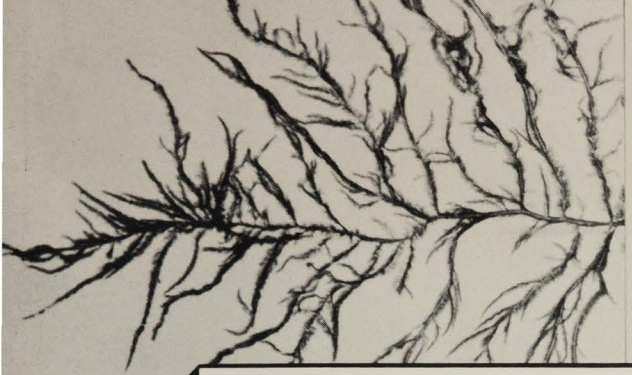
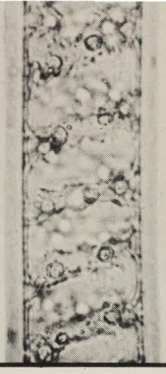

PHYCOLOGICAL STUDIES

III. The Taxonomy of Certain Ulotrichacean Algae

KARL R. MATTOX AND HAROLD C. BOLD

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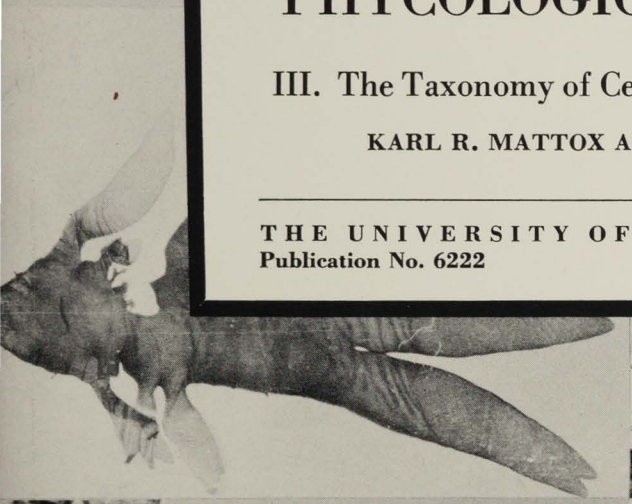

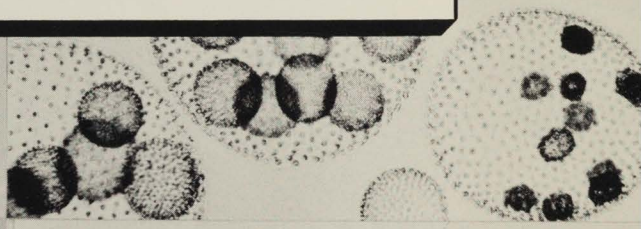
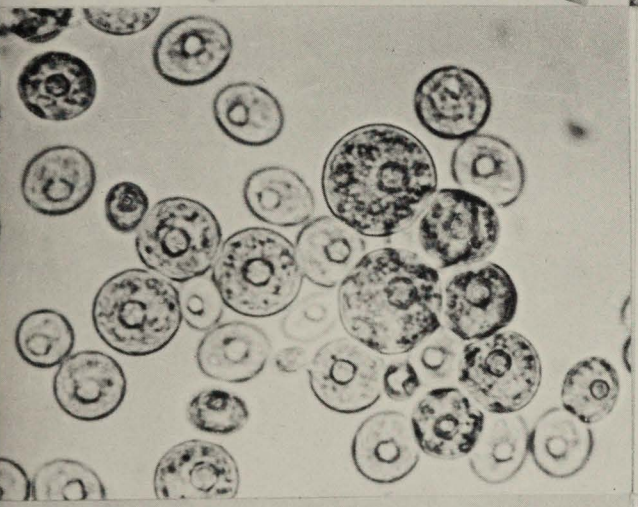

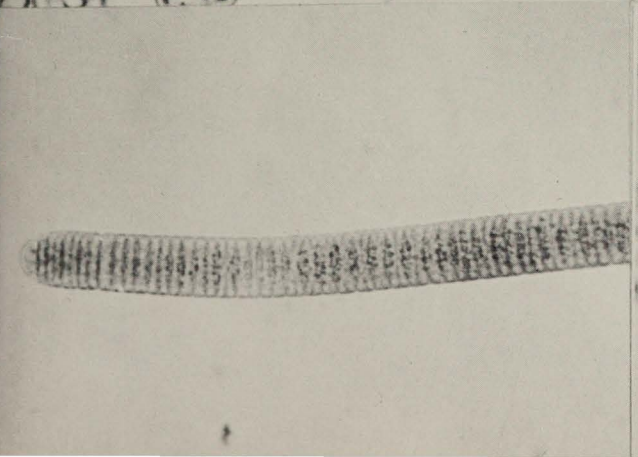
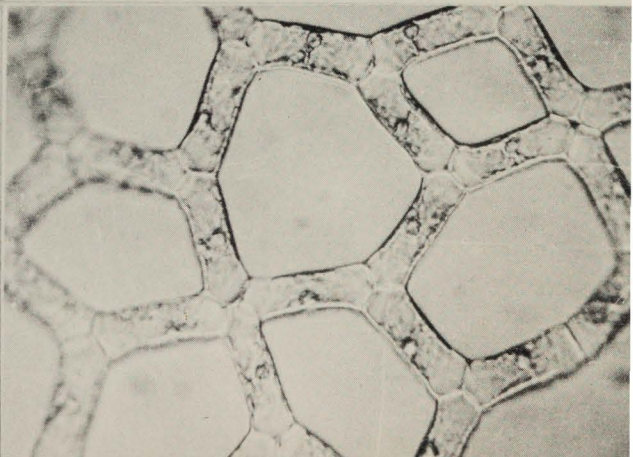

Phycological Studies

III. The Taxonomy of Certain Ulotrichacean Algae

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Introduction

The family Ulotrichaceae includes those normally unbranched and uniseriate, filamentous Chlorophyceae that have uninucleate cells with parietal, band or plate-like plastids. Asexual reproduction may be vegetative by fragmentation or by the production of akinetes, aplanospores, or bi- or quadriflagellate zoospores; sexual reproduction is by the fusion of isogamous or slightly anisogamous gametes.

Because generic differences are based largely or exclusively on vegetative characters, considerable confusion has arisen regarding the limits of certain genera presently included within the family. Particularly troublesome in this respect are the genera *Ulothrix* Kützing, *Uronema* Lagerheim, *Hormidium* Kützing, and *Stichococcus* Nägeli. About other genera in the family there seems to be less confusion, primarily because each appears to have one or more attributes less subject to variation, but also because they are less frequently encountered and, therefore, not so often studied. Hare (1961) has recently called attention to difficult aspects of these genera.

It has been a primary objective of this study to determine which attributes of the organisms likely to be included in the above-named genera are valid taxonomically and which are not. The principal tool employed has been the culture method; many isolates have been intensively and repeatedly examined in axenic culture. Comparative observations have been made on cultures of various ages grown in different media under standard environmental conditions.

A second objective of this study has been to provide information concerning the taxonomic utility, if any, of certain physiological differences among morphologically similar organisms. To implement this objective, the experimental organisms have been grown in nutrient solutions containing several different organic carbon and nitrogen sources and on various solid media, and their relative apparent growth rates have been compared.

This investigation is intended to provide a firm basis for the taxonomy of ulotrichacean algae, a taxonomy founded on morphological and physiological attributes which have been shown to be reliable. The absolutely essential role of the culture method in the taxonomic disposition of these algae has been demonstrated.

Materials and Methods

The algae studied during this investigation were isolated from various sources as indicated in Table 1. Although a number of isolates were studied briefly from only unialgal cultures (not listed in Table 1), those that were examined intensively later were purified to axenic state by one of several methods and then maintained on one or more of the following media:

1. Modified Bristol's solution (Bold, 1949), prepared as follows: 6 stock solutions 400 ml in volume were employed, each containing one of the following salts in the concentration 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

To 940 ml of distilled water were added 10 ml of each stock solution and 1.0 ml of each of the 4 stock, trace-element solutions prepared as stated by Chantanachat and Bold (1962).

2. Soil-water tubes, prepared as follows: a very small amount of $CaCO_3$ (less than 0.01 g) was placed in the bottom of a Pyrex culture tube (13 × 100 mm). To this was added about ¼ in. of soil[3] and enough distilled water to fill the tube 2/3 full. The tubes were then autoclaved for ½ hr and allowed to settle for about 48 hr before use.

3. Soil-water extract was prepared by autoclaving a kilogram of soil in a liter of distilled water and filtering the supernatant. To make soil extract agar, 40 ml of the filtrate and 15 g of agar were added to 960 ml of Bristol's solution. Soil-water extract has also been employed in liquid media in combination with Bristol's solution. The most common dilutions were 2 parts Bristol's to 1 part soil-water extract and 9 parts Bristol's to 1 part soil-water extract.

4. Proteose-peptone agar was prepared by adding 1.0 g of proteose-peptone (Difco) and 15 g agar to 1 liter of Bristol's solution.

Methods of isolation and purification were as follows:

1. It was possible in a few cases to plate out raw collections in Bristol's agar and later to pick up fungus- and bacteria-free filaments.

2. Algae obtained from soil samples were isolated and purified by the following method: approximately 10 g of soil were added to a 125-ml flask containing about 60 ml of sterile Bristol's solution. The flask was then incubated in a culture room under fluorescent illumination of 250 ft-c maintained on a 12-hr light, 12-hr dark cycle at a temperature of 22 C. These conditions are hereinafter referred to as "standard conditions." Algae became apparent in the flasks in 1–2 weeks. Some

[3] Soil from a garden in Nashville, Tennessee, is used routinely in this laboratory and at the Culture Collection of Algae at Indiana University.

TABLE 1. *Sources and isolators of organisms studied*

Organism	Source	Isolator
Ulothrix gigas (Vischer) comb. nov.	I.U. 174[a]	Vischer
U. minuta sp. nov	I.U. 330	Pringsheim
U. acuminata sp. nov.		
f-10[b]	soil	Bold
f-24	lake	Bold
f-26	lake	Bold
f-30	stream	Mattox
f-31	stream	Mattox
f-32	stream	Mattox
U. fimbriata Bold	I.U. 638	Bold
U. belkae sp. nov.	stream	Mattox
U. confervicola (Lagerh.) comb. nov.	I.U. 331	Pringsheim
U. sp.	I.U. 420	Pringsheim
Hormidium flaccidum A. Braun.		
f-2	soil	Mattox
f-3	soil	Johnston
f-4	soil	Johnston
f-8	soil	Johnston
f-12	soil	Johnston
f-16	soil	Johnston
f-23	soil	Johnston
f-57	atmosphere	Brown
f-83	soil	Bischoff
f-86	soil	Bischoff
f-93	soil	Bischoff
321	I.U. 321	Pringsheim
322	I.U. 322	Pringsheim
323	I.U. 323	Pringsheim
623	I.U. 623	Lewin
f-34	stream	Mattox
f-48	soil	Ridgway
H. subtilissimum (Rabenh.) comb. nov.		
f-70	soil	Bischoff
f-74	soil	Bischoff
462	I.U. 462	Lewin
H. sterile Deason & Bold	soil	Deason
Stichococcus bacillaris Näg.		
176	I.U. 176	Pringsheim
314	I.U. 314	Vischer
419	I.U. 419	Algeus
315	I.U. 315	Krüger
S. mirabilis	I.U. 316	Pringsheim
S. chodati (Bial.) Heering	soil	Johnston

[a] Culture Collection of Algae at Indiana University.

[b] Numbers such as f-10, f-2, etc. refer to the writers' isolation numbers.

of the liquid containing the algae was then plated out in Bristol's agar, and single filaments were later picked up with a fine platinum needle and transferred to soil-water tubes. After being transferred to liquid Bristol's, these unialgal cultures were later purified by one of the following methods. (a) A thriving culture was obtained, centrifuged, washed for 2 hr in a 4% solution of Tween-80 (Atlas Powder Co., Wilmington, Delaware), rinsed 5 times in sterile distilled water, and plated out in 1.5% Bristol's agar. Two–5 days later, bacteria-free filaments were picked up, whenever possible, and transferred to fresh plates of Bristol's agar. (b) Several isolates were washed and rinsed in the above manner and then plated out in 1.5% Bristol's agar containing 5000 units buffered crystalline Squibb Penicillin G potassium and 1000 gamma crystalline Squibb Dihydro Streptomycin Sulfate per ml. Two–5 days later, bacteria-free filaments were transferred, when possible, to fresh plates of Bristol's agar.

3. Most of the algae obtained from streams were isolated into unialgal culture by transferring single filaments from the raw collection to soil-water tubes. The algae were purified later by method 2a or 2b.

Pure cultures were grown routinely under standard conditions on Bristol's agar slants in cotton-plugged tubes. These were regularly tested for purity in one or more of the following media: (1) Yeast-extract agar (1.0 g yeast extract and 15 g agar/liter of Bristol's); (2) Nutrient Agar (Difco); (3) Nutrient Broth (Difco); (4) A. C. Broth (Difco); and (5) Thioglycollate Broth (Difco).

The morphology of the organisms was studied in fresh mounts from 1-week-old cultures grown under standard conditions on Bristol's agar or in liquid Bristol's solution. Observations were made also on cultures grown for 2 weeks, 1 month, and 2 months in flasks of liquid Bristol's solution. India ink and Methylene blue were employed to determine which, if any, of the organisms investigated has a sheath. Aqueous iodine (I_2-KI) was used to establish the presence of starch and as an aid in the determination of the nuclear and pyrenoid conditions. Sudan IV was used to verify the presence of lipids. Colony characteristics were recorded from colonies grown for 2 weeks on 60-mm Petri dishes of Bristol's agar. Zoosporogenesis and zoospore germination were studied from hanging-drop preparations maintained, in some cases, for 24 hr at low temperatures (5–7 C). The above techniques are considered fundamental to the taxonomic study of these organisms.

In addition, a number of physiological tests have been employed in a search for taxonomically useful supplementary attributes. These techniques are not original with the writers, but have been modified from earlier work (Deason and Bold, 1960; Bold and Parker, 1962) for use with the algae presently under consideration. The following is a list of the materials used in the preparation of media for physiological tests, with the concentration indicated in grams/liter. Media prepared with items 1–11 were employed as liquids, and tests were made in Pyrex glass culture tubes (13 × 100 mm or 18 × 150 mm). Media prepared with items 12–16 were solidified with 1.5% Difco agar before use.

(1)	A. C. Broth (Difco)	34.0
(2)	Thioglycollate Broth (Difco)	29.3
(3)	Nutrient Broth (Difco)	8.0
(4)	Sulfathiazole (Lilly)	0.4
(5)	D glucose (Mallinckrodt)	5.0
(6)	Sodium acetate (Mallinckrodt)	5.0
(7)	L(+) arabinose (Mann)	5.0
(8)	D(−) ribose (Mann)	5.0
(9)	D fructose (Gen. Biochem.)	5.0
(10)	D(+) xylose (Mann)	5.0
(11)	Heikol detergent (Heinicke)	0.1, 0.01, & 0.001
(12)	Crystal violet (Difco)	0.1, 0.04, & 0.02
(13)	Starch (Baker)	1.0
(14)	Eosin Methylene blue (Difco)	36.0
(15)	Milk	50.0
(16)	Butter	10.0

All media were prepared with Bristol's solution as a base except those involving items 1, 2, and 3 which were made with distilled water. Media prepared with items 4, 11, 14, 15, and 16 were not differentiating and were not employed beyond preliminary experiments. Inocula for all tests had been grown for 2 weeks on Bristol's agar slants under standard conditions. Solid media were streaked directly in inoculation using a wire loop, an effort being made to introduce uniform amounts of inoculum. The inocula for liquid tests were washed from the agar slant into a tube of sterile distilled water, broken up with a di SON tegrator (system 80—Ultrasonic Industries, Inc., Albertson, N.Y.), and introduced into the test vessel by means of a sterile, disposable, glass-transfer pipette (Clay-Adams, Inc., New York 10, N.Y.). Test media 1–4 were used in small tubes (13 × 100 mm) and received 5 drops of inoculum. Test media 5–11 were dispensed into large tubes (18 × 150 mm) and received 10 drops of inoculum. All tests were observed 2 weeks after inoculation with apparent amounts of growth being described as excellent, good, fair, trace, or none. These were determined on the basis of arbitrary standards recorded on Kodachrome slides for repeated reference.

Bristol's solution was used throughout the investigation not only for routine cultivation of stock cultures but also as a control in most physiological tests. Media made with items 1, 2, and 3, originally used to test stock cultures for purity, were later employed as differential test media.

Crystal violet has long been used by bacteriologists as a selective bacteriostatic agent. Preliminary experiments with this compound during the present investigation have indicated that it may be useful as a differentiating agent with these and other algae.

Starch agar was employed to test for the production of extracellular enzymes. The test was performed by streaking the organisms on the surface of a 0.01%

starch-agar plate. After 2 weeks' incubation, the plates were flooded with aqueous I_2-KI and observed for the presence or absence of a clear halo around the colony. Although the results are most often striking, they have not yet been completely reproducible. The explanation of these inconsistencies is not yet available.

Finally, each organism investigated has been tested for susceptibility to each of 43 different antibiotic agents. This was done by using Multidisk Sensitivity Discs obtained from Consolidated Laboratories, Inc., Chicago Heights, Illinois. To perform the test, 7 Petri dishes were seeded with a heavy suspension of the alga to be tested and mixed with sterile, cooled-but-liquid 1.5% Inoagar (Oxoid, Consolidated Lab.). One of the 7 Multidisks (Fig. 53) was placed on each plate. The tests were read 2 weeks after inoculation; only definitely clear zones of inhibition were recorded as positive tests. A list of the antibiotic agents used and their concentrations are given in Table 2. The agents will hereafter be referred to by their abbreviations.

Taxonomic History

The genus *Ulothrix* was established by Kützing in 1833 with the publication of a description for *U. tenuissima*. In the same paper, Kützing also transferred 4 species from the genus *Conferva* L. to the new genus. The following description was given for *Ulothrix:*

"Fila (simplica, affixa) sine muco matricali, attenuata, rigidiusculis, tranquilla, articulata, geniculis annularibus, annulis remotiusculis. Massa sporacea interna plerumque in fascias vel globulos collabens."

In 1863, Rabenhorst listed 16 species of *Ulothrix,* but by 1868 he had abandoned that generic name in favor of *Hormiscia* Fries (1835). DeToni (1889) continued the use of the name *Hormiscia* and included in his description of the genus the facts that the plastid is parietal and laminate and that reproduction may be by means of quadriflagellate macrozoospores or by the fusion of biflagellate "microzoospores."

