoid in the cells (text-fig. 3, B). As the colonies grew older, more pyrenoids were formed, and they were formed *de novo*. In no case was the original pyrenoid observed to divide. The new pyrenoids make their appearance as hazy round bodies, which gradually increase in size and finally take a definite form. The newly formed pyrenoid is much smaller than the original single one. The pyrenoids arise successively, and when three or four pyrenoids are present they are generally of slightly different sizes and the original pyrenoid can be distinguished for a long time by its larger size. The type with a single pyrenoid may be considered as more primitive, and the form which *normally* has a larger number of pyrenoids as the more advanced.

The dimensions of the full-grown thirty-two-celled colonies were 55–64 μ broad and 63–74 μ long (55×64 μ, 57×63 μ, 57×68 μ, 61×68 μ, 64×74 μ). The cells measured 11–15 μ in breadth and were 12–14·8 μ long.

EUDORINA ELEGANS Ehrenberg. (Text-fig. 2 ; Pl. **28.** fig. 17.)

This occurs in smaller and larger pieces of water among bigger algae and as stray individuals in the plankton, but it is found in the greatest profusion in the rain-water pools, to the water of which it very often imparts a green colour. Most of my observations confirm what has already been recorded, but a certain number of supplementary data are given in the following. The coenobia are generally elliptic-oblong to broadly elliptic. Very often the anterior pole is broadly rounded, while the posterior one is somewhat truncate (text-fig. 2, A, L). The rotation of the colonies during forward progression is generally in a clockwise direction as seen from the anterior end, but the same colony may quite often rotate in the opposite direction. Grove (1915) found the individuals of *Eudorina illinoisensis* (*Pleodorina illinoisensis* Kofoid) collected in Great Britain showing clockwise rotation, unless their movement was in some way obstructed, when as they receded they rotated in the opposite direction, Carter (1858, p. 238) observed rotation in both directions in *Eudorina*

colonies from Bombay, and Kofoid (1898, pp. 282–3) made the same observation on his material of *Eudorina illinoisensis*.

In my material I very often found a few stray colonies *moving without any rotation whatever*, their movement being so striking that it always arrested my attention. The movement was usually very rapid, compared with the normal rotating colonies. The cause of this peculiar movement has not been elucidated.

The eye-spots were concavo-convex with the concavity directed outwards, and, as has often been described, were largest in the four cells of the front tier and became smaller and smaller towards the posterior end, sometimes being so small as hardly to be seen.

The cells of thirty-two-celled colonies were arranged in the familiar five tiers of 4, 8, 8, 8, and 4 cells from front to back, the cells of the three middle rows alternating with one another. In the sixteen-celled colonies there are four tiers of four cells each and the cells of the four tiers alternate with one another.

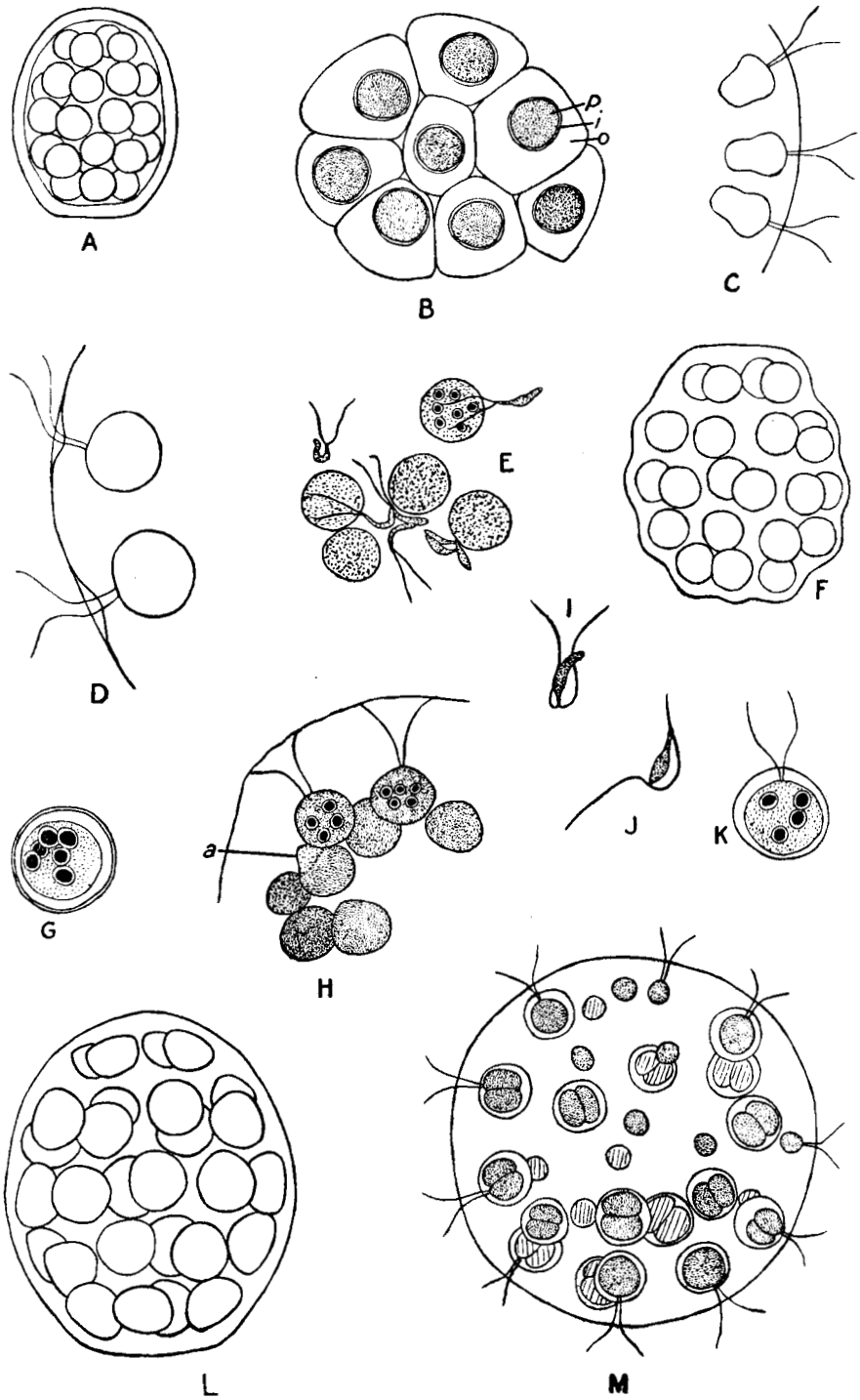
Each cell has a very thick wall which consists of a broad gelatinous portion on the outside (text-fig. 2, B, *o*) and a thin, comparatively firm innermost layer (text-fig. 2, B, *i*) immediately next to the protoplast (cf. p. 336). The cell-walls are seen clearly on staining with dilute methylene-blue or toluidine-blue.

The colonies vary very much in their general appearance. In some the cells are placed close together, as in text-fig. 4, A. In others the cells are more widely separated and a considerable area of clear gelatinous matrix is seen between them. Further, the cells may be placed either at a certain distance from the periphery of the colony or very close to the surface. In the latter case the bounding mucilage is often very compact and somewhat refractive, and the gelatinous matrix (of the formalin material) is readily stained by an aqueous solution of erythrosine, which does not colour the mucilage in the other types of colonies.

Mammillary processes were seen in living material on most of the colonies in a few collections, but in others none of the individuals showed this feature. This suggests that the presence of processes is characteristic of distinct races. The colonies possessing these processes were generally ellipsoidal and not subglobose.

In most specimens the boundary of the mucilage, when examined under high power, was seen to be slightly though definitely depressed just in front of each cell, about on a level with the point of exit of the cilia (text-fig. 2, D); a very similar condition is seen in Hartmann's figures of *Eudorina elegans* (Hartmann, 1921, tab. 1, figs. 3 & 4). This appearance is visible in living, as well as in preserved, material. In other living colonies the boundary of the mucilage appears to be slightly wavy following the contour of the cells (text-fig. 2, F), so that it is slightly bulged out opposite each cell; this undulation was more pronounced towards the posterior end. It is possible that the posterior mamillose processes are simply an exaggeration of this feature.

TEXT-FIG. 2.



EXPLANATION OF TEXT-FIG. 2.

Eudorina elegans Ehrenberg.

A, F, L, different types of colonies. B, groups of cells showing the gelatinous walls: *p*, protoplast; *i*, innermost layer of wall; *o*, outer gelatinous layer of wall. C, portion of a colony with pyriform cells. D, small part of a colony showing a depression in the gelatinous envelope opposite each cell. E, spermatozoids swarming round ova. G, oospore. H, loosened ova in the diffluent mucilage of the colony; cilia still present on two ova. I and J, spermatozoids showing metaboly. K, ovum before gelatinisation of the inner layer of the wall. M, colony with gonidia and somatic cells irregularly scattered.

(A & M, × 280; B, × 440; C, × 540; D, × 800; E & L, × 435; F, × 325; G–K, × 870.)

The shape of the protoplasts as observed in living material also varies somewhat. Usually they are spherical, but sometimes they are slightly broadened in a plane parallel to the outer surface of the colony. In some colonies the cells have the outer surface somewhat flattened, while the inner one is rounded, the cell in side view appearing somewhat hemispherical (text-fig. 2, L). In one colony, found during the examination of formalin material, the cells were elongated at right angles to the surface and somewhat narrowed towards the inside, with the inner and outer surfaces broadly rounded, so that the whole cell had a pear-shaped appearance (text-fig. 2, C). The cells distantly resembled old ones of *Pandorina morum* f. *major*, but they were arranged as in a typical *Eudorina*, being disposed separately in the periphery of the mucilage.

In the absence of pure cultures it is impossible to say whether certain of these forms could be regarded as distinct races. But it may be mentioned that most of the individuals of any single collection tended to conform to one particular type in respect of the crowded or scattered disposition of the cells, the general shape of the colonies (whether broadly or narrowly ellipsoidal or subglobose), the presence or absence of mammillary processes, the shape of the cells, etc. In every collection, however, there were usually a few colonies belonging to one or more of the other forms.

Whatever value these may have, they are of interest in suggesting possible steps in the evolution of *Eudorina*. Those forms in which the cells are crowded in the centre may be regarded as less advanced than those in which they are placed further apart and nearer the periphery. All my specimens of *Eudorina illinoisensis*—a decidedly more advanced form than *E. elegans*—conform to the second of these types.

Although all the cells of a colony of *E. elegans* may appear to be equal in size, a careful examination shows that, in most full-grown colonies, the four front cells are *very slightly* smaller than the rear ones. This fact has been recorded by Hartmann (1921, p. 227) and by Pascher (1927, p. 49) in European material. During asexual reproduction all the thirty-two cells sometimes form thirty-two-celled daughter-colonies, though sixteen-celled colonies are frequently formed, especially by the four front cells.

A single thirty-two-celled colony of *Eudorina elegans* was found, in which seventeen of the cells (i.e. about half) had enlarged and were undergoing their first divisions, while the remainder were quite small and evidently somatic (text-fig. 2, M). The gonidial and somatic cells were distributed without order throughout the colony, except in the foremost tier where three small cells could be recognised. This specimen resembles the two isolated colonies found by W. & G. S. West (1906, pp. 104–5, pl. 10. figs. 7 & 8) in the plankton of Lough Mawmeen in Ireland, although in these specimens the gonidia had not yet divided.

Sexual reproduction was observed in three different years. The colonies were always dioecious. In the male and female colonies all the cells usually became antheridia or oogonia, though a few cells occasionally formed daughter-colonies, especially one or more of the front four cells (Pascher, 1927, pp. 62, 436–7).

The first indication of commencing sexual reproduction is an increasing softening of the gelatinous matrix accompanied by a swelling of the colonies. The cells lie loosely in the soft mucilage and numerous foreign particles are seen adhering to its surface. In this condition the colonies float up to the surface in large numbers and form a green scum.

The antheridial clusters, after struggling for a while inside the loose gelatinous matrix of the parent colony, finally escape. Many of them were seen swarming round the female colonies and even round the individual oogonia, the soft condition of the gelatinous matrix of the female colony rendering the entry of the spermatozoid clusters easy. The individual spermatozoids of the clusters soon separate. They appear to undergo change of shape during movement, as the greatest width is found sometimes at one and sometimes at the other end of the body (text-fig. 2, I, J).

In female colonies approaching maturity the oogonia lie loosely in the softened matrix. In this condition they still retain the cilia and the firmer inner layer of the wall, although the gelatinous outer portion has by this time lost its individuality and become merged in the general matrix (text-fig. 2, K). Soon after, the remaining portion of the wall evidently gelatinises leaving the naked protoplasts of the ova, still bearing the cilia, embedded in the soft mucilage (text-fig. 2, H). At this stage they perform very slow oscillatory movements from side to side, or occasionally a short forward movement inside the mucilage. Finally, the cilia are lost and the ova become quiescent. Most of the ova are spherical, but in the preserved material some appeared to be slightly beaked, with an individual hyaline area near the beak suggesting a receptive spot (text-fig. 2, H, *a*).

During these changes numerous spermatozoids were swarming around the egg-cells and some were seen actively attacking the ova, although many were lying motionless next to them (text-fig. 2, E ; Pl. **28.** fig. 17). Nevertheless, though they were kept under observation from 10 a.m., when the spermatozoid clusters generally escaped, until late in the afternoon when the sperms became

quiescent, no instance of actual fertilisation or any stage in fusion was seen. Since the spermatozoids became quiescent in the evening, they were not further observed during the night. But, on the next day, numerous egg-cells surrounded by definite and fairly thick walls were found in the material (text-fig. 2, G). Evidently fertilisation, if it took place at all, must have ensued during the night. Carter (1858, p. 240) likewise did not see the actual process of fertilisation in his Bombay material of *Eudorina*, though he found the spermatozoids swarming round the egg-cells. Merton (1908, p. 445) and Chatton (1911, p. 309) observed the same stages, but failed to see actual fertilisation, nor did Grove (1915, p. 177) observe it in *Eudorina illinoisensis*. Thus five observers have seen the stages leading to fertilisation, but none have observed the actual process. If Schreiber (1925, pp. 362–3) had not described the formation of four protoplasts in the germination of the zygote of *Eudorina*, which renders a reduction division and a previous fusion probable, one would be tempted to suspect parthenogenesis in *Eudorina*.

Dimensions.—Colonies 72–118 μ long and 60–102 μ broad ($60\times72\ \mu$, $60\times77\ \mu$, $63\times72\ \mu$, $72\times91\ \mu$, $74\times99\ \mu$, $83\times94\ \mu$, $91\times106\ \mu$, $85\times101\ \mu$, $102\times118\ \mu$). Cells 11–19 μ in diameter. Oospores 19–23 μ in diameter.

EUDORINA ILLINOISENSIS (Kofoid) Pascher (*Pleodorina illinoisensis* Kofoid).* (Pl. **28.** fig. 1.)

This alga is often found in large numbers in the rain-pools at Madras, generally associated with other Volvocales like *Eudorina elegans*, *Pandorina morum*, *Chlamydomonas* spp., etc. The colonies are ellipsoidal or somewhat subglobose, usually without mammillary processes. The movements are like those of *E. elegans*, described on p. 330.

The colonies usually contain thirty-two cells, but sixteen-celled colonies are frequently met with. In the young condition all the cells of a colony are very nearly equal, but in the older colonies the four anterior cells are always smaller than the others. In the colonies of a single collection, however, all gradations can be found between those in which the difference is slight and those in which it is well marked ; these differences are no doubt due to differences of age. As in *E. elegans*, the anterior cells possess the biggest eye-spots.

The cells of the young colonies contain only a single pyrenoid. As the cells enlarge more pyrenoids are formed, and, up to a certain stage, the number of pyrenoids increases uniformly in all the cells, generally until two to four are present. After that, pyrenoid-formation ceases in the four anterior cells, but continues for some time in the others, so that ultimately the remaining cells contain as many as nine or ten pyrenoids. The accessory pyrenoids are formed *de novo*, as in *Pandorina* and *E. elegans*. Grove (1915, p. 173) found the same features in his English material of *E. illinoisensis*, but states that the cells of the youngest colonies have no pyrenoids. The youngest

* Pascher, 1927, p. 443. With respect to the reference of *Pleodorina illinoisensis* to *Eudorina*, cf. p. 339.

colonies in my material, however, always showed a small pyrenoid in each cell.

