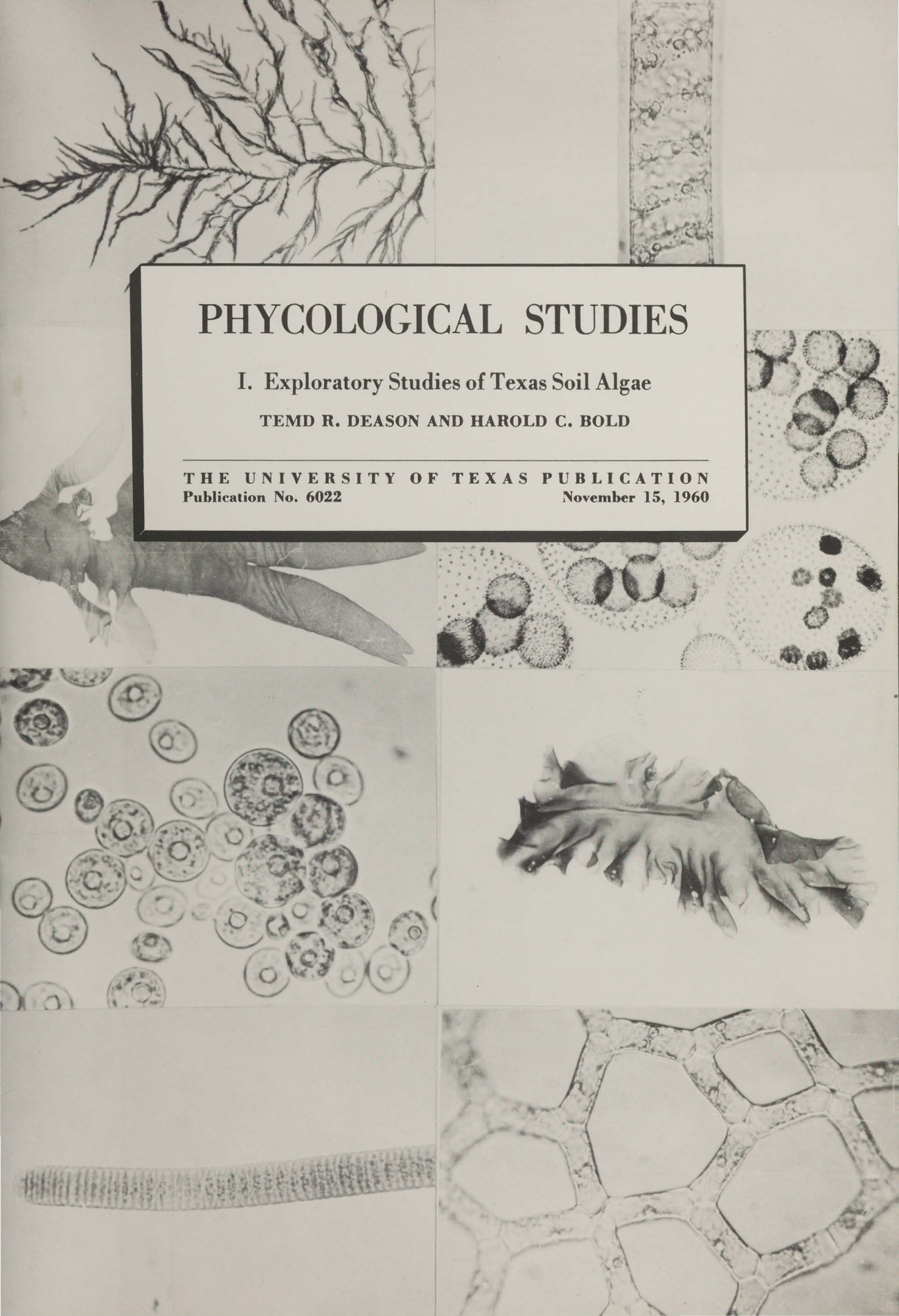

PHYCOLOGICAL STUDIES

I. Exploratory Studies of Texas Soil Algae

TEMD R. DEASON AND HAROLD C. BOLD

THE UNIVERSITY OF TEXAS PUBLICATION
Publication No. 6022 November 15, 1960

Phycological Studies

I. Exploratory Studies of Texas Soil Algae

by

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PUBLISHED BY THE UNIVERSITY TWICE A MONTH. SECOND CLASS POSTAGE
PAID AT AUSTIN, TEXAS

Acknowledgments

The writers are deeply grateful to Professors Walter R. Herndon of the University of Alabama and Richard C. Starr of Indiana University, and to Professors Benjamin C. Tharp, B. L. Turner and Ralph E. Alston of The University of Texas for their helpful criticisms and suggestions. We wish also to thank Dr. Hannah T. Croasdale, Dartmouth College, for preparing the Latin diagnoses. Finally, we gratefully acknowledge financial support from the Publications Fund of the Graduate School of The University of Texas and from the National Science Foundation through Grant No. NSF-G 6373.

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Introduction

The need for prolonged study of unialgal cultures under standard conditions as the prerequisite for elucidating their taxonomy has been emphasized by one of us and co-workers (Bold, 1950; 1958), Trainor and Bold (1953), Starr (1955), Herndon (1958a, 1958b), and Arce and Bold (1958). Starr (1955) established reliable criteria for the delimitation of the unicellular, spherical, zoospore-producing genera of the Chlorococcales. Herndon (1958a) extended these criteria to the vegetatively dividing Chlorosphaerales. It is now possible for a careful observer, using the methods of the above-cited authors and the criteria of Starr (1955), to identify members of the Chlorococcales and Chlorosphaerales to the generic level, even though he might not be familiar with these groups.

The identification of species is considerably more difficult and time-consuming. Initially, the goal of this research was to investigate the soil algal flora of the Carrizo Sands formation of Texas which extends from the northeast corner to the southwestern portion of the state and to compare this flora with that from other localities and soils in Texas. After several months of intensive preliminary work, it became evident that this goal was an unattainable one at this point in the development of soil algal taxonomy, because it was necessary first to develop and standardize techniques to facilitate the identification of large numbers of organisms. More specifically, it has become increasingly apparent that criteria in addition to strictly morphological ones must be used, if possible, to delimit species. Therefore, the development of better techniques and the discovery of additional criteria for the delimitation of species of soil algae and the application of these techniques and criteria to the study and formal description of a number of isolates from Texas soil have been the immediate goals of this investigation. Data obtained by application of these techniques and criteria to continuing studies of the algal flora of Texas will be presented in subsequent reports in this series.

Materials and Methods

The algae described in this paper were isolated from the Carrizo Sands Formation of Caldwell County, Texas, and from samples collected in Williamson County, Texas. A 10-g. portion of each soil sample was placed in a sterile 125-ml. Erlenmeyer flask and enriched with 50 ml. of modified Bristol's solution (Bold, 1949). This medium was used with and without agar for routine cultivation of most of the algae described in this paper. The medium was prepared as follows: six stock solutions 400 ml. in volume were employed, each containing one of the following salts in the concentrations listed:

$NaNO_3$	10.0 g.	K_2HPO_4	3.0 g.
$CaCl_2$	1.0 g.	KH_2PO_4	7.0 g.
$MgSO_4 \cdot 7H_2O$	3.0 g.	NaCl	1.0 g.

To 940 ml. of distilled water were added 10 ml. of each stock solution and 1.0 ml. of each of the four stock, trace-element solutions prepared as follows:

Stock Designation	*Salt*	*Stock Concentration (g./l)*
B	H_3BO_3	11.42
H-Fe	$FeSO_4 \cdot 7H_2O$	4.98
	$ZnSO_4 \cdot 7H_2O$	8.82
	$MnCl_2 \cdot 4H_2O$	1.44
H_5	MoO_3	0.71
	$CuSO_4 \cdot 5H_2O$	1.57
	$Co(NO_3)_2 \cdot 6H_2O$	0.49
EDTA	EDTA	50.0
	KOH	31.0

The flasks were placed under fluorescent illumination of 250 ft.-c. intensity in a culture room which is maintained at a temperature of 22°C. The illumination is controlled by an automatic timing device which regulates the period of illumination (12 hours light, 12 hours darkness). (These conditions of temperature and illumination are hereinafter referred to as "standard conditions.") A phototactic ring of green algae appeared in these enrichment cultures within one to two weeks, and additional algae grew on the surface of the submerged soil.

Isolations were made from the enrichment cultures by the usual methods of streaking, plating, and by single-cell isolations (Bold, 1942; Pringsheim, 1946) using finely drawn-out "Disposable Pasteur Capillary Pipettes" obtainable from Scientific Products, Division of American Hospital Supply Corporation, Evanston, Illinois.

Other media used routinely were: soil-extract agar, soil-water tubes, and proteose peptone agar. Soil-extract agar was prepared by autoclaving a kilogram of soil in a liter of distilled water. Forty ml. of the supernatant and 15 g. of Difco agar were then added to 940 ml. of Bristol's solution to make soil-extract agar. Soil-

water tubes were prepared by placing a pinch of $CaCO_3$ in the bottom of a Pyrex test tube (18 × 150 mm.), then adding ¼ to ½ inch of soil and filling the tube approximately 2/3 full with distilled water. The tubes were steamed for one hour on each of three successive days. Proteose peptone agar was prepared by adding 1.0 g. of proteose peptone and 15 g. of agar to 1.0 liter of Bristol's solution.

Morphology and life cycles of the organisms were studied in fresh mounts from two-week-old cultures grown under standard conditions on Bristol's agar. Observations on fresh mounts were supplemented by the use of preparations with India ink, methylene blue, Sudan IV, and aqueous iodine (I_2-KI). India ink and methylene blue were used to determine the extent of wall layers and matrices. Sudan IV was used to determine the presence of lipids. Aqueous iodine was used as an aid in determining the nuclear condition, characteristics of flagella, and presence or absence of starch and pyrenoids. The Azure-A stain (Delamater, 1951), as modified by Buffaloe (1958), and the aceto-carmine technique were used to supplement observations, made on living material, of the nuclear conditions. The procedure employed with the Azure-A stain was as follows: cells were affixed to clean glass slides by means of egg albumen and placed in Carnoy's solution (acetic acid-absolute alcohol 1:3 v/v). After the slides had remained in the fixative for at least 30 minutes, they were washed in distilled water and immersed in 1 N HCl at 60°C. for from four to ten minutes. The slides were then placed directly in a 0.25% aqueous Azure-A solution, to which one drop of thionyl chloride had been added (just before use) for each 10 ml. of dye. After approximately two hours, excess stain was removed from the slides by two rapid rinses in distilled water, excess water was wiped off, and the slides were quickly placed in absolute ethyl alcohol. The Coplin jar containing the slides was then stored in the freezing compartment of a refrigerator for 12 hours in order to effect dehydration. The slides were then passed through a mixture of equal parts of absolute alcohol and xylol, then pure xylol, after which they were mounted in Permount and covered.

The fixative for the aceto-carmine stain was that of Johansen (1940), as modified by Cave and Pocock (1956), and further modified by Bischoff (1959). The modified fixative was prepared as follows:

Iodine	0.25 g.	Formalin	24.0 ml.
Potassium iodide	1.0 g.	Distilled water	400.0 ml.
Acetic acid, Glacial	4.0 ml.		

Cells were affixed to the slides as previously described and immersed in the fixative for two to four hours. Then the excess fixative was drained off and two drops of aceto-carmine, prepared by the method described by Cave and Pocock (1951), were placed on the fixed material. After the addition of a coverslip, the slide was heated over a low flame until vapor arose from the stain. Observations were made immediately and no permanent aceto-carmine slides were prepared.

The above-described techniques, with many variations, have now been estab-

lished as fundamental and necessary ones in any taxonomic study of soil algae. The following techniques, although not original with the writers, have been applied to these organisms with the purpose of providing more information on the biology of soil algae and perhaps providing additional reliable taxonomic criteria, particularly for the delimitation of species. These techniques include a study of colony morphology (macroscopic and under low magnification), color changes on Bristol's agar upon aging, growth in nutrient broth, sensitivity to antibiotics, growth in media containing various nitrogen and carbon sources, and growth factors including vitamins and amino acids.

In order to study the differences in gross colony morphology which might be of taxonomic significance, a loopful of cells from a liquid suspension was streaked in circular fashion on solidified Bristol's agar in small (60 × 15 mm.) Petri dishes. After two weeks' growth under standard conditions, the colony characteristics including color and topography were observed and recorded. Observations were made both macroscopically and with the low power (20.0 X) magnification of a Bausch and Lomb stereoscopic binocular microscope using reflected light. Photomicrographs of colony characteristics were made at a magnification of 17.5 X. Terms used to describe colony characteristics are rough or smooth, and dry, dull-shiny, or shiny.

Bacteria-free cultures on Bristol's agar slants were maintained in triplicate Pyrex test tubes, 18 × 150 mm., plugged with cotton, and/or Bakelite-capped tubes. Observations were made on these stock cultures at two-week, one-month, and two-month intervals to determine color changes associated with the age of the cultures.

Inoculations from stock cultures were made into Difco nutrient broth tubes to assure that they remained bacteria-free, and to test the ability of the algae to grow in this medium. Observations to determine bacterial contamination were made after 48 hours, and comparative observations of algal growth in nutrient broth were made after two weeks' growth under standard conditions. The previously mentioned conditions of temperature and light were maintained. It was found that this method alone of revealing bacterial contamination was not satisfactory. In addition, it was necessary to examine the cultures microscopically, and if doubt arose as to the purity of the cultures, nutrient agar plates were streaked with a loop of cells from the culture concerned. Some contaminating bacteria which did not appear in nutrient broth tubes grew abundantly on Bacto nutrient agar plates.

High concentration Difco Bacto-Unidisks (Fig. 132) were placed aseptically on the surface of Bristol's agar "pour" plates previously inoculated with a heavy suspension of the test organisms. After two or three weeks, the plates were examined to determine the sensitivity of the organisms to antibiotics. The antibiotic Unidisks had been impregnated with the following antibiotics in the concentrations listed:

Aureomycin	30 mcg.	Terramycin	30 mcg.
Penicillin	10 units	Erythromycin	15 mcg.
Chloromycetin	30 mcg.	Tetracycline	30 mcg.
Polymyxin B	30 mcg.	Di'streptomycin	100 mcg.

This procedure is hereinafter referred to as the Unidisk test.

Experiments to determine growth-stimulation effects, growth-factor requirements, and/or inhibition, were carried out with bacteria-free cultures, reagent grade chemicals (except where indicated), and sulfuric acid-washed Pyrex glassware. Each organism tested was grown in Bristol's solution (entirely inorganic) prior to inoculation into other media. Test media were the following:

B-1 Bristol's solution (control).
B-2 An equivalent molarity of nitrogen in the form of ammonium chloride was substituted for sodium nitrate in Bristol's solution.
B-3 An equivalent amount of nitrogen in the form of ammonium nitrate was substituted for sodium nitrate in Bristol's solution.
B-4 0.5% sodium acetate in Bristol's solution.
B-5 0.5% maltose in Bristol's solution.
B-6 0.5% glucose in Bristol's solution.
B-7 0.5% arabinose in Bristol's solution.
B-9 An equivalent percentage of urea was substituted for the sodium nitrate in Bristol's solution.
B-10 0.5% xylose in Bristol's solution.
B-11 10.0 mg. vitamin-free Casamino acids/l. in Bristol's solution.
B-12 Two drops of the following sterile vitamin stock solution in 50 ml. of sterile Bristol's solution: 10 μg/l. thiamine, 10 μg/l. pantothenic acid, 0.1 μg/l. biotin, 0.012 μg/l. cyanacobalamin.
B-12a 0.2% asparagine to replace $NaNO_3$ in Bristol's solution.

Media B-2, B-3 and B-12 were not differentiating in effect on the algae tested in this investigation and will not be referred to again.

The bacteria-free algae were inoculated into 50 ml. of sterile medium in triplicate 125-ml. Erlenmeyer flasks by means of sterile pipettes. As uniform as possible inoculum was taken from a suspension of cells growing in Bristol's solution. Duplicate sets of B-6 flasks were inoculated. One of these sets was placed with the other media under the standard conditions of light and temperature. The other was stored in darkness at approximately the same temperature. After two or three weeks, estimates were made of the amount of growth in all flasks. This was described by the adjectives excellent, good, fair, trace, or none.

Drawings were made with the aid of a Zeiss drawing device and their magnifications are given in the legends. Photomicrographs were taken with a 35-mm. Zeiss Ikonta camera. Final magnifications of photomicrographs are also indicated in the accompanying legends.

Observations

A. CHLOROPHYCEAE

Seven isolates of *Chlamydomonas*, order Volvocales, family Chlamydomonadaceae, have been investigated.

CHLAMYDOMONAS actinochloris sp. nov. (Figs. 1–3).

Cellulae 11–20 μ long., 4–9 μ lat., in "agare" cultie non cohaerentes. Papilla nulla, membrana tenuis, protoplastam adpressa. Chloroplastis solida, radiatim incisa, uno pyrenoideo centrali perspicuo atque stigmate minuto anteriore praedita. Vacuolae contractiles duae; nucleus anterior. Flagella aeque longa ac longitudo cellulae vel paulo longiora.

Reproductio per bipartitiones endogenas cellularum immobilium ad cellulas-filias (plerumque) quatuor formandas. Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (T-1-2-2-A) seiunctae e solo in querceto in latere orientali Viae "95" admodum versus septentrionem a fine meridionali loci Williamson County, Texas dicti, 31 m. Oct., an 1957; necnon in culturam puram (C-2-14) sieuncta e solo sub *Quercus marilandica,* in formatione Carrizo Sands nomine, distante 5.1 milia passum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

It has been an unusual experience, up to the present, for investigators of soil algae to isolate the same organism from different soil samples. However, *C. actinochloris* was isolated both from Caldwell County (C-2-14) and Williamson County (T-1-2-2-A) soils. Cells of this alga are ellipsoidal to subglobose, and are characterized by a massive, radiating chloroplast with a single central, axial pyrenoid (Figs. 1–3). Mature cells are 18 to 20 μ long and 7 to 9 μ in width. The flagella approximate cell length or slightly exceed it. The small stigma is anterior as are the clearly visible nucleus and the two contractile vacuoles. The cell wall is thin and closely adpressed to the protoplast. A papilla is absent. The cells usually lack flagella on agar, except in freshly transferred cultures. No matrices (Fig. 75) are demonstrable with India ink or methylene blue. Flagella develop within one hour when cells from two-week-old Bristol's agar cultures are transferred to liquid media.

Reproduction is accomplished by repeated endogenous bipartitions (Fig. 3) to form (usually) 8 daughter cells which are liberated soon after the cessation of division in liquid media but which behave as aplanospores on agar media. Sexual reproduction has not been observed.

Colonies on Bristol's agar are smooth and shiny. Cells from two-month-old cultures on Bristol's agar remain green and the cell walls are thickened in a unipolar or bipolar fashion. No measurable growth occurs in nutrient broth,[1] and the alga is inhibited by all antibiotics tested except penicillin and chloromycetin. Growth is excellent in Bristol's solution (B-1) and B-1 supplemented with glucose (B-6). In

[1] Isolate C-2-14 differs from T-1-2-2-A in that the former does not grow in nutrient broth.

the following pages, Bristol's liquid supplemented with glucose will be referred to simply as glucose, Bristol's solution supplemented with acetate will be referred to as acetate, etc. Growth is good in acetate, maltose, and xylose; fair in arabinose; trace in glucose in darkness; and no growth occurs in acetate in darkness.

Study of the organism here called *C. actinochloris* at first suggested such species as *C. stellata* Dill, *C. rotula* Playfair, *C. sectilis* Korchikoff, and *C. augustae* Skuja. It differs from all of these, however, in lacking a papilla, in size, and in other respects. It also differs from *C. radiata* Deason and Bold (Figs. 4, 5), a description of which follows, in that the nucleus of *C. radiata* is posterior. No sexual fusions were noted when *C. actinochloris* and *C. radiata* were grown together.

In the writers' opinion, *C. rotula* was erroneously placed in the subgenus Euchlamydomonas by Pascher (1927). Because it possesses a single central pyrenoid embedded in a "Chromatophor (im Prinzipe) H-förmig" rather than in a "Chromatophor im Prinzipe topfförmig," it belongs in the subgenus Aglöe, as do *C. stellata*, *C. radiata*, and *C. actinochloris*.

CHLAMYDOMONAS radiata sp. nov. (Figs. 4, 5; 76, 132).

Cellulae ellipsoideae, utroque polo late rotundato, ad ovatas. Plastis asteroidea, segmentis a parte pyrenoidei centralis radiantibus. Cellula duas vacuolae contractiles anteriores, stigma anterius, nucleum posteriorem, duo flagella quasi aequa longitudini cellulae habens. Papilla atque matrix nullae. Cellulae 12–21.0 μ long., 7.5–13.0 μ lat.

Reproductio per divisionem cellularum immobilium ad 2–16 partes, raro plures, effecta.

Origo: Cellulae in culturam puram (C-1-10) seiunctae ex exemplo soli sub *Quercum stellatam,* in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This alga was isolated into bacteria-free culture (C-1-10) from a soil sample from the Carrizo Sands formation 5.1 miles east of McMahan, Caldwell County, Texas. The cells are ellipsoidal to ovoid; when ellipsoidal, both poles are broadly rounded. The plastid is asteroidal with the segments radiating from the region of the central pyrenoid. There are two anterior contractile vacuoles, an anterior stigma, a posterior nucleus, and two flagella of approximately cell length. No papilla is present and the alga is never embedded in a matrix. Cell length ranges from 13 to 21 μ and width from 7 to 13 μ. The alga remains green for two months on Bristol's agar slants and only slight thickening of the wall layers occurs. Two-week-old colonies on Bristol's agar are dull-shiny and have a smooth surface.

Asexual reproduction (Fig. 76) is by division of non-motile cells to form 2–16 products, rarely more. Sexual reproduction has not been observed.

Only trace amounts of growth of the alga are detectable in nutrient broth and it is distinctly inhibited by polymyxin B. It is slightly inhibited by aureomycin, ter-

ramycin, erythromycin, and tetracycline (Fig. 132). Chloromycetin, penicillin, and dihydrostreptomycin do not appear to have any inhibitory effect on *C. radiata.* Growth is excellent in maltose, glucose, and xylose. Growth is good in acetate and fair in arabinose. Only trace amounts of growth occur in glucose in darkness; no growth occurs in acetate in darkness, or in urea.

