AN INVESTIGATION, IN CERTAIN SPECIES OF HORMIDIUM AND RELATED GENERA, OF SOME OF THE CHARACTERS COMMONLY USED IN THEIR IDENTIFICATION.

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Abstract.

Clonal cultures of algae identified as Hormidium, Ulothrix, or Stichococcus species show variation in characters commonly used in distinguishing between the genera. Both chloroplast size and shape, and the amount of pigmentation vary with the cultural conditions and appear to depend on the nutrient supply. Pyrenoids were generally clearer in culture than under natural conditions. Culture made the detection of two pyrenoids per cell possible in one clone and the development of clearly visible pyrenoids appears to depend on nutrition. The character of absence of pyrenoids for separating the genus Stichococcus from Hormidium is therefore considered of doubtful value. Reproduction by motile cells could not be obtained although the methods suggested by Klebs and others were used.

Clonal cultures of Hormidium, Ulothrix, and Stichococcus show variation in the characters commonly used in the identification of the species. Cell measurements alter by as much as + or - 20% when the algae are grown in a different culture solution. The formation of a silky film is a useful character only if considered in conjunction with the culture solution. Constriction of the filaments and wall thickness also vary when the algae are grown in different culture solutions.

The lack of precision which marks the boundaries of the genera Hormidium, Ulothrix and Stichococcus is emphasized

for by suitable culture algae can be pushed over the boundaries.

Clones of algae identified as Uronema species retained the special characters of the genus under all conditions under which they were studied. Reproduction by motile cells occured readily and the terminal cells were always acuminate. The validity of the genus is supported.

I. INTRODUCTION.

INTRODUCTION

Hormidium and the related genera Ulothrix, Stichococcus and Uronema are taxonomically troublesome. There is disagreement on the exact limits of the genera and some doubt whether all four genera are valid. Many species have been described but few are satisfactory entities.

The genera are usually separated by features of reproduction or features which are only developed typically in filaments from motile reproductive cells. Stichococcus is distinguished by the absence of motile reproductive cells and by reproduction by fragmentation which gives filaments with rounded cells at both ends. Hormidium, Ulothrix and Uronema all have motile reproductive cells but only Uronema has a pointed apical cell. Hormidium and Ulothrix both have a rounded apical cell but are separated by the zoospores of characteristically different form in the two genera. Reproduction by motile cells is however only infrequently recorded or inadequately described for many species of Ulothrix and Hormidium and in some species may never occur. This makes the separation of these genera from one another and from Stichococcus difficult. The majority of identifications and even some initial diagnoses are made from vegetative material using morphological characters to decide on the genus. This is probably the main reason

for confusion in limits of the genera and for disagreements on the assigning of species to genera.

Morphological characters such as cell and chloroplast size are used to separate the species in all the four genera. The variation and therefore reliability of the morphological characters has not been fully investigated. The algae of this group being very similar to one another in morphological features, uninvestigated variations may be the main cause of confusion among the species. Contributory causes are inadequate knowledge of the morphological features of different phases of the life history of particular species and association of filaments of more than one variety.

In this investigation it was expected that growing the algae in unialgal culture and examining them over a period of time would resolve the confusion. Work was limited to plants with slender filaments of 20 ~~μin~~ and under because these have proved the most troublesome to identify. Individual filaments from collections of different appearance were selected as representatives of different species and grown in a variety of different media.

The use of clonal cultures as representatives of different varieties or species simplifies the problem by ensuring that the subject of study is not a mixture. It

was hoped firstly that reproduction mechanisms would occur naturally or would be induced to occur readily by special methods such as those of Klebs. It was hoped that the occurrence of reproduction would then be of use in determining and classifying the algae and in clarifying the four genera. Secondly, it was hoped that the cultures would indicate the extent of variation in morphological character within certain limits of variation in environment and that the cultures would reveal various constant and well marked characters which would not only be diagnostic of the species but would also throw light on the nature of the species in the group. Thirdly, it was hoped that further examination of features such as fragmentation might lead to an explanation of differences found in their description.

The hopes for the investigation proved largely ~~in~~ vain. Except in one group (Uronema) already well defined, reproduction by motile cells failed to occur. Many of the morphological characters used to separate the genera and species were found to be unreliable since they vary with the composition of the culture medium. The cultures did not reveal constant and well marked characters which could be used for separating the species. The cultures did possess a certain degree of stability in certain media in respect of some morphological characters usually regarded

as specific. Although the cultures possessed a certain degree of character of their own they could not be sharply differentiated and did not fall into convincing specific groups. They corresponded to existing diagnoses only in a rather vague imprecise way. The investigation of the morphological characters helps in indicating the possible extent of variation in these characters and shows how confusion in identification and in general descriptions of particular features could arise through incomplete knowledge of variation within a species.

II. HISTORICAL SURVEY.

HISTORICAL SURVEY.

The most recent descriptions of species of the genera Ulothrix, Hormidium, Stichococcus and Uronema are by Hazen (1902) Collins (1909), Heering (1914) and Prescott (1951). Fritsch and West list the British species but do not fully describe them. These systematic works are at least partly based on those of earlier Phycologists, for instance those of Kützing, (1833, 1843, 1849), Rabenhorst (1868) and De Toni (1889).

All four genera Ulothrix, Hormidium, Stichococcus and Uronema are recognised by Fritsch and West (1927) and an extract from the key given by them as an aid to the identification of the genera in the Ulotrichales will indicate the type of characters on which the genera are commonly separated.

1a. Filaments not regularly fragmenting into individual cells - - - 2

b. Filaments readily fragmenting into the individual cells which are more or less cylindrical - - - STICHOCOCCUS

2a. Filaments elongated without a specially differentiated apical cell - - - 3

b. Filaments short with an attenuate apical cell, epiphytic - - - URONEMA

3a. Chloroplast annular or plate shaped usually extending round more than half the circumference of the cell and occupying its whole length, zoospores 4 or 2 ciliate, aquatic - - ULOTHRIX

b. Chloroplast elliptical or circular in outline, often occupying only half the length of the cell zoospores 2 ciliate, threads readily fragmenting, terrestrial or aquatic - - - HORMIDIUM

The main characters used for separating the genera are firstly methods of reproduction and secondly the type of terminal and basal cells and the chloroplast. The following characters, taken from the works of Fritsch and West (1927) Heering (1914) and Prescott (1951) give the differences in fuller form.

Ulothrix

Filaments fixed by a special basal cell at least when young.

Chloroplast ring or plate shaped extending round more than half the circumference of the cell and with one or more pyrenoids.

Reproduction by 4 ciliate macrozoospores and 2 or 4 ciliate microzoospores produced more than one in a cell (also by aplanospores, akinetes and gametes).

Hormidium

Filaments without a special basal cell (sometimes attached by secondarily developed rhizoids).

Chloroplasts usually occupying about half the length of the cell, circular or elliptical in outline, mostly with one pyrenoid.

Reproduction by 2 ciliate dorsiventrally flattened zoospores produced singly in the cells (also by aplanospores, akinetes and gametes and by fragmentation).

Stichococcus

Filaments without special basal cells, readily fragmenting into individual cells.

Chloroplasts frequently occupying only about half the cell wall, without a pyrenoid.

Reproduction solely by fragmentation.

Uronema.

Filaments fixed by a basal attaching disc, terminal cell acuminate. Chloroplast a parietal plate with one or more pyrenoids. Reproduction by 4 ciliate zoospores (also by aplanospores and gamates).

The recognition of these four genera is not universal and different generic names have been used. The generic name Ulothrix was first used by Kützing (1833a) for Ulothrix zonata. He is also responsible for the erection

of Hormidium as a separate genus (1843) although in his various works (1843, 1845, 1849) different views are taken. In his final work, Species Algarum (1849) Kützing removed two of the three species he had first put in Hormidium and added others to make Hormidium a section under Ulothrix and not a genus. The definition of Hormidium given on the previous page is due to Klebs (1896) that of Kützing being less complete; but it would probably include the species of Kützings section Hormidium. Rabenhorst (1868) and De Toni (1889) used Hormiscia as synonymous with Ulothrix as originally defined by Kützing (1843). Hormiscia Fries (1835) was originally a genus of two species now included in Urospora and the use of Hormiscia in a different sense, as a synonym of Ulothrix, is contrary to the rules of nomenclature and has been abandoned.

Stichococcus was established by Naegeli (1849) who described Stichococcus bacillaris. Many recent authors, for instance Heering and Prescott, recognize this genus as defined by Naegeli but others give different limits to Stichococcus. Hazen and Collins recognise only three genera. Hazen pointed out that zoospore production occurs only occasionally in species described under Hormidium and that one cannot definitely say that such reproduction never occurs in

species described under Stichococcus. He therefore united the two genera under the name Stichococcus as the earlier name. Kützing (1849) although maintaining Naegeli's description of Stichococcus bacillaris considered the alga to have affinities with the Protococcales and renamed it Protococcus bacillaris. This view of Stichococcus bacillaris is not now generally accepted and disagreement only arises on whether Hazens view is correct or incorrect.

Uronema was established as a genus by Lagerheim in 1887 when he found and described Uronema confervicolum. Arguments about its validity have not been resolved. Gaidukov (1903) questioned the validity of the genus because he observed Uronema-type pointed tips in his form of Ulothrix flaccida. Fritsch and Rich (1929) regarded the absence of accuminate tips in some of their Uronema specimens as being supporting evidence for Gaidukov's conclusions and thought that the pointed tips described for Ulothrix flaccida were really the shrivelled up remnants of intercalary cells which sometimes persisted on the broken up lengths of filaments. Mitra (1947) who found all gradations from acuminate to rounded tips in Uronema terrestre believes Uronema and Ulothrix to be distinct genera but to have close affinities.

The current view seems to be that Hazen erroneously

enlarged the genus Stichococcus and that Uronema is a valid genus. Four genera Ulothrix, Hormidium, Uronema and Stichococcus with the characters listed on page 5 are recognized although considerable differences arise in the species included.

Most authors describe fewer species than did Kutzing but it should be pointed out that some species have on further investigation been united or transferred to still other genera. De Toni in particular reduced many of Kützings species to varietal rank. Differences in the placing of a species arise with the acceptance of different generic limits but even Authors who accept the generic limits given on page 5 disagree in their practice in placing a species in a genus. For example Heering placed Ulothrix subtilis Kütz. in Hormidium but Fritsch and West disagreed believing that it has the Ulothrix type of zoospore. However, they expressed the view that two species might have been included in Kützings original descriptions, one a Ulothrix and the other a Hormidium species. Stichococcus bacillaris Naegeli is maintained by several authors but Heering believed that the alga described as S.bacillaris has a feebly perceptible pyrenoid and therefore transferred it to Hormidium as Hormidium pseudostichococcus.

The different species of each genus are distinguished

by the morphological characters, but it must be noted that different authors give these characters very different weight. The characters most frequently used are cell size, chloroplast size and shape, the shape of the cells (i,e whether barrel shaped, the filament being constricted at the cross septa) the amount of fragmentation, the number of pyrenoids, the presence of "knee bends" with rhizoid like outgrowths and the wall thickness. In certain cases species are distinguished by their behaviour in culture, in particular by whether they form a silky film at the surface of the culture medium. The differences between the species recognized by Heering (1914) may be illustrated by an extract from his key.

I. Cells up to 10μ wide. Chromatophore usually with one pyrenoid.

1 Cells up to 5μ wide.

a. Cells 4-5μ wide ----- Ulothrix subtilissima

b. Cells 2-4μ wide ----- U. limnetica

2 Cells 5-10μ wide

a. Mucilaginous covering in layers. Filament attached by a mucilaginous base. -- U.mucosa

b. Mucilaginous covering may be present. Filament fixed by an elongated basal cell.--

Cells 5-7μ wide ----- U variabilis

Cells 7-10μ wide ----- U.tenerrima

II. Cells over 10μ wide, Chromatophore usually with 2 or more pyrenoids.

1. Membrane thin

A. Cells 10-14μ wide ---- U.oscillarina

B. Cells 15-28μ wide ---- U.tenuissima

2. Membrane thick often distinctly stratified

A. Filament usually slightly constricted in the vegetative state 9-15μ wide -- U.moniliformis

B. Filament only constricted at time of zoospores formation Cells 13-16μ (up to 18μ) wide - 1-2 times as long -- U.aequalis

C. Filament of varying form, 11-72μ wide, usually 30-40μ -- U.zonata

The use of characters occuring in culture is restricted to a certain species of Hormidium and the chief way in which these species differ can also be shown by Heering's key.

I. In culture in nutrient solutions forms a silky film

1. Cultures on glucose agar do not become slippery and glistening

A. Cells 5.5-7μ wide ----- Hormidium nitens.

B. Cells 6.5-8μ wide ----- H.crassum

2. Cultures on glucose agar becoming slippery and glistening. ----- H.lubricum.

II. In culture in nutrient solutions not forming a silky film ----- H.flaccidum

The original diagnoses disclose only slight specific distinctions and offer little hope of sharp dividing lines. In addition later descriptions of particular species by various authors do not exactly correspond with one another and with the original diagnosis. This results in widespread confusion. For instance Lind (1932) identified algae studied by her as Ulothrix rorida Thuret but stated that she suspected that similar algae had been identified as U.aequalis Kütz. or U.oscillarina by other Phycologists. Lund (1946) drew attention to the fact that Phycologists studying soil algae tended either to describe only Hormidium flaccidum or to describe only H.nitems and he pointed out the difficulty of distinguishing between the two species under the usual conditions used (i.e. absence of cultures in liquids). Lund also mentions Phillipson's view that Ulothrix subtilis var variabilis as described by Bristol (1920), Ulothrix subtilis, U.variabilis, U.tenerrima as described by Gistl (1931-1933), Moore and Karrer (1919), and Moore and Carter (1926) all correspond to Hormidium flaccidum.

Although identification of algae in this group depends mainly on morphological features, the number of investigations of the morphology of members at the genera Hormidium and Ulothrix is small, Klebs (1896) studied Ulothrix zonata (Web. and Mohr) Kütz. and Hormidium flaccidum A.Br. sensu lato and is mainly responsible for the differentiation of species

on behaviour in culture. H.nitens Menegh,emend. Klebs is separated from H. flaccidum A.Br. sensu strict. by the formation of a silky film at the surface of the culture medium by the former only. Piercy (1926) investigated a form of Hormidium flaccidum which although terrestrial she identified as forma aquatica. Hazen (1902) dealt with the species of Hormidium and Ulothrix then recorded in the U.S.A. He made collections of as many algae as possible and tried by careful observation and comparison to decide on the distinctness of the various forms and then identified them as far as possible with described species. He does not appear to have kept unialgal cultures but did keep samples of material in glass cylinders,with muslin covered ends,anchored in a running brook. Chodat (1909, 1913) was responsible for further studies of algae in culture and described H.lubricum. and H.crassum. which are scarcely known out of culture. Further work on algae in culture was carried out by Strøm (1929) who investigated the effect of pH of the medium on the growth of four morphologically similar forms of Hormidium flaccidum.

Studies of particular aspects of these algae have also been made. Accounts of fragmentation were given by Benecke (1898) and by Vischer (1926). Woodhead and Jane (1946) reported the occurence in nature of special thickening of

the cell wall in Ulothrix zonata and Hormidium flaccidum. Wall thickening is also reported in Ulothrix mucosa Thuret (Heering 1914), Hormidium rivulare (Printz 1927), H.mucosum (Lund 1946) and H.crenulatum (Fritsch and John 1942). Investigation of reproduction has been made in only a limited number of species, Viz: U.zonata (Klebs 1896, Dodel Port 1876, Regel 1923, Grosse 1931, Lind 1932) U.rorida (Lind 1932) U.oscillarina (Gross 1931) and U.variabilis (Cholnoky 1932) The descriptions in reproduction in Hormidium are confined chiefly to those given by nineteenth century workers (eg Klebs) Later workers (Piercy, Lund) failed to observe reproduction in species for which it had been previously described even though they were using methods similar to those of the earlier investigators.

The total number of Uronema species that have been described is very small. The extent of descriptions of their morphology vary considerably and are confined to the descriptions given by the Phycologists who established the species. The species described are Uronema confericolum Lagerheim (1886), Uronema elongatum Hodgetts (1921), Uronema simplicissinium (Reinsch.) Lagerheim (1886), U. gigas Vischer (1933), U.indicum Ghose (1920), U.terrestre Mitra (1947).

Investigation of Stichococcus species is connected with study of Hormidium since as mentioned on page 8. these genera are united by certain phycologists. An assessment of the distinguishing characters of many described species of the genus Stichococcus was made by Grintzesco and Peterfi (193-). They used behaviour in culture to separate some species and mention morphological variation. A comprehensive list of the species of Hormidium, Ulthrix, and Stichococcus as given by various phycologists would be excessively long and confusing. As an indication of the type of differences that occur in descriptions and in naming algae a short summary (Appendix 1) is given of the characters of algae falling within the group investigated.

III. GENERAL ACCOUNT OF MATERIAL AND METHODS.

GENERAL ACCOUNT OF MATERIAL AND METHODS

A. MATERIAL

Source

Collections of alga were made from different types of habitat - the soil surface, still and flowing water, and exposed surfaces of stone and wood. The thirteen sources of collections of alga are briefly described in Table 1. Named algae were also received from the Cambridge Culture Collection.

Identification.

Preliminary identification of the algae on collection was made by comparison of their characters with the descriptions of recognized species. The collections of alga may often contain several different species or varieties growing together. It was assumed that certain collections containing filaments of widely different width, although showing little or no variation in other characters, consisted of one or more species. Since keys based on the width of the filaments have been devised by Heering (1914) and since it seems probable that species will give a variation curve ~~more~~ with only one mode or maximum, any discontinuities in measurements of width in a sample were taken as indicating the limits of size of different species.

Measurements of width of filament in the thirteen collections showed that twenty one collections of various forms (varieties or species) had been made. Comparison of their characters with one another and with the descriptions of recognised species showed that some collections from different places consisted of the same alga. The material did not necessarily show all the features needed for definite identification and very few of the algae studied fitted the descriptions of recognised species exactly. At least twelve distinct species or varieties were represented by the collections of alga. The descriptions of the algae in the various collections, the tentative identifications and reasons for these determinations are given in Table 1. Some of the algae are illustrated in Figs. 1 - 4.

The algae from Cambridge were already identified but I do not agree with all these determinations and their identification is also discussed in Table 1.

Cultures

A total of thirty seven clones were successfully isolated. All the types of habitat were represented but successful isolations were not made from all the collections, Eighteen of the twenty one collections of different forms were however represented and all the twelve distinct forms

were isolated.

In naming clones the type of habitat of the original source was indicated by a preliminary letter as follows

T - - - Terrestrial

A - - - Aquatic, in still or slow running water

R - - - Aquatic, in swift running water

E - - - Aerial (ie on exposed surfaces above soil level)

U - - - Aquatic, attached algae-Uronema species.

Each form in these groups was then indicated by a number and duplicates (ie separate isolations of one form from a particular source) by a small letter. The list of cultures kept is included in Table 1.

Since I do not agree with the identification of all the algae from Cambridge,cultures of these algae although indicated by a specific name have (C) after it.

TABLE 1.

