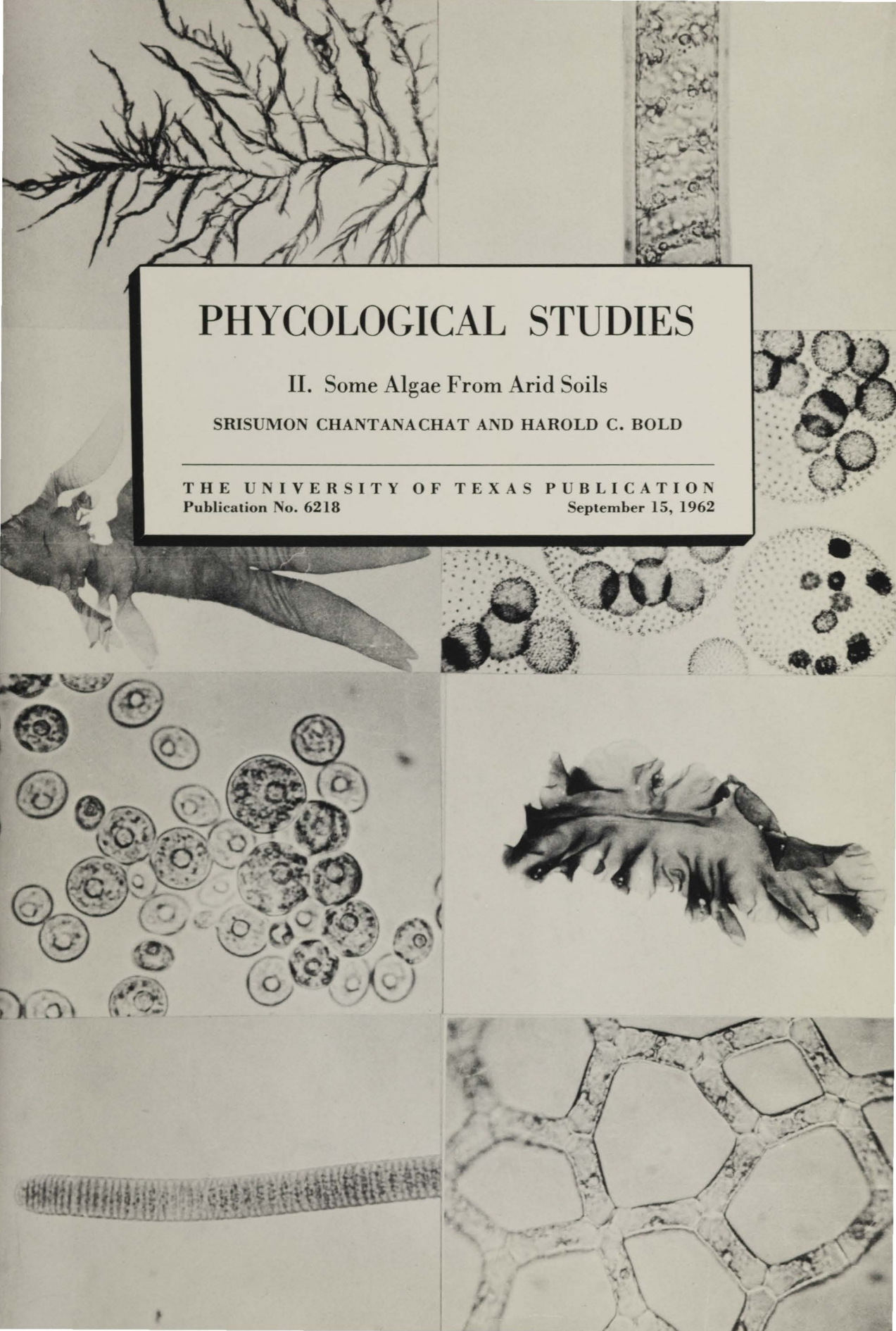

PHYCOLOGICAL STUDIES

II. Some Algae From Arid Soils

SRISUMON CHANTANACHAT AND HAROLD C. BOLD

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Phycological Studies

II. Some Algae From Arid Soils

by

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Introduction

Although there have been a number of studies of algae of various types of soil, few have dealt especially with arid or desert soils. Killian and Fe'her (1939) made a survey of algae in soil of the Sahara Desert, North Africa, and L. Moewus (1953) reported on some algae isolated from semi-desert soils around Broken Hill, New South Wales, Australia. Because preliminary studies of the soil algal flora of certain sand samples from Saudi Arabia indicated that algae were present even in soils from a region of such low rainfall, it occurred to the writers that further investigation of the algae in other arid soils might be of interest. Such an investigation, on a limited scale, has been made possible by the cooperation of a number of people to whom grateful appreciation is herewith expressed.

Materials and Methods

The algae described in this paper were isolated from samples of desert soils collected from various places, including Saudi Arabia, Arizona, Utah, Israel, and Mexico.

For studying the soil algae present in each sample, 15-g portions were placed in sterile 125-ml Erlenmeyer flasks in quadruplicate, each containing 50 ml of: (1) modified Bristol's solution (Bold, 1949); (2) Kratz and Myer's (1955) medium D; (3) Knop's solution (Bold, 1942); and (4) sea water, respectively. The last was used because of evidence that some of the soils were from marine deposits containing high concentration of salts.

Modified Bristol's solution was used with and without agar for routine cultivation of the algae described in this paper. The media were prepared as follows:

Modified Bristol's solution (Bold, 1949)

Six stock solutions in 400 ml of distilled water were employed. Each contained one of the following salts in the amounts listed:

$NaNO_3$	10.0 g	K_2HPO_4	3.0 g
$CaCl_2 \cdot 2H_2O$	1.0 g	KH_2PO_4	7.0 g
$MgSO_4 \cdot 7H_2O$	3.0 g	NaCl	1.0 g

10 ml of each stock solution were added to 936 ml of distilled water and then 1.0 ml of each of the 4 stock trace-element solutions per liter of macro-element solution was introduced.

The stock trace-element solutions used were the following:

(1) EDTA stock solution: 50 g EDTA plus 31 g KOH per liter.

(2) Fe stock solution: 4.98 g $FeSO_4 \cdot 7H_2O$ per liter of acidified H_2O. (Make acidified H_2O by adding 1.0 ml conc H_2SO_4 to 999 ml distilled water.)

(3) Boron stock solution: 11.42 g H_3BO_3 per liter.

(4) H_5 stock solution[3]:

$ZnSO_4 \cdot 7H_2O$	8.82 g
$MnCl_2 \cdot 4H_2O$	1.44 g
MoO_3	0.71 g
$CuSO_4 \cdot 5H_2O$	1.57 g
$Co(NO_3)_2 \cdot 6H_2O$	0.49 g

all per liter of acidified[4] H_2O (acidified as described under Fe stock)

Kratz and Myer's (1955) Medium D

$NaNO_3$	1.0 g per liter
K_2HPO_4	1.0 g per liter
$MgSO_4 \cdot 7H_2O$	0.15 g per liter
$Ca(NO_3)_2 \cdot 4H_2O$	0.010 g per liter
EDTA	0.050 g per liter
$Fe(SO_4)_3 \cdot 6H_2O$	0.004 g per liter
H_5 micro-elements[3]	1.0 ml of stock solution, see (4) above

This medium is used for the cultivation of some blue-green algae and has a pH of about 8.0. This high pH inhibits development of many bacteria.

Knop's solution (Bold, 1942)

Part A		Part B	
$Ca(NO_3)_2$	4 g	KNO_3	1.0 g
Distilled water	500 ml	KH_2PO_4	1.0 g
		$MgSO_4 \cdot 7H_2O$	1.0 g
		Distilled water	500 ml

Parts A and B were mixed just before using. Micro-elements were added as they were to the other solutions.

The flasks with culture medium, having been inoculated with soil samples, were placed in the culture room at a temperature of 22°C under fluorescent illumination controlled by an automatic timing device (12 hr light, 12 hr darkness). These conditions of cultivation are hereinafter referred to as "standard conditions." A phototactic ring of green, zoospore-producing algae appeared within 2 or 3 weeks in many of the flasks, and additional algae grew on the surface of liquid and on the submerged soil.

Isolations of organisms which developed were made by several methods, including direct removal of cells with capillary pipettes and plating-out dilute suspensions in 2% Bristol's agar. Algal colonies appeared in the agar within 1 week. Single colonies of different kinds of algae were isolated by using finely drawn-out "Disposable Pasteur Capillary Pipettes" or platinum needles. Aliquots of each colony selected were inoculated into a small tube of Bristol's solution and also into a soil-water tube. The latter were prepared by placing a pinch of $CaCO_3$ in the bottom of a Pyrex test tube (13 × 100 mm), then adding 1/4–1/2 inch of soil[5] and filling the tube approximately 2/3 full with distilled water. The tubes were steamed for 1 hr on each of the 3 successive days and inoculated after they had cooled and cleared. These unialgal cultures showed macroscopically visible growth under standard conditions about 2 weeks after inoculation.

[3] From Kratz and Myers, 1955.

[4] Myers, personal communication.

[5] Soil from a garden in Nashville, Tennessee.

In order to study the morphology and life cycle of the organisms, and especially the distinctive physiological attributes, it was necessary to obtain bacteria-free cultures. The algae described in this paper were purified with the aid of 4% Tween-80 (Atlas Powder Co., Wilmington, Del.). The procedures employed were as follows: (1) a heavy suspension of the organisms (actively growing cultures) was placed in a 12-ml centrifuge tube and centrifuged for 1 or 2 min at 2,000 RPM until the organisms had sunk to the bottom of the tube. The supernatant was then decanted. (2) 8 ml of 4% Tween-80 (prepared by adding 4 ml Tween-80 to 96 ml sterile distilled water) was then poured into the tube; the latter was then placed in a Disontegrator[6] for 1 min in order to separate the clumps. The organisms then were left in the Tween-80 for 2–3 hr. It was found that frequently shaking the tube at this stage was helpful in attaining pure cultures. (3) The organisms were then centrifuged again and the Tween-80 supernatant discarded. (4) After washing 5 times with sterile distilled water, with alternating centrifugations, the organisms apparently were rendered largely bacteria-free. (5) Isolations were made from these purified suspensions by streaking, plating, and single-cell isolation. (Bold, 1942; Pringsheim, 1946, 1950).

Bacteria-free cultures on Bristol's agar slants were maintained in triplicate Pyrex tubes (18 ×150 mm), plugged with cotton or in Bakelite-capped tubes. These are hereinafter referred to as "stock cultures." Macroscopic observations were made on these stock cultures at 2-week, 1-month, and 2-month intervals to record color changes associated with the age of the cultures, and microscopic studies of cell morphology were recorded in drawings and photomicrographs.

The morphology and life cycle of the organisms were studied in fresh mounts from 2-week-old cultures grown under standard conditions both in Bristol's solution and on Bristol's agar. Observations on fresh mounts were supplemented by using India ink and Methylene-blue to determine the extent of wall layers. Sudan IV was used to demonstrate the presence of lipid, and aqueous iodine (I_2-KI) to determine the nuclear condition, characteristics of flagella, and presence or absence of starch and pyrenoids. The acetocarmine technique was used to supplement observation of the nuclear condition in living cells. For this purpose, the procedure employed was as follows: cells were affixed to clean glass slides by means of egg albumin and immersed in the fixative for 12–24 hr (or fixed overnight). The freshly prepared fixative contained the following: 3 parts of absolute alcohol; 1 part of glacial acetic acid; $FeCl_3 \cdot 6H_2O$ then was added until mixture became light brown. The excess fixative was drained off and several drops of acetocarmine, prepared by the method of Cave and Pocock (1951), were placed on the fixed material. After the addition of the coverslip, the slide was heated over a low flame until vapor arose from the stain. Observation of the preparation was made immediately.

[6] Disontegrator System 80. Ultrasonic Industries, Inc., Albertson, L.I., N.Y.

For providing more information on the taxonomy of soil algae, especially for the delimitation of species, several techniques and supplementary attributes were employed, as suggested, in part, by Deason and Bold (1960) and by Bold and Parker (1962). These include a study of colony characteristics (macroscopic and under low magnification); color changes on Bristol's agar upon aging; growth in different agar media, i.e., Proteose-peptone agar, soil-extract agar, 0.1% yeast extract agar, Bacto Nutrient agar, Bacto Nutrient Broth, Bacto A C Broth, Bacto Thioglycollate medium and 0.04% sulfathiazole in Bristol's solution; sensitivity to antibiotics, sulfonamides, and other agents, and, finally, growth in media containing various carbon sources.

In order to study the differences in gross colony characteristics, a loopful of cells from 2-week-old stock cultures was streaked in circular fashion on the surface of Bristol's agar in small Petri dishes. After 2 weeks' growth under standard conditions, the colony characteristics, including color and topography, were observed and recorded. Observations were made both macroscopically and microscopically with low power (14×) magnification of a stereoscopic binocular microscope using reflected light. Photomicrographs of colony characteristics were made for the record.

For comparative studies of growth in different solutions mentioned above, media were prepared as follows: Proteose-peptone agar was made by adding 1.0 g of proteose-peptone and 15.0 g agar to 1.0 liter of Bristol's solution. Soil-extract agar was prepared by adding 40 ml of soil-water supernatant (prepared by autoclaving a kilogram of soil in a liter of distilled water) and 15.0 g of Difco agar to 960 ml of Bristol's solution. Yeast-extract agar was made by adding 1.0 g of yeast extract and 15.0 g of Difco agar to 1 liter of Bristol's solution. Inoculations from 2-week-old stock cultures were streaked into small tubes (13 ×100 mm) of proteose-peptone agar, soil-extract agar, yeast-extract agar, and Bacto-Nutrient Agar slants. After 2 weeks under standard conditions, estimation of the amount of growth was made and recorded. This was described by the adjectives "excellent," "good," "fair," "trace," or "none," based on standards used by Bold and Parker (1962).

In maintaining the pure cultures, inoculations from stock cultures were made routinely into Bacto Nutrient-Broth tubes, AC broth and Thioglycollate media to insure that the cultures remained bacteria-free. Observations to determine bacterial contamination were made after 48 hr and subsequently. For comparative observations and to test the ability of each alga to grow in these media, the observations were made after 2 weeks' growth under standard conditions.

In the light of the work of Parker, Bold, and Deason (1961) and Bold and Parker (1962), study was made of the effects on the algae herein described, in light and in darkness, of adding carbon sources to Bristol's solution. Each organism tested was grown on Bristol's agar slants for 2 weeks. In order to get as uniform-as-possible an inoculum, the organisms to be tested were carefully washed off the agar slants into screw-cap tubes with sterile Bristol's solution. Uniform amounts of the

suspensions were used to inoculate the several algae into other media. Test media included the following: Bristol's solution (control); 0.75% arabinose, fructose, glucose, ribose, xylose, and sodium acetate, each in Bristol's solution.

The bacteria-free cultures were inoculated into the tubes (18 ×150 mm) of sterile medium in duplicate by means of sterile disposable Pasteur pipettes. One of these sets was placed under standard conditions, while the other was placed in darkness at approximately the same temperature. After 2 weeks, and periodically thereafter, estimates were made of the amount of growth in these tubes. These were described by the adjectives "excellent," "good," "fair," "trace" or "none," as stated previously.

To determine the sensitivity of the organisms to antibiotics, sulfonamides, and other agents, the procedure employed was as follows: High-concentration "Multidisks" were placed aseptically on the surface of Bristol's agar (prepared by using deionized water and Ion-agar instead of distilled water and Difco agar) "pour" plates previously "seeded" with a heavy suspension of 2-week-old cultures of the organisms to be tested. After 2 weeks' growth under standard conditions, observation was made of the occurrence of zones of inhibition. The "Multidisks" had been impregnated before use with the substances and at the concentrations listed in Table 1.

TABLE 1. *Certain inhibitory agents, manufacturer's*[a] *abbreviations and concentrations used in "Multidisks."*

Code number	Antibiotic agents		Concentration
11–102A	Chlortetracycline	(A+)	30 mcg
	Amphotericin B	(AB+)	100 mcg
	Bacitracin	(B+)	10 units
	Chloramphenicol	(C+)	30 mcg
	Carbomycin	(CA+)	15 mcg
	Colistin sulfate	(CS+)	10 mcg
11–102B	Demethylchlortetracycline	(D+)	30 mcg
	Erythromycin	(E+)	15 mcg
	Kanamycin	(K+)	30 mcg
	Neomycin	(N+)	50 mcg
	Novobiocin	(NV+)	30 mcg
	Nystatin	(NS+)	100 units
11–102C	Oleandomycin	(OL+)	15 mcg
	Penicillin	(P+)	10 units
	Polymyxin-B	(PB+)	300 units
	Paromomycin	(PM+)	300 mcg
	Ristocetin	(R+)	30 mcg
	Syncillin	(SY+)	10 mcg

TABLE 1. (continued)

11–102D	Cycloserine	(CY+)	30 mcg
	Dihydrostreptomycin	(S+)	10 mcg
	Staphcillin	(SC+)	30 mcg
	Oxytetracycline	(T+)	30 mcg
	Triacetyloleandomycin	(TAO+)	15 mcg
	Tetracycline	(TE+)	30 mcg
11–102E	Sulfisomidine	(EL+)	300 mcg
	Sulfisoxazole	(G+)	300 mcg
	Sulfamethoxypyridazine	(KY+)	300 mcg
	Triclobisonium chloride	(TR+)	1 mg
	Viomycin	(V+)	10 mcg
	Vancomycin	(VA+)	30 mcg
11–102F	Sulfadimethoxine	(MA+)	300 mcg
	Sulfadiazine	(SD+)	300 mcg
	Sulfamerazine	(SM+)	300 mcg
	Triple-sulfa	(SSS+)	300 mcg
	Sulfathiazole	(ST+)	300 mcg
	Thiosulfil	(TH+)	300 mcg
11–102G	Furaltadone	(AL+)	100 mcg
	Nitrofurantoin	(F+)	100 mcg
	Nitrofurazone	(FC+)	100 mcg
	Furazolidone	(FR+)	100 mcg
	Iso Nicotinic acid hydrazide	(IH+)	25 mcg
	Methenamine mandelate	(M+)	2.5 mg
	Para amino salicylic acid	(PS+)	100 mcg

[a] Consolidated Laboratories, Chicago Heights, Illinois.

In addition to the agents tested with "Multidisks," 0.04% sulfathiazole in Bristol's solution also was employed, as it had been by Bold and Parker (1962).

The drawings were made with the aid of Spencer Camera Lucida and the photomicrographs were taken with a Bausch and Lomb microscope with apochromatic objectives and a Zeiss-Winkel camera attachment.

Observations

A. GENERAL ASPECTS OF THE ARID-SOIL ALGAL FLORA

Isolations into unialgal cultures were made from 21 samples of arid soils from different places, including Saudi Arabia, Utah, Arizona, Israel, and Mexico. It was

found that among the algae which developed were genera of the Chlorococcaceae, Chlorosphaeraceae, Xanthophyceae, and Cyanophyceae. The origins of the samples and organisms isolated are listed in Table 2.

From Table 2 it is clear that the most prevalent genus of algae in arid soils is *Chlorosarcinopsis*, a chlorosphaeralean genus. Certain of the taxa listed in the table have been isolated into pure culture and have been investigated intensively; they are described in the following account. Others await further study.

B. ORGANISMS NEW TO SCIENCE OR OTHERWISE OF INTEREST

NEOCHLORIS **oleoabundans** sp. nov. (Fig. 1–8; 73–75)

Cellulae sphaericae, 6–25μ diam., sine matrice gelatinosa. Choroplastus parietalis poculiformis quasi dimidium tantummodo superficiei cellulae obducens; duo (raro unum vel tria) pyrenoidea in quasi omni cellula matura. Membranae cellularum in culturis vel aliquot mensium aetate semper tenues, interdum, autem, incrassationem unipolarem bulliformem praebentes. Cellulae uninucleatae.

Reproductio per zoosporas (4–8) (raro 16) ex una cellula effectas, bipartitione iterata enascentes. Zoosporae ovatae 3.6–2.7μ, duo flagella aequa, stigma anterius, duas vacuolas contractiles anteriores, nucleum anteriorem et pyrenoideum posterius habentes, quiescentes sphaericae factae. Aplanosporae (4–8–16) quoque effectae.

Reproductio sexualis non observata.

Origo: E tumulis arenae Lat. 21°15′E, Long. 55°15′E, in loco Rub al Kali, Saudi Arabia dicto.

Cult. num. Ch-1.

Starr (1955) erected the genus *Neochloris* to include chlorococcalean algae with the following attributes: (1) vegetative cells with a hollow, cup-like chloroplast; (2) having at least a single pyrenoid in the chloroplast of the vegetative cells; and (3) producing zoospores without a wall and with 2 equal flagella.

A species of this genus, apparently hitherto undescribed, was isolated into bacteria-free culture during this investigation from a soil sample collected from the top of sand dunes Lat. 21°15′N, Yong. 55°15′E in Rub al Khali, Saudi Arabia.

The vegetative cells, which cohere in irregular masses, are spherical throughout their development (Fig. 1). Cell size is variable (Fig. 73) and a maximum diameter of 25μ has been observed. In actively growing cultures, the cell walls are characteristically thin and smooth; they do not thicken with age, but in cultures 2 or 3 months old, some of the cells may have a bubble-like thickening at one pole (Fig. 3).

