The Morphology, Cytology and Life History of Urococcus Insignis (Hass.) Kutz.

by Beverley Cobb Richards

1962

Submitted to the Department of Botany and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

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Lyle H. Thompson
Instructor in Charge

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For the Department

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INTRODUCTION

Urococcus insignis was first described by Hassall (1849) under the generic name Haematococcus. Since then, the genus has had three different names, has appeared in three separate algal divisions and has been classified in five different families.

The only modern study of the genus has been by Prof. R. H. Thompson, who first found the genus in Kansas on the under surface of a dripping sandstone ledge near Yates Center in March, 1949 (unpublished). He noted that the alga fulfilled the description of the species as given by Richter except that none of the juvenile cells were green. Instead, all cells were deep brown and contained one to several red or orange-red globules of oil. On culturing the alga, Dr. Thompson found that a thecate zoospore was formed. Following Thompson's suggestion, Smith (1950) transferred Urococcus to the order Dinocapsales in the family Gloeodiniaceae.

Since Smith's publication, authors have varied in their treatment of Urococcus. The purpose of this thesis is to clarify the standing of Urococcus insignis and to prove that it is a rightful member of the Dinophyceae. In order to do this, studies were made of the morphology, cytology and the life history of the organism. Collections

were taken from Lake Pegan, near Yates Center, Kansas and more frequently from an area, called Hole-in-the-Rock, near Baldwin, Kansas. It is to be expected that any suitably moist and shaded sandstone rock should support colonies of Urococcus insignis.

MATERIALS AND METHODS

COLLECTIONS AND CULTURING: All collections were taken as scrapings. The collections were then taken to the laboratory, placed in petri dishes and flooded with distilled water. Within one to three days, zoospores were released. The zoospores were micropipetted to various media.

Soil extract was used both as a liquid medium and as a base for solid media. A mixture of 500 grams of sandy soil and one liter of distilled water was autoclaved and filtered. The solution was then diluted in the ratio of one to four parts of distilled water. A commercial nutrient preparation, Plant-tab, was added to each liter of soil extract.

Of the solid media used, a 1.5% solution of Difco Bacto-agar and the extract base proved to be the most satisfactory. It was possible to obtain unialgal cultures on this medium but the growth of the alga was too slow. For some cultures, it was over two months before any

divisions were observed. In most of the cultures, contaminant growth was so rapid that further study of the material was impossible. For this reason, zoospores were transferred directly to the soil extract medium.

Again, the soil extract was effective but growth of the organism was slow. Better results were obtained by leaving the material in the original petri dish and allowing zoospores to accumulate on the sides of the dish. The zoospores gradually shed their thecas, developed layers of gelatinous sheathing and matured into vegetative cells. Whenever zoospores were needed, the material was dried and then reflooded. Cells in all stages of reproduction and growth then became available.

FIXATION: Most of the cytological studies were based upon living cells which were stained on the slide without previous fixation. Fixation tended to harden the outer sheaths but various fixation solutions were used successfully. The three best solutions were Carnoy's 3:1 alcohol-acetic acid fixative, Carnoy's 6:3:1 alcohol-chloroform-proprionic acid fixative and a solution of ethanol-methanol-chloroform-acetone and proprionic acid in the ratio 4:2:2:1:1. The material was left in the fixative until almost colorless. In some vials, a few drops of ferric-proprionate were added as a mordant. If the chromosomes were still obscured

by the massed chromatophores, either acetone or 45% propionic acid was introduced under the coverslip. Zoospores were killed by placing them over iodide crystals for two to three minutes and then treating with a five percent sodium hypochlorite solution to cause shedding of the theca.

STAINING PROCEDURES: Two stains were found to be the most effective. Propriono-carmine was used for staining nucleoli, heterochromatic areas and for determining the stage of the chromosomes. After the carmine was introduced under the coverslip, the slide was gently heated over an alcohol lamp and then pressure was applied to the coverslip. This process was repeated until the chromosomes were stained and spread the desired amount. The only disadvantage with carmine was that chromatids were poorly defined.

For more careful observations of chromatids, the Fuelgen technique was used. Both fixed and living material was used, but the living material proved to be the better. Cells were scraped from the side of the petri dish, placed in a small beaker of 1.0 N. hydrochloric acid and hydrolized at 60 degrees for seven minutes. The cells were allowed to settle, the acid decanted and the cells washed with distilled water or they were filtered and washed. The material was then placed in a vial with Fuelgen stain and put in a refrigerator for at least one hour. After decanting,

the cells were mounted in propriono-carmine, heated and pressure was applied.

Orcein was also used as a stain but was later discarded because protoplasmic staining was too heavy.

Slides were made permanent by heating until the carmine steamed, then quickly lifting the coverslip and placing a drop of venetian-turpentine dissolved in proprionic acid on the slide before replacing the coverslip. On some slides, the venetian-turpentine solution was drawn under the coverslip by using filter paper.

In order to demonstrate spindle fibers, cells were placed in fixative to which mordant had been added and kept refrigerated for one month. Cells were examined at intervals during the month but fibers were not demonstrated. Fumes of osmic acid were also used with no success.

Cells were subjected to prolonged drying, light and dark conditions and refrigeration in order to discover the tolerance of the genus. Constant light or dark produced little change in the organism. After prolonged drying for one or two months, the cells were still viable but a longer time than this usually resulted either in death of the cells or in a loss of ability to produce zoospores. The alga survived the refrigeration with no apparent effects.

Cells were also grown in weak giberrellin and sandy-soil extract solutions. After a period of two months, the

cells appeared healthy but the chromosomes seemed to be in a perpetual interphase.

REVIEW OF LITERATURE

Hassall (1845, fide 1849) described Urococcus insignis as a species of Haematococcus, a genus erected by Agardh (1828) to include cells with red coloring. Hassall divided Haematococcus into three subgenera. He described the genus as having variously sized spherical or oval cells, each with one or more concentric vesicles, and as being capable of multiplying either by division or by "granules" formed within the parent cells. The subgenus Ouracoccus was described as having cells terminally embedded in a mucous prolongation or "stalk" which formed the mass of the plant. In Haematococcus insignis (subgenus Ourococcus), the stalk was transversely corrugated or ringed and contained at the end one or two very large, blood-red, spherical granules surrounded by one to many concentric envelopes. The genus was placed in the family Protococceae of the Chlorophyta.

Kützing (1849) erected the genus Urococcus to hold four of the species previously described by Hassall in the subgenus Ouracoccus as Haematococcus allmanni, H. cryptophila, H. insignis and H. hookeriana. To the original description, Kützing added that the stalk may be shortened or obsolete. He did not mention a habitat for Urococcus insignis although he repeated Hassall in saying that the

three other species were collected from the sides of moist rocks. He placed the genus in the family Palmelleae in the Chlorophyta.

Jenner (1866) removed Haematococcus sanguineus and H. cryptophyllus from Urococcus and placed these species under Gloeocapsa, a genus in the Cyanophyta. He described Gloeocapsa and Urococcus as being separate but similar genera in one evolutionary line. Urococcus, the more advanced form, exhibited a multicellular condition derived from the continued formation of one cell within another. Jenner explained the striations, described by Hassall, as outlines of cells and the peduncle or stalk as a stem derived from cells growing in successively higher planes. The usual color of Urococcus as observed by Jenner was green or white although he did find some blood-red cells.

Rabenhorst (1868) mentioned that Urococcus occurred in Europe and that the cells were Gloeocapsa-like.

The first extensive study of Urococcus insignis was by Richter (1886), who collected it on a moist rock in 1885. He described the juvenile cells as green, from three to five microns in diameter and as occurring in a palmella condition in gelatinous colonies. He observed that as the cells grew, they exhibited colors ranging from red to brown and demonstrated a stalk formation. In the mature state, Urococcus cells were described as spherical, from 20-53 microns in diameter, or, when oblong, 21 microns

wide and 35 or more microns in length. Two divisions were observed to have taken place resulting in colonies of four or rarely eight cells.

Richter also observed what he interpreted as sexual differentiation in "golden-yellow cells with elliptically-split, rod-like particles of one to one and one-half microns". He saw no sexual union of cells. Bleisch (fide Richter, 1886), who observed the same phenomenon, recorded that the whole cell content formed a swarm cell with two flagella. Bleisch described this formation for Protococcus macrococcus, which was growing with Urococcus, but Richter felt that the swarmer belonged to Urococcus.

Richter doubted that the stalk was a good character since he observed it on dead or dying cells only. He suggested that the under-most concentric rings were stronger than the upper, thus giving one the impression of a stalk. After dying, the envelope separated and formed a long stalk by the gelatinization of the inner surface. This surface took up water and became swollen to form a stalk. Richter's views were in opposition to Braun's (fide Richter, 1886) theory of separate but cohesive rings which were formed through-out the vegetative cycle.

Wolle (1887) made no mention of Richter's study but instead recognized the genus according to Rabenhorst (1868). He made special note of Rabenhorst's phrase "Gloeocapsa-like" and remarked that Urococcus was merely an occasional

condition or arrested development of Gloeocapsa. He further stated that Gloeocapsa was but an intermediate or polymorphous spore condition of Sirosiphon; therefore, Urococcus was in reality a spore condition of Sirosiphon. He retained Urococcus in the family Palmellaceae.

De-Toni (1889) summarized the genus according to Richter's description. He added that the cells may be from 33-75 microns in diameter with the sheath. He also discussed Richter's rod-shaped "particles", but no cell sexuality was admitted. A variety, U. insignis var. ferrugineus Lagerh. (De-Toni, 1889) was described in which the cells were rusty-yellow with a cell diameter of 28-66 microns or up to 130 microns with the sheath. De-Toni also kept Urococcus in the family Palmellaceae.

Wille (1897) placed Urococcus under the Pleurococcaceae of the Chlorophyta but noted that the true position of the genus was uncertain. He thought that Urococcus-like cells were developments of other organisms, especially Peridinium and Chlamydomyxa, a genus no longer recognized.

Hieronymous (1898) reduced Urococcus or Haematococcus insignis to synonymy with Chroococcus macrococcus, a member of the Myxophyceae, which he considered a juvenile form of Chlamydomyxa.

West, W. and G. S. West (1907) described an aquatic species, Urococcus tropicus, from India. The species, described from preserved material sent to the authors,

was characterized by thick walled cells, 19-36.5 microns long and 13-33 microns wide, which were single or in small colonies with hyaline stalks. The cells were described as being conspicuously lamellose and as containing one, rarely two, pyrenoids. The two pyrenoid condition was illustrated as occurring primarily in a panduriform-shaped cell. None of the cells contained the red-brown pigment which is characteristic of other species of Urococcus.

To Richter's description of Urococcus, Collins (1909) added that the cells varied from green to some shade of red or yellow, that a large, granular, bell-shaped chromatophore was present, and that a pyrenoid was absent. He described the lamellated wall as ultimately breaking at one side but remaining attached at the other, forming a stipe-like prolongation of about the same breadth as the cell. Collins reported on three species, U. foslieanus, a marine form, U. hookerianus and U. insignis. The latter two species differed in cell size from 6-15 microns for U. hookerianus to 25-50 microns in diameter for U. insignis without the sheaths. In the early stage, U. insignis was described as like Gloeocystis with cells originally three to five microns in diameter but increasing in size and number in the gelatinous colonies until the cells were about ten microns in diameter. The cells then became free and developed the lamellate wall and, later, the stipe.

Collins agreed with Richter that the formation of the stipe did not occur until the period of active vegetation was past; for the greater part of its existence, the species developed like a Gloeocystis.

Collins reduced Urococcus insignis variety ferrugineus to a form of U. foslieanus Hansgirg. and mentioned that the latter species was probably a marine form of U. insignis. Collins placed the entire genus Urococcus in the Chaetophoraceae for lack of a "better place to put it". Reasons for the inclusion were that the cells had the same structure, some species produced zoospores and aplanospores and all had regular vegetative cell division. Still, Collins wrote that Urococcus was a doubtful genus which ultimately would be absorbed in Gloeocystis.

