

# NOTES ON POONA ALGAE—I. *PLEODORINA CALIFORNICA* SHAW

M. S. BALAKRISHNAN AND MEERA BONDRE (*nec* GOLE)

*Department of Botany, University of Poona, Poona-7 India.*

## ABSTRACT

Details of structure and reproduction in *Pleodorina californica* Shaw var. *californica* (Chlorophyta—Volvocales), reported for the first time from India, are described. The authors' observations are in general agreement with those of previous workers who have studied the genera *Eudorina* and *Pleodorina*. Points of special interest and the taxonomy of the genus *Pleodorina* Shaw are critically discussed.

During collections of fresh water algae in and around Poona, *Pleodorina californica* Shaw has been frequently encountered. It often occurred as a partial bloom in masonry garden ponds and temporary pools. Advantage was taken of its rather frequent occurrence, to make a study of its structure and reproduction. The results of these studies are presented briefly below.

The material on which most of the study is based was collected during the month of October 1970. The alga was quite abundant up to the third week of the month but later it gradually became less abundant and totally disappeared in November. Collections remained in a healthy condition when maintained in the laboratory for nearly a fortnight. Most of the observations were made from living material maintained thus. Material killed with dilute Lugol's iodine and with or without subsequent staining with eosin was used for supplementing the observations already made from living material, for camera lucida drawings and photomicrography. Stock material was preserved in 4% formalin.

## VEGETATIVE ORGANISATION

Fully developed adult colonies are spherical to ellipsoidal in shape, consisting of 32, 64 or 128 cells. The cells are more or less evenly distributed within the mucilaginous envelope and not arranged in distinct tiers as in *Eudorina* Ehr. Cells in the anterior half of the colony are small and sterile somatic cells. Cells of the posterior half are larger and reproductive 'gonidial' cells (Text-figs. 1, 2, 4).

The cells are typically chlamydomonadine in appearance, having two anterior flagella, stigma and a cup-shaped chloroplast in which are embedded one or more pyrenoids. The two flagella pass outside through holes in the mucilaginous matrix of the colony and are little divergent (Text-fig. 10). The eyespot, which is situated a little below the anterior end, gradually decreases in size as we go from the anterior to the posterior end of the colony (Text-fig. 14, A, B, C, D). Eye spots are also present in the gonidial cells but they are very small and inconspicuous as compared to those of the somatic cells (Text-figs. 11A,B). Often the eye spot is so much obscured by the cell contents in a mature gonidial cell that even with some effort it is not distinguishable.

In the somatic cells there is usually a single pyrenoid but sometimes two or three are also seen. In such cells, however, one of the pyrenoids is large and the other two are smaller in size. As the colonies grow in size the gonidial cells enlarge and the cup-shaped chloroplast becomes more or less dense and almost completely fills up the cell cavity. The pyrenoids also increase in number. A fully developed gonidial cell may have 5 to 14 pyrenoids. According to GOLDSTEIN (1964), in the usually unipyrenoidal cells of *Eudorina unicocca* Smith var. *peripheralis* Goldstein, just prior to daughter colony formation small pyrenoids arise *de novo* near the periphery of the chloroplast giving rise to a condition intermediate between a unipyrenoidal and a multipyrenoidal condition somewhat similar to what we have reported here. Mature asexual colonies (Text-fig. 1) with fully developed gonidial cells are 120 to 400  $\mu$  in diameter.

#### ASEXUAL REPRODUCTION

After reaching their maximum size the gonidial cells begin to divide and produce daughter colonies. Cell division begins more or less simultaneously in all the reproductive cells of a colony. The first two divisions are longitudinal and radial, and are at right angles to each other (Text-fig. 2). Further divisions lead to the formation of a somewhat cup-shaped plakea with its broad opening facing the periphery of the colony. In some cases the lips of the plakea come close together and instead of a broad opening a slit is developed. At the commencement of the inversion, the lips of the plakea draw apart and gradually fold back. A little later, as the 'hat' stage develops, the marginal cells elongate and also start developing flagella while the central ones remain humped as a dome for a short while. The folding back of the lips goes on still further until they come close together again on the other side and a spheroid develops. The opening is obliterated as the cells now start enlarging.