Source and no. of form	Description	Identification	Cultures
Terrestrial Chobham Common, Surrey. Algae occur on damp gravelly soil in areas of heath disturbed by Tanks and reseeded with grasses.	1 Wefts of filaments on damp soil. Cell 4-6μ wide by 2-4 times as long. Cell wall thin. Chloroplast reaching total length of cell and more than half circumference with one distinct pyrenoid of moderate size Filaments long and not constricted.	Ulothrix subtilissima Very similar to the description by Heering 1914 but the habitat is different No attaching disc was observed but Heering does not mention one in his specific description and Petersen 1935 says that Bolte considers Ulothrix subtilissima to be a soil alga	T.1.a T.1.b
	2 Wefts of filaments on damp soil. Cells 8-11μ wide by ½ -1 times as long. Most walls thin but some thicker. a few H pieces Chloroplast reaching total length of cell by more than half circumference, with one large distinct pyrenoid filaments long and not constricted.	Hormidium flaccidum A.Br. Under natural conditions there appears to be only one form -similar to that described by Heering as Hormidium flaccidum sensu ampl. This is a wide description and Heering splits it up into several species and forms with the aid of cultural behaviour.	T.2.a T.2.b T.3.a T.3.b T.3.c
	3 Wefts of filaments on damp soil. Cells 12-18μ wide by ½ -1 times as long. Cell wall two layered and with H pieces chloroplast reaching total length and more than half circumference of the cell Filaments long and not constricted Cell wall does not dissolve in chlor-	Hormidium mucosum Boy.Pet. Similar to description by Lund 1946 but is slightly smaller. The alga differs little from that described by Fritsch and John 1942 as H.crenulatum but they describe a stratified cell wall Lund separates the species by the reaction with chlor-zinc-iodide.	T.4

Source and No. of form		Description	Identification	Cultures
		zinc-iodide without a cellulose reaction.	Fritsch and John do not mention this test.	
Aquatic 1 Royal Holloway College, Surrey. Southwest pond.	1	Free floating flocculent masses in standing water. Cells 4½-6½μ by 3/4-1½ times as long. Cell wall thin. Chloroplast reaching total length of cell by more than half circumference, with one indistinct pyrenoid per cell. Filaments long and not constricted.	Ulothrix variabilis Kütz. If it is assumed that this alga was originally attached by a basal cell the alga could be identified as U. variabilis and is as described by Prescott 1951. If this assumption is not made the probable identification would be HORMIDIUM subtile as described by Heering. nb. Kirchner lists U.variabilis as a variety - H subtilis var variabilis.	A.1 a,b,c,d.
Aquatic 2 Chobham Common Surrey. Sphagnum bog near Sunningdale.	1	Free floating in standing water. Cells 3½-4½μ wide by 2-9 times as long. Cell wall thin Chloroplast not reaching whole length of cell, pale One indistinct pyrenoid per cell. Filaments fairly long and not constricted.	Stichococcus scopulinus Hazen. The very small width of the filaments and the length of the cells very nearly correspond to those given in description by Hazen 1902. None of the other described species are described as having such long cells.	A.2 a,b.
Aquatic 3 Aberystwyth Alga occurred in a collection of Draparnaldia of unstated source, sent for class practical work	1	Cells 4½-5½μ wide by 1-4 times as long. Cell wall thin. Chloroplast covering total length of cell wall and more than half circumference with one large distinct pyrenoid. Filaments fairly long and not constricted. Received in collection of Draparnaldia and already forming a silky film.	Hormidium lubricum Chodat. The silky film present when the alga was received, although not in culture, corresponds together with the other characters to those given for H.lubricum by Heering. 1914	A.3 a,b.

Source and No. of form		Description	Identification	Cultures
Aquatic 4 River Churnet, Yorks. Alga collected by Trent River Board from a noncalcareous stream, the Churnet.	1	From a stream. Cells 5-7½μ wide by 1-2 times as long. Cell wall moderately thick. Chloroplast angular and contracted into one corner of the cell. One small pyrenoid not always distinct. Filaments long, constricted at the cross walls only with "Kneebends" at intervals.	Hormidium subtile (Kutz) Heering. This alga is most nearly like that described as H. subtile by Heering but the irregularity of the chloroplasts is similar to that described for U. subtilis var variabilis Kirchn.	A.4 a,b.
AQUATIC 5 Virginia Water, Windsor Great Park, Surrey.	1	From irrigated stones at side of waterfall. Cells 6-7½μ wide by 1-2 times as long. Chloroplast covering total length and more than half the circumference of the cell. One large pyrenoid per cell. Filaments long, not constricted but with "Kneebends" ~~Cell wall thin, outgrowths.~~ and rhizoid-like outgrowths. Cell wall thin.	Hormidium rivulare - Kutz Form 2 This alga is covered by the description of H. rivulare as given by Heering 1914. but is too small to be identified as such by other Authors. The size is nearer that of H. fluitans? (Gay) Heering but the filaments do not break up readily.	R.1 a,b,c.
AQUATIC 5 as above	2	From irrigated stones at side of waterfall. Cells 7½-12½μ wide by 1-2 times as long. Chloroplast covering total length of the cell and more than half the circumference. One large pyrenoid. Cell wall sometimes 2 layered. Filaments long, not constricted and with only a few slight kneebends and rhizoid-like outgrowths.	Hormidium rivulare - Kutz Form 3. This alga is covered by the descriptions of H. rivulare given by a number of Authors and shows the typical features of "Kneebends" and rhizoid like outgrowths.	R.2 a,b.

Source and No. of Form		Description	Identification	Cultures
AQUATIC 6 Royal Holloway College Surrey, Alga from Stream indirectly connected with S.W. pond.	1	From rapidly running stream. Cells 5-6μ wide by 4-1 times as long. Cell wall thin but with irregular brown "papery" coat and H pieces of same substance. Chloroplast covering total length of cell wall and more than half the circumference. One pyrenoid not always clear. Filaments long not constricted but with kneebends and rhizoid like outgrowths.	Hormidium rivulare Kutz Form 1. This alga corresponds to the description of H.rivulare given by Heering (1914) but is too small to be identified as such if the description given by other authors are followed. It is nearer H.subtile (Kutz) Heering in size but has kneebends and rhizoid outgrowths.	R.3
"	2	Cells 8½-9½μ wide by 1-2 times as long. Cell wall thin but cemented to the substratum at intervals. Chloroplast covering the total length of the cell wall and more than half the circumference, with one pyrenoid. Filaments long, not constricted but with kneebends and rhizoid-like outgrowths.	Hormidium rivulare - Kutz No clones were isolated from this alga, so it was unimportant to decide on whether it was a distinct form. It falls within descriptions of H.rivulare form 3 but not such a wide range of filament size was found.	none (referred to as X.1)
"	3	Cells 14-15μ wide by 1-2 times as long. Cell wall rather thick. Chloroplast as above. Filaments long not constricted, no kneebends or rhizoid outgrowths.	Ulothrix tenuissima - Kutz. This alga is nearest Ulothrix tenuissima although only one pyrenoid per cell was seen Lind has neglected pyrenoid number in some of her identifications.	none (referred to as X.2)

Source and No. of form.		Description	Identification	Cultures
AQUATIC 7 Royal Holloway College, Surrey. Concrete slab in stream below the swimming pool.	1.	On concrete slab in fast running water. Cells 5-6μ wide by 1-4 times as long. Cell wall thin but irregularly coated with brown papery substance. Chloroplast covering the total length of the cell wall and more than half the circumference. One pyrenoid. Filaments long, not constricted but with kneebends and rhizoid outgrowths.	Hormidium rivulare Kutz. Form 1 This alga resembles the alga described on the previous page as H. rivulare form 1.	none (referred to as X.3)
AQUATIC 7 as above	2	Habitat as above. Cells 6½-9½μ wide by 1-2 times as long. Cell wall thin cemented to substrate at intervals. Chloroplast as above. Filament as above	Hormidium rivulare Kutz Form 2 This alga resembles that described on the previous page but the size differs slightly and overlaps that of form 3	none (referred to as X.4)
"	3	Habitat as above. Cells 10-12½μ wide by 1-2 times as long. Other characters as 2 above.	Hormidium rivulare Kutz. Form 4 For convenience this is described as a separate form but it has a size within that described for form 3.	R.4 a,b,c.
"	4	Habitat as above. Cells 15-19μ wide by 1-2 times as long. Cell wall thick and two layered. Chloroplast covering total length of cell wall and more than half the circumference. One large pyrenoid. Filament long and not constricted, very slight kneebends and outgrowths.	Ulothrix tenuissima Kutz. The size of this alga is greater than that given for Hormidium species other than H. mucosum and H. crenulatum and the appearance is nearer that described for U. tenuissima.	R.5 a,b,c.

Source and No. of form		Description	Identification	Cultures.
AERIAL 1 Malham York. Alga from fallen damp tree trunk near Malham Cove.	1	From damp tree. Cells 5-6μ wide by 1-2 times as long. Cell wall moderately thick. Chloroplast dark green and rather square in outline, covering total length. Pyrenoid difficult to see, probably one. Filaments short and dissociating No marked con-traction only of this appearance when separating.	Hormidium nitens Menegh. emend Kleb's The alga is nearest H. nitens. but cultural behaviour is usually used to separate this species from H. flaccidum The wall thickness resembles that of H. dissectum.	E.1
AERIAL 2 Alga collected from damp wall and sent to Professor Jane for identification	1	From damp stones. Cells 5-6μ wide by 1-2 times as long. Cell wall thin Chloroplast covering total length and more than half circumference of cell. One clear pyrenoid. Filaments of moderate length and some fragmentation occurs	Hormidium nitens. Menegh emend Kleb's This alga differs from that described above in filament length and in wall thickness but is still more like H. nitens than like other species.	E.2

Source and Name.				
Hormidium flaccidum CAMBRIDGE CULTURE COLLECTION		Cells 4½-6μ wide by 1-2 times as long. Cell wall thin. Chloroplast covering the total length of the cell wall and more than half the circumference. One clear pyrenoid. Filaments short and fragmenting, not constricted forming a silky film, in liquid culture.	H. nitens Menegh. emend Klebs. The formation of a silky film in cultures makes identification of this alga as Hormidium flaccidum unsuitable unless the name is given in the wide sense - H. flaccidum A. Br sensu lato as described by Heering 1914. As H. nitens is apparently also used by Cambridge the alga is better identified as H. nitens. Memegh emend. Klebs.	H. flaccidum (C)

Source and Name.	Description	Identification	Culture.
U. nitens. CAMBRIDGE CULTURE COLLECTION.	Cells $2\frac{1}{2}$-3μ wide by 1-2 times as long. Cell wall thin. Chloroplast covering the total length of the cell wall and more than half the circumference. One pyrenoid very feebly perceptible. Filaments not constricted but consisting of very short few celled fragments.	H. pseudostichococcus (Naegeli) Heering. The size of this alga and the absence of a sheath film in the cultures make the identification as H. nitens very unlikely. The alga is probably H. pseudostichococcus and does not differ from Heerings description. Whether this corresponds to S. bacillaris could not be decided.	H. nitens (C)
Ulothrix subtilissima CAMBRIDGE CULTURE COLLECTION.	Cells 5-6μ wide by 1-2 times as long. Cell wall thin. Chloroplast covering total length of cell wall and more than half the circumference. One pyrenoid. Filaments long not constricted.	Ulothrix subtilissima Rabenh. This alga is similar to that described under this name by Heering and resembles T.1 No specialised basal cell was found in this clone also.	U. subtilissima
Uronema gigas CAMBRIDGE CULTURE COLLECTION	15μ wide by $\frac{3}{4}$-$1\frac{1}{2}$ times as long. Cell wall thin Chloroplast covering total length of cell wall and more than half circumference, 2-3 pyrenoids per cell. Filaments very long with basal attaching disc and acuminate apex.	The characters of the alga confirm the identification as Uronema gigas	Uronema gigas (C)
Uronema confervicolum CAMBRIDGE CULTURE COLLECTION	Not forming regular filaments but masses of cells with tapering ends The masses of cells sometimes occuring as if germinating zoospores remained in old filament Chloroplast indefinite in outline. Pyrenoids indistinct. Occasionally one pyrenoid discernable.	The culture received on agar was obviously growing abnormally. In subsequent liquid cultures the alga formed filaments which were similar to Uronema gigas (C)	Uronema confervicolum (C)

Source and No. of form	Description	Identification	Cultures.
Stour, Kent Alga collected from snails shells by Miss. M. Campion.	Cells 62-87u wide by about as long as wide Filaments fairly long with basal disc and pointed apical cell Chloroplast reaching whole length of cell and more than half circumference. Filaments slightly constricted at the cross septa.	Possibly Uronema gigas but is rather small	U.1
Fern Dale District. Belham Fern. Alga collected from snails shells by Miss. M. Campion.	Cells 40-50µ wide at tip to 62-68µ at base of filament 2-4 times as long as wide at tip 1-2 times as long as wide at base. Pyrenoids usually one sometimes 2 per cell.	Uronema confervicolum This alga corresponds to the description of Uronema confervicolum Lagerheim in all respects except that he states that 2 pyrenoids per cell are commoner than one.	U.2
Malham Yorks-on Glacial Drift near the Field Station.	Cells 5.0-6.2u wide by 1-2 times as long Filaments fairly long with basal attaching disc and acuminate end cell. Chloroplast mostly reaching whole length of cell and surrounding more than half the circumference. Pyrenoids indistinct. Probably one per cell.	Uronema confervicolum ~~Nageli~~ Naegeli. This alga most nearly corresponds with descriptions of U. confervicolum although two pyrenoids per cell were not detected.	U.3

B. CULTURAL METHODS

Isolation of the algae

Clones were isolated from most of the collections. They are listed in Table 1. Single filaments were taken from the collections of alga using sterile glass needles to pick up the filaments. They were passed through a series of washes of sterile distilled water in sterile watch glasses enclosed in sterile petri dishes. Finally they were transferred to sterile Soil Solution. A number of parallel isolations were made from each collection of alga.

Culture vessels

Cultures in liquid media were,,when first established, kept in 1" diameter specimen tubes plugged with cotton wool. Later cultures were kept in 5 centimetre deep petri dishes or in 250 mls., Pyrex conical flasks plugged with cotton wool.

The vessels were not treated with chromic acid type cleansers before use as these may be injurious to algae (Harvey 1949). If deposits of lime were present these were removed with hydrochloric acid. The vessels were then washed in hot water with a detergent and then rinsed under a running tap. Finally they were rinsed with distilled water and left to dry. All utensils were sterilised by autoclaving at 15 lbs pressure per sq.inch above normal

atmospheric pressure for 20 minutes. They were wrapped in paper during autoclaving to prevent excessive condensation on their surfaces.

Subcultures.

Cultures were subcultured at intervals of approximately four weeks. A "massive" sample was taken and transferred to fresh culture medium in a fresh vessel. Normal precautions were taken to maintain sterility.

Aeration of cultures.

An experiment on the effect of aeration and consequent stirring was carried out. A series of cultures in 5 cms., petri dishes was aerated from a small electric pump. Two tubes in parallel were run off from the pump and from each a series of side arms were run to the cultures. Each arm and the free ends of the main tubes could be closed down with springclips until the flow through each culture was similar. The ends of the side tubes dipped into the cultures under a slightly tilted lid so the cultures were open to contamination from the air (the use of cotton wool plugged tubes or flasks would eliminate this difficulty but were not available at the time.) The side arms were loosely plugged with cotton wool to help in filtering the air. Twenty cultures could be aerated at approximately the same rate of bubbling.

Other environmental conditions

Preliminary experiments were carried out growing the

cultures in artificial light of controlled intensity and duration in thermostatically controlled incubators (20-25 degrees C), and in the uncontrolled but differing temperature and light ranges provided by outdoor, cool greenhouse, and laboratory conditions. Satisfactory initial growth of clones was only obtained in the laboratory in a North facing window at the normal lab. temperature (of 15 degrees centigrade in winter-rising to 20-25 in summer). It was decided to limit work to the effect of the culture solution (nutrients) and grow the alga in the prevailing laboratory conditions ie. with normal daylight and temperature, the former showing normal daily and seasonal variation, the latter maintained by heating within the range 15-25 degrees and normally not higher than 20 degrees.

Nutrient Solutions.

Earlier workers used solutions which were modifications, usually dilutions of the water culture solutions for higher plants. Knops water culture solution has been widely used (eg. Klebs 1896, Gaidukov 1903) and Vischer (1933) states that it is this solution, under the name of Detmer, which was used by Detmer (1912), Chodat (1913), Kufferath (1930), and others. Detmers solution appears however to differ in that potassium chloride is substituted for potassium nitrate. Other solutions of mineral salts have been

specially devised for algae and are commonly used and described by the names of the originators - Benecke, Molisch, Bjeirinck, Moore, Pringsheim, Chu, and many others.

There is also a tendency to use water extracts of soil alone or with the addition of particular salts, under the name of Soil solution. The exact methods of preparation differ and anyway the solution has the considerable disadvantage that its composition is unknown and is not exactly repeatable. The advantage claimed is that growth is better and more normal.

Only a selection of a few of the great number of culture media which have been devised and used for various algae, were used. The selection included Knops (Maclean and Cooke 1941)

Molischs	(Pringsheim 1946 pg 35)
Pringsheims	(Pringsheim 1946 pg 35)
Moores	(Poulton 1930)
Beneckes	(Maclean and Cooke 1941)
Bjeirincks	(Pringsheim 1946 pg. 35)
Herveys	(Hervey 1949)
Godwards	(Godward 1941) (this is a modified Chu solution)
Uspenskaja	(Uspenskaja 1925)
Soil solution	(Bold 1942)

A fuller account of these nutrient solutions and of

any modifications is given in Appendix II.

In addition to these usual solutions "modified" solutions and a series of solutions intermediate in composition between Pringsheim's and Nolisch's solutions were used. Modified solutions were similar to that described by Hervey (1949) as "Hervey's modified" in that they contained an addition of Soil solution. Knops modified, Molisch's modified, etc were prepared by mixing equal quantities of Knop's, Molisch's etc with soil solution. The composition of the intermediate solutions between Pringsheim's and Molisch's solutions are given in the table in the Appendix.

The addition of particular substances, generally of organic nature, to induce better growth or zoospore formation, has been suggested (Pringsheim 1946) Certain additions were made in this way to Soil solution. Yeast extract (.2%), Dextrose (0.2%), dibasie potassium phosphate (0.02%), Calcium bicarbonate (.005-0.02%) were used.

The pH of all the culture solutions depends on the salts included, and varies as they are utilized. All are approximately neutral or slightly acid pH 5.0 - 7.0 and in attempts to obtain zoospore production a wider range of pH was required. Alteration of pH was made by one of two methods.

1. The use of acid and alkali - Hydrochloric acid and sodium hydroxide.
2. The use of buffer solutions - Clark and Lubs buffer and Acetic acid/Acetate buffer.

The buffers were made up as given in the appendix and used with an equal quantity of nutrient solution. Under these conditions they did not give the exact pH stated there and changed with time but over a limited period of culture they gave a wide range of pH and the only aim in their use was to grow the alga in solutions of widely differing pH in case this was limiting zoospore production.

IV. INVESTIGATION OF CHARACTERS USED IN IDENTIFICATION.

REPRODUCTION BY MOTILE CELLS

The absence of reproduction by motile cells is one of the characters used to separate off Stichococcus from related genera. Characters of the zoospores and gametes are used to separate the genera Ulothrix and Hormidium although reproduction by motile cells has not been described for all the species. Particular minor characters eg., Aplanospore shape, are used in specific descriptions.

A. METHODS.

Reproduction by motile cells occured in all the Uronema clones (U.1, U.2, U.3, U.gigas (C), U.confervicolum (C).) without the use of special methods. These species have been adequately described for taxonomic use and were not studied further. The aim was to obtain reproduction by motile cells in the Hormidium and Ulothrix clones, since it is in this section that features of reproduction are particularly useful.

Variation in culture solution, pH and aeration, were all used in attempts to obtain the production of motile cells in clonal culture. The methods of Klebs and others, that is change in the strength of the medium and the use of darkness, were employed without success. Briefly summarizing the transfers were made as follows:-

1. From Knops solution to more dilute solutions - 1/2, 1/3,

1/6. 1/12, of the original strength (6%)

2. From Knops solution to sterile distilled water.

3. As 1. above but cultures kept in the dark - separate subcultures examined after 1, 2, 3, 4, 5, days.

4. As 2. above but in the dark - separate sub cultures examined after 1, 2, 3, 4, 5, days.

5. From soil solution to more dilute solutions - 1/2, 1/4, 1/8, 1/16

6. From soil solution to distilled water.

7. As 5 above but in the dark - separate sub cultures removed after 1, 2, 3, 4, and 5 days.

8. As 6 above but in the dark - separate sub cultures removed after 1, 2, 3, 4, and 5 days.

Observations were made on all the cultures during the day and examinations of certain clones was also made during the night. Clones T.1, T.2, T.3, T.4, R.3, A.4 and freshly collected material of all these except A.4 were examined at intervals on nights during March 1952, November 1952, and May 1953. Since there was no indication that zoospores and gamete formation would occur in the majority of the clones and since there is no certainty that zoospores and gamete formation can ever occur, attention was transferred to an investigation of the variation in morphological characters.