The very careful study of Hazen (1902) clearly established the validity of the generic name *Ulothrix* and indicated the sense in which the name *Hormiscia* should be employed. Furthermore, the description which Hazen gave for the genus *Ulothrix* remains in use today.

Fritsch (1935) gave a quite complete account of the structure and reproduction of *Ulothrix* but pointed out the confusion which existed in the taxonomy of ulotrichacean algae at that time. Such relatively recent authorities as Smith (1950) and Prescott (1951) have continued to employ the generic name *Ulothrix* in essentially the sense of Hazen, so that the genus may be said presently to include those unbranched, filamentous Chlorophyceae with uninucleate cells and parietal band or girdle-shaped plastids with 1 or more pyrenoids. Asexual reproduction in the genus is by fragmentation or by means of quadriflagellate zoospores;

TABLE 2. *Agents used in testing comparative sensitivity of certain ulotrichacean algae*

Code No.	Agent	Abbr	Concentration
11–102A	Chlortetracycline	(A+)	30 μg
	Amphotericin B	(AB+)	100 μg
	Bacitracin	(B+)	10 units
	Chloramphenicol	(C+)	30 μg
	Carbomycin	(CA+)	15 μg
	Colistin Sulfate	(CS+)	10 μg
11–102B	Demethylchlortetracycline	(D+)	30 μg
	Erythromycin	(E+)	15 μg
	Kanamycin	(K+)	30 μg
	Neomycin	(N+)	50 μg
	Novobiocin	(NV+)	30 μg
	Nystatin	(NS+)	100 units
11–102C	Oleandomycin	(OL+)	15 μg
	Penicillin	(P+)	10 units
	Polymyxin-B	(PB+)	300 units
	Paromomycin	(PM+)	300 μg
	Ristocetin	(R+)	30 μg
	Syncillin	(SY+)	10 μg
11–102D	Cycloserine	(CY+)	30 μg
	Dihydrostreptomycin	(S+)	10 μg
	Staphcillin	(SC+)	30 μg
	Oxytetracycline	(T+)	30 μg
	Triacetyloleandomycin	(TAO+)	15 μg
	Tetracycline	(TE+)	30 μg
11–102E	Sulfisomidine	(EL+)	300 μg
	Sulfisoxazole	(G+)	300 μg
	Sulfamethoxypyridazine	(KY+)	300 μg
	Triclobisonium Chloride	(TR+)	1 μg
	Viomycin	(V+)	10 μg
	Vancomycin	(VA+)	30 μg
11–102F	Sulfadimethoxine	(MA+)	300 μg
	Sulfadiazine	(SD+)	300 μg
	Sulfamerazine	(SM+)	300 μg
	Triple Sulfa	(SSS+)	300 μg
	Sulfathiazole	(ST+)	300 μg
	Thiosulfil	(TH+)	300 μg
11–102G	Furaltadone	(AL+)	100 μg
	Nitrofurantoin	(F+)	100 μg
	Nitrofurazone	(FC+)	100 μg
	Furazolidone	(FR+)	100 μg
	Iso Nicotinic Acid Hydrazide	(IH+)	25 μg
	Methenamine Mandelate	(M+)	2.5 μg
	Para Amino Salicylic Acid	(PS+)	100 μg

sexual reproduction, when present, is by the fusion of biflagellate isogametes.

The genus *Uronema* Lagerheim, erected in 1887, has been considered suspect by most phycologists almost from the time of its origin. DeToni (1889) separated *Uronema* from *Hormiscia* (*Ulothrix*) on the basis of the attenuate apical cell of the former but considered it a questionable distinction. It should be noted that Kützing's original description of *Ulothrix* included, "Fila . . . attenuata." However, it is not clear to which part of the filament this referred.

Hazen (1902) did not include *Uronema* in his work, no doubt because no species had been described from the United States at that time. Gaidukow (1903) reported that, at times, *Ulothrix flaccida* var. *genuina* Hansgirg can develop filaments with pointed ends, and, therefore, decided that *Uronema* should not be considered a valid genus. Heering (1914), however, did not consider this report free of doubt and retained the genus, distinguishing it from *Ulothrix* on the basis of filament length and shape of the terminal cell. Printz (1927) gave a brief description of *Uronema* in an appendix to his section on the Ulotrichaceae but noted that: "Es bedarf weitere Untersuchungen, um festzustellen, ob die Gattung *Uronema* aufrechtzuerhalten ist, oder ob sie eine Sektion von *Ulothrix* darstellt." Similarly, Fritsch (1935) doubted that *Uronema* could be maintained as a separate genus because all described species, except *U. confervicolum*, lack 1 or more of the distinctive attributes.

However, Pankow (1960) has carefully reviewed the literature concerning *Uronema*, has given a full discussion of the controversy in which the genus has been involved, and has decided that the genus with its 6 species should be retained. He considers as distinctive attributes the discoidal holdfast produced by a basal cell which retains its chromatophore and which may take part in zoosporogenesis and the irregularly limited and lobed chromatophore bands, which are often perforate. Thus, the most recent worker on the subject has concluded that the genus should be retained.

The histories of the genera *Hormidium* Kützing and *Stichococcus* Nägeli are so interrelated that it seems best to present them together. *Hormidium* was first named by Kützing in Linnaea (1843a) and contained 3 species. Later that year, Kützing listed the same 3 species and published descriptions for them in his *Phycologia Generalis*. In *Phycologia Germanica* (1845), he added 3 more species to the genus, but in his *Species Algarum* (1849) he removed the first 3 species to other genera.[4] The 3 species described in 1845, *H. murale*, *H. parietinum*, and

[4] Strict application of the International Code of Botanical Nomenclature (Art. 64, 1961) would require that the name *Hormidium* be rejected as a later homonym. However, Article 14 of the Code provides for exceptions to the strict adherence to the rules. According to that article, names which have been in general use in the 50 years following their publication may be retained as *nomina conservanda*. A proposal, therefore, will be made that the name *Hormidium* Klebs 1896 be conserved and the name *Hormidium* Kützing 1843a be rejected. Personal communication with Dr. P. C. Silva indicates that such a proposal is reasonable.

H. crenulatum, remained the basis of the genus until 1891. They were recorded by Hansgirg (1888) and DeToni (1889) as the only valid species in the genus. However, Gay (1891) concluded that all of the aerial, filamentous algae which had been included in the genus *Ulothrix* should be separated and attributed to 2 genera: *Schizogonium* and *Stichococcus*. She also included in *Stichococcus* those forms which had been in *Hormidium* and classified *Stichococcus* in the Protococcaceae.

Stichococcus had been erected by Nägeli in 1849 with the publication of a description for *S. bacillaris*. Nägeli (1849), Rabenhorst (1863), and DeToni (1889) classified *Stichococcus* in the Palmellaceae, but Hansgirg and Borzi (1890) considered *S. bacillaris* to be only a form of *Ulothrix flaccida*. This seems to be the first recognition of the affinities of *Stichococcus* with the Ulotrichaceae.

When Klebs (1896) found zoospores in *Stichococcus flaccidus* (Kütz.) Gay, he revived the name *Hormidium* to accommodate those species of *Stichococcus* which produce motile cells. Hazen (1902) agreed with Klebs that the forms then included in the genera *Hormidium* and *Stichococcus* belonged in the Ulotrichaceae, but he followed Gay in abandoning the generic name *Hormidium*. Thus, Hazen included in *Stichococcus* both forms that do and those that do not produce motile cells and forms that have and those that do not have pyrenoids.

However, Hazen's treatment of these genera has not been generally accepted. Heering (1914) retained both *Hormidium* (with 1 pyrenoid/cell) and *Stichococcus* (without pyrenoids). Printz (1927) also included *Hormidium* (with biflagellate zoospores) and *Stichococcus* (without zoospores) in the Ulotrichaceae. Fritsch (1935), Smith (1933, 1950), and Prescott (1951) all included both genera in the family. Criteria used by these authorities to distinguish between the 2 genera are given below (comparable criteria are numbered similarly):

Hormidium:

1. With 1 pyrenoid/cell and biflagellate zoospores ..... Fritsch (1935)
2. Chloroplast ½ cell length ..... Smith (1933)
3. Filaments more than 10 cells long, without basal cell ..... Smith (1950)
4. Chloroplast ½ cell length ..... Prescott (1951)

Stichococcus:

1. Without pyrenoid and without motile cells ..... Fritsch (1935)
2. Chloroplast extending whole cell length ..... Smith (1933)
3. Filaments never more than 10 cells, ends not pointed ..... Smith (1950)
4. Chloroplast as long as the cell or nearly so ..... Prescott (1951)

In summary, it is clear that the limits of the genera to be considered in this investigation are not well defined. Concerning the taxonomy of the Ulotrichaceae, Fritsch (1935) has said:

> . . . it remains doubtful whether *Uronema* can really be regarded as an independent genus . . . The other Ulotrichaceae are even less completely studied. *Hormidium* . . . is

often confused with *Ulothrix*, and in the present state of our knowledge the exact limits of the two genera are difficult to define.

Smith (1950) concluded his treatment of the family Ulotrichaceae with the following statement:

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

As previously stated, it has been a principal objective of this investigation to provide information regarding the precise limits of the genera considered.

Observations and Experimental Results

Approximately 100 unialgal cultures, 39 of them axenic (Table 1), have been studied during the present investigation. This study has revealed that several attributes of these organisms, formerly considered taxonomically valid at the generic level, are not constant and, therefore, not reliable for taxonomic purposes. The investigation has also shown that certain other attributes of the organisms being considered are constant and taxonomically useful. These will be discussed later.

In addition to living material, a representative number of herbarium specimens from the Chicago Natural History Museum have been examined. In many cases, however, the critical generic attributes are not preserved in dried specimens (Fig. 1–8). Therefore, an exhaustive survey of the many hundreds of such specimens available was not attempted. Much of the material preserved on slides by the late Dr. Tracy Hazen was also examined, but this, although better preserved than dried material (Fig. 4), does not display all of the generic attributes, especially the dynamic ones. It must be said, then, that nearly all of the information provided by this study has been gained by comparative examination of living material, all of it grown in unialgal culture, much of it in axenic culture.

Of the genera here considered, *Hormidium* and *Stichococcus* have most often been confused. Hazen (1902) abandoned the name *Hormidium* and included in *Stichococcus* all forms which previously had been in both genera. Later phycologists, however, have continued to use both names and have employed the attributes of filament length, plastid length relative to cell length, and degree of filament dissociation to separate the 2 genera. All of the above characteristics are so variable, in the writers' experience, as to be not useful in distinguishing genera.

In clonal cultures of *Hormidium* and of *Stichococcus*, filament length can vary from 2 to an almost indefinite number of cells (Fig. 44, 46). Similarly, in clonal cultures of species of the 2 genera, the length of the plastid relative to the length of the cell can also vary from less than ½ to cell length (Fig. 40, 48).

Finally, while it is generally true that filaments of *Stichococcus* dissociate more readily than those of *Hormidium*, it is not always true that filaments of *Stichococcus* will be short and those of *Hormidium* long. Filaments of 50 cells are not uncommon in *S. bacillaris* (Fig. 46), but such long filaments are rare in *H. sterile* (Fig. 44).

The genus *Uronema* has been a controversial one almost since its first characterization by Lagerheim (1887). Some phycologists consider it to be valid, while others believe it should be treated as a section under the genus *Ulothrix*. The present investigation supports the belief that *Uronema* should be included within the genus *Ulothrix*.

The shape of the apical cell, filament length, and characteristics of the holdfast and basal cell are attributes that have been employed to distinguish *Uronema* from *Ulothrix*. In the literature, *Uronema* has been said to have a pointed apical cell, filaments of limited growth (Smith, 1950), and peculiar disc-like holdfasts. However, here again clonal cultures have shown clearly that some germlings have pointed apical cells and others rounded apical cells (Fig. 11, 28). All of the writers' cultures of *Ulothrix* and *Uronema* show some degree of filament dissociation, but there certainly is no consistent and/or genetic limit to the length of filaments (Fig. 16). *Ulothrix* and *Uronema* are alike in having quadriflagellate zoospores that produce holdfasts upon germination. While it is true that there are differences in the structure of the holdfasts, these seem to be of degree, not of kind. Other evidence, to be presented later, also supports the contention that those organisms up to the present included in *Uronema* should really be in the genus *Ulothrix*.

Thus, this investigation has shown that several generic-level attributes of the organisms considered are not useful or reliable taxonomically, even though some authorities have thought them to be so. More important in a positive sense, however, is the fact that this investigation has shown that a number of other characteristics of these organisms are consistent and, therefore, taxonomically useful. It is now possible to divide these organisms into 3 genera, namely, *Ulothrix, Hormidium*, and *Stichococcus*. Using more subtle attributes, both morphological and physiological, it has been possible to distinguish a number of species within each genus from among the cultures available. These genera and species will now be discussed in some detail.

I. THE GENUS ULOTHRIX KÜTZING EMEND.

The nomenclatural validity of the name *Ulothrix* is certainly now accepted by all phycologists. The questions to be considered here are: (1) Should *Uronema* be maintained as a separate genus or combined with *Ulothrix?* (2) What are the generic limits of *Ulothrix?*

The most recently published description of a new species of *Uronema* appears

to be that by Mitra (1947). That paper contains a very thorough account of the structure and reproduction of an organism isolated to unialgal culture from Indian soil. In addition, considerable attention is given to the taxonomic disposition of the organism. Mitra states that, in view of facts elucidated by the use of culture techniques, the only attributes which separate *Uronema* from *Ulothrix* are the acuminate apical cells and the distinctive holdfasts of the former, but adds that in filaments derived from aplanospores or akinetes (of *Uronema terrestre* Mitra, particularly), both of these attributes are lacking. Considering this observation, Mitra's conclusion that the genus *Uronema* should be retained seems illogical.

As Mitra mentions, many authorities (Gaidukow, 1903; Oltmanns, 1904; Wille, 1909; and Fritsch, 1935) have doubted the validity of *Uronema*. Observations presented earlier in this contribution, as well as observations of other workers, indicate that there are no consistent morphological attributes which are useful in separating the 2 genera. Furthermore, isolates of the 2 genera are quite similar physiologically when compared in axenic culture. In the writers' opinion, the 2 genera should, therefore, be combined under *Ulothrix*.

As mentioned earlier, it is possible to separate the organisms presently being investigated into 3 groups or genera. The attributes which are especially useful in making this separation are the plastid morphology, the number of pyrenoids in each cell, the presence or absence of holdfasts, and the characteristics of the motile cells, if any are produced. Considering these attributes, the generic description of *Ulothrix* should be further emended to exclude those organisms with: (1) plate-shaped plastids that have but a single pyrenoid and are not lobed along the longitudinal margins; and (2) biflagellate zoospores that secrete no holdfast upon germination. The writers' concept of the genus *Ulothrix* is as follows:

Ulothrix Kützing, 1833 (including *Hormiscia* Fries, 1835, and *Uronema* Lagerheim, 1887).

Filaments of this genus are normally unbranched, always uniseriate, and consist of uninucleate cells with parietal, band-like or girdling plastids that encircle all, or nearly all, of the cell lumen and typically contain more than 1 pyrenoid. Incompletely encircling plastids are lobed along the longitudinal margins. Filaments lack a gelatinous sheath.

Asexual reproduction may be vegetative by fragmentation or by the production of akinetes, aplanospores, or quadriflagellate zoospores which always secrete a holdfast upon germination. Sexual reproduction involves the fusion of biflagellate isogametes (Dodel, 1876; Klebs, 1896; Grosse, 1931).

Six species of *Ulothrix*, 3 of them new, have been studied intensively in the present investigation. These will be discussed individually in the following account. Tables listing supplementary attributes of these organisms and a key to these 6 species will be included following the individual accounts of these species.

ULOTHRIX **gigas** comb. nov. (Fig. 11–15)

(*Uronema gigas* Vischer)

A single culture of this species, apparently isolated and named by Vischer himself, was obtained from the Culture Collection of Algae at Indiana University (no. 174, Starr, 1960) and studied in axenic culture. It conforms closely to Vischer's original description (1933), although cell size is somewhat different. A description of the writers' material follows.

A 1-week-old culture, grown in liquid Bristol's solution, is composed mostly of rather short germlings, many of which have apiculate apical cells, a characteristic attribute of the organism (Fig. 14). The basal cell tapers slightly to a hyaline holdfast which is composed of a flat disc subtended by a hemispherical bulbous portion (Fig. 13). Individual cells are 5–10 μ in diameter and 8–20 μ in length. Each has a band-shaped plastid which encircles all, or nearly all, of the cell lumen and contains 2 or more pyrenoids (Fig. 12). The plastid, which is lobed along the longitudinal margins when the band is incomplete, extends to, or nearly to, the terminal walls (Fig. 12).

Quadriflagellate zoospores are formed singly within the cells and escape through a pore in the lateral wall (Fig. 15). Although they may change shape somewhat while emerging, the zoospores do not become spherical at quiescence. Each has an anterior stigma, a median nucleus, and a cup-shaped plastid which is often deeply incised and lobed, and contains 1–several pyrenoids. The apiculate, posterior pole of the zoospore becomes the apiculate apical cell of the germling, while the anterior pole of the zoospore secretes the holdfast. The stigma may often be seen in the basal cell of 3- or 4-celled germlings.

Filaments from 1-month-old Bristol's liquid cultures may be indefinitely long and rarely have slight constrictions at the cross walls. Grown for 2 weeks in axenic culture on Bristol's agar, this organism has a green colony with a moist, wavy surface. After 1 month, most of the colony is still green, but it has a thinner, yellow-green margin. The surface is dry and wavy. The apiculate apical cell of most germlings of *U. gigas* distinguishes it from all other species of *Ulothrix* here considered except *U. belkae*. *Ulothrix gigas*, however, lacks the thick-walled, akinete-like and dumbbell-shaped cells of *U. belkae*.

ULOTHRIX **minuta** sp. nov. (Fig. 16–21)

This organism was obtained from the Culture Collection of Algae at Indiana University (no. 330, Starr, 1960). It is there labeled *Uronema barlowii*, but Dr. Pringsheim, the author and isolator, considers the name dubious (personal communication), and the writers have found no published description. The name therefore, appears to be a nomen nudum. Pankow (1960), in his careful review of the literature of *Uronema*, makes no mention of *U. barlowii*. The name is, there-

fore, probably invalid. Because this isolate is unlike any previously described species of *Ulothrix* or *Uronema*, it is here described as a new species.