The chloroplast is parietal and cup-like, and the single nucleus lies in a small area of colorless cytoplasm. Young vegetative cells recently derived from zoospores have a single pyrenoid, but, as the cells increase in size, 2 elongate pyrenoids are always present, these resulting from division of the original one (Fig. 2, 73). This

TABLE 2. *Samples of arid soils, their pH, and algae observed and/or isolated therefrom*

Origin of soil samples	pH	Organisms
Sand from top of sand dunes Lat. 21° 15′N, Long. 55° 15′E. Saudi Arabia	8.0	*Neochloris oleoabundans* sp. nov. *Scytonema* sp. *Chlamydomonas* sp.
Rippled sand near base of sigmoidal dunes Lat. 20° 40′N, Long. 54° 40′E. Saudi Arabia	8.3	*Scenedesmus* sp. *Chlorosarcinopsis* sp. *Chlamydomonas* sp. (palmelloid stage) *Scytonema* sp.
As-Sanam, S.E. of Batnah Lat. 21° 46′N, Long. 53° 10′E. Saudi Arabia	8.2	*Chlorosarcinopsis gelatinosa* sp. nov. *Anabaena* sp. *Nostoc* sp.
As-Sanam, surface of Zibar, Lat. 21° 46′N, Long. 53° 10′E. Saudi Arabia	8.1	*Chlorosarcinopsis* sp. *Spongiochloris incrassata* sp. nov. *Bracteacoccus* sp. *Chlamydomonas* sp. *Oscillatoria* sp.
Large sigmoidal dunes near Lat. 20° 40′N, Long. 54° 40′E. 20 miles above Sabkhah. Saudi Arabia	8.1	*Chlorosarcinopsis* sp. *Chlamydomonas* sp. *Anabaena* sp.
Top of Sigmoidal ridge Lat. 20° 25′N, Long. 53° 40′E. Saudi Arabia	8.0	*Chlamydomonas* sp. *Chlorosarcinopsis* sp. *Oscillatoria* sp.
Six miles north of St. John, Arizona, elevation 5800 ft.	7.8	*Bracteacoccus* sp. *Chlorococcum* sp. *Chlorosarcinopsis* sp. *Spongiochloris minor* sp. nov.
Near Apache Junction, Arizona, elevation 1900 ft.	7.8	*Chlorosarcinopsis* sp. *Chlorococcum diplobionticoideum* sp. nov. *Chlamydomonas* sp.
Phoenix, Arizona	7.2	*Protosiphon* sp. *Chlorosarcinopsis eremi* sp. nov. *Bracteacoccus* sp. *Chlamydomonas* sp. *Chlorococcum* sp. *Nostoc.* sp.
North of Moab, Grand County, Utah	7.6	*Chlorosarcinopsis* sp. *Chlorosarcina brevispinosa* sp. nov. *Chlamydomonas* sp.

TABLE 2. (continued)
Samples of arid soils, their pH, and algae isolated therefrom

Origin of soil samples	pH	Organisms
Near Superior, Pinal County, Arizona	8.1	*Bracteacoccus* sp. *Chlorosarcina longispinosa* sp. nov. *Chlorococcum* sp. *Chlamydomonas* sp. *Chlorosarcinopsis* sp.
Four miles north of Glade, Arizona	7.1	*Chlorosarcinopsis* sp. *Chlamydomonas* sp. *Tetraspora* sp. *Oscillatoria* sp.
North of Samalayuca, Chihuahua, Mexico. (The west side of the highway)	8.0	*Radiosphaera* sp. *Bracteacoccus* sp. *Chlorococcum* sp. *Chlorosarcinopsis* sp. *Scytonema* sp.
North of Samalayuca, East side of the highway, Chihuahua, Mexico	7.8	*Chlorosarcinopsis* sp. *Chlorococcum* sp. *Chlamydomonas* sp. Diatoms.
South of Samalayuca, East side of the highway, Chihuahua, Mexico.	7.2	*Chlorosarcinopsis* sp. *Chlorococcum* sp. *Chlamydomonas* sp. *Nostoc* sp. *Anabaena* sp.
Loess soil, Sdeh Boku, Israel	8.3	Diatoms.
Coarse Sandy soil near Sodom (environment of Dead Sea), Israel	7.5	*Chlamydomonas* sp. *Chlorococcum* sp. *Oscillatoria* sp.
Hawmada soil near Sodom, Israel	7.4	*Chlorosarcinopsis* sp. *Oscillatoria* sp. *Lyngbya* sp.
Sandy soil, Gevuloth, Israel	8.0	*Chlorosarcinopsis* sp. *Spongiochloris* sp. *Scytonema* sp. xanthophycean alga
Marly soil (environment of Dead Sea), Israel	8.1	*Chlorosarcinopsis* sp. *Chlamydomonas* sp. *Anabaena* sp.
Rich organic soil, Ein Arus, Israel	7.4	*Chlorosarcinopsis* sp. *Chlamydomonas* sp. *Oscillatoria* sp.

feature is very characteristic of the species. The pyrenoids, of course, are obscured by starch and oil in cells from cultures from the stationary phase of growth.

In cells of cultures 3 weeks old and older, it has been possible, by use of Sudan IV, to demonstrate a considerable amount of oil. As the cultures age, these oil droplets increase in size and finally coalesce, thus crowding the chloroplast to one pole of the cell (Fig. 4–6, 74).

Asexual reproduction is accomplished by means of zoospores (Fig. 7) and aplanospores formed by successive bipartition. Zoospore size is between 2 and 3.5μ in width and 3.6 and 4.5μ in length. The individual ovoid zoospore possesses a single nucleus, a parietal chloroplast with a posterior pyrenoid, an anterior stigma, 2 anterior contractile vacuoles, and 2 flagella of equal length (Fig. 8). The zoospores at quiescence become spherical (*Protosiphon*-type; Starr, 1955) (Fig. 1). The flagella, stigmata, and contractile vacuoles disappear, and the young vegetative cells then begin to grow.

Reproduction is also effected by aplanospore formation. The aplanospores resemble zoospores soon after quiescence. They are liberated by rupture of the parent wall.

Sexual reproduction has not been observed.

Two-week-old colonies on Bristol's agar are dull-shiny and homogeneous except for lines made at inoculation (Fig. 75). Cultures remain green for as long as 3 months. Additional supplementary attributes will be compared with those of other known species in Tables 4–7.

Up to date, 8 species of *Neochloris* have been described as follows:

N. aquatica (type species)	Starr, 1955
N. gelatinosa	Herndon, 1958b
N. terrestris	Herndon, 1958b
N. fusispora	Arce and Bold, 1958
N. pyrenoidosa	Arce and Bold, 1958
N. minuta	Arce and Bold, 1958
N. alveolaris	Bold, 1958
N. pseudoalveolaris	Deason and Bold, 1960

Cells from pure cultures of *N. oleoabundans* were compared carefully, with respect to both morphological and physiological attributes, with the previously described species of *Neochloris*. The morphological comparisons (Table 3) were made of material grown under standard conditions. From these comparisons, it became clear that the organism isolated from Arabian sand is unlike all of those described before the present investigation was undertaken.

Supplementary attributes in the genus Neochloris

Supplementary attributes investigated in most recent studies of soil algae in our laboratory (Deason and Bold, 1960; Bold and Parker, 1962) were not studied in

TABLE 3. *Morphological attributes of species of Neochloris*

Organism	Vegetative cell size	Cell wall (at various phases of growth)	No. of nuclei	Position and no. of pyrenoids	Nature of zoospores
N. aquatica	13.5μ	outer wall layer not thickening markedly[b] (o.w.n.t)	multi-nucleate[a]	1, excentric	5.5μ long, 2.5μ wide, elongate
N. alveolaris	25μ	(o.w.n.t)	uni-nucleate	1, excentric	8–10μ long 4–5μ wide, fusiform
N. fusispora	22μ	(o.w.n.t)	multi-nucleate	1, excentric	12–22.5μ long, 2–3μ wide, elongate, pointed both ends
N. gelatinosa	17μ–37μ	(o.w.n.t) prominent, common gelatinous matrix present	multi-nucleate	1, or more, excentric	5–9μ long 2.5–4μ wide, elongate
N. minuta	18μ	outer wall layer thickening markedly with age	multi-nucleate	1, excentric	3–5μ long 2–3μ wide, almost spherical
N. oleoabundans	6μ–25μ	(o.w.n.t)	multi-nucleate	1 (young cells), 2, or rarely 3 (mature cells), excentric	3.6μ long 2.7μ wide, ovoid
N. pseudoalveolaris	25μ	(o.w.n.t)	uni-nucleate	1, excentric	8–10μ long 4–5μ wide, pointed anterior; rounded posterior
N. pyrenoidosa	13μ–25μ up to 45μ	outer wall layer thickening markedly with age	multi-[a] nucleate	1–5, excentric	7–10μ long 3–5μ wide, compressed
N. terrestris	35μ–45μ up to 75μ	(o.w.n.t)	uni-nucleate	1, excentric	5–12μ long 2–6μ wide, elongate

[a] Nuclear number increases gradually as the cell ages.
[b] In stationary-phase cultures

previously described species of *Neochloris*. Accordingly, the writers have included in this investigation a comparative study of the known species of *Neochloris* with respect to supplementary attributes. This involved, as a necessary prerequisite, purification of the cultures of *N. gelatinosa, N. pyrenoidosa,* and *N. terrestris,* not previously available in this condition. The results of comparative studies of certain supplementary attributes are summarized in Tables 4–7. It is clear from these data that the supplementary attributes are useful in distinguishing among the several species of *Neochloris*. As indicated in Table 5, ribose and xylose markedly inhibited growth of most species of *Neochloris*.

TABLE 4. *Colonial attributes of 2-week-old cultures of Neochloris grown under standard conditions on Bristol's agar; color of aging (stationary-phase cells) on Bristol's agar slants*

Species	Macroscopic	14×, transmitted light	
N. aquatica	dry	rough (delicate, reticulate, vermiform)	yellow-green
N. alveolaris	dry	homogeneous (slightly granular)	green
N. fusispora	dry	delicate, reticulate, rugose; adherent to agar surface	green-orange
N. gelatinosa	shiny	homogeneous, with dark lines, uniform translucent dots	orange at the edge of slant
N. minuta	shiny	homogeneous	light green
N. oleoabundans	dull-shiny	homogeneous	green
N. pseudoalveolaris	dull-shiny	homogeneous	green
N. pyrenoidosa	dull-shiny	homogeneous	green with orange tinge
N. terrestris	dry	homogeneous	orange at the edge of slants

With respect to the Multidisk test (Table 7), the following conclusions are drawn: (1) all 9 species were inhibited by Paromomycin, Triclobisonium choride and Methenamine mandelate. (2) Dihydrostreptomycin slightly inhibited only 2 species. (3) Oxytetracycline, Sulfadimethoxine, and sulfadiazine each slightly inhibited only 1 species. (4) None of the 9 species is inhibited by chlortetracycline, Bacitracin, Chloramphenicol, Novobiocin, Oleandomycin, Penicillin, Ristocetin, Syncillin, Cycloserine, Staphcillin, Triacetyloleandomycin, Tetracycline, Sulfisomi-

TABLE 5. *Effects of certain carbon compounds*[a] *on growth of Neochloris species in light*

Species	Bristol's solution	Arabinose	Glucose	Fructose	Sodium acetate	Ribose	Xylose
N. aquatica	good +	fair	good	fair	none	good	fair
N. alveolaris	trace +	trace	good	good	fair	trace	trace
N. fusispora	fair	trace +	fair +	trace −	none	trace	none
N. gelatinosa	fair +	fair	fair	good	fair	fair	trace
N. minuta	fair	none	fair +	good	none	good	fair
N. oleoabundans	fair	none	fair	none	none	trace −	none
N. pseudoalveolaris	trace	trace	good	good	fair	trace	trace
N. pyrenoidosa	fair	trace	trace +	trace −	trace −	trace	trace
N. terrestris	fair	trace +	fair +	fair	fair +	trace +	trace −

[a] Added at a concentration of 0.75% to Bristol's solution.

TABLE 6. *Comparative growth species of Neochloris on/in certain complex media*

Species	Yeast-extract agar	Proteose-peptone agar	Bacto nutrient agar	Bacto thio-glycollate med.	Bacto AC broth	Bacto nutrient broth
N. aquatica	good	excellent	fair	trace	fair	fair
N. alveolaris	excellent	excellent	fair	fair	trace −	trace
N. fusispora	fair	good	fair	trace	trace +	fair
N. gelatinosa	fair	fair	fair	fair −	none	fair +
N. minuta	good	excellent	good	trace	trace	good
N. oleoabundans	trace	good	trace	good	fair +	trace −
N. pseudoalveolaris	excellent	fair	fair	trace −	none	trace
N. pyrenoidosa	fair	good	fair	trace	none	trace
N. terrestris	good	excellent	excellent	good +	fair	fair

TABLE 7. *Sensitivity of species of Neochloris to certain agents*[a]

	11-102A						11-102B					
	(A +)	(AB +)	(B +)	(C +)	(CA +)	(CS +)	(D +)	(E +)	(K +)	(N +)	(NV +)	(NS +)
N. aquatica	....	+	....	....	....	.. .	. ..	....	....	....	.. .	....
N. alveolaris	....	.. .	....	.. .	....	. .	. ..	.. .	....	....	....	....
N. fusispora	....	+	....	....	....	+	. ..	....	+	....	....	+
N. gelatinosa	....	+	....	....	....	+	. ..	+	+	.. .	.. .	....
N. minuta	....	+	....	.. .	+	. .	+	+	+	....	....	+
N. oleoabundans	....	+	....	....	+	. .	.. .	.. .	. .	....	+	+
N. pseudoalveolaris	.. .	+	. .	....	+	....	.. .	. ..	....	+	....	. .
N. pyrenoidosa	.. .	+	....	.. .	....	+	. ..	. ..	+	+	....	. .
N. terrestris	....	+	....	....	.. .	+	.. .	....	+	+	....	.. .

TABLE 7.—*(continued)*

	11–102C						11–102D					
	(OL +)	(P +)	(PB +)	(PM +)	(R +)	(SY +)	(CY +)	(S +)	(SC +)	(T +)	(TPO +)	(TE +)
N. aquatica	....	....	....	+	....	....	....	....	....	....	....	....
N. alveolaris	....	....	....	+	....	....	....	....	....	....	....	....
N. fusispora	....	....	+	+	....	....	....	....	....	....	....	....
N. gelatinosa	....	....	+	+	....	....	....	+	....	....	....	....
								(sl)				
N. minuta	....	....	....	+	....	....	....	+	....	+	....	....
N. oleoabundans	....	....	+	+	....	....	....	....	....	....	....	....
N. pseudoalveolaris	....	....	....	+	....	....	....	+	....	....	....	....
								(sl)				
N. pyrenoidosa	....	....	+	+	....	....	....	....	....	....	....	....
N. terrestris	....	....	+	+	....	....	....	....	....	....	....	....

	11–102E						11–102F					
	(EL +)	(G +)	(KY +)	(TR +)	(V +)	(VA +)	(MA +)	(SD +)	(SM +)	(SSS +)	(ST +)	(TH +)
N. aquatica	....	....	....	+	....	....	....	....	....	....	....	....
N. alveolaris	....	....	....	+	....	....	....	....	....	....	....	....
N. fusispora	....	....	....	+	....	....	....	....	....	....	....	....
N. gelatinosa	....	....	....	+	....	....	....	....	....	....	....	....
N. minuta	....	....	....	+	....	....	....	....	....	....	....	....
N. oleoabundans	....	....	....	+	....	....	....	....	....	....	....	....
N. pseudoalveolaris	....	....	....	+	....	....	+	+	....	....	....	....
							(sl)	(sl)				
N. pyrenoidosa	....	....	....	+	....	....	....	....	....	....	....	....
N. terrestris	....	....	....	+	....	....	....	....	....	....	....	....

	11–102G							sulfathiazole (0.04%)
	(AL +)	(F +)	(FC +)	(FR +)	(IH +)	(M +)	(PS +)	
N. aquatica	....	....	+	....	....	+	....	trace
N. alveolaris	+	+	....	+	....	+	....	trace
N. fusispora	+	+	....	+	....	+	+	trace +
N. gelatinosa	....	+	....	+	....	+	....	trace +
N. minuta	+	+	....	+	....	+	+	good
							(sl)	
N. oleoabundans	+	+	+	+	....	+	+	trace —
N. pseudoalveolaris	+	+	....	+	....	+	+	trace
N. pyrenoidosa	+	+	+	+	....	+	....	trace —
			(sl)					
N. terrestris	+	+	+	+	....	+	+	trace
			(sl)					

[a] The agents and the abbreviations for them are listed in Table 1; + indicates inhibition; .... indicates no inhibition; (sl) means slight inhibition.

dine, Sulfisoxozole, Sulfamethoxypyridazine, Viomycin, Vancomycin, Sulfamerazine, Triple sulfa, Sulfathiazole, Thiosulfil and Isonicotinic acid hydrazide. (5) No 2 species of the 9 tested with the Multidisks had identical inhibition patterns.

In order to facilitate identification of isolates of *Neochloris*, the key of Arce and Bold (1958) has been modified and extended to include all presently known taxa, as follows:

Key to the species of *Neochloris* Starr

1. Cells, except those recently originating from zoospores, aggregated in a common matrix ..... *N. gelatinosa* Herndon (1958b)
1. Mature cells lacking a common matrix 2
   2. Individual matrix of watery consistency, present in young cells; cells attaining 75μ ... *N. terrestris* Herndon (1958b)
   2. Individual matrix absent 3
3. Outer wall layer thickening markedly with age 4
3. Outer wall layer not thickening markedly with age 6
   4. Cells attaining a maximum size of 18μ; zoospores almost spherical ..... *N. minuta* Arce & Bold (1958)
   4. Cells exceeding 18μ; zoospores elongate 5
5. Cells up to 45μ in diameter; zoospores pointed anteriorly ..... *N. pyrenoidosa* Arce & Bold (1958)
5. Cells up to 22μ in diameter; zoospores pointed at both ends ..... *N. fusispora* Arce & Bold (1958)
   6. Cells multinucleate ..... *N. aquatica* Starr (1955)
   6. Cells uninucleate 7
7. Mature cells usually with 2 pyrenoids ..... *N. oleoabundans* sp. nov.
7. Mature cells usually with 1 pyrenoid 8
   8. Colony dry, pyrenoid elongate-ellipsoidal ..... *N. alveolaris* Bold (1958)
   8. Colony dull-shiny, pyrenoid isodiametric ..... *N. pseudoalveolaris* Deason & Bold, (1960)

CHLOROCOCCUM **diploibionticoideum** sp. nov. (Fig. 9–20; 76–86)

Planta diplobiontica, i.e., cellulae vegetativae diploideae atque haploideae probabiliter in populatione, diploideae sphaericae, saepe membranas crassas habentes, ad 26μ diam., chloroplastum parietalem poculiformem, unico pyrenoideo praeditum, praebentes; haploideae Chlamydamonadoideae binae quaternaeque intra membranam parentalem; cellulae-filiae ad zoosporas aut aplanosporas efficiendas divisiones insequentes saepe subeuntes aut magnitudine graditim auctae et per rupturam membranae parentalis directe liberatae.

Reproductio per coniunctionem isogametarum bipartitione successiva cellularum vegetativarum diploidearum atque aplanosporarum factarum. Gametae 14–18μ long., 7–9μ lat., ovatae et typi *Chlamydomonadis* (membranam habentes) chloroplastum parietalem, unicum pyrenoideum posteriorem, nucleum anteriorem, duas

vacuolas contractiles, stigma et duo flagella longitudine aequa quasi aeque longa ac corpus cellulae habentes. Gametae per parthenogenesim ad cellulas haploideas Chlamydamonadoideas binas quaternasque efficiendas saepe evolutae, hae aggregatae aequae magnitudine ac cellulae vegetativae diploideae (dissimiles hucusque cellulis haploideis in *C. diplobiontico*).
Origo: Ex exemplo soli prope locum Apache Junction, Arizona dictum.
Cult. num. Ch-9.

The genus *Chlorococcum,* according to Starr (1955) possesses the following 3 distinctive attributes: (1) a hollow, parietal chloroplast with or without a surface opening; (2) 1 or more pyrenoids; (3) biflagellate zoospores which do not become spherical at quiescence. The organism here described as a new species, *Chlorococcum diplobionticoideum*, was isolated into bacteria-free culture from a soil sample collected near Apache Junction, Arizona. This alga seems to be strikingly similar in life cycle to *C. diplobionticum* Herndon (1958b) but differs from the latter sufficiently to be considered a separate taxon. In the life cycle of these organisms, both haploid and diploid phases seem to occur simultaneously. The diploid vegetative cells, which originate as zygotes, may develop thickened walls and become spherical as they enlarge (up to 36μ), a typical *Chlorococcum*-like attribute (Fig. 76, 81, 85). These presumably diploid vegetative cells have a hollow, cup-like chloroplast with a single pyrenoid and nucleus (Fig. 10). At maturity, in actively growing cultures, they undergo successive bipartitions to form a large number (ca. 32) of biflagellate gametes (Fig. 12, 76), which are liberated by rupture of the parent cell wall.

The individual gametes (Fig. 13, 77) are ovoidal, 14–18μ long and 7–9μ wide. Each has 2 anterior flagella, about body length, an anterior nucleus, stigma, 2 contractile vacuoles, and a parietal chloroplast with posterior pyrenoid. These motile cells swim for only an hour or so, and many of them unite isogamously (Fig. 16–20; 80, 81). Inasmuch as the cultures studied were clonal, the organisms must be considered homothallic. Soon after plasmogamy, the walls of the gametes are shed posteriorly, and a new wall is secreted by the zygote (Fig. 20, 81). Flagella, stigmata, and contractile vacuoles disappear shortly. One of the 2 pyrenoids contributed by gametes begins to decrease in size, the latter a possible manifestation that one gamete plastid is disintegrating. The zygotes, as noted above, are actively photosynthetic; they enlarge, and function as vegetative cells.

The fate of gametes which fail to unite in sexual reproduction is illustrated in (Fig. 14, 82). Soon after cessation of motility, these gametes undergo bipartition(s) to form 2 or 4 *Chlamydomonas*-like daughter cells (Fig. 14, 82–84), as in *Chlorococcum diplobionticum.* These daughter cells remain within the parental wall. After some growth, each daughter cell may undergo further division to form either haploid gametes or aplanospores. Those which fail to produce gametes or aplanospores may be liberated directly from the parental wall.

In addition to the formation of gametes by the diploid vegetative cells, under certain conditions, aplanospores may develop (Fig. 78). The latter may undergo gametogenesis while still enclosed within the original parental cell wall (Fig. 15).