B. OBSERVATIONS

Although motile cells were never seen, observations were made which made it possible to infer that in some cases such reproduction by motile cells had occured. Samples of the following clones T.2, T.3, T.4, R.1, R.3, A.1, A.4 had a few filaments with empty cells with conspicuous pores. The contents of a few cells in these clones rounded off but failed to emerge. A very small number of spores (probably zoospores which failed to emerge) germinated in situ to form short filaments of a few cells. Examples of these features by which it was inferred that reproduction by motile cells may occur are shown in Figure 5.

THE SHAPE OF THE TERMINAL AND BASAL CELLS OF THE FILAMENTS.

The type of terminal and basal cells is an important generic character and is used as described on page 5.

A. METHOD.

The shape of terminal and basal cells was recorded for the various clones studied and since the terminal and basal cells in a clonal culture may be the result of fragmentation a record was also made of whether motile cells had been formed.

B. OBSERVATIONS.

General description.

The shapes of the basal and terminal cells differed in the various clones. Either the clones had basal cells with attaching discs and acuminate terminal cells characteristic of the genus Uronema or they had rounded basal cells and terminal cells which were never of the acuminate shape so constantly found in the Uronema species.

I) Filaments fixed by basal attaching disc and with terminal cells acuminate.

The clones U.1, U.2, U.3 and Uronema gigas (C), always contained filaments with basal attaching discs and an acuminate terminal cell. This form, typical of Uronema species, is illustrated in figure 4 . When subcultures were made from old bleached cultures, motile cells were

always formed in the fresh solution, and grew into young plants with basal attaching discs and acuminate terminal cells. When green filaments were transferred to fresh solution motile reproductive cells were not always produced but continued vegetative growth and fragmentation resulted in free floating filaments with both ends generally rounded. Clone U.confervicolum (C) when received from Cambridge was in the form of fragments mainly of very irregular filaments as shown in figure 4D , with many cells wedge shaped in section, ie. with rather pointed ends, projecting in all directions. After continual subcultering some subcultures were obtained which behaved in a typical manner and formed the normal filaments of a Uronema species. This clone finally became as constant in character as the other Uronema clones.

II) Filaments without acuminate end cells.

All the other clones studied (T.1, T.2, T.3, T.4, A.1, A.2, A.3, A.4, R.1, R.2, R.3, R.4, R.5, E.1, E.2,) were of Ulothrix or Hormidium type without acuminate apical cells. Clones T.1, T.2, T.3, A.1, A.2, A.4, R.3, probably formed motile reproductive cells though these were never seen in cultures. The terminal and basal cells were generally both rounded. However in a few of the filaments of clones T.3, T.4, A.4, all of which normally formed long filaments, the

ends had projecting cell walls (cf., pg. 46.) The remains of these broken intercalary cells were often crushed and might in a cursory examination have been confused with acuminate end cells. Small short filaments of 2-3 cells, probably germlings, found in clones T.2, T.3, as shown in figure 5 . had a very slightly tapering end. No tapering cells as acuminate as those found in Uronema were found. In clone R.5, very long filaments with attaching discs and rounded ends occured and the number of ends seen in a sample was consequently small.

Effect of culture solutions on the shape of the end cells of the filaments.

The use of different culture solutions had no effect on the type of basal and terminal cells found nor did culturing itself make any difference. In Uronema clones acuminate apical cells were constantly found; in other clones they were always absent. The shape of the terminal and basal cells of clones grown in culture and the type of end cells at the time of collection were similar. As the Uronema species all showed such constancy in the formation of acuminate terminal cells and since the other clones never had acuminate cells this character was useful for separating them. The difficulty of examining the small fragments present in the natural habitat prevented the assessment of

the shape of the end cells in many algae until they were in culture, this was specially so for attached species (U.1, U.2, U.3,) which were browsed by animals.

CHLOROPLAST SIZE AND SHAPE.

The character of the chloroplast (cf. pg, 4) may be used as a generic character and its use emphasized in differentiating between Hormidium and Ulothrix. It is also used as a specific character in a few cases.

A. METHOD.

It was difficult to express the chloroplast size and shape in terms of measurement as the chloroplast is always a parietal plate lying close to the cylindrical surface of the cell wall. Records were made of the percentage of cells in which the chloroplast covered the entire cell wall in one plane and an estimate was also made of the proportion of the circumference of the cell wall covered by the widest part of the chloroplast. Observations were made on the Hormidium and Ulothrix species only, because in differentiating between these genera the emphasis has been put on whether the chloroplast is "ring or plate shaped and usually encircling more than half the circumference and frequently occupying the whole length of the cell" or "an elliptical or circular plate generally occupying about

half the length of the cell."

B. OBSERVATIONS.

NATURAL CONDITIONS.

Under natural conditions the chloroplast, in the majority of the algae studied, was remarkably similar. The chloroplast extended the full length of the cell and was elliptical or a more or less rectangular plate with the longitudinally lying edges rather rounded. The widest part of the chloroplast surrounded about three quarters of the cell circumference (ie Cylindrical face) A.2, differed in that the chloroplast was very small in relation to the cell size.— the shape usually described as Hormidium type, only half or less of the length of the cell wall was covered and about half of the circumference. A.4, differed in that the chloroplasts were irregular in outline and frequently were contracted into a corner of the cell.

CULTURAL CONDITIONS.

In cultures grown in soil solution the chloroplasts were generally similar in size and shape to those found under natural conditions. The exceptions were A.2, and A.4, those clones which had small chloroplasts under natural conditions. In culture in soil solution these two had chloroplasts similar to the other algae. This will be described as "normal" for convenience.

In cultures in solutions made up from known amounts of chemicals, solutions such as Knops and Molisch's, certain of the algae studied developed chloroplast of the Hormidium type. These algae were T.2, A.1, A.4, R.1, E.2,. Table 2. summarizes the observations. The variability of the chloroplast was in a few sufficient to alter the entire appearance of the algae from the type commonly described for Ulothrix species to that commonly described for Hormidium species. In the majority of the algae studied there was absence of variation in size and shape of the chloroplast. Both the variation within some clones and the absence of difference between most clones prevent this character being useful in differentiating between the algae.

Table 2 — Effect of culture on the chloroplast.

Clone	Chloroplast shape under natural conditions.	Chloroplast shape under various cultural conditions. In Soil Solution			In Molisch's Solution			In Knops Solution		
		Description	1	2	Description	1	2	Description	1	2
T.1	normal.	normal	100.	100(54)	normal	100.	100(36)	normal	100.	100(33)
T.2	normal.	normal.	92.	100	Hormidium type	55.	94	Hormidium type	52	50
T.3	normal	normal	100.	100	normal	100.	100	normal	100.	100
T.4	normal	normal	100.	100	-	-		-	-	
A.1	normal	normal	100.	100	Hormidium type	50.	80	Hormidium type	45	86
A.2	Hormidium type	normal	100.	100	normal	100.	100(15)	normal	100.	100
A.3	normal	normal	100.	100(30)	normal	95.	100(78)	normal	100.	100(41)
A.4	Contracted	normal	94.	100(8)	Reduced	78.	100	Hormidium type	70.	100
E.1	normal	normal	100.	100	normal	100.	100	normal	100.	100
E.2	normal	normal	100.	100(26)	normal	100.	100	Hormidium type	76	98
R.1	normal	normal	100.	100(30)	Hormidium type	30.	90	Hormidium type	80	95
R.2	normal	normal	100.	100	-					
R.3	normal	normal	100.	100(28)	-					
R.4	normal	normal	100.	100(30)	-					
R.5	normal	normal	100.	100	-					

Column 1 shows the percentage of cells in which the chloroplast reaches the entire length of the cell.

Column 2 shows the percentage of cells in which the chloroplast surrounds are half or more of the circumference. In brackets as shown the percentage, if any, in which the chloroplast surrounds $\frac{3}{4}$ or more.

FRAGMENTATION.

Stichococcus is separated from related genera by the great tendency to fragment so that only few celled filaments are found. The amount of fragmentation is also used as a specific character in the genus Hormidium.

A. METHOD.

Following Benecke, Petersen, and others, fragmentation was recorded as rapid or slow fragmentation. In rapid fragmentation filaments break up into short lengths, the dissociation appearing to be caused by separation of the middle lamellae and rounding off of neighbouring cells. The term rapid fragmentation is slightly deceptive in that it does not occur suddenly and rapidly but may be a continuous slow process. In slow fragmentation the filaments break up into long lengths and the ends of the resulting filaments show remnants of a projecting cell wall.

B. OBSERVATIONS.

General description

i) RAPID FRAGMENTATION

Rapid fragmentation resulted in filaments of varied lengths although all were relatively short. The number of cells in filaments were counted and filament length fell naturally into groups without any intermediates. Filaments were of less than 10 cells, 20-30 cells long, or

several hundred cells long. No filaments approached the length of filaments in cultures in which slow fragmentation was recorded. In the latter, filaments were several centimetres long. In the majority of cultures one particular length seemed to predominate. Three types of rapid fragmentation were therefore distinguished.

1) Filaments absent or composed of less than 10 cells.
2) Filaments short consisting of about 25 cells.
3) Filaments of several hundred cells.

A few cultures contained filaments of all the lengths from few cells to several hundred cells. These were described as type 4.

The filaments showed the characteristic zig zag appearance because of the loose connection of cells or the close juxtaposition of short recently separated filaments. There was generally no sign of thickening of any kind, while staining with methylene blue did not show any special accumulation of stainable material at the cross walls.

b) Slow Fragmentation.

In slow fragmentation the filaments remained several cms. long the occasional breaks had projecting walls. There was generally a thickened cell wall and occasional "H"

pieces intercalary in the filament but not at the ends of filaments. Free floating "H" pieces were not found. It did not seem as if the thickenings were directly associated with fragmentation. On applying methylene blue dead or disorganised cells stained first, the outer layer of the wall and the "H" pieces stained gradually. Material between the cell walls, comparable with Piercy's accumulations of stainable material, occurred but could not be differentiated clearly from small "H" pieces and thickened outer cell walls. The ends of the filaments always showed the projecting pieces of wall described by Lund (1940). These Lund attributed to the remaining outer wall, separation of the middle lamella between the inner walls having occurred as in rapid fragmentation. Since projections were found in filaments where a double cell wall was not present, they must, at least in this case, represent torn cells as described by Petersen. Disorganised cells were often bent or the neighbouring cells projected into them. It appeared that tearing did occur.

Effect of culture solutions on fragmentation.

The various algae investigated showed varying amounts of fragmentation when growing in the different media and when compared with one another.

Table 3 summarizes the results for three solutions and

sterile distilled water. Water was used because it has been suggested that lack of nutrients causes fragmentation. The type of rapid fragmentation is indicated. The classification was normally based on estimation but table 4 shows numerical records of the number of filaments of different length for certain of the clones. These counts clearly show the difference in length of the predominating filaments in the types 1, 2, 3, 4 of table 3. Types 3, 2, 1 could be clearly separated but type 4 (a mixture of filaments of all lengths) might approach type 2 or type 1 in having a high proportion of filaments of 25 or a few cells.

Table 3 Effect of Culture Solutions on Fragmentation

Clone.	Type of Fragmentation in Soil Soln	Type of Fragmentation in Knops soln.	in Molishs soln	In distilled H_2O
E.1	Rapid (3)	Rapid (3)	Rapid (3)	No further fragmentation.
E.2	Rapid (3) & slow	Rapid (3) &slow	Rapid (3) &slow	-
E.3	Rapid (3) " "	Rapid (3) "	Rapid (3) "	-
E.4	slow	-	slow	-
A.3	Rapid (3)	Rapid (3)	Rapid (3)	-
A.2	Rapid (1)	Rapid (2)	Rapid (3)	-
A.3	Rapid (4)	Rapid (2)	Rapid (4)	-
A.4	None	Rapid (3)	Rapid (3)	None
E.1	None	-	-	None
.2	None	-	-	None
.3	Rapid (3)	-	-	No further fragmentation
E.4	None	-	-	-
.	None	-	-	-
.1	Rapid (1)	Rapid (1)	Rapid (1)	-
.2	Rapid (4)	Rapid (4)	Rapid (4)	-
N.Flaccidum (C)	Rapid (2)	Rapid (2)	Rapid (2)	-
N.nitens (C)	Rapid (1)	Rapid (2)	Rapid (2)	-
N.subtilissima (C)	Rapid (2)	Rapid (2)	Rapid (2)	-

A blank (-) indicates that the clone was not grown in that particular soln

Table 4 Number of filaments of different lengths in cultures showing different types of fragmentation

Clone.	Type of fragmentation.	Nutrient soln.	Percentage of filaments of various lengths (Predominating filaments underlined.) Less than 10 cells.	Approx 25 cells.	100 cells.
A.1	Rapid (3)	Soil soln.	24	12	64
A.2	Rapid (3)	Molisch's soln.	12	0	88
A.4	Rapid (3)	" "	18	24	58
A.3	Rapid (4)	Soil soln.	70	23	7
A.3	Rapid (2)	Knops soln	0	92	8
E.2	Rapid (4)	Soil soln.	36	48	16
A.2	Rapid (2)	Knops soln	8	85	7
A.2	Rapid (1)	Soil soln.	100	0	0

Rapid fragmentation was more characteristic of certain clones than of others and absence of fragmentation was characteristic of some. The marked effect of the medium on fragmentation in certain clones, particularly A2 where the general form was entirely altered, indicates the possible extent to which environmental conditions may affect the amount of fragmentation. In all cases where the clone was transferred to sterile distilled water growth was arrested and fragmentation did not occur either at once or later.

Further investigation of fragmentation - the effect of a series of solutions on one clone.

A series of solutions intermediate between Pringheim's and Molisch's solutions and having the composition given in the Appendix were set up. The solutions were inoculated with roughly equal portions of clone A.4 and examined after 4 weeks. Table 5 records whether fragmentation was present or absent.

TABLE 5

Further investigation of the effect of the culture solution on one clone

Solution No.	Main Alteration In Composition	Fragmentation. P-Present A-Absent.
1.	Pringsheims soln minus iron	P
2.	Pringsheims soln	P
3.	Pringsheims soln, minus iron, plus phosphate	P
4.	Pringsheims soln, minus iron, plus conc. phosphate	P
5.	Pringsheims soln, minus iron, plus phosphate (K_2HPO_4)	P
6.	Pringsheims soln, plus phosphate as in 3.	P
7.	Pringsheims soln, plus phosphate as in 4.	P
8.	Pringsheims soln, plus phosphate as in 5.	P
9.	Pringsheims soln plus $MgSO_4$	P
10.	Pringsheims soln minus iron, plus $MgSO_4$ & PO_4 as in 3.	P
11.	Pringsheims soln minus iron, plus $MgSO_4$ & PO_4 as in 4.	P
12.	Pringsheims soln minus iron, plus $MgSO_4$ & PO_4 as in 5.	P
13.	Pringsheims soln plus $MgSO_4$ plus phosphate as in 3.	A
14.	Pringsheims soln plus $MgSO_4$ plus phosphate as in 4.	A
15.	Pringsheims soln plus $MgSO_4$ plus phosphate as in 5.	A
16.	Molischs soln minus most $MgSO_4$	A
17.	Molischs soln minus some $MgSO_4$	A
18.	Molischs soln	P
19.	Molischs soln minus $CaSO_4$	P
20.	Molischs soln minus iron	P
21.	Molischs soln minus $CaSO_4$ & iron	P
22.	Molischs soln minus $MgSO_4$ & $(NH_4)_2HPO_4$, ~~& HPO_4~~ plus KNO_3.	P
23.	Molischs soln minus $(NH_4)_2HPO_4$ plus KNO_3 & K_2HPO_4	P
24.	Molischs soln minus $(NH_4)_2HPO_4$ plus more KNO_3, cone K_2HPO_4	P

No fragmentation occurred when $MgSO_4$ and additional phosphate were added to Pringsheim's solution or when the $MgSO_4$ in Molisch's solution was reduced. The formulae of the solutions in which fragmentation did not occur is listed below:-

No.	$MgSO_4$	KNO_3	$(NH_4)_2HPO_4$	$CaCl_2$	$FeSO_4$	K_2HPO_4	$CaSO_4$
13	0.2	0.2	0.2	trace	trace	-	-
14	0.2	0.2	0.4	trace	trace	-	-
15	0.2	0.2	0.2	trace	trace	0.2	0.4
16	0.001	-	0.8	-	trace	0.4	0.4
17	0.2	-	0.8	-	trace	0.4	0.4

Fragmentation occurred when $MgSO_4$ and different phosphate concentrations were added, but $FeSO_4$ not included. When other salts are removed from Molisch's solution as well as reducing the $MgSO_4$ concentration fragmentation again occurred.

THE effect of aeration (and the resulting stirring) on fragmentation

Aeration of certain clones was carried out as described on pg.29. The results are given in Table 6.

Table 6.

Aeration.	Nutrient coln.	Clone	Percentage of filament of different Filament length.			
			1-10 cells.	approx.25.	several 100	sev cms.
Aerated.	Knops	A.2	7	85	8	0
Not aerated	"	"	13	87	0	0
Aerated	Molisch	A.2	0	12	88	0
Not aerated	"	"	14	16	70	0
Aerated	Knops	A.2	13	87	0	0
Not aerated	"	"	7	85	8	0
Aerated	Molisch	A.2	14	16	70	0
Not aerated	"	"	0	12	88	0
Aerated	Soil Soln	A.4	0	0	0	100
Not aerated	" "	"	0	0	0	100
Aerated	Soil Soln	A.3	75	15	10	0
Not aerated	" "	A.3	70	23	7	0

In the clones studied aeration appears to have no effect on fragmentation.

PYRENOIDS.

The absence of pyrenoids is used as a generic character for separating Stichococcus from related genera. The number of pyrenoids present per cell is also used as a specific character in the genera Ulothrix, Uronema, and Hormidium.

METHOD.

The number of pyrenoids per cell was recorded in the cells selected for measurement of size (cf. pg 56) Similar examinations were also made after staining with iodine in potassium iodide but this did not reveal any further pyrenoids.

OBSERVATIONS

General description.

One pyrenoid was detected in the majority of cells of the algae growing under natural conditions. Frequently the pyrenoid was very indistinct and particularly so in the algae from which clones A.1, A.2, A.4, E.1, R.3, were isolated. It was in these algae too that a pyrenoid was not detected in every cell. The pyrenoids were large and oval in shape (elongated in the direction of the longitudinal acis of the cell) and were visible as a glistening or slightly more opaque area in the chloroplast. The differences under natural conditions were insufficient to

be of use in separating the algae.

Effect of culture solutions on the size and visibility of pyrenoids.

In culture the pyrenoid and chloroplast may be obscured by droplets presumably of oil. Such droplets occurred sporadically but infrequently in cultures of T.1, T.2, T.3, A.1, A.2, A.3, A.4, E.1, E.2, R.1, R.2, R.3, R.4, H.Flaccidum (C) H.nitens (C), Uronema gigas (C), U.confervicolum (C), U.1, U.2, U.3, but their formation could not be correlated with any known alteration in conditions and was not recorded. The pyrenoids were generally more distinct in culture than under natural conditions and were noticeably so in the flourishing cultures in Soil soln. with the exception of only A.2 and H. nitens (C). These two fragment in soil solution, in Molisch's solution however they too showed clearer pyrenoids.

Although culture made the pyrenoids easier to see, it made no difference to the size and shape or to the number of pyrenoids per cell detectable except in clone R.5. In culture it was possible to detect two pyrenoids per cell in 75% of the cells of this clone, although the use of the pyrenoids for differentiating between the genera and species was not practicable the pyrenoids were clearer in the cultured algae and this was of use in confirming the naming of R.5 as a Ulothrix species with two pyrenoids per cell.

IV CELL MEASUREMENTS

A. METHOD.

Measurements were made using a microscope with 150 mm tube length a 1/6 inch objective and a x18 ocular containing a graduated scale. At this magnification one division of the scale corresponded approximately to 1.24μ. Estimations of size were made to the nearest half division.

Before taking a sample with a sterile needle the culture was mixed by gentle shaking. The samples were mounted in the culture solution on a slide and covered with a coverslip in the normal way.

Measurements of width of the cells were made for cells along a line transect (say right to left of the field) in filaments crossing this line at about a right angle (ie top to bottom of the field). It was hoped by this method to sample different filaments as it seemed reasonable to assume that, with care in mounting, filaments would be unlikely to be completely bent back on themselves.