Transfer of this organism to fresh, liquid culture medium (Bristol's) induces prolific zoosporogenesis, so that a 1-week-old culture is composed mostly of germlings that usually have acuminate, but *never* apiculate, apical cells (Fig. 19). The basal cell tapers slightly to a holdfast very similar to that of *U. gigas* (Fig. 18). Individual cells are 5–6 μ in diameter and 4–6 times that long. Each has a single, parietal, band-shaped plastid that encircles all, or nearly all, of the cell lumen, and extends to, or nearly to, both terminal walls. Two–several pyrenoids are present in each cell (Fig. 18).

The quadriflagellate zoospores are nearly always produced singly by each cell (Fig. 21), although, rarely, a cell may produce 2. The zoospores, which vary in shape, escape through pores in the lateral walls; the latter then deliquesce leaving only the end walls, with very short segments of the adjacent lateral walls as remnants of the filament (Fig. 20). This behavior of the cell walls at zoosporogenesis is uniquely characteristic (among the species studied) of *U. minuta*. Each zoospore has a large anterior-to-median stigma, a median nucleus, and a cup-shaped plastid which is often deeply incised and contains 1–several pyrenoids. The zoospore settles on its anterior pole which secretes a gelatinous holdfast. It is the shape of the posterior pole of the zoospore which determines the shape of the apical cell of the germling.

Filaments from a 1-month-old culture usually have slight constrictions at the cross walls (Fig. 17) and may be indefinite in length. The colony of this organism is similar at all ages to that of *U. gigas*. Of the species here studied, *U. minuta* is most like *U. acuminata*. It is, however, smaller in diameter than *U. acuminata* and has zoospores with equatorial nuclei and anterior-to-median stigmata, while the zoospores of *U. acuminata* have anterior nuclei and median stigmata.

Ulothrix **acuminata** sp. nov. (Fig. 22–27)

Six different isolates of this organism have been studied in axenic culture. The sources of these isolates were a soil sample taken from an oak woods in Williamson County, Texas (f-10); the leaf of a *Myriophyllum* plant in Lake Travis, Texas (f-24); the leaf of an *Elodea* plant growing in a lily pool at Barton Springs Park, Austin, Texas (f-26); and rocks in Sandy Creek at the foot of Enchanted Rock, Llano County, Texas (f-30, 31, & 32). Careful examination of these 6 isolates has convinced the writers that, in spite of their diverse origins, they represent a single species, and they are here described as the new species, *Ulothrix acuminata*, since they do not correspond to any previously described species.

Filaments from a 1-week-old liquid Bristol's culture are usually not more than 10 cells long (Fig. 22). Apical cells of germlings may be rounded but are most often acuminate (Fig. 24). They are *never* apiculate. Basal cells secrete a gelatinous

holdfast composed of a thin, disc-like layer and a hemispherical portion below (Fig. 23). The plastid is a parietal band, lobed along its longitudinal margin, which incompletely encircles the cell lumen and contains 2 or more pyrenoids (Fig. 23). Individual cells range from 6 to 9.5 μ in diameter and are 1–4 times that long, depending on the interval since cell division.

Asexual reproduction in this organism may be vegetative by fragmentation, or by the production of quadriflagellate zoospores (Fig. 26) which are formed singly within the cells and escape through a pore in the lateral wall (Fig. 27). The zoospores are usually ellipsoidal and have a longitudinally split, cup-shaped plastid, a median stigma, and an anterior nucleus which lies within a hyaline area. The stigma can often be seen in the basal cell of 3- or 4-celled germlings.

Grown for 30 days in a flask of liquid Bristol's, the filaments may become indefinitely long with slight constrictions at the cross walls (Fig. 25) and with cellular details often obscured by a heavy accumulation of starch (Fig. 25). Two-week-old, axenic colonies on Bristol's agar are dark green and have dry, wavy surfaces. One-month-old colonies may or may not develop a lighter, yellow-green margin. As previously mentioned, among the species of *Ulothrix* studied during this investigation, *U. acuminata* is most similar to *U. minuta*. It differs from *U. minuta* in the respects listed following the description of that species as well as in its growth rate in several of the media employed in physiological tests, especially sodium acetate, arabinose, A.C. broth, and nutrient broth.

ULOTHRIX FIMBRIATA Bold. (Fig. 9, 19)

This species has recently been carefully described from cultured material (Bold, 1958). All of the writers' observations have been made on a subculture from the type culture obtained from the Culture Collection of Algae at Indiana University (no. 638, Starr, 1960). The culture at hand agrees in all respects with the original description.

The fimbriate plastid, which completely encircles the cell lumen and usually contains several pyrenoids (Fig. 10), is the most distinguishing feature of the species. As a result of the present investigation, additional physiological attributes are now known for *U. fimbriata,* and these are given in the accompanying tables. Grown for 2 weeks on Bristol's agar in axenic culture, the organism has a green colony with a moist, wavy surface (Fig. 9). A bright-orange color, occasioned by the presence of accessory pigments in the thick-walled hypnospores, develops at the margin of the colony by the end of 1 month. This attribute, along with the formation of hypnospores, also serves to distinguish *U. fimbriata* from all other species of *Ulothrix* studied in the present investigation.

ULOTHRIX **belkae** sp. nov.[5] (Fig. 28–32)

This organism was isolated into axenic culture from a stream that runs through

[5] The writers consider it a privilege to name this organism in honor of Professor Ethel C. Belk of Miami University, Oxford, Ohio.

Hancock Park, Austin, Texas. Because it is unlike any previously described species of *Ulothrix*, it is here described as a new taxon.

Filaments of this species, as is the case with all other species of *Ulothrix* here investigated, may be indefinite in length. Cell diameter increases with the age of a filament from 5 to 11.5 μ, cells of the older, broader filaments being relatively shorter (Fig. 31). In the younger, narrower filaments, the plastid incompletely encircles the cell lumen and is lobed along the longitudinal margins (Fig. 28), while in the older, wider filaments the plastid is a complete band. In both cases, the plastid reaches the terminal cell walls and contains 2 or more pyrenoids.

Quadriflagellate zoospores are produced singly within the cells and escape through a pore in the lateral wall. Each has an anterior stigma, a median nucleus, and produces a holdfast at the anterior pole upon germination. The zoospores have cup-shaped plastids which are often deeply incised and contain 1–several pyrenoids. Apical cells of germlings may be rounded, broadly or narrowly pointed, or even apiculate (Fig. 29). Holdfasts (Fig. 29) are also variable in form, but they do not have the disc and bulb organization of many other species of the genus.

Filaments from a 1-month-old or older culture may have markedly thickened cell walls. Thick-walled, akinete-like cells may also develop in filaments of this age (Fig. 32). It is also not uncommon to see cells, in filaments from old cultures, which are narrower at the center than at the ends (Fig. 30). Of the species of *Ulothrix* examined during the present investigation, these unique, dumbbell-shaped cells have been observed only in this species. Filaments from old cultures may also have constrictions at the cross walls (Fig. 31).

A 2-week-old colony on Bristol's agar is dark green with a dry, wavy surface. The colony has the same appearance at 1 month. *Ulothrix belkae* is unique, among the species of *Ulothrix* studied, in having the characteristic, dumbbell-shaped and thick-walled akinete-like cells in filaments from 1-month-old or older cultures.

ULOTHRIX **confervicola** comb. nov.

(*Uronema confervicolum* Lagerh.)

A single isolate of this organism was obtained from the Culture Collection of Algae at Indiana University (no. 331, Starr, 1960). The material agrees with Lagerheim's original description (1887) with the exception that filaments may become indefinitely long. In conformity with the writers' conclusion that the genus *Uronema* should be abandoned, this species is now given the new name *Ulothrix confervicola.*

This species has filaments which may be indefinite in length, and it is unique among the species of *Ulothrix* here investigated in having very little, if any, vegetative reproduction by fragmentation. Cells are 5–6 μ in diameter and 2–3 times that long, with parietal, band-shaped plastids that encircle somewhat less than the entire cell lumen and usually contain 2 pyrenoids. Apical cells are nearly always

acuminate; basal cells taper slightly to a disc-like holdfast which does not have a hemispherical portion below.

The quadriflagellate zoospores, which are rarely produced, do not form a phototactic ring at the surface of the culture medium. In fact, a culture of this species growing in a flask of liquid Bristol's solution produces a characteristic mat of filaments on the bottom of the culture vessel. It has not been possible, unfortunately, to observe enough zoospores to adequately characterize them here.

One-month-old filaments are very long and have no constrictions at the cross walls. There is usually very little starch accumulation in 1-month-old filaments, and cell walls do not thicken in filaments of that age. A 2-week-old colony on Bristol's agar is green with a dry, wavy surface. It has the same appearance at 1 month.

ULOTHRIX sp.

Culture no. 420 (Starr, 1960), labeled *Uronema terrestre* Mitra, from the Culture Collection of Algae at Indiana University, has also been studied during this investigation. Because it differs in so many respects from Mitra's original description of *Uronema terrestre,* it cannot be considered an isolate of that species. However, it has not been possible for the writers to completely characterize this isolate because of the extreme difficulty with which zoosporogenesis is evoked. Although this isolate is unlike any other species of *Ulothrix* here examined or previously described, it is not described as a new species because it has not yet been completely characterized. Final disposition of the organism must await further investigation.

In contrast to cells of *Uronema terrestre*, which are only 3–5 μ in diameter, cells of this isolate are 5.5–9.0 μ in diameter and 1–4 times that long. They have parietal, band-shaped plastids which encircle all, or nearly all, of the cell lumen, contain 1–several pyrenoids, and extend from 1/3 to the entire cell length.

Reproduction by fragmentation is common in this isolate but zoospores are very rarely produced, again in sharp contrast to *Uronema terrestre,* as described by Mitra. In a liquid culture of this organism, there is never a phototactic ring at the surface, but, instead, all filaments occur in a tufted mat on the bottom of the culture vessel. Of the species of *Ulothrix* examined during this investigation, only *U. fimbriata, U. confervicola,* and this isolate fail to produce phototactic rings at the surface of liquid flask cultures. *Ulothrix fimbriata* is easily distinguished from the other two by its fimbriate plastid and bright-orange pigmentation in 1-month-old colonies. *Ulothrix confervicola* is distinguished from this isolate by having cells 5–6 μ in diameter and in lacking vegetative reproduction by fragmentation.

Results of experiments performed with these species of *Ulothrix* are summarized in Tables 3–6.

During the early part of this investigation, media with the organic carbon sources (Table 3) were used in an attempt to differentiate the taxa (Bold and Parker, 1962) and were prepared in the following manner. A 0.5% Bristol's

TABLE 3. *Growth of Ulothrix species in Bristol's solution supplemented with various carbon sources*[a]

Organism	Glucose	Na Acetate	Arabinose	Ribose	Fructose	Xylose
U. acuminata						
f-10	good[b]	trace	good	good	good	good
f-24	good	fair	good	good	good	good
f-26	good	fair	good	good	good	good
f-30	good	trace	fair	good	good	good
f-31	good	fair	good	good	good	good
f-32	good	trace	fair	good	good	good
U. fimbriata	trace	none	trace	none	trace	trace
U. minuta	excel	excel	trace	good	good	fair
U. confervicola	fair	fair	fair	fair	fair	trace
U. gigas	fair	none	trace	fair	good	good
U. belkae	good	good	fair	good	good	good
U. sp.	good	trace	trace	good	good	fair

[a] All at 0.5% concentration in Bristol's solution.

[b] The apparent amount of growth is described as none, trace, fair, good or excellent on the basis of arbitrary standards as recorded on Kodachrome slides.

TABLE 4. *Growth of Ulothrix species in/on several undefined, differential media*

Organism	A.C. broth	Thioglycollate broth	Nutrient broth	0.01% starch agar[a]
U. acuminata				
f-10	none	trace	trace	halo
f-24	none	trace	fair	halo
f-26	none	trace	trace	halo
f-30	none	fair	trace	halo
f-31	none	fair	trace	halo
f-32	none	trace	trace	halo
U. fimbriata	trace	trace	trace	halo
U. minuta	good	fair	good	halo
U. confervicola	none	none	good	halo
U. gigas	trace	trace	good	halo
U. belkae	trace	trace	fair	halo
U. sp.	trace	trace	trace	halo[b]

[a] After the organisms had grown for 2 weeks on the surface of the starch agar, the surface was flooded with aqueous I_2-KI. The presence of a clear zone around the colony, an evidence of extracellular starch digestion, is recorded as a halo.

[b] Slight.

solution of each compound was prepared, tubed, and autoclaved for 15 min. After cooling, the tubes were inoculated and incubated under standard conditions for 2 weeks. Observations of apparent amounts of growth were then recorded. It became apparent, after these tests had been repeated 3 or 4 times, that the results were only about 75% reproducible. Therefore, 2 experiments were devised in an attempt to determine the possible causes of the observed inconsistencies.

To make certain that traces of detergent ("Heikol," Heinicke Co.) in the culture tubes, left by inadequate rinsing, were not responsible for the inconsistencies, 40 tubes were carefully cleaned and dried. These were then divided into 4 sets of 10 tubes each. One set was filled with Bristol's solution as a control. The second set was filled with Bristol's + 100 ppm detergent; the third set received Bristol's + 10 ppm detergent; and the fourth set received Bristol's + 1 ppm detergent. Ten representative algae were then selected, and each was inoculated into 1 tube from each set. After 2 weeks, incubation under standard conditions, it was apparent that all organisms had grown at about the same rate in all 4 tubes. It was concluded, therefore, that detergent contamination was not responsible for the observed inconsistencies.

It was also suspected that the method of sterilization of the carbon sources might have some effect upon the growth of the algae (Finkelstein and Lankford, 1957), and a different method of sterilization was tried. By this method, the clean, dry tubes were plugged and sterilized in a dry oven. At the same time, a concentrated distilled-water solution of the carbon source and enough Bristol's solution to fill 40 tubes (20-ml/tube) were autoclaved for 15 min in separate flasks. After cooling, the 2 solutions were mixed, and the mixture was introduced aseptically into the tubes. The latter were allowed to incubate at room temperature for 1 week, contaminated tubes being discarded before inoculation. With certain combinations of algae and carbon sources, this method of sterilization gave results somewhat different from the earlier method of sterilization. All work to date also indicates that these results are repeatable. Therefore, in later tests this method of sterilization was used exclusively. The significance of these experiments with respect to the taxonomy of representatives of the genus *Ulothrix* will be considered in the *Discussion* section of this paper.

Key to the species of *Ulothrix* studied

1. Cell walls thickening with age, especially to form thick-walled, akinete-like cells; hypnospores sometimes present 2
1. Cell walls never thickening with age 3
   2. Plastids with fimbriate margins; 1-month-old colony with bright-orange hypnospores ..... *U. fimbriata* Bold.
   2. Plastids without fimbriate margins; 1-month-old colony remaining entirely green ..... *U. belkae* sp. nov.

3. Filaments never with constrictions at the cross walls; zoospores infrequent under standard conditions of culture, not producing a phototactic ring in liquid culture ..... *U. confervicola* comb. nov.
3. Filaments developing at least slight constrictions at the cross walls by 1 month; zoospores abundant, always producing a phototactic ring in liquid culture 4
   4. Apical cells of germlings *often* apiculate ..... *U. gigas* comb. nov.
   4. Apical cells of germlings *never* apiculate 5
5. Zoospores with an anterior stigma and median nucleus; cells of filaments 5–6 μ in diameter ..... *U. minuta* sp. nov.
5. Zoospores with a median stigma and anterior nucleus; cells of filaments 6–9.5 μ in diameter ..... *U. acuminata* sp. nov.

The following brief descriptions, which indicate the distinguishing attributes of the species of *Ulothrix* studied during this investigation, are presented as a supplement to the above key. For more complete description see the text, pages 20–24.

ULOTHRIX FIMBRIATA Bold.

Filaments indefinite in length, attached or free-floating; cells 10–12 $\mu \times$ 24–106 μ, somewhat tumid; apical cell variable in form, not apiculate; fimbriate plastid distinctive, usually containing several pyrenoids.

Quadriflagellate zoospores borne singly, 10 $\times$ 14 μ in size, escaping through lateral pores, attachment at anterior pole. Thick-walled, orange-pigmented hypnospores in older filaments; 2-week colony green, moist, undulate; bright-orange pigmentation appearing at 1 month.

TABLE 5. *Growth of Ulothrix species on different concentrations of crystal-violet agar*

Organism	1:10,000	1:25,000	1:50,000	1:100,000
U. acuminata				
f-10	trace	trace	trace	fair
f-24	trace	trace	trace	trace
f-26	trace	trace	trace	fair
f-30	trace	trace	trace	trace
f-31	trace	trace	trace	trace
f-32	trace	trace	trace	trace
U. fimbriata	trace	trace	trace	trace
U. minuta	trace	trace	trace	trace
U. confervicola	trace	trace	trace	trace
U. gigas	trace	trace	trace	trace
U. belkae	trace	trace	trace	trace
U. sp.	trace	trace	trace	trace

TABLE 6. *Comparative sensitivity of Ulothrix species to several antibotic agents*[a]

Organism	AB+	CS+	K+	N+	PB+	S+	AL+	FC+	FR+	IH+	PS+
U. acuminata											
f-10	+	+	....	+	+	....	+	....	+	....	+
f-24	....	+	....	+	+	....	+	+	+	+	+
f-26	....	+	....	+	+	....	+	....	+	....	+
f-30	....	+	....	+	+	....	+	....	+	....	+
f-31	....	+	....	+	+	....	+	+	+	+	+
f-32	....	+	....	+	+	....	+	+	+	+	+
U. fimbriata	+	+	....	+	+	+	+	+	+	+	+
U. minuta	....	+	....	+	+	....	+	+	+	+	+
U. confervicola	....	+	....	+	+	....	+	+	+	+	+
U. gigas	+	+	....	+	+	....	+	....	+	....	+
U. belkae	....	+	....	+	+	....	+	+	+	+	+
U. sp.	+	+	....	+	+	....	+	+	+	+	+

[a] Of 43 antibiotic agents tested, only these 11 were differential with respect to the organisms studied; + indicates a definitely clear zone of inhibition as indicated in Fig. 53.

Represented by culture no. 638, Culture Collection of Algae, Indiana University.

ULOTHRIX **belkae** sp. nov.

Filamenta longitudine indefinita, senescentia a 5 ad 11.5 μ spissescentia, cellulis latioribus relative brevioribus quam cellulis angustis. Plastis in filamentis iunioribus angustis lumen cellulae non omnino circumdans, marginibus longitudinalibus lobatis praedita. In filamentis vetustioribus latioribusque taeniam perfectam habens. Plastis dissepimenta plerumque attingens, duo vel plura pyrenoidea continens.