Apart from the markedly smaller dimensions of the front cells, the remainder also usually show a gradual, though very slight, increase in size from the anterior to the posterior end. Thus, the cells of the third tier are slightly larger than those of the second, and those of the fourth are larger than those of the third. The cells of the fifth (the posterior) tier, however, are generally slightly smaller than those of the fourth and sometimes also exhibit a smaller number of pyrenoids. Grove mentions the gradual increase in size of the cells from the anterior to the posterior end, but states that often, though not always, the cells of the posterior tier are the largest in the colony. In all the colonies examined by me, however, the cells of the fourth tier were the largest in the colony.

Measurements of the cells of four colonies (in μ).

	Colony I.	Colony II.	Colony III.	Colony IV.
Cells of 1st tier	13·5–14	10·5–11·3	11–11·7	9·75–15·25
2nd ,, ...	17	11–16·5	14·5–18	17·25
3rd ,, ...	17·7	16·5	19·25	17·25
4th ,, ...	18·5	16·5	19·25	17·35
5th ,, ...	16·5	15·75	17·25–19	16·5

Kofoid (1898, p. 276) observed hexagonal reticulations round the cells of the colonies after treatment with aqueous methylene-blue. Grove (1915, pp. 171–2) makes the following remarks with reference to this feature :— ' I could not succeed in demonstrating any hexagonal reticulations round the cells with the methylene-blue which Kofoid recommends for the purpose, until I adopted the expedient of pressing upon the cover-glass so as to expel the cells ; then five rows of faintly outlined irregular hexagons could sometimes be detected. But this was an artefact, produced by the mutual pressure of the gelatinous capsules surrounding each cell at a certain stage.'

I was able to see such reticulations around the cells very distinctly, both in *E. elegans* and in *E. illinoisensis*, after treatment with either aqueous methylene-blue or aqueous toluidine-blue (text-fig. 2, B). The reticulation around each protoplast really represents the outer edge of the gelatinous layer of the cell-wall, which is bounded, immediately adjacent to the protoplast, by a fairly firm inner layer (cf. p. 331). When a colony is stained with aqueous methylene or toluidine blue, the outermost edge of the gelatinous layer takes up the stain quicker than the rest of the wall, and hence the more or less polygonal contour stands out rather prominently for some little time. Later, when the stain becomes more uniformly distributed, the reticulation is not so distinct. The reticulation is thus really due to the mutual pressure of the gelatinous walls of the cells upon one another, as Grove suggests, but it is not clear why he regards it as an artefact.

Sexual reproduction.—The distribution of antheridia and oogonia was not uniform. Some colonies were definitely male, others were monoecious. None were purely female. In the male colonies the four anterior cells either failed

to divide or underwent very slow division into a few cells, while the remaining cells formed antheridia. Sometimes a few of these latter divided to form sixteen-celled daughter-colonies. In the monoecious colonies, twenty-eight posterior cells became oogonia, while the four anterior cells usually developed into antheridia, but sometimes one (Pl. **28.** fig. 1) or more, or even all, of them divided to form sixteen-celled daughter-colonies. Some of the twenty-eight posterior cells may develop full-sized (i.e., thirty-two-celled) daughter-colonies, but in none of the monoecious specimens examined did they give rise to antheridia or to sixteen-celled coenobia. Similarly, the four anterior cells never formed full-sized thirty-two-celled coenobia, not did they ever become oogonia. Grove's experience with his English material was similar.

Carter (1858, pl. 8, fig. 4) in the Bombay material of *E. elegans* observed the four front cells forming antheridia, while the remainder became oogonia, but this has not been recorded by any subsequent worker. His specimens were probably monoecious forms of *E. illinoisensis*.

The following are examples of a few of the colonies observed by me :—

(*a*) Monoecious colonies (28 oogonia in all cases).

Specimen 1. Front tier 4 antheridia.
,, 2. ,, 2 undivided cells, 2 antheridia.
,, 3. ,, 3 undivided cells, 1 antheridium.
,, 4. ,, 1 undivided cell, 1 dividing into an antheridium, 2 antheridia.
,, 5. ,, 3 antheridia, one 16-celled colony.
,, 6. ,, no antheridia, four 16-celled colonies.

(*b*) Male colonies (when the cells were in the early stages of division, it was not possible to judge whether they would have become antheridia or daughter-colonies, but all the data below were obtained from collections containing colonies with ripe sexual cells and devoid of normal asexual colonies).

Specimen 1. 1st tier 2 in 4-celled stage, 2 in 8-celled stage.
2nd ,, 6 antheridia, 2 in 8-celled stage.
3rd ,, 7 antheridia, 1 in 16-celled stage.
4th ,, 8 antheridia.
5th ,, 4 antheridia.
,, 2. 1st ,, 4 undivided.
2nd ,, 1 undivided, 1 degenerate, 6 in 16-celled stage.
3rd ,, 7 in 16-celled stage, 1 degenerate.
4th ,, 8 in 16-celled stage.
5th ,, 3 in 16-celled stage, 1 degenerate.
,, 3. 1st ,, 4 undivided.
2nd ,, 1 in 2-celled stage, 2 undivided, 1 in 4-celled stage, 4 in 8-celled stage.
3rd ,, 1 in 4-celled stage, 3 in 8-celled stage, 4 in 16-celled stage.
4th ,, 8 in 16-celled stage.
5th ,, 4 in 16-celled stage.

Specimen 4. 1st tier 4 undivided.
2nd ,, 8 antheridia.
3rd ,, 8 antheridia.
4th ,, 8 antheridia.
5th ,, 4 antheridia.

The sexual cells resembled those of *E. elegans*, and the spermatozoids were seen swarming round the egg-cells, though actual fertilisation was not observed.

Since Kofoid described his *Pleodorina illinoisensis*, repeated doubts have been expressed as to the validity of the species. While some have accepted it with a measure of suspicion, others have expressed the view that it should be regarded as a *Pleodorina*-state of *Eudorina elegans*. Grove suggested that the species was not yet fixed, being still in process of evolution. All previous workers, however, have regarded *E. illinoisensis* as essentially characterised by the somatic nature of the front four cells and have found this not to be universal. For, while sometimes they are truly somatic, they are mostly capable of division. But, though the species appears ill circumscribed when tested from this point of view, it shows a very definite character of its own, when its reproductive vagaries are carefully analysed.

This character, which gives it a distinct specific and perhaps even a generic status, is afforded by the markedly different behaviour of the front four cells in every colony, as compared with that of the remainder. Thus, (1) when the rear cells become oogonia, the front cells become antheridia ; (2) when the rear cells become oogonia, or full-sized thirty-two celled coenobia, the front cells become either antheridia or form small-sized sixteen-celled coenobia ; or, (3) when the rear cells form antheridia, the front cells fail to divide or divide at a late stage into a few cells. The behaviour of the front four cells is always quite distinct from that of the remainder and *on this basis* this form can be established as a distinct species. A tendency in this direction is observable even in *E. elegans* (cf. p. 333, and Pascher, 1927, p. 437).

Grove found that starving the colonies by cultivating them in distilled water induced the formation of numerous antheridia. This perhaps indicates that, when accumulation of food is prevented or vitality lowered, development of oogonia is prevented. Looked at from this point of view, it may be said that the behaviour of the front four cells of the *E. illinoisensis* colony in nature is in every instance one step, as it were, behind that of the remainder. They do not appear to possess the same reproductive efficiency. It is conceivable that they are so specialised, in relation to light-perception and the work of steering the colony, that their vitality for reproductive purposes has become lessened, so that, as compared with *E. elegans*, in which every cell is capable of developing into an oogonium or full-sized colony, these cells have lost part of their reproductive capacities.

The diagnosis of *Eudorina illinoisensis* should, therefore, be modified to read as follows :—

Colony of thirty-two or often sixteen cells, resembling *Eudorina elegans*

Ehrenb., but the front four cells smaller (in a greater or lesser degree) than the remaining ones, and either purely somatic or reproducing differently from the remaining cells of the same colony.

Much of the present uncertainty as regards the specific status of *Pleodorina illinoisensis* is due to Kofoid's description of the species being based on specimens whose full life-history he was not able to follow owing to the short period during which they were available. If he had seen the reproductive stages that have since been recorded, he would not have assumed that the front four cells were invariably purely somatic. The few divisions of the front cells that he did observe, he evidently regarded as aberrant or as exceptions to the general rule. So far as I am aware, no forms have subsequently been recorded answering strictly to the letter of Kofoid's definition of *Pleodorina illinoisensis*.

Pascher has included this alga under *Eudorina* as *E. illinoisensis*, because the front four cells are capable of division and because the cells are arranged in tiers as in *E. elegans*. Its removal from *Pleodorina* is certainly justified, but *E. illinoisensis* cannot be regarded as conforming, in the strictest sense, to the past definition of *Eudorina*, since in some male colonies the four front cells (or some of them) are truly somatic and do not divide. Moreover, while in *Eudorina* proper every cell in the female colony can become an oogonium, the front four cells of *E. illinoisensis* cannot develop into oogonia. Pascher (1927, pp. 439, 443) has consequently given an emended diagnosis of *Eudorina* to embrace Kofoid's *Pleodorina illinoisensis*. *E. illinoisensis* possibly exhibits a step in the direction of *Pleodorina*.

Dimensions of the Indian material :—

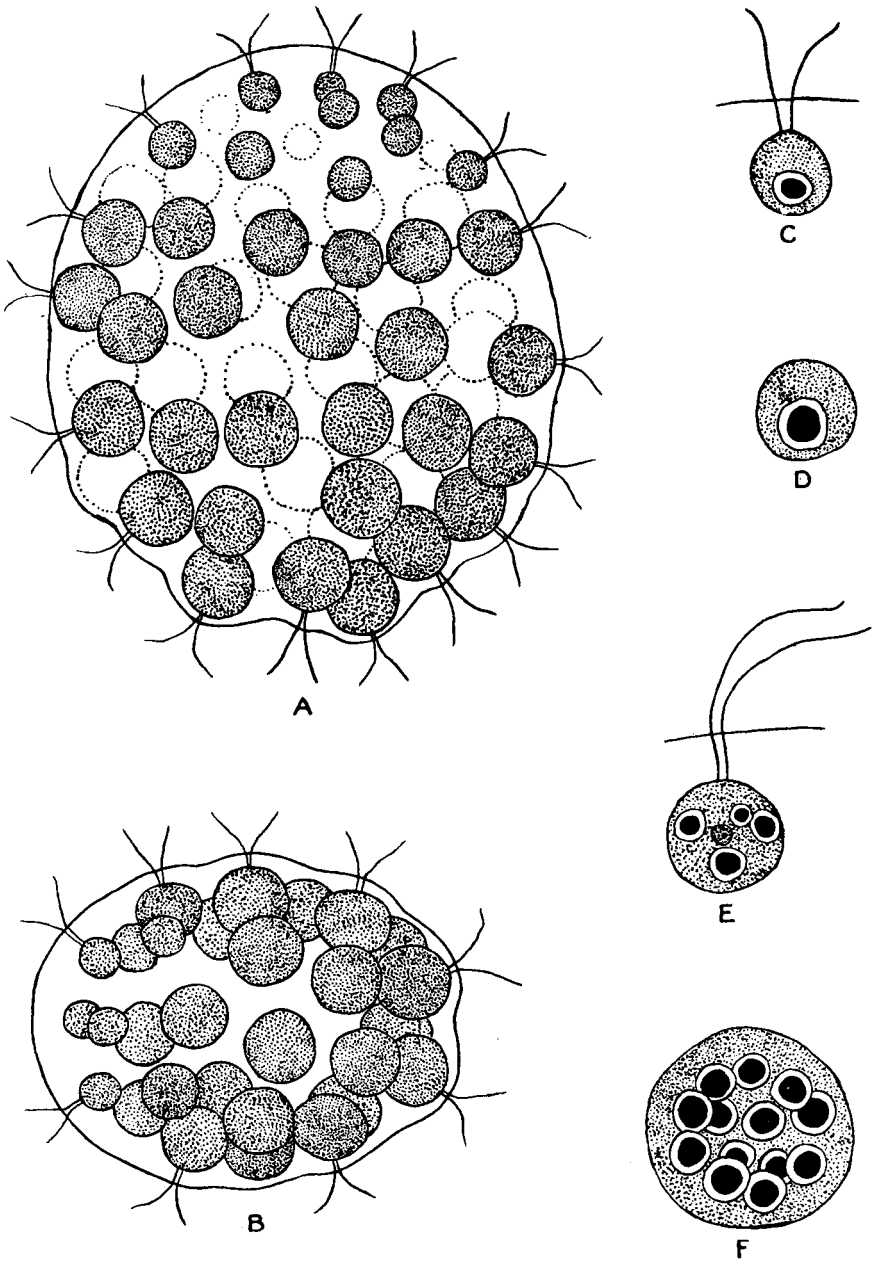
Thirty-two-celled colony 110–140 μ long and 95–118 μ broad (110$\times$95 μ, 120$\times$100 μ, 120$\times$95 μ, 128$\times$102 μ, 138$\times$101 μ, 130$\times$109 μ, 110$\times$100 μ, 130$\times$104 μ, 132$\times$110 μ, 140$\times$112 μ, 140$\times$110 μ, 140$\times$120 μ). Sixteen-celled full-grown colony 96$\times$71 μ. Sixteen-celled young colony 55$\times$30 μ.

The dimensions of the cells are given on p. 336.

EUDORINA INDICA, sp. n. (Text-fig. 3 ; Pl. **28.** figs. 2, 12, 13.)

This alga was first collected by Dr. Sampathkumaran in a rain-water pool at Talguppa in Mysore Province, and subsequently by me in a similar pool at Madras. The colonies contained sixty-four cells which were arranged in seven tiers of 4, 8, 12, 12, 12, 12, and 4 cells respectively, from the anterior to the posterior end (text-fig. 3, A, B). The colony was ellipsoidal to subglobose, and the cells were similar to those of *Eudorina elegans* in shape. The cells of the first four-celled and second eight-celled tiers were decidedly smaller than the remainder, although those of the second tier were larger than those of the first. In the following four twelve-celled tiers, composed of relatively larger cells, there was a gradual though slight increase in size in passing from the anterior to the posterior end. The posterior tier of four cells, however, was an exception to this rule, the cells being slightly smaller than those of the

TEXT-FIG. 3.



Eudorina indica, sp. n.

A, 64-, and B, 32-celled colonies; C, an anterior, and D, a posterior, cell from a young colony; E, F, the same from a mature colony. Pyrenoids black.
(A & B, ×400; C–F, ×1225.)

penultimate tier. In illustration of this the dimensions of two colonies are given (in μ) :—

(1) Full-grown ellipsoidal colony, 175×131.
Cells of the 1st tier 8·75–12.
,, ,, 2nd tier 12–14.
,, ,, 3rd–6th tier 16–23·5.
,, ,, 7th tier 20.

(2) Smaller colony, 140×110.
Cells of the 1st tier 10.
,, ,, 2nd ,, 10–12·5.
,, ,, 3rd ,, 14–16.
,, ,, 4th–6th tiers 16–17·5.
,, ,, 7th tier 17.

As in other cases, the eye-spots of the first tier are the largest and those of the succeeding tiers are progressively smaller, the posterior tier having extremely minute eye-spots or none whatever. Each cell has more than one pyrenoid when fully grown, but the cells of the two front tiers contain only a few pyrenoids, while those of the remaining tiers have up to sixteen pyrenoids (text-fig. 5, E, F). The following are the numbers of pyrenoids in the cells of a single full-grown colony :—

1st and 2nd tiers	3 pyrenoids in each cell.
3rd tier	8–10 pyrenoids in each cell.
4th–7th tier	12–16 pyrenoids in each cell.

