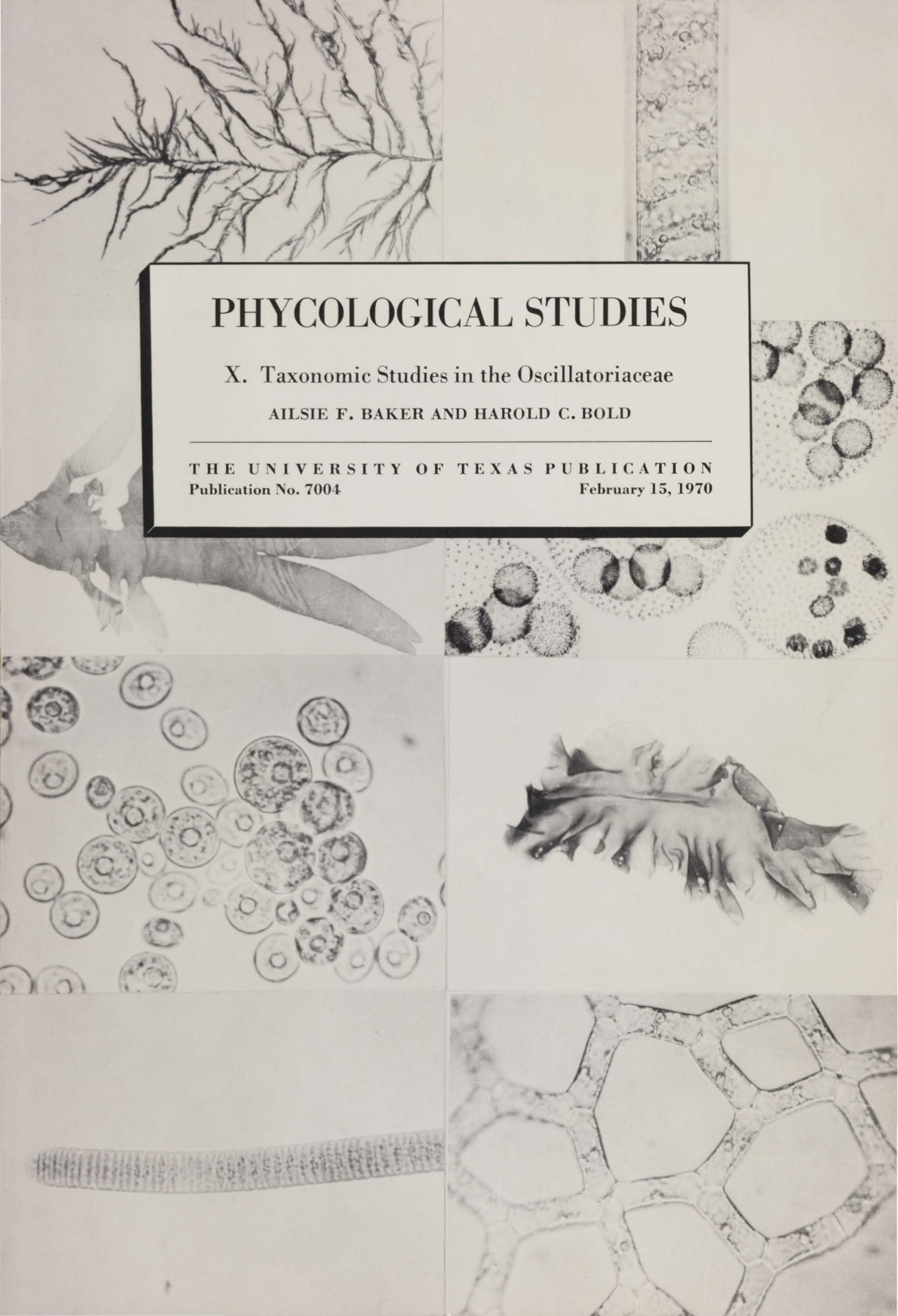

PHYCOLOGICAL STUDIES

X. Taxonomic Studies in the Oscillatoriaceae

AILSIE F. BAKER AND HAROLD C. BOLD

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Phycological Studies

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The University of Texas at Austin

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Introduction

The primary concern of this investigation was to examine in culture a number of isolates of blue-green algae belonging to the family Oscillatoriaceae to see which, if any, characteristics would prove consistent and, therefore, taxonomically useful.

Members of the family Oscillatoriaceae (Suborder Oscillatorineae, Order Oscillatoriales, Division Cyanophyta) are characterized by the filamentous, uniseriate organization of the cells and by the occurrence of reproduction by fragmentation (formation of hormogonia) only. Specialized cells such as akinetes and heterocysts are absent. The species are found in a great variety of geographical areas.

The problems encountered in trying to identify many blue-green algae, even in a general way, are numerous. Though the blue-green algae are generally conceded to have many affinities with the bacteria (Echlin and Morris, 1965; Pringsheim, 1968), they have only rarely been studied by or characterized on the basis of any microbiological techniques. Taxonomy depends upon the type specimen method. Taxa are described from natural collections, and keys for identification are frequently based on habitats, especially the type habitat, even though further collections may have considerably expanded the original distribution of the species. The occurrence of environmental variation has long been recognized, but until recently (Drouet and Daily, 1956; Drouet, 1962, 1963, 1964, 1968) it has not been seriously considered in the taxonomy of the group; also the consideration of variation was and is primarily field-based with few cultural studies supporting it.

Reports of variation among the Oscillatoriaceae were published by Agardh, Kützing, Hansgirg, and other nineteenth-century workers, but some of the supposed variation appears to have been inferred from observations of mixed populations. Gomont (1892) recognized that there could be some modification of the alga by the environment, but he thought reports of transformations of one species into a completely different one were the result of incorrect observations. In his monograph he retained a number of genera and species at least partly because he thought that uniting a large number of genera would result in one genus of such magnitude that it would be impossible to work with.

In the twentieth century Crow (1923, 1924, 1925, 1928) grew numerous and varied members of the Cyanophyta in the laboratory and also examined them in the field. With different concentrations of organic materials he observed variation in the structure of the trichome and of the sheath. Despite the numerous variations which he found, he retained the idea that real groups (or species) do occur under field conditions, and he did not attempt to revise the classification of any group although in some genera he could quite possibly have done so. Canabaeus (1929), studying the effects of various salt concentrations on the akinetes of *Anabaena*,

found that environmentally induced variation was indeed possible. She failed, however, to offer an alternative, more stable character for taxonomic use.

The variability in the false-branching genera has been investigated by several workers. Jaag (1943) collected *Scytonema* from very wet, moist, and very dry habitats; in the very wet places the sheath formation was that of the genus *Petalonema* and in the moist and dry habitats two different species of *Scytonema* occurred. He also found intermediates in the expected localities and accordingly grouped all three species as stata of *Scytonema myochrous*. The status designation (an infraspecific taxon applied to ecologically varying forms) has been used from time to time by other European workers (Golubić, 1965b; Komarek, 1958) in attempts either to describe more fully very large and diverse genera or possibly to avoid the problem of variation within the species.

Variation in numbers and type of branching in *Tolypothrix* was investigated by Hollerbach (1928) and, more recently, by Stein (1963).

Golubić and Kann (1967), on the basis of many measurements and statistical calculations, have concluded that cell measurements can be used to distinguish species in some cases. In *Tolypothrix distorta* v. *penicillata* they decided the significant cellular differences could not be attributed solely to ecological variation and advocated treating the two variants as separate species according to the original descriptions. Golubić (1965a) had earlier compared variability in marine Oscillatorias; based on variation in granulation, spiraling of trichomes, and terminal cell shape, he divided them into two groups of species.

Schwabe (1960, 1964) fully described the problems in blue-green algal systematics and investigated relative stability of various characteristics in *Plectonema* spp. and in *Oscillatoria acutissima* in the field and, to a more limited extent, in the laboratory. He concluded that there were definite species in *Plectonema*, that ecological variation, while present, was not nearly as prevalent as in *Lyngbya* and in some other genera, and that the taxonomic value of false-branching was limited. In his paper on *O. acutissima* (1964) he discussed the criteria given in various monographs and seen in his own investigations but refrained from a conclusive description due to lack of knowledge of variation.

Other experimental, morphologically oriented works with various blue-green algae have included those of McLachlan, Hammer, and Gorham (1963) and Gorham, *et al.* (1964) on *Aphanizomenon* and *Anabaena*, respectively. These investigators have examined the effects of various nutrients and of pH upon several strains of these genera. Pearson and Kingsbury (1966) used four species (*Gloeocapsa montana, Anabaena cylindrica, Calothrix membranacea*, and *Lyngbya* sp.) of four different families in an investigation devised to test induction of morphological variation. The characteristics of all varied, but in their isolates *Anabaena* and *Gloeocapsa* were changed less than *Lyngbya* and *Calothrix*.

Demeter (1956) investigated 13 species (eight genera) with regard to variation in different media and in different concentrations of several components of the

media. He noted the different plant-mass forms on agar and liquid and for the species he investigated, plant-mass form seemed to correspond with other generic attributes. He also found that increasing concentrations of salts generally produced changes in morphology.

One ecologically and culturally coordinated investigation of the type called for by Pringsheim (1967) is that of Sorensen and Conover (1962) on the *Lyngbya confervoides* mats occurring in lagoons along the Texas coast. They identified five layers in the mats and cultured samples from them. Differences in appearance of the protoplasm and in sheath coloration and in thickness were observed that were constant for each zone in the field and under simulated culture conditions.

Nostoc and *Anabaena* were extensively studied in culture by Kantz (1966). Kantz and Bold (1969) found that motility and configuration of the plant-mass correlated well with taxonomic position and that these attributes were much more constant in culture than some of the classical characteristics usually cited for these genera.

Sharp (1969) observed three strains of *Schizothrix calcicola* from three ecologically varying tropical marine habitats. He concluded that they were separate, infraspecific entities, but the exact meaning of his discussion is not clear.

The correlation of environmental variation with ultrastructural variation has only begun. Peat and Whitton (1967) found great variation in the lamellar structure of *Chlorogloea fritschii* at different ages and with or without a combined nitrogen source. They also (1969) studied the variation in a species of *Oscillatoria* in culture and in the field; finding several cultural forms of the field species which they thought might correlate with seasonal variation in the field.

It is no longer necessary to demonstrate that changes can occur in blue-green algae, for this is common knowledge.

The need is for defining conditions which cause changes and for studying the range of possible variations—provided, of course, that someone can determine how much is "possible." The necessity of experimental work for future systematic evaluation of blue-green algae has been noted, among others, by Schwabe (1960, 1964), Koster (1966), Padmaja and Desikachary (1967), Allen and Stanier (1968) and Kantz and Bold (1969).

Materials and Methods

The cultures used in this investigation were obtained primarily by isolation from soil samples and from freshwater, air, and marine collections. Cultures available from the Culture Collection of Indiana University were also used as well as some isolated by Dr. Chase Van Baalen. Table 1 summarizes the sources and isolators of the cultures used.

The culture medium most frequently used was Bold's Basal Medium—1N BBM (Bischoff and Bold, 1963) and modifications thereof, including 3N BBM (Brown and Bold, 1964), BBM-K (Kantz and Bold, 1969), and 3N BBMT (Groover and Bold 1969). Other media employed were biphasic soil water (Pringsheim, 1946), Shen-X (Shen, 1966), Kratz and Myers' Medium D—KMD (Kratz and Myers, 1955), C_8-10, a modification of Katz and Myers' Medium C (Van Baalen, 1967), and von Stosch's enriched sea water—VSE (von Stosch, 1964). The formulae for these media are given below.

1N BBM: 10 ml each of the following six major element stocks and 1 ml each of the four minor element solutions are added to 936 ml deionized water. The major element stock solutions are made by dissolving the indicated amount of salt in 400 ml deionized water.

$NaNO_3$	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

The minor element solutions are made as follows:

1. 50 g NaEDTA and 31 g KOH are dissolved in 1 liter deionized water.
2. 4.98 g $FeSO_4 \cdot 7H_2O$ are dissolved in 1 liter acidified water. Acid water is made by adding 1 ml concentrated H_2SO_4 to 999 ml deionized water.
3. 11.42 g H_3BO_3 are dissolved in 1 liter deionized water.
4. The following are dissolved in 1 liter deionized water:

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

3N BBM: This medium is made just as in 1N BBM except that 30.0 g $NaNO_3$ instead of 10.0 g, are used in the stock solution of this salt.

BBM-K: 5 ml each of the following major element stocks and 1 ml each of the BBM minor element solutions are added to 966 ml deionized water. The pH is adjusted to 7.5 with 1N HCl. The major element stocks are made by dissolving the indicated amount of each salt in 400 ml deionized water.

$NaNO_3$	10.0 g	NaCl	7.0 g
$CaCl_2 \cdot 2H_2O$	1.0 g	Tris (trihydroxy-methyl-	
$MgSO_4 \cdot 7H_2O$	3.0 g	aminomethane	20.0 g
K_2HPO_4	3.0 g		

3N BBMT: 10 ml of Tris stock and of the 3N BBM macro- and microelement stocks are added to 926 ml deionized water. The pH is adjusted to the desired point with 1N HCI. The Tris stock is made by dissolving 25 g Tris in 1 liter deionized water.

Biphasic Soil water: A small pinch of $CaCO_3$ and approximately one-fourth inch of garden soil are placed in the bottom of a test tube which is then filled three-fourths full with deionized water and autoclaved.

Shen-X: 1 ml of each of the following major element stocks as well as 1 ml of each of the BBM minor element solutions are added to about 600 ml deionized water. The volume is then brought to 950 ml, and the pH is adjusted to 7.2 with 1N HCl. Deionized water is then added to bring the total volume to 1 liter. The stocks are made by dissolving the indicated amount of salt in 100 ml deionized water.

$Co(NH_2)_2$	2.0 g	Na_2SiO_3	1.0 g
$CaCl_2 \cdot 2H_2O$	10.0 g	KCl	0.055 g
$MgSO_4 \cdot 7H_2O$	5.0 g	K_2HPO_4	5.0 g
Na_2CO_3	2.0 g		

KMD: 10 ml of each of the major element stocks cited below are added to 800 ml deionized water; 1 ml of H_5 microelement solution (= minor element solution No. 4 of BBM) is also added, and the total volume is brought to 1 liter with deionized water. The stocks are made by dissolving the indicated amount of each of the following salts in 500 ml deionized water:

$MgSO_4 \cdot 7H_2O$	7.5 g	$NaNO_3$	50.0 g
K_2HPO_4	50.0 g	EDTA	2.5 g
$Ca(NO_3)_2 \cdot 4H_2O$	0.05 g	$Fe_2(SO_4)_3 \cdot 6H_2O$	0.2 g

C9-10: 10 ml of each of the stock solutions are added to 800 ml deionized water; 1 ml of the A_5 microelement solution is also added. The total volume is brought to 1 liter with deionized water, and the pH is adjusted to 8.0 with 1N HCl. The stock solutions are made by dissolving the indicated amount of each of the following salts in 500 ml deionized water:

$MgSO_4 \cdot 7H_2O$	12.5 g	Na_2EDTA	8.25 g
K_2HPO_4	2.5 g	$Fe_2(SO_4)_3 \cdot 6H_2O$	0.2 g
$Ca(NO_3)_2 \cdot 4H_2O$	1.25 g	glycylglycine	50.0 g
KNO_3	50.0 g		

TABLE 1. *Sources of Cultures of Oscillatoriaceae*

No.	Isolate	Source	Isolator
1	*Schizothrix calcicola* v. *vermiformis*	soil, Univ. Texas campus, Austin	Baker
2	*Schizothrix calcicola* v. *compacta*	soil, Takamatsu Plateau, Japan	Groover
3	*Schizothrix calcicola* v. *densa*	soil, Welsh Bog, Elkhart Co., Ind.	Archibald
4	*Microcoleus lyngbyaceus*	pond, west of Austin, Travis Co., Texas	Baker
5	*Porphyrosiphon notarisii*	pool, Univ. Texas greenhouse, Austin	Baker
6	*Schizothrix calcicola* v. *discreta*	soil, Brackenridge Field Tract, Austin	Baker
7	*Schizothrix calcicola* v. *glomerulata*	soil, Brackenridge Field Tract, Austin	Baker
8	*Schizothrix calcicola* v. *diffusa*	soil, Univ. Texas campus, Austin	Baker
9	*Schizothrix calcicola*	Austin Sewage Disposal Pond	Baker
10	*Schizothrix calcicola* v. *radiata*	pond, Biol. Lab. Bldg., UT, Austin	Baker
11	*Schizothrix calcicola* v. *vermiformis*	Mobile, Alabama	Baker
12	*Microcoleus lyngbyaceus* v. *vermiformis*	soil, Pampa, Texas	Baker
16	*Schizothrix calcicola*	temporary pond, Brackenridge Field Tract, Austin	Baker
17	*Microcoleus vaginatus* v. *fuscus*	soil, Univ. Texas greenhouse, Austin	Baker
18	*Schizothrix calcicola* v. *vaginata*	sand, Marine Inst., Port Aransas, Texas	Baker
19	*Schizothrix calcicola* v. *vermiformis*	soil, Austin Texas	Baker
20	*Schizothrix calcicola*	contaminant in culture of *Selaginella*	Baker
21*	*Schizothrix calcicola*	pond, west of Austin, Travis Co., Texas	Baker
22*	*Oscillatoria lutea* v. *auxotrophica*	Rock Garden Pool, Zilker Park, Austin	Baker
23*	*Oscillatoria lutea*	soil, Brackenridge Field Tract, Austin	Baker

No.	Isolate	Source	Isolator
24*	*Oscillatoria lutea* v. *auxotrophica*	growing with *Typha* spp., UT greenhouse, Austin	Baker
25*	*Oscillatoria lutea* v. *contorta*	temporary pond, Brackenridge Field Tract, Austin	Baker
26*	*Microcoleus vaginatus* v. *fuscus*	contaminant of *Marchantia* culture	Van Baalen
27*	*Schizothrix calcicola* v. *minuta*	brackish water, east coast, N.Y.	Van Baalen
30	*Schizothrix calcicola* v. *olivacea*	soil, roadside, Rt. 77, LaVaca Co., Texas	Baker
32	*Schizothrix arenaria* v. *vermiformis*	soil, roadside, Rt. 77, LaVaca Co., Texas	Baker
33	*Microcoleus vaginatus* v. *araneaformis*	soil, roadside, Rt. 77, LaVaca Co., Texas	Baker
34	*Microcoleus vaginatus* v. *fuscus*	stream, Landa Park, New Braunfels, Texas	Baker
35	*Microcoleus irriguus*	stream, Landa Park, New Braunfels, Texas	Baker
38	*Oscillatoria lutea* v. *scabra*	soil, near Sacramento, California	Baker
39	*Porphyrosiphon notarisii*	sand, beach, Monterey, California	Baker
40	*Porphyrosiphon notarisii* v. *canus*	roadside ditch, Rt. 16, west of Sacramento, California	Baker
43	*Schizothrix calcicola* v. *discreta*	sulfur spring, Dripping Springs, Tex.	Baker
45	*Schizothrix calcicola* v. *glabra*	sulfur spring, Dripping Springs, Tex.	Baker
46	*Schizothrix calcicola* v. *fusca*	sulfur spring, Dripping Springs, Tex.	Baker
K2	*Schizothrix calcicola* v. *circinalis*	serpentine soil, near Austin, Texas	Kantz
K6	*Schizothrix calcicola*	soil, between Kingsville and Brownsville, Texas	Kantz
K17			
K18*	*Schizothrix calcicola*	petri dish exposed to air, Austin, Texas	Kantz
K44*			
K63			
K21	*Schizothrix calcicola* v. *densa*	petri dish exposed to air, Austin, Texas	Kantz

No.	Isolate	Source	Isolator
K27	*Microcoleus vaginatus cyano-viridis*	petri dish exposed to air, Austin, Texas	Kantz
K35	*Schizothrix calcicola*	unknown	Kantz
K36	*Schizothrix calcicola* v. *actinoformis*	unknown	Kantz
K38	*Schizothrix calcicola* v. *vaginata*	petri dish exposed to air, Austin, Texas	Kantz
K39	*Schizothrix calcicola* v. *densa*	petri dish exposed to air, Austin, Texas	Kantz
K43	*Schizothrix calcicola* v. *nitida*	petri dish exposed to air, Austin, Texas	Kantz
K51	*Schizothrix calcicola*	unknown	Kantz
K52	*Schizothrix calcicola*	soil, between Kingsville and Brownsville, Texas	Kantz
K55	*Microcoleus vaginatus* v. *conicus*	unknown	R. Smith
K56	*Schizothrix calcicola* v. *vaginata*	petri dish exposed to air, Austin, Texas	Kantz
K57*	*Schizothrix calcicola* v. *vermiformis*	unknown	Kantz
K58	*Schizothrix calcicola* v. *scabella*	soil, between Kingsville and Brownsville, Texas	Kantz
K59	*Schizothrix calcicola*	petri dish exposed to	Kantz
K60	v. *vermiformis*	air, Austin, Texas	
K61	*Schizothrix calcicola* v. *actinoformis*	petri dish exposed to air, Austin, Texas	Kantz
K62	*Schizothrix calcicola* v. *fuscoviridis*	petri dish exposed to air, Austin, Texas	Kantz
K107*	*Schizothrix calcicola* v. *spiralis*	petri dish exposed to air, Austin, Texas	Kantz
K120	*Schizothrix calcicola*	petri dish exposed to	Kantz
K123	v. *mucosa*	air, Austin, Texas	
K182*	*Schizothrix calcicola* v. *mucosa*	soil, Hawaii	Kantz
I386	*Oscillatoria lutea* v. *contorta*	Indiana University Culture Collection (IUCC) No. B386 as *O. chalybea* Maertens	Manten
I390	*Oscillatoria lutea* v. *contorta*	IUCC No. LB390 as *O. formosa* Bory	Pringsheim
1426*	*Schizothrix calcicola* v. *glomerulata*	IUCC No 426 as *Phormidium luridum* v. *olivacea* Boresch	Boresch

No.	Isolate	Source	Isolator
I427*	*Schizothrix calcicola* v. *glomerulata*	IUCC No. B427 as *Phormidium faveolarum* Gomont	De Marz
I428	*Microcoleus vaginatus* v. *radiatus*	IUCC No. B428 as *Oscillatoria tenuis* Agardh	Manten
I482*	*Schizothrix calcicola* v. *glomerulata*	IUCC No. B482 as *Plectonema notatum* Schmidle	Dyar
I485*	*Schizothrix calcicola* v. *amorpha*	IUCC No. 485 as *Phormidium* sp.	Allen
I487*	*Schizothrix calcicola*	IUCC Nos. 487, 488	Dyar
I488	v. *globerulata*	as *Lyngbya* sp.	
I596	*Schizothrix calcicola*	IUCC Nos. B596, 597	Dyar
I597*	v. *glomerulata*	*Plectonema boryanum* Gomont	
I598*	*Schizothrix calcicola* v. *glomerulata*	IUCC No. 598 as *Plectonema calothricoides* Gomont	Allen
I617	*Microcoleus vaginatus* v. *fuscorubens*	IUCC No. B 617 as *Symploca muscorum*	Hughes
I621*	*Microcoleus vaginatus*	IUCC No. 621 as *Lyngbya* sp.	Lewin
I622	*Microcoleus vaginatus* v. *undulatus*	IUCC No. 622 as *Lyngbya* sp.	Lewin
I1270	*Microcoleus vaginatus* v. *fuscus*	IUCC No. 1270 as *Oscillatoria prolifera*	Lewin
I1306	*Microcoleus vaginatus* v. *funiformis*	IUCC No. LB1306 as *Oscillatoria amoena* (Kütz). Gomont (Göttingen LB 1459–7)	unknown
I1309	*Microcoleus vaginatus* v. *funiformis*	IUCC No. LB1309 as *Oscillatoria animalis* (Göttingen LB1459–6)	unknown
I1546	*Microcoleus vaginatus* v. *glaber*	IUCC No. 1546 as *Lyngbya* sp.	M. M. Allen
I1547	*Microcoleus vaginatus* v. *glaber*	IUCC No. 1547 as *Lyngbya kuetzingii* Schmidle	Pringsheim

* Indicates axenic culture.

The A_5 microelement solution is made by dissolving the following amounts of salts in 1 liter deionized water:

H_3BO_3	2.86 g	MoO_3 (85%)	0.0177 g
$MnCl_2 \cdot 7H_2O$	1.81 g	$CuSO_4 \cdot 5H_2O$	0.079 g
$ZnSO_4 \cdot 7H_2O$	0.222 g		

VSE: 1 ml of each of the salt and vitamin stock solutions is added to 1 liter of natural seawater, which has been filtered through No. 1 filter paper. The final solution is autoclaved at 15 psi for 25 min. The stock solutions are made by dissolving the indicated amount of each salt listed below 200 ml deionized water:

$NaNO_3$	8.5 g	$MnCl_2 \cdot 4H_2O$	3.9 mg
$Na_2HPO_4 \cdot 12H_2O$	2.15 g	$Na_2EDTA \cdot 2H_2O$	0.74 g
$FeSO_4 \cdot 7H_2O$	55.6 mg		

The vitamins listed below are dissolved in a single aliquot of 200 ml deionized water:

biotin	0.2 mg	B_{12} soln (1,000 γ/ml)	0.2 ml
thiamine-HCl	40.0 ml		

At the beginning of the investigation two drops (0.1 ml) vitamin B_{12} solution (1000 γ/ml solution) and 10 ml 100× Eagle's vitamin solution were added routinely to the media. In an attempt to define more precisely the nutrition of the isolates, vitamins were omitted from the media for 3 months. The deterioration of some of the isolates during this time led to including vitamins once again in the media.

Cultures were maintained on agar-solidified 3N BBM medium (1.5% Difco Bacto-Agar) under standard conditions of light and temperature (150–300 ft c, 12–12 hr light-dark cycle, 22°C).

Unialgal cultures from soil samples were generally obtained by plating out aliquots of suspensions of the soil in sterile water on 3N BBM or BBM-K plates. Parts of the developing plant-masses were transferred to fresh media. Single hormogonia or minute portions of the plant-mass were used to establish stock cultures. For some samples, a slurry of deionized water and the sample was prepared; this was sprayed directly onto a 3N BBM or BBM-K plate from which unialgal plant-masses were subsequently transferred to new media. Freshwater and some marine collections were placed in liquid 3N BBM or BBM-K or on agar plates. Single filaments were isolated as they moved out from the center of the inoculum or were taken directly from the original collection, washed in 5–10 drops of sterile, deionized water, and placed in 3N BBM or BBM-K media.

Growth of solitary filaments frequently was increased when washed agar was used; possibly the increased growth was due to removal of peroxides or phenolic compounds (Van Baalen, 1965; Gorham, in Kantz and Bold, 1969). Washed agar is prepared by solidifying 1 liter deionized water with 32 g agar. This is cut

into small pieces, about 1–2 cm square, and immersed in deionized water. The water is changed about 10 times over a period of 72 hr. The water is then poured off, and the agar is mixed with 1 liter of double-strength medium and prepared for either plates or tubes (Kantz and Bold, 1969). The advantages of washing agar were equaled by the use of C_g-10 medium, which, as Van Baalen (1967) discussses, was designed especially to facilitate growth of single cells of blue-green algae. Peroxide formation is eliminated and there is no precipitation at pH 8.0. Growth of very short single filaments or trichomes was excellent in the isolates tested.

The axenic cultures used were all clonal; not all of the unialgal cultures were, however. Several isolates were received in an axenic state. Other cultures were purified by several methods. The spraying technique of Wiedeman, Walne, and Trainor (1964), with some slight modifications, was first used. With this method the material in an actively growing culture is borken up using a diSONtegrator unit (if agar slants are used, the slant is flooded with sterile water, the plant material scraped off, and then broken up). The material is then placed in a sterile centrifuge tube and centrifuged for a short time at a low speed. The supernatant is poured off, and 5–8 ml sterile water and a drop of Tween 80 are added. The tube is then placed in the diSONtegrator unit or on a test-tube stirrer for 1–2 min. The material is left in the Tween 80 solution for about 20 min; it is stirred or sonicated several times during this period. The tube is then centrifuged again, the supernatant poured off, and fresh sterile water is added. Sonication or stirring, centrifugation, and addition of fresh water follow. This step is repeated 7–10 times. After the successive washings, the remaining material is aspirated onto an agar plate. The plate is incubated under standard conditions for 3 days to 1 week; at the end of this period any filaments appearing uncontaminated are transferred to new media. The difficulty in breaking up the plant-mass with the diSONtegrator unit is the main drawback to this method.

Some axenic cultures were obtained by isolating single hormogonia that moved rapidly away from the plant-mass. Repeated isolations were made. This method was also used after apparently clean cultures had been obtained by spraying to insure clonal isolates.

The most successful method for obtaining axenic cultures was one developed by Dr. Chase Van Baalen[1] and used in his laboratory. This combines sonication and ultra-violet irradiation. Filaments from an actively growing culture are placed in distilled water and broken up with a sonicator (Branson 100 unit) to the point where most fragments are 1, 2, or 3 cells in length. The cell-liquid suspension is then placed on a magnetic stirrer in an open petri dish under an ultra-violet light source for 5 min (19 in from a GE 15T8 rod). At the end of this time small portions of the suspension are drawn into capillary tubes and pipetted into melted

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agar, and pour-plates are made. The plates are immediately returned to light (non-fluorescent) and allowed to incubate for 48–72 hr. At that time axenic filaments can be selected with a dissecting microscope and transferred to new media.