In the stationary phase of growth, many of the cells develop excentrically thickened walls (Fig. 11), which may reach 7μ in thickness. Cells from such cultures are bright orange and filled with oil droplets.

Two-week-old Bristol's agar (Fig. 86) are dry and coarse-granular; 3-month-old cultures remain green, except the thick-walled cells which become bright orange. Additional supplementary attributes which serve to distinguish *C. diplobiontic-oideum* from *C. diplobionticum* are shown in Tables 8, 9. With respect to colony

TABLE 8. *Colonial attributes of 2-week-old cultures of Chlorococcum diplobionticum and C. diplobionticoideum grown under standard conditions on Bristol's agar; color of aging (stationary-phase) cultures on Bristol's-agar slant*

Species	Macroscopic	20×, transmitted light	Color at 3-months old
C. diplobionticum	dull, dry	rough, slightly vermiform	green[a]
C. diplobionticoideum	dry	coarse granular	green[b]

[a] Diploid cells orange.
[b] Thick-walled cells orange.

TABLE 9. *Effects of certain carbon compounds*[a] *on growth of C. diplobionticum and C. diplobionticoideum in light.*

Species	Bristol's solution	Arabinose	Fructose	Sodium acetate	Ribose	Xylose
C. diplobionticum	good	good	trace	trace	fair	trace
C. diplobionticoideum	trace	trace —	none	none	trace —	none[b]

[a] Added at a concentration of 0.75% to Bristol's solution.

[b] Probably requires growth factor(s).

characters, there are only minor differences; but with respect to growth in organic carbon sources, several differences are evident.

With respect to the Multidisk tests, the following conclusions are drawn:

(1) Chlortetracycline, Carbomycin, Demethylchlortetracycline, Erythromycin, Dihydrostreptomycin, each inhibited only growth of *C. diplobionticoideum*.

(2) Nitrofurazone and Iso-nicotinic acid hydrazide each inhibited only the growth of *C. diplobionticum*.

(3) Amphotericin B, Colistin sulfate, Kanamycin, Neomycin, Nystatin, Polymyxin-B, Paromomycin, Furaltadone, Nitrafurantoin, Methenamine mandelate and Para-amino-salicylic acid each inhibited growth of both species.

As noted above, *Chlorococcum diplobionticoideum* is very similar in its morphology and life cycle to *C. diplobionticum* Herndon (1958b). With respect to the latter, both seem to have in their population diploid and haploid cells.[7] However, there is a striking difference between the 2 organisms. In *C. diplobionticum*, the haploid cells, which originate from single gametes, increase very little in size, and thereupon release their 2 (typically) division products. In contrast, in *C. diplobionticoideum* the haploid cells, which also originate from single gametes, fail to release their 2 or 4 division products, but, instead, increase gradually in size, ultimately equaling the diploid cells in this respect. Further divisions to form either gametes or aplanospores occur, or each daughter cell may be liberated directly from the parental cell wall.

One might well contrast the ephemeral and transitory nature of the haploid cells in *C. diplobionticum* with the persistent haploid phase of *C. diplobionticoideum;* and, furthermore, in light of this, one might consider the former to exhibit a heteromorphic and the latter an isomorphic diplobiontic life cycle. Cytological study, however difficult, is strongly indicated for these anomalous taxa of the genus *Chlorococcum*.

SPONGIOCHLORIS Starr

The generic attributes of *Spongiochloris,* as defined by Starr (1955), are: (1) chloroplast net-like; (2) with 1 or more pyrenoids; (3) zoospores becoming spherical at quiescence. Two new species of *Spongiochloris* have been studied during the present investigation.

SPONGIOCHLORIS **minor** sp. nov. (Fig. 21–28; 87–92)

Cellulae omnis aetatis sphaericae; chloroplastus cellulae iuvenis parietalis poculiformis, gradatim spongiosus reticulatus transformatus; cellulae ad 40μ diam., membranis aetate paululum incrassatis, non stratifactis; cellulae maturae multinucleatae.

Reproductio asexualis per zoosporas aplanosporasque fissione progrediente effectas, membrana parentali rupta liberatas. Zoosporae ovate chloroplastum parietalem, stigma anterius, nucleum, duas vacuolas contractiles et flagella habentes, 8.85μ long., 3–3.5μ lat.; pyrenoideum posterius; zoosporae quiescentes sphaericae factae.

Reproductio sexualis non observata.

Origo. a loco distante 6 milia passuum ab oppido North St. John, Arizona dicto.

Cult. num. Ch-8.

The organism herein described as a new species, *Spongiochloris minor,* was isolated into bacteria-free culture from a soil sample collected 6 miles from North

[7] The cytological evidence on this point is not yet adequate for either organism.

St. John, Arizona. Its generic attributes are clearly those of the genus *Spongiochloris* as set forth by Starr (1955). The vegetative cells are spherical at all ages (Fig. 87). Cells with a maximum diameter of 40μ have been observed. The cell wall is thin, but it may thicken slightly in cultures 1 month old or older.

The chloroplast of *S. minor* varies in appearance with age. Young vegetative cells have a parietal chloroplast (Fig. 21), but as the cells increase in size, the chloroplast segments (Fig. 22), finally becoming a net-like structure. The chloroplasts of mature cells are typically net-like (Fig. 23, 88). The net-like character of the chloroplast becomes less distinct as the cells age because of the presence of starch and oil in large quantities (Fig. 89). A single, excentric pyrenoid is always embedded in the chloroplast; occasionally 2 or more are present. In older cells of *S. minor,* there may be many pyrenoids resulting from fragmentation of the original one (Fig. 24).

The vegetative cells of *S. minor* are uninucleate only when very young (Fig. 21); as the cells enlarge, they become multinucleate (Fig. 23). The nuclei can be demonstrated with aqueous iodine (I_2-KI) and acetocarmine.

Asexual reproduction is accomplished by means of aplanospores (Fig. 25–26, 90) and zoospores (Fig. 27), formed by progressive cleavage of the protoplast. The zoospores (Fig. 28) are ovoid, 8μ in average length and 3μ in average width. They are of the *Protosiphon* type (Starr, 1955) in that they become spherical immediately at quiescence (Fig. 91). Individual zoospores possess an anterior stigma, 2 anterior contractile vacuoles, 2 flagella of equal length, a single anterior nucleus and a parietal chloroplast with posterior pyrenoid (Fig. 28).

The aplanospores and zoospores vary in number, depending on the size of the cell which produced them; young aplanospores are 4–6μ in diameter. They are released by rupture of the parent wall.

Sexual reproduction has not been observed.

Two-week-old colonies on Bristol's agar are dry (macroscopically) and minutely glomerulate (Fig. 92). Comparison of *S. minor* with other known species of *Spongiochloris* has been made in Tables 10–14.

SPONGIOCHLORIS **incrassata** sp. nov. (Fig. 29–35; 93–96)

Cellulae omnis aetatis sphaericae, chloroplastus cellulae iuvenis parietalis poculiformis, in plastidem spongiosam reticulatam, filia in superficie relative tenuia, prope centrum grossa habentem, gradatim transformatus; cellulae 54μ diam. attingentes; membranae in culturis duarum hebdomadum aetate manifeste incrassatae stratifactaeque; cellulae maturae multinucleatae.

Reproductio asexualis per zoosporas aplanosporasque fissione progrediente effectas, membrana parentali rupta liberatas. Zoosporae ovatae, chloroplastum parietalem, pyrenoideum posterius, stigma anterius, duas vacuolas contractiles et flagella habentes, 7–8μ long., 2–4μ lat., quiescentes sphaericae factae.

TABLE 10. *Morphological attributes of known species of Spongiochloris*

Organisms	Vegetative cell size	Cell wall	Nature of chloroplast	Position and no. of pyrenoids	Nature of zoospores
S. excentrica	30μ–60μ	not thickening markedly (bubble-like thickening may be present)	net-like (coarse strands)	1, excentric	15μ long × 2μ wide, elongate
S. incrassata	38μ–54μ	outer wall layer thick and stratified (3μ or more)	net-like (fine strand at surface, coarse at the center)	1, central	7μ long × 2μ wide, ovoid
S. lamellata	80μ	outer wall layer thick (25μ), composed of several layers	sponge-like rather than net-like	several, excentric	9–13μ long × 3–3μ wide, elongate
S. minor	36μ–40μ	outer wall layer thickening markedly with age (2.7μ)	net-like (coarse strands)	several, excentric	8–8.5μ long × 3–3.5μ wide, ovoid
S. spongiosa	30μ–100μ	outer wall layer not thickening markedly	net-like (very fine strands)	1, central	15μ long × 2μ wide, elongate

Reproductio sexualis non observata.
Origo: a loco As-Sanam, Lat. 21°46′ N, Long. 53°10′ E, Saudi Arabia dicto.
Cult. num. Ch-4.

The organism here described as a second new species of *Spongiochloris, S. incrassata,* was isolated into bacteria-free culture from a soil sample collected from As-Sanam. Lat. 21°46′ N, Long. 53°10′ E Saudi Arabia.

The vegetative cells are spherical and vary in size (Fig. 93). Cells with a maximum diameter of 54μ have been observed. The cell wall is markedly thickened and stratified even in 2-week-old cultures (Fig. 29, 94, 95) and may be as much as 2 or 3μ thick.

The chloroplast of *Spongiochloris incrassata* varies in appearance with age. Young vegetative cells, recently developed from zoospores, have a parietal chloro-

TABLE 11. *Colonial attributes of 2-week-old cultures of Spongiochloris grown under standard conditions on Bristol's agar; color of aging (stationary-phase) cells on Bristol's agar slants*

Species	Macroscopic appearance	14×, transmitted light	Color at 3 months
S. excentrica	very shiny	smooth, homogeneous	yellowish-green
S. incrassata	dry	scabellate, cracked into irregular polygonal patches	brick-red flecks on green
S. lamellata	dry	homogeneous, but granular	orange at the edge of slant
S. minor	dry	homogeneous, minutely glomerulate	entirely bright orange
S. spongiosa	dry	homogeneous, but granular	green tinged with orange

TABLE 12. *Effects of certain carbon compounds*[a] *added to Bristol's solution on growth of Spongiochloris species in light*

Species	Bristol's solution	Arabinose	Glucose	Fructose	Sodium acetate	Ribose	Xylose
S. excentrica	good	trace	fair+	fair	fair	fair	trace
S. incrassata	trace[c]	trace	fair	trace+	trace−	trace−	trace−
S. lamellata	fair	trace−	trace+	fair−	trace−	fair	trace
S. minor	trace+[c]	trace	fair+[b]	fair−	fair−	trace+	none
S. spongiosa	fair	trace	good	trace	none	trace	trace

[a] Added at a concentration of 0.75% to Bristol's solution.
[b] Color changed to orange.
[c] Probably has growth factor requirement.

TABLE 13. *Comparative growth of Spongiochloris species on/in certain complex media*

Species	Yeast-extract agar	Proteose-peptone agar	Bacto nutrient agar	Bacto thio-glycollate med.	Bacto AC broth	Bacto nutrient broth
S. excentrica	excellent	excellent	good	excellent	trace	fair
S. incrassata	fair	fair	fair	good	trace−	trace+
S. lamellata	fair	fair	trace	trace−	trace−	trace
S. minor	fair	good	fair	fair−	trace	fair−
S. spongiosa	fair	good	trace	trace	trace−	trace−

plast (Fig. 30); later, as the cells increase in size, the chloroplast segments (Fig. 31), finally becoming a net-like configuration which is relatively fine-stranded at the surface of the cell, becoming coarse at the center (Fig. 32). The nature of the plastid, of course, becomes obscured as the cells age because of the presence of starch and oil in large quantities.

TABLE 14. *Sensitivity of Spongiochloris species to certain agents*[a]

	11–102A						11–102B					
	(A +)	(AB +)	(B +)	(C +)	(CA +)	(CS +)	(D +)	(E +)	(K +)	(N +)	(NV +)	(NS +)
S. excentrica	....	+	....	....	....	+	....	....	+	+	....	....
S. incrassata	....	+	....	....	....	+	....	....	....	+	....	....
S. lamellata	....	+	....	....	....	+	....	....	+	+	....	....
S. minor	....	+	....	....	....	+	+	+	+	+	....	....
							(sl)	(sl)				
S. spongiosa	....	+	....	....	....	+	....	....	+	+	....	+

	11–102C						11–102D					
	(OL +)	(P +)	(PB +)	(PM +)	(R +)	(SY +)	(CY +)	(S +)	(SC +)	(T +)	(TPO +)	(TE +)
S. excentrica	....	....	+	+	....	....	....	....	....	....	....	....
S. incrassata	....	....	+	+	....	....	....	....	....	....	....	....
S. lamellata	....	....	+	+	....	....	....	....	....	....	....	....
S. minor	....	....	+	+	....	....	....	+	....	....	....	....
S. spongiosa	....	....	+	+	....	....	+	+	....	....	....	....
							(sl)	(sl)				

	11–102E						11–102F					
	(EL +)	(G +)	(KY +)	(TR +)	(V +)	(VA +)	(MA +)	(SD +)	(SM +)	(SSS +)	(ST +)	(TH +)
S. excentrica	....	....	....	+	....	....	....	....	....	....	....	....
S. incrassata	....	....	....	+	....	....	....	....	....	....	....	....
S. lamellata	....	....	....	+	....	....	....	....	....	....	....	....
S. minor	....	....	....	+	....	....	....	....	....	....	....	....
S. spongiosa	....	....	....	+	....	....	....	....	....	....	....	....

	11–102G							
	(AL +)	(F +)	(FC +)	(FR +)	(IH +)	(M +)	(PS +)	0.04% sulfathiazole
S. excentrica	....	+	....	+	....	+	....	trace
S. incrassata	+	+	....	+	....	+	....	trace +
S. lamellata	....	+	....	....	....	+	....	fair
S. minor	+	+	+	+	+	+	+	trace −
S. spongiosa	....	+	....	....	....	+	+	fair

[a] The agents and the abbreviations for them are listed in Table 1; + indicates inhibition; .... indicates no inhibition; (sl) means slight inhibition.

The pyrenoid of the young vegetative cells is parietal when the plastid is entirely parietal. As the latter becomes more complex and as its net-like character becomes apparent, the pyrenoid becomes central. At least one pyrenoid always is embedded in the chloroplast (Fig. 29–32, 93); occasionally 2 or more are present.

The cells are uninucleate only when very young (Fig. 93, 95); as they enlarge, they become multinucleate (Fig. 29). The nuclei are suspended within the hyaline cytoplasm of the living cells.

Asexual reproduction is accomplished by means of aplanospores and zoospores (Fig. 33, 34) formed by progressive cleavage of the protoplast. The zoospores are ovoid, 7–8μ in length and 2–4μ in width. The individual zoospore possesses 2 flagella of equal length, 2 anterior contractile vacuoles, an anterior stigma, a single anterior nucleus and a parietal chloroplast with a posterior pyrenoid.

The most prevalent, obvious, and characteristic method of multiplication of the organism is by aplanospore formation; zoosporogenesis is less frequently encountered. The aplanospores and zoospores vary in number, depending on the size of the cell which produced them.

Sexual reproduction has not been observed.

Two-week-old colonies on Bristol's agar are dry, rugose and cracked (Fig. 94). Additional supplementary attributes are listed in Tables 11–14.

In appraising the morphological and supplementary attributes of *Spongiochloris minor* and *S. incrassata*, comparative study of these attributes in the other known species was included.

Table 10 summarizes the morphological and supplementary attributes of all the known species of *Spongiochloris* which have been studied comparatively in this investigation. Appraisal of the morphological and supplementary attributes of *S. minor* and *S. incrassata* has impelled the writers to consider these 2 isolates of *Spongiochloris* from desert soils as new species.

Morphologically, *S. incrassata* and *S. minor* are closest to *S. spongiosa* Starr. They differ from each other especially in cell size and thickening of the outer wall layers in stationary phase cultures.

With respect to supplementary attributes, several points are noteworthy. For example, the color of *S. minor* becomes orange in Bristol's solution supplemented with glucose, while the other species remain green. Growth of *S. excentrica* is excellent on yeast-extract agar, Proteose-peptone agar and Bacto Thioglycollate media (Table 13), in contrast to that of the other species.

With respect to the "Multidisk" tests (Table 14), the following conclusions can be drawn:

(1) All 5 species are inhibited by Amphotericin B, Colistin sulfate, Neomycin, Polymyxin-B, Paromomycin, Triclobisonium chloride, Nitrofurantoin and Methenamine mandelate.

(2) Demethylchlortetracycline, Erythromycin, Nystatin, Cycloserine, Nitrofurazone and Iso-nicotinic acid hydrazide each inhibited only 1 species.

(3) Dihydrostreptomycin, Furaltadone, and Para-amino-salicylic acid inhibited only 2 species.

(4) No 2 species of the 5 subjected to the "Multidisk" test had identical patterns of sensitivity.

The following key will aid in distinguishing all the currently known species of *Spongiochloris.*

Key to the species of *Spongiochloris* Starr

1. Cell wall thickening markedly with age 2
1. Cell wall not thickening markedly with age 4
   2. Mature cells with 1 pyrenoid ..... *S. incrassata* sp. nov.
   2. Mature cells with more than 1 pyrenoid 3
3. Young cells coherent by their cell walls; 3-month-old cultures on Bristol's agar becoming orange at the margin of colony ..... *S. lamellata* Deason & Bold (1960)
3. Young cells isolated, separate; 3-month-old cultures on Bristol's agar becoming bright orange all over ..... *S. minor* sp. nov.
   4. Pyrenoid central in mature cells ..... *S. spongiosa* Starr (1955)
   4. Pyrenoid excentric in mature cells ..... *S. excentrica* Starr (1955)

RADIOSPHAERA *dissecta* (Korschikoff) Starr. (Fig. 97–104)

Two organisms isolated from arid soils clearly possess all the generic attributes of *Radiosphaera,* namely: (1) vegetative cells with an asteroid chloroplast consisting of peripheral lobes joined with a central mass; (2) vegetative cells with at least 1 pyrenoid; (3) production of *Chlamydomonas*-type (walled) zoospores which do not immediately become spherical at quiescence.

Two organisms were isolated into bacteria-free culture from soil samples from 2 different collections. One (Ch-14) was isolated from a sample collected from the west side of the highway, North of Samalayuca, Chihuahua, Mexico. The other (K-9) was isolated from a sample of algae from South Dakota dusts (courtesy of Mr. R. M. Brown, Jr., Department of Botany, The University of Texas). The 2 organisms were carefully studied and compared with *R. dissecta* (Korschikoff) Starr (1955). The 3 taxa are morphologically alike. The vegetative cells are non-motile and spherical, except that young cells (those recently derived from walled zoospores) are ellipsoidal (Fig. 97). As the cells age, they gradually become spherical. Cell size is variable (Fig. 103) and, as the cultures age, cells up to 150μ in diameter are not rare, although smaller cells are always present. The cell wall is slightly thickened with age. The chloroplast is asteroidal, consisting of peripheral lobes joined with a central mass (Fig. 98). In young cells, the chloroplast is parietal and the lobes are simple. As the cells increase in size, the periphery of the chloroplast becomes more lobed, the lobes being joined with the central portion which contains a single pyrenoid. The pyrenoid appeared to be composed of many radiating, crystal-like bodies. The nature of chloroplast and pyrenoid is obscured in old cultures because of the presence of oil and starch granules (Fig. 104). Young vegetative cells are uninucleate, but as the cells increase in size, they become multinucleate; the nuclei lie between the lobes of the chloroplast.

Asexual reproduction is accomplished by means of zoospores (Fig. 99–102)

and aplanospores formed by progressive cleavage of the protoplast. They are of the *Chlamydomonas*-type (Starr, 1955), possess a thin wall, parietal chloroplast with posterior pyrenoid, single anterior nucleus, anterior stigma, 2 flagella of equal length and 2 contractile vacuoles. The zoospores retain their shape upon quiescence.

Reproduction is also effected by aplanospore formation. The aplanospores resemble immature vegetative cells. They are liberated by rupture of the parental cell wall.

Sexual reproduction has not been observed.

At the time (1955) that Starr worked with *Radiosphaera*, physiological attributes had not been considered in the taxonomy of chlorococcalean algae. In the present investigation, the writers have compared certain supplementary attributes in Starr's strain and with those in the 2 newly isolated ones. The results indicated that although morphologically indistinguishable, the 3 isolates differ physiologically.

Tables 15–18 show that with respect to colony characters (dry and rough), the 3 organisms are similar, but with respect to sensitivity to antibiotics and growth in organic media (in the light), some differences are evident. There are only minor differences in tests with organic carbon, but with yeast-extract agar and sensitivity to antibiotics, greater differences among the isolates are apparent.

Chlorosarcinopsis Herndon

Two isolates of *Chlorosarcinopsis* have been studied intensively in the present investigation and described as new taxa.

TABLE 15. *Colonial attributes of 2-week-old cultures of Radiosphaera grown under standard conditions on Bristol's agar; color of aging (stationary-phase) on Bristol's agar slants*

Species	Macroscopic	14×. transmitted light	Color at 3 months
R. dissecta	dry	rough, (coarse granular)	light green
R. dissecta (K-9)	dry	rough, (coarse granular)	light green
R. dissecta (Ch-14)	dry	rough, (coarse granular)	light green

TABLE 16. *Effects of certain carbon compounds*[a] *on the growth of Radiosphaera in light*

Species	Bristol's solution	Arabinose	Glucose	Fructose	Sodium acetate	Ribose	Xylose
R. dissecta	fair	fair	trace +	trace −	trace −	trace +	none
R. dissecta (K-9)	fair	fair +	trace +	trace	none	fair +	fair −
R. dissecta (Ch-14)	trace	trace −	trace −	none	none	trace −	trace −

[a] Added at a concentration of 0.75% to Bristol's solution.