CHLAMYDOMONAS akinetos sp. nov. (Figs. 6, 7; 77–79).

Cellulae iuvenes anguste ellipsoideae, postice aliquanto latiores et saepe asymmetricales; cellulae vetustiores ovatae aut subsphericae. Cellulae vegetativae 12–27 μ long., 4.5–23 μ lat., membranis incrassatis, akineta saepe factae. Cellula chloroplastidem poculiformem, fissura longitudinali atque pyrenoideo equatoriali lateralique praeditam, atque flagella quasi aequa longitudini cellulae, atque stigma atque duas vacuolas contractiles anteriores atque nucleum posteriorem habens. Papilla matricesque nullae. Partes divisionis modo liberatae c. 12 μ long., akineta matura usque ad 27 μ long.

Reproductio asexualis per divisiones ad 2–32 vel plures partes formandas effecta. Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C-1-11) seiunctae ex exemple soli sub *Quercum stellatam,* in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

The young cells (Fig. 77) of *Chlamydomonas akinetos* sp. nov., also isolated from the Carrizo Sands, are narrowly ellipsoidal, somewhat broader posteriorly and often asymmetrical (Fig. 6). Older cells are ovoid or subspherical (Fig. 7). Vegetative cells often thicken their walls, develop a large central vacuole, and are transformed into akinetes (Fig. 78). The plastid is parietal, often with a longitudinal fissure, and has embedded within it an equatorial and lateral pyrenoid (Figs. 6, 77). This *Chlamydomonas* thus belongs in the sub-genus Chlamydella, section Monopleura. Motile cells possess two flagella of approximately cell length and a small anterior stigma. They also have, like the non-motile cells, two anterior contractile vacuoles (present in all but the largest cells) and a posterior nucleus. No papilla is present and no matrix is secreted. Cells range in length from 12 μ (newly released division products) to approximately 27 μ (mature akinetes). The range in width is from 4.5 μ to 23.0 μ. Two-month-old cultures are yellow-green and the cell wall layers thicken to 1.5 μ. The alga forms dry, smooth colonies on Bristol's agar after two weeks' growth under standard conditions.

Asexual reproduction occurs when cells become non-motile and divide to form 2 to 32 or more products (Fig. 78). Sexual reproduction has not been observed.

With respect to supplementary attributes, only trace amounts of growth can be observed in nutrient broth, and the alga is inhibited by all the antibiotics used except aureomycin, penicillin, and chloromycetin. Growth is excellent in B-1 and

maltose; fair in acetate and glucose; occurs in trace amounts in acetate in darkness, glucose in darkness, and arabinose. No growth occurs in urea or xylose.

Cells of young cultures of *C. akinetos* are somewhat similar to *C. kniepii* Moewus (1931) and *C. parvula* Gerloff (1940). However, the asymmetrical cells of *C. akinetos* are much more narrowly ellipsoidal than those of *C. kniepii* and do not form a *Palmella* stage, which cells of *C. parvula* are reported to do. Mature cells of *C. akinetos* are considerably larger than either of the other above-mentioned species.

CHLAMYDOMONAS pyrenoidosa sp. nov. (Figs. 8, 9; 80, 132).

Cellulae ellipsoideae, saepe, autem, asymmetricae, 16–27 μ long., 6–23 μ lat. Chloroplastis poculiformis, fissuras interdum habens, 1–5 pyrenoidea lateralia continens. Flagella divergentia, a dimidio usque ad totam longitudinem cellulae attigentia. Cellula duas vacuolas contractiles anteriores, stigma parvum anterius atque nucleum posteriorem habens. Papilla adest. Matrices nullae. Partes divisionis duae ad octo, raro plures.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C-2-4) seiunctae ex exemplo soli sub *Quercum marilandicum*, in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This alga was isolated into bacteria-free culture (C-2-4) from a soil sample collected from under *Quercus marilandica* from the Carrizo Sands formation 5.1 miles east of McMahan, Caldwell County, Texas. It is ellipsoidal but often asymmetrical. The cells have cup-shaped plastids with occasional dissections or fissures, and one to five lateral pyrenoids. It, therefore, belongs in the sub-genus Pleiochloris. Each motile cell has two anterior contractile vacuoles, a small anterior stigma, two divergent flagella which vary in length from half the length of a cell to the whole length, and a posterior nucleus (Figs. 8, 9). The papilla is broadly rounded in one aspect and sharply pointed in the other. There are two to eight division products (Fig. 80), rarely more, and cell length reaches 27 μ; cell width may attain 23 μ. No matrices are formed. Colonies two weeks old on Bristol's agar are smooth and are dull-shiny. Two-month-old cultures retain their green color and only slight thickening of the cell wall occurs. This alga does not resemble closely any other described species of *Chlamydomonas*.

Sexual reproduction was not observed.

Chlamydomonas pyrenoidosa does not grow in nutrient broth and is inhibited by all antibiotics tested except chloromycetin and penicillin (Fig. 132). Growth is good in B-1, and only fair in maltose, glucose, and xylose. Trace amounts of growth appeared in arabinose, and no growth occurred in acetate, glucose in darkness, or in urea.

CHLAMYDOMONAS aggregata sp. nov. (Figs. 10–13; 81, 82, 132).

Cellulae 5.6–10 μ long., 3–4 μ lat., ovatae cylindricaeve. Papilla nulla, membrana tenuis, flagella duo quasi aeque longa ac longitudo corporis. Chloroplastis parietalis cingulum imperfectum uno pyrenoideo equatoriali-anteriore atque stigmate anteriore praeditum formans. Duae vacuolae contractiles anteriores; nucleus semper posterior.

Cellulae in "Bristol's agar" atque in aliquot medeis liquidis strata in quibus cellulae immobiles endogene in 4 vel 8 cellulas-filias dividunt formantes; matrix communis nulla, i.e., cellulae non vere palmelloideae, periodus dum movent brevis. Cellulae homothallicae, zygotae magnitudine ad 16 μ diam. crescentes, membranis levibus.

Origo: Cellulae in culturam puram (T-1-12) e solo in querceto aperto seiunctae, in latere orientali Viae "95" admodum versus septentrionem a fine meridionali loci Williamson County, Texas dicti, m. Dec., an. 1957.

This organism was isolated into pure culture (T-1-12) from the soil sample which contained *Chlorococcum ellipsoideum* and *C. scabellum* (to be described below). It clearly belongs to the subgenus Chlamydella of *Chlamydomonas* and to the section Chlorogoniella. The cells of *C. aggregata* are small, usually about 5–10 μ long $\times$ 3–4 μ wide. The cells are elongate-cylindrical or ovoidal, with a delicate cell wall. The flagella are approximately the same length as the cell body and usually directed anteriorly when at rest. Each cell contains a parietal chloroplast which only partially covers the cell surface (Figs. 10–12). The single pyrenoid is equatorial or slightly anterior and the stigma is anterior. The nucleus is constantly posterior. A papilla is absent.

On Bristol's agar, in two-week-old cultures, the cells are always in masses in which the component cells contain groups of four or eight daughter cells (Fig. 81). Staining with methylene blue did not demonstrate a common matrix. The parent cell walls are coherent on agar media, but the individual clusters readily separate when mounted in liquid media. In Bristol's liquid and in soil-water supernatant, motility continues for some time, but on agar, the non-motile condition prevails. Colonies on Bristol's agar are shiny and rough. Two-month-old cultures remain green, and the cells do not thicken their walls appreciably.

Chlamydomonas aggregata is homothallic. When the cultures were first isolated, zygotes in all stages of development (Figs. 13, 82) were present. These underwent enlargement before the beginning of the dormant period. The walls of mature zygotes were not ornamented and the details of the protoplast were obscured by oil and starch. It has not been possible to evoke the sexual stage of this organism in subsequent cultures, but the sexual phase is described for the record.

Chlamydomonas aggregata is the only species of *Chlamydomonas* tested which grows fairly well in nutrient broth. The alga is inhibited by all the antibiotics of the Unidisk test except penicillin and chloromycetin (Fig. 132). Growth is excellent in B-1, maltose, glucose, arabinose, urea, and xylose. Growth is good in acetate,

occurs in trace amounts in acetate in darkness, and does not occur in glucose in darkness.

Search of the literature (Pascher, 1927; Gerloff, 1940; and Ettl, 1959; and Ettl and Ettl, 1959, among other sources) has failed to reveal an organism identical with *C. aggregata* which, accordingly, has been described as a new taxon. As noted above, its affinities are clearly in the subgenus Chlamydella, section Chlorogoniella. Use of the keys in Pascher's and Gerloff's works leads one to *C. microscopica* G. S. West, to *C. inflexa* and *C. minuta* of Pringsheim (1930) and to *C. mucicola* Schmidle. The present organism differs from each of these in several attributes.

CHLAMYDOMONAS appendiculata sp. nov. (Figs. 14–17; 83–85; 136).

Cellulae 7.5–12 μ long. $\times$ 3–9 μ lat., maiores cellulae immobiles, *Chlorococco* similes. Cellulae in "Bristol's agar" cultae palmelloideae, in matrice communi inclusae; cellulae singulae in culturis vetustioribus, coria exteriora stratifacta asymmetrice incrassata saepe tubiformia habentes. Chloroplastis cellularum e culturis laete crescentibus cingulum imperfectum, pyrenoideo medio atque stigmate anteriore praeditum, formans. Nucleus posterior, duae vacuolae contractiles anteriores Membrana in cellulis mobilibus delicata, postea incrassata facta.

Reproductio per bipartitionem endogenam in octo (plerumque) vel plures cellulas-filias effecta. Cellulae-filiae si liberatae mobilissimae, aut velut aplanosporae sine flagellis retentae.

Reproductio sexualis non observata.

Origo: Cellulae in cultura sine bacteriis (T-2-5) seiunctae, e solo horti in praedio Martinii, distante 2.5 milia passuum versus septentrionem a fine loci Williamson County, Texas dicti, ad Viam "95," an. 1957.

This organism was isolated into bacteria-free culture (T-2-5) from soil from a vegetable garden at the Martin farm, 2.5 miles north of the Williamson County line on the west side of State Highway 95. Cells in Bristol's agar cultures are embedded in a watery, gelatinous common matrix (Fig. 83) which stains readily with methylene blue. In cultures on this medium a month or more old, as cell division ceases, the individual cells develop stratified thickenings of the outer wall layer (Figs. 14, 85), which sometimes are unipolar and suggestive of those of *Hormotilopsis* (Trainor and Bold, 1953; Arce and Bold, 1958). Most cells of two-week-old and older cultures on Bristol's agar are without flagella, but these develop when the cells are mounted in liquid (Fig. 15). Actively motile cells are 7.5–12 μ in length and 3–9 μ in width. They are ovoid-ellipsoidal and often rather truncate anteriorly. The cell wall is delicate and closely adpressed to the protoplast in motile cells. The chloroplast is a parietal, incomplete girdle with a single median pyrenoid and anterior stigma. The nucleus is posterior and the two contractile vacuoles anterior; a papilla is absent. Colonies are shiny and smooth (Fig. 136). Two-month-old cultures remain green and the cells do not thicken their wall layers.

Motility is of short duration even in liquid media. As the cells settle, they increase

in size, their outer wall layers thicken and the cells become *Chlorococcum*-like (Figs. 16, 84). Such cells undergo endogenous bipartitions to form 8 (usually) or more daughter cells (Figs. 17, 84). Sexual reproduction has not been observed.

This alga, unlike *C. aggregata*, does not grow in nutrient broth. It is seemingly inhibited by all the antibiotics except chloromycetin. However, it is possible that penicillin does not inhibit the alga but that the inhibitory effects of neighboring discs extended into the penicillin zone. Growth is excellent in B-1, maltose and glucose; good in urea; fair in acetate; occurs in trace amounts in acetate in darkness, and no growth occurs in glucose in darkness, in arabinose or in xylose.

The present organism clearly belongs to the subgenus Chlamydella, section Chlorogoniella of *Chlamydomonas* because of its girdle-like chloroplast and lateral pyrenoid. Its affinities are with the non-papillate species, but it differs from those described in the literature in its characteristically aggregated organization, in the posterior position of its nucleus and in the asymmetric thickening of the outer wall layer. Accordingly, it has been described as a new taxon.

CHLAMYDOMONAS typica sp. nov. (Figs. 18, 19; 86–89).

Cellulae 9–18 μ long., 8–12 μ lat., multae minores, ovato-ellipsoideae. Papilla nulla, membrana perspicua. Duo flagella quasi aeque longa ac corpus cellulae. Coria exteriora cellularum in "Bristol's agar" culturam spissescentia, cellulis, autem, non cohaerentibus, i.e., matrice communi nulla, non palmelloideis. Chloroplastis urceolata, parte basali crassa, aequatorem cellulae saepe attingens, pyrenoideo posteriore axiali praedita. Cellula duas vacuolas contractiles atque stigma medium ad paululum anterius habens.

Reproductio per bipartitiones ad cellulas-filias quatuuor (plerumque) vel plures formandas. Periodus dum movent in media liquida prorogatus.

Origo: Cellulae in culturis sine bacteriis (T-2-11) seiunctae, e solo horti in praedio Martinii, distante 2.5 milia passuum versus septentrionem a fine loci Williamson County, Texas dicti, ad Viam "95," an. 1957.

This alga was isolated into pure culture (T-2-11) from the same soil sample as *C. appendiculata*. It grows well on Bristol's agar, but, except in freshly transferred cultures, the cells are without flagella. Mature vegetative cells (Figs. 18, 86) are 17 μ in length and 12 μ in width. Clearly a member of the subgenus Euchlamydomonas, *C. typica* has a vase-like chloroplast with a massive basal thickening containing a pyrenoid. The basal portion of the plastid reaches almost to the equator of the cell. In optical section (Fig. 18), a single nucleus is apparent in the colorless cytoplasm as are two anterior contractile vacuoles. The stigma is clearly visible and its position is slightly anterior to equatorial. The two flagella are equal in length to the cell body. The cell wall is of moderate thickness (Fig. 18), and a papilla is lacking. A distinct matrix is present (Figs. 87–89).

Endogenous bipartitions of non-motile cells occur to form four (usually) daugh-

ter cells (Figs. 19, 86). The division products, after liberation, gradually increase to the size characteristic of the species.

Colonies on Bristol's agar are shiny and smooth. Cells of cultures two months old remain grass green and thicken their walls to 3 μ. The alga does not grow in nutrient broth and is inhibited by all of the antibiotics tested except chloromycetin. Growth of *C. typica* is excellent in B-1, glucose, arabinose, urea, and xylose. No growth occurred in acetate (in light or in darkness) or in glucose in darkness. It is of interest that agar upon which this alga has been growing becomes lavender.

Sexual reproduction has not been observed.

Use of the keys in Pascher (1927) and Gerloff (1940) leads one to *C. lismorensis* Playfair, an organism not available for comparison in living cultures, but one from which the present organism is clearly distinct, having a thicker cell wall and typically deeper basal portion to the chloroplastid and a pyrenoid which is present in every vegetative cell. Furthermore, *C. typica* is rounded at the anterior pole. Inasmuch as the present isolate could not be clearly identified as an already described species, it is here described as a new taxon.

Although no considerable difficulties are usually encountered, in distinguishing species of *Chlamydomonas* (in culture) on a morphological basis, it is interesting to note that these species also may be readily distinguished on the basis of physiological criteria and cultural characteristics. The real value of these criteria probably will be realized by comparative study of genera related to *Chlamydomonas*. An example of physiological differences between morphologically similar species is the case of *C. actinochloris* and *C. radiata*. The colonies of *C. actinochloris* are shiny, while those of *C. radiata* produce only a dull shine (almost dry). *Chlamydomonas actinochloris* is inhibited by dihydrostreptomycin, while *C. radiata* is not. Furthermore, *C. actinochloris*, in contrast to *C. radiata*, grows fairly well in urea.

Table 1 summarizes the results of growing several species of *Chlamydomonas* in the differential media series. These data indicate that species of *Chlamydomonas* which are relatively more distinct from each other morphologically differ to a greater extent in physiological attributes than those more closely related on morphological evidence. Among the species tested, acetate, arabinose, urea, and xylose proved to be the best media for distinguishing species in the genus *Chlamydomonas*. Growth in nutrient broth was uniformly poor, except in the case of *C. aggregata* which also differed from the other species in producing rough colonies on Bristol's agar.

A number of chlorococcalean algae also occurred in the soil samples studied. Discussion of these follows.

CHLOROCOCCUM ellipsoideum sp. nov. (Figs. 20–25; 90–92).

Cellulae iuvenes ovatae ad ellipsoideas, sphericae nisi muto compressae dum maturuerunt; multa zoosporangia culturis 2-septimararum aetate in "Bristol's agar" cultis propria; zoosporangia in culturis vetustioribus per aplanosporangia

TABLE 1

Comparative Growth of Some Species of *Chlamydomonas* in Various Media

	B-1	Acetate (light)	Acetate (dark)	Maltose	Glucose (light)
C. actinochloris	Excel.*	Good	None	Good	Excel.
C. radiata	Excel.	Good	None	Excel.	Excel.
C. akinetos	Excel.	Fair	Trace	Excel.	Fair
C. pyrenoidosa	Good	None	None	Fair	Fair
C. aggregata	Excel.	Good	Trace	Excel.	Excel.
C. appendiculata	Excel.	Fair	Trace	Excel.	Excel.
C. typica	Excel.	None	None	Excel.	Excel.

	Glucose (dark)	Arabinose	Urea	Xylose
C. actinochloris	Trace	Fair	Fair	Good
C. radiata	Trace	Fair	None	Excel.
C. akinetos	Trace	Trace	None	None
C. pyrenoidosa	None	Trace	None	Fair
C. aggregata	None	Excel.	Excel.	Excel.
C. appendiculata	None	None	Good	None
C. typica	None	Excel.	Excel.	Excel.

* Excellent.

gradatim substituta. Zoosporae biflagellatae (2–32 in unoquoque sporangio) per bipartitionem successivam enascentes, 7–9 × 2.5–3 μ, duas vacuolas contractiles, unum pyrenoideum, nucleum posteriorem atque stigma anterius habentes. Cellulae in culturis 2 vel plurium mensium aetate in "Bristol's agar" oleum luteum copiosum praebentes, corium membranae exterius stratifactum atque usque ad 5 μ incrassatum.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (T-1-3) e solo in querceto aperto seiunctae, in latere orientali Viae "95" admodum versus septentrionem a fine meridionali loci Williamson County, Texas dicti, m. Oct., an. 1957.

The genus *Chlorococcum* is characterized by the following attributes: cells with a single, parietal chromatophore with one or more pyrenoids and zoospores which do not become spherical immediately after the motile period.

An organism somewhat like *Chlorococcum minutum* Starr was isolated into pure culture (T-1-3) from a sample of sandy soil in an open oak wood on the east side of Highway 95 just north of the southern boundary of Williamson County. The sample was collected on October 31, 1957. Careful and prolonged study of two isolates of this organism with *C. minutum* have compelled the writers to describe the isolates as a new taxon, *C. ellipsoideum*.

In Bristol's agar cultures two weeks old, *C. ellipsoideum*, like *C. minutum*, forms shiny colonies which are quite smooth. On slants of Bristol's agar, the alga changes from green to yellow-orange, in two-month-old and older cultures. The outer wall layer of the cells from such cultures may become as great as 5 μ in thickness (Figs. 21, 92) and the protoplast becomes obscured by rather large droplets of orange-yellow oil (as revealed by Sudan IV). The thickened outer wall layer is concentrically stratified.

Cells from two-week-old cultures on Bristol's agar, grown under standard conditions, present a different appearance (Figs. 20, 22; 90, 91). As is typical of the genus *Chlorococcum*, such cultures contain cells varying greatly in size (up to 26 μ in diameter), depending on their age; i.e., there is always marked lack of synchrony of development. The ellipsoidal shape, characteristic of growing vegetative cells, is maintained for an unusually long time in this species so that only fully mature cells, no longer increasing in size, are spherical (Fig. 91). These are covered by a delicate cellulose wall and have a prominent outer wall layer. It is the latter which is greatly augmented in cultures in the stationary phase of growth (Fig. 92). A hollow, parietal chloroplastid with a single pyrenoid is present in the cells, as is a single nucleus in the colorless cytoplasm, within the chloroplast.

As compared with two-week-old cultures of other species of *Chlorococcum*, a striking attribute of *C. ellipsoideum* is the abundance of zoosporangial cells (Fig. 90) which contain 2–4–8–16 or 32 zoospores, 8 and 16 being the usual number. The abundance of zoosporangia also especially characterizes *C. scabellum* Deason and Bold (to be described below) and *C. perforatum* Arce and Bold. After the liberation of zoospores, the empty zoosporangial walls are long persistent (Fig. 90) in bacteria-free cultures of *C. ellipsoideum*. When thick-walled cells form zoospores, the outer wall layer enlarges markedly and persists long after zoospore discharge. Discharge of zoospores within a vesicle has not been observed.

The zoospores themselves (Fig. 23) apparently are walled, 7–9 $\mu \times$ 2.5–3.0 μ, with two anterior contractile vacuoles, an anterior stigma and posterior nucleus. As is typical of *Chlorococcum*, their motile period continues for some time. Upon becoming quiescent, the zoospores remain ellipsoidal and gradually grow into vegetative cells.

In cultures on Bristol's agar, more than two weeks of age, aplanospore production (Fig. 25) supplants zoospore formation. Aplanospores, like zoospores, are produced in multiples of two. Sexual reproduction was not observed, even after mixing clonal cultures.

The following supplementary attributes are recorded herewith for *C. ellipsoideum*. Growth in two-week-old nutrient broth cultures is excellent, as it is also in Bristol's solution without agar; excellent growth also occurs in glucose, arabinose, and urea. Good growth occurs in acetate, but only fair growth occurs in asparagine. Finally, growth of *C. ellipsoideum* is markedly inhibited by dihydrostreptomycin and slightly by aureomycin in the Unidisk test.