Cultures were checked for bacterial and fungal contaminants over a period of 2–3 weeks, on a number of different media, including: Nutrient broth (Difco); Nutrient agar (Difco); Proteose peptone agar (1 g/liter 3N BBM or BBM-K and 15 g agar); Yeast extract agar (5 g/liter dionized water and 15 g agar); Ma't extract agar (30 g/liter dionized water and 15 g agar); Thioglycollate broth (Difco); and Sabaraud dextrose agar.

Descriptions of the plant mass of the algal isolates were made at 2, 4, and 8 weeks; morphological descriptions were based on cultures from 1 week to 6 months of age. Measurements of cells were made with a Bausch and Lomb filar micrometer.

Stains for sheaths included methylene blue, chlor-zinc-iodide, India ink, and ruthenium red (0.02% aqueous solution).

Colors of the plant-masses at different ages were determined using an Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) Centroid Color Name Chart, Standard Sample No. 2106. Numbers and names of colors given in the descriptions and tables refer to this chart.

Photomicrographs were taken using a Zeiss "Ikon" camera attached to a Bausch and Lomb monocular microscope equipped with apochromatic objectives; and with a Kodak "Pony" camera attached to a Wild Heerbrugg stereoscopic binocular microscope. Other photographs were made with a Zeiss Super Contraflex, 35 mm, single-lense reflex camera with adaptable close-up lenses.

Taxonomic Criteria

Genera of the family Oscillatoriaceae have classically been distinguished (until Drouet's [1968] revision of the family, to be discussed below) one from another primarily on the basis of the presence or absence of a sheath. In addition to types of sheaths, the appearance of the filament, the habitat, and the color have been used as generic characteristics. At the specific level, cell size, cellular inclusions, habitat, and cell shape (especially of the terminal cells of filaments) have been the basis of differentiation among species.

The basic terminology of the taxonomy of the family is as follows. A *trichome* (Fig. 1B) is the aggregate of cells resulting from divisions of a cell in one plane only. The cells of trichomes secrete various colloidal substances. In many cases these products form a visible colloidal matrix about the trichome that is called the *sheath.* The latter (Fig. 1A, 1C) may be firm, soft, colorless or pigmented, homogeneous or layered (lamellated), distinct or diffluent or confluent. A *filament* (Fig. 1A, 1C) is a trichome surrounded by a sheath. A *hormogonium* (Fig. 1C) is a motile, usually short (2–50 cells), trichome. A *terminal cell* (Fig. 1D-H) is the one occurring at either end of a trichome; it may be rounded, pointed, capitate, rostrate, or

bent. The outer wall of the distal end of the terminal cell may be thicker than that of other walls (Fig. 1B). The trichome may be constricted at the crosswalls, the constrictions being very slight, or so great as to result in barrel-shaped cells in the trichome (Fig. 1B). The constrictions frequently are not conspicuous when the sheath is present. *Cellular inclusions,* at the level of light microscopy, include *granules* and *vacuoles* (Fig. 1A-B). Granules may be dark or translucent and scattered throughout the cell and/or aligned along either side of the crosswalls and along the periphery of the cells. Vacuoles may be black or reddish, irregularly shaped *gas vacuoles* (pseudovacuoles) or, in unhealthy cells, colorless rounded ones. The term *plant-mass* refers to the macroscopic aggregation of trichomes and/or filaments and hormogonia. There are many types, the most common and basic of which in agar culture are vermiform, minutely glomerulate, and compact (Figs. 3, 14, 37). The plant-mass types are more completely characterized in the descriptions of the isolates.

The taxonomic nomenclature of the family Oscillatoriaceae begins with that given by Gomont in the *Monographie des Oscillariées* (Gomont, 1892). Gomont recognized 15 genera, characterized mainly by the type of sheath and the disposition of the trichomes within the sheath (whether one or several, falsely branched, coiled or straight).

Geitler (1932) compiled the descriptions of the known genera, which had by then increased to 22. The type of sheath, morphology of the trichome, and habitat were the distinguishing characteristics used by Gomont and Geitler. The latter's publication, however, was largely a compilation and does not include taxonomic revisions.

The publication by Drouet (1968) of *Revision of the Classification of the Oscillatoriaceae* is the first revision since Gomont's. After studying an enormous number of herbarium specimens, including many of the type specimens, Drouet retained six genera. These genera were characterized by cytological differences—primarily arrangement of the granules in the cells and the morphology of the terminal cell. The morphology of sheath and trichome were considered to be too variable to be reliable taxonomically. Each of the 23 species recognized by Drouet has very broad morphological limits.

During the course of the present investigation, numerous specimens have been collected, many of which were established in unialgal and/or axenic cultures (Table 1). Observations over a 2-year period have repeatedly shown that while the Gomontian system of classification may have included unreliable characteristics, that of Drouet seemingly does also.

Perhaps the most variable characteristic in culture was that of granulation. The larger (in trichome width) isolates all contained distinct granules in the cells, but the number and arrangement varied considerably in the cells of individual trichomes. Since the presence or absence of dark granules along the crosswalls is a prime generic distinction in Drouet's system, difficulties in identification were

greatly compounded by these variations. Drouet, recognizing that some variation in granulation does occur, suggests staining with I_2KI followed by $ZnCl_2$ as a method of clearly demonstrating granules that were not already visible. Identifications were made on the assumption that granules were characteristic if they had ever been observed in an isolate.

The morphology of the terminal cell is more constant in culture than the granulations along crosswalls (the granules represent stored metabolites and thus variation in them is not surprising). Fluctuations in terminal cells did occur, but usually several observations of a culture at different ages revealed the pattern of development from rounded (newly formed) to the capitate, conical, etc. type characteristic of a given taxon. There are several isolates in which the thickened outer wall of the terminal cell was never observed or in which it was only very rarely observed; in spite of this, these isolates were assigned to the genus *Microcoleus* upon the recommendation of Dr. Drouet.

In culture, the sheath was in our experience as reliable and constant as the morphology of the terminal cell and considerably more reliable than the presence and disposition of granules. The isolates herein described included three types, with respect to sheaths: (1) those in which some sheaths were always seen, no matter how young the culture; these algae would thus be considered as *Lyngbya sensu* Gomont; (2) those in which sheaths were never seen, no matter how old the culture (*Oscillatoria sensu* Gomont); and (3) those in which sheaths appeared with age. The consistency of the sheath was also constant in most isolates.

The form of the plant-mass on agar also proved to be quite constant and accordingly taxonomically significant. Several different types of plant-mass were consistently observed in some instances for a single morphological type of trichome. That these plant-mass types are truly indicative of other differences seems to be borne out by the rudimentary physiological analysis herein conducted. A given plant-mass configuration could not, however, be correlated with a given species or genus as these are currently delimited by Drouet (1968) or as they were by Gomont (1892). Plant-mass type is related to motility and to other, in part undetermined, attributes. It is affected by light, pH, and mineral nutrition, but it is always, apparently, constant under a constant set of conditions. Plant-mass form is used in the descriptions of the isolates given in this dissertation as an infraspecific attribute. Since the nomenclature follows that of Drouet, the sheath type is also herein considered an infraspecific characteristic.

Descriptions of Certain Isolates of the Oscillatoriaceae in Culture

The descriptions of the isolates used in this investigation are based on observations of 2- to 4-week-old cultures grown on agar-solidified 3N BBM medium. As is discussed in the section on comparative studies with several media, there are

constant differences in plant-mass form, cell structure, and trichome structure in different liquid or solid media. The need for standard growth conditions as a basis for descriptions is thus obvious.

The attempt was made to identify the isolates with previously described species as summarized by Drouet (1968). For each isolate or group of isolates the generic and specific names listed are taken from Drouet's publication. The varietal names were appended during the course of this investigation to recognize certain isolates with constantly occurring attributes.

The brief discussion of the genera that follows is based on Drouet's descriptions; his complete description of each species represented in these isolates is also given. The additional descriptions of the isolates of each species are based on the differences observed in culture during this investigation. Keys to the varieties of each species are given following the descriptions.

The genera of Oscillatoriaceae recognized in Drouet's revision of the family are *Spirulina, Schizothrix, Oscillatoria, Porphyrosiphon, Microcoleus,* and *Arthrospira. Spirulina* is unicellular, or at least crosswalls can not be distinguished. The other five genera have been separated by Drouet on the basis of terminal cell form and type of granulation in the cells. *Schizothrix* is not granulated along the crosswalls, the outer wall of the terminal cell is never thickened, and only the terminal cell, if any, is attenuated. If the ends of the trichomes do taper, and the other characteristics remain as in *Schizothrix,* the genus is *Porphyrosiphon. Oscillatoria* includes those organisms without granules along the crosswalls that have terminal cells with thickened outer walls. Two additional genera are distinguished by the presence of many granules along each side of the crosswall; of these, *Arthrospira* includes those organisms in which the terminal cell has a thin outer wall and *Microcoleus* those in which the outer wall is thickened.

Species of *Schizothrix, Microcoleus, Porphyrosiphon,* and *Oscillatoria* were studied during this investigation.

Schizothrix calcicola (Agardh) Gomont

The most widely encountered taxon in this work was the unwieldy complex designated by Drouet as *Schizothrix calcicola.* Isolates with the cell structure described for this species were obtained from soils, freshwater ponds, marine and brackish environments, and from the air. The species is distinguished from others of the genus by Drouet by its small cellular diameter and its rounded terminal cell.

The 52 isolates studied in culture during this investigation, which, according to Drouet's system are *Schizothrix calcicola,* are described as 21 different taxa in this publication on the basis of morphological and physiological studies. The type of the plant-mass is the single most obvious attribute which varies consistently among the isolates, but there is also variation in cell shape, presence or absence of a sheath, and type of sheath, if present. For purposes of this investigation the most commonly encountered of the various isolates of *S. calcicola* is considered to represent the

species; others are listed as varieties. The distinctness of the varieties (both in *S. calcicola* and other species) is often much more apparent from macro- or microscopic observation, or from photographs, than from the written descriptions. Whether or not more widespread collection and subsequent culturing would always yield this form as the most common remains to be seen.

Drouet (1968) described *Schizothrix calcicola* as follows:

> Trichomes blue-green, yellow-green, yellow, olive, brown, red, violet, or gray-green, cylindrical or torulose or somewhat constricted at the cross walls, 0.2–3.5 μ (4.5 μ)[1] in diameter, here and there and in part increasing or decreasing in diameter, straight or curving or spiraled, capable of determinate or indeterminate growth in length, breaking by means of the destruction of an intercalary cell or by constriction at a cross wall. Cells quadrate or shorter, sometimes longer, than broad, 0.2–6 μ long, the protoplasm homogeneous or granulose, often pseudovacuolate, often with one or two granules at either side of a cross wall. Terminal cell at first cylindrical, becoming bulbose, often enlarged, the outer membrane not thickened, at first quasitruncate, then becoming rotund or rarely excentrically swollen. Sheath material hyaline, sometimes developing yellow, brown, blue, violet, or red pigments, often turning blue in chlorzinc-iodide. Plant consisting of long or short naked trichomes, or of trichomes in a homogeneous or laminose mucus, or of solitary or few or many trichomes within a more or less discrete cylindrical, often branched, sheath.

Descriptions of the isolates representing this species are given below.

Schizothrix calcicola (Agardh) Gomont (Figs. 2–4). Isolates 9, 16, 21, K6, K17, K18, K35, K44, K51, K52, K63

The plant-mass is rough-vermiform macroscopically (Fig. 2). At 14X, curved, smooth bundles (Fig. 3) of filaments are easily distinguished at the edges, extending outward from the central, densely vermiform mat. The color of the plant-mass is deep yellowish-green (132) at 2 weeks, becoming some shade of olive-green (125, 126) at 4 weeks.

The cells (Fig. 4) are 1.5–3.0 μ wide and 1.5–3.0 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls, but the constrictions are often not apparent in the filamentous condition. The cell contents may be homogeneous, or the central portion may appear as a bright translucent spot, or (as cultures age) several distinct granules may appear in the protoplast. The sheaths are colorless and narrow (to 0.5 μ wide).

Isolates 1, 8, 10, 11, 19, K2, K57, K59, and K60 are indistinguishable from the above-described isolates when observed microscopically, but they are characterized by different plant-mass types. There are slight, but ever-present, physio-

[1] Parts of the description enclosed in parentheses indicate additions to it. Isolate 30 was identified by Dr. Drouet as *S. calcicola*; the cells, however, are wider than 3.5 μ. Several other isolates also at times were wider than 3.5 μ.

logical differences in K57 as compared to K44 and 21, which are the only isolates of these groups that have been thus investigated. These isolates and their variations from *S. calcicola* are summarized in the several varieties proposed below.

Schizothrix calcicola (Agardh) Gomont v. **vermiformis** var. nov. Fig. 5.
Isolates 1, 11, 19, K57, K59, K 60

Varietas a specie typica differens eo quod magnificatione 14X pars centralis massae plantarum aspera apparet.

The plant-mass is very similar to that of isolates listed as belonging to *S. calcicola,* but a separation seems to be tenable as there are certain small, but consistent, differences. The plant-mass is macroscopically a dense vermiform mat with rough, curving bundles of filaments visible at the edges (Fig. 5). At 14X, the central part of the plant-mass appears rough. The color of the plant-mass is deep yellow-green (132) at 2 weeks and dark olive-green (126) at 4 weeks. The change in color from yellowish-green to reddish-orange (51), which is the ultimate color of both groups, is much slower than in the first group of isolates.

Schizothrix calcicola (Agardh) Gomont v. **diffusa** var. nov. Figs. 6–7.
Isolate 8

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata diffusa et sine proprietate.

The plant-mass is macroscopically nondescript. At 14X, it appears rough and dense in the center (Fig. 7) with very thin bundles of filaments at the edge (Fig. 6). Growth on agar is usually more diffuse than in the two previously discussed taxa. The color of the plant-mass is deep yellow-green (118) at 2 weeks and deep yellowish-green (138) at 4 weeks.

Schizothrix calcicola (Agardh) Gomont v. **circinalis** var. nov. Figs. 8–9.
Isolate K 2

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata tegetem asperam format; singula trichomata anfractus arctos efficientia.

The plant-mass is macroscopically a rough mat. At 14X, it appears as a mounded sheet of filaments except at the extreme edge where rough ropes of filaments may be seen (Fig. 8). The color of the plant-mass is deep yellow-green (118) at 2 weeks, and medium olive-green (125) at 4 weeks.

Single trichomes from time to time form tight coils on the agar (Fig. 9). Granules are rarely observed in the cells.

Schizothrix calcicola (Agardh) Gomont v. **radiata** var. nov. Figs. 10–11.
Isolate 10

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata radiata, fasciculis filamentorum et ad centrum et ad marginem arcte appressis.

The plant-mass is macroscopically radially arranged, with the bundles of filaments tightly appressed both at the center and at the edge (Fig. 10). At 14X, the ropes of filaments are rough, frequently confluent with one another, and centripetally oriented instead of curving in all directions (Fig. 11). The color of the plant-mass is very dark yellowish-green (138) at 2 weeks and dark olive-green (126) at 4 weeks.

The remaining isolates falling into the *Schizothrix calcicola* group vary from the "type" of the species not only in plant-mass but also have at least one other consistent difference. A large group of isolates obtained from the Indiana University Culture Collection belong in this caterogry. These isolates (I426, I427, I482, I485, I487, I488, I596, I597, I598) were identified originally (Starr, 1964) as members of three different genera (*Plectonema, Lyngbya*, and *Phormidium*). It would be interesting to have descriptions of these isolates when they were first taken into culture to see if there were differences then which in a common environment are no longer expressed. The possibility of a "laboratory species" was seriously considered until isolate 7 was studied; it has the same plant-mass characteristics and cell structure and has exhibited them in culture continuously from the time of its isolation.

Two plant-mass types occur among these algae (I485 is one type, the rest are the other) from the Culture Collection of Algae at Indiana University. The cell morphology is the same in all except for minor and somewhat variable differences. The maximum width of the trichomes in I598 is always narrower than that in the other isolates, but the width falls into the lower limits of the range of the rest. Sheaths are not of general occurrence; they are present to a limited extent in I485, I597, and I426. The general descriptions of this group of isolates are given below under *S. calcicola* v. *glomerulata* and v. *amorpha.*

Schizothrix calcicola (Agardh) Gomont v. **glomerulata** var. nov. Figs. 12–15. Isolates 7, I426, I427, I482, I487, I488, I596, I597, I598

Varietas a specie typica differens eo quod mass plantarum sine microscopo observata in area centrali densa, fasciculis trichomatum arcuatis ad marginem; magnificatione 14X observata, pars centralis minute glomerulata apparet; cellulae interdum dolioformes; culturae post 2–3 hebdomades omnino e hormogoniis plerumque compositae.

The plant-mass macroscopically has a dense central area with curving bundles of trichomes at the edges (Fig. 12). At 14X, the center appears minutely glomerulate (Fig. 13); the bundles of trichomes appear zigzagged and fade into single trichomes, which may be very intricately curved, at the margins (Fig. 14). The color of the plant-mass is deep, to very dark, yellowish-green (132/138) at 2 weeks and dark olive-green (126) at 4 weeks.

The cells (Fig. 15) are 1.5–4.0 μ (1.5–2.5 μ in I598) wide and 1.0–4.0 μ long. There are very great variations in width of cells in the same trichome and of tri-

chomes in the same culture. The terminal cell is rounded. The trichomes are constricted at the crosswalls; constrictions may be slight or pronounced. At times the cells are slightly barrel-shaped. The cell contents may have one central translucent zone or several translucent granules (3–4-week-old cultures and older.) The cultures are generally in a hormogonial state after 2–3 weeks (except for 7, I427 and I598). Sheaths occur in older cultures of I426 and I597 and are colorless, narrow, and soft.

Schizothrix calcicola (Agardh) Gomont v. **amorpha** var. nov. Figs. 16–17.
Isolate I485

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata sine proprietate; vaginae rare in culturis vel in periodo immobili, sporadice, autem, apparentes.

The plant-mass is macroscopically nondescript. At 14X, it appears rough and dense in the center and has rough bundles of filaments at the edges which fade into single filaments (Figs. 16–17). The color of the plant-mass is deep yellowish-green (132) at 2 weeks and medium olive-green (125) at 4 weeks.

Sheaths occur only on some individuals in cultures 3 to 4 weeks old and older.

The following isolates are distinctive either in plant-mass type or cell morphology or both.

Schizothrix calcicola (Agardh) Comont v. **vaginata** var. nov. Figs. 18–19.
Isolates 18, K38, K56

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata sine proprietate; filamenta vaginas solidissimas persistentes (usque ad 0.8 μ lat.) habentia.

The plant-mass is macroscopically nondescript. At 14X, it is rough; thin bundles of filaments are seen at the edges (Fig. 18). The color of the plant-mass is deep yellowish-green (132) at 2 weeks and very dark yellowish-green (138) at 4 weeks.

The cells (Fig. 19) are 2.0–3.5 μ wide and 1.2–2.5 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls. The cells have one to several dark or translucent granules. Gas vacuoles have been observed in isolate K56 in very small numbers. The sheaths are very firm, up to 0.8 μ wide, and colorless.

Schizothrix calcicola (Agardh) Gomont v. **olivacea** var. nov. Figs. 20–21.
Isolate 30

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata sine proprietate; cellulae alquantulo minores (1.2–3.0 μ lat., 1.0–2.2 μ long.) quam in planta typica; trichomata saepe in spiram contorta aut undulata.

The plant-mass is macroscopically nondescript and has feathery, thin bundles of filaments radiating from the central mat (Fig. 20). At 14X, the individual

filaments may be seen growing among the bundles of filaments. The color of the plant-mass is dark olive-green (126) at 2 and 4 weeks.

The cells (Fig. 21) are 3.7–4.5 μ wide and 1.5–3.0 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls, but the constrictions are not apparent in the filamentous condition. The cells may contain several dark and translucent granules. The trichomes are frequently undulate. The sheaths are firm, 0.5–1.0 μ wide, and colorless to pale yellow-brown.

Schizothrix calcicola (Agardh) Gomont v. **minuta** var. nov. Figs. 22–24. Isolate 27

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata sine proprietate; magnificatione 14 × observata ut massa fasciculorum tenuium asperorumque filamentorum visa. Massa plantarum profunde flavovirens 117(118) pos post duas quattuorque hebdomades.

The plant-mass is macroscopically nondescript. At 14X, it appears as a mass of thin, rough bundles of filaments (Figs. 22–23). The color of the plant-mass is deep yellow-green (117/118) at 2 and 4 weeks.

The cells (Fig. 24) are 1.2–2.0 μ wide and 1.0–2.2 μ long. The terminal cell is rounded. The trichomes are slightly constricted at the crosswalls. The cells frequently contain several translucent and dark granules. Some of the trichomes may be spiraled or undulate. The sheaths are narrow and colorless.

Schizothrix calcicola (Agardh) Gomont v. **actiniformis** var. nov. Figs. 25–26. Isolate K36, K61

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata radiata; cellulae 2.5–4.5 μ lat., 1.0–3.0 μ long.; trichomata per 3.6 cellulas ultra vaginam typice extensa; vagina solida (usque ad 0.5 μ lat.), sine colore ad dilute flavam.

The plant-mass is macroscopically radial with ropes of filaments swirling close to the center of the plant-mass (Fig. 25). The color of the plant-mass is deep yellowish-green (132) at 2 weeks and dark olive-green (126) at 4 weeks.

The cells (Fig. 26) are 2.5–4.5 μ wide and 1.0–3.0 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls; constrictions are easily seen in the trichomes which characteristically extend for 3–6 cells beyond the sheath. The cell contents are granular. False branches may occur sparsely as the culture ages. The sheaths are firm, distinct (up to 0.5 μ wide), and colorless to pale yellow.

Schizothrix calcicola (Agardh) Gomont v. **fuscoviridis** var. nov. Figs. 27–28. Isolate K62

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata radiata, usque ad duas menses semper atro-virides; cellulae 2.5–4.0 μ lat., 1.5–2.5 μ long., vaginae nullae.

The plant-mass is macroscopically radial. At 14X, straight bundles of trichomes are seen to radiate from a dense, rough central mass (Fig. 27). The color of the plant-mass is dark olive-green (126) at 2 and 4 weeks.

The cells (Fig. 28) are 2.5–4.0 μ wide and 1.5–2.5 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls. The cell contents may be granular or the central portion of the cell may appear as a translucent area. Sheaths have not been observed.

Schizothrix calcicola (Agardh) Gomont v. **spiralis** var. nov. Figs. 29–30.
Isolate K107

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata radiata; trichomata vaginis distinctis (usque ad 0.8 μ lat.) praedita; trichomata cum aut sine vagina, per tantam longitudinem in spiram perspicue contorta.

The plant-mass is macroscopically radially arranged with very short bundles of filaments extending out from the central mat (Fig. 29). The color of the plant-mass is very dark yellowish-green (138) at 2 and 4 weeks.

The cells (Fig. 30) are 2.0–3.0 μ wide and 1.0–2.5 μ long. The terminal cell is rounded. The trichomes may be very slightly constricted at the crosswalls. The cell contents may include a translucent zone or several translucent granules. The sheaths are 0.2–0.8 μ wide, firm, and colorless. The filaments are distinctly spiraled along their entire length.

Schizothrix calcicola (Agardh) Gomont v. **densa** var. nov. Figs. 31–32.
Isolates 3, K21, K39

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata compacta, non patens; magnificatione 14X observata aspectu aspera; cellulae saepe piluliformes; vagina solida (usque ad 1.0 μ lat.), sine colore.

The plant-mass is macroscopically compact. At 14X, it is rough (Fig. 31). The color of the plant-mass is very dark yellowish-green (138) at 2 and 4 weeks.

The cells are 2.0–4.0 μ wide and 1.0–2.5 μ long (Fig. 32). The terminal cell is rounded. The trichomes are constricted at the crosswalls and may be bead-like in appearance. In very old cultures some false branches occur. The sheaths are up to 1.0 μ wide, colorless, and firm.

Schizothrix calcicola (Agardh) Gomont v. **scabella** var. nov. Figs. 33–34.
Isolate K58

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata compacta, non patens; magnificatione 14X observata, penicilli erecti e superficie emergentes.

The plant-mass is macroscopically compact. At 14X, it appears as a dense, rough mat with tufts of filaments on the surface (Fig. 33). The color of the plant-mass is very dark olive-green (128) at 2 and 4 weeks.

The cells (Fig. 34) are 2.5–3.0 μ wide and 1.5–2.5 μ long. The terminal cell is rounded. The trichomes are frequently slightly constricted; this is usually not apparent in the filamentous condition. The cells contain several granules; occasionally one or two dark granules are seen on either side of the crosswalls. The sheaths are firm, colorless and 0.2–0.5 μ wide.

Schizothrix calcicola (Agardh) Gomont v. **compacta** var. nov. Figs. 35–36. Isolate 2

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata compacta, non patens; magnification 14X observata minute glomulata; cellulae 2.5–3.5 μ lat., 1.5–4.0 μ long.; culturae vetustiones (4–8 hebdomadum) omnino e hormongoniis brevibus saepe consistantes; vaginae nullae.

The plant-mass is macroscopically compact. At 14X, it appears minutely glomerulate except at the extreme edges where some intricately curved and coiled bundles of trichomes are seen (Fig. 35). The color of the plant-mass is very dark yellowish-green (138) at 2 and 4 weeks.

The cells (Fig. 36) are 2.5–3.5 μ wide and 1.5–4.0 μ long. The terminal cell is rounded. The trichomes are slightly constricted; the cells may be barrel-shaped. Older cultures (4–8 weeks) frequently consist entirely of short hormogonia. The central area of the cell frequently appears as a translucent zone. Sheaths have not been observed.

Schizothrix calcicola (Agardh) Gomont v. **nitida** var. nov. Figs. 37–38. Isolate K43

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata compacta, non patens, atque butyracea; magnificatione 14X observata, minute glomerulata.

The plant-mass is macroscopically compact. At 14X, it is a dense, undulate to minutely glomerulate mass (Fig. 37). The color of the plant-mass is dark olive-green (126) at 2 weeks, very dark olive-green (128) at 4 weeks.

The cells (Fig. 38) are 1.5–2.0 μ wide and 1.5–2.5 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls. The cell contents may be granular. Sheaths have not been observed, but the butyrous consistency of the plant-mass would seem to indicate the presence of a colloidal material.

Schizothrix calcicola (Agardh) Gomont v. ***mucosa*** var. nov. Figs. 39–40. Isolate K120, K123, K182

Varietas a specie typica differens eo quod trichomata in materia communi colloidali dispersa, vaginis singulis distinctis raris.

The plant-mass is vermiform macroscopically with many curved bundles of trichomes (Fig. 39). At 14X, among the bundles, growing both on and into the agar,

solitary trichomes occur. The color of the plant-mass is very dark yellowish-green (138) at 2 weeks and deep yellowish-green (118) at 4 weeks. The trichomes are positively phototactic.

The cells (Fig. 40) are 2.0–4.0 μ wide and 1.5–4.0 μ long. The terminal cell is rounded. The trichomes are constricted at the crosswalls; the constrictions may be so great as to give a bead-like appearance to the cells. The cells may have several translucent granules. Distinct sheaths are of very rare occurrence, but diffuse colloidal material may usually be demonstrated with methylene blue stain. In isolate K182 sheath material is better defined; in cultures 3–4 weeks old staining with methylene blue or India ink frequently revealed wide sheaths with several trichomes scattered through them.

Schizothrix calcicola (Agardh) Gomont v. **glabra** var. nov. Figs. 41–42.
Isolate 45

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata tegetem densam levemque format; cellulae 2.0–2.5 μ lat., 2.5–4.5 μ long.; unica granula translucens in utraque cellulae extremitate saepe reperta; vaginae distinctae non apparent, multa materia colloidalis diffusa, autem, adest.

The plant-mass is macroscopically a smooth, dence mat, which shows bundles of trichomes in several layers in the center and single trichomes at the edges (Fig. 41). At 14X, the trichomes and bundles at the margin appear rough. The color of the plant-mass is deep yellowish-green (132) at 2 weeks and deep yellow-green (118) at 4 weeks.

The cells (Fig. 42) are 2.0–2.5 μ wide and 2.5–4.5 μ long (usually 3.5–4.0 μ long). The terminal cell is rounded. The cells frequently contain one translucent granule at either end near the crosswalls; there may also be several granules scattered throughout the cell. The crosswalls are often hard to distinguish. The trichomes are not constricted at the crosswalls. Distinct sheaths have not been observed, but diffuse colloidal material may usually be demonstrated with methylene blue stain. The trichomes adhere very firmly to one another, and a large sheet-like portion of the plant-mass is easily removed from an agar surface.

Schizothrix calcicola (Agardh) Gomont v. **fusca** var. nov. Figs. 43–45.
Isolate 46

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata in centro confertissima brevisque; ad marginem fasciculos filamentorum arcuatas praebens; brunnea.

