TULANE STUDIES IN ZOOLOGY AND BOTANY

Volume 15, Number 3 March 24, 1969

PLANOPHILA TERRESTRIS, A NEW GREEN ALGA FROM TENNESSEE SOIL

ROBERT D. GROOVER[1] and SISTER ADRIAN MARIE HOFSTETTER, O.P.[2]

Department of Botany
The University of Texas, Austin

ABSTRACT

A new chlorosphaeracean alga, *Planophila* **terrestris,** is described from Tennessee soil.

Hofstetter (1968) has reported on the distribution and identification of previously described soil algae from twelve areas in Shelby County, Tennessee. During this investigation an organism tentatively identified as a new chlorosphaeracean alga was isolated into unialgal culture. Because of the first author's special interest in the Chlorosphaeraceae (*sensu* Herndon, 1958), the organism was studied jointly during the summer of 1967.

Unialgal cultures were maintained in Pringsheim's soil-water medium (Starr, 1964). Axenic cultures were obtained using the techniques of Brown and Bischoff (1962). The axenic cultures were maintained and studied in Bold's Basal Medium (BBM) with and without 1.5 per cent agar (Brown and Bold, 1964). The cultures were grown in a culture room under standard conditions, namely 300 ft c light (cool-white fluorescent) on a 12-hr light, 12-hr dark cycle at 22 C.

Morphological observations were made routinely on cultures varying in age from one day to three months. Acetocarmine, Haidenhain's haematoxylin, aqueous iodine (I_2KI), India Ink, and Sudan IV were used to elucidate certain aspects of the cellular morphology. The photomicrographs were made with a Bausch and Lomb microscope with a 35-mm Zeiss-Winkel camera attachment.

OBSERVATIONS

Planophila **terrestris** sp. nov.

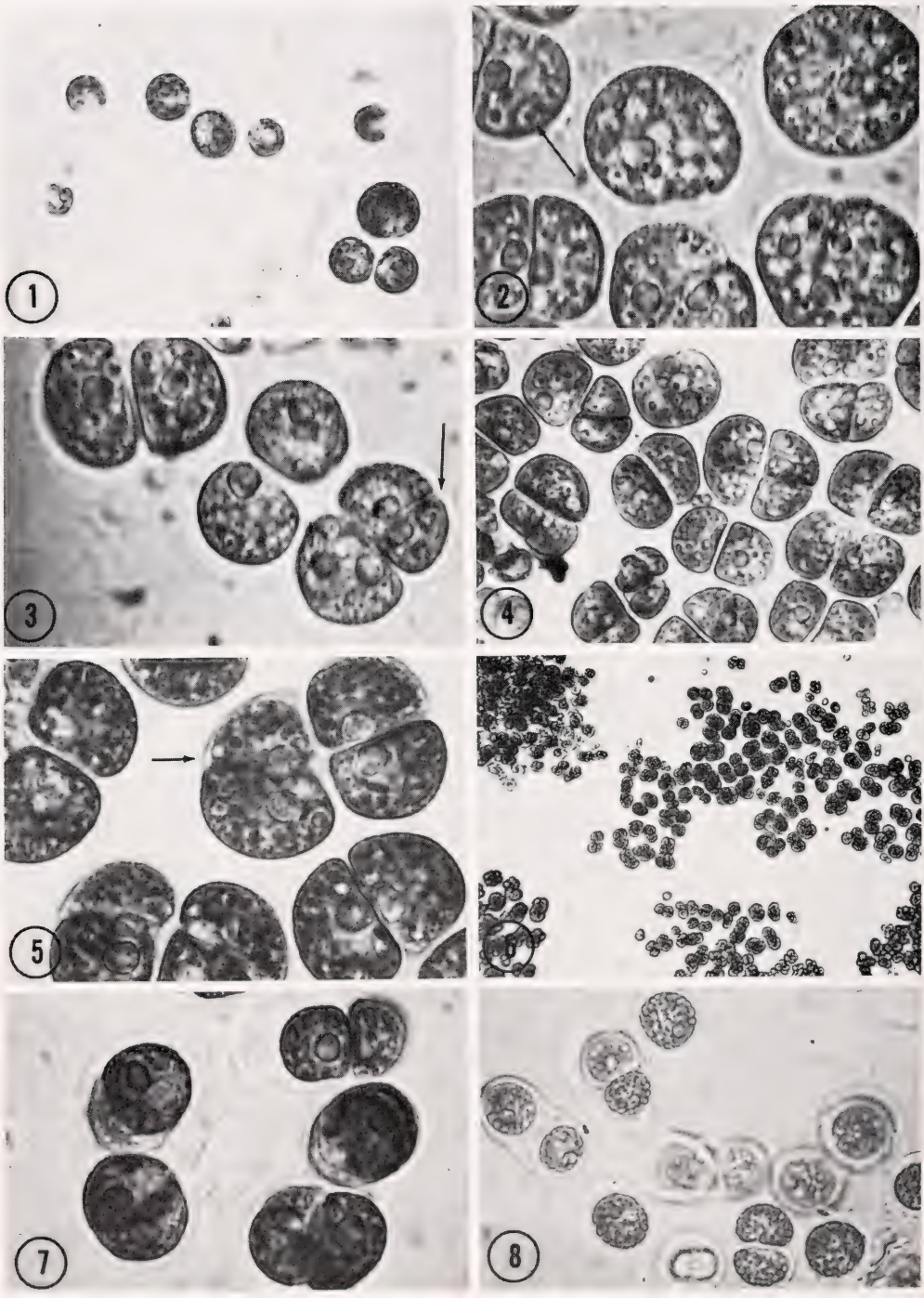
(Figs. 1-16)