TABLE 17. *Comparative growth of Radiosphaera on/in certain complex media*

Species	Yeast-extract agar	Proteose-peptone agar	Bacto nutrient agar	Bacto AC broth	Bacto thio-glycollate med.	Bacto nutrient broth
R. dissecta	good	fair +	fair +	trace —	fair	trace
R. dissecta (K-9)	trace	fair	trace —	none	trace	none
R. dissecta (Ch-14)	trace	trace —	trace —	trace —	trace	none

TABLE 18. *Sensitivity of isolates of Radiosphaera to certain agents*[a]

	11–102A						11–102B					
	(A +)	(AB +)	(B +)	(C +)	(CA +)	(CS +)	(D +)	(E +)	(K +)	(N +)	(NV +)	(NS +)
R. dissecta	+	+	....	....	....	+	+	+	+	+	....	+
R. dissecta (K-9)	....	+	....	....	....	+	+	....	+	+	....	+
R. dissecta (Ch- 4)	....	....	....	....	....	+	....	....	+	+	....	+

	11–102C						11–102D					
	(OL +)	(P +)	(PB +)	(PM +)	(R +)	(SY +)	(CY +)	(S +)	(SC +)	(T +)	(TAO +)	(TE +)
R. dissecta	....	....	+	+	....	....	....	+	....	+	....	....
R. dissecta (K-9)	....	....	+	+	....	....	....	+	....	+	....	....
R. dissecta (Ch-14)	....	....	+	+	....	....	....	+	....	....	....	....

	11–102E						11–102F					
	(EL +)	(G +)	(KY +)	(TR +)	(V +)	(VA +)	(MA +)	(SD +)	(SM +)	(SSS +)	(ST +)	(TH +)
R. dissecta	....	....	....	+	....	....	....	....	....	....	....	....
R. dissecta (K-9)	....	....	....	+	....	....	....	....	....	....	....	....
R. dissecta (Ch-14)	....	....	....	+	....	....	....	....	....	....	....	....

	11–102G							
	(AL +)	(F +)	(FC +)	(FR +)	(IH +)	(M +)	(PS +)	0.04% sulfathiazole
R. dissecta	+	+	....	+	....	+	+	fair
R. dissecta (K-9)	+	+	....	+	....	+	....	fair
R. dissecta (Ch-14)	+	+	....	+	....	+	....	trace

[a] The agents and the abbreviations for them are listed in Table 1; + indicates inhibition; .... indicates no inhibition; (sl) means slight inhibition.

CHLOROSARCINOPSIS **gelatinosa** sp. nov. (Fig. 36–42; 105–108)

Cellulae typice in fasciculis tridimensionalibus, in matrice communi inclusae; cellulae tantummodo iuvenes (e zoosporis nuper oriundae) singulares, hae sphaericae 6–10μ diam. Cellulae uninucleatae chloroplastum parietalem poculiformen et pyrenoideum immersum typice excentricum habentes. Membrana cellulae semper tenuis.

Reproductio asexualis per zoosporas typice 4–8 in unaquaque cellula bipartitionibus successivis effectas; zoosporae ovatae 6–8μ long., 2–5μ lat., duo flagella anteriora aequa, vacuolas contractiles nucleum stigmaque anterius et chloroplastum parietalem, pyrenoideo posteriore praeditum, habentes; zoosporea quiescentes sphaericae factae.

Reproductio sexualis non observata.

Origo: a loco as-Sanam, air-strip surface of Zibar; Lat. 21°46′ N, Long. 53°10′ E, Saudi Arabia dicto.

Cult. num.: Ch-5.

The organism herein described as a new species, *Chlorosarcinopsis gelatinosa,* was isolated into bacteria-free culture from a soil sample collected from As-Sanam, air-strip surface at Zibar, Lat. 21°46′ N, Long. 51°10′ E, Saudi Arabia.

Vegetative cells are spherical when solitary, although when in groups they may be angular as the result of mutual compression (Fig. 107). Young solitary vegetative cells attain a diameter of 6–10μ (Fig. 36). They are spherical, possess a parietal chloroplast and an excentric pyrenoid; the cytoplasm is granular and usually contains abundant starch granules. The cells are uninucleate (Fig. 36). As they approach 6–10μ in diameter, they either undergo zoosporogenesis or divide vegetatively (Fig. 38–40; 106). Vegetative cell division occurs in actively growing cultures in Bristol's liquid or agar media. Cytokinesis is preceded by division of the pyrenoid and by nuclear division. A partition is laid down across the center of the parent cell, dividing it into 2 daughter cells (Fig. 37) which remain in close association with the parent cell wall. Vegetative growth is resumed by the daughter cells which, in turn, may undergo cell division. The direction of each vegetative division is, usually, perpendicular to the preceding, so that packets of cells are produced (Fig. 39, 105). Cells in these packets may partially dissociate, but the groups of cells are quite characteristic, especially in older cultures (2-month-old cultures). The packets in this organism are composed typically of 2 or 4 cells usually lying in 1 plane, and they adhere in large clusters with a common gelatinous matrix (Fig. 105).

The cell wall is delicate. Aqueous mounts in India ink and Methylene blue of material from 1-month-old cultures demonstrate the presence of an external, gelatinous layer, a common matrix (as defined by Deason and Bold, 1960).

Cells of cultures 1-month-old become bright orange, and it has been possible, by the use of Sudan IV, to demonstrate a considerable amount of oil in such cells. The cells are uninucleate throughout their development (Fig. 36, 37, 40, 42). This attribute usually is evident from observations of living cells but has been confirmed by preparations stained by aqueous I_2-KI and acetocarmine.

Asexual reproduction is accomplished by dissociation of the cell packets and by zoospore formation. The zoospores arise by successive bipartitions of the protoplast (Fig. 40). Usually 4–8 zoospores are produced by each parent cell. The zoospores

are liberated by rupture of the parent wall (Fig. 106). They are of the *Protosiphon*-type (Starr, 1955). Individual zoospores (Fig. 41) are 6–8μ in length and 3–5μ in width. They are elongate with the anterior pole slightly pointed. Each has 2 flagella of equal length, a single anterior nucleus, parietal chloroplast with posterior pyrenoid, an anterior stigma and 2 contractile vacuoles. At quiescence, they become spherical and then begin vegetative growth.

Sexual reproduction has not been observed.

Two-week-old colonies on Bristol's agar are dry, scabellate vermiform, and adhere to the agar surface (Fig. 108). Three-month-old cultures become bright orange. Additional supplementary attributes are compared with those of other known species in Tables 20–23.

CHLORSARCINOPSIS **eremi** sp. nov. (Fig. 43–46, 109–112).

Cellulae in fasciculis tridimensionalibus ad structuram pseudofilamentosam formandam typice ordinatae; matrix communis nulla; cellulae tantummodo iuvenes (e zoosporis nuper oriundae) singulares, hae sphaericae 6–8μ diam.; cellulae uninucleatae, chloroplastum parietalem poculiformem et pyrenoideum typice excentricum habentes; membrana cellulae semper tenuis.

Reproductio asexualis per zoosporas typice 8–16μ in unaquaque cellula bipartitionibus successivis effectas. Zoosporae ovatae 9–11μ long., 4–6μ lat., duo flagella aequa duas vacuolas contractiles nucleum anteriorem stigma et chloroplastum parietalem, pyrenoideo posteriore praeditum, habentes; zoosporae quiescentes sphaericae factae.

Reproductio sexualis non observata.

Origo: ex exemplo soli a loco Phoenix, Arizona dicto.

Cult. num. Ch-10.

The second species of *Chlorosarcinopsis* investigated was isolated into bacteria-free culture from a soil sample collected from Phoenix, Arizona. Its vegetative cells are spherical when solitary, but when aggregated they may become angular as the result of mutual compression. Young, solitary vegetative cells attain a diameter of 6–8μ (Fig. 43). The cell wall is relatively thin at all ages. Aqueous mounts in India ink and Methylene blue have failed to demonstrate a gelatinous matrix.

The chloroplast is parietal with a single excentric pyrenoid (Fig. 43–45, 110). The plastids of young vegetative cells possess a unipolar opening. This opening is not usually evident in mature cells. Each cell contains a single nucleus in the central cytoplasm. As the cultures age, pyrenoid and nucleus become obscured because of the formation of abundant starch granules and oil.

Asexual reproduction is accomplished by zoospores formed by successive bipartitions (Fig. 109). The zoospores vary in shape and average between 4 and 6μ in width and 9 and 11μ in length. Each zoospore possesses 2 flagella of equal length, 2 anterior contractile vacuoles, an anterior stigma and nucleus, and a parietal

TABLE 19. *Morphological attributes of known species of Chlorosarcinopsis*

Species	Vegetative cell size	Cell wall	Habit of growth under standard conditions	Nature of akinete-like cells	Nature of zoospores
C. aggregata	13.6μ	thin, common matrix absent	packets of 2–4 cells	none	8–22μ long × 2–3.4μ wide, elongate
C. dissociata	15–25μ	thin, common matrix absent	cells dissociated, packets irregular	thick wall size 25–30μ	6–15μ long × 3–6μ wide, spherical to elongate
C. eremi	6–8μ	thin, common matrix absent	slightly dissociate, packets regular arranged to form pseudofilamentous groups	none	9–11μ long × 4–6μ wide, ovoid
C. gelatinosa	6–10μ	thin, common matrix present	packets regular	none	6–8μ long × 3–5μ wide, elongate
C. minor	12μ	thin, common matrix absent	packets regular	none	7–12μ long × 3–5μ wide, fusiform

TABLE 20. *Colonial attributes of 2-week-old cultures of Chlorosarcinopsis grown under standard conditions on Bristol's agar; color of aging (stationary-phase cultures) on Bristol's-agar slants*

Species	Macroscopic	14×, transmitted light	Color at 3 months
C. aggregata	dry	minutely glomerulate; plain margin	orange flecks on green
C. dissociata	dull-shiny	nerved, venose	green flecks on orange
C. eremi	dry	rough; adhere closely to agar surface	entirely orange
C. gelatinosa	dry	rugose vermiform; closely to agar surface	bright orange
C. minor	dry	minutely glomerulate; radiate margin	orange flecks on green

TABLE 21. *Effects of certain carbon compounds*[a] *on growth of Chlorosarcinopsis species in light*

Species	Bristol's solution	Arabinose	Glucose	Fructose	Sodium acetate	Ribose	Xylose
C. aggregata	good	good	good[b]	good	fair+	good	none
C. dissociata	excellent	good	excellent[b]	good[b]	none	good	fair+
C. eremi	good	fair	good	fair	fair	fair	trace+
C. gelatinosa	fair+	trace+	good	fair−	fair	trace	trace−
C. minor	excellent	fair	good+	good	trace	excellent	none

[a] Added at a concentration of 0.75% to Bristol's solution.
[b] With orange ring on top of the tube.

TABLE 22. *Comparative growth of Chlorosarcinopsis species on/in certain complex media*

Species	Yeast-extract agar	Proteose-peptone agar	Bacto nutrient agar	Bacto thio-glycollate med.	Bacto AC broth	Bacto nutrient broth
C. aggregata	good	excellent	fair	fair	excellent[a]	fair
C. dissociata	fair	good	fair	fair+	excellent[a]	fair
C. eremi	trace	trace	trace+	excellent	excellent	fair
C. gelatinosa	trace+	trace	trace	excellent[b]	fair	trace+
C. minor	good	good	fair	excellent[c]	trace+	fair

[a] Excellent growth only at the bottom of the tube.
[b] Excellent growth at the top.
[c] Excellent growth at junction of aerobic and anaerobic layers; fair at top.

chloroplast with posterior pyrenoid. At quiescence, the zoospores become spherical and the flagella, contractile vacuoles and stigmata disappear. The zoospores then begin vegetative growth.

Reproduction is also effected by vegetative cell division, which results in the formation of many-celled packets, usually in 1 plane (Fig. 45, 110). It is quite typical in this organism, even in older cultures (1 month old), that the cells remain in packets which are arranged in pseudofilamentous configurations (Fig. 111). In stationary-phase cultures, however, the cells become somewhat dissociated, but most remain together.

Sexual reproduction has not been observed.

Two-week-old cultures on Bristol's agar are dry and depress the agar surface (Fig. 112). Three-month-old cultures become orange. Additional supplementary attributes are compared with those of other known species in Tables 20–23.

In addition to *Chlorosarcinopsis gelatinosa* and *C. eremi,* 3 other species of *Chlorosarcinopsis* were available for comparative study in culture. They are *C. minor* (Herndon, 1958a), *C. dissociata* (Herndon, 1958a) and *C. aggregata* (Arce and Bold, 1958). The writers have included a comparative study of all the known species of *Chlorosarcinopsis* with respect to the supplementary attributes. This

TABLE 23. *Sensitivity of Chlorosarcinopsis species to certain agents*[a]

	11-102A						11-102B					
	(A +)	(AB +)	(B +)	(C +)	(CA +)	(CS +)	(D +)	(E +)	(K +)	(N +)	(NV +)	(NS +)
C. aggregata	....	+	....	....	....	+	....	....	+	+	....	+
C. dissociata	....	+	....	....	....	+	....	....	+	+	....	+
C. eremi	....	+	....	....	....	+	....	....	+	+	....	....
C. gelatinosa	....	+	....	....	....	+	....	....	+	+	....	....
C. minor	....	....	....	....	....	+	....	....	....	....	....	....

	11-102C						11-102D					
	(OL +)	(P +)	(PB +)	(PM +)	(R +)	(SY +)	(CY +)	(S +)	(SC +)	(T +)	(TPO +)	(TE +)
C. aggregata	....	....	+	+	....	....	....	....	....	....	....	....
C. dissociata	....	....	+	+	....	....	+ (sl)	+ (sl)	....	....	....	....
C. eremi	....	....	....	+	....	....	....	+	....	....	....	....
C. gelatinosa	....	....	+	+	....	....	....	....	....	....	....	....
C. minor	....	....	+	+	....	....	....	....	....	....	....	....

	11-102E						11-102F					
	(EL +)	(G +)	(KY +)	(TR +)	(V +)	(VA +)	(MA +)	(SD +)	(SM +)	(SSS +)	(ST +)	(TH +)
C. aggregata	....	....	....	+	....	....	....	....	....	....	....	....
C. dissociata	....	....	....	+	....	....	....	....	....	....	....	....
C. eremi	....	....	....	+	....	....	....	....	....	....	....	....
C. gelatinosa	....	....	....	+	....	....	....	....	....	....	....	....
C. minor	....	....	....	+	....	....	....	....	....	....	....	....

	11-102G							
	(AL +)	(F +)	(FC +)	(FR +)	(IH +)	(M +)	(PS +)	0.04% sulfathiazole in Bristol's solution
C. aggregata	+ (sl)	+	+	+	....	+	....	excellent
C. dissociata	....	+	....	+	....	+	....	trace
C. eremi	+	+	....	+	....	+	....	trace
C. gelatinosa	....	+	....	+	....	+	....	trace
C. minor	....	+	....	+	....	+	....	trace

[a] The agents and the abbreviations for them are listed in Table 1; + indicates inhibition; .... indicates no inhibition; (sl) means slight inhibition.

involved, as a necessary prerequisite, purification of the cultures of *C. minor* and *C. dissociata* not previously available in the axenic condition. On the basis of both morphological (Table 19) and physiological attributes (Tables 20–23), it is clear that *C. gelatinosa* and *C. eremi* differ from each other and from the species previously described.

With respect to colonial attributes, the color change is noticeable. With respect to defined media, several points are noteworthy. For example, arabinose and ribose inhibited growth only in 1 species, *C. gelatinosa*. Xylose inhibited growth of 4 of the 5 species of *Chlorosarcinopsis*. The color of 2 species changed in Bristol's solution supplemented with glucose and fructose. Furthermore, the 2 new species did not grow well on yeast-extract agar, Proteose-peptone agar and Bacto Nutrient Agar. With respect to the "Multidisk" tests, the following conclusions are drawn:

(1) All 5 species were inhibited by Colistin sulfate, Paromomycin, Triclobisonium chloride, Nitrofurantoin, Furazolidone and Methenamine mandelate.

(2) Nitrofurazone, Cycloserine each inhibited only 1 species.

(3) Nystatin, Dihydrostreptomycin, Furaltadone each inhibited only 2 species.

Key to the species of *Chlorosarcinopsis* Herndon

1. Cells not remaining aggregated in *Sarcina*-like packets ..... *C. dissociata* Herndon (1958)
1. At least some cells remaining aggregated in obvious *Sarcina*-like packets even in old cultures 2
   2. Cells remaining aggregated in packets of more than 4, usually arranged in 2 or 3 planes ..... *C. minor*. Herndon (1958)
   2. Cells remaining aggregated in packets of 4 or more cells, usually in 1 plane 3
3. Common matrix present in cultures in stationary phase of growth ..... *C. gelatinosa* sp. nov.
3. Common matrix absent in cultures in stationary phase of growth 4
   4. Packets of cells not arranged to form pseudo-filamentous configuration ..... *C. aggregata* Arce and Bold (1958)
   4. Packets of cells arranged to form pseudo-filamentous configuration ..... *C. eremi* sp. nov.

CHLOROSARCINA Gerneck.

The genus *Chlorosarcina*, according to Vischer (1933) and Herndon (1958a) includes green algae with the following attributes: (1) a cup-like parietal chloroplast; (2) without a pyrenoid; and (3) zoospores (wall-less) which become spherical immediately upon quiescence. Two new species of this genus are described below and subsequently compared with the only other species available in culture, namely, *Chlorosarcina stigmatica* Deason (1959) (Tables 24–27).

CHLOROSARCINA **brevispinosa** sp. nov. (Fig. 47–53; 113–121)

Cellulae singulares, aut in aggregationibus pseudofilamentosis, tractatae multo frigore (e.g.,–8°C per unam habdomadem, deinde ad solutionem Bristolii novam translatae) fasciculas tridimensionales Sarcinoideas formentes. Cellulae singulares

usque ad 25μ diam., membranis etiam in culturis vestustioribus non perspicue incrassatis; matrix communis semper nulla. Chloroplasti cellularum vegetativarum typice parietales poculiformes bipartiti sine pyrenoideis; cellulae uninucleatae; cultura in incrementi periodo immobili sublutea facta; akineta, spinis brevibus (usque ad 4μ long.) praedita, culturis senescentibus abunde facta, ad aplanosporas zoosporasque (?) formandas germinantia, membrana akineti per fissuram aequatoriam aperta.

Reproductio quoque per zoosporas aplanosporasque bipartitionibus successivis effectas. Zoosporae ovatae, 6–11μ long., 3–5μ lat., chloroplastum parietalem duas vacuolas contractiles anteriores et nucleum anteriorem habentes, stigmata nulla; zoosporae quiescentes sphaericae factae.

Reproductio sexualis non observata.

Origo: e solo deserti versus septentriones a loco Moab, Grand County, Utah dicto. Cult. num. Ch-11.

The organism herein described as a new species, *C. brevispinosa,* was isolated into bacteria-free culture from a soil sample collected from the desert north of Moab, Grand County, Utah. The vegetative cells are spherical when solitary (Fig. 47–49, 113–115) although pseudofilamentous configurations may be formed (Fig. 117). If cultures of this organism are stored in the freezing compartment of an electric refrigerator at 8°C for 1 week and then transferred to Bristol's solution, they respond by undergoing intensified vegetative cell divisions to form *Sarcina*-like packets. These are absent in cultures grown at 22–25°C. Young, solitary, vegetative cells (those recently derived from zoospores) have diameters of 4–7μ, while mature cells may reach 25μ in diameter. The cell wall remains relatively thin at all ages. Some of the cells, however, rapidly increase in size, attaining a diameter of 30–35μ. The cell walls of these are slightly thickened (3–4μ), and their outer wall layers begin to form spiny protuberances (Fig. 49–50, 113, 114, 116, 117). These cells are properly termed akinetes. Cell size is variable in any population, as is typical of all zoosporiferous chlorococcalean algae, because of differences in the ages of the component cells.

The chloroplast of vegetative cells is parietal and cup- or bowl-like and is typically bipartite (Fig. 48). Occasionally, as during zoosporogenesis, more lobes are present. It has been possible by use of aqueous I_2-KI to demonstrate abundant starch granules in these cells, but pyrenoids are absent. The chloroplasts of the akinetes are different. They are more or less massive and a considerable amount of oil can be demonstrated within them (Fig. 50) by use of Sudan IV. A single nucleus is present within the hyaline cytoplasm of the living cells (Fig. 47–49).

Asexual reproduction occurs by means of aplanospores and zoospores (Fig. 51–53, 118–120) formed by successive bipartitions of vegetative cells. The morphology of zoospores is similar to that of *Protosiphon* zoospores (Starr, 1955). They range from 6 to 11μ in length and from 3 to 5μ in width. Each zoospore (Fig. 52) is

biflagellate with the flagella of body length; 2 anterior contractile vacuoles, an anterior nucleus and a parietal chloroplast are present. Stigma and pyrenoid are absent. The number of zoospores in each cell is less than 16, usually 4 or 8. The zoospores are liberated by rupture of the parent wall (Fig. 51). The motile period of the zoospores is apparently short (less than 1 hr). Soon after quiescence, zoospores rapidly become spherical to form tiny vegetative cells (Fig. 47); the flagella and contractile vacuoles soon disappear.

Sometimes, some of the spiny akinetes produce aplanospores which resemble zoospores at quiescence. These aplanospores are released by rupture of the spiny wall (Fig. 53, 118). The spiny akinetes apparently represent the resistant phase of this alga.

At 2 weeks, cultures of *Chlorosarcina brevispinosa* consist largely of single cells (Fig. 115), although pseudofilamentous branches may be formed (Fig. 117). As the cultures age, these cells dissociate and the number of spiny akinetes increases; 2-week-old cultures in darkness tend to form abundant spiny akinetes.

Sexual reproduction has not been observed.

Two-week-old colonies on Bristol's agar are dry and rough (Fig. 121). Three-month-old cultures become tinged with orange. Additional supplementary attributes are considered comparatively with those of known species in Tables 25–28.