The plant-mass is macroscopically very dense and smooth in the center with many curving bundles of filaments at the edge (Fig. 43). At 14X, the bundles of trichomes are smooth (Fig. 44). The color of the plant-mass is dark olive-brown (96) at 2 and 4 weeks.

The cells (Fig. 45) are 2.0–2.5 μ wide and 1.5–2.5 μ long. The terminal cell is

rounded to slightly conical. The cells contain no distinct granules. The trichomes are slightly constricted at the crosswalls; the crosswalls appear very thick. Sheaths are not ubiquitous, but they are narrow (0.3 μ wide) and colorless when they are seen.

Schizothrix calcicola (Agardh) Gomont v. **discreta** var. nov. Figs. 46–48. Isolates 6, 43

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata massas rotundatas discretas ad marginem massae centralis confluentis praebet; trichomata ad dissepimenta valde constricta; vaginae nullae.

The plant-mass macroscopically shows discrete, round masses at the margin; the central area is a confluent mass of indeterminate shape (Figs. 46–47). At 14X, a few trichomes are seen growing between the central and exterior portions. The color of the plant-mass is deep yellowish-green (132) at 2 weeks and medium olive-green (125) at 4 weeks. The isolate exhibits negative photoaxis.

The cells (Fig. 48) are 1.8–2.5 μ wide and 1.5–3.5 μ long. The terminal cell is slightly rounded; several dark granules usually occur in an otherwise clear area in the outer end of the terminal cell. The trichomes are distinctly constricted at the crosswalls. There is a dark granule at each end of the cell usually; chromatoplasm appears to be located mainly at the periphery of the cell. Sheaths have not been observed.

Schizothrix arenaria (Berkeley) Gomont

Schizothrix arenaria is distinguished from *S. calcicola* by the elongate, conical, terminal cell in contrast to a rounded one no longer than the others of the trichome.

The species was described by Drouet (1968) as follows:

Trichomes blue-green, yellow-green, olive, brown, red, violet, or gray-green, predominantly constricted at the crosswalls, often torulose-cylindrical, 1–6 μ in diameter, here and there and in part increasing or decreasing in diameter, straight or curving or spiraled, capable of indeterminate growth in length, breaking by means of the destruction of an intercalary cell or by separation of adjacent cells, becoming abruptly conical at the tips. Cells quadrate or longer than broad, 2–10 μ long, the protoplasm homogeneous or granulose, rarely pseudovacuolate, with rarely a single granule developing on either side of a cross wall. Terminal cells becoming acutely or obtusely conical, rarely more or less cylindric-conical, the outer wall not becoming thickened. Sheath material hyaline or in part becoming yellow or brown, rarely red, violet, or blue, not at all or only here and there turning blue in chlor-zinc-iodide. Plant consisting of long or short naked trichomes, or of trichomes in a homogeneous or laminose mucus, or of one, few, or many trichomes in a more or less discrete cylindrical, often sheath.

Descriptions of the two isolates of this species studied in culture are given below.

Schizothrix arenaria (Berkeley) Gomont (Figs. 49–51.)
Isolate 20

The plant-mass is macroscopically distinguishable by the presence of small dark dots scattered over the surface (Fig. 49). At 14X, these dots are seen to be tightly coiled ropes of trichomes. The ropes of trichomes are elsewhere arranged in an undulate manner (Fig. 50). The color of the plant-mass is medium olive-green (125) at 2 weeks and dark olive-green (126) at 4 weeks.

The cells (Fig. 51) are 1.5–2.0 μ wide and 2.5–6.5 μ long. The terminal cell is long, tapered, and may bend back and forth. The trichomes are constricted at the crosswalls. The cell contents include one dark granule (very rarely two) at each end of the cell next to the crosswall. Sheaths are of very infrequent occurrence and are narrow, soft, and colorless.

Schizothrix arenaria (Berkeley) Gomont v. **vermiformis** var. nov. Figs. 52–53.
Isolate 32

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis.

The plant-mass is microscopically vermiform with curved and coiled bundles of filaments. At 14X, the bundles are seen to be smooth to confluent (Fig. 52). The color of the plant-mass is deep yellow-green (118) at 2 and 4 weeks.

The cells (Fig. 53) are 1.5–2.5 μ wide and 3.5–6.0 μ long. The terminal cell is long and slightly conical. The trichomes are slightly constricted at the crosswalls. One large dark granule occurs at either end of the cell; in very long cells one or two granules may be seen in the center of the cell—perhaps an indication of imminent cell division. Sheaths are narrow and colorless.

A key to above-characterized isolates of *Schizothrix* follows.

A KEY TO SPECIES OF *SCHIZOTHRIX* IN CULTURE[1,2]

1. Terminal cell hemispherical to almost spherical ..... *S. calcicola.*
1. Terminal cell blunt- to acute-conical ..... *S. arenaria.*

KEY TO THE VARIETIES OF SCHIZOTHRIX ARENARIA

1. Plant-mass showing macroscopically small dark dots (tightly coiled trichomes) scattered over the surface ..... *S. arenaria.*
1. Plant-mass macroscopically without any small dark dots scattered over the surface. ..... *S. arenaria* v. *vermiformis.*

KEY TO THE VARIETIES OF SCHIZOTHRIX CALCICOLA

1. Plant-mass vermiform 2
1. Plant-mass otherwise 4
   2. Plant-mass appearing rough or minutely glomerulate in the center except when young (vermiform then), becoming distinctly vermiform at the margin ..... *S. calcicola v. vermiformis.*
   2. Plant-mass vermiform over the entire surface at all times 3

[1] The keys to the species are taken from Drouet (1968).
[2] The keys to the varieties are based on 2 to 4-week-old cultures grown on 3N BBM agar under standard conditions.

3. Trichomes with narrow, but firm, sheaths ..... *S. calcicola.*
3. Trichomes occurring in a mass of colloidal material, sometimes several trichomes loosely arranged in a sheath ..... *S. calcicola* v. *mucosa.*
4. Plant-mass minutely glomerulate 5
4. Plant-mass otherwise 6
5. Plant-mass distinctly minutely glomerulate in the center; minutely glomerulate and "zigzagging" bundles of trichomes at the margin ..... *S. calcicola* v. *glomerulata.*
5. Plant-mass minutely glomerulate and compact ..... *S. calcicola* v. *compacta.*
6. Plant-mass compact; rough or smooth 7
6. Plant-mass otherwise 10
7. Plant-mass brownish-black ..... *S. calcicola* v. *scabella.*
7. Plant-mass green 8
8. Plant-mass smooth ..... *S. calcicola* v. *nitida.*
8. Plant-mass rough 9
9. Plant-mass very compact and rough; filaments straight or (if older) irregularly curved with very firm sheaths ..... *S. calcicola v. densa.*
9. Plant-mass an extended, rough, dense mat; filaments straight and at times tightly coiled with thin sheaths ..... *S. calcicola* v. *circinalis.*
10. Plant-mass distinctly radially arranged 11
10. Plant-mass otherwise 14
11. Trichomes noticeably spiraled along their entire length ..... *S. calcicola* v. *spiralis.*
11. Trichomes not spiraled, but straight 12
12. Plant-mass with long, tightly appressed bundles of trichomes radiating from the inoculum ..... *S. calcicola* v. *radiata.*
12. Plant-mass with central compact mass and short bundles of trichomes radiating from it. 13
13. Trichomes with firm, distinct sheaths ..... *S. calcicola* v. *actinoformis.*
13. Trichomes without sheaths ..... *S. calcicola* v. *fuscoviridis.*
14. Plant-mass dense and smooth in center with bundles of filaments visible at the margin 15
14. Plant-mass otherwise 16
15. Plant-mass green ..... *S. calcicola* v. *glabra.*
15. Plant-mass brown ..... *S. calcicola v. fusca.*
16. Plant-mass with distinct globular masses at the margin ..... *S. calcicola* v. *discreta.*
16. Plant-mass otherwise—nondescript—not included in any of the above descriptions 17
17. Sheaths occurring only rarely and then in older cultures ..... *S. calcicola* v. *amorpha.*
17. Sheaths occurring regularly and abundantly 18
18. Trichomes (filaments) spiraled or undulate in part ..... *S. calcicola* v. *minuta.*
18. Trichomes (filaments) straight 19
19. Sheaths thin, frequently seen only where empty, to 0.5 μ wide ..... *S. calcicola* v. *diffusa.*
19. Sheaths very firm, easily seen, to 1.0 μ wide 20

20. Trichomes generally no wider than 3.5 μ (most about 3.0 μ) ; sheaths colorless ..... *S. calcicola* v. *vaginata.*
20. Trichomes generally 4.0–4.5 μ wide, sheaths colorless and becoming pale yellow-brown with age ..... *S. calcicola* v. *olivacea.*

Microcoleus vaginatus (Vaucher) Gomont

Most of the fast-growing, extremely motile isolates studied in this investigation were identified as *Microcoleus vaginatus.* The species is distinguished by the conspicuously attenuated ends of the trichomes, according to Drouet (1962, 1968). Isolates were both terrestrial and aquatic in origin.

Drouet (1968) described the species as follows:

> Trichomes blue-green, yellow-green, olive, brown, red, violet, or gray-green cylindrical, rarely constricted at the cross walls, 2.5–9 μ in diameter, straight or curving or spiraled, capable of growth to an indeterminate length, breaking by means of the destruction of an intercalary cell or rarely by separation of two cells at a cross wall, attenuated through usually several cells at the tips, the tips not rarely capitate. Cells quadrate or shorter or longer than the diameter, 1–10 μ long, the protoplasm homogeneous or granulose, often pseudovacuolate, the cross walls lined on either side with a layer of granules. Terminal cell conical, hemispherical, truncate-cylindrical, the outer membrane becoming thickened into a rigid cone, cup, or convex disc. Sheath material hyaline, not at all or only in part and here and there turning blue in chlor-zinc-iodide. Plant consisting of long or short naked trichomes, or of trichomes in a homogeneous or laminose mucus, or of single, few, or many trichomes within a more or less discrete cylindrical, often branched, sheath.

The plant-mass types and the morphology of the cells and trichomes encountered in these isolates divided them into ten groups, which are described below.

Microcoleus vaginatus (Vaucher) Gomont (Figs. 54–57.)
Isolate I621

The plant-mass is vermiform macroscopically with curved bundles of filaments (Fig. 54). At 14X, among the bundles, growing both on and into the agar, solitary filaments are seen. The bundles may be rugose (Fig. 55), thus resulting in a striped appearance when the plant-mass is examined at low magnification, or they may be rough (Fig. 56). The color of the plant-mass is deep yellowish-green (132) at 2 weeks and yellowish-brown (74) at 4 weeks.

The cells (Fig. 57) are 5.0–7.0 μ wide and 2.0–5.0 μ long. The terminal cell is rounded, pointed, or rarely capitate; the terminal one to five cells may taper. The trichomes are usually not constricted at the crosswalls, but slight constrictions occur sometimes. The cell contents are granular; dark granules may occur at the crosswalls. The sheaths are narrow (0.8 μ), firm, and colorless.

Microcoleus vaginatus (Vaucher) Gomont v. **glaber** var. nov. Figs. 58–59.
Isolates I1546, I1547

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis, multos fasciculos trichomatum convolutos praebens; magnificatione 14X fasciculi leves apparentes.

The plant-mass is macroscopically vermiform with curved and coiled thin bundles of trichomes. At 14X, the bundles are seen to be smooth, and solitary trichomes among them are not numerous (Fig. 58). The color of the plant-mass is deep yellow-green (118) aat 2 weeks and medium olive-green (125) at 4 weeks.

The cells (Fig. 59) are 3.5–4.5 μ wide and 2.0–4.0 μ long. The terminal cell is rounded or rostrate and may bend and have a thickened outer wall; the end several cells may taper (to 2.0 μ wide). The trichomes are not constricted at the crosswalls. The cell contents are granular and translucent granules occur throughout, including either side of the crosswalls. Sheaths have not been observed.

Microcoleus vaginatus (Vaucher) Gomont v. **undulatus** var. nov. Figs. 60–62.
Isolate I622

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis, fasciculos trichomatum crassos habens; trichomata post circa duas hebdomades hic illic abrupte inflectunt, ut fasciculi torulosi videantur.

The plant-mass is macroscopically vermiform with thick bundles of trichomes, which from about 2 weeks on, are abruptly curved at intervals, giving a knobby appearance to the bundles (Fig. 60). At 14X, the bundles are smooth and solitary trichomes are present among them (Fig. 61). The color of the plant-mass is dark olive-green (126) at 2 and 4 weeks.

The cells (Fig. 62) are 5.0–7.0 μ wide and 2.5–5.0 μ long. The terminal cell is rounded, capitate, or pointed; the outer wall may be thickened. The end few cells may taper (to 4.0 μ wide). The trichomes are not constricted at the crosswalls. The cell contents are granular; dark granules may occur along either side of the crosswalls as well as in the central portion of the cell. The crosswalls are often indistinct. Sheaths have not been observed.

Microcoleus vaginatus (Vaucher) Gomont v. **cyano-viridis** var. nov. Figs. 63–64.
Isolate K27

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis, colore semper atrovirens usque ad duas hebdomades.

The plant-mass is vermiform macroscopically with curved bundles of filaments. At 14X, solitary filaments may be seen among the bundles, growing both on and into the agar (Fig. 63). The color of the plant-mass is medium olive-green (125) at 2 weeks and deep yellow-green (118) at 4 weeks.

The cells (Fig. 64) are 4.5–6.0 μ wide and 1.8–5.0 μ long. The terminal cell is

rounded and very slightly tapered or (rarely) rostrate or capitate; the outer wall is occasionally slightly thickened. The terminal 1–5 cells may taper (to 2.0–3.5 μ wide). The trichomes are not constricted at the crosswalls. The cell contents are granular; dark granules occur at the crosswalls frequently and dark or translucent ones in the center of the cell. The sheaths are firm, distinctly visible (to 1.0 μ wide), and colorless.

Microcoleus vaginatus (Vaucher) Gomont c. **funiformis** var. nov. Figs. 65–66. Isolates I1306, I1309

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata ad centrum dense vermiformis, fasciculos filamentorum longos crassos radiantes habens.

The plant-mass macroscopically has a densely vermiform center from which radiate long, frequently thick, curving bundles of filaments. At 14X, many solitary filaments are seen among the smooth bundles (Fig. 65). The color of the plant-mass is deep yellow-green (118) at 2 weeks and dark olive-green (126) at 4 weeks.

The cells (Fig. 66) are 4.5–5.0 μ wide and 2.0–5.0 μ long. The terminal cell is conical or rounded; the end few cells may taper (to 3.0 μ wide). Thickened terminal cells are of very rare occurrence. The trichomes are not constricted at the crosswalls. The cell contents are granular; dark granules occur throughout the cell and may be arranged in lines along either side of the crosswalls. Sheaths are present in cultures more than 2 weeks old; they are firm, colorless, and narrow.

Microcoleus vaginatus (Vaucher) Gomont v. **conicus** var. nov. Figs. 67–68. Isolate K 55

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis, fasciculis filamentorum levibus; cellula terminalis conica.

The plant-mass is vermiform macroscopically with curved bundles of filaments. At 14X, the bundles of filaments appear smooth, and numerous solitary filaments are also seen (Fig. 67). The color of the plant-mass is dark olive-green (126) at 2 and 4 weeks.

The cells (Fig. 68) are 6.0–8.5 μ wide and 2.0–5.0 μ long. The terminal cell is rounded or conical; the end several cells may taper (to 4.0 μ wide). The outer wall of the terminal cell may infrequently be slightly thickened. The trichomes are frequently slightly constricted at the crosswalls. The cell contents are granular; at times dark granules are seen on either side of each crosswall. The sheaths are 0.5–1.5 μ wide, firm and colorless.

Microcoleus vaginatus (Vaucher) Gomont v. **fuscus** var. nov. Figs. 69–72. Isolates 17, 26, 34, I1270

Varietas a specie typica differens eo quod massa plantarum colore fusco-brunnea.

The plant-mass is macroscopically vermiform with curved bundles of filaments (Fig. 69). At 14X, many of the bundles appear angularly curved, and numerous solitary filaments are seen among them (Fig. 70). The color of the plant-mass is dark olive-brown (96) at 2 and 4 weeks.

The cells are 5.5–8.5 μ wide and 2.0–5.5 μ long. The terminal cells are widely rounded, capitate (Fig. 71), or conical; the outer wall is frequently thickened. The end few cells often taper (to 4.0 μ wide). The cells contain granules scattered throughout the protoplasm; sometimes there may be dark granules along either side of the crosswalls (Fig. 72). The sheaths are 0.5–1.5 μ wide, firm, colorless, and sometimes lamellated.

Microcoleus vaginatus (Vaucher) Gomont v. **fuscorubens** var. nov. Figs. 73–75. Isolate I617

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata e fasciculis trichomatum curvatis convolutisque, quae post ca. duas hebdomades fimbriata videntur, composita.

The plant-mass is macroscopically composed of curving and coiling bundles of filaments (Fig. 73). At 14X, these bundles appear smooth to somewhat rough, and solitary filaments, growing both on and into the agar, are seen (Fig. 74). These solitary filaments are so numerous as to give a pale color to the agar. The color of the plant-mass is deep yellow-brown (78) at 2 and 4 weeks.

The cells (Fig. 75) are 6.0–8.5 μ wide and 2.5–4.5 μ long. The terminal cell is rounded and is infrequently thickened; the end few cells may taper (to 4.0 μ wide). The trichomes are not constricted at the crosswalls. The cell contents are granular; translucent and a few dark granules may be seen throughout the cell. In 3N BBM, the granules were not observed in rows along either side of the crosswalls, but they were thus seen in 3N BBM at a lowered initial pH (5.0 and 6.0). The sheaths are narrow (0.3 μ wide), firm, and colorless to pale yellow.

Microcoleus vaginatus (Vaucher) Gomont v. **radiatus** var. nov. Figs. 76–78. Isolate I428

Varietas a specie typical differens eo quod massa plantarum sine microscopo observata radiata, fasciculos trichomatum distinctos divergentes qui post duas hebdomades fimbriati videntur, habens.

The plant-mass is macroscopically radial with distinct, divergent bundles of trichomes that appear fimbriate from about 2 weeks on (Fig. 76). At 14X, the fringed look is seen to be the result of intricately curved trichomes (Fig. 77). The color of the plant-mass is very dark yellowish-green (138) at 2 weeks and deep yellow-green (118) at 4 weeks.

The cells (Fig. 78) are 4.0–5.5 μ wide and 3.5–5.0 μ long. The terminal cell is rounded or conical; the end few cells may taper. Thickened outer walls of the

terminal cell have not been seen. The trichomes are not constricted at the crosswalls. The cell contents are granular; granules often occur along each side of the crosswalls. Sheaths have not been observed.

Microcoleus vaginatus (Vaucher) Gomont v. **araneaformis** var. nov. Figs.79–80.
Isolate 33

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata arachnoidea; cellulae 7.0–10.0 μ lat., 2.5–4.0 μ long.

The plant-mass is macroscopically arachnoid. At 14X, the individual filaments and bundles of filaments are seen (Fig. 79). The color of the plant-mass is very dark yellowish-green (138) at 2 and 4 weeks.

The cells (Fig. 80) are 7.0–10.0 μ wide and 2.5–4.0 μ long. The terminal cell is rounded. The trichomes are not constricted at the crosswalls. The cells contain numerous granules in a random arrangement and along either side of the crosswalls. Some sheaths are always seen though they never seem to be ubiquitous; they are to 1.0 μ wide and colorless.

Microcoleus lyngbyaceus (Kützing) Crouan

This species is distinguished by Drouet (1968) from the other species by the densely granular protoplasm. This characteristic is best demonstrated by isolate 4 when growing under apparently less than optimal conditions. The dense granules are not, however, present when growth is good, except in scattered cells.

Drouet (1968) described the species as follows:

Trichomes blue-green, yellow-green, yellow, olive, brown, red, violet, or gray-green, cylindrical, constricted (sometimes only here and there or in parts) at the cross walls, 3.5–8 μ in diameter, here and there or in part decreasing or increasing in diameter, straight or curving or spiraled, capable of growth to an indeterminate length, breaking by means of the destruction of intercalary cells or by the separation of cells at a crosswall, cylindrical or long- or short-attenuate at the tips. Cells commonly shorter than broad, up to 15 times as short as broad, rarely quadratic, 1.5–8 μ long, the protoplasm homogeneous or granulose, often pseudovacuolate, the cross walls and side walls lined with a layer of granules. Terminal cell rotund, the outer membrane at first thin, becoming thickened in a depressed-rotund, depressed-hemispherical, or depressed-conical shape. Sheath material hyaline, often developing yellow or brown pigments, not at all or only in part turning blue in chlor-zinc-iodide. Plant consisting of long or short naked trichomes, or of trichomes in a homogeneous or laminose mucus, or of one or few or many trichomes in a more or less discrete, often branched, cylindrical sheath.

The two isolates belonging to this species are described as two varieties based on plant-mass form, size, and nature of the sheath in the descriptions given below.

Microcoleus lyngbyaceus (Kützing) Crouan Figs. 81–83, 127.
Isolate 4

The plant-mass is macroscopically arachnoid (Fig. 81). At 14X, the individual filaments may be seen (Fig. 82). The color of the plant-mass is very dark yellowish-green (138) at 2 and 4 weeks.

The cells (Fig. 83) are 16.0–24.0 μ wide and 2.5–7.0 μ (mostly about 4.0 μ) long. The terminal cell is broadly rounded; thickened outer walls have not been observed. The trichomes are not constricted at the crosswalls. The cells contain many dark and translucent granules, which may appear at times to form almost a solid mass in the cells (Fig. 127). The sheaths are 1.0–4.0 μ wide, colorless to slightly yellow, lamellated (4–8 layers), and firm.

Microcoleus lyngbyaceus (Kützing) Crouan v. **vermiformis** var. nov. Figs. 84–86.
Isolate 12

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis; cellulae 5.0–6.0 μ lat., 1.0–2.5 μ long.

The plant-mass is vermiform macroscopically (Fig. 84) with many fine curved bundles of filaments (Fig. 85). At 14X, many solitary filaments can also be seen growing both on and into the agar. The color of the plant-mass is deep yellowish-green (118) at 2 and 4 weeks.

The cells (Fig. 86) are 5.0–6.0 μ wide and 1.0–2.5 μ long. The terminal cell is rounded and may contain many dark granules; thickened outer walls have not been observed. The trichomes are not constricted at the crosswalls. The cell contents are granular; granules are usually distributed throughout the cell, but dark granules may also occur at the crosswalls and along the sides of the cells. The sheaths are 0.5–1.5 μ wide, firm, and colorless.

Microcoleus irriguus (Kützing) Drouet

This species is distinguished from the two other species by Drouet (1968) by the very slight attenuation of the tips of the trichomes.

Drouet described the species as follows:

Trichomes blue-green, yellow-green, olive, brown, red, violet, or gray-green, cylindrical, constricted here and there only lightly at the crosswalls, 6–18 μ in diameter, here and there and in part increasing or decreasing in diameter, straight or curving or spiraled, capable of growth to an indeterminate length, breaking by means of the destruction of an intercalary cell or by the separation of two cells at a cross wall, the tips not at all or scarcely attenuated, the apices truncate. Cells longer or shorter than broad, 3–15 μ long, the protoplasm homogeneous, sometimes pseudovacuolate, the cross walls covered on either side with a layer of granules. Terminal cell cylindrical, the outer membrane depressed-hemispherical or broadly depressed-conical, becoming thickened in age. Sheath material hyaline, not or only in part becoming blue when placed in

chlor-zinc-iodide. Plant consisting of long or short naked trichomes or of trichomes in a homogeneous or laminose mucus or of one or few or many trichomes within a more or less discrete cylindrical, often branched, sheath.

One isolate was studied and is described below.

Microcoleus irriguus (Kützing) Drouet Figs. 87–88.
Isolate 35

The plant-mass is macroscopically arachnoid. At 14X, many separate, non-coiling filaments are seen, but only a few bundles of filaments occur (Fig. 87). The color of the plant-mass is medium olive-green at 2 and 4 weeks.

The cells (Fig. 88) are 5.0–8.0 μ wide and 4.0–6.0 μ long. The terminal cell is slightly and shallowly pointed, and the outer wall may be thicker than other walls. Dark granules occur along both sides of the crosswalls and some are scattered throughout the cell. The sheaths are 0.5–1.0 μ wide, colorless, and firm. A key to these isolates of *Microcoleus* follows.

A KEY TO SPECIES OF *MICROCOLEUS* IN CULTURE

1. Dense protoplasm and granules extending along the lateral walls of each cell as well as along the crosswalls ..... *M. lyngbyaceus.*
1. Dense protoplasm and granules along the crosswalls only 2
   2. Trichomes becoming at most slightly attenuated at the tips ..... *M. irriguus.*
   2. Trichomes becoming conspicuously attenuated at the tips ..... *M. vaginatus.*

KEY TO THE VARIETIES OF MICROCOLEUS LYNGBYACEUS

1. Plant-mass macroscopically arachnoid ..... *M. lyngbyaceus.*
1. Plant-mass macroscopically vermiform ..... *M. lyngbyaceus* v. *vermiformis.*

KEY TO THE VARIETIES OF MICROCOLEUS VAGINATUS

1. Plant-mass macroscopically vermiform 2
1. Plant-mass otherwise 9
   2. Plant-mass brown 3
   2. Plant-mass some shade of green 4
3. Plant-mass macroscopically consisting of curving, spreading bundles of filaments ..... *M. vaginatus* v. *fuscus.*
3. Plant-mass macroscopically consisting of many coils of bundles of filaments ..... *M. vaginatus* v. *fuscorubens.*
   4. Plant-mass macroscopically consisting of bundles of trichomes having an undulate appearance ..... *M. vaginatus* v. *undulatus.*
   4. Plant-mass macroscopically consisting of bundles of trichomes that are not undulate 5
5. Many solitary filaments present among the bundles of filaments, giving distinctive background color to the bundles 6
5. Some solitary filaments present, but never enough to impart distinct color to the agar 7

6. Bundles of filaments thick, generally radiating from the inoculum ..... *M. vaginatus* v. *funiformis.*
6. Bundles of filaments thin, generally growing equally over the entire surface ..... *M. vaginatus* v. *cyano-viridis.*
7. Plant-mass dark olive-green, becoming some shade of yellow only after 6–8 weeks ..... *M. vaginatus* v. *conicus.*
7. Plant-mass lighter shade of green, beginning (unless in low light) to turn yellow at 3–4 weeks 8
8. Trichomes without sheaths: usually 3.5–4.0 μ (sometimes to 4.5 μ) wide ..... *M. vaginatus* v. *glabrus.*
8. Trichomes with sheaths, at least in part; usually 5.0–6.4 μ (to 7.0 μ) wide ..... *M. vaginatus.*
9. Plant-mass with undulate bundles of trichomes radiating from the inoculum ..... *M. vaginatus* v. *radiatus.*
9. Plant-mass with straight bundles of trichomes radiating from the inoculum ..... *M. vaginatus* v. *araneaformis.*

Porphyrosiphon notarisii (Meneghini) Kützing

The species is delimited by Drouet by its rotund to conical terminal cells that are not thickened along the outer wall. The separation from *P. kurzii* (not represented among these isolates) is based on the longer cells (3–15 μ) in *P. notarisii*; cell length in *P. kurzii* does not exceed 4 μ.

Drouet (1968) described *Porphysosiphon notarisii* as follows:

Trichomes blue-green, yellow-green, olive, brown, red, violet, or gray-green, cylindrical, commonly constricted (at least here and there) at the cross walls, 3–40 μ in diameter, here and there and in part increasing or decreasing in diameter, straight or curving or spiraled, capable of indeterminate growth in length, breaking by means of the destruction of an intercalary cell or by separation of cells at a cross wall, at the ends evidently long-attenuated through several cells. Cells shorter or longer than broad, (2.0 μ) 3–15 μ long, the protoplasm homogeneous or granulose, sometimes pseudovacuolate, the cross walls not granulated. Terminal cell at first hemispherical, becoming obtuse- or acute-conical, the outer membrane not thickened. Sheath material at first hyaline, later often developing yellow, brown, red, violet, or blue pigments, mostly turning blue in chlor-zinc-iodide. Plant consisting of long or short naked trichomes, or of trichomes in a homogeneous or laminose mucus, or of one to many trichomes in a more or less discrete, often branched, cylindrical sheath.

Three isolates, from freshwater and marine environments, were studied in culture. They are described as two varieties below.

Porphyrosiphon notarisii (Meneghini) Kützing. Figs. 89–91.
Isolates 5, 39

The plant-mass is macroscopically extremely and irregular convoluted (Fig. 89). At 14X, the bundles of trichomes appear very smooth and among them soli-

tary trichomes are seen growing both on and into the agar (Fig. 90). The color of the plant-mass is very dark yellowish-green (138) at 2 weeks and dark olive-green (108) at 4 weeks.

The cells (Fig. 91) are 4.0–5.0 μ wide and 2.0–3.5 μ long. The terminal cell may be conical or rounded; it often curves or hooks. The end few cells may be tapered (to 2.5 μ wide). The trichomes are not constricted at the crosswalls. The cell contents are granular, but no dark granules are seen either centrally or at the crosswalls. Sheaths may occur in older (4–8 weeks) cultures where they are very soft, colorless (apparent only if stained with methylene blue or India ink), and never seem to be common.