Cellulae vegetativae sphericae, 6-15.2 μ diam ad paululum ellipsoideas, 14-18 μ × 18-26 μ; membrana cellulae tenuis levisque, senescens paululum spissescens, chloroplastus parietalis, in cellulis maturis perforatus; unatres pyrenoides omni in cellular; cellulae per crescentiam uninucleatae; vacuolae contractiles in cellulis vegetativis observatae; fasciculi cellularum duo-quattuor-cellulares, plerumque due-dimensionales, per divisiones cellularum vegetativarum successivas effecti; cellulae vetustae numerosas guttulas olei minutas habentes; cellulae brunneo-aurantiae; culturae senescentes colonias *Hormotilopsis*-formes effectae.

Reproductio per dissociationem fasciculorum cellularum aut per zoosporas quadriflagellatas aut per aplanosporas; zoosporae elongatae, per bipartitionem iteratam effectae, 3-6 μ lat. × 7-16 μ long., sine membrana, nucleum anteriorem, stigma anterius lineare, duas vacuolas contractiles anteriores,

[1] Present address: Department of Biology, Tulane University, New Orleans, Louisiana 70118.
[2] Present address: Siena College, Memphis, Tennessee 38117.

The authors express their sincere appreciation to Dr. H. C. Bold for reading the manuscript.



Figures 1-8. *Planophila terrestris.*
Figures 1-6. Cells from actively growing cultures on 3N BBM agar.
Figure 1. Young cells; note parietal plastid, one pyrenoid per cell, and variation in cell size. × 600.

pyrenoidem posteriorem aut interdum mediam, et plastidem parietalem habentes; zoosporogenesis per examinantem directam interdum effecta.

Reproductio sexualis non observata.

Massa plantalis in agaro 3N BBM dicto culta aetate 2 et 4 hebdomadum sine microscopio observata sicca, magnificatione 14 × observata aspera (minute glomerulata), consistencia butyracea, in culturis veteribus in periodo immobili brunneo-aurantia facta.

Origo: Plantae e solo horti culto in loco Memphis, Shelby County, Tennessee dicto. m. Nov., 1966 lectae; numerus culturae R-81.