Measurements of length were taken by determining the longest and shortest cells within an arbitary distance along the filaments whose width had been taken. The distance chosen was the field of view and one field on either side of this. These lengths have been termed the maximum and minimum lengths in the subsequent tables.

The number of measurements taken varied. In clonal

cultures it was never less than 25 and in collections of material from natural conditions it was never less than 100 measurements as in the latter case there may be more than one species present and the range in width is greater. The measurements have not been treated statistically but where 25 and 100 measurements were taken for the same clone exactly similar limits for width and length were obtained.

B. ~~RESULTS~~ OBSERVATIONS.

UNDER NATURAL CONDITIONS

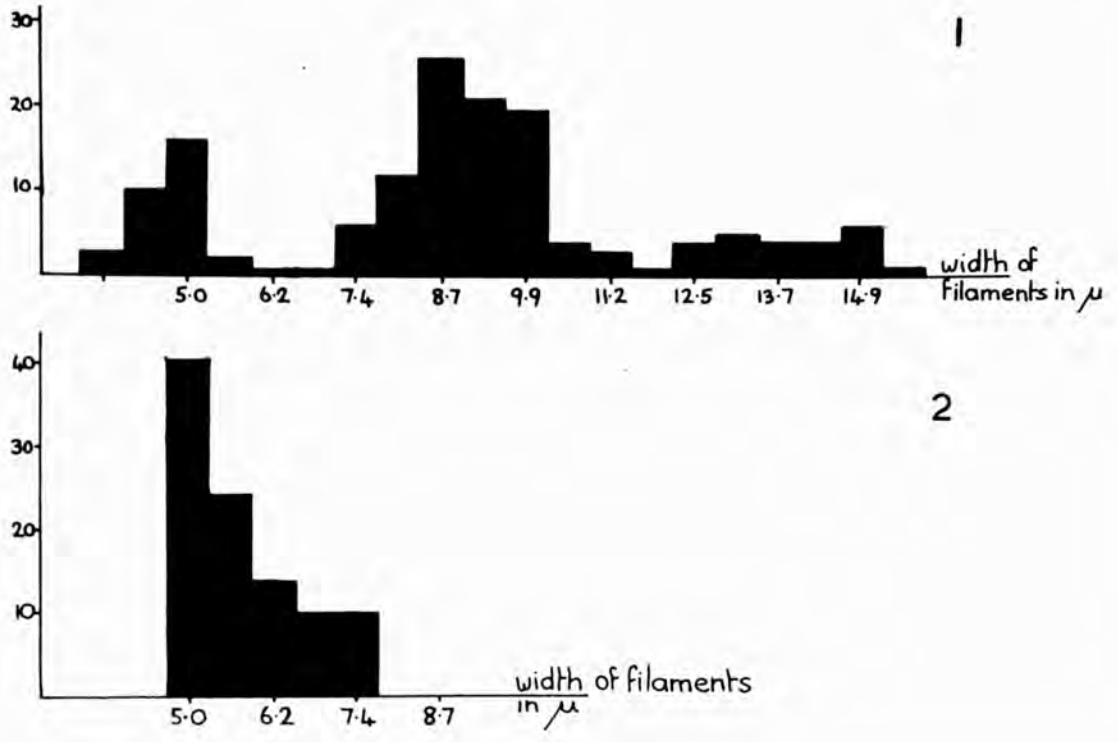
Since collections of alga did not necessarily consist of one species only, measurements of width, in particular, may cover a wide range when the number of filaments of different width is expressed as a histogram, two types are obtained as shown in Histograms 1 and 2. The histogram for the alga from the River Churnet (Source Aquatic 4.) has only one maximum. The histogram for a collection of alga from Chobham Common (Source-Terrestrial 1.) shows several maxima or modes which probably indicate the presence of more than one species or variety. If the species do not overlap in their range of width it would be easy to determine the limits of their size. As will be seen from the histogram it is only possible to estimate their limits by the position of minima. The collection

here was taken to be three species approximately 3.5-6.5μ, 8.5-11.5μ, and 11.5-15.5μ wide.

Similar histograms were constructed for other collections of alga but are not included. The size of forms or species collected is listed below.

Terrestrial	1.	T 1a.	3.7 - 5.2 μ wide
		T 2 a,b, T 3 a,bc	6.8 - 11.2 μ wide
		T 4	11.8 - 15.5 μ wide
AQUATIC	1	A1	4.3 - 5.8μ wide
AQUATIC	2	A2	3.7 - 4.3μ wide
AQUATIC	3	A3	4.3 - 5.6μ
AQUATIC	4	A4	5.0 - 7.4μ
AQUATIC	5	R1	1.2 - 7.4 μ
		R2	7.4 -12.4 μ
AQUATIC	6	R3	5.0 - 6.2 μ
		X1	8.7 - 9.3 μ
		X2	14.0 -15.0 μ
AQUATIC	7	X3	5.0 - 6.2μ
		X4	6.8 - 8.2μ
		R4	10.0 -12.4μ
		R5	14.9 -18.6μ
AERIAL	1	E1	5.0 - 6.2μ
AERIAL	2	E2	5.0 - 6.2μ
URONEMA	1	U1	6.2 - 8.7μ
URONEMA	2	U2	4.0 - 6.8μ
URONEMA	3	U3	5.0 - 6.2μ

Histograms 1 and 2.



1. Histogram of measurements of width of cells in collection of algæ from Chobham Common (Terrestrial 1) The histogram shows several maxima or modes.

2. Histogram of measurements of width of cells in a collection of algæ from River Chirnet (aquatic 4.) The histogram shows one maximum or mode.

UNDER CULTURAL CONDITIONS

In addition to measurements of the algae under natural conditions measurements were made of all the algae in clonal culture in Soil Solution and of many in other solutions, in particular in Knops and Molischs solutions. The final range in width of the cells in μ and the maximum and minimum length of cells in terms of the width (ie. 2 times as long as wide etc) is given in Table 7.

After the establishment of clones from a single filament or after the transfer of clonal material to a different solution, there was always a gradual change in width until a final constant range for each clone in the particular solution was obtained. The time taken for this final constant range to be reached was approximately 9 months in cultures started from single filaments and 6 months in cultures started from large samples of clonal material. Thus measurements for clone T.1 at 6, 9, 12, and 24 months after establishment were 5.0-5.6μ, 4.3-5.6μ, 4.3-5.6μ, 4.3-5.6μ respectively and for transfers of clonal material to Knops solution after 6 months in Soil solution the measurements 3,6,9,12, and 18 months later were 3.7-4.3μ 3.7-4.6μ, 3.7-4.3μ, 3.7-4.3μ, 3.7 -4.3μ. It is these final constant ranges in width which are given in the table Maximum and minimum length did not vary after 3 months and no determinations were made before this as there was insufficient material for continual sampling.

TABLE 7

Conditions	Natural		Soil Soln.		Knops Soln.		Molischs Soln.	
Name of Clone.	Width in μ	Length in terms of width	Width in μ (mean)	Length in terms of width	Width in μ (mean)	Length in terms of width	Width in μ (mean)	Length in terms of width
T.1.a.	3.7-6.2	1-2	4.3-5.6 (5.0)	1-2	3.7-4.3 (4.1)	2-4	3.7-5.0 (4.1)	2-6
T.1.b.	3.7-6.2	1-2	4.3-6.2 (5.2)	1-2	3.7-4.3 (4.1)	2-4	3.7-5.0 (4.1)	2-6
T.2.a.			7.4-8.7. (7.8)	1-2			6.2-7.4 (6.8)	$1\frac{1}{2}$-4
T.2.b.			6.8-8.7 (8.0)	1-2	5.0-7.4 (6.1)	1-4	5.0-8.1 (6.2)	$1\frac{1}{2}$-5
T.3.a.	6.8-11.2	$\frac{1}{2}$-1	8.7-11.2 (9.8)	$\frac{1}{2}$-1	7.4-8.7 (7.9)	$\frac{3}{4}$-$1\frac{1}{2}$	7.4-8.7 (7.9)	1-2
T.3.b.			8.7-11.2 (9.8)	$\frac{1}{2}$-1	7.4-8.7 (7.9)	1-2	7.4-8.7 (7.9)	1-2
T.3.c.			8.7-11.2 (9.7)	$\frac{1}{2}$-1	7.4-9.3 (8.0)	1-2	7.4-8.7 (7.9)	1-2
T.4	11.8-15.5	$\frac{1}{2}$-1	13.6-14.9 (14.3)	$\frac{1}{2}$-1	Dies		12.4-14.9 (13.4)	$\frac{1}{2}$-$1\frac{1}{4}$
A.1	4.3-6.8 (5.4)	$\frac{3}{4}$-$1\frac{1}{2}$	5.0-6.2 (5.3)	1-2	4.3-5.0 (4.6)	$1\frac{1}{2}$-3	4.3-6.2 (5.1)	$1\frac{1}{2}$-3
A.2	3.7-4.3 (4.1)	2-9	4.3-5.0 (4.9)	1-2	4.3-5.0 (4.8)	1-$2\frac{1}{2}$	4.3-5.0 (4.8)	1-3
A.3	4.3-5.6 (4.8)	1-4	4.3-5.6 (4.8)	1-4	3.7-4.3 (4.0)	$1\frac{1}{2}$-4	3.7-5.0 (4.0)	$1\frac{1}{2}$-4
A.4	5.0-7.4 (5.7)	1-2	5.0-6.2 (5.3)	$1\frac{1}{2}$-4	4.3-5.6 (4.8)	$1\frac{1}{2}$-4	5.0-6.2 (5.3)	$1\frac{1}{2}$-4
R.1a	6.2-7.4 (7.2)	1-2	7.4-8.7 (7.9)	$\frac{3}{4}$-2	5.6-7.4 (6.8)	$1\frac{1}{2}$-3	6.2-7.4 (7.2)	$1\frac{1}{2}$-3
R.1b.	6.2-7.4 (6.8)	1-2	7.4-8.7 (8.1)	$\frac{3}{4}$-2	6.2-7.4 (7.2)	$1\frac{1}{2}$-3	6.2-7.4 (7.2)	$1\frac{1}{2}$-3
R.1c	6.2-7.4 (6.8)	1-2	7.4-8.7 (7.9)	$\frac{3}{4}$-2	6.2-7.4 (7.2)	$1\frac{1}{2}$-3	6.2-7.4 (7.2)	$1\frac{1}{2}$-3
R.2	7.4-12.4 (9.6)	$\frac{1}{2}$-1	9.3-11.2 (10-1)	$\frac{1}{2}$-$1\frac{1}{2}$	6.8-8.7 (7.9)	$2\frac{1}{3}$-2	6.8-8.7 (7.9)	$2\frac{1}{3}$-2
R.3	5.0-6.2 (5.5)	1-2	5.0-6.2 (5.5)	1-2	-		-	
R.4	10-12.4	1-2	10-12.4	1-2	-		-	
R.5	14.9-18.6	1-2	14.9-16.6	1-2	-		-	

Examination of the table shows that there was no one solution which gave the same final width and length of cells as found in nature.

Most clones when grown in Soil solution remained of the same general width and length as under natural conditions but some showed differences and all showed restriction in the range of width. The clones showing noticeable changes in width were A.2, R.1, and R.2.

Most clones grown in Knops solution showed narrower and longer cells than those found in culture in soil solution or under natural conditions.

All the terrestrial algae (T.1, T.2, T.3, T.4,) and all the aquatic algae except A.2 (A.1, A.3, A.4, R.1, R.2,) showed this smaller size in Knops solution.

Similar results were obtained when clones were grown in Molischs solution that is the cells were longer and narrower than those of clones grown in Soil solution or of algae under natural conditions.

From results confined to these three solutions only, it appears that the solutions of known composition prepared from mineral salts give smaller plants. Other nutrient solutions were used for certain of the clones and it was found that they did not necessarily affect the clones in the same way or affect all clones equally. Histogram 3 shows results for clones T.1, T.2, T.3, T.4, in the three

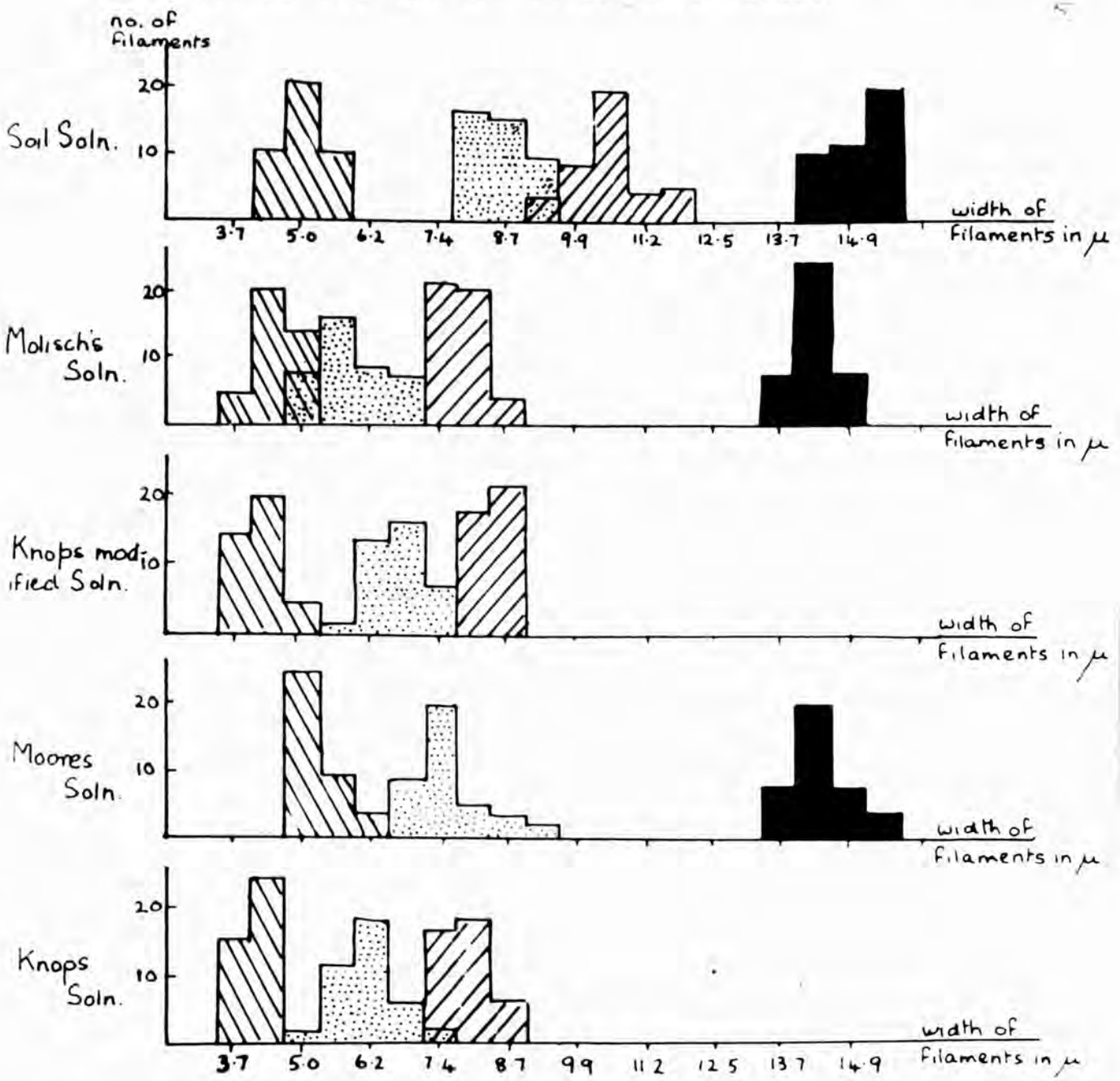
solutions already mentioned and in Moores Solution and Knops modified solution.

In the histogram measurements for algae originally growing together are plotted on the same scale so that the extent to which the size in different forms coincides can be easily seen. It will be exceedingly difficult to separate T.1, T.2, T.3, when they are growing together using size as the main character.

Histogram 5.

Measurements of width of cells filaments in clones T1, T2, T3, and T4 grown in various solutions.

Measurements for the different clones in one solution are plotted on the same horizontal scale to indicate how size of the clones overlaps.
Measurements for different culture solutions are plotted directly above one another to show the change in size that occurs for any one particular clone.



All results indicated that the nature of the culture solution affects the cell measurements, that no one solution will give the size found under natural conditions for all algae, that some solutions affect the size of all the clones in one general direction (e.g making them all smaller than in nature) and that some affect the different clones in different ways.

FORMATION OF A SILKY FILM.

This is an important character for separating some species of Hormidium.

A. METHOD.

The presence or obsence of a silky film was recorded for material growing in different culture solutions.

B. OBSERVATIONS.

General Description.

The term "silky film" is applied in this investigation to a layer of floating filaments lying closely side by side in swirling lines. The whole film when viewed at an angle is glistening (rather like moire silk). This is distinct from knots of floating filaments frequently held at the surface by bubbles of oxygen in a rapidly photosynthesising culture and also from the film of young germlings which may form at the surface of the medium in culture of Uronema species. These latter two films never appear silky with

a characteristic lustre.

Effect of culture solutions on the formation of a silky film.

Only certain of the clonal cultures showed a silky film whatever the solution used. Certain clonal cultures forming a silky film in one solution did not form a silky film when other solutions were used. Table 8 records the behaviour of clonal cultures in three solutions and of certain non-clonal cultures in Soil solution. The time interval before the formation of a silky film is also included. The time taken for the film to appear varies in various clones. It must be noted that the interval is not for one culture but for a series of subcultures of the one algal clone. This may slow down film formation but since a "massive" sample was transferred, once a film was present it invariably occurred in the subculture. The time taken for a film to appear in clonal cultures was never less than eight weeks but these cultures were started from a single filament and were initially slow in starting growth and for several weeks after establishment remained as a single short submerged filament. Non-clonal cultures of large samples of material (although grown in larger quantities of nutrient solution) quickly formed a silky film

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TABLE 6.

Effect of the culture solution on the formation of a silky film.

Clone	In Soil Soln. P - Present A - Absent	Time in mths.	In Molisch soln.	Time in mths.	In Knops soln.	Time in mths	Width of filament in silky film.
T.1.	A		A		A		-
T.2.	P	12	A		A		5.0 -8.7μ
T.3	A		A		A		-
T.4	A		A		A		-
A.1	P	9	P	5	P	6	5.6 - 6.2μ
A.2	A		P	14	P	6	4.3 - 6.2μ
A.3	P	12	P	6	P	6	5.0u
A.4	A		A		P	10	5.0 - 7.4μ
R.1	A		A		A		-
R.2	A		A		A		-
R.3	P	6	-		-		5.0 - 6.2μ
R.4	A		A		A		-
R.5	A		A		A		-
E.1	A		-		A		-
E.2	P	2	P	2	P	2	5.0 - 6.2μ
H. Flaccidum (C)	P	1	P	1	P	1	5.0 - 6.2μ
H.nitens (C)	A		A		A		-
U. subtilissima (C)	P	3	A		-		5.0 - 6.2μ
Nonclonal Cultures							
R.3	P	½	-		-		5.0 - 6.2μ
T1.+ T2.+ T3.+ T4.	P	½	-		-		6.2 - 8.7μ

The algae forming a silky film at least in certain culture solutions were one terrestrial alga (T.2) the four aquatic algae of still water (A1, A2, A3, A4,) the subaerial alga (E.2) and one alga of swift flowing water (R.3). All were of small size but differ slightly in width of the filaments. All had short filaments. A2 and A4 although forming a silky film in certain media did not do so in Soil Solution. These two algae differed from the other clones in their fragmentation behaviour in this solution, A4 having long filaments, A2 single or few celled fragments.

Confirmation of Results.

Certain clones formed a silky film in only certain nutrient solutions and the time for the formation was several weeks. In view of these unexpected results and to prove that the silky film was not an aerial contaminant or a mutant the following experiments were carried out:-

1. Transfer experiments.

a. The silky films formed in Molisch's and Knop's solutions by clones A2 and A4 were transferred to Soil Solution.

b. Submerged filaments of A2 and A4 grown in Soil Solution were transferred to Molisch's and Knop's solutions.

c. The silky film formed in Soil Solution by T2 was transferred to Molisch's and Knop's solutions.

d The submerged filaments formed by T2 in Molisch's and Knop's solutions were transferred to Soil Solution.