CHLOROSARCINA **longispinosa** sp. nov. (Fig. 54–61; 122–128)

Cellulae singulares aut in incrementi periodo immobili fasciculas cubicas efficientes; periodus pseudofilamentosa tantummodo tempore frigoris gravis (e.g., —8°C per unam hebdomadem) evocata, cellulae deinde ad solutionem Bristolii novam translatae. Cellulae singulares usque ad 12μ diam. Membranes senescentes non perspicue incrassatae, matrice communi nulla; chloroplastus cellularum vegetativarum typice parietalis poculiformis multipartitus (plerumque quadripartitus) sine pyrenoideis; cellulae uninucleatae. Culturae in periodo incrementi immobili subluteae factae; akineta membranas crassas necnon spinas longas (usque ad 9μ) habentia, culturis senescentibus abunde producta, sine luce per duas hebdomades saltem ad aplanosporas aut fasciculum quatuor cellularum aut zoosporas (?) formandas germinantia.

Reproductio quoque per zoosporas, bipartitione successiva cellularum vegetativarum plerumque 4 vel 8 in unaquaque cellula effectas; zoosporae ovatae, 8–10μ long., 3–5μ lat., duo flagella aequa duas vacuolas contractiles nucleum anteriorem et chloroplastum parietalem habentes; stigmata pyrenoideaque nulla; zoosporae quiescentes sphaericae factae.

Reproductio sexualis non observata.

Origo: e solo deserti, e regione loci Superior, Pinal County, Arizona dicti collecta. Cult. num. Ch-12.

The second species of *Chlorosarcina* here described as a new taxon was isolated

into bacteria-free culture from a soil sample collected from near Superior, Pinal County, Arizona.

Vegetative cells of *C. longispinosa* are spherical when solitary (Fig. 54, 122), or pseudofilamentous (Fig. 123), or both types may be present in a given culture. The cells occur typically in *Sarcina*-like configurations or packets formed by vegetative cell division (Fig. 55, 61, 124, 126). The cell wall of vegetative cells is thin and does not thicken with age. The cell walls of the spiny akinetes, present also in this species, are slightly thickened, and the outer layers develop prominent, elongate spines (Fig. 59, 60, 125). The latter may be as long as 5–9μ. This characteristic alone (although there are others) can be used to distinguish *C. brevispinosa* from *C. longispinosa*.

The chloroplast of vegetative cells is parietal and divided into several (usually 4) large segments (Fig. 55). Abundant starch granules may be present. As the cultures age, the chloroplasts become less distinct because of the production of oil and starch in large quantities. This is true, especially for the spiny cells (akinetes) at all ages. The alga is uninucleate (Fig. 54, 55, 59, 61) throughout development.

Asexual reproduction is accomplished by means of zoospores formed by successive bipartitions (Fig. 56, 58); the zoospores (Fig. 58) are ovoid or slightly pointed at both ends, 8–10μ in length and 3–5μ in width. Each zoospore possesses 2 flagella of equal length, 2 anterior contractile vacuoles, an anterior nucleus and parietal chloroplast. The zoospores are liberated by the rupture of the parent wall (Fig. 61). At quiescence, they become spherical (*Protosiphon*-type [Starr, 1955]) and begin vegetative growth (Fig. 54, 122).

Sometimes some of the spiny akinetes produced 4-cell packets. These packets are released by rupture of the spiny wall (Fig. 61, 127). This evidently usually occurs in old and dry cultures (more than 6 months old).

Sexual reproduction has not been observed.

Two-week-old colonies on Bristol's agar are dry and rough (Fig. 128). Three-month-old cultures become tinged with orange. Additional supplementary attributes are compared with those of other known species in Tables 24–28.

Three species of the genus *Chlorosarcina,* as delimited by Vischer (1933) and Herndon (1958a), are now available for comparative study in bacteria-free cultures. These are: *Chlorosarcina stigmatica* Deason (1959), *C. brevispinosa* and *C. longispinosa*. The last 2 species are in one respect intermediate between chlorosphaeralean and chlorococcalean algae. For example, although vegetative cell division (*sensu* Fritsch [1935] and Herndon [1958], *non* Smith [1950]) is characteristic of chlorosphaeralean algae, this attribute is not readily observable in *C. brevispinosa* except following cold treatment (−8°C for 1 week). On the other hand, vegetative cell division occurs at ordinary temperatures in *C. stigmatica* and *C. longispinosa,* with the result that characteristic *Sarcina*-like packets are formed. Thus, depending on environmental conditions, the unicellular (chlorococcalean) or packet phase (chlorosphaeralean) may prevail.

Of the 3 species here assigned to *Chlorosarcina,* both *C. brevispinosa* and *C. longispinosa* may form short filaments or pseudofilaments. This attribute might be considered adequate to exclude these 2 species from *Chlorosarcina,* but the writers have been conservative in this connection. Similarly, although cells of the genus *Chlorosarcina* clearly possess single, cup-like parietal plastids (according to Herndon, 1958a), as exemplified by *C. stigmatica,* the plastids of *C. brevispinosa* and *C. longispinosa* are parietal and cup-like, *but* bipartite (*C. brevispinosa*) or multipartite (*C. longispinosa*). In the last instance, this suggests the disc-like plastids of the genus *Bracteacoccus*; but, here again, the writers have been conservative in not erecting a new genus to include taxa with segmented plastids, preferring to treat these 2 organisms as species of *Chlorosarcina.*

With respect to morphological and physiological attributes, the 3 species of *Chlorosarcina* now available in culture may be compared as shown in Tables 24–28.

It is clear from the data in Table 24 that these 3 species differ consistently in a number of morphological attributes.

TABLE 24. *Morphological attributes in species of Chlorosarcina*

Organisms	Vegative cell size, diam. (solitary)	Cell wall	Nature of chloroplast	Habit of growth under standard conditions	Nature of akinetes	Nature of zoospores
C. brevispinosa	25μ	common matrix absent	cuplike, bipartite	mostly solitary or pseudo-filamentous packets of cells developing at low temperatures	35μ in diam. with short-spines (2–4μ)	stigma absent, 6–11μ long × 3–5μ wide, ovoid
C. longispinosa	12μ	common matrix absent	cuplike and multi-partite	*Sarcina*-like packets or solitary	18μ–25μ in diam. with long spines (5–9μ)	stigma absent, 8–10μ long × × 3–5μ wide, ovoid
C. stigmatica	12μ	common matrix present	cuplike, parietal	*Sarcina*-like packets	none	with protuberant stigma, 8μ long × 3.5μ wide, spindle-shaped

TABLE 25. *Colonial attributes of 2-week-old cultures of Chlorosarcina grown under standard conditions on Bristol's agar; and color of aging (stationary-phase cells) on Bristol's-agar slants*

Species	Macroscopic	14×, transmitted light	Color at 3 months
C. brevispinosa	dry	rough, (coarse granular)	tinged with orange
C. longispinosa	dry	rough, (coarse granular)	tinged with orange
C. stigmatica	dry	rough	orange

TABLE 26. *Effects of certain carbon compounds*[a] *on growth of Chlorosarcina species in light*

Species	Bristol's solution	Arabinose	Glucose	Fructose	Sodium acetate	Ribose	Xylose
C. brevispinosa	good	trace	fair	trace	trace	trace	none
C. longispinosa	fair+	trace	good	trace−	none	trace−	none
C. stigmatica	good	fair	fair	good	none	trace	fair−

[a] Added at a concentration of 0.75% to Bristol's solution.

Supplementary characteristics for these organisms are summarized in Tables 25–28. With respect to colony characters, there are only minor differences, but with respect to growth in organic carbon sources and growth in complex media, other differences are evident. With respect to the "Multidisk" tests, these 3 organisms show differences in inhibition patterns.

Friedmannia[8] gen. nov.

Cellulae sphaericae chloroplastum parietalem cavub sine pyrenoideo omnibus periodis habentes, bipartitione successiva ad (plerumque) 4 cellulas-filias tetrahedrales formandas multiplicantes. Cellulae-filiae vicissim ad fasciculas cellularum tetrahedralium similium formandas saepe denuo divisae, hae cellulae aut membrana parentali rupta liberatae aut zoosporas aplanosporasve biflagellatas formantes; zoosporae sine membrana, quiescentes sphaericae factae (typi *Protosiphonis*).

F. israeliensis sp. nov. (Fig. 62–72, 129–136)

Cellulae magnitudine variantes, aggregatis quaternis 12–18μ diam.; zoosporae stigma anterius minutum duas vacuolas contractiles et nucleum aequatorialem ad posteriorem habentes; colonia duarum hebdomadum aetate in "Bristol's agar" (statu consueto) (magnificatione 20 plo) sicca asperaque aspectu.

Reproductio sexualis non observata.

[8] Nomen huius algae Clar. Dr. I. Friedmann, Department of Botany, The Hebrew University, Jerusalem, Israel, honorat.

TABLE 27. *Comparative growth of Chlorosarcina species on/in certain complex media*

Species	Yeast-extract agar	Proteose-peptone agar	Bacto nutrient agar	Bacto thio-glycollate med.	Bacto AC broth	Bacto nutrient broth
C. brevispinosa	good	good	good	trace	trace −	fair +
C. longispinosa	fair	fair	good	trace	trace −	fair +
C. stigmatica	fair	excellent	trace −	trace	none	trace −

	11-102A						11-102B					
	(A +)	(AB +)	(B +)	(C +)	(CA +)	(CS +)	(D +)	(E +)	(K +)	(N +)	(NV +)	(NS +)
C. brevispinosa	....	....	....	....	....	+	....	....	+	+	....	....
C. longispinosa	....	....	....	....	....	+	....	....	+	+	....	....
C. stigmatica	....	+	....	....	+	+	+	+	....	....	....	+

	11-102C						11-102D					
	(OL +)	(P +)	(PB +)	(PM +)	(R +)	(SY +)	(CY +)	(S +)	(SC +)	(T +)	(TAO +)	(TE +)
C. brevispinosa	....	....	+	+	....	....	....	+ (sl)	....	....	....	....
C. longispinosa	....	....	+	+	....	....	....	+ (sl)	....	....	....	....
C. stigmatica	....	....	+	+	....	....	....	+ (sl)	....	....	....	....

	11-102E						11-102F					
	(EL +)	(G +)	(KY +)	(TR +)	(V +)	(VA +)	(MA +)	(SD +)	(SM +)	(SSS +)	(ST +)	(TH +)
C. brevispinosa	....	....	....	+	....	....	....	....	....	....	....	....
C. longispinosa	....	....	....	+	....	....	....	....	....	....	....	....
C. stigmatica	....	....	....	+	....	....	....	....	....	....	....	....

	11-102G							
	(AL +)	(F +)	(FC +)	(FR +)	(IH +)	(M +)	(PS +)	0.04% sulfathiazole in Bristol's solution
C. brevispinosa	+	+	....	+	....	+	....	trace
C. longispinosa	....	+	....	....	....	+	....	trace
C. stigmatica	+	+	+	+	+	+	+	fair

[a] The agents and the abbreviations for them are listed in Table 1; + indicates inhibition; .... indicates no inhibition; (sl) means slight inhibition.

Origo: cultura pura per Clar. Dr. I. Friedmann, e lapide (avdat, Negev Desert) seiuncta.
Cult. num. Ch-28.

A culture of the organism herein described as new genus and species, *Friedmannia israeliensis,* of the Chlorosphaerales, was sent to the writers by Dr. I. Fried-

mann, Department of Botany, The Hebrew University, Jerusalem, Israel, in February, 1961, and has been maintained in bacteria-free culture since.

Friedmannia, although a non-motile organism, reproduces by biflagellate zoospores (Fig. 62, 133). These are ovoid, with pointed anterior poles, and lack a wall. Individual zoospores range from 5 to 8μ in length and from 3 to 5μ in width. Their chloroplast is parietal, their nucleus usually equatorial to slightly posterior, and the contractile vacuoles are anterior. A pyrenoid is absent from the zoospores and they have a tiny anterior stigma. The flagella are equal in length to the cell body. The zoospores are motile only briefly (less than ½ hr) and, upon cessation of motility, they rapidly become spherical (Fig. 63).

These young cells increase somewhat in size and then undergo successive bipartitions to form tetrads of cells (Fig. 64, 65, 129), as in *Borodinella.* These daughter cells really are more like autospores (see below) than like aplanospores, the latter being potentially zoospores. After some growth, each of the 4 cells may divide to form a second generation (Fig. 130), and this may be repeated to form loose clusters of cells. As the cultures age, these clusters may become dissociated into separate, individual cells (Fig. 131). In this respect, therefore, the vegetative cells are like autospores, from which they differ, however, in that a portion of their cell walls seems to include part of the original parent wall.

In older cultures (2 months old) in Bristol's solution, the cells undergo zoosporogenesis (Fig. 66–72; 132–133), a phenomenon which is absent from 2-week-old cultures and restricted to older cultures (beginning at 6 weeks). The postponement of zoosporogenesis in this alga is unparalleled in other unicellular algae in the writers' experience. Cells which undergo zoosporogenesis may vary in size (Fig. 135); it usually occurs when the cells approach a diameter of approximately 10–14μ. In this process, nuclear division apparently occurs without ensuing cytokinesis, and the parietal plastid undergoes division (Fig. 66–68). The zoospores arise by progressive cleavage (Fig. 67, 68).

The zoospores are liberated from the parent wall within a vesicle (Fig. 69–71) which is forced out of the cell by swelling of a colloidal substance between the vesicle and the outermost layer of the cell wall. The substance fills the cell after the vesicle has been completely extruded. The zoospores become actively motile and swim about within the vesicle (Fig. 71). They are eventually liberated by its rupture. At quiescence, the zoospores become spherical (Fig. 63) (*Protosiphon*-type, [Starr, 1955]). The flagella, stigma and contractile vacuoles disappear, and the young cells then begin vegetative growth.

Under certain conditions, some of the cells produce aplanospores, approximately 8–16 in number (Fig. 72, 134). These aplanospores resemble zoospores soon after quiescence. They are retained within the parent wall which expands as the aplanospores enlarge. Aplanospores are liberated by rupture of the parent wall. They are more numerous than the daughter cells which are produced by vegetative cell division (*sensu* Fritsch [1935)], Herndon [1958a], and Deason and Bold [1960]).

The tetrad cells differ clearly in number and origin from aplanospores.

Sexual reproduction has not been observed.

Two-week-old colonies in Bristol's agar are rough and dry (Fig. 136) and dark green. Three-month-old cultures become tinged with orange. Growth is good in Bristol's solution supplemented with glucose and fructose; fair in Bristol's with sodium acetate, ribose and xylose, and trace in arabinose. Growth is excellent on yeast-extract and proteose-peptone agars, good on nutrient agar, fair in AC broth and Nutrient broth, trace in Thioglycollate medium and 0.4% sulfathiazole in Bristol's solution. The alga is inhibited by Amphotericin, Neomycin, Paromomycin, Triclobisonium chloride, Nitrofurantoin and Methenamine mandelate.

Friedmannia has been classified as a chlorosphaeralean alga because of the characteristic vegetative cell division of the organism into tetrahedral quartets (Fig. 65, 129). This same attribute characterizes the genus *Borodinella* Miller and at least 3 taxa presently included in *Chlorococcum* (*C. aplanosporum* Arce and Bold, *C. intermedium* Deason and Bold, and *C. tetrasporum* Arce and Bold). Brown[9] is at present making an intensive comparative study of these species along with 8 or more *Borodinella*-like algae; he has recently reached the tentative conclusion that these 3 species of *Chlorococcum* should be removed from the genus (Brown, personal communication). *Friedmannia* differs from the *Chlorococcum* species and from *Borodinella* and Brown's other isolates in lacking pyrenoids. Although slightly suggestive of *Protococcus, Friedmannia,* of course, produces zoospores, unlike the former. Accordingly, it has been described as a new genus.

C. FACULTATIVE HETEROTROPHY

The 12 organisms isolated from various arid soils, and other related species previously described, were studied to test their capacity to grow in complete darkness as long as certain organic substances are supplied. The substances, used were: glucose, fructose, sodium acetate, arabinose and ribose. These carbon sources were added, at a concentration of 0.75%, to duplicate tubes of Bristol's solution and stored in darkness for 2 months. This experiment was repeated with the sugars autoclaved separately from the basal medium and subsequently added aseptically.

The results are summarized in Table 29.

From these data, the following conclusions may be drawn:

(1) Of the 27 organisms tested for facultative heterotrophy, 12 grow to a degree described herein as "fair," "good" or "excellent," in one or several carbon sources; that is, they clearly are facultatively heterotrophic.

(2) Of all the carbon sources used, xylose alone did not support heterotrophic growth. Ribose supported such growth in the case of only 1 organism (*Neochloris minuta*).

[9] Personal communication from Mr. R. Malcolm Brown, Jr., Department of Botany, The University of Texas.

TABLE 29. *Effect of certain carbon compounds*[a] *on growth of certain unicellular Chlorophyceae in darkness*

Organisms	Bristol's solution	Arabinose	Glucose	Fructose	Sodium acetate	Ribose	Xylose
Neochloris aquatica	none	fair	fair	trace	none	trace	trace
N. alveolaris	none	none	good	fair	fair	trace	trace
N. fusispora	none	trace	trace	trace	none	trace −	none
N. gelatinosa	trace	trace	trace	trace	trace	trace	trace −
N. minuta	trace	none	none	none	none	fair	none
N. oleoabundans	trace −	none	none	none	none	none	none
N. pseudoalveolaris	trace −	trace −	trace −	trace −	trace −	trace −	trace −
N. pyrenoidosa	trace −	none	none	none	none	none	none
N. terrestris	trace −	trace −	fair −	trace −	trace −	trace	trace −
Chlorococcum diplobionticoideum	none	none	none	none	none	none	none
Spongiochloris excentrica	trace	trace	good	good	trace	trace	trace −
S. incrassata	trace −	trace −	none	none	trace −	none	none
S. lamellata	none	none	none	none	none	none	none
S. minor	none	none	fair	none	none	none	none
S. spongiosa	trace	none	good	none	none	none	none
Radiosphaera dissecta	trace −	none	trace −	none	none	none	none
R. dissecta(K-9)	trace −	trace −	none	none	none	none	none
R. dissecta(CH-14)	none	none	none	none	none	none	none
Chlorosarcinopsis aggregata	trace −	trace	good	good	trace	trace −	none
C. dissociata	trace	trace	excellent	good	none	trace	none
C. eremi	none	trace	excellent	good	good	none	none
C. gelatinosa	none	none	none	none	none	none	none
C. minor	none	none	excellent	trace	none	none	none
Chlorosarcina brevispinosa	trace −	none	none	none	none	trace −	none
C. longispinosa	trace −	trace −	trace −	trace −	none	none	none
C. stigmatica	trace −	trace −	none	none	none	none	none
Friedmannia israeliensis	trace +	fair	good	excellent	fair	trace +	trace

[a] Added at a concentration of 0.75% to Bristol's solution.

(3) Only 2 species, namely *Neochloris aquatica* and *Friedmannia israeliensis* could use arabinose appreciably in darkness.

(4) *Neochloris aquatica, N. terrestris,* and *Spongiochloris minor* grow to "fair" degree in glucose in darkness. *Neochloris alveolaris, Spongiochloris incrassata, S. spongiosa, Chlorosarcinopsis aggregata,* and *Friedmannia israeliensis* grow better

("good") and *Chlorosarcinopsis dissociata, C. eremi* and *C. minor* grow excellently ("excellent") in Bristol's solution supplemented with glucose in darkness. All but one species of *Chlorosarcinopsis* are facultatively heterotrophic when supplied with glucose.

(5) Growth of *Neochloris alveolaris* is "fair," that of *Spongiochloris excentrica* and *Chlorosarcinopsis dissociata* is "good," and *Friedmannia israeliensis* grows excellently in Bristol's with fructose in darkness.

(6) Growth of *Neochloris alveolaris* and of *Friedmannia israeliensis* is "fair," while growth of *Chlorosarcinopsis eremi* is "good" in Bristol's with sodium acetate in darkness.

(7) The newly described *Chlorococcum diplobionticoideum* is similar to other species of the genus (Parker, Bold, and Deason, 1961) in its failure to grow heterotrophically on the carbon sources supplied.

Summary

Enrichment cultures made from samples of arid soils from Saudi Arabia, Mexico, Arizona, Utah, and Israel have been studied with respect to their algal flora, as it developed in such cultures. General observations of such cultures have been summarized in tabular form. In addition, on the basis of comparison of both morphological and supplementary attributes, 1 new genus and 9 new species of Chlorophyceae isolated from various arid soil samples have been described as follows:

Order Chlorococcales
  Family Chlorococcaceae
    Neochloris oleoabundans sp. nov.
    Chlorococcum diplobionticoideum sp. nov.
    Spongiochloris minor sp. nov.
    Spongiochloris incrassata sp. nov.
Order Chlorosphaerales
  Family Chlorosphaeraceae
    Chlorosarcinopsis gelatinosa sp. nov.
    Chlorosarcinopsis eremi sp. nov.
    Chlorosarcina brevispinosa sp. nov.
    Chlorosarcina longispinosa sp. nov.
    Friedmannia israeliensis gen. et. sp. nov.

In addition, 2 isolates of *Radiosphaera* have been described and studied comparatively with the only other isolates available. Although the 2 taxa are morphologically similar to each other and to the previously described species *R. dissecta* Starr (1955), they differ among each other with respect to supplementary attributes.

Finally, it has been determined by experiments with pure cultures that certain chlorophycean species can lead a facultatively heterotrophic existence in darkness, and preserve their green color, when supplied with several carbon sources, includ-

ing glucose, fructose, sodium acetate, arabinose and ribose. None can do so in xylose.

All of the organisms which are described as new to science have been grown in bacteria-free condition and have been deposited in the Culture Collection of Algae, Indiana University, Bloomington, Indiana. Herbarium specimens have been deposited in the Chicago Natural History Museum.

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CORRECTION

Chlamydomonas carrizoensis Deason et Bold, nom. nov. (Basionym: *Chlamydomonas pyrenoidosa* Deason et Bold [1960],[1] non *Chlamydomonas pyrenoidosa* Schiller [1952][2]).