Porphyrosiphon notarisii (Meneghini) Kützing v. **canus** var. nov. Figs. 92–94. Isolate 40

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata fasciculos trichomatum in centro arcuatos convolutosque qui recti radiatique fiunt, habet; cinereo-brunnea.

The plant-mass macroscopically has curved and coiled bundles of trichomes in the center that become straight and radiate from the center of the plant-mass (Fig. 92). At 14X, the bundles are seen to be smooth and many solitary trichomes occur (Fig. 93). The color of the plant-mass is dark gray-yellow (91) at 2 and 4 weeks.

The cells (Fig. 94) are 5.0–6.0 μ wide and 3.0–4.0 μ long. The terminal cell is conical or rounded; the end few cells may taper (to 3.5 μ wide). The trichomes are not constricted at the crosswalls. The cell contents are granular; distinct translucent and dark granules are seen throughout the cells. Sheaths have not been observed.

These isolates may be distinguished as follows.

KEY TO THE VARIETIES OF PORPHYROSIPHON NOTARISII

1. Plant-mass green ..... *P. notarissi.*
1. Plant-mass gray-brown ..... *P. notarissi* v. *canus.*

Oscillatoria lutea Agardh

The species is distinguished by Drouet (1968) by the general occurrence of short cells and by the slight attenuation of the end of the trichomes. Several of the isolates herein described are frequently more than slightly attenuated at the ends, but they seem to be more like this species than any other.

Drouet (1968) described the species as follows:

Trichomes blue-green, yellow-green, olive, brown, red, violet, or gray-green, cylindrical, scarcely constricted here and there at the cross walls, 2.5–10 μ in diameter, here and there and in part increasing or decreasing in diameter, straight or curving or

spiraled, indeterminate in growth in length, fragmenting by means of the destruction of an intercalary cell or by separation of cells at a cross wall, cylindrical or attenuating somewhat through one or more cells at tips. Cells as long as or shorter than broad, often very short, 1–7 long, the protoplasm homogeneous or granulose, rarely pseudovacuolate, the cross walls not granulated. Terminal cell broadly truncate-conical, the outermembrane at first thin, becoming thickened in age, convex-platelike. Sheath material hyaline, often turning blue in chlor-zinc-iodide. Plant consisting of long or short trichomes, naked or in a homogeneous or laminose mucus, or solitary or few or many in more or less discrete, often branched sheaths.

The isolates of this species studied in culture were grouped as follows.

Oscillatoria lutea Agardh Figs. 95–97.
Isolate 23

The plant-mass is vermiform macroscopically with curved bundles of filaments. At 14X, the bundles of filaments appear smooth, and solitary filaments may also be seen growing both on and into the agar (Figs. 95–96). The color of the plant-mass is deep yellowish-green (132) at 2 weeks and dark olive-green (126) at 4 weeks.

The cells (Fig. 97) are 5.0–7.0 μ wide and 1.0–4.0 μ long. The terminal cell is rounded or capitate, and the outer wall may be thicker than that in other cells. The end few cells taper frequently (to 4.0 μ wide). In old cultures pointed terminal cells may occur. The trichomes are not constricted at the crosswalls. Gas vacuoles seem to be of sparse occurrence. Sheaths appear with age; they are colorless and to 2.0 μ wide.

Oscillatoria lutea Agardh v. **auxotrophica** var. nov. Fig. 98.
Isolates 22, 24

Varietas a specie typica differens eo quod in cultura increscens vitamino "B_{12}" eget.

The plant-mass form is the same as that of isolate 23 (*O. lutea*).

The cells (Fig. 98) are 5.0–7.0 μ wide and 1.0–3.0 μ long. The terminal cell is capitate, rounded, or (rarely) rostrate; the outer wall is thicker than other walls. The end few cells may taper (to 3.0 μ wide). In old cultures pointed terminal cells may occur. The trichomes are only rarely slightly constricted at the crosswalls. The cells contain granules scattered throughout the protoplasm; vacuoles may occur in old cultures and in vitamin-deficient media. Gas vacuoles occur at alkaline pH's to a limited extent on agar (and much more commonly in liquid). The sheaths are narrow in young cultures (0.5 μ), becoming wider (to 2.0 μ) with age, and colorless.

Isolates 22 and 24 differ only slightly morphologically from isolate 23 (more capitate cells, more sheaths, some constrictions at the crosswalls), but several physiological differences were also noted. The most important of these was that isolate

23, although possibly enhanced by addition of vitamin B_{12} to the medium, did not require it for continued growth in axenic culture as did isolates 22 and 24.

Oscillatoria lutea Agardh v. **contorta** var. nov. Figs. 99–102.
Isolates 25, I386, I390.

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata vermiformis, amenta atque anfractus arctos fasciculorum filamentorum, praebens; magnificatione 14X observata, fasciculi leves apparent.

The plant-mass is macroscopically vermiform with loops and tight coils of bundles of filaments (Fig. 99). At 14X, the bundles appear very smooth, and few solitary filaments are seen among them (Fig. 100–101). The color of the plant-mass is deep yellow-green (132) at 2 weeks and medium to dark olive-green (125, 126) at 4 weeks.

The cells (Fig. 102) are 4.0–7.0 μ wide and 1.5–6.0 μ long. The terminal cell is rounded (some with thickened outer walls), capitate, or rostrate. In older cultures the terminal cells may be pointed. The end few cells may taper (to 3.0 μ wide). The trichomes are at times slightly constricted at the crosswalls. The cells contain granules scattered throughout the protoplasm; gas vacuoles occur frequently. Sheaths are soft to firm, colorless, and in older cultures are to 4.0 μ wide.

Oscillatoria lutea Agardh v. **scabra** var. nov. Figs. 103–104.
Isolate 38

Varietas a specie typica differens eo quod massa plantarum sine microscopo observata conferte vermiformis; magnificatione 14X visa, fasciculi filamentorum asperi apparentes.

The plant-mass is macroscopically densely vermiform. At 14X, rough curving and coiling bundles of filaments are seen; numerous solitary filaments also occur (Fig. 103). The color of the plant-mass is deep yellowish-green (118) at 2 weeks and very dark yellow-green (138) at 4 weeks.

The cells (Fig. 104) are 3.0–5.0 μ wide and 2.0–4.0 μ long. The terminal cell is rounded to slightly conical; the outer wall may be thickened. The trichomes are not or only very slightly constricted at the crosswalls. Granules occur randomly dispersed throughout the cells. With age, trichomes become extremely contorted and twisted within the sheaths. The sheaths are narrow (0.2 μ) at first, becoming wider with age (to 1.0 μ wide), colorless, and frequently lamellated.

The isolates of *O. lutea* may be identified with the aid of the following key:

KEY TO THE VARITIES OF OSCILLATORIA LUTEA

1. Plant-mass macroscopically vermiform with curved bundles of filaments 2
1. Plant-mass macroscopically vermiform with many coils of filaments .....
O. lutea v. *contorta.*

2. Curved bundles of filaments smooth 3
2. Curved bundles of filaments rough. ..... *O. lutea v. scabra.*
3. Organisms B_{12} auxotrophs ..... *O. lutea v. auxotrophica.*
3. Organisms not B_{12} auxotrophs ..... *O. lutea.*

Physiological Studies

Various physiological studies were performed both for the taxonomic aid they might give and for any contribution to the knowledge of blue-green algal physiology they might make. The selection of the basal medium, the comparison of unialgal and axenic cultures, and changes in cultures due to aging are first discussed. Growth in various carbon and nitrogen sources, with and without vitamins, production of extracellular enzymes, and sensitivity to crystal violet dye, and pH changes are also included in this section.

Selection of the Basal Medium

Several media were employed in an attempt to find the one most suitable to the growth of the most isolates. These media included BBM-K, Shen-X, KMD, C_R-10, 1N BBM, and 3N BBM; the formulae for these media are given in the Materials and Methods.

Most isolates were originally grown on BBM-K, a Tris-buffered, more dilute variation of 1N BBM which Kantz and Bold (1969) in their investigation of *Nostoc* and *Anabaena* found to be the most satisfactory medium for the isolates studied. Cultures of Oscillatoriaceae grown in this medium had moderate-to-excellent growth during the first 2 weeks, but the colors of the algae were generally pale, and within 3 weeks definite yellowing began in the great majority. By 4 weeks, almost all were yellow or orange with only a small amount of green coloration left. Microscopically, the cells had numerous granules, both dark and translucent. The growth on agar was generally very diffuse; plant-mass types in some isolates varied considerably from the 3N BBM type herein designated as the definitive one.

Shen-X, another dilute culture medium, was also used in preliminary testing; it caused even more rapid change of color in isolates (from green to red-orange in most cases) than did BBM-K. Growth was minimal. By 2 weeks color change was complete in most isolates (isolate 27 was a noticeable exception; it remained green although there was no detectable growth). The isolates were not dead, however, for when transferred to BBM-K or 1N BBM they became green and started to grow within a few days.

KMD (Kratz and Myers' Medium D) was also tested. Growth was moderate-to-good when the cultures were 2 to 4 weeks old. In flasks, growth tended to result in long filaments which formed large, loose masses in the culture flasks. Growth in the other media usually resulted in a large number of clumps of filaments and a membranous layer on the surface of the liquid. KMD thus resulted in a more

"natural" macroscopic appearance of many of the isolates. Microscopically, however, no significant variation from the other media could be seen in the cells when the cultures were in a healthy state. The deterioration (color change, formation of hormogonia, dead cells) of most of the cultures after about 4 weeks was the principal drawback to use of KMD for long-term observations.

C_g-10 proved to be an excellent medium for growth of single filaments at time of isolation (see Materials and Methods) and also supported good growth of many isolates without the early deterioration of cultures seen in KMD.

The same type of growth was observed in 1N BBM and 3N BBM. It was found that 1N BBM supported vigorous growth longer than BBM-K. This increased growth was suspected to be due to the increased amount of nitrogen, a view supported by the results of doubling or tripling the nitrate stock of BBM-K. The use of 3N BBM further enhanced growth; cultures in tightly capped tubes remained green and continued to grow for several months. The amount of growth (macroscopically observed) was greater in 3N BBM over a long period of time than in other media. Although color changes began at about 4–5 weeks, frequently cultures several months old were still—at least in part—green. Plant-mass in 1N BBM had more definite form than in BBM-K (although in all media, plant-mass form was constant for the medium).

Cell morphology and plant-mass form were not modified when the isolates were grown in Tris-buffered 3N BBM (BBMT) at an initial pH of 7.0–7.2.

Comparison of Unialgal and Axenic Cultures

Statements have been made, from time to time, that the presence of bacteria and fungi in cultures negates their morphological value or at least their morphological constancy. All of the axenic isolates used in this work, except I621 and 27, were either received in unialgal condition or were isolated from field collections. Observations thus were made on cultures of the same organisms in both the unialgal and axenic states. The presence of an excessive number of fungi in a culture can modify the plant-mass form and the morphology of the trichomes. In such a case, the fungus often kills the alga eventually. The presence of bacteria was not seen to have any effect on the morphology of the trichomes. To check this conclusion, 10 isolates were recontaminated with a bacterium isolated from isolate 38 and with *Aerobacter aerogenes*. Growth through several transfers showed no differences between the axenic control and the newly contaminated culture except in isolate K107. There the difference was only in the amount of growth (K107 grew better when contaminated with the bacterium from isolate 38 than without bacteria or with *Aerobacter aerogenes*); the plant-mass form and the morphology of the cells were unchanged.

The use of axenic cultures is always preferable and is mandatory for most physiological studies. Such cultures are necessary for completely defining variation in any

organism, but in the writers' experience, it did not appear that unialgal cultures in the laboratory were any more "abnormal" or subject to change than either axenic cultures or organisms growing in nature. They are, in fact, much less variable than the latter.

Comparisons of Cultures at Various Ages

The most striking change in the oscillatoriacean isolates as they age is that in color. Most, when grown on BBM agar plates under standard conditions, are initially yellowish-green, olive-green, or brown. Change in color in many cultures begins in about 3 weeks; the process may be hastened by excessive drying of the agar, by higher light intensity, and by reducing the nitrogen in the medium. Some of the isolates (among them K107, K58, 4) exhibit little, if any, change in color even if cultures are maintained for 2 or 3 months. Most, however, eventually (1–3 months) become some shade of reddish-brown, yellow, or brown. Table 2 sum-

Table 2. *Change in Color of 20 Isolates of Oscillatoriaceae on 3N BBM Agar Between 2 and 12 Weeks' Growth*

No.	2 wk	12 wk
2	138 (dark yellowish-green)	126 (dark olive-green)
4	138 (dark yellowish-green)	138 (dark yellowish-green)
5	138 (dark yellowish-green)	95 (medium olive-brown)
7	132 (deep yellowish-green)	74 (soft yellowish-brown)
10	138 (dark yellowish-green)	51 (deep orange)
12	118 (deep yellowish-green)	74 (soft yellowish-brown)
20	125 (medium olive-green)	125 (medium olive-green)
22	132 (deep yellowish-green)	126 (dark olive-green)
26	96 (dark olive-brown)	94 (light olive-brown)
30	126 (dark olive-green)	95 (medium olive-brown)
K51	132 (deep yellowish-green)	51 (deep orange)
K55	126 (dark olive-green)	95 (medium olive-brown)
K107	138 (dark yellow-green)	94/126 (light olive brown/ dark olive-green)
K182	138 (dark yellowish-green)	74 (soft yellowish-brown)
I386	132 deep yellowish-green)	126 (dark olive-green)
I485	132 (deep yellowish-green)	126/95 (dark olive-green/ medium olive-brown)
I597	132 (deep yellowish-green)	74 (soft yellowish-brown)
I621	132 (deep yellowish-green)	74 (soft yellowish-brown)
I622	126 (dark olive-green)	69 (deep orange-yellow)
I1306	118 deep yellow-green)	74 (soft yellowish-brown)

TABDE 3. *Color of 10 Isolates of Oscillatoriaceae at 2 and 4 Weeks on 3N BBM, BBM-K, and KMD Agar Media*

No.	3N BBM		BBM-K		KMD	
	2 wk	4 wk	2 wk	4 wk	2 wk	4 wk
1	118	132	117/84	74	102/84	125
4	138	138	138	138	138	138
5	138	108/84	118	85	137	84
10	138	132	120	74	120	125
12	118	118	125	84	120	68
17	96	96	96	96	96	96
19	132	126	118	85	137	84
24	132	126	132	84	117	125
39	118	118	118	84	118	108/100
K107	138	138	138	75	138	126

68 = soft orange yellow
74 = soft yellowish brown
75 = deep yellowish brown
84 = soft yellow
85 = deep yellow
96 = dark olive brown
100 = deep greenish yellow
102 = medium greenish yellow
108 = dark olive
117 = soft yellow green
118 = deep yellow green
120 = medium yellow green
125 = medium olive green
126 = dark olive green
132 = deep yellowish green
137 = dark yellowish green
138 = very dark yellowish green

marizes color change in some of the isolates during 2 to 12 weeks growth.

Although no special effort to affect the colors of the isolates was made, it was found that the various media often resulted in different patterns, both of initial and of terminal color. Table 3 summarizes the colors at 2 and 4 weeks of 10 isolates grown on 3N BBM, BBM-K, and KMD media. As can be seen from the table, there are isolates which have the same color in all three media (17, 34, 4).

If colors and change in color are to be used as a characteristic of the species or variety, it is necessary to define the culture conditions. Not only the medium used but also the form of the medium have a definite effect on the color. Growth in liquid media or on slants almost always results in slower change in color than in petri dishes, probably as a result of greater available moisture in the culture tubes.

The necessity of defining the age of the culture when the plant-mass is described was emphasized by Groover and Bold (1969). The attributes of shinyness and dryness have not been used in the current study, so that this change, frequently observed in green microalgae, has been eliminated. Gross and complete changes were not observed in the plant-mass of these isolates from 2 weeks on. The plant-

mass may continue to grow for 8 or more weeks, and as it does so, it not only becomes much larger in diameter but also much thicker. Therefore, in many isolates there tends to be a blurring of some characteristics, as the originally distinct ropes of trichomes become more confluent, rather than a definite change.

Thirty of the isolates were observed microscopically at weekly intervals for periods up to 3 months. Several patterns of morphological changes in the trichomes and filaments were noted in these isolates grown in BBM and variants thereof. Variation in liquid and solid media was also observed (Figs. 108–109).

The first type of change was characteristic of *Schizothrix calcicola* v. *vermiformis* (isolates 16, 21, K18, K44, K57). As the culture aged, crosswalls became less distinct (beginning at about 6 weeks), until they were almost entirely invisible in many filaments at 10–12 weeks. The central bright area of the cell resolved into several granular inclusions. Short false branches were also formed as the trichomes continued to grow, but the sheaths apparently did not expand after about 6–8 weeks (Fig. 105).

Cultures of *Schizothrix calcicola* v. *glomerulata* (isolates I426, I482, I487, I596, I597) became almost entirely hormogonial, with trichomes mostly 2æ10 cells long between 4 and 7 weeks of age (Fig. 106). Other isolates (I598, I427, 7) that have the same plant-mass type and cell size and cell shape remain filamentous.

The third type of change was apparent in isolates that were fairly large, fast-growing, and extremely motile (isolates 22, 23, 24, 25, I621, I386). The sheaths at first were either not seen or were very thin and infrequent in occurrence. The terminal cells were rounded, capitate, or rostrate. The sheaths appeared at 2 or 3 weeks in either liquid or agar-solidified media and became thicker from that time on. By the time the culture was 6–8 weeks old, the sheaths were frequently one-half to two-thirds as wide as the trichome itself. Staining with methylene blue or India ink did not reveal lamellation. The terminal cells of some of these trichomes became extremely pointed (Fig. 107). Isolate I622 developed pointed (cuspidate) terminal cells, but sheaths were not observed.

Isolate K55 appeared progressively more constricted at the crosswalls with age.

Isolates I1270, 26, K55, and K27 varied in the distribution of granules in the cells (whether central or along the crosswalls or both), but no correlation with age of cultures was established; their presence or absence seemed to be quite random (Figs. 71–72).

The cells of the remaining isolates studied over an extended period of time (4, 6, 27, K62, K107, K182) did not show any noticeable morphological changes.

It is interesting to note that many of the changes associated with age also appeared in young cultures grown at higher pH's (8.0–9.0). Whether the changes could be retarded by maintaining a lower pH (in BBM the pH may change from 6.5 to 10.0 depending on the alga), and whether the effects at higher pH's are influenced by the type of buffer, remain to be elucidated.

Effects of Various Carbon Sources

The assimilation of assorted carbon sources in light and dark in the blue-green algae has been investigated by numerous workers. Early work was in part stimulated by the isolation into pure culture of endophytic species and a subsequent desire to demonstrate the presence of heterotrophic growth as an operative system in these algae.

Reports of heterotrophic growth in a blue-green alga (*Nostoc*) were first given by Bouilhac (1898). Pringsheim (1913) was unsuccessful, however, in attempts to grow *Oscillatoria brevis, O. tenuis,* and *Nostoc* sp. in the dark. Harder (1917), Winter (1935), and Allison, Hoover, and Morris (1937) demonstrated heterotrophy in several strains of *Nostoc* with glucose as the carbon source. Enhancement of growth in light with various carbon sources was reported in all the above-cited investigations. The main result of these studies was to implant widely the idea that blue-green algae are usually heterotrophic. As more species have been examined, heterotrophy has been found to be the exception, not the rule.

Allen (1952) found only six isolates (including one previously known) to be heterotrophic. These included *Oscillatoria, Lyngbya, Phormidium, Plectonema,* and *Nostoc* species; growth was very slow on a glucose-yeast autolysate medium. Kratz and Myers (1955) grew Allison's strain of *Nostoc muscorum* in the dark, but were not able to grow another strain of the same species or several other species heterotrophically. Further reports of heterotrophy continue to appear in a wide range of blue-green species (Kiyohara, *et al.*, 1960; Fay, 1965; Kantz and Bold, 1968) although the occurrence of any true heterotrophic growth in blue-green algae is also questioned (Holm-Hansen, 1967; Van Baalen, personal communication).

The assimilation in light of various carbon sources has been investigated by Allison, *et al.* (1937), Hoare and coworkers (1965, 1967), Carr and Pearce (1966), Carr, *et al.* (1967), Pearce and Carr (1969), and Kantz and Bold (1969). Glucose and fructose have generally been found to increase growth, although many other compounds (acetate, ribose, galactose, etc.) can be assimilated by some organisms. Failure to assimilate a compound occurring in one of the metabolic pathways is generally thought to be due to permeability limitations (Kratz and Myers, 1955; Fogg, 1956b), but such limitations have been questioned following work with cell-free preparations in which assimilation did not occur (Holm-Hansen, 1968).

Comparative studies of carbon nutrition of several species have rarely been undertaken; in two such studies (Van Baalen, 1962; Kantz and Bold, 1969) different patterns of carbon utilization have been found in the same morphological species, emphasizing again that it is tenuous to base discussions of physiological attributes unquestioningly upon results concerning one or a few species, varieties, or strains.

Clean culture tubes (100 mm × 10 mm) were soaked for 3 days in deionized

water which was changed daily. The tubes were then dried, plugged with cotton, and autoclaved. The control medium was 3N BBMT: eight carbon sources were tested by preparing eight aliquots of media, each containing the equivalent amount of carbon of a 1% glucose solution. The compounds tested were glucose, fructose, *D*- and *L*-arabinose, ribose, xylose, pyruvate (sodium salt), and acetate (sodium salt). The carbon equivalents were, in each case, dissolved in 20 ml of 3N BBMT and added by Millipore filtration to 980 ml of autoclaved 3N BBMT. The media were pipetted into the tubes, which were allowed to incubate at room temperature for 3 days so that bacterial contamination could be detected.

Two separate sets of tubes were prepared; eight of each medium were inoclulated with each axenic isolate for each set. The inocula were taken from the surface of 3-week-old 3N BBM agar slants. Sterilized mineral oil was added to half of the tubes to simulate anaerobic conditions. One duplicate set of aerobic and anaerobic tubes was placed in the culture room under standard conditions and incubated for 3 weeks. The other set was stored in the dark and incubated for a total of 6

TABLE 4. *Growth of Certain Axenic Isolates of Oscillatoriaceae in Media With Various Carbon Sources in Light*

Isolate	No.	Control		Ribose		Fructose		Glucose		D-arab.		L-arab.		Xylose		Pyruv.		Acetate	
		a	an	a	an	a	an	a	an	a	an	a	an	a	an	a	an	a	an
O. lutea	23	G+	N	F	T	N	N	G	F-	T	T	T	T	N	N	N	N	N	N
O. lutea v. auxotrophica	22	G	N	G	T	N	N	E	T	F	T	F	F	N	T	N	N	N	N
O. lutea v. auxotrophica	24	E	N	F	T	N	N	F	T	T	N	G	T	T	N	N	N	N	N
O. lutea v. contorta	25	E	N	F	T	N	N	G	F	F	T	F	T	T	T	N	N	N	N
M. vaginatus	I621	T	N	E	T	F	F	G	T	T+	N	G	N	T	N	N	N	N	N
M. vaginatus v. fuscus	26	G	N	E	F	N	N	E	F	F	T	F	N	T	T	N	N	T	N
S. calcicola	21	G	N	E	G+	G+	T+	G+	T+	F	N	T	N	F	N	N	N	N	N
S. calcicola	K18	G	N	E	G+	G	F	G+	F	F	N	T	N	F	N	N	N	N	N
S. calcicola	K44	F+	N	E	G+	G	F	G	F	T	N	T	N	F	N	N	N	N	N
S. calcicola v. vermiformis	K57	G-	N	E	G	G	F	G	T	F	N	F-	N	N	N	N	N	N	N
S. calcicola v. minuta	27	E	N	N	N	N	N	E+	G	N	N	N	N	F	T	T	G	G-	N
S. calcicola v. spiralis	K107	E	T	G	T	G	F	G	T	E	N	N	N	G	N	N	N	N	N
S. calcicola v. mucosa	K182	F	N	G	N	F	N	F	N	F+	N	T	N	F	N	N	N	N	N
S. calcicola v. glomerulata	I426	F+	N	E	G	G	F	G+	T	F	N	F	N	N	N	N	N	N	N
S. calcicola v. glomerulata	I427	F	N	E	G+	G	G	G	G	F	N	F	N	N	N	T	T	N	N
S. calcicola v. glomerulata	I482	G	N	G	E	G+	E	G	E	F	T-	T+	N	N	N	N	N	T	N
S. calcicola v. glomerulata	I487	G	N	E	G+	G+	F	G+	F	F	N	F	T	T	N	N	N	N	N
S. calcicola v. glomerulata	I597	G	N	E	G	G	F+	G	F	F+	N	F+	N	F	N	N	N	N	N
S. calcicola v. glomerulata	I598	G	N	E	E	G	F+	G+	T	G-	N	G-	N	N	N	N	N	N	N
S. calcicola v. amorpha	I485	F	N	E	G+	G	G	F	T	F	N	F	N	F-	N	N	N	N	N

E = Excellent growth (covering the sides of the culture tube and surface of the medium)
G = Good growth F = fair growth T = Trace growth N = No growth
all values based on macroscopic observations

[a]Growth of the isolates in darkness was very limited: 23, glucose, an, T+; 22, glucose, an, T; 25, glucose, a, T, an, F; K18, ribose, a, F, fructose, a, F; K44, ribose, a, T+; K57, ribose, a, T+, fructose, a, T+, glucose, a, T+; I427, fructose, T; I482, D-arab., an, F. Tests not discussed in this dissertation showed that only 22 in glucose survived a second transfer.

weeks. Table 4 summarizes growth of the isolates as macroscopically determined at the end of 3 weeks.

Growth in the control medium (3N BBMT) was rated as "trace" to "excellent" in comparison to growth in other media; most isolates were considered to show "good" growth (Fig. 110–112). There was no growth in any isolate in 3N BBMT in the dark, and in the light under anaerobic conditions only K107 showed a trace amount of growth.

Growth of all but four of the isolates (23, 24, 25, K107) was enhanced by at least one of the carbon sources. Ribose increased growth in isolates 21, 26, 1621, K18, K44, K57, K182, I426, I427, I482, I485, I487, I597, and I598 under aerobic conditions. Anaerobic conditions in the presence of ribose resulted in increased growth in all of the above except 26, I621, and K 182. Growth in the dark in ribose-supplemented medium was observed for K18, K44, K57 (aerobically), I487 (anerobically—a minute amount), and I598 (both aerobically and anerobically.

Glucose enhanced growth in isolates 22, 27, I426, I427, and anerobically in I482. Fructose increased the amount of growth in isolates I426, I427, and anerobically in I482. Isolate 23 grew slightly anaerobically, and isolate K57 grew slightly aerobically in glucose in the dark. Isolate 22 continued growth in the dark after a transfer to new media. K57 showed a "trace" amount of growth in fructose aerobically in the dark. Isolates 22, 23, 24, 25, and 26 were entirely inhibited in fructose, and isolate I621 grew only slightly.

Both *D- and L*-arabinose did not increase growth, or inhibited it, in most isolates in the light, although all grew with these sources except for K107 in *L*-arabinose. There were only "trace" amounts of growth in five of the isolates (22, 23, 25, 26, I582 in *D*-arabinose; 22, 23, 24, 25, I487 in *L*-arabinose), and no growth of any occurred in darkness.

Xylose supported at least some growth in three-fourths of the isolates, and in K107 growth was rated "good." Isolates 22, 25, 26, 27 grew slightly anerobically in xylose. No growth of any of the isolates occurred in darkness.

Pyruvate and acetate both supported growth in isolates 26 and 27. Isolate 27 grew quite well aerobically in acetate and anerobically in pyruvate: isolate 26 grew only slightly. Isolate I427 grew slightly in pyruvate both anerobically and aerobically, and isolate I482 grew slightly aerobically in acetate. No other isolates grew at all in either medium in light and none grew in the dark.

The inhibition of certain of the isolates by fructose and the enhancement of many by ribose to a far greater degree than by either glucose or fructose are the most distinctive patterns of carbon utilization seen in these isolates. Heterotrophy is definitely not a pronounced attribute in any, if indeed, it is present at all. The several isolates of a species (as K18, K44, 21—*Schizothrix calcicola*; I482, I487, 1597—*S. calcicola* v. *glomerulata*; 22, 24—*Oscillatoria lutea* v. *glomerulata*) showed identical or very similar patterns.

Effects of Various Nitrogen Sources

Investigations of blue-green algal nitrogen metabolism have been made for over 50 years. Since the confirmation of the occurrence of nitrogen fixation in some blue-green algae (Drewes, 1928), the majority of work has been related to this phenomenon; reviews of the subject have been made by Fogg (1956a, 1962), Nicholas (1963), and Stewart (1966). Economic aspects have not been ignored either (Singh, 1961). Reports of nitrogen fixation in the Oscillatoriaceae (Copeland, 1932) have since been disproven, although there has been recent interest in *Trichodesmium* (= *Oscillatoria*) as a possible nitrogen fixer (Dugdale, Goering, and Ryther, 1964); the capacity for nitrogen fixation seems to be found only in heterocystous genera. Fay *et al.* (1968) are of the opinion that the heterocyst may actually be the location of nitrogen fixation in blue-green algae.