2. Exposure experiments.

A series of vessels previously used for algal cultures were sterilised the normal way and sterile medium inserted in the same way as for normal algal cultures. Four dishes for each of the nutrient solutions - Soil Solution, Molisch's and Knop's were prepared and exposed in the laboratory in the following way -

a. Two dishes of each solution exposed for one hour.

b. Two dishes of each solution exposed continually.

The exposed vessels were examined after two and four weeks.

The transfer experiments gave results entirely consistent with those recorded in Table 8. That is a silky film appeared in the transfers b and d while the silky film was lost, submerged filaments being formed, in the transfers a and c.

In the exposure experiments the evidence was against aerial contamination. No silky film formed in any of the exposed solutions. Contaminents were found particularly Chlorella sp., Penicillium spp., and other fungi and bacteria but no filamentous algae were ever found.

CONSTRICTION OF THE FILAMENTS.

This is regarded as a minor specific character although it is the chief character used on separating Ulothrix moniliformis from other Ulothrix species of similar width e.g. U.aequalis.

A. METHOD.

The presence (in more than 30% of the filaments) or absence of constriction of the filaments at the cross septa was recorded in filaments selected as when making cell width measurements (See p. 56). The extent of constriction was recorded by measuring the width of the cells at the cross septa and midway between the cross septa.

B. OBSERVATIONS

General Description.

In constricted filaments the cells appeared barrel shaped and wider at their mid-point. The percentage of filaments constricted was normally about 50% of the total, wider filaments being constricted. Thus in A4 the results given below were obtained:-

Width of filaments.	Percentage of filaments of this width in a sample	Percentage of filaments of this width which are constricted.	
5.0μ	34%	30	) Total
5.6μ	28%	36	) percentage
6.2μ	20%	60	) of filaments
6.8μ	6%	66	) in a sample
7.4μ	12%	100	) which are
			) constricted

Figure 5 shows the extent of constriction in the clones A1, T.3, T.2, R.2. A4 is shown in figure 1. The average difference (for 25 measurements) in the width of the cross section mid midway between cross-walls was 1.2μ this being approximately 15% wider at the centre of the cell than at the cross-septa.

Effects of culture solutions on constriction of the filaments.

Presence or absence of constriction in the filaments of both clonal ~~and non-clonal~~ isolates grown in 3 nutrient solutions is recorded in Table 9.

TABLE 9

Constriction of the filaments.

Clone	Soil soln.	Knop's soln.	Molisch's soln.
T.1	absent	absent	absent
T.2	present	absent	absent
T.3	present	absent	absent
T.4	absent	------	absent
A.1	present	absent	absent
A.2	absent	absent	absent
A.3	absent	absent	absent
A.4	absent	absent	absent
R.1	absent	absent	absent
R.2	absent	absent	absent
R.3	present	absent	absent
R.4	absent	------	-------
R.5	absent	------	-------
E.1	absent	absent	-------
E.2	absent	absent	absent

Constriction of the filaments was not a common or constant character of the alga studied. Only four clones E.2, T.3, A.1, and R.3 had constricted filaments and these clones only

showed constriction of the filaments when grown in sulture in Soil solution. None of these algae had constricted filaments when growing under natural conditions, only A.4 showed constricted filaments at the time of collection.

Further investigation of the effect of the nutrient solution on the shape of the cells was carried out using A.3 in non clonal cultures in Benecke's, Bjeirinck's Godwards, Hervey's, Knop's, Molisch's and Pringsheim's solutions. Constriction of the filaments was once again not a constant character and was present only in Bjeirinck's and Goward's solutions. (The algae died in Benecke's solution.)

THICKENING OF THE CELL WALL.

This is an important specific character in that certain species eg. Hormidium mucosum Boy. Pet. and H.crenulatum Kutz. are mainly characterised by their cellwall. It is not however a generally useful character it's use being restricted to the differentiating of a few species.

A. METHOD

Records were made of the presence or absence and type of thickening in the algae. Microchemical tests were carried out after the method of Woodhead and Jane (1941). Methylene blue, Schultze's solution, and Ruthenium red were employed, with inclusive results. Such tests on small algae are often unsatisfactory because it is difficult to see the slight colour changes which occur so that negative results mean little.

B. OBSERVATIONS.

General description.

Wall thickening was of three types.

1. Filaments with 2 layered wall and localized thickenings (H pieces).

Filaments showed an outer layer of translucent colourless appearance and variable thickness. The inner wall of normal appearance, was of constant width. Localized thickenings

of the cross septa, the "H" pieces described by Woodhead and Jane and others, were rather regularly arranged. Thus in a particular filament of clone T.3 in Soil solution the number of cells between the H pieces were successively 10, 6, 4, 6, 13, 9, 7, 3, 5, 4, 4, 4, 4, 6, 10, 16, 4, 4, 6, Inclusive results were obtained when micro chemical tests were employed. The outer wall and "H" pieces stained blue in Methylene blue and the inner wall stained indistinctly purple in Chlorezinc-iodide (Schultze's soln) and in some cases Ruthenium Red stained the outer wall slightly as if a pellicle were present. This does not conflict with the results of Woodhead and Jane who concluded that the "H" pieces and outer wall were not cellulose but mucilaginous and similar to one another.

2. GENERAL THICKENING OF THE WALL, WITHOUT LAYERS BUT WITH "H" PIECES.

Generally thickened walls in which an outer layer was not discernible were also found. Small H pieces of similar nature to those described above also occurred at infrequent intervals. It was impossible to decide whether this was distinct from the thickening described above or merely represented a poorer development of a similar thickening.

3. Irregular thickening of the cell wall with "H" pieces. Irregular thickening of the cell wall accompanied by

regularly occuring "H" pieces both not translucent but brown and almost opaque were found in certain clones. The thickening, which was irregular wrinkled patches, and the H pieces both have a similar appearance to the brown basal attaching discs found in certain algae (eg Uronema). The colour and opacity prevented satisfactory microchemical tests.

The effect of the culture solution on the wall thickening. The Table 10 summarizes the results for the clones studied. The number 1, 2, or 3 indicates thickening of the type described above under these numbers.

TABLE 10.

The effect of the Culture Solution on wall thickness.

Clone.	Type of wall under various conditions			
	Natural.	Cultural Soil Soln.	Cultural Knop's Soln.	Cultural Molisch's Soln.
N.1	Thin	Thin	Thin	Thin
N.2	Some type 2 but most thin.	50% thickened, most type 2 some type 1	Thin	Thin
N.3		All thickened, most type 2 some type 1	Thin	Thin
N.4	Type 1	Type 1	-	Type 1
A.1	Thin	Thin	Thin	Thin
A.2	Thin	Thin	Thin	Thin
A.3	Thin	Thin	Thin	Thin
A.4	Thin	Thin	Thin	Thin
N.1	Moderately thick	Thin	Thin	Thin
N.2	Thin	Thin	Thin	Thin
T.1	Thin	Thin	Thin	Thin
N.2	Most thin, some type 1	Most type 2, some type 1	Thin	Thin
R.3	Type 3	Type 3	-	-
R.4	Thin	Mostly type 2	-	-
R.5	Type 1	Type 1	-	-
H. flaccidum (C)	-	Thin	Thin	Thin
H. nitens (C)	-	Thin	Thin	Thin
U. subtilissima- (C)		Thin	Thin	Thin

(A blank (-) indicates that the clone was not examined under these conditions.)

Thickening of the cell wall was not a constant or common character in the algae studied. Only six clones showed thickening of any kind in culture. Of these only T.4 had thick walls in all three solutions investigated, and the thickness of the outer wall varied being greatest in soil solution. R.5 which seemed to be a similar alga to T.4 was only grown in Soil solution.

Further investigation of the effect of the culture solution on wall thickness.

Clone T.4 was grown in a further series of nutrient media - Hervey's modified, and Moores solution and in Soil solution with added dextrose, or yeast extract. Figure 7 . shows the extent of the wall thickening in representative filaments. The outer wall was very variable in thickness reaching its maximum extent in cultures in Soil solution with added dextrose or yeast extract. These results further supported the view that although the ability to form a thickened wall may be a character restricted to some species the actual thickness of the wall is not a constant character.

V. DISCUSSION.

DISCUSSION.

The present use of reproduction and morphological characters in the taxonomy of Hormidium and its allies has been summarized in the historical survey. Reproduction by motile cells, an important generic character, could not be studied further. Motile reproductive cells were not obtained in most clones. In the absence of motile cells all stages in the normal life history may not be obtained and the work on the variation in morphological characters is consequently open to the criticism that the full range in variation, even within the limited environmental conditions was not obtained. Failure to obtain reproduction using the classical methods has been commented on frequently by other phycologists and the absence of it in this investigation was not unexpected. Failure to obtain reproduction frequently casts doubt on the separation and identification of Stichococcus on the basis of absence of motile cells.

Although separation of the genera by features of reproduction could not be studied, clear cut differentiation between Uronema and other clones was possible using morphological characters. In culture the former clones retained their clear cut character of pointed apices and specialised basal cells and normally reproduced by motile cells. No other alga developed true Uronema apices.

The shrivelled ends of broken filaments (Brand 1913) could never have been confused with Uronema. These results contradict the suggestion that Uronema is a mere stage in the development of ordinary Ulothrix species (Gaidukov 1903) I would support Mitras view that Uronema is a distinct genus.

The use of other morphological characters, commonly used in both generic and specific descriptions, was less reliable. Variation in the chloroplast was observed when different culture solutions were used. Similar variation with habitat has been commented on frequently yet emphasis is still placed on the size and shape of the chloroplast in many descriptions of Hormidium and Ulothrix. It seems to me that not only should richness or poverty of the habitat be taken into account when assessing the significance of the chloroplast but that the relations of the length ~~of~~ to the width of the cells should be considered. The chloroplast is often described as small when it reaches only part of the length of the cell yet in a long cell its size may be the same as that of a chloroplast reaching the entire length of a short cell. Similarly amount of fragmentation has been shown to vary with the nature of the culture solution. It is possibly related to some extent to lack of nutrients. Absence of fragmentation in distilled water may well be

explained by the complete cessation of growth. The descriptions of earlier workers could generally be applied to the clones studied but accumulations of material of the type described by Piercy, although possibly present, were not associated with fragmentation, neither do I consider that Lunds description of Hormidium mucosum explains Petersens statement on projection of cell walls. Both broken cellulose membranes and projecting outer walls left after the separation of the middle lamellae may occur although only one may be observed by one worker.

Considerable changes in the distinctness of pyrenoids were observed. The pyrenoids were clearly visible in cultures and this resulted in the detection of a second pyrenoid in cells of clone R.5 and of one pyrenoid per cell in the alga sent by Cambridge as Hormidium nitens. The alga from Cambridge was identified as Stichococcus bacillaris Naegeli when received but in later cultures the presence of one pyrenoid per cell could only be reconciled with identification as Hormidium pseudostichococcus Heering. It seems that the presence of a discernable pyrenoid depends to some extent on nutrition and the use of absence of pyrenoids for separating off a genus is unreliable. Further investigation of the nature and constancy of pyrenoids is needed before deciding whether the character can justifiably be used to separate Stichococcus and Hormidium. Establishment

of the fact that changes in the number of visible pyrenoids do occur is useful in that it partly justifies the practice of discounting differences in pyrenoid number which has been followed in some investigations of Ulothrix species. The variation found in width and length of cells, though slight, is important since the identification of species is commonly made with artificial keys based on these measurements. Variation in size in this group has only been discussed by Chantesco and Peterfi (1934). They found that they could use size taxonomically in Stichococcus as it was possible to distinguish four groups based on the relation of length to breadth. In the Hormidium species studied a similar grouping was not always possible because of variations in measurements for algae grown in culture. Thus A.2 had cells 10 times as long as broad when collected but only slightly longer than broad when grown in Soil solution. It is clear at least in Hormidium that the length of the cell is not always reliable as a taxonomic character of a species and it seems to me that its value generally is likely to be rather limited.

The use of special characters shown in culture is confined to Hormidium and Stichococcus and only one such character, the formation of a silky film, was studied. The results described show that the ability to form a silky film may be more widespread than has been previously

suggested and, that, since several dissimilar clones formed films and behaved differently in various solutions, it is an unreliable character. If formation of a silky film is considered A.3, A.4, R.3, and A.1, would all have been identified as Hormidium nitens although they differed from one another in other respects. The film did not consist of germinating zoospores (Chodat and Meering 1914) and it seems that in particular culture solutions certain clones from filaments which can float in that medium. The characters taxonomic value seems doubtful and it will be unreliable until further investigated.

Other characters investigated are of limited taxonomic use. The occurrence of Barrel shaped cells (ie constricted filaments) in the vegetative state as well as when about to reproduce is considered characteristic of Ulothrix moniliformis Since constricted filaments were found in the vegetative filaments of clones which could not have been identified as Ulothrix moniliformis this character cannot be entirely reliable. Kneebends (using this term in the restricted sense to describe angles formed by firmly attached as opposed to loosely attached or apposed cells) were found only in algae collected from swift flowing water. The kneebends and rhizoid like outgrowths were substantially lost in culture. It has been suggested that rhizoids are

produced as a result of irritation by the substrata. These results would support this. The real assessment of the taxonomic value could not be made but it did appear that algae collected from swift flowing water soon closely resembled algae of similar size from other habitats when they were cultured under similar conditions. General thickening of the cell wall and localized thickenings or H pieces have also been recorded for a limited number of species but is important in distinguishing between H. mucosum and H. crenulatum. In this investigation wall thickening and the occurrence of H pieces were relatively common. The variability of wall thickness was considerable and H pieces and wall thickness do not appear to be generally useful taxonomically. In the separation of H.mucosum and H. crenulatum this investigation may lead to further confusion in that T.4 had a two layered wall yet did not give the reaction expected of H. mucosum, with chlor-zinc-iodide.

The figures given by Lund, and Fritsch and John differ so little that doubt exists in my mind as to their separate existence of these two species. Puymaly (1924 - not seen) is reported as having established that H. mucosum is a dry soil form of H.flaccidum. In this investigation the thickening of walls was greater in liquid culture than in collections of

algae from damp soil surfaces and it seems unlikely that dryness alone causes this form. The reason for wall thickening was not investigated but it is interesting to note that Livingstone (1900) attributes differences in thickness of mucilage and wall thic ness in palmelloid Stigeoclonium to differences in the osmotic pressure of his culture media. The formation of H pieces was not investigated and no explanation is commonly advanced. Heerings statement that in Ulothrix mucosa four or eight daughter cells lie between the cross septa of the old filament seems to have been largely overlooked. The general appearance of clones studied in this investigation is that which would be expected if H pieces are the remnants of old long established cell walls. Evidence has been advanced in support of Priestley and Scotts view, that in higher plants, daughter cells secrete their own cell wall and remain within the parent one (Elliot 1891 and Wardrop 1952) and in view of this, further investigation of the formation of H pieces might yield interesting results.

The present investigation has not led to results which supports the current use of morphological characters in separating the genera and species of the group of Hormidium allies studied. Indeed the results may be said to show that the criteria used are much less safe than the authors using

them had realised. I do not go as far as to state that the characters used are valueless but that they are at present unsafe and their use must continue to lead to conflict and mistake. I consider that much more investigation of these characters under experimental conditions is needed and I think that a return of the clone culture to the wild conditions might sometimes lead to valuable results.

The present work has shown that culture solutions of different composition produce growths of considerably different character. The possibility of varying culture solutions is endless and at present we know very little of the principles involved.

VI. SUMMARY.

SUMMARY.

Clones were isolated from eighteen collections of alga from various types of habitat. Thirtyseven clones were studied as representatives of different species. These clones were not all different from one another and did not necessarily represent true species but at least twelve distinct forms were present.

The clones were grown under similar light and temperature conditions but parallel cultures were grown in different nutrient solutions and in a few instances with other factors, such as aeration, varied. Observations were made on the effect of these various conditions on the behaviour of the algae in an attempt to investigate the reliability of characters commonly used to distinguish between Hormidium, Ulothrix, Uronema and related genera and between the species of these genera. These characters are considered separately.

1. Reproduction

Clones which clearly were, by their other features, Uronema species readily produced motile spores but in clones of Ulothrix and Hormidium asexual and sexual reproduction by motile spores was not obtained sufficiently frequently for observation and often not at all. The common method of reproduction in these clones was by fragmentation. This failure to obtain reproduction readily by motile cells raises

doubts as to the value, at least in practice if not on theory, of differentiating between Hormidium and Stichococcus on the basis of presence or absence of motile reproductive cells.

2. Terminal and basal cells.

Certain clones had acuminate terminal cells and basal cells with attaching discs throughout the entire period of culture and thus showed the essential characters of Uronema species. The Uronema species stand out as a satisfactory group and the shape of the terminal cell is a satisfactory character for identifying the genus.

3. Chloroplast size and shape.

The chloroplast was always a parietal plate but in all the clones studied the interpretation of the shape (ie an elliptical or circular plate, or collar-shaped) depended on its size in relation to the cell. The size relative to the cell length proved a most unreliable character and the filament from the wild with notably small chloroplasts formed large ones in culture. Chloroplast size and shape appeared to depend entirely on nutrition.

4. Fragmentation.

Fragmentation was more characteristic of some clones than others but the extent of it was highly dependent on the culture solution. Fragmentation did not appear to be

associated with wall thickening or with accumulations of any special material. Aeration did not affect fragmentation. Culture of clones shows that the average length of filaments or frequency of fragmentation depends on the composition of the nutrient solution. In experiments with one clone (A.4) fragmentation occurred freely when the nutrient solution had a low phosphate concentration, no iron or high sulphate concentration. Fragmentation occurring under the unknown conditions of nature has been used as a specific and even as a generic character. This would clearly be safer if regard were paid to nutrient supply.

5. Pyrenoids.

The development of clearly visible pyrenoids depended on nutrition. They were clearer in the better nourished flourishing algae and were generally clearer in culture. All the clones showed a clear pyrenoid in culture but not all did so when collected. Culture made the detection of two pyrenoids per cell possible in one clone.

The character of absence of pyrenoids for separating the genus Stichococcus from Hormidium is of doubtful value since clearly visible pyrenoids may be absent at the time of collection of the alga although detectable in culture. The results are perhaps taxonomically helpful in that differences in pryenoid number may be partly discounted in

determining species, a practice which has been followed in some investigations of Ulothrix species.

6. Cell Measurements

When a clone was grown in different culture solutions the mean length and width of the cells (and also the extreme measurements) gradually differed and each solution finally produced a steady mean. No one culture solution was discovered which would provide for all clones, cells of the same size as they had at the time they were collected. The alteration of final mean in different culture solutions was sometimes as much as + or - 20%. Since the mean size is greatly used in discriminating between species I regard differences of less than this with suspicion.

7. Silky Film.

The formation of silky film was characteristic of certain clones in certain solutions only. Some clones form silky films in every solution used while others formed one in a particular solution only. Formation of a silky film is a useful character if considered in conjunction with the culture solution. Its use as a specific character is limited to a few species and its validity and value will depend on whether its formation is also limited to these species.

8. Constriction of the Filaments

Only a few clones ever had constricted filaments. Constricted filaments in those clones were not found under all conditions. Thus certain clones were constricted in Soil solution but not in other culture solutions or in the wild, another clone which had constricted filaments in the wild lost constrictions in all the cultures established.

The presence of constrictions is used as a distinguishing character for a few species. The present work shows that its use is unreliable unless the conditions of life are taken into account.

9. Wall thickness

Wall thickness varied between different clones but depended on the nature of the culture solution. The presence of H pieces proved a stable feature in certain clones but in other clones were variably developed and did not occur under all conditions. H pieces were only found in culture in clones in which at least some collections from the wild showed H pieces. Although wall thickness and the H pieces were variable in development, the ability to form them may be a valid specific character. The investigation did not show the conditions necessary for their greatest development but the development of H. pieces in liquid culture discounts the idea that has been put forward that they are caused by dryness alone.

This investigation for all its negative results, does support the validity of Uronema. The species investigated retained their special characters under all conditions under which they were studied. Further no other alga, however cultured assumed the Uronema character. On the other hand the lack of precision which marks the boundaries of the genera Stichococcus, Hormidium, and Ulothrix is emphasized for by suitable culture many algae can be pushed over the boundaries.

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APPENDIX 1.