The most significant work on the genus after Richter was published by Klebs (1912). In erecting the new genus Gloeodinium, Klebs considered forms originally described under Urococcus. He collected a water form of Urococcus insignis which was described by Schmidle as variety regularis. The cells were at first pure green, then became blood-red and had a thick, partly clear, laminated, gelatinous envelope without a stalk formation. The red color of the old cells was derived from a red oil-like substance which covered green chromatophores. Klebs removed this form from the genus Urococcus to the family Phytodiniaceae in the Peridineae. He called the organism

Gloeodinium montanum since it occurred in Sphagnum bogs of mountains. Klebs left the point unanswered whether U. hookerianus should be placed with Gloeodinium or left in the genus Urococcus.

Schilling (1913) took note of Klebs description of Gloeodinium montanum and stated that Urococcus hookerianus was probably the same organism, but he did not mention Urococcus insignis.

West (1916) in discussing the family Phytodiniaceae as erected by Klebs claimed that Gloeodinium montanum was very similar to Chroococcus macrococcus Rabenh. and moreover was found in similar habitats. He considered C. macrococcus to be a true member of the Myxophyceae and a synonym of Urococcus insignis but not of Ouracoccus insignis Hass. He did not explain his reasons for separating Urococcus from Ouracoccus.

West and Fritch (1927) reported that Chroococcus macrococcus (syn. Urococcus insignis (Hass.) Kütz.) was a common form from the bogs of moorland and upland districts which sometimes occurred abundantly amongst submerged Sphagnum. The cell contents were described as golden to dark brown with a cell diameter of 25-51 microns without the sheath or 41-78 microns including the sheath. The envelope was described as thick and lamellose with the outer layers often splitting off.

In a later book, Fritsch (1935) evidently retained the synonymy of Urococcus insignis and Chroococcus macrococcus as no further mention was made of Urococcus. Fritsch did quote Geitler (1932) as reporting that C. macrococcus was a member of the Dinophyceae and probably identical to Gloeodinium montanum.

In 1950, for the first time, two authors placed Urococcus in the Dinophyceae. Huber-Pestalozzi recorded that Ouracoccus Hass. (Urococcus Kütz.) was a synonym of Gloeodinium and placed the genus in the order Dinocapsales, family Gloeodiniaceae.

Smith (1950), on the suggestion of Prof. R. H. Thompson, placed Urococcus in the Dinocapsales as a separate genus from Gloeodinium. The two genera were separated by the layers of the cellular envelope. Urococcus was described as possessing a gelatinous envelope with many concentric layers which later became uneven by the formation of incompletely encircling layers. Gloeodinium was described as having either a homogeneous cellular envelope or one with few layers. Smith further reported that the central protoplast of Urococcus was darker brown than the peripheral portion but he could not determine whether the coloration were due to a single chromatophore or to many crowded ones. The protoplast was also said to contain numerous starch granules and a few large to small red globules of oil. Reproduction was listed as usually being by cell division but a naked

gymnodinoid zoospore was possible.

Prescott (1951, 1961) kept Urococcus in the family Palmellaceae, order Tetrasporales. In another publication (Prescott, 1954), however, he stated that the genus might be moved to the Dinophyceae.

Thompson (1959), following the treatment of Smith (1950), retained the genus in the family Gloeodiniaceae, order Dinocapsales. He differentiated Urococcus and Gloeodinium by the lack of stalk-like sheathing in the latter.

Anderson and Walker (1920) reported the genus Urococcus (which they placed in the Pleurococcaceae) from Cherry County, Nebraska. The genus was also collected by Taft (1949) from wet cliff faces in Hocking County, Ohio.

Hue (1905) reported Urococcus from the cephalodium of Lepolichen coccophora, a lichen. Tiffany (1938, 1951) repeated the information but Ahmadjian (1958) doubted the listing of Urococcus as a symbiont.

MORPHOLOGY OF THE VEGETATIVE CELL

The cells of Urococcus insignis are 30-83 microns in diameter or 41-100 microns including the sheath. After division, cells are ellipsoidal, 29-46 microns long and 25-29 microns wide without the sheath. The ellipsoidal cells usually become spherical after growth but may retain the ellipsoidal shape until escaping from the common sheath (Fig. 9).

None of the juvenile cells are bright green. The color is olive green to deep brown to orange and only in dying cells is the pigment a grass-green. The cells contain numerous small starch granules and a few large or small reddish or yellow-green globules of oil. The shape of the chromatophores is difficult to determine. Richter (1886) reported spherical chromatophores in varying sizes while Collins described them as large, granular and bell-shaped. In the material of the present study, some healthy cells appear to have elliptical chromatophores radially distributed and extending to the periphery while others have the brown coloring concentrated at the center but with radiating processes. In still other cells, especially dying ones, no organization of chromatophores is apparent; the chromatophores appear to become diffuse and are then obscured by oil globules and starch granules.

Each of the cells is surrounded by a firm gelatinous envelope which may be single or have two to many concentric lamellae. The cells are usually solitary, but two to four cells, rarely five to eight, each with a conspicuous sheath, may lie in a common sheath derived from the original cell (Fig. 10). As the cell grows older, it exhibits several layers of excentric sheathing (Fig. 5). These layers are the beginning of the "stalk"-like process. As the cells grow and divide, the layered sheathing diverges into a branched system which may hold five or six terminal cells (Figs. 3, 4).

When kept in a water culture, the stalk is usually lost and, quite frequently, the concentric sheathing also. Richter suggested that the stalk was not a true character but a result of optical illusion caused by some cells being raised higher than others. He did not observe any live cells with a stalk but he did notice that after the death of cultured cells, a long stalk was often present. Braun (fide Richter, 1886) thought that the stalk was formed during the entire vegetative period and represented a cohesive stratification of ring-like structures. Richter, however, thought the stalk represented the taking up of water by the older envelope. In such a case, the inner surface of the envelope became swollen with water and slid out into a stalk formation.

From observing the stalk and its later dissolution, it seems likely that Braun was correct in describing the stalk as representing cohesive layers of a mucous sheath which may be formed throughout the vegetative period. As the cell matures and grows in diameter, it is forced upward from its original sheathing and secretes more gelatinous layers. Eventually four to eight cells may be found in a common, branched stalk. Richter was incorrect in thinking that the stalk was a result of cell death because young, healthy cells are often observed with a stalk. As the stalk is lost by cells grown in a water culture, it is probable that it represents a protective

devise which is capable of water retention. Its existence, then, depends upon the environment and not the internal condition of the cell. The use of the term "stalk" is in itself incorrect because it implies a unified, functional structure. The prolongation is merely a mass of discarded, excentric sheathing. The term "stalk" should be used only for convenience.

Within the cell, no evidence of a pusule, a type of vacuole described as characteristic of the dinoflagellates by Graham (1951), has been found. Other vacuoles, pyrenoids and stigmas are also missing from the Urococcus insignis cell.

The morphology of the nonmotile stage of species of Urococcus presents a problem. Urococcus insignis is separated from other members of the genus primarily by size and by habitat. Presently only four species are recognized. Of these, U. fosilieanus Hansg., a marine form, is said to have a diameter of 15-25 microns (Taylor, 1937), U. tropicus, an aquatic species, measures 19-36.5 microns in length, U. hookerianus varies from 13-60 microns and U. insignis is reported to be 33-75 microns in diameter without the sheaths. The size of living cells of Urococcus insignis from the sandstone ledge at Baldwin, however, embraces the whole range.

Of the two aquatic species, U. fosilieanus is marine and possibly differs physiologically, at least, from the fresh-water forms. Little, however, is known of the true

habitat of U. tropicus. The presence of a stalk suggests that it occurred in a non-aquatic habitat, perhaps on a moist rock, rather than in a lake. The description of a pyrenoid strongly resembles that of the large, prominant nucleus characteristic of Urococcus, and the panduriform cell containing two pyrenoids is identical to the elongation figure of a U. insignis cell after nuclear division (Fig. 7). In order to adequately compare U. tropicus, which was described from material preserved in a "weak carbolic acid" solution, to the other members of the genus, however, living material must be examined.

Urococcus insignis and U. hookerianus were originally described from the same kind of habitat. As the descriptions for the two species are almost identical, there seems little reason for separating the two. At least, a complete examination of both species should be made.

MORPHOLOGY OF THE ZOOSPORE

Urococcus insignis zoospores are thecate, 26-46 microns long, 15-30 microns wide and have numerous, radial, brown, elliptical or band-shaped chromatophores. A girdle incompletely encircles the cell in a downward, left-hand spiral (Fig. 12). The sulcus either terminates posteriorly in a pocket or disappears before reaching the posterior end (Fig. 13). There are two unequal flagella: one transverse and the other a trailing flagellum equal to the cell length

beyond the posterior end. There is no eyespot. The anterior two-thirds of the cell is broadly rounded and broader than the posterior one-third. The latter attenuates slightly and is abruptly truncated or slightly concave at the extremity (Fig. 11).

The zoospore may escape from its theca (Fig. 15) before entering the vegetative phase but usually secretes the first sheath within the theca. In order to see the thecal plates, then, it is necessary to either search for a free theca or to apply pressure on the coverslip to expel the zoospore from the theca. For this reason, many drawings are composites and therefore may not be exact in all measurements. Whole thecas can often be obtained by inserting a five percent sodium hypochlorite solution under the coverslip. The resulting thecas are slightly swollen but entact and free from the zoospore.

Dr. Thompson (unpublished) noted that the zoospore plates from his first collection area near Yates Center differed in number from later collections taken near Baldwin. On the Yates Center material, he observed a plate arrangement of four apicals, two anterior intercalarys, seven precingulars, seven postcingulars, one posterior intercalary and two antapicals (Fig. 1). In the Baldwin material, he noted four apicals, three series of anterior intercalarys totaling seven plates, seven precingulars, seven postcingulars, two posterior intercalarys and two

Fig. 1: The illustration of the third generation zoospore from the Yates Center material is taken from the unpublished works of Dr. R. H. Thompson. The formula for the plate arrangement of this smaller zoospore is 4', 2A, 7", 7"', 1P, 2"". (x 1560)

LEGEND:

'.....Apical

A.....Anterior Intercalary

".....Precingular

"'.....Postcingular

P.....Posterior Intercalary

"".....Antapical

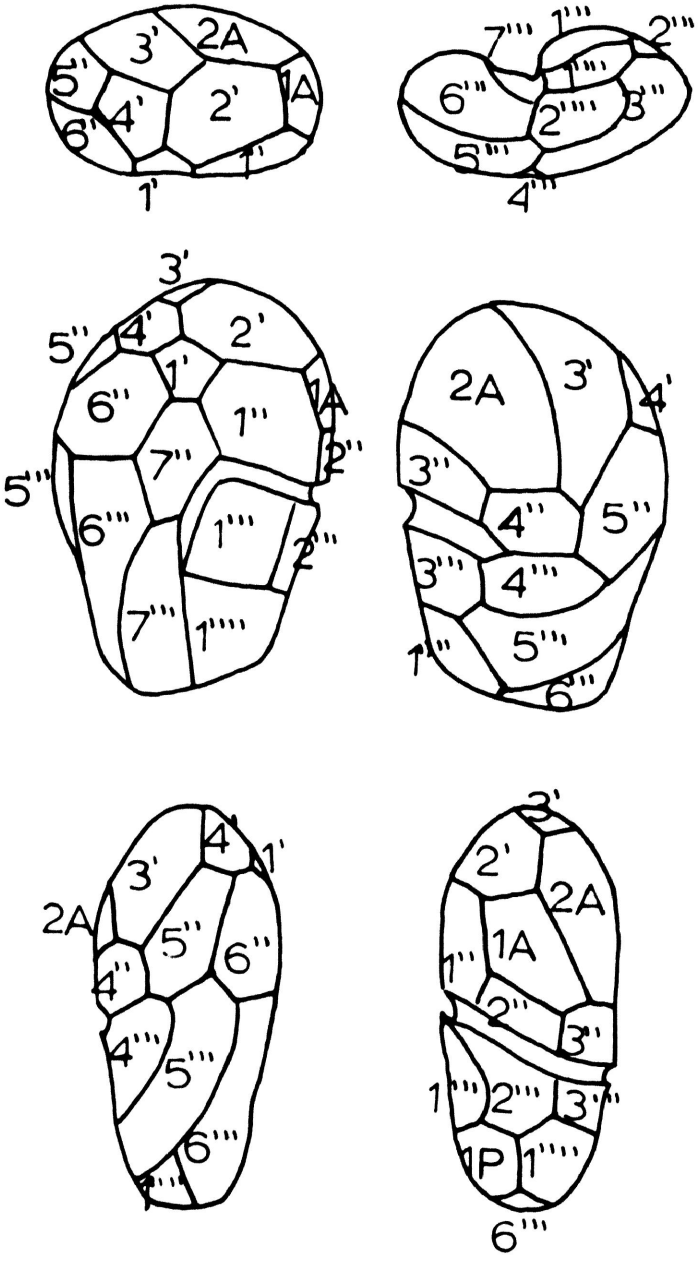
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Fig. 2: Illustration of the second generation zoospore from the Hole-in-the-Rock area. This plate arrangement and larger size of the zoospore was also found in the material from Yates Center. The formula for the plate arrangement is 4', 3A, 3AA, 1AAA, 7", 7"', 2P, 2"".
(x 1455)

LEGEND:

'.....Apical

A.....1st series of anterior intercalarys

AA.....2nd series of anterior intercalarys

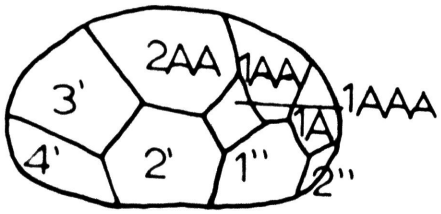
AAA.....3rd series of anterior intercalarys

".....Precingular

"'.....Postcingular

P.....Posterior intercalary

"".....Antapical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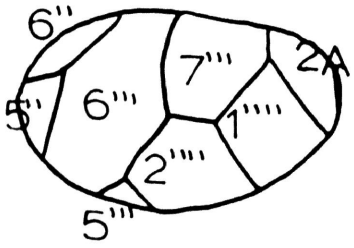
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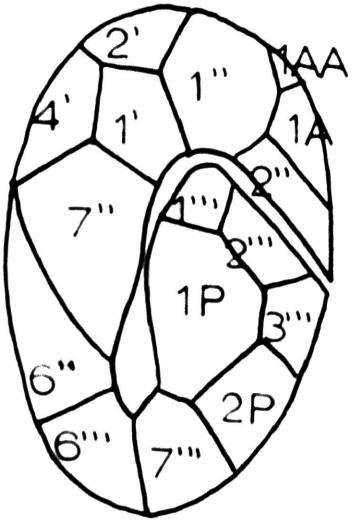
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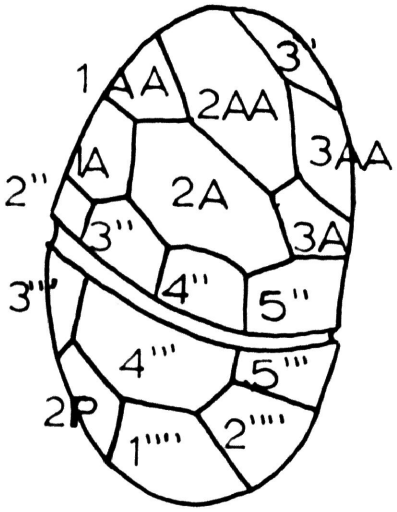
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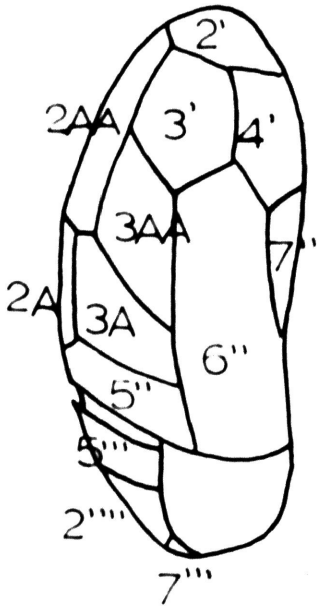
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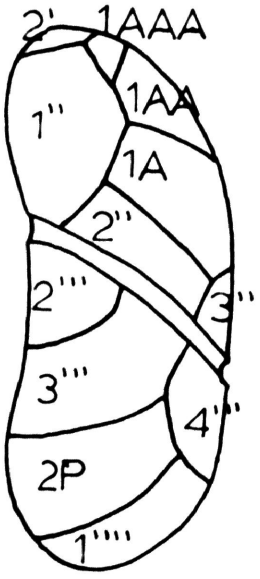
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antapicals. The Baldwin material used in the present study verifies Dr. Thompson's findings (Fig. 2), although several variations were observed. The suture between precingular plates three and four is often absent forming a long, straight line between the combined plates and the second anterior intercalary. The suture between the first plate of the second and third series of anterior intercalarys is also frequently missing. Several sutures may be altered, particularly in the three series of anterior intercalarys.

The larger zoospores of the Baldwin area are the result of two divisions while those of the Yates Center material are the result of three divisions. The larger cell size (15-30 microns wide and 26-46 microns long), the additional plates and the earlier generation suggests a possible origin from a many-plated thecate form such as described by Woloszynska (1930) for Hemidinium nasutum var. tatrica Wolosz. (Thompson, unpublished)

The theca of the Urococcus zoospore is very delicate and allows for some change in shape of the cell. While the theca is "lifted" and swollen as the zoospore comes to rest and appears ovoid when empty, on the active zoospore there is a characteristic, and apparently relatively permanent, shallow crease across precingular plates four to seven from the dorsal end of the semi-girdle to

the sulcus on the ventral surface. The transverse flagellum terminates before this shallow "extension" of the girdle.

LIFE HISTORY OF UROCOCCUS

In Kansas, Urococcus occurs on moist, sandstone ledges. When collected it is found either solitary or with other cells in a gelatinous sheath. Usually it is associated with species of Coccomyxa, Gloeocapsa, Cylindrocystis and other occasional desmids. In a culture dish, Urococcus is found to perpetuate the palmelloid condition through cell division (Figs. 6-9). This mode of reproducing may continue indefinitely. When conditions within the culture chamber are changed, as through flooding the material, zoospores are formed. It is assumed that this condition also exists in nature.

The large cells of Urococcus reproduce by two successive divisions to produce four cells. Each resulting cell develops a sheath and the group is often retained in the original sheathing (Fig. 9). These cells are at first ellipsoidal and later become spherical. If the palmelloid habit is continued, each cell secretes successive excentric sheaths as it grows in size and diverges on its own gelatinous stalk (Figs. 2, 3). If the alga is to reproduce by motile cells, then the four cells are released by a dissolution of the old colonial sheathing. In the Yates Center material, an additional division results in

eight cells which may be released as zoospores. The zoospores are formed from the second generation in the Baldwin organisms.

The zoospores, which are produced one to two days after flooding, swim about for a period of approximately one hour before coming to rest upon the side of the culture dish. Once settled, they shed their thecas or secrete a sheath within them, form concentric sheathing and start growth and cell division. Depending upon the condition of the culture, they either produce zoospores or form colonies as in the palmelloid state (Figs. 16-18).

As with the other dinoflagellates, there is no definite proof of sexual reproduction. On several occasions, a conjugation-like figure of vegetative cells similar to that reported by Hall (1925b) for Ceratium hirundinella has been observed. Zoospores have also been photographed in a state resembling conjugation (Fig. 43). The cytoplasm of the zoospores is usually connected but studies of the nuclei have been inconclusive. Other cells of Urococcus have been observed in which the nucleus of the parent cell had divided, and the daughter nuclei migrated to opposite ends of the elongated cell and were undergoing a second division, still within the parent cell. It is supposed that the cell represented the mitotic formation of four cells without immediate cytokinesis, but it could also have represented meiotic divisions.

As will be discussed later, chromosome counts afford ittle proof of either the stability of the number of hromosomes or the presence of a meiotic division. It is ossible, however, that some of the counts represent meiotic umbers. One photograph in particular does suggest that eiotic divisions are present (Fig. 42). Several chromo- omes were out of the camera field and an accurrate count as impossible, but the figures do seem to represent eiotic pairing.

The great size variation of both motile and nonmotile orms also leads to a speculation of sexual differentiation.

NUCLEAR CYTOLOGY

Few workers have studied nuclear division in the linoflagellates. Most of the studies have concentrated on the marine genus Oxyrrhis and species of Ceratium. Hall (1925a) described a neuromotor system in Oxyrrhis consisting of two flagella each of which ended in a bleph- aroplast and with a rhizoplast extending from each bleph- aroplast to an extra-nuclear centrosome. He described a characteristic chromomeric "string-of-beads" and a single spherical nucleolus. Chromosomes of Oxyrrhis were found to be parallel and numbered approximately 40. Hall further observed a paradesmose, a delicate fibril drawn out between the daughter centrosomes as they separated,

and remarked that the fibril was a characteristic morphological feature for the mitotic dinoflagellate.

Hall also reported that the nuclear membrane in Oxyrrhis, as reported for all dinoflagellates, persisted throughout the entire mitotic process. He observed that the "endosome" (nucleolus) divided by constriction during metaphase. Chromosomes were seen to split longitudinally and form V-shaped structures at metaphase through anaphase. The further unfolding of the V-shaped chromosomes on an "equatorial plate" caused a densely stained "cylinder" of parallel chromosomes in late metaphase.

Hall (1925b) also reported on mitosis in Ceratium hirundinella. He again ascertained that a longitudinal splitting occurred in which the separating daughter chromosomes unfolded to form an end-to-end joined pair of chromatids on the metaphase plate. At first the chromosomes retained their parallel organization, but this was soon lost.

Köhler-Wieder (1937) examined the mitotic cycle of Peridinium willei and Glenodinium pulvisculus. In both genera he observed the chromatids in pairs either rod-shaped or sometimes curved, and parallel in arrangement. During mitosis, the "beaded" chromatids were arranged parallel to each other and spiraled in the longer axis of the division figure.

During anaphase, the chromatids appeared to "glide" past one another until they were separated into two groups.

This position in late anaphase was the only one in which Köhler-Wieder reported the chromosomes unsplit into chromatids. By early telophase, the chromosomes were observed longitudinally divided. The interphase nucleus contained rods which were more or less organized as pairs. He called the contents of the interphase nucleus heterochromatin.

Skoczylas (1958) reported the complete mitotic cycle of Ceratium cornutum and certain other dinoflagellates. For the first time in the dinoflagellates, a spindle fiber was reported. Skoczylas verified Köhler-Wieder's observation of the presence of chromatids in every stage except late anaphase and further noted the presence of probably six to ten nucleolar-organizing chromosomes. The acromatic structure, or spindle formation, he determined as not only the cause for chromosome movement but also responsible for the construction of the resting nucleus. He described a conspicuous turning movement of the chromosomes which was demonstrated throughout the entire mitosis cycle and which weakened the formation of a matrix. Skoczylas further described an extreme ball or coil stage organized after the cyst formation as not belonging to the mitotic cycle but instead leading to meiosis.

In reference to Skoczylas's work, von Stosch (1958) developed a possible explanation for the chromosome coiling in the dinoflagellates. He referred to the plectonemic coiling as obscuring two mechanically different conditions.