Pringsheim (1913), in the first investigation definitely known to use axenic cultures of blue-green algae, studied the effects of a number of organic nitrogen sources on *Oscillatoria brevis, O. tenuis,* and *Nostoc* sp. and found that a wide range supported growth. Maertens (1914), working with various species, and Harder (1917), using an endophytic *Nostoc punctiforme,* also successfully grew their isolates with many nitrogen sources.

Allen (1952) studied the effect of various cultural conditions upon a large number of blue-green algae, including several Oscillatoriaceae (*Oscillatoria, Phormidium, Lyngbya*). She grew isolates on a variety of nitrogen sources and found NO_3^- and NH_4^+ to be used by all, some amino acids by most, and casein by many. The prevalence of NO_3^- and NH_4^+ as good nitrogen sources has been confirmed by many studies since then (Magee and Burris, 1954; Kratz and Myers, 1955; Pintner and Provasoli, 1958; Van Baalen, 1962; McLachlan and Gorham, 1962; Volk and Phinney, 1968; Kantz, 1968).

Urea has been found to be a good source of nitrogen, though rarely as good as NO_3^-, by Kratz and Myers (1955), Van Baalen (1962), McLachlan and Gorham (1962), and Kantz (1968), while Volk and Phinney (1968) found urea inhibited growth in a strain of *Anabaena spiroides.* These investigations have also pointed out that some of the amino acids support growth, although more seem not to, and results vary widely with the organism used.

In addition to these studies on the effect of various nitrogen sources on the growth of some of the blue-green algae, a few have also been made in relation to ecological or morphological characteristics. Nitrogen fixation studies have, of course, been the subject of most. The limitation of growth due to available nitrogen in the coccoid *Microcystis aeruginosa* was demonstrated by Gerloff and Skoog (1957). Nitrogen cycles, primarily in nitrogen fixing species, in freshwater areas are discussed by Billaud (1967). Fogg (1949), in his study of heterocysts in *Anabaena cylindrica,* found that the formation of heterocysts was inhibited by NH_4^+ and that heterocyst formation was usually inhibited by the presence of much

available combined nitrogen. The same general results were obtained by Mickelson, Davis, and Tischer (1967). The effect of inorganic nitrogen sources on morphology of *Anabaena doliolum* was investigated by Singh and Srivasteva (1968).

The possible use of differential growth in a range of nitrogen sources as a supplementary taxonomic attribute has been considered by several investigators of the green and blue-green algae (Cain, 1966; Smith and Bold, 1966; Groover and Bold, 1969; Kantz and Bold, 1969).

Growth of the axenic isolates used in this investigation in various inorganic and organic nitrogen sources was tested to see if results of such tests might be of taxonomic significance, and to ascertain what the effects of the various nitrogen sources on the morphology of the isolates might be.

Clean culture tubes (100 mm × 10 mm) were soaked for 3 days in deionized water which was changed daily. The tubes were then dried, plugged with cotton, and autoclaved. The control medium was 1N BBMT; an amount of nitrogen equal to that of the $NaNO_3$ in 1N BBM was added to BBMT-N in preparing the other media. Eleven other sources, including gelatin, were tested as well as growth in the absence of combined nitrogen. The nitrogen equivalents were, in each case, dissolved in 10–25 my deionized water and added by Millipore filtration to the sterile BBMT-N made up to 975–990 ml. The pH of the BBMT-N was 7.2; Table 5 gives the initial pH of each medium. The media were pipetted into the tubes and allowed to incubate at room temperature for 3 days so that bacterial contamination, if any, could be detected.

Two sets of duplicate tubes were inoculated. The inocula were grown in BBM-K, which contains half as much NO_3 as 1N BBMT, in an attempt to minimize the effects of residual nitrogenous compounds. That this method does, indeed, reduce carry-over effects into the new media was seen in the test run with no combined nitrogen source. Some cultures transferred from 3N BBM to BBM-N showed fair growth at first although color changes soon began, and growth appeared to have ceased in all cultures after 1 week. Cultures transferred from BBM-K, however, showed no or only an extremely minute amount of growth for the first day or two.

Where only "fair" or "trace" growth was recorded, subsequent transfers on these media might result in eventual death of the isolate; such transfers were not made.

The amounts of growth in the various media as macroscopically determined, and the pH (except for gelatin and in the absence of nitrogen) at the end of 3 weeks' growth under standard conditions are summarized in Table 5.

All of the isolates grew well with $NaNO_3$ as the nitrogen source; the pH of all the media increased during growth. With the exception of isolate K107, the various pH increases appear relative to the amount of growth; K107 grows more slowly than the other isolates, yet the terminal pH (8.3) was the second highest of the group. Microscopic examination revealed that the trichomes appeared generally to have a low percentage of abnormal or irregularly shaped cells and few

distinct granular inclusions. They corresponded in morphology to the descriptions of the isolates given earlier in this report.

Gelatin also was a good nitrogen source for all the isolates tested. All cultures showed growth, graded as "good" or "excellent," both throughout the tube and on its surface. Further discussion of growth in gelatin is deferred to a later section.

Ammonium nitrate and ammonium sulfate, 1X urea, and 10X urea supported growth rated as "good" or "excellent" in most isolates. In many, however, the cell morphology appeared quite different from that in $NaNO_3$ in that numerous granules and irregular cells frequently occurred. One group of isolates (21, K18, K44, K57, 27) did not show much difference in morphology in any of these media as compared to $NaNO_3$. The pH in NH_4NO_3 and $(NH_4)_2SO_4$ was lowered somewhat in all but isolate K182, where there was no change; in urea, there was usually an increase in pH, although a decrease was found in some isolates, and in some the pH remained stable.

All isolates grew in $NaNO_2$, but except for isolates 24 and I482, macroscopic growth was less—sometimes substantially so—than in $NaNO_3$. Some large numbers of cellular inclusions (isolates 22, 23, 24); others were indistinguishable from the set grown in $NaNO_3$ (21, I426, I482).

Uric acid supported "excellent" growth in two isolates, "good" growth in nine, and "fair" growth in nine. Cells were frequently irregular, however; many hormogonia were seen, and the cells were often brownish-green or somewhat bleached.

Growth in casamino acids, succinamide, and uracil was graded as "good" to "no" growth. Casamino acids supported "fair" growth in six, "trace" growth in eleven, and "no" growth in three cases. With succinamide, five isolates were considered to show "good" growth, eight "fair," and seven "trace" growth. The cultures were in many cases (see Table 5) yellowing at the end of 3 weeks in these media. Microscopically, they also appeared to have aged prematurely in that crosswalls were frequently indistinguishable; many hormogonia and dead cells were present; and false branches were seen in those isolates which had been found to produce them with age. Dense granular inclusions were of rare occurrence.

Hydrolysis of Gelatin

The use of gelatin hydrolysis as a supplementary attribute has been a common test for some time in bacteriological studies; application of the test to a wide variety of algae by several workers has shown that many algae, freshwater and marine, secrete an extracellular gelatinase (Tanner, 1923; Pringsheim, 1951; Allen, 1952; Tazawa and Miwa, 1953; Kessler and Czygan, 1967; Groover and Bold, 1969; Kantz and Bold, 1969).

The gelatin medium was prepared by first melting 100 g Difco gelatin in 400 ml deionized water, then pouring the gelatin into dialyzing tubes and soaking them in deionized water for 24 hr with four changes of water to remove soluble nitrogenous

TABLE 5. *Growth of Certain Axenic Isolates of Oscillatoriaceae in Media with Various Nitrogen Sources*

Isolate	No.	NO_3(7.2)[a]	NO_2(7.2)	NH_4NO_3(7.1)	$(NH_4)_2SO_4$(7.1)	cas acid(7.2)	uric acid(7.2)	uracil(7.4)	succinamide(7.4)	urea-1X(7.3)	urea-10X(7.3)	urea-100X(7.3)	gel	-N
O. lutea	23	E(7.9)[b]	G(7.5)	E(6.8)	E(6.9)	T*(7.0)	G(7.1)	F(7.2)	G(7.2)	E(7.3)	T(7.2)	N(7.2)	E	N
O. lutea v. auxotrophica	22	E(7.7)	F(7.5)	E(6.8)	G(6.9)	T*(7.0)	E(7.1)	T+*(7.1)	F*(7.2)	G(7.3)	G(7.7)	N(7.3)	G	N
O. lutea v. auxotrophica	24	E(7.8)	E(7.6)	E(6.8)	G(6.8)	F*(6.9)	G(7.0)	F+(7.1)	F*(7.0)	G(7.2)	E(7.1)	N(7.1)	G	N
O. lutea v. contorta	25	E(7.5)	T(7.2)	T(6.8)	T(6.9)	F*(6.9)	G(7.0)	G*(7.0)	G*(7.0)	F(7.2)	T(7.1)	N(7.1)	G	N
M. vaginatus	I621	E(7.7)	T(7.2)	T(6.9)	T(6.9)	T(7.0	F(7.0)	F*(7.0)	F(7.1)	G(7.2)	N(7.1)	N(7.2)	E	N
M. vaginatus v. fuscus	26	E(7.8)	T(7.2)	T(6.8)	F(6.8)	N(7.2)	E*(7.1)	G(7.1)	G-(7.1)	F(7.3)	T(7.1)	N(7.2)	E	N
S. calcicola	21	E(7.7)	G+(7.5)	E(6.8)	G+(6.9)	T(7.0)	G+(7.0)	G+(7.0)	T(7.1)	E(7.4)	E(7.4)	N(7.2)	E	N
S. calcicola	K18	E(7.7)	G(7.6)	E(6.8)	E(6.9)	T(7.0)	F(7.1)	F*(7.2)	F*(7.1)	E(7.3)	E(7.4)	N(7.2)	E	N
S. calcicola	K44	E(7.9)	G(7.5)	E(6.7)	E(6.4)	T(7.0)	F(7.2)	F*(7.2)	F*(7.1)	E(7.5)	E(7.3)	N(7.3)	E	N
S. calcicola v. vermiformis	K57	E(7.6)	G(7.5)	F(6.8)	F(6.9)	N(6.9)	G(7.0)	F(7.1)	T(7.0)	E*(7.5)	G(7.7)	N(7.1)	E	N
S. calcicola v. minuta	27	E(7.9)	F(7.6)	E(6.8)	E(6.8)	T(7.0)	G(7.1)	G(7.1)	G(7.2)	E(7.6)	E(7.8)	T(7.3)	G	N
S. calcicola v. spiralis	K107	E(8.3)	T(7.2)	T(7.0)	T(7.1)	T*(7.1)	G*(7.1)	G*(7.1)	F*(7.2)	T(7.1)	G(7.4)	N(7.1)	G	N
S. calcicola v. mucosa	K182	G(7.5)	F(7.4)	G(6.9)	G(7.0)	N(7.1)	F(7.1)	G(7.2)	F(7.2)	F(7.6)	F(7.1)	N(7.3)	G	N
S. calcicola v. glomerulata	I426	E(7.9)	T(7.4)	G(6.8)	E(6.8)	F(7.0)	F(7.2)	F(7.2)	T(7.2)	G(7.4)	G(7.3)	N(7.2)	E	N
S. calcicola v. glomerulata	I427	E(8.0)	F(7.6)	G(6.8)	E(6.7)	T(7.0)	F(7.1)	F(7.2)	T(7.1)	G(7.8)	G(7.9)	N(7.1)	E	N
S. calcicola v. glomerulata	I482	E(8.0)	E(7.7)	G(6.7)	E(6.5)	F(7.1)	G(7.1)	G*(7.1)	G*(7.2)	E(7.7)	G(7.2)	N(7.3)	E	N
S. calcicola v. glomerulata	I487	E(7.9)	F(7.6)	G(6.8)	E(6.8)	T(7.1)	F(7.1)	T(7.1)	T(7.1)	G(7.4)	G(7.3)	N(7.2)	E	N
S. calcicola v. glomerulata	I597	E(7.9)	F(7.5)	G(6.8)	E(6.9)	T(7.0)	F(7.1)	T(7.1)	T(7.1)	G(7.4)	G(7.3)	N(7.3)	E	N
S. calcicola v. glomerulata	I598	E(8.5)	G(7.8)	E(6.8)	E(6.3)	F*(7.0)	G(7.1)	F*(7.2)	F*(7.1)	E(7.4)	E(7.2)	N(7.3)	E	N
S. calcicola v. amorpha	I485	E(7.9)	T(7.5)	G(6.8)	G(6.9)	F(7.0)	F(7.2)	F*(7.1)	T(7.1)	G(7.8)	G(7.2)	N(7.2)	E	N

E = Excellent growth (covering the sides of the culture tube and surface of the medium) G = Good growth F = Fair growth T = Trace growth
N = No growth all values based on macroscopic observations

[a] pH of the medium at time of inoculation
[b] pH of the medium after 3 weeks growth under standard conditions
* indicates yellowing or redness of alga by end of 3 wee[k]

substances in the solution. The BBM stocks for 1 liter (except for $NaNO_3$) were added to deionized water to make 600 ml of solution. The dialyzed gelatin was added to this BBM, melted, and poured into tubes. The latter were then autoclaved and allowed to resolidify. Deep stab inoculations were made of each of the 20 axenic isolates. Duplicate tubes were inoculated for two separate tests. Inocula were taken from agar slants.

All of the isolates grew well in the medium, using gelatin as the sole nitrogen source. Isolates K 107, K18, and K182 entirely hydrolyzed the medium within 6–9 weeks. Isolate I485 showed slight hydrolysis at 13 weeks, as did isolate I598 at 14 weeks. At 18 weeks, isolates I426, I427, and I487 showed hydrolysis at the top of the tube. No further change was observed in any isolate after 18 weeks.

Isolates I426, I427, I482, I485, I487, I597, I598, K107, and K182 secreted a blue pigment into the medium. Isolates K44 and K57 secreted a blue-green pigment, and isolate 26 secreted a purplish-red pigment. These pigments were also secreted when the isolates were grown on nutrient agar or proteose-peptone agar, although apparently always in lesser quantities. Secretion of pigments on complex organic media was also noted by Kantz (1968) in *Nostoc* and *Anabaena*. Here, as in his isolates, appearance does not signify a breakdown of cells.

Isolates 22, 23, and 24 all grew well throughout the tubes, but in 3–4 weeks isolate 23 became colorless. Isolates 22 and 24, which are very similar morphologically to 23, remained green and continued to grow for several months.

Hydrolysis of Starch

The presence or absence of extracellular amylasic activity has been used as a supplementary taxonomic criterion by several investigators of the Chlorophycophyta and Cyanophycophyta (Mattox and Bold, 1962; Bischoff and Bold, 1963; Cain, 1963; Brown and Bold, 1964; Wiedeman, 1964; Smith and Bold, 1966; Groover and Bold, 1969; Kantz and Bold, 1969). Harder (1917) found that starch could be used as a carbon source in both light and dark by an endophytic *Nostoc punctiforme*. The digestion of a soluble starch into diffusible compounds is the result of enzymatic reactions of the α- and β- amylases; these amylases are secreted by many microorganisms. It is a matter of some question whether the enzymes and mechanisms are really the same in all cases.

Soluble potato starch (0.1 g/liter) was added to 3N BBM and solidified with 1.5% agar. The autoclaved sterile solution was poured into sterile petri dishes, which, after solidifying, were inoculated in the center with a small amount of each of the 20 axenic isolates. Duplicate plates were inoculated for three separate tests.

After 2 weeks' growth under standard conditions, the plates were flooded with a dilute I_2-KI solution, followed by washing with deionized water.

All the isolates showed at least a small colorless zone. In some cases the size of the zone appeared to be correlated with the amount of growth; thus, the agar on which isolates 26 and I621 grew was completely colorless as they entirely covered the plate while that of K107, which grew only slightly, was deep blue except for

a narrow halo around the plant-mass. Other isolates did, however, seem to show differential responses. Isolates K18, K44, and 21 showed complete hydrolysis of the starch. Isolate K57, which is identical morphologically to K18, K44, and 21 although it has a slightly different plant-mass form and color-change pattern, showed a wide halo, but also a clear band of unhydrolyzed starch. While isolates 22, 23, 24, and 25 grew well on the starch agar, they showed only slight hydrolysis. Isolate 27, which is not fast-growing on the starch media, nevertheless showed a wide halo. Isolate K182, I426, I427, I482, I485, I487, I597, and I598 all showed clear halos.

The presence of amylases reacting similarly to those of green algae in the blue-green algae is not unexpected as cyanophycean starch has been shown to have the same properties as does amylopectin of "higher" plants.

Sensitivity to Crystal Violet

The differential inhibitory effect of adding various concentrations of crystal violet to standard media has been used as a supplementary taxonomic attribute by Bold and coworkers for a number of species of green algae with varying degrees of success (Mattox and Bold, 1962; Brown and Bold, 1964; Groover and Bold 1969).

The crystal violet plates were prepared by adding 10 different concentrations of crystal violet to 3N BBM. A stock solution was made by dissolving 1 g crystal violet (Difco) in 100 ml deionized water. The stock was used to prepare media with the following concentrations of crystal violet: 0.00001%, 0.00002%, 0.000045%, 0.0001%, 0.0002%, 0.00045%, and 0.001% Three additional solutions were made by weighing the necessary amounts of crystal violet and adding them directly to the 3N BBM to give final concentrations of 0.002%, 0.0045%, and 0.01% per liter.

The solutions were solidified with 15 g agar, autoclaved, poured into small sterile petri dishes, and inoculated at once with the axenic isolates. They were then placed in the culture room and allowed to grow for 2 weeks. Tests were done twice in duplicate. Table 6 summarizes the growth of the isolates after 2 weeks.

The blue-green algae tested here appear on the whole to be somewhat more sensitive to crystal violet than the green algae which have been heretofore investigated. The greatest degree of resistance (growing on 0.002% CV agar) was shown by isolates 22 and 24 (Fig. 116), considered on all criteria to be duplicate isolates of *Oscillatoria leuta* v. *auxotrophica*. The only isolates (23 and I482) that grew on 0.001% CV agar and not above were different species (*Oscillatoria lutea* and *Schizothrix calcicola* v. *glomerulata*). The most sensitive organisms (Fig. 115) were isolates 21, 26, 27, K57, and K107, none of which grew above a concentration of 0.0001%; these isolates (except for 21 and K57) are all quite distinct from one another in several other respects. Seven isolates grew on 0.002% CV agar, but not above it (Fig. 114): four grew on 0.00045% CV agar, but not above it (Fig. 113). The differences in growth by one concentration of duplicate isolates of a variety or a species (K18 and 21, I482 and I487, etc.) are almost certainly not sig-

TABLE 6. *Inhibition of Axenic Oscillatoriaceae on Crystal Violet Agar at Different Concentrations*

Isolate	No.	Concentration (%)									
		.00001	.00002	.000045	.0001	.0002	.00045	.001	.002	.0045	.01
O. lutea	23	G	G	G-	T	T	T	T	N	N	N
O. lutea v. auxotrophica	22	G	G	G	G	T	T	T	T-	N	N
O. lutea v. auxotrophica	24	G	G-	T	T	T-	T-	T-	T-	N	N
O. lutea v. contorta	25	G+	G	T	T	T	T	N	N	N	N
M. vaginatus	I621	G	G-	G-	T	T	T	N	N	N	N
M. vaginatus v. fuscus	26	G	T	T	T	N	N	N	N	N	N
S. calcicola	21	E	G+	G	T	N	N	N	N	N	N
S. calcicola	K18	E	G+	G	T	T-	N	N	N	N	N
S. calcicola	K44	E	G+	G	T	T-	N	N	N	N	N
S. calcicola v. vermiformis	K57	E	G	G	T	N	N	N	N	N	N
S. calcicola v. minuta	27	E	G+	G	T	N	N	N	N	N	N
S. calcicola v. spiralis	K107	G	G	G	T	N	N	N	N	N	N
S. calcicola v. mucosa	K182	G+	G	G-	T	T-	N	N	N	N	N
S. calcicola v. glomerulata	I426	E	E	G+	T	T-	N	N	N	N	N
S. calcicola v. glomerulata	I427	E	E	G+	T	T-	N	N	N	N	N
S. calcicola v. glomerulata	I482	G+	G	G	G-	T	T-	T-	N	N	N
S. calcicola v. glomerulata	I487	G+	G+	G	T	T	T-	N	N	N	N
S. calcicola v. glomerulata	I597	E	E	G	T	T	N	N	N	N	N
S. calcicola v. glomerulata	I598	E	G	G	T	T	T-	N	N	N	N
S. calcicola v. amorpha	I485	G	G	G-	T	T-	N	N	N	N	N

E = Excellent growth--same as control (3N BBM)
G = Good growth
T = Trace growth; T- indicates original inoculum remained green, but that no or only a very few trichomes grew away from it
N = No growth

nificant. The only obvious separation seems to be that most of the larger isolates grew at slightly higher concentrations than did the smaller ones. Isolates 26 and I482 are exceptions to this generalization.

Effect of Vitamins

Three vitamins, B_{12}, thiamine, and biotin, have been shown to be growth factors required singly or in combination by a number of algae. Reviews of auxotrophy in the algae have been made by Lewin (1961) and Droop (1962). In the blue-green algae, reports have been mainly of studies of marine species (Pintner and Provasoli, 1958; Van Baalen, 1961, 1962). Van Baalen's study of 15 isolates revealed a B_{12} requirement in more than half of them. Kantz and Bold (1969) reported an isolate of *Anabaena utermohli* in which growth was greatly enhanced by the addition of a vitamin mixture, but whether this represented an absolute requirement was not determined.

Preliminary tests were made to establish whether any of the axenic isolates had a vitamin requirement. The cultures were transferred from 3N BBM + B_{12} + Eagle's vitamin mixture to 3N BBM without vitamins. Successive transfers were

then made to the vitamin-free medium. For the first month and a half no differences in growth were noted. By the end of 3 months, during which three transfers had been made, three isolates, 22, 24, and 25, showed only minute amounts of growth on agar plates. Microscopic observation showed that even filaments in 1- or 2-week-old cultures contained numerous granules and vacuoles and had very thick sheaths.

The isolates were transferred again to four sets of media: 3N BBM, 3N BBM + B_{12}, 3N BBM + Eagle's, 3N BBM + B_{12} + Eagle's. Both liquid and agar-solidified media were used. After this transfer, isolates 22, 24, and 25 were no longer viable on 3N BBM without vitamins. The other isolates showed no difference on agar with any of the four media (Fig. 117). Isolates 22, 24, and 25 showed greatly increased growth in 3N BBM + B_{12} and in 3N BBM + B_{12} + Eagle's. Reciprocal transfers were then made, and good growth of the isolates continued only on those media containing B_{12} (Fig. 118). After two successive transfers on 3N BBM + B_{12}, the cultures were once again flourishing.

Growth in liquid media showed more differences than that on agar. After 2 weeks, isolates K18 and K44 showed slightly more growth in all enriched media than in 3N BBM. Isolates K107, 22, 24, 25, 26, and I621 grew better in B_{12} and in B_{12} + Eagle's than in other media. Isolates I426, I427, I482, I485, I487, I597, and I598 showed slightly more growth in 3N BBM + B_{12} + Eagle's than in the other media, but as growth was very good in all, macroscopic determination of differences was difficult.

Some of the unialgal isolates were tested on 3N BBM without vitamins, 3N BBM + B_{12}, and 3N BBM + B_{12} + Eagle's during the course of this investigation. Isolates 5, I386, and I390 did not show significant growth after three to four successive transfers not containing B_{12}. Whether some of the other unialgal isolates might have some vitamin requirement, or at least show increased growth with vitamins, if they were in axenic culture or had different contaminants in the unialgal state is impossible to determine without further work.

The results from these tests are similar to other, previous ones with blue-green algae in that no absolute requirement for any vitamin except B_{12} was demonstrated, although something in the Eagle's mixture enhances growth in liquid media of several of the isolates. Continued growth of three isolates (22, 24, and 25) in axenic culture was impossible to maintain without the addition of B_{12}, but which analogues are necessary is not known.

Effects of Varying the pH

One of the classical tenets of culturing blue-green algae has been the requirement for alkaline growth conditions. For some organisms, a pH as high as 10.0 has been considered optimal (Allen, 1952; Kratz and Myers, 1955; Gerloff and Skoog, 1957). More recently, the use of better buffer systems has allowed for lower pH ranges to be tolerated by some of the same organisms (McLachlan and Gorham,

1962). Since the basal medium for the morphological studies in this investigation was 3N BBM, the pH of which falls in the range of 6.5–6.8, and since blue-green algae are encountered in a wide range of habitats, all of which certainly do not continually have high pH values, comparative studies of some of the isolates at various pH's are of interest.

Seven sets of media were prepared with initial pH's of 4.0, 5.0, 6.0, 6.5 (3N BBM), 7.0, 8.0, and 9.0. Those at pH 4.0 and 5.0 were citrate-buffered; those at 7.0, 8.0, and 9.0 were Tris-buffered. The control, 3N BBM, and the 6.0 medium were phosphate-buffered. Terminal pH's (taken after 3 weeks growth) in most cases were only slightly higher than the initial pH in the citrate- and Tris-buffered medium, while those of the phosphate-buffered media often rose to 8.0–9.0.

Twenty isolates, 10 axenic and 10 unialgal, were selected; they were isolates 3, 4, 18, 23, 25, 26, 27, 43, K18, K44, K55, K57, K58, K107, K182, I427, I487, I617, I621, and I622. These isolates included representatives of a wide range of morphology and also had been isolated from a wide range of habitats (see Table 1).

Duplicate flasks were inoculated for two separate tests and incubated under standard conditions. Macroscopic and microscopic observations were made at the end of 3 weeks. Table 7 summarizes the appearance of the flasks after 3 weeks. As can be seen, despite the good growth normally seen in 3N BBM, growth in many isolates was as good or better at pH 7.0–9.0 as at 6.5 (isolates 4, I487, K44, K57, K107, K182). Others (18, 25, 26, 27, 3, K18, K58) had excellent growth at points anywhere between 5.0 and 7.0–9.0. A few (K55, 23, 43, I621, I622) were sharply delimited at one or two pH's very close to that of 3N BBM. K58 survived in all but the lowest pH, but growth was slow in all, and differences could not be seen within 2–3 weeks. I617 grew excellently at 5.0, 6.0, and 6.5 and only enough to be considered "fair" above 6.5.

Macroscopically, the plant-mass varied regularly with pH in some of the isolates. Growth in K44 and K18 was filamentous with a thin layer of filaments forming on the surface of the media and on the sides and bottom of the flask at pH 6.0–7.0, but discrete clumps of the algae occurred at 8.0 and 9.0 (Fig. 120). Isolate I617 was reddish-brown at 5.0 and 6.0, but distinctly gray-brown at 6.5. Isolate I622 was bright green at 6.5 and 7.0, olive-brown at 8.0 and 9.0. These changes in color were accompanied by changes in the cell or trichome morphology.

The original inocula of isolates 18, K44, and K57 remained green at pH 4.0, but no growth occurred. Isolate 3 was the only one which grew even slightly at 4.0 in the 3-week period. It had been isolated from a bog soil sample. While growth was better at the higher pH's, there was tolerance of the acidic media as well as of the alkaline ones.

There were numerous microscopic differences observed in some of the isolates at different pH's. No visible differences, however, were observed in isolates 18, K44, K18, K58, 27, and 43 in any of the media.

TABLE 7. *Growth of Certain Isolates of Oscillatoriaceae at Various pH's at 3 Weeks*

Isolate	pH						
	4.0	5.0	6.0	6.5	7.0	8.0	9.0
3	T	E	E	E	E	G	F
4	N	T	T	T	G	E	E
18	N	T	E	E	E	E	E
23	N	N	N	G	E	T	T
25	N	N	N	G	G	F	F
26	N	N	N	G	G	F	F
27	N	N	N	E	E	E	E
43	N	T	E	G	G	G	G
K18	N	N	T	E	E	E	E
K44	N	N	T	G	G	E	E
K55	N	T	T	E	G	T	T
K57	N	N	T	F	G	F	G
K58	N	G	G	G	G	G	F
K107	N	N	N	G	G	E	E
K182	N	N	T	T	G	G	G
I427	N	N	F	G	G	G	E
I487	N	N	T	G	G	E	E
I617	N	E	E	E	F	F	F
I621	N	N	N	E	G	N	N
I622	N	N	N	E	E	F	F

E = excellent G = good F = fair T = trace N = no growth as macroscopically and microscopically determined with reference amount of growth, plant-mass color, and morphology

Isolate I427 at 6.0 (the lowest at which it grew) had many terminal, short, curving branches one to three cells long (Fig. 119). These branches were also seen in I426 in ammonium sulfate medium, but they were not elsewhere observed. Isolates I427 and I487 had many crooked trichomes and irregularly shaped cells

in lower pH's while at higher ones cells were very regularly arranged.

A striking characteristic of isolates 23 and 25 was the presence of gas vacuoles in alkaline media and their almost complete absence below 7.0 (Figs. 121–122).