Comparison of *Chlorococcum ellipsoideum* with other known species of *Chlorococcum* in living cultures indicates its affinity with *C. minutum* Starr, as noted above. This is suggested by such similar attributes as cell size, abundance of free-swimming zoospores in the cultures, zoospore morphology and similarities with respect to supplementary attributes such as colony characteristics, growth in nutrient broth and in the series of differential media. However, a number of important dissimilarities also are revealed by comparison, as follows. Isogamous sexual reproduction, so prevalent and readily observable in cultures of *C. minutum*, is absent in *C. ellipsoideum*. Furthermore, formation and release of zoospores (gametes) in *C. minutum* are clearly immediately sequential, as evidenced by the rarity with which one encounters zoosporangia. In *C. ellipsoideum*, in contrast, there is obviously an interval between zoospore formation and their liberation, as evidenced by the characteristically abundant occurrence of zoosporangia.

However, the most convincing evidence that *C. ellipsoideum* and *C. minutum* are different taxa is provided by comparative observation of cells from two-month-old Bristol's agar-slant cultures and even older cultures (up to six months old) (Fig. 92). Cells of *C. minutum* in such cultures undergo asymmetric augmentation of the outer wall layer (Fig. 75) which, in some cases, is so pronounced as to suggest the unipolar slime of *Hormotilopsis* (Trainor and Bold, 1953; Arce and Bold, 1958).

CHLOROCOCCUM scabellum sp. nov. (Figs. 26–29; 93–95).

Cellulae iuvenes ovatae ad ellipsoideas, sphericae, nisi mutuo compressae, dum maturuerunt, usque ad 20 μ diam. Cellulae in culturis 2–3 septimanarum in "Bristol's agar" aliquot generationibus aplanosporarum cohaerentibus complicatae, zoosporangia minus frequentia. Zoosporae biflagellatae (2–16 in unoquoque sporangia) per bipartitionem successivam enascentes, 7–9 $\times$ 2.5–3 μ, duas vacuolas contractiles, unum pyrenoideum, nucleum posteriorem atque stigma anterius habentes. Cellulae in culturis 2 vel plurium mensium aetate, in "Bristol's agar" olei lutei plenae, unaquaque cellula unam gulullam excentricam magnam (necnon minores) habente, corium exterius membranae stratifactum usque ad 5 μ crass.

Reproductio sexualis non observata.

Origo: cellulae in culturam puram (T-1-8) e solo in querceto aperto seiunctae, in latere orientali Viae "95" admodum versus septentrionem a fine meridionale loci Williamson County, Texas dicti, m. Dec., an. 1957.

From the same soil sample that yielded *C. ellipsoideum*, another species of *Chlorococcum*, namely, *C. scabellum*, was isolated into bacteria-free culture (T-1-8). This organism forms a very shiny stratum on Bristol's agar two weeks after inoculation. Its cells display certain similarities to those of *C. ellipsoideum, C. minutum* Starr, and *C. echinozygotum* Starr, but, whereas all of these form smooth, homogeneous colonies on Bristol's agar at two weeks, those of *C. scabellum* are clearly rough. Upon this and evidence from other comparisons, T-1-8 is here described as

a new taxon, *C. scabellum*. The specific epithet alludes to the texture[1] of the colony on Bristol's agar. Two-month-old or older cultures on Bristol's agar slants become yellow-orange. Cells from such two- to six-month-old cultures contain one or more prominent, orange-colored oil droplets; in many, there is characteristically one large, excentric droplet in addition to smaller droplets. The outer layer of the cell wall of these algae is concentrically stratified and reaches 5 μ in thickness (Fig. 95).

Cells from Bristol's agar cultures two weeks old are very coherent and remain aggregated because of the stickiness of their outer wall layers. No matrix is demonstrable. The spherical, mature, vegetative cells range from 16 to 20 μ in diameter. The outer wall layer is well developed (Figs. 26, 27). The plastid is parietal and cup-like with a single pyrenoid. There is usually a unipolar opening in the chloroplast through which the single nucleus is visible (Fig. 26).

Zoospores, which arise in multiples of two by bipartition of the protoplast of vegetative cells (Fig. 24), are about 7–9 $\mu \times$ 2.5–3.0 μ, with two anterior contractile vacuoles, an anterior stigma, and a posterior nucleus. The cells retain their ellipsoidal shape (Fig. 29) at the termination of the motile period and only become spherical as they mature into vegetative cells. Sexual reproduction has not been observed.

Aplanospores (Fig. 93), 4–8 per cell, are produced in abundance in *C. scabellum*. The cell wall of the aplanosporangium tends to persist, so that rather complex associations of cells, representing several generations of aplanospores, are characteristic of *C. scabellum*, and their coherence probably explains the rugose configuration of the populations on agar.

The following additional attributes of *C. scabellum* have been recorded. Excellent growth occurs in nutrient broth as well as in repeated transfers in Bristol's solution. Good growth occurred also in acetate, glucose, and urea; growth was only fair, in contrast, in arabinose and in asparagine. Growth of *C. scabellum* was inhibited only by dihydrostreptomycin, among the antibiotics tested.

The range of size of vegetative cells of *C. scabellum* is similar to that of *C. minutum* Starr and *C. ellipsoideum*, so that careful comparison is required to distinguish the three organisms. *Chlorococcum scabellum*, like *C. ellipsoideum*, lacks the isogamous sexuality of *C. minutum* and similarly lacks the irregular thickening of the outer wall layer which occurs in the latter. It differs from *C. ellipsoideum* in the marked coherence of its cells in complexes arising from aplanosporangia and in the paucity of zoosporangia even in two-week-old cultures. The occurrence of large excentric droplets (in addition to smaller ones) of orange-colored oil in cells from two-month-old and older cultures is in contrast to the few small droplets of oil in *C. minutum* and to the numerous smaller, yellow-orange droplets in similar cells of *C. ellipsoideum*. The rough colonies of *C. scabellum* are

[1] Other species of *Chlorococcum* also form rough colonies but differ in other attributes from the present isolate (Bold and Parker, personal communication).

in marked contrast to the homogeneous ones of *C. ellipsoideum* and *C. minutum*. Finally, *C. scabellum* is inhibited only by dihydrostreptomycin in the Unidisk test, thus differing further from *C. ellipsoideum* and *C. minutum*.

CHLOROCOCCUM intermedium sp. nov. (Figs. 30–32; 96–97, 132).

Cellulae inuvenes antice ovatae acutaeque, sphericae factae ut maturescunt. Cellulae maturae uninucleatae, vacuolis contractilibus praeditae, usque ad 20 μ diam. Divisiones cellulae cellulas quaternas efficientes, generationibus cellularum quaternarum sequentibus aggregatas parenchymatas interdum formantibus. Matrix denotata nulla.

Zoosporae aplanosporaeque per bipartitiones abrupte successivas formatae. Zoosporae elongatae asymmetricae, polo posteriore rotundato, polo anteriore acuto; zoosporae stigma anterius, duas vacuolas contractiles anteriores, nucleum posteriorem, duo flagella longiora quam cellula habentes, usque ad 9.5 μ long., 3.7 μ diam. ellipsoideae manentes admodum cum quiescunt.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C-1-13) seiunctae ex exemplo soli sub *Quercum stellatam*, in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This organism was isolated into pure culture (C-1-13) from the Carrizo Sands formation of Caldwell County. Young cells of *C. intermedium* (Fig. 32) are ovoid and acute anteriorly. The cells become spherical as they mature (Fig. 30). The parietal plastid contains at least one pyrenoid and the cells are uninucleate. Contractile vacuoles also are present in mature cells. The latter reach a diameter of approximately 20 μ when grown on Bristol's agar under standard conditions. Cell divisions, which might be interpreted as vegetative (see discussion which follows), usually occur before this size is attained. These divisions give rise to tetrads of aplanospores (Fig. 96) or occasionally to few-celled parenchymatous aggregates. However, many cells of the same generation do not divide to form tetrads or parenchymatous aggregates but develop like a typical *Chlorococcum*, ultimately producing zoospores and aplanospores. No matrix is demonstrable with India ink. Colonies on Bristol's agar are smooth, with a dull shine. Two-month-old cultures are bright orange and the cells thicken their walls unilaterally to almost 5 μ.

Rapidly successive bipartitions result in the formation of zoospores (Fig. 97) and true aplanospores. The zoospores (Fig. 31) are elongate and asymmetrical with the posterior pole rounded and the anterior acute. As many as 16 zoospores have been observed within a single cell. Zoospores have a parietal plastid, two flagella which exceed the cell body in length, an anterior stigma, two anterior contractile vacuoles, and a posterior nucleus. Zoospores attain a length of 9.5 μ with a diameter of 3.7 μ and do not become spherical at the end of the motile period.

Sexual reproduction has not been observed.

This alga grows in trace amounts in nutrient broth and is inhibited strongly by dihydrostreptomycin and polymyxin B, and to a lesser degree by terramycin, tetracycline, and erythromycin (Fig. 132). Growth is excellent in B-1, maltose, and glucose; good in arabinose; fair in acetate; occurs in trace amounts in urea, xylose, and acetate in darkness, and no growth occurs in glucose in darkness.

This organism differs from *Chlorococcum tetrasporum* and *C. aplanosporum*, which it most resembles, especially in the shape of the maturing vegetative cells and in the zoospore. The zoospores of *C. intermedium* are smaller than those of the former species and possess longer flagella in relation to body size. The maturing cells of *C. intermedium* are acute anteriorly, while this is not true of *C. aplanosporum* and *C. tetrasporum*. Contractile vacuoles can be observed in the mature vegetative cells of all three organisms (although not reported by Arce and Bold, 1958, in *C. tetrasporum*). In cultures of the same age and grown under the same conditions, the mature cells of *C. aplanosporum* are larger than those of *C. tetrasporum* and *C. intermedium*. The nucleus of the zoospore of *C. aplanosporum* is anterior, while that of *C. tetrasporum* and *C. intermedium* is posterior. Further discussion of the supplementary attributes of *C. aplanosporum* and *C. tetrasporum* will be published by Bold and Parker (in manuscript).

Prolonged observations on organisms from cultures of *Chlorococcum tetrasporum*, *C. aplanosporum*, *C. intermedium*, *Spongiococcum tetrasporum*, and *Chlorosarcinopsis minor* have led to a reappraisal of Herndon's definition of the term "vegetative cell division." Herndon based his definition on that of Fritsch (1935) which is as follows:

> . . . that type of division in which the parent cell ordinarily divides without rupture or gelatinisation of its wall, the two units produced by division of the protoplast being merely separated by the development between them of a strip of membrane which is joined laterally to the membrane of the parent.

Herndon's (1958a) definition, as derived from that of Fritsch, states:

> The three organisms . . . herein described as possessing *vegetative cell division*, differ from those described above in that the parent wall remains closely contiguous with the two daughter protoplasts at the completion of cytokineses, there being no immediate rupture or dissolution of the parental wall.

The degree of contiguity of the daughter cell wall with the parent wall varies in both the Chlorococcales and Chlorosphaerales. There is not, in every individual of a culture, immediate rupture or dissolution of the parent wall in chlorococcalean zoosporangia and aplanosporangia, and immediate rupture or dissolution of the parent wall can be observed under certain conditions of culture in members of

the Chlorosphaerales. Intermediate forms emphasize this overlapping and must be dealt with conservatively at least until definite affinities can be established by physiological or other means.

Herndon recognizes the problem of precisely defining vegetative cell division and his current comments (personal communication) are as follows:

As I conceive the phenomenon of vegetative cell division, it is a sequence of events in which the order in which the events occur, as well as the duration of each, are of prime importance in recognizing the phenomenon. The steps of the sequence and their duration are open to subjective evaluation, and in the twilight zone between unicellular and multicellular algae, I believe that the phenomenon can be recognized usually only on a comparative basis using populations (cultures).

While the recognition of vegetative cell division is subjective in part, I do not believe that it is any more so than many of the other criteria that we use (often under the label "objective"). When a population of a chlorococcalean and one of a chlorosphaeralean alga are compared under the same conditions of growth, the attribute that I call vegetative cell division is recognizable whether or not its limits are finite. If intermediates straddle our human categories, we have at least the alternatives either of changing our concept of the category or of making new categories. As I see it, this is simply a matter of the "best judgment" of those concerned (keeping in mind the reasons requiring the category in the first place).

Herndon emphasizes the contiguity of the cell wall of the daughter cell with that of the parent, and the formation of an incipient, multicellular thallus (personal communication). An incipient thallus may be recognizable under certain conditions in cultures of *Chlorosarcinopsis minor*, but this is hardly true in the case of *C. dissociata*. The attribute of contiguity may be a useful one in delimiting taxa, but, as the writers see it, the contiguity of the walls is not necessarily essential for vegetative cell division. The important difference between vegetatively dividing Chlorosphaerales and non-vegetatively dividing Chlorococcales is the fact that in a typical chlorosphaeralean alga, divisions are comparatively slow and a cell wall is deposited after each nuclear division. The pattern is: cell growth, nuclear and cell division, cell growth, nuclear and cell division, etc., etc., etc. The rapid divisions which result in zoospore and aplanospore formation occur usually only after transfer to fresh medium in chlorosphaeralean algae. In a typical chlorococcacean alga, the pattern is cell growth without cell division (but sometimes with nuclear division), ultimately followed by rapid divisions (successive bipartitions or progressive cleavage) to form zoospores and aplanospores; here no cell walls are formed until all cytokineses have been completed, and indeed, often not until the products have been released and become quiescent *after* motility (algae with *Protosiphon*-type zoospores).

In the organisms which might be considered intermediate (*C. tetrasporum*, *C. aplanosporum*, *C. intermedium*, *Spongiococcum tetrasporum*), tetrads of cells and

often pseudo-parenchymatous aggregates appear to be derived from vegetative cell divisions. The question then is, are these products formed step-wise by mitosis and cytokinesis, each followed by intervening cell-wall deposition, or are they formed by rapidly occurring divisions, and is cell-wall deposition delayed until all cytokineses have been completed? Since very few two-celled stages can be found in these "intermediates," it can be assumed that the tetrads were formed by rapid divisions and were "intended" to be zoospores. These tetrads of aplanospores may divide in the same way to form more aplanospores and thus parenchymatous aggregates much like those formed by true vegetative divisions. Thus, in the writers' opinion, the intermediates should be relegated to the Chlorococcales.

NEOCHLORIS pseudoalveolaris sp. nov. (Figs. 33–37; 98–100).

Cellulae sphericae usque ad 25 μ diam., sine matricibus mucosis in culturis laete crescentibus; cellulae in culturis 2–3 mensium aetate in matrice communi solida inclusae. Chromatophorus cavus, parietalis craterioformisque, aperturam habens, in qua cytoplasma sine colore saepe alveolare atque unus nucleus visibiles; unum pyrenoideum manifestum, plerumque isodiametricum. Membranae cellularum in culturis 2–3 mensium aetate non perspicue spissescentes.

Reproductio per 4–8–16 cellulas-filias immobiles in aggregationibus irregularibus cohaerentes cum e cellulis parentibus liberantur. Cellulae iuvenes in medis liquidis aut in "agar" humido quoque zoosporas formantes, 8–16 zoosporis ex unaquaque cellula per bipartitiones iteratas enascentibus. Zoosporae 10 μ long. $\times$ 5 μ lat., antice rotundatae, postice acuminatae, biflagellatae, ac ut videtur, sine membrana, unum pyrenoideum, nucleum anteriorimedium, duas vacuolas contractiles atque stigma anterius habentes, sphericae factae statim cum quiescunt.

Origo: Cellulae in culturam puram (T-1-2A) e solo in querceto aperto seiunctae, in latere orientali Viae "95" admodum versus septentrionem a fine meridionali loci Williamson County, Texas dicto, m. Oct., an. 1957.

Neochloris pseudoalveolaris was isolated into bacteria-free culture (T-1-2-A) from a sample of sandy soil collected in an open oak wood on the east side of State Highway 95 just north of the southern boundary of Williamson County on October 31, 1957. The organism grows well both on modified Bristol's agar and in Bristol's solution without agar. This alga clearly possesses the generic attributes of *Neochloris* as set forth by Starr (1955), namely, cells each with a hollow parietal chloroplast, with a pyrenoid, and with apparently wall-less zoospores which become spherical immediately after the motile period (Fig. 36, 37). As in the case with all chlorococcacean algae, populations show considerable variation in cell size (Fig. 98). The spherical individuals from two-week-old Bristol agar cultures become as large as 25 μ in diameter. Although a great majority of cells are spherical, some individuals are irregular or ellipsoidal in shape and possess unipolar thickenings of the outer wall layer. The pyrenoid is very conspicuous in actively growing cells from two-week-old Bristol's agar cultures and tends to be isodiametric unless it

and the surrounding chloroplasts are in division (Fig. 33). In actively growing cultures the single nucleus is usually clearly visible in the hyaline cytoplasm which is often alveolar. Living cells mounted from two-week-old Bristol's agar cultures tended to cohere in irregular groups, but immersion in India ink failed to reveal a matrix. However, in cultures on the same medium two to three months old, a rather firm, common matrix is visible even without India ink. In such old cultures the cell wall layers do not thicken strikingly (Fig. 99).

The prevalent method of reproduction seems to be by formation of daughter cells which might appropriately be termed autospores (Fig. 100), inasmuch as they represent miniature individuals and are probably not potentially motile. Four, eight or sixteen autospores are formed, and in subsequent growth they rupture the parental cell wall which does not persist. Various numbers of cells cohere in irregular groups, apparently because of the stickiness of their walls, inasmuch as no common matrix is demonstrable. Colonies are smooth and dull-shiny. Two-month-old cultures remain green and the cells thicken their cell walls only slightly.

Reproduction also is effected by zoospore formation (Figs. 35, 36). This mode of reproduction is restricted to very young vegetative cells or clustered autospores especially when cultures are frequently transferred to fresh, moist agar media. Zoospores, approximately 8 to 16 in number, arise by repeated bipartitions of the cells and upon release are motile for only a short period. The zoospores usually are released in a vesicle and are rounded posteriorly and pointed anteriorly. Zoospore dimensions are approximately 8–10 μ long $\times$ 4–5 μ wide. Each zoospore (Fig. 36) possesses two flagella approximately equal to the cell body in length, two contractile vacuoles, an anterior stigma, an equatorial or slightly anterior nucleus, and a parietal canoe-shaped plastid in which a minute pyrenoid sometimes is visible. As the end of the motile period approaches, the flagella are withdrawn and the cells rather violently change their shape to the spherical (Fig. 37); this results in the bending of the canoe-shaped plastid into one that is somewhat cup-shaped.

Up to date, six species of *Neochloris* have been described (Starr, 1955; Herndon, 1958b; Arce and Bold, 1958; Bold, 1958). Comparison of the attributes of the present organism with those of other described species of the genus *Neochloris* indicates that its affinities lie most closely with *Neochloris alveolaris* (Bold, 1958). However, comparison of the morphological and physiological attributes of clonal populations of these organisms grown under standard conditions on several sets of media reveals that *N. alveolaris* grows heterotrophically in darkness and other minor differences occur.

Both organisms are inhibited by dihydrostreptomycin and neither organism grows well in nutrient broth. In two-week-old Bristol's agar cultures, the colonies of *N. pseudoalveolaris* are dull-shiny, while those of *N. alveolaris* are decidedly dry. Furthermore, successive cell generations in *N. alveolaris* cling together in rather regular groups, autospores remaining clustered after their liberation, and, in turn,

forming new generations of autospores, as noted above. Coherence is irregular in *N. pseudoalveolaris*. The pyrenoids in cells of *N. alveolaris* are elongate-ellipsoidal and not nearly as clearly visible as are the more isodiametric pyrenoids of *N. pseudoalveolaris*. In cultures on Bristol's agar two to three months old, another striking difference becomes apparent: the cells of *N. pseudoalveolaris* secrete a rather firm and clearly delimited common matrix, as noted above, while those of *N. alveolaris* tend not to cohere, and no matrix is demonstrable with India ink.

SPONGIOCOCCUM multinucleatum sp. nov. (Figs. 38–42; 101–104).

Cellulae vegetativae iuvenes ovatae pyriformesve; cellulae maturae ad 50 μ, sphericae pyriformesve. Cellulae multinucleatae, nucleis singulis in cavis chloroplastidis, cellulae vegetativae sine vacuolis contractilibus matricibusque, cohaerentes et ad superficie "agar" adhaerentes.

Reproductio asexualis per formationem zoosporaraum aplanosporarumque, zoosporis tenuibus ellipsoideisque, priusquam ad 8.4 μ long., 3.2 μ lat. Chloroplastis parietalis, pyrenoideo usque dum zoospora quiescit non perspicuo; zoosporae duas vacuolas contractiles anteriores, stigma anterius, nucleum paululo anteriorem ad medium, duo flagella paululo longiora quam cellula habentes. Nonnullae zoosporae sphericae factae admodum dum quiescunt, maiore ex parte, autem, semper ellipsoideae manentes.

Reproductio sexualis non observata.

Origo: Cellulae in culturam (T-1-5) puram e solo in querceto aperto seiunctae, in loco Williamson County, Texas dicto, m. Dec., an. 1957 et cellulae in culturam puram (C-1-14) seiunctae ex exemplo soli sub *Quercum stellatam*, in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This is the second example of the isolation of the same organism from two locations, namely, the black calcareous soil of Williamson County (T-1-5) and the Carrizo Sands of Caldwell County (C-1-14).

Young vegetative cells of this alga are spherical, ovoid or pyriform (Figs. 42, 101), and contain a parietal plastid with one pyrenoid. The cells are circular in polar view. Only one nucleus is present in young cells, but nuclear number increases with age and size of the cell. Mature vegetative cells (Figs. 38, 39, 102) contain a sponge-like plastid with a large pyrenoid, and a number of nuclei lie singly in cavities in the chloroplast which contain colorless cytoplasm. Cells up to 50 μ in diameter have been observed. Contractile vacuoles are absent in these cells. Two-month-old cultures maintained on Bristol's agar are flecked with orange patches. No significant thickening of wall layers occurs with age, except at the narrow pole of pyriform cells, and no individual or common matrix can be detected with India ink or methylene blue. The cells do adhere tightly to an agar surface and are coherent.