[1] Deason, T. R., and H. C. Bold. 1960. Phycological Studies. I. Exploratory studies of Texas soil algae. Univ. Texas Publ. 6022.

[2] Schiller, J. 1952. Neue Mikrophyten aus dem Neusiedler See und benachbarter Gebiete. Österr. Bot. Zeitschr. 99: 100–117.

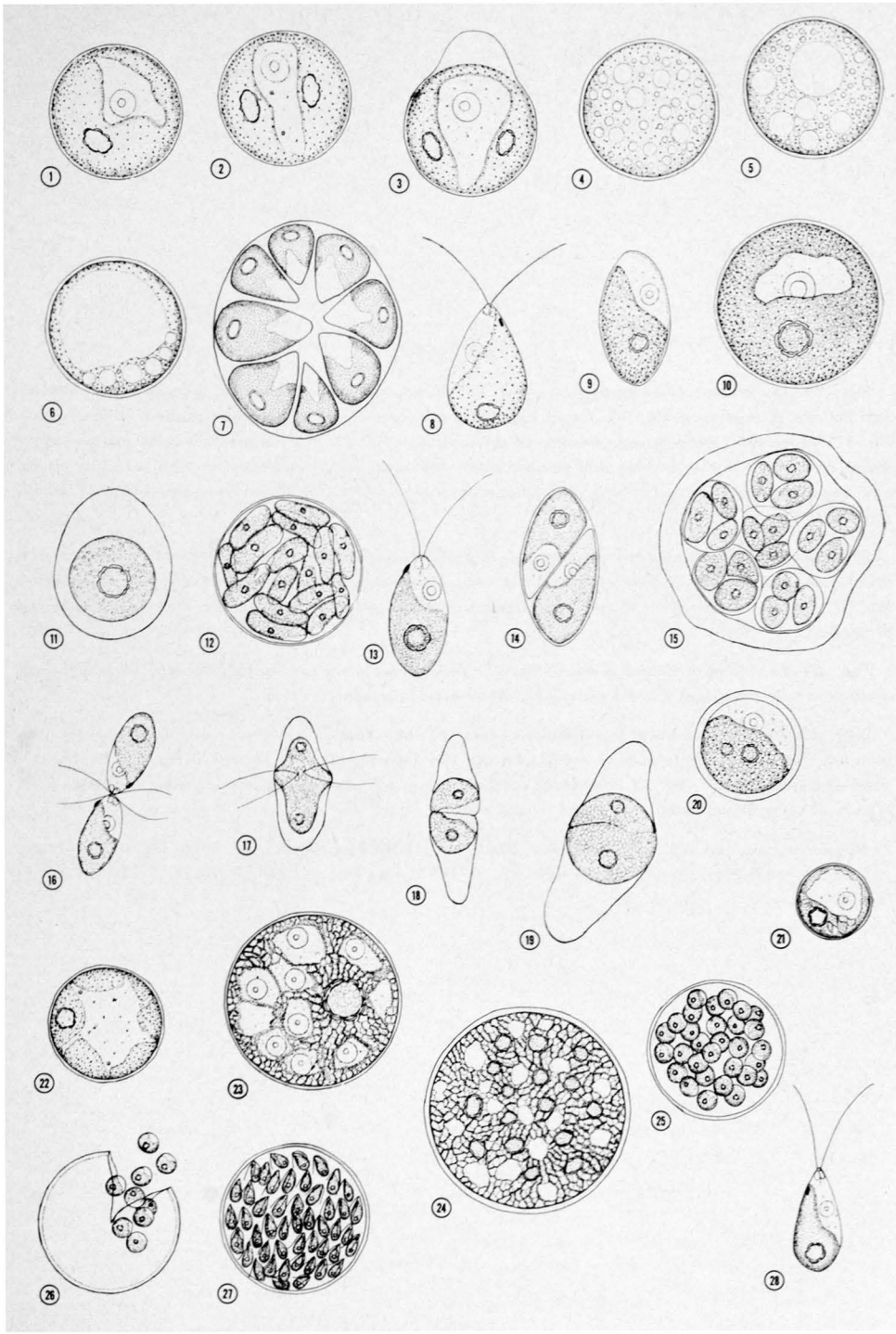
Figs. 1–28

Figs. 1–8. *Neochloris oleoabundans*.—Fig. 1. Vegetative cell; note single pyrenoid.—Fig. 2. Mature vegetative cell; note 2 pyrenoids.—Fig. 3. Mature cell from culture in stationary phase of growth; note bubble-like thickening at one pole.—Figs. 4–6. Mature cells from 6-week-old culture; note abundant oil droplets.—Fig. 7. Zoosporangium optical section.—Fig. 8. Individual zoospore.

Figs. 9–20. *Chlorococcum diplobionticoideum*.—Fig. 9. Young vegetative cell recently derived from zoospore.—Fig. 10. Mature vegetative cell—Fig. 11. Mature vegetative cell from culture in stationary phase of growth; note thickening of outer wall layer—Fig. 12. Zoosporangium (gametangium)—Fig. 13. Motile individual (either zoospore or gamete)—Fig. 14. Oblique division of non-motile individual.—Fig. 15. Aplanospores within parental wall—Fig. 16–19. Fusion of isogametes—Fig. 20. Mature zygote; note 1 pyrenoid beginning to degenerate.

Figs. 21–28. *Spongiochloris minor*.—Fig. 21. Young vegetative cell; note parietal chloroplast, excentric pyrenoid and single nucleus.—Fig. 22. Vegetative cell; note early formation of net-like chloroplast.—Fig. 23. Mature vegetative cell; note net-like chloroplast, multinucleate condition.—Fig. 24. Mature vegetative cell from culture in stationary phase of growth; note several pyrenoids.—Figs. 25–26. Aplanospores and their liberation.—Fig. 27. Zoosporangium.—Fig. 28. Individual zoospore.

Magnifications: Figs. 1–3, 7, × 3125; Figs. 4–6, × 2084; Fig. 7, × 4167; Figs. 9, 13, 16–18, 22, × 875; Figs. 10, 12, × 625; Fig. 11, × 937; Figs. 14, 19, 20, × 1250; Figs. 15, 24, × 1042; Fig. 21, × 458; Fig. 23, × 521; Figs. 25, 26, × 292; Fig. 27, × 730; Fig. 28, × 1170.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Figs. 29–53

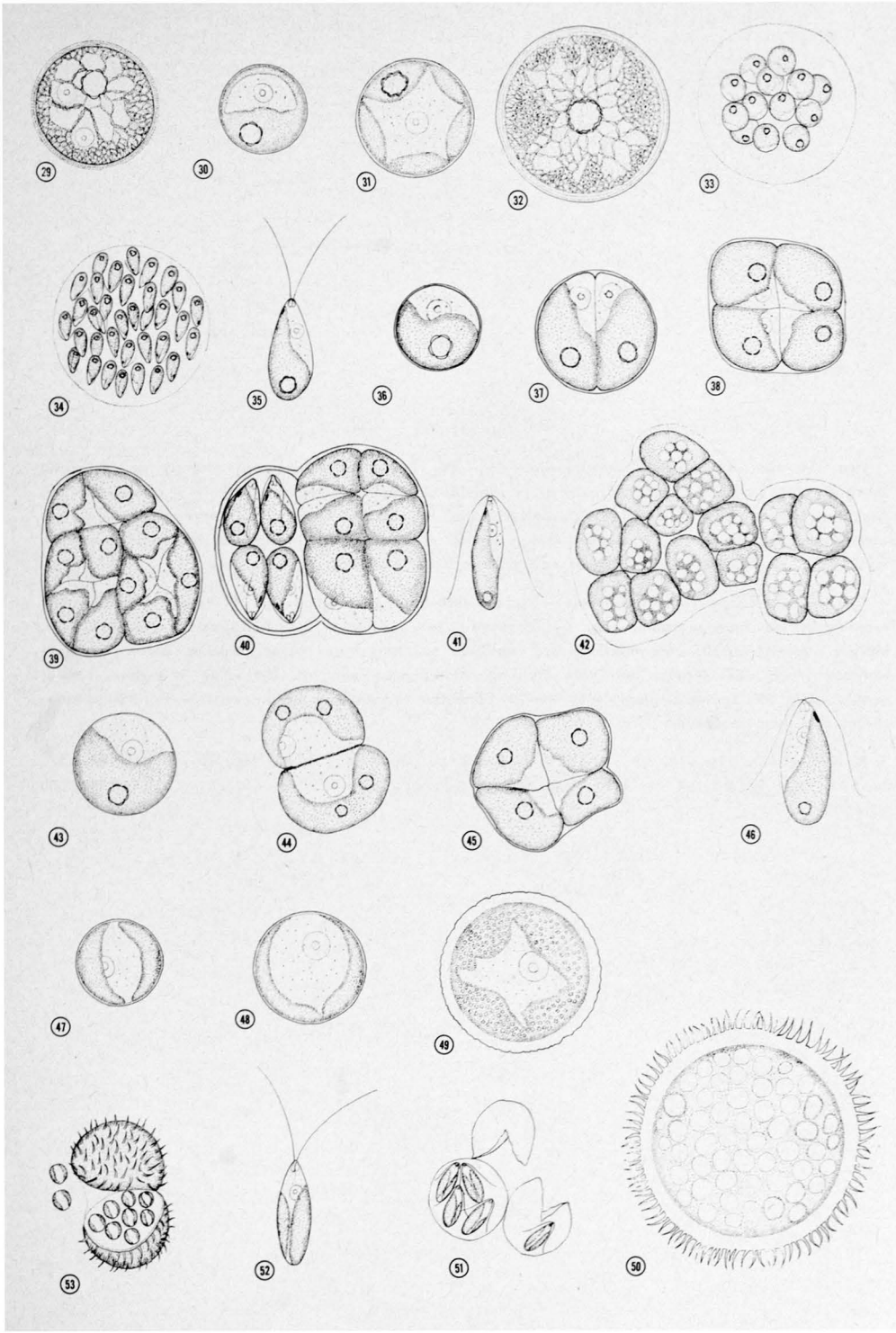
Figs. 29–35. *Spongiochloris incrassata*.—Fig. 29. Mature vegetative cell; note multinucleate condition and net-like chloroplast.—Fig. 30. Young vegetative cell; note single nucleus and parietal chloroplast.—Fig. 31. Vegetative cell; note segmentation of chloroplast.—Fig. 32. Mature cell from cultur ein stationary phase of growth; note thickening and striated outer wall layer, net-like chloroplast with central pyrenoid and abundant starch granules.—Fig. 33. Aplanosporangium.—Fig. 34. Zoosporangium.—Fig. 35. Single zoospore.

Figs. 36–42. *Chlorosarcinopsis gelatinosa*.—Fig. 36. Young vegetative cell.—Fig. 37. Vegetative cell division.—Figs. 38–39. Packets of cells.—Fig. 40. Zoosporogenesis.—Fig. 41. Individual zoospore.—Fig. 42. Packets of cells from culture in stationary phase of growth; note common matrix; abundant oil droplets.

Figs. 43–46. *Chlorosarcinopis eremi*.—Fig. 43. Young vegetative cell.— Figs. 44–45. Vegetative cell division to form packets of 2 and 4 cells.—Fig. 46. Individual zoospore.

Figs. 47–53. *Chlorosarcina brevispinosa*.—Figs. 47–48. Young vegetative cell; note parietal and bipartite chloroplast, single nucleus.—Figs. 49–50. Development of spiny akinete.—Fig. 51. Zoosporangium and empty cells.—Fig. 52. Individual zoospore.—Fig. 53. Liberation of aplanspores; note equatorial rupture of spiny akinete wall.

Magnifications: Figs. 29, 33, 34, 44–46, 50–51, × 1250; Figs. 30, 35, × 1170; Fig. 31, × 2500; Fig. 32, ×1562; Figs. 36–40, 43, 47–49, 52, × 1875; Fig. 41, × 2084; Fig. 42, × 1042; Fig. 53, × 625.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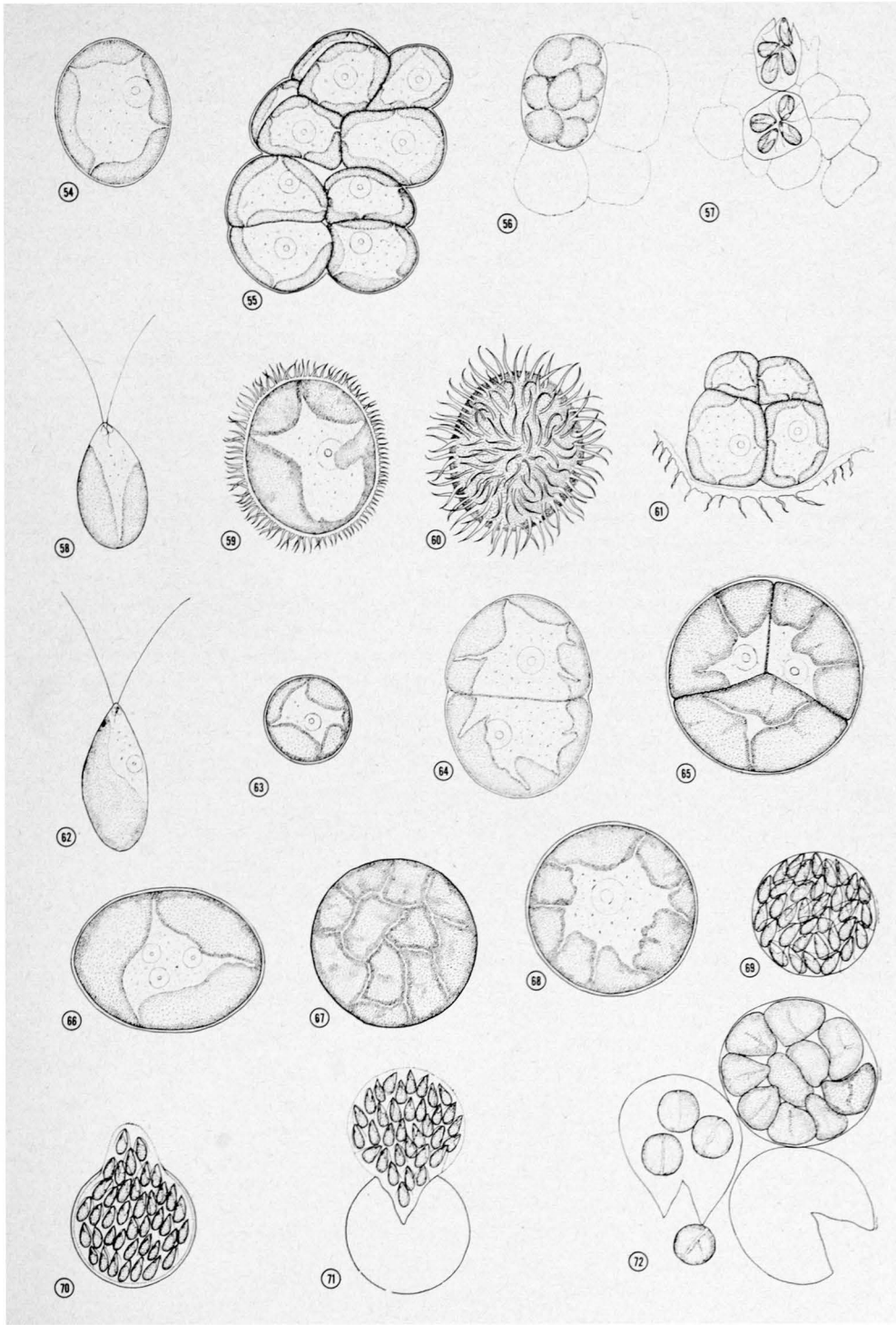
Figs. 54–72

Figs. 54–61. *Chlorosarcina longispinosa*.—Fig. 54. Young vegetative cell recently derived from zoospore; note single nucleus and multipartite parietal chloroplast.—Fig. 55. Packets of cells.—Fig. 56. Zoospore formation; note dividing chloroplast.—Fig. 57. Zoospore within zoosporangium.—Fig. 58. Individual zoospore.—Fig. 59. Median optical section of spiny akinete.—Fig. 60. The same in surface view.—Fig. 61. Rupture of spiny akinete wall; note packet of 4 cells.

Figs. 62–72. *Friedmannia israeliensis*.—Fig. 62. Individual zoospore.—Fig. 63. Young vegetative cell recently derived from zoospore.—Fig. 64. Vegetative cell division.—Fig. 65. Tetrad of cells.—Fig. 66. Mature vegetative cell; note multinucleate condition resulting from nuclear division before zoospore formation.—Fig. 67. Mature cell; note dividing chloroplasts.—Fig. 68. The same in median optical section.—Fig. 69. Zoosporangium.—Fig. 70–71. Liberation of zoospores within vesicle.—Fig. 72. Aplanospores and their liberation.

Magnifications: Figs. 54, 58, × 5000; Figs. 55, 61, 69–71, × 1150; Fig. 56, × 938; Fig. 57, × 625; Figs. 59, 60, 62, × 3125; Figs. 63, 64, × 1875; Figs. 65, 67, 68, 72, × 2084; Fig. 66, × 1562.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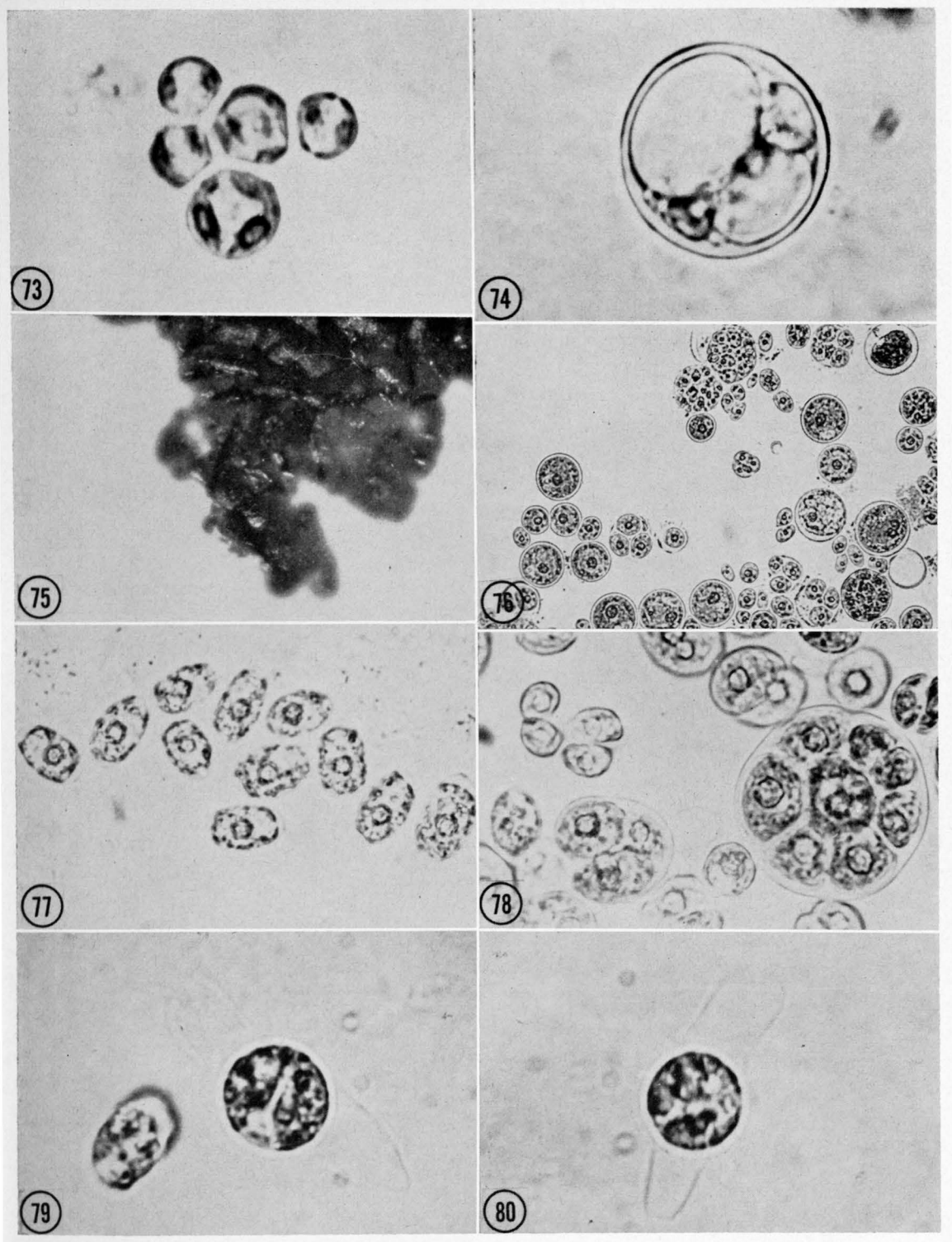
Figs. 73–80

Figs. 73–75. *Neochloris oleoabundans*.—Fig. 73. Mature vegetative cell; note 2 pyrenoids in each cell and variation in cell size.—Fig. 74. Cell from 6-week-old culture; note abundant oil droplets in protoplast. —Fig. 75. A portion of 2-week-old colony on Bristol's agar.

Figs. 76–80. *Chlorococcum diplobionticoideum*.—Fig. 76. General view; note the formation of biflagellate gametes and thickened outer wall layer of diploid cells.—Fig. 77. Young (haploid) vegetative cells recently derived from motile cells.—Fig. 78. Aplanospore formation.—Fig. 79. Early stage of zygote; note empty walls of gametes shed posteriorly.—Fig. 80. Mature zygote.