After inversion is complete, the adult configuration is gradually developed in the young daughter colonies. The cells which were closely adpressed enlarge and move apart and get more or less regularly spaced within the mucilaginous investment. After attaining the adult configuration the daughter colonies enlarge for a while and simultaneously the size of the parent colony also increases due to distension of the envelope. Consequently, colonies containing fully mature daughter colonies are much larger than sterile adult colonies, ranging between 440  $\mu$  and 510  $\mu$  in diameter (Text-fig. 3).

The details of asexual reproduction as observed by us are in agreement with the observations of other workers who have studied the genera *Eudorina* Ehr. and *Pleodorina* Shaw.

Daughter colonies are finally liberated by gelatinisation and disintegration of the mother colony. After liberation, the daughter colonies continue to enlarge further and in a short while an increase in the size of the cells in the posterior half also becomes noticeable. Thus, in the daughter colonies differentiation starts only after their liberation from the mother colony. The freshly liberated colonies have all the cells alike and later on, as the colonies grow, differentiation starts gradually. There is apparently no definite correlation between the size of the colony and the degree of differentiation. Thus, some colonies attain a fairly large size without any differentiation into sterile and fertile halves (Text, fig. 8), and often small-sized colonies show clear differentiation (Text-fig. 4). Therefore, young colonies without any differentiation of cells show a wide variation in size (Text-figs. 7, 8), ranging from 40  $\mu$  to 120  $\mu$  in diameter.

## SEXUAL REPRODUCTION

The colonies are heterothallic, with male and female colonies separate. However, quite often asexual and sexual reproduction were combined; and in these cases, daughter colonies occurred intermingled with the antherozoid clusters or eggs in the male and female colonies respectively.

Mature male colonies with fully developed antherozoid clusters measure from 258  $\mu$  to 348  $\mu$  in diameter and mature female colonies with ripe eggs from 300  $\mu$  and 400  $\mu$ . In the male colonies, the clusters of antherozoids are formed by successive division of the antheridium. As in asexual reproduction, here also there is plakea formation and inversion (Text-fig. 5).

POCOCK (1933) who studied *Volvox*, was the first to recognize that inversion takes place also during the development of the antherozoid bundles in the Volvocales. Later, inversion was observed during the development of the antherozoid clusters in *E. elegans* Ehr. by IYENGAR and DORAISWAMI (see: DORAISWAMI, 1940) and in *E. indica* Iyengar by DORAISWAMI (1940). In *P. sphaerica* Iyengar (IYENGAR & RAMNATHAN, 1951) also, inversion takes place during the development of the antherozoid bundles. PASCHER (1927), however, does not refer to any inversion in case of *P. californica*. TIFFANY (1935) only mentions that antherozoids occur in cup-shaped clusters rather than flat plates. PAVILLARD (1952) who bases his account apparently on CHATTON's (1911) studies, refers to inversion during daughter colony formation but does not mention it during formation of antherozoid clusters. Our observations show there is distinct inversion during the formation of antherozoid clusters in this species, similar to what has been reported for *P. sphaerica* by IYENGAR and RAMANATHAN (1951).

Entire clusters of antherozoids are liberated as such and they swim in the water for a while, breaking up only when in the vicinity of a female colony. The antherozoids apparently enter the female colony through the holes formed by the gelatinisation of the colonial envelope at the posterior end.

The antherozoids are highly metabolic, changing shape constantly. They are spindle shaped with a narrow anterior and a slightly broader posterior portion. Because of the metabolic nature a variation is seen in the dimensions of the antherozoids. They are 12-16  $\mu$  long and 2-5  $\mu$  broad at the broadest portion. The two long flagella are inserted a little below the anterior beak-line portion. The chloroplast is pale yellowish green and possesses a single pyrenoid. Incidentally, it may be mentioned that in CHATTON's figures of antherozoids, the flagella are shown as though starting from the tip of the antherozoid and not a little below as here or as in *P. sphaerica* (see: IYENGAR & RAMNATHAN, 1951, p. 220, fig. 20).

In the female colonies, cells of the posterior half function as 'eggs'\*. These cells enlarge considerably and become bright green in colour, but there is no other perceptible change in the protoplast itself. The wall, however, gelatinizes considerably, so that ripe 'eggs' ready for fertilization appear as rounded bright green biflagellate protoplasts inside enlarged vesicles. (Text-fig. 13). It may be mentioned here that the somatic cells of ripe female colonies also often show this type of vesicular development.