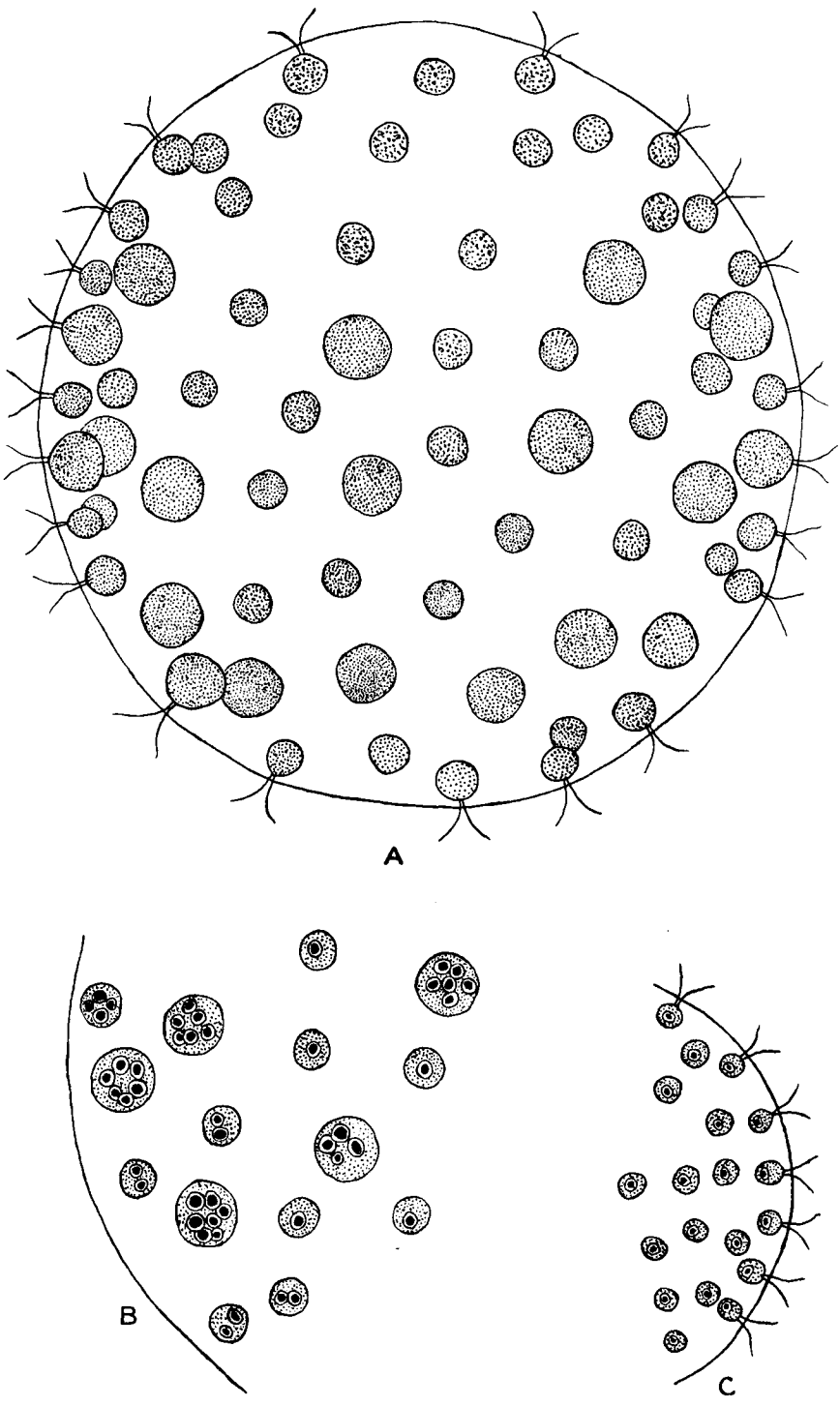
The posterior cells in full-grown colonies are generally crowded with pyrenoids.

The colonies, when young, have all the cells nearly of the same size, each with one pyrenoid (text-fig. 3, C, D). As the colony grows larger, all the cells increase in size equally for a time, and the number of pyrenoids increases by new ones arising *de novo*. After a certain stage is reached, the cells of the first tier undergo no further increase in size and no further pyrenoids are formed in them, and soon after the same thing happens with respect to the second tier of cells. Increase in size and formation of additional pyrenoids continue, however, in the cells of the remaining tiers for some time.

Only one colony was observed in which the cells were undergoing division (Pl. **28.** fig. 2). The twelve anterior cells, belonging to the first and second tiers, were not dividing. Of the twelve cells of the third tier, three were not dividing, one had divided into two, another into four, and another into eight cells. The remaining cells of this tier and all the cells of the two immediately following were dividing to form daughter-coenobia, whilst those nearest the posterior end were forming antheridia. The antheridia usually had thirty-two spermatozoids in the bundle, although one bundle contained only sixteen cells. No other reproductive stages were observed in the material.

This alga is more advanced than *E. illinoisensis*, and in some respects stands between it and *Pleodorina californica*. Unfortunately, little is known about

TEXT-FIG. 4.



Pleodorina sphaerica, sp. n.

A, a mature colony; B, portion of a mature colony with gonidia and somatic cells; C, portion of a young colony. Pyrenoids black. (All × 495.)

its reproduction, but the one case observed indicates that the front twelve cells do not divide, while most of the others do so. It is possible that in this alga the front twelve cells are truly somatic.

In the same collection were a number of colonies having thirty-two cells and resembling those of *E. illinoisensis* (Pl. **28.** fig. 13 ; text-fig. 3, B), but the cells of the front two tiers were smaller than those of the remainder, and those of the first tier were smaller than those of the second. These are evidently smaller (thirty-two-celled) colonies of *E. indica*.

PLEODORINA SPHAERICA, sp. n. (Text-fig. 4 ; Pl. **28.** figs. 4, 5.)

This new species of *Pleodorina* was found sparsely scattered among other algae in a pool on a hill-slope at Vandalur near Madras. The alga is of special interest in having numerous gonidial cells interspersed among somatic cells.

The colony is spherical or nearly spherical, and contains 128 cells, which are arranged in no special manner near the periphery. The full-grown colony measures 187–210 μ in diameter. One part of the colony, presumably the anterior portion, forming about one-fourth to one-fifth of the whole, is free from gonidia. In the remaining part a large number of gonidia are found scattered among somatic cells (text-fig. 4, A, B ; Pl. **28.** figs. 4, 5). The somatic cells measure about 9–11 μ and the gonidial cells 15–17 μ in diameter. The detailed structure of the cells was that typical of *Eudorina* and *Pleodorina*. Eye-spots could not be detected in the preserved material.

The cells of the young colonies are all equal in size and contain a single pyrenoid (text-fig. 4, C), and all the cells exhibit uniform increase in size up to a certain stage. Then the somatic cells cease to grow, and only those continue to enlarge which are to become gonidia. In these cells further pyrenoids are formed, while in the somatic cells there is no increase in the number of pyrenoids or only one or two additional ones are formed. In the full-grown colonies the number of pyrenoids in the somatic cells is one to two, sometimes three, while in the gonidial cells there are six to eight pyrenoids (text-fig. 4, B). Unfortunately, no division-stages were found in the material, and, although the pool was searched during the next two years, the alga was not found again.

This alga, in the irregular disposition of its cells and the limited number of gonidia, is clearly allied to *Pleodorina californica* Shaw (1894, pp. 279–283), but appears to be more advanced. In *Eudorina illinoisensis* the front four cells show signs of becoming somatic. In *E. indica*, where the colony is slightly larger, a bigger proportion of the cells (i.e. twelve out of sixty-four) become somatic. In *Pleodorina californica* there is still further advance, not only in the size of the colony and the number of its cells, but also in the fact that nearly half the total number of cells are somatic. In actual fact practically the whole anterior half of the colony is somatic, while the posterior half is gonidial. In the present alga, while a small area of the anterior portion is again somatic as in *P. californica*, a still further advance is indicated by the fact that a large number of the posterior cells have become somatic. In other

words, the process of sterilisation has invaded even the posterior region, which in *Eudorina illinoisensis*, *E. indica*, and *Pleodorina californica* is purely gonidial. In fact, *P. sphaerica* shows a condition very closely approaching that of *Volvox*, where usually a relatively small number of gonidial cells are found scattered among the somatic cells in the posterior region of the colony. But for the limited number of cells, this alga might be classed under *Volvox*.

The evolution of certain *Volvox*-forms from a *Pleodorina*-like ancestor, especially from a form like *P. californica*, is probable. *P. sphaerica* is of interest in this connection in providing yet another important link, showing how sterilisation has invaded even the purely gonidial posterior region of a *P. californica*, and has thus brought about a condition similar to that of *Volvox*.

The alga here described has a definite number of cells, and the gonidial cells are scattered among the somatic cells. There are no protoplasmic connections. It could thus be placed under *Besseyosphaera*, as Shaw (1916, pp. 253–4) defines this genus. But *Besseyosphaera* is akin to *Volvox*, if not a *Volvox* itself, being a large form with about a thousand cells, while the present alga with its small size and limited number of cells falls naturally into the series *Gonium* (4, 8, 16 cells), *Pandorina* (8, 16, 32 cells), *Eudorina* (8, 16, 32, 64 cells), and *Pleodorina* (64, 128 cells). There is still a big gap between *Pleodorina* with the limited number of cells and even the smallest species of *Volvox* with numerous cells. I am therefore of the opinion that the alga should be included in *Pleodorina*, though this will necessitate a slight modification of the present diagnosis of the genus.

The progressive series of forms discussed in the preceding pages may be summarised as follows :—

(1) *Pandorina morum* (8- and 16-celled solid colonies ; cells with a single pyrenoid).
(2) *Pandorina morum* f. *major* (8–16- and 32-celled solid colonies ; cells with many pyrenoids).
(3) *Eudorina elegans* (16- and 32-celled hollow colonies, with cells placed peripherally ; cells with many pyrenoids).
(4) *Eudorina illinoisensis* (16- and 32-celled hollow colonies ; front 4 cells progressing towards sterilisation).
(5) *Eudorina indica* (32- and 64-celled hollow colonies ; front 12 cells somatic).
(6) *Pleodorina californica* (64- and 128-celled hollow colonies ; cells of front half somatic, remainder gonidial).
(7) *Pleodorina sphaerica* (128-celled hollow colony ; as in *P. californica*, but many cells even in the posterior region somatic).
(8) *Volvox* (colony very large and composed of numerous cells ; somatic cells very numerous as compared with the gonidia).

VOLVOX PROLIFICUS, sp. n. (Text-fig. 5, A–G ; text-fig. 7, J ; text-fig. 8, A ; text-fig. 9, C ; Pl. **28.** figs. 3, 6, 9, 20, 23.)

This species was collected by Mr. M. S. Raghava Chari in a pool in the bed of the river Nagari, near Tirupati in South India. The pool was on the point

of drying up, but at the time of collection the alga occurred in such enormous numbers as to make the water appear bright green, even from a distance.

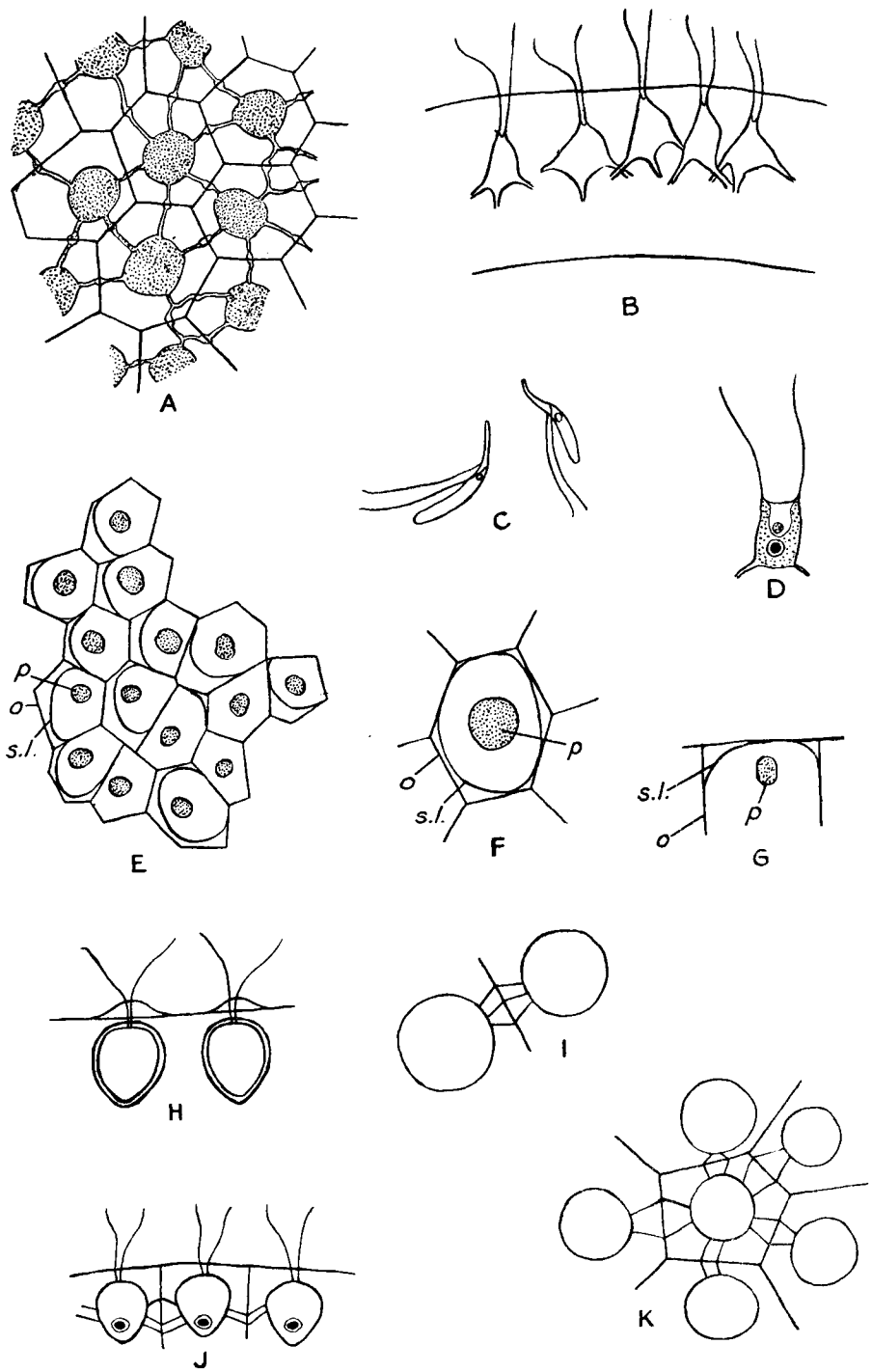
The colonies of *V. prolificus* are either asexual or sexual, the sexual colonies being in the main dioecious. The asexual and female colonies are globose or subglobose, while the male are subglobose to ellipsoidal. The female colonies are slightly broadened at the anterior and somewhat narrowed at the posterior end (Pl. **28.** fig. 3). Often a few (two to five) daughter-individuals are seen in a male or female colony. A single asexual colony was found to contain a number of well-developed oogonia and oospores, and one very old male colony contained a single ripe oospore. The number of cells in the colony is 9,000 to 25,000. The asexual colonies measure from 400 to 1010 μ in diameter, the female from 400 to 1090 μ, and the male from 410 to 800 μ.

In surface-view the boundaries of the cells are pentagonal or hexagonal in outline (text-fig. 5, A). In optical section they are found to be about 30 μ deep with parallel sides. The protoplasts of the colony thus lie between two curved contours representing the outer surface of the colony and the slightly curved inner surfaces of the cells bounding the large central hollow. The cells possess much the same structure as those of *V. globator* (Meyer, 1896, pp. 187–217). There are usually five protoplasmic connections, often there are four, and occasionally six (text-fig. 5, A ; text-fig. 8, A). In an optical section of the colony they are seen to arise from the sides of the protoplast and to spread out horizontally, but in a slightly downward direction (text-fig. 5, B). In younger colonies the main body of the protoplast is rounded in surface-view and pear-shaped in side-view, with the pointed end directed outwards. In the older colonies, however, it appears irregularly rounded in surface-view and narrowly pear-shaped or wedge-shaped in side-view (text-fig. 5, B, D). The cilia, especially in the wedge-shaped cells, are placed well apart from each other.

In very old colonies of *V. prolificus* the protoplasmic connections disappear and the protoplasts constitute rounded masses in the centre of the cells, while the thick gelatinous walls appear about to disorganise. In such old colonies one sees, in surface-view at a high focus, internal to the polygonal boundary-walls, a second closely apposed membrane which has a rounded outline and is readily distinguishable only at the corners (text-fig. 5, E, F). In optical section this second membrane appears rounded on the outer surface, so as to leave a small space between it and the bounding membrane on either side or more frequently on one side only (text-fig. 5, G).

The protoplasts are more densely crowded on the posterior than on the anterior side of the colony, the distance between them at the posterior and anterior ends being 1·5 μ and 2–3 μ respectively. The protoplasts are 5–6 μ in diameter, and, in the preserved material, appear sometimes slightly larger in the young than in the full-grown colonies. The protoplast contains a somewhat bell-shaped chloroplast in which is embedded a single pyrenoid. The single nucleus is situated in the centre of the protoplast within the cup-shaped hollow of the chloroplast (text-fig. 5, D).