MAIN CHARACTERS OF HORMIDIUM AND ULOTHRIX SPECIES (OF SMALL FILAMENT WIDTH) AS GIVEN BY MAIN AUTHORS MENTIONED IN INTRODUCTION.

1. Hormidium pseudostichococcus (Naegeli) Heering (probably synonymous with Stichococcus bacillaris Naegeli)
2. Hormidium subtile (Kutz) Heering. (probably synonymous with Stichococcus subtilis (Kutz) Klercker and Ulothrix subtilis Kutz.)
3. Hormidium rivulare Kutz. (synonymous with Stichococcus rivularis (Kutz) Hazen)
4. Hormidium fluitans (Gay) Heering (= Stichococcus fluitans Gay)
5. Hormidium flaccidum
   Descriptions under H.flaccidum A.Br. sensu ampl.
   H.flaccidum A.Br. sensu strict.
   Stichococcus flaccidum (Kutz) Gay
6. Hormidium nitens Menegh emend Klebs (= Stichococcus nitens as described by Bristol)
7. Hormidium crassum. Chodat
8. Hormidium dissectum. Chodat
9. Hormidium lubricum Chodat
10. Hormidium mucosum Boy.let.
11. Hormidium crenulatum Kütz. (= Hormidiopsis crenulata (Kutz) Heering)
12. Hormidium Klebsii G. M. Smith
13. Stichococcus scopulinus Hazen. (= Gloeotila scopulina (Hazen) Heering. As Stichococcus Hazen includes Hormidium Kütz and the alga is stated to have a pyrenoid it may be a Hormidium species)
14. Ulothrix subtilissima Rabenh.
15. Ulothrix variabilis Kutz. (= U.subtilis var variabilis Kirchner but Bristol probably uses this name for Hormidium flaccidum)
16. Ulothrix tenerrima Kutz.
17. Ulothrix moniliformis Kutz.
18. Ulothrix subconstricta West.
19. Ulothrix rorida Thuret.
20. Ulothrix tenuissima Kutz.
21. Ulothrix oscillarina Kutz.

1. Hormidium pseudostichococcus. (Naegeli) Heering

AUTHOR & Name given to algae	HABITAT & FORM	SIZE	MUCUS	HABIT SIZE CELL SHAPE	CHROMATOPHORE	CHROMATOPHORE	OTHER FEATURES
Collins S.bacillaris Naegeli	Damp ground, rocks, flower pots. Crisped and floccose masses	2.5-3μ x 1-4 times as long	-	2-4 cells long.	-	Elliptical thin & pale	Forms confervoidea has long filaments
Prescott. S.bacillaris.	Most aerial substrates.	2-.5μ wide x 3-8μ long.	-	Filaments slightly constricted	-	Pale green Parietal plate or folded disc covering a small portion of the wall.	Cells loosely connected
Hazen S.bacillaris	Fine short filaments (2-24 cells) Damp earth rocks, flower pots etc.	2.5-3μ wide x 1-4 times as long	-	Cylindrical cells but slightly constricted at ends.	-	Elliptical thin & pale	Very readily disintegrating
Heering.	Green coating to wet walls, trees etc. Longer filaments in water	2.5-3μ x 1-4 times as long (Occasionally 4½)	Very delicate	Single cells or short filaments, slightly constricted	very pale pyrenoid	Pale yellow green. Elliptical to circular	Easily disintegrating single cells become ellipsoidal finally 3-4u wide by 2-1 times as long
Bristol. S.bacillaris	Very short filaments - not more than 3 cells Soil.	2.2.5μ x 6-9μ long.	-	1-3 celled	No pyrenoid	Parietal, covering about half surface of cell.	
Grintzesco & Peterfi. S.bacillaris.		2.5-3μ wide by 5-12u long	-	Mostly one celled or loosely united in 3 etc.	No pyrenoid	Dark green	Great tendency to fragment

2. Hormidium subtile (Kutz) Heering. (Blanks indicate no mention of character in author's description)

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOID	CHROMATOPHORE	OTHER FEATURES
Heering	Slippery tufts in dripping water, waterfalls running water, at pumps. Interwoven mass of filaments in standing water	5-7μ by 1½-3 times as long	Delicate	Long filaments	Distinct but small, one per cell.	Elliptical or circular	Rarely disintegrates strongly. Akinetes 7x9μ. Zoospores seldom observed
Hazen Stichococcus subtilis (Kutz) Klercker.	Extended bright green lubricous masses. On moist cliffs, rocks in cascades, watering troughs, quiet waters - all the year round	5-6.5(8)μ by 1-3 times as long.	Thin	Long filaments Not constricted	Rather small one	Elliptical	Cells break up for vegetative reproduction less readily than in other Stichococcus species. Zoospores formed freely at times - not in very weather.
Collins. S. subtilis (Kutz) Klercker	On rocks in running water, water-troughs and pools Extensive bright green lubricous masses. Mainly in spring	5-6.5 (8) μ x 1-3 times as long	Thin	Long cylindrical filaments	Small, one.	Elliptical	Liable to decay to fragment.
Prescott S. subtilis (Kutz.) Klercker	In shallow water	5-7 (8)μ wide by 7-20μ long	-	Filaments long or short, cylindrical cells not constricted	One pyrenoid	Elliptical parietal plate	
Bristol Ulothrix subtilis (Kutz)	In soil	4-5μ wide by 1 times as long	-	Short filaments of 12 or more cells.	-	-	-

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3. Hormidium rivulare Kutz.

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOID	CHLOROPLAST	CELL DIVISION.
Collins S. rivularis (Kutz) Hazen	On rocks and earth, in rapid streams, Bright green tufts	8-11μ wide by 1-2 times as long	-	Filament of few cells. G frequently geniculate Rhizoidal branches Cells somewhat swollen.	One pyrenoid per cell large.	Orbicular to rhomboidal clearly marked.	Not easily breaking up.
Hazen S. rivularis (Kutz) Heering.	Elongated bright green tufts. On rock and earth in rapids of grassy meadow streams	8-11μ wide by 1-2 times as long	Rather thick walled	Filaments of 1-3 cells developing rhizoidal hooks from the terminal cell and from those of the knees. Somewhat constricted cells.	One large pyrenoid	Orbicular to elliptical or rhomboidal with clear outline.	Not easily breaking up. Zoospores not formed freely and only empty cells seen.
Heering H. rivulare Kutz.	In strongly flowing water. Bright green submerged tufts	4-11μ wide by 1-3 times as long	Relatively thick.	Long filaments often with kneebends. Rhizoid like formations from the end cell and knee cells. Somewhat constricted.	One clear pyrenoid	Circular to elliptical	Not easily disintegrating

4. Hormidium fluitans (Gay) Heering

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	FIRST CELL	CHLOROPLAST	LIFE FEATURES
Collins S.fluitans Gay	Yellowish green crisped and interwoven on smooth rocks swept by rapid water from cascades.	6.5-9μ by 1-3 times as long.	-	Crisped and somewhat geniculate	Inconspicuous nearly concealed by chromatophore	Large and thick	Strong tendency to break up in very short time when removed to quiet water.
Hazen S.fluitans Gay	Yellowish green often crisped and inter-woven filaments torulose some-times geniculate. In cascades, on oblique surfaces of rocks - sprayed or with film of water.	6.5-9μ by 1-3 times as long.	-	Crisped or torulose sometimes geniculate cells slightly constricted	Dull one.	Large and opaque	Very readily breaks up into single cells. Reproduction by zoospores infrequent.
Heering H.fluitans	Short or many celled filaments. Short yellow green turf in spray from waterfalls or in irrigated positions.	6.5-9μ by 1-3 times as long.	-	Cells usually slightly barrel shaped.	-	-	Easily disintegrates Zoospore formation seldom.

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5. Hormidium flaccidum

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOID	CHLOROPLAST	OTHER CHARACTERS
Hazen *S.flaccidus* (Kutz) Gay	Short filaments forming floccose masses or interwoven strata on wet rocks and bark of trees.	6-9.5u (occasionally 6-14u) by $\frac{1}{4}$-2 times as long.	Thicker than in *S.subtilis*	Filaments short. Cells generally somewhat bunid.	-	-	Reproduction by zoospores fre uent.
Collins *S.flaccidus*	Filaments short forming floccose or interwoven masses. Wet rocks soil, bark of trees.	6-9.5u by $\frac{1}{4}$-1 times as long occasionally 2 x as long.	Cell wall fairly thick.	Cells somewhat swollen.	One large pyrenoid per cell.	Broad.	
Piercy *H.flaccidum* forma aquatica	On soil	9-13u wide x 2/3-2$\frac{1}{2}$ times as long.	-	Long filaments (about 1,400 cells)	-	late shaped bright green covers total length of cell and about 2/3 circumference curved outline to one or both longitudinal cages	Forms aplanospores

5. Hormidium flaccidum contd.

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	PLASTID SIZE & CELL SHAPE	PYRENOID	ZYGOTE	OTHER FEATURES
Heering H.flaccidum A.Br. sensu ampl.	On ice, standing water, dripping water, running water, damp places, trees.	5-14u x 1-3 times as long ($\frac{1}{4}$-$\frac{1}{2}$)			Large Distinct pyrenoid	Bi-.	Zoospores. Aplanospores. Gametes. Disintegration of cell.
H.flaccidum A.Br. Sensu strict.		6-14 x $\frac{1}{4}$-3 times as long	-	Four ribs. Sometimes incised at the cross walls.	One		
forma typica (5-9u) α	Meereseis & Norway (Wille)	5 - 9 by 1-3	-		One		Basal cell acuminate. Gametes 1 or 2 in the cell.
β	Mucilaginous masses on rocks Norway (Wille)	6-9 x 1-2 ($\frac{1}{4}$-$\frac{1}{2}$)	-		One		Vegetative cells aplanospores gametes and hypnetine stage - Trochiscia like
δ		6.5-8 x	-	Loose spirals winding and twisting round one another in culture.	One		
formatumida	short filaments Wet rocks, trees ? = S.flaccidus	6-9.5 x $\frac{1}{4}$-2	Relatively thick	Somewhat swollen	One pyrenoid	Rare	Zoospores or gametes Resembles H. flaccatum.

5 Hormidium flaccidum contd.

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	CHROMATOPHORES	OTHER CONTENTS	OTHER FEATURES
forma montana (Hormiscia flaccida var montana Hansgirg)		8-14μ by 1-1½ (½-2) times as long	Glutinous adhering particles	-	-	Broadish flat yellowish green	
forma aquatica	Long filaments in standing water	10-14μ x 1½-2	delicate	-	Weakly visible	Light green.	

6Y. Hormidium nitens Menegh. [illegible] [illegible]

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOID	CHROMATOPHORE	REPRODUCTION
Heering	Filaments may be very long (20 cms) Green film on stones, walls, flower pots etc.	5.5-7µ by 1-3 times as long			One		Zoospores and resting akinete [illegible] formed form a silky film, filaments [illegible]
Fritsch & John H. nitens.	From soil cultures	5.7-10µ by shorter than broad to 3 times as long		Short, 2-10 celled	One pyrenoid often elongate	Parietal with [illegible] of [illegible] Leaves ½ of wall free	[illegible] silky film [illegible]
Bristol Stichococcus nitens	From soil cultures	5-6µ x 8-15µ long		Single cells or 2-4 celled Occasionally long filaments	Single pyrenoid 2 in cells about to divide	Single parietal chloroplast covers 2/3 circumference of cell and total length	Disarticulation by splitting apart and rounding off of cells. Parallel filaments form a stratum.

N.B. Hazen states that as he did not study fresh material he could not question the distinctness of the species but he found that the Exsiccata scarcely distinguishable from S. subtilis.

78. Hormidium crassum Chodat

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	CHLOROPLAST & CELL CONTENTS	PYRENOID	COLOR REACT	OTHER CHARACTERS
Heering H. crassum Chodat	Scarcely known out of culture	6.5-7.5μ (8) x 15-20u long			Distinct pyrenoid		Forms silky tufts in liquid culture. On glucose agar grows faster than H. nitens and H. flaccidum

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8. Hormidium dissectum Chodat.

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	PLASTID SIZE & CELL PLATES	PYRENOID	CHEMICAL TEST	LIFE CYCLE NOTES
Heering Hormidium dissectum Chodat	Dark green film on trees and other substrates	7-9μ x 1-1½ times as long	Somewhat thicker than in other aerial spp	Short rils scarcely 1C cells.	One pyrenoid		rils, often heavily bent & easily disintegrating. Only known method of separation disintegration akinetes.
In culture behaves much as H. flaccidum							

29. Hormidium lubricum Chodat

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	PLASMA & CELL PLATE	PYRENOID	NUCLEUS	OTHER CHARACTERS
Heering H.lubricum	Scarcely known out of culture	5-6μ x 8-25μ long	Delicate	Long, not easily disintegrating fils.	One pyrenoid not always distinct.	Large but scarcely covering a side of the cell wall. A little incised at edge.	Slimy film in liquid culture. Slippery & glistening on agar-Glucose.

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10. Hormidium mucosum Boy. Pet.

AUTHOR and Name	HABITAT & ECOL	SIZE	WALL	CHLOROPLAST SIZE & CELL WALL	PYRENOID	CONJUGATION	OTHER FEATURES
Lund H. mucosum.	In soils in company with H. flaccidum	15-20u x 9-22 long	two layered wall - not stratified (outer layer mucilaginous)	Outer wall dissolves in chlor-zinc iodide without giving cellulose reaction	Commonly 1. not always visible even after staining.	Band shape but very variable	Fragmentation by separation of middle lamellae, often swellings (plug) at those points. of U. tenuissima as described by Bristol.
Heering Ulothrix mucosa Thuret		8-10u by 1-2 times as long	Strongly thickened cross walls between 4-8 daughter cells Mucilage sheath		Usually one pyrenoid		4 ciliate microzoospores
Bristol? U. tenerrima	In soil	18u x 10 - 17u			One pyrenoid (two pyrenoids practically in one cell only)	Whole length x 2/3 circumference	

11/. Hormidium crenulatum Kutz. Sec. Brand.

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SHAPE & CELL SHAPE	CONTENTS	CHLOR. MASS	OTHER FEATURES
Fritsch & John H. crenulatum (Kutz)	Elongate fils. on soil	10-20μ x ½-1 times as long	Thick and stratified An uneven edge. Occasionally H pieces (One or wall rather thin)	Long	Distinct with starch sheath	Chl. with rounded edges dense ..sometimes. appears axile	No tendency to fragment Occasional long divisions
DITTO var β	Found in liquid culture.	10-24μ	Thick, more or less laminated walls	Usually constricted at the cross walls as in H. moniliformis Kutz.	As in typical	As in typical form	Occasional long divs. Occ. cells round off to give pinnates.
Collins Schizogonium crenulatum (Kutz) Gay nb.	On moist wood etc - fils. forming a thin stratum. Bright or dull green	11-14μ x 1	Wall thick. walls between cells quite thick	Cells swollen Filaments more or less moniliform or crenulate	One pyrenoid	Central stellate chl	Cells occasionally duplicate

Described by Heering as Hormidiopsis crenulata.

1/2. Hormidium Klebs"11 G.M.Smith 1933

AUTHOR and NAME	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOID	CHROMATOPHORE	OTHER FEATURES
Prescott	Spagnum bogs Roadside ditches.	5.8-6μ x 15.6-25μ long		Not constricted	? no mention pyrenoid	A parietal plate covering only small portion of wall.	

14.3. Stichococcus scopulinus Hazen (Gen. Torr. Bot. Club 1902)

AUTHOR and Name	Habitat & Form	Size	Wall	Filament Size & Cell Shape	Pyrenoid	Chloroplast	Other Features
Hazen.	Long fils form bright green lubricous masses. Dripping rocks	3-3.5μ x 1-10 times as long	Very thin	Cells cylindrical, not constricted	Not distinct	Narrow pale green without a distinct pyrenoid	Zoospores 1 cell more or less than veg. repro
Prescott S. scopulinus Hazen	Stones & Soil	3-4μ x up to 30μ long		Long cells, no constriction	Indistinct pyrenoid	A long folded plate	Could not differentiate clearly from S. bacillaris [illegible] idea
Collins S. scopulinus Hazen	Long fils form bright green lubricous masses on dripping rocks	3-3.5μ x 1-10 times as long	Very thin		Indistinct	Plate filaments	When collected soon breaks up to give abund. zoospores
Heering Gloeotila scopulinus (Hazen) Heering.	Bright green slippery mass	3-3.5μx 1-12 times as long	Very thin	Not constricted	Without distinct pyrenoid	Cell, light green.	Zoospores more common than disintegration

1/4. Ulothrix subtilissima Rabenhorst ~~Flora Europaea algarum aquae dulcis et submarinae~~

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	CHLOR.	MICRO. ASS.	OTH. CHARACTERS
Heering U. subtilissima Rabenh.	Standing and flowing water and in plankton	4-5μ by 1-2 or 1-5 times as long			Distinct parietal		Akinetes by reduction unknown
Prescott	Long slender fils. free floating or attached	4-5μ by 11-14.8μ long		Cells v. slightly inflated and constricted at cross wall.	One parietal plate length of cell.		

165. Ulothrix variabilis (Kutz) Kütz. Spec. Alg. 346 1849

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	SHAPE & SIZE [illegible]	PYRENOID	CHROMATOPHORE	OTHER CHARACTERS
Hazen	Brooks and stagnant water	5-6µ x $\frac{1}{2}$-$1\frac{1}{2}$ times as long	Very thin and delicate	Cells often square in optical sect.	One small but distinct	[illegible] rarely cover-ing more than [illegible] cell wall. [illegible] as a rectangular plate. Some-times angular mass in corner.	
Heering U. variabilis	Pale green flocculent masses in flowing or standing water	5-7µ x $\frac{1}{2}$-$1\frac{1}{2}$ times as long (or 2)	Very thin	Cylindrical		About [illegible] the cell wall. [illegible] square or rectangular plate often irregular. [illegible] angular mass in corner of cell.	Reproduction littl known. Micro-zoospores 2-4/ cell. Aplanospores [illegible]/cell.
Collins "	Floccose masses in brooks and quiet waters.	5-6µ x $\frac{1}{2}$-$1\frac{1}{2}$ times as long	Very thin and delicate	Cells cylindrical	Single small	Occupying about half wall often irregular in shape and position.	
Prescott U. variabilis	Long slender entangled fils forming cottony masses	4.5-6µ x up to 15µ long		Cells cylindrical without constrictions	One pyrenoid (?2)	Folded parietal plate $\frac{1}{2}$-2/3 length of cell.	
Bristol U. subtilis var. variabilis	From soil Probably =H. flaccidum	8-9µ x 6-17µ long			Single lge. pyrenoid not always seen in winter	Parietal chl. total length x 2/3 circum.	After time break up into few celled fragments.

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176. ULOTHRIX TENERRIMA Kutz 1843 Phycologia generalis

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOID	CHLOROPLAST	OTHER FEATURES
Heering		7-9µ (10) x 2/3-1⅓ times as long	Very thin, somewhat mucilaginous		One pyrenoid	Girdle shaped (good nutrients covering more than wall or covering one side (Bad conditions)	Reproduction chiefly by 2-celled or Hormospora stage. Microzoospores arise singly rare.
Collins	Light green silky masses often of considerable length	7-9µ x 2/3 -1⅓ times as long	Very thin	Cells cylindrical	Single pyrenoid	Lonate or contracted to one side	Near U. variabilis but larger
Hazen	Light green silky or floccose masses often 1 dm long. Iron fountain basin - watering trough	7.5-9µ x 2/3-1⅓ times as long	Very thin	Cells cylindrical	One pyrenoid	Lonate or contracted to one side	

18.7 ULOTHRIX MONILIFORMIS Kütz Spec.Alg. 347 1849

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	SPECIES	CHROMATOPHORE	OTHER OBSERVATIONS
Hazen		11-14μ	thickened	More or less crisped or torulose.			
Collins		11-14μ x as long as broad	thick walls	crisped or torulose			? a fruiting condition of another species.
Heering U. moniliformis Kutz.	Filaments light green. In standing water in small pools bogs and moors.	9-14μ x as long or shorter	Cell wall thick	Fils. more or less curled up. Distinctly constricted.		Usually ellipsoidal yellow green occ. band shaped	Reproduction through akinetes only observed.