Reproductio asexualis per fragmentationem aut per zoosporas quadriflagellatas singulatim formatas; zoosporae per porum membrana in laterali liberatae. Omnes zoosporae stigmate anteriore, nucleo medio, et plastide poculiformi longitudinaliter incisa, unum ad aliquot pyrenoidea continente praeditae. Polus anterior zoosporae germinatae hapteron hyalinum secernens; hapteron forma varians, sine, autem, organisatione disci bulbique specierum multarum aliarum generis. Cellulae germinum spicales rotundatae, late angusteve acuminatae apiculataeve.

Filamenta in cultura "Bristol's" liquida unius mensis aetate membranas incrassatas et/aut cellulas akinetiformes membranis crassis praeditas interdum habentia; cellulae propriae utraque in extremitate inflatae etiam interdum visae; constrictiones mediocres ad dissepimenta perspicuae. Colonia in "Bristol's agars" post duas hebdomades atroviridis superficiem siccam undulatam praebens.

Reproductio sexualis non observata.

Planta e rivulo in loco Hancock Park, Austin, Texas dicto seiuncta.

Nomen huius speciei Dr. Ethel C. Belk, Miami University, Oxford, Ohio, honorat.

Filaments indefinitely long; increasing in diameter, with age, from 5 to 11.5 μ; plastid incompletely encircling cell lumen in younger cells, a complete band in older cells, containing 2 or more pyrenoids.

Quadriflagellate zoospores borne singly, escaping through lateral pores; each zoospore with an anterior stigma, median nucleus, and cup-shaped plastid; anterior pole of zoospore secreting holdfast upon germination; apical cell of germling rounded, acuminate, or apiculate; holdfast lacking disc and bulb organization.

Thickened walls, thick-walled, akinete-like, and characteristic dumbbell-shaped cells sometimes present in older filaments. Two-week colony dark green, dry, undulate.

Type culture deposited in the Culture Collection of Algae, Indiana University.

Ulothrix confervicola comb. nov.
(*Uronema confervicolum* Lagerh.)

Filaments indefinite in length, almost always attached, lacking constrictions at cross walls; cells 5–6 $\mu \times$ 10–18 μ with incompletely encircling, band-shaped plastids containing 2 or more pyrenoids; apical cells usually acuminate; basal cells with disc-like holdfast. Zoospores rarely produced, not phototactic; 2-week colony green, dry, undulate.

Repesented by culture no. 331, Culture Collection of Algae, Indiana University.

Ulothrix gigas comb. nov.
(*Uronema gigas* Vischer)

Filaments indefinite in length, attached or free-floating; cells 5–10 $\times$ 8–20 μ; apical cell of germling often apiculate; holdfast with disc and bulb organization; plastid band-shaped, longitudinally lobed and incomplete or complete, containing 2 or more pyrenoids.

Quadriflagellate zoospores borne singly, escaping through lateral pores; each zoospore with an anterior stigma, median nucleus, and longitudinally incised, cup-shaped plastid; posterior pole of zoospore often apiculate, anterior pole secreting holdfast; stigma persistent in basal cell of germling. Two-week colony green, moist, undulate.

Represented by culture no. 174, Culture Collection of Algae, Indiana University.

Ulothrix **minuta** sp. nov.

Cellulae 5–6 μ diam., 4–6 plo longiores quam latae; omnis cellula plastidem unicam taeniaformem parietalem, duo vel plura pyrenoidea continentem, habens;

plastis lumen cellulae fere aut omnino circumdans, ad dissepimenta fere aut omnino attingens.

Reproductio per zoosporas quadriflagellatas 1 vel 1 omnibus in cellulis factas, per porum in membrana laterali emergentes, hac deinde deliquescente solum dissepimenta cum segmentis membranarum lateralium brevissimis, ut vestigia filamentorum propria relinquente. Zoosporae stigmatibus magnis anterioribus ad media, nucleis mediis, et plastidibus poculiformibus saepe profunde incisis, unum ad aliquot pyrenoidea continentibus, praeditae. Haptera hyalina germinum e strato tenui disciformi per partem hemisphericam subtenso composita; forma poli zoosporae posterioris formam saepe acuminatam cellulae apicalis germinis determinans.

Filamenta unius mensis aetate longitudine indefinita, constrictiones mediocres ad dissepimenta plerumque habentia; colonia duarum hebdomadum in "Bristol's agar" viridis, superficiem humadam undulatamque praebens.

Reproductio sexualis non observata.

Origo: Cultura #330 e Collectione Culturum Algarum, Indiana University, Bloomington, Indiana. Origo ultima ignota.

One-month-old filaments indefinitely long with slight constrictions at the cross walls; apical cell of germling often acuminate, *never* apiculate; basal cell with disc and bulb holdfast; cells 5–6 × 20–35 μ; plastid a complete, or nearly complete, band with 2–several pyrenoids.

Quadriflagellate zoospores borne 1 or 2/cell, escaping through lateral pores; lateral walls deliquescing after zoosporogenesis; zoospores with anterior to median stigmata, median nuclei, and deeply incised, cup-shaped plastids containing 1–several pyrenoids; anterior pole or zoospore secreting holdfast. Two-week colony green, moist, undulate.

Represented by culture no. 330, Culture Collection of Algae, Indiana University.

ULOTHRIX **acuminata** sp. nov.

Filamenta 6–9.5 μ diam., cellulate 1–4 plo longiores quam latae, plastidibus parietalibus taeniaformibus non omnino circumdantibus praeditae, plastides secundum margines laterales lobatae, duo vel plura pyrenoidea continentes. Cellulae germinum apicales saepe acuminatae *numquam* apiculatae; cellulae basales hapteron hyalinum, compositum e strato tenui disciformi per partem hemisphericam subtenso, secernentes.

Reproductio per fragmentationem aut per zoosporas quadriflagellatas intra cellulas singulatim formatas, per poros in membranis lateralibus emergentes; zoosporae plerumque ellipsoideae, plastidibus poculiformibus longitudinaliter fissis, stigmatibus medis, et nucleis anterioribus praeditae. Stigma in cellula germinis basali saepe persistens.

Filamenta unius mensis aetate e culturis liquidis longitudine indefinita, constrictiones mediocres ad dissepimenta habentia; proprietates cellulae per accumulationem densam amyli saepe celata; colonia duarum hebdomadum aetate viridis, superficiem siccam undulatamque praebens.

Reproductio sexualis non observata.

Origo: Planta ex exemplo soli e querceto in loco Williamson County, Texas, dicto seiuncti.

Filaments similar to those of *U. minuta*; cells 6–9.5 × 6–38 μ; plastid an incompletely encircling, longitudinally lobed band.

Quadriflagellate zoospores borne singly, escaping through lateral pores; each zoospore with a median stigma, anterior nucleus, and longitudinally split, cup-shaped plastid; stigma persistent for a time in basal cell of germling. Two-week colony dark green, dry, undulate; 1-month colony sometimes with lighter margin.

Type culture deposited in the Culture Collection of Algae, Indiana University.

II. THE GENUS HORMIDIUM KLEBS 1896 (NON KÜTZ. 1843) EMEND.

The taxonomic history of the genus *Hormidium* has already been discussed. During the present investigation, 21 isolates of the genus, representing 3 species, have been intensively examined in axenic culture. A description of the genus and of the investigated species follows.

Hormidium Klebs 1896 (non *Hormidium* Kütz. 1843a) emend.

Filaments of this genus are uniseriate and unbranched and consist of uninucleate cells with single, parietal, plate-shaped plastids that encircle ½–¾ of the cell lumen (Fig. 37, 41) and contain 1 distinct pyrenoid (Fig. 38). The plastid may be only ½ the length of the cell or may approach cell length (Fig. 40) and is not lobed along its longitudinal margins (Fig. 37). The nucleus commonly lies in a cytoplasmic bridge opposite the plastid (Fig. 39). Filaments lack a gelatinous sheath.

The most common method of reproduction in the genus is fragmentation of the filaments. Biflagellate zoospores, which are rarely formed, are produced singly in the cells and escape through a circular pore. They lack a stigma and produce no holdfast upon germination. Sexual reproduction is said to involve the fusion of biflagellate isogametes (Wille, 1912).

To distinguish species within the genus, physiological as well as morphological attributes have been employed. Tables listing the physiological attributes follow the descriptions of the species.

HORMIDIUM FLACCIDUM A. Braun. (Fig. 33–42)

As indicated in Table 1, 17 isolates of this species have been examined. Because none of these is clearly distinct, morphologically, from the others, it is thought best

to consider these isolates a single taxon. There are, to be sure, physiological differences among these isolates, but that is to be expected considering that they have been isolated from diverse habitats.

Of the 17 isolates here considered, special mention must be made of the 4 cultures obtained from the Culture Collection of Algae at Indiana University. Of these, as listed in the catalogue of the Culture Collection (Starr, 1960), number 623 is listed as *Hormidium* sp., number 323 is listed as *H. stoechidum* but no authority is given, number 321 is listed as *H. barlowii,* but Dr. Pringsheim, the isolator, considers this last name dubious (personal communication). Thus, only number 322, listed as *H. flaccidum* A. Br., seems to have a valid name. Furthermore, the writers have found no significant differences among the 4 cultures. It thus seems reasonable to conclude that these cultures could well be merely different isolates of the same species and they are here so considered on the basis of prolonged, comparative study.

The cells of *Hormidium flaccidum* range in diameter from 5 to 8 μ and cell length is ½–2½ times the diameter. All isolates make good growth on Bristol's agar and at 2 weeks have green colonies with wavy, dull-shiny surfaces (Fig. 33). Grown for 1 month in flasks of liquid Bristol's solution, the filaments may be relatively short and straight (Fig. 34), twisted (Fig. 35), or woven into rope-like strands (Fig. 36).

Many of these isolates grow slowly, if at all, in liquid Bristol's solution, but most grow well if small amounts of a vitamin mixture (Vitamins Eagle 100, Difco) are added to the flasks prior to inoculation. Apparently the agar supplies some growth factor for most isolates of this species.

Hormidium subtilissimum comb. nov. (Fig. 43)

(*Ulothrix subtilissima* Rabenh.)

Three isolates of this organism, 1 labeled *Ulothrix subtilissima* in the catalogue of the Culture Collection of Algae at Indiana University (Starr, 1960), have been examined. The 3 are without doubt different isolates of the same species and certainly belong in the genus *Hormidium*.

Cells of *H. subtilissimum* are 5–6 μ in diameter and ½–2 times that long. The organism grows very slowly in liquid Bristol's solution but makes good growth on Bristol's agar. Two-week-old colonies are dark green and have a rough, dry surface. This attribute, among others, distinguishes the species from all other species of the genus here investigated. Filaments from 1-month Bristol's liquid medium are straight or twisted but never occur in rope-like strands (Fig. 43). This species has a pattern of physiological attributes unlike any other isolate with which it might be confused.

HORMIDIUM STERILE Deason and Bold. (Fig. 44)

This species has recently been described from axenic culture (Deason and Bold, 1960). It is distinguished from all other species here considered by its smaller cell

size (Fig. 44), smooth, shiny colony, and generally shorter filaments (Fig. 44).

The significance of the data on supplementary attributes, summarized in Tables 7–10, will be considered in the *Discussion* section of this paper.

TABLE 7. *Growth of Hormidium species in Bristol's solution supplemented with various carbon sources*[a]

Organism	Glucose	Na Acetate	Arabinose	Ribose	Fructose	Xylose
H. flaccidum						
f-2	fair[b]	good	good	fair	fair	fair
f-3	fair	fair	fair	fair	fair	fair
f-4	fair	fair	fair	fair	fair	fair
f-8	fair	fair	fair	fair	fair	fair
f-12	fair	fair	trace	trace	fair	trace
f-16	fair	fair	fair	fair	fair	fair
f-23	trace	trace	fair	fair	fair	fair
f-57	trace	trace	trace	trace	trace	trace
f-83	trace	trace	trace	trace	trace	trace
f-86	good	good	fair	fair	fair	fair
f-93	good	good	fair	fair	fair	fair
321	fair	fair	fair	good	good	good
322	good	good	good	fair	fair	fair
323	good	good	good	good	good	fair
623	fair	fair	fair	fair	fair	fair
f-34	excel	fair	good	excel	excel	excel
f-48	trace	trace	trace	trace	trace	trace
H. subtillissimum	trace	trace	trace	trace	trace	trace
H. sterile	trace	trace	trace	trace	trace	trace

[a] All at 0.5% concentration in Bristol's solution.

[b] The apparent amount of growth is described as none, trace, fair, good, or excellent on the basis of arbitrary standards as recorded on Kodachrome slides.

Key to the species of *Hormidium* studied

1. Two-week-old colony on Bristol's agar with a wavy, moist surface ..... *H. flaccidum* A. Br.
1. Two-week-old colony on Bristol's agar with a rough, dry surface or with a smooth, shiny surface 2
   2. Two-week-old colony with a rough, dry surface ..... *H. subtilissimum* comb. nov.
   2. Two-week-old colony with a smooth, shiny surface ..... *H. sterile* Deason and Bold

The species of *Hormidium* studied during this investigation are morphologically quite similar. The following brief descriptions are included here, as a supplement

TABLE 8. *Growth of Hormidium species in/on several undefined, differential media*

Organism	A.C. broth	Thioglycollate broth	Nutrient broth	0.01% starch agar[a]
H. flaccidum				
f-2	excel	fair	good	no halo
f-3	fair	trace	good	no halo
f-4	fair	trace	good	no halo
f-8	fair	trace	good	no halo
f-12	trace	fair	fair	no halo
f-16	fair	trace	good	no halo
f-23	trace	trace	fair	no halo
f-57	trace	trace	trace	no halo
f-83	trace	trace	trace	no halo
f-86	good	good	good	no halo
f-93	good	good	good	no halo
321	fair	trace	fair	no halo
322	fair	trace	fair	no halo
323	excel	excel	fair	no halo
623	trace	fair	fair	no halo
f-34	excel	good	good	slight halo
f-48	none	none	trace	no halo
H. subtilissimum	trace	trace	fair	no halo
H. sterile	fair	trace	fair	halo

[a] After the organisms had grown for 2 weeks on the surface of the starch agar, the surface was flooded with aqueous I_2-KI. The presence of a clear zone around the colony, an evidence of extracellular starch digestion, is recorded as a halo.

to the above key, to indicate some of the significant differences observed among these species.

HORMIDIUM FLACCIDUM A. Br.

Cells 5–8 × 3–20 μ; 1-month-old filaments straight, twisted, or in rope-like strands; growth in Bristol's solution trace or fair; 2-week-old colony green, wavy, dull-shiny.

Represented by culture no. 322 and by nos. 321, 323, and 623, Culture Collection of Algae, Indiana University.

Hormidium subtilissimum comb. nov.

(*Ulothrix subtilissima* Rabenh.)

Cells 5–6 × 3–12 μ; 1-month-old filaments straight or twisted, not in rope-like strands; growth in Bristol's solution trace; 2-week-old colony green, rough, dry.

Represented by culture no. 462, Culture Collection of Algae, Indiana University.

TABLE 9. *Growth of Hormidium species on different concentrations of crystal-violet agar*

Organism	1:10,000	1:25,000	1:50,000	1:100,000
H. flaccidum				
f-2	trace	trace	excel	excel
f-3	trace	good	excel	excel
f-4	trace	good	excel	excel
f-8	trace	good	excel	excel
f-12	good	excel	excel	excel
f-16	trace	fair	excel	excel
f-23	fair	good	excel	excel
f-57	trace	trace	excel	excel
f-83	trace	fair	excel	excel
f-86	trace	fair	excel	excel
f-93	trace	good	excel	excel
321	trace	good	excel	excel
322	trace	good	excel	excel
323	trace	good	excel	excel
623	trace	trace	excel	excel
f-34	trace	excel	excel	excel
f-48	trace	trace	trace	trace
H. subtilissimum	trace	fair	good	excel
H. sterile	fair	excel	excel	excel

HORMIDIUM STERILE Deason and Bold.

Cells 4–5 × 12–15 μ; filaments readily dissociating; growth in Bristol's solution trace; 2-week-old colony green, smooth, shiny.

Type culture deposited in Culture Collection of Algae, Indiana University; no published number.

III. THE GENUS STICHOCOCCUS NÄGELI EMEND.

The genus *Stichococcus* was established by Nägeli in 1849. In the original description, Nägeli does not mention pyrenoids or motile cells. However, after Gay (1891) removed all of the aerial, filamentous algae from the genus *Ulothrix,* Hazen (1902) included in *Stichococcus* algae with motile cells in their life cycles and pyrenoids in the plastids as well as organisms which lack pyrenoids and motile cells. Careful examination of many isolates has convinced the writers that those isolates with a single, distinct pyrenoid in each plastid and motile cells in the life cycle form a group clearly distinct from those isolates that lack pyrenoids and motile cells. Accordingly, the generic description of *Stichococcus* has been emended to exclude those organisms which have pyrenoids and which produce motile cells.

Filaments of this genus are uniseriate and unbranched and consist of uninucleate

TABLE 10. *Comparative sensitivity of Hormidium species to several agents*[a]

Organism	AB+	CS+	K+	N+	PB+	S+	AL+	FC+	FR+	IH+	PS+
H. flaccidum											
f-2	....	....	+	+	+	....	+	+	+	+	+
f-3	....	....	+	+	+	....	+	....	+	....	....
f-4	....	....	+	+	+	....	+	....	+	....	+
f-8	....	....	+	+	+	....	+	+	+	....	....
f-12	....	....	+	+	+	....	....	....	+	....	+
f-16	....	...	+	+	+	....	+	....	+	....	+
f-23	....	....	+	+	+	....	+	+	+	+	+
f-57	....	....	+	+	+	....	+	+	+	+	+
f-83	....	....	+	+	+	....	+	....	+	....	....
f-86	....	....	+	....	....	....	....	....	+	....	....
f-93	....	....	+	+	....	....	+	+	+	....	+
321	....	....	+	+	+	....	+	+	+	....	....
322	....	+	+	+	+	....	+	....	+	....	+
323	....	....	+	+	+	....	+	+	+	....	+
623	....	....	+	+	+	....	+	+	+	....	+
f-34	....	+	+	+	+	....	+	+	+	....	....
f-48	....	....	+	+	....	....	+	+	+	+	+
H. subtilissimum	....	....	+	+	+	....	+	+	+	....	+
H. sterile	....	....	+	....	....	....	....	+	+	....	+

[a] Of 43 antibiotic agents tested, only these 11 were differential with respect to the organisms studied; + indicates a definitely clear zone of inhibition as indicated in Fig. 53.

cells, with rounded ends, that have parietal, plate-shaped plastids which lack pyrenoids and incompletely encircle the cell lumen. Fragmentation of the filaments is common and often results in an almost unicellular population. Motile cells and sexual reproduction apparently are absent, and, of the species available in culture, none has a gelatinous sheath.