TEXT-FIG. 5.



EXPLANATION OF TEXT-FIG. 5.

A–G. *Volvox prolificus*, sp. n.

A, surface-view; B, optical section showing the form of protoplasts; C, spermatozoids; D, single cell with chloroplast, pyrenoid, and nucleus; E, surface-view of cells from a very old colony; F, a single cell of E enlarged; G, optical section of the upper portion of a cell of E (diagrammatic). *p*, protoplast; *o*, outer layer of cell; *s.l.*, second layer of wall.

H–K. *V. dissipatrix* (Shaw), comb. nov.

H, cells showing protrusion of the gelatinous layer; I, and K, protoplasmic connections between cells in surface-view; J, the same in optical section.

(A, C, F, H, K, × 1200; D, E, × 640; B, × 810; I, × 1950; J, × 975.)

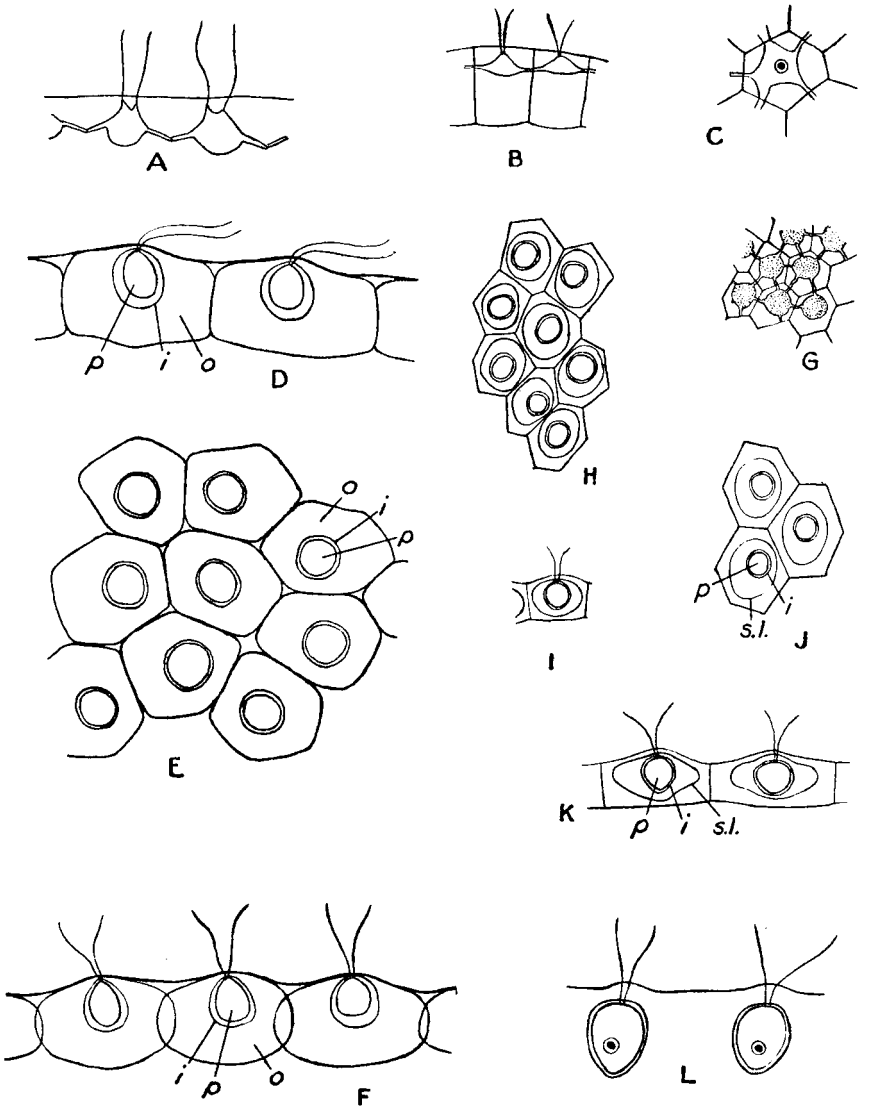
V. prolificus differs from all hitherto-described species of *Volvox* in the fact that the reproductive cells are differentiated continuously throughout the existence of the colony.* In fact, most of the cells appear to retain the capacity to take on reproductive functions at any stage in the life of the colony, whereas in the other species of the genus, once the gonidia or sexual cells are differentiated the other cells remain definitely somatic. The reproductive cells are, however, restricted to the posterior half of the colony in the manner customary in *Volvox*.

Asexual colonies.—The continuous differentiation of reproductive cells is not so noticeable in the asexual as in the sexual colonies, owing to the relatively small number of gonidia formed. Side by side with developing daughter-coenobia, however, one always finds a small number of enlarging cells, which are growing into further gonidia. In very old colonies containing a considerable number of daughter-coenobia, moreover, the latter are usually of very different sizes, some being very much smaller than others and comprising a much smaller number of cells (text-fig. 7, J). The difference in size and cell-number is evidently due to their successive formation. The total number of daughter-coenobia formed varies between four and twenty, the usual number being six to twelve. Very young coenobia are not completely spherical, but slightly ellipsoidal. At the time of birth they measure about 153–230 μ in diameter. The gonidia commence to differentiate before the birth of the colony.

Male colonies.—Formation of antheridia begins in very young male colonies which are of small size, and more and more antheridia are produced throughout life, so that in the older and larger male colonies one finds antheridia in all, from the earliest to the latest, stages of development. In a young colony one finds antheridia with spermatozoids ready to escape, and a certain number of developing antheridia scattered over the posterior half. In slightly older colonies there are maturing antheridia and a number of spaces representing the

* In the male colonies of some species (*V. perglobator* Powers and *V. Rousseleti* West var. *lucknowensis*, cf. p. 350) there is a successive formation of antheridia, though not as marked as in *V. prolificus*.

TEXT-FIG. 6.



Cell-structure in species of *Volvox*.

A–C. *Volvox globator* Ehrenberg var. *maderaspatensis*, nov.

A, cells in optical section. B, the same, showing the limits of the cell-membranes. C, cell in surface-view.

D–F. *V. Carteri* Stein.

D, cells in optical section showing limits of walls; E, cells in surface-view; F, optical section of cells from a very old colony.

G, *V. Rousseleti* West var. *lucknowensis*, nov., cells in surface-view.

H, *V. africanus* West, surface-view of cells drawn from Shaw's material.

I–L. *V. africanus* West f. *minor*, nov.

I, cell in optical section from the posterior end of a young colony; J, surface-view of cells from the anterior end of an older colony; K, optical section of cells in the anterior end of an older colony; L, cells showing the protrusion of the gelatinous envelope opposite the protoplasts.

(A–F, L, × 915; G–J, × 480; K, × 765.)

empty antheridia from which the spermatozoid bundles have escaped ; at the same time there are again a number of cells in the early stages of antheridium-formation. The same state of affairs is met with even in very old colonies about to disintegrate. There can therefore be no doubt that successive crops of antheridia are formed in this species of *Volvox*. The number of antheridia in young colonies is small (up to twenty-five) ; in the older colonies more numerous antheridia (up to fifty-five) are observed, quite apart from those which have already liberated their spermatozoids and are no longer clearly recognisable.

The antheridia are round and disc-shaped, from 38 to 40 μ in diameter, and form numerous (about 256) spermatozoids arranged in a single layer (Pl. **28.** fig. 20). The male cells, so far as could be ascertained in preserved material, are narrow, elongate, spindle-shaped structures, about 13–15 μ long and about 0·9 μ broad. The cilia are attached at a certain distance from the anterior end, the part of the body in front of their point of attachment being narrowed into a beak. Below the point of insertion there is a round space, evidently a contractile vacuole (text-fig. 5, C).

Female colonies.—The oogonia are about 80–150 in number in young colonies and gradually increase in number as the colonies grow older and larger, reaching 500 or more in the fully developed colonies. In the young female colonies a quantity of mature oogonia are found, as well as a number of enlarging cells scattered over the posterior half which no doubt represent developing oogonia. In older colonies, and even in such as are breaking up, oogonia in various stages of development are still to be found side by side with others containing ripe oospores.

Very young female colonies, however, appear to be bisexual, for every such colony had two to six spaces in it which evidently represent the positions occupied by antheridia from which the spermatozoids have escaped ; a few spermatozoids are, in fact, often still to be seen in these spaces. Numerous young colonies, including daughter-colonies inside the mother, have been carefully examined, but actual antheridia could not be found in any of them. In several young female colonies, however, division-stages of enlarged cells have been observed, but whether these would have given rise to antheridia or merely to daughter-coenobia it is impossible to say. The number of such dividing cells was two to six, i.e. equal to the number of presumed antheridia, and it is therefore probable that they represent the developing antheridia.

The ripe oospore has two walls—the inner smooth, the outer spinous. The spines are strong and conical (5–7 μ long), with a fairly broad base. In median optical section fourteen to sixteen spines are visible around the periphery of the spore. Without the spines the oospores measure 30–35 μ in diameter (text-fig. 9, C ; Pl. **28.** fig. 23). The spines are fully developed only in ripe spores, the younger ones having blunter and shorter spines, while in the earliest stages the spore has a crenate or even a smooth wall. All these stages can be seen in one and the same colony.

Development of reproductive cells.—As the cells enlarge to form gonidia, antheridia, or oogonia, they gradually project into the central hollow of the coenobium and ultimately come to lie below the general surface, although the outer portion of their gelatinous wall still occupies its former position between the other cells of the colony. This is as in other species. There is no rupture of the general surface at these points. In this species the protoplasts of the reproductive cells, however, are surrounded by a very wide mucilaginous envelope, formed from the original gelatinous wall by enlargement and several times exceeding the protoplasts in width. The large mucilage-envelope surrounding them is readily stained with alcoholic safranin, or dilute aqueous methylene or toluidine blue. When these reproductive cells are remote from each other, the envelopes appear as rounded vesicles, but when they are closely crowded the envelopes become angular through mutual pressure, and on staining appear like internal reticulations. This is best seen in a well-developed female colony with numerous oogonia (Pl. **28.** fig. 9).

In old colonies about to disintegrate, the gelatinous envelopes of the somatic cells become diffluent, and appear as a broad transparent layer of homogeneous mucilage which reaches far into the interior of the colony. This is very clearly seen in the asexual colonies in which the daughter-coenobia, with their own individual gelatinous envelope still intact, lie in the broad homogeneous mucilage derived from the gelatinous envelopes of the somatic cells.

VOLVOX ROUSSELETI West var. LUCKNOWENSIS, nov. (Text-fig. 6, G; text-fig. 7, F; text-fig. 8, B; text-fig. 9, G; Pl. **28.** figs. 8, 11.)

I am indebted to Prof. Fritsch for placing this material at my disposal. It was collected by Mr. A. R. Roa in Lucknow in 1929, but no further information about the habitat is available.

The colonies are subglobose to ellipsoid, and measure 510–629×544–765 μ (510×544 μ, 510×612 μ, 527×561 μ, 544×595 μ, 629×765 μ, 697×731 μ, 697×765 μ); they contain 6,000 to 8,000 cells. They are either asexual, male or female, female colonies being present in the largest numbers. Up to ten spaces can be seen in the asexual and female colonies, so that the very young coenobia of these types contain a few antheridia. A few gonidia or daughter-coenobia (up to five) are sometimes seen in the antheridial or female colonies.

The protoplasts are rounded to somewhat broadly stellate in surface-view (text-fig. 8, B), and measure 5·5–7·5 μ in diameter. The protoplasmic connections are thin, but not delicate; they were often disintegrating all over the colony. In an optical median section of the colony the protoplasmic connections appear to arise a little below the middle of the protoplast. The actual outline of the cells is hexagonal or pentagonal in surface-view (text-fig. 6, G) and rectangular in side-view. The height of the cell (equivalent to the thickness of the gelatinous matrix of the coenobium) is 25–29 μ. The distance between the centres of adjacent cells at the anterior and posterior ends of one colony were 13 μ and 9·5 μ respectively.

Asexual colonies.—Three to seven gonidia may be seen in the asexual colonies, some being larger than the others. The larger ones are nearer the middle and the smaller nearer the posterior end of the colony (text-fig. 7, F).

Female colonies.—These are slightly narrowed towards the posterior and broadly rounded at the anterior end (Pl. **28.** fig. 8). The number of oogonia varies between 100 and 159, and they are found throughout the colony except for about the anterior fifth. The oospore has an inner smooth and an outer spinous wall. The spines are normally rather short and broadly conical (text-fig. 9, G), but all gradations from oospores with a truly crenate wall to such as bear broadly conical spines are seen in the same coenobium. The crenate walls do not, however, appear to belong to spores which are immature. Thus, in one specimen, the spores with crenate walls had already formed the inner (smooth) layer, while those with spinous walls had not yet formed this layer. The former were evidently the older. The spiny oospores are 32–33·5 μ wide without the spines, the spines being 3·7–5·5 μ long. The crenate oospores measure 33–35 μ in diameter.

Male colonies.—The male colonies form from twenty to sixty antheridia, which are about 40 μ in diameter. The antheridia produce sperm-globoids, which are at first spherical, but become flattened when fully grown, measuring about 19×40 μ. The number of spermatozoids appears to be 256. No free spermatozoids were seen in the material. The antheridia are found in all stages of development in the male colonies, from the just enlarging somatic cell to the large undivided antheridium and from this in all stages of division to the fully formed sperm globoid (Pl. **28.** fig. 11). Such a condition has been reported by Powers (1908, p. 164) as occurring frequently in *V. perglobator*.

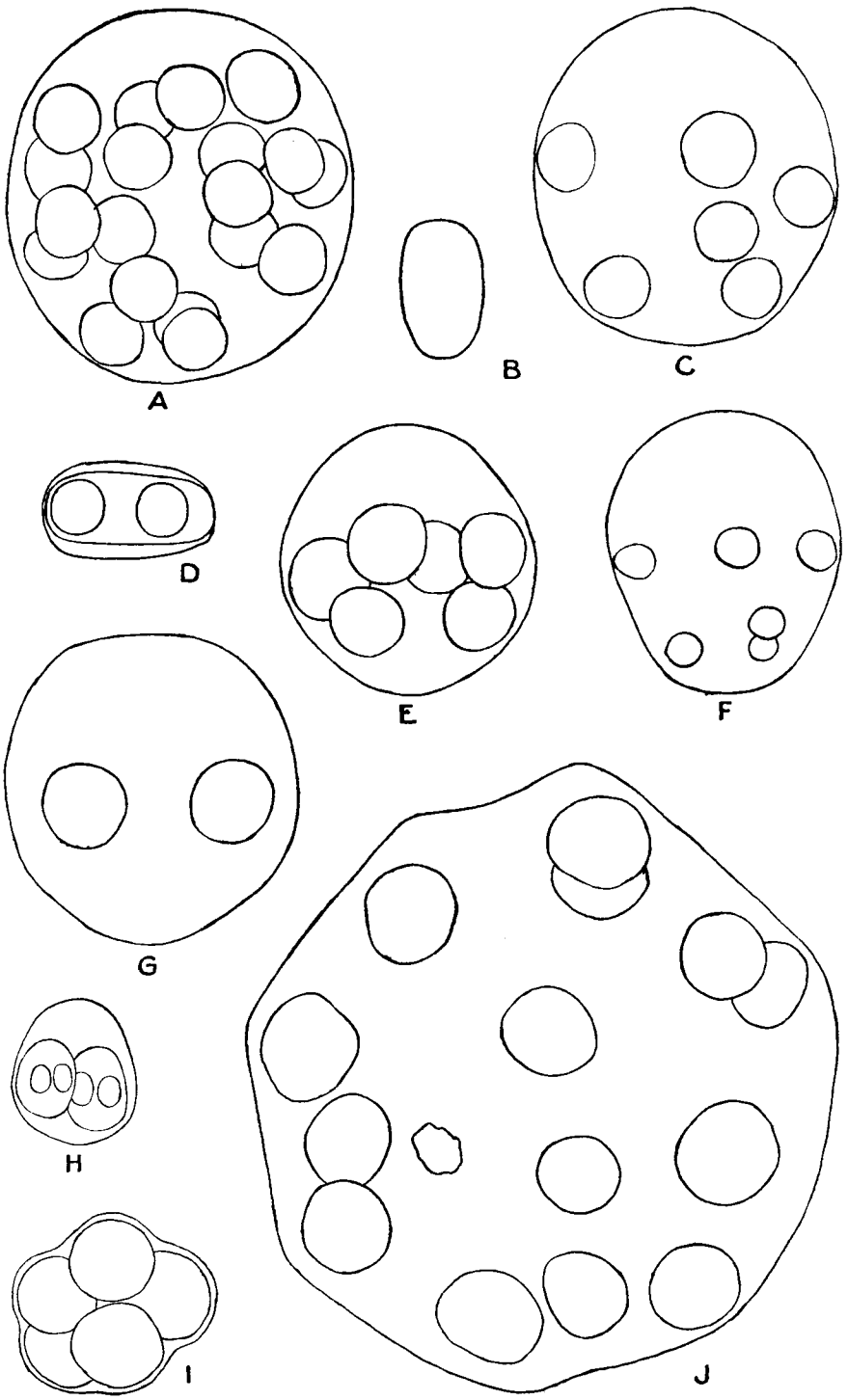
The female colonies resemble those of *V. Rousseleti* West (1918, pp. 425–8, pls. 29 & 30) in their general shape, and there is also similarity in the cells and protoplasmic connections, as well as in the dioecious habit. The colonies, however, are not so large as those of *V. Rousseleti*, and consist of a smaller number of cells. Moreover, the spines on the oospores are not at all like those of *V. Rousseleti*, but resemble more those of *V. globator* in being short and broadly conical. In the presence of a number of crenate oospores, in the antheridia being found in all stages of development, and in the dioecious nature of the colonies, it comes near to *V. perglobator* Powers, but it differs from this species in the frequent spiny walls of the oospores and in the rather different type of cell-structure which Powers describes for his species.