At this stage the posterior part of the colony envelope also gelatinises and becomes lobed and the mucilage in the posterior part becomes a little more fluid presumably to allow free movement of the antherozoids. Thus, female colonies containing ripe 'eggs'

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\*The term 'egg' is used to be in conformity with the terminology of other workers who have described sexual reproduction in the colonial volvocales.

are easy to locate because of the characteristic dark green colour of the eggs and the irregularly wavy colonial envelope at the posterior end.

The antherozoids were observed actively swimming inside the female colonies in large numbers (Text-fig. 6). Some of these could be seen browsing about the egg cells and some even kept swimming inside the enlarged vesicles for some time though how they actually entered could not be made out. Contact with the egg is made by the anterior end of the antherozoid (Text-figs. 16, 17). A little later the antherozoid becomes attached to the egg all along its length (Text-fig. 18), and fuses in this position. In one instance the two flagella of an antherozoid were observed projecting outside at the place where it fused with the 'egg' (Text-fig. 19).

Often in such ripe female colonies undergoing fertilisation, some of the antherozoids were also attracted towards the somatic cells in the anterior half, and occasionally 3 or 4 antherozoids were seen attached to a single somatic cell (Text-fig. 15A and 15B). This was observed in several colonies, but actual fusion of the antherozoids with these somatic cells was, however, not seen. A phenomenon of this type does not appear to have been observed so far. As mentioned a little earlier, in ripe female colonies the somatic cells often show gelatinization of the walls and consequent vesicular development in a manner similar to the 'egg' cells. This feature, as well as the fact that there is still some attraction of antherozoids to these somatic cells, would appear to indicate that though much reduced in size, these cells have not totally lost their sexual reproductive potential.

As may be seen from what has been said above, in the Poona form of *P. californica* the 'egg' retains its flagella at the time of fertilization (and is in fact a macrogamete) as has been reported previously for *Eudorina* (MERTON, 1908; MEYER, 1935; POCOCK, 1937; IYENGAR, 1937; DORAISWAMI 1940), *P. californica* (CHATTON, 1911) and *P. sphaerica* (IYENGAR & RAMANATHAN, (1951). In all these instances, therefore, we have a very advanced type of heterogamy bordering on oogamy but not true oogamy (see also: PASCHER, 1927; IYENGAR 1951).

However, it is to be noted that SMITH (1950) accepts TIFFANY's (1935) account as indicating that true oogamy occurs in *P. californica*, apparently because TIFFANY's figures of the female gametes do not show flagella. TIFFANY himself uses the terms 'female gametes' and 'eggs' interchangeably and does not say anything regarding flagella. (TIFFANY, 1935, pp. 141, 142, figs. 1-4). GOLDSTEIN (1964) however, specifically mentions that his *E. californica* v. *californica* (in which he includes the alga depicted by Tiffany in his figure 2 showing fertilization) has biflagellate eggs. Goldstein, however, says nothing about the female gametes of the monoecious form depicted by Tiffany in his figure 4, which is considered as a distinct variety viz. *E. californica* v. *tiffanyi* Goldstein. Thus there appears to be little justification of Smith's assumption. *P. californica* and *P. sphaerica* are both, therefore, heterogamous; the heterogamy, however, is more advanced than that seen in *Eudorina*. For, in *Eudorina* spp. the mucilaginous envelope gelatinizes prior to fertilization so that the 'eggs', which retain the capacity for movement, are fertilized either when they are lying loose in this matrix or after having moved slightly away from the general matrix of the coenobium and then getting surrounded by swarming antherozoids (see: DORAISWAMI, 1940, p. 134). In *P. californica*, however, our studies show that though flagellate, the female gamete does not exhibit any movement and is fertilized *in situ*. The same is true of *P. sphaerica* also (IYENGAR & RAMANTHAN, 1951, pp. 221, 22).