STICHOCOCCUS BACILLARIS Näg. (Fig. 45–49)

Four isolates, all from the Culture Collection of Algae at Indiana University, have been carefully studied, and the writers conclude that they must be considered a single species. These isolates, as listed (Starr, 1960), are: 314, *S. bacillaris* Näg.; 419, *S. bacillaris* Näg.; 315, *S. chloranthus* Krüger, and 176, *Hormidium nitens* Menegh.

Filaments of *Stichococcus bacillaris* may attain a length of 50 cells, although they are rarely longer than 10 cells (Fig. 46, 47). Most cells occur singly. Individual cells of an axenic culture, grown for 1 week on Bristol's agar, have broadly rounded poles and a single, parietal, plate-like plastid that encircles ½–¾ of the cell lumen and extends for all, or nearly all, of the cell length (Fig. 48). There are

no distinct pyrenoids in living cells, but staining with strong iodine may reveal traces of an incipient or vestigial pyrenoid in a few cells. Cells range in diameter from 2.3 to 3.3 μ and are 1–3 times that long.

Young cells have very little reserve photosynthate, but after growing for a month in liquid Bristol's, they exhibit a marked accumulation of starch and have lipid-containing droplets at the ends of the cells not covered by the plastid (Fig. 49). There are no specialized apical or basal cells and no motile cells are produced by this alga. The only method of reproduction is cell division followed by separation of the daughter cells or by fragmentation of longer filaments. Two-week-old colonies are green and have a smooth, shiny surface. The colony appearance is the same at 1 month (Fig. 45).

STICHOCOCCUS CHODATI (Bial.) Heering. (Fig. 50)

This organism was isolated into axenic culture from the Blackland Prairie soil of Hayes County, Texas. When grown in pure culture for 1 week on Bristol's agar, nearly all cells occur singly and filaments are never more than 2 or 3 cells long. Individual cells of this organism are similar to those of *S. bacillaris* in the form and structure of the plastid but differ in cell size and shape. Cells of this alga are 2.0–2.5 μ in diameter, only 1–1½ times that long, and ellipsoidal in shape (Fig. 50).

Cellular morphology changes little with age except for the development of droplets, similar to those of *S. bacillaris*, which also appear to be lipid, as indicated by Sudan IV. A definite accumulation of starch can also be observed in cells a month or more old. The only method of reproduction is cell division. There are no motile cells. Two-week-old colonies are green with a smooth, shiny surface and the colonies have the same appearance at 1 month.

STICHOCOCCUS MIRABILIS Lagerheim. (Fig. 51–52)

The isolate of this organism studied is number 316 (Starr, 1960) from the Culture Collection of Algae at Indiana University. The alga is essentially unicellular and rarely produces filaments of more than 2 cells. Individual cells have a single, parietal, plate-like plastid, which lacks a distinct pyrenoid, encircles ½–¾ of the cell lumen and extends for ½ to the entire cell length (Fig. 51).

In this species cells are 2.3–3.3 μ in diameter but may be up to 30 μ long (Fig. 52) in sharp contrast to both of the other species here discussed. No motile cells or specialized apical or basal cells have been observed. The only method of reproduction is cell division. Two-week-old colonies are green and have a smooth, dry surface; they do not change in appearance at 1 month.

The significance of the data on supplementary attributes, summarized in Tables 11–14, will also be considered in the *Discussion* section of this paper.

Key to the species of *Stichococcus* studied

1. Individual cells up to 30 μ long ..... *S. mirabilis*
1. Individual cells never more than 15 μ long 2

2. Cells 2.3–3.3 μ in diameter; sometimes united into filaments of 40–50 cells ..... *S. bacillaris*
2. Cells 2.0–2.5 μ in diameter; never united into filaments of more than 5 cells ..... *S. chodati*

TABLE 11. *Growth of Stichococcus species in Bristol's solution supplemented with various carbon sources*[a]

Organism	Glucose	Na Acetate	Arabinose	Ribose	Fructose	Xylose
S. bacillaris						
176	good[b]	fair	fair	fair	good	trace
314	excel	good	fair	fair	good	trace
419	excel	fair	fair	fair	good	trace
315	excel	good	fair	fair	good	trace
S. mirabilis	excel	fair	fair	fair	good	trace
S. chodati	fair	trace	trace	trace	trace	trace

[a] All at 0.5% concentration in Bristol's solution.

[b] The apparent amount of growth is described as none, trace, fair, good or excellent on the basis of arbitrary standards as recorded on Kodachrome slides.

TABLE 12. *Growth of Stichococcus species in/on several undefined, differential media*

Organism	A.C. broth	Thioglycollate broth	Nutrient broth	0.01% starch agar[a]
S. bacillaris				
176	fair	good	good	no halo
314	fair	fair	fair	no halo
419	trace	good	fair	no halo
315	trace	good	fair	no halo
S. mirabilis	fair	fair	fair	no halo
S. chodati	none	good	trace	no halo

[a] After the organisms had grown for 2 weeks on the surface of the starch agar, the surface was flooded with aqueous I_2-KI. The presence of a clear zone around the colony, an evidence of extracellular starch digestion, is recorded as a halo.

TABLE 13. *Growth of Stichococcus species on different concentrations of crystal-violet agar*

Organism	1:10,000	1:25,000	1:50,000	1:100,000
S. bacillaris				
176	trace	trace	fair	fair
314	trace	trace	trace	trace
419	trace	trace	trace	trace
315	trace	trace	trace	trace
S. mirabilis	trace	trace	fair	fair
S. chodati	trace	trace	good	good

TABLE 14. *Comparative sensitivity of Stichococcus species to several antibiotic agents*[a]

Organism	AB+	CS+	K+	N+	PB+	S+	AL+	FC+	FR+	IH+	PS+
S. bacillaris											
176	....	+	+	+	+	....	....	....	....	....	....
314	....	+	+	+	+	....	....	....	....	....	....
419	....	+	+	+	+	....	....	....	+	....	....
315	....	+	+	+	+	....	....	....	....	....	....
S. mirabilis	....	+	+	+	+	+	+	....	+	....	....
S. chodati	....	+	+	+	+	....	....	....	....	....	+

[a] Of 43 antibiotic agents tested, only these 11 were differential with respect to the organisms studied; + indicates a definitely clear zone of inhibition as indicated in Fig. 53.

The species of *Stichococcus* studied during this investigation are easily distinguished on the basis of filament length and cell size, among other attributes. For additional supplementary attributes see Tables 11–14.

STICHOCOCCUS BACILLARIS Nägeli.

Filaments up to 50 cells long: cells 2.3–3.3 μ in diameter, 3–10 μ long.

Represented by culture nos. 314, 419, 315, 176, Culture Collection of Algae, Indiana University.

STICHOCOCCUS CHODATI (Bial.) Heering.

Filaments rarely more than 2 or 3 cells long; cells 2.0–2.5 μ in diameter, 2.0–4.5 μ long.

Culture to be deposited in the Culture Collection of Algae, Indiana University.

STICHOCOCCUS MIRABILIS Lagerh.

Filaments rarely more than 2 or 3 cells long; cells 2.3–3.3 μ in diameter, 5–30 μ long.

Represented by culture no. 316, Culture Collection of Algae, Indiana University.

Discussion

The primary objectives of this investigation have been two-fold: (1) to determine how many genera are represented by the organisms studied in culture, with the realization that these organisms would be classified by most phycologists in the genera *Ulothrix, Uronema, Hormidium,* and *Stichococcus;* and (2) to define the limits of these genera as precisely as possible as a basis for future work with additional species. By careful, prolonged examination of, and experimentation with, many axenic cultures, it has been possible to achieve both of these objectives.

The study of a number of named herbarium specimens of ulotrichacean algae

has forced upon the writers the conclusion that, in order for a taxonomic study of this type to be significant, the necessity for the extensive examination of cultured material is absolute. Furthermore, it is necessary to study the organisms at many different ages and conditions of culture, for attributes that are present in young material may be obscured in older material, while other attributes appear only in older material. For example, the morphology of the plastid and the number of pyrenoids are critical attributes which are often obscured by the accumulation of reserve photosynthate in older cells and not preserved in herbarium specimens (Fig. 1–8). On the other hand, thick-walled hypnospores (in *Ulothrix fimbriata*), dumbbell-shaped cells (in *U. belkae*), and rope-like strands of filaments (in *Hormidium flaccidum*) can rarely, if ever, be observed in young cultures.

Although a number of the attributes of the organisms studied have been shown to be variable, this investigation has revealed that several of the characteristics of these organisms are reliable and taxonomically useful. In the writers' opinion, 3 clearly distinct and well-defined genera are apparent among the organisms studied. These genera will now be discussed in the light of the observations and experimental results of this investigation.

I. THE GENUS ULOTHRIX KÜTZING EMEND.

The genus *Ulothrix* is distinguished by its band-shaped plastid which nearly, or completely, encircles the cell lumen. Incompletely encircling plastids are lobed along the longitudinal margins. The plastid in *Ulothrix* typically contains more than 1 pyrenoid, althought a few cells may be observed which have only 1.

In addition, the production of quadriflagellate zoospores, which have red stigmata, may be rather easily evoked in most species of the genus studied during this investigation. These zoospores always produce a holdfast upon germination.

All of the species of *Ulothrix* studied make excellent growth in an inorganic, liquid culture medium (Bristol's solution) and appear to be completely photoautotrophic. None of the species of *Ulothrix* studied by the writers makes better than trace amounts of growth on any concentration of crystal-violet agar used in physiological tests, with the exception of 2 isolates of *U. acuminata*, which make fair growth on crystal-violet agar at a concentration of 1:100,000. None of the species of *Ulothrix* studied by the writers is inhibited by Kanamycin, but all of the isolates of *Hormidium* and *Stichococcus* studied by the writers are inhibited by that antibiotic.

The combination of attributes summarized above serves to distinguish the genus *Ulothrix* (including the genus *Uronema*) from the other genera of ulotrichacean algae under consideration here. In the writers' opinion, the attributes of plastid morphology and number of pyrenoids are fundamental. Therefore, it is recommended that the generic description of *Ulothrix* be emended to exclude all previously described species of *Ulothrix* which have plate-shaped plastids that encircle less than the entire cell lumen, are not lobed along the longitudinal margins,

and contain but a single, distinct pyrenoid. Species with these attributes should be transferred to the genus *Hormidium*.

Within the genus *Ulothrix* there are a number of morphological attributes which are useful in distinguishing species. Among these are: filament diameter, shape of the apical cell of germlings, degree of thickening of cell walls with age, characteristics of the zoospores, behavior of the cell walls at zoosporogenesis, presence of unique kinds of cells (hypnospores, akinetes, dumbbell-shaped cells), and pigmentation. Employing these and other morphological attributes, the writers have been able to distinguish 6 species of *Ulothrix,* 3 of them new, among the isolates available in axenic culture. In addition to these morphological attributes, a number of supplementary ones have been discovered as the result of a series of experiments performed during this investigation. These supplementary attributes and the experiments by which they were discovered will be considered on pages 42–45.

II. THE GENUS HORMIDIUM KLEBS 1896 (NON KÜTZING 1843) EMEND.

The most characteristic attribute of the genus *Hormidium* is the single, parietal, plate-shaped plastid which is present in each cell. The plastid always incompletely encircles the cell lumen, is not lobed along the longitudinal margins, and contains 1 distinct pyrenoid. The plastid differs from that of *Stichococcus* in having a pyrenoid, and from that of *Ulothrix* in having only 1 pyrenoid and in lacking lobing along its longitudinal margins.

It is difficult to evoke zoosporogenesis in the cultures of *Hormidium*. The zoospores, when produced, are biflagellate[6] and lack a stigma. They do not produce a holdfast upon germination, nor do they ever give rise to filaments with anything but rounded apical cells.

Of the writers' cultures of *Hormidium*, only isolate f-34 makes better than fair growth in the completely inorganic Bristol's solution. Preliminary experiments have shown that the addition of small amounts of a vitamin mixture to Bristol's solution provides a culture medium in which many of the isolates of *Hormidium* can make excellent growth. However, time has not permitted a more thorough study to determine which vitamin (or vitamins) is required. It seems probable to the writers that many of their isolates of *Hormidium* may require, or at least partially require, a growth factor. In this connection, it may be significant that the isolates of *Hormidium* studied during this investigation were almost exclusively terrestrial in origin, the notable exception being isolate f-34, which, surprisingly, makes excellent growth in Bristol's solution. On the other hand, the isolates of *Ulothrix* studied, which grow luxuriantly in inorganic media, were aquatic in origin, except perhaps those organisms obtained from the Culture Collection of Algae at Indiana University, whose ultimate origin is not known to the writers.

[6] This is not meant to exclude from the genus any organisms, which may be discovered in the future, which produce quadriflagellate zoospores but are in other respects *Hormidium*-like.

Two other striking physiological differences between *Ulothrix* and *Hormidium* are to be noted. All isolates of *Hormidium* are clearly inhibited by Kanamycin and all, except isolate f-48, make excellent growth on crystal-violet agar in concentrations of 1:50,000 and 1:100,000. These readily apparent, physiological differences between *Ulothrix* and *Hormidium* indicate to the writers a fundamental difference in the metabolism of the 2 genera. The mechanism of this difference, although interesting, has been reserved for later study.

Within the genus *Hormidium* the matter of distinguishing species is a little more difficult than in the case of *Ulothrix*. Of the species studied by the writers, *H. sterile* is easily distinguished by its smaller cell size and smooth, shiny colony. The difference between *H. subtilissimum* and *H. flaccidum* are distinct but subtle. The rough, dry colony of *H. subtilissimum* is quite unlike the wavy, moist colony of *H. flaccidum*. Cell diameter and the behavior of the filaments in older cultures also serve to distinguish these 2 species. Thus, in *Hormidium,* as in *Ulothrix,* species can be distinguished on a purely morphological basis. Additional supplementary attributes of the genus *Hormidium*, also useful at the specific level, will be discussed on pages 42–45.

III. THE GENUS STICHOCOCCUS NÄGELI EMEND.

The genus *Stichococcus* appears to be the simplest of the 3 genera investigated by the writers. It has cells which contain parietal, plate-shaped plastids that incompletely encircle the cell lumen, are not lobed along the longitudinal margins, and lack pyrenoids. No motile cells have been observed in any of the writers' cultures.

With respect to growth in Bristol's solution and on crystal-violet agar, this genus cannot be so neatly characterized as *Ulothrix* and *Hormidium*. It is, however, similar to *Hormidium* in being inhibited by Kanamycin. To emend the genus *Stichococcus,* it is necessary only to remove from it those organisms which have pyrenoids and motile cells.

Specific differences within the genus depend, in part, upon the attributes of cell diameter, cell length, and filament length. It must be noted here again that species within this genus can be delimited using morphological attributes. Additional supplementary attributes of this genus, and of *Ulothrix* and *Hormidium*, will be discussed next.

IV. SUPPLEMENTARY ATTRIBUTES IN THE TAXONOMY OF ULOTRICHACEAN ALGAE

Investigations concerning the use of supplementary attributes in the taxonomy of the algal genus *Chlorococcum* Meneghini have recently been summarized by Bold and Parker (1962). Other workers (Arce, 1956; Deason and Bold, 1960; Chantanachat, 1962; Chantanachat and Bold, 1962) have used methods similar to those reported by Bold and Parker as aids in the classification of related but

different genera. During the present investigation, the writers have further extended and modified these methods for use with ulotrichacean algae.

The addition of various organic carbon sources[7] to inorganic Bristol's solution provides culture media which may be inhibitory with respect to the growth of the organisms investigated. During this investigation arabinose, fructose, glucose, ribose, sodium acetate, and xylose have been employed, in combination with Bristol's solution, as the organic carbon components of differential culture media. These carbon sources were selected for use on the basis of past experience as being most likely to provide taxonomically useful tools. The data recorded in Tables 3, 7, and 11 are based on at least 5 different experiments done in duplicate tubes.

Examination of Tables 3, 7, and 11 reveals several interesting points which might be considered here:

1. The 6 isolates of *Ulothrix acuminata*, although taken into culture from widely separated places, are quite similar with respect to their rates of growth in Bristol's solution supplemented with these carbon sources.
2. There are differences among the 17 isolates of *Hormidium flaccidum*, with respect to their rates of growth in the presence of the several carbon sources, but no isolate is clearly distinct from the others.
3. All of the carbon sources used inhibit the growth of 1 or more of the isolates studied.
4. *Ulothrix fimbriata*, *Hormidium flaccidum* isolates f-48, f-57, and f-83, *H. subtilissimum*, and *H. sterile* are inhibited by all of the organic carbon sources employed.
5. The differential inhibition of the various species of *Ulothrix*, *Hormidium*, and *Stichococcus* by the carbon sources employed provides supplementary attributes useful in the taxonomy of these genera. The mechanism of inhibition of algae by certain carbon sources is currently under investigation.

The use of the undefined media, A.C. Broth, Nutrient Broth, and Thioglycollate Broth, originally intended to verify the purity of stock cultures, as differential culture media has also been attempted during this investigation. With these media, each isolate was inoculated to duplicate tubes on at least 3 separate occasions. Each of these undefined media has been shown to inhibit the growth of 1 or more of the isolates studied, and, therefore, these media are also useful in the elucidation of supplementary attributes in the ulotrichacean genera *Ulothrix*, *Hormidium*, and *Stichococcus*.

Starch agar (0.01% starch in Bristol's agar) was employed during this study to investigate the possibility that differences in production of extracellular digestive enzymes by the organisms being studied might be taxonomically useful. The

[7] None of the ulotrichacean algae studied was facultatively heterotrophic with any of the carbon sources used.

appearance of a clear halo around a 2-week-old colony after flooding with iodine (I_2-KI) has been assumed to indicate the production of such enzymes by a given organism. Although it is not yet possible to get perfectly repeatable results with this technique, one interesting condition has been brought to light. All of the investigated species of *Ulothrix* usually have a halo, but of the species of *Hormidium*, only *H. sterile* and *H. flaccidum* isolate f-34, and none of the species of *Stichococcus* typically have a halo. This may be further evidence of a fundamental difference in metabolism between *Ulothrix* and the other 2 genera. Furthermore, the exceptions noted within the genus *Hormidium* may prove to be taxonomically significant. More work is needed to perfect this technique, but the writers consider that such investigations would prove beneficial.