The form under discussion, therefore, combines characters of both *V. perglobator* and *V. Rousseleti*, but it appears to stand closer to the latter and may for the present be described as a new variety of *V. Rousseleti* under the name of var. *lucknowensis*.

VOLVOX GLOBATOR Ehrenberg var. MADERASPATENSIS, nov. (Text-fig. 6, A–C ; text-fig. 7, G ; text-fig. 8, C ; text-fig. 9, A ; Pl. **28.** figs. 10, 15, 24.)

This alga was collected by Dr. T. Ekambaram in a pool inside a *Casuarina*-plantation near Elliot Beach in Madras during the winter monsoon season

TEXT-FIG. 7.



EXPLANATION OF TEXT-FIG. 7.

Asexual colonies and disposition of daughter-coenobia in species of *Volvox*.

A, *V. Carteri* Stein f. *nagariensis*, nov.; B, *V. africanus* West f. *minor*, nov. (side-view); C, *V. dissipatrix* (Shaw), comb. nov.; D, *V. africanus* West f. *minor*, nov. (view from anterior end); E, *V. Carteri* Stein; F, *V. Rousseleti* West var. *lucknowensis*, nov.; G, *V. globator* Ehrenberg var. *maderaspatensis*, nov.; H, *V. africanus* West f. *minor*, nov.; I, *V. Merrilli* Shaw f. (?); J, *V. prolificus*, sp. n. (A–H, *J*, × 60; I, × 48.)

of 1930. It occurred sufficiently abundantly to give the water a green colour.

The asexual coenobia were subglobose, while the sexual ones were elliptic to elliptic-obovate, being slightly broader at the posterior than at the anterior end. The asexual colonies attained to 693 μ in diameter, while the sexual ones measured as much as 640×759 μ. The protoplasts were 5·5–7·5 μ in diameter; they were somewhat rounded when young, though later becoming angular where the protoplasmic connections arose and often appeared quite stellate. In optical section they were more or less rounded, though horizontally extended (text-fig. 6, A, B). The protoplasmic connections were often long and thin, but not very fine. The boundary of the cell-wall was pentagonal or often hexagonal in surface-view and rectangular in side-view (text-fig. 6, C, B). The number of cells in the colony was 6,000 to 11,000.

The asexual coenobia generally produced only two to four gonidia, usually only two (text-fig. 7, G), which were placed on opposite sides in the median portion of the coenobium, a little towards the posterior end. They are not differentiated in the young coenobia, which reach about 180–204 μ in diameter before birth.

The sexual coenobia (Pl. **28.** fig. 15) were monoecious and protandrous. They contained seven or eight antheridia, but only a few specimens in the material showed them, their former position in most of the colonies being represented by spaces. The oogonia were 18–38 in number and were as usual confined to the posterior portion, the front two-fifths of the coenobium generally not containing any. The oospores differed from those of the type in being clothed with strong, sharp, conical spines, which were 8–13 μ long and often slightly curved at the tip, the curvature not always being in the same direction. The inner layer of the membrane was smooth (text-fig. 9, A; Pl. **28.** fig. 24). The oospores were 35–42 μ in diameter without the spines, fourteen to fifteen spines being visible around the periphery, when viewed in optical section.

This alga forms a link between *V. globator* and *V. Merrilli* Shaw. In the small number of antheridia and oogonia it resembles *V. globator*, but in the character of the protoplasts, with the often long and moderately thin protoplasmic connections, in the long sharp spines of its oospores, and in the smaller size of the latter, it resembles *V. Merrilli*. The very small number of gonidia and their arrangement are features peculiar to the form under discussion. It may be seen from the above that it might be regarded as a variety of *V. globator* from one point of view and of *V. Merrilli* from another. Since it thus combines

the characters of both species and presents some peculiarities of its own, it might be justifiable to regard it as a distinct species. But the small number of its antheridia and oogonia, and the general appearance of its colonies incline me to rank it as a variety of *V. globator*, which I shall call var. *maderaspatensis*. Some of the existing species of *Volvox* with protoplasmic connections may, as more information about them becomes available, prove to be only varieties of *V. globator*.

VOLVOX MERRILLI Shaw (1922, pp. 492–496), forma (?). (Text-fig. 7 ; text-fig. 8, D ; text-fig. 9, F.)

This form was found in a pool in a *Casuarina*-plantation near the sea-coast at Seven Pagodas, near Madras. The pool was somewhat shaded by the trees, and only a small amount of direct sun-light reached the water. The alga occurred very sparsely and was collected by pouring the water through a broad funnel covered with bolting silk. The material thus obtained proved to be very old and in a very poor condition, most of the colonies being much shrivelled and about to disintegrate. Only a few colonies were found in which the details could be made out.

Only asexual colonies with fully developed daughter-coenobia and female colonies with ripe oospores were present. The former were globose to subglobose, while the sexual colonies were ellipsoid. The asexual colonies measured 495–750 μ in diameter, while the sexual colonies were 412–500$\times$580–660 μ (412$\times$580 μ, 490$\times$590 μ, 500$\times$660 μ). The number of cells, as far as could be recognised in the poor material, was 5,000 to 11,000.

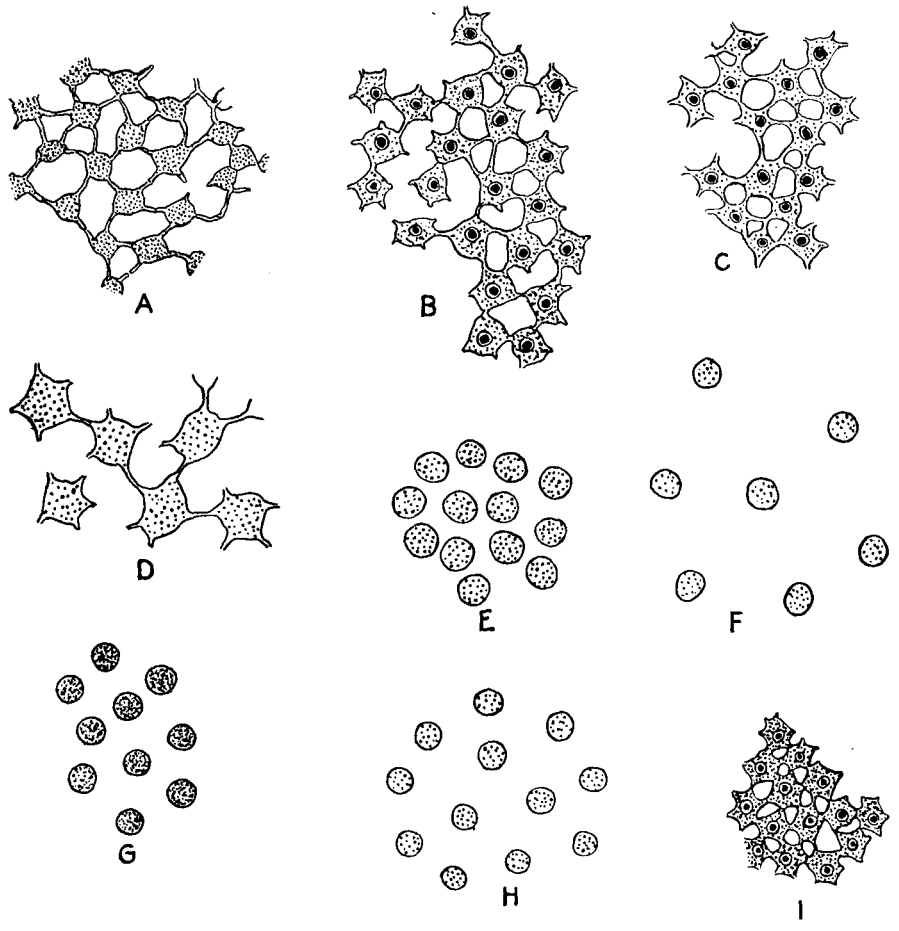
The protoplasts, as seen in surface-view, were round when young, but gradually became angular opposite the protoplasmic connections (text-fig. 8, D). In side-view the protoplasts were pear-shaped, the protoplasmic connections starting from very near their base. The connections were thin, but not very delicate, and were disintegrating except in a few places. The protoplasts measured 5–7 μ in diameter when young, but the older ones appeared slightly smaller, being 4–6·5 μ in diameter. Even in very old colonies the protoplasts were very close together, the distance between the lateral surfaces of the adjacent protoplasts being 1·5 μ at the posterior end and 2–3 μ at the anterior end of the colony.

Asexual colonies.—The only asexual colonies found contained well-developed daughter-coenobia (generally four to six), which were large and so closely packed inside the parent that they were closely invested by the latter, which presented a distended lobed appearance (text-fig. 7, I). These daughter-coenobia measured 220–250 μ in diameter.

Sexual colonies.—All the sexual colonies observed were female, and no evidence was obtained of the presence of any antheridia in these colonies, but in view of the poor condition of the material this could not be established with absolute certainty. If, as seems probable, there were separate male colonies, they must have disintegrated some time previously, after the escape

of the spermatozoids. It is probable therefore that the alga was dioecious. The number of oospores in the colonies was 50–128 (50, 64, 75, 78, 128 in different colonies). The ripe oospores were clothed with strong conical spines,

TEXT-FIG. 8.



Surface-view of the protoplasts of diverse species of *Volvox*.

A, *V. prolificus*, sp. n.; B, *V. Rousseleti* West var. *lucknowensis*, nov.; C, *V. globator* Ehrenberg var. *maderaspatensis*, nov.; D, *V. Merrilli* Shaw f. (?); E, *V. dissipatrix* (Shaw), comb. nov.; F, *V. Carteri* Stein f. *nagariensis*, nov.; G, *V. africanus* West f. *minor*, nov.; H, *V. Carteri* Stein; I, *V. globator* Ehrenberg. (A–C, E–I, × 590; D, × 1115.)

broad below and tapering to a sharp point above, often slightly curved at the tip (text-fig. 9, F). The length of the spines was 9·5–10·5 μ. The spores without the spines measured 34–39 μ in diameter, fourteen to sixteen spines

being visible at the periphery in optical section. The oospores were distributed over most of the colony, except for a very small area (about one-sixth or less of the whole colony) near the anterior end.

In the form of the protoplasts, in the character of the oospores, in the number of oogonia, and to some extent in the size of the coenobia, this form comes very close to *V. Merrilli* Shaw. The great distention of the asexual coenobia by the daughter-individuals giving them a lobed appearance is very characteristic. The mature asexual coenobium of *V. Merrilli* with the daughter-individuals fully developed was, however, not present in Shaw's material, the largest daughter-coenobia found by him being 80 μ in diameter (Shaw, 1922, p. 495). Since some uncertainty also remains as regards the monoecious or dioecious character of my material, it is not easy to say whether the form here described is identical with *V. Merrilli*.

VOLVOX GLOBATOR (L.) Ehrenberg. (Text-fig. 8, I ; text-fig. 9, B.)

This species was collected by Dr. M. A. Sampathkumaran in a pool near Bangalore, occurring in such large numbers as to give a greenish colour to the water. The globose to subglobose coenobia measured 264–408 μ in diameter and contained 2,000 to 8,000 cells. The somatic protoplasts were star-shaped and measured 4–7 μ in diameter, the protoplasmic connections being fairly thick and appearing as prolongations of the angles of the protoplasts (text-fig. 8, I). The cell-walls formed a pentagonal or hexagonal pattern in surface-view, and appeared rectangular in side-view. In the asexual colonies up to seven daughter-coenobia were formed.

The sexual colonies contained oogonia only, but in each a few (up to five) spaces could be found, evidently representing the positions of former antheridia from which the spermatozoids had escaped. The colonies were therefore monoecious and protandrous. The number of oogonia was eighteen to thirty-five. The ripe oospores had a smooth inner and a spinous outer membrane, the short and conical spines having a broad base (text-fig. 9, *B*). The spores measured 35–44 μ without the spines, which were 3·5–7 μ long, the average length being about 5 μ ; fourteen to sixteen spines could be counted around the periphery in optical section.

VOLVOX DISSIPATRIX (Shaw), comb. nov. (*Copelandosphaera dissipatrix* Shaw *). (Text-fig. 5, H–K ; text-fig. 7, C ; text-fig. 8, E ; text-fig. 9, H.)

This alga was collected by Dr. M. A. Sampathkumaran from a greenish pool near Bangalore. There were present subglobose asexual and monoecious sexual colonies ; the latter were ellipsoidal when young, though later subglobose, slightly longer than broad and somewhat narrower at the posterior end. The asexual coenobia reached 935 μ (697 × 748 μ, 842 × 867 μ, 867 × 935 μ) and the sexual ones 1037 μ (646 × 697 μ, 842 × 884 μ, 952 × 1037 μ) in diameter. On one occasion two sexual colonies, which were 1495 μ and 1815 μ in diameter

* Shaw, 1922 *a*.

respectively, were found, but very old colonies often measured nearly 2 mm. in diameter. The number of cells in the sexual coenobia was 12,000 to 20,000, and in the asexual ones 14,000 to 26,000.