Asexual reproduction is accomplished by the release of zoospores and aplano-

phores formed by progressive cleavage (Figs. 40, 104). The zoospores are slender and ellipsoidal, between 7.5 μ and 8.4 μ in length and 2.3 μ and 3.2 μ in width. Zoospores just emerging from the zoosporangium are pointed anteriorly; as they swim, the anterior pole becomes rounded. The chloroplast in the zoospore is parietal with one pyrenoid which becomes visible only after cessation of motility. The zoospores have two equal flagella, an anterior linear stigma, and two anterior contractile vacuoles. The nucleus is slightly anterior of the median line. The zoospores of this alga differ from those of all other spherical, zoospore-producing chlorococcalean algae thus far described in that some of them became spherical at the end of the motile period (*Protosiphon*-type) while others remain ellipsoidal (*Chlamydomonas*-type) (Starr, 1955). However, since a far greater number of the zoospores remain ellipsoidal upon quiescence, they will be considered *Chlamydomonas*-type for purposes of classification.

Spongiococcum multinucleatum grows well in nutrient broth and forms dry, rough, colonies on Bristol's agar under standard conditions. It is inhibited by polymyxin B and dihydrostreptomycin. Growth is excellent in B-1, maltose, glucose, and urea. Growth is good in acetate and arabinose; fair in glucose in darkness, and only trace amounts of growth occur in acetate in darkness or in xylose.

Spongiococcum multinucleatum differs from other recently described (Deason, 1959) species of this genus in the following attributes: possession of many nuclei in mature vegetative cells; zoospore and aplanospore formation by progressive cleavage; and the behavior of the zoospores upon quiescence, some remaining ellipsoidal and some becoming spherical.

SPONGIOCOCCUM excentricum sp. nov. (Figs. 43–45; 105–107).

Cellulae vegetative ellipsoideae; cellulae maturae sphaericae, ad 21 μ, uninucleatae, nucleum in cavis chloroplastidis habentes; cellulae neque distincte cohaerentes neque ad superficiem "agar" adhaerentes, pyrenoideum semper excentricum habentes.

Reproductio asexualis per formationem zoosporarum aplanosporarumque, zoosporis tenuibus ellipsoideisque, usque ad 6 μ long., 3.2 μ lat. Chloroplastis parietalis; pyrenoidus zoosporae perspicuus; zoosporae duas vacuolas contractiles anteriores, stigma anterius, nucleum posteriorem, duo flagella paululo longiora quam cellula ipsu habentes.

Reproductio sexualis non observata.

Origo: Cellulae in culturam (501) ex aqua aquarii translatae, Iowa City, Iowa. August, 1957.

Although *Spongiococcum excentricum* was not isolated from Texas soil, a description of it is included here because the occasion to study the organism arose at the same time that *Spongiococcum multinucleatum* and other species of the genus were being investigated comparatively. *Spongiococcum excentricum* was sent to the writers in bacteria-free culture (501) by Dr. Harold H. Kuehn, Senior

Research Mycologist of the Grain Processing Corporation of Muskateen, Iowa. He had submitted the organism to two other phycologists who disagreed regarding its identity. Comparative study of the organism (501) with other species of *Spongiococcum* grown under standard conditions indicated clearly that it differed from the other three species.

Young vegetative cells of this alga (those recently derived from zoospores) are ellipsoidal but, as in the genus *Chlorococcum,* gradually become spherical as they increase in size. Each contains a parietal chloroplast with a single pyrenoid. The latter is prominent and eccentric in the young cells and, unlike the pyrenoids of other species in the genus, it remains characteristically eccentric in the mature cells. In his description of *S. alabamense,* one of us (Deason, 1959) reported that the pyrenoids in young cells of this species were eccentric and that they might be slightly so in the mature cells. Careful comparison of mature cells of the present organism with those of *S. alabamense* indicates that the eccentricity of the pyrenoid in *S. excentricum* is much more marked and consistent (Figs. 43, 44; 105, 106). The cells of *S. excentricum* are uninucleate, the nucleus lying within one of are areas of colorless cytoplasm within the sponge-like plastid. Two-month-old cultures maintained on Bristol's agar are green. No significant thickening of the outer wall layer occurs in cells in cultures two months old. Material mounted in Bristol's solution from two-week-old Bristol's agar culture is slightly coherent. The cells may be embedded in a rather diffuse common matrix in such cultures.

Two-week-old colonies on Bristol's agar are dull-shiny (not dry in appearance), especially on thicker parts of the mass, upon macroscopic examination. With transmitted light at a magnification of approximately 20 × the plant mass on the agar surface is rather homogeneous but granular with slightly rugose margins. Mature vegetative cells in two-week-old Bristol's agar cultures reached a maximum diameter of approximately 21 μ.

Reproduction is by zoospore and aplanospore formation in this organism (Figs. 45; 106, 107). The zoospores are approximately 6 μ long by 3 μ in width and their flagella exceed the body in length. Each contains two anterior contractile vacuoles and anterior stigma, the latter embedded in a parietal chloroplast with a single median to post-median pyrenoid. The single nucleus is post-median to posterior. The number of zoospores produced in each cell is variable and as few as two may develop from smaller cells. The zoospores in this species, in contrast to those of *S. multinucleatum,* always remain ellipsoidal upon the cessation of motility.

Sexual reproduction was not observed in this species.

With respect to supplementary characters, *Spongiococcum excentricum* clearly differs in its combination of attributes from other species in the genus. Growth of *S. excentricum,* in Bristol's solution supplemented with glucose in darkness, is very good. The alga is sensitive to both polymyxin B and dihydrostreptomycin.

The known species of *Spongiococcum* may be compared with respect to certain supplementary characteristics as follows:

APPEARANCE OF THE COLONY

Spongiococcum alabamense	Rough, shiny
S. tetrasporum	Smooth, dull-shiny
S. multinucleatum	Rough, dry, adhering firmly to agar surface
S. excentricum	Rough, dull-shiny

COLOR ON TWO-MONTH-OLD BRISTOL'S AGAR SLANTS

S. alabamense	Orange at edges of agar
S. tetrasporum	Green
S. multinucleatum	Orange flecks on green
S. excentricum	Green

DEGREE OF THICKENING OF OUTER WALL LAYER OF TWO-MONTH-OLD CULTURES ON BRISTOL'S AGAR SLANTS

S. alabamense	Few cells with thickened wall layers
S. tetrasporum	Pronounced unipolar thickening
S. multinucleatum	Slight thickening
S. excentricum	No significant thickening

GROWTH IN NUTRIENT BROTH

S. alabamense	Fair, with phototactic ring
S. tetasporum	No growth
S. multinucleatum	Fair, with phototactic ring
S. excentricum	Good, with phototactic ring

INHIBITION BY ANTIBIOTICS

S. alabamense	Polymyxin B, dihydrostreptomycin
S. tetrasporum	Polymyxin B
S. multinucleatum	Polymyxin B, dihydrostreptomycin
S. excentricum	Polymyxin B, dihydrostreptomycin

Growth responses of three species of *Spongiococcum* are recorded in Table. 2.

TABLE 2

Growth responses of three species of *Spongiococcum* in various media

	S. alabamense	*S. tetrasporum*	*S. multinucleatum*
B-1	Excellent	Good	Excellent
Acetate	Trace	Trace	Good
Acetate (dark)	Trace	Trace	Trace
Maltose	Excellent	Good	Excellent
Glucose	Excellent	Good	Excellent
Glucose (dark)	No growth	No growth	Fair
Arabinose	Excellent	Good	Good
Urea	Excellent	Good	Excellent
Xylose	Trace	Fair	Trace

Figure 133 shows the results of one experiment involving *S. alabamense*.

A key to the currently known species of *Spongiococcum* follows:

1. Pyrenoid markedly excentric to almost parietal, even in mature cells ..... *S. excentricum*
1. Pyrenoid usually central in mature cells[1] 2
   2. Vegetative cells multinucleate ..... *S. multinucleatum*
   2. Vegetative cells uninucleate 3
3. Aplanospores characteristically in tetrads; colonies smooth on Bristol's agar at two weeks ..... *S. tetrasporum*
3. Aplanospores not in tetrads, colorless; colonies rough on Bristol's agar at two weeks ..... *S. alabamense*

SPONGIOCHLORIS lamellata sp. nov. (Figs. 46–48; 108–110, 135).

Cellulae iuvenes sphericae, chloroplastidem parietalem habentes; cellulae maturae sphericae, usque ad 80 μ diam. Cellulae per membranam saepe cohaerentes, matrices, autem, nullae. Membranae zoosporangii usque ad 25 μ crass. saepe tumescentes. Membrana cellulae ex aliquot stratis composita. Cellulae maturae multinucleatae; vacuolae contractiles nullae.

Reproductio asexualis per formationem 100 vel plurim zoosporarum amoeboidearum elongatarum effecta. Zoosporae plastidem parietalem dissectam, guttullis pinguibus saepe praeditam, atque duas vacuolas contractiles anteriores, atque nucleum medium, atque stigma anterius habentes. Flagella longiora quam longitudo cellulae, usque ad 13 μ long., 3.7 μ lat. Zoospora spherica facta cum quiescit.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C–9–7) seiunctae ex exemplo soli sub *Quercum stellatam*, in formatio Carrizo Sands nomine, distante 7.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This organism also was isolated into bacteria-free culture (C–9–7) from the Carrizo Sands. Since its cells possess sponge-like chloroplasts, one or more pyrenoids, and *Protosiphon*-type zoospores, it belongs to the genus *Spongiochloris* Starr. Young cells are spherical and have a parietal chloroplast (Fig. 48). The chloroplast of mature cells, although sponge-like rather than net-like, does not differ essentially from that described by Starr (1955). The spherical vegetative cells (Figs. 46, 108) may attain a diameter of 80 μ. Young cells cohere by their cell walls, and these connections persist throughout the lives of the cells. However, no extensive matrix is present. The cell wall is composed of several layers, the outer ones sloughing off as the inner are added. The cell wall may attain a thickness of

[1] The position of the pyrenoid is variable in *S. alabamense* from central to somewhat excentric; it is not, however, consistently excentric as in *S. excentricum*. Furthermore, 2-week-old colonies of the latter on Bristol's agar are homogeneous, not rough.

25 μ when old cells liberate zoospores (Fig. 109). Only very young cells are uninucleate; mature cells are multinucleate. No contractile vacuoles were observed in mature cells. Colonies are dry and rough (Fig. 135). Cultures two months old are orange at the edges of the colony and, although cell walls may swell during zoosporogenesis after transfer to fresh medium, they are not significantly thicker than at one month.

Asexual reproduction is by formation of 100 or more zoospores (Fig. 109) or aplanospores. The zoospores are elongate (Fig. 47) and shaped very much like those of *Spongiochloris excentrica*, as figured by Starr (1955). They become spindle-shaped as they swim. Zoospores are amoeboid at first and are liberated through a pore or tear in the cell wall (Figs. 109, 110). The fat globules within the cell are not broken down in zoospore formation and the smaller drops are sometimes incorporated in the posterior region of zoospores (Fig. 47b). The larger drops may remain within the persistent wall of the zoosporangium or may flow out with the zoospores. The zoospore contains a parietal chloroplast which may be dissected, an anterior stigma, two anterior contractile vacuoles, two flagella longer than body length, and a median nucleus. Zoospores reach a maximum length of approximately 13 μ the average being about 9 μ. The maximum width is approximately 3.7 μ, with an average of about 3.0 μ. Zoospores become spherical at the end of the motile period.

No sexual reproduction has been observed.

No growth of this alga occurs in nutrient broth and it is inhibited by polymyxin B and erythromycin. Growth is excellent in glucose; good in B-1, maltose and urea; fair in arabinose; occurs in trace amounts in xylose and glucose in darkness; and no growth occurs in acetate.

This alga differs from the known species, *Spongiochloris spongiosa* Starr and *S. excentrica* Starr, in size of both vegetative cells and zoospores and thickness of the cell wall even in relatively young cultures (one month old or less); finally, the cells of *S. lamellata* often have several pyrenoids, unlike those of the other two species. The following key distinguishes the species:

1. Mature cells with more than one pyrenoid ..... *S. lamellata*
1. Mature cells usually with only one pyrenoid 2
   2. Pyrenoid central in mature cells ..... *S. spongiosa*
   2. Pyrenoid excentric in mature cells ..... *S. excentrica*

CHARACIUM polymorphum Trainor and Bold (1953) (Figs. 49–53; 111).

Characium polymorphum, described as a new taxon seven years ago, has not been isolated since its original description until it appeared in an enrichment culture of a soil sample from the Martin farm, 2.5 miles north of the Williamson County line, on the west side of State Highway 95, during the present investigation. The type species was isolated from cultivated soil in Lowndes County, Georgia.

Comparison of the Georgia strain with the present isolate (T-2-4) in bacteria-free cultures grown under identical conditions clearly established their similarity. The Texas isolate of *C. polymorphum* is recorded in Figures 49–50 and 111, some of which illustrate the typically rapid stalk formation from the anterior end of the zoospore soon after its loss of motility. Figure 111 shows a group of young individuals which arose from zoospores not liberated from the parent cell.

HORMIDIUM sterile sp. nov. (Figs. 54, 55, 112).

Planta unicellularis aut filamentose usque ad ca. 25 cellulas long. Cellulae uninucleatae, chloroplastis laminata per totam cellulae longitudinem extensa, plus quam dimidium periferiae circumdans, uno pyrenoideo spherico oblongove praedita. Cellulae usque ad 15 μ long., 6 μ lat.; constrictiones ad dissaepimenta nullae, poli cellularum in forma unicellulari rotundati.

Reproductio nisi per fragmentationes non observata.

Origo: Cellulae in culturam puram (C-1-26) seiunctae ex examplo soli sub *Quercum stellatam,* in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This alga also was isolated from a soil sample from the Carrizo Sands formation. A bacteria-free culture (C-1-26) maintained on Bristol's agar for two weeks, consists of single uninucleate cells with rounded poles or 2-10-celled, unbranched filaments (Figs. 54, 55; 112). Filaments of 25 cells in length have been observed occasionally. The chloroplasts have one ovoid pyrenoid, are laminate, and encircle slightly more than one-half the circumference of the cell (Figs. 54, 55; 108). The cells may attain a length of 15 μ and a width of 6 μ. There are no constrictions at the cross-walls. No motile cells have been observed despite the usual manipulations designed to evoke their formation. These attributes differ from those of any known species of *Hormidium* or *Stichococcus*. This alga grows well on Bristol's agar and forms smooth, dull-shiny colonies after two weeks' growth under standard conditions. Two-month-old cultures remain green, and the cells do not thicken their walls. *Hormidium sterile* grows well in soil-water medium in which bacteria are present but poorly or not at all in other liquid media used in this investigation. Agar apparently contains a growth factor necessary for this alga.

It was difficult to decide upon the taxonomic disposition of this organism since considerable disagreement has centered around forms sometimes placed in the genus *Hormidium* and sometimes in the genus *Stichococcus*. *Hormidium* was erected by Kützing in 1843 and contained three species which were later removed to other genera. Thus, Gay (1891) abandoned the name *Hormidium*. Hazen (1902), in tracing the history of these genera, states:

There are, however, several species among those referred to *Hormiscia (Ulothrix)* by DeToni (1889) which form, together with certain more recently described species, a

group possessing characters which furnish good reason for their separation from *Ulothrix*. These were placed by Gay in the genus *Stichococcus* Nageli (1849) because of their tendency, in common with *S. bacillaris,* toward aerial life and vegetative reproduction. Because of the supposed absence of reproduction by zoospores, this genus was placed with the Protococcaceae rather than the Ulotrichaceae. Klerker (1896) went a step farther, and added to *Stichococcus* a form which he supposed to be *Ulothrix subtilis* Kütz., a species which is very generally aquatic.

Now in one of the best known *Stichococcus* species, *S. flaccidus* (Kütz.) Gay, Klebs (1896) found zoospores (there seems to be no reason for doubting the correctness of the determination of the species). He therefore revived the genus *Hormidium,* because of his objection to the name *Stichococcus* as implying affinities with the Protococcaceae.

Hazen (1902) followed Gay (1891) in abandoning *Hormidium* and recognized *Stichococcus* which he distinguished from the other genera of the Ulotrichaceae as follows: "Filaments not attached; chromatophore a parietal plate or disc, with one pyrenoid." However, he included forms without pyrenoids. Hazen further stated that zoospores had been observed in all species which he placed in the genus *Stichococcus,* except the type species, *Stichococcus bacillaris.*

Heering (1914) followed Kleb's (1896) revival of the genus *Hormidium,* placing forms without pyrenoids in the genus *Stichococcus.*

In 1933, Smith described both genera as having pyrenoids and motile cells, distinguishing them on the following basis, "Chloroplast extending whole length of cell–*Stichococcus.* Chloroplast half as long as cell–*Hormidium.*"

Fritsch (1935) separated the genera on the basis of the absence of a pyrenoid in *Stichococcus* and the tendency to fragmentation in this organism. Smith (1950) emphasized the same criteria but added the attribute of reproduction by fragmentation only. In view of Smith's (1933) discussion of the genera, his later (1950) comments on the treatment of the genera by others seem to be a bit harsh when he wrote: "Some phycologists[1] expand the generic concept (of *Stichococcus*) to include most species of *Hormidium* but this inclusion of zoosporic forms whose chloroplasts have pyrenoids is illogical."

Prescott (1951) apparently follows Smith (1933) in recognizing both genera and including species with pyrenoids in both genera.

Although *Hormidium sterile* has attributes of *Ulothrix* (plastid encircling more than one-half the circumference of the cell), *Hormidium* (unattached, with pyrenoid), and *Stichococcus* (unattached, chloroplast extending to the terminal walls, reproduction by fragmentation), it has been placed in the genus *Hormidium* almost arbitrarily, because it is free-floating and has a pyrenoid in its plastid.

The above summary of the taxonomic history of these species and genera and the resulting dilemma regarding taxonomic disposition of the present organism

[1] Gay, 1891; Hazen, 1902.

emphasize the need for further work in the Ulotrichaceae to define adequately the limits of the genera of this family. Smith (1933, 1950) states:

> Generic differences among the Ulotrichaceae are based upon the structure of the cells and structure of the filament. Since the structure of the filaments is subject to considerable variation in certain genera, there is more or less disagreement among phycologists as to the precise generic limits in certain cases.

This disagreement would undoubtedly be resolved by investigations based on the culture method, and use of physiological as well as morphological criteria to delimit the taxa.

PSEUDOSCHIZOMERIS caudata gen. et sp. nov. (Figs. 56–63; 113, 114).

Filamenta non ramosa, longitudine variabilia, divisiones longitudinales et obliquas necnon transversas habentia, per hapteron e cellula basali formatum affixa. Cellulae uninucleatae; chloroplastis laminata, plus quam dimidium cellulae circumdans ac usque ad parietes terminales attingens, plerumque pyrenoideo uno praedita; cellulae usque ad 20.0 μ long., 12 μ lat., maiore ex parte minores; matrices nullae.

Reproductio asexualis per zoosporas quadriflagellatas obovatas usque ad c. 15 μ long., 9 μ lat. effecta. Zoosporae chloroplastidem parietalem pyrenoideo ab anteriore ad posterius variante praeditam, atque 4 vacuolas contractiles anteriores atque stigma anterius habentes; flagella longiora quam cellula. Singula zoospora ex una cellula effecta, per porum membranae liberata. Hapteron elementarium ad polum anteriorem zoosporae quiescentis formatum.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C-1-6) seiunctae ex exemplo soli sub *Quercum stellatam*, in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This alga, a member of the Ulotrichaceae, was isolated into soil-water medium (C-1-6) from the Carrizo Sands formation. At first, it produced long, unbranched filaments. Longitudinal and oblique, as well as transverse, divisions of the cells occurred, so that the filaments attained an irregular, pluriseriate condition (Figs. 63, 113). Young filaments looked much like those of other ulotrichalean algae. However, as the filaments aged, swelling of the cell wall materials and longitudinal divisions caused the cells to appear to be embedded at random in a matrix (Fig. 63). When the alga was purified of bacteria and placed on Bristol's agar, it lost the ability to form filaments over a few cells in length. Subsequent transfers to soil-water medium, proteose agar, and to soil-extract agar, among other media, did not restore the former capacity for filament formation. Only after repeated transfers in Bristol's liquid, over a period of several months, was this capacity expressed. The

tendency to form filaments is especially noticeable in urea (B-9). The filaments, which are reminiscent of the genus *Schizomeris,* are composed of uninucleate cells which possess a napkin-ring type plastid encircling more than one half the cell (Figs. 56, 57, 114). The plastid, which has characteristic processes (Fig. 57), possesses one, or occasionally two, pyrenoids. Filaments are free-floating in liquid media but produce rudimentary holdfasts which are formed by the basal cell (Figs. 56, 114). The cells attain a maximum length of 20 μ and a maximum width of 12 μ, but the majority of cells is smaller. Two-week-old colonies on Bristol's agar are shiny and smooth. Two-month-old cultures become orange around the edges, and cells of these cultures thicken their wall layers only slightly.

The only means of reproduction observed has been the formation of obovoid, quadriflagellate zoospores. The zoospore (Fig. 59) has a parietal plastid with a pyrenoid, the position of which varies from anterior to posterior, an anterior stigma, four anterior contractile vacuoles, and four apically inserted flagella which are longer than the cell body. Zoospores may attain a maximum length of about 15 μ and a maximum width of 9 μ. A single zoospore is produced by each cell, and is liberated through a pore in the cell wall (Fig. 58). After a short period of motility, the zoospore withdraws its flagella and begins to elongate and divide (Figs. 60–62). A holdfast is formed at the anterior pole of the quiescent zoospore. Stigmata are often persistent.

Among the supplementary attributes recorded for this alga are the following: it does not grow in nutrient broth; it is inhibited by polymyxin B and dihydrostreptomycin. Growth is good in B-1, maltose, and urea; fair in acetate and glucose; and no growth occurs in acetate in darkness, glucose in darkness, arabinose, or xylose.

PLEURASTRUM erumpens sp. nov. (Figs. 64, 65; 115–120).