A further advance over *Eudorina* is also shown by the behaviour of the coenobia during reproduction. In *Eudorina*, during reproduction the coenobium becomes immobile. There

is also a gradual gelatinization of the envelope and daughter coenobia or antheridia or eggs as the case may be, lie loose in a fluid gelatinous matrix. In *P. californica*, as our observations have shown, and in *P. sphaerica* (IYENGAR & RAMANATHAN, 1951) the coenobia remain active and motile during reproduction in a manner similar to *Volvox*. Also, there is no gelatinisation of the colony envelope and reproductive colonies retain their original subspherical shape. Commenting on this feature, IYENGAR and RAMANATHAN (1951, p. 223) say that "this persistence of the colony in an active condition for some time even after the reproductive stage in the present alga (i.e. *P. sphaerica* auth.) is a natural consequence of the large amount of sterilization, since the large number of vegetative cells in the colony continue to live and keep the colony intact and motile for some time after the reproductive stage is over and until they themselves die".

The fully ripe zygotes are more or less spherical, with a multilayered wall which is somewhat irregular in outline. They are 30-40  $\mu$  in diameter.

The Poona form of *P. californica* has slightly larger zygotes than the French or U. S. forms. The dimensions of the zygote in the present form approach those of *P. sphaerica* (IYENGAR & RAMANATHAN, 1951) but the zygote wall is smooth and not with an ornamented middle layer as in *P. sphaerica*. It may be mentioned here that PAVILLARD (1952, apparently after CHATTON, 1911) describes the zygote in *P. californica* as having a finely granulated membrane and TIFFANY (1935; p. 142, fig. 4) describes zygospores as having smooth, finely granular or irregularly thickened walls.

## DISCUSSION

The genus *Pleodorina* was established by SHAW in 1894 with a single species, *P. californica* Shaw. Since SHAW's original report, *P. californica* has been reported by many workers in the West and by FRITSCH (1904) and CROW (1923) from Ceylon. KHAN (1970) has recently recorded this alga from Dehra Dun.

CHATTON (1911) published the first detailed account of reproduction in this species, based on his observations on material collected from Banyuls-sur Mer in France. Later, TIFFANY (1935) also made a study of this alga collected from an island in Lake Erie, U.S.A. and added some observations. More recently, GERISCH (1959) and GOLDSTEIN (1964) have made valuable contributions to our knowledge of structure and reproduction of this alga in nature as well as in cultures.

SHAW (1894) based the genus *Pleodorina* on the large number of cells in the coenobia (64-128) and the fact that cells in the anterior 1/2 or 1/3 of the colony were smaller in size and non reproductive.

Based on this concept, KOFOID (1898) described a second species, *P. illinoisensis* Kofoid, from the plankton of the Illinois river, with 32-celled colonies, in which the four cells of the anterior tier were markedly reduced in size and usually did not participate in reproduction. CHODAT (1902), however, considered *P. illinoisensis* to be just a physiological form of *E. elegans* Ehr. and reduced it to synonymy with *E. elegans*.

Reexamining the question, PASCHER (1927) drew attention to the general morphological similarity between *E. elegans* and *P. illinoisensis* particularly the arrangement of cells in regular tiers. He also observed that occasionally in *E. elegans* itself the anterior four cells often divide tardily, lagging behind the other cells. PASCHER (1927, pp. 439, 449) was of the opinion that as it showed very close similarity to *Eudorina* and none to *P. californica*, Kofoid's alga should be considered a distinct species of *Eudorina* rather than *Pleodorina* and he made the new combination—*E. illinoisensis* (Kofoid) Pascher.

After describing details of structure and reproduction of *E. illinoisensis* from Madras, IYENGAR (1933) also discussed at length the validity of this species. Supporting Pascher, he stressed that the first 4 cells in a coenobium were not only smaller than the rest but differed also in their behaviour i.e. they were either purely somatic or reproduced differently from the remaining cells of the same colony. Iyengar felt that this difference in behaviour of the front tier of four cells further distinguished *E. illinoisensis* from *E. elegans*. (see, however, GOLDSTEIN, 1964, p. 5). IYENGAR (1933) also described a new species of *Eudorina*—*E. indica*, having 32/64-celled colonies, with the first two tiers of cells smaller in size. DORAISWAMI (1940) showed that the anterior cells of *E. indica* Iyengar, while normally somatic, could at times function as gonidia.