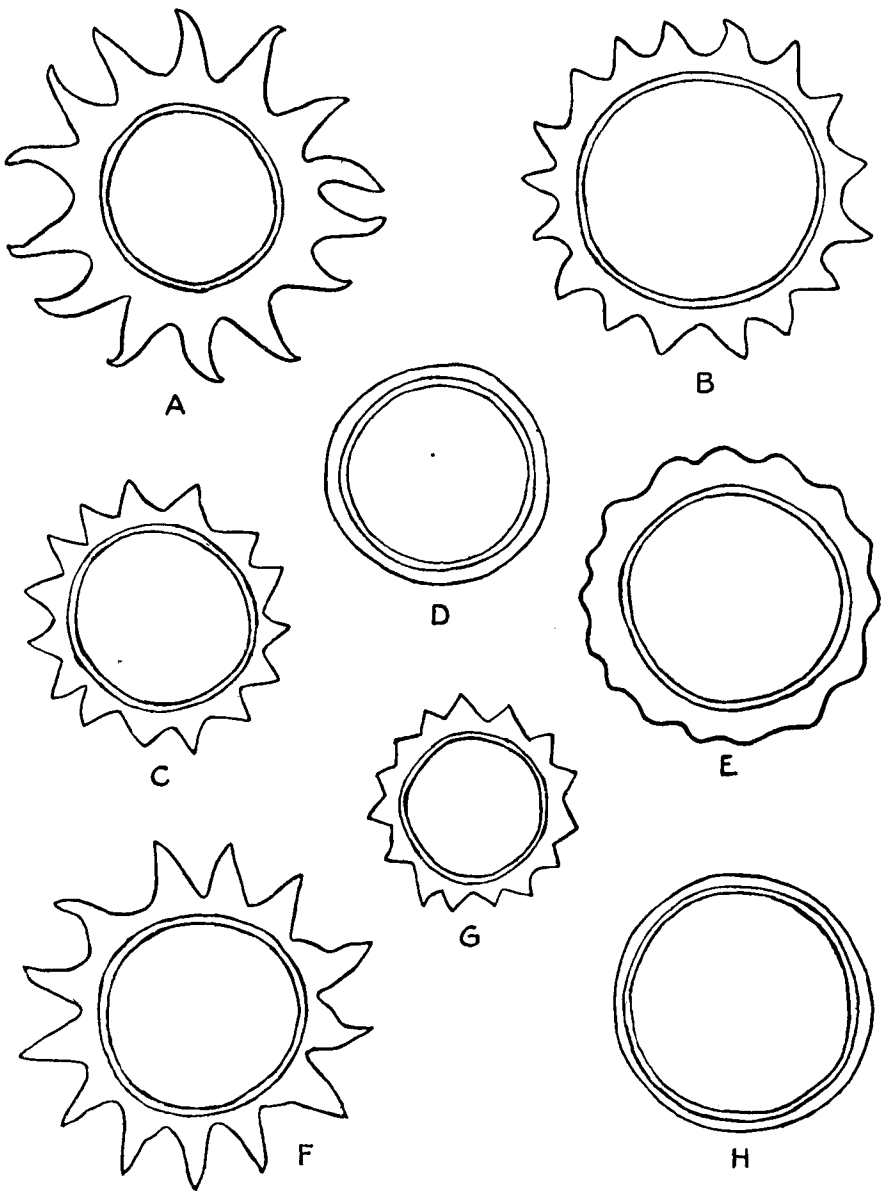
The protoplasts are round in surface-view (text-fig. 5, K, I; text-fig. 8, E) and elliptic-ovate or broadly pear-shaped (with the broader end directed outwards) in side-view (text-fig. 5, H, J); they are 6 μ wide and 7·5 μ high. The distance between the centres of adjacent protoplasts varies between 8 and 22 μ, as one passes from the posterior to the anterior end of the colony. The cell-wall consists of a delicate but comparatively firm layer immediately next to the protoplast (text-fig. 5, H) and a broad gelatinous outer layer with a definite boundary. The outlines of the cell-walls are generally pentagonal or hexagonal in surface-view (text-fig. 5, K) and rectangular in side-view. A single pyrenoid can be seen in each protoplast towards the posterior end (text-fig. 5, J). Under high magnifications a very slight bulging of the gelatinous envelope is recognisable on the outside of each protoplast (text-fig. 5, H). The protoplasts at first sight appear to have no protoplasmic connections, but under very high magnifications, after staining with aqueous methylene-blue or toluidine-blue or alcoholic safranin, extremely fine and delicate connections are recognisable between the cells. They are much finer than the cilia. Adjacent protoplasts are generally connected by two such strands (sometimes three), which often meet at a slight angle when they reach the boundary of the wall (text-fig. 5, I–K).

Asexual colonies.—From three to ten daughter-coenobia are formed inside the mother-colony, the usual number being 4–6 (text-fig. 7, C). The shape of the daughter-coenobia varies between ellipsoid and globose, and before liberation they measure 221–323 × 247–382 μ. Each escapes from the parent colony through a separate circular to elliptical hole, as Shaw (1922 *a*, p. 217) noticed in his Philippine material. The gonidia of the daughter-coenobia differentiate at a rather late stage, but before the new colony escapes from the parent.

Sexual colonies.—The front fourth of the colony is free from sexual reproductive bodies. Only a few antheridia (up to six) are formed in the sexual coenobia, and these are found nearer the anterior end. The antheridia form round sperm-platelets, which measure 37–40 μ in diameter and 10–11 μ in depth. The number of spermatozoids appears to be about 256. The number of oogonia is about seventy to ninety and the oospores have a smooth double wall (text-fig. 9, H) and measure 37–40 μ in diameter.

The alga just described agrees in most respects with *Copelandosphaera dissipatrix* Shaw. The dimensions of the asexual coenobia and the number of daughter-coenobia formed, however, appear to be somewhat smaller than in the Philippine material. Shaw describes this species as lacking protoplasmic connections between the cells. Through the kindness of Prof. Fritsch I was able to examine some of the material of *Copelandosphaera dissipatrix* distributed by Shaw, and found that, after staining in the way described on

TEXT-FIG. 9.



Oospores of diverse species of *Volvox*.

A, *V. globator* Ehrenberg var. *maderaspatensis*, nov.; B, *V. globator* Ehrenberg; C, *V. prolificus*, sp. n.; D, *V. africanus* West f. *minor*, nov.; E, *V. Carteri* Stein; F, *V. Merrilli* Shaw f. (?); G, *V. Rousseleti* West var. *lucknowensis*, nov.; H, *V. dissipatrix* (Shaw), comb. nov. (All × 785.)

p. 357, delicate protoplasmic strands were recognisable under high magnifications (×2,000 to 3,000), also in his material. These connections, however, are easily missed.

After discovering them in this species, I was led to apply the same methods to other forms in which the cells are believed to be devoid of protoplasmic connections. I examined in this way the following species :—*V. africanus* (Shaw's Philippine material and my own, cf. below), *V. Carteri* (own material, cf. p. 362), *V. Carteri* var. *manillana* (Shaw's material), *V. Carteri* f. *nagariensis* (own material, cf. p. 364), and a *Volvox* collected by Mr. D. J. Scourfield, I.S.O., from Epping Forest and showing much resemblance to *V. mononae* G. M. Smith (1920, p. 99, pl. 18, fig. 1). In none of these forms could protoplasmic connections be detected, although in each coenobia of very different ages (from very young to very old) were examined.

Meyer (1896, p. 200, pl. 8, fig. *a*) states that he found delicate protoplasmic connections in the unborn daughter-colonies of *V. tertius*, although he could not find them in the older colonies. The presence of connections in the young coenobia of *V. tertius* may constitute a recapitulation of a phylogenetic feature. In that case we should have to regard the species of *Volvox*, devoid of protoplasmic connections, as the more specialised and as derived from those possessing such connections.

The discovery of protoplasmic connections in *Copelandosphaera dissipatrix* brings this species nearer Shaw's *Janetosphaera*. It would be interesting to know whether the second species (*V. spermatosphaera* Powers), which Shaw includes under *Copelandosphaera* as *C. spermatosphaera* (Powers) Shaw, also possesses these protoplasmic connections.

VOLVOX AFRICANUS West f. MINOR, nov. (Text-fig. 6, I–K ; text-fig. 7, B, D, H ; text-fig. 8, G ; text-fig. 9, D ; Pl. **28.** figs. 14, 21, 22.)

This alga was collected by Dr. M. A. Sampathkumaran in a small pool on the top of the Nandhi Hill, near Bangalore, in the Mysore Province.

The colonies were asexual or sexual, the latter being unisexual or hermaphrodite. The asexual colonies were ovoid to ellipsoidal, the posterior end being broadly rounded and somewhat distended by the contained daughter-colonies, while the anterior end which was free from daughter-colonies was much narrower (text-fig. 7, H ; Pl. **28.** fig. 21). The sexual colonies were ellipsoidal, the female with a slightly broader anterior end (Pl. **28.** figs, 14, 22). All the three kinds of colonies are slightly flattened along an antero-posterior plane. When mounted in water on a slide, they rest on one of their flattened surfaces, so that only their broader surfaces are seen, but when crowded some of them rest on their narrower surfaces and the flattening of the coenobium is manifest. Occasional colonies are strongly flattened, but the majority are only slightly compressed ; the shape of a cross-section at right angles to the plane of flattening would probably be broadly oblong-elliptic (text-fig. 7, B, D).

The asexual coenobia measured 187×238–$289\times416\ \mu$, the female and bisexual 170×204–$331\times374\ \mu$, and the male 190×231–$248\times306\ \mu$. The number of cells in the colony varied from 1,400 to 3,000.

The protoplasts are round in surface-view (text-fig. 6, J; text-fig. 8, G) and pear-shaped or ovoid (with the broader end directed outwards) in side-view (text-fig. 6, I, K, L). They measure 5–6·5 μ in diameter. They are more closely placed at the posterior than at the anterior end of the colony, the distance in one colony between the edges of the protoplasts being 9 μ at the anterior and 2–3·5 μ at the posterior end. There are no protoplasmic connections between the cells. The cell-walls are well seen when stained with aqueous methylene- or toluidine-blue or with alcoholic safranin. The last-named stain, although it tends to distort the shape of the colony, brings out the details fairly clearly. In surface-view the outermost part of the cell-walls forms a pentagonal or hexagonal network, within each compartment of which a second elliptic membrane is discernible separated by a wide space from the central rounded protoplast (text-fig. 6, J). Immediately adjacent to the protoplast is found a delicate membrane representing the innermost layer of the gelatinous wall. The same features are seen in the side-view, where the outline of the cell as a whole appears rectangular (text-fig. 6, I, K). The exact morphological significance of the second elliptic layer of the envelope is not clear.

In older colonies the outline of the outermost common envelope, as seen in optical section under higher magnifications, presents a slightly wavy appearance owing to its being slightly bulged out opposite each cell (text-fig. 6, L). This feature was more prominent at the anterior than at the posterior end.

Asexual colonies.—The asexual colonies usually form two daughter-coenobia (text-fig. 7, D, H; Pl. **28.** fig. 21), although one, three, or four are frequently found. When two are produced, they are arranged opposite each other in the middle region of the parent colony. The fully developed daughter-coenobia finally come to lie near the broad posterior end of the latter, so that the posterior poles of the two elongate daughter-individuals are very close to the posterior end of the mother-colony (text-fig. 7, H; Pl. **28.** fig. 21). When three daughter-individuals are formed, the third is placed behind and between the two median coenobia, a little towards the posterior end. When there are four coenobia, two are located in the middle opposite each other, and the remaining two behind these and alternating with the first pair. The median pair of coenobia are always the largest, when more than two are present. The young coenobia are slightly compressed and ovoid in form, with their long axis parallel to the longitudinal axis of the parent coenobium. A thin gelatinous vesicle, the remains of the gelatinous wall of the gonidium, is seen round each daughter-coenobium.

Sexual colonies.—The sexual colonies were not as numerous as the asexual ones, and the male colonies were very rare. The latter contain 50–180 antheridia which occur over nearly the whole of the male colony, only about the anterior eighth (or even less) being free from antheridia (Pl. **28.** fig. 22).

The antheridia are thus very crowded and it is difficult to count their exact number. They measure up to 20 μ in diameter, while the platelets of spermatozoids measure up to 22 μ in diameter and 22×13 μ in side-view. There were 128 spermatozoids in each antheridium. No free spermatozoids were observed, though the cilia were fully formed on the cells in the platelets. The spermatozoids in the bundle measured 8–10 μ long and about 1·5 μ broad.

Some colonies were purely female, but most of them were bisexual containing from one to three, sometimes four, antheridia in the middle region of the coenobium. The number of oogonia in the female colonies varied between six and twenty-two (though in one colony as many as fifty-five oogonia were found), the bisexual colonies generally containing more oogonia than the purely female ones. The ripe oospores have a smooth wall of two layers and measure 30–39 μ in diameter.

As the above account shows, the Indian form agrees very closely with *Volvox africanus* as originally described by West (1910, 1918), and as subsequently amplified by Shaw (1923) from Philippine material. Neither West nor Shaw noted the slightly flattened nature of the coenobia. I have been able to examine one of West's slides made from the original material of *V. africanus* *, and also the material distributed by Shaw under the name of *Merillosphaera africana*. In both the slight flattening was observable, though not to the same extent as in the Indian alga.

West, in his first paper (1910), stated that there were no protoplasmic connections, but in the second paper (1918, p. 426) the occurrence of such connections is mentioned in the tabular statement; this was probably an error. Shaw found no protoplasmic connections in his material, nor does the Indian alga show any (cf. also p. 359).

West pointed out that the gonidia in the daughter-coenobia were well differentiated long before birth and, as noted by Shaw, they attain considerable size before they divide. Both West and Shaw observed a few colonies of the third generation with gonidia already differentiated, so that four generations were represented in one individual. In my material colonies showing three generations are extremely common, and there are occasional ones in which four generations are represented.

Though the Indian alga shows very close agreement in all essential respects with the African and Philippine material, there are some minor differences. The colonies of the Indian alga are much smaller than those previously described, measuring only $238–408 \times 187–289$ μ, whereas the African one measured $345–610 \times 295–480$ μ and the Philippine form $345–600 \times 295–500$ μ. The asexual colonies in the African and Philippine material are somewhat narrowed instead of being broadly rounded posteriorly, and the daughter-coenobia are not so close to the posterior end, as in the Indian alga. While the number of gonidia and daughter-coenobia formed in the Indian alga is generally two, West's photographs show three to four, and in the above-mentioned slide

* By the courtesy of Prof. W. Stiles, F.R.S.

four to six are present. Shaw records one to eight daughter-coenobia. The Indian alga also differs in the small number of oogonia and in the smaller size of the oospores, although Shaw records a smaller number of oogonia (12–32) than West found in the African material (70–80).

The small form, occasionally found by Shaw (1923, pp. 205–208, pl. 6, figs. 43–45) in a pure condition, is probably identical with the Indian alga, with which Shaw's description and photographs agree in many ways. Thus, he describes the coenobia as sometimes broader than long, and measuring 150–270×160–240 μ; asexual coenobia having usually two daughter-coenobia; oogonia fifteen to twenty-seven in number; and the oospores 35–43 μ in diameter. Unfortunately, the specimens he photographed were very much contracted in the venetian turpentine mount, so that one cannot get a good idea of the shape of the colonies from the photographs. The posterior end of the colony in the photograph, however, appears to be broadened somewhat as in the Indian alga. I consider this alga a new form of *V. africanus* West, which may be named f. *minor*.

VOLVOX CARTERI Stein.* (Text-fig. 6, D, E, F; text-fig. 7, E; text-fig. 8, H; text-fig. 9, E; Pl. **28.** figs, 16, 19, 25.)

This alga was collected by Mr. M. O. T. Iyengar in January 1916 in some small pools, on the point of drying up, in the bed of a tank in Mylapore, Madras.

The coenobia are asexual, male or female. The asexual coenobia are globose to subglobose, and measure 460–630×485–646 μ (460×485 μ, 560×578 μ, 578×612 μ, 630×646 μ). The female coenobia are subglobose, and measure up to 578×612 μ. The male coenobia are very small, ellipsoid to globose, and attain only to 221×238 μ. The asexual colonies have 3,000 to 11,000 cells, the female 2,000 to 3,000, and the male 500 to 700.

The protoplasts are round in surface-view and are 4·5–5·5 μ wide and 5·5–6·5 μ high (text-fig. 6, E; text-fig. 8, H). The distance between the edges of the adjacent protoplasts is 3·5–5·5 μ at the posterior and 8·5 μ at the anterior end of the colony. No protoplasmic connections could be detected, although the same methods to render them visible were used as were successful in *V. dissipatrix*. The outlines of the actual cells are pentagonal or hexagonal in surface-view (text-fig. 6, E) and somewhat rectangular in side-view (text-fig. 6, D). The actual depth of the cells is 14–16 μ from outer to inner wall. The cell-wall consists of a firm though delicate inner layer, immediately surrounding the protoplast, and of a broad gelatinous outer portion. In the preserved material the protoplasts have contracted somewhat, and the inner layer of the wall can be seen as a delicate ring round each protoplast.

Asexual coenobia.—From three to eight daughter-colonies are found in the asexual coenobia, but the usual number is four to six. Generally four gonidia,

* *Volvox globater* Carter non Ehrenberg (Carter, 1859, pp. 2–5, 18, 19, pl. i. figs. 1, 3, 4, 7, 8, & 10); *Volvox Carteri* Stein (1859–83, Abth. iii. p. 134); *Merillosphaera Carteri* (Stein) Shaw var. *typica* Shaw (Shaw, 1922, xxi, p. 87 et seq.).

dividing to form daughter-coenobia, are arranged more or less equidistantly in the middle region of the parent colony, while the remainder are situated towards the posterior end and alternate with the others (text-fig. 7, E ; Pl. **28.** fig. 16). The middle four are usually larger than the others, which appear to be younger, and are often merely represented by undivided gonidia, at a time when the middle ones are well-developed daughter-coenobia, whose gonidia are already differentiated. The daughter-coenobia inside the parent measure 165–238 μ in diameter. The young individuals found in the asexual coenobia in my material were all either male or asexual or partly asexual and partly male. None were female.