He also described a transitory spiral which originated de novo during anaphase only and caused an alteration in the size of the chromosomes.

Dodge (1960) described the dinoflagellate chromosomes as always stainable and without centromeres. He reported a chromosome number of 30 to 60. He mentioned the absence of a spindle and the parallel separation at anaphase with the free ends moving towards the pole.

The only discussion of cytology of Urococcus prior to the present one was done by Richter (1886), who observed a "sexual differentiation in golden-yellow cells in which elliptically divided rod-like strings of one to one and one-half microns were found". He did not, however, observe sexual union.

Urococcus presents a series of mitotic phases which are difficult to interpret:

A) The cells do not seem to have any specific time for division; at any given time of the day or night, one is able to find cell division. In a culture dish with thousands of cells, then, it is impossible to know what stage the cells may be entering. As mentioned earlier, the cell sometimes remains undivided throughout the second nuclear division so that two nuclei, both in anaphase, may be observed.

B) The chromosomes of the anaphase appear very similar to those of prophase. Thus it is necessary to count chromosomes

to ascertain the stage. This is not always satisfactory because the number varies.

C) In working with fixed material, the sheath is often so thick that application of pressure without the subsequent ruin of the material is difficult. Also, some cells cannot be broken. On the other hand, in unfixed mounts, the addition of de-staining acids, slight pressure, or even gentle heating is often sufficient to cause cell breakage and loss of chromosome material.

D) It is difficult to separate the cells from the sand grains. For this reason, most material for cytological work was collected from the side of the petri dishes.

E) The chromosomes themselves tend to stick together in a coiled ball.

The mitotic cycle for Urococcus insignis is as follows:

INTERPHASE: The chromosomes of the interphase are first noticed as being short series of deeply stained dots or as rods of various lengths tightly packed into the nucleus (Fig. 19). There is one fairly large, spherical nucleolus in each nucleus. In later interphase, the chromosomes are visible as long, fuzzy, coiled strands. They are too indefinite to photograph. It is unknown whether the heterochromatic regions exhibited are due to differential coiling, partial formation of a matrix or because of intervening centromeric regions. The regions were demonstrated

in all stains except Fuelgen. The entire nucleus is surrounded by a clearly defined nuclear membrane in interphase.

PROPHASE: In early prophase, the coils of the interphase begin to contract and become hidden by the presence of a matrix (Figs. 21-25). The differential staining seen in interphase chromosomes becomes outstanding in prophase and accounts for the phraseology "beaded chromosomes" usually found in descriptions of dinoflagellate chromosomes (Figs. 24-26).

By mid-prophase, if not earlier, the chromatids are usually visible. Often, however, chromatids are also observed in interphase. Evidently the chromatids become twisted together in this early stage to form relational coils. These coils, while loose and often visible in interphase, become tighter in early prophase and usually obscure the chromatids. By mid- or late-prophase, however, the coils are relaxed, demonstrating the chromatids again, and a second type of coiling is visible. This type of coiling, involving the chromosome as a whole, is called somatic or standard coiling. In the prophase and extending into anaphase, then, at least two types of coiling are present: the chromatids are first twisted together in relational coiling and, secondly, the twisted chromatids form larger somatic coils.

METAPHASE: By metaphase, the coiled chromosomes are aligned parallel to each other against the periphery of the nuclear membrane (Fig. 28). No spindle formation as was described by Skoxzylas (1958) has been found. Therefore, it is impossible to know whether the downward, winding pattern of the chromosomes is due to a longitudinally spiraled metaphase plate, a spindle formation or if the chromosomes are merely confined within the limits of a nuclear membrane. The oblique, but parallel, alignment of the chromosomes is broken by several to many horizontal chromosomes. These chromosomes are usually more sinuous in shape than are the oblique ones.

Many chromosomes also exhibit a coarse coiling which is the result of relaxing the somatic coils. After division in anaphase, these late somatic gyres, now called relic coils, become straightened.

The differential staining, described above in both interphase and prophase, is still present on many of the metaphase chromosomes.

ANAPHASE: In early anaphase, several types of separation are found. The longitudinal "splitting" from one end to another, as described by Hall (1925 a, b) and Köhler-Wieder (1937), is shown by the many Y- to V-shaped chromosomes in both metaphase and anaphase (Figs. 34, 37). Also present are chromosomes whose ends are still fastened

together as described by Skoczylas (1958). Still other chromosomes complete a parallel separation such as described by Godward (1954) for Spirogyra and by King (1960) for certain desmids (Figs. 34-36, 38). A few, small, deep-staining fragments of chromosomes are also noticable. The possibilities presented by the many types of separation will be discussed later.

In later anaphase, the lengthening of the nucleus is an indication of the period of chromatid separation. The nuclear membrane as such is no longer visible in anaphase. Some chromosomes apparently move by the action of one end, others (primarily short chromosomes) move in a V-shape or an S-shape, and still others move parallel to each other. The only evidence of polarity is that the chromosomes eventually stop at opposite ends of the nuclear area. Whether the opposing formation is due to spindle fibers or limitations of an obscure nuclear membrane is unknown. The nucleolus is often present in anaphase (Fig. 36).

TELOPHASE: Early telophase appears to be a mass of long chromosomes with the parallel arrangement at first present but later lost. Chromatids are at times visible. By late-telophase the chromosomes appear as shortened rods (Fig. 41). From this point, a nuclear membrane and a nucleolus are clearly reorganized. During the reorganization,

the daughter nuclei migrate from the center of the cell to opposite extremities. Cytokinesis does not necessarily follow telophase.

In dividing, some cells elongate and constrict in the median section; others retain their original shape.

DISCUSSION: In most prophase nuclei of Urococcus insignis, an average number of 40 chromosomes has been counted. By late anaphase, however, over 51 chromosome-like structures per nucleus have been counted. In most prophase and nearly all anaphase cells, the chromosomes range in size from less than one micron to more than 21 microns in length. In shape, they vary from rod- to V- or S-shaped. At first glance, the sinuous chromosomes appear to be a result of relic coiling, but the latter disappears in early anaphase or before. The disorder of the metaphase "plate", the short telophase chromosomes and the long late interphase and early prophase chromosomes need further explanation as do the "beaded" chromosomes of interphase, prophase and metaphase.

Perhaps the most obvious answer to the problem of interpreting the cytology of Urococcus is the presence of diffuse or polycentric chromosomes. Several authors have reported this condition in algae. One of the most noteworthy was Godward (1954) who worked with Spirogyra species.

Several parallels may be drawn between the work on Spirogyra and that on Urococcus:

A) Godward listed four different types of chromosomes in Spirogyra but only the first two will be discussed. The first type listed by Godward were large, sinuous-shaped, "sticky" chromosomes; the chromatids were parallel at anaphase and separated at metaphase throughout their length. Godward assumed these chromosomes were polycentric.

In the second type, the chromosomes were of medium and small sizes without stickiness; parallel and normal separation of chromatids occurred. Both the polycentric and single centromere conditions, then, were present.

As described earlier, variation from long, rod-shaped to shorter, sinuous-shaped to much smaller S-, V-, and dot-shaped chromosomes are found in Urococcus. These either separate normally, from one end, or they separate parallel to each other.

B) Spirogyra chromosomes demonstrated coiling not unlike Urococcus. The spirals in Spirogyra were noticed in mid-prophase and ended in loose spirals in anaphase. The latter coiling appears to be similar to the relic spirals in Urococcus.

C) The transition from interphase to metaphase of both genera was similar. Prophase chromosomes of Spirogyra exhibited stained blocks as do those of Urococcus.

Godward explained the blocks as being derived from the chromocenters of the resting stage.

Godward preferred to consider the Spirogyra chromosomes as polycentric rather than as having diffuse centromeres for the same reasons as would follow in Urococcus:

A) In Spirogyra, alternate staining areas of the long chromosomes in prophase was found to have a pattern corresponding to the interphase chromocenters. The unstained blocks, or heterochromatic regions, of both Spirogyra and Urococcus could represent regions containing a centromere. Godward was able to ascertain that the chromocenters were constant in number and arrangement.

B) Studying the shape of the medium-sized chromosomes in early anaphase, Godward noted that apparently some chromosomes had two or three centromeres while others possessed a single centromere. The same chromosome shapes are present in the Urococcus anaphase.

C) In many species Godward noted the presence of numerous dot-like chromosomes which were similar to parts of the other types of chromosomes. These she surmized to be loose centromere units. Again, the same dot-like structures are present in Urococcus.

King (1960) described parallel disjunction of chromatids at anaphase in desmids and inferred that a "non-localized" type of centromeric organization of the chromosomes was present. He noted that the chromosomes also had a banded

appearance in prophase which later disappeared. He also found great variation in chromosome number within the same species in Cosmarium botrytis and Netrium digitus and within the same clone in Cosmarium cucumis. He decided that this condition had come about through transverse division or through fusion of chromosomes. Only by the possession of a "non-localized" centromere could transverse division occur without the loss of chromosome fragments. The "non-localized" centromere or polycentric chromosome could also arise through an end-to-end joining of chromosome parts.

Nordenskiöld (1961) in reference to Luzula campestris, a member of the Juncaceae, mentioned that, as is usual in the case of diffuse centromeres, the size of the chromosome was always reduced when the number of chromosomes was increased. This condition of the fragmenting of a polycentric chromosome is variously called agmatoploidy, endonuclear polyploidy or pseudoploidy.

The theory of transverse division of chromosomes in dinoflagellates has been reported previously by Entz (1921) (fide Hall, 1925). Entz recorded the chromosomes of Ceratium hirundinella as splitting longitudinally into chromatids, then fusing end-to-end to form long, compound chromosomes. The multiple chromosomes became arranged parallel to each other in the metaphase plate.

In Urococcus, it seems reasonable to suppose that a limited transverse breakage of a few chromatids occurs in

metaphase. Several cells have demonstrated the breaking away of parts of the chromatids. Further breakage may occur in anaphase and telophase.

The chromosomes of Urococcus are apparently polycentric. The condition of agmatoploidy thus allows for a variance in movement to the poles and a differing chromosome number. The fragments may either continue in the Urococcus cell as separate entities or may reunite with other chromosomes or fragments.

CONCLUSION

Both cytological and morphological evidence supports the placement of Urococcus insignis in the Dinophyceae. The varying types of chromosome separation, the parallel arrangement of "beaded" chromosomes as well as the large nucleus with a single nucleolus characteristic of Urococcus are found in all dinoflagellates which have been examined. The approximate number of 40 chromosomes has been reported previously for Oxyrrhis (Hall, 1925a) and is well within the range of 30-60 chromosomes reported for the Dinophyceae by Dodge (1960).

The discovery of a thecate zoospore along with known morphological characters necessitated the placement of Urococcus in the family Gloeodiniaceae of the order Dinocapsales. Although Urococcus has been rightly placed in

the family, perhaps a further study of Gloeodinium montanum (Klebs), the only other genus in the order, should be undertaken. The key character separating Urococcus and Gloeodinium (Smith, 1950; Thompson, 1959) has been the number of layers in the cellular envelope. Gloeodinium montanum, a fresh-water form, has few concentric rings and no stalk whereas Urococcus insignis, a lithophyte, has many concentric rings and a stalk. When Urococcus cells are grown in a water culture, however, the cells tend to lose their concentric sheathing and the stalk. Furthermore, after division and sometimes for long periods, Urococcus retains the subspherical or ellipsoidal shape as described for Gloeodinium. Before it would be possible to make any definite comparisons, the zoospore of Gloeodinium should be studied in order to prove whether it is thecate or truly naked, as the original descriptions report. If the zoospore is thecate, the genus should probably be reduced to synonymy under Urococcus.