In the very same paper, Iyengar has described a new species of *Pleodorina*, viz. *P. sphaerica*, in which the majority of cells (up to 95% in some cases) were somatic, a condition which he considered close to *Volvox*. Iyengar's discussion and figures indicate that he, like Pascher, considered arrangement of cells in tiers a characteristic feature of *Eudorina*, distinguishing it from *Pleodorina*.

GERISCH (1959), working with cultures demonstrated that the small, presumably somatic, anterior cells of *P. californica* were capable of asexual reproduction. Confirming this, GOLDSTEIN (1964) emphasized that it was the somatic nature of the anterior cells and not the reduced size alone which was considered the diagnostic character by Shaw. Consequently Goldstein felt that *P. californica* should be assigned to the genus *Eudorina* as *E. californica* (Shaw) Goldstein; he also reduced *E. indica* Iyengar to synonymy with his *E. californica*. Goldstein further merged *P. westii* Tiffany also into *E. californica* as he considered mammillations as a trivial character.

As already pointed out, our material of *P. californica* showed a tendency for posterior mammillations. Such loving or mammillations of the envelope have also been described by various authors for species of *Eudorina* (e.g. *E. indica*; IYENGAR, 1933, p. 340, fig. 3). Therefore, in our opinion Goldstein's stand in this respect is quite justified.

Commenting on TIFFANY'S (1935) observations on homothallism and variations, Goldstein expressed the opinion that Tiffany probably was dealing with two distinct varieties of *E. californica*, namely (1) a dioecious form which Goldstein considered to be the typical variety of the species and named *E. californica* v. *californica* and (2) a monoecious form which was considered a new variety and designated as *E. californica* v. *tiffanyi*. The Poona form, therefore, is apparently identical with Goldstein's *E. californica* v. *californica*. It may be pointed out that Goldstein considers *P. sphaerica* Iyengar also as a species of *Eudorina* and tentatively maintains it as a distinct species—*E. sphaerica* (Iyengar) Goldstein though he feels that *E. sphaerica* and *E. californica* might be conspecific.

PASCHER (1927) has stressed that *Pleodorina* is distinct from *Eudorina* in that the cells are not arranged in clear transverse series. He apparently feels that this feature is as important a diagnostic character of the genus, as the fact that the anterior one-third or half of the coenobium consists of small somatic cells.

The original diagnosis of *Pleodorina* Shaw as typified by *P. californica* Shaw gives the cell numbers in a coenobium as normally 128 and occasionally 64. CHATTON (1911) and PAVILLARD (1952) have also said that colonies of *Pleodorina* normally have 128 cells. However, other workers have reported greater variations in cell numbers. TIFFANY (1935) reported 32 cells in his *P. westii* (previously reported as a 32 celled variant of *P. californica* by WEST and WEST, 1905), and 32, more often 64 and 128 cells in *P. californica*. GERISCH (1959) remarks that while normally coenobia of *P. californica* are 64 or 128 celled they can be occasionally 32 celled also.

More recently, GOLDSTEIN (1964) has recorded 32-celled colonies as not infrequent in clonal cultures of his *E. californica*. As reported already, in the Poona form also the cell number varies from 32 to 128, though 64-celled colonies are the most frequent. In Iyengar's *P. sphaerica* the coenobia usually have 128 cells but 64-celled coenobia are not infrequent. (IYENGAR, 1933; IYENGAR & RAMANATHAN, 1957). Thus perhaps it would be more appropriate to consider *Pleodorina* Shaw as possessing coenobia with 32-64-128 cells (see also: SMITH, 1950).

Species of *Pleodorina* (*S. lat.*) have often been cited as illustrations of progressive sterilization. In *P. illinoisensis* the first tier of four cells is often sterile. In *P. indica* (vid SMITH, 1950; BOURRELLY, 1966) the first tier or the first two tiers (4-12 cells) are sterile. In *P. westii* Tiffany the anterior 16 cells are sterile. In *P. californica*, the type species, the number varies. SHAW (1894) in his original report said that 50-62% of the cells in a coenobium were sterile. CHATTON (1911) reported that the ratio of somatic to generative cells was 1:1 in the Banyuls-sur-Mer form which he studied. GERISCH (1959) and GOLDSTEIN (1964) report fluctuation in the number of somatic cells ranging from 30% to nearly 50% of the cells in the Coenobia. Finally, in *P. sphaerica*, the number of somatic cells in a coenobium ranges usually from 66 to 80% or more; Iyengar reports an instance where 61 out of 64 cells (95%) in a coenobium were somatic, a condition very close to that in *Volvox*, a genus which *Pleodorina* resembles in the large number and irregular arrangement of cells in a coenobium.