Isolate I621 was characterized by dark granules along the crosswalls in 3N BBM and at 7.0, but at 8.0 and 9.0 numerous granules were scattered throughout the cells (Figs. 123–124). In I617 granules along the crosswalls occurred at 5.0 and 6.0, but not in 3N BBM in this experiment—3N BBM contained dark granules scattered throughout the cells. Isolate K55 showed the normal morphology at 6.5 and 7.0, but in other media cells were constricted at the crosswalls; sheaths were quite thick; and vacuoles occurred at 8.0 and 9.0. Isolate 4 at 6.5 was almost entirely hormogonial; at 7.0 it was filamentous, and the cells were generally black from numerous granules. At 8.0, about half the cells had dense granules, and at 9.0 only a few cells were densely granulate (Fig. 125).

Isolates which did not grow well at the higher pH's generally appeared prematurely aged; granules, vacuoles, copious sheaths, and extensive hormogonia formation were characteristic.

Discussion

This report summarizes an investigation of variation in certain Oscillatoriaceae, an investigation primarily concerned with coordinating variation with taxonomy. The data herein summarized have shown that constant characteristics may be obtained under constant conditions.

The problems encountered in trying to classify members of this family according to the classical system of Gomont (1892) were alleviated by the revision of the family by Drouet in 1968, but a whole new set of difficulties was precipitated at the same time. There are fewer choices to make, fewer answers to arrive at, and fewer chances of deciding that the organism in question really corresponds to the description, since the descriptions allow for almost every conceivable variation. An organism, however, can frequently be collected and identified quite rapidly as one of the 23 species of Oscillatoriaceae recognized by Drouet. If the isolate is merely kept in the laboratory or if it is grown in culture, and if it is observed repeatedly, changes may occur in the characteristics which were apparent when the identification was made. If these variations can occur in the laboratory, it is only reasonable to assume that they also can occur somewhere in the field. It would thus seem somewhat irrational to dismiss summarily all cultures that vary from the "natural" states as revealing abnormalities and unnatural phenomena, a conclusion voiced by some phycologists. Some cultures continue to look exactly as they did when collected; some seemingly grow better, since some collections are certainly made from what must be less than optimal habitats. Some cultures do deteriorate, for there is no one common best laboratory habitat any more than there is one common outdoor habitat. That differences are not necessarily found under different conditions,

however, is seen in the example of isolate 5 and 39 (*Porphyrosiphon notarisii*). Isolate 5 was collected in a pool in Austin, Texas; isolate 39 was growing on a beach in Monterey, California. They looked alike when observed at the time of collection, and the similarities were maintained in culture, both on agar (3N BBM) and in liquid (3N BBM or VSE—von Stosch's enrichment of sea water). On the other hand, two isolates (12 and 34), *Microcoleus lyngbyaceus* v. *vermiformis* and *M. vaginatus* v. *fuscus,* respectively, represent in culture very different forms from those originally collected. When collected, many trichomes were in a single sheath; isolate 12 was identified as *Microcoleus* (*sensu* Gomont), and isolate 34 were identified as *Schizothrix mexicana* (*sensu* Gomont and also according to Drouet). In these cultures in the laboratory only one trichome per sheath was ever observed, and in the case of *S. mexicana,* there were cytological changes. It will be of interest to determine what culture conditions are necessary to maintain the multiple-trichome forms of Gomont's genera *Microcoleus, Schizothrix, Symploca,* etc. Also unknown is whether other isolates identical in culture to 34 (17, 26, I1270) would, if transplanted to field locations, assume the "typical" *Schizothrix mexicana* form.

It has been noted elsewhere (Cox and Bold, 1966; Kantz and Bold, 1969) that the current interest in soil algae, which rarely appear in the natural state in great enough quantity to be identified by classical methods, has led to a need for some additions to methods of classification. A corollary to this idea is seen in that enrichment cultures could easily give rise to a "bloom" of a species described (as in many of the descriptions given in Geitler's work) as occurring only as single cells or filaments. Drouet has overcome the need for using habitat as a key characteristic in all genera except *Oscillatoria,* and it is, of course, possible that *O. erythraea* (described as "marine planktonts, forming water blooms") is a well-defined species confined to the marine plankton. If it is, it seems possible that there are other such habitat-limited species as well, which possibility does not seem to have been emphasized by Drouet.

It is not now considered unreasonable to have to follow a chrysophycean, chlorophycean, or phaeophycean alga through its life history or to have to find numerous stages of the carporporophytic spore development of a red alga in order to determine the order, family, genus, or species of the organism. Even though in the blue-green algae, especially in the homocystous ones, there is little to the life history, it appears quite probable from the current work that it might be wise to observe a blue-green algal isolate over a period of several weeks before finally deciding what species it is.

What part physiological studies could come to have in blue-green algal taxonomy is at present unknown. Most physiological investigations have been made with one or a few isolates and have often been concerned with basic processes that have been shown to be quite similar in all. The presence of nitrogen-fixing strains of several species is one of the few exceptions. The possibility of using supplementary

physiological and cultural characteristics, as in the Actinomycetes, for example, has been considered only rarely. Such aspects of taxonomy will most probably not be explored to any noticeable extent until blue-green algal taxonomy is thought of less in terms of vascular plants and more in terms of microorganisms.

The physiological studies summarized herein represented no great departure from established data on similar organisms. The various physiological tests employed were of little taxonomic value in themselves, but they were considered as further support for the reality of some of the varieties that had been erected on the basis of morphological data. The several differences between the autotrophic *Oscillatoria lutea* (isolate 23) and the auxotrophic variety, *O. lutea* v. *auxotrophica* (isolates 22 and 24), were the best indications that physiological strains exist and must be considered in laboratory studies.

A method of classification based on cultures, while no doubt a great asset to the experimental botanist, would leave the field botanist somewhat at a disadvantage, unless or until correlation of characteristics and their relations to the environment are worked out. Such correlations will have to be based not only on laboratory cultures and field observations, but also upon transplants of known cultures to the field in a manner similar to that of Cox and Bold's (1966) investigation of *Stigeoclonium*. In order to be a practical tool, a future classification (and definitely an "ideal" one) must be established that combines both field characteristics from preliminary determination and characteristics of organisms grown in the laboratory under specified conditions.

Summary

Eighty-two isolates of Oscillatoriaceae, representing the genera *Schizothrix, Microcoleus, Porphyrosiphon*, and *Oscillatoria*, were obtained from soil samples, air samples, marine, and freshwater habitats. They were studied in culture in several defined media; the basal medium selected for further morphological and physiological studies was 3N BBM. Comparisons of unialgal and axenic cultures showed bacterial contamination had no effect on the morphology of the trichome, filament, or on the plant-mass type, but that extensive fungal contamination had detrimental effects. The isolates studied exhibited definite patterns of aging (color change, sheath and trichome changes) associated with alteration of the medium. Cultures were grown on basal medium supplemented with 11 alternate nitrogen sources and with nine carbon sources. Slight heterotrophic growth was initially seen in eight isolates. Growth of the isolates was enhanced in the light by addition of carbon compounds, most frequently by ribose or glucose. All the axenic isolates hydrolyzed starch to varying degrees, but only eight hydrolyzed gelatin. Sensitivity to crystal violet of the axenic isolates was also tested. Three of the axenic isolates exhibited a B_{12} requirement, and growth of several others was enhanced by addition of vitamins to the basal medium. Several unialgal isolates also appeared to have

a vitamin requirement. Varying the initial pH of the basal medium resulted in great variation in plant-mass type and in trichome/filament morphology. Many of the changes seen at higher pH's were comparable to those seen as cultures grown in 3N BBM age.

On the basis of morphological observations, the type of plant-mass, form of the sheaths, and morphology of the terminal cell of the trichomes were determined to be the most constant characteristics, while type of granulation and vacuolation, cell size and shape were quite variable. All morphological attributes were seen to vary under differing conditions, but the plant-mass type, sheath, and terminal cell were constant under each set of conditions, although the mature terminal cell type was frequently somewhat difficult to find. The other attributes did not appear constantly under any of the tested conditions. The diverse isolates included in each large species were classified as 41 varieties mainly on the basis of the plant-mass type. Keys to the isolates studied in culture have been prepared and the cultures of the organisms herein discussed have been deposited in the Culture Collection of Algae at Indiana University. Herbarium specimens have been deposited at the Chicago Field Museum of Natural History.

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Illustrations

FIGURE 1

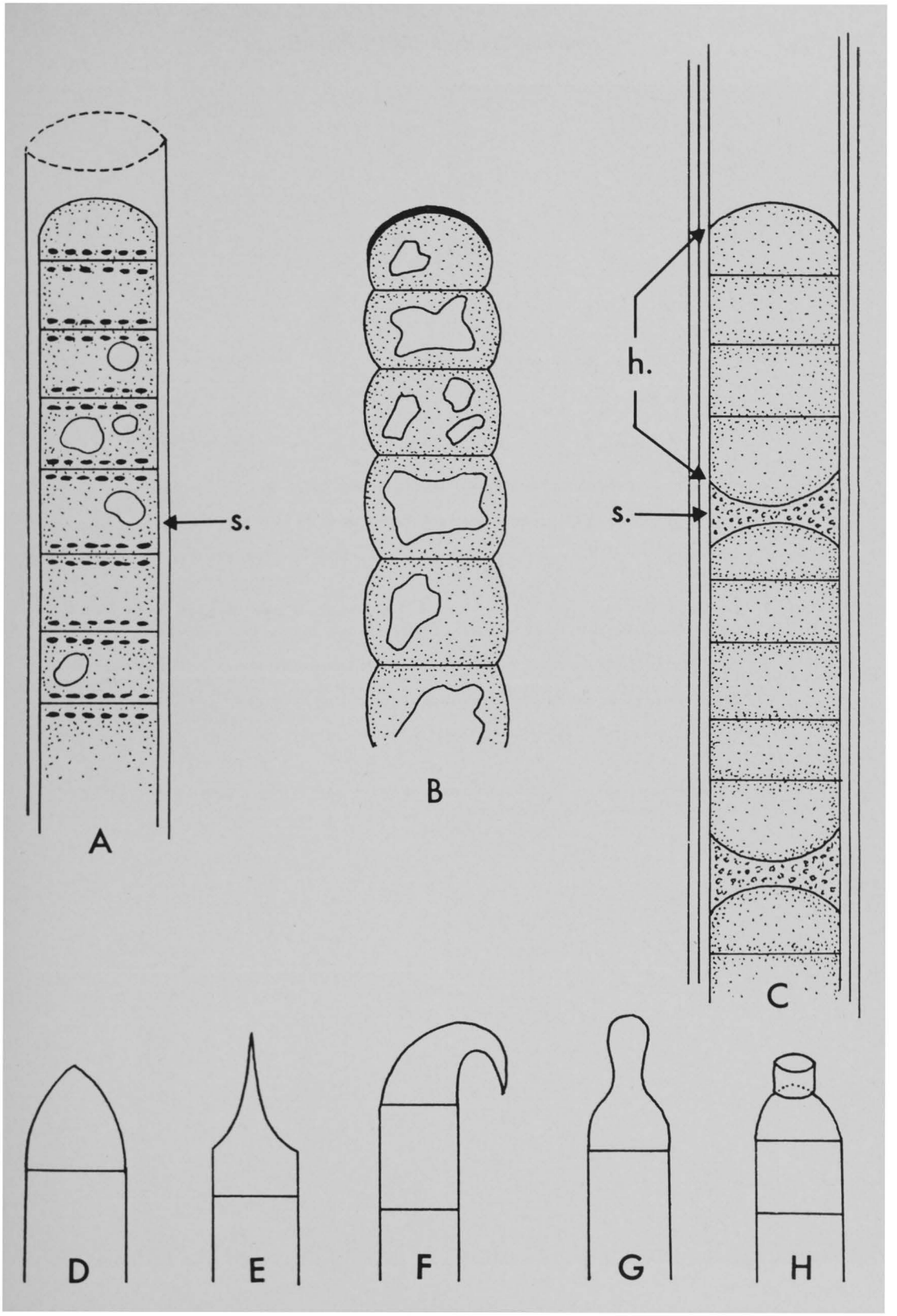
Diagrams illustrating terminology used in describing the morphology of Oscillatoriaceae

A. Diagrammatic representation of a trichome with simple sheath (i.e., filament), with dark granules along the crosswalls, and colorless vacuoles typical of very old or unhealthy cells.

B. Diagrammatic representation of trichome with cells constricted at the crosswalls; with gas vacuoles, and outer wall of rounded terminal cell thickened.

C. Diagrammatic representation of filament containing hormogonia separated one from another by dead cells; sheath lamellated.

D-H. Diagrammatic representations of various types of terminal cells: D, conical; E, pointed; F, bent; G, rostrate; H, capitate.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FIGURES 2–9

Fig. 2. *Schizothrix calcicola*, Macroscopic view of the plant-mass.

Fig. 3. *Schizothrix calcicola*. Portion of a 2-week-old plant-mass on 3N BBM agar, X 14.

Fig. 4. *Schizothrix calcicola*. Filaments and trichome of a 2-week-old culture grown on 3N BBM agar, X 900.

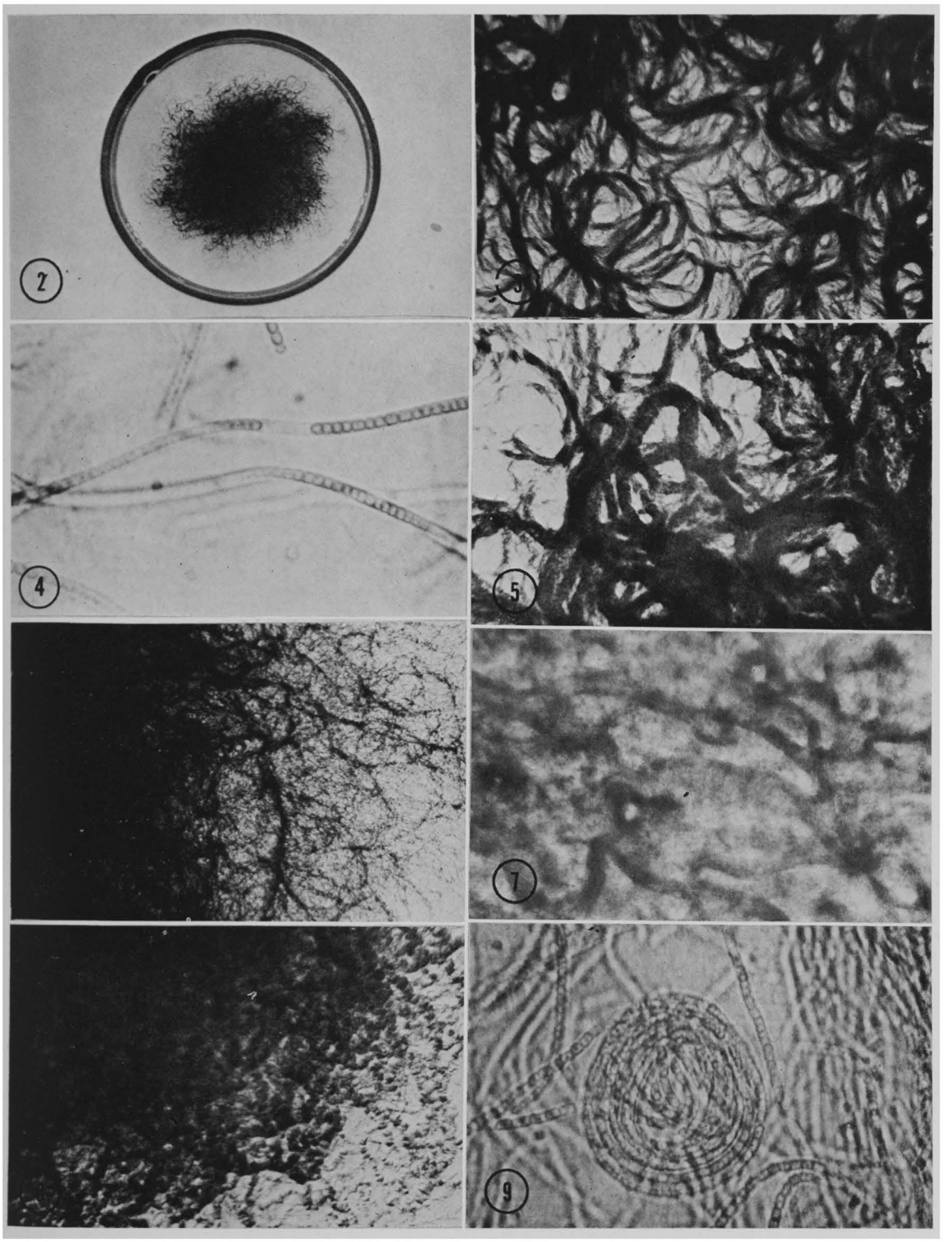
Fig. 5. *Schizothrix calcicola* v. *vermiformis*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, showing rough texture of bundles of filaments as contrasted to *S. calcicola* (fig. 2), X 14.

Fig. 6. *Schizothrix calcicola* v. *diffusa*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 7.

Fig. 7. *Schizothrix calcicola* v. *diffusa*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 8. *Schizothrix calcicola* v. *circinalis*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, x 14.

Fig. 9. *Schizothrix calcicola* v. *circinalis*. Filaments of a 2-week-old culture grown on 3N BBM agar showing coiled filaments that are often seen, X 1100.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FIGURES 10–17

Fig. 10. ***Schizothrix calcicola* v. *radiata*. Macroscopic view of the plant-mass.**

Fig. 11. ***Schizotrix calcicola* v. *radiata*. Portion of a 2-week-old plant-mass grown on 3N BBM X 14. X 14.**

Fig. 12. ***Schizothrix calcicola* v. *glomerulata*. Macroscopic view of the plant-mass.**

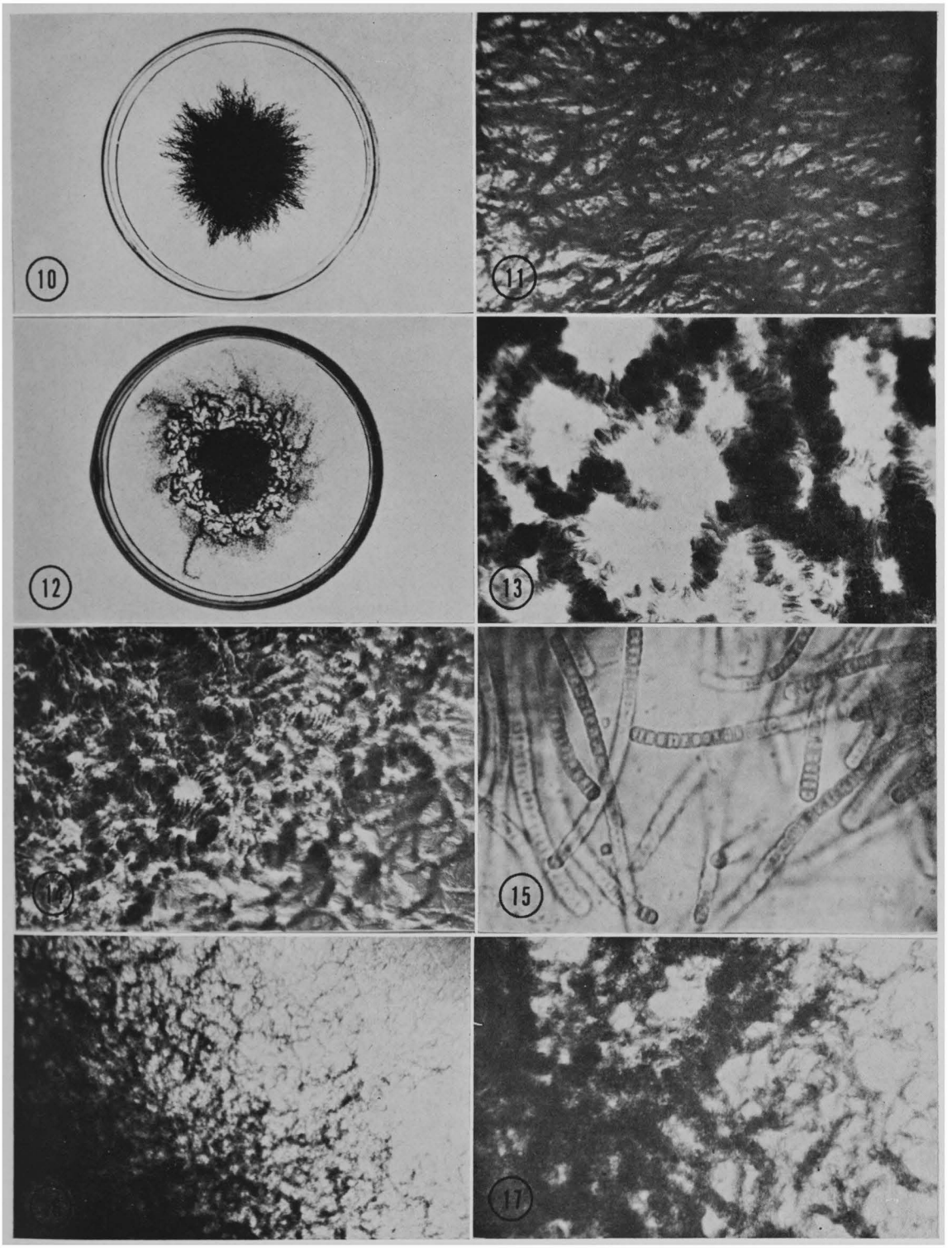
Fig. 13. ***Schizothrix calcicola* v. glomerulata. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 14. ***Schizothrix calcicola* v. *glomerulata*. Central portion of a 2-week-old plant-mass grown on 3N BBM agar showing minutely glomerulate and zigzagging bundles of trichomes, X 14.**

Fig. 15. ***Schizothrix calcicola* v. *glomerulata*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 7.**

Fig. 16. ***Schizothrix calcicola* v. *amorpha*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 7.**

Fig. 17. ***Schizothrix calcicola* v. *amorpha*. Central portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**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FIGURES 18–25

Fig. 18. *Schizothrix calcicola* v. *vaginata*. Portion of the plant-mass of a 2-week-old culture grown on 3N BBM agar, X 14.

Fig. 19. *Schizothrix calcicola* v. *vaginata*. Filaments and trichomes of a 2-week-old culture grown on 3N BBM agar, X 935.

Fig. 20. *Schizothrix calcicola* v. *olivacea*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

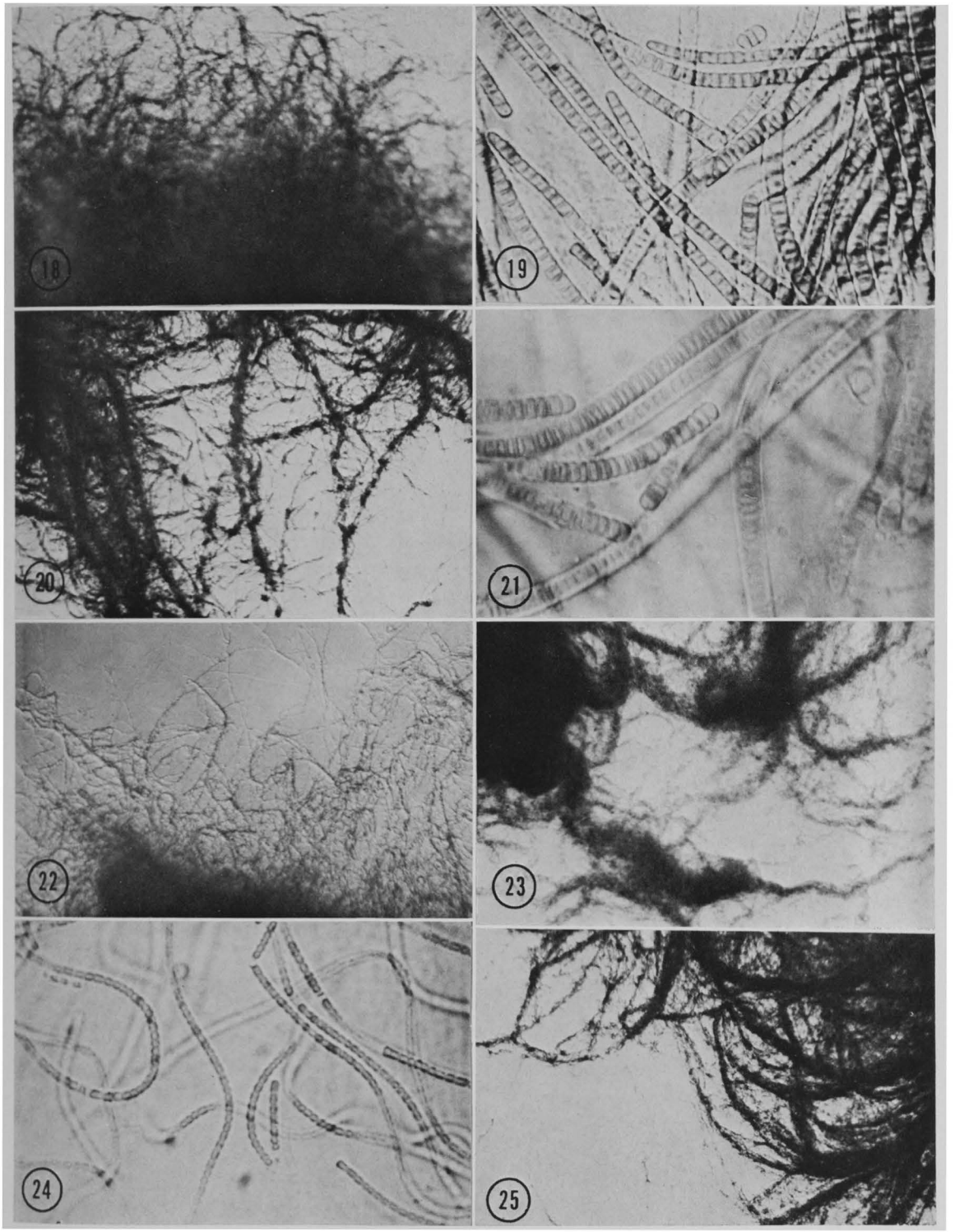
Fig. 21. *Schizothrix calcicola* v. *olivacea*. Filaments and trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 22. *Schizothrix calcicola* v. *minuta*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 25.

Fig. 23. *Schizothrix calcicola* v. *minuta*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 25.

Fig. 24. *Schizothrix calcicola* v. *minuta*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 25. *Schizothrix calcicola* v. *actiniformis*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 14.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FIGURES 26–33

Fig. 26. *Schizothrix calcicola* v. *actiniformis*. Filament of a 3-week-old culture grown on 3N BBM agar, showing trichome extending beyond the sheath, X 1000.

Fig. 27. *Schizothrix* v. *fuscoviridis*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fg. 28. *Schizothrix calcicola* v. *fuscoviridis*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

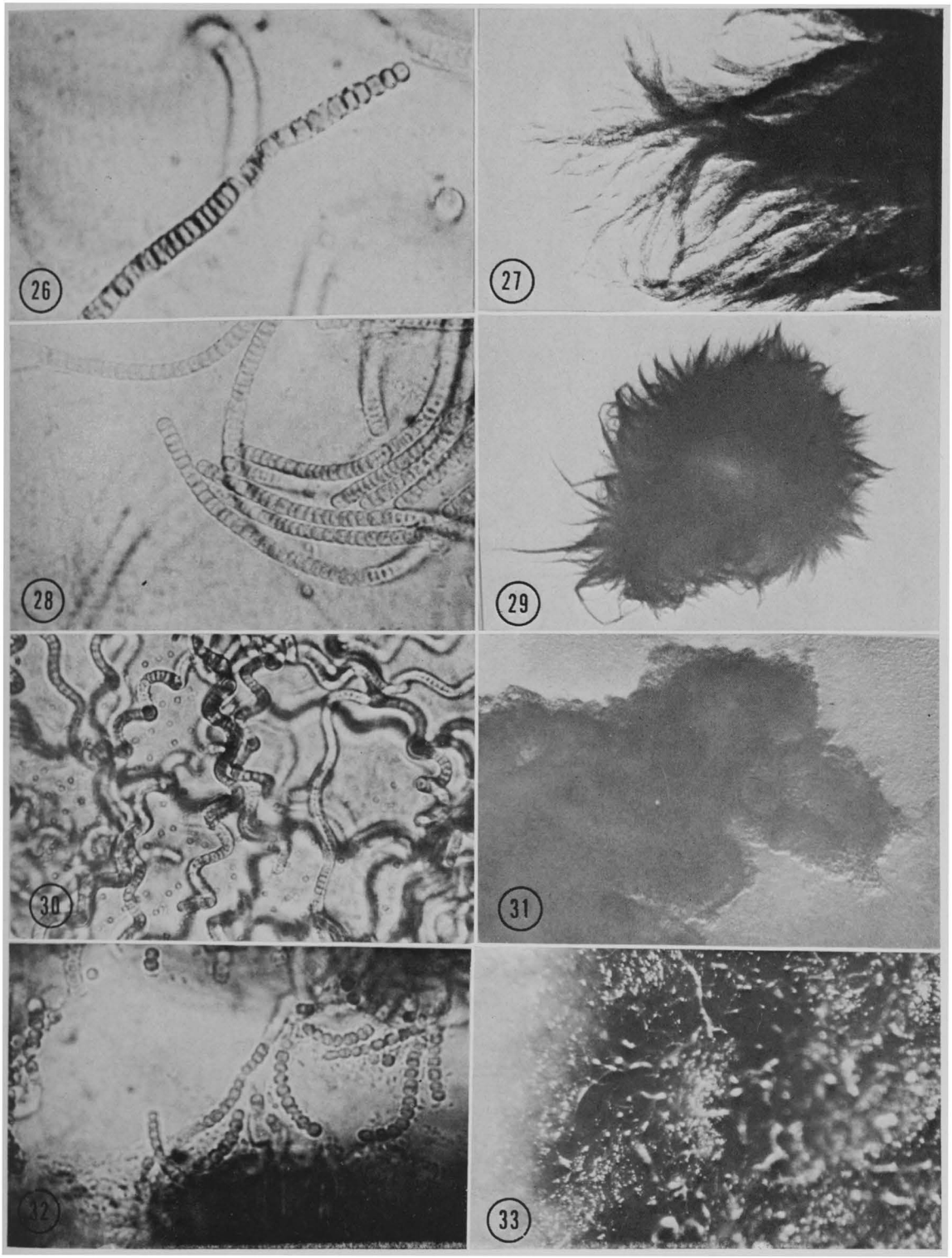
Fig. 29. *Schizothrix calcicola* v. *spiralis*. View of the plant-mass at 2 weeks, X 14.