Planta vel unicellularis vel/et ramoso-filamentosa; cellulae singulae sub-sphericae aut clavatae, uninucleatae, plastidem parietalem, pyrenoideo uno vel pluribus praeditam habentes. Cellulae filamenti cylindricae, dolioformes aut subsphericae, plastidem laminatam, praecipue in cellulis cylindricis habentes. Cellulae dolioformes et cylindricae usque ad 15 μ long., 9.0 μ lat.; cellulae subsphericae usque ad 27 μ diam.

Zoosporae aplanosporaeque per cytokinesem celerem formatae. Zoosporae usque centum vel plus elongatae complanataeque, antice acuminatae postice rotundatae, duo flagella longitudine aequa, stigme anterius mediusve, nucleum posteriorem, duas vacuolas contractiles plerumque medias habentes; zoosporae usque ad. 9.6 μ long., 3.7 μ lat., per porum terminalem displodenter dimissae, aut sphericae aut elongatae factae admodum cum quiescunt.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C-1-4) seiunctae ex exemplo soli sub *Quercum stellatam,* in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This chaetophoracean alga, isolated into pure culture (C-1-4) from the Carrizo Sands, consists of single cells or often of branching filaments, or both (Figs. 115, 116). The single cells are uninucleate and possess a parietal chloroplast with one or more pyrenoids. The cells of the filaments, especially the terminal ones, have distinctly laminate plastids (Fig. 117). The filaments are limited in length in bacteria-free cultures. Size and shape of the cells vary considerably; cylindrical to barrel-shaped or sub-spherical types are common (Fig. 115, 117, 118). Barrel-shaped cells average 14 μ in length and 9 μ in width. Sub-spherical cells may reach 27 μ in diameter. Cells on two-month-old slants of Bristol's agar are dark green, and their outer wall layers thicken to a maximum of 3.7 μ. The colonies are dry and rough.

Rapid karyokinesis in the uninucleate vegetative cells, followed by cytokinesis, results in the formation of zoospores or aplanospores. A single zoosporangium may contain 100 or more zoospores (Figs. 64, 116, 119) each of which possesses a parietal chloroplast with an inconspicuous pyrenoid. The pyrenoid becomes more conspicuous as the cell matures. Two contractile vacuoles are present, usually in a median position (Fig. 64). An anterior or median stigma and a posterior nucleus also are present. The zoospores, which are usually elongate, are pointed anteriorly, flattened, rounded posteriorly, and bear two flagella of equal length. The zoospore wall is very flexible, for the motile cell may exhibit amoeboid change of form. Zoospores leave the zoosporangium through a pore at one pole of the cell (Figs. 116, 119). They move out slowly at first and later, explosively. They usually become spherical at the end of the motile period (Fig. 65) but sometimes do not (Fig. 120). Zoospores attain a length of 9.6 μ and a width of 3.7 μ. As the cells grow, there develop within them one or more spherical bodies (Fig. 118) which are not vacuoles and do not stain positively with Sudan IV. Direction of division is unrestricted except when filaments are formed. Filaments are initiated when a portion of a cell is walled off and succeeding divisions are in a single plane. The chloroplasts of cells not in filaments contain prominent pyrenoids, but cells of filaments possessing laminate chloroplasts sometimes must be stained with iodine to demonstrate the pyrenoid. Branching usually is initiated at an angle of approximately 90 degrees (Figs. 115, 117) by cell division in a new direction.

Sexual reproduction has not been observed.

Growth is uniformly sparse in antibiotic test plates three weeks after inoculation. No specific inhibition could be noted. Growth is only fair in nutrient broth and also in B-1, maltose, glucose, and urea. Only trace amounts of growth occurred in acetate in darkness, glucose in darkness, and in arabinose. No growth occurred in xylose. The results of one experiment appear in Figure 134.

Pleurastrum erumpens differs from *P. terrestre* (Fritsch and John, 1942), the species which it most closely resembles, in that the filamentous habit is more pronounced, the zoospores are rounded posteriorly rather than fusiform, as usually is the case in *P. terrestre,* which is the more motile of the two species (e.g., it produced

zoospores at two weeks on Bristol's agar); the stigma of the zoospore of *P. erumpens* is larger than that of *P. terrestre,* and the nucleus of the zoospore of *P. erumpens* is posterior, while that of *P. terrestre* is anterior. Growth of *P. terrestre* in the series of differential media does not vary significantly from that of *P. erumpens.* However, colonies of *P. terrestre* are grass-green, while colonies of *P. erumpens* are dark green on the same medium.

B. XANTHOPHYCEAE (HETEROKONTAE)

Of the organisms isolated from soil in the present investigation, two members of the Xanthophyceae are discussed herewith.

BOTRYDIOPSIS arhiza Borzi (Figs. 66–70; 121–125).

Botrydiopsis arhiza was isolated into bacteria-free culture (T-1-2-9-B) from a sample of soil from an oak woods on the east side of State Highway 95, just north of the Williamson County line.

The organism grows well on Bristol's agar, in soil-water media and especially well on Bristol's agar fortified with soil-water supernatant. The genus *Botrydiopsis* is characterized (Pascher, 1939) as possessing free-living, non-motile spherical cells in which there is marked increase in cell size from the minute, spherical derivative of the zoospore (4–6 μ diameter) (Fig. 68) to the mature vegetative cell (Figs. 66, 121, 122) (said to reach 80 μ in diameter). Some individuals (Fig. 123) may be almost ellipsoidal-cylindrical, but the vast majority are spherical. The cell membrane, according to Pascher, may become echinate, although this has not been observed in the present isolate. Furthermore, in cultures in soil-water and Bristol's agar of varying ages, cell size has not exceeded 35 μ. The chloroplasts of immature cells are discoidal segments; those of older cells are somewhat elongate, narrower, and curved segments and largely peripheral (Figs. 66, 69; 121, 122). Their orientation, with respect to the cell surface, is apparently variable (cf. Figs. 121, 122 with 124). As the cells mature, nuclear number increases, as in *Botrydium* and *Protosiphon,* and mature cells contain large numbers of nuclei just within the peripheral chloroplasts (Fig. 125). The center of the cell lumen is filled with colorless cytoplasm rich in granular materials (Fig. 66) which exhibit vigorous Brownian movement and often appear red.

Reproduction by zoospore formation occurs in rapidly growing cultures of *Botrydiopsis arhiza* in freshly transferred material. Rapid cleavages result in the formation of large numbers of zoospores. These (Fig. 67) are typically xanthophycean, 7.8 μ long $\times$ 3–4 μ wide and undergo amoeboid changes of form. The zoospores contain 1–3 chloroplasts, a single nucleus and two contractile vacuoles. They lack a stigma which is prominent in other species. Motility is of short duration and the zoospores become spherical immediately upon quiescence (Fig. 68). This is followed by marked increase in cell size and nuclear number, alluded to above. In cultures on Bristol's agar, more than two weeks old, zoospore production is re-

placed by aplanospore formation (Fig. 124). Sexual reproduction has not been observed.

On Bristol's agar cultures, two weeks old, *B. arhiza* is dry, as it appears macroscopically. Under a magnification of 20 × with transmitted light, the plant mass is minutely glomerulate. Cells mounted from this medium do not cohere, and India ink indicates that both common and individual matrices are absent.

The problem of specific identification within the genus *Botrydiopsis* is not a simple one. Pascher (1939) and Vischer (1945) recognize only four well-defined species, namely *B. arhiza* Borzi, *B. anglica* Fritsch and John, *B. intercedens* (Pringsheim) Vischer, and *B. alpina* Vischer, although several others have been described. Inadequacy of description in the past of organisms from nature and mixed cultures have contributed to the confusion of taxa in this genus. It is clear, for example, that culture #87 of the Culture Collection of Algae, Indiana University, cannot be *B. arhiza* Borzi, which it is labeled, not only because of its lesser cell size, but especially because its zoospores have stigmata which are absent from the zoospores of *B. arhiza* Borzi, according to Pascher. As Pascher himself emphasizes (1939, p. 393: "Jedenfalls bedürfen vor allem die Erdformen unter den *Botrydiopsis*-arten einer kritischen und Klaren Erfassung"); the soil species require careful, comparative investigation. Such a study is in progress.

PSEUDOBUMILLERIOPSIS pyrenoidosa gen. et sp. nov. (Figs. 71–74; 126–132).

Cellulae bacilliformes usque ad 40 μ long., et 12 μ lat., aut in filamentis nonramosis brevibus coniunctae, nucleos unum ad aliquot et plastides laminatas unam ad aliquot, amnibus pyrenoideo praeditis, habentes. Matrices nullae.

Reproductio asexualis formatione 1–16 zoosporarum aplanosporarumve per dissolutionem membranarum parentium superpositarum effecta. Zoosporae duo flagella longitudine satis imparia, inter se satis remota inserta, atque chloroplastidem parietalem, stigma anterius, atque vacuolas contractiles parvas duas vel plures habentes. Zoosporae amoeboideae aut celeriter mobiles, forma variantes, ad 12 μ long. sphericae abrupte factae cum quiescunt.

Reproductio sexualis non observata.

Origo: Cellulae in culturam puram (C-1-19) seiunctae ex exemplo soli sub *Quercum stellatam,* in formatio Carrizo Sands nomine, distante 5.1 milia passuum versus orientem a loco dicto McMahan, Caldwell County, Texas. 11 m. Sept. an. 1958.

This organism was isolated into bacteria-free culture (C-1-19) from the Carrizo Sands. It is a rod-shaped, xanthophycean alga with broadly rounded poles (Figs. 71, 126) and laminate plastids with pyrenoids (Figs. 126, 131). The cells possess one to several nuclei (Fig. 127), depending on the size of the cell. Cells attain a maximum length of 40 μ and a maximum width of 12 μ. However, most of the cells are smaller (26 μ × 9.5 μ). Cell walls are composed of overlapping portions (Fig.

74). Occasionally, short filaments are formed. No matrix is demonstrable. Two-week-old colonies on Bristol's agar are smooth and dry. Cultures which have been maintained for two months on agar are green, and no significant thickening of the cell walls occurs.

Asexual reproduction is by formation of zoospores or aplanospores (Figs. 72, 73, 128, 129). Zoospores vary considerably in shape and are often flattened. They have a parietal plastid, two flagella which are quite unequal in length (Fig. 73) and inserted some distance apart, an anterior stigma, and two or more small contractile vacuoles. Zoospores are about 8–12 μ in length. One to sixteen zoospores may be released from a single cell. Release of the zoospores is effected by the dissolution of the parent walls where they overlap (Figs. 72, 128). Occasionally, zoospores are not released and, as aplanospores, they form a new wall within the parent cell (Fig. 130). Zoospores may be rapidly motile or exhibit slow, amoeboid movements. After a short period of motility, they become spherical (Fig. 129).

Sexual reproduction has not been observed.

The alga does not grow in nutrient broth, and it is inhibited by polymyxin B and dihydrostreptomycin (Fig. 132). Growth of the alga is good in B-1; fair in glucose; occurs in trace amounts in maltose and glucose in darkness; and no growth occurs in other media tested.

The present organism most clearly resembles the genus *Bumilleriopsis* Printz, but it has been classified as a new genus, *Pseudobumilleriopsis,* because pyrenoids are lacking in *Bumilleriopsis.*

Summary

This paper summarizes investigations of the algal flora in soils of the Carrizo Sands, Caldwell County and of Williamson County, Texas. From approximately 400 isolates, 20 organisms are discussed, of which 18 are described as new species, two of them typifying new genera. The organisms comprising the last group have been grown in bacteria-free condition and type cultures have been deposited in the Culture Collection of Algae, Indiana University, Bloomington, Indiana. Herbarium specimens have been placed in the Herbaria of The University of Texas and of the Chicago Natural History Museum. The organisms described herein for the first time are:

CHLOROPHYCEAE

Chlamydomonas actinochloris sp. nov.
Chlamydomonas radiata sp. nov.
Chlamydomonas akinetos sp. nov.
Chlamydomonas pyrenoidosa sp. nov.
Chlamydomonas aggregata sp. nov.
Chlamydomonas appendiculata sp. nov.
Chlamydomonas typica sp. nov.

Chlorococcum ellipsoideum sp. nov.
Chlorococcum scabellum sp. nov.
Chlorococcum intermedium sp. nov.
Neochloris pseudoalveolaris sp. nov.
Spongiococcum multinucleatum sp. nov.
Spongiococcum excentricum sp. nov. (from Iowa)
Spongiochloris lamellata sp. nov.
Hormidium sterile sp. nov.
Pseudoschizomeris caudata gen. et sp. nov.
Pleurastrum erumpens sp. nov.

XANTHOPHYCEAE

Pseudobumilleriopsis pyrenoidosa gen. et sp. nov.

As an aid to taxonomic characterization, special attention was devoted to devising techniques which would provide additional differentiating criteria. The data obtained have, in fact, helped to clarify the classification of several groups of soil algae. Although the determination of the genera of the spherical, unicellular Chlorococcales has been facilitated by the establishment of reliable criteria by Starr (1955), the determination of species by exclusively morphological attributes remains difficult. The writers have investigated attributes other than strictly morphological, including colony characters, color of two-month-old and older cultures grown on standard media under standardized conditions, sensitivity to antibiotics, and growth in differential media.

The supplementary attributes found useful for identifying chlorococcalean algae were employed also in the studies of volvocalean, ulotrichalean and xanthophycean organisms described in this report.

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Figs. 1–16.

Figs. 1–3.—*Chlamydomonas actinochloris*.—Fig. 1. Vegetative individual in median optical section (m.o.s.).—Fig. 2. Vegetative cell in surface view.—Fig. 3. Eight young daughter cells enclosed within parental membrane.

Figs. 4, 5.—*Chlamydomonas radiata*.—Fig. 4. Vegetative cell in median optical section.—Fig. 5. The same in surface view.

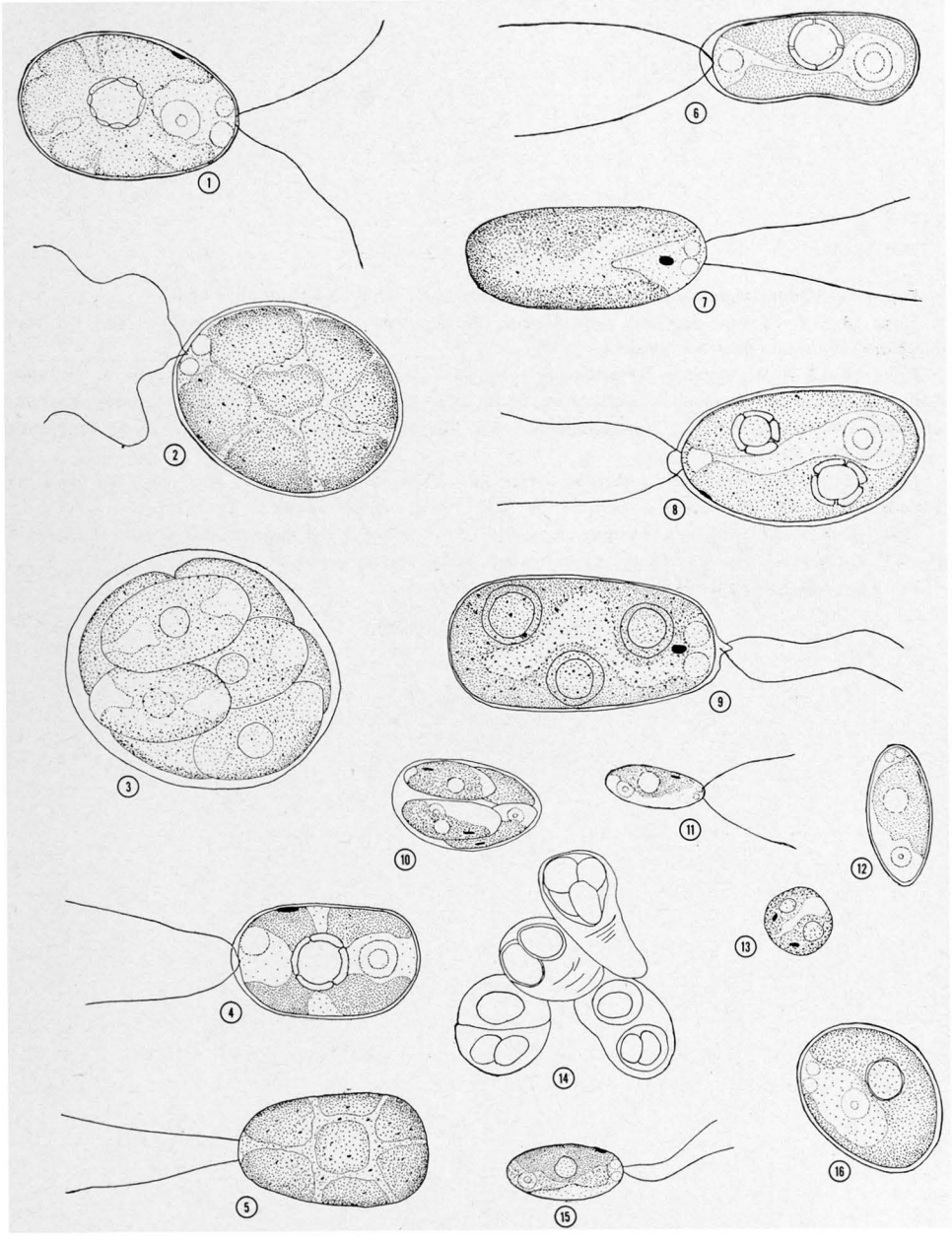
Figs. 6, 7.—*Chlamydomonas akinetos*.—Fig. 6. Vegetative cell in m.o.s.—Fig. 7. The same in surface view.

Figs. 8, 9.—*Chlamydomonas pyrenoidosa*.—Fig. 8. Vegetative cell in m.o.s.—Fig. 9. The same in surface view.

Figs. 10–13.—*Chlamydomonas aggregata*.—Fig. 10. Four daughter cells within parental cell wall.—Fig. 11. Immature motile individual.—Fig. 12. Enlarging non-motile individual prior to cell division.—Fig. 13. Recently formed zygote.

Figs. 14–16.—*Chlamydomonas appendiculata*.—Fig. 14. Outline drawing of cells from culture in stationary phase of growth showing unipolar thickening of outer wall layer.—Fig. 15. Motile individual.—Fig. 16. Non-motile individual just prior to cell division.

(Magnification, × 2000, except Fig. 14 which is × 1200.)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Figs. 17–33.

Fig. 17.—*Chlamydomonas appendiculata*. Daughter cells within parental envelope.

Figs. 18, 19.—*Chlamydomonas typica*.—Fig. 18. Vegetative individual in m.o.s.—Fig. 19. Four daughter cells within parental cell wall.

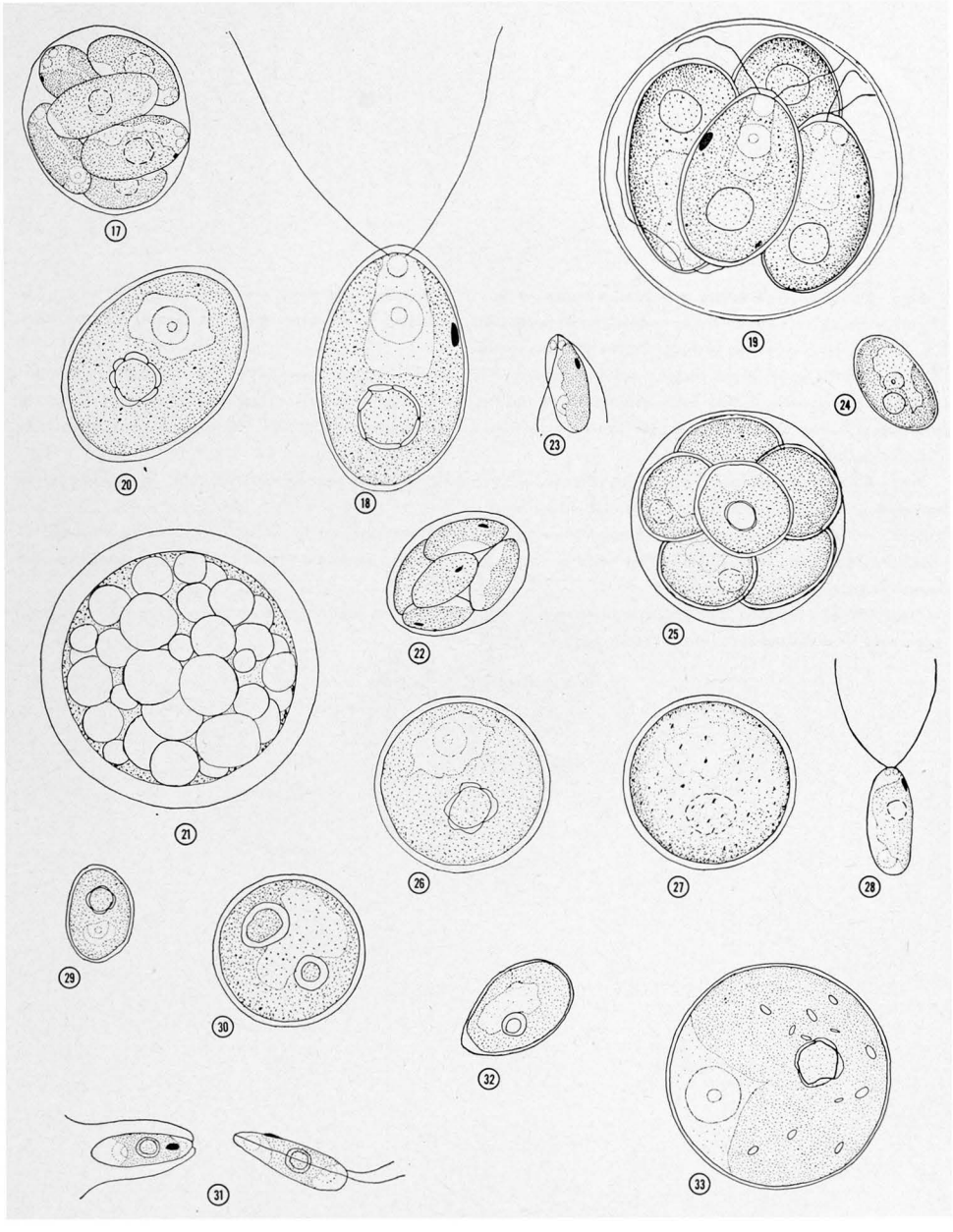
Figs. 20–25.—*Chlorococcum ellipsoideum*.—Fig. 20. Almost mature vegetative cell, in m.o.s.—Fig. 21. Mature cell from culture in stationary phase of growth; note abundant oil droplets and thickening of outer wall layer.—Fig. 22. Zoosporangium.—Fig. 23. Single zoospore.— Fig. 24. Young vegetative cell.—Fig. 25. Aplanosporangium.