The writers are inclined to accept PASCHER's (1927) and IYENGAR's (1933) concept of the genus *Pleodorina* and feel that it can be clearly distinguished from *Eudorina* by the following characters: (a) cells not arranged in regular tiers, (b) a fairly large number (30—95%) of the cells in a coenobium small in size and usually non-reproductive, and (c) large biflagellate female gametes which are immobile and are fertilized *in situ*.

Thus circumscribed, the genus would include the following taxa:

1. ***Pleodorina californica*** Shaw, 1894, *Bot. Gaz.* **19**: 279-83, p. 27.  
*Eudorina californica* (Shaw) Goldstein, 1964, *J. Protozool.* **11**: 11.
- 1 a. ***Pleodorina californica*** Shaw var. ***californica***  
*Eudorina californica* (Shaw) Goldstein var. *californica* Goldstein, 1964, *J. Protozool.* **11**: 11, figs. 22, 23, 24 (excl. *Eudorina indica* Iyengar, 1933).
- 1 b. ***Pleodorina californica*** Shaw var. ***tiffanyi***  
*Pleodorina californica* Shaw *sensu* Tiffany, 1935, *Arch. Protistenk.* **85**: 141-2, fig. 4.  
*Eudorina californica* (Shaw) Goldstein var. *tiffanyi* Goldstein, 1964, *J. Protozool.* **11**: 13.
2. ***Pleodorina sphaerica*** Iyengar, 1933, *J. Linn. Soc. Lond.* **49**: 370, text-fig. 4; pl. 28, figs. 4 and 5.  
*Eudorina sphaerica* (Iyengar) Goldstein, 1964, *J. Protozool.* **11**: 13.

GOLDSTEIN (1964) has reduced *Eudorina indica* Iyengar to synonymy with his *E. californica* v. *californica*. *E. indica* shows a distinctly tiered arrangement of cells in contrast to the irregular arrangement accepted as characteristic of the genus *Pleodorina* by PASCHER (1927), IYENGAR (1933) and by us. The figures of *E. indica* given by IYENGAR (1933) and DORAISWAMI (1940) compare well with Goldstein's figures for other species of *Eudorina* (GOLDSTEIN, 1964, figs. 1-5, 9, 13-16, 19-21). In Goldstein's figure 22 (a 32-celled coenobium of *E. californica* cited by him to support his synonymization), there is no such tiered arrangement as also in his figures 23 and 24 of the same species. In our opinion, therefore, *E. indica* Iyengar is a valid species of *Eudorina*.

*Pleodorina westii* Tiffany (1935, p. 142-3, fig. 1) has been synonymized with *E. cali-*

*fornica* (incl. *E. indica* Iyengar) by GOLDSTEIN (1964). Tiffany's figure clearly shows a tiered arrangement of cells in the 32-celled coenobium, the first two tiers consisting of small, somatic cells. In the writers' opinion *P. westii* Tiffany should be considered conspecific with *E. indica* Iyengar.