Fig. 30. *Schizothrix calcicola* v. *spiralis*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 835.

Fig. 31. *Schizothrix calcicola* v. *densa*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 32. *Schizothrix calcicola* v. *densa*. Filaments and trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 33. *Schizothrix calcicola* v. *scabella*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, showing tufts of filaments, X 14.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FIGURES 34–41

Fig. 34. *Schizothrix calcicola* v. *scabella*. Trichomes and filaments of a 3-week-old culture grown on 3N BBM agar, X 1000.

Fig. 35. *Schizothrix calcicola* v. *compacta*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 36. *Schizothrix calcicola* v. *compacta*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

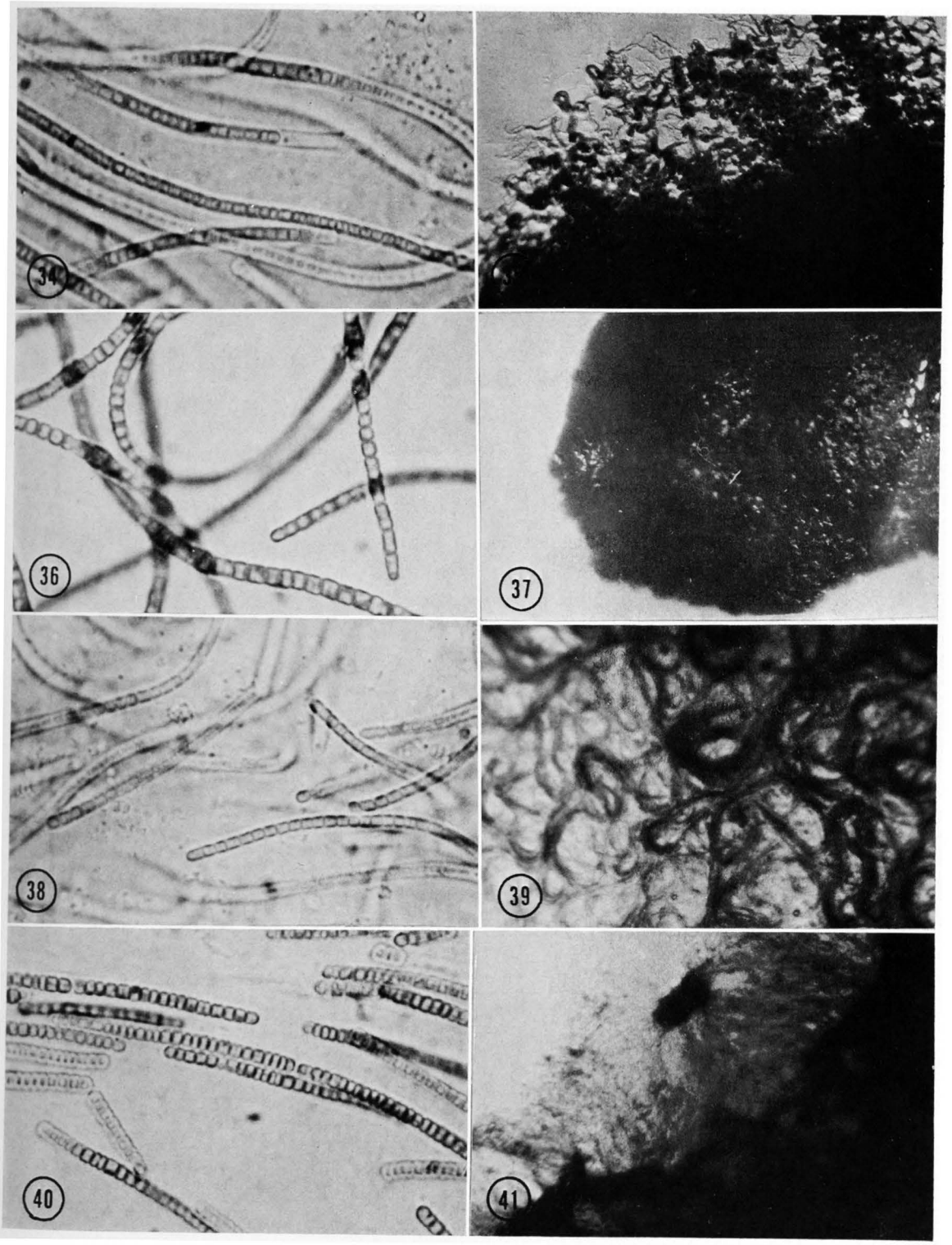
Fig. 37. *Schizothrix calcicola* v. *nitida*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 38. *Schizothrix calcicola* v. *nitida*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 39. *Schizothrix calcicola* v. *mucosa*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 40. *Schizothrix calcicola* v. *mucosa*. Trichomes of a 3-week-old culture grown on 3N BBM agar, X 900.

Fig. 41. *Schizothrix* v. *glabra*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.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FIGURES 42–49

Fig. 42. ***Schizothrix calcicola*** **v. *glabra*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 43. ***Schizothrix calcicola*** **v. *fusca*. Macroscopic view of the plant-mass.**

Fig. 44. ***Schizothrix calcicola*** **v. *fusca*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

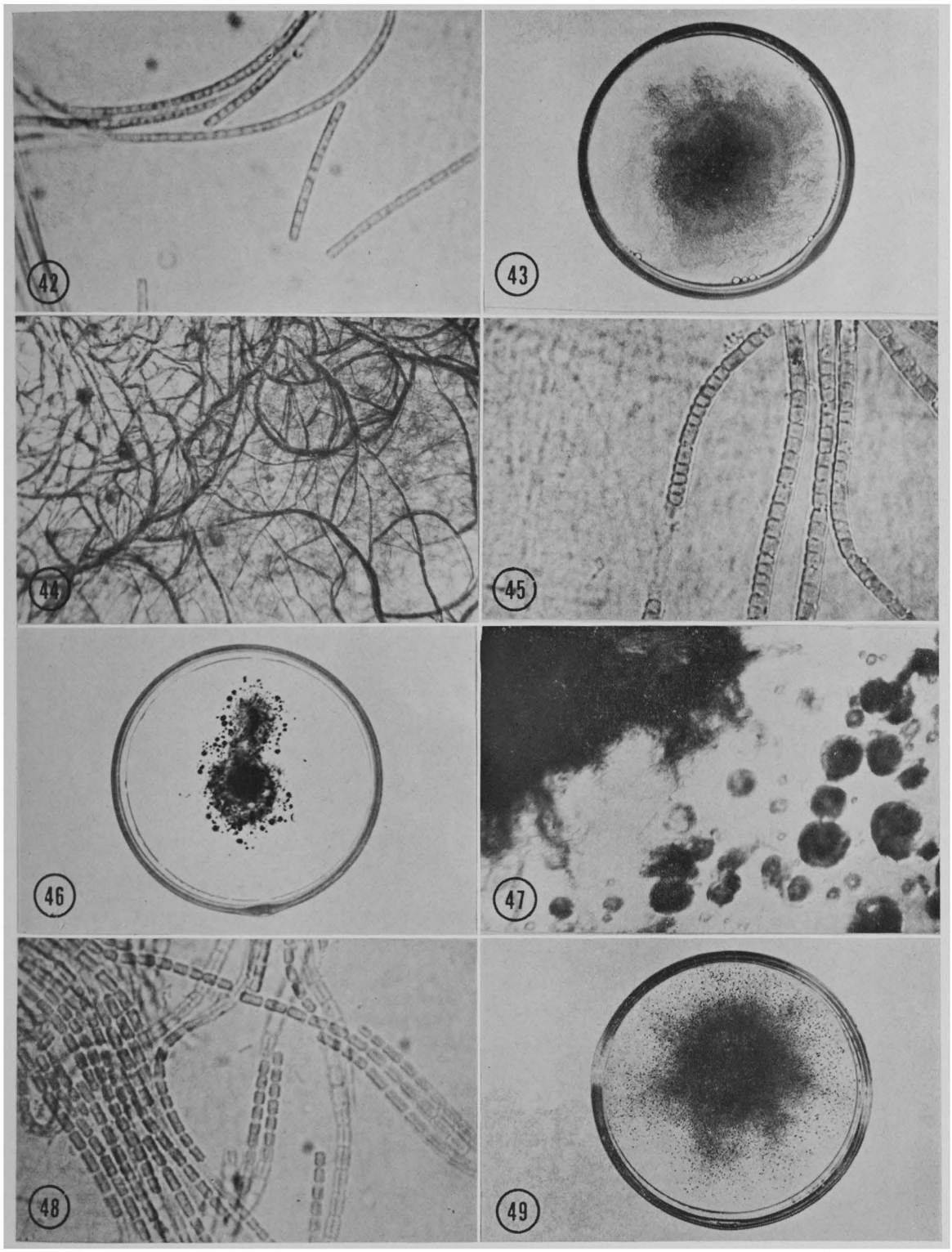
Fig. 45. ***Schizothrix calcicola*** **v. *fusca*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 835.**

Fig. 46. ***Schizothrix calcicola*** **v. *discreta*. Macroscopic view of the plant-mass.**

Fig. 47. ***Schizothrix calcicola*** **v. *discreta*. Portion of the plant-mass of a 3-week-old culture grown on 3N BBM agar, X 14.**

Fig. 48. ***Schizothrix calcicola*** **v. *discreta*. Trichomes of a 3-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 49. ***Schizothrix arenaria*****. Macroscopic view of the plant-mass.**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FIGURES 50–57

Fig. 50. *Schizothrix arenaria*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 51. *Schizothrix arenaria*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 52. *Schizothrix arenaria* v. *vermiformis*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

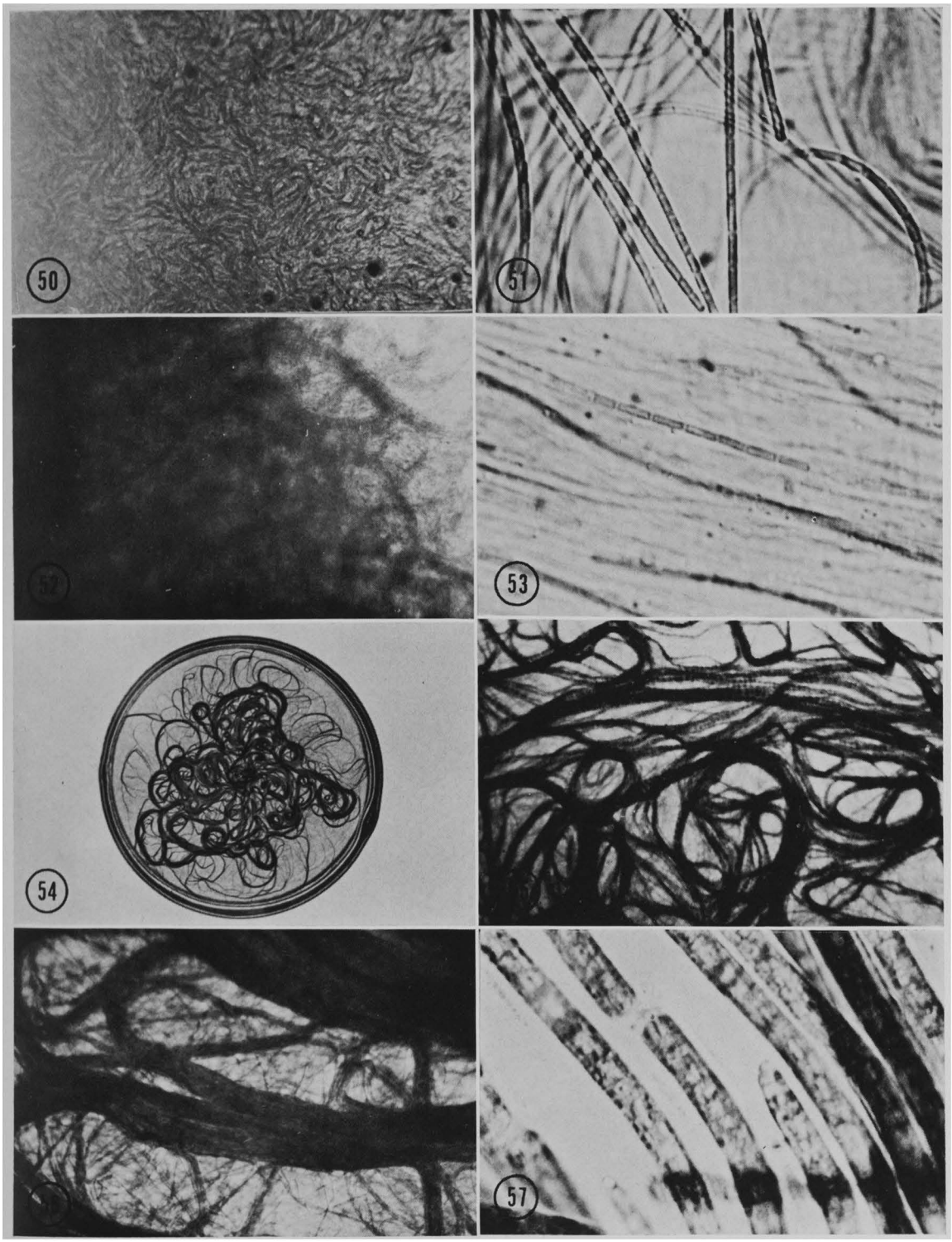
Fig. 53. *Schizothrix arenaria* v. *vermiformis*. Filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 54. *Microcoleus vaginatus*. Macroscopic view of the plant-mass.

Fig. 55. *Microcoleus vaginatus*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, showing rugose bundles of filaments, X 7.

Fig. 56. *Microcoleus vaginatus*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, showing rough bundles of filaments, X 14.

Fig. 57. *Microcoleus vaginatus*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.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FIGURES 58–65

Fig. 58. *Microcoleus vaginatus* v. *glaber*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 59. *Microleus vaginatus* v. *glaber*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 60. *Microcoleus vaginatus* v. *undulatus*. Macroscopic view of the plant-mass.

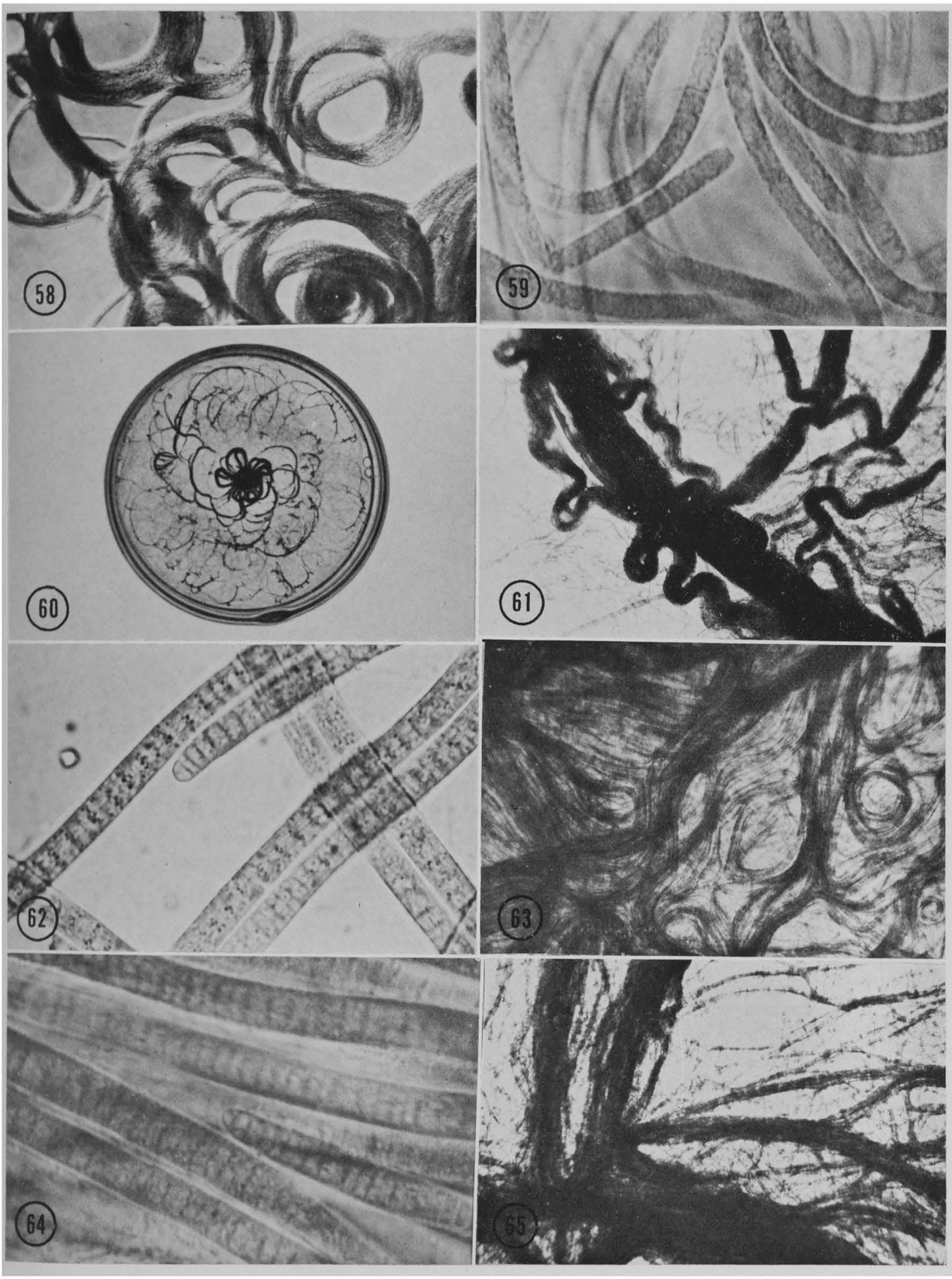
Fig. 61. *Microcoleus vaginatus* v. *undulatus*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, showing knobby or undulate bundles of trichomes, X 14.

Fig. 62. *Microcoleus vaginatus* v. *undulatus*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 63. *Microcoleus vaginatus* v. *cyano-viridis*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 64. *Microcoleus vaginatus* v. *cyano-viridis*. Filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 65. *Microcoleus vaginatus* v. *funiformis*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.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FIGURES 66–73

Fig. 66. ***Microcoleus vaginatus*** **v. *funiformis*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 67. ***Microcoleus vaginatus*** **v. *conicus*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 68. ***Microcoleus vaginatus*** **v. *conicus*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.**

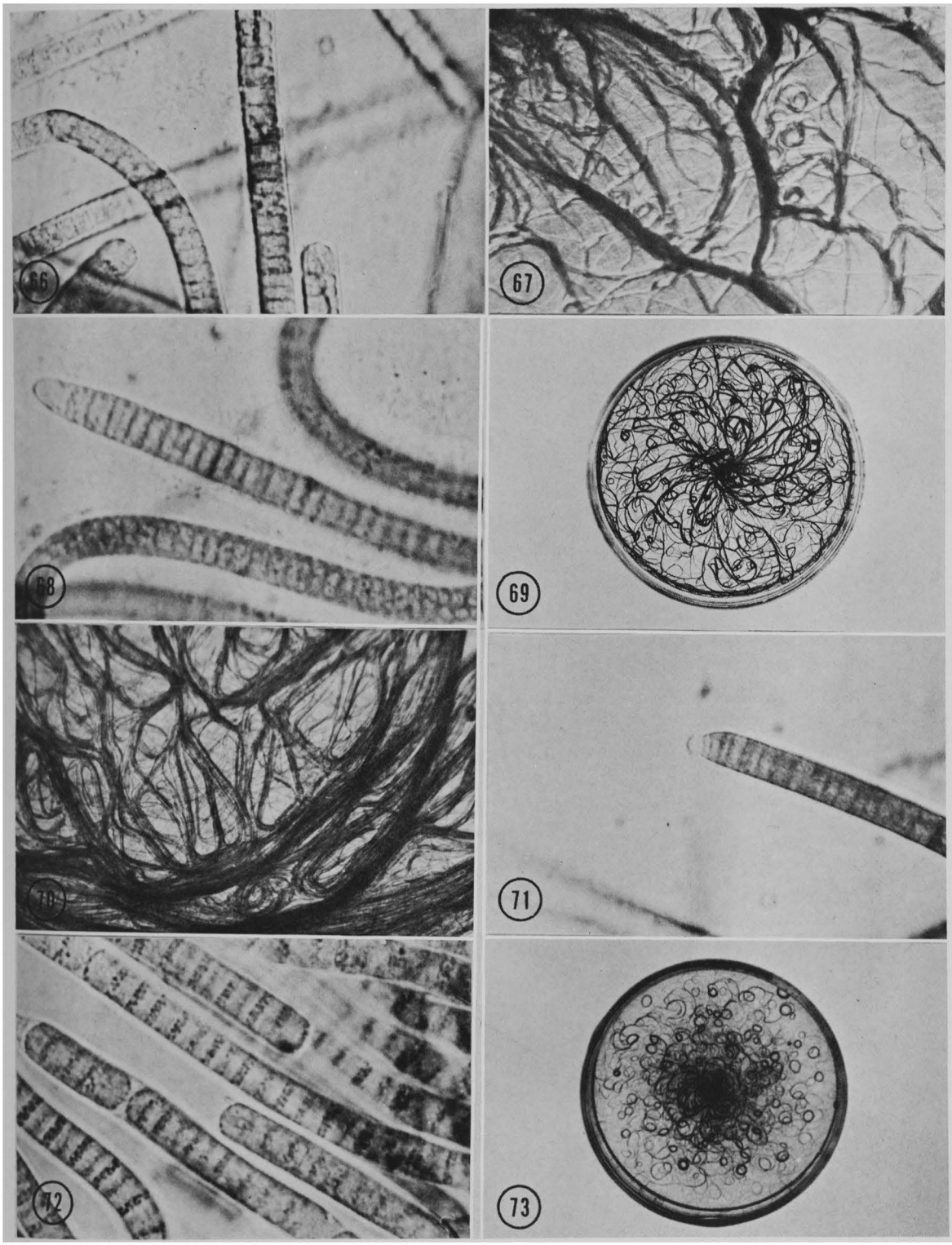
Fig. 69. ***Microcoleus vaginatus*** **v. *fuscus*. Macroscopic view of the plant-mass.**

Fig. 70. ***Microcoleus vaginatus*** **v. *fuscus*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 9.**

Fig. 71. ***Microcoleus vaginatus*** **v. *fuscus*. Trichome of a 3-week-old culture grown on 3N BBM agar, showing tapering end and capitate terminal cell, X 1000.**

Fig. 72. ***Microcoleus vaginatus*** **v. *fuscus*. Trichomes and filaments of a 3-week-old culture grown on 3N BBM agar, showing dark granules that may occur along each side of the crosswalls, X 1000.**

Fig. 73. ***Microcoleus vaginatus*** **v. *fucsorubens*. Macroscopic view of the plant-mass.**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FIGURES 74–81

Fig. 74. *Microcoleus vaginatus* v. *fuscorubens*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 75. *Microcoleus vaginatus* v. *fuscorubens*. Trichome of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 76. *Microcoleus vaginatus* v. *radiatus*. Macroscopic view of the plant-mass.

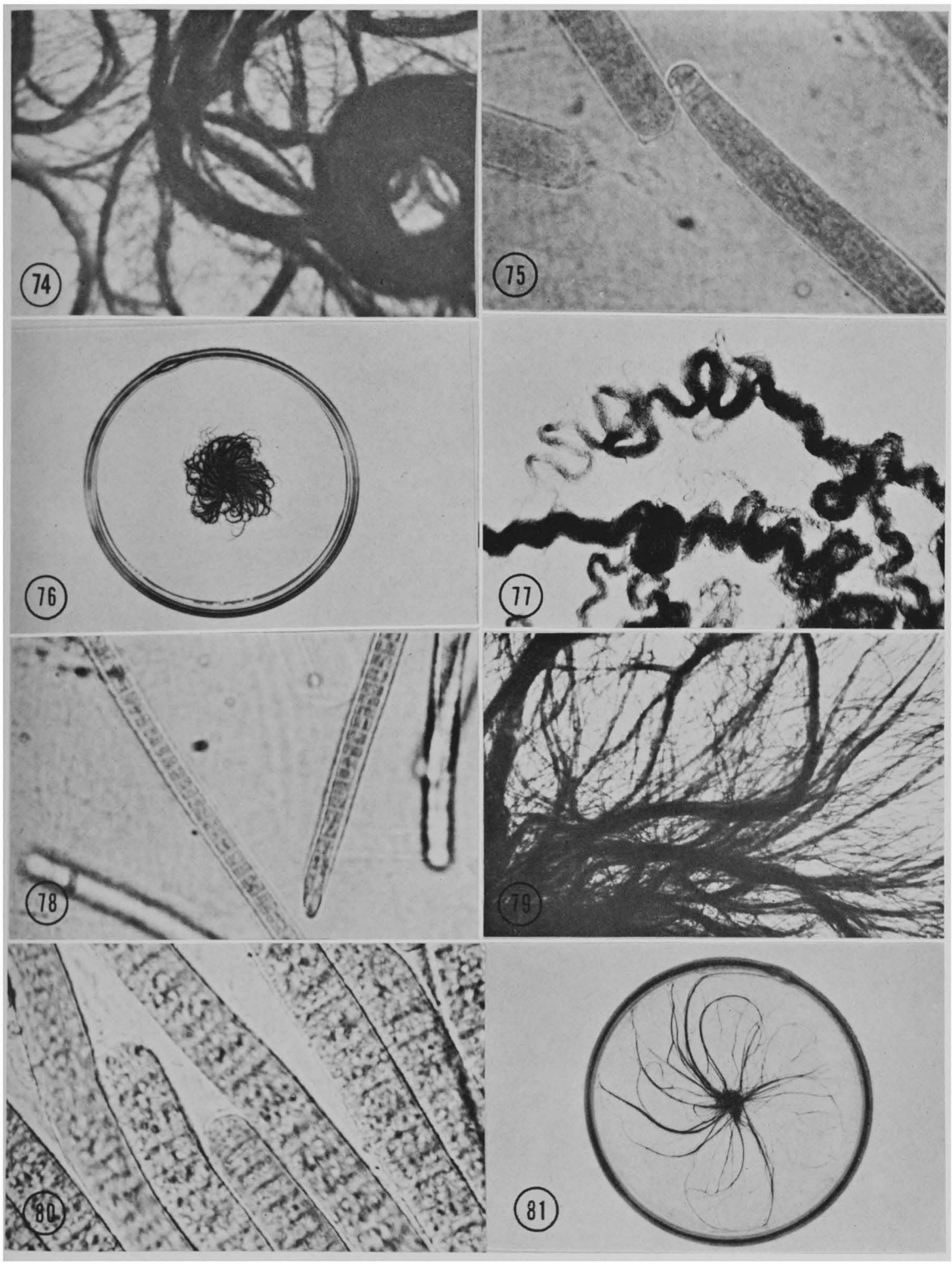
Fig. 77. *Microcoleus vaginatus* v. *radiatus*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, showing knobby or undulate portions of the bundles of trichomes, X 14.

Fig. 78. *Microcoleus vaginatus* v. *radiatus*. Trichomes of a 3-week-old culture grown on 3N BBM agar, X 1000.

Fig. 79. *Microcoleus vaginatus* v. *araneaformis*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 14.

Fig. 80. *Microcoleus vaginatus* v. *araneaformis*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.