Female coenobia.—These were very scantily represented as compared with the asexual coenobia. From 17 to 29 oogonia were observed (Pl. **28.** fig. 19). The oospores had a smooth inner wall and a wavy crenate outer wall (text-fig. 9, E ; Pl. **28.** fig. 25). The spores measure 42–47 μ in diameter.

Male coenobia.—There were young male coenobia inside the asexual colonies, but only a few were found free. The antheridia begin to divide in the daughter-coenobia, while they are still inside the mother. There were about seventy-five and these occupied almost the whole of the colony, except at the extreme anterior end. The undivided antheridial cells are about 14 μ wide. The antheridial cells, in the few free male colonies observed, were dividing into two or four cells, but fully formed antheridia were not seen in the material.

The alga just described resembles very closely the one collected by Carter (1859) in Bombay, and described by him under the name of *V. globator.* Stein (1859–83) later established Carter's form as a new species, *V. Carteri,* basing his diagnosis on Carter's account of the Bombay *Volvox.*

The dimensions of Carter's alga are given in fractions of inches, but reckoned in micromillimetres they are as follows :—The adult spherical or nearly spherical asexual coenobium measures 770 μ, the female coenobium 608 μ, the male 270 μ, and the young daughter-coenobia about 192 μ. The daughter-colonies are stated to be very regularly arranged and generally to be eight in number. They contain, while still inside the parent, well-differentiated gonidia, reaching up to 85 μ before division. The cells are described as globular, and Carter's figures show that they were devoid of protoplasmic connections, and that the innermost layer of the cell-wall had a structure somewhat similar to that above described for my specimens. Carter found either male or female daughter-coenobia along with asexual ones in the same individual, but did not find the male and female coenobia in the same parent. He also points out that one, several, or all the daughter-individuals of a colony may be male. The female coenobia contained thirty to fifty oogonia, the oospores being 40 μ in diameter, and having a slightly wavy outline.

This description agrees so closely with that of the Madras *Volvox* that the two are no doubt identical. The female daughter-coenobia within the parent-colony are described by Carter as attaining double the size of a male daughter-coenobium, but my material did not enable me to check this point.

Since Carter described this *Volvox*, it has only been once recorded. Playfair (1918, p. 527, pl. 56, figs. 21, 22) records it from the Lismore District. He describes the oospores as wavy when young, but spiny when fully developed. He has a drawing of the spiny oospore, but does not give any figures or photographs of the colonies, nor does he give any proper description of them. The oospores examined by me were fully ripe, with both walls well developed, and the membrane was invariably crenate. Shaw found the same in the Philippine material referred to a variety (cf. below). In the absence of a proper description and figures or photographs of the colonies, it is not possible to be sure that Playfair's *Volvox* was really *V. Carteri*. It must be pointed out that he did not see Carter's paper, but identified his *Volvox* from the casual reference to the crenate oospores of *V. Carteri* by Lemmermann (1904, p. 105). The oospores in all species of *Volvox* having spiny oospores have wavy walls when young and develop the spines when they become older.

VOLVOX CARTERI Stein forma NAGARIENSIS, nov. (Text-fig. 7, A; text-fig. 8, F; Pl. **28.** fig. 18.)

Occasional colonies of this alga were found among those of *V. prolificus* in the pool in the bed of the river Nagari. Only globose asexual coenobia were present, but these were sufficiently characteristic to warrant a reference to *V. Carteri*. They measured up to 1003 μ in diameter and comprised up to 8,000 cells.

The protoplasts were round in surface-view (text-fig. 8, F) and pear-shaped in side-view. They measured 6–7 μ in diameter and up to 9 μ in length. The cell-wall shows the structure described on p. 362. The distance between the centres of adjacent cells in an old colony was up to 30 μ. There were no protoplasmic connections. The number of daughter-coenobia was large, sometimes as many as twenty-one, the usual number being about fifteen (text-fig. 7, A; Pl. **28.** fig. 18). Their arrangement could not be easily recognised. The daughter-coenobia were slightly ellipsoidal and measured from $123 \times 138\ \mu$ to $340 \times 357\ \mu$ before liberation. The daughter-coenobia were all asexual and contained well-developed gonidia, those in one daughter-coenobium about to escape measuring 26–30 μ in diameter at a time when the somatic protoplasts were only 4–5 μ in diameter.

This form differs from *V. Carteri* Stein and from *V. Carteri* Stein forma *manilana* (*Merillosphaera Carteri* (Stein) Shaw var. *manilana* Shaw*), and *V. Carteri* Stein forma *Weismanniana* comb. nov. (*V. Weismannia* Powers †; *Merillosphaera Carteri* (Stein) Shaw var. *Weismannia* (Powers) Shaw ‡) in the larger dimensions of the asexual coenobia and in the larger number of daughter-

* Shaw, 1922 *b*, pp. 90–104, 120–1.
† Powers, 1908, pp. 152–162, 172–5.
‡ Shaw, 1922 *b*, pp. 107–110, 121.

colonies produced. It appears to be no more than a form of *V. Carteri* Stein, which may be named f. *nagariensis*.

THE CLASSIFICATION OF THE SPECIES OF VOLVOX

The cell-structure in all species of *Volvox* shows a considerable degree of uniformity. The cells have thick gelatinous walls, the outlines of which as a result of mutual pressure form a polygonal network in surface-view. The second membrane, between the external and internal boundaries of the wall, above recorded for certain species of *Volvox* without protoplasmic connections, is at present difficult to explain, nor is it clear how far it is represented in other species. The relatively small protoplasts, though varying somewhat in shape, appear to show a rather uniform structure. The main difference in cell-structure among the species lies in the presence or absence of protoplasmic connections.

Shaw has classified the existing species of *Volvox* into two groups, according as protoplasmic connections are present or absent between the cells. The species lacking protoplasmic connections he has placed under four genera, mainly based on the stage at which the gonidia differentiate in the embryos. Such differences, while they appear constant for a given species, scarcely seem sufficient to warrant the establishment of separate genera, in view of the general similarity in cell-structure. Moreover, *Copelandosphaera* has been shown above to possess protoplasmic connections (cf. p. 359), while the character (viz. the migration of the gonidia from without into the interior in the young embryo) on which *Campbellosphaera* (Shaw, 1919) was mainly based, is due to the inversion of the embryo, a phenomenon which is now known to occur in all colonial members of Volvocales.

The fundamental features, apart from cell-structure, on which the species of *Volvox* can be distinguished are the distribution of the sexes, the numbers of the reproductive cells, and to some extent the disposition of the gonidia, especially when there are few of these. *V. prolificus* is essentially distinguished by the consecutive formation of all three kinds of reproductive cells and by their large numbers.

A further feature that is apparently of systematic importance is the character of the oospore, although further experience is wanted to assess the value of the relatively slight differences between the spines on the spores of diverse species.

It may be emphasised that the description of the forms of *Volvox* dealt with above is based mostly on the investigation of a considerable bulk of material and that this has displayed a considerable constancy in certain features —even, sometimes, in minor particulars. Until extensive culture-experiments have been carried out, however, it is scarcely possible to say how far some of them may represent local habitat-forms. In any case their careful description has, I hope, added to our knowledge of the forms of *Volvox* as they occur in nature.

ORGANISMS OBSERVED ON PANDORINA, EUDORINA, AND VOLVOX

(1) A unicellular epiphyte resembling *Craniocystis bipes* Korschikoff (Printz, 1927) was often seen on the colonies of *Pandorina morum*, *Eudorina elegans*, and *E. illinoisensis* (text-fig. 10, E, G), without apparently doing any harm to them. Usually only one or two individuals were found on a colony, and these were restricted to the posterior portion. The shape of the chloroplast of the epiphyte could not be clearly made out, but it contained two or three pyrenoids. The cells were enveloped by two membranes, the inner fairly firm, and the outer very thin and delicate. The outer wall bore at its base two very small, slightly toothed processes, by means of which the epiphyte was attached to the outer gelatinous sheath of the colonies (text-fig. 10, G). In the type-species the attaching processes are not toothed, but appear to be disc-shaped. Empty envelopes with the apex ruptured (text-fig. 10, E) were often found, suggesting reproduction by motile spores. The full-grown cells measured about 18 μ high and 20 μ broad.

(2) In another unicellular epiphyte (text-fig. 10, A, F), found on the colonies of *Volvox prolificus*, the cell was rounded in surface-view, while in side-view it was broadly ovate, with the apical portion beaked, as in text-fig. 10, A. The chloroplast appeared to be bell-shaped, with the opening directed towards the point of attachment. Two to four pyrenoids were present in the chloroplast. In a few cells the protoplast had divided into 8, 16, or 32 parts. The cells measured 16–18 μ in diameter and were 14–16 μ high.

(3) A Chytridiaceous parasite (text-fig. 10, B–D, I) was commonly observed on the colonies of *Pandorina morum*, *Eudorina elegans*, and *E. illinoisensis*. The external part of the parasite was somewhat globose with a rounded apex, and from it a thin haustorial process extended into a cell of the host, gradually absorbing the contents, while the part outside the host became bigger. The lower end of the haustorium, in contact with the host-cell, also becomes slightly swollen (text-fig. 10, B). The protoplast of the cell attacked gradually shrivels, and finally only a minute residue is left. The parasite may attack several cells of a colony successively, and it is not uncommon to find colonies with one or more cells missing through its activities (text-fig. 10, B–D). The empty ruptured envelope of the parasite is often found attached to such colonies. The rupture is broad and apical (text-fig. 10, B, D). It seems probable that the contents of the fungal cell escape as motile spores, which in their turn attack other colonies, but the actual escape of spores has not been observed. The fully developed fungal cell was 16 μ broad and 14 μ high, the haustorial process being about 9 μ long.

This parasite closely resembles *Rhizidium Pandorinae* (Wille) Fischer (Fischer, 1892, i, Abt. iv. 1892, p. 109=*Chytridium Pandorinae* Wille, 1884, p. 46), which Wille has recorded as attacking *Pandorina morum* in South America. The American fungus differs from the Indian one, however, in the fact that the apex of the cell is produced into a kind of beak, and that the zoospores escape

by a lateral orifice (text-fig. 10, H). The Indian form may be regarded as a new variety, var. *globosa**, of Wille's form, differing in the broadly rounded apex and the spores escaping by a wide apical aperture.

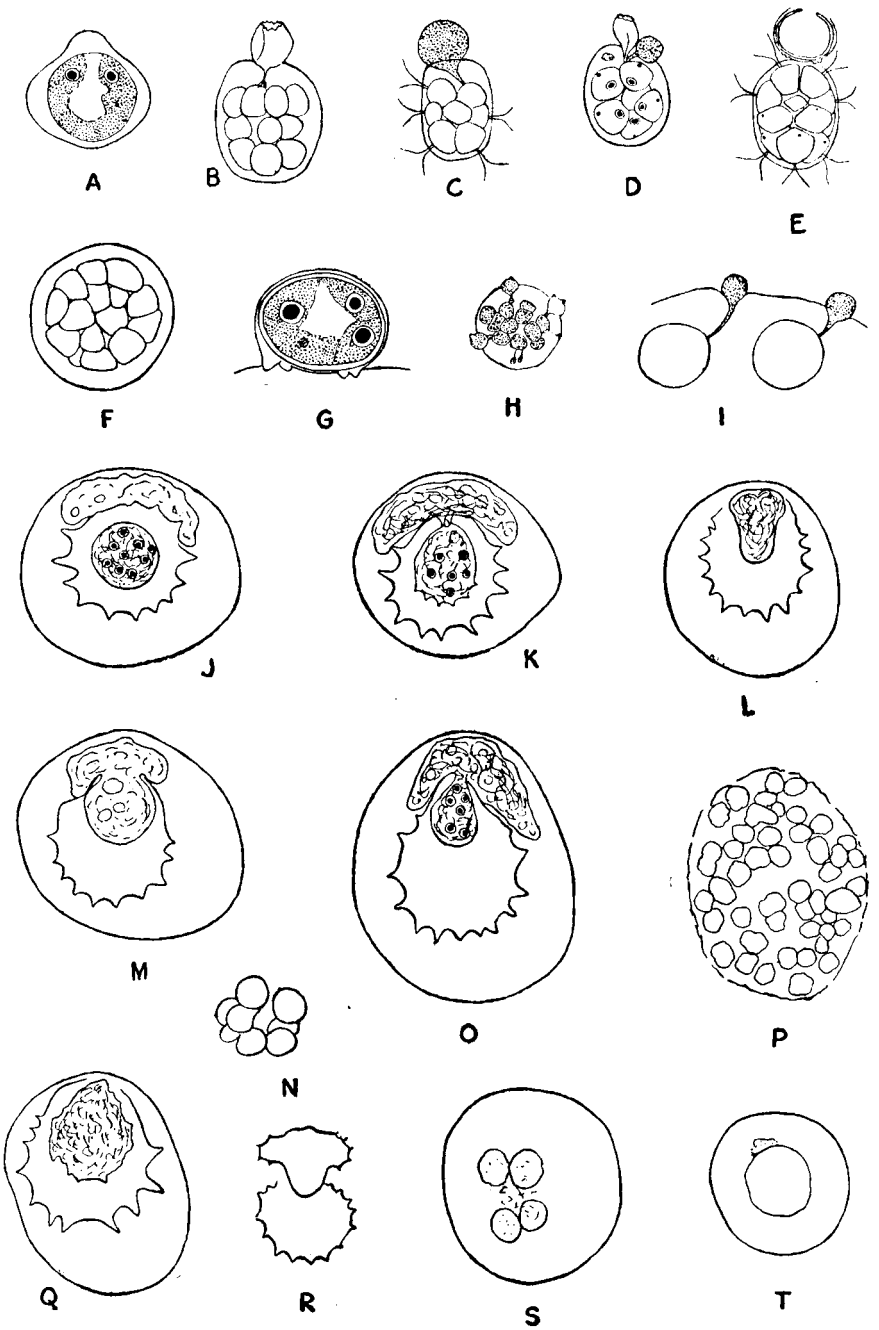
(4) In the formalin material of *Eudorina elegans* the cells of certain colonies showed a peculiar appearance. The cilia were crumpled or shrivelled up close to the surface of the colony, looking like a singed hair. When stained with erythrosine, the cells showed a dense cobweb-like covering of somewhat dark matted threads which did not take up the stain. Often the cells were completely surrounded by this covering, but sometimes it occurred only on one side. At a later stage the cells appear to lose their rounded shape and become somewhat flattened on their inner side, the two lower edges being slightly bent outwards. When all the cells assume this shape, they appear to touch one another. This at first gives the impression of a *Eudorina* colony in which the cells have developed protoplasmic connections. The fluffy covering around the cells does not appear to consist of fungal hyphae, but rather to be of bacterial (?) nature. The cilia appear first to be attacked, changing into a loose, more or less crumpled mass in which numerous rod- and dot-like fragments are to be seen. The disease seems to progress through the cilia into the colony and then to spread around the cilia. I was not able to detect any other details of structure and my main object in referring to this case is to point out the deceptive resemblance of the diseased alga to one having protoplasmic connections.

(5) In preserved material of *Volvox globator* var. *maderaspatensis*, the oospores in some colonies were attacked by an amoeboid parasite (*Vampyrella* ?) (text-fig. 10, J–M, O, Q, R). When the colony is stained with alcoholic safranin the parasite is at first seen closely attached to one side of the spiny oospores, within the gelatinous outer envelope of the latter (text-fig. 10, J). One or two processes then pierce the spiny wall and penetrate into the contents (text-fig. 10, K). Since an inner wall was not observable in these oospores, the parasite evidently attacks them before its formation. Finally the whole parasite enters the oospore (text-fig. 10, M), engulfing and finally absorbing the contents (text-fig. 10, Q). After some time it again escapes from the envelope of the oospore (text-fig. 10, L, R ; Pl. **28.** fig. 10).

(6) In certain old colonies of *Volvox prolificus*, the protoplast of one or more oospores showed peculiar features, evidently as a result of the attack of an amoeboid parasite, which was often seen attacking the young oospores. Such oospores fail to form walls, but undergo great enlargement while still enclosed in the wide mucus-wall, which has also increased in size. The contents are very dense. These enlarged cells had in one instance divided into four (text-fig. 10, S), and in several instances into eight (text-fig. 10, N) and more large irregularly rounded cells of unequal sizes, which are often loosely grouped as an irregular hollow sphere and present an unhealthy appearance (text-fig. 10, P).

* *Rhizidium Pandorinae* (Wille) Fischer var. *globosa* nov. A typo differt apice latissimo et poro terminale.