#### AGKNOWLEDGEMENTS

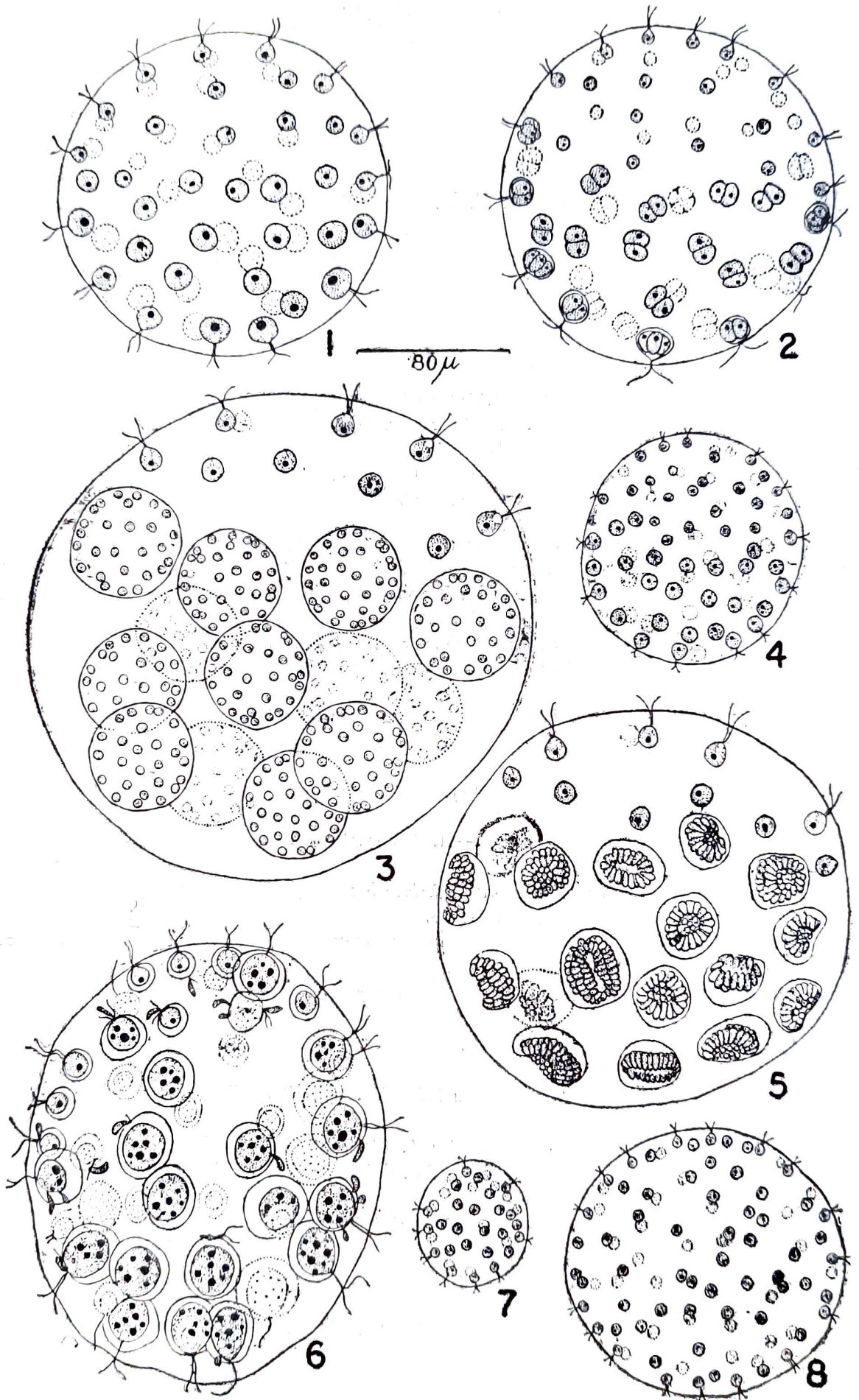
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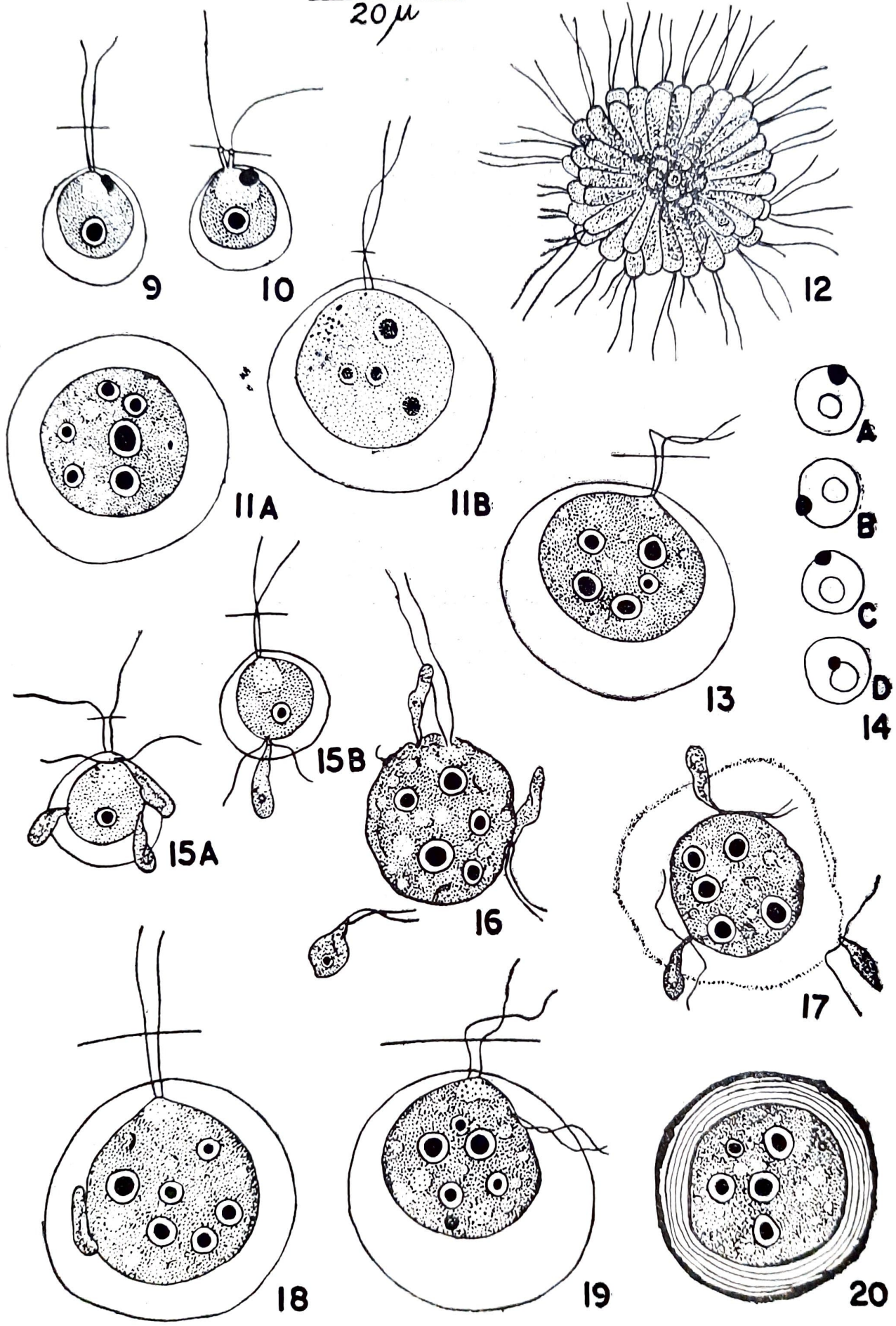
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\*Not seen in the original.