Magnifications: Fig. 73, × 700; Fig. 74, × 1365; Fig. 75, × 30; Fig. 76, × 230; Fig. 77; × 800; Fig. 78, × 750; Fig. 79, × 440; Fig. 80, × 275.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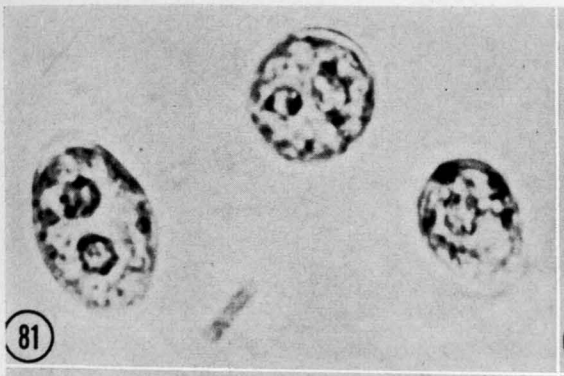
Figs. 81–88

Fig. 81–86. *Chlorococcum diplobionticoideum*.—Fig. 81. Zygote; note 1 pyrenoid beginning to degenerate.—Fig. 82. Oblique division of nonmotile gametes (zoospores) to form 2 daughter cells.—Fig. 83. Two daughter cells within parental wall.—Fig. 84. Four daughter cells increasing in size.—Fig. 85. General view from 2-week-old culture on Bristol's agar.—Fig. 86. A portion of 2-week-old colony on Bristol's agar.

Figs. 87–88. *Spongiochloris minor*.—Fig. 87. Group of vegetative cells showing variation in cell size.—Fig. 88. Mature vegetative cell; note net-like chloroplast.

Magnifications: Fig. 81, × 400; Fig. 82, × 1336; Fig. 83, × 1100; Fig. 84, × 756; Fig. 85, × 810; Fig. 86, × 30; Fig. 87, × 530; Fig. 88, × 1100.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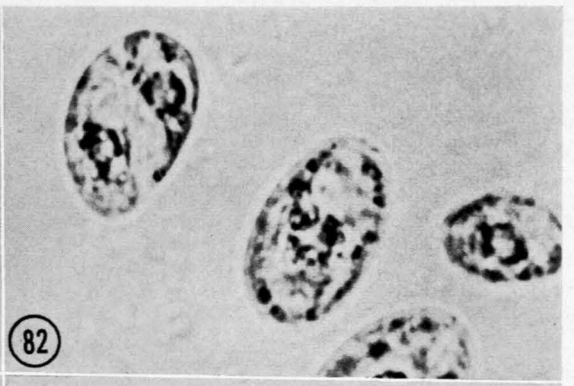
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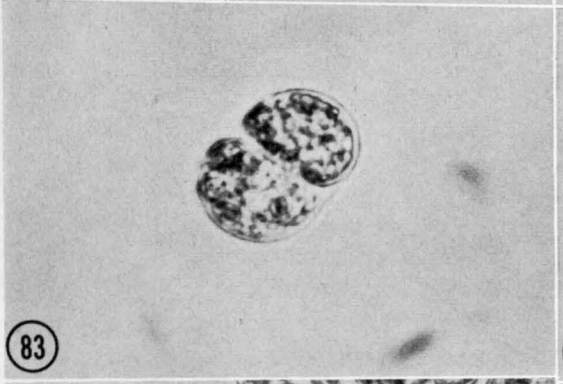
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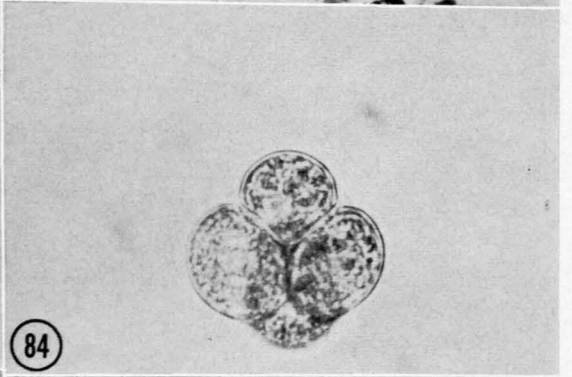
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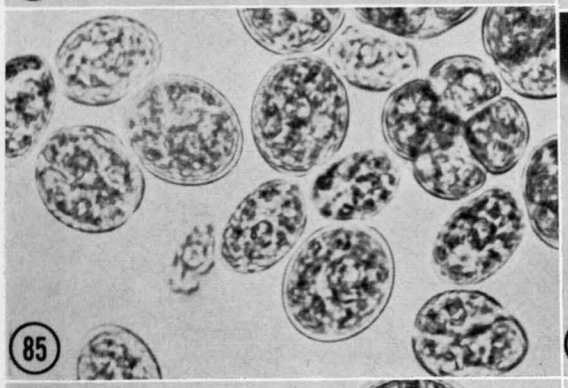
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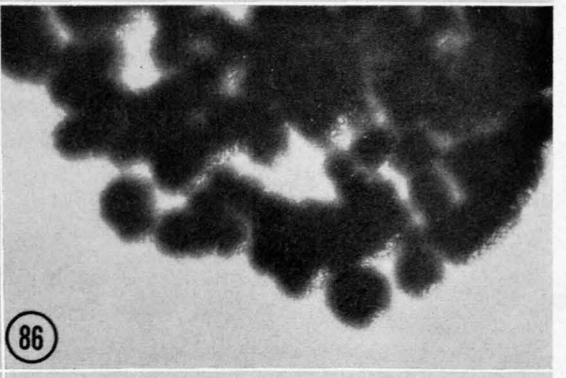
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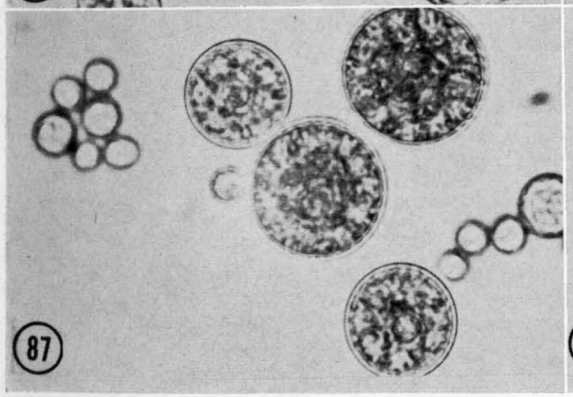
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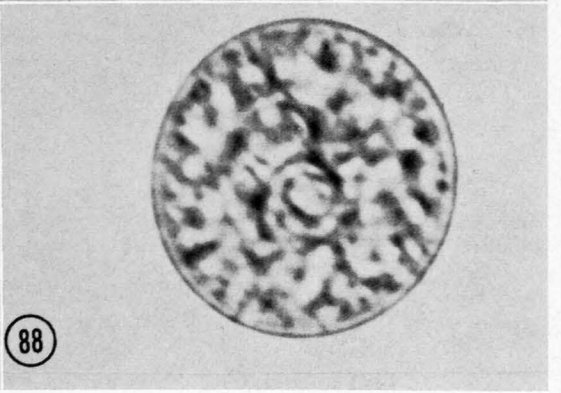
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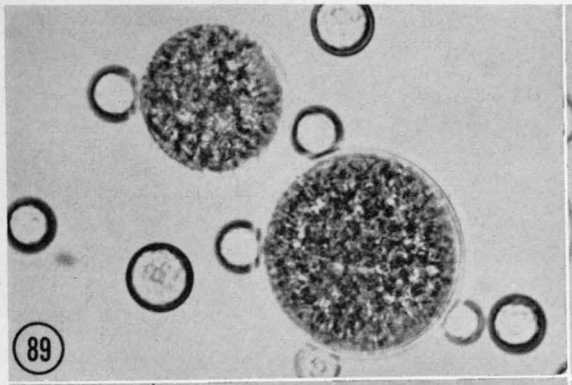
Figs. 89–96

Figs. 89–92. *Spongiochloris minor*.—Fig. 89. Cell from culture in stationary phase of growth; note obscured chloroplast and abundant starch granules.—Fig. 90. Aplanosporangium.—Fig. 91. Young vegetative cells recently derived from zoospores; note cup-like chloroplast.—Fig. 92. A portion of 2-week-old colony on Bristol's agar.

Figs. 93–96. *Spongiochloris incrassata*.—Fig. 93. Vegetative cells; note central pyrenoid and variation in cell size.—Fig. 94. Mature vegetative cells.—Fig. 95. Mature cell; note thickening and striated outer wall layer and abundant starch granules.—Fig. 96. A portion of 2-week-old colony on Bristol's agar.

Magnifications: Fig. 89, × 675; Fig. 90, × 825; Fig. 91, × 1050; Fig. 92, × 30; Fig. 93, × 675; Fig. 94, × 625; Fig. 95, × 780; Fig. 96, × 30.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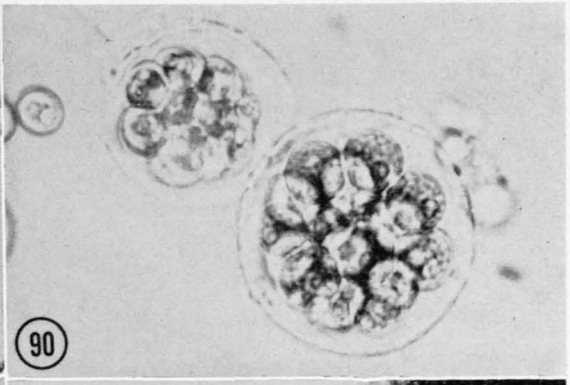
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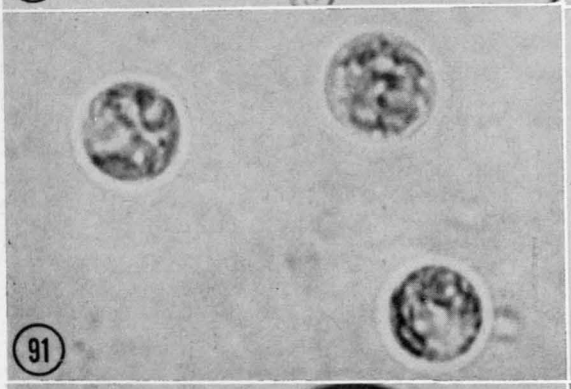
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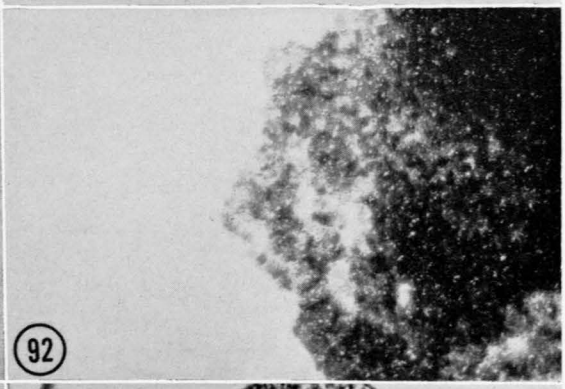
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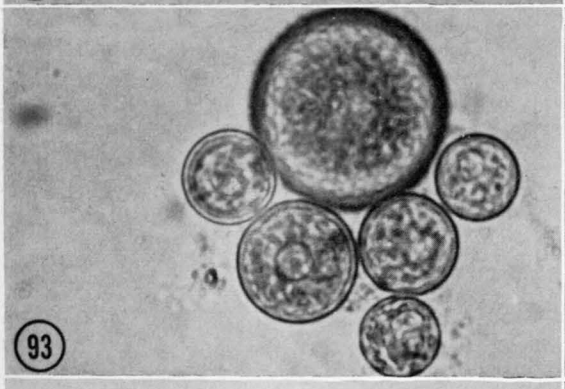
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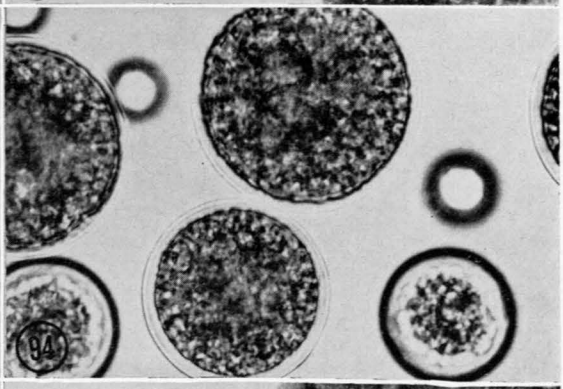
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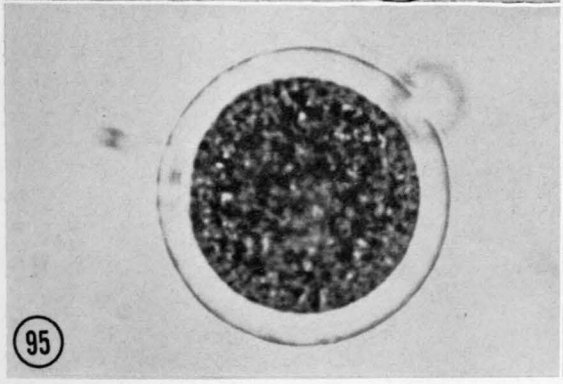
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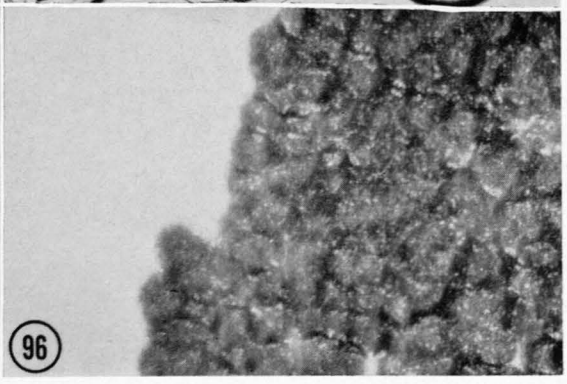
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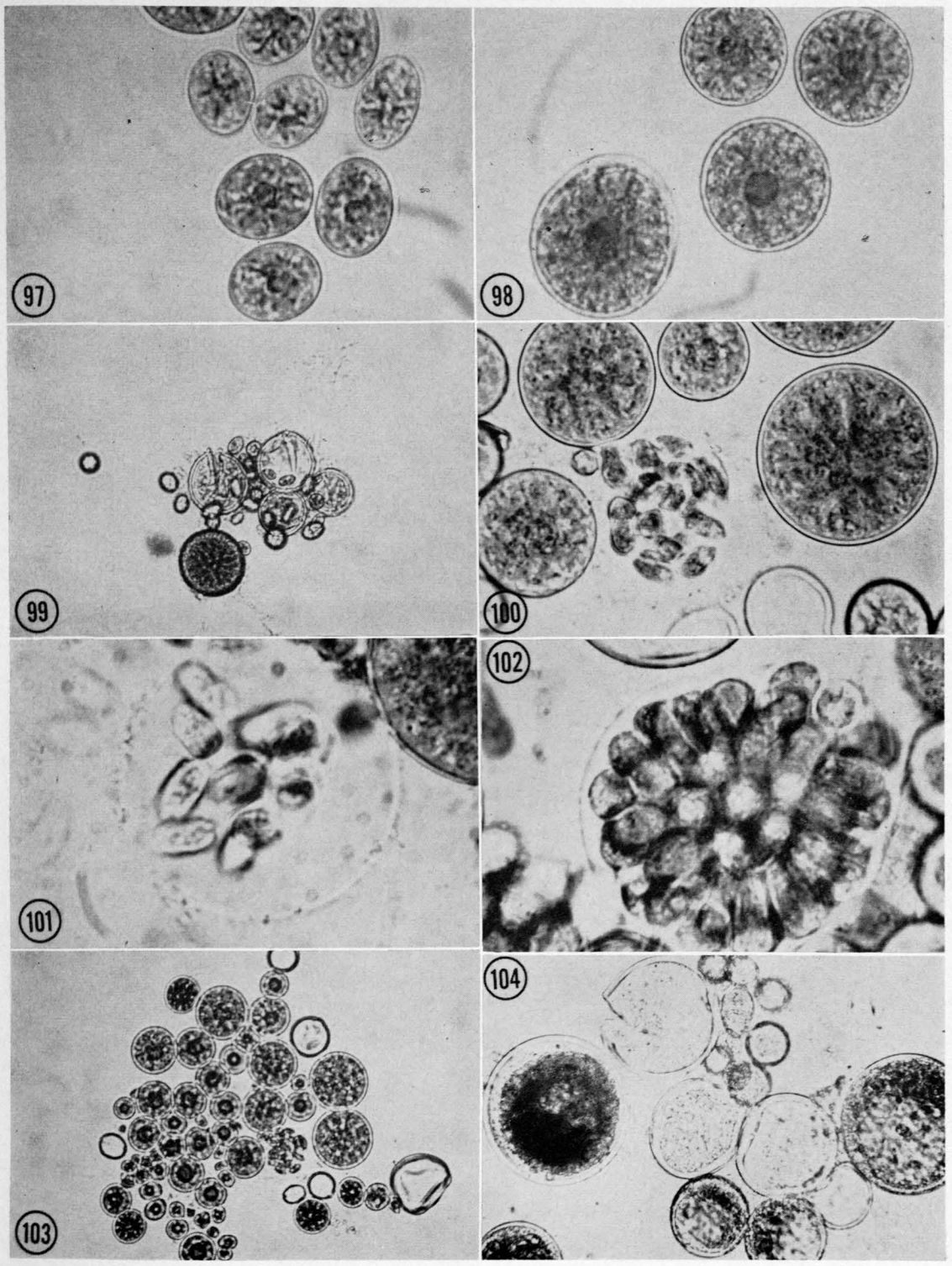
Figs. 97–104

Figs. 97–104. *Radiosphaera dissecta* Starr.—Fig. 97. Young vegetative cells recently derived from zoospores.—Fig. 98. Mature vegetative cells; note asteroid chloroplast with central pyrenoid.—Fig. 99. Vegetative cell, zoospores and empty cells.—Fig. 100. Zoospores within zoosporangium.—Fig. 101. Enlarged view of zoosporangium.—Fig. 102. Liberation of zoospores.—Fig. 103. General view from 2-week-old culture.—Fig. 104. Cells from culture in stationary phase of growth; note obscured chloroplast.

Magnifications: Fig. 97, × 900; Fig. 98, × 405; Fig. 99, × 250; Fig. 100, × 580; Fig. 101, × 800; Fig. 102, × 930; Fig. 103, × 180; Fig. 104, × 447.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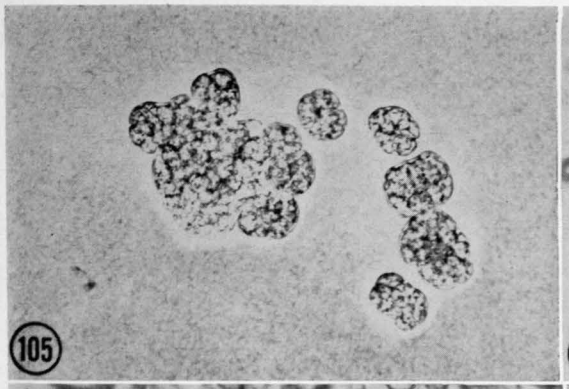
Figs. 105–112

Figs. 105–108. *Chlorosarcinopsis gelatinosa*.—Fig. 105. Packet of cells mounted in India ink to show common matrix.—Fig. 106. Enlarged view of zoosporangia.—Fig. 107. Young vegetative cells showing the result of mutual compression.—Fig. 108. A portion of 2-week-old colony on Bristol's agar.

Figs. 109–112. *Chlorosarcinopsis eremi*.—Fig. 109. Zoospores.—Fig. 110. Vegetative cell division.—Fig. 111. Packets of cells forming a pseudofilamentous configuration.—Fig. 112. A portion of 2-week-old colony on Bristol's agar.