SUMMARY

Urococcus insignis (Hass.) Kütz. is a brown-pigmented, sandstone-dwelling, tegumented, solitary or colonial alga which is capable of reproducing either by continued cell division or by the formation of zoospores. Contrary to present day descriptions, the zoospore is thecate with a plate arrangement of four apicals, two anterior intercalary plates, seven precingulars, seven postcingulars, one posterior intercalary and two antapicals for zoospores resulting from three divisions. Zoospores resulting from two divisions may have two additional series of anterior intercalary plates and another posterior intercalary. The plate arrangements may vary due to loss and altering of sutures.

The base chromosome number of *Urococcus insignis* is about 40, but the number may range up to 51. The typical dinoflagellate "beaded" chromosomes are visible in interphase, prophase and metaphase. In anaphase, the chromosomes are variously shaped: dots, small or long rods, V- and S-shapes may be present. In late telophase, the chromosomes appear as innumerable, small, rod and curved chromosomes. The beaded chromosomes, the varying chromosome number, the differing shapes of the anaphase chromosomes and the many small, late telophase chromosomes possibly indicate that the chromosomes are polycentric and agmatoploidy has occurred.

According to descriptions alone, it seems likely that Urococcus insignis and U. hookerianus (Bark. and Hass.) are the same species. The growth habits of U. insignis in water cultures also leads to the possible combination of Gloeodinium montanum Klebs and U. insignis.

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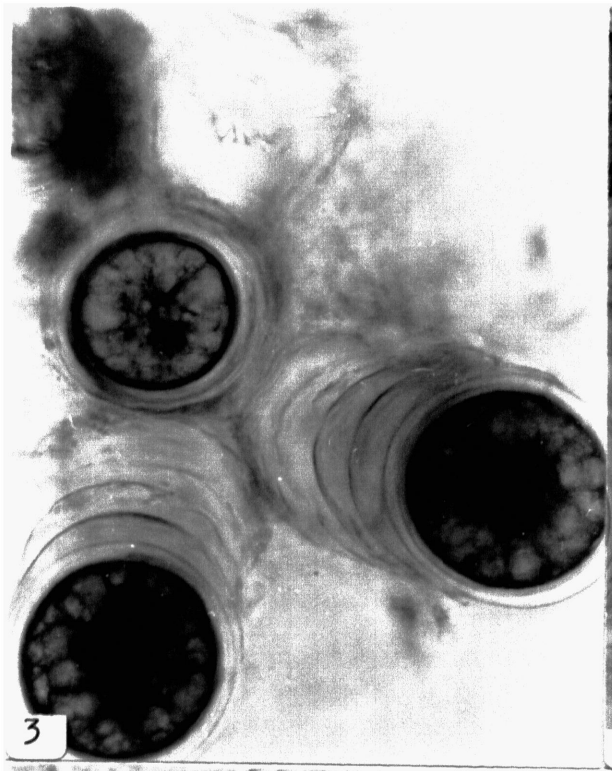
Fig. 3. Healthy cells and "stalk" of Urococcus insignis. (x 400, water mount)

Fig. 4. Dissolution of the "stalk" of the palmella stage. (x 400, water mount)

Fig. 5. Unequal concentric sheathing of a single cell. (x 400, water mount)

Fig. 6. Nuclear division of the nonmotile cell. (x915, carmine)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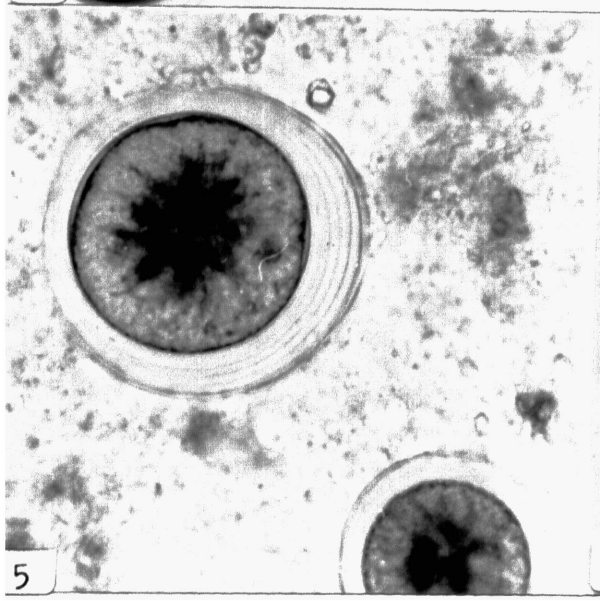
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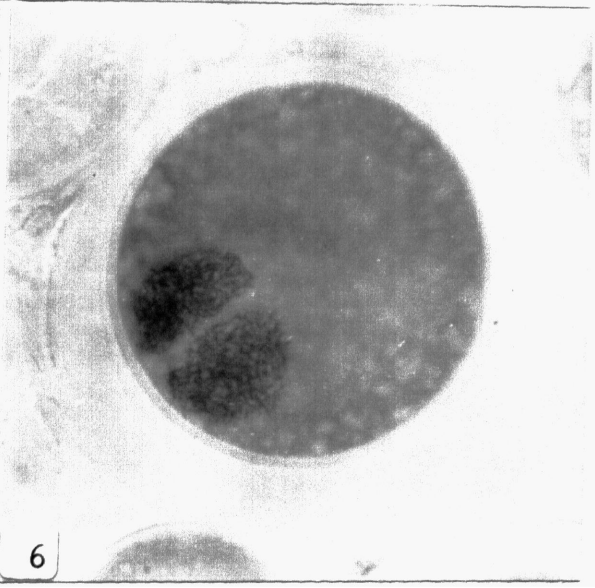
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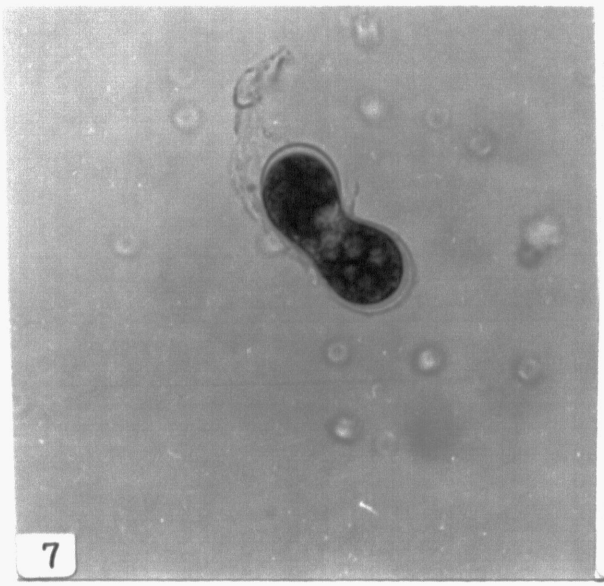
Fig. 7. Elongation and beginning of cell division. (x 400, water mount)

Fig. 8. Cleavage of a vegetative cell. (x 400, carmine)

Fig. 9. Group of cells demonstrating normal spherical cells and recently divided elliptical cells. (x 400, water mount)

Fig. 10. Group of four cells still in the original parental sheathing. (x 400, water mount)



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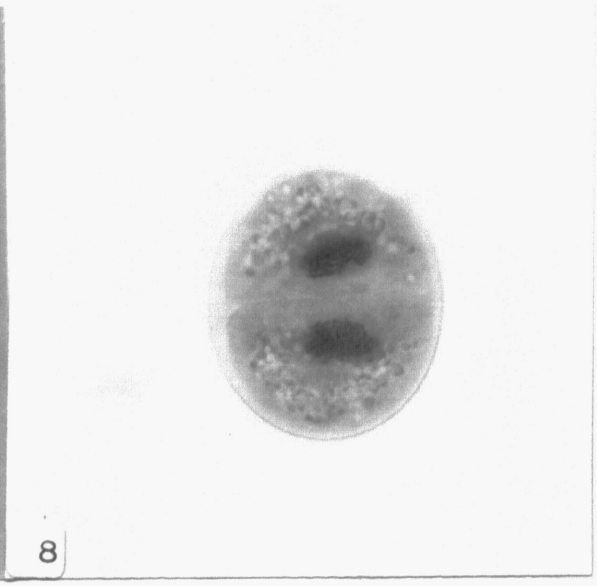

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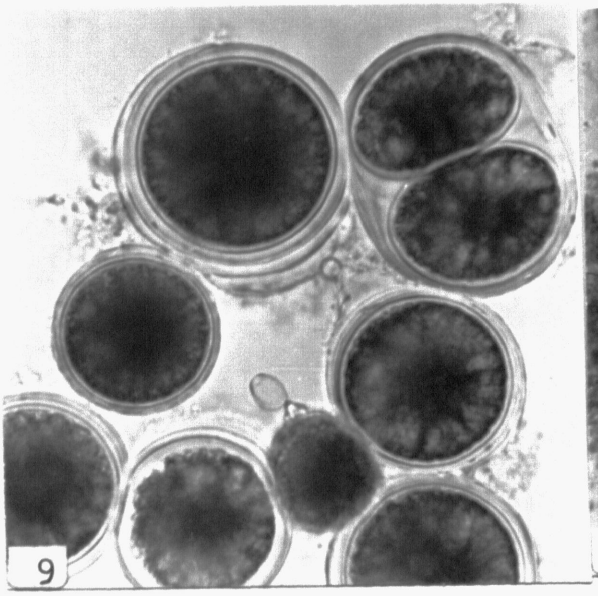

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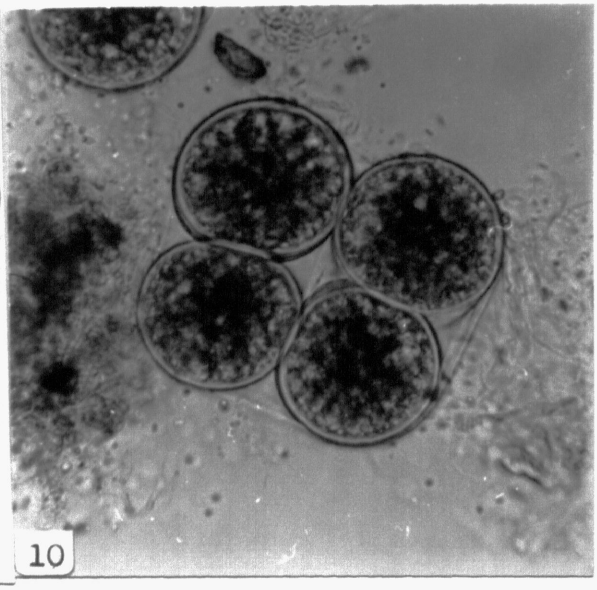


10

Fig. 11. Dorsal aspect of the zoospore (x 400, water mount)

Fig. 12. Side view of the zoospore showing the downward spiraling girdle. (x 400, water mount).

Fig. 13. Ventral surface of the zoospore showing the termination of the sulcus. (x 400, water mount).

Fig. 14. Group of zoospores showing size variation. (x 400, water mount).

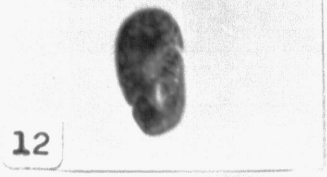
Fig. 15. Shedding of the theca by the zoospore. (x 915, water mount).

Fig. 16. Beginning of elongation. (x 400, water mount)

Fig. 17. Further elongation of the zoospore prior to division. (x 400, water mount).

Fig. 18. Formation of the cell after division of the zoospore. (x 400, water mount).