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Text-figs. 1-8. *Pleodorina californica* Shaw.

1. A 64-celled colony showing the distribution of somatic and gonidial cells into distinct anterior and posterior halves.
2. The 2-4 stage of division of the gonidial cells. Note the simultaneous division of all the gonidial cells.
3. Daughter colony formation.
4. A daughter colony in which cell differentiation has started.
5. Male colony showing clusters of antherozoids in various stages of development.
6. Oblique posterior view of a mature female colony showing fertilisation.
7. A newly liberated daughter colony in which differentiation is yet to start and all the cells are alike.
8. A daughter colony which has grown considerably in size and still does not show any cell differentiation.

Text-figs. 9-20. *P. californica* Shaw.

9. Side view of a vegetative cell from the anterior part of the colony showing two flagella and the large eye spot.
10. A vegetative cell stained with eosin showing the two tubular structures in the mucilaginous matrix through which the flagella emerge outside.
- 11A and 11B. Surface and side views respectively of the mature gonidial cells showing the very small eye spots. (in 3A the flagella have not been shown).
12. A cluster of fully developed antherozoids
13. A mature female gamete. ('egg').
- 14 A—D. Four somatic cells, to show the progressive reduction in size of the eye spots.
- 15 A—B. Vegetative cells showing antherozoids attached to them.
- 16, 17. 'Eggs' showing two antherozoids already attached and a third one being attracted towards them.
18. A later stage in fertilization showing an antherozoid laterally adpressed to the 'egg'.
19. An 'egg' immediately after fertilization still showing the two flagella of the antherozoid which has fused with it.
20. A fully ripe zygote.