Crystal violet has long been used by bacteriologists as a differential bacteriostatic agent. To the writers' knowledge it has not been previously employed in algal taxonomy. Crystal violet has proven to be most useful, during the present investigation, in providing an additional attribute by which the 3 genera here being considered may be distinguished. Considering only the isolates studied during this investigation, crystal violet appears to have no usefulness in distinguishing species within the genus *Ulothrix* and only limited usefulness in distinguishing species within the genera *Hormidium* and *Stichococcus*. The writers are convinced, however, that the use of crystal violet should be considered in further investigations of algal taxonomy in which the elucidation of additional supplementary attributes is desirable or necessary.

Finally, during the present investigation, 43 antibiotic agents[8] have been tested in an effort to determine whether or not the differential sensitivity of the organisms studied to these agents might be of taxonomic significance. It must be reported here that many of these agents are not differential with respect to the organisms under consideration. Thus, Chlortetracycline, Bacitracin, Chloramphenicol, Carbomycin, Demethylchlortetracycline, Erythromycin, Novobiocin, Nystatin, Oleandomycin, Penicillin, Ristocetin, Syncillin, Cycloserine, Staphcillin, Oxytetracycline, Triacetyloleandomycin, Tetracycline, Sulfisomidine, Sulfisoxazole, Sulfamethoxypyridazine, Viomycin, Vancomycin, Sulfadimethoxine, Sulfadiazine, Sulfamerazine, Triple Sulfa, Sulfathiazole, and Thiosulfil fail to inhibit (at the concentrations used) any of the organisms studied, and Paromomycin, Triclobisonium Chloride, Nitrofurantoin, and Methenamine Mandelate inhibit (at the concentrations used) all of the organisms studied. Only those antibiotic agents indicated in Tables 6, 10, and 14 are differentially inhibitory (at the concentrations used) with respect to the organisms studied. The writers, however, are unwilling to attach taxonomic significance to this differential sensitivity for 2 reasons: (1) there occurs as much variation within the different isolates of a single species, i.e., in *Ulothrix acuminata, Hormidium flaccidum,* and *Stichococcus bacillaris,* as occurs among the several other species of a given genus (Tables 6, 10, 14, for example).

[8] All obtained from Consolidated Laboratories, Chicago Heights, Illinois.

Because the various isolates of these species have been shown to be morphologically and otherwise physiologically very similar, the writers remain skeptical about the significance of the differences indicated by the antibiotics. (2) They are especially concerned with the value of differential sensitivity to antibiotics as a taxonomic tool in view of their inability to obtain reproducible results with different lots of the antibiotics and in view of similar difficulties reported by Bold and Parker (1962). There is good reason to believe that with standardization of the antibiotic discs by their several manufacturers, these discs may become a valuable adjunct to phycological taxonomy.

Typical responses of the organisms studied to crystal-violet agar and to a few antibiotic agents are shown in Fig. 53, 54. The critical morphological features of the genera considered in this investigation are illustrated diagrammatically in Figs. 55–57.

The use of the culture method in phycology has been criticized by some phycologists on the grounds that it is not possible to observe the "normal" morphology of organisms grown in culture. That this is untrue has been demonstrated by many workers. In fact, it is becoming increasingly apparent that with certain algal taxa use of the culture method is essential if one wishes to observe the maximum possible number of reliable morphological criteria. Such is the case with the organisms studied during the present investigation. It is, of course, apparent that no useful supplementary attributes can be recorded without experimentation with cultured material.

It is desirable, ideally, in a taxonomic investigation of the type herein reported, to combine the study of natural collections and herbarium specimens with that of organisms grown in culture. Insofar as possible, that has been done during the present investigation. It must be noted, however, that direct study and identification of most algae in soil is fruitless, if not impossible. Finally, with respect to the organisms studied by the writers, many important attributes unfortunately are not often preserved in dried herbarium specimens.

Summary and Conclusions

This investigation has involved the critical examination of representatives of the ulotrichacean genera *Ulothrix* Kützing, *Uronema* Lagerheim, *Hormidium* Klebs, and *Stichococcus* Nägeli. Approximately 100 unialgal cultures, 39 of them axenic, have been intensively studied under standard conditions of culture. The primary objectives of this investigation have been two-fold: (1) to determine how many valid genera are represented by the organisms now classified in the above-named genera, and (2) to define, as precisely as possible, the limits of the valid genera and of species within them. A secondary objective has been the elucidation of taxonomically significant supplementary attributes in the organisms studied.

To implement the first objectives, the appropriate literature was carefully re-

viewed, a number of herbarium specimens were examined, and all of the axenic cultures available to the writers were intensively and repeatedly studied at various ages and under various conditions of culture. With respect to these objectives the following conclusions can be drawn:

1. The genus *Uronema* should be abandoned inasmuch as there are no reliable morphological attributes which separate it from the genus *Ulothrix*. Validly described species of *Uronema*, previously published, should be transferred to the genus *Ulothrix*.

2. The genus *Ulothrix* should be emended to include only those ulotrichacean algae with parietal, complete or incomplete band-shaped plastids that typically contain more than 1 pyrenoid.

3. A proposal will be made to have the generic name *Hormidium* Klebs 1896 conserved. The generic description of *Hormidium* should be emended to include only those ulotrichacean algae which have parietal, incompletely encircling, plate-shaped plastids that typically contain only 1 pyrenoid.

4. The generic description of *Stichococcus* should be emended to exclude those organisms that have motile cells and plastids with pyrenoids.

5. Within each of the above-named genera a number of species can be distinguished on purely morphological evidence. Three new species of the genus *Ulothrix* have been described. These are:

Ulothrix **acuminata** sp. nov.
Ulothrix **belkae** sp. nov.
Ulothrix **minuta** sp. nov.

Furthermore, on the basis of the present investigation a number of taxa have been transferred, as follows:

Uronema confercicolum Lagerheim to **Ulothrix confervicola** comb. nov.
Uronema gigas Vischer to **Ulothrix gigas** comb. nov.
Ulothrix subtissima Rabenhorst to **Hormidium subtilissimum** comb. nov.

With respect to the secondary objective, a series of experiments, designed to reveal taxonomically significant supplementary attributes, has been performed. The following conclusions have resulted from these experiments:

6. Characteristic differences noted in 2-week-old colonies of the organisms studied are taxonomically useful supplementary attributes.

7. The use of various organic carbon sources, in combination with Bristol's solution, and of several undefined media, as differential culture media, has provided additional supplementary attributes, advantageous in the taxonomic disposition of the organisms studied.

8. Starch agar and crystal-violet agar also provide useful supplementary attributes.

9. The differential inhibition of the organisms studied by a number of antibiotic agents must be interpreted as having limited significance, in the present instance, in view of differences recorded among the various isolates of some species, the inability to obtain reproducible results with different lots of the same agents, and similar difficulties reported by other workers.

In light of the entire investigation herein reported, one final conclusion must be recorded.

10. The absolutely essential role of the culture method in the taxonomic disposition of the organisms studied has been unequivocally demonstrated.

In spite of their demonstrated inadequacies (Fig. 1–8), herbarium specimens of the taxa discussed in this paper have been prepared and deposited in the Chicago Natural History Museum. Axenic cultures of newly described taxa have been deposited in the Culture Collection of Algae, Indiana University.

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Figs. 1–8

Photomicrographs of rehydrated herbarium specimens of certain ulotrichacean algae.

Fig. 1. Undetermined species of *Ulothrix* from a specimen (#614) in the Cryptogamic Herbarium, Chicago Natural History Museum. × 350.

Figs. 2,3. *Ulothrix tenuissima (Kütz)* Kütz No. 380 [T. E. Hazen Herbarium]. Fig. 2, × 500; Fig. 3, × 2600. (Chicago Nat. History Museum[a], Cryptogamic Herbarium, coll. & det. T .E. Hazen 8 May, 1900).

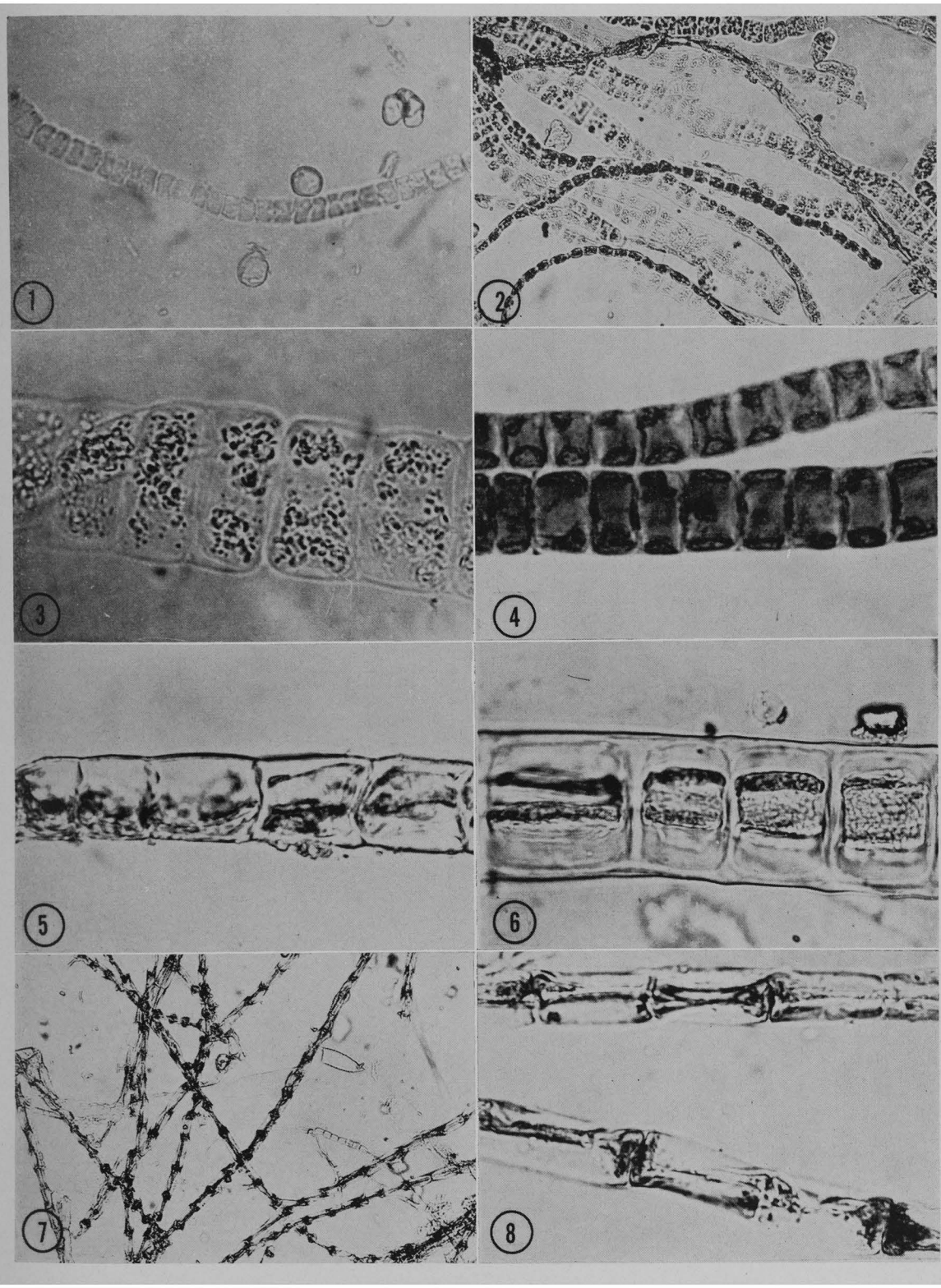
[a] We are grateful to Dr. Patricio Ponce de León for the loan of herbarium specimens and for providing certain data in connection with the same.

Fig. 4. *U. tenuissima* No. 380, from a slide prepared by the late Dr. T. E. Hazen. (Chicago Nat. History Museum, Cryptogamic Herbarium).

Fig. 5. *Ulothrix zonata* (Web. & Mohr) Kütz. (Specimen No. 962840, Chicago Nat. History Museum, Cryptogamic Herbarium. No. 19a, PBA, Collins, Holden and Setchell.)

Fig. 6. *Ulothrix zonata* (Web. & Mohr) Kütz. (Specimen No. 1131853 Chicago Nat. History Museum, Cryptogamic Herbarium. Coll. Fred A. Barkley, 7 July, 1943).

Figs 7, 8. *Nronema confervicolum* Lagerh. (#910 from the Herbarium of Francis Wolle; coll. Wittrock and Nordstedt. Fig. 7, × 200; Fig. 9 × 900).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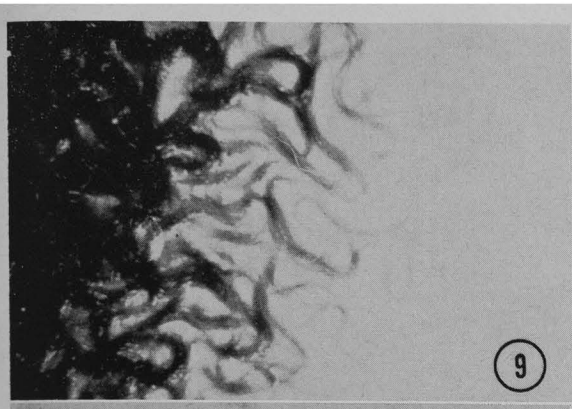
Figs. 9–16

Figs. 9, 10. *Ulothrix fimbriata*.—Fig. 9. Two-week-old colony on Bristol's agar, × 80.—Fig. 10. One-week-old filament; note fimbriate plastid, × 950.

Figs. 11–15. *Ulothrix gigas*.—Fig. 11. Cluster of germlings showing rounded and pointed apical cells, × 270.—Fig. 12. Young filament showing 2 pyrenoids in each cell, × 550.—Fig. 13. Basal cells of young filaments showing holdfasts with disc and bulb organization, × 1600.—Fig. 14. Apiculate apical cell, × 2000.—Fig. 15. Portion of a filament after zoosporogenesis, note pores, × 1000.

Fig. 16. *Ulothrix minuta*. One-month-old filaments, indefinite in length, × 100.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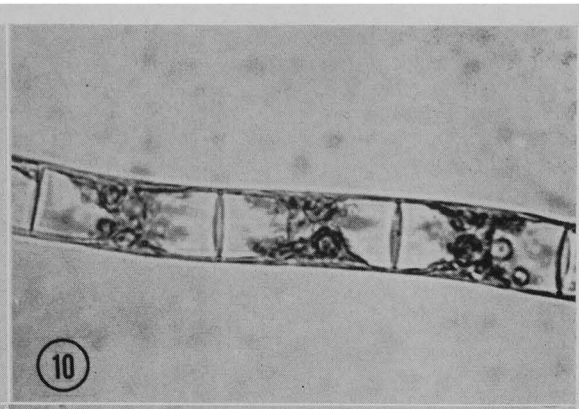
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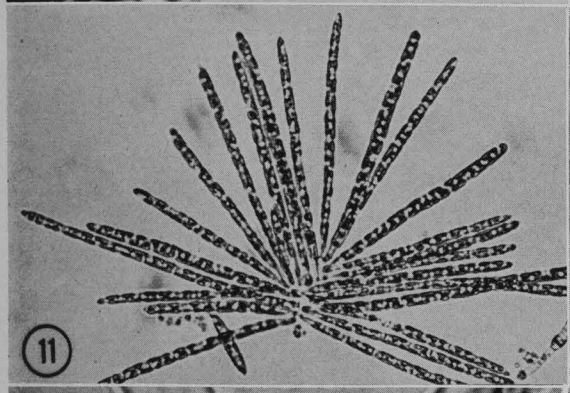
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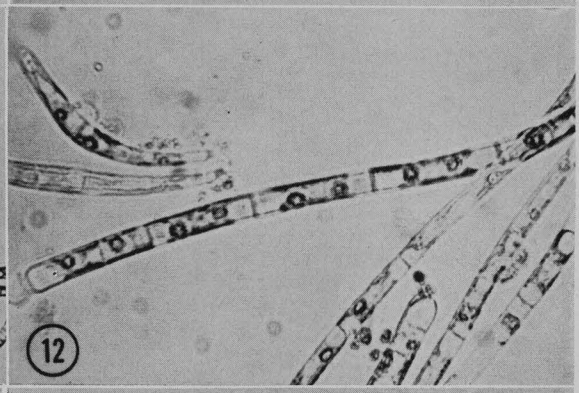
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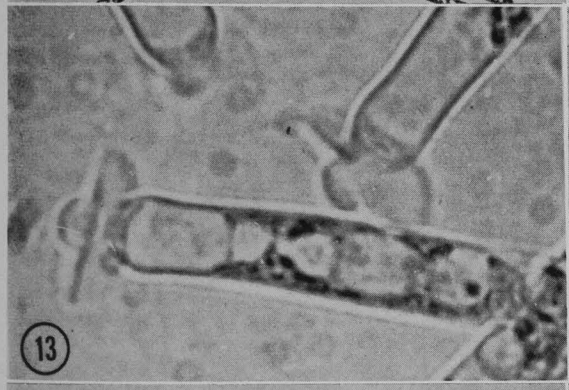
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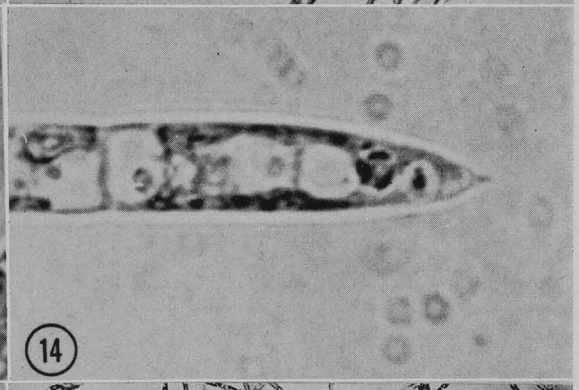
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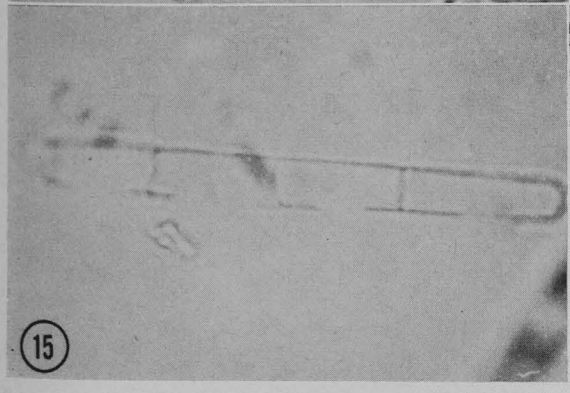
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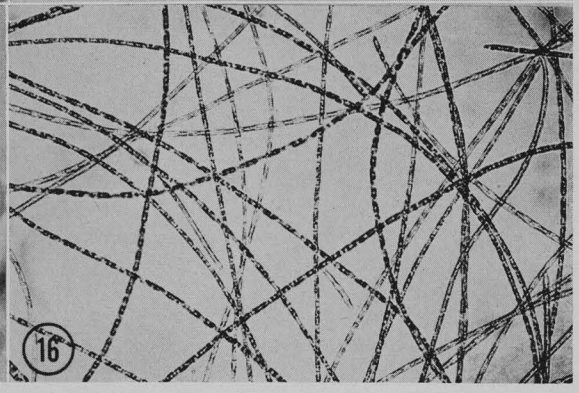
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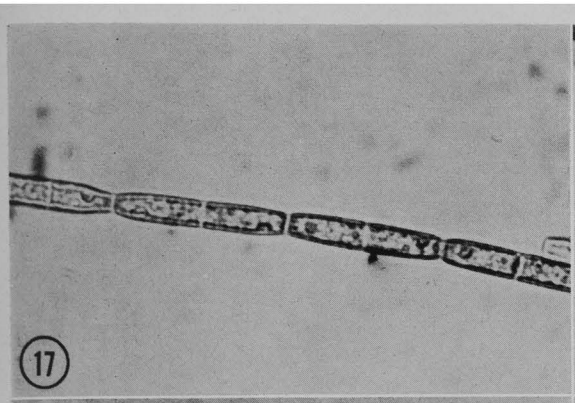
Figs. 17–24

Figs. 17–21. *Ulothrix minuta*.—Fig. 17. One-month-old filament with constrictions at the cross walls, × 660.—Fig. 18. Basal cells of young filament showing holdfast, × 660.—Fig. 19. Acuminate apex of germling, × 1800.—Fig. 20. Filament during zoospore release; note indication of characteristic wall remnants which remain, × 900.—Fig. 21. Portion of filament just prior to zoospore release, × 900.