Figs. 26–29.—*Chlorococcum scabellum*.—Fig. 26. Vegetative cell, m.o.s.—Fig. 27. The same in surface view.—Fig. 28. Single zoospore.—Fig. 29. Young vegetative cell.

Figs. 30–32.—*Chlorococcum intermedium*.—Fig. 30. Vegetative cell in m.o.s. just prior to division.—Fig. 31. Zoospores.—Fig. 32. Young vegetative cell, accuminate at one pole.

Fig. 33.—*Neochloris pseudoalveolaris*. Vegetative cell in m.o.s

(Magnification, × 2000.)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Figs. 34–53.

Figs. 34–37.—*Neochloris pseudoalveolaris*.—Fig. 34. Mature vegetative cell, surface view.—Fig. 35. Young vegetative cell or aplanospore with zoospores escaping in vesicle.—Fig. 36. Single zoospore.—Fig. 37. Young vegetative cell just derived from zoospore.

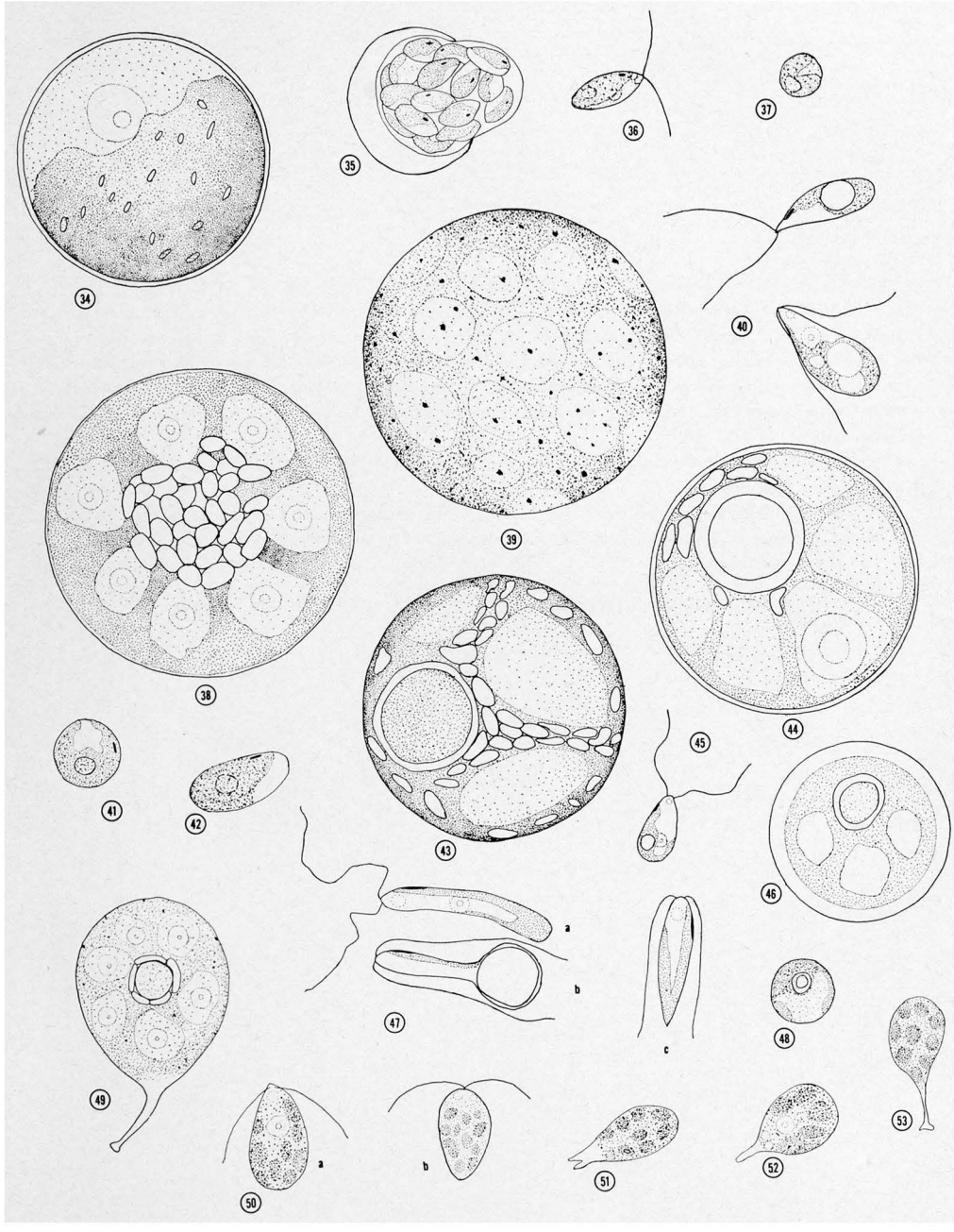
Figs. 38–42.—*Spongiococcum multinucleatum*.—Fig. 38. Mature vegetative cell in m.o.s.; granules in center are starch grains surrounding pyrenoid; nuclei occupy colorless cytoplasm in spongy areas of *chloroplast*.—Fig. 39. The same, surface view.—Fig. 40. Zoospores.—Figs. 41, 42. Young vegetative cells recently derived from zoospores.

Figs. 43–45.—*Spongiococcum excentricum*.—Figs. 43, 44. Mature vegetative cells in surface view and optical section respectively.—Fig. 45. Zoospore.

Figs. 46–48.—*Spongiochloris lamellata*.—Fig. 46. Vegetative cell in m.o.s.—Fig. 47. Variation of zoospore form, the individual at (b) with a large posterior oil droplet—Fig. 48. Young vegetative cell recently derived from zoospore.

Figs. 49–53.—*Characium polymorphum*.—Fig. 49. Vegetative individual in m.o.s.—Figs. 50–53. Zoospore and its development into a young vegetative cell.

(Magnification, × 2000.)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Figs. 54–70.

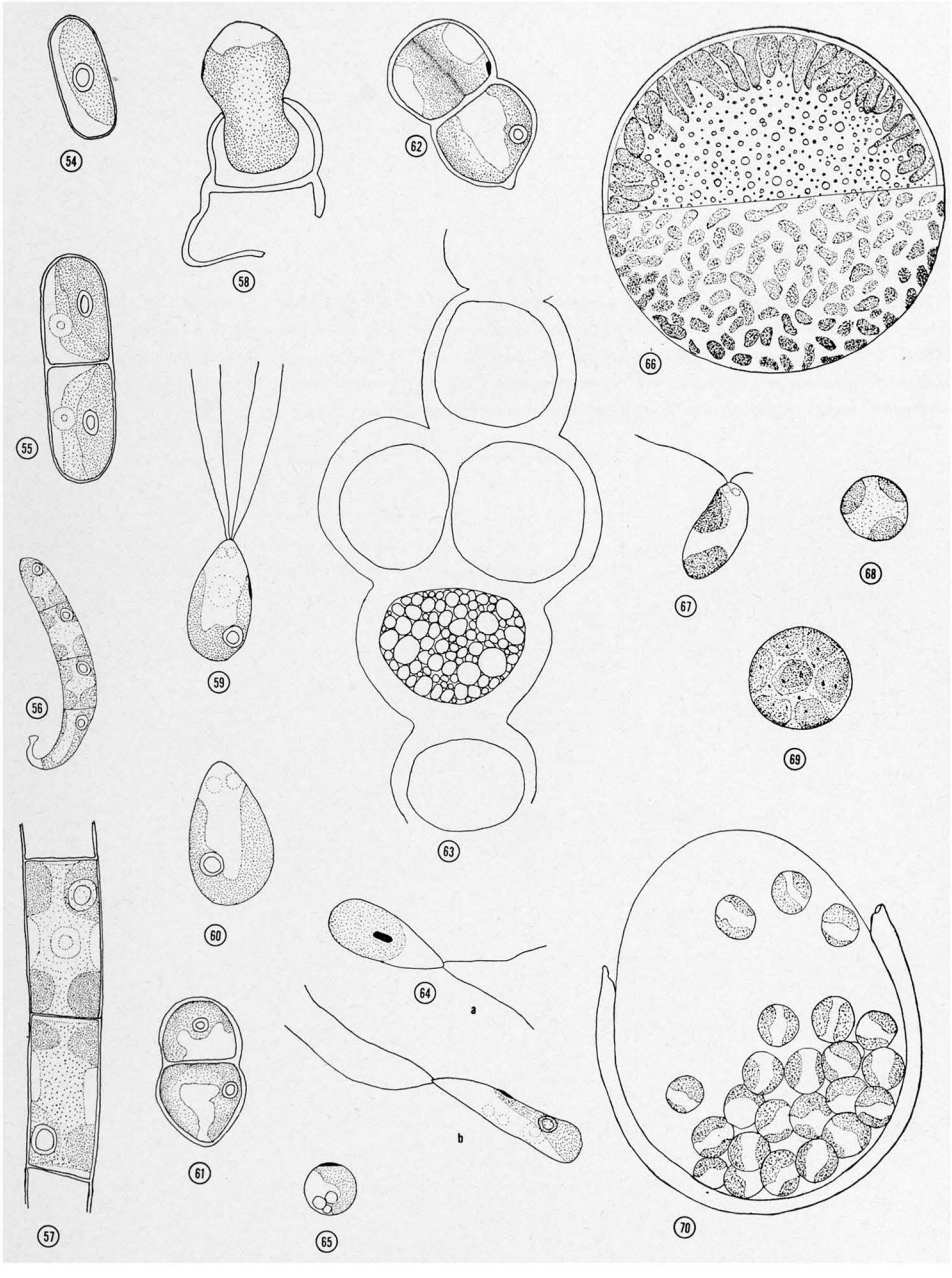
Figs. 54–55.—*Hormidium sterile*.—Fig. 54. Unicellular segment.—Fig. 55. Two-celled filament.

Figs. 56–63.—*Pseudoschizomeris caudata*.—Fig. 56. Young vegetative individual; note processes on plastid.—Fig. 57. Segment of preceding at higher magnification.—Fig. 58. Escape of zoospore.—Fig. 59. Single motile zoospore.—Figs. 60–62. Development of young vegetative individual from zoospore; note persistent stigma.—Fig. 63. Pseudopalmelloid organization of plant from old soil-water culture; note thickened outer wall layers and numerous (orange-colored) oil droplets (protoplasts of all but one cell omitted).

Figs. 64, 65.—*Pleurastrum erumpens*.—Fig. 64. Zoospores.—Fig. 65. Young vegetative cell recently derived from a zoospore.

Figs. 66–70.—*Botrydiopsis arhiza*.—Fig. 66. Maturing vegetative cell, the upper portion in m.o.s., the lower in surface view.—Fig. 67. Single zoospore.—Figs. 68, 69. Immature vegetative cells.—Fig. 70. Aplanosporangium.

(Magnifications, × 2000, except Fig. 56, × 800, and Figs. 61, 62, × 1600.)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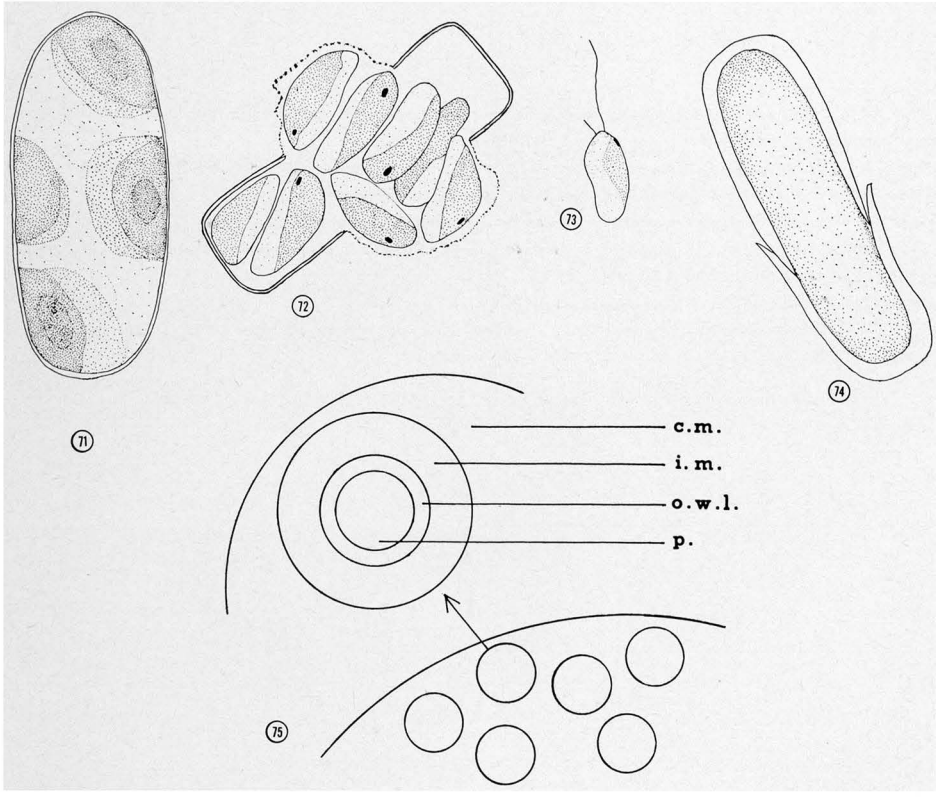
Figs. 71–75.

Figs. 71–74.—*Pseudobumilleriopsis pyrenoidosa*.—Fig. 71. Vegetative cell.—Fig. 72. Zoosporogenesis.—Fig. 73 Single zoospore.—Fig. 74. Cell treated with 10% KOH showing two-parted wall.

Fig. 75.—Diagrammatic representation of organization of surface membranes in unicellular algae (adapted from Arce, 1957).—c.m., common matrix; i.m., individual matrix; o.w.l., outer wall layer; p., protoplast; below a group of cells embedded in a common matrix.

(Magnification, × 2000.)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c.m.
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Figs. 76–83.

Fig. 76.—*Chlamydomonas radiata*. Vegetative cells and daughter cell formation; note radiate chloroplast with axial pyrenoid.

Figs. 77–79.—*Chlamydomonas akinetos*.—Fig. 77. Young vegetative cell showing parietal plastid and pyrenoid.—Fig. 78. Single akinete.—Fig. 79. Daughter cell formation.

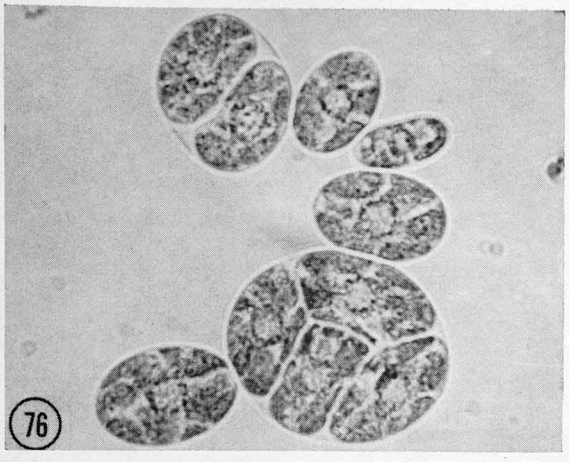
Fig. 80.—*Chlamydomonas pyrenoidosa*. Vegetative cells forming daughter cells.

Fig. 81.—*Chlamydomonas aggregata*. General view of portion of two-week-old culture on Bristol's agar; a majority of cells forming daughter cells.

Fig. 82.—*Chlamydomonas aggregata*; zygotes.

Fig. 83.—*Chlamydomonas appendiculata*; general view of portion of agar colony after two weeks' growth on Bristol's agar, mounted in India ink to show matrix.

(Magnification × 1200, except Fig. 77, × 2400; Figs. 80, 81, 83, × 600.)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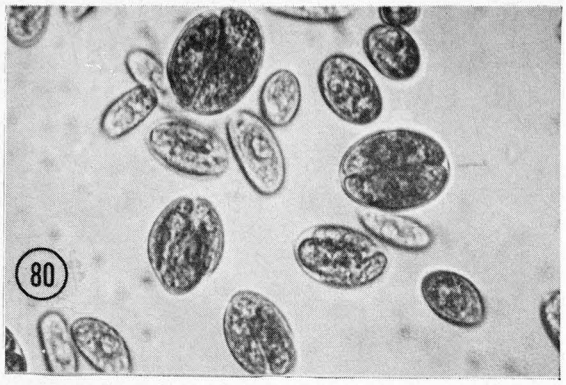
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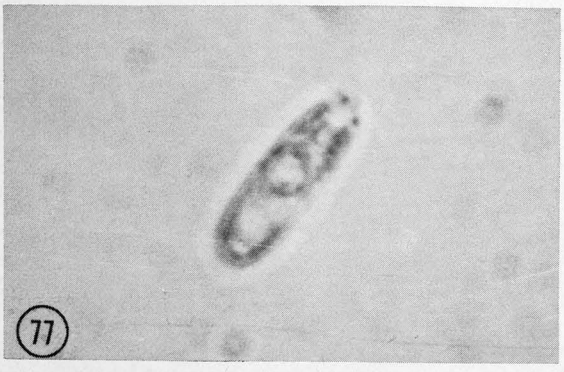
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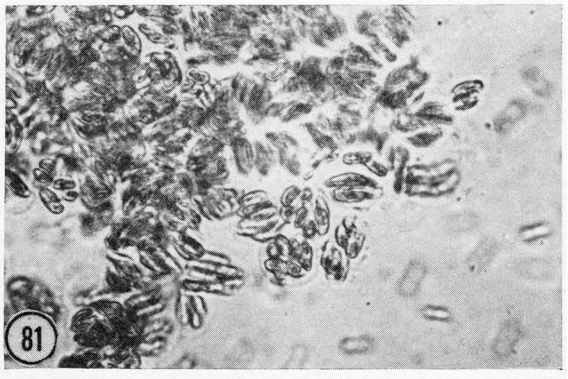
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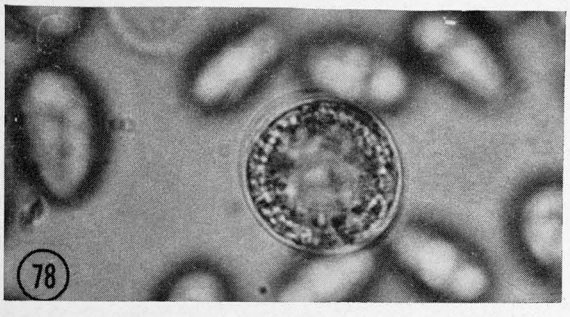
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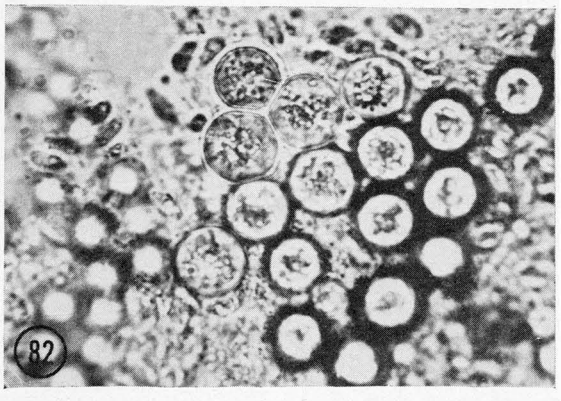
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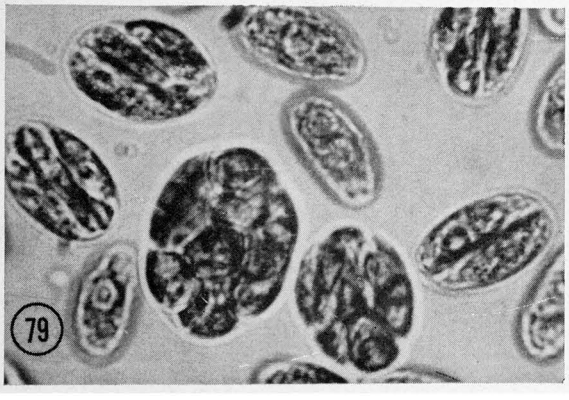
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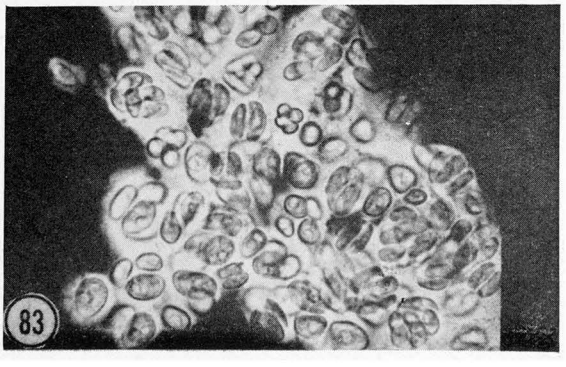
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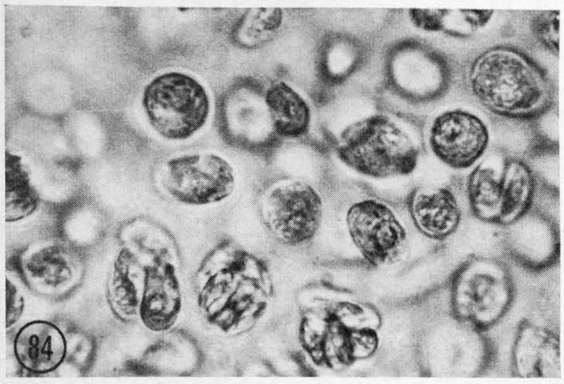
Figs. 84–91.

Figs. 84–85.—*Chlamydomonas appendiculata*.—Fig. 84. Non-motile cells; division stages.—Fig. 85. Cells from older culture stained with methylene blue; note unipolar thickenings on some of the cells (upper center).