Magnifications: Fig. 105, × 750; Fig. 106, × 4150; Fig. 107, × 1130; Fig. 108, × 30; Fig. 109, × 1365; Fig. 110, × 2500; Fig. 111, × 700; Fig. 112, × 30.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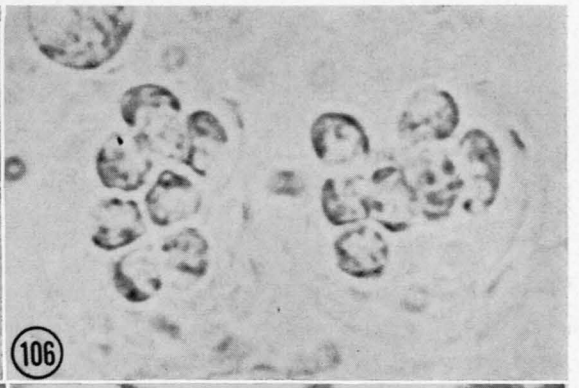
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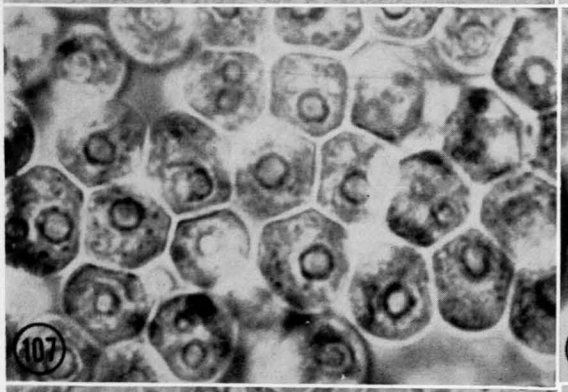
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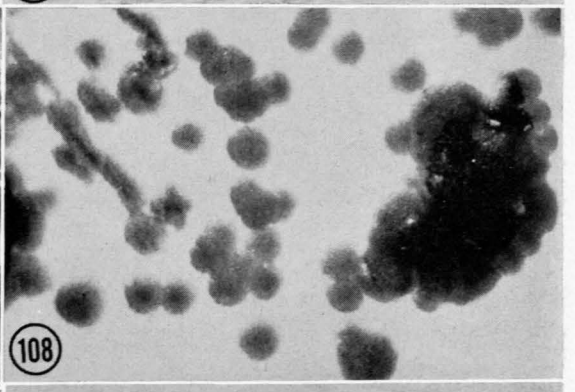
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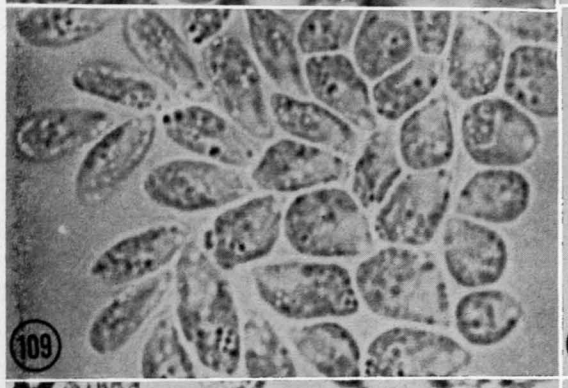
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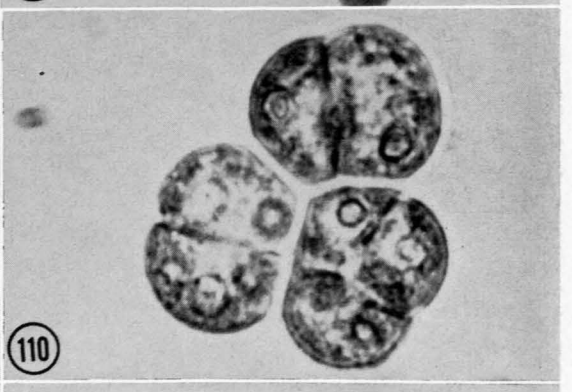
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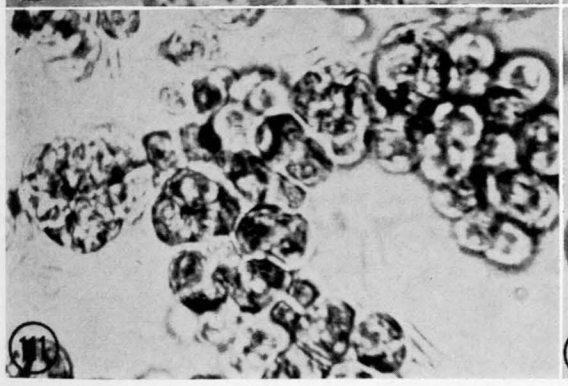
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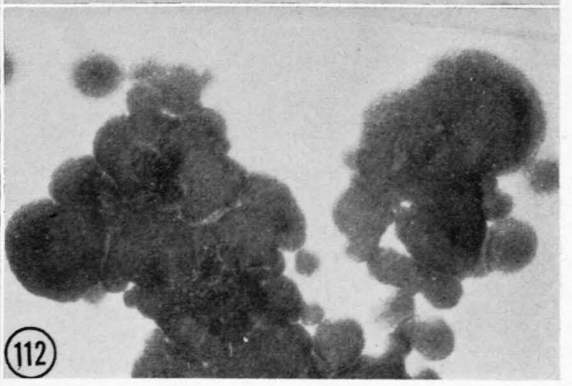
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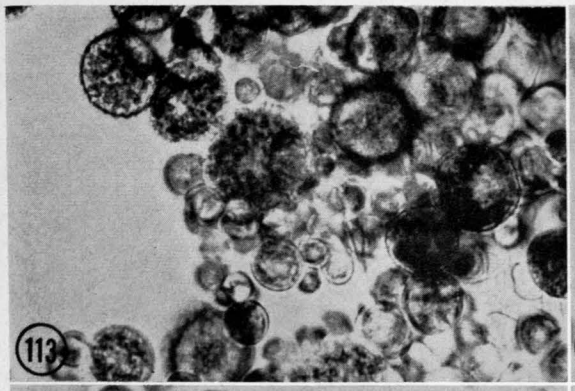
Figs. 113–120

Figs. 113–120. *Chlorosarcina brevispinosa*.—Fig. 113. General view of 2-week-old culture on Bristol's agar.—Fig. 114. Pseudofilamentous phase and spiny akinete.—Fig. 115. Young vegetative cells recently derived from zoospores.—Fig. 116. Enlarged view of akinete; note short-spiny wall, abundant starch granules.—Fig. 117. Surface view of spiny akinete and pseudofilamentous configuration.—Fig. 118. Liberation of aplanospores; note equatorial rupture of spiny akinete wall.—Fig. 119. Spiny akinete which produced packets of cells (under low temperature)—Fig. 120. Zoosporangium.

Magnifications: Fig. 113, × 357; Fig. 114, × 647; Fig. 115, × 1000; Fig. 116, × 940; Fig. 117, × 600; Fig. 118, × 650; Fig. 119, × 505; Fig. 120, × 850.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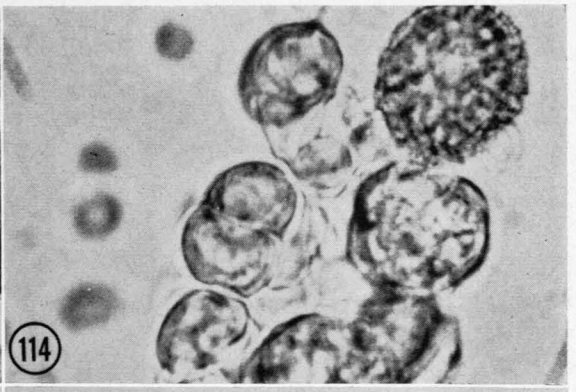
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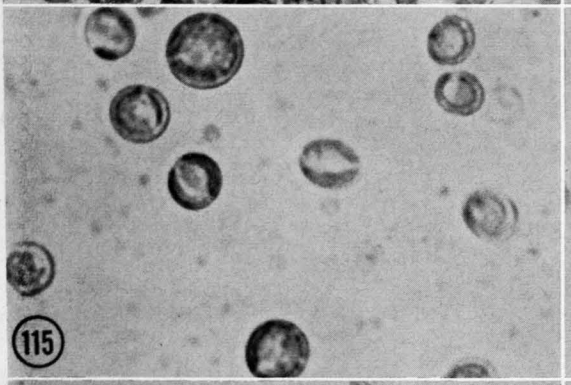
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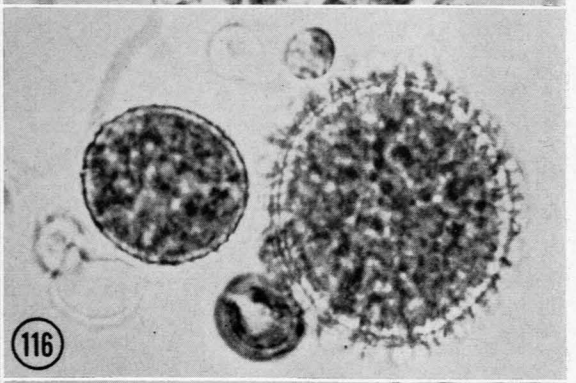
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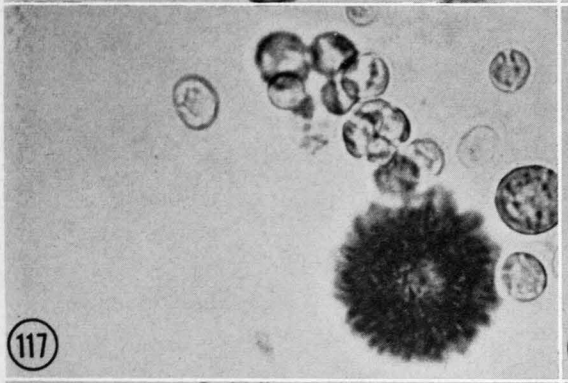
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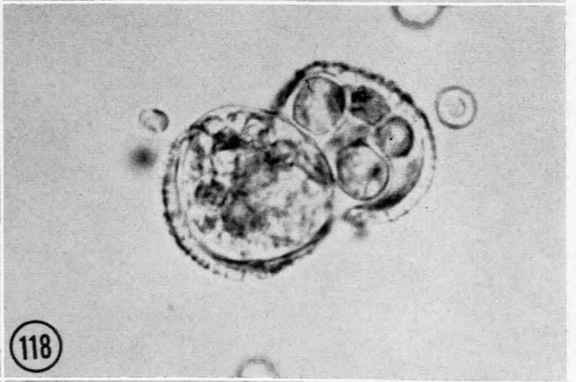
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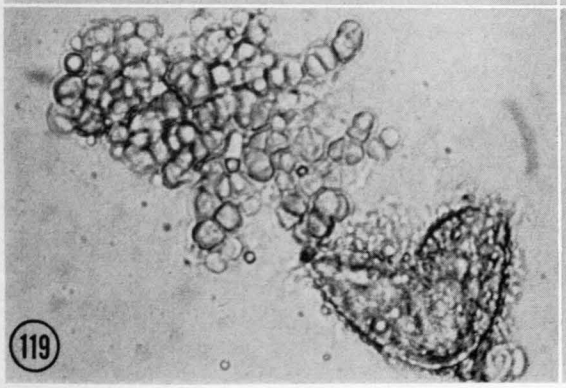
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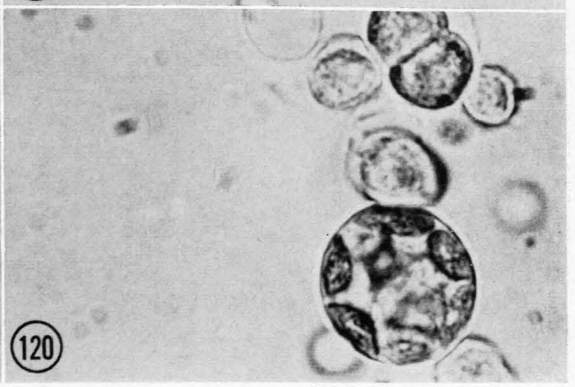
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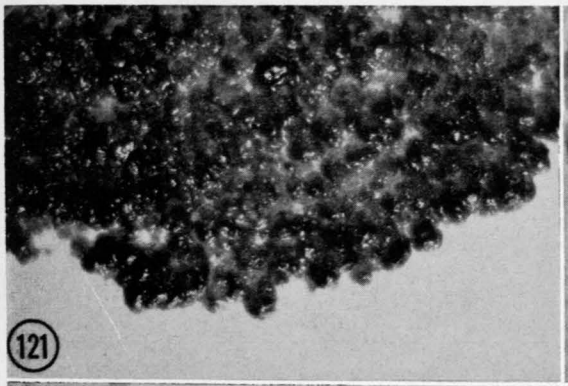
Figs. 121–128

Fig. 121. *Chlorosarcina brevispinosa*. A portion of 2-week-old colony on Bristol's agar.

Fig. 122–128. *Chlorosarcina longispinosa*.—Fig. 122. Young vegetative cells recently derived from zoospores.—Fig. 123. A portion of pseudofilamentous configuration formed under low temperature.—Fig. 124. General view of 2-week-old culture; note spiny akinetes and packets of cells.—Fig. 125. Enlarged view of spiny akinetes; note long spines.—Fig. 126. Cells from culture in stationary phase of growth; note packets of cells dissociating.—Fig. 127. Rupture of spiny akinete; note packets of cells inside.—Fig. 128. A portion of 2-week old colony on Bristol's agar.

Magnifications: Fig. 121, × 30; Fig. 122, × 1098; Fig. 123, × 530; Fig. 124, ×170; Fig. 125, × 720; Fig. 126, × 550; Fig. 127, × 600; Fig. 128, × 30.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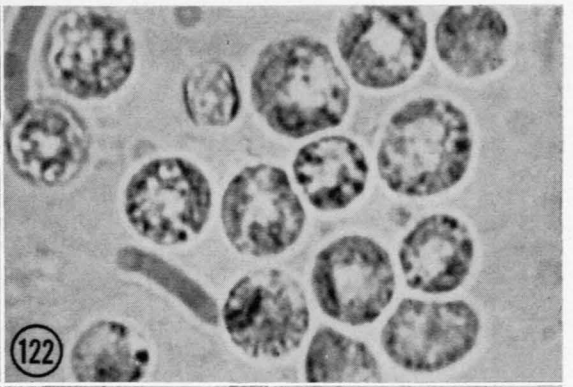
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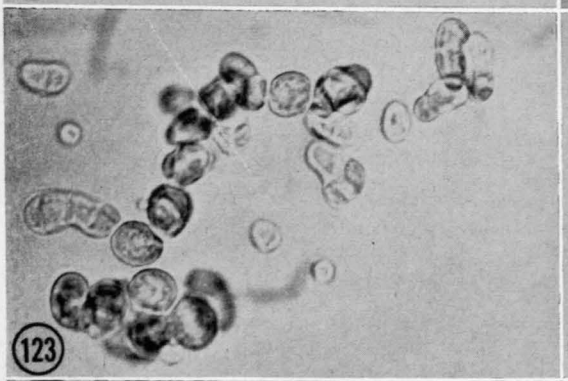
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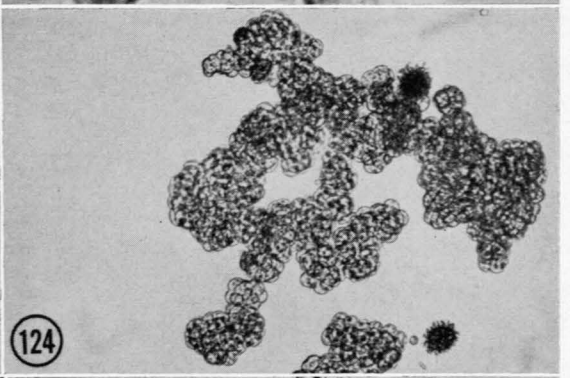
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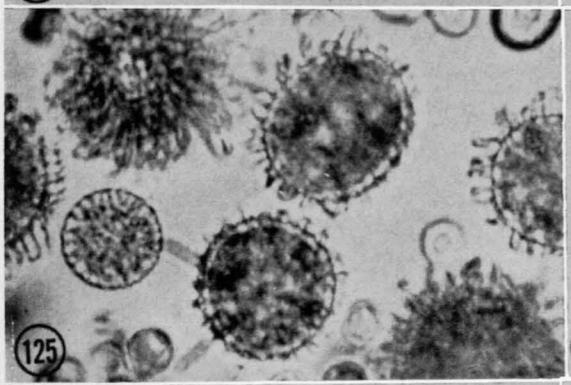
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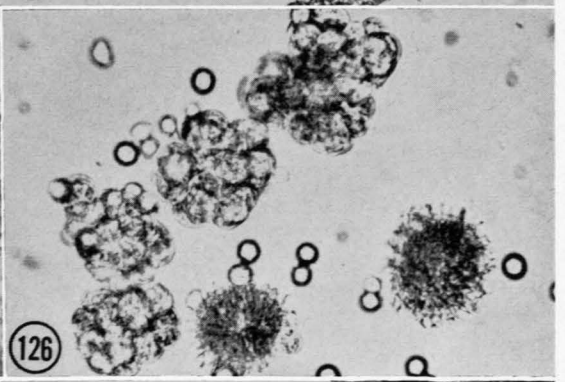
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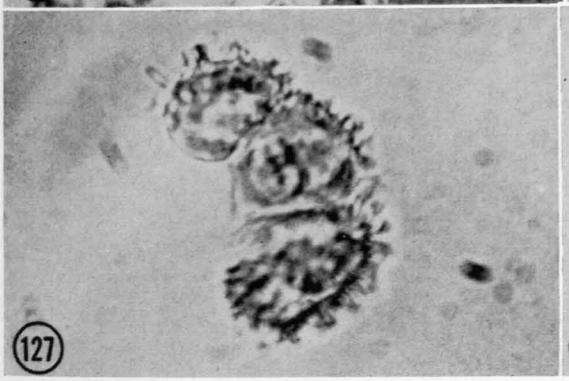
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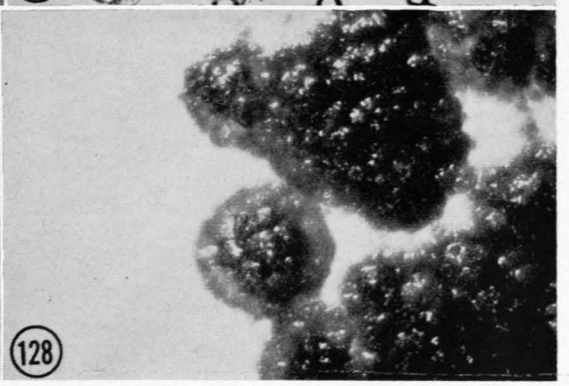
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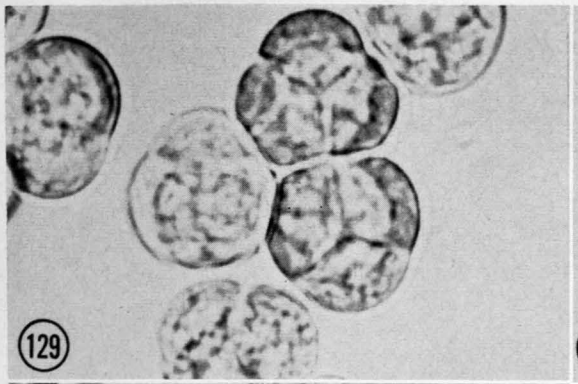
Figs. 129–136

Figs. 129–136. ***Friedmannia israeliensis.*****—Fig. 129. Tetrads of cells.—Fig. 130. Further division of tetrads to form 8 daughter cells.—Fig. 131. Dissociation of tetrads into individual cells.—Fig. 132. Enlarged view of zoosporangium.—Fig. 133. Individual zoospore.—Fig. 134. Aplanospores and their liberation.—Fig. 135. Mature vegetative cells; note division of parietal plastid before zoospore formation.—Fig. 136. A portion of 2-week-old colony on Bristol's agar.**

Magnifications: Fig. 129, × 1110; Fig. 130, × 1020; Fig. 131, × 1365; Fig. 132, × 2140; Fig. 133, × 1875; Fig. 134, × 1850; Fig. 135, × 1560; Fig. 136, × 30.



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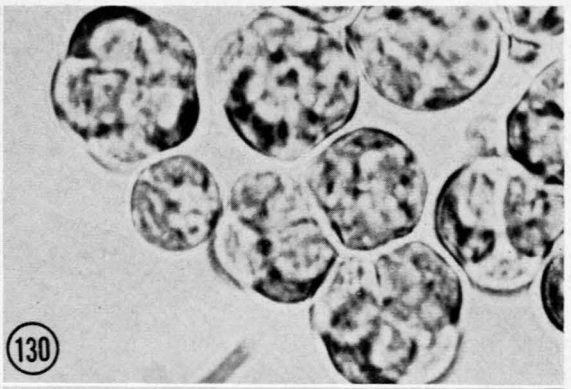

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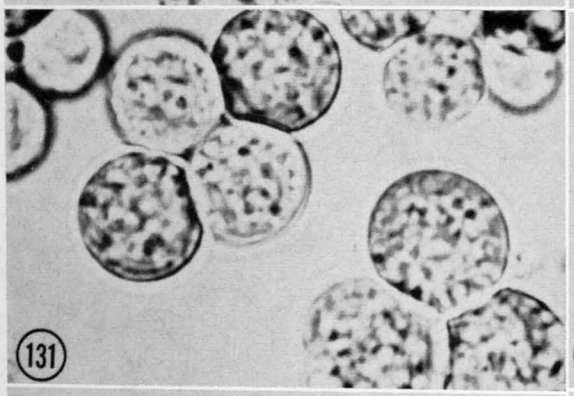

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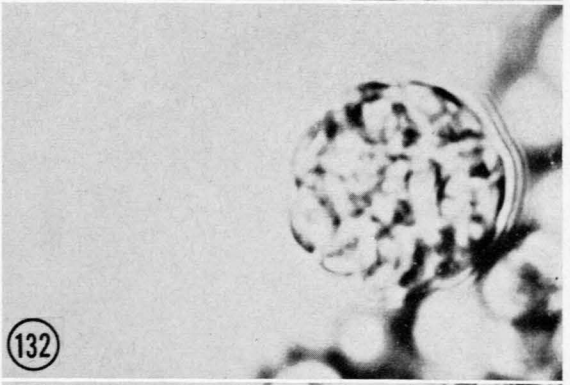

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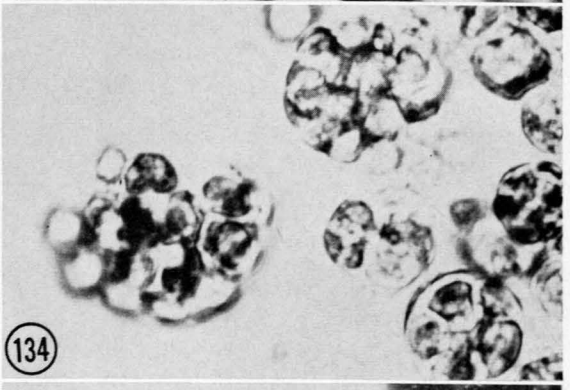

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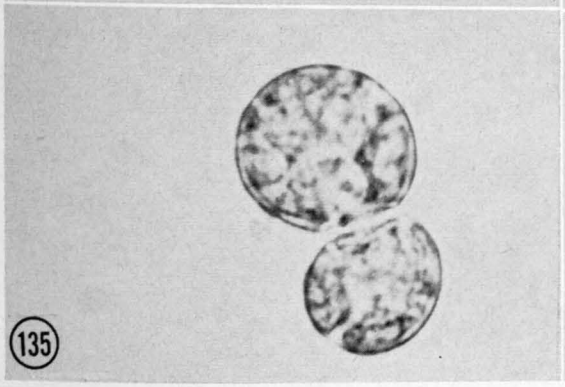

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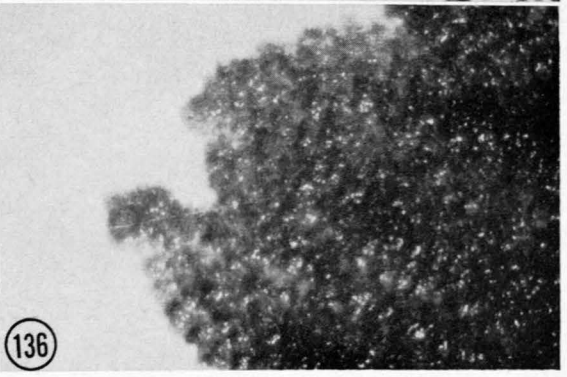

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