Figs. 22–24. *Ulothrix acuminata*.—Fig. 22. One-week-old germlings, × 180.—Fig. 23. Young germlings showing holdfasts and typical, incompletely encircling plastids with several pyrenoids, × 530.—Fig. 24. Acuminate apical cell, × 800.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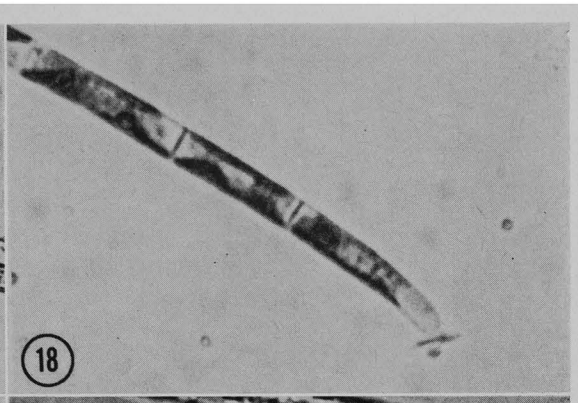
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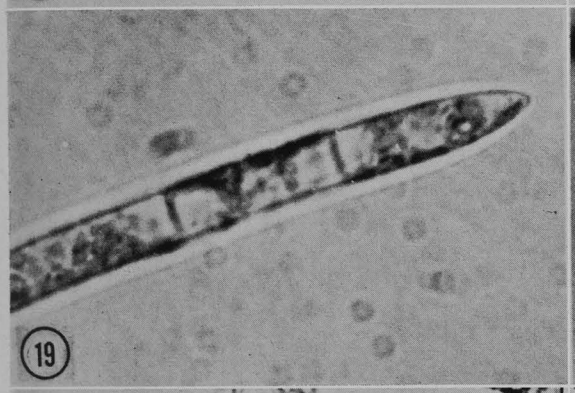
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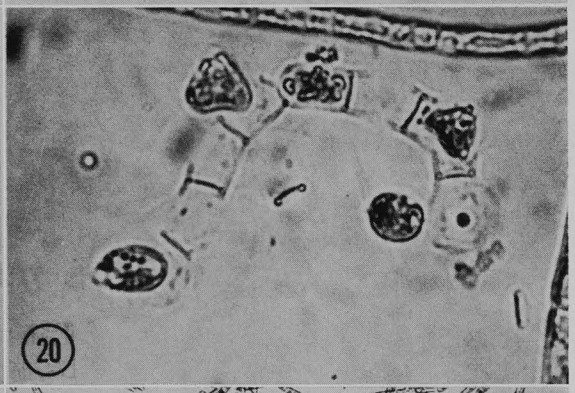
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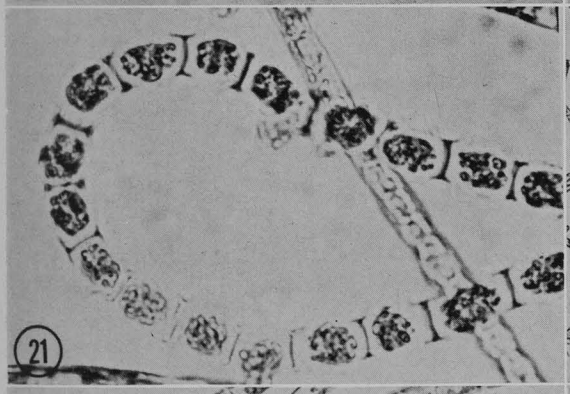
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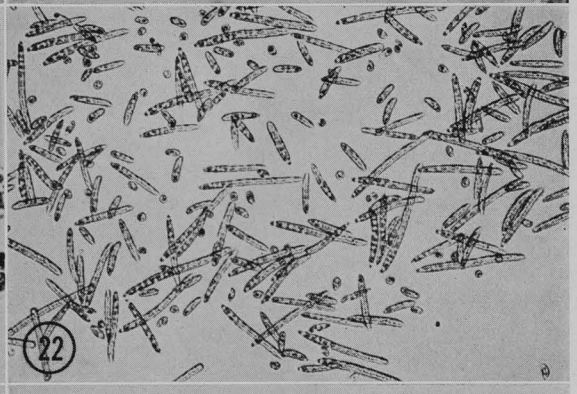
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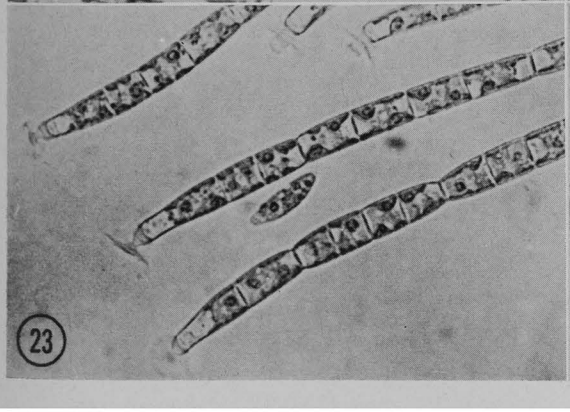
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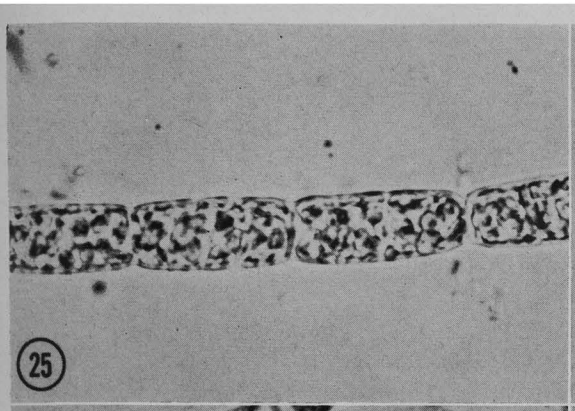
Figs. 25–32

Figs. 25–27. *Ulothrix acuminata*.—Fig. 25. One-month-old filament with a heavy accumulation of starch and slight constriction at the cross walls, × 1000.—Fig. 26. Zoospore showing 4 flagella, × 1500. —Fig. 27. Zoospore release, × 750.

Figs. 28–32. *Ulothrix belkae*.—Fig. 28. Germlings with pointed and rounded apical cells, × 700. —Fig. 29. Apiculate apical cell and typical holdfast, × 730.—Fig. 30. Dumbbell-shaped cells in one-month-old filament, × 100.—Fig. 31. One-month-old filament with heavy starch accumulation, × 800. Fig. 32. Thick-walled, akinete-like cells, × 850.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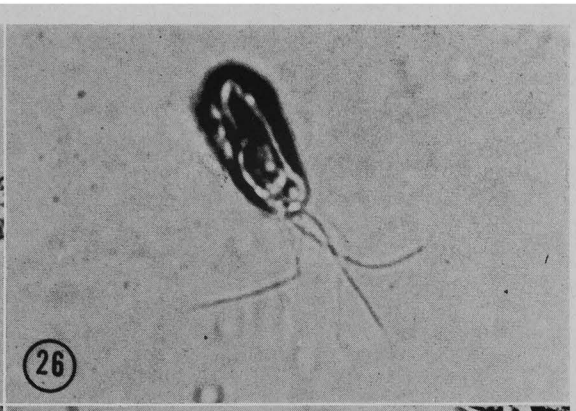
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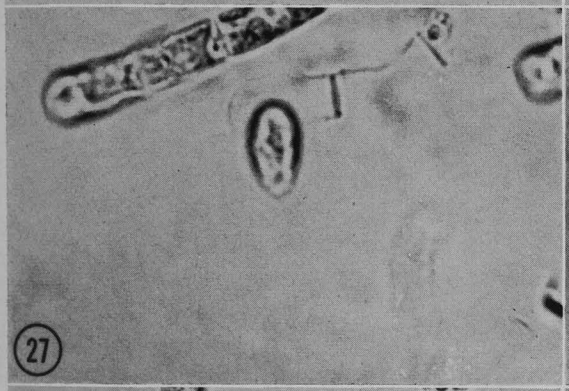
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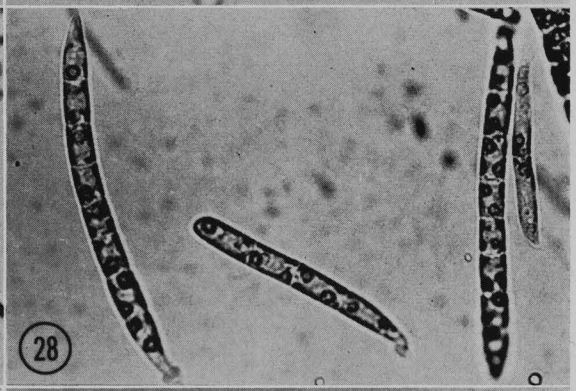
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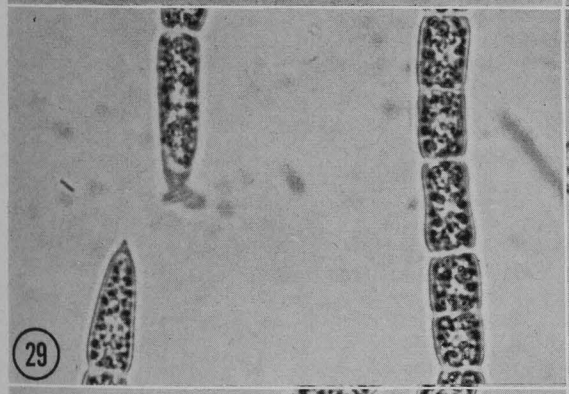
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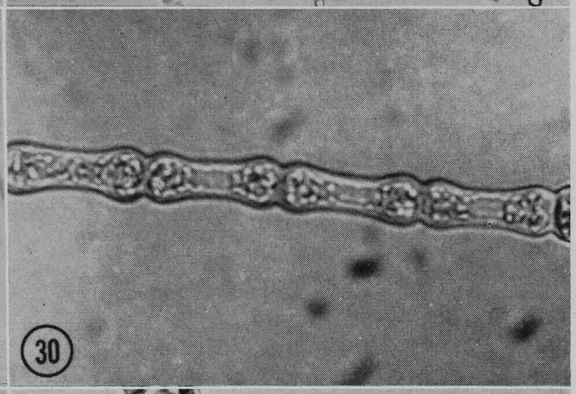
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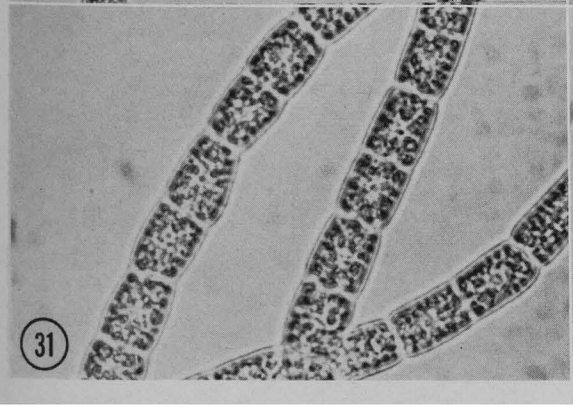
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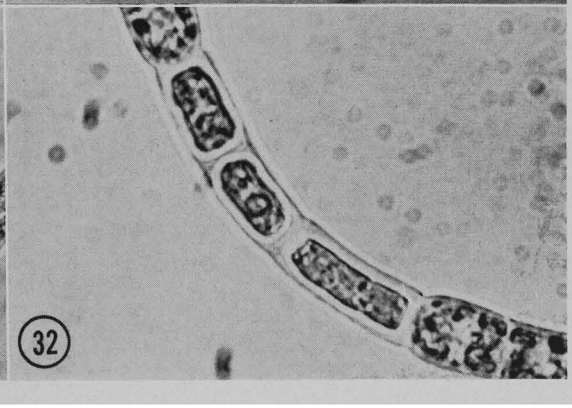
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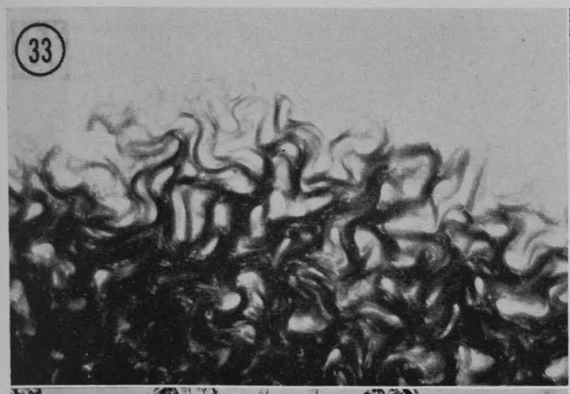
Figs. 33–40

Figs. 33–40. *Hormidium flaccidum*.—Fig. 33. Two-week-old colony on Bristol's agar, × 20.—Fig. 34. One-month-old filaments; most straight or slightly curved, × 200.—Fig. 35. One-month-old filaments; most greatly twisted, × 200.—Fig. 36. One-month-old filaments; note rope-like strands, × 200.—Fig. 37. One-week-old filaments; note plastid morphology, × 700.—Fig. 38. One-week-old filaments; note 1 distinct pyrenoid, × 750.—Fig. 39. One-week-old filaments stained with aqueous I_2-KI to show nuclei, × 1500.—Fig. 40. One-month-old filaments; note variable plastid length and some cells filled with starch, × 750.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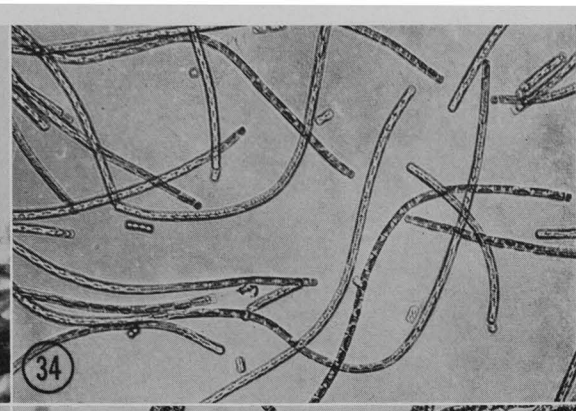
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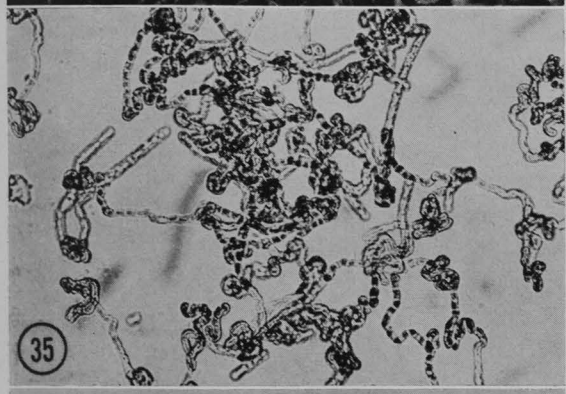
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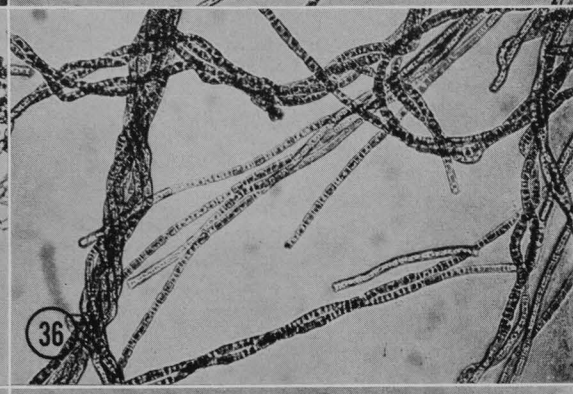
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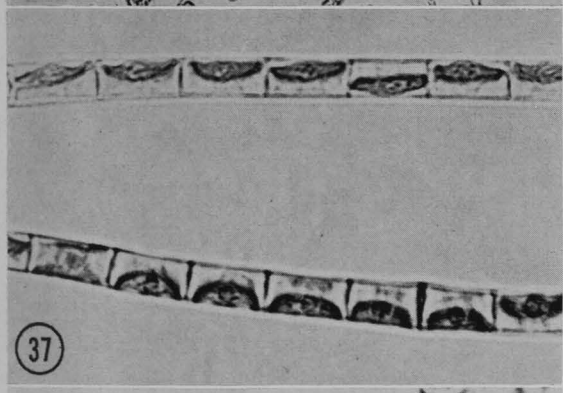
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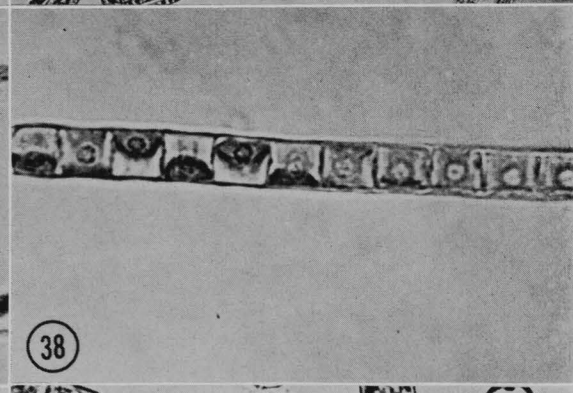
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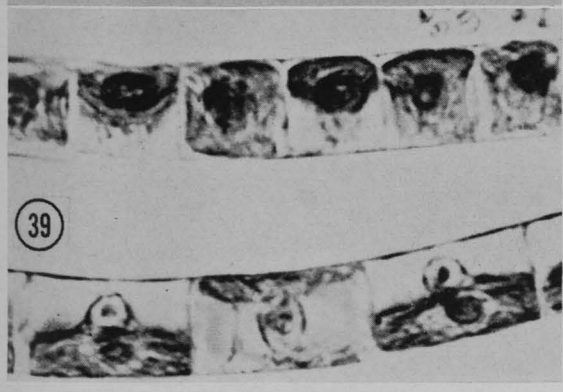
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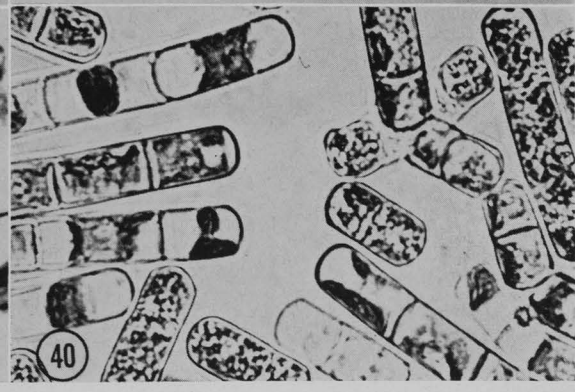
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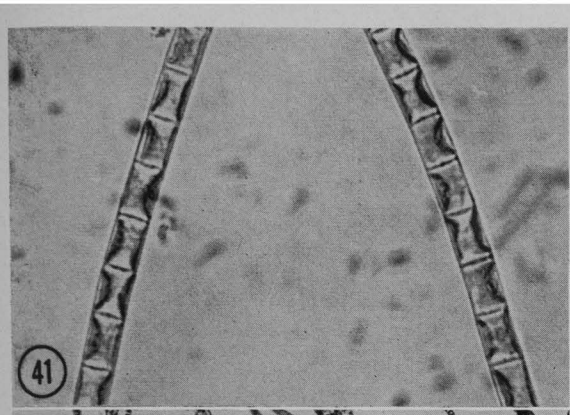
Figs. 41–48