In actively growing cultures, young vegetative cells, recently derived from zoospores, are spherical to slightly ellipsoidal (Fig. 1). Such cells, generally 6-11 μ in diameter, attain a diameter of 15.2 μ in cultures 4 days old. The chloroplast in young cells is typically parietal and covers most of the cell surface (Fig. 1). The plastid soon becomes perforate (Fig. 2) and contains from one to three pyrenoids (Figs. 1-5); the cells are uninucleate. Young cells contain numerous colorless lipid droplets (Fig. 2). One or two contractile vacuoles have been observed in vegetative cells. The walls of cells in actively growing cultures are thin and smooth (Figs. 1-5) but become slightly thickened with age (Figs. 8 and 9). Solitary cells which fail to divide vegetatively or to undergo zoosporogenesis may be slightly ellipsoidal, 14-18 × 18-26 μ.

Vegetative cell division (*sensu* Herndon, 1958) is characteristic of log-phase cultures. During cytokinesis a partition is laid down across the center of the parent cell, dividing it into two daughter cells which remain in close association with the parent cell wall. The daughter cells, in turn, may undergo vegetative cell division. The direction of each division is usually perpendicular to the preceding division giving two-dimensional, four-cell packets (Figs. 2-6). Not infrequently, three-dimensional, cuboidal, eight-celled packets are produced. The packets may dissociate either partially or entirely to form diads or solitary cells.

In stationary-phase cultures, a unilateral deposition of wall materials results in the formation of branching *Hormotilopsis*-like colonies (Figs. 7 and 8). Cells from such cultures are brownish-orange due to the presence of colored oil droplets.

Asexual reproduction is by zoospores and aplanospores (Figs. 10-15). Both solitary cells and those in packets may produce zoospores by successive bipartition of the maternal protoplast. Usually four (Fig. 10), sometimes two or eight, zoospores are produced by each parent cell. Zoospores are released through a pore formed by a gelatinization of the zoosporangial wall. Occasionally the protoplast of a single cell behaves as a single zoospore (Fig. 11). In such cases the remains of the parent wall are often visible in the culture medium (Fig. 11). The zoospores, elongate in shape, are 3-6 μ wide and 7-16 μ long. Each has a parietal plastid, an anterior nucleus, an anterior linear stigma, two contractile vacuoles, and a posterior, sometimes medial, pyrenoid. The zoospores have four flagella of equal length (Fig. 12). They lack a wall (*Protosiphon*-type, Starr, 1955) and become spherical at quiescence. The period of motility is several hours. The eyespot is persistent and may be observed for several hours after the zoospore has become nonmotile. The zoospores may be rapidly motile or exhibit slow, amoeboid movements.

Aplanospores (Figs. 14 and 15) are formed in the same manner as zoospores and are morphologically similar. They develop a wall prior to their release by a gelatinization and rupture of the aplanosporangial wall.

Macroscopically the plant mass of *Planophila terrestris* on 3N BBM[1] agar is dry; at 14× it is rough and glomerulate (Fig.

[1] BBM with 3× the usual amount of $NaNO_3$.

←

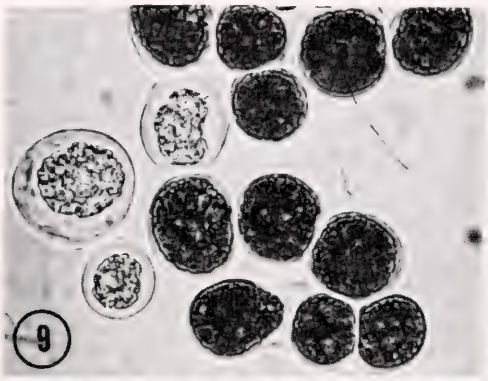
Figure 2. Cells showing perforate condition of chloroplast. The numerous droplets (arrow) in the cells are Sudan-IV-positive and probably lipid. × 1500.

Figures 3, 4. Typical packets. Note cells with more than one pyrenoid and dividing cell (arrow). Figure 3, × 1250; Figure 4, × 800.

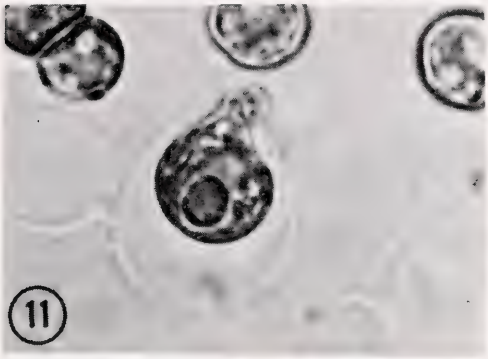
Figure 5. Cells illustrating contiguity of the parent wall around packet (arrow). × 1200.