Figs. 86–89.—*Chlamydomonas typica*.—Fig. 86. General view of vegetative cells and daughter cell formation; note contractile vacuoles in several individuals.—Fig. 87. Motile cell mounted in India ink; note individual matrix and median stigma.—Fig. 88. Portion of two-week-old Bristol's agar population mounted in India ink; note palmelloid condition.—Fig. 89. Portion of preceding, enlarged.

Figs. 90–91.—*Chlorococcum ellipsoideum*.—Fig. 90. General view of two-week-old Bristol's agar culture; note numerous zoosporangia.—Fig. 91. Same as preceding; cells mounted in India ink; note persistent, empty cell walls, and numerous zoosporangia.

(Magnification × 1200, except Figs. 85, 88, 90, 91, × 600)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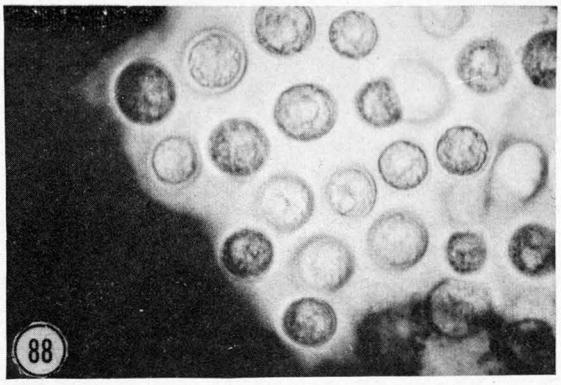
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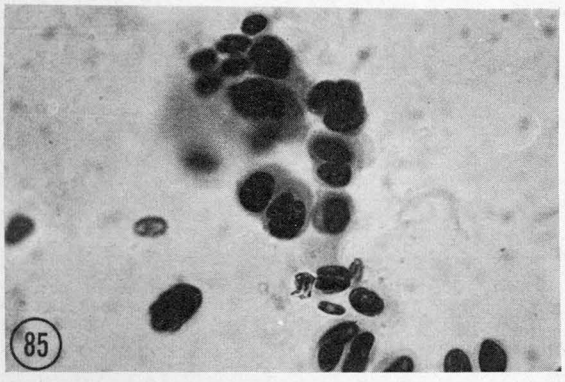
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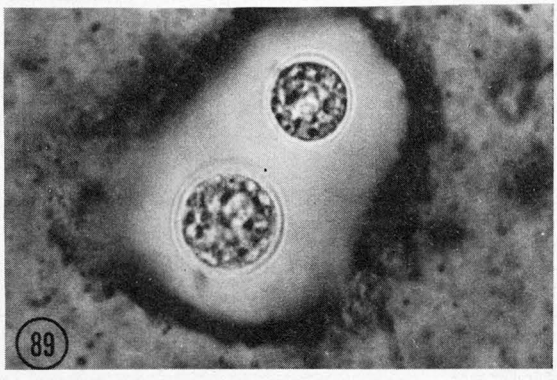
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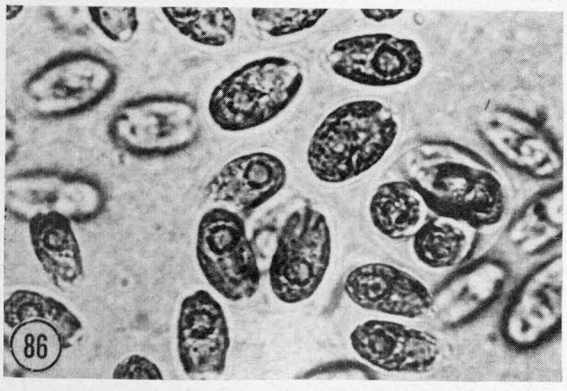
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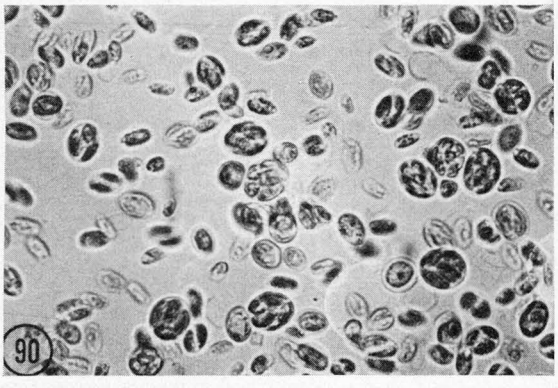
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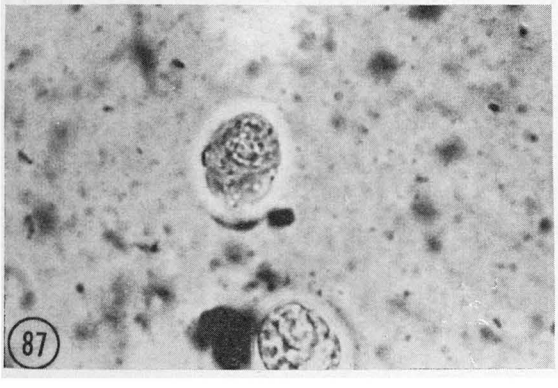
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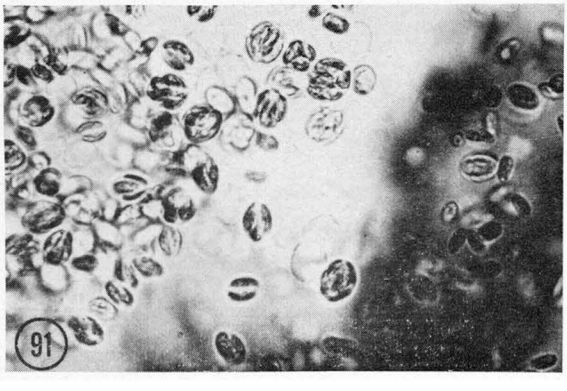
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Figs. 92–99.

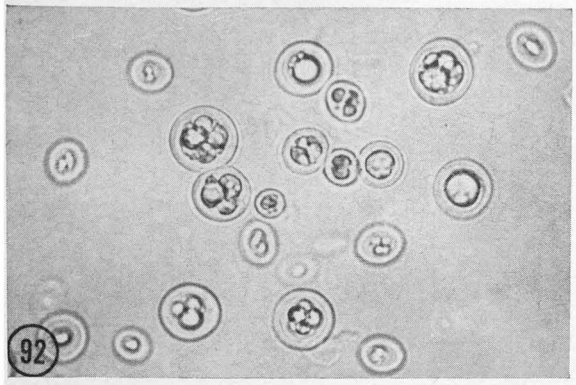
Fig. 92.—*Chlorococcum ellipsoideum*.—Cells from six-month-old culture; note thickening of outer wall layer, abundant oil droplets, and presence of ellipsoidal cells.

Figs. 93, 94.—*Chlorococcum scabellum*.—Fig. 93. Cells from two-month-old culture: vegetative cells and aplanosporangia.—Fig. 94. Same as preceding undergoing zoosporogenesis; note thickening of outer wall layer.—Fig. 95. Cells from six-month-old culture on Bistol's agar; note pronounced thickening of outer wall layer.

Figs. 96–97.—*Chlorococcum intermedium*.—Fig. 96. Cells from two-week-old culture on Bristol's agar; note young vegetative cells, cells divided to form tetrahedral groups of four and zoosporangium above.—Fig. 97. Portion of similar culture enlarged; note zoosporangium at left slightly out of focus.

Figs. 98–99.—*Neochloris pseudoalveolaris*.—Fig. 98. Cells from two-week-old Bristol's agar culture; note massive chloroplasts which are dividing in several individuals.—Fig. 99. Cells from six-month-old culture on Bristol's agar; note slight thickening of outer wall layer and abundant oil droplets in protoplasts.

(Magnification, $\times$1200, except Figs. 92, 94, 95, 98 which are $\times$ 600.)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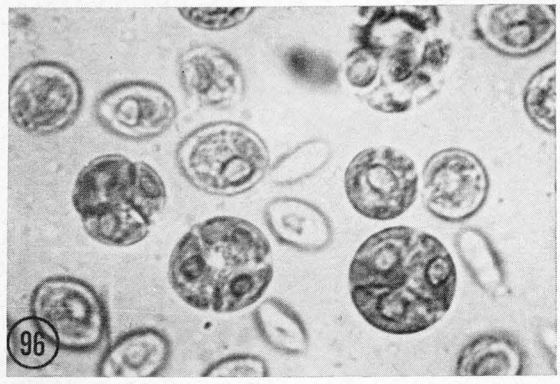
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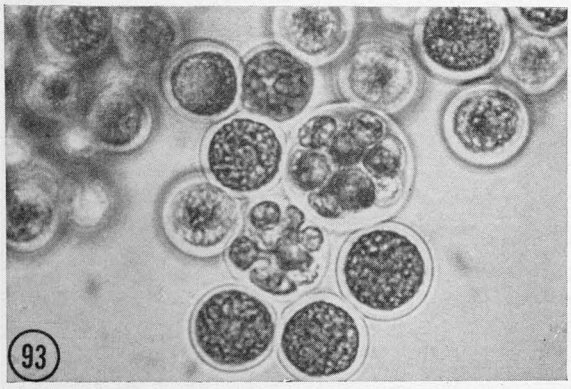
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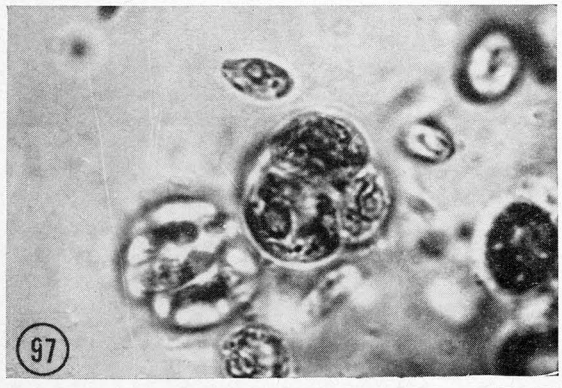
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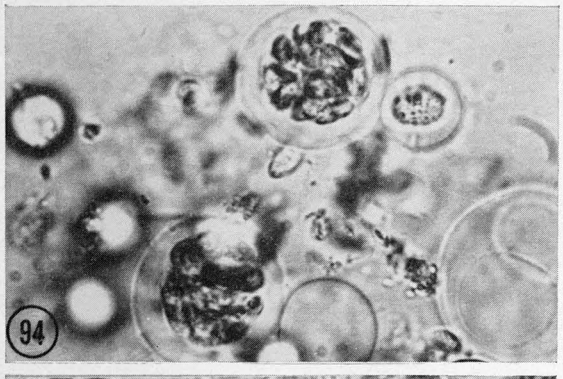
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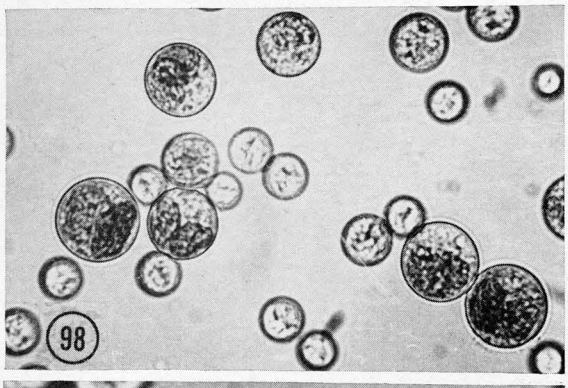
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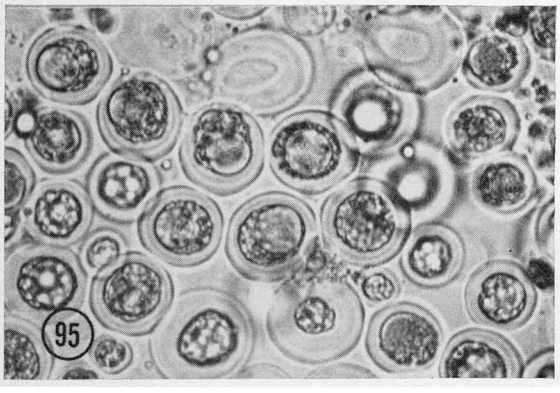
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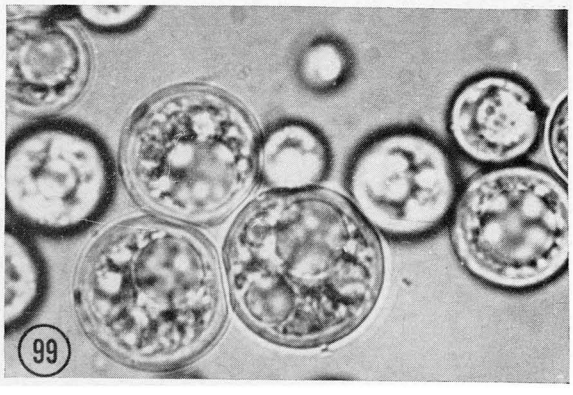
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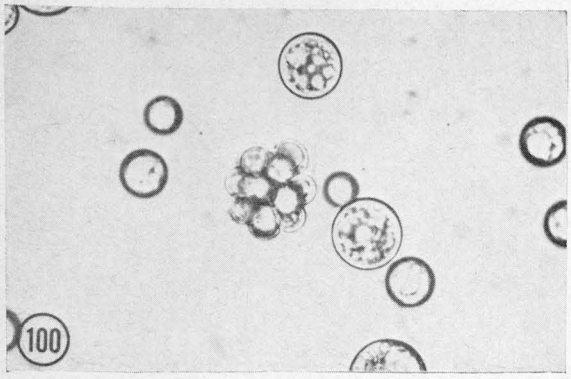
Figs. 100–107.

Fig. 100.—*Neochloris pseudoalveolaris*. General view showing cluster of autospores.

Figs. 101–104.—*Spongiococcum multinucleatum*.—Fig. 101. Young cells developing from zoospores; note spherical cells in which the sponge-like nature of the chloroplast is apparent.—Fig. 102. Almost mature vegetative cell showing central pyrenoid and spongy nature of the chloroplast.—Fig. 103. Portion of two-week-old Bristol's agar culture under low magnification; note rough appearance.—Fig. 104. Group of vegetative cells and zoospores with several empty zoosporangia.

Figs. 105–107.—*Spongiococcum excentricum*.—Fig. 105. Vegetative cells.—Fig. 106. Vegetative cells and zoosporangium.—Fig. 107. Aplanosporangium liberating aplanospores.

(Magnification, × 1200, except Figs. 101, 104, 107 which are × 600 and Fig. 103, × 20.)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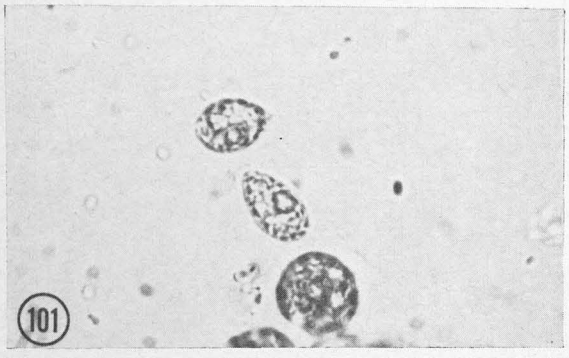
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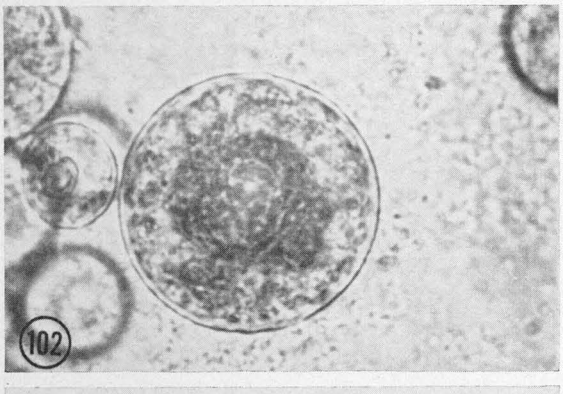
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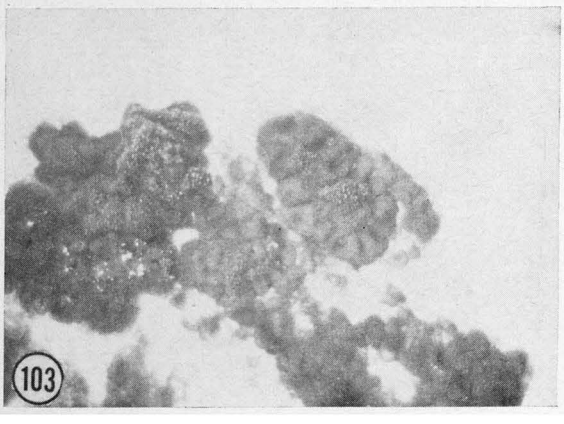
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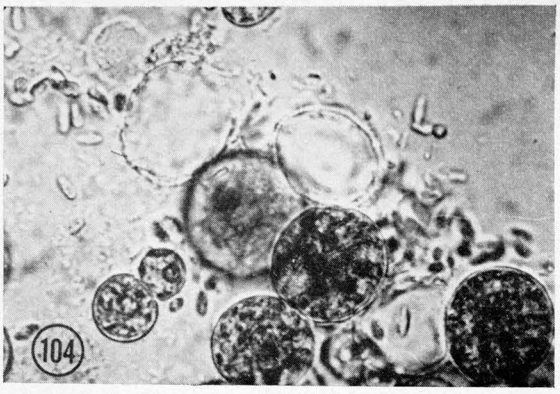
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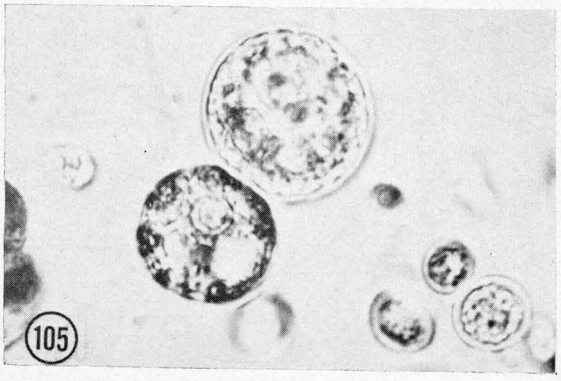
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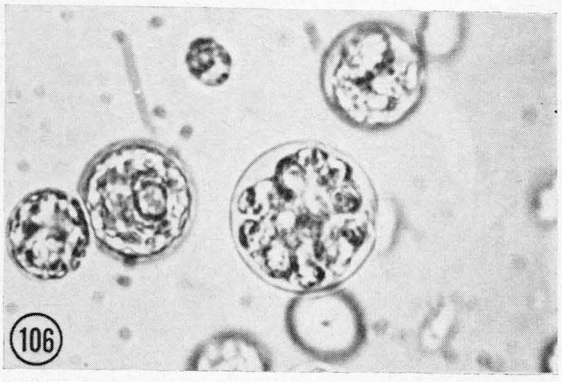
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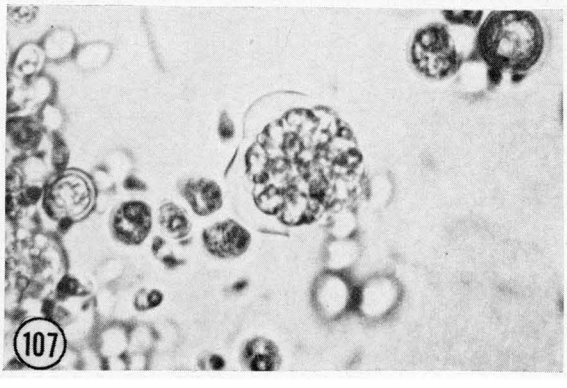
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Figs. 108–115.

Figs. 108–110.—*Spongiochloris lamellata*.—Fig. 108. Mature vegetative cell; note lamellate wall layers.—Fig. 109. Liberation of zoospores.—Fig. 110. Enlarged view of zoosporangium in which a number of zoospores have been retained; note stigma in one.

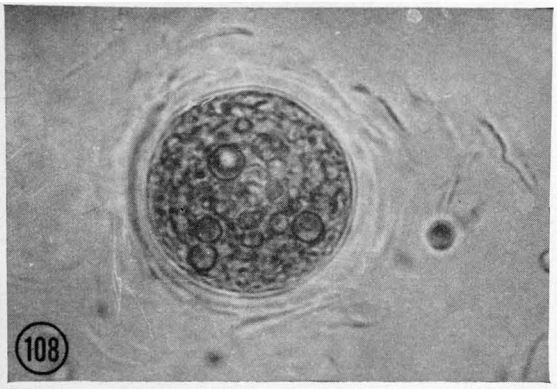
Fig. 111.—*Characium polymorphum*. Young cells which arose from zoospores that failed to escape from parental cell wall.

Fig. 112.—*Hormidium sterile*. Short filament and single cells.

Figs. 113–114.—*Pseudoschizomeris caudata*.—Fig. 113. Uniseriate and pluriseriate individuals.—Fig. 114. Young plants which have developed from zoospores; note caudate, holdfast-like bases.

Fig. 115.—*Pleurastrum erumpens*. Filamentous habit.