Figs. 41, 42. *Hormidium flaccidum*.—Fig. 41. One-week-old filaments, with plastids encircling, in some cases, about ¾ of the cell lumen, × 750.—Fig. 42. One-week-old filaments; note rounded apical cells and nuclei in cytoplasmic bridges, × 750.

Fig. 43. *Hormidium subtilissimum*. Twisted filaments from a one-month-old culture, × 150.

Fig. 44. *Hormidium sterile*. One-week-old; note preponderance of single cells with a few short filaments, × 400.

Figs. 45–48. *Stichococcus bacillaris*.—Fig. 45. Two-week-old colony on Bristol's agar, × 20.—Fig. 46. One-month-old culture with some rather long filaments, × 200.—Fig. 47. One-week-old culture; note majority of single cells, × 200.—Fig. 48. One-week-old cells from an agar slant culture, × 1200.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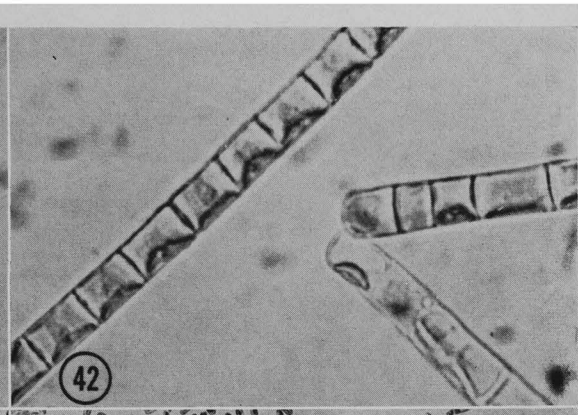
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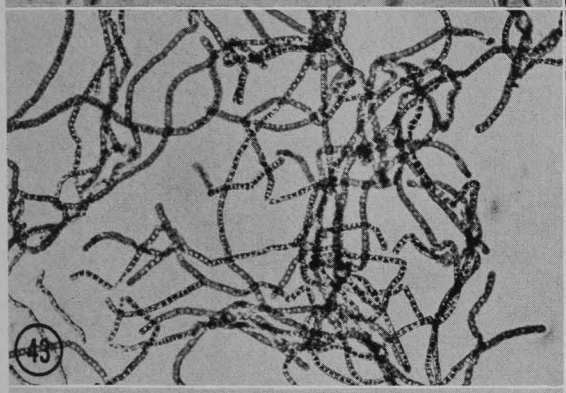
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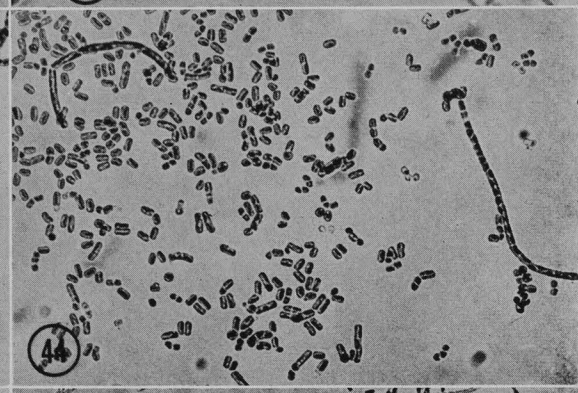
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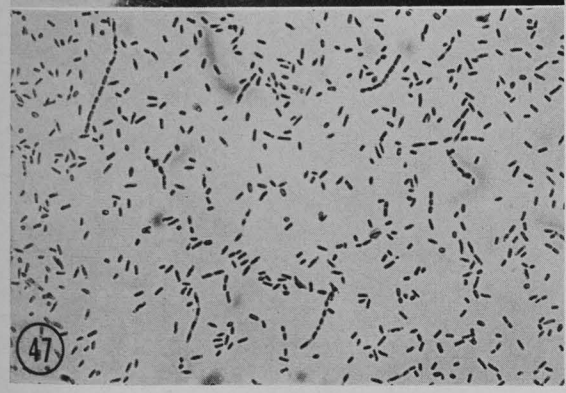
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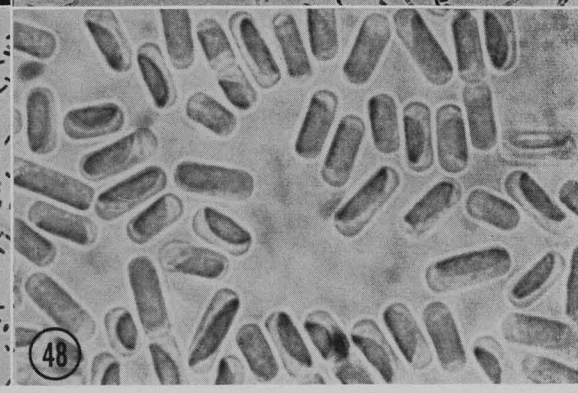
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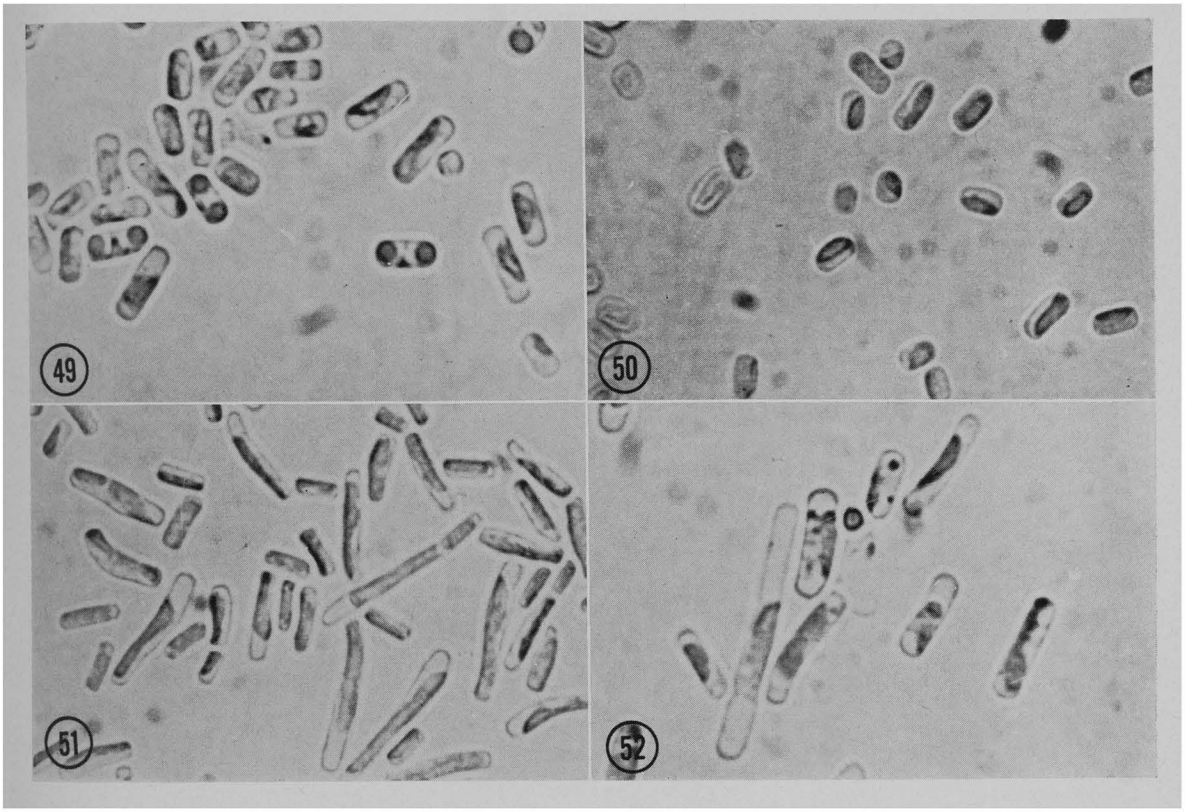
Figs. 49–52

Fig. 49. *Stichococcus bacillaris*. Two-month-old cells; note oil droplets at the ends of some cells, × 1200.

Fig. 50. *Stichococcus chodati*. Typical cells from a one-week-old culture, × 1200.

Figs. 51, 52. *Stichococcus mirabilis*.—Fig. 51. Cells from a one-week-old culture; note variable cell length, ×900.—Fig. 52. Cells from a two-month-old liquid culture, × 1200.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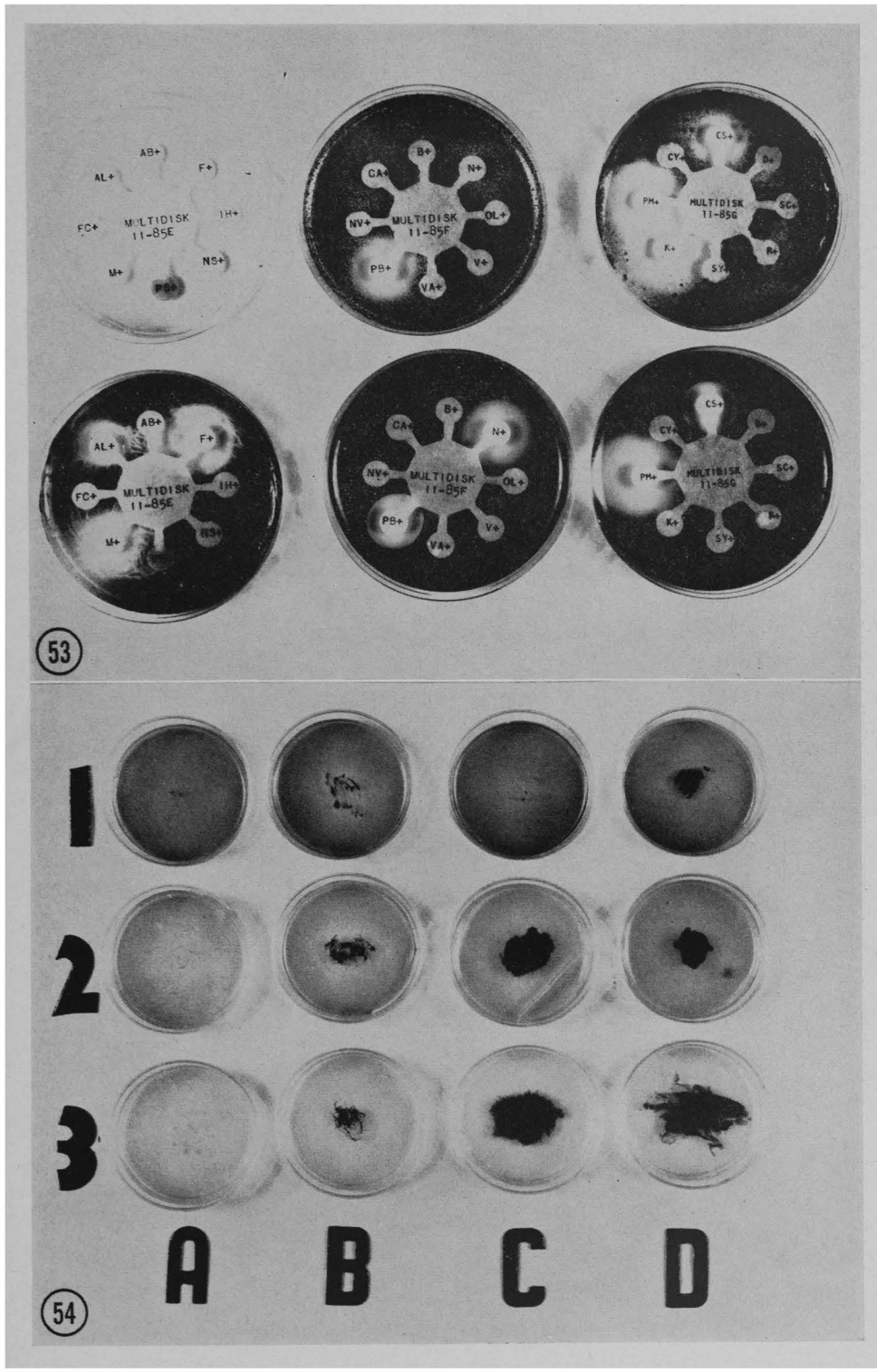
Figs. 53, 54

Fig. 53. Representative examples of the inhibition of selected organisms by certain antibiotic agents.

Fig. 54. Typical growth patterns of 4 different algae on 3 different concentrations of crystal-violet agar.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AL+
F+
FC+
MULTIDISK
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1H+
NS+
M+
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CA+
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NV+
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11-85F
OL+
PB+
V+
VA+
CS+
CY+
PM+
MULTIDISK
11-85G
SC+
K+
SY+
53
1
2
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A
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D
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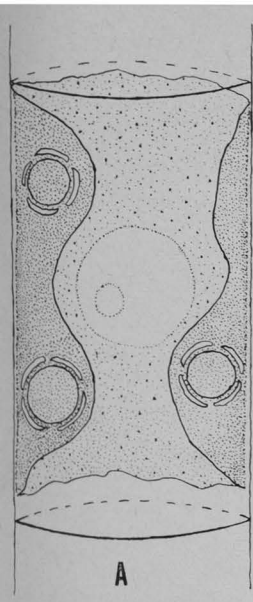
Figs. 55–57

Fig. 55. Diagrammatic representation of a *Ulothrix* cell.—A. Surface view.—B. Median optical section.—C. Transverse section.

Fig. 56. Diagrammatic representation of a *Hormidium* cell.—A. Surface view.—B. Median optical section.—C. Transverse section.

Fig. 57. Diagrammatic representation of a *Stichococcus* cell.—A. Surface view.—B. Median optical section.—C. Transverse section.



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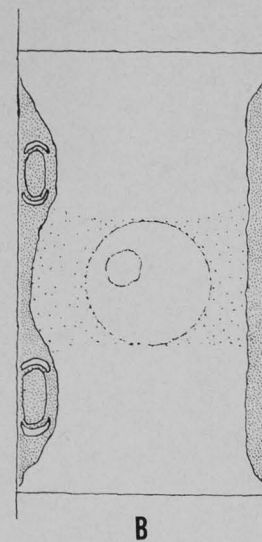

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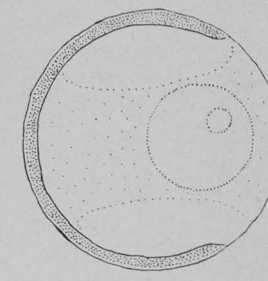

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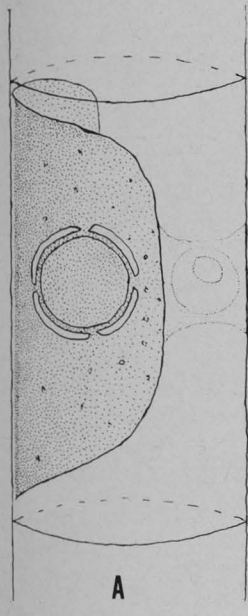

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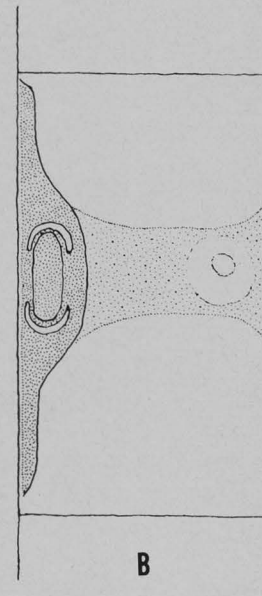

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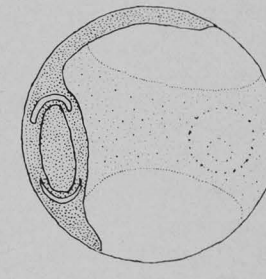

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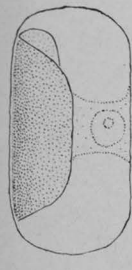

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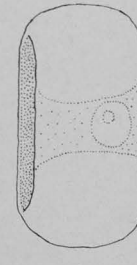

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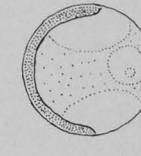

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