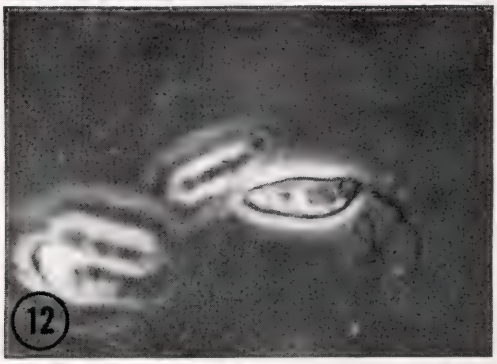
Figure 6. Cells arranged in typical packets. × 100.

Figures 7, 8. Cells from stationary-phase cultures on 3N BBM agar; note branched *Hormotilopsis*-like organization. Figure 7, × 700; Figure 8, × 500.

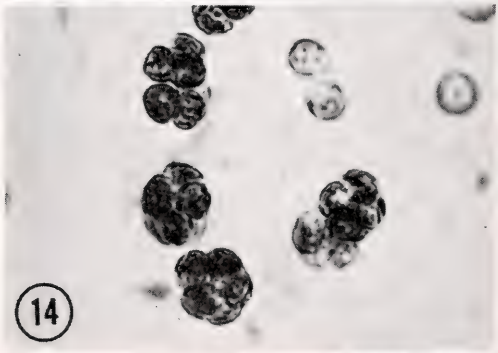

9


10

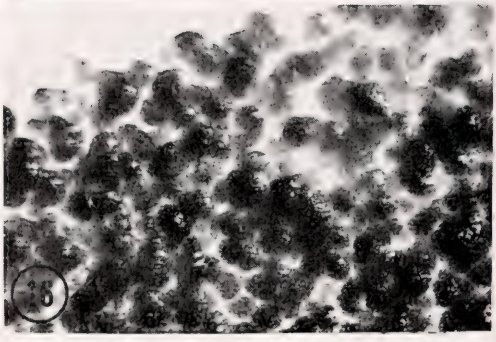

11


12


13


14


15


16

16); its consistency on agar is butyrous. Actively growing cultures are green but change with age through various shades to a brownish-orange.

Discussion

Only a small number of green, nonfilamentous algae reproducing by quadriflagellate zoospores have been described. In two genera, *Fernandinella* Chodat (1922) and *Tetraciella* Pascher and Petrova (1930), the cells are typically pyriform. Fott and Kalina (1965) consider the two genera to be synonymous. In the tetrasporalean alga *Hormotilopsis* Trainor and Bold (1953), persistent packets are absent. The genus *Planophila* Gerneck (1907) is characterized by spherical cells which divide vegetatively in two directions to form flat (i.e., two-dimensional), two-eight-celled packets. In determining the taxonomic position of the organism herein described, the descriptions and illustrations of the four above-named genera and their species were studied in the original references and also in several other sources: Wille (1911), Arce and Bold (1958), Reisigl (1964), Fott and Kalina (1965), and Bourrelly (1966).

The morphological attributes of this organism, though not in complete agreement with the generic descriptions of any of the four genera, are sufficiently close to *Planophila* to warrant its classification in this genus. The isolate described here differs distinctly from the species of this genus treated by Gerneck (1907) and Reisigl (1964) and accordingly, it is described as a new taxon.

The majority of species of *Planophila* have their cells in a flat, biseriate packet. The packets of *P. terrestris* are frequently cuboidal but this characteristic is not of sufficient importance to remove it from the genus. The cuboidal packets of cells, the presence of a *Hormotilopsis*-like stage, and the much greater length of the zoospores distinguish *P. terrestris* from the other three species.