18. ULOTHRIX SUBCONSTRICTA (Algological Notes XLV - XVII J.Bot. 53 73-84 1915)

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	STIGMATA	MICROGONIDIA	OTHER FEATURES
Prescott	Planktonic	5-7.9μ x 10-16u long	Some-times agelatinous sheath.	Slightly inflated cells moderately constricted at cross walls.	Sometimes one pyrenoid	About 2/3 media region.	

19. Ulothrix rorida Thuret

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	STEROID	CHLOROPLAST	CELL PECULIARITIES
Lind.	On stones in turbid water (Jan-Mar). Ponds.	14-20u and 13-18u x as long as wide.		Fils, 5" long	One gyrosi[illegible]	[illegible]rms a [illegible]mplete [illegible]nd.	Holdfast colourless also secondary rhizoids. U. oscillarina as described by Gross. Microzoospores (4 cilia) (2), 3-4 per cell. Gametes 16 or more/cell. bicilinte.

270. <u>ULOTHRIX TENUISSIMA</u> Kutz.

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	FILAMENT SIZE & CELL SHAPE	PYRENOIDS	CHLOROPLAST	OTHER FEATURES
Prescott		16-20µ x up to 1	Thin wall	Not constricted	2 or several pyrenoids	Broad band encircling about 2/3 circumference of cell	
Collins	Filaments dark green	15-20 (25)µ x about ½ diam	Thinner than U. Zonata	Cylindrical (except when fruiting)		A broad band.	
Hazen	Running water in brooks - watering troughs. Dark green	15-20 (25)µ x ½ as long or shorter		Not at all constricted.		[illegible]	Zoosporiferous cells somewhat moniliform
Heering U. tenuissima Kütz	Dark green turf in moving water (partic. mountainous regions)	15-25µ (or 14-30µ) ½ or c. ¼ times as long. (1. in young)	Thin	Not constricted	2 or more pyrenoids	Broad band	[illegible] ores in somewhat swollen filaments

~~22~~21. ULOTHRIX OSCILLARINA Kutz (Phyc. Germ. 197 1845)

AUTHOR and Name	HABITAT & FORM	SIZE	WALL	PROTOPLAST & CHROMATOPHORE	PYRENOID	CHROMATOPHORE	OTHER FEATURES
Hazen	In a ditch	11μ x ¼-½ times as long (1)				Chromatophore a broad band	
Collins	Soft mucilaginous masses quiet or slow running water	11μ x ¼-½ (rarely 1)	mucilaginous			A broad band	(A plant of summer)
Heering U. oscillarina Kutz.	Vivid green, smooth slimy turf on irrigated stones in dripping and standing water and larger flowing waters	Mostly 11μ occ. 10-14μ x ¼-½ times as long.	Thin. easily becoming mucilaginous		Usually with two or more pyrenoids ?	A broad girdle form band occupying most or ⅔ of the circumference	Little known differentiated from U. tenuissima by small width)
Gross U. oscillarina Kutz.	From rivers	8-9-20μ			One pyrenoid		Hind identifies this with her U. rorida. Kuret.
	Identified as nearest Heerings description.						

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APPENDIX II. CULTURE SOLUTIONS

The formulae of the solutions used is given below with a reference to the source of the formula and notes on any modifications made.

KNOPS SOLUTION

The composition of the solution as given by Mclean & Cook (1941) is :-

1.	KNO_3	1 gramme
2.	$MgSO_4\ 7H_2O$	1 gramme
3.	$Ca(NO_3)_2$	3 grammes
4.	K_2HPO_4	1 gramme
5.	$FeCl_3$	1 drop of 1%

1000 mls of distilled water

The solution was made up at $\frac{1}{4}$ this strength. The salts 1, 2 and 4 were dissolved in 500 mls of the water, 3 was dissolved in the other 500 mls and the solutions autoclaved separately and mixed when cooled.

MOLISCHS SOLUTION

The composition of this solution was as given by Pringsheim 1946 pg 35.

1.	$(NH_4)_2 H_2O_4$	0.8 grammes
2.	K_2HPO_4	0.4 "
3.	$MgSO_4\ 7H_2O$	0.4 "
4.	$CaSO_4$	0.4 "
5.	$FeSO_4\ 7H_2O$	1 drop of 1% in 100 mls

1000 mls of distilled water.

1 and - were dissolved in 500 mls of water, 3, 4, and 5 in the other 500 mls. The Solutions were autoclaved separately.

BENECKS SOLUTION

The composition as given by Mclean and Cook (1941) is

$Ca(NO_3)_2$	0.5 grammes
$MgSO_4\ 7H_2O$	0.1 "
K_2HPO_4	0.2 "
$FeCl_3$	a trace

1000 mls of distilled water.

This is not the "Beneckes" solution used by some phycologists. Some workers use Beneckes agar minus the agar and this is similar to Bjeirincks solution (cf. below and discussion by Bold 1942).. The solution given here is as in Benecke 1898a. Bot. Ztg. 56.

BJEIRINCKS SOLUTION

The composition as given by Pringsheim (1948 pg 35) is:

$NH_4\ NO_3$	1 grm
$K_2\ HPO_4$	0.2 "
$MgSO_4\ 7H_2O$	0.1 "
$FeCl_3$	0.001 "

1000 mls of water.

This solution is quoted as being that of Bjeirinck (1898 Zbl. Bakt. 2:4) but Bold giving the same reference includes

$CaCl_2$ $2H_2O$ 0.01 gms but not $FeCl_3$.

HERVEYS SOLUTION.

The composition as given by Hervey (1949) is :

KNO_3	400 p.pm	$MnSO_4$ $4H_2O$	15 p.pm
K_2HPO_4	50 p.pm	$Fe(NH_4)_2(SO_4)_2$ $4H_2O$	20 p.pm
$MgSO_4$ $7H_2O$	250 p.pm	Na Si O_3 $9H_2O$	500 p.pm
$CaCl_2$ $2H_2O$	20 p.pm	KI	10 p.pm
K_2CO_3	500 p.pm	Distilled water	1000 mls.

$NaSiO_3$ $9H_2O$ was omitted as it was not available. As the culture solution was not to be used for diatoms the omission of silicate was not considered important.

GODWARDS SOLUTIONS

The composition as given by Godward (1941) is :

NH_4Cl	0.00003 grms	This solution is a modified
K_2HPO_4	0.08 "	Chu solution, K_2SiO_3 was omitted
$MgSO_4$	0.08 "	as in Hervey's solution and
Na_2SO_4	0.058 "	for the same reason
K_2SiO_3	0.0025 "	
$CaCO_3$	0.01 "	
$FeCl_3$	0.00003 "	
KNO_3	0.25 "	

MOORES SOLUTION

The composition of this solution as given by Poulton (1930) is:

NH_4NO_3	0.5 grms
KH_2PO_4	0.2 "
$CaCl_2$	0.1 "
$MgSo_4$ $7H_2O$	0.2 "

$FeSO_4$ $7H_2O$ a trace

1000 mls distilled water

This solution is only one of those used by Moore (Moore & Carter 1926. Moore & Karrer 1919)

PRINGSHEIM'S Solution.

The composition as given by Pringsheim 1946 pg 25 is:

KNO_3	2%
$(NH_4)_2$ HPO_4	0.002%
$MgSO_4$ $7H_2O$	0.001%
$CaCl_2$ $6H_2O$	0.00005%
$FeCl_3$	0.00005%

This is only one of solutions used by Pringsheim.

USPENSKAJA'S SOLUTION.

This solution as given by Uspenski and Uspenskaja 1925 has the composition:-

KNO_3	0.025 grms
$MgSO_4$	0.025 "
$Ca(NO_3)2$	0.100 "
KH_2 PO_4	0.025 "
K_2CO_3	0.0345 "
$Fe(SO_4)_2$	0.00125 "

SOIL SOLUTION

Soil solution was prepared in the following way:

A soil extract was first prepared. 300 grammes of garden or arable field soil was weighed as collected but collection was not made immediately after rain. The soil was autoclaved with 600 mls of distilled water in a Pyrex flask at a pressure of 20 lbs per square inch for thirty minutes. The resulting liquid was immediately filtered, the first filtrate being returned as a clear filtrate was not obtained until soil partly blocked the filter paper. The filtrate, which was collected over night was made up to 500 mls. This soil extract was then diluted to make up soil solution, 150 mls. of soil extract and 0.15 gms of potassium nitrate were made up to 1000 mls, with distilled water. This solution was then autoclaved at 20 lbs. pressure for twenty minutes.

This method is a modification of the method described by Bold (1942).

Solutions with adjusted pH

Solutions with different pH were made by mixing 50 ccs of buffers solution with 50 ccs of nutrient solution.

The composition of the buffers solutions is given in the table below.

pH	M/5 K_2HPO_4	M/5 NaOH	
6.0	50 ccs.	5.64 ccs	Made up to 200 ccs in each case.
6.5	50 ccs.	15.07 ccs	
7.0	50 ccs	29.34 ccs	
7.5	50 ccs	41.24 ccs	
8.0	50 ccs.	46.85 ccs	
	0.2N Acetic acid	0.2n sodium acetate	
4.0	40. ccs	10 ccs	
4.5	28.8 ccs	21.2 ccs	Made up to 100 ccs in each case.
5.0	15.0 ccs	35.0 ccs	
5.5	6.0 ccs	44.0 ccs	
6.0	1.9 ccs	48.1 ccs	

Series of Culture solutions intermediate between Pringsheims and Molischs.

The compositions of these solutions is tabulated below.

No. of Soln.	$MgSO_4 7H_2O$	KNO_3	$(NH_4)_2HPO_4$	$CaCl_2 6H_2O$	$FeSO_4 7H_2O$	K_2HPO_4	$CaSO_4$
1	0.01	0.2	0.02	trace	-	-	-
2	0.01	0.2	0.02	trace	trace	-	-
3.	0.01	0.2	0.2	trace	-	-	-
4	0.01	0.2	0.4	trace	-	-	-
5	0.01	0.2	0.2	trace	-	0.2	-
6	0.01	0.2	0.2	trace	trace	-	-
7	0.01	0.2	0.4	trace	trace	-	-
8	0.01	0.2	0.2	trace	trace	0.2	-
9	0.2	0.2	0.02	trace	trace	-	-
10	0.2	0.2	0.2	trace	-	-	-
11	0.2	0.2	0.4	trace	-	-	-
12	0.2	0.2	0.2	trace	-	0.2	-
13	0.2	0.2	0.2	trace	trace	-	-
14	0.2	0.2	0.4	trace	trace	-	-
15	0.2	0.2	0.2	trace	trace	0.2	-
16	0.01	-	0.8	-	trace	0.4	0.4
17	0.2	-	0.8	-	trace	0.4	0.4
18	0.4	-	0.8	-	trace	0.4	0.4
19	0.4	-	0.8	-	trace	0.4	-
20	0.4	-	0.8	-	-	0.4	0.4
21	0.4	-	0.8	-	-	0.4	-
22	0.2	0.2	0.02	trace	trace	-	-
23	0.4	0.2	-	-	trace	0.4	0.4
24	0.4	0.2	-	-	trace	0.6	-

ALL WEIGHTS IN GRAMS

The phosphate Salts were dissolved in 500 cc distilled water. The other salts were dissolved in a separate 500 ccs of water and the two solutions autoclaved separately. They were mixed when cold.

FIGURES 1-8

Figure. 1.

Form of the algae collected from natural surroundings.

Camera lucida drawings of small portions of the filaments.

x 1200.

A. Source Aquatic 1. ? Ulothrix variabilis (Clones A.1.)

B. Source Aquatic 2. ? Stichococcus scopulinus (Clones A.2.)

C. Source Aquatic 3. ? Hormidium lubricum (Clones A.3.)

D. Source Aquatic 4. ? Hormidium subtile (Clones A.4.)

Figure 1

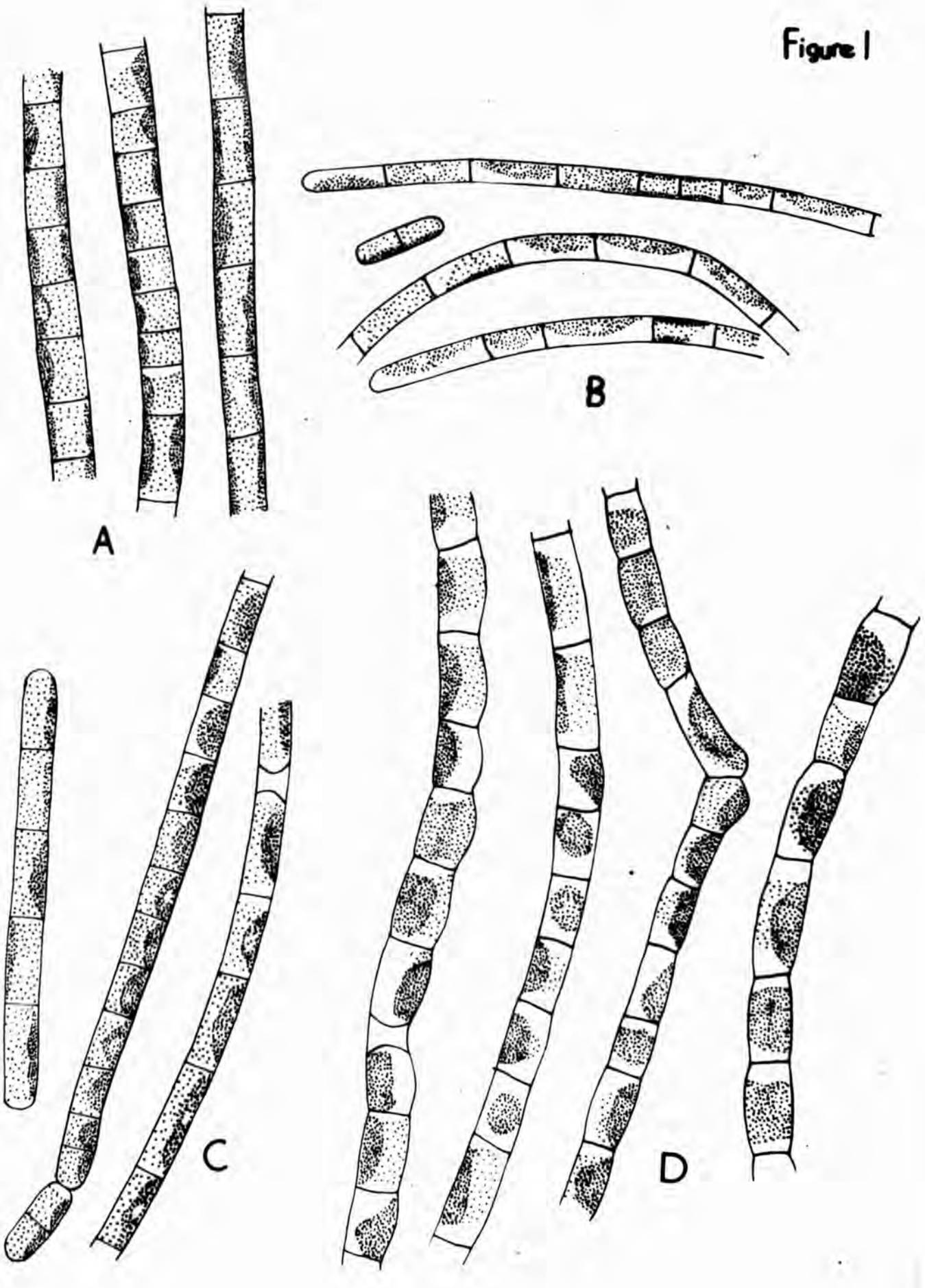

Figure. 2.

Form of the algae collected from natural surroundings.

Camera lucida drawings of small portions of filaments.

x 1200

M. Source Aquatic 5. ? Hormidium rivulare (form 2).
(Clones R.1)

L. Source Aquatic 5. ? Hormidium rivulare (form 3).
(Clones R.2)

O. Source Aquatic 6. ? Hormidium rivulare (form 1).
(Clones R.3)

P. Source Aquatic 6. ? Hormidium rivulare (form 4)
(Clones R.4)

Fig. 2

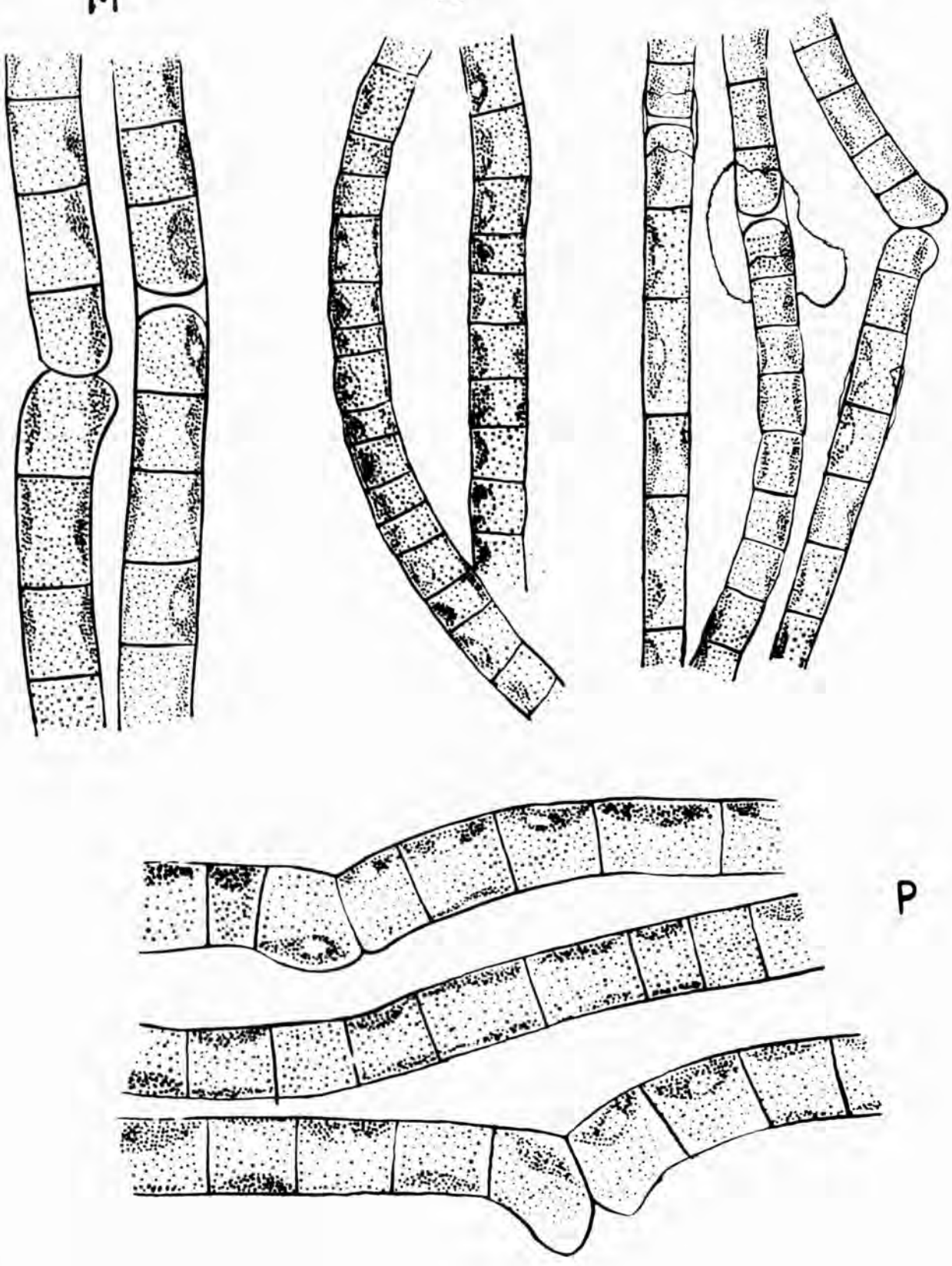

Figure. 3.

Form of the algae collected from natural surroundings.

Camera lucida drawings of small portions of filaments.

x1200

A. Source Terrestrial 1. 1 - ? Ulothrix subtilissima

2 - ? Hormidium flaccidum

♂ - ? Hormidium mucosum

(Clones T1,T2,T3,T4.)