TEXT-FIG. 10.



EXPLANATION OF TEXT-FIG. 10.

A, F, unicellular epiphytic alga on *V. prolificus*: A, from the side; F, from the surface, showing division of protoplast.

B–D, I. *Rhizidium Pandorinae* (Wille) Fischer var. *globosa*, nov.

C vegetation stage on *Pandorina morum*; B, empty cell of parasite after escape of contents; D, the two stages side by side; I, young individuals attacking cells of *Eudorina elegans*.

E, G. *Craniocystis bipes* Korschikoff f. (?); G, mature cell; E, empty cell.

H, *Rhizidium Pandorinae* (Wille) Fischer on *Pandorina morum* (after Wille).

J–M, O, Q, R, successive stages of an amoeboid parasite (*Vampyrella* ?) attacking oospores of *Volvox globator* Ehrenberg var. *maderaspatensis* (see text p. 367).

N, P, S, T, oospores of *Volvox prolificus* dividing within the mother-colony, a pathological condition, probably due to the attack of an amoeboid parasite: T, the parasite in contact with the oospore; S, N, P, division of the protoplast of the oospore into 4, 8, and numerous parts respectively. Pyrenoids black.

(A, F, G, × 1200; B–E, × 400; H, × 190; I, × 745; J–T, × 390.)

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(7) Rotifers are often seen infesting colonies of *Eudorina elegans*, *E. illinoisensis*, and those of species of *Volvox*. One species commonly attacked the colonies of *Volvox prolificus*. Sometimes as many as six individuals were seen inside a colony, but usually there were only one or two. Even very young colonies, sometimes still inside the parent, were occupied by this rotifer. Many of the colonies were partly devoured by the rotifers.

(8) On one occasion a kind of blood-worm was seen devouring large numbers of colonies of *Eudorina illinoisensis* kept in a dish in the laboratory at Madras.

(9) A protozoan epiphyte, resembling a small shortly stalked species of *Vorticella*, was found in large numbers on the surface of *Volvox globator* var. *maderaspatensis*. Animal epiphytes have so far not been recorded on *Volvox*.

DIAGNOSES OF NEW SPECIES, VARIETIES, AND FORMS

PANDORINA MORUM Bory f. MAJOR, nov. (Text-fig. 1, N, O, P, U, V; Pl. **28.** fig. 7.)

Familiis e 32 vel saepe e 16 cellulis constantibus, pyrenoidibus 3–4 in cellulis maturis, plus numerosis in cellulis vetustis. Fam. 32-cell., 55–64 μ lat., 63–74 μ long. (55×63 μ, 57×63 μ, 57×68 μ, 61×68 μ, 64×74 μ); cellulae pyriformes, 11–15 μ lat., 12–14·8 μ long.

Hab. In rain-water pools along with other motile algae, in Madras.

EUDORINA INDICA, sp. n. (Text-fig. 3; Pl. **28.** figs. 2, 12, 13.)

Familiis ellipsoideis, e cellulis 64 constantibus, cellulis in seriebus transversis 7 cum 4, 8, 12, 12, 12, 12 et 4 cellulis dispositis; iis series anterioris multo minoribus quam iis series secundae et iis series secundae minoribus quam iis serium reliquarum; cellulis serium anteriorum 2 probabiliter somaticis, iis serium reliquarum propagativis; pyrenoidibus ad 4 in cellulis serium anteriorum, ad 16 in cellulis serium reliquarum.

Familia 140–175 μ long., 110–131 μ lat.; cellulis series anterioris 8·75–12 μ lat., ser. secund. 10–14 μ lat., ser. reliq. 14–23·5 μ lat.

Hab. Talguppa, Mysore Province, in a rain water pool (*M. A. Sampathkumaran*) ; Madras, in a rain-water pool.

PLEODORINA SPHAERICA, sp. n. (Text-fig. 4 ; Pl. **28.** figs. 4, 5.)

Familiis fere sphaericis, a cellulis 128 irregulariter in parte peripherica massae gelatinosae dispositis ; cellulis somaticis numerosis, in tota familia inter gonidia sparsis ; pyrenoidibus 1–3 in cellulis somaticis, ad 8 in gonidiis ; propagatione asexuali et sexuali ignota.

Fam., 187–210 μ lat. ; cell. somat., 9–11 μ lat. ; gonid., 15–17 μ lat.

Hab. Among other algae in a pool on a hill-slope at Vandalur, near Madras.

VOLVOX PROLIFICUS, sp. n. (Text-fig. 5, A–G ; text-fig. 7, J ; text-fig. 8, A ; text-fig. 9, C ; Pl. **28.** figs. 3, 6, 9, 20, 23.)

Familiis dioicis, in parte anteriori leviter dilatatis, in parte posteriori angustatis, asexualibus et femineis subglobosis vel globosis, masculinis ellipsoideis aut subglobosis, cellulis 9,000–25,000 ; protoplastis irregulariter rotundatis, 5–6 μ lat., processibus cytioplasmaticis angustis, in cellulis senioribus tenuibus et elongatis. Cellulis propagativis continue et successive per vitam in familiis efformatis. Familiis filialibus 4–20, plerumque 6–12, leviter ellipsoideis, 153–230 μ lat. ante nationem, gonidiis in familiis filialibus vix ante nationem efformatis ; antheridiis olim efformatis, 25–55, rotundatis, discoideis, 38–40 μ lat., spermatozoideis 13–15 μ long. et 0·8–1 μ lat., ciliis post apicem insertis ; oogoniis 100–500 ; antheridiis paucis in familiis juvenalibus asexualibus vel femineis. Oosporis membrana externa cum spinis conicis robustis, 5–7 μ longis et membrana interna laevi munitis. Fam. asex., 400–1010 μ ; fam. masc., 400–800 μ ; fam. fem., 400–1070 μ ; oosp. sine spin., 30–35 μ lat.

Hab. In a drying pool in the bed of the River Nagari, near Tirupati, in South India (*M. S. Raghava Chari*).

VOLVOX ROUSSELETI West var. LUCKNOWENSIS, nov. (Text-fig. 6, G ; text-fig. 7, F ; text-fig. 8, B ; text-fig. 9, G ; Pl. **28.** figs. 8, 11.)

Familiis dioicis, subglobosis vel ellipsoideis, cellulis 6,000–8,000 ; protoplastis late stellatis, 5·5–7·5 μ lat., processibus cytioplasmaticis tenuibus. Familiis filialibus ad 7 ; oogoniis numerosis, 160 vel pluribus ; antheridiis 20–60, 38–42, 5 μ lat., spermatozoideis ca. 256. Oosporis cum spinis brevibus, late conicis, 3·7–5·5 μ longis, munitis vel saepe cum membrana undulata.

Fam., 510–629$\times$544–765 μ ; oosp. sine spin. 32–33·5 μ lat., oosp. crenat. 34–35 μ.

Hab. Lucknow (*A. R. Roa*).

VOLVOX GLOBATOR (L.) Ehrenberg var. MADERASPATENSIS, nov. (Text-fig. 6, A–C ; text-fig. 7, G ; text-fig. 8, C ; text-fig. 9, A ; Pl. **28.** figs. 10, 15, 24.)

Familiis monoicis, asexualibus subglobosis, sexualibus ellipticis vel ellipticoovatis, cellulis 6,000–11,000 ; protoplastis irregulariter rotundatis vel stellatis,

processibus cytioplasmaticis tenuibus longis. Familiis filialibus 2–4, plerumque 2, in lateribus oppositis posteriore dispositis ; antheridiis paucis, jusqua 8 ; oogoniis 13–38. Oosporis cum spinis conicis robustis acutis saepe leviter curvatis, 8–13 μ longis, vestitis.

Fam. asex. ad 693 μ lat., fam. sex. ad 640×759 μ ; oosp. sine spin. 35–42 μ lat.

Hab. In a pool inside a *Casuarina*-plantation near Elliot Beach, Madras (*T. Ekambaram*).

VOLVOX AFRICANUS West f. MINOR, nov. (Text-fig. 6, I–K ; text-fig. 7, B, D, H ; text-fig. 8, G ; text-fig. 9, D ; Pl. **28.** figs. 14, 21, 22.)

Familiis dioicis vel monoicis, leviter deplanatis, asexualibus ovoideis vel ellipsoideis in parte posteriori late et in parte anteriori anguste rotundatis, sexualibus ellipsoideis, cellulis 1,400–3,000 ; protoplastis a superficie visis rotundatis, a latere visis pyriformibus in parte exteriori dilatatis, processus cytioplasmatices absunt. Familiis filialibus 2–4, elongato-ovoideis, plerumque 2 solum, in media parte familiae cum polis prope partem posteriorem familiae maternae dispositis ; antheridiis numerosissimis, in tota familia praeter sextam partem anteriorem dispositis, spermatozoideis 128 ; familiis femineis saepe bisexualibus, cum antheridiis paucis (ad 4) in media parte ; oogoniis 6–22, interdum ad 59. Oosporis cum membrana laevi.

Fam. asex., 187×238–289×416 μ ; fam. masc., 190×231–248×306 μ ; fam. fem., 170×204–331×374 μ ; lat. oospor., 30–39 μ.

Hab. In a small pool on the top of the Nandhi Hill, near Bangalore, in Mysore Province (*M. A. Sampathkumaran*).

VOLVOX CARTERI Stein f. NAGARIENSIS, nov. (Text-fig. 7, A ; text-fig. 8, F ; Pl. **28.** fig. 18.)

Familiis asexualibus solum notatis, globosis, cellulis ad 8,000, typo similis, sed familiis filialibus numerosis, ad 21, plerumque 15 ; protoplastis 6–7 μ lat., ad 9 μ long., a superficie visis rotundatis, a latere visis pyriformibus in parte exteriori dilatatis, processus cytioplasmatices absunt. Familiis filialibus subellipsoideis, 123×138–136×153 μ, gonidiis bene efformatis.

Diam. fam., ad 1003 μ.

Hab. In a drying pool in the bed of the River Nagari near Tirupati, South India, occurring sparsely among *Volvox prolificus* (*M. S. Raghava Chari*).

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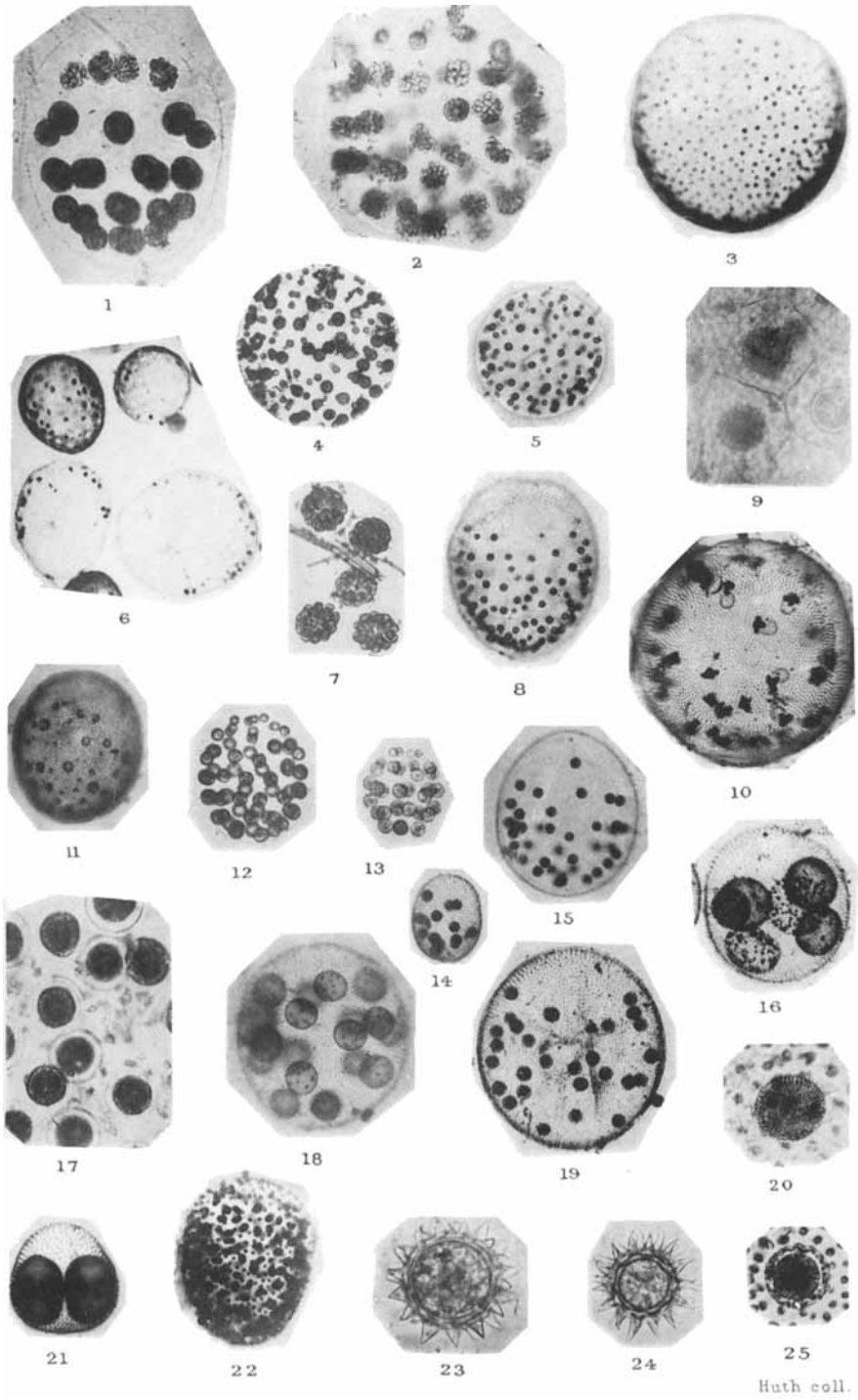

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EXPLANATION OF PLATE 28.

Fig. 1. *Eudorina illinoisensis* (Kofoid) Pascher, monoecious colony; the four front cells forming three antheridia and one 16-celled colony, the remaining cells forming oogonia. (× 240.)

Fig. 2. *Eudorina indica*, sp. n., cells dividing to form antheridia and daughter-colonies; the anterior cells are not dividing. (× 150.)

Fig. 3. *Volvox prolificus*, sp. n., female colony. (× 30.)

Fig. 4. *Pleodorina sphaerica*, sp. n., colony with mature gonidia. (× 115.)

Fig. 5. Colony of *Pleodorina sphaerica*, sp. n., with the gonidia beginning to enlarge. (× 35).

Fig. 6. *Volvox prolificus*, sp. n., showing diverse sizes of male colonies. (× 22.)

Fig. 7. *Pandorina morum* Bory f. *major*, nov. (× 100.)

Fig. 8. *Volvox Rousseleti* West var. *lucknowensis*, nov., female colony. (× 35.)

Fig. 9. *Volvox prolificus*, sp. n., showing polygonal outlines formed by the membranes of the oogonia. (× 165.)

Fig. 10. *Volvox globator* Ehrenberg var. *maderaspatensis*, nov., amoeboid parasites escaping from the oospores. (× 40.)

Fig. 11. *Volvox Rousseleti* West, var. *lucknowensis*, nov., male colony. (× 41.)

Figs. 12 & 13. *Eudorina indica*, sp. n., 64-celled and 32-celled colonies respectively (× 100.)

Fig. 14. *Volvox africanus* West f. *minor*, nov., female colony. (× 40.)

Fig. 15. *Volvox globator* Ehrenberg var. *maderaspatensis*, nov., colony with oospores. (× 30.)

Fig. 16. *Volvox Carteri* Stein, with three male and three asexual daughter-coenobia. (× 40.)

Fig. 17. *Eudorina elegans* Ehrenberg., spermatozoids around egg-cells. (× 520.)

Fig. 18. *Volvox Carteri* Stein f. *nagariensis*, nov. (× 30.)

Fig. 19. *Volvox Carteri* Stein., colony with oospores. (× 55.)

Fig. 20. *Volvox prolificus*, sp. n., antheridium. (× 265.)

Fig. 21. *Volvox africanus* West f. *minor*, nov., asexual colony with daughter-individuals. (× 45.)

Fig. 22. The same, male colony. (× 63.)

Fig. 23. *Volvox prolificus*, sp. n., oospore. (× 400.)

Fig. 24. *Volvox globator* Ehrenberg var. *maderaspatensis* nov., oospore. (× 240.)

Fig. 25. *Volvox Carteri* Stein, oospore. (× 210.)