(Magnification, × 1200, except Figs. 109, 111, 115 which are × 600.)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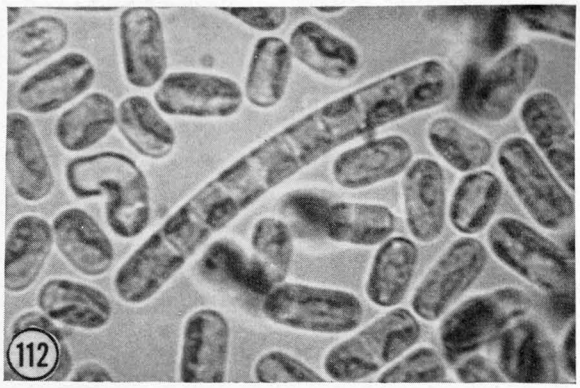
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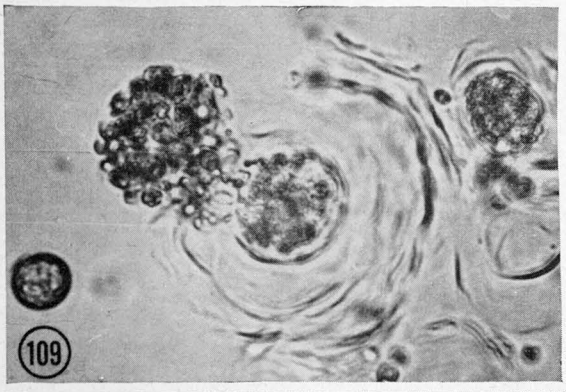
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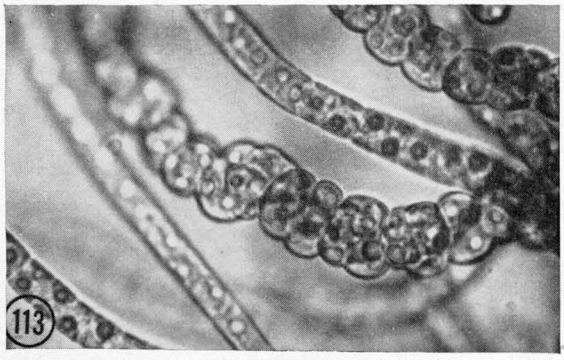
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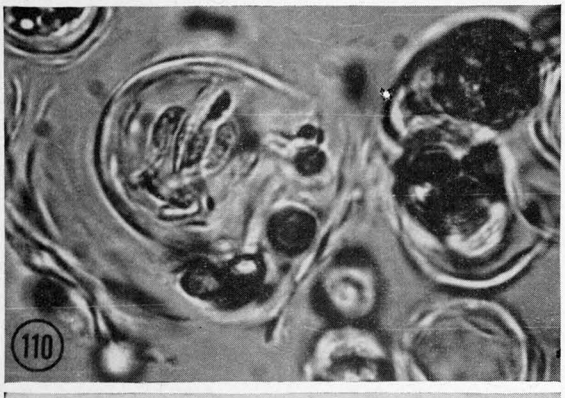
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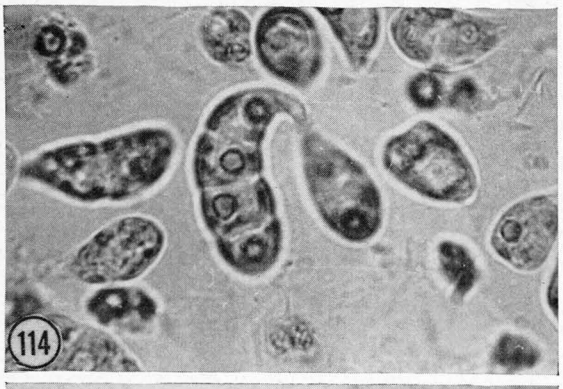
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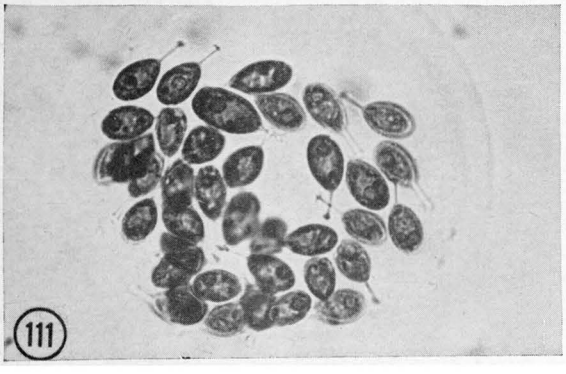
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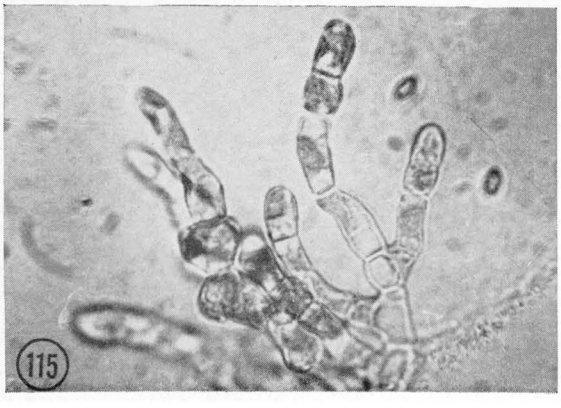
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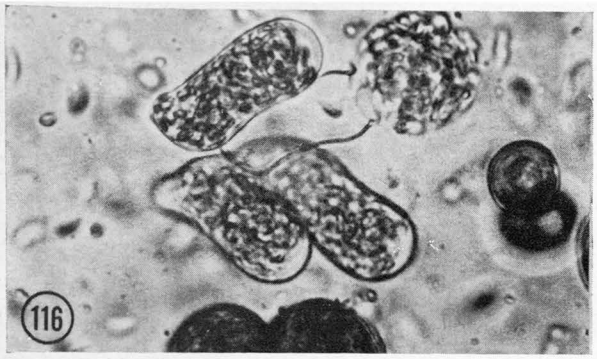
Figs. 116–123.

Figs. 116–120.—*Pleurastrum erumpens*.—Fig. 116. Unicellular stage undergoing zoosporogenesis.—Fig. 117. Detail of filamentous phase showing parietal girdle-like chloroplasts.—Fig. 118. Sub-spherical cells, one of which is undergoing zoosporogenesis.—Fig. 119. Liberation of zoospores in a vesicle.—Fig. 120. Cluster of zoospores beginning to develop into vegetative cells; in this case the zoospores have not become spherical upon quiescence.

Figs. 121–123.—*Botrydiopsis arhiza*.—Fig. 121. Vegetative cell in surface view; note numerous chloroplasts.—Fig. 122. The same in optical section; note central granular cytoplasm.—Fig. 123. Sub-cylindrical vegetative cell.

(Magnification, × 1200, except Figs. 116, 119, 120 which are × 600.)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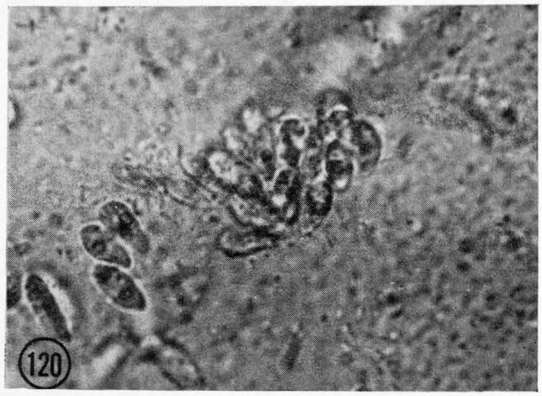
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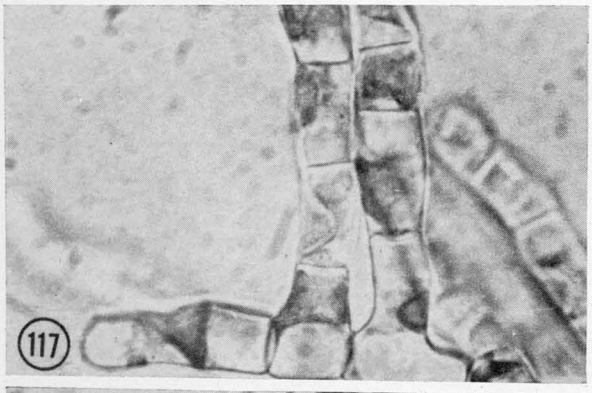
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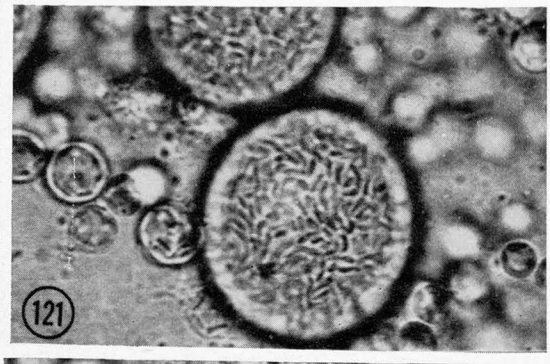
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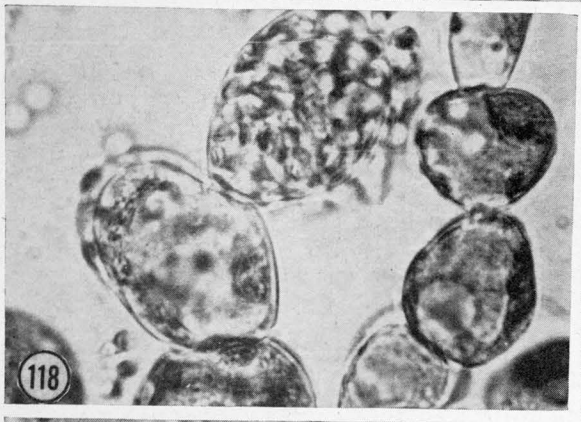
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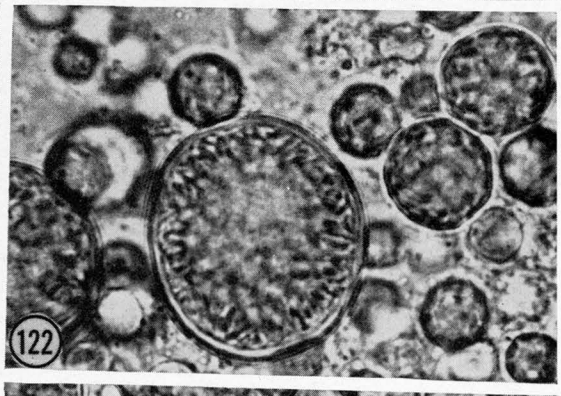
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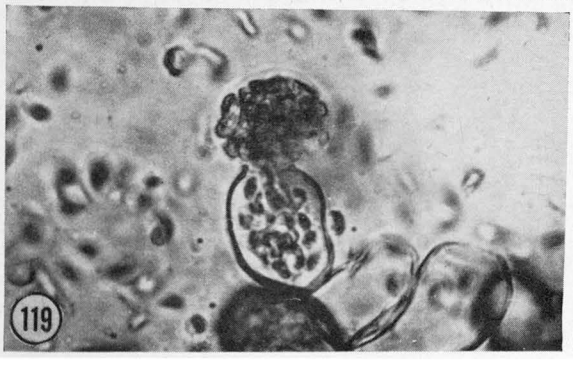
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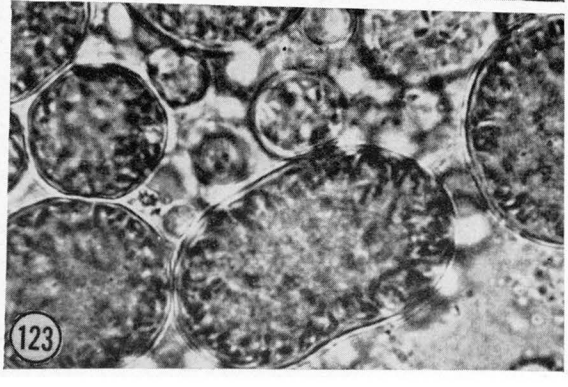
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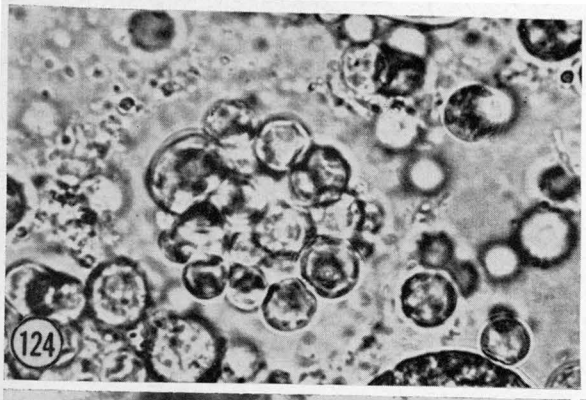
Figs. 124–131.

Figs. 124–125.—*Botrydiopsis arhiza*.—Fig. 124. Aplanosporangium; note variation in aplanospore size.—Fig. 125. M.o.s. and surface views of vegetative cells stained in the Azure-A nuclear stain showing multinucleate condition.

Figs. 126–131.—*Pseudobumilleriopsis pyrenoidosa*.—Fig. 126. Vegetative cells; note parietal plastids.—Fig. 127. Cell stained with Azure A; note multinucleate condition.—Fig. 128. Liberation of zoospores.—Fig. 129. Motile zoospore (note heterokontan flagella in one above) and zoospores which have recently become quiescent.—Fig. 130. Double wall formed by retention of zoospore within parental cell; the former ultimately liberated zoospores.—Fig. 131. Cell stained with iodine; note pyrenoids.

(Magnifications, × 1200, except Figs. 127, 128 which are × 600.)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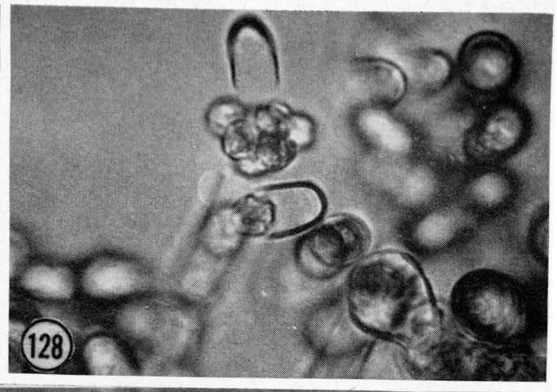
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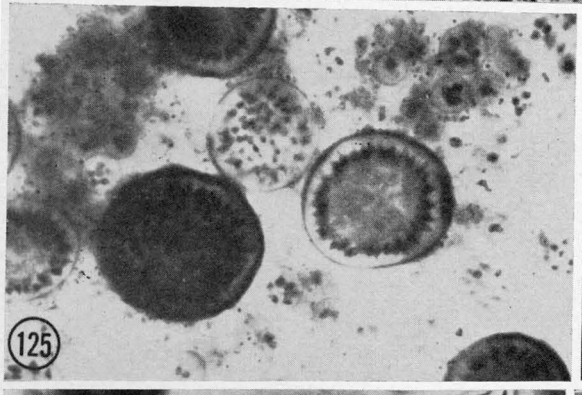
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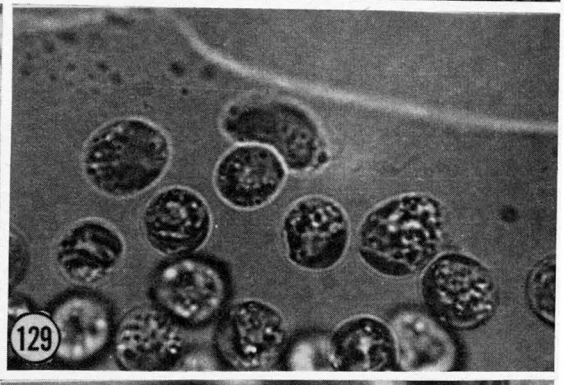
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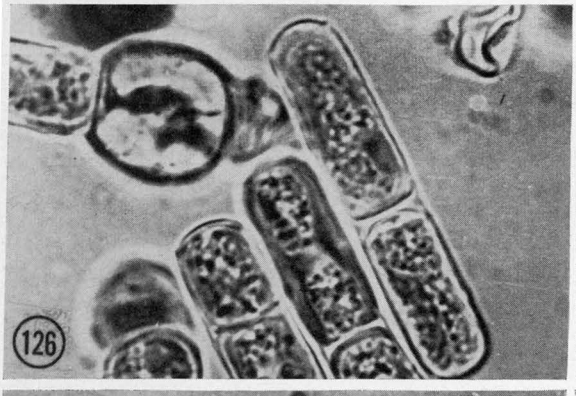
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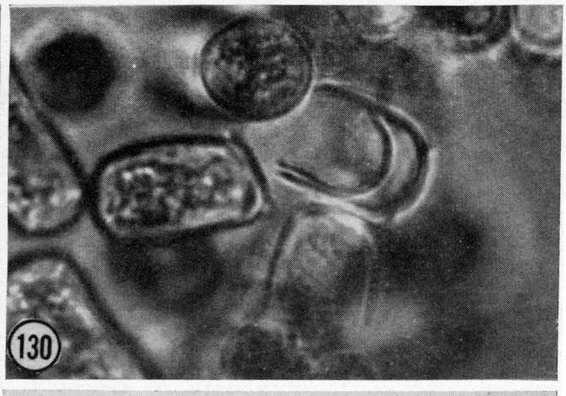
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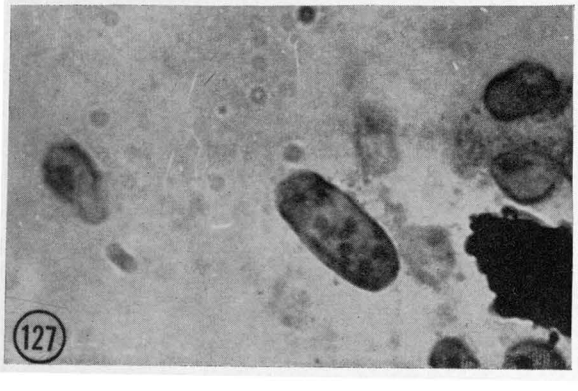
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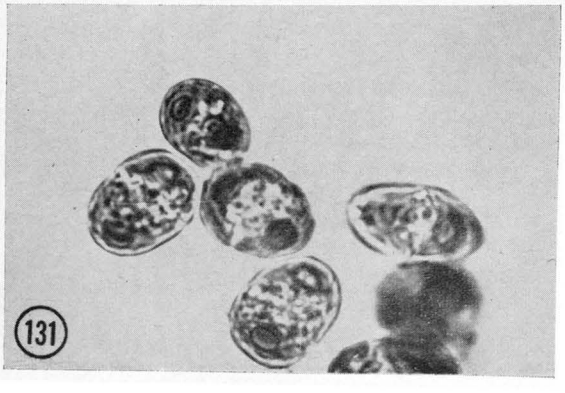
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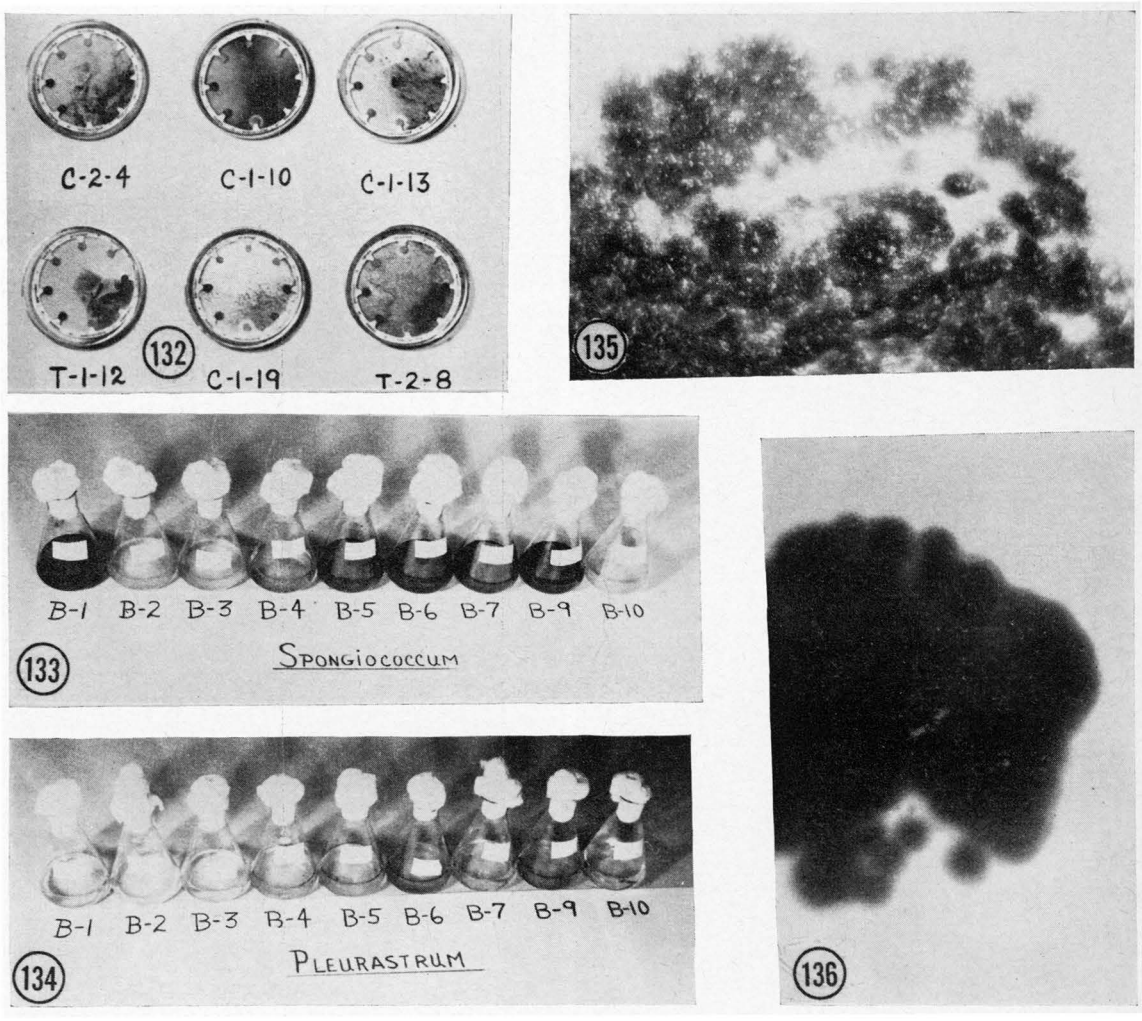

Figs. 132–136.

Fig. 132. Six examples of the "Unidisk test": C-2-4, *Chlamydomonas pyrenoidosa;* C-1-10, C. *radiata;* C-1-13, *Chlorococcum intermedium;* T-1-12, *Chlamydomonas aggregata;* C-1-19, *Pseudobumilleriopsis pyrenoidosa;* T-2-8, *Chlorosarcinopsis* sp.

Fig. 133, 134. Growth of a species of *Spongiococcum* and of *Pleurastrum*, respectively, in differential media series.

Fig. 135.—*Spongiochloris lamellata;* portion of two-week-old colony on Bristol's agar under low magnification; note roughness.

Fig. 136.—*Chlamydomonas appendiculata;* portion of smooth, two-week-old colony on Bristol's agar under low magnification.

(Magnifications, Figs. 135, 136, × 30.)