B. Source Aquatic 7. ? Ulothrix tenuissima
(Clones R.5)

Fig. 3

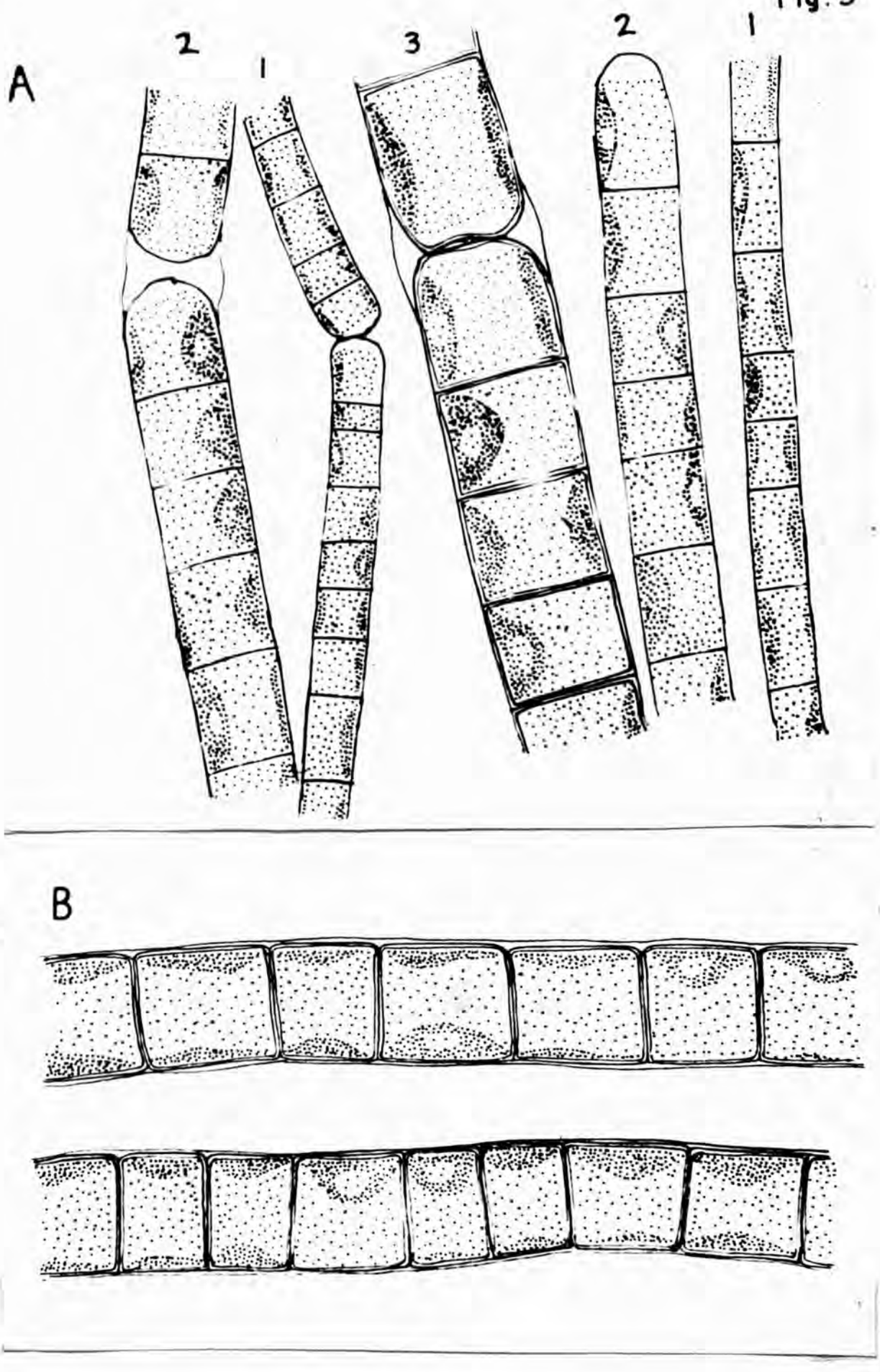

Figure. 4.

Form of algae identified as Uronema species.

Camera lucida drawings of small portions of filaments

x 1200.

A. Source U.1 ? Uronema gigas (Clones U.1)

B. Source U.2 ? U.confervicolum (Clones U.2)

C. Source U.3 ? U.confervicolum (Clones U.3)

D. Cambridge Culture Collection, U.confervicolum
(Clones U.confervicolum (C)).
This alga formed normal filaments in cultures in liquid media and would then have been identified as U.gigas

E. Cambridge Culture Collection, U.gigas
(Clones U.gigas (C)).

Fig. 4

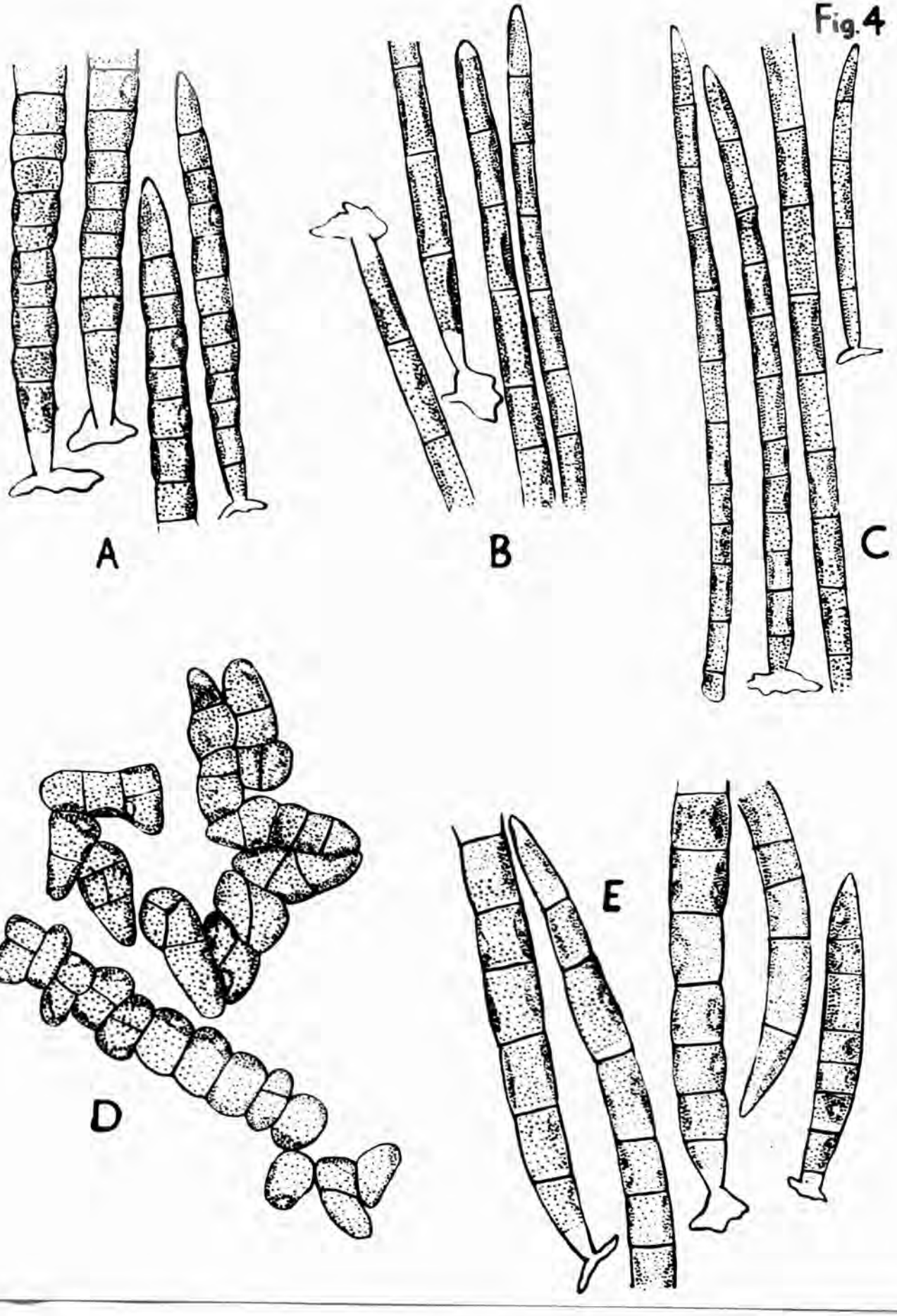

Figure. 5.

Possible indications of reproduction .

Camera lucida drawings of small portions of filaments.

x 800

A. Clone T.3. grown in soil solution.

B. Clone A.1. grown in soil solution.

Filaments show pores and rounded off cell contents.
The short tapering filaments may be germlings.

Fig.5

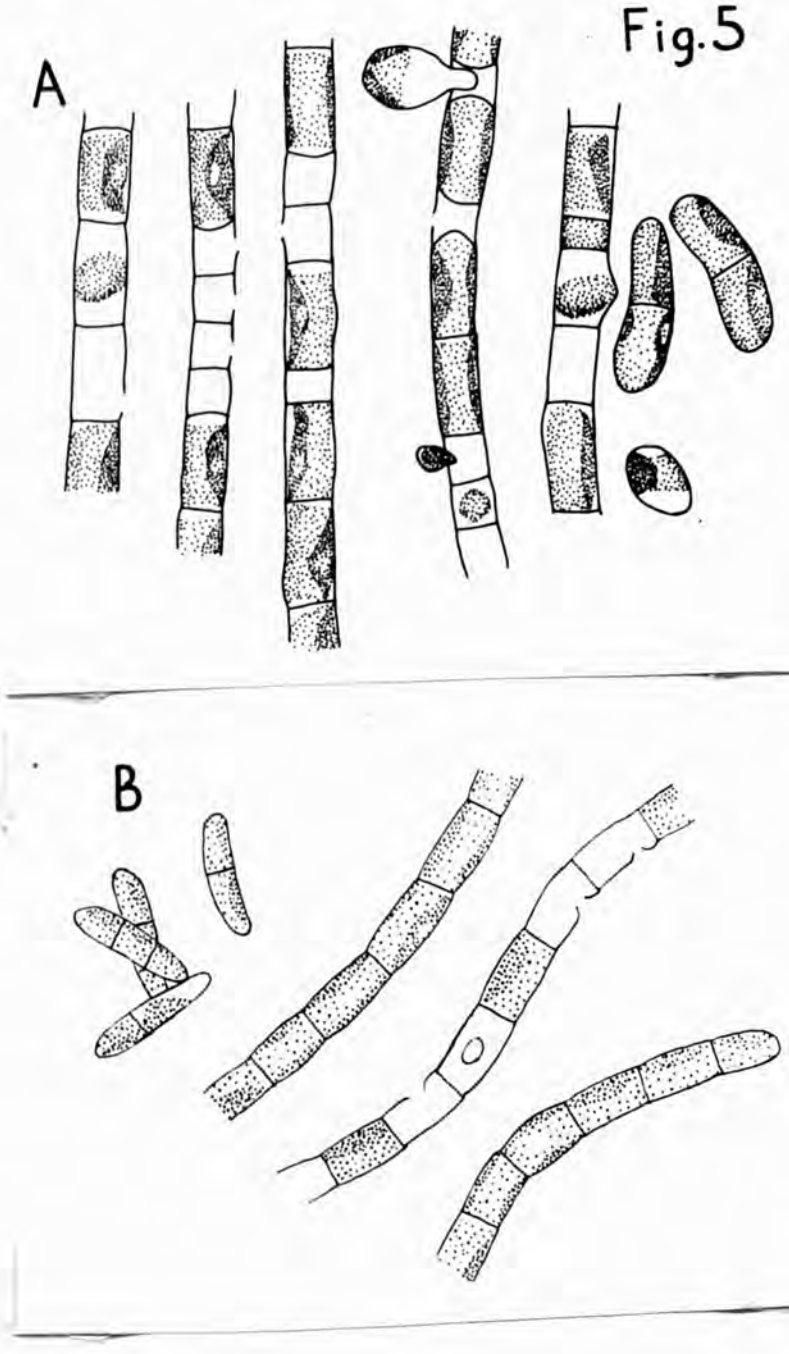

Figure 6.

Constriction of the filaments.

Camera lucida drawings of small portions of filaments

x 1200.

A. Clone A.1. grown in soil solution.

B. Clone R.3. 1 & 2 grown in Godwards solution.
3 grown in Pringsheims solution.

C. Clone T.3. grown in soil solution.

D. Clone T.4. grown in soil solution.

Fig.6

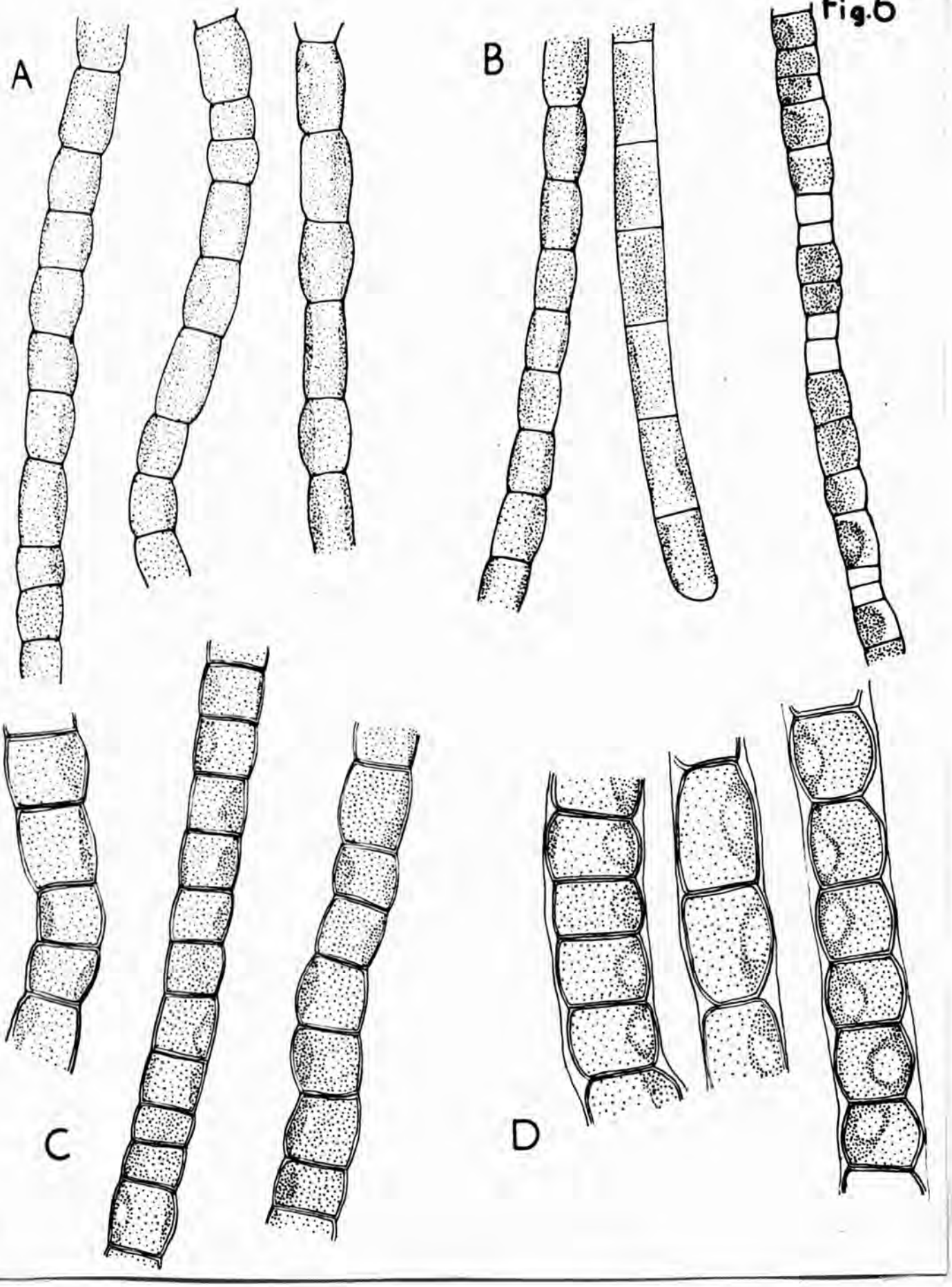

Figure 7.

Variation in wall thickening.

Camera lucida drawings of small portions of filaments.

x 1200.

W. Clone T.4. grown in Knops modified solution.

X. Clone T.4. Grown in soil solution.

Y. Clone T.4. grown in soil soln., with added dextrose.

Z. Clone T.4. grown in soil soln., with added yeast extract.

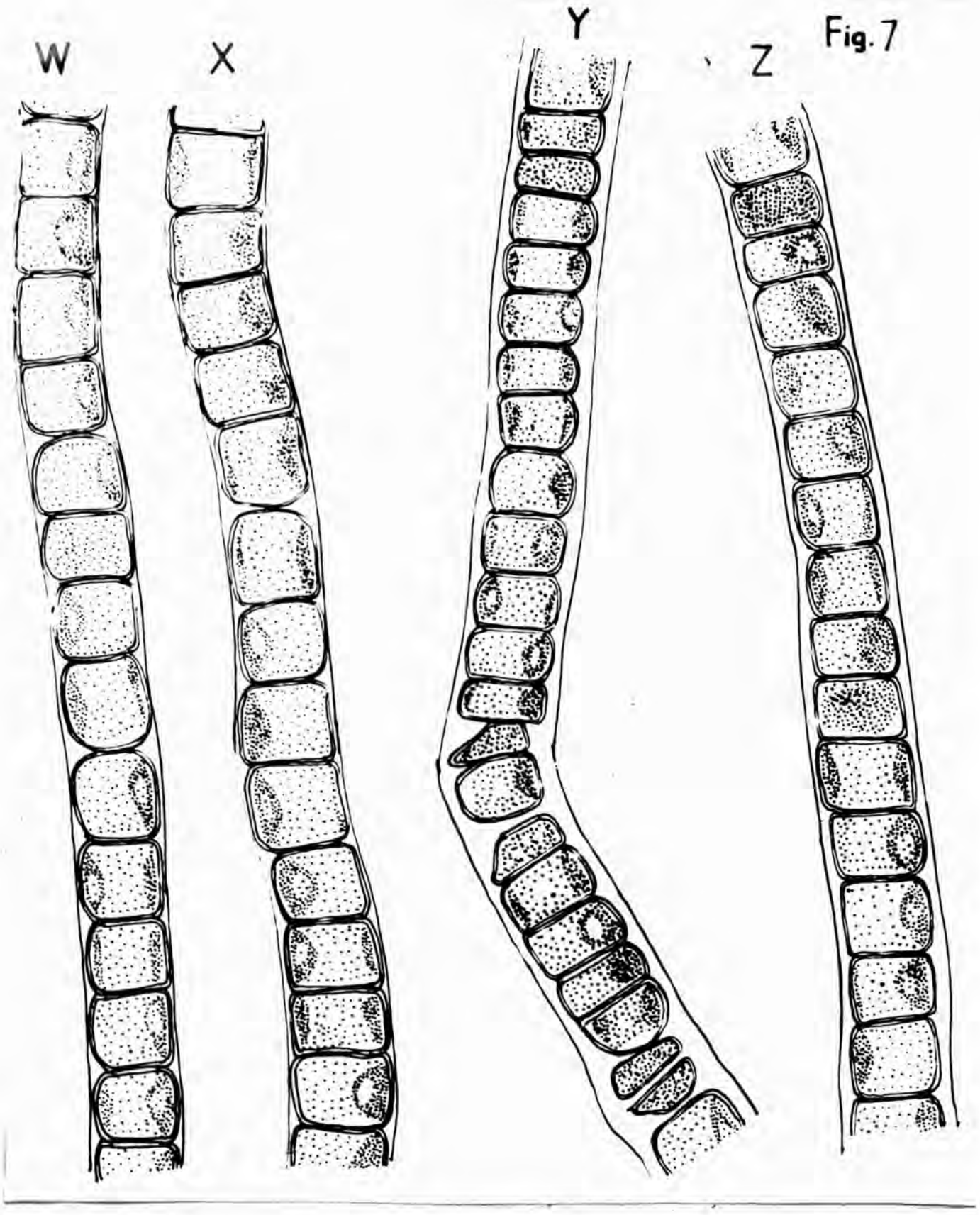

Fig. 7

Figure 8.

Wall thickening.

Free hand drawings of clone T.4. stained in methylene blue.

Material staining blue is stippled.

L. Shows material between some cross septa.

M. Shows H piece and two layered wall in some parts.

N. Shows H piece and completely two layered wall.

Fig. 8