11



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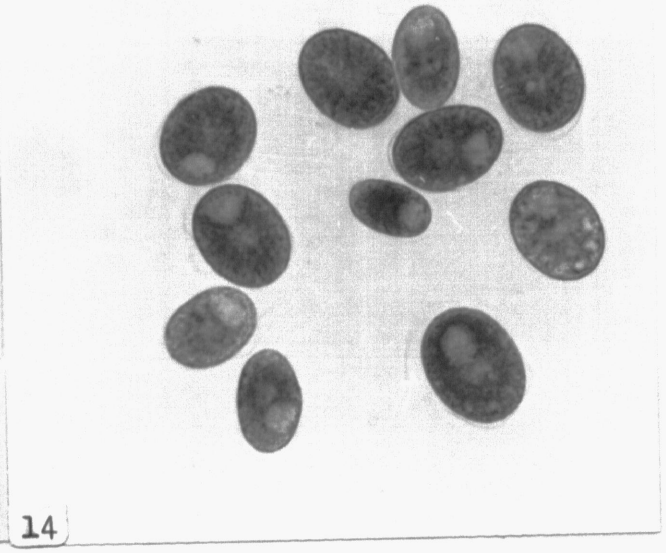

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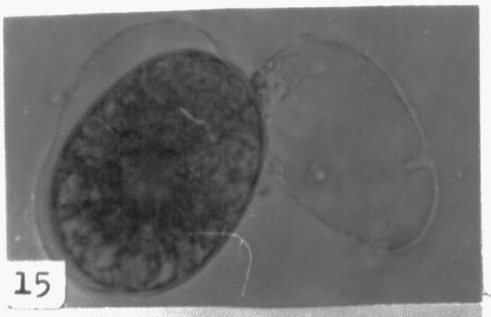

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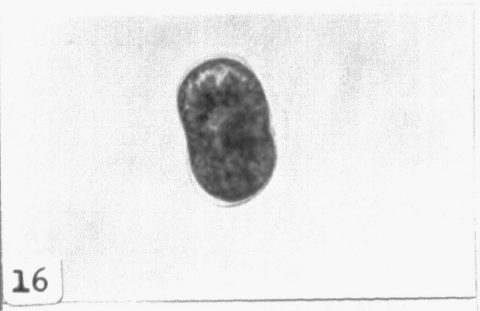

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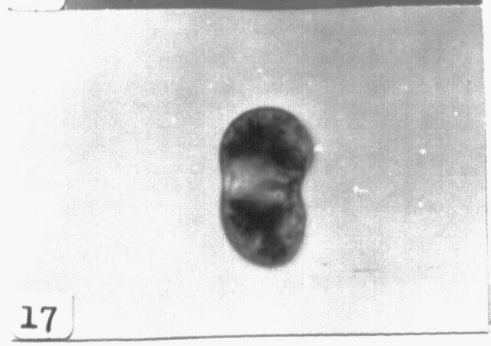

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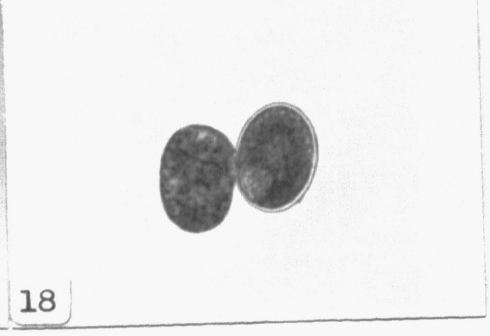


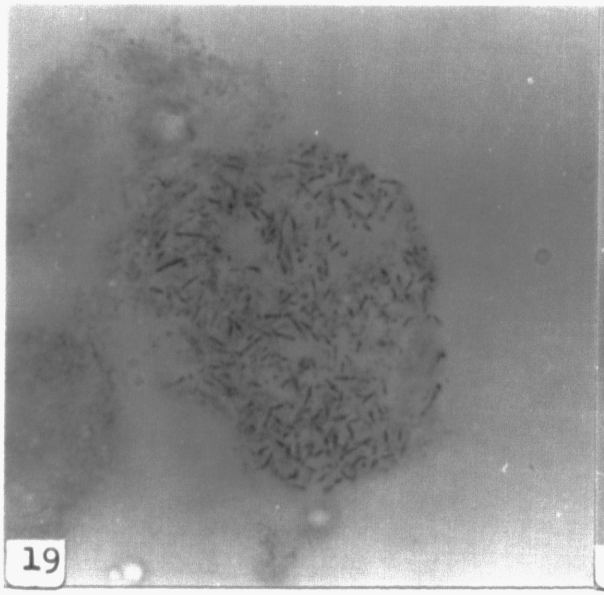
18

Fig. 19. Interphase with many, short, staining areas of chromosomes. The nucleolus is not visible. (x 915, carmine)

Fig. 20. Somatic coiling of prophase chromosomes. (x 915, carmine)

Fig. 21. Prophase chromosomes exhibiting somatic coiling. (x 915, carmine)

Fig. 22. Somatic coils. (x 915, carmine)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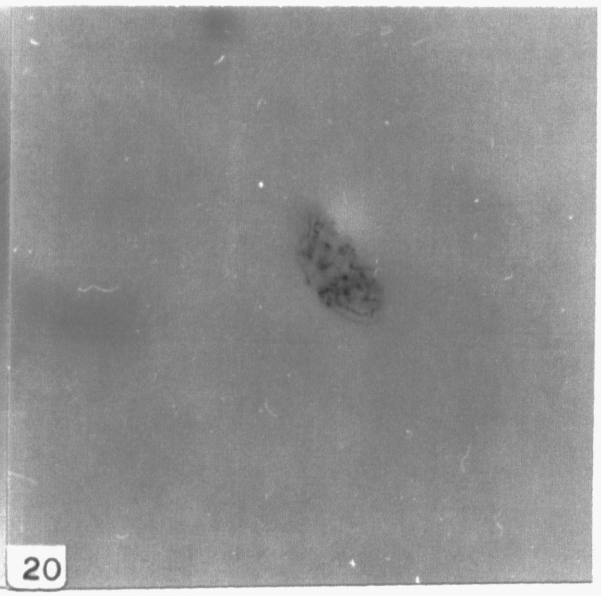
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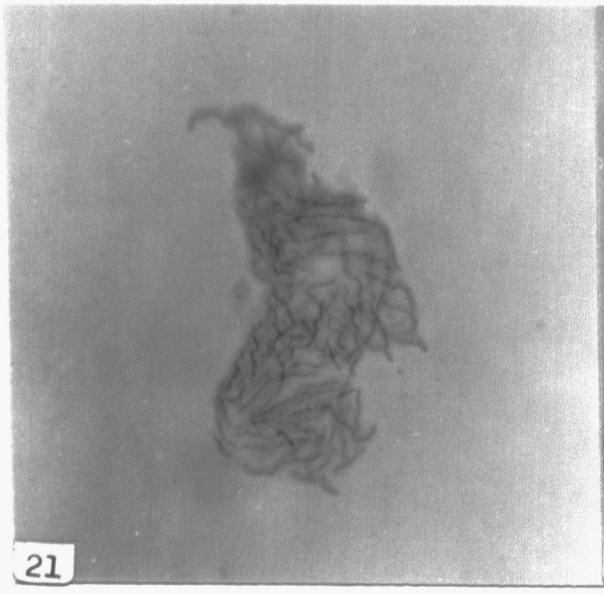
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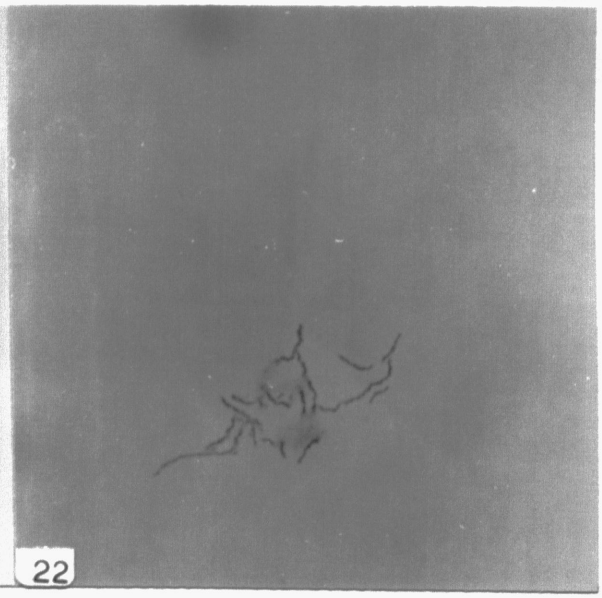
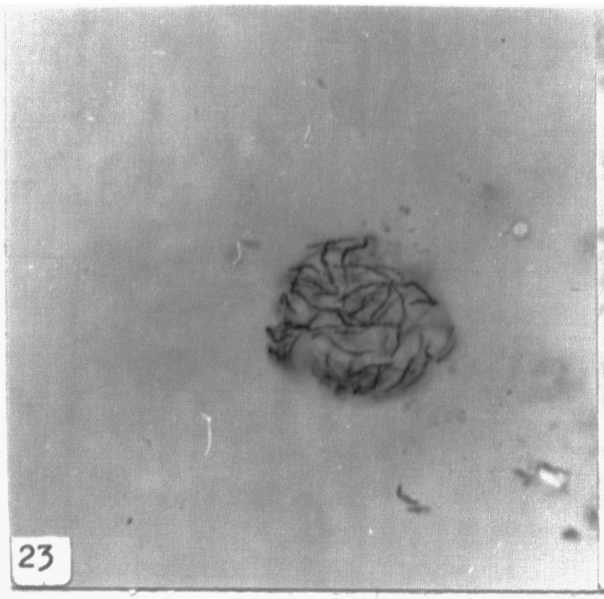

22

Fig. 23. Tightening of somatic coils demonstrating early heterochromatic areas. (x 915, ferric-carmine)

Fig. 24. Further tightening of coils showing typical dinoflagellate "beaded" chromosomes. (x 915, carmine).

Fig. 25. Prophase. Note the nucleolus (top, center) and the "beaded" chromosomes. (x 915, carmine)

Fig. 26. Nucleolus and nucleolar-organizer chromosome. (x 915, carmine)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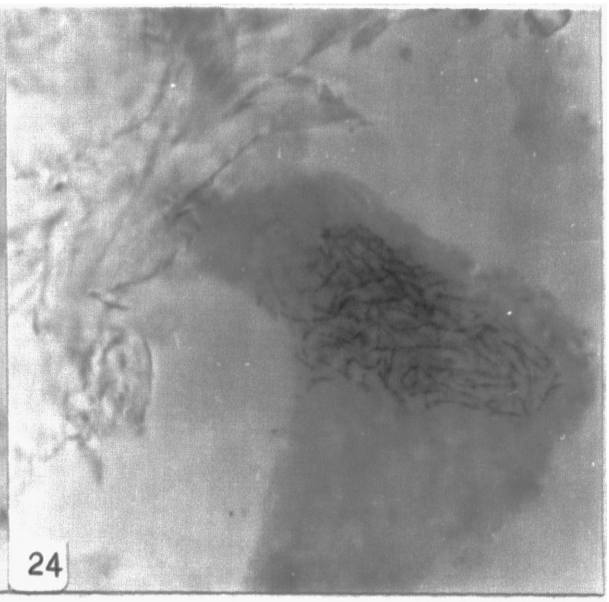
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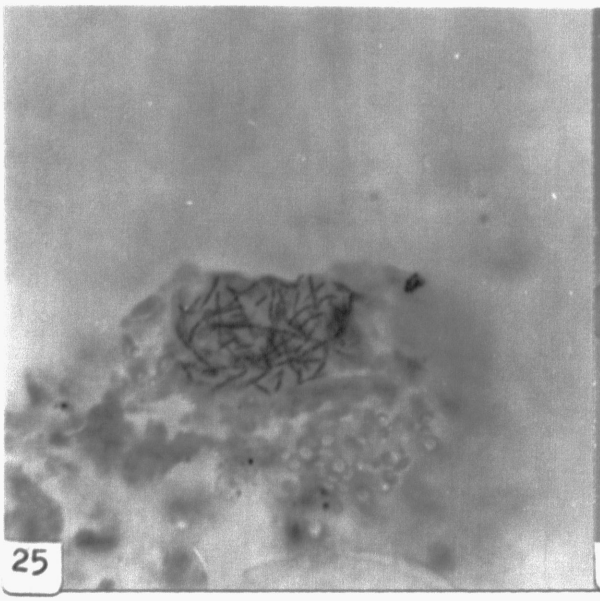
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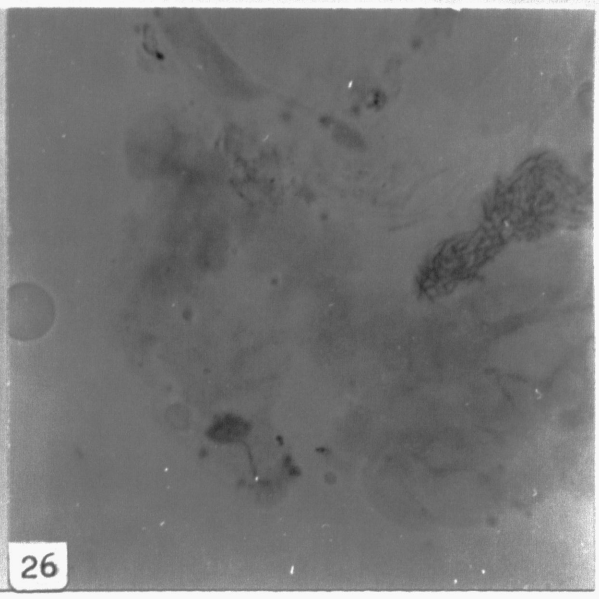
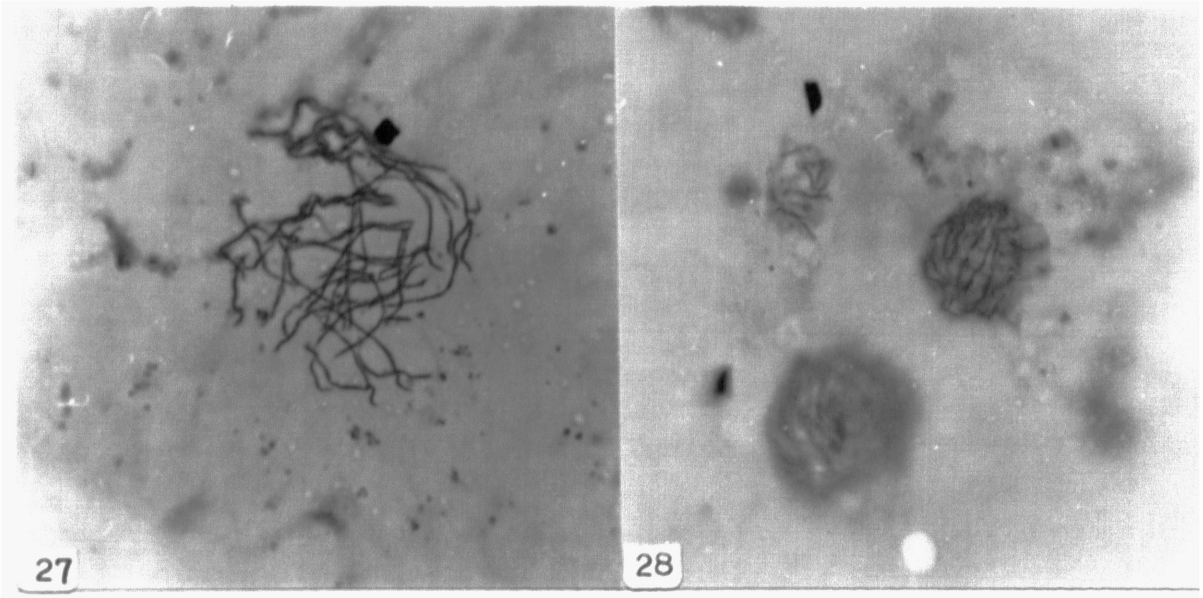

26

Fig. 27. Uncoiling of both somatic and relational coiling. (x 915, Feulgen)

Fig. 28. The center nucleus shows an early polarity of metaphase chromosomes. Although no spindle fibers were ever demonstrated, all early metaphase and anaphase chromosomes exhibited a definite parallel pattern. (x 915, Feulgen)

Fig. 29. Metaphase alignment showing the beginning of chromosome separation. (x 915, Feulgen)

Fig. 30. Division of chromosomes. (x 915, Feulgen)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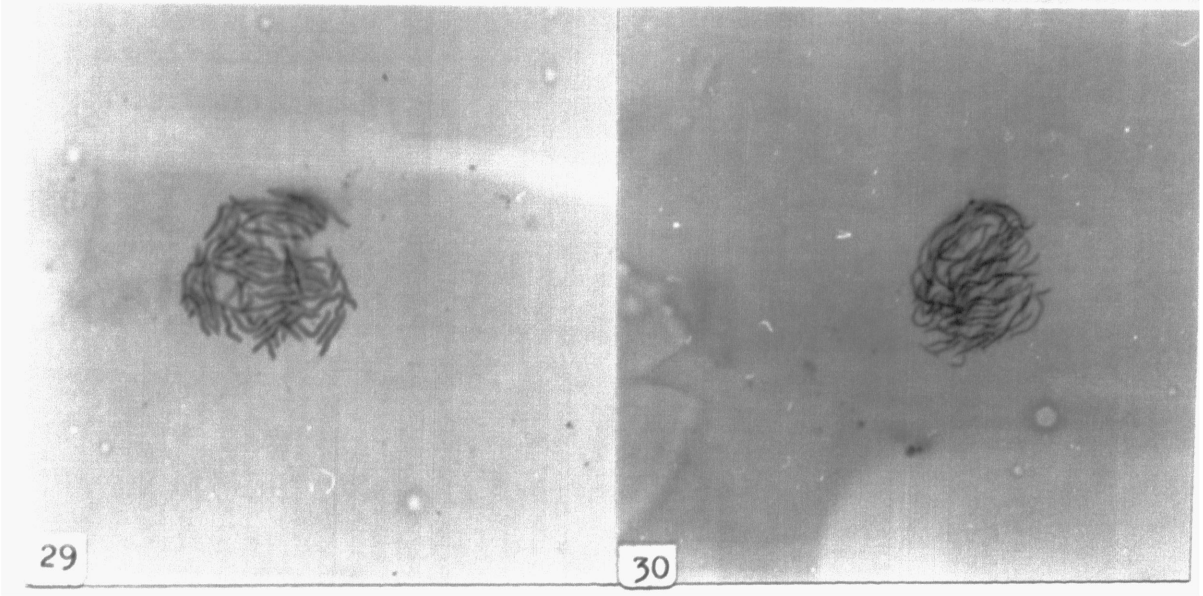
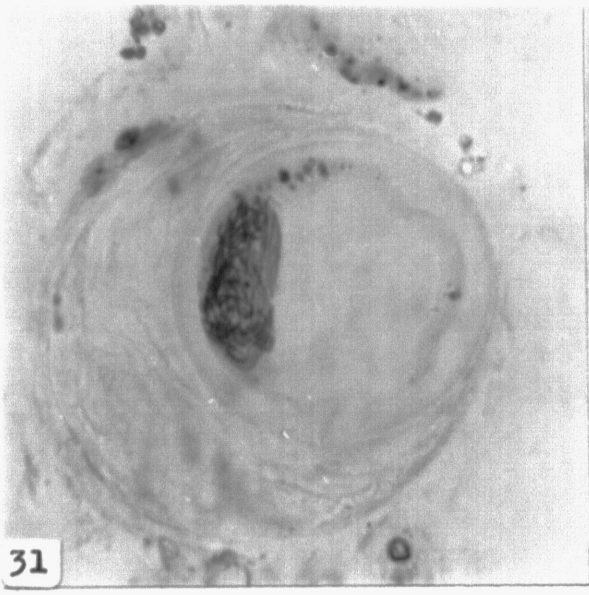

29
30

Fig. 31. Early parallel arrangement and separation of chromatids. (x 915, Feulgen)

Fig. 32. Early anaphase showing separation of chromatids. (x 915, Feulgen)

Fig. 33. Early anaphase showing both vertical and horizontal arrangement of chromatids. V- and Y-shaped chromosomes are a result of longitudinal splitting of the chromosome from one end to the other. (x 915, Feulgen)

Fig. 34. Nucleus immediately after separation showing both parallel and longitudinal splitting of chromatids. (x 915, carmine)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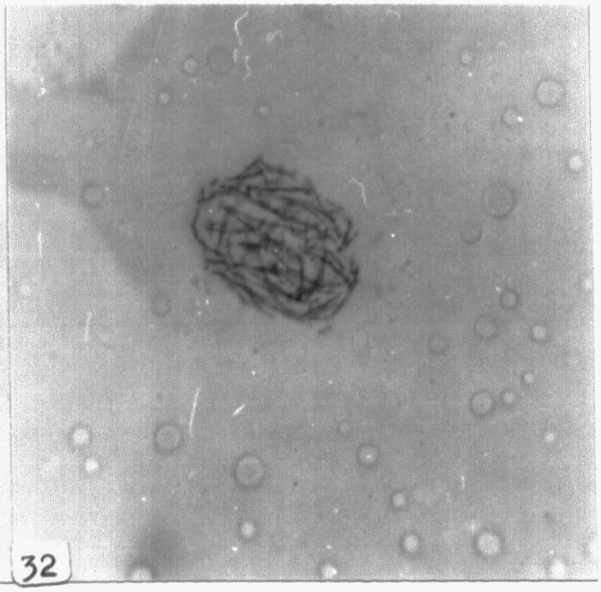
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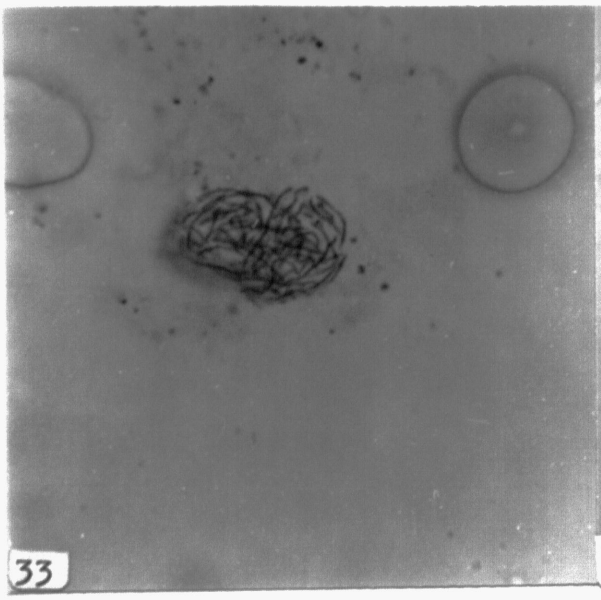
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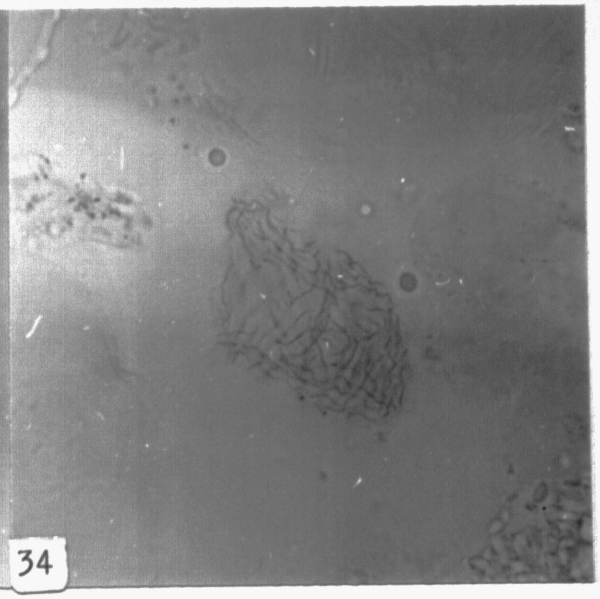
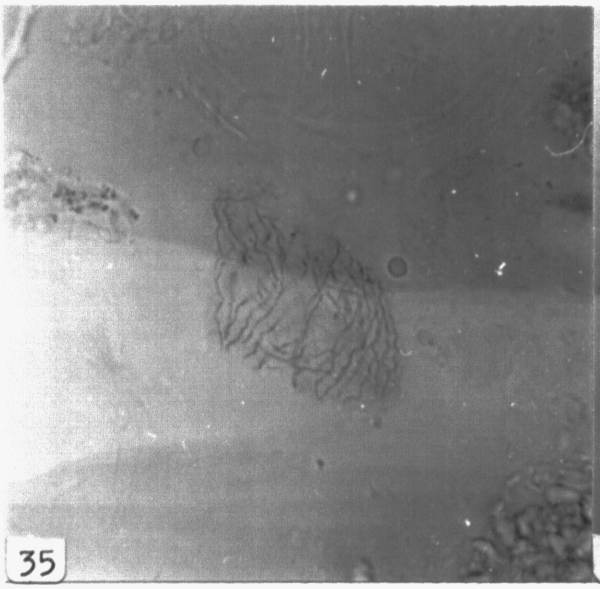

34

Fig. 35. Another focal level of the nucleus in photomicrograph #34.

Fig. 36. Two anaphase nuclei from the same cell. Nucleus on the left has a nucleolus in the center; the chromatids are uncoiling in the lower right-hand corner. The nucleus on right shows V- and Y-shaped chromatid division. A nucleolus is in the upper right-hand corner. (x 915, carmine)

Fig. 37. Partial nucleus with both longitudinal and parallel separation of chromatids. (x 915, Feulgen)

Fig. 38. Parallel separation of chromatids. (x 915, Feulgen)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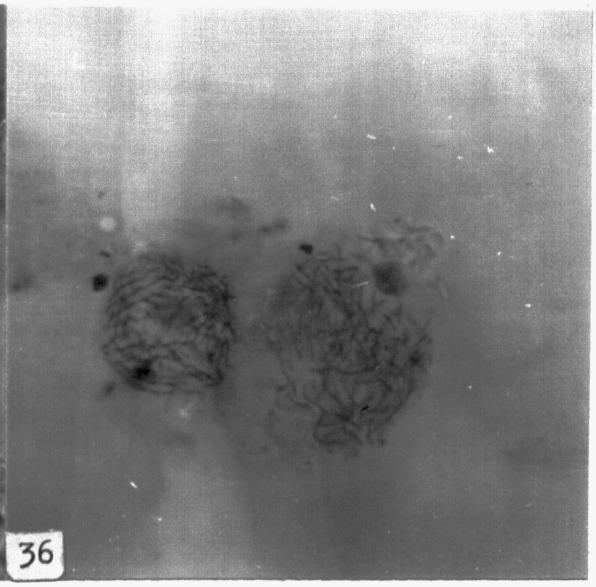
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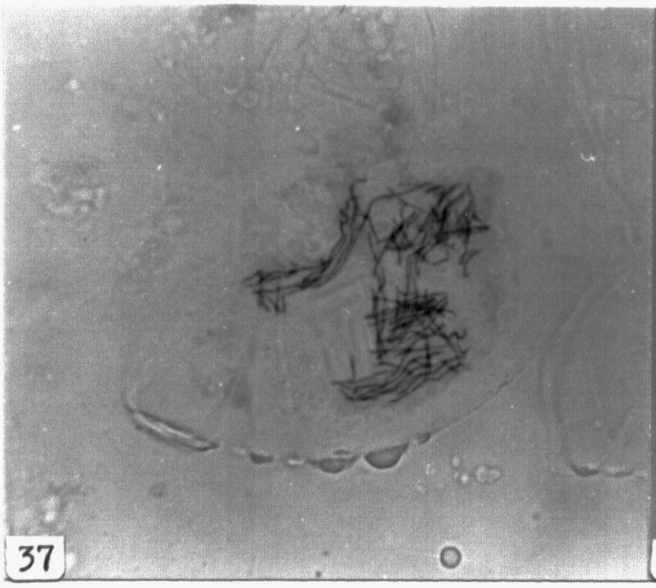
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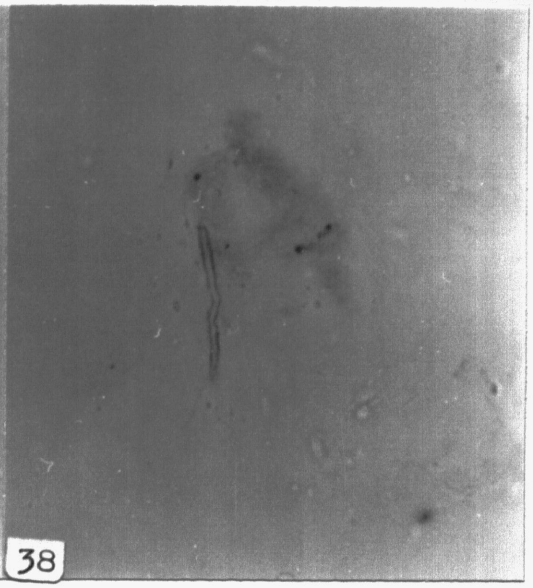
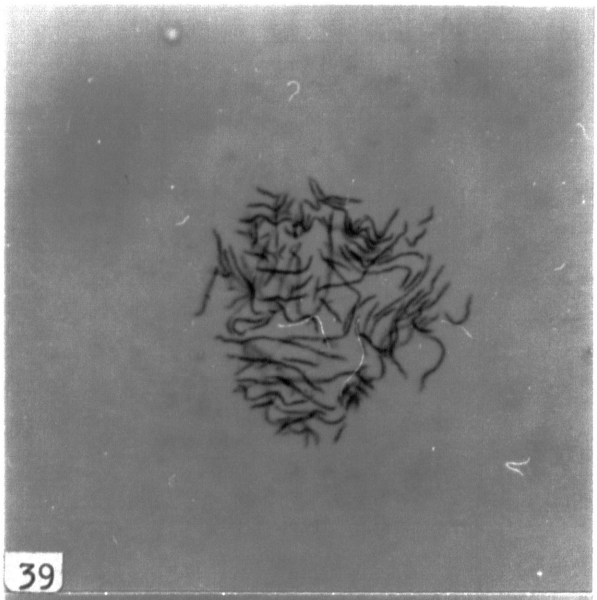

38

Fig. 39. View of late anaphase showing the shape of the chromosomes. (x 915, Feulgen)

Fig. 40. Early telophase with long, tangled chromosomes. (x 915, Feulgen)

Fig. 41. Late telophase with chromosomes mostly appearing as short rods. The nuclear membran has been re-constituted in the upper telophas figure. A nucleolus is evident in the upper part of the lower telophase figure. (x 915, carmine)



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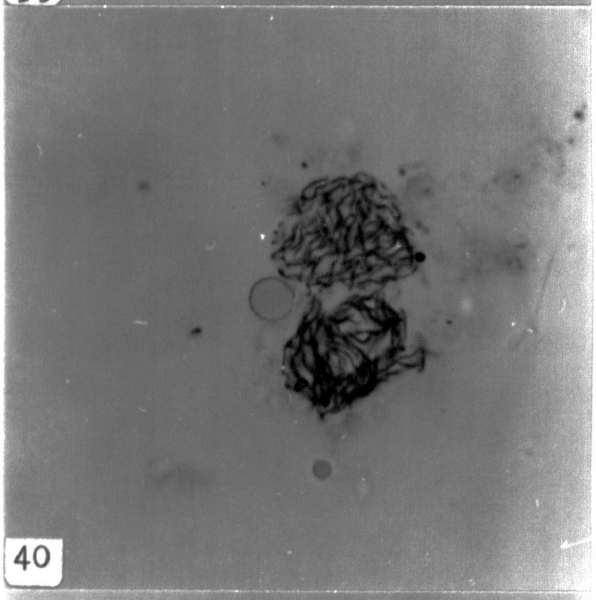

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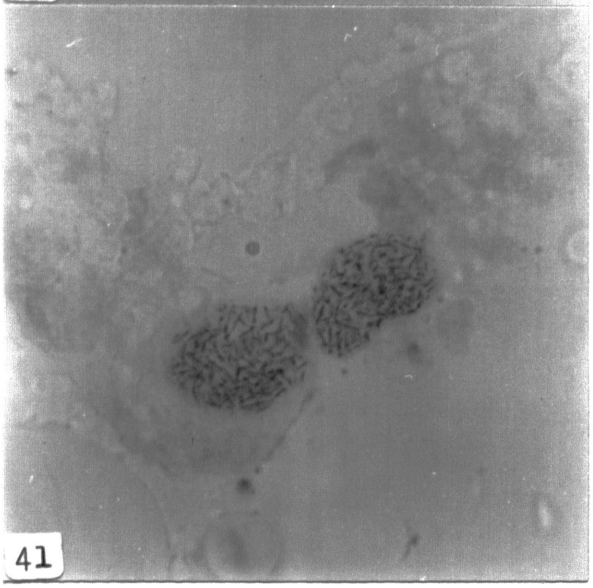
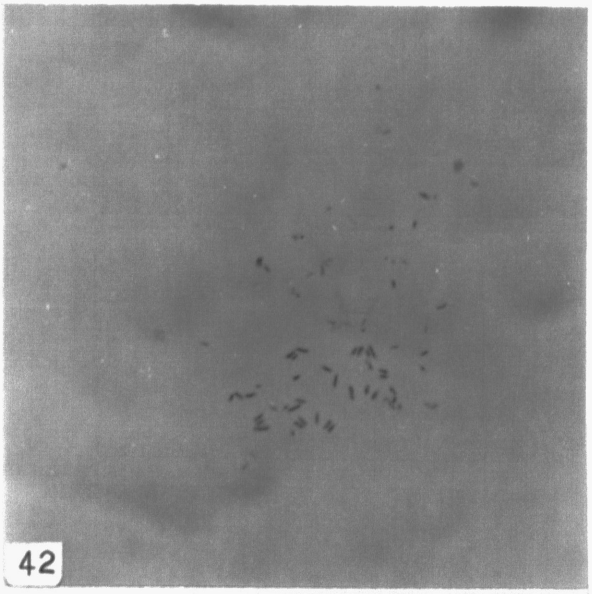


41

Fig. 42. Meiotic-like pairing of Urococcus insignis. (x 915, carmine)

Fig. 43. Two zoospores which have formed a conjugation-like figure. Note the discarding of the thecas. (x 915, water mount)



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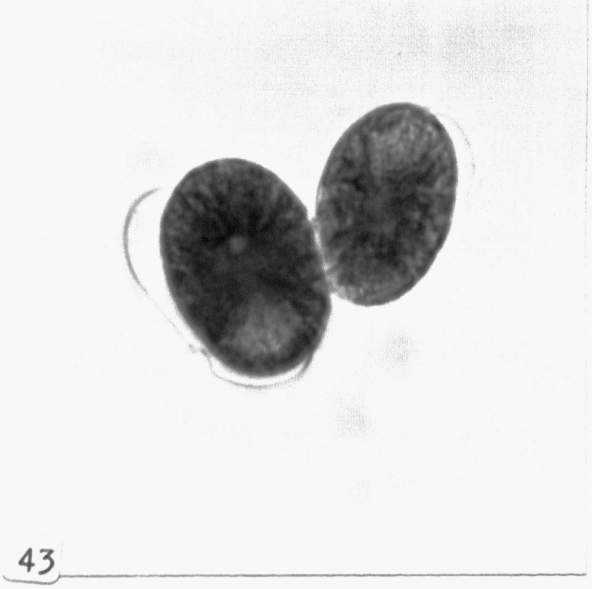

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