The species of *Planophila* may be distinguished as follows:

1. Stationary-phase cultures forming *Hormotilopsis*-like stages; zoospores elongate, up to 16 μ in length ..... *P. terrestris* sp. nov.
1. *Hormotilopsis*-like stage not reported; zoospores less than 10 μ long 2
   2. Zoospores without an eyespot ..... *P. asymmetrica* (Gerneck) Wille (1911)
   2. Zoospores with an eyespot 3
3. Solitary cells 6-8 μ in diameter; pyrenoids two; zoospores 6.5 μ long, 4 μ wide ..... *P. bipyrenoidosa* Reisigl (1964)
3. Solitary cells 5-15 μ (-22) μ in diameter; pyrenoid one; zoospores almost spherical, 6 μ in diameter ..... *P. laetevirens* Gerneck (1907)

A culture of *Planophila terrestris* has been deposited in the Culture Collection of Algae, Indiana University, Bloomington, Indiana and an herbarium specimen in the Field Museum of Natural History, Chicago, Illinois.

References Cited

Arce, G. and H. C. Bold. 1958. Some Chlorophyceae from Cuban soils. Amer. Jour. Bot. 45:492-503.

Bourrelly, P. 1966. Les algues d'eau douce. Algues vertes. N. Boubée Cie. Paris.

Brown, R. M., Jr. and H. W. Bischoff. 1962. A new and useful method for obtaining axenic cultures of algae. Phycol. News Bull. 15:43-44.

———, and H. C. Bold. 1964. Phycological Studies V. Comparative studies of the algal genera *Chlorococcum* and *Tetracystis*. Univ. Texas Publication No. 6417.

Chodat, R. 1922. Materiaux pour l'histoire des

←

Figures 9-16. *Planophila terrestris*.

Figure 9. Cells from stationary-phase cultures on 3N BBM agar; note thickened cell walls. × 725.

Figures 10-15. Cells from actively growing cultures on 3N BBM agar.

Figure 10. Zoospores prior to release, four per sporangium (arrow). × 1200.

Figure 11. Direct swarming; note liberation of entire protoplast as a single zoospore and persistence of parental walls. × 2000.

Figure 12. Single zoospore. Note quadriflagellate condition and position of pyrenoid. Phase-contrast. × 1500.

Figure 13. Zoospore. Note parietal plastid, position of pyrenoid, and position of eyespot (arrow). × 1800.

Figures 14, 15. Aplanosporangia; eight aplanospores per parent cell in Figure 14 and four per cell in Figure 15. Figure 14, × 300; Figure 15, × 1000.

Figure 16. Surface view of plant mass; 2-week-old culture on 3N BBM agar. × 16.

algues de la Suisse. Bull. Soc. Bot. Geneve 13:66-114.

Fott, B. and T. Kalina. 1965. Zur Klarung einiger tetrakonten Grünalgen. Preslia 37: 369-379.

Gerneck, R. 1907. Zur Kenntnis der niederen Chlorophyceen. Beih. Bot. Centralbl. 2(21): 221-290.

Herndon, W. R. 1958. Studies on chlorophaeracean algae from soil. Amer. J. Bot. 45:298-308.

Hofstetter, Sister A. M. 1968. A preliminary report on the algal flora of soils from selected areas of Shelby County. Jour. Tenn. Acad. Sci. 43(1):20-21.

Petrova, J. 1930. Eine neue festsitzende Protococcalengattung (*Tetraciella* nov. gen.). Arch. Protistenk. 71:550-566.

Reisigl, H. 1964. Zur Systematik und Ökologie alpiner Bodenalgae. Österr. Bot. Z. 4: 402-499.

Starr, R. C. 1955. A comparative study of *Chlorococcum* Meneghini and other spherical zoospore producing genera of the Chlorococcales. Indiana Univ. Publication Sci. Ser. No. 20.

———. 1964. The culture collection of algae at Indiana University. Amer. Jour. Bot. 51:1013-1044.

Trainor, F. and H. C. Bold. 1953. Three unicellular Chlorophyceae from soil. Amer. Jour. Bot. 40:758-767.

Wille, N. 1911. Chlorophyceen. In: Engler und Prantl, Die naturlichen Pflanzenfamilien. 1. Aufl., Nachtrag.