Fig. 81. *Microcoleus lyngbyaceus*. Macroscopic view of the plant-mass.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FIGURES 82–89

Fig. 82. ***Microcoleus lyngbyaceus*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 25.**

Fig. 83. ***Microcoleus lyngbyaceus*. Trichome and filament of a 2-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 84. ***Microcoleus lyngbyaceus* v. *vermiformis*. Macroscopic view of the plant-mass.**

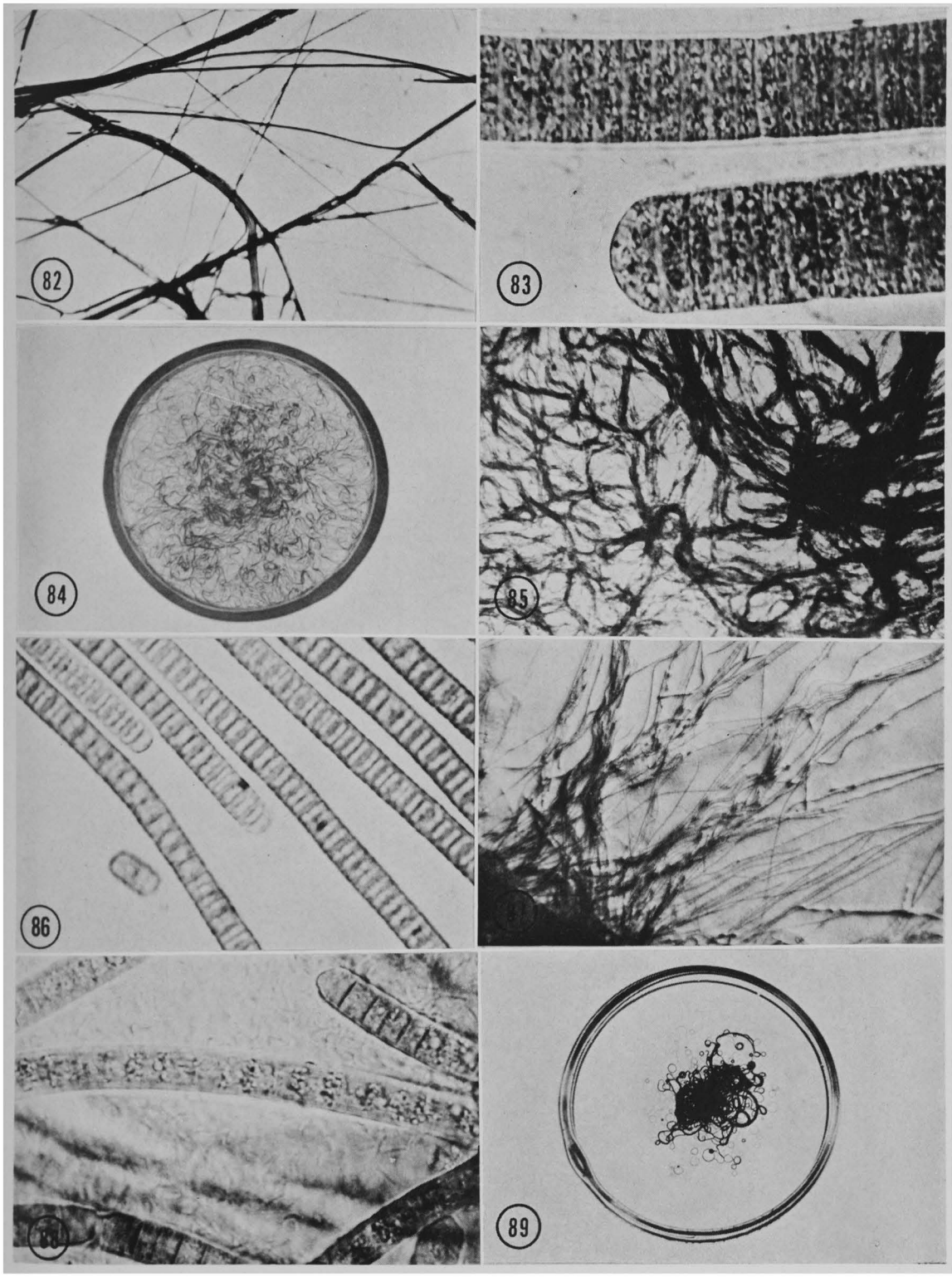
Fig. 85. ***Microcoleus lyngbyaceus* v. *vermiformis*. Portion of a 3-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 86. ***Microcoleus lyngbyaceus* v. *vermiformis*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 87. ***Microcoleus irriguus*. Portion of the plant-mass of a 3-week-old culture grown on 3N BBM agar, X 25.**

Fig. 88. ***Microcoleus irriguus*. Trichomes and filaments of a 3-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 89. ***Porphyrosiphon notarisii*. Macroscopic view of the plant-mass.**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FIGURES 90–97

Fig. 90. ***Porphyrosiphon notarisii*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 91. ***Porphyrosiphon notarisii*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 92. ***Porphyrosiphon notarisii* v. *canus*. Macroscopic view of the plant-mass.**

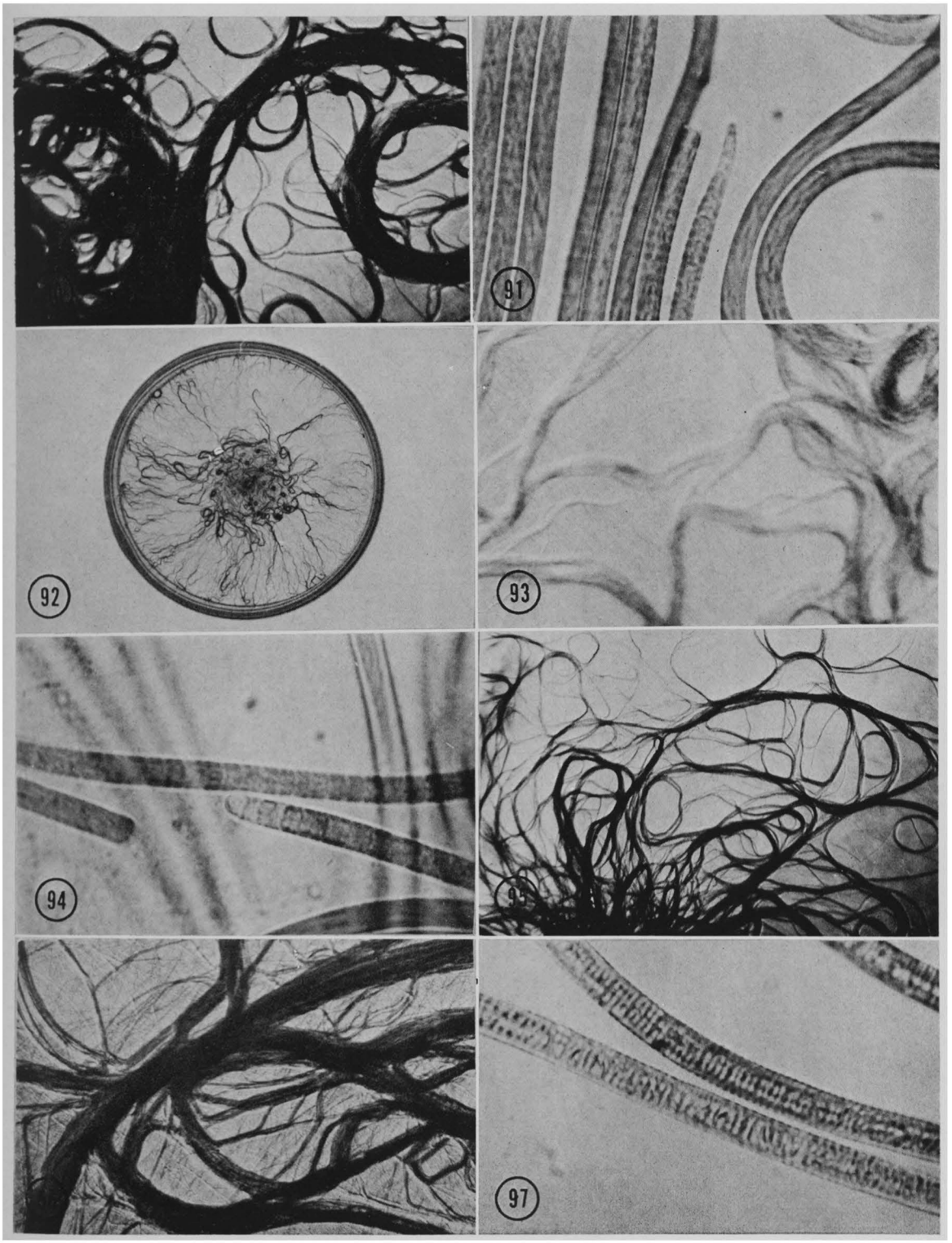
Fig. 93. ***Porphyrosiphon notarisii* v. *canus*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 94. ***Porphyrosiphon notarisii* v. *canus*. Trichomes of a 2-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 95. ***Oscillatoria lutea*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 9.**

Fig. 96. ***Oscillatoria lutea*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 97. ***Oscillatoria lutea*. Trichomes of a 3-week-old culture grown on 3N BBM agar, X 1000.**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FIGURES 98–104

Fig. 98. ***Oscillatoria lutea*** **v. *auxotrophica*. Trichomes of a 2-week-old culture grown on 3N BBM agar, showing typical capitate terminal cell, X 1000.**

Fig. 99. ***Oscillatoria lutea*** **v. *contorta*. Macroscopic view of the plant-mass.**

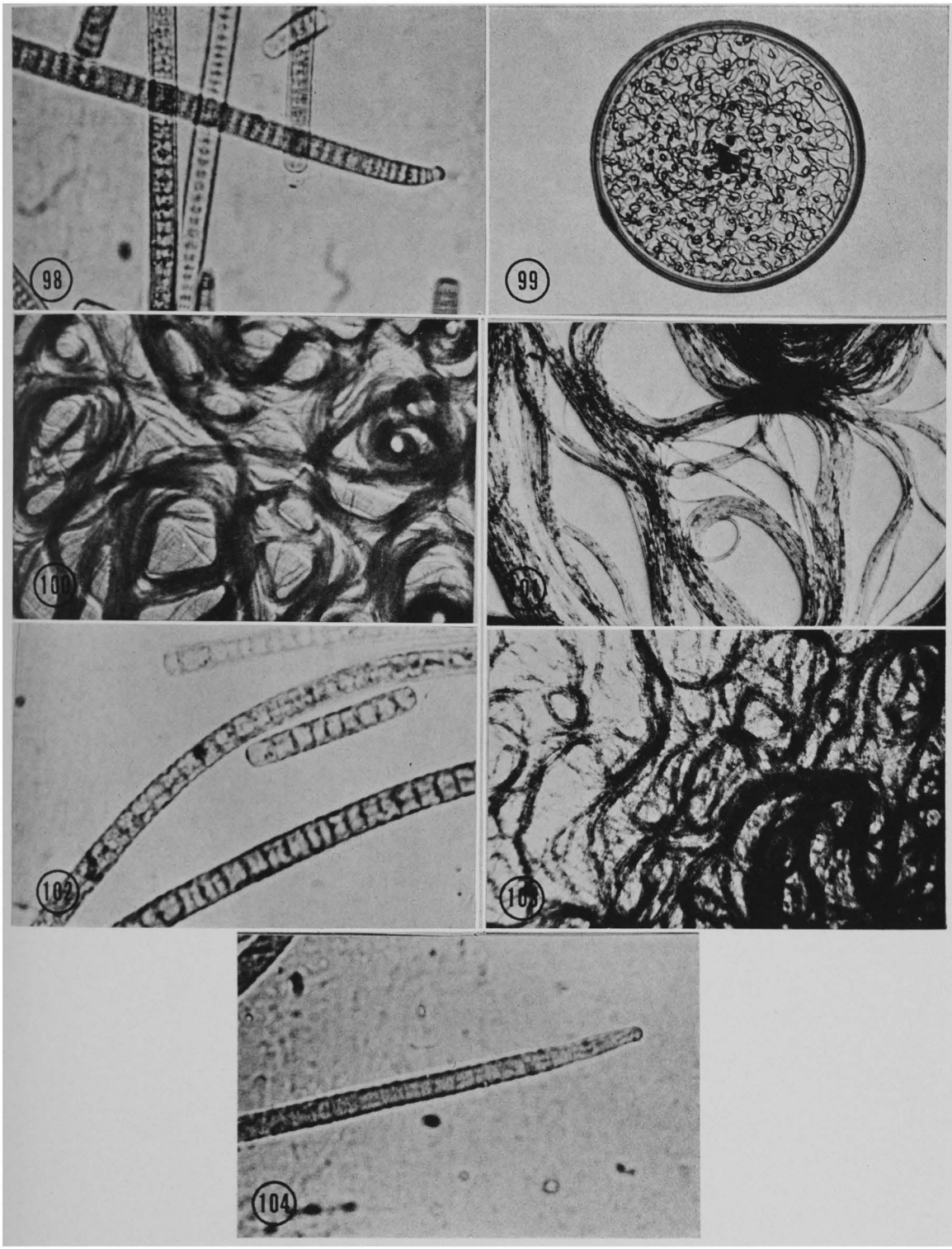
Fig. 100. ***Oscillatoria lutea*** **v. *contorta*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 9.**

Fig. 101. ***Oscillatoria lutea*** **v. *contorta*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 60.**

Fig. 102. ***Oscillatoria lutea*** **v. *contorta*. Trichomes and filaments of a 3-week-old culture grown on 3N BBM agar, X 1000.**

Fig. 103. ***Oscillatoria lutea*** **v. *scabra*. Portion of a 2-week-old plant-mass grown on 3N BBM agar, X 14.**

Fig. 104. ***Oscillatoria lutea*** **v. *scabra*. Trichomes and filaments of a 2-week-old culture grown on 3N BBM agar, X 1000.**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FIGURES 105–112

Fig. 105. ***Schizothrix calcicola.*** **Isolate K18 at 12 weeks (3N BBM agar), showing short false branches that begin to be seen at about 6 weeks, X 1000.**

Fig. 106. ***Schizothrix calcicola*** **v.** ***glomerulata.*** **Isolate 1487 at 12 weeks (3N BBM agar), showing hormogonia which compose the entire culture, X 875.**

Fig. 107. ***Oscillatoria lutea*** **v.** ***auxotrophica.*** **Isolate 22 at 12 weeks (3N BBM agar), showing wide sheaths, pointed terminal cells, and large granular bodies that occur from 6 weeks, X 1000.**

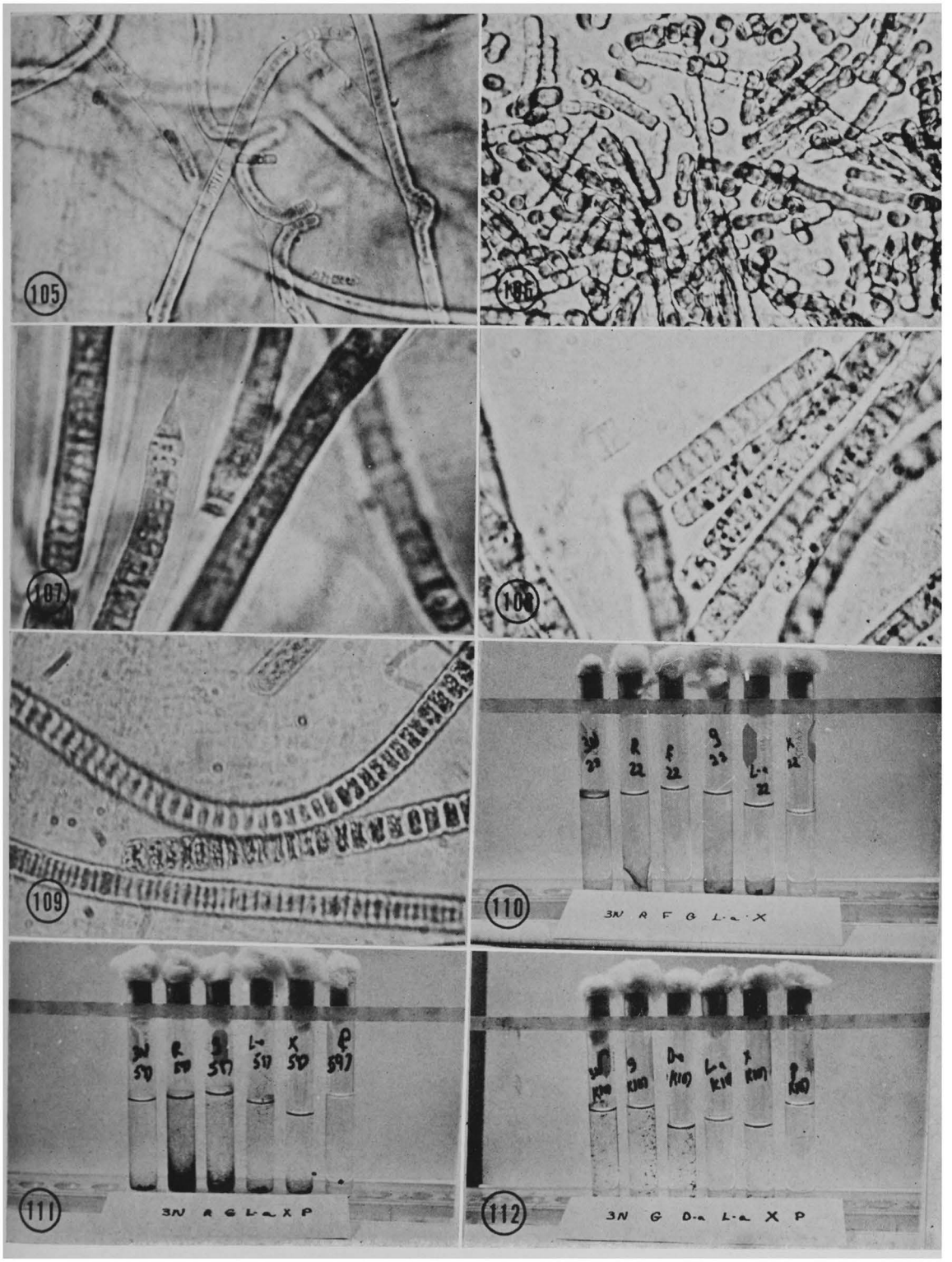
Fig. 108. ***Oscillatoria lutea*** **v.** ***contorta.*** **Isolate 1386 at 6 weeks (3N BBM agar slant), X 1000.**

Fig. 109. ***Oscillatoria lutea*** **v.** ***contorta.*** **Isolate 1386 at 6 weeks (3N BBM liquid), X 1000.**

Fig. 110. ***Oscillatoria lutea*** **v.** ***contorta.*** **Isolate 25. Growth in 3N BBM supplemented with various carbon sources. Left to right: control (3N BBM), ribose, fructose, glucose, *L*-arabinose, xylose, pyruvate.**

Fig. 111. ***Schizothrix calcicola*** **v.** ***glomerulata.*** **Isolate 1597. Growth in 3N BBM supplementeed with various carbon sources. Left to right: control (3N BBM), ribose, glucose, *L*-arabinose, xylose, pyruvate.**

Fig. 112. ***Schizothrix calcicola*** **v.** ***spiralis.*** **Isolate K107. Growth in 3N BBM supplemented with various carbon sources. Left to right: control (3N BBM), glucose, *D*-arabinose, *L*-arabinose, xylose, pyruvate.**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3N R G L-a X P
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3N G D-a L-a X P

FIGURES 113–118

Fig. 113. ***Microcoleus vaginatus.*** **Isolate 1621. Differing amounts of growth on 3N BBM crystal violet agar. Concentration of crystal violet ranges from 0 % (3N BBM) to 0.0001 % (7).**

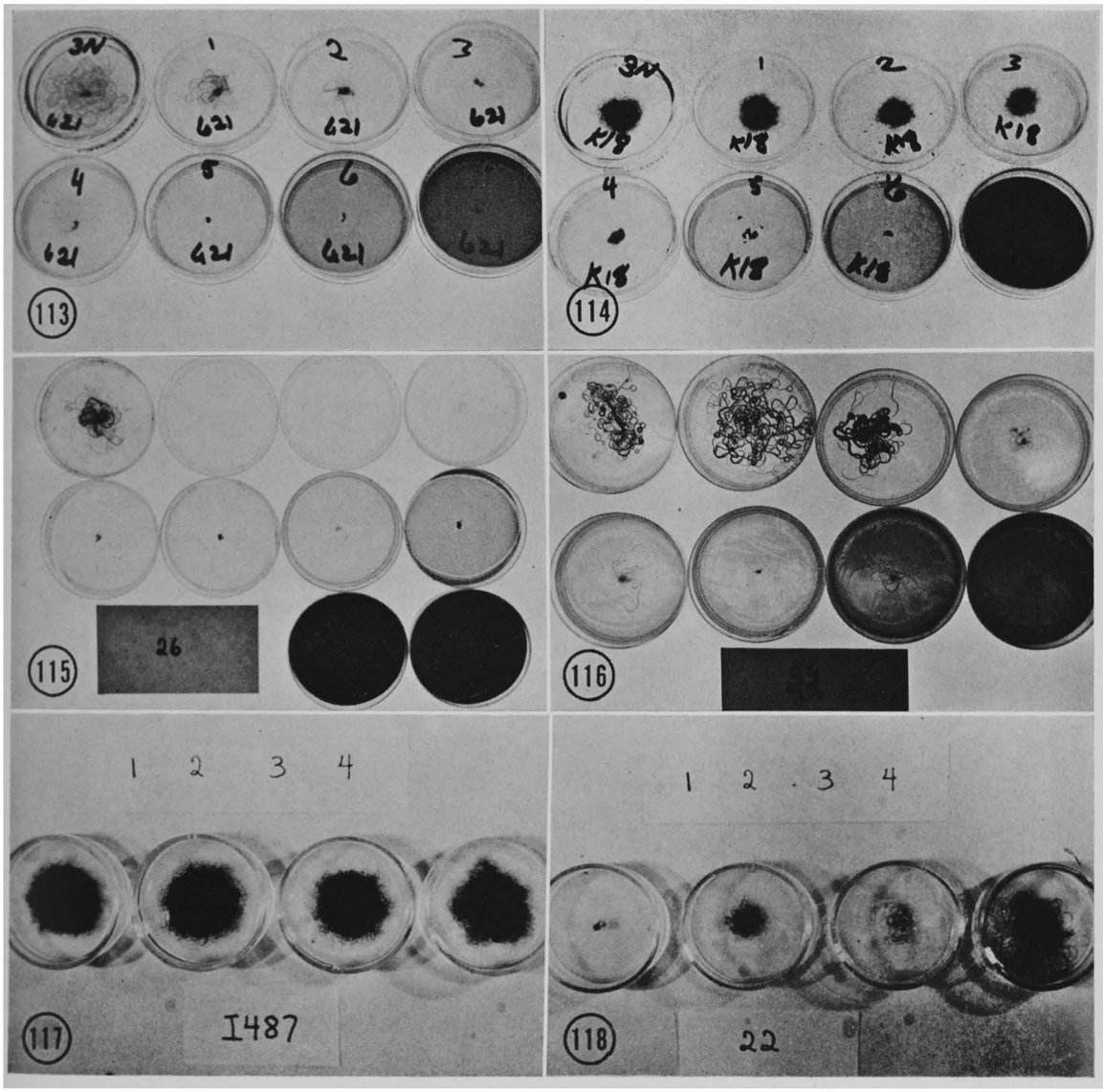
Fig. 114. ***Schizothrix calcicola.*** **Isolate K18. Differing amounts of growth on 3N BBM crystal violet agar. Concentration of crystal violet ranges from 0 % (3N BBM) to 0.0001 % (7).**

Fig. 115. ***Microcoleus vaginatus*** **v.** ***fuscus.*** **Isolate 26. Differing amounts of growth on 3N BBM crystal violet agar. Concentration of crystal violet ranges from 0.00001 % to 0.001 %.**

Fig. 116. ***Oscillatoria lutea*** **v.** ***auxotrophica.*** **Isolate 22. Differing amounts of growth on 3N BBM crystal violet agar. Concentration of crystal violet ranges from 0.00001 % to 0.001 %.**

Fig. 117. ***Schizothrix calcicola*** **v.** ***glomerulata.*** **Isolate 1487. Growth at 3 weeks on (left to right) 3N BBM (transferred from 3N BBM), 3N BBM + B_{12} (transferred from 3N BBM), 3N BBM (transferred from 3N BBM + B_{12}), and 3N BBM + B_{12} (transferred from 3N BBM + B_{12}).**

Fig. 118. ***Oscillatoria lutea*** **v.** ***auxotrophica.*** **Isolate 22. Growth at 3 weeks on (left to right) 3N BBM (transferred from 3N BBM), 3N BBM + B_{12} (transferred from 3N BBM), 3N BBM (transferred from 3N BBM + B_{12} (transferred from 3N BBM + B_{12}).**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FIGURES 119–125

Fig. 119. ***Schizothrix calcicola*** **v.** ***glomerulata*****. Isolate 1427 grown in 3N BBM at pH 6.0, showing very short terminal branch, X 1100.**

Fig. 120. ***Schizothrix calcicola*****. Isolate K44 grown in 3N BBM at pH 8.0, showing discrete clumps of filaments that occur at higher pH's, X 100.**

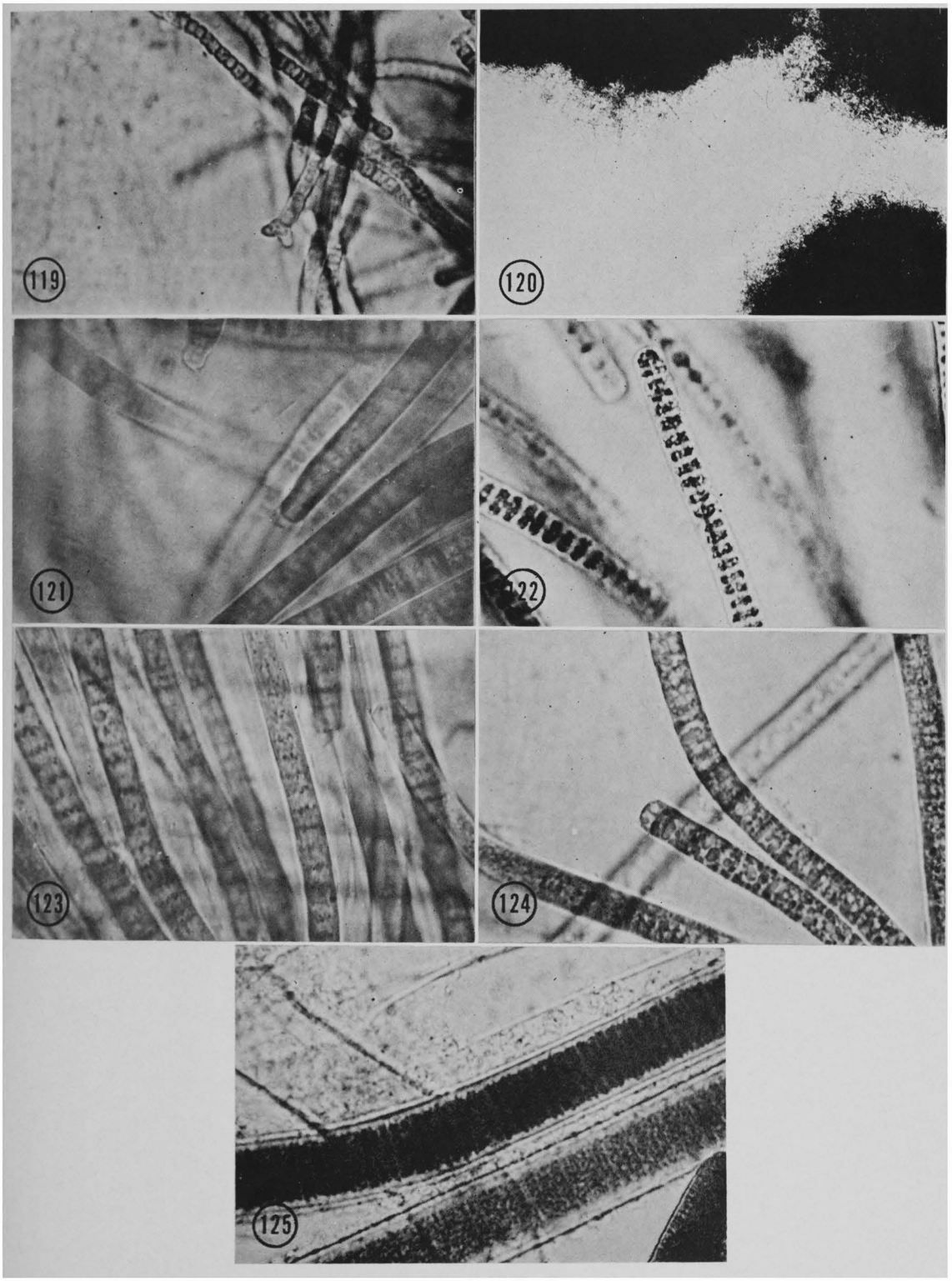
Fig. 121. ***Oscillatoria lutea*****. Isolate 23 grown in 3N BBM at pH 6.5, X 1000.**

Fig. 122. ***Oscillatoria lutea*****. Isolate 23 grown in 3N BBM at pH 7.0, showing gas vacuoles that were numerous at 7.0 and above, X 1000.**

Fig. 123. ***Microcoleus vaginatus*****. Isolate 1621 grown in 3N BBM at pH 6.5, showing distinct dark granules along either side of the crosswalls, X 1000.**

Fig. 124. ***Microcoleus vaginatus*****. Isolate 1621 grown in 3N BBM at pH 8.0, showing coarsely granular nature of the protoplasm, X 1000.**

Fig. 125. ***Microcoleus lyngbyaceus*****. Isolate 4 grown in 3N BBM at pH 7.0, showing densely granular filament characteristically found at lower pH's and lightly granular filament characteristic of growth at higher pH